a = 6.130 (6) Å, b = 8.224 (6) Å, c = 13.108 (11) Å, α = 95.71 (6), β = 102.16 (7), and γ = 99.74 (7)°, with V = 630.4 (9) Å^3, Z = 1, and d_{calcd} = 1.282 g/cm^3. A total of 1743 independent reflections were measured in the $\theta/2\theta$ mode to $2\theta_{max}$ = 45°. Corrections were applied for Lorentz and polarization effects, but not for absorption. The structure was solved by direct methods with the aid of the program SHELXTL[31] and refined with a full matrix least squares.[31] The 336 parameters refined include the coordinates and anisotropic thermal parameters for all non-hydrogen atoms. Carbon hydrogens used a riding model in which the coordinate shifts of the carbons were applied to the attached hydrogens, and C-H = 0.96 Å, angle H-C-H = 109.5°, and U(H) = 1.1U_{eq}(C). The remaining hydrogens were refined isotropically. The final R values for the 1652 observed reflections with $F_o > 3\sigma(|F_o|)$ were R = 0.036 and wR = 0.041, where $s = 1/[\sigma^2(|F_o| + g(F_o)^2]$ and g = 0.00023. Tables of coordinates, bond distances, and bond angles, and anisotropic thermal parameters, have been deposited with the Crystallographic Data Center, Cambridge CB2 1EW, England.

Acknowledgment. This work was supported by Grant DA-03776 from the National Institute on Drug Abuse (W.D.B.) and the Dystonia Medical Research Foundation (W.D.B. and J.M.W.).

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Lipophilic Analogues of Sparsomycin as Strong Inhibitors of Protein Synthesis and Tumor Growth: A Structure-Activity Relationship Study

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Fourteen derivatives of sparsomycin (1) were synthesized. Six of them were prepared following a novel synthetic route starting from the L-amino acid alanine. Some physicochemical properties, viz. lipophilicity and water solubility, of selected derivatives were measured. The biological activity was tested in vitro in cell-free protein synthesis inhibition assays, in bacterial and tumor cell growth inhibition assays, and in the L1210 leukemia in vivo model in mice. Also for selected drugs the acute toxicity in mice was determined. Ribosomes from both an eukaryotic and a prokaryotic organism were used in the protein synthesis inhibition systems. A linear correlation between the lipophilicity parameters measured was observed. Water solubility and drug toxicity in mice were found to be linearly correlated with lipophilicity. All the derivatives studied are more lipophilic than 1. The deshydroxysparsomycin analogues (**30–33**) showed an interesting phenomenon: increase in hydrophobicity was accompanied by a considerable increase in water solubility. We found that an increase in hydrophobicity of the drug as a result of replacing the SMe group of 1 with larger alkylthio groups causes an increase in the biological activity of the drug. However, not only the hydrophobicity but also shape and size of the substituent are important; in the homologous series **1–9–10–11–12**, **21–22–23–24**, and **30–31–32–33**, highest protein synthesis inhibitory and in vitro cytostatic activity is found with compounds **11**, **23**, and **32**, respectively, and in comparison with the highly active *n*-butyl compound **10**, the isomeric *tert*-butyl compound **13** is rather inactive. Polar substituents replacing the SMe group, i.e. Cl in **17** and **35**, also render the molecule inactive. Substituting the bivalent sulfur atom for a methylene group decreases the drug's activity. This effect can be compensated for by increasing the length of the alkylsulfinyl side chain. The agreement between the resuts derived from cell-free and "in vivo" tests is good. The assays using ribosomes of bacterial and eukaryotic organisms give similar results although the latter seem to be more sensitive to changes in hydrophobicity of the drug. Our results confirm the presence of a hydrophobic region at the peptidyl transferase center of the ribosome; the interaction of sparsomycin with this region is more pronounced in the eukaryotic particles. The sparsomycin analogues **11**, **23**, and **30** show the highest antitumor activity against L1210 leukemia in mice, their median T/C values are 386, 330, and 216%, respectively. Sparsomycin (1), showing a T/C value of 117%, is only marginally active against this tumor. The analogues tested are 5–100 times less toxic than 1.

Sparsomycin (1)[1] is a broad-spectrum antibiotic active against parasites,[2] viruses,[3,4] fungi,[5] and bacteria.[6,7] The antibiotic has achieved prominence in the past 25 years as a tool to study the protein biosynthesis machinery. Since the isolation of sparsomycin from *Streptomyces sparsogenes* in 1962, much attention was focused on its early observed antitumor activity.[5]

Recently, both we[8,9] and Helquist[10] succeeded in developing total syntheses of sparsomycin. In 1984, our synthetic route to sparsomycin allowed the preparation of the first analogue, i.e octylsparsomycin (**12**), which proved to be 3 times more active against L1210 leukemia in vitro

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(1) For a review, see: Ottenheijm, H. C. J.; van den Broek, L. A. G. M.; Ballesta, J. P. G.; Zylicz, Z. In *Progress in Medicinal Chemistry*, Ellis, G. P., West, G. B., Eds.; Elsevier: Amsterdam, 1986; Vol. 23, pp 219–268.
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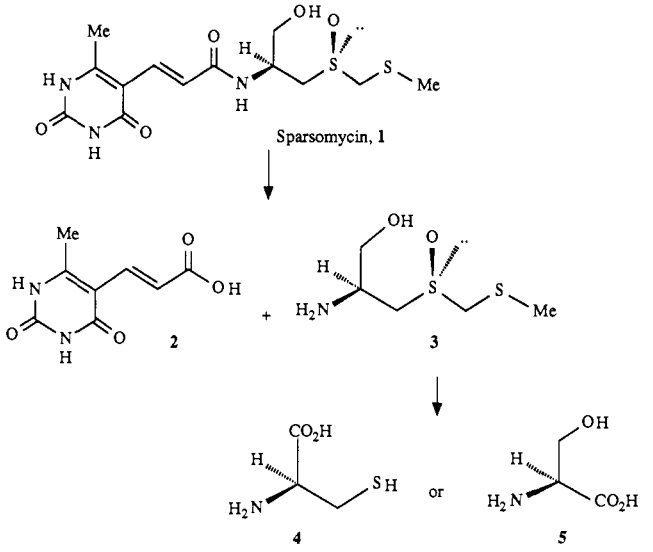
Scheme I. Retrosynthetic Analysis of Sparsomycin

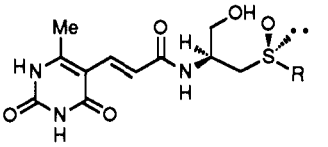


than the natural product itself.[11] Three years later, a structure–activity relationship study was published[12] in which the biological activity of 14 analogues of sparsomycin was studied in both cell-free and whole-cell systems. Each derivative possessed not more than two structural modifications of the sparsomycin molecule. Increase of the lipophilicity of the molecule was found to have a marked effect on the biological activity of the antibiotic. A correlation was found between protein synthesis inhibition and antitumor activity.

Sparsomycin blocks the peptidyl transferase center in the large ribosomal subunit by interfering with the binding of substrates.[13,14] It has been shown to inhibit the interaction of substrates with the peptidyl transferase A site, while stimulating at the same time the binding of substrates at the P site.[15,16] This mutual interaction was proposed to be due to an allosteric effect.[1] The high activity of hydrophobic derivatives of sparsomycin in protein synthesis inhibition assays[12] indicates the existence of an hydrophobic region in the peptidyl transferase center.[17] It was proposed that this site may also be involved in the binding of hydrophobic amino acyl residues of the amino acyl-tRNA molecules at the ribosomal A site.

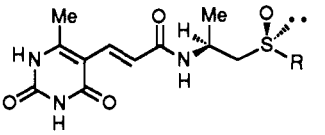
The synthesis of 14 derivatives of sparsomycin is described in this paper, i.e. compounds **9–11**, **13**, **14**, **18**, **22**, **23**, **30–33**, **35**, and **38**. Six of these analogues, i.e. **30–33**, **35**, and **38**, were prepared via a novel synthetic route starting from L-alanine. The analogue having the hydroxymethyl group replaced by a methyl group (**30**) is 5 times more active than sparsomycin against L1210 leukemia in vitro, whereas the activity of the compounds having the hydroxymethyl moiety substituted for larger alkyl or aralkyl groups[18] is equal to or even slightly less that of 1. Therefore, we felt the need to study systematically the relationship between physicochemical properties, i.e. lipophilicity and water solubility, of a series of analogues of sparsomycin and their biological activity. The inhibition of protein synthesis in five different cell-free systems, cell growth inhibition of *Escherichia coli* cells and of two human tumor cell lines, inhibition of colony formation of murine leukemia cells, and the toxicity and antitumor activity in mice was investigated for the newly prepared analogues of sparsomycin. Both prokaryotic as well as eukaryotic cell-free systems were chosen for this study. The results described in this paper are a firm basis for selection of the best drugs to be investigated in further pharmacological and toxicological studies.

Table I. Sparsomycin Derivatives



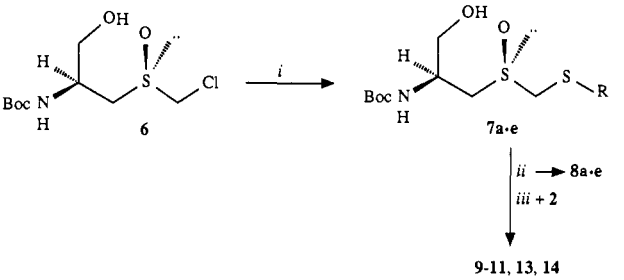
compd	R	compd	R
9	$CH_2S(CH_2)_2Me$	**17**[12]	CH_2Cl
10	$CH_2S(CH_2)_3Me$	**18**	$CH_2SCH_2CH_2NHC(O)Me$
11	$CH_2S(CH_2)_4Me$	**21**[12]	$(CH_2)_2Me$
12[11]	$CH_2S(CH_2)_7Me$	**22**	$(CH_2)_5Me$
13	CH_2SCMe_3	**23**	$(CH_2)_6Me$
14	$CH_2SC_6H_{11}$	**24**[12]	$(CH_2)_9Me$

Table II. Sparsomycin Derivatives



compd	R
30	CH_2SMe
31	CH_2SEt
32	$CH_2S(CH_2)_3Me$
33	$CH_2S(CH_2)_7Me$
35	CH_2Cl
38	$(CH_2)_2Me$

Scheme II[a]



[a] (i) NaSR; (ii) TFA, ion exchange; (iii) $ClCO_2$-*i*-Bu, Et_3N. a, R = $(CH_2)_2Me$; b, R = $(CH_2)_3Me$; c, R = $(CH_2)_4Me$; d, R = CMe_3; e, R = C_6H_{11}.

Results and Discussion

Chemistry. According to the retrosynthetic scheme of sparsomycin (1, Scheme I), the molecule can be built up

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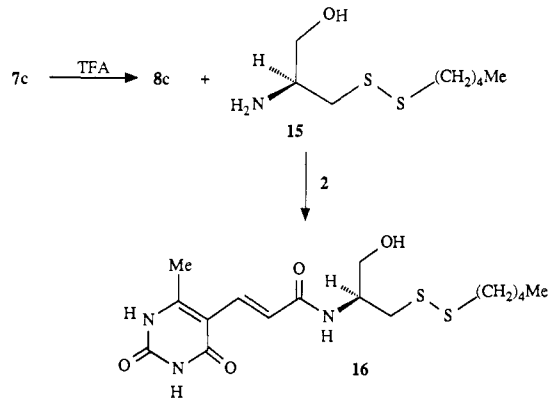
Scheme III



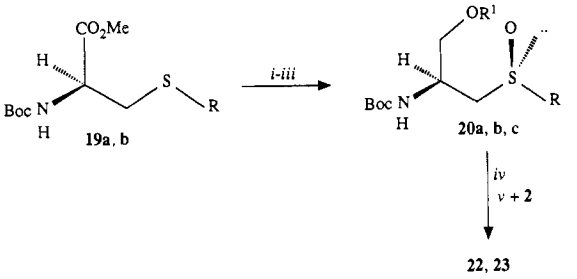
from either the amino acid D-cysteine (**4**) or L-serine (**5**). Both amino acids have been employed successfully for total syntheses of the natural product.[8,10] The approach starting from the L-amino acid is particularly intriguing as it opens the way to sparsomycin analogues having alkyl or aralkyl substituents at the chiral carbon atom. We have employed this approach for the synthesis of compounds **30–33**, **35**, and **38** (Table II). The homologous series **1–9–10–11–12** (Table I), **21–22–23–24** (Table I), and **30–31–32–33** (Table II) each contain at the sulfoxide moiety alkyl side chains of increasing length allowing the detailed study of the effect of increased lipophilicity of this pharmacophore on the biological activity.

All of the variations introduced into the molecule concern modifications of the amine fragment **3** (Scheme I). The first series of analogues of sparsomycin retaining the hydroxymethyl function is listed in Table I. The synthesis of compounds **9–11**, **13**, and **14** was accomplished as depicted in Scheme II. These derivatives are characterized by substitution of the methyl group on the bivalent sulfur atom by other—more lipophilic—alkyl chains. The synthesis of the chloromethyl sulfoxide **6** has been described earlier.[8] Substitution of the chlorine atom by the corresponding mercaptide (90% yield), deprotection of the amine (quantitative yield) by treatment with TFA, and coupling of the resulting amine with the acid **2** (25–35% yield) according to the isobutyl chloroformate mixed anhydride methode furnished the desired sparsomycin derivatives **9–11**, **13**, and **14**.

In the synthesis of pentylsparsomycin (**11**) on a large scale (20 mmol of **6**) it was possible to isolate a byproduct, characterized as the disulfide sparsomycin analogue **16** (Scheme III). The rearrangement of the monoxodithioacetal moiety into the disulfide functionality must have occurred during removal of the Boc protecting group of **7c** with TFA to give compound **15** (Scheme III). This reaction is known for the preparation of mixed disulfides[19,20] and proceeds via a sulfenate ester intermediate with loss of formaldehyde.

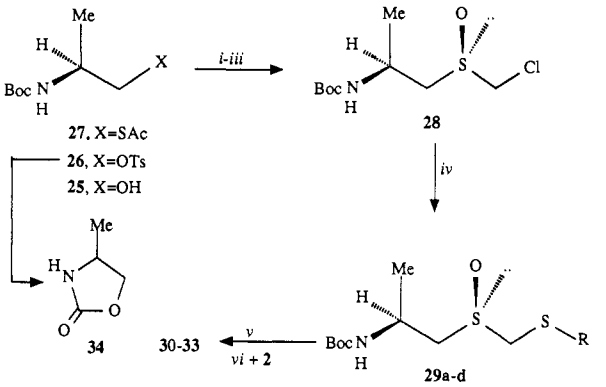
Compound **18** (Table I) was obtained in the following way. After removal of the Boc group of **6** by treatment with TFA and coupling of the resulting amine with the acid **2** using isobutyl chloroformate, compound **17** was prepared.[12] This compound was allowed to react in liquid ammonia with the sodium salt of *N*-acetylcysteamine, prepared in situ by reduction of *N,N*-diacetylcystamine with sodium in liquid ammonia, to give the desired analogue **18** in 23% yield.[21]

Scheme IV[a]



[a] (i) $LiBH_4$; (ii) $NaIO_4$; (iii) separation; (iv) TFA, ion exchange; (v) $ClCO_2$-*i*-Bu. **a**, R = $(CH_2)_5Me$, R^1 = H; **b**, R = $(CH_2)_6Me$, R^1 = H; **c**, R = $(CH_2)_6Me$, R^1 = $Si(Me)_2$-*t*-Bu.

Scheme V[a]



[a] (i) Cl_2, Ac_2O; (ii) CH_2N_2; (iii) separation; (iv) NaSR; (v) TFA, ion exchange; (vi) $ClCO_2$-*i*-Bu, Et_3N. **a**, R = Me; **b**, R = Et; **c**, R = $(CH_2)_3Me$; **d**, R = $(CH_2)_7Me$.

