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Synthesis of neplanocin F analogues as potential antiviral agents

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Abstract—Neplanocin F is a natural carbocyclic nucleoside. Herein, we describe the synthesis and antiviral activity of (±)-5′-deoxyneplanocin F analogues. The key intermediate **4**, synthesized from the commercially available (±)-2-azabicyclo[2.2.1]-hept-5-en-3-one (ABH), was utilized to prepare the target nucleosides. Among the target compounds, 5′-deoxyneplanocin F adenine exhibited moderate anti-HIV activity in human lymphocytes without any marked cytotoxicity.

1. Introduction

Nucleoside analogues have played important roles in the treatment of cancer and viral diseases.[1] However, the development of drug resistance and toxicity by the prolonged use of these compounds warrant further development of more selective and less toxic nucleoside analogues. Carbocyclic nucleosides, in which an oxygen atom in the carbohydrate ring is replaced by a methylene group, have drawn much attention as potential chemotherapeutic agents (Fig. 1).[2] A number of carbocyclic nucleosides have been synthesized and some have shown potent antiviral and anticancer activity.[3] For example, the guanosine analogue carbovir[4] and its prodrug abacavir[5] exhibit potent anti-HIV activity and abacavir has been approved by the US FDA. Entecavir, a synthetic carbocyclic nucleoside, shows potent anti-hepatitis B virus (HBV) activity. Recently it has been approved for the treatment of chronic HBV infection.[6] It is interesting to note that all of these potent carbocyclic nucleosides have a double bond as a common structural feature.

A natural carbocyclic nucleoside neplanocin A displays potent antiviral and anticancer activity.[7] The antiviral activity is attributed to the inhibition of *S*-adenosyl-L-homocysteine hydrolase (Adohcy hydrolase),[7,8] which catalyzes the transmethylation reaction in viral mRNA synthesis. The cytotoxic effect could be derived from phosphorylation of the primary hydroxyl group at its 6′-position by the adenosine kinase to its triphosphate, which interferes with the host-cell RNA synthesis and metabolizes to *S*-neplanocylmethionine.[9,10] Neplanocin A is easily deaminated to an inactive inosine-congener by the ubiquitous adenosine deaminase, resulting in a reduced therapeutic potency as well as its half-life in vivo.[11]

Although neplanocin A has a broad antiviral spectrum, it has limitations as a useful chemotherapeutic agent due to its cytotoxicity and a short half-life in vivo. Thus, it is of interest to synthesize neplanocin analogues which maintain the activity but reduce its toxicity. Neplanocin F,[12] which has a specific double bond position and is devoid of 3′-hydroxy group, is significantly less cytotoxic than neplanocin A.[13] Given its lack of cytotoxicity and similarity of neplanocin A, neplanocin F analogues may be potential antiviral agents. In this connection, neplanocin F, as a lead compound, was used to synthesize novel neplanocin analogues as potential chemotherapeutic agents. Herein, we report the synthesis and anti-HIV activity of neplanocin F analogues.

2. Results and discussion

2.1. Chemistry

Neplanocin F analogues, **9** and **12**, were synthesized as outlined in (Scheme 1). The intermediate **4** was synthesized by modification of the reported procedure,[14] which enabled the introduction of different heterocyclic

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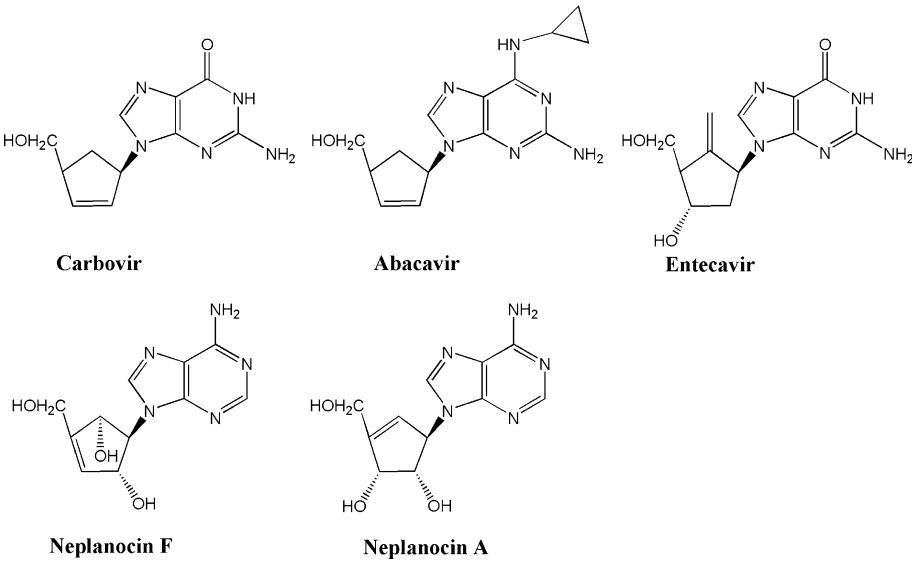


Figure 1. Purine carbocyclic nucleosides with a double bond.

moieties. *N*-Boc-protected compound **2** was prepared by the treatment of (±)-2-azabicyclo[2.2.1]-hept-5-en-3-one (ABH) with $(Boc)_2O$ in the presence of Et_3N and DMAP.[15] Epoxidation of **2** with *m*-CPBA and sodium bicarbonate in dichloromethane gave a single isomer, trans-epoxide **3** plus the starting material **2** (1:1 via ^1H NMR). Without separation of compounds **3** and **2**, treatment of crude mixture with a catalytic amount of sodium methoxide yielded **4** in 39% yield in two steps.[14] Removal of the Boc group of **4** using trifluoroacetic acid in dichloromethane followed by the condensation with 4,6-dichloro-5-nitropyrimidine in the presence of Et_3N gave the pyrimidine intermediate **5** (98%).[16] Reduction of **5** with stannous (II) chloride afforded the amino compound **6** in 89% yield. Cyclization of compound **6** with triethyl orthoformate in the presence of a catalytic amount of methanesulfonic acid provided the 6-chloropurine derivative **7** (50%). Treatment of **6** with a mixture of stannous (II) chloride and triethyl orthoformate failed to give the desired compound **7**.[17]

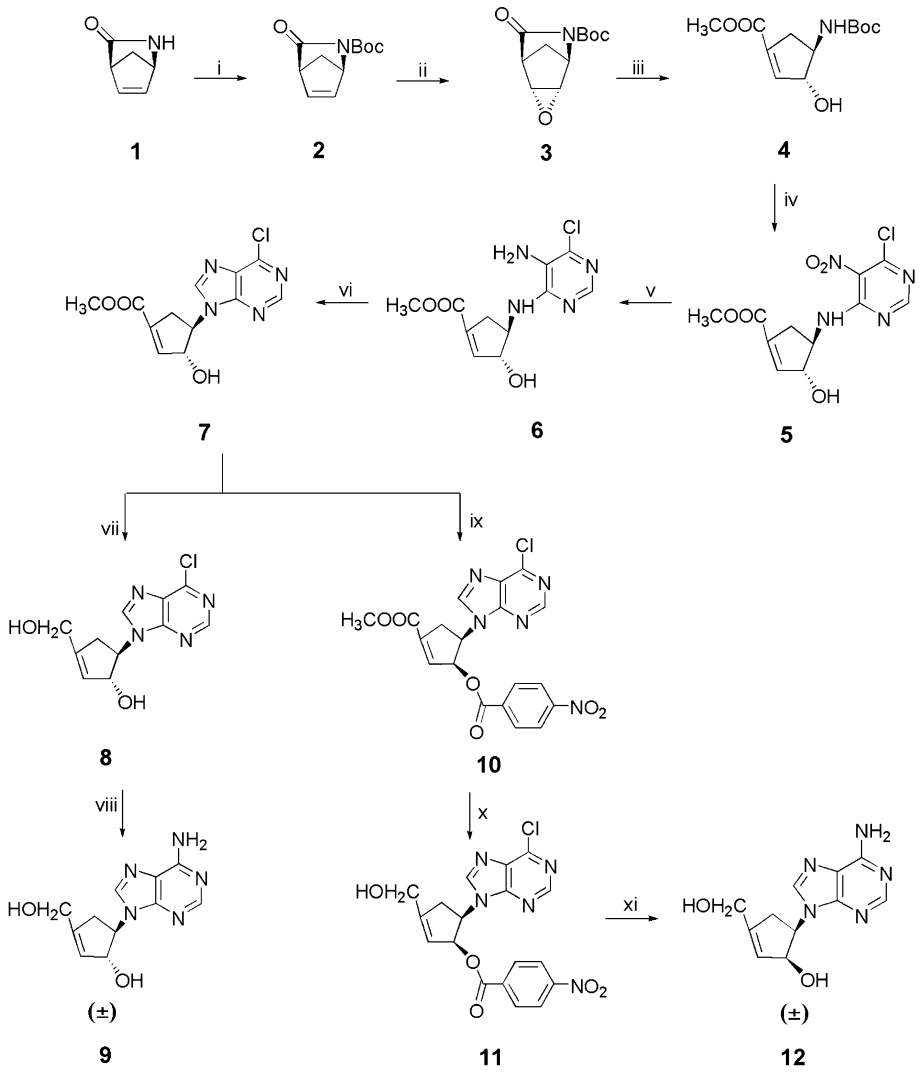
Reduction of ester **7** by DIBAL-H gave the corresponding alcohol **8** (40%). Compound **8** was treated with methanolic ammonia in a steel bomb at 110 °C to furnish racemic compound **9** in 99% yield. Stereochemical inversion at the 2′-hydroxyl position of **7** was conduced by the Mitsunobu reaction to yield **10** (77%).[18] Reduction of **10** followed by treatment with methanolic ammonia gave racemic compound **12** (36% yield in two steps).

