Synthesis and Antiviral Activity of Deoxy Analogs of 1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) as Potent and Selective Anti-HIV-1 Agents[1]

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Received July 20, 1992

The effect of substitution in the acyclic structure of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)-thymine (HEPT) on anti-HIV-1 activity was investigated by synthesizing a series of deoxy analogs and related compounds. Preparation of 1-[(2-alkyloxyethoxy)methyl]-6-(phenylthio)thymine (**2–4**) derivatives was carried out based on alkylation of HEPT with primary alkyl halides. Preparation of the 1-[(alkyloxy)methyl]-6-(phenylthio)thymine (**26–31**) and 1-[(alkyloxy)methyl]-6-(arylthio)-2-thiouracil (**32–45**) derivatives was carried out on the basis of LDA lithiation of 1-[(alkyloxy)-methyl]thymine (**9–14**) and 1-[(alkyloxy)methyl]-2-thiouracil (**15–25**) followed by reaction with diaryl disulfides. The oxidative hydrolysis of the 2-thiouracil derivatives gave 1-[(alkyloxy)methyl]-6-(arylthio)uracil derivatives (**46–57**). 1-Alkyl-6-(phenylthio)thymine (**59–61**) derivatives were prepared on the basis of alkylation of 6-(phenylthio)thymine (**58**). Methylation of the hydroxyl group of HEPT did not affect the anti-HIV-1 activity of HEPT. Substitution of the 1-(2-hydroxyethoxy)methyl group by ethyl, butyl, methoxymethyl, (propyloxy)methyl, and (butyloxy)-methyl groups somewhat improved the original anti-HIV-1 activity of HEPT. Substitution with ethoxymethyl and (benzyloxy)methyl groups further potentiated the activity [EC_{50}: 1-(ethoxy-methyl)-6-(phenylthio)thymine (**27**), 0.33 μM; 1-[(benzyloxy)methyl]-6-(phenylthio)thymine (**31**), 0.088 μM]. When the 5-methyl group of **27** and **31** was replaced by an ethyl or an isopropyl group, the anti-HIV-1 activity was improved remarkably [EC_{50}: 5-ethyl-1-(ethoxymethyl)-6-(phenylthio)-uracil (**46**), 0.019 μM; 5-ethyl-1-[(benzyloxy)methyl]-6-(phenylthio)uracil (**52**), 0.0059 μM; 5-isopropyl-1-(ethoxymethyl)-6-(phenylthio)uracil (**55**), 0.012 μM; 5-isopropyl-1-[(benzyloxy)methyl]-6-(phenylthio)uracil (**56**), 0.0027 μM]. Introduction of two *m*-methyl groups into the phenylthio ring also potentiated the activity.

Human immunodeficiency virus type 1 (HIV-1)[2,3] is the causative agent of acquired immunodeficiency syndrome (AIDS), which is characterized as a systemic and fatal disorder. 3′-Azido-3′-deoxythymidine (AZT) is a thymidine analog that suppresses HIV-1 replication and is currently a licensed compound available for the treatment of patients with AIDS.[4,5] Despite its clinical efficacy, long-term administration of AZT often leads to toxic side effects, such as bone marrow suppression.[6] A purine dideoxynucleoside, 2′,3′-dideoxyinosine (DDI),[7] has recently been approved as an alternative drug for the patients who do not tolerate AZT, yet it also has unfavorable side effects.[8] AZT and DDI act as inhibitors of viral reverse transcriptase

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(1) Part of this work has appeared in a preliminary communication: Tanaka, H.; Baba, M.; Saito, S.; Miyasaka, T.; Takashima, H.; Sekiya, K.; Ubasawa, M.; Nitta, I.; Walker, R. T.; Nakashima, H.; De Clercq, E. Specific Anti-HIV-1 "Acyclonucleosides" Which Cannot Be Phosphorylated: Synthesis of Some Deoxy Analogues of 1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)thymine. *J. Med. Chem.* **1991**, *34*, 1508–1511.

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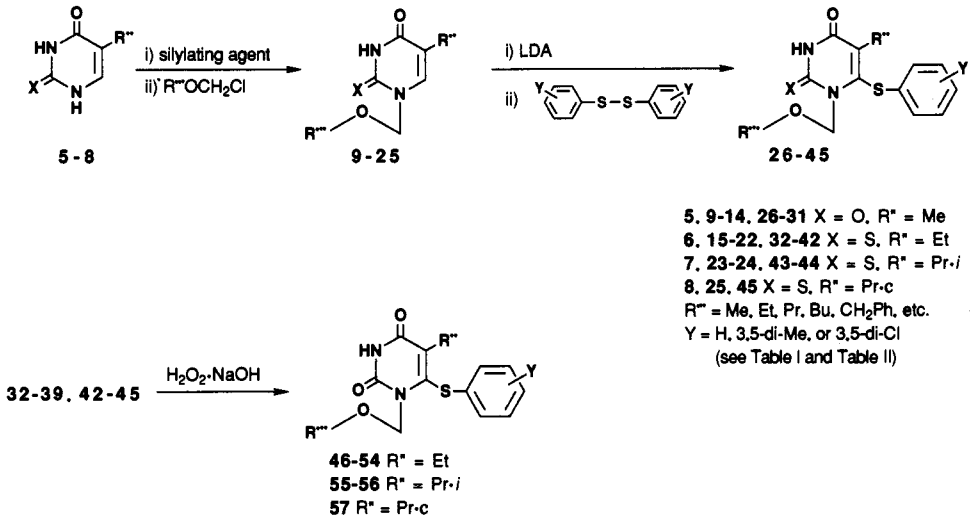
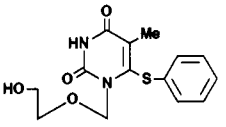
Scheme I



after being phosphorylated to their 5′-triphosphates.[9,10] Such 5′-triphosphates may also interact with the host cellular DNA polymerases.[11] This nonspecific action appears to contribute to the toxic side effects of this class of compounds. Therefore, it is still necessary to find new compounds having low toxicity and, preferably, a different mode of inhibition of viral replication.

