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Synthesis and Antiviral Activities of Novel 2′,4′- or 3′,4′-Doubly Branched Carbocyclic Nucleosides as Potential Antiviral Agents

In this study, a series of 2′,4-′ or 3′,4′-doubly branched carbocyclic nucleosides (**11**, **12**, **19**, and **20**) were synthesized from simple acyclic ketone derivatives as starting materials. The installation of the 4′-quaternary carbon needed was carried out using a [3,3]-sigmatropic rearrangement. In addition, the introduction of a methyl group in the 2′- or 3′-position was accomplished by either Grignard reaction or Horner-Wadsworth-Emmons reaction with triethyl-2-phosphonopropionate, respectively. Bis-vinyl was successfully cyclized using a Grubbs' catalyst II. The natural bases (adenine, cytosine) were coupled efficiently using a Pd(0) catalyst. Although all the synthesized compounds were assayed against several viruses, only the cytosine analogue **20** showed moderate antiviral activity against the human cytomegalovirus.

Keywords: Carbocyclic nucleosides; [3,3]-sigmatropic rearrangement; Horner-Wadsworth-Emmons reaction, Antiviral agents

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Introduction

Carbocyclic nucleosides [1, 2, 3], where a carbon atom replaces the oxygen atom of the sugar moiety, have emerged as a promising class of nucleosides with interesting antiviral and antitumor activities. Natural as well as synthetic carbocyclic nucleosides such as abacavir [4] and entecavir [5] (Figure 1) have shown interesting antitumor activities and antiviral activities against the human cytomegalovirus (HCMV) [6], herpes virus [7], hepatitis B virus [8], and human immunodeficiency virus (HIV) [9, 10].

However, the toxicitiy associated with these nucleosides as well as the emergence of resistant viral strains has prompted nucleoside chemists to search for additional novel and structurally diverse compounds with minimal overlapping resistance and toxicity profiles. These compounds have a higher metabolic stability against the nucleoside phosphorylases [11] because of the absence of a glycosidic bond. Various carbocyclic nucleosides are also believed to be potent inhibitors of the cellular enzyme, *S*-adenosyl-*L*-homocysteine (AdoHcy) hydrolase, which is very important in regulating the *S*-adenosylmethionine (SAM)-dependent methylation reactions, and has emerged as a specific target for the reversible hydrolysis of the AdoHcy linkage to adenosine and homocysteine [12, 13]. The inhibition of the enzyme in intact cellular systems results in the accumulation of AdoHcy; a higher concentration of AdoHcy suppresses the enzyme activity by acting as a product inhibitor of the AdoMet-dependent methylation reaction [14, 15]. Methyltransferases are essential for the maturation of mRNA. Therefore, inhibiting the methyl transferases by blocking the AdoHcy metabolism can disrupt viral mRNA maturation. AdoHcy inhibitors usually display a broad-spectrum of antiviral activity. In view of these interesting biological

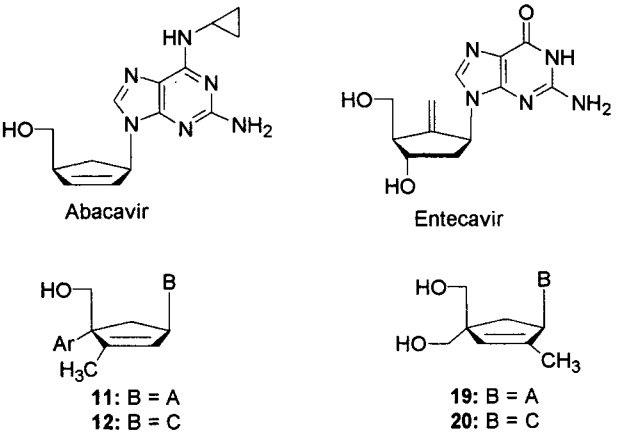

Figure 1. Structures of olefinic carbocyclic nucleosides and target nucleosides

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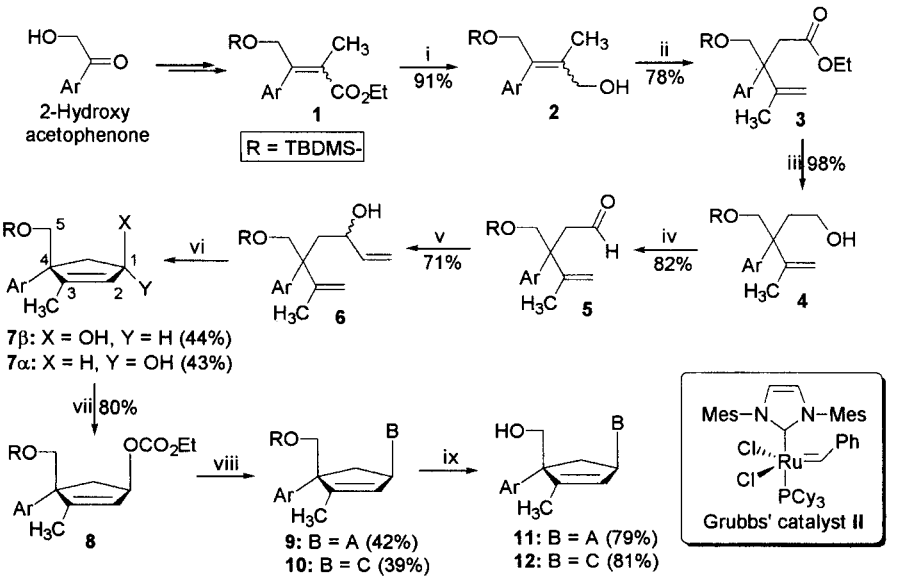


