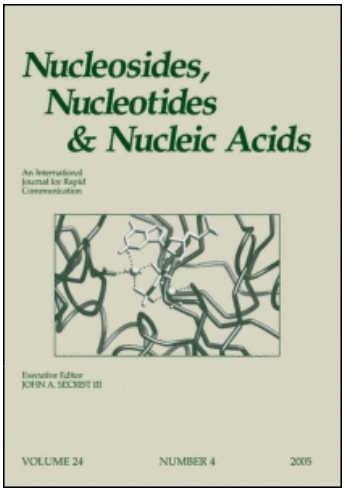

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF ARA AND 2'-DEOXY-CYCLOPENTENYL CYTOSINE NUCLEOSIDE ANALOGUES[1]

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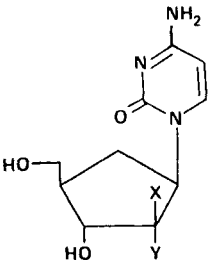
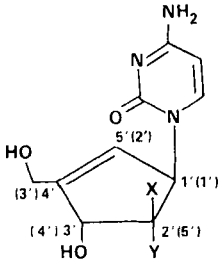
Abstract. The cytosine analogue of Neplanocin A, cyclopentenyl cytosine (CPE-C, 4), has significant antitumor and antiviral activity. Two closely related analogues modified at the 2'-position, ara-CPE-C (6) and 2'-deoxy-CPE-C (5), have been synthesized from the corresponding uracil derivative CPE-U. Both compounds were devoid of cytotoxicity against L1210 leukemia in vitro. Ara-CPE-C displayed antiviral activity against influenza type A_2 but was not very potent.

The carbocyclic cytidine nucleoside (carbodine, 1) and its closely related cyclopentenyl cytosine analogue (CPE-C, 4) are both endowed with significant antitumor and antiviral activities.[2-4] In several antitumor and antiviral assays, however, the cyclopentene-containing carbocyclic, CPE-C, consistently displayed greater potency and superior biological





1, X = H, Y = OH

2, X = Y = H

3, X = OH, Y = H

4, X = H, Y = OH

5, X = Y = H

6, X = OH, Y = H

activity.[2-4] In our laboratory, we are interested in exploring whether this difference extends to other closely related members of the CPE-C family.

Molecular modifications performed on the carbocyclic moiety of carbodine (1) by Shealy and coworkers demonstrated the superiority of the "ara" configuration (compound 3) in relation to the "ribo" configuration (compound 1) in terms of antitumor activity and potency against L1210 leukemia in mice.[2] The "2-deoxy"[1] (compound 2) and "3-deoxy" configurations, in turn, completely lacked antitumor activity at comparable doses and schedules of administration.[5] In terms of antiherpetic activity against HSV-1 and HSV-2, the order was reversed for the first two compounds: the "ribo" analogue was superior to the "ara", as ascertained from its viral rating (VR) and minimum inhibitory concentration (MIC_{50}).[3] The "2′-deoxy" analogue still retained a significant amount of antiviral activity, albeit at a higher dose, and the "3′-deoxy" analogue was completely inactive.[3]

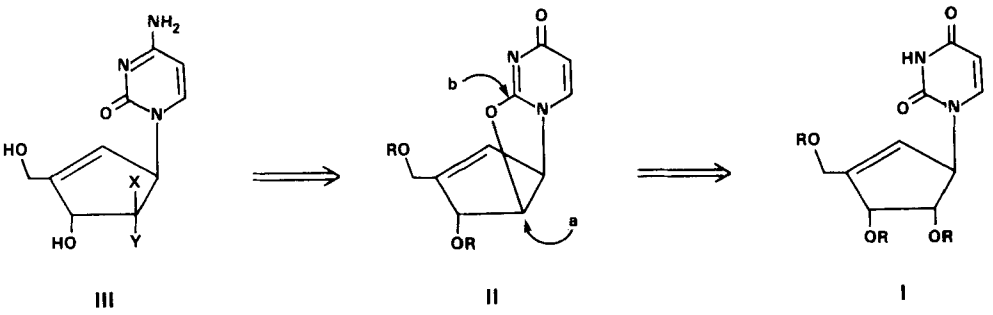
Based on these observations we became interested in the synthesis of the corresponding "ara" (compound 6) and "2′-deoxy" (compound 5) analogues of the highly potent unsaturated, carbocyclic nucleoside CPE-C (4) in order to correlate these structural changes with antiviral and antitumor activities.

A preliminary account of this work has been presented[6] and the two identified target compounds, 2′-deoxy-CPE-C (5) and ara-CPE-C (6) have also been reported by Arita and coworkers.[7]

