DOI 10.1002/ardp.200500187 Arch. Pharm. Chem. Life Sci. 2005, 338, 528–533

Synthesis and Antiviral Evaluation of Novel Exo-methylene Acyclic Nucleosides and Phosphonic Acid Nucleosides

Aihong Kim, Joon Hee Hong

College of Pharmacy, Chosun University, Kwangju, Republic of Korea

This paper describes a very simple synthesis route of novel acyclic nucleosides and phosphonic acid nucleosides. The condensation of the mesylates **6** and **17** with the natural nucleosidic bases (A, C, U, T) under nucleophilic substitution (K_2CO_3, 18-*Crown*-6, DMF) and deprotection afforded the target nucleosides (**11**, **12**, **13**, **14**) and phosphonic acid nucleosides (**22**, **23**, **24, 25**). In addition, these compounds were evaluated for their antiviral properties against various viruses. Uracil derivative **24** shows significant anti-HCMV activity (EC_{50} = 10.24 μM).

Keywords: PMEA; Entecavir; Nucleosides; Phosphonic acid nucleoside; Antiviral agents

Received: August 8, 2005; Accepted: September 9, 2005

Introduction

The discovery of acyclovir [1] as an antiherpes agent ignited the search for new antiviral nucleosides with a disconnected chain resulting from the omission of various bonds from the pentose or cyclopentane rings. During the past twenty years, many new synthetic schemes for various acyclic nucleoside analogues [2] have been reported, and many of these molecules have exhibited promising antiviral activity [3−5]. The recent approval of bis(POC)PMPA, **1** [6] by the FDA as an anti-HIV agent warrants further study for acyclic nucleosides as chemotherapeutic agents.

One of acyclic phosphonic acid nucleosides, PMEA **2** [7], shows a broad spectrum of antiviral activity and is effective against hepatitis B virus (HBV) [8], human immunodeficiency virus (HIV) [9], and also the herpes simplex virus (HSV) [10]. Unlike nucleoside analogues, a phosphonic acid nucleoside has the advantage of skipping the requisite first phosphorylation which is a crucial step for the activation of nucleosides [11].

Recently, D- and L-carbocyclic nucleosides with exocyclic methylene in place of furanose oxygen were synthesized and evaluated for antiviral activities, among which D-guanine derivative entecavir (**3**) was very active on HBV and was 100 times more potent than clinically available drug lamivudine [12].

In view of the interesting results reported for acyclic nucleic acid derivative and exocyclic methylene nucleoside, the aim of this study was to synthesize novel acyclic methylene nucleosides and nucleotides.

Correspondence: Joon Hee Hong, College of Pharmacy, Chosun University, Kwangju 501-759, Republic of Korea. Phone: +82 62 230-6378, Fax: +82 62 222-5414, e-mail: hongjh@mail.chosun.ac.kr

Results and discussion

For the synthesis of acyclic nucleosides, the commercially available 2-methylene-propane-1,3-diol **4** was selected as a starting material. As shown in Scheme 1, the synthetic route is very simple and straightforward. A monosilylation of the diol **4** afforded the allylic alcohol derivative **5**. The hydroxyl group of compound **5** was mesylated by treating with methanesulfonyl chloride (MsCl) in anhydrous CH_2Cl_2 to give the key intermediate **6**, which was coupled with the nucleosidic bases such as adenine, cytosine, uracil, or thymine, under nucleophilic substitution conditions (K_2CO_3, 18-Crown-6, DMF) [13] to give the acyclic nucleoside derivatives **7**−**10**. The silyl protecting groups were removed by tetrabutylammonium fluoride (TBAF) to give the desired nucleosides **11**−**14**.

For the synthesis of phosphonic acid nucleosides, the hydroxyl group of **5** was phosphonated by treating them with diisopropyl bromomethylphosphonate in anhydrous DMF to give the phosphonate intermediate **15** (Scheme 2). Desilylation and sequential mesylation of corresponding hydroxyl group of **16** gave key intermediate **17**, which was also coupled with nucleosidic bases (adenine, cytosine, uracil, thymine) under similar S_N2 substitution conditions to give the acyclic phosphonate nucleoside derivatives **18**−**21**, respectively. Although a small quantity of the N^7-isomer of **18** (less than 12%) of the adenine base was present, they could be readily differentiated (UV (MeOH) λ_{max} 279 nm) and also readily separated by column chromatography [14].