The structure of compounds **22** and **23** is characterized by replacement of the thiomethyl fragment by alkyl chains of increasing length. Their straightforward synthesis is described in Scheme IV. From *N*-Boc-*S*-alkylcysteine methyl ester **19** the N-protected cysteinol sulfoxide **20** was easily obtained in three steps in 44–49% overall yield according to procedures published earlier.[12] Again, deprotection of the amine (quantitative yield) by treatment with TFA and coupling of the resulting amine with the acid **2** (24–33% yield) using isobutyl chloroformate afforded the derivatives **22** and **23**.

The second series of deshydroxy analogues comprises compounds **30–33**, **35**, and **38** (Table II). Their synthesis was achieved as follows. The amino acid L-alanine was transformed into the corresponding tosylate **26** (four steps, 72% overall yield), following known procedures[8] (Scheme V). The tosylate is unstable and should not be stored. Recrystallization of **26** yielded the cyclic urethane **34**.

The next step in the reaction sequence is the introduction of the sulfur atom. Recently, a method using cesium thiocarboxylates was published[22] for the conversion of alcohols into thiols via subsequently the corresponding mesylate and thioacetate. This method was found to be successful for our purposes. Upon treatment of **26** with CsSAc in DMF, the thioacetate **27** was formed in 80% yield. The thioacetate was converted into a nearly 1:1 mixture of sulfoxide diastereomers by treatment with

(19) Kishi, Y.; Fukuyama, T.; Nakatsuka, S. *J. Am. Chem. Soc.* **1973**, *95*, 6490.
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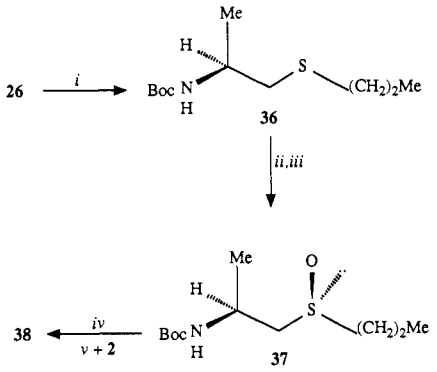
(22) Strijtveen, B.; Kellogg, R. M. *J. Org. Chem.* **1986**, *51*, 3664.

Scheme VI[a]



[a] (i) $NaS(CH_2)_2Me$; (ii) mCPBA; (iii) separation; (iv) TFA; (v) Et_3N, Pfp, DCC.

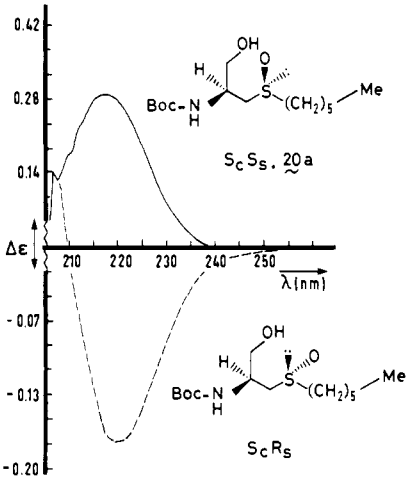


Figure 1.

chlorine and acetic anhydride and subsequently with diazomethane.[23] This reaction proceeds in yields ranging from 40 to 50%.[24] From this mixture the α-chloro sulfoxide **28** was isolated by fractionated crystallization from ethyl acetate. After substitution of the chlorine atom in **28** by the corresponding mercaptide (88–98% yield), deprotection of the amine function (quantitative yield) by treatment with TFA, and coupling of the resulting amine with the acid **2** employing isobutyl chloroformate, the desired analogues **30–33** were obtained in yields ranging between 37% and 71%.

Compound **35** was prepared from compound **28** (Scheme V). After removal of the Boc protecting group by treatment with TFA (quantitative yield) and coupling with the acid **2** using isobutyl chloroformate, compound **35** was obtained in 26% yield.

The straightforward synthesis of compound **38** is presented in Scheme VI. This compound has the bivalent sulfur atom in **30** substituted by a methylene group as is the case with compound **21** versus **1**. Reaction of the tosylate **26** with sodium 1-propanethiolate afforded the substitution product **36** in 92% yield. Oxidation of **36** with mCPBA[25] gave a nearly 1:1 mixture of the sulfoxide **37** and

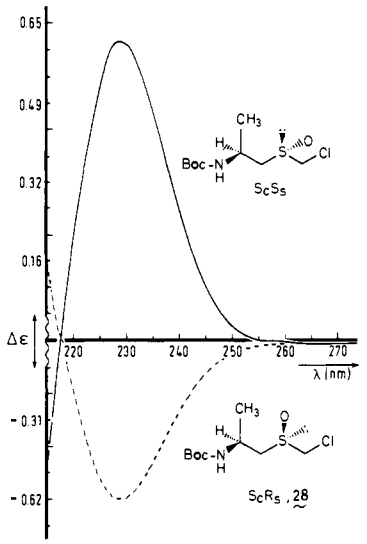


Figure 2.

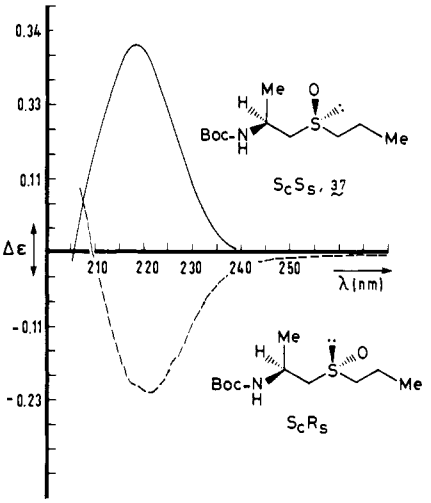


Figure 3.

its corresponding *S* epimer (not shown) in 91% yield. After separation of **37** from its diastereomeric counterpart, the Boc protecting group of **37** was removed by treatment with TFA. The amine was coupled with the acid **2** via its pentafluorophenyl ester,[26] prepared in situ by reaction of **2** with pentafluorophenol and DCC, to give compound **38** in 72% overall yield. This pentafluorophenyl ester amide coupling procedure is superior in yield in comparison with the isobutyl chloroformate mixed anhydride procedure.

CD Spectroscopy. The assignment of the chirality at the sulfoxide sulfur atom of the newly prepared compounds deserves some comment. Previously, we showed that for sparsomycin (**1**)[8] as well as for several other α-functionalized sulfoxides[9,21,27] circular dichroism (CD) spectroscopy can be employed in the assignment of the absolute configuration of the sulfoxide sulfur atom.

The rule of thumb used is that in the absence of strongly perturbing groups, a negative sign of the Cotton effect—centered at the sulfoxide absorption band at 220–240 nm—correlates with an *R* configuration whereas a positive sign correlates with an *S* configuration.[28]

(23) Kee, M.-L.; Douglass, I. B. *Org. Prep. Proc.* **1970**, *2*, 235.

(24) The diastereomeric cysteinol chloromethyl sulfoxides **6** can be separated easily by a combination of crystallization from EtOAc and silica gel column chromatography (eluent MeOH/CH_2Cl_2, 7:93). The "correct" stereoisomer possessing the S_cR_s configuration crystallizes from EtOAc contrary to its S_cS_s diastereomeric counterpart. This procedure also works well for obtaining compound **28**.

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(26) Kisfaludy, L.; Schön, I. *Synthesis* **1983**, 325.

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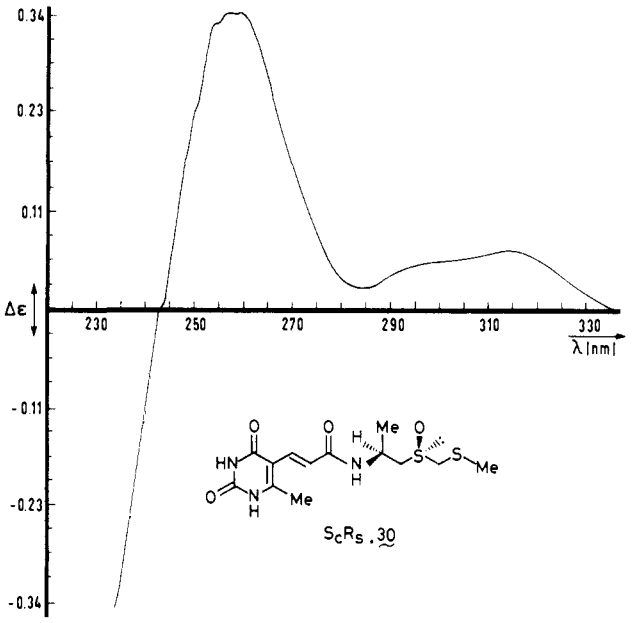

Figure 4.

Table III. Inhibition of Peptide Bond Formation

	ED_{50}, μM				
	polyPhe synthesis		modified fragment reaction		Puromycin reaction
compd	*E. coli*	*S. cerevisiae*	*E. coli*	*S. cerevisiae*	*E. coli*
1[12]	8.5	5.2	3.2	2.8	0.10
9	3.0	7.0	5.5	7.5	0.08
10	1.8	1.0	5.1	4.8	0.06
11	1.0	0.17	2.6	2.7	0.03
12[12]	1.6	1.3	60	300	0.25
13	31	900 (47)[a]	27.5	100	0.31
14	6.6	5.1	5.2	12	0.04
17[12]	450	220	7.6	17	0.65
18	34	32	60	56	1.0
21[12]	140	42	4.5	6.2	0.23
22	0.75	0.14	7.5	5.0	0.18
23	0.48	0.10	9.0	5.8	0.04
24[12]	1.8	0.20	5000 (30)[a]	700 (41)[a]	0.08
30	14.6	50	18	39	0.25
31	12	11.1	10.3	10.4	0.07
32	3.0	1.9	12.1	15	0.05
33	8.5	1.4	46	60	0.46
35	86	inact[b]	inact[b]	400	6.6
38	80	200	NT[c]	NT[c]	0.38

[a] Extrapolated values; the figures in parentheses show the percent of inhibition at 500 μM. [b] Inactive at the highest concentration tested, i.e. 100 μM. [c] NT, not tested.

The CD spectra of the sulfoxides **20a**, **28**, and **37**, precursors of sparsomycin, and their *S* epimers and of deshydroxysparsomycin **30** are shown in Figures 1–4, respectively. We employed the above-mentioned rule to establish the absolute configuration of the sulfoxide function of these compounds.[29]

(28) This rule was formulated first for methyl alkyl sulfoxides by Mislow and co-workers: Mislow, K.; Green, M. M.; Laur, P.; Melillo, J. T.; Simmons, T.; Ternay, A. L. *J. Am. Chem. Soc.* **1965**, *87*, 1958. Later on it was found to be still applicable for compounds like *S*-methylcysteine *S*-oxides possessing an additional chiral center: Axelrod, M.; Bickart, P.; Goldstein, M. L.; Green, M. M.; Kjaer, A.; Mislow, K. *Tetrahedron Lett.* **1968**, 3249.

(29) This assignment of the stereochemistry of the sulfoxide function was confirmed in several instances by data from in vitro assays on both *S* epimers of a compound; only drugs having the absolute configuration at sulfur as depicted for 1 show biological activity, e.g. the *S* epimers of compounds **12**, **18**, and **38** are biologically inactive in all assays described in this report (unpublished results).

Table IV. Physicochemical Parameters

compd	R_m	log k^0	log *P*	water solubility, mg/mL
1	−0.638	−0.676	−1.713	3.8
9	−0.261	0.117	−0.665	1.5
10	−0.028	0.777	0.143	0.57
11	0.160	1.168	0.600	0.28
12	0.895	3.333	>2.424	0.013
22	0.235	1.111	0.410	0.89
23	0.560	1.771	1.021	0.22
30	−0.404	−0.209	−0.699	18.0
31	−0.190	0.079	−0.301	8.8
32	0.218	1.290	0.819	0.94
33	1.151	3.533	>2.424	0.015

Comparison of Figures 2 and 4 shows that the sign of the Cotton effect at 220–240 nm of compound **30** is not influenced by the other absorption bands of the molecule.[8] The Cotton effect in the 260–270-nm region in Figure 4 is due to the amide chromophore.

Biological Activity. The structures of the compounds studied in this paper are shown in Tables I and II. The biological activity of the sparsomycin analogues was tested first in three in vitro and four "in vivo" assays (Table III). The polyuridylic acid dependent synthesis of polyphenylalanine and the "puromycin reaction" under two different conditions were investigated in cell-free systems using ribosomes from *E. coli* or *Saccharomyces cerevisiae*.

The physicochemical properties, i.e. lipophilicity and water solubility, are given in Table IV. Linear regression equations describing the linear correlation between various physicochemical parameters studied and between lipophilicity and toxicity are shown in Table V.

As "in vivo" assays, the inhibition of *E. coli* cell growth in liquid medium, of T24 bladder carcinoma and WiDr colon carcinoma cell growth in tissue culture, and of colony formation of L1210 murine leukemia cells in soft agar were studied (Table VI). Subsequently, of selected analogues, the acute toxicity in mice and antitumor activity in L1210 leukemia bearing mice were determined.

On the basis of their structure the compounds studied (see Tables I and II) can be devided into three classes, i.e. a homologous series of sparsomycin analogues (**1–9–10–11–12**), of desthiosparsomycin analogoues (**21–22–23–24**), and of deshydroxysparsomycin analogues (**30–31–32–33**). One representative of each class, i.e. compounds **11**, **23**, and **30**, has attracted our attention because these compounds in relation to sparsomycin combine a high antitumor activity with a low toxicity in vivo.

Compounds **21**, **22**, **23**, and **24** are structurally related to compounds **1**, **10**, **11**, and **12**, respectively. In the former compounds the bivalent sulfur atom has been replaced by a methylene group. Compounds **30**, **32**, and **33** carry identical side chains as compounds **1**, **10**, and **12**, respectively. However, the hydroxymethyl group of the latter compounds has been replaced by a methyl group.

In Vitro Tests. Looking at the results of the polyphenylalanine synthesis assay given in Table III, it can be concluded that an increase in the length of the aliphatic side chain at the sulfur containing end of the molecule results in a higher inhibitory capacity. In the three homologous series investigated, i.e. **1–9–10–11–12**, **21–22–23–24**, and **30–31–32–33**, an optimum in activity was observed for compounds **11**, **23**, and **32**, respectively.

Substitution of the bivalent sulfur atom in **1** by a methylene group to give **21** decreases the activity in the polyPhe assay seriously. The activity seen in the other assays is however much less affected. This loss of activity can be fully compensated for by increasing the alkyl side chain length in **21** with four carbon atoms to give **23**.

Table V. Linear Regression Equations[a]

	$y = ax + b$						
entry	y	x	b	a	r	n	compd
A	log k^0	log P	−0.63	0.92	0.986	11	**1, 9–12, 22, 23, 30–33**
B	log k^0	R_m	−0.30	0.40	0.989	11	**1, 9–12, 22, 23, 30–33**
C	R_m	log P	0.07	2.28	0.981	11	**1, 9–12, 22, 23, 30–33**
D	R_m	Cn	−0.90	0.22	0.998	5	**1, 9–12**
E	R_m	Cn	−0.64	0.22	0.999	4	**30–33**
F	log k^0	log Sol	0.51	−1.55	−0.973	7	**1, 9–12, 22, 23**
G	log k^0	log Sol	1.28	−1.23	−0.999	4	**30–33**
H	log k^0	log LD_{50}	−0.06	0.49	0.937	6	**1, 10–12, 22, 23**
I	log k^0	log LD_{50}	0.66	0.47	0.858	3	**30–32**

[a] Abbreviations used: a, slope; b, intercept; r, correlation coefficient; n, number of compounds used in linear regression analysis; Cn, number of carbon atoms (see text); Sol, water solubility.