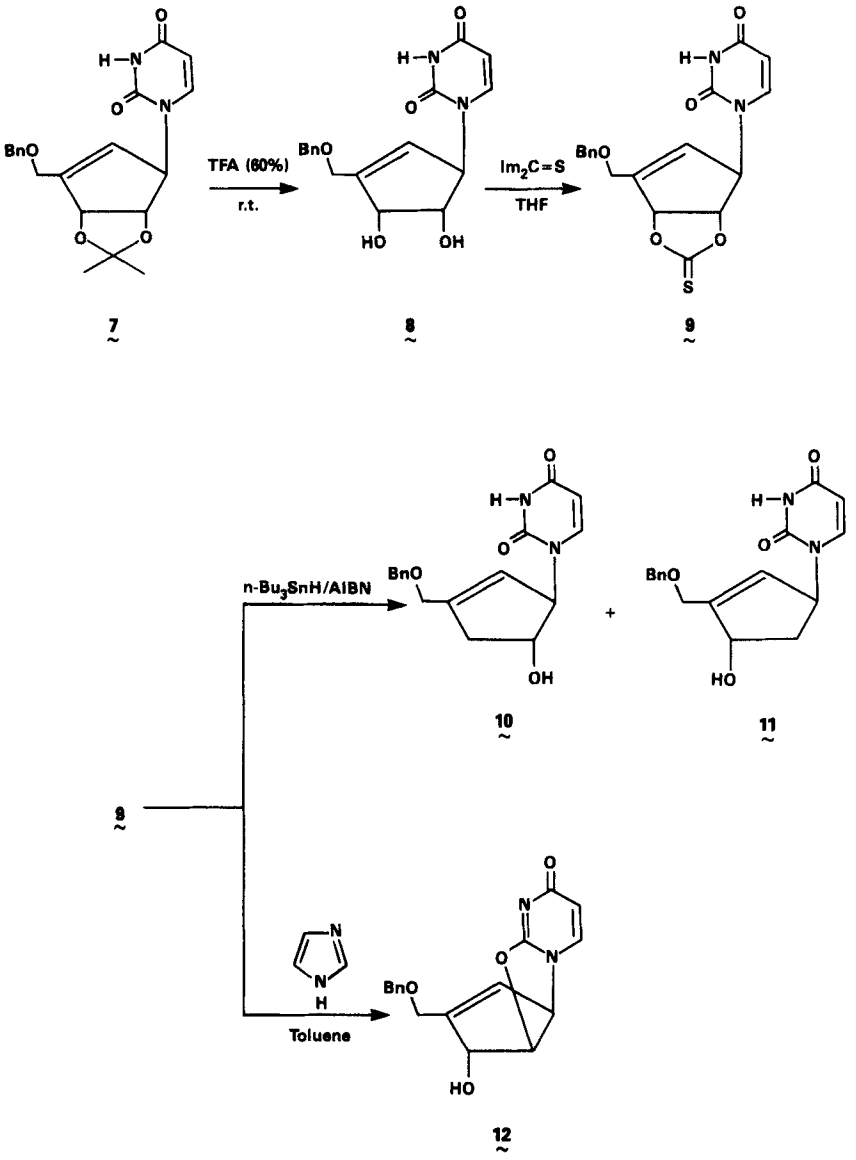
In the present communication we report on our synthetic approach which produces the target compounds 5 and 6 from a common 2,2′-anhydro intermediate and discuss the cytotoxic and antiviral properties of these compounds.

Results and Discussion

A logical starting point for our synthesis was the known carbocyclic nucleoside CPE-U (I, R = H) which we had prepared earlier.[4] With the introduction of suitable protective groups, the 2,2′-anhydro intermediate (II), generated from I, was to be converted to either the "2′-deoxy" (III, X = Y = H) or "ara" configuration (III, X = OH, Y = H) by selective opening of the oxide bridge (route a vs. route b).



Specifically, compound 7[4] was deblocked to the dihydroxy intermediate 8 and reaction of this compound with N,N-thiocarbonyldiimidazole gave the expected cyclic thiocarbonate derivative 9 (SCHEME 1). Preliminary attempts to use this compound to generate the 2'-deoxy intermediate 11, following homolytic cleavage of the C-O bond with tri-n-butyltin hydride, gave an inseparable mixture of the desired 2'-deoxy isomer (11) and the more abundant 3'-deoxy isomer (10), which resulted from the radical deoxygenation at the more reactive allylic position. Thus, formation of the 2,2'-anhydro intermediate (12) appeared to be the best approach. For this route a reliable proof of structure for the desired 2,2'-anhydro intermediate (12), capable of discriminating between 12 and the competing 2,3'-anhydro isomer, was required. It is known that 2,3'-anhydro nucleosides derived from uridine and cytidine are formed only with difficulty and that the reaction of 5'-O-trityl uridine with N,N-thiocarbonyldiimidazole gives exclusively the 2,2'-anhydro derivative.[8] Such preference in favor of the 2,2'-anhydro compound also seems to extend to saturated carbocyclic nucleosides.[9,10] However, it is possible that the unsaturation in the cyclopentene carbocyclic nucleoside 9 could change this preference due to a different puckering of the carbocyclic moiety. The imidazole-catalyzed formation of the anhydro compound (SCHEME 1) proceeded with the exclusive formation of only one isomer. The 2D NMR COSY spectra (FIG. 1) provided convincing evidence in favor of the formation of the 2,2'-anhydro intermediate 12. The characteristic olefinic broad singlet at δ 6.00 (notice the strong coupling with the $6'_{a,b}$ protons) helps to identify the H-1' doublet at δ 5.47, which in turn identifies the H-2' doublet at δ 5.15. After D_2O exchange (data not shown), the higher field doublet at δ 4.72 collapses to a singlet as the OH doublet (δ 5.90) disappears.