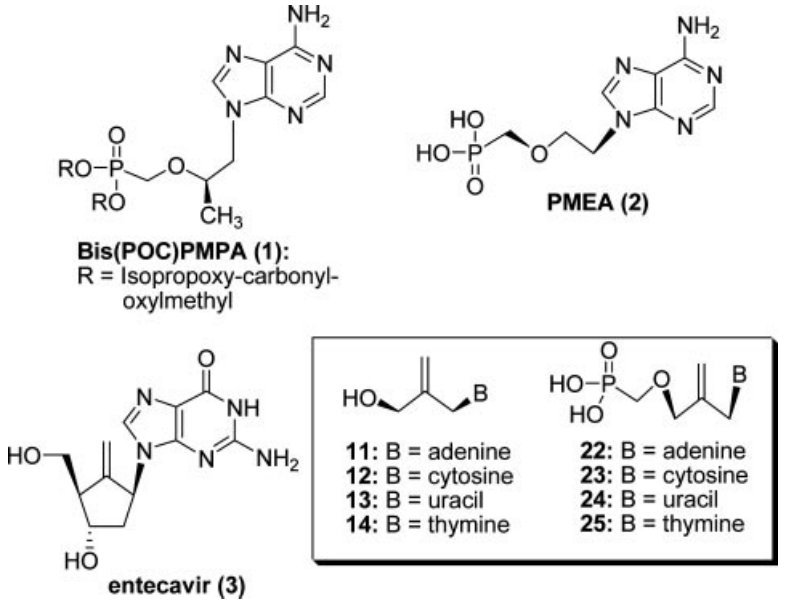
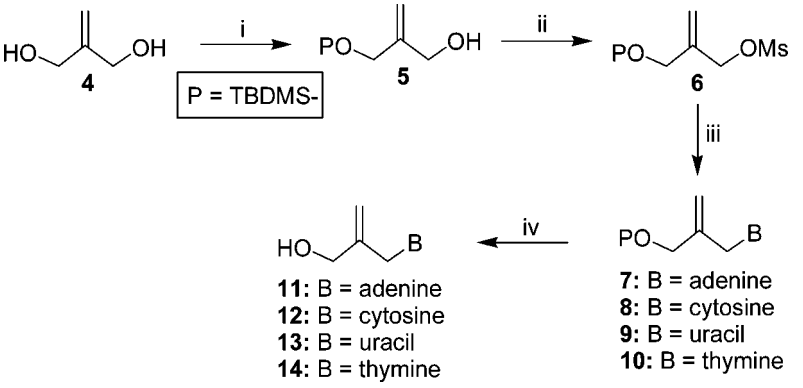


Figure 1. Synthesis rationale for the target nucleosides.



Reagents: i) TBDMSCl, imidazole, CH_2Cl_2; ii) MsCl, TEA, CH_2Cl_2; iii) nucleosidic bases (A, C, U, T), 18-Crown-6, K_2CO_3, DMF; iv) TBAF, THF.

Scheme 1. Synthesis of methylene acyclic nucleosides.

Isopropyl groups of phosphonates **18**–**21** were readily hydrolyzed using trimethylsilylbromide (CH_3SiBr) [15] to give the target nucleoside phosphonic acids **22**–**25**.

