Total Synthesis of the Antibiotic Sparsomycin, a Modified Uracil Amino Acid Monoxodithioacetal†

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The total syntheses of sparsomycin (1), a naturally occurring antibiotic and antitumor substance, and its three stereomers **65–67** are described for the first time. In a convergent approach, the carboxylic acid **2** and the amine **3** were synthesized followed by amide formation (Scheme I). The acid **2** was prepared (23% yield) from 6-methyluracil (**12**) by coupling the aldehyde **19** with the phosphorane **20** (Scheme III). The synthesis of the amine **3**, especially challenging because of the monoxodithioacetal moiety, was accomplished by the reaction of a cysteine α-halo sulfoxide derivative **8** with sodium methylmercaptide (Scheme II, route B). Alternatively, oxidation of the dithioacetals **23–26** was unsatisfactory, yielding predominantly the undesired regioisomers **27B–30B** (Table I). Procedures are given for the preparation and separation of the α-halo sulfoxide diastereomers **33, 35, 36–41,** and **52–54**. By use of these procedures, the amino alcohol monoxodithioacetals **3** and **60** were prepared in five steps (40% yield) from the D-cystine derivative **59** having the S_C chirality of sparsomycin (Scheme VII). Finally, sparsomycin (1) and the S_C diastereomer **67** were prepared (40% yield) by mixed anhydride coupling of **2** with **3** and **60**, respectively (Schemes I and X). In addition, syntheses of the R_C enantiomer **65** and corresponding diastereomer **66** are described (Scheme IX). The CD spectra of 1 and its three stereomers are also discussed.

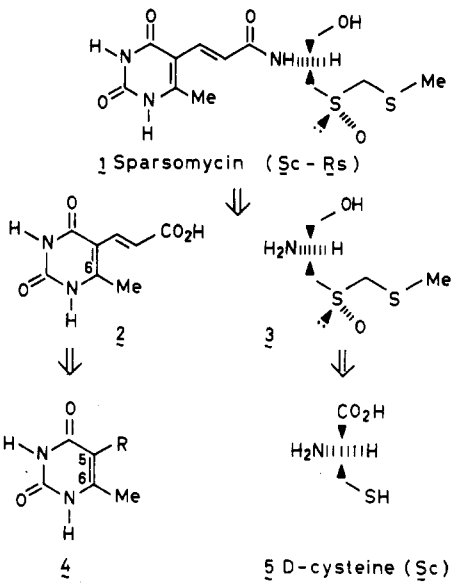
Sparsomycin (1), a metabolite of *Streptomyces sparsogenes*[1] or *Streptomyces cuspidosporus*,[2] has attracted much attention because of its activity against various tumors,[3,4] bacteria[2-4], fungi,[5] and viruses[6] and for studies[7,8] on inhibiting protein biosynthesis. On the basis of spectroscopic and degradation studies[9] the presently accepted structure 1 was proposed by Wiley and MacKellar. The chiral carbon atom was shown to have the *S* configuration; however, the chirality of the sulfoxide sulfur atom was not determined.

Although sparsomycin in limited amounts is accessible from natural sources,[10] a total synthesis would be desirable for several reasons. First, a synthesis would confirm the assigned structure and would allow the chirality of the sulfoxide to be determined. Second, an efficient synthesis would provide sparsomycin in quantities sufficient for further clinical testing and other studies of its biological activity. Third, small alterations in a flexible synthesis might permit the preparation of a number of analogues for structure–activity studies. Finally, a synthesis of 1 constitutes a challenge, because among its several functionalities is that of the formaldehyde monoxodithioacetal function $RS(O)CH_2SCH_3$. This moiety is rarely encountered[11] in nature but has recently attracted much attention because of its synthetic utility.[12]

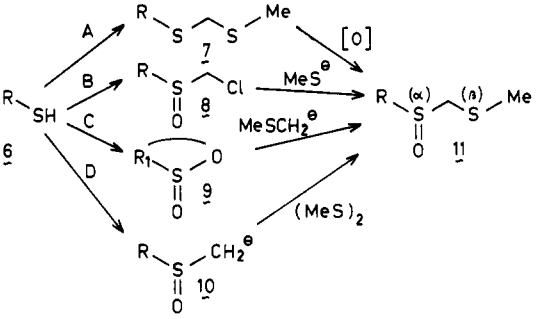
The synthesis of (*S*)-deoxosparsomycin by us[13] and others[14,15] had substantiated structure 1; however, no total synthesis of this antibiotic was reported until recently when Helquist[16] and we[17,18] each described in preliminary reports different routes to the R_C enantiomer of sparsomycin. In addition, the sulfoxide could be assigned[19] the *R* configuration as depicted in structure 1 (Scheme I). This assignment is based on chiroptical studies and X-ray crystallographic analysis of precursors of sparsomycin (vide infra). The present publication presents in detail our synthetic approaches to sparsomycin and its three stereoisomers. These syntheses confirm the Wiley and MacKellar structure and should provide a practical source of sparsomycin and its analogues for further study of its biological activity.

Strategy. Sparsomycin (1) may be considered as an amide derived by the coupling of the β-(6-methyluracilyl)acrylic acid (**2**) and the amine **3** (Scheme I). The latter can be viewed as a derivative of D-cysteine (**5**) having

Scheme I



Scheme II



† Dedicated to Professor Dr. R. J. F. Nivard on the occasion of his 60th birthday.

(1) A. D. Argoudelis, R. R. Herr, *Antimicrob. Agents Chemother.*, 505 (1962).
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(3) L. Slechta, "Antibiotics I". D. Gottlieb, P. D. Shaw, Eds., Springer Verlag, New York, 1967, p 410.
(4) T. F. Brodasky, *J. Pharm. Sci.*, **52**, 233 (1963); K. E. Price, R. E. Buck, and J. Lein, *Antimicrob. Agents Chemother.*, 505 (1964).

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Scheme III

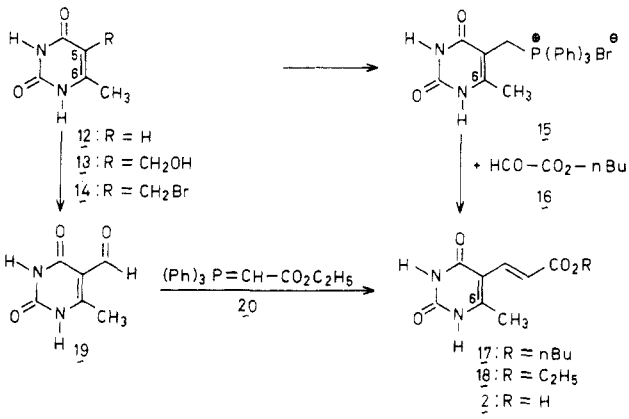

Chart I

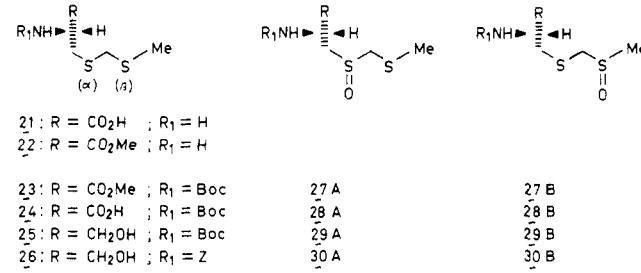

Table I. Conversion of 23-26 into 27A-30A and 27B-30B

	overall yield,[a] %	rel yield,[b] % A	B
23 → 27	92	20	80
24 → 28	78	16	84
25 → 29	96	33	67
26 → 30	92	25	75

[a] After column chromatography. [b] Based on ^1H NMR spectroscopy before chromatography.

a reduced CO_2H function and its sulfhydryl function alkylated and oxidized.

Component **2** could be prepared in two ways by using a Wittig condensation of a C(5)-substituted 6-methyluracil (**4**). More challenging was the synthesis of component **3**, since the unsymmetrical monoxodithioacetal moiety is acid labile[12] and is also capable of undergoing the thermal- or base-induced β eliminations for which sulfoxides are prone.

Two fundamentally different approaches are reported here. Initially we studied the regioselective oxidation of a dithioacetal (**7**) derived from cysteine **6** (Scheme II, route A). Our second approach (route B) employed the reaction of an α-chloro sulfoxide derivative of cysteine (**8**) with sodium methylmercaptide. A third approach (route C), featuring sultines **9** as intermediates, will be subject of a future report.[20] A fourth approach (route D) has been explored successfully by Helquist,[16,21] who employed the sulfenylation of an α-sulfinyl carbanion **10**. Routes B–D have in common the introduction of the β-sulfur atom subsequent to the oxidation of the α-sulfur atom. Incidentally these four approaches also represent general methods for the preparation of carbonyl compounds.[12]

Acid Component 2. The two procedures developed[13] for the preparation of the β-(6-methyluracilyl)acrylic acid (**2**) commenced from 5-(hydroxymethyl)-6-methyluracil (**13**, Scheme III). This alcohol was prepared from commercial 6-methyluracil (**12**) with formaldehyde and aqueous NaOH by a variation of Kircher's method.[22] Yields of 70–80% could be reached if these reagents were used in molar ratios of 1:3:2. Treatment of **13** with HBr in glacial acetic acid gave **14**[23] (79% yield), which upon reaction with $(C_6H_5)_3P$ in DMF yielded quantitatively the phosphonium salt **15**. *n*-Butyl glyoxylate (**16**) was prepared in variable yields from *n*-butyl dimethoxyacetate by distillation from P_2O_5. A more satisfactory preparation of **16** was the oxidation[24] of dibutyl tartrate with $NaIO_4$ according to Atkinson.[25] In contrast to their report, however, we find that the hemihydrate of **16** is actually isolated. From this, **16** may be obtained by distillation from P_2O_5. The Wittig coupling of **15** with **16** in DMF gave **17** in low yields (5–15%), regardless of the reaction conditions and bases used. This low yield might be explained by deprotonation of either the uracil nitrogen or the C-(6)-CH_3 group of **15** to give an *exo*-methyleneuracil derivative and $(C_6H_5)_3P$. Indeed, the latter could be detected on TLC, along with the expected $(C_6H_5)_3PO$. The overall yield by the route **12** → **15** → **17** was only 6%.

In a variation of the Wiley–MacKellar procedure[9] we employed the inverse of the previous Wittig reaction, i.e., coupling of **19** with **20**, for a more satisfactory synthesis of **2**. The aldehyde **19** was prepared by us from **13** (63% yield) by reaction with $K_2S_2O_8$ and a trace of $AgNO_3$.[26,27]

(5) S. P. Owen, A. Dietz, and G. W. Caminer, *Antimicrob. Agents Chemother.*, 772 (1962).
(6) L. Thiry, *J. Gen. Virol.*, **2** (part 1), 143 (1968).
(7) A. E. Smith and D. T. Wigle, *Eur. J. Biochem.*, **35**, 566 (1973).
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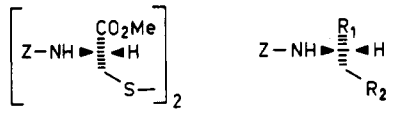
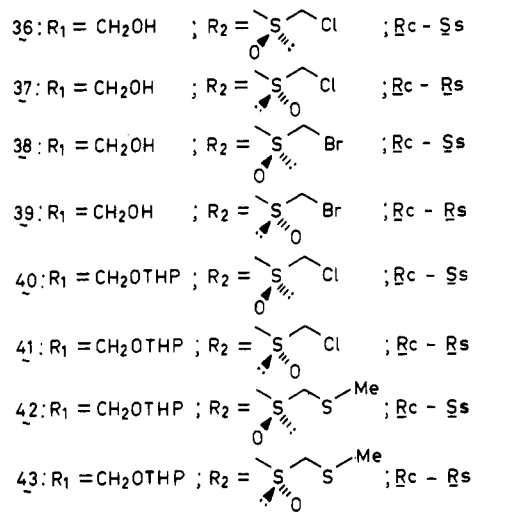
(9) (a) P. F. Wiley and MacKellar, *J. Am. Chem. Soc.*, **92**, 417 (1970); (b) P. F. Wiley and MacKellar, *J. Org. Chem.*, **41**, 1858 (1976).
(10) The isolation of sparsomycin by cultivation of fungi has been patented. From *Streptomyces sparsogenes*, see: Upjohn Co., British Patent 974 541 (1964); *Chem. Abstr.*, **62**, 5855*d* (1965). From *Streptomyces cuspidosporus*, see: E. Higashide, M. Shibata, T. Hasegawa, and K. Mizuno, Japanese Patent 7 134 196 (1971); *Chem. Abstr.*, **76**, 2549 (1972).
(11) The only other natural example is γ-glutamylmarasmine: R. Gmelin, H.-H. Luxa, K. Roth, and G. Höfle, *Phytochemistry* **15**, 1717 (1976).
(12) The dithioacetal monoxide is a masked carbonyl compound, whose carbanion can serve as an acyl anion equivalent. See: F. I. K. Ogura and G. Tsuchihashi, *Tetrahedron Lett.*, 3151 (1971). In addition, their acidolytic cleavage can be used to prepare unsymmetric disulfides, see: Y. Kishi, T. Fukuyama, and S. Nakatsuka, *J. Am. Chem. Soc.*, **95**, 6490 (1973); B. Zwanenburg and P. Kielbasinski, *Tetrahedron*, **35**, 169 (1979).
(13) For a preliminary report, see: H. C. J. Ottenheijm, S. P. J. M. van Nispen and M. J. Sinnige, *Tetrahedron Lett.*, 1899 (1976).
(14) C. C. L. Lin and R. J. Dubois, *J. Med. Chem.*, **20**, 337 (1977).
(15) C. K. Lee and R. Vince, *J. Med. Chem.*, **21**, 176 (1978).
(16) P. Helquist and M. S. Shekhani, *J. Am. Chem. Soc.*, **101**, 1057 (1979).
(17) H. C. J. Ottenheijm and R. M. J. Liskamp, *Tetrahedron Lett.*, 387 (1978).
(18) H. C. J. Ottenheijm, R. M. J. Liskamp, and M. W. Tijhuis, *Tetrahedron Lett.* 387 (1979).
(19) H. C. J. Ottenheijm, R. M. J. Liskamp, P. Helquist, J. W. Lauher, and M. Shekhani, *J. Am. Chem. Soc.*, **103**, 1720 (1981).
(20) R. M. J. Liskamp, H. J. M. Zeegers, and H. C. J. Ottenheijm, manuscript in preparation.
(21) P. Helquist, M. S. Shekhani, and D.-R. Hwang, manuscript in preparation.
(22) W. Kircher, *Justus Liebigs Ann. Chem.*, **385**, 293 (1911). See also ref 9b. In addition, it was found that Cline's procedure for this reaction gave only polymeric material: R. E. Cline, R. M. Fink, and K. Fink, *J. Am. Chem. Soc.*, **81**, 2521 (1959).
(23) Y. P. Shvachkin and L. A. Syrtsova, *Zh. Obshch. Khim*, **34**, 2159 (1964); *Chem. Abstr.*, **61**, 9575*h* (1964).
(24) In our hands preparation of a pure sample of **16** according to F. J. Wolf, J. Wyland, N. J. Leonard, and L. A. Miller, *Org. Synth.*, **35**, 18 (1966), failed.
(25) C. M. Atkinson, C. W. Brown, and J. C. E. Simpson, *J. Chem. Soc.*, 26 (1956).
(26) R. Brossmer and D. Ziegler, *Chem. Ber.*, **102**, 2877 (1969).
(27) Most of the conventional reagents for the conversion of alcohols into aldehydes were found to be unsatisfactory; for instance, the chromic oxide oxidation used by Wiley and MacKellar[9b] gave a 20% yield only.

