HOH·HCl, 4229-44-1; $(MeO)_3CH$, 149-73-5; O_2NCH_3, 75-52-5; $EtOCH{=}C(CN)_2$, 123-06-8; $Me_2NCH(OMe)_2$, 4637-24-5; $NCCH_2CN$, 109-77-3; $EtOCH{=}C(Me)CHO$, 42588-57-8; $BrCH_2CO_2Me$, 96-32-2; $Me_2NCH_2CO_2Me$, 7148-06-3; $Me_2NCH_2CO_2H$·HCl, 2491-06-7; $O_2NCH_2CH{=}NOH$, 5653-21-4; cyclopropylamine, 765-30-0; morpholine, 110-91-8.

Supplementary Material Available: Table IV, antitumor data for inactive analogues of *N*-methylformamide against the TLX5 lymphoma in mice. Table V, antitumor data for inactive analogues of *N*-methylformamide against the M5076 reticulum cell sarcoma in mice (2 pages). Ordering information is given on any current masthead page.

Reactive 5′-Substituted Thymidine Derivatives as Potential Inhibitors of Nucleotide Biosynthesis

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Fourteen derivatives of thymidine substituted at the 5′-position with haloacetamido (**2**–**4**), 2- and 3-bromopropionamido (**5** and **6**), bromoacetoxy (**7**), *O*-mesylglycolamido (**8**), bromo- and chloro-*N*-methylacetamido (**10** and **11**), bromomethanesulfonamido (**12**), ethyloxamido (**13**), 4- and 3-(fluorosulfonyl)benzamido (**14** and **15**), and (phenoxycarbonyl)amino (**16**) groups have been synthesized and evaluated as potential inhibitors of enzymes that metabolize purine and pyrimidine nucleosides. Rates of reaction of these nucleosides with mercaptoethanol at pH 7 were compared and related to biological activity. Compounds **2**, **3**, and **7** were cytotoxic to H.Ep.-2 and L1210 cells in culture and 5′-(bromo- and iodoacetamido)-5′-deoxythymidine (**2** and **3**) showed good activity against P388 leukemia in mice.

A current research program in this laboratory involves the preparation of nucleosides containing chemically reactive groups attached to C-5′ that may act as irreversible inhibitors of enzymes that act on the corresponding nucleotides.[1-5] The rationale for this work has been described in a previous paper.[1] During the course of investigating a variety of reactive groups at this position, including nitrosoureido, α-bromoacetamido, (phenoxycarbonyl)amino, (fluorosulfonyl)benzamido, and (halomethyl)keto, the bromoacetamido substituent was one of the groups found to impart significant toxicity.[2,4] The α-haloacyl group is of particular interest since it has a broad scope of reaction with enzyme nucleophilic groups, being capable of reacting with about half of the possible enzyme amino acids having a third functional group.[6,7] The 5′-deoxy-5′-(haloacetamido)thymidines **2**, **3**, and **4** have been prepared,[5] and the bromo amide (**2**, BAT) has been found to be cytotoxic to H.Ep.-2 and L1210 cells in culture and produced 71% ILS in the P388 mouse leukemia screen.[4] BAT has also been found to be an irreversible inhibitor of thymidylate synthase purified from L1210 cells.[5] The inhibitory effects of these halo amides is in the order BAT > **3** > **4**, which corresponds to their cytotoxic effects in L1210 cells. This paper describes the synthesis and evaluation of other BAT analogues with variations in the reactive group at the 5′-position.

The successful preparation of the haloacetamides **2** and **4** from **1** and the corresponding 4-nitrophenyl haloacetates[5] suggested a similar approach for the synthesis of the 2-bromopropionamide **5**. Acylation of the sodium salt of 4-nitrophenol with 2-bromopropionyl chloride gave 4-nitrophenyl 2-bromopropionate (**17**)[8] which was used to selectively acylate the amino group of **1**[9] prepared from 5′-*O*-tosylthymidine[10] to give an 88% yield of pure **5**. Synthesis of the 3-bromopropionamide derivative **6** was prompted by the reported ability of 3-bromopropionic acid to act as an enzyme-alkylating agent.[11,12] The activated ester *N*-[(3-bromopropionyl)oxy]succinimide (**18**) was prepared in 72% yield from *N*-hydroxysuccinimide, 3-bromopropionic acid, and DCC. This ester was not, however, sufficiently activated to cleanly acylate the amino group of **1** and give an acceptable yield of **6**. A successful synthesis of **6** was achieved by careful addition of 3-bromopropionyl chloride at −20 °C to a solution of **1** and *N*,*N*-diethylaniline in CH_2Cl_2. The 5′-*O*-bromoacetate **7** was considered as a desirable candidate for screening because its spatial requirements are almost identical with those of BAT. The selective acylation of the 5′-OH of thymidine with bromoacetyl bromide was carried out by a procedure previously described[13] to give a 26% yield of the ester **7**. The presence of methanesulfonate groups in the clinically useful anticancer agent busulfan[14] suggested replacement of the bromine of BAT with the methanesulfonate group. This replacement was carried out by heating a mixture of BAT with silver methanesulfonate

