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 68 000 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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Registry No. **2**, 117068-45-8; **3a**, 117143-02-9; **3b**, 117068-50-5; **4**, 117068-40-3; **5**, 117068-41-4; **6**, 117068-42-5; **6** (*O*-mesyl derivative), 117068-43-6; **7**, 117068-44-7; **8**, 117068-46-9; **9**, 117068-47-0; **10**, 117068-48-1; **11**, 117068-49-2; $Ph_3COCH_2CH_2OH$, 18325-45-6; HC≡CCOOMe, 922-67-8; $HOCH_2COOMe$, 96-35-5; $HSCH_2CH_2OH$, 60-24-2; thymine, 65-71-4.

(24) McDougal, J. S.; Cort, S. P.; Kennedy, M. S.; Cabridilla, C. D.; Feorino, P. M.; Francis, D. P.; Hicks, D.; Kalyanaramen, V. S.; Martin, L. S. *J. Immunol. Methods* **1985**, *76*, 171.
(25) Spira, T. J.; Bozeman, L. H.; Halman, R. C.; Warfield, D. I.; Phillips, S. K.; Feorino, P. M. *J. Clin. Microbiol.* **1987**, *25*, 97.
(26) Schinazi, R.; Peters, J.; Williams, C. C.; Chance, D.; Nahmias, A. J. *Antimicrob. Agents Chemother.* **1982**, *22*, 499.
(27) Schinazi, R. F.; Chou, T. C.; Scott, R. J.; Yao, X.; Nahimas, A. J. *Antimicrob. Agents Chemother.* **1986**, *30*, 491.

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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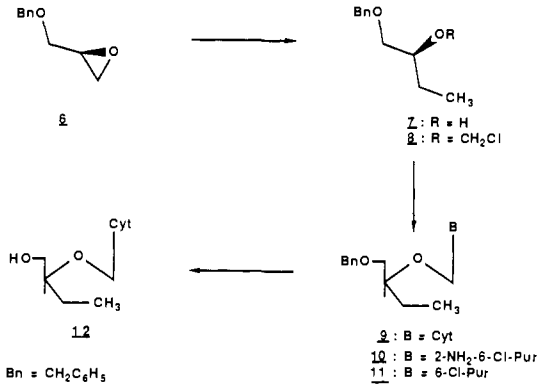
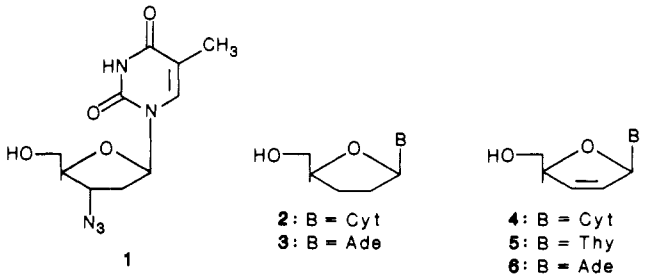
Scheme I



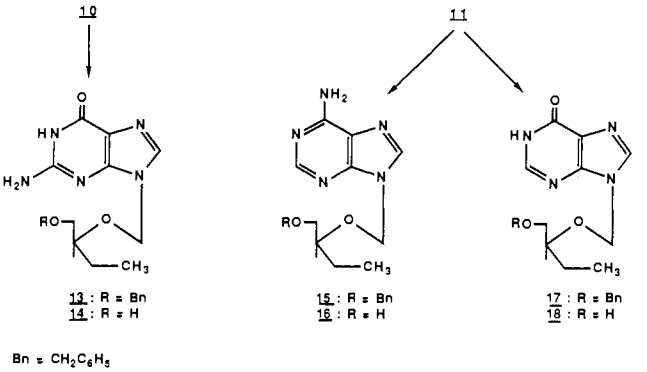
known to inhibit the replication of HIV. Among these 2′,3′-dideoxycytidine (**2**, ddCyd) and 2′,3′-dideoxyadenosine (**3**, ddAdo) appear to be the leading candidates for further evaluation.[2] Other 2′,3′-dideoxynucleoside derivatives have since been prepared and tested as potential antivirals.[3-5]



