Synthesis and Potent Anti-HIV-1 Activity of Novel 6-Benzyluracil Analogues of 1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)thymine

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Ethyl 2-alkyl-4-aryl-3-oxobutyrates were synthesized from the corresponding arylacetonitriles and 2-bromo esters. Condensation of the butyrates with thiourea followed by treatment with chloroacetic acid afforded the 5-alkyl-6-(arylmethyl)uracils. Condensation of the uracils with acetals using trimethylsilyl triflate (TMS triflate) as a catalyst gave acyclic 5-alkyl-6-(arylmethyl)uracil derivatives. 6-Benzyl-5-ethyluracil was also condensed with methyl 5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-3-*O*-(phenoxythiocarbonyl)-α,*β*-D-*erythro*-pentofuranoside, followed by Barton reduction and deprotection, to give the anomers of 6-benzyl-5-ethyl-2′,3′-dideoxyuridine. Alkylation of the uracils with alkyl chloromethyl sulfides gave new thio analogues of HEPT. All new N^1-substituted uracils were tested for activity against HIV-1, and the thio analogues were found extremely potent.

The discovery of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) as a compound with potent and selective *in vitro* activity against human immunodeficiency virus type 1 (HIV-1)[1] has led to the synthesis of many new analogues,[2−6] of which 6-benzyl-1-(ethoxymethyl)-5-isopropyluracil (MKC-442) has been chosen as a candidate for clinical trials with AIDS patients.[6] Syntheses of the 6-benzyl analogues[5−7] have usually been done by condensation of the sugar moiety with a 5-substituted uracil[8,9] followed by lithiation in the 6-position, reaction with benzoyl chloride, subsequent reduction of the carbonyl group, and eventually deprotection of the sugar moiety. The 5- and 6-substituents of the uracil with respect to HIV-1 inhibition are now well established,[5,6,8−10] but there are still unexplored possibilities in changing the functionality in the sugar moiety. The lithiation step can be an obstacle of doing this. Recently, we have reported a new strategy for the synthesis of acyclouridine derivatives by condensation of 6-benzyl-5-ethyluracil with acetals or 1,3-dioxolanes.[11] The steric hindrance exerted by the 6-substituent and the buttressing effect of the 5-substituent were believed to result in lower yields of the N-1 alkylation product. Also bulky alkylating reagents were expected to reduce the yield of this type of product. In particular, we were interested in synthesizing the N^1-(ethylthio)methyl analog of MKC-442 because such a compound is not likely to be synthesized by the route of lithiation in the 6-position of 1-(ethylthiomethyl)uracils, because the most acidic protons here are expected to the ones on the methylene group next to sulfur which can stabilize the neighboring anion by its d orbitals.

Chemistry

The 3-oxo esters **1a**−**c** were prepared according to the method of Danel *et al.*[11] by reaction of phenylacetonitrile with ethyl 2-bromobutyrate or ethyl 2-bromo-3-methylbutyrate, or by reaction of 3,5-dimethylphenylacetonitrile with ethyl 2-bromobutyrate. The so formed 3-oxo esters **1a**−**c** were converted by reaction with thiourea and sodium in ethanol into a 2-thiouracil which was refluxed with chloroacetic acid overnight to give 5-alkyl-6-(arylmethyl)uracils **2a**−**c**.[11] Silylation[12] of the uracils **2a**,**c** with 1,1,1,3,3,3-hexamethyldisilazane (HMDS) was done prior to condensation with acetals or 1,3-dioxolanes. The condensation reaction with acetals was accomplished under the Vorbrüggen condition[13] using trimethylsilyl trifluoromethanesulfonate (TMS triflate) as a Lewis acid catalyst to give high yields of the desired N^1-substituted nucleosides **3** (Scheme 1). When racemic mixtures were obtained, no attempts were made to separate the enantiomers. Compounds **3a**−**c** have previously been prepared by the same method.[11] For compound **3d** the method was modified by silylation *in situ* of **2b** with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) in chloroform followed by reaction with chloromethyl ethyl ether.

Treatment of 2-deoxy-D-ribose (**4**) with HCl in methanol,[14] followed by selective 5-O-protection with *tert*-butylchlorodiphenylsilane in dry pyridine using 4-(*N*,*N*-dimethylamino)pyridine (DMAP) as catalyst[14,15] and finally reaction with phenoxythiocarbonyl chloride[16] afforded the methyl 5-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-3-*O*-(phenoxythiocarbonyl)-α,*β*-D-*erythro*-pentofuranoside (**5**). Condensation of compound **5** with silylated 6-benzyl-5-ethyluracil using TMS triflate as catalyst gave an anomeric mixture of the corresponding nucleoside (α/*β* = 1/2). After separation of the anomers, each of them were subjected to a Barton deoxygenation[16] followed by deprotection of the silyl group at 5′-O using tetrabutylammonium fluoride to give the anomers of 6-benzyl-5-ethyl-2′,3′-dideoxyuridine **6a**,**b** (Scheme 2).

