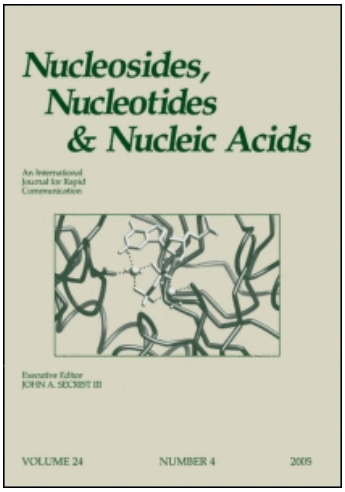

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Hirofumi Furusho[a]; Takahiro Ogata[a]; Akihisa Kato[a]; Yoshie Sato[a]; Tohru Endo[a]; Akira Kaji[b]
[a] Research Institute for Molecular Genetics, TSUMURA &CO., Ibaraki, Japan [b] Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

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SYNTHESIS AND ANTIHERPETIC ACTIVITIES OF SEVERAL ACYCLIC ANALOGUES OF GUANOSINE

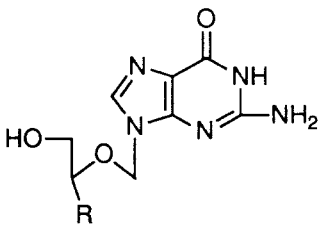
Hirofumi Furusho[a], Takahiro Ogata[a], Akihisa Kato[a], Yoshie Sato[a], Tohru Endo[a], and Akira Kaji[b*]

[a]Research Institute for Molecular Genetics, TSUMURA & CO., 3586 Yoshiwara, Ami-Machi, Inashiki-Gun, Ibaraki 300-11, Japan, and [b]Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104-6076, USA.

Abstract: Several acyclic analogues of guanosine, 2'-deoxy-2',3'-secoguanosine (**3**), 3'-deoxy-2',3'-secoguanosine (**4**), and 2',3'-dideoxy-2',3'-secoguanosine (**5**) were synthesized from guanosine. In addition, the 3',5'-cyclic phosphate (**21**) and 3',5'-cyclic methylphosphonates (**22a,b**) of **3** were also prepared. At concentrations up to 300 μM none of these compounds had significant antiherpetic activity in antiviral assays in vitro.

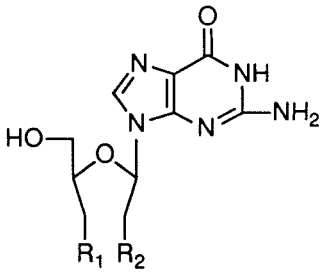
Among the acyclic analogues of guanosine, 9-(2-hydroxyethoxymethyl) guanine (**1**, acyclovir)[1,2] and 9-(1,3-dihydroxy-2-propoxymethyl)guanine (**2**, DHPG)[3-8] are compounds that have established antiherpetic activity. The selective toxicity of acyclovir to herpes viruses is based on the specific action against the viral enzymes related to nucleic acid synthesis.[1] DHPG is known to be effective against cytomegalovirus, a virus that acyclovir has only slight activity against. Because of the dramatic antiherpetic activities of these compounds, various analogues of this type have been synthesized.[9,10] However, none of these analogues has proved to be a better antiherpetic agent than acyclovir or DHPG. In addition, 2',3'-Secoguanosine[11-15] showed no appreciable antiherpetic activity, despite the fact that this compound resembles guanosine more closely than DHPG. Although 2',3'-seconucleoside analogues have been known for three decades,[12] there is renewed interest in them as potential antiviral or antitumor agents.[13-19]

To study the effect that alkyl groups at the C-1' or C-4' position of 2',3'-secoguanosine have on antiherpetic activities, we have synthesized several acyclic analogues of guanosine, **3**, **4**, and **5**.



1: R=H

2: R=CH_2OH



3: R_1=OH , R_2=H

4: R_1=H , R_2=OH

5: R_1=R_2=H

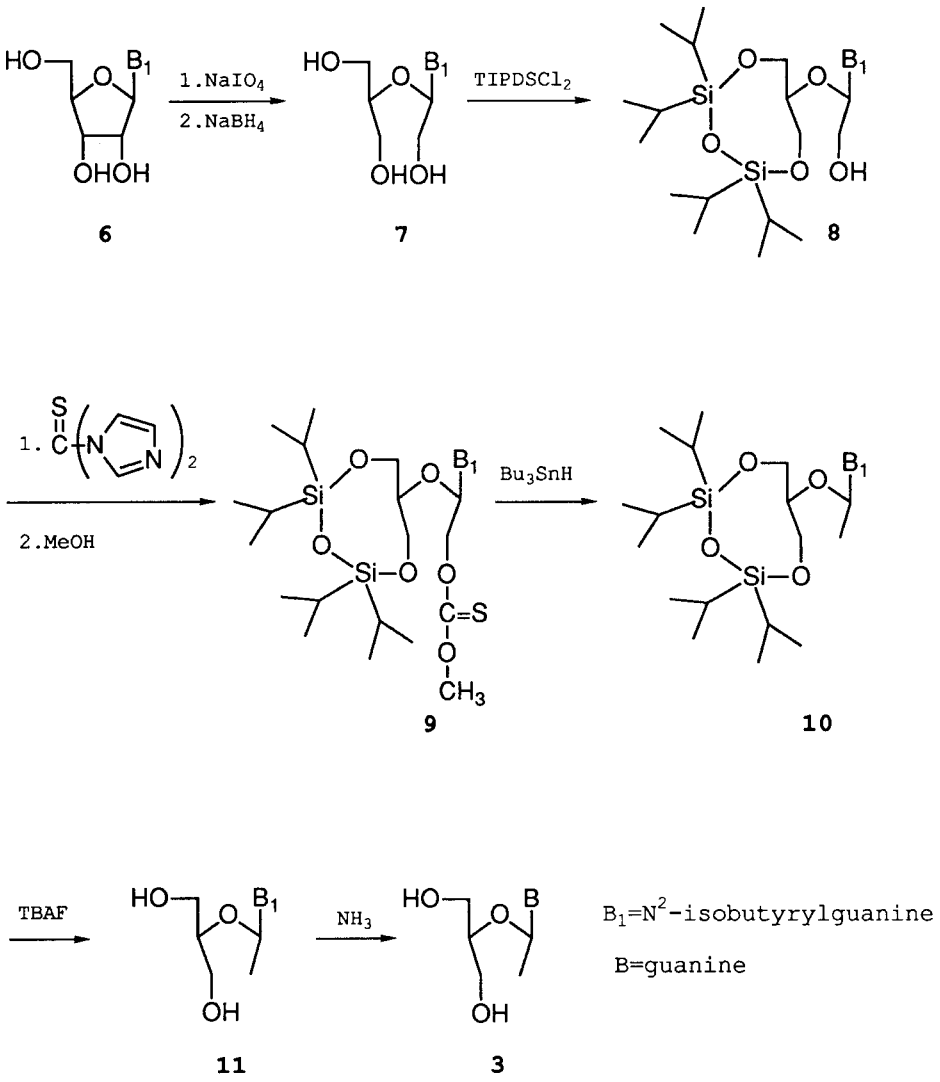
To avoid the problem with chirality at the C-1' and C-4' positions, naturally occurring guanosine was used as the starting material.