We have previously reported that 1-[(2-hydroxyethoxy)-methyl]-6-(phenylthio)thymine (1; HEPT) had potent and

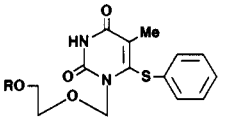


1; HEPT

selective in vitro activity against HIV-1.[12] It is interesting that HEPT does not inhibit the replication of animal retrovirus or even HIV-2.[13] HEPT can be regarded as an acyclonucleoside analog, yet its triphosphate does not interact with HIV-1 reverse transcriptase.[12] In addition, HEPT does not compete with [^{3}H-*methyl*]thymidine for phosphorylation by thymidine kinase derived from MT-4 cells.[13] We recently found that HEPT itself proved inhibitory to recombinant HIV-1 reverse transcriptase.[14] These results prompted us to synthesize HEPT analogs which cannot be phosphorylated. In the present article, we report the synthesis and anti-HIV-1 activity of deoxy HEPT analogs. The structure–activity relationships of these compounds are also described.

Chemistry

Initially, preparation of *O*-alkyl derivatives of HEPT was carried out. Selective *O*-alkylation of HEPT was accomplished by using 1.0 equiv of alkyl halides, such as MeI, pentyl iodide, and benzyl bromide, in the presence of 2.1 equiv of NaH in THF at room temperature to give **2**–**4** in 87%, 21%, and 80% yields, respectively.



2 R = Me
3 R = C_5H_{11}-*n*
4 R = CH_2Ph

We have already reported that the lithiation approach is highly efficient for synthesizing 6-substituted pyrimidine acyclonucleosides.[12,15–17] As shown in Scheme I, a series of analogs lacking a hydroxyl function in the acyclic structure of HEPT was synthesized from the corresponding pyrimidine bases (**5**–**8**) by employing the lithiation strategy as a key reaction step.

The preparation of 1-[(alkyloxy)methyl]thymines and 5-alkyl-1-[(alkyloxy)methyl]uracils, used as substrates for the lithiation, deserves some comment. When thymine (**5**) was treated with *N*,*O*-bis(trimethylsilyl)acetamide

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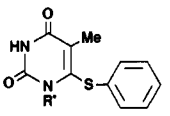
(BSA; 2.2 equiv) in CH_2Cl_2 at room temperature followed by reaction with the alkyl chloromethyl ether (1.2 equiv) in the presence of Bu_4NI (0.1 equiv) under reflux, the 1-[(alkyloxy)methyl]thymines (**9–14**) were obtained in good yield.[18] In contrast, similar treatment of 5-alkyl-2-thiouracils (**6–8**)[19] gave the corresponding products in uniformly low yield.[20] The yields were slightly improved by applying an alternative method published by Kim and co-workers.[21] Thus, when compounds **6–8** were silylated with 1,1,1,3,3,3-hexamethyldisilazane in the presence of a catalytic amount of $(NH_4)_2SO_4$ at refluxing temperature and then reacted with the alkyl chloromethyl ether (1.2 equiv) in the presence of CsI (1 equiv), compounds **15–25** were obtained in 16–43% yield.

The C-6 position of compounds **9–25** was lithiated with LDA (lithium diisopropylamide, 2.2 equiv) in THF below −70 °C for 1 h and the resulting lithiated species was then allowed to react with the diaryl disulfide (1.5 equiv). This reaction gave the desired 6-(phenylthio)thymine derivatives (**26–31**) and 5-alkyl-6-(arylthio)-2-thiouracils (**32–45**). The 5-alkyluracil analogs (**46–57**) were prepared from compounds **32–39** and **42–45** by oxidative hydrolysis (H_2O_2 in aqueous NaOH) of the thione function.[22]

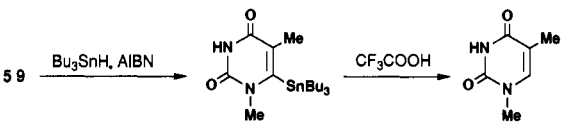
To simplify further the acyclic structure of HEPT, analogs having a primary alkyl group at the N-1 position were also prepared. Acid hydrolysis of HEPT in concentrated aqueous HCl–MeOH at 80 °C afforded 6-(phenylthio)thymine (**58**) in 24% yield. This compound was



58 R' = H
59 R' = Me
60 R' = Et
61 R' = Bu

allowed to react with MeI (1.0 equiv) in the presence of K_2CO_3 (1.0 equiv) to give a monomethylated (13%) and a dimethylated (34%) product after column chromatography on silica gel. When the monomethyl derivative was subjected to a radical-mediated desulfurizative stannylation followed by protonolysis according to the published method,[23] 1-methylthymine was obtained (Scheme II).[24] This confirmed the monomethyl derivative to be 1-methyl-6-(phenylthio)thymine (**59**). Following this alkylation method, compounds **60** (10%) and **61** (18%) were also prepared.

Scheme II



AIBN: 2,2'-azobisisobutyronitrile

Results of the Anti-HIV Assay and Discussion

Anti-HIV-1 activity and cytotoxicity of HEPT analogs synthesized in the present study are summarized in Table III, together with those of HEPT and AZT. The procedure to measure anti-HIV-1 activity has been described previously.[25] The HTLV-III_B strain of HIV-1 and MT-4 cells were used for the assay.

Among the *O*-alkyl derivatives of HEPT, the *O*-methyl derivative (**2**) retained the original activity of HEPT with a slight increase in cytotoxicity. This result suggested that, unlike acyclovir, HEPT did not require an hydroxy group in its acyclic structure. As can be seen from the data for compounds **3** and **4**, the presence of a bulkier *O*-alkyl group resulted in almost complete loss of the activity.

Reflecting the above-mentioned positive result for compound **2**, deoxy HEPT analogs 1-[(alkyloxy)methyl]-6-(phenylthio)thymines (**26–31**) showed a significant activity, except for compound **30** which had a [2-(trimethylsilyl)ethoxy]methyl group in the acyclic portion. It should be mentioned that a remarkable increase in anti-HIV-1 activity was accomplished at this stage, as is shown in the case of compound **27** (EC_{50} = 0.33 μM), which corresponded to the genuine deoxy analog of HEPT, and for 1-[(benzyloxy)methyl]-6-(phenylthio)thymine (**31**, EC_{50} 0.088 μM). In terms of SI value, the latter analog was now almost equivalent to AZT.