Table VI. Biological Activity

	whole cell: cell growth inhibition				in vivo	
		ID_{50}, μM				
compd	*E. coli*, ED_{50}, μM	T24	WiDr	L1210	LD_{50}, mg/kg	L1210 T/C, %
1	11.0	0.91	0.92	0.48	0.26	117
9	5.0	0.65	0.89	0.14	NT	NT
10	3.0	0.23	0.31	0.10	2.33	156
11	1.0	0.28	0.40	0.06	4.7	386
12	4.4	0.06	0.22	0.10	29.5	151
13	15.0	NT[a]	NT	2.50	NT	NT
14	7.5	NT	NT	0.60	NT	NT
17	20.0	NT	NT	0.29	NT	NT
18	100	NT	NT	1.90	NT	NT
21	36.0	NT	NT	0.75	NT	NT
22	3.0	0.35	0.39	0.14	1.6	135
23	1.4	0.16	0.28	0.15	6.1	330
24	3.0	NT	NT	0.14	NT	NT
30	5.6	0.44	0.58	0.07	2.33	216
31	8.5	0.34	0.48	0.15	8.7	184
32	2.5	0.09	0.16	0.05	16.5	164
33	15.0	0.14	0.28	0.03	NT	NT
35	7.5	NT	NT	0.15	NT	NT
38	9.5	NT	NT	0.45	NT	NT

[a] NT, not tested.

Comparison of the activity data of compounds **23** and **11** shows that replacement of the sulfur atom in **11** by a methylene group no longer has a substantial effect on the biological activity. In a similar way, substitution of the bivalent sulfur atom in **30** to give compound **38** also reduces the activity of the drug in the polyPhe assay in particular. Of the hydroxy-containing sparsomycin analogues (Table I), the *S*-oxo-*S*-*n*-heptyl derivative **23** possesses the highest affinity for the ribosomal peptidyl transferase.

The *S. cerevisiae* system seems to be more sensitive than the bacterial system to an increase in hydrophobicity of the drug. This result, as previously reported with other sparsomycin analogues,[18] indicates that these drugs can be used as probes to detect structural differences in the ribosomes derived from prokaryotic and eukaryotic cells, and more specifically of the hydrophobic pocket of the peptidyl transferase center.[17,30,31] Our results suggest that hydrophobic interactions during the binding of the aminoacyl moiety of the aa–tRNA at the A site of the peptidyl transferase are more pronounced in the eukaryotic particles.

The results for compounds **13**, **14**, **17**, **18**, and **35** indicate that in addition to hydrophobicity also the shape, size, and polarity of the substituents attached to the sulfoxide group influence the activity of the compound. In spite of the highly hydrophobic character of its *tert*-butyl group, **13** is rather inactive, especially when compared with the *n*-butyl derivative **10**. The same is true, although to a lesser extent, for compound **14**; compare its activity with that of analogues **11** and **12**.

The presence of polar groups in the substituent attached to the sulfoxide group affects the activity of the drug seriously as is exemplified clearly in the case of analogue **18**. Although this analogue carries a linear chain of approximately the same length as pentylsparsomycin **11**, it is rather inactive probably due to the presence of the amide group. Substitution of the SMe group in **1** or **30** by a chlorine atom, giving **17** and **35**, respectively, renders these compounds very inactive.

A similar relation between lipophilicty of the drug and its activity was observed in the puromycin reaction assay. However, the differences in activity among the various compounds are less pronounced than in the polyPhe synthesis assay. This is probably due to the fact that sparsomycin itself is a much better inhibitor of this reaction than of polyPhe synthesis. An increase in the affinity of the drug for the ribosome probably affects less the binding of puromycin to the ribosome than the binding of aa-tRNA to the ribosome.[12]

The third cell-free test used is the "modified fragment reaction". In the homologous series studied, i.e. **1–9–10–11–12**, **21–22–23–24**, and **30–31–32–33**, no clear-cut correlation exists between the activity and the length of the sulfoxide substituent. In fact, the more hydrophobic compounds, e.g. **12** and **24**, are very inactive in this assay. This result confirms our previous conclusion[12] that the

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modified fragment reaction, in spite of being extensively used in studies on the mode of action of antibiotics,[32,33] has serious shortcomings for comparing antibiotics of very different hydrophobicity.

Physicochemical Properties. The lipophilicty of the sparsomycin compounds can be well characterized by the experimentally simple determination of R_m values (Table IV). Excellent linear correlations were found (entries A, B, and C of Table V) between this parameter and the other lipophilicity parameters measured, i.e. log k^0 and log P. The sparsomycin analogues **1**, **10**, and **12** have R_m, log k^0, and log P values that are lower than those of the corresponding deshydroxysparsomycin derivatives **30**, **31**, and **32**, respectively (Table IV). The linear correlation calculated between the R_m values of the series **1**, **9–12**, and **30–33** versus the number of carbon atoms attached to the bivalent sulfur atom is given in entries D and E, respectively, of Table V. It can be seen that these linear regression lines have equal slopes. The difference in the R_m values of two corresponding structures in these series, e.g. **1** and **30**, is equal to the effect of a single methylene group.

Water solubility of the drugs (Table IV) correlates linearly with all lipophilicity parameters used; in entries F and G of Table V only the correlation with log k^0 is shown for the series **1**, **9–12**, **22**, **23**, and **30–33**, respectively. Surprisingly, the deshydroxysparsomycin derivatives **30**, **32**, and **33** are better water soluble than the corresponding sparsomycin analogues **1**, **10**, and **12**, respectively, despite their increased lipophilicity. For example, compound **30** is 5 times more water soluble than sparsomycin (**1**) itself. We do not have an explanation for this interesting phenomenon.

The pK_a values of sparsomycin (**1**) (8.97 ± 0.02) and deshydroxysparsomycin **30** (9.02 ± 0.02) were measured.[34] From this we conclude that the hydroxy function of sparsomycin is poorly dissociated since the pK_a values are virtually the same.

In Vivo Tests. In Table VI the activity of the sparsomycin analogues in "in vivo" test is shown. The correlation between the results from in vitro (Table III) and "in vivo" asays is reasonably good, and similar conclusions can be drawn as mentioned in the discussion on the in vitro assays studied.

The activity of the sparsomycin analogues **1**, **9–12**, **22**, and **23** against the two human tumor cell lines (T24 bladder carcinoma and WiDr colon carcinoma) is related to the lipophilicity of the compounds. In general, an increase of the lipophilicity results in an increase of the activity.

The change in the hydrophobicity of the molecule must also affect the permeability properties of the drug, and some discrepancies between the data obtained in vitro and "in vivo" are probably due to this fact. In this respect it is interesting to note that the most potent inhibitor of the L1210 clonogenic assay, i.e. **33**, is somewhat less active than sparsomycin in *E. coli* cell growth inhibition. Substitution of the hydroxymethyl group in **1** and **21** for a methyl group to give **30** and **38**, respectively, decreases the in vitro activity yet increases the "in vivo" activity. Moreover, **17** and **35**, which are very inactive in vitro are as good or even better inhibitors than sparsomycin "in vivo". These results suggest the possibility that in addition to altering cell permeability, some of the sparsomycin derivatives may act on processes other than protein synthesis in intact cells.[1,12]

Considering the data all together, they confirm clearly the existence of a hydrophobic region on the peptidyl transferase center of prokaryotic and eukaryotic ribosomes with which sparsomycin interacts. This domain is probably involved in the binding of the hydrophobic lateral residues of the amino acyl moiety of aa-tRNA.[30,31] It seems reasonable to think that the presence of this region will help to discriminate among the aa-tRNA molecules favoring the binding of those carrying amino acids with hydrophobic side chains. This might have an effect on the rate of chain elongation affecting less the synthesis of proteins with a low ratio of hydrophobic to hydrophilic amino acids.

Comparison of the data obtained for compounds **1**, **9–12** in the polyPhe assay, in the L1210 clonogenic assay, and in the L1210 in vivo system shows that there is an optimum in activity for pentylsparsomycin **11**. This optimum in activity is most pronounced in the in vivo L1210 system.

Substitution of the bivalent sulfur atom by a methylene group (compare the data of compound **22** vs **10** and **23** vs **11**, respectively) has no serious effect on the in vitro and in vivo biological activity. The in vivo antitumor activity of compounds **22** and **23** is somewhat lower than that of compounds **10** and **11**, respectively. In both cases a surprisingly better T/C ratio was obtained by extension of the side chain with a single methylene group.

In the deshydroxysparsomycin series **30–33** an optimum in activity was observed for compound **32** in the protein synthesis inhibition assay and in the growth inhibition assays of T24 and WiDr tumor cells. In this series compound **31** has the lowest activity in the L1210 clonogenic assay. Despite the lower affinity for the ribosome of compounds **30** and **31** in comparison with sparsomycin (**1**) itself, these analogues are more active in vivo than **1** against L1210 leukemia.

Acute Toxicity. We observed a striking linear relationship between drug toxicity in mice and lipophilicity; an increase of the lipophilicity results in a decrease of the toxicity. The correlations found for the sparsomycin compounds **1**, **10–12**, **22**, **23** and for the deshydroxysparsomycin compounds **30–32** are depicted in entries H and I, respectively, of Table V. Removal of the hydroxy group decreases the drug's toxicity considerably; compare the data of compounds **30** vs **1** and **32** vs **10**, respectively (Table VI). Comparison of the LD_{50} values of compounds **22** vs **10** and **23** vs **11**, respectively, shows that substitution of the bivalent sulfur atom by a methylene group has a variable effect on in vivo toxicity.

A rationale for the decreased toxicity of the more lipophilic analogues could be the following. Pentylsparsomycin **11** has a total body clearance in beagle dogs that is twice that of sparsomycin (**1**).[35] A more rapid clearance permits on one hand the administration of higher doses of the drug, but on the other hand produces a lower AUC (area under the curve). In other words, exposure of the body to the analogues decreases as the lipophicity of the latter increases. Consequently, **11** is less toxic than **1**. The increased volume of distribution and increased protein binding of these lipophilic derivatives may explain further the increase of the LD_{50} value.

The role of the substituent at the chiral carbon atom of sparsomycin on the biological activity in vitro has been investigated earlier.[18] It was concluded that the affinity for the ribosome and the antitumor activity "in vivo" are hardly affected by replacement of this substituent by larger

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(35) Unpublished results.

alkyl or aralkyl groups or by its O-methylation or O-acylation. Some of these changes even cause a slight decrease in activity. Only when the hydroxymethyl group is replaced by a methyl group an increase in the activity in the L1210 clonogenic assay was observed (Table VI). This study shows that also in vivo deshydroxysparsomycin **30** is a better antitumor drug than sparsomycin (Table VI). Unfortunately, substitution of the methylthio group in **30** by larger alkylthio groups, as in compounds **31** and **32**, does not give rise to an increase of the in vivo antitumor activity, as observed in the series **1–9–10–11**.

Conclusion

Our results show that it is possible to improve the protein synthesis inhibitory activity of sparsomycin by increasing the alkyl side chain length at the sulfur substituent. These results and a better knowledge of the peptidyl transferase center will probably enable us to design more active antibiotics in the future. In this sense it is interesting to note that the observed structure–activity relationship can probably hold true for other drugs acting on the same ribosomal active center displaying a similar mode of action such as amicetin, gougerotine, actinobolin, blasticidin S, and anthelmycin.[36] This possibility is worthwhile to explore.

The therapeutic index of sparsomycin was improved considerably by minor modification of the molecular structure. The drug with an *n*-pentyl side chain attached to the bivalent sulfur atom, i.e. **11**, shows the highest antitumor activity in mice. *S*-Oxo-*S*-*n*-heptylsparsomycin **23** and deshydroxysparsomycin **30** also pair high antitumor activity with low toxicity in comparison with the unmodified antibiotic. Compound **30** shows the unique feature of combining an increased lipophilicity with a considerable increase in water solubility.

Experimental Section

Biological Activity. All tests used have been described in detail elsewhere.[11,12] The peptidyl transferase activity was measured by the polyphenylalanine synthesis assay, the "modified fragment reaction", and the puromycin reaction. The "modified fragment reaction" was performed under the same conditions as described for the fragment reaction[32] but with intact *N*-acetyl-Phe-tRNA instead of its terminal pentanucleotide as substrate. The ED_{50} values obtained for sparsomycin with either the *N*-acetyl-aa-tRNA or its terminal pentanuleotide fragment as substrate are very similar (unpublished results). ED_{50} represents the 50% effective dose. In the in vitro tests ribosomes from *E. coli* MRE600 or *S. cerevisiae* Y166 were used.

The activity data on compounds **1**, **12**, **17**, **21**, and **24** are taken from ref 12. These data are shown here for completeness.

In Vitro Cytotoxicity. As a cell growth inhibition assay the growth inhibition of *E. coli* MRE600 in liquid medium was studied.[12] The cytotoxicity against two human tumor cell lines was determined following a new automated technique for large-scale drug cytotoxicity testing, i.e. the PIT (propidium iodide, Ink, Triton X-100) method.[37] The 50% inhibitory dose (ID_{50}) was calculated. In vitro antitumor activity was determined in the L1210 murine leukemia clonogenic assay in soft agar.[11] The 50% inhibitory dose (ID_{50}) was calculated.

Toxicity in Mice. The toxicity of each drug was determined following the NCI protocol.[38] The tests were done on male CDF_1 mice and each drug, suspended or dissolved in 0.9% NaCl, was injected ip daily × 9. There were five dose levels and 10 mice per dose level. The LD_{50} values (milligrams/kilogram per injection) were calculated with use of Weils' tables.[39] The 95% confidence interval was usually within 15–20% of the value shown. These data were reported elsewhere.[40]

Antitumor Activity in Mice. The antitumor activity of the drugs against L1210 leukemia was determined in male CDF_1 mice inoculated with 10^5 vital L1210 cells ip on day 0. Animals were treated with drugs dissolved or suspended in 0.9% NaCl ip on days 1–9. The median survival time of each treated group (T) was compared with that of the control group (C). The results are expressed as a T/C × 100% ratio at the optimal dose (this dose is approximately equal to the LD_{10} value). These data as well as the results of activity tests against other murine tumors (i.e. P388 leukemia, RC carcinoma, and B16 melanoma) were reported elsewhere.[40]

Physicochemical Properties. Chromatography. R_f values were determined by high-performance thin-layer chromatography (hptlc) using Merck RP-8 F_{254s} plates (eluent 60% methanol/water). R_m values were calculated from these R_f values by employment of the following formula: $R_m = \log (1/R_f - 1)$.

Drug concentrations were determined by using a semiautomated high-performance liquid chromatography (HPLC) system from Kratos Analytical (Ramsey, NY). This system consisted of two Spectroflow 400 pumps, Multiport Streamswitch with two six-way valves (Rheodyne), a 757 UV–vis detector, and a DS 610 multirange strip chart recorder. The connection of the sampling valves for the back flushing method has been described before.[41] The whole system was operated by means of an HPLC solvent programmer (9224, Kipp Analytical) equipped with a digitial time indicator.

The columns used were a concentration column (5 cm × 3 mm i.d.) filled with LiChrosorb RP-8 (10 μm particle size) and an analytical column (10 cm × 4.6 mm i.d.) filled with CP Spher C8 (8 μm particle size); both packing materials were obtained from Chrompack B.V. (Middelburg).

Solvent A (flow 1.3 mL/min), used for sample concentration and cleaning of the columns, consisted of filtered tap water. Solvent B (flow 1.0 mL/min), used to flush back the sample from the concentration column through the analytical column, consisted of 0.1 M phosphoric acid buffer (pH 6.7) with different amounts of methanol (Fisons, HPLC grade) added.

Determination of log *k* Values by HPLC. For each drug the retention time (t_r) was determined on HPLC for at least five methanol concentrations (see above). In general, the results for each drug could be described by the following equation:[42] $\log k' = \log k^0 + bM$, where k' = the capacity factor at a certain percentage of organic solvent in the eluent, k^0 = the capacity factor obtained by extrapolation to 0% of organic solvent in the eluent, b = slope, and M = percent of methanol in the eluent. The capacity factor k' is calculated from the difference between the retention time (t_r) of the drug and the retention time (t_0) of the unretained substance: $\log k' = \log (t_r - t_0) - \log t_0$.

Octanol/Water Partition Coefficient. The partition coefficients (P) of the drugs were measured by mixing 1 mL of a solution containing 12 μg/mL of a drug dissolved in demineralized water and 1 mL of 0.1 M phosphoric acid buffer (pH 7.4) saturated with 2 mL of 1-octanol (Merck). After mixing for 1 min on a Vortex mixer and centrifugation at 1400*g* for 10 min, 1 mL of the water phase was analyzed by HPLC (see above). Increasing the time of mixing did not change log P. Each test was repeated three times and each sample was determined in duplicate. The coefficient P was calculated from the following formula: $P = (C - C_w)/C_w$, where C = total concentration of drug in both phases and C_w = concentration of drug in the water phase.