It is well known that both c-AMP and c-GMP play very important roles in the metabolic control of various organisms.[20] It appears that c-AMP and c-GMP may play opposite roles in these control mechanisms. It has also been reported that 2'-nor-cGMP,which is the 3',5'-cyclic phosphate of DHPG,has a wide spectrum of anti-DNA-virus activities.[21] However,the effectiveness of 2'-nor-cGMP may not be related to the physiological role of c-AMP or c-GMP. The exact mechanism of the selective toxicity of 2'-nor-cGMP toward the DNA viruses is not well understood.

In this paper,we also report the syntheses of phosphate analogue **21** and methylphosphonate analogues **22a,b** of **3**. We attempted to synthesize a better antiviral agent by introducing a methylphosphonyl group instead of phosphoryl group with the hope that a methylphosphonyl group would increase the permeability of **22a,b** into cells.

CHEMISTRY. The target compound **3** was synthesized via selective protection of 3'- and 5'-hydroxy groups of N^2-isobutyryl-2',3'-secoguanosine(**7**) with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TIPDSCl_2$) as outlined in Scheme I. It was previously reported by Jones et al.[17] that selective protection of the 3'- and 5'-hydroxy groups of 2',3'-secouridine was achieved using an isopropylidene group. Since isopropylidene group requires acidic conditions for its removal,this method did not appear to be suitable for the syntheses of 2',3'-secoguanosine analogues which may be relatively unstable under acidic

Scheme I



conditions. On the other hand, TIPDS seemed to be suitable as a protecting group because it is easily removed by TBAF under mild conditions.[22] The selective nature of this protecting group towards 3'- and 5'-hydroxy groups in the presence of 2'-hydroxy group was supported by the finding that no 2',3'-protected or 2',5'-protected isomers were detected in the reaction mixture for the conversion of **7** to 3',5'-O-silylated derivative **8**. It should be pointed out that a similar

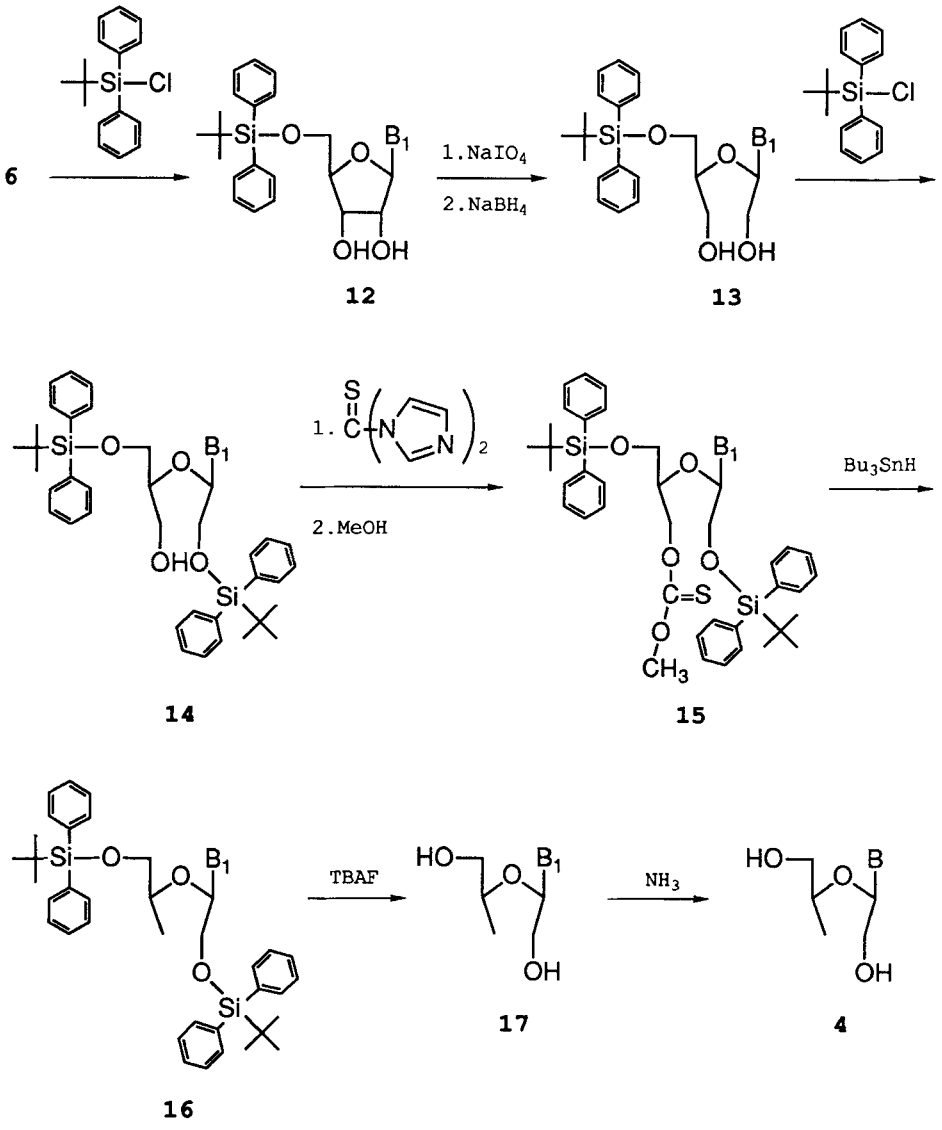
selectivity was not observed with a cytidine analogue in a separate experiment. Thus, N^4-benzoyl-2',3'-secocytidine gave a complex mixture of products when reacted with $TIPDSCl_2$ under similar conditions (data not shown). These results suggest that the selectivity of $TIPDSCl_2$ to 3'- and 5'-hydroxy groups is influenced by the nucleoside base. Similar influence of the base group on the reactivity of 2'-hydroxy group was suggested by Beaton et al..[18] It appears that there is steric hindrance at C-2' of the purine 2',3'-seconucleoside analogues.

