filtrate was then cooled to 10 °C. The CrO_3 solution was then added to a cooled solution of **29** at such a rate that the temperature did not rise above 15 °C. The mixture was then stirred at room temperature for 4 h, diluted to 3 times its volume with icewater, and then cooled to 25 °C. This was followed by extraction three times with EtOAc. The combined fractions were concentrated to 150 mL and washed twice with 10% NaOH and twice with water, followed by drying over $MgSO_4$ and concentration to dryness. Analysis by TLC (silica gel, EtOAc) showed the presence of three compounds, with R_f values of 0.88, 0.43, and 0.35. The mixture was then fractionated on a silica gel column, which was eluted with EtOAc. The first compound eluted was the starting material. This was followed by 0.6 g (22%) of **40** and then 1.2 g (44%) of **41**. The latter melted at 249–250 °C: MS ($M^+ + 1$) 275; NMR (Me_2SO-d_6) δ 2.21 (s, 3, CH_3), 2.68 (m, 2, 6-CH_2), 3.41 (m, 2, 5-CH_2), 7.91 (s, 1, 8-H). Anal. ($C_{12}H_{10}N_4O_4$), C, H, N.

2-Amino-6,7-dihydro-8-nitroindeno[5,6-*d*]imidazol-5-(1*H*)-one Hydrochloride (42). A suspension of **41** (0.5 g, 1.8 mmol) in 2 N HCl (15 mL) was heated to boiling. The material went into solution after 20 min. After another 15 min the mixture was chilled and the precipitate was isolated and recrystallized from dilute EtOH plus a few drops of HCl: wt 460 mg (97%) of **42**; mp 250 °C dec; MS ($M^+ + 1$) 233; NMR (Me_2SO-d_6) δ 3.52 (m, 2, 6-CH_2), 3.76 (m, 2, 7-CH_2), 7.80 (s, 1, 4-H), 8.82 (br s, 2, NH_2). Anal. ($C_{10}H_8N_4O_3$·HCl) C, H, N, Cl.

2′-Amino-1′,5′,6′,7′-tetrahydro-8′-nitrospiro[1,3-dithiolane-2,5′-indeno[5,6-*d*]imidazole] (43). To a solution of **42** (0.4 g, 1.5 mmol) in CF_3COOH was added 6 mL of ethanedithiol. The mixture was stirred at room temperature for 2 h, and the solvents were evaporated under vacuum. The resultant syrup was added to 50 mL of EtOAc and a few drops of concentrated NH_4OH was added to adjust to pH to 8. The solution was shaken three times with 50-mL portions of water and dried over $MgSO_4$, and the solvent was removed. The yellowish solid was recrystallized from EtOAc: wt 0.35 g (73%) of **43**; mp 240 °C; MS ($M^+ + 1$) 309; NMR (Me_2SO-d_6) δ 2.45 (t, 2, 6′-CH_2), 2.60 (t, 2, 7′-CH_2), 3.42 (m, 4, $S(CH_2)_2S$), 6.70 (s, 2, NH_2) 7.32 (s, 1, 4′-H), 11.50 (br s, 1, NH). Anal. ($C_{12}H_{12}N_4O_2S_2$) C, H, N, S.

2′-Amino-1′,5′,6′,7′-tetrahydro-8′-nitrospiro[1,3-dioxolane-2,5′-indeno[5,6-*d*]imidazole] (44). A solution of **42** (1.0 g, 3.7 mmol) in 10 mL of ethylene glycol was prepared by heating the mixture, and 30 mL of benzene was then added. The biphasic mixture was refluxed for 20 h, with continuous removal of water, with use of a Dean Stark trap. The mixture was then poured into 10 mL of icewater and extracted three times with 50-mL portions of EtOAc. The combined extracts were washed with water and evaporated to dryness under reduced pressure. The residue was extracted with EtOAc: wt residue 1.0 g (95%) of **44**; mp 300 °C dec; MS ($M^+ + 1$) 277; NMR (Me_2SO-d_6) δ 2.25 (t, 2, 6-CH_2), 3.1 (t, 2, 7-CH_2), 4.05 (m, 4, $O(CH_2)_2O$), 6.47 (br s, 2, NH_2), 7.35 (s, 1, 4′-H), 11.5 (br s, 1, 1′-NH). Anal. ($C_{12}H_{12}N_4O_4$·0.4H_2O) C, H, N.

2′,4′-Diamino-1′,5′,6′,7′-tetrahydrospiro[1,3-dithiolane-2,7′-indeno[5,6-*d*]imidazole] (6). A solution of **43** (0.1 g, 0.3 mmol) in EtOH (20 mL) was hydrogenated over 10% Pd/C (60 mg) at 30 psi until 3 equiv of H_2 was consumed. The catalyst was removed and the filtrate evaporated to dryness. The resulting solid was recrystallized from 50% aqueous EtOH to give 45 mg (53%) of **6**: mp 210–212 °C dec; MS (FAB) ($M^+ + 1$) 279; NMR (Me_2SO-d_6) δ 2.58 (m, 2, 5′, and 6′-CH_2), 3.40 (m, 4, $S(CH_2)_2S$), 4.50 (br s, 2, NH_2), 7.9 (s, 2, NH_2), 8.60 (s, 1, 8′-H), 11.30 (br s, 1, NH). Anal. ($C_{12}H_{14}N_4S_2$·0.2 H_2O) C, H, N, S.

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Synthesis and Anti-HIV Activity of 2-, 3-, and 4-Substituted Analogues of 1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT)

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Several analogues of a new lead for anti-HIV-1 agents, 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT), in which the C-2, N-3, or C-4 position was modified were synthesized. These involve 2-thiothymine (**11**), 2-thiouracil (**12**), 4-thiothymine (**17**), 4-thiouracil (**18**), 5-methylcytosine (**27**), and cytosine (**28**) derivatives. Preparation of N-3-substituted derivatives (**29** and **30**) of HEPT was also carried out. Among these analogues, compound **11** exhibited excellent activity against HIV-1 HTLV-III_B strain with an EC_{50} value of 0.98 μM, which is 7-fold more potent than that of HEPT. Removal of the 5-methyl group in compound **11** results in total loss of activity. Other compounds did not show any anti-HIV-1 activity. The 4-thio derivatives **17** and **18** were found to be rather cytotoxic. When compound **11** was evaluated for its inhibitory effects on another HIV-1 strain, HTLV-III_{RE}, and two HIV-2 strains, LAV-2_{ROD} and LAV-2_{EHO}, it proved equally inhibitory to HTLV-III_{RF}, whereas both HIV-2 strains were insensitive to the compound.

