Synthesis and Anti-HIV-1 Activity of Carbocyclic Versions of Stavudine Analogues Using a Ring-closing Metathesis

Lian Jin Liu, Ok Hyun Ko, and Joon Hee Hong*

*BK21-Project Team, College of Pharmacy, Chosun University, Gwangju 501-759, Korea. *E-mail: hongjh@chosun.ac.kr*
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An efficient synthetic route for carbocyclic versions of stavudine analogues and their evaluation on antiviral activity are described. The construction of an ethynylated quaternary carbon at the 4'-position of carbocyclic nucleosides was accomplished using Claisen rearrangement of **11** and ring-closing metathesis (RCM) of dienyne **14** as key transformations. An antiviral evaluation of the synthesized compounds, **20**, **21**, **22**, and **25** against HIV-1, HSV-1, HSV-2, and HCMV showed that only the guanine analogue **25** is moderately active against HIV-1 in the MT-4 cell line (EC_{50} = 11.91 μmol).

Key Words : Carbocyclic nucleoside, Antiviral agents, Ring-closing metathesis, Claisen rearrangement

Introduction

Replacement of the furanose ring oxygen atom with carbon is of particular interest because the resulting carbocyclic nucleosides[1] have greater metabolic stability against chemical or enzymatic hydrolysis,[2] which cleaves the glycosidic bond of nucleosides. Many carbocyclic nucleosides have antiviral and anticancer activity because the cyclopentane ring of these compounds can emulate a furanose moiety. Carbocyclic nucleosides are also potent inhibitors of the cellular enzyme, *S*-adenosyl-*L*-homocysteine (AdoHcy) hydrolase, which regulates *S*-adenosylmethionine (SAM)-dependent methylation reactions, and are specific targets for the reversible hydrolysis of the AdoHcy linkage to adenosine and homocysteine.[3] The recent discovery of olefinic carbocyclic nucleosides, such as carbovir[4] and abacavir,[5] which are potential anti-HIV agents, has increased interest in the search for novel carbocyclic nucleosides, whereas their side effects[6] and the emergence of drug-resistant mutants are lasting concerns to be solved.[7]

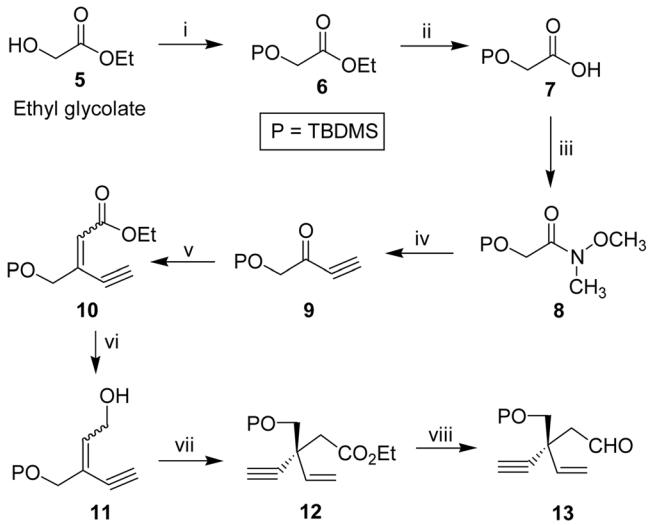
Recent reports that thymidine analogues with 4'-azido **1**[8] and 4'-cyano groups **2**[9] show significant inhibitory activity against HIV proliferation have stimulated the synthesis of 4'-substituted nucleoside analogues to lead to the discovery of 4'-ethynylated stavudine **3**[10] and thiostavudine **4**[11] analogues which turned out to be efficient antiviral and antitumor agents.

Stimulated by these interesting SAR (structure activity relationship), we describe herein the synthesis of a novel class of nucleosides containing 4'-ethynyl carbocyclic nucleosides and their antiviral profile.

Results and Discussion

As depicted in Scheme 1, we hypothesized that ring-closing metathesis (RCM) of proper divinyls **14**, which could be readily synthesized via sequential reactions, such as Claisen rearrangement and Grignard addition starting from ethyl glycolate **5**, would produce ethynylated cyclopentene **15**β.

Silyl protection of the alcohol of the commercially available starting material **5** followed by hydrolysis gave carboxylic acid derivative **7**, which was transformed to the Weinreb amide **8** by the treatment of DCC and DMAP coupling reagents.[12] Conversion of the amide to the propargyl ketone derivative **9** was successful under the usual carbonyl addition conditions (propargylMgBr, THF, 0 °C). Treatment of **9** with triethylphosphonoacetate[13] provided α,β-unsaturated ethyl ester **10** as a cis/trans isomeric mixture. These isomers do not need separating because they merge into one isomer **12** after Claisen rearrangement. Addition of the diisobutylaluminum hydride (DIBALH) to **10** provided the allylic alcohol **11**, which was subjected to a regular Johnson's orthoester Claisen rearrangement[14] with triethyl orthoacetate to yield the γ,δ-unsaturated ester **12**.