(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. *Biochem. Biophys. Acta*, in press.
(6) Baker, B. R.; Santi, D. V.; Coward, J. R.; Shapiro, H. S.; Jordaan, J. H. *J. Heterocycl. Chem.* **1966**, *3*, 425 and included references.
(7) Vallee, B. L.; Riordan, J. F. *Annu. Rev. Biochem.* **1969**, *38*, 733.
(8) Bischoff, C. A. *Chem. Ber.* **1906**, *39*, 3854.
(9) Horwitz, J. P.; Tomson, A. J.; Urbanski, J. A.; Chua, J. *J. Org. Chem.* **1962**, *27*, 3045.
(10) Reist, E. J.; Benitez, A.; Goodman, L. *J. Org. Chem.* **1964**, *29*, 554.
(11) Okamoto, M.; Morino, Y. *Biochemistry* **1972**, *11*, 3188, 3196.
(12) Harada, M.; Irie, M. *J. Biochem. (Tokyo)* **1973**, *73*, 705.
(13) Agarwal, K. L.; Dhar, M. M. *Experientia* **1965**, *21*(8), 432.
(14) Haddow, A.; Timmis, G. M. *Lancet* **1953**, *264*, 207.

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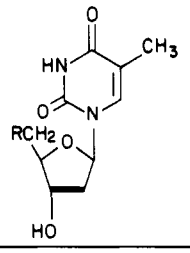
Table I. Chemical Reactivity and Biological Activity



	R	~$T_{1/2}$[a]	cytotoxicity I_{50},[b] μM H.Ep.-2	cytotoxicity I_{50},[b] μM L1210	P388 in vivo dose, mg/kg[c]	P388 in vivo % ILS[d]
1	NH_2					
2	$BrCH_2CONH$	6 min	8	14	100	71
3	ICH_2CONH	<60 s	8	16	50	34
4	$ClCH_2CONH$	3 h	25	130	200	9
5	$CH_3CHBrCONH$	4 h	>50	>110	200	5
6	$BrCH_2CH_2CONH$	16 h	>50	>100	200	0
7	$BrCH_2CO_2$	<30 s	3	6	50	17
8	$CH_3SO_3CH_2CONH$	55 min		>100		
9	CH_3NH					
10	$BrCH_2CON(CH_3)$	<40 s		120		
11	$ClCH_2CON(CH_3)$	9 min		>120		
12	$BrCH_2SO_2NH$	>30 days	>50	>100		
13	EtO_2CCONH	31 min	>60	>120		
14	4-$(SO_2F)C_6H_4CONH$	5 min		>90	100	15[e]
15	3-$(SO_2F)C_6H_4CONH$	10 min	>50	>90	100	0
16	PhOCONH	9 days		>110		
	$BrCH_2CONH_2$	5 min		1	10	25

[a] $T^{1/2}$ = half-life as estimated by TLC of a 0.25–0.5% solution of compound in MeOH mixed 1:1 with 1% mercaptoethanol in pH 7 buffer; loss of alkylating activity was determined with 4-(4-nitrobenzyl)pyridine spray. [b] I_{50} is the concentration that produces 50% inhibition of cloning of H.Ep.-2 cells over a 12-day period relative to growth in the controls. [c] Single injection on days 1–5. [d] Percent increase in life span relative to untreated controls. [e] Single injection on days 1, 5, and 9.

in sulfolane to give a 57% yield of the mesyl derivative **8**.

The effect of placing an alkyl substituent on the amide nitrogen of BAT was demonstrated by synthesis and evaluation of the *N*-methylhaloacetamides **10** and **11**. 5'-Deoxy-5'-(methylamino)thymidine (**9**) was prepared in near theoretical yield by treatment of 5'-*O*-tosylthymidine[10] with methylamine at 30 °C. Acylation of the methylamino group of **9** with 4-nitrophenyl bromoacetate was much less selective than in the acylation of **1** and resulted in a low yield (14%) of isolated **10**. Acylation of **9** with the more reactive chloroacetyl chloride gave a somewhat better yield (40%) of the chloroacetamide **11**.

Replacement of the carboxamide group of BAT with a sulfonamide group gave compound **12** which is similar to BAT in physical dimensions but chemically quite different since the bromine of **12** is extremely resistant to nucleophilic displacement.[15] This sulfonamide was prepared in 55% yield by acylation of **1** with bromomethanesulfonyl chloride.[16] The ethyl oxamate **13** was prepared in 89% yield from **1** and diethyl oxalate. This type of activated ester is known to react readily with amines under mild conditions.[17] The reported use of 5'-*O*-[4-(fluorosulfonyl)benzoyl]adenosine[18] and 5'-*O*-[4-(fluorosulfonyl)benzoyl]guanosine[19] as affinity labels for catalytic sites of enzymes suggested the preparation of the (fluorosulfonyl)benzamides **14** and **15**. Reaction of **1** with 4- and 3-(fluorosulfonyl)benzoyl chloride gave respectively 88% and 85% yields of **14** and **15**. A similar reaction of **1** with phenyl chloroformate gave the phenylurethane **16**.

The stability of these reactive nucleosides in pH 7 buffer containing 0.5% mercaptoethanol was examined to determine the relationship, if any, to biological activity (Table I). A similar study of the stability of nitrosoureas in pH 7 buffer indicated that reactivity could frequently be correlated directly with biological activity.[1] The inhibitory activity of α-haloacyl compounds might be expected to be more closely related to their ability to react with thiols or sulfides as in cysteine or methionine.[7] The half-lives were determined by TLC using 4-(4-nitrobenzyl)pyridine spray to follow loss of alkylating activity.