Water Solubility. An excessive amount of each drug was shaken overnight with 5 mL of demineralized water at room temperature (19.5 ± 0.3 °C). The drug suspension was filtered through a paper filter (Rundfilter, no. 595, Schleicher & Schull). The last milliliter of the filtrate was collected and the concen-

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tration was determined by HPLC (see above). Each experiment was performed twice.

Dissociation Constant pK_a. The pK_a values of **1** and **9** were determined following the method of Hurwitz and Liu.[43] The UV absorption of several samples was measured at 328 nm (DU-7 UV–vis spectrophotometer, Beckman). Samples with the same drug concentration dissolved in different borax and phosphate buffers (pH 3.5–12) were prepared and analyzed. The pK_a value was calculated from the following formula: $pK_a = pH - \log (A - A_a/A_b - A)$, where pH = pH of the buffer, A = absorbance at a specific pH, A_a = absorbance in acidic solution (pH 3.5), and A_b = absorbance in basic solution (pH 12). The pK_a value is expressed as a mean of the results of each sample ($n = 10$) ± SD.

Synthesis. Melting points (mp) were determined on a Reichert hot stage and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker WH-90 spectrometer with Me_4Si as an internal standard. Mass spectra (MS, m/z, relative intensity) were recorded on a double-focusing VG 7070E mass spectrometer. Circular dichroism (CD) spectra were recorded on an Autodichrograph Mark V apparatus (Jobin Yvon). For determination of the specific rotation ($[\alpha]^{20}_D$), a Perkin–Elmer 241 polarimeter was used. Thin-layer chromatography (TLC) was carried out on Merck precoated silica gel F-254 plates (thickness 0.25 mm). Spots were visualized with an UV lamp, ninhydrin, or Cl_2-TDM.[44] for column chromatography, Merck silica gel H (type 60) was used. For gel filtration, Merck Fractogel TSK HW-40(F) was used. Ion-exchange chromatography was carried out on Amberlite IRA-410.

(S_CR_S)-*N*-(*tert*-Butyloxycarbonyl)-*S*-oxo-*S*-[(*n*-propylthio)methyl]cysteinol (7a). This compound was prepared in 75% yield from the α-chlorosulfoxide **6** (683 mg, 2.5 mmol) by treatment with $NaS(CH_2)_2Me$ which was prepared in situ by reaction of Na (2.5 mmol) with freshly distilled 1-propanethiol (2.75 mmol) in 15 mL of EtOH, following the procedure that has been described earlier:[8] TLC R_f 0.38 (eluent MeOH/$CHCl_3$, 7:93); ^{1}H NMR ($CDCl_3$) δ 1.01 (t, J = 7.2 Hz, 3 H, CH_2CH_3), 1.44 (s, 9 H, *t*-Bu), 1.68 (sextet, J = 7.4 Hz, 2 H, $CH_2CH_2CH_3$), 2.72 (t, J = 7.3 Hz, 2 H, SCH_2), 2.99 and 3.31 (AB part of ABX spectrum, 8 lines, J_{AX} = 6.6 Hz, J_{BX} = 6.0 Hz, J_{AB} = 13.3 Hz, 2 H, $CHCH_2S(O)$), 3.73–3.94 (m, 2 H, CH_2OH), 3.80 (s, 2 H, $S(O)CH_2S$), 3.89–4.31 (m, 1 H, $CHCH_2$), 5.48 (br d, J = 7 Hz, 1 H, NH); CI MS m/z 312 (32, M + H), 256 (22, $C_8H_{18}NO_4S_2$), 212 (30, $C_7H_{18}NO_2S_2$), 89 (100, C_4H_9S). Anal. ($C_{12}H_{25}NO_4S_2$) C, H, N.

(S_CR_S)-*N*-(*tert*-Butyloxycarbonyl)-*S*-oxo-*S*-[(*n*-butylthio)methyl]cysteinol (7b). This compound was prepared in 74% yield from **6** (542 mg, 2.0 mmol) by treatment with $NaS(CH_2)_3Me$ which was prepared in situ by reaction of Na (2 mmol) with freshly distilled 1-butanethiol (2.2 mmol) in 15 mL of EtOH, following the procedure that has been described earlier.[8] TLC R_f 0.40 (eluent MeOH/$CHCl_3$, 7:93); ^{1}H NMR ($CDCl_3$) δ 0.91 (br t, J = 6.7 Hz, 3 H, CH_2CH_3), 1.23–1.80 (m, 4 H, $CH_2CH_2CH_3$), 1.44 (s, 9 H, *t*-Bu), 2.73 (t, J = 7.1 Hz, 2 H, SCH_2CH_2), 2.96 and 3.29 (AB part of ABX spectrum, 8 lines, J_{AX} = 6.7 Hz, J_{BX} = 6.0 Hz, J_{AB} = 13.3 Hz, 2 H, $CHCH_2S(O)$), 3.63–3.93 (m, 2 H, CH_2OH), 3.80 (s, 2 H, $S(O)CH_2S$), 3.93–4.24 (m, 1 H, $CHCH_2$), 5.44 (br d, J = 7 Hz, 1 H, NH); CI MS m/z 326 (26, M + H), 270 (26, $C_9H_{20}NO_4S_2$), 226 (31, $C_8H_{20}NO_2S_2$), 103 (100, $C_5H_{11}S$). Anal. ($C_{13}H_{27}NO_4S_2$) C, H, N.

(S_CR_S)-*N*-(*tert*-Butyloxycarbonyl)-*S*-oxo-*S*-[(*n*-pentylthio)methyl]cysteinol (7c). This compound was prepared in 78% yield from **6** (542 mg, 2.0 mmol) by treatment with $NaS(CH_2)_4Me$ which was prepared in situ by reaction of Na (2.0 mmol) with freshly distilled 1-pentanethiol (2.2 mmol) in 15 mL of EtOH, following the procedure that has been described earlier:[8] TLC R_f 0.42 (eluent MeOH/$CHCl_3$, 7:93); ^{1}H NMR ($CDCl_3$) δ 0.90 (t, J = 7.0 Hz, 3 H, CH_2CH_3), 1.18–1.92 (m, 6 H, $(CH_2)_3CH_3$), 1.44 (s, 9 H, *t*-Bu), 2.72 (t, J = 7.0 Hz, 2 H, SCH_2CH_2), 3.00 and 3.32 (AB part of ABX spectrum, 8 lines, J_{AX} = 6.7 Hz, J_{BX} = 6.0 Hz, J_{AB} = 13.3 Hz, 2 H, $CHCH_2S(O)$), 3.63–3.90 (m, 2 H, CH_2OH), 3.79 (s, 2 H, $S(O)CH_2S$), 3.93–4.24 (m, 1 H, $CHCH_2$), 5.48 (br d, J = 7 Hz, 1 H, NH); CI MS m/z 340 (26, M + H), 284 (26, $C_{10}H_{22}NO_4S_2$), 240 (31, $C_9H_{22}NO_2S_2$), 117 (100, $C_6H_{13}S$). Anal. ($C_{14}H_{29}NO_4S_2$) C, H, N.

(S_CR_S)-*N*-(*tert*-Butyloxycarbonyl)-*S*-oxo-*S*-[(*tert*-butylthio)methyl]cysteinol (7d). This compound was prepared in 85% yield from **6** (393 mg, 1.4 mmol) by treatment with NaS-*t*-Bu which was prepared in situ by reaction of Na with freshly distilled 2-methyl-2-propanethiol in EtOH, following the procedure that has been described earlier:[8] TLC R_f 0.31 (eluent MeOH/CH_2Cl_2, 7:93); ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 9 H, S-*t*-Bu), 1.44 (s, 9 H, O-*t*-Bu), 3.09 and 3.22 (AB part of ABX spectrum, 8 lines, J_{AX} = 7.0 Hz, J_{BX} = 6.1 Hz, J_{AB} = 13.4 Hz, 2 H, $CHCH_2S(O)$), 3.20–3.58 (m, 1 H, OH), 3.80 and 3.91 (AB q, J_{AB} = 13.6 Hz, 2 H, $S(O)CH_2S$), 3.58–4.36 (m, 3 H, $CHCH_2OH$), 5.20–5.62 (br d, 1 H, NH); CI MS exact mass calcd for $C_{13}H_{28}NO_4S_2$ m/z 326.1460 (M + H), found 326.1451; MS m/z 326 (10), 270 (20, $C_9H_{20}NO_4S_2$), 226 (15, $C_8H_{20}NO_2S_2$), 103 (100, $C_5H_{11}S$), 57 (87, C_4H_9). Anal. ($C_{13}H_{27}NO_4S_2$) C, H, N.

(S_CR_S)-*N*-(*tert*-Butyloxycarbonyl)-*S*-oxo-*S*-[(cyclohexylthio)methyl]cysteinol (7e). This compound was prepared in 85% yield from **6** (399 mg, 1.5 mmol) by treatment with $NaSC_6H_{11}$ which was prepared in situ by reaction of Na with freshly distilled cyclohexyl mercaptan in EtOH, following the procedure that has been described earlier:[8] TLC R_f 0.44 (eluent MeOH/CH_2Cl_2, 7:93); ^{1}H NMR ($CDCl_3$) δ 1.08–2.16 (m, 10 H, $(CH_2)_5$), 1.46 (s, 9 H, *t*-Bu), 2.69–3.13 (m, 1 H, SCH), 3.07 and 3.20 (AB part of ABX spectrum, 8 lines, J_{AX} = 7.0 Hz, J_{BX} = 6.1 Hz, J_{AB} = 13.5 Hz, 2 H, $CHCH_2S(O)$), 3.13–3.58 (m, 1 H, OH), 3.58–3.93 (m, 2 H, CH_2OH), 3.83 (s, 2 H, $S(O)CH_2S$), 3.93–4.33 (m, 1 H, $CHCH_2OH$), 6.22–6.56 (br d, 1 H, NH); CI MS exact mass calcd for $C_{15}H_{30}NO_4S_2$ m/z 352.1616 (M + H), found 352.1612; MS m/z 352 (12), 296 (20, $C_{11}H_{22}NO_4S_2$), 252 (14, $C_{10}H_{22}NO_2S_2$), 129 (100, $C_7H_{13}S$), 57 (34, C_4H_9). Anal. ($C_{15}H_{29}NO_4S_2$) C, H, N.

(S_CR_S)-*S*-Oxo-*S*-[(*n*-propylthio)methyl]cysteinol (8a). The compound was prepared in 87% yield from compound **7a** (553 mg, 1.78 mmol) by treatment with TFA at 0 °C and subsequent ion-exchange column chromatography on Amberlite following the procedure that has been described earlier:[8] ^{1}H NMR ($CDCl_3$) δ 1.01 (t, J = 7.1 Hz, 3 H, CH_2CH_3), 1.69 (sextet, J = 7.5 Hz, 2 H, CH_2CH_3), 2.06 (br s, 3 H, NH_2, OH), 3.02–3.67 (m, 4 H, $CHCH_2S(O)$, SCH_2CH_2), 3.33–3.65 (m, 3 H, CH_2OH, $CHCH_2$), 3.79 (s, 2 H, $S(O)CH_2S$). Anal. ($C_7H_{17}NO_2S_2$) C, H, N.

(S_CR_S)-*S*-Oxo-*S*-[(*n*-butylthio)methyl]cysteinol (8b). Following the procedure that has been described earlier,[8] the Boc amino protecting group of compound **7b** (480 mg, 1.48 mmol) was removed by treatment with TFA at 0 °C and subsequent ion-exchange column chromatography on Amberlite to yield the desired compound in 89% yield: ^{1}H NMR ($CDCl_3$) δ 0.92 (t, J = 6.8 Hz, 3 H, CH_2CH_3), 1.18–1.77 (m, 4 H, $(CH_2)_2CH_3$), 2.47–3.01 (m, 7 H, SCH_2CH_2, $CHCH_2S(O)$, NH_2, OH), 3.36–3.70 (m, 3 H, CH_2OH, $CHCH_2$), 3.78 (s, 2 H, $S(O)CH_2S$); CI MS m/z 226 (100, M + H, $C_8H_{19}NO_2S_2$), 136 (14, $C_4H_{10}NO_2S$), 103 (37, $C_5H_{11}S$).

(S_CR_S)-*S*-Oxo-*S*-[(*n*-pentylthio)methyl]cysteinol (8c). The compound was prepared in 73% yield from compound **7c** (583 mg, 1.77 mmol) by treatment with TFA at 0 °C and subsequent ion-exchange column chromatography on Amberlite following the procedure that has been described earlier:[8] ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 6.5 Hz, 3 H, CH_2CH_3), 1.09–1.49 (m, 4 H, $(CH_2)_2CH_3$), 1.63 (quintet, J = 7.4 Hz, 2 H, SCH_2CH_2), 1.89 (br s, 3 H, NH_2, OH), 2.73 (t, J = 7.3 Hz, 2 H, SCH_2CH_2), 2.82–3.18 (m, 2 H, $CHCH_2S(O)$), 3.34–3.71 (m, 3 H, CH_2OH, $CHCH_2$), 3.77 (s, 2 H, $S(O)CH_2S$); CI MS m/z 240 (36, M + H), 136 (65, $C_4H_{10}NO_2S$), 117 (100, $C_6H_{13}S$). Anal. ($C_9H_{21}NO_2S_2$) C, H, N.

(S_CR_S)-*S*-Oxo-*S*-[(*tert*-butylthio)methyl]cysteinol (8d). This compound was prepared from **7d** (353 mg, 1.1 mmol) by treatment with TFA and ion-exchange column chromatography on Amberlite according to the procedure that has been described earlier[8] to give the desired product in 90% yield: TLC R_f 0.32 (eluent MeOH/$CHCl_3$, 1:4); ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 9 H, *t*-Bu), 1.82 (br s, 2 H, NH_2), 2.81 and 2.93 (AB part of ABX spectrum, 8 lines, J_{AX} = 8.4 Hz, J_{BX} = 3.9 Hz, J_{AB} = 13.2 Hz, 2 H, $CHCH_2S(O)$), 2.82–3.27 (m, 1 H, OH), 3.27–3.71 (m, 3 H, $CHCH_2OH$), 3.76 and 3.91 (AB q, J_{AB} = 14.1 Hz, 2 H, $S(O)CH_2S$); CI MS exact mass calcd for $C_8H_{20}NO_2S_2$ m/z 226.0935 (M + H), found 226.0935; MS m/z 226 (100), 136 (46, $C_4H_{10}NO_2S$), 103 (83, $C_6H_{11}S$), 57 (78, C_4H_9).

(43) Hurwitz, A. R.; Liu, S. T. *J. Pharm. Sci.* **1977**, *66*, 624.
(44) von Arx, E.; Faupel, M.; Brugger, M. *J. Chromatogr.* **1976**, *120*, 224.

(S_CR_S)-S-Oxo-S-[(cyclohexylthio)methyl]cysteinol (8e). This compound was prepared from **7e** (353 mg, 1.0 mmol) by treatment with TFA and ion-exchange column chromatography on Amberlite according to the procedure that has been described earlier[8] to give the desired product in 99% yield: TLC R_f 0.35 (eluent MeOH/$CHCl_3$, 1:4); ^1H NMR ($CDCl_3$) δ 1.06–2.29 (m, 10 H, $(CH_2)_5$), 1.97 (br s, 2 H, NH_2), 2.56–3.18 (m, 4 H, SCH, OH, $CHCH_2S(O)$), 3.32–3.72 (m, 3 H, $CHCH_2OH$), 3.67 and 3.73 (AB q, J_{AB} = 14.2 Hz, 2 H, $S(O)CH_2S$); CI MS exact mass calcd for $C_{10}H_{22}NO_2S_2$ m/z 252.1092 (M + H), found 252.1093; MS m/z 252 (9), 136 (27, $C_4H_{10}NO_2S$), 129 (100, $C_7H_{13}S$), 83 (84, C_6H_{11}).