The compounds were identified by comparison of similar NMR data,[11,16] ^{1}H-^{1}H-COSY, and ^{1}H-nuclear Overhauser effects (NOE). NOE of **7a** proved it to be a *β* anomer as irradiation of 4′-H resulted in 2% NOE in 1′-H. N^1-Glycosylation was confirmed by NOE in the CH_2Ph group of **7a** (CH_2, 4%) when 1′-H was irradiated. The anomeric configuration of the corresponding α anomer **7b** was determined from its precursor **6b**. A decisive feature for the anomeric configuration of **6b** was irradiation of 3′-H which resulted in 3% NOE in 2*β*′-H

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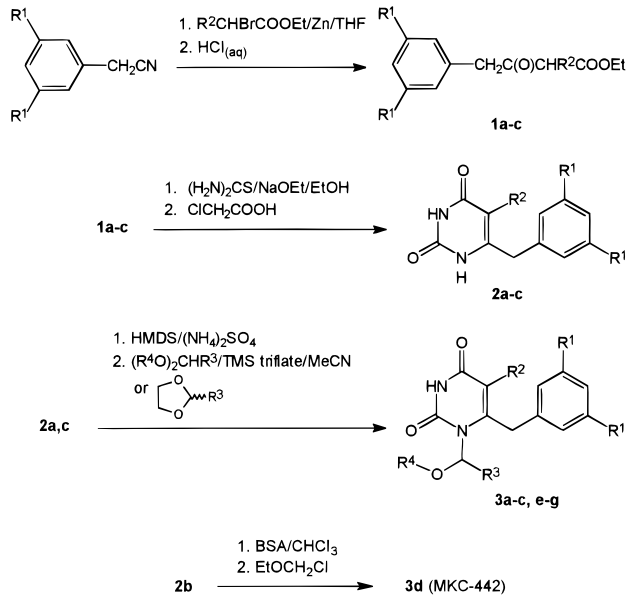
Scheme 1



1,2	R^1	R^2
a	H	Et
b	H	*i*-Pr
c	Me	Et

3	R^1	R^2	R^3	R^4
a	H	Et	Me	Me
b	H	Et	Me	Et
c	H	Et	Me	CH_2CH_2OH
d	H	*i*-Pr	H	Et
e	Me	Et	H	Et
f	Me	Et	Me	Me
g	Me	Et	Et	Et

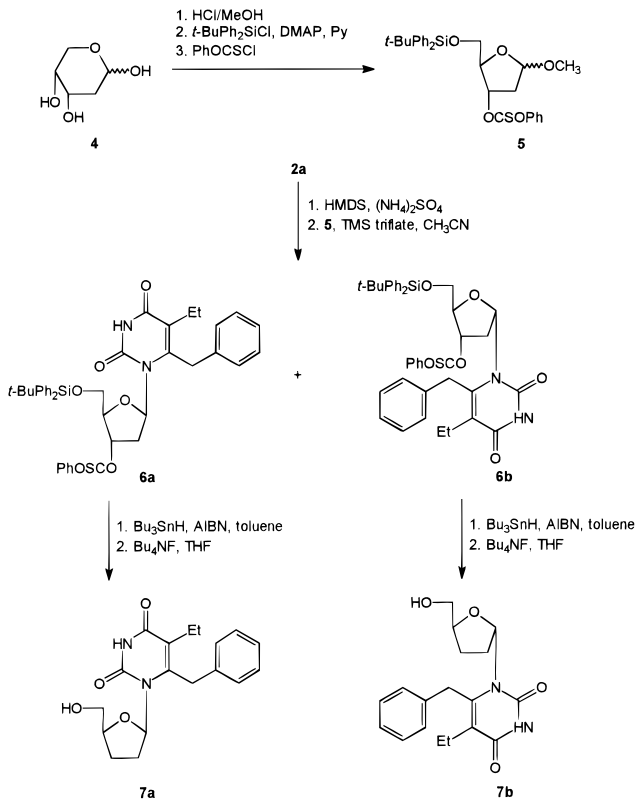
whereas irradiation of 1′-H gave 5% NOE in 2β′-H. N^1-Glycosylation was confirmed by NOE in the CH_2Ph group of **6b** (CH_2, 2%) when 1′-H was irradiated. The α and β anomer assignments of compounds **7a** and **7b** were also confirmed by observing 4′-H in the α anomer at lower field in ^{1}H-NMR than 4′-H in the corresponding β anomer.[17]

Alkylation of compounds **2a**−**c** using alkyl chloromethyl sulfides and K_2CO_3 in DMF gave a mixture of N^1- and N^3-substituted and disubstituted uracils in a typical molar ratio of 2:2:5, but only the 1-((alkylthio)methyl)uracils **8a**−**f** (Scheme 3) were isolated in a state of purity and in very low yields (4−10%). Much higher yields 69−78% were obtained for compounds **8a**,**c**,**e** when synthesized under the Vorbrüggen conditions.[13] The uracils **2a**−**c** were silylated with HMDS prior to condensation with methylthiomethyl acetate using TMS triflate as the catalyst.

