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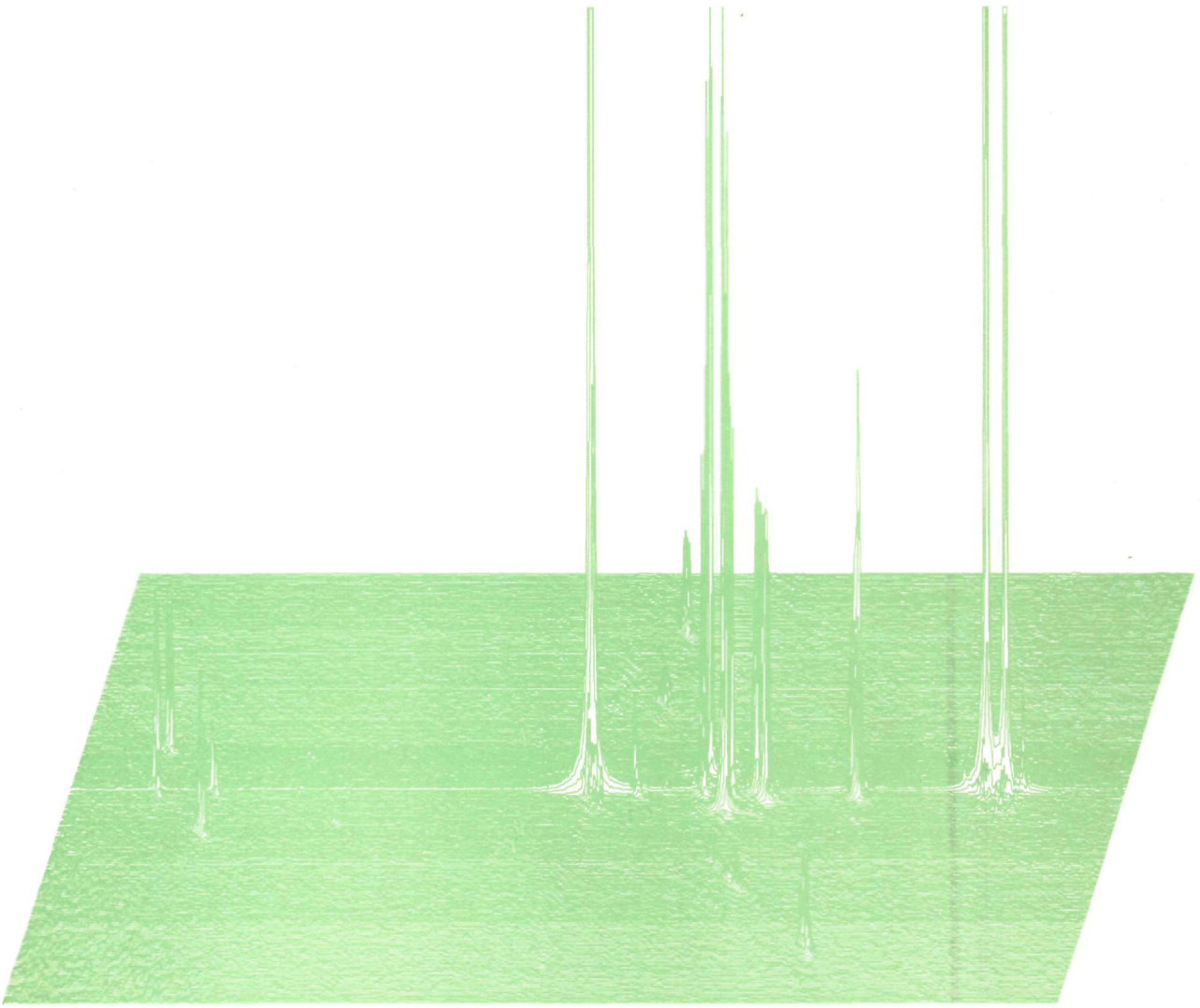

ROB M.J. LISKAMP

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
IN DE WISKUNDE EN NATUURWETENSCHAPPEN
AAN DE KATHOLIEKE UNIVERSITEIT VAN NIJMEGEN
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. DR. J.H.G.I. GIESBERS
VOLGENS HET BESLUIT VAN HET COLLEGE VAN DEKANEN
IN HET OPENBAAR TE VERDEDIGEN
OP VRIJDAG 10 DECEMBER 1982
DES NAMIDDAGS OM 4 UUR

DOOR

ROBERTUS MATTHIAS JOSEPH LISKAMP
GEBOREN TE NIJMEGEN

NIJMEGEN 1982

Dit proefschrift vormt de neerslag van vier jaar wetenschappelijk onderzoek. Ik wil iedereen - en dat waren er zeer velen - bedanken, die hieraan gedurende die tijd door hun stimulerende discussie of door de feitelijke uitvoering deel hadden. Zij gaven mede richting en vorm, niet alleen organisch maar ook biochemisch/biologisch en fysisch chemisch, aan het verrichte onderzoek. Daarnaast wil ik hen bedanken die een bijdrage geleverd hebben aan de onmisbare technische uitvoering van het in dit proefschrift beschreven onderzoek. En tenslotte wil ik ook hen bedanken die aan de vormgeving van het proefschrift hun medewerking hebben verleend.

Tekeningen : Wim van Luijn en de afdeling illustratie
Type-werk : Dorine Hesselink-Balvert
Afbeelding op de omslag: Een 'stacked plot' van een 2D-spin-echo correlated spectrum van synthetisch sparsomycine verzorgd door Wim Guijt

This research was supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

Het leven is een pijpkaneel.....

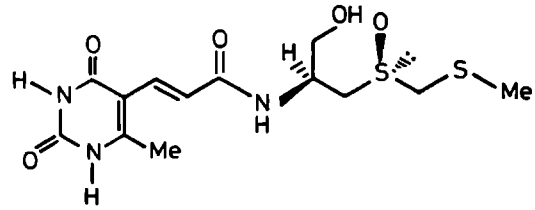
CONTENTS

CHAPTER I

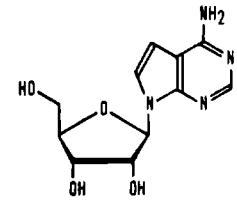
GENERAL INTRODUCTION

INTRODUCTION TO THE CHAPTERS

The isolation of sparsomycin 1, next to tubericidin (sparsomycin A) 2[1], from a *Streptomyces Sparsogenes* broth was reported by Argoudelis and Herr in 1962[2,3]. More recently this compound has also been obtained from *Streptomyces Cuspidosporus*[4,5]. After its isolation it was roughly characterized (UV, IR, elementary analysis, specific rotation and molecular weight) by Argoudelis and Herr[2].



1 Sparsomycin ($S_C - R_S$)



2 tubericidin

Subsequently a first evaluation of its biological activity was carried out by Owen, Dietz and Camiener[6]. In their experiments sparsomycin was found to be active against KB human epidermoid carcinoma cells in tissue culture. The compound was very active against these mammalian cells (KB cell protein synthesis was inhibited 50% (ID_{50}) at a concentration of 0.05 µg/ml), and moderately active against a variety of gram negative and gram positive bacteria as well as against fungi. *In vivo* data showed inhibition of growth of several tumors as assayed from tumor diameter measurements or from tumor weights. The toxicological screening of sparsomycin indicated an acute LD_{50} value of 2.4 mg/kg in mice. These data about the biological activity were probably the immediate cause to test sparsomycin in a phase I clinical study in 1964[7]. In this study with 5 patients who had all advanced carcinomas or sarcomas, the drug was daily administered i.v. for an intended period of 42 days. The dose given ranged from 0.085 mg/kg - 0.24 mg/kg. Two patients noted difficulty of vision, one after 13 days of treatment (total dose 12 mg) and one after 15 days of treatment (total dose 7.5 mg), whereafter treatment was stopped.

It was conceived, that the primary biological activity of sparsomycin was due to a strong inhibition of the protein synthesis rather than inhibition of DNA or RNA synthesis. Its effect on the protein synthesis was demonstrated by the decline of the protein synthesis of intact prokaryotic cells [4,6,8-12], eukaryotic cells[13-20] - including transformed[6,8,9,19,20] and/or virus infected cells[14,22]; and in various cell-free systems (prokaryotes[13,23-26,34], eukaryotes[27-41]).

The behavior of sparsomycin with regard to its inhibitory action and influence on the polyribosomes has also been investigated *in vivo*[42-47].

There is ample evidence[48,49] that sparsomycin inhibits the protein synthesis by interacting with the peptidyltransferase center of ribosomes, the devices of the cell, where the protein synthesis actually takes place. The peptidyltransferase center is responsible for the cruxial event in the elongation step of the protein synthesis in which the peptidyl chain of the peptidyl-tRNA is transferred to the aminoacyl-tRNA.

The pronounced inhibition of the protein synthesis may be - at least partly - responsible for several reported secondary actions of sparsomycin, such as the inhibition of the RNA-synthesis[13], the DNA-synthesis[16] and perhaps also for the reported ocular toxicity of sparsomycin[6,50]. Because sparsomycin is a selective and effective inhibitor of the protein synthesis, it has been used as a tool in a number of other biochemical studies[15,51-60].

Our interest in sparsomycin was roused by the discrepancy that existed between the wealth of information on the biological activity as well as the biochemical mechanism of action (*vide supra*) on one hand and the limited knowledge of the organic chemistry of the molecule on the other hand: when we started our research on sparsomycin there was no total synthesis of the molecule available.

To our opinion the development of a flexible synthesis or synthetic methodologies for a molecule possessing an interesting biological activity is an absolute prerequisite for thorough studies on the biological activity and/or biochemical mechanisms of interaction. This can also be concluded from the work on - especially - puromycin (Chapter V), on chloramphenicol and on lincomycin, which interfere with the peptidyltransferase center too.

As a consequence we started our investigations on sparsomycin, directing our first efforts towards a total synthesis of this molecule. A total synthesis was feasible since the structure of sparsomycin - excepting the chirality of the sulfoxide moiety - had been elucidated by Wiley and MacKellar[61] in 1970. Our purpose was to develop a flexible synthesis that would enable us to prepare a wide variety of analogs. It was planned to use these analogs in structure activity relationship studies (see Chapter V) to determine:

a the structural features and stereochemical requirements, essential for an optimal biological activity

b the molecular mechanism of action of sparsomycin and its analogs.

In addition, our aim was to study whether structural modifications in sparsomycin might yield a molecule with more selective biochemical and farmacological properties e.g. a molecule that will penetrate preferentially into a transformed cell.

Furthermore, our experiences with the syntheses of analogs of sparsomycin will be used to develop a synthesis of a sparsomycin analog, containing a radioactive label as well as an affinity label. The latter may give rise to covalent bond(s) with (a) molecule(s) of its site of interaction. Studies with this affinity analog of sparsomycin might be valuable for an further understanding of ribosome structure and function.

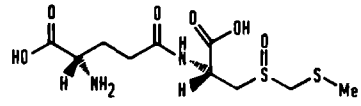
The guiding principle in structure-activity relation studies and in affinity label studies has to be the awareness of the existence of a close relation between the structure as well as the chemistry of a molecule and its biological activity. This implies a 'bio-organic chemical' attitude: hypotheses have to be formulated based on results obtained from studies on the biological activity of an effector molecule and on available information about the biochemical/biological structure of the effector's target (e.g. the ribosome). These hypotheses must lead to the design of molecular models.

Although the biological activity of sparsomycin was an important reason for directing our synthetic efforts toward this molecule, there were other reasons for embarkment on a total synthesis. First, the development of a total synthesis would allow the determination of the absolute configuration of the sulfoxide-sulfur atom of sparsomycin. Second, the development of a total synthesis enabled us to get an impression about the organic chemistry of several of sparsomycin's separate functionalities (sulfoxide function, mono-oxodithioacetal moiety, hydroxy function). In the course of a total synthesis the molecule is composed from building bricks, fragments of the molecule. Each fragment contains less functionalities than the molecule as a whole, thus facilitating study of the chemistry of a particular functionality.

This information may be useful for speculating on the metabolic stability of the different fragments of the molecule. Among others, this is of importance for the preparation of a radioactively labelled sparsomycin analog for *in vivo* experiments. Furthermore on basis of the chemistry of the various functionalities it will be possible to speculate about the molecular mechanism of the biological activity.

As was mentioned above, there was no total synthesis of sparsomycin available. However, there have appeared some publications on the synthesis of analogs of sparsomycin ever since[62-65] (see also Chapter V). These analogs have been used in structure activity relationship studies[66]. Our efforts resulted in the first total synthesis of sparsomycin[66], shortly thereafter followed by a total synthesis of Helquist and Shekhani[67]. Subsequently, we developed a second route[69] to sparsomycin which required the development of exploratory chemistry. A new synthesis of functionalized γ-sultines was developed. The sultine approach is an attractive alternative route to the synthesis of sparsomycin and might be the method of choice for the preparation of certain analogs. The preparation of the sultine synthon allowed us to study its virtually unexplored chemistry. We have shown that functionalized sultines lead to compounds, that are otherwise accessible with difficulty.

Finally, the part of the total synthesis dealing with the cysteinol mono-oxo-dithioacetal moiety, offers a starting point as well as a handle for a total synthesis of γ-glutamylmarasmin[70] 3, a secondary fungus metabolite too. To our knowledge γ-glutamylmarasmin and sparsomycin are the only natural products containing the intruiging mono-oxodithioacetal function.



3 γ-glutamyl marasmin

A brief outline of the contents of the thesis seems opposite here. In Chapter II the absolute configuration of the sulfoxide-sulfur atom of sparsomycin is assigned, which completes the earlier described[61] structure elucidation. Chapter III and IV deal with total syntheses of sparsomycin and analogs. The total synthesis which is outlined in Chapter III features the employment of an α-halogensulfoxide as a cruxial synthon, whereas the synthesis in Chapter IV features the employment of a functionalized sultine. Subsequently, in Chapter V the site of interaction of sparsomycin, the peptidyltransferase center is discussed, followed by a study of structure-activity relationships of sparsomycin and analogs as well as an evaluation of sparsomycin's antitumor activity. Chapter VI and VII consist of the first results of further studies on the chemistry of functionalized sultines. In Chapter VI a conformational analysis of functionalized sultines is presented; in Chapter VII the flash vacuum thermolysis of functionalized sultines is discussed. The contens of Chapter VIII consists of preliminary results of the synthesis of the marasmin fragment of γ-glutamylmarasmin.

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INTRODUCTION TO THE CHAPTERS

The elucidation of the structure of sparsomycin by Wiley and MacKellar on the basis of spectroscopic methods and chemical degradation studies did not include the chirality of the sulfoxide moiety.

In Chapter II we describe the determination of the absolute configuration of sparsomycin's sulfoxide-sulfur atom. The assignment is based on CD (Circular Dichroism) spectroscopic studies of synthetic precursors of the enantiomer of sparsomycin, and confirmation by an X-ray crystallographic study. The observation that the sign as well as the amplitude of the Cotton effect in the CD spectra are dependent upon the nature of the substituent, may be of value in determining the absolute configuration of other functionalized sulfoxides.

In Chapter III the first total synthesis of sparsomycin and its three stereoisomers, as well as the synthesis of S-deoxy-sparsomycin, is described. This synthesis is the definitive proof of the structure of sparsomycin. Starting compounds in our approach are the simple, commercially available compounds 6-methyluracil and D- or L-cystine.
In this study several problems inherent to the reactivity of the various functionalities of the molecule, were encountered. The main challenge in the synthesis of sparsomycin was the presence of the cysteinol mono-oxodithio-acetal fragment. It possesses several functionalities which require protection and deprotection at the right time and in the right way. In addition the lability of the mono-oxodithioacetal moiety limites the number of possible approaches.
In the synthesis of the other fragment, the β-(6-methyl-uracilyl)acrylic acid and its subsequent coupling with the amine fragment the poor solubility of the uracil derivatives had to be overcome.

Another approach to the synthesis of sparsomycin and analogs is presented in Chapter IV. This approach features an β-amino-γ-sultine as intermediate for the preparation of the cysteinol mono-oxodithioacetal fragment. This second method for the preparation of sparsomycin, via an amino sultine, is based on the development of a useful synthesis of functionalized sultines, the separation of diastereomers and a study of their virtually unexplored chemistry. This study includes ring opening reactions, which can be performed by cleavage of either the C-O or the S-O bond, and the stereochemistry of the latter reaction. Other aspects of the chemistry of functionalized sultines are mentioned in Chapters VI and VII.

Chapter V consists of four sections.
The first section is devoted to back-ground information on sparsomycin's receptor site i.e. the peptidyltransferase center. A good understanding of the protein synthesis as well as of the role of the peptidyltransferase center in this process is a prerequisite to get insight into the activity of sparsomycin on a molecular level. The main function of the peptidyl-transferase center, i.e. the actual attachement of subsequent amino acids to the growing peptide chain is explained. The peptidyltransferase activity *in vitro* can be assayed among others by the fragment reaction.
In section 2, preliminary results of a study on structure-activity relation-ships of sparsomycin and analogs employing this fragment reaction, are given. In section 2 these results are compared with those, obtained from testing the compounds against tumor cells (Leukemia L1210 cells) in an *in vitro* clonogenic assay. The latter results are described in section 3.
In section 4 the available data on the antineoplastic activity of sparsomycin are evaluated. In addition, present and future investigations on the anti-tumor activity of sparsomycin and analogs are discussed.

The preparation and separation of diastereomeric β-amino-γ-sultine deriva-tives have been described (Chapter IV). It is known that chiral sulfur compounds generally have a high inductory power. In initial experiments (Chapter IV) we noticed that chiral induction occurred in a ring opening reaction with a prochiral nucleophile.

Study of the conformations, which are present in solution, may give insight into the chiral induction process. For that reason, among others, we determined the conformations in solution of the sultines at issue, by 500 MHz ^{1}H-NMR spectroscopy and through use of a generalized Karplus equation as well as the pseudo-rotation concept. The results of this study are described in Chapter VI.
In addition, the solid state conformation of one isomer, as determined by X-ray crystallographic analysis, is discussed.

In Chapter VII the flash vacuum thermolysis (FVT) of β-amino-γ-sultines is described. We were intrigued by the possibility of preparing compounds by thermal extrusion of sulfur, which might be otherwise accessible with difficulty.
Furthermore we were interested in the flash vacuum thermolysis reaction of functionalized sultines as compared to the reaction of non-functionalized sultines. As a first experiment we carried out the flash thermolysis of 4-(benzamido)-γ-sultine. FVT of the compound led to a mixture of products, an allylamide being the main product. A mechanism that explains the formation of these products is proposed. Support for this proposal has been found by FVT of a deuterated sultine.

In Chapter VIII preliminary results of four synthetic approaches to the marasmin part of the γ-glutamylmarasmin are described. So far, γ-glutamylmarasmin and sparsomycin are the only natural products containing a mono-oxodithioacetal moiety related to cysteine. However, in γ-glutamylmarasmin the cysteine moiety possesses a carboxylic acid function, whereas sparsomycin has a methylene hydroxy group. We were interested whether we could apply the synthetic methodologies developed for the synthesis of the cysteinol mono-oxodithioacetal fragment of sparsomycin to the synthesis of marasmin. If so we might be able to prepare marasmin-sparsomycin, i.e. sparsomycin having a carboxylic acid function. In addition a synthesis of marasmin would enable us to elucidate the absolute configuration of the sulfinate sulfuratom. We have not yet completed the synthesis of marasmin; however, of the four approaches, the α-chlorosulfoxide approach seems promising. This approach originates from our total synthesis of sparsomycin (Chapter III), but cannot be simply applied to the synthesis of marasmin.

CHAPTER II

ABSOLUTE CONFIGURATION OF SPARSOMYCIN. A CHIROPTICAL STUDY OF SULFOXIDES.

Absolute Configuration of Sparsomycin. A Chiroptical Study of Sulfoxides[1]

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Abstract: Sparsomycin (**1**) is a naturally occurring compound possessing a wide range of biological activity, including antitumor and antibiotic activity. The R_C enantiomer **1*** and a diastereomer **2** have previously been synthesized. The absolute configuration of **1** was determined by CD spectroscopic studies of precursors of **1***. For several intermediates in the synthesis, the sign of the Cotton effect could be employed in the assignment of the configuration of the sulfoxide sulfur atom by extension of the principles established by Mislow and Snatzke. Sparsomycin was thus assigned the S_C,R_S configuration. The assignment was confirmed by single-crystal X-ray crystallographic studies of a precursor (**5**) of (R_C)-sparsomycin (**1***).

Sparsomycin (**1**), which was originally isolated as a metabolite of *Streptomyces sparsogenes*,[2] has attracted considerable interest because of its biological activity against various tumors,[3,4] bacteria,[4,5] fungi,[6] and viruses[7] and because of its use in studying protein biosynthesis,[8] a process which is inhibited by sparsomycin.[9] The structure (**1**), as first reported in 1970,[10] contains one chiral carbon atom which was shown to possess the *S* configuration and a chiral sulfur atom of the sulfoxide group for which the configuration was not determined. Structure–activity relationship studies have shown[11] that the activity of sparsomycin is dependent upon the configuration of the chiral carbon atom as well as on the presence of the sulfoxide function. However, the influence of the configuration of the sulfoxide sulfur atom has not been determined. Therefore we decided to establish the absolute configuration of sparsomycin's sulfoxide atom so that further work can proceed on studying its structure–activity relationships.

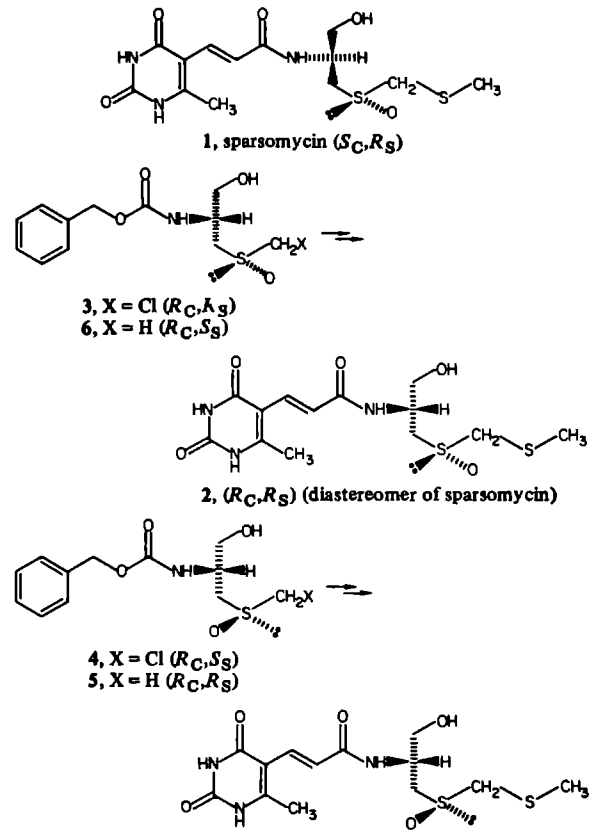
There is a large number of naturally occurring sulfoxides for which the configurations have been determined.[12] Particularly intriguing among these compounds are toxins obtained from poisonous mushrooms of the genus *Amanita*; whereas the compounds of one sulfoxide configuration are very lethal, the compounds of the opposite configuration are inactive up to rather high dose levels.[13]

Recently, we have reported two routes for the total synthesis of the enantiomer (**1***) and diastereomer (**2**) of sparsomycin. One approach is based[14] upon the conversion of the α-chloro sulfoxides **3** and **4** into a diastereomer (**2**) and the enantiomer (**1***), respectively, of sparsomycin. The other route involves[15] sulfenylation of the methyl sulfoxides **6** and **5** to also yield **2** and **1***, respectively. We now wish to report the determination of the absolute configuration of sparsomycin (**1**) by use of *chiroptical studies* and *single-crystal X-ray analysis*. We also wish to report our finding of a possible correlation between the sign of the Cotton effect exhibited by certain chiral sulfoxides involved in this work and the *R,S* designation of their configurations.

CD Spectra of Sulfoxides. Mislow et al. have shown that a correlation exists between the absolute configuration of methyl alkyl sulfoxides and their optical activity; in the absence of strongly perturbing groups, a negative Cotton effect, centered at the absorption band near 200 mμ in acetonitrile, correlates with the *R* configuration.[16,17] This rule was found to still be applicable when the alkyl group itself is also chiral but not strongly perturbing,[18] as is the case with *S*-methylcysteine *S*-oxide.[19]

We decided to study the Cotton effect of our sulfoxides through the use of CD spectra. In comparison with ORD spectra, CD spectra have the advantage of giving information about individual electronic transitions without background effects.[20] The CD

Scheme I



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(1) Part of this work was presented at the 178th National Meeting of the American Chemical Society, Washington, D.C., Sept 1979; American Chemical Society: Washington, D.C., 1979; ORGN 127, and at the meeting of the Netherlands Foundation for Chemical Research (SON), Lunteren, November 5, 1979.

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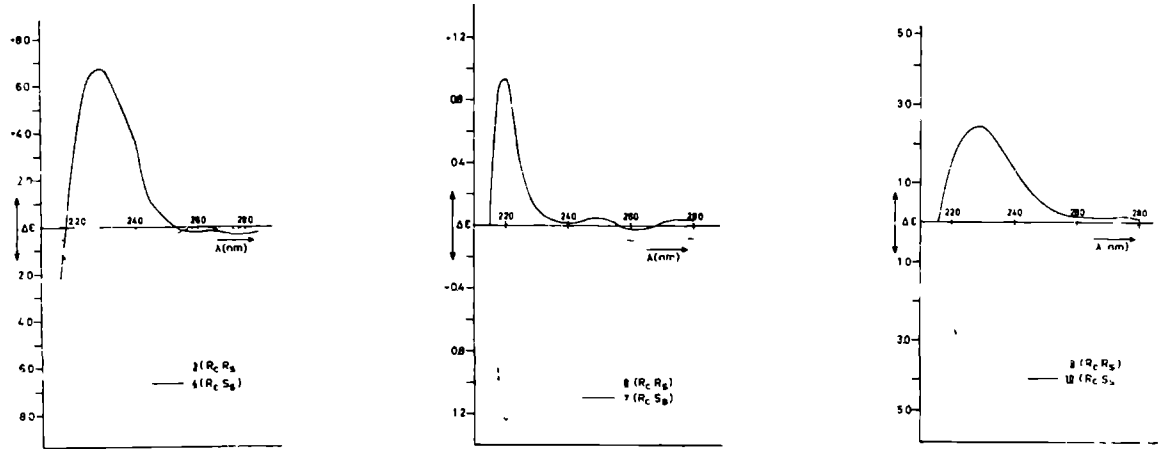
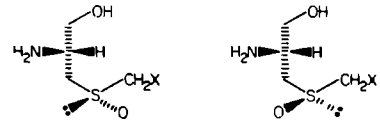


Figure 1. CD spectra of compounds **3**, **4**, and **7**–**10**

spectra of the previously mentioned precursors **3** and **4** of the sparsomycin system as well as of the intermediates **7**–**10** are shown



7, X = H (R_C,S_S) **8**, X = H (R_C,R_S)
9, X = SCH_3 (R_C,R_S) **10**, X = SCH_3 (R_C,S_S)

in Figure 1 The ^1H NMR spectra of the compounds **9** and **10**

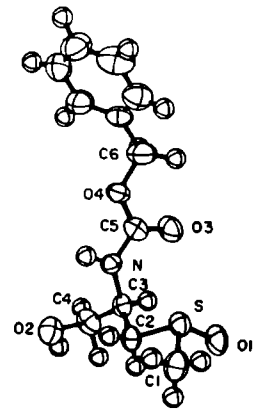


Figure 2. The molecular structure of **5** showing the R_C,R_S configuration

in the presence of a chiral shift reagent showed that the enantiomeric purity is greater than 95%

Of principal importance are the sulfoxides **4** and **5** First of all, these compounds display ABX patterns for the NC*H*C*H*$_2$S(O) segments in their ^1H NMR spectra[21] which are very similar to the corresponding portion of the spectrum of sparsomycin (**1**) but quite different from the corresponding portions of the spectra of compounds **3** and **6** Furthermore, compounds **4** and **5** can each be converted into the enantiomer (**1***) of sparsomycin[22] by use of our earlier synthetic sequences [14,15] Therefore, if the absolute configuration of **4** and a derivative of **5**, i e , **8**, were to be determined, the absolute configuration of **1***, and thus of **1**, would be revealed

By application of Mislow's rule,[16] the sulfur atom of **8**, a *methyl* alkyl sulfoxide, can be assigned the *R* configuration because of the negative sign of the Cotton effect observed for this compound (see Figure 1) From this measurement, we can conclude that the precursor of **8**, i e , **5**, also has the *R* configuration, and therefore **1***, derived from **5**, has the *S* configuration at the sulfoxide sulfur atom This change in nomenclature is due to the reversal in the priority assignments for the sulfur substituents in going from **8** or **5** to **1*** Consequently, the conclusion is reached that **1*** has the R_C,S_S configuration and that *sparsomycin* (*1*) *has the* S_C,R_S *configuration* as depicted in the structural drawing. This assignment was confirmed by a single-crystal X-ray structure

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(22) For the purpose of developing our initial routes to sparsomycin, we chose to prepare the R_C enantiomer which is derivable from commonly available L-cysteine Recently completed is the synthesis of **1**, having the natural configuration from D-cysteine and from L serine

determination of **5** (vide infra)

Intriguing is that the sign of the Cotton effect of the α-chloro sulfoxide **4** is the opposite of that of **8** Whereas the sulfur atoms of these two compounds have *geometrically analogous arrangements* of their substituents, they have *opposite R,S designations*, merely because of *reversed priority assignments* of their substituents So that this point could be pursued further, the CD spectra of compounds **3**, **7**, **9**, and **10** were also examined, note that in the compounds **7**–**10** the possibly perturbing benzyloxycarbonyl group is absent [23] From studying all of the spectra shown in Figure 1, the following conclusions may be reached.

(i) For the sulfoxides **3**, **4**, and **7**–**10** the sign of the Cotton effect centered at the absorption band in the region 220–230 mμ is not influenced by the configuration of the α-carbon atom [24]

(ii) The sign of the Cotton effect changes by introduction of a chloro or alkylmercapto group on the methyl group which leads to a change in the *R,S* assignment of the sulfoxide

The phenomenon of the correlation of the sign of the Cotton effect with configuration has been explained by Snatzke, at least for several other chromophores, through use of qualitative MO theory [25]

Without further examples of the effect of heteroatomic substituents in addition to the two types studied here, we would certainly be premature in stating that the effect that we have observed to date in a few limited cases will ultimately be found to be a general phenomenon for substituted sulfoxides Clearly, more complete investigations are necessary to determine whether simple correlations exist between configurations and Cotton effect of nonmethyl sulfoxides Further studies of this nature are continuing in our laboratories

X-ray Analysis. So that the assignment of configuration based upon CD spectra could be confirmed, a single-crystal X-ray structure determination of **5** was performed [26] This compound crystallizes from water as long, thin, orthorhombic needles in the form of a monohydrate with 2 molecules/asymmetric unit A crystal was mounted in a sealed capillary filled with the supernatant liquid from the recrystallization Mounting by more conventional techniques resulted in the rapid deterioration of the crystal, apparently due to loss of the water of hydration. The structure of one of the two unique molecules is shown in Figure 2 By reference to the chiral carbon atom of the *R* configuration, the chiral sulfur atom can readily be seen to possess the *R* configuration also [28]

Conclusion

We have determined the absolute configuration of sparsomycin (**1**) by a combination of chiroptical, X-ray crystallographic, and chemical techniques Further work may now be directed toward determining the relationship between the sulfoxide configuration and the biological activity of sparsomycin Also, the principles delineated in this paper may be applied to the structural investigation of other compounds such as γ-glutamyl-marasmine[29] that are related to sparsomycin

Experimental Section

Circular dichroism spectra were measured with a Dichrograph II apparatus (Roussel-Jouan, France) The concentrations varied between 7.0×10^{-4} mol L^{-1} and 5.9×10^{-3} mol L^{-1}, acetonitrile was used as solvent [30] For the ^1H NMR spectra a Bruker WH-90 was used with Me_4Si as an internal standard Thin-layer chromatography (TLC) was carried out with the use of Merck plates which were precoated with silica gel F-254 or silica gel 60 F-254 silanised, thickness 0.25 mm Spots were visualized with a UV hand lamp, iodine vapor, and, in the case of amines and amides, with ninhydrin TDM,[31] respectively

***N*-((Benzyloxy)carbonyl)-*S*-(chloromethyl)cysteinol *S*-Oxides 3 and 4 and *N*-((Benzyloxy)carbonyl)-*S*-methylcysteinol *S*-Oxides 5 and 6.** The syntheses of compounds **3**–**6** have been described before,[14a,15] detailed experimental descriptions are presented elsewhere [32,33]

***S*-Methylcysteinol *S*-Oxides 7 and 8 and *S*-((Methylthio)methyl)-cysteinol *S*-Oxides 9 and 10.** Protection of the alcohol function of **3** or **4** with the THP group and subsequent treatment with sodium methylmercaptide gave the N,O-protected derivatives of **9** and **10**, respectively, these conversions have been described previously [14a]

Removal of the N-Protecting Group. Ammonia was condensed until complete dissolution of the compound occurred After removal of the external cooling bath, sodium was added carefully to the refluxing ammonia solution[34] until the blue color persisted for a few minutes The solvent was evaporated subsequent to the 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 under slightly increased pressure (10 cmHg) on silica gel (Merck 60-H) When CH_2Cl_2/CH_3OH (v/v) was used as eluent in a ratio of 9:1 the O-protected derivatives of **9** or **10** were isolated in 10–38% yield Subsequent elution with CH_2Cl_2/CH_3OH (85:15, v/v) gave the O-protected derivatives of **7** or **8** in 20–30% yield The product ratios **7**:**9** and **8**:**10** varied from experiment to experiment All compounds were homogeneous on TLC (CH_2Cl_2/MeOH, 75:25, v/v ^1H NMR ($CDCl_3$) **7**-OTHP 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), **8** OTHP 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, 1 H, OC(*H*)O), **9**-OTHP 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 = 13.5 Hz, S(O)-CH_2S), 4.58 (br s, 1 H, OC(*H*)O), **10**-OTHP 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, CCH_2O, H_2NCH, OCH_2CH_2), 3.67 and 3.84 (AB spectrum, 2 H, J = 13.5 Hz, S(O)CH_2S), 4.60 (br s, 1 H, OC(*H*)O)

Removal of the O-Protecting Group. A solution of the O-protected dithioacetal-*S*-oxides **7**, **8**, **9**, or **10** in ethanol, the pH of which was adjusted at 3 with 0.1 N aqueous HCl, was refluxed The reaction, which took about 1.5 h, was monitored by TLC (silanised silica gel, eluent $CHCl_3$/MeOH saturated with NH_3, 9:1, v/v) When the reaction was complete, solid carbonate was added, and the resulting suspension was stirred overnight at room temperature Filtration and subsequent concentration to dryness gave a colorless oil which was extracted twice with acetonitrile Evaporation of the solvent gave the unprotected amino alcohol in quantitative yield All four compounds thus prepared were homogeneous on TLC (silanised silica gel, eluent as used for monitoring the reaction) The enantiomeric purity of **9** and **10** was determined by ^1H NMR spectroscopy in $CDCl_3$ A racemic mixture[22] of **9** showed in the presence of tris[3-((trifluoromethyl)hydroxymethylene)-D-campho-

(23) Amide and urethane bonds have an n,π^* transition at 220–250 m, so that the Cotton effects observed for **3** and **4** must be the result of two chromophores, one inherently chiral, the other inherently symmetric but chiral perturbed

(24) This conclusion is in accordance with the observations made with *S*-methylcysteine-*S*-oxide, see ref 19

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(26) Crystals of **5** belong to the orthorhombic space group $P2_12_12_1$ with a = 13.4448 (3) Å, b = 41.950 (6) Å, c = 5.102 (2) Å, and Z = 8 There are two $C_{12}H_{17}SNO_4 \cdot H_2O$ formula units per asymmetric unit Data were collected on an Enraf Nonius CAD4A diffractometer with use of Cu Kα X-radiation in the range $0 < 2\theta \leq 90°$ The structure was solved by using the MULTAN direct method programs and refined to values of 0.029 and 0.036 for the 343 variables and 1237 observations with $F_o > 3\sigma(F_o)$ The computing was carried out on a PDP 1145 computer with use of the Enraf-Nonius Structure Determination Package developed by Okaya and Frenz [27]

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(28) Supplementary material

(29) Gmelin, R, Luxa, H H, Roth, K, Höfle, G *Phytochem* **1976**, *15*, 1717–1721

(30) Although CD Spectra of amino-containing compounds are commonly obtained from solutions to which acid has been added, we were precluded from following this procedure because of the acid-sensitivity of our compounds, especially the alkylmercapto sulfoxides which, as a general class, are very well-known to undergo facile acid-catalyzed hydrolysis (a) Ogura, K, Tsuchihashi, G, *Tetrahedron Lett* **1971**, 3151–3154 (b) Richman, J E, Herrmann, J L, Schlessinger R H *Ibid* **1973**, 3267–3270 (c) Schill, G, Jones, P R *Synthesis* **1974**, 117–118 If we had been able to use acidic conditions, a complication may have been the quite different basicities of the amines **7**–**10** compared to those of the carbamates **3** and **4** Furthermore, we have already commented on the apparent absence of effects arising from the configuration of the α-carbon atom and, to a limited extent, changes in the nature of substituents about this position

(31) Arx, E von, Faupel, M, Brugger, M *J Chromatogr* **1976**, *120*, 224–228

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rato]ytterbium(III) two well-separated signals for the SCH_3 group The more downfield shifted signal could be assigned to the R_C,S_S enantiomer, the other one to the S_C,S_S compound The same phenomenon was observed with a racemic mixture of **10** According to this method, compounds **9** and **10** were found to be optically pure With the methyl sulfoxides **7** and **8**, no chemical shift difference could be observed in the presence of the shift reagent used or with the Pr or Eu analogues ^{1}H NMR ($CDCl_3/CD_2Cl_2$) **7** δ 2 64 (s, 3 H, S(O)CH_3), 2 84 (d, 2 H, CH_2S(O)), 3 30–3 71 (m, 3 H, CHCH_2O), **8** δ 2 63 (s, 3 H, S(O)CH_3) 2 55–3 02 (m, 2 H, CH_2S(O)), 3 30–3 80 (m, 3 H, CHCH_2O), **9** δ 2 33 (s, 3 H, SCH_3), 2 87 and 3 05 (8 lines, AB part of ABX spectrum, 2 H, J_{AB} = 13 Hz, J_{AX} = 5 Hz, J_{BX} = 6 Hz, 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_2S), **10** δ 2 34 (s, 3H, SCH_3), 2 80–3 00 (AB part of ABX spectrum, 2H, CHCH_2S(O)), 3 33–3 71 (m, 3H, CHCH_2O), 3 73 and 3 81 (AB spectrum, 2H, J = 13 8 Hz, S(O)CH_2S)

Compounds 7 and 8 from 6 and 5, Respectively. The N-protected alcohol **5** or **6**[15,33] were treated with sodium in liquid ammonia as described for the preparation of the O-protected derivatives of **7–10** When the reaction was complete and no sodium consumed anymore, slightly more than 1 equiv of ammonium chloride was added, after which the solvent was evaporated The residue was extracted twice with acetonitrile, and subsequently the solvent was evaporated The amino alcohols **8** and **7**, both obtained in 80% yield, were identical (TLC, ^{1}H NMR) with those obtained above

Acknowledgment. We are grateful to the National Institutes of Health, DHEW, for the financial support of this research (Grant No CA20701) Part of the investigations was supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organisation for the Advancement of Pure Research (ZWO) We thank Mr W van Berkel (Department of Biochemistry, Agricultural University of Wageningen) for his helpful assistance in the recording of the CD spectra

Supplementary Material Available: Tables of crystal data, scattering factors, bond distances and bond angles, positional and thermal parameters, and calculated and observed structure factor amplitudes (17 pages) Ordering information is given on any current masthead page

CHAPTER III

TOTAL SYNTHESIS OF THE ANTIBIOTIC SPARSOMYCIN, A MODIFIED URACIL AMINO ACID MONO-OXODITHIOACETAL.