Reagents: i) Dibal-H, CH_2Cl_2, -20 °C; ii) Triethylorthoacetate, propionic acid, 135-140 °C; iii) Dibal-H, CH_2Cl_2, -10 °C; iv) PCC, 4A MS, CH_2Cl_2, rt; v) CH_2=CHMgBr, THF, -78 °C; vi) Grubbs' catalyst II, CH_2Cl_2, rt, overnight; vii) $ClCO_2Et$, DMAP, pyridine, rt, overnight; viii) Bases (adenine, cytosine), $Pd_2(dba)_3.CHCl_3$, P(O-*i*-Pr)$_3$, NaH, THF/DMSO, reflux, overnight; ix) TBAF, THF, rt.

Scheme 1. Synthesis of 3′,4′-doubly branched carbocyclic nucleosides.

and chemical properties of the olefinic carbonucleosides, we have synthesized novel 2′,4′- or 3′,4′-doubly branched olefinic carbonucleosides and assayed them as potential antiviral agents.

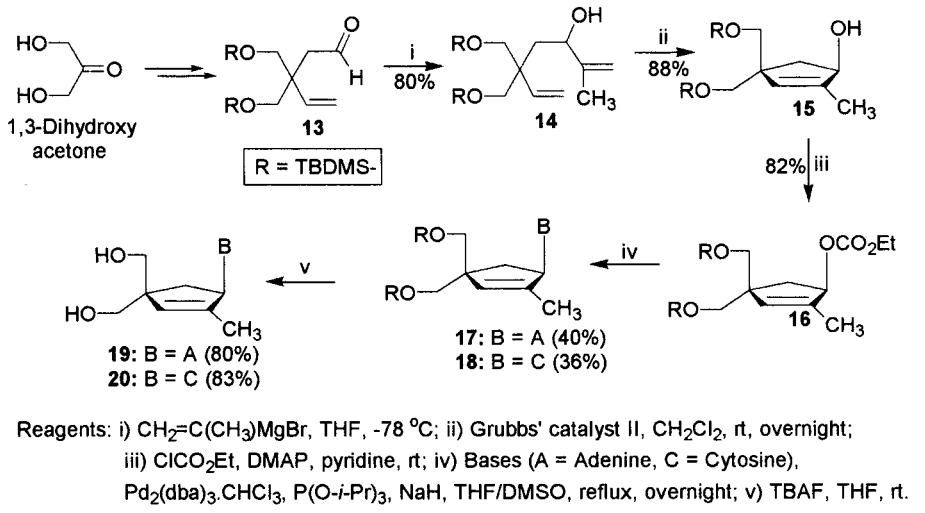
Chemistry

As shown in Scheme 1, for the synthesis of the 3′,4′-doubly branched target nucleosides, α,β-unsaturated ester derivative **1** was selected as the starting compound, which was readily synthesized from 2-hydroxyacetophenone by a method reported previously [16, 17]. Without separation, the ester **1** was reduced using diisobutylaluminum hydride (DIBALH) and the resulting alcohol was subjected to a [3,3]-sigmatropic rearrangement [18, 19] using triethylorthoacetate to give the g,d-unsaturated esters **3** in a 71% two-step yield. The addition of DIBALH to a solution of the esters **3** in CH_2Cl_2 at −10°C gave the alcohol **4**, which was subjected to oxidation conditions using PCC (pyridinium chlorochromate). The resulting aldehyde **5** was subjected to a Grignard reaction by CH_2 = CHMgBr to yield the bis-olefin **6** as a stereoisomeric mixture.

The bis-olefin **6** was subjected to the well-known ring-closing metathesis conditions [20, 21, 22] using a Grubbs' catalyst II [(Im)Cl_2PCy_3RuCHPh] to provide the stereoisomers, **7α** and **7β**, in equal amounts. The relative stereochemistry of compounds **7α** and **7β** was unambiguously determined based on the NOE correlations between the proximal hydrogen and methylene group (H-1, vs. H-5). Unlike compound **7β**, the NOE correlation was observed between the H-1 vs. H-5 of compound **7α**.

In order to couple the cyclopentenol with the bases (A = adenine, C = cytosine) using a palladium(0) catalyst [23, 24], the cyclopentenol **7β** was transformed to the ethoxycarbonyl derivative **8** using ethyl chloroformate in a high yield of 80%. Compound **8** was coupled with an adenine anion generated by NaH/DMSO with the [tris(dibenzylidene-acetone)-dipalladium(0)-chloroform] adduct to give the compounds **9** and **10**. The required stereochemistry of the nucleosides **9** and **10** were successfully controlled from the β-configuration of compounds **8** via a Pd(0) catalyzed π−allyl complex mechanism. Compounds **9** and **10** were desilylated by treating the compounds with tetrabutylammonium fluoride (TBAF) to give the final nucleosides **11** and **12** in high yield.