Scheme 1. Synthesis route of aldehyde intermediate **13**. Reagents: i) TBDMSCl, CH_2Cl_2, imidazole; ii) KOH, EtOH; iii) *N*-methyl hydroxylamine hydrochloride, DCC, DMAP, TEA; iv) propargylmagnesium bromide, THF; v) Triethylphosphonoacetate, NaH, THF; vi) DIBALH, CH_2Cl_2; vii) Triethylorthoacetate, propionic acid, overnight, 135-140 °C; viii) DIBALH, toluene, –78 °C.

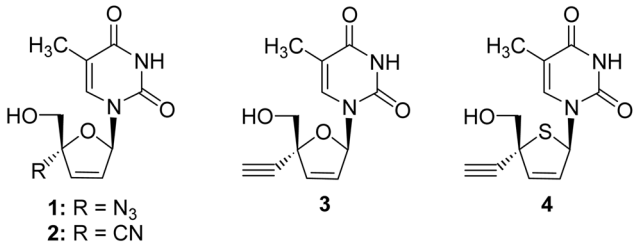


Figure 1. Structures and rationale of target 4'-ethynylated nucleosides.

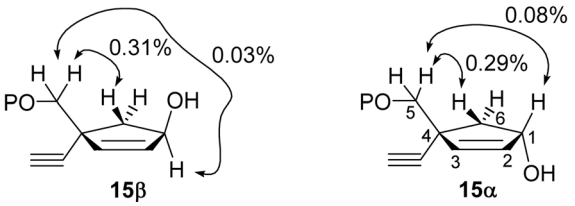
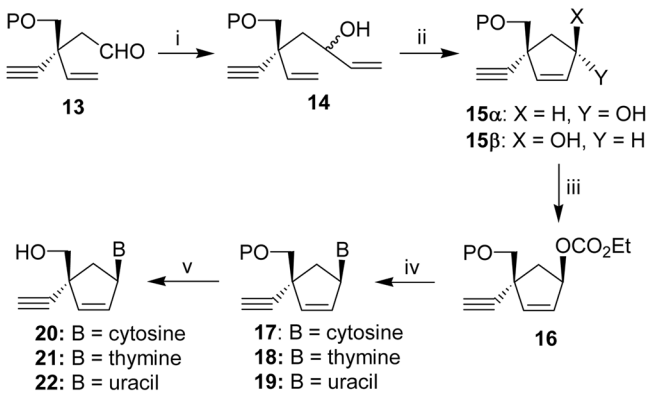


Figure 2. NOE comparisons of compound **15**α and **15**β.

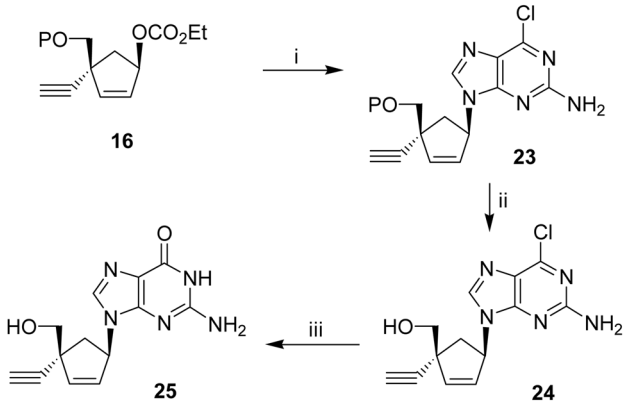
Direct reduction of the ester **12** to the aldehyde **13** was successfully accomplished by slow addition of DIBALH in the toluene solvent system at −78 °C. The aldehyde **13** was subjected to carbonyl addition by CH_2=CHMgBr to give divinyl **14**.

Divinyl **14** was subjected to standard RCM[15] conditions using a second-generation Grubbs catalyst to provide the diene metathesis product **15**α/**15**β as well as enyne metathesis product, which were readily separated by simple silica gel column chromatography. The correct configurations of **15**α and **15**β were assigned based on NOE comparisons. Upon the irradiation of C_5-H, different NOE pattern was observed at the protons of compound **15** [C_1-H (0.03%) & C_6-Hβ (0.31%)], from those of compound **15** [C_1-H (0.08%) & C_6-Hβ (0.29%)] (Figure 2).