The half-lives varied from <1 min for the iodoacetamide **3**, the bromoacetate ester **7**, and *N*-methyl BAT **10** to >30 days for the bromomethanesulfonamide (**12**). Although the data in Table I shows no close correlation between reactivity and biological activity, the biologically active compounds tested have half-lives in the range of <30 s to 6 min. The most active compound in the P388 murine leukemia screen[20] is BAT with 71% ILS and a half-life of 6 min, the most stable of the biologically active compounds. Bromoacetamide with a similar half-life of 5 min and the bromo ester **7** with a half-life of <30 s were more cytotoxic than BAT to H.Ep.-2[21] and L1210[22] cells in culture but were toxic and inactive in vivo. The high cytotoxicity and low in vivo activity of **7** can be explained by in vivo hydrolysis of the ester of **7** to highly cytotoxic bromoacetic acid (I_{50} is 4 μM in H.Ep.-2 and 6 μM in L1210). Increasing the half-life to 9 min (compound **11**) resulted in loss of

(15) Bordwell, F. G.; Jarvis, B. B. *J. Org. Chem.* **1968**, *33*, 1182.
(16) Truce, W. E.; Abraham, D. J.; Son, P. *J. Org. Chem.* **1967**, *32*, 990.
(17) Elliott, R. D.; Johnston, T. P. *J. Med. Chem.* **1969**, *12*, 507.
(18) Pal, P. K.; Wechter, W. J.; Colman, R. F. *J. Biol. Chem.* **1975**, *250*, 8140.
(19) Pal, P. K.; Reischer, R. J.; Wechter, W. J.; Colman, R. F. *J. Biol. Chem.* **1978**, *253*, 6644.
(20) Geran, R. I.; Greenberg, N. H.; Macdonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*, 9.
(21) Bennett, L. L., Jr.; Allan, P. A.; Carpenter, J. W.; Hill, D. L. *Biochem. Pharmacol.* **1976**, *25*, 517.
(22) Thayer, P. W.; Himmelfarb, P.; Watts, G. L. *Cancer Chemother. Rep., Part 2* **1971**, *2*, 1.

Table II. Effect of BAT on Incorporation of Nucleoside Precursors into DNA and RNA in L1210 Cells in Culture

	percent incorporation[a]		
precursor	DNA	RNA	protein
[C^3H_3]dThd	22	...	
[6-^{3}H]dUrd	4	...	
[5-^{3}H]Urd[b]	1	102	
[5-^{3}H]Cyd[b]	15	91	
[8-^{14}C]Ade	14	44	
[8-^{14}C]Hyp	11	65	
[4,5-^{3}H]L-Leu[c]			54

[a] Incorporation of labeled precursors into RNA, DNA, and protein is measured as nCi/10^5 cells and expressed as percent of control. [b] Incorporated into DNA as [5-^{3}H]deoxycytidylate. [c] Incorporated into protein of TCA-insoluble fraction.

activity. Addition of a methyl group to the amide nitrogen of BAT resulted in a >10-fold increase in reactivity and a >8-fold decrease in cytotoxicity. Replacement of the bromine of BAT with iodine gave increased reactivity with no appreciable change in cytotoxicity and some loss in P388 in vivo activity. All of the other modifications in BAT resulted in loss of biological activity.

In addition to the cytotoxicity data and in vivo P388 data shown in Table I, BAT was also found to give an 81% ILS in a methotrexate resistant strain of P388, a 59% ILS in a 5-fluorouracil resistant strain of P388, and a 73% ILS in an *ara*-C resistant strain of P388,[23] all at a dose of 100 mg/kg (qd 1–5). The lack of cross resistance of these cell lines to BAT indicate a different mechanism of action from methotrexate, 5-fluorouracil, or *ara*-C.

The mechanism of action of BAT was investigated by determining the extent to which it inhibited the incorporation of labeled nucleoside precursors into DNA and RNA.[24] It is evident from results summarized in Table II that at 25-nm concentration, BAT was an effective inhibitor of incorporation of precursors into DNA. In the same experiments, BAT did not significantly inhibit incorporation of labeled uridine or cytidine into RNA. Incorporation of purines into DNA was inhibited to a greater extent than was their incorporation into RNA, and incorporation of [^{3}H]leucine into protein was 50% inhibited under these conditions. These results led us to focus on effects of BAT on DNA synthesis. Ribonucleotide reductase activity of L1210 cells was inhibited only at high concentrations of inhibitor, and DNA polymerase activity from L1210 cells was not significantly inhibited. However, thymidylate synthase activity in intact L1210 cells was inhibited, and evidence was obtained for irreversible inactivation of the isolated enzyme.[5] No inhibition of metabolism of thymidine to dTMP, dTDP, or dTTP was observed, but incorporation of thymidine into DNA was inhibited by BAT, as already noted. The mechanism for this inhibition of thymidine incorporation into DNA in the absence of inhibition of DNA polymerase is not yet understood. It is possible that the cell-free enzyme is not inhibited whereas polymerase activity is inhibited in the intact cell or that there may be a yet unrecognized site of inhibition of DNA synthesis by this agent.