SCHEME 1

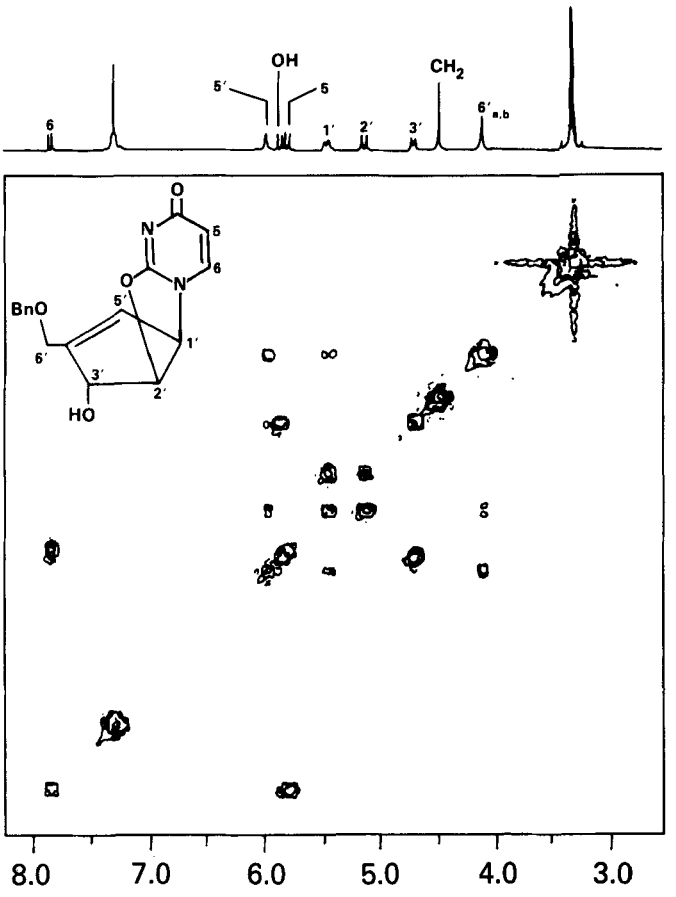
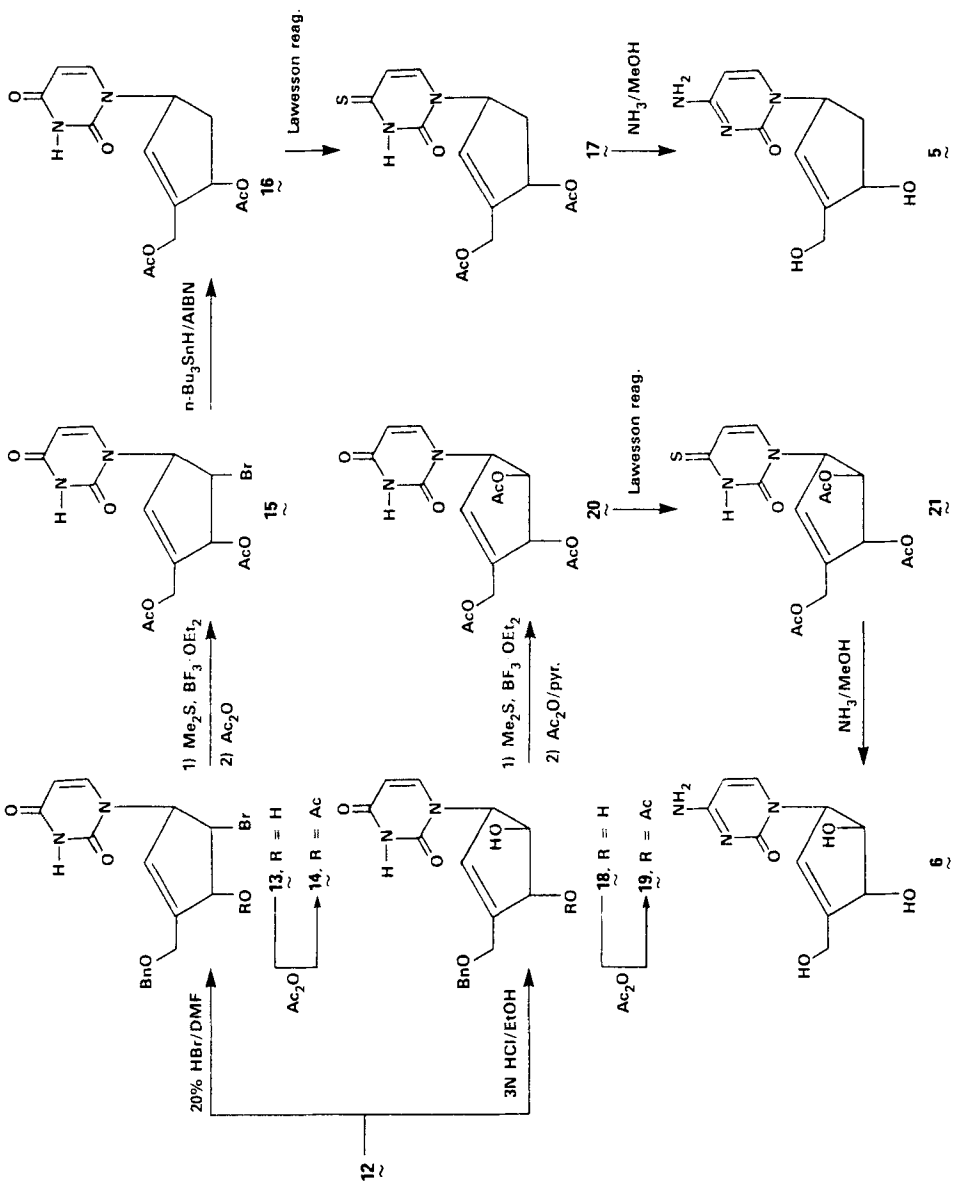


FIGURE 1. 2D-NMR COSY of anhydro-C-nucleoside 12 in Me_2SO-d_6

These observations clearly identify the signal at δ 4.72 as the H-3' proton on the carbon bearing the hydroxyl group, since with the exception of the strong coupling to the OH proton, this signal shows no other off-diagonal cross peaks.

