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Syntheses, molecular structures, and antiviral activities of 1- and 2-(2′-deoxy-D-ribofuranosyl)cyclohepta[*d*][1,2,3]triazol-6(1*H*)-ones and 1-(2′-deoxy-D-ribofuranosyl)cyclohepta[*b*]pyrrol-8(1*H*)-one

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ABSTRACT

The synthesis of a novel class of 2′-deoxyribonucleosides possessing tropone-fused nitrogen heterocycles as nucleobases was developed. The reaction of alkali metal salts of 1*H*-cyclohepta[*d*][1,2,3]triazol-6-one with 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-ribofuranosyl chloride (α-chlorosugar) afforded an anomeric mixture of N1- and N2-coupled glycosylation products. Good stereospecificity in relation to the amount of the β-anomer was achieved in the less polar solvent DME. The reactions of the sodium salt of 1*H*-cyclohepta[*b*]pyrrol-8-one and its 3-methyl derivative with an α-chlorosugar in DME gave the β-anomer of the N1-coupled glycosylation products. The glycosylation products were treated with NaOMe in MeOH to produce the desired 2′-deoxyribonucleosides. X-ray structural analyses of the three nucleosides prepared confirmed their anomeric configuration and revealed that their sugars were puckered. The β-anomers of the nucleosides had weak antiviral activities for herpes simplex virus type 1 and herpes simplex virus type 2 in comparison with those of acyclovir (ACV). These nucleosides showed no cytotoxicity on a lung (A549) and two colon (HT-29 and HCT-116) cancer cell lines.

1. Introduction

A number of fused seven-membered aromatics, such as azaazulenes[1] and troponoids condensed with a heterocyclic ring,[2] have been synthesized, and some of them exhibit biological activities.[1a,d,e,2b] The nucleoside coformycin, which has a 3,6,7,8-tetrahydro-1,3,4,6-tetraazaazulene ring, is a potent inhibitor of adenosine deaminase.[3] Nucleosides with a fused seven-membered azaaromatic system are interesting building blocks for a novel class of nucleoside analogs. Synthesis of β-ribo- and β-arabinonucleosides (**1**–**4**) with 1-azaazulen-2(1*H*)-one, 1,3-diazaazulen-2(1*H*)-one and its thione, together with the α-anomers of **3** and **4** have been reported (Fig. 1).[4] The biological activity of triazoles[5] and pyrroles[6] and the biochemical and pharmacodynamic interest in troponoid-fused triazoles[7] and pyrroles[8] prompted us to synthesize nucleoside analogs with 1*H*-cyclohepta[*d*][1,2,3]triazol-6-one **5**, 1*H*-cyclohepta[*b*]pyrrol-8-one **6**, and its methyl derivative **7** as nucleobases. For the sugar moiety of the analog, 2-deoxy-D-ribose was employed because of its importance and usefulness in the field of nucleoside chemistry[9] and because of the facile access to 2′-deoxyribonucleosides by using a simple glycosylation reaction.[10] We report herein the glycosylation of **5**–**7** with 2-deoxy-3,5-di-*O*-

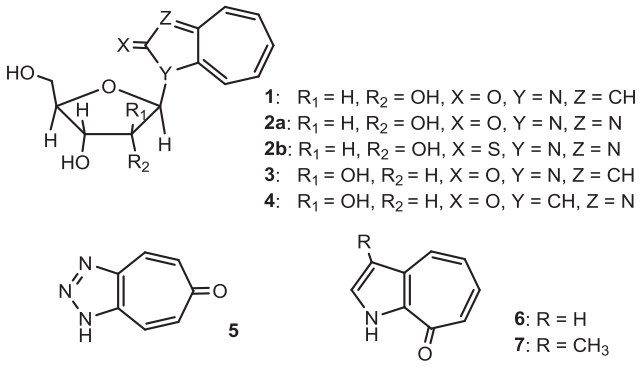

Fig. 1. Molecular structures of β-ribo- and β-arabinonucleosides with 1-aza- and 1,3-diazaazulen-2(1*H*)-one (thione) **1**–**4** together with tropone-fused nitrogen heterocycles **5**–**7**.

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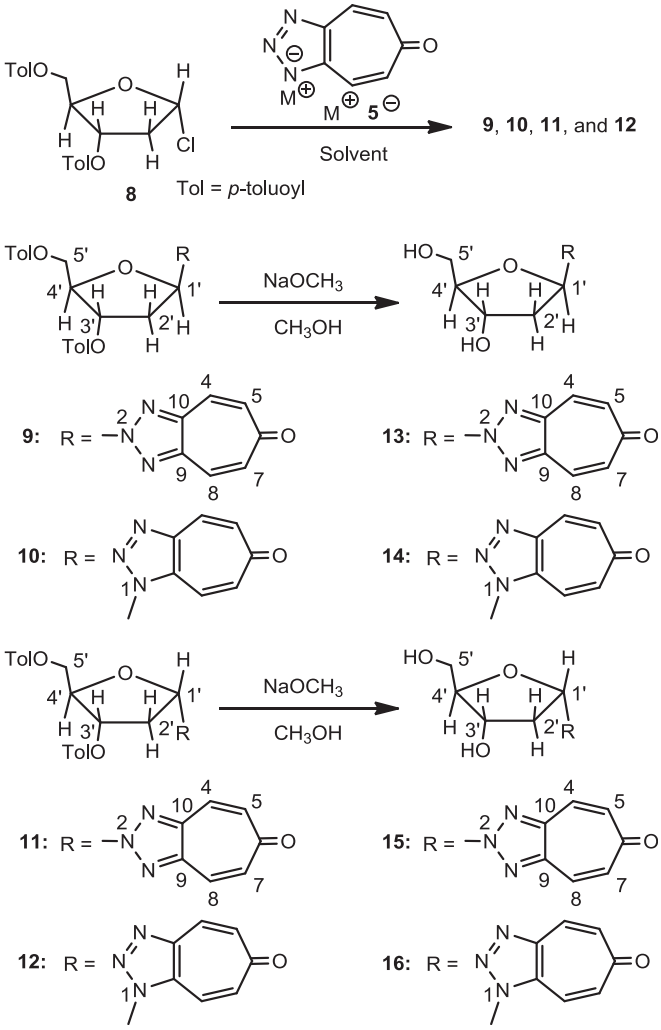
(*p*-toluoyl)-α-D-ribofuranosyl chloride (α-chlorosugar) **8**,[10a] deprotection of the glycosylation products, their molecular structures based on X-ray structural analyses, and their antiviral and anticellular activities.

2. Results and discussions

1*H*-Cyclohepta[*d*][1,2,3]triazol-6-one **5**, 1*H*-cyclohepta[*b*]-pyrrol-8-one **6**, and its methyl derivative **7** were prepared following reported methods.[11,12] Alkali metal salts of **5** were easily prepared by treating it with an equimolar amount of LiOH, NaOH, $NaHCO_3$, or KOH in water, followed by evaporation to dryness.

