1-(3,5-*O*-ALKYLIDENE-2-DEOXY-4-*C*-HYDROXYMETHYL-α-L-*threo*-PENTOFURANOSYL)URACILS

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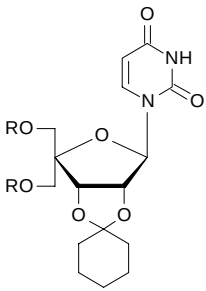
1-(2,3-*O*-Cyclohexylidene-4-*C*-hydroxymethyl-α-L-lyxofuranosyl)uracil (**1**) was converted in seven steps into 1-(2-deoxy-4-*C*-hydroxymethyl-α-L-*threo*-pentofuranosyl)uracil (**8**) and further into 1-(2-deoxy-4-*C*-hydroxymethyl-3,5-*O*-isopropylidene-α-L-*threo*-pentofuranosyl)uracil (**9**). Successive benzoylation, removal of the isopropylidene group, reaction with acetaldehyde diethyl acetal, and debenzoylation afforded (*R*)- and (*S*)-1-(2-deoxy-3,5-*O*-ethylidene-4-*C*-hydroxymethyl-α-L-*threo*-pentofuranosyl)uracil (**10a** and **10b**, respectively). Reaction of 1-(2-deoxy-4-*C*-triphenylmethyloxymethyl-α-L-*threo*-pentofuranosyl)uracil (**14**) with dichloromethane under conditions of phase transfer, followed by detritylation, afforded 1-(2-deoxy-4-*C*-hydroxymethyl-3,5-*O*-methylidene-α-L-*threo*-pentofuranosyl)uracil (**15**). Compound **14** was obtained from the derivative **8** by partial silylation, tritylation and desilylation. The absolute configuration of the isomeric ethylidene derivatives **10a** and **10b** was determined by NMR spectroscopy and the population of the deoxypentofuranose ring conformers was derived from the vicinal coupling constants *J*(H,H). The obtained results were compared with energy calculations. Neither of the prepared nucleoside analogues was active *in vitro* against HIV-1 and HIV-2.

Key words: 4′-*C*-Hydroxymethyluridine; Alkylidene derivatives; Conformational analysis.

Some of the recently synthesized 4′-*C*-substituted nucleosides, such as 4′-*C*-azido-2′-deoxythymidine[1,2], 4′-*C*-cyano-2′-deoxythymidine[3] and 1-(3,5-anhydro-2-deoxy-4-*C*-hydroxymethyl-α-*threo*-pentofuranosyl)thymine[4] exhibit marked activity against HIV. Also 1-(2,5-dideoxy-4-*C*-hydroxymethyl-α-L-*threo*-pentofuranosyl)thymine, 1-(5-azido-2,5-dideoxy-4-*C*-hydroxymethyl-α-L-*threo*-pentofuranosyl)thymine, 1-(2-deoxy-4-*C*-hydroxymethyl-α-L-*threo*-pentofuranosyl)thymine[4] and 9-(2-deoxy-4-*C*-hydroxymethyl-α-L-*threo*-pentofuranosyl)adenine[5] are active but their activity is lower. Obviously, the activity is connected with the presence of a hydroxy group in position 3′, derivatives with an electron-withdrawing group in position 4′-C being the most active. On the other hand, also the 3′,5′-anhydro derivative without any hydroxyl in the position 3′ is reported to be highly active.

The objective of the present study has been to synthesize 3′,5′-*O*-alkylidene derivatives of 1-(2-deoxy-4-*C*-hydroxymethyl-α-L-*threo*-pentofuranosyl)uracil and to investigate the effect of an alkylidene grouping on the virostatic activity of these compounds.



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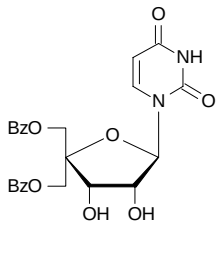

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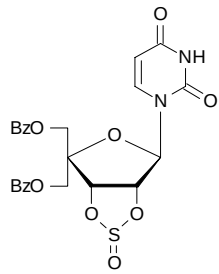

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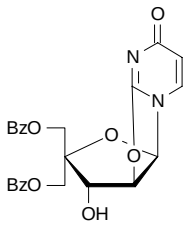

5

	R^1	R^2	R^3
6	Bz	Bz	Cl
7	Bz	Bz	H
8	H	H	H
11	Bz	H	H
12	H	TBDPS	H
13	TBDPS	TBDPS	H
14	Tr	H	H

	R^1	R^2
9	CH_3	CH_3
10a	H	CH_3
10b	CH_3	H
15	H	H

TBDPS = *tert*-Butyldiphenylsilyl

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Infrared spectra were recorded on a Zeiss UR 20 spectrometer (wavenumbers in cm^{-1}). Proton NMR spectra of compounds **2**, **3**, **5–9** and **11–15** were taken on a Varian UNITY-200 spectrometer in CD_3SOCD_3 and referenced to the solvent residual signal (δ 2.50). ^1H and ^{13}C NMR spectra of the isomers **10a** and **10b** were measured on a Varian UNITY-500 instrument in CD_3SOCD_3 and $CDCl_3$. 2D-ROESY spectra of these compounds in CD_3SOCD_3 (mixing time 250 ms) were used for determination of their absolute con-

figuration. The calculations were run on an SGI Power Challenge computer at the Computer Centre of the Academy of Sciences of the Czech Republic using Gaussian 94 program package[6]. Column chromatography was performed on silica gel 30–60 μm (Service Laboratories of this Institute) and thin-layer chromatography (TLC) on Silufol UV 254 sheets (Kavalier, Votice). The solvents were evaporated at bath temperature 30–60 °C/2 kPa and the compounds were dried over phosphorus pentoxide at 13 Pa.

