Acyclic Analogues of 3′-Azido-3′-deoxythymidine as Potential Antiviral Agents. Nucleoside Synthesis by Michael Addition

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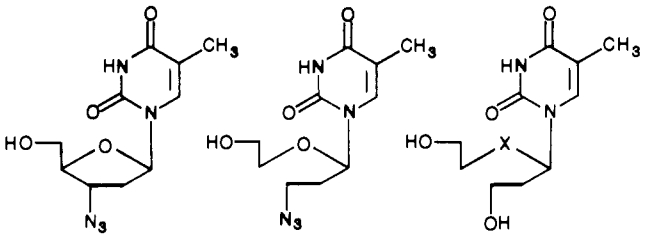
An acyclic derivative of 3′-azido-3′-deoxythymidine, (*R,S*)-1-[1-(2-hydroxyethoxy)-3-azidopropyl]thymine (**2**), has been synthesized by a path involving Michael-type addition. Related thymidine analogues lacking the C(3′)–C(4′) bond were similarly obtained. The method provides a versatile new route to nucleoside analogues. The new compounds were found to be essentially inactive against human immunodeficiency virus type 1 (HIV-1) and herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) in vitro when tested up to 100 μM.

The acquired immune deficiency syndrome (AIDS) epidemic has stimulated the search for agents capable of arresting the causative virus, human immunodeficiency virus type 1 (HIV-1). Among the numerous candidates proposed[1-3] the nucleoside analogue 3′-azido-3′-deoxythymidine (**1**, AZT) is at present the only drug receiving wide clinical usage. Despite its efficacy[4,5] AZT suffers from serious disadvantages. Side effects include headaches, lowered white-cell counts,[4] and suppression of bone marrow cell formation.[6] Additionally, its short half-life in the body necessitates frequent administration to maintain therapeutically effective levels.[7] These considerations, as well as the costliness of the drug, have underlined the urgent need for new, selective antiretroviral drugs. Recently, several 2′,3′-dideoxy- and 2′,3′-unsaturated-2′,3′-dideoxynucleosides have shown promising in vitro activity.[6,8,9]

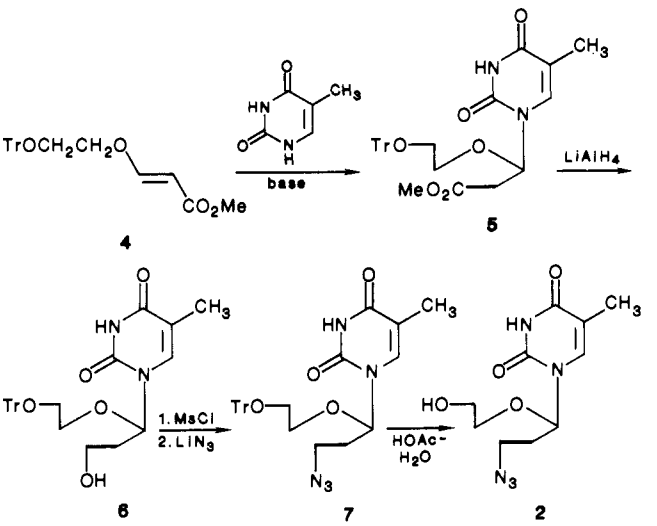
Antiherpetic activity of various acyclic nucleoside analogues,[10] e.g. acyclovir[11] and ganciclovir,[12] is well established and has given rise to effective antiviral drugs. Acyclic derivatives of AZT, on the other hand, have not as yet been described. In view of the above-mentioned activity, acyclonucleosides related to AZT appeared to be attractive synthetic targets for the development of new anti-HIV compounds. Compound **2** ("acyclo-AZT") was of particular interest due to its close structural similarity to AZT; **2** differs from **1** only in the scission of the C(3′)–C(4′) bond. This paper describes the preparation of racemic **2** by a new and general synthetic route. The corresponding acyclothymidine **3a** and its thio congener **3b** are also reported, as well as in vitro HIV-1 and HSV-1, HSV-2 test results.