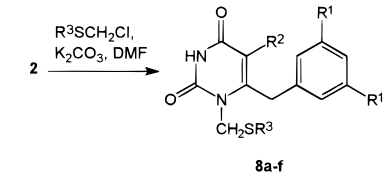
Results of the Anti-HIV Assay and Discussion

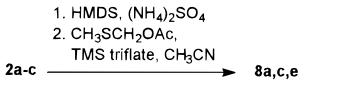
The HIV-1 strain HTLV-IIIB and MT-4 cells were used in our assay to investigate the anti-HIV-1 activity and cytotoxicity of HEPT analogues synthesized in the present study. The results are summarized in Table 1 together with those of AZT. Besides the commercially available AZT, we synthesized MKC-442 (**3d**) and E-EBU-dM (6-(3,5-dimethylbenzyl)-1-(ethoxymethyl)-5-ethyluracil) (**3e**) and used them as references for the biological activity. We found that the analogues **8** with oxygen replaced with sulfur showed comparable activities and selectivities with those found for MKC-

Scheme 2



Scheme 3





8	a	b	c	d	e	f
R^1	H	H	H	H	Me	Me
R^2	Et	Et	*i*-Pr	*i*-Pr	Et	Et
R^3	Me	Et	Me	Et	Me	Et

442,[3,6,7,18,19] E-EBU (6-benzyl-1-(ethoxymethyl)-5-ethyluracil)[2,3,7] and E-EBU-dM.[1−3,7] Compounds **8a**,**d**,**f** showed the highest activities and selectivities, but without showing a clear picture of the structure−activity relationship. Compound **8a** was in our study even more potent than MKC-442 and E-EBU-dM. It is surprising to find **8a**, with an acyclic (methylthio)methyl group, as the most active compound since no highly active compounds with a methoxymethyl counterpart have yet been reported. We are now doing further testing of the sulfur compounds **8** against resistant HIV-1 mutants, and these studies will be reported in due time.

For compounds **3**, we were also testing the effect of changing R^3 = H in the E-EBU type of compounds into R^3 = alkyl. The effect was rather dramatic as can be seen by comparing **3e** (E-EBU-dM) with **3g** (R^3 = Et) with a decrease in activity against HIV of about 5 powers. A similar change in activity was observed for E-EBU with the reported activity ED_{50} = 0.041 μM[7]

Table 1. Antiviral Activity of HEPT Analogues **3a**−**g**, **7a**,**b**, and **8a**−**f** and AZT against HIV-1 in MT-4 Cells

compd	ED_{50},[a] μM	CD_{50},[b] μM	SI[c]
3a	>100		
3b	>100		
3c	>100		
3d	0.005	141	28000
3e	0.004	100	25000
3f	2	100	50
3g	15	130	8.7
7a	37	52	1.4
7b	0.5	1	2
8a	0.002	32	16000
8b	0.040	37	925
8c	0.020	37	1850
8d	0.006	37	6200
8e	0.050	52	1040
8f	0.004	68	17000
AZT	0.040	52	1300

[a] Effective dose of compound, achieving 50% inhibition of HIV-1 antigen production in MT-4 cultures. [b] Cytotoxic dose of compound, required to reduce the proliferation of normal uninfected MT-4 cells by 50%. [c] Selectivity index: ratio CD_{50}/ED_{50}. ED_{50} and CD_{50} are expressed as the mean values of three independent determinations.

when compared with **3b**. In this case a change of R^3 from hydrogen to methyl resulted in a completely inactive compound at 100 μM.

Compound **7a** can be considered as a hybrid between E-EBU and 2′,3′-dideoxy-5-ethyluridine (D2EtU),[20] and therefore two possible modes of action against HIV were conceivable, both as a nucleoside and as a non-nucleoside HIV reverse transcriptase inhibitor. However, we failed to find a molecule with such properties since compound **7a** was inactive against HIV-1 in MT-4 cells. In fact, compound **7a** has the same structural feature at the anomeric center as the inactive compounds **3a**−**c** and the weakly active compounds **3f**,**g**.

Experimental Section

The ^{1}H-NMR spectra were recorded on a Bruker A 250 FT NMR spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). Silica gel (0.040−0.063 mm) and analytical silica gel TLC plates 60 F_{254} were purchased from Merck. Tetrahydrofuran (THF) was distilled from sodium/benzophenone prior to use.

3-Oxo Esters 1b,c. General Procedure. Activated zinc dust (18 g, 275 mmol) was suspended in dry THF (125 mL) at reflux, and a few drops of ethyl 2-bromo ester were added to initiate the reaction. After the appearance of a green color (ca. 45 min), the arylacetonitrile (4.50 mmol) was added in one portion followed by dropwise addition of ethyl 2-bromo ester (10 mmol) over a period of 1 h. The reaction mixture was refluxed for an additional 10 min, diluted with THF (375 mL), and quenched with aqueous K_2CO_3 (50%, 54 mL). Rapid stirring for 45 min gave two distinct layers. The THF layer was decanted, the residue was washed with THF (2 × 100 mL), and the combined THF fractions were treated with aqueous HCl (10%, 50 mL) at room temperature for 45 min. The mixture was concentrated *in vacuo*, diluted with CH_2Cl_2 (300 mL), and washed with saturated aqueous $NaHCO_3$ (200 mL). The organic phase was dried (Na_2SO_4) and evaporated *in vacuo* to give an oily residue, which was used without further purification for the synthesis of compound **2b**,**c**. Further purification of compounds **1b**,**c** could be achieved by chromatography on silica gel (200 g) with petroleum ether (bp 60−80 °C)/Et_2O (95:5).