Our previous studies concerning the structure-activity relationships of HEPT analogs suggested that the following modifications at the base moiety would potentiate its original activity: (1) Replacement of the 2-oxo function with a thione function.[16] (2) Substitution at the meta position of the 6-phenylthio ring with two chlorine atoms or two methyl groups.[17] (3) Replacement of the 5-methyl group with a bulkier alkyl group.[17] The molecular design of compounds **32–57** was based on these findings as well as the results from the present study of deoxy HEPT analogs.

With respect to these compounds (**32–57**), again there was a general trend that a bulkier acyclic portion made the compound less active. To improve the EC_{50} value, both ethoxymethyl and (benzyloxy)methyl groups seemed to be the most suitable; further substitution uniformly increased the EC_{50} value, as can be seen from the data of compounds **35–37** and **40–42**.

Compound **46**, having an ethyl group at the C-5 position, was 10 times more potent than its 5-methyl counterpart (**27**). The corresponding 2-thio analog (**32**) had a similar EC_{50} value as that of compound **46** but was much more cytotoxic. Although the above-mentioned meta substitution at the 6-phenylthio ring was certainly effective in improving activity, comparison of toxicity data for com-

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(20) For example, reaction of 5-ethyl-2-thiouracil with BSA (2.2 equiv) and the following reaction with chloromethyl ethyl ether (1.2 equiv) gave a complex mixture of products. After chromatographic purification, the desired 1-(ethoxymethyl)-5-ethyl-2-thiouracil was obtained in only 15% yield.

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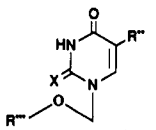
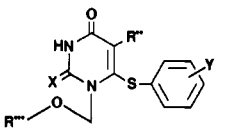
Table I. Physical Properties of Compounds **9–25**[a]



compd	X	R″	R‴	recrystn solv	% yield	mp, °C	formula	analysis
9	O	Me	Me	EtOAc	54	142–143	$C_7H_{10}N_2O_3$	C, H, N
10	O	Me	Et	*i*-PrOH–Et_2O	62	108–109	$C_6H_{12}N_2O_3$	C, H, N
11	O	Me	Pr	*i*-PrOH	63	141–142	$C_6H_{14}N_2O_3$	C, H, N
12	O	Me	Bu	*i*-PrOH	49	128–129	$C_{10}H_{16}N_2O_3$	C, H, N
13	O	Me	$CH_2CH_2SiMe_3$	EtOAc-hexane	87	136–137	$C_{11}H_{20}N_2O_3Si$	C, H, N
14	O	Me	CH_2Ph	EtOAc-hexane	86	133–134	$C_{13}H_{14}N_2O_3$	C, H, N
15	S	Et	Et	*i*-PrOH	37	111–112	$C_9H_{14}N_2O_2S$	C, H, N, S
16	S	Et	*i*-Pr	*i*-PrOH	25	124–125	$C_{10}H_{18}N_2O_2S$	C, H, N, S
17	S	Et	c-Hex	*i*-PrOH	34	123–124	$C_{13}H_{20}N_2O_2S$	C, H, N, S
18	S	Et	Hex-c-CH_2	*i*-PrOH	36	96–97	$C_{14}H_{22}N_2O_2S$	C, H, N, S
19	S	Et	CH_2Ph	*i*-PrOH	36	94	$C_{14}H_{18}N_2O_2S$	C, H, N, S
20	S	Et	$CH_2C_6H_4$(4-Me)	*i*-PrOH	23	110–111	$C_{15}H_{18}N_2O_2S$	C, H, N, S
21	S	Et	$CH_2C_6H_4$(4-Cl)	*i*-PrOH	16	101–102	$C_{14}H_{15}ClN_2O_2S$	C, H, N, S
22	S	Et	CH_2CH_2Ph	*i*-PrOH	32	129–130	$C_{15}H_{18}N_2O_2S$	C, H, N, S
23	S	*i*-Pr	Et	*i*-PrOH	31	93–94	$C_{10}H_{18}N_2O_2S$	C, H, N, S
24	S	*i*-Pr	CH_2Ph	*i*-PrOH	31	104–105	$C_{15}H_{18}N_2O_2S$	C, H, N, S
25	S	C-Pr	Et	*i*-PrOH	43	123–124	$C_{10}H_{14}N_2O_2S$	C, H, N, S

[a] Other physical data (UV, mass spec, ^1H NMR, and elemental analyses) of compounds **9–25** are presented in the supplementary material.

Table II. Physical Properties of Compounds **26–57**[a]



