Viscometric Measurements. Viscometry was performed with Cannon-Ubbelohde semimicro dilution viscometer cells (Cannon Instrument Co., State College, PA). The temperature was maintained at 25 ± 0.1 °C by a Cannon constant temperature bath. Calf thymus DNA (0.7–1.5 mM in nucleotide residues) in 0.01 M sodium phosphate–0.10 M sodium chloride–0.1 mM EDTA (pH 7.0) was titrated with successive increments of the desired analogue dissolved in the same buffer. After each addition, the solution was thoroughly mixed, and the viscosity was measured a minimum of 5 times. Each experimental point is the average of these determinations. The relative viscosity (the ratio of the viscosity at any given analogue concentration to the viscosity of that DNA solution prior to the addition of any analogue) was then plotted as a function of the molar ratio of analogue to DNA phosphate.

Cell Culture Experiments. Mouse leukemia L1210 cells were maintained in suspension culture in RPMI 1640 medium supplemented with 10% fetal bovine serum (Calbiochem, LaJolla, CA) and incubated at 37 °C in an atmosphere of 5% carbon dioxide. Compounds were assayed for biological activity based on the effects of the analogues on cell number or on the ability of treated cells to form clones once removed from the presence of the compound.

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Registry No. **2a**, 85318-76-9; **2b** (X = I), 83579-43-5; **2c** (X = I), 85318-77-0; **4**, 2227-79-4; **5a**, 70-23-5; **5b**, 76275-88-2; **5c**, 80337-80-0; **6a**, 59937-01-8; **6b**, 7113-08-8; **6c**, 85318-71-4; **7a**, 85318-72-5; **7b**, 85318-73-6; **7c**, 85318-74-7; **8b**, 14002-51-8; **9b**, 85318-75-8; poly(dA-dT), 26966-61-0; 3-(methylthio)propylamine, 4104-45-4; 4,4′-biphenylcarboxylic acid, 92-92-2.

3′-Amino-2′,3′-dideoxyribonucleosides of Some Pyrimidines: Synthesis and Biological Activities

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3′-Amino-2′,3′-dideoxyribonucleosides of thymine, uracil, and 5-iodouracil (**1–3**) were synthesized from the corresponding 2′-deoxyribonucleosides via the *threo*-3′-chloro and the *erythro*-3′-azido derivatives. Corresponding aminonucleosides of 5-bromouracil, 5-chlorouracil, and 5-fluorouracil (**4–6**) were synthesized enzymatically with 3′-amino-2′,3′-dideoxythymidine as the aminopentosyl donor and thymidine phosphorylase (EC 2.4.2.4) as the catalyst. 3′-Amino-2′,3′-dideoxycytidine (**7**) was synthesized by amination of the 3′-azido precursor of 3′-amino-2′,3′-dideoxyuridine. The biological activity of 3′-amino-2′,3′-dideoxy-5-fluorouridine (**6**) was notable among this group of aminonucleosides. It had an ED_{50} of 10 μM against adenovirus and was not appreciably cytotoxic to mammalian cells in culture. It also had activity against some Gram-positive bacteria but not against a variety of Gram-negative bacteria. The other aminonucleosides (**1–5** and **7**) lacked or exhibited weak antiviral and antibacterial activities. The only compounds in this group that were appreciably toxic to mammalian cells in culture were the thymidine and deoxycytidine analogues (**1** and **7**).

Only a few 3′-amino-2′,3′-dideoxyribonucleosides of pyrimidines have been reported. The first such nucleosides synthesized contained the thymine, 5-methylcytosine, and *N*-butyl-5-methylcytosine moieties.[1] 3′-Amino-2′,3′-dideoxythymidine (**1**) was found to have appreciable antitumor activity but only weak antiherpes virus activity.[1c,2] Later, the corresponding nucleoside of 5-(2-bromovinyl)-uracil was reported to have appreciable activity against herpes simplex virus (type I).[3] Recently, the uracil (**2**) and cytosine (**7**) congeners were reported to have considerable antitumor activity.[4]

In this study, the 3′-amino-2′,3′-dideoxyribonucleosides of the 5-halogenouracils, as well as that of thymine, uracil, and cytosine, were synthesized. The ability of these aminonucleosides to inhibit the growth of mammalian cells and a variety of viruses and bacteria in vitro was assessed.

Results and Discussion

Chemistry. The synthetic routes for the 3′-amino-2′,3′-dideoxyribonucleosides listed in Table I are summarized in Scheme I. The abbreviated names provided in Table I are used below. 3′-NH_2-dThd (**1**) and 3′-NH_2-dUrd (**2**) were prepared from the corresponding 2′-deoxyribonucleosides. The initial step was tritylation of the 5′-hydroxy groups by reaction with trityl chloride in pyridine at 100 °C.[5] Next, chlorination in the 3′-position was achieved by reaction with triphenylphosphine and carbon tetrachloride in dimethylacetamide.[6] After the 5′-hydroxy group was deblocked by treatment with 80% acetic acid, the 1-(3-chloro-2,3-dideoxy-β-D-*threo*-pentofuranosyl) derivatives were obtained. Their *threo* configuration was confirmed by NMR (dd for $H_{1'}$).[6,7] These *threo*-3′-chloro derivatives were converted to the corresponding *erythro*-3′-azido derivatives by displacement with lithium azide.