Compound **4** was synthesized via 2',5'-bis-O-silylated alcohol **14** as outlined in Scheme II. In order to synthesize **4** it was necessary to protect the 2'-hydroxy group of diol **13** selectively. This selective protection was accomplished with *t*-butyldiphenylsilyl groups.[23] It was not unreasonable to expect that because of the bulkiness of *t*-butyldiphenylsilyl group at the 5'-position of **13**, the 3'-hydroxy group of **13** would react more slowly with a second molecule of *t*-butyldiphenylsilyl chloride than the 2'-hydroxy group. In fact, the 3',5'-bis-O-silylated compound was not isolated, but **14** and the 2',3',5'-tris-O-silylated compound were obtained in 15% and 28% yield, respectively, suggesting that the reaction of **13** with *t*-butyldiphenylsilyl chloride proceeded first at the 2'-position followed by the reaction at the 3'-position.

Compound **5** was synthesized via bis-methylthionocarbonate **18** as outlined in Scheme III. During the synthesis of 2',3'-dideoxy compound **19**, two partially deoxygenated compounds (**19a** and **19b**), **13**, and other unidentified products were formed as by-products. Preparation of **5** from **19** was accomplished by desilylation with TBAF followed by ammonolysis in 5% overall yield from **18**. Deprotection of **19a** and **19b** in the same manner as **19** gave **3** (2.4% from **18**) and **4** (4.9% from **18**), respectively.

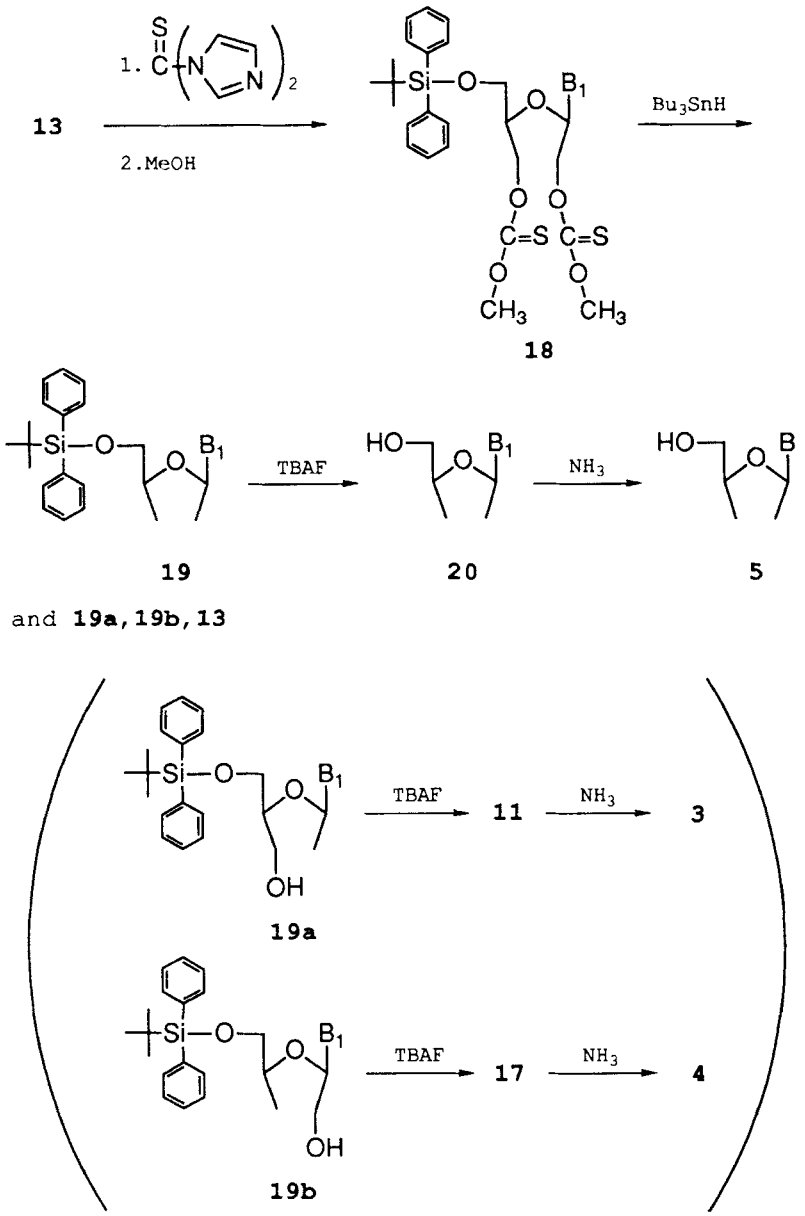
Compounds **21** and **22a,b** were synthesized as outlined in Scheme IV. Tolman et al.[21] reported the synthesis of 2'-nor-cGMP by the reaction of DHPG with phosphorus oxychloride in 7% yield. Alternatively, Marugg et al. used 2-chlorophenyl-O,O-bis(1-benzotriazolyl)phosphate[24] for the synthesis of oligodeoxyribonucleotides. Since the yield of the phosphorylation reaction in the latter case was far better than in the former, the latter method was adopted for the synthesis of **21**. Compound **21** was obtained in good overall yield (69%) after deprotection. Similarly, compounds **22a,b** were synthesized in good yield (75%). In this reaction, it was important to use a low concentration of both **3** and methyl-O,O-bis(1-benzotriazolyl)phosphonate to avoid condensing two molecules of **3** with the phosphorylating agent. This phosphorylating reagent had been used by Marugg et al. for the synthesis of uridine-3',5'-cyclic methylphosphonate.[24] Interestingly enough, only one diastereomer was obtained under their conditions. In support of their result, a separate experiment in our laboratory involving adenosine and

Scheme II



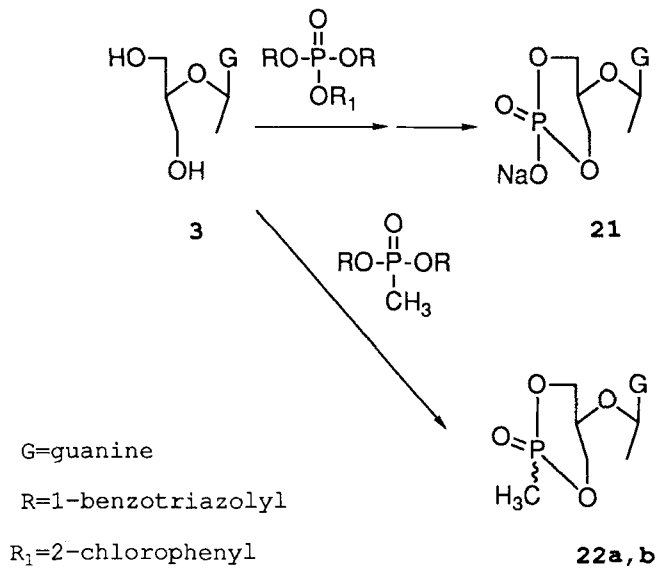
methyl-O,O-bis(1-benzotriazolyl)phosphonate yielded only one diastereomer(data not shown). In contrast,two diastereomers were obtained in the reaction of **3** with this phosphorylating reagent. It appears that phosphorylations with methyl-O,O-bis(1-benzotriazolyl) phosphonate proceed with less stereoselectivity in the case of acyclic nucleosides.

