spectral measurements and Mr. T. Matsuyama for technical assistance.

Registry No. 1, 83465-22-9; 2, 38231-86-6; 3, 32780-32-8; 6, 83480-29-9; 7, 83470-49-9; 8a, 101540-73-2; 8b, 101540-74-3; 9a, 83541-26-8; 9b, 83541-27-9; 10a, 101540-71-0; 10b, 101540-72-1; 11, 101540-75-4; 12, 83471-08-3; 13, 83470-38-6; 14, 83470-52-4; 15, 83470-45-5; 16, 83470-43-3; 17, 101540-76-5; 18, 101540-77-6; 19, 82920-27-2; 20, 83470-79-5; 21, 89859-43-8; 22, 89859-46-1; 23a, 89859-79-0; 23b, 90024-25-2; 24a, 89859-81-4; 24b, 90024-26-3; 25a, 80943-41-5; 25b, 89920-24-1; 26a, 89859-74-5; 26b, 89920-25-2; 27, 89859-47-2; 28, 89859-86-9; 29, 89859-87-0; 30, 89859-88-1; 31, 89859-89-2; 32, 89859-90-5; 33a, 89860-05-9; 33b, 89920-31-0; 34a, 89860-06-0; 34b, 89920-32-1; methyl 2,3-*O*-cyclohexylidene-6-deoxy-α-D-glucopyranoside, 89859-42-7; methyl 6-*O*-acetyl-2,3-*O*-cyclohexylidene-α-D-glucopyranoside, 89859-45-0; *N*-(benzyloxycarbonyl)validamine, 85281-05-6.

Supplementary Material Available: ^{1}H NMR data of compounds **6**, **8a,b–10a,b**, **19–22**, **23a,b**, **24a,b**, **32**, **33a,b**, and **34a,b**, and ^{13}C NMR data of compounds **23a**, **24a**, **25a,b**, and **26a** (8 pages). Ordering information is given on any current masthead page.

Structural Studies on Bioactive Compounds. 4.[1] A Structure–Antitumor Activity Study on Analogues of *N*-Methylformamide

E. Nicholas Gate, Michael D. Threadgill,* Malcolm F. G. Stevens, David Chubb, Lisa M. Vickers, Simon P. Langdon, John A. Hickman, and Andreas Gescher

Cancer Research Campaign Experimental Chemotherapy Group, Pharmaceutical Sciences Institute, Aston University, Aston Triangle, Birmingham B4 7ET, U.K. *Received July 11, 1985*

A series of derivatives of *N*-methylformamide (NMF), an experimental antitumor agent, has been prepared, having the general formula $R^3C(X)NR^1R^2$ where R^1 = H, CH_3, CD_3, CH_2CF_3, CH_2CH_2Cl, cyclopropyl, C_2H_5, CH_2OH, CH_2OR, $CH_2N(CH_3)_2$; R^2 = H, CH_3; R^3 = H, CF_3, CCl_3, CH_3, Ph, $NHCH_3$, $N(CH_3)_2$; and X = O, S, NH. A further short series of "push–pull" olefins of the general formula $R^1R^2C{=}CHNR^3R^4$ has been synthesized where R^1 = H, CH_3 and R^2 = H, NO_2, CN, CHO, CH_3 and R^3 = H and R^4 = H, CH_3, morpholino. These compounds have been tested for activity against the M5076 ovarian sarcoma and the TLX5 lymphoma in mice. NMF was by far the most potent agent of both series with activity against both tumors. Some other compounds showed weak activity, but there is a rigorous structural requirement for activity and most analogues were inactive. Certain members of the series exist as equilibrium mixtures of rotamers about the amide or pro-amide bonds as shown by NMR.

The antitumor activity of *N*-methylformamide (NMF; NSC 3051: **1**) in experimental use was first described[2] in 1953. A subsequent clinical trial[3] in five patients was terminated when indications of hepatotoxicity intervened. We have shown that the hepatotoxicity of NMF toward mice can be minimized if the drug is scheduled in divided doses;[4] moreover, optimum antitumor activity is elicited if the drug is administered in a chronic schedule.[4] On the basis of these preclinical studies, a new phase 1 trial was conducted, and the dose-limiting toxicities were hyperbilirubinemia, nausea, and malaise. Remarkably the agent has no myelosuppressive activity in rodents or in man.[5] Beneficial effects of **1** in combination with conventional (myelosuppressive) antitumor agents have been demonstrated against rodent experimental tumors.[6] The drug is now in phase 2 trial particularly against lung and colon tumors since the compound is very active against the NCI lung (LX-1), colon (CX-1), and mammary (MX-1) human tumor xenografts implanted in mice.[7]

Earlier studies on analogues of NMF tested against the Ehrlich ascites[8] and sarcoma 180 tumors[2] revealed that only the simplest amides, NMF, and formamide **2** had antitumor activity. We have screened a range of formamides, thioformamides, acetamides, benzamides, ureas, thioureas, guanidines, enamines, and vinylogous amides **3–35** and some related compounds **36–39** against either the TLX5 lymphoma or the M5076 reticulum cell sarcoma (or both). These tumors are sensitive to a range of agents that have an *N*-alkyl group bearing an electron-withdrawing substituent. The TLX5 lymphoma is especially sensitive to nitrosoureas,[9] triazenes,[10] and the recently discovered imidazotetrazines[11] whereas the M5076 tumor is additionally responsive to the 1,3,5-triazine series based on hexamethylmelamine.[12] Structure–activity studies in the aforementioned agents have confirmed a requirement for either an *N*-methyl or *N*-(2-haloethyl) fragment for optimum antitumor activity. It was of interest, therefore, to investigate whether or not there are similar structural