2′,3′-Unsaturated analogues of dideoxynucleosides have also been reported to be effective anti-HIV agents. 2′,3′-DehydroddCyd (**4**) proved to be equally effective as ddCyd in protecting HIV-infected ATH8 cells.[6] The didehydrothymidine analogue (**5**) was also found to be a potent and selective inhibitor of HIV.[7,8] The 2′,3′-unsaturated analogue of 2′,3′-dideoxyadenosine (**6**) was reported to be moderately active against HIV-1 effects in ATH8 cells,[9] but also less toxic to uninfected cells.

In a recent report on the general properties of HIV reverse transcriptase, Cheng et al. showed that certain acyclonucleoside triphosphates had significant inhibitory activity against this enzyme. These compounds had IC_{50} against human DNA polymerase that were 10–15 times greater than those against the viral reverse transcriptase.[10] These findings prompted us to prepare the title compounds for evaluation as potential anti-HIV agents.

Scheme II



Chemistry. The chirality at C-4′ in 2′,3′-dideoxynucleosides is *S*, which could be arrived at in the 1′,2′-seco analogues by starting with (*S*)-*O*-benzylglycidol,[11] as depicted in Scheme I. Epoxide **6** was obtained by a minor modification of the Mitsunobu reaction[12] whereby the product is distilled off under vacuum as the reaction mixture of (*S*)-1-*O*-benzylglycerol, triphenylphosphine, and diethyl azodicarboxylate is heated at 130 °C. This modification is applicable to a wide variety of vicinal diols[13] and is more efficient than the multistep process reported earlier[11] for the synthesis of compound **6**. Copper-catalyzed addition of methylmagnesium chloride[14] to **6** proceeded regiospecifically and in high yields to give (2*S*)-1-*O*-benzylbutane-1,2-diol (**7**). Chloromethylation[15] of **7** followed by coupling with the proper persilylated base[16] furnished the protected 1′,2′-seco nucleosides (**9**–**11**). Proof that alkylation took place at N-9, rather than N-7, was derived from UV data which were in agreement with literature reports (see the Experimental Section).[17] The target cytidine analogue (**12**) was obtained by debenzylation of **9** with Pearlman's catalyst.[18]

The guanosine, adenosine, and inosine analogues (**14**, **16**, and **18**) were prepared as shown in Scheme II. Aqueous base hydrolysis of **10** afforded the 5′-benzylated guanosine **13**, from which the target 1′,2′-*seco*-dideoxyguanosine was obtained by catalytic transfer hydrogenation over palladium hydroxide on charcoal.[18] The remaining 1′,2′-*seco*-dideoxyadenosine and inosine were derived from 6-chloropurine **11**. While ammonolysis furnished adenosine **15**, base hydrolysis yielded the corresponding inosine analogue **17**. Debenzylation of **15** and **17** furnished the target compounds **16** and **18**, respectively.

(1) Furman, F. A.; Fyfe, J. A.; St. Clair, M. H.; Weinhold, K.; Rideout, J. L.; Freeman, G. A.; Nusinoff-Lehrman, S.; Bolognesi, D. P.; Broder, S.; Mitsuya, H.; Barry, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 8333.

(2) Cooney, D. A.; Ahluwalia, G.; Mitsuya, H.; Fridland, A.; Johnson, M.; Hao, Z.; Dalal, M.; Balzarini, J.; Broder, S.; Johns, D. G. *Biochem. Pharmacol.* **1987**, *36*, 1765.

(3) Herdewijn, P.; Balzarini, J.; De Clercq, E.; Pauwels, R.; Masanori, B.; Broder, S.; Vanderhaeghe, H. *J. Med. Chem.* **1987**, *30*, 1270.

(4) Herdewijn, P.; Pauwels, R.; Baba, M.; De Clercq, E. *J. Med. Chem.* **1987**, *30*, 2131.

