105310-78-9; **XI** (X = *p*-F, $R_1 = R_2 = C_2H_5$), 105310-79-0; **XI** (X = *p*-CH_3, $R_1 = R_2 = C_2H_5$), 105310-80-3; **XI** (X = H, $R_1 = R_2 = C_6H_7$), 105335-55-5; **XI** (X = H, $R_1 = R_2 = C_4H_9$), 105310-81-4; **XI** (X = H, $R_1 = R_2 = C_6H_{13}$), 105310-82-5; **XI** (X = H, $R_1 = C_2H_5$, $R_2 = (CH_2)_2OH$), 105310-83-6; **XI** (X = H, $R_1 = R_2 = C_6H_7$-*i*), 105310-84-7; **XI** (X = H, $R_1 = R_2 = (CH_2)_4$), 105310-85-8; *cis*-**XI** (X = H, $R_1 = R_2 = CH(CH_3)(CH_2)_2CH(CH_3)$), 105310-86-9; *trans*-**XI** (X = H, $R_1 = R_2 = CH(CH_3)(CH_2)_2CH(CH_3)$), 105370-62-5; *trans*-**XI** (X = H, $R_1 = R_2 = CH(CH_3)(CH_2)_3CH(CH_3)$), 105310-87-0; *cis*-**XI** (X = H, $R_1 = R_2 = CH(CH_3)(CH_2)_3CH(CH_3)$), 105370-63-6; **XIV**, 22613-97-4; **XIV** (tosyl ether), 105310-92-7; **VIV** (phthalimide deriv.), 105310-93-8; $H_3CNH(CH_2)_2C_6H_5$, 589-08-2; *N*-methylpiperazine, 109-01-3; phthalimidoacetyl chloride, 6780-38-7.

Supplementary Material Available: Tables containing physical properties of synthetic intermediates: (*Z*)-1-aryl-2-(halomethyl)cyclopropanecarboxylic acids esters (IV), (*Z*)-1-aryl-2-(phthalimidomethyl)cyclopropanecarboxylic acids esters (V), (*Z*)-1-aryl-2-(bromomethyl)cyclopropanecarboxylic acids (IX), (*Z*)-1-aryl-2-(bromomethyl)cyclopropanecarbonyl chlorides (X), (*Z*)-1-aryl-2-(phthalimidomethyl)cyclopropanecarboxamides (XI) (5 pages). Ordering information is given on any current masthead page.

Structure–Activity Relationships of Sparsomycin and Its Analogues. Inhibition of Peptide Bond Formation in Cell-Free Systems and of L1210 and Bacterial Cell Growth⊥

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The biological activity of 14 analogues of sparsomycin (**1**) was studied in cell-free systems of *Escherichia coli*, *Saccharomyces cerevisiae*, and *Sulfolobus solfataricus* by measuring the inhibition of protein synthesis. The inhibition of L1210 colony formation in soft agar and bacterial cell growth in solid as well as in liquid medium was also examined. Each analogue possesses not more than two structural modifications of the sparsomycin molecule. This enabled us to determine unambiguously several structural and stereochemical features that are required for an optimal biological activity in these assays. Sparsomycin, having the S_CR_S chirality, is the most potent of the four possible stereoisomers. The results obtained with compounds **5–7** indicate that the presence of an oxygen atom on the S(α) atom is essential. Substitution of the bivalent sulfur atom by a CH_2 group (**10**) or of the SCH_3 moiety by a Cl atom (**12**) affects the activity of the molecule partially. Compound **12** is surprisingly active against intact cells. Substitution of the C(6)-CH_3 group by a H (**14**) reduces the activity of the molecule. Isomerization of the trans double bond into the cis double bond yields *cis*-sparsomycin (**15**), which is inactive. The hydrophobic derivatives **8**, **9**, and **11** are considerably more active than sparsomycin; thus the ribosomal binding site for sparsomycin may have a hydrophobic character.

Sparsomycin (**1**)[1] is a potent inhibitor of protein synthesis, and there is ample evidence that its site of interaction is the larger ribosomal subunit, where it prevents peptide transfer by interfering with the peptidyl transferase center.[2–6] Sparsomycin 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. This mutual interaction might be due to an allosteric effect.[7] It was shown recently that the sulfoxide moiety of sparsomycin is important for its activity.[8,9] It was observed, moreover, that the proper oxidation state of the sulfur atom of sparsomycin is important for the enhanced inhibition of peptidyl transferase that is observed when *Escherichia coli* polysomes are preincubated with the drug; this effect was called the "preincubation effect".[8,10] It was proposed that a peptidyl transferase mediated Pummerer rearrangement of the sulfoxide moiety of sparsomycin is responsible for this preincubation effect.[8] This mechanistic rationale of the mode of action of sparsomycin was corroborated recently by studying the four possible stereoisomers of sparsomycin as inhibitors of peptide bond formation in *E. coli* polysomes.[11] It was found that only the S_CR_S stereoisomer (**1**) places the sulfoxide moiety in the same orientation as the nitrogen–carbon bond of the natural substrate, i.e., L-aminoacyl-tRNA, thus inducing the Pummerer rearrangement. This stresses the structural similarity of the drug and the natural substrate of peptidyl transferase.

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⊥ Dedicated to Dr. B. Witkop on the occasion of his 70th birthday.

(1) Sparsomycin is a metabolite of *Streptomyces sparsogenes* (Argoudelis, A. D.; Herr, R. R. *Antimicrob. Agents Chemother.* **1962**, 780) and of *Streptomyces cuspidosporus* (Higashide, E.; Hasegawa, T.; Shibata, M.; Mizuno, K.; Akaide, H. *Takeda Kenkyusho Nempo* **1966**, *25*, 1; *Chem. Abstr.* **1967**, 66, 54238).
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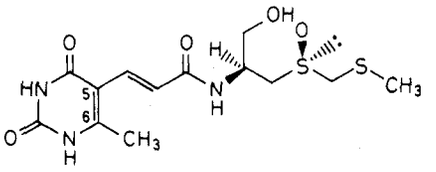
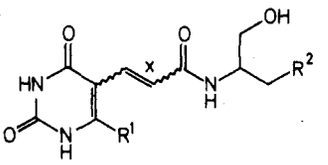


Sparsomycin (1)

Sparsomycin manifests its action in intact prokaryotic cells,[12-14] eukaryotic cells[12,15]—including transformed[16-19] and/or virus-infected cells[20,21]—and in various cell-free systems.[12,22-26] The behavior of sparsomycin with regard to its inhibitory action and its influence on hepatic polysomes has also been investigated in vivo.[27-29] Since sparsomycin has been shown to be active against transformed cells and various tumors, it has been investigated as a potential cytostatic drug. A phase I clinical trial of sparsomycin, however, revealed eye toxicity.[30,31]

Recently, both we[32,33] and Helquist[34,35] succeeded in developing total syntheses of sparsomycin. Most of the structure–activity relationship studies of sparsomycin that appeared[12,36-42] prior to its first total synthesis were concerned with analogues in which several structural parameters had been varied simultaneously; mixtures of stereoisomers were frequently studied. This allowed only a limited interpretation of the results with regard to the role of the various structural fragments.

Table I. Sparsomycin and Its Analogues



compd	R^2	chirality at C	chirality at S
R^1 = Me, X = trans			
1	$S(O)CH_2SCH_3$	*S*	*R*
2	$S(O)CH_2SCH_3$	*R*	*S*
3	$S(O)CH_2SCH_3$	*S*	*S*
4	$S(O)CH_2SCH_3$	*R*	*R*
5	SCH_2SCH_3	*S*	
6	SCH_2SCH_3	*R*	
7	$SCH_2S(O)CH_3$	*S*	*R/S*
8	$S(O)CH_2S(CH_2)_7CH_3$	*S*	*R*
9	$S(O)CH_2SCH_2Ph$	*S*	*R*
10	$S(O)CH_2CH_2CH_3$	*S*	*R*
11	$S(O)CH_2(CH_2)_8CH_3$	*S*	*R*
12	$S(O)CH_2Cl$	*S*	*R*
13	$S(O)CH_2Cl$	*S*	*S*
R^1 = H, X = trans			
14	$S(O)CH_2SCH_3$	*S*	*R*
R^1 = Me, X = cis			
15	$S(O)CH_2SCH_3$	*S*	*R*

To obtain an unambiguous interpretation, we prepared recently eight analogues of sparsomycin (i.e., **2–8** and **15**) possessing not more than two structural modifications of the sparsomycin molecule.[9]

The cytostatic activity of sparsomycin and these eight analogues against intact murine leukemia L1210 cells in an in vitro clonogenic assay was studied.[9] We found that the cytostatic activity changed significantly by modifying some of the substituents of sparsomycin. To determine whether the observed structure–activity relationship reflects the affinity of the compounds studied for the peptidyl transferase center, we decided to investigate the activity of these analogues and six additional ones (i.e., **9–14**) in cell-free protein-synthesizing systems. The results are reported here. A prokaryotic as well as an eukaryotic cell-free system was chosen for this study, and in both cases the fragment reaction[47] for peptide bond formation as well as the polyphenylalanine synthesis assay was used.