Experimental Section

All evaporations were carried out in vacuo with a rotary evaporatory 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-(4-nitrobenzyl)pyridine (NBP) reagent. All analytical samples were TLC homogeneous. Melting points were determined with a Kofler Heizbank apparatus unless otherwise specified. Purifications by "flash chromatography"[25] were carried out on Merck silica gel 60 (230–400 mesh) using the slurry method of column packing. 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 ($\epsilon \times 10^{-3}$ M^{-1} cm^{-1}). The NMR spectra of compounds 1–4, 8–11, and 15 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 5–7, 12–14, and 16 were determined with a Varian XL-100-15 spectrometer operating at 100.1 MHz. Chemical shifts (δ) quoted in the case of multiplets are measured from the approximate center. The mass spectral data were obtained with a Varian-MAT 311A mass spectrometer in the field desorption (FD), fast atom bombardment (FAB), or 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. Compound 12 was purified on a Model 7924 Chromatatron, Harrison Research, Palo Alto, CA.

5′-(2-Bromopropionamido)-5′-deoxythymidine (5). A solution of 1[9] (241 mg, 1.00 mmol) prepared from 5′-*O*-tosylthymidine[10] in DMAC (15 mL) was cooled in an ice bath, treated with 17 (252 mg, 1.05 mmol), and stirred at 25 °C for 35 min. The solution was evaporated to dryness under high vacuum at 25 °C and the residue triturated with Et_2O (4 × 25 mL). The crystalline product was collected by filtration and washed with Et_2O: yield 330 mg (88%); mp 230 °C dec; UV (H_2O) λ_{max} ($\epsilon \times 10^{-3}$ M^{-1} cm^{-1}), in pH 1, 267 nm (9.65), in pH 7, 267 (9.94), in pH 13, 267 (7.67); ^{1}H NMR δ 1.67 (d, 3, CH_3CBr), 1.82 (s, 3, CH_3), 2.11 (m, 2, $H_{2'}$), 3.37 (m, 2, $H_{5'}$), 3.78 (m, 1, $H_{4'}$), 4.17 (m, 1, $H_{3'}$), 4.57 (m, 1, CHBr), 5.32 (m, 1, $O_{3'}H$), 6.16 (t, 1, $H_{1'}$), 7.48 (s, 1, H_6), 8.42 (t, 1, $NHCH_2$), 11.11 (s, 1, H_3). Anal. ($C_{13}H_{18}BrN_3O_5$) C, H, N.

5′-(3-Bromopropionamido)-5′-deoxythymidine (6). A solution of 3-bromopropionyl chloride (150 μL, 1.49 mmol) in anhydrous CH_2Cl_2 (6 mL) was added dropwise over a period of 15 min to a stirred solution of 1 (300 mg, 1.25 mmol) and *N*,*N*-diethylaniline (237 μL, 1.49 mmol) in anhydrous DMAC (18 mL) at −20 °C under N_2. The solution was stirred at 25 °C for 40 min and evaporated to dryness under high vacuum at 25 °C. The residue after stirring with $CHCl_3$ (36 mL) and refrigeration gave a solid which was collected and washed with cold $CHCl_3$: yield 413 mg (83%); mp ca. 215 °C dec; UV (H_2O) λ_{max} ($\epsilon \times 10^{-3}$ M^{-1} cm^{-1}), in pH 1 and pH 7, 266 nm (10.3), in pH 13, 265 (8.1); ^{1}H NMR δ 1.82 (s, 3, CH_3), 2.09 (m, 2, $H_{2'}$), 2.7 (q, 2, CH_2CO), 3.36 (m, 2, $H_{5'}$), 3.70 (m, 3, $BrCH_2$, $H_{4'}$), 4.17 (m, 1, $H_{3'}$), 6.15 (t, 1, $H_{1'}$), 7.49 (s, 1, H_6), 8.19 (t, 1, $NHCH_2$), 11.28 (s, 1, H_3), 1.97, 2.95 (DMAC). Anal. ($C_{13}H_{18}BrN_3O_5 \cdot 0.25Me_2NCHO$) C, H, N.

5′-*O*-(Bromoacetyl)thymidine (7). A stirred suspension of thymidine (1.00 g, 4.12 mmol) and pyridine (0.8 mL) in anhydrous MeCN (160 mL) was cooled in an ice bath, treated dropwise over a period of 45 min with a solution of bromoacetyl bromide (0.576 mL, 6.61 mmol) in MeCN (40 mL), and stirred at 25 °C for 2 h. The solution was evaporated at 25 °C in vacuo to give a syrup which was triturated with Et_2O (2 × 100 mL). A solution of the resulting solid in MeOH was applied to 8 Brinkman 2-mm silica gel F-254 plates (8 × 8 in.) and developed with $CHCl_3$–MeOH (9:1). The major band at R_f 0.6 was extracted with EtOH (300 mL) at 25 °C and the extract evaporated to dryness in vacuo. A solution of the residue in EtOH (20 mL) was filtered and refrigerated. The crystalline 7 was collected and washed with cold EtOH: yield 388 mg (26%); mp 165 °C (lit.[13] mp 159–160 °C); UV (EtOH) λ_{max} ($\epsilon \times 10^{-3}$ M^{-1} cm^{-1}), in pH 1, 266 nm (9.61), in pH 7, 266 (9.45), in pH 13, 266 (7.30); ^{1}H NMR δ 1.81 (s, 3, CH_3), 2.16 (m, 2, $H_{2'}$), 3.94 (m, 1, $H_{4'}$), 4.21 (s, CH_2Br), 4.27 (m, $H_{3',5'}$),

(23) Schabel, F. M., Jr.; Skipper, H. E.; Trader, M. W.; Laster, W. R., Jr.; Griswold, D. P., Jr.; Corbett, T. H. *Cancer Treat. Rep.* **1983**, *67* (No. 10), 905; **1984**, *68* (No. 2), 453.
(24) Brockman, R. W.; Shaddix, S. C.; Williams, M.; Struck, R. F. *Cancer Treat. Rep.* **1976**, *60*, 1317.
(25) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

5.41 (m, 1, $O_{3'}H$), 6.20 (t, 1, $H_{1'}$), 7.43 (m, 1, H_6), 11.30 (s, 1, H_3). Anal. ($C_{12}H_{15}BrN_2O_6$) C, H, N.