Ethyl 2-isopropyl-3-oxo-4-phenylbutyrate (1b): yield 1.03 g (92%) as an oil; ^{1}H NMR ($CDCl_3$) δ 0.85 (3H, d, J = 6.6 Hz, CH_3), 0.94 (3H, d, J = 6.7 Hz, CH_3), 1.22 (3H, t, J = 7.1 Hz, CH_3), 2.43−2.49 (1H, m, CH), 3.32 (1H, d, J = 9.4 Hz, CH), 3.78 (2H, s, CH_2Ph), 4.12 (2H, q, J = 7.2 Hz, CH_2), 7.17−7.35 (5H, m, aryl); ^{13}C NMR ($CDCl_3$) δ 14.01 (CH_3), 20.22 (CH_3), 20.49 (CH_3), 28.40 (CH), 49.40 (CH_2Ph), 61.08 (OCH_2), 65.75 (CH), 127.07, 128.54, 129.62, 133.16 (aryl), 168.83 (C-1), 202.11 (C-3).

Ethyl 2-ethyl-4-(3,5-dimethylphenyl)-3-oxobutyrate (1c): yield 1.06 g (90%); ^{1}H NMR ($CDCl_3$) δ 0.85 (3H, t, J = 7.4 Hz, CH_3), 1.24 (3H, t, J = 7.2 Hz, CH_3), 1.86 (2H, m, CH_2), 2.28 (6H, s, $2CH_3$), 3.46 (1 H, t, J = 7.2 Hz, CH), 3.73 (2H, s, CH_2Ph), 4.15 (2H, q, J = 7.1 Hz, CH_2), 6.80 (2H, s, aryl), 6.89 (1H, s, aryl); ^{13}C NMR ($CDCl_3$) δ 11.6 (CH_3), 13.9 (CH_3), 21.0 (CH_3), 21.4 ($2CH_3$), 48.8 (CH_2Ph), 59.3 (OCH_2), 61.0 (CH), 127.2, 128.6, 132.9, 138.0 (aryl), 169.4 (C-1), 202.6 (C-3).

5-Alkyl-6-(arylmethyl)uracils 2b,c. General Procedure. Sodium (2 g) was dissolved in anhydrous EtOH (45 mL), and thiourea (4.63 g, 60 mmol) and compounds **1b**,**c** (4.0 mmol) were added to the clear solution. The reaction mixture was refluxed for 6 h and evaporated *in vacuo* at 40−50 °C until nearly dryness and the residue redissolved in H_2O (40 mL). The 2-thiouracil was precipitated by addition of concentrated aqueous HCl (7 mL) and subsequent acidification to pH 4 with glacial acetic acid. The precipitated 2-thiouracil was desulfurized by suspension in 10% aqueous chloroacetic acid (100 mL) and subsequent reflux overnight. After cooling to room temperature, the precipitate was filtered off, washed with cold EtOH and Et_2O, and finally dried *in vacuo* to give compounds **2b**,**c**.

6-Benzyl-5-isopropyluracil (2b): yield 701 mg (72%); mp 231−233 °C; ^{1}H NMR (DMSO-d_6) δ 1.07 (6H, d, J = 6.9 Hz, $2CH_3$), 2.49−2.52 (1H, m, CH), 3.78 (2H, s, CH_2Ph), 7.18−7.36 (5H, m, aryl), 10.68−10.77 (br s, 2NH); ^{13}C NMR (DMSO-d_6) δ 19.99 ($2CH_3$), 26.29 (CH), 35.21 (CH_2Ph), 113.79 (C-5), 126.46, 127.88, 128.46, 136.99 (aryl), 148.45 (C-6), 150.94 (C-2), 163.77 (C-4). Anal. ($C_{14}H_{16}N_2O_2\cdot0.5H_2O$) C, H, N.

6-(3,5-Dimethylbenzyl)-5-ethyluracil (2c): yield 712 mg (69%); mp 216−218 °C; ^{1}H NMR (DMSO-d_6) δ 0.85 (3H, t, J = 7.3 Hz, CH_3), 2.25 (2H, q, J = 7.1 Hz, CH_2), 2.25 (6H, s, $2CH_3$), 3.67 (2H, s, CH_2Ph), 6.86 (3H, s, aryl), 10.64 (1H, s, NH), 10.96 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 13.32 (CH_3), 17.51 (CH_2), 20.76 ($2CH_3$), 111.13 (C-5), 125.73, 127.96, 136.54, 137.42 (aryl), 148.66 (C-6), 150.82 (C-2), 164.40 (C-4). Anal. ($C_{15}H_{18}N_2O_2\cdot0.25H_2O$) C, H, N.