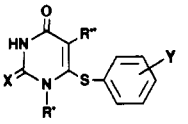
compd	X	R″	R‴	Y	recrystn solv	% yield	mp, °C	formula	analysis
26	O	Me	Me	H	EtOAc	73	148–150	$C_{13}H_{14}N_2O_3S$	C, H, N, S
27	O	Me	Et	H	EtOH	85	134	$C_{14}H_{16}N_2O_3S$	C, H, N, S
28	O	Me	Pr	H	EtOAc–hexane	77	136–138	$C_{15}H_{18}N_2O_3S$	C, H, N, S
29	O	Me	Bu	H	EtOAc–hexane	65	118–120	$C_{16}H_{20}N_2O_3S$	C, H, N, S
30	O	Me	$CH_2CH_2SiMe_3$	H	hexane	57	87–89	$C_{17}H_{24}N_2O_3SSi$	C, H, N, S
31	O	Me	CH_2Ph	H	EtOH–H_2O	58	157–161	$C_{19}H_{18}N_2O_3S$	C, H, N, S
32	S	Et	Et	H	EtOAc–hexane	88	106–109	$C_{15}H_{18}N_2O_2S_2$	C, H, N, S
33	S	Et	Et	3,5-Me_2	EtOAc–hexane	63	155–156	$C_{17}H_{22}N_2O_2S_2$	C, H, N, S
34	S	Et	Et	3,5-Cl_2	EtOAc–hexane	42	112–116	$C_{15}H_{16}Cl_2N_2O_2S_2$	C, H, N, S
35	S	Et	*i*-Pr	H	EtOAc–hexane	63	141–142	$C_{16}H_{20}N_2O_2S_2$	C, H, N, S
36	S	Et	c-Hex	H	EtOAc–hexane	71	139–141	$C_{19}H_{24}N_2O_2S_2$	C, H, N, S
37	S	Et	CH_2-c-Hex	H	EtOAc–hexane	72	134–135	$C_{20}H_{26}N_2O_2S_2$	C, H, N, S
38	S	Et	CH_2Ph	H	EtOH	89	151–153	$C_{20}H_{20}N_2O_2S_2$	C, H, N, S
39	S	Et	CH_2Ph	3,5-Me_2	EtOAc-hexane	66	158–160	$C_{22}H_{24}N_2O_2S_2$	C, H, N, S
40	S	Et	$CH_2C_6H_4$(4-Me)	H	EtOAc–hexane	59	157–160	$C_{21}H_{22}N_2O_2S_2$	C, H, N, S
41	S	Et	$CH_2C_6H_4$(4-Cl)	H	EtOAc–hexane	36	132–134	$C_{20}H_{19}ClN_2O_2S_2$	C, H, N, S
42	S	Et	CH_2CH_2Ph	H	EtOAc-hexane	32	136–138	$C_{21}H_{22}N_2O_2S_2$	C, H, N, S
43	s	*i*-Pr	Et	H	EtOAc–hexane	70	138–140	$C_{16}H_{20}N_2O_2S_2 \cdot {}^1/_6H_2O$	C, H, N, S
44	S	*i*-Pr	CH_2Ph	H	EtOAc–hexane	31	165–168	$C_{21}H_{22}N_2O_2S_2$	C, H, N, S
45	S	c-Pr	Et	H	EtOAc–hexane	85	123–124	$C_{16}H_{18}N_2O_2S_2$	C, H, N, S
46	O	Et	Et	H	EtOAc–hexane	77	123–125	$C_{15}H_{18}N_2O_3S$	C, H, N, S
47	O	Et	Et	3,5-Me_2	EtOAc–hexane	85	165–166	$C_{17}H_{22}N_2O_3S$	C, H, N, S
48	O	Et	Et	3,5-Cl_2	EtOAc–hexane	56	159–162	$C_{15}H_{16}Cl_2N_2O_3S \cdot {}^1/_3H_2O$	C, H, N, S
49	O	Et	*i*-Pr	H	EtOAc–hexane	81	119–121	$C_{16}H_{20}N_2O_3S \cdot {}^1/_5H_2O$	C, H, N, S
50	O	Et	c-Hex	H	EtOAc–hexane	79	167–171	$C_{19}H_{24}N_2O_3S$	C, H, N, S
51	O	Et	Hex-c-CH_2	H	EtOAc–hexane	83	115–116	$C_{20}H_{26}N_2O_3S$	C, H, N, S
52	O	Et	CH_2Ph	H	EtOAc–hexane	77	110–112	$C_{20}H_{20}N_2O_3S$	C, H, N, S
53	O	Et	CH_2Ph	3,5-Me_2	EtOAc–hexane	81	156–158	$C_{22}H_{24}N_2O_3S \cdot {}^1/_5H_2O$	C, H, N, S
54	O	Et	CH_2CH_2Ph	H	EtOAc–hexane	75	108–110	$C_{21}H_{22}N_2O_3S$	C, H, N, S
55	O	*i*-Pr	Et	H	EtOAc–hexane	65	112–114	$C_{16}h_{20}N_3O_3S \cdot {}^1/_3H_2O$	C, H, N, S
56	O	*i*-Pr	CH_2Ph	H	EtOAc–hexane	66	138–141	$C_{21}H_{22}N_2O_3S \cdot {}^1/_6H_2O$	C, H, N, S
57	O	c-Pr	Et	H	EtOAc–hexane	91	94–97	$C_{16}H_{18}N_2O_3S$	C, H, N, S

[a] Other physical data (UV, Mass, ^1H NMR, and elemental analyses) of compounds **26–57** are presented in Supplementary Material.

pounds **33** and **34** and compounds **47** and **48** clearly showed that analogs substituted with *m*-dimethyl groups provided much better SI values than those having *m*-dichloro substituents. The 5-isopropyl derivatives (**43**, **44**, **55**, and **56**) were as potent as their 5-ethyl counterparts, yet introduction of the 5-cyclopropyl group (**45** and **57**) weakened the activity.

6-(Phenylthio)thymine (**58**), the base moiety of HEPT, did not show any anti-HIV-1 activity. Although 1-methyl-6-(phenylthio)thymine (**6**) was also inactive, introduction of an ethyl or butyl group into the N-1 position of compound **58** significantly contributed to the anti-HIV-1 activity, as is shown by the EC_{50}s of compounds **60** and **61**. It could be assumed from this result that even the

Table III. Inhibition of HIV-1 Recplication in MT-4 Cells by the Deoxy Analogs of HEPT



compd	X	R′	R″	Y	EC_{50},[a] μM	CC_{50},[b] μM	SI[c]
2	O	$CH_2OCH_2CH_2OMe$	Me	H	8.7	299	34
3	O	$CH_2OCH_2CH_2OC_5H_{11}$-*n*	Me	H	>55	55	<1
4	O	$CH_2OCH_2CH_2OCH_2Ph$	Me	H	≥20	45	2.3
26	O	CH_2OMe	Me	H	2.1	244	116
27	O	CH_2OEt	Me	H	0.33	231	700
28	O	CH_2OPr	Me	H	3.6	147	41
29	O	CH_2OBu	Me	H	4.7	83	18
30	O	$CH_2OCH_2CH_2SiMe_3$	Me	H	>32	32	<1
31	O	CH_2OCH_2Ph	Me	H	0.088	95	1080
32	S	CH_2OEt	Et	H	0.026	81	3120
33	S	CH_2OEt	Et	3,5-Me_2	0.0044	>100[d]	>22700
34	S	CH_2OEt	Et	3,5-Cl_2	0.013	45	3460
35	S	CH_2-*i*-Pr	Et	H	0.22	>100[d]	>455
36	S	CH_2O-c-Hex	Et	H	1.6	223	139
37	S	CH_2OCH_2-c-Hex	Et	H	0.35	>100[d]	>286
38	S	CH_2OCH_2Ph	Et	H	0.0078	>100[d]	>12800
39	S	CH_2OCH_2Ph	Et	3,5-Me_2	0.0069	>20[d]	>2900
40	S	$CH_2OCH_2C_6H_4$(4-Me)	Et	H	0.078	>20[d]	>256
41	S	CH_2OCH_2(4-Cl)	Et	H	0.012	>20[d]	>1670
42	S	$CH_2OCH_2CH_2Ph$	Et	H	0.091	>20[d]	>220
43	S	CH_2OEt	*i*-Pr	H	0.014	>100[d]	>7140
44	S	CH_2OCH_2Ph	*i*-Pr	H	0.0068	>20[d]	>2940
45	S	CH_2OEt	c-Pr	H	0.095	46	484
46	O	CH_2OEt	Et	H	0.019	161	8500
47	O	CH_2OEt	Et	3,5-Me_2	0.0054	>100[d]	>18500
48	O	CH_2OEt	Et	3,5-Cl_2	0.0074	45	6080
49	O	CH_2O-*i*-Pr	Et	H	0.34	143	421
50	O	CH_2O-c-Hex	Et	H	4.0	>100[d]	>25
51	O	CH_2OCH_2-c-Hex	Et	H	0.45	17	38
52	O	CH_2OCH_2Ph	Et	H	0.0059	34	5800
53	O	CH_2OCH_2Ph	Et	3,5-Me_2	0.0032	>20[d]	>6250
54	O	$CH_2OCH_2CH_2Ph$	Et	H	0.096	38	396
55	O	CH_2OEt	*i*-Pr	H	0.012	106	8830
56	O	CH_2OCH_2Ph	*i*-Pr	H	0.0027	>20[d]	>7410
57	O	CH_2OEt	c-Pr	H	0.1	224	2240
58	O	H	Me	H	>250	250	<1
59	O	Me	Me	H	>150	150	<1
60	O	Et	Me	H	2.2	94	43
61	O	Bu	Me	H	1.2	89	74
1 (HEPT)	O	$CH_2OCH_2CH_2OH$	Me	H	7.0	740	106
AZT					0.003	7.8	2600