5'-Deoxy-5'-(*O*-mesylglycolamido)thymidine (8). A mixture of **2**[5] (362 mg, 1.00 mmol) and silver methanesulfonate (1.22 g, 6.00 mmol) in sulfolane (15 mL) was heated in an oil bath at 78 °C for 1 week, filtered hot, diluted with toluene (50 mL), and refrigerated. The supernatant was decanted from a brown gum, diluted with toluene (44 mL), and refrigerated to give a solid which was collected, dried, triturated with EtOH (1 mL), collected, and washed with EtOH: yield 186 mg (47%), mp 174 °C. Elemental analysis and ^1H NMR indicated a partial solvate with sulfolane and H_2O: UV (MeOH) λ_{max} ($\epsilon \times 10^{-3}$ M^{-1} cm^{-1}), in pH 1 and pH 7, 266 nm (9.92), in pH 13, 266 (7.48); ^1H NMR δ 1.81 (s, 3, CH_3C), 2.08, 3.00 (m, t, sulfolane), 3.26 (s, 3, CH_3S), 3.40 (m, 2, CH_2N), 3.79 (m, 1, $H_{4'}$), 4.18 (m, 1, $H_{3'}$), 4.68 (s, 2, CH_2O), 6.16 (t, 1, $H_{1'}$), 7.50 (s, 1, H_6), 8.35 (t, 1, NH), 11.29 (s, 1, H_3); MS (FD), *m/e* 378 ($(M + 1)^+$). Anal. ($C_{13}H_{19}N_3O_8S \cdot 0.15C_4H_8O_2S \cdot 0.1H_2O$) C, H, N.

5'-Deoxy-5'-(methylamino)thymidine (9). A solution of 5'-*O*-tosylthymidine[10] (2.03 g, 5.13 mmol) in liquid CH_3NH_2 (30 mL) was stirred in a glass-lined stainless steel bomb at 30 °C for 64 h and the excess CH_3NH_2 allowed to volatilize off. A solution of the residue in a minumum of 20:10:1 $CHCl_3$–MeOH–NH_4OH was applied to a flash column (silica gel, 125 g) and eluted with the same solvent. The product fraction containing some *p*-toluenesulfonic acid was further purified as above on 80 g of silica gel using MeOH as the solvent. The product fraction was evaporated under high vacuum to a chromatographically pure foam: yield 1.67 g (99%); UV (MeOH) λ_{max} ($\epsilon \times 10^{-3}$ M^{-1} cm^{-1}), in pH 1, 265 nm (9.74), in pH 7, 265 (9.87), in pH 13, 266 (7.67); ^1H NMR δ 1.78 (s, 3, CCH_3), 2.0–2.2 (m, 2, $H_{2'}$), 2.32 (s, 3, NCH_3), 2.68 (d, 2, NCH_2), 3.17 (s, CH_3 of MeOH), 3.78 (m, 1, $H_{4'}$), 4.17 (m, 1, $H_{3'}$), 6.13 (t, 1, $H_{1'}$), 7.64 (s, 1, H_6); MS (FD), *m/e* 256 ($(M + 1)^+$). Anal. ($C_{11}H_{17}N_3O_4 \cdot 0.5CH_3OH$) C, H, N.

5'-(Bromo-*N*-methylacetamido)-5'-deoxythymidine (10). A solution of **9** (255 mg, 1.00 mmol) in DMAC (10 mL) was cooled in an ice bath, treated with 4-nitrophenyl bromoacetate[26] (273 mg, 1.05 mmol) and stirred at 25 °C for 16 h. The reaction mixture was evaporated at 25 °C in vacuo and the residue triturated with Et_2O (2 × 20 mL), dissolved in a minimum of $CHCl_3$–MeOH (6:1), applied to a flash column (silica gel, 100 g), and developed with the same solvent to give 75 mg of an NBP positive product which was further purified on 25 g of silica gel as above to give, after trituration of the evaporated product fraction with Et_2O, pure **10**: yield 51 mg (14%); mp 199 °C; UV (MeOH) λ_{max} ($\epsilon \times 10^{-3}$ M^{-1} cm^{-1}), in pH 1 and pH 7, 265 nm (10.0), in pH 13, 265 (7.52); ^1H NMR δ 1.80, 1.81 (s, s, 3, CCH_3), 2.0–2.4 (m, 2, $H_{2'}$), 2.87, 3.05 (s, s, 3, NCH_3), 3.55 (m, 2, NCH_2), 3.90 (m, 1, $H_{4'}$), 4.13 (m, $H_{3'}$), 4.15 (s, 2, CH_2Br), 6.16 (m, 1, $H_{1'}$), 7.53 (s, 1, H_6), 11.29, 11.32 (s, s, 1, H_3); MS (FD), *m/e* 376 ($(M + 1)^+$). Anal. ($C_{13}H_{18}BrN_3O_5$) C, H, N.