Synthesis of pyrimidine (**19** and **21**) and guanine (**23**) derivatives is outlined in (Scheme 2). Conversion of the intermediate **4** to its corresponding alcohol **13** by DIBAL-H in THF at −78 °C failed. A modified method, which utilized a strong Lewis acid, boron trifluoride diethyl etherate, and DIBAL-H, provided the alcohol **13** (95%).[19] Esterification of **13** gave the corresponding ester **14** (96%). Removal of the Boc group from **14** under acidic condition, followed by coupling the resulting amine with methacryloyl isocyanate, gave compound **15** (57%).[20] Cyclization of **15** in the presence of methanolic ammonia produced the uracil derivative **16** in 80% yield, which was protected with TBDMS group to yield **17** (99%). The uracil derivative **17** was converted to the cytosine derivative **18**[21] in 86 % yield by the treatment of 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) and Et_3N followed amination by ammonium hydroxide. Desilylation of the protected derivative **18** afforded the racemic cytosine derivative **19** (93%).

The racemic thymine derivative **21** was also prepared by the condensation of 3-methoxy-2-methylacryloyl isocyanate[22] with the appropriate amino compound. The corresponding amine, which was prepared from **14**, was condensed with *N*-(4,6-dichloro-5-nitropyrimidin-2-yl)acetamide,[23] followed by reduction and ring closure with triethyl orthoformate in the presence of concentrated HCl and a catalytic amount of methanesulfonic acid. The racemic compound **23** was obtained by treatment of the corresponding 6-chloropurine analog with a mixture of mercaptoethanol and sodium methoxide in methanol under reflux in 96% yield.

2.2. Antiviral activity

Anti-HIV-1 activity of the synthesized compounds was evaluated in human peripheral blood mononuclear (PBM) cells and the results are summarized in Table 1. Among all the synthesized nucleosides, the adenine derivative showed moderate activity against HIV-1. The inversion of 2′-hydroxy position (compound **12**) resulted in complete loss of activity. All final compounds did not exhibit significant cytotoxicity. In addition, none of the target compounds showed any marked antiviral activity against Vaccinia, Cowpox, Human Cytomegalovirus, Dengue, Yellow Fever, Herpes simplex virus 1 and 2, Tacaribe, Punta Toro, and West Nile virus. These results showed that the specific double bond position in these compounds may greatly contribute to low cytotoxicity. However with this group of compounds, there is also a reduced inhibition of Adohcy hydrolase (except compound **9**).



Scheme 1. Synthesis of adenine derivatives. Reagents and conditions: (i) $(Boc)_2O$, DMAP, Et_3N, CH_2Cl_2, rt, 3 h, 97%; (ii) *m*-CPBA, CH_2Cl_2, refluxed, 2 h; (iii) MeONa, MeOH, rt, overnight, 39% in two steps; (iv) a—CF_3COOH, CH_2Cl_2, 0 °C, 2.5 h; b—Et_3N, EtOH, 4,6-dichloro-5-nitropyrimidine, 0 °C, 2 h, 98%; (v) $SnCl_2{\cdot}2H_2O$, EtOH; 80 °C, 10 min, 89%; (vi) $CH(OCH_3)_3$, CH_3SO_3H, rt, 1 h, 50%; (vii) DIBAL-H, THF, CH_2Cl_2, −78 °C, 4 h, 40%; (viii) NH_3/CH_3OH, 110 °C, 24 h, 99%; (ix) *p*-Nitrobenzoic acid, DIAD, Ph_3P, THF, 0 °C, 6 h, 77%; (x) DIBAL-H, THF, CH_2Cl_2, −78 °C, 1 h, 40%; (xi) NH_3/CH_3OH, 100 °C, 18 h, 91%.

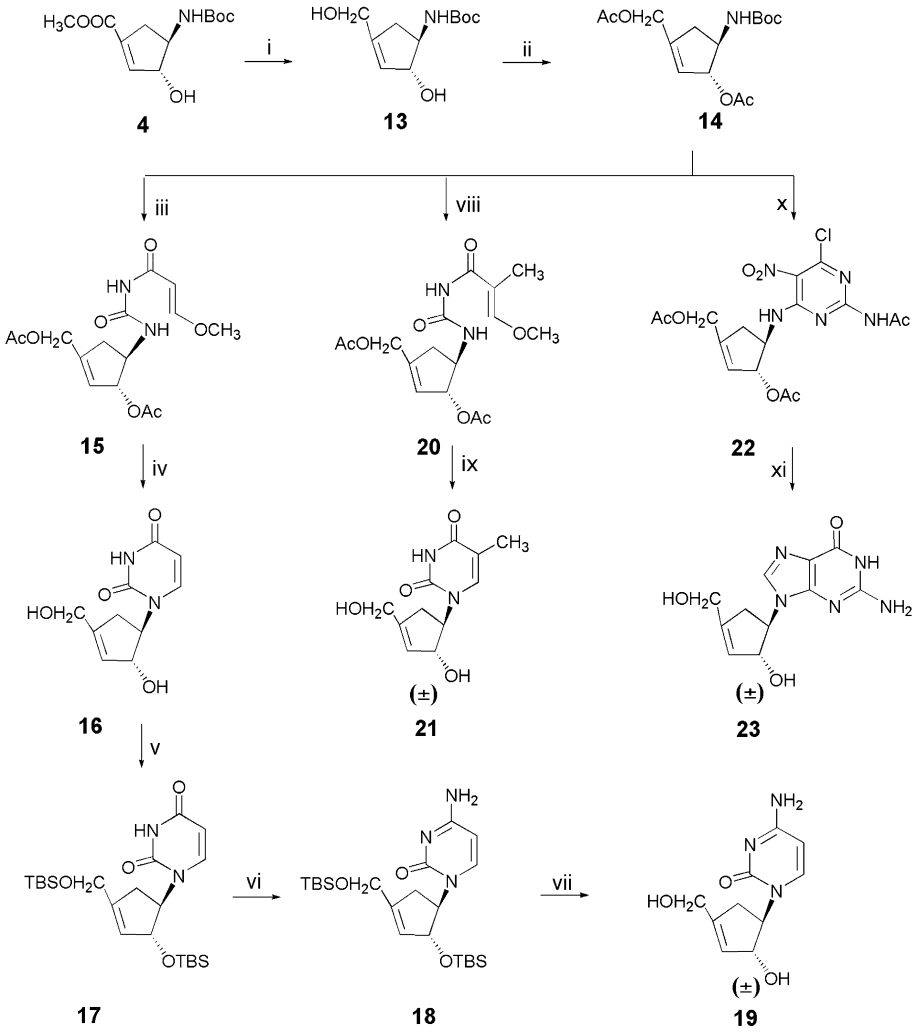
In conclusion, we have developed a simple and efficient method to synthesize (±)-5′-deoxy-neplanocin F analogues. Compound **9** exhibited moderate anti-HIV activity without significant cytotoxicity up to 100 μM. Further optimization of neplanocin F is currently underway.