Following nucleophilic ring-opening at C-2' with bromide (route a), the resulting bromo compound 13 was subsequently treated with acetic anhydride to give 14 (SCHEME 2). Debenzylation of 14 according to the method of Fuji et al.,[11] with Me_2S and $BF_3.OEt_3$, proceeded satisfactorily. However, isolation of the product proved difficult unless one formed the di-O-acetyl derivative 15. Homolytic cleavage of the C-Br bond in compound 15 by tri-n-butyltin hydride/AIBN gave the desired 2'-



SCHEME 2

TABLE 1. Inhibition of L1210 Cell Growth by CPE-C Nucleosides.

	% Inhibition Concentration (μM)				ID_{50} (μM)
	0.1	1	10	100	
CPE-C (4)	>90	>90	>90	>90	0.03
2'-d-CPE-C (5)	<10	<10	<10	<10	>100
ara-CPE-C (6)	<10	<10	<10	<10	>100

deoxy intermediate 16. Thiation with Lawesson's reagent generated the thio compound 17 which was then treated with saturated methanolic ammonia under pressure to give the target 2'-deoxy-CPE-C (5).

Cleavage at C-2 of the base (route b) was accomplished by acid hydrolysis (SCHEME 2) and treatment with acetic anhydride afforded the fully protected compound 19. Debenzylation of 19 with Me_2S/BF_3, followed by *in situ* reacetylation of the liberated hydroxyl function, gave the protected ara-CPE-U derivative 20. This compound was converted to ara-CPE-C (6) by the sequence of reactions already described for the synthesis of 2'-deoxy-CPE-C (SCHEME 2).

Initial *in vitro* cytotoxicity tests against L1210 leukemia cells in culture revealed that both "2'-deoxy" and "ara" configurations in the CPE-C class of carbocyclic nucleosides produced compounds devoid of any detectable cytotoxicity. As seen in TABLE 1, the simple removal or inversion of the critical 2'-OH group completely abrogated the potent cytoxicity observed with CPE-C.

In preliminary antiviral assays the "2'-deoxy" analogue was devoid of antiviral activity, against both herpes and influenza viruses (TABLE 2). The "ara" analogue, however, demonstrated activity only against influenza type A_2 virus (TABLE 2). Despite the fact that the MIC_{50} was rather high, the compound appeared to be relatively non-toxic with a minum toxic concentration above 1000 μg/mL.

As published by Shealy and coworkers, the saturated cyclopentane compounds, 2'-deoxycarbodine (2) and ara-carbodine (3), displayed activity against the same herpes virus and in the same assay system with

TABLE 2. Antiviral Activities of CPE-C Nucleosides Against DNA and RNA Viruses

	DNA Virus[a] Herpes Simplex Type 1 (E-377, TK+)		RNA Virus[b] Influenza Type A_2 (Aichi/2/68)	
	VR[c]	MIC_{50}[d]	VR	MIC_{50}
CPE-C (4)	3.8	0.3	2.4	18.2
2′-d-CPE-C (5)	0	---	0	---
ara-CPE-C (6)	0	---	1.3	173.2

[a]Vero cells (African green monkey kidney cells) were used as host cells.
[b]MDCK (Madin-Darby canine kidney cells) were used as host cells.
[c]For the definition of VR see reference 12.
[d]Minimum inhibitory concentration that causes 50% reduction in virus-induced cytopathogenicity.

VR values of 1.2 (MIC_{50} = 196 μg/mL) and 1.3 (MIC_{50} = 20 μg/mL), respectively.[3,12] When tested against the influenza virus, however, only ara-carbodine showed marginal activity having a VR value of 0.8 (MIC_{50} = 312 μg/mL).[3] Therefore, both 3 and 6 appear rather poor when compared to carbodine (VR = 3.5, MIC_{50} = 2.6 μg/mL)[3] or CPE-C (VR = 3.8, MIC_{50} = 0.3 μg/mL)[4] in the influenza test system.

From the standpoint of structure-activity correlations it is obvious that for the saturated series a departure from the "ribo" configuration is not detrimental to biological activity in general. The present results indicate that this situation has no parallel in the cyclopentene series where both changes drastically reduce antiviral activity and cytotoxic properties.

