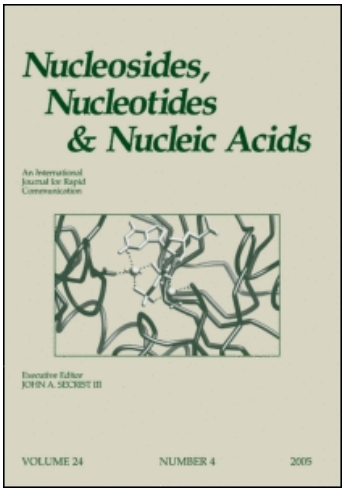

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Synthesis of 5-Substituted-1-[[2-Hydroxy-1-(hydroxymethyl)ethoxy]methyl]cytosines.

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Abstract. 5-Substituted cytosine analogues of 9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]guanine (BIOLF-62) have been synthesized and tested against HSV-1.

We have been developing a series of nucleoside analogues based on a novel acyclonucleoside structure[1,2]. One of these compounds, the guanine analogue known as BIOLF-62, has shown significant activity as an antiherpetic agent[2-6]. Changes in the purine or pyrimidine rings of acyclonucleosides have been shown to give rise to wide variations in biological activity[7,8]. We wish to report the synthesis of the 5-substituted cytosine analogues (4a-e) of BIOLF-62 and their activity as antiherpetic agents.

The initial route to compounds 4 was modelled on the procedure used to prepare BIOLF-62[2]. The chloromethyl ether 1 was condensed with the trimethylsilylated[9] bases 2a-c using either stannic chloride[10] or tetra-n-butylammonium iodide[2] as catalysts. Yields were lower (39 and 34% for 3a and 3b) using stannic chloride than when $(nBu)_4NI$ was employed (90% for 3b).

The removal of the benzyl groups through catalytic hydrogenation proved to be somewhat of a problem. In the mildest conditions of catalytic transfer hydrogenation using palladium black and cyclohexene, yields of 4a-b were 23 and 79% respectively. The bond between N-1 and the side chain is very sensitive to cleavage and unless great care is