1 **2** **3a**: X = O; **b**: X = S

A route to acyclic nucleosides lacking the C(3′)–C(4′) bond has been described by Bryant and co-workers.[13] The adenine analogue of **3a** was thus obtained by alkylation of the 6-chloropurine anion with a suitably substituted and protected α-iodo ether (generated in situ from a 1,3-dioxolane and trimethylsilyl iodide[14]), followed by ammonolysis and deprotection. Although potentially general, the method does not appear to be well suited to the preparation of **2**. A new approach was therefore investigated.

Scheme I



Among nucleoside-forming reactions (e.g. heterocycle anion alkylation, Vorbruggen glycosylation[15]) Michael-type

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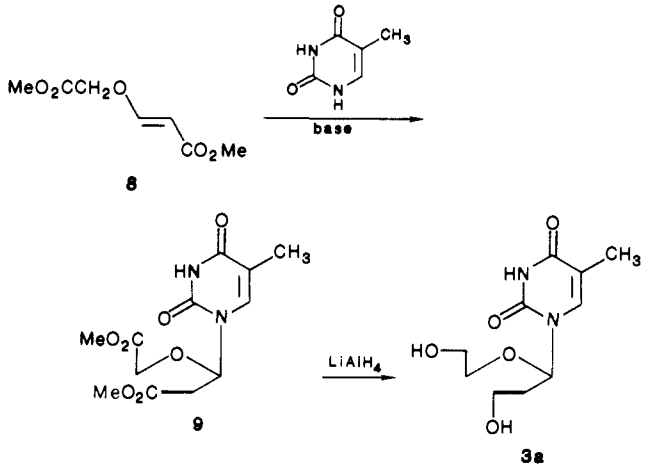
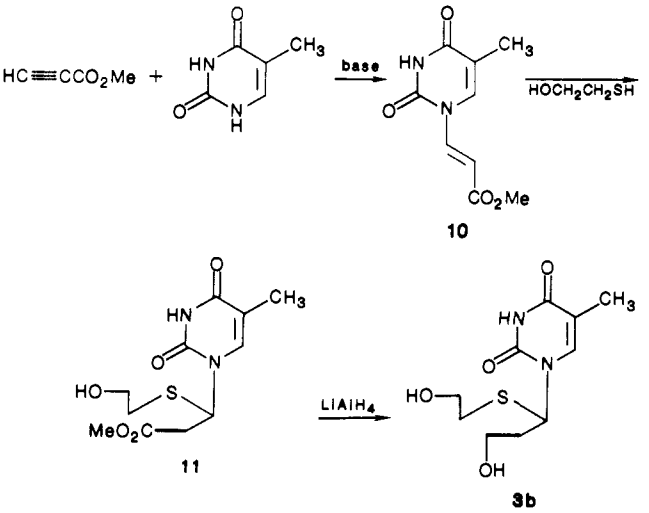
Scheme II



Scheme III



addition has been largely neglected. Scattered examples of purine[16] and pyrimidine[17] alkylation with simple Michael acceptors have appeared, but the reaction has not been exploited for the synthesis of novel nucleosides. However, Michael-type addition of heterocyclic bases to appropriate acceptors, followed by carbonyl reduction, provides a direct route to 2′-deoxynucleoside analogues. Depending on acceptor substituents and the degree of unsaturation, a variety of nucleoside-related products may be prepared; many of these would be difficult, if not impossible, to obtain by conventional methods.[18]

Compound **2** was synthesized via Michael-type addition, as outlined in Scheme I. The required azide–alcohol functionality of **2** was achieved by protecting the (ultimate) terminal hydroxyl by tritylation prior to Michael addition. To this end the protected Michael acceptor, methyl (*E*)-3-[2-(trityloxy)ethoxy]propenoate (**4**), was prepared (88%) by addition of 2-(trityloxy)ethanol to methyl propiolate. Following elaboration of the azide group, the alcohol was unmasked in a manner similar to that used to prepare **1**.[19]