Scheme III



ANTIVIRAL TESTING. The novel series of acyclic analogues of guanosine, **3**, **4**, **5**, **21**, and **22a,b** were tested against herpes simplex viruses type 1(HSV-1) and type 2(HSV-2) in cell culture(Vero cells) and found to be inactive up to 300 μM. None of these compounds were toxic

Scheme IV



for the cell monolayers up to 300 μM. It has already been reported that 2',3'-secoguanosine and analogues having an alkyl group at the 1'-position were inactive against herpes viruses.[14,15] 5'-Deoxy-2',3'-secoguanosine,which is a diastereomer of compound **4** with respect to C-4',was synthesized by McGee and Martin[14] and reported to be inactive against HSV-1 and HSV-2 in vitro. After we completed the syntheses of **3,4,5,21,**and **22a,b,**Kalman and Houston independently reported the synthesis of **3** without experimental detail. Their results also showed that compound **3** was inactive against HSV-1 and HSV-2 in vitro.[25] These results imply that in these types of acyclic guanosine analogues,the presence of an alkyl group at the 1'-position renders these compounds completely inactive as antiherpetic agents. We are currently studying the exact mechanism by which the alkyl group at the 1'-position effectively abolishes the antiherpetic activity.

EXPERIMENTAL SECTION

General Methods. Melting points(uncorrected) were determined by a Yanagimoto MP-500D micro melting point apparatus. ^{1}H and ^{13}C NMR spectra were recorded by a Bruker AM-500 or JEOL FX-90Q instrument using tetramethylsilane as an internal reference. ^{31}P NMR spectra were recorded by a Bruker AM-500 using 85% D_3PO_4 as an external reference. Chemical shifts are reported as δ(ppm) and the signal splitting is

described as s(singlet),d(doublet),t(triplet),q(quartet),m(multiplet), and b(broad). Positive ion fast atom bombardment(FAB) mass spectra were obtained by a JEOL DX-300 mass spectrometer. Flash column chromatography was carried out with silica gel(Kieselgel 60 type 9385, 230-400 mesh ASTM,Merck). Thin layer chromatography was performed with Merck Kieselgel 60 F254 analytical sheets. The UV spectra were measured in water by a Shimadzu UV-260 spectrophotometer. Specific rotations were measured with a JASCO DIP-360 digital polarimeter. Preparative HPLC was carried out by a Shimadzu LC-8A with reverse phase columns of 5 cm or 10 cm diameter.

N^2-Isobutyrylguanosine(6). This compound was prepared from guanosine and isobutyryl chloride in 78% yield according to the published procedure.[26] The synthesized compound had a melting point of 146-148°C(lit.[26] 146-148°C).

N^2-Isobutyryl-2',3'-secoguanosine(7).[27] A mixture of **6** (17.67 g,50 mmol),$NaIO_4$(18.91 g,88.4 mmol),and water(884 mL) was stirred for 1.5 h at room temperature. Ethylene glycol(1 M,53 mL) was added and stirring was continued for 30 min. The volume of the solution was adjusted to 2 L by the addition of water,followed by the addition of $NaBH_4$(17.67 g,467 mmol) and stirring was continued for 10 min at 0°C. Treatment with $NaBH_4$ for more than 10 min under these conditions accelerated the formation of 2',3'-secoguanosine. The solution was mixed with 1 M HCOOH(884 mL) and stirred for 1 h to destroy excess $NaBH_4$. The solution was neutralized with 1 M NH_4OH and dried in vacuo. The resulting residue was subjected to HPLC(5%-40% CH_3CN in H_2O,linear gradient) to give **7**(7.6 g,43%). ^1H NMR(CD_3OD) δ 8.15(s,1,C(8)H),5.97 (t,1,J=5.4 Hz,C(1')H),4.00(d,2,J=5.2 Hz,2'-CH_2),3.90-3.30(m,5, C(3',4',5')H),2.90-2.60(m,1,CH),1.23(d,6,J=6.8 Hz,isobutyryl methyl); FAB-MS m/z 356$(M+1)^+$.

N^2-Isobutyryl-3',5'-O-TIPDS-2',3'-secoguanosine(8). A mixture of **7**(4.05 g,11.4 mmol),$TIPDSCl_2$(3.95 mL,12.5 mmol),and dry pyridine(50 mL) was stirred for 4 h at -30°C under an argon atmosphere. The reaction mixture was partitioned between chloroform and water. The organic layer was evaporated under reduced pressure and the resulting residue was applied to a column of silica gel. Compound **8**(3.16 g,46%) was eluted with chloroform-methanol(20:1). ^1H NMR($CDCl_3$) δ 12.00(s,1, NH),9.52(s,1,NH),7.84(s,1,C(8)H),5.68(t,1,J=5.5 Hz,C(1')H),4.16-3.36 (m,7,C(2',3',4',5')H),2.96-2.56(m,1,CH),1.27(d,6,J=6.8 Hz,isobutyryl methyl),1.06-0.97(m,28,isopropyl); FAB-MS m/z 598$(M+1)^+$.

N^2-Isobutyryl-2'-O-methoxythiocarbonyl-3',5'-O-TIPDS-2',3'-secoguanosine(9). A mixture of **8**(4.75 g,7.9 mmol),1,1'-thiocarbonyl diimidazole[28](2.13 g,11.9 mmol),and dry DMF(47 mL) was stirred for 2 days at room temperature under an argon atmosphere. The solvent was evaporated in vacuo,dry methanol(47 mL) was added to the residue,and the mixture was stirred for 2 h at 60°C. The reaction mixture was partitioned between chloroform and water. The organic layer was evaporated under reduced pressure and the resulting residue was applied to a column of silica gel. Elution with chloroform-methanol (50:1) gave **9**(4.0 g,75%). ^{1}H NMR($CDCl_3$) δ 12.17(s,1,NH),9.22(s,1,NH), 7.89(s,1,C(8)H),6.02(t,1,J=5.6 Hz,C(1')H),4.78(d,2,J=5.5 Hz,2'CH_2), 3.99(s,3,OCH_3),4.08-3.44(m,5,C(3',4',5')H),2.96-2.56(m,1,CH),1.28 (d,6,J=6.8 Hz,isobutyryl methyl),1.20-0.88(m,28,isopropyl); FD-MS m/z 672$(M+1)^+$.

