Notes

Pyrimidine Acyclic Nucleosides. 1-[(2-Hydroxyethoxy)methyl]pyrimidines as Candidate Antivirals

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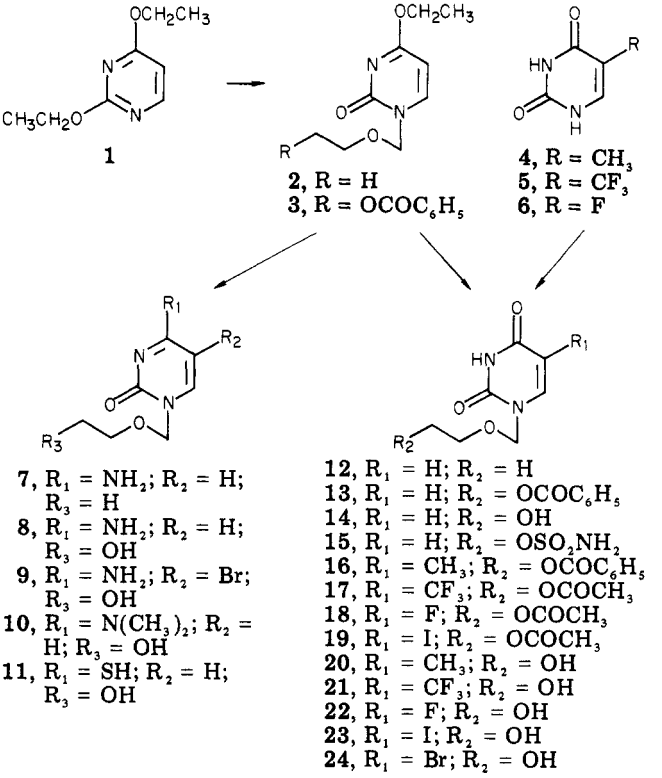
A number of pyrimidine acyclic nucleosides were synthesized and tested for activity against herpes simplex virus type 1. Synthesis of 1-[(2-hydroxyethoxy)methyl]cytosine (8) and 1-[(2-hydroxyethoxy)methyl]uracil (14) was accomplished in two or three steps from 2,4-diethoxypyrimidine and 2-(benzoyloxy)ethoxymethyl chloride. The 5-methyl (20), 5-(trifluoromethyl) (21), and 5-fluoro (22) analogues of 14 were available in two steps from the appropriate bis(trimethylsilyl)ated 5-substituted uracil and 2-(acetoxymethoxy)ethyl acetate or 2-(benzoyloxy)ethoxymethyl chloride. Bromination of 8 and 14 or iodination of 14 gave the 5-halogeno-1-[(2-hydroxyethoxy)methyl]pyrimidines 9, 23, and 24. These pyrimidine acyclic nucleosides exhibited little or no activity against herpes simplex virus type 1 or against a range of other DNA and RNA viruses. This is compatible with their lack of substrate properties toward herpes simplex virus induced thymidine kinase.

The potent antiherpetic drug acyclovir [9-[(2-hydroxyethoxy)methyl]guanine, Zovirax], has emerged from a program initiated to synthesize nucleoside analogues in which the cyclic carbohydrate is replaced with an acyclic side chain.[1,2] Acyclovir possesses potent antiviral activity in cells infected with herpes simplex virus type 1 (HSV-1), but it is essentially nontoxic to uninfected host cells.[2,3] Acyclovir, which is a substrate for HSV-1 encoded thymidine kinase, is phosphorylated to the monophosphate preferentially in HSV-1 infected cells with subsequent inhibition of viral replication.[2,4] It was reasoned that acyclic nucleoside analogues of biologically important pyrimidine cyclic nucleosides might also possess interesting biological activity.[5] Therefore, a number of pyrimidine analogues of the purine acyclic nucleoside, acyclovir, have now been synthesized and evaluated for their antiviral activity against HSV-1 and several other DNA or RNA viruses.