3. Experimental

Melting points were taken on a Mel-temp II apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Varian Mercury 400 spectrometer at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR or Varian Inova 500 MHz spectrometer at 500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR with tetramethylsilane as the internal standard. Chemical shifts (δ) are reported in parts per million (ppm) and signals 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 spectrophotometer. 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 chromatography. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

3.1. (±)-*N*-*tert*-Butyloxycarbonyl-2-azabicyclo[2.2.1]hept-5-en-3-one (2)

$(Boc)_2O$ (4.8 g, 22 mmol) was added to a solution of (±)-2-azabicyclo[2.2.1]-hept-5-en-3-one (2.2 g, 20 mmol), DMAP (20 mg, 0.16 mmol), and triethylamine (3 mL) in dichloromethane (20 mL) at 0 °C. After addition, the reaction mixture was warmed to room temperature and stirred for 3 h. Then the reaction mixture was poured into water and extracted with dichloromethane



Scheme 2. Synthesis of pyrimidine and guanine derivatives. Reagents and conditions: (i) DIBAL-H, $BF_3{\cdot}Et_2O$, CH_2Cl_2, −78 °C, 2.5 h, 95%; (ii) Ac_2O, pyridine, CH_2Cl_2, 0 °C, 3 h, 96%; (iii) a—CF_3COOH, CH_2Cl_2, 0 °C, 2.5 h; b—Et_3N, methacryloyl isocyanate, rt, overnight, 57%; (iv) NH_4OH, CH_3OH, 100 °C, 16 h, 80%; (v) TBDMSCl, imidazole, DMF, rt, 6 h, 99%; (vi) a—TPSCl, Et_3N, 24 h; b—NH_4OH, 3 h, 86%; (vii) 3 N HCl/CH_3OH, 0 °C, 2 h, 93%; (viii) a—CF_3COOH, CH_2Cl_2, 0 °C, 2.5 h; b—Et_3N, 3-methoxy-2-methylacryloyl isocyanate, toluene, 0 °C, 3 h, 45%; (ix) NH_4OH, CH_3OH, 85 °C, 24 h, 89%; (x) a—CF_3COOH, CH_2Cl_2, 0 °C, 2.5 h; b—*N*-(4,6-dichloro-5-nitropyrimidin-2-yl)acetamide, Et_3N, EtOH, 3 h, 77%; (xi) a—$SnCl_2{\cdot}2H_2O$, EtOH, 60 °C, 30 min; b—$CH(OCH_3)_3$, CH_3SO_3H, rt, 3 h, in two steps, 68%; c—$HSCH_2CH_2OH$, CH_3ONa, CH_3OH, reflux, 7 h, 96%.

Table 1. In vitro antiviral activity and toxicity of synthesized nucleosides

Compound	Nucleoside	Cytotoxicity (IC_{50}, μM) in		
	HIV-1_{LAI} (EC_{50}, μM)	PBM	Vero	CEM
9	13.8	>100	>100	>100
12	>100	>100	>100	>100
19	>100	>100	>100	>100
21	>100	>100	>100	>100
23	>100	>100	>100	>100
AZT	0.0029	>100	50.6	14.3

(3× 20 mL). The organic layers were combined and dried over magnesium sulfate, filtered, and concentrated. The residue was purified by silica gel chromatography with hexane/ethyl acetate (100:1) to obtain **2** (white solid, 4.1 g, 97%).The NMR is the same as the literature.[9] ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.50 (s, 9 H, Boc), 2.14 (d, 1H, J = 8 Hz, 7-H), 2.34 (d, 1H, J = 8.5 Hz, CH, 7-H), 3.38 (m, 1H, CH, 4-H), 4.95 (m, 1H, CH, 1-H), 6.65 (t, J = 3.5 Hz, CH, 6-H), 6.88 (dd, J = 2 Hz, CH, 5-H).

3.2. (±)-7-Oxo-3-oxa-6-aza-tricyclo[3.2.1.0^{2,4}]octane-6-carboxylic acid *tert*-butyl ester (3)

A suspension of **2** (5 g, 23.89 mmol), *m*-CPBA (15.7 g, 70%, 63.68 mmol), and sodium bicarbonate (21.6 g, 250 mmol) in dichloromethane (150 mL) was stirred overnight at room temperature followed by refluxing for 2 h. The reaction mixture was cooled to room temperature, filtered through Celite, and washed with dichloromethane (2× 40 mL). The combined filtrate was successively washed with saturated sodium bicarbonate (2× 30 mL), followed by saturated sodium sulfite (10 mL), then the organic layer was dried over magnesium sulfate and concentrated. The residue was purified

by column chromatography with hexane/ethyl acetate (50:1) to afford an oily product (3.8 g) which was used in the next step without further purification.

3.3. (±)-*trans*-1-*tert*-Butoxycarbonylamino-2-hydroxycyclopent-4-enecarboxylic acid methyl ester (4)

A catalytic amount of sodium methoxide (0.5 M, 4 mL, 2 mmol) was added to a suspension of crude mixture (3.8 g) in methanol (30 mL) at 0 °C. The solution was warmed to room temperature and stirred overnight. Acetic acid (0.5 mL) was added and the mixture was evaporated in vacuo. Dichloromethane (100 mL) was added and washed with saturated sodium bicarbonate (10 mL), dried over magnesium sulfate, and concentrated. The residue was purified by silica gel chromatography with ethyl acetate/hexane (1:2 to 1:1) to obtain **4** (2.4 g, 39% in two steps): ^{1}H NMR of the compound **4** is the same as the literature.[5] mp 238–240 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.46 (s, 9H, CH_3, Boc), 2.30 (ddt, J = 16 Hz, J = 8 Hz, J = 2 Hz, 1H, CH, 5′-H), 3.02 (app dd, J = 16 Hz, J = 8 Hz, 1H, CH, 5′-H), 3.76 (s, 3H, $COOCH_3$,), 3.95 (m, 1H, CH, 1-H), 4.41 (br s, 1H, OH), 4.82 (m, 1H, CH, 2-H), 5.00 (br s, 1H, NH), 6.65 (br s, 1H, 3-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 28.33, 35.80, 51.87, 60.95, 80.53, 83.57, 133.75, 142.50, 157.30 and 164.85; Anal. Calcd for $C_{12}H_{19}NO_5$: C, 56.02; H, 7.44; N, 5.44. Found: C, 56.09; H, 7.55; N, 5.27; MS: $[M+Na]^+$ = 280.11. Found: 280.08.

3.4. (±)-*trans*-1′-(6-Chloro-5-nitropyrimidin-1′-yl-amino)-2′-hydroxycyclopent-4′-enecarboxylic acid methyl ester (5)

Trifluoroacetic acid (2 mL) was added to a solution of **4** (0.5 g, 1.94 mmol) in dichloromethane (5 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 2.5 h. The solvents were evaporated in vacuo and the residue was dissolved in a mixture of ethanol (20 mL) and triethylamine (6 mL) at 0 °C, then 4,6-dichloro-5-nitropyrimidine (0.51 g, 2.62 mmol) was added. The reaction mixture was stirred for 2 h at 0 °C, then poured into water (120 mL) and extracted with ethyl acetate (3× 20 mL). The organic layer was dried over magnesium sulfate and concentrated. The residue was purified by silica gel chromatography with ethyl acetate/hexane (1:3 to 2:3) to obtain **5** (yellow solid, 0.6 g, 98%): ^{1}H NMR (500 MHz, CD_3COCD_3): δ 2.65 (m, 1 H, CH, 5′-H), 3.18 (m, 1H, CH, 5′-H), 3.77 (d, 3H, $COOCH_3$), 4.87 (m, 1H, OH), 5.16 (s, 1H, CH, 1′-H), 6.68 (s, 1H, CH, 2′-H), 8.23 (s, 1H, CH, 3′-H), 9.74 (s, 1H, CH, 2-H), 11.30 (br s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 36.5, 52.1, 61.2, 80.1, 115.9, 133.7, 144.2, 152.3, 155.3, 158.6 and 164.8.