The synthesis of the 2′,4′-doubly branched carbocyclic nucleosides was initiated using aldehyde **13** as the starting material, which was also readily prepared from 1,3-dihydroxyacetone with a similar procedure described for synthesizing compound **5** [16, 17]. As shown in Scheme 2, introduction of the methyl branch



Scheme 2. Synthesis of 2′,4′-doubly branched carbocyclic nucleosides.

in the 2′-position could be accomplished by adding isopropenylmagnesium bromide [CH_2 = C(CH_3)MgBr] to aldehyde **13**. Using a similar procedure as described for compound **8**, the allylic coupling substrate **16** could be synthesized via successive ring-closing metathesis and ethoxycarbonylation reactions. The desired nucleosides **19** and **20** were successfully synthesized by the coupling of the bases, adenine and cytosine, using a Pd(0) catalyst and desilylation.

Antiviral activity studies

All synthesized compounds were tested against several viruses such as the HIV (MT-4 cells), HSV-1 (CCL81 cells), HSV-2 (CCL-81 cells), and HCMV (AD-169, Davis cells). All the compounds synthesized exhibited neither excellent antiviral activity nor any cytotoxicity when tested up to 100 μg/mL. However, it is interesting to note that only the cytosine analogue **20** exhibited moderate antiviral activity against the HCMV (Table 1), indicating that this virus might allow the conformationally rigid sugar moiety for phosphorylation as well as for DNA polymerase, which is unlike other viruses. In addition, the adenine analogue **11** showed weak antiviral activity against the HSV-1. For the evaluation of anti-HCMV activity, HCMV strains AD-169 (ATTCC VR-583) and Davis (ATCC VR-807) and a standard CPE inhibition assay were used. HEL 299 cells (human embryonic lung fibroblast) served for the cytotoxicity assay.

In summary, a concise synthetic method for synthesizing 2′,4′- or 3′,4′-doubly branched carbocyclic nucleo-

Table 1. The antiviral activities of the synthesized compounds.

compound	HIV-1 EC_{50}(μg/mL)	HSV-1 EC_{50}(μg/mL)	HSV-2 EC_{50}(μg/mL)	HCMV EC_{50}(μg/mL)	cytotoxicity IC_{50}(μg/mL)
11	>100	>56.2	>100	>100	>100
12	>100	>100	>100	>100	>100
19	>100	>100	>100	>100	>100
20	>100	>100	>100	>21.6	>100
AZT	0.001	ND	ND	ND	1.15
Ganciclovir	ND	1.66	1.66	ND	>10
Ribavirin	ND	ND	ND	15.50	300.00

ND−not determined.

sides from simple a-hydroxy ketone derivatives was developed in this study. The scope of this methodology along with its application to other nucleosides is currently under investigation. When the synthesized compounds were tested against several viruses such as the HIV-1, HSV-1, HSV-2, and HCMV, only the cytosine analogue **20** exhibited moderate activity against HCMV.

Acknowledgments

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Experimental

All the chemicals were of reagent grade and were used as purchased. All the moisture-sensitive reactions were performed in an inert atmosphere with either N_2 or Ar using distilled dry solvents. The elemental analyses were performed using an Elemental Analyzer System (Profile HV-3). The NMR spectra were recorded on a Bruker 300 MHz Fourier transform spectrometer (Bruker, Karlsruhe, Germany).

*(E) and (Z)-4-(t-Butyldimethylsilyloxy)-3-phenyl-2-methyl-but-2-enoic acid ethyl ester (**1**)*

To a suspension of sodium hydride (60% in mineral oil, 1.11 g, 27.7 mmol) in distilled THF at 0 °C, triethyl 2-phosphonopropionate (5.94 mL, 27.7 mmol) was added dropwise and with constant stirring at room temperature for 30 min. 2-(t-Butyldimethylsilyloxy)-acetophonone (6.93 g, 27.7 mmol) was added to this mixture and stirred for 2 h. The solution was neutralized with saturated ammonium chloride 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, 1:20) to give **1** (7.8 g, 85%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) as mixture δ 7.54−7.19 (m, 5H), 4.71, 4.53 (s, s, 2H), 4.33 (q, *J* = 6.9 Hz, 2H), 3.89 (q, *J* = 6.9 Hz, 2H), 2.14, 0.84 (s, s, 3H), 1.21, 0.97 (dt, *J* = 6.9, 6.9 Hz, 3H), 0.85, 0.67 (s, s, 9H), 0.04, 0.02 (s, s, 6H); Anal calc. for $C_{19}H_{30}O_3Si$: C, 68.22; H, 9.04. Found: C, 68.34; H, 8.90.

*(E) and (Z)-4-(t-Butyldimethylsilyloxymethyl)-4-phenyl-2-methyl-but-2-en-1-ol (**2**)*

To a solution of compound **1** (10 g, 29.89 mmol) in CH_2Cl_2 (350 mL), DIBALH (62.7 mL, 1.0 M solution in hexane) was added slowly at −20 °C, and stirred for 1 h at the same temperature. To the resulting mixture, methanol (60 mL) was added. The mixture was stirred at room temperature for 3 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 the allylic alcohol **2** (7.95 g, 91%) as a colorless oil: as a isomeric mixture for ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.44-7.02 (m, 5H), 4.51 (d, *J* = 5.4, 2H), 4.36 (d, *J* = 4.2 Hz, 1H), 4.02 (s, 1H), 0.89 (s, 6H), 0.82 (s, 3H), 0.04 (s, 4H), 0.02 (s, 2H); Anal calc. for $C_{17}H_{28}O_2Si$: C, 69.81; H, 9.65. Found: C, 69.61; H, 9.54.

