mL), 2 precipitated as a powder, which was collected by filtration (28 mg, 71%): UV (MeOH λ_{max} 277.0 nm (ϵ 3150); ^{1}H NMR (Me_2SO-d_6) δ 9.33 (1 H, s, H-2), 9.06 (1 H, s, H-6), 8.84 (1 H, m, H-4), 4.85 (1 H, d, H-1′, $J_{1',2'}$ = 6.70 Hz), 4.47 (3 H, s, Me), 3.93–3.22 (5 H, m, H-2′,3′,4′,5′,5″); MS, m/e 269 (M – iodide, 26), 255, [(M – CH_3I)H^+, 100].

Acknowledgment. This investigation was supported in part by funds from the National Cancer Institute, NIH, USDHHS (Grant No. CA-08748 and 33907).

Reactive 5′-Substituted 2′,5′-Dideoxyuridine Derivatives as Potential Inhibitors of Nucleotide Biosynthesis

Robert D. Elliott,* Pamela S. Pruett, R. Wallace Brockman, and John A. Montgomery

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35255. *Received September 2, 1986*

5′-(Bromoacetamido)-2′,5′-dideoxyuridine (3) and derivatives (8, 10, 12, and 14) substituted at the 5-position with bromo, iodo, fluoro, and ethyl groups have been synthesized as potential inhibitors of enzymes that metabolize pyrimidine nucleosides. Also prepared were 2′,5′-dideoxyuridine derivatives (4–6) substituted at the 5′-position with 2-bromopropionamido, iodoacetamido, and 4-(fluorosulfonyl)benzamido groups. Compounds 3, 5, 8, 12, and 14 were examined for effect on macromolecular synthesis in L1210 leukemia cells in culture and compared with 5′-(bromoacetamido)-5′-deoxythymidine (1, BAT), a compound with demonstrated cytotoxicity and activity in vivo against P388 murine leukemia. Compounds 3, 8, 12, and 14 inhibited DNA synthesis without significant inhibition of RNA synthesis, and protein synthesis was affected less than DNA synthesis. Compounds 3, 5, 6, 8, 10, 12, and 14 were cytotoxic to H.Ep.-2 and L1210 cells in culture, and 3, 5, 8, and 12 showed activity in the P388 mouse leukemia screen.

In the course of synthesizing and evaluating nucleosides containing reactive groups attached at C-5′ that can act as irreversible enzyme inhibitors,[1-6] major emphasis has been placed on the preparation of nucleosides closely related to 5′-(bromoacetamido)-5′-deoxythymidine (1, BAT), a compound that showed significant activity (71% ILS) in the P388 mouse leukemia screen.[6] A study of the effects of BAT on macromolecular synthesis in L1210 cells in culture showed that BAT did not inhibit intermediary metabolism of dThd to dTMP, dTDP, and dTTP but did selectively inhibit incorporation of pyrimidine nucleoside precursors into DNA.[4] BAT is also an effective inhibitor of thymidylate synthase from L1210 cells.[5] The bromoacetamide group of BAT has been replaced with a variety of other reactive groups,[6] but none of the 13 thymidine analogues prepared had better in vivo activity than BAT. The present paper describes the synthesis and evaluation of 2′,5′-dideoxyuridines with H, Et, Br, F, and I at the 5-position of the pyrimidine ring and several reactive groups, principally bromoacetamide, at the 5′-position of the sugar.

