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# Enantioselective synthesis and antiviral activity of purine and pyrimidine cyclopentenyl C-nucleosides

Jagadeeshwar R. Rao,<sup>a</sup> Raymond F. Schinazi<sup>b</sup> and Chung K. Chu<sup>a,\*</sup>

<sup>a</sup>The University of Georgia College of Pharmacy, Athens, GA 30602, USA

<sup>b</sup>Emory University School of Medicine/Veterans Affairs and Center for AIDS Research, Decatur, GA 30033, USA

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**Abstract**—The enantiomerically pure carbocyclic purine and pyrimidine C-nucleosides **1–4** were synthesized via the key intermediate, 2,3-(isopropylidenedioxy)-4-(trityloxymethyl)-4-cyclopenten-1-ol (**5**), which was prepared from D-ribose in eight steps. Synthesized compounds were evaluated as potential antiviral agents against HIV, SARS-CoV, Punta Toro, West Nile, and Cowpox viruses. However, only 9-deazaanplanocin A (**1**) exhibited moderate anti-HIV activity.

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## 1. Introduction

Synthesis of C-nucleoside and carbocyclic nucleoside analogs has been inspired by their interesting biological activities as well as chemical and enzymatic stability. C-Nucleosides are a unique class of compounds in which the heterocycle is connected to the sugar moiety by a C–C bond instead of a C–N bond of the natural nucleosides. As a result, they are resistant to the chemical and enzymatic hydrolytic cleavage of the glycosidic bond. C-Nucleosides have received considerable attention due not only to their chemical stability, but also for their interesting biological activities of naturally occurring compounds such as showdomycin, formycins, oxazinomycin, pyrazomycin, etc.<sup>1</sup> Also several biologically active synthetic C-nucleosides such as 9-deazaadenosine,<sup>2</sup> pseudoisocytidine,<sup>3</sup> and thiazofurin<sup>4</sup> have been reported to possess both antitumor and antiviral activities. Carbocyclic nucleosides are another class of metabolically stable nucleosides, in which a methylene group replaces the oxygen in the furan ring of the natural nucleosides.<sup>5</sup> The natural carbocyclic nucleosides such as neplanocin<sup>6</sup> and aristeromycin<sup>7</sup> were isolated from microorganisms and were found to possess various biological activities including antiviral and antitumor activities. The synthetic carbocyclic nucleosides, abacavir,<sup>8</sup> entecavir,<sup>9</sup> and

lobucavir,<sup>10</sup> have shown promising antiviral activities. Recently, abacavir and entecavir were approved by the FDA as an anti-HIV agent and anti-hepatitis B virus agent, respectively.

On the basis of these chemical and biological properties of C-nucleosides and carbocyclic nucleosides, it was of interest to synthesize hybrid nucleosides, carbocyclic C-nucleosides. Although some carbocyclic nucleosides and C-nucleosides are naturally occurring, so far no natural carbocyclic C-nucleosides have been identified. The history of the synthesis of carbocyclic C-nucleosides dates back to 1960s.<sup>11</sup> Despite the long history of both carbocyclic and C-nucleosides, only a few carbocyclic C-nucleosides have been synthesized,<sup>12</sup> probably due to the synthetic difficulties of these nucleosides. As part of our ongoing program toward synthesis of novel nucleosides, we were exploring the synthesis of cyclopentenyl carbocyclic nucleosides with unnatural bases.<sup>13</sup> Therefore, it was of great interest to synthesize optically

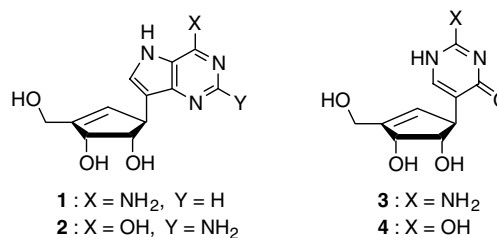


Figure 1. Structure of target compounds **1–4**.

**Keywords:** Carbocyclic C-nucleosides; Enantioselective synthesis; Antiviral activity.

\* Corresponding author. Tel.: +1 706 542 5379; fax: +1 706 542 5381; e-mail: dchu@rx.uga.edu

active carbocyclic C-nucleosides, possessing a cyclopentenyl moiety as analogs of neplanocin. Herein, we report the enantiomeric synthesis of purine and pyrimidine cyclopentenyl C-nucleosides **1–4** (Fig. 1) from the key intermediate, 2,3-(isopropylidenedioxy)-4-(trityloxymethyl)-4-cyclopenten-1-ol (**5**), and their antiviral activities.