[a] Effective concentration of compound required to achieve 50% protection of MT-4 cells against the cytopathic effect of HIV-1. [b] Cytotoxic concentration of compound required to reduce the viability of mock-infected MT-4 cells by 50%. [c] Selectivity index: ratio of CC_{50}/EC_{50}. [d] Higher concentrations could not be achieved because of crystallization of the compound in the culture medium.

presence of an oxygen atom in the acyclic portion of HEPT is not essential.

The present modification at the 1-position of the pyrimidine moiety of HEPT indicated that the (2-hydroxyethoxy)methyl group could be replaced by certain deoxy substituents. It should be noted that a combination of the aforementioned modifications at the 2-, and 5-, and 6-positions and the present result involving modification at the 1-position brought about a marked increased in the anti-HIV-1 activity without increasing the cytotoxicity of the compounds.

In the next set of experiments, 6-(arylthio)-5-ethyluracil derivatives **32**, **33**, **38**, **46**, **47**, and **52** were examined for their inhibitory effects on the replication of HIV-1 (HTLV-III_B) in peripheral blood lymphocytes (PBLs). These compounds proved highly effective against HIV-1 in PBLs (Table IV). In addition to the HTLV-III_B, an AZT-resistant variant (A023D)[26] of HIV-1 was also highly susceptible to the inhibitory effects of compounds **32**, **33**, **38**, **46**, **47**, and **52**. Whereas these compounds inhibit the replication of A012D at a similar EC_{50}s as that of HTLV-III_B, the susceptibility of A012D to AZT was 100-fold lower than that of the HTLV-III_B strain (Table IV). As previously noted with HEPT,[13] these potent HIV-1 inhibitors had no effect on the replication of LAV-2_{ROD} strain of HIV-2 at their nontoxic concentrations to the host cells.

Although HEPT and its analogs (i.e. **27**, **28**, **31**, **46**, and **52**) are inhibitory to HIV-1 reverse transcriptase, HEPT triphosphate is totally inactive against this enzyme.[12,14] These results suggest that HEPT does not need to be phosphorylated intracellularly to interact with the HIV-1 reverse transcriptase. In fact, compounds **27**, **28**, **31**, **46**, and **52** have no hydroxyl group which can be phosphorylated, and yet are potent inhibitors of HIV-1 reverse transcriptase. HEPT and these compounds behave similarly to the benzodiazepine (TIBO) derivatives[27] in that they are highly specific and potent inhibitors of HIV-1,

(26) Larder, B. A.; Darby, G.; Richman D. D. HIV with Reduced Sensitivity to Zidovudine (AZT) Isolated During Prolonged Therapy. *Science (Washington, D.C.)* **1989**, *243*, 1731-1734.

Table IV. Inhibition of HIV-1 and HIV-2 Replication in MT-4 Cells and Peripheral Blood Lymphocytes (PBL) by 5-Ethyluracil Derivatives[a]

compd	virus	strain	cell	EC_{50}, μM	CC_{50}, μM
32	HIV-1	$HTLV\text{-}III_B$	PBL	0.013	35
	HIV-1	A012D	MT-4	0.015	81
	HIV-2	$LAV\text{-}2_{ROD}$	MT-4	>50	50
33	HIV-1	$HTLV\text{-}III_B$	PBL	0.0022	33
	HIV-1	A012D	MT-4	0.0027	>100[b]
	HIV-2	$LAV\text{-}2_{ROD}$	MT-4	>100	>100[b]
38	HIV-1	$HTLV\text{-}III_B$	PBL	0.0037	28
	HIV-1	A012D	MT-4	0.0050	>100[b]
	HIV-2	$LAV\text{-}2_{ROD}$	MT-4	>100	>100[b]
46	HIV-1	$HTLV\text{-}III_B$	PBL	0.011	93
	HIV-1	A012D	MT-4	0.015	146
	HIV-2	$LAV\text{-}2_{ROD}$	MT-4	>146	146
47	HIV-1	$MTLV\text{-}III_B$	PBL	0.0018	56
	HIV-1	A012D	MT-4	0.0027	>100[b]
	HIV-2	$LAV\text{-}2_{ROD}$	MT-4	>100	>100[b]
52	HIV-1	$HTLV\text{-}III_B$	PBL	0.0015	43
	HIV-1	A012D	MT-4	0.0029	30
	HIV-2	$LAV\text{-}2_{ROD}$	MT-4	>30	30
AZT	HIV-1	$HTLV\text{-}III_B$	PBL	0.0014	26
	HIV-1	A012D	MT-4	0.3	7.8
	HIV-2	$LAV\text{-}2_{ROD}$	MT-4	0.0028	7.8

[a] The antiviral activity and cytotoxicity of the compounds were expressed as the EC_{50} for virus-infected cells and the CC_{50} for mock-infected cells, respectively. [b] Higher concentrations could not be achieved because of crystallization of the compound in the culture medium.

but do not interfere with the replication of HIV-2. As the TIBO derivatives are also assumed to interact with HIV-1 reverse transcriptase,[27] it is quite possible that HEPT analogs and TIBO derivatives share a similar mechanism of action. It would now seem imperative to elucidate how these apparently unrelated classes of anti-HIV-1 compounds interact with reverse transcriptase at the molecular level.