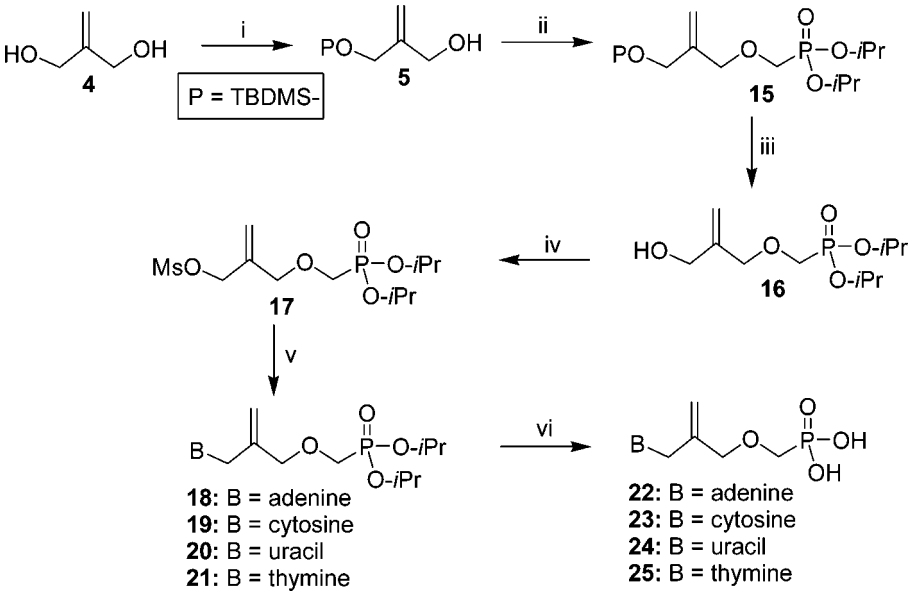
Results and discussion

The antiviral assays against several viruses such as HIV (MT-4 cells), HSV-1 (CCL81 cells), HSV-2 (CCL-81 cells), and human cytomegalo virus, HCMV, (AD-169) were performed. As shown in Table 1, none of the tested compounds showed excellent antiviral activity except for the uracil nucleotide **24**, which exhibited significant anti-HCMV activity (EC_{50} = 10.24 μmol) without any cytotoxicity up to 100 μM.

Conclusion

This study performed the synthesis and biological evaluation of novel methylene acyclic nucleosides and nucleotides, respectively. None of the synthesized compounds exhibited good antiviral activity except for the uracil nucleotide derivative **24**, which showed significant anti-HCMV activity. The finding of some antiviral activity for compound **24** suggests that this class of phosphonic acid acyclic nucleoside analogues may be studied in more detail.

Full Paper



Reagents: i) TBDMSCl, imidazole, CH_2Cl_2; ii) Diisopropyl bromomethylphosphonate, LiOt-Bu, LiI, DMF; iii) TBAF, THF; iv) MsCl, TEA, CH_2Cl_2; v) Bases, K_2CO_3, 18-Crown-6, DMF; vi) $(H_3C)_3SiBr$, CH_2Cl_2.

Scheme 2. Synthesis of methylene acyclic phosphonic acid nucleosides.

Table 1. The antiviral activities of the synthesized compounds.

Comp.	HIV-1 EC_{50} [μM]	HSV-1 EC_{50} [μM]	HSV-2 EC_{50} [μM]	HCMV EC_{50} [μM]	Cytotoxicity CC_{50} [μM]
11	> 100	> 100	> 100	68.76	> 100
12	> 100	> 100	> 100	> 100	> 100
13	47.8	> 100	> 100	> 100	> 100
14	> 100	> 100	> 100	> 100	> 100
22	> 100	> 100	> 100	> 100	> 100
23	> 100	> 100	> 100	> 100	> 100
24	> 100	43.27	> 100	10.24	> 100
25	> 100	> 100	> 100	54.71	> 100
AZT	0.0005	–	–	–	1.10
GCV	–	–	–	0.85	> 10
ACV	–	0.15	–	–	> 100

–, Not Determined; EC_{50} (M), Concentration required to inhibit 50% of virus induced cytopathicity; CC_{50} (μM), Concentration required to reduce cell viability by 50%.

Acknowledgments

The authors wish to acknowledge Dr. C-K. Lee (Korea Research Institute of Chemical Technology) for the antiviral assays.

Experimental

General

All the chemicals were of reagent grade and were used as purchased. All the moisture-sensitive reactions were performed in an inert atmosphere of either N_2 or Ar using distilled dry solvents. The melting points were determined using a Mel-temp II laboratory device (Laboratory Devices, Holliston , MA, USA) and were uncorrected. The NMR spectra were recorded on a JEOL JNM-LA 300 MHz-NMR spectrometer (Jeol, Tokyo, Japan). 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 (Beckman, South Pasadena, CA, USA). The elemental analyses were performed using an Elemental Analyzer System (Leco-932, St.Joseph, MI, USA). TLC was performed on Uniplates (silica gel) purchased from Analtech Co. (Newark, DE, USA) The dry THF was obtained by distillation from Na and benzophenone when the solution became purple.

