mitochondrial enzyme from the peripheral blasts of patients with chronic lymphocytic leukemia. The viral dThd kinases were extracted from dThd kinase deficient HeLa (BU-25) cells infected with HSV-1 (strain KOS) or HSV-2 (strain 333). The enzymes were purified by affinity chromatography.[39-41] To estimate the inhibition constants of the compounds, several concentrations of dThd were employed; the methods were described previously.[42] To determine the ability of these compounds to act as alternative substrates for dThd kinases, the assay of Dobersen and Greer was employed.[46] The concentration of [γ^{32}P]ATP was 0.5 mM, and the concentration of substrate was 0.4 mM. The relative phosphorylation is based on dThd as 100%. The results are presented in Table II.

Acknowledgment. This work was supported by research grants from the National Cancer Institute, from the NIH (CA-06695-21 to T.J.B.), and from the American Cancer Society (CH-20K to T.J.B. and CH-29 to Y.-C.C.).

Registry No. **1a**, 38353-06-9; **1b**, 79387-69-2; **2a**, 83768-01-8; **2b**, 88350-65-6; **3a**, 96245-83-9; **3b**, 96245-84-0; **4a**, 96245-85-1; **4b**, 96245-86-2; **5a**, 96245-87-3; **5b**, 93265-81-7; **6a**, 96245-88-4; **6b**, 96245-89-5; **7b**, 96245-90-8; **8**, 21740-23-8; α-**9**, 96291-74-6; β-**9**, 96291-75-7; α-**10**, 96291-76-8; β-**10**, 4258-01-9; **11a**, 96245-91-9; **11b**, 96245-92-0; α-Y, 96245-93-1; β-Y, 96245-94-2; α-Z, 96245-95-3; β-Z, 96245-96-4; 2-pyrimidinone, 557-01-7; 2-deoxy-D-ribose, 533-67-5.

(46) Dobersen, M. J.; Greer, S. *Anal. Biochem.* **1975**, *67*, 602.

N-[[(Mercaptoacetyl)amino]benzoyl]glycines as Mucolytic Agents

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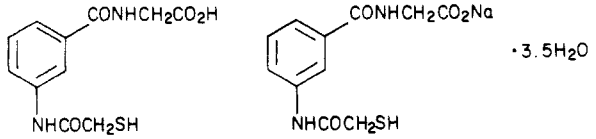
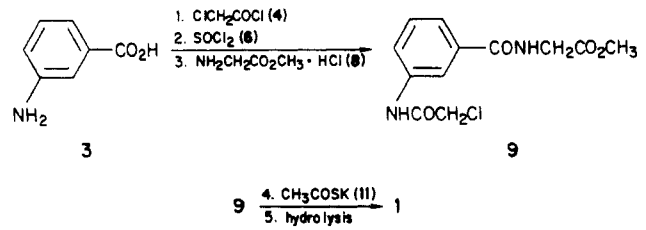
m- and *p*-aminobenzoic acids were converted to the title compounds by sequential use of $ClCH_2COCl$, $SOCl_2$, glycine methyl or ethyl ester, AcSK, and hydrolysis. The title compounds and a number of salts were compared for mucolytic activity, toxicity, stability, and hygroscopicity. When compared to *N*-acetyl-L-cysteine (NAC), the compounds exhibit several times the in vitro mycolytic activity of NAC on a molar basis. The most promising candidate appears to be the sodium salt $3.5H_2O$ **2** of the meta series.

Extensive investigations[1-4] followed the discovery of *N*-acetyl-L-cysteine (NAC)[5,6] as a mucolytic agent. The present paper deals with the preparation of the meta (**1**) and para (**21**) isomers of the title compounds (Table I) as well as six stable salts of **1** (Table II). The mucolytic activity of the representative sulfhydryl compounds is demonstrated (Tables III and IV). Compound **1** and some of its salts, especially the sodium salt $3.5H_2O$ **2**, were investigated in more detail.