Chemistry. The 1-[(2-hydroxyethoxy)methyl]pyrimidines in Scheme I were prepared in two or three steps using the Hilbert–Johnson reaction[6] or by the trimethylsilyl modification of the Hilbert–Johnson reaction. The intermediate 2-oxo-4-ethoxy-1,2-dihydropyrimidines 2 and 3 were hydrolyzed to the uracils 12 and 13. The UV spectra substantiated that these compounds were the 1-isomers.[7] When 3 was treated with ammonia, dimethylamine, or sodium hydrosulfide,[8] the pyrimidine acyclic nucleosides 8, 10, and 11 were obtained. The 5-methyl-, 5-(trifluoromethyl)-, and 5-fluorouracil analogues 16–18 were prepared from 4–6 by the trimethylsilyl modification of the Hilbert–Johnson reaction. The benzoyl or acetyl protective group on 13 and 16–18 were removed with ammonia, methylamine, sodium methoxide, and dimethylamine, respectively, to give the pyrimidine acyclic nucleosides 14 and 20–22. The UV spectra confirmed that these compounds were the 1-substituted isomers.[7,9–11] Bromination of 8 and 14 gave the 5-bromo analogues 9 and 24. Iodination of 14 with iodine monochloride in acetic

Scheme I



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acid afforded ester **19**, which was transesterified with sodium methoxide to give **23**. The sulfamoyl ester **15** was prepared as a possible phosphate mimic.[12]

Biological Results and Discussion

Thirteen of these pyrimidine acyclic nucleosides (**7**, **10–15**, **17–19**, and **21–23**) were tested for cytotoxicity against Detroit 98 and mouse L cells in cell culture.[13] None exhibited any significant cytotoxicity when tested at 10^{-4} M.

When **7–12** and **14–24** were tested by means of plaque-inhibition tests against HSV-1 in cell culture,[1,14,15] only **8**, **9**, and **19** were active at 50 μg per disk. When the activity was quantitated using plaque-reduction assays,[1] none reduced plaque formation at 100 μM. Thus, all of these pyrimidine acyclic nucleosides are at least 1000-fold less active than the clinically useful purine acyclic nucleoside, acyclovir (Zovirax).[1-3] Little or no activity was observed against two other DNA viruses (vaccinia virus, adenovirus type 5) or the RNA viruses rhinovirus 1B, measles, and mengo virus.[1]

Most of these compounds have been studied by Fyfe et al. as substrates and/or inhibitors of HSV-1 encoded thymidine kinase.[4,16] Several compounds (**13**, **20**, **21**, **23**, and **24**) had fair affinity for the kinase, showing 45–56% inhibition at 300 μM, but only **20** and **24** were weak substrates for the HSV-1 thymidine kinase. The uniform lack of significant antiviral activity, especially of **21** and **23**, contrasts sharply with the good antiherpetic activity of the analogous cyclic pyrimidine nucleosides, 5-(trifluoromethyl)-2′-deoxyuridine and 5-iodo-2′-deoxyuridine, which are excellent substrates for the viral-encoded thymidine kinase.[4] The lack of good substrate properties for HSV-1 encoded thymidine kinase is sufficient to explain the absence of antiherpetic activity with these pyrimidine acyclic nucleosides.

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block or a Thomas-Hoover Uni-Melt and are uncorrected. UV spectra were measured on a Unicam SP 800 spectrophotometer. NMR data were recorded on a Varian XL-100-15-FT and T-60 spectrometers using Me_4Si as an internal standard. Each analytical sample had spectral data compatible with its assigned structure, gave combustion values for C, H, and N within 0.4% of theoretical, and moved as a single spot on TLC. TLC were performed on Eastman Chromagram sheets of silica gel with fluorescent indicator.

1-(Ethoxymethyl)uracil (12) and 1-(Ethoxymethyl)cytosine (7). A solution of 5.0 g (29.7 mmol) of **1**[17] and 3.5 g (37.2 mmol) of chloromethyl ethyl ether was stirred at ambient temperature under reduced pressure (~20 mmHg) for 24 h. The reaction mixture was chromatographed on a silica gel column by elution with CH_2Cl_2–MeOH mixtures to give 6.4 g of a mixture containing 1-(ethoxymethyl)-2-oxo-4-ethoxy-1,2-dihydropyrimidine (**2**) and 1,3-bis(ethoxymethyl)uracil. A suspension of 4.0 g of this mixture in 15 mL of H_2O and 1 mL of concentrated HCl was stirred at ambient temperature for 45 min. The reaction was spin evaporated in vacuo, and the residual white solid was chromatographed on a silica gel column by elution with $CHCl_3$–Me_2CO mixtures to give 1.3 g (19%) of **1,3-bis(ethoxymethyl)uracil**: mp 59–60 °C (H_2O); TLC (15% EtOAc–C_6H_6); UV (0.1 N HCl) λ_{max} 271 nm (ε 7800); NMR ($CDCl_3$) δ 7.35 (d, 1 H, J = 8 Hz, C-6), 5.80 (d, 1 H, J = 8 Hz, C-5), 5.42 (s, 2 H, NCH_2O), 5.19 (s, 2 H, NCH_2O), 3.65 (q, 2 H, J = 7 Hz, CH_3CH_2), 3.62 (q, 2 H, J = 7 Hz, CH_3CH_2), 1.25 (t, 6 H, J = 7 Hz, CH_3). Anal. ($C_{10}H_{16}N_2O_4$) C, H, N.