In the search for more selective and effective agents against human immunodeficiency virus (HIV),[1,2] which is the causative agent of the acquired immunodeficiency syndrome (AIDS), a large number of nucleoside analogues have been synthesized and investigated for their antiviral activities.[3,4] Among these, 3′-azido-3′-deoxythymidine[5] (AZT) has already been approved for use for patients with AIDS. 2′,3′-Dideoxyinosine (DDI), which is less toxic than

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[⊥] Rega Institute.

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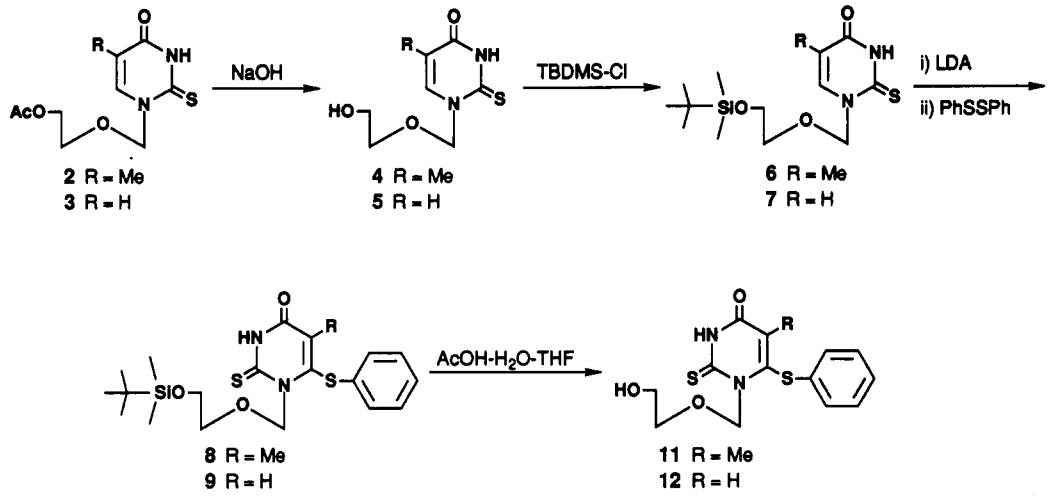
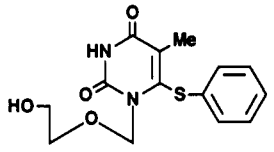
Scheme I



AZT,[6] is another promising candidate for therapy of AIDS. The 2′,3′-dideoxynucleosides act as potent inhibitors of HIV reverse transcriptase (RT) after being phosphorylated by cellular kinases to their 5′-triphosphates.[7] These triphosphates may also interact with host cellular DNA polymerases, and therefore contribute to the toxic side effects of this class of compounds. As a result, it would seem imperative to search for new classes of compounds that have potent anti-HIV activity, low toxicity, and possibly different modes of action.

We have recently reported that novel 6-substituted acyclouridine derivatives have potent anti-HIV-1 activity in various T4 cell cultures.[8-11] 1-[(2-Hydroxyethoxy)-methyl]-6-(phenylthio)thymine (1; HEPT) appears to be



1; HEPT

as active and selective as DDI against HIV-1 replication in vitro. HEPT is interesting in that it is only inhibitory to HIV-1 and its triphosphate does not interact with HIV-1 RT.[8,9]

We have also described the structure–activity relationships of 5- or 6-substituted derivatives of HEPT, wherein a ring structure at the C-6 position and a methyl group at the C-5 position of the pyrimidine moiety seem to be important determinants for the anti-HIV-1 activity. In the present article, synthesis of C-2, N-3, or C-4 modified analogues of HEPT and their anti-HIV activity are described.

Chemistry

We have reported a series of papers on the lithiation of nucleosides, which provide general access to the modification of their base moieties.[8,11-20] In these studies, we found that the metalation of suitably protected uridine and acyclouridine nucleosides took place at the C-6 position in an essentially regiospecific manner when lithium diisopropylamide (LDA) was used as a lithiating agent. As the resulting C-6 lithiated species readily react with a wide range of electrophiles, this gives a general method for the preparation of their 6-substituted derivatives.

Based on the above lithiation tactics, preparation of 6-(phenylthio)-2-thioacyclouridine derivatives was carried out (Scheme I). Compounds **2** and **3**[21] were deacetylated to give the respective free 2-thioacyclouridines (**4** and **5**). For the LDA lithiation, the hydroxyl group of compounds **4** and **5** was protected with *tert*-butyldimethylsilyl (TBDMS) group to afford compounds **6** and **7**. Compounds **6** and **7** were treated with LDA (2.5 equiv, below

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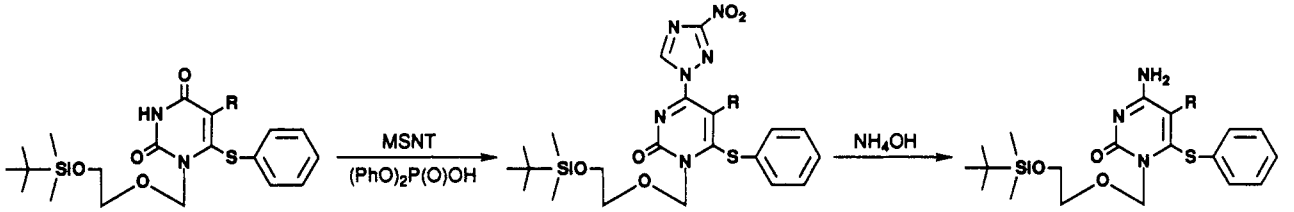
Scheme II

