filtered saturated solutions. The solubilities were generally found to exceed 10% w/v.

In addition to be useful for parenteral or ophthalmic administration the novel prodrugs may be applied to improve the peroral or rectal bioavailability of slightly water-soluble drugs. Thus, whereas allopurinol and acyclovir are both highly nonlipophilic compounds with log P values[44] of −0.55 and −1.47, respectively, and also poorly soluble in water (0.5 mg mL^{-1} for allopurinol and 1.2 mg mL^{-1} for acyclovir) prodrug derivatives of the present type with both increased lipophilicity and solubility can readily be designed as exemplified with the derivatives **29** (log P = 1.13), **31** (log P = 0.53), **32** (log P = 0.97), and **13** (log P = −0.05). It is apparent that the lipophilicity of the prodrug derivatives can be readily modified or controlled by the appropriate selection of the amino group both in terms of amine basicity,[45] and hence degree of ionization at physiological pH, and in terms of hydrophobicity of the substituents on the nitrogen atom. Indeed, preliminary experiments in rabbits have shown that the allopurinol prodrugs **29** and **32** are much better absorbed than allopurinol itself upon rectal administration.

In conclusion, N-substituted (3- or 4-aminomethyl)benzoate esters are shown to be a potentially useful biolabile and solution-stable prodrug type for drugs containing hydroxyl groups or NH-acidic groups, in the latter case with the corresponding N-hydroxymethyl or, in general, N-(α-hydroxylalkyl) derivatives as a synthetic "handle". The esters are highly water soluble at pH 1–6 and combine a high stability in weakly acidic aqueous solution with a rapid rate of hydrolysis in plasma.

(44) P is the partition coefficient between octanol and 0.05 M phosphate buffer of pH 7.4.

(45) For example, (morpholinomethyl)benzoate esters possess pK_a values of 6.0–6.1 at 25 °C whereas (N,N-dimethylaminomethyl)benzoate esters have pK_a values around 7.8 as determined by titration.

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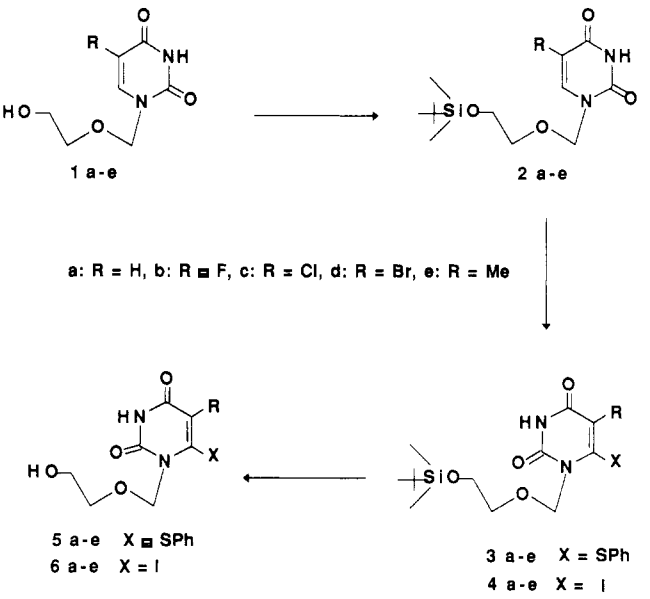
A Novel Lead for Specific Anti-HIV-1 Agents: 1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)thymine

Sir:

As a result of the clinical efficacy of 3′-azido-3′-deoxythymidine (AZT, retrovir) in the treatment of AIDS (acquired immunodeficiency syndrome),[1-3] a large number of nucleoside analogues,[4-17] including acyclonucleosides,[18-20]

Scheme I



have been synthesized and evaluated as potential drug candidates against this disease.

Base-modified pyrimidine nucleoside analogues, so far synthesized in the above context,[13-17] have always been substituted at the C-5 position, presumably because of the ease of substitution at this position. Consequently, to the best of our knowledge, no information seems to be available concerning the anti-HIV (human immunodeficiency virus) activity of C-6 substituted derivatives.

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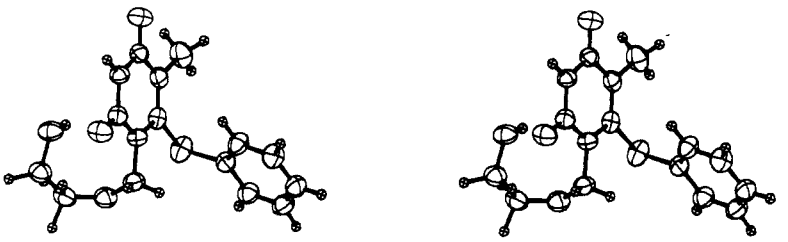


Figure 1. ORTEP stereoview of **5e**.