2'-Deoxy-N^2-isobutyryl-3',5'-O-TIPDS-2',3'-secoguanosine(10). A mixture of **9**(2.3 g,3.4 mmol),AIBN(561 mg,3.4 mmol),Bu_3SnH(4.6 mL,17.1 mmol),and dry dioxane(85 mL) was refluxed with stirring for 1.5 h under an argon atmosphere.[29] The reaction mixture was partitioned between chloroform and water. The organic layer was evaporated in vacuo and the resulting residue was applied to a column of silica gel. Compound **10**(1.13 g,57%) was eluted with chloroform-methanol(50:1). ^{1}H NMR($CDCl_3$) δ 12.12(bs,1,NH),9.20(bs,1,NH),7.90(s,1, C(8)H),5.83(q,1,J=5.7 Hz,C(1')H),4.08-3.44(m,5,C(3',4',5')H),2.96-2.48 (m,1,CH),1.71(d,3,J=5.7 Hz,2'-CH_3),1.27(d,6,J=6.8 Hz,isobutyryl methyl),1.20-0.80(m,28,isopropyl); FD-MS m/z 582$(M+1)^+$.

2'-Deoxy-N^2-isobutyryl-2',3'-secoguanosine(11). Compound **10**(2.3 g,3.97 mmol) was added to 1 M TBAF in THF(52 mL) and the solution was stirred for 10 min at room temperature. The reaction mixture was evaporated under reduced pressure and the resulting residue was subjected to reverse phase HPLC(5%-40% CH_3CN in H_2O,linear gradient) to give **11**(0.74 g,63%). ^{1}H NMR($CDCl_3$) δ 12.08(bs,1,NH),10.56 (bs,1,NH),8.06(s,1,C(8)H),6.11(q,1,J=5.9 Hz,C(1')H),3.92-3.28(m,5, C(3',4',5')H),2.88-2.64(m,1,CH),1.75(d,3,J=6.2 Hz,2'-CH_3),1.25(d,6, J=6.8 Hz,isobutyryl methyl); FAB-MS m/z 340$(M+1)^+$.

2'-Deoxy-2',3'-secoguanosine(3). A mixture of **11**(700 mg,2.06 mmol),28% NH_3(35 mL),and pyridine(35 mL) was stirred for 3 days at room temperature. The reaction mixture was evaporated in vacuo and the resulting residue was subjected to reverse phase HPLC(5%-40% CH_3CN in H_2O,linear gradient) to give **3**(0.54 g,97%). ^{1}H NMR(CD_3OD) δ 7.94(s,1, C(8)H),6.01(q,1,J=6.1 Hz,C(1')H),3.74(dd,1,J=4.3 Hz,11.8 Hz,CH_2),3.62 (dd,1,J=5.6 Hz,11.8 Hz,CH_2),3.54-3.50(m,1,C(4')H),3.40-3.38(m,2,CH_2),

1.72(d,3,J=6.1 Hz,CH_3); ^{13}C NMR(CD_3OD) δ 159.40(6),155.47(2),152.85(4), 137.24(8),117.50(5),81.39(1'),80.50(4'),62.70(CH_2),62.34(CH_2),22.20(2'); FAB-MS m/z 270(M+1)$^+$; mp 181 °C dec; UV λmax(H_2O)252 nm(ε 1.20 x 10^4); $[\alpha]^{27}_D$ 12.27°(c 1.33,H_2O); Anal. Calcd. for $C_{10}H_{15}N_5O_4 \cdot H_2O$: C, 41.81; H, 5.96; N, 24.38. Found: C, 41.91; H, 6.13; N, 24.17.

N^2-Isobutyryl-5'-O-*t*-butyldiphenylsilylguanosine(12). A mixture of **6**(17.66 g,50 mmol),imidazole(13.62 g,200 mmol), *t*-butyldiphenylsilyl chloride(39 mL,150 mmol),and dry DMF(140 mL) was stirred for 3 days at room temperature under an argon atmosphere. The reaction mixture was partitioned between chloroform and water. The organic layer was evaporated under reduced pressure and the resulting residue was applied to a column of silica gel. Elution with chloroform-methanol(20:1) gave **12**(14.0 g,47%). ^1H NMR($CDCl_3$) δ 12.47(s,1,NH),11.20 (s,1,NH),7.95(s,1,C(8)H),7.76-7.20(m,10,C_6H_5),5.96(d,1,J=4.4 Hz, C(1')H),4.88-3.68(m,5,C(2',3',4',5')H),3.28-2.80(m,1,CH),1.22(d,6,J=6.6 Hz,isobutyryl methyl),0.97(s,9,*t*-butyl); FAB-MS m/z 592(M+1)$^+$.

N^2-Isobutyryl-5'-O-*t*-butyldiphenylsilyl-2',3'-secoguanosine(13). A mixture of **12**(8.88 g,15 mmol),$NaIO_4$(5.69 g, 26.6 mmol),18-crown-6(7.02 g,26.6 mmol),MeOH(900 mL),and H_2O(100 mL) was stirred for 2 h at room temperature. Ethylene glycol(1 M,15.8 mL) was added and the mixture was stirred for 30 min. The solution was cooled in an ice bath,$NaBH_4$(5.1 g,135 mmol) was added,and the solution was stirred for 5 min. To destroy excess $NaBH_4$,1 M HCOOH(255 mL) was added to the solution and stirring was continued for 1 h at 0°C. The reaction mixture was neutralized with 1 M NH_4OH and evaporated in vacuo. The resulting residue was subjected to reverse phase HPLC(30%-100% CH_3CN in H_2O,linear gradient) to give **13**(6.2 g,70%). ^1H NMR (CD_3OD) δ 8.02(s,1,C(8)H),7.56-7.16(m,10,C_6H_5),5.89(t,1,J=5.7 Hz, C(1')H),4.08-3.24(m,7,C(2',3',4',5')H),2.88-2.52(m,1,CH),1.21(d,6, J=6.8 Hz,isobutyryl methyl),0.90(s,9,*t*-butyl); FAB-MS m/z 594(M+1)$^+$.

