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Synthesis and biological evaluation of novel 2′,3′,4′-triply branched carbocyclic nucleosides as potential antiviral agents

Novel 2′,3′,4′-triply branched carbocyclic nucleosides were synthesized in this study. The introduction of two methyl groups in the 2′- and 3′-position was accomplished by a Horner-Wadsworth-Emmons reaction and isopropenyl magnesiumbromide addition, respectively. The construction of the required 4′-quaternary carbon was carried out using a [3,3]-sigmatropic rearrangement. Bis-vinyls were successfully cyclized using a Grubbs' catalyst II. The natural bases (adenine, cytosine) were efficiently coupled using a Pd(0) catalyst. The antiviral activities of the synthesized compounds were evaluated against HIV-1, HSV-1, HSV-2 and HCMV. Compound **30** displayed moderate anti-HCMV activity (EC_{50} = 30.1 μg/mL), without exhibiting any cytotoxicity at up to 100 μM.

Keywords: Antiviral Agents; Triply Branched Carbocyclic Nucleosides; [3,3]-Sigmatropic Rearrangement

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Introduction

Emerging drug-resistant virus strains and toxicity are major problems in antiviral chemotherapy, and a number of structurally modified nucleosides have been synthesized to overcome these drawbacks. Recently, several branched nucleosides were synthesized and evaluated as potent antitumor or antiviral agents. Among them, 4′-*C*-hydroxymethyl-thymidine **1** [1], 4′-*C*-ethenyl-2′-deoxycytidine **2** [2], 3′-*C*-methyladenosine **3** [3] and 2′-*C*-methyl-2′-deoxycytidine **4** [4], which have additional branches at the 2′-, 3′- or 4′-position, were reported to exhibit potent antiviral and antitumor activities (Figure 1).

More fundamental modifications of the pentofuranose moiety, such as carbocyclic nucleosides, have been reported to be compatible with their antiviral activities. Carbocyclic nucleosides [5] are a group of compounds that are structurally similar to the natural nucleosides where the furanose oxygen is replaced by a methylene group. The replacement of the oxygen on the furanose ring by carbon is of particular interest because the resulting carbocyclic nucleosides possess a greater metabolic stability to phosphorylase [6], which cleaves the glycosidic bond of nucleosides. Since the cyclopentane ring of the carbocyclic nucleosides can emulate the furanose moiety, a number of these compounds exhibit interesting biological activities, particularly in the areas of antiviral and anticancer chemotherapy. The recent discovery of olefinic carbocyclic nucleosides such as abacavir **5** [7] and entecavir **6** [8] as potential antiviral agents has given a strong impetus to the search for novel nucleosides in this class of compounds. Carbocyclic nucleosides are also believed to be potent inhibitors of the cellular enzyme *S*-adenosyl-*L*-homocysteine (AdoHcy) hydrolase, which is very important for 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 [9]. The inhibition of this enzyme on 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 [10]. Methyltransferases are essential for the maturation of mRNA. Therefore, inhibiting the methyl transferases by blocking the AdoHcy metabolism can disrupt the maturation of viral mRNA. AdoHcy inhibitors usually display a broad spectrum of antiviral activities. In addition, this mechanism might be exploited in some form of a combination therapy, with the nucleosides having a different mechanism of action.

Encouraged by these interesting structures and the biological activities of branched nucleosides as well as of olefinic carbocyclic nucleosides, a novel class of nucleosides comprising branched carbocyclic nucleo-

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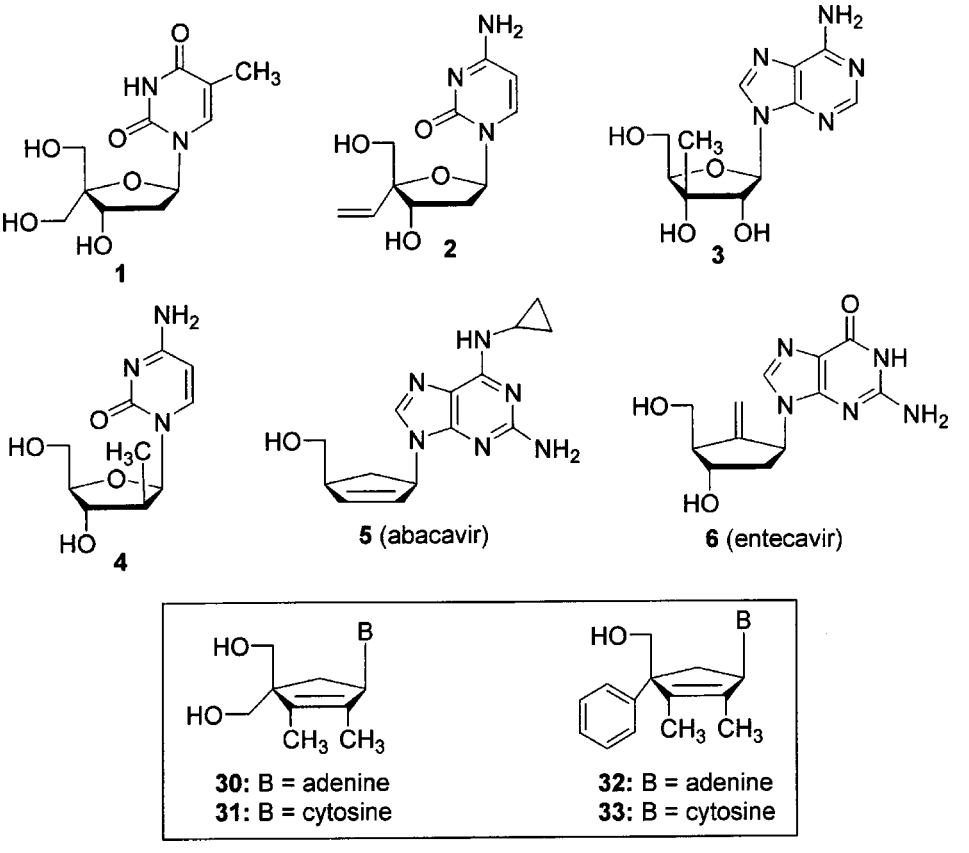


Figure 1. Structures of olefinic carbocyclic nucleosides, branched furanose nucleosides and target nucleosides.

sides with additional branches at the 2′-, 3′- and 4′-position was synthesized.