Metal salts of **5** were reacted with α-chlorosugar **8** in acetone, DMF, or DME at room temperature, affording four glycosylation products **9**–**12**, as shown in Scheme 1 and Table 1. These four products were separated by using column chromatography and/or preparative thin layer chromatography. The structure of **10**, determined from X-ray analysis, which is described below, is a glycosylation product of the β-anomer formed through N1-coupling of **5** and allowed us to confirm the assignment of the ^{1}H NMR spectrum of **10**. Anomeric structures of **9**, **11**, and **12** were determined from their ^{1}H NMR spectra as follows. (1) 2′-Deoxyribose moieties of **9**–**12** were assigned on the basis of the characteristic H1′, H2′, H3′, H4′, and H5′ peaks of the sugar groups and those of the protecting groups. (2) The structures of cyclohepta[*d*]triazol moieties were determined on the basis of the ^{1}H NMR data of **9**–**12**, as shown in Table 2. Anomers **9** and **11** are coupling products of **5** at the N2 position, *i.e.*, 2-(D-ribofuranosyl)cyclohepta[*d*][1,2,3]triazol-8(1*H*)-one, because of equivalent H4 and H8 and H5 and H7 protons, whereas anomers **10** and **12** are coupling products of **5** at the N1 position, *i.e.*, 1-(D-ribofuranosyl)cyclohepta[*d*][1,2,3]triazol-8(1*H*)-one, on the basis of the two pairs of AX type protons (H4 and H5 and H7 and H8). (3) Anomeric configurations of **9**, **11**, and **12** were determined on the basis of the coupling patterns of the peaks for the H1′ proton of the β-anomer of **10**, as shown in Table 2. Anomer **9**, of which the H1′ signal appeared as a triplet-like signal, like **10**, was assigned to have a β-D configuration, whereas anomers **11** and **12**, of which the H1′ signal appeared as doublets of doublets, were assigned to have α-D configurations. These assignments agree with those of related nucleosides.[14]



Scheme 1. Synthesis of 2′-deoxyribonucleosides **13**–**16** by reacting the alkali metal salt of 1*H*-cyclohepta[*d*][1,2,3]triazol-6-one **5** with **8**, followed by deprotection.

As shown in Table 1, the glycosylation products from 1*H*-cyclohepta[*d*][1,2,3]triazol-6-one **5** were obtained in relatively good yields. From the viewpoint of the anomeric stereoselectivity, β-anomers **9** and **10** and α-anomers **11** and **12** were obtained in comparative yields in moderately polar solvents, such as acetone and dimethylformamide (DMF) (entries 1–3). The relative ratios of the yields of the β-anomers to those of the α-anomers depended on the metal counter cation of the salt of **5** (entries 2 and 3). Good β-stereoselectivities were achieved in less polar DME (entries 4 and 5). The relative yield of β-anomer **9** to α-isomer **11** was comparable with that of β-anomer **10** to α-isomer **12** under each reaction condition (entries 1–5). The above results were explained in terms of the easy anomerization of α-chlorosugar **8** in polar solvents.[10d,15] Concerning the regioselectivity of the glycosylation reaction, the ratios of the yield of N2-coupling products **9** and **11** to those of N1-coupling products **10** and **12** were dependent on the metal cations (entries 2 and 3) and solvent polarity (entries 1–4), as shown in Table 1. The ratios were larger in less polar solvents, such as DME. A similar substitution reaction involving **5** and *O*-(2,4-dinitropheny) hydroxylamine, giving products substituted with N2- and N1-amino groups, has been reported.[16] In order to deprotect the 3′,5′-di-*O*-(*p*-toluoyl) glycosylation products, **9**–**12** were reacted with $NaOCH_3$ in CH_3OH to afford **13**–**16** in moderate to good yields with liberation of methyl *p*-toluate. The structures of **13**–**16** were confirmed by using ^{1}H NMR spectroscopy.

Glycosylation product **10** was recrystallized from benzene/ether to give single crystals suitable for X-ray analysis. The X-ray structure of **10**, which is in a β-D configuration, is shown in Fig. 2.[13]

In order to synthesize 2′-deoxyribonucleosides with cycloheptapyrrolone as a nucleobase, sodium salts of 1*H*-cyclohepta[*b*]pyrrol-8-one **6** and its methyl derivative **7** were prepared by reacting **6** and **7** with equimolar amounts of $NaOC_2H_5$ in anhydrous ethanol, followed by evaporation to dryness. The reactions of 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-ribofuranosyl chloride **8** with $Na^+\cdot\mathbf{6}^-$ and $Na^+\cdot\mathbf{7}^-$ afforded glycosylation products **17** and **18** in 65% and 70% yields, respectively. In a similar manner to the conversion of **9** and **10** to **13** and **14**, **17** and **18** were treated with $NaOCH_3$ in CH_3OH to afford **19** and **20** in 90% and 67% yields, respectively (Scheme 2).

X-ray quality single crystals of 2′-deoxyribonucleosides of **18** and **19** were obtained by recrystallization from benzene/hexane and methanol/dichloromethane, respectively. X-ray structures of **18** and **19**, shown in Figs. 3 and 4, show that they are glycosylation products formed through N1-coupling of **6** and **7** and that they have β-D configurations. In addition, **17** and **20** were confirmed to be β-anomers. The β-configuration of **17** was assigned on the basis of the H1′ signal in the ^{1}H NMR spectrum, which was a triplet, as shown in Table 2. Moreover, the anomeric configuration of **18** was maintained during deprotection under mild basic conditions.

The X-ray structures of **10**, **18**, and **19** exhibit important conformational features. Considering the similarities of tropone-fused nitrogen heterocycles **5** and **6** to purine bases, the *syn*- and *anti*-

Table 1
Yields of the glycosylation products **9**–**12** under various conditions

Entry	Metal cation of **5**	Solvent[a]	Total yield (%) of **9** and **11** (ratio of **9**:**11**)	Total yield (%) of **10** and **12** (ratio of **10**:**12**)	Total yield (%) of **9**, **10**, **11**, and **12**
1	Li^+	Acetone	41.3 (39:61)	34.5 (34:66)	75.8
2	Li^+	DMF	49.5 (45:55)	38.4 (45:55)	87.9
3	Na^+	DMF	34.9 (72:28)	45.9 (69:31)	80.8
4	Na^+	DME	60.0 (98:2)	25.5 (95:5)	85.5
5	K^+	DME	61.7 (99:1)	25.1 (96:4)	86.8

[a] DMF (dimethylformamide) and DME (dimethoxyethane).

Table 2
Selected ^{1}H chemical shifts (δ, ppm) and coupling constants (Hz) of the glycosylation products **9**–**12**, **17**, and **18**

Compound	H1′ of sugar moiety[a,b]	H4 and H8 of **9**, **10**, **11**, and **12**[a,b]	H5 and H7 of **9**, **10**, **11**, and **12**[a,b]
9	6.60 (t, $J\approx6.0$)	7.50 (d, 2H, J=12.2)	6.82 (d, 2H, J=12.2)
10	6.57 (t, $J\approx6.0$)	7.66 (d, J=12.2)	6.89 (dd, J=12.2, 2.1)
		7.75 (d, J=11.9)	6.86 (dd, J=11.9, 2.1)
11	6.59 (dd, J=6.9, 1.7)	7.56 (d, 2H, J=11.3)	6.85 (d, 2H, J=11.3)
12	6.60 (dd, J=7.3, 1.8)	7.82 (d, J=12.2)	6.93 (dd, J=12.2, 2.1)
		7.85 (d, J=12.2)	6.97 (dd, J=12.2, 2.1)
17	7.61 (br. t, $J\approx6.6$)		
18	7.65 (br. t, $J\approx6.6$)		

[a] Solvent: $CDCl_3$.
[b] ^{1}H NMR chemical shift δ/ppm (coupling constant/Hz).