EXPERIMENTAL SECTION

All chemical reagents were commercially available. Melting points were determined on a Thomas-Hoover melting point apparatus and

are uncorrected (recrystallization solvents are indicated parenthetically after melting points). Proton NMR spectra were recorded on a Varian XL-200 instrument. Proton chemical shifts are expressed as δ values with reference to Me_4Si. Positive-ion fast atom bombardment (FAB) mass spectra were obtained on a VG 7070E mass spectrometer that was equipped with a FAB ion source. Elemental Analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

Biological Tests. In vitro cytotoxicity evaluations were performed by Dr. James D. Moyer of the Laboratory of Biological Chemistry, DTP, DCT, NCI. Antiviral evaluations were conducted under NIH purchase order 263-MD-610174 at Southern Research Institute, Birmingham, AL, under the direction of Dr. William Shannon and Ms. Gussie Arnett.

(1R,4R,5S)-1-[3'-[(Benzyloxy)methyl]-4',5'-dihydroxy-2'-cyclopenten-1'-yl]uracil (8). A solution of 7[4] (4.26 g, 11.5 mmole) in 40 mL of 60% trifluoroacetic acid was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (silica gel, EtOAc/MeOH, 30:1) to afford 3.31 g (87%) of 8 as a white foam; ^1H NMR (Me_2SO-d_6) δ 3.90 (m, 1 H, H-5'), 4.08 (br s, 2 H, H-6'$_{a,b}$), 4.30 (m, 1 H, H-4'), 4.51 (s, 2 H, CH_2Ph), 4.95 (d, J = 6.1 Hz, 1 H, OH), 5.07 (d, J = 6.8 Hz, 1 H, OH), 5.30 (br s, 1 H, H-1'), 5.56 (d, J = 8.0 Hz, H-5), 5.62 (s, 1 H, H-2'), 7.1-7.50 (m, 5 H, Ph, H-6). Anal. Calcd for $C_{17}H_{18}N_2O_5 \cdot 0.33\ H_2O$: C, 60.71; H, 5.59; N, 8.33. Found: C, 60.82; H, 5.95; N, 8.32.

(1R,4R,5S)-1-[3'-[(Benzyloxy)methyl]-4',5'-O-thiocarbonyl-2'-cyclopenten-1'-yl]uracil (9). A solution of 8 (3.17 g, 9.6 mmol) in dry THF (80 mL) was treated with 1,1'-thiocarbonyldiimidazole (2.56 g, 14.4 mmol) under a nitrogen atmosphere and the resulting mixture stirred at room temperature overnight. After removal of the solvent under reduced pressure, the residue was partitioned between chloroform and water. The organic layer was washed with brine, dried ($MgSO_4$), filtered, and concentrated under vacuum. The crude product was purified by column chromatography (silica gel, EtOAc/petroleum ether, 1:20) to give 3.29 g (92%) of the cyclic thiocarbonate 9, mp 105-7°C (EtOAc/petroleum ether); ^1H NMR ($CDCl_3$) δ 4.25 (br s, 2 H, H-6'$_{a,b}$), 4.57 (br s, 2 H, CH_2Ph),

5.04 (br s, 1 H, H-1′), 5.48 (d, J = 6.0 Hz, 1 H, H-5′), 5.76 (dd, J = 8.0 Hz, J′ = 2.0 Hz, 1 H, H-5), 5.87 (br s, 1 H, H-2′), 5.97 (d, J = 6.0 Hz, 1 H, H-4′), 7.10 (d, J = 8.0 Hz, 1 H, H-6), 7.34 (br s, 5 H, Ph), 8.42 (br s, 1 H, NH). Anal. Calcd for $C_{18}H_{16}N_2O_5 \cdot 0.20\ H_2O$: C, 57.50; H, 4.40; N, 7.45. Found: C, 57.88; H, 4.32; N, 7.04.

(1R,4R,5R)-1-[3′-[(Benzyloxy)methyl]-2,5′-anhydro-4′-hydroxy-2′-cyclopenten-1′-yl]-4(1H)-pyrimidinone (12). A suspension of the cyclic thiocarbonate **9** (1.79 g, 4.8 mmol) in dry toluene (60 mL) was heated at 100°C for 2 h under nitrogen in the presence of imidazole (0.33 g, 4.18 mmol). The solvent was then removed under reduced pressure and the residue chromatographed (silica gel, EtOAc/MeOH, 7:1) to give 1.12 g (75%) of the anhydro compound **12** as a crystalline solid, mp 193-94°C (acetone/petroleum ether); ^{1}H NMR (Me_2SO-d_6) δ 4.13 (s, 2 H, H-6′$_{a,b}$), 4.50 (s, 2 H, CH_2Ph), 4.72 (d, J = 6.4 Hz, 1 H, H-4′, converted to a singlet after D_2O exchange), 5.15 (d, J = 7.0 Hz, 1 H, H-5′), 5.47 (d, J = 7.0 Hz, 1 H, H-1′), 5.81 (d, J = 7.4 Hz, 1 H, H-5), 5.90 (d, J = 6.4 Hz, 1 H, OH), 6.00 (br s, 1 H, H-2′), 7.32 (s, 5 H, Ph), 7.87 (d, J = 7.4 Hz, 1 H, H-6). Anal. Calcd for $C_{17}H_{16}N_2O_4$: C, 65.38; H, 5.16; N, 8.97. Found: C, 65.43; H, 5.47; N, 8.80.