## 2. Results and discussion

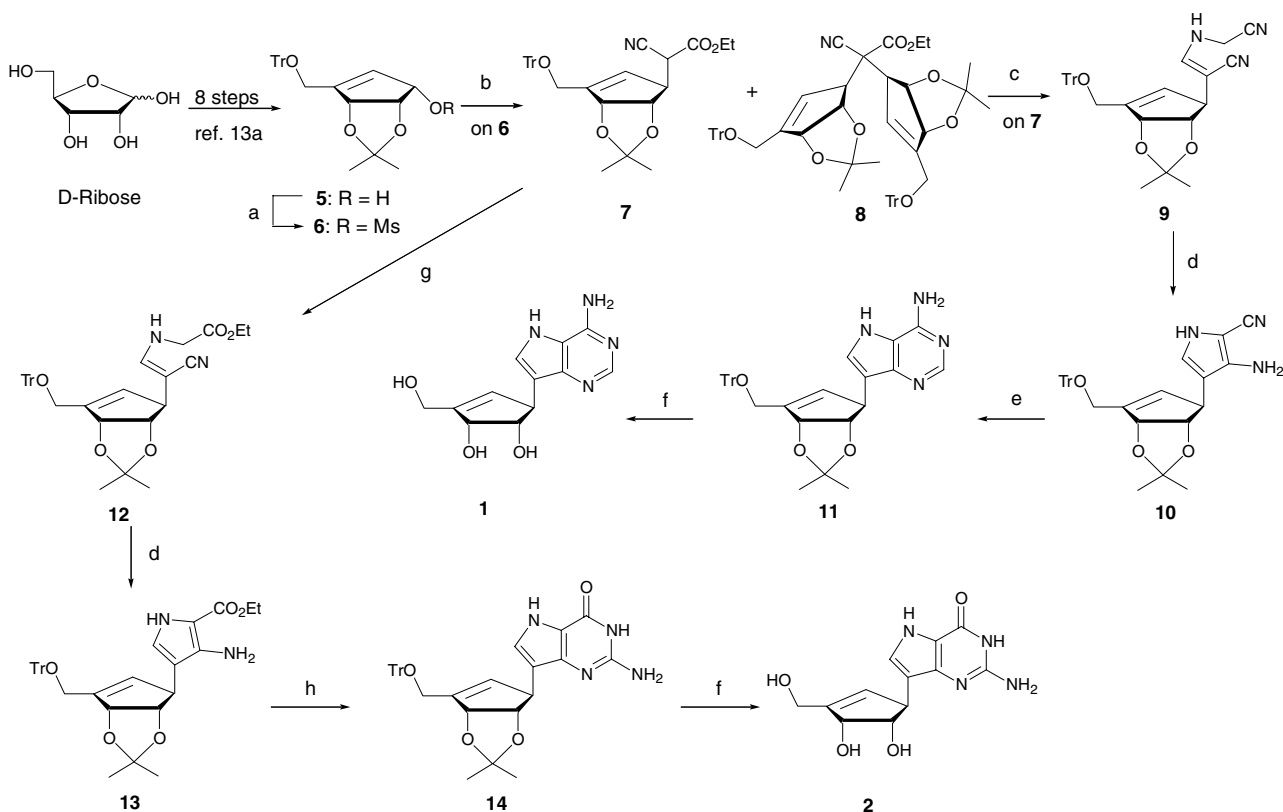
### 2.1. Synthesis

For the synthesis of target purine and pyrimidine carbocyclic C-nucleosides, we utilized the key intermediate **5** as a chiral starting material, which was prepared from D-ribose in eight steps using recently reported procedure from our group.<sup>13a</sup> In order to synthesize 9-deazaneplanocin A (**1**) (Scheme 1), the key intermediate **5** was converted to mesylate **6**, followed by SN<sub>2</sub> reaction with ethylcyanoacetate in the presence of sodium hydride to give compound **7** in 52% yield plus disubstituted product **8** in 10% yield. The optimal temperature was found to be 55 °C for this reaction, since at 70 °C the starting material decomposed. The assignment of β-configuration of **7** was performed by proton NOE experiments.<sup>13a</sup> Selective reduction of ester group of **7** with DIBAL-H, followed by condensation with aminoacetonitrile bisul-

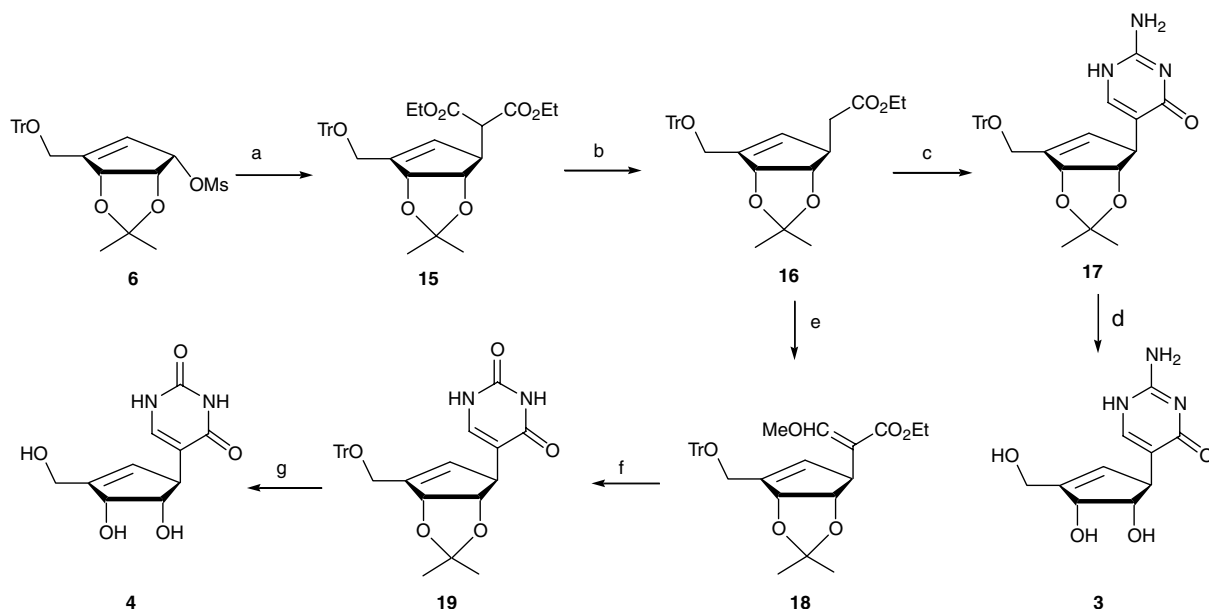
fate in the presence of sodium acetate, gave the pyrrole precursor **9**.<sup>12a</sup> Protection of the amino nitrogen of **9** as a carbamate with ethylchloroformate followed by cyclization catalyzed by 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) and subsequent deprotection of carbamate with sodium carbonate led to the substituted pyrrole **10**. Condensation of **10** with formamidine acetate in ethanol yielded the protected 9-deazaneplanocin **11** in 61%, which was deprotected with methanolic hydrochloric acid to afford the target compound, 9-deazaneplanocin A (**1**).

To synthesize the carbocyclic 9-deazaguanosine (**2**) (Scheme 1), substituted ethylcyanoacetate **7** was treated with DIBAL-H followed by condensation with ethylglycinate hydrochloride in the presence of sodium acetate to provide **12**. N-Protection of **12** with ethyl chloroformate, followed by ring closure with DBU and subsequent deprotection of carbamate, provided **13**. Treatment of **13** with N-benzoylisothiocyanate and methyl iodide, followed by treatment with methanolic ammonia in a steel bomb at 95 °C, gave the protected carbocyclic 9-deazaguanosine (**14**) in 64% yield. Deprotection of **14** with methanolic hydrochloric acid gave the target compound **2**.

The carbocyclic isocytosine (**3**) was prepared in four steps from the intermediate **6** (Scheme 2). The SN<sub>2</sub> reac-



**Scheme 1.** Reagents and conditions: (a) MsCl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 1 h; (b) NCCH<sub>2</sub>CO<sub>2</sub>Et, NaH, THF, rt then to 55 °C, 40 h; (c) i—DIBAL-H, Et<sub>2</sub>O, −78 °C, 30 min; ii—H<sub>2</sub>NCH<sub>2</sub>CN · H<sub>2</sub>SO<sub>4</sub>, NaOAc·3H<sub>2</sub>O, MeOH, rt, 24 h; (d) i—ClCO<sub>2</sub>Et, DBU, CH<sub>2</sub>Cl<sub>2</sub> 0 °C then to reflux, 6 h; ii—K<sub>2</sub>CO<sub>3</sub>, MeOH, rt, 1 h; (e) HC(=NH)NH<sub>2</sub>·AcOH, EtOH, reflux, 8 h; (f) i—12% HCl/MeOH, rt, 2 h; ii—NaHCO<sub>3</sub>/MeOH, rt, 2 h; (g) i—DIBAL-H, Et<sub>2</sub>O, −78 °C, 30 min; ii—H<sub>2</sub>NCH<sub>2</sub>CO<sub>2</sub>Et·HCl, NaOAc·3H<sub>2</sub>O, MeOH, rt, 24 h; (h) i—BzN=C=S, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 1 h; ii—MeI, DBN, CH<sub>2</sub>Cl<sub>2</sub>, rt, 2 h; iii—NH<sub>3</sub>/MeOH, 95 °C, 16 h.



**Scheme 2.** Reagents and conditions: (a)  $\text{CH}_2(\text{CO}_2\text{Et})_2$ , NaH, THF, 38 °C, 48 h; (b) LiCl, DMSO, 175 °C, 3 h; (c) i—LDA, THF, –78 °C, 2 h then ethyl formate, 12 h; ii—guanidine hydrochloride, NaOEt, EtOH, reflux, 12 h; (d) 10% HCl/MeOH, 2 h; (e) i—LDA, THF, –78 °C, 2 h then ethyl formate, 12 h; ii—MeI, DMF, rt, 4 h; (f) urea, KO<sup>t</sup>Bu, THF, reflux, 48 h; (g) i—10% HCl/MeOH, 2 h; ii—NaHCO<sub>3</sub>/MeOH, rt, 2 h.

tion on **6** with diethylmalonate in the presence of sodium hydride gave **15** in 72% yield. Mono-decarbomethoxylation of **15** in the presence of lithium chloride in DMSO gave **16**<sup>14</sup> in 86% yield, which was then treated with lithium diisopropylamide and ethyl formate in THF, followed by condensation with guanidine carbonate in the presence of sodium ethoxide in ethanol yielded **17** in 64%. Deprotection of **17** with methanolic hydrochloric acid gave the HCl salt of target compound **3**.