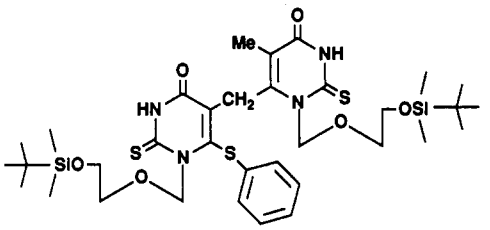


19 R = Me
20 R = H

21 R = Me
23 R = H

22 R = Me
24 R = H

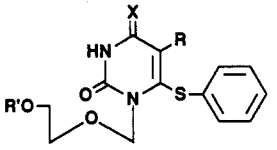
−70 °C, for 1 h) and then subjected to reaction with diphenyl disulfide (2.0 equiv, below −70 °C, for 5 min) to give the respective products **8** (79%) and **9** (64%) as shown in Scheme I. These results confirm that the lithiation also works with the 2-thiouracil system.[22] It is worth mentioning that, in the reaction of compound **6**, a side product (**10**) was also isolated in 17% yield, which apparently resulted from an addition-elimination between compound **8** and its 5-methyl-dissociated species. The formation of



10

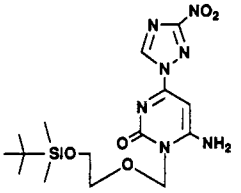
such a product could be prevented by using a limited amount of LDA. In fact, when 2.0 equiv of LDA was used, we were unable to detected more than trace amount of compound **10**. Desilylation of compounds **8** and **9** in AcOH–THF–H_2O gave the corresponding free 2-thioacyclouridines **11** and **12**, respectively.

For the preparation of the 4-thio counterparts, *O*-benzoyl derivatives (**13** and **14**) of the preformed 6-(phenylthio)-acyclonucleosides were treated with 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane 2,4-disulfide (Lawesson's reagent).[23] Following deacylation of the products (**15** and **16**), the target compounds (**17** and **18**) were obtained.



13 R = Me, R' = PhCO, X = O
14 R = H, R' = PhCO, X = O
15 R = Me, R' = PhCO, X = S
16 R = H, R' = PhCO, X = S
17 R = Me, R' = H, X = S
18 R = R' = H, X = S

As we encountered difficulty in lithiating a cytosine-nucleoside,[24] the alternative of the introduction of an amino group to the C-4 position of compounds **19** and **20** by the published method[25,26] was examined. Thus, compound **19** was reacted with 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT) in pyridine in the presence of diphenyl phosphate (Scheme II). The reaction was very sluggish and ca. 30% of compound **19** remained even after 7 days. Moreover, the product **21** turned out to be unstable to column chromatographic conditions, leaving a mixture of ca. 6:4 of compounds **19** and **21**. The mixture was treated with aqueous ammonia in dioxane. This allowed the isolation of compound **22** in 26% yield from compound **19**. A 42% yield of the starting material (**19**) was also recovered. In contrast to the case of compound **20**, the 4-(3-nitro-1,2,4-triazol-1-yl) derivative (**23**) was obtained from compound **20** in 86% yield as crystals after silica gel column chromatography. However upon ammonolysis, it produced three products. On the basis of their mass and ^1H NMR spectra and analyses, the structures were confirmed to be **24** (57%), **25** (21%), and **26** (20%). The cytosine analogues **27** and **28** were obtained after desilylation of compounds **22** and **24**.



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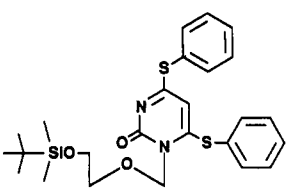

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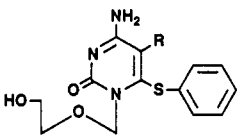
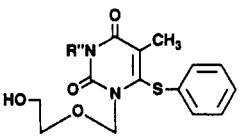


27 R = Me
28 R = H



29 R" = Me
30 R" = $PhCH_2$

Finally, preparation of N-3 methyl (**29**) and N-3 benzyl (**30**) derivatives were carried out by alkylation of HEPT with MeI or benzyl bromide in DMF in the presence of diisopropylethylamine.

Antiviral Activities

Anti-HIV-1 activity and cytotoxicity of the compounds synthesized in this study are shown in Table I. Compound **11** has an excellent inhibitory effect against HIV-1 (HTLV-III_B strain) in MT-4 cells. Its 50% antiviral effective concentration (EC_{50}) is 0.98 μM, which is 7-fold more potent than that of HEPT. Compound **11** also exhibited the activity against HTLV-III_B with the EC_{50} value of 2.9 μM in peripheral blood lymphocytes (PBL). None of other thio analogues (**12**, **17**, and **18**) was inhibitory of HIV-1 replication. In addition 4-thio derivatives **17** and **18** were rather cytotoxic. The cytosine analogues (**27** and

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Table I. Inhibition of HIV-1 and HIV-2 Replication in MT-4 Cells and Peripheral Blood Lymphocytes (PBL) by HEPT Analogues

compd	virus	strain	cell	EC_{50},[a] μM	CC_{50},[b] μM
11	HIV-1	$HTLV\text{-}III_B$	MT-4	0.98	123
			PBL	2.9	292
		$HTLV\text{-}III_{RF}$	MT-4	2.5	
	HIV-2	$LAV\text{-}2_{ROD}$	MT-4	>100	
		$LAV\text{-}2_{EHO}$	MT-4	>100	
12	HIV-1	$HTLV\text{-}III_B$	MT-4	>198	198
17	HIV-1	$HTLV\text{-}III_B$	MT-4	>27	27
18	HIV-1	$HTLV\text{-}III_B$	MT-4	>4.1	4.1
27	HIV-1	$HTLV\text{-}III_B$	MT-4	>91	91
28	HIV-1	$HTLV\text{-}III_B$	MT-4	>250	>250
29	HIV-1	$HTLV\text{-}III_B$	MT-4	>374	>374
30	HIV-1	$HTLV\text{-}III_B$	MT-4	>40	40
HEPT	HIV-1	$HTLV\text{-}III_B$	MT-4	7.0	740
			PBL	7.9	640
		$HTLV\text{-}III_{RF}$	MT-4	8.1	
	HIV-2	$LAV\text{-}2_{ROD}$	MT-4	>250	
		$LAV\text{-}2_{EHO}$	MT-4	>250	
AZT	HIV-1	$HTLV\text{-}III_B$	MT-4	0.0030	7.8
	HIV-2	$LAV\text{-}2_{ROD}$	MT-4	0.0028	
DDA	HIV-1	$HTLV\text{-}III_B$	MT-4	6.3	>500
	HIV-2	$LAV\text{-}2_{ROD}$	MT-4	7.2	