Chart II



31 32: $R_1 = CO_2Me$; $R_2 = S(O)Cl$
33: $R_1 = CO_2Me$; $R_2 = S(O)CH_2Cl$
34: $R_1 = CO_2Me$; $R_2 = S(O)OMe$
35: $R_1 = CO_2Me$; $R_2 = S(O)CH_2Br$



36: $R_1 = CH_2OH$; R_2 ; Rc - Ss
37: $R_1 = CH_2OH$; R_2 ; Rc - Rs
38: $R_1 = CH_2OH$; R_2 ; Rc - Ss
39: $R_1 = CH_2OH$; R_2 ; Rc - Rs
40: $R_1 = CH_2OTHP$; R_2 ; Rc - Ss
41: $R_1 = CH_2OTHP$; R_2 ; Rc - Rs
42: $R_1 = CH_2OTHP$; R_2 ; Rc - Ss
43: $R_1 = CH_2OTHP$; R_2 ; Rc - Rs

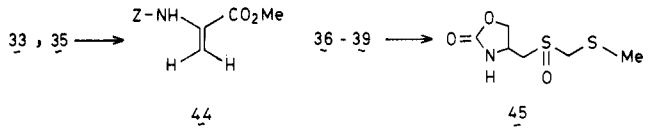
Coupling of **19** with **20** gave **18** (41% yield); the overall yield by this route (**12** → **19** → **18**) is 23%.

Alkaline hydrolysis of **17** or **18** gave quantitatively the acid **2**, identical with the product obtained by Wiley and MacKellar.[9]

Amine Component 3. Routes A and B (Scheme II) were explored initially by using the more readily available L-cysteine (*R* configuration) as the starting material.

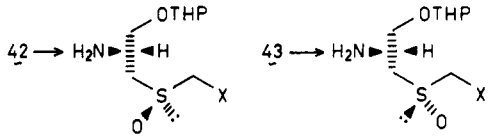
Route A. L-Cystine was reduced with sodium in liquid NH_3, treated with chloromethyl methyl sulfide[28] and acidified to give **21** (Chart I) in 61% yield. The amino acid ester **22** was prepared in 87% yield by treatment with CH_3OH and $SOCl_2$, followed by deprotonation with $(C_2H_5)_3N$. Compound **22** was used for the preparation of enantiomeric (*S*)-deoxosparsomycin (**62**) via **61** as is described below. The ester **61** and the alcohol **62** are difficult to purify because of their high polarity. Therefore, the regioselective oxidation **7** → **11** was studied on the more easily handled cysteine derivatives having the conventional (benzyloxy)carbonyl (Z) or (*tert*-butyloxy)carbonyl (Boc) N-protecting groups. In order to study the influence of group R on the regioselectivity of the oxidation, we prepared the acid **24** and the alcohols **25** and **26** in addition to **23**. Compound **23** was prepared from **22** by standard techniques. Protection of the amino function of **21** and reduction ($LiBH_4$) of **23** afforded **24** and **25**, respectively. The alcohol **26** was prepared from the corresponding N-protected ester by reduction ($LiBH_4$). Treatment of the dithioacetals **23**–**26** with 1 equiv of $NaIO_4$ gave a mixture of the corresponding monoxodithioacetals **27A**–**30A** and **27B**–**30B**. Table I shows that the less hindered β-sulfur atom is attacked preferentially. In addition, it can be concluded that on oxidation of compounds **23**–**25**, the ratio of **A** to **B** varies slightly but significantly depending upon the nature of R, reaching a maximum for R = CH_2OH.[29]

Scheme IV



44 45

Scheme V



46: X = SCH_3; Rc - Ss 47: X = SCH_3; Rc - Rs
48: X = H 49: X = H

Structures **27A**–**30A** and **27B**–**30B** were assigned by means of spectroscopy and an independent synthesis of **29A** (vide infra). As a mixture of all possible stereoisomers of **A** and **B** were formed, e.g., **25** (R_C) → **29A** (R_C–R_S) + **29** (R_C–S_S) + **29B** (R_C–R_S) + **29B** (R_C–S_S), in which the desired regioisomers **A** were by far the minor components, this route was temporarily abandoned.[30]

Route B. The α-chloro sulfoxide **8** was prepared[31] by reaction of CH_2N_2 with a sulfinyl chloride,[32,33] synthesized from the corresponding disulfide with Cl_2 and acetic anhydride.[34] Thus, treatment of *N*-[(benzyloxy)carbonyl]-L-cystine methyl ester (**31**) with 3 equiv of Cl_2 in the presence of Ac_2O gave the sulfinyl chloride **32** (Chart II) as a stable, white solid. Reaction of **32** with dry CH_2N_2 gave, according to ^1H NMR spectroscopy, a mixture of the two diastereomeric α-chloro sulfoxides **33** (R_C,R_S) and **33** (R_C,S_S). If an undried etheral CH_2N_2 solution was used, up to 30% of the sulfinate ester **34** could be isolated besides **33**. The above procedure with a slight but crucial modification was applied to the preparation of the α-bromo sulfoxides **35** (R_C,R_S) and **35** (R_C,S_S) in that **32** was added dropwise to a solution of CH_2N_2 and LiBr in ether–THF.[35] The reverse order of addition gave mainly dimeric products of unknown structure.

The nucleophilic displacement of halogen in **33** or **35** by CH_3S^- was expected to proceed normally as it is a known reaction[36] for α-chloro sulfoxides. However, treatment of **33** or **35** with CH_3SNa gave the dehydroamino acid derivative **44** as the main product (Scheme IV). This β

(28) This is a general method for the preparation of S-alkylated cysteine derivatives according to P. J. E. Brownlee, M. E. Cox, B. O. Handford, J. C. Marsden, and G. T. Young, *J. Chem. Soc.*, 3832 (1964).

(29) We are inclined to contribute this effect to anchimeric assistance; if R = CH_2OH, a cyclic intermediate can be formulated, which is six or eight membered, depending upon the involvement of S(α) or S(β), respectively. As the six-membered ring is favored, this should lead to oxidation at S(α). Similar cyclic intermediates have been formulated for the oxidation of 1,2-diols with $NaIO_4$, e.g., H. O. House in "Modern Synthetic Reactions", 2nd ed., W. A. Benjamin, New York, 1972, p 354.

(30) A search for a method for the regio- and diastereoselective oxidation of dithioacetals **25** and **26** is in progress. In addition, we are studying the regioselective reduction of dithioacetal *S*(α),*S*(β)-dioxides; so far this approach has failed since the reaction conditions used induced β eliminations to yield **44**.

(31) Attempts to convert a suitably protected *S*-methyl-L-cysteine *S*-oxide derivative into the corresponding compound **8** by a known procedure [S. Iriuchimja and G. Tsuchihashi, *Synthesis* 558 (1970); N. Kumeda, J. Nokanu, and M. Kinoshito, *Bull. Chem. Soc. Jpn.*, **49**, 256 (1976)] failed. Nor was it possible to prepare a *S*(halomethyl)-L-cysteine derivative from CH_2X_2 (X = Cl or Br) and cysteine; regardless of the reaction conditions used, the corresponding dithioacetal $RSCH_2SR$ was formed: A. G. van Veen, *Recl. Trav. Chim. Pays-Bas*, **54**, 493 (1935).

(32) E. Ayca, *Istanbul Univ. Fen Fak. Mecm., Seri C*, **22**, 371 (1957).

(33) We thank Professor Venier for bringing this reaction, developed for the preparation of simple α-halosulfoxides, to our attention: C. G. Venier, H.-H. Hsieh, and H. J. Barager, *J. Org. Chem.*, **38**, 17 (1973).

(34) I. B. Douglass and R. V. Norton, *J. Org. Chem.*, **33**, 2104 (1968).

(35) C. G. Venier and H. J. Barager III, *J. Chem. Soc., Chem. Commun.*, 319 (1973).

(36) K. Ogura and G. Tshuchihashi, *J. Chem. Soc., Chem. Commun.*, 1689 (1970).

elimination reaction could be prevented by reduction of the ester function of **33** or **35** with $LiBH_4$ in monoglyme to yield a mixture of the diastereomeric alcohols **36**, **37** and **38**, **39**, respectively. Whereas the diastereomers of **33** or **35** could not be separated, the alcohols **36** (21% yield) and **37** (34% yield) could be separated easily by column chromatography. The yields given are based on **31**. The assignment of the absolute configurations will be discussed below.

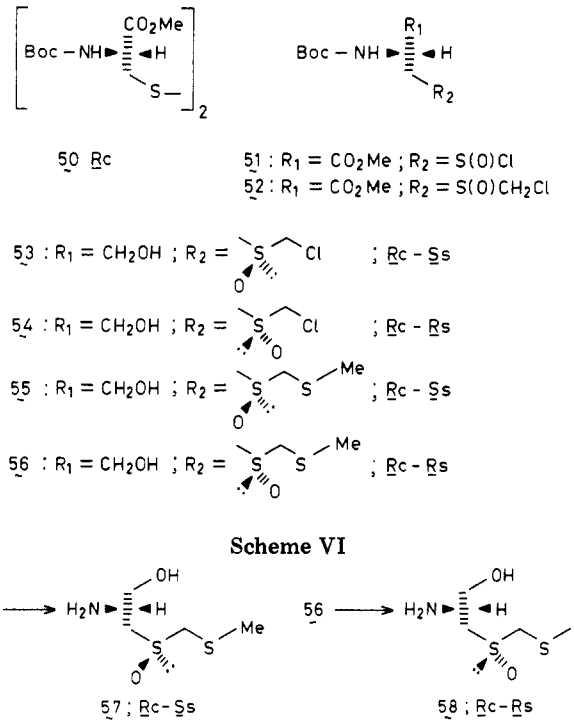
Direct conversion of **36–39** into the desired corresponding monoxodithioacetals **42** and **43** failed: treatment with CH_3SNa in C_2H_5OH gave the cyclic urethane **45** (Scheme IV) as the main product (30% yield). To circumvent this cyclization, we protected the alcohol function of **36** or **37** with the tetrahydropyranol group[37] and obtained **40** or **41**, respectively. Finally, treatment of **40** and **41** with 1.2 molar equiv CH_3SNa[38] gave the monoxodithioacetals **42** and **43**, respectively, in quantitative overall yield.

At this stage the N-protecting group, introduced to avoid side reactions in the preceding steps, had to be removed.[39] We had noted that **42** and **43** were stable to liquid NH_3,[40] so du Vigneaud's procedure[41] for removal of the Z group was applied. When **42** or **43** in refluxing NH_3 was treated carefully[42] with Na in liquid NH_3, the desired amines **46** and **47** were isolated (Scheme V), though in low yields (20–30%). These amines were used for the first synthesis of the enantiomer **65** and diastereomer **66**, respectively, of sparsomycin (**1**) (vide infra).