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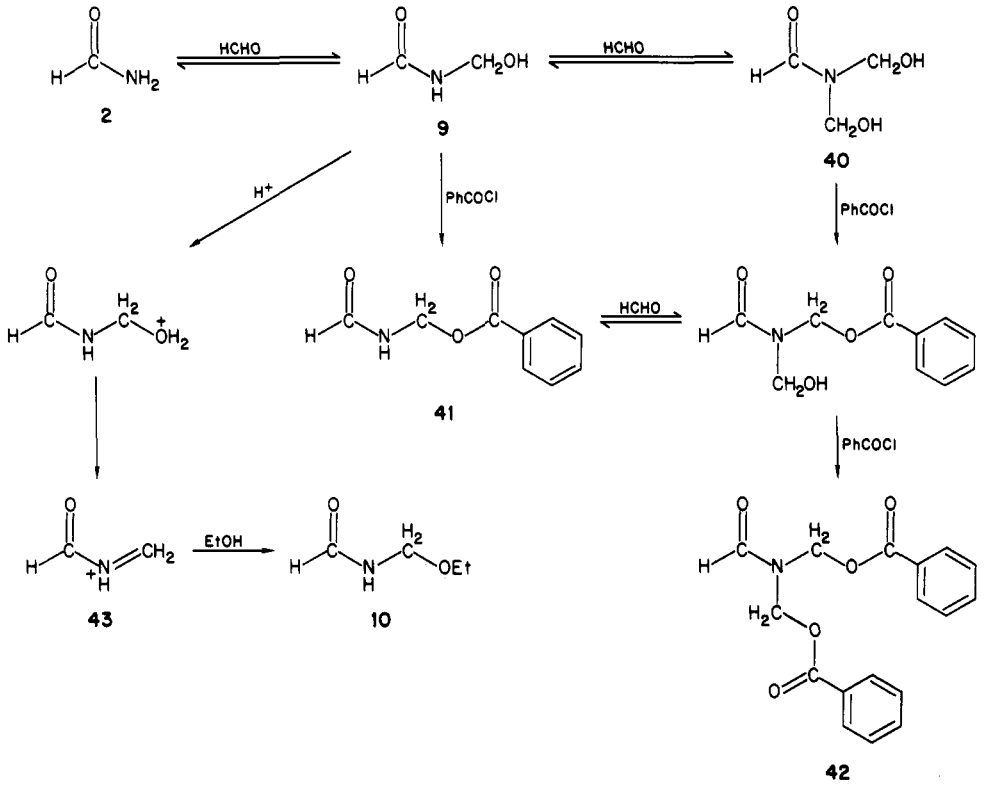
Scheme I



requirements in a series of analogues of NMF.

Chemistry

Preparation of the simple *N*-alkylformamides **3–6** and *N*-hydroxy-*N*-methylformamide (**15**) was achieved by formylation of the appropriate amine by alcoholic ethyl formate in the presence of an inorganic base. However 2,2,2-trifluoroethylamine proved to be insufficiently nucleophilic toward this agent, and a more vigorous method, previously used by us[13] for the synthesis of *N*-methyl-[^{14}C]formamide involving controlled fusion of sodium [^{14}C]formate with the amine hydrochloride, was employed for the synthesis of **7**. The isomeric *N*-methyltrifluoroacetamide (**18**) was prepared directly from methylamine and trifluoroacetic anhydride. Attempts to alkylate NMF **1** or formamide **2** at nitrogen using a variety of conditions, including the use of the alumina-supported potassium fluoride catalyst reported by Yamawaki,[14] were unsuccessful.

Hydroxymethylation of formamide and NMF with formaldehyde in the presence of a catalytic amount of base gave the carbinolamides **9** and **12**, respectively; these colorless oils were thermally unstable and could not be purified by distillation. In contrast, the carbinolamide **22** could be crystallized to purity.[15] The major contaminant in **12** (shown by ^{1}H NMR) was 5% of the starting NMF whereas the NMR spectrum (^{1}H and ^{13}C) of *N*-(hydroxymethyl)formamide (**9**) showed numerous impurities including, probably, *N*,*N*-bis(hydroxymethyl)formamide (**40**). The involvement[16] of **40** in the $HCONH_2/HCHO/HCONHCH_2OH$ system is also indicated in the reaction of **9** with benzoyl chloride in which both the expected monoester **41** and bis[(benzoyloxy)methyl]formamide (**42**) are formed. Two pathways are possible for the production of **42** (Scheme I): the preformed *N*,*N*-bis(hydroxymethyl)formamide (**40**) might be esterified by benzoyl chloride: alternatively, the intermediate monoester **41** could be hydroxymethylated by formaldehyde and subsequently benzoylated. Esterification of *N*-(hydroxymethyl)-*N*-methylformamide (**12**) proceeded straightforwardly to give **13** and **14**. The ether **10** was prepared from *N*-(hydroxymethyl)formamide (**9**) in acidic ethanol, the mechanism presumably involving trapping of the intermediate[17] *N*-formylmethyleneiminium ion (**43**) by ethanol (Scheme I).

The aminal **11** was synthesized by the Einhorn reaction[18] from dimethylamine, formaldehyde, and formamide. In this case, the iminium ion intermediate arises[19] from condensation of formaldehyde with the secondary amine rather than with formamide. The aminal was quaternized to afford the trimethylammonium iodide **44** in order to enhance the leaving-group ability of the amino group. A variety of basic conditions was examined in an effort to effect condensation of the salt with *N*-acetyl-L-cysteine: NMR analysis of the products confirmed the presence of *N*-acetyl-*S*-(formamidomethyl)cysteine (**45**), but a pure sample could not be isolated from the mixtures.

N,*N*-Dimethylglycine (**37**) was prepared in two efficient steps: substitution of bromide in methyl bromoacetate by dimethylamine was followed by acid hydrolysis of the ester to give the hydrochloride salt of **37** in 76% overall yield.