[a] Effective concentration of compound required to achieve 50% protection of MT-4 cells against the virus-induced cytopathic effect or 50% reduction of p24 antigen in culture supernatant of PBL. [b] Cytotoxic concentration of compound required to reduce the viability of mock-infected MT-4 cells or the incorporation of [5-^{3}H]Urd into RNA of mock-infected PBL by 50%.

28) as well as the N-3 substituted HEPTs (29 and 30) were also inactive.

The above results suggest that substitution of oxygen by sulfur at the C-2 position of the uracil moiety potentiates the antiviral activity, while modification at the C-4 position decreases it with a concomitant increase in the cytotoxicity. In our previous study, we have demonstrated that replacement of the 5-methyl group of HEPT by hydrogen removes its anti-HIV-1 activity.[8] This is also the case with the 2-thio analogues of HEPT. The presence of a hydrogen at the N-3 position seems to be essential for these compounds to exert activity.

In another series of experiments, compound 11 was further evaluated for its inhibitory effects on another HIV-I strain, $HTLV\text{-}III_{RF}$ (from a patient of Haitian descent),[27] and two HIV-2 strains, $LAV\text{-}2_{ROD}$[28] and $LAV\text{-}2_{EHO}$.[29] HIV-1 and HIV-2 are related human retroviruses, but they differ in genomic sequence, antigenic properties and in the size of their proteins.[28-30] Like HEPT, compound 11 proved equally inhibitory of $HTLV\text{-}III_{RF}$, whereas both HIV-2 strains were insensitive to the compound (Table I).

Although an investigation is being undertaken to determine the mode of action of HEPT and compound 11, it is highly possible that both compounds act on the same target in the virus replicative cycle. HEPT does not interfere with an early event (*i.e.* adsorption, penetration, or uncoating) of the replicative cycle of HIV-1.[9] From time of addition experiments, it seems that HEPT interacts at a stage of the replicative cycle that corresponds well to the reverse transcription process. Furthermore, neither HEPT nor compound 11 suppress virus production in chronically HIV-1 infected MOLT-4 cells (data not shown), suggesting that a late event of the replicative cycle may not also be excluded as a target of these compounds for HIV-1. Besides the higher anti-HIV-1 activity, we have recently observed that oral bioavailability of compound 11 in rats is higher than that of HEPT.[10] Thus compound 11 may be the most promising candidate for AIDS chemotherapy among the 6-substituted acyclouridine derivatives so far synthesized. The preclinical toxicology studies of compound 11 are currently being undertaken.

Experimental Section

Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a AC-250 Bruker NMR spectrometer at 250 MHz with tetramethylsilane (TMS) used as the internal standard; chemical shifts are recorded in parts per million (ppm). UV spectra were recorded with a Shimadzu UV-260 spectrophotometer. Mass spectra were obtained with a Hitachi M-80A spectrometer in the normal EI mode or the negative-ion SIMS mode. Column chromatography was carried out on Merck Silica gel 60 H. TLC was performed on silica gel (precoated silica gel plate 60 F_{254}, Merck). Elemental analyses were performed on a Perkin-Elmer 240-C elemental analyzer.

General Procedure for the Preparation of 1-[(2-Hydroxyethoxy)methyl]-2-thiouracil Derivatives (4 and 5). A solution of the 2 or 3 (3.13 mmol) in EtOH (5 mL) was treated with aqueous 1 N NaOH (5 mL). The mixture was stirred for 10 min at room temperature. After neutralization with cation exchange resin Dowex 50W-X8 (H^+), the solution was filtered and evaporated to dryness. The residue was crystallized from EtOH to give the 1-[(2-hydroxyethoxy)methyl]-2-thiouracil derivative.

1-[(2-Hydroxyethoxy)methyl]-2-thiothymine (4): yield 89%; mp 138.5–140.5 °C; UV (MeOH) λ_{max} 280 nm (ε 16 000); ^{1}H NMR (Me_2SO-d_6) δ 1.82 (d, J = 1.0 Hz, 3 H, 5-Me), 3.49 (dt, J = 5.3, 4.9 Hz, 2 H, $HOCH_2CH_2O$), 3.57 (t, J = 4.9 Hz, 2 H, $HOCH_2CH_2O$), 4.68 (t, J = 5.3 Hz, 1 H, OH), 5.55 (s, 2 H, NCH_2O), 7.74 (q, J = 1.0 Hz, 1 H, 6-H), 12.60 (br, 1 H, NH). Anal. ($C_8H_{12}N_2O_3S$) C, H, N, S.

1-[(2-Hydroxyethoxy)methyl]-2-thiouracil (5): yield 92%; mp 156 °C; UV (MeOH) λ_{max} 276 nm (ε 14 000); ^{1}H NMR (Me_2SO-d_6) δ 3.48 (dt, J = 5.3, 4.7 Hz, 2 H, $HOCH_2CH_2O$), 3.57 (t, J = 4.7 Hz, 2 H, $HOCH_2CH_2O$), 4.69 (t, J = 5.3 Hz, 1 H, OH), 5.56 (s, 2 H, NCH_2O), 5.97 (d, J = 7.9 Hz, 1 H, 5-H), 7.82 (d, J = 7.9 Hz, 1 H, 6-H), 12.64 (br, 1 H, NH). Anal. ($C_7H_{10}N_2O_3S$) C, H, N, S.