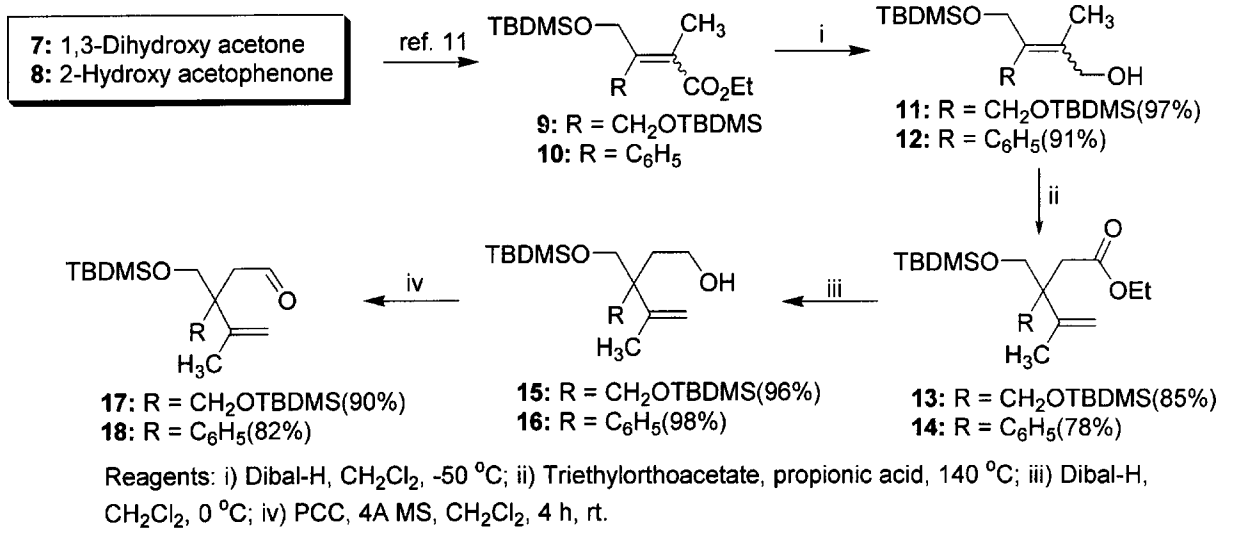
Chemistry

As shown in Scheme 1, the α,β-unsaturated ester derivatives **9** and **10**, which were readily synthesized from 1,3-dihydroxy acetone and 2-hydroxy acetophenone, respectively, by a previously reported method (Scheme 1), were selected as the starting compounds for the synthesis of the 2′,3′,4′-triply branched target nucleosides [11].

Without separation, the esters **9** and **10** were reduced using diisobutylaluminum hydride (DIBALH), and the resulting allylic alcohols **11** and **12** were subjected to a [3,3]-sigmatropic rearrangement using triethylorthoacetate to give the γ,δ-unsaturated esters **13** and **14** at a high yield. The addition of DIBALH to a solution of the esters **13** and **14** in CH_2Cl_2 at 0 °C gave the alcohols **15** and **16**, which were subjected to oxidation conditions using PCC. The resulting aldehydes **17** and **18** were subjected to a Grignard reaction with CH_2=C(CH_3)MgBr to yield the bis-olefins **19** and **20** as stereoisomeric mixtures.

The bis-olefins **19** and **20** were subjected to the well-known ring-closing metathesis conditions [12] using a Grubbs' catalyst II [(Im)Cl_2PCy_3RuCHPh], giving the compound **21** and the stereoisomers **22** and **23** in almost equal amounts. The relative stereochemistry of compounds **22** and **23** was unambiguously determined based on the NOE correlations between the proximal hydrogen and the methylene group (H-1 *vs.* H-5). Unlike compound **22**, a strong NOE correlation was observed between the H-1 *vs.* H-5 of compound **23** (2.2% NOE) (Figure 2).

In order to couple the cyclopentenol with the bases (A = adenine, C = cytosine) using a simple nucleophilic substitution-type reaction, compounds **21** and **23** were subjected to a mesylation reaction (MsCl, TEA, CH_2Cl_2). Unexpectedly, the reactions had a low yield, and the results were not reproducible. Therefore, attention was turned to a palladium(0)-catalyzed reaction procedure [13].



Scheme 1. Synthesis of key intermediates **17** and **18**.

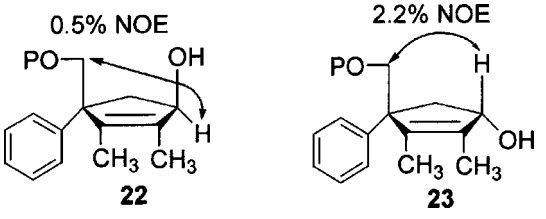


Figure 2. NOE study of compound **22** and **23**.