Under appropriate conditions, base-catalyzed addition of thymine to **4** afforded adduct **5** in excellent yield (83%, based on thymine). For this and related Michael-type additions, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was found to be an excellent catalyst. Due to the reversibility of Michael-type addition,[20] highest yields of **5** were obtained with large molar excesses (7–10-fold) of the α,β-unsaturated ester. Product separation and recovery of excess **4** thus required column chromatography. A minor side product, methyl (*E*)-3-thymin-1-ylpropenoate (**10**), formed by β-elimination of 2-(trityloxy)ethanol from **5**, was minimized by conducting the addition at room temperature. The position of alkylation (N-1) parallels that reported for related pyrimidine Michael-type additions to acrylonitrile.[17]

Lithium aluminum hydride reduction of ester **5** afforded **6** (81%). A sample of **6**, deprotected by treatment with 80% HOAc–H_2O, gave acyclothymidine **3a** identical in all respects with the compound prepared as shown below in Scheme II. Conversion of **6** to the protected azide (**7**) was accomplished by standard procedures. Detritylation of **7** (80% HOAc–H_2O) then produced racemic acyclo-AZT (**2**). The assigned structure is in agreement with the NMR and IR spectra and elemental analysis.

A similar but simpler sequence was used to prepare **3a** (Scheme II). Methyl (*E*)-3-[(methoxycarbonyl)methoxy]propenoate (**8**), conveniently obtained from methyl glycolate and methyl propiolate, served well as a Michael acceptor for thymine. The resulting adduct **9** (82%) was then reduced to the desired product **3a** (62%). Diester **8** has been successfully employed in Michael-type additions with adenine and 2-amino-6-chloropurine; acyclic adenine and guanine nucleosides corresponding to **3a** were thus prepared.[18]

Antiviral activity has been reported for certain thio analogues of acyclonucleosides.[21,22] Accordingly, synthesis of the thio analogue of **3a** (**3b**) was undertaken. Although the method of Scheme II (with **8** replaced by the methyl thioglycolate–methyl propiolate adduct) would provide a direct route to **3b**, an alternative approach involving a different Michael addition–reduction sequence was exam-

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ined (Scheme III). Base-catalyzed addition of thymine to methyl propiolate gave methyl (*E*)-3-thymin-1-ylpropenoate (**10**), a new type of unsaturated nucleoside derivative. With thymine and propiolate a single product (trans by NMR) was obtained; both cis and trans isomers have been isolated from the corresponding additions with adenine and 2-amino-6-chloropurine.[18] As anticipated, compound **10** functioned well as a Michael acceptor with 2-mercaptoethanol, affording adduct **11**. Hydride reduction then gave **3b**.

Schemes I–III indicate the utility of Michael-type addition in nucleoside analogue synthesis. The method has allowed facile synthesis of structures difficult to obtain by conventional techniques. It thus provides a new alternative route to novel nucleosides. The great diversity of Michael acceptor molecules[20] suggests the wide potential of the method. Further applications to cyclic and unsaturated systems, as well as to non-carbonyl Michael acceptors, will be reported in the near future.

Antiviral Testing. Antiviral and cytotoxicity assays of the new acyclic pyrimidine nucleosides (**2, 3a, 3b, 9–11**) against HIV-1 in human peripheral blood mononuclear (PBM) cells and against HSV-1 and HSV-2 in Vero (African Green Monkey) cells were performed. The results indicated that any modification in the sugar moiety resulted in a marked reduction of antiviral activity when compared to the known anti-HIV-1 and HSV nucleosides analogues, AZT and acyclovir (ACV). When tested against HIV-1, whereas compounds **2, 3, 3b,** and **9** were inactive up to 100 μM, compounds **10** and **11** showed slight inhibition (median effective concentration was 76 and 87 μM, respectively). None of the compounds were active against HSV-1 and HSV-2 or exhibited toxic effects in uninfected PBM and Vero cells when tested up to 100 μM. Studies on the anticytomegalovirus activity of these compounds is ongoing.