So far several studies have been reported that deal with structure–activity relationships of sparsomycin and analogues in cell-free systems.[8,11,12,26,36,37,39] In all of these studies the puromycin assay[43] was used. Therefore, in order to correlate our results with those obtained previously, we also used the puromycin reaction. Next to the in vitro data, the results obtained in in vivo growth inhibition tests using several Gram-positive and Gram-negative bacteria are reported. Finally, the inhibitory activity

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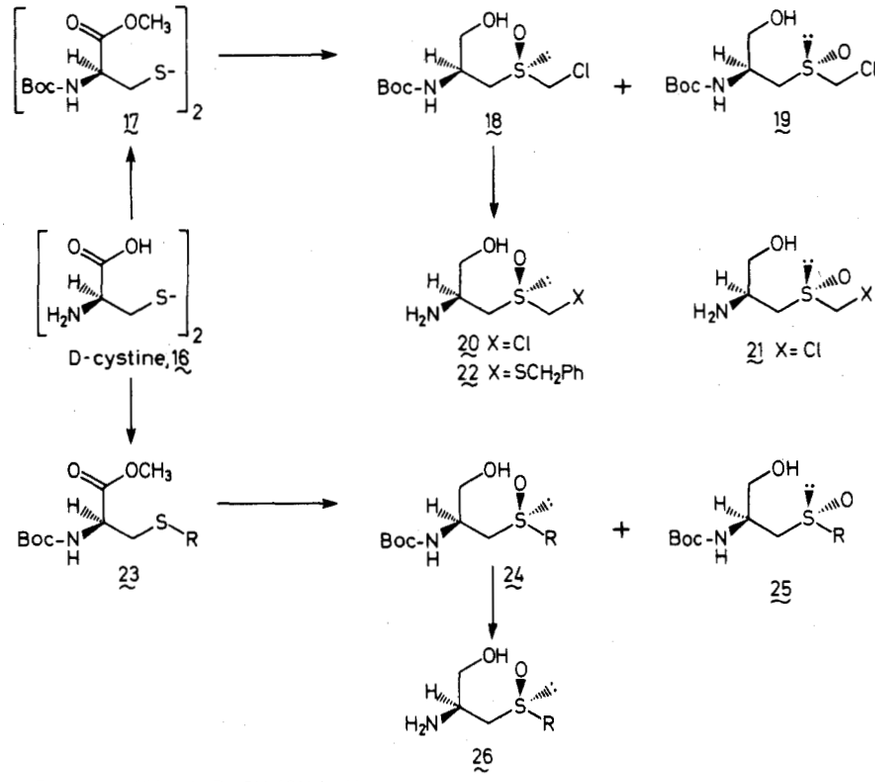
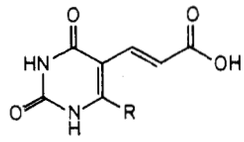
Scheme I



of the six newly synthesized analogues against lymphocytic leukemia L1210 cells in vitro was studied.

Results

Chemistry. The compounds studied are listed in Table I. The syntheses of analogues **2–8** and **15** as well as the rationale for their selection have been described before.[9] Compound **9**, benzylsparsomycin, was prepared as described earlier[9] for the synthesis of octylsparsomycin (**8**). The α-chloro sulfoxide **18** was used as a key intermediate in the synthesis (see Scheme I). The diastereomeric sulfoxides **18** and **19** were separated by column chromatography on silica gel. Substitution of the chlorine atom in **18** by SCH_2Ph and subsequent removal of the amino protecting group yielded the amine **22**. This compound was coupled in a mixed anhydride procedure with acid **27**[32] to give analogue **9**. For the preparation of compounds **10** and **11** the S-oxidated and S-alkylated cysteinol derivatives **24a** and **24b** were used. The sulfoxides **24** and **25** were easily obtained from the corresponding sulfide by oxidation with sodium metaperiodate and subsequent separation of the resulting diastereomers by column chromatography on silica gel. Removal of the N-protection group in **24a** and **24b** yielded compounds **26a** and **26b**, respectively. The amino alcohols **26a** and **26b** were coupled with acid **27** to give the analogues **10** and **11**. By coupling of **20** and **21** with acid **27**, the sparsomycin derivatives **12** and **13** were obtained. Compound **14**, uracilsparsomycin, was obtained by the same reaction sequence used for the preparation of **1**: now uracil acrylic acid **28**[45] was used instead of **27**.



27 R=CH_3
28 R=H

Biological Activity. The assays used and results obtained are described briefly here.

Inhibition of Polyphenylalanine Synthesis[44] (See Table II). Sparsomycin (**1**) and analogues **2–15** have been tested in a cell-free system for phenylalanine polymerization with use of ribosomes and supernatant factors from three different species, i.e., *Saccharomyces cerevisiae*, *Escherichia coli*, and *Sulfolobus solfataricus*, belonging to the eukaryotes, eubacteria, and archaebacteria, respectively. In these species, three analogues show a considerably higher activity than sparsomycin, i.e., octylsparsomycin (**8**), benzylsparsomycin (**9**) and *S*-oxo-*S*-decylsparsomycin (**11**). Compounds **10** and **12** show some activity in this assay. *S. solfataricus* is rather insensitive to sparsomycin as it is to most antibiotics;[46] in this species only the analogues **9** and **11** have ED_{50} values lower than 100 μM. The activity of compound **15** is commented on in the Discussion. Further, the polymerization assay does not discriminate between the activities of the various less active analogues in this assay (i.e., **2–7** and **12–14**).

Inhibition of the Fragment Reaction (See Table II). The simplest model assay for peptide bond formation is the fragment reaction.[47] In this test the ribosome catalyzes the transfer reaction between the donor substrate, i.e., *N*-acetyl-Leu-ACCAC(U), and puromycin in the absence of both mRNA and even the small ribosomal subunit. The reaction requires, however, the addition of an organic solvent. This assay has been used extensively to study the mode of action of several antibiotics.[3]

Our results are depicted in Table II. A comparison of the activity of the compounds tested in the polyphenylalanine synthesis assay and in the fragment reaction demonstrates that in both assays sparsomycin is quite active. This does not hold for the analogues; compounds **10** and **12** are very active in the fragment reaction, as well as in all but one of the other assays reported in Table II. Only in the polyphenylalanine synthesis assay these com-

Table II. Inhibition of Peptide Bond Formation and L1210 Leukemia Colony Formation by Sparsomycin and Its Derivatives

	ED_{50}, μM						
	polyphenylalanine[a] synthesis in:			fragment reaction[a] in:		puromycin[a] reaction in	
compd	*E. coli*	*S. cerevisiae*	*S. solfataricus*	*E. coli*	*S. cerevisiae*	*E. coli*	ID_{50}, μM: L1210
1	8.5	5.2	900	3.2	2.8	0.1	0.40[b]
2	2000	2000		1000	1000	100	300[b]
3	2000	2000		700	65	13	5.8[b]
4	2000	2000		1000	650	40	300[b]
5	2000	2000		550	150	7	12.8[b]
6	2000	2000		1000	550	1000	300[b]
7	2000	2000		750	350	160	180[b]
8	1.6	1.3		60	300	0.25	0.10[b]
9	0.6	0.6	60	7.6	2.6	0.13	0.11
10	140	42	1000	4.5	6.2	0.23	0.75
11	1.8	0.2	30	5000	700	0.08	0.14
12	450	220	5000	7.6	17	0.65	0.29
13	2000	2500	5000	5000	5000	5000	30
14	600	1000		30	50	50	12.7
15[c]	22.5	10		14.5	32.5	0.4	0.85[b]

[a] The values given are the average of three experiments and have an average error of ±20%. [b] The data are from ref 9. [c] A mixture of **1** and **15** (ratio 2:3) was used.[9]

Table III. Effect of Different Organic Solvents on Inhibition of the Fragment Reaction by Sparsomycin and Three Analogues[a]

	percent of inhibition by:				
	A			B	
solvent	1, 10 μM	9, 10 μM	11, 100 μM	1, 20 μM	8, 200 μM
methanol	57	25	44	64	42
ethanol	91	29	35	92	30
2-propanol	64	20	12	76	6
butanol	86	54	15		

[a] The percent of inhibition at the given drug concentration is shown. In experiment A 100% activity in the absence of drug corresponds to 2300, 1670, 1510, and 760 cpm in case of methanol, ethanol, 2-propanol, and butanol, respectively. In experiment B 100% activity in the absence of drug corresponds to 1110, 905, and 925 cpm in case of methanol, ethanol, and 2-propanol, respectively. In all cases the final concentration of the organic solvent in the reaction mixture was 33%.

pounds are considerably less active than sparsomycin. An opposite effect can also be found in Table II. In the fragment reaction the hydrophobic analogues **8**, **9**, and **11** are as active (i.e., **9**) or considerably less active than sparsomycin. In the polyphenylalanine synthesis—and as a matter of fact in all the other assays mentioned in Table II—these analogues are more active than sparsomycin. So it appears that in all but one assay an increase in lipophilicity of a compound is accompanied by an increase of activity of that compound; only the fragment reaction fails to demonstrate this correlation.