The cyclopentenols **21** and **22** were transformed to the ethoxycarbonyl derivatives **24** and **25** using ethyl chloroformate. Compounds **24** and **25** were coupled with the adenine and cytosine anion generated by NaH/DMSO with the [tris(dibenzylidene-acetone)-dipalladium(0)-chloroform] adduct to give the compounds **26**–**29** (Scheme 2). The required stereochemistry of the nucleosides **28** and **29** was successfully controlled from the b-configuration of compound **25** via a Pd(0)-catalyzed π–allyl complex mechanism. Compounds **26**–**29** were desilylated by treating them with tetrabutylammonium fluoride (TBAF) to give the final nucleosides **30**–**33** at high yields.

Antiviral activity studies

The synthesized compounds **30**, **31**, **32** and **33** were tested against several viruses, such as HIV (MT-4 cells), HSV-1 (CCL81 cells), HSV-2 (CCL81 cells), and HCMV (AD-169, Davis cells). All the compounds synthesized showed neither excellent antiviral activity nor any cytotoxicity when tested at up to 100 μg/mL. However, the adenine analogue **30** did show moderate antiviral activity against HCMV (Table 1), indicating that this virus might allow the sugar moiety for phosphorylation as well as for DNA polymerase, which is different from other viruses. In addition, the adenine analogue **32** also had weak antiviral activity against HIV-1 (MT-4 cells). The HCMV strains AD-169 (ATTCC VR-583) and Davis (ATCC VR-807) were used to evaluate the anti-HCMV activity, and a standard CPE inhibition assay was used. HEL 299 (human embryonic lung fibroblast) cells were used for the cytotoxicity assay.

In summary, a novel synthetic method for 2′,3′,4′-triply branched carbocyclic nucleosides from simple α-hydroxy carbonyl derivatives was developed. When the synthesized compounds were tested against several viruses such as HIV-1, HSV-1, HSV-2 and HCMV, only the adenosine analogue **30** exhibited moderate antiviral activity against HCMV. The lack of antiviral activity of these racemic compounds is presumably associated with their unfavorable conformations for the phosphorylation occurring during the nucleotide activation process. However, the information obtained in the present study will be useful for the development of a novel carbonucleoside. Studies toward this end and to clarify the mechanism are underway.

Acknowledgments

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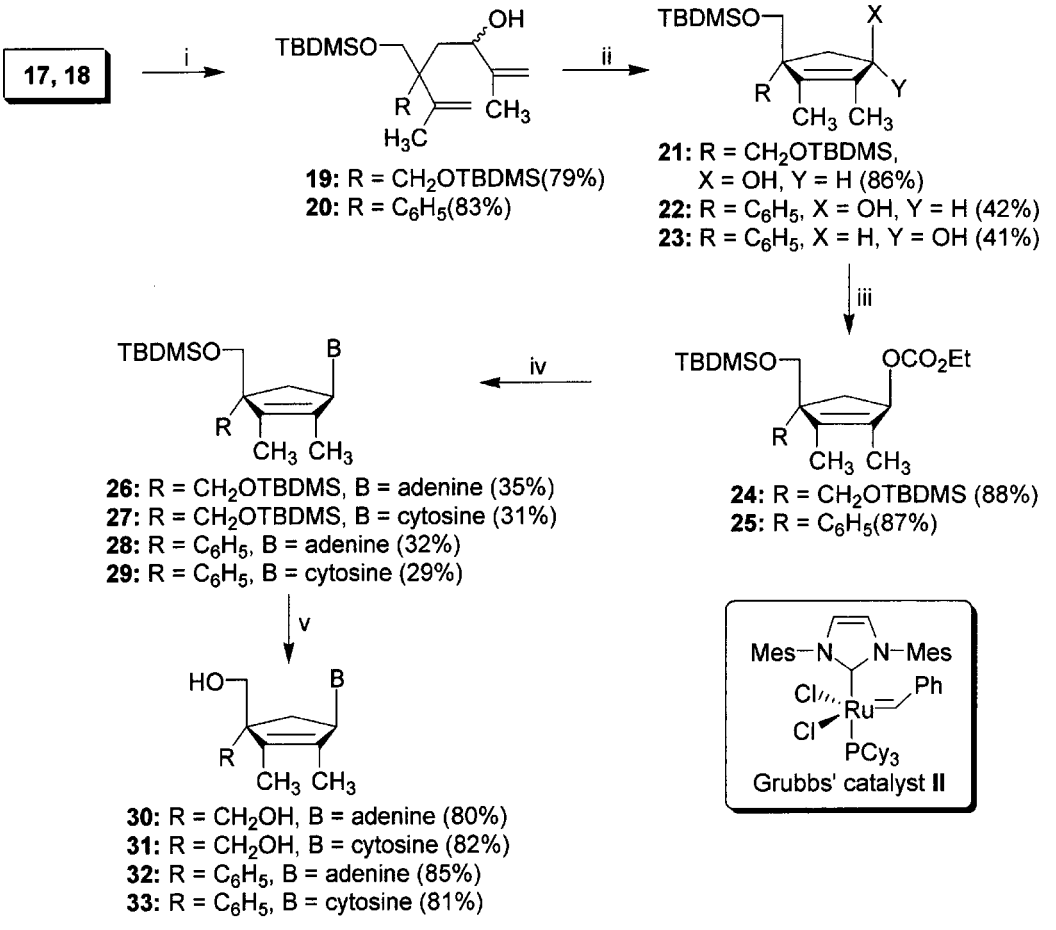

Reagents: i) $CH_2=C(CH_3)MgBr$, THF, -78 °C; ii) Grubbs' catalyst II, benzene, reflux, overnight; iii) $ClCO_2Et$, DMAP, pyridine, rt, overnight; iv) Bases (A = adenine, C = cytosine), $Pd_2(dba)_3.CHCl_3$, $P(O\text{-}i\text{-}Pr)_3$, NaH, THF/DMSO, reflux, overnight; v) TBAF, THF, rt.