Studies on pharmacokinetics, metabolism, disposition, and toxicology are indispensable before the novel 6-substituted acyclouridine derivatives could be advocated for clinical use in the prophylaxis or therapy of AIDS (or ARC). These studies are in progress. A comparative test of AZT and compound **46** for their effects on murine bone marrow progenitor cells clearly demonstrated that compound **46** did not suppress the in vitro proliferation of the cells at concentrations up to 10 μM.[28] With AZT, however, approximately 50 and 95% inhibition of colony formation was observed at a concentration of 1 and 10 μM, respectively.[28] These results indicate that the HEPT analogs, unlike AZT, may not cause bone marrow suppression in vivo. Because of their anti-HIV-1 potency, their effectiveness against AZT-resistant strains of HIV-1 and their low toxicity for bone marrow cells, we believe that these compounds may constitute highly promising candidates for use in the chemotherapy of AIDS.

Experimental Section

Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. ^1H NMR spectra were recorded at 250 MHz on a AC-250 Bruker NMR spectrometer using tetramethylsilane 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 taken on a Hitachi M-80A spectrometer. Silica gel column chromatography was carried out on Merck Silica gel 60 H. Octadecylsilyl (ODS) silica gel column chromatography was carried out on MCI Gel ODS IMY (Mitsubishi Kasei Corp., Tokyo, Japan). TLC was performed on silica gel (precoated silica gel plate 60 F254, Merck). Elemental analyses were performed on a Perkin-Elmer 240-C elemental analyzer.

General Procedure for the O-Alkylation of HEPT. To a solution of HEPT (2.46 g, 8 mmol) in THF (20 mL) was added NaH (60% in oil, 0.67 g, 16.8 mmol) under a nitrogen atmosphere at 0 °C. The mixture was stirred for 1 h at room temperature and alkyl halide (8 mmol) was added to the mixture. The reaction mixture was stirred for 14 h. The solution was added to aqueous 1 N HCl (30 mL) and the mixture extracted with EtOAc (30 mL). The organic layer was washed with saturated $NaHCO_3$ solution (20 mL) and then with brine (20 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness. The residue was purified by chromatography on silica gel ($CHCl_3$) and then crystallized from a suitable solvent.

1-[(2-Methoxyethoxy)methyl]-6-(phenylthio)thymine (2). MeI was used as the alkylating agent: yield 87%; mp 102–104 °C (EtOH–H_2O); UV (MeOH) λ_{max} 275 (ε 8000), 244 nm (ε 10 000); MS *m/z* 322 (M^+); ^1H NMR ($CDCl_3$) δ 2.03 (s, 3 H, 5-Me), 3.33 (s, 3 H, OMe), 3.40–3.78 (m, 4 H, OCH_2CH_2O), 5.60 (s, 2 H, NCH_2O), 7.16–7.37 (m, 5 H, SPh), 8.50 (br, 1 H, NH). Anal. ($C_{15}H_{18}N_2O_4S$) C, H, N, S.

1-[[2-(Pentyloxy)ethoxy]methyl]-6-(phenylthio)thymine (3). Pentyl iodide was used as the alkylating agent: yield 21%; mp 76–78 °C (EtOH–H_2O); UV (MeOH) λ_{max} 274 (ε 8100), 244 nm (ε 10 200); MS *m/z* 378 (M^+); ^1H NMR ($CDCl_3$) δ 0.88 [t, J = 6.8 Hz, 3 H, $O(CH_2)_4CH_3$], 1.19–1.72 [m, 6 H, $OCH_2(CH_2)_3CH_3$], 2.02 (s, 3 H, 5-Me), 3.30–3.62 (m, 4 H, OCH_2CH_2O), 3.40 [t, J = 6.8 Hz, 2 H, $OCH_2(CH_2)_3CH_3$], 5.61 (s, 2 H, NCH_2O), 7.12–7.58 (m, 5 H, SPh), 8.83 (br, 1 H, NH). Anal. ($C_{19}H_{26}N_2O_4S$) C, H, N, S.

1-[[2-(Benzyloxy)ethoxy]methyl]-6-(phenylthio)thymine (4). Benzyl bromide was used as the alkylating agent: yield 80%; mp 107–109 °C (Et_2O–hexane); UV (MeOH) λ_{max} 274 (ε 7800), 244 nm (ε 9800); MS *m/z* 398 (M^+); ^1H NMR ($CDCl_3$) δ 2.00 (s, 3 H, 5-Me), 3.46–3.86 (m, 4 H, OCH_2CH_2O), 4.50 (s, 2 H, OCH_2Ph), 5.60 (s, 2 H, NCH_2O), 7.12–7.38 (m, 10 H, Ph × 2), 8.50 (br, 1 H, NH). Anal. ($C_{21}H_{22}N_2O_4S$) C, H, N, S.

General Procedure for the Preparation of 1-[(Alkyloxy)methyl]thymine Derivatives 9–14. A suspension of **5** (25 g, 0.2 mol) and *N,O*-bis(trimethylsilyl)acetamide (109 mL, 0.44 mol) in CH_2Cl_2 (250 mL) was stirred for 2 h at room temperature. To the resulting solution, Bu_4NI (0.74 g, 2 mmol) and alkyl chloromethyl ether (0.24 mol) were added. The mixture was heated under reflux with stirring for 2 h and allowed to cool to room temperature. The reaction mixture was poured into saturated $NaHCO_3$ solution (100 mL) and ice (50 mL) and stirred for 30 min. The organic layer was washed with brine (150 mL), dired over $MgSO_4$, filtered, and concentrated to dryness. The residue was crystallized from a suitable solvent to give 1-[(alkyloxy)methyl]thymines (**9–14**).