3.5. (±)-*trans*-1′-(5-Amino-6-chloro-pyrimidin-1′-yl-amino)-2′-hydroxycyclopent-4′-enecarboxylic acid methyl ester (6)

A solution of **5** (110 mg, 0.34 mmol) and stannous chloride dihydrate (0.4 g, 1.77 mmol) in ethanol (2 mL) was stirred at 80 °C for 10 min. The solvent was removed in vacuo. Dichloromethane (10 mL) was added to the residue and washed with saturated sodium bicarbonate (2× 10 mL). The organic layer was dried over magnesium sulfate and concentrated. The residue was purified by silica gel chromatography with ethyl acetate/hexane (1:1) to obtain **6** (yellow solid, 88 mg, 89%). This compound was directly used without further purification.

3.6. (±)-*trans*-1′-(6-chloropurin-9-yl)-2′-hydroxy-4′-methoxycarbonyl-cyclopent-3′-ene (7)

A solution of **6** (0.2 g 0.7 mmol), triethyl orthoformate (2 mL), and methanesulfonic acid (2 drops) was stirred at room temperature for 1 h. The reaction mixture was diluted with ethyl acetate (20 mL), washed with saturated sodium bicarbonate (2× 10 mL), dried over magnesium sulfate, and concentrated. The residue was purified with silica gel chromatography with ethyl acetate/hexane (1:1) to obtain **7** (white solid, 0.1 g, 50%): mp 188–190 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.18 (ddt, J = 2.5 Hz, J = 8.5 Hz, J = 15.25 Hz, 1H, CH, 5′-H) 3.46 (dd, J = 8.5 Hz, J = 15.25 Hz, 1H, CH, 5′-H), 3.83 (s, 3H, $COOCH_3$), 4.81 (dt, J = 6.5 Hz, J = 8.5 Hz, 1H, CH, 1′-H), 5.13 (br s, 1H, OH), 5.44 (dt, J = 2 Hz, J = 4 Hz, 1H, CH, 2′-H), 6.83 (br s, 1H, 3′-H), 8.15 (s, 1H, CH, 8-H), 8.76 (s, 1H, CH, 2-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 34.55, 52.18, 65.57, 80.69, 132.49, 133.69, 141.78, 144.19, 151.54, 151.80, 152.02 and 164.08; Anal. Calcd for $C_{11}H_{13}ClN_4O_3$: C, 48.91; H, 3.76; N, 19.01. Found: C, 49.03; H, 3.84; N, 19.07; UV (CH_3OH): λ_{max} 212, 264.

3.7. (±)-*trans*-1′-(6-Chloropurin-9-yl)-2′-hydroxy-4′-hydroxymethyl-cyclopent-3′-ene (8)

DIBAL-H (1 M in hexane, 0.08 mL) was added to a solution of **7** (0.12 g, 0.4 mmol) in THF (2 mL) and dichloromethane (2 mL). The mixture was cooled to −78 °C, and more DIBAL-H (1 M in hexane, 1.6 mL, 1.6 mmol) was added. The reaction mixture was stirred at −78 °C for 4 h. Ethyl acetate (0.88 mL) was added and warmed to room temperature, then Rochelle salt (3.5 mL) was added, stirred for 2 h at room temperature, and extracted with ethyl acetate (3× 20 mL). The organic layer was dried over magnesium sulfate and concentrated. The residue was purified by preparative TLC with ethyl acetate/acetone (1:1) to obtain **8** (40 mg, 40%): mp 170 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.83 (m, 2H, CH_2, 5′-H), 4.05 (s, 2H, CH_2, 6′-H), 4.91 (m, 1H, CH, 1′-H), 4.99 (s, 1H, OH), 5.18 (s, 1H, CH, 2′-H), 5.46 (s, 1H, OH), 5.66 (d, J = 1.5 Hz, 1H, CH, 3′-H), 8.81 (s, 1H, CH, 8-H), 8.83 (s, 1H, CH, 2-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 36.63, 60.28, 64.90, 79.87, 126.32, 131.93, 145.11, 147.39, 149.64, 151.73 and 152.44; Anal. Calcd for $C_{11}H_{11}ClN_4O_2$: C, 49.54; H, 4.16; N, 21.01. Found: C, 49.62; H, 4.31; N, 20.80; UV (CH_3OH): λ_{max} 207, 264; HRMS: $[M+H]^+$: 267.0651. Found: 267.0652.

3.8. (±)-*trans*-1′-(6-Aminopurin-9-yl)-2′-hydroxy-4′-hydroxymethyl-cyclopent-3′-ene (9)

Compound **8** (99 mg, 0.37 mmol) in saturated methanolic ammonia (40 mL) was stirred at 110 °C for 24 h. The

solvent was removed in vacuo, and the residue was purified by silica gel chromatography with dichloromethane/methanol (5:1) to obtain **9** (white solid, 91 mg, 99%): mp 240 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.79 (m, 2H, CH_2, 5′-H) 4.04 (m, 2H, CH_2, 6′-H), 4.75 (s, 1H, CH, 1′-H), 4.96 (s, 1H, OH), 5.15 (m, 1H, CH, 2′-H), 5.43 (m, 1H, OH), 5.64 (s, 1H, CH, 3′-H), 7.25 (s, 2H, NH_2), 8.15 (s, 1H, CH, 8-H), 8.20 (s, 1H, CH, 2-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 36.72, 60.33, 64.29, 79.71, 119.87, 126.51, 140.78, 145.00, 149.98, 152.57 and 156.55; Anal. Calcd for $C_{11}H_{13}N_5O_2$: C, 53.43; H, 5.30; N, 28.32. Found: C, 53.41; H, 5.34; N, 28.33; UV (H_2O): λ_{max} 260 (ε 9999) (pH 7); 260 (ε 9970) (pH 11); 259 (ε 9894) (pH 2); HRMS (M^+H): 248.1147. Found: 248.1123; MS $[M+Na]^+$: 270.0966. Found: 270.0977.

3.9. (±)-*cis*-1′-(6-Chloropurin-9-yl)-4′-methoxycarbonyl-2′-(*p*-nitrobenzoyl)oxy-cyclopent-3′-ene (10)

Diisopropyl azodicarboxylate (0.57 mL, 2.85 mmol) was added to a solution of **7** (0.5 g, 1.7 mmol), *p*-nitrobenzoic acid (0.5 g, 3 mmol), and triphenylphosphine (0.75 g, 2.85 mmol) at 0 °C. After stirring for 6 h, dichloromethane (30 mL) was added and the resulting mixture was washed with saturated sodium bicarbonate (3× 15 mL). The organic layer was dried over magnesium sulfate and concentrated. The residue was purified by silica gel chromatography with ethyl acetate/hexane (1:1) to obtain **10** as a white solid (580 mg, 77%): mp 186–187 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.45 (m, 2H, CH_2, 5′-H), 3.88 (d, 3H, CH_3), 5.78 (m, 1H, CH, 1′-H), 6.26 (d, 1H, CH, 2′-H), 6.96 (s, 1H, CH, 3′-H), 7.72 (d, 2H, Ph), 8.15 (d, 2H, Ph), 8.23 (d, 1H, CH, 8-H), 8.71 (d, 1H, CH, 2-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 35.87, 52.53, 54.37, 123.71, 130.48, 131.30, 136.48, 140.06, 144.15, 150.00, 150.82, 151.39, 152.07, 152.27, 163.18 and 163.60; Anal. Calcd for $C_{19}H_{14}ClN_5O_6$: C, 51.42; H, 3.18; N, 15.78. Found: C, 51.54; H, 3.12; N, 15.74; UV (CH_3OH): λ_{max} 207, 259.