Scheme 2. Synthesis of targeted nucleosides.

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)
30	>100	>100	>100	**30.1**	>100
31	>100	>100	>100	>100	>100
32	61.0	78.5	>100	>100	>100
33	>100	>100	>100	>100	>100
AZT	0.001	ND	ND	ND	1.17
Ganciclovir	ND	2.10	2.10	ND	>10
Ribavirin	ND	ND	ND	12.60	300.00

ND: not determined.

Experimental

All the chemicals were of reagent grade and were used without further purification. All the moisture-sensitive reactions were performed in an inert atmosphere with either N_2 or Ar, using distilled dry solvents. The melting points were determined using a Mel-temp II laboratory device and were uncorrected. The NMR spectra were recorded on a JEOL JNM-LA 300 spectrometer. The chemical shifts are reported in parts per million (δ), and the signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and dd (doublet of doublets). The UV spectra were obtained using a Beckman DU-7 spectrophotometer. The elemental analyses were performed using an Elemental Analyzer System (Profile HV-3). TLC was performed on Uniplates (silica gel) purchased from Analtech Co. The dry THF was obtained by distillation from Na and benzophenone when the solution became purple.

*4,4′-Bis-(t-butyldimethylsilyloxy)-2-methyl-but-2-en-1-ol (**11**)*

To a solution of compound **9** (10 g, 24.83 mmol) in CH_2Cl_2 (300 mL), DIBALH (52.1 mL, 1.0 M solution in hexane) was added slowly at −50 °C and stirred for 2 h at the same temperature. To the resulting mixture, methanol (50 mL) was 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 : 7) to give the alcohol **11** (8.68 g, 97%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.25 (s, 4H), 4.17 (s, 2H), 1.71 (s, 3H), 0.93 (s, 18H), 0.05 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 141.21, 125.39, 64.85, 59.40, 58.70, 25.83, 20.34, 18.24, −5.35; Anal. calc. for $C_{18}H_{40}O_3Si_2$: C 59.94; H 11.18. Found: C 59.77; H 11.21.

*(E)- and (Z)-4-(t-Butyldimethylsilyloxy)-2,3-dimethyl-but-2-en-1-ol (**12**)*

Compound **12** was prepared from compound **10** using the method described for compound **11**: yield 91%; ^{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, 9H), 0.04 (s, 6H); Anal. calc. for $C_{17}H_{28}O_2Si$: C 69.81; H 9.65. Found: C 69.61; H 9.54.

*3,3′-Bis-(t-butyldimethylsilyloxymethyl)-4-methyl-pent-4-enoic acid ethyl ester (**13**)*

A solution of the allylic alcohol **11** (15 g, 41.58 mmol) in triethyl orthoacetate (300 mL) and 1.5 mL of propionic acid was heated at 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 : 50) to give compound **13** (15.22 g, 85%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.87 (s, 1H), 4.62 (s, 1H), 4.05 (q, *J* = 7.5 Hz, 2H), 3.65 (dd, *J* = 15.6, 9.0 Hz, 4H), 2.41 (s, 2H), 1.61 (s, 3H), 1.12 (t, *J* = 7.5 Hz, 3H), 0.94 (s, 18H), 0.02 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) d 171.92, 139.76, 114.48, 64.67, 59.88, 45.98, 36.84, 25.85, 20.34, 18.25, 14.25, −5.26; Anal. calc. for $C_{22}H_{46}O_4Si_2$: C 61.34; H 10.76. Found: C 61.12; H 10.60.

*(±)-3-(t-Butyldimethylsilyloxymethyl)-3-phenyl-4-methyl-pent-4-enoic acid ethyl ester (**14**)*

Compound **14** was prepared from compound **12** using the method described for compound **13**: yield 78%; ^{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.

*3,3′-Bis-(t-butyldimethylsilyloxymethyl)-4-methyl-pent-4-enol (**15**)*

To a solution of compound **13** (8.5 g, 19.73 mmol) in CH_2Cl_2 (300 mL), DIBALH (41.43 mL, 1.0 M solution in hexane) was added slowly at 0 °C and stirred for 30 min at the same temperature. To the resulting mixture, methanol (40 mL) was added. The mixture was then 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 : 25) to give compound **15** (7.09 g, 96%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.82 (s, 1H), 4.62 (s, 1H), 3.57 (dd, *J* = 12.9, 9.6 Hz, 6H), 1.69 (s, 3H), 1.64 (dd, *J* = 6.0, 3.6 Hz, 2H), 0.87 (s, 18H), 0.04 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 146.45, 112.24, 63.41, 59.16, 47.99, 33.63, 25.81, 20.30, 18.16, −5.57; Anal. calc. for $C_{20}H_{44}O_3Si_2$: C 61.79; H 11.41. Found: C 61.52; H 11.55.

*(±)-3-(t-Butyldimethylsilyloxymethyl)-3-phenyl-4-methyl-pent-4-enol (**16**)*

Compound **16** was prepared from compound **14** using the method described for compound **15**: yield 98%; ^{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.