Experimental Section

General Methods. Melting points (uncorrected) were taken with an Electrothermal apparatus. NMR (^{1}H) spectra were determined with a Varian 360 instrument (60 MHz) using tetramethylsilane as an internal reference. Infrared spectra (KBr) were obtained with a Perkin-Elmer Model 1420 spectrophotometer. Thin-layer chromatography employed silica gel plates (EK-F254) and Merck silica gel (230–400 mesh) was used for column chromatography. Elemental analyses were performed by Atlantic Micro Lab, Atlanta, GA.

Methyl (*E*)-3-[2-(Trityloxy)ethoxy]propenoate (4). A solution of 2-(trityloxy)ethanol[23] (13.5 g, 44.5 mmol), triethylamine (4.49 g, 44.5 mmol), and methyl propiolate (3.73 g, 44.5 mmol) in anhydrous Et_2O (160 mL) was stirred at room temperature for 67 h. Removal of solvent and triethylamine gave a viscous oil that slowly crystallized on standing (15.9 g, 92%). Recrystallizations from petroleum ether (30–60 °C)–benzene (2:1) gave **4**, mp 86–86.5 °C. NMR ($CDCl_3$): δ 7.78 (d, 1, J = 12.5 Hz, H), 7.4 (m, 15, Ar H), 5.32 (d, 1, J = 12.5 Hz, H), 3.9, 3.3 (m's, 4, CH_2CH_2), 3.75 (s, 3, CH_3). Anal. ($C_{25}H_{24}O_4$) C, H.

(*R,S*)-Methyl 3-Thymin-1-yl-3-[2-(trityloxy)ethoxy]propanoate (5). A suspension of thymine (1.50 g, 11.9 mmol), **4** (18.5 g, 47.6 mmol), DBU (100 μL, 0.7 mmol) in acetonitrile (100 mL) and dimethyl sulfoxide (10 mL) was stirred at room temperature for 3 weeks by which time a solution was obtained. Acetic acid (0.5 mL) was added and solvent removed under reduced pressure. The resulting semisolid was chromatographed over silica gel (150 g). Elution with petroleum ether–EtOAc (2:1) afforded 5.08 g (83%) of a white solid. Recrystallizations from EtOAc–petroleum ether (2:1) gave **5**, mp 172–173 °C. NMR ($CDCl_3$): δ 9.55 (s br, 1, NH), 7.30 (m, 16, Ar H, C(6)H), 6.12 (t, 1, J = 6.3 Hz, C(1')H), 3.68 (s, 3, OCH_3), 3.7–3.4, 3.4–3.1 (m's, 2, 2, CH_2CH_2), 2.83 (d, 2, J = 6.3 Hz, C(2')H), 1.90 (s, 3, C(5)CH_3). Anal. ($C_{30}H_{30}N_2O_6$) C, H, N.

(*R,S*)-1-[1-[2-(Trityloxy)ethoxy]-3-hydroxypropyl]thymine (6). Under a N_2 atmosphere a solution of **5** (1.48 g, 2.88 mmol) in anhydrous THF (65 mL) was added dropwise to a suspension of lithium aluminum hydride (0.23 g, 6.0 mmol) in THF (35 mL). Immediately after addition, TLC (EtOAc) showed only product. Water (0.392 mL, 21.8 mmol) was added cautiously, and after stirring 1 h, the suspension was filtered by gravity, and residual solids were washed with CH_2Cl_2. Evaporation of the filtrates gave **6** (0.569 g). The residual solids were suspended in CH_2Cl_2 (70 mL) to which glacial HOAc (2 mL) had been added. Filtration followed evaporation gave additional **6** (0.571 g; total, 1.140 g, 81%). TLC (EtOAc, $CHCl_3$–MeOH (9:1)) showed this material to be pure despite its broad melting point (110–136 °C), which was unchanged after three recrystallizations from 50% aqueous acetone and vacuum drying. NMR ($CDCl_3$): δ 9.5 (v br, NH), 7.3 (m, 16, Ar H and C(6)H), 5.95 (t, 1, J = 7 Hz, C(1')H), 3.9–3.1 (m's, ca. 7, OCH_2 and OH), 2.05 (m, 2, C(2')H), 1.92 (s, 3, CH_3). Anal. ($C_{29}H_{30}N_2O_5 \cdot {}^1/_3H_2O$) C, H, N.