1-(5-*O*-Benzoyl-4-*C*-benzoyloxymethyl-2,3-*O*-cyclohexylidene-α-L-lyxofuranosyl)uracil (**2**)

A solution of benzoyl chloride (5.5 ml, 47 mmol) in pyridine (30 ml) was added at 0 °C during 2 h to a stirred solution of 1-(2,3-*O*-cyclohexylidene-4-*C*-hydroxymethyl-α-L-lyxofuranosyl)uracil[7] (**1**; 7.08 g, 20 mmol) in pyridine (50 ml). The mixture was set aside at 0 °C for 30 min, mixed with methanol (3 ml) and after 15 min the solvent was evaporated. The residue was dissolved in ethyl acetate (250 ml), washed successively with water (100 ml), 5% hydrochloric acid (to acidic reaction of the washings), and 5% sodium hydrogen carbonate solution. After drying over magnesium sulfate and evaporation, the product was crystallized from 2-propanol to give 10.09 g (90%) of benzoyl derivative **2**, m.p. 198.5–199.5 °C. For $C_{30}H_{30}N_2O_9$ (562.5) calculated: 64.05% C, 5.38% H, 4.98% N; found: 64.12% C, 5.39% H, 5.14% N. ^{1}H NMR spectrum (200 MHz): 1.25–1.80 m, 10 H (H-cyclohexylidene); 4.56 s, 2 H (CH_2O); 4.64 s, 2 H (CH_2O); 5.12 d, 1 H, J(3′,2′) = 6.7 (H-3′); 5.30 dd, 1 H, J(2′,1) = 2.2, J(2′,3′) = 6.7 (H-2′); 5.53 d, 1 H, J(5,6) = 7.9 (H-5); 5.97 d, 1 H, J(1′,2′) = 2.2 (H-1′); 7.47–7.57 m, 4 H, 7.62–7.73 m, 3 H and 7.93–8.02 m, 4 H (H-6, H-arom.); 11.47 s, 1 H (NH).

1-(5-*O*-Benzoyl-4-*C*-benzoyloxymethyl-α-L-lyxofuranosyl)uracil (**3**)

A solution of cyclohexylidene derivative **2** (8.44 g, 15 mmol) in 90% aqueous trifluoroacetic acid (50 ml) was set aside at room temperature for 1 h and then poured slowly into a stirred mixture of sodium hydrogen carbonate (49 g) and water (200 ml). The precipitated crystalline material was collected and washed with water. Yield 7.11 g (98%) of compound **3**, m.p. 204–206 °C (2-propanol). For $C_{24}H_{22}N_2O_9$ (482.4) calculated: 59.75% C, 4.60% H, 5.81% N; found: 59.52% C, 4.49% H, 6.05% N. ^{1}H NMR spectrum (200 MHz): 4.31–4.67 m, 6 H (2 × CH_2O, H-2′, H-3′); 5.58–5.63 m, 2 H (H-5, OH); 5.71 d, 1 H, J = 5.2 (OH); 5.96 d, 1 H, J(1′,2′) = 7.0 (H-1′); 7.46–7.73 m, 7 H and 7.94–7.99 m, 4 H (H-6, H-arom.); 11.41 s, 1 H (NH); after exchange with D_2O: 5.56 d, 1 H, J(5,6) = 7.9 (H-5).

2,2′-Anhydro-1-(5-*O*-benzoyl-4-*C*-benzoyloxymethyl-α-L-xylofuranosyl)uracil (**5**)

Thionyl chloride (2 ml) was added to a stirred suspension of dibenzoate **3** (4.82 g, 10 mmol) in acetonitrile (50 ml) and the mixture was stirred at 40 °C for 1 h. After concentration to half of the original volume, the residue was diluted with ethyl acetate (200 ml) and the solution was washed with cold 5% sodium hydrogen carbonate solution (2 × 50 ml) and cold water (50 ml), dried over magnesium sulfate and the solvent was evaporated. The residue was dissolved in acetonitrile (40 ml) and 1,8-diazabicyclo[5.4.0]undec-7-ene (2.5 ml) was added. After standing at room temperature overnight, the solution was diluted with chloroform (200 ml), washed with water (3 × 50 ml), dried over magnesium sulfate and the solvent was evaporated. Crystallization of the residue afforded 2.94 g (63%) of anhydronucleoside **5**, m.p. 230–232 °C. Another portion (370 mg; 8%) of the product was obtained from the mother liquors by chromatography on a column of silica gel (50 g) in ethyl acetate–acetone–ethanol–water (40 : 6 : 3 : 1). For $C_{24}H_{20}N_2O_8$ (464.4) calculated: 62.07% C, 4.34% H, 6.03% N; found: 61.83% C, 4.22% H, 6.22% N. ^{1}H NMR spectrum (200 MHz): 4.43–4.67 m, 5 H (2 × CH_2O, H-3′); 5.41 dd, 1 H, J(2′,1′) = 6.0, J(2′,3′) = 2.9 (H-2′); 5.83 d, 1 H, J(5,6) = 7.5 (H-5); 6.47 d, 1 H, J(1′,2′) = 6.0 (H-1′); 6.52 d, 1 H, J(OH,3′) = 5.6 (3′-OH); 7.44–8.05 m, 11 H (H-6,

H-arom.); after exchange with D_2O: 4.41 s, 2 H (CH_2O); 4.44 d, 1 H and 4.59 d, 1 H, *J*(gem) = 11.5 (CH_2O); 4.63 d, 1 H, *J*(2′,3′) = 2.9 (H-3′).

1-(5-*O*-Benzoyl-4-*C*-benzoyloxymethyl-2-chloro-2-deoxy-α-L-lyxofuranosyl)uracil (**6**)

A solution of anhydro derivative **5** (4.64 g, 10 mmol) in 1 M HCl in dimethylformamide (20 ml) was heated at 100 °C for 40 min. After evaporation, the residue was codistilled with xylene (2 × 20 ml), dissolved in ethyl acetate (100 ml), the solution was washed with 5% sodium hydrogen carbonate (50 ml), dried over magnesium sulfate and the solvent was evaporated. Yield 4.86 g (97%) of chloro derivative **6** as a solid foam. For $C_{24}H_{21}ClN_2O_8$ (500.9) calculated: 57.55% C, 4.23% H, 7.08% Cl, 5.59% N; found: 57.31% C, 4.40% H, 6.81% Cl, 5.31% N. ^{1}H NMR spectrum (200 MHz): 4.52–4.68 m, 5 H (2 × CH_2O, H-3′); 5.09 dd, 1 H, *J*(2′,1′) = 8.8, *J*(2′,3′) = 5.1 (H-2′); 5.73 dd, 1 H, *J*(5,NH) = 2.1, *J*(5,6) = 7.9 (H-5); 6.20 d, 1 H, *J*(1′,2′) = 8.8 (H-1′); 6.43 d, 1 H, *J*(OH,3′) = 6.2 (3′-OH); 7.44–7.78 m, 7 H and 7.94–7.98 m, 4 H (H-6, H-arom.); 11.54 d, 1 H, *J*(5,NH) = 2.1 (NH).