First, we attempted the mesylation of **15**α because mesylate is an excellent reactive intermediate for the replacement of free hydroxyl groups with nucleoside bases. To our surprise, the mesylate that appeared in the reaction mixture disappeared during the work-up, resulting in decomposition into an unidentifiable byproduct and requiring an alternative coupling method. Alternatively, we turned out attention to Palladium(0)-catalyzed reactions of allylic carbonate.[16] To this end, cyclopentenol **15**β was transformed to **16** using ethyl chloroformate, which was coupled with pyrimidine nucleosidic base (cytosine, thymine, uracil) anions generated by NaH/DMSO with use of catalyst [tris(dibenzylideneacetone)-dipalladium(0)-chloroform] adduct to provide nucleoside analogues **17**-**19**. Removing the silyl protection groups of **17**-**19** was performed by the treatment of tetrabutylammonium fluoride (TBAF) to yield final nucleosides **20**-**22** (Scheme 2). Similarly, the guanine derivative was synthesized by coupling the same intermediate **16** as used in the preparation of pyrimidine analogues. The silicon protection group of compound **23** was removed by treatment with TBAF to produce compound **24**. Treatment of compound **24** with 2-mercaptoethanol and sodium methoxide in



Scheme 2. Synthesis route of taregt pyrimidine nucleosides. Reagents: i) vinylMgBr, THF; ii) Grubbs catalyst (II), CH_2Cl_2; iii) $ClCO_2Et$, pyridine, DMAP; iv) pyrimidine nucleosidic bases, $Pd_2(dba)_3$·$CHCl_3$, P(O-*i*-Pr)$_3$, NaH, THF/DMSO; v) TBAF, THF.



Scheme 3. Synthesis route of taregt purine nucleoside. Reagents: i) 2-amino-6-chloropurine, $Pd_2(dba)_3$·$CHCl_3$, P(O-*i*-Pr)$_3$, NaH, THF/DMSO; ii) TBAF, THF; iii) (a) 2-mecaptoethanol, NaOMe, MeOH, (b) CH_3COOH.

methanol, followed by hydrolysis with acetic acid, gave the desired nucleoside **25** (Scheme 3).

Antiviral activity studies. Compounds, **20**, **21**, **22**, and **25** were tested against HIV-1 (MT-4 cells), HSV-1 (CCL81 cells), HSV-2 (CCL-81 cells), and HCMV (AD-169, Davis cells). Among them, only guanine analogue **25** exhibited moderate antiviral activity against HIV-1 (Table 1); and the thymine analogue **21** showed weak antiviral activity against HCMV. The assay involved the killing of T4-lymphocytes by HIV-1. T4 lymphocytes (MT-4 cell line) were exposed to HIV at a virus-to-cell ratio of approximately 0.05 and treated with the compounds, dissolved in dimethylformamide, at doses ranging from 10^{-8} to 10^{-4}. A complete cycle of virus reproduction is necessary to obtain the required cell killing (incubation at 37 °C in a 5% carbon dioxide atmosphere for 6 days). Uninfected cells with the compounds served as a toxicity control, whereas the infected and uninfected cells without the compound served as basic control.[17]

Compared to **3** and **4**, it is surprising that their corresponding carbacyclic analog **21** did not show any noticeable activity. Investigation on the cause of this unexpected SAR would be an interesting topic as a guidance for further

Table 1. Antiviral activity of the synthesized compounds

	HIV-1 EC_{50} (μmol)	HSV-1 EC_{50} (μmol)	HSV-2 EC_{50} (μmol)	HCMV EC_{50} (μmol)	cytotoxicity CC_{50} (μmol)
20	90	>100	>100	>100	90
21	45.7	98	>100	19.3	98
22	99	>100	>100	99	>100
25	**11.91**	88	>100	36.4	99
D4T	0.05	ND	ND	ND	20
GCV	ND	ND	ND	0.8	>10
ACV	ND	0.2	ND	ND	>100

D4T: Stavudine; GCV: Ganciclovir; ACV: Acyclovir. ND: Not Determined. EC_{50} (μM): Concentration required to inhibit 50% of the virus-induced cytopathicity. CC_{50} (μM): Concentration required to reduce cell viability by 50%

development of carbacyclic derivatives. In summary, we developed an efficient synthetic method to yield 4'-ethynyl carbocyclic nucleosides starting from ethyl glycolate. Based on this strategy, the syntheses of other nucleosides with different nucleobases are in progress in our laboratory.

Experimental Section

Melting points were determined on a Mel-temp II laboratory device and are uncorrected. NMR spectra were recorded on a JEOL 300 Fourier transform; chemical shifts are reported in parts per million (δ) and signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and dd (doublet of doublets). UV spectra were obtained on a Beckman DU-7 spectrophotometer. The elemental analyses were performed using an Elemental Analyzer System (EA1112). TLC was performed on Uniplates (silica gel) purchased from Analtech Co. All reactions were performed under a nitrogen atmosphere unless otherwise specified. Dry dichloromethane, benzene, and pyridine were obtained by distillation from CaH_2. Dry THF was obtained by distillation from Na and benzophenone immediately prior to use.

(*tert*-Butyldimethylsilyloxy)-acetic acid ethyl ester (6): To a solution of ethyl glycolate **5** (10.0 g, 0.09 mol) and imidazole (8.80 g, 0.14 mol) in CH_2Cl_2 (200 mL), TBDMSCl (15.9 g, 0.10 mol) was added slowly at 0 °C, and stirred for 5 h at the same temperature. The reaction solvent was evaporated under reduced pressure. The residue was extracted twice with diethyl ether and water. The combined organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:20) to give compound **6** (19.9 g, 95%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.12 (s, 2H), 4.08 (q, J = 6.9 Hz, 2H), 1.17 (t, J = 6.9 Hz, 3H), 0.81 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 171.54, 61.80, 60.66, 25.69, 18.35, 14.12, –5.50.