Preparation of the carbocyclic pseudouridine analog **4** was accomplished from **16** in three steps (Scheme 2). Compound **16** was treated with lithium diisopropylamide and ethylformate, followed by methyl iodide in

DMF, to give the, compound **18**<sup>15</sup> in 64% yield, which was condensed with urea in the presence of potassium *tert*-butoxide to afford **19**. Deprotection of **19** with methanolic hydrochloric acid gave the target compound **4** in 72% yield. All the compound structures were assigned based on NMR, UV, mass spectroscopy, and elemental analysis.

## 2.2. Biological results and conclusions

The synthesized carbocyclic C-nucleosides **1–4** were tested for anti-HIV activity as well as cytotoxicity using AZT as the positive control, and the results are summarized in Table 1. Anti-HIV activity was determined in

**Table 1.** In vitro anti-HIV-1<sub>LAI</sub> activity and toxicity of target nucleosides **1–4**

Compound	Anti-HIV-1 <sub>LAI</sub> activity		Cytotoxicity (IC <sub>50</sub> , μM)		
	EC <sub>50</sub> (μM)	EC <sub>90</sub> (μM)	PBM	CEM	Vero
<b>1</b>	2.0 ± 1.1 <sup>a</sup>	43.2 ± 28.8 <sup>a</sup>	15.6	10.6	>100
<b>2</b>	>100	>100	>100	>100	>100
<b>3</b>	>100	>100	>100	>100	>100
<b>4</b>	>100	>100	>100	>100	>100
AZT	0.0029	0.02	>100	14.3	50.6

<sup>a</sup> Standard deviation.

**Table 2.** Antiviral activities of synthesized nucleosides **1–4** against SARS, Punta Toro, Cowpox, and West Nile viruses

Compound	SARSCoV (μM)		Punta Toro (μM)		WNV (μM)		Cowpox (μM)	
	EC <sub>50</sub>	IC <sub>50</sub>	EC <sub>50</sub>	IC <sub>50</sub>	EC <sub>50</sub>	IC <sub>50</sub>	EC <sub>50</sub>	IC <sub>50</sub>
<b>1</b>	ND	ND	ND	ND	>100	>100	>100	>100
<b>2</b>	>100	>100	2.5	>100	>100	>100	>100	>100
<b>3</b>	>100	>100	>100	>100	11	>100	>100	>100
<b>4</b>	49	>100	65	>100	>100	>100	>100	>100
Neplanocin A	ND	ND	ND	ND	>51	ND	8.4	>300

ND, not determined.

human peripheral blood mononuclear (PBM) cells infected with HIV-1 (strain *LAI*). Among the synthesized nucleosides, only 9-deazaneplanocin A (**1**) showed moderate anti-HIV-1 activity ( $EC_{50}$  2.0  $\mu$ M) and the other compounds did not display any anti-HIV activity. Compounds **1–4** were also evaluated against a wide variety of viruses, including vaccinia, cowpox, severe acute respiratory syndrome (SARS), Punta Toro virus, West Nile virus, yellow fever, herpes simplex virus 1 and 2, varicella zoster virus, Tacaribe, Venezulean equine encephalitis, Dengue, and hepatitis C. Compounds **2** ( $EC_{50}$  2.5  $\mu$ M) and **4** ( $EC_{50}$  65  $\mu$ M) showed moderate activity against Punta Toro virus (strain Adames, LLC-MK2 cell line) without any cytotoxicity. Compound **3** was slightly active against West Nile virus (New York isolate) ( $EC_{50}$  11  $\mu$ M) and compound **4** ( $EC_{50}$  49  $\mu$ M) showed marginal activity against SARS-CoV (Table 2). None of other compounds showed any antiviral activity against all other tested viruses.

In summary, enantioselective synthesis of purine and pyrimidine cyclopentenyl C-nucleosides **1–4** was achieved from the common key intermediate **5**, which was prepared from commercially available D-ribose. Synthesized compounds were tested as potential antiviral agents. However, only 9-deazaneplanocin A (**1**) exhibited moderate anti-HIV activity.

### 3. Experimental

#### 3.1. Materials and methods

Melting points were determined on a Mel-temp II apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Varian Inova 500 spectrometer at 500 MHz for  $^1\text{H}$  NMR and 125 MHz for  $^{13}\text{C}$  NMR with tetramethylsilane as the internal standard. Chemical shifts ( $\delta$ ) are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). UV spectra were recorded on a Beckman DU-650 spectrometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. High-resolution mass spectra were recorded on a Micromass Autospec high-resolution mass spectrometer. TLC was performed on Uniplates (silica gel) purchased from Analtech Co. Column chromatography was performed using silica gel 60 (220–440 mesh) for flash chromatography or silica gel G (TLC grade, >440 mesh) for vacuum flash column chromatography or Radisep amine functionalized column (Combiflash, Isco, Inc.). Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

**3.1.1. (1S,2R,3R)-[2,3-(Isopropylidenedioxy)-4-(trityloxymethyl)-4-cyclopenten-1-yl]-methane sulfonate (6).** To a solution of **5** (12 g, 28.0 mmol) in anhydrous  $\text{CH}_2\text{Cl}_2$  (100 mL),  $\text{Et}_3\text{N}$  (11.64 mL, 84.0 mmol) was added under  $\text{N}_2$  atmosphere at 0 °C. After 5 min, methanesulfonyl chloride (3.24 mL, 42.0 mmol) was slowly added to the reaction mixture at 0 °C and then stirred for 1 h at room temperature. To the reaction mixture,  $\text{Et}_3\text{N}$  (20 mL) was added until the solution became basic and then solvent was removed under reduced pressure.

The residue was purified by silica gel chromatography (hexane–EtOAc = 8:2–7:3) to give compound **6** (13.2 g, 93%) as a white solid. Mp 92 °C;  $[\alpha]_{\text{D}}^{25}$  –10.72 (*c* 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  7.45–7.42 (m, 6H, Tr-H), 7.32–7.22 (m, 9H, Tr-H), 6.02 (d, 1H,  $J$  = 2 Hz, H-6), 5.43 (m, 1H, H-1), 4.86 (m, 2H, H-2, 3), 3.96 (d, 1H,  $J$  = 15.2 Hz, H-5a), 3.69 (d, 1H,  $J$  = 15.2 Hz, H-5b), 3.15 (s, 3H,  $-\text{CH}_3$ ), 1.37 (s, 3H,  $-\text{CH}_3$ ), 1.35 (s, 3H,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  147.8, 143.7, 128.5, 127.9, 127.2, 124.0, 113.5, 86.4, 80.8, 77.2, 61.0, 39.0, 27.5, 27.1; HRMS-ESI ( $m/z$ ): ( $\text{M}+\text{H}$ ) $^+$  calcd for  $\text{C}_{29}\text{H}_{30}\text{O}_6\text{S}$ , 507.1842; found, 507.1843.