The effect of the organic solvent, used in the fragment reaction, on the inhibition of peptide bond formation was studied in greater detail. The results shown in Table III indicate that the inhibitory activity of the drugs is affected by the polarity of the medium in the reaction mixture.

Inhibition of the Reaction of *N*-Acetyl-Phe-tRNA with Puromycin (See Table II). A convenient way to study the ribosomal function is by the use of the antibiotic puromycin, an analogue of the aminoacyladenosine end of tRNA. Thus, in the formation of polylysylpuromycin[48-50] and acetylphenylalanylpuromycin,[51] sparsomycin acts as a competitive inhibitor of the peptidyl transferase reaction with respect to puromycin. This reaction, in the presence of the cognate mRNA and 70S ribosomes, is another standard assay for peptide bond formation and useful in the study of antibiotics.[52] This assay is considerably more sensitive to the drugs studied than the two systems previously described. The large differences in activity between the derivatives found in the polymerization assay and to a lesser extent in the fragment reaction are markedly reduced in this assay. Compounds **8**–**11** show comparable activity to sparsomycin itself.

L1210 Clonogenic Assay (See Table II). The inhibition of colony formation of leukemia L1210 cells in soft agar medium (0.3%) by sparsomycin (**1**) and the analogues **2**–**8** and **15** was used as a first evaluation of the antitumor activity of these drugs.[9] It was observed that there is a good correlation between the in vitro and in vivo activity of drugs tested. Thus, the in vitro system is of predictive value for the leukemia L1210 in vivo system in the mouse, which is generally used in standard screening of compounds of potential interest. In Table II the ID_{50} values of the analogues **9**–**14** and the previously reported values[9] of compounds **1**–**8** and **15** are presented. Only the lipophilic analogues (i.e., **8**, **9**, and **11**) and, surprisingly, also compound **12** are more active than sparsomycin in this assay.

Inhibition of Bacterial Growth (See Table IV). Several Gram-positive and Gram-negative species were assayed. The inhibitory capacity of the antibiotics on the growth of these cells was tested in bacterial cultures by using the agar plate technique. In the case of *E. coli* MRE600 the inhibition test was also carried out in liquid medium; the results are summarized in Table IV. As expected, the extent of inhibition depends not only on the organism but also on the antibiotic used. Compounds **1** and **9**–**14** were also tested against *Pseudomonas aeruginosa* but were found to be inactive.

Discussion

Of the four stereoisomers **1**–**4** tested, sparsomycin (**1**) having the S_CR_S chirality is by far the most potent inhibitor of protein biosynthesis. This confirms the previously made observations about the requirement for the proper configuration at the two chiral centers of sparsomycin for its activity: the S_CR_S stereoisomer is the strongest inhibitor of protein synthesis in a cell-free system as measured by the puromycin reaction on polysomes[11] and has the highest cytostatic activity against intact murine

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Table IV. Inhibition of Bacterial Growth by Sparsomycin and Its Derivatives[a]

compd	*E. coli* MRE600 liquid[b]	*E. coli* MRE600	*E. coli* AB301	*E. coli* N281	*E. coli* W3110	*Bacillus cereus*	*Bacillus subtilis*	*Sarcina lutea*	*Salmonella typhimurium*	*Corynebacterium serosis*	*Staphylococcus aureus*
1	1 (11)	1 (14)	1 (14)	1 (18)	1 (14)	1 (10)	1 (7)	1 (10)	1 (6)	1 (20)	1 (7)
2	0.01	0	0	0	0	0	0	0	0	0	0
3	0.92	1	0.6	0.8	0.6	0.3	0	0.3		0.1	0
4	0.32	0.5	0.2	0.1	0.1	0.1	0	0		0	0
5	1.8	1.5	0.4	0.6	0.6	0.6	0.9	0.6		0.5	trace
6	0.01	0	0	0	0	trace	0	0		0	0
7	0.40	0.6	0.2	0.4	0.2	trace	0	0		0	0
8	2.5	0.7	0	0	0	0.1	0.1	1.2		0.1	
9	1	1.2	0	0.3	0.1	0.9	0	3.3	0	1	trace
10	0.30	0.7	1	1	0.7	1	0.6	1.2	0.9	0.4	0.7
11	3.6	1.1	0	0	0	1.9	2.4	3.6	0	0.6	0
12	0.55	0.8	1.1	1	0.7	2	1.2	1.2	1.2	0.5	0.7
13	0.01	0	0	0	0	0	0	0	0	0	0
14	0.27		0.9	0.5	0.7	0.3	trace	0	0	0	0
15	0.47	0.7	0.7	0.8	0.8	0.7	0.4	0.8		0.7	0.4

[a] The diameter of the inhibition zone on agar plates was used as a measure for the activity of each drug. The numbers given express the ratio of the diameter measured for a drug and the diameter measured for sparsomycin. The diameter (in millimeters) of the inhibition zone for sparsomycin is given in parentheses. [b] The numbers express the ratio of the ED_{50} value of sparsomycin and the ED_{50} value of the analogue; ED_{50} represents the dose in μM that inhibits growth to 50% of control growth. The ED_{50} value for sparsomycin is given in parentheses.

leukemia L1210 cells[9] (see Table II).

Confirmation of this conclusion as far as the chiral carbon atom is concerned is found in the ID_{50}, and bacterial growth inhibition values of the *S*-deoxo analogues **5** and **6**; compound **5** having the *S* configuration at the chiral carbon atom is substantially more active than its enantiomer **6**.

A comparison of the ID_{50}, ED_{50}, and bacterial growth inhibition data of compounds **1** vs. **3** and **12** vs. **13** (see Tables II and IV) stresses the importance for the correct chirality at the sulfoxide sulfur atom. Particularly, analogue **13** is totally inactive in all the assays tested.

The low activity of the *S*-deoxo analogue **5** is remarkable, since a racemic mixture of **5** showed a comparable activity to sparsomycin in the puromycin reaction.[36,37] Our results employing the puromycin reaction as well as all other assays used (see Table II) indicate that compound **5** is less active than sparsomycin. This discrepancy in results indicates that one has to be careful in the comparison and interpretation of data obtained in different systems. Another example that underlines this view can be found in Table II. Comparison of the ED_{50} values of **1**, **3**, and **5** obtained in the fragment reaction in *S. cerevisiae* suggests that the presence of an oxygen atom on S(α) increases the activity, whereas this reaction in *E. coli* indicates that the stereochemistry of the sulfoxide sulfur atom is more crucial than its oxidation state.

The importance of the sulfoxide moiety of sparsomycin has been alluded to in the past.[37] A comparison of the ID_{50} and ED_{50} values of **1**, **3**, and **7** indicates that the proper position of the sulfoxide moiety in the cysteinol side chain, S(α) vs. S(β), is important. In addition, analogue **7** shows a diminished activity compared to the corresponding *S*-deoxo analogue **5**, suggesting an adverse effect of a sulfoxide function at the S(β) position in both the *S. cerevisiae* cell-free system employing the fragment reaction and in the puromycin reaction.

The results obtained with **15** suggest that this compound is inactive. Starting from this assumption, the experimental data for **15** are in accordance with calculated ID_{50} and ED_{50} values based on pure **1** and on the observed ratio **1**/**15** = 2:3. Previously, we had estimated that **15** has still considerable activity.[9] This conclusion had to be corrected as it is the result of an error in calculation. Future experiments will be necessary to clarify the activity of pure *cis*-sparsomycin (**15**). Analogue **14** lacking the C(6)-methyl group shows low activity in all tests, indicating that this methyl group is important for an optimal activity.