conformations were assigned in relation to the arrangement about the glycosidic C1′–N1 bond and depend on whether the tropone nucleus lies above the plane of the sugar ring or points away from it. The glycosidic torsion angle (χ) (O1′–C1′–N1–C9) was determined to be 54.1(6)° for **10** (*syn*-conformation), 161.7(3)° for **18** (*anti*-conformation), and −121.3(2)° for **19** (*anti*-conformation). X-ray structures of *anti*-conformers **18** and **19** show that the H1′ protons are close to the tropone oxygen O8 (interatomic C1′–O8 and H1′–O8 distances were 2.764 and 2.267 Å for **18** and 2.844 and 2.108 Å for **19**, respectively). Although the tropone-fused heterocycle ribonucleosides **10** and **17** have similar frameworks, except for a tropone carbonyl group, the H1′ protons of **17** and its methyl derivative **18** are shifted downfield (1.04–1.08 ppm) in comparison to that of **10**, as shown in Table 2. The H1′ protons of **19** and **20** are downfield (0.67–0.69 ppm) from that of **14**. Furthermore, 2′-deoxyuridine and 2′-deoxycytidine in solution have been reported

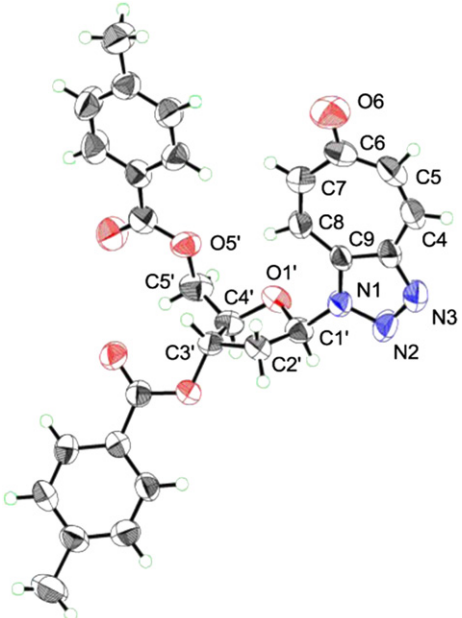
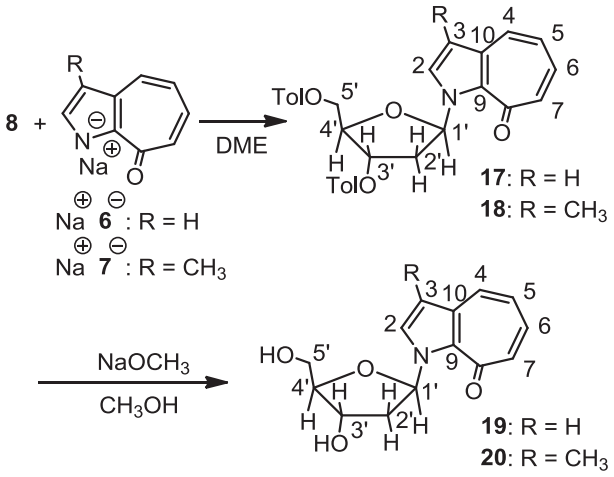


Fig. 2. ORTEP diagram of **10**. Thermal ellipsoids are drawn at the 50% probability level. Selected atoms were numbered according to the nomenclature.



Scheme 2. Reactions of the sodium salts of 1*H*-cyclohepta[*b*]pyrrol-8-one **6** and its methyl derivative **7** with 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-ribofuranosyl chloride **8**, affording glycosylation products **17** and **18**, respectively, and deprotection of the glycosylation products.

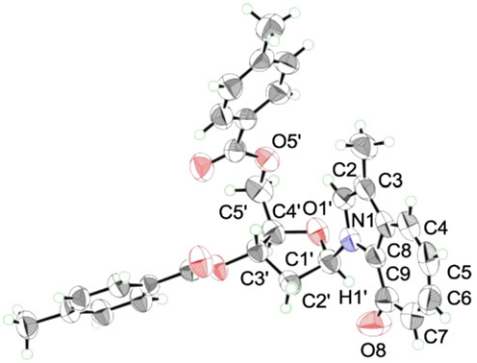


Fig. 3. ORTEP diagram of **18**. Thermal ellipsoids are drawn at the 50% probability level. Selected atoms were numbered according to the nomenclature.

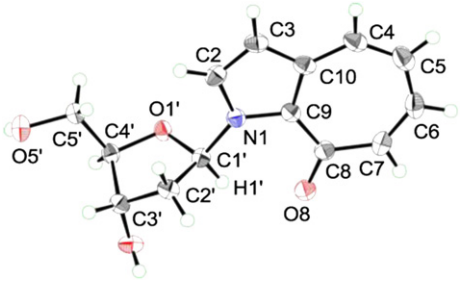


Fig. 4. ORTEP diagram of **19**. Thermal ellipsoids are drawn at the 50% probability level. Selected atoms were numbered according to the nomenclature.

to prefer the *anti*-conformation.[17] Taking into account that the tropone oxygen atoms (O8) of **17**–**20** are closer to the sugar ring than the C2 carbonyl oxygens of pyrimidine nucleosides are, the downfield shifts of the H1′ protons of cyclohepta[*b*]pyrrol

nucleosides and the X-ray structures of **18** and **19**, described above, indicate that **17–20** prefer the *anti*-conformation in solution.

In relation to the 2′-deoxyribose unit, the O5′-C5′–C4′ side chains of **10** and **18** are arranged in *gauche–gauche* conformations, and the dihedral angles O5′–C5′–C4′–C3′ were determined to be 56.8(5)° and 55.1(5)°, respectively.[18] In contrast, the O5′–C5′–C4′ side chain of **19** is in a *trans–gauche* conformation, and the dihedral angle was determined to be −74.7(2)°.[18] For the sugar puckering, there are three possible conformations, as shown in Fig. 5.[19] **10** has an O1′-*endo* conformation, whereas **18** has a C3′-*endo* conformation (**10**: the pseudorotation phase angle (P)=91.8° (envelope like) and the maximum puckering amplitude (ν_m)=41.4°); **18**: P=17.2° (half-chair like) and ν_m=37.6°.[20] In contrast, **19** takes a C2′-*endo* conformation (P=176.4° (half-chair like) and ν_m=38.9°).[20] It should be noted that the O1′-*endo* and C3′-*endo* classes of sugar puckers of **10** and **18** are rarely found in 2′-deoxyribonucleosides. The C2′-*endo* mode of puckering in **19** is comparable with the features of 2′-deoxyribopurines and 2′-deoxyribopyrimidines.[21] The bond lengths of sugar rings (O1′–C1′ bond lengths) (1.405(5) Å for **10**, 1.417(5) Å for **18**, and 1.440(2) Å for **19**) are shorter than the C4′–O1′ bond lengths (1.440(5) Å for **10**, 1.440(5) Å for **18**, and 1.447(3) Å for **19**), showing that the anomeric effect is due to hyperconjugation [$n_{O1'} \rightarrow \sigma^*_{C1'-N1}$] occurring in these β-nucleosides.[22]

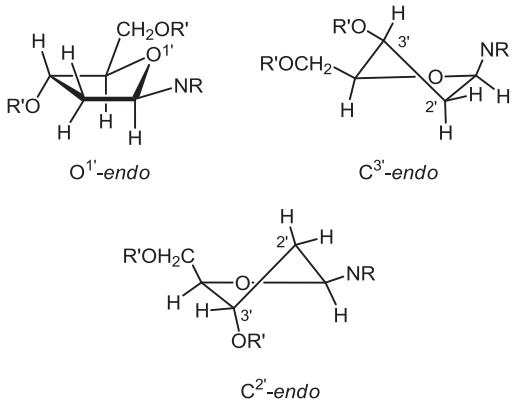


Fig. 5. Possible conformations of 2′-deoxyribonucleosides.