(5) Marquez, V.; Tseng, C. K.-H.; Kelley, J. A.; Mitsuya, H.; Broder, S.; Roth, J. S.; Driscoll, J. S. *Biochem. Pharmacol.* **1987**, *36*, 2719.

(6) Balzarini, J.; Pauwels, R.; Herdewijn, P.; De Clercq, E.; Cooney, D. A.; Kang, G.-J.; Dalal, M.; Johns, D. J.; Broder, S. *Biochem. Biophys. Res. Commun.* **1986**, *140*, 735. Lin, T. S.; Schinazi, R. F.; Chen, M. S.; Kinney-Thomas, E.; Prusoff, W. H. *Biochem. Pharmacol.* **1987**, *36*, 311.

(7) Baba, M.; Pauwel, R.; Herdewijn, P.; De Clercq, E.; Desmyster, J.; Vandeputte, M. *Biochem. Biophys. Res. Commun.* **1987**, *142*, 128.

(8) Lin, T. S.; Schinazi, R. F.; Prusoff, W. H. *Biochem. Pharmacol.* **1987**, *36*, 2713.

(9) Mitsuya, H.; Matsukura, M.; Broder, S. In *AIDS. Modern Concepts and Therapeutic Challenges*; Broder, S., Ed.; Marcel Dekker: New York, 1987; pp 303–333.

(10) Cheng, Y.-C.; Dutschman, G. E.; Bastow, K. F.; Sarngadharan, M. G.; Ting, R. Y. C. *J. Biol. Chem.* **1987**, *262*, 2187.

(11) Takano, S.; Kazuhiko, S.; Goto, E.; Michiyasu, H. *Synthesis* **1983**, 116.

(12) Mitsunobu, O. *Synthesis* **1981**, 1.

(13) Abushanab, E.; Vemishetti, P.; Leiby, R. W.; Singh, H. K.; Mikkilineni, A. B.; Wu, D.C.-J.; Saibaba, R.; Panzica, R. P. *J. Org. Chem.* **1988**, *53*, 2598.

(14) Fouquet, G.; Schlosser, M. *Angew. Chem., Int. Ed. Engl.* **1974**, *13*, 82.

(15) Martin, J. C.; Dvorak, C. A.; Smee, D. F.; Matthews, T. R.; Verheyden, J. P. H. *J. Med. Chem.* **1983**, *26*, 759.

(16) Wittenburg, E. *Z. Chem.* **1964**, *4*, 303.

(17) *CRC Handbook of Biochemistry*, 2nd ed.; The Chemical Rubber Co.: Boca Raton, FL, 1970; pp G23, G36.

(18) Anantharamaiah, G. M.; Sivanandaiah, K. M. *J. Chem. Soc., Perkin Trans.* **1977**, *1*, 490.

Biological Testing. ATH8 cells were infected with HIV (2×10^3 virus particles per cell) and were treated with compounds **12**, **14**, **16**, and **18** at concentrations ranging from 1 to 100 M. While ddAdo showed complete protection at concentration of 20 M, none of the above compounds showed similar effects against HIV.

The fact that other acyclic nucleoside triphosphates had inhibitory activity against HIV reverse transcriptase[10] suggests that the title compounds do not appear to be substrates for a kinase and were not phosphorylated to the corresponding nucleotides.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded on Varian EM-390 spectrometer. The chemical shifts are expressed in parts per million with respect to tetramethylsilane. Mass spectral data were obtained by the FAB ionization method on an MAT 731 mass spectrometer. Silica gel (Merck, grade 60, 230–240 mesh, 60A) suitable for column chromatography was purchased from Aldrich. TLC plates were run on precoated (0.2 mm) silica gel 60 F-254 plates manufactured by EM Laboratories, Inc. and short-wavelength light (254 nm) was used to detect the UV-adsorbing spots. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Repeated attempts to obtain correct elemental analyses for certain compounds were not successful. In these cases purities were ascertained by reverse-phase HPLC analysis (80% MeOH in 0.01 M KH_2PO_4, pH 6.0) on a Whatman Partisil 5 ODS-3 column. Furthermore molecular formulae were confirmed by high-resolution mass spectral analyses.