Acyclic Uracil Derivatives 3e−g. General Procedure. A mixture of compound **2** (1.0 mmol), HMDS (5 mL), and $(NH_4)_2SO_4$ (10 mg) was heated under reflux for 2 h. The mixture was concentrated at room temperature under reduced pressure to obtain the silylated base as a pale yellow solid. Anhydrous CH_3CN (10 mL) was added, and the solution was stirred at −45 °C. TMS triflate (0.24 g, 1.05 mmol) was added to the mixture followed by dropwise addition of the appropriate acetal or 1,3-dioxolane (2.0 mmol). The reaction was quenched after 2−3 h and neutralized by addition of saturated aqueous $NaHCO_3$ at −45 °C and evaporated *in vacuo* at room temperature to dryness. The residue was extracted with dry Et_2O (2 × 25 mL), and the ether extracts were evaporated under reduced pressure to give compound **3** after silica column chromatography with $CHCl_3$. In some cases compound **3** was further purified by preparative TLC ($CHCl_3$).

6-(3,5-Dimethylbenzyl)-1-(1-ethoxymethyl)-5-ethyluracil (3e): yield 345 mg (94%); mp 159−61 °C (lit.[5] mp 160−160.7 °C).

6-(3,5-Dimethylbenzyl)-5-ethyl-1-(1-methoxyethyl)-uracil (3f): yield 320 mg (87%); mp 153−154 °C dec; ^{1}H NMR (DMSO-d_6) δ 0.85 (3H, t, J = 7.2 Hz, CH_3), 1.35 (3H, d, J = 6.3 Hz, CH_3), 2.18−2.23 (8H, m, CH_2, $2CH_3$), 3.09 (3H, s, OCH_3), 4.09 (1H, d, J = 17.2 Hz, CH_2Ph), 4.24 (1H, d, J = 17.2 Hz, CH_2Ph), 5.88 (1H, d, J = 5.1 Hz, CH), 6.76 (2H, s, aryl), 6.85 (1H, s, aryl), 11.32 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 12.99 (CH_3), 18.34 (CH_2), 20.57 (CH_3), 20.75 ($2CH_3$), 33.16 (CH_2Ph), 55.73 (OCH_2), 84.80 (CH), 115.93 (C-5), 124.91, 127.73, 136.91, 137.49 (aryl), 148.66 (C-6), 151.12 (C-2), 162.72 (C-4). Anal. ($C_{18}H_{24}N_2O_3$) C, H, N.

6-(3,5-Dimethylbenzyl)-1-(1-ethoxypropyl)-5-ethyluracil (3g): yield 248 mg (72%); mp 131−133 °C; ^{1}H NMR ($CDCl_3$) δ 0.84 (3H, t, J = 7.3 Hz, CH_3), 0.94 (3H, t, J = 7.0

Hz, CH_3), 1.65−1.73 (2H, m, CH_2), 2.26 (6H, s, $2CH_3$), 3.41 (2H, q, J = 7.0 Hz, OCH_2), 4.13 (1H, d, J = 16.7 Hz, CH_2Ph), 4.33 (1H, d, J = 16.8 Hz, CH_2Ph), 6.07 (1H, br s, NCHO), 6.66 (2H, s, aryl), 6.84 (1H, s, aryl), 9.98 (1H, br s, NH); ^{13}C NMR ($CDCl_3$) δ 9.80 (CH_3), 13.12 (CH_3), 14.33 (CH_3), 18.76 (CH_2), 21.20 ($2CH_3$), 28.53 (CH_2), 33.76 (CH_2Ph), 65.35 (OCH_2), 89.09 (CH), 117.83 (C-5), 124.93, 128.14, 136.47, 138.19 (aryl), 149.77 (C-6), 152.30 (C-2), 163.49 (C-4). Anal. ($C_{20}H_{28}N_2O_3 \cdot 0.5H_2O$) C, H, N.

6-Benzyl-1-(ethoxymethyl)-5-isopropyluracil (3d). Compound **2b** (0.2 g, 0.8 mmol) was suspended in $CHCl_3$ (10 mL), and to this suspension was added bis(trimethylsilyl)acetamide (BSA) (0.35 g, 1.72 mmol). The mixture became clear after stirring at room temperature for 10 min. To this solution was added chloromethyl ethyl ether (0.15 g, 1.16 mmol) and stirring continued until no change in the amount of the starting material could be noticed on TLC. After evaporation of the solvent *in vacuo* the resulting syrup was chromatographed on silica gel with $CHCl_3$ to afford 200 mg (80%) as crystals; mp 108−110 °C (lit.[18] mp 109−110 °C).