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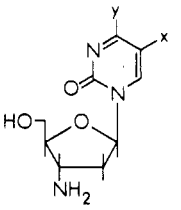
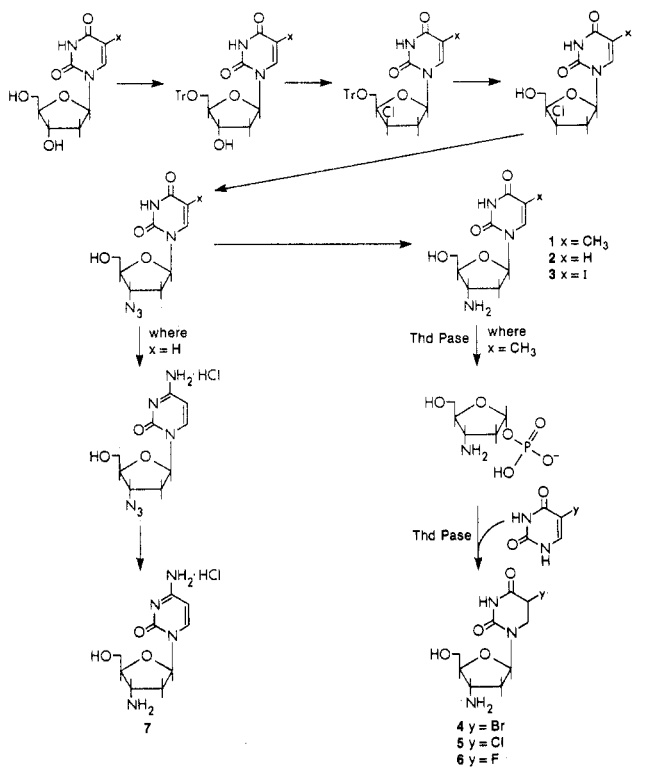
Table I. Biological Activities of Some Pyrimidine 3′-Amino-2′,3′-dideoxyribonucleosides



no.	abbreviated name	X	Y	antiviral ED_{50}, μM adenovirus	antibacterial MIC, μM *Staph. aureus*	cytotoxicity ED_{50}, μM human D-98	cytotoxicity ED_{50}, μM mouse L
1	3′-NH_2-dThd	CH_3	OH	>100	>400	100	3
2	3′-NH_2-dUrd	H	OH	>100	>400	>100	>100
3	3′-NH_2-IUdR	I	OH	>100	>400	>100	>100
4	3′-NH_2-BrUdR	Br	OH	100	>400	>100	>100
5	3′-NH_2-ClUdR	Cl	OH	100	>200	>100	100
6	3′-NH_2-FUdR	F	OH	10	4	>100	100
7	3′-NH_2-dCyd	H	NH_2	>100	>400	3	1

Scheme I



Subsequent catalytic hydrogenation in aqueous methanol with 5% palladium on carbon resulted in the desired 3′-amino-2′,3′-dideoxyribonucleosides. A detailed description is provided under Experimental Section for each intermediate in the synthesis of 3′-NH_2-dUrd (**2**). Similar procedures were followed for the synthesis of **1**.

The synthetic procedures for **3** were modified from those for **1** and **2** because of the presence of the 5-iodo substituent. The tritylation of the 5′-hydroxy group was performed under milder reaction conditions in methylene chloride and pyridine.[8] The temperature was 25 °C instead of 100 °C, and the reaction time was 12 days instead of 18 h. Deblocking the 5′-hydroxy was performed under neutral conditions with zinc bromide in methylene chloride at 24 °C.[8] Displacement of the 3′-chloro with lithium azide was performed in dimethyl sulfoxide. Reduction of the azido intermediate to the product (**3**) was achieved with triphenylphosphine in dioxane.[9]

The other 5-halogenouracil congeners [3′-NH_2-BrUdR (**4**), 3′-NH_2-ClUdR (**5**), and 3′-NH_2-FUdR (**6**)] were prepared by the enzyme-catalyzed transfer of the aminopentosyl moiety of 3′-NH_2-dThd (**1**) to the appropriate 5-halogenouracil (Scheme I). Such pentosyl transfers occur via an α-pentose 1-phosphate intermediate.[10] This phosphate ester was generated in situ by the phosphorolysis of 3′-NH_2-dThd (**1**). Both the phosphorolytic and synthetic reactions were catalyzed by thymidine phosphorylase purified from *Escherichia coli*. The effectiveness of this enzymatic method was a function of the 5-substituent; the relative order was F > Cl > Br > I. Attempts to synthesize the iodo congener (**3**) by this method did result in detection of small amounts of product by TLC [cellulose with acetonitrile–5 N NH_4OH (85:15), R_f 0.2]. However, **3** was not isolated from these reactions.