General Procedure for the Preparation of 1-[[2-[(*tert*-Butyldimethylsilyl)oxy]ethoxy]methyl]-2-thiouracil Derivatives (6 and 7). A mixture of 4 or 5 (3 mmol), DMF (10 mL), imidazole (245 mg, 3.6 mmol), and *tert*-butyldimethylsilyl chloride (TBDMS Cl) (543 mg, 3.6 mmol) was stirred at room temperature. After 14 h, the reaction mixture was poured into H_2O (50 mL). The resulting precipitate was collected on a filter and washed with saturated $NaHCO_3$ solution (3 × 50 mL) and H_2O (3 × 50 mL). The precipitate was dried in vacuo and crystallized from toluene–hexane to give the desired TBDMS derivative.

1-[[2-[(*tert*-Butyldimethylsilyl)oxy]ethoxy]methyl]-2-thiothymine (6): yield 89%; mp 86–87 °C; UV (MeOH) λ_{max} 280 nm (ε 16 000); ^{1}H NMR ($CDCl_3$) δ 0.08 (s, 6 H, Me_2Si), 0.90 (s, 9 H, Me_3C), 1.98 (d, J = 1.3 Hz, 3 H, 5-Me), 3.71, 3.78 (A_2B_2, 4 H, $SiOCH_2lH_2O$), 5.65 (s, 2 H, NCH_2O), 7.36 (q, J = 1.3 Hz, 1 H, 6-H), 9.64 (br, 1 H, NH). Anal. ($C_{14}H_{26}N_2O_3SSi$) C, H, N, S.

1-[[2-[(*tert*-Butyldimethylsilyl)oxy]ethoxy]methyl]-2-thiouracil (7): yield 69%; mp 132.5–133.5 °C; UV (MeOH) λ_{max} 276 nm (ε 14 000); ^{1}H NMR ($CDCl_3$) δ 0.08 (s, 6 H, Me_2Si), 0.90 (s, 9 H, Me_3C), 3.71, 3.79 (A_2B_2, 4 H, $SiOCH_2CH_2O$), 5.63 (s, 2 H, NCH_2O), 6.01 (dd, J = 7.9 Hz, 2.3 Hz, 1 H, 5-H), 7.55 (d, J = 7.9 Hz, 1 H, 6-H), 9.42 (br, 1 H, NH). Anal. ($C_{13}H_{24}N_2O_3SSi$) C, H, N, S.

General Procedure for the Preparation of 6-(Phenylthio)-2-thiouracil Derivatives (8 and 9). LDA (4.29 mmol) dissolved in THF (6.3 mL) was placed in a three-necked flask fitted with a nitrogen inlet adapter, a thermometer, and a rubber septum. To this solution, under a nitrogen atmosphere 6 or 7

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(1.58 mmol) dissolved in THF (6.3 mL) was added at a rate such that the temperature did not exceed −70 °C. After the mixture had been stirred for 1 h, diphenyl disulfide (690 mg, 3.16 mmol) in THF (5 mL) was added maintaining the temperature below −70 °C. The mixture was stirred for 15 min below −70 °C, then quenched with AcOH (0.42 mL), and allowed to warm to room temperature. The whole was evaporated to dryness, and the residue was purified by chromatography ($CHCl_3$) and crystallized from EtOAc–hexane to give the 6-(phenylthio) derivative.

1-[[2-[(*tert*-Butyldimethylsilyl)oxy]ethoxy]methyl]-6-(phenylthio)-2-thiothymine (8): yield 79%; mp 87.5–88.5 °C; ^{1}H NMR ($CDCl_3$) δ 0.05 (s, 6 H, Me_2Si), 0.88 (s, 9 H, Me_3C), 1.98 (s, 3 H, 5-Me), 3.70–3.78 (m, 4 H, $SiOCH_2CH_2O$), 6.20 (s, 2 H, NCH_2O), 7.18–7.36 (m, 5 H, SPh), 9.38 (br, 1 H, NH). Anal. ($C_{20}H_{30}N_2O_3S_2Si$) C, H, N, S. The side product **10** was also obtained in 17% yield: NMR ($CDCl_3$) δ 0.03, 0.05 (s × 2, 6 H × 2, Me_2Si × 2), 0.87, 0.89 (s × 2, 9 H × 2, Me_3C × 2), 1.80 (s, 3 H, 5-Me), 3.60–3.75 (m, 8 H, $SiOCH_2CH_2O$ × 2), 3.82 (s, 2 H, CH_2), 6.04, 6.21 (s × 2, 2 H × 2, NCH_2O × 2), 7.13–7.39 (m, 10 H, SPh × 2). After removal of the TBDMS groups, the molecular ion peak could be observed by negative SIMS: m/z 573 $[M - H]^-$.

1-[[2-[(*tert*-Butyldimethylsilyl)oxy]ethoxy]methyl]-6-(phenylthio)-2-thiouracil (9): yield 61%; mp 116–117 °C; ^{1}H NMR ($CDCl_3$) δ 0.10 (s, 6 H, Me_2Si), 0.93 (s, 9 H, Me_3C), 3.80–3.92 (m, 4 H, $SiOCH_2CH_2O$), 5.24 (s, 1 H, 5-H), 6.17 (s, 2 H, NCH_2O), 7.49–7.60 (m, 5 H, SPh), 9.52 (br, 1 H, NH). Anal. ($C_{19}H_{28}N_2O_3S_2Si$) C, H, N, S.

General Procedure for the Deprotection of the TBDMS Group. The TBDMS protected derivative (1 mmol) was dissolved in 10 mL of THF–AcOH–H_2O (2:2:1, v/v/v). The solution was stirred at room temperature for 14 h and evaporated to dryness. The residue was coevaporated with toluene (3 × 10 mL) and crystallized from a suitable solvent to give the corresponding free acyclonucleoside.

1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)-2-thiothymine (11): yield 91%; mp 120 °C (toluene); UV (MeOH) λ_{max} 284 nm (ε 22 000); MS m/z 324 (M^+); ^{1}H NMR ($CDCl_3$) δ 1.88 (t, J = 6.1 Hz, 1 H, OH), 2.05 (s, 3 H, 5-Me), 3.65–3.81 (m, 4 H, $HOCH_2CH_2O$), 6.17 (s, 2 H, NCH_2O), 7.19–7.39 (m, 5 H, SPh), 9.69 (br, 1 H, NH). Anal. ($C_{14}H_{16}N_2O_3S_2$) C, H, N, S.