1-(5-*O*-Benzoyl-4-*C*-benzoyloxymethyl-2-deoxy-α-L-*threo*-pentofuranosyl)uracil (**7**)

To a stirred solution of chloro derivative **6** (5.0 g, 10 mmol) in dioxane (25 ml), heated at 100 °C, were added 1 M solution of tributylstannane in toluene (15 ml) and 2,2′-azobis(2-methylpropionitrile) (200 mg). After heating for 40 min, the mixture was evaporated and the residue was mixed with light petroleum (100 ml). The precipitate was collected and chromatographed on a column of silica gel (250 g) in ethyl acetate. Crystallization from 2-propanol gave 3.30 g (71%) of deoxy derivative **7**, m.p. 174–175.5 °C. For $C_{24}H_{22}N_2O_8$ (466.4) calculated: 61.80% C, 4.75% H, 6.01% N; found: 62.00% C, 4.77% H, 6.01% N. ^{1}H NMR spectrum (200 MHz): 2.28–2.57 m, 2 H (2 × H-2′); 4.48–4.67 m, 5 H (2 × CH_2O, H-3′); 5.33 dd, 1 H, *J*(5,6) = 7.9, *J*(5,NH) = 1.5 (H-5); 5.78 d, 1 H, *J*(OH, 3′) = 4.9 (3′-OH); 6.31 dd, 1 H, *J*(1′,2′) = 6.4, *J*(1′,2″) = 7.0 (H-1′); 7.48–7.56 m, 4 H, 7.61–7.71 m, 3 H and 7.95–8.00 m, 4 H (H-6, H-arom.); 11.36 brs, 1 H (NH).

1-(2-Deoxy-4-*C*-hydroxymethyl-α-L-*threo*-pentofuranosyl)uracil (**8**)

A solution of dibenzoyl derivative **7** (3.26 g, 7 mmol) in 0.1 M methanolic sodium methoxide (50 ml) was set aside at room temperature for 2 days. After neutralization with Dowex 50 (H^+ form), the ion exchanger was filtered and washed with methanol. The filtrate was taken down, the residue washed with ether and crystallized from 2-propanol to give 1.57 g (87%) of compound **8**, m.p. 168–169.5 °C. ^{1}H NMR spectrum (200 MHz): 2.18 dd, 2 H, *J*(2′,1′) = 6.7, *J*(2′,3′) = 5.0 (H-2′, H-2″); 3.38–3.60 m, 4 H (2 × CH_2O); 4.32 q, 1 H, *J*(3′,OH) = 4.9, *J*(2′,3′) ≈ *J*(2″,3′) = 5.0 (H-3′); 4.45 t, 1 H, *J*(OH,CH_2) = 5.8 (CH_2OH); 4.99 t, 1 H, *J*(OH,CH_2) = 5.2 (CH_2OH); 5.12 d, 1 H, *J*(OH,3′) = 4.9 (3′-OH); 5.63 d, 1 H, *J*(5,6) = 8.2 (H-5); 6.19 t, 1 H, *J*(1′,2′) ≈ *J*(1′,2″) = 6.7 (H-1′); 7.91 d, 1 H, *J*(6,5) = 8.2 (H-6); 11.25 s, 1 H (NH).

1-(4-*C*-Hydroxymethyl-3,5-*O*-isopropylidene-α-L-*threo*-pentofuranosyl)uracil (**9**)

To a stirred suspension of free nucleoside **8** (516 mg, 2 mmol) in a mixture of acetone (8 ml) and 2,2-dimethoxypropane (3 ml) was added sulfuric acid (3 drops). After standing for 1 h, dimethylformamide (4 ml) was added and the solution was allowed to stand for 15 min at room temperature. The solution was neutralized with finely ground sodium hydrogen carbonate, the insoluble portion was filtered off and washed with acetone. The combined filtrates were evaporated, the residue was codistilled with xylene and mixed with ether. The crystalline product was collected and washed with ether to give 567 mg (95%) of isopropylidene derivative **9**, m.p. 222–225 °C. For

$C_{13}H_{18}N_2O_6$ (298.3) calculated: 52.34% C, 6.08% H, 9.39% N; found: 52.37% C, 6.13% H, 9.29% N. IR spectrum ($CHCl_3$): 3 629 (OH); 3 391 (NH); 1 717, 1 695 (C=O); 2 993, 2 884 (CH_3); 1 384, 1 363 ($C(CH_3)$); 1 177, 1 156, 1 117, 1 097, 1 077, 1 048 (C–O). ^{1}H NMR spectrum (200 MHz): 1.30 s, 3 H and 1.33 s, 3 H ($C(CH_3)_2$); 2.09–2.29 m, 2 H (H-2′, H-2″); 3.42–3.63 m, 4 H (2 × CH_2O); 4.37 d, 1 H, J(3′,2′) = 3.4 (H-3′); 5.30 t, 1 H, J(OH,CH_2) = 5.2 (CH_2OH); 5.67 d, 1 H, J(5,6) = 8.2 (H-5); 6.29 dd, 1 H, J(1′,2′) = 8.5, J(1′,2″) = 6.1 (H-1′); 7.91 d, 1 H, J(6,5) = 8.2 (H-6); 11.34 s, 1 H (NH).