J Org Chem 1981, *46*, 3273-3283

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

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Received October 17, 1980

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

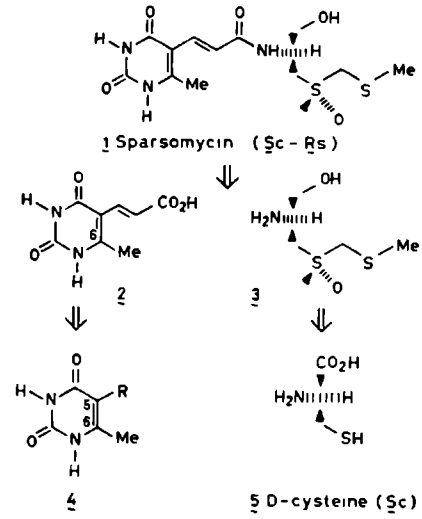
Sparsomycin (1), a metabolite of *Streptomyces sparsogenes*[1] or *Streptomyces cuspidosporus*,[2] has attracted much attention because of its activity against various tumors,[3,4] bacteria[2-4], fungi,[5] and viruses[6] and for studies[7,8] on inhibiting protein biosynthesis. On the basis of spectroscopic 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]

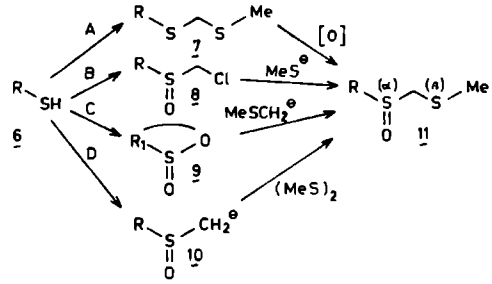
The synthesis of (*S*)-deoxosparsomycin by us[13] and others[14,15] had substantiated structure 1; however, no total synthesis of this antibiotic was reported until recently when Helquist[16] and we[17,18] each described in preliminary reports different routes to the R_C enantiomer of sparsomycin. In 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*, 780 (1962)
(2) E. Higashide, T Hasegawa, M Shibita, K Mizuno, and H Akaike, *Takeda Kenkyusho Nempo*, **25**, 1 (1966), *Chem Abstr*, **66**, 54328 (1967).
(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)

Scheme III

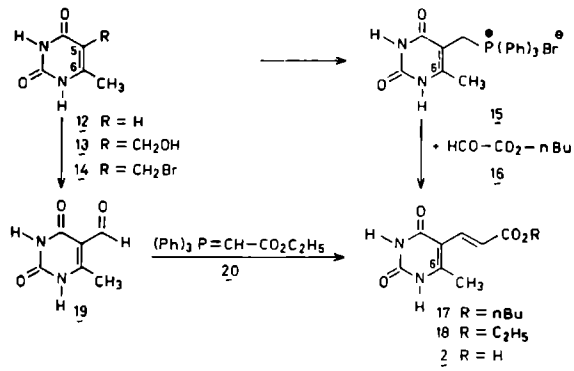


Chart I

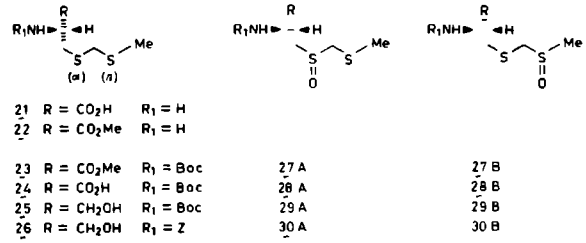


Table I. Conversion of 23–26 into 27A–30A and 27B–30B

	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 ^{1}H 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.

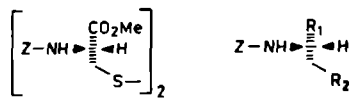
Two fundamentally different approaches are reported here. Initially we studied the regioselective oxidation of a dithioacetal (7) derived from cysteine 6 (Scheme II, route A). Our second approach (route B) employed the reaction of an α-chloro sulfoxide derivative of cysteine (8) with sodium methylmercaptide. 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]

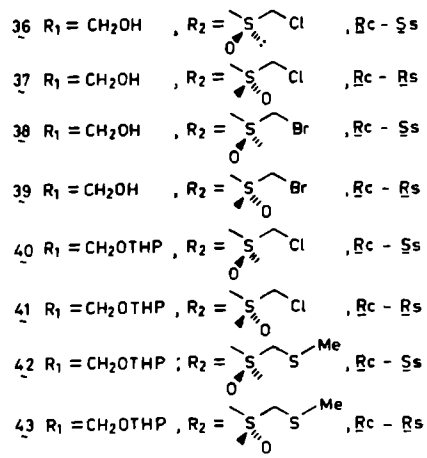
(5) S P Owen, A Dietz, and G W Camiiner, *Antimicrob Agents Chemother*, 772 (1962)
(6) L Thiry, *J Gen Virol*, 2 (part 1), 143 (1968)
(7) A E Smith and D T Wigle, *Eur J Biochem*, 35, 566 (1973)
(8) I H Goldberg, *Cancer Chemoth Rep, Part 1* 58, 479 (1974), D Vasquez, *FEBS Lett*, 40 (Suppl), S63 (1974)
(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, 5855d (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, 9575h (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

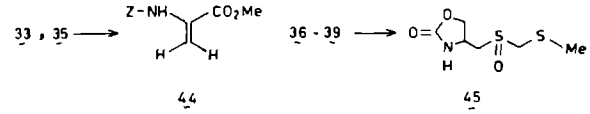
Coupling of **19** with **20** gave **18** (41% yield); the overall yield by this route (**12** → **19** → **18**) is 23%.

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.

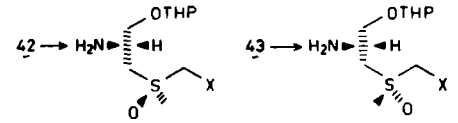
Route A. L-Cystine was reduced with sodium in liquid NH_3, treated with chloromethyl methyl sulfide[28] and acidified to give **21** (Chart I) in 61% yield. The amino acid ester **22** was prepared in 87% yield by treatment with CH_3OH and $SOCl_2$, followed by deprotonation with $(C_2H_5)_3N$. Compound **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 Iriuchijima 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.

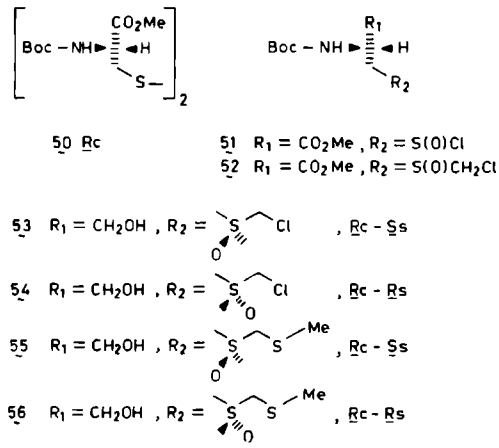
Direct conversion of **36** **39** into the desired corresponding monoxodithioacetals **42** and **43** failed treatment with CH_3SNa in C_2H_5OH gave the cyclic urethane **45** (Scheme IV) as the main product (30% yield). To circumvent this cyclization, we protected the alcohol function of **36** or **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



50 Rc

51 $R_1 = CO_2Me$, $R_2 = S(O)Cl$

52 $R_1 = CO_2Me$, $R_2 = S(O)CH_2Cl$

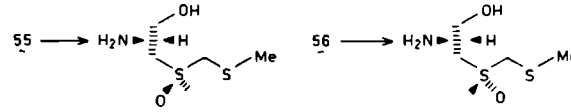
53 $R_1 = CH_2OH$, R_2 = , Rc - Ss

54 $R_1 = CH_2OH$, R_2 = , Rc - Rs

55 $R_1 = CH_2OH$, R_2 = , Rc - Ss

56 $R_1 = CH_2OH$, R_2 = , Rc - Rs

Scheme VI



57, Rc-Ss **58** Rc-Rs

$RS(O)CH_2SCH_3$ 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)

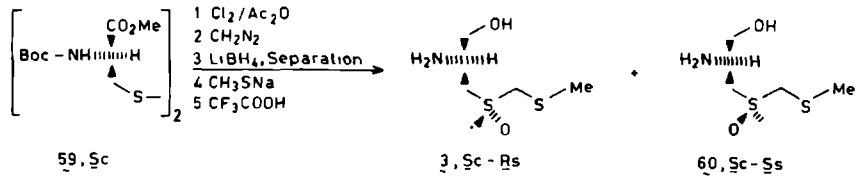
(42) The procedure of H Nesvadba and H Roth, *Monatsh Chem*, **98**, 1432 (1967), was applied with a simplified apparatus

(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).

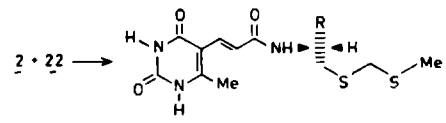
(45) Whereas the HCl-catalyzed racemization of sulfoxides is well established, we are not aware of a precedent for this reaction E Ciuffarin, et al, *J Chem Res, Synop*, 270 (1978)

Scheme VII



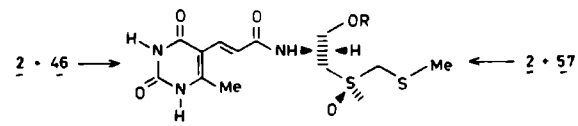
59, Sc 3, Sc - Rs 60, Sc - Ss

Scheme VIII

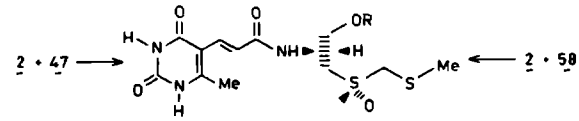


61 R = CO_2Me
62 R = CH_2OH

Scheme IX

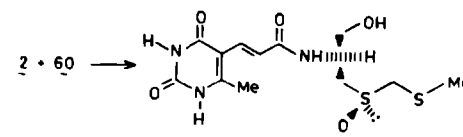


63 : R = THP
65 : R = H, Rc - Ss



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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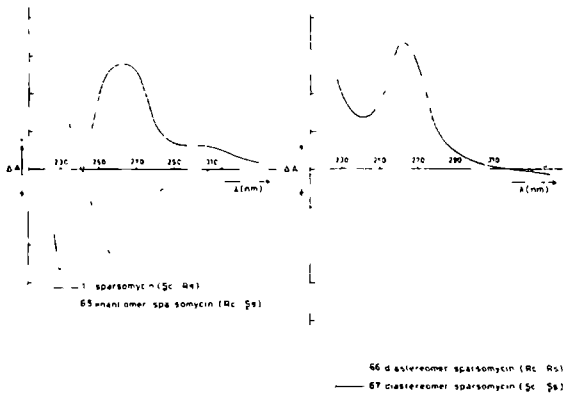

Figure 1. CD spectra of compounds **1** and **65–67** in acetonitrile.

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 Kofler 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

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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 quantitative 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)-

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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 sulfoxide 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 Wunsch, 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 $C_{13}H_{18}N_3O_4S_2$: 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 2.30 (s, 3 H, SCH_3), 2.41 (s, 3 H, C(6)CH_3), 3.21 (d, 2 H, $CHCH_2S(O)$), 3.77 (m, 2 H, $CHCH_2OH$), 3.95 and 4.11 (AB spectrum, J = 13.8 Hz, 2 H, $S(O)CH_2SCH_3$), 4.47 (m, 1 H, $CHCH_2OH$), 7.06 and 7.40 (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 2.30 (s, 3 H, SCH_3), 2.38 (s, 3 H, C(6)CH_3), 3.15 and 3.39 (AB part of ABX spectrum, 8 lines, J_{AB} = 14 Hz, J_{AX} = 4.6 Hz, J_{BX} = 7.2 Hz, 2 H, $CHCH_2S(O)$), 3.78 (d, 2 H, $CHCH_2OH$), 3.98 and 4.15 (AB spectrum, J = 14 Hz, 2 H, $S(O)CH_2S$), 4.46 (m, 1 H, $CHCH_2OH$), 7.03 and 7.41 (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.

Acknowledgment. We thank Professor P. Helquist for sharing his results with us prior to publication and Dr. T. F. Spande for reading and critisizing the manuscript. Part of the investigations was supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

Registry No. **1**, 1404-64-4; **2**, 28277-67-0; **3**, 77826-38-1; **12**, 626-48-2; **13**, 147-61-5; **14**, 60484-35-7; **15**, 60484-36-8; **16**, 6295-06-3; **17**, 60484-39-1; **18**, 28277-68-1; **19**, 24048-74-6; **20**, 1099-45-2; **21**, 56010-93-6; **22**, 77826-39-2; **22**·HCl, 56010-94-7; **23**, 77826-40-5; **24**, 77826-41-6; **25**, 77826-42-7; **26**, 77826-43-8; **27A**, 77826-44-9; **27B**, 77826-45-0; **28A**, 77826-46-1; **28B**, 77826-47-2; **29A**, 77826-48-3; **29B**, 77826-49-4; **30A**, 77880-66-1; **30B**, 77826-50-7; **31**, 3693-95-6; **32**, 68836-00-0; **33**, 68835-92-7; **34**, 77826-51-8; **35**, 77826-52-9; **36**, 77398-52-8; **37**, 72843-11-9; **38**, 77826-53-0; **39**, 77826-54-1; **40**, 77880-67-2; **41**, 77880-68-3; **42**, 77880-69-4; **43**, 77880-70-7; **44**, 21149-17-7; **45**, 68835-95-0; **46**, 69977-63-5; **47**, 70005-50-4; **48**, 69977-62-4; **49**, 70005-49-1; **50**, 77826-55-2; **51**, 77826-56-3; **52**, 77826-57-4; **53**, 77826-58-5; **54**, 77826-59-6; **55**, 77880-71-8; **56**, 77880-72-9; **57**, 77330-21-3; **58**, 77330-20-2; **59**, 77826-60-9; **60**, 77826-61-0; **61**, 77826-62-1; **62**, 60484-34-6; **63**, 77880-73-0; **64**, 77880-74-1; **65**, 77880-75-2; **66**, 77880-76-3; **67**, 77880-77-4; sodium dimethoxyacetate, 77826-63-2; *n*-butyl dimethoxyacetate, 60484-37-9; di-*n*-butyl tartrate, 87-92-3; L-cystine, 56-89-3; sodium methylmercaptide, 5188-07-8.

CHAPTER IV

SYNTHESIS AND RING-OPENING REACTIONS OF FUNCTIONALIZED SULTINES. A NEW APPROACH TO SPARSOMYCIN.

Synthesis and Ring-Opening Reactions of Functionalized Sultines. A New Approach to Sparsomycin

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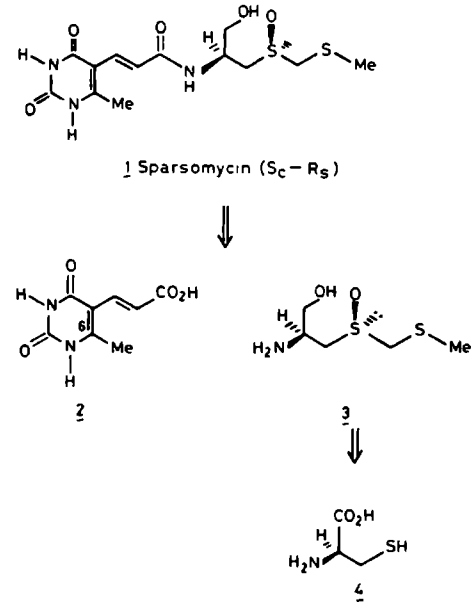
Received July 31, 1981

An efficient route leading to the functionalized five-membered cyclic sulfinate esters (γ-sultines) **14a,b** and **21a,b** is described on the basis of the reaction of the N-protected cystinol derivatives **11** and **20**, respectively, with *N*-chlorosuccinimide (NCS) and AcOH. Ring-opening reactions of the sultines can be performed in two ways. Treatment of **14** with Cl_2 or NCS/HCl gives the sulfonyl chloride **15** by cleavage of the C–O bond. Selective cleavage of the S–OR bond occurs when **14** or **21** is treated with $MeSCH_2Li$, *n*-BuLi, or $C_6H_5C(Li)(H)CN$ to yield the hydroxyalkyl sulfoxides **22–26**. These nucleophilic ring-opening reactions are stereospecific and proceed with inversion at the sulfoxide sulfur atom. The synthesis of **23a** constitutes a new approach to the antibiotic sparsomycin (**1**). Circular dichroism measurements can be employed in the assignment of absolute configurations to the sulfoxide sulfur atom of the sultines **14a,b** and **21a,b** (patents pending).

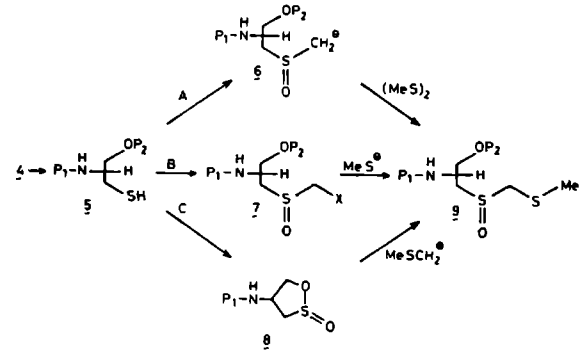
The antibiotic sparsomycin (**1**)[1] may be considered as an amide derived from the acid component **2** and the amine component **3** (Scheme I). The latter can be viewed as a derivative of D-cysteine (**4**), having its CO_2H function reduced and its SH function alkylated and oxidized. Recently, in the course of total syntheses of **1**, two approaches were developed to prepare protected derivatives of **3**, as represented by formula **9** (Scheme II). Helquist[2] applied successfully the sulfenylation of the α-sulfinyl carbanion **6**, prepared, in turn, from D-cysteine **4** (route A), while we[3,4] employed the reaction of the cysteinol α-halo sulfoxide derivative **7** with sodium methylmercaptide (route B).

An attractive alternative to these routes might feature the nucleophilic ring opening of a cyclic sulfinate ester or γ-sultine, **8** (approach C). Such an intermediate has a sulfur atom activated toward nucleophilic attack and simultaneously provides protection for the alcohol function. We report the viability of this approach: we have synthesized sultines of type **8** and have studied their ring-opening reactions with nucleophiles. In the course of our synthetic work on sulfur-containing natural products we have become interested in sultines for several reasons. In contrast to their lactone counterparts they are inherently chiral, so that in reactions with racemic nucleophiles (e.g., $R_1R_2R_3Cl^-$) ring opening will be a diastereoselective process. As will be shown below, these ring-opening reactions may proceed by cleavage of either the S–O bond or the C–O bond. Oxidation of sultines gives sultones which have a utility of their own in synthetic organic chemistry.[5]

Scheme I



Scheme II



Synthesis of γ-Sultines 8

In contrast to the extensive literature which exists on the preparation of sulfinate esters[6] only a limited number of sultines have been described.[7] To our knowledge, no synthesis of *functionalized* sultines, e.g., **8**, have yet been reported.

For the preparation of **8** we examined first the method used[8,9] for the conversion of 1-mercaptopropan-3-ols into

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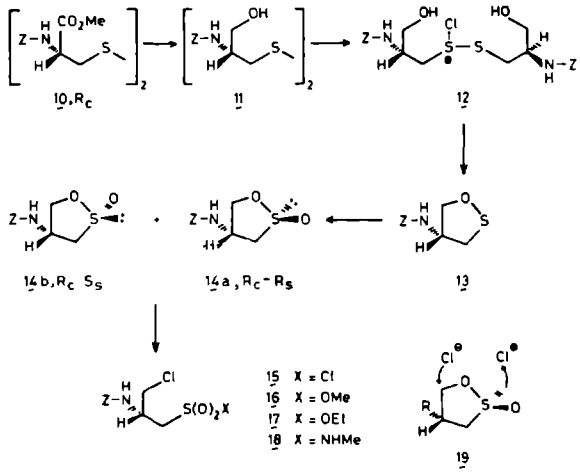
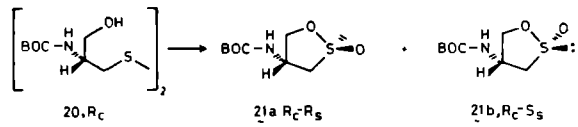
(8) Givens, E N, Hamilton, L A *J Org Chem* **1967**, *32*, 2857

Scheme III



Scheme IV



γ-sultines as it allowed us to use cysteinol as the starting material Thus, the N-protected L-cystinol derivative **11**, prepared from **10** by $LiBH_4$ reduction followed by I_2 oxidation, was treated with 3 equiv of Cl_2 and an excess of AcOH (Scheme III). However, instead of the expected compound **14** the sulfonyl chloride **15** was formed (78% yield). The structure of **15** was secured by spectroscopic data and the formation of the derivatives **16–18**. We then reexamined the reaction conditions. When the reaction, which was monitored by TLC, was stopped just as **15** began to appear, in addition to starting material **11**, the sultines **14a** and **14b** could be isolated, albeit in low yields (7% and 28%, respectively). The molecular structures of **14a** and **14b** were assigned by spectroscopy and the preparation of derivatives; the absolute configuration of the sulfoxide sulfur atoms[10] was assigned by X-ray analysis of a homologue (vide infra). The two diastereomers showed a surprisingly large difference in R_f value on chromatography and were readily separated by silica gel chromatography, compound **14a** being the more polar component.[11]

Treatment of the isolated sultines with Cl_2 resulted in quantitative formation of **15**. The formation of **14** and **15** from **11** can be rationalized by the following sequence. Chlorine oxidation of **11** produces first the sulfonium ion **12**, which, by subsequent intramolecular displacements by the hydroxyl functions, leads to 2 mol of the sultene **13**.[12] This, on oxidation with Cl_2/AcOH, gives the sultines **14a** and **14b**. Finally, **15** is formed by cleavage of the C–O bond, a reaction known to proceed with sulfinate esters[13] and with some sultines[7] in the presence of halogens. This ring-opening reaction might be rationalized as depicted in **19**.

Apparently, ring opening of **14** to **15** and the oxidation steps that lead to **14** are competing reactions with similar rates in the Cl_2/AcOH procedure. We reasoned that the use of another oxidizing agent, generating Cl^+ and having a less nucleophilic counterion, might prevent the ring opening. Consequently, we studied, in turn, SO_2Cl_2 and *N*-chlorosuccinimide (NCS).[14] Reaction of **11** with 3 equiv of SO_2Cl_2 gave an intractable reaction mixture. Oxidation with NCS, however, was more successful; treatment of **11** with AcOH and 3 equiv of NCS gave **14a** and **14b** (1:1 ratio) in 90% yield. Thus it appears that under these reaction conditions no Cl_2 is being formed. With an excess (10 equiv) of NCS, compound **15** was again isolated as the sole product. The sultines were found to be unreactive toward NCS alone. Thus the production of **15** must be caused by reaction of the sultines with Cl^-/NCS or Cl_2. Reaction of **11** with 3 equiv as well as with an excess (10 equiv) or *N*-bromosuccinimide (NBS) gave the sultines **14a** and **14b** (1.1 ratio) in 86% yield. Here the use of an excess of NBS gave no detectable amounts of the ring-opened product.[15]

Earlier we had shown[4] in the synthesis of sparsomycin (**1**) that the *tert*-butoxycarbonyl (Boc) group was preferred for the N-protection of **9**. Therefore, we also prepared the sultines **21a** and **21b** (88% yield, 1:1 ratio) by treatment of **20** with 3 equiv of NCS, an excess of AcOH, and, to avoid removal of the acid-labile Boc group, 3 equiv of pyridine (Scheme IV). Structures **21a** and **21b** were assigned to the more polar and less polar diastereomeric products, respectively, by means of spectroscopy and an X-ray crystallographic analysis of **21a**.[17] These structures were also supported by conversion of the products into derivatives (vide infra).

The ^1H NMR spectra of **14a,b** and **21a,b** deserve some comment. Previous studies have shown that protons in a 1,3-syn-diaxial relationship to a sulfinyl oxygen atom experience deshielding, which has been referred to as the syn-axial effect.[18] This effect has been discussed for four-,[19] five-,[7] and six-membered[18,20] ring systems and caused a $\Delta\delta$ of 0.6–1.1 ppm. This effect was not observed in **14a,b** and **21a,b**; the ^1H NMR spectra showed nearly the same δ value for the C(4) proton in either a syn relationship with the sulfinyl oxygen (**21a**, δ 4.79) or an anti position (**21b**, δ 4.85). This indicates that the syn-axial effect may not be applicable to substituted γ-sultines, where substituents assume pseudoaxial and pseudoequatorial positions.[21]

(9) Dodson, R. M., Hammen, P. D., Davis, R. A. *J. Chem. Soc., Chem. Commun* **1968**, 9

(10) The *R/S* nomenclature as adopted for sulfinate esters (Mislow, K., Green, M. M., Laur, P., Melillo, J. T., Simmons, T., Ternay, A. L. *J. Am. Chem. Soc* 1965, *87*, 1958) has been followed here, in that the S–O "double" bond has a lower priority than the S–OR bond

(11) The conformations of **14a** and **14b** are being studied by NMR and may provide some explanation for the large difference in polarity

(12) While sultenes have been proposed before as reactive intermediates, only recently has the first stable sultene been prepared, see Astrology, G. W.; Martin, J. C. *J. Am. Chem. Soc* **1977**, *99*, 4390 and references cited therein

(13) Douglass, I. B. *J. Org. Chem* **1965**, *30*, 633; **1974**, *39*, 563

(14) Both reagents have been used for the conversion of *tert*-butyl hydroxyalkyl sulfoxides into sultines (see ref 7)

(15) This interesting difference in reactivity of the sultines **14** toward NBS and NCS might be explained by a polarization difference between their N–halogen bonds, the N–Br bond is less polarized than the N–Cl bond,[16] so that NBS may not be reactive enough to cause ring opening of **14**. We noticed that during the NBS reaction Br_2 was formed From this it may follow that the sultines **14** are stable toward Br_2 but reactive toward Cl_2

(16) Lumbroso, H., Gasco, L.; Malèn, C *Bull Chem Soc Fr* **1951**, *15*, 823.

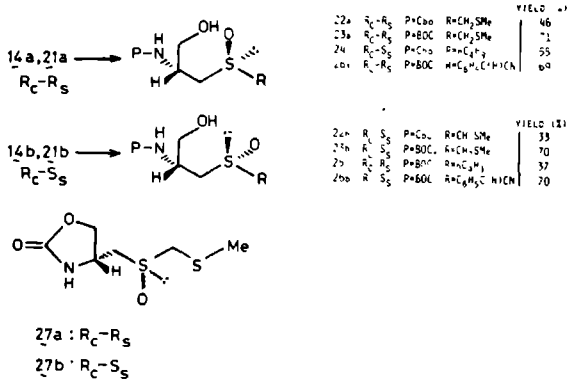
(17) A detailed discussion of the X-ray crystallographic analysis of **21a** and a conformational analysis of **21a** and **21b** based on NMR spectroscopy will be the subject of a future report

(18) Harpp, D. N., Gleason, J. G. *J Org Chem* **1971**, *36*, 1314 and references cited therein

(19) Johnson, C. R., Siegl, W. O. *Tetrahedron Lett* **1969**, 1879

(20) Buchanan, G. W., Sharma, N. K., De Reinach Hirtzbach, F., Durst, T. *Can J Chem* **1977**, *55*, 44

Scheme V



Nucleophilic Ring-Opening Reactions of Sultines

The most widely used procedure for the synthesis of sulfoxides of high optical purity involves the reaction of an optically active sulfinate ester with a Grignard reagent, the Andersen synthesis.[22] Recently, this method has been used by Colombo et al.[23] for the synthesis of optically active thioacetal monosulfoxides by reaction of an optically active sulfinate ester with (alkylthio)methyllithium. These reactions are stereospecific and proceed with inversion at sulfur.[24] Nucleophilic ring opening of sultines has been reported only twice. Grignard reagents[25] as well as organocopper–lithium reagents[26] gave the corresponding sulfoxide alcohols. Although the stereochemistry of these ring-opening reactions has not been rigorously established, it has been discussed and assumed[25] by analogy to open-chain sulfinates to proceed also with inversion at sulfur.

We found that Colombo's approach[23] was also applicable for the conversion of **8** into **9**. Thus, reaction of **14** and **21** at −78 °C with 3 equiv of (methylthio)methyllithium, prepared according to Peterson,[27] gave the desired dithioacetal monoxides **22** and **23** (Scheme V; the yields for **22** have not been optimized). The known[4] compounds **27a** and **27b** were found as side products (19% and 11% yields, respectively) when the reaction mixtures of **14** → **22** were not acidified rapidly after completion.

The formation of **22** and **23** was found to be stereospecific; no trace of the corresponding diastereomers was found. Since the absolute configuration of sultine **21a**[17] as well as of the ring-opened products **22** and **23**[28] has been rigorously established, we can now conclude that in analogy to open-chain sulfinates, *sultines undergo nucleophilic ring-opening reactions with inversion at sulfur.*[29]

The reactivity of **14** and **21** toward other nucleophiles was also studied. Reaction of **14a** and **21b** with *n*-butyllithium gave the sulfoxides **24** and **25**, respectively. In an attempt to prepare other α-functionalized sulfoxides in addition to **22** and **23**, compounds **21a** and **21b** were treated with lithium benzylcyanide[30] to give the α-cyano sulfoxides **26a** and **26b**, respectively. In each case diastereomers having different configurations at the C(H)(CN) carbon atom were formed in unequal amounts: for **26a** the ratio was 1/2; for **26b** the ratio was 9/11 (the stereochemistry is undetermined). This shows that asymmetric induction by the chiral sulfur atom is at work. So far, optically active α-cyano sulfoxides have been virtually unexplored.[31]

Treatment of **21b** with 2 equiv of NaOMe in MeOH gave a mixture of the starting material and **21a** in a 1:1 ratio. This epimerization can be explained by a transesterification reaction of the ring-opened R_C–R_S methyl sulfinate ester;[32] ring closure of the latter gives **21a**. None of the ring-opened methyl sulfinate esters could be isolated.

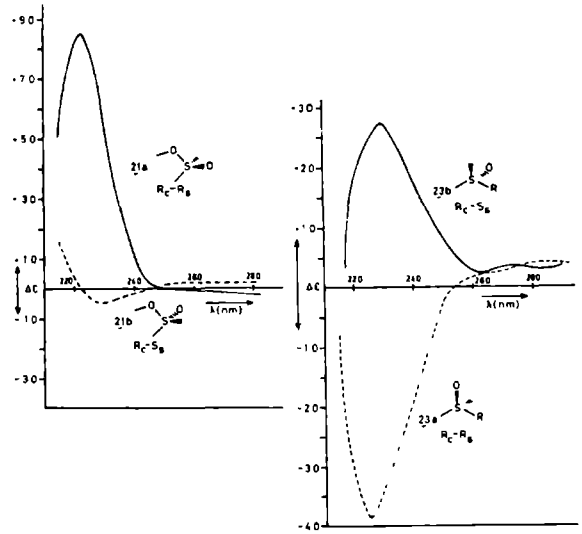


Figure 1. CD spectra of **21a,b** and **23a,b** in acetonitrile.

CD Spectra of 21 and 23

Previously we showed that for sparsomycin[4] as well as for several α-functionalized sulfoxides used as synthetic intermediates[28] that CD can be employed in the assignment of the configuration of the sulfoxide sulfur atom; a negative sign of the Cotton effect centered at the S(O) absorption band in the 220–230-nm region correlates with an *R* configuration (as in **22a** and **23a**) and a positive sign with an *S* configuration (as in **22b** and **23b**). Examples in which CD spectra have been applied to sulfinates or sultines are so few[25] as to allow no generalizations. The CD spectra of the sultines **21a** and **21b** were measured and compared with those of **23a** and **23b** (Figure 1).

For **21a** and **21b** a striking difference is observed in the magnitude of rotational strength, whereas the bands for the sulfoxides **23a** and **23b** have nearly the same amplitude. This may be rationalized as follows. In the region of 220–240 nm each spectrum consists of a composite

(21) This syn-axial effect was applied incorrectly by Sharma et al.,[7] who assigned erroneous structures to γ-sultines having a phenyl substituent at C(4).

(22) Andersen, K. K. *Tetrahedron Lett.* **1962**, 93. Andersen, K. K.; Foley, J.; Perkins, R.; Gaffield, W.; Papanikalaou, N. *J. Am. Chem. Soc.* **1964**, *86*, 5637. Andersen, K. K. *Int. J. Sulfur Chem. Part B*, **1971**, *6*, 69.

(23) Colombo, L.; Gennare, C.; Narisano, E. *Tetrahedron Lett.* **1978**, 3861.

(24) Axelrod, M.; Bickart, P.; Jacobus, H.; Green, M. M.; Mislow, K. *J. Am. Chem. Soc.* **1968**, *90*, 4835.

(25) Pirkle, W. H.; Hoekstra, M. S. *J. Am. Chem. Soc.* **1976**, *98*, 1832.

(26) Harpp, D. N.; Vines, S. M.; Montillier, J. P.; Chan, T. H. *J. Org. Chem.* **1976**, *41*, 3987.

(27) Peterson, D. J. *J. Org. Chem.* **1967**, *32*, 1717.

(28) Ottenheijm, H. C. J.; Liskamp, R. M. J.; Helquist, P.; Lauher, J. W.; Shekhani, M. *J. Am. Chem. Soc.* **1981**, *103*, 1720.

(29) Whereas the ring-opening reactions of **14** and **21** proceeded with inversion, the *R*/*S* nomenclature does not change in those cases where there is a reversal in the priority assignments for the sulfur substituents, e.g., in going from **14** to **22**, or **21** to **23**, and **26**. See also ref 10.

