4'-Ethynyl Carbocyclic Nucleosides

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

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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 diyne 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 (EC₅₀ = 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 nucleosides¹ 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 carbovir⁴ and abacavir,⁵ which are potential anti-HIV agents, has increased interest in the search for novel carbocyclic nucleosides, whereas their side effects⁶ and the emergence of drug-resistant mutants are lasting concerns to be solved.⁷

Recent reports that thymidine analogues with 4'-azido¹⁸ and 4'-cyano groups²⁹ show significant inhibitory activity against HIV proliferation have stimulated the synthesis of 4'-substituted nucleoside analogues to lead to the discovery of 4'-ethynylated stavudine³⁰ and thio stavudine³¹ 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 15β.

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 triethylphosphonoacetate¹³ 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 with triethyl orthoacetate to yield the γ,δ-unsaturated ester 12.

Scheme 1. Synthesis route of aldehyde intermediate 13. Reagents: i) TBDMSCl, CH₂Cl₂, imidazole; ii) KOH, EtOH; iii) N-methyl hydroxylamine hydrochloride, DCC, DMAP TEA; iv) propargylmagnesium bromide, THF; v) Triethylphosphonoacetate, NaH, THF; vi) DIBALH, CH₂Cl₂; vii) Triethylthioacetate, propionic acid, overnight, 135-140 °C; viii) DIBALH, toluene, ~78 °C.
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 CH₂=CHMgBr to give divinyl 14.

Divinyl 14 was subjected to standard RCM¹⁵ conditions using a second-generation Grubbs catalyst to provide the diene metathesis product 15 as well as enyne metathesis product, which were readily separated by simple silica gel column chromatography. The correct configurations of 15α and 15β were assigned based on NOE comparisons.

Upon the irradiation of C₅-H, different NOE pattern was observed at the protons of compound 15α \[C₁-H (0.03\%) & C₆-H (0.29\%)] from those of compound 15β \[C₁-H (0.08\%) & C₆-H (0.29\%)] (Figure 2).

First, we attempted the mesylation of 15α 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 our attention to Palladium(0)-catalyzed reactions of allylic carbonate.¹⁶ To this end, cyclopentenol 15β was transformed to 16 using ethyl chloroformate, which was coupled with pyrimidine nucleosidic bases, Pd₂(dba)₃·CHCl₃, P(O-i-Pr)₃, NaH, THF/DMSO; v) TBAF, THF.

![Figure 1](image1.png)

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

![Figure 2](image2.png)

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

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 research.
development of carbocyclic derivatives. In summary, we developed an efficient synthetic method to yield 4'-ethyl 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 and dT (5.0 g, 26.2 mmol) in a anhydrous CH2Cl2 (150 mL), N, O-dimethyldiarylamino 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 NaHCO3 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: 1H NMR (CDCl3, 300 MHz) δ 4.48 (s, 2H), 3.57 (s, 3H), 3.05 (s, 3H), 0.80 (s, 9H), 0.02 (s, 6H); 13C NMR (CDCl3) 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 2 h. The ketone was neutralized with AcOH (3 mL) and poured into H2O (150 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 MgSO4, 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: 1H NMR (CDCl3, 300 MHz) δ 4.34 (s, 2H), 2.98 (s, 1H), 0.85 (s, 9H), 0.01 (s, 6H); 13C NMR (CDCl3) δ 196.31, 82.87, 79.43, 73.43, 25.76, 18.34, -5.58; Anal. calcd. for C10H18O2Si: C, 82.87; H, 9.15. Found: C, 80.45; H, 9.07.

4'-Ethynyl Carbocyclic Nucleosides

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<th>Antiviral activity of the synthesized compounds</th>
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(E) and (Z)-3-(tert-Butyldimethylsilyloxy)methyl-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 CH2Cl2 (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 12 (3.50 g, 16.7 mmol) in dry CH2Cl2 (150 mL) and concentrated in vacuum. The residue was extracted with EtOAc/H2O and the organic layer was dried over Na2SO4, filtered, and concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:25) to give 12 (6.05 g, 84%) as a colorless oil: 1H NMR (CDCl3, 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), 1.48 (dd, J = 13.4, 7.2 Hz, 1H), 1.48 (dd, J = 13.4, 7.2 Hz, 1H), 1.37 (s, 9H), 0.86 (m, 9H), 0.02 (m, 6H); 13C NMR (CDCl3) δ 172.6, 171.19, 143.54, 114.50, 80.76, 77.65, 69.34, 61.32, 49.35, 25.78, 18.76, 13.76, −5.76; Anal. calcld. for C14H24O2Si: C, 69.33; H, 10.26. Found: C, 69.37; H, 10.26.