(*R*)- and (*S*)-1-(2-Deoxy-4-*C*-hydroxymethyl-3,5-*O*-ethylidene-α-L-*threo*-pentofuranosyl)uracil (**10a** and **10b**)

A. A solution of monobenzoyl derivative **11** (725 mg, 2 mmol) in a mixture of dimethylformamide (2.5 ml) and acetaldehyde diethyl acetal (2.5 ml) was acidified with concentrated sulfuric acid (80 μl), stirred at room temperature for 2 days and then neutralized with aqueous ammonia. The obtained crystalline product was recrystallized from water to give 320 mg (56%) of ethylidene derivative **10a**, m.p. 246–249 °C. For $C_{12}H_{16}N_2O_6$ (284.3) calculated: 50.70% C, 5.67% H, 9.85% N; found: 50.67% C, 5.74% H, 9.65% N. IR spectrum ($CHCl_3$): 3 628 (OH); 3 391 (NH); 1 716, 1 694 (C=O); 1 635 (C=C); 2 958 (CH_3); 1 142, 1 127, 1 088, 1 065 (C–O). For ^{1}H NMR spectrum (500 MHz) see Table I.

The combined mother liquors were concentrated and chromatographed on a column of silica gel (70 g) in ethyl acetate–acetone–ethanol–water (105 : 15 : 3 : 2). The second fraction on crystallization afforded another 33 mg (6%) of the product **10a**. The residue after evaporation of the first fraction was layered with ether and the crystalline product was collected to give 74 mg (13%) of the isomer **10b**, m.p. 193–196 °C. For $C_{12}H_{16}N_2O_6$ (284.3) calculated: 50.70% C, 5.67% H, 9.85% N; found: 50.64% C, 5.70% H, 9.84% N. IR spectrum ($CHCl_3$): 3 631, 3 454 (OH); 3 392 (NH); 1 716, 1 693 (C=O); 1 635 (C=C); 2 965, 2 986, 2 881 (CH_3); 1 151, 1 126, 1 109, 1 089, 1 072, 1 054 (C–O). For ^{1}H NMR spectrum (500 MHz) see Table I.

B. Concentrated sulfuric acid (25 ml) was added to a stirred mixture of deoxy derivative **8** (129 mg, 0.5 mmol), dimethylformamide (0.8 ml) and acetaldehyde diethyl acetal (0.8 ml), and the formed solution was allowed to stand at room temperature for 2 h. TLC of the mixture in ethyl acetate–acetone–ethanol–water (40 : 6 : 3 : 1) exhibited five UV-absorbing spots (R_F 0.33, 0.41, 0.53, 0.65 and 0.74). The mixture was concentrated and chromatographed on a column of silica gel (20 g) in the above-mentioned solvent system. The fraction of R_F 0.41 gave 31 mg (22%) of (*R*)-ethylidene derivative **10a** and the fraction of R_F 0.53 afforded 13 mg (9%) of (*S*)-ethylidene derivative **10b**.

1-(4-*C*-Benzoyloxymethyl-2-deoxy-α-L-*threo*-pentofuranosyl)uracil (**11**)

Triethylamine (0.3 ml) was added to a stirred mixture of isopropylidene derivative **9** (597 mg, 2 mmol), acetonitrile (6 ml) and benzoyl cyanide (290 mg). After 20 min, methanol (0.3 ml) was added and after another 10 min the solvent was evaporated. The residue was mixed with 80% aqueous methanol (10 ml) and Dowex 50 (H^+ form; 1.6 ml) and the mixture was refluxed for 2 h. The ion exchanger was removed by filtration of the hot mixture and washed with methanol (5 ml). Upon cooling, the combined filtrates afforded 286 mg (39%) of benzoyl derivative **11**. The mother liquors on crystallization from methanol gave another portion (271 mg; 37%) of the product, m.p. 120–121 °C. For $C_{17}H_{18}N_2O_7$ (362.3) calculated: 56.35% C, 5.01% H, 7.73% N; found: 56.07% C, 5.22% H, 7.49% N. ^{1}H NMR spectrum (200 MHz): 2.32 m, 2 H (H-2′, H-2″); 3.59–3.75 m, 2 H (H-5′, H-5″); 4.36–4.50 m, 3 H (H-3′, CH_2O); 4.75 t, 1 H, J(OH,5′) ≈ J(OH,5″) = 5.7 (5′-OH); 5.41 d, 1 H, J(OH,3′) = 4.9 (3′-OH); 5.47 dd, 1 H, J(5,NH) = 1.8, J(5,6) = 7.9 (H-5); 6.23 t, 1 H, J(1′,2′) = J(1′,2″) = 6.7 (H-1′); 7.50–7.73 m, 4 H and 7.97–8.01 m, 2 H (H-6, H-arom.); 11.32 d, 1 H, J(NH,5) = 1.8 (NH); after exchange with D_2O: 3.62 d, 1 H, J(5′,5″) = 11.8 (H-5′); 3.67 d, 1 H, J(5″,5′) = 11.8 (H-5″); 4.35 d,