**3.1.2. (2R/S,1'S,2'S,3'R)-Ethyl-2-[2',3'-(isopropylidenedioxy)-4'-(trityloxymethyl)-4'-cyclopenten-1'-yl]-2-cyanoacetate (7) and (1'S,2'S,3'R)-ethyl-2-bis[2',3'-(isopropylidenedioxy)-4'-(trityloxymethyl)-4'-cyclopenten-1'-yl]-2-cyanoacetate (8).** To a solution of NaH (1.87 g, 78.2 mmol) in THF (200 mL) was added ethylcyanoacetate (8.33 mL, 78.2 mmol) in THF (100 mL) under nitrogen atmosphere at 0 °C. After 1 h, **6** (13.2 g, 26.0 mmol) in THF (0.8 L) was added to the reaction mixture. The mixture was stirred at 50 °C for 40 h and then cooled to room temperature,  $\text{H}_2\text{O}$  (100 mL) was added and extracted with ethyl acetate (2  $\times$  300 mL). The combined organic layers were dried over  $\text{MgSO}_4$ , concentrated, and then purified on silica gel column chromatography (hexane–EtOAc = 9:1–8:2) to give compound **7** (7.09 g, 52%) and compound **8** (2.43 g, 10%) as white solids. Compound **7**: mp 50 °C;  $[\alpha]_{\text{D}}^{26}$  –14.45 (*c* 0.5,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  7.45–7.43 (m, 6H, Tr-H), 7.31–7.22 (m, 9H, Tr-H), 5.87 (d, 1H,  $J$  = 11 Hz, H-6'), 5.08 (d, 1H,  $J$  = 5.5 Hz, H-3'), 4.68 (d, 0.55H,  $J$  = 6.0 Hz, H-2'), 4.62 (d, 0.45H,  $J$  = 6.0 Hz, H-2'), 4.31 (m, 2H,  $-\text{OCH}_2-$ ), 3.84 (d, 1H,  $J$  = 15 Hz, H-5'a), 3.70 (m, 2H, H-5'b,  $-\text{CHCN}$ ), 3.48 (br d, 1H,  $J$  = 2.5 Hz, H-1'), 1.35–1.31 (m, 9H,  $-\text{CH}_3$ ,  $-\text{C}(\text{CH}_3)_2$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  165.0, 164.8, 147.27, 147.25, 143.9, 128.6, 128.5, 127.9, 123.4, 123.2, 115.2, 115.1, 111.4, 111.3, 87.1, 84.4, 84.3, 81.9, 81.4, 63.19, 63.14, 61.4, 51.8, 51.5, 41.2, 40.8, 27.4, 26.0, 25.9, 14.0; HRMS-ESI ( $m/z$ ): ( $\text{M}+\text{Na}$ ) $^+$  calcd for  $\text{C}_{33}\text{H}_{33}\text{NO}_5$ , 546.2256; found, 546.2248.

Compound **8**: mp 87 °C;  $[\alpha]_{\text{D}}^{26}$  –18.13 (*c* 1,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  7.45–7.43 (m, 12H, Tr-H), 7.32–7.21 (m, 18H, Tr-H), 6.22 (s, 1H, H-6') 5.78 (s, 1H, H-6''), 5.10 (d, 1H,  $J$  = 5.5 Hz, H-3'), 5.01 (d, 1H,  $J$  = 6.0 Hz, H-3''), 4.91 (d, 1H,  $J$  = 6.0 Hz, H-2'), 4.63 (d, 1H,  $J$  = 6.0 Hz, H-2''), 4.33 (m, 2H,  $-\text{OCH}_2-$ ), 3.88 (d, 1H,  $J$  = 15.5 Hz, H-5'a), 3.82 (d, 1H,  $J$  = 14.5 Hz, H-5''a), 3.74 (d, 1H,  $J$  = 15 Hz, H-5'b), 3.72 (d, 1H,  $J$  = 15 Hz, H-5''b) 3.64 (br s, 1H, H-1'), 3.51 (s, 1H, H-1'') 1.38, 1.35 (2  $\times$  s, 2  $\times$  3H,  $-\text{C}(\text{CH}_3)_2$ ), 130, 1.22 (2  $\times$  s, 2  $\times$  3H,  $-\text{C}(\text{CH}_3)_2$ ). 1.31 (t, 3H,  $J$  = 6.5 Hz,  $-\text{CH}_3$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  167.3, 148.0, 147.4, 143.9, 143.8, 128.6, 128.5, 127.9, 127.1, 122.7, 121.7, 117.1, 111.4, 111.1, 87.1, 84.1, 83.9, 81.4, 81.1, 63.3, 61.5, 57.2, 56.4, 55.5, 27.1, 27.3, 26.1, 25.7, 14.2; HRMS-ESI ( $m/z$ ): ( $\text{M}+\text{H}$ ) $^+$  calcd for  $\text{C}_{61}\text{H}_{59}\text{NO}_8$ , 933.4319; found, 933.4317.

**3.1.3. (1'S,2'S,3'R)-3-Amino-2-cyano-4-(2',3'-(isopropylidenedioxy)-4'-(trityloxymethyl)-4'-cyclopenten-1'-yl)-1H-pyrrole (10).** To a solution of **7** (3.50 g, 6.68 mmol) in anhydrous ether (60 mL), DIBAL-H (1 M in hexane, 13.36 mL, 13.36 mmol) was added at  $-78^{\circ}\text{C}$  over 10 min. The mixture was stirred at  $-78^{\circ}\text{C}$  for 30 min and quenched with MeOH (90 mL) at  $-78^{\circ}\text{C}$ . The mixture was stirred for 30 min and concentrated under reduced pressure. The resultant solid cake was suspended in EtOAc–MeOH (30:2) and then stirred for 2 h and filtered. After removal of the filtrate under reduced pressure, the residue was dissolved in MeOH (70 mL). Aminoacetonitrile bisulfate (2.57 g, 16.71 mmol), NaOAc·3H<sub>2</sub>O (2.18 g, 16.04 mmol) were added and the mixture was stirred at rt for 24 h. The solvent was evaporated and the residue was co-evaporated with MeOH (3× 60 mL). The obtained residue was suspended in EtOAc (100 mL) and stirred for 30 min. After filtration and concentration, the residue was purified using silica gel column chromatography (hexane–EtOAc = 8:2–7:3) to give compound **9** (1.54 g, 45%) as a white solid. To a solution of **9** (1.54 g, 2.97 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (75 mL), DBU (8.89 mL, 59.5 mmol) and ethyl chloroformate (2.26 g, 20.8 mmol) were added at 0 °C and the mixture was heated to reflux for 12 h. After removal of the solvent, the residue was dissolved in MeOH (30 mL) and water (1 mL), and then treated with K<sub>2</sub>CO<sub>3</sub> (55 mg, 0.42 mmol). The new mixture was stirred for 1 h at rt and solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (hexane–EtOAc = 8:2) to give **10** (730 mg, 21% from **7**) as a white solid. Mp 92 °C;  $[\alpha]_{\text{D}}^{25} - 18.37$  (c 0.5, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\text{max}}$ , nm: 249; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  7.87 (s, 1H, NH), 7.46 (m, 6H, Tr-H), 7.27 (m, 9H, Tr-H), 6.42 (s, 1H, H-5), 5.97 (s, 1H, H-6'), 5.09 (d, 1H,  $J = 5.5$  Hz, 1H, H-3'), 4.49 (d, 1H,  $J = 5.5$  Hz, H-2'), 3.90 (d, 1H,  $J = 14$  Hz, H-5'a), 3.79 (s, 1H, H-1'), 3.73 (d, 1H,  $J = 14$  Hz, H-5'b), 3.71 (br s, 2H, NH<sub>2</sub>), 1.40 (s, 3H, CH<sub>3</sub>), 1.31 (s, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  150.0, 143.9, 143.2, 141.6, 128.6, 127.8, 127.18, 127.12, 120.9, 114.6, 113.7, 111.2, 87.1, 85.0, 84.7, 61.4, 47.0, 27.5, 26.0; HRMS-ESI ( $m/z$ ): (M+Na)<sup>+</sup> calcd for C<sub>33</sub>H<sub>31</sub>N<sub>3</sub>O<sub>3</sub>, 540.2262; found, 540.2286.