Syntheses

2-(tert-Butyl-dimethyl-silanyloxymethyl)prop-2-en-1-ol ***5***

To a stirred solution of compound **4** (3.5 g, 39.72 mmol) and imidazole (4.05 g, 59.58 mmol) in CH_2Cl_2 (150 mL), *t*-butyldimethylsilyl chloride (6.28 g, 41.7 mmol) was added at 0°C. The mixture was stirred at the same temperature for 4 h, and quenched by adding a $NaHCO_3$ aqueous solution (10 mL). The mixture was extracted using EtOAc (200 mL), dried over $MgSO_4$, filtered, and then concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:7) to give **2** (6.27 g, 78%) as a colorless syrup: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.99 (m, 2H), 4.15 (s, 2H), 4.07 (s, 2H), 0.83 (s, 9H), 0.03 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 147.43, 111.04, 65.05, 64.59, 25.83, 18.26, −5.46.

Methanesulfonic acid 2-(tert-butyl-dimethyl-silanyloxymethyl)allyl ester ***6***

To a solution of the alcohol **5** (2.2 g, 10.87 mmol) in anhydrous CH_2Cl_2 (50 mL), anhydrous triethylamine (4.0 mL) and MsCl (1.48 g, 12.98 mmol) was added at 0°C. The mixture was stirred at the same temperature for 4 h, and quenched by a cold saturated $NaHCO_3$ aqueous solution (4.0 mL). The mixture was extracted with CH_2Cl_2 (200 mL)/water (200 mL) twice. The combined organic layer were dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (EtOAc/hexane, 4:1) to give **6** (2.31 g, 76%) as a colorless syrup: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.26 (s, 1H), 5.19 (s, 1H), 4.65 (s, 2H), 4.12 (s, 2H), 2.96 (s, 3H), 0.82 (s, 9H), 0.03 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 142.23, 116.76, 69.91, 63.02, 37.45, 25.78, 18.81, −5.76.

9-[2-(tert-Butyl-dimethyl-silanyloxymethyl)allyl ester] adenine ***7***

A solution of the mesylate **6** (430 mg, 1.53 mmol), K_2CO_3 (519 mg, 3.93 mmol), 18-crown-6 (331 mg, 1.25 mmol), and adenine (305.5 mg, 2.25 mmol) in dry DMF (15 mL) was stirred overnight at 85−90°C. The mixture was cooled to room temperature and concentrated in high vacuum. The residue was diluted with brine (70 mL) and extracted with CH_2Cl_2 (150 mL × 3). 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 (MeOH/CH_2Cl_2, 1:10) to give compound **7** (146 mg, 30%) as a white solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.35 (s, 1H), 7.80 (s, 1H), 5.62 (s, 1H), 5.22 (s, 1H), 4.78 (s, 2H), 4.11 (s, 2H), 0.87 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 155.45, 153.24, 143.92, 141.01, 113.51, 64.11, 45.65, 25.45, 18.43, −5.54.

1-[2-(tert-Butyl-dimethyl-silanyloxymethyl)allyl ester] cytosine ***8***

Cytosine derivative **8** was synthesized from **6** by the similar procedure as described for **7**: yield 37% as a white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.74 (d, J = 6.6 Hz, 1H), 5.74 (d, J = 6.6 Hz, 1H), 5.27 (s, 1H), 4.95 (s, 1H), 4.41 (s, 2H), 4.15 (s, 2H), 0.91 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 165.31, 155.80, 147.61, 146.80, 109.54, 64.21, 49.72, 25.67, 18.82, −5.23.