(S_CR_S)-2-[(E)-β-(6-Methyl-5-uracilyl)acrylamido]-3-[[(n-propylthio)methyl]sulfinyl]propanol (9). This compound was prepared in 18% yield (93 mg) from compounds **8a** (335 mg, 1.71 mmol) following the same procedure as described for the preparation of compound **12**:[11] TLC R_f 0.37 (eluent MeOH/$CHCl_3$, 1:4); ^1H NMR (D_2O) δ 0.89 (d or t, J = 6.9 Hz, 3 H, CH_2CH_3), 1.53 (sextet, J = 7.3 Hz, 2 H, CH_2CH_3), 2.34 (s, 3 H, C(6)-CH_3), 2.66 (t, J = 7.5 Hz, 2 H, SCH_2CH_2), 2.92–3.36 (m, 2 H, $CHCH_2S(O)$), 3.40–3.89 (m, 3 H, $CHCH_2$, CH_2OH), 3.91 and 4.05 (AB q, J_{AB} = 14.0 Hz, 2 H, $S(O)CH_2S$), 6.99 and 7.37 (AB q, J_{AB} = 15.5 Hz, 2 H, CH=CH); FAB MS m/z 390 (M + H, $C_{15}H_{24}N_3O_5S_2$); $[\alpha]^{20}_D$ +80° (c 0.08, MeOH/H_2O, 1:1).

(S_CR_S)-2-[(E)-β-(6-Methyl-5-uracilyl)acrylamido]-3-[[(n-butylthio)methyl]sulfinyl]propanol (10). This compound was prepared in 16% yield (82 mg) from compound **8b** (284 mg, 1.27 mmol) following the same procedure as described for the preparation of compound **12**:[11] TLC R_f 0.37 (eluent MeOH/$CHCl_3$, 1:4); ^1H NMR (CD_3OD) δ 0.94 (br t, J = 6.5 Hz, 3 H, CH_2CH_3), 1.18–1.83 (m, 4 H, $(CH_2)_2CH_3$), 2.38 (s, 3 H, C(6)-CH_3), 2.80 (t, J = 6.9 Hz, 2 H, SCH_2CH_2), 2.97–3.35 (m, 2 H, $CHCH_2S(O)$), 3.72 (d, J = 5.1 Hz, 2 H, CH_2OH), 3.90 and 4.04 (AB q, J_{AB} = 13.8 Hz, 2 H, $S(O)CH_2S$), 4.33–4.71 (m, 1 H, $CHCH_2$), 7.26 and 7.47 (AB q, J_{AB} = 15.4 Hz, 2 H, CH=CH); FAB MS m/z 404 (10, M + H), 103 (32, $C_5H_{11}S$); $[\alpha]^{20}_D$ + 85° (c 0.22, MeOH/H_2O, 1:1). Anal. ($C_{16}H_{25}N_3O_5S_2 \cdot {}^1/_2H_2O$) C, H, N.

(S_CR_S)-2-[(E)-β-(6-Methyl-5-uracilyl)acrylamido]-3-[[(n-pentylthio)methyl]sulfinyl]propanol (11). Via the same procedure as described for the preparation of compound **12**,[11] this compound was prepared in 40% yield (212 mg) from compound **8c** (314 mg, 1.6 mmol): TLC R_f 0.45 (eluent MeOH/$CHCl_3$, 1:4); ^1H NMR (CD_3OD) δ 0.92 (br t, J = 7 Hz, 3 H, CH_2CH_3), 1.12–1.54 (m, 4 H, $(CH_2)_2CH_3$), 1.54–1.88 (m, 2 H, SCH_2CH_2), 2.40 (s, 3 H, C(6)-CH_3), 2.79 (t, J = 6.5 Hz, 2 H, SCH_2CH_2), 2.95–3.35 (m, 2 H, $CHCH_2S(O)$), 3.72 (d, J = 5.1 Hz, 2 H, CH_2OH), 3.91 and 4.05 (AB q, J_{AB} = 14.0 Hz, 2 H, $S(O)CH_2S$), 4.28–4.69 (m, 1 H, $CHCH_2$), 7.26 and 7.47 (AB q, J_{AB} = 15.3 Hz, 2 H, CH=CH); FAB MS m/z 418 (11, M + H), 284 (7, $C_{11}H_{14}N_3O_4S$), 179 (40, $C_8H_7N_2O_3$), 165 (12, $C_6H_{13}OS_2$), 153 (9, $C_7H_9N_2O_2$), 117 (57, $C_6H_{13}S$); $[\alpha]^{20}_D$ +82° (c 0.11, MeOH/H_2O, 1:1). Anal. ($C_{16}H_{27}N_3O_5S_2 \cdot H_2O$) C, H, N.

(S_CR_S)-2-[(E)-β-(6-Methyl-5-uracilyl)acrylamido]-3-[[(tert-butylthio)methyl]sulfinyl]propanol (13). This compound was prepared in 30% yield (112 mg) from compound **8d** (206 mg, 0.91 mmol) following the same procedure as described for the preparation of compound **12**.[11] The crude reaction product was purified by a combination of chromatography on Fractogel (eluent MeOH/H_2O, 1:1) and countercurrent distribution (eluent $CHCl_3$/CCl_4/MeOH/buffer, 8:3:8:5; buffer, 19.3 g of NH_4OAc and 28.6 mL of HOAc in a total volume of 1L of water; 240 transfers in 10 mL bank): TLC R_f 0.41 (eluent MeOH/$CHCl_3$, 1:4); ^1H NMR (CD_3OD) δ 1.42 (s, 9 H, t-Bu), 2.39 (s, 3 H, C(6)-CH_3), 3.02 and 3.35 (AB part of ABX spectrum, 8 lines, J_{AX} = 9.9 Hz, J_{BX} = 3.7 Hz, J_{AB} = 13.2 Hz, 2 H, $CHCH_2S(O)$), 3.75 (d, J = 5.1 Hz, 2 H, CH_2OH), 4.03 (s, 2 H, $S(O)CH_2S$), 4.38–4.76 (m, 1 H, $CHCH_2$), 7.29 and 7.49 (AB q, J_{AB} = 15.4 Hz, 2 H, CH=CH); $[\alpha]^{20}_D$ +85° (c 0.125, MeOH/H_2O, 1:1); FAB MS m/z 404 (11, M + H, $C_{16}H_{26}N_3O_5S_2$), 226 (35, $C_8H_{20}NO_2S_2$), 179 (15, $C_8H_7N_2O_3$), 103 (100, $C_5H_{11}S$).

(S_CR_S)-2-[(E)-β-(6-Methyl-5-uracilyl)acrylamido]-3-[[(cyclohexylthio)methyl]sulfinyl]propanol (14). Following the same procedure as described for the preparation of compound **12**,[11] this compound was prepared in 40% yield (152 mg) from compound **8e** (221 mg, 0.88 mmol). The crude reaction product was purified by a combination of gel filtration on Fractogel and countercurrent distribution (420 transfers in 10 mL bank) as described for the preparation of compound **13**: TLC R_f 0.47 (eluent MeOH/$CHCl_3$, 1:4); ^1H NMR (CD_3OD) δ 0.77–2.26 (m, 10 H, $(CH_2)_5$), 2.35 (s, 3 H, C(6)-CH_3), 2.68–3.04 (m, 1 H, CHS), 3.02 and 3.28 (AB part of ABX spectrum, 8 lines, J_{AX} = 10.0 Hz, J_{BX} = 4.2 Hz, J_{AB} = 13.3 Hz, 2 H, $CHCH_2S(O)$, 3.72 (d, J = 5.1 Hz, 2 H, CH_2OH), 3.93 and 4.03 (AB q, J_{AB} = 13.9 Hz, 2 H, $S(O)CH_2S$), 4.33–4.68 (m, 1 H, $CHCH_2S(O)$), 7.24 and 7.45 (AB q, J_{AB} = 15.4 Hz, 2 H, CH=CH); $[\alpha]^{20}_D$ +81° (c 0.130, MeOH/H_2O, 1:1); FAB MS m/z 430 (10, M + H, $C_{18}H_{28}N_3O_5S_2$), 252 (33, $C_{10}H_{22}NO_2S_2$), 179 (14, $C_8H_7N_2O_3$), 129 (100, $C_7H_{13}S$).

N-[(E)-β-(6-Methyl-5-uracilyl)acryloyl]-S-(pentylthio)-D-cysteinol (16). This compound was isolated as a by-product (50 mg) of a large-scale synthesis (2 g) of pentylsparsomycin (11): TLC R_f 0.72 (eluent MeOH/H_2O, 1:4); ^1H NMR (CD_3OD) δ 0.94 (br t, 1.17–1.58 (m, 4 H), $(CH_2)_2CH_3$), 1.58–1.91 (m, 2 H, SCH_2CH_2), 2.39 (s, 3 H, C(6)-CH_3), 2.76 (t, J = 6.9 Hz, 2 H, SCH_2CH_2), 2.80 and 3.07 (AB part of ABX spectrum, 8 lines, J_{AX} = 6.0 Hz, J_{BX} = 4.4 Hz, J_{AB} = 13.8 Hz, 2 H, $CHCH_2S$), 3.69 (br d, J = 4.8 Hz, 2 H, CH_2OH), 4.17–4.54 (m, 1 H, $CHCH_2$), 7.26 and 7.45 (AB q, J_{AB} = 15.3 Hz, 2 H, CH=CH); FAB MS m/z 388 (38, M + H), 284 (16, $C_{11}H_{14}N_3O_4S$), 252 (10, $C_{11}H_{14}N_3O_4$), 210 (26, $C_8H_{20}NOS_2$), 179 (100, $C_8H_7N_2O_3$), 153 (16, $C_7H_9N_2O_2$), 117 (71, $C_6H_{13}S$); $[\alpha]^{20}_D$ +128.5° (c 0.135, MeOH/H_2O, 1:1). Anal. ($C_{16}H_{25}N_3O_4S_2 \cdot {}^1/_2H_2O$) C, H, N, S.

(S_CR_S)-2-[(E)-β-(6-Methyl-5-uracilyl)acrylamido]-3-[[[[2-[(1-oxoethyl)amino]ethyl]thio]methyl]sulfinyl]-propanol (18). This compound was prepared in 23% yield (15 mg) by reaction of **17** (53 mg, 0.15 mmol) with the sodium salt of N-acetylcysteamine (24 mg, 0.16 mmol) in liquid NH_3 for 1.5 h at −78 °C. The thiolate was prepared by reduction of the corresponding disulfide with 2 equiv of Na in liquid NH_3. The reaction vessel was kept under an argon atmosphere throughout the reaction. After evaporation of the ammonia, the reaction product was taken up in water and the pH was adjusted to 7 with a 2 N HCl solution. Gel filtration over Fractogel (eluent water) was carried out for purification of the product: TLC R_f 0.11 (eluent MeOH/$CHCl_3$, 1:4); ^1H NMR (D_2O) δ 1.89 (s, 3 H, C(O)CH_3), 2.32 (s, 3 H, C(6)-CH_3), 2.81 (t, J = 6.5 Hz, 2 H, SCH_2CH_2), 2.98–3.18 (AB part of ABX spectrum, 4 lines, 2 H, $CHCH_2S(O)$), 3.33 (t, J = 6.5 Hz, 2 H, SCH_2CH_2), 3.71 (d, J = 5.2 Hz, 2 H, CH_2OH), 3.93 and 4.09 (AB q, J_{AB} = 13.9 Hz, 2 H, $S(O)CH_2S$), 4.26–4.64 (m, 1 H, $CHCH_2$), 6.99 and 7.31 (AB q, J_{AB} = 15.3 Hz, 2 H, CH=CH); FAB MS m/z 433 (2%, M + H, $C_{16}H_{25}N_4O_6S_2$), 179 (3%, $C_8H_7N_2O_3$).

N,N-Diacetylcystamine.[45] This compound was prepared by a modified procedure known from the literature[45] by reaction of cystamine dihydrochloride (1.8 g, 8 mmol) with Ac_2O (4.9 g, 48 mmol) in 5 mL of pyridine. After stirring for 1 h at room temperature, a clear solution was formed. Evaporation in vacuo yielded the crude reaction product which was purified by column chromatography on silica gel (eluent MeOH/CH_2Cl_2, 3:97) to give the pure compound in 83% yield: mp 90 °C; TLC R_f 0.24 (eluent MeOH/$CHCl_3$, 1:10); ^1H NMR ($CDCl_3$) δ 2.00 (s, 3 H, C(O)CH_3), 2.81 (t, J = 6.0 Hz, 2 H, CH_2S), 3.58 (q, J = 6.0 Hz, 2 H, $NHCH_2$), 6.48 (br s, 1 H, NH); CI MS exact mass calcd for $C_8H_{17}N_2O_2S_2$ m/z 237.0731 (M + H), found 237.0724; MS m/z 237 (72), 118 (100, C_4H_8NOS). Anal. ($C_8H_{16}N_2O_2S_2$) C, H, N.

N-(tert-Butyloxycarbonyl)-S-n-hexyl-D-cysteine Methyl Ester (19a). This compound was prepared in quantitative yield from the hydrochloride of S-n-hexyl-D-cysteine methyl ester (19.6 g, 76.7 mmol) by treatment with di-tert-butyl dicarbonate following the procedure that has been described earlier.[8] TLC R_f 0.84 (eluent MeOH/CH_2Cl_2, 3:97); ^1H NMR ($CDCl_3$) δ 0.88 (br t, J = 7.0 Hz, 3 H, CH_2CH_3), 1.46 (s, 9 H, t-Bu), 1.14–1.83 (m, 8 H, $SCH_2(CH_2)_4$), 2.52 (t, J = 7.0 Hz, 2 H, SCH_2CH_2), 2.95 (d, J = 5.1 Hz, 2 H, $CHCH_2S$), 3.75 (s, 3 H, CO_2CH_3), 4.31–4.66 (m, 1 H, $CHCH_2S$), 5.14–5.44 (m, 1 H, NH); CI MS exact mass calcd for $C_{15}H_{30}NO_4S$ m/z 320.1896 (M + H), found 320.1888; MS m/z 320 (17), 264 (68, $C_{11}H_{22}NO_4S$), 220 (100, $C_{10}H_{22}NO_2S$), 203 (55, $C_{10}H_{19}O_2S$).

The hydrochloride of S-n-hexyl-D-cysteine methyl ester was prepared from S-n-hexyl-D-cysteine (16.6 g, 81.0 mmol) by treatment with $SOCl_2$ in MeOH following the procedure that has

(45) Conrad, R. A.; Cullinan, G. J.; Gerzon, K.; Poore, G. A. *J. Med. Chem.* **1979**, *22*, 391.

been described earlier[8] to yield the desired compound in 96%: TLC R_f 0.79 (eluent *s*-BuOH/NH_4OH, 55:22); ^1H NMR (CD_3OD) δ 0.92 (br t, 3 H, CH_2CH_3), 1.11–1.90 (m, 8 H, $SCH_2(CH_2)_4$), 2.61 (t, J = 6.8 Hz, 2 H, SCH_2CH_2), 3.00–3.09 (m, 2 H, $CHCH_2S$), 3.84 (s, 3 H, CO_2CH_3), 4.13–4.46 (m, 1 H, $CHCH_2S$). Anal. Calcd for $C_{10}H_{22}NO_2SCl$: C, 46.95; H, 8.67; N, 5.48. Found: C, 46.38; H, 8.56; N, 5.43.

S-*n*-Hexyl-D-cysteine was prepared from D-cystine (10.0 g, 41.6 mmol) and 1-bromohexane with Na in liquid NH_3 according to a procedure described earlier[12] in 98% yield: TLC R_f 0.60 (eluent *n*-BuOH/HOAc/H_2O, 4:1:1); CI MS exact mass calcd for $C_9H_{20}NO_2S$ m/z 206.1215 (M + H), found 206.1221; MS m/z 206 (24), 189 (100, $C_9H_{19}NOS$). Anal. Calcd for $C_9H_{19}NO_2S$: C, 52.65; H, 9.33; N, 6.82. Found: C, 51.77; H, 9.12; N, 6.86.