(30) Kaiser, E. M.; Hauser, C. R. *J. Am. Chem. Soc.* **1966**, *88*, 2348.

(31) After completion of our study on the reaction of sultines with a prochiral nitrile, the preparation of α-cyano sulfoxides from sulfinate esters was reported: Annunziato, R.; Cinquini, M.; Colonna, S.; Cozzi, F. *J. Chem. Soc., Perkin Trans. 1* **1981**, 614.

(32) For the methanolysis of sulfinate esters, see: Darwish, D.; Noreyko, J. *Can. J. Chem.* **1965**, *43*, 1366.

chromophore, which includes an inherently symmetric but chirally perturbed amide band as well as an inherently chiral sulfoxide band. With the sulfoxides **23a** and **23b** and other sulfoxides we have studied before,[4] the contribution due to the chiral carbon atom is small, so that their CD curves are nearly mirror images. This behavior contrasts with that of the sultines **21a** and **21b**, where the contribution of the chiral carbon is evidently considerably larger and on the same order of magnitude as that of the sulfoxide atom. As yet we have no explanation for this increase of rotational strength of the amide chromophore. Nevertheless, the CD curves of **21a** and **21b** allow the conclusion that the sign of the band which is due to the sulfoxide chromophore is positive and negative, respectively. From this it follows that the correlation between the sign of the Cotton effect and the absolute configuration of the sulfoxide sulfur atom in unsubstituted sultines is identical with that mentioned above for α-functionalized sulfoxides: *compounds that have a geometrical arrangement as depicted in **21a** and **23b** have a positive sign of the Cotton effect, and their stereomers **21b** and **23a** a negative one.*[29] This implies that the nucleophilic ring-opening reactions of sultines **14** and **21** that lead to α-functionalized sulfoxides are accompanied by a change in the sign of the Cotton effect.[33] In addition, it can be concluded that at least for γ-sultines, CD can be employed in the assignment of the absolute configuration of the sulfoxide sulfur atom. Whereas this method is as direct and reliable as the method of ^{1}H NMR using chiral fluoro alcohols,[25] we have to urge caution when sultines having an additional chiral center are studied; the strong effect of coupling of both chromophores could lead to an alteration in the sign of the 220–240-nm band; i.e., both sulfoxide diastereomers could have cotton effects of the same sign.

In summary, we have shown that sultines can undergo ring-opening reactions either by cleavage of the C–O bond or by cleavage of the S–OR bond. Previously, we have shown[4] that **23a** is easily converted into (R_C)-**3** and can be subsequently coupled with **2** to give (R_C)-sparsomycin Thus, the sequence of reactions **20** → **21a** → **23a** constitutes a new approach to sparsomycin (**1**).

Work is in progress on determining the inductive power of the chiral sulfur atom of the sultines in ring-opening reactions with racemic nucleophiles. Also, the general appplicability of the CD rule for sultines as delineated in this paper will be studied.

Experimental Section

^{1}H NMR spectra were measured on a 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. ^{13}C NMR spectra were measured on a Bruker WP-60 spectrometer IR spectra were measured with a Perkin-Elmer spectrophotometer, Model 997, and UV spectra on a Perkin-Elmer spectrophotometer, Model 555 Circular dichroism spectra were measured with a Dichrograph II apparatus (Roussel-Jouan).

Mass spectra were obtained with a double-focusing Varian Associates SMI-B spectrometer. Melting points were taken on a Kofler 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), with the following solvent systems. (A) $MeOH/CH_2Cl_2$, 1/9 v/v; (B) $MeOH/CH_2Cl_2$, 6/94 v/v, (C) $MeOH/CH_2Cl_2$, 4/96 v/v, (D) $MeOH/CH_2Cl_2$, 3/97 v/v. Spots were visualized with an UV lamp, iodine vapor, ninhydrin, or Cl_2–TDM[34] For column chromatography Merck silica gel H (type 60) was used The Miniprep LC (Jobin Yvon) was used for preparative HPLC

Z-L-Cystinol (11). To a stirred, cooled (−78 °C) solution of sodium borohydride (6 81 g, 180 mmol) and lithium iodide (24 09 g, 180 mmol) in 600 mL of dry dimethoxy ethane (DME) was added methyl ester **10**, prepared according to the procedure of Gustus,[35] in one portion The reaction mixture was allowed to warm to room temperature and then stirred until the reaction was complete, as monitored by TLC (system A) The solution was neutralized to pH 7 with an aqueous solution of 1 N HCl with ice cooling Stirring was continued for 1 h at room temperature, after which time the volume was reduced to half its volume A methanolic solution 0 1 M in iodine and 0 2 M in pyridine was added until a faint yellow color of iodine persisted The excess of iodine was destroyed by adding a few crystals of $Na_2S_2O_5$ After evaporation of DME and methanol in vacuo, water and dichloromethane were added The aqueous layer was extracted three times with dichloromethane and twice with ethyl acetate The combined organic layers were dried (Na_2SO_4), and the solvent was evaporated in vacuo After recrystallization of the residue from ethyl acetate, 12 53 g (87% yield) of **11** was obtained. This material was homogenous on TLC (R_f 0 17, solvent system B): NMR (CD_3OD) δ 2.67–3.13 (m, 2 H, CH_2S), 3 63 (br d, 2 H, CH_2O), 3 80–4 10 (m, 1 H, $CHCH_2$), 5 07 (s, 2 H, $C_6H_5CH_2$), 7 32 (s, 5 H, C_6H_5), IR (KBr) 3300, 1695, 1680, 1535 cm^{-1} Anal. Calcd for $C_{22}H_{28}N_2O_6S_2$ C, 54 98; H, 5.87, N, 5 83 Found C, 55.27, H, 5 84, N, 5 65.

4-[(Benzyloxycarbonyl)amino]-1,2-oxathiolane 2-Oxide (14a,b). To a stirred solution of Z-cystinol (**11**, 4 80 g, 10 mmol) in 100 mL of glacial acetic acid was added a solution of *N*-chlorosuccinimide (4 01 g, 30 mmol) in 150 mL of glacial acetic acid dropwise at room temperature The reaction mixture was stirred overnight After completion of the reaction as monitored by TLC (solvent system B) the acetic acid was evaporated in vacuo at room temperature The residue was dissolved in 400 mL of dichloromethane and 15 mL of water The organic layer was separated and dried, and the solvent evaporated in vacuo The residue was dried and then chromatographed over silica (eluant $MeOH/CH_2Cl_2$, 0 5/99 5 v/v) to yield **14a** (45%) and **14b** (45%) The synthesis of **14a** and **14b** with *N*-bromosuccinimide (3 equiv or more) was carried out as described above After evaporation of acetic acid, residual bromine was removed by dissolving the residue in methanol and evaporation of the solvent in vacuo, this was repeated twice. Column chromatography of the residue gave **14a** (43% yield) and **14b** (43% yield)

14a: mp 87 °C (AcOEt–hexane) R_f 0 43 (solvent system B); NMR δ 3 34 and 3 07 (AB part of ABX spectrum, 8 lines, J_{AB} = 13 9 Hz, J_{AX} = 6.2 Hz, J_{BX} = 2 4 Hz, 2 H, $CH_2S(O)$), 4 38–4.52 and 4 62–4 88 (m, 3 H, $CHCH_2O$), 5 09 (s, 2 H, $C_6H_5CH_2$), 5 45 (br d, 1 H, NH), 7.33 (s, 5 H, C_6H_5), IR (KBr) 3310, 1720, 1530, 1110 cm^{-1}; exact mass calcd for $C_{11}H_{13}NO_4S$ 255 147, found 255 149. Anal Calcd for $C_{11}H_{13}NO_4S$: C, 51.75, H, 5 13; N, 5 49 Found. C, 51 89, H, 5.11; N, 5 35

14b R_f 0 64 (solvent system B), NMR δ 2.91 and 3 20 (AB part of ABX spectrum, 8 lines, J_{AB} = 13.9 Hz, J_{AX} = 6.2 Hz, J_{BX} = 2 4 Hz, 2 H, $CH_2S(O)$), 4 58 and 4 75 (AB part of ABX spectrum, 8 lines, J_{AB} = 9 6 Hz, J_{AX} = 5 7 Hz, J_{BX} = 1 9 Hz, 2 H, CH_2O), 4 98 (m, 1 H, $CHCH_2O$), 5 10 (s, 2 H, $C_6H_5CH_2$), 6.36 (br d, 1 H, NH), 7 33 (s, 5 H, C_6H_5); IR (KBr) 3320, 1690, 1545, 1108 cm^{-1}; exact mass calcd for $C_{11}H_{13}NO_4S$ 255 147, found 255 147. Anal. Calcd for $C_{11}H_{13}NO_4S$. C, 51 75; H, 5 13; N, 5.49 Found: C, 51 42, H, 5 20, N, 5 35

3-Chloro-2-[(benzyloxycarbonyl)amino]-1-propanesulfonyl Chloride (15). To a stirred solution of Z-cystinol (**11**, 1.44 g, 3 mmol) in 40 mL of glacial acetic acid was added a solution of chlorine (1 59 g, 22 mmol) in 15 mL of dry, ethanol-free dichloromethane in small portions at room temperature After the addition was complete, the reaction mixture was stirred for 2 h at room temperature, after which time the excess of chlorine was removed by a stream of argon Evaporation of the solvent at room temperature in vacuo gave **15** 78% yield, R_f 0 25 (solvent system

(33) We anticipate that ring-opening reactions that lead to alkyl sulfoxides will proceed with conservation of the sign of the Cotton effect This prediction is based upon our observation[28] that the sign of the Cotton effect of alkyl sulfoxides $(RS(O)R_1$, is opposite that of the corresponding α-functionalized derivatives $(RS(O)CH(X)(R_2)$

(34) Von Arx, E, Faupel, M, Brugger, M *J Chromatogr* **1976**, *120*, 224

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

B), NMR δ 3 67–4 00 (m, 2 H, CH_2SO_2Cl), 4 00–4 10 (br d, 2 H, CH_2Cl), 4 44–4 82 (m, 1 H, $CHCH_2Cl$), 5 13 (s, 2 H, $C_6H_5CH_2$), 5 59 (br d, 1 H, NH), 7 35 (s, 5 H, C_6H_5), IR (Nujol) 3340, 1695, 1380, 1360, 1350, 1175 cm^{-1}, mass spectrum, *m/e* 325, 327, 329 (M^+)

Methyl 3 Chloro-2-[(benzyloxycarbonyl)amino]-1-propanesulfonate (16). The sulfonyl chloride **15** was converted in 43% yield to **16** by chromatography over silica gel with solvent system C R_f 0 82 (solvent system C), NMR δ 3 45–3 52 (AB part of ABX spectrum, 2 H, $CH_2S(O_2)$), 3 75 and 3 92 (AB part of ABX spectrum, J_{AX} = 4 2 Hz, J_{BX} = 5 4 Hz, J_{AB} = 11 4 Hz, CH_2Cl), 3 90 (s, 3 H, OCH_3), 4 41 (m, 1 H, $CHCH_2Cl$), 5 12 (s, 2 H, $C_6H_5CH_2$), 5 69 (br d, 1 H, NH), 7 35 (s, 5 H, C_6H_5), IR (Nujol) 3320, 1695, 1545, 1350, 1330, 1180 cm^{-1}, exact mass calcd for $C_{12}H_{16}ClNO_5S$ 321 252, found 321 251 Anal Calcd for $C_{12}H_{16}ClNO_5S$ C, 44 79, H, 5 01, N, 4 35 Found C, 44 55, H, 4 80, N, 4 11

Ethyl 3-Chloro-2-[(benzyloxycarbonyl)amino]-1-propanesulfonate (17). The sulfonyl chloride **15** (1 52 g, 4 7 mmol) was dissolved in 200 mL of chloroform containing ethanol and solid sodium carbonate The reaction mixture was stirred overnight Subsequently the precipitate was removed and the solvent evaporated in vacuo The residue was recrystallized from chloroform/hexane to give the ethyl sulfonate **17** 40% yield, mp 133–134 °C, NMR δ 1 40 (t, 3 H, $SO_3CH_2CH_3$), 3 44–3 51 (AB part of ABX spectrum, 2 H, CH_2SO_2), 3 75 and 3 92 (AB part of ABX spectrum, J_{AX} = 4 3 Hz, J_{BX} = 5 5 Hz, J_{AB} = 11 4 Hz, 2 H, CH_2Cl), 4 32 (q, 2 H, CH_2CH_3), 4 46 (m, 1 H, $CHCH_2Cl$), 5 12 (s, 2 H, $C_6H_5CH_2$), 5 65 (br d, 1 H, NH), 7 35 (s, 5 H, C_6H_5), IR (Nujol) 3315, 1690, 1545, 1345, 1330, 1180, 1170 cm^{-1}, mass spectrum, *m/e* 335, 337 (M^+) Anal Calcd for $C_{13}H_{18}ClNO_5S$ C, 46 50, H, 5 40, N, 4 17 Found C, 46 11, H, 5 26, N, 4 35

***N*-Methyl-3-chloro 2 [(benzyloxycarbonyl)amino]-1-propanesulfonamide (18).** To a stirred and chilled (0 °C) solution of the sulfonyl chloride **15** (200 mg, 0 61 mmol) in dry, ethanol-free chloroform was added a solution of methylamine in benzene (1 22 mmol) The solution was stirred at room temperature overnight, the salt was removed by filtration, and the solvent was evaporated in vacuo The crude product was next purified by column chromatography (solvent system D) to give the sulfonamide **18** 62% yield, R_f 0 61 (solvent system B), NMR δ 2 74 (d, 3 H, $NHCH_3$), 3 34 (d, 2 H, CH_2SO_2), 3 71 and 3 87 (AB part of ABX spectrum, J_{AX} = 4 0 Hz, J_{BX} = 5 0 Hz, J_{AB} = 11 3 Hz, 2 H, CH_2Cl), 4 41 (m, 1 H, $CHCH_2Cl$), 4 71 (br, 1 H, $NHCH_3$), 5 12 (s, 2 H, $C_6H_6CH_2$), 5 51 (br d, 1 H, NH), 7 36 (s, 5 H, C_6H_5), IR (KBr) 3320, 1690, 1535, 1330, 1155 cm^{-1}, exact mass calcd for $C_{12}H_{17}ClN_2O_4S$ 320 2675, found 320 266 Anal Calcd for $C_{12}H_{17}ClN_2O_4S$ C, 44 93, H, 5 34, N, 8 73 Found C, 44 83, H, 5 21, N, 8 46

***N* (*tert*-Butoxycarbonyl)-L-cystinol (20).** *N*-(*tert*-butoxycarbonyl)-L cystine methyl ester (7 03 g, 15 mmol), prepared as described earlier,[4] was reduced with lithium borohydrate [sodium borohydride (3 41 g, 90 mmol) and lithium iodide (12 05 g, 90 mmol) in 200 mL of dry DME] as described for the preparation of **11** The workup, however, was modified due to the acid lability of the N-protecting group the pH was adjusted to 5 by addition of aqueous 1 N $KHSO_4$ to the stirred and cooled (0 °C) solution Sometimes a sticky mass precipitated before neutralization was complete In that case the solvent was evaporated in vacuo, the residue dissolved in methanol/water (1/1 v/v), and the neutralization then completed The oxidation with iodine was carried out as described for the preparation of **11** Subsequently, the methanol was evaporated in vacuo, and water and ethyl acetate were added The aqueous phase was extracted five times with ethyl acetate The collected organic layers were washed with brine and dried (Na_2SO_4), and the solvent was evaporated in vacuo The residue was recrystallized from methanol/water to give **20** 87% yield mp 124–125 °C, R_f 0 23 (solvent system A), NMR (CD_3OD) δ 1 41 (s, 9 H, *t*-Bu), 2 85 (d, 2 H, CH_2S), 3 46–4 05 (m, 3 H, $CHCH_2O$), IR (KBr) 3360, 3600–3100, 1685, 1525 cm^{-1} Anal Calcd for $C_{16}H_{32}N_2O_6S_2$ C, 46 58, H, 7 82, N, 6 79 Found C, 46 78, H, 7 92, N, 6 87

4-[(*tert* Butoxycarbonyl)amino] 1,2-oxathialane 2-Oxide (21a,b). A solution of *N*-chlorosuccinimide (4 01 g 30 mmol) in 150 mL of glacial acetic acid was added dropwise to a solution of *N*-(*tert*-butoxycarbonyl)-L-cystinol (**20**, 4 12 g, 10 mmol) and pyridine (2 4 g, 30 mmol) in 100 mL of glacial acetic acid By use of the procedure as described for **14a** and **14b**, **21a** and **21b** were isolated in a 1/1 ratio (86% yield)

21a mp 127 °C (CH_2Cl_2–CCl_4), R_f 0 43 (solvent system B), ^1H NMR δ 1 44 (s, 9 H, *t*-Bu), 3 14 and 3 45 (AB part of ABX spectrum, J_{AX} = 2 9 Hz, J_{BX} = 6 0 Hz, J_{AB} = 13 9 Hz, 2 H, CH_2S), 4 33–4 98 (m, 2 H, CH_2O), 4 79 [m, 1 H (covered by CH_2O), $CHCH_2O$], 5 05 (br, 1 H, NH), IR (KBr) 3325, 1683, 1530, 1102 cm^{-1}, ^{13}C NMR (CD_2Cl_2) δ 28 4 (CH_3)$_3$C), C–N covered by CD_2Cl_2 signals, 67 4 (CS), 80 7 (CH_3)$_3$C), 77 6 (COS(O)), 155 2 (C(O)N), exact mass calcd for $C_7H_{12}NO_4S$ (M^+ CH_3) 206 139, found 206 139 Anal Calcd for $C_8H_{15}NO_4S$ C, 43 42, H, 6 83, N, 6 33 Found C, 43 46, H, 6 90, N, 6 13

21b mp 135–136 °C (CH_2Cl_2) R_f 0 64 (solvent system B), ^1H NMR δ 1 43 (s, 9 H, *t*-Bu), 2 94 and 3 22 (AB part of ABX spectrum, J_{AX} = 1 3 Hz, J_{BX} = 6 6 Hz, J_{AB} = 9 7 Hz, 2 H, CH_2S), 4 59 and 4 76 (AB part of ABX spectrum, J_{AX} = 1 9 Hz, J_{BX} = 5 5 Hz, J_{AB} = 9 7 Hz, 2 H, CH_2O), 4 85 (m, 1 H, $CHCH_2O$), 6 06 (br, 1 H, NH), ^{13}C NMR (CD_2Cl_2) δ 28 5 ((CH_3)$_3$C), 50 9 (C N), 62 8 (CS), 80 2 (CH_3)$_3$C), 83 0 (COS(O)), 155 2 (C(O)N), IR (KBr) 3325, 1725, 1525, 1115 cm^{-1}, exact mass calcd for $C_7H_{12}NO_4S$ (M^+ - CH_3) 206 139, found 206 139 Anal Calcd for $C_8H_{15}NO_4S$ C, 43 42, H, 6 83, N, 6 33 Found C, 43 56, H, 6 85, N, 6 29

***N*-(Benzyloxycarbonyl)-*S* oxo *S* [(methylthio) methyl] L cysteinol (22a,b) and *N*-(*tert* Butoxy carbonyl) *S*-oxo-*S* [(methylthio)methyl] L-cysteinol (23a,b).** The anion of dimethyl sulfide was prepared in a manner analogous to the method described by Peterson et al [27] TMEDA (0 70 g, 0 91 mL, 6 0 mmol), freshly distilled dimethyl sulfide (0 37 g, 0 44 mL, 6 0 mmol), and 2 mL of freshly distilled THF, respectively, were brought via a syringe into a 50-mL, cooled (0 °C), round-bottomed flast (equipped with a septum) containing 3 75 mL of a 1 6 M solution of *n* butyllithium in hexane (6 mmol) The resulting solution was stirred at room temperature for 4 h, cooled to −30 °C, and added dropwise to a cooled (−78 °C) solution of the sultine **14a,b** (510 mg, 2 0 mmol) in 6 mL of freshly distilled THF Subsequently, the reaction mixture was stirred at −70 °C for 30 min and at room temperature for 30 min, and then rapidly quenched at 0 °C with 5 mL of a saturated aqueous solution of $KHSO_4$ Immediately thereafter the pH of the mixture was adjusted to 6–8 by addition of solid sodium carbonate Ethyl acetate was added, and then the aqueous layer was extracted four times with ethyl acetate The combined organic layers were dried (Na_2SO_4), and the solvent was evaporated in vacuo The residue was chromatographed under slightly elevated pressure (10 mmHg, eluant MeOH/CH_2Cl_2, 5/95 v/v) to give **22a** (46% yield) and **22b** (33% yield), respectively Compounds **23a** and **23b** were obtained in 71% and 70% yields, respectively, after preparative HPLC (solvent system C) and were identical in every aspect with the corresponding compounds obtained earlier [4]

22a mp 93 °C (CH_2Cl_2–hexane), R_f 0 35 (solvent system A), NMR (CD_2Cl_2) δ 2 27 (s, 3 H, SCH_3), 2 96 and 3 33 (AB part of ABX spectrum, J_{AX} = 4 9 Hz, J_{BX} = 5 8 Hz, J_{AB} = 13 4 Hz), 3 60–3 95 (m, 2 H, CH_2OH), 3 71 and 3 84 (AB spectrum covered by CH_2OH, 2 H, J_{AB} = 13 5 Hz, $S(O)CH_2S$), 4 26 (m, 1 H, $CHCH_2O$), 5 09 (s, 2 H, $C_6H_5CH_2$), 5 94 (br d, 1 H, NH), 7 34 (s, 5 H, C_6H_5), IR (KBr) 3335, 1680, 1530, 1006 cm^{-1}, exact mass calcd for $C_{13}H_{19}NO_4S_2$ 317 256, found 317 256 Anal Calcd for $C_{13}H_{19}NO_4S_2$ C, 49 19, H, 6 03, N, 4 41 Found C, 49 15, H, 6 03, N, 4 34

22b mp 162 °C (CH_2Cl_2–hexane), R_f 0 32 (solvent system A), NMR (CD_2Cl_2) δ 2 29 (s, 3 H, SCH_3), 2 97 and 3 21 (AB part of ABX spectrum, J_{AX} = 7 0 Hz, J_{BX} = 6 4 Hz, J_{AB} = 13 2 Hz, 2 H, $CHCH_2S(O)$), 3 73–4 00 (m, 4 H, $CHCH_2O$ and $S(O)CH_2S$), 4 16 (m, 1 H, $CHCH_2O$), 5 09 (s, 2 H, $C_6H_5CH_2$), 5 70 (br, 1 H, NH), 7 35 (s, 5 H, C_6H_5), IR (KBr) 3330, 1695, 1538, 1025, 1015 cm^{-1}, exact mass calcd for $C_{13}H_{19}NO_4S_2$ 317 256, found 317 257 Anal Calcd for $C_{13}H_{19}NO_4S_2$ C, 49 19, H, 6 03, N, 4 41 Found C, 49 16, H, 5 95, N, 4 16

2 Oxo 4 [[[(methylthio)methyl]sulfoxo]methylene]oxazolidine (27a,b). The ring-opening reaction of the sultine **14a** or **14b** was carried out as described above for the preparation of **22a** and **22b** with a slightly different workup Instead of being quenched with a saturated aqueous solution of $KHSO_4$, the reaction mixture was stirred overnight with solid $KHSO_4$ Methanol was then added and the mixture stirred for another 2 h at room temperature After removal of the salts by filtration, the filtrate

was concentrated to dryness, and the residue chromatographed on silica (eluant $MeOH/CH_2Cl_2$, 5/95 v/v) to give **27a** (19%) and **27b** (11%), respectively.

27a: R_f 0.24 (solvent system A); NMR (CD_2Cl_2) δ 2.31 (s, 3 H, SCH_3), 2.89–3.29 (br d, 2 H, $CHCH_2S$), 3.77 and 3.90 (AB spectrum, J_{AB} = 13.8 Hz, 2 H, $S(O)CH_2S$), 4.10 and 4.69 (s, 3 H, $CHCH_2O$), 6.40 (br s, 1 H, NH); IR (Nujol) 3250, 1745, 1710, 1040 cm^{-1}; mass spectrum m/e 209 (M^+).

27b: R_f 0.22 (solvent system A); NMR (CD_2Cl_2) δ 2.32 (s, 3 H, SCH_3), 2.94–3.29 (m, 2 H, $CHCH_2S$), 3.78 and 3.86 (AB spectrum, J_{AB} = 13.6 Hz, 2 H, $S(O)CH_2S$), 4.17–4.69 (m, 3 H, $CHCH_2O$), 6.84 (br s, 1 H, NH); IR (Nujol) 3240, 1760, 1710, 1045 cm^{-1}; mass spectrum, m/e 209 (M^+).

***N*-(Benzyloxycarbonyl)-*S*-oxo-*S*-*n*-butyl-L-cysteinol (24) and *N*-(*tert*-Butoxycarbonyl)-*S*-oxo-*S*-*n*-butyl-L-cysteinol (25).** A cooled (CO_2/2-propanol) solution of the *n*-butyllithium–TMEDA complex, prepared by adding TMEDA (523 mg, 0.68 mL, 4.5 mmol) to a solution of *n*-butyllithium in hexane (4.5 mmol), was added to a stirred, cooled (–78 °C) solution of the sultine **14a** (383 mg, 1.5 mmol) or **21b** (331 mg, 1.5 mmol) in 5 mL of freshly distilled, dry THF. The reaction mixture was stirred at –70 °C for 30 min and at room temperature for another 30 min. The workup was carried out as described to the preparation of **22** and **23**. Compounds **24** and **25** were obtained after HPLC (solvent system C) in yields of 55% and 37%, respectively.

24: R_f 0.33 (solvent system A); NMR δ 0.95 (t, 3 H, CH_2CH_3), 1.10–2.0 (m, 4 H, $S(O)CH_2CH_2CH_2CH_3$), 2.52–3.29 (m, 4 H, $CH_2S(O)CH_2$), 3.57–3.97 (m, 2 H, $CHCH_2O$), 3.97–4.40 (m, 1 H, $CHCH_2O$), 5.09 (s, 2 H, $C_6H_5CH_2$), 5.88 (br, 1 H, NH), 7.34 (s, 5 H, C_6H_5); IR (KBr) 3430, 3200, 1715, 1510, 1060 cm^{-1}; exact mass calcd for $C_{15}H_{23}NO_4S$ 313.225, found 313.226.

25: R_f 0.32 ($MeOH/CH_2Cl_2$, 9/91 v/v); NMR (CD_2Cl_2) δ 0.97 (t, 3 H, CH_2CH_3), 1.42 (s, 9 H, *t*-Bu), 1.22–1.93 (m, 4 H, $S(O)CH_2CH_2CH_2CH_3$), 2.62–3.21 (m, 4 H, $CH_2S(O)CH_2$), 3.78 (t, 2 H, CH_2OH), 3.87–4.27 (m, 1 H, $CHCH_2O$), 5.44 (br, 1 H, NH); IR (KBr) 3430, 1710, 1530, 1060 cm^{-1}; mass spectrum, m/e 222 (M^+ – *t*-Bu). Anal. Calcd for $C_{12}H_{25}NO_4S$: C, 51.59; H, 9.02; N, 5.01. Found: C, 51.53; H, 8.97; N, 4.99.

***N*-(*tert*-Butoxycarbonyl)-*S*-oxo-*S*-(cyanobenzyl)-L-cysteinol (26a,b).** The anion of benzyl cyanide[30] was prepared by addition of benzyl cyanide (0.72 mL, 703 mg, 6 mmol) to 3.75 mL of a cooled (0 °C) 1.6 M solution of *n*-butyllithium (6.0 mmol) in hexane; 15 mL of freshly distilled, chilled THF was then added to dissolve the anion. The resulting, yellow-colored solution was added dropwise to a stirred, cooled (–78 °C) solution of **21a** or **21b** (442 mg, 2 mmol) in 5 mL of freshly distilled THF. Subsequently, the reaction mixture was stirred for 30 min at –70 °C and for another 30 min at room temperature. The workup was carried out as described for **22** and **23**. Compounds **26a** and **26b** were obtained in yields of 69% and 70%, respectively, after HPLC (solvent system D).

26a: R_f 0.31 (solvent system A); NMR δ 1.39 and 1.48 (2 s, 9 H, *t*-Bu), 3.02–3.59 (m, 2 H, $CHCH_2S(O)$), 3.59–3.89 (m,2 H, $CHCH_2O$), 4.09 (m, 1 H, $CHCH_2O$), 5.22 and 5.37 (2 s, 1 H, S(O)CHCN), 5.4 (br d, 1 H, NH), 7.47 (s, 5 H, C_6H_5); IR (KBr) 3460, 2240, 1680, 1520, 1050 cm^{-1}; mass spectrum, m/e 281 (M^+ – C_4H_9). Anal. Calcd for $C_{16}H_{22}N_2O_4S$: C, 56.79; H, 6.55; N, 8.28. Found: C, 56.98; H, 6.62; N, 8.28.

26b: R_f 0.28 (solvent system A); NMR δ 1.40 and 1.44 (2 s, 9 H, *t*-Bu), 2.87–3.42 (m, 2 H, $CHCH_2S(O)$, 3.78 (br d, 2 H, $CHCH_2O$), 4.09 (m, 1 H, $CHCH_2O$), 4.96 and 5.17 (2 s, 1 H, S(O)CHCN), 5.3 (br, 1 H, NH), 7.44 (s, 5 H, C_6H_5); IR (KBr) 3450, 2240, 1685, 1525, 1050 cm^{-1}; mass spectrum, m/e 312 (M^+ – CN). Anal. Calcd for $C_{16}H_{22}N_2O_4S$: C, 56.79; H, 6.55; N, 8.28. Found: C, 56.82; H, 6.51; N, 8.23.

Acknowledgment. We thank Dr. T. F. Spande and Professor Dr. R. J. F. Nivard for reading and critisizing the manuscript. Part of the investigations was supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

Registry No. **10**, 3693-95-6; **11**, 79409-89-5; **14a**, 79409-90-8; **14b**, 79464-58-7; **15**, 79409-91-9; **16**, 79409-92-0; **17**, 79409-93-1; **18**, 79409-94-2; **20**, 79409-95-3; **21a**, 79409-96-4; **21b**, 79464-59-8; **22a**, 79464-60-1; **22b**, 79464-61-2; **23a**, 77880-72-9; **23b**, 77880-71-8; **24**, 79409-97-5; **25**, 79409-98-6; **26**, 79409-99-7; **27a**, 79410-00-7; **27b**, 79410-01-8; *N*-(*tert*-butoxycarbonyl)-L-cystine methyl ester, 79410-02-9.

CHAPTER V

1. INTERACTIONS OF SPARSOMYCIN WITH THE PEPTIDYL-TRANSFERASE CENTER OF RIBOSOMES
2. INHIBITION OF THE 'PROTEIN' SYNTHESIS IN YEAST (SACCHAROMYCES CEREVISAE) CELL-FREE SYSTEMS BY SPARSOMYCIN AND ANALOGS; PRELIMINARY RESULTS OF A STRUCTURE-ACTIVITY RELATIONSHIP STUDY
3. STRUCTURE-ACTIVITY RELATIONSHIPS OF SPARSOMYCIN AND ANALOGS; OCTYLSPARSOMYCIN: THE FIRST ANALOG MORE ACTIVE THAN SPARSOMYCIN
4. THE ANTITUMOR ACTIVITY OF SPARSOMYCIN.

1. *INTERACTIONS OF SPARSOMYCIN WITH THE PEPTIDYLTRANSFERASE CENTER OF RIBOSOMES*

INTRODUCTION

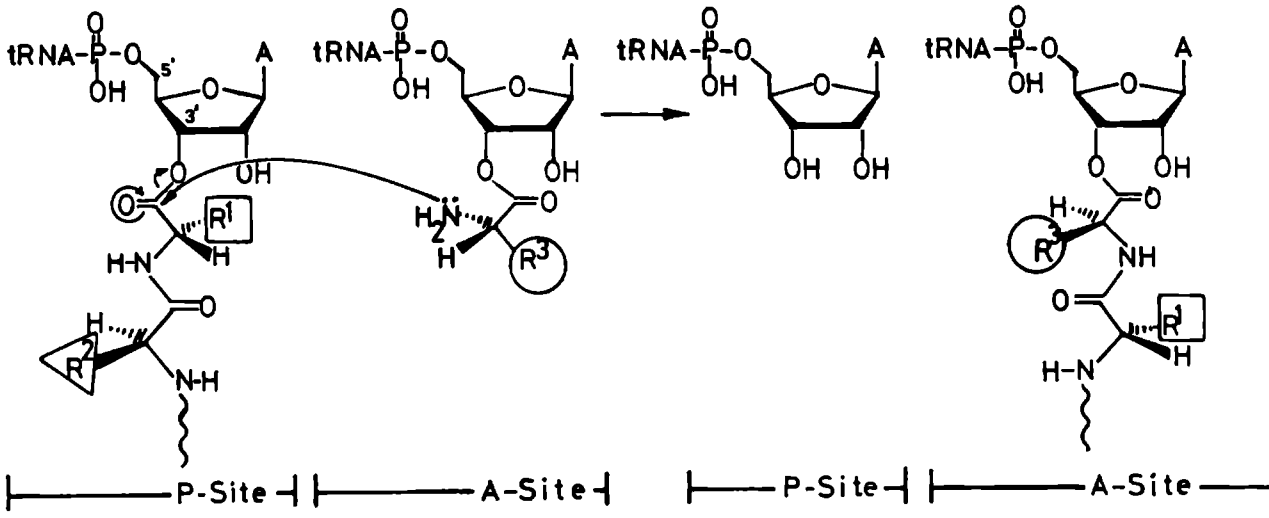
There is ample evidence[1] that sparsomycin inhibits the protein synthesis by interfering with the peptidyltransferase center. For an understanding of sparsomycin's site of interaction, this section is devoted to information about the peptidyltransferase center. Beside inhibition of the protein synthesis, sparsomycin influences the protein synthesis in several other ways. First, even in the absence of messenger RNA[1], it enhances non-enzymatic binding of N-acetyl-AA-tRNA(N-Ac-Phe-tRNA) or N-acetyl-AA-tRNA fragments (N-Ac-Leu-ACCAC) to the donor(peptidyl)site of the ribosome.
Second, sparsomycin shows a remarkable effect on the ribosome as a whole, by preventing ribosomal subunit exchange, which is essential for the continuation of the protein synthesis[9b,25]. Third, sparsomycin causes extensive disaggregation of polyribosomes into monomers, - that still contain peptides[2] - *in vivo* but not *in vitro*[2,3].

THE PEPTIDYLTRANSFERASE CENTER OF RIBOSOMES: FUNCTION AND LOCALIZATION

The region of the ribosome, that catalyzes synthesis of peptide bonds is refered to as the peptidyltransferase center. More precisely it catalyzes the transfer of the nascent peptide chain from the peptidyl-tRNA, located at the P-site(peptidyl site) of the ribosome, to the aminoacyl(AA)-tRNA, located at the A-site(aminoacyl site). In organic chemical terms the peptidyl transfer represents an ester aminolysis (equation 1, Scheme I), namely the nucleophilic attack by the amino group of the AA-tRNA-molecule on the ester function of the peptidyl group, with as leaving group the empty -tRNA-molecule.

Peptidyl-tRNA + AA-tRNA ⟶ tRNA-3'OH + Peptidyl-AA-tRNA (equation 1)

Scheme I



Although the designation 'ase' and an enzyme nomenclature number (E.C. 2.3.2.12[4]), suggest that peptidyltransferase is an enzyme, it has to be treated as a hypothetical enzyme since the aforementioned activity cannot be attributed to one or more defined ribosomal protein(s). Therefore we favor the use of the term 'peptidyltransferase center'.

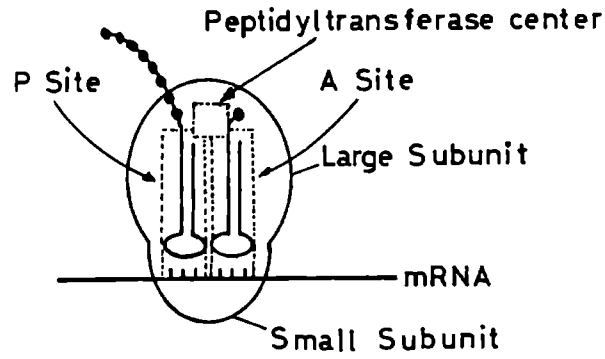
Apart from peptide bond formation the peptidyltransferase center is involved in the process of termination by catalyzing hydrolysis of the ester bond in the peptidyl-tRNA[5] (Weissbach[6]), as is depicted in equation 2.

$$\text{Peptidyl-tRNA} + H_2O \longrightarrow \text{tRNA-3'OH} + \text{Peptide} \quad \text{(equation 2)}$$

Furthermore there is evidence that the course of peptide bond formation is connected with the translocation event[5], the last stage of the elongation cycle consisting of the relative movement along the messenger RNA towards its 3' end over a distance of one codon.