General Procedure for the Preparation of 5-Alkyl-1-[(alkyloxy)methyl]-2-thiouracil Derivatives 15–25. A suspension of 5-alkyl-2-thiouracil (**6–8**; 10 mmol) and $(NH_4)_2SO_4$ (200 mg) in 1,1,1,3,3,3-hexamethyldisilazane (30 mL) was heated under reflux with stirring for 15 h. Excess silylating reagent was removed in vacuo. The residual oil was dissolved in MeCN (50 mL), and to the solution was added alkyl chloromethyl ether (12 mmol) and CsI (2.6 g, 10 mmol). The mixture was heated under reflux with stirring for 2 h and allowed to cool to room temperature. The reaction mixture was added to H_2O (50 mL) and extracted with EtOAc (150 mL). The organic layer was washed with saturated $NaHCO_3$ solution (50 mL) and concentrated to dryness. The residue was purified by chromatography on silica gel ($CHCl_3$) and then crystallized from 2-propanol to give 5-alkyl-1-[(alkyloxy)methyl]-2-thiouracils (**15–25**).

General Procedure for the Preparation of 5-Alkyl-1-[(alkyloxy)methyl]-6-(arylthio)uracil Derivatives 26–45. To a solution of LDA (4.4 mmol) in THF (10 mL) was added

(27) Pauwels, R.; Andries, K.; Desmyter, J.; Schols, D.; Kukla, M. J.; Breslin, H. J.; Raeymaeckers, A.; Van Gelder, J.; Woestenborghs, R.; Heykants, J.; Schellekens, K.; Janssen, M. A. C.; De Clercq, E.; Janssen, P. A. J. Potent and Selective Inhibition of HIV-1 Replication in vitro by a Novel Series of TIBO Derivatives. Nature (*London*) **1990**, *343*, 470–474.

(28) Umezu, K.; Tsurufuji, M.; Yuasa, S.; Tsutsui, N.; Yabuuchi, S.; Ikeda, Y. No Toxic Effects of a New Anti-HIV-1 Agent 6-Substituted Acyclouridine Derivative on Murine Bone Marrow Cell Growth. *Abstracts of Papers*; 7th International Conference on AIDS; Florence, Italy; Instituto Superiore di Sanità: Rome, Italy, 1991; Abstract W.A.1010.

1-[(alkyloxy)methyl]uracil (9–25; 2 mmol) in THF (8 mL) under a nitrogen atmosphere at a rate such that the temperature did not exceed −70 °C. After the mixture was stirred for 1 h, diaryl disulfide (3 mmol) dissolved in THF (5 mL) was added, the temperature being maintained below −70 °C. The mixture was stirred for 1 h below −70 °C and allowed to warm to room temperature. The solution was neutralized with concentrated HCl, poured into brine (20 mL), and extracted with EtOAc (30 mL). The organic layer was washed with saturated $NaHCO_3$ solution (20 mL) and then with brine (20 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness. The residue was purified by chromatography on silica gel ($CHCl_3$–hexane; 8:2, v/v) and then crystallized from a suitable solvent.

General Procedure for the Preparation of 5-Alkyl-1-[(alkyloxy)methyl]-6-(arylthio)uracils 46–57. To a suspension of the 5-alkyl-1-[(alkyloxy)methyl]-6-(arylthio)-2-thiouracil derivatives (10 mmol) in aqueous 1 N NaOH (80 mL) was added 35% H_2O_2 (6 mL, 60 mmol) and the mixture stirred at room temperature. After 1 h, the reaction mixture was neutralized with concentrated HCl. 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 EtOAc–hexane to give the target compounds.

6-(Phenylthio)thymine (58). To a solution of HEPT (1.30 g, 4.2 mmol) in MeOH (20 mL) was added aqueous concentrated HCl (3 mL) and the mixture was stirred at 80 °C (bath temperature) for 40 min. The solution was allowed to cool to room temperature and evaporated to dryness. The residue was purified by chromatography on silica gel ($CHCl_3$–MeOH 20:1, v/v) and then crystallized from EtOAc–MeOH to give 0.24 g (24%) of **58**: mp 238–242 °C; UV (MeOH) λ_{max} 288 (ϵ 9100), 245 nm (ϵ 7200); MS *m/z* 234 (M^+); ^1H NMR (Me_2SO-d_6) δ 1.89 (s, 3 H, 5-Me), 7.25–7.43 (m, 5 H, SPh), 10.9, 11.2 (br × 2, 1 H × 2, NH × 2). Anal. ($C_{11}H_{10}N_2O_2S$) C, H, N, S.

General Procedure for the Preparation of 1-Alkyl-6-(phenylthio)thymine Derivatives 59–61. A mixture of **58** (200 mg, 0.85 mmol), Me_2SO (2 mL), alkyl iodide (0.85 mmol), and K_2CO_3 (117 mg, 0.85 mmol) was stirred at 80 °C for 2 h and then allowed to cool to room temperature. After neutralization with concentrated HCl, the mixture was evaporated to dryness. The residue was purified by chromatography on silica gel ($CHCl_3$). The fraction containing the monoalkyl derivative was concentrated and the residue was crystallized from a suitable solvent.

1-Methyl-6-(phenylthio)thymine (59). MeI was used as the alkylating agent: yield 13%; mp 229–230 °C ($CHCl_3$); UV (MeOH) λ_{max} 286 (ϵ 8600), 242 nm (ϵ 8900); MS *m/z* 248 (M^+); ^1H NMR ($CDCl_3$) δ 2.21 (s, 3 H, 5-Me), 3.44 (s, 3 H, 1-Me), 7.13–7.43 (m, 5 H, SPh), 9.09 (br, 1 H, N^3H). Anal. ($C_{12}H_{12}N_2O_2S \cdot {}^1/_8H_2O$) C, H, N, S. Dimethyl-6-(phenylthio)thymine was also isolated in 34% yield.