(*tert*-Butyldimethylsilyloxy) acetic acid (7): A solution of KOH (2.57 g, 59.5 mmol) in EtOH (20 mL) was slowly added to a solution of **6** (10.0 g, 45.7 mmol) in EtOH (200 mL) at 0 °C. The mixture was stirred overnight at rt and concentrated under reduced pressure. The residue was dissolved in water (200 mL) and carefully neutralized with *c*-HCl solution to pH 3-4. The solution was extracted with EtOAc two times. The organic layer was washed with brine and dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:3) to give **7** (7.84 g, 90%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.12 (s, 2H), 0.82 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 171.02, 61.90, 25.55, 18.71, –5.57.

2-(*tert*-Butyldimethylsilyloxy)-*N*-methoxy-*N*-methyl-acetamide (8): To a solution of acid derivative **7** (5.00 g, 26.2 mmol) in a anhydrous CH_2Cl_2 (150 mL), *N*,*O*-dimethylhydroxylamine hydrochloride (3.06 g, 31.4 mmol), DCC (6.48 g, 31.4 mmol), DMAP (317 mg, 2.60 mmol) and triethylamine (3.18 g, 31.4 mmol) were sequentially added to the reaction mixture. The solution was stirred overnight at rt. After addition of methanol (5 mL) and acetic acid (5 mL), the mixture was stirred for 1 h and neutralized with saturated aqueous $NaHCO_3$ solution. The resulting solid was filtered off through a short pad of Celite and the filtrate was concentrated in vacuum. The resulting residue was purified by silica gel column chromatography (EtOAc/hexane, 1:1.5) to give Weinreb amide **8** (5.21 g, 85%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.48 (s, 2H), 3.57 (s, 3H), 3.05 (s, 3H), 0.80 (s, 9H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 171.63, 61.00, 52.63, 31.67, 25.54, 18.49, -5.61.

1-(*tert*-Butyldimethylsilyloxy)-but-3-yn-2-one (9): Ethynylmagnesium bromide (32.8 mL, 0.5 M solution in THF) was slowly added to a solution of Weinreb amide **8** (3.20 g, 13.7 mmol) in dry THF (70 mL) at 0 °C and stirred for 5 h at the same temperature. The mixture was quenched with saturated NH_4Cl (16 mL), and the reaction mixture was slowly warmed to room temperature. The mixture was extracted with EtOAc (2 × 100 mL). The combined organic layer was dried over $MgSO_4$, filtered, and evaporated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:10) to give **9** (1.87 g, 69%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.34 (s, 2H), 2.98 (s, 1H), 0.85 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 196.31, 82.87, 79.43, 73.43, 25.76, 18.34, -5.58; Anal. calcd. for $C_{10}H_{18}O_2Si$: C, 60.56; H, 9.15. Found: C, 60.45; H, 9.07.

(*E*) and (*Z*)-3-(*tert*-Butyldimethylsilyloxymethyl)-pent-2-en-4-ynoic acid ethyl ester (10): To a suspension of sodium hydride (0.40 g, 16.7 mmol) in distilled THF (100 mL) was added drop wise triethyl phosphonoacetate (3.74 g, 16.7 mmol) at 0 °C and the mixture was stirred at room temperature for 2 h. The ketone **9** (3.31 g, 16.7 mmol) was added to this mixture and stirred for 2 h. The solution was neutralized with AcOH (3 mL) and poured into H_2O (150 mL) and extracted with EtOAc. The organic layer was washed with brine and dried over anhydrous $MgSO_4$, filtered and evaporated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:12) to give **10** (3.22 g, 72%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.22 (s, 1H), 4.50 (s, 2H), 4.21 (q, J = 7.2 Hz, 2H), 3.09 (s, 1H), 1.31 (t, J = 7.2 Hz, 2H), 0.84 (s, 9H), 0.02 (s, 6H).

(*E*) and (*Z*)-3-(*tert*-Butyldimethylsilyloxymethyl)-pent-2-en-4-yn-1-ol (11): DIBALH (35.2 mL, 1.0 M solution in hexane) was slowly added to a solution of **10** (4.50 g, 16.7 mmol) in CH_2Cl_2 (150 mL) at −20 °C, and stirred for 1.5 h at the same temperature. Methanol (35 mL) was added to the mixture. The mixture was stirred at room temperature for 2 h, and the resulting solid was filtered through a Celite pad. The filtrate was concentrated under vacuum and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:7) to give alcohol **11** (3.41 g, 90%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.17 (t, J = 1.8 Hz, 1H), 4.31 (d, J = 6.6 Hz, 2H), 4.08 (s, 2H), 3.10 (s, 1H), 0.86 (m, 9H), 0.01 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 139.74, 135.52, 123.81, 83.65, 79.05, 64.79, 60.90, 25.78, 18.56, -5.50.