(1R,4R,5S)-1-[3′-[(Benzyloxy)methyl]-4′-hydroxy-5′-bromo-2′-cyclopenten-1′-yl]-uracil (13). A solution of the anhydro compound **12** (0.584 g, 1.87 mmol) in dry DMF (6 mL) was heated to 100°C under nitrogen in an oil bath. Ten minutes later, the heated solution was treated with an HBr solution (20% in DMF) and the resulting mixture was stirred at the same temperature for a total of 4 h. After cooling to room temperature, the reaction mixture was diluted with chloroform and washed with water and saturated brine. The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue obtained was chromatographed (silica gel, EtOAc/petroleum ether, 3:1) to afford 0.61 g (83%) of compound **13** as a colorless foam; ^{1}H NMR ($CDCl_3$) δ 4.24 (s, 2 H, H-6′$_{a,b}$), 4.32 (t, J = 6.0 Hz, 1 H, H-5′), 4.59 (s, 2 H, CH_2Ph), 4.67 (d, J = 6.0 Hz, 1 H, H-4′), 5.67 (m, 1 H, H-1′), 5.72 (d, J = 8.0 Hz, 1 H, H-5), 5.85 (br s, 1 H, H-2′), 7.02 (d, J = 8.0 Hz, 1 H, H-6), 7.35 (br s, 5 H, Ph), 8.20 (br s, 1 H, NH). Anal. Calcd for $C_{17}H_{17}N_2BrO_4 \cdot 0.6\ H_2O$: C, 50.54; H, 4.54; N, 6.93; Br, 19.78. Found: C, 50.26; H, 4.52; N, 6.72; Br, 19.83.

(1R,4R,5S)-1-[3'-[(Benzyloxy)methyl]-4'-acetoxy-5'-bromo-2'-cyclopenten-1'-yl]uracil (14). A solution of 13 (0.61 g, 1.55 mmol) in 7 mL of pyridine was treated with acetic anhydride (0.75 mL) and the resulting mixture stirred at room temperature for 1 h. Pyridine was azeotropically removed with isopropyl alchohol under vacuum and the residue was purified by flash chromatography (silica gel, EtOAc/petroleum ether, 1.5:1). The product-containing fractions were combined and evaporated to give 0.634 g (94%) of compound 14 as a crystalline solid, mp 58-62°C (ether/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 2.11 (s, 3 H, $COCH_3$), 4.11 (s, 2 H, H-6'$_{a,b}$), 4.33 (t, J = 6.0 Hz, 1 H, H-5'), 4.56 (s, 2 H, $C\underline{H}_2Ph$), 5.80 (m, 2 H, H-1', H-5), 5.85 (d, J = 6.0 Hz, 1 H, H-4'), 5.96 (d, J = 1. 5 Hz, 1 H, H-2'), 7.00 (d, J = 8.0 Hz, 1 H, H-6), 7.33 (br s, 5 H, Ph), 8.42 (br s, 1 H, NH). Anal. Calcd for $C_{19}H_{19}BrN_2O_5 \cdot 0.1\ H_2O$: C, 52.21; H, 4.43; N, 6.41; Br, 18.28. Found: C, 52.33; H, 4.43; N, 6.43; Br, 17.98.

(1R,4R,5S)-1-[3'-[(Acetoxy)methyl]-4'-acetoxy-5'-bromo-2'-cyclopenten-1'-yl]uracil (15). A solution of compound 14 (0.226 g, 0.52 mmol) in anhydrous 1,2-dichloroethane (15 mL) was treated with Me_2S (0.97 g, 15.6 mmol) and $BF_3 \cdot OEt_2$ (1.48 g, 10.4 mmol) under nitrogen. After stirring the resulting solution at room temperature for 7 h, 0.15 mL of acetic anhydride was added and stirring was continued for 0.5 h more. After the addition of 5 mL of 1,2-dichloromethane, water was added and the organic layer was washed further with water and brine, dried ($MgSO_4$) and reduced to dryness under reduced pressure. The residue was purified by flash chromatography (silica gel, EtOAc/petroleum ether, 2:1) to give 0.151 g (75%) of compound 15, mp 64-68°C (ether/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 2.10 (s, 3 H, $COCH_3$), 2.16 (s, 3 H, $COCH_3$), 4.42 (t, J = 6.0 Hz, 1 H, H-5'), 4.70 (s, 2 H, H-6'$_{a,b}$), 5.69 (dm, 1 H, H-1'), 5.78 (dd, J = 8.0 Hz, J' = 1.7 Hz, 1 H, H-5), 5.84 (d, J = 6.0 Hz, 1 H, H-4'), 5.96 (br s, 1 H, H-2'), 7.04 (d, J = 8.0 Hz, 1 H, H-6), 8.86 (br s, 1 H, NH). Anal. Calcd for $C_{14}H_{15}N_2BrO_6$: C, 43.43; H, 3.91; N, 7.23; Br, 20.64. Found: C, 43.51; H, 3.98; N, 7.18; Br, 20.77.