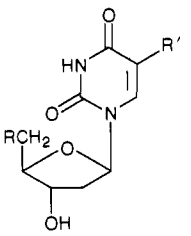
5′-Amino-2′,5′-dideoxyuridine (2) was prepared via the corresponding tosylate and azide[7] and selectively bromoacetylated on the amino group with 4-nitrophenyl bromoacetate[8] to give a 93% yield of the bromoacetamide 3. A similar reaction of 2 with 4-nitrophenyl 2-bromopropionate[6] gave an 89% yield of the bromopropionamide 4. The iodoacetamide 5 was synthesized by reaction of 2 with the activated ester *N*-(iodoacetoxy)succinimide.[9] The reported use of 4-(fluorosulfonyl)benzoyl derivatives of adenosine[10] and guanosine[11] as affinity labels suggested the preparation of the (fluorosulfonyl)benzamide 6. Reaction of 2 with 4-(fluorosulfonyl)benzoyl chloride in the presence of *N*,*N*-diisopropylethylamine gave an 85% yield of 6. Direct bromination of 2 in acetic acid gave 7·HBr, which was converted with aqueous NH_4OH to the free amine 7 in 52% yield. This procedure is an improvement over a previously described conversion of 2 to 7 via the 5-mercuriacetate.[12] Reaction of 7 with 4-nitrophenyl bromoacetate by the usual procedure gave an 85% yield of the bromoacetamide 8. The corresponding 5′-amino-2′,5′-dideoxy-5-iodouridine (9) was prepared from 2 via the 5′-mercuriacetate[12] and converted in 93% yield to the bromoacetamide 10. A similar reaction of 5′-amino-2′,5′-dideoxy-5-fluorouridine[13] (11) with 4-nitrophenyl bromoacetate gave a 63% yield of 12. 5′-Amino-2′,5′-dideoxy-5-ethyluridine (13) was prepared from 2′-deoxy-5-ethyluridine[14] via the 5′-tosyl and 5′-azido intermediates[15] and treated with 4-nitrophenyl bromoacetate to give a 94% yield of the bromoamide 14.

Biological and Biochemical Data. The cytotoxicities of these reactive nucleosides to H.Ep.-2[16] and L1210[17] cells in culture were determined (Table I) and compared with

(1) Montgomery, J. A.; Thomas, H. J.; Brockman, R. W.; Wheeler, G. P. *J. Med. Chem.* **1981**, *24*, 184.
(2) Elliott, R. D.; Brockman, R. W.; Montgomery, J. A. *J. Med. Chem.* **1981**, *24*, 350.
(3) Montgomery, J. A.; Thomas, H. J.; Brockman, R. W.; Elliott, R. D. *J. Med. Chem.* **1984**, *27*, 680.
(4) Brockman, R. W.; Shaddix, S. C.; Rose, L. M.; Elliott, R. D.; Montgomery, J. A. *Proc. Am. Assoc. Cancer Res.* **1984**, *25*, 1426.
(5) Sani, B. P.; Vaid, A.; Cory, J. G.; Brockman, R. W.; Elliott, R. D.; Montgomery, J. A. *Biochim. Biophys. Acta* **1986**, *881*, 175.
(6) Elliott, R. D.; Brockman, R. W.; Montgomery, J. A. *J. Med. Chem.* **1986**, *29*, 1052.
(7) Benz, E.; Elmore, N. F.; Goodman, L. *J. Org. Chem.* **1965**, *30*, 3067.
(8) Russell, D. W. *Biochem. J.* **1961**, *78*, 696.
(9) Hampton, A.; Slotin, L. A.; Chawla, R. R. *J. Med. Chem.* **1976**, *19*, 1279.
(10) Pal, P. K.; Wechter, W. J.; Colman, R. F. *J. Biol. Chem.* **1975**, *250*, 8140.
(11) Pal, P. K.; Reischer, R. J.; Wechter, W. J.; Colman, R. F. *J. Biol. Chem.* **1978**, *253*, 6644.
(12) Lin, T. S.; Neenan, J. P.; Cheng, Y. C.; Prusoff, W. H. *J. Med. Chem.* **1976**, *19*, 495.
(13) Lin, T. S.; Prusoff, W. H. *J. Med. Chem.* **1978**, *21*, 106.
(14) 2′-Deoxy-5-ethyluridine was kindly supplied by Dr. Don Ilse of Ortho Pharmaceutical (Canada) Ltd.
(15) Gauri, K. K.; Kiefer, G. German Patent 3 002 197, 1981.
(16) Bennett, L. L., Jr.; Allan, P. A.; Carpenter, J. W.; Hill, D. L. *Biochem. Pharmacol.* **1976**, *25*, 517.
(17) Thayer, P. W.; Himmelfarb, P.; Watts, G. L. *Cancer Chemother. Rep., Part 2* **1971**, *2*, 1.