2',5'-O,O-Bis(*t*-butyldiphenylsilyl)-N^2-isobutyryl-2',3'-secoguanosine(14). A mixture of **13**(594 mg,1 mmol),imidazole(272 mg, 4 mmol),*t*-butyldiphenylsilyl chloride(312 μL,1.2 mmol),and dry DMF(10 mL) was stirred for 2 days at room temperature under an argon atmosphere. The reaction mixture was partitioned between chloroform and water. The organic layer was evaporated under reduced pressure and the resulting residue was applied to a column of silica gel. Compound **14** (128 mg,15%) was eluted with chloroform-methanol(30:1). As by-products, 2',3',5'-tris-O-silylated compound(299 mg,28%) and depurinated product (127 mg) were obtained(data not shown),however 3',5'-bis-O-silylated compound was not isolated. ^1H NMR($CDCl_3$) δ 12.21(s,1,NH),10.14(s,1,NH),

7.80(s,1,C(8)H),7.68-7.12(m,20,C_6H_5),6.16(t,1,J=5.7 Hz,C(1')H), 4.24-3.44(m,7,C(2',3',4',5')H),2.96-2.56(m,1,CH),1.21(d,6,J=6.8 Hz, isobutyryl methyl),0.93(s,18,*t*-butyl); FAB-MS m/z 833$(M+1)^+$.

2',5'-O,O-Bis(*t*-butyldiphenylsilyl)-N^2-isobutyryl-3'-O-methoxythiocarbonyl-2',3'-secoguanosine(15). A mixture of **14** (128 mg,0.154 mmol),1,1'-thiocarbonyldiimidazole(41 mg,0.231 mmol),and dry DMF(1 mL) was stirred for 2 days at room temperature under an argon atmosphere. The reaction mixture was evaporated in vacuo. Dry methanol (1 mL) was added to the residue and stirring was continued for 2 h at 60°C. The reaction mixture was partitioned between chloroform and water. The organic layer was evaporated under reduced pressure and the resulting residue was applied to a column of silica gel. Elution with chloroform-methanol(100:1) gave **15**(87 mg,62%). ^{1}H NMR($CDCl_3$) δ 11.99 (s,1,NH),8.84(s,1,NH),7.73(s,1,C(8)H),7.68-7.12(m,20,C_6H_5),5.82(t,1, J=5.7 Hz,C(1')H),4.96-4.40(m,2,3'-CH_2),4.01(s,3,OCH_3),4.08-3.44(m,5, C(2',4',5')H),2.48-2.44(m,1,CH),1.23(d,6,J=6.8 Hz,isobutyryl methyl), 0.96(s,9,*t*-butyl),0.92(s,9H,*t*-butyl); FD-MS m/z 907$(M+1)^+$.

2',5'-O,O-Bis(*t*-butyldiphenylsilyl)-3'-deoxy-N^2-isobutyryl-2',3'-secoguanosine(16). A mixture of **15**(86 mg,0.095 mmol),AIBN(16 mg,0.095 mmol),Bu_3SnH(128 μL,0.48 mmol),and dry dioxane (3 mL) was refluxed with stirring for 2 h under an argon atmosphere. The reaction mixture was partitioned between chloroform and water. The organic layer was evaporated in vacuo and the resulting residue was applied to a column of silica gel. Compound **16**(33 mg,43%) was eluted with chloroform-methanol(100:1). ^{1}H NMR($CDCl_3$) δ 11.88(s,1,NH),8.28(s, 1,NH),7.81(s,1,C(8)H),7.68-7.12(m,20,C_6H_5),5.68(t,1,J=5.7 Hz,C(1')H), 4.08-3.04(m,5,C(2',4',5')H),2.72-2.32(m,1,CH),1.22(d,6,J=6.8 Hz, isobutyryl methyl),1.21(d,3,J=6.8 Hz,3'-CH_3),0.96(s,9,*t*-butyl),0.92 (s,9,*t*-butyl); FD-MS m/z 817$(M+1)^+$.

3'-Deoxy-N^2-isobutyryl-2',3'-secoguanosine(17). Compound **16**(30 mg,0.037 mmol) was added to 1 M TBAF in THF(3 mL) and the solution was stirred for 10 min at room temperature. The reaction mixture was evaporated under reduced pressure and the resulting residue was subjected to reverse phase HPLC(5%-40% CH_3CN in H_2O,linear gradient) to give **17**(6.8 mg,50%). ^{1}H NMR($CDCl_3$) δ 12.24(s,1,NH),10.68 (s,1,NH),8.21(s,1,C(8)H),5.78(t,1,J=5.1 Hz,C(1')H),4.16-3.20(m,5, C(2',4',5')H),3.20-2.72(m,1,CH),1.25(d,6,J=6.8 Hz,isobutyryl methyl), 1.18(d,3,J=6.8 Hz,3'-CH_3); FAB-MS m/z 340$(M+1)^+$.

3'-Deoxy-2',3'-secoguanosine(4). A mixture of **17**(6.7 mg, 0.020 mmol),28% NH_3(0.5 mL),and pyridine(0.5 mL) was stirred for 3

days at room temperature. The reaction mixture was evaporated in vacuo and the resulting residue was subjected to reverse phase HPLC(5%-40% CH_3CN in H_2O,linear gradient) to give **4**(4.4 mg,83%). ^1H NMR(CD_3OD) δ 7.92(s,1,C(8)H),5.75(t,1,J=5.3 Hz,C(1')H),3.88(d,2,J=5.3 Hz,2'-CH_2), 3.55-3.51(m,1,C(4')H),3.41(dd,1,J=11.7 Hz,6.4 Hz,C(5')H),3.37(dd,1, J=11.7 Hz,4.4 Hz,C(5')H),1.21(d,3,J=6.2 Hz,3'-CH_3); ^{13}C NMR(CD_3OD) δ 159.40(6),155.50(2),153.46(4),138.02(8),117.31(5),82.83(1'),75.78(4'), 66.63(CH_2),64.49(CH_2),15.73(3'); FAB-MS m/z 270$(M+1)^+$; mp 241 °C dec; $[\alpha]^{26}_D$ 44.4°(c 0.33,H_2O); Anal. Calcd. for $C_{10}H_{15}N_5O_4 \cdot H_2O$: C, 41.81; H, 5.96; N, 24.38. Found: C, 41.87; H, 5.70; N, 24.29.

2',3'-O,O-Bis(methoxythiocarbonyl)-N^2-isobutyryl-5'-O-t-butyldiphenylsilyl-2',3'-secoguanosine(18). A mixture of **13** (2.97 g,5 mmol),1,1'-thiocarbonyldiimidazole(4.46 g,25 mmol),and dry DMF(17 mL) was stirred for 2 days at 60°C under an argon atmosphere. The reaction mixture was evaporated under reduced pressure. Dry methanol(50 mL) was added to the residue and stirring was continued for 2.5 h at room temperature. The reaction mixture was partitioned between chloroform and water. The organic layer was evaporated in vacuo and the resulting residue was applied to a column of silica gel. Elution with chloroform-methanol(100:1) gave **18**(1.37 g,37%). ^1H NMR($CDCl_3$) δ 12.26 (s,1,NH),10.74(s,1,NH),7.75(s,1,C(8)H),7.68-7.20(m,10,C_6H_5),6.11(t,1, J=5.5 Hz,C(1')H),4.96-4.56(m,4,C(2',3')H),4.04(s,3,OCH_3),3.97(s,3, OCH_3),3.92-3.36(m,3,C(4',5')H),3.04-2.64(m,1,CH),1.23(d,6,J=5.9 Hz, isobutyryl methyl),0.97(s,9,*t*-butyl); FD-MS m/z 742$(M+1)^+$.