Continued elution gave **12**: yield 1.7 g (34%); mp 125–126 °C (C_6H_6); TLC (15% EtOAc–C_6H_6); UV (0.1 N HCl + 9.5% EtOH) λ_{max} 258.5 nm (ε 9200); UV (0.1 N NaOH + 9.5% EtOH) λ_{max} 258 nm (ε 6400); NMR ($CDCl_3$) δ 7.38 (d, 1 H, J = 8 Hz, C-6), 5.80 (d, 1 H, J = 8 Hz, C-5), 5.18 (s, 2 H, NCH_2O), 3.63 (q, 2 H, J = 7 Hz, CH_3CH_2), 1.20 (t, 3 H, J = 7 Hz, CH_3). Anal. ($C_7H_{10}N_2O_3$) C, H, N.

The remaining 2.4 g of the mixture of **2** and 1,3-bis(ethoxymethyl)uracil was refluxed in 28% aqueous ammonia for 3 h and left at ambient temperature for 12 h, and the solid was collected to give crude **7**. The filtrate was acidified with 0.1 N HCl and extracted with CH_2Cl_2. The aqueous layer was made basic with 0.1 N NaOH and again extracted with CH_2Cl_2. The organic layer was concentrated, and the residue was combined with the crude **7**, isolated by filtration, and recrystallized to give 1.6 g (31%) of **7**: mp 248–250 °C dec (H_2O); TLC (10% MeOH–$CHCl_3$); UV (0.1 N HCl) λ_{max} 276 nm (ε 10 600); NMR (D_2O) δ 7.70 (d, 1 H, J = 7 Hz, C-6), 6.00 (d, 1 H, J = 7 Hz, C-5), 5.20 (s, 2 H, NCH_2O), 3.65 (q, 2 H, J = 6 Hz, CH_3CH_2), 1.20 (t, 3 H, J = 6 Hz, CH_3). Anal. ($C_7H_{11}N_3O_2$) C, H, N.

1-[(2-Hydroxyethoxy)methyl]cytosine (8). A solution of 9.4 g (29.5 mmol) of **3** (prepared as described for **13**) in 50 mL of MeOH saturated with NH_3 was heated at 80 °C for 18 h in a stainless-steel Paar bomb. The solution was flash evaporated to a red oil, which was treated with charcoal in hot MeOH, filtrated, and diluted with EtOAc to give **8**: yield 3.1 g (62%); mp 158–159 °C (MeOH–EtOAc); UV (1 N HCl) λ_{max} 276 nm (ε 10 900); UV (1 N NaOH) λ_{max} 268 nm (ε 7400); NMR (Me_2SO-d_6) δ 7.90 (d, 1 H, J = 7 Hz, C-6), 7.35 (br s, 2 H, NH_2), 6.06 (d, 1 H, J = 7 Hz, C-5), 5.16 (s, 2 H, OCH_2N), 4.70 (br s, 1 H, OH), 3.53 (br s, s with D_2O, 4 H, CH_2CH_2). Anal. ($C_6H_{11}N_3O_3$) C, H, N.

1-[(2-Hydroxyethoxy)methyl]-5-bromocytosine (9). Following the procedure of Frisch and Visser[18] a solution of 1.39 g (7.5 mmol) of **8** in 140 mL of glacial AcOH and 100 mL of dry pyridine was cooled to −10 °C. The solution was illuminated for 15 min with a 100-W Westinghouse projection spotlight secured 9 cm from the top of the solution. An ice-cold solution of Br_2 (1.3 g, 8.1 mmol) in CCl_4 was added, and the reaction was brought to room temperature over 3 h and then flash evaporated. The residue was extracted with MeOH. The extracts were filtrated, concentrated to a small volume, and diluted with EtOAc to give 1.35 g of a crystalline benzoyl ester of **9**. This material was reacted with MeOH containing 5% NH_3 for 3 days at ambient temperature and flash evaporated: yield 0.5 g (25%); mp 170–172 °C dec (MeOH); UV (EtOH) λ_{max} 289 nm (ε 5000); UV (0.1 N HCl) λ_{max} 297 nm (ε 10 000). Anal. ($C_7H_{10}BrN_3O_3$) C, H, N.