(±)-3-(*tert*-Butyldimethylsilyloxymethyl)-3-ethynyl-pent-4-enoic acid ethyl ester (12): A solution of allylic alcohol **11** (5.50 g, 24.3 mmol) in triethyl orthoacetate (150 mL) and 0.2 mL of propionic acid was heated at 135-140 °C overnight with stirring for the distillation of ethanol. The excess triethyl orthoacetate was distilled off and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:25) to give **12** (6.05 g, 84%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.92 (d, J = 9.8 Hz, 1H), 5.80 (d, J = 10.4 Hz, 1H), 5.31 (d, J = 1.4 Hz, 1H), 4.02 (q, J = 6.9 Hz, 2H), 3.64 (d, J = 9.6 Hz, 1H), 3.51 (d, J = 9.6 Hz, 1H), 2.30 (d, J = 7.8 Hz, 1H), 2.24 (d, J = 7.8 Hz, 1H), 1.98 (s, 1H), 0.84 (s, 9H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 171.75, 143.54, 114.50, 80.76, 77.65, 69.34, 61.32, 49.35, 25.76, 18.76, 13.76, −5.76; Anal. calcd. for $C_{16}H_{28}O_3Si$: C, 64.82; H, 9.52. Found: C, 65.03; H, 9.67.

(±)-3-(*t*-Butyldimethylsilyloxymethyl)-3-ethynyl-pent-4-enal (13): To a solution of **12** (2.50 g, 8.43 mmol) in toluene (40 mL), DIBALH (6.18 mL, 1.5 M solution in toluene) was added slowly at −78 °C, and stirred for 15 minutes at the same temperature. To the mixture, methanol (7 mL) was added. The mixture was stirred at room temperature for 1.5 h, and the resulting solid was filtered through a Celite pad. The filtrate was concentrated under vacuum and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:20) to give **13** (1.29 g, 61%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.80 (m, 1H), 5.85 (d, J = 10.0 Hz, 1H), 5.70 (d, J = 9.4 Hz, 1H), 5.33 (d, J = 8.0 Hz, 1H), 3.79 (s, 2H), 2.93 (m, 2H), 2.01 (s, 1H), 0.83 (s, 9H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 202.78, 143.32, 113.76, 81.39, 78.61, 69.55, 48.43, 25.78, 18.72, −5.76; Anal. calcd. for $C_{14}H_{24}O_2Si \cdot 0.5$ Hx: C, 69.33; H, 10.26. Found: C, 69.49; H, 10.40.

(*rel*)-(3*R* and *3S*,5*S*)-5-(*t*-Butyldimethylsilyloxymethyl)-5-ethynyl-hepta-1,6-dien-3-ol (14): To a solution of **13** (4.20 g, 16.6 mmol) in dry THF (100 mL) was slowly added vinyl magnesiumbromide (19.9 mL, 1.0 M solution in THF) at −78 °C. After 4 h, saturated NH_4Cl solution (20 mL) and water (100 mL) was sequentially added, and the reaction mixture was slowly warmed to rt. The mixture was extracted with EtOAc (2 × 120 mL). The combined organic layer was dried over $MgSO_4$, filtered, and evaporated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:18) to give **14** (3.50 g, 75%) as colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.08-5.70 (m, 2H), 5.41-5.17 (m, 4H), 4.27 (m, 1H), 3.52 (m, 2H), 2.02 (m, 1H), 1.69-1.57 (m, 2H), 0.82 (m, 9H), 0.02 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 142.91, 140.54, 114.76, 114.64, 112.12, 111.99, 88.32, 73.45, 68. 57, 68.43, 67.09, 41.12, 41.35, 30.06, 25.43 (m), 18.70, −5.71 (m); Anal. calcd. for $C_{16}H_{28}O_2Si \cdot 0.5$ EtOAc: C, 66.62; H, 9.94. Found: C, 66.68; H, 9.96.

