(38) Results to be published. State 4 basal respiration of Ehrlich ascites cells was depressed 38% by helenalin and 48% by tenulin at 0.2 mg. State 3 ADP stimulated respiration was reduced 40% in the presence of helenalin and 30% of tenulin (p = 0.001 for both drugs).

(39) G. W. Snedecor in "Statistical Methods", The Iowa State College Press, Ames, Iowa, 1956, Section 2.

(40) Reaction of equimolar cyclopentenone and L-cysteine in 0.067 phosphate buffer–D_2O (pH 7.4) for 20 min gave the same product (**6**) as analyzed by NMR.

Pyrimidinylpropenamides as Antitumor Agents. Analogues of the Antibiotic Sparsomycin

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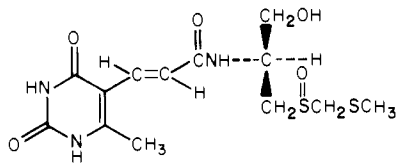
A series of pyrimidinylpropenamides **9** and their oxidation products **10** was prepared, as analogues of sparsomycin (**1**), for antitumor evaluation. Syntheses involved condensation of the appropriate amino alcohol **5** with acid **8**. The resulting sulfides **9** were then oxidized with $NaIO_4$ or H_2O_2 to sulfoxides **10**. Activity was studied in lymphocytic leukemia P-388 and KB cell culture. With the exception of the *n*-decyl analogue, all of the deoxygenated compounds **9** were inactive regardless of the stereochemical form. In the sulfoxide series **10**, those compounds prepared with an L configuration at the asymmetric carbon were also inactive. The completely racemic sulfoxides, on the other hand, displayed substantial antitumor activity (ILS = 37–61% in P-388; ED_{50} = 1.2–2.4 μg/ml in KB) suggesting that both the presence of a sulfoxide moiety and a D configuration at the chiral carbon atom were structural requirements for a positive antitumor response. There appeared to be a large tolerance for the group substituted at the sulfoxide moiety, however.

Sparsomycin (**1**) is a fermentation product of *Streptomyces sparsogenes*, first isolated in 1962.[1] Soon after this, **1** was subjected to several preliminary biological tests where it displayed a broad spectrum of moderate in vitro activity against bacteria and moderate antifungal activity.[2] It also showed moderate to high inhibition in several in vivo tumor systems such as the Walker carcinosarcoma 256 and the sarcoma 180 solid tumor.[2]

Its biological activity appears to be primarily due to inhibition of protein synthesis, and this inhibition has been substantiated.[3,4] Further work[5] indicated that its mechanism of action in the *Escherichia coli* system is on the 50S ribosome subunit, where it prevents peptide transfer by interfering with the function of the enzyme peptidyl transferase. Clinical testing of sparsomycin, however, revealed a severe eye toxicity, limiting the



1

usefulness of **1** in cancer chemotherapy.[6] Consequently, a synthetic program was initiated in our laboratories in an attempt to exploit the antitumor activity of sparsomycin in a molecule presenting more selective pharmacological properties while determining the minimum structural and stereochemical requirements necessary for antitumor activity.

Our initial investigations involved the synthesis of several N-substituted 3-aryl-2-propenamides **2** which proved to be essentially inactive.[7] In this report, we wish

trans-ArCH=CHCONHCH(CH_2OH)$CH_2SCH_2SCH_3$

2

to relate the synthesis and biological activity of various stereochemical forms of the pyrimidinylpropenamides **9** and their oxidation products **10**.

Chemistry. The synthetic route employed for the preparation of the intermediate amino alcohols **5** is outlined in Scheme I. Reduction of cystine in the appropriate stereochemical form with Na and liquid ammonia followed by addition of the substituted chloride (iodide for **3h** and bromide for **3e**) generated the amino acids (**3a,b,d–j**). Compound **3c** was purchased from the Aldrich Chemical Co. These materials were converted into their methyl or ethyl ester hydrochlorides **4** by thionyl chloride in methanol (**4a–c**) or ethanol (**4d–h**) or by ethanol saturated with gaseous HCl (**4i,j**). Lithium borohydride treatment conveniently led to the desired amino alcohols **5a–j** isolated as the hydrochloride salts.