*(±)-3-(t-Butyldimethylsilyloxymethyl)-3-phenyl-4-methyl-pent-4-enoic acid ethyl ester (**3**)*

A solution of the allylic alcohol **2** (10 g, 34.18 mmol) in triethyl orthoacetate (150 mL) and 1.0 mL of propionic acid was heated at 135−140 °C overnight with constant stirring to allow for the removal of ethanol. An excess of triethyl orthoacetate was removed by distillation and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:40) to give compound **3** (9.66 g, 78%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.51−7.24 (m, 5H), 5.09 (s, 1H), 4.94 (s, 1H), 4.29 (d, *J* = 9.0 Hz, 1H), 4.10 (d, *J* = 9.0 Hz, 1H), 4.05 (q, *J* = 6.9 Hz, 1H), 3.22 (d, *J* = 6.9 Hz, 2H), 2.95 (d, *J* = 6.9 Hz, 1H), 1.61 (s, 3H), 1.20 (t, *J* = 6.9 Hz, 3H), 0.87 (s, 9H), 0.03 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.78, 147.14, 143.40, 127.85, 126.48, 126.19, 112.04, 65.84, 59.87, 51.38, 37.91, 25.67, 18.47, 17.84, 14.12, −5.83; Anal calc. for $C_{21}H_{34}O_3Si$: C, 69.56; H, 9.45. Found: C, 69.31; H, 9.50.

*(±)-3-(t-Butyldimethylsilyloxymethyl)-3-phenyl-4-methyl-pent-4-enol (**4**)*

To a solution of compound **3** (8.0 g, 22.06 mmol) in CH_2Cl_2 (200 mL), DIBALH (46.33 mL, 1.0 M solution in hexane) was added slowly at −10 °C, and stirred for 30 min at the same temperature. To the mixture, methanol (40 mL) was then added. 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, 1:20) to give compound **4** (6.93 g, 98%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.46−7.34 (m, 5H), 5.25 (s, 1H), 5.14 (s, 1H), 4.21 (d, *J* = 9.3 Hz, 1H), 4.02 (d, *J* = 9.3 Hz, 1H), 3.80 (dd, *J* = 6.9, 6.6 Hz, 2H), 2.61 (dd, *J* = 13.5, 6.9 Hz, 1H), 2.38 (dd, *J* = 13.2, 6.6 Hz, 1H), 1.68 (s, 3H), 1.00 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 147.59, 144.17, 127.93, 127.06, 126.05, 112.46, 67.53, 59.58, 51.29, 35.42, 25.69, 20.61, 18.06, −5.90; Anal calc. for $C_{19}H_{32}O_2Si$: C, 71.19; H, 10.06. Found: C, 70.98; H, 9.89.

*(±)-3-(t-Butyldimethylsilyloxymethyl)-3-phenyl-4-methyl-pent-4-enal (**5**)*

To a solution of compound **4** (7.0 g, 21.83 mmol) in CH_2Cl_2 (150 mL), 4Å molecular sieves (12.7 g) and PCC (11.76 g, 54.55 mmol) were added slowly at 0 °C, and stirred for 4 h at room temperature. To the mixture, excess diethyl ether (600 mL) was then added. The mixture was stirred vigorously for 3 h at the same temperature, and the resulting solid was filtered through a short silica gel column. The filtrate was concentrated under vacuum and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:50) to give compound **5** (5.70 g, 82%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.64 (m, 1H), 7.35−7.21 (m, 5H), 5.15 (d, *J* = 2.4 Hz, 1H), 5.09 (s, 1H), 4.05 (d, *J* = 9.6 Hz, 1H), 3.90 (d, *J* = 9.6 Hz, 1H), 2.99 (dd, *J* = 13.2, 3.0 Hz, 1H), 2.84 (dd, *J* = 13.2, 3.2 Hz, 1H), 1.55 (s, 3H), 0.84 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 203.05, 146.09, 142.60, 127.88, 126.77, 113.53, 68.58, 51.45, 47.38, 25.76, 20.39, 18.17, −5.84; Anal calc. for $C_{19}H_{30}O_2Si$: C, 71.64; H, 9.49. Found: C, 71.44; H, 9.40.

*(rel)-(3R and 3S,5S)-5-(t-Butyldimethylsilyloxymethyl)-5-phenyl-6-methyl-hepta-1,6-dien-3-ol (**6**)*

To a solution of compound **5** (6.0 g, 18.83 mmol) in dry THF (200 mL) vinylmagnesium bromide (22.59 mL, 1.0 M solution

in THF) was added slowly at -78 °C. After 3 h, a saturated NH_4Cl solution (22 mL) was added, and the reaction mixture was warmed slowly to room temperature. The mixture was extracted with EtOAc (2 × 250 mL). The combined organic layer was dried over $MgSO_4$, filtered, and evaporated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:30) to give compound **6** (4.63 g, 71 %) as a colorless oil: as a diastereomeric mixture for ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.45−7.32 (m, 5H), 6.17−5.97 (m, 1H), 5.43−5.11 (m, 4H), 4.36−4.18 (m, 2H), 2.56−2.42 (m, 1H), 2.36−2.27 (m, 1H), 1.69, 1.62 (s, s, 3H), 0.97 (s, 9H), 0.03, 0.01 (s, 6H); 13C NMR ($CDCl_3$, 75 MHz) δ 149.34, 146.75, 144.26, 143.95, 141.92, 128.13, 127.15, 126.20, 113.48, 112.34, 70.01, 69.50, 69.03, 66.64, 52.10, 51.42, 41.01, 40.69, 25.72, 20.94, 20.81, 18.11, −5.70, −5.92; Anal calc. for $C_{21}H_{34}O_2Si$: C, 72.78; H, 9.89. Found: C, 72.58; H, 9.74.