The peptidyltransferase center is localized in the large ribosomal subunit (50S subunit of prokaryotes; 60S subunit of eukaryotes) and cannot be separated from this subunit. As is expected from its 'transfer' function (equation 1, Scheme I) the peptidyltransferase center should be localized somewhere in between the A-site and the P-site. Therefore the localization of the peptidyltransferase center in the simplest model of a ribosome can be represented as in Figure 1.

Fig 1 Representation of the ribosome and localization of peptidyltransferase center



Considerable information about the three dimensional structure, spatial arrangement of ribosomal proteins and - indirectly - the localization of the functional domains on the ribosomal surface is derived from immuno-electron-microscopic studies (M. Stöffler, R. Bald, B. Kastner, M. Luhrmann, M. Stöffler-Meilicke, G. Tischendorf[6]). With the aid of this technique the three dimensional representation of the *E-coli* ribosome and its functional domains were studied. As a result the peptidyltransferase center appears to be located in a domain as depicted in Figure 2. In another representation of the ribosome[8], - also derived from electronmicroscopic studies by Lake[8] - the peptidyltransferase center is suggested to be a domain as depicted in Figure 3. However, Lake[3] studied only one protein present in the peptidyltransferase center, whereas Stöffler *et al.* have studied several proteins present in this center, so that most of the border of the peptidyltransferase center in the model of Lake is unknown.

Fig 2 Shape of the peptidyltransferase center according to the model of Tischendorf, Zeichhardt and Stöffler[7]

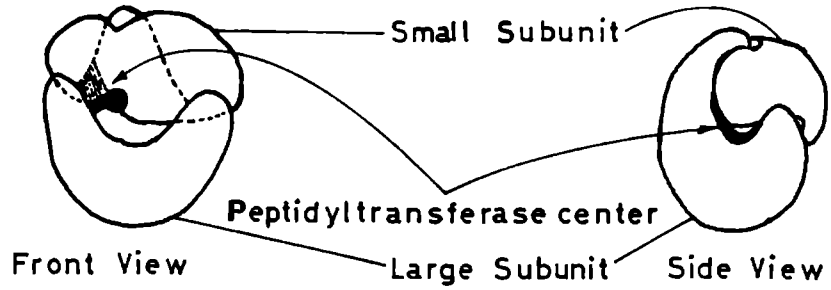
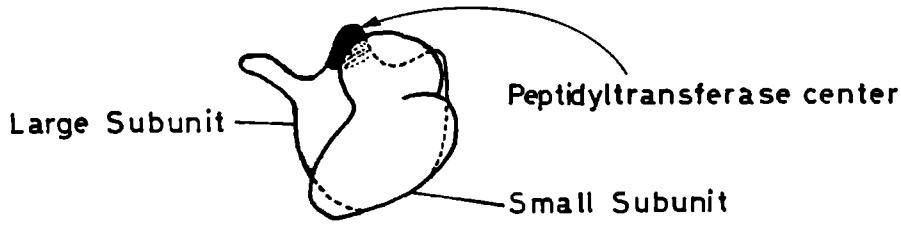


Fig 3 Shape of the peptidyltransferase center according to the model of Lake[8]



PROTEINS PRESENT IN THE PEPTIDYLTRANSFERASE CENTER AND INVOLVED IN ITS ACTIVITY

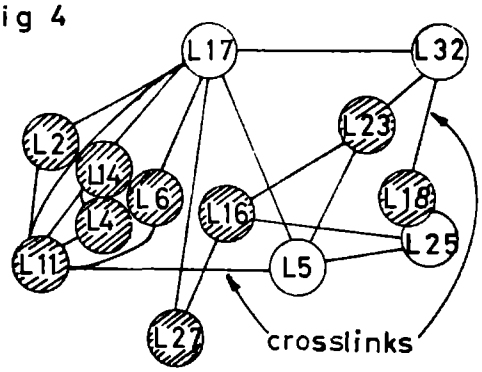
In addition to three RNA molecules (5S, 16S and 23S) prokaryotic ribosomes contain 50-60 proteins. In *E-coli* the small ribosomal subunit has 21 proteins, which are designated S1, S2 etc., while the large ribosomal subunit has 32 different proteins (L1, L2, etc.). Eukaryotic ribosomes are considerably larger: they contain about 70-80 proteins, and an extra molecule of RNA as well[9].

Through use of the method of affinity labeling (Cooperman[6,10]) information has been obtained about the location of functional sites, such as the peptidyltransferase center. In these studies radioactively labeled compounds, which also contain an affinity label, are used. The affinity label enables the molecule to form covalent bond(s) with (a) molecule(s) at its site of interaction.

One should realize that the extensively used method of affinity labeling is a topographical technique, i.e. it identifies ribosomal proteins at or near the peptidyltransferase center. It is therefore possible that the components thus identified may not even include the structural elements exerting the peptidyltransferase activity. Analogs of antibiotics (chloramphenicol, puromycin, lincomycin), which interfere with the peptidyltransferase activity, or analogs of aminoacyl-tRNA (AA-tRNA) have been used in affinity label studies of the peptidyltransferase center. Cooperman examined critically the affinity labeling studies and drew the conclusion that the proteins on the peptidyltransferase site of *E-coli* ribosome are L2, L11, L15, L18, L23, L27 and less securely L14 and L16[10].

The relative position of ribosomal proteins including the proteins present in the peptidyltransferase center can be derived from cross-linking experiments. Figure 4 shows some results of these experiments (R.R. Traut, J.M. Lambert, G. Boileau, J.W. Kenny[6]). The shaded sferes indicate proteins that constitute the peptidyltransferase center.

Fig 4



Beside the method of affinity labeling, partial reconstitution as well as chemical modification have been frequently used in the study of the peptidyltransferase center. Although the experiments using the different methods, yield partly contradictory results, protein L11 and L16 seem to be important in the peptidyltransferase activity[11].

Much less is known about the proteins, which are involved in the peptidyltransferase center/activity of eukaryotes[13]. Studies with rat liver ribosomes lead to partly contradictory results[12].

Finally, the role of the 5S RNA in the peptidyltransferase activity has to be discussed[14,16]. Erdmann showed[15] that 50S ribosomal subunits reconstituted in the absence of 5S RNA possess a strongly reduced peptidyltransferase activity. Thus 5S RNA is important but not essential for peptidyltransferase activity (K.H. Nierhaus[6]). Although the 5S RNA appears to be situated in a functionally important part of the 50S subunit, namely at the interface within a cluster of proteins, which includes L16 - essential - and L11 - important - for peptidyltransferase activity, it cannot yet be assigned a precise function[16].

ASSAY OF THE PEPTIDYLTRANSFERASE ACTIVITY IN VITRO

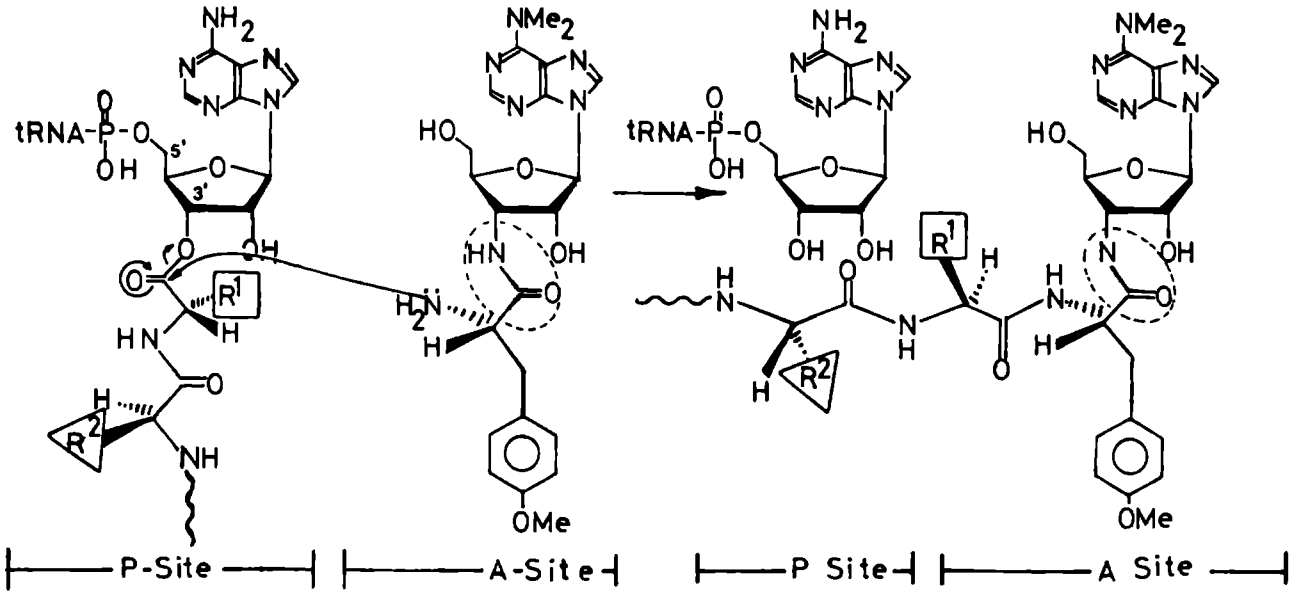
The peptidyltransferase activity may be assayed by determination of one of both products of the reaction as depicted in equation 1 and Scheme I. Often, puromycin is employed, instead of AA-tRNA(analogs), as acceptor substrate for the peptidyl chain (*vide infra*, equation 3).

Peptidyl-tRNA + Puromycin ⟶ tRNA-3'OH + Peptidyl-puromycin (equation 3)

Peptidyl-puromycin + AA-tRNA ⟶✕ tRNA-3'OH + Peptidyl-AA-tRNA (equation 4)

Puromycin, an inhibitor of protein synthesis in both prokaryotic and eukaryotic systems, has some resemblances[10b] with AA-tRNA and can therefore be used as an analog (Scheme II). The amide bond in puromycin may be an essential feature that prevents further chain elongation, once the puromycin has been attached to the peptidyl chain to give a peptidyl-puromycin adduct. For further elongation the amide bond of the aminosugar has to be cleaved. This fission of the amide bond is energetically less favored than the fission of the ester bond, which connects the peptidyl chain to the tRNA molecule (equation 4).

Scheme II Reaction of puromycin with peptidyl-tRNA



Further elongation of the peptidylpuromycin adducts is also prevented because the adducts diffuse away from the ribosome. The amount of formed peptidylpuromycin adducts is a measure of the peptidyltransferase activity.

As a donor substrate i.e. the molecule that furnishes the peptidylmoiety, peptidyl-tRNA and a range of analogs can be used. To facilitate the determination of the amount of puromycin adducts, either the peptidyl-tRNA (analog) or puromycin is radioactively labeled. Mostly the donor substrate (the peptidyl-tRNA analog) is labeled.

In the frequently used 'fragment' reaction - developed by Vazquez, Monro and co-workers[17] - an oligonucleotide fragment of the tRNA molecule, carrying an N-acetyl (radioactively labeled) amino acid at the 3' end, is used as a donor substrate. Another widely used donor substrate is N-acetyl [^{14}C]Phe-tRNA[18], or other N-acylated labeled amino acid tRNA's[19-21]

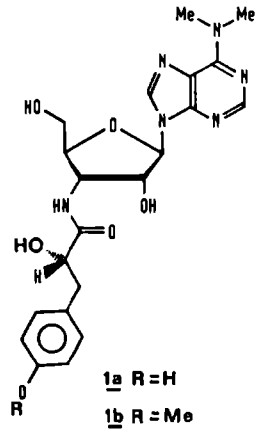
This assay of peptidyltransferase activity in which puromycin is used is denoted as the 'puromycin reaction'[22].

INTERACTIONS OF SPARSOMYCIN WITH THE RIBOSOME

Sparsomycin is a competitive inhibitor of puromycin in the 'puromycin reaction'. In contradistinction to puromycin, the molecular mechanism underlying the action of sparsomycin on the peptidyltransferase center, causing an inhibition of the protein synthesis is completely unknown. Before embarking on the synthesis of analogs, in order to study the molecular mechanism of action, it is sensible to speculate about possible interactions of the sparsomycin molecule with the ribosome.

The inhibition of the protein synthesis caused by sparsomycin may be the result of a non-covalent interaction or the formation of a covalent bond. In the latter case the covalent bond may be formed either with tRNA molecules - as is the case with puromycin - or with essential sites of the peptidyltransferase center. In case of a non-covalent interaction, the transfer of the peptidyl chain might be blocked either by interaction with the receptor site of the peptidyltransferase center or the site(s) of attachement of the tRNA molecules.

A working-hypothesis for a covalent interaction might be that sparsomycin reacts with peptidyl-tRNA's in a similar way as puromycin. One might expect the hydroxy function of sparsomycin to enforce a similar nucleophilic displacement on the ester-carbonyl moiety of the peptidyl-tRNA molecule. Support for this assumption is found in studies with the hydroxy analogs[23] 1a, 1b of puromycin. The hydroxy puromycins react in an N-formylmethionine fragment reaction[24] to give N-formylmethionyl-oxypuromycin adducts. In section 3 of this chapter the synthesis of an analog of sparsomycin having an acylated hydroxy function, is described. This analog is unable to induce the aforementioned nucleophilic displacement with the peptidyl-tRNA molecule. The results presented in section 3 of this chapter indicate that the hydroxy function is not involved in the molecular mechanism of action.



1a R=H

1b R=Me

Other hypotheses can be formulated in which a reactive species is proposed that is generated from sparsomycin, giving rise to the formation of a covalent bond with ribosomal components. This reactive species has to be formed by metabolic processes involving enzymes or enzyme systems. Because sparsomycin also manifests its action in ribosomal systems without additional enzymes or enzyme systems present, these hypotheses are improbable. Thus it seems that the activity of sparsomycin is not due to formation of a covalent bond.

So far no satisfactory molecular mechanism can be offered explaining the inhibitory action of sparsomycin on the protein synthesis.

SPARSOMYCIN AND OTHER INHIBITORS OF THE PEPTIDYLTRANSFERASE ACTIVITY

Other inhibitors[1] of the peptidyltransferase activity include gougerotin, griseoviridin, althiomycin, actinobolin, amicitin, blasticidin S, chloramphenicol, lincomycin, spiramycin III, streptogramin A, anisomycin, tenuazonic acid, trichodermin and althiomycin. The first six compounds act in both prokaryotic and eukaryotic systems, whereas chloramphenicol, lincomycin, althiomycin, spiramycin III and the streptogramin A antibiotics are only active in prokaryotes and anisomycin, tenuazonic acid, trichodermin are only active in eukaryotes. Of this serie chloramphenicol and lincomycin are the best known antibiotics, because they are widely used against bacterial infections. Furthermore they have - like puromycin - extensively been used for preparing peptidyltransferase affinity label reagents[10]. Of all the compounds mentioned in this section, beside puromycin, sparsomycin is the best known compound interacting with the peptidyltransferase center of eukaryotic systems. In addition sparsomycin appears to be the most effective in these systems[1].

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INHIBITION OF THE PROTEIN SYNTHESIS IN YEAST (SACCHAROMYCES CEREVISIAE) CELL-FREE SYSTEMS BY SPARSOMYCIN(1) AND ANALOGS; PRELIMINARY RESULTS OF A STRUCTURE-ACTIVITY RELATIONSHIP STUDY

The contents of this section and that of section 3 of this Chapter consist of the first results of a planned, extensive study on the relationships between the structure of sparsomycin and analogs on one hand and the mode of action as well as the antineoplastic activity on the other hand.
The inhibition of the protein biosynthesis by natural sparsomycin (1a), synthetic sparsomycin (1b) and seven of the analogs listed in Table I was determined in the fragment reaction, which is described in section 1 of this Chapter. In addition, the inhibitory activity of the same compounds in a polyphenylalanine synthesizing system was measured. In the latter system phenylalanine-tRNA is used as the acceptor substrate instead of puromycin, whereas N-acetylphenylalanine-tRNA is used as the donor substrate (see section 1). The assays were carried out by Dr D. Vazquez and Dr J.P.G. Ballesta*.
The concentration of sparsomycin or an analog causing 50% inhibition of the protein synthesis relative to the control cell-free system is denoted as ED_{50}.
There is a close resemblance between the results obtained with cell-free systems (Table I) and those obtained from testing the compounds against intact cells (leukemia L1210 cells) as listed in Table I of section 3.
From the results of the fragment reaction the following conclusions can be drawn. Comparison of the ED_{50} values of 1a and 1b shows that synthetic sparsomycin (1b) is practically as active as natural sparsomycin (1a). This is not surprising since 1a and 1b are chemically identical. The necessity of an S configuration of sparsomycin's chiral carbon atom for an optimal activity can be concluded unambiguously from a comparison of the inhibitory activities of 1 and 4. Conformation of this conclusion is found in the ED_{50} values of 12 and 13; compound 12 - having an S configuration of the chiral carbon atom - is about three times as active as its enantiomer 13. Compound 3, which differs only from sparsomycin (1a and 1b) in having the opposite chirality of the sulfoxide-sulfur atom, has about one fourth of the activity of the natural product; from this we conclude that the R configuration of the sulfoxide-sulfur atom is important but not a requisite for the activity.
The importance of the oxidation state of the α sulfur atom can be derived from a comparison of the ED_{50} values of 1 and 3 on one hand and 12 on the other hand. The compounds having a sulfoxide function at the α-position are three to ten times more active than the compound having a bivalent sulfur atom at this position. Comparison of the activities of 1 and 14 demonstrates that the proper position of the sulfoxide moiety in the cysteinol side chain - i.e. in α-position - is of importance too.
Finally, the relatively low ED_{50} value of the mixture of 1b and 16 (ratio 2/5) suggests that isosparsomycin (16) has a considerable activity; the ED_{50} value, that is calculated from the ED_{50} value of the mixture, amounts to 14 μg/mL. This is in contrast with the results from the assay with L1210 cells, where isosparsomycin (16) showed a very low activity, if any (see Table I, section 3). In the polyphenylalanine synthesizing, cell-free yeast system, only sparsomycin (1a and 1b) and the mixture of 16 and 1b showed appreciable activity.

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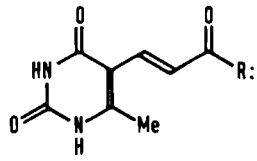
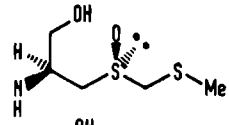
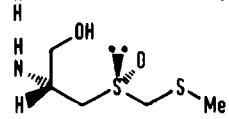
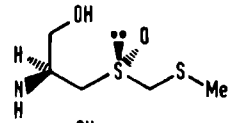
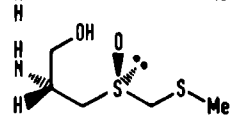
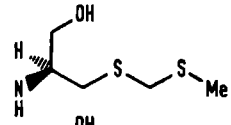
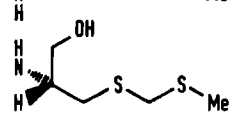
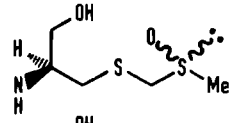
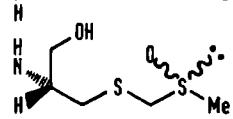
Apparently, this cell-free system does not discriminate between the activity of the various less active analogs at the concentrations tested. So far, we have no explanation for this observation, the more so as the results of the fragment reaction assay show a good correlation with the results obtained in intact cell systems.

Table I



R:

R:		chirality		ED_{50} Fragment Reaction µg/mL	ED_{50} Fragment Reaction M×10^5	ED_{50} polyphe * System µg/mL	ED_{50} polyphe * System M×10^5
	1	S_C - R_S	1a	6	1.7	1.8	0.5
			1b	7	2.0	1.8	0.5
	2	R_C - S_S		1084	300	–	–
	3	S_C - S_S		24	6.5	–	–
	4	R_C - R_S		240	65	–	–
	12	S_C		60	17.5	–	–
	13	R_C		207	60	–	–
	14	S_C - R_S / S_C - S_S		123	34	–	–
	15	R_C - R_S / R_C - S_S		not tested		not tested	
isosparsomycin	16**	S_C - R_S		11	3.0	225	62

*The highest dose tested was 1450 µg/mL (2×10^{-3} M); no value is reported for compounds showing no activity at this dose.

**A mixture of 40% sparsomycin(1) and 60% isosparsomycin(16) was tested in the assay (see section 3).

CHAPTER V.3.

STRUCTURE-ACTIVITY RELATIONSHIPS OF SPARSOMYCIN AND ANALOGS; OCTYLSPARSOMYCIN: THE FIRST ANALOG MORE ACTIVE THAN SPARSOMYCIN

STRUCTURE ACTIVITY RELATIONSHIPS OF SPARSOMYCIN AND ANALOGS; OCTYLSPARSOMYCIN: THE FIRST ANALOG MORE ACTIVE THAN SPARSOMYCIN

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Abstract. The analogs 2-4, 12-17 and 20 of sparsomycin(1) were synthesized and their cytostatic activity was studied in an *in vitro* clonogenic L1210 assay, by measuring the inhibition of colony formation. The activity of an analog, expressed as an ID_{50} value was compared to that of sparsomycin (Table I). Each analog possesses not more than two structural modifications of the sparsomycin molecule 1. This enabled us to determine unambiguously the structural and stereochemical features that are required for an optimal biological activity in this assay. It is shown that the S configuration of the chiral carbon atom and the presence of an oxygen atom on the (α) sulfur atom are essential for an optimal activity, whereas the R chirality of the sulfoxide-sulfur atom of sparsomycin is of importance. Isomerization of the E-double bond into the Z-double bond yields isosparsomycin(16), which has a drastically decreased activity. This finding is noteworthy as we observed that authentic sparsomycin(1) is contaminated by isosparsomycin(16). The hydroxy function is probably not involved in the molecular mechanism of action, as acylation of this function does not affect the activity of sparsomycin. In addition, the cytostatic activity seems to be related to the lipophilicity of the effector molecule; octylsparsomycin 19 was shown to be three times as effective as sparsomycin. In our assay, this analog has a comparable activity to that of the clinically used antitumor agents 5-fluoro uracil and adriamycin.

INTRODUCTION

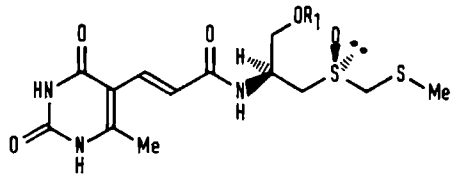
The development of a flexible synthesis or synthetic methodologies for (a) structural fragment(s) of a particular molecule possessing an interesting or important biological activity, is a prerequisite for thorough studies on the biological activity and/or biochemical mechanism of interaction.

An outstanding example, which underlines this view, is sparsomycin[1] 1. The structure activity relation studies, which have appeared so far[2-4], were hampered by the absence of a total synthesis. As a consequence they concern analogs, in which several structural parameters had been varied simultaneously[5], thus allowing only a limited interpretation of the results with regard to the role of the various structural fragments.

The interpretation and comparison of the available information on structure activity relationships of sparsomycin encounters another difficulty: the biological activity of the analogs has been determined in different systems *in vitro*: KB cell culture[2] and cell-free ribosomal systems[3], *in vivo*: P-388 system and Walker 256 system[4]. This hampers comparison of the results.

Sparsomycin[1] 1 has attracted widespread attention because of its biological activity.

This activity is primarily due to a strong inhibition of the protein biosynthesis resulting in a decline of the protein synthesis and concomitant biological effects. There is ample evidence[6] that sparsomycin has its site of interaction in the large ribosomal subunit, where it prevents peptide transfer by interfering with the peptidyltransferase center. Sparsomycin manifests its action in intact prokaryotic cells[7], eukaryotic cells[8] - including transformed[2,9] and/or virus infected cells[10] - and in various cell-free systems[11]. The behavior of sparsomycin with regard to its inhibitory action and its influence on the polyribosomes has also been investigated *in vivo*[12]. Since sparsomycin had been shown to be active against transformed cells *vide supra* and various tumors[9a] , it has been investigated as a potential cytostatic compound. A clinical trial of sparsomycin, however revealed eye toxicity[13].



1 R=H : Sparsomycin (S_C-R_S)

17 R=  : O-Acetyl sparcomycin (S_C-R_S)

Recently, both we[15,16] and Helquist and Shekhani[17] succeeded in developing a total synthesis of sparsomycin. In addition we were able to synthesize analogs[15] in order to carry out structure-activity relation studies. The synthesis of analogs also seems promising for studies of the molecular mechanism of action of sparsomycin. Moreover attempts will be made to develop a molecule with more selective biochemical and pharmacological properties, while determining the minimum structural and stereochemical requirements for the anti-tumor activity.

As a first contribution to the realization of these aims, we wish to report here the synthesis of a number of relevant analogs and the investigation of their activity against lymphocytic leukemia L1210 cells *in vitro*[14].

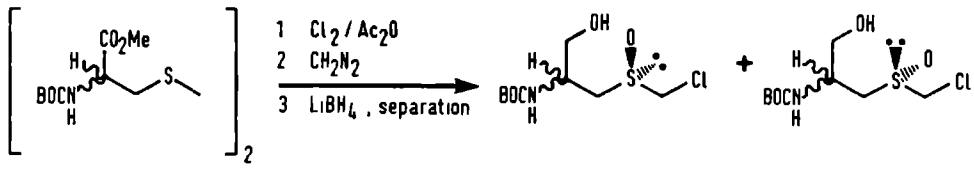
RESULTS and DISCUSSION

Choice and synthesis of the analogs

One of the synthetic routes, which we have developed earlier[15] for the preparation of sparsomycin 1[18] and analogs 2-4 (Table I), features the employment of the cysteinol α-chlorosulfoxide (6a-b, 7a-b) as a cruxial synthon (Scheme I). This synthon is prepared in three steps starting from BOC-D-cystine methylester S_C 5a or BOC-L-cystine methylester R_C 5b (Scheme I). As was described earlier[15] reaction of the resulting stereoisomers 6a, 7a, 6b and 7b with sodiummethylmercaptide leads - after deprotection with trifluoroacetic acid and coupling with the uracil acrylic acid fragment 11 - to sparsomycin 1 and its stereoisomers 3, 4 and 2 respectively. The analogs 2-4 allow us to study the dependence of the biological activity on the absolute configuration of the chiral carbon atom as well as the sulfoxide sulfur atom.

Compounds 12, 13, 14 and 15 (Table I) were included in order to evaluate the role of the oxidation state of the sulfur atom, as well as the influence of the position of the sulfoxide moiety: S(α) or S(β).

Scheme I



5a (S_C)
5b (R_C)

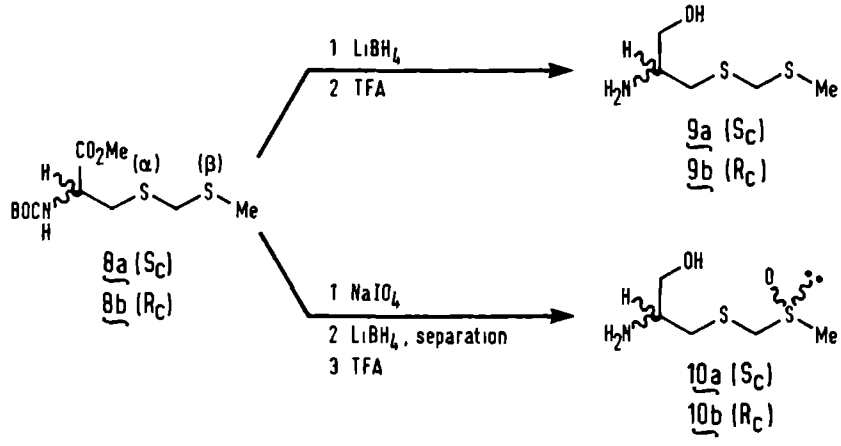
6a (S_C-R_S)
6b (R_C-R_S)

7a (S_C-S_S)
7b (R_C-S_S)

To circumvent partial racemization of the chiral carbon atom during the synthesis of the S-deoxo analogs 12 and 13, we first reduced 8a and 8b to the corresponding amino alcohols with lithium borohydrate (Scheme II). The proton at the chiral carbon atom in the amino alcohol is less acidic; as a result the chiral carbon atom is less prone to racemization. Subsequently, the BOC group was removed by TFA and amino alcohols 9a and 9b (Scheme II) were coupled to the uracil acrylic acid fragment 11 to give 12 and 13 in 31% and 28% yield, respectively.

We prepared 14 and 15 starting from 8a and 8b in four steps (Scheme II). Oxidation with sodium metaperiodate of 8a and 8b, resulted in formation of the regio isomer containing an (β)sulfoxide in excess over the (α)sulfoxide regio isomer (ratio β/α ca. 4/1). These regioisomers were separated by HPLC after reduction of the ester function. Removal of the N-protecting group led to 10a and 10b respectively, which were coupled in a mixed anhydride procedure with 11 to give 14 (54%) and 15 (47%) respectively.

Scheme II



8a (S_C)
8b (R_C)

9a (S_C)
9b (R_C)

10a (S_C)
10b (R_C)

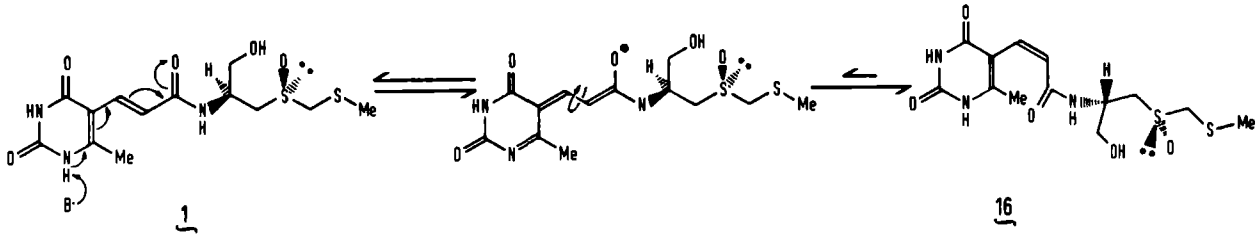
In the course of the synthesis of sparsomycin[15], we invariably noticed a by-product, which appeared after the last step - i.e. the coupling procedure - in the total synthesis. The by-product - albeit present in a small amount - is hardly to remove from sparsomycin, by chromatography or gelfiltration.

By ^{1}H-NMR it was shown that this product was the cis-isomer of sparsomycin, i.e. 16 (Scheme III). As perusal of the NMR spectrum of an authentic sample of sparsomycin[20] showed also the presence of a significant amount of the cis-isomer we decided to investigate this phenomenon more extensively. It has been shown before by Wiley and MacKellar[21], that irradiation of sparsomycin with a fluorescent desk lamp for a long period (7 days) resulted in the formation of cis-isomerized sparsomycin ('isosparsomycin') 16 in 20% yield. In repeating the irradiation experiment with some modification of the experimental conditions, we found, by monitoring the trans-cis conversion by NMR, that irradiation for 20 minutes with a 300nm lamp is sufficient to produce a mixture of isosparsomycin and sparsomycin in a ratio of 4/1. Longer periods of irradiation did not result in complete conversion to isosparsomycin. Although isosparsomycin is likely to be thermodynamically less stable than sparsomycin, it is present in large excess in the irradiation mixture at the photo-equilibrium situation (Scheme III). This is probably due to a change in the UV spectrum in going from sparsomycin to isosparsomycin: the λmax changes from 300nm to 290nm; in addition, the molar absorbance decreases to about one third. In order to avoid isomerization, sparsomycin should be preferentially stored as a solid in the dark, because we noticed that the compound in solution isomerizes slowly when exposed to daylight or laboratory TL-light[22]. In addition, we noticed that alkaline solutions of sparsomycin always contain a higher percentage of isosparsomycin. This result is in accordance with the finding that upon irradiation in the presence of base sparsomycin isomerizes more rapidly to isosparsomycin. We suppose that this base catalyzed isomerization involves an intermediate as depicted in Scheme III.

To study the biological activity of isosparsomycin[23], we prepared a mixture of 40% sparsomycin(1) and 60% isosparsomycin(16), by irradiation[23].

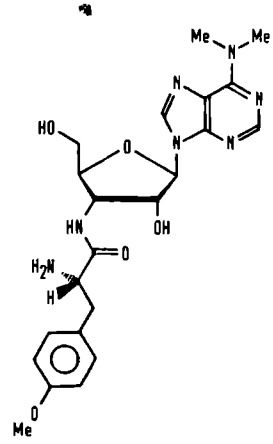
The (base-catalyzed) radiation induced isomerization is also observed with the stereoisomers 2-4 of sparsomycin, the S-deoxy analogs 12 and 13, and the pseudo-sparsomycin analogs 14 and 15.

Scheme III



The inclusion of compound 17, the O-acylated derivative of sparsomycin also deserves further comment. The reason for assigning a possible role to the hydroxy function originates from the vast amount of work on an other inhibitor of the protein biosynthesis namely, puromycin[24] (18). Puromycin and sparsomycin interact on the same site of the ribosome. In addition, they share some structural features. Both contain a nucleotide base residue, enabling them to interact with ribosomal and/or messenger RNA. Furthermore, both contain a modified amino acid part, which is the structural feature ultimately responsible, for preventing the continuation of the protein synthesis as has been proven for puromycin.

The molecular mechanism underlying the blocking of the protein synthesis by puromycin features a S_N2 like nucleophilic attack of the amino group of this molecule on the carbonyl moiety of the peptidyl-tRNA, resulting in the formation of peptidyl-puromycin adducts. Analogs of puromycin, which contain a hydroxy group instead of an amino function, also display a nucleophilic reaction with the peptidyl-tRNA , to form peptidyl-oxypuromycin adducts[25].



18 puromycin

Sparsomycin might enforce a similar nucleophilic displacement as the hydroxy puromycin analog. This proposed mode of molecular action can be studied by blocking the hydroxy group of sparsomycin.

The O-acetyl analog 17 was prepared by treatment of sparsomycin(1) with acetylchloride and triethylamine in 30% yield.

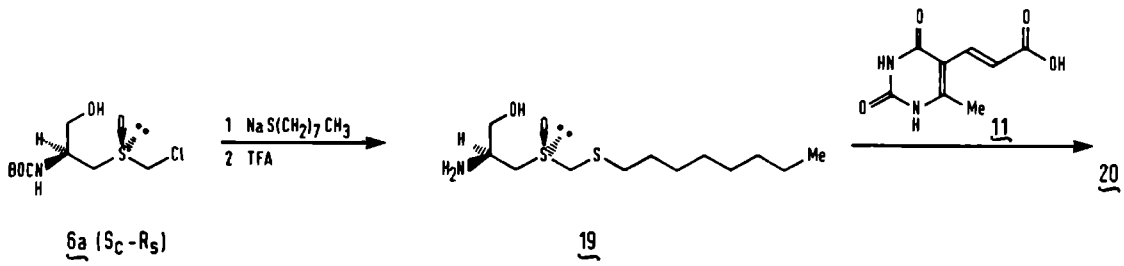
Beside the aforementioned modifications of sparsomycin, variations leading to a less polar molecule are important. It has been shown[26] that sparsomycin displays no activity against intact reticulocytes. This has been ascribed[26] to the inability of sparsomycin to penetrate into these cells, which might be due to its polar character.

Therefore the octyl analog of sparsomycin 20 has been chosen to study whether an increased lipophilicity will result in an increase of the biological activity, because diffusion of the effector molecule into the cell might be facilitated. Furthermore, we observed[27] in pharmacokinetic experiments in the dog, that sparsomycin has a short half life of elimination (70 minutes). A more lipophilic compound might have a longer half life and thus may be used in smaller quantities to reach a certain plasma level. The octyl analog 20 was prepared using the α-chlorosulfoxide 6a, which was converted to the cysteinol mono-oxodithioacetal with sodiumoctylthiolate (Scheme IV). Deprotection with trifluoroacetic acid at 0 °C and subsequent deprotonation with an ionexchange resin gave the amino alcohol 19 in 85% yield, which was coupled thereafter to the uracil acrylic acid fragment 11 to give 20 in 37% yield.

Biological activity

Colony assays are widely used to measure the response of established lines of animal and human tumor cells treated with cytotoxic agents. Recently, for example, the Raji cell culture line of Burkitt's lymphoma[28] has been used to determine the effect of several anticancer drugs on the ability of these cells to form colonies in soft ager. The results also suggest that established human tumor cell lines may be useful for the screening of new anticancer drugs.

Scheme IV



6a (S_C-R_S) 19

The growth of tumor colonies in soft agar from primary human tumor explants[29] is even more promising. Preliminary results indicate that the assay is 90-95% accurate in predicting clinical resistance and 60-65% in predicting a clinical response[30,31]. Furthermore, this assay is of potential importance as a screening test for new antitumor agents[32].