Synthesis of the "push–pull" olefins and vinylogous amides **29–35** involved condensation of the appropriate activated methyl and methylenic substrates with a dialkoxymethyl electrophile. Synthetic and conformational studies on nitro enamines **29–31** have been described by

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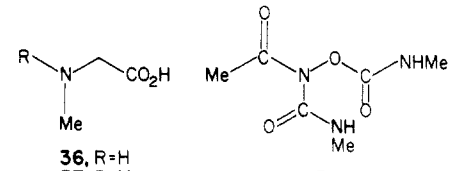
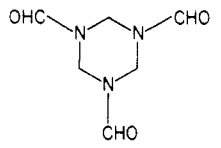


36, R=H
37, R=Me

38



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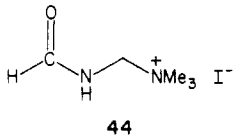

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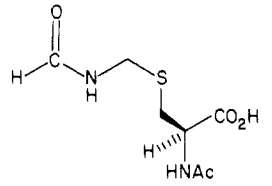

45

us previously.[20] The morpholine analogue **32** was prepared in a three-component condensation (nitromethane, morpholine, and triethyl orthoformate). The ^1H NMR spectrum of this compound confirms *E* geometry about the C=C bond in solution in dimethyl sulfoxide ($J_{C1H,C2H}$ = 11 Hz); this coupling constant is diagnostic of trans coupling in **30** and **31**.[20] Treatment of preformed 2-cyano-3-ethoxypropenenitrile with methylamine in a conjugate addition–elimination reaction afforded the monomethyl dicyano enamine **33** whereas use of malononitrile as the activated methylenic reactant in condensation with dimethylformamide dimethyl acetal furnished the dimethyl analogue **34**. Attempts to prepare 2-nitro-3-(substituted amino)propenenitriles through condensation using the relatively unstable nitroacetonitrile (**46**) were unsuccessful.

The vinylogous amide **35** was formed in good yield from treatment of 3-methoxy-2-methylpropenal with methylamine again via a conjugate addition–elimination reaction. Proton NMR spectroscopy of **35** in chloroform showed the *E* conformation about the pro-amide C–N bond ($J_{C3H,NH}$ = 14 Hz). Also, compound **33** is shown to be a 5:1 mixture of rotamers in dimethyl sulfoxide with the *E* conformer ($J_{vinylCH,NH}$ = 15 Hz) about the pro-amide bond predominating over the *Z* form ($J_{vinylCH,NH}$ = 9 Hz).

Antitumor Activity

Of the range of amides screened against the TLX5 lymphoma (Table II), only NMF (**1**) (optimum T/C 191% at 800 mg kg^{-1}), its CD_3 analogue **3** (T/C 142% at 400 mg kg^{-1} day^{-1}), and the *N*-ethyl homologue **4** (T/C 137% at 800 mg kg^{-1} day^{-1}) display activity. The M5076 tumor was completely inhibited by NMF at a dose of 200 mg kg^{-1} day^{-1} for 17 days (Table III), but, of the other congeners of NMF tested, only formamide (**2**), *N*,*N*-dimethylformamide (DMF, **8**), *N*-(hydroxymethyl)formamide (**9**), and *N*-methylbenzamide (**21**) showed marginal activity. Particularly noteworthy is the inactivity of *N*-(2-chloroethyl)formamide (**5**) against the TLX5 lymphoma, which contrasts with the marked activity elicited by the 2-chloroethyl derivatives in the nitrosourea,[9] triazene,[10] and imidazotetrazine[11] series and suggests that the formamides may operate by a nonalkylating mechanism.

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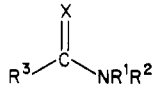
Table I



	R_1	R_2	R_3	X
1	Me	H	H	O
2	H	H	H	O
3	CD_3	H	H	O
4	Et	H	H	O
5	CH_2CH_2Cl	H	H	O
6	cyclopropyl	H	H	O
7	CH_2CF_3	H	H	O
8	Me	Me	H	O
9	CH_2OH	H	H	O
10	CH_2OEt	H	H	O
11	CH_2NMe_2	H	H	O
12	CH_2OH	Me	H	O
13	CH_2OAc	Me	H	O
14	CH_2O_2CPh	Me	H	O
15	Me	OH	H	O
16	Me	Me	H	S
17	Me	H	Me	O
18	Me	H	CF_3	O
19	Me	Me	Me	O
20	CH_2OH	H	CCl_3	O
21	Me	H	Ph	O
22	CH_2OH	H	Ph	O
23	OH	H	NH_2	O
24	Me	H	NHMe	O
25	Me	Me	NMe_2	O
26	Me	H	NHMe	S
27	Me	Me	NMe_2	S
28	Me	Me	NMe_2	NH
29	H	H	H	$CHNO_2$
30	Me	H	H	$CHNO_2$
31	Me	Me	H	$CHNO_2$
32	$-CH_2CH_2OCH_2CH_2-$		H	$CHNO_2$
33	Me	H	H	$C(CN)_2$
34	Me	Me	H	$C(CN)_2$
35	Me	H	H	C(Me)CHO

Many *N*-alkyl xenobiotics are metabolized to the corresponding α-hydroxy derivatives, some of which give rise to electrophiles.[16] Thus, the possibility[21] that NMF might require in vivo metabolic activation to *N*-(hydroxymethyl)formamide (**9**), or a conjugate thereof, was given initial credence when the carbinolamide was shown to possess potent inhibitory activity against the TLX5 lymphoma in vitro: however, this effect may have been mediated[21] by traces of cytotoxic formaldehyde that contaminated the sample of **9**. Attempts to purify the sample of **9** by fractional distillation led to fragmentation of the carbinolamide into starting materials, formaldehyde, and formamide. Four *N*-hydroxymethyl compounds **9** (a metabolite of NMF in mice),[22] **12** (the major metabolite of dimethylformamide in rodents),[23] and the carbinolamides **20** and **22**, derived from trichloroacetamide and benzamide, respectively, were all devoid of activity against the TLX5 lymphoma in vivo (Table II). Nevertheless, compound **9** showed marginal activity against the M5076 tumor (Table III), albeit at a very high dose (1600 mg kg^{-1} daily × 17). The disappointing in vivo results with these compounds may reflect an inherent lack of activity of *N*-hydroxymethyl amides per se, although *N*-hydroxymethyl compounds of the melamine[24] and triazene[10] series are at least