The results on analogues **8**, **9**, and **11** indicate that the addition of a large hydrophobic group at the sulfur-containing end of the sparsomycin molecule increases the activity of the drug considerably. These analogues are considerably more active than the unmodified drug in both the polymerization and in the L1210 colony formation assays. Comparison of the activity of compounds **1**, **8**, **10**, and **11** suggests that the presence of the bivalent sulfur atom is not required for activity when the added hydrophobic side chain is sufficiently large. The activity of compound **10**, in which the bivalent sulfur atom has been substituted by a CH_2 group, is partially affected (see Table II). Substitution of the SCH_3 moiety by a Cl atom (**12**) also reduces the activity of the molecule.

Remarkably, the hydrophobic drugs **8**, **9**, and **11**, although quite active in the polymerization assay, are almost inactive in the fragment reaction. A possible explanation for these observations can be found in the reaction conditions used for this reaction. The reaction is carried out in the presence of 33% of aqueous ethanol. Organic solvents altering the polarity of the medium are known[61,62]

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to affect the affinity of substrates for the ribosome. In particular, lipophilic substrates are expected to be most sensitive to this effect. If so, changing the polarity of the solvent would change the affinity of one particular drug for the ribosome. Moreover, this change would be dependent upon the lipophilicity of the analogue. The data from Table III clearly show that the inhibitory activity of the drug is indeed dependent upon the polarity of the organic solvent. For compounds 8 and 11 a correlation was found in that an increase in lipophilicity of the alcohol used resulted in a decrease in activity of the drug. Care is necessary when using the fragment reaction to compare analogues with different physicochemical properties.

In spite of the high activity in the polyphenylalanine synthesis assay, the hydrophobic derivatives 8, 9, and 11 are roughly equivalent to sparsomycin when tested in the puromycin reaction. Sparsomycin is known[63-65] to compete with the acceptor substrate for binding to the peptidyl transferase A site. These results can be rationalized as follows.[7] Sparsomycin has structural similarities with the 3′ end of the acceptor tRNA molecule. The binding of sparsomycin to a site on the peptidyl transferase center that partially overlaps with the interaction site for the aminoacyladenine moiety of the aminoacyl-tRNA is the most straightforward mechanism of action. The bound drug will totally block the interaction of small-size substrates, such as the 3′-terminal fragments and puromycin. It is also able to disturb the correct positioning of the 3′ end of the large aminoacyl-tRNA molecule. In both cases, the formation of the peptide bond will be inhibited. It should be pointed out here that the polyphenylalanine synthesis assay may reflect inhibition of several particular reactions whereas the puromycin reaction is a model of formation of a single peptide bond.

This hypothesis of overlapping binding sites agrees with the competitive nature of the puromycin reaction inhibition by sparsomycin. It is compatible with a high sensitivity of this reaction to sparsomycin ($ED_{50} \sim 0.1$ μM), considering the low affinity of puromycin for the ribosome ($K_a \sim 100$ μM).[56] In the case of the amino acid polymerization assay, the higher affinity of aminoacyl-tRNA for ribosomes ($K_a \sim 1$ μM) explains the lower sensitivity of this reaction toward sparsomycin ($ED_{50} \sim 1$ μM).

The results obtained using the more hydrophobic derivatives of sparsomycin support the view that the binding sites of the aminoacyl moiety of aminoacyl-tRNA and sparsomycin overlap at the peptidyl transferase.[3] The high sensitivity of the phenylalanine polymerization assay to sparsomycin analogues carrying a hydrophobic group at the sulfur-containing end of the molecule suggests that these compounds interact with a region on the peptidyl transferase that probably functions as the recognition site for the lateral chains of the hydrophobic amino acids during protein synthesis. The existence of such a region at the peptidyl transferase is supported by data obtained by using different aminoacyl-A-C fragments as acceptor substrates.[53a] It has been reported[53b] that Phe-A-C, probably the most hydrophobic of these substrates, shows the lowest K_m(app) in the peptide bond forming reaction, suggesting that it has the highest affinity for the ribosome.

Recent data indicate that demethylation of the tyrosine residue in puromycin abolishes the activity of this antibiotic.[54] So, apparently, puromycin displays like sparsomycin a hydrophobic interaction with the ribosome. On the basis of our results, we anticipate that increasing the hydrophobicity at the tyrosine end of puromycin will increase the inhibitory activity of the molecule. We are presently synthesizing such puromycin derivatives.

The data from the peptide bond formation assays correlate well (except for compound 12) with those on inhibition of L1210 colony formation (see Table II). This correlation—not found with prokaryotic cells (vide infra)—confirms that in eukaryotic cells inhibition of protein synthesis is the main effect of sparsomycin and derivatives. Support for this is found by the isolation of a mouse mammary carcinoma cell line resistant to the antibiotic blasticidin S.[18] This mutant cell line—altered in the 60S ribosomal subunit—shows some cross-resistance to sparsomycin.

The effect of sparsomycin and analogues on the growth of several bacteria in the solid phase, as well as in liquid medium, was studied. The results of this study, which are summarized in Table IV, allow the following conclusions.

(1) Only a partial correlation exists between the observed structure–activity relationship in whole-cell systems and that observed in the cell-free peptide bond formation assays. The relationship between the chiralities at the carbon and the sulfur atom and the antibacterial activity correlates, for all species tested so far, to that observed in the peptide bond formation assays; compare the activities of compounds 1–6, 12, and 13, shown in Tables II and IV.

(2) In most of the bacteria tested there is no correlation between the activity of a given drug in peptide bond formation assays and the antibacterial activity. The hydrophobic analogues 9 and 11 show high activity in the polymerization and puromycin reaction but are inactive against *E. coli* AB301, *E. coli* W3110, *S. aureus*, and *S. typhimurium* in the bacterial growth inhibition assay. This inactivity might be the result of permeability barriers.

(3) Conversely, compounds 3, 5, 7, and 12, which show a low activity in the peptide bond formation assays, are surprisingly active against *E. coli* MRE600 and N281. Besides, compound 12 showed in the L1210 test a remarkable high activity and is very active against practically all species tested. One might interpretate this result on the basis of changes in the permeability properties of these derivatives, facilitating their penetration through the cell membrane. However, this explanation is unlikely, since the growth inhibition observed would then have required a too high concentration of the analogue inside the cell to be realistic.

It is worthwhile to note that in initial studies on the mode of action of sparsomycin on bacteria, the release of amino acids to the growth medium was reported.[55] On the basis of this and of the above-mentioned results, it is questionable whether alterations in cell membrane permeability are associated with cell growth inhibition. Therefore, a direct action of the drug on the membrane permeability cannot be excluded.

(4) The antibacterial activity of analogues 5, 8, and 11 depends on the species as well as on the strain used. The strain dependency is illustrated by the fact that, of the four *E. coli* strains tested, only the growth of strain MRE600 is inhibited by compounds 8 and 11. Compound 5 is only against this strain more active than sparsomycin. Presently, ignorance of the permeability mechanisms for sparsomycin as well as that of the detailed cell wall structures of the different bacterial species and strains precludes an interpretation of these data.

So far, we have not been able to assess unambiguously the role of the hydroxy function of sparsomycin. In order

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to achieve this, the O-methylated derivative of **1**, as well as analogues in which the hydroxyl group has been replaced by hydrogen, has been prepared. The activities of these compounds will be the subject of a future report.

Experimental Section

Biological Activity. The peptidyl transferase activity was measured by the polyphenylalanine synthesis assay, the fragment reaction, and the puromycin reaction. In all the in vitro tests, i.e., polyphenylalanine synthesis, fragment reaction, and puromycin reaction, ribosomes from *E. coli* MRE600 and *S. cerevisiae* Y166 were used.

The reaction mixture for **polyphenylalanine synthesis** was slightly different for *E. coli*, *S. cerevisiae*, and *S. solfataricus*.

E. coli. The reaction mixture (50 μL) contained 50 mM Tris-HCl, pH 7.6, 15 mM $MgCl_2$, 90 mM KCl, 15 mM β-mercaptoethanol, 1 mM GTP, 10 mM ATP, 0.2 mg/mL polyuridylic acid, 1.5 mg/mL tRNA, 0.3 mM ribosomes, 2.5 mg/mL phosphoenol pyruvate, 20 μg/mL pyruvate kinase, the required concentration of sparsomycin or its analogue, and 5 μL of supernatant fraction S-100. Subsequently, 30 μM [^{3}H]phenylalanine (about 60 cpm/pmol) was added to start the reaction. Incubation was at 37 °C for 30 min. The reaction was stopped by the addition of 1 mL of 10% TCA, and the samples were filtered through glass fiber filters. The filters were washed with 10 mL of cold 10% TCA, dried, and counted for radioactivity.