1-[(2-Hydroxyethoxy)methyl]-*N*,*N*-dimethylcytosine (10). To an ice-cold solution of 20.0 g (65 mmol) of **3** in 50 mL of MeOH was added 100 g (2.2 mol) of Me_2NH. The reaction was heated at 65 °C for 18 h in a stainless-steel Paar bomb and then flash evaporated to a red oil that was chromatographed on silica gel by elution with $CHCl_3$–MeOH mixtures to give a crude product. Rechromatography on silica gel by elution with EtOAc–MeOH mixtures gave 4.6 g (33%) of **10**, which was a single spot on TLC. Recrystallization gave the analytical sample: mp 120–121 °C (MeOH–EtOAc–Et_2O); UV (EtOH) λ_{max} 277 nm (ε 10 900); NMR (Me_2SO-d_6) δ 7.70 (d, 1 H, J = 8 Hz, C-6), 6.00 (d, 1 H, J = 7 Hz, C-5), 5.10 (s, 2 H, OCH_2N), 3.50 (br s, 4 H, CH_2CH_2), 3.05 (s, 6 H, CH_3). Anal. ($C_9H_{15}N_3O_3$) C, H, N.

1-[(2-Hydroxyethoxy)methyl]-4-thiouracil (11). The procedure of M. J. Robins and S. R. Naik[8] was used with little modification to transform 15 g (47 mmol) of **3** into crude product. The mixture was purified by chromatography on silica gel by elution with $CHCl_3$–MeOH to give an oily solid. This mixture

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was extracted with hot $CHCl_3$, which was cooled to give **11**: yield 8.5 g (85%); mp 110–112 °C ($CHCl_3$); UV (MeOH–HCl) λ_{max} 328 nm (ϵ 16 500), 248 (3800); UV (MeOH–NaOH) λ_{max} 319 nm (ϵ 15 800), 248 (3800); NMR (Me_2SO-d_6) δ 12.5 (br s, 1 H, NH), 7.60 (d, 1 H, J = 7 Hz, C-6), 6.25 (d, 1 H, J = 7 Hz, C-5), 5.10 (s, 2 H, OCH_2N), 3.55 (br s, 4 H, CH_2CH_2). Anal. ($C_7H_{10}O_3N_2S$) C, H, N.

1-[[2-(Benzoyloxy)ethoxy]methyl]uracil (13). A solution of 0.62 g (4.5 mmol) of **1**[17] and 1.0 g (4.6 mmol) of 2-(benzoyloxy)ethoxymethyl chloride[1,19] was stirred at ambient temperature for 24 h under reduced pressure. The reaction mixture was chromatographed on silica gel by elution with $CHCl_3$ to give 1.08 g (74% yield) of the intermediate **3**. Compound **3** was hydrolyzed with 3.5 mL of 0.7 N HCl for 1.5 h at ambient temperature. It was then flash evaporated to an oil, which was chromatographed on silica gel by elution with CH_2Cl_2–Me_2CO mixtures to give **13**: yield 0.4 g (30%); mp 84–85 °C (C_6H_6); UV (0.1 N HCl + 9.5% EtOH) λ_{max} 258.5 nm (ϵ 8600); UV (0.1 NaOH + 9.5% EtOH) λ_{max} 258 nm (ϵ 7000); NMR ($CDCl_3$) δ 8.2–7.1 (m, 6 H, C-6, ArH), 5.70 (d, 1 H, J = 7 Hz, C-5), 5.22 (s, 2 H, OCH_2N), 4.6–3.8 (m, 4 H, CH_2CH_2). Anal. ($C_{14}H_{14}N_2O_5$) C, H, N.