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Table II. Antitumor Activity of *N*-Methylformamide and Its Active Analogues[a] against the TLX5 Lymphoma[b] in Mice

no.	dose range tested, mg kg^{-1} day^{-1}	optimal dose,[c] mg kg^{-1} day^{-1}	mean death day[d] (±SD)	control death day (±SD)	T/C × 100%
1	800–100	800	17.6 (0.9)	9.2 (0.4)	191
		400	16.4 (1.3)	9.2 (0.4)	178
		200	12.4 (1.1)	9.2 (0.4)	135
		100	11.0 (0.7)	9.2 (0.4)	120
2	1200–50	800	11.2 (1.3)	9.1 (0.3)	123
3	800–50	400	18.8 (1.5)	13.2 (0.8)	142[e]
4	800–100	800	12.6 (1.3)	9.2 (0.4)	137
31	400–25	100	15.4 (1.3)	11.1 (0.3)	139

[a] Analogues **5**, **7–15**, **17**, **20**, **22**, **23**, **29**, **30**, **32–39** were found to be inactive against this tumor. The data for these compounds are presented in the supplementary material (see below). [b] The TLX5 lymphoma was passaged at 7-day intervals by ip injection of 2×10^5 cells into female CBA/ca mice (18–23 g). For antitumor tests 2×10^5 cells were injected subcutaneously into the inguinal region of female CBA/ca mice (18–23 g). Drugs were dissolved in physiological saline or Me_2SO/arachis oil (1:9) and administered daily for 5 days by ip injection commencing 72 h after tumor implantation. The mean day of death of groups of five animals were recorded and survival times of treated animals (T) compared with untreated controls (C). A value of T/C (×100%) of >125 is considered significant antitumor activity. [c] Except for compound 1 where a range of doses is described. [d] Value at optimal dose. [e] T/C × 100% for NMF (1) at this dose was 139 in this experiment.

Table III. Antitumor Activity of *N*-Methylformamide and Its Active Analogues[a] against the M5076 Reticulum Cell Sarcoma[b] in Mice

no.	dose range tested, mg kg^{-1} day^{-1}	LD_{10},[c] mg kg^{-1} day^{-1}	LD_{50},[c] mg kg^{-1} day^{-1}	optimal dose,[d] mg kg^{-1} day^{-1}	mean tumor volume (±SD)	control tumor volume (±SD)	T/C × 100%
1	400–6.25	220	300	200	NM	2.7 (0.5)	0
				100	0.3 (0.1)	2.7 (0.5)	11
				50	0.9 (0.2)	2.7 (0.5)	33
				25	2.1 (0.6)	2.7 (0.5)	70
2	400–100	200	270	200	0.8 (0.3)	2.1 (0.6)	38
8	1500–600	1130	1280	1000	1.0 (0.2)	2.5 (0.5)	40
9	2500–600	1580	1930	1500	0.8 (0.1)	2.1 (0.6)	38
21	800–100	450	650	400	0.8 (0.2)	1.9 (0.5)	42
31	200–25	55	75	50	0.5 (0.2)	1.5 (0.3)	35
38	150–6.25	53	63	50	0.9 (0.1)	2.4 (0.3)	38

[a] Analogues **4**, **6**, **12**, **16–19**, **24–28** were found to be inactive against this tumor. The data for these compounds are presented in the supplementary material (see below). [b] Fragments of tumor were obtained from donor BDF_1 mice (18–23 g). These were pooled, homogenized, and diluted with saline to produce a suspension of 10^7 cells mL^{-1}. Cells (10^6 in 0.1 mL) were injected intramuscularly into the left hind legs of groups of five female BDF_1 mice (18–23 g). Drugs were administered daily for 17 days by ip injection commencing 24 h after tumor implantation. Mean tumor volumes (in cm^3) were determined 24 days after tumor implantation. Tumor diameters were measured by calipers and the volumes calculated according to the following formula: volume = $(l \times w^2)/2$ where l is the longest tumor diameter and w the diameter perpendicular to the long axis. The volume of the leg without tumor is 0.1–0.2 cm^3 when measured in this manner. Tumors smaller than 0.2 cm^3 are "nonmeasurable" (NM). The mean tumor volumes of test animals (T) was compared to those of controls (C). A value of T/C × 100% of <42% is considered significant antitumor activity.[6] [c] Lethal dose values were estimated on day 24 from the number of survivors in the drug-treated groups. [d] Except for compound 1 where a range of doses is described.

as active as their *N*-methyl analogues. Alternatively, the inactivity of the *N*-hydroxymethyl derivatives in the formamide series may be due to pharmacokinetic problems associated with their high polarity. Successful chemical efforts to prepare lipophilic prodrug modifications of *N*-(hydroxymethyl)formamide (**9**) did not lead to biological activity. Thus, *N*-(ethoxymethyl)formamide (**10**), *N*-[(dimethylamino)methyl]formamide (**11**), and 1,3,5-triformylhexahydro-1,3,5-triazine (**39**) were all inactive in vivo against the TLX5 lymphoma as were the acetyl (**13**) and benzoyl (**14**) derivatives of *N*-(hydroxymethyl)-*N*-methylformamide (**12**).

Possibly, metabolic hydroxylation of NMF represents a bioinactivation as far as antitumor activity is concerned,[21] but its involvement in the hepatotoxicity of the drug cannot be excluded. Metabolic N-hydroxylation was also considered, but the possible product of such a pathway, *N*-hydroxy-*N*-methylformamide (**15**), a known teratogen,[25] proved inactive against the TLX5 lymphoma as did another *N*-hydroxy compound, the clinically used hydroxyurea **23**.