1 **2**

Scheme I (See Table I, 5, 7, 9, 12)



3 **9** **9** → **1**

Chemistry. The synthesis[7] of compound **1** involved a five-step reaction (Scheme I). In step 1, anhydrous NaOAc in HOAc served as an excellent acid acceptor to give the *N*-chloroacetyl derivative **5**[8d] (Table I) in 92% yield. The preparation of the acid chloride **7** (Table I) was performed in a relatively small volume of $SOCl_2$–$CHCl_3$ by the batchwise addition of the benzoic acid derivative **5**. The glycine methyl ester derivative **9** was prepared in the reaction between **7** and glycine methyl ester hydrochloride (**8**)[9] in in the presence of $NaHCO_3$ and aqueous CH_3OH as solvent. The thiol ester **12** (Table I) was prepared in the reaction between **9** and AcSK **11**[10] in methanolic solution. Both ester moieties (one thio) of the key intermediates **12** were hydrolyzed in aqueous methanolic NaOH solution to yield the meta isomer **1** in an acceptable

(1) (a) Martin, T. A.; Sheffner, A. L. Neth Appl. 6608252, 1966; *Chem. Abstr.* **1967**, *67*, 53897n or U.S. Patent 3340147, 1967. (b) Martin, T. A.; Causey, D. H.; Sheffner, A. L.; Wheeler, A. G.; Corrigan, J. R. *J. Med. Chem.* **1967**, *10*, 1172.

(2) (a) Martin, T. A.; Causey, D. H.; Corrigan, J. R. *J. Med. Chem.* **1968**, *11*, 625. (b) Martin, T. A. *J. Med. Chem.* **1969**, *12*, 950.

(3) Martin, T. A. U.S. Patents (a) 3647834, 1972; *Chem. Abstr.* **1972**, *76*, P158364a and (b) 3749770, 1973; *Chem. Abstr.* **1973**, *79*, P83474n.

(4) Martin, T. A.; Comer, W. T. (a) Fr. Patent 2161910, 1973; *Chem. Abstr.* **1974**, *80*, 3554w; Ger. Patent 2249054, 1973; *Chem. Abstr.* **1973**, *79*, 32106m or U.S. Patents 3809697, 1974 and 3898338 1975; (b) U.S. Patent 4005222, 1977; *Chem. Abstr.* **1977**, *86*, P161300p or U.S. Patents 4096277, 1978; 4132802, **1979**; and 4178301, **1979**.

(5) Acetyl-L-cysteine is the generic name for Mucomyst, Mead Johnson and Co.

(6) (a) Martin, T. A.; Corrigan, J. R.; Waller, C. W. *J. Org. Chem.* **1965**, *30*, 2839. (b) Martin, T. A.; Waller, C. W. U.S. Patent 3184505, 1965; *Chem. Abstr.* **1965**, *63*, P7107d. (c) Sheffner, A. L. *Pharmacotherapeutica* **1965**, *1*, 46. (d) Hamlow, E. E.; Martin, T. A. Ger. Patent 2305271, **1973**; *Chem. Abstr.* **1973**, *79*, P139653q or U.S. Patent 3965167, 1976. (e) Sheffner, A. L. U.S. Patent 3091569, 1963; *Chem. Abstr.* **1963**, *59*, 11661e.

(7) Martin, T. A. U.S. Patents (a) 4093739, 1978; *Chem. Abstr.* **1978**, *89*, P163958v and (b) 4132803, 1979; *Chem. Abstr.* **1979**, *90*, P187348t.

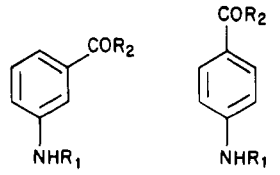
(8) (a) This compound **31** was prepared similarly to preparative method H. (b) Friedman, M. "The Chemistry and Biochemistry of the Sulfhydryl Group in Amino Acids, Peptides and Proteins", 1st ed.; Pergamon Press: New York, 1973; Chapters 3, 8. (c) Reference 8b, pp 292–293. (d) *Beilstein 14(I)*, 562.

(9) The use of glycine ethyl ester gives **10** in 76% yield.

(10) The use of intermediate **10** and EtOH as reaction solvent gives **13** in 84% yield.

0022-2623/85/1828-0910$01.50/0

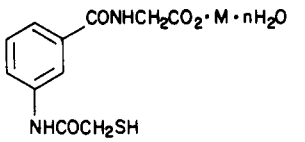
Table I. Products and Their Intermediates