Scheme I

$$NH_2CHCH_2SSCH_2CHNH_2 \xrightarrow[2.\ RX]{1.\ Na,\ NH_3(l)} NH_2CH(CO_2H)CH_2SR$$
$$CO_2H \qquad CO_2H \qquad\qquad \textbf{3}$$

$$\xrightarrow[HCl(g),\ R'OH]{SOCl_2,\ R'OH\ or} HCl\cdot NH_2CH(CO_2R')CH_2SR$$

4, R' = CH_3 or CH_2CH_3 (see text for appropriate group)

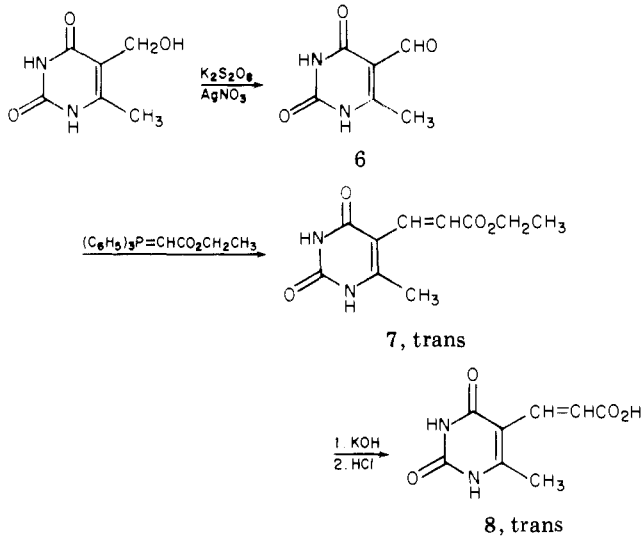
$$\xrightarrow[2.\ HCl(g)]{1.\ LiBH_4} HCl\cdot NH_2CH(CH_2OH)CH_2SR$$

5

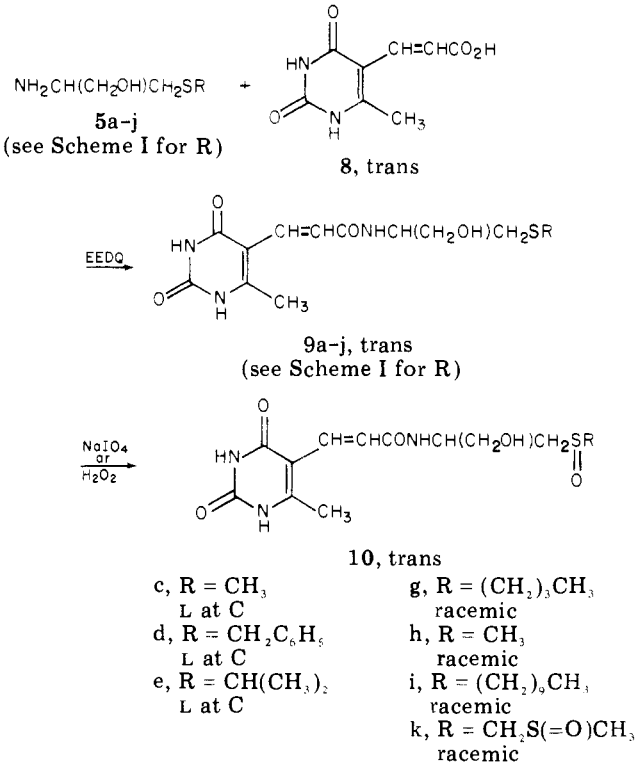
a, R = CH_2SCH_3 L stereoisomer	f, R = CH_2SCH_3 DL mixture
b, R = $(CH_2)_3CH_3$ L stereoisomer	g, R = $(CH_2)_3CH_3$ DL mixture
c, R = CH_3 L stereoisomer	h, R = CH_3 DL mixture
d, R = $CH_2C_6H_5$ L stereoisomer	i, R = $(CH_2)_9CH_3$ DL mixture
e, R = $CH(CH_3)_2$ L stereoisomer	j, R = CH_2SCH_3 D stereoisomer