2',3'-Dideoxy-2',3'-secoguanosine(5). A mixture of **18**(1.98 g,2.67 mmol),AIBN(877 mg,5.34 mmol),Bu_3SnH(7.18 mL,26.7 mmol),and dry dioxane(60 mL) was refluxed with stirring for 1 h under an argon atmosphere. The reaction mixture was partitioned between chloroform and water. The organic layer was evaporated under reduced pressure and the resulting residue was applied to a column of silica gel. An oily mixture(1.04 g) of 2',3'-dideoxy-N^2-isobutyryl-5'-O-*t*-butyldiphenylsilyl-2',3'-secoguanosine(**19**) and **19a** was eluted with chloroform-methanol(50:1). Further elution with chloroform-methanol (20:1) gave oily **19b**(0.31 g).

An oily mixture(1.04 g) of **19** and **19a** was added to 1 M TBAF in THF(20 mL) and the solution was stirred for 20 min at room temperature. The reaction mixture was evaporated in vacuo and the resulting residue was subjected to reverse phase HPLC(10%-45% CH_3CN in H_2O,linear gradient) to give oily 2',3'-dideoxy-N^2-isobutyryl-2',3'-secoguanosine (**20**)(455 mg) and oily **11**. Oily **19b** was desilylated by the same manner as **19** to give oily **17**.

A mixture of crude **20**(455 mg),28% NH_3(20 mL),and pyridine(20 ml) was stirred for 3 days at room temperature. The reaction mixture was evaporated under reduced pressure and the resulting residue was subjected to reverse phase HPLC(10%-45% CH_3CN in H_2O,linear gradient) to give **5**(36 mg,5% yield from **18**). Ammonolysis of oily **11** and **17** by the same manner as **20** gave **3**(2.4% yield from **18**) and **4**(4.9% yield from **18**),respectively. ^1H NMR(CD_3OD) δ 7.94(s,1,C(8)H),5.89(q,1,J=6.0 Hz, C(1')H),3.51-3.44(m,1,C(4')H),3.38-3.32(m,2,5'-CH_2),1.68(d,3,J=6.1 Hz, 2'-CH_3),1.18(d,3,J=6.2 Hz,3'-CH_3); ^{13}C NMR(CD_3OD) δ 159.40(6), 155.49(2),152.92(4),137.23(8),117.40(5),79.53(1'),75.36(4'),66.70(5'), 22.60(2'),15.97(3'); FAB-MS m/z 254$(M+1)^+$; mp 224 °C dec; $[\alpha]^{26}_D$ 24.1° (c 0.15,H_2O); Anal. Calcd. for $C_{10}H_{15}N_5O_3 \cdot 1.7H_2O$: C, 42.31; H, 6.53; N, 24.67. Found: C, 42.54; H, 6.44, N, 24.40.

2'-Deoxy-2',3'-secoguanosine-3',5'-cyclic phosphate(21). Compound **3**(50 mg,0.19 mmol) was added to a mixture of 2-chlorophenyl-O,O-bis(1-benzotriazolyl)phosphate(97.8 mg,0.22 mmol) and dry dioxane (5 mL) and the solution was stirred for 30 min at room temperature. The reaction mixture was partitioned between chloroform and water. The organic layer was evaporated to dryness in vacuo. The resulting residue was added to a mixture of syn-pyridine-2-aldoxime(611 mg,5 mmol), N^1,N^1,N^3,N^3-tetramethylguanidine(518 mg,4.5 mmol),dry dioxane(5 mL),and dry CH_3CN(5 mL). The solution was stirred for 2 days at room temperature. Dowex 50W cation-exchange resin(1 g,H^+ form) was added to the reaction mixture and it was shaken vigorously for 1 min. The resin was removed by filtration and the filtrate was evaporated under reduced pressure. The resulting residue was subjected to reverse phase HPLC(3%-23% CH_3CN in 0.05 M triethylammonium acetate,linear gradient) followed by treatment with sodium perchlorate to give **21**(Na salt; 45.3 mg,69%). ^1H NMR(CD_3OD) δ 7.95(s,1,C(8)H),5.96(q,1,J=6.1 Hz,C(1')H),4.43-4.31 (m,2,CH_2),4.14-4.09(m,1,CH_2),3.91-3.84(m,1,CH_2),3.54-3.52(m,1,C(4')H), 1.75(d,3,J=6.1 Hz,2'-CH_3); ^{31}P NMR(D_2O/85% D_3PO_4) δ 1.89; FAB-MS m/z 354$(M+1)^+$.

2'-Deoxy-2',3'-secoguanosine-3',5'-cyclic methylphosphonate(22a,b). Compound **3**(48 mg,0.18 mmol) was added to a mixture of methyl-O,O-bis(1-benzotriazolyl)phosphonate(69.7 mg,0.21 mmol) and dry dioxane(4 mL). The mixture was stirred for 30 min at room temperature then evaporated to dryness in vacuo and the resulting residue was subjected to reverse phase HPLC(3%-23% CH_3CN in H_2O,linear gradient) to give two diastereomers,**22a**(34.5% yield) and **22b**(40.8% yield),respectively. The diastereomer that eluted first is referred to as **22a** and the other as **22b**. The absolute configurations of **22a** and **22b** at phosphorus atoms are unknown.

22a: ^{1}H NMR(CD_3OD) δ 7.98(s,1,C(8)H),5.99(q,1,J=6.1 Hz,C(1')H),4.76-4.69(m,1,CH_2),4.49-4.45(m,1,CH_2),4.36-4.32(m,1,CH_2),4.13-4.06(m,1,CH_2),3.67-3.65(m,1,C(4')H),1.80(d,3,J=6.1 Hz,2'-CH_3),1.58(d,3,J=17 Hz,P-CH_3); ^{31}P NMR(D_2O/85% D_3PO_4) δ 27.76; FAB-MS m/z 330$(M+1)^+$; mp 186-187 °C; Anal. Calcd. for $C_{11}H_{16}N_5O_5P \cdot H_2O$: C, 38.05; H, 5.22; N, 20.17. Found: C, 37.76; H, 5.12; N, 19.86.