TABLE I
Proton and carbon-13 NMR parameters of compounds **10a** and **10b**

^{1}H NMR										^{13}C NMR		
	δ, ppm					*J*, Hz					δ, ppm	
Proton	**10a** (*R*)		**10b** (*S*)		*J*(H,H)	**10a** (*R*)		**10b** (*S*)		Carbon	**10a** (*R*)	**10b** (*S*)
	DMSO	$CDCl_3$	DMSO	$CDCl_3$		DMSO	$CDCl_3$	DMSO	$CDCl_3$		DMSO	DMSO
H-5	5.64	5.73	5.63	5.75	H-5,H-6	8.1	8.0	8.1	8.1	C-2	150.80	150.60
H-6	7.95	7.62	7.85	7.67	H-5,NH	~0	2.3	~0	2.3	C-4	163.33	163.37
NH	11.33	7.91	11.30	8.10	H-1′,H-2′	9.5	9.4	4.6	6.9	C-5	101.96	102.07
H-1′	6.26	6.21	6.20	6.26	H-1′,H-2″	5.4	5.3	7.5	6.5	C-6	140.73	140.88
H-2′	2.37	2.69	2.18	2.43	H-2′,H-2″	13.4	13.2	14.1	14.0	C-1′	85.66	83.46
H-2″	2.17	2.40	2.78	2.60	H-2′,H-3′	3.9	4.1	8.0	7.0	C-2′	38.08	33.48
H-3′	4.4	4.40	4.60	4.64	H-2″,H-3′	<0.5	~0.9	7.2	4.1	C-3′	76.75	71.63
H-5′a	3.39	3.68	3.66	4.00	H-5′a,H-5′b	[a]	11.4	12.1	11.5	C-4′	81.19	79.80
H-5′b	3.39	3.62	3.56	3.80	H-5′a,OH	[b]	5.6	5.4	6.1	C-5′	63.40	62.06
OH	5.27	2.54	5.30	2.37	H-5′b,OH	[b]	4.6	5.4	3.9	$O–CH_2$	68.07	65.67
O–CH–O	4.64	4.66	5.07	5.09	O–CHaHb	12.7	12.5	11.7	11.7	O–CH–O	95.71	92.54
CH_3	1.21	1.36	1.19	1.33	CH,CH_3	5.0	5.1	5.0	5.1	CH3	20.90	19.94
$O–CH_2–$	3.85	4.06	3.67	3.66								
	3.76	3.68	3.59	3.55								

[a] *J*-Value could not be determined due to chemical shift equivalence of protons. [b] Only the sum of *J*(H-5′a,OH) + *J*(H-5′b,OH) = 10.2 Hz could be obtained.

1 H, *J*(gem) = 11.9 and 4.42 d, 1 H, *J*(gem) = 11.9 (CH_2O); 4.46 t, 1 H, *J*(3′,2′) = 5.8, *J*(3′,2″) = 6.1 (H-3′).

1-[5-*O*-*tert*-Butyldiphenylsilyl-4-*C*-(*tert*-butyldiphenylsilyloxymethyl)-2-deoxy-α-*threo*-pentofuranosyl]uracil (**13**) and 1-(5-*O*-*tert*-Butyldiphenylsilyl-2-deoxy-4-*C*-hydroxymethyl-α-*threo*-pentofuranosyl)uracil (**12**)

To a solution of free deoxy derivative **8** (1.03 g, 4 mmol) and imidazole (544 mg, 8 mmol) in dimethylformamide (10 ml) was added *tert*-butylchlorodiphenylsilane in 8 portions (0.2 ml each, 6 mmol) in the course of 6 h. Dimethylformamide was evaporated, the residue was dissolved in ethyl acetate (100 ml), washed with water (2 × 20 ml) and dried over magnesium sulfate. After evaporation of the solvent, the residue was chromatographed on a column of silica gel (200 g) in ethyl acetate–toluene (8 : 1). The faster moving fraction afforded 1.50 g (51%) of disilyl derivative **13** as a solid foam. For $C_{42}H_{50}N_2O_6Si_2$ (735.0) calculated: 68.63% C, 6.86% H, 3.81% N; found: 68.90% C, 6.99% H, 3.57% N. ^{1}H NMR spectrum (200 MHz): 0.93 s, 0.95 s and 0.99 s, 18 H (2 × $C(CH_3)_3$); 2.13–2.36 m, 2 H (H-2′, H-2″); 3.82 d, 1 H, *J*(gem) = 11.3 and 3.89 d, 1 H, *J*(gem) = 11.3 (CH_2O); 3.80–3.88 m, 2 H (H-5′, H-5″); 4.41–4.50 m, 1 H (H-3′); 5.32 dd, 1 H, *J*(5,NH) = 1.9, *J*(5,6) = 8.0 (H-5); 5.40 d, 1 H, *J*(OH,3′) = 4.6 (3′-OH); 6.26 t, 1 H, *J*(1′,2′) = *J*(1′,2″) = 6.4 (H-1′); 7.32–7.51 m, 12 H and 7.58–7.72 m, 9 H (H-6, H-arom.); 11.34 d, 1 H, *J*(NH,5) = 1.9 (NH).

The slower fraction gave 940 mg (47%) of monosilyl derivative **12** as a solid foam. For $C_{26}H_{32}N_2O_6Si$ (496.6) calculated: 62.88% C, 6.50% H, 5.64% N; found: 62.60% C, 6.65% H, 5.89% N. ^{1}H NMR spectrum (200 MHz): 0.99 s, 9 H ($C(CH_3)_3$); 2.23 t, 2 H, *J*(2′,1′) = *J*(2′,3′) = 4.6 (H-2′, H-2″); 3.63 d, 2 H, *J*(CH_2,OH) = 5.2 (CH_2O); 3.74 d, 1 H, *J*(5′,5″) = 10.9 (H-5′), 3.77 d, 1 H, *J*(5″,5′) = 10.9 (H-5″); 4.39 q, 1 H, *J*(3′,2′) = *J*(3′,2″) = *J*(3′,OH) = 4.6 (H-3′); 5.09 t, 1 H, *J*(OH,CH_2) = 5.2 (CH_2OH); 5.24 d, 1 H, *J*(OH,3′) = 4.6 (3′-OH); 5.66 dd, 1 H, *J*(5,NH) = 2.1, *J*(5,6) = 7.9 (H-5); 6.25 t, 1 H, *J*(1′,2′) = *J*(1′,2″) = 4.6 (H-1′); 7.37–7.48 m, 6 H and 7.67–7.73 m, 4 H (H-arom.); 7.91 d, 1 H, *J*(6,5) = 7.9 (H-6); 11.30 d, 1 H, *J*(NH,5) = 2.1 (NH).