Oxidation of 5-hydroxymethyl-6-methyluracil with potassium persulfate and silver nitrate[8] (Scheme II) gave the 5-formyl derivative **6**. Treatment with carbethoxymethylenetriphenylphosphorane[9] in DMF at 90 °C followed by KOH–EtOH hydrolysis[9] of the resulting ester **7** yielded the uracilacrylic acid **8** exclusively in the trans configuration.

Scheme II



Scheme III



This intermediate was subsequently coupled with the appropriate amino alcohol **5** as the free base by means of the versatile reagent *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ)[10] as shown in Scheme III, resulting in the formation of the pyrimidinylpropenamides (**9a–j**).[11] Several of these were converted to their sulfoxide analogues. Specifically, compounds **9c–e** were oxidized with sodium metaperiodate to the diastereomeric mixtures **10c–e** with an L configuration at the asymmetric carbon atom. Since the absolute configuration of sparsomycin at the sulfur asymmetric center has never been determined, no direct attempt was made to separate these mixtures or to determine their stereoisomer compositions.

Under similar conditions, the completely racemic derivatives **10g** and **10h** were prepared from the appropriate sulfides **9g** and **9h**.

Conversion of the *n*-decyl analogue **9i** to racemic **10i** could not be accomplished by periodate oxidation but utilization of 30% H_2O_2 did prove successful.

Disulfoxide **10k** in racemic form was smoothly prepared from the corresponding disulfide **9f** and 2 mol of sodium metaperiodate.

Table I. Antitumor Activity[a]

Compd no.	P-388 lymphocytic leukemia[b,c] Dose[d]	P-388 lymphocytic leukemia[b,c] T/C[e,f]	KB cell culture, ED_{50}[g,h]
9i	100[i]	155	
10g	5.0[j]	161	1.2[k]
10h	256[j]	137	1.5[k]
10i	6.25[i]	153	
10k	5.0[j]	137	2.4[i]
Sparsomycin	0.3[j]	156	0.08[j]

[a] Protocols and tumor systems described in ref 12. [b] QD 1–9 treatment. [c] Drug given intraperitoneally. [d] In mg/kg. [e] T/C = (treated survival/control survival) × 100. [f] All T/C results reported for the active compounds (see text for definition of activity) have been reproduced in a minimum of one additional experiment to give values not more than 8% lower than the value shown. All actives are confirmed active (at least on additional active test). [g] ED_{50} = dose in μg/ml that inhibits growth to 50% of control growth. [h] All ED_{50} results reported for the new active compounds (see text for definition of activity) have been reproduced in a minimum of one additional experiment to give values not more than 100% higher than the value shown. All actives are confirmed (at least one additional active test). [i] H_2O + Tween 80 as vehicle. [j] H_2O as vehicle. [k] Propylene glycol as vehicle. [l] Saline as vehicle.

Biological Results. The antimitotic activity of the synthetic propenamides **9** and **10** was measured in lymphocytic leukemia P-388 and KB cell culture by standard protocols of the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health.[12] Compounds are considered active if they give reproducible T/C activity[12] values in the P-388 leukemia system equal to or greater than 125% where T/C represents the ratio of the mean or median survival times of the treated animals over those of the control animals expressed as a percentage. In the case of KB cell culture, an ED_{50} (dose that inhibits growth to 50% of control growth) equal to or less than 4 μg/ml is considered active.