1-[2-(tert-Butyl-dimethyl-silanyloxymethyl)allyl ester] uracil ***9***

Uracil derivative **9** was synthesized from **6** by the similar procedure as described for **7**: yield 30% as a white solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.43 (br s, 1H), 7.21 (d, J = 7.6 Hz, 1H), 5.75 (d, J = 7.6 Hz, 1H), 5.35 (s, 1H), 5.20 (s, 1H), 4.54 (s, 2H), 4.32 (s, 2H), 0.88 (s, 8H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 165.56, 153.82, 145.78, 141.21, 119.55, 103.27, 64.38, 49.25, 25.34, 18.82, −5.53;

1-[2-(tert-Butyl-dimethyl-silanyloxymethyl)allyl ester] thymine ***10***

Thymine derivative **10** was synthesized from **6** by the similar procedure as described for **7**: yield 27% as a white solid; δ 8.74 (br s, 1H), 7.05 (s, 1H), 5.41 (s, 1H), 5.10 (s, 1H), 4.56 (s, 2H), 4.21 (s, 2H), 1.56 (s, 3H), 0.90 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 165.23, 152.76, 143.33, 141.90, 119.72, 106.45, 64.78, 48.99, 25.61, 18.58, 13.12, −5.34.

9-[2-(Hydroxymethyl)allyl ester] adenine ***11***

To a solution of **10** (250 mg, 0.78 mmol) in tetrahydrofuran (10 mL) tetrabutylammonium fluoride (1.17 mL, 1.0 M solution in THF) was added at 0°C and stirred for 5 h at room temperature. The reaction mixture was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1:6) to give **11** (129 mg, 81%) as a white solid; mp. 179−181°C; UV (H_2O) λ_{max} 262.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.15 (s, 1H), 8.05 (s, 1H), 7.08 (br s, 2H), 5.52 (s, 1H), 5.17 (s, 1H), 4.99 (s, 1H), 4.65 (s, 2H), 4.13 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 154.98, 153.24, 149.60, 145.92, 141.01, 118.51, 110.76, 64.11, 45.65; Anal. calcd. for $C_9H_{11}N_5O$: C, 52.67; H, 5.40; N, 34.13. Found: C, 52.90; H, 5.52; N, 34.10.

1-[2-(Hydroxymethyl)allyl ester] cytosine **12**

Cytosine nucleoside **12** was synthesized from **8** by the similar procedure as described for **11**: yield 70% as a white solid; mp. 168−170°C; UV (H_2O) λ_{max} 272.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.44 (d, J = 6.9 Hz, 1H), 6.98 (br d, 2H), 5.04 (s, 1H), 4.95 (t, J = 5.4 Hz, 1H), 4.68 (s, 1H), 4.22 (s, 2H), 3.84 (d, J = 5.1 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 165.87, 155.81, 146.20, 145.84, 113.78, 109.53, 62.67, 49.52; Anal. calcd. for $C_8H_{11}N_3O_2$: C, 53.03; H, 6.12; N, 23.19. Found: C, 52.91; H, 6.22; N, 23.11.

1-[2-(Hydroxymethyl)allyl ester] uracil ***13***

Uracil nucleoside **13** was synthesized from **9** by the similar procedure as described for **11**: yield 74% as a white solid; mp. 166−169°C; UV (H_2O) λ_{max} 262.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.25 (br s, 1H), 7.47 (d, J = 7.4 Hz, 1H), 5.73 (d, J = 7.4 Hz, 1H), 5.45 (s, 1H), 5.18 (s, 1H), 4.50 (s, 2H), 4.22 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 165.68, 152.24, 145.61, 143.83, 118.24, 102.90, 63.76, 49.28; Anal. calcd. for $C_8H_{10}N_2O_3$: C, 52.74; H, 5.53; N, 15.38. Found: C, 52.90; H, 5.68; N, 15.46.

1-[2-(Hydroxymethyl)allyl ester] thymine **14**

Thymine nucleoside **14** was synthesized from **10** by the similar procedure as described for **11**: yield 79% as a white solid; mp. 164−165°C; UV (H_2O) λ_{max} 266.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.69 (br s, 1H), 7.12 (s, 1H), 5.38 (s, 1H), 5.09 (s, 1H), 4.50 (s, 2H), 4.18 (s, 2H), 1.55 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 165.62, 153.87, 144.38, 141.45, 118.72, 103.26, 64.78, 49.12, 13.21; Anal. calcd. for $C_9H_{12}N_2O_3$: C, 55.09; H, 6.16; N, 14.28. Found: C, 54.91; H, 5.97; N, 14.11.