*3,3′-Bis-(t-butyldimethylsilyloxymethyl)-4-methyl-pent-4-enal (**17**)*

To a solution of compound **15** (5.0 g, 12.86 mmol) in CH_2Cl_2 (100 mL), 4-Å molecular sieves (7.5 g) and PCC (6.93 g, 32.15 mmol) were added slowly at 0 °C and stirred for 4 h at room temperature. To the resulting mixture, excess diethyl ether (500 mL) was added. The mixture was then vigorously stirred for 2 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 **17** (4.47 g, 90%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.65 (m, 1H), 4.96 (s, 1H), 4.75 (s, 1H), 3.64 (dd, *J* = 13.5, 9.3 Hz, 4H), 2.43 (s, 2H), 1.76 (s, 3H), 0.84 (s, 18H), 0.04 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 202.89, 145.02, 113.28, 65.01, 48.62, 45.44, 25.79, 20.42, 18.17, −5.67; Anal. calc. for $C_{20}H_{42}O_3Si_2$: C 62.12; H 10.95. Found: C 62.33; H 10.81.

*(±)-3-(t-Butyldimethylsilyloxymethyl)-3-phenyl-4-methyl-pent-4-enal (**18**)*

Compound **18** was prepared from compound **16** using the method described for compound **17**: yield 82%; ^{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)-5,5′-Bis-(t-butyldimethylsilyloxymethyl)-2,6-dimethyl-hepta-1,6-dien-3-ol (**19**)*

To a solution of compound **17** (6.0 g, 15.5 mmol) in dry THF (150 mL), isopropenyl magnesiumbromide (23.27 mL, 1.0 M solution in THF) was added slowly at −78°C. After 3 h, a saturated NH_4Cl solution (20 mL) was added, and the reaction mixture was then slowly warmed 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 : 45) to give compound **19** (5.25 g, 79%) as a colorless oil: as a diastereomeric mixture for ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.93 (d, *J* = 8.4 Hz, 2H), 4.71 (d, *J* = 10.8 Hz, 2H), 4.10 (d, *J* = 11.1 Hz, 1H), 3.80 (d, *J* = 9.9 Hz, 1H), 3.71 (d, *J* = 9.9 Hz, 1H), 3.57 (d, *J* = 9.9 Hz, 1H), 3.49 (d, *J* = 9.9 Hz, 1H), 3.47 (s, 1H), 1.71 (s, 3H), 1.68 (s, 3H), 1.55 (m, 2H), 0.86 (s, 18H), 0.04 (s, 12H); 13C NMR ($CDCl_3$, 75 MHz) δ 148.35, 146.47, 112.85, 109.81, 64.32, 63.49, 48.37, 37.47, 25.82, 20.39, 18.17, 17.86, −5.58; Anal. calc. for $C_{23}H_{48}O_3Si_2$: C 64.42; H 11.28. Found: C 64.60; H 11.39.

*(rel)-(3R and 3S,5S)-5-(t-Butyldimethylsilyloxymethyl)-5-phenyl-2,6-dimethyl-hepta-1,6-dien-3-ol (**20**)*

Compound **20** was prepared from compound **18** using the method described for compound **19**: yield 83%; as a diastereomeric mixture for ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.28 (m, 5H), 5.11 (s, 1H), 5.01 (s, 1H), 4.96 (s, 1H), 4.80 (s, 1H), 4.16 (d, *J* = 6.3 Hz, 1H), 4.1 (d, *J* = 10.5 Hz, 1H), 2.78 (d, *J* = 2.7 Hz, 1H), 2.41 (dd, *J* = 14.4, 2.4 Hz, 1H), 2.19 (dd, *J* = 14.4, 8.1 Hz, 1H), 1.74 (s, 3H), 1.58 (s, 3H), 0.85 (s, 9H), 0.09 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 149.36, 148.34, 143.91, 128.00, 127.18, 126.14, 112.32, 109.84, 77.19, 72.50, 66, 32, 51.46, 39.86, 25.71, 20.96, 18.10, 17.92, −5.85; Anal. calc. for $C_{22}H_{36}O_2Si$: C 73.28; H 10.06. Found: C 70.41; H 10.32.

*(±)-4,4′-Bis-(t-butyldimethylsilyloxymethyl)-2,3-dimethyl-cyclopent-2-enol (**21**)*

To a solution of compound **19** (2.5 g, 5.8 mmol) in dry benzene (5 mL), Grubbs' catalyst II (51 mg, 0.06 mmol) was added. The reaction mixture was refluxed overnight and then concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1 : 15) to give the cyclopentenol **21** (1.99 g, 86%) as colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) d 4.20 (dd, *J* = 10.8, 7.2 Hz, 1H), 3.62 (d, *J* = 9.3 Hz, 1H), 3.50 (dd, *J* = 9.9, 5.4 Hz, 2H), 3.40 (d, *J* = 9.9 Hz, 1H), 2.74 (d, *J* = 10.2 Hz, 1H), 1.95 (dd, *J* = 14.1, 7.5 Hz, 1H), 1.67 (s, 3H), 1.51 (s, 3H), 0.85 (s, 18H), 0.04 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) d 137.10, 135.24, 77.87, 67.19, 64.93, 57.13, 41.07, 25.71, 18.07, 11.56, 10.42, −5.67; Anal. calc. for $C_{21}H_{44}O_3Si_2$: C 62.94; H 11.07. Found: C 63.11; H 10.92.