0022-2623/87/1830-0927$01.50/0

Table I. Biological Activity of 5′-Substituted 2′-Deoxyuridines



no.	R	R′	cytotoxicity I_{50},[a] μM H.Ep.-2	cytotoxicity I_{50},[a] μM L1210	P388 in vivo dose,[b] mg/kg	P388 in vivo % ILS[c]
1	$BrCH_2CONH$	Me	8	14	100	71
2	NH_2	H				
3	$BrCH_2CONH$	H	11	14	100	57
4	$CH_3CHBrCONH$	H	>55	>100	100	5
5	ICH_2CONH	H	28	35	100	44
6	$4\text{-}(SO_2F)C_6H_4CONH$	H	10	36	200	6
7	NH_2	Br				
8	$BrCH_2CONH$	Br	1	6	50	28
9	NH_2	I				
10	$BrCH_2CONH$	I	2	6	50	21
11	NH_2	F				
12	$BrCH_2CONH$	F	16	5	100	33
13	NH_2	Et				
14	$BrCH_2CONH$	Et	1	9	200	17

[a] I_{50} is the concentration that produces 50% inhibition of proliferation of L1210 cells in suspension culture in 48 h or 50% inhibition of colony formation of H.Ep.-2 cells exposed to drug over a 12-day period relative to growth in the controls. [b] Single daily intraperitoneal dose on days 1–5 after implant of 10^6 P388 cells. [c] Percent increase in life span relative to untreated controls.

Table II. Comparison of Effects of Equimolar Concentrations (25 μM) of 5′-Haloacetamido Derivatives of 2′-Deoxyuridine on Macromolecular Synthesis in L1210 Cells in Culture

	incorporation of labeled substrate (% of control)[a]				
	DNA			RNA,	protein,
no.	[^{3}H]dThd[b]	[6-^{3}H]dUrd	[5-^{3}H]Urd[c]	[5-^{3}H]Urd	[4,5-^{3}H]-L-Leu
1	22	4	1	102	54
3	87	62	32	118	74
5	99	87	94	97	89
8	35	47	17	106	64
10	75	47	60	87	90
12	85	3	10	99	77
14	15	18	27	74	63

[a] For detailed results of a representative experiment, see Figure 1. Data are presented as percent of control after 4-h incubation of cells with radiolabeled substrates. [b] [C^3H_3]Thymidine. [c] [5-^{3}H]Urd is incorporated into DNA as [5-^{3}H]deoxycytidylate.

that of BAT. The compounds were also examined for activity against P388 leukemia in vivo.[18] Replacement of the 5-methyl group of BAT with hydrogen (**3**) resulted in no appreciable change in cytotoxicity and only slight reduction in the survival time in the P388 screen. Increasing the size of the 5-substituent to Et (**14**) did not significantly alter the inhibitory effect in tumor cells in culture but did result in loss of activity in vivo relative to compounds **1** and **3**. Replacement of the 5-Me of BAT with Br, I, or F (**8**, **10**, and **12**) resulted in retention of activity in cell culture with a decrease in activity against P388 leukemia in vivo. Replacement of the bromoacetamide of **3** with iodoacetamide (**5**) resulted in decreased activity both in cell culture and in vivo. Replacement of the bromoacetamido moiety with 4-(fluorosulfonyl)benzamide (**6**) or 2-bromopropionamide (**4**) resulted in loss of activity in vivo.

A study was made of the effects of selected 5′-haloacetamido derivatives of 2′-deoxyuridine on macromolecular synthesis in L1210 leukemia cells in culture. The method has been described;[19] representative results of effects of inhibitors on incorporation of precursors into macromolecules are presented in Figure 1. Results of a comparison of the effects of equimolar concentrations of a series of 5′-haloacetamido derivatives of 5-substituted 2′-deoxyuridines are summarized in Table II. The 5-methyl (**1**), 5-ethyl (**14**), 5-fluoro (**12**), and 5-bromo (**8**) derivatives were more inhibitory to incorporation of precursors into DNA than into RNA and protein. The 5-methyl, 5-ethyl, and 5-bromo derivatives at a concentration of 25 μM produced significant inhibition of incorporation of [^{3}H]dThd into DNA whereas the 5-F derivative (**12**), a strong inhibitor of incorporation of [6-^{3}H]dUrd and [5-^{3}H]Urd into DNA, did not inhibit significantly the incorporation of [^{3}H]dThd (Table I; Figure 1). The compound with no substituent on the pyrimidine moiety (**3**) was less inhibitory to Urd and dUrd incorporation and did not significantly inhibit dThd incorporation into DNA.

A specific inhibitor of dTMP synthase would be expected to inhibit incorporation of [6-^{3}H]dUrd into DNA without inhibition of incorporation of [^{3}H]dThd. Inhibition of incorporation of [5-^{3}H]Urd into DNA (as deoxycytidylate) can be inhibited as a consequence of inhibition

(18) Geran, R. I.; Greenberg, N. H.; Macdonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*, 9.