S. cerevisiae. The test was carried out as indicated above for *E. coli*. The phophoenol pyruvate and pyruvate kinase were, however, replaced by creatine phosphate (20 mM) and creatine phosphokinase (50 μg/mL). The incubation was performed at 30 °C for 30 min, and the samples were processed as described above.

Under these conditions the control samples polymerized 5–14 and 3–8 molecules of phenylalanine per ribosome derived from *E. coli* and *S. cerevisiae*, respectively. Ribosomes and supernatant factors from *E. coli* and *S. cerevisiae* were prepared according to standard procedures.[57,58]

S. solfataricus. The reaction mixture (63 μL) contained 15 mM Tris-HCl, pH 7.3, 6 mM NH_4Cl, 18 mM magnesium acetate, 1 mM dithiothreitol, 3 mM spermine, 2.4 mM ATP, 1.6 mM GTP, 0.16 mg/mL poly(U), 20 μM [^{3}H]phenylalanine, 0.3 μM ribosomes, and 5 μL of supernatant fraction S-100. The incubation was carried out at 75 °C for 40 min, and the samples were processed as described above for *E. coli*.

Ribosomes and supernatant factors from *S. solfataricus* were prepared as has been described earlier.[46]

The **fragment reaction** was carried out in 150 μL of 33 mM Tris-HCl, pH 7.4, 270 mM KCl, 13 mM magnesium acetate containing 1 mg/mL ribosomes, 2 mM puromycin, 1 pmol of *N*-acetyl-[^{3}H]Leu-ACCAC(U), the required concentration of sparsomycin or its analogue, and 33% methanol. The reaction was initiated by the addition of the alcohol and allowed to proceed at 0 °C for 30 min and was then stopped by the addition of 100 μL of 0.3 M sodium acetate, pH 5.5, saturated with $MgSO_4$. The samples were extracted with 1.5 mL of ethyl acetate, and 1 mL of the organic phase was checked for radioactivity. The 3′-terminal pentanucleotide *N*-acetyl-[^{3}H]Leu-ACCAC(U) was prepared from *N*-acetyl-[^{3}H]Leu-tRNA by ribonuclease T1 as described by Monro.[47]

The **puromycin reaction** was carried out in 25 μL of 30 mM KCl, 50 mM NH_4Cl, 30 mM Tris-HCl, pH 7.8, 20 mM $MgCl_2$, 10 pmol of ribosomes, 100 μg/mL poly(U), and 15 pmol of *N*-acetyl-[^{3}H]Phe-tRNA. The reaction mixture was incubated for 30 min at 37 °C, and then 1 μL of 20 mM puromycin was added and incubation was followed for an additional 5 min. To the samples were added 250 μL of 0.1 M sodium carbonate and 0.7 mL of ethyl acetate, the samples were shaken for 1 min centrifuged to separate the phases, and 0.5 mL of the upper organic phase was taken for estimating the radioactivity.

Inhibition of bacterial growth was tested in solid as well as in liquid medium. For the test on agar plates, petri dishes containing 15 mL of LB medium (10 g/L bactotryptone, 5 g/L yeast extract, and 5 g/L NaCl) were covered with 5 mL of the same medium (kept melted at 40 °C) containing 40 μL of a culture of the required bacterium at approximately an A_{550} of 2. After solidification, 3-mm filter disks containing 4 μL of a 10 mM solution of the antibiotic in 50% EtOH/Me_2SO were placed on the surface of the agar, and the plates were incubated at 37 °C for 24 h.

For the test in liquid medium, *E. coli* MRE600 was grown in LB medium supplemented with 0.2% of glucose to exponential phase (A_{560} = 0.1) and distributed in 1-mL aliquots in tubes containing the required amount of antibiotic. The tubes were incubated at 37 °C for 4 h and subsequently diluted with 3 mL of 0.1% of sodium azide to stop growth, and the absorption at 550 nm was measured. The absorption of the medium without addition of antibiotic was considered as 100% growth.

Synthesis. ^{1}H NMR spectra were measured on a Bruker WH-90 spectrometer with Me_4Si or $Me_3SiCD_2CD_2CO_2Na$ as an internal standard. For determination of the specific rotation, a Perkin-Elmer 241 polarimeter was used. Thin-layer chromatography (TLC) was carried out by using Merck precoated F-254 plates (thickness 0.25 mm). Spots were visualized with a UV lamp, ninhydrin, and Cl_2-TDM.[66] For column chromatography, Merck silica gel H (type 60) was used. Mass spectra were recorded on a double-focusing VG 7070E mass spectrometer.

(S_CR_S)-2-[β-(6-Methyl-5-uracilyl)acrylamido]-3-[[(benzylthio)methyl]sulfinyl]propanol (9; Benzylsparsomycin). This compound was obtained in 60% yield by coupling **22** (2.78 mmol with **27** in a mixed anhydride procedure following the procedure described earlier[9] for the synthesis of octylsparsomycin (**8**). The compound was homogeneous on TLC (R_f 0.53 MeOH/$CHCl_3$, 1:4); ^{1}H NMR (D_2O) δ 2.42 (s, 3 H, C(6)-CH_3), 2.77–3.22 (m, 2 H, $CHCH_2S(O)$), 3.64–3.77 (m, 2 H, CH_2OH), 3.88 (s, 2 H, SCH_2Ph), 3.89 and 4.02 (AB q, J_{AB} = 14.0 Hz, 2 H, $S(O)CH_2S$), 4.55–4.78 (m, 1 H, $CHCH_2OH$), 7.03 and 7.47 (AB q, J_{AB} = 16.0 Hz, 2 H, CH=CH), 7.33 (br s, 5 H, Ph); FAB MS, *m/e* 438 (M^+ + 1); $[\alpha]^{25}_D$ +121° (*c* 0.135, H_2O). Anal. Calcd for $C_{19}H_{23}N_3O_5S_2 \cdot 2H_2O$: C, 48.19; H, 5,74; N, 8.87. Found: C, 48.88; H, 5.30; N, 8.81.

(S_CR_S)-2-[β-(6-Methyl-5-uracilyl)acrylamido]-3-[*n*-propylsulfinyl]propanol (10; *S*-Oxo-*S*-propylsparsomycin). Compound **10** was prepared in 52% yield by coupling **26a** (0.65 mmol) with **27** in a mixed anhydride procedure as has been described earlier[9] for the preparation of **8**: TLC R_f 0.27 (eluent MeOH/$CHCl_3$, 1:4); ^{1}H NMR (D_2O) δ 1.06 (t, *J* = 7.6 Hz, 3 H, CH_2CH_3), 1.80 (hextet, *J* = 8.0 Hz, 2 H, $CH_2CH_2CH_3$), 2.40 (s, 3 H, C(6)-CH_3), 2.93 (t, *J* = 7.5 Hz, $CH_2CH_2CH_3$), 3.10 (d, *J* = 3.0 Hz, 2 H, $CHCH_2S(O)$), 3.70 and 3.77 (AB part of ABX spectrum, 7 lines, J_{AX} = 3.6 Hz, J_{BX} = 6.3 Hz, J_{AB} = 11.4 Hz, 2 H, CH_2OH), 4.36–4.64 (m, 1 H, $CHCH_2OH$), 7.04 and 7.40 (AB q, J_{AB} = 15.6 Hz, 2 H, CH=CH); FAB MS, *m/e* 344 (M^+ + 1); $[\alpha]^{25}_D$ +98.9° (*c* 0.185, H_2O). Anal. Calcd for $C_{14}H_{21}N_3O_5S \cdot 2H_2O$: C, 44.31; H, 6.64; N, 11.07. Found: C, 44.31; H, 6.43; N, 11.04

(S_CR_S)-2-[β-(6-Methyl-5-uracilyl)acrylamido]-3-[*n*-decylsulfinyl]propanol (11; *S*-oxo-*S*-decylsparsomycin). This compound was obtained in 13% yield by coupling **26b** (1.19 mmol) with **27** in a mixed anhydride procedure following the procedure described earlier[9] for the preparation of **8**. The compound was homogeneous on TLC (R_f 0.47, MeOH/$CHCl_3$, 1:4); FAB MS, *m/e* 441 (M^+); $[\alpha]^{25}_D$ +57° (*c* 0.175, MeOH); ^{1}H NMR (D_2O + Na_2CO_3) δ 0.71–0.93 (m, 3 H, CH_2CH_3), 1.04–1.48 (m, 16 H, $(CH_2)_8CH_3$), 2.38 (s, 3 H, C(6)-CH_3), 2.82–3.24 (m, 4 H, $CHCH_2S(O)$, $S(O)CH_2CH_2$), 3.64–3.84 (m, 3 H, $CHCH_2OH$), 6.97 and 7.61 (AB q, J_{AB} = 15.0 Hz, 2 H, CH=CH). Anal. Calcd for $C_{21}H_{38}N_3O_5S_2 \cdot 2H_2O$: C, 52.81; H, 8.22; N, 8.79. Found: C, 51.38; H, 7.33; N, 8.67.