*(rel)-(1R,4R)-4-(t-Butyldimethylsilyloxymethyl)-4-phenyl-2,3-dimethyl-cyclopent-2-ene (**22**) and (rel)-(1S,4R)-4-(t-butyldimethylsilyloxymethyl)-4-phenyl-2,3-dimethyl-cyclopent-2-ene (**23**)*

Compounds **22** and **23** were prepared from compound **20** using the method described for compound **21**: yield for **22** (42%) and for **23** (41%). Compound **22**; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.17−6.94 (m, 5H), 4.28 (dd, *J* = 10.8, 7.2 Hz, 1H), 3.95 (d, *J* = 9.0 Hz, 1H), 3.81 (d, *J* = 9.6 Hz, 1H), 2.75 (d, *J* = 10.8 Hz, 1H), 2.17 (dd, *J* = 14.1, 7.2 Hz, 1H), 1.67 (s, 3H), 1.23 (s, 3H), 0.79 (s, 9H), −0.02 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 145.94, 138.74, 136.13, 128.41, 126.17, 78.66, 65.29, 59.63, 49.06, 25.93, 18.44, 11.84, 10.61, −5.59; Anal. calc. for $C_{20}H_{32}O_2Si$: C 72.23; H 9.70. Found: C 72.38; H 9.58. Compound **23**; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.25−7.08 (m, 5H), 4.55 (s, 1H), 3.99 (d, *J* = 9.3 Hz, 1H), 3.77 (d, *J* = 9.3 Hz, 1H), 2.61 (dd, *J* = 13.8, 7.5 Hz, 1H), 1.71 (s, 3H), 1.65 (dd, *J* = 13.5, 3.9 Hz, 1H), 1.35 (s, 3H), 0.80 (s, 9H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 146.23, 137.78, 136.91, 128.20, 127.47, 112.50, 79.43, 66.23, 58.97, 47.65, 25.49, 18.09, 11.48, 11.15, −5.48; Anal. calc. for $C_{20}H_{32}O_2Si$: C 72.23; H 9.70. Found: C 72.08; H 9.77.

*(rel)-(1R,4R)-1-Ethoxycarbonyloxy-4,4′-(t-butyldimethylsilyloxymethyl)-2,3-dimethyl-cyclopent-2-ene (**24**)*

To a solution of compound **21** (3.0 g, 7.48 mmol) in anhydrous pyridine (15 mL), ethyl chloroformate (1.72 mL, 11.22 mmol) and DMAP (110 mg, 0.9 mmol) were added. The reaction mixture was stirred overnight at room temperature. The reaction mixture was quenched using a saturated $NaHCO_3$ solution (1 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 : 30) to give compound **24** (3.1 g, 88%) as a colorless syrup: ^{1}H NMR ($CDCl_3$, 300 MHz) d 5.64 (dd, *J* = 7.0, 4.6 Hz, 1H), 4.16 (q, *J* = 7.6 Hz, 2H), 3.54 (m, 4H), 2.92 (dd, *J* = 13.6, 7.6 Hz, 1H), 1.78 (s, 3H), 1.68 (d, *J* = 13.6 Hz, 1H), 1.51 (s, 3H), 1.33 (t, *J* = 7.6 Hz, 3H), 0.86 (s, 18H), 0.05 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) d 155.32, 137.43, 134.76, 83.56, 67.45, 65.11, 63.21, 58.32, 40.91, 25.78, 18.44, 14.78, 11.67, 10.34, −5.54; Anal. calc. for $C_{24}H_{48}O_5Si_2$: C 60.97; H 10.23. Found: C 61.21; H 10.06.

*(rel)-(1R,4R)-1-Ethoxycarbonyloxy-4-(t-butyldimethylsilyloxymethyl)-4-phenyl-2,3-trimethyl-cyclopent-2-ene (**25**)*

Compound **25** was prepared from compound **22** using the method described for compound **24**: yield 87%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.17−6.98 (m, 5H), 5.57 (dd, *J* = 7.2, 4.6 Hz, 1H), 4.18 (q, *J* = 7.8 Hz, 2H), 3.82 (d, *J* = 9.4 Hz, 1H), 3.70 (d, *J* = 9.3 Hz, 1H), 2.61 (dd, *J* = 14.2, 7.6 Hz, 1H), 1.87 (d, *J* = 14.2 Hz, 1H), 1.65 (s, 3H), 1.34 (t, *J* = 7.8 Hz, 3H), 1.30 (s, 3H), 0.85 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.36, 145.71, 138.89, 136.76, 128.25, 127.41, 112.54, 83.13, 66.20, 63.65, 59.23, 48.65, 25.59, 18.19, 14.21, 11.68, 11.12, −5.65; Anal. calc. for $C_{23}H_{36}O_4Si$: C 68.27; H 8.97. Found: C 68.34; H 9.09.

*(rel)-(1′R,4′R)-9-[4,4′-Bis-(t-butyldimethylsilyloxymethyl)-2,3-dimethyl-cyclopent-2-en-1-yl] adenine (**26**)*

To pure NaH (70.2 mg, 2.92 mmol) in anhydrous DMSO (10.0 mL), adenine (402 mg, 2.94 mmol) was added. The reaction mixture was stirred for 30 min at 50−55°C and cooled to

room temperature. Simultaneously, P(O-*i*-Pr)$_3$ (1.44 mL, 3.3 mmol) was added to a solution of $Pd_2(dba)_3{\cdot}CHCl_3$ (69 mg, 37.5 μmol) in anhydrous THF (10.0 mL), which was stirred for 40 min. To the adenine solution in DMSO, a catalyst solution of THF and compound **24** (1.24 g, 2.64 mmol) dissolved in anhydrous THF (9 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 : 15) to give compound **26** (478 mg, 35%) as a white solid: mp 186–188 °C; UV (MeOH) λ_{max} 260.5 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.27 (s, 1H), 7.97 (s, 1H), 5.67 (dd, *J* = 8.6, 4.6 Hz, 1H), 3.71 (m, 4H), 2.63 (dd, *J* = 13.8, 7.8 Hz, 1H), 1.92 (d, *J* = 13.8, 1H), 1.77 (d, 3H), 1.51 (s, 3H), 0.86 (s, 18H), 0.06 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.46, 152.90, 142.44, 138.82, 136.36, 68.45, 67.31, 59.45, 57.13, 39.17, 25.89, 18.32, 11.61, 10.67, −5.51; Anal. calc. for $C_{26}H_{47}N_5O_2Si_2$: C 60.30; H 9.15; N 13.52. Found: C 60.54; H 9.31; N 13.44.