(19) Brockman, R. W.; Shaddix, S. C.; Williams, M.; Struck, R. F. *Cancer Treat. Rep.* **1976**, *60*, 1317.

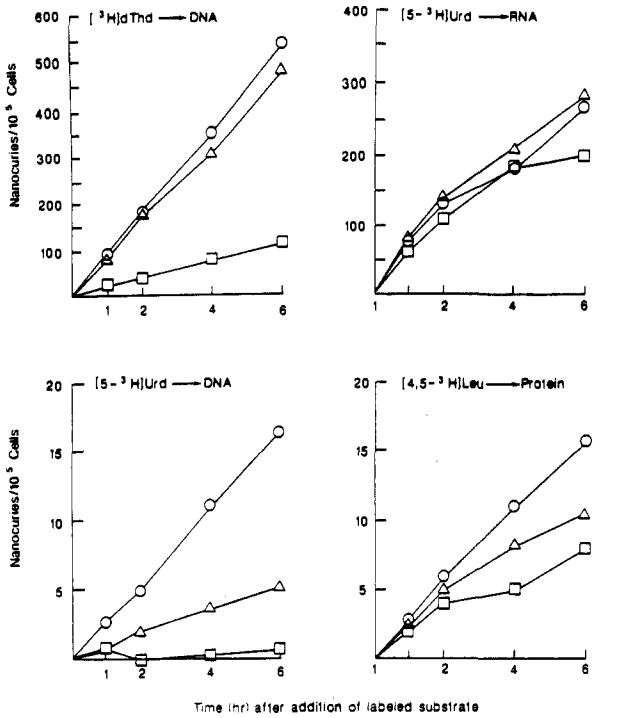


Figure 1. Comparison of the effects of equimolar concentrations (25 μM) of 1 and 12 on macromolecular synthesis in L1210 cells in culture. Inhibitors were added to L1210 cells in suspension culture 30 min prior to addition of labeled substrates. Samples were removed for analysis at the time periods indicated. Control (○); compound 1 (25 μM) (□); compound 12 (25 μM) (△).

of dTMP synthase with resultant deficiency in dTTP in treated cells. Compound **12** was an effective inhibitor of the incorporation of [6-^{3}H]dUrd into DNA but was less effective than **1** or **14** in inhibiting [^{3}H]dThd incorporation. This result suggests that **12** may be a more specific inhibitor of dTMP synthase in intact cells than **1** or **14**. Another possibility is that compound **12** is cleaved to 5-fluorouracil (5-FU), which, after its metabolism to FdUMP, specifically inhibits dTMP synthase. Results of an examination of the effects of 5-FU (5–25 μM) showed inhibition (60–93%) of [6-^{3}H]dUrd but an increase (12–21% above control) in [^{3}H]dThd incorporation into DNA. The apparent increase in incorporation of [^{3}H]dThd into DNA is attributable to a decreased intracellular dTMP pool with consequent increased specific activity of intracellular dTMP. It is evident from these results that **12** is not completely cleaved to 5-FU, but partial cleavage is not ruled out.

We observed that **1** did not inhibit the metabolism of [^{3}H]dThd to dTMP, dTDP, and dTTP but that it did inhibit [^{3}H]dThd incorporation into DNA.[4,6] However, **1** did not inhibit DNA polymerase isolated from L1210 cells (data not presented). Thymidine failed to prevent or reverse **1**-induced inhibition of proliferation of L1210 cells. Thus, **1** inhibits the process of DNA synthesis by some mechanism(s) in addition to its known inhibition of dTMP synthase.