The starting material for the synthesis of 3′-NH_2-dCyd (**7**) was 3′-azido-2′,3′-dideoxyuridine, an intermediate in the synthesis of **2**. This nucleoside was aminated in the 4-position by using formamide and ammonium sulfate in hexamethyldisilazane.[11] Subsequent catalytic hydrogenation gave 3′NH_2-dCyd (**7**).

Biology. 3′-NH_2-FUdR (**6**) had an ED_{50} of 10 μM against adenovirus (Table I). 3′-NH_2-ClUdR (**5**) and 3′-NH_2-BrUdR (**4**) were an order of magnitude less potent. None of the other aminonucleosides (**1–3** and **7**) exhibited appreciable activity toward adenovirus. No antiherpes activity in vitro (ED_{50} > 100 μM) was detected with any of the aminonucleosides listed in Table I. This is in marked contrast to the related aminonucleoside, 5′-amino-2′,5′-dideoxy-5-iodouridine.[12]

With three RNA viruses (influenza, measles, and rhinovirus), none of the nucleosides (**1–7**) exhibited appreciable activity in vitro except for some weak activity of **2** against rhinovirus.

The aminonucleosides listed in Table I were also tested for antibacterial activity. Against some Gram-positive

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organisms (*Streptococcus faecalis*, *Streptococcus agalactiae*, and *Staphylococcus aureus*), the minimal inhibitory concentrations (MIC) with 3'-NH_2-FUdR (**6**) ranged from 4 to 20 μM. With *Bacillus megaterium*, this value was 40 μM. However, no activity was found against the Gram-positive organisms, *Candida albicans* and *Streptococcus pyrogenes* (MIC > 200 μM). None of the other nucleosides (**1**, **2**, and **4–7**) listed in Table I had activity (MIC > 200 μM) toward these Gram-positive bacteria. A variety of Gram-negative organisms (*Vibrio cholerae*, *Pasteurella multocida*, *Salmonella typhimurium*, *Salmonella typhosa*, *Shigella flexneri*, *Escherichia coli*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Proteus vulgaris*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*) were not significantly (MIC > 200 μM) inhibited by any of the nucleosides (**1–7**) in Table I.

3'-NH_2-dCyd (**7**) was the only nucleoside listed in Table I that was appreciably toxic to human D-98 cells in culture. With mouse L cells, although **7** was still the most toxic nucleoside, some of the other nucleosides were also cytotoxic. 3'-NH_2-dThd (**1**) was one-third as toxic as **7**. 3'-NH_2-ClUdR (**5**) and 3'-NH_2-FUdR (**6**) were also inhibitory but only at relatively high concentrations. The other nucleosides (**2–4**) were not appreciably inhibitory. These findings on the toxicity of 3'-NH_2-dThd (**1**) and 3'-NH_2-dCyd (**7**) are in agreement with earlier observations.[2,4]

In the same system used to determine the cytotoxicity of the nucleosides listed in Table I, 5-fluorouracil had an ED_{50} against both D-98 and L cells of 1 μM. This finding suggests that 3'-NH_2-FUdR (**6**) was not readily cleaved to the pyrimidine base in these cells. Compound **6** was also nontoxic in vivo. Young mice dosed with this nucleoside on days 1, 5, and 9 (450 mg/kg, ip) gained weight at a normal rate. Further, **6** was inactive against the P-388/0 mouse tumor at 675 mg/kg administered three times on days 1, 5, and 9 after implantation. No evidence of toxicity was observed at this dose.

It can be concluded that among the group of aminonucleosides listed in Table I, 3'-NH_2-FUdR (**6**) is unique in that it shows desirable selectivity in its biological activities. It exhibits antiviral and antibacterial activities at concentrations that were well below those necessary to inhibit the growth of mammalian cells.

Experimental Section

Cytotoxicity Evaluation. Cells originally derived from human sternal marrow (D-98) and mouse connective tissue (L) were cultured, and inhibition by nucleosides was tested as previously described.[13]

Antiviral Activity. The plaque-inhibition test[14] was adapted to the plaque assays previously described for herpes type 1,[15] influenza A/Sweden,[16] measles,[17] and rhinovirus 1-B.[18] Activity against adenovirus type 5 was measured in plaque-reduction assays. Adenovirus plaques were produced in monolayers of NCTC 2544 cells[19] that were overlaid with nutrient agarose containing a range of doubling concentrations of test compound. Plaque counts were expressed as a percentage of the control value and plotted against the logarithm of the compound concentration to yield a dose–response line from which the ED_{50} could be determined. Details of this procedure will be published elsewhere (J.W.T.S.).