1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)-2-thiouracil (12): yield 74%; mp 150–151 °C (toluene); UV (MeOH) λ_{max} 284 nm (ε 24 000); MS m/z 310 (M^+); ^{1}H NMR ($CDCl_3$) δ 2.02 (t, J = 6.1 Hz, 1 H, OH), 3.78–3.95 (m, 4 H, $HOCH_2CH_2O$), 5.25 (d, J = 2.4 Hz, 1 H, 5-H), 6.17 (s, 2 H, NCH_2O), 7.46–7.59 (m, 5 H, SPh), 9.36 (br, 1 H, NH). Anal. ($C_{13}H_{14}N_2O_3S_2$) C, H, N, S.

General Procedure for the Benzoylation of an Alcohol. To a solution of the alcohol (0.4 mmol) in pyridine (2 mL) was added benzoyl chloride (0.07 mL, 0.6 mmol), and the solution was stirred for 2 h at room temperature. The mixture was poured into saturated $NaHCO_3$ solution (10 mL) and extracted with EtOAc (3 × 10 mL). The organic layer was washed with saturated $NaHCO_3$ solution (3 × 10 mL) and then brine (3 × 10 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness. The residue was coevaporated with toluene (3 × 10 mL) and crystallized from toluene–hexane to give the benzoyl derivative.

1-[[2-(Benzoyloxy)ethoxy]methyl]-6-(phenylthio)thymine (13). HEPT was used as the starting material. Compound **13** was obtained in 99% yield: mp 136–136.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.97 (s, 3 H, 5-Me), 3.92 (t, J = 4.7 Hz, 2 H, $COOCH_2CH_2O$), 4.39 (t, J = 4.7 Hz, 2 H, $COOCH_2CH_2O$), 5.63 (s, 2 H, NCH_2O), 7.15–7.29 (m, 5 H, SPh), 7.43 [dd, J = 8.4, 7.4 Hz, 2 H, COPh(*m*)], 7.57 [tt, J = 7.4, 1.4 Hz, 1 H, COPh(*p*)], 8.02 [dd, J = 8.4, 1.4 Hz, 2 H, COPh(*o*)], 8.11 (br, 1 H, NH). Anal. ($C_{21}H_{20}N_2O_5S$) C, H, N, S.

1-[[2-(Benzoyloxy)ethoxy]methyl]-6-(phenylthio)uracil (14). 1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)uracil[12] was used as the starting material. Compound **14** was obtained in 85% yield: mp 150–151 °C; ^{1}H NMR ($CDCl_3$) δ 4.02 (t, J = 4.6 Hz, 2 H, $COOCH_2CH_2O$), 4.54 (t, J = 4.6 Hz, 2 H, $COOCH_2CH_2O$), 4.96 (d, J = 2.2 Hz, 1 H, 5-H), 5.63 (s, 2 H, NCH_2O), 7.40–7.58 [m, 8 H, SPh, COPh(*m,p*)], 8.08 [dd, J = 9.6, 1.5 Hz, 2 H, COPh(*o*)], 8.14 (br, 1 H, NH). Anal. ($C_{20}H_{18}N_2O_5S$) C, H, N, S.

General Procedure for the Preparation of 6-(Phenylthio)-4-thiouracil Derivatives (15 and 16). To a solution of **13** or **14** (0.24 mmol) in toluene (1.5 mL) was added Lawesson's reagent (49 mg, 0.12 mmol), and the mixture was stirred at 100 °C for 2 h. The solution was allowed to cool to room temperature, poured into saturated $NaHCO_3$ solution (10 mL), and extracted with EtOAc (3 × 10 mL). The organic layers was washed with saturated $NaHCO_3$ solution (3 × 10 mL) and then with brine (3 × 10 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness. The residue was purified by chromatography (EtOAc–hexane; 4:6, v/v) and crystallized from EtOH to give the 4-thiouracil derivative.

1-[[2-(Benzoyloxy)ethoxy]methyl]-6-(phenylthio)-4-thiothymine (15): yield 69%; mp 110.5–111.5 °C; ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3 H, 5-Me), 3.96, 4.40 (A_2B_2, 4 H, $COOCH_2CH_2O$), 5.68 (s, 2 H, NCH_2O), 7.15–7.28 (m, 5 H, SPh), 7.42 [dd, J = 8.4, 7.5 Hz, 2 H, COPh(*m*)], 7.55 [tt, J = 7.5, 1.4 Hz, 1 H, COPh(*p*)], 8.01 [dd, J = 8.4, 1.4 Hz, 2 H, COPh(*o*)], 10.66 (br, 1 H, NH). Anal. ($C_{21}H_{20}N_2O_4S_2$) C, H, N, S.

1-[[2-(Benzoyloxy)ethoxy]methyl]-6-(phenylthio)-4-thiouracil (16): yield 78%; mp 157–158 °C; ^{1}H NMR ($CDCl_3$) δ 4.02, 4.54 (A_2B_2, 4 H, $COOCH_2CH_2O$), 5.63 (s, 2 H, NCH_2O), 5.72 (d, J = 1.3 Hz, 1 H, 5-H), 7.26–7.62 [m, 8 H, SPh, COPh(*m,p*)], 8.07 [dd, J = 8.4, 1.4 Hz, 2 H, COPh(*o*)], 9.02 (br, 1 H, NH). Anal. ($C_{20}H_{18}N_2O_5S$) C, H, N, S.

General Procedure for the Deprotection of the Benzoyl Group. A solution of the benzoyl-protected derivative (0.17 mmol) in THF (1 mL) and EtOH (5 mL) was treated with aqueous 1 N NaOH (0.5 mL). The mixture was stirred for 2 h at room temperature. After neutralization with aqueous 2 N HCl (0.25 mL), the solution was evaporated to dryness. The residue was extracted with EtOAc (20 mL) and water (20 mL). The organic layer was washed with saturated $NaHCO_3$ solution (3 × 10 mL) and then with brine (3 × 10 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness. The residue was crystallized from toluene to give the corresponding free acyclonucleoside.