3. Antiviral and anticellular activities

For pre-clinical information, we evaluated the antiviral effects of **13**, **14**, **19**, and **20** on herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2). The antiviral activities on HSV-1 and HSV-2 in Vero cells were determined by using plaque reduction assays. Compounds **13**, **14**, **19**, and **20** had weak to no antiviral activities in comparison with those of acyclovir (ACV). After treatment with 50 μM solutions of **13**, **14**, **19**, **20**, and ACV, the plaque numbers of HSV-1 decreased to approximately 75%, 100%, 85%, 85%, and 0% of the vehicle control (DMSO), respectively, where the plaque numbers of the treatment with the vehicle control was defined as 100%. On the other hand, after treatment with 50 μM solutions of **13**, **14**, **19**, **20**, and ACV, the plaque numbers of HSV-2 decreased to approximately 95%, 90%, 70%, 100%, and 0% of the vehicle control, respectively. In addition, all compounds had weak cytotoxic activities toward Vero cells. The CC_{50} values of **13**, **14**, **19**, and **20** as well as ACV were all over 100 μM.

A lung (A549) and two colon (HT-29 and HCT-116) cancer cell lines were treated with 0.01 nM–10 μM solutions of **13–16**, **19**, and **20** for 96 h. After treatment, live cell numbers were measured and compared with that of a non-treated control. In this assay, compounds **13–16**, **19**, and **20** had no effect on cell proliferation and survival regardless of the concentration tested (IC_{50}>10 μM).[23]

4. Conclusion

We synthesized hitherto unknown 2′-deoxyribonucleosides with 1*H*-cyclohepta[*d*][1,2,3]triazol-6-one and 1*H*-cyclohepta[*b*]pyrrol-8-one as nucleobases. The reaction of 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-ribofuranosyl chloride **8** with alkali metal salts of 1*H*-cyclohepta[*d*][1,2,3]triazol-6-one **5** in polar or slightly polar solvents afforded anomeric mixtures of N2- and N1-coupling products, i.e., **9** (N2 coupled, β-), **10** (N1 coupled, β-), **11** (N2 coupled, α-) and **12** (N1 coupled, α-). Good β-stereoselectivities were achieved using the less polar solvent DME. Furthermore, the reaction of **8** with the sodium salts of 1*H*-cyclohepta[*b*]pyrrol-8-one **6** and its 3-methyl derivative **7** afforded the corresponding glycosylation products **17** (N1 coupled, β-) and **18** (N1 coupled, β-), respectively. **9–12**, **17** and **18** were deprotected with $NaOCH_3$ in methanol to afford the corresponding 2′-deoxyribonucleosides **13–16**, **19** and **20**, respectively. The anomeric structures of **10**, **18**, and **19** and sugar ring conformations (O1′-*endo* for **10**, C3′-*endo* for **18**, and C2′-*endo* for **19**) were determined by using X-ray analysis. Compounds **13**, **14**, **19**, and **20** showed weak to no antiviral activities in comparison with those of acyclovir (ACV) for herpes simplex virus type 1 and herpes simplex virus type 2, although **13–16**, **19**, and **20** showed no cytotoxicity toward a lung (A549) and two colon (HT-29 and HCT-116) cancer cell lines (0.01 nM–10 μM).

5. Experimental section

5.1. General

^{1}H and ^{13}C NMR spectra were recorded on JEOL 270 and LA-500 and BRUKER B-500 spectrometers. Chemical shift values are given in δ (ppm) relative to an internal $SiMe_4$ standard or residual solvent, and ^{1}H NMR signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). High resolution mass spectra were acquired on a JEOL JMS-T100LC mass spectrometer. IR spectra were recorded on PerkinElmer Spectrum Two FT-IR spectrometer. Column chromatography was carried out using Merck silica gel 60, 70–230, and 230 mesh ASTM and Daiso silica gel 1001W. Thin layer chromatography was carried out using Merck TLC silica gel $60F_{254}$. Preparative layer chromatography was carried out using Merck PLC plates (20 cm×20 cm, Silica gel $60F_{254}$, Layer=1 mm). Melting points were determined with a Yanaco MP-500D melting point apparatus. Elemental analyses were performed with Yanako CHN Corder MT-5. All reactions described below were performed under an argon or nitrogen atmosphere and monitored by TLC.

*5.1.1. 2- and 1-[2′-Deoxy-3′,5′-di-O-(p-toluoyl)-β-D-ribofuranosyl]-cyclohepta[d][1,2,3]triazol-6(1H)-one (**9** and **10**) and 2- and 1-[2′-deoxy-3′,5′-di-O-(p-toluoyl)-α-D-ribofuranosyl]cyclohepta-[d][1,2,3] triazol-6(1H)-one (**11** and **12**).* Entry 1, starting from the lithium salt of **5** and **8** in anhydrous acetone.

To a solution of the lithium salt of **5**, prepared by evaporation of a solution of **5** (0.181 g, 1.23 mmol) and $LiOH \cdot H_2O$ (0.0517 g, 1.23 mmol) in water (3 mL) to dryness in vacuo, followed by drying at 110 °C, in anhydrous acetone (30 mL) was added **8** (0.320 g, 0.823 mmol). The mixture was stirred at room temperature for two days. After evaporation of the reaction mixture to dryness under reduced pressure below 50 °C, the residue was dissolved in CH_2Cl_2 (20 mL). After filtration, the filtrate was evaporated under reduced pressure, and the residue was loaded on a silica gel column, packed with SiO_2 deactivated with 10% H_2O, and eluted with CH_2Cl_2/Et_2O (v:v=95:5) to give a mixture of **9** and **11** (0.170 g, 41.3%, **9**:**11**=39:61) and a mixture of **10** and **12** (0.142 g, 34.5%, **10**:**12**=34:66).

Entry 2, starting from the lithium salt of **5** and **8** in anhydrous DMF.

To a solution of the lithium salt of **5**, prepared by evaporation of a solution of **5** (0.174 g, 1.18 mmol) and $LiOH \cdot H_2O$ (0.0483 g,

1.18 mmol) in water (3 mL) to dryness in vacuo, followed by drying at 110 °C, in anhydrous DMF (15 mL) was added **8** (0.308 g, 0.792 mmol). The reaction mixture was stirred at room temperature for two days and then worked up in a similar manner to that of entry 1, affording a mixture of **9** and **11** (0.196 g, 49.5%, **9**:**11**=45:55) and a mixture of **10** and **12** (0.152 g, 38.4%, **10**:**12**=45:55).

Entry 3, starting from the sodium salt of **5** and **8** in anhydrous DMF.