1-(Benzyloxy)-2(*S*)-butanol (7). Methylmagnesium chloride (72 mL, 0.219 mol) was added to a 0.2 M solution of lithium tetrachlorocuprate in dry tetrahydrofuran (6.5 mL) and dry ether (80 mL) and the mixture cooled to −78 °C. To this cooled solution was added epoxide **6** (12 g, 73 mmol) in dry ether (200 mL) and the resulting solution was stirred at −78 °C for 3 h and left overnight to warm up to room temperature. The solvents were evaporated at room temperature, and saturated aqueous ammonium chloride solution (250 mL) was added to the residue and the mixture stirred for 0.5 h. The reaction mixture was extracted with ether (150 mL), and the ether extract was dried and concentrated to give the liquid product: yield 13.15 g (99%); $[\alpha]^{25}_D$ −4.35° (*c* 4.525, EtOH); ^{1}H NMR ($CDCl_3$) δ 7.23 (s, 5 H, Ar), 4.46 (s, 2 H, $ArCH_2$), 3.8–3.43 (m, 1 H, C*H*OH), 3.43–3.1 (m, 2 H, CH_2O), 2.73 (br s, 1 H, OH), 1.63–1.13 (m, 2 H, CH_2CH_3), 0.9 (t, 3 H, J = 6.5 Hz, CH_2CH_3). Anal. ($C_{11}H_{16}O_2$) C, H.

1-[[1-(Benzyloxy)-2(*S*)-butoxy]methyl]cytosine (9). Cytosine (3.7 g, 0.33 mol) was suspended in hexamethyldisilazane (HMDS, 60 mL), ammonium sulfate (0.1 g) was added, the mixture was refluxed overnight, and the resulting clear solution was concentrated in vacuo to give a liquid. This was dissolved in dry 1,2-dichloroethane (150 mL) along with chloromethyl ether **8** (7.5 g, 3.2 mmol) and *tert*-butylammonium iodide (0.2 g) and the mixture was refluxed for 4.5 h. At the end of this period MeOH (47 mL) and water (12 mL) were added to precipitate the unreacted base, and the solution was filtered. The filtrate was concentrated and was taken up in CH_2Cl_2 (150 mL) and dried over anhydrous Na_2SO_4. Removal of solvent afforded a gum, which was chromatographed on a column of silica gel (400 g). The product was obtained by eluting with hexane–EtOAc (85:15): yield 4.8 g (48%); $[\alpha]^{25}_D$ −12.43° (*c* 3.45, EtOH); mp 163 °C; ^{1}H NMR (DMSO-d_6) δ 10.16 (br s, 1 H, NH), 9.0 (br s, 1 H, NH), 7.6 (d, 1 H, J = 9 Hz, H-5), 7.2 (br s, 5 H, Ar), 6.2 (d, 1 H, J = 9 Hz, H-6), 5.2 (br s, 2 H, OCH_2N), 4.43 (s, 2 H, OCH_2Ar), 3.8–3.5 (m, 1 H, CHO), 3.5–3.3 (m, 2 H, CH_2O), 1.66–1.23 (m, 2 H, CH_2CH_3), 0.86 (t, 3 H, J = 6.5 Hz, CH_2CH_3); exact mass calcd 303.1583, found 303.1576.