Methyl 5-*O*-(*tert*-Butyldiphenylsilyl)-2-deoxy-3-*O*-(phenoxythiocarbonyl)-α,*β*-D-*erythro*-pentofuranoside (5). 2-Deoxy-D-ribose (**4**, 2.5 g, 18.7 mmol) was dissolved in MeOH (25 mL), and a solution of 1 mol % of HCl in MeOH (50 mL) was added. After 20 min the reaction mixture was quenched with pyridine (2 mL) and evaporated *in vacuo*. The oily residue was dissolved in dry pyridine (20 mL), DMAP (244 mg, 2 mmol) and *tert*-butylchlorodiphenylsilane (5.50 g, 20 mmol) were added, and the reaction was mixture stirred for 2 h. Then phenoxythiocarbonyl chloride (3.45 g, 20 mmol) was added in small portions, and the reaction mixture was stirred for an additional 12 h, quenched by addition of MeOH (1 mL), and evaporated *in vacuo*. The residue was partitioned between $CHCl_3$ (50 mL) and H_2O (50 mL). The organic phase was dried (Na_2SO_4) and evaporated *in vacuo*. After chromatographic workup on silica gel (100 g) using the gradient 0−25% Et_2O in petroleum ether (bp 60−80 °C), compound **5** was obtained as a colorless oil (7.50 g, 77%). ^{1}H and ^{13}C NMR data are in accordance with those in ref 16.

6-Benzyl-5-ethyl-2′,3′-dideoxyuridine (7a) and Its α Anomer 7b. Condensation of compound **2a** (465 mg, 2.0 mmol) with compound **5** (1.04 g, 2.0 mmol) was done using the same method as for the acyclic uracil derivatives **3e**−**g**. The anomeric mixture (α/β = 1/2) was separated with preparative TLC using the gradient 5−30% Et_2O in petroleum ether (bp 60−80 °C) to give 430 mg of β anomer **6a** and 234 mg of α anomer **6b**. Each of the anomers (0.3 mmol) and α,α′-azoisobutyronitrile (AIBN, 23 mg, 0.15 mmol) were dissolved with stirring in anhydrous toluene (15 mL) under nitrogen. Tributyltin hydride (0.25 mL, 0.94 mmol) was added, and stirring was continued for 3 h at 80 °C. The solvent was evaporated, and the residue was dissolved in $CHCl_3$ (25 mL), washed with saturated aqueous $NaHCO_3$ (2 × 25 mL) and water (2 × 25 mL), and dried over Na_2SO_4. The solvent was evaporated *in vacuo* and the resulting residue dissolved in 1.1 M tetrabutylammonium fluoride in THF (10 mL). The reaction mixture was stirred for 2 h at room temperature and evaporated *in vacuo* and the residue dissolved in $CHCl_3$ (25 mL). The organic phase was washed with H_2O (2 × 50 mL), dried (Na_2SO_4), and evaporated *in vacuo*. The β anomer **7a** and the α anomer **7b** were obtained, respectively, after preparative TLC using MeOH/CH_2Cl_2 (2:98) as eluent.

Compound 7a: yield 32 mg (10%) as a foam; ^{1}H NMR ($CDCl_3$) δ 1.04 (3H, t, J = 7.4 Hz, CH_3), 1.45−1.57 (1H, m, 2′α-H), 1.67−1.79 (1H, m, 3′α-H), 2.22−2.64 (4H, m, 2′β-H, 3′β-H, CH_2), 3.61 (1H, dd, J = 3.3, 12.0 Hz, 5′a-H), 3.85 (1H, d, J = 11.2 Hz, 5′b-H), 3.97 (1H, d, J = 17.2 Hz, CH_2Ph), 4.03−4.09 (1H, m, 4′-H), 4.24 (1H, d, J = 17.2 Hz, CH_2Ph), 5.63 (1H, dd, J = 5.3, 7.8 Hz, 1′-H), 7.11−7.40 (5H, m, aryl), 9.69 (1H, br s, NH); ^{13}C NMR ($CDCl_3$) δ 13.69 (CH_3), 19.33 (CH_2), 25.48 (C-3′), 29.08 (C-2′), 34.80 (CH_2), 63.99 (C-5′), 81.10 (C-4′), 88.65 (C-1′), 116.85 (C-5), 127.33, 127.42, 129.99, 135.13 (aryl), 148.71 (C-6), 150.70 (C-2), 163.11 (C-4); FAB MS ($CHCl_3$, 3-nitrobenzyl alcohol) m/z = 331 (M + H$^+$).

Compound 7b: yield 23 mg (7%) as a foam; ^{1}H NMR ($CDCl_3$) δ 1.04 (3H, t, J = 7.4 Hz, CH_3), 1.43−1.68 (2H, m, 2′α-H, 3′α-H), 2.11−2.60 (4H, m, 2′β-H, 3′β-H, CH_2), 3.39 (1H, dd, J = 5.5, 11.9 Hz, 5′a-H), 3.67 (1H, dd, J = 3.1, 11.8 Hz, 5′b-H), 3.93 (1H, d, J = 17.2 Hz, CH_2Ph), 4.20 (1H, d, J = 17.2 Hz, CH_2Ph), 4.61−4.71 (1H, m, 4′-H), 5.73−5.79 (1H, m, 1′-H), 7.11−7.39 (5H, m, aryl); ^{13}C NMR ($CDCl_3$) δ 13.78 (CH_3), 19.29 (CH_2), 27.48 (C-3′), 29.71 (C-2′), 34.74 (CH_2Ph), 64.75 (C-5′), 82.72 (C-4′), 89.01 (C-1′), 116.32 (C-5), 127.40, 127.45, 129.25, 135.13 (aryl), 148.93 (C-6), 150.57 (C-2), 163.33 (C-4); FAB MS ($CHCl_3$, 3-nitrobenzyl alcohol) m/z = 331 (M + H$^+$).