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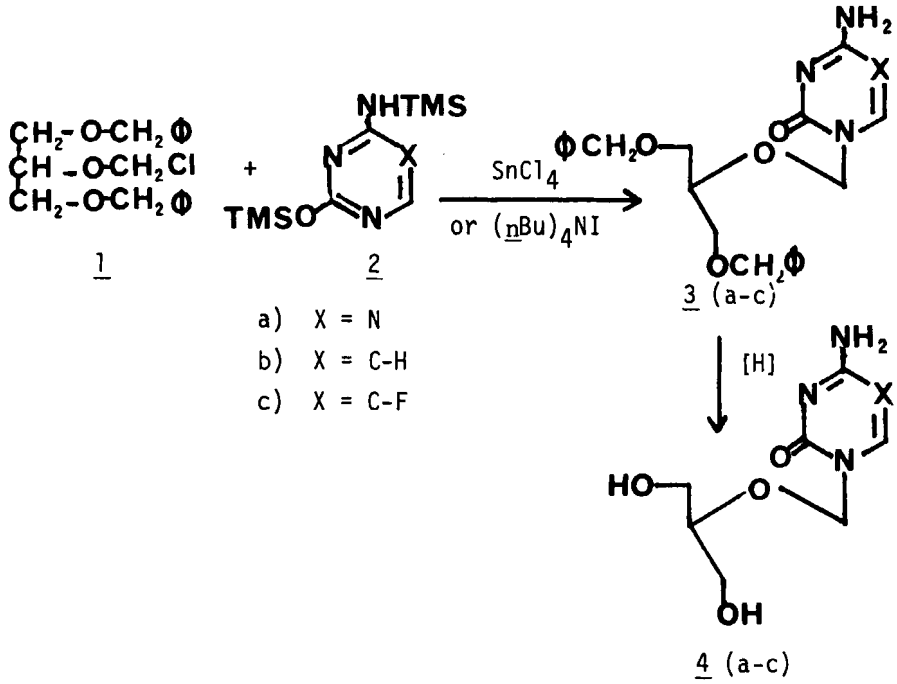
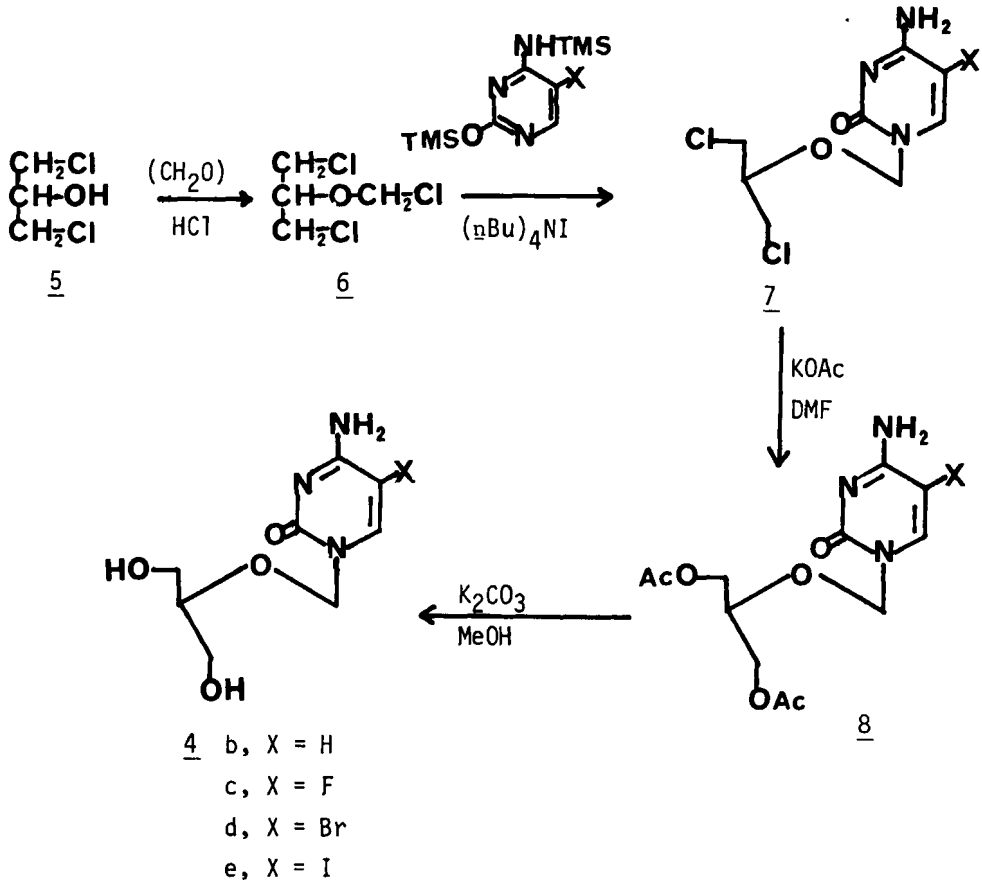
taken, total cleavage of the cytosine ring results. With 5-halocytosines catalytic hydrogenation gave complete cleavage of the cytosine ring from the molecule.



An alternative route to the cytosine analogues is shown below. 1,3-dichloro-2-propanol (5) was converted to the chloromethyl derivative 6. Direct condensation of 6 with the trimethylsilylated cytosines 2 (b-e) in the presence of $(n\text{-Bu})_4NI$ gave the desired products 7 (b-e) in yields of 80, 99, 81 and 67% respectively. Compound 7 was converted into the acetoxy derivatives 8 using potassium acetate in DMF. Removal of the acetyl groups with potassium carbonate in methanol gave the desired compound 4 (b-e), all of which were white solids.

None of the compounds 4 a-e approached BIOLF-62 in activity against HSV-1. The ED-50's vs. HSV-1 are shown below.

Compound	Virus ED-50 (vs HSV-1, μg/ml).[3,4]
4a	35
4b	> 100
4c	> 320
4d	> 320
4e	> 320
BIOLF-62	0.12



Experimental

General Methods. Thin-layer chromatographic data (R_f values) are recorded from Merck Kieselgel 60F 254 analytical sheets. Column chromatography was performed using Merck silica gel 60 (230-240 mesh) packed in glass columns using 15 g of silica per gram of crude material. Cytosine bases were purchased from Sigma Chemical Co. UV Spectra were recorded on a Cary 17 spectrometer. Nuclear Magnetic Resonance spectra were recorded using Varian XL-200 and T60A spectrometers.