[2-(tert-Butyl-dimethyl-silanyloxymethyl)allyloxymethyl]-phosphonic acid diisopropyl ester ***15***

To a solution of **5** (2.14 g, 10.6 mmol) in 11 mL of DMF LiI (108 mg, 0.79 mmol) was added at 25°C. LiO*t*-Bu (17.1 mL of 1.0 M solution in THF, 17.1 mmol) and a solution of diisopropyl bromomethylphosphonate (3.75 g, 14.4 mmol) in 10 mL of DMF were slowly and simultaneously added to the reaction mixture for 5 h at 60°C under anhydrous condition. The mixture was quenched by adding water (80 mL), and the organic solvents (THF) were re-

moved *in vacuo*. The aqueous layer was extracted with EtOAc (3 × 150 mL). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:2) to give **15** (2.58 g, 64%) as a colorless syrup: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.11 (m, 2H), 4.71 (m, 2H), 4.53 (s, 2H), 4.19 (s, 2H), 3.73 (d, J = 7.8 Hz, 2H), 1.36 (m, 12H), 0.86 (s, 9H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 149.21, 112.35, 70.23, 66.32, 65.34, 64.50, 25.82, 23.45, 18.21, −5.56.

(2-Hydroxymethyl-allyloxymethyl)phosphonic acid diisopropyl ester ***16***

Compound **16** was prepared using a similar procedure as described for **11**: yield 80% as a colorless syrup; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.09 (m, 2H), 4.75 (m, 2H), 4.44 (s, 2H), 4.21 (s, 2H), 3.70 (d, J = 8.0 Hz, 2H), 1.35 (m, 12H); 13C NMR ($CDCl_3$) δ 146.45, 115.43, 69.23, 66.72, 65.56, 63.23, 23.67.

Methanesulfonic acid 2-(diisopropoxy-phosphorylmethoxymethyl)-allyl ester ***17***

Mesylate **17** was prepared from **16** by the procedure as described for **6**: yield 78% as a colorless syrup; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.12 (m, 2H), 4.77 (m, 2H), 4.61 (s, 2H), 4.33 (s, 2H), 3.73 (d, J = 8.2 Hz, 2H), 3.03 (s, 3H), 1.37 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 149.21, 118.21, 71.02, 67.56, 65.45, 63.89, 36.45, 23.81.

9-[2-(Diisopropoxy-phosphorylmethoxymethyl)allyl] adenine ***18***

Adenine derivative **18** was synthesized from **17** using the reaction condition as described for **7**: yield 35% as a yellow syrup; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.33 (s, 1H), 7.78 (s, 1H), 5.60 (s, 1H), 5.19 (s, 1H), 4.79 (s, 2H), 4.75 (m, 2H), 4.08 (s, 2H), 3.74 (d, J = 8.0 Hz, 2H), 1.36 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 155.38, 153.17, 143.16, 140.91, 70.57, 65.56, 64.11, 45.21, 23.81; Anal. calcd. for $C_{16}H_{26}N_5O_4P$: C, 50.13; H, 6.84; N, 18.27. Found: C, 49.97; H, 6.72; N, 18.11.

1-[2-(Diisopropoxy-phosphorylmethoxymethyl)allyl] cytosine ***19***

Cytosine derivative **19** was prepared from compound **17** using the method described for synthesizing compound **7**: yield 30% as a yellow syrup; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.32 (d, J = 6.9 Hz, 1H), 5.71 (d, J = 6.9 Hz, 1H), 5.25 (s, 1H), 4.98 (s, 1H), 4.70 (m, 2H), 4.43 (s, 2H), 4.12 (s, 2H), 3.74 (d, J = 7.9 Hz, 2H), 1.35 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 165.78, 155.89, 147.32, 146.81, 108.43, 95.45, 70.25, 65.87, 64.15, 50.64, 23.66; Anal. calcd. for $C_{15}H_{26}N_3O_5P$: C, 50.13; H, 7.29; N, 11.69. Found: C, 49.90; H, 7.12; N, 11.81.