2-Amino-6-chloro-9-[[1-(benzyloxy)-2(*S*)-butoxy]methyl]purine (10). 2-Amino-6-chloropurine (5.65 g, 33 mmol) was suspended in HMDS (100 mL), ammonium sulfate (0.1 g) was added, and the mixture was heated at reflux for overnight with exclusion of moisture. The resulting clear solution was concentrated at reduced pressure to give a pale yellow solid. Mercuric cyanide (8.9 g, 35 mmol) and dry benzene (120 mL) were added to the above solid and heated to reflux. To this refluxing solution was added the chloromethyl ether **8** in dry benzene (20 mL), and refluxing was continued for 3 h under a N_2 atmosphere. The benzene was removed under diminished pressure and the residue was stirred with CH_2Cl_2 (200 mL). The solution was collected by filtration and washed twice with 30% aqueous solution of potassium iodide (200 mL), twice with 10% aqueous potassium carbonate solution (300 mL), and finally with water (300 mL). The organic layer was dried, the solvent was evaporated, and the resulting yellow liquid was charged on a silica gel column. The desired product was obtained by eluting with hexane–EtOAc (6:4): yield 4.11 g (43%); $[\alpha]^{25}_D$ −35.64° (*c* 2.04, EtOH); ^{1}H NMR ($CDCl_3$) δ 7.8 (s, 1 H, H-8), 7.2 (s, 5 H, Ar), 5.5 (AB q, 2 H, J = 12 Hz, OCH_2N), 4.5 (s, 2 H, OCH_2Ar), 3.8–3.33 (m, 3 H, OCH_2CHO), 1.6–1.16 (m, 2 H, CH_2CH_3), 0.7 (t, 3 H, J = 6.5 Hz, CH_2CH_3). Anal. ($C_{17}H_{20}N_5O_2Cl$) C, H, N, Cl.

6-Chloro-9-[[1-(benzyloxy)-2(*S*)-butoxy]methyl]purine (11). To a suspension of sodium hydride (0.76 g, 32 mmol) in dry acetonitrile (100 mL) was added 6-chloropurine (5 g, 32 mmol) and the mixture was stirred for 2 h at room temperature. 1-(Benzyloxy)-2(*S*)-(chloromethoxy)butane (7.5 g) in dry acetonitrile (20 mL) was added to the above suspension under nitrogen and stirred. After 15 h of stirring, the reaction mixture was filtered and the filtrate was concentrated in vacuo to give a liquid. Purification by silica gel (400 g) column chromatography, eluting with hexane–EtOAc (85:15), provided the 9-alkylated 6-chloropurine derivative: yield 5.07 g (44.5%); $[\alpha]^{25}_D$ −22.86° (*c* 1.645, EtOH); ^{1}H NMR ($CDCl_3$) δ 8.7, 8.2 (2 s, 2 H, H-8, H-2), 7.2 (s, 5 H, Ar), 5.7 (AB q, 2 H, J = 12 Hz, OCH_2N), 4.4 (s, 2 H, OCH_2Ar), 3.83–3.5 (m, 1 H, CHO), 3.43 (d, 2 H, J = 4.5 Hz, CH_2O), 1.63–1.1 (m, 2 H, CH_2CH_3), 0.7 (t, 3 H, J = 6.5 Hz, CH_2CH_3). Anal. ($C_{17}H_{19}O_2N_4Cl$) C, H, N, Cl.

1-[(1-Hydroxy-2(*S*)-butoxy)methyl]cytosine (12). Compound **9** (1 g, 4.6 mmol) was dissolved in a mixture of EtOH (50 mL) and cyclohexene (10 mL). To this solution $Pd(OH)_2/C$ (20%, 1 g) was added and the resulting suspension was refluxed for 3 h. Filtration of the catalyst followed by evaporation of the solvent furnished a white solid: yield 0.65 g (98%); mp 95 °C; $[\alpha]^{25}_D$ −8.74 (*c* 1.27, EtOH); UV λ_{max} (pH 1.3) 278 nm, (ϵ 1.28 × 10^4), λ_{max} (pH 11) 272 (ϵ 8.7 × 10^3), λ_{max} (EtOH) 282 (ϵ 1.32 × 10^4); ^{1}H NMR ($CDCl_3$) δ 9.96 (br s, 1 H, NH), 8.83 (br s, 1 H, NH), 8.01 (d, 1 H, J = 9 Hz, H-5), 6.15 (d, 1 H, J = 9 Hz, H-6), 5.2 (s, 2 H, OCH_2), 3.5–3.2 (m, 3 H, CH_2OH, $CHOCH_2$), 1.56–1.16 (m, 2 H, CH_2CH_3), 0.8 (t, 3 H, J = 6.5 Hz, CH_2CH_3); exact mass calcd 213.1113, found 213.1108.