5'-(Chloro-*N*-methylacetamido)-5'-deoxythymidine (11). A solution of **9** (300 mg, 1.18 mmol) and *N,N*-diisopropylethylamine (216 μL, 1.24 mmol) in DMAC (10 mL) at 0 °C was treated in small portions (microsyringe) with chloroacetyl chloride (98.8 μL, 1.24 mmol) and stirred at 25 °C for 70 min. The solution was evaporated in vacuo to a syrup which was triturated with Et_2O (2 × 10 mL) to give a semisolid which was dissolved in a minimum of $CHCl_3$–MeOH (1:1), applied to a flash column (silica gel, 25 g), and developed with $CHCl_3$–MeOH (7:1). The major NBP positive fraction was evaporated and the residue triturated with Et_2O to give a semisolid (273 mg) which was further purified as above on a second column (25 g) to give, after trituration of the evaporated product fraction with Et_2O, a crystalline solid: yield after drying at 56 °C, 157 mg (40%); mp ~171 °C; UV (MeOH) λ_{max} ($\epsilon \times 10^{-3}$ M^{-1} cm^{-1}), in pH 1 and pH 7, 265 nm (9.53), in pH 13, 266 (7.26); ^1H NMR δ 1.81, 1.82 (s, s, 3, CCH_3), 2.0–2.4 (m, 2, $H_{2'}$), 2.89, 3.04 (s, s, 3, N-CH_3), 3.55, 3.57 (s, s, 2, N-CH_2), 3.90 (m, 1, $H_{4'}$), 4.14 (m, 1, $H_{3'}$), 4.41 (s, 2, CH_2Cl), 5.34, 5.41 (d, d, 1, $O_{5'}H$), 6.16 (m, 1, $H_{1'}$), 7.53, 7.54 (s, s, 1, H_6), 11.29, 11.32 (s, s, 1, H_3); MS (FD), *m/e* 332 ($(M + 1)^+$). Anal. ($C_{13}H_{18}ClN_3O_5$) C, H, N.

5'-(Bromomethanesulfonamido)-5'-deoxythymidine (12). A solution of **1** (241 mg, 1.00 mmol) and triethylamine (209 μL, 1.50 mmol) in DMF (5 mL) was cooled in an ice bath and treated with bromomethanesulfonyl chloride[16] (140 μL, 1.50 mmol). The solution was stirred at 25 °C for 32 min, refrigerated, filtered to remove triethylamine hydrochloride, and evaporated to a gum under high vacuum. A solution of the gum in MeOH (2 mL) was applied to 2 Chromatatron plates (4-mm layer of Merck silica gel 60 PF_{254} containing gypsum) and eluted with 7:1 CH_2Cl_2–MeOH. The product fraction (R_f 0.3 in 7:1 $CHCl_3$–MeOH) was evaporated in vacuo and the residue triturated with Et_2O to give a crystalline product which was collected, washed with Et_2O, and dried: yield 220 mg (55%); mp ca. 183 °C (Mel-Temp); UV (H_2O) λ_{max} ($\epsilon \times 10^{-3}$ M^{-1} cm^{-1}), in pH 1, 265 nm (9.80), in pH 7, 266 (9.67), in pH 13, 266 (7.50); ^1H NMR δ 1.81 (s, 3, CH_3), 2.11 (m, 2, $H_{2'}$), 3.28 (m, 2, $H_{5'}$), 3.80 (m, 1, $H_{4'}$), 4.19 (m, 1, $H_{3'}$), 4.82 (s, 2, CH_2Br), 5.31 (d, 1, $O_{3'}H$), 6.18 (t, 1, $H_{1'}$), 7.53 (s, 1, H_6), 7.89 (t, 1, $N_{5'}H$), 11.30 (s, 1, 3-NH); MS (FAB), *m/e* 398 ($(M + 1)^+$). Anal. ($C_{11}H_{16}BrN_3O_6S \cdot 0.3H_2O$) C, H, N.

5'-Deoxy-5'-(ethyloxamido)thymidine (13). A solution of **1** (300 mg, 1.24 mmol) in DMAC (10 mL) was treated with diethyl oxalate (337 μL, 2.48 mmol) and *N,N*-diisopropylethylamine (216 μL, 1.24 mmol), stirred for 3 days and evaporated to dryness at 25 °C under high vacuum. An extract of the residue in $CHCl_3$ (20 mL) was filtered and evaporated in vacuo to a white solid which was triturated with Et_2O (10 mL), collected, washed with Et_2O, and dried: yield 378 mg (89%); UV (H_2O) λ_{max} ($\epsilon \times 10^{-3}$ M^{-1} cm^{-1}), in pH 1 and pH 7, 267 nm (9.30), in pH 13, 267 (7.09); ^1H NMR δ 1.27 (t, 3, CH_3 of Et), 1.81 (s, 3, 5-CH_3), 2.10 (m, 2, $H_{2'}$), 3.36 (m, 2, $H_{5'}$), 3.86 (m, 1, $H_{4'}$), 4.22 (m, 3, $H_{3'}$, CH_2 of Et), 5.31 (s, 1, $O_{3'}H$), 6.14 (t, 1, $H_{1'}$), 7.48 (s, 1, H_6), 9.00 (t, 1, $N_{5'}H$), 11.26 (s, 1, 3-NH); MS (EI), *m/e* 341 ($(M)^+$). Anal. ($C_{14}H_{19}N_3O_7$) C, H, N.