no.	R_1	R_2	prep[a]	recrystn[b]	yield, %	mp, °C	formula	anal.[e]
5	$COCH_2Cl$	OH	A	O	92, 77	228–231	$C_9H_8ClNO_3$	C, H, N
7	$COCH_2Cl$	Cl	B		80	106–108	$C_9H_7Cl_2NO_2$	
9	$COCH_2Cl$	$NHCH_2CO_2CH_3$	C	P	77	122–127	$C_{12}H_{13}ClN_2O_4$	C, H, N
10	$COCH_2Cl$	$NHCH_2CO_2C_2H_5$	C	Q	76	119–121	$C_{13}H_{15}ClN_2O_4$	C, H, N
12	$COCH_2SCOCH_3$	$NHCH_2CO_2CH_3$	D	P	86	90–92	$C_{14}H_{16}N_2O_5S$	C, H, N
13	$COCH_2SCOCH_3$	$NHCH_2CO_2C_2H_5$	E	Q	84	111–114	$C_{15}H_{18}N_2O_5S$	C, H, N
14	$COCH_2SH$	$NHCH_2CO_2C_2H_5$	F	Q	93	84–85	$C_{13}H_{16}N_2O_4S$	C, H, N
1	$COCH_2SH$	$NHCH_2CO_2H$	G		89	207–209[c]	$C_{11}H_{12}N_2O_4S$	C, H, N, SH
16	disulfide of 1		H	R	79	189.5–191.5	$C_{22}H_{22}N_4O_8S_2$	C, H, N
17	$COCH_2Cl$	OH	A[d]		95	225.5–258.5	$C_9H_8ClNO_3$	C, H, N
18	$COCH_2Cl$	Cl	B		66	131–135	$C_9H_7Cl_2NO_2$	
19	$COCH_2Cl$	$NHCH_2CO_2C_2H_5$	C	S	72	172–174	$C_{13}H_{15}ClN_2O_4$	
20	$COCH_2SCOCH_3$	$NHCH_2CO_2C_2H_5$	E	S	53	169–170	$C_{15}H_{18}N_2O_5S$	C, H, N
21	$COCH_2SH$	$NHCH_2CO_2H$	G		90	217.5–218.5[c]	$C_{11}H_{12}N_2O_4S$	C, H, N, SH

[a] The preparative methods are given in the Experimental Section. [b] Recrystallization solvents: O = EtOAc, P = CH_3OH, Q = anhydrous EtOH, R = DMF–H_2O, S = EtOH. [c] With decomposition. [d] Prepared similar to aq method A, except $^1/_6$ mol of K_2HPO_4 is included in the reaction mixture and also CH_2Cl_2 as the cosolvent. [e] See Experimental Section for the limits.

Table II. Salts of 1



no.	M	*n*	prep[a]	yield, %	mp,[b] °C	formula	anal.[e]
22	Na	1	*c*		117–165	$C_{11}H_{11}N_2O_4SNa·H_2O$	C, H, N, SH
23	Na	$1^1/_2$	I	93	74–124	$C_{11}H_{11}N_2O_4SNa·1^1/_2H_2O$	C, H, N, SH
24	Na	2	I[d]	66	108–136	$C_{11}H_{11}N_2O_4SNa·2H_2O$	C, H, N, SH
2	Na	$3^1/_2$	J	88	68–120	$C_{11}H_{11}N_2O_4SNa·3^1/_2H_2O$	C, H, N, SH
25	K	1	K	93	175	$C_{11}H_{11}N_2O_4SK·H_2O$	C, H, N, SH
26	NH_4	0	L	88	144–154	$C_{11}H_{11}N_2O_4S·NH_4$	C, H, N, SH
27	NH_4	$1^1/_2$	M	79	56–119	$C_{11}H_{11}N_2O_4S·NH_4·1^1/_2H_2O$	C, H, N, SH
28	$NH_3(CH_2)_2OH$	0	N	93	144–160	$C_{11}H_{11}N_2O_4S·C_2H_8NO$	C, H, N, SH
29	$NH_2(CH_2H_2OH)_2$	0	N	82	64–74.5	$C_{11}H_{11}N_2O_4S·C_4H_{12}NO_2$	C, H, N, SH
30	$NH_3(CH_2)_2NH_3$	0	N	70	204.5–205.5	$(C_{11}H_{11}N_2O_4S)_2·C_2H_{10}N_2$	C, H, N, SH

[a] The preparative methods are given in the Experimental Section. [b] With decomposition. [c] On drying **24** at 60 °C under vacuum over $CaCl_2$. [d] Dried only 1 h in the vacuum over $CaCl_2$. [e] See Experimental Section for the limits.

Table III. Comparative in Vitro Mucolytic Activities of 1 and NAC Using Human Sputum[a]

	% viscosity reduction at the following time, min		
treatment	5	10	15
saline[b]	26	20	2
1 (0.03 M)[c]	64	84	90
NAC (0.03 M)[c]	27	38	31

[a] Pooled human sputum, adjusted to pH 7.5. [b] 0.9%, w/v solution. [c] pH 7.5 solutions in 0.9% saline.