1-[2-(Diisopropoxy-phosphorylmethoxymethyl)allyl] uracil ***20***

Uracil derivative **20** was prepared from compound **17** using the method described for synthesizing compound **7**: yield 28% as a yellow syrup; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.40 (br s, 1H), 7.17 (d, J = 7.8 Hz, 1H), 5.73 (d, J = 7.8 Hz, 1H), 5.36 (s, 1H), 5.18 (s, 1H), 4.72 (m, 2H), 4.57 (s, 2H), 4.40 (s, 2H), 3.71 (d, J = 7.8 Hz, 2H), 1.36 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 163.23, 151.70, 143.34, 141.25, 118.56, 102.56, 70.56, 66.21, 64.71, 49.71, 23.56; Anal. calcd. for $C_{15}H_{25}N_2O_6P$: C, 50.00; H, 6.99; N, 7.77. Found: C, 49.80; H, 7.13; N, 7.89.

1-[2-(Diisopropoxy-phosphorylmethoxymethyl)allyl] thymine ***21***

Thymine derivative **21** was prepared from compound **17** using the method described for synthesizing compound **7**: yield 25% as a yellow syrup; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.60 (br s, 1H), 6.96 (s, 1H), 5.34 (s, 1H), 5.17 (s, 1H), 4.71 (m, 2H), 4.57 (s, 2H), 4.19 (s, 2H), 3.72 (d, J = 7.8 Hz, 2H), 1.78 (s, 3H), 1.35 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 164.20, 151.81, 144.21, 142.67, 118.87, 108.12, 70.78, 65.28, 64.49, 49.44, 23.61, 12.98; Anal. calcd. for $C_{16}H_{27}N_2O_6P$: C, 51.33; H, 7.27; N, 7.48. Found: C, 51.41; H, 7.17; N, 7.61.

9-[2-(Hydroxymethyl)-allyloxymethylphosphonic acid] adenine ***22***

To a solution of the phosphonate **18** (150 mg, 0.39 mmol) in 10 mL of anhydrous methylene chloride $(CH_3)_3SiBr$ (0.567 g, 3.74 mmol) was added. The mixture was refluxed overnight and concentrated *in vacuo*. The residue was partitioned between distilled water and washed out by CH_2Cl_2. The aqueous layer was dried by freezer dryer to give **22** (92 mg, 79%) as a yellow solid: UV (H_2O) λ_{max} 261.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.11 (s, 1H), 8.07 (s, 1H), 5.07 (s, 1H), 5.02 (t, J = 5.4 Hz, 1H), 4.75 (s, 2H), 3.91 (d, J = 5.7 Hz, 2H), 3.74 (d, J = 8.2 Hz, 2H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ 155.97, 152.52, 149.58, 145.54, 141.03, 118.49, 110.63, 65.27, 61.97, 44.50.

1-[2-(Hydroxymethyl)-allyloxymethylphosphonic acid] cytosine ***23***

Cytosine nucleotide **23** was prepared from **19** using the method as described for **22**: yield 69% as a yellow solid; UV (H_2O) λ_{max} 271.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) d 7.46 (d, J = 6.9 Hz, 1H), 7.06 (br d, 2H), 5.64 (d, J = 6.9 Hz, 1H), 5.47 (s, 1H), 4.95 (t, J = 5.4 Hz, 1H), 4.68 (s, 1H), 4.22 (s, 2H), 3.86 (d, J = 5.1 Hz, 2H), 3.72 (d, J = 7.8 Hz, 2H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ 165.88, 155.80, 146.28, 145.84, 109.71, 93.48, 65.61, 62.05, 49.51.