Experimental Section

All evaporations were carried out in vacuo with a rotary evaporator or by short-path distillation into a dry ice–acetone cooled receiver under high vacuum. Analytical samples were normally dried in vacuo over P_2O_5 at room temperature for 16 h. Analtech precoated (250 μm) silica gel G(F) plates were used for TLC analyses; the spots were detected by irradiation with a Mineralight and by charring after spraying with saturated $(NH_4)_2SO_4$. Reactive halo and mesyl derivatives were also detected on TLC plates by spraying with 4-(nitrobenzyl)pyridine (NBP) reagent. All analytical samples were TLC homogeneous. Melting points were determined with a Kofler Heizbank apparatus unless otherwise specified. The UV absorption spectra were determined in 0.1 N HCl (pH 1), pH 7 buffer, and 0.1 N NaOH (pH 13) with a Cary 17 spectrophotometer: the maxima are reported in nanometers ($\epsilon \times 10^{-3}$). The NMR spectra of compounds **12** and **14** were determined with a Nicolet NMC NT-300NB spectrometer operating at 300.65 MHz in Me_2SO-d_6 with tetramethylsilane as an internal reference. NMR spectra of compounds **3–8** and **10** were determined with a Varian XL-100-15 spectrometer operating at 100.1 MHz. Chemical shifts (δ, in ppm) quoted in the case of multiplets are measured from the approximate center. The mass spectrum of **7** was obtained with a Varian-MAT 311A mass spectrometer in the electron-impact (EI) mode. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

5′-(Bromoacetamido)-2′,5′-dideoxyuridine (3). A solution of **2**[7] (227 mg, 1.00 mmol) in dimethylacetamide (DMAC; 15 mL) was cooled in an ice bath, treated with 4-nitrophenyl bromoacetate[8] (273 mg, 1.05 mmol), stirred at 25 °C for 30 min, and evaporated to a syrup at 25 °C under high vacuum. Trituration of the syrup with Et_2O (4 × 25 mL) gave a solid, which was collected, washed with Et_2O, and dried: yield 324 mg (93%); mp ca. 167 °C; UV (H_2O) λ_{max} ($\epsilon \times 10^{-3}$) in pH 1 and pH 7, 261 (8.32); in pH 13, 260 (6.41); ^{1}H NMR δ 2.13 (q, 2, $H_{2'}$), 3.35 (m, 2, $H_{5'}$), 3.77 (m, $H_{4'}$), 3.88 (s, 2, CH_2Br), 4.14 (m, 1, $H_{3'}$), 5.63 (q, 1, H_5), 6.13 (t, 1, $H_{1'}$), 7.66 (d, 1, H_6), 8.43 (m, 1, N_5H), 11.30 (s, 1, H_3). Anal. ($C_{11}H_{14}BrN_3O_5$) C, H, N.

5′-(2-Bromopropionamido)-2′,5′-dideoxyuridine (4). The title compound, mp 197 °C, was prepared in 89% yield from **2** (340 mg, 1.50 mmol) and 4-nitrophenyl 2-bromopropionate[6] (382 mg, 1.59 mmol) by the procedure described for the preparation of **3**. The final product was in addition washed with $CHCl_3$ (20 mL) before drying: UV (H_2O) λ_{max} ($\epsilon \times 10^{-3}$) in pH 1, 260 (9.14); in pH 7, 262 (9.04); in pH 13, 260 (6.82); ^{1}H NMR δ 1.66 (d, 3, CH_3), 2.11 (q, 2, $H_{2'}$), 3.34 (m, 2, $H_{5'}$), 3.76 (m, 1, $H_{4'}$), 4.14 (m, 1, $H_{3'}$), 4.56 (q, 1, CHBr), 5.31 (m, 1, $O_{3'}$H), 5.62 (d, 1, H_5), 6.12 (t, 1, $H_{1'}$), 7.65 (d, 1, H_6), 8.39 (m, 1, N_5H), 11.28 (s, 1, H_3). Anal. ($C_{12}H_{16}BrN_3O_5$) C, H, N.

2′,5′-Dideoxy-5′-(iodoacetamido)uridine (5). A solution of **2** (150 mg, 0.660 mmol) in DMAC (10 mL) was cooled in an ice bath, treated with *N*-(iodoacetoxy)succinimide[9] (196 mg, 0.693 mmol), stirred at 25 °C for 33 min, and evaporated to a syrup at 25 °C under high vacuum. A solution of the syrup in acetone (5 mL) was filtered and evaporated to a syrup, which was triturated with $CHCl_3$ (20 mL) to give a white powder, which was collected, washed with $CHCl_3$, and dried: yield 215 mg (82%); mp 174 °C dec; UV (H_2O) λ_{max} ($\epsilon \times 10^{-3}$) in pH 1 and pH 7, 261 (9.41); in pH 13, 261 (7.37); ^{1}H NMR δ 2.11 (q, 2, $H_{2'}$), 3.33 (m, $H_{5'}$), 3.66 (s, CH_2I), 3.72 (m, $H_{4'}$), 4.12 (m, 1, $H_{3'}$), 5.30 (m, 1, $O_{3'}$H), 5.64 (q, 1, H_5), 6.12 (t, 1, $H_{1'}$), 7.66 (d, 1, H_6), 8.40 (t, 1, N_5H), 11.30 (s, 1, H_3). Anal. ($C_{11}H_{14}IN_3O_5$) C, H, N.