Antibacterial Activity. Tests for susceptibility of bacteria were carried out with the replica inoculating apparatus of Steers, Foltz, and Graves.[20] The replicator delivers approximately 0.004 mL of each inoculum. The inocula for the tests were obtained from overnight cultures in Wellcotest broth (Wellcome Reagents Ltd., Wellcome Research Laboratories, Beckenham, England). Serial threefold dilutions of the compounds were incorporated into Wellcotest sensitivity test agar supplemented with 7% lysed, defibrinated horse blood. Plates were seeded with the replicator with appropriately diluted cultures (undiluted to 10^{-3} dilutions, depending upon the microorganism). The plates were read for growth after incubation for 18 h at 37 °C; the minimum inhibitory concentration (MIC) was taken as the lowest concentration on which there was no discernible growth.

Enzyme Catalyst. Thymidine phosphorylase (EC 2.4.2.4) was purified from *Escherichia coli* and assayed as previously described.[21] One unit of enzyme activity catalyzed the phosphorolysis of 1 μmol of thymidine per minute. Enzyme was added to reaction mixtures as a solution in 10 mM potassium phosphate buffer, pH 6.8, containing 3250 units/mL.

Physical Characterization of Compounds. Melting points were determined with a Thomas Hoover apparatus and are uncorrected. UV spectra were recorded on a Varian Superscan 3. A Varian XL-100 or FT-80A provided the NMR spectra in Me_2SO-d_6. Microanalyses were performed by Atlantic Micro Labs. All compounds analyzed within ±0.4% of calculated values.

3'-NH_2-dThd (**1**),[1] the only compound listed in Table I for which the details of synthesis are not provided, softened at 174 °C and melted at 182–185 °C (lit.[1a,c] mp 187–187.5; 160–161 °C); $[\alpha]^{23}_D$ 22.3° (*c* 0.835, H_2O) [lit.[1a] $[\alpha]^{23}_D$ 20° (*c* 0.64, H_2O)]; NMR δ 7.75 (d, 1 H, *J* = 1.1 Hz, 6 H), 6.08 (t, 1 H, *J* = 5.9 Hz, 1 H), 3.48 (m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 2.02 (m, 2 H, $H_{2'}$), 1.765 (d, 3 H, *J* = 1.0 Hz, 5-CH_3).

The NMR spectra of **1–7** all showed a very broad peak in the region of δ 5. The integrations were indicative of a composite signal due to the protons of the 3'-amino, 5'-hydroxy, and water present.

Syntheses. 5-Fluorouracil, 5-chlorouracil, 5-bromouracil, and the 2'-deoxyribonucleosides of thymine, uracil, and 5-iodouracil were purchased from Sigma Chemical Co. Pyridine was stored over KOH. Dimethylacetamide and methanol were dried over molecular sieves. Lithium azide was dried at 80 °C for 2 h in vacuo.

Solvents were removed by evaporation under reduced pressure in a rotary evaporator. Bio-Rex 70 (50–100 mesh, H_+ form) was purchased from Bio-Rad Laboratories, Richmond, VA. This resin in a weakly acidic cation exchanger containing carboxylic acid exchange groups on a macroreticular acrylic polymer lattice. The silica gel used was no. 60, particle size 0.04–0.063 mm, purchased from E. Merck, Darmstadt, Germany. Except where noted, elution of the silica gel columns was performed under a pressure of 10 psi.[22]

1-(3-Chloro-2,3-dideoxy-β-D-*threo*-pentofuranosyl)-2,4-(1*H*,3*H*)-pyrimidinedione. 2'-Deoxyuridine was tritylated by reaction with trityl chloride in pyridine at 100 °C for 18 h.[5] The resulting 2'-deoxy-5'-*O*-(triphenylmethyl)uridine (12.58 g, 26.7 mmol) and triphenylphosphine (15.73 g, 60 mmol, 2.25 equiv) were dissolved in 31 mL of dimethylacetamide. Carbon tetrachloride, 63 mL, was then added. The reaction mixture was allowed to stir at 24 °C for 18 h. TLC (ethyl acetate on silica gel plates) showed that no starting material remained. The reaction was quenched with 25 mL of methanol. Carbon tetrachloride and methanol were then removed in vacuo. The remaining dimethylacetamide solution was poured into 500 mL of water at