(S_CR_S)- and (S_CS_S)-2-[β-(6-Methyl-5-uracilyl)acrylamido]-3-[(chloromethyl)sulfinyl]propanol (12 and 13; Chlorosparsomycin). These compounds were prepared from the corresponding α-chloro sulfoxides **18** (0.34 mmol) and **19** (1 mmol), respectively. The Boc group was removed with trifluoroacetic acid according to the procedure that has been described earlier.[32] Treatment with 1 equiv of triethylamine gave **20** and **21**, respectively, which were coupled with **27** in a mixed anhydride procedure following the procedure described earlier[9] for the preparation of **8**. The yields were 31% and 20% for compounds **12** and **13**, respectively. Both compounds were homogeneous on TLC (**12**, R_f 0.15; **13**, R_f 0.19; MeOH/$CHCl_3$, 1:4).

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For **12**: ^{1}H NMR (D_2O) δ 2.40 (s, 3 H, C(6)-CH_3), 3.10 and 3.33 (AB part of ABX spectrum, 7 lines, J_{AX} = 3.6 Hz, J_{BX} = 11.7 Hz, J_{AB} = 14.4 Hz, 2 H, CHCH_2S(O)), 3.75 (d, J = 3.0 Hz, 2 H, CH_2OH), 3.98–4.53 (m, 1 H, CHCH$_2$OH), 4.81 and 4.96 (AB q, J_{AB} = 12.0 Hz, 2 H, S(O)CH_2Cl), 7.05 and 7.41 (AB q, J_{AB} = 16.2 Hz, 2 H, CH=CH); FAB MS, m/e 350 (M^+ + 1); $[\alpha]^{25}_D$ +102° (c 0.14, H_2O). Anal. Calcd for $C_{12}H_{16}N_3O_5SCl\cdot{}^1/_2H_2O$: C, 40.17; H, 4.77; N, 11.71. Found: C, 39.94; H, 4.46; N, 11.43.

For **13**: ^{1}H NMR (D_2O) δ 2.40 (s, 3 H, C(6)-CH_3), 3.22 and 3.38 (AB part of ABX spectrum, 8 lines, J_{AX} = 5.4 Hz, J_{BX} = 9.0 Hz, J_{AB} = 13.5 Hz, 2 H, CHCH_2S(O)), 3.76 (d, J = 2.5 Hz, 2 H, CH_2OH), 3.98–4.53 (m, 1 H, CHCH$_2$OH), 4.90 and 5.04 (part of AB q, 2 H, S(O)CH_2Cl), 7.04 and 7.40 (AB q, J_{AB} = 16.2 Hz, 2 H, CH=CH); FAB MS, m/e 350 (M^+ + 1); $[\alpha]^{25}_D$ +14° (c 0.2, H_2O). Anal. Calcd for $C_{12}H_{16}N_3O_5SCl\cdot{}^1/_2H_2O$: C, 40.18; H, 4.77; N, 11.71. Found: C, 40.20; H, 4.45; N, 11.67.

($S_C R_S$)-2-[β-(5-Uracilyl)acrylamido]-3-[[(methylthio)-methyl]sulfinyl]propanol (14; Uracilsparsomycin). Compound **14** was obtained in 30% yield by coupling **20** (0.5 mmol) with **28** in a mixed anhydride procedure following the procedure[9] described for the synthesis of **8**: TLC R_f 0.20 (eluent MeOH/$CHCl_3$, 1:4); ^{1}H NMR (D_2O) δ 2.26 (s, 3 H, SCH_3), 3.14 and 3.22 (AB part of ABX spectrum, 5 lines, J_{AX} = 9.9 Hz, J_{BX} = 4.5 Hz, J_{AB} = 13.5 Hz, 2 H, CHCH_2S(O)), 3.60–3.82 (m, 2 H, CH_2OH), 3.95 and 4.10 (AB q, J_{AB} = 14.1 Hz, 2 H, S(O)CH_2S), 4.36–4.62 (m, 1 H, CHCH$_2$OH), 6.98 and 7.22 (AB q, J_{AB} = 16.0 Hz, 2 H, CH=CH), 7.88 (s, 1 H, C(6)-H); FAB MS, m/e 348 (M^+ + 1); $[\alpha]^{25}_D$ +88° (c 0.171, MeOH/H_2O, 1:1). Anal. Calcd for $C_{12}H_{17}N_3O_5S_2\cdot H_2O$: C, 39.44; H, 5.24; N, 11.49. Found: C, 40.27; H, 5.05; N, 10.85.

***S*-Oxo-*S*-[(benzylthio)methyl]-D-cysteinol (22).** This compound was prepared in 98% yield by removal of the Boc group from the corresponding N-protected compound (2.84 mmol) by treatment with trifluoroacetic acid following the procedure that has been described earlier.[32] The reaction product was used without further purification for the synthesis of **9**.

N-(*tert*-Butyloxycarbonyl)-*S*-oxo-*S*-[(benzylthio)methyl]-D-cysteinol was prepared from **18** (10.9 mmol) by treatment with sodium (10.9 mmol) and freshly distilled benzyl mercaptan in 60 mL of dry ethanol. The reaction mixture was worked up following the procedure that has been described earlier[9] for compound **8** to give the desired compound in 96% yield: TLC R_f 0.29; (eluent MeOH/$CHCl_3$, 7:93); ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9 H, *t*-Bu), 2.97 and 3.22 (AB part of ABX spectrum, 8 lines, J_{AX} = 6.6 Hz, J_{BX} = 6.0 Hz, J_{AB} = 13.2 Hz, 2 H, CHCH_2S(O)), 3.60 and 3.64 (part of AB q, 2 H, S(O)CH_2S), 3.73 (m, 2 H, CH_2OH), 3.88 (s, 2 H, SCH_2Ph), 4.06 (m, 2 H, CHCH_2OH), 5.51 (d, J = 9.0 Hz, 1 H, NH), 7.28 (br s, 5 H, Ph). Anal. Calcd for $C_{16}H_{25}NO_4S_2$: C, 53.46; H, 7.01; N, 3.90. Found: C, 53.46; H, 6.99; N, 3.92.

***N*-(*tert*-Butyloxycarbonyl)-*S*-propyl-D-cysteine Methyl Ester (23a).** This compound was prepared in 92% yield from the hydrochloride of *S*-propyl-D-cysteine methyl ester (10 mmol) by treatment with di-*tert*-butyl pyrocarbonate following the procedure that has been described earlier:[32] TLC R_f 0.80 (eluent MeOH/$CHCl_3$, 3:97); ^{1}H NMR ($CDCl_3$) δ 0.97 (t, J = 7.0 Hz, 3 H, CH_2CH_3), 1.45 (s, 9 H, *t*-Bu), 1.48–1.80 (m, 2 H, $CH_2CH_2CH_3$), 2.51 (t, J = 7.0 Hz, 2 H, SCH_2CH$_2$), 2.95 (d, J = 5.0 Hz, 2 H, CHCH_2S), 3.77 (s, 3 H, CO_2CH_3), 4.37–4.67 (m, 1 H, CHCH$_2$S), 5.33 (br d, 1 H, NH). Anal. Calcd for $C_{12}H_{23}NO_4S$: C, 51.96; H, 8.36; N, 5.03. Found: C, 51.76; H, 8.33; N, 4.97.

The hydrochloride of *S*-propyl-D-cysteine methyl ester was prepared from *S*-propyl-D-cysteine (20 mmol) by treatment with thionyl chloride in methanol following the procedure that has been described earlier[32] to yield the desired compound in 95%: TLC R_f 0.75 (eluent *sec*-BuOH/NH_4OH, 55:22); ^{1}H NMR (CD_3OD) δ 1.02 (t, J = 7.0 Hz, 3 H, CH_2CH_3), 1.64 (sextet, J = 7.0 Hz, 2 H, CH_2CH$_3$), 2.60 (t, J = 7.0 Hz, 2 H, SCH_2CH$_2$), 3.10 and 3.13 (AB part of ABX spectrum, 8 lines, J_{AX} = 4.8 Hz, J_{BX} = 6.9 Hz, J_{AB} = 13.5 Hz, 2 H, CHCH_2S), 3.86 (s, 3 H, CO_2CH_3), 4.31 (X part of ABX spectrum, 4 lines, J_{AX} + J_{BX} = 11.9 Hz, 1 H, CHCH$_2$S).