2′,5′-Dideoxy-5′-(4-(fluorosulfonyl)benzamido)uridine (6). A stirred solution of **2** (200 mg, 0.881 mmol) and *N*,*N*-diisopropylethylamine (153 μL, 0.881 mmol) in DMAC (4 mL) was treated with 4-(fluorosulfonyl)benzoyl chloride (246 mg, 1.10 mmol), stirred for 4 h, and evaporated to dryness at 25 °C under high vacuum. The residue was triturated with Et_2O (3 × 10 mL) and then 0.1 N HCl (10 mL) to give a white powder, which was collected, washed with H_2O and then Et_2O, and dried: yield, 310 mg (85%); mp 225 °C dec; UV (EtOH) λ_{max} ($\epsilon \times 10^{-3}$) in pH 1, 226 (15.9), 255 (12.8); in pH 7, 226 (16.2), 255 (31.1); in pH 13, 228 (21.6), 265 sh (8.95); ^{1}H NMR δ 2.19 (m, 2, $H_{2'}$), 3.56 (m, 2, $H_{5'}$), 3.93 (m, 1, $H_{4'}$), 4.27 (m, 1, $H_{3'}$), 5.36 (m, 1, $O_{3'}$H), 5.65 (dd, 1, H_5), 6.15 (t, 1, $H_{1'}$), 7.73 (d, 1, H_6), 8.25 (m, 4, C_6H_4), 9.02 (t, 1, N_5H), 11.32 (s, 1, H_3). Anal. ($C_{16}H_{16}FN_3O_7S$) C, H, N.

5′-Amino-5-bromo-2′,5′-dideoxyuridine (7). A solution of **2** (600 mg, 2.64 mmol) in acetic acid (60 mL) at 10 °C was treated dropwise with a solution of Br_2 (149 μL, 2.90 mmol) in acetic acid (6 mL), stirred at 25 °C for 30 min, and refrigerated for 16 h. The solvent was removed at 25 °C in vacuo and the residue triturated with Et_2O (25 mL). A solution of the resulting solid in EtOH (17 mL) was filtered and diluted with Et_2O (175 mL) to give a precipitate of **7**·HBr. A solution of the dried salt (926 mg) in H_2O (5 mL) was filtered, treated with 14.8 N NH_4OH (0.4 mL), and

refrigerated. The white crystalline precipitate was collected, washed with a minimum of cold H_2O, and dried: yield 422 mg (52%); mp 220 °C dec (lit.[12] mp 189–191 °C dec); UV (H_2O) λ_{max} ($\epsilon \times 10^{-3}$) in pH 1, 277 (8.82); in pH 7, 276 (8.52); in pH 13, 276 (6.39); ^{1}H NMR δ 2.17 (m, $H_{1'}$), 2.76 (d, $H_{5'}$), 3.70 (m, $H_{4'}$), 4.19 (m, $H_{3'}$), 6.09 (t, $H_{1'}$), 8.45 (s, H_6); MS (EI), m/e 306 $(M + 1)^+$. Anal. ($C_9H_{12}BrN_3O_4$) C, H, N.

5-Bromo-5′-(bromoacetamido)-2′,5′-dideoxyuridine (8). The title compound, mp 190 °C dec, was prepared in 85% yield from **7** (300 mg, 0.980 mmol) by the procedure described for the preparation of **3**. The analytical sample required drying at 100 °C; UV (EtOH) λ_{max} ($\epsilon \times 10^{-3}$) in pH 1, 279 (8.62); in pH 7, 278 (8.43); in pH 13, 276 (5.77); ^{1}H NMR δ 2.17 (m, 2, $H_{2'}$), 3.38 (m, 2, $H_{5'}$), 3.77 (m, 1, $H_{4'}$), 3.88 (s, 2, CH_2Br), 4.14 (m, 1, $H_{3'}$), 5.30 (s, 1, $O_{3'}H$), 6.08 (t, 1, $H_{1'}$), 8.04 (s, 1, H_6), 8.45 (m, 1, N_5H), 11.83 (s, 1, H_3). Anal. ($C_{11}H_{13}Br_2N_3O_5$) C, H, N.