To a solution of the sodium salt of **5,** prepared by evaporation of a solution of **5** (0.221 g, 1.50 mmol) and 2 N aqueous NaOH (0.75 mL) in water (2 mL) to dryness in vacuo, followed by drying at 110 °C, in anhydrous DMF (30 mL) was added **8** (0.407 g, 1.05 mmol). The reaction mixture was stirred at room temperature for two days and then worked up in a similar manner to that of entry 1, affording a mixture of **9** and **11** (0.183 g, 34.9%, **9**:**11**=72:28) and a mixture of **10** and **12** (0.241 g, 45.9%, **10**:**12**=69:31).

Entry 4, starting from the sodium salt of **5** and **8** in anhydrous DME.

To a solution of the sodium salt of **5**, prepared by evaporation of a solution of **5** (0.209 g, 1.42 mmol) and 2 N aqueous NaOH (0.71 mL) in water (2 mL) to dryness in vacuo, followed by drying at 110 °C, in anhydrous dimethoxyethane (DME) (30 mL) was added **8** (0.369 g, 0.949 mmol). The reaction mixture was stirred at room temperature for two days and then worked up in a similar manner to that of entry 1, affording a mixture of **9** and **11** (0.285 g, 60.0%, **9**:**11**=98:2) and a mixture of **10** and **12** (0.121 g, 25,5%, **10**:**12**=95:5).

Entry 5, starting from the potassium salt of **5** and **8** in anhydrous DME.

To a solution of the potassium salt of **5**, prepared by evaporation of a solution of **5** (0.187 g, 1.27 mmol) and 1 N aqueous KOH (1.27 mL) in water (2 mL) to dryness in vacuo, followed by drying at 110 °C, in anhydrous DME (50 mL) was added **8** (0.329 g, 0.846 mmol). The reaction mixture was stirred at room temperature for two days and then worked up in a similar manner as that of entry 1, affording a mixture of **9** and **11** (0.261 g, 61.7%, **9**:**11**=99:1) and a mixture of **10** and **12** (0.106 g, 25.1%, **10**:**12**=96:4).

*5.1.1.1. Isolation of **9**.* To a mixture of **9** and **11** (0.285 g, **9**:**11**=98:2) was added Et_2O (20 mL). The resulting colorless crystals of **9** were collected by filtration (0.238 g) and were recrystallized from CH_3CN to give colorless plates: mp 170.8–171.2 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.96 (d, 2H, J=8.2 Hz, ArH), 7.90 (d, 2H, J=8.2 Hz, ArH), 7.50 (d, 2H, J=12.2 Hz, H4, H8), 7.28 (d, 2H, J=8.2 Hz, ArH), 7.19 (d, 2H, J=8.2 Hz, ArH), 6.82 (d, 2H, J=12.2 Hz, H5, H7), 6.60 (t, 1H, $J\approx$6.0 Hz, H1′), 5.96 (m, 1H, H3′), 4.72 (m, 1H, H5′b), 4.69 (m, 1H, H4′), 4.53 (m, 1H, H5′a), 3.43 (ddd, 1H, J=14.3, 6.7, 5.2 Hz, H2′b), 2.82 (ddd, 1H, J=14.3, 6.7, 4.9 Hz, H2′a), 2.44 (s, 3H, CH_3), 2.40 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.18, 166.03, 165.85, 145.89, 144.43, 143.89, 136.14, 129.74, 129.22, 129.02, 128.61, 126.80, 126.35, 92.75, 83.62, 74.49, 63.55, 37.41, 21.68, 21.62; IR (ATR) 1723, 1703 ($\nu_{C=O}$ of ester), 1626, 1608, 1598 cm^{-1} ($\nu_{C=O}$ and $\nu_{C=C}$ of tropone); Anal. Calcd for $C_{28}H_{25}O_6N_3$: C, 67.33; H, 5.04; N, 8.41%. Found: C, 67.56; H, 4.95; N, 8.45%.

*5.1.1.2. Isolation of **10**.* A mixture of **10** and **12** (0.121 g, **10**:**12**=95:5) was recrystallized from C_6H_5/Et_2O to afford **10** (0.068 g) as pale yellow prisms: mp 148.7–149.8 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.96 (d, 2H, J=7.9 Hz, ArH), 7.78 (d, 2H, J=7.9 Hz, ArH), 7.75 (d, 1H, J=11.9 Hz, H4 or H8), 7.66 (d, 1H, J=12.2 Hz, H8 or H4), 7.28 (d, 2H, J=7.9 Hz, ArH), 7.20 (d, 2H, J=7.9 Hz, ArH) 6.89 (dd, J=12.2, 2.1 Hz, 1H, H7 or H5), 6.86 (dd, J=11.9, 2.1 Hz, 1H, H5 or H7), 6.57 (t, 1H, $J\approx$6.0 Hz, H1′), 5.88 (m, 1H, H3′), 4.71 (m, 1H, H4′), 4.57 (dd, 1H, J=12.2, 4.3 Hz, H5′b), 4.39 (dd, 1H, J=12.2, 4.9 Hz, H5′a), 3.90 (dt, 1H, J=14.3, 6.1 Hz, H2′b), 2.93 (ddd, 1H, J=14.3, 6.1, 4.1 Hz, H2′a), 2.44 (s, 3H, CH_3), 2.40 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 187.28, 165.81, 146.86, 144.52, 144.02, 138.09, 135.28, 134.89, 130.28, 129.74, 129.59, 129.25, 129.11, 126.33, 126.26, 120.80, 87.33, 84.11, 74.40, 63.16, 36.27, 21.68, 21.61; IR (ATR) 1708 ($\nu_{C=O}$ of ester), 1609, 1591, 1557 cm^{-1} ($\nu_{C=O}$ and $\nu_{C=C}$ of tropone); Anal. Calcd for $C_{28}H_{25}O_6N_3$: C, 67.33; H, 5.04; N, 8.41%. Found: C, 67.18; H, 4.91; N, 8.43%.

*5.1.1.3. Isolation of **11**.* A mixture of **9** and **11** (0.311 g, **9**:**11**=38:62) was separated by using column chromatography with SiO_2 deactivated with 10% H_2O and CH_2Cl_2 as an eluent. Several eluted fractions, checked with TLC, were collected. After drying the collected fractions, compound **11** (0.025 g) was obtained as colorless crystals and was recrystallized from C_6H_6/Et_2O, affording colorless plates: mp 137.0–137.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.94 (d, 2H, J=7.9 Hz, ArH), 7.74 (d, 2H, J=8.2 Hz, ArH), 7.56 (d, 2H, J=11.3 Hz, H4, H8), 7.25 (d, 2H, J=7.9 Hz, ArH), 7.16 (d, 2H, J=8.2 Hz, ArH) 6.85 (d, 2H, J=11.3 Hz, H5, H7), 6.59 (dd, 1H, J=6.9, 1.7 Hz, H1′), 5.63 (m, 1H, H3′), 4.97 (m, 1H, H4′), 4.69 (dd, 1H, J=12.2, 3.7 Hz, H5′b), 4.61 (dd, 1H, J=12.2, 4.1 Hz, H5′a), 3.18 (dt, 1H, J=15.0, ~1.7 Hz, H2′b), 3.09 (dt, 1H, J=15.0 and 6.9 Hz, H2′a), 2.42 (s, 3H, CH_3), 2.40 (s, 3H, CH_3). HRMS (ESI+) m/z $C_{28}H_{25}O_6N_3Na$ $(M+Na)^+$ calcd 522.16410. obsd: 522.16576.