**3.1.4. (1'S,2'S,3'R)-4-Amino-7-[2',3'-(isopropylidenedioxy)-4'-(trityloxymethyl)-4'-cyclopenten-1'-yl]-5H-pyrrolo[3,2-d]pyrimidine (11).** A solution of **10** (350 mg, 0.67 mmol) and formamidine acetate (352 mg, 3.38 mmol) in EtOH (20 mL) was heated under reflux for 8 h. The solvent was removed by reduced pressure and the residue was purified by silica gel column chromatography (CH<sub>2</sub>Cl<sub>2</sub>–MeOH = 98:2) to give **11** (224 mg, 61%) as a white solid. Mp 132 °C;  $[\alpha]_{\text{D}}^{25} - 16.12$  (c 0.40, MeOH); UV (MeOH)  $\lambda_{\text{max}}$ , nm: 274; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 500 MHz)  $\delta$  10.82 (s, 1H, NH), 8.11 (s, 1H, H-2), 7.46–7.25 (m, 16H, Tr-H, H-6), 6.75 (s, 2H, –NH<sub>2</sub>), 5.98 (s, 1H, H-6'), 5.22 (d, 1H,  $J = 5.5$  Hz, H-3'), 4.65 (d, 1H,  $J = 6$  Hz, H-2'), 4.16 (s, 1H, H-1'), 3.70 (d, 1H,  $J = 13.5$  Hz, H-5'a), 3.60 (d, 1H,  $J = 14$  Hz, H-5'b), 1.31 (s, 3H, –CH<sub>3</sub>), 1.24 (s, 3H, –CH<sub>3</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 125 MHz)  $\delta$  150.6, 150.3, 145.0, 144.1, 141.2, 128.7, 128.5, 128.4, 127.6,

125.6, 115.5, 114.1, 110.4, 86.8, 85.0, 84.7, 61.4, 46.1, 27.9, 26.3; HRMS-ESI ( $m/z$ ): (M+H)<sup>+</sup> calcd for C<sub>34</sub>H<sub>32</sub>N<sub>4</sub>O<sub>3</sub>, 545.2553; found, 545.2558; Anal. Calcd for C<sub>34</sub>H<sub>32</sub>N<sub>4</sub>O<sub>3</sub>: C, 74.98; H, 5.92; N, 10.29; found: C, 74.96; H, 5.88; N, 10.01.

**3.1.5. (1'S,2'S,3'R)-4-Amino-7-(2',3'-dihydroxy-4'-hydroxymethyl-4'-cyclopenten-1'-yl)-5H-pyrrolo[3,2-d]pyrimidine (9-deazaneplanocin) (1).** A solution of **11** (200 mg, 0.36 mmol) in 12% methanolic HCl (10 mL) was stirred at rt for 2 h. The solvent was evaporated under reduced pressure and the residue was co-evaporated with MeOH (3× 30 mL). The product was dissolved in MeOH (15 mL), neutralized with solid NaHCO<sub>3</sub>, stirred for 2 h, filtered, and concentrated to dryness under reduced pressure. The residue was purified by column chromatography (amine functionalized silica gel) (CH<sub>2</sub>Cl<sub>2</sub>–MeOH = 5:1) to give **1** (60 mg, 63%) as a white solid. Mp 202 °C;  $[\alpha]_{\text{D}}^{26} - 129.56$  (c 0.30, MeOH); UV (H<sub>2</sub>O)  $\lambda_{\text{max}}$ , nm: 276 (18,530, pH 2), 275 (11,760, pH 7), 274 (11,720, pH 11); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 500 MHz)  $\delta$  10.76 (s, 1H, NH), 8.11 (s, 1H, H-2), 7.33 (d, 1H,  $J = 2.0$  Hz, H-6), 6.73 (s, 2H, NH<sub>2</sub>), 5.83 (d, 1H,  $J = 2$  Hz, H-6'), 5.41 (br s, 1H, OH), 4.75 (t, 1H,  $J = 5.5$  Hz, OH), 4.53 (d, 1H,  $J = 6.5$  Hz, OH), 4.37 (t, 1H,  $J = 5.5$  Hz, H-3'), 4.08 (m, 3H, H-2',5'a,b), 3.92 (d, 1H,  $J = 2.5$  Hz, H-1'); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 125 MHz)  $\delta$  150.6, 149.9, 145.3, 144.9, 129.1, 125.6, 116.1, 114.1, 78.3, 73.6, 58.9, 46.0; HRMS-ESI ( $m/z$ ): (M+Na)<sup>+</sup> calcd for C<sub>12</sub>H<sub>14</sub>N<sub>4</sub>O<sub>3</sub>, 263.1148; found, 263.1170; Anal. Calcd for C<sub>12</sub>H<sub>14</sub>N<sub>4</sub>O<sub>3</sub>·0.8H<sub>2</sub>O: C, 52.03; H, 5.69; N, 20.02; found: C, 52.03; H, 5.69; N, 19.98.

**3.1.6. (1'S,2'S,3'R)-[3-Amino-4-(2',3'-(isopropylidenedioxy)-4'-(trityloxymethyl)-4'-cyclopenten-1'-yl)-1H-pyrrole]-2-carboxylic acid ethyl ester (13).** To a solution of **7** (5.2 g, 9.93 mmol) in anhydrous ether (80 mL), DIBAL-H (1 M in hexane, 19.8 mL) was added at  $-78^{\circ}\text{C}$  over 10 min. The mixture was stirred at  $-78^{\circ}\text{C}$  for 30 min and quenched with MeOH (90 mL) at  $-78^{\circ}\text{C}$ . The mixture was stirred for 30 min and concentrated under reduced pressure. The resultant solid cake was suspended in EtOAc–MeOH (30:2) and then stirred for 1 h and filtered. After removal of the filtrate under reduced pressure, the residue was dissolved in MeOH (70 mL). Ethylglycinate hydrochloride (3.32 g, 23.8 mmol) and NaOAc·3H<sub>2</sub>O (3.37 g, 24.8 mmol) were added and the mixture was stirred at rt for 24 h. The solvent was evaporated and the residue was co-evaporated with MeOH (3× 60 mL). The obtained residue was suspended in EtOAc (100 mL) and stirred for 30 min. After filtration and concentration under vacuum, the residue was purified using silica gel column chromatography (hexane–EtOAc = 8:2–7:3) to give compound **12** (2.12 g, 38%) as a white solid. To a solution of **12** (2.12 g, 3.75 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (75 mL) were added DBU (11.23 mL, 75.14 mmol) and ethyl chloroformate (2.50 mL, 26.30 mmol) at 0 °C and then the mixture was heated to reflux for 6 h. After removal of the solvent, the residue was dissolved in MeOH (30 mL) and water (1 mL), and then treated with K<sub>2</sub>CO<sub>3</sub> (50 mg). This mixture was stirred for 1 h at rt and solvent was

removed under reduced pressure. The residue was purified by silica gel column chromatography (hexane–EtOAc = 4:1) to give **13** (0.986 g, 18% from **7**) as a white solid. Mp 86 °C;  $[\alpha]_{\text{D}}^{25}$  – 16.94 (*c* 0.50, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 7.49–7.46 (m, 6H, Tr-H), 7.32–7.22 (m, 9H, Tr-H), 6.45 (br s, 1H, H-5), 6.0 (s, 1H, H-6'), 5.10 (d, 1H, *J* = 5.5 Hz, H-3'), 4.53 (d, 1H, *J* = 6.5 Hz, H-2'), 4.31 (q, 2H, –CH<sub>2</sub>), 3.90 (d, 1H, *J* = 14 Hz, H-5'a), 3.81 (s, 1H, H-1'), 3.73 (d, 1H, *J* = 14 Hz, H-5'b), 1.41 (s, 3H, –CH<sub>3</sub>), 1.35 (t, 3H, *J* = 7 Hz, –CH<sub>3</sub>), 1.31 (s, 3H, –CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 162.1, 144.0, 142.7, 128.6, 127.8, 127.5, 127.0, 120.2, 113.2, 111.0, 87.05, 85.2, 84.8, 61.4, 47.0, 27.6, 26.0, 14.7; HRMS-ESI (*m/z*): (M+Na)<sup>+</sup> calcd for C<sub>35</sub>H<sub>36</sub>N<sub>2</sub>O<sub>5</sub>, 587.2521; found, 587.2438.