Table IV. Comparative in Vitro Mucolytic Activities of 1, 21, and 1 Salts on Pooled Human Sputa[a,b]

addition	compd form	% viscosity reduction at 15 min	rel act., %
1	solution	60	100
2	powder	56	94
21	solution	73[c]	
27	powder	59	99
30	powder	44	74

[a] Adjusted to pH 7.0. Neutralized solutions of 1 or powders of the salts were employed. [b] Final concentrations of 1 and its salts were 10 mM. [c] Compared to 0.06 M NAC with percent viscosity reduction of 15.

state of purity.[11] However, the thiol ester moiety may be selectively hydrolyzed; for example, the ethyl ester **13**, which is the equivalent of the methyl ester **12**, was treated with a sodium ethoxide solution to give the mercapto ethyl ester **14** (Table I) and ethyl acetate.

Compound **1** was oxidized with iodine in the presence of piperazine to its disulfide. This insoluble piperazinium salt **15** served as an easy mode of purification. On acidification of **15**, the disulfide **16** was obtained. The intermediates **17–20** in the para series and the para isomer **21** (Table I) were prepared in the same manner described for the synthesis of the meta isomer **1**.

Six different salts of **1** (Table II) were prepared. In general, the reaction solvents included CH_3OH, THF, or aqueous THF. The Na salt was isolated as three metastable hydrates: mono **22**, sesqui **23**, and di **24**, and as the stable hydrate, $3.5H_2O$ **2**. The K salt exists as the monohydrate **25**. The NH_4 salt was isolated in either the anhydrous **26** or the sesquihydrate **27** form. Two other alkylammonium salts, **28** and **29**, and the bissalt with

(11) In general, analytically pure product **1** is obtained when the methanolic reaction solution of **12** or **13** is treated with carbon prior to reaction.

Table V. Powder Stability of Micronized Sodium Salt of *N*-[3-[(Mercaptoacetyl)amino]benzoyl]glycine–3.5-Hydrate (2)

storage conditions		% disulfide[a]					
temp, °C	head space						
		6 days	8 days	15 days	26 days	35 days	
room temp	air	7.6	7.4	7.7	7.3	7.7	
	O_2	7.4	7.2	7.3	7.1	7.2	
		4 days	7 days	14 days	27 days		41 days
40	air	7.2	8.1	7.8	8.8		9.0
	O_2	8.9	8.8	9.2	10.0		10.4
			7 days	16 days	28 days		
50	air		9.9	14.9	23.5		
	O_2		15.4	34.2			

[a] At zero days the disulfide content was 6.5%.

Table VI. Powder Stability of Micronized Ammonium Salt of *N*-[3-[(Mercaptoacetyl)amino]benzoyl]glycine Sesquihydrate (27)

storage conditions		% disulfide				
temp, °C	head space	0 days	7 days	14 days	34 days	42 days
room temp	air	3.7	3.8	3.4	4.7	3.9
	O_2	3.7	3.7	4.2	4.1	3.8
40	air	3.7	4.8	6.0	9.7	10.1
	O_2	3.7	5.4	9.0	29.4	67.4
50	air	3.7	10.3	14.8	26.2	27.6
	O_2	3.7	16.5	21.7	31.0	45.0

Table VII. Powder Stability of the Ethanolamine Salt of *N*-[3-[(Mercaptoacetyl)amino]benzoyl]glycine (28)

storage conditions		% disulfide					
temp, °C	head space	0 days	8 days	15 days	27 days	42 days	56 days
room temp	air	3.4	4.0	3.2	3.5	3.6	
	O_2	3.4	3.2	3.2	3.3	3.5	3.5
40	air	3.4	3.7	3.8	4.4	5.3	
	O_2	3.4	3.5	3.7	4.1	3.8	4.5
50	air	3.4	4.6	4.8	7.6	11.1	14.0
	O_2	3.4	3.9	4.4	10.7	14.4	26.2

ethylenediamine **30** were also prepared in anhydrous form.

Mucolytic Activity.[12] Compound **1** and its salts, especially the Na **2**, NH_4 **27**, and ethylenediammonium **30**, are 8–14 times[13] more active than NAC on a molar basis. Tables III and IV give a summary of these data. It is noted that **1** and its salts **2**, **27**, **30** give quite similar mucolytic potencies. When compared to NAC, these compounds show a more rapid onset of mucolysis and exhibit their activity over a broader pH range. The mucolytic potency of the para isomer **21** appears to be quite similar to that exhibited by the meta isomer **1**.