Detritylation of 6. A sample of **6** (72 mg, 0.15 mmol) was heated over steam with 80% (aqueous) HOAc (0.4 mL) for 20 min, diluted with H_2O (0.4 mL), cooled, and filtered. The collected solid was trityl alcohol by TLC comparison with an authentic sample. The filtrate was evaporated and the residue recrystallized from acetone. Product (22 mg, 60%) was obtained which proved identical with **3a** by TLC with three different solvent systems (EtOAc; $CHCl_3$–MeOH 9:1 and 7:3).

(*R,S*)-1-[1-[2-(Trityloxy)ethoxy]-3-azidopropyl]thymine (7). Methanesulfonyl chloride (0.80 mL, 1.18 g, 10.3 mmol) was added dropwise to a stirred ice-cold solution of **6** (1.59 g, 3.25 mmol) in anhydrous pyridine (9 mL). After standing overnight at 5 °C, the mixture was cooled to 10 °C, treated with water (0.7 mL), and stirred cold for 1 h. The product mixture was added to an ice-water mixture and blended at high speed. The resulting amorphous solid was collected and dried under vacuum (3 h) to give crude product (1.69 g, 94%), mp 64–68 °C. TLC (EtOAc) showed a single new component, and the NMR ($CDCl_3$) was consistent with the methanesulfonate derivative of **6**. On standing 3 weeks at room temperature, this material completely decomposed, as indicated by TLC (EtOAc).

A stirred solution of the crude methanesulfonate (1.65 g, 2.99 mmol) and LiN_3 (0.40 g, 8.2 mmol) in DMF (7 mL) was heated at 70–75 °C for 2 h. The amber solution was added dropwise to a vigorously stirred ice–water mixture (40 g, 40 mL). The white precipitate was collected and dried to give **7** (1.32 g, 86%). Recrystallization from Et_2O–petroleum ether (4:1) gave product, mp 159 °C dec. NMR ($CDCl_3$): δ 7.3 (m, 16, Ar H, C(6)H), 5.87 (t, 1, J = 6 Hz, C(1')H), 3.78–3.13 (m's, 6, C(3',4',5')H), 2.10 (m, 2, C(2')H), 1.92 (s, 3, CH_3). Anal. ($C_{29}H_{29}N_5O_4$) C, H, N.

(*R,S*)-1-[1-(2-Hydroxyethoxy)-3-azidopropyl]thymine (2). A suspension of **7** (1.10 g, 2.15 mmol) in 80% HOAc (H_2O) (6 mL) was refluxed 15 min, cooled, and diluted with H_2O (1 mL). After 1 h at 5 °C, the precipitated solid (TrOH by TLC) was filtered, and the filtrate was stirred with charcoal and refiltered. The charcoal was rinsed in portions with water (50 mL), and the combined filtrates were evaporated under reduced pressure (35 °C) to a yellow solid (0.408 g, 70%), which was further purified by chromatography (petroleum ether–EtOAc) and recrystallization (acetone–petroleum ether), mp 98–99 °C dec. NMR ($CDCl_3$): δ ca. 9.8 (v br, NH), 7.27 (s, 1, C(6)H), 5.92 (t, 1, J = 6.5 Hz, C(1')H), 4.0–3.3 (m's, 7, C(3',4',5')H, OH), 2.0 (m, 2, C(2')H), 1.95 (s, 3, CH_3). IR (KBr): 2118 cm^{-1}. Anal. ($C_{10}H_{15}N_5O_4$) C, H, N.