(±)-3-(tert-Butyldimethylsilyloxy)methyl-3-ethynyl-pent-4-en-1-ol (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 slowly added 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: 1H NMR (CDCl3, 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), 0.85 (s, 9H), 0.02 (s, 6H); 13C NMR (CDCl3) δ 202.78, 143.32, 113.76, 81.39, 78.61, 69.55, 48.43, 25.78, 18.72, −5.76; Anal. calcld. for C14H24O2Si: 0.5 Hx; C, 69.33; H, 10.26. Found: C, 69.49; H, 10.40.

(1R,4S)-1-Ethoxy carbonyloxy-4-(tert-Butyldimethylsilyloxy)methyl-4-ethynyl-cyclopent-2-ene (16): Ethyl chlorofominate (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 refluxed overnight and concentrated in vacuum. The residue was extracted with EtOAc/H2O and the organic layer was dried over MgSO4, 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: 1H NMR (CDCl3, 300 MHz) δ 6.79-6.59 (m, 2H), 5.84 (d, J = 6.4 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 (s, J = 7.4 Hz, 3H), 0.84 (s, 9H), 0.01 (s, 6H); 13C NMR (CDCl3) 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. calcld. for C17H28O4Si·1.0 EtOAc: C, 66.68; H, 9.58. Found: C, 66.48; H, 9.51.
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, PO(O-Pr)3 (0.07 mL, 0.22 mmol) was added to a solution of Pd2(dba)3-CHCl3 (4.60 mg, 2.50 mmol) in anhydrous THF (5.0 mL), which was stirred for 30 min. To the nucleoside base solution of DMSO was sequentially added catalyst solution of THF and water (3 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/H2O, 0.5:1) to give 17 (118 mg, 39%) as a white solid: 1H NMR (CDCl3, 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), 3.59 (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); 13C NMR (CDCl3, 75 MHz) δ 164.56, 151.49, 147.88, 144.12, 136.58, 134.25, 126.54, 123.13, 119.19, 114.75, 111.35, 109.67, 73.93, 73.37, 69.64, 52.38, 43.37, −5.61; Anal. calcd. for C18H28N2O3Si: C, 63.30; H, 7.83; N, 7.57.

(1R,4'R)-9-[4-(Butyldimethylsilyloxy)ethyl]-4-ethylcylopent-2-en-1-yl] thymine (18): The thymine nucleoside analogue was synthesized from 16 as described for 17: yield 30%; 1H NMR (CDCl3, 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); 13C NMR (CDCl3) δ 164.21, 156.67, 154.39, 144.21, 127.88, 93.71, 89.56, 71.42, 69.54, 55.62, 42.32, 25.76, 18.66, −5.61; Anal. calcd. for C19H28N2O3Si: C, 60.55; H, 5.72; N, 11.09.

(1R,4'R)-9-[4-(Hydroxymethyl)-4-ethyl-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%; 1H NMR (DMSO-d6, 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); 13C NMR (DMSO-d6) δ 164.16, 152.54, 147.88, 144.21, 128.02, 102.08, 89.54, 73.45, 69.29, 57.47, 44.38; Anal. calcd. for C13H12ClN5O·0.5 MeOH: C, 53.03; H, 4.61; N, 22.91. Found: C, 52.90; H, 4.56; N, 22.80.
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/CH2Cl2, 1:4) to give compound 25 (53.0 mg, 60%) as a solid: mp 180-183; UV (H2O) λ max 253.0 nm; 1H NMR (DMSO-d6, 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); 13C NMR (DMSO-d6) 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 C13H13N5O2·1.0H2O: C, 53.97; H, 5.23; N, 24.21. Found: C, 54.11; H, 5.30; N, 24.17.

References
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