For a first evaluation of the relation between structure and antitumor activity of sparsomycin and analogs, we used an *in vitro* clonogenic assay of leukemia L1210 cells. Study of the activity of sparsomycin and relevant analogs against tumor cells derived from primary human tumors is under present investigation[33]. The leukemia L1210 *in vivo* system (in the mouse) is generally used in standard screening of compounds of potential interest[32,34]. However, for certain (semi)quantitative studies the L1210 *in vitro* system is more sensitive and more practical. This system has been used in suspension culture[35] and in soft agar medium[36,37].

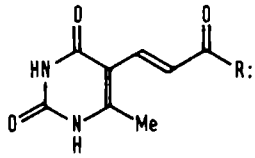
In this study we used leukemia L1210 cells in soft agar medium (0.3%) in an *in vitro* clonogenic assay. It has been observed before (P. Lelieveld, unpublished observations), that there is a good correlation between the *in vitro* and *in vivo* activity of the drugs tested. Thus, the *in vitro* system is of predictive value for the *in vivo* system. Inhibition of L1210 colony formation by sparsomycin and analogs was determined for several concentrations and the dose causing 50% inhibition of colony formation ID_{50} relative to untreated control cells was calculated. The results are collected in Table I.

DISCUSSION

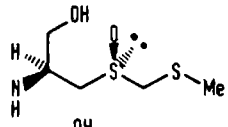
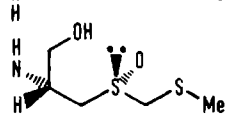
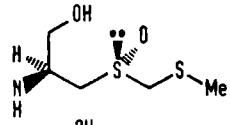
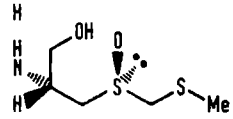
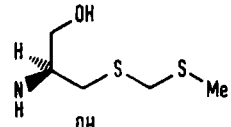
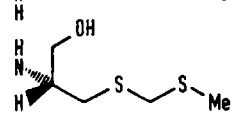
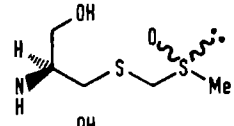
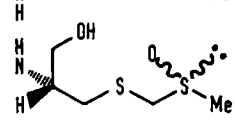
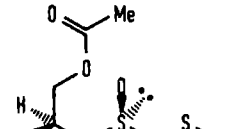
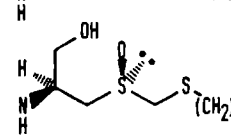
Comparison of the ID_{50} value of 1 with that of 4 unambigeously demonstrates the necessity of a S configuration of the chiral carbon atom (S_C) for an optimal biological activity). This has been suggested earlier by Vince *et al*[3] and by Lin and Dubois[2]. However, their conclusion was based on experiments with analogs containing more than one modification. Confirmation of this conclusion is found in the ID_{50} values of the S-deoxy analogs 12 and 13 and of the pseudo analogs 14 and 15: compounds having an S configuration of the chiral carbon atom have a significantly lower ID_{50} value.

The higher ID_{50} value of the analog 3, which only differs with sparsomycin in having the opposite chirality of the sulfoxide-sulfur atom, clearly demonstrates the importance of an R configuration of the sulfoxide-sulfur atom (R_S). A similar difference in biological activity between molecules, which only differ in chirality of the sulfoxide-sulfur atom has been observed - although evaluated *in vivo* - with Amanita toxins[38]. 6-Methoxy-α-amanitin, having a sulfoxide-sulfur atom with the R configuration, is at least ten times more toxic than the corresponding compound with the S configuration.

›le I



R:

R:		chirality	ID_{50} (µg/mL)*
	1	$S_C - R_S$	0.15
	2	$R_C - S_S$	—
	3	$S_C - S_S$	2.1
	4	$R_C - R_S$	—
	12	S_C	4.4
	13	R_C	—
	14	$S_C - R_S$ / $S_C - S_S$	66
	15	$R_C - R_S$ / $R_C - S_S$	—
	16**	$S_C - R_S$	0.3
	17	$S_C - R_S$	0.15
	20	$S_C - R_S$	0.05

* The highest dose tested was 100 µg/mL; no value is reported for compounds showing no activity at this dose.

** A mixture of 40% sparsomycin(1) and 60% isosparsomycin(16) was tested in the assay.

The importance of the presence of the oxygen atom on S(α) can be derived from a comparison of the ID_{50} values of 1 and 3 on one hand and 12 on the other hand. This is in accordance with the findings of Lin and Dubois[2], who demonstrated that the biological activity - if any - of the synthetically more accessible monosulfide analogs (R=cysteinol-S-alkyl) is invariably lower than the biological activity of the corresponding sulfoxide analogs (R=cysteinol-S(O)-alkyl).

The importance of the proper position of the sulfoxide moiety in the cysteinol side chain of 1, is demonstrated by comparing the ID_{50} values of 1 and 14: when the positions of the sulfoxide function and the sulfur atom are reversed,the biological activity is markedly reduced. In addition, the pseudo-sparsomycin analog 14 possesses a diminished activity compared to the corresponding S-deoxy-analog 12, suggesting an adverse effect of a sulfoxide function at this position of the molecule.

Although study of the biological activity of the separate diastereomers of 14 might give additional insight into the role of the stereochemistry of the (β)sulfoxide function, no attempts were made to separate these diastereomers as this would not lead to a more active antitumor agent. Comparison of the ID_{50} value of 14 with those of 1 and 3 shows that the mono-oxodithioacetal moiety as such does not determine the biological activity. Apparently a bivalent (β)sulfur atom is preferable for an optimal activity.

The ID_{50} value of the mixture of 1 and 16 (ratio 2/3) is about two times the ID_{50} value of pure 1. This suggests that isosparsomycin(16) has a very low activity, if any. However, this result should be interpreted with caution, because of the error present in this biological assay. It will only be regarded as indicative and future experiments will be necessary to clarify the activity of sparsomycin(16) itself[23].

The ID_{50} value of the octyl-analog of sparsomycin i.e. 20 is three times lower than that of sparsomycin itself. This result supports our assumption that an increase of sparsomycin's lipophilicity, facilitates cell penetration. The high activity, expressed as a low ID_{50} value, demonstrated in this L1210 clonogenic assay, is comparable to those found for the clinically used cytostatic compounds 5-fluorouracil and adriamycin which have ID_{50} values of 0.04 μg/mL and 0.03 μg/mL respectively in the same assay. (P. Lelieveld, unpublished observations).

At first glance a comparison of the ID_{50} value of 1 and the O-acetyl-analog 17 suggests that the hydroxy function of sparsomycin is not involved in the molecular mechanism, underlying the inhibitory action of sparsomycin on the protein synthesis (*vide supra*). However, this result has to be confirmed by cell-free experiments, because in this clonogenic assay the compound is in continuous contact with the L1210 cells and the components of the medium. This continuous contact may lead to (metabolic)hydrolysis of the O-acetyl-analog to give sparsomycin, so that the observed activity may be partly due to the presence of - liberated - sparsomycin. In cell-free experiments the compound to be tested is present in the reaction mixture for a shorter period and no metabolism is likely to take place.

Under present investigation is the antitumor activity of sparsomycin and octylsparsomycin in mouse against leukemia L1210 as well as solid tumors. The results will indicate whether the *in vitro* prediction of the higher activity of octylsparsomycin compared to sparsomycin (*vide supra*) reflects the (*in vivo*) situation.

EXPERIMENTAL PART

Biological activity

The L1210 *in vitro* clonogenic assay used in this study is an improved variant of the method described earlier[36,39] for the growth into colonies of L1210 cells in soft agar medium.

From a suspension culture, 100 L1210 cells were plated into 35 mm culture dishes (Falcon), containing 1 ml of soft agar growth medium and the compound to be tested in appropriate concentrations. The soft agar growth medium consisted of Dulbecco's medium supplemented with 20% horse serum, 60 µmol 2-mercaptoethanol, 20 mg/ml L-asparagine, 75 mg/ml DEAE dextran (molecular weight $2x10^6$) and 0.3% bacto agar (Difco). The culture dishes were incubated at 37 °C in an atmosphere of 10% CO_2 in humidified air for 8 days. After this period of continuous drug exposure, colonies were counted and dose-effect curves were made. From these curves the drug dose causing 50% inhibition of colony formation (ID_{50}) relative to untreated control cells was calculated.

Synthesis

^{1}H-NMR spectra were measured on a Varian Associates Model T-60 or a Bruker WH-90 spectrometer with Me_2Si or $Me_3SiCD_2CD_2CO_2Na$ as an internal standard. UV spectra were measured on a Perkin-Elmer spectrophotometer, Model 555. For determination of the specific rotation, a Perkin-Elmer 241 polarimeter was used. The irradiation experiments were carried out in a Rayonet RPR 100 or RPR 200 photochemical reactor, fitted with 300 nm lamps,in pyrex tubes. 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 TDM. For column chromatography, Merck silica gel H type 60 was used. A miniprep LC (Jobin Yvon) was used for preparative HPLC.

Sparsomycin(1); sparsomycin enantiomer 2; sparsomycin diastereomer 3; sparsomycin diastereomer 4

Compounds 1, 2, 3 and 4 were prepared as described earlier[15]. However, the reactions were carried out under the exclosure of light and the purification of the end-product was changed: the crude products were chromatographed over silica gel by HPLC (eluent MeOH/CH_2Cl_2/NH_4OH, 80/20/0.2 v/v),followed by gelfiltration over Sephadex LH 20 (eluent H_2O/MeOH, 15/85 v/v).

S-[(methylthio)methyl]-D-cysteinol(9a); S-[(methylthio)methyl]-L-cysteinol(9b)

The ester function of 8a and 8b (885 mg, 3 mmol) was reduced with lithium-borohydrate as has been described earlier[15]. The N-protected cysteinol derivative was purified by HPLC (eluent MeOH/CH_2Cl_2, 5/95 v/v). Subsequently, the BOC group was removed by trifluoroacetic acid as described for the preparation of 19 (*vide infra*) to give 9a (40%) and 9b (50%), respectively. 9a and 9b: R_f 0.56 (eluent sec-BuOH/NH_4OH, 5/2 v/v); NMR (CD_2Cl_2) δ2.18 (s, 3H, SCH_3), 2.73-2.93 (m, 2H, $CHCH_2S$), 3.67 (s, 2H, SCH_2S), 3.69-3.89 (m, 3H, $CHCH_2OH$).

S-[(methylthio-oxo)methyl]-D-cysteinol(10a); S-[(methylthio-oxo)methyl]-L-cysteinol(10b)

Compounds 8a and 8b (932 mg, 3 mmol) were oxidized with sodium metaperiodate followed by reduction with lithiumborohydrate according to procedures described earlier[15]. The regio isomers containing an (β)sulfoxide were separated from those with an (α)sulfoxide function by HPLC (eluent MeOH/CH_2Cl_2, 5/95 v/v). The desired compounds had a lower R_f value on TLC than the corresponding regio isomers. No attempts were made to separate the N-protected diastereomers of 10a (S_cR_s-S_cS_s) or 10b (R_cR_s-R_cS_s). Deprotection of the aminofunction was achieved by treatment with trifluoroacetic acid, as is described for the preparation of 19 (*vide infra*). The products were obtained in 85% yield.

10a and 10b: R_f 0.42 (eluent sec-BuOH/NH_4OH, 5/2 v/v); NMR (CD_2Cl_2) δ2.6 (s, 3H, SCH_3), 2.67-3.20 (m, 2H, $CHCH_2S(O)$), 3.20-3.62 (m, 3H, $CHCH_2OH$), 3.20-3.98 (AB spectrum partly covered by $CHCH_2OH$ signals, 2H, $S(O)CH_2S$).

S-deoxo-(S_c)sparsomycin(12); S-deoxo-(R_c)sparsomycin(13).

Compounds 12 and 13 were obtained by coupling of (E)-3-(2,4-dioxo-6-methyl-5-pyrimidyl)acrylic acid (11)[15] with 9a and 9b, respectively (each 250 mg, 1.5 mmol), in a mixed anhydride procedure as has been described earlier[15] for the preparation of 1-4.

The yields were 31% and 28% respectively after HPLC (eluent MeOH/CH_2Cl_2, 9/91 v/v).
12: R_f 0.51 (eluent MeOH/$CHCl_3$, 1/4 v/v); $[\alpha]_D^{25}$ + 82° (c 0.205, water); NMR(D_2O) δ2.18 (s, 3H, SCH_3), 2.42 (s, 3H, C(6)CH_3), 2.76 and 3.03 (AB part of ABX spectrum, 8 lines, J_{AX} = 8 Hz, J_{BX} = 5 Hz, J_{AB} = 14 Hz, 2H, $CHCH_2S(O)$), 3.59-3.88 (m, 2H, CH_2OH), 3.78 (s, 2H, SCH_2S), 4.07-4.37 (br. 1H, $CHCH_2OH$), 7.08 and 7.41 (AB spectrum, J_{AB} = 15.5 Hz, 2H, HC=CH).
13: was identical in every aspect with 12, excepting $[\alpha]_D^{25}$, which had a value of -76° (c 0.225, water).

S_C-pseudosparsomycin(14); R_C-pseudosparsomycin(15).

Compounds 14 and 15 were obtained by coupling of 11 with 10a and 10b, respectively (each 275 mg, 1.5 mmol) in a mixed anhydride procedure as has been described earlier[15]. The yields of 14 and 15 were 54% and 47% respectively.
14: R_f 0.14 (eluent MeOH/$CHCl_3$, 1/4 v/v), $[\alpha]_D^{25}$ + 89° (c 0.218, water); NMR (D_2O) δ2.42 (s, 3H, C(6)CH_3), 2.77 (s, 3H, S(O)CH_3), 2.77-3.27 (m, 2H, $CHCH_2S(O)$), 3.60-3.90 (AB part of ABX spectrum, 2H, CH_2OH), 3.91-4.16 (AB spectrum or two doublets, 2H, $SCH_2S(O)$), 7.09 and 7.40 (AB spectrum, J_{AB} = 15.5 Hz, HC=CH).
Compound 15 was identical with 14 in every aspect excepting $[\alpha]_D^{25}$, which had a value of -82° (c 0.174, water).

Isosparsomycin(16)

A solution of sparsomycin(1) (30 mg, 0.08 mmol) in 20 ml of water was irradiated at 300 nm in a pyrex tube for 50 minutes. Subsequently, the solvent was removed by freeze-drying. The isosparsomycin(16)/sparsomycin(1) ratio was shown to be 3/2, as was determined by NMR, from the ratio of integration of the signals due to the cis HC=CH protons (δ 6.26 and 6.55) on one hand, and the signals due to the trans HC=CH protons (δ 7.07 and 7.41) on the other hand. This ratio corresponds to the ratio of integration of the C(6)CH_3 (δ 2.17) signal in isosparsomycin and the C(6)CH_3 (δ 2.40) signal in sparsomycin. Except for these signals, the remaining signals in the ^{1}H-NMR spectrum of 16 and 1 coincide. R_f 0.17, for comparison R_f 1 : 0.21 (eluent, MeOH/$CHCl_3$, 1/4 v/v); R_f 0.22, for comparison R_f 1 : 0.35 (eluent n-BuOH/EtOH/H_2O, 70/27/3 v/v).

O-acetyl-sparsomycin(17)

To a solution of sparsomycin(1) (46.8 mg, 0.13 mmol) in 5 ml of dry pyridine was added 3 ml (0.39 mmol) of a 0.13 M solution of acetylchloride in dichloromethane. The reaction was stirred overnight at room temperature. After completion of the reaction as was monitored by TLC (eluent MeOH/$CHCH_3$, 1/4 v/v), 2 ml of dry ethanol was added. Subsequently the solvent was evaporated in vacuo and the crude product was chromatographed over silica gel 60 H (HPLC, eluent MeOH/CH_2Cl_2, 12/88 v/v). The product was obtained after final purification over Sephadex LH20 (eluent H_2O/MeOH, 15/85 v/v) in 30% yield. No attempts were made to improve the yield. R_f 0.64 (eluent MeOH/$CHCl_3$, 1/4 v/v); NMR (D_2O) δ2.13 (s, 3H, C(O)CH_3), 2.31 (s, 3H, SCH_3), 2.43 (s, 3H, C(6)CH_3), 3.07-3.46 (AB part of ABX spectrum, 2H, $CHCH_2S(O)$), 3.96 and 4.18 (AB spectrum, J_{AB} = 14 Hz, 2H, S(O)CH_2S), 4.20-4.44 (m, 2H, $CHCH_2O$), 4.44-4.70 (m, 1H, $CHCH_2O$), 7.04 and 7.43 (AB spectrum, J_{AB} = 15.5 Hz, 2H, HC=CH).

S-oxo-S-[(octylthio)methyl]-D-cysteinol(19)

A solution of sodium octylthiolate[40] (353 mg, 2.1 mmol), of which the purity was checked as described earlier[15] in 10 ml of dry ethanol was added at once to a stirred solution of the chloro-sulfoxide 6a (542 mg, 2 mmol) in 10 ml of dry ethanol. The preparation of 6a has been reported earlier[15]. Argon had been passed through both solutions for 15 minutes. The reaction mixture was stirred overnight at room temperature. After completion of the reaction, as was monitored by TLC (eluent MeOH/CH_2Cl_2, 1/9 v/v), the solvent was evaporated, and water (5 ml) and dichloromethane (30 ml) were added. Removal of the turbidness, due to finely divided sodium chloride, could be achieved by stirring with Na_2SO_4 for about 1 h. Filtration and removal of the solvent afforded the BOC-protected S-oxo-S (octylthio)methyl -D-cysteinol in 85% yield.

R_f 0.45 (eluent MeOH/CH_2Cl_2, 1/9 v/v); NMR($CDCl_3$) δ 0.67-2.16 (m, 15H, $(CH_2)_6CH_3$), 1.44 (s, 9H, t-Bu), 2.56-2.82 (m, 2H, $SCH_2(CH_2)_6CH_3$), 2.96 and 3.22 (AB part of ABX spectrum, 8 lines, $J_{AX} = J_{BX} = 6$ Hz, $J_{AB} = 14$ Hz, 2H, $CHCH_2S(O)$), 3.71-3.97 (m, 4H, CH_2OH, $S(O)CH_2S$), 3.97-4.28 (br, 1H, $CHCH_2OH$), 5.22-5.33 (br, 1H, NH); Anal. Calcd. for $C_{17}H_{35}NS_2O_4$: C, 53.53; H, 9.29; N, 3.67. Found: C, 53.68; H, 9.31; N, 3.70. For removal of the BOC group, the compound (190 mg, 0.5 mmol) was dissolved in 10 ml of trifluoroacetic acid. The solution was stirring for 30 minutes at 0 °C, after which the trifluoroacetic acid was evaporated in vacuo at room temperature. The residue was dried in vacuo over for 1 h and then dissolved in a minimal amount of water. The solution was placed on a ion-exchange column (Amberlite IRA-410, 20-50 mesh OH^- form). Elution with water and removal of the solvent by freeze-drying gave 19 in 90% yield. R_f 0.40 (eluent MeOH/$CHCl_3$, 1/4 v/v); NMR(CD_2Cl_2) δ 0.70-2.20 (m, 15H, $(CH_2)_6CH_3$), 2.60-2.85 (m, 2H, $SCH_2(CH_2)_6CH_3$), 2.95 and 3.23 (AB part of ABX spectrum, 8 lines, $J_{AX} = J_{BX} = 6$ Hz, $J_{AB} = 14$ Hz, 2H, $CHCH_2S(O)$, 3.70-3.98 (m, 4H, CH_2OH, $S(O)CH_2S$), 3.98-4.35 (br, 1H, $CHCH_2OH$).

Octylsparsomycin 20

Compound 20 was prepared by a mixed anhydride procedure as follows. To a stirred, cooled (0 °C) solution of the acid 11 (112 mg, 0.66 mmol) and triethylamine (86 mg, 0.86 mmol) in 5 ml of THF/DMF (1/1 v/v) was added ethylchloroformate (103 mg, 0.86 mmol). Stirred was continued at 0 °C for 4 h. Subsequently a solution of the amino alcohol 19 (140 mg, 0.5 mmol) in 5 ml of THF/DMF (1/1 v/v) was added dropwise. The reaction was stirred at room temperature for 48 h, under the exclosure of light. The solvents were removed in vacuo at room temperature. The crude product was chromatographed over silica gel 60H by HPLC (eluent MeOH/CH_2Cl_2, 1/4 v/v), followed by gelfiltration over Sephadex LH-20 (eluent H_2O/MeOH, 15/85 v/v). 20 was obtained in 17% yield. R_f 0.43 (eluent MeOH/$CHCl_3$, 1/4 v/v), $[\alpha]_D^{25}$ 3.3° (c 0.095, MeOH/H_2O, 1/1 v/v); NMR(D_2O/K_2CO_3) δ 0.58-2.33 (m, 15H, $(CH_2)_6CH_3$), 2.35 (s, 3H, $C(6)CH_3$), 2.55-2.84 (m, 2H, $SCH_2(CH_2)_6CH_3$), 2.84-3.49 (m, 2H $CHCH_2S(O)$), 3.49-3.87 (m, 2H, $CHCH_2OH$), 3.87-4.16 (br, s, 2H, $S(O)CH_2S$), 4.40-4.67 (m, 1H, $CHCH_2OH$), 6.95 and 7.61 (AB spectrum, $J_{AB} = 16$ Hz, HC=CH).

ACKNOWLEDGEMENT

Parts of the investigations were supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO) and the Netherlands Cancer Foundation, Koningin Wilhelmina Fonds.

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4. *THE ANTITUMOR ACTIVITY OF SPARSOMYCIN*

EVALUATION OF THE AVAILABLE DATA

Clinical Phase I Study

In 1964[1] sparsomycin was selected for a Phase I clinical study on the basis of its activity against KB cells (the KB cell culture is a cell line derived from human epidermoid carcinoma of the mouth) as well as its moderate inhibitory activity in a number of *in vivo* tumor systems[2]. In this study, sparsomycin was daily administered i.v. for an intended period of 42 days to 5 patients who had all far advanced carcinoma for which no specific therapy was available. The total dose given ranged from 0.085 to 0.24 mg/kg. Two patients noted difficulty of vision, one after 13 days of treatment (total dose 12 mg) and one after 15 days of treatment (total dose 7.5 mg), whereafter treatment was stopped.

The complaints consisted of ring scotomas, i.e. ring shaped blind spots. This impaired vision and the wide spread degeneration of retinal pigment epithelium observed at necropsy indicated a severe ocular toxicity[1,3].

Necropsy of other organs of the 5 patients revealed no changes related to drug toxicity[1].

Ocular changes have not been observed in laboratory animals including rat, dog and monkey. These results have been referred to by MacFarlane *et al.*[3]; however, neither references nor detailed information are given in this report.

The Phase I Study was terminated pending additional preclinical studies.

It was expected that determination of the structural formula of sparsomycin in conjuction with animal studies might further define the pathophysiology of this ocular toxicity. In addition, it was stated[1] that preclinical studies of sparsomycin's activity should include studies of ocular toxicity. However, to our best knowledge the number of publications concerning additional preclinical studies, that have appeared ever since, is limited. We have no conclusive explanation for this sudden decline of interest in sparsomycin as an antineoplastic agent. We are inclined to assume that further research was hampered because of two reasons: first, the reported ocular toxicity and second, a possible lack of sufficient material. The sparsomycin used had been obtained through tedious isolation procedures from a fermentation broth[4-7].

IN VITRO AND IN VIVO EXPERIMENTS

In vitro experiments

Sparsomycin has attracted much attention because of its activity against transformed cells[8,9,11] as well as its activity against various tumors[2,9,10]. For 50% inhibition of KB cell growth 0.08 µg/mL of sparsomycin is sufficient[2,8,9]. Experiments with normal cells from a Chinese hamster fibroblast line showed[12] that sparsomycin was most cytotoxic to cells in the S-phase. During the S-phase DNA synthesis takes place. Beside inhibiting the protein synthesis, sparsomycin inhibited markedly the DNA synthesis. This has been ascribed to an interruption of the histone synthesis, which is tightly coupled to DNA synthesis[12]. Although it has been shown that sparsomycin is active against transformed and normal cells (Chapter I, general introduction), no information is available on the selectivity of sparsomycin against transformed cells as compared to normal cells.

In vivo experiments

As mentioned above sparsomycin showed a moderate inhibition in a number of *in vivo* tumor systems[2]. In these experiments with laboratory animals having implanted tumors sparsomycin manifested its effects at a dose level of 0.5 mg/kg.

In the *in vivo* systems p-388 lymphocytic leukemia[9] and Walker carcinoma 256[10] sparsomycin showed its activity at a dose level of 0.3-0.8 mg/kg. In a study[13] with rats bearing intra hepatically transplanted hepatomas, the responses to sparsomycin, in terms of changes in the polyribosome pattern and inhibition of protein synthesis (*in vitro* and *in vivo*) were evaluated relative to the responses in the host livers. In general, the host livers responded much more than the hepatomas did, which might be partly attributed to a difference in blood supply to the transplanted hepatomas[13].

Toxicity

An acute LD_{50} value (lethal dose for 50% of the animals) of 2.4 mg/kg in mice has been reported for sparsomycin. However, new experiments[18] indicate recently a considerable lower toxicity; the observed LD_{50} value is about 20 mg/kg. Sparsomycin, when administered intraperitoneally to mice (20 µg/20 g of body weight), induced marked disaggregation of hepatic polyribosomes and inhibited incorporation of [^{14}C]-leucine into hepatic proteins by 90%[14]. Disaggregation was not observed *in vitro*[14]. These results, the aforementioned ocular toxicity and effect on normal cells indicate that sparsomycin is toxic. Its toxicity is a consequence of its ability to inhibit the protein synthesis. In general, toxicity is an intrinsic property of antineoplastic agents. The question to be answered by further research is whether the therapeutic index of sparsomycin is large enough for clinical studies.

PRESENT AND FUTURE INVESTIGATIONS ON THE ANTITUMOR ACTIVITY OF SPARSOMYCIN AND ANALOGS

An interesting aspect of the activity of sparsomycin is, that it inhibits the protein synthesis (Chapter I and this Chapter, sections 1 and 2), whereas all clinically used antitumor agents act more or less on the DNA or RNA level. Therefore, sparsomycin might be a valuable antineoplastic agent for use in multiple drug schedules. We suppose that the mode of action of sparsomycin features another aspect; many of the clinically used antitumor agents have also carcinogenic properties. We expect that this adverse effect is absent with sparsomycin. The syntheses developed for sparsomycin enable us to prepare sufficient quantities for further biomedical studies. In addition, more elaborate structure-activity relationship studies will be performed to develop compounds with more selective biochemical and pharmacological properties while determining the minimum structural and stereochemical requirements for the biological activity.

Now that it has been shown that sparsomycin as well as octylsparsomycin possess a high activity in an *in vitro* L1210 clonogenic assay (this Chapter, section 3), we and others[15-17] decided that renewed investigations on the antitumor activity of sparsomycin are called for. The following experiments are planned. Sparsomycin and octylsparsomycin will be tested against primary human tumor cells in the human tumor cell assay[16].

Furthermore the compounds will be tested in mice against implanted leukemia L1210 and solid tumors [18].

For further preclinical studies of sparsomycin and analogs evaluation of their pharmacological properties in laboratory animals is essential. No data of pharmacokinetics or metabolism of sparsomycin have been published so far. A first pharmacokinetic study in the dog showed that sparsomycin has a short half-time of elimination [19], namely 70 min. In addition, the recovery of sparsomycin is only 25% in 24 h, although sparsomycin is rather soluble in water. Octylsparsomycin is more active in the L1210 clonogenic assay (this Chapter, section 3) and might have the advance of a longer half-time of elimination, because of an increased lipophilicity. This is under present investigation[15,17].

For closer examinations of the pharmacological properties (metabolism, distribution, excretion, mass balance) and ocular toxicity of sparsomycin[20], we have planned to prepare radio-actively labeled sparsomycin.

Radio-actively labeled sparsomycin derivatives will also be used to study the penetration of these compounds into transformed cells as compared to normal cells. This might give information on the selectivity on the cellular level. Finally, based on results of these experiments and further preclinical investigations in laboratory animals, it will be decided whether sparsomycin will be reintroduced into clinical studies[15].

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CHAPTER VI

CONFORMATIONAL ANALYSIS OF FUNCTIONALIZED SULTINES BY NUCLEAR MAGNETIC RESONANCE AND X-RAY CRYSTALLOGRAPHY; APPLICATION OF A GENERALIZED KARPLUS EQUATION.

CONFORMATIONAL ANALYSIS OF FUNCTIONALIZED SULTINES BY NUCLEAR MAGNETIC RESONANCE AND X-RAY CRYSTALLOGRAPHY; APPLICATION OF A GENERALIZED KARPLUS EQUATION

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Abstract. The *solid state* conformation of an N-protected sultine 2 was determined by X-ray crystallography, which allowed also the assignment of the R configuration of the sulfinate sulfur atom. In addition the conformation of compounds 1 and 2 *in solution* is reported. This conformational analysis was based on the application of a new, empirical generalization of the classical Karplus equation. Application of equation 1 and 4 allowed the expression of vicinal coupling constants - obtained by 500 MHz NMR spectroscopy - in proton-proton torsion angles ϕ_{HH}. Using the concept of pseudorotation (equation 1, 2a-d) the puckering and conformation of the sultine ring of 1 and 2 was quantitatively described.

It was shown that in $CDCl_3$ at 233K or 300K compound 1 is present as a twist chair conformer, which can be denoted as $^4T_5$ (Scheme I). In Me_2SO-d^6 compound 1 is engaged in an equilibrium between this $^4T_5$ conformer and a ^3_2T conformer. Compound 2 in $CDCl_3$ at 233K is engaged in two conformational equilibria, a slow and a fast one on the NMR time scale. The slow equilibrium between a major component and a minor component is due to hindered rotation in the urethane side chain.

In the fast equilibrium the five-membered ring is engaged in an equilibrium between a twisted chair conformer (^4_3T) and an envelope shaped conformer ($_1E$, see Scheme II). The slow equilibrium is not observed in Me_2SO-d^6 at 300K or in $C_2D_2Cl_4$ at 383K. The effects that might play a role in determining the conformations of 1 and 2 in solution are the gauche effect (Figure 7), the anomeric effect (Figure 8) and hydrogen bonding. The solid state conformation of 2, which is governed by hydrogen bond formation (see Figure 1), can be described as $_3E$ (Figure 3). Thus, a comparison of both the solution conformer and the solid state conformer of 2 (Scheme II and Figure 3, respectively) shows a remarkable difference.

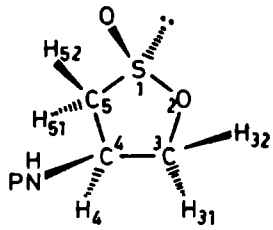
INTRODUCTION

Recently, we reported[2] an efficient route leading to the functionalized, five-membered cyclic sulfinate esters - γ-sultines - 1-3. It was concluded that nucleophilic ring-opening reactions of these sultines proceed with inversion at sulfur[2]. To draw this conclusion, the absolute configuration of one of the sultines had to be established rigorously.

Here we report a detailed discussion of the X-ray crystallographic, configurational and conformational analysis of 2.

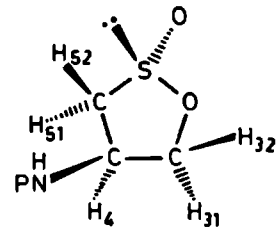
We noticed[2] that the chiral sulfur atom causes asymmetric induction in ring-opening reactions with a prochiral nucleophile[3]. For a fundamental understanding of this chiral induction, insight into the conformation of the sultines *in solution* is a prerequisite.

Therefore, beside the crystallographic analysis of the solid state conformation, an analysis of the conformation of 2 and 3 in solution will be given. This analysis is a new application of a generalized form of the Karplus equation, which has been formulated by one of us[4] and which has been used successfully[5].



R_C-S_S

1 : P=BOC



R_C-R_S

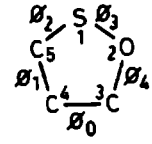
2 : P=BOC
3 : P=Cbo
4 : P=$C_6H_5(CH_2)_2CO$

METHODS

Pseudorotation Analysis of the Sultine Ring. The conformation of a five-membered ring in general is conveniently described by using the concept of pseudorotation[6,7]. The *endo*cyclic torsion angles Φ_j of a five-membered ring are interrelated via the pseudorotation equation 1.

$$\Phi_j = \Phi_m \cos(P + 4\pi j/5) \text{ in which } j = 0\text{-}4 \quad (1)$$

The puckering and conformation of the sultine ring may thus be quantitatively described by two parameters: P - the phase angle of pseudorotation and Φ_m - the puckering amplitude. The endocyclic torsion angle opposite to the sulfur atom is defined as the reference angle Φ_0, the remaining torsion angles Φ_1, Φ_2, Φ_3 and Φ_4 are designated counterclockwise along C4-C5, C5-S1, S1-O2 and O2-C3, respectively (see structure 5).



5

By application of a new empirical generalization of the classical Karplus equation (*vide infra*, equation 4), experimental vicinal ^{1}H-NMR coupling constants can be correlated with proton-proton torsion angles ϕ_{HH}. These torsion angles are intimately related to the endocyclic torsion angles Φ_j, which, on their turn determine the pseudorotation parameters P and Φ_m (equation 1). Therefore, in order to determine the conformation of the sultine ring in terms of the pseudorotation concept, correlations between the pseudorotation parameters (P and Φ_m) and the proton-proton torsion angles are called for.

A complication arises however, because this pseudorotation equation 1 can only be regarded as near-exact for *equilateral* five-membered rings such as cyclopentane.

In heterocyclic systems with varying endocyclic bond distances, as is pertinently the case for the sultine ring under study (bond distances of 1.40-1.83 Å, Table 1), equation 1 is expected to *reproduce* the endocyclic torsion angles - and hence the exocyclic proton-proton torsion angles - only with limited accuracy. This expectation is indeed borne out by experiment as the torsion angles recalculated from the pseudorotation parameters for the sultine ring in the solid state (*vide infra*) show deviations up to 2.5° when compared with the experimentally observed torsion angles. This situation was only recently remedied by de Leeuw *et al*[8] by applying two correction factors to the pseudorotation equation 1. This corrected pseudorotation equation yields the following correlations between the proton-proton torsion angles ϕ_{HH} and the pseudorotation parameters governing the conformation of the sultine ring:

$$\phi_{31-4} = -3.9 + 1.0033\ \Phi_m\cos(P-2.48) \quad (2a)$$
$$\phi_{32-4} = -123.3 + 1.0033\ \Phi_m\cos(P-2.48) \quad (2b)$$
$$\phi_{4-51} = 4.0 + 1.0206\ \Phi_m\cos(P+140.72) \quad (2c)$$
$$\phi_{4-52} = 123.2 + 1.0206\ \Phi_m\cos(P+140.72) \quad (2d)$$

Obviously, by combining equations 1 and 2a-2d, it will suffice - at least in theory - to determine two vicinal coupling constants in order to give a full description of the five-membered ring in terms of P and Φ_m. When more coupling constants and hence more torsion angles are known, the system is overdetermined and 'best' values for P and Φ_m may be obtained either by averaging or, preferably, by least-squares fitting of the data.

A complication may arise when the five-membered ring under study is engaged in a fast equilibrium between two distinct conformers; then the experimental coupling constants ^3J represent time-average values that are linearly related to the coupling constants of the individual conformers J(I) and J(II) and their populations as expressed by equation 3.

$$^3J_{exp} = xJ(I) + (1-x)J(II) \quad (3)$$

where x is the mole fraction of conformer I.

In this case the complete conformational analysis entails the determination of five independent parameters P(I), Φ_m(I), P(II), Φ_m(II) and x from the observed coupling constants. This objective was realized by an iterative least-squares computer program (written in PASCAL) devised to obtain the best fit of the equilibrium parameters to the experimental coupling constants. At this point it should be mentioned that for the sultine ring under study the system is in fact underdetermined as only four observables - *i.e.* coupling constants - are available from experiment for the calculation of Φ_m(I), P(I), Φ_m(II), P(II) and x. Therefore, within each minimization one or more parameters must be constrained to reasonable values. In the following sections it will be demonstrated that the procedure outlined above yields a consistent conformational analysis of the sultine rings in solution.

Determination of the Proton-Proton Torsion Angles. To obtain the proton-proton torsion angles, necessary for the pseudorotation analysis of the sultine ring (eqs 1, 2a-2d), a new emperical generalization[4] of the classical Karplus equation was used.