It has been shown recently[22] that the formyl carbon of NMF is extensively metabolized, being excreted by mice as carbon dioxide. The inactivity of a series of compounds where the formyl hydrogen is replaced by methyl or substituted methyl groups (**17–20**) or amino and substituted amino groups (**23–28**) and the corresponding inactivity of derivatives where the formyl oxygen is replaced by sulfur (**16**, **26–27**) attests to the critical influence of the formyl group in mediating the activity of NMF. Compounds **21** and **38** do, however, possess marginal activity against the M5076 sarcoma. Replacement of the formyl group by substituted ethenyl residues to afford "push–pull" olefins **29–34** and the vinylogous NMF **35** led to loss of activity, except in the case of *N*,*N*-dimethyl-2-nitroethaneamine (**31**), which inhibited both tumors, although not markedly with T/C × 100% = 139 on TLX5 and T/C × 100% = 35 on M5076 experiments. Interestingly, the corresponding monomethyl compound **30**, arguably a closer analogue of NMF (with a 9:1 *Z*/*E* ratio of rotamers about the MeNH–C bond),[20] is inactive against the TLX5 lymphoma (Table II).

In conclusion, NMF (**1**) is the only compound in the series reported with good inhibitory activity against both the TLX5 lymphoma and M5076 reticulum cell sarcoma in vivo; any structural modification has a dyschemotherapeutic effect. These results are consistent with an earlier study[8] on the effects of formamides against the Ehrlich ascites tumor, which demonstrated that there is a rigorous structural requirement for antitumor activity. Other tumors do respond to certain of the agents described here: worthy of mention is the inhibitory activity of NMF (**1**),[26]

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N-(hydroxymethyl)formamide (**9**),[21] *N*-(acetoxymethyl)-*N*-methylformamide (**13**), and carecamide (**38**)[26] against the MX-1 mammary xenograft. Moreover, recent interest in NMF stems from the recognition that it is a prototype of a new class of low molecular weight antitumor agent capable of inducing terminal differentiation of human tumor cell lines such as the human promyelocytic leukemia HL60,[27] a property that it shares with a number of the congeners described in this paper. Results of these studies will be described in a future paper.[28]

Experimental Section

Unless otherwise stated, NMR spectra were obtained at 60 MHz with Varian EM360A and Varian A60A spectrometers. Other NMR spectra were acquired at 80 MHz and 220 MHz with Bruker WP80 and Perkin-Elmer R34 instruments, respectively. IR spectra were recorded with Pye Unicam SP200 and Perkin Elmer 1310 spectrometers. Melting points are uncorrected.

N-(Hydroxymethyl)-2,2,2-trichloroacetamide (**20**) was a kind gift of Professor Bundgaard (Copenhagen, Denmark) and *N,O*-bis-(*N*-methylcarbamoyl)acetohydroxamic acid (**38**, carecamide) was donated by the Drug Resources Branch of the National Cancer Institute. *N*-(Trideuteriomethyl)formamide (**3**),[13] *N*-(acetoxymethyl)-*N*-methylformamide (**13**),[15] *N*-(hydroxymethyl)benzamide (**22**),[15] 2-nitroethenamine (**29**),[20] *N*-methyl-2-nitroethenamine (**30**),[20] and *N,N*-dimethyl-2-nitroethenamine (**31**)[20] were prepared as previously described by us.

***N*-Ethylformamide (4).** Ethyl formate (74.0 g, 1.0 mol) was added to 70% aqueous ethylamine with cooling, and the mixture was stirred at ambient temperature for 2 h. Distillation gave *N*-ethylformamide (70.0 g, 96%) as a colorless liquid, bp 195–198 °C (lit.[29] bp 197–199 °C).

***N*-(2-Chloroethyl)formamide (5).** Sodium (2.30 g, 100 mmol) was dissolved in anhydrous ethanol (100 mL). Ethyl formate (25 mL), anhydrous Na_2CO_3 (20 g), and 2-chloroethylamine hydrochloride (8.15 g, 70 mmol) were added at −5 °C during 30 min. Stirring was continued at ambient temperature for 20 h before the mixture was filtered. Distillation of the filtrate afforded the formamide (5.50 g, 51%) as a colorless liquid: bp 98–100 °C (2 mm) (lit.[30] bp 79–95 °C (2 mm)); IR (liquid film) 3290, 1670, and 1520 cm^{-1}; NMR ($CDCl_3$) δ 3.5–3.7 (m, 4 H, CH_2CH_2), 7.5 (br, 1 H, NH), and 8.0–8.3 (m, 1 H, CHO); MS, *m/z* 109/107 (M^+).

***N*-Cyclopropylformamide (6).** Cyclopropylamine (11.4 g, 200 mmol) was added to a mixture of ethyl formate (50 mL), anhydrous Na_2CO_3 (30 g), and anhydrous ethanol (50 mL) at −5 °C, and the mixture was stirred at ambient temperature for 20 h. *N*-Cyclopropylformamide was isolated as for **5** above as a colorless liquid (10.0 g, 59%), bp 86–88 °C (3 mm); IR (liquid film) 3260 and 1670 cm^{-1}; NMR (220 MHz, $CDCl_3$) δ 0.9–1.1 (m, 4 H, CH_2CH_2), 2.6–2.85 (m, 1 H, cyclopropyl 1-H), 8.18 (s, 0.6 H, CHO of *Z* rotamer), and 8.29 (d, *J* = 12 Hz, 0.4 H, CHO (*E*)); MS, *m/z* 85 (M^+). Anal. (C_4H_7NO) C, H, N.

***N*-(2,2,2-Trifluoroethyl)formamide (7).** A mixture of sodium formate (680 mg, 10 mmol) and 2,2,2-trifluoroethylamine hydrochloride (1.35 g, 10 mmol) was heated gently under reflux for 20 min in the apparatus described previously[18] and then distilled. This product was redistilled (Kugelrohr) to give *N*-(2,2,2-trifluoroethyl)formamide (1.05 g, 83%) as a colorless liquid: bp 70–80 °C (3 mm); IR (liquid film) 3310, 1680, and 1540 cm^{-1}; NMR (220 MHz, $CDCl_3$) δ 3.96 (dq, *J* = 6.5 and 10 Hz, 1.8 H, CH_2CF_3 of *Z* rotamer), 8.11 (d, *J* = 11.5 Hz, 0.1 H, CHO (*E*)), 8.23 (s, 0.9 H, CHO (*Z*)), and 8.75 (br, 1 H, NH); MS, *m/z* 127 (M^+). Anal. ($C_3H_4F_3NO$) C, H, N.