As a part of our studies of the utilization of the lithiation reaction for the modification of the base moiety of nucleosides,[21-32] we were interested in synthesizing 6-substituted uracil acyclonucleosides based on our own strategy using LDA (lithium diisopropylamide) as a lithiating agent. This methodology has already been successfully used in the modification of uridine[21,23] and 2'-deoxyuridine.[24]

In the present communication, we report the synthesis and anti-HIV-1 activity of 6-phenylthio and 6-iodo derivatives of 1-[(2-hydroxyethoxy)methyl]uracil. Our choice of these 6-substituents was motivated by the chemical evidence that 6-(phenylthio)-[33] and 6-iodouridine[34] derivatives were highly susceptible to nucleophilic addition–elimination reactions at the C-6 position. Thus, some reactivity could be expected, even in a physiological environment.

Chemistry. 1-[(2-Hydroxyethoxy)methyl]uracil (**1a**) and its 5-substituted analogues (**1b–e**) were prepared according to the published procedure.[35] The hydroxyl group in **1** was silylated with *tert*-butyldimethylsilyl chloride in DMF in the presence of imidazole to give **2** (Scheme I).

Lithiation of these protected acylonucleosides (**2**) was carried out in THF below −70 °C by using 2.5 equiv of LDA, and the resulting clear solution of the C-6 lithiated species was subsequently treated with the corresponding electrophile (2.0 equiv), diphenyl disulfide, or iodine, at −70 °C for 1 h. Column chromatographic purification of the reaction mixture gave the respective 6-phenylthio (**3a**, 81%; **3b**, 92%; **3c**, 26%; **3d**, 42%; **3e**, 73%) and 6-iodo (**4a**, 76%; **4b**, 94%; **4c**, 68%; **4d**, 61%; **4e**, 79%) derivatives (the yields were not optimized).

Table I. Inhibition of HIV-1 Replication in MT-4 Cells by Compounds **5** and **6**

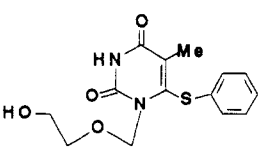
compd	EC_{50},[a] μM	CC_{50},[b] μM	SI[c]
5a	>500	1850	<3.7
5b	>100	288	<2.9
5c	>30	100	<3.4
5d	>20	44	<2.2
5e	7.0	740	106
6a	>30	75	<2.5
6b	>15	32	<2.1
6c	>6	13	<2.2
6d	>5	11.5	<2.3
6e	>80	400	<5
AZT	0.016	20	1250
ddCyd	0.3	40	133
ddAdo	6.3	890	141

[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}.

The desired 6-substituted acylonucleosides (**5** and **6**) were produced in high yields upon desilylation of **3** or **4** in THF containing aqueous acetic acid. Compounds **5** and **6** were all obtained in crystalline form and gave physical data (elemental analyses, MS, ^1H NMR, and UV) consistent with their structures.

The data of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (**5e**) are representative: mp 123–124 °C (EtOAc/MeOH); MS *m/z* 308 (M^+); ^1H NMR (Me_2SO-d_6, 400 MHz) δ 1.83 (s, 3 H, 5-Me), 3.37–3.41 (m, 2 H, $HOCH_2CH_2O$), 3.48 (t, 2 H, $HOCH_2CH_2O$), 4.57 (t, 1 H, OH), 5.42 (s, 2 H, NCH_2O), 7.27–7.38 (m, 5 H, SPh), 11.67 (br, 1 H, NH); UV λ_{max} (MeOH) 244 (ε 9600) and 275 nm (ε 8400). Anal. ($C_{14}H_{16}N_2O_4S$) C, H, N.

The structure of **5e** was further confirmed by X-ray crystallography and is depicted in Figure 1 by an ORTEP stereoview. Detailed data and discussion of X-ray crystallographic analysis will be published elsewhere.