All T/C results reported for the active compounds have been reproduced in a minimum of one additional experiment to give values not more than 8% lower than the value shown.

ED_{50} results have also been reproduced but display more variability. Results up to 100% higher were found. Except for **9i** (see Table I), all of the sulfides **9a–f,h–j** were found to be inactive when tested in vivo or in vitro. (See paragraph at end of paper regarding supplementary material.) (Compound **9g** was directly converted to **10g** and not screened.) Several of the sulfoxide analogues **10** were active, however, and the results are also reported in Table I where they are compared to the parent, sparsomycin.

Discussion

The pyrimidinylpropenamides **9** retained a sufficient portion of the sparsomycin structure to warrant their preparation and testing as potential antitumor agents. Although it had been established that sparsomycin possessed a D configuration at the asymmetric carbon atom,[9] our initial analogues **9a–e** were prepared in the L form in order to test the dependency of antitumor activity on absolute configuration.

The lack of anticancer action of these analogues, most notably **9a**, a deoxygenated version of sparsomycin in the enantiomeric form, suggested the possibility of a config-

Table II. Physical and Chemical Data

Compd no.	R	R_1	Stereochemistry	Yield, %	Mp, °C	Optical rotation, $[\alpha]_D$ (temp, °C; concn, g/100 ml; solvent)	Mol formula	Analyses
			$NH_2CH(CO_2R_1)CH_2SR$					
3f[a]	CH_2SCH_3	H	DL	77	215 dec		$C_5H_{11}NO_2S_2$	
3i[b]	$(CH_2)_9CH_3$	H	DL	70	225		$C_{13}H_{27}NO_2S$	C, H, N, S
3j[a]	CH_2SCH_3	H	D	68	170-174 dec	+11.30 (27, 1.04, 1 N HCl)	$C_5H_{11}NO_2S_2$	
4b[c]	$(CH_2)_3CH_3$	CH_3	L	79	129-131	+15.83 (26, 1.16, EtOH)	$C_8H_{17}NO_2S$·HCl	C, H, N
4f[c]	CH_2SCH_3	CH_2CH_3	DL	64	96.5		$C_7H_{15}NS_2O_2$·HCl	C, H, N
4h[c]	CH_3	CH_2CH_3	DL	29	138-140		$C_6H_{13}NO_2S$·HCl	C, H, N
4i[a,c]	$(CH_2)_9CH_3$	CH_2CH_3	DL	60	175 dec, wetting at 100		$C_{15}H_{31}NO_2S$·HCl	
4j[a,c]	CH_2SCH_3	CH_2CH_3	D	68	96-101	+15.59 (26, 1.09, EtOH)	$C_7H_{15}NO_2S_2$·HCl	

[a] Combustion analysis not performed; compound identified by NMR or IR. See Experimental Section for spectral data. [b] Prepared in NH_3(l)-EtOH mixture. [c] As an HCl salt.

Table III. Physical and Chemical Data

Compd no.	R	Stereochemistry	Yield, %	Mp, °C	Optical rotation, $[\alpha]_D$ (temp, °C; concn, g/100 ml; solvent)	Mol formula	Analyses
					$HCl·NH_2CH(CH_2OH)CH_2SR$		
5a	CH_2SCH_3	L	20	113-118	−14.80 (25, 1.10, EtOH)	$C_5H_{13}NOS_2$·HCl	C, H, N
5b	$(CH_2)_3CH_3$	L	19	98-101	+0.44 (27, 0.91, EtOH)	$C_7H_{17}NOS$·HCl	C, H, N
5c	CH_3	L	24	79-83	−7.71 (27, 0.96, EtOH)	$C_4H_{11}NOS$·HCl	C, H, N
5d	$CH_2C_6H_5$	L	60	140-142	−43.73 (25, 1.02, EtOH)	$C_{10}H_{15}NOS$·HCl	C, H, N
5e	$CH(CH_3)_2$	L	62	125-130	+4.40 (26, 0.99, EtOH)	$C_6H_{15}NOS$·HCl	C, H, N
5f	CH_2SCH_3	DL	62	118-121		$C_5H_{13}NOS_2$·HCl	C, H, N
5i[a]	$(CH_2)_9CH_3$	DL	63	85-90		$C_{13}H_{29}NOS$·HCl	