(1R,4R)-1-[3'-[(Acetoxy)methyl]-4'-acetoxy-2'-cyclopenten-1'-yl]uracil (16). A solution of compound 15 (0.115 g, 0.30 mmol) in 4 mL of toluene was added dropwise to a refluxing solution of tri-n-butyltin hydride

(0.13 g, 0.45 mmol) and azabis(isobutyrylnitrile) (AIBN, 0.01 g, 0.06 mmol) in 5 mL of toluene which was kept under nitrogen. Refluxing continued for 2 h and the solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (silica gel, EtOAc/petroleum ether, 7:1) to give 0.071 g (77 %) of compound **16**, mp 123-24°C (ether/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 2.08 (s, 3 H, $COCH_3$), 2.12 (s, 3 H, $COCH_3$), 2.00-2.20 (m, 1 H, H-5′$_a$), 2.40-2.60 (m, 1 H, H-5′$_b$), 4.72 (s, 2H, H-6′$_{a,b}$), 5.74 (dd, J = 8.0 Hz, J′ = 2.0 Hz, 1 H, H-5), 5.80-5.90 (m, 3 H, H-1′, H-2′, H-4′), 7.02 (d, J = 8.0 Hz, 1 H, H-6), 8.57 (br s, 1 H, NH). Anal. Calcd for $C_{14}H_{16}N_2O_6$: C, 54.54; H, 5.23; N, 9.09. Found: C, 54.82; H, 5.36; N, 9.06.

(1R,4R)-1-[3′-[(Acetoxy)methyl]-4′-acetoxy-2′-cyclopenten-1′-yl]-4-thioxouracil (17). A solution of compound **16** (0.12 g, 0.39 mmol) in dry toluene (15 mL) was treated with 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (Lawesson's reagent, 0.174 g, 0.43 mmol) and the resulting solution was heated at 100°C and stirred at that temperature for 1.5 h under nitrogen. The solvent was evaporated under reduced pressure and the residue purified by flash column chromatography (silica gel, EtOAc/petroleum ether, 1:1), to give 0.1 g (79 %) of **17** as a yellowish foam-like solid; ^{1}H NMR ($CDCl_3$) δ 2.02 (s, 3 H, $COCH_3$), 2.06 (s, 3 H, $COCH_3$), 2.15-2.25 (m, 1 H, H-5′$_a$), 2.40-2.60 (m, 1 H, H-5′$_b$), 4.66 (s, 2 H, H-6′$_{a,b}$), 5.70-5.90 (m, 3 H, H-1′, H-2′, H-4′), 6.34 (dd, J = 7.4 Hz, J′ = 1.9 Hz, 1 H, H-5), 6.80 (d, J = 7.4 Hz, 1 H, H-6), 9.79 (br s, 1 H, NH). This compound was used directly for the next reaction without further purification.

(1R,4R)-1-[3′-(Hydroxymethyl)-4′-hydroxy-2′-cyclopenten-1′-yl]cytosine (5). Compound **17** (0.10 g, 0.31 mmol) was dissolved in 25 mL of saturated methanolic ammonia and the solution kept in a stainless steel bomb at 80°C for 24 h. The solvent was removed under reduced pressure and the residue purified by C-18 reversed phase column chromatography using water as eluant. Fractions were monitored by UV (254 nm) and the collected fractions were lyophilized to afford 0.033 g (48%) of **5** as an amorphous powder; ^{1}H NMR (D_2O) δ 2.10 (m, 1 H, H-5′$_a$), 2.18 (m, 1 H, H-5′$_b$), 4.20 (s, 2 H, H-6′$_{a,b}$), 4.80 (br d, 1 H, H-4′), 5.50 (br s, 1 H, H-1′), 5.60 (s, 1 H, H-2′), 5.75 (d, J = 8.0 Hz, 1 H, H-5), 7.20 (d, J =

8.0 Hz, 1 H, H-6); FAB mass spectrum, m/z (relative intensity) 316 (MH + glycerol, 13), 224 (MH^+, 53), 112 (b + 2H, 100). Anal. Calcd for $C_{10}H_{13}N_3O_3 \cdot 0.75H_2O$: C, 50.73; H, 6.17; N, 17.75. Found: C, 50.82; H, 6.04; N, 17.78.

(1R,4R,5R)-1-[3'-[(Benzyloxy)methyl]-4',5'-dihydroy-2'-cyclopenten-1'-yl]uracil (18). A solution of the anhydride **12** (1 g, 3.2 mmol) in absolute ethanol (60 mL) was treated with 60 mL of 3 N HCl and the resulting solution was stirred for 3 days at room temperature. Ethanol was evaporated under reduced pressure and the remaining aqueous solution was extracted with chloroform and neutralized with conc. ammonium hydroxyde. The solid powder obtained after lyophilization was extracted with acetone and filtered. The organic solution was reduced to dryness and the solid residue was purified by flash column chromatography (silica gel, EtOAc/MeOH, 15:1) to give 0.95 g (90 %) of **18** as a colorless foam; ^1H NMR (Me_3SO-d_6) δ 4.00 (m, 1 H, H-5'), 4.12 (s, 2 H, H-$6'_{a,b}$), 4.34 (d, J = 5.9 Hz, 1 H, H-4'), 4.52 (s, 2 H, CH_2Ph), 5.25 (d, J = 6.6 Hz, 1 H, 4'-OH), 5.34 (d, J = 4.9 Hz, 1 H, 5'-OH), 5.40-5.60 (m, 2 H, H-1', H-5), 5.63 (s, 1 H, H-2'), 7.08 (d, J = 8.0 Hz, H-6), 7.34 (m, 5 H, Ph), 11.19 (s, 1 H, NH). Anal. Calcd for $C_{17}H_{18}N_2O_5 \cdot 0.5H_2O$: C, 60.19; H, 5.64; N, 8.26. Found: C, 60.17; H, 5.51; N, 8.35.