5′-(Bromoacetamido)-2′,5′-dideoxy-5-iodouridine (10). The title compound, mp 217 °C dec, was prepared in 93% yield from 5′-amino-2′,5′-dideoxy-5-iodouridine (**9**)[12] (155 mg, 0.424 mmol) by a modification of the procedure described for the preparation of **3**. A greater volume of DMAC (20 mL) and longer reaction time (80 min) were required for this reaction. The reaction mixture was filtered before evaporation; UV (EtOH) λ_{max} ($\epsilon \times 10^{-3}$) in pH 1, 287 (6.75); in pH 7, 287 (6.39); in pH 13, 278 (4.61); ^{1}H NMR δ 2.15 (m, 2, $H_{2'}$), 3.35 (m, $H_{5'}$), 3.75 (m, 1, $H_{4'}$), 3.89 (s, 2, CH_2Br), 4.13 (s, 1, $H_{3'}$), 5.30 (d, 1, $O_{3'}H$), 6.03 (t, 1, $H_{1'}$), 8.01 (s, 1, H_6), 8.44 (m, 1, N_5H), 11.68 (s, 1, H_3). Anal. ($C_{11}H_{13}BrIN_3O_5$) C, H, N.

5′-(Bromoacetamido)-2′,5′-dideoxy-5-fluorouridine (12). A solution of **11**[13] (270 mg, 1.10 mmol) in DMAC (5 mL) was treated with 4-nitrophenyl bromoacetate (300 mg, 1.16 mmol) and stirred at 25 °C for 40 min. The solution, containing unreacted **11** and bromoacetate (~40% by TLC), was treated with *N,N*-diisopropylethylamine (87.1 μL, 0.50 mmol), stirred for 30 min, and evaporated to dryness under high vacuum. The residue was triturated and stirred with Et_2O (2 × 10 mL) followed by $CHCl_3$ (2 × 20 mL) to give a homogeneous powder, which was collected, washed with $CHCl_3$, and dried: yield 252 mg (63%); melting point indefinite; UV (MeOH) λ_{max} ($\epsilon \times 10^{-3}$) in pH 1, 267 (8.75); in pH 7, 266 (10.20); in pH 13, 268 (6.81); ^{1}H NMR δ 2.14 (m, 2, $H_{2'}$), 3.36 (m, $H_{5'}$), 3.76 (m, 1, $H_{4'}$), 3.87 (s, 2, CH_2Br), 4.12 (m, 1, $H_{3'}$), 5.33 (m, 1, $O_{3'}H$), 6.10 (t, 1, $H_{1'}$), 7.98 (d, 1, J_{H_6,F_5} = 6.8 Hz, H_6), 8.47 (m, 1, N_5H). Anal. ($C_{11}H_{13}BrFN_3O_5$) C, H, N.

5′-(Bromoacetamido)-2′,5′-dideoxy-5-ethyluridine (14). A solution of **13**[15] (300 mg, 1.18 mmol) in DMAC (15 mL) was cooled in an ice bath, treated with 4-nitrophenyl bromoacetate (321 mg, 1.24 mmol), and stirred at 25 °C for 35 min. The resulting solution was evaporated to dryness at 25 °C under high vacuum and the residue triturated with Et_2O (3 × 30 mL), collected, washed with Et_2O, and dried: yield 416 mg (94%); mp 237 °C dec; UV (MeOH) λ_{max} ($\epsilon \times 10^{-3}$) in pH 1, 266 (9.80); in pH 7, 267 (9.46); in pH 13, 266 (7.18); ^{1}H NMR δ 1.05 (t, 3, CH_3), 2.12 (m, 2, $H_{2'}$), 2.26 (q, 2, CH_2CH_3), 3.34 (m, 2, $H_{5'}$), 3.76 (m, 1, $H_{4'}$), 3.38 (s, 2, CH_2Br), 4.15 (m, 1, $H_{3'}$), 5.32 (d, 1, $O_{3'}H$), 6.15 (t, 1, $H_{1'}$), 7.38 (s, 1, H_6), 8.45 (t, 1, N_5H), 11.28 (s, 1, H_3). Anal. ($C_{13}H_{18}BrN_3O_5$).