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5 °C. The solution was stirred, and after it settled, the aqueous phase was decanted from a dark green oil, which was dissolved in chloroform and extracted with water. The organic phase was decreased in volume in vacuo and then applied to a column of silica gel (7.5 × 15 cm). After an initial elution with chloroform, elution with 1% methanol in chloroform removed the tritylated derivative of the product, 1-[3-chloro-2,3-dideoxy-5-*O*-(triphenylmethyl)-β-D-*threo*-pentofuranosyl]-2,4(1*H*,3*H*)-pyrimidinedione. After the solvents were removed in vacuo, the resulting oil was heated in 80% acetic acid on a steam bath for 20 min to remove the trityl group. While the oil cooled, a precipitate formed, which was removed by filtration, and the volume of the filtrate was decreased in vacuo. The resulting oil was applied to a column of silica gel (5 × 15 cm). The product was eluted with ethyl acetate. After removal of the solvent and crystallization from ethyl acetate/methanol, 3.1 g (47% yield) of product was obtained: mp 172–174 °C; UV λ_{max} at pH 1, 264 nm; at pH 13, 262 nm; NMR δ 7.74 (d, 1 H, $J_{5,6}$ = 8.1 Hz, H_6), 5.69 (d, 1 H, H_5), 6.04 (dd, 1 H, J = 3.2 and 7.8 Hz, $H_{1'}$), 4.78 (ddd, 1 H, $J_{3',4'}$ = 3.5 Hz, $H_{3'}$), 4.16 (dt, 1 H, $J_{4',5'}$ = 5.6 Hz, $H_{4'}$), 3.71 (d, 2 H, $H_{5'}$), 3.03 (ddd, 1 H, $J_{2'a,2'b}$ = 15.5 Hz, $H_{2'a}$), 2.27 (ddd, 1 H, $J_{2'b,3'}$ = 1.5 Hz, $H_{2'b}$). Anal. ($C_9H_{11}ClN_2O_4$) C, H, Cl, N.

3′-Azido-2′,3′-dideoxyuridine. 1-(3-Chloro-2,3-dideoxy-β-D-*threo*-pentofuranosyl)-2,4(1*H*,3*H*)-pyrimidinedione (2.9 g, 11.8 mmol) was reacted with lithium azide (1.15 g, 23.5 mmol) in hexamethylphosphoramide (10 mL). The reaction proceeded for 18 h at 90 °C. The reaction mixture was then poured into water and extracted with an equal volume of chloroform. The organic phase was dried with magnesium sulfate. After the chloroform was removed in vacuo, the product was crystallized from ethyl acetate/methanol. A second crystallization from water gave 0.78 g of product (26% yield): mp 166.5–168.5 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, 262 (9.6) nm; at pH 13, 262 (6.8) nm; NMR δ 7.84 (d, 1 H, $J_{5,6}$ = 8.1 Hz, H_6), 5.64 (d, 1 H, H_5), 6.08 (t, 1 H, $H_{1'}$), 4.3 (m, 1 H, $H_{3'}$), 3.83 (m, 1 H, $H_{4'}$), 3.6 (d, 2 H, $H_{5'}$), 2.34 (m, 2 H, $H_{2'}$). Anal. ($C_9H_{11}N_5O_4$) C, H, N.

3′-Amino-2′,3′-dideoxyuridine (2, 3′-NH$_2$-dUrd). 3′-Azido-2′,3′-dideoxyuridine (0.3 g, 1.3 mmol) was dissolved in 35 mL of ethanol and reduced in a Parr hydrogenator with 5% palladium on carbon (25 mg) for 1.5 h at 24 °C. The product was purified by chromatography on a column of Bio-Rex 70 (5 × 25 cm). The column was washed with water, and then the product was eluted from the column with 1 N NH_4OH. Removal of solvent from the appropriate fractions in vacuo gave 0.125 g (46% yield) of product: mp 149–158 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, 262 (8.6) nm; at pH 13, 263 (7.2) nm; NMR δ 7.90 (d, 1 H, $J_{5,6}$ = 8.0 Hz, H_6), 5.60 (d, 1 H, H_5), 6.07 (t, 1 H, $H_{1'}$), 3.47 (m, $H_{3'}$, $H_{5'}$), 2.05 (m, 2 H, $H_{2'}$). Anal. ($C_9H_{13}N_3O_4$) C, H, N.