Regardless of conditions used for the deblocking of **42** or **43**, a ninhydrin-positive compound was isolated as the main product (30–40% yield). Structure **48** and **49**, respectively, were assigned to these byproducts on the basis of independent syntheses (see Experimental Section). These products, formed by a reductive scission of the C–S bond, were found to be identical with the intermediates used by Helquist[16] in approach D (Scheme II). Monitoring the Na/NH_3 reaction by TLC revealed that Z removal and C–S bond cleavage are competing reactions. Because of the inefficiency and poor reproducibility of this reaction, we decided to prepare a derivative of **42** and **43** having an N-protecting group, whose cleavage conditions are more compatible with the monoxodithioacetal moiety.

The base-labile [[(methylsulfonyl)ethyl]oxy]carbonyl (Msc) group[43] and the (trichloroethoxy)carbonyl (Toc) group,[44] removable under neutral conditions, were found to be unsuitable; their removal was accompanied to a large extent by β elimination. We had noticed, however, that the THP group of **63** and **64** could be removed under mildly acidic conditions (vide infra), indicating that the

Chart III



Scheme VI

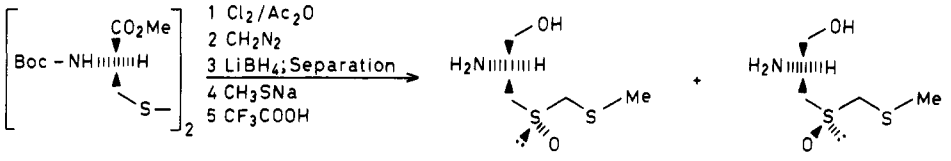
$RS(O)CH_2SCH_3$ function had a greater acid stability than we had originally anticipated. Therefore we chose to synthesize the amine fragment by employing the (*tert*-butyloxy)carbonyl (Boc) group which is removable in CF_3COOH at 0 °C. Thus, [(*tert*-butyloxy)carbonyl]-L-cystine methyl ester **50** (Chart III) was converted into the α-chloro sulfoxides **53** and **54** (47% yield, ratio 1:2) via intermediates **51** and **52** as has been described for the syntheses of **36** and **37**. It is noteworthy that the Boc group is stable under the reaction conditions employed for the synthesis of **51**, i.e., treatment with Ac_2O and Cl_2 and the attendant formation of AcCl. Compounds **53** and **54** could be separated easily and were each converted nearly quantitatively into the previously prepared (Chart I, **29A**) monoxodithioacetals **55** and **56** by treatment with CH_3SNa.

Finally, the amino alcohols **57** and **58** were prepared quantitatively by treatment of **55** and **56**, respectively, with CF_3COOH at 0 °C and subsequent deprotonation with an ion-exchange resin (Scheme VI).

The conversions **53** → **55** → **57** and **54** → **56** → **58** deserve further comment. First, when the reactions are done in reverse order, i.e., removal of the Boc group prior to the substitution reaction, unidentified side products are formed in addition to the desired compound during the last reaction. A possible side reaction might be the intramolecular displacement of chloride by the amine function. Second, it is unnecessary to protect the alcohol groups of **53** and **54** prior to treatment with CH_3SNa, as these alcohols, unlike **36** and **37**, do not form **45**. Third, care has to be taken to avoid epimerization of the sulfoxide sulfur atom. It was noticed that during silica gel chromatography of the crude reaction mixture of **54** → **56**, the diastereomer **55** is formed. As compounds **55** and **56** are not interconvertable by chromatography on silica gel in the absence of NaCl, the formation of **55** evidently arises by a silica gel/NaCl-catalyzed epimerization of the sulfoxide sulfur atom.[45] Finally, ^1H NMR spectra of the

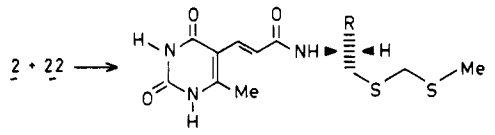
(37) J. H. van Boom and J. D. M. Herscheid, *Synthesis*, **3**, 169 (1973).
(38) The quality of the CH_3SNa was found to be crucial for the success of this reaction. Of all the procedures used, only the reaction of MeSSMe with Na in liquid NH_3 according to F. E. Williams and E. Giebauer-Fuelnegg, *J. Am. Chem. Soc.*, **53**, 352 (1931), gave CH_3SNa that fulfilled our requirements. The purity was checked gravimetrically by reaction of 2,4-dinitrofluorobenzene with the mercaptide to yield its 2,4-dinitrobenzene derivative.
(39) For removal of the Z group the usual acid cleavage had to be avoided, because of the acid-labile monoxodithioacetal group. In addition, catalytic hydrogenation of compounds containing bivalent sulfur generally fails, due to catalyst poisoning.
(40) Palladium-catalyzed hydrogenation in liquid NH_3 according to J. Meienhofer and K. Kuromizu, *Tetrahedron Lett.*, 3259 (1974), gave only starting material.
(41) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).
(42) The procedure of H. Nesvadba and H. Roth, *Monatsh. Chem.*, **98**, 1432 (1967), was applied with a simplified apparatus.
(43) G. Tesser and I. C. Balvert-Geers, *Int. J. Pept. Protein Res.*, **7**, 295 (1975).
(44) T. B. Windholz and D. B. R. Johnston, *Tetrahedron Lett.*, 2555 (1967).
(45) Whereas the HCl-catalyzed racemization of sulfoxides is well established, we are not aware of a precedent for this reaction: E. Ciuffarin, et al., *J. Chem. Res., Synop.*, 270 (1978).

Scheme VII



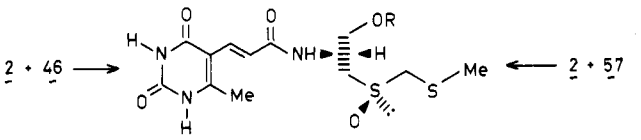
59; Sc 3; Sc - Rs 60; Sc - Ss

Scheme VIII

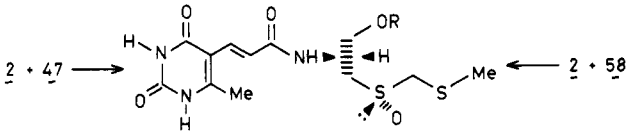


61 : R = CO_2Me
62 : R = CH_2OH

Scheme IX

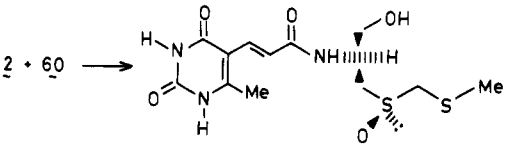


63 : R = THP
65 : R = H; Rc - Ss



64 : R = THP
66 : R = H; Rc - Rs

Scheme X



67 Sc - Ss

amino alcohols **57** and **58** in the presence of a chiral shift reagent showed that their enantiomeric purity is greater than 95%. From this we concluded that no racemization or epimerization had occurred during the reactions leading to **57** or **58**.

Having established this, the amino compounds **3** and **60** were prepared in five steps, starting from the D-cystine derivative **59** having the S_C chirality (Scheme VII) as occurs in sparsomycin (**1**). The overall yield of this sequence of five reactions is 40%.

The absolute configuration of the sulfoxide sulfur atom of **1** was determined[19] by CD studies of the intermediates **36**, **37**, **57**, and **58** as well as compounds **48–49** after THP removal. This assignment has been confirmed[19] by X-ray analysis of the *N*-carbobenzyloxy derivative **48**, lacking the THP group.

Coupling of the Fragments. (*S*)-Deoxosparsomycin. Coupling of **2** with **22** was achieved by means of dicyclohexylcarbodiimide (DCC) and hydroxybenztriazole (HOBT) in DMF, yielding **61** in 60% yield (Scheme VIII). Selective reduction of the ester function with $LiBH_4$ in monoglyme gave the enantiomer of deoxosparsomycin **62** in 63% yield after column chromatography (Sephadex LH-20). The ^1H NMR spectrum was similar to that reported for **1**, except for the presence of a singlet at δ 3.96, due to the SCH_2S protons.

Sparsomycin (1) and Its Three Stereoisomers 65–67. The reagents DCC and HOBT again were used for the coupling of the O-protected amino alcohols **46** and **47** with **2**, providing **63** and **64**, respectively, in 40–50% yields (Scheme IX). The THP group could be removed by refluxing a slightly acidified ethanol solution of **63** and **64** for 15 min to give **65** and **66**, respectively, in 70–80% yields after column chromatography (Sephadex LH-20). To avoid tedious purification of the polar compounds **65** and **66**, the precursors **63** and **64**, respectively, were carefully chromatographed (silica gel). Compound **65** proved to be identical in all respects with sparsomycin[46] (**1**), but, as expected, it exhibits a negative specific rotation. Thus **65** is the enantiomer and **66** the R_C diastereomer of **1**. Compounds **65** and **66** are easily distinguished by different R_f values on TLC and by their ^1H NMR spectra: **65** shows three lines for the $CHCH_2S(O)$ protons, whereas **66** displays the eight lines typical of the AB part of an ABX spectrum. Both compounds show the four lines of an AB spectrum for the $S(O)CH_2S$ protons, however, their chemical shift values differ slightly but significantly. According to these criteria, and to the specific rotation of **65**, no epimerization occurs during the coupling and deprotection reactions.

An alternative route to **65** and **66** is the coupling of **2** with the amino alcohols **57** and **58**, respectively (Scheme IX). While this approach avoids the protection of the alcohol function, it has the disadvantage that reactants and product are poorly soluble in typical organic solvents; in addition, purification of the end products **65** and **66** is a cumbersome task. A variety of coupling procedures including EEDQ,[47] IIDQ,[48] Woodward's L reagent,[49] *p*-nitrophenyl trifluoroacetate,[50] DCC/HOBT,[51] DCC/HONSu,[52] and ethyl chloroformate[53] were studied in an attempt to improve the yield of this step. The last two gave optimal results, in that yields were acceptable (33–40%) and that complete separation of side products was possible by reverse-phase HPLC or chromatography on Sephadex LH-20.

Finally, sparsomycin (**1**) and the S_C diastereomer **67** were prepared in 33% and 40% yields, by coupling **2** with the amino alcohols **3** and **60**, respectively, by the mixed

(46) We are grateful for Dr. P. F. Wiley (Upjohn Co.) for supplying us with a sample of authentic sparsomycin.

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(48) Y. Kiso, Y. Kai, and H. Yayima, *Chem. Pharm. Bull.*, **21**, 2507 (1973).
(49) D. J. Woodman and A. I. Davidson, *J. Org. Chem.*, **38**, 4288 (1973).
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(53) T. Wieland and H. Bernard, *Justus Liebigs Ann. Chem.*, **572**, 190 (1951).

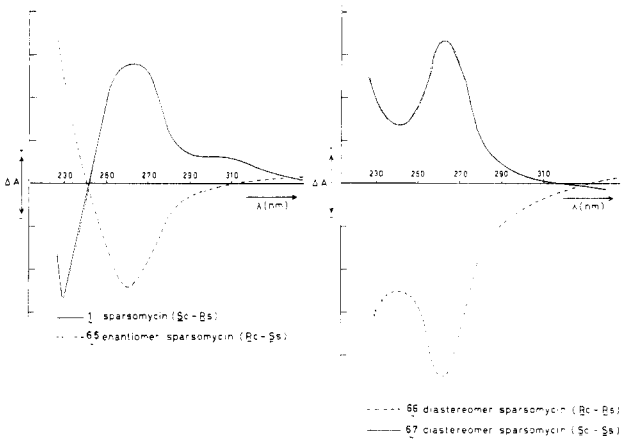

Figure 1. CD spectra of compounds 1 and **65–67** in acetonitrile.

anhydride method (Schemes I and X). Compound 1 thus obtained was identical in all respects with an authentic sample[46] of sparsomycin, whereas **67** was identical with diastereomer **66**, differing only in the sign of specific rotation. The yield of this six-step synthesis of 1 is 16% based on **59**.

The CD spectra of sparsomycin (1) and its stereomers **65–67** are shown in Figure 1. It appears that the sulfoxide and amide chromophores can be considered separately; the sign of the Cotton effect at 230 nm (sulfoxide function) is not influenced by the sign of the band at 265 nm (amide function). The sign of the Cotton effect in the region 230–240 nm is a criterion of the chirality of the sulfoxide function: a positive sign correlates with an S_S configuration and a negative sign with an R_S configuration. This agrees with our earlier findings[19] on precursors for 1. The Cotton effect at 260–270 nm is due to an n,π^* transition of the amide bond. The sign of this band is determined by the chirality of the carbon atom; a positive sign correlates again with an S_C configuration and a negative sign with an R_C configuration.

Discussion

The syntheses of 1 and its stereoisomers **65–67** described herein provide definite proof of sparsomycin's structure. In addition, they made possible the assignment of the chirality of the sulfoxide sulfur atom. The most efficient route involved coupling **2** with the amino alcohol **3**, prepared from [(*tert*-butyloxy)carbonyl]-D-cystine methyl ester **59**. The overall yield (16%) of this six-step approach, while being acceptable, is lowered chiefly by the inefficiency (40%) of the coupling of **2** with **3**. Unfortunately, all of the coupling reactions of **2** with the amines described in this report proceeded in low yields. This might be due to a decrease in nucleophilicity of the α,β-unsaturated carboxylate anion of **2** and to acylation of the uracil ring.