1-(2-Deoxy-4-*C*-triphenylmethyloxymethyl-α-L-*threo*-pentofuranosyl)uracil (**14**)

A solution of silyl derivative **12** (993 mg, 2 mmol) and triphenylmethyl chloride (670 mg, 2.4 mmol) in pyridine (8 ml) was heated at 100 °C for 1 h. After cooling, the solution was diluted with ethyl acetate (50 ml), washed with water (3 × 20 ml), dried over magnesium sulfate, and the solvent was evaporated. The residue was dissolved in tetrahydrofuran (8 ml) and 1 M solution of tetrabutylammonium fluoride in tetrahydrofuran (2.2 ml) was added. After standing for 1 h at room temperature, the solution was diluted with ethyl acetate (100 ml), washed with water (2 × 30 ml), dried over magnesium sulfate and the solvent was evaporated. Crystallization from toluene afforded 770 mg (77%) of trityl derivative **14**, m.p. 176–179 °C. For $C_{29}H_{28}N_2O_6$ (500.5) calculated: 69.58% C, 5.64% H, 5.60% N; found: 69.69% C, 5.64% H, 5.41% N. ^{1}H NMR spectrum (200 MHz): 2.08–2.21 m, 1 H (H-2′); 2.28–2.41 m, 1 H (H-2″); 3.11 d, 1 H, *J*(gem) = 9.8 and 3.27 d, 1 H, *J*(gem) = 9.8 (CH_2O); 3.56 d, 2 H, *J*(5′,OH) = 5.5 (H-5′,H-5″); 4.47 m, 1 H, *J*(3′,2′) = 5.5, *J*(3′,2″) = 6.4, *J*(3′,OH) = 5.2 (H-3′); 4.56 t, 1 H, *J*(OH,5′) = *J*(5″,OH) = 5.5 (5′-OH); 5.29 d, 1 H, *J*(5,6) = 8.2 (H-5); 5.31 d, 1 H, *J*(OH,3′) = 5.2 (3′-OH); 6.18 t, 1 H, *J*(1′,2′) = 5.8, *J*(1′,2″) = 6.4 (H-1′); 7.24–7.42 m, 15 H (H-arom.); 7.58 d, 1 H, *J*(6,5) = 8.2 (H-6); 11.30 s, 1 H (NH).

1-(2-Deoxy-3,5-*O*-methylene-α-L-*threo*-pentofuranosyl)uracil (**15**)

To a boiling solution of trityl derivative **14** (600 mg, 1.2 mmol) and benzyltrimethylammonium chloride (370 mg, 2 mmol) in dichloromethane (15 ml) was added 30% aqueous sodium hydroxide

(4 ml) and the mixture was stirred at reflux for 12 h. The aqueous layer was separated and the organic one diluted with dichloromethane (50 ml) and shaken with water (30 ml). The formed emulsion was neutralized with dilute hydrochloric acid, the organic layer was separated, washed with water (2 × 30 ml), dried over magnesium sulfate and the solvent was evaporated. Chromatography of the residue on a column of silica gel (25 g) in ethyl acetate–toluene (2 : 1) afforded 390 mg of the tritylated intermediate which was refluxed with 80% aqueous acetic acid (4 ml) for 15 min. After standing at 5 °C for 2 h, the separated trityl alcohol was removed by filtration and washed with 80% aqueous acetic acid. The combined filtrates were evaporated, the residue was codistilled with water and crystallized from 2-propanol to give 178 mg (55%) of compound **15**, m.p. 208–211 °C. For $C_{11}H_{14}N_2O_6$ (270.2) calculated: 48.89% C, 5.22% H, 10.37% N; found: 48.82% C, 5.22% H, 10.19% N. IR spectrum ($CHCl_3$): 3 626 (OH); 3 391 (NH); 1 713, 1 695 (C=O); 1 633 (C=C); 1 133, 1 122, 1 104, 1 090, 1 061 (C–O). ^{1}H NMR spectrum (200 MHz): 2.21 brdd, 1 H, J(2″,1′) = 5.5, J(2″,2′) = 13.4, J(2″,3′) < 1 (H-2″); 2.39 ddd, 1 H, J(2′,1′) = 9.2, J(2′,2″) = 13.4, J(2′,3′) = 4.0 (H-2′); 3.40 d, 2 H, J(5′,OH) = 4.3 (H-5′, H-5″); 3.72 d, 1 H, J(gem) = 12.6 and 3.86 d, 1 H, J(gem) = 12.6 (CH_2O); 4.39 brd, 1 H, J(3′,2′) = 4.0, J(3′,2″) < 1 (H-3′); 4.56 d, 1 H, J(gem) = 6.1 and 4.90 d, 1 H, J(gem) = 6.1 (OCH_2O); 5.29 t, 1 H, J(OH,5′) = J(OH,5″) = 4.3 (OH); 5.64 d, 1 H, J(5,6) = 8.2 (H-5); 7.63 dd, 1 H, J(1′,2′) = 9.2, J(1′,2″) = 5.5 (H-1′); 7.94 d, 1 H, J(6,5) = 8.2 (H-6); 11.33 s, 1 H (NH).

RESULTS AND DISCUSSION

Preparation of 3′,5′-Alkylidene Derivatives

The starting compound, 1-(2,3-*O*-cyclohexylidene-4-*C*-hydroxymethyl-α-L-lyxofuranosyl)-uracil[7] (**1**), was benzoylated to give dibenzoyl derivative **2** which was converted into compound **3** by hydrolysis of the cyclohexylidene grouping with 90% aqueous trifluoroacetic acid. Treatment of derivative **3** with thionyl chloride afforded the cyclic sulfite **4** which, without isolation, was reacted with 1,8-diazabicyclo[5.4.0]undec-7-ene in acetonitrile. The obtained product, anhydro derivative **5**, was converted into the chloro derivative **6** by treatment with 1 M hydrogen chloride in dimethylformamide at 100 °C. Reduction of **6** with tributylstannane gave deoxydibenzoyl derivative **7**. The free deoxy derivative **8** was prepared by methanolysis with methanolic sodium methoxide. Reaction of compound **8** with 2,2-dimethoxypropane in the presence of sulfuric acid as catalyst afforded in high yield (95%) the isopropylidene derivative **9**. A similar reaction with acetaldehyde diethyl acetal was much slower and, moreover, led to a mixture of at least five products, due to the presence of the reactive 4′-*C*-hydroxymethyl group. From the reaction mixture we isolated (*R*)-ethylidene derivative **10a** and (*S*)-ethylidene derivative **10b** in the yield of 22% and 9%, respectively. To achieve higher yields of **10a** and **10b**, it was necessary to protect the 4′-*C*-hydroxymethyl group with an acid-stable group. Therefore, we prepared 4′-*C*-benzoyloxymethyl derivative **11** by benzoylation of isopropylidene derivative **9** and subsequent removal of the isopropylidene grouping. Reaction of **11** with acetaldehyde diethyl acetal, debenzoylation of the obtained products and chromatography on silica gel afforded the (*R*)-ethylidene derivative **10a** in 62% yield and the (*S*)-isomer **10b** in 13% yield.