S-Propyl-D-cysteine was prepared from D-cysteine (20.8 mmol) and 1-bromopropane with sodium in liquid ammonia according to procedures described earlier[32,37,38,59,60] in 98% yield: TLC R_f 0.26 (eluent *sec*-BuOH/NH_4OH, 55:22); ^{1}H NMR (D_2O) δ 0.93 (t, J = 6.5 Hz, 3 H, CH_2CH_3), 1.62 (sextet, J = 7.0 Hz, 2 H, $CH_2CH_2CH_3$), 2.57 (t, J = 7.0 Hz, 2 H, SCH_2CH$_2$), 3.01 and 3.07 (AB part of ABX spectrum, 8 lines, J_{AX} = 4.5 Hz, J_{BX} = 7.5 Hz, J_{AB} = 14.9 Hz, 2 H, CHCH_2S), 3.91 (X part of ABX spectrum, 4 lines, J_{AX} + J_{BX} = 11.5 Hz, 1 H, CHCH$_2$S). Anal. Calcd for $C_6H_{13}NO_2S$: C, 44.15; H, 8.02; N, 8.58. Found: C, 44.08; H, 7.96; N, 8.60.

***N*-(*tert*-Butyloxycarbonyl)-*S*-decyl-D-cysteine Methyl Ester (23b).** This compound was prepared in 92% yield from the hydrochloride of *S*-decyl-D-cysteine methyl ester (15 mmol) by treatment with di-*tert*-butyl pyrocarbonate following the procedure that has been described earlier:[32] TLC R_f 0.47 (eluent MeOH/$CHCl_3$, 1:99); ^{1}H NMR ($CDCl_3$) δ 0.88 (br t, J = 7.2 Hz, 3 H, CH_2CH_3), 1.15–1.71 (br s, 16 H, $(CH_2)_8CH_3$), 1.46 (s, 9 H, *t*-Bu), 2.53 (t, J = 7.8 Hz, 2 H, SCH_2CH$_2$), 2.95 (d, J = 2.5 Hz, 2 H, CHCH_2S), 3.75 (s, 3 H, CO_2CH_3), 4.36–4.64 (m, 1 H, CHCH$_2$S), 5.33 (br d, 1 H, NH). Anal. Calcd for $C_{19}H_{37}NO_4S$: C, 60.76; H, 9.93; N, 3.73. Found: C, 60.63; H, 9.93; N, 3.68.

The hydrochloride of *S*-decyl-D-cysteine methyl ester was prepared in 90% yield from *S*-decyl-D-cysteine (18 mmol) by treatment with thionyl chloride in methanol following the procedure that has been described earlier:[32] TLC R_f 0.68 (eluent *sec*-BuOH/NH_4OH, 55:22); ^{1}H NMR (CD_3OD) δ 0.84 (br t, J = 7.9 Hz, 3 H, CH_2CH_3), 1.15–1.73 (br s, 16 H, $(CH_2)_8CH_3$), 2.55 (br t, J = 7.2 Hz, 2 H, SCH_2CH$_2$), 3.00 and 3.11 (AB part of ABX spectrum, 8 lines, J_{AX} = 4.8 Hz, J_{BX} = 6.6 Hz, J_{AB} = 14.4 Hz, 2 H, CHCH_2S), 3.82 (s, 3 H, CO_2CH_3), 4.24 (X part of ABX spectrum, 4 lines, J_{AX} + J_{BX} = 11.7 Hz, 1 H, CHCH$_2$S). Anal. Calcd for $C_{14}H_{30}NO_2SCl$: C, 53.91; H, 9.69; N, 4.49. Found: C, 52.65; H, 9.54; N, 4.39.

S-Decyl-D-cysteine was prepared in 98% yield from D-cysteine (20.8 mmol) and 1-bromodecane with sodium in liquid ammonia according to procedures described earlier:[32,37,38,59,60] TLC R_f 0.36 (eluent *sec*-BuOH/NH_4OH, 55:22). The reaction product was used for esterification as described above without purification.

***N*-(*tert*-Butyloxycarbonyl)-*S*-oxo-*S*-propyl-D-cysteinol (24a and 25a).** These compounds were prepared by reduction of *N*-(*tert*-butyloxycarbonyl)-*S*-oxo-*S*-propyl-D-cysteine methyl ester (4 mmol) with lithium borohydride following the procedure that has been described earlier.[32] Separation of the diastereomers by column chromatography on silica gel (eluent MeOH/CH_2Cl_2, 3:97) afforded **24a** and **25a** in 37% and 35% yield, respectively. Both compounds were homogeneous on TLC (**24a**, R_f 0.19; **25a**, R_f 0.22; MeOH/$CHCl_3$, 7:93).

For **24a**: ^{1}H NMR ($CDCl_3$) δ 1.08 (t, J = 7.0 Hz, 3 H, CH_2CH_3), 1.42 (s, 9 H, *t*-Bu), 1.84 (sextet, J = 7.0 Hz, 2 H, CH_2CH$_3$), 2.47 (br s, 1 H, CH_2OH), 2.60–3.15 (m, 4 H, CHCH_2S(O), S(O)CH_2CH$_2$), 3.68–3.98 (AB part of ABX spectrum, 2 H, CHCH_2OH), 3.98–4.24 (m, 1 H, CHCH$_2$OH), 5.46 (br d, 1 H, NH). Anal. Calcd for $C_{11}H_{23}NO_4S$: C, 49.79; H, 8.74; N, 5.28. Found: C, 49.82; H, 8.64; N, 5.16.

For **25a**: ^{1}H NMR ($CDCl_3$) δ 1.10 (t, J = 7.2 Hz, 3 H, CH_2CH_3), 1.44 (s, 9 H, *t*-Bu), 1.63–2.16 (8 lines, 2 H, CH_2CH_3), 2.79 (d of t, J = 7.5 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} = 3.1 Hz, J_{AB} = 13.5 Hz, 2 H, CHCH_2S(O)), 3.56–3.91 (AB part of ABX spectrum, 2 H, CHCH_2OH), 3.98–4.38 (m, 1 H, CHCH$_2$OH), 5.52 (br d, J = 7 Hz, 1 H, NH). Anal. Calcd for $C_{11}H_{23}NO_4S$: C, 49.79; H, 8.74; N, 5.28. Found: C, 49.48; H, 8.70; N, 5.16.

N-(*tert*-Butyloxycarbonyl)-*S*-oxo-*S*-propyl-D-cysteine methyl ester was prepared by oxidation of the corresponding sulfide **23a** (5.2 mmol) with sodium metaperiodate according to the procedure that has been described earlier[32] to give the desired product as a mixture of diastereomers in 93% yield: TLC R_f 0.15 (eluent MeOH/$CHCl_3$, 5:95); ^{1}H NMR (CD_3OD) δ 1.08 (t, J = 7.0 Hz, 3 H, CH_2CH_3), 1.44 (s, 9 H, *t*-Bu), 1.77 (sextet, J = 7.0 Hz, 2 H, CH_2CH$_3$), 2.48–2.88 (m, 2 H, S(O)CH_2CH$_2$), 2.98–3.36 (m, 2 H, CHCH_2S(O)), 3.80 (s, 3 H, CO_2CH_3), 4.51–4.87 (m, 1 H, CHCH$_2$S(O)), 5.51–5.91 (br d, 1 H, NH). Anal. Calcd for $C_{12}H_{23}NO_5S$: C, 49.13; H, 7.90; N, 4.77. Found: C, 49.11; H, 7.85; N, 4.71.

***N*-(*tert*-Butyloxycarbonyl)-*S*-oxo-*S*-decyl-D-cysteinol (24b and 25b).** These compounds were prepared by reduction of *N*-(*tert*-butyloxycarbonyl)-*S*-oxo-*S*-decyl-D-cysteine methyl ester (4 mmol) with lithium borohydride following the procedure that has been described earlier.[32] Separation of the diastereomers by column chromatography on silica gel (eluent MeOH/CH_2Cl_2, 4:96) afforded **24b** and **25b** in 37% and 35% yield, respectively. Both

compounds were homogeneous on TLC (**24b**, R_f 0.24; **25b**, R_f 0.27; MeOH/$CHCl_3$, 5:95).