[a] Combustion analysis not performed; compound identified by NMR or IR. See Experimental Section for spectral data.

urational dependency, although the alternative requirement for an oxygenated sulfur could not be eliminated. The observed inactivity of the racemic compounds **9f** and **9h** provided more evidence for the latter; this was further substantiated on observation of inactivity with **9j**, *S*-deoxysparsomycin, possessing the same stereochemistry at carbon as the parent compound.

Our attention then turned to the preparation of the sulfoxide analogues **10c–e** with carbon in the L configuration. The resultant inactivity of these compounds in the P-388 tumor system again suggested that carbon configuration played an important role in the antitumor activity of sparsomycin. It thus appeared that both the presence of a sulfoxide moiety and a D configuration at carbon were required in order to observe activity in sparsomycin or its analogues.

This was demonstrated with the racemic sulfoxide **10h**, active in the P-388 screen. This compound was isolated as a mixture of stereoisomers which, of course, contains diastereomeric forms with carbon in the appropriate D configuration. It will be recalled that **10c** with carbon in the enantiomeric L form was found to be inactive.

Racemic sulfoxides **10g,i,k** also revealed activity in the P-388 tumor indicating a large tolerance for the group substituted at the sulfoxide moiety and further substantiating the structure–activity relationship previously referred to.

The unexpected activity of the *S*-decyl sulfide **9i** is not readily explained at this time but is undoubtedly related to the highly lipophilic side chain at sulfur which could alter drug transport across cell membranes and result in a different mechanism of action. It is hoped that further synthetic analogues will clarify this.

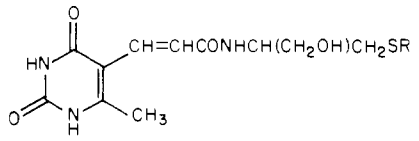
In general, the sparsomycin molecule possesses very specific structural features necessary for antitumor activity although it is evident that some variations at the sulfoxide moiety are tolerated. This has led to some biologically active analogues but, unfortunately, properties superior to the parent were not demonstrated.

Experimental Section

All melting points are uncorrected and recorded on a Thomas-Hoover capillary melting point apparatus. Optical rotations were obtained with a Perkin-Elmer 141 polarimeter and combustion analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Infrared spectra were recorded as paraffin oil mulls on a Perkin-Elmer 521 spectrophotometer and nuclear magnetic resonance spectra were obtained on a Varian A-60A spectrometer. Chemical shifts are given in parts per million (δ) downfield from tetramethylsilane, the internal standard. When several compounds were prepared by comparable procedures, only one representative example is included in this section. Reference should be made to Tables II–V for supplementary information on new compounds. Although all new compounds were identified by NMR and IR spectroscopy, several were not fully characterized and have not been included in the tables. Previously reported compounds which were resynthesized and agreed with literature properties have also been deleted from the tables. Satisfactory elemental analyses (±0.4% of calculated values) are indicated by elemental symbols in Tables II–V. Compounds **6–8** were prepared by literature procedures.[8,9]

S-Alkylated Cysteines (3). Compound **3c** was purchased from the Aldrich Chemical Co. All remaining analogues were prepared by the procedure previously outlined for **3a**.[7] Compound