3.10. (±)-*cis*-1′-(6-Chloropurin-9-yl)-4′-hydroxymethyl-2′-(*p*-nitrobenzoyl)oxy-cyclopent-3′-ene (11)

DIBAL-H (1 M in hexane, 0.4 mL, 0.4 mmol) was added to a solution of **10** (40 mg, 0.09 mmol) in THF (0.4 mL) and dichloromethane (0.4 mL) at −78 °C. The reaction mixture was stirred for 1 h; ethyl acetate (1 mL) was added and warmed to room temperature. Saturated Rochelle salt (0.6 mL) was added and the mixture was stirred for another 3 h, extracted with ethyl acetate (4× 20 mL), dried over magnesium sulfate, and concentrated. The residue was purified by silica gel chromatography with ethyl acetate/hexane/dichloromethane (10:3:10) to obtain **11** (15 mg, 40%): mp 177–178 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.90 (dd, J = 8 Hz, J = 16.8 Hz, 1H, CH, 5′-H) 3.46 (dd, J = 7.2 Hz, J = 16.2 Hz, 1H, CH, 5′-H), 4.18 (m, 2H, CH_2, 6′-H), 5.17 (t, J = 7.6 Hz, 1H, CH, 1′-H), 5.62 (dd, J = 7.6 Hz, J = 14.4 Hz, 1H, OH), 5.86 (d, J = 3 Hz, 1H, CH, 2′-H), 6.00 (m, 1H, CH, 3′-H), 7.73 (m, 2H, Ph), 8.17 (m, 2H, Ph), 8.74 (s, 1H, CH, 2-H), 8.87 (s, 1H, CH, 8-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 35.27, 55.48, 60.26, 78.42, 119.90, 124.13, 130.77, 131.23, 134.66, 147.70, 149.54, 150.76, 152.06, 152.89, 154.01 and 163.68; Anal. Calcd for $C_{18}H_{14}ClN_5O_5$: C, 52.00; H, 3.39; N, 16.84. Found: C, 52.18; H, 3.45; N, 16.75; UV (CH_3OH): λ_{max} 207, 260.

3.11. (±)-*cis*-1′-(6-Aminopurin-9-yl)-2′-hydroxy-4′-hydroxymethyl-cyclopent-3′-ene (12)

A suspension of **11** (37 mg, 0.088 mmol) in 10 mL saturated methanolic ammonia was stirred in a steel bomb at 100 °C for 18 h, cooled to room temperature, concentrated, and purified by silica gel chromatography with ethyl acetate/methanol (10:1) to obtain **12** (20 mg, 91%): mp 240 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.72 (dd, J = 8 Hz, J = 16 Hz, 1H, CH, 5′-H), 2.87 (dd, J = 7.5 Hz, J = 16 Hz, 1H, CH, 5′-H), 4.10 (d, J = 1.5 Hz, 2H, CH_2, 6′-H), 4.59 (t, J = 5.5 Hz, 1H, CH, 1′-H), 4.90 (d, J = 6.5 Hz, 1H, OH), 5.01 (t, J = 5 Hz, 1H, CH, 2′-H), 5.09 (q, J = 7.5 Hz, 1H, OH), 5.74 (d, J = 1.5 Hz, 1H, CH, 3′-H), 7.20 (br s, 2H, NH_2), 8.14 (s, 1H, CH, 8-H), 8.16 (s, 1H, CH, 2-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 36.30, 55.58, 60.34, 73.45, 118.82, 125.47, 141.11, 147.96, 150.44, 152.51 and 156.26; Anal. Calcd for $C_{11}H_{13}N_5O_2{\cdot}0.31H_2O$: C, 52.25; H, 5.43; N, 27.70. Found: C, 52.26; H, 5.32; N, 27.81; UV (H_2O): λ_{max} 260 (ε 14,956) (pH 7), 260 (ε 14,956) (pH 11), 259 (ε 14,550) (pH 2); MS $[M+H]^+$: 248.11, Found: 248.10.

3.12. (±)-*trans*-1-*tert*-Butoxycarbonylamino-2-hydroxy-4-hydroxymethyl-cyclopent-3-ene (13)

DIBAL-H (1 M in dichloromethane, 6.1 mL, 6.1 mmol) was added to a solution of **4** (0.27 g, 1.04 mmol), $BF_3{\cdot}Et_2O$ (0.2 mL, 1.6 mmol) in dichloromethane (6 mL) at −78 °C and the mixture was stirred for 2.5 h. Saturated Rochelle salt (10 mL) was added, then warmed to room temperature, and stirred for another 3 h. The mixture was extracted with ethyl acetate (3× 20 mL). The organic layer was dried over magnesium sulfate and concentrated under reduced pressure. The residue was purified by silica gel chromatography with ethyl acetate/methanol (10:1) to give **13** (white solid, 0.23 g, 95%): mp 108-110 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.36 (s, 9H, CH_3, Boc), 1.89 (dd, J = 6.4 Hz, J = 16 Hz, 1H, CH, 5-H), 2.45 (m, 1H, CH, 5-H), 3.66 (t, J = 6.4 Hz, 1H, CH, 1-H), 3.86 (d, J = 1.6 Hz, 2H, CH_2, 6-H), 4.41 (m, 1H, CH, 2-H), 4.72 (t, J = 5.6 Hz, 1H, OH), 4.81 (d, J = 6.4 Hz, 1H, OH), 5.39 (d, J = 1.2 Hz, 1H, CH, 3-H), 7.00 (d, J = 8 Hz, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 28.80, 37.96, 60.31, 60.49, 77.99, 80.65, 126.54, 145.17 and 155.95; Anal. Calcd. for $C_{11}H_{19}NO_4{\cdot}0.4H_2O$: C, 55.87; H, 8.44; N, 5.92. Found: C, 55.77; H, 8.17; N, 5.89; MS: $[M+Na]^+$: 252.12. Found: 252.09.

3.13. (±)-*trans*-1-*tert*-Butoxycarbonylamino-2-acetyloxy-4-acetyloxymethylcyclopent-3-ene (14)

DMAP (100 mg) and acetic anhydride (5 mL, 56.6 mmol) were added to a solution of **13** (2.2 g, 9.59 mmol) in dichloromethane (20 mL) and pyridine

(20 mL) at 0 °C. The mixture was stirred for 3 h and then evaporated in vacuo. The residue was dissolved in ethyl acetate (30 mL) then washed with dilute HCl (1 N, 2× 10 mL), water (10 mL), and saturated sodium bicarbonate (2× 15 mL), dried over magnesium sulfate, and concentrated in vacuo to obtain **14** (oil, 2.9 g, 96%): ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37 (s, 9H, CH_3, Boc), 1.99 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 2.14 (m, 1H, CH, 5-H), 2.83 (m, 1H, CH, 5-H), 4.10 (s, 1H, NH), 4.55 (s, 2H, CH_2, 6-H), 5.12 (br s, 1H, CH, 1-H), 5.48 (t, J = 2 Hz, 1H, CH, 2-H), 5.59 (t, J = 2 Hz, 1H, CH, 3-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.71, 21.07, 28.34, 38.64, 56.81, 62.24, 79.61, 84.04, 124.22, 143.32, 155.50, 170.48 and 171.00; Anal. Calcd for $C_{15}H_{23}NO_6$: C, 57.50; H, 7.40; N, 4.47. Found: C, 57.58; H, 7.49; N, 4.51; MS: $[M+Na-H]^+$: 335.13. Found: 335.11.