1-((Alkylthio)methyl)uracils 8a−f: Procedure A. To a suspension of the uracil **2** (2 mmol) and K_2CO_3 (0.55 g, 4 mmol) in anhydrous DMF (10 mL) was added chloromethyl alkyl sulfide (2 mmol) and the mixture was stirred vigorously for 4 days. The solvent was removed *in vacuo* and the residue coevaporated with toluene (2 × 10 mL). The residue was extracted with $CHCl_3$ (2 × 10 mL) and the $CHCl_3$ phase evaporated *in vacuo* to give compound **8** after being chromatographed using preparative TLC with $CHCl_3$.

Procedure B. The uracil **2** (2 mmol) was refluxed for 2 h in HMDS (10 mL) in the presence of $(NH_4)SO_4$ (10 mL) followed by evaporation *in vacuo*. The resulting residue was dissolved in MeCN (10 mL), the solution cooled to −40 °C, and TMS triflate (2 mmol) added. The mixture was cooled to −60 °C, and (methylthio)methyl acetate (6 mmol) was added. The temperature of the mixture was allowed to increase slowly. When the temperature had reached 4 °C after 15 h, the mixture was quenched by addition of a saturated aqueous $NaHCO_3$ (5 mL) and evaporated *in vacuo*. The resulting residue was purified using preparative TLC with $CHCl_3$ as eluent to give compounds **8** in 69−78% yield.

6-Benzyl-5-ethyl-1-((methylthio)methyl)uracil (8a): procedure A, yield 41 mg (7%); procedure B, yield 452 mg (78%); mp 185−188 °C; ^{1}H NMR ($CDCl_3$) δ 1.08 (3H, t, J = 7.4 Hz, CH_3), 2.28 (3H, s, SCH_3), 2.50 (2H, q, J = 7.4 Hz, CH_2), 4.17 (2H, s, CH_2Ph), 4.79 (2H, s, NCH_2S), 7.10−7.38 (5H, m, aryl), 9.76 (1H, s, NH); ^{13}C NMR ($CDCl_3$) δ 13.87 (CH_3), 15.72 (SCH_3), 19.30 (CH_2), 34.12 (CH_2Ph), 46.90 (NCH_2S), 117.32 (C-5), 127.32, 127.54, 129.37, 134.58 (aryl), 148.60 (C-6), 151.78 (C-2), 163.11 (C-4). Anal. Calcd ($C_{15}H_{18}N_2O_2S \cdot 0.25H_2O$): C, 61.10; H, 6.32; N, 9.50. Found: C, 61.60; H, 6.26; N, 9.16.

6-Benzyl-5-ethyl-1-((ethylthio)methyl)uracil (8b): procedure A, yield 60 mg (10%); yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.08 (3H, t, J = 7.4 Hz, CH_3), 1.27 (3H, t, J = 7.4 Hz, CH_3), 2.50 (2H, q, J = 7.4 Hz, CH_2), 2.76 (2H, q, J = 7.4 Hz, CH_2), 4.16 (2H, s, CH_2Ph), 4.81 (2H, s, NCH_2S), 7.10−7.38 (5H, m, aryl), 9.93 (1H, s, NH); ^{13}C NMR ($CDCl_3$) δ 13.88 (CH_3), 14.94 (SCH_3), 19.28 (CH_2), 26.55 (SCH_2), 34.21 (CH_2), 45.05 (NCH_2S), 117.27 (C-5), 127.29, 127.50, 129.34, 134.61 (aryl), 148.63 (C-6), 151.72 (C-2), 163.24 (C-4).

6-Benzyl-5-isopropyl-1-((methylthio)methyl)uracil (8c): procedure A, yield 25 mg (4%); procedure B, yield 456 mg (75%); mp 134−136 °C; ^{1}H NMR ($CDCl_3$) δ 1.3 (6H, d, J = 6.9 Hz, $2CH_3$), 2.29 (3H, s, SCH_3), 2.86−2.97 (1H, m, CH), 4.19 (2H, s, CH_2Ph), 4.81 (2H, s, NCH_2S), 7.10−7.39 (5H, m, aryl), 9.40 (1H, s, NH); ^{13}C NMR ($CDCl_3$) δ 15.77 (SCH_3), 20.51 ($2CH_3$), 28.44 (CH), 34.17 (CH_2Ph), 47.10 (NCH_2S), 120.18 (C-5), 127.29, 127.48, 129.35, 134.74 (aryl), 148.01 (C-6), 151.75 (C-2), 162.14 (C-4). Anal. Calcd ($C_{16}H_{20}N_2O_2S \cdot 0.25H_2O$): C, 62.21; H, 6.69; N, 9.07. Found: C, 62.75; H, 6.74; N, 8.75.