(*rel*)-(1*R*,4*S*)-4-(*t*-Butyldimethylsilyloxymethyl)-4-ethynyl-cyclopent-2-enol (15β); and (*rel*)-(1*S*,4*S*)-4-(*t*-Butyldimethylsilyloxymethyl)-4-ethynyl-cyclopent-2-enol (15α): A second-generation Grubbs catalyst (153 mg 0.18 mmol) was added to a solution of **14** (1.55 g, 5.54 mmol) in dry CH_2Cl_2 (20 mL). The reaction mixture was refluxed overnight and cooled to room temperature. The mixture was concentrated in a vacuum, and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:10) to give cyclopentenol **15β** (293 mg, 21%) and **15α** (307 mg, 22%) as colorless oils. Cyclopentenol **15β**: ^{1}H NMR ($CDCl_3$, 300 MHz) d 6.00-5.92 (m, 5H), 4.54 (m, 1H), 3.68 (d, J = 9.4 Hz, 1H), 3.51 (d, J = 9.4 Hz, 1H), 2.30 (dd, J = 13.2, 6.8 Hz, 1H), 1.99 (s, 1H), 1.59 (dd, J = 8.4, 6.8 Hz, 1H), 0.84 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 141.10, 134.65, 80.76, 78.49, 73.35, 68.99, 52.54, 45.38, 25.65, 18.57, −5.62; Anal. calcd. for $C_{14}H_{24}O_2Si$: C, 66.61; H, 9.58. Found: C, 66.70; H, 9.68. Cyclopentenol **15α**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.79-5.68 (m, 2H), 4.82 (dd, J = 6.6, 1.4 Hz, 1H), 3.37 (s, 2H), 2.28 (dd, J = 13.4, 7.2 Hz, 1H), 2.01 (s, 1H), 1.48 (dd, J = 13.4, 7.2 Hz, 1H), 0.83 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 140.97, 132.82, 89.57, 75.54, 71.69, 69.12, 52.43, 44.28, 25.76, 18.72, −5.78; Anal. calcd. for $C_{14}H_{24}O_2Si$: C, 66.61; H, 9.58. Found: C, 66.48; H, 9.51.

(*rel*)-(1*R*,4*S*)-1-Ethoxy carbonyloxy-4-(*t*-butyldimethylsilyloxymethyl)-4-ethynyl-cyclopent-2-ene (16): Ethyl chloroformate (1.65 mL, 17.3 mmol) and DMAP (102 mg, 0.84 mmol) were added to a solution of **15β** (2.18 g, 8.65 mmol) in anhydrous pyridine (15 mL). The reaction mixture was stirred overnight at room temperature. The reaction mixture was quenched with saturated $NaHCO_3$ solution (1.5 mL) and concentrated in vacuum. The residue was extracted with EtOAc/H_2O and the organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:12) to give **16** (2.1 g, 75%) as a colorless syrup: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.41-6.36 (m, 2H), 5.50 (dd, J = 6.4, 1.4 Hz, 1H), 4.29 (q, J = 7.4 Hz, 2H), 3.86 (d, J = 9.6 Hz, 1H), 3.79 (d, J = 9.6 Hz, 1H), 2.43 (dd, J =14.0, 7.8 Hz, 1H), 2.17 (dd, J = 14.0, 6.8 Hz, 1H), 2.09 (s, 1H), 1.31 (t, J = 7.4 Hz, 3H), 0.84 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 154.95, 143.99, 128.51, 88.72, 84.03, 73.58, 71.12, 64.52, 50.78, 41.49, 25.59, 18.67, 14.62, −5.57; Anal. calcd. for $C_{17}H_{28}O_4Si \cdot 1.0$ EtOAc: C, 61.13; H, 8.79. Found: C, 61.11; H, 8.64.

(*rel*)-(1'*R*,4'*S*)-9-[4-(*t*-Butyldimethylsilyloxymethyl)-4-ethynyl-cyclopent-2-en-1-yl] cytosine (17): Cytosine (109 mg, 0.98 mmol) was added to pure NaH (23.5 mg, 0.98

mmol) in anhydrous DMSO (6.00 mL). The reaction mixture was stirred for 30 min at 50-55 °C and cooled to room temperature. Simultaneously, P(O-*i*-Pr)$_3$ (0.07 mL, 0.22 mmol) was added to a solution of $Pd_2(dba)_3$·$CHCl_3$ (4.60 mg, 2.50 mmol) in anhydrous THF (5.0 mL), which was stirred for 30 min. To the nucleosidic base solution of DMSO was sequentially added catalyst solution of THF and **16** (286 mg, 0.88 mmol) dissolved in anhydrous THF (5 mL). The reaction mixture was stirred overnight at refluxing temperature and quenched with water (3 mL). The reaction solvent was removed in a vacuum. The residue was purified by silica gel column chromatography (MeOH/Hexane/EtOAc, 0.1:1:5) to give **17** (118 mg, 39%) as a white solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.31 (d, J = 7.0 Hz, 1H), 6.06 (d, J = 5.4 Hz, 1H), 5.96 (m, 1H), 5.54 (d, J = 7.0 Hz, 1H), 5.39 (dd, J = 6.4, 1.4 Hz, 1H), 3.81 (d, J = 9.2 Hz, 1H), 3.75 (d, J = 9.0 Hz, 1H), 2.67 (dd, J = 13.8, 8.0 Hz, 1H), 2.22 (dd, J = 13.8, 6.6 Hz, 1H), 2.05 (s, 1H), 0.85 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 165.72, 156.67, 145.39, 144.21, 127.88, 93.71, 89.56, 71.42, 69.54, 55.62, 42.32, 25.67, 18.66, −5.61; Anal. calcd. for $C_{18}H_{27}N_3O_2Si$·1.0 MeOH: C, 57.62; H, 7.89; N, 10.61. Found: C, 57.42; H, 7.79; N, 10.73.