Work is presently in progress to improve the coupling procedure (e.g., using diethyl cyanophosphate[54]), to streamline the synthesis of **11** via the sultine approach (route C), to prepare a series of analogues of 1, and to synthesize γ-glutamylmarasmine.[11]

Experimental Section

^{1}H NMR spectra were measured on Varian Associates Model T-60 or a Bruker WH-90 spectrometer with Me_4Si or *t*-BuOH as an internal standard. $CDCl_3$ was used as the solvent unless stated otherwise. IR spectra were measured with a Perkin-Elmer spectrophotometer, Model 997, and UV spectra on a Perkin-Elmer spectrophotometer, Model 555. For determination of the specific rotation, a Perkin-Elmer 241 polarimeter was used. Circular dichroism spectra were measured with a Dichrograph II apparatus (Roussel-Jouan, France).

Mass spectra were obtained with a double-focusing Varian Associates SMI-B spectrometer. Melting points were taken on a Köfler hot stage (Leitz-Wetzlar) and are uncorrected. Thin-layer chromatography (TLC) was carried out by using Merck precoated silica gel F-254 plates (thickness 0.25 mm). Spots were visualized with an UV lamp, iodine vapor, ninhydrin, and TDM. For column chromatography, Merck silica gel H (typ 60) was used. A Miniprep LC (Jobin Yvon) was used for preparative HPLC.

5-(Hydroxymethyl)-6-methyluracil (13). This synthesis is a modification of Kircher's procedure,[22] which was found to be highly erratic.

6-Methyluracil (**12**; 5.2 g, 40 mmol) was dissolved in 64 mL of a 5% aqueous NaOH solution with stirring and gentle heating; to this solution was added 10 g of a 37% aqueous formaldehyde solution. This reaction mixture was stirred for 16 h at room temperature. The resulting precipitate was filtered off and washed with cold water; subsequently the precipitate was dissolved in the minimal amount of water of 80 °C. If necessary, the resulting solution was filtered in order to remove polymeric byproducts. To the clear solution was carefully added concentrated sulfuric acid until neutral pH.

Finally, the solution was kept at 4 °C for 4 h during which the alcohol **13** crystallized. Filtration, washing with ice-cold water, recrystallization from water, and drying in vacuo for 6 h at 80 °C yielded 73% of **13** (4.5 g). The compound was homogeneous on TLC (R_f 0.43, *sec*-BuOH saturated with water): NMR (Me_2SO-d_6) δ 2.1 (s, 3 H, CH_3), 4.0 (s, 2 H, CH_2OH); IR (KBr) 3400, 1700, 1665, 1110 cm^{-1}. Anal. Calcd for $C_6H_8N_2O_3$: C, 46.15; H, 5.16; N, 17.94. Found: C, 45.93; H, 5.15; N, 17.71.

5-(Bromomethyl)-6-methyluracil[23] (14). A solution of pulverized **13** (2.0 g, 13.0 mmol) in 40% HBr/AcOH (40 mL) was stirred with the exclusion of atmospheric moisture at 100 °C for 16 h. Subsequently, the reaction mixture was kept at 4 °C for 16 h during which **14** crystallized. The precipitate was collected and washed with dry ether to yield **14** (2.2 g, 78%) which is very hygroscopic and had to be stored in a desiccator over KOH: mp 310 °C dec; ^{1}H NMR ($AsCl_3$) δ 2.1 (s, 3 H, C(6)CH_3), 4.1 (s, 2 H, CH_2Br); positive Beilstein test.

5-(Methyltriphenylphosphonium)-6-methyluracil Bromide (15). A solution of $P(Ph)_3$ (3.0 g, 11 mmol) in dry DMF (10 mL) was added at room temperature to a stirred solution of **14** (2.0 g, 9 mmol) in 18 mL of dry DMF; the temperature of the reaction mixture increased to 35–40 °C. Stirring was continued at room temperature for 2 h and finally at 70° C for 1 h. The reaction mixture was cooled at room temperature, and subsequently dry ether was added dropwise while the solution was being stirred. The resulting precipitate was sticky at the beginning but upon continued stirring turned into a crystalline mass. Filtration and washing with dry ether gave **15**: 98% yield (4.3 g); mp 270 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.6 (s, 3 H, C(6)CH_3), 3.8 (d, 2 H, CH_2P), 6.2 (m, 15 H, $(Ph)_3$); IR (KBr) 1715 and 1665 cm^{-1}. Anal. Calcd for $C_{24}H_{22}BrN_2O_2 \cdot H_2O$: C, 57.79; H, 4.84; N, 5.61. Found: C, 57.28; H, 4.94; N, 5.60.

***n*-Butyl Glyoxalate (16). From *n*-Butyl Dimethoxyacetate.** Sodium dimethoxyacetate was prepared from dichloroacetic acid as has been described by Moffett et al.[55] The solvents were evaporated in vacuo, and the residue was dissolved in ether and 5% aqueous $KHSO_4$ solution. The water layer was extracted two times with ether, and then the organic layers were dried (Na_2SO_4) and concentrated in vacuo. The residue was dissolved in *n*-BuOH containing HCl, and the resulting solution was stirred at room temperature for 16 h. The reaction mixture was then cooled to 0 °C, and the excess acid was neutralized to approximately pH 7 by addition of solid $NaHCO_3$. The mixture was filtered, and then ether and 5% aqueous $NaHCO_3$ solution were added. The organic layer was dried (Na_2SO_4), and subsequently the ether was removed by distillation. Finally the residue was distilled under reduced pressure by using a Vigreux column to yield *n*-butyl dimethoxyacetate: bp 100 °C (20 mm); ^{1}H NMR

(54) S. Jew, S. Terashima, and K. Koga, *Tetrahedron*, **35**, 2337 (1979).

(55) R. B. Moffett, C. C. Price, and C. E. Scott, *Org. Synth.*, **35**, 59 (1955).

δ 1.3 (m, 7 H, $OCH_2C_3H_7$), 3.3 (s, 6 H, 2 OCH_3), 4.15 (t, 2 H, OCH_2), 4.8 (s, 1 H, $HC(OMe)_2$). This compound was distilled over P_2O_5 (approximately 1 g/g of the acetal) to give **16** in yields ranging from 10% to 30%; bp 65–68 °C (20 mm). The ester is not stable and polymerizes within 24 h, even when stored at −20 °C: ^1H NMR δ 1.4 (m, 7 H, $OCH_2C_3H_7$), 4.3 (t, 2 H, OCH_2), 8.3 (s, 1 H, HC(O)).

From Dibutyl Tartrate. Di-*n*-butyl tartrate [bp 150 °C (2 mm)] was prepared from the acid and *n*-BuOH with a catalytic amount of boron trifluoride etherate. The ester was converted into the hemihydrate of **16** according to Atkinson et al.;[25] fractions in the boiling range 60–75 °C (20 mm) [lit. bp 55 °C (14 mm)] were collected: IR ($CHCl_3$) 3500 cm^{-1} (OH); ^1H NMR (only characteristics) δ 4.4–5.5 (m, 2 H, HOC(**H**)OC(**H**)OH). The hemihydrate, which is a stable compound, was converted into **16** by distillation over P_2O_5 (1.4 g/1.0 g of hemihydrate); **16** was isolated in 48% yield as a light yellow liquid.

***n*-Butyl (*E*)-3-(2,4-Dioxo-6-methyl-5-pyrimidinyl)acrylate (17).** A solution of KO-*t*-Bu (2.4 g, 20 mmol) in DMF (40 mL) was added dropwise to a stirred and cooled (−30 °C) solution of **15** (4.8 g, 10 mmol) as well as **16** (2.6 g, 20 mmol) in DMF (60 mL) at such a rate that the temperature of the reaction mixture stayed at −30 °C. Stirring was continued for 5 h at −30 °C and then 24 h at room temperature, after which the excess base was neutralized to ca. pH 7 by addition of AcOH. Subsequently the solvent was removed under reduced pressure to leave a sticky residue. Chromatography on a Sephadex LH-20 column with MeOH/H2O (85/15 v/v) as eluent gave **17**: 10% yield (250 mg); mp 265 °C dec; ^1H NMR (Me_2SO-d_6) δ 1.2 (m, 7 H, $OCH_2C_3H_7$), 2.3 (s, 3 H, C(6)CH_3), 4.1 (t, 2 H, OCH_2), 6.7 and 7.3 (AB spectrum, 2 H, J = 16 Hz, (*E*)-HC=CH); IR (KBr) 1730, 1710, 1645, 1615, and 1590 cm^{-1}.

5-Formyl-6-methyluracil (19). To a stirred, hot (90–100 °C) solution of **13** (6.24 g, 40 mmol) in 150 mL of water was added potassium persulfate (10.8 g, 40 mmol) all at once. The resulting clear solution was allowed to cool slowly to 40 °C, after which a catalytic amount of silver nitrate (0.1 g, 0.6 mmol) was added. The reaction mixture was stirred at room temperature for 16 h; within 30 min of stirring the first crystalls of the aldehyde **19** appeared. The precipitate was collected and recrystallized from water to yield 4 g (63%) of **19** after drying in vacuo for 6 h at 80 °C. The compound was homogeneous on TLC (R_f 0.60; $MeOH/CH_2Cl_2$, 1/4 v/v) and gave the same spectroscopic data as reported by Wiley and MacKellar.[9b] Anal. Calcd for $C_6H_6N_2O_3 \cdot H_2O$: C, 41.86; H, 4.68; N, 16.27. Found: C, 41.62; H, 4.61; N, 16.03.

Ethyl (*E*)-3-(2,4-Dioxo-6-methyl-5-pyrimidinyl)acrylate (18). This compound was prepared by a slight modification of Wiley's procedure.[9b] (Carbethoxymethylene)triphenylphosphorane (**20**; 15.8 g, 45 mmol) was added to a stirred solution of the aldehyde **19** (7.0 g, 45 mmol) in 110 mL of dry DMF. Stirring was continued for 3 days at room temperature after which a solution of **20** (1.6 g, 4.5 mmol) in 11 mL of dry DMF was again added. Finally the solution was stirred at room temperature for 24 h. Then ca. 2 mL of glacial acetic acid was added, and the solution was evaporated to dryness in vacuo at 60 °C. The residue was dissolved in the minimal amount of boiling methanol. The solution was cooled and kept at 0 °C. The crystalls were collected and washed with cold methanol to give the ester **18**: 40% yield; mp 302–303 °C (lit.[9b] mp 299–302 °C); TLC R_f 0.70 ($MeOH/CH_2Cl_2$, 1/4 v/v). Anal. Calcd for $C_{10}H_{12}N_2O_4$: C, 53.57; H, 5.39; N, 12.49. Found: C, 53.34; H, 5.40; N, 12.47.

(*E*)-3-(2,4-Dioxo-6-methyl-5-pyrimidinyl)acrylic Acid (2). The ester **18** (6.5 g, 29 mmol) was dissolved in ca. 250 mL of methanol and 250 mL of dioxane. Upon addition of 30 mL of an aqueous 4 N NaOH solution a precipitate appeared; therefore, water was added until complete dissolution. The reaction mixture was stirred at 40 °C for 16 h. After completion of the reaction, which was monitored by TLC (eluent $MeOH/CH_2Cl_2$, 1/4 v/v), the pH of the mixture was adjusted to 1–2 with 4 N aqueous HCl. Subsequently, the volume was reduced to ca. 50 mL by evaporation of the solvent in vacuo at 40 °C. The precipitate was collected by filtration or centrifugation to give **2**: 90% yield (5.1 g); mp 270 °C dec (lit.[9b] mp 265 °C); TLC R_f 0.18 ($MeOH–CH_2Cl_2$, 1/4 v/v); spectroscopical data were essentially identical with those reported by Wiley.[9b] Anal. Calcd for $C_8H_8N_2O_4$: C, 48.98; H, 4.11; N, 14.28. Found: C, 48.93; H, 4.13; N, 14.03.

***S*-[(Methylthio)methyl]-L-cysteine (21).** To a stirred solution of L-cystine (24.0 g, 0.1 mol) in 500 mL of liquid ammonia (−33 °C) were added small pieces of sodium metal until the blue color persisted for a few minutes. Then freshly distilled chloromethyl methyl sulfide (19.3 g, 0.2 mol) was added dropwise. After this the ammonia was evaporated at room temperature and the residue dissolved in the minimal amount of water. The solution was adjusted to pH 5 with a 6 N aqueous solution of HCl. The formed precipitate of **21** was filtered off and washed with cold water, ethanol, and ether successively. Drying in vacuo afforded the cysteine derivative **21**: 70% yield (12.7 g); TLC R_f 0.44 (*n*-BuOH/HOAc/H_2O, 4/1/1 v/v); NMR (D_2O/LiOD) δ 2.25 (s, 3 H, SCH_3), 2.95 (m, 2 H, $CHCH_2$), 3.25–3.65 (m, 1 H, $CHCH_2$), 3.75 (s, 2 H, SCH_2S); mp 220 °C dec. Anal. Calcd for $C_5H_{11}NO_2S_2$: C, 33.13; H, 6.12; N, 7.73. Found: C, 32.75; H, 5.98; N, 7.81.