1-[(2-Hydroxyethoxy)methyl]uracil (14). A solution of 5.0 g (17.2 mmol) of crude **13** in 60 mL of MeOH saturated with NH_3 was left for 15 h at ambient temperature in a stainless-steel Paar bomb. The solution was flash evaporated to give **14**: yield 1.8 g (56%); mp 136–137 °C (MeOH–EtOAc); UV (0.1 N HCl + 9.5% EtOH) λ_{max} 258.5 nm (ϵ 9100); UV (0.1 N NaOH + 9.5% EtOH) λ_{max} 258.5 nm (ϵ 6000); NMR (Me_2SO-d_6) δ 11.3 (br s, 1 H, NH), 7.66 (d, 1 H, J = 8 Hz, C-6), 5.60 (d, 1 H, J = 8 Hz, C-5), 5.08 (s, 2 H,OCH_2N), 4.6 (br s, 1 H, OH), 3.5 (m, 4 H, CH_2CH_2). Anal. ($C_7H_{10}N_2O_4$) C, H, N.

1-[[2-(Sulfamoyloxy)ethoxy]methyl]uracil (15). A dry solution of 2.46 g (13.2 mmol) of **14** in 100 mL of 1,2-dimethoxyethane was stirred with 3.0 g (26 mmol) of sulfamoyl chloride in the presence of 8 g of 3 Å molecular seives at ambient temperature for 48 h. The reaction was flash evaporated to a residue that was chromatographed on silica gel by elution with $CHCl_3$–MeOH to give **15**: yield 0.80 g (23%); mp 139–140 °C (MeOH); UV (EtOH) λ_{max} 259 nm (ϵ 8000); NMR (Me_2SO-d_6) δ 11.2 (br s, 1 H, NH), 7.70 (d, 1 H, J = 8 Hz, C-6), 7.50 (br s, 2 H, NH_2), 5.63 (d, 1 H, J = 8 Hz), C-5), 5.13 (s, 2 H, OCH_2N), 4.13–3.78 (m, 4 H, CH_2CH_2). Anal. ($C_7H_{11}N_3O_6S$) C, H, N.

1-[[2-(Benzoyloxy)ethoxy]methyl]-5-methyluracil (16). A mixture of 6.30 g (50 mmol) of thymine (**4**), 100 mL of hexamethyldisilazane, and 1 mL of trimethylsilyl chloride was refluxed with stirring under nitrogen for 20 h. The resultant solution was spin evaporated in vacuo to an oil, which was twice dissolved in 1,2-dichloroethane and reevaporated. To the residual oil in 50 mL of 1,2-dichloroethane was added 10.60 g (49.4 mmol) of 2-(benzoyloxy)ethoxymethyl chloride[1,19] under a blanket of N_2. The solution was cooled on ice and 5 mL (44 mmol) of anhydrous $SnCl_4$ in 50 mL of 1,2-dichloroethane was added rapidly dropwise. The resultant cloudy solution was stirred at ambient temperature for 17 h. The reaction was poured over 300 mL of a saturated aqueous $NaHCO_3$ solution and shaken in a separatory funnel. The resultant emulsion was diluted with 500 mL of 1,2-dichloroethane and filtered. The organic layer was separated, dried ($MgSO_4$), and spin evaporated in vacuo. The residual oil crystallized under cyclohexane containing a little EtOAc: yield 9.29 g (61%); mp 94–99 °C, which contained ~15% of a second isomer. Several recrystallizations from EtOAc gave pure material: yield 2.30 g (15%); mp 115–116 °C; TLC (C_6H_6–EtOH, 10:1, v/v); UV (0.1 N HCl + 20% EtOH) λ_{max} 267 nm (ϵ 8400); UV (0.1 N NaOH + 20% EtOH) λ_{max} 265 nm (ϵ 6950); NMR (Me_2SO-d_6) δ 11.34 (br s, 1 H, NH), 8.10–7.33 (2 m, 6 H, C-6 and ArH), 5.13 (s, 2 H, NCH_2O), 4.40 and 3.85 (2 m, 4 H, CH_2CH_2), 1.72 (s, 3 H, CH_3). Anal. ($C_{15}H_{16}H_2O_5$) C, H, N.