***N*-(*tert*-Butyloxycarbonyl)-*S*-*n*-heptyl-D-cysteine Methyl Ester (19b).** This compound was prepared in quantitative yield from the hydrochloride of *S*-*n*-heptyl-D-cysteine methyl ester (15.3 g, 56.9 mmol) by treatment with di-*tert*-butyl dicarbonate following the procedure that has been described earlier:[8] TLC R_f 0.76 (eluent MeOH/CH_2Cl_2, 3:97); ^1H NMR ($CDCl_3$) δ 0.88 (br t, J = 5.4 Hz, 3 H, CH_2CH_3), 1.05–1.84 (m, 10 H, $SCH_2(CH_2)_5$), 1.44 (s, 9 H, *t*-Bu), 2.51 (t, J = 7.0 Hz, 2 H, SCH_2CH_2), 2.98 (d, J = 5.1 Hz, 2 H, $CHCH_2S$), 3.71 (s, 3 H, CO_2CH_3), 4.33–4.67 (m, 1 H, $CHCH_2S$), 5.51 (br d, J = 7.8 Hz, 1 H, NH); CI MS exact mass calcd for $C_{16}H_{32}NO_4S$ m/z 334.2052 (M + H), found 334.2045; MS m/z 334 (5), 278 (44, $C_{12}H_{24}NO_4S$), 234 (100, $C_{11}H_{24}NO_2S$), 217 (71, $C_{11}H_{21}O_2S$).

The hydrochloride of *S*-*n*-heptyl-D-cysteine methyl ester was prepared from *S*-*n*-heptyl-D-cysteine (12.6 g, 57.4 mmol) by treatment with $SOCl_2$ in MeOH following the procedure that has been described earlier[8] to yield the desired compound in 99%: TLC R_f 0.43 (eluent MeOH/CH_2Cl_2, 3:97); ^1H NMR (CD_3OD) δ 0.89 (br t, J = 6.2 Hz, 3 H, CH_2CH_3), 1.04–1.81 (m, 10 H, $SCH_2(CH_2)_5$), 2.59 (t, J = 7.1 Hz, 2 H, SCH_2CH_2), 3.02–3.12 (AB part of ABX spectrum, 4 lines, 2 H, $CHCH_2S$), 3.87 (s, 3 H, CO_2CH_3), 4.20–4.33 (X part of ABX spectrum, 4 lines, $J_{AX} + J_{BX}$ = 11.9 Hz, 1 H, $CHCH_2S$). Anal. Calcd for $C_{11}H_{24}NO_2SCl$: C, 48.96; H, 8.97; N, 5.19. Found: C, 48.00; H, 8.92; N, 5.09.

S-*n*-Heptyl-D-cysteine was prepared from D-cystine (7.6 g, 31.4 mmol) and 1-bromoheptane with Na in liquid NH_3 according to a procedure described earlier[12] in 93% yield: TLC R_f 0.47 (eluent *n*-BuOH/HOAc/H_2O, 4:1:1); CI MS exact mass calcd for $C_{10}H_{22}NO_2S$ m/z 220.1371 (M + H), found 220.1368; MS m/z 220 (21), 203 (100, $C_{10}H_{21}NOS$). Anal. ($C_{10}H_{21}NO_2S$) C, H, N.

***(S_CS_S)*-*N*-(*tert*-Butyloxycarbonyl)-*S*-oxo-*S*-*n*-hexylcysteinol (20a).** This compound was prepared by oxidation of *N*-(*tert*-butyloxycarbonyl)-*S*-*n*-hexyl-D-cysteinol (18.6 g, 64.0 mmol) with $NaIO_4$ according to the procedure that has been described earlier[12] to give the desired product as a mixture of diastereomers in quantitative yield. The stereochemical structure of the S_cR_s compound is not shown in Scheme IV. The diastereomers were separated by repeated column chromatography on silica gel (eluent MeOH/CH_2Cl_2, gradient 3:97–5:95). The diastereomers were obtained in roughly equal amounts. Both compounds were homogeneous on TLC (S_cS_s, R_f 0.28; S_cR_s, R_f 0.31; eluent MeOH/CH_2Cl_2, 7:93).

For the S_CS_S compound (**20a**): ^1H NMR ($CDCl_3$) δ 0.90 (br t, J = 5.7 Hz, 3 H, CH_2CH_3), 1.11–2.33 (m, 8 H, $(CH_2)_4CH_3$), 1.44 (s, 9 H, *t*-Bu), 2.77 (d of t, J = 7.5 Hz, 2 H, $S(O)CH_2CH_2$), 2.90–3.18 (m, 2 H, $CHCH_2S(O)$), 3.35–3.69 (m, 1 H, OH), 3.69–4.24 (m, 3 H, $CHCH_2OH$), 5.50 (br d, J = 7.3 Hz, 1 H, NH); CI MS exact mass calcd for $C_{14}H_{30}NO_4S$ m/z 308.1896 (M + H), found 308.1891; MS m/z 308 (27), 252 (49, $C_{10}H_{22}NO_4S$), 234 (35, $C_{10}H_{20}NO_3S$), 208 (100, $C_9H_{22}NO_2S$); $[\alpha]^{20}_D$ +40.2° (*c* 0.132, MeCN); CD spectrum (MeCN), at 217.5 nm a single positive Cotton effect was observed ($\Delta\epsilon$ = +0.28). Anal. Calcd for $C_{14}H_{29}NO_4S$: C, 54.69; H, 9.51; N, 4.56. Found: C, 55.42; H, 9.58; N, 4.51.

For the S_CR_S compound: ^1H NMR ($CDCl_3$) δ 0.90 (br t, J = 5.7 Hz, 3 H, CH_2CH_3), 1.08–2.22 (m, 8 H, $(CH_2)_4CH_3$), 1.44 (s, 9 H, *t*-Bu), 2.80 (d of t, J = 7.5 Hz, 2 H, $S(O)CH_2CH_2$), 3.01 and 3.15 (AB part of ABX spectrum, 8 lines, J_{AX} = 5.8 Hz, J_{BX} = 3.2 Hz, J_{AB} = 13.5 Hz, 2 H, $CHCH_2S(O)$), 3.52–3.96 (m, 2 H, CH_2OH), 3.96–4.47 (m, 2 H, $CHCH_2OH$), 5.52 (br d, J = 6.3 Hz, 1 H, NH); CI MS exact mass calcd for $C_{14}H_{30}NO_4S$ m/z 308.1896 (M + H), found 308.1900; MS m/z 308 (11), 252 (41, $C_{10}H_{22}NO_4S$), 234 (37, $C_{10}H_{20}NO_3S$), 208 (100, $C_9H_{22}NO_2S$); mp 141.5–142.5 °C; $[\alpha]^{20}_D$ +31.2° (*c* 0.138, MeCN); CD spectrum (MeCN), at 220.5 nm a single negative Cotton effect was observed ($\Delta\epsilon$ = −0.18).

***N*-(*tert*-Butyloxycarbonyl)-*S*-*n*-hexyl-D-cysteinol.** This compound was prepared by reduction of *N*-(*tert*-butyloxycarbonyl)-*S*-*n*-hexyl-D-cysteine methyl ester (20.6 g, 65.0 mmol) with $LiBH_4$ following the procedure that has been described earlier[8] to give the desired product in quantitative yield: TLC R_f 0.64 (eluent MeOH/CH_2Cl_2, 7:93); ^1H NMR ($CDCl_3$) δ 0.89 (br t, J = 5.6 Hz, 3 H, CH_2CH_3), 1.06–1.56 (m, 8 H, $CH_2(CH_2)_4$), 1.44 (s, 9 H, *t*-Bu), 1.56–2.32 (m, 1 H, OH), 2.56 (t, J = 6.9 Hz, 2 H, SCH_2CH_2), 2.70 (d, J = 6.0 Hz, 2 H, $CHCH_2S$), 3.56–3.94 (m, 3 H, $CHCH_2OH$), 4.78–5.18 (m, 1 H, NH); CI MS exact mass calcd for $C_{14}H_{30}NO_3S$ m/z 292.1946 (M + H), found 292.1937; MS m/z 292 (13), 236 (57, $C_{10}H_{22}NO_3S$), 192 (100, $C_9H_{22}NOS$), 175 (86, $C_9H_{19}OS$).

***(S_CS_S)*-*N*-(*tert*-Butyloxycarbonyl)-*S*-oxo-*S*-*n*-heptylcysteinol (20b).**[46] This compound was prepared by oxidation of *N*-(*tert*-butyloxycarbonyl)-*S*-*n*-heptyl-D-cysteinol (15.1 g, 49.5 mmol) with $NaIO_4$ according to the procedure that has been described earlier[12] to give the desired product as a mixture of diastereomers in 96% yield. The structure of the S_cR_s compound is not shown in Scheme IV. The diastereomers were separated by repeated column chromatography on silica gel (eluent MeOH/CH_2Cl_2, gradient 3:97–5:95). The diastereomers were obtained in roughly equal amounts. Both compounds were homogeneous on TLC (S_cS_s, R_f 0.39; S_cR_s, R_f 0.40; eluent MeOH/CH_2Cl_2, 7:93).

For the S_CS_S compound (**20b**): ^1H NMR ($CDCl_3$) δ 0.88 (br t, J = 5.4 Hz, 3 H, CH_2CH_3), 1.09–2.09 (m, 10 H, $(CH_2)_5CH_3$), 1.42 (s, 9 H, *t*-Bu), 2.49 (br s, 1 H, OH), 2.78 (d of t, J = 7.2 Hz, 2 H, $S(O)CH_2CH_2$), 2.91–3.27 (m, 2 H, $CHCH_2S(O)$), 3.64–3.93 (m, 2 H, CH_2OH), 3.93–4.28 (m, 1 H, $CHCH_2$), 5.50 (br d, J = 7.0 Hz, 1 H, NH); CI MS exact mass calcd for $C_{15}H_{32}NO_4S$ m/z 322.2052 (M + H), found 322.2054; MS m/z 322 (9), 266 (34, $C_{11}H_{24}NO_4S$), 248 (29, $C_{11}H_{22}NO_3S$), 222 (56, $C_{10}H_{24}NO_2S$), 57 (100, C_4H_9). Anal. ($C_{15}H_{31}NO_4S$) C, H, N.

For the S_CR_S compound: ^1H NMR ($CDCl_3$) δ 0.89 (br t, J = 5.6 Hz, 3 H, CH_2CH_3), 1.09–2.18 (m, 10 H, $(CH_2)_5CH_3$), 1.43 (s, 9 H, *t*-Bu), 2.80 (d of t, J = 7.3 Hz, 2 H, $S(O)CH_2CH_2$), 3.00 and 3.14 (AB part of ABX spectrum, 8 lines, J_{AX} = 6.0 Hz, J_{BX} = 3.4 Hz, J_{AB} = 13.5 Hz, 2 H, $CHCH_2S(O)$), 3.61–3.98 (m, 2 H, CH_2OH), 3.98–4.44 (m, 2 H, $CHCH_2OH$), 5.52 (br d, J = 6.6 Hz, 1 H, NH); CI MS exact mass calcd for $C_{15}H_{32}NO_4S$ m/z 322.2052 (M + H), found 322.2048; MS m/z 322 (14), 266 (31, $C_{11}H_{24}NO_4S$), 248 (43, $C_{11}H_{22}NO_3S$), 222 (54, $C_{10}H_{24}NO_2S$), 57 (100, C_4H_9). Anal. ($C_{15}H_{31}NO_4S$) C, H, N.

***N*-(*tert*-Butyloxycarbonyl)-*S*-*n*-heptyl-D-cysteinol.** This compound was prepared by reduction of *N*-(*tert*-butyloxycarbonyl)-*S*-*n*-heptyl-D-cysteine methyl ester (18.6 g, 56.0 mmol) with $LiBH_4$ following the procedure that has been described earlier[8] to give the desired product in 92% yield: TLC R_f 0.62 (eluent MeOH/CH_2Cl_2, 7:93); ^1H NMR ($CDCl_3$) δ 0.90 (br t, J = 5.7 Hz, 3 H, CH_2CH_3), 1.03–2.00 (m, 10 H, $SCH_2(CH_2)_5$), 1.44 (s, 9 H, *t*-Bu), 2.00–2.43 (m, 1 H, OH), 2.55 (t, J = 6.8 Hz, 2 H, SCH_2CH_2), 2.70 (d, J = 6.0 Hz, 2 H, $CHCH_2S$), 3.49–4.10 (m, 3 H, $CHCH_2OH$), 4.78–5.44 (m, 1 H, NH); CI MS exact mass calcd for $C_{15}H_{32}NO_3S$ m/z 306.2103 (M + H), found 306.2094; MS m/z 306 (28), 250 (77, $C_{11}H_{24}NO_3S$), 206 (100, $C_{10}H_{24}NOS$), 189 (77, $C_{10}H_{21}OS$).

***(S_CS_S)*-2-[(*E*)-β-(6-Methyl-5-uracilyl)acrylamido]-3-(*n*-hexylsulfinyl)propanol (22).** This compound was prepared in 33% yield (380 mg) by coupling *S*-oxo-*S*-hexylcysteinol (619 mg, 3.0 mmol) with **2** in a mixed-anhydride procedure using isobutyl chloroformate as has been described earlier:[11] TLC R_f 0.33 (eluent MeOH/$CHCl_3$, 1:4); ^1H NMR (CD_3OD) δ 0.91 (br t, 3 H, CH_2CH_3),

(46) Separation of the diastereomeric sulfoxides **20** can be effected by chromatography on silica gel using as eluent MeOH/CH_2Cl_2, 7:93. By preparing the corresponding *tert*-butyldimethylsilyl ether of **20b**, i.e. **20c**, the sulfoxides could be separated more easily on silica gel with ether/hexane 7:3 as eluent. We observed that the relative position of both diastereomers on TLC changed when ether/hexane was used as eluent. Chromatography under pressure (0.5 atm) resulted in substantial hydrolysis of the silyl ether (unpublished results).

1.16–1.70 (m, 6 H, $(CH_2)_3CH_3$), 1.70–1.97 (m, 2 H, $S(O)CH_2CH_2$), 2.36 (s, 3 H, C(6)–CH_3), 2.86 (br t, J = 7.2 Hz, 2 H, $S(O)CH_2CH_2$), 3.03 (br d, J = 7.2 Hz, 2 H, $CHCH_2S(O)$), 3.68 (br d, J = 5.7 Hz, 2 H, CH_2OH), 4.35–4.75 (m, 1 H, $CHCH_2$), 7.20 and 7.42 (AB q, J_{AB} = 15.2 Hz, 2 H, CH=CH); FAB MS m/z 386 (46, M + H, $C_{17}H_{28}N_3O_5S$), 252 (19, $C_{11}H_{14}N_3O_4$), 208 (100, $C_9H_{22}NO_2S$), 179 (73, $C_8H_7N_2O_3$), 74 (58, C_3H_8NO); MS m/z 384 (100, M – H, $C_{17}H_{26}N_3O_5S$); $[\alpha]^{20}_D$ +80.8° (c 0.104, MeOH/H_2O, 1:1).

(S_CS_S)-2-[(*E*)-β-(6-Methyl-5-uracilyl)acrylamido]-3-(*n*-heptylsulfinyl)propanol (23). This compound was prepared in 24% yield (330 mg) by coupling *S*-oxo-*S*-heptylcysteinol (750 mg, 3.4 mmol) with **2** in a mixed-anhydride procedure using isobutyl chloroformate as has been described earlier.[11] The crude reaction product was purified by a combination of gel filtration on Fractogel (eluent MeOH/H_2O, 1:1) and countercurrent distribution (eluent $CHCl_3$/CCl_4/MeOH/buffer, 7:2:8:4; buffer: 19.3 g of NH_4OAc and 28.6 mL of HOAc in a total volume of 1 L of water; 560 transfers in 25 mL bank): TLC R_f 0.35 (eluent MeOH/$CHCl_3$, 1:4); ^{1}H NMR (CD_3OD) δ 0.90 (br t, 3 H, CH_2CH_3), 1.16–1.67 (m, 8 H, $(CH_2)_4CH_3$), 1.67–1.80 (m, 2 H, $S(O)CH_2CH_2$), 2.33 (s, 3 H, C(6)-CH_3), 2.83 (t, J = 7.5 Hz, 2 H, $S(O)CH_2CH_2$), 3.02 (br d, J = 7.0 Hz, 2 H, $CHCH_2S(O)$), 3.67 (br d, J = 5.0 Hz, 2 H, CH_2OH), 4.29–4.69 (m, 1 H, $CHCH_2$), 7.20 and 7.41 (AB q, J_{AB} = 15.0 Hz, 2 H, CH=CH); FAB MS m/z 400 (48, M + H, $C_{18}H_{29}N_3O_5S$), 252 (23, $C_{11}H_{14}N_3O_4$), 222 (84, $C_{10}H_{24}NO_2S$), 179 (100, $C_8H_7N_2O_3$), 74 (60, C_3H_8NO); $[\alpha]^{20}_D$ +73.1° (c 0.104, MeOH/H_2O, 1:1).