***S*-[(Methylthio)methyl]-L-cysteine Methyl Ester (22).** The hydrochloride of **22** was prepared from **21** by the well-known method of treatment with methanol and thionyl chloride. The product was obtained in 87% yield. Free amino ester **22** was prepared in situ by treatment with 1 equiv of triethylamine. Hydrochloride of **22**: TLC R_f 0.72 (*sec*-BuOH/NH_4OH, 55/22 v/v); NMR (Me_2SO-d_6) δ 2.1 (s, 3 H, SCH_3), 3.15 (d, 2 H, $CHCH_2$), 3.7 (s, 3 H, CO_2CH_3), 3.8 (s, 2 H, SCH_2S), 4.2 (t, 1 H, $CHCH_2$).

***N*-[(*tert*-Butyloxy)carbonyl]-*S*-[(methylthio)methyl]-L-cysteine Methyl Ester (23).** This compound was prepared in 84% yield from the hydrochloride of **22** as described for the synthesis of **50**: TLC R_f 0.85 ($MeOH/CH_2Cl_2$, 3/97 v/v); NMR δ 1.45 (s, 9 H, $C(CH_3)_3$), 2.15 (s, 3 H, SCH_3), 3.1 (m, 2 H, $CHCH_2$), 3.65 (s, 2 H, SCH_2S), 3.75 (s, 3 H, CO_2CH_3), 4.5 (br s, 1 H, $CHCH_2$), 5.35 (br d, 1 H, NH).

***N*-[(*tert*-Butyloxy)carbonyl]-*S*-[(methylthio)methyl]-L-cysteine (24).** This compound was prepared in 89% yield from **21** as described for the synthesis of **50**: TLC R_f 0.53 ($MeOH/CH_2Cl_2$, 15/85 v/v); NMR δ 1.45 (s, 9 H, $C(CH_3)_3$), 2.15 (s, 3 H, SCH_3), 3.00–3.15 (m, 2 H, $CHCH_2S$), 3.65 (s, 2 H, SCH_2S), 4.35–4.65 (m, 1 H, $CHCH_2S$), 5.35–5.65 (br, 1 H, NH), 9.80 (s, 1 H, CO_2H).

***N*-[(*tert*-Butyloxy)carbonyl]-*S*-[(methylthio)methyl]-L-cysteinol (25).** Compound **25** was prepared in 64% yield from **23** by reduction with lithium borohydride as described for the preparation of **53** and **54**: TLC R_f 0.56 ($MeOH/CH_2Cl_2$, 6/94 v/v); NMR δ 1.44 (s, 9 H, $C(CH_3)_3$), 2.18 (s, 3 H, SCH_3), 2.73–2.93 (m, 2 H, $CHCH_2S$), 3.67 (s, 2 H, SCH_2S), 3.69–3.89 (br s, 3 H, $CHCH_2OH$), 5.07 (br s, 1 H, NH). Anal. Calcd for $C_{10}H_{21}NO_3S_2$: C, 44.92; H, 7.92; N, 5.14. Found: C, 44.56; H, 7.87; N, 5.14.

***N*-[(Benzyloxy)carbonyl]-*S*-[(methylthio)methyl]-L-cysteinol (26).** Starting from the hydrochloride of **22**, we introduced the (benzyloxy)carbonyl group using its *N*-hydroxyphthalimide derivative.[56] The N-protected ester thus obtained was reduced in 56% yield as described for the preparation of **53** and **54**: TLC R_f 0.65 ($MeOH/CH_2Cl_2$, 1/9 v/v); NMR δ 2.13 (s, 3 H, SCH_3), 2.84 (d, 2 H, $CHCH_2S$), 3.64 (s, 2 H, SCH_2S), 3.76 (d, 2 H, $CHCH_2OH$), 3.87 (m, 1 H, $CHCH_2OH$), 5.11 (s, 2 H, $C_6H_5CH_2$), 5.35 (br d, 1 H, NH), 7.33 (s, 5 H, C_6H_5). Anal. Calcd for $C_{13}H_{19}NO_3S_2$: C, 51.80; H, 6.35; N, 4.65. Found: C, 51.67; H, 6.33; N, 4.65.

Compounds 27A–30A and 27B–30B. Oxidation of **23–26** was performed with 1 equiv of sodium metaperiodate by using the following procedure. To a stirred, cooled (0 °C) solution of the cysteine derivative **23**, **24**, **25**, or **26** (4 mmol) in 12 mL of acetonitrile was added dropwise a solution of sodium metaperiodate (856 mg, 4 mmol) in 12 mL of water. The reaction mixture was stirred at 4 °C until completion of the reaction, as was monitored by TLC (about 16 h). The precipitate consisting of sodium iodate was removed by filtration, and ethyl acetate was added. After separation of the organic layer the aqueous layer was extracted three times with ethyl acetate. The collected organic layers were washed with brine, dried (Na_2SO_4), and evaporated in vacuo. Relative yields of **A** and **B** compounds are based on the NMR spectra of the mixtures; determined were the ratios of integration of the $S(O)CH_2SCH_3$ signal (ca. δ 2.3) and the $SCH_2S(O)CH_3$ signal (ca. δ 2.7), each of which appeared as a singlet. The overall

(56) E. Th. M. Wolters, Thesis, Nymegen, 1973.

yields were determined after purification by HPLC.

***N*-[(Benzyloxy)carbonyl]-L-cysteine Methyl Ester Sulfinyl Chloride (32).** (*Z*)-L-Cystine methyl ester (**31**; 3.22 g, 6 mmol), prepared according to a procedure of Gustus,[57] and acetic anhydride (1.22 g, 12 mmol) were dissolved in 25 mL of ethanol-free dichloromethane. The solution was stirred and cooled at −10 °C. In a separate 100-mL flask, containing 25 mL of dry, ethanol-free dichloromethane cooled to −10 °C, was introduced dry gaseous chlorine until about 1.3–1.4 g of chlorine had been dissolved (the theoretically necessary amount of chlorine was 1.28 g, 18 mmol). This solution of chlorine was added dropwise to the former solution 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 stirred at room temperature for 1 h. Subsequently, dichloromethane and excess of chlorine were evaporated at room temperature at water-pump pressure and the acetyl chloride at oil-pump pressure. The sulfinyl chloride **32** thus isolated appeared as colorless oil, which eventually solidified: NMR δ 7.36 (s, 5 H, C_6H_5), 6.0 (br, 1 H, NH), 5.15 (s, 2 H, $C_6H_5CH_2$), 4.82 (m, 1 H, $HCCO_2CH_3$), 4.0 (m, 2 H, $CH_2S(O)Cl$), 3.8 (s, 3 H, CO_2CH_3); IR ($CHCl_3$) 1745, 1720, 1125 cm^{-1}.

***N*-[(Benzyloxy)carbonyl]-*S*-oxo-*S*-(chloromethyl)-L-cysteine Methyl Ester (33).** A solution of the sulfinyl chloride **32** (2.43 g, 8 mmol) in the minimal amount of dichloromethane or DME was added dropwise to a stirred, dried (over KOH pellets) solution of excess diazomethane in ether. During this the reaction mixture was kept at 0 °C. After completion of the addition of the sulfinyl chloride the reaction mixture was stirred for 1 h at room temperature. The excess of diazomethane was removed by adding a few drops of acetic acid, after which the solvent was removed in vacuo. The product thus obtained was used without purification for the next experiment. Purification could be achieved by column chromatography (eluent $MeOH/CH_2Cl_2$, 25/975 v/v) to yield **33** (80%): TLC R_f 0.38 ($MeOH/CH_2Cl_2$, 4/96 v/v); NMR δ 7.36 (s, 5 H, C_6H_5), 5.9 (br, 1 H, NH), 5.14 (s, 2 H, $C_6H_5CH_2$), 4.76 (m, 1 H, $HCCO_2CH_3$), 4.49 (AB spectrum, 2 H, $S(O)CH_2Cl$), 3.80 (s, 3 H, CO_2CH_3), 3.42 (AB part of ABX spectrum, 2 H, $CHCH_2S(O)$); IR (CH_2Cl_2) 1740, 1720, 1055 cm^{-1}; mass spectrum, *m/e* 335, 333 (M^+).

***N*-[(Benzyloxy)carbonyl]-*S*-oxo-*S*-methoxy-L-cysteine Methyl Ester (34).** If the above-mentioned reaction was carried out with an undried diazomethane solution, the sulfinate ester **34** was isolated (0.77 g, 30% yield) after column chromatography besides the α-chloro sulfoxide **33** (0.88 g, 35% yield). For **34**: TLC R_f 0.55 ($MeOH/CH_2Cl_2$, 4/96 v/v); NMR δ 7.36 (s, 5 H, C_6H_5), 5.85 (br, 1 H, NH), 5.14 (s, 2 H, $C_6H_5CH_2$), 4.76 (m, 1 H, $CHCO_2CH_3$), 3.76 (s, 3 H, $S(O)OCH_3$), 3.29 (AB part of ABX spectrum, 2 H, $CHCH_2S(O)$); IR (CH_2Cl_2) 1120 cm^{-1}; mass spectrum, *m/e* 315 (M^+).

***N*-[(Benzyloxy)carbonyl]-*S*-oxo-*S*-(bromomethyl)-L-cysteine Methyl Ester (35).** Lithium bromide (552 mg, 6.4 mmol), dried in vacuo at 110 °C, was dissolved in THF, freshly distilled from sodium hydride. To this solution a dried (KOH) solution of diazomethane (7.2 mmol) in ether was added. To the resulting stirred solution was added a solution of **32** (1.9 g, 6 mmol) in ethanol-free dichloromethane dropwise at 0 °C. After completion of the addition excess diazomethane was removed by a stream of argon, and subsequently water and dichloromethane were added. The aqueous layer was extracted twice with dichloromethane. The combined organic layers were dried (Na_2SO_4) and evaporated to dryness in vacuo at room temperature to yield the crude α-bromo sulfoxide **35** (1.87 g, 82%), which was used without further purification: TLC R_f 0.58 ($MeOH/CH_2Cl_2$, 1/9 v/v); NMR δ 7.38 (s, 5 H, C_6H_5), 5.9 (br, 1 H, NH), 5.16 (s, 2 H, $C_6H_5CH_2$), 4.76 (m, 1 H, $CHCO_2Me$), 4.36 (m, 2 H, $S(O)CH_2Br$), 3.83 (s, 3 H, CO_2CH_3), 3.4 (m, 2 H, $CHCH_2S(O)$); mass spectrum, *m/e* 377, 379 (M^+).

***N*-[(Benzyloxy)carbonyl]-*S*-oxo-*S*-(chloromethyl)-L-cysteinol (36 and 37).** To a stirred, cooled (−78 °C) solution of sodium borohydride (0.91 g, 24 mmol) as well as lithium iodide (3.21 g, 24 mmol) in 200 mL of dry DME was added the ester **33** (2.67 g, 8 mmol) all at once. The reaction mixture was allowed to warm up at room temperature and then was stirred for 2 h. After completion of the reaction, as was monitored by TLC ($MeOH/CH_2Cl_2$, 1/9 v/v), the solution was neutralized to pH 7 with an aqueous solution of 1 N HCl under ice cooling. After this, stirring was continued for 1 h at room temperature. Subsequent to evaporation of the DME in vacuo, water and dichloromethane were added. The aqueous layer was extracted three times with dichloromethane and twice with ethylacetate. The combined organic layers were dried (Na_2SO_4) and evaporated in vacuo. The residue was chromatographed on silica gel (eluent $MeOH/CH_2Cl_2$, 6/94 v/v) to give the alcohols **37** (807 mg, 33%) and **36** (513 mg, 21%). The yields are based on **31**. Both compounds were homogeneous on TLC (**37**, R_f 0.27; **36**, R_f 0.24; $MeOH/CH_2Cl_2$, 1/9 v/v).

For **36**: NMR δ 3.16 (d, 2 H, $CHCH_2S(O)$), 3.87 (d, 2 H, CH_2OH), 4.16 (m, 1 H, $CHCH_2OH$), 4.40 and 4.54 (AB spectrum, J = 11.1 Hz, 2 H, $S(O)CH_2Cl$), 5.11 (s, 2 H, $C_6H_5CH_2$), 5.67 (br d, 1 H, NH), 7.34 (s, 5 H, C_6H_5); IR (KBr) 3330, 1680, 1020 cm^{-1}; mass spectrum, *m/e* 305, 307 (M^+). Anal. Calcd for $C_{12}H_{16}ClNO_4S$: C, 47.14; H, 5.27; N, 4.58. Found: C, 47.49; H, 5.25; N, 4.40.

For **37**: NMR δ 3.09 and 3.31 (AB part of ABX spectrum, 8 lines, J_{AX} = 5.4 Hz, J_{BX} = 5.7 Hz, J_{AB} = 13.3 Hz, 2 H, $CHCH_2S(O)$), 3.78 (AB spectrum, J_{AB} = 12.5 Hz, 2 H, CH_2OH), 4.13 (m, 1 H, $CHCH_2OH$), 4.47 and 4.57 (AB spectrum, J_{AB} = 11.2 Hz, 2 H, $S(O)CH_2Cl$), 5.11 (s, 2 H, $C_6H_5CH_2$), 5.84 (br, d, 1 H, NH), 7.34 (s, 5 H, C_6H_5); IR (KBr) 3340, 1695, 1040 cm^{-1}; mass spectrum, *m/e* 305, 307 (M^+). Anal. Calcd for $C_{12}H_{16}ClNO_4S$: C, 47.14; H, 5.27; N, 4.58. Found: C, 46.48; H, 5.21; N, 4.43.