(*rel*)-(1'*R*,4'*S*)-9-[4-(*t*-Butyldimethylsilyloxymethyl)-4-ethynyl-cyclopent-2-en-1-yl] thymine (18): The thymine nucleoside analogue **18** was synthesized from **16** as described for **17**: yield 30%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.29 (br s, 1H), 7.15 (s, 1H), 6.11 (d, J = 5.2 Hz, 1H), 6.00-5.93 (m, 2H), 5.35 (m, 1H), 3.76 (d, J = 9.0 Hz, 1H), 3.60 (d, J = 9.0 Hz, 1H), 2.59 (dd, J = 14.0, 7.8 Hz, 1H), 2.18 (dd, J = 14.0, 6.8 Hz, 1H), 2.03 (s, 1H), 1.55 (s, 3H), 0.86 (s, 9H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 164.21, 151.70, 143.59, 142.29, 128.21, 109.39, 88.43, 73.39, 69.43, 56.19, 41.54, 25.60, 18.59, 12.30, −5.62; Anal. calcd. for $C_{19}H_{28}N_2O_3Si$: C, 63.30; H, 7.83; N, 7.77. Found: C, 63.43; H, 7.70; N, 7.62.

(*rel*)-(1'*R*,4'*S*)-9-[4-(*t*-Butyldimethylsilyloxymethyl)-4-ethynyl-cyclopent-2-en-1-yl] uracil (19): The uracil nucleoside analogue **19** was obtained from **16** as described for **17**: yield 28%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.35 (br s, 1H), 7.20 (d, J = 7.8 Hz, 1H), 6.05 (dd, J = 5.4, 1.8 Hz, 1H), 5.93-5.88 (m, 2H), 5.68-5.59 (m, 2H), 3.69 (d, J = 9.2 Hz, 1H), 3.51 (d, J = 9.2 Hz, 1H), 2.40 (dd, J = 14.0, 7.8 Hz, 1H), 2.09-2.00 (m, 2H), 0.85 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 163.86, 151.21, 147.30, 143.50, 127.39, 101.47, 89.38, 74.32, 70.55, 57.78, 43.19, 25.67, 18.59, −5.73; Anal. calcd. for $C_{18}H_{26}N_2O_3Si$·0.5 EtOAc: C, 61.50; H, 7.74; N, 7.17. Found: C, 61.44; H, 7.61; N, 7.16.

(*rel*)-(1'*R*,4'*R*)-9-[4-(Hydroxymethyl)-4-ethynyl-cyclopent-2-en-1-yl] cytosine (20): TBAF (0.43 mL, 1.0 M solution in THF) was added to a solution of **17** (99.0 mg, 0.27 mmol) in THF (5 mL) at 0 °C. The mixture was stirred overnight at room temperature and concentrated. The residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1:5) to give **20** (50.0 mg, 74%) as a white solid: mp 164-167 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.39 (d, J = 7.2 Hz, 1H), 6.99 (br d, 2H), 6.08 (dd, J = 5.6, 1.2 Hz, 1H), 5.97 (d, J = 5.6 Hz, 1H), 5.56-5.49 (m, 2H), 4.97 (t, J = 5.4 Hz, 1H), 3.68 (d, J = 9.2 Hz, 1H), 3.59 (d, J = 9.2 Hz, 1H), 2.51 (dd, J = 14.0, 8.2 Hz, 1H), 2.06 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 165.42, 155.78, 146.49, 143.93, 128.37, 92.37, 88.54, 73.43, 68.99, 54.32, 43.41; Anal. calcd. for $C_{12}H_{13}N_3O_2$·1.0 H_2O: C, 57.82; H, 6.06; N, 16.85. Found: C, 57.99; H, 5.97; N, 16.80.

(*rel*)-(1'*R*,4'*R*)-9-[4-(Hydroxymethyl)-4-ethynyl-cyclopent-2-en-1-yl] thymine (21): The thymine carbocyclic nucleoside analogue **21** was synthesized from **18** by the procedure described for **20**: yield 69%; mp 160-163 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.19 (br s, 1H), 7.18 (s, 1H), 6.13 (d, J = 5.4 Hz, 1H), 6.98-5.91 (m, 2H), 5.38 (m, 1H), 4.90 (t, J = 5.4 Hz, 1H), 3.65 (d, J = 9.2 Hz, 1H), 3.52 (d, J = 9.2 Hz, 1H), 2.42 (dd, J = 14.2, 7.6 Hz, 1H), 2.01-1.95 (m, 2H), 1.52 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 164.56, 151.49, 144.50, 143.79, 128.51, 108.90, 89.31, 72.49, 69.77, 54.54, 43.48, 12.28; Anal. calcd. for $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.38. Found: C, 63.53; H, 5.92; N, 11.43.