***N*-(Ethoxymethyl)formamide (10).** A mixture of 40% aqueous KOH (2 mL, 14 mmol), formamide (45 g, 1 mol), and paraformaldehyde (33 g, 1.1 mol HCHO) was stirred for 30 min before ethanol (200 mL) and concentrated H_2SO_4 (3 mL) were added. The reaction mixture was stirred for a further 6 h and filtered. Anhydrous diethyl ether (25 mL) was added to the filtrate. The whole was allowed to stand over $NaHCO_3$ for 20 h, filtered, and concentrated under reduced pressure. The residue, in water (50 mL), was extracted with dichloromethane (5 × 100 mL). The combined extracts were washed with water and dried (Na_2SO_4) and the solvents evaporated under reduced pressure. Distillation gave *N*-(ethoxymethyl)formamide (22.0 g, 21%) as a colorless liquid: bp 108–120 °C (1 mm) (lit.[31] bp 71–82 °C (0.01–0.1 mm)); IR (liquid film) 3450 and 1670 cm^{-1}; NMR ($CDCl_3$) δ 1.16 (3 H, t, *J* = 7 Hz, CH_3), 3.53 (m, 2 H, CH_2CH_3), 4.7 (m, 2 H, NCH_2O), 7.1 (br, 1 H, NH), and 8.27 (m, 1 H, CHO); MS, *m/z* 103 (M^+).

***N*-(Dimethylaminomethyl)formamide (11).** Aqueous formaldehyde (37%, 112 g) was added dropwise to a mixture of formamide (45 g, 1 mol) and 40% aqueous dimethylamine (70 g) at 0 °C. The whole was stirred at ambient temperature for 20 h. Distillation furnished *N*-(dimethylaminomethyl)formamide (68.3 g, 67%) as a colorless oil: bp 100 °C (3 mm) (lit.[32] bp 62–65 °C (0.05 mm)); IR (liquid film) 3300 and 1650 cm^{-1}; NMR ($CDCl_3$) δ 2.25 (m, 6 H, $N(CH_3)_2$), 4.0 (m, 2 H, NCH_2N), 7.1 (br, 1 H, NH), and 8.2 (m, 1 H, CHO); MS, *m/z* 102 (M^+).

***N*-(Hydroxymethyl)-*N*-methylformamide (12)** was prepared by the method of Grady and Stott.[33] ^1H NMR showed the product to contain 5% *N*-methylformamide (**1**). The former decomposed on attempted distillation. *N*-(Hydroxymethyl)formamide (**9**) was similarly prepared.[33]

***N*-[(Benzoyloxy)methyl]-*N*-methylformamide (14).** *N*-(Hydroxymethyl)-*N*-methylformamide (**11**; 95%; 9.0 g, 96 mmol) and benzoyl chloride (11.5 mL) were stirred together in 5% aqueous NaOH (120 mL) at 0 °C for 30 min. The oily lower layer was washed with aqueous $NaHCO_3$. Distillation furnished the ester (5.2 g, 29%) as a colorless oil: bp 154 °C (3 mm) (lit.[34] bp 111–123 °C (0.02 mm)); IR (liquid film) 1720 and 1690 cm^{-1}; NMR (220 MHz; $(CD_3)_2SO$) δ 2.94 (s, 2.55 H, NCH_3 of *Z* rotamer), 3.14 (s, 0.45 H, NCH_3 (*E*)), 5.64 (s, 0.3 H, NCH_2 (*E*)), 5.72 (s, 1.7 H, NCH_2 (*Z*)), 7.66 (m, 3 H, Ar 3-, 4-, and 5-H), 8.08 (m, 2 H, Ar 2- and 6-H), 8.29 (s, 0.15 H, CHO (*E*)), and 8.70 (m, 0.85 H, CHO (*Z*)).

***N*-Hydroxy-*N*-methylformamide (15).** To *N*-methylhydroxylamine hydrochloride (835 mg, 10 mmol), in methanol (20 mL), was added sodium methoxide (540 mg, 10 mmol), followed by ethyl formate (20 mL). The whole was stirred for 16 h before being filtered through diatomaceous earth. The solvents were evaporated from the combined filtrate and methanol washings. Distillation of the residue gave the hydroxamic acid (610 mg, 81%) as a colorless oil: bp 140–147 °C (1 mm) (lit.[35] bp 60 °C (0.005 mm)); IR (liquid film) 2300 (br) and 1670 cm^{-1}; NMR ($CDCl_3$) δ 3.20 (br s, 3 H, CH_3), 7.8–8.2 (m, 1 H, CHO), and 9.73 (s, 1 H, OH).

***N*-Methyl-2,2,2-trifluoroacetamide (18).** Excess methylamine was passed through a solution of trifluoroacetic anhydride (12.6 g, 60 mmol) in diethyl ether (400 mL) during 2 h. The mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was distilled at 3 torr to give *N*-methyl-2,2,2-trifluoroacetamide (4.44 g, 58%) as a white solid: mp 45 °C (lit.[36] bp 80 °C (3 mm), mp 48–49.5 °C); IR (Nujol mull) 3320, 1700, and 1560 cm^{-1}; NMR ($CDCl_3$) δ 2.95 (d, 3 H, *J* = 5 Hz, NCH_3) and 7.2 (br, 1 H, NH).