Under the above-mentioned conditions, no reaction was observed between benzoyl derivative **11** and dimethoxymethane. The formation of the 1,3-dioxane ring by reaction of the corresponding diol with formaldehyde[8] requires conditions under which a cleavage of the nucleoside bond may occur. Although the reaction of 1,3-diols with methylene sulfate[9] takes place under milder conditions, it is performed in an aqueous medium. Therefore, we made use of the base-catalyzed reaction of dichloromethane with the deoxy derivative **8** containing an alkali-stable protecting group at the 4′-*C*-hydroxymethyl group. The nucleoside **8** was converted into a mixture of monosilyl derivative **12** and disilyl derivative **13** by reaction with *tert*-butylchlorodiphenylsilane. The higher reactivity of the 5′-hydroxy group has already been mentioned in the literature[4]. The monosilyl derivative **12** was separated by chromatography on silica gel and was converted into the trityl derivative **14** by tritylation and subsequent desilylation. The reaction of **14** with dichloromethane was performed under conditions of phase transfer catalysis. Final detritylation gave the 3′,5′-*O*-methylene derivative **15** in 55% yield.

Determination of Absolute Configuration of Ethylidene Derivatives **10a** *and* **10b**

The absolute configuration at the O–**C**H(CH_3)–O carbon atom was determined by 2D-ROESY spectra, using the medium range crosspeaks of the O–**C**H(CH_3)–O proton in both isomers. In the case of compound **10b**, this proton (δ 5.07) gives a crosspeak with the H-2′ proton (δ 2.78) which indicates the (*S*)-configuration. On the other hand, in the spectrum of **10a** the analogous proton (δ 4.60) affords a crosspeak with the H-3′ proton (δ 4.40), showing thus the (*R*)-configuration.

Conformational Analysis of Ethylidene Derivatives **10a** *and* **10b** *by NMR Spectroscopy*

The conformation of the deoxyribofuranose ring can be determined by analysis of vicinal coupling constants of the deoxyribose protons. The large number of NMR and X-ray data available shows that the pentose rings in nucleosides and nucleotides usually exist as an equilibrium mixture of conformers[10,11] of the N- and S-type. Using the principle of pseudorotation of deoxyribofuranose ring[10], a model assuming two rapidly interconverting conformers (leading to population-weighed averaging of the observed NMR parameters), and a generalized Karplus equation[12], one can derive the pseudorotation parameters (phase pseudorotation angle, puckering amplitude and molecular fraction) of both the conformers present[13,14]. The pseudorotation parameters were calculated using a programme[15] similar to the programme PSEUROT (ref.[16]). As input data we used the experimental values of the four coupling constants $J(1',2')$, $J(1',2'')$, $J(2',3')$ and $J(2'',3')$. The theoretical values of J(H,H) were calculated using a generalized Karplus equation with empirically adjusted parameters for the CH_2CH frag-

ment[12] and the corresponding corrections for electronegativity of the substituents and their relative orientation to the geminal hydrogen. The puckering amplitude was optimized in the range 30 to 50° in 1° steps, assuming that its value is the same in both conformers ($\Phi_N = \Phi_S$). The pseudorotation phase angles (P_N and P_S) for both conformers were changed in the whole range of the possible values (–90 to +90° and +90 to 270°) in 1° steps, and the conformer population, expressed by molar fractions X_N and X_S, in the range 0 to 1 in 0.01 steps. The results obtained for individual combinations of the pseudorotation parameters were evaluated as differences between the found and calculated values for the individual constants *J*(H,H), the decisive criterion being the sum of absolute values of these differences (Σ_{diff}).

Conformational analysis of the deoxyribofuranose ring was performed for the isomers **10a** and **10b** using the values obtained in DMSO and $CDCl_3$. The results are summarized in Table II. For the (*R*)-isomer **10a** the corresponding constants *J*(H,H) are almost the same (Table I) which indicates analogous conformational behaviour in both solvents. The calculations have shown a clear preference (about 90%) for a $C_{2'}$ *endo* type conformation with phase angle $P_S \approx 180°$. The presence of a small amount (about 10%) of the conformer with phase angle P_N –89° leads to somewhat lower values of Σ_{diff} (compare values of Σ_{diff} for equilibria A ⇌ B and D ⇌ E with the values for the single conformer C or F in Table II). As seen from Table II, for the conformational equilibria A ⇌ B and D ⇌ E (in DMSO or $CDCl_3$) the observed and calculated *J*(H,H) values agree very well, except for *J*(2″,3′) where the observed values (<1 Hz) are lower than the minimum values calculated from the generalized Karplus equation. Such problem of lower experimental values has already been observed by other authors[15,17].

In contrast to the (*R*)-isomer **10a**, in the case of the (*S*)-isomer **10b** the *J*(H,H) values in DMSO markedly differ from those in $CDCl_3$ which indicates a different conformational behaviour in these two solvents. Calculations have shown that in DMSO a $C_{3'}$ *endo* conformer with $P_N \approx 7°$ predominates (about 59%), the minor conformer having $P_S \approx 94°$ (about 41%) (conformational equilibrium G ⇌ H in Table II). On the other hand, in $CDCl_3$ the markedly predominating conformer is of the $C_{2'}$ *endo* type, with $P_S \approx 148°$ (about 64%), the minor conformer having $P_N \approx 10°$ (36%) (equilibrium I ⇌ J in Table II). The accord of the calculated and found values of *J*(H,H) is excellent (Σ_{diff} 0.05 and 0.04 Hz for DMSO and $CDCl_3$, respectively).