(*rel*)-(1'*R*,4'*R*)-9-[4-(Hydroxymethyl)-4-ethynyl-cyclopent-2-en-1-yl] uracil (22): The uracil nucleoside analogue **22** was synthesized from **19** using the deprotection procedure described for **20**: yield 75%; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.21 (br s, 1H), 7.21 (d, J = 7.6 Hz, 1H), 6.08 (d, J = 5.6 Hz, 1H), 6.01-5.93 (m, 2H), 5.59-5.50 (m, 2H), 3.64 (d, J = 9.2 Hz, 1H), 3.55 (d, J = 9.2 Hz, 1H), 2.38 (dd, J = 14.0, 7.6 Hz, 1H), 2.02 (s, 1H), 1.90 (dd, J = 14.0, 6.8 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 164.10, 152.54, 147.88, 144.21, 128.02, 102.08, 89.54, 73.45, 69.29, 57.47, 44.38; Anal. calcd. for $C_{12}H_{12}N_2O_3$·0.5 MeOH: C, 60.47; H, 5.68; N, 11.28. Found: C, 60.55; H, 5.72; N, 11.09.

(*rel*)-(1'*R*,4'*S*)-9-[4-(*t*-Butyldimethylsilyloxymethyl)-4-ethynyl-cyclopent-2-en-1-yl] 2-amino-6-chloropurine (23): The purine nucleoside analogue **23** was synthesized with the condensation reaction method described for **17**: yield 28%; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.95 (s, 1H), 6.08 (d, J = 5.6 Hz, 1H), 5.98 (dd, J = 4.8, 1.4 Hz, 1H), 5.47 (dd, J = 5.2, 1.8 Hz, 1H), 3.62 (d, J = 9.2 Hz, 1H), 3.54 (d, J = 9.2 Hz, 1H), 2.47 (dd, J =14.0, 7.6 Hz, 1H), 2.04-1.92 (m, 2H), 0.86 (s, 9H), 0.02 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 159.20, 154.31, 151.10, 143.89, 143.11, 126.54, 125.23, 90.02, 74.42, 69.54, 58.21, 43.58, 25.72, 18.58, −5.62; Anal. calcd. for $C_{19}H_{26}ClN_5OSi$: C, 56.49; H, 6.49; N, 17.34. Found: C, 56.58; H, 4.35; N, 17.27.

(*rel*)-(1'*R*,4'*S*)-9-[4-(Hydroxymethyl)-4-ethynyl-cyclopent-2-en-1-yl] 2-amino-6-chloropurine (24): The nucleoside analogue **24** was obtained from **23** as described for **20**; yield 62%; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.89 (s, 1H), 6.10 (dd, J = 5.4, 1.4 Hz, 1H), 6.02 (dd, J = 5.2, 1.6 Hz, 1H), 5.50 (m, 1H), 4.91 (t, J = 5.2 Hz, 1H), 3.58 (d, J = 9.2 Hz, 1H), 3.49 (d, J = 9.2 Hz, 1H), 2.50 (dd, J = 14.2, 7.8 Hz, 1H), 2.07-1.99 (m, 2H), 0.86 (s, 9H); ^{13}C NMR (DMSO-d_6) δ 159.65, 153.98, 150.87, 143.21, 142.79, 125.42, 124.21, 89.64, 73.43, 69.11, 57.42, 42.28; Anal. calcd. for $C_{13}H_{12}ClN_5O$·0.5 MeOH: C, 53.03; H, 4.61; N, 22.91. Found: C, 52.90; H, 4.56; N, 22.80.

(*rel*)-(1'*R*,4'*S*)-9-[4-(Hydroxymethyl)-4-ethynyl-cyclopent-2-en-1-yl] guanine (25): 2-Mercaptoethanol (0.14

mL, 1.90 mmol) and NaOMe (1.76 mL, 1.76 mmol, 1.0 M solution in MeOH) was added to a solution of compound **24** (95.6 mg, 0.33 mmol) in MeOH (10 mL), and heated overnight under reflux. After cooling, the reaction mixture was neutralized with a few drops of glacial AcOH and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($MeOH/CH_2Cl_2$, 1:4) to give compound **25** (53.0 mg, 60%) as a solid: mp 180-183; UV (H_2O) λ_{max} 253.0 nm; ^1H NMR (DMSO-d_6, 300 MHz) δ 10.80 (br s, 1H), 7.95 (s, 1H), 6.56 (br s, 2H), 6.87 (d, J = 6.2 Hz, 1H), 6.14 (d, J = 5.6 Hz, 1H), 6.07 (dd, J = 5.0, 1.4 Hz, 1H), 5.48 (m, 1H), 4.93 (t, J = 5.4 Hz, 1H), 3.42 (d, J = 9.0 Hz, 1H), 3.31 (d, J = 9.0 Hz, 1H), 2.45 (dd, J = 14.0, 8.4 Hz, 1H), 2.05-1.98 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 157.58, 154.32, 152.57, 143.56, 136.36, 124.98, 117.39, 88.98, 72.87, 69.32, 58.43, 43.65; Anal. calcd. for $C_{13}H_{13}N_5O_2 \cdot 1.0H_2O$: C, 53.97; H, 5.23; N, 24.21. Found: C, 54.11; H, 5.30; N, 24.17.

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