*(rel)-(1′R,4′R)-1-[4,4′-Bis-(t-butyldimethylsilyloxymethyl)-2,3-dimethyl-cyclopent-2-en-1-yl] cytosine (**27**)*

Compound **27** was prepared from compound **24** using the method described for synthesizing compound **26**: yield 31%; mp 176–178 °C; UV (MeOH) λ_{max} 271.0 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.72 (d, *J* = 7.4 Hz, 1H), 5.72 (dd, *J* = 7.8, 4.6 Hz, 1H), 5.62 (d, *J* = 7.4 Hz, 1H), 3.72 (dd, *J* = 13.6, 7.2 Hz, 1H), 3.44 (d, *J* = 8.6 Hz, 1H), 3.21 (d, *J* = 8.6 Hz, 1H), 2.64 (dd, *J* = 14.2, 7.6 Hz, 1H), 2.12 (d, *J* = 14.2 Hz, 1H), 1.73 (s, 3H), 1.41 (s, 3H), 0.87 (s, 18H), 0.05 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.42, 155.45, 143.67, 144.78, 132.45, 93.81, 68.32, 67.45, 61.18, 58.62, 35.80, 25.40, 18.76, 11.71, 10.78, −5.66; Anal. calc. for $C_{25}H_{47}N_3O_3Si_2$: C 60.80; H 9.59; N 8.51. Found: C 60.67; H 9.41; N 8.40.

*(rel)-(1′R,4′R)-9-[4-(t-Butyldimethylsilyloxymethyl)-4-phenyl-2,3-dimethyl-cyclopent-2-en-1-yl] adenine (**28**)*

Compound **28** was prepared from compound **25** using the method described for synthesizing compound **27**: yield 32%; mp 194–196 °C; UV (MeOH) λ_{max} 261.5 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.36 (s, 1H), 8.12 (s, 1H), 7.26–6.99 (m, 5H), 5.70 (dd, *J* = 8.8, 6.2 Hz, 1H), 3.85 (d, *J* = 8.6 Hz, 1H), 3.64 (d, *J* = 8.6 Hz, 1H), 2.65 (dd, *J* = 13.8, 6.8 Hz, 1H), 2.15 (d, *J* = 13.8 Hz, 1H), 1.71 (s, 3H), 1.26 (s, 3H), 0.83 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.76, 152.81, 146.22, 141.54, 139.21, 137.32, 136.65, 129.43, 126.21, 66.45, 59.60, 49.12, 25.72, 18.21, 11.45, 10.31, −5.40; Anal. calc. for $C_{25}H_{35}N_5OSi$: C 66.78; H 7.85; N 15.57. Found: C 66.80; H 7.78; N 15.42.

*(rel)-(1′R,4′R)-1-[4-(t-Butyldimethylsilyloxymethyl)-4-phenyl-2,3-dimethyl-cyclopent-2-en-1-yl] cytosine (**29**)*

Compound **29** was prepared from compound **25** using the method described for synthesizing compound **28**: yield 29%; mp 182–184 °C; UV (MeOH) λ_{max} 271.5 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.52 (d, *J* = 7.2 Hz, 1H), 7.27–7.04 (m, 5H), 5.85 (br s, 1H), 5.52 (d, *J* = 7.2 Hz, 1H), 3.89 (d, *J* = 8.6 Hz, 1H), 3.62 (d, *J* = 8.6 Hz, 1H), 2.76 (dd, *J* = 13.8, 7.8 Hz, 1H), 2.13 (d, *J* = 13.8 Hz, 1H), 1.76 (s, 3H), 1.33 (s, 3H), 0.83 (s, 9H), 0.03 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.12, 155.72, 146.20, 144.667, 138.80, 137.34, 128.19, 127.02, 112.51, 93.74, 66.23, 58.97, 47.65, 25.49, 18.09, 11.53, 10.09, −5.42; Anal. calc. for $C_{24}H_{35}N_3O_2Si$: C 67.72; H 8.29; N 9.87. Found: C 67.91; H 8.02; N 9.92.

*(rel)-(1′R,4′R)-9-Bis-[4,4′-(hydroxymethyl)-2,3-dimethyl-cyclopent-2-en-1-yl] adenine (**30**)*

To a solution of compound **26** (200 mg, 0.386 mmol) in THF (5 mL), TBAF (0.58 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 **30** (89 mg, 80%) as a white solid: mp 186–188 °C; UV (H_2O) λ_{max} 261.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.24 (s, 1H), 8.07 (s, 1H), 7.20 (br s, 2H D_2O exchangeable), 5.62 (br s, 2H), 4.83 (br s, 2H D_2O exchangeable), 3.82 (dd, *J* = 13.6, 6.6 Hz, 2H), 3.35 (d, *J* = 8.6 Hz, 2H), 2.71 (dd, *J* = 13.6, 8.0 Hz, 1H), 2.12 (d, *J* = 13.6 Hz, 1H), 1.72 (s, 3H), 1.11 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 155.71, 153.10, 143.76, 139.06, 135.63, 67.61, 66.21, 58.41, 55.78, 38.12, 11.12, 10.07; Anal. calc. for $C_{14}H_{19}N_5O_2$: C 58.12; H 6.62; N 24.21. Found: C 58.34; H 6.60; N 24.29.