1-[(2-Acetoxyethoxy)methyl]-5-(trifluoromethyl)uracil (17). A mixture of 0.690 g (3.83 mmol) of 5-(trifluoromethyl)uracil (**5**), 20 mL of hexamethyldisilazane, and 0.20 mL of trimethylsilyl chloride was refluxed with stirring for 1.5 h under nitrogen. The clear solution was cooled and evaporated to a viscous oil. To the oil, under a blanket of nitrogen, was added 20 mL of CH_2Cl_2, 0.77 g (4.4 mmol) of 2-(acetoxymethoxy)ethyl acetate,[20] and 1.0 mL (0.38 mmol) of a 10% solution of anhydrous $SnCl_4$ in CH_2Cl_2. The solution was stirred at room temperature for 48 h and then diluted with 1 mL of Et_3N and 9 mL of EtOH. The solution was spin evaporated in vacuo to an oil, which was partitioned between CH_2Cl_2 and H_2O. The layers were separated, and the organic layer was dried ($CaCl_2$) and spin evaporated to a clear oil that crystallized. The solid was recrystallized from EtOAc–hexane to give 0.60 g (55%); mp 91–100 °C. Recrystallization from CH_2Cl_2–hexane gave 0.31 g (27%): mp 98–100 °C; TLC (EtOAc–hexanes, 5:3, v/v); UV (0.1 N HCl + 20% EtOH) λ_{max} 258 nm (ϵ 9700); UV (0.1 N NaOH + 20% EtOH) λ_{max} 257 nm (ϵ 6300); NMR (Me_2SO-d_6) δ 11.90 (br s, 1 H, NH), 8.44 (d, 1 H, C-6), 5.18 (s, 2 H, NCH_2O), 4.09 and 3.75 (2 m, 4 H, CH_2CH_2), 1.98 (s, 3 H, CH_3). Anal. ($C_{10}H_{11}F_3N_2O_5$) C, H, F, N.

1-[(2-Acetoxyethoxy)methyl]-5-fluorouracil (18). A mixture of 4.91 g (37.7 mmol) of 5-fluorouracil (**6**), 100 mL of hexamethyldisilazane, and 1.8 mL (15 mmol) of trimethylsilyl chloride was refluxed with stirring for 3 h under N_2. The clear solution was cooled and evaporated to an oil. To the oil, under a blanket of N_2, was added 140 mL of CH_2Cl_2, 14.0 g (79.5 mmol) of 2-(acetoxymethoxy)ethyl acetate,[20] and 1.0 mL (0.38 mmol) of a 10% solution of anhydrous $SnCl_4$ in CH_2Cl_2. After 18 h at ambient temperature, an additional 6 mL of the $SnCl_4$ solution was added. The mixture was refluxed with stirring for 5 days and then diluted with 1.6 mL of Et_3N and 20 mL of EtOH. The reaction was spin evaporated in vacuo to an oil, which are partitioned between CH_2Cl_2 and H_2O. The resultant solid was removed by filtration, the layers were separated, and the organic layer was evaporated to give additional solid. The combined crops were recrystallized from EtOH: yield 3.82 g (46%); mp 140–145 °C. Two more recrystallizations from EtOH–H_2O gave the analytical sample: yield 1.90 g (23%); mp 142–146 °C; TLC (C_6H_6–EtOH, 10:1, v/v): UV (0.1 N HCl + 20% EtOH) λ_{max} 266 nm (ϵ 8700), UV (0.1 N NaOH + 20% EtOH) λ_{max} 266 nm (ϵ 6400); NMR (Me_2SO-d_6) δ 11.83 (br s, 1 H, NH), 8.12 (d, 1 H, J = 6.6 Hz, C-6), 5.06 (s, 2 H, NCH_2O), 4.08 and 3.73 (2 m, 4 H, CH_2CH_2), 1.99 (s, 3 H, CH_3). Anal. ($C_9H_{11}FN_2$) C, H, N.

1-[(2-Acetoxyethoxy)methyl]-5-iodouracil (19). A solution of 1.08 g (5.80 mmol) of **14** in 10.0 g (6.1 mmol) of a 10% solution of iodine monochloride in AcOH was stirred at ambient temperature for 5 h. The reaction was spin evaporated in vacuo to an oil, which was dissolved in EtOH–EtOAc and applied to a silica gel column. The product was eluted with a gradient of hexane to EtOAc–hexane (1:1). The appropriate fractions were combined and evaporated to give **19**: yield 0.92 g (44%); mp 114–118 °C. Recrystallization from EtOH–Et_2O gave the analytical sample: yield 0.66 g (32%); mp 120–122 °C; TLC (EtOAc–hexanes, 2:1, v/v); UV (0.1 N HCl + 20% EtOH) λ_{max} 284 nm (ϵ 7800); UV (0.1 N NaOH + 20% EtOH) λ_{max} 276.5 nm (ϵ 5600); NMR (Me_2SO-d_6) δ 11.60 (br s, 1 H, NH), 8.24 (s, 1 H, C-6), 5.09 (s, 2 H, NCH_2O), 4.11 and 3.70 (2 m, 4 H, CH_2CH_2), 1.99 (s, 3 H, CH_3). Anal. ($C_9H_{11}IN_2O_5$) C, H, N.