***N*-(*tert*-Butyloxycarbonyl)-L-alaninol (25).** This compound was prepared in 90% yield from the ester (2.44 g, 12 mmol) by reduction with $LiBH_4$ in dry DME analogously to the method described earlier.[8] The product was used for the next reaction without further purification: ^{1}H NMR ($CDCl_3$) δ 1.14 (d, J = 6.6 Hz, 3 H, $CHCH_3$), 1.44 (s, 9 H, *t*-Bu), 2.71 (br s, 1 H, CH_2OH), 3.45 and 3.55 (AB part of ABX spectrum, 7 lines, J_{AX} = 4.7 Hz, J_{BX} = 7.4 Hz, J_{AB} = 10.1 Hz, 2 H, CH_2OH), 3.47–3.95 (m, 1 H, $CHCH_3$), 4.68 (br d, 1 H, NH). Anal. ($C_8H_{17}NO_3$) C, H, N.

***N*-(*tert*-Butyloxycarbonyl)-L-alanine Methyl Ester.** This compound was prepared in 93% yield from the hydrochloride of L-alanine methyl ester (13.2 g, 94.6 mmol) by treatment with di-*tert*-butyl dicarbonate following the procedure that has been described earlier.[8] TLC R_f 0.80 (eluent MeOH/$CHCl_3$, 3:97); ^{1}H NMR ($CDCl_3$) δ 1.36 (d, J = 5 Hz, 3 H, $CHCH_3$), 1.50 (s, 9 H, *t*-Bu), 3.76 (s, 3 H, CO_2CH_3), 4.03–4.65 (m, 1 H, $CHCH_3$), 5.50 (br d, 1 H, NH).

(*S*)-*O*-(*p*-Tolylsulfonyl)-*N*-(*tert*-butyloxycarbonyl)-1-hydroxy-2-aminopropane (26). To solution of the alcohol **25** (11.4 mmol) in 76 mL of dry pyridine was added tosyl chloride (12.5 mmol) at –10 °C. The reaction mixture was stirred at 4 °C overnight. After completion of the reaction as was monitored by TLC (eluent MeOH/$CHCl_3$, 5:95), most of the pyridine was removed by evaporation in vacuo at room temperature. The residue was dissolved in 200 mL of CH_2Cl_2 and subsequently washed two times with a 2 N $KHSO_4$ solution to remove pyridine and then with water. The organic layer was dried (Na_2SO_4) and the solvent was evaporated in vacuo at room temperature. The crude product was obtained as a white solid in 87% yield. The crystalline product was not purified by recrystallization because of the instability of this compound. In attempts to recrystallize the product, it decomposes even at room temperature into the cyclic urethane **34**. For **26**: TLC R_f 0.79 (eluent MeOH/$CHCl_3$, 5:95); ^{1}H NMR ($CDCl_3$) δ 1.15 (d, J = 6.5 Hz, 3 H, $CHCH_3$), 1.37 (s, 9 H, *t*-Bu), 2.44 (s, 3 H, $PhCH_3$), 3.67–4.11 (m, 1 H, $CHCH_3$), 3.95 (br s, 2 H, CH_2O), 4.53 (br d, 1 H, NH), 7.22 and 7.73 (AB q, J_{AB} = 8 Hz, 4 H, Ph-H); CI MS m/z 330 (M + H, $C_{15}H_{24}NO_5S$).

(*S*)-*S*-Acetyl-*N*-(*tert*-butyloxycarbonyl)-1-mercapto-2-aminopropane (27). To a suspension of Cs_2CO_3 (18.5 g, 57 mmol) in 60 mL of dry DMF was added freshly distilled thioacetic acid (9.0 g, 119 mmol). Previously, nitrogen had been passed through the suspension for 15 min. When the cesium carbonate was dissolved, the crude tosylate **26** (28.8 g, 96 mmol) dissolved in 40 mL of dry DMF was added. The reaction mixture was stirred in the dark for 16 h at room temperature and was kept under a nitrogen atmosphere. The reaction was monitored by TLC (eluent diisopropyl ether/hexane, 1:1). After completion of the reaction, the solvent was evaporated in vacuo. The residue was dissolved in 300 mL of CH_2Cl_2 and washed with water. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The crude reaction product was subjected to column chromatography (silica gel, eluent diisopropyl ether/hexane, 1:1) to give **27** in 80% yield. The yield is based on the alcohol **25**: TLC R_f 0.37 (eluent diisopropyl ether/hexane, 1:1); ^{1}H NMR ($CDCl_3$) δ 1.15 (d, J = 6.5 Hz, 3 H, $CHCH_3$), 1.44 (s, 9 H, *t*-Bu), 2.35 (s, 3 H, $C(O)CH_3$), 3.02 (d, J = 6 Hz, 2 H, $CHCH_2$), 3.59–4.03 (m, 1 H, $CHCH_2$), 4.73 (br d, J = 8 Hz, 1 H, NH). Anal. ($C_{10}H_{19}NO_3S$) C, H, N.

(S_CR_S)-1-[(Chloromethyl)sulfinyl]-2-[(*tert*-butyloxycarbonyl)amino]propane (28). This compound was prepared with some modifications of the procedure described earlier.[8] Thioester **27** (14.0 g, 60 mmol) and Ac_2O (6.12 g, 60 mmol) were dissolved in 150 mL of dry CH_2Cl_2. The solution was stirred and cooled to –10 °C. Subsequently, a solution of 8.9 g of dry gaseous Cl_2 (the theoretically necessary amount of chlorine was 120 mmol, 8.4 g) in 20 mL of dry CH_2Cl_2—cooled at –50 °C—was added via a connecting tube. The temperature of the reaction mixture was kept below 0 °C. After the addition had been completed, the cooling was removed and the reaction mixture was allowed to warm up and was stirred for 1 h at room temperature. The so-prepared sulfinyl chloride precipitated. Subsequently, 200 mL of dry CCl_4 was added, and the organic solvents were evaporated in vacuo at room temperature. The residue was stripped with another 200 mL of dry CCl_4 to remove the AcCl. The resulting sulfinyl chloride was dissolved in 150 mL of dry CH_2Cl_2 and added dropwise over a period of 3 h to a stirred, cooled, and dried (KOH pellets) solution of excess CH_2N_2 in Et_2O. During the reaction the temperature was kept at 0 °C. The reaction mixture was stirred overnight at room temperature. The solvents were evaporated in vacuo and the crude product was purified by column chromatography on silica gel (eluent MeOH/CH_2Cl_2, gradient from 0% to 2% of MeOH) to give the diastereomeric α-chloro sulfoxides in 41% overall yield. The S_cS_s diastereomer was formed in slight excess as appeared from the ^{1}H NMR spectrum of the reaction product. The structure of the S_cS_s compound is not shown in Scheme V. Separation of the diastereomeric α-chloro sulfoxides, a tedious task, was achieved by crystallization from EtOAc to yield the pure S_CR_S compound. Subsequent column chromatography of the residue on silica gel (eluent *i*-Pr_2O/MeOH, 93:7) yielded the pure S_cS_s diastereomer next to a considerable amount of the other diastereomer. This procedure was carried out repeatedly.

For the S_CR_S compound (**28**): mp 177–178 °C; TLC R_f 0.23 (eluent *i*-Pr_2O/MeOH, 93:7); ^{1}H NMR ($CDCl_3$) δ 1.42 (d, J = 5 Hz, 3 H, $CHCH_3$), 1.44 (s, 9 H, *t*-Bu), 3.00 and 3.17 (AB part of ABX spectrum, 7 lines, J_{AX} = 9.0 Hz, J_{BX} = 5.4 Hz, J_{AB} = 13.5 Hz, 2 H, $CHCH_2S(O)$), 3.69–4.35 (m, 1 H, $CHCH_3$), 4.39 and 4.49 (AB q, J_{AB} = 11 Hz, 2 H, $S(O)CH_2Cl$), 5.08 (br d, 1 H, NH); CI MS m/z 256 (M + H). Anal. ($C_9H_{18}NO_3SCl$) C, H, N; CD spectrum (MeCN), at 229 nm a single negative Cotton effect was observed ($\Delta\epsilon$ = –0.6).

For the S_CS_S compound: mp 130 °C; TLC R_f 0.25 (eluent diisopropyl ether/methanol, 93:7); ^{1}H NMR ($CDCl_3$) δ 1.34 (d, J = 6.6 Hz, 3 H, $CHCH_3$), 1.42 (s, 9 H, *t*-Bu), 2.89 and 3.18 (AB part of ABX spectrum, 8 lines, J_{AX} = 9.9 Hz, J_{BX} = 5.4 Hz, J_{AB} = 13.5 Hz, 2 H, $CHCH_2S(O)$), 3.79–4.24 (m, 1 H, $CHCH_3$), 4.57 (s, 2 H, $S(O)CH_2Cl$), 4.73 (br d, 1 H, NH); CI MS m/z 256 (M + H); CD spectrum (MeCN), at 229 nm a single positive Cotton effect was observed ($\Delta\epsilon$ = +0.6). Anal. ($C_9H_{18}NO_3SCl$) C, H, N.

(S_CR_S)-1-[[(Methylthio)methyl]sulfinyl]-2-[(*tert*-butyloxycarbonyl)amino]propane (29a). Following the procedure that has been described earlier,[8] **28** (3.6 mmol) was allowed to react with NaSMe in EtOH to give the desired compound in 98% yield: TLC R_f 0.18 (eluent EtOAc/hexane, 3:1); ^{1}H NMR ($CDCl_3$) δ 1.42 (d, J = 6 Hz, 3 H, $CHCH_3$), 1.44 (s, 9 H, *t*-Bu), 2.33 (s, 3 H, SCH_3), 2.85 and 3.13 (AB part of ABX spectrum, 8 lines, J_{AX} = 8.1 Hz, J_{BX} = 4.5 Hz, J_{AB} = 13.5 Hz, 2 H, $CHCH_2S(O)$), 3.69 and 3.75 (AB q, J_{AB} = 13.5 Hz, 2 H, $S(O)CH_2S$), 3.97–4.48 (m, 1 H, $CHCH_3$), 4.97–5.40 (m, 1 H, NH); EI MS m/z 268 (M + H). Anal. Calcd for $C_{10}H_{21}NO_3S_2$: C, 44.92; H, 7.92; N, 5.24. Found: C, 45.77; H, 8.04; N, 5.25.

(S_CR_S)-1-[[(*n*-Butylthio)methyl]sulfinyl]-2-[(*tert*-butyloxycarbonyl)amino]propane (29c). This compound was prepared in 95% yield from **28** (1.17 g, 4.6 mmol) by treatment with $NaS(CH_2)_3Me$ which was prepared in situ by reaction of Na (4.6 mmol) with freshly distilled 1-butanethiol (5.0 mmol) in 20

mL of EtOH. The reaction mixture was worked up following the procedure that has been described earlier:[8] TLC R_f 0.42 (eluent MeOH/$CHCl_3$, 3:97); ^1H NMR ($CDCl_3$) δ 0.93 (t, J = 6 Hz, 3 H, CH_2CH_3), 1.23–1.80 (m, 4 H, $CH_2CH_2CH_3$), 1.41 (d, J = 6 Hz, 3 H, $CHCH_3$), 1.44 (s, 9 H, t-Bu), 2.74 (t, J = 7 Hz, 2 H, SCH_2CH_2), 2.82 and 3.20 (AB part of ABX spectrum, 8 lines, J_{AX} = 7.0 Hz, J_{BX} = 4.4 Hz, J_{AB} = 13.0 Hz, 2 H, $CHCH_2S(O)$), 3.76 (s, 2 H, $S(O)CH_2S$), 4.02–4.43 (m, 1 H, $CHCH_2$), 5.06–5.41 (m, 1 H, NH); CI MS exact mass calcd for $C_{13}H_{28}NO_3S_2$ m/z 310.1511 (M + H), found 310.1512; MS m/z 310 (37), 254 (88, $C_9H_{20}NO_3S_2$), 210 (70, $C_8H_{20}NOS_2$), 103 (100, $C_5H_{11}S$). Anal. ($C_{13}H_{27}NO_3S_2$) C, H, N.

(S_CR_S)-1-[[(*n*-Octylthio)methyl]sulfinyl]-2-[(*tert*-butyloxycarbonyl)amino]propane (29d). This compound was prepared in quantitative yield from **28** (315 mg, 1.23 mmol) by treatment with $NaS(CH_2)_7Me$ which was prepared in situ by reaction of Na (1.35 mmol) with freshly distilled 1-octanethiol (1.48 mmol) in 10 mL of EtOH. The reaction mixture was worked up following the procedure that has been described earlier:[8] TLC R_f 0.57 (eluent MeOH/$CHCl_3$, 1:4); ^1H NMR ($CDCl_3$) δ 0.81 (br t, J = 6 Hz, 3 H, CH_2CH_3), 1.20 (br s, 10 H, $(CH_2)_5CH_3$), 1.25 (d, 3 H, $CHCH_3$), 1.37 (s, 9 H, t-Bu), 1.40–1.78 (m, 2 H, SCH_2CH_2), 2.66 (t, J = 7.2 Hz, 2 H, SCH_2CH_2), 2.71 and 3.09 (AB part of ABX spectrum, 8 lines, J_{AX} = 7.2 Hz, J_{BX} = 5.4 Hz, J_{AB} = 12.9 Hz, 2 H, $CHCH_2S(O)$), 3.68 (s, 2 H, $S(O)CH_2S$), 3.91–4.31 (m, 1 H, $CHCH_3$), 5.31 (br d, J = 7 Hz, 1 H, NH). Anal. ($C_{17}H_{35}NO_3S_2 \cdot {}^1/_2H_2O$) C, H, N.

(S_CR_S)-1-[[(Methylthio)methyl]sulfinyl]-2-[(*E*)-β-(6-methyl-5-uracilyl)acrylamido]propane (30). This compound was prepared in the following way. The Boc protecting group of **29a** was removed by reaction with TFA at 0 °C.[8] After concentration in vacuo, the resulting oil was used subsequently for coupling. To a solution of **2** (784 mg, 4.0 mmol) in 5 mL of DMF at 0 °C was added Et_3N (404 mg, 4.0 mmol) and isobutyl chloroformate (544 mg, 4.0 mmol). After 5 min, a solution of **29a** (664 mg, 4.0 mmol) in 10 mL of DMF was added. The reaction mixture was stirred in the dark at 0 °C for 16 h. Evaporation of the solvent and gel filtration on Fractogel (eluent MeOH/H_2O, 85:15) afforded the desired compound **30** in 71% yield (based on the α-chloro sulfoxide **33a**): TLC R_f 0.53 (eluent MeOH/$CHCl_3$, 1:4); ^1H NMR (D_2O) δ 1.37 (d, J = 6.0 Hz, 3 H, $CHCH_3$), 2.26 (s, 3 H, SCH_3), 2.37 (s, 3 H, C(6)-CH_3), 3.11 and 3.19 (AB part of ABX spectrum, 5 lines, J_{AX} = 5.4 Hz, J_{BX} = 3.6 Hz, J_AB = 13.5 Hz, 2 H, $CHCH_2S(O)$), 3.90 and 4.06 (AB q, J_{AB} = 13.5 Hz, 2 H, $S(O)CH_2S$), 4.31–4.62 (m, 1 H, $CHCH_3$), 7.00 and 7.30 (AB q, J_{AB} = 16.0 Hz, 2 H, CH=CH); FAB MS m/z 346 (11, M + H), 179 (10, $C_8H_7N_2O_3$), 168 (5, $C_5H_{14}NOS_2$); MS m/z 344 (100, M − H); $[\alpha]^{20}_D$ +80° (c 0.155, MeOH/H_2O, 1:1). Anal. ($C_{13}H_{19}N_3O_4S_2$) C, H, N.