***N*-[(Benzyloxy)carbonyl]-*S*-oxo-*S*-(bromomethyl)-L-cysteinol (38 and 39).** Reduction of the ester **35** (1.4 g, 3.7 mmol) was carried out as described for the preparation of **36** and **37** to yield a crude mixture of **38** and **39** (1.12 g, 86%). Purification by column chromatography (eluent, $MeOH/CH_2Cl_2$, 7/93 v/v) gave the mixture of **38** and **39** in 63% yield (0.82 g); the two diastereomers could not be separated, in contrast to the corresponding chloro sulfoxides. **38** and **39**: TLC R_f 0.41 ($MeOH/CH_2Cl_2$, 1/9 v/v); NMR (CD_3OD) δ 7.36 (s, 5 H, C_6H_5), 5.09 (s, 2 H, $C_6H_5CH_2$), 4.6 (m, br, 2 H, $S(O)CH_2Br$) δ 4.07 (br m, 1 H, $CHCH_2OH$), 3.62 (d, 2 H, CH_2OH), 3.10 (m, 2 H, $CHCH_2S(O)$); mass spectrum *m/e* 349, 351 (M^+).

***N*-[(Benzyloxy)carbonyl]-*S*-oxo-*S*-(chloromethyl)-*O*-(tetrahydropyranyl)-L-cysteinol (40).** Dihydropyran (666 mg, 7.9 mmol) was added dropwise to a stirred solution of **36** (764 mg, 2.5 mmol) and *p*-toluenesulfonic acid monohydrate (38 mg, 0.2 mmol) in 17 mL of dioxane. After the mixture was stirred at room temperature for 90 min, the reaction was complete, as judged by TLC ($MeOH/CH_2Cl_2$, 6/94 v/v). The pH of the mixture was adjusted to about 8–9 by addition of a solution of ammonia in methanol after which the solvent was evaporated in vacuo. The residue was dissolved in dichloromethane, and this solution was washed with 20 mL of an aqueous saturated sodium bicarbonate solution. The organic layer was dried (Na_2SO_4) and evaporated in vacuo to dryness to yield **40** as a pale yellow semisolid in quantitation yield: TLC R_f 0.28 ($MeOH/CH_2Cl_2$, 4/96 v/v); NMR δ 1.6 (m, 6 H, $OCH_2(CH_2)_3$), 2.9–3.2 (br d, 2 H, $CHCH_2S(O)$), 3.2–4.1 (m, 5 H, OCH_2CH_2 and $CHCH_2O$), 4.3 (d, 2 H, $S(O)CH_2Cl$), 4.5 (br s, 1 H, OC(H)O), 5.0 (s, 2 H, $C_6H_5CH_2$), 5.3–6.1 (br, 1 H, NH), 7.2 (s, 5 H, C_6H_5).

***N*-[(Benzyloxy)carbonyl]-*S*-oxo-*S*-(chloromethyl)-*O*-(tetrahydropyranyl)-L-cysteinol (41).** O-Protection of **37** was carried out as described for the synthesis of **40** to yield **41** quantitatively: TLC R_f 0.28 ($MeOH/CH_2Cl_2$, 4/96 v/v); NMR δ 1.6 (m, 6 H, $OCH_2(CH_2)_3$), 2.7–3.8 (m, 2 H, $CHCH_2S(O)$, 3.8–4.2 (m, 5 H, OCH_2CH_2 and $CHCH_2O$), 4.5 (br s, 3 H, $S(O)CH_2Cl$, OC(H)O), 5.0 (s, 2 H, $C_6H_5CH_2$), 5.4–6.0 (br, 1 H, NH), 7.1 (s, 5 H, C_6H_5).

Sodium Methylmercaptide. Sodium methylmercaptide was prepared by treatment of dimethyl disulfide with sodium in liquid ammonia according to a published procedure.[38] Before each experiment the quality was assayed gravimetrically as follows. To a solution of 1 equiv of sodium methylmercaptide in the minimal amount of dry ethanol was added all at once a solution of 1.5 equiv of 1-fluoro-2,4-dinitrobenzene (FDNB) in the minimal amount of dry ethanol. Immediately the 1-(methylmercapto)-

(57) E. L. Gustus, *J. Org. Chem.*, **32**, 3425 (1967).

2,4-dinitrobenzene crystallized. Subsequently the mixture was refluxed for 30 min, after which the 1-(methylmercapto)-2,4-dinitrobenzene was allowed to crystallize at 4 °C for 16 h. Finally the bright yellow crystals were collected and weighted; mp 128 °C. Preferentially the reagent is stored in a desiccator over KOH in an argon atmosphere. Batches containing less than 95% sodium methylmercaptide were discarded.

***N*-[(Benzyloxy)carbonyl]-*S*-oxo-*S*-[(methylthio)methyl]-*O*-(tetrahydropyranyl)-L-cysteinol (42).** A solution of sodium methylmercaptide (693 mg, 9.9 mmol), the purity of which was checked as described above, in 100 mL of dry ethanol was added at once to a stirred solution of the α-chloro sulfoxide **40** (3.24 g, 8.3 mmol) in 100 mL of dry ethanol. Argon had been passed through both solutions for 15 min. The reaction mixture was stirred at 50 °C and monitored by TLC ($MeOH/CH_2Cl_2$, 1/9 v/v). The reaction took about 2.5–5 h, depending on the quality of the sodium methylmercaptide. If the reaction did not proceed to completeness, an additional quantity of sodium methylmercaptide (175 mg, 2.5 mmol) was added. After completion, the solvent was evaporated, and water and dichloromethane were added. The organic layer was washed with water to neutral pH and dried (Na_2SO_4). Removal of a slight turbidness, due to finely devided sodium chloride, could be achieved by stirring with Na_2SO_4 for 1 h. Filtration and removal of the solvent afforded quantitatively (3.33 g) the monoxodithioacetal **42**, which was homogeneous on TLC (R_f 0.22, $MeOH/CH_2Cl_2$, 6/94 v/v): NMR δ 4.6 (m, 6 H, $OCH_2(CH_2)_3$), 2.3 (s, 3 H, SCH_3), 2.8–3.3 (br t, 2 H, $CHCH_2$), 3.7 (br s, 2 H, $S(O)CH_2SCH_3$), 3.3–4.3 (m, 5 H, $CHCH_2O$, OCH_2CH_2), 4.5 (br s, 1 H, OC(H)O), 5.0 (s, 2 H, $C_6H_5CH_2$), 7.2 (s, 5 H, C_6H_5); mass spectrum, *m/e* 401 (M^+). Anal. Calcd for $C_{18}H_{27}NO_5S_2$: C, 53.84; H, 6.78; N, 3.49. Found: C, 53.70; H, 6.79; N, 3.68.

***N*-[(Benzyloxy)carbonyl]-*S*-oxo-*S*-[(methylthio)methyl]-*O*-(tetrahydropyranyl)-L-cysteinol (43).** This compound was prepared quantitatively from **41** (2.38 g, 6.1 mmol) as has been described for the preparation of **42**. Its R_f value was identical with and the ^1H NMR spectrum very similar to those of **42**: R_f 0.22 ($MeOH/CH_2Cl_2$, 6/94 v/v); NMR δ 1.6 (m, 6 H, $OCH_2(CH_2)_3$), 2.3 (s, 3 H, SCH_3), 2.8–4.3 (m, 7 H, $CHCH_2O$, $CHCH_2$, OCH_2CH_2), 3.7 (br d of AB spectrum, 2 H, $S(O)CH_2SCH_3$), 4.5 (br s, 1 H, OC(H)O), 5.0 (s, 2 H, $C_6H_5CH_2$), 7.2 (s, 5H, C_6H_5); mass spectrum, *m/e* 401 (M^+). Anal. Calcd for $C_{18}H_{27}NO_5S_2$: C, 53.84; H, 6.78; N, 3.49. Found: C, 53.40; H, 6.70; N, 3.58.

Methyl *N*-[(Benzyloxy)carbonyl]-2-aminoacrylate (44). A two-phase system consisting of a solution of sodium methyl mercaptide (54 mg, 0.75 mmol) and tetraethylammonium chloride (124 mg, 0.75 mmol) in the minimal amount of water and a solution of **36** or **37** (50 mg, 0.15 mmol) in the minimal amount of chloroform was stirred at room temperature for 4 h, after which the organic layer was separated and dried (Na_2SO_4). According to NMR and TLC (R_f 0.91; $MeOH/CH_2Cl_2$, 4/96 v/v) the α-chloro sulfonide was quantitatively converted to the dehydroamino acid derivative **44**: NMR δ 3.83 (s, 3 H, CO_2CH_3), 5.16 (s, 2 H, $C_6H_5CH_2$), 5.79 (d, 1 H, C=CH), 6.24 (s, 1 H, C=CH), 7.37 (s, 5 H, C_6H_5).

2-Oxo-4-[[[(methylthio)methyl]sulfoxo]methyl]oxazolidine (45). Sodium methylmercaptide (96 mg, 1.4 mmol) was added all at once to a stirred solution of **38** (210 mg, 0.6 mmol) in the minimal amount of acetonitrile/water (1/1 v/v). After the mixture was stirred for 24 h at 40 °C, dichloromethane was added, and the aqueous layer was extracted twice with dichloromethane. The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. After column chromatography (eluent $MeOH/CH_2Cl_2$, 7/93 v/v) benzyl alcohol was isolated in addition to the cyclic urethane derivative **45**: yield 38 mg (30%); NMR (CD_2Cl_2) δ 2.29 (s, 3 H, SCH_3), 3.04 (m, 2 H, $CH_2S(O)$), 3.82 (AB spectrum, 2 H, $S(O)CH_2SCH_3$), 4.51 (m, 2 H, $CH_2OC(O)$), 6.13 (br s, 1 H, NH); IR (CH_2Cl_2) 1755, 1060 cm^{-1}, mass spectrum, *m/e* 209 (M^+).

***S*-Oxo-*S*-[(methylthio)methyl]-*O*-(tetrahydropyranyl)-L-cysteinol (46).** The N,O-protected dithioacetal *S*-oxide **42** (321 mg, 0.8 mmol) was placed in a three-walled reaction vessel, which was cooled with chilled methanol (−33 °C). Ammonia was condensed until complete dissolution of the compound. After removal of the external cooling, a solution of sodium in ammonia was added dropwise to the refluxing ammonia solution by using a simplified version of a published procedure.[42] Once the blue color persisted for a few minutes, the source of sodium was removed. The solvent was evaporated subsequent to addition of a few crystals of ammonium chloride. The residue thus obtained was extracted twice with chloroform. Evaporation of the solvent gave a yellow oil, which was chromatographed. Elution with $MeOH/CH_2Cl_2$ (1/9 v/v) gave **46** in 10–38% yield. Subsequent elution with $MeOH/CH_2Cl_2$ (15/85 v/v) gave **48** in 20–30% yield. The product ratio varied from experiment to experiment. Both compounds were homogeneous on TLC.

For **46**: TLC R_f 0.29 ($MeOH/CH_2Cl_2$, 1/9 v/v); NMR δ 1.60 (m, 6 H, $OCH_2(CH_2)_3$), 2.33 (s, 3 H, SCH_3), 2.85–2.89 (AB part of ABX spectrum, 2 H, $CH_2S(O)$), 3.55 (m, 5 H, $CHCH_2O$, H_2NCH, OCH_2CH_2), 3.67 and 3.84 (AB spectrum, 2 H, J_{AB} = 13.5 Hz, $S(O)CH_2SCH_3$), 4.60 (br s, 1 H, OC(H)O); IR($HCCl_3$) 3400, 1120, 1020 cm^{-1}.

For **48**: TLC R_f 0.12 ($MeOH/CH_2Cl_2$, 1/9 v/v); NMR δ 1.62 (m, 6 H, $OCH_2(CH_2)_3$), 2.63 (s, 3 H, $S(O)CH_3$), 2.80 (m, 2 H, $CH_2S(O)$), 3.53 (m, 3 H, $CHCH_2O$), 3.74 (m, 2 H, OCH_2CH_2), 4.60 (br s, OC(H)O)); IR ($HCCl_3$) 3400, 1120, 1020 cm^{-1}.

***S*-Oxo-*S*-[(methylthio)methyl]-*O*-(tetrahydropyranyl)-L-cysteinol (47).** This compound was prepared from **43** as has been described for the preparation of **46**. The yields of **47** (10–38%) and of the byproduct **49** (20–30%) varied from experiment to experiment.

For **47**: TLC R_f 0.24 ($MeOH/CH_2Cl_2$, 1/9 v/v); NMR δ 1.60 (m, 6 H, $OCH_2(CH_2)_3$), 2.33 (s, 3 H, SCH_3), 2.60–3.27 (m, 2 H, $CH_2S(O)$), 3.47 (m, 3 H, $CHCH_2O$), 3.71 (m, 2 H, OCH_2CH_2), 3.67 and 3.89 (AB spectrum, 2 H, J_{AB} = 13.5 Hz, $S(O)CH_2SCH_3$), 4.58 (br s, 1 H, OC(H)O)); IR ($HCCl_3$) 3400, 1120, 1020 cm^{-1}.