22b: ^{1}H NMR(CD_3OD) δ 7.97(s,1,C(8)H),5.99(q,1,J=6.1 Hz,C(1')H),4.63-4.52(m,2,CH_2),4.42-4.38(m,1,CH_2),4.05-3.98(m,1,CH_2),3.82-3.79(m,1,C(4')H),1.80(d,3,J=6.1 Hz,2'-CH_3),1.61(d,3,J=18 Hz,P-CH_3); ^{31}P NMR (D_2O/85% D_3PO_4) δ 30.53; FAB-MS m/z 330$(M+1)^+$; mp 152-153 °C; Anal. Calcd. for $C_{11}H_{16}N_5O_5P \cdot 1.1\ H_2O$: C, 37.85; H, 5.26; N, 20.06. Found: C, 38.20; H, 5.24; N, 19.74.

Antiherpetic and Cytotoxic Assays in vitro. The novel acyclic analogues of guanosine **3**,**4**,**5**,**21**,and **22a**,**b** were evaluated for activities against HSV-1(strain F) and HSV-2(strain UW) in Vero cells by methods and procedures described previously[30] with some modifications. Vero cells(2.5×10^4 cells/0.1 mL/well) were incubated in 96 well tissue-culture grade microtiterplates(Coster) at 37 °C for 24 h in a 5% CO_2 atmosphere. The cells were infected with HSV-1 or HSV-2(MOI of 0.1) and the testing compounds(4 to 300 μM) were added at the time of the infection. Inhibition of viral cytopathic effect was examined after 72 h of incubation at 37 °C in a 5% CO_2 atmosphere. While DHPG showed a 73% inhibition of the cytopathic effect of HSV-2 at a concentration of 13 μM,none of the above compounds had a significant antiherpetic activity.

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REFERENCES

(1)Elion,G.B.;Furman,P.A.;Fyfe,J.A.;De Miranda,P.;Beauchamp,L.;Schaeffer,H.J. *Proc.Natl.Acad.Sci.U.S.A.* **1977**,*74*,5716.
(2)Schaeffer,H.J.;Beauchamp,L.;De Miranda,P.;Elion,G.B.;Bauer,D.J.;Collins,P. *Nature(London)* **1978**,*272*,583.
(3)Smith,K.O.;Galloway,K.S.;Kennell,W.L.;Ogilvie,K.K.;Radatus,B.K. *Antimicrob.Agents Chemother.* **1982**,*22*,55.
(4)Ogilvie,K.K.;Cheriyan,U.O.;Radatus,B.K.;Smith,K.O.;Galloway,K.S.;Kennell,W.L. *Can.J.Chem.* **1982**,*60*,3005.
(5)Ashton,W.T.;Karkas,J.D.;Field,A.K.;Tolman,R.L. *Biochem.Biophys.Res.Commun.* **1982**,*108*,1716.
(6)Field,A.K.;Davies,M.E.;De Witt,C.;Perry,H.C.;Liou,R.;Germershausen,J.;Karkas,J.D.;Ashton,W.T.;Johnston,D.B.R.;Tolman,R.L. *Pro.Natl.Acad.Sci.U.S.A.* **1983**,*80*,4139.

(7)Martin,J.C.;Dvorak,C.A.;Smee,D.F.;Mattews,T.R.;Verheyden,J.P.H.; *J.Med.Chem.* **1983**,*26*,759.
(8)Smee,D.F.;Martin,J.C.;Verheyden,J.P.H.;Matthews,T.R.; *Antimicrob.Agents Chemother.* **1983**,*23*,676.
(9)Chu,C.K.;Cutler,S.J. *J.Heterocycl.Chem.* **1986**,*23*,289.
(10)Remy,R.J.;Secrist III,J.A. *Nucleosides & Nucleotides* **1985**,*4*,411.
(11)Since this type of compounds can be considered to be ribonucleosides which lack a C2'-C3' bond,they are called conveniently as 2',3'-seconucleosides as has already been proposed by McCormick and McElhinney. McCormick,J.E.;McElhinney,R.S. *J.Chem.Research(s)* **1981**,12.
(12)Khym,J.X.;Cohn,W.E. *J.Am.Chem.Soc.* **1960**,*82*,6380.
(13)Lerner,L.M. *Carbohydro.Res.* **1984**,*127*,141.
(14)McGee,D.P.C.;Martin,J.C. *Can.J.Chem.* **1986**,*64*,1885.
(15)Keller,P.M.;Fyfe,J.A.;Beauchamp,L.;Lubbers,C.M.;Furman,P.A.; Schaeffer,H.J.;Elion,G.B. *Biochem.Pharmacol.* **1981**,*30*,3071.
(16)Jones,A.S.;Walker,R.T.;Wyatt,P.G.;Balzarini,J.;De Clercq,E. *J.Chem.Research(s)* **1985**,336.
(17)Jones,A.S.;McClean,M.J.;Tanaka,H.;Walker,R.T.;Balzarini,J.; De Clercq,E. *Tetrahedron* **1985**,*41*,5965.
(18)Beaton,G.;Jones,A.S.;Walker,R.T. *Tetrahedron* **1988**,*44*,6419.
(19)Hirota,K.;Tomishi,T.;Maki,Y. *Chem.Pharm.Bull.* **1988**,*36*,1298.
(20)Nathanson,J.A.;Kebabian,J.W. Cyclic Nucleotides II; Springer-Verlag:Berlin,Heidelberg,New York,1982.
(21)Tolman,R.L.;Field,A.K.;Karkas,J.D.;Wagner,A.F.;Germershausen,J.; Crumpacker,C.;Scolnick,E.M. Biochem.*Biophys.Res.Commun.* **1985**,*128*,1329.
(22)Markiewicz,W.T. *J.Chem.Research(s)* **1979**,24.
(23)Hanessian,S.;Lavallee,P. *Can.J.Chem.* **1975**,*53*,2975.
(24)Marugg,J.E.;De Vroom,E.;Dreef,C.E.;Tromp,M.;Van der Marel,G.A.; Van Boom,J.H. *Nucleic Acids Res.* **1986**,*14*,2171.
(25)Kalman,T.I.;Houston,D.M. *Nucleosides & Nucleotides* **1989**,*8*,899.
(26)Ohtsuka,E.;Nakagawa,E.;Tanaka,T.;Markham,A.F.;Ikehara,M. *Chem.Pharm.Bull.* **1978**,*26*,2998.
(27)Khym,J.X. *Biochemistry* **1963**,*2*,344.
(28)Prisbe,E.J.;Martin,J.C. *Synth.Commun.* **1985**,*15*,401.
(29)Barton,D.H.R.;McCombie,S.W. *J.Chem.Soc.,Perkin Trans.1.* **1975**,1574.
(30)Huffman,J.H.;Sidwell,R.W.;Khare,G.P.;Witkowski,J.T.;Allen,L.B.; Robins,R.K. *Antimicro.Agents Chemother.* **1973**,*3*,235.

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