***N*-(2-Nitroethenyl)morpholine (32).** A mixture of trimethoxymethane (29.6 g, 279 mmol), morpholine (8.7 g, 100 mmol), nitromethane (30.5 g, 500 mmol), and toluene-4-sulfonic acid (500 mg) was boiled under reflux for 1 h. Evaporation of

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excess reagents under reduced pressure and passage of the residue through a short column of silica gel in CH_2Cl_2 followed by recrystallization from EtOH afforded *N*-(2-nitroethenyl)morpholine (10.4 g, 73%) as yellow needles: mp 142.5 °C (lit.[37] mp 140–141 °C); IR (KBr) 1625 cm^{-1}; NMR ($(CD_3)_2SO$) δ 3.63 (m, 8 H, CH_2), 7.00 (d, 1 H, J = 11 Hz, 2-H), and 8.30 (d, 1 H, J = 11 Hz, 1-H).

2-Cyano-3-(methylamino)propenenitrile (33). Excess methylamine was passed into a suspension of 2-cyano-3-ethoxypropenenitrile (1.22 g, 10 mmol) in diethyl ether (50 mL) at 0 °C and the whole was stirred at ambient temperature for 20 h. Evaporation of the solvent and excess methylamine under reduced pressure and recrystallization of the residue from anhydrous EtOH yielded 2-cyano-3-(methylamino)propenenitrile (770 mg, 72%) as orange microcrystals: mp 191–194 °C (lit.[38] mp 191–192 °C); IR (KBr) 3250, 2220, 2200, and 1640 cm^{-1}; NMR ($(CD_3)_2SO$) δ 2.97 (d, J = 4 Hz, 2.5 H, NCH_3 of *E* rotamer about vinyl–N bond), 3.07 (m, 0.5 H, NCH_3 (*Z*)), 7.53 (d, J = 9 Hz, 0.17 H, CH (*Z*)), 7.83 (d, J = 15 Hz, 0.83 H, CH (*E*)), and 8.8 (br, 1 H, NH); MS, m/z 107 (M^+).

2-Cyano-3-(dimethylamino)propenenitrile (34). Dimethylformamide dimethyl acetal (4.76 g, 40 mmol) and propanedinitrile (2.64 g, 40 mmol) were stirred together for 5 h and then concentrated under reduced pressure to give a yellow solid. Chromatography (silica gel, ethyl acetate) furnished the amino nitrile (1.0 g, 21%) as a white solid: mp 80.5–82 °C (lit.[39] mp 82–83 °C); IR (KBr) 2220, 2210, and 1650 cm^{-1}; NMR ($CDCl_3$) δ 3.23 (s, 3 H, NCH_3), 3.37 (s, 3 H, NCH_3), and 7.1 (s, 1 H, CH); MS, m/z 121 (M^+).

2-Methyl-3-(methylamino)propenal (35). Ethanolic methylamine (33%, 12.5 mL, 135 mmol) was added dropwise to 3-ethoxy-2-methylpropenal (11.4 g, 10 mmol) at 0 °C and the whole was stirred at ambient temperature for 20 h. Distillation gave a yellow oil (bp 114–118 °C (2 mm)), which was redistilled to afford 2-methyl-3-(methylamino)propenal (5.7 g, 58%): bp 114–116 °C (1 mm); mp 55 °C; IR 3260, 1660, and 1580 cm^{-1}; NMR (80 MHz, $CDCl_3$) δ 1.65 (s, 3 H, CCH_3), 3.07 (d, J = 3 Hz, 3 H, NCH_3), 6.54 (br, 1 H, NH), 6.86 (d, J = 14 Hz, 1 H, NCH), and 8.84 (s, 1 H, CHO); MS, m/z 99 (M^+). Anal. (C_5H_9NO) C, H, N.

***N*,*N*-Dimethylglycine (37).** Dimethylamine hydrochloride (50 g) was dissolved in H_2O (100 mL) and diethyl ether (400 mL) was added at 0 °C followed by sufficient anhydrous K_2CO_3 to dry the organic phase. Filtration gave an ethereal solution of dimethylamine to which was added methyl bromoacetate (15.3 g, 100 mmol), and the mixture was allowed to stand at ambient temperature for 20 h. The precipitated dimethylamine hydrobromide was removed by filtration and the filtrate was concentrated at atmospheric pressure. Distillation of the residue gave methyl (dimethylamino)acetate (9.13 g, 77%) as a colorless liquid: bp 45–51 °C (20 mm) (lit.[40] bp 50–54 °C (30 mm)); IR (liquid film) 1740 cm^{-1}. The above ester (9.13 g, 77 mmol) was boiled under reflux in 11 M hydrochloric acid for 24 h before evaporation of the excess reagent and drying at 10 torr in the presence of sodium hydroxide pellets gave *N*,*N*-dimethylglycine hydrochloride (10.70 g, 99%) as a white powder: mp 187–189 °C (lit.[41] mp 186–189 °C); IR (liquid film) 2930 (br) and 1740 cm^{-1}; NMR ($CDCl_3$) δ 2.9 (s, 6 H, $N(CH_3)_2$), 4.3 (s, 2 H, NCH_2), and 7.4 (br, 2 H, NH and OH).

1,3,5-Triformylhexahydro-1,3,5-triazine (39). Acetic anhydride (21.4 g) was added during 20 min to formic acid (9.7 g, 211 mmol) at −5 °C. Hexamethylenetetramine (6.7 g, 48 mmol) was added during 1.5 h such that the temperature remained below 10 °C, and then the mixture was stirred at ambient temperature for 2 h. Water (11 mL) was added and the mixture was neutralized by addition of K_2CO_3. After evaporation of the solvents under reduced pressure, the residue was extracted with $CHCl_3$. Drying (Na_2SO_4) and evaporation of the solvent furnished a white solid which was recrystallized from anhydrous EtOH to yield the hexahydrotriazine (2.4 g, 22%) as an hygroscopic white solid: mp 170.5–171.5 °C (lit.[42] mp 171–172 °C); IR 1710 and 1675 cm^{-1}; NMR ($CDCl_3$) δ 5.2 (s, 6 H, NCH_2N) and 8.25 (s, 3 H, CHO); NMR (CF_3CO_2H) δ 5.5 (s, 6 H, NCH_2N) and 8.46 (m, 3 H, CHO); MS, m/z 171 (M^+). Anal. ($C_6H_9N_3O_3$) C, H, N.