9-[[1-(Benzyloxy)-2(*S*)-butoxy]methyl]guanine (13). A solution containing compound **10** (2.0 g, 5.5 mmol), MeOH (120 mL), NaOH (0.7 mol), and water (8.2 mL) was heated under reflux for 1.5 h. After evaporation of the solvent, water (150 mL) was added and the solution was acidified, resulting in the formation of a precipitate which was filtered off and crystallized from a mixture of DMSO–H_2O: yield 1.1 g (50%); mp 215 °C; $[\alpha]^{25}_D$ −35.12 (*c* 1.085, DMF); ^{1}H NMR ($CDCl_3$) δ 7.76 (s, 1 H, H-8), 7.2 (br s, 5 H, Ar), 6.4 (br s, 2 H, NH_2), 5.3 (s, 2 H, OCH_2N), 4.4 (s, 2 H, OCH_2), 3.8–3.5 (m, 1 H, CHO), 3.35 (d, 2 H, J = 4.5 Hz, OCH_2), 1.5–1.1 (m, 2 H, CH_2CH_3), 0.66 (t, 3 H, J = 6.5 Hz, CH_2CH_3). Anal. ($C_{17}H_{21}N_5O_3$) C, H, N.

9-[(1-Hydroxy-2(*S*)-butoxy)methyl]guanine (14). The same procedure described earlier for debenzylation was applied to compound **13** to give the product in 96% yield; mp 270 °C; $[\alpha]^{25}_D$ −29.54° (*c* 1.035, DMF); UV λ_{max} (pH 1.3) 255 nm (ϵ 1.3 × 10^4), 278 (ϵ 1.1 × 10^4), λ_{max} (pH 11) 255 (ϵ 1.2 × 10^4), 268 (ϵ 1.26 × 10^4), λ_{max} (EtOH) 255 (ϵ 1.28 × 10^4); ^{1}H NMR (DMSO-d_6) δ 10.65 (s, 1 H, NH), 7.85 (s, 1 H, H-8), 6.5 (s, 2 H, NH_2), 5.5–5.4 (br s, 2 H, OCH_2N), 3.55–3.25 (m, 3 H, CH_2OH, CHO), 1.5–1.2 (m, 2 H, CH_2CH_3), 0.65 (t, 3 H, J = 6.5 Hz, CH_2CH_3); exact mass calcd 253.1175, found 253.1167. Anal. ($C_{10}H_{15}N_5O_3 \cdot {}^1/_2H_2O$) C, H, N.

9-[[1-(Benzyloxy)-2(*S*)-butoxy]methyl]adenine (15). Chloropurine **11** (1.52 g, 4.3 mmol) was dissolved in liquid ammonia (50 mL) and heated in a bomb at 100 °C for 15 h. At the end of this period ammonia was allowed to evaporate and the residue was taken up in chloroform (50 mL) and washed with water. The organic phase was dried and evaporated to give **15**, as a white solid: yield, 1.4 g (97%); mp 82 °C; $[\alpha]^{25}_D$ −33.78° (*c* 2.735, EtOH); ^{1}H NMR ($CDCl_3$) δ 8.3, 7.9 (2 s, 2 H, H-8, H-2), 7.3–7.1 (m, 7 H, Ar, NH_2), 5.8–5.5 (m, 2 H, OCH_2N), 4.43 (s, 2

H, OCH_2), 3.86-3.56 (m, 2 H, CHO), 3.42 (d, 2 H, J = 4.5 Hz, CH_2O), 1.6-1.16 (m, 2 H, CH_2CH_3), 0.66 (t, 3 H, J = 6.5 Hz, CH_2CH_3). Anal. ($C_{17}H_{21}N_5O_2$), C, H, N.