*5.1.1.4. Isolation of **12**.* A mixture of **10** and **12** (19 mg, **10**:**12**=1.0:1.1) was chromatographed on four PL plates. After repeated development of the plates with CH_2Cl_2 for 2 days, **10** (9 mg) was obtained from the first migrating zone, and **12** (9 mg) was obtained from second migrating zone as a waxy solid: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.94 (d, 2H, J=7.9 Hz, ArH), 7.85 (d, 1H, J=12.2 Hz, H4 or H8), 7.82 (d, 1H, J=12.2 Hz, H8 or H4), 7.81 (d, 2H, J=7.9 Hz, ArH), 7.27 (d, 2H, J=7.9 Hz, ArH), 7.22 (d, 2H, J=7.9 Hz, ArH), 6.97 (dd, 1H, J=12.2, 2.1 Hz, H5 or H7), 6.93 (dd, 1H, J=12.2, 2.1 Hz, H7 or H5), 6.60 (dd, 1H, J=7.3 and 1.8 Hz, H1′), 5.70 (m, 1H, H3′), 4.70 (dd, 1H, J=11.3, 3.1 Hz, H5′b), 4.67 (m, 1H, H4′), 4.62 (dd, 1H, J=11.3, 3.9 Hz, H5′a), 3.80 (dt, 1H, J=15.3, 1.8 Hz, H2′b), 3.12 (dt, 1H, J=15.3, 7.3 Hz, H2′a), 2.43 (s, 3H, CH_3), 2.40 (s, 3H, CH_3).

*5.1.2. 2-(2′-Deoxy-β-D-ribofuranosyl)cyclohepta[d][1,2,3]triazol-6(1H)-one (**13**).* To a solution of **9** (75 mg, 0.15 mmol) in MeOH (2 mL) was added a solution of NaOMe in MeOH (0.0913 mmol/mL, 1.97 mL). The mixture was stirred at room temperature for one day (TLC control) and then concentrated under reduced pressure. The residue was separated by using column chromatography with SiO_2 deactivated with 10% MeOH and with CH_2Cl_2 as the eluent to afford methyl toluate (38 mg, 84%) and CH_2Cl_2/MeOH (v:v=9:1) to afford **13** (41 mg, 98%) as colorless crystals. **13** was recrystallized from CH_2Cl_2/MeOH, affording colorless needles: mp 127.5–128.0 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.80 (dd, 2H, J=12.2, 1.2 Hz, H4, H8), 6.79 (dd, 2H, J=12.2, 1.2 Hz, H5, H7), 6.46 (dd, 1H, J=6.7, 4.3 Hz, H1′), 5.45 (d, 1H, J=4.9 Hz, OH3′), 4.78 (t, 1H, J=5.5 Hz, OH5′), 4.50 (m, 1H, H3′), 3.89 (m, 1H, H4′), 3.51 (m, 1H, H5′b), 3.43 (m, 1H, H5′a), 2.83 (m, 1 H, H2′b), 2.45 (m, 1H, H2′a); ^{13}C NMR (125 MHz, CD_3OD): δ 190.38, 147.07, 136.69, 131.11, 94.65, 90.14, 72.44, 63.77, 40.95; IR (ATR) 3308 (OH), 1621, 1583 cm^{-1} ($\nu_{C=O}$ and $\nu_{C=C}$ of tropone); HRMS (ESI+) m/z $C_{12}H_{13}N_3O_4Na$ $(M+Na)^+$ calcd 286.08038, obsd 286.08039.

*5.1.3. 1-(2′-Deoxy-β-D-ribofuranosyl)cyclohepta[d][1,2,3]triazol-6(1H)-one (**14**).* To a solution of **10** (74 mg, 0.148 mmol) in MeOH (2 mL) was added a solution of NaOMe in MeOH (0.0913 mmol/mL 1.95 mL). The mixture was stirred at room temperature for one day (TLC control). The reaction mixture was worked up in a manner similar to that of **13**, affording methyl toluate (33 mg, 74%) and **14** (39 mg, 98%) as colorless crystals, which was recrystallized from EtOAc to afford colorless needles: mp 135.0–136.5 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.98 (d, 1H, J=12.2 Hz, H4 or H8), 7.92 (d, 1H, J=12.2 Hz, H8 or H4), 6.91 (dd, 1H, J=12.2, 2.1 Hz, H5 or H7), 6.81 (dd, 1H, J=12.2, 2.1 Hz, H7 or H5), 6.70 (dd, 1H, J=6.4, 4.9 Hz,

H1′), 5.47 (d, 1H, *J*=4.6 Hz, OH3′), 4.75 (t, 1H, *J*=5.3 Hz, OH5′), 4.50 (m, 1H, H3′), 3.92 (m, 1H, H4′), 3.43 (m, 1H, H5′b), 3.26 (m, 1H, H5′a), 3.14 (m, 1H, H2′b), 2.45 (m, 1H, H2′a); ^{13}C NMR (125 MHz, CD_3OD): δ 189.78, 147.81, 138.29, 136.68, 135.87, 132.37, 124.23, 90.24, 88.68, 72.30, 63.28, 40.03; IR (ATR) 3310 (OH), 1604, 1548 cm^{-1} ($\nu_{C=O}$ and $\nu_{C=C}$ of tropone); HRMS (ESI+) *m*/*z* $C_{12}H_{13}N_3O_4Na$ $(M+Na)^+$ calcd 286.08038, obsd 286.07619.

*5.1.4. 2-(2′-Deoxy-α-D-ribofuranosyl)cyclohepta[d][1,2,3]triazol-6(1H)-one (**15**).* A solution of NaOMe in MeOH (0.045 mmol/mL, 0.42 mL) was added to **11** (7.8 mg, 0.016 mmol). The mixture was stirred at room temperature for one day (TLC control). The reaction mixture was worked up in a manner similar to that of **13**, affording **15** (2.3 mg, 55%) as a colorless waxy solid: ^{1}H NMR (500 MHz, CD_3OD): δ 7.82 (d, 2H, *J*=12.1 Hz, H4, H8), 6.87 (d, 2H, *J*=12.1 Hz, H5, H7), 6.47 (dd, 1H, *J*=7.3, 3.8 Hz, H1′), 4.40 (m, 1H, H3′), 4.25 (m, 1H, H4′), 3.78 (dd, 1H, *J*=12.2, 3.1 Hz, H5′b), 3.66 (dd, 1H, *J*=12.2, 4.6 Hz, H5′a), 2.93 (dt, 1H, *J*=14.2, 7.6 Hz, H2′b), 2.75 (ddd, 1H, *J*=14.2, 5.1, 3.8 Hz, H2′a); HRMS (ESI+) *m*/*z* $C_{12}H_{13}N_3O_4Na$ $(M+Na)^+$ calcd 286.08038, obsd 286.08020.