(S_CR_S)-1-[[(Ethylthio)methyl]sulfinyl]-2-[(*E*)-β-(6-methyl-5-uracilyl)acrylamido]propane (31). This compound was prepared in 60% overall yield from **28** (600 mg, 2.35 mmol) following the procedures which have been described for compound **30** but using NaSEt instead of NaSMe for the substitution reaction. Alternatively, from **28** (750 mg, 2.94 mmol) 930 mg (88% overall yield) of **31** was obtained with use of 1 equiv of the pentafluorophenyl ester of **2**, which was prepared in situ by reaction of **2** with pentafluorophenol and DCC in DMF,[24] in the amide coupling reaction and otherwise similar reaction conditions as used in the preparation of **30**: TLC R_f 0.55 (eluent MeOH/$CHCl_3$, 1:4); ^1H NMR (D2O) δ 1.19 (t, J = 7.5 Hz, 3 H, CH_2CH_3), 1.33 (d, J = 7.2 Hz, 3 H, $CHCH_3$), 2.33 (s, 3 H, C(6)-CH_3), 2.65 and 2.79 (AB q, J_{AB} = 7.3 Hz, 2 H, CH_2CH_3), 1.96 and 2.10 (AB part of ABX spectrum, 8 lines, J_{AX} = 10.1 Hz, J_{BX} = 3.4 Hz, J_{AB} = 13.5 Hz, 2 H, $CHCH_2S(O)$), 3.94 and 4.08 (AB q, J_{AB} = 14.1 Hz, 2 H, $S(O)CH_2S$), 4.33–4.66 (m, 1 H, $CHCH_2$), 6.96 and 7.33 (AB q, J_{AB} = 15.5 Hz, 2 H, CH=CH); FAB MS m/z 360 (78, M + H, $C_{14}H_{22}N_3O_4S_2$), 181 (10, $C_6H_{15}NOS_2$), 179 (100, $C_8H_7N_2O_3$), 75 (22, C_3H_7S); $[\alpha]^{20}_D$ +83° (c 0.16, MeOH/H_2O, 1:1).

(S_CR_S)-1-[[(*n*-Butylthio)methyl]sulfinyl]-2-[(*E*)-β-(6-methyl-5-uracilyl)acrylamido]propane (32). This compound was prepared in 37% overall yield (620 mg) (based on the chloromethyl sulfoxide **28**) from the amine (1.33 g, 4.30 mmol), following the same procedures that have been described for the preparation of compound **30**: TLC R_f 0.66 (eluent MeOH/$CHCl_3$, 1:4); ^1H NMR (CD_3OD) δ 0.77 (br t, J = 6.3 Hz, 3 H, CH_2CH_3), 1.20 (d, J = 6.9 Hz, 3 H, $CHCH_3$), 1.10–1.68 (m, 4 H, $CH_2CH_2CH_3$), 2.18 (s, 3 H, C(6)-CH_3), 2.60 (t, J = 7.2 Hz, 2 H, SCH_2CH_2), 2.79 and 3.03 (AB part of ABX spectrum, J_{AX} = 9.3 Hz, J_{BX} = 4.4 Hz, J_{AB} = 13.0 Hz, 2 H, $CHCH_2S(O)$), 3.70 and 3.83 (AB q, J_{AB} = 13.6 Hz, 2 H, $S(O)CH_2S$), 4.21–4.57 (m, 1 H, $CHCH_3$), 7.02 and 7.27 (AB q, J_{AB} = 15.0 Hz, 2 H, CH=CH); FAB MS m/z 388 (17, M + H), 179 (16, $C_8H_7N_2O_3$), 103 (30, $C_5H_{11}S$); $[\alpha]^{20}_D$ +80° (c 0.115, MeOH/H_2O, 1:1). Anal. ($C_{16}H_{25}N_3O_4S_2 \cdot H_2O$), C, H, N.

(S_CR_S)-1-[[(*n*-Octylthio)methyl]sulfinyl]-2-[(*E*)-β-(6-methyl-5-uracilyl)acrylamido]propane (33). Following the same procedures which have been described for the preparation of compound **30**, this compound was prepared in 50% overall yield (272 mg) (based on the α-chloro sulfoxide **28**) from the amine (340 mg, 1.24 mmol): TLC R_f 0.71 (eluent MeOH/$CHCl_3$, 1:4); ^1H NMR (CD_3OD) δ 0.91 (br t, J = 5.5 Hz, 3 H, CH_2CH_3), 1.28 (br s, 10 H, $(CH_2)_5CH_3$), 1.37 (d, J = 8.0 Hz, 3 H, $CHCH_3$), 1.52–1.82 (m, 2 H, SCH_2CH_2), 2.35 (s, 3 H, C(6)-CH_3), 2.77 (t, J = 7.0 Hz, 2 H, SCH_2CH_2), 3.30 (d, J = 5.0 Hz) and 3.17 (d, J = 9.0 Hz, AB part of ABX spectrum, 2 H, $CHCH_2S(O)$), 3.88 and 3.95 (AB q, J_{AB} = 13.5 Hz, 2 H, $S(O)CH_2S$), 4.42–4.51 (m, 1 H, $CHCH_3$), 7.18 and 7.42 (AB q, J_{AB} = 16.0 Hz, 2 H, CH=CH); FAB MS m/z 444 (2, M + H), 207 (18, $C_9H_{19}OS_2$), 179 (3, $C_8H_7N_2O_3$), 159 (4, $C_9H_{19}S$); $[\alpha]^{20}_D$ +93° (c 0.102, MeOH). Anal. ($C_{20}H_{33}N_3O_4S_2$) C, H, N.

2-Oxo-4-methyloxazolidine (34). This compound was prepared in quantitative yield from **26** by refluxing for 30 min in CH_2Cl_2: TLC R_f 0.28 (eluent MeOH/$CHCl_3$, 5:95); ^1H NMR ($CDCl_3$) δ 1.27 (d, J = 5.9 Hz, 3 H, $CHCH_3$), 3.78–4.19 (m, 2 H, $CHCH_2$), 4.29–4.64 (m, 1 H, CH), 6.98 (br s, 1 H, NH); EI MS m/z 101 (M, $C_4H_7NO_2$).

(S_CR_S)-1-[(Chloromethyl)sulfinyl]-2-[(*E*)-β-(6-methyl-5-uracilyl)acrylamido]propane (35). This compound was prepared in 26% overall yield (695 mg) from compound **28** (2.04 g, 8.0 mmol) following the same procedures that have been described for the preparation of **30**: TLC R_f 0.43 (eluent MeOH/$CHCl_3$, 1:4); ^1H NMR (D_2O) δ 1.37 (d, J = 6.9 Hz, 3 H, $CHCH_3$), 2.37 (s, 3 H, C(6)-CH_3), 2.95–3.41 (AB part of ABX spectrum, 2 H, $CHCH_2$), 4.32–5.12 (m, 3 H, CH, CH_2Cl), 7.01 and 7.39 (AB q, J_{AB} = 15.6 Hz, 2 H, CH=CH); FAB MS m/z 334 (20, M + H, $C_{12}H_{17}N_3O_4SCl$), 256 (100, $C_{10}H_{10}N_3O_3Cl$), 222 (81, $C_{10}H_{12}N_3O_3$), 179 (24, $C_8H_7N_2O_3$), 156 (22, $C_4H_{11}NOSCl$); $[\alpha]^{20}_D$ +90° (c 0.115, MeOH/H_2O, 1:1).

1-(*n*-Propylthio)-2-[(*tert*-butyloxycarbonyl)amino]propane (36). A solution of the tosylate **26** (5.2 g, 15.8 mmol) in 15 mL of dry EtOH was added to a solution of $NaS(CH_2)_2Me$ in 15 mL of dry EtOH which was prepared in situ by reaction of 1-propanethiol (1.14 g, 15 mmol) and Na (380 mg, 16.5 mmol). The entire reaction vessel was kept carefully under an argon atmosphere. The reaction mixture was stirred at room temperature during the night. The solvent was removed in vacuo and the product was extracted between ether and water. The water layer was once extracted with ether, and the combined organic layers were twice extracted with water and once with a saturated NaCl solution. After drying ($MgSO_4$) and concentration in vacuo a yellow oil was obtained. After a quick column chromatographic procedure on silica gel using CH_2Cl_2 as eluent, 3.2 g (92% overall yield) of **36** was obtained as as colorless oil: TLC R_f 0.57 (eluent i-Pr_2O); ^1H NMR ($CDCl_3$) δ 0.98 (t, J = 7.2 Hz, 3 H, CH_2CH_3), 1.19 (d, J = 6.5 Hz, 3 H, $CHCH_3$), 1.43 (s, 9 H, t-Bu), 1.54–1.86 (m, 2 H, CH_2CH_3), 2.52 (t, J = 7.2 Hz, 2 H, SCH_2CH_2), 2.54 and 2.68 (AB part of ABX spectrum, 8 lines, J_{AX} = 4.0 Hz, J_{BX} = 2.5 Hz, J_{AB} = 11.9 Hz, 2 H, $CHCH_2S$), 3.83 (septet, J = 6.5 Hz, 1 H, CH), 4.44–4.84 (br d, 1 H, NH); CI MS exact mass calcd for $C_{11}H_{24}NO_2S$ m/z 234.1528 (M + H), found 234.1524; MS m/z 234 (14), 178 (38, $C_7H_{16}NO_2S$), 134 (31, $C_6H_{16}NS$), 117 (100, $C_6H_{13}S$), 57 (23, C_4H_9).

(S_CS_S)-1-(*n*-Propylsulfinyl)-2-[(*tert*-butyloxycarbonyl)amino]propane (37). This compound and its S epimer were synthesized in approximately equal amounts by oxidation of **36** (1.95 g, 8.37 mmol) in 40 mL of CH_2Cl_2 with mCPBA[23] (2.2 g, 12.8 mmol) at 0 °C for 10 min in 91% combined yields. The two diastereomers could be separated by a combination of column chromatography on silica gel (eluent THF/i-Pr_2O, 1:1) and crystallization from $CHCl_3/Et_2O$: TLC R_f 0.56 (**37**, S_CS_S) and 0.61 (S_CR_S) (eluent THF).

For the S_CS_S compound **37**: mp 139–140 °C; ^1H NMR ($CDCl_3$) δ 1.07 (t, J = 7.2 Hz, 3 H, CH_2CH_3), 1.38 (d, J = 5.4 Hz, 3 H, $CHCH_3$), 1.41 (s, 9 H, t-Bu), 1.82 (sextet, J = 7.3 Hz, 2 H, CH_2CH_3), 2.46–2.96 (m, 2 H, $S(O)CH_2CH_2$), 2.83 (d, J = 5.7 Hz,

2 H, $CHCH_2$), 4.17 (septet, J = 6.6 Hz, 1 H, CH), 5.36 (br d, J = 7 Hz, 1 H, NH); CI MS m/z 250 (2, M + H), 194 (5, $C_7H_{16}NO_3S$), 150 (34, $C_6H_{16}NOS$), 57 (100, C_4H_9), 44 (32, CO_2); $[\alpha]^{20}_D$ +40.8 (c 0.12, MeOH). CD spectrum (MeCN), at 216 nm a single positive Cotton effect was observed ($\Delta\epsilon$ = +0.31). Anal. ($C_{11}H_{23}NO_3S$) C, H, N.

For the S_CR_S compound: mp 131–132 °C; ^{1}H NMR ($CDCl_3$) δ 1.08 (t, J = 7.2 Hz, 3 H, CH_2CH_3), 1.37 (d, J = 6.7 Hz, 3 H, $CHCH_3$), 1.41 (s, 9 H, t-Bu), 1.63–2.03 (m, 2 H, CH_2CH_3), 2.76 (t, J = 8 Hz, 2 H, $S(O)CH_2CH_2$), 2.89 (d, J = 6.6 Hz, 2 H, $CHCH_2$), 4.88 (septet, J = 6.8 Hz, 1 H, CH), 4.96 (br d, J = 6.6 Hz, 1 H, NH); CI MS m/z 250 (1, M + H), 194 (1, $C_7H_{16}NO_3S$), 150 (22, $C_6H_{16}NOS$), 57 (100, C_4H_9), 44 (23, CO_2); $[\alpha]^{20}_D$ +20.7° (c 0.135, MeOH); CD spectrum (MeCN), at 221 nm a single negative Cotton effect was observed ($\Delta\epsilon$ = −0.21). Anal. ($C_{11}H_{23}NO_3S$) C, H, N.

(S_CS_S)-1-(*n*-Propylsulfinyl)-2-[(*E*)-β-(6-methyl-5-uracilyl)acrylamido]propane (38). The *N*-Boc protecting group of **37** (530 mg, 2.13 mmol) was removed by stirring with 10 mL of TFA for 30 min at 0 °C. After evaporation of the excess TFA, stripping twice with EtOH and drying in vacuo, the amine was dissolved in 20 mL of DMF and the solution was neutralized with Et_3N. Subsequently, Et_3N (0.3 mL, 1 equiv) and the pentafluorophenyl ester of **2** (771 mg, 2.13 mmol), which was prepared in situ by reaction of **2** with pentafluorophenol and DCC in DMF,[24] were added, and the reaction mixture was stirred in the dark for 18 h at room temperature. Concentration in vacuo and gel filtration of the crude reaction product on Fractogel (eluent MeOH/H_2O, 1:1) afforded 502 mg (72% yield) of a white material after lyophilization from HOAc and drying in an exsiccator on KOH: TLC R_f 0.39 (eluent MeOH/$CHCl_3$, 1:4); ^{1}H NMR (D_2O) δ 1.04 (t, J = 7.4 Hz, 3 H, CH_2CH_3), 1.36 (d, J = 6.7 Hz, 3 H, $CHCH_3$), 1.77 (sextet, J = 7.4 Hz, 2 H, CH_2CH_3), 2.34 (s, 3 H, C(6)-CH_3), 2.89 (t, J = 7.4 Hz, 2 H, $S(O)CH_2CH_2$), 3.06 (d, J = 6.7 Hz, 2 H, $CHCH_2$), 4.42 (quintet, J = 6.6 Hz, 1 H, CH), 6.96 and 7.30 (AB q, J_{AB} = 15.4 Hz, 2 H, CH=CH); FAB MS m/z 328 (7, M + H, $C_{14}H_{22}N_3O_4S$), 179 (11, $C_8H_7N_2O_3$), 150 (53, $C_6H_{16}NOS$); $[\alpha]^{20}_D$ +98.3° (c 0.115, MeOH).

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Water-Soluble Third Generation Antitumor Platinum Complexes, [2,2-Bis(aminomethyl)-1,3-propanediol-*N*,*N*']-[1,1-cyclobutanedicarboxylato(2-)-*O*,*O*']platinum(II) and [1,1-Cyclobutanedicarboxylato(2-)-*O*,*O*'][tetrahydro-4*H*-pyran-4,4-dimethanamine-*N*,*N*']platinum(II)

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The synthesis, stability, and antitumor activity of a series of water-soluble third generation platinum(II) complexes have been described. Among these complexes, [2,2-bis(aminomethyl)-1,3-propanediol-*N*,*N*'][1,1-cyclobutanedicarboxylato(2-)-*O*,*O*']platinum(II) and [1,1-cyclobutanedicarboxylato(2-)-*O*,*O*'](tetrahydro-4*H*-pyran-4,4-dimethanamine-*N*,*N*')platinum(II) have shown the greatest promise for further investigation and are currently under clinical evaluation.

cis-Diamminedichloroplatinum(II) (cisplatin)[1] is one of the most effective oncolytic agents against cancers of the testes, ovaries, bladder, and head and neck.[2-4] It is also an important adjunct for cancers of cervix, lung, and breast.[2] Its most spectacular success has been in the treatment of testicular cancer,[3] a form of cancer previously resistant to any therapy but now considered to be curable in most cases. However, cisplatin has three drawbacks which limit its usefulness: (1) it has severe toxicities[5-7] such as nephrotoxicity, nausea/vomiting, myelosuppression, ototoxicity, and neuologic complications, (2) it only affects a narrow range of tumors, and (3) it causes the develop-

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