1-[(2-Hydroxyethoxy)methyl]-5-methyluracil (20). A solution of 0.870 g (2.86 mmol) of **16** and 20 mL of 40% aqueous methylamine was heated on a steam bath for 15 min. The reaction was cooled and spin evaporated in vacuo. The residual syrup was triturated with Et_2O to give a solid, which was digested with Et_2O to remove *N*-methylbenzamide. The white solid was collected and washed with Et_2O: yield 0.495 g (86%); mp 138–140 °C. Recrystallization from EtOAc gave analytically pure material: yield 0.375 g (65%); mp 139–140 °C; TLC (C_6H_6–EtOH, 1:1, v/v); UV (0.1 N HCl) λ_{max} 265 nm (ϵ 8700), UV (0.1 N NaOH) λ_{max} 265 nm (ϵ 6500); NMR (Me_2SO-d_6) δ 11.23 (br s, 1 H, NH), 7.56 (s, 1 H, C-6), 5.07 (s, 2 H, NCH_2O), 4.61 (br s, 1 H, OH), 3.51 (s, 4 H, CH_2CH_2), 1.77 (s, 3 H, CH_3). Anal. ($C_8H_{12}N_2O_4$) C, H, N.

1-[(2-Hydroxyethoxy)methyl]-5-(trifluoromethyl)uracil (21). A solution of 1.00 g (3.38 mmol) of **17**, 0.411 g (7.34 mmol) of sodium methoxide, and 10 mL of dry MeOH was heated on the steam bath for 0.5 h. The solution was cooled and neutralized with Bio-Rad AG 50W-X4 cationic resin. The resin was removed by filtration and washed with MeOH. The combined filtrate and wash was concentrated to an oil, which solidified under EtOAc.

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The product was purified by flash chromatography[21] on silica gel using 40% hexane in EtOAc. The appropriate fractions were combined, evaporated, and recrystallized from EtOAc–hexane: yield 0.23 g (27%); mp 142–143.5 °C; TLC (toluene–EtOH, 4:1, v/v); UV (0.1 N HCl + 20% EtOH) λ_{max} 258.5 nm (ϵ 8800); UV (0.1 N NaOH + 20% EtOH) λ_{max} 255.5 nm (ϵ 5500); NMR (Me_2SO-d_6) δ 11.87 (br s, 1 H, NH), 8.44 (d, 1 H, C-6), 5.18 (s, 2 H, NCH_2O), 4.66 (t, 1 H, OH), 3.53 (s, 4 H, CH_2CH_2). Anal. ($C_8H_9F_3N_2O_4$) C, H, F, N.

5-Fluoro-1-[(2-hydroxyethoxy)methyl]uracil (22). A solution of 1.690 g (6.86 mmol) of **18**, 150 mL of a 10% solution of dimethylamine in EtOH, and 15 mL of water was heated on the steam bath for 3 h. The volatiles were evaporated to give a solid that was triturated with Et_2O. The residue was recrystallized from EtOH: yield 1.59 g; mp 144–156 °C. Several recrystallizations from EtOH gave the analytical sample: yield 0.45 g (32%); mp 155–157 °C; TLC (toluene–EtOH, 3:1, v/v); UV (0.1 N HCl + 20% EtOH) λ_{max} 266 nm (ϵ 7700), UV (0.1 N NaOH + 20% EtOH) λ_{max} 266 nm (ϵ 5600); NMR (Me_2SO-d_6) 11.82 (br s, 1 H, NH), 8.11 (d, 1 H, J = 6.6 Hz, C-6), 5.06 (s, 2 H, NCH_2O), 4.64 (br s, 1 H, OH), 3.52 (s, 4 H, CH_2CH_2). Anal. ($C_7H_9FN_2O_4$) C, H, F, N.