5'-Deoxy-5'-[4-(fluorosulfonyl)benzamido]thymidine (14). A solution of **1** (150 mg, 0.622 mmol) and *N,N*-diisopropylethylamine (108 μL, 0.622 mmol) in DMAC (3 mL) under N_2 was treated in small portions with 4-(fluorosulfonyl)benzoyl chloride (173 mg, 0.778 mmol) and stirred for 4 h. The solution was evaporated to dryness at 25 °C under high vacuum and the residue triturated with Et_2O (3 × 12 mL) and then 0.1 N HCl (10 mL). The white powder was collected, washed with H_2O, and dried: yield 233 mg (88%); mp 243 °C; UV (EtOH) λ_{max} ($\epsilon \times 10^{-3}$ M^{-1} cm^{-1}), in pH 1, 222 nm (17.8), 260 (12.4), in pH 7, 222 (17.7), 260 (12.2), in pH 13, 227 (22.7), 265 sh (9.80); ^1H NMR δ 1.78 (s, 3, CH_3), 2.16 (m, 2, $H_{2'}$), 3.60 (t, 2, $H_{5'}$), 3.93 (m, 1, $H_{4'}$), 4.29 (m, 1, $H_{3'}$), 5.38 (s, 1, $O_{3'}H$), 6.16 (t, 1, $H_{1'}$), 7.52 (s, 1, H_6), 8.25 (m, 4, C_6H_4), 9.02 (t, 1, $N_{5'}H$), 11.28 (s, 1, 3-NH). Anal. ($C_{17}H_{18}FN_3O_7S$) C, H, N.

5'-Deoxy-5'-[3-(fluorosulfonyl)benzamido]thymidine (15). Reaction of **1** (150 mg) with 3-(fluorosulfonyl)benzoyl chloride by the procedure used for the preparation of **14** gave an 85% yield of **15**: mp 208 °C; UV (EtOH) λ_{max} ($\epsilon \times 10^{-3}$ M^{-1} cm^{-1}), in pH 1, 267 nm (9.97), in pH 7, 266 (9.59), in pH 13, 266 (7.68); ^1H NMR δ 1.76 (s, 3, CH_3), 2.15 (m, 2, $H_{2'}$), 3.36 (s, H_2O), 3.60 (m, 2, $H_{5'}$), 3.92 (m, 1, $H_{4'}$), 4.28 (m, 1, $H_{3'}$), 5.38 (m, 1, $O_{5'}H$), 6.18 (t, 1, $H_{1'}$), 7.53 (s, 1, H_6), 7.93 (t, 1, 4H-Ph), 8.33, 8.43 (d, d, 2, 4H- and 6H-Ph), 8.57 (s, 1, 2H-Ph), 9.11 (m, 1, $N_{5'}H$), 11.31 (s, 1, H_3); MS (FAB), *m/e* 428 ($(M + 1)^+$). Anal. ($C_{17}H_{18}FN_3O_7S \cdot 0.5H_2O$) C, H, N.

5'-Deoxy-5'-[(phenoxycarbonyl)amino]thymidine (16). A stirred solution of **1** (150 mg, 0.622 mmol) and *N,N*-diisopropylethylamine (108 μL, 0.622 mmol) in DMAC (3 mL) was treated in portions (microsyringe) over 5 min with phenyl chloroformate (87.3 μL, 0.684 mmol), stirred for 2 h, and evaporated to a syrup at 25 °C under high vacuum. Addition of $CHCl_3$ (10 mL) to the syrup gave a crystalline product which was collected, washed successively with $CHCl_3$, H_2O, and Et_2O, and then recrystallized from hot MeOH (ca. 1 mL) to give pure **16**: yield 79 mg (35%); mp 196 °C; UV (EtOH) λ_{max} ($\epsilon \times 10^{-3}$ M^{-1} cm^{-1}), in pH 1 and pH 7, 266 nm (9.49) in pH 13, 232 (16.8), 267 (8.49); ^1H NMR δ 1.76 (s, 3, CH_3), 2.12 (m, 2, $H_{2'}$), 3.35 (m, 2, $H_{5'}$), 3.84 (m, 1, $H_{4'}$), 4.22 (m, 1, $H_{3'}$), 5.32 (d, 1, $O_{3'}H$), 6.18 (t, 1, $H_{1'}$), 7.0–7.48 (m, 5, C_6H_5), 7.52 (s, 1, H_6), 7.94 (5, 1, $N_{5'}H$), 11.27 (s, 1, 3-NH). Anal. ($C_{17}H_{19}N_3O_6$) C, H, N.

4-Nitrophenyl 2-Bromopropionate (17). A vigorously stirred solution of sodium *p*-nitrophenoxide (7.26 g, 45.1 mmol) in 1 N NaOH (54.2 mL, 54.2 mmol) and H_2O (300 mL) was cooled in an ice bath and treated dropwise with 2-bromopropionyl chloride

(26) Russell, D. W. *Biochem. J.* **1961**, *78*, 696.