**3.1.7. (1'S,2'S,3'R)-9-(2',3'-(Isopropylidenedioxy)-4'-(trityloxymethyl)-4'-cyclopenten-1'-yl)-9-deazaguanosine (14).** *N*-Benzoylisothiocyanate (0.214 mL, 1.59 mmol) was added to a solution of **13** (0.6 g, 1.06 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) at 0 °C. The reaction mixture was stirred for 1 h at rt and concentrated under vacuum. The residue was purified by silica gel column chromatography (hexane–EtOAc = 4:1) to give a residue (1.55 g). After being dried under high vacuum, the residue (1.55 g, 2.12 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) and treated consecutively with DBN (1.39 mL, 11.68 mmol) and MeI (0.30 mL, 4.67 mmol). The resulting reaction mixture was stirred for 2 h at rt, diluted with CH<sub>2</sub>Cl<sub>2</sub> (30 mL), washed with water (2× 20 mL), dried over MgSO<sub>4</sub>, and concentrated. The residue was purified by silica gel column chromatography (hexane–EtOAc = 4:1) to give a residue. The residue was dissolved in MeOH (10 mL), saturated with NH<sub>3</sub> (g) at –78 °C in a steel bomb under anhydrous conditions. The mixture was then heated for 16 h at 95 °C under tight sealing. After being cooled to rt, the mixture was concentrated under vacuum and the residue was purified by silica gel chromatography (hexane–EtOAc = 1:4) to give **14** (380 mg, 64%) as a white solid. Mp 168 °C;  $[\alpha]_{\text{D}}^{25}$  – 6.51 (*c* 0.50, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz) δ 7.48–7.44 (m, 6H, Tr-H), 7.31–7.20 (m, 9H, Tr-H), 6.89 (s, 1H, H-8), 5.93 (s, 1H, H-6'), 5.20 (d, 1H, *J* = 7 Hz, H-3'), 4.60 (d, 1H, *J* = 6.5 Hz, H-2'), 4.11 (s, 1H, H-1'), 3.80 (d, 1H, *J* = 14 Hz, H-5'a), 3.74 (d, 1H, *J* = 14 Hz, H-5'b), 1.34 (s, 3H, –CH<sub>3</sub>), 1.28 (s, 3H, –CH<sub>3</sub>); HRMS-ESI (*m/z*): (M+H)<sup>+</sup> calcd for C<sub>34</sub>H<sub>32</sub>N<sub>4</sub>O<sub>4</sub>, 561.2503; found, 561.2501; Anal. Calcd for C<sub>34</sub>H<sub>32</sub>N<sub>4</sub>O<sub>4</sub>: C, 72.84; H, 5.75; N, 9.99; found: C, 72.77; H, 5.69; N, 9.98.

**3.1.8. (1'S,2'S,3'R)-9-(2',3'-Dihydroxy-4'-hydroxymethyl-4'-cyclopenten-1'-yl)-deazaguanosine (2).** A solution of **14** (220 mg, 0.39 mmol) in 12% methanolic HCl (10 mL) was stirred at rt for 2 h. The solvent was evaporated under reduced pressure and the residue was co-evaporated with MeOH (3× 30 mL). The product was dissolved in MeOH (15 mL), neutralized with solid NaHCO<sub>3</sub>, stirred for 2 h, filtered, and concentrated to dryness under reduced pressure. The residue was purified by combiflash column chromatography (amine functionalized silica gel) (CH<sub>2</sub>Cl<sub>2</sub>–MeOH = 5:1) to give **2** (76 mg, 70%) as a white solid. Mp 198 °C;

$[\alpha]_{\text{D}}^{25}$  – 99.9 (*c* 0.25, MeOH); UV (H<sub>2</sub>O) λ<sub>max</sub>, nm: 273 (14,946, pH 2), 272 (12,040, pH 7), 270 (11,003, pH 11); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 500 MHz) δ 11.16 (s, 1H, NH), 6.90 (s, 1H, H-8), 6.24 (s, 2H, NH<sub>2</sub>), 5.76 (d, 1H, *J* = 1.5 Hz, H-6'), 4.78 (br s, 1H, –NH), 4.34 (d, 1H, *J* = 5.5 Hz, H-3'), 4.05 (t, 2H, *J* = 16 Hz, H-5'a,b), 3.94 (t, 1H, *J* = 6.0 Hz, H-2'), 3.75 (d, 1H, *J* = 2.5 Hz, H-1'), 3.42 (br s, 3H, 3× OH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 125 MHz) δ 155.6, 151.8, 145.4, 144.8, 128.2, 123.8, 115.3, 113.2, 78.5, 73.2, 59.0, 46.0; HRMS-ESI (*m/z*): (M+Na)<sup>+</sup> calcd for C<sub>12</sub>H<sub>14</sub>N<sub>4</sub>O<sub>4</sub>, 301.0912; found, 301.0921; Anal. Calcd for C<sub>12</sub>H<sub>14</sub>N<sub>4</sub>O<sub>4</sub>·0.2H<sub>2</sub>O: C, 51.13; H, 5.15; N, 19.88; found: C, 51.12; H, 5.15; N, 19.84.

**3.1.9. (1'S,2'S,3'R)-2-[2',3'-(isopropylidenedioxy)-4'-(trityloxymethyl)-4'-cyclopenten-1'-yl]-2-diethyl-malonate (15).**

To a solution of NaH (1.98 g, 82.9 mmol) in THF (200 mL), diethyl malonate (20.9 mL, 138.17 mmol) in THF (100 mL) was added under nitrogen atmosphere at rt. After 1 h, compound **6** (14 g, 27.6 mmol) in THF (0.8 L) was added to the reaction mixture. The mixture was stirred at 38 °C for 48 h and then cooled to room temperature, H<sub>2</sub>O (100 mL) was added and extracted with ethyl acetate (2× 300 mL). The combined organic layers were dried over MgSO<sub>4</sub>, concentrated, and then purified on silica gel column chromatography (hexane–EtOAc = 9:1) to give compound **15** (11.35 g, 72%) as a colorless liquid.  $[\alpha]_{\text{D}}^{25}$  – 11.68 (*c* 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 7.45–7.42 (m, 6H, Tr-H), 7.30–7.20 (m, 9H, Tr-H), 5.86 (d, *J* = 1 Hz, 1H, H-6'), 5.01 (d, 1H, *J* = 7.5 Hz, H-3'), 4.70 (d, 1H, *J* = 7 Hz, H-2'), 4.20 (m, 4H, –OCH<sub>2</sub>–), 3.80 (d, 1H, *J* = 18.5 Hz, H-5'a), 3.62 (d, 1H, *J* = 18.5 Hz, H-5'b), 3.46 (m, 2H, H-1', –CH–), 1.35 (s, 3H, –CH<sub>3</sub>), 1.31 (s, 3H, –CH<sub>3</sub>), 1.29–1.24 (m, 6H, 2× CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 168.2, 168.1, 144.4, 143.9, 128.5, 127.9, 127.8, 127.0, 125.8, 110.6, 86.8, 84.5, 82.0, 61.6, 61.5, 61.3, 54.2, 50.9, 27.5, 26.0, 14.1, 14.0; HRMS-ESI (*m/z*): (M+Na)<sup>+</sup> calcd for C<sub>35</sub>H<sub>38</sub>O<sub>7</sub>, 593.2515; found, 593.2519.