6-Benzyl-1-((ethylthio)methyl)-5-isopropyluracil (8d): procedure A, yield 38 mg (6%); mp 136−138 °C; ^{1}H NMR ($CDCl_3$) δ 1.27 (3H, t, J = 7.4 Hz, CH_3), 1.30 (6H, d, J = 7.0 Hz, $2CH_3$), 2.78 (2H, q, J = 7.4 Hz, CH_2), 2.86−2.97 (1H, m, CH), 4.18 (2H, s, CH_2Ph), 4.83 (2H, s, NCH_2S), 7.10−7.39 (5H, m, aryl), 9.49 (1H, s, NH); ^{13}C NMR ($CDCl_3$) δ 14.98 (CH_3), 20.52 ($2CH_3$), 26.65 (CH), 28.43 (SCH_2), 34.28 (CH_2Ph), 45.31 (NCH_2S), 120.15 (C-5), 127.28, 127.46, 129.34, 134.76 (aryl), 148.05 (C-6), 151.67 (C-2), 162.24 (C-4). Anal. ($C_{17}H_{22}N_2O_2S \cdot 0.25H_2O$) C, H, N.

6-(3,5-Dimethylbenzyl)-5-ethyl-1-((methylthio)methyl)uracil (8e): procedure A, yield 37 mg (6%); procedure B, yield 438 mg (69%); mp 112−114 °C; ^{1}H NMR ($CDCl_3$) δ 1.08 (3H, t, J = 7.4 Hz, CH_3), 2.28 (9H, s, SCH_3, $2CH_3$), 2.48 (2H, q, J = 7.4 Hz, CH_2), 4.08 (2H, s, CH_2), 4.83 (2H, s, NCH_2S), 6.69 (2H, s, aryl), 6.90 (1H, s, aryl), 9.78 (1H, s, NH); ^{13}C NMR ($CDCl_3$) δ 13.89 (CH_3), 15.75 (CH_3), 19.30 (CH_2), 21.24 ($2CH_3$),

33.98 (CH_2Ph), 46.95 (NCH_2S), 117.18 (C-5), 125.02, 129.18, 134.37, 139.06 (aryl), 148.92 (C-6), 151.87 (C-2), 163.23 (C-4).

6-(3,5-Dimethylbenzyl)-5-ethyl-1-((ethylthio)methyl)-uracil (8f): procedure A, yield 46 mg (7%); mp 145−147 °C; ^{1}H NMR ($CDCl_3$) δ 1.08 (3H, t, J = 7.4 Hz, CH_3), 1.28 (3H. t, J = 7.4 Hz, CH_3), 2.28 (6H, s, $2CH_3$), 2.49 (2H, q, J = 7.4 Hz, CH_2), 2.77 (2H, q, J = 7.4 Hz, CH_2), 4.08 (2H, s, CH_2Ph), 4.83 (2H, s, NCH_2S), 6.69 (2H, s, aryl), 6.90 (1H, s, aryl), 9.82 (1H, s, NH); ^{13}C NMR ($CDCl_3$) δ 13.89 (CH_3), 14.96 (CH_3), 19.29 (CH_2), 21.23 ($2CH_3$), 26.57 (SCH_2), 34.07 (CH_2Ph), 45.12 (NCH_2S), 117.13 (C-5), 124.99, 129.16, 134.39, 139.02 (aryl), 148.96 (C-6), 151.76 (C-2), 163.31 (C-4). Anal. ($C_{18}H_{24}N_2O_2S$) C, H, N.

Virus and Cells. The HIV-1 strain HTLV-IIIB[21] was propagated in H9 cells[22] at 37 °C, 5% CO_2 using RPMI 1640 with 10% heat-inactivated fetal calf serum (FCS) and antibiotics (growth medium). Culture supernatant was filtered (0.45 nm), aliquotted, and stored at −80 °C until use.

Inhibition of HIV-1 Replication. Compounds were examined for possible antiviral activity against HIV-1 using MT-4 cells as target cells. For screening studies, MT-4 cells were incubated with virus (0.005 MOI) for 2 h, washed, and thereafter added in a proportion of 1:10 to uninfected cells, which had been preincubated in growth medium containing the test compound for 2 h. Cultures were maintained with the test compound for 6 days in parallel with virus-infected control cultures without compound added. Expression of HIV in the culture medium was quantitated by HIV-1 antigen detection ELISA.[23] Compounds mediating less than 30% reduction of antigen expression were considered without biological activity. Compounds mediating a reduction of 30% or more were examined for cytotoxic effect using concentration-dependent inhibition of MT-4 cell proliferation as measure of cytotoxicity using the MTT assay as previously described.[24] A 30% inhibition of cell growth relative to control cultures was considered significant.

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