*(rel)-(1R,4R)-4-(t-Butyldimethylsilyloxymethyl)-3-methyl-4-phenyl-cyclopent-2-enol (**7β**); and (rel)-(1S,4R)-4-(t-Butyldimethylsilyloxymethyl)-3-methyl-4-phenyl-cyclopent-2-enol (**7α**)*

To a solution of compound **6** (4.0 g, 11.54 mmol) in dry CH_2Cl_2 (15 mL), Grubbs' catalyst II (102 mg 0.12 mmol) was added. The reaction mixture was stirred overnight, and concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:10) to give the cyclopentenols **7β** (1.6 g, 44 %) and **7α** (1.58 g, 43 %) as colorless oil. **7β:** ^{1}H NMR ($CDCl_3$, 300 MHz) d 7.16-6.95 (m, 5H), 5.67 (s, 1H), 4.47 (t, *J* = 9.3 Hz, 1H), 3.95 (d, *J* = 9.6 Hz, 1H), 3.84 (d, *J* = 9.6 Hz, 1H), 2.27 (dd, *J* = 14.4, 6.9 Hz, 1H), 1.96 (d, *J* = 14.1 Hz, 1H), 1.34 (s, 3H), 0.71 (s, 9H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) d 145.73, 145.03, 126.97, 126.30, 126.14, 126.03, 74.47, 64.98, 59.02, 50.29, 25.73, 18.03, 12.99, −5.68; Anal calc. for $C_{19}H_{30}O_2Si$: C, 71.64; H, 9.49. Found: C, 71.56; H, 9.52. **7α**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.26−7.03 (m, 5H), 5.65 (s, 1H), 4.73 (br s, 1H), 3.91 (d, *J* = 9.6 Hz, 1H), 3.80 (d, *J* = 9.6 Hz, 1H), 2.65 (dd, *J* = 13.8, 7.2 Hz, 1H), 1.77 (dd, *J* = 18.8, 3.6 Hz, 1H), 1.37 (s, 3H), 0.79 (s, 9H), 0.03 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 147.64, 145.54, 128.34, 127.20, 126.77, 126.07, 66.12, 59.17, 48.66, 25.76, 18.15, 13.63, −5.52; Anal calc. for $C_{19}H_{30}O_2Si$: C, 71.64; H, 9.49. Found: C, 71.79; H, 9.30.

*(rel)-(1R,4R)-1-Ethoxycarbonyloxy-4-(t-butyldimethylsilyloxymethyl)-4-phenyl-3-methyl-cyclopent-2-ene (**8**)*

To a solution of compound **7β** (3.0 g, 9.41 mmol) in anhydrous pyridine (30 mL) ethyl chloroformate (1.8 mL, 18.83 mmol) and dimethylaminopyridine (220 mg, 1.8 mmol) were added. The reaction mixture was stirred overnight at room temperature. The reaction mixture was quenched using a saturated $NaHCO_3$ solution (5 mL) and concentrated under vacuum. The residue was extracted with EtOAc, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:40) to give compound **8** (2.94 g, 80 %) as a colorless syrup: ^{1}H NMR ($CDCl_3$, 300 MHz) d 7.34−7.22 (m, 5H), 5.98 (s, 1H), 5.52 (dd, *J* = 6.7, 3.0 Hz, 1H), 4.31 (q, *J* = 7.0 Hz, 2H), 3.88 (d, *J* = 9.3 Hz, 1H), 3.81 (d, *J* = 9.3 Hz, 1H), 2.34 (dd, *J* = 13.6, 6.4 Hz, 1H), 2.12 (dd, *J* = 13.6, 3.6 Hz, 1H), 1.56 (s, 3H), 1.43 (t, *J* = 7.0 Hz, 3H), 0.89 (s, 9H), 0.03 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.47, 146.12, 138.72, 134.12, 128.23, 126.77, 125.79, 85.67, 71.46, 64.11, 58.21, 40.31, 25.74, 18.34, 14.23, 13.89, −5.34; Anal calc. for $C_{22}H_{34}O_4Si$: C, 67.65; H, 8.77. Found: C, 67.70; H, 8.62.