1-[2-(Hydroxymethyl)-allyloxymethylphosphonic acid] uracil ***24***

Uracil nucleotide **24** was prepared from compound **20** using the method described for synthesizing compound **22**: yield 65% as a yellow solid; UV (H_2O) λ_{max} 261.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.40 (br s, 1H), 7.15 (d, J = 7.6 Hz, 1H), 5.70 (d, J = 7.6 Hz, 1H), 5.21 (s, 1H), 5.11 (s, 1H), 4.97 (t, J = 5.6 Hz, 1H), 4.21 (s, 2H), 3.85 (s, 2H), 3.71 (d, J = 8.0 Hz, 2H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ 163.65, 152.45, 142.89, 141.23, 110.43, 101.34, 65.48, 63.56, 49.78.

1-[2-(Hydroxymethyl)-allyloxymethylphosphonic acid] thymine **25**

Thymine nucleotide **25** was prepared from compound **21** using the method described for synthesizing compound **22**: yield 76% as a yellow solid; UV (H_2O) λ_{max} 267.5 nm ; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.12 (s, 1H), 5.24 (s, 1H), 5.12 (s, 1H), 4.99 (t, J = 5.4 Hz, 1H), 4.27 (s, 2H), 3.87 (s, 2H), 3.73 (d, J = 7.8 Hz, 2H), 2.02 (s, 3H); ^{13}C NMR (DMSO-d_6, 300 MHz) δ 164.67, 152.34, 144.56, 142.81, 113.57, 105.62, 65.27, 64.12, 49.67, 13.11.

Evaluation of anti-HCMV activity and cytotoxicity

The anti-HCMV activities and cytotoxicities were determined as described elsewhere [16].

References

[1] H. J. Schaeffer, L. Beauchamp, P. de Miranda, G. B. Elion, D. J. Bauer, *Nature* **1978**, *272*, 583−585.

[2] E. S. H. el Ashry, Y. El Kilany, Acylonucleosides: part 1−3 in *Advances in Heterocyclic Chemisty*, Academic Press **1997**, Vol. *67−69*.

[3] E. De Clercq, J. Descamps, P. de Somer, A. Holy, *Science* **1978**, *200*, 563−566.

[4] J. C. Martin, C. A. Dvorak, D. F. Smee, T. R. Mattews, J. P. Verheyden, *J. Med. Chem*. **1983**, *26*, 759−761.

[5] K. O. Smith, K. S. Galloway, W. L. Kennell, K. K. Oglivie, B. K. Radatus, *Antimicrob. Agents Chemother.* **1982**, *22*, 55−61.

[6] M. N. Arimilli, C. U. Kim, J. Dougherty, A. Mulato, R. Oliyai, J. P. Shaw, K. C. Cundy, N. Bischofberger, *Antivir. Chem. Chemother*. **1997**, *8*, 557−564.

[7] E. De Clercq, T. Sakuma, M. Baba, R. Pauwels, J. Balzarini, I. Rosenberg, A. Holy, *Antiviral Res.* **1987**, *8*, 262−272.

[8] B. L. Robbins, R. V. Srinivas, N. Bischofberger, A. Fridland, *Antimicrob. Agents Chemother*. **1998**, *42*, 612−617.

[9] S. Noble, K. L. Goa, *Drugs*, **1999**, *58*, 479−487.

[10] J. E. Starrett, D. R. Tortolani, J. Russell, M. J. M. Hitchcock, V. Whiterock, J. C. Martin, M. M. Mansuri, *J. Med. Chem*. **1994**, *37*, 1857−1864.

[11] N. Bischofberger, R. J. Jones, *Antiviral Res.* **1995**, *27*, 1−17.

[12] S. Levine, D. Hernandez, G. Yamanaka, G. S. Zhang, R. Rose, S. Weinheimer, R. J. Colonno, *Antimicrob. Agents Chemother*. **2002**, *46*, 2525−2532.

[13] G. S. Jeon, V. Nair, *Tetrahedron*, **1996**, *52*, 12643−12650.

[14] R. P. Panzica, R. J. Rousseau, R. K. Robins, L. B. Townsend, *J. Am. Chem. Soc*. **1972**, *94*, 4708−4714.

[15] H. I. El Subbagh, S. Racha, E. Abushanab, R. P. Panzica, *J. Org. Chem*. **1996,** *61*, 890−894.

[16] O. H. Ko, J. H. Hong, *Arch. Pharm. Pharm. Med. Chem*. **2004**, *337*, 579−586.