Desulfurizative Stannylation and Successive Protonolysis of 59. A mixture of **59** (5 mg, 0.02 mmol), 2,2′-azobisisobutyronitrile (1 mg), and tributyltin hydride (27 μL, 0.1 mmol) in dry benzene (2 mL) was refluxed for 4 h. After evaporation of the solvent, the residues was treated with 50% aqueous CF_3COOH (5 mL) at room temperature for 14 h. An aliquote of the mixture was purified by TLC ($CHCl_3$–MeOH 10:1, v/v) and the sole product obtained was analyzed by UV spectroscopy: (H_2O) λ_{max} 273 nm, (1 N NaOH) λ_{max} 271 nm. These data are identical with those of 1-methylthymine.[24]

1-Ethyl-6-(phenylthio)thymine (60). EtI was used as the alkylating agent: yield 10%; mp 158–160 °C (acetone–hexane); UV (MeOH) λ_{max} 286 (ϵ 10 000), 239 nm (ϵ 10 000); MS *m/z* 262 (M^+); ^1H NMR ($CDCl_3$) δ 1.18 (t, J = 7.0 Hz, 3 H, CH_2Me), 2.08 (s, 3 H, 5-Me), 4.13 (q, J = 7.0 Hz, 2 H, CH_2Me), 7.13–7.38 (m, 5 H, SPh), 8.22 (br, 1 H, NH). Anal. ($C_{13}H_{14}N_2O_2S$) C, H, N, S.

1-Butyl-6-(phenylthio)thymine (61). BuI was used as the alkylating agent: yield 18%; mp 124–127 °C (EtOAc); UV (MeOH) λ_{max} 286 (ϵ 10 000), 240 nm (ϵ 9800); MS *m/z* 290 (M^+); ^1H NMR ($CDCl_3$) δ 0.89 (t, J = 7.3 Hz, 3 H, $CH_2CH_2CH_2Me$), 1.26–1.35 (m, 4 H, $CH_2CH_2CH_2Me$), 2.11 (s, 3 H, 5-Me), 4.03 (q, J = 7.8 Hz, 2 H, $CH_2CH_2CH_2Me$), 7.16–7.38 (m, 5 H, SPh), 8.43 (br, 1 H, NH). Anal. ($C_{15}H_{18}N_2O_2S$) C, H, N, S.

Antiviral Assay Procedures. The activity of the compounds against HIV-1 (HTLV-III_B strain) and HIV-2 (LAV-2_{ROD} strain) replication was based on the inhibition of virus-induced cytopathic effect in MT-4 cells as previously described.[25] Briefly, virus stocks were titrated in MT-4 cells and expressed as 50% cell culture infective dose ($CCID_{50}$). MT-4 cells were suspended in culture medium at 1 × 10^5 cells/mL and infected with HIV at a multiplicity of infection (MOI, ratio of $CCID_{50}$ to cell number) 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. The test compounds were dissolved in dimethyl sulfoxide at 50 mM or higher. 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.[29] Activity of the compounds against the AZT-resistant clinical isolate of HIV-1 (A012D) was determined by the amount of HIV-1 p24 antigen in the culture supernatant [using a sandwich ELISA kit (Abbott)] on day 4 after infection of MT-4 cells.

The assay procedure for measuring the anti-HIV-1 activity of the compounds in peripheral blood lymphocytes (PBLs) was also based on the quantitative detection of HIV-1 p24 antigen in the culture supernatant using a sandwich ELISA kit (Abbott). Phytohemagglutinin-stimulated PBL (1 × 10^6 cells/mL) were infected with HIV-1 (HTLV-III_B) at a MOI of 0.2 and cultured at 37 °C in the presence of various concentrations of the test compounds. On day 4 after virus infection, the cells were subcultured at a ratio of 1:5 with fresh culture medium containing appropriate concentrations of the compounds. The assay was performed on day 7 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 cells, as monitored by the MTT method.[29]

Acknowledgment. Generous financial support (to H. Tanaka) from the Naito Foundation is gratefully acknowledged. This work has also been supported by a British Council Collaborative Research Project (to H. Tanaka and R.T.W.) and by the Grant-in-Aid for Scientific Research on Priority Areas (No. 03242104) from the Ministry of Education, Science and Culture, Japan. HIV-2 (LAV-2_{ROD}) was kindly provided by Dr. L. Montagnier (Pasteur Institute, Paris, France), while the AZT-resistant variant (A012D) was obtained through the AIDS Research and Reference Reagent Program, AIDS program, NIAID, Bethesda, MD (contributor: Dr. D. D. Richman).

Supplementary Material Available: Physical data (UV, mass spectra, ^1H NMR, and elemental analyses) of compounds **2–4** and **9–61** (14 pages). Ordering information is given on any current masthead page.

Registry No. 1, 123027-56-5; **2**, 132774-35-7; **3**, 136160-16-2; **4**, 132774-36-8; **5**, 65-71-4; **6**, 34171-37-4; **7**, 18718-34-8; **8**, 59698-21-4; **9**, 136160-46-8; **10**, 132774-37-9; **11**, 144410-10-6; **12**, 144410-11-7; **13**, 144410-12-8; **14**, 80140-17-6; **15**, 144410-13-9; **16**, 144410-14-0; **17**, 144410-15-1; **18**, 144410-16-2; **19**, 144410-17-3; **20**, 144410-18-4; **21**, 144410-19-5; **22**, 144410-20-8; **23**, 144410-21-9; **24**, 144410-22-0; **25**, 144410-23-1; **26**, 136160-17-3; **27**, 132774-39-1; **28**, 133563-27-6; **29**, 136160-31-1; **30**, 144410-24-2; **31**, 132774-43-7; **32**, 136011-43-3; **33**, 136011-45-5; **34**, 144410-25-3; **35**, 136160-39-9; **36**, 136160-41-3; **37**, 144410-26-4; **38**, 136160-24-2; **39**, 136105-78-7; **40**, 136160-28-6; **41**, 136160-43-5; **42**, 136160-45-7; **43**, 136160-33-3; **44**, 136160-35-5; **45**, 136160-37-7; **46**, 132774-45-9; **47**, 136011-44-4; **48**, 144410-27-5; **49**, 136160-38-8; **50**, 136160-40-2; **51**, 136160-42-4; **52**, 132774-46-0; **53**, 136105-76-5; **54**, 136160-44-6; **55**, 136160-32-2; **56**, 136160-34-4; **57**, 136160-36-6; **58**, 136160-18-4; **59**, 136160-19-5; **60**, 136160-20-8; **61**, 136160-21-9; dimethyl-6-(phenylthio)thymine, 144410-28-6.

(29) Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. Rapid and Automated Tetrazolium-Based Colorimetric Assay for the Detection of Anti-HIV Compounds. *J. Virol. Methods* **1988**, *20*, 309–322.