Toxicities. The preliminary toxicity studies with the Na salt $3.5H_2O$ **2** indicate a relatively nontoxic substance substantially free of other pharmacological action. No deaths were observed in mice or rats that received oral doses up to 5000 mg/kg. The LD_{50} of an intravenous (iv) solution of **2** in mice was found to be 1304 mg/kg. When **2** was administered up to 100 mg/kg to dogs, no remarkable effects were noted; however, on repeated dose study, sporadic emesis occurred. When **1** was intubated into rats at levels of 0, 45, 141, or 450 mg/kg/day (po) for 28 days, no untoward effects were noted.

The para isomer **21**, when dosed to white mice up to 500 mg/kg (ip) and up to 2000 mg/kg (po), shows low-potency central nervous system (CNS) depression. In dogs at 10 mg/kg (iv), **21** exhibits some parasympatholytic activity.

Stability Studies.[7] To determine whether **1** or its powdered salts **2**, **27**, and **28** are stable to normal atmospheric conditions, i.e., do not oxidize to the disulfide **16**, samples were stored with air or oxygen from room temperature (24–27 °C) to 50 °C and for 41, 42, and 56 days, respectively. The contents were analyzed at time intervals by HPLC. The three salts (see Tables V–VII) are relatively stable at room temperature under air or oxygen. However, at 40 °C both **2** and **28** are substantially more stable than **27**, which shows a relative instability after 14 days.

Table VIII. Stability of **2**, **27**, and NAC·NH_3 as 5% Solution in D_2O

	% disulfide			
days	**2** (air)[a]	**27** (air)[a]	NAC·NH_3 (air)[a]	**27** (N_2)[b]
0	3.0	1.6	10.8	4.7
1	5.7	7.0	15.7	5.6
2		15.4	18.7	
3	29.4			
4	28.2			4.5
5		29.6	20.1	4.3
6		36.2	21.5	
7	48.4	40.2	19.8	4.3
8	51.6	45.8	23.4	3.6
9	59.3	50.4	23.5	
10	59.9			
11	61.7			6.5
12		59.4	24.6	6.8
13		64.9	26.4	4.8
14				9.5
15		69.8	29.1	7.7
16		70.7	31.4	
17	72.8			
18				8.6
19		78.5	33.3	6.9
20	77.4	79.9	41.5	7.4

[a] On the indicated days, the NMR tubes were opened momentarily, shaken, and stored at 37 °C. [b] Trace quantities of ethylenediaminetetraacetic acid disodium salt were included in this solution.

(12) Lieberman, J. *Am. Rev. Resp. Dis.* **1968**, *97*, 654, 662.
(13) The compounds were compared in an appropriate crossover study against pooled sputum.

Table IX. Hygroscopicity of *N*-[3-[(Mercaptoacetyl)amino]benzoyl]glycine Salts at 58% Relative Humidity

salt	initial mol of H_2O of hydration	days	moisture gain, %	hydroscopicity
sodium **23**	1.5	6	9.0	yes
sodium **2**	3.5	10	−0.05	no
potassium **25**	1	10	2.1	yes
ammonia **27**	1.5	10	−0.28	no
ethanolamine **28**	0	12	2.1	yes
diethanolamine **29**	0	11	7.7	yes

Similarly, stability studies were determined on **2** and **27** in D_2O solution by NMR with L-*N*-acetylcysteine ammonium salt (NAC·NH_3)[6d] and its disulfide **31**[8a] as standard references. The percentage of disulfide formed by air oxidation during 20 days is given in Table VIII. As expected, the oxidative results for **2** and **27** are quite similar. After 20 days, the disulfide content was about 80%, which indicates that **2** and **27** are less stable than NAC·NH_3 (about 40% disulfide). However, when **27** was evaluated again, except that the solution contained a trace of ethylenediaminetetracetic acid disodium salt and was stored under nitrogen, only about 2–3% of disulfide formed during the 20 days. When compared to NAC, the data relative to the more potent mucolytic compounds, i.e., **1** or **2**, but less stable to oxidation are in agreement[8b] with our pK_a values for the sulfhydryl group: **1**, 8.84; NAC, 9.70.

Hygroscopicity.[7] As insufflation is a desired mode of administering these salts, the medicament must be relatively nonhygroscopic. Compounds **2**, **23**, **25**, **27**, **28**, and **29** were subjected to a relative humidity of 58% for 6–12 days. The sodium salt $3.5H_2O$ **2** and the ammonium salt sesquihydrate **27** are relatively nonhygroscopic (see Table IX).