*5.1.5. 1-(2′-Deoxy-α-D-ribofuranosyl)cyclohepta[d][1,2,3]triazol-6(1H)-one (**16**).* A solution of NaOMe in MeOH (0.060 mmol/mL 0.55 mL) was added to **12** (13.8 mg, 0.0276 mmol). The mixture was stirred at room temperature for one day (TLC control). The reaction mixture was worked up in a manner similar to that of **13**, affording methyl toluate (8.3 mg, 95%) and **16** (5.4 mg, 74%) as a colorless oil: ^{1}H NMR (270 MHz, CD_3OD): δ 8.09 (d, 1H, *J*=12.2 Hz, H4 or H8), 7.94 (d, 1H, *J*=12.0 Hz, H8 or H4), 6.99 (dd, 1H, *J*=12.2, 2.3 Hz, H5 or H7), 6.92 (dd, 1H, *J*=12.0, 2.3 Hz, H7 or H5), 6.68 (dd, 1H, *J*=7.2, 3.8 Hz, H1′), 4.46 (m, 1H, H3′), 4.09 (m, 1H, H4′), 3.76 (dd, 1H, *J*=12.2, 3.2 Hz, H5′b), 3.67 (dd, 1H, *J*=12.2, 4.4 Hz, H5′a), 3.14 (ddd, 1 H, *J*=14.2, 4.6, 3.8 Hz, H2′b), 2.94 (dt, 1H, *J*=14.2, 7.2 Hz, H2′a); HRMS (ESI+) *m*/*z* $C_{12}H_{13}N_3O_4Na$ $(M+Na)^+$ calcd 286.08038, obsd 286.08130.

*5.1.6. 1-[2′-Deoxy-3′,5′-di-O-(p-toluoyl)-β-D-ribofuranosyl]cyclohepta[b]pyrrol-8(1H)-one (**17**).* To a solution of the sodium salt of **6**, prepared by evaporation of a solution of **6** (0.182 g, 1.25 mmol) and $NaOC_2H_5$ (1.06 mmol/mL EtOH solution, 1.2 mL) in absolute EtOH (5 mL) to dryness, followed by drying at 100 °C, in anhydrous DME (30 mL) was added **8** (0.348 g, 0.895 mmol). The reaction mixture was stirred for two days at room temperature and then filtered. The filtrate was evaporated under reduced pressure. The residue was separated by using column chromatography with SiO_2 deactivated with 10% H_2O and with CH_2Cl_2 as the eluent to afford **17** (0.291 g, 65.2% for compound **8** used) as a colorless oil and with CH_2Cl_2/Et_2O (*v*:*v*=9:1) to recover the remaining **6** (0.071 g, 39.0% for compound **6** used). Compound **17**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.99 (d, 2H, *J*=8.2 Hz, ArH), 7.90 (d, 2H, *J*=8.2 Hz, ArH), 7.81 (d, 1H, *J*=3.1 Hz, H2), 7.61 (br. t, 1H, *J*≈6.6 Hz, H1′), 7.47 (d, 1H, *J*=10.7 Hz, H4), 7.26 (d, 2H, *J*=8.2 Hz, ArH), 7.21 (d, 2H, *J*=8.2 Hz, ArH), 7.13 (dd, 1H, *J*=12.5, 8.2 Hz, H6), 7.04 (d, 1H, *J*=12.5 Hz, H7), 6.72 (dd, 1H, *J*=10.7, 8.2 Hz, H5), 6.56 (d, *J*=3.1 Hz, H3), 5.60 (m, 1H, H3′), 4.75 (dd, 1H, *J*=12.0, 3.5 Hz, H5′b), 4.73 (dd, 1H, *J*=12.0, 3.8 Hz, H5′a), 4.64 (m, 1H, H4′), 3.12 (ddd, 1H, *J*=14.3, 5.6, 2.6 Hz, H2′b), 2.43 (m, 1H, H2′a), 2.43 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), ^{13}C NMR (125 MHz, $CDCl_3$): δ 177.89, 166.21, 166.12, 144.22, 144.03, 136.84, 134.91, 134.63, 133.23, 132.25, 129.83, 129.63, 129.17, 129.16, 126.72, 126.51, 126.10, 123.70, 111.06, 89.13, 82.87, 74.65, 64.25, 41.83, 21.67, 21.62; IR (ATR) 1715 ($\nu_{C=O}$ of ester), 1631, 1612, 1573, 1555 cm^{-1} ($\nu_{C=O}$ and $\nu_{C=C}$ of tropone); Anal. Calcd for $C_{30}H_{27}O_6N$: C, 72.42; H, 5.47; N, 2.82%. Found: C, 72.04; H, 5.40; N, 2.59%.

*5.1.7. 1-[2′-Deoxy-3′,5′-di-O-(p-toluoyl)-β-D-ribofuranosyl]-3-methylcyclohepta[b]pyrrol-8(1H)-one (**18**).* To a solution of the sodium salt of **7**, prepared by evaporation of a solution of **7** (0.243 g, 1.53 mmol) and NaOEt (1.06 mmol/mL EtOH solution, 1.5 mL) in absolute EtOH (5 mL) to dryness, followed by drying at 100 °C, in anhydrous DME (20 mL) was added **8** (0.423 g, 1.09 mmol). The reaction mixture was stirred for two days at room temperature and then filtered. The filtrate was evaporated under reduced pressure. The residue was separated by using column chromatography with SiO_2 deactivated with 10% H_2O and with CH_2Cl_2 as the eluent to give **18** (0.392 g, 70.3% for compound **8** used) as colorless crystals and with CH_2Cl_2/EtOAc (*v*:*v*=9:1) to recover the remaining **7** (0.111 g, 45.7% for compound **7** used). Compound **18** was recrystallized from MeOH to give colorless needles: mp 151.9–152.3 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.98 (d, 2H, *J*=8.2 Hz, ArH), 7.93 (d, 2H, *J*=8.4 Hz, ArH), 7.65 (br. t, 1H, *J*≈6.6 Hz, H1′), 7.59 (s, 1H, H2), 7.46 (d, 1H, *J*=10.7 Hz, H4), 7.28 (d, 2H, *J*=8.4 Hz, ArH), 7.22 (d, 2H, *J*=8.2 Hz, ArH), 7.15 (dd, 1H, *J*=12.5 and 8.2 Hz, H6), 7.03 (d, 1H, *J*=12.5 Hz, H7), 6.75 (dd, 1H, *J*=10.7, 8.2 Hz, H5), 5.62 (m, 1H, H3′), 4.80 (dd, 1H, *J*=12.1, 3.2 Hz, H5′b), 4.70 (dd, 1H, *J*=12.1, 3.6 Hz, H5′a), 4.61 (m, 1H, H4′), 3.05 (ddd, 1H, *J*=14.3, 5.6, 2.7 Hz, H2′b), 2.43 (s, 3H, CH_3), 2.42 (m, 1H, H2′a), 2.40 (s, 3H, CH_3), 2.14 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 177.84, 166.27, 166.19, 144.25, 144.07, 136.81, 135.46, 134.65, 130.81, 130.55, 129.87, 129.66, 129.22, 129.20, 126.83, 126.57, 124.74, 123.01, 118.68, 88.74, 82.76, 74.63, 64.18, 41.74, 21.71, 21.67, 9.80; IR (ATR) 1712 ($\nu_{C=O}$ of ester), 1629, 1612, 1564, 1551 cm^{-1} ($\nu_{C=O}$ and $\nu_{C=C}$ of tropone); Anal. Calcd for $C_{31}H_{29}O_6N$: C, 72.78; H, 5.71; N, 2.74%. Found: C, 72.67; H, 5.74; N, 2.75%.