For **49**: R_f 0.10 ($MeOH/CH_2Cl_2$, 1/9 v/v); NMR δ 1.62 (m, 6 H, $OCH_2(CH_2)_3$), 2.66 (s, 3 H, $S(O)CH_3$), 2.83 (d, 2 H, $CH_2S(O)$), 3.52 (m, 3 H, $CHCH_2O$), 3.78 (m, 2 H, OCH_2CH_2), 4.59 (br s, 1 H, OC(H)O); IR ($HCCl_3$) 3400, 1120, 1020 cm^{-1}.

***S*-Oxo-*S*-methyl-*O*-(tetrahydropyranyl)-L-cysteinol (48 and 49).** From *N*-[(benzyloxy)carbonyl]-*S*-methyl-L-cysteine methyl ester, oxidation to the sulfoxide was performed as follows. A solution of sodium metaperiodate (4.28 g, 20 mmol) in 35 mL of water was added dropwise to a stirred solution of *N*-[(benzyloxy)carbonyl]-*S*-methyl-L-cysteine methyl ester in 25 mL of acetonitrile. Subsequently the reaction mixture was stirred at room temperature for 48 h, after which the solvents were evaporated in vacuo. Dichloromethane was added and the precipitate consisting of sodium iodate was removed by filtration. The solvent was evaporated in vacuo, and the residue was crystallized from methanol ether to give *N*-[(benzyloxy)carbonyl]-*S*-oxo-*S*-methyl-L-cysteine methyl ester: 92% yield; NMR δ 2.62 (s, 3 H, $S(O)CH_3$), 3.26 (m, 2 H, $CHCH_2S(O)$), 3.75 (s, 3 H, CO_2CH_3), 4.63 (m, 1 H, $CHCH_2S(O)$), 5.13 (s, 2 H, $C_6H_5CH_2$), 6.30 (br d, 1 H, NH), 7.33 (s, 5 H, C_6H_5).

The thus prepared sulfoxide (567 mg, 2 mmol) was added to a stirred and cooled (0 °C) solution of sodium borohydride (113 mg, 3 mmol) and lithium iodide (400 mg, 3 mmol) in 15 mL of dry DME. The reaction mixture was stirred at room temperature. After completion of the reaction, as was shown by TLC ($MeOH/CH_2Cl_2$, 1/9 v/v), the solution was neutralized with an aqueous 2 N HCl solution under ice-cooling. Stirring was continued for 1 h at room temperature. The solution was concentrated in vacuo to a small volume and extracted twice with dichloromethane. The combined organic layers were dried (Na_2SO_4), and the solvent was removed to give the two diastereomeric *N*-[(benzyloxy)carbonyl]-*S*-oxo-*S*-methyl-L-cysteinols: 56% yield (288 mg); NMR δ 2.55 and 2.58 2 s, 3 H, $S(O)CH_3$), 2.83–3.13 (m, 2 H, $CHCH_2S(O)$), 3.53–3.83 (m, 3 H, CH_2OH), 3.83–4.33 (m, 1 H, $CHCH_2S(O)$), 5.0 (s, 2 H, $C_6H_5CH_2$), 5.97 (br d, 1 H, NH), 7.23 (s, 5 H, C_6H_5). The crude product was used as such in the reaction with dihydropyran, yielding quantitatively the diastereomers of the O-protected derivatives. This reaction was carried out as described for the preparation of **40**: NMR δ 1.6 (m, 6 H, $OCH_2(CH_2)_3$), 2.55 and 2.60 (2 s, 3 H, $S(O)CH_3$), 2.83–3.13 (m, 2 H, $CHCH_2S(O)$), 3.2–4.2 (m, 5 H, $CHCH_2O$, OCH_2CH_2), 4.5 (br s, 1 H, OC(H)O), 5.01 (s, 2 H, $C_6H_5CH_2$), 7.22 (s, 5 H, C_6H_5). Removal of the Z group was accomplished as described for the preparation of **46**. The two diastereomeric *S*-methyl sulfoxides thus prepared were separated on a silica gel column (MeOH/

CH_2Cl_2, 1/9 v/v). The stereomer having the highest R_f value on TLC (CH_2Cl_2/MeOH, 4/1 v/v) was found to be identical (TLC, ^1H NMR) with compound **48**, obtained in the Z removal reaction leading to **46**. The second stereomer was identical with **49**.

***N*-[(*tert*-Butyloxy)carbonyl]-L-cystine Methyl Ester (50).** Starting from L-cystine methyl ester hydrochloride, we prepared **50** with di-*tert*-butyl pyrocarbonate analogous to the method described[58] for unesterified amino acids. To a stirred and cooled (0 °C) solution of L-cystine methyl ester hydrochloride (3.41 g, 10 mmol) and an aqueous 1 N NaOH solution (20 mL, 20 mmol) in 30 mL of dioxane/water (2/1 v/v) was added di-*tert*-butyl pyrocarbonate (4.8 g, 22 mmol). Subsequently the cooling was removed and the reaction mixture stirred for 24 h at room temperature. After concentration of the reaction mixture to a small volume, ethyl acetate was added, and the solution was carefully acidified with an aqueous 2 N potassium hydrogen sulfate solution to pH 3. The organic layer was separated and the aqueous layer extracted twice with ethyl acetate. The collected organic layers were washed with water and with brine and dried (Na_2SO_4). Evaporation of the ethyl acetate in vacuo and recrystallization from ethyl acetate/hexane gave **50**, in 85% yield, which was homogeneous on TLC (R_f 0.40; MeOH/CH_2Cl_2, 3/97 v/v): mp 96–97 °C; NMR δ 1.45 (s, 9 H, *t*-Bu), 3.16 (d, 2 H, $CHCH_2S$), 3.78 (s, 3 H, CO_2CH_3), 4.60 (m, 1 H, $CHCH_2S$), 5.38 (br d, NH). Anal. Calcd for $C_{18}H_{32}N_2O_8S_2$: C, 46.14; H, 6.88; N, 5.98. Found: C, 46.39; H, 6.96; N, 5.93.

***N*-[(*tert*-Butyloxy)carbonyl]-*S*-oxo-*S*-chloro-L-cysteine Methyl Ester (51).** Compound **51** was prepared from **50** (7.96 g, 17 mmol) as described for the synthesis of **32**. The product obtained, a light yellow oil, was used without further purification for the preparation of **52**: NMR (CD_2Cl_2) δ 1.44 (s, 9 H, *t*-Bu), 3.81 (s, 3 H, CO_2CH_3), 3.96 (t, 2 H, $CHCH_2S(O)$), 4.75 (m, 1 H, $CHCH_2S(O)$).

***N*-[(*tert*-Butyloxy)carbonyl]-*S*-oxo-*S*-(chloromethyl)-L-cysteine Methyl Ester (52).** A solution of the sulfinyl chloride **51** (14.0 g, 34 mmol) in 200 mL of dichloromethane or THF was added dropwise over a period of 3 h to a cooled (0 °C) dried (KOH) solution of diazomethane (ca. 39 mmol) in ether. After the workup as described for the preparation of **33**, the oily substance thus obtained (R_f 0.27; MeOH/CH_2Cl_2, 3/97 v/v) was used without further purification for the preparation of **53** and **54**: NMR δ 1.45 (s, 9 H, *t*-Bu), 3.38 (AB part of ABX spectrum, 8 lines, 2 H, $CHCH_2S(O)$), 3.80 (s, 3 H, CO_2CH_3), 4.50 (AB spectrum, 2 H, $S(O)CH_2Cl$), 4.63 (m, 1 H, $CHCH_2S(O)$).

***N*-[(*tert*-Butyloxy)carbonyl]-*S*-oxo-*S*-(chloromethyl)-L-cysteinol (53 and 54).** The ester **52** (34 mmol) was reduced with lithium borohydride, which was prepared from sodium borohydride (3.86 g, 102 mmol) and lithium iodide (13.65 g, 102 mmol), as described for the preparation of **36** and **37**. The workup had to be changed: the pH was adjusted to 5 by addition of an aqueous 1 N citric acid solution to the stirred and cooled (0 °C) solution. Before neutralization was complete a sticky mass precipitated sometimes. In that case the solvent was evaporated in vacuo, after which the residue could be dissolved in methanol/water (1/1 v/v) and the neutralization could be completed. After extraction followed by drying and evaporation of the solvent, separation of the diastereomers was performed by column chromatography on silica gel with MeOH/CH_2Cl_2 (7/93 v/v) as the eluent to yield 17% (1.57 g) of **53** and 30% (2.77 g) of **54**. Both compounds were homogeneous on TLC (**53**, R_f 0.33; **54**, R_f 0.36; MeOH/CH_2Cl_2, 1/9 v/v).

For **53**: NMR δ 1.45 (s, 9 H, *t*-Bu), 2.91–3.42 (AB part of ABX spectrum, 2 H, $CHCH_2S(O)$), 3.64–3.93 (br t, 2 H, CH_2OH), 3.93–4.29 (m, 1 H, $CHCH_2OH$), 4.44 and 4.55 (AB spectrum, J_{AB} = 11.1 Hz, $S(O)CH_2Cl$), 5.24–5.58 (br d, 1 H, NH); IR (KBr) 1690, 1170, 1040, 670 cm^{-1}; mass spectrum, *m/e* 271, 273 (M^+); mp 139–140 °C (CH_2Cl_2–hexane). Anal. Calcd for $C_9H_{18}ClNO_4S$: C, 39.78; H, 6.68; N, 5.15. Found: C, 39.79; H, 6.38; N, 5.13.

For **54**: NMR δ 1.45 (s, 9 H, *t*-Bu), 3.11 and 3.41 (AB part of ABX spectrum, 8 lines, 2 H, J_{AB} = 13.5 Hz, $J_{AX} = J_{BX}$ = 5.6 Hz, $CHCH_2S(O)$), 3.60–3.93 (m, 2 H, CH_2OH), 3.93–4.33 (m, 1 H, $CHCH_2OH$), 4.54 and 4.61 (AB spectrum, J = 11.3 Hz, 2 H, $S(O)CH_2Cl$), 5.22–5.64 (br d, 1 H, NH); IR (KBr) 1700, 1170, 1040, 670 cm^{-1}; mass spectrum, *m/e* 271, 273 (M^+); mp 108–109 °C (CH_2Cl_2–hexane). Anal. Calcd for $C_9H_{18}ClNO_4S$: C, 39.78; H, 6.68; N, 5.15. Found: C, 39.79; H, 6.71; N, 4.94.

***N*-[(*tert*-Butyloxy)carbonyl]-*S*-oxo-*S*-[(methylthio)-methyl]-L-cysteinol (55).** The monoxodithioacetal **55** was prepared from the α-chloro sulfoxide **53** (1.086 g, 4 mmol) as has been described for the synthesis of **42**. The reaction was now carried out at room temperature and monitored by TLC (MeOH/$HCCl_3$, 1/9 v/v). In addition, the workup was changed: after 24 h, the reaction was complete, and the mixture was filtrated over Celite. The solvent was evaporated in vacuo after which the residue was dissolved in dichloromethane. Residual sodium chloride was removed by washing with ca. 5 mL of water. The organic layer was dried by being stirred for 2 h with Na_2SO_4. By doing this, residual NaCl could be filtered off together with the desiccant. Evaporation of the solvent gave **55** in 85% yield, which was homogeneous on TLC (R_f 0.34; MeOH/CH_2Cl_2, 1/9 v/v): NMR δ 1.45 (s, 9 H, *t*-Bu), 2.33 (s, 3 H, SCH_3), 3.03 and 3.28 (AB part of ABX spectrum, 8 lines, J_{AB} = 13.3 Hz, $J_{AX} = J_{BX}$ = 6.3 Hz, 2 H, $CHCH_2S(O)$), 3.73 and 3.83 (AB spectrum, J = 13.5 Hz, 2 H, $S(O)CH_2SCH_3$), 3.67–3.76 (m, covered by AB spectrum, 2 H, CH_2OH), 3.76–4.31 (m, 1 H, $CHCH_2OH$), 5.36 (br d, 1 H, NH); IR (KBr) 1690, 1250, 1170, 1030 cm^{-1}; mass spectrum, *m/e* 227 (M^+ – isobutene). Anal. Calcd for $C_{10}H_{21}NO_4S_2$: C, 42.38; H, 7.47; N, 4.94. Found: C, 42.56; H, 7.53; N, 5.22.

***N*-[(*tert*-Butyloxy)carbonyl]-*S*-oxo-*S*-[(methylthio)-methyl]-L-cysteinol (56).** The monoxodithioacetal **56** was prepared from **54** as has been described for the preparation of **55**. If the crude product was chromatographed over silica gel instead of being worked up as described for **55**, an epimerization of the sulfoxide sulfur atom was observed (see text). Compound **56** was obtained in 85% yield and was homogeneous on TLC (R_f 0.38; MeOH/CH_2Cl_2, 1/9 v/v): NMR δ 1.45 (s, 9 H, *t*-Bu), 2.33 (s, 3 H, SCH_3), 2.98 and 3.47 (AB part of ABX spectrum, 8 lines, J_{AB} = 13.5 Hz, J_{AX} = 3.8 Hz, J_{BX} = 5.8 Hz, 2 H, $CHCH_2S(O)$), 3.76 and 3.88 (AB spectrum, J = 13.8 Hz, 2 H, $S(O)CH_2SCH_3$), 3.65–3.95 (m, 2 H, covered by AB spectrum, CH_2OH), 5.44 (br d, 1 H, NH); IR (KBr) 1700, 1260, 1170, 1060 cm^{-1}; mass spectrum, exact mass calculated for $C_6H_{13}NO_4S_2$ ($M^+ - C_4H_8$) *m/e* 227.2096, found *m/e* 227.2118. Anal. Calcd for $C_{10}H_{21}NO_4S_2$: C, 42.38; H, 7.47; N, 4.94. Found: C, 42.38; H, 7.39; N, 4.88.