3.14. (±)-*trans*-1′-[3-(3-Methoxyacryloyl)-ureido]-2′-acetoxy-4′-acetoxymethylcyclopent-3′-ene (15)

The free amine derivative was prepared from **14** (0.2 g, 0.63 mmol) by treatment with trifluoroacetic acid (0.8 mL) in dichloromethane (2 mL) at 0 °C for 2.5 h, followed by evaporation in vacuo. Thionyl chloride (2 mL) was added to a solution of 3-methoxyacrylic acid (1.6 g, 15.6 mmol) in dry dichloromethane (13 mL), refluxed for 3 h, and concentrated to dryness. The residue was dissolved in dry toluene (6 mL) and silver cyanate (4 g, 26.6 mmol) was added. The suspension was refluxed for 30 min, cooled to 0 °C. The supernatant liquor was transferred to the above free amine derivative in dichloromethane (20 mL) and triethylamine (2 mL). The mixture was stirred at 0 °C, then slowly warmed to room temperature and stirred for overnight. The reaction mixture was washed with saturated sodium bicarbonate (2× 10 mL), dried and purified by silica gel chromatography with ethyl acetate/hexane (1:2) to afford **15** (120 mg, 57%): mp 160–162 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.05 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.35 (m, 1H, CH, 5′-H), 2.88 (dd, 1H, CH, 5′-H), 3.73 (s, 3H, CH_3), 4.37 (m, 1H, CH, 1′-H), 4.59 (dd, J = 14 Hz, 2H, CH_2, 6′-H), 5.33 (d, J = 12.5 Hz, 1H, CH, 2′-H), 5.69 (br s, 2H, CH, 3′-H, 5-H), 7.66 (d, J = 12.5 Hz, 1H, CH, 6-H), 9.03 (d, J = 7 Hz, 1H, NH), 9.68 (d, J = 12.5 Hz, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.73, 21.04, 38.34, 55.73, 57.91, 62.21, 83.92, 97.59, 124.57, 143.20, 155.05, 163.68, 167.94, 170.48 and 170.67; Anal. Calcd for $C_{15}H_{20}N_2O_7$: C, 52.94; H, 5.92; N, 8.23. Found: C, 52.88; H, 5.88; N, 8.19; MS: $[M+Na]^+$: 363.11. Found: 363.08.

3.15. (±)-*trans*-1′-(2′-Hydroxy-4′-hydroxymethylcyclopent-3′-enyl)uracil (16)

A suspension of **15** (0.1 g, 0.29 mmol), ethanol (2 mL), and NH_4OH (28%, 5 mL) was heated at 80–100 °C in a steel bomb for 16 h. The solution was evaporated and the crude product was purified by silica gel chromatography with dichloromethane/methanol (4:1) to obtain **16** (white solid, 52 mg, 80%): mp 188–189 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.32 (dd, J = 7.2 Hz, J = 16.0 Hz, 1H, CH, 5′-H) 2.55 (dd, J = 8.8 Hz, J = 16.2 Hz, 1H, CH, 5′-H), 3.93 (m, 2H, CH_2, 6′-H), 4.53 (m, 1H, CH, 1′-H), 4.79 (m, 1H, OH), 4.86 (t, J = 5.6 Hz, 1H, CH, 2′-H), 5.24 (d, J = 6 Hz, 1H, OH), 5.50 (dd, J = 1.6 Hz, J = 3.2 Hz, 1H, CH, 3′-H), 5.53 (dd, J = 2 Hz, J = 8 Hz, 1H, CH, 6-H), 7.55 (d, J = 7.6 Hz, 1H, CH, 5-H), 11.24 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 35.85, 60.31, 66.16, 79.16, 101.81, 126.45, 144.21, 145.51, 151.49 and 163.80; Anal. Calcd for $C_{10}H_{12}N_2O_4$: C, 53.57; H, 5.39; N, 12.49. Found: C, 53.62; H, 5.27; N, 12.50; UV (CH_3OH): λ_{max} 208, 267; MS: $[M+H]^+$: 247.0674. Found: 247.0687.

3.16. (±)-*trans*-1′-(2′-*tert*-Butyldimethylsilyloxy-4′-(*tert*-butyldimethylsilyloxymethyl)cyclopent-3-enyl)uracil (17)

TBDMSCl (0.12 g, 0.78 mmol) was added to a solution of **16** (70 mg, 0.31 mmol) and imidazole (74 mg, 1.08 mmol) in DMF (1 mL). The mixture was stirred at room temperature for 6 h, diluted with ethyl acetate (10 mL), washed with water (10 mL), dried over magnesium sulfate, and concentrated and purified by silica gel chromatography with ethyl acetate/hexane (2:1) to obtain **17** (white solid, 0.14 g, 99%): mp 150–151 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (s, 3H, CH_3), 0.03 (s, 3H, CH_3), 0.11 (s, 6H, CH_3), 0.86 (s, 9H, CH_3), 0.93 (s, 9H, CH_3), 2.51 (dd, J = 7 Hz, J = 16.2 Hz, 1H, CH, 5′-H), 2.60 (d, J = 8.5 Hz, J = 15.2 Hz, 1H, CH, 5′-H), 4.21 (s, 2H, CH_2, 6′-H), 4.62 (m, 1H, CH, 1′-H), 5.14 (m, 1H, CH, 2′-H), 5.56 (d, J = 1 Hz, 1H, CH, 3′-H), 5.63 (d, J = 8 Hz, 1H, CH, 5-H), 7.72 (d, J = 7.5 Hz, 1H, CH, 6-H), 11.36 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 0.00, 0.02, 0.40, 0.50, 23.12, 23.36, 31.00, 31.15, 39.61, 66.79, 72.10, 85.11, 106.79, 130.93, 147.24, 149.67, 149.75, 156.34 and 168.64; Anal. Calcd for $C_{22}H_{40}N_2O_4Si_2$: C, 58.36; H, 8.91; N, 6.19. Found: C 53.38; H 8.91; N, 6.12; UV (CH_3OH): λ_{max} 206, 267; MS: $[M+Na]^+$: 475.24. Found: 475.22.

3.17. (±)-*trans*-1′-(2′-*tert*-Butyldimethylsilyloxy-4′-(*tert*-butyldimethylsilyloxymethyl)cyclopent-3′-enyl)cytosine (18)

A solution of **17** (0.38 g, 0.84 mmol), TPSCl (0.8 g, 2.6 mmol), and Et_3N (0.37 mL) in acetonitrile (10 mL) at room temperature was stirred for 24 h. Next NH_4OH (28%, 2 mL) was added, the mixture was stirred for 3 h and evaporated under vacuum. The residue was dissolved in ethyl acetate (30 mL). The organic layer was washed with saturated sodium bicarbonate (2× 10 mL), dried over magnesium sulfate, and concentrated and purified by silica gel chromatography with ethyl acetate/methanol (10:1 to 3:1) to obtain **18** (white solid, 0.32 g, 86%): mp 136–138 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.0 (s, 3H, CH_3), 0.03 (s, 3H, CH_3), 0.14 (s, 6H, CH_3), 0.89 (s, 9H, CH_3), 0.97 (s, 9H, CH_3), 2.55 (m, 1H, CH, 5′-H), 2.63 (dd, J = 7.5 Hz, J = 15.5 Hz, 1H, CH, 5′-H), 4.24 (s, 2H, CH, 6′-H), 4.48 (q, J = 7.5 Hz, 1H, CH, 1′-H), 5.26 (m, 1H, CH, 2′-H), 5.58 (d, J = 1.5 Hz, 1H, CH, 3′-H), 5.73 (d, J = 7 Hz, 1H, CH, 5-H), 7.06 (d, J = 35.5 Hz, 2H, NH_2), 7.62 (d, J = 7.5 Hz, 1H, CH, 6-H); ^{13}C NMR (125 MHz,

$CDCl_3$): δ 0.00, 0.01, 0.47, 0.51, 23.14, 23.36, 31.05, 31.15, 39.85, 66.87, 74.24, 85.01, 98.83, 131.08, 149.77, 150.70, 161.00 and 170.93; Anal. Calcd for $C_{22}H_{41}N_3O_3Si_2$: C, 58.49; H, 9.15; N, 9.30. Found: C, 58.29; H, 9.17; N, 9.17; UV (CH_3OH): λ_{max} 206, 276; MS: $[M+H]^+$: 452.27. Found: 452.26.