Experimental Section

Except for compounds **7**, **15**, and **18** (not run), the IR and NMR spectra were consistent with the structures. Except for **7**, **15**, **18**, and **19**, the elemental analyses values were determined. Where analyses are indicated for C, H, and N, analytical values are within ±0.4% of the calculated values. When SH is indicated, SH analyses are obtained by the *N*-ethylmaleimide reagent[8c] and are within ±0.6% of calculated values. Melting points are uncorrected (Thomas-Hoover capillary apparatus). In general, all preparative operations involving sulfhydryl compounds are carried out in an atmosphere of N_2, using deionized H_2O.

A. 3-[(Chloroacetyl)amino]benzoic Acid (5).[8d] After dissolution first of 19 g (0.23 mol) of NaOAc and then of 13.7 g (0.1 mol) of **3** in 150 mL of HOAc by warming at 70 °C, 11.3 g (0.1 mol) of **4** was added slowly at 40–45 °C. As the reaction mixture thickens, 75 mL of $CHCl_3$ was added. After a stirring period at 26 °C for 16 h, the solid was collected, washed, and slurried with dilute HCl to give 19.6 g (92%) of **5**.

The product **5** may be prepared similarly in 77% yield: chloroacetyl chloride (**4**; 22.7 g, 0.2 mol) and 20 mL of 40% NaOH were added simultaneously during 0.5 h to a cold (0–5 °C) mixture of 27.4 g (0.2 mol) of **3** and 140 mL of 1.43 N NaOH. The reaction mixture was first stirred at 26 °C for 3 h and then acidified at 10–15 °C with 40 mL of 6 N HCl. The solid was collected, washed with H_2O, and dried in vacuo at 60 °C; yield, 32.9 g (77%).

B. 3-[(Chloroacetyl)amino]benzoyl Chloride (7).[7] Compound **5** (150 g, 0.7 mol) was added in three equal portions at 0.5-h intervals to a warm (55–60 °C) solution of 250 mL of $SOCl_2$, 50 mL of $CHCl_3$, and 6 drops of pyridine. After the addition is complete, the reaction mixture was warmed at a temperature of 55–60 °C for 2 h, cooled, and diluted with 500 mL of Skellysolve F (bp 30–60 °C). The precipitate that formed was collected, washed with Skellysolve F, and dried under vacuum to give 143 g (80%) of **7**.

C. *N*-[3-[(Chloroacetyl)amino]benzoyl]glycine Methyl Ester (9).[7] To a mixture of **8** (358 g, 2.85 mol) and 2.4 L of H_2O was added $NaHCO_3$ (239.4 g, 2.85 mol). The reaction mixture was stirred at room temperature for 0.5 h, then diluted with 1 L of CH_3OH, and cooled to 0 °C, while $NaHCO_3$ (239.4 g, 2.85 mol) was added followed by portionwise addition of **7** (650 g, 2.8 mol) during 0.5 h. Considerable foaming takes place when approximately half of **7** had been added and was controlled by periodic addition of small quantities of CH_3OH. The reaction mixture was stirred for 16 h, diluted with 800 mL of H_2O, and then stirred for an additional 0.5 h to provide a solid, which was collected, washed with 500 mL of 50% CH_3OH, 2 L of H_2O, and 500 mL of 50% CH_3OH; yield, 626.7 g (77%).

D. *N*-[3-[[(Acetylthio)acetyl]amino]benzoyl]glycine Methyl Ester (12).[7] A solution of **11** was prepared by adding 495 mL of 2 N CH_3OH–KOH (0.99 mol) duuring a period of 15 min to a solution of 74.3 mL (79.1 g, 1.04 mol) of CH_3COSH and 500 mL of CH_3OH while a temperature of 1–5 °C was maintained. This solution was added during 0.5 h to a mixture of **9** (267.5 g, 0.94 mol) in 2 L of CH_3OH while a temperature of 0–5 °C was maintained. The reaction mixture was first stirred for 1 h and then at 40–45 °C for 2 h and finally cooled and filtered. The solid was washed with 50% CH_3OH, H_2O, and 50% CH_3OH; yield, 262.4 g (86%).

E. *N*-[3-[[(Acetylthio)acetyl]amino]benzoyl]glycine Ethyl Ester (13). A total of 6 g (0.053 mol) of **11** was added at 10–15 °C to a mixture of 15 g (0.05 mol) of **10** and 200 mL of 50% EtOH. The reaction mixture was heated at 55–60 °C for 16 h. On cooling, an oil separated. It was slurried with H_2O to give 14.2 g (84%) of solid.