1-[[2-Hydroxy-1-(hydroxymethyl)ethoxy]methyl]-5-azacytosine (4a)

5-Azacytosine (5 g, 44.6 mmole) and ammonium sulfate (100 mg) were suspended in 1,1,1,3,3,3-hexamethyldisilazane (HMDS, 40 ml). The mixture was heated at reflux for 20 min at which point an additional 100 mg of ammonium sulfate was added. After a further 20 min at reflux the solu-

tion was clear. Solvents were then removed at reduced pressure and the white residue was dissolved in 1,2-dichloroethane (100 ml) and stannic chloride (3.5 ml) was added. At this point the chloromethyl ether[2] (1) was added and the solution was stirred at room temperature for 14 h. The reaction mixture was then poured into an aqueous sodium bicarbonate solution. Chloroform was added and the whole mixture was filtered through celite. The chloroform layer was separated, washed with water and dried over sodium sulfate. The solvent was concentrated to a small volume (~ 20 ml) and applied to a silica gel column (14.5 x 6.5 cm) which was eluted first with chloroform (450 ml) and then with 5% methanol in chloroform. The fractions containing the desired product were pooled and the solvent removed at reduced pressure. The residue crystallized from ethanol to give a total of 6.21 g of 1-[[2-benzyloxy-1-(benzyloxymethyl)ethoxy]methyl]-5-azacytosine (3a, 39%, mp 120-122°C, λ max (nm), 251 (pH1), 241 (pH7). 250(pH13)). The NMR spectrum in $CHCl_3$ showed signals with δ(ppm) of 3.52 (d, 4H, -$\underline{CH_2}$CH$\underline{CH_2}$-), 4.02 (m, 1H, -CH), 4.45 (s, 4H, 2 x PhCH_2-), 5.30 (s, 2H, -CH_2N-), 7.27 (m, 10H, 2 x Ph) and 8.07 (s, 1H, H-6).

Compound 3a (6.43 g, 16.2 mmole) was dissolved in ethanol (200 ml) and palladium oxide (8g) and cyclohexene (100 ml) were added. The stirred mixture was brought to a gentle reflux for a total of 25 h. The solvent was collected by filtration and the residue was washed with hot 95% ethanol. The filtrate and washings were combined and evaporated to leave a residue that crystallized from ethanol to give 4a (23%, mp 189.5-191°C). The product had λmax (nm) of 250 (pH1), 220, 245 (H_2O) and 248 (pH13). The NMR spectrum in CD_3OD showed signals at δ(ppm) of 3.43-3.83 (m,5H), -$\underline{CH_2}$-$\underline{CH}$-$\underline{CH_2}$-), 5.37 (s, 2H, -CH_2N-) and 8.27 (s, 1H, H-6).

Anal. calc'd for $C_7H_{12}N_4O_4$: C, 38.89; H, 5.59; N, 25.91. Found: C, 39.60; H, 5.35; N, 26.02.

1-[[2-Hydroxy -1-(hydroxymethyl)ethoxy]methyl]cytosine (4b)

(a) Cytosine (5 g, 45 mmole) was treated with HMDS and ammonium sulfate as described above. The product was condensed with 1 using stannic chloride as above. Compound 3b was obtained by silica gel chromatography using 5% methanol in chloroform. After crystallization from ethanol 5.32 g (34%) of pure 1-[[2-benzyloxy-1-(benzyloxymethyl)-

ethoxy]methyl]cytosine (3b) was obtained (mp 129.5-131°C, λ max (MeOH, nm) 279 (pH2), 272 (H_2O) and 272 (pH14). The NMR spectrum in $CDCl_3$ gave signals with δ(ppm) of 3.60 (d, 4H, $-CH_2CH-CH_2-$), 4.05(m, 1H, -CH<), 4.52(s, 4H, 2xPhCH_2-), 5.23(s, 2H, $-CH_2N-$), 5.70(d, 1H, H-5, $J_{5,6}$= 7.5 Hz), 7.26 (d, 1H, H-6), 7.35(s, 2H, $-NH_2$).