*(rel)-(1′R,4′R)-9-[4-(t-Butyldimethylsilyloxymethyl)-4-phenyl-3-methyl-cyclopent-2-en-1-yl] adenine (**9**)*

To pure NaH (46.8 mg, 1.96 mmol) in anhydrous DMSO (9.0 mL), adenine (268 mg, 1.96 mmol) was added. The reaction mixture was stirred for 30 min at 50-55 °C and cooled to room temperature. Simultaneously, $P(O\text{-}i\text{-}Pr)_3$ (0.96 mL, 2.2 mmol) was added to a solution of $Pd_2(dba)_3{\cdot}CHCl_3$ (46 mg, 25 mmol) in anhydrous THF (8.0 mL), which was stirred for 40 min. To the adenine solution in DMSO, the catalyst solution of THF and compound **8** (687 mg, 1.76 mmol) dissolved in anhydrous THF (5 mL) were added slowly. The reaction mixture was stirred overnight at a refluxing temperature and quenched with water (5 mL). The reaction solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography ($MeOH/CH_2Cl_2$, 1:10) to give compound **9** (322 mg, 42 %) as a white solid: mp 192−195 °C; UV (MeOH) l_{max} 260.5 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) d 8.33 (s, 1H), 8.03 (s, 1H), 7.36−7.26 (m, 5H), 5.81 (s, 1H), 5.56 (dd, *J* = 7.2, 6.0 Hz, 1H), 3.70 (s, 2H), 2.61 (dd, *J* = 13.8, 7.8 Hz, 1H), 1.92 (dd, *J* = 13.8, 6.4 Hz, 1H), 1.47 (s, 3H), 0.87 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.46, 152.91, 145.26, 139.31, 138.47, 135.34, 128.34, 126.62, 70.24, 61.38, 57.45, 42.94, 25.98, 18.52, 13.71, −5.37; Anal calc. for $C_{24}H_{33}N_5OSi$: C, 66.17; H, 7.64; N, 16.08. Found: C, 65.88; H, 7.51; N, 16.21.

*(rel)-(1′R,4′R)-1-[4-(t-Butyldimethylsilyloxymethyl)-4-phenyl-3-methyl-cyclopent-2-en-1-yl] cytosine (**10**)*

Compound **10** was prepared from compound **8** using the method described for synthesizing compound **9**: Yield 39 %; mp 170−173 °C; UV (MeOH) λ_{max} 272.5 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) d 7.81 (d, *J* = 7.2 Hz, 1H), 7.30-7.26 (m, 5H), 6.00 (s, 1H), 5.91 (d, *J* = 7.2 Hz, 1H), 5.78 (dd, J = 6.8, 5.6 Hz, 1H), 3.62 (dd, *J* = 13.2, 7.8 Hz, 2H), 2.78 (dd, *J* = 13.6, 7.6 Hz, 1H), 2.32 (dd, *J* = 13.6, 5.6 Hz, 1H), 1.70 (s, 3H), 0.90 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.41, 154.63, 157.48, 146.50, 139.81, 133.89, 128.71, 127.23, 124.44, 98.34, 83.12, 71.67, 58.72, 42.50, 25.71, 18.45, 14.33, −5.45; Anal calc. for $C_{23}H_{33}N_3O_2Si$: C, 67.11; H, 8.08; N, 10.21. Found: C, 67.32; H, 8.11; N, 10.39.

*(rel)-(1′R,4′R)-9-[4-(Hydroxymethyl)-4-phenyl-3-methyl-cyclopent-2-en-1-yl] adenine (**11**)*

To a solution of compound **9** (130 mg, 0.298 mmol) in THF (3 mL), TBAF (0.45 mL, 1.0 M solution in THF) at 0 °C was added. The mixture was stirred at room temperature for 4 h, and concentrated. The residue was purified by silica gel column chromatography ($MeOH/CH_2Cl_2$, 1:5) to give compound **11** (75 mg, 79 %) as a white solid: mp 190−193 °C; UV (MeOH) λ_{max} 260.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.35 (s, 1H), 8.11 (s, 1H), 7.30−7.22 (m, 5H), 7.16 (br s, 2H, D_2O exchangeable), 5.83 (s, 1H), 5.59 (br s, 1H), 4.74 (t, *J* = 6.6 Hz, 1H, D_2O exchangeable), 3.82 (d, *J* = 5.6 Hz, 2H), 2.58 (dd, *J* = 13.6, 7.2 Hz, 1H), 1.89 (dd, *J* = 13.6, 6.6 Hz, 1H), 1.43 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 155.62, 153.32, 145.25, 139.95, 138.21, 135.21, 128.67, 127.23, 69.31, 61.12, 58.56, 42.12, 14.12; Anal calc. for $C_{18}H_{19}N_5O$: C, 67.27; H, 5.96; N, 21.79. Found: C, 67.40; H, 5.88; N, 21.67.

*(rel)-(1′R,4′R)-1-[4-(Hydroxymethyl)-4-phenyl-3-methyl-cyclopent-2-en-1-yl] cytosine (**12**)*

Compound **12** was prepared from compound **10** using the method described for synthesizing compound **11**: yield 81 %; mp 174−176 °C; UV (H_2O) λ_{max} 272.5 nm; ^{1}H NMR (DMSO-

d_6, 300 MHz) d 7.78 (d, J = 7.2 Hz, 1H), 7.30−7.21 (m, 5H), 7.03 (br d, 2H, D_2O exchangeable), 5.81 (d, J = 7.2 Hz, 1H), 5.79 (s, 1H), 5.70 (br s, 1H), 4.70 (t, J = 6.8 Hz, 1H, D_2O exchangeable), 3.71 (dd, J = 13.6, 7.6 Hz, 2H), 2.68 (dd, J = 13.8, 7.6 Hz, 1H), 2.30 (dd, J = 13.8, 6.8 Hz, 1H), 1.56 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 166.31, 155.56, 158.11, 146.98, 140.54, 134.31, 128.98, 128.56, 124.99, 98.87, 83.87, 72.45, 58.69, 42.50, 13.65, −5.78; Anal calc. for $C_{17}H_{19}N_3O_2$: C, 68.67; H, 6.44; N, 14.13. Found: C, 68.50; H, 6.38; N, 14.25.