1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)-4-thiothymine (17): yield 55%; mp 126–128 °C; UV (MeOH) λ_{max} 356 nm (ε 16 000), 246 nm (e 11 000); MS m/z 324 (M^+); ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 3 H, 5-Me), 3.63–3.71 (m, 4 H, $HOCH_2CH_2O$), 5.84 (s, 2 H, NCH_2O), 7.19–7.38 (m, 5 H, SPh), 10.26 (br, 1 H, NH). Anal. ($C_{14}H_{16}N_2O_3S_2$) C, H, N, S.

1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)-4-thiouracil (18): yield 68%; mp 164–165 °C; UV (MeOH) λ_{max} 352 nm (ε 23 000), 274 nm (ε 7400); MS m/z 310 (M^+); ^{1}H NMR ($CDCl_3$) δ 2.01 (br, 1 H, OH), 3.80 (s, 4 H, $HOCH_2CH_2O$), 5.61 (s, 2 H, NCH_2O), 5.78 (d, J = 1.9 Hz, 1 H, 5-H), 7.49–7.61 (m, 5 H, SPh), 9.16 (br, 1 H, NH). Anal. ($C_{13}H_{14}N_2O_3S_2$) C, H, N, S.

General Procedure for the Preparation of the 4-(3-Nitro-1,2,4-triazol-1-yl)-6-(phenylthio)pyrimidin-2-one Derivatives (21 and 23). To a solution of **19**[8] or **20**[8] (2.37 mmol) in pyridine (12 mL) were added 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT) (727 mg, 2.45 mmol) and diphenyl phosphate (61.3 mg, 0.245 mmol), and the solution was stirred at room temperature. After a period, water (0.5 mL) was added and after a further period of 30 min, the mixture was evaporated to dryness and coevaporated with toluene (3 × 10 mL). The residue was purified by chromatography ($CHCl_3$–hexane; 7:3, v/v) to give 4-(3-nitro-1,2,4-triazol-1-yl-6-(phenylthio)pyrimidin-2-one derivative.

1-[[2-[(*tert*-Butyldimethylsilyl)oxy]ethoxy]methyl]-4-(3-nitro-1,2,4-triazol-1-yl)-5-methyl-6-(phenylthio)pyrimidin-2-one (21). The reaction period was 7 days and ca. 70% of the starting material (**19**) was converted into **21**. After chromatography on silica gel, a mixture of **19** and **21** (ca. 6:4) was obtained. The mixture was used in the next reaction without further purification, because of instability of **21** on silica gel.

1-[[2-[(*tert*-Butyldimethylsilyl)oxy]ethoxy]methyl]-4-(3-nitro-1,2,4-triazol-1-yl)-6-(phenylthio)pyrimidin-2-one (23): yield 71%; mp 66–69 °C (crystallized from hexane); ^{1}H NMR ($CDCl_3$) δ 0.09 (s, 6 H, Me_2Si), 0.91 (s, 9 H, Me_3C), 3.83 (s, 4 H, $SiOCH_2CH_2O$), 5.85 (s, 2 H, NCH_2O), 6.26 (s, 1 H, 5-H), 7.60–7.74 (m, 5 H, SPh), 9.22 (s, 1 H, 5″-H). Anal. ($C_{21}H_{28}N_6O_5SSi$) C, H, N, S.

General Procedure for the Preparation of the 6-(Phenylthio)cytosine Derivatives (22 and 24). To a solution of **21** or **23** (0.85 mmol) in dioxane (10 mL) was added concentrated

aqueous ammonia (d 0.88, 4 mL). The solution was stirred at room temperature for 6 h, evaporated to dryness and coevaporated with toluene (3 × 10 mL). The residue was purified by chromatography ($CHCl_3$–MeOH; 98:2, v/v) and crystallized from EtOAc to give the 6-(phenylthio)cytosine derivative.

1-[[2-[(*tert*-Butyldimethylsilyl)oxy]ethoxy]methyl]-5-methyl-6-(phenylthio)cytosine (22): yield 26% (from **19**); mp 142–145 °C; ^{1}H NMR ($CDCl_3$) δ 0.03 (s, 6 H, Me_2Si), 0.86 (s, 9 H, Me_3C), 2.01 (s, 3 H, 5-Me), 3.68 (s, 4 H, $SiOCH_2CH_2O$), 5.69 (s, 2 H, NCH_2O), 7.13–7.33 (m, 5 H, SPh). Anal. ($C_{20}H_{31}N_3O_3SSi$) C, H, N, S. Compound **19** was also recovered in 43% yield.

1-[[2-[(*tert*-Butyldimethylsilyl)oxy]ethoxy]methyl]-6-(phenylthio)cytosine (24): yield 57%; mp 242–244 °C; MS *m/z* 350 (M^+ – Bu-*t*); ^{1}H NMR ($CDCl_3$) δ 0.08 (s, 6 H, Me_2Si), 0.91 (s, 9 H, Me_3C), 3.75–3.81 (m, 4 H, $SiOCH_2CH_2O$), 4.87 (s, 1 H, 5-H), 5.67 (s, 2 H, NCH_2O), 7.49–7.61 (m, 5 H, SPh). Anal. ($C_{19}H_{29}N_3O_3SSi$) C, H, N, S. Two side products (**25** and **26**) were also obtained.

6-Amino-1-[[2-[(*tert*-butyldimethylsilyl)oxy]ethoxy]methyl]-4-(3-nitro-1,2,4-triazol-1-yl)pyrimidin-2-one (25): yield 21%; mp 244–245 °C; MS *m/z* (M^+ – Bu-*t*); ^{1}H NMR (Me_2SO-d_6) δ 0.01 (s, 6 H, Me_2Si), 0.83 (s, 9 H, Me_3C), 3.60, 3.69 (A_2B_2, 4 H, $SiOCH_2CH_2O$), 5.47 (s, 2 H, NCH_2O), 6.24 (s, 1 H, 5-H), 8.21 (br, 2 H, NH_2), 9.51 (s, 1 H, 5″-H). Anal. ($C_{15}H_{25}N_7O_5Si$) C, H, N.