Compound 3b (5.59 g, 14.1 mmole) was treated with palladium black and cyclohexene in ethanol at reflux for 3h. The product was collected as in 4a and crystallized from methanol without the need for chromatography to give 4b (2.41 g, 79%, mp 140-141°C, λ max (nm) of 275 (pH2), 268 (H_2O), 268 (pH12)). The NMR spectrum in CD_3OD showed signals with δ(ppm) at 3.62 (m, 5H, $-CH_2CHCH_2-$), 5.30 (s, 2H, $-CH_2N-$), 5.87 (d, 1H, H-5, $J_{5,6}$= 7.5 Hz) and 7.63 (d, 1H, H-6).

Anal. Calc'd for $C_8H_{13}N_3O_4$: C, 44.64; H, 6.09; N, 19.53. Found: C, 44.34; H, 6.08; N, 19.44.

(b) This procedure was repeated except that a catalytic amount of tetra-n-butylammonium iodide was added in place of stannic chloride. The condensation reaction was heated at reflux for 45 min and the product isolated by silica gel chromatography. Compound 3b was obtained in 88.5% yield.

Attempted Preparation of 1-[[2-Hydroxy-1-(hydroxymethyl)ethoxy]methyl]-5-fluorocytosine (4c) from 1

5-Fluorocytosine (1g, 7.75 mmole) was treated with HMDS as before and condensed with 1 using $(nBu)_4NI$ (0.005 eq). The product was collected by silica gel column chromatography using ethyl acetate and methanol (95:5). 1-[[2-Benzyloxy-1-(benzyloxymethyl)ethoxy]methyl]-5-fluorocytosine (3c) was obtained in 84% yield (1.2 g, mp 90-91°C, λ max (nm) in MeOH: 291(pH2), 281, 243(pH7), 282, 243(pH14)). The NMR in $CDCl_3$ showed signals at δ(ppm) of 3.23 (d, 4H, $-CH_2CH-CH_2-$), 3.65 (m, 1H, -CH), 4.48 (s, 4H, PhCH_2-), 5.27 (bs, 2H, $-CH_2N-$), 7.27 (s, 12H, Ph and $-NH_2$), 7.38 (d, 1H, H-6, J = 6 Hz).

Catalytic hydrogenation of 3c under conditions that removed the benzyl groups gave rise to 5-fluorocytosine and cytosine as the only products.

Preparation of 1,3-dichloro-2-chloromethoxypropane (6)

This preparation is virtually identical to the preparation of 1[2]. 1,3-Dichloro-2-propanol (10 g) was dissolved in dichloromethane (100 ml)

in a 250 ml, three-neck, round-bottom flask. Paraformaldehyde (2.45 g) was added and the mixture was cooled in an ice-salt bath. (All solvents and systems were kept anhydrous). Dry hydrogen chloride gas was bubbled slowly into the solution with stirring for 4h. Additional paraformaldehyde (2.45 g) was added and the reaction was continued for 2h. At this point addition of hydrogen chloride was stopped and anhydrous calcium chloride (5 g) was carefully added. The reaction mixture was filtered quickly through celite. The filtrate was concentrated under high vacuum. The NMR in $CDCl_3$ (δ(ppm) of 3.73 (d, 4H, $-CH_2Cl$), 4.07 (m, 1H, -CH), and 5.53 (s, 2H, $-OCH_2Cl$)) indicated that the material was about 80% pure. The product was used without further purification.

General Preparation of 1-[[2-chloro-1-(chloromethyl) ethoxy]methyl]-cytosines (7).

The silylated cytosine base (prepared as described previously) was condensed with an excess (1.5 eq) of compound 6 using $(nBu)_4NI$ as catalyst in methylene chloride as solvent. The products 7 were collected from silica gel column chromatography using methylene chloride - methanol 10:1 as solvent. Properties of compounds 7 are collected in the Table 1.