In this generalized equation the standard Karplus equation is extended with a correction term in order to describe the influence of electronegative substituents on vicinal coupling constants in an explicit way. Each H-C-C-H fragment in the ring of compounds 1 and 2 carries three non-hydrogen substituents in which case the generalized equation takes the form:

$$^3J_{HH} = 13.22\cos^2\phi - 0.99\cos\phi + \Sigma\Delta\chi_i\{0.87-2.46\cos^2(\xi_i\phi + 19.9|\Delta\chi_i|)\} \quad (4)$$

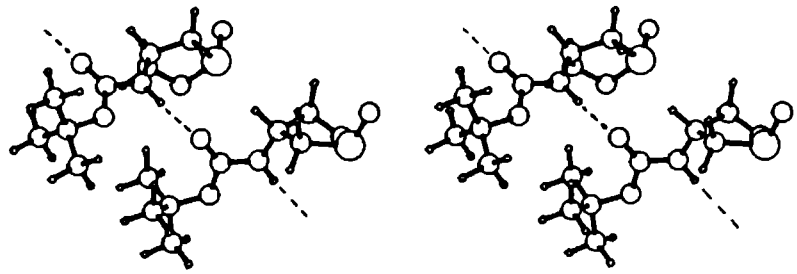
The first two terms describe the dependency of the vicinal coupling constant ($^3J_{HH}$) in the H-C-C-H fragment under study on the Klyne-Prelog signed proton-proton torsion angle[9] (4). The remaining terms account for the dependency of $^3J_{HH}$ on the electronegative substituents S_i; these terms depend on the torsion angle ϕ, on the difference in electronegativity between the substituents S_i and hydrogen on the Huggins scale[10] ($\Delta\chi_i$), and finally on the orientation of the substituent S_i with respect to the coupling protons[4] (ξ_i).

RESULTS

X-Ray Crystallographic Configurational and Conformational Analysis of 2. So that the configuration of the sulfoxide atom and the solid state conformation of the ring could be determined, a single crystal X-ray structure determination of 2 was performed (see also experimental section). This compound crystallized from dichloromethane/carbon tetrachloride as monoclinic needles with two crystallographically independent molecules in the unit cell, see Figure 1.

Bond distances (Table 1) and angles of these two molecules agree with each other without significant differences. The structure of the molecules is shown in Figure 2. By reference to the chiral carbon atom having the R-configuration (see experimental section), the chiral sulfur atom can readily be seen to possess the R-configuration[11] too.

Fig 1 Stereoscopic view of 2 in the unit cell



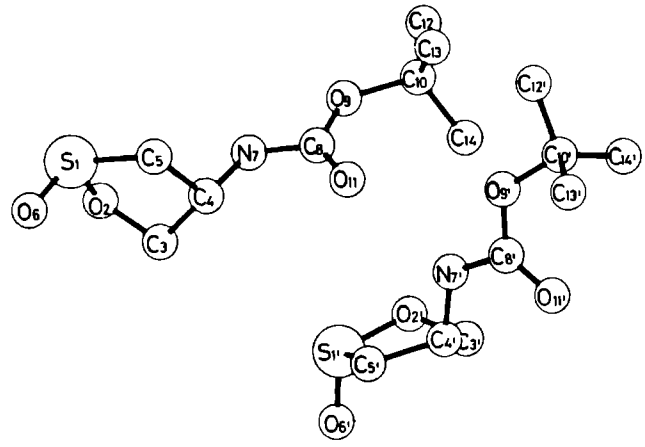
The C5-S1 bond is, as expected, the longest bond in the ring of both molecules in the unit cell; its values (1.780Å and 1.833Å) are similar to th se reported for non-functionalized sultines[12,13] .

In Figure 1 the presence of intermolecular hydrogen bonds between NH and C=O is indicated. The observed O-N distances are 2.88Å and 2.93Å and the corresponding N-H-O angles are 156° and 150°, respectively. Consequently, the hydrogen bonds can be classified as moderately strong[14]. Due to these hydrogen bonds the molecules form straight chains throughout the crystal.

Table 1 BOND DISTANCES (Å) OF 2

Atoms	d	<d>	Atoms	d
S1 -06	1.466(7)	1.460	S1' -06'	1.453(7)
S1 -02	1.586(7)	1.592	S1' -02'	1.598(6)
S1 -C5	1.780(7)	1.807	S1' -C5'	1.833(9)
02 -C3	1.426(11)	1.415	02' -C3'	1.403(8)
C3 -C4	1.524(11)	1.515	C3' -C4'	1.505(11)
C4 -C5	1.525(11)	1.534	C4' -C5'	1.543(10)
C4 -N7	1.402(9)	1.418	C4' -N7'	1.434(9)
N7 -C8	1.356(7)	1.355	N7' -C8'	1.354(7)
C8 -011	1.189(7)	1.195	C8' -011'	1.201(7)
C8 -09	1.332(7)	1.327	C8' -09'	1.321(8)
09 -C10	1.484(7)	1.483	09' -C10'	1.482(7)
C10-C12	1.503(9)	1.495	C10'-C12'	1.487(10)
C10-C13	1.491(10)	1.512	C10'-C13'	1.533(11)
C10-C14	1.513(10)	1.502	C10'-C14'	1.490(11)

Fig 2 X-ray analysis of 2 showing the R_C-R_S configuration



P =334.7°
Φ_m = 44.6°

P =332.7°
Φ_m = 44.7°

The conformation of the ring of 2 in the unit cell can be described in terms of pseudorotation as has been outlined above (eq 1). As a result the two sultine rings have phase angles of pseudorotation (P) of 334.7° and 332.7° with puckering-amplitudes (Φ_m) of 44.6° and 44.7°, respectively. Both conformations are almost envelope shaped, with C3 out of the plane, pointing into the direction opposite to the C4-N bond, a conformation which is denoted[15] by $_3$E (Figure 3).

An alternative way to describe the envelope shaped conformation is the dihedral angle between the planes through the atoms [C4, C5, S1, 02] and [C4, C3, 02]; this value is 41° for both molecules in the unit cell.

^{1}H-NMR spectra of 1 and 2. The proton chemical shift values of 1 in $CDCl_3$ at 233K are collected in Table 2. The corresponding observed coupling constants are listed in Table 3.

Fig 3 Representation of 2 in the solid state showing the envelop $_3E$ shape

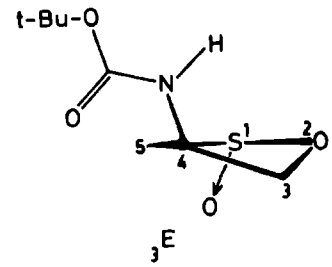


Table 2 CHEMICAL SHIFT DATA (PPM) OF 1 IN DIFFERENT SOLVENTS AND AT DIFFERENT TEMPERATURES

Proton	$CDCl_3$ 233 K	$CDCl_3$ 300 K	Me_2SO-d^6 300 K
H_{31}	4.659	4.623	4.572
H_{32}	4.810	4.749	4.511
H_4	4.955	4.899	4.433
H_{51}	3.021	2.972	3.481
H_{52}	3.294	3.201	2.994
H_N	6.154	6.012	7.040
$H_{t\text{-}Bu}$	1.438	1.437	1.390

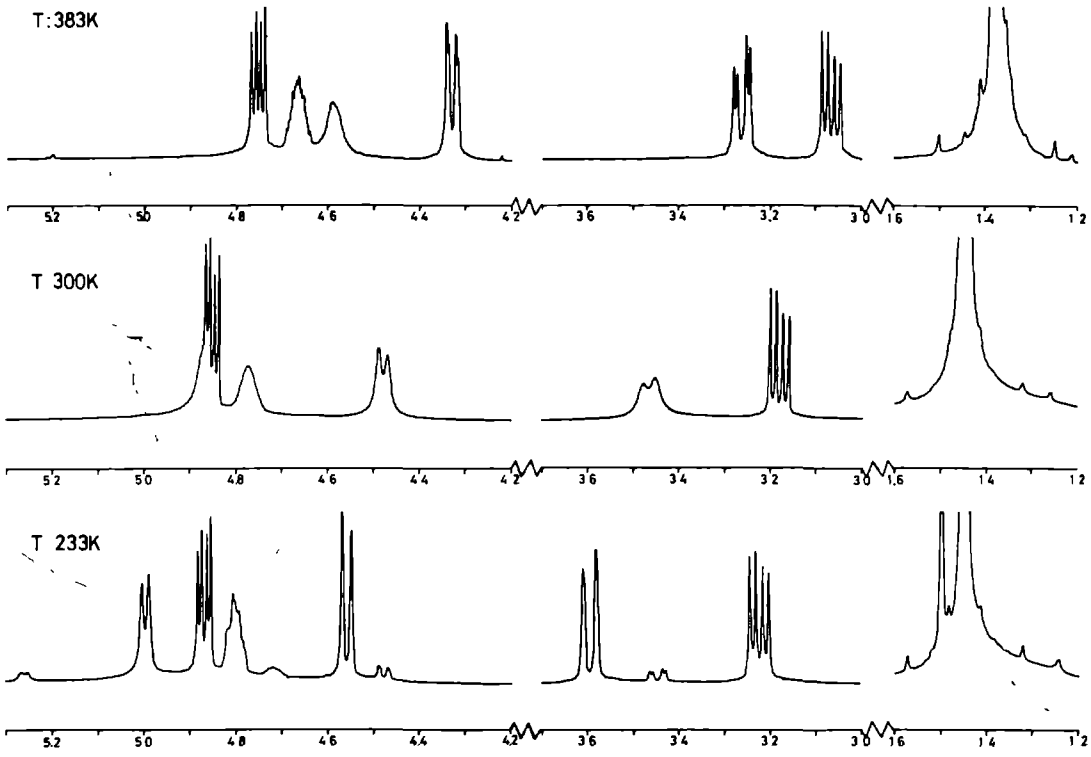
Table 3 COUPLING DATA (Hz) OF 1 IN DIFFERENT SOLVENTS AND AT DIFFERENT TEMPERATURES

	$CDCl_3$, 233K			$CDCl_3$, 300K			Me_2SO-d^6, 300K		
Coupling	$J_{exp.}$	$J_{calc.}$	Δ_J	$J_{exp.}$	$J_{calc.}$	Δ_J	$J_{exp.}$	$J^*_{calc.}$	Δ_J
H_{31-4}	5.82	5.80	0.02	5.93	5.96	-0.03	6.68	6.72	-0.04
H_{32-4}	1.52	1.48	0.04	1.74	1.54	0.20	5.22	5.26	-0.04
H_{51-4}	7.00	7.05	-0.05	7.21	7.18	0.03	8.21	8.12	0.09
H_{52-4}	0.90	1.22	-0.32	0.99	1.22	-0.23	3.53	3.50	0.03
H_4-NH	9.9			8.2			6.0		
H_{31-32}	-9.67			-9.67			-8.92		
H_{51-52}	-13.14			-13.15			-13.41		

*Calculated for a conformational equilibrium (see text)

The 500 MHz spectra of 2 deserve some comment; the spectrum in $CDCl_3$ at 300K is depicted in Figure 4 and shows the simultaneous presence of both sharp and broad resonances.
The broad resonances at δ 3.47 and 4.48 were taken indicative for chemical shift exchange phenomena. This conception was indeed confirmed by experiment; at lower temperature (233K), the broad lines sharpen up to give two sets of signals with an integration ratio of about 9:1 (Figure 4). The gaussian enhanced spectrum of 2 at 233K is represented in Figure 5.

Fig 4 *500 MHz ^{1}H-NMR spectrum of 2 in $CDCl_3$ at 233K, at 300K and in $C_2D_2Cl_4$ at 383K*



Perusal of this spectrum shows that the sharp resonances are also splitted into sets of signals, displaying a similar integration ratio; however, the chemical shift differences are very small (0.01 ppm). From these observations we concluded that 2 is engaged in a slow equilibrium between two conformers; the conformer with the larger integration values for all resonances is called the *major* component and the second conformer is called the *minor* component.

We have solid evidence that the bipartition of 2 in these two components originates from different conformations in the side chain and not from different ring conformations (*vide infra*). The chemical shift data and coupling data for the major and minor component of 2 are collected in Tables 4 and 5.
These data and those presented in Tables 2 and 3 were obtained from computer simulated spectra. As an example the simulated spectrum of the major and the minor component of 2 in $CDCl_3$ at 233K is shown in Figure 5.

The assignment of the signals in the spectrum of 2 at 233K (Figures 4 and 5) to individual protons is based on the following rationale. The urethane proton is easily recognized by its broad doublet appearance at 5 ppm and the fact that it exchanges after the addition of D_2O/TFA to the sample. The singlet at high field is due to the t-butyl protons. The signals in the 3.20 - 3.60 ppm region originate from the protons H_{51} and H_{52}; each proton gives rise to a doublet of doublets.

Fig 5 *Gaussian enhanced experimental spectrum of 2 at 233K in $CDCl_3$ and the corresponding simulated spectra of the major and minor component*

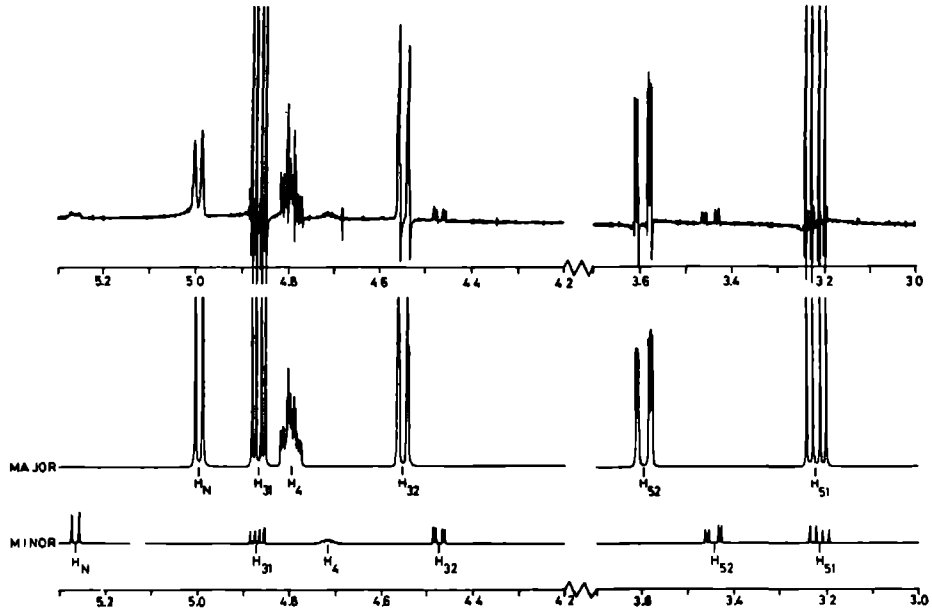


Table 4 *CHEMICAL SHIFT DATA (ppm) OF 2 IN DIFFERENT SOLVENTS AND AT DIFFERENT TEMPERATURES*

proton	$CDCl_3$,233K minor	$CDCl_3$,233K major	$CDCl_3$ 300K	$C_2D_2Cl_4$ 383K	Me_2SO-d^6 300K
H_{31}	4.869	4.865	4.847	4.746	4.626
H_{32}	4.475	4.554	4.474	4.323	4.332
H_4	4.717	4.798	4.78	4.660	4.566
H_{51}	3.216	3.920	3.176	3.062	3.067
H_{52}	3.443	3.590	3.461	3.256	3.432
H_N	5.259	4.994		4.581	7.327
H_{t-Bu}	1.495	1.445	1.445	1.383	1.388

The doublets of doublets in the 4.55 - 4.87 ppm region are due to the protons H_{31} and H_{32}; in this region are also located the multiplets due to the H_4 proton in the major and minor component. This global assignment is in accordance with the chemical shift data reported for the closely related compound 1,2 oxathiolane 2,2-dioxide (propane-sultone) and, moreover, consistent with all observed geminal and vicinal coupling constants reported in Table 5.

Assignment of the individual geminal protons at C3 and C5 is based on coupling constant considerations.

Table 5 COUPLING DATA (Hz) OF 2 IN DIFFERENT SOLVENTS AND AT SEVERAL TEMPERATURES

coupling	$CDCl_3$, 233K						$C_2D_2Cl_4$, 383K			Me_2SO-d^6, 300K		
	minor component			major component								
	J_{exp}	J_{calc}	Δ_J	J_{exp}	J_{calc}	Δ_J	J_{exp}	J_{calc}	Δ_J	J_{exp}	J_{calc}	Δ_J
H_{31-4}	4.83	5.13	-0.32	4.34	4.35	-0.01	5.16	5.47	-0.31	5.17	5.62	-0.45
H_{32-4}	2.27	2.27	-0.00	1.25	1.71	-0.46	2.61	2.62	-0.01	2.82	2.86	-0.04
H_{51-4}	6.61	6.47	0.14	6.69	6.59	0.10	6.71	6.58	0.13	6.79	6.60	0.19
H_{52-4}	3.59	3.39	0.20	2.25	2.01	0.24	4.03	3.91	0.12	4.30	4.12	0.18
H_4-NH	7.94			7.95			6.00			4.99		
H_{31-32}	-10.00			-10.16			-9.82			-9.25		
H_{51-52}	-13.82			-14.23			-13.67			-13.66		
H_{32-52}				0.99								

Using the appropriate equations 1 and 2a-2d together with the equation 4 the dependency of each of the four vicinal coupling constants on the phase angle of pseudorotation P is easily computed for two values of the puckering amplitude, *viz.* 35° and 45°[17]. The resulting coupling constants profiles are shown in Figure 6.

Fig 6 Calculated coupling constant profiles ^3JHH (Hz) for 1 and 2 as a function of the pseudorotation phase angle P. Solid curves: Φ_m 35° dashed curves: Φ_m 45°

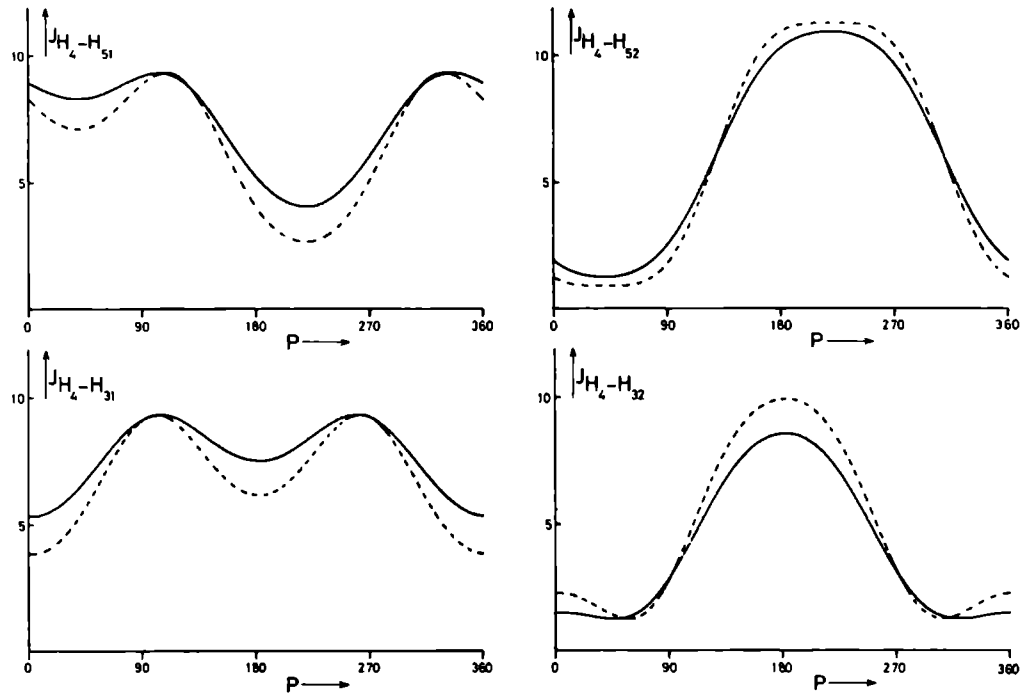
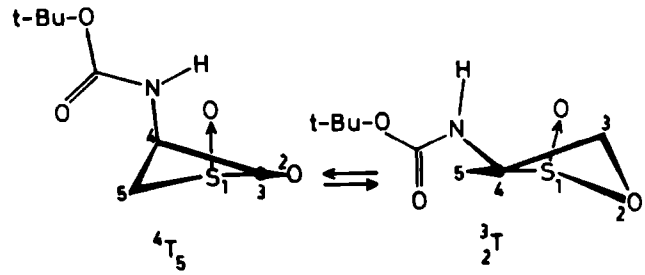


Figure 6 allows an assignment of the C3 and the C5 protons; only the H_{32} and H_{52} protons can adopt - for certain ranges of P - coupling constants values less than or equal to 2 Hz. Consequently, those signals that correspond to a vicinal coupling constant of 2 Hz (see Table 5) can be assigned to the protons H_{32} and H_{52}.

The same line of reasoning can be used to assign the signals in the spectra of 1,(see Tables 2 and 3),with the exception of the spectrum in Me_2SO-d^6 at 300K. In the latter solvent protons H_{31} and H_{32} display vicinal coupling constants of roughly the same magnitude (≈6 Hz). In this case we had recourse to a solvent-mixture technique: by recording the NMR-spectra in mixtures of $CDCl_3$ and Me_2SO-d^6 of varying composition it was shown that the H_{31} and H_{32} protons as well as the H_{51} and H_{52} protons interchange position in the spectrum upon increasing Me_2SO-d^6 content in the solvent mixture. No other assignment leads to acceptable structures in the conformational analysis (*vide infra*).

Solution conformation of 1. The results of the pseudorotation analysis of 1 in $CDCl_3$ at 233K show that the five-membered ring is best described in terms of a single conformation characterized by $P=25^\circ$, $\Phi_m=34^\circ$ ($^4T_5$) Scheme I,

Scheme I *Solution conformers of 1 present in $CDCl_3$ at 233K (1a) in $CDCl_3$ at 300K (1b) and in Me_2SO-d^6, at 300K (1c and 1d)*



1a,1b,1c 1d

see also Table 6 (conformer 1a). Raising the temperature to 300K hardly affects the experimental coupling constants: again a comparable conformational system described by $P=26^\circ$, $\Phi_m=33^\circ$ (Table 6, conformer 1b) is found.

Substantial changes in the vicinal coupling constants are induced, however, when 1 is dissolved in Me_2SO-d^6 (300K). In this case, the observed couplings cannot be rationalized in terms of one conformation for the five-membered ring, as pilot-calculation yielded unacceptably high differences between observed and calculated coupling constants (up to 1 Hz) for the ultimately 'best' single conformer. Therefore, the possibility of a conformational equilibrium (see 'Methods') was considered. In order to reduce the number of parameters to be extracted from the coupling constant data, the involved puckering amplitudes $\Phi_m(I)$ and $\Phi_m(II)$ were fixed to the arbitrary value of 35°[18].

Indeed, the constrained calculation strongly indicates the existence of a conformational equilibrium characterized by $P(I)=17^\circ$, $P(II)=148^\circ$ and x=0.54 (x=molefraction of conformer I). Moreover, good agreement between calculated and observed coupling constants is noted (all $\Delta J's < 0.1$ Hz). The correspondence between one of these conformers ($P(I)=17^\circ$, $\Phi(I)=35^\circ$, ^4E) and the solution conformation 1a in $CDCl_3$ ($P=25^\circ$, $\Phi_m=34^\circ$, $^4T_5$) is striking, the geometrical differences between both five-membered rings are differences of degree, not of kind.

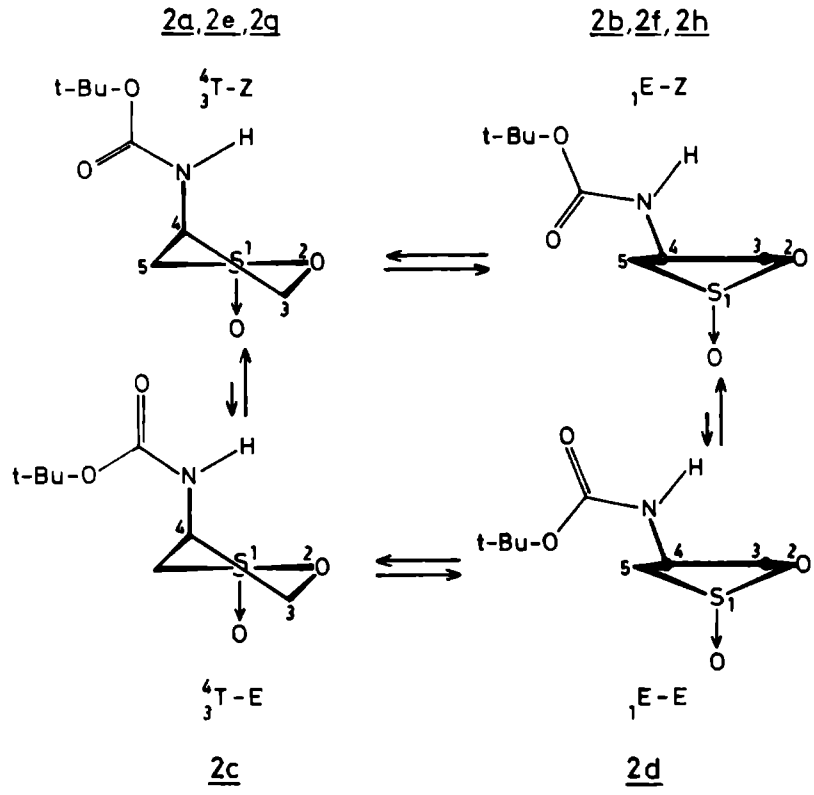
Therefore, it is not very surprising that introduction of the latter conformation 1a as one of the components of the conformational equilibrium yields - after pseudorotational analysis - an equilibrium of 1c ($^4T_5$) and 1d (^3_2T) characterized by P(I)=25°, Φ_m(1)=34°, P(II)=146°, Φ_m(II)=40° and x=0.56 (Table 6). This reproduces the observed coupling constants well (Table 3).

Table 6 *PSEUDOROTATION PARAMETERS OF 1 IN DIFFERENT SOLVENTS AND AT DIFFERENT TEMPERATURES*

			P(I)	Φ_m(I)	x		P(II)	Φ_m(II)
$CDCl_3$	233K	1a: $^4T_5$	25°	34°	1			
$CDCl_3$	300K	1b: $^4T_5$	26°	33°	1			
Me_2SO-d^6	300K	1c: $^4T_5$	25°	34°	0.56	1d: ^3_2T	146°	40°

Solution conformation of 2. The ^{1}H-NMR spectrum of 2 in $CDCl_3$ at 233K shows the presence of a *major* component and a *minor* component (*vide supra*). The *major* and the *minor* component are proposed to be characterized by a *trans* urethane bond ($^4_3$T-Z and $_1$E-Z, Scheme II) and a *cis* urethane bond ($^4_3$T-E and $_1$E-E, Scheme II), respectively.

Scheme II *Solution conformers of 2 present in $CDCl_3$ at 233K (2a–2d), in Me_2SO-d^6 at 300K (2e and 2f) and in $C_2D_2Cl_4$ at 383K (2g, 2h)*



Indirect evidence can be offered for this presumption:

a. calculations showed (*vide infra*) that nearly the same conformers of the sultine ring were present both in the major and the minor component. This indicates that the sultine ring itself can be excluded as the cause for the chemical exchange phenomena
b. the bipartition in a major and a minor component does not originate from different conformations about the C4-N bond, as in both components vicinal proton-proton couplings H_4-C-N-H on one hand and geminal ^{13}C4-NH coupling constants on the other hand are very alike
c. the occurence of a major and a minor component is intimately related to the presence of a urethane-bond. The NMR-spectra of the N-benzyl oxycarbonyl-sultine 3 at 233K and 300K display similar features. However, no chemical exchange phenomena evinced from the corresponding spectra of 4 which is a 'carbon-analogue' of 3
d. several examples of molecules containing a urethane bond are known, which due to the C-N-C(O) bond can exist as a pair of rotamers (see 'Discussion').

Attention is now drawn to the sultine ring of the *minor* component. Model calculation in which the *solid-state* pseudorotation parameters of 2 ($P=334^{o}$, $\Phi_m=44.7^{o}$, *vide supra*) were introduced as a constraint did not yield satisfactory results; the residual r.m.s. deviations between the observed couplings and those calculated for the ultimately 'best' conformational equilibrium remained unacceptably high (>1 Hz). Therefore, the experimental coupling constant data of the minor component were subjected to a pseudorotational analysis along the lines described for 1 in Me_2SO-d^6 (*vide supra*). The phase-angles of pseudorotation and molefractions in the conformational equilibrium were optimized for arbitrary puckering amplitudes, after which the puckering amplitudes were optimized. This was followed by a re-optimization of the phase-angles and mole-fraction.
This procedure resulted in the following description of the conformational equilibrium between conformers 2c and 2d: $P(I)=7^{o}$, $\Phi_m(I)=45^{o}$, $P(II)=270^{o}$, $\Phi_m(II)=54^{o}$, and x=0.76 (Table 7). Calculated values for the coupling constants of the ring protons in this equilibrium are listed in Table 5 and show indeed good agreement (residual rms-deviation between calculated and observed couplings = 0.23 Hz).

Similar pseudorotational analyses were carried out for the coupling constant data of the *major* component. Again, when guided by the *solid-state* conformation of 2, no satisfactory solution was reached. However, upon introduction of the most populated conformer 2d of the *minor* component (*vide supra*), the analysis smoothly optimized to a biased equilibrium of 2a and 2b (Scheme II) characterized by $P(I)=5^{o}$, $\Phi_m(I)=45^{o}$, $P(II)=262^{o}$, $\Phi_m(II)=45^{o}$ and x=0.91 (Table 7). Subsequently, the effect of raising the temperature on the equilibrium between the major and minor component was studied, since the ^{1}H-NMR spectrum in $CDCl_3$ at 300K shows coalescence phenomena. In order to record the ^{1}H-NMR spectra at elevated temperatures, the compound was dissolved in di-deuterotetrachloroethane ($C_2D_2Cl_4$). At 383K, the minor and major component are rapidly interconverting, yielding a 'time-averaged' spectrum, see Figure 4.
A description of the five-membered ring in terms of an equilibrium between two conformers 2g and 2h (Scheme II) represented by the parameters $P(I)=5^{o}$, $\Phi_m(I)=45^{o}$, $P(II)=268^{o}$, $\Phi_m(II)=54^{o}$ and x=0.71 matches the experimental couplings constants very well (rms=0.20 Hz, see Table 7).

Remarkably, no coalescence phenomena or separation into two components could be observed when the NMR spectrum of 2 was recorded in Me_2SO-d^6 at 300K (Tables 4 and 5). Either the *cis-trans* isomerization of the urethane bond is fast on the NMR time-scale under these conditions (*cf* the situation in $C_2D_2Cl_4$ at 383K) or the equilibrium is completely one-sided in favour of one of the aforementioned isomers. No unambiguous choice between these two possibilities can be made from the data at hand.

Apart from this, the pseudorotational analysis of the sultine ring in this solvent appeared to be rather straightforward, yielding an equilibrium between conformers 2e and 2f (see Scheme II) with P(I)=5°, Φ_m(I)=45°, P(II)=265°, Φ_m(II)=50° and x=0.68 (see Table 7).

Table 7 PSEUDOROTATION PARAMETERS OF 2 IN DIFFERENT SOLVENTS AT DIFFERENT TEMPERATURES

			P(I)	Φ_m(I)	x		P(II)	Φ_m(II)	RMS
$CDCl_3$	233K	2a: $^4_3$T-Z	5°	45°	0.91	2b: $_1$E-Z	262°	45°	0.23
		2c: $^4_3$T-E	7°	45°	0.76	2d: $_1$E-E	270°	54°	0.30
Me_2SO-d^6	300K	2e: $^4_3$T	5°	45°	0.68	2f: $_1$E	265°	50°	0.30
$C_2D_2Cl_4$	383K	2g: $^4_3$T	5°	45°	0.71	2h: $_1$E	268°	54°	0.20

DISCUSSION

Although the results reported in this study allow only a qualitative insight into the specific intramolecular interactions that govern the conformational behaviour of the five-membered sultine-ring, the data at hand reveal several interesting points. The molecular model constructed for the $^4T_5$-conformer of 1 (*i.e.* the solution conformation in $CDCl_3$, 1a, Scheme I) suggests the facile formation of an intramolecular hydrogen-bond between the NH proton and the oxygen atom of the *syn* S→O moiety. Sundry experimental findings support this concept:

a. the N-H absorption-bond in the IR-spectrum of 1 in $CHCl_3$ is found at 30 cm^{-1} lower frequency with respect to the corresponding bond in the IR-spectrum of 1 having the C-N and S→O bonds in an *anti*-arrangement (Figure 2). A similar difference in IR-absorption frequency (± 40 cm^{-1}) has been observed in the case of 5-hydroxy-1,3-dioxane[19] and has been assigned to the presence of an intramolecular hydrogen-bond
b. the NH proton in the 500 MHz NMR-spectrum of 1 in $CDCl_3$ resonates at significant lower field (ca. 1 ppm, *cf* Tables 2 and 4) compared to the corresponding proton in 2
c. the proposed intramolecular bond in 1 in $CDCl_3$ requires an anti-periplanar position of the C4-H and N-H protons. The vicinal coupling constant between these protons (^3J (H_4-NH)=9,9 Hz) even exceeds the maximum value (9.45 Hz) predicted by the Karplus equation for H-C-N-H fragments given by Ramachandran *et al*[20]. This indicates a complete anti-periplanar position at 233K of the protons under discussion.
   Upon raising the temperature to 300K the magnitude of the latter coupling constant diminishes to 8.2 Hz, concomitantly the urethane-amide proton shifts to higher field. These observations betoken a certain amount of disruption of the intramolecular hydrogen-bonding[21] according to the pseudorotational analysis given in the preceding section; however, the change in temperature has virtually no effect on the conformation of the sultine-ring, inferring that the formation of the intramolecular hydrogen bond does not play a dominant role in determining the conformation of the five-membered ring.

Thus, although the intramolecular hydrogen bond stabilizes the $^4T_5$-conformation of the sultine-ring of 1 to a certain extent, we seek the main driving force for this conformation in the so-called *gauche*-effect. This is the well-documented[22] preference for *gauche* over *anti*-geometry in X-C-C-Y fragments in which X and Y represent electronegative substituents. It has been pointed out that the conformational behaviour of five-membered ring sugars in nucleosides and nucleotides [5c] as well as in the five-membered protine ring[5d] can be rationalized by assuming a predominant *gauche* stabilization between the endocyclic hetero-atom and *exo*cyclic substituents.

We propose that the conformational behaviour of the sultine ring of 1 can be understood on the same grounds; as in the $^4T_5$-conformation of the sultine ring the *exo*cyclic C-N-bond occupies a *gauche* orientation with respect to the *endo*cyclic C-S and C-O bonds. From the observation that in chloroform no other conformation than the $^4T_5$-conformer could be detected (*vide infra*), it follows that the (double) *gauche* stabilization in the sultine-ring amounts to >2 kcal/mole. This lower limit estimate is in accordance with data[5d] available for the *gauche* effect in N-C-C-O fragments (2.0 kcal/mole), to which the *gauche* stabilization for the N-C-C-S(O) fragment should be added.

This conformational behaviour of 1 changes drastically when the compound was dissolved in Me_2SO-d^6: next to the aforementioned 1c-$^4T_5$ conformer a second (1d) (^3_2T) conformer becomes populated to approximately an equal extent. This finding may be explained in terms of a diminished *gauche* effect[24] in the more polar solvent Me_2SO.

In addition, the anomeric effect was taken into account. In Figure 7 the Newman projections along the O2-S1 bond of the conformers 1a-d are depicted. It is noted that in the ^3_2T-conformation (1d) the S→O bond of the sultine ring approaches an antiperiplanar orientation with respect to one of the ring-oxygen lone-pairs, suggesting a stabilization on the basis of the anomeric effect[25,26]. In Figure 8 this anomeric effect is visualized. Finally, Me_2SO is a strong hydrogen bond accepting solvent capable of competing with the intramolecular hydrogen bond-accepting sulfinyl function. Indeed, a reduced intramolecular hydrogen bonding tendency is reflected by the - relatively - low ^3J (H_4-NH) value (Table 3).

In the discussion of the conformational analysis of 2 an additional observation has to be taken into account, i.e. the occurrence of a minor ('*cis*', E-configuration) and a major ('*trans*', Z-configuration) component at low temperature (233K). Molecules like 1-3, containing a urethane moiety can indeed exist as a pair of rotamers of the C-N-C(O) bond. This has been observed earlier with urethanes in general[27-32] and more particularly with urethanes derived from amino acids[31-35], amino acid esters[33,38,39] and peptides[33-39] containing the BOC or Cbo group as N-protecting group. Often the rotamers were found to be present in unequal amounts[29,31,34,39] as is the case in this study too. The *trans* rotamers (Z-configuration) is expected to be more stable and is therefore present in excess over the *cis*-rotamer (E-configuration).