5e

Biological Results. The procedure to measure anti-HIV-1 activity in MT-4 cells has been described previously.[36] Either mock-infected or HIV-1-infected MT-4 cells were incubated in the presence of various concentrations of test compounds and the number of viable cells was determined by the MTT [3-(4,5-dimethylthiazol-2-

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Table II. Inhibitory Effect of **5e** and Its Triphosphate on HIV-1 Reverse Transcriptase (RT) Activity[a]

compd	IC_{50},[b] μM	
	poly(rA)·oligo(dT)[c]	poly(rC)·oligo(dG)[c]
5e	>500	150
5e triphosphate	280	500
AZT triphosphate	0.034	ND[d]

[a] The assay procedure has been described previously.[40] [b] 50% Inhibitory concentration. [c] Template–primer used for assay. [d] Not determined.

yl)-2,5-diphenyltetrazolium bromide] method[37] on day 5 after virus infection.

The anti-HIV-1 activity and cytotoxicity of the 6-substituted uracil acyclonucleosides (**5a–e** and **6a–e**) are shown in Table I together with those of AZT, 2′,3′-dideoxycytidine (ddCyd), and 2′,3′-dideoxyadenosine (ddAdo). Among the compounds prepared in the present study, 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (**5e**) turned out to be the most selective anti-HIV-1 agent with a selectivity index of 106. When the activity of **5e** was compared to that of known anti-HIV-1 nucleosides, AZT and ddCyd were more active but more toxic than **5e** in MT-4 cells. In terms of activity and cytotoxicity, **5e** was almost comparable to ddAdo, which is currently undergoing clinical trials.

When **5e** was examined for its inhibition of HIV-2 (ROD) under the same assay conditions as those used for HIV-1, it had no activity at concentrations up to 250 μM. This compound was also found to be ineffective against simian immunodeficiency virus (SIV) induced cytopathogenicity in MT-4 cells, simian AIDS related virus (SRV) induced giant-cell formation in Raji cell cultures, and Moloney murine sarcoma virus (MSV) induced transformation of murine C3H/3T3 embryo fibroblasts (data not shown). Thus, **5e** can be considered to be a highly specific anti-HIV-1 agent.

It is also interesting that **5e** did not compete with [^{3}H-Me]thymidine for phosphorylation by thymidine kinase derived from MT-4 cells, and its anti-HIV-1 activity in MT-4 cells was not affected by the addition of 25 μM thymidine or 250 μM thymidine plus 1 mM 2′-deoxycytidine. Further studies to investigate the metabolic fate of **5e** are now in progress using radiolabeled **5e**.

Finally, **5e** itself and its triphosphate[38] were evaluated for inhibition of HIV-1 reverse transcriptase (RT).[39] As can be seen from the results shown in Table II, irrespective of the template–primer used, neither **5e** nor its triphosphate inhibited RT at concentrations much higher than that of the EC_{50} of **5e** (7.0 μM, see Table I) for HIV-1 replication in MT-4 cells.

In conclusion, the results obtained in the present study clearly indicate that compound **5e**, which can be regarded as a nucleoside analogue, manifests its anti-HIV-1 activity through a mechanism different from that so far known for other nucleoside analogues. Since **5e** was neither markedly toxic against human bone marrow cells in vitro at 100 μM concentration, which is 20 times that at which AZT is significantly toxic (5 μM), nor inhibitory to murine leukemia L1210, human B-lymphoblast Raji, and T-lymphoblast Molt/4F cell proliferation at the same concentration, we believe our results may lead to discovery of a new area in which to search for anti-HIV-1 agents that may be considerably less toxic than those currently being investigated.

Registry No. **1a**, 78097-04-8; **1b**, 77474-50-1; **1c**, 81777-50-6; **1d**, 78097-11-7; **1e**, 68724-11-8; **2a**, 121749-94-8; **2b**, 121749-95-9; **2c**, 121749-96-0; **2d**, 121749-97-1; **2e**, 121749-98-2; **3a**, 123027-47-4; **3b**, 123027-48-5; **3c**, 123027-49-6; **3d**, 123027-50-9; **3e**, 123027-51-0; **4a**, 121749-89-1; **4b**, 121749-90-4; **4c**, 121749-91-5; **4d**, 121749-92-6; **4e**, 121749-93-7; **5a**, 123027-52-1; **5b**, 123027-53-2; **5c**, 123027-54-3; **5d**, 123027-55-4; **5e**, 123027-56-5; **6a**, 121749-84-6; **6b**, 121749-85-7; **6c**, 121749-86-8; **6d**, 121749-87-9; **6e**, 121749-88-0.

Supplementary Material Available: Physical data of compounds **5a–e** and **6a–e** (3 pages). Ordering information is given on any current masthead page.

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