TABLE 1

Properties of Compounds 7 and 8

Compound	Yield (%)	Melting Point(°C)	max(MeOH,nm) pH2	pH7	pH14	NMR (δ,ppm) $-(CH_2)_2CH$	$-CH_2N-$	H-6
7b	80	gum	279	269 239	268 239	3.70[a]	5.33	7.55 d,J=7.5Hz
7c	88	215-216	288	277 240	277 240	3.73[b]	5.15	7.90 d,J=6 Hz
7d	81	160-162	297	292	292	3.58[b]	5.12	7.93
7e	67	179-180	309	293	293	3.70[a]	5.33	7.95
8b	68	147-148	279	268 239	268 240	4.07[c]	5.20	7.33 d,J=7.5 Hz
8c	74	168-169	291	281 243	282 243	4.10[c]	5.25	7.42 d,J=6 Hz
8d	34	gum	293 248	291 248	290 248	4.13[c]	5.27	7.60
8e	52	gum	308	290 254	292 255	4.16[c]	5.30	7.63

[a] CD_3OD-$CDCl_3$(1.1)

[b] DMSO

[c] $CDCl_3$-CD_3OD-D_2O(5:1:0.1)

General Preparation of 1-[[2-Acetoxy-1-(acetoxymethyl)ethoxy]methyl]-cytosines (8)

Compound 7 (3 mmole) was dried by repeated distillation of toluene and was then mixed with potassium acetate (12 mmole) in DMF (15 ml) and the mixture was heated at reflux for 2h under nitrogen. The solvent was removed at reduced pressure and the residue was chromatographed on silica gel (67 x 2 cm) using methylene chloride-methanol (16:1). The product 8 was collected and crystallized from methanol. Properties are collected in the Table 1.

General Conversion of Compounds 8 to 4

Compound 8 (1.5 mmole) was dissolved in methanol (15 ml) and potassium carbonate (5 mg) was added. The solution was stirred at room temperature for 20 h and carefully neutralized with Dowex 50W x 8 (H^+ form). The solution was collected by filtration, concentrated at reduced pressure and applied to a silica gel column (62 x 1.3 cm) and eluted with methylene chloride-methanol (2:1). The products were generally collected by crystallization from methanol.

The yield of 4b was 63% and the product was identical to that described previously.

Compound 4c was obtained in 38% yield (mp 134-135 C, λ max (MeOH, nm): 288 (pH2), 278, 243 (pH7), 280, 244 (pH14)). The NMR in acetone-d6-D_2O showed signals at δ (ppm) of 3.65 (d, 4H, $HOCH_2$-), 5.33 (bs, 2H, -CH_2N), 7.93 (d, 1H, H-6, J = 6 Hz).

Anal. Calc'd for $C_8H_{12}N_3O_4F$: C, 41.20; H, 5.19; N, 18.02. Found: C, 40.38; H, 5.37; N, 17.87.

1-[[2-Hydroxy-1-(hydroxymethyl)ethoxy]methyl]-5-bromocytosine (4d) was obtained in 88% yield (mp 195°C, λmax(MeOH, nm): 300 (pH2), 286, 243 (pH7), 287, 243 (pH14). The NMR in CD_3OD-D_2O showed signals at δ (ppm) of 3.6 (d, 4H, $HOCH_2$-), 3.93 (m, 1H, -CH), 5.27 (bs, 2H, -CH_2N-), 7.98 (s, 1H, H-6).

Anal. Calc'd for $C_8H_{12}N_3O_4Br \cdot H_2O$: C, 30.79; H, 4.52; N, 13.46. Found: 30.62; H, 4.75; N, 13.29.

1-[[2-Hydroxy-1-(hydroxymethyl)ethoxy]methyl]-5-iodocytosine (4e) was obtained in 38% yield (mp 177-178°C, max (MeOH, nm): 306 (pH2), 285, 248 (pH14). The NMR in acetone-d6-D_2O showed signals at (ppm) of 2.62 (b, 4H, $HOCH_2$), 5.33 (bs, 2H, -CH_2N-), 8.13 (s, 1H, H-6).

Anal. Calc'd for $C_8H_{12}N_3O_4I$: C, 28.17; H, 3.55; N, 12.32. Found: C, 28.24; H, 3.29; N, 12.19.

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