(5.00 mL, 49.6 mmol) over a period of 5 min. The mixture was stirred in an ice bath for an additional 5 min and the crude product collected, washed with H_2O, and dried in vacuo. A solution of the solid (4.78 g) in cyclohexane (100 mL) was filtered and refrigerated to give crystalline **14** which was collected and washed with cold cyclohexane: yield 3.28 g (25%); mp 45 °C (lit.[8] mp 42-46 °C). Anal. ($C_9H_8BrNO_4$) C, H, N.

***N*-[(3-Bromopropionyl)oxy]succinimide (18).** *N*-Hydroxysuccinimide (4.97 g, 43.1 mmol), dried at 56 °C in vacuo, 2-bromopropionic acid (6.60 g, 43.1 mmol), and DCC (8.90 g, 43.1 mmol) were added successively under N_2 with stirring to anhydrous ethyl acetate (1.2 L). After 20 h, the solution was filtered under N_2 and evaporated at 25 °C in vacuo to a syrup. A solution of the syrup in EtOAc (20 mL) was filtered and evaporated under high vacuum to a crystalline solid which was triturated with EtOH (50 mL), collected, washed with EtOH, and dried: yield 7.72 g (72%); mp 86 °C. Anal. ($C_7H_8BrNO_4$) C, H, N.

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Synthesis and Biological Activity of Resolved C-10 Diastereomers of 10-Methyl- and 10-Ethyl-10-deazaminopterin

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Synthesis and evaluation of the antitumor drugs 10-methyl- and 10-ethyl-10-deazaminopterin (**15a,b**) were previously reported for the diastereomeric mixtures, lacking resolution at the C-10 position. In order to assess biological properties of the individual diastereomers, the C-10 isomers of 4-amino-4-deoxy-10-methyl- and 10-ethyl-10-deazapteroic acids (**13a,b**) were prepared by total synthesis. Coupling with L-glutamate afforded the appropriate diastereomers of the title compounds. Biochemical, transport, and cell growth inhibitory properties in L1210 cells and folate-dependent bacteria were measured. Differences were generally less than 2-fold between diastereomeric pairs, but a factor of 3 was noted for *d*,L-**15b** vs. *l*,L-**15b** in inhibition of DHFR from L1210 cells and in cytotoxicity toward L1210 cells. An in vivo comparison of the isomers of **15b** with racemic compound against L1210 in mice did not show a significant efficacy difference (ILS) among the compounds. However, *d*,L-**15b** showed an acute toxicity about 2.5 times that of *l*,L-**15b**.

In a previous paper[1] we reported the synthesis, in vitro observations on bacterial and L1210 cells, and antileukemic activity in L1210 bearing mice for 10-methyl- and 10-ethyl-10-deazaminopterin (**15a,b**). The latter drug has been found to be considerably more efficacious than methotrexate or 10-deazaminopterin in a number of experimental murine tumor models.[2-4] More recently it was shown to cause regressions in human mammary, lung, and colon tumor xenografts in nude mice.[5] Clinical trials have been initiated[6] for this agent, whose primary advantage appears to lie in its enhanced differential penetration and polyglutamylation in tumor vs. normal tissue.[7] The enhanced transport takes place via an active-transport protein in the cell wall and represents one of the few examples whereby an antitumor drug takes advantage of a fundamental difference in the nature of this transport system between tumor and normal cells. The advantage of an enhanced polyglutamation of the compound once it enters the cell may result from diminished efflux of the polyglutamate species from the cell and also its increased inhibitory potency for folate-dependent thymidine and purine synthetic enzymes.

The 10-alkyl-10-deazaminopterin molecules have two chiral centers, namely, at the 10-position and the α-carbon of the glutamate moiety. The latter is conveniently fixed as the L isomer by incorporation of L-glutamate into the synthetic scheme. However, our synthetic route previously reported[1] afforded compounds that were completely ra-

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(1) DeGraw, J. I.; Brown, V. H.; Tagawa, H.; Kisliuk, R. L.; Gaumont, Y.; Sirotnak, F. M. *J. Med. Chem.* **1982**, *25*, 1227.
(2) Sirotnak, F. M.; DeGraw, J. I.; Chello, P. L.; Moccio, D. M.; Dorick, D. *Cancer Treat. Rep.* **1982**, *66*, 351.
(3) Sirotnak, F. M.; DeGraw, J. I.; Moccio, D. M.; Samuels, L. L.; Goutas, L. J. *Cancer Chemother. Pharmacol.* **1984**, *12*, 18.
(4) Sirotnak, F. M.; DeGraw, J. I.; Schmid, F. A.; Goutas, L. J.; Moccio, D. M. *Cancer Chemother. Pharmacol.* **1984**, *12*, 26.
(5) Schmid, F. A.; Sirotnak, F. M.; Otter, G. M.; DeGraw, J. I. *Cancer Treat. Rep.* **1985**, *69*, 551.
(6) Wertheim, M. S.; Kris, M. G.; Gralla, R. J.; O'Connell, J. P.; Kinahan, J. J.; Cibas, I. R.; Williams, L.; Bauer, T.; Farag, F. M.; Fanucchi, M. P.; Young, C. W. 76th Meeting, American Association of Cancer Research, Houston, TX, May 22-25, 1985, paper 704.
(7) Sirotnak, F. M.; DeGraw, J. I. In *Folate Antagonists as Therapeutic Agents*; Sirotnak, F. M., Ed.; Academic: New York, 1984; Vol. 2, pp 43-91.

0022-2623/86/1829-1056$01.50/0