F. *N*-[3-[(Mercaptoacetyl)amino]benzoyl]glycine Ethyl Ester (14). A mixture of 9 g (0.0266 mol) of **13** and 150 mL of anhydrous EtOH was warmed at 50 °C until a solution resulted and then a solution of 0.7 g (0.03 mol) of Na dissolved in 50 mL of anhydrous EtOH was added at 15–20 °C during 15 min. The reaction mixture was stirred at 25 °C for 4 h and then acidified in the cold (5–10 °C) with 5 mL of 7.2 N EtOH–HCl. The NaCl was collected (1.7 g). The filtrate was first concentrated and the resulting oil was then stirred with EtOAc–Skellysolve B to give on cooling 7.3 g (93%) of product.

G. *N*-[3-[(Mercaptoacetyl)amino]benzoyl]glycine (1).[7] To a warm solution of 280 g (0.863 mol) of **12** and 2.5 L of CH_3OH was added DARCO G-60. This mixture was stirred for 10 min and the charcoal was collected and washed with CH_3OH. The filtrate was cooled at 5–15 °C while 1.1 L (2.75 mol) of 10% NaOH was added during 20 min. The reaction mixture was stirred at 26 °C for 2 h and then cooled to 5 °C and acidified with 500 mL (3 mol) of 6 N HCl. The suspension was stirred in the cold for 20 min. The solid was collected and washed first with 50% CH_3OH, then H_2O, and finally with 50% CH_3OH; yield, 205.3 g (89%).

H. 2,2′-Dithiobis[*N*-[3-[[(carboxymethyl)amino]carbonyl]phenyl]acetamide] (16). To a cold mixture (15–20 °C) of 26.8 g (0.1 mol) of **1** and 200 mL of CH_3OH were added simultaneously during 20 min a solution of 25.4 g (0.1 mol) of I_2 and 400 mL of CH_3OH and a solution of 20 g (0.103 mol) of piperazine hexahydrate and 400 mL of CH_3OH. The reaction mixture was stirred for 64 h at 25 °C. The solid **15** was collected and washed first with CH_3OH and then with 50% aqueous CH_3OH. The solid was slurried with 250 mL of H_2O and acidified with 100 mL of 1 N HCl. The suspension was stirred at 25 °C for 18 h. The white solid was collected, washed with H_2O, and air-dried; yield, 21.1 g (79%) of **16**.

Salts of 1. I. Na Salt Sesquihydrate 23.[7] A batch of 1.5 mL of 10% NaOH was slowly added to a mixture of 1 g (0.00373 mol) of **1** and 1 mg of disodium edetate in 20 mL of THF with cooling. The reaction mixture was allowed to stand for 0.5 h. The solid was collected, washed with THF, and dried for 16 h in vacuo over $CaCl_2$ to provide 1.1 g (93%) of **23**.

J. Na Salt $3.5H_2O$ 2.[7] A batch of 65 mL (0.1625 mol) of 10% NaOH was slowly added to a mixture of 43 g (0.16 mol) of **1** and 230 mL of THF with cooling at 10–20 °C. The reaction mixture was warmed to about 25 °C and a trace of solid was collected. The filtrate was diluted with 480 mL of THF. After 2 h, the solid was collected, washed with THF, and air-dried to give 50 g (88%) of **2**.

Exposure of **22** (Table II, footnote *c*) to air for 3 days provided this stable hydrate.

K. K Salt Hydrate 25.[7] A total of 8.65 mL of 2.5 N (0.0186 mol) of KOH was added slowly to a mixture of 5 g (0.0186 mol) of 1 and 5 mg of disodium edetate in 40 mL of THF. After the mixture warmed to 45 °C to give a complete solution, 200 mL of THF was added to give the solid. The mixture was first cooled with a dry ice–EtOH bath to promote crystallization and then allowed to stand at 25 °C for 16 h. The solid was collected, washed with THF, and dried in vacuo over $CaCl_2$ to give 5.6 g (93%) of **25**.

L. Ammonium Salt 26.[7] To a mixture of 0.8 g (0.003 mol) of 1 and 8 mL of CH_3OH was added dropwise 1.1 mL (0.0033 mol) of 3 N NH_4OH under N_2. The N_2 atmosphere was maintained and solvent was removed by warming at 40–45 °C. The residual solid was stirred with 2-PrOH for 16 h, collected on a filter, washed with acetone, and dried in a vacuum desiccator over KOH to give 0.75 g (88%) of **26**.