*(±)-5,5′-Bis-(t-butyldimethylsilyloxymethyl)-2-methyl-hepta-1,6-dien-3-ol (**14**)*

Compound **14** was prepared from compound **13** using isopropenylmagnesium bromide instead of vinylmagnesium bromide following the method described for synthesizing compound **6:** yield: 80%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.75 (dd, J = 17.7, 11.1 Hz, 1H), 5.09 (d, J = 11.4 Hz, 1H), 4.98 (d, J = 18.0 Hz, 1H), 4.90 (s, 1H), 4.49 (s, 1H), 4.14 (d, J = 9.6 Hz, 1H), 3.63 (s, 2H), 3.43 (dd, J = 12.9, 9.6 Hz, 2H), 1.65 (s, 3H), 1.48 (m, 2H), 0.83 (s, 18H), 0.03 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 148.34, 141.39, 114.72, 109.79, 71.49, 66.86, 65.19, 46.15, 40.40, 25.85, 18.25, −5.51; Anal calc. for $C_{22}H_{46}O_3Si_2$: C, 63.71; H, 11.18. Found: C, 63.78; H, 11.29.

*(±)-4,4′-Bis-(t-butyldimethylsilyloxymethyl)-2-methyl-cyclopent-2-enol (**15**)*

Compound **15** was prepared from compound **14** using the method described for synthesizing compounds **7β** and **7α**: yield: 88%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.19 (s, 1H), 4.21 (dd, J = 11.1, 7.5 Hz, 1H), 3.57 (d, J = 9.3 Hz, 1H), 3.47 (d, J = 9.3 Hz, 1H), 3.40 (d, J = 9.6 Hz, 1H), 3.34 (d, J = 9.6 Hz, 1H), 1.86 (dd, J = 14.4, 7.5 Hz, 1H), 1.73 (s, 1H), 1.51 (d, J = 14.4 Hz, 1H), 0.82 (s, 18H), 0.03, 0.02 (s, s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 144.54, 130.13, 78.33, 68.25, 66.95, 55.79, 41.56, 41.56, 25.98, 18.54, 13.94, −5.52; Anal calc. for $C_{20}H_{42}O_3Si_2$: C, 62.12; H, 10.95. Found: C, 62.33; H, 10.83.

*(±)-1-Ethoxycarbonyloxy-4,4′-bis-(t-butyldimethylsilyloxymethyl)-2-methyl-cyclopent-2-ene (**16**)*

Compound **16** was prepared from compound **15** using the method described for synthesizing compound **8:** yield 82%; ^{1}H NMR ($CDCl_3$, 300 MHz) d 5.50 (s, 1H), 5.45 (dd, J = 7.5, 4.2 Hz, 1H), 4.19 (q, J = 7.8 Hz, 2H), 3.52−3.41 (m, 4H), 2.17 (dd, J = 13.8, 7.5 Hz, 1H), 1.72 (s, 3H), 1.60 (dd, J = 14.1, 3.6 Hz, 1H), 1.30 (t, J = 7.8 Hz, 3H), 0.86 (s, 18H), 0.01 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.28, 139.01, 134.51, 85.05, 66.36, 65.93, 63.72, 55.41, 37.28, 25.87, 18.26, 14.30, 13.92, −5.50; Anal calc. for $C_{23}H_{46}O_5Si_2$: C, 60.21; H, 10.11. Found: C, 60.02; H, 10.20.

*(±)-9-[4-Bis-(t-butyldimethylsilyloxymethyl)-2-methyl-cyclopent-2-en-1-yl] adenine (**17**)*

Compound **17** was prepared from compound **16** using the method described for synthesizing compound **9:** yield 40%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.32 (s, 1H), 7.85 (s, 1H), 5.67 (s, 1H), 5.55 (dd, J = 15.0, 7.2 Hz, 1H), 3.64 (d, J = 9.9 Hz, 1H), 3.57 (d, J = 9.9 Hz, 1H), 3.44 (s, 2H), 2.44 (dd, J = 14.4, 9.6 Hz, 1H), 1.85 (dd, J = 14.2, 5.4 Hz, 1H), 1.51 (s, 3H), 0.85 (s, 18H), 0.02 (s, 16H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.37, 152.90, 150.27, 139.28, 138.59, 134.77, 119.65, 66.66, 61.63, 56.06, 38.20, 25.99, 18.46, 13.79, −5.40; Anal calc. for $C_{25}H_{45}N_5O_2Si_2$: C, 59.60; H, 9.00; N, 13.90. Found: C, 59.72; H, 9.11; N, 13.70.

*(±)-1-[4-Bis-(t-butyldimethylsilyloxymethyl)-2-methyl-cyclopent-2-en-1-yl] cytosine (**18**)*

Compound **18** was prepared from compound **16** using the method described for synthesizing compound **9:** yield 36%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.00 (d, J = 5.7 Hz, 1H), 6.04 (d, J = 5.1 Hz, 1H), 5.74 (dd, J = 7.8, 3.9 Hz, 1H), 5.48 (s, 1H), 3.53-3.43 (m, 4H), 2.23 (dd, J = 8.4, 7.2 Hz, 1H), 1.74 (s, 3H), 1.58 (dd, J = 14.4, 3.6 Hz, 1H), 0.88 (s, 18H), 0.03 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.42, 164.57, 157.59, 140.30, 133.16, 98.92, 83.25, 66.60, 66.05, 55.12, 37.79, 25.93, 18.30, 14.22, −5.50; Anal calc. for $C_{24}H_{45}N_3O_3Si_2$: C, 60.08; H, 9.45; N, 8.76. Found: C, 59.81; H, 9.27; N, 8.65.