Acknowledgment. This investigation was supported by Grant CA23173 awarded by the National Cancer Institute, National Institutes of Health. We are indebted to Dr. W. R. Laster, Jr., for the in vivo screening data, to D. J. Adamson for H.Ep.-2 and L1210 data, and to Dr. W. C. Coburn, Jr., and other members of the Molecular Spectroscopy Section of Southern Research Institute who performed the microanalytical and spectral determinations.

Registry No. 1, 50700-63-5; **2**, 35959-38-7; **3**, 106928-99-8; 4, 106929-00-4; **5**, 106929-01-5; **6**, 106929-02-6; **7**, 58349-32-9; **7**·Br, 106929-07-1; **8**, 106929-03-7; **9**, 56045-73-9; **10**, 106929-04-8; 11, 56512-26-6; **12**, 106929-05-9; **13**, 90760-95-5; 14, 106929-06-0; 4-nitrophenyl bromoacetate, 19199-82-7; 4-nitrophenyl 2-bromopropionate, 56985-87-6; *N*-(iodoacetoxy)succinimide, 39028-27-8; 4-(fluorosulfonyl)benzoyl chloride, 402-55-1.

N-Methyl Derivatives of the 5-HT_2 Agonist 1-(4-Bromo-2,5-dimethoxyphenyl)-2-aminopropane

Richard A. Glennon,*† Milt Titeler,‡ Mark R. Seggel,† and Robert A. Lyon‡

Department of Medicinal Chemistry, School of Pharmacy, Medical College of Virginia/Virginia Commonwealth University, Richmond, Virginia 23298, and Department of Pharmacology and Toxicology, Albany Medical College, Albany, New York 12206. *Received September 15, 1986*

1-(4-Bromo-2,5-dimethoxyphenyl)-2-aminopropane (DOB) is a serotonin (5-HT) agonist that displays a high affinity and selectivity for a certain population of central 5-HT binding sites (i.e., 5-HT_2 sites). In the present study, (a) an enantiomeric potency comparison was made for the optical isomers of DOB and (b) the activity of *N*-monomethyl-, *N,N*-dimethyl-, and *N,N,N*-trimethyl-DOB was examined. (*R*)-(−)-DOB (K_i = 0.39 nM) was found to have 6 times greater affinity than its *S*-(+) enantiomer at [^{3}H]DOB-labeled (rat cortical homogenates) 5-HT_2 sites; N-methylation of racemic DOB resulted in a decrease in affinity that was at least 1 order of magnitude per methyl group. Similar results were obtained in an in vivo drug discrimination paradigm with rats as subjects and (*R*)-(−)-DOB (0.2 mg/kg) as the training drug. Thus, the *R*-(−) isomer of DOB is more active than its *S*-(+) enantiomer and than any of the possible *N*-methyl derivatives of DOB, both with respect to affinity at central 5-HT_2 binding sites and with respect to potency in the behavioral (i.e., stimulus generalization) studies.

We have recently demonstrated that certain 4-substituted derivatives of 1-(2,5-dimethoxyphenyl)-2-aminopropane are serotonin (5-HT) agonists with a high affinity and selectivity for a particular population of central 5-HT binding sites (i.e., 5-HT_2 sites).[1-4] Amongst the most potent and selective of these agents is 1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane (DOB; **1**),[5] which may now be considered a prototypic 5-HT_2 agonist. More recently, we have introduced [^{3}H]DOB as a radioligand for selectively labeling these sites.[6] To date, an enantiomeric

† Medical College of Virginia/Virginia Commonwealth University.
‡ Albany Medical College.

(1) Glennon, R. A.; Young, R.; Rosecrans, J. A. *Eur. J. Pharmacol.* **1983**, *91*, 189.
(2) Glennon, R. A.; Hauck, A. E. *Pharmacol. Biochem. Behav.* **1985**, *23*, 937.
(3) Shannon, M.; Battaglia, G.; Glennon, R. A.; Titeler, M. *Eur. J. Pharmacol.* **1984**, *102*, 23.
(4) Glennon, R. A.; Titeler, M.; McKenney, J. D. *Life Sci.* **1984**, *35*, 2505.
(5) Glennon, R. A.; McKenney, J. D.; Lyon, R. A.; Titeler, M. *J. Med. Chem.* **1986**, *29*, 194.

0022-2623/87/1830-0930$01.50/0