**3.1.10. (1'R,2'S,3'R)-Ethyl-2-[2',3'-(isopropylidenedioxy)-4'-(trityloxymethyl)-4'-cyclopenten-1'-yl]-acetate (16).**

To a solution of **15** (7.5 g, 13.4 mmol) in DMSO (50 mL), LiCl (1.67 g, 39.4 mmol) and H<sub>2</sub>O (2 drops) were added. The mixture was heated up to 175 °C for 3 h. After cooling the mixture, H<sub>2</sub>O (50 mL) was added and extracted with ethyl acetate (2× 200 mL), washed with brine, dried over MgSO<sub>4</sub>, and concentrated under reduced pressure. The product was purified using silica gel column chromatography (hexane–EtOAc = 9:1) to give **16** (5.69 g, 86%) as a white solid. Mp 119 °C;  $[\alpha]_{\text{D}}^{25}$  – 6.58 (*c* 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 7.46 (m, 6H, Tr-H), 7.30–7.20 (m, 9H, Tr-H), 5.86 (s, 1H, H-6'), 5.01 (d, 1H, *J* = 5.5 Hz, H-3'), 4.48 (d, 1H, *J* = 5.5 Hz, H-2'), 4.15 (q, 2H, –OCH<sub>2</sub>–), 3.83 (d, 1H, *J* = 14.5 Hz, H-5'a), 3.65 (d, 1H, *J* = 14.5 Hz, H-5'b), 3.19 (t, 1H, *J* = 6 Hz, H-1'), 2.40 (dd, 2H, *J* = 2, 7.5 Hz, –CH<sub>2</sub>–), 1.36 (s, 3H, –CH<sub>3</sub>), 1.31 (s, 3H, –CH<sub>3</sub>), 1.26 (t, 3H, *J* = 7 Hz, –CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 171.9, 144.0, 142.7, 128.6, 128.2, 127.8, 127.0, 110.6, 86.9, 84.5, 83.7, 61.4, 60.6,

47.3, 38.0, 27.6, 26.1, 14.2; HRMS-ESI ( $m/z$ ): (M+H)<sup>+</sup> calcd for C<sub>32</sub>H<sub>34</sub>O<sub>5</sub>, 499.2484; found, 499.2479.

**3.1.11. (1'S,2'S,3'R)-5-(2',3'-(Isopropylidenedioxy)-4'-(trityloxymethyl)-4'-cyclopenten-1'-yl)-isocytosine (17).**

To a solution of lithium diisopropylamide (2 M in hexane, 3.95 mL) in THF (20 mL), a solution of **16** (2.63 g, 5.27 mmol) in THF (40 mL) was added at –78 °C under nitrogen atmosphere. The mixture was stirred at the same temperature for 1 h and to this was added ethyl formate (1.69 mL, 21.09 mmol). After stirring at –78 °C for an additional hour, the mixture was stirred at rt for 12 h. To the mixture satd NH<sub>4</sub>Cl solution (50 mL) was added and extracted with EtOAc (2× 200 mL), washed with water (50 mL) and brine solution (50 mL), dried over MgSO<sub>4</sub>, and evaporated to dryness to obtain a crude residue. To a solution of guanidine carbonate (2.70 g, 14.99 mmol) in EtOH (50 mL), EtONa/EtOH (21 wt% solution, 6.47 mL, 19.99 mmol) was added and stirred for 10 min. To this mixture, the above-obtained residue in ethanol (10 mL) was added and refluxed for 12 h. The mixture was filtered, concentrated, and purified using silica gel column chromatography (EtOAc–MeOH = 95:5) to give **17** (1.78 g, 64 %) as a white solid. Mp 156 °C;  $[\alpha]_D^{25} + 18.59$  (*c* 0.8, MeOH); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 500 MHz)  $\delta$  10.93 (s, 1H, NH), 7.44–7.27 (m, 16H, Tr-H, H-6), 6.46 (s, 2H, NH<sub>2</sub>), 5.78 (s, 1H, H-6'), 5.08 (d, 1H, *J* = 5.5 Hz, H-3'), 4.40 (d, 1H, *J* = 5.5 Hz, H-2'), 3.76 (s, 1H, H-1'), 3.67 (d, 1H, *J* = 14.0 Hz, H-5'a), 3.57 (d, 1H, *J* = 14.0 Hz, H-5'b), 1.27 (s, 3H, –CH<sub>3</sub>), 1.22 (s, 3H, –CH<sub>3</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 125 MHz)  $\delta$  162.9, 155.7, 144.2, 142.8, 128.5, 128.4, 127.5, 126.9, 114.8, 110.0, 86.8, 84.7, 84.4, 61.6, 48.4, 28.0, 26.4; HRMS-ESI ( $m/z$ ): (M+Na)<sup>+</sup> calcd for C<sub>32</sub>H<sub>31</sub>N<sub>3</sub>O<sub>4</sub>, 544.2212; found, 544.2262; Anal. Calcd for C<sub>32</sub>H<sub>31</sub>N<sub>3</sub>O<sub>4</sub>: C, 73.68; H, 5.99; N, 8.06; found: C, 73.57; H, 5.95; N, 8.01.

**3.1.12. (1'S,2'S,3'R)-5-(2',3'-Dihydroxy-4'-hydroxymethyl-4'-cyclopenten-1'-yl)-isocytosine (3).**

A mixture of **17** (250 mg) and 10% methanolic hydrogen chloride (10 mL) solution was stirred at rt for 2 h. Concentration in vacuum gave a white solid which was co-evaporated with EtOH (3× 5 mL). The residue was triturated three times with 5 mL of ether, the compound **3** was obtained as a white powder (112 mg, 85%). Mp 186 °C (color change);  $[\alpha]_D^{25} - 186.65$  (*c* 0.1, MeOH); UV (H<sub>2</sub>O)  $\lambda_{max}$ , nm: 264 (6507, pH 2), 290 (4217, pH 7), 279 (5737, pH 11); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 500 MHz)  $\delta$  12.42 (s, 1H, NH), 8.28 (br s, 2H, NH<sub>2</sub>), 7.34 (s, 1H, H-6), 5.49 (d, 1H, *J* = 1.5 Hz, H-6'), 4.56 (br s, 3H, 3× OH), 4.31 (d, 1H, *J* = 5.0 Hz, H-3'), 4.05 (m, 2H, H-5'a,b), 3.90 (t, 1H, *J* = 5.0 Hz, H-2'), 3.56 (d, 1H, *J* = 2.5 Hz, H-1'); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 125 MHz)  $\delta$  160.8, 152.6, 147.7, 137.1, 125.4, 117.8, 76.1, 73.7, 58.9, 47.8; HRMS-ESI ( $m/z$ ): (M+H)<sup>+</sup> calcd for C<sub>10</sub>H<sub>13</sub>N<sub>3</sub>O<sub>4</sub>, 240.0985; found, 240.0963; Anal. calcd for C<sub>10</sub>H<sub>13</sub>N<sub>3</sub>O<sub>4</sub>·HCl: C, 43.57; H, 5.12; N, 15.24; found: C, 43.46; H, 5.09; N, 15.03.