***S*-Oxo-*S*-[(methylthio)methyl]-L-cysteinol (57).** A solution of **55** (1.273 g, 4.5 mmol) in 50 mL of TFA was stirred at 0 °C for 30 min, after which the TFA was evaporated in vacuo at room temperature. The residue was dried in vacuo over KOH for 1 h and then dissolved in a minimal amount of water. The solution was placed on an ion-exchange column (Amberlite IRA-410, 20–50 mesh, $^-$OH form). Elution with water and removal of the solvent by freeze-drying gave **57** in a quantitative yield (823 mg). The product thus obtained was homogeneous on TLC (R_f 0.31; *sec*-BuOH/NH_4OH, 5/2 v/v): NMR ($CDCl_3/CD_2Cl_2$) δ 2.34 (s, 3 H, SCH_3), 2.88–3.00 (AB part of ABX spectrum, 2 H, $CHCH_2S(O)$), 3.33–3.71 (m, 3 H, $CHCH_2O$), 3.73 and 3.81 (AB spectrum, 2 H, J = 13.8 Hz, $S(O)CH_2SCH_3$). The enantiomeric purity of **57** was determined in $CDCl_3$ as follows: a racemic mixture, prepared by mixing **57** and **3** (vide intra), showed in the presence of tris-[3-[(trifluoromethyl)hydroxymethylene]-*d*-camphorato]ytterbium(III) two well-separated signals for the SCH_3 group. According to this method, **57** was found to be optically pure. Anal. Calcd for $C_5H_{13}NO_2S_2$: C, 32.76; H, 7.15; N, 7.64. Found: C, 32.52; H, 7.19; N, 7.63.

***S*-Oxo-*S*-[(methylthio)methyl]-L-cysteinol (58).** The amino alcohol **58** was prepared quantitatively from **56** (636 mg, 2.25 mmol) as has been described for the preparation of **57**. The product obtained was homogeneous on TLC (R_f 0.30; *sec*-BuOH/NH_4OH, 5/2 v/v). The enantiomeric purity was checked as reported for **57** and was found to be larger than 95%: NMR δ 2.34 (s, 3 H, SCH_3), 2.87 and 3.05 (AB part of ABX spectrum, 8 lines, J_{AB} = 13 Hz, J_{AX} = 5 Hz, J_{BX} = 6 Hz, 2 H, $CHCH_2S(O)$), 3.33–3.71 (m, 3 H, $CHCH_2O$), 3.72 and 3.86 (AB spectrum, 2 H, J = 13.5 Hz, $S(O)CH_2SCH_3$). Anal. Calcd for $C_5H_{13}NO_2S_2$: C, 32.76; H, 7.15; N, 7.64. Found: C, 32.79; H, 7.15; N, 7.52.

***S*-Oxo-*S*-[(methylthio)methyl]-D-cysteinol (3 and 60).** [(*tert*-Butyloxy)carbonyl]-D-cystine methyl ester (**59**) was prepared from D-cystine methyl ester hydrochloride as described for the synthesis of the enantiomeric compound **50**. Compound **59** (2.75

(58) L. Moroder, A. Hallet, E. Wünsch, O. Keller, and G. Wersin, *Hoppe-Seyler's Z. Physiol. Chem.*, **357**, 1651 (1976).

g, 8.1 mmol) was converted in five steps into the amino alcohols **3** and **60** as described for the synthesis of their enantiomers **57** and **58**, respectively. The overall yields were 14% and 26%, respectively, based on **59**. Both compounds were identical (TLC, ^{1}H NMR) with their antipodes and were enantiomerically homogeneous. Anal. Calcd for $C_5H_{13}NO_2S_2$: C, 32.76; H, 7.15; N, 7.64. Found for **3**: C, 32.31; H, 7.20; N, 7.53. Found for **60**: C, 32.48; H, 7.23; N, 7.48.

Methyl 2-[β-(6-Methyl-5-uracilyl)acrylamido]-3-[[(methylthio)methyl]sulfido]propionate (61). Triethylamine (0.53 mL, 3.8 mmol) was added to a solution of the hydrochloride of **22** (880 mg, 3.8 mmol) in 6 mL of dry DMF. The precipitated triethylamine hydrochloride was filtered off, and the filtrate was added to a stirred solution of the acrylic acid **2** (500 mg, 2.5 mmol) and hydroxybenztriazole (415 mg, 3.1 mmol) in 5 mL of dry DMF. Then DCC (525 mg, 2.5 mmol) was added all at once to the cooled (−15 °C) reaction mixture. Stirring was continued at room temperature for 16 h. Subsequently the reaction mixture was cooled to −10 °C and then filtered. The solvent of the filtrate was removed in vacuo at 50 °C, after which column chromatography (eluent MeOH/CH_2Cl_2, 4/96 v/v) of the residue gave the amide **61**: 60% yield; TLC R_f 0.64 (MeOH/CH_2Cl_2, 2/8 v/v); NMR (Me_2SO-d_6) δ 2.22 (s, 3 H, SCH_3), 2.44 (s, 3 H, C(6)CH_3), 3.15 (m, 2 H, $CHCH_2S$), 3.8 (s, 3 H, CO_2CH_3), 3.9 (s, 2 H, SCH_2S), 4.72 (m, 1 H, $CHCO_2CH_3$), 7.25 and 7.45 (AB spectrum, J = 16 Hz, 2 H, HC=CH), 8.7 (br d, 1 H, HN); IR (KBr) 1730, 1690, 1670, 1615 cm^{-1}; mp 180–182 °C (water).

***S*-Deoxo-(*R*)-sparsomycin (62).** The ester **61** (150 mg, 0.4 mmol) was reduced with lithium borohydride as described for the preparation of **36** and **37**. After removal of the solvent in vacuo, the product was purified by gel filtration over Sephadex LH-20 (eluent H_2O/MeOH, 15/85 v/v) to yield **62** (63%). The product was homogeneous on TLC (R_f 0.51, MeOH/CH_2Cl_2, 1/4 v/v): NMR (Me_2SO-d_6) δ 2.32 (s, 3 H, SCH_3), 2.5 (s, 3 H, C(6)CH_3), 3.0 (m, 2 H, $CHCH_2S$), 3.75 (m, 2 H, CH_2OH), 4.0 (s, 2 H, SCH_2S), 4.25 (m, 2 H, $CHCH_2OH$), 7.30 and 7.54 (AB spectrum, J = 16 Hz, 2 H, HC=CH), 8.25 (br d, 1 H, HNCH); IR (KBr) 1725, 1655, 1605 cm^{-1}; mp 221–222 °C. Anal. Calcd for $C_{13}H_{19}N_3O_4S_2$: C, 45.40; H, 5.45; N, 12.04. Found: C, 45.20; H, 5.54; N, 12.16.

Sparsomycin Enantiomer 65. From 46. The coupling procedure was analogous to the procedure which has been described for the preparation of **61**. A coupling of O-protected amino alcohol **46** (200 mg, 0.75 mmol) with the acid **2** (164 mg, 0.84 mmol) gave after workup and column chromatography (eluent MeOH/CH_2Cl_2, 8/92 v/v) the amide **63**: 45% yield TLC R_f 0.34 (MeOH/CH_2Cl_2, 1/9 v/v). A solution of this product (130 mg, 0.29 mmol) in 7 mL of ethanol to which was added 70 μL of an 0.1 N aqueous HCl solution was refluxed for 15 min. Then the solution was neutralized with ammonium hydrogen carbonate and the solvent evaporated in vacuo. Gel filtration over Sephadex LH-20 (eluent H_2O/MeOH, 15/85 v/v) gave **65**, in 75% yield, which was homogeneous on TLC (R_f 0.28; MeOH/CH_2Cl_2, 1/4 v/v): NMR (D_2O) δ 2.71 (s, 3 H, SCH_3), 2.82 (s, 3 H, C(6)CH_3), 3.62 (d, 2 H, $CHCH_2S(O)$), 4.18 (m, 2 H, $CHCH_2OH$), 4.36 and 4.52 (AB spectrum, J = 13.8 Hz, 2 H, $S(O)CH_2SCH_3$), 4.88 (m, 1 H, $CHCH_2OH$), 7.47 and 7.81 (AB spectrum, J = 15.6 Hz, 2 H, HC=CH); IR (KBr) 1715, 1660, 1600, 1015 cm^{-1}; UV (MeCN) λ_{max} 297 nm; $[\alpha]^{25}_D$ −60° (*c* 0.47, water). Anal. Calcd for $C_{13}H_{19}N_3O_5S_2$: C, 43.20; H, 5.30; N, 11.63. Found: C, 43.61; H, 5.20; N, 11.43.

From 57. To a stirred, cooled (0 °C) solution of the acid **2** (383 mg, 1.95 mmol) and triethylamine (218 mg, 2.15 mmol) in 25 mL of THF/DMF (1/1 v/v) was added ethyl chloroformate (212 mg, 1.95 mmol). Stirring was continued at 0 °C for 4 h. Subsequently, a solution of the amino alcohol **57** (275 mg, 1.5 mmol) in 25 mL of THF/DMF (1/1 v/v) was added dropwise. The reaction mixture was stirred at room temperature for 48 h, and then the solvents were removed in vacuo at room temperature. The product thus obtained was purified by gel filtration with Sephadex LH-20 (eluent H_2O/MeOH, 15/85 v/v) to yield **65** (48%).

Sparsomycin Diastereomer 66. From 47. The coupling procedure was analogous to the procedure used for the preparation of **61**. Reaction of the O-protected amino alcohol **47** (104 mg, 0.38 mmol) and the uracilylacrylic acid **2** (82 mg, 0.42 mmol) gave after workup and column chromatography (eluent MeOH/CH_2Cl_2, 8/92 v/v) **64**: 45% yield; TLC R_f 0.38 (MeOH/CH_2Cl_2, 1/9 v/v). Deprotection of the alcohol function and purification of the reaction mixture was done as has been described for the synthesis of **65**. The diastereomer **66** was obtained in 74% yield: TLC R_f 0.32 (MeOH/CH_2Cl_2, 1/4 v/v); NMR (D_2O) δ 2.71 (s, 3 H, SCH_3), 2.79 (s, 3 H, C(6)CH_3), 3.56 and 3.80 (AB part of ABX spectrum, 8 lines, J_{AB} = 14 Hz, J_{AX} = 4.6 Hz, J_{BX} = 7.2 Hz, 2 H, $CHCH_2S(O)$), 4.19 (d, 2 H, $CHCH_2OH$), 4.39 and 4.56 (AB spectrum, J = 14 Hz, 2 H, $S(O)CH_2S$), 4.87 (m, 1 H, $CHCH_2OH$), 7.44 and 7.82 (AB spectrum, J = 15.6 Hz, 2 H, HC=CH); IR (KBr) 1710, 1660, 1600, 1010 cm^{-1}; UV (MeCN) λ_{max} 298 nm; $[\alpha]^{25}_D$ −59° (*c* 0.59, water). Anal. Calcd for $C_{13}H_{19}N_3O_5S_2$: C, 43.20; H, 5.30; N, 11.63. Found: C, 43.57; H, 5.45; N, 11.17.

From 58. Diastereomer **66** was prepared in 35% yield from the amino alcohol **58** (357 mg, 1.95 mmol) and the uracilylacrylic acid **2** (392 mg, 2.0 mmol) as described for the preparation of **65**.

Sparsomycin (1). Sparsomycin was prepared in 33% yield from the amino alcohol **3** (46 mg, 0.25 mmol) and the uracilylacrylic acid **2** (64 mg, 0.325 mmol) as described for the preparation of **65**; $[\alpha]^{25}_D$ +75° (*c* 0.245, water) (lit.[1] $[\alpha]^{25}_D$ +69° (*c* 0.5, water)). The product thus obtained showed the same spectral characteristics (^{1}H NMR, IR, UV) as **65** except for the CD curve (see Figure 1), whereas it was identical with an authentic sample of sparsomycin.[46] Anal. Calcd for $C_{13}H_{19}N_3O_5S_2$: C, 43.20; H, 5.30; N, 11.63. Found: C, 43.51; H, 5.43; N, 11.27.

Sparsomycin Diastereomer 67. Compound **67** was prepared from the amino alcohol **60** (92 mg, 0.5 mmol) and the acid **2** in 40% yield as has been described for the preparation **65**. The product thus obtained was identical with **66**, except for the sign of the specific rotation and the CD curve (see Figure 1); $[\alpha]^{25}_D$ +48° (*c* 0.175, water). Anal. Calcd for $C_{13}H_{19}N_3O_5S_2$: C, 43.20; H, 5.30; N, 11.63. Found: C, 42.89; H, 5.36; N, 11.34.

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