M. Ammonium Salt Sesquihydrate 27.[7] A total of 7.2 mL (0.0216 mol) of 3 N NH_4OH was slowly added to a mixture of 5 g (0.0186 mol) of 1, 5 mg of disodium edetate, and 40 mL of THF. The resulting solution was diluted with 300 mL of THF. The reaction mixture was first cooled with a dry ice–EtOH bath to promote crystallization and then stirred for 16 h. The solid was collected, washed with THF, and air-dried to give 4.6 g (79%) of **27**.

N. (2-Hydroxyethyl)ammonium Salt 28.[7] A solution of 1.13 mL (1.15 g, 0.0188 mol) of ethanolamine and 10 mL of THF was added to a mixture of 5 g (0.0186 mol) of 1, 5 mg of disodium ededate, and 46 mL of 87% aqueous THF. The reaction mixture was first warmed to 40–45 °C to give a solution and then 200 mL of THF was added to precipitate the product. The product was isolated as described in preparative method M. However, the compound was dried in vacuo over $CaCl_2$ to afford 5.7 g (93%) of **28**.

Preparation of Pooled Human Sputum Substrate. Frozen sputa from patients with chronic bronchitis or asthma were thawed at room temperature and kept chilled on ice. The sputa were mixed by homogenization in a Potter-Elvehjem homogenizer (three strokes) and combined in a large Erlenmeyer flask. Pooled sputa were again homogenized, subdivided into tubes (ca. 18 mL), and stored at −20 °C.

Mucolytic Testing.[12] Frozen pooled sputum was thawed, mixed in a Potter-Elvehjem homogenizer (10 strokes), and kept on ice. Sputum was adjusted to the desired pH, and data were obtained with use of a Wells-Brookfield microviscometer set to a clearance of 5×10^{-4} in. and fitted with a pneumatic transducer. A 2-mL aliquot of the sputum is placed in the center of a viscometer plate and allowed to equilibrate to 37 °C for 2 min. The cone is then rotated at gradually increasing speed up to 100 rpm during 2 min. The rotation is reduced to give a convenient reading for 1 min. The test compounds were added as dry powders or as a 0.2-mL aliquot of solution and readings were recorded for 15 min.

Calculation of Percent Mucolysis. The effects of the various sulfhydryl compounds on sputum viscosity were calculated from the following equation:

$$\% \text{ mucolysis} = \left(\frac{V_1 - V_2}{V_1}\right) \times 100$$

where V_1 represents the initial sputum viscosity reading and V_2 represents the sputum viscosity reading at specified times after compound addition to the sputum.

Stability Studies in D_2O Solution. A total of 50 mg of the compounds (**2**, **27**, NAC·NH_3) was placed in the NMR tube and 1 mL of D_2O was added. The extent of disulfide formation, determined by a Varian T-60 NMR spectrometer, is given in Table VIII from 0 to 20 days. L-*N*,*N*-Diacetylcystine ammonium (1:2) salt sesquihydrate **31**[8a] was employed as reference material. The values on the indicated days were determined by the difference in the areas of the methylene loss in the CH_2SH region, NAC δ 2.84 (Me_2SO-d_6) and **26** δ 3.25 (D_2O), and the methylene gain in the $(SCH_2)_2$ region, **31** δ 3.11 (Me_2SO-d_6) and **16** δ 3.81 (Me_2SO-d_6).

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Synthesis of the Antileukemic Agents 5,10-Dideazaaminopterin and 5,10-Dideaza-5,6,7,8-tetrahydroaminopterin[1]

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Total syntheses from pyridine precursors of 5,10-dideazaaminopterin (1) and 5,10-dideaza-5,6,7,8-tetrahydroaminopterin (2) are described. These compounds exhibit significant in vivo activity against L1210 leukemia that is comparable to that observed with methotrexate.

Despite the established position of methotrexate as a clinical agent for the treatment of lymphocytic leukemia and choriocarcinoma, its extreme toxicity, coupled with its ineffectiveness against most other types of human cancers,[2,3] have provided continuing incentives for the development of less toxic drugs with more selective transport properties that might be effective against a broader range of human cancers. Various deaza analogues of folic acid, methotrexate, and aminopterin (the 10-desmethyl derivative of methotrexate) have shown particular promise; 10-deazaaminopterin, for example, is a

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