9-[(1-Hydroxy-2(*S*)-butoxy)methyl]adenine (16). The procedure mentioned earlier for debenzylation was applied to compound **15** to give the product in 95% yield; mp 130 °C; $[\alpha]^{25}_D$ -14.59° (*c* 1.295, EtOH); UV λ_{max} (pH 1.3) 256 nm (ϵ 1.4 × 10^4), λ_{max} (pH 11) 260 (ϵ 1.4 × 10^4), λ_{max} (EtOH) 260 (ϵ 1.25 × 10^4); ^1H NMR (DMSO-d_6) δ 8.6, 8.5 (2 s, 2 H, H-8, H-2), 5.7 (s, 2 H, OCH_2N), 3.6-3.3 (m, 3 H, H_2OH, CHO), 1.5-1.2 (m, 2 H, CH_2CH_3), 0.65 (t, 3 H, J = 6.5 Hz, CH_2CH_3); exact mass calcd 237.1226, found 237.1224.

9-[[1-(Benzyloxy)-2(*S*)-butoxy]methyl]hypoxanthine (17). Chloropurine **11** (1.66 g, 4.7 mmol) was dissolved in methanol-water (50 mL, 6 mL) containing NaOH (16 g, 0.4 mol) and the solution was heated to reflux for 2 h. At the end of this period, methanol was removed under diminished pressure and the residue was diluted with water (100 mL), neutralized with acetic acid, and extracted with $CHCl_3$ (60 mL). Concentration of the dry organic phase afforded a solid, which was recrystallized from ethyl acetate, to furnish the product: yield 0.8 g (57.3%); mp 160 °C; $[\alpha]^{25}_D$ -37.86° (c 1.565, EtOH); ^1H NMR ($CDCl_3$) δ 13.1 (br s, 1 H, NH), 8.16, 7.9 (2 s, 2 H, H-8, H-2), 7.2 (s, 5 H, Ar), 5.8-5.5 (m, 2 H, OCH_2N), 4.43 (s, 2 H, CH_2O), 3.8-3.5 (m, 1 H, CHO), 3.43 (d, 2 H, J = 4.5 Hz, CH_2O), 1.66-1.26 (m, 2 H, CH_2CH_3), 0.7 (t, 3 H, J = 6.5 Hz, CH_2CH_3). Anal. ($C_{17}H_{20}N_4O_3$) C, H, N.

9-[(1-Hydroxy-2(*S*)-butoxy)methyl]hypoxanthine (18). The procedure mentioned earlier for debenzylation was applied to compound **17** to give the product in 91.3% yield: mp 170 °C; $[\alpha]^{25}_D$ +19.6° (*c* 1.145, H_2O); UV λ_{max} (pH 1.3) 248 nm (ϵ 1.3 × 10^4), λ_{max} (pH 11) 253 (ϵ 1.35 × 10^4), λ_{max} (EtOH) 245 (ϵ 1.22 × 10^4) 273 (ϵ 6.3 × 10^3); ^1H NMR (DMSO-d_6) δ 8.25, 8.1 (2 s, 2 H, H-8, H-2), 5.7-5.55 (br s, 2 H, OCH_2N), 3.6-3.5 (m, 1 H, CHO), 3.5-3.35 (m, 2 H, CH_2OH), 1.5-1.2 (m, 2 H, CH_2CH_3), 0.65 (t, 3 H, J = 6.5 Hz, CH_2CH_3). Anal. ($C_{10}H_{14}N_4O_3$) C, H, N.