(1R,4R,5R)-1-[3'-[(Benzyloxy)methyl]-4',5'-diacetoxy-2'-cyclopenten-1'-yl]uracil (19). This compound was prepared by the same procedure used to prepare compound **14**. Compound **19** (0.896 g, 94% yield) was obtained from **18** (0.76 g, 2.3 mmol); mp 170-171°C (EtOH); ^1H NMR ($CDCl_3$) δ 1.96 (s, 3 H, $COCH_3$), 2.05 (s, 3 H, $COCH_3$), 4.12 (AB multiplet, 2 H, H-$6'_{a,b}$), 4.60 (AB multiplet, 2 H, CH_2Ph), 5.42 (m, 1 H, H-5'), 5.66 (dd, J = 8.0 Hz, J' = 2.0 Hz, 1 H, H-5), 5.76 (br s, 1 H, H-4'), 5.80-5.90 (m, 2 H, H-1', H-2'), 7.03 (d, J = 8.0 Hz, 1 H, H-6), 7.30 (m, 5 H, Ph), 8.75 (br s, 1 H, NH). Anal. Calcd for $C_{21}H_{22}N_2O_7$: C, 60.86; H, 5.35; N, 6.76. Found: C, 60.98; H, 5.73; N, 6.58.

(1R,4R,5R)-1-[3'-[(Acetoxy)methyl]-4',5'-diacetoxy-2'-cyclopenten-1'yl]uracil (20). This compound was obtained from compound **19** (0.216 g, 0.53 mmol) following an identical procedure as described for the synthesis of **15**. This procedure afforded 0.131 g (69%) of **20**, mp 151-

152°C (ether/petroleum ether) after flash column chromatography (silica gel, EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 3 H, $COCH_3$), 2.11 (s, 3 H, $COCH_3$), 2.12 (s, 3 H, $COCH_3$), 4.70 (AB multiplet, 2 H, H-6'$_{a,b}$), 5.43 (dd, J = 6.6 Hz, J' = 2.6 Hz, 1 H, H-5'), 5.70 (dd, J = 8.0 Hz, J' = 2.4 Hz, 1 H, H-5), 5.80 (br s, 1 H, H-4'), 5.86 (br s, 1 H, H-2'), 5.91 (m, 1 H, H-1'), 7.04 (d, J = 8.0 Hz, 1 H, H-6), 8.38 (br s, 1 H, NH). Anal. Calcd for $C_{16}H_{18}N_2O_8 \cdot 0.5H_2O$: C, 51.20; H, 5.10; N, 7.46. Found: C, 51.18; H, 4.91; N, 7.64.

(1R,4R,5R)-1-[3'-[(Acetoxy)methyl]-4',5'-diacetoxy-2'-cyclopenten-1'-yl]-4-thioxouracil (21). Treatment of compound **20** (0.103 g, 0.28 mmol) with Lawesson's reagent as described for the synthesis of **17**, afforded 0.099 g (92%) of **21** as a yellowish foam after purification by flash column chromatography (silica gel, EtOAc/petroleum ether, 1:1); ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 3 H, $COCH_3$), 2.11 (s, 3 H, $COCH_3$), 2.12 (s, 3 H, $COCH_3$), 4.71 (AB multiplet, 2 H, H-6'$_{a,b}$), 5.45 (dd, J = 6.9 Hz, J' = 2.7 Hz, 1 H, H-5'), 5.80-6.00 (m, 3 H, H-4', H-2', H-1'), 6.37 (dd, J = 7.7 Hz, J' = 1.8 Hz, H-5), 6.86 (d, J = 7.7 Hz, 1 H, H-6), 9.31 (br s, 1 H, NH). This compound was used in the next and final reaction without any further purification.

(1R,4R,5R)-1-[3'-(Hydroxymethyl)-4',5'-dihydroxy-2'-cyclopenten-1'-yl]cytosine (6). Treatment of **21** (0.099 g, 0.26 mmol) with a solution of concentrated methanolic ammonia, under pressure, as described for the synthesis of compound **5**, afforded 0.039 g (63%) of **6** as a white lyophilized powder (purified by C-18 reversed phase chromatography using water as eluant); ^{1}H NMR (D_2O) δ 3.59 (br s, 2 H, H-6'$_{a,b}$), 3.63 (partially hidden m, 1 H, H-5'), 3.88 (br s, 1 H, H-4'), 4.90 (m, 1 H, H-1'), 5.08 (br s, 1 H, H-2'), 5.24 (d, J = 7.4 Hz, 1 H, H-5), 6.63 (d, J = 7.4 Hz, 1 H, H-6); FAB mass spectrum, m/z (relative intensity) 332 (MH + glycerol, 13), 240 (MH^+, 89), 112 (b + 2H, 100). Anal. Calcd for $C_{10}H_{13}N_3O_4 \cdot 0.25H_2O$: C, 49.27; H, 5.58; N, 17.24. Found: C, 49.48; H, 5.57; N, 17.29.

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REFERENCES

1. Throughout the discussion in this article the numbering system used to describe the carbocyclic nucleoside analogues will be the same as the one employed for conventional nucleosides (numbers outside parenthesis in structure 4). However, in the Experimental Section the numbering system is changed in order to described these compounds as cyclopentenyl derivatives (numbers inside parenthesis in structure 4).
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