The equilibrium between the major and minor component of 2 is slow on the NMR time scale at 233K, so that the separate rotamers can be observed (Figure 4). At 300K a situation near coalescence is observed and at 383K in $C_2D_2Cl_4$ the interconversion of rotamers is rapid. Assuming that 300K is about the temperature of coalescence a rough estimate for the activation energy for rotation around the C-N-bond ($\Delta G^{\ddagger}$) gives a value of 15 kcal/mole. This compares favourably with studies on several urethanes[27,28]. (The major as well as the minor component consist of an equilibrium between a twist-chair 2a,2b (^4_3T) and envelope 2b,2d ($_1$E-)conformer (Scheme II). The twist-chair conformer is present in excess in the minor as well as in the major component (mole fraction 0.76 and 0.91, respectively, see Table 7). We attribute the stabilization of this conformer ^4_3T to a double gauch effect: the C-N bond has a gauche position to the *endo*cyclic C-O and C-S bonds. In the E-conformer the only stabilization that is remarked at first glance is the anomeric effect (Figure 7).

Finally, a comparison of the conformations of 2, which are present in solution (Scheme II) with the solid state conformation (Figure 3) shows that the latter differs from the conformers in solution. The solid state conformation is almost envelope shaped with the C3-atom as the most puckered atom. In contradistinction the conformations in solution are of twist-chair shape (2a,2e,2g,2c) or envelope shaped (2b,2f,2h,2d) with the S-atom as the puckered atom. Obviously the intermolecular interactions in the solid state - especially the intermolecular hydrogen bonding (Figure 1) force the molecule into a certain conformation, which does not need to be the most stable one in solution.

In summary: both diastereomers 1 and 2 behave in a remarkably consistent way: in $CDCl_3$ the presence of the *exo*cyclic C-N-bond dominates the conformational behaviour by exerting a (double) gauche stabilization which leads to a $^4T_3$ conformation for 1 and to a (predominant) ^4_3T-conformation for 2. In Me_2SO-d^6 this gauche effect is counterbalanced by the anomeric effect and/or the gauche effect diminished in this more polar solvent.

This study adds a new example[40] to the growing row of molecules showing that solid state and solution conformers can differ. Work is in progress to evaluate the influence of the conformation of functionalized sultines on ring-opening reactions[1] and reactions of the α-sulfinyl carbanion with electrophiles.

Fig 7 Newman projection along the O2-S1 bond of the conformers of compounds 1 and 2

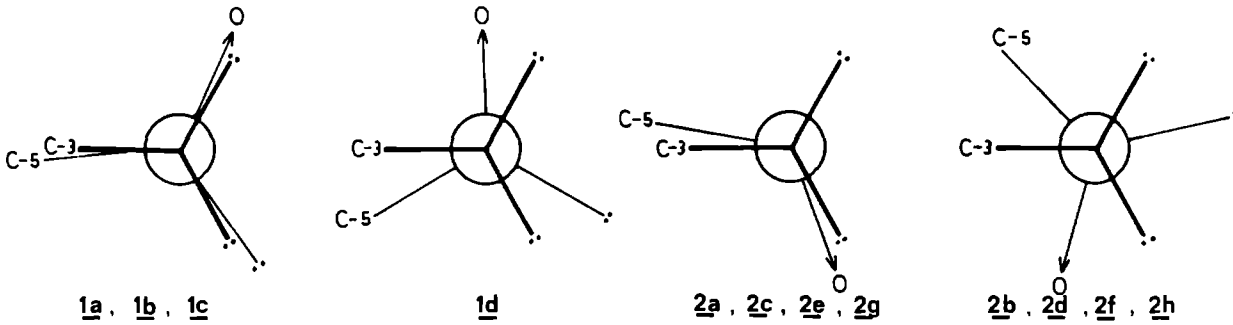
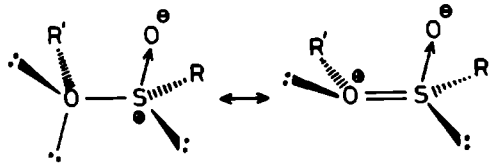


Fig 8 Visualization of the anomeric effect



EXPERIMENTAL PART

Synthesis

Compounds 1, 2 and 3 were synthesized as has been described before[2].

2R-4R-4[(3-phenylpropanoyl)amino]-1,2-oxathiolane 2-oxide (4)

This compound has been prepared from N-(3-phenylpropanoyl)-L-cystine methyl ester as described[1] for the preparation of 1 and 2 from the corresponding N-protected cystine ester. R_f 0.19 ($MeOH/CH_2Cl_2$, 5/95, v/v). 500 MHz ^{1}H-NMR ($CDCl_3$) at 300K: δ2.455 (m, 2H, $C\underline{H}_2C(O)$), 2.938 (t, 2H, $C_6H_5C\underline{H}_2$), 7.158-7.305 (m, 5H, C_6H_5); 4.309 (H_{32}), 4.763 (H_{31}), 4.913 (H_4), 3.084 (H_{51}), 3.258 (H_{52}) and 5.977 (H_N).
Coupling constants (Hz): H_{31-4}: 4.60, H_{32-4}: 1.69, H_{51-4}: 6.74, H_{52-4}: 2.89, H_4-N_H: 7.20, H_{31-32}: -9.98, H_{51-52}: -14.06.

^{1}H-NMR spectroscopy

^{1}H-NMR spectra at 500 MHz were recorded in the Fourier Transform mode on a Bruker WM500 spectrometer interfaced with an ASPECT-2000 computer for data-accumulation (32K datapoints).

The spectrometer was field-frequency locked on the deuterium resonance of the solvent ($CDCl_3$, $C_2D_2Cl_4$ or Me_2SO-d^6), the sample temperature was regulated with a VT-1000 temperature controller. If necessary, a Lorentz Gauss transformation was carried out before a Fourier transformation, yielding Gaussian enhanced spectra[41,42]. All relevant spectra were analyzed by standard iterative simulation techniques using the LAOCOON-III-like PANIC program[43] for the abovementioned ASPECT computer.

Chemical shift data (relative to tetramethylsilane as internal reference) and coupling constants for the investigated compounds are presented in Tables 2-5. Based on the excellent agreement between observed and simulated spectra, the accuracy of the values of the chemical shifts and of the coupling constants can be considered better than 0.001 ppm and 0.1 Hz, respectively.

X-ray crystallography

A crystal of about 0.05x0.05x0.40 mm was used for X-ray measurements. The colourless crystals are monoclinic. Unit cell and space group data were obtained from diffractometer measurements. Accurate unit cell dimensions were determined by a least-squares method from 25 general reflections, measured with CuKα radiation (graphite crystal monochromator, λ=1.5418Å). Crystal data are listed in Table 8.

Table 8 CRYSTAL DATA

Molecular formula	$C_8H_{15}NO_4S$
FW	221.275
Crystal system	monoclinic
Space group	$P2_1$
a	9.604(1)Å
b	11.652(1)Å
c	9.949(1)Å
β	90.93(1)°
V_c	1113.3Å^3
Z	4
D_c	1.32 gcm^{-3}
F(000)	471.9
μ(CuKα)	24.74 cm^{-1}

The intensities of 8098 reflections, the full sphere with sin θ/λ<0.600, were measured on a CAD4 diffractometer, using the ω-2θ scan with a scan range of 0.80 + 0.14 tan θ°.

Intensity and orientation control reflections were measured every 30 min. and used to correct the data for primary beam intensity fluctuations and crystal decay (~20%). Symmetry equivalent reflections were averaged resulting in 4068 independent reflections, 2341 of which were considered 'observed', I > 3σ(I) counting statistics. Lorentz and polarization corrections were applied but no absorption correction. The structure was solved by direct methods (MULTAN) using the PDP-8/CSP[44] programs and refined by full-matrix least-squares to an R-value of 0.08. Then, based on the anomalous dispersion of S, the absolute configuration was established by comparison of the observed and calculated Bijvoet differences[45]. Refinement was continued; hydrogen atoms could be located in a difference Fourier synthesis but were included at calculated positions. Anisotropic thermal parameters were used for all non-hydrogen atoms. The quantity minimized was $\bar{Z}\, w(|F_o|-K|F_c|)^2$ with $2=(\sigma_c^2 + 0.0025\, F_o^2)^{-1}$; σ_c^2 from counting statistics. The final R-value is 0.051 for 252 variables and 2341 contributing reflections. A final difference map showed no significant resulting density.

The atomic scattering factors for S, O, N and C were those of Cromer and Mann[46] and for those of Stewart *et al*[47]. CSP[44] and X-RAY[48] programs were used for the calculations. The molecular structure and the atomic numbering are shown in Figure 2. The final atomic parameters are given[49] in Table 9.

Table 9 FINAL POSITIONAL PARAMETERS (10^4 for non-hydrogen-, 10^3 for H-atoms) AND THERMAL PARAMETERS 10^2

	Molecule -1-					Molecule -2-			
Atom	x	y	z	U_{eq}	Atom	x	y	z	U_{eq}
S1	1192	7241	9819	8.9(1)	S1'	888	10558	4705	8.4(1)
C5	2178	7168	8323	7.2(2)	C5'	1003	9487	3362	8.9(3)
C4	2075	8334	7631	7.1(2)	C9'	2477	11102	2873	6.9(3)
C3	784	8876	3239	10.1(4)	C4'	2244	9864	2500	6.2(2)
C8	4131	9236	6760	4.8(1)	C8'	4338	8888	1776	5.0(2)
C10	6376	10176	6315	4.9(2)	C10'	6617	7988	1445	5.0(2)
C12	7343	10811	7256	7.0(2)	C12'	7551	7366	2404	7.1(3)
C13	7045	9124	5768	8.7(3)	C13'	6089	7170	343	10.0(3)
C14	5852	10968	5212	7.8(3)	C14'	7276	9051	920	9.5(3)
O6	-70	6560	9571	10.6(3)	O6'	-334	11221	4316	12.3(3)
O2	844	8562	9624	9.1(2)	O2'	2279	11197	4262	7.5(2)
O11	3888	8955	5631	5.9(2)	O11'	4157	9155	620	7.7(2)
O9	5224	9837	7209	5.4(2)	O9'	5412	8313	2275	6.1(2)
N7	3265	9019	7792	6.6(2)	N7'	3450	9170	2767	5.8(2)
H51	325	694	852		H51'	127	865	386	
H52	174	650	764		H52'	6	941	284	
H4	198	826	654		H32'	175	1167	230	
H32	-16	852	773		H31'	353	1138	259	
H31	76	980	807		H4'	195	980	143	
H7	348	935	872		H7'	366	890	70	
H121	824	1114	676		H121'	853	715	194	
H122	681	1155	775		H122'	780	792	328	
H123	772	1027	811		H123'	706	660	277	
H131	630	862	519		H131'	694	678	-17	
H132	793	932	518		H132'	550	646	84	
H133	742	853	659		H133'	539	759	-35	
H141	528	1171	562		H141'	752	962	180	
H142	671	1130	464		H142'	825	885	46	
H143	514	1054	451		H143'	662	952	25	

1. The numbering of the non-hydrogen atoms is as indicated in Figure 2. The numbering of the hydrogen atoms (not shown in the Figure) is determined by the atoms to which they are attached e.g. H_{121}, H_{122} and H_{123} denote three protons attached to atom 12, a C-atom
2. H atoms were assigned a fixed μ-value of 9.00Å^2. E.s.d.'s are between 2×10^{-4} and $9.\times10^{-4}$ for non H atoms.

ACKNOWLEDGEMENT

We thank Prof.dr. C. Altona and Drs. F.A.A.M. de Leeuw for sharing unpublished data and for stimulating discussions.
We thank Mr B. Zweers for preparing compound 4.

This research was supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

NMR spectra were recorded at the Dutch National 500/200 MHz hf-NMR facility at Nijmegen.

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    ${}^{x}E$: envelope with atom x puckered downward
    ${}^{y}_{x}T$: twist chair, with atom y puckered up and atom x puckered downward, the degree of puckering is about the same. If atom x is less puckered than atom y, ${}^{y}T_{x}$ is used. The C4-N bond is defined as being upward.

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CHAPTER VII

FLASH VACUUM THERMOLYSIS OF FUNCTIONALIZED γ-SULTINES.

FLASH VACUUM THERMOLYSIS OF FUNCTIONALIZED γ-SULTINES

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Abstract. The flash vacuum thermolysis (FVT) of the 4-(benzamido)-γ-sultines 5a and 5b is shown to lead to a mixture of the allylamide 6 and enamides 7 and 8, the allylamide being the main product. This reaction involves a novel migration of the benzamido group, which is proposed to proceed as depicted in path c, Scheme IV.

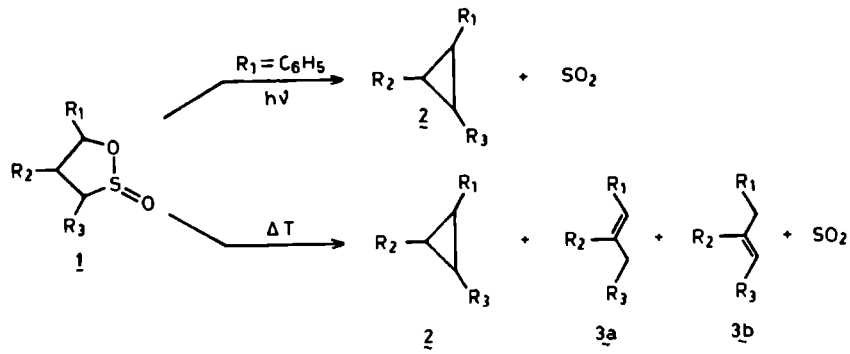
This proposed mechanism features heterolytic bond fission, accompanied by neighbouring group participation. Support for this proposal has been found by FVT of 5a-d_2 (Scheme IV).

INTRODUCTION

During the last decade several aspects of the chemistry of cyclic sulfinate esters (sultines) received incidental attention, but all those studies[1,2] concerned sultines containing only phenyl or simple alkyl substituents or sultines condensed with aromatic rings. Recently, we reported[3] for the first time an efficient route to functionalized cyclic sulfinate esters, *viz.* the N-protected β-amino-γ-sultines, and showed that nucleophilic ring-opening reactions can be performed by selective cleavage of either the S-O or the C-O bond.

Durst *et al.* have studied the photochemical[4] and thermolytic[5] breakdown of non-functionalized γ-sultines 1. Photolysis was only observed with sultines having a γ-phenyl substituent and gave phenylcyclopropanes 2 (Scheme I). Thermolysis gave the alkenes 3a and 3b beside 2[6].

Scheme I

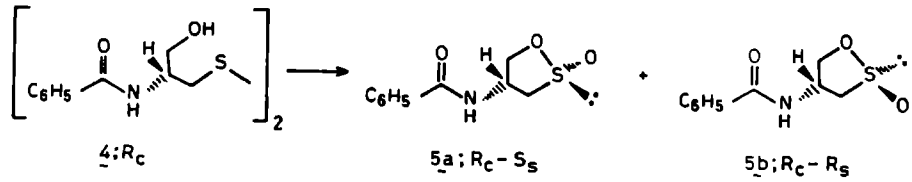


The authors assumed that thermolysis of 1 proceeds *via* an intermediate diradical by consecutive cleavage of the C-O and C-S bond[5]. It seemed worthwhile to investigate whether thermolysis of N-protected β-amino-γ-sultines leads to functionalized analogs of 2 and 3.

RESULTS and DISCUSSION

The N-protected amino γ-sultines 5a and 5b were prepared from N-benzoyl-L-cystinol 4 by treatment with N-chlorosuccinimide and AcOH[3] (Scheme II). The two diastereomers 5a and 5b were readily separated by silica gel chromatography. Their thermolytic behaviour was studied by flash vacuum thermolysis (FVT) at 0.05 mmHg through a quartz tube heated by a tube furnace. The products were collected on a cold finger and analysed by thin layer chromatography, ^{1}H-NMR spectroscopy and mass spectroscopy.

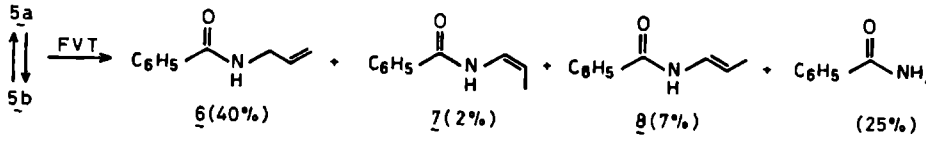
Scheme II



Interestingly, the sultines 5a and 5b epimerise at the sulfur atom at 130 °C, which is the preheating temperature of the samples[7]. As a consequence, thermolysis of 5a and 5b gave identical reaction mixtures. At 700 °C the compounds fragmented as shown in Scheme III. At higher temperatures a considerable amount of unidentifiable products was formed whereas at 650 °C the starting material was not completely converted. Other variations in reaction conditions yielded invariably the allylamide 6 as the main product: when the reaction tube was filled with quartz wool (600 °C, 0.05 mmHg) or quartz chips (625 °C, 0.09 mmHg, N_2 flow) compound 6 was isolated in yields of 45% and 50%, respectively.

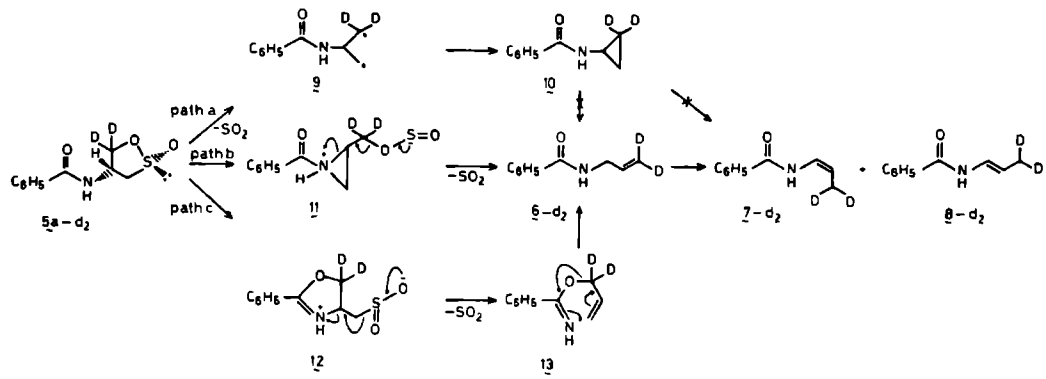
In variation with the results of Durst *et al*[5] (Scheme I), we could not detect cyclopropane derivatives.

Scheme III



The formation of 6 via an intermediate biradical 9 and a cyclopropane derivative 10, as proposed by Durst *et al.* for non-functionalized γ-sultines (Scheme I), could be ruled out by the following experiment. Thermolysis of benzamidocyclopropane 10 at 700 °C yielded a mixture of 7 and 8; none of the allylamide 6 could be detected. Conclusive evidence which allowed us to rule out pathway a for the formation of 6 as well as of 7 and 8 was obtained by thermolysis of the labelled sultine 5a-d_2 (Scheme IV). Thermolysis of 5a-d_2 at 700 °C gave the compounds 6-d_2, 7-d_2 and 8-d_2, all having the label at the terminal carbon atom. These structures were secured by ^{1}H-NMR spectroscopy. Pathway a fails to explain these results, as the intermediacy of 10 would inevitably lead to scrambling of the label in 6-d_2, 7-d_2 and 8-d_2.

Scheme IV

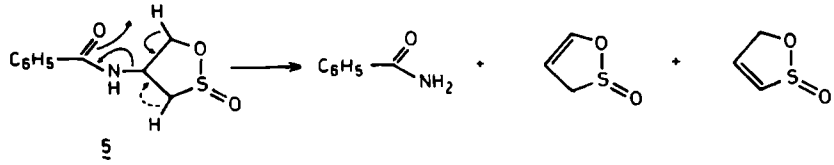


Apparently, the formation of 6-8 by thermolysis of 5 involves migration of the amide function. Pathways b and c (Scheme IV) might explain this rearrangement. Path b features intramolecular nucleophilic attack by the amide *nitrogen* on the C-S bond leading to the aziridine intermediate 11; expulsion of SO_2 gives 6-d_2. Alternatively, intramolecular nucleophilic attack on the C-O bond by the amide *oxygen* (path c) gives the oxazoline intermediate 12. Extrusion of SO_2 from the latter yields 13, which gives 6-d_2 via a Claisen type rearrangement[9]. Compounds 7-d_2 and 8-d_2 may have been formed on their turn from 6-d_2 by a H-shift. This could be substantiated as follows: thermolysis of 6-d_2 at 700 °C gave a mixture of 7-d_2 and 8-d_2, beside starting material.

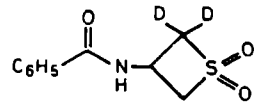
At present we have no conclusive evidence permitting a choice between pathways b and c. However, we are inclined to favour pathway c for two reasons. First, in 5 the C-O bond is more polarized than the C-S bond, and thus more susceptible to a nucleophilic attack. Second, attack by the amide nitrogen on the C-S bond (path b) would lead to a strained transition state, in which the nucleophile and the leaving group hardly can take apical positions.

The formation of benzamide (Scheme III) can be explained as depicted in Scheme V. This mechanism is analogous to the well-documented[10] thermolytic fragmentation of amides having a C(O)-N-C-C-H moiety. As this fragmentation is in competition with path c and/or path b, the occurence of benzamide explains the relatively low yields of compounds 6-8.

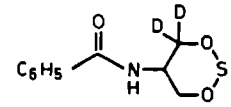
Scheme V



The mechanisms as depicted in paths b and c (Scheme IV) deserve some further comment. First, one might argue that - prior to attack by N or O - the sultine 5a-d_2 rearranges to the sulfone 14 or the sulfoxylate 15. However, the formation of 6-d_2 excludes the intermediacy of these structures in any conceivable mechanism, as they would lead to an allylamide 6 having a scrambled label[11].



14



15

Second, the proposed mechanistic pathways b and c (Scheme IV) involve *heterolytic* bond fissions, whereas radical pathways are rather generally encountered in pyrolytic reactions[15]. However, there is limited evidence for the occurence of charged intermediates in thermolysis[15,16]. These examples concern polar molecules containing moieties which are able to accommodate charge, as is the case with 5 too. Heterolytic bond fission is expected to take place primarily at the polar surface of the reaction tube[17]. Loss of deuterium, when observed in pyrolytic reactions, can be expected also to occur in a surface process.

We argued that as a consequence, loss of deuterium in the conversion of 5a-d_2 into 6-d_2 might be indicative of the reaction to take place - at least partly - at the surface and would thus support the proposed polar mechanism. Therefore, we determined by mass spectroscopy the percentage of mono-deuterated (d_1) and non-deuterated (d_0) compounds 5a-d_2 and 6-d_2 relative to the corresponding dideuterated (d_2) compounds. For 5a-d_2 and 6-d_2 the ratios $d_2/d_1/d_0$ were found to be 100/26/2 and 100/41/6, respectively. This loss of deuterium is significant and suggests that the mechanism for the conversion of 5 into 6 is a polar one.

CONCLUSIONS

The above findings suggest that thermolysis of 5a or 5b involves a novel migration of the benzamide group to yield 6-8. The proposed mechanisms (Scheme IV) feature neighbouring group participation by the amide nitrogen (path b) or amide oxygen (path c) in the heterolytic fission of the C-S or C-O bond, respectively. We favor pathway c above pathway b for the aforementioned reasons. Support has been found for the heterolytic nature of the bond fission.

Whereas thermolysis of non-functionalized γ-sultines led to cyclopropanes among other products[5], we could not detect benzamidocyclopropane in the reaction mixture. Experiments to refine the proposed mechanism are being sought.

In addition, we plan to study the FVT of other functionalized sultines and sultones. Presently, the influence of the N-protecting group on the behaviour of sultines in FVT is under investigation. Also synthetic applications of this route to allylamides 6 are being studied[18]. Finally, the synthesis of sparsomycin starting from a sultine[3,19] can be optimized now, by making use of the finding that sultines 5a and 5b undergo a clean epimerization at 120-130 °C[7].

EXPERIMENTAL PART

^{1}H-NMR spectra were measured on a Varian Associates Model T-60 or a Bruker WH-90 spectrometer with Me_4Si as internal standard. IR spectra were measured with a Perkin-Elmer spectrophotometer, Model 997. Mass spectra were obtained with a double-focusing Varian Associates SMI-B spectrometer and with a Finnigan 3100 Gas chromatograph/mass spectrometer.

Melting points were taken on a Kőfler hot stage (Leitz-Wetzlar) and are uncorrected. Thin layer chromatography (TLC) was carried out by using Merck precoated silicagel F254 plates (thickness 0.25 mm). Spots were visualized with an UV lamp, ninhydrin or Cl_2-TDM[20]. For column chromatography Merck silica gel H (type 60) was used. The Miniprep LC (Jobin-Yvon) was used for preparative HPLC.

Flash Vacuum Thermolysis (FVT) was carried out in standard equipment in a horizontal assembly as described in Chapter II of ref. 15. The quartz tube (outer diameter 1.75 cm) was heated over a length of 17.5 cm. The products were collected on a cold finger cooled with isopropanol/CO_2. The preheating temperature was 130 °C. The reported yields are after HPLC column chromatography.

N-benzoyl-L-cystinol (4)

N-benzoyl-L-cystine methylester (10.0 g, 21 mmol) was reduced with lithium borohydrate [sodium borohydride (4.75 g, 125 mmol) and lithium iodide (21.34 g, 125 mmol)] in 500 ml of DME as described earlier[3] for the preparation CbO-L-cystinol. The work-up had to be slightly modified, however, due to the poor solubility of the reaction products in DME. The pH was adjusted to 5 by addition of an aqueous 1N HCl solution to the stirred and cooled (0 °C) solution. DME was evaporated *in vacuo*, the residue dissolved in 500 ml of methanol/water (1/1, v/v), and then oxidized with iodine as described[3] for CbO-L-cystinol. Subsequently, the methanol was evaporated *in vacuo*, and water and dichloromethane were added. The aqueous phase was extracted five times with 400 ml portions of dichloromethane.

The collected organic layers were dried (Na_2SO_4) and residual iodine was removed by stirring with $Na_2S_2O_5$. The residue was recrystallized from methanol/water to give 4: 56% yield; m.p. 194 °C; R_f 0.27 (eluent MeOH/CH_2Cl_2, 1/9, v/v); NMR (CD_3OD) δ3.00 (ABX, 2H, CHC$\underline{H}_2$S), 3.63 (d, 2H, C$\underline{H}_2$OH), 4.11-4.52 (m, 1H, C$\underline{H}$CH$_2$OH), 7.20-7.89 (m, 5H, C_6H_5); IR (KBr) 3380, 3300, 1640, 1530 cm^{-1}. Anal. Calcd for $C_{20}H_{24}N_2O_4S_2$: C, 57.12; H, 5.75; N, 6.56. Found: C, 56.75; H, 5.79; N, 6.51.

N-benzoyl-L-dideuterocystinol (4-d_2)

This compound was prepared as has been described for the preparation of 4. Instead of sodiumborohydride, sodiumborodeuteride was used. The compound was obtained in 60% yield and was identical in every aspect with 4, except for the C$\underline{H}_2$OH signal in the NMR spectrum, due to residual protons (ca 18% by integration). Anal. Calcd. for $C_{20}D_4H_{20}N_2O_4S_2$: C, 56.58; N, 6.60. Found: C, 56.58; N, 6.46.

4-(Benzamido)-1,2-oxothiolane-2-oxide (5a, 5b)

Compounds 5a and 5b were prepared from N-benzoyl-L-cystinol 4 (3.50 g, 8.3 mmol) and N-chlorosuccinimide (3.34 g, 25 mmol) in 150 ml of glacial acetic acid as has been reported earlier[3] for other N-protected aminosultines. HPLC (eluent MeOH/CH_2Cl_2, 5/95, v/v) gave 5a (43%) which was homogeneous by TLC (MeOH/CH_2Cl_2, 1,9 v/v). Compound 5b thus obtained was still contaminated with succinimide; both compounds have nearly identical R_f values on TLC. Purification was achieved by repeated extraction of a dichloromethane solution of the mixture with 0.1 N $NaHCO_3$ solution. After drying (Na_2SO_4) and evaporation of the solvent, sultine 5b was obtained in 32% yield.

5a: R_f 0.77 (eluent MeOH/CH_2Cl_2, 1/9, v/v); NMR ($CDCl_3$) δ3.06 and 3.32 (AB part of ABX spectrum, J_{AX} = 1.2 Hz, J_{BX} = 6.3 Hz, J_{AB} = 13.2 Hz, 2H, CH_2S), 4.67 and 4.84 (AB part of ABX spectrum, J_{AX} = 1.7 Hz, J_{BX} = 5.4 Hz, J_{AB} = 9.9 Hz, 2H, CH_2O), 5.22-5.60 (m, 1H, $CHCH_2O$), 7.17-8.11 (m, 6H, C_6H_5 and NH); IR (KBr) 3280, 1640, 1535 and 1060 cm^{-1} ; mass spectrum: m/e 225 (M^+), 161 ($-SO_2$); Anal. Calcd. for $C_{10}H_{11}NO_3S$: C, 53.32; H, 4.92; N, 6.22. Found: C, 53.12; H, 4.86; N, 6.19.

5b: R_f 0.40 (eluent MeOH/CH_2Cl_2, 1/9, v/v); NMR ($CDCl_3$), δ3.24 and 3.60 (AB part of ABX spectrum, J_{AX} = 3 Hz, J_{BX} = 6.6 Hz, J_{AB} = 14 Hz, 2H, CH_2S), 4.60 and 4.89 (AB part of ABX spectrum, J_{AX} = 1.5 Hz, J_{BX} = 4.6 Hz, J_{AB} = 10 Hz, 2H, CH_2O), 5.00-5.29 (m, 1H, $CHCH_2O$), 6.87 (d, 1H, NH), 7.16-7.84 (m, 5H, C_6H_5); IR (KBr) 3300, 1650, 1530 and 1030 cm^{-1}; mass spectrum: 225 (M^+), 161 ($-SO_2$); Anal. Calcd. for $C_{10}H_{11}NO_3S$: C, 53.32; H. 4.92; N, 6.22. Found: C, 53.26; H, 4.90; N, 6.22.

4-(Benzamido)-5,5-dideutero-1,2-oxothiolane 2-oxide (5a-d_2, 5b-d_2)

The synthesis of 5a-d_2 and 5b-d_2 from 4-d_2 was carried out as described above for 5a and 5b, yielding the compounds in 43% and 29%, respectively.

5a-d_2: R_f 0.77 (eluent MeOH/CH_2Cl_2, 1/9, v/v); NMR ($CDCl_3$), δ3.06 and 3.32 (AB part of ABX spectrum, J_{AX} = 1.2 Hz, J_{BX} = 6.3 Hz, J_{AB} = 13.2 Hz, 2H, CH_2S), 4.55-5.02 (residual protons CH_2O; 15% by integration), 5.44 (d of t, 1H, $CHCH_2O$), 7.17-8.11 (m, 6H, C_6H_5 and NH); mass spectrum: m/e 227 (M^+), 163 ($-SO_2$); Anal. Calcd. for $C_{10}D_2H_9NO_3S$: C, 52.85; N, 6.16. Found: C, 52.67 N, 6.08.

5b-d_2: R_f 0.40 (eluent MeOH/CH_2Cl_2, 1/9, v/v); NMR ($CDCl_3$), δ3.24 and 3.60 (AB part of ABX spectrum, J_{AX} = 3 Hz, J_{BX} = 6.6 Hz, J_{AB} = 14 Hz, 2H, CH_2S). 4.59 and 4.89 (residual protons CH_2O; 15% by integration), 5.14 (d of t, 1H, $CHCH_2O$), 6.82 (d, 1H, NH), 7.16-7.84 (m, 5H, C_6H_5); mass spectrum m/e 227 (M^+), 163 ($-SO_2$). Anal. Calcd. for $C_{10}D_2H_9NO_3S$: C, 52.85; N, 6.16. Found: C, 53.03; N, 6.28.

3-Benzamido-propene-1 (6), 3-benzamido-Z-propene-2 (7), 3-benzamido-E-propene-2 (8)

The FVT of 5a or 5b (0.5 g, 2.2 mmol) was carried out as described above. The products were separated by HPLC (eluent CH_2Cl_2) to give 6, 7 and 8 in yields of 40-50%, 2% and 7%, respectively.

6: R_f 0.66 (MeOH/CH_2Cl_2, 1/9, v/v); NMR ($CDCl_3$), δ4.08 (Y_2 part of $ABXY_2$-spectrum J_{AY} = J_{BY} = 1.6 Hz, J_{XY} = J_{Y-NH} = 5.6 Hz, 2H, $NHCH_2$), 5.17 and 5.24 (AB part of $ABXY_2$ spectrum, J_{AB} = 3.2 Hz, J_{AX} = 17.2 Hz, J_{BX} = 10 Hz, 2H, C = CH_2), 5.95 (X-part of $ABXY_2$, spectrum 1H, CH_2CH), 6.16-6.76 (br, 1H, NH), 7.07-7.93 (m, 5H, C_6H_5); IR ($CHCl_3$) 3460, 3360, 1660, 1520, 995 and 930 cm^{-1}; mass spectrum: m/e 161 (M^+).

7: R_f 0.81 (MeOH/CH_2Cl_2, 1/9, v/v); NMR ($CDCl_3$), δ 1.70 (X_3 part of ABX_3 spectrum, d of d; J_{BX} = 6.9 Hz, J_{AX} = 1.7 Hz, 3H, $CHCH_3$); 4.95 (B part of ABX_3 spectrum, J_{AB} = 8.4 Hz, 1H, $CHCH_3$), 6.93 (A part of ABX_3 spectrum, J_{AX} = 1.8 Hz, J_{A-NH} = 10.0 Hz, 1H, NHCH), 7.30-7.96 (m, 6H, C_6H_5, NH); mass spectrum: m/e 161 (M^+).

8: R_f 0.73 (MeOH/CH_2Cl_2, 1/9, v/v); NMR ($CDCl_3$), δ1.74 (X_3 part of ABX_3 spectrum, d of d, J_{BX} = 6.8 Hz, J_{AX} = 1.6 Hz, 3H, $CHCH_3$), 5.31 (B part of ABX_3 spectrum, J_{AB} = 13.8 Hz, 1H, $CHCH_3$), 6.97 (A part of ABX_3 spectrum, J_{A-NH} = 10.0 Hz, 1H, NHCH), 7.30-8.00 (m, 6H, C_6H_5, NH); mass spectrum: m/e 161 (M^+).

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ACKNOWLEDGEMENT

We thank Mr J.M.J. Verlaak for his assistence in carrying out the FVT experiments, Professor Dr B. Zwanenburg and Dr A.J.H. Klunder for making available the FVT-apparatus and Mr H.H.K. Brinkhof for the ^{1}H-NMR spectra and helpful discussions.
We are indebted to Mr J. Zwinselman and Professor Dr N. Nibbering (University of Amsterdam) for the electron impact and field ionization mass spectra.
Part of the investigations was supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

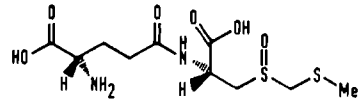
CHAPTER VIII

APPROACHES TO THE MARASMIN FRAGMENT OF γ-GLUTAMYL MARASMIN.

APPROACHES TO THE MARASMIN FRAGMENT OF γ-GLUTAMYLMARASMIN

INTRODUCTION

More than 70 γ-glutamyl derivatives of amino acids and amines have been isolated from plants, including mushrooms (Basidomycetes), during the last twenty years[1]. Among these are γ-glutamyl derivatives of sulfur containing protein amino acids and γ-glutamyl derivatives of sulfur or selenium containing non-protein amino acids. Six γ-glutamyl derivatives of sulfur and two γ-glutamyl derivatives of selenium containing non-protein amino acids have been identified so far.