3′-Amino-2′,3′-dideoxy-5-iodouridine (3, 3′-NH$_2$-IUdR). 2′-Deoxy-5-iodouridine[23] (10 g, 28 mmol) was dissolved in 140 mL of pyridine. Methylene chloride (2500 mL) was added, and a precipitate formed. Powdered 4Å molecular sieves (55 g) and trityl chloride (9.45 g, 34 mmol) were added. The reaction mixture was stirred at 24 °C for 12 days. TLC on silica gel with ethyl acetate indicated that the reaction was complete (R_f of dUrd and product 0.5 and 0.15). The reaction mixture was filtered, and the solvent removed from the filtrate in vacuo. The resulting oil was applied to a column of silica gel (7.5 × 15 cm). 2′-Deoxy-5-iodo-5′-*O*-(triphenylmethyl)uridine was eluted with ethyl acetate. After the solvent was removed in vacuo, 15.4 g (91% yield) of this tritylated nucleoside was obtained. A portion of this material (5.26 g, 8.39 mmol) was converted to 1-[3-chloro-2,3-dideoxy-5-*O*-(triphenylmethyl)-β-D-*threo*-pentofuranosyl]-5-iodo-2,4-(1*H*,3*H*)-pyrimidinedione by reaction with triphenylphosphine (5.2 g) and carbon tetrachloride (22.5 mL) in 20 mL of dimethylacetamide as described above with the corresponding uracil congener. Removal of the trityl group was achieved under neutral conditions with zinc bromide in methylene chloride at 24 °C.[8] After the solvent was removed in vacuo, the resulting oil was applied to a column of silica gel (5 × 18 cm). The product was eluted with ethyl acetate. Application to another column of silica gel (5 × 18 cm), elution with chloroform–methanol (9:1), and solvent removal gave 2 g (61% yield) of 1-(3-chloro-2,3-dideoxy-β-D-*threo*-pentofuranosyl)-5-iodo-2,4(1*H*,3*H*)-pyrimidinedione, suitable for further use. A portion of this material (1.8 g, 4.6 mmol) was dissolved in 40 mL of dimethyl sulfoxide and treated with lithium azide (0.92 g, 18.7 mmol) at 90 °C for 48 h. After the solvent was removed, the resulting oil was dissolved in water and extracted with 2 vol ethyl acetate. The extract was dried over magnesium sulfate, and the solvent was removed in vacuo. The resulting oil was applied to a column of silica gel (2.5 × 18 cm). The product was eluted with ethyl acetate, product-containing fractions were combined, and the solvent was removed to give 3′-azido-2′,3′-dideoxy-5-iodouridine (0.64 g, 36% yield). A portion of this material (0.3 g, 0.79 mmol) was dissolved in 23 mL of dioxane and treated with triphenylphosphine (0.53 g, 2 mmol) for 3 h at 23 °C. Water (12 mL) was added, and this mixture was maintained at 50 °C for 18 h. After the solvents were removed, the resulting oil was partitioned between water and benzene. The aqueous phase was evaporated to an oil and applied to a column of silica gel (2.5 × 18 cm). A mixture of methylene chloride, methanol, and water (5:4:1) was used as the eluent. Solvent removal from appropriate fractions gave the final product (0.069 g, 25% yield for the last step; 5% overall yield from 2′-deoxy-5-iodouridine): mp 188–190 °C; UV λ_{max} at pH 1, 286 nm; at pH 13, 277 nm; NMR δ 8.49 (s, 1 H, H_6), 5.98 (t, 1 H, J = 6 Hz, $H_{1'}$), 3.5 (m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 2.07 (dd, 2 H, J = 6 and 2 Hz, $H_{2'}$). Anal. ($C_9H_{12}N_3O_4I$) C, H, N, I.

3′-Amino-2′,3′-dideoxy-5-bromouridine (4, 3′-NH$_2$-BrUdR). 5-Bromouracil (2.25 g, 12 mmol), 1 (1.38 g, 5.7 mmol), and KH_2PO_4 (1.3 mmol) were suspended in 130 mL of water. The pH of the suspension was adjusted to 7.0 with acetic acid. Thymidine phosphorylase (13 000 units) was added, and the reaction mixture was maintained at 37 °C. After 9 days, 5.5 mL of dimethylformamide and more enzyme (60 000 units) were added. After an additional 4 days, the mixture was filtered, and solvent was removed from the filtrate. The residue was extracted with 20 mL of chloroform–methanol–2.5 N NH_4OH (5:4:1). Insoluble material was removed by filtration, and the filtrate was applied to a column of silica gel (2.5 × 30 cm) and eluted with the chloroform–methanol–NH_4OH mixture by gravity. This chromatography step was repeated twice. Solvent was removed from appropriate fractions. The residue was dissolved in water and applied to a column of Bio-Rex 70 (2.5 × 22 cm). After the resin was washed with water, the product was eluted with 2 N NH_4OH. Product-containing fractions were lyophilized to give 0.12 g (7% yield on basis of 1 used) of product: mp 101–103 °C; UV λ_{max} at pH 1, 278 nm; at pH 11, 277 nm; NMR δ 8.51 (s, 1 H, H_6), 6.01 (dd, 1 H, $H_{1'}$), 3.47 (m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 2.08 (m, 2 H, $H_{2'}$). Anal. ($C_9H_{12}B_RN_3O_4$) C, H, N.

3′-Amino-2′,3′-dideoxy-5-chlorouridine (5, 3′-NH$_2$-ClUdR). 5-Chlorouracil (2 g, 13.6 mmol), 3′-NH_2-dThd (1, 1.38 g, 5.7 mmol), and KH_2PO_4 (1.3 mmol) were suspended in 130 mL of water. The pH of the suspension was adjusted to 7.0 with acetic acid. Thymidine phosphorylase (9750 units) was then added. After 8 days at 37 °C, the reaction mixture was filtered. Solvent was removed from the filtrate, and the residue was extracted with 35 mL of chloroform–methanol–2.5 N NH_4OH. (4:5:1). After filtration of the resulting suspension, the filtrate was applied to a column of silica gel (2.5 × 30 cm). The product was eluted by gravity with the solvent mixture described above. Removal of solvent from product-containing fractions was followed by a repetition of the chromatographic step, except that the proportions in the solvent mixture were altered (5:4:1). Product-containing fractions eluted from this column were further purified by chromatography on Bio-Rex 70 as described above for 4. Impurities still present in the sample thus obtained were removed by repeating the last two chromatographic steps. Product (0.14 g) was obtained in 9% yield on the basis of 1 used: mp 108–112 °C; UV λ_{max} at pH 1, 276 nm; at pH 13, 275.5 nm; NMR δ 8.43 (s, 1 H, H_6), 6.01 (dd, 1 H, $H_{1'}$), 3.51 (m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 2.07 (m, 2 H, $H_{2'}$). Anal. ($C_9H_{12}ClN_3O_4 \cdot 0.75H_2O$) C, H, N, Cl.