*(rel)-(1′R,4′R)-1-Bis-[4,4′-(hydroxymethyl)-2,3-dimethyl-cyclopent-2-en-1-yl] cytosine (**31**)*

Compound **31** was prepared from compound **27** using the method described for synthesizing compound **30**: yield 82%; mp 171–174 °C; UV (H_2O) λ_{max} 271.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) d 7.76 (d, *J* = 7.2 Hz, 1H), 7.14 (br d, 2H D_2O exchangeable), 5.70 (br s, 1H), 5.61 (d, *J* = 7.2 Hz, 1H), 4.83 (br s, 2H D_2O exchangeable), 3.80 (d, *J* = 14.2, 6.6 Hz, 1H), 3.47 (d, *J* = 14.2 Hz, 1H), 3.33 (dd, *J* = 13.2, 7.6 Hz, 2H), 2.74 (dd, *J* = 12.2, 7.8 Hz, 1H), 2.32 (d, *J* = 12.4 Hz, 1H), 1.68 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 165.77, 155.67, 144.78, 142.78, 132.25, 93.78, 67.90, 68.12, 61.22, 58.25, 35.45, 11.23, 11.02; Anal. calc. for $C_{13}H_{19}N_3O_3$: C 58.85; H 7.22; N 15.84. Found: C 58.67; H 7.31; N 15.60.

*(rel)-(1′R,4′R)-9-[4-(t-Hydroxymethyl)-4-phenyl-2,3-trimethyl-cyclopent-2-en-1-yl] adenine (**32**)*

Compound **32** was prepared from compound **28** using the method described for synthesizing compound **31**: yield 85%; mp 190–192 °C; UV (H_2O) λ_{max} 261.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.30 (s, 1H), 8.07 (s, 1H), 7.26–6.99 (m, 5H), 7.12 (br s, 2H D_2O exchangeable), 5.77 (dd, *J* = 8.6, 4.2 Hz, 1H), 4.81 (br s, 1H D_2O exchangeable), 3.81 (d, *J* = 8.8 Hz, 1H), 3.54 (d, *J* = 8.8 Hz, 1H), 2.68 (dd, *J* = 13.8, 8.6 Hz, 1H), 2.25 (d, *J* = 13.8 Hz, 1H), 1.79 (s, 3H), 1.34 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 155.61, 153.13, 146.28, 141.92, 138.12, 137.45, 135.10, 129.43, 125.91, 67.467, 58.54, 48.21, 11.40, 10.23; Anal. calc. for $C_{19}H_{21}N_5O$: C 68.04; H 6.31; N 20.88. Found: C 67.88; H 6.41; N 20.91.

*(rel)-(1′R,4′R)-1-[4-(t-Butyldimethylsilyloxymethyl)-4-phenyl-2,3-dimethyl-cyclopent-2-en-1-yl] cytosine (**33**)*

Compound **33** was prepared from compound **29** using the method described for synthesizing compound **32**: yield 81%; mp 178–181 °C; UV (H_2O) λ_{max} 272.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.59 (d, *J* = 7.4 Hz, 1H), 7.29–7.08 (m, 5H), 5.81 (dd, *J* = 8.6, 4.5 Hz, 1H), 5.52 (d, *J* = 7.4 Hz, 1H), 3.73 (d, *J* = 8.4 Hz, 1H), 3.52 (d, *J* = 8.4 Hz, 1H), 2.66 (dd, *J* = 14.2, 7.2 Hz, 1H), 2.13 (d, *J* = 14.2 Hz, 1H), 1.82 (s, 3H), 1.38 (s, 3H), 0.88; ^{13}C NMR (DMSO-d_6, 75 MHz) δ 165.70, 154.99, 146.28, 144.61, 137.34, 136.41, 128.65, 126.81, 112.88, 93.31, 66.90, 59.05, 48.54, 11.571, 10.21; Anal. calc.

for $C_{18}H_{21}N_3O_2$: C 69.43; H 6.80; N 13.49. Found: C 69.29; H 6.72; N 13.52.

Evaluation of anti-HCMV activity

A standard CPE inhibition assay was used [14]. HEL cells in the 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 of adsorption at 37 °C, the liquid was aspirated off to remove the unabsorbed viruses, and 100 μL of MEM/2% FBS containing a compound was applied to each well in duplicate for each concentration and incubated for a further 6 days. The antiviral activity was measured either microscopically or fluorometrically. For the microscopical observations, the cells were fixed with 70% ethanol, stained with a 2.5% Giemsa solution for 2 h, rinsed with distilled water and air-dried. The antiviral activity is expressed as the EC_{50}, *i.e.* the concentration required to inhibit virus-induced CPE by 50%. The EC_{50} values were estimated from the semi-logarithmic graphic plots of the percentage of CPE as a function of the concentration of the test compound. For the fluorometric assay [15], the cells were washed twice with 100 μL 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 solution was removed by aspiration, and each well was washed with 100 μL 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 the host cell growth and on viability was assayed both microscopically and fluorometrically using propidium iodide staining (PI; Sigma, St. Louis, MO, USA). In order to measure the cytostatic effects, the HEL cells were seeded at 3000 cells/well in 96-well plates in 100 μL MEM/10% FBS. The cells were incubated at 37 °C for 1 day in order to let them attach to the plates. Different dilutions of the test compounds were added, and the cells were incubated for a further 3 days at 37 °C. To each well, 100 μL PI (40 μg/mL, diluted with the medium) was added, followed by 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), and the concentration of the compound responsible for a 50% inhibition of cell growth was calculated and expressed as CS_{50} (50% cytostatic effect).

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

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