3.18. (±)-*trans*-1′-(2′-Hydroxy-4′-hydroxymethylcyclopent-3′-enyl)cytosine (19)

Aqueous HCl (3 N, 2 mL) was added to solution of **18** (260 mg, 0.57 mmol) in methanol (12 mL) at 0 °C. The reaction mixture was stirred for 2 h at 0 °C, neutralized by saturated sodium bicarbonate (8 mL), and evaporated in vacuo at room temperature. The residue was purified by silica gel chromatography with ethyl acetate/methanol (10:1 to 3:1) to obtain **19** (white solid, 120 mg, 93%): mp 220 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.38 (dd, J = 6 Hz, J = 16 Hz, 1H, CH, 5′-H), 2.56 (dd, J = 8.5 Hz, J = 16 Hz, 1H, CH, 5′-H), 3.97 (m, 2H, CH_2, 6′-H), 4.52 (m, 1H, CH, 1′-H), 4.87 (m, 1H, OH), 4.88 (t, J = 6 Hz, 1H, CH, 2′-H), 5.17 (d, J = 6 Hz, 1H, OH), 5.54 (d, J = 1.5 Hz, 1H, CH, 3′-H), 5.68 (d, J = 7 Hz, 1H, CH, 5-H), 7.00 (app d, J = 42.5 Hz, 2H, NH_2), 7.50 (d, J = 7.5 Hz, 1H, CH, 6-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 36.24, 60.39, 67.52, 79.30, 93.98, 126.61, 144.91, 145.60, 156.15 and 165.87; Anal. Calcd for $C_{10}H_{13}N_3O_3$: C, 53.80; H, 5.87; N, 18.82. Found: C, 53.89; H, 6.01; N, 18.71; UV (H_2O): λ_{max} 275 (ε 9390) (pH 7), 275 (ε 9220) (pH 11), 284 (ε 13,819) (pH 2); MS: $[M+H]^+$: 224.10. Found: 224.09.

3.19. (±)-*trans*-1′-[3-(3-Methoxy-2-methyl-acryloyl)-ureido]-2′-acetoxy-4′-acetoxymethylcyclopent-3′-ene (20)

The free amine derivative was prepared from **14** (2 g, 6.3 mmol) by treatment with trifluoroacetic acid (8 mL) in dichloromethane (20 mL) at 0 °C for 2.5 h, followed by evaporation in vacuo. Oxalyl chloride (3 mL) was added dropwise to a solution of 3-methoxy-2-methylacryloyl acid (3.6 g, 31 mmol) in anhydrous dichloromethane (20 mL). The mixture was stirred at room temperature for 1 h, evaporated in vacuo. The residue was dissolved in toluene (20 mL) and silver cyanate (8.6 g, 57 mmol) was added. The mixture was heated to 80–100 °C for 40 min and then cooled to room temperature. The supernatant liquor was added to the prepared free amine derivative in dichloromethane (40 mL) and triethylamine (10 mL) at −78 °C. After addition, the mixture was warmed to 0 °C, stirred for 3 h, diluted with ethyl acetate (50 mL), and washed with water (2× 20 mL) and saturated sodium bicarbonate (20 mL). After filtration, the solvent was dried over magnesium sulfate, filtered again, and concentrated. The residue was purified by silica gel chromatography with ethyl acetate/hexane (1:1) to give **20** as a white solid (1.03 g. 45%): mp 138–139 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.64 (d, J = 1 Hz, 3H, CH_3) 2.03 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.27 (dd, J = 5.5 Hz, J = 16.25 Hz, 1H, CH, 5′-H), 2.77 (dd, J = 8.0 Hz, J = 16.75 Hz, 1H, CH, 5′-H), 3.83 (s, 3H, CH_3), 4.25 (m, 1H, CH, 1′-H), 4.64 (s, 2H, CH_2, 6′-H), 5.62 (m, 2H, CH, 2′-H, 3′-H), 7.49 (d, J = 1.5 Hz, 1H, CH, 6-H), 8.93 (d, J = 7 Hz, 1H, NH), 9.80 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 9.30, 20.99, 21.31, 38.17, 55.64, 61.58, 62.24, 83.77, 107.38, 124.04, 144.19, 154.04, 158.70, 169.99, 170.49 and 170.61; Anal. Calcd for $C_{16}H_{22}N_2O_7$: C, 54.23; H, 6.26; N, 7.91. Found: C, 54.51; H, 6.46; N, 7.97; MS: $[M+H]^+$: 355.15. Found: 355.14.

3.20. (±)-*trans*-1′-(2′-Hydroxy-4′-hydroxymethylcyclopent-3′-enyl)thymine (21)

A solution of **20** (250 mg, 0.705 mmol) in NH_4OH (28%, 8 mL) and MeOH (8 mL) was heated in a steel bomb at 85 °C for 24 h. The mixture was concentrated in vacuo and the residue was purified by silica gel chromatography with dichloromethane/methanol (4:1) to give **21** (white solid, 0.15 g, 89%): mp 200 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.79 (s, 3H, CH_3), 2.37 (dd, J = 7.5 Hz, J = 16 Hz, 1H, CH, 5′-H), 2.58 (dd, J = 8 Hz, J = 16 Hz, 1H, CH, 5′-H), 3.96 (m, 2H, CH, 6′-H), 4.61 (dd, J = 7.0 Hz, 1H, CH, 1′-H), 4.87 (m, 1H, OH), 4.92 (t, J = 6.0 Hz, 1H, CH, 2′-H), 5.27 (d, J = 5.5 Hz, 1H, OH), 5.55 (s, 1H, CH, 3′-H), 7.50 (s, 1H, CH, 6-H), 11.27 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 12.48, 35.82, 60.34, 65.78, 79.10, 109.47, 126.53, 139.75, 145.29, 151.46 and 164.39; Anal. Calcd for $C_{11}H_{14}N_2O_4 \cdot 0.19H_2O$: C, 54.67; H, 6.0; N, 11.59. Found: C, 54.71; H, 6.06; N, 11.44; UV (H_2O): λ_{max} 273 (ε 9554) (pH 7); 271 (ε 7755) (pH 11); 273 (ε 15,129) (pH 2); MS: $[M+Na]^+$: 261.10. Found: 261.08.

3.21. (±)-*trans*-9-(2′-Hydroxy-4′-hydroxymethylcyclopent-3′-enyl)guanine (23)

The free amine derivative was prepared from **14** (0.2 g, 0.63 mmol) by treatment with trifluoroacetic acid (0.8 mL) in dichloromethane (2 mL) at 0 °C for 2.5 h, followed by evaporation in vacuo. *N*-(4,6-Dichloro-5-nitropyrimidin-2-yl)acetamide (0.2 g, 0.8 mmol) was added to a solution of prepared free amine derivative in anhydrous ethanol (5 mL) and triethylamine (3 mL) at 0 °C. The resulting solution was stirred for 3 h at room temperature and concentrated in vacuo. The residue was dissolved in ethyl acetate (20 mL), washed with water (10 mL) and brine (10 mL). Organic layer was dried over magnesium sulfate, filtered and evaporated. The crude product was purified by silica gel chromatography with dichloromethane/ethyl acetate (1:1) to obtain **22** (210 mg, 77%). This compound was directly used in the next step without further purification.

A mixture of **22** (200 mg, 0.46 mmol) and stannous chloride dihydrate (1.0 g, 4.6 mmol) in ethanol (5 mL) was heated at 60 °C for 30 min, cooled to room temperature, diluted with ethyl acetate (40 mL), the mixture was then poured into saturated sodium bicarbonate (20 mL), filtered and washed with ethyl acetate (2× 10 mL). The organic layer was separated and dried over magnesium sulfate and concentrated to give the crude amino compound. To a suspension of the above amino compound in triethyl orthoformate (3 mL) was added 5 drops

methanesulfonic acid and concd HCl (0.2 mL). The reaction mixture was stirred for 3 h, then diluted with ethyl acetate (50 mL), washed with saturated sodium bicarbonate (2× 30 mL) and brine (20 mL). The organic layer was separated and dried over magnesium sulfate and evaporated in vacuo. The crude product was purified by silica gel chromatography with ethyl acetate/hexane (1:1) to obtain 6-chloropurine derivative (0.13 g, 68% in two steps).