Methyl (*E*)-3-[(Methoxycarbonyl)methoxy]propenoate (8). Compound **8** was prepared from methyl glycolate and methyl propiolate as described for **4** and recrystallized from petroleum ether–benzene, mp 64–66 °C. NMR ($CDCl_3$): δ 7.53 (d, 1, J = 13 Hz, H), 5.24 (d, 1, J = 13 Hz, H), 4.47 (s, 2, CH_2), 3.80, 3.68 (s's, 3, 3, CH_3's). Anal. ($C_7H_{10}O_5$) C, H.

(*R,S*)-Methyl 3-[(Methoxycarbonyl)methoxy]-3-thymin-1-ylpropanoate (9). A suspension of thymine (0.631 g, 5.00 mmol), **8** (6.51 g, 37.5 mmol), methyl glycolate (0.450 g, 5.00 mmol), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (76 μL, 0.5 mmol) in acetonitrile (100 mL) was stirred 5 days at room temperature. Acetic acid (0.4 mL) was added, the solvent removed under re-

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duced pressure, and the residue chromatographed. Elution with petroleum ether–EtOAc (1:1) gave a white solid (1.23 g, 82%), which was recrystallized from petroleum ether–EtOAc (1:2), mp 140–141 °C. NMR (DMSO-d_6): δ 11.32 (s, 1, NH), 7.62 (s, 1, C(6)H), 6.04 (t, 1, J = 6.5 Hz, C(1′)H), 4.17 (s, 2, CH_2O), 3.63 (s, 6, CH_3's), 3.01 (d, 2, J = 6.5 Hz, C(2′)H), 1.78 (s, 3, C(5)CH_3). Anal. ($C_{12}H_{16}N_2O_7$) C, H, N.

(*R*,*S*)-1-[1-(2-Hydroxyethoxy)-3-hydroxypropyl]thymine (3a). Compound **9** was reduced as described for **6**. The residual solids were rinsed with absolute EtOH, and following evaporation the crude product was chromatographed. Elution with 3% MeOH–CH_2Cl_2 gave **3a** (62%), which was recrystallized from EtOAc, mp 115–116 °C. NMR (DMSO-d_6): δ 11.3 (s br, 1, NH), 7.52 (s, 1, C(6)H), 5.80 (t, 1, J = 6.5 Hz, C(1′)H), 4.7–4.4 (m's, 2, D_2O exch, OH's), 3.8–3.3 (m's, 6, C(3′,4′,5′)H), 2.1–1.8 (m, 2, C(2′)H), 1.82 (s, 3, CH_3). Anal. ($C_{10}H_{16}N_2O_5$) C, H, N.

Methyl (*E*)-3-Thymin-1-ylpropenoate (10). A suspension of thymine (1.26 g, 10.0 mmol), methyl propiolate (3.78 g, 45.0 mmol), and DBU (50 μL, 0.3 mmol) in acetonitrile (120 mL) was stirred at room temperature for 93 h, cooled (5 °C) for 2 h, and then filtered. The solid was washed well with acetone to give **10** (0.83 g, 40%) and recrystallized from EtOAc–MeOH (1:1), mp 269–270 °C. NMR (DMSO-d_6): δ 11.3 (s br, 1, NH), 8.05 (s, 1, C(6)H), 8.05 (d, 1, J = 14.7 Hz, H), 6.28 (d, 1, J = 14.7 Hz, H), 3.71 (s, 3, OCH_3), 1.83 (s, 3, C(5)CH_3). Anal. ($C_9H_{10}N_2O_4$) C, H, N.