***N*-[(Benzoyloxy)methyl]formamide (41) and *N*,*N*-Bis[(benzoyloxy)methyl]formamide (42).** To *N*-(hydroxymethyl)formamide (**4**; 10.0 g, 133 mmol) and triethylamine (20 mL) in THF (100 mL) at 0 °C was added dropwise benzoyl chloride (17 mL). The mixture was stirred for 1 h at 0 °C, then filtered and concentrated under reduced pressure. The residue, in $CHCl_3$, was washed with $NaHCO_3$ and water and dried (Na_2SO_4), and the solvents were evaporated under reduced pressure. Chromatography (silica gel, chloroform) gave *N*-[(benzoyloxy)methyl]formamide (240 mg, 1%): mp 86–88 °C from aqueous MeOH; IR (KBr) 3250, 3150, 1710, and 1690 cm^{-1}; NMR (220 MHz, $CDCl_3$) δ 5.52 (d, J = 8.5 Hz, 0.67 H, CH_2 of *E* rotamer), 5.58 (d, J = 8.5 Hz, 1.33 H, CH_2 (*Z*)), 6.94 (br, 0.33 H, NH (*E*)), 7.07 (br, 0.67 H, NH (*Z*)), 7.52 (m, 2 H, Ar 3- and 5-H), 7.66 (m, 1 H, Ar 4-H), 8.11 (m, 2 H, Ar 2- and 6-H), 8.35 (s, 0.67 H, CHO (*Z*)), and 8.48 (0.33 H, d, J = 11 Hz, CHO (*E*)); MS, m/z 180 (M + 1). Anal. ($C_9H_9NO_2$) C, H, N. Evaporation of the solvents from earlier eluates afforded *N*,*N*-bis[(benzoyloxy)methyl]formamide (100 mg, 0.2%) as a colorless oil: IR (liquid film) 1720 and 1710 cm^{-1}; NMR ($CDCl_3$) δ 5.75 and 5.8 (2 x s, 4 H, CH_2), 7.3 (m, 6 H, 3-, 4-, and 5-H), 7.75 (m, 4 H, 2- and 6-H), 8.63 (s, 1 H, CHO). Anal. ($C_{17}H_{15}NO_5$) C, H, N.

***N*-(Formamidomethyl)trimethylammonium Iodide (44).** A mixture of iodomethane (10 mL) and *N*-[(dimethylamino)methyl]formamide (**9**; 5.1 g, 50 mmol) was stirred in diethyl ether at 0 °C for 2 h. The evaporation residue was recrystallized from EtOH to give *N*-(formamidomethyl)trimethylammonium iodide (5.9 g, 58%) as a white solid: mp 149–151 °C (lit.[43] mp 151–156 °C dec); IR (KBr) 3300 and 1695 cm^{-1}; NMR ($(CD_3)_2SO$) δ 3.1 (9 H, s, $N(CH_3)_3$), 4.75 (d, J = 7 Hz, 1.6 H, NCH_2N of *Z* rotamer), 5.00 (d, J = 7 Hz, 0.4 H, NCH_2N (*E*)), 8.45 (s, 0.8 H, CHO (*Z*)), 8.57 (m, 0.2 H, CHO (*E*)), and 9.4 (br, 1 H, NH).

Nitroacetonitrile (46). Freshly distilled thionyl chloride (4.3 mL) was added dropwise to a solution of 2-nitroacetaldoxime[44] (6.0 g, 58 mmol) in boiling anhydrous diethyl ether (40 mL) during 5 min, and the mixture was boiled under reflux for a further 1 h. Filtration and concentration under reduced pressure gave a yellow oil which, in diethyl ether, was washed with water and dried ($CaCl_2$), and the solvents were evaporated to give nitroacetonitrile (2.9 g, 58%) as a brown oil: IR (liquid film) 1560 and 1350 cm^{-1}; NMR ($CDCl_3$ + $(CD_3)_2SO$; 3:1) δ 5.85 (s, 2 H, CH_2); MS, m/z 120 (M^+).

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Registry No. **1**, 123-39-7; **2**, 75-12-7; **3**, 87955-92-8; **4**, 627-45-2; **5**, 24589-68-2; **6**, 58644-54-5; **7**, 34005-37-3; **8**, 68-12-2; **9**, 13052-19-2; **10**, 38952-30-6; **11**, 28919-10-0; **12**, 20546-32-1; **13**, 1608-69-1; **14**, 5129-78-2; **15**, 21239-12-3; **16**, 758-16-7; **17**, 79-16-3; **18**, 815-06-5; **19**, 127-19-5; **20**, 34891-76-4; **21**, 613-93-4; **22**, 6282-02-6; **23**, 127-07-1; **24**, 96-31-1; **25**, 632-22-4; **26**, 534-13-4; **27**, 2782-91-4; **28**, 80-70-6; **29**, 29270-77-7; (*Z*)-**30**, 86602-47-3; (*E*)-**30**, 86602-50-8; (*E*)-**31**, 73430-27-0; (*E*)-**32**, 101419-83-4; **33**, 79080-32-3; **34**, 16849-88-0; **35**, 101419-84-5; **36**, 107-97-1; **37**, 1118-68-9; **38**, 81424-67-1; **39**, 58793-59-2; **41**, 101419-85-6; **42**, 101419-86-7; **44**, 52322-57-3; **46**, 13218-13-8; HCO_2Et, 109-94-4; $Cl(CH_2)_2NH_2$·HCl, 870-24-6; HCO_2Na, 141-53-7; $F_3CCH_2NH_2$·HCl, 373-88-6; MeN-

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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

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

Kettering-Meyer Laboratory, Southern Research Institute, P.O. Box 55305, Birmingham, Alabama 35255.
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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

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