For **24b**: ^1H NMR ($CDCl_3$) δ 0.88 (br t, J = 6.0 Hz, 3 H, CH_2CH_3), 1.27 (br s, 14 H, $(CH_2)_7CH_3$), 1.42 (s, 9 H, t-Bu), 1.59–1.97 (m, 2 H, $S(O)CH_2CH_2$), 2.77 (d of t, J = 8.0 Hz, 2 H, $S(O)CH_2CH_2$), 2.89–3.21 (AB part of ABX spectrum, 7 lines, 2 H, $CHCH_2S(O)$), 3.47 (br t, J = 6.0 Hz, 1 H, CH_2OH), 3.64–3.99 (AB part of ABX spectrum, 8 lines, 2 H, $CHCH_2OH$), 3.89–4.26 (m, 1 H, $CHCH_2OH$), 5.50 (br d, 1 H, NH). Anal. Calcd for $C_{18}H_{37}NO_4S$: C, 59.47; H, 10.26; N, 3.85. Found: C, 59.46; H, 10.34; N, 3.89.

For **25b**: ^1H NMR ($CDCl_3$) δ 0.88 (br t, J = 6 Hz, 3 H, CH_2CH_3), 1.27 (s, 14 H, $(CH_2)_7CH_3$), 1.43 (s, 9 H, t-Bu), 1.56–2.10 (m, 2 H, $S(O)CH_2CH_2$), 2.82 (d of t, J = 8 Hz, 2 H, $S(O)CH_2CH_2$), 3.02 and 3.16 (AB part of ABX spectrum, 8 lines, J_{AX} = 6.0 Hz, J_{BX} = 3.7 Hz, J_{AB} = 13.5 Hz, 2 H, $CHCH_2S(O)$), 3.59–3.92 (AB part of ABX spectrum, 7 lines, 2 H, $CHCH_2OH$), 3.98–4.44 (m, 1 H, $CHCH_2OH$), 5.59 (br d, J = 7 Hz, 1 H, NH). Anal. Calcd for $C_{18}H_{37}NO_4S \cdot {}^1/_2H_2O$: C, 58.03; H, 10.01; N, 3.76. Found: C, 57.72; H, 10.08; N, 3.73.

N-(*tert*-Butyloxycarbonyl)-*S*-oxo-*S*-decyl-D-cysteine methyl ester was prepared by oxidation of the corresponding sulfide **23b** (5 mmol) with sodium metaperiodate according to the procedure that has been described earlier[32] to give the desired product as a mixture of diastereomers in 99% yield: TLC R_f 0.31 (eluent MeOH/$CHCl_3$, 3:97); ^1H NMR ($CDCl_3$) δ 0.86 (br t, J = 6.0 Hz, 3 H, CH_2CH_3), 1.26 (br s, 14 H, $(CH_2)_7CH_3$), 1.44 (s, 9 H, t-Bu), 1.53–1.73 (m, 2 H, $S(O)CH_2CH_2$), 2.73 (d of t, J = 7.0 Hz, 2 H, $S(O)CH_2CH_2$), 3.06–3.31 (m, 2 H, $CHCH_2S(O)$), 3.77 (s, 3 H, CO_2CH_3), 4.53–4.82 (m, 1 H, $CHCH_2S(O)$), 5.51–5.86 (m, 1 H, NH). Anal. Calcd for $C_{19}H_{37}NO_5S$: C, 58.28; H, 9.52; N, 3.58. Found: C, 58.56; H, 9.54; N, 3.50.

S-Oxo-S-propyl-D-cysteinol (26a). This compound was prepared from **24a** by treatment with trifluoroacetic acid according to the procedure that has been described before:[32] TLC R_f 0.46 (eluent *sec*-BuOH/NH_4OH, 55:22); ^1H NMR (D_2O) δ 1.08 (t, J = 7.5 Hz, 3 H, CH_2CH_3), 1.80 (sextet, J = 8.0 Hz, 2 H, CH_2CH_3), 2.93 (t, J = 8.0 Hz, 2 H, $S(O)CH_2CH_2$), 2.83 and 3.00 (AB part of ABX spectrum, 8 lines, J_{AX} = 5.4 Hz, J_{BX} = 11.7 Hz, J_{AB} = 13.2 Hz, 2 H, $CHCH_2S(O)$), 3.20–3.47 (m, 1 H, $CHCH_2OH$), 3.60 and 3.66 (AB part of ABX spectrum, 8 lines, J_{AX} = 9.0 Hz, J_{BX} = 2.7 Hz, J_{AB} = 11.4 Hz, 2 H, $CHCH_2OH$).

S-Oxo-S-decyl-D-cysteinol (26b). This compound was prepared from **24b** by treatment with trifluoroacetic acid according to the procedure that has been described earlier:[32] TLC R_f 0.62 (eluent *sec*-BuOH/NH_4OH, 55:22); ^1H NMR (CD_2Cl_2) δ 0.73–0.95 (m, 3 H, CH_2CH_3), 1.29 (br s, 8 H, $(CH_2)_4CH_3$), 1.70 (br s, 8 H, $S(O)CH_2(CH_2)_4$), 2.58–2.93 (m, 4 H, $CHCH_2S(O)$, $S(O)CH_2CH_2$), 3.48–3.68 (m, 1 H, $CHCH_2OH$), 3.75 (s, 2 H, $CHCH_2OH$).

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Registry No. **2**, 77880-75-2; **3**, 77880-77-4; **4**, 77880-76-3; **5**, 61787-30-2; **6**, 60484-34-6; **7** (isomer 1), 105498-95-1; **7** (isomer 2), 105498-96-2; **8**, 88001-59-6; **9**, 91922-13-3; **10**, 105431-37-6; **11**, 105431-38-7; **12**, 105431-39-8; **13**, 105431-40-1; **14**, 105456-27-7; **15**, 58462-97-8; **18**, 88001-57-4; **19**, 105431-41-2; **20**, 105431-42-3; **21**, 105431-43-4; **22**, 91922-11-1; **22** (*N*-BOC), 91922-10-0; **23a**, 105431-44-5; **23a** (sulfoxide) (isomer 1), 105456-28-8; **23a** (sulfoxide) (isomer 2), 105431-55-8; **23b**, 105431-45-6; **23b** (sulfoxide) (isomer 1), 105431-56-9; **23b** (sulfoxide) (isomer 2), 105431-57-0; **24a**, 105431-46-7; **24b**, 105431-47-8; **25a**, 105431-48-9; **25b**, 105431-49-0; **26a**, 105431-50-3; **26b**, 105431-51-4; **27**, 28277-67-0; **28**, 57412-59-6; $(BOC)_2O$, 24424-99-5; benzyl mercaptan, 100-53-8; D-cysteine, 921-01-7; 1-bromopropane, 106-94-5; 1-bromodecane, 112-29-8; *S*-propyl-D-cysteine, 85955-34-6; *S*-decyl-D-cysteine, 105431-52-5; *S*-propyl-D-cysteine methyl ester hydrochloride, 105431-53-6; *S*-decyl-D-cysteine methyl ester hydrochloride, 105431-54-7.

Comparison of Aminoglycoside Antibiotics with Respect to Uptake and Lethal Activity in *Escherichia coli*

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Forty-five aminoglycoside antibiotics and related compounds were compared for their ability to induce the accumulation of dihydrostreptomycin in *Escherichia coli* K12. The common aminoglycosides and a streptothricin antibiotic all induced enhanced uptake within a relatively narrow concentration range. These concentrations were lethal to the bacteria. Comparison of aminoacyl derivatives of tobramycin and apramycin, the latter synthesized utilizing transition-metal cations to selectively control the site of substitution, revealed that 1-*N*-aminoacyl modifications resulted in an increased ability to induce enhanced uptake. 2′-*N*-Aminoacyl modifications were also effective at inducing enhanced uptake, albeit without noticeable improvement over parent. The findings from this structure–activity comparison support the proposition that aminoglycosides share a common critical target (most likely the ribosome), which, when acted upon, results in both drug accumulation and killing.

Aminoglycoside antibiotics are accumulated by an active energy-requiring transport mechanism in bacteria.[1,2] Bryan and Kwan[3] have described this transport as consisting of an initial ionic binding followed by two energy-dependent phases (EDP-I and -II). EDP-I is a slow, energized uptake phase during which the drug promotes its own uptake. The resulting rapid, linear rate of uptake is defined as EDP-II. There is considerable evidence that the induction of EDP-II requires interaction between the drug and a sensitive ribosome.[3-5]

Aminoglycoside-induced EDP-II appears to be associated with the drug-induced killing event(s). There are

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