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(24) ^{13}C NMR spectra of pyrimidine 2′-deoxyribonucleosides otherwise similar to **6** showed the $C_{3'}$ peak at δ 70 rather than at δ 50. In contrast, the other pentose carbons gave similar shifts with the 2′-deoxyribonucleosides and with **6**. This indicated the presence of the amino group on the 3′-carbon of **6**.

3′-Amino-2′,3′-dideoxy-5-fluorouridine (6, 3′-NH_2-FUdR). 5-Fluorouracil (7.07 g, 54 mmol), **1** (4.1 g, 17 mmol), and KH_2PO_4 (3.5 mmol) were suspended in 393 mL of water. The pH of the suspension was adjusted to 6.5. Thymidine phosphorylase (30 000 units) was then added, and the suspension was maintained at 37 °C for 3 days. More enzyme (40 000 units) and water (200 mL) were then added. After 2 more days at 37 °C, the reaction mixture was filtered, and the volume of the filtrate was decreased to 150 mL in vacuo. The resulting suspension was filtered, and the solvent was removed from the filtrate. The residue was extracted with 35 mL of chloroform–methanol–2.5 N NH_4OH (4:5:1). After centrifugation of the suspension, the supernatant fluid was applied to a column of silica gel (5 × 15 cm), and the solvent mixture described above was used as the eluent. Solvent was removed from those fractions containing product. This provided 1.4 g (29% yield on the basis of 1 used): mp 119–121 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, 268 (8.06) nm; at pH 13, 269 (6.01) nm; $[\alpha]^{20}_D$ 58.65° (*c* 1.0, DMF); ^{1}H NMR δ 8.32 (d, 1 H, *J* = 7.4 Hz, H_6), 6.03 (m, 1 H, $H_{1'}$), 3.62 (m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 2.1 (m, 2 H, $H_{2'}$); ^{13}C NMR δ 157.2 (d, J_{CF} = 25.9 Hz, C_4), 149.0 (s, C_2), 139.8 (d, J_{CF} = 229.5 Hz, C_5), 124.8 (d, J_{CF} = 34.7 Hz, C_6), 87.6 (s, $C_{4'}$), 84.3 (s, $C_{1'}$), 60.1 (s, $C_{5'}$), 50.0 (s, $C_{3'}$),[24] 40.7 (s, $C_{2'}$). Anal. ($C_9H_{12}FN_3O_4 \cdot 2H_2O$) C, H, N.

3′-Azido-2′,3′-dideoxycytidine. 3′-Azido-2′,3′-dideoxyuridine (2.2 g, 8.7 mmol), formamide (0.693 mL, 17.4 mmol), hexamethyldisilazane (27.5 mL, 130.5 mmol), and ammonium sulfate (0.13 g, 1 mmol) were combined in a steel bomb and stirred in a 120 °C oil bath for 71 h. After cooling, the contents of the bomb were dissolved in methanol and refluxed for 6 h. After removal of the solvents in vacuo, the resulting foam was applied to a column of silica gel (2.5 × 23 cm). The column was washed with a mixture of ethyl acetate and methanol (85:15). Solvent was removed from the fractions containing the product and then applied to a second column of silica gel (5 × 18 cm). The gel was washed with a mixture of chloroform and methanol (85:15). The product was then eluted with a mixture having a higher proportion of methanol (3:2). After the solvent was removed, the residue was dissolved in methanol and 1 equiv of HCl in dioxane was added. Ethyl ether precipitated the product (1.29 g, 51% yield) as the HCl salt from the solution: mp 174.5–176.5 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, 279 (13.4) nm; at pH 13, 271 (8.6) nm; NMR δ 8.22 (d, 1 H, $J_{5,6}$ = 7.9 Hz, H_6), 6.16 (d, 1 H, H_5), 6.0 (t, 1 H, $H_{1'}$), 4.32 (m, 1 H, $H_{3'}$), 3.92 (m, 1 H, $H_{4'}$), 3.64 (m, 2 H, $H_{5'}$). Anal. ($C_9H_{12}N_6O_3 \cdot HCl$) C, H, Cl, N.