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Structure-Activity Relationships among Methoctramine-Related Polymethylene Tetraamines. Chain-Length and Substituent Effects on M-2 Muscarinic Receptor Blocking Activity[1]

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Several polymethylene tetraamines related to methoctramine (1) were prepared and evaluated for their blocking activity on M-2 muscarinic receptors in guinea pig atria and ileum. It turned out that antimuscarinic potency depends on the following parameters: (a) nature of the substituent on both inner and outer nitrogens and (b) carbon chain length separating the inner nitrogens as well as the inner and outer nitrogens. Optimum activity at cardiac M-2 muscarinic receptors was associated with the chain lengths present in 1, that is, eight methylenes between the inner nitrogens and six methylenes between the inner and outer nitrogens. With regard to the substituents, replacement of the benzylic moiety of 1 by a 2-furyl or a 5-methyl-2-furyl nucleus resulted in enhanced potency toward cardiac M-2 muscarinic receptors. In fact, furtramine (**18**) and mefurtramine (**19**) proved to be more potent and more selective than 1. Moreover, N-methylation of the four nitrogens of 1 gave different effects: methylation of the outer nitrogens, giving **22**, caused a significant decrease in activity whereas methylation of the inner nitrogens, yielding **23**, resulted in an increase in activity in both atria and ileum.

Increasing evidence indicates multiple subtypes for muscarinic receptors. A first subdivision of muscarinic receptors into at least two subtypes was advanced on the basis of their affinity for pirenzepine.[2,3] Thus, muscarinic receptors with high affinity for pirenzepine were named M-1 while those with low affinity for pirenzepine were classified as M-2 muscarinic receptors. Recent cloning studies have confirmed this subclassification as the M-1 muscarinic receptor of the cerebral cortex and the M-2 muscarinic receptor of the heart proved to be distinct gene products and to have different amino acid sequences.[4-7] Furthermore, two additional muscarinic receptor subtypes that appear to be different from M-1 and M-2 types have been cloned from rat brain.[7] However, matters were further complicated by the observation that certain antagonists have different affinity for muscarinic receptors previously classified as M-2 types. For example, 4-DAMP[8-10]

(1) Preliminary results were presented at the International Symposium on Muscarinic Cholinergic Mechanisms, Tel Aviv, November 30–December 4, 1986, Abstracts, p 29, and published in: Quaglia, W.; Cassinelli, A.; Melchiorre, C. In *Muscarinic Cholinergic Mechanisms*; Cohen, S., Sokolovsky, M., Eds.; Freund Publishing House Ltd.: London, 1987; p 302.

(2) Hammer, R.; Berrie, C. P.; Birdsall, N. J. M.; Burgen, A. S. V.; Hulme, E. C. *Nature (London)* **1980**, *283*, 90.

(3) Hammer, R.; Giachetti, A. *Life Sci.* **1982**, *31*, 2991.

(4) Kubo, T.; Fukuda, K.; Mikami, A.; Maeda, A.; Takahashi, H.; Mishina, M.; Haga, T.; Haga, K.; Ichiyama, A.; Kangawa, K.; Kojima, M.; Matsuo, H.; Hirose, T.; Numa, S. *Nature (London)* **1986**, *323*, 411.

(5) Kubo, T.; Maeda, A.; Sugimoto, K.; Akiba, I.; Mikami, A.; Takahashi, H.; Haga, T.; Haga, K.; Ichiyama, A.; Kangawa, K.; Matsuo, H.; Hirose, T.; Numa, S. *FEBS Lett.* **1986**, *209*, 367.

(6) Peralta, E. G.; Winslow, J. W.; Peterson, G. L.; Smith, D. H.; Ashkenazi, A.; Ramachandran, J.; Schimerlik, M. I.; Capon, D. J. *Science (Washington, D.C.)* **1987**, *236*, 600.

(7) Bonner, T. I.; Buckley, N. J.; Young, A. C.; Brann, M. R. *Science (Washington, D.C.)* **1987**, *237*, 527.

(8) Barlow, R. B.; Berry, K. J.; Glenton, P. A. M.; Nikolaou, N. M.; Soh, K. S. *Br. J. Pharmacol.* **1976**, *58*, 613.

(9) Barlow, R. B.; Kitchen, R. *Br. J. Pharmacol.* **1982**, *77*, 549.

0022-2623/89/1832-0079$01.50/0