1-[(2-Hydroxyethoxy)methyl]-5-iodouracil (23). A solution of 0.500 g (1.41 mmol) of **19**, 0.170 g (3.15 mmol) of sodium methoxide, and 20 mL of dry MeOH was heated on a steam bath with protection from moisture for 2 h. The solution was cooled and neutralized with Bio-Rad AG 50W-X4 cationic resin. The resin was removed by filtration and washed with MeOH. The combined filtrate and wash was decolorized with Norit and concentrated to 10 mL. The resultant crystals were collected and dried: yield 0.32 g (72%); mp 171–173 °C. Recrystallization from MeOH gave the analytical sample: yield 0.260 g (59%); mp 172–174 °C; TLC (toluene–EtOH, 3:1, v/v); UV (0.1 N HCl + 20% EtOH) λ_{max} 285 nm (ϵ 6900); UV (0.1 N NaOH + 20% EtOH) λ_{max} 278 nm (ϵ 4800); NMR (Me_2SO-d_6) δ 11.67 (br s, 1 H, NH), 8.24 (s, 1 H, C-6), 5.09 (s, 2 H, NCH_2O), 4.65 (br t, 1 H, OH), 3.51 (s, 4 H, CH_2CH_2). Anal. ($C_7H_9IN_2O_4$) C, H, I, N.

1-[(2-Hydroxyethoxy)methyl]-5-bromouracil (24). To a solution of 2.0 g (10.7 mmol) of **14** in 200 mL of H_2O was added 200 mL of Br_2-saturated H_2O over 10 min when the color persisted. The solution was flash evaporated to a residue that was chromatographed on silica gel by elution with $CHCl_3$–MeOH mixtures. The fractions containing product were evaporated, dissolved in MeOH, and diluted with EtOAc to give **24**: yield 1.08 g (38%); mp 147–148 °C (MeOH-EtOAc); UV (EtOH) λ_{max} 276 nm (ϵ 8400); NMR (Me_2SO-d_6) δ 11.8 (br s, 1 H, NH), 8.30 (s, 1 H, C-6), 4.2 (br s, 1 H, OH), 3.55 (s, 4 H, CH_2CH_2). Anal. ($C_7H_9N_2O_4Br$) C, H, N.

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Inhibitors of Phenylethanolamine *N*-Methyltransferase and Epinephrine Biosynthesis. 3. Bis[tetrahydroisoquinoline]s[1,2]

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7,8-Dichloro-1,2,3,4-tetrahydroisoquinoline (SK&F 64139) is a potent inhibitor of phenylethanolamine *N*-methyltransferase (IC_{50} = 10 μM) that may have therapeutic utility in man. A series of related compounds in which two 7,8-dichloro-1,2,3,4-tetrahydroisoquinoline molecules have been bridged from nitrogen to nitrogen by an unbranched alkyl chain have been prepared and have demonstrated potent inhibitory properties (0.08 to 2 μM). In contrast, simple substitution on the nitrogen of 7,8-dichloro-1,2,3,4-tetrahydroisoquinoline with a variety of substituents gives compounds with greatly diminished inhibitory potencies (IC_{50} = 2 to >100 μM) relative to SK&F 64139. Kinetic studies with a C_6 analogue have shown that it is competitive with respect to phenylethanolamine and uncompetitive with respect to *S*-adenosylmethionine. The increased potency of some of the bis analogues relative to that seen with the tetrahydroisoquinolines having larger alkyl groups on nitrogen suggests that several of the bis compounds show supplemental or cooperative binding to the enzyme, presumably as a result of the second tetrahydroisoquinoline moiety.

Phenylethanolamine *N*-methyltransferase (PNMT) is the enzyme which catalyzes the final step in epinephrine biosynthesis. It is mainly localized in the adrenal medulla, although it is also found in much lower concentration in other organs.[3] Physiologically, PNMT catalyzes the transfer of a methyl group from *S*-adenosylmethionine (SAM) to norepinephrine (NE) to yield epinephrine and *S*-adenosylhomocysteine (Scheme I). Under resting conditions, the levels of circulating epinephrine are quite low and it is not a significant regulatory hormone.[4,5] However, when the organism is subjected to stress, large quantities of epinephrine are released into the blood stream and this initiates physiological changes, which are associated with the "fight or flight syndrome", which prepare the body to cope with the stressor situation. It is possible that under certain circumstances the incresed metabolic and or physiological activities induced by the efflux of epinephrine from the adrenal medulla may be detrimental to people who are predisposed to disease states such as anxiety or ischemic heart disease.[6] Thus, an agent that can selectively inhibit the final step in epinephrine biosynthesis could be of potential therapeutic utility in the treatment of these disorders.

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