**3.1.13. (1'S,2'S,3'R)-Ethyl-2-(2',3'-(isopropylidenedioxy)-4'-(trityloxymethyl)-4'-cyclopenten-1'-yl)-3-methoxy acrylate (18).**

To a solution of lithium diisopropylamide (2 M in hexane, 3.95 mL) in THF (20 mL) was added a solution of **16** (2.63 g, 5.27 mmol) in THF (40 mL) at –78 °C under nitrogen atmosphere. The mixture was stirred at the same temperature for 1 h and then ethyl formate (1.69 mL, 21.09 mmol) was added. After stirring at –78 °C for an additional hour, the mixture was warmed up to 0 °C and allowed to stir for 12 h at rt. The resulting mixture was evaporated to dryness and the residue was dissolved in dry DMF (15 mL) and treated with MeI (0.98 mL, 15.83 mmol) under nitrogen. After stirring for 4 h at rt, DMF was removed under reduced pressure and water (30 mL) was added, extracted with ethyl acetate (2× 100 mL). The combined organic layers were washed with brine (50 mL), dried over MgSO<sub>4</sub>, concentrated under reduced pressure, and purified by silica gel column chromatography (hexane–EtOAc = 9:1) to give **18** (1.82 g, 64%) as a white solid. Mp 58 °C;  $[\alpha]_D^{27} - 64.26$  (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  7.45 (m, 6H, Tr-H), 7.36 (s, 1H, =CH–OMe), 7.30–7.20 (m, 9H, Tr-H), 5.71 (s, 1H, H-6'), 5.16 (d, 1H, *J* = 5.6 Hz, H-3'), 4.68 (d, 1H, *J* = 6 Hz, H-2'), 4.12 (q, 2H, –OCH<sub>2</sub>), 4.04 (br s, 1H, H-1'), 3.83 (s, 3H, –OCH<sub>3</sub>), 3.82 (d, 1H, *J* = 14.0 Hz, H-5'a), 3.64 (d, 1H, *J* = 14.8 Hz, H-5'b), 1.38 (s, 3H, –CH<sub>3</sub>), 1.31 (s, 3H, –CH<sub>3</sub>), 1.23 (t, 3H, *J* = 6.8 Hz, –CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz)  $\delta$  167.3, 159.6, 144.2, 141.9, 128.6, 127.8, 126.9, 126.5, 110.3, 110.2, 86.9, 86.2, 84.3, 61.8, 61.6, 59.9, 47.1, 27.7, 26.2, 14.3; HRMS-ESI ( $m/z$ ): (M+H)<sup>+</sup> calcd for C<sub>34</sub>H<sub>36</sub>O<sub>6</sub>, 541.2591; found, 541.2590.

**3.1.14. (1'S,2'S,3'R)-5-(2',3'-(Isopropylidenedioxy)-4'-(trityloxymethyl)-4'-cyclopenten-1'-yl)uracil (19).**

A mixture of urea (133 mg, 2.22 mmol) and KO<sup>t</sup>Bu (246 mg, 2.22 mmol) in THF (5 mL) was stirred at rt for 30 min and then **18** (0.6 g, 1.11 mmol) in THF (8 mL) was added under N<sub>2</sub> atmosphere. The mixture was heated under reflux for 48 h and evaporated under reduced pressure. The product was purified using silica gel column chromatography (hexane–EtOAc = 8:2) to give **19** (208 mg, 36%) as a white solid. Mp 135 °C;  $[\alpha]_D^{25} + 14.69$  (*c* 0.55, MeOH); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 500 MHz)  $\delta$  11.15 (s, 1H, NH), 10.78 (s, 1H, NH), 7.42–7.26 (m, 15H, Tr-H), 6.97 (s, 1H, H-6), 5.75 (s, 1H, H-6'), 5.05 (d, 1H, *J* = 6 Hz, H-3'), 4.38 (d, 1H, *J* = 6 Hz, H-2'), 3.72 (s, 1H, H-1'), 3.66 (d, 1H, *J* = 13.5 Hz, H-5'a), 3.56 (d, 1H, *J* = 13.5 Hz, H-5'b), 1.25 (s, 3H, –CH<sub>3</sub>), 1.20 (s, 3H, –CH<sub>3</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 125 MHz)  $\delta$  164.3, 151.7, 144.2, 143.8, 138.3, 128.5, 128.4, 127.6, 126.0, 112.4, 110.2, 86.8, 84.5, 83.9, 61.5, 48.0, 27.8, 26.4; HRMS-ESI ( $m/z$ ): (M+Na)<sup>+</sup> calcd for C<sub>32</sub>H<sub>30</sub>N<sub>2</sub>O<sub>5</sub>, 545.2052; found, 545.2058.

**3.1.15. (1'S,2'S,3'R)-5-(2',3'-Dihydroxy-4'-hydroxymethyl-4'-cyclopenten-1'-yl)uracil (4).**

A mixture of **19** (200 mg) and 10% methanolic HCl (10 mL) solution was stirred at rt for 2 h. Concentration under reduced pressure gave a white solid, which was co-evaporated with EtOH (3× 3 mL). The residue was dissolved in methanol (5 mL), neutralized with solid NaHCO<sub>3</sub>, and stirred for 2 h. The mixture was filtered and purified using amine functionalized silica gel column chromatography (CHCl<sub>3</sub>–MeOH = 8.5:1.5). The compound **4**



(65 mg, 72%) was obtained as a white powder. Mp 192 °C;  $[\alpha]_{\text{D}}^{25} - 144.6$  (c 0.15, MeOH); UV (H<sub>2</sub>O)  $\lambda_{\text{max}}$ , nm: 263 (8400, pH 2), 262 (8800, pH 7), 285 (6500, pH 11); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 500 MHz)  $\delta$  11.16 (s, 1H, NH), 10.75 (s, 1H, NH), 7.01 (s, 1H, H-6), 5.89 (d, 1H, *J* = 2 Hz, H-6'), 4.52 (br s, 3H, 3 × OH) 4.26 (d, 1H, *J* = 5.0 Hz, H-3'), 4.10 (t, 2H, *J* = 13.5 Hz, H-5'a,b), 3.96 (t, 1H, *J* = 5.0 Hz, H-2'), 3.82 (d, 1H, *J* = 2.5 Hz, H-1'); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 125 MHz)  $\delta$  164.8, 151.6, 147.7, 139.1, 127.4, 125.8, 76.1, 73.7, 58.9, 47.8; HRMS-ESI (*m/z*): (M+H)<sup>+</sup> calcd for C<sub>10</sub>H<sub>12</sub>N<sub>2</sub>O<sub>5</sub>, 240.0746; found, 240.0743; Anal. calcd for C<sub>10</sub>H<sub>12</sub>N<sub>2</sub>O<sub>5</sub>: C, 50.00; H, 5.04; N, 11.66; found: C, 49.89; H, 5.03; N, 11.67.

### 3.2. Antiviral assay

HIV drug susceptibility assay<sup>16</sup> and cytotoxicity assay<sup>17</sup> (PBM, CEM and Vero cells) were performed as previously described.

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