*5.1.8. 1-(2′-Deoxy-β-D-ribofuranosyl)cyclohepta[b]pyrrol-8(1H)-one (**19**).* To a solution of **17** (75 mg, 0.151 mmol) in anhydrous MeOH (2 mL) was added a solution of NaOMe in MeOH (0.0913 mmol/mL, 1.98 mL). The mixture was stirred at room temperature for one day (TCL control). The reaction mixture was worked up in a manner similar to that of **13**, affording methyl toluate (42 mg, 93%) and **19** (35 mg, 90%) as colorless prisms: mp 182.0–183.5 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.07 (d, 1H, *J*=2.4 Hz, H2), 7.60 (d, 1H, *J*=10.7 Hz, H4), 7.37 (br. t, 1H, *J*≈5.8 Hz, H1′), 7.19 (dd, 1H, *J*=12.5, 8.5 Hz, H6), 6.88 (d, 1H, *J*=12.5 Hz, H7), 6.77 (dd, 1H, *J*=10.7, 8.5 Hz, H5), 6.75 (d, *J*=2.4 Hz, H3), 5.26 (d, 1H, *J*=4.0 Hz, OH3′), 5.07 (t, *J*=4.7 Hz, OH5′), 4.25 (m, 1H, H3′), 3.86 (m, 1H, H4′), 3.65 (m, 1H, H5′b), 3.60 (m, 1H, H5′a), 2.40 (m, 1H, H2′b), 2.09 (m, 1H, H2′a); ^{13}C NMR (125 MHz, DMSO-d_6): δ 176.92, 136.32, 134.53, 134.15, 133.28, 131.59, 127.61, 123.44, 110.94, 87.51, 87.34, 69.58, 61.10, 43.53; IR (ATR) 3288 (OH), 1628, 1567, 1547 cm^{-1} ($\nu_{C=O}$ and $\nu_{C=C}$ of tropone); HRMS (ESI+) *m*/*z* $C_{14}H_{15}NO_4Na$ $(M+Na)^+$ calcd 284.08988, obsd 284.08921.

*5.1.9. 1-(2′-Deoxy-β-D-ribofuranosyl)-3-methylcyclohepta[b]pyrrol-8(1H)-one (**20**).* To a solution of **18** (80 mg, 0.156 mmol) in anhydrous MeOH (2 mL) was added a solution of NaOMe in MeOH (0.0913 mmol/mL, 2.05 mL). The mixture was stirred at room temperature for one day (TLC control). The reaction mixture was worked up in a manner similar to that of **13**, affording methyl toluate (31 mg, 66%) and **20** (29 mg, 67%) as colorless needles: mp 186.5–188.0 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.87 (s, 1H, H2), 7.55 (d, 1H, *J*=11.0 Hz, H4), 7.39 (br. t, 1H, *J*≈6.3 Hz, H1′), 7.20 (dd, 1H, *J*=12.5, 8.2 Hz, H6), 6.87 (d, 1H, *J*=12.5 Hz, H7), 6.80 (dd, 1H, *J*=11.0, 8.2 Hz, H5), 5.24 (d, 1H, *J*=4.0 Hz, OH3′), 5.04 (t, 1H, *J*=5.2 Hz, OH5′), 4.24 (m, 1H, H3′), 3.78 (m, 1H, H4′), 3.62 (m, 2H, H5′a, H5′b), 2.36 (m, 1H, H2′b), 2.24 (s, 3H, CH_3), 2.08 (m, 1H, H2′a); ^{13}C NMR (125 MHz, DMSO-d_6): δ 176.80, 136.25, 134.66, 134.49, 130.57, 129.93, 126.06, 122.82, 117.89, 87.43, 86.89, 69.84, 61.29, 43.29, 9.72; IR (ATR) 3300 (OH), 1604, 1548 cm^{-1} ($\nu_{C=O}$ and $\nu_{C=C}$ of tropone); HRMS (ESI+) *m*/*z* $C_{15}H_{17}NO_4Na$ $(M+Na)^+$ calcd 298.10553, obsd 298.10411.

5.2. X-ray crystallography

X-ray data were acquired on a Bruker Smart APEX diffractometer equipped with a CCD area detector with graphite-

monochromated Mo Kα radiation (λ=0.71073 Å). The structure was solved by using direct methods (SHELXTL) and refined by using full-matrix least-squares method on F^2 (SHELXL-97). Non-hydrogen atoms were refined anisotropically, and hydrogen atoms were placed using AFIX instructions. Crystallography data has been deposited at the Cambridge Crystallography Data Center with deposition number of 869269, 869270, and 869271 for **10**, **18**, and **19**. Copies of this information may be obtained free of charge from The CCDC, 12 Union road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

Crystal data for **10**. $C_{28}H_{25}N_3O_6$, M=499.51; orthorhombic, space group $P2_12_12_1$; a=6.1410(6), b=16.8267(16), c=23.706(2), α=90°, β=90°, γ=90°, V=2449.6(4) Å^3; Dc (Z=4)=1.354 g cm^{-3}; T=293 K, 8981 collected reflections, 3492 reflections with $I>2\sigma(I)$, R_1=0.0509, wR_2=0.112.

Crystal data for **18**. $C_{31}H_{29}NO_6$, M=511.55; orthorhombic, space group $P2_12_12_1$; a=5.8101(12), b=14.674(3), c=30.356(6), α=90°, β=90°, γ=90°, V=2588.9(9) Å^3; Dc (Z=4)=1.313 g cm^{-3}; T=293 K, 9459 collected reflections, 3734 reflections with $I>2\sigma(I)$, R_1=0.0449, wR_2=0.110.

Crystal data for **19**. $C_{14}H_{15}NO_4$, M=261.27; monoclinic, space group $P2_1$; a=11.733(4), b=4.6709(15), c=12.052(4), α=90°, β=112.443(5)°, γ=90°, V=610.5(3) Å^3; Dc (Z=2)=1.345 g cm^{-3}; T=293 K, 2624 collected reflections, 1655 reflections with $I>2\sigma(I)$, R_1=0.0326, wR_2=0.0837.

5.3. Determination of antiviral and anticellular activities

The antiviral activities of the compounds against the HF strain of herpes simplex virus type 1 (HSV-1) and SAVAGE strain of herpes simplex virus type 2 (HSV-2) in Vero cells were determined by using a plaque reduction assay. Vero cells were cultivated with DMEM supplemented with 10% fetal bovine serum (DMEM-10% FBS). Vero cells seeded in a 6-well tissue culture plate were cultivated for two days, and then the cell monolayers were washed once with a phosphate buffered saline solution (PBS). Vero cell sheets were inoculated with about 100 PFU of HSV-1 or HSV-2 in 0.2 mL of DMEM-0%FBS. After adsorption for 60 min, the inoculum was removed, and HSV-infected cell sheets in duplicated wells were fed with DMEM-10%FBS containing 0.5% methylcellulose and serially diluted test compounds. After incubating for 2–3 days in a 5% CO_2 humidified incubator at 37 °C, the maintenance medium was removed. Then the cell sheets were stained with 1% crystal violet in 50% methanol, and the plaque numbers were counted. The plaque number for the treatment with the vehicle control (DMSO) was defined as 100%. Determination of anticellular activities of compounds.

Cells were seeded in a 96-well tissue culture plate at 1×10^4 cells per well. After 1 day, the cells were fed again with DMEM-10%FBS containing an appropriate amount of the test compound. After 2 days, the number of viable cells was estimated by using a cell-counting kit-8 (Dojindo). The optical density of the sample was measured at 450 nm with a microplate spectrophotometer and expressed as a percentage of the value of the untreated cells (defined as 100%). The value of CC_{50}, a 50% cytotoxic concentration of drug, was calculated from the plot of the drug concentration versus the percentage of live cells.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2012.04.109.

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