Calculation of Conformer Population in (R)- and (S)-Ethylidene Derivatives **10a** *and* **10b**

The calculations were performed at the DFT level using B3LYP functional[18–20]. The basis set was of 6-31G** quality[21–24].

Eight structures (four conformers each of *R* and *S* configurations) were preoptimized using semiempirical PM3 method and then fully optimized at the B3LYP/6-31G**

TABLE II

Conformational analysis of deoxypentofuranosyl ring in compounds **10a** and **10b** from the proton NMR data in DMSO and $CDCl_3$

Compound	Solvent	Conformation	Phase angle P_N/P_S	Puckering amplitude Φ_N/Φ_S	Mole fraction X_N/X_S	J (calc) – J (obs)					Calculated torsion angles H,H[a], °			
						$J(1',2')$	$J(1',2'')$	$J(2',3')$	$J(2'',3')$	Σ_{diff}	$\phi(1',2')$	$\phi(1',2'')$	$\phi(2',3')$	$\phi(2'',3')$
10a (*R*)	DMSO	A/B	–89/181	48/48	0.10/0.90	0.09	0.00	0.11	1.98	2.18	92/161	–29/40	3/–48	124/72
		C	–/178	44/44	–/1.00	0.83	0.01	0.12	1.42	2.38	–/159	–/38	–/–44	–/76
	$CDCl_3$	D/E	–89/179	48/48	0.12/0.88	0.07	0.02	0.04	1.61	1.74	92/162	–29/40	3/–48	124/72
		F	–/174	43/43	–/1.00	1.00	0.00	0.11	0.91	2.02	–/160	–/39	–/–43	–/78
10b (*S*)	DMSO	G/H	7/94	37/37	0.59/0.41	–0.01	0.01	0.01	0.00	0.05	94/146	–27/25	41/0	162/120
	$CDCl_3$	I/J	–10/148	33/33	0.36/0.64	0.02	0.00	0.00	0.01	0.04	91/155	–29/34	37/–27	157/93

[a] Torsion angles H,H for individual conformers were calculated from phase angle (P) and puckering amplitude (Φ) using the relations for deoxyribose: $\phi(1',2') = 121.4 + 1.03\Phi \cos(P - 144)$; $\phi(1',2'') = 0.9 + 1.02\Phi \cos(P - 144)$; $\phi(2',3') = 2.4 + 1.06\Phi \cos P$; $\phi(2'',3') = 122.9 + 1.06\Phi \cos P$.

level of theory. The relative populations of the conformers were estimated using Boltzmann statistics, employing energies of the optimized structures (Fig. 1 and Table III).

For the (*R*)-isomer **10a**, the calculated relative populations and the H,H torsion angles are in good accord with the values derived from the NMR spectra. In the case of the (*S*)-isomer **10b**, whose population is solvent-dependent, the calculated values agreed well with those derived from the NMR spectra in $CDCl_3$ (compare the calcu-

TABLE III
Calculated relative populations (R.P.) at room temperature and torsion angles H,H (ϕ) of the conformers

Compound	Comformation	ΔE kJ mol^{-1}	R.P. %	Torsion angles H,H			
				ϕ(1′,2′)	ϕ(1′,2″)	ϕ(2′,3′)	ϕ(2″,3′)
10a	*R*1	34.278	0.0	88.4	–33.7	28.2	150.0
	*R*2	0.0	100	157.5	34.2	–42.7	80.6
	*R*3	40.580	0.0	88.9	–33.2	35.9	159.3
	*R*4	44.275	0.0	101.1	–19.3	28.9	149.8
10b	*S*1	0.0	54.0	153.2	30.9	–33.2	88.3
	*S*2	11.609	0.5	157.3	34.0	–42.3	80.9
	*S*3	31.712	0.0	89.8	–31.9	32.0	155.5
	*S*4	0.427	45.0	113.9	–5.8	24.6	144.9

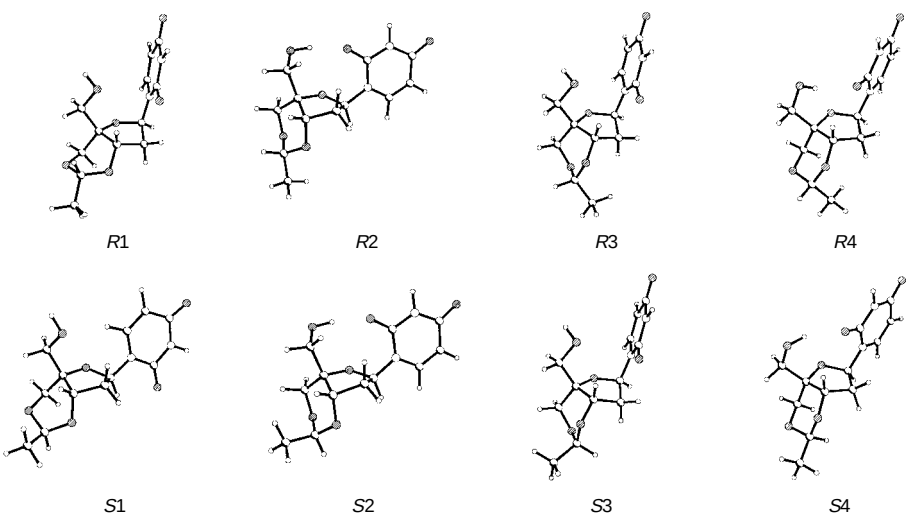

FIG. 1
Calculated minima on the energy hyperplane for the (*R*)- and (*S*)-isomers

lated torsion angles for conformer B or C in Table II with those in conformer *R*2 in Table III, and torsion angles in conformers G and H or I and J in Table II with those in conformers *S*4 and *S*1 in Table III).

Antiviral Assays

None of the prepared compounds was significantly active *in vitro*[25] against HIV.

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