1 γ-glutamyl marasmin

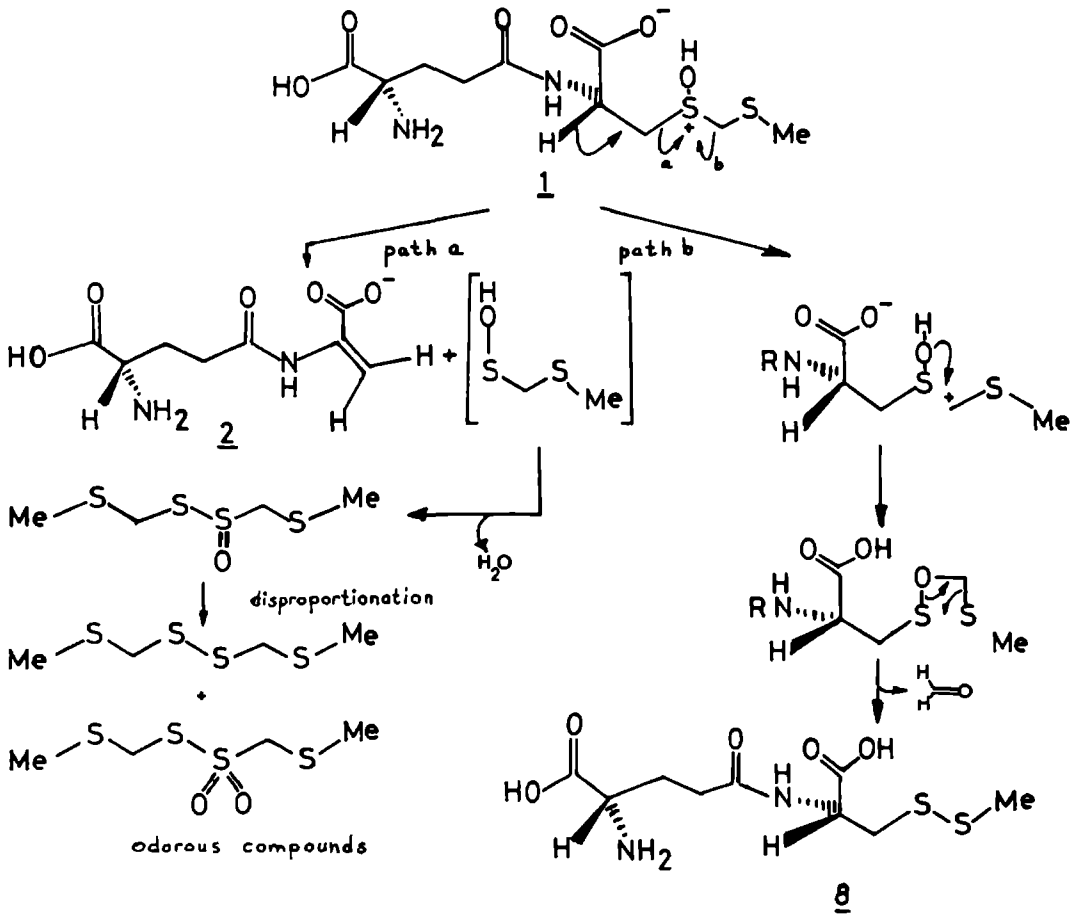
γ-Glutamylmarasmin[1], an N-γ-glutamyl-S-substituted cysteine derivative, has attracted our interest, because it possesses a mono-oxodithioacetal moiety. To our knowledge, 1 and sparsomycin are the only natural products containing this moiety.

γ-Glutamylmarasmin was isolated[2] from the Basidomycetes mushrooms, *Marasminus alliaceus*, *M. scorodonius*, and *M. prasiosmus*, which are known for their garlic like odour. Its structure was established[2,3] by chemical and enzymic degradation studies as well as by spectroscopic methods.

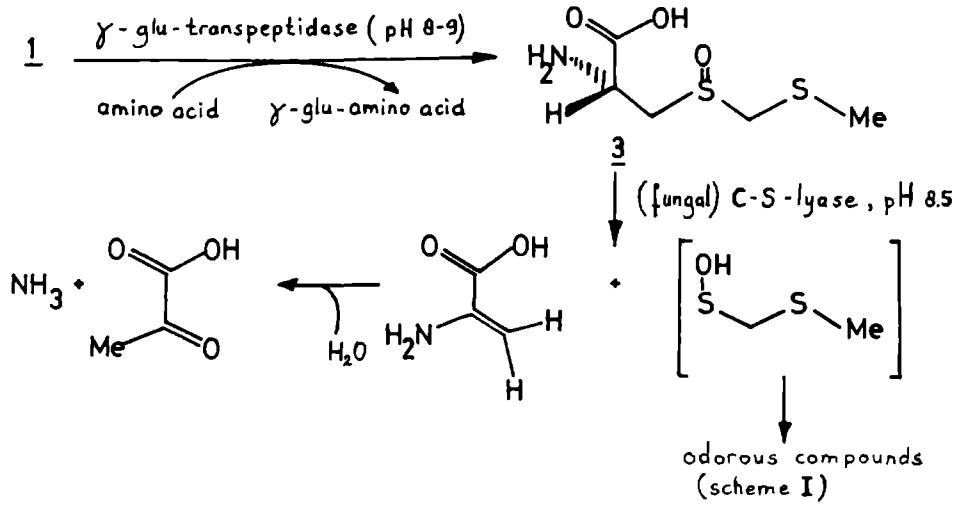
γ-Glutamylmarasmin is readily soluble in water, giving a slightly acidic solution. In solution the compound gradually decomposes what causes the typical odour of the parent mushrooms. This odour is ascribed to products derived from the sulfenic acid $MeSCH_2SOH$, which is formed by an acid catalyzed β-elimination reaction[4] of 1 (Scheme I, path a), with concomitant formation of γ-glutamyl-dehydroalanin 2. In the mushroom a similar degradation takes place by the fungal C-S lyase[2,5]. This reaction takes place at higher pH(8.5) and only after cleavage of the γ-glutamyl residue (Scheme II) by γ-glutamyl-transpeptidase (E.C. 2.3.2.2.), resulting in the formation of marasmin 3. In the latter reaction the γ-glutamyl residue is transferred to an acceptor e.g. another amino acid[6]. The C-S lyase induced β-elimination is characteristic for many S-substituted derivatives[11] of γ-glutamyl-cysteine compounds and the corresponding sulfoxides. Noteworthy examples are (epi)lentinic acid[7,8] 4,5; γ-glutamyl-S-(prop-1-enyl)-cysteine-R-sulfoxide[9] 6 and γ-glutamyl-S-(prop-1-enyl)-cysteine-S-sulfoxide[10] 7. Via consecutive transpeptidation and lyase mediated[11] β-elimination, these compounds also give rise to the formation of pyruvic acid, ammonia and a variety of sulfur compounds (Scheme III[12]).

Beside the aforementioned acid-catalyzed β-elimination of γ-glutamyl-marasmin, the compound might undergo an acid-catalyzed decomposition of the mono-oxodithioacetal moiety, as depicted in Scheme I, path b. In this reaction pathway the sulfoxide is protonated too, but now the protonation is followed by cleavage of the other C-S(O) bond. Subsequently the intermediate sulfenate ester is formed, which looses formaldehyde, leaving the mixed disulfide 8. This decomposition of the mono-oxodithioacetal moiety[13] - which is of value for the preparation of mixed disulfides[14,15] - requires the presence of a strong acid.

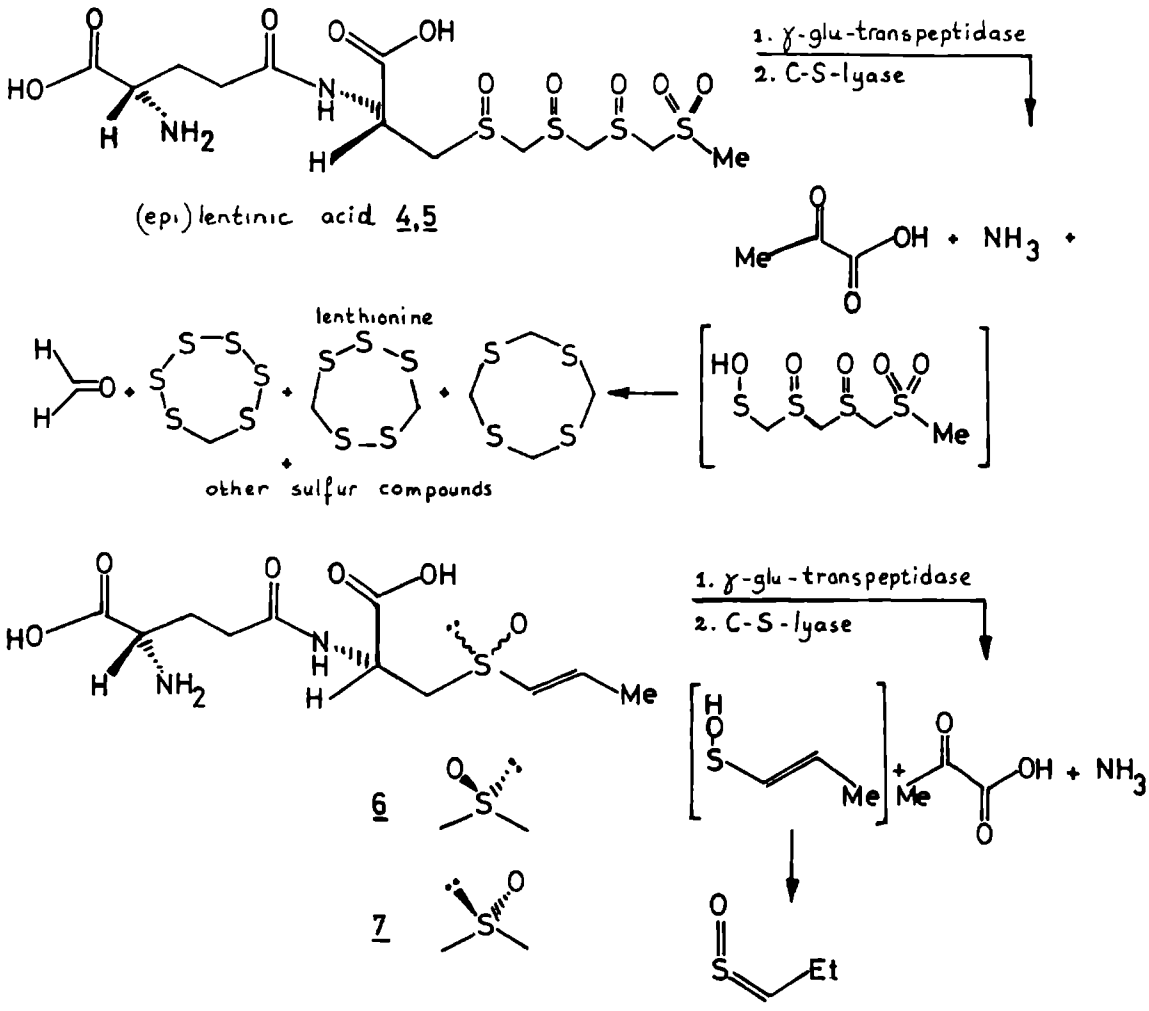
Scheme I acid catalyzed β-elimination and decomposition of the mono-oxodithioacetal moiety of 1



Scheme II (fungal)C-S lyase induced β-elimination of 1



Scheme III C-S lyase induced β-elimination of S-substituted-sulfoxide-γ-glutamyl-cysteine derivatives



Consequently, the use of strong acids has to be avoided in synthetic routes leading to γ-glutamylmarasmin[1].

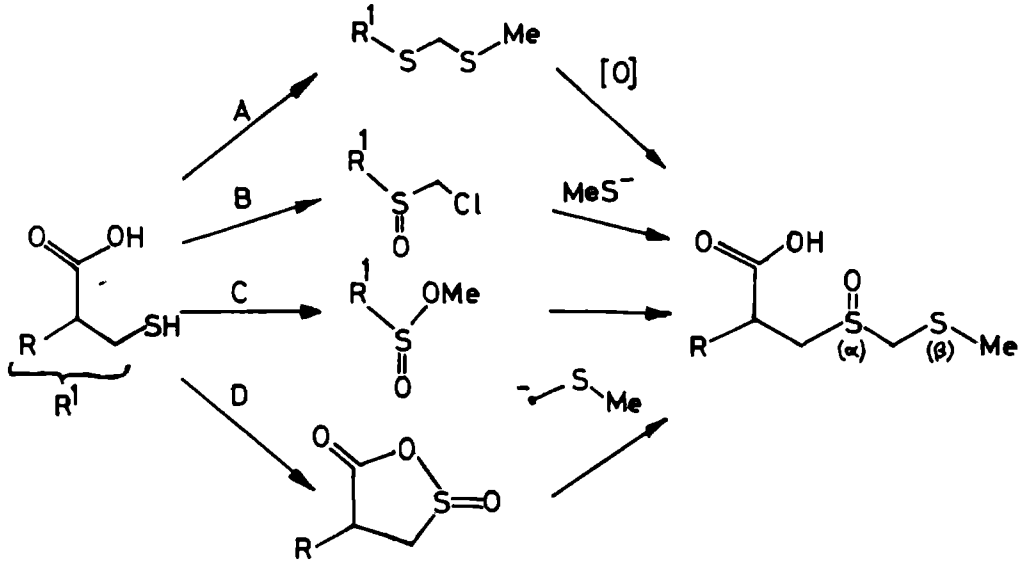
The motive to start an investigation into a total synthesis of γ-glutamylmarasmin emanated from our experiences, gained in the synthetic studies on sparsomycin (Chapter II-IV). We were interested to investigate whether the synthetic methodologies developed for the introduction of the mono-oxodithioacetal moiety of sparsomycin could be applied to the preparation of 1. In further studies the construction might be considered of a sparsomycin analog, in which the cysteinol mono-oxodithioacetal fragment of sparsomycin is replaced by S_C-marasmin 3. Furthermore, we could expect that successful completion of synthetic route of 1, would lead to the elucidation of the absolute configuration of the sulfoxide-sulfur atom of marasmin.

STRATEGY AND SYNTHETIC APPROACHES

As will be clear from the enzymatic degradation pattern (Scheme II) of γ-glutamylmarasmin, this compound can be considered as a dipeptide derived from L-glutamic acid and the cysteine-mono-oxodithioacetal fragment, marasmin[3]. We planned the coupling procedure toward the end of the total synthesis. Standard methods are available for the preparation of γ-glutamyl derivatives[1,16]. The most suitable method for the preparation of γ-glutamyl-marasmin seems to be coupling of marasmin with N-phtaloyl-L-glutamic anhydride, which is known to react predominantly at the γ-carbonyl function. The N-protecting group can be removed by hydrazinolysis.

More challenging is the synthesis of the marasmin fragment. Several approaches can be envisaged for its preparation (3, Scheme IV). The four approaches depicted in Scheme IV are discussed here in succession.

Scheme IV

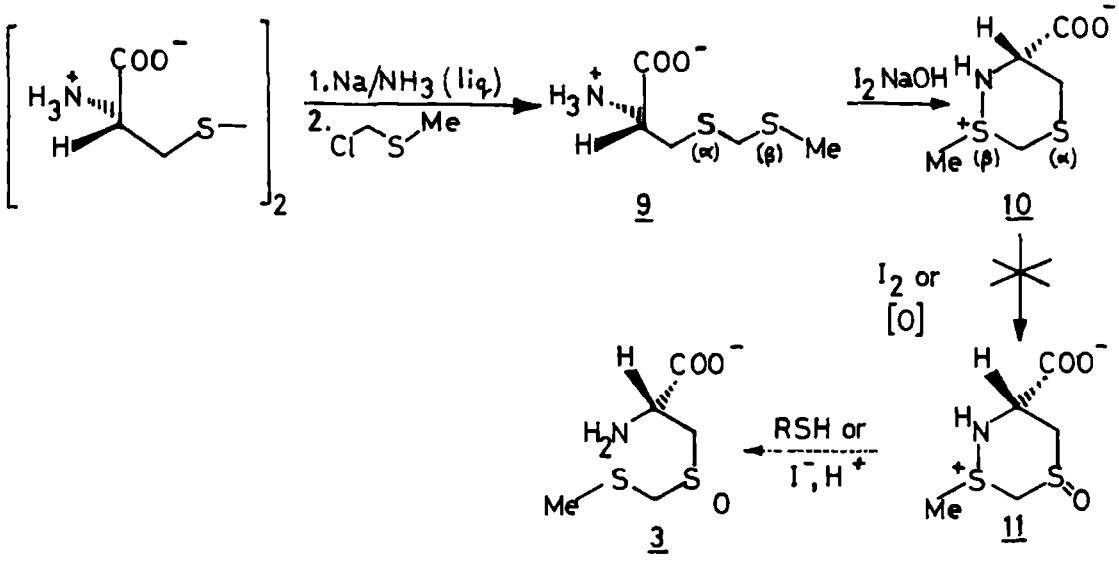


ROUTE A

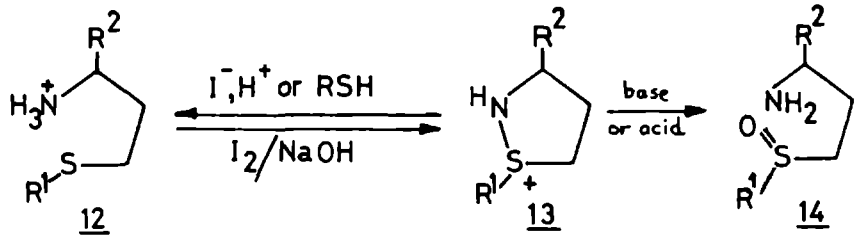
As was described earlier (Chapter III[17]) oxidation of the dithioacetal (Chapter III, compounds 23-26) failed to yield predominantly the regio isomer, occuring in sparsomycin. A conceivable route to obtain the desired regio isomer from the dithioacetal 9 is depicted in Scheme V. This synthetic route proceeds via the intermediate 10, in which the β-sulfur atom is protected intramolecularly by the amine nitrogen as a sulfilimine[18] moiety. As the β-sulfur atom is protected, it will no longer be susceptible to oxidation and therefore the α-sulfur atom might be oxidized. Subsequently the β-sulfur atom can be deprotected with hydrogen sulfide or a thiol (*vide infra*) to give marasmin.

The proposed synthesis of the six-membered ring sulfilimine 10 is based on the well documented[19] synthesis of the corresponding five-membered ring sulfilimines 13 (Scheme VI). These compounds have been prepared from methionine or other S-substituted homo cysteine derivatives 12 (R_1=Me,Et, C_6H_5,$C_6H_5CH_2$; R_2=COOH) in an oxidation reaction with iodine at neutral to slightly alkaline pH. Especially the preparation and chemistry of dehydromethionine 13 (R_1=Me, R_2=COOH) have found attention[19,20]. Treatment of 13 with hydrogen sulfide[20c], a thiol[19,21] or KI in the presence of acid yields the starting material, whereas treatment with aqueous base or acid gives the corresponding sulfoxides 14[19].

Scheme V elaboration of route A



Scheme VI

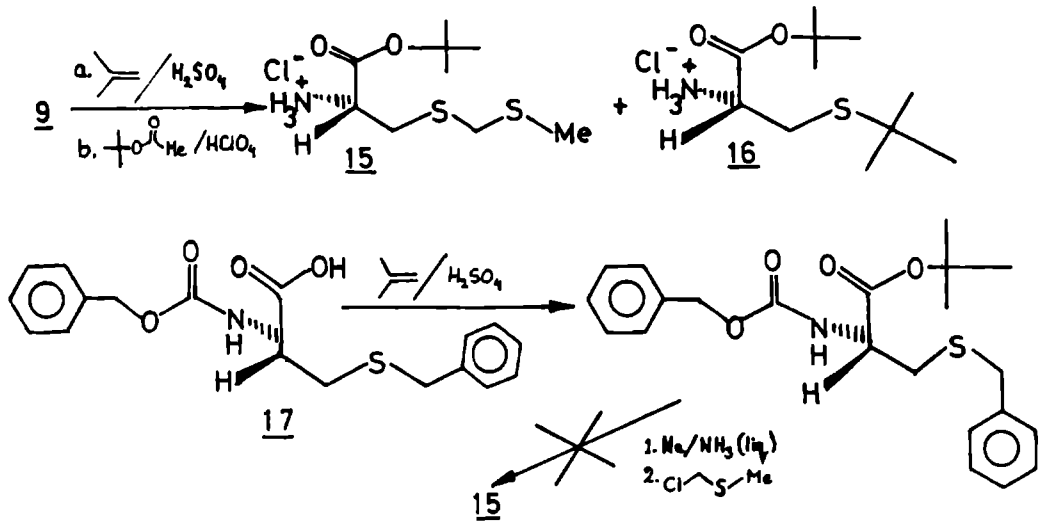


Compound 10 was obtained from 9 - which was prepared as described earlier (Chapter III[17]) - by treatment with one equivalent of iodine. During the reaction an aqeous solution of sodium hydroxide was added, to maintain a pH of 7 - 8.5. Addition of a second equivalent of iodine did not result in the formation of the sulfoxide 11. The use of other oxidizing agents (sodium metaperiodate, hydrogen peroxide) failed also to yield 11. These failures were partly attributed to the poor solubility of 10. In order to obtain a sulfilimine derivative with an increased solubility, we decided to prepare the t-butyl ester of 9, i.e. 15. However, so far we have not been able to develop a satisfactory synthesis of 15 (Scheme VII).

Treatment of 9 with isobutylene/sulfuric acid[22] or t-butyl acetate/perchloric acid[23] gave compound 16 beside the desired t-butyl ester 15, both isolated as hydrochloric acid salts. Attempts to separate the free amino esters of 15 and 16 were unsuccessful.

Another approach to 15 is outlined in the second entry of Scheme VII: the t-butyl ester i.e. 18 was prepared from the commercially available 17 with isobutylene/sulfuric acid, according to the procedure of Anderson[25]. Subsequently, we studied the simulteneous removal of the N- as well as the S-protecting group by treatment with sodium in liquid ammonia[24]. It was expected that subsequent addition of chloromethyl methyl sulfide would yield 15.

Scheme VII *a: ratio* 1 6
b: ratio 2 1



Unfortunately deprotection gave rise to a mixture of products, among which β-elimination products were present. In a final attempt to prepare 15, we tried to reduce the t-butyl ester of cystine - prepared according to the aforecited methods[22,23] - with sodium borohydride in methanol or ethanol, followed by treatment of the formed thiolate with chloromethyl methyl sulfide. However, beside some β-elimination product, the starting compound was recovered.

In view of these poor results, this - albeit at first glance elegant - route for the preparation of marasmin was abandoned temporarily.

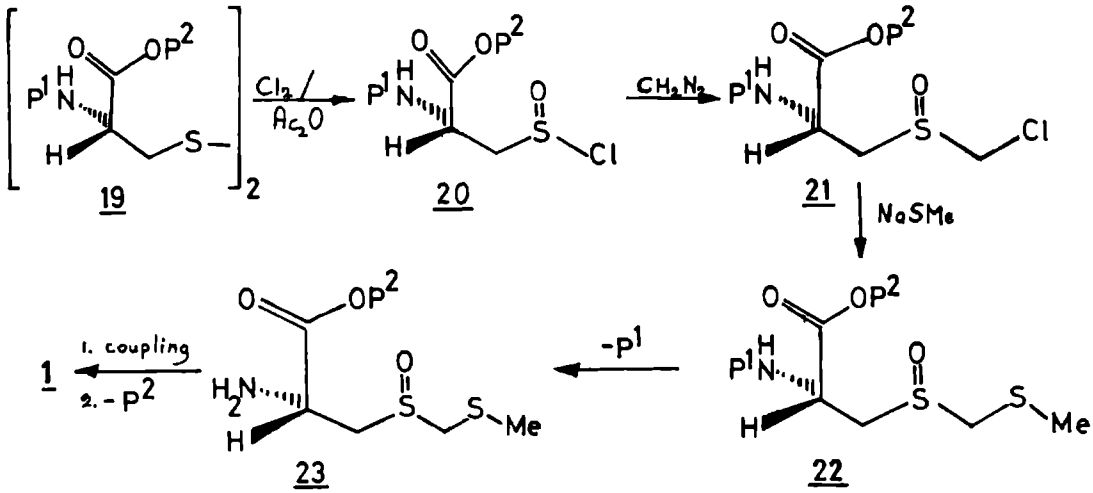
ROUTE B

We had recourse to a route, which we had developed earlier (Chapter III[17]) for the synthesis of the cysteinol mono-oxodithioacetal moiety of sparsomycin. An adaptation of this route to the synthesis of γ-glutamylmarasmin is represented in Scheme VIII. It is obvious that for the synthesis of 22 according to this scheme, the amino group as well as the carboxylic function[26] of 19 have to be protected; the required protecting groups are denoted as P_1 and P_2, respectively. These protecting groups have to be removed selectively and in succession, as appears from Scheme VIII. Furthermore, to avoid base induced racemization and β-elimination, the protecting groups have to be acid-labile or removable under neutral conditions, without affecting the mono-oxodithioacetal moiety. Finally, as a matter of course the protecting groups must be stable to the conditions used for the introduction of the mono-oxodithioacetal moiety.

Therefore the t-butyloxycarbonyl (BOC) group and the 2-trimethylsilylethyl (Tmse) group were chosen for P_1 and P_2 respectively (Scheme IX). The BOC-group can be removed by treatment with HCl in trifluoroethanol without affecting the Tmse group; the Tmse group is removed by treatment with fluoride ions, also in the presence of the BOC-group[28].

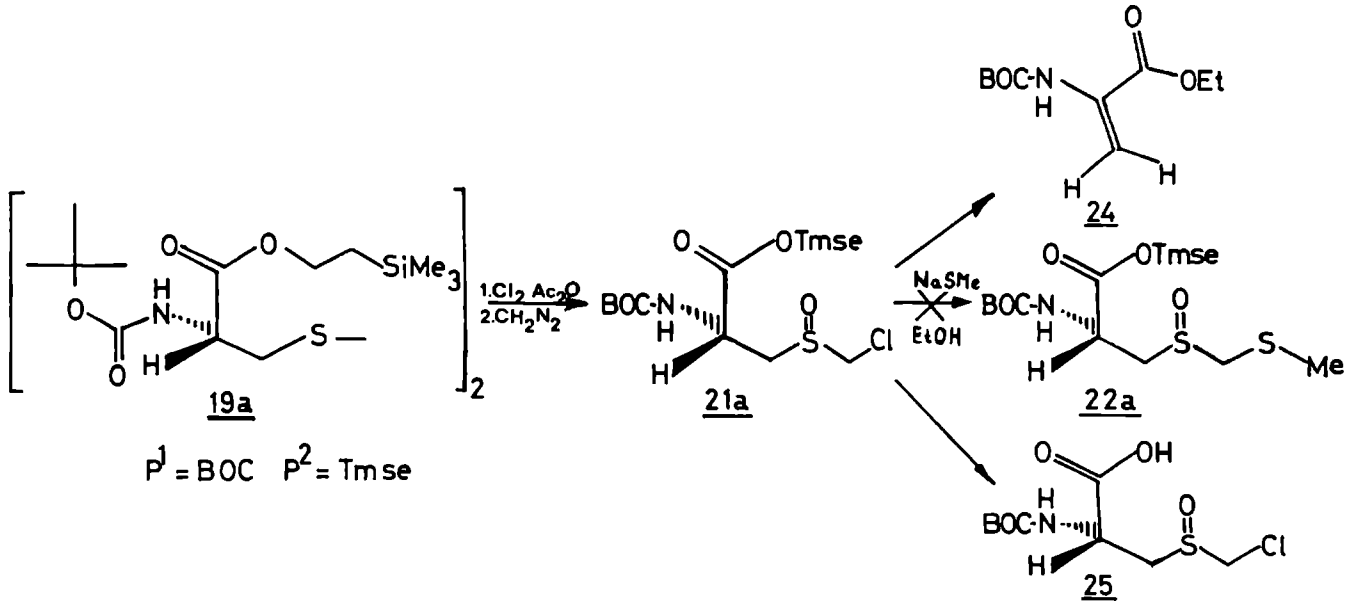
The cystine derivative 19a (Scheme IX) was prepared from cystine by subsequent introduction of the BOC-group with di-tert-butyl-pyrocarbonate[29] and the Tmse group in a DCC coupling procedure of N-BOC-cystine[28a] with 2-trimethylsilylethanol. Treatment of 19a with chlorine and acetic anhydride, followed by reaction with diazomethane afforded 21a in 48% yield.

Scheme VIII



Reaction of 21a with sodium methylmercaptide in ethanol to substitute the chlorine atom, resulted in the formation of 24 instead of the desired 22a. 24 is the product of a β-elimination and a transesterification reaction. Reaction of 21a with sodium methylmercaptide in DMF instead of ethanol also led to β-elimination. This apparent inclination to β-elimination must be ascribed to the presence of an ester function; the C(α) proton is acidic, which in combination with the leaving group character of the $S(O)CH_2Cl$ moiety facilitates the observed β-elimination. To circumvent β-elimination we decided to remove the ester function prior to the reaction with sodium methylmercaptide, because the α-proton in the carboxylic acid is less acidic. By treatment with a large excess of tetra-n-butylammonium fluoride the Tmse group was slowly removed. However, during the working-up procedure the BOC-group was largely removed from 25. So far we have no satisfactory explanation for this observation. Treatment of the crude reaction mixture, that resulted from the desylilation procedure with excess sodium methylmercaptide failed to yield the mono-oxodithioacetal.

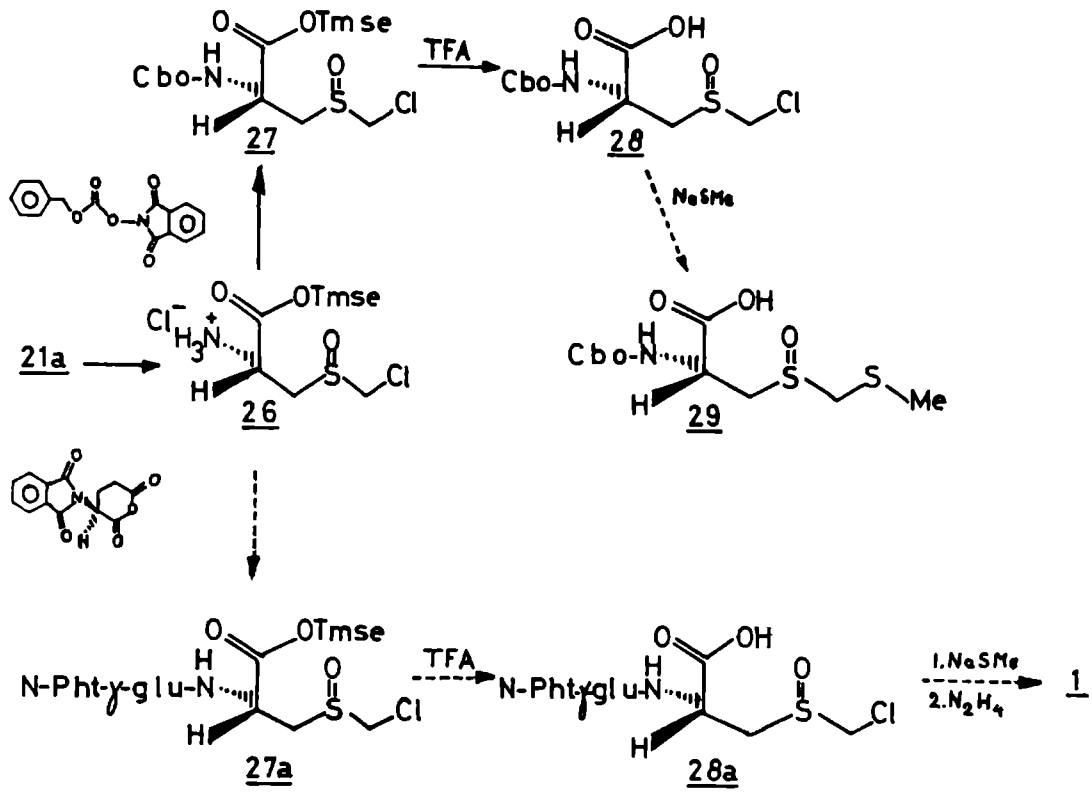
Scheme IX



In view of the apparent lability of the BOC-group in 25, during or after the procedure for removal of the Tmse group and the β-elimination caused by sodium methylmercaptide, it seemed sensible to reconsider the sequence of reactions. In Scheme X an altered sequence is depicted that takes these problems into account. The sequence of reactions 21a→26→27→28 has been realized so far. First, the BOC-group of 21a was removed to yield 26. Subsequently the more stable Z-group was introduced to give 27, whereafter the carboxylic acid is deprotected to give 28. This compound is a suitable model compound to study the substitution of the chlorine atom by the methylthiolate anion. If this substitution reaction succeeds, the route will be repeated using N-phthaloyl-L-glutamic anhydride instead of N-oxy-(benzyloxy) carbonyl-phthalimide. This route features introduction of the γ-glutamyl fragment prior to completion of the marasmin fragment.

The substitution reaction of the chlorine atom in 28 is under present investigation.

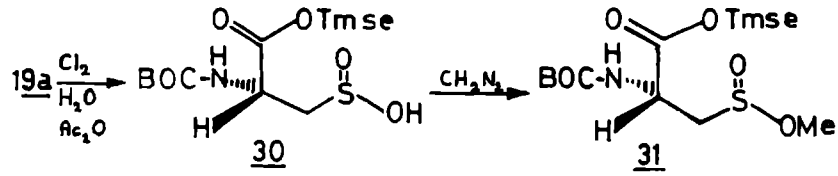
Scheme X



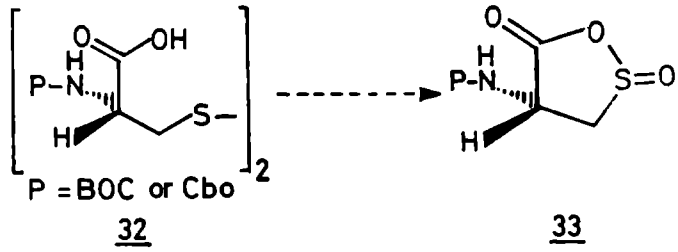
ROUTES C AND D

Two other possible routes are depicted in Scheme IV. Both routes have in common the use of the anion of dimethylsulfide. This anion has been used earlier (Chapter IV[30]) in a ring-opening reaction of a functionalized sultine, in order to complete the cysteinol mono-oxodithioacetal fragment of sparsomycin. In the routes C and D the anion might also be used for the synthesis of the mono-oxodithioacetal moiety. Route C (Scheme IV) features the employment of a sulfinate ester, whereas the synthetic intermediate in route D is an carboxylic-sulfinic anhydride. For the preparation of the sulfinate ester 29 from 19a, wet chlorine, instead of dry chlorine in the preparation of 21, was used. The resulting sulfinic acid 30 was esterified with diazomethane to the sulfinate ester 31 (Scheme XI). Attempts to complete the mono-oxodithioacetal moiety in the aforecited[30] way with the anion of dimethyl sulfide resulted in the formation of a β-elimination product. Removal of the Tmse group from 29 in order to obtain a better substrate *viz.* a compound with a less acidic α-proton, gave rise to similar problems as encountered in the removal of the Tmse group from 21a (*vide supra*).

Scheme XI



In the proposed route D the preparation of the substrate 33 for the ring-opening reaction with the anion of dimethyl sulfide turned out to be the bottle-neck. Attempts to synthesize the carboxylic-sulfinic anhydride 33 from Z- or BOC-cystine 32, either with chlorine[26,27] or with N-chloro-succinimide under varying conditions failed.



In summary, experiences gained in the synthesis of the cysteinol mono-oxo-dithioacetal fragment of sparsomycin cannot be simply used for the preparation of marasmin. Although we have not yet completed the synthesis of marasmin, we expect that at least one of the approaches discussed in this chapter will lead to a total synthesis in due time. Route A seems the most promising one, as for completion of the synthesis of marasmin by this route only the last step, namely the substitution of the chlorine atom, has to be successful.

EXPERIMENTAL PART

The used equipment and materials have been described in Chapter III. Thin layer chromatography was carried out on Merck precoated silica gel F254 plates (thickness 0.25 mm), unless stated otherwise.

Compound 10

Compound 10 was prepared analogous to the procedure, used for the preparation of 13, by Lambeth and Swank[19].
A solution of iodine (1.27 g, 5 mmol) in methanol was added in aliquots to a suspension of 5-(methylthio)methyl -L-cysteine 9[17], in 20 mL of methanol/water (1/1 v/v). The pH was kept between 7.0 and 8.5 by the dropwise addition of 4N NaOH. After completion of the reaction (3-4 h), the methanol was evaporated and the remaining solution placed on an ion-retardation resin column (50-100 mesh, Biorad), to remove the sodium iodide. The column was eluted with water and the fractions containing 10 were combined and evaporated to dryness. Residual water was removed by dissolving the compound in dry ethanol, followed by evaporation of the solvent. After repeating this procedure the compound was dried *in vacuo* over P_2O_5.
The compound was obtained as a mixture of diastereomers in 90% yield. TLC: R_f 0.27 and 0.31 (Merck cellulose plates, thickness 0.1 mm; eluent acetonitrile-ethylene glycol-0.1 M ammonium acetate, 70/15/15, v/v); R_f 0.11 (eluent n-BuOH-HOAc-H_2O, 4/1/1 v/v). The spots were visualized by spraying with a 10% solution of potassium iodide in 0.5 N HCl.
Compound 10 did not give a colour reaction with ninhydrin. NMR(D_2O) δ 2.4-3.1 (m, 2H, $CHCH_2S$), 3.1-3.5 (m, 1H, $CHCH_2S$), 3.3 (two s, 3H, SCH_3), 3.65-4.25 (m, 2H, SCH_2S). Compound 10 could be converted again to 9 by passing hydrogensulfide through a solution of 10 in methanol for 15 min. The resulting precipitate was filtrated and washed with ether.

Compounds 15 and 16

The t-butyl esters 15 and 16 were prepared according to procedures described earlier[22,23] for the preparation of the t-butyl esters from free amino acids. Relative yields of 15 and 16 are based on the ratio of integration of the O-t-Bu (δ 1.7) and the S-t-Bu (δ 1.5) signals.

N-[(benzyloxy)carbonyl]-S-benzyl-L-cysteine-tert-butyl ester(