2-Mercaptoethanol (0.06 mL, 0.86 mmol) and sodium methoxide (0.5 M, 1.68 mL, 0.84 mmol) were added to a solution of 6-chloropurine derivative (40 mg, 0.098 mmol) in methanol (4 mL). The reaction mixture was refluxed for 7 h, cooled to room temperature, neutralized by 3 N HCl (0.3 mL), and evaporated in vacuo. The crude product was purified by silica gel chromatography with dichloromethane/methanol (2:1) to give white solid (24 mg, 96%): mp 250 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.58 (dd, J = 7 Hz, J = 17 Hz, 1H, CH, 5′-H), 2.73 (dd, J = 8.5 Hz, J = 15.2 Hz, 1H, CH, 5′-H), 4.01(s, 2H, CH_2, 6′-H), 4.55 (q, J = 7 Hz, 1H, CH, 1′-H), 5.00 (t, J = 5 Hz, 1H, CH, 2′-H), 5.05 (s, 1H, OH), 5.43 (d, J = 5.5 Hz, 1H, OH), 5.61 (s, 1H, CH, 3′-H), 6.75 (s, 2H, NH_2), 7.73 (s, 1H, CH, 8-H), 10.87 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 37.08, 60.29, 63.49, 79.73, 117.55, 126.62, 136.82, 145.00, 151.66, 153.94 and 157.26; Anal. Calcd for $C_{11}H_{13}N_5O_3{\cdot}1.03H_2O$: C, 46.88; H, 5.39; O, 24.85. Found: C, 46.89; H, 5.17; N, 24.85; UV (H_2O): λ_{max} 252 (ε 11,902) (pH 7); 266 (ε 10,327) (pH 11); 255 (ε 11,331) (pH 2); HRMS: $[M+H]^+$: 264.109. Found: 264.107.

3.22. Cell culture assays for HIV-1

Antiviral and cytotoxicity assays were conducted as described by Schinazi et al.[24] HIV-1 antiviral assays were performed in activated primary human peripheral blood mononuclear (PBM) cells.

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References and notes

1. De Clercq, E. *Nat. Rev. Drug Disc.* **2002**, *1*, 13.
2. Agrofoglio, L.; Suhas, E.; Farese, A.; Condom, R.; Challand, S. R.; Earl, R. A.; Guedj, R. *Tetrahedron* **1994**, *50*, 10611; Crimmins, M. T. *Tetrahedron* **1998**, *54*, 9229.
3. Hayashi, S.; Norbeck, D. W.; Rosenbrook, W.; Fine, R. L.; Matsukura, M.; Plattner, J. J.; Border, S.; Mitsuya, H. *Antimicrob. Agents Chemother.* **1990**, *34*, 287;; Huryn, D.; Okabe, M. *Chem. Rev.* **1992**, *92*, 1745.
4. Vince, R.; Hua, M. *J. Med. Chem.* **1990**, *33*, 17.
5. Daluge, S. M. U.S. Patent 5,034,394, 1991.
6. Bissachi, G. S.; Chao, S. T.; Bachard, C.; Daris, J. P.; Innaimo, S.; Jacobs, G. A.; Kocy, O.; Lapionte, P.; Martel, A.; Merchant, Z.; Slusarchyk, W. A.; Sundeen, J. E.; Young, M. G.; Colonno, R.; Zahler, R. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 127.
7. (a) Hayashi, M.; Yaginuma, S.; Yoshioka, H.; Nakatsu, K. *J. Antibiot.* **1981**, *34*, 675; (b) De Clercq, E. *Antimicrob. Agents Chemother.* **1985**, *28*, 84.
8. (a) Ueland, P. M. *Pharmacol. Rev* **1982**, *34*, 223; (b) Wolfe, M. S.; Borchardt, R. T. *J. Med. Chem.* **1991**, *34*, 1521.
9. De Clercq, E. *Biochem. Pharmacol.* **1987**, *36*, 2567.
10. (a) Bennett, L. L., Jr.; Allan, P. W.; Rose, L. M.; Comber, R. N.; Secrist, J. A., III *Mol. Pharmacol.* **1986**, *29*, 383; (b) Inaba, M. K.; Tsukagoshi, N. S.; Sakurai, Y. *Cancer Res.* **1986**, *46*, 1063; (c) Glazer, R. I.; Knode, M. C. *J. Biol. Chem.* **1984**, *259*, 12964.
11. (a) Shuto, S.; Yaginuma, O. T.; Toriya, M.; Hosoya, M.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1992**, *35*, 324; (b) Obara, T.; Shuto, S.; Saito, Y.; Snoeck, R.; Andrei, G.; Balzarini, J.; Clercq, E. D.; Matsuda, A. *J. Med. Chem.* **1996**, *39*, 3847.
12. (a) Hayashi, M.; Yaginuma, S.; Muto, N.; Tsujino, M. *Nucleic Acids Res.* **1980**, Symposium Series No.*8*, s 65; (b) Comin, M. J.; Leitofuter, J.; Rodriguez, J. B. *Tetrahedron* **2002**, *58*, 3129.
13. Bodenteich, M.; Marquez, V. E.; Hallows, W. H.; Goldstein, B. M. *J. Org. Chem.* **1992**, *57*, 2071.
14. (a) Smith, M. E. B.; Derrien, N. *PCT Int. Appl.* **2001**, 38 pp. WO 2001017952 A1 20010315; (b) Smith, M. E. B.; Derrien, N.; Lloyd, M. C.; Taylor, S. J. C.; Chaplin, D. A.; McCague, R. *Tetrahedron Lett.* **2001**, *42*, 1347.
15. (a) Toyota, A.; Habutani, C.; Katagiri, N.; Kaneko, C. *Tetrahedron Lett.* **1994**, *35*, 5665; (b) Katagiri, N.; Ito, Y.; Takuya, S.; Maruyama, T.; Sato, Y.; Kaneko, C. *Nucleosides Nucleotides* **1996**, *15*, 631.
16. Levy, D. E.; Bao, M.; Cherbavaz, D. B.; Tomlinson, J. E.; Sedlock, D. M.; Homcy, C. J.; Scarborough, R. M. *J. Med. Chem.* **2003**, *46*, 2177.
17. Guan, H.-P.; Ksebati, M. B.; Cheng, Y.-C.; Drach, J. C.; Kern, E. R.; Zemlicka, J. *J.Org. Chem.* **2000**, *65*, 1280.
18. (a) Trost, B. M.; Madsen, R.; Guile, S. D.; Brown, B. *J. Am. Chem. Soc.* **2000**, *122*, 5947; (b) Trost, B. M.; Madsen, R.; Guile, S. D. *Tetrahedron Lett.* **1997**, *38*, 1707.
19. Moriwake, T.; Hamano, S.; Miki, D.; Saito, S.; Torii, S. *Chem. Lett.* **1986**, *5*, 815.
20. Lapkin, I. I.; Fotin, V. V. *Zh. Org. Khim.* **1986**, *22*, 738.
21. Komatsu, H.; Morizane, K.; Kohno, T.; Tanikawa, H. *Org. Process Res. Dev.* **2004**, *8*, 564.
22. (a) Walkup, R. D.; Obeyesekere, N. U. *Synthesis* **1987**, *7*, 607; (b) Rene, C.; Yvonne, V. S. *Tetrahedron* **1995**, *51*, 7193.
23. Temple, C., Jr.; Smith, B. H.; Montgomery, J. A. *J. Org. Chem.* **1975**, *40*, 3141.
24. (a) Schinazi, R. F.; Sommadossi, J. P.; Saalmann, V.; Cannon, D. L.; Xie, M.-W.; Hart, G. C.; Smith, G. A.; Hahn, E. F. *Antimicrob. Agents Chemother.* **1990**, *34*, 1061; (b) Stuyver, L. J.; Lostia, S.; Adams, M.; Mathew, J.; Pai, B. S.; Grier, J.; Tharnish, P.; Choi, Y.; Chong, Y.; Choo, H.; Chu, C. K.; Otto, M. J.; Schinazi, R. F. *Antimicrob. Agents Chemother.* **2002**, *46*, 3854.