Table IV. Physical and Chemical Data

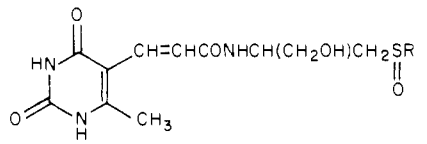


trans

Compd no.	R	Stereochemistry	Yield, %	Mp, °C	Optical rotation, [α]D (temp, °C; concn, g/100 ml; solvent)	Mol formula	Analyses
9a	CH_2SCH_3	L	62	219-222[a]	−75.92 (26, 0.98, Me_2SO)	$C_{13}H_{19}N_3O_4S_2$	C, H, N
9b	$(CH_2)_3CH_3$	L	40	230-235 dec	−53.94 (25, 1.00, Me_2SO)	$C_{15}H_{23}N_3O_4S$	C, H, N
9c	CH_3	L	50	218-223	−77.96 (26, 0.94, Me_2SO)	$C_{12}H_{17}N_3O_4S$	C, H, N
9d	$CH_2C_6H_5$	L	60	204-206	−56.83 (25, 0.96, Me_2SO)	$C_{18}H_{21}N_3O_4S$	C, H, N
9e	$CH(CH_3)_2$	L	53	230-240 dec	−61.18 (25, 0.81, Me_2SO)	$C_{14}H_{21}N_3O_4S$	C, H, N
9f	CH_2SCH_3	DL	67	228-232 dec		$C_{13}H_{19}N_3O_4S_2$	C, H, N
9g	$(CH_2)_3CH_3$	DL	50	239-241 dec		$C_{15}H_{23}N_3O_4S$	C, H, N
9h	CH_3	DL	43	218-223		$C_{12}H_{17}N_3O_4S$	C, H, N
9i	$(CH_2)_9CH_3$	DL	50	250-255 dec		$C_{21}H_{35}N_3O_4S$	C, H, N
9j	CH_2SCH_3	D	63	210-215 dec	+74.95 (26, 1.02, Me_2SO)	$C_{13}H_{19}N_3O_4S_2$	C, H, N

[a] Lit.[11] mp 233 °C.

Table V. Physical and Chemical Data



trans

Compd no.	R	Stereochemistry	Yield, %	Mp, °C	Optical rotation, [α]D (temp, °C; concn, g/100 ml; solvent)	Mol formula	Analyses
10c[a]	CH_3	L at C	62	172, wetting at 140	−72.77 (25, 0.96, H_2O)	$C_{12}H_{17}N_3O_5S\cdot H_2O$	C, H, N
10d[a,b]	$CH_2C_6H_5$	L at C	45	228-233 dec, wetting at 140	−56.09 (25, 0.95, Me_2SO)	$C_{18}H_{21}N_3O_5S\cdot H_2O$	C, H, N
10e[a]	$CH(CH_3)_2$	L at C	75	208-212 dec, wetting at 160	−77.98 (25, 0.94, H_2O)	$C_{14}H_{21}N_3O_5S\cdot H_2O$	C, H, N
10g[b]	$(CH_2)_3CH_3$	Racemic	78	192 dec, wetting at 163		$C_{15}H_{23}N_3O_5S$	H, N; C[c]
10h[a]	CH_3	Racemic	49	190-205 dec, wetting at 170		$C_{12}H_{17}N_3O_5S\cdot H_2O$	C, H, N
10i	$(CH_2)_9CH_3$	Racemic	71	228-232 dec, wetting at 145		$C_{21}H_{35}N_3O_5S$	C, H, N
10k[b,d]	$CH_2S(=O)CH_3$	Racemic	33	196-200 dec		$C_{13}H_{19}N_3O_6S_2\cdot 2H_2O$	C, H, N

[a] As a monohydrate. [b] Prepared in H_2O-1,4-dioxane solution. [c] C: calcd, 50.40; found, 49.88. [d] As a dihydrate.

3f: IR 2060 (NH_3), 1580 cm^{-1} (CO_2). Compound **3j**: IR 2100 (NH_3), 1600 cm^{-1} (CO_2).