3′-Amino-2′,3′-dideoxycytidine (7, 3′-NH_2-dCyd). 3′-Azido-2′,3′-dideoxycytidine hydrochloride (0.42 g, 1.45 mmol) was converted to the product by catalytic hydrogenation as described above for **2**. Purification was achieved by chromatography on a column of silica gel (1.25 × 15 cm) with chloroform–methanol–water (5:4:1) as the eluent. The yield was 0.28 g (65%) of a hygroscopic powder: mp 180 °C dec; UV λ_{max} at pH 1, 278 nm; at pH 13, 272 nm; NMR δ 7.82 (d, 1 H, $J_{5,6}$ = 7.5 Hz, H_6), 5.72 (d, 1 H, H_5), 7.1 (br s, 2 H, 4-NH_2), 6.15 (t, 1 H, $H_{1'}$), 3.5 (m, $H_{3'}$, $H_{4'}$, $H_{5'}$), 2.15 (m, 2 H, $H_{2'}$). Anal. ($C_9H_{15}N_4O_3 \cdot HCl \cdot 2H_2O$) C, H, Cl, N.

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Registry No. 1, 52450-18-7; 2, 84472-86-6; 3, 85236-89-1; 4, 85236-93-7; 5, 85236-94-8; 6, 85236-95-9; 7·HCl, 85236-97-1; 1-(3-chloro-2,3-dideoxy-β-D-*threo*-pentofuranosyl)-2,4(1*H*,3*H*)pyrimidinedione, 85236-87-9; 2′-deoxyuridine, 951-78-0; 2′-deoxy-5′-*O*-(triphenylmethyl)uridine, 14270-73-6; 1-[3-chloro-2,3-dideoxy-5-*O*-(triphenylmethyl)-β-D-*threo*-pentofuranosyl]-2,4-(1*H*,3*H*)pyrimidinedione, 85236-88-0; 3′-azido-2′,3′-dideoxyuridine, 84472-85-5; 2′-deoxy-5-iodouridine, 54-42-2; 2′-deoxy-5-iodo-5′-*O*-(triphenymethyl)uridine, 15414-61-6; 1-[3-chloro-2,3-dideoxy-5-*O*-(triphenymethyl)-β-D-*threo*-pentofuranosyl]-5-iodo-2,4-(1*H*,3*H*)-pyrimidinedione, 85236-90-4; 1-(3-chloro-2,3-dideoxy-β-D-*threo*-pentofuranosyl)-5-iodo-2,4(1*H*,3*H*)-pyrimidinedione, 85236-91-5; 3′-azido-2′,3′-dideoxy-5-iodouridine, 85236-92-6; 5-bromouracil, 51-20-7; thymidine phosphorylase, 9030-23-3; 5-chlorouracil, 1820-81-1; 5-fluorouracil, 51-21-8; 3′-azido-2′,3′-dideoxycytidine hydrochloride, 85236-96-0.

4,5,6,7-Tetrahydroisothiazolo[5,4-*c*]pyridin-3-ol and Related Analogues of THIP. Synthesis and Biological Activity

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The thio analogues of the GABA (γ-aminobutyric acid) agonist THIP (4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol), the GABA uptake inhibitor THPO (4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol), and the glycine antagonist THAZ (5,6,7,8-tetrahydro-4*H*-isoxazolo[4,5-*d*]azepin-3-ol) have been synthesized and tested biologically on single neurons in the cat spinal cord and in vitro by using synaptic membrane preparations obtained from rat brains. In contrast to THIP, thio-THIP (4,5,6,7-tetrahydroisothiazolo[5,4-*c*]pyridin-3-ol, **5**) was only a weak GABA agonist. Thio-THPO (4,5,6,7-tetrahydroisothiazolo[4,5-*c*]pyridin-3-ol, **10**) was slighlty weaker than THPO as an inhibitor of GABA uptake in vitro, and these two compounds were approximately equipotent in enhancing the inhibition of the firing of cat spinal neurons by GABA. Like THAZ and structurally related bicyclic isoxazole zwitterions, thio-THAZ (5,6,7,8-tetrahydro-4*H*-isothiazolo[4,5-*d*]azepin-3-ol, **15**) was an antagonist at glycine receptors on cat spinal neurons. The *I*/*U* ratios, which reflect the ability of neutral amino acids to penetrate the blood–brain barrier (BBB), were calculated for **5** (*I*/*U* = 16), **10** (63), and **15** (200). These low *I*/*U* ratios, compared with the findings that THIP (*I*/*U* = 500 or 1500) and THPO (*I*/*U* = 2500) enter the brain after systemic administration, suggest that the thio analogues may penetrate the BBB very easily.

γ-Aminobutyric acid (GABA) is an inhibitory transmitter concerned with the control of neuronal activity in virtually all regions of the mammalian central nervous system,[1-3] and impaired transmission at such GABA-ergic synapses may be important in a number of neurological and psychiatric disorders.[4-6] As a consequence, com-

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