(*R*,*S*)-Methyl 3-[(2-Hydroxyethyl)thio]-3-thymin-1-ylpropanoate (11). A mixture of **10** (0.420 g, 2.00 mmol), 2-mercaptoethanol (1.0 mL, 15 mmol), DBU (15 μL, 0.1 mmol), and THF (5 mL) was stirred overnight at room temperature. Glacial HOAc (3 drops) was added to the clear solution and the volatile material removed under reduced pressure. The amorphous residue was taken up in boiling EtOAc, diluted with petroleum ether, and refrigerated. Crystals of **11** were collected (0.477 g, 83%), mp 127–129 °C; recrystallized from EtOAc, mp 130–131 °C. NMR (DMSO-d_6): δ 11.2 (s, 1, NH), 7.73 (s, 1, C(6)H), 6.00 (t, 1, J = 7 Hz, C(1′)H), 3.65 (s, 3, OCH_3), 3.53 (t, 2, J = 6 Hz, CH_2O), 3.08 (d, 2, J = 7 Hz, C(2′)H), 2.60 (t, 2, J = 6 Hz, CH_2S), 1.92 (s, 3, C(5)CH_3). Anal. ($C_{11}H_{16}N_2O_5S$) C, H, N, S.

(*R*,*S*)-1-[1-[(2-Hydroxyethyl)thio]-3-hydroxypropyl]thymine (3b). Compound **11**, reduced as described for **6**, gave **3b** (52%). The product crystallized with difficulty from Et_2O–petroleum ether, mp dec > 107 °C. NMR (DMSO-d_6): δ 11.4 (s, 1, NH), 7.68 (s, 1, C(6)H), 5.90 (t, 1, J = 7 Hz, C(1′)H), 4.7–4.4 (m's, D_2O exch, OH's), 3.7–3.3 (m's, 4, C(3′,5′)H), 2.58 (t, 2, CH_2S), 2.1–1.8 (m, 2, C(2′)H), 1.83 (s, 3, CH_3). Anal. ($C_{10}H_{16}N_2O_4S$) C, H, N, S.

Antiviral and Cytotoxicity Assays. (a) HIV-1. Three-day-old mitogen-stimulated PBM cells (10^6 cells/mL) from healthy donors that were hepatitis and HIV-1 seronegative were infected with HIV-1 (strain LAV) at a concentration of about 100 $TCID_{50}$ per mL and cultured in the presence and absence of various concentrations of compounds. The drugs were added about 45 min after infection. Five days after infection the supernatant was clarified and the virus pelleted. The reverse transcriptase activity associated with the disrupted virus was determined. The methods used for culturing the PBM cells, harvesting the virus, and determining the reverse transcriptase activity were those described by McDougal et al.[24] and Spira et al.[25] except that fungizone was not included in the medium. The virus-infected control had about 68000 dpm/mL of reverse transcriptase activity. The blank and uninfected cell control values were about 300 and 1300 dpm, respectively. PBM cells from three different donors were used in these studies.

The effect of drugs on the growth of uninfected human PBM cells was also established. Mitogen-stimulated PBM cells (3.8×10^5 cells/mL) were cultured in the presence and absence of drugs under the same conditions as those used for the antiviral assays described above. The cells were counted daily for 5 days by using the trypan blue exclusion method.

(b) HSV-1 and HSV-2. The newly synthesized nucleosides were evaluated for activity against HSV-1 (strain F) and HSV-2 (strain G) by a plaque reduction assay in Vero cells, using methodologies described previously.[26] Cytotoxicity assays were conducted in rapidly dividing Vero cells, as previously described.[26] The median effective concentration was determined by the median effect method.[27]

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1′,2′-*seco*-Dideoxynucleosides as Potential Anti-HIV Agents

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1′,2′-*seco*-2′,3′-Dideoxycytidine (**12**), -guanosine (**14**), -adenosine (**16**), and -inosine (**18**) were prepared from (*R*)-benzylglycidol as potential anti-HIV agents. When compared to ddAdo in protecting ATH8 cells, they were found to be inactive.

A number of sugar-modified nucleosides have been reported to exhibit antiviral activity against the human immunodeficiency virus (HIV). 3′-Azido-3′-deoxythymidine (**1**), the first drug to have clinical utility, is believed to exert its activity as a triphosphate by inhibiting the viral reverse transcriptase.[1] Several 2′,3′-dideoxynucleosides are also

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