S-Alkylated Cysteine Ester Hydrochlorides (4). All compounds were prepared by one of the alternate procedures reported for **4a**.[7] (See Chemistry section of this report for method and alcohol used.) Compound **4l**: IR 2020 (NH_3), 1740 cm^{-1} (C=O ester). Compound **4j**: NMR (Me_2SO-d_6) δ 1.11 (t, 3 H, CH_2CH_3), 2.02 (s, 3 H, SCH_3), 3.20 (d, 2 H, $HCCH_2$), 3.86 (s, 2 H, SCH_2S), 4.26 (m, 3 H, CH_2CH_3 and $HCCH_2$), 9.28 (br, 3 H, NH_3).

(-)-2-Amino-3-benzylthio-1-propanol Hydrochloride (5d). To a solution of 5.34 g (0.25 mol) of $LiBH_4$ in 410 ml of THF was added 29.6 g (0.12 mol) of L-*S*-benzylcysteine ethyl ester hydrochloride (**4d**) under ice cooling. After addition the mixture was stirred at room temperature for 23 h and then cooled in an ice bath and 82.2 ml of 6 N HCl was added dropwise. The mixture was then refluxed 0.5 h and concentrated under vacuum to remove THF. The aqueous phase was treated with 50% NaOH to pH 11, extracted with $CHCl_3$, and dried over K_2CO_3. Hydrogen chloride gas was bubbled in until saturated and solvent removed to yield 16.8 g (60%) of **5d**. Recrystallization from EtOAc–2-propanol gave an analytical sample: mp 140–142 °C; $[\alpha]^{25}D$ −43.73° (*c* 1.02, EtOH).

Compound **5i**: NMR (Me_2SO-d_6) δ 1.00 [m, 19 H, $(CH_2)_8CH_3$], 2.70 (m, 4 H, $HCCH_2$ and SCH_2), 3.20 (m, 1 H, $HCCH_2$), 3.70 (d, 2 H, CH_2O), 5.40 (br, 1 H, OH), 8.20 (br, 3 H, NH_3).

(*E*)-(-)-*N*-[1-(Hydroxymethyl)-2-[[(methylthio)methyl]-thio]ethyl]-3-(1,2,3,4-tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinyl)-2-propenamide (9a). A mixture of 4.69 g (0.024 mol) of 6-methyl-5-uracilylacrylic acid (**8**) and 7.31 g (0.029 mol) of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) in 150 ml of DMF was stirred at 35 °C until solution formed. Then 4.0 g (0.024 mol) of **5a** (as the free base) in 21 ml of DMF was added, followed by 20 ml of H_2O. The solution was stirred at 35 °C for 3 days. The solvent was removed under vacuum and the oil obtained triturated with Et_2O to a solid. Recrystallization from H_2O gave 5.1 g (62%) of **9a**. An analytical sample was prepared by recrystallization from EtOH: mp 219–222 °C; $[\alpha]^{26}D$ −75.92° (*c* 0.98, Me_2SO) (lit.[11] mp 233 °C).

(*E*)-(−)-*N*-[2-(**Methylsulfinyl**)-1-(**hydroxymethyl**)**ethyl**]-3-(1,2,3,4-tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinyl)-2-**propenamide Monohydrate (10c)**. To 5.4 g (18.0 mmol) of sulfide **9c** slurried in 114 ml of H_2O at 25 °C was added dropwise 3.91 g (18.3 mmol) of sodium metaperiodate in 57 ml of H_2O. After addition, the solution was stirred at room temperature for 2 h, then diluted with MeOH to 900 ml, cooled to 10 °C, and filtered and the solid discarded. The filtrate was then evaporated under vacuum and the residue chromatographed over silica gel Woelm (dry column grade, activity III) with absolute EtOH to give 3.5 g (62%) of **10c**. Recrystallization from EtOH gave an analytical sample: mp 172 °C with wetting at 140 °C; $[\alpha]^{25}D$ −72.77° (*c* 0.96, H_2O).