*(±)-9-[4,4′-Bis-(hydroxymethyl)-2-methyl-cyclopent-2-en-1-yl]adenine (**19**)*

Compound **18** was prepared from compound **17** using the method described for synthesizing compound **11:** yield 80%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.17 (s, 1H), 8.14 (s, 1H), 7.19 (br s, 2H, D_2O exchangeable), 5.61 (s, 1H), 5.53 (t, J = 7.8 Hz, 1H), 4.72 (br s, 2H, D_2O exchangeable), 3.52−3.40 (m, 4H), 2.42 (dd, J = 13.5, 9.3 Hz, 1H), 1.94 (dd, J = 14.4, 6.3 Hz, 1H), 1.47 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.98, 152.32, 149.48, 139.25, 137.83, 134.26, 118.78, 65.40, 65.00, 55.81, 37.36, 13.48; Anal calc. for $C_{13}H_{17}N_5O_2$: C, 56.71; H, 6.22; N, 25.44. Found: C, 56.80; H, 6.29; N, 25.31.

*(±)-1-[4,4′-Bis-(hydroxymethyl)-2-methyl-cyclopent-2-en-1-yl]cytosine (**20**)*

Compound **20** was prepared from compound **18** using the method described for synthesizing compound **11:** yield 83%; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.94 (d, J = 6.0 Hz, 1H), 7.20 (br s, 1H, D_2O exchangeable), 6.98 (br s, 1H, D_2O exchangeable), 5.87 (d, J = 5.9 Hz, 1H), 5.70 (dd, J = 7.6, 4.0 Hz, 1H), 5.45 (s, 1H), 4.71 (br s, 2H, D_2O exchangeable), 3.50−3.39 (m, 4H), 2.22 (dd, J = 13.8, 6.8 Hz, 1H), 1.72 (s, 3H), 1.52 (dd, J = 13.8, 3.8 Hz, 1H); 13C NMR (DMSO-d_6, 75 MHz) δ 166.17, 165.27, 157.78, 141.37, 133.88, 97.45, 82.43, 66.12, 65.78, 54.23, 38.46, 13.45; Anal calc. for $C_{12}H_{17}N_3O_3$: C, 57.36; H, 6.82; N, 16.72. Found: C, 57.46; H, 6.79; N, 16.98.

Evaluation of anti-HCMV activity

Standard CPE inhibition assay was used [25]. HEL cells in stationary phase were infected with the virus at a multiplicity of infection of 2−4 $CCID_{50}$ per well of 96%-well plates. After 2 h adsorption at 37°C, the liquid was aspirated off to remove the unadsorped viruses and 100 μL of MEM/2% FBS containing a compound was applied to each well in duplicate for each concentration and further incubated for 6 days. Antiviral activity was measured microscopically or fluorometrically. For microscopical observation the cells were fixed with 70% ethanol, stained with 2.5% Giemsa solution for 2 h, rinsed with distilled water, and air-dried. Antiviral activity was expresses as the EC_{50}, or the concentration required to inhibit virus-induced CPE by 50%. EC_{50} values were estimated from semilogarithmic graphic plots of the percentage of CPE as a function of the concentration of the test compound. For fluorometric assay [26], the cells were washed twice with 100 μL of phosphate-buffered saline (PBS). To each well 100 μL of 5 μg/mL fluorescein diacetate (FDA, Sigma) was added and the plates were incubated for 30 min at 37°C. The FDA solu-

tion was removed by aspiration and each well was washed with 100 μL of PBS. The fluorescence intensity (as absolute fluorescent units, AFU) in each well was measured with a fluorescent microplate reader equipped with a 485-nm excitation filter and a 538-nm emission filter.

Cytotoxicity assay

The effect of the test compounds on host cell growth and on viability was assayed microscopically and fluorometrically by using propidium iodide (PI; Sigma, St. Louse, MI, USA). To measure cytostatic effects, HEL cells were seeded at 3000 cell/well in 96-well plates in 100 μL of MEM/10% FBS. The cells were allowed to attach to the plates by incubation at 37 °C for 1 day, different dilutions of the test compounds were added and the cells were further incubated for 3 days at 37 °C. 100 μL of 40 μg/mL PI diluted with medium was added each well following incubation at room temperature for 60 min in the dark to allow dye penetration. Fluorescence was measured using a fluorescence microplate reader (544-nm excitation; 620-nm emission), the concentration of compounds responsible for 50% inhibition of cell growth was calculated and expressed as CS_{50} (50% cytostatic effect).

Cytocidal assay was performed as a control experiment for the antiviral assay. It was carried out simultaneously with the antiviral assay described previously using mock instead of virus for infection, and cell viability was measured using PI instead of FDA. The concentration of compounds responsible for 50% reduction of cell viability was calculated and expressed as CC_{50} (50% cytocidal effect).

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