1-[[2-[(*tert*-Butyldimethylsilyl)oxy]ethoxy]methyl]-4,6-(diphenylthio)pyrimidin-2-one (26): yield 20%; mp 57–59 °C (crystallized from hexane); MS *m/z* 500 (M^+); ^{1}H NMR ($CDCl_3$) δ 0.08 (s 6 H, Me_2Si), 0.90 (s, 9 H, Me_3C), 3.77 (s, 4 H, $SiOCH_2CH_2O$), 4.98 (s, 1 H, 5-H), 5.67 (s, 2 H, NCH_2O), 7.22–7.39 (m, 10 H, SPh × 2). Anal. ($C_{25}H_{32}N_2O_3S_2Si \cdot {}^1/_5H_2O$) C, H, N, S; N: calcd, 5.55; found, 6.06.

Following the general procedure for the deprotection of TBDMS group, **27** and **28** were prepared from **22** and **24**, respectively.

1-[(2-Hydroxyethoxy)methyl]-5-methyl-6-(phenylthio)cytosine (27): yield 79%; mp 217 °C (EtOH); UV (MeOH) λ_{max} 302 nm (ε 7400), 244 nm (ε 13 000); MS *m/z* 307 (M^+); ^{1}H NMR (Me_2SO-d_6) δ 1.90 (s, 3 H, 5-Me), 3.28–3.49 (m, 4 H, $HOCH_2CH_2O$), 4.56 (t, *J* = 5.2 Hz, 1 H, OH), 5.45 (s, 2 H, NCH_2O), 7.04, 7.56 (br × 2, 1 H × 2, NH_2), 7.20–7.37 (m, 5 H, SPh). Anal. ($C_{14}H_{17}N_3O_3S$) C, H, N, S.

1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)cytosine (28): yield 86%; mp 202 °C (EtOH); UV (MeOH) λ_{max} 293 nm (ε 12 000); MS *m/z* 293 (M^+); ^{1}H NMR (Me_2SO-d_6) δ 3.45–3.55 (m, 4 H, $HOCH_2CH_2O$), 4.65 (t, *J* = 5.5 Hz, 1 H, OH), 5.05 (s, 1 H, 5-H), 5.43 (s, 2 H, NCH_2O), 6.96–7.05 (br, 2 H, NH_2, 7.51–7.66 (m, 5 H, SPh). Anal. ($C_{13}H_{15}N_3O_3S$) C, H, N, S.

General Procedure for the Preparation of the 3-Alkyl Derivatives (29 and 30). To a solution of HEPT (617 mg, 2 mmol) in DMF (4 mL) were added diisopropylethylamine (0.38 mL, 2.2 mmol) and the respective alkyl halide (2.2 mmol). The mixture was stirred at room temperature for 16 h and evaporated to dryness. The residue was dissolved in EtOAc, washed with aqueous NH_4Cl, H_2O, and brine. The organic layer was dried over $MgSO_4$, filtered, and evaporated to dryness. The residue was purified by column chromatography ($CHCl_3$) and crystallized from EtOAc–hexane to give the N-3 alkylated derivative.

1-[(2-Hydroxyethoxy)methyl]-3-methyl-6-(phenylthio)-thymine (29): yield 47%; mp 74–75 °C; UV (MeOH) λ_{max} 275 nm (ε 7900), 243 nm (ε 11 000); MS *m/z* 322 (M^+); ^{1}H NMR ($CDCl_3$) δ 2.13 (s, 3 H, 5-Me), 3.41 (s, 3 H, N-Me), 3.65 (s, 4 H, $HOCH_2CH_2O$), 5.63 (s, 2 H, NCH_2O), 7.17–7.38 (m, 5 H, SPh). Anal. ($C_{15}H_{18}N_2O_4S$) C, H, N, S. The starting material was also recovered in 42% yield.

3-Benzyl-1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)-thymine (30). This compound was obtained as a colorless syrup: yield 30%; UV (MeOH) λ_{max} 275 nm (ε 7800), 243 nm (ε 11 000); MS *m/z* 398 (M^+); ^{1}H NMR ($CDCl_3$) δ 2.08 (s, 3 H, 5-Me), 3.61 (s, 4 H, $HOCH_2CH_2O$), 5.14 (s, 2 H, NCH_2Ph), 5.59 (s, 2 H, NCH_2O), 7.16–7.51 (m, 10 H, Ph × 2). Anal. ($C_{21}H_{22}N_2O_4S$) C, H, N, S. The starting material was also recovered in 36% yield.

Antiviral Assay Procedures. Two strains of HIV-1 ($HTLV\text{-}III_B$ and $HTLV\text{-}III_{RF}$) and two strains of HIV-2 ($LAV\text{-}2_{ROD}$ and $LAV\text{-}2_{EHO}$) were used in the anti-HIV assays. The assays were based on the inhibition of virus-induced cytopathic effect in MT-4 cells as previously described.[31] Briefly, MT-4 cells were suspended in culture medium at 1 × 10^5 cells/mL and infected HIV at a multiplicity of infection (MOI) of 0.02. Immediately after virus infection, 100 μL of the cell suspension was brought into each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. After a 4-day incubation at 37 °C, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[32]

The assay procedure for measuring the anti-HIV-1 activity of the compounds in peripheral blood lymphocytes (PBL) was based on the quantitative detection of HIV-1 p24 antigen in the culture supernatant by using a sandwich ELISA kit (Abbott). Phytohemagglutinin-stimulated PBL (1 × 10^6 cells/mL) were infected with HIV-1 ($HTLV\text{-}III_B$) at a MOI of 0.02 and cultured at 37 °C in the presence of various concentration of the test compounds. On day 4 and 7 after virus infection, the cells were subcultured at a ratio of 1:2 with fresh culture medium containing appropriate concentrations of the compounds. The assay was performed on day 10 after virus infection.

Cytotoxicity of the compounds was assessed in parallel with their antiviral activity. It was based on the viability of mock-infected MT-4 by the MTT method[32] and the incorporation of [5-^{3}H]Urd into RNA of mock-infected PBL.

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