(*E*)-(±)-*N*-[2-(*n*-**Decylsulfinyl**)-1-(**hydroxymethyl**)-**ethyl**]-3-(1,2,3,4-tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinyl)-2-**propenamide (10i)**. To 3.0 g (7.04 mmol) of sulfide **9i** in 150 ml of H_2O was added 6.6 ml (77 mmol) of 30% H_2O_2 and the mixture stirred at room temperature. After 7 days, another 3.3 ml of 30% H_2O_2 was added. This was repeated after 6 more days and the mixture stirred 1 week longer and then filtered. Recrystallization from EtOH gave 2.2 g (71%) of **10i**, mp 228–232 °C dec with wetting at 145 °C.

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Supplementary Material Available: biological data on inactive target compounds (1 page). Ordering information is given on any current masthead page.

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(11) While this manuscript was in preparation we became aware of the work of H. C. J. Ottenheijm, S. P. J. M. van Nispen, and M. J. Sinnige, *Tetrahedron Lett.*, **22**, 1899 (1976), who reported the synthesis of *S*-deoxy-L-sparsomycin (**9a**) by a slightly modified procedure. Their melting point, however, is in closer agreement with our *S*-deoxy-DL-sparsomycin (**9f**). It should be noted that the authors did not report an optical rotation for their sample.
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Potential Antitumor Agents. Some Sulfur-Substituted Derivatives of α- and β-2′-Deoxythioguanosine

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A series of ten S-substituted derivatives of the α and β anomers (**1a** and **1b**) of 2′-deoxy-6-thioguanosine has been prepared by S-alkylation of the parent nucleosides and/or by mercaptide displacement reactions on 6-chloro intermediates. Against L1210 murine leukemia all β anomers were active but potency was reduced relative to **1b**. Most *S*-alkyl α anomers were inactive in this test. Limited testing against P388 murine leukemia showed all α-anomer derivatives to be inactive but the β anomers were more effective than the parent. S-Substitution sharply reduced acute toxicity in both series. In vitro DNA and RNA synthesis inhibition data are also reported. The antitumor activity of these derivatives and of the 2′,5′-di-*O*-acetyl derivatives of **1a** and **1b** against lymphoid leukemia L1210 is reported. Some results with the lymphocytic leukemia P388 and an in vitro assay of the inhibition of nucleic acid synthesis are also given.

2′-Deoxythioguanosine (**1b**),[1] originally designed to by-pass some of the mechanisms of resistance to thioguanine (6-TG),[2a] has demonstrated antitumor activity[2] and is being considered as a candidate for clinical trials.[3] Its α anomer, **1a**,[1,4] is unique because it is the only nucleoside α anomer found to date to have significant in vivo antitumor activity.[2a,5] Laboratory and preclinical toxicology studies[6] show that **1a** is much less toxic than **1b** in mice, rats, dogs, and monkeys. LePage and his colleagues have found that both **1a** and **1b** are phosphorylated by extracts of many murine and human neoplasms, including neoplastic bone marrows. However, extracts of normal bone marrows, with one exception, did not phosphorylate **1a**,[7] therefore suggesting that this anomer has some selectivity for tumors. Tamaoki and LePage also have suggested that **1a** may be a unique tool for the study of DNA replication since it terminates chain elongation[8] when incorporated into DNA.

Because of these interesting properties of **1a** and **1b**, other investigators have prepared the related seleno compounds,[9a] the Se-substituted derivatives,[9b] and the 6-amino-2-methylthiopurin-9-yl deoxyribonucleosides.[9c] We have synthesized the series of S-substituted derivatives shown in Scheme I and Table I.

The specific S-substituents were chosen because they are able to impart desirable properties to the analogous purine ribonucleosides. *S*-Methyl-6-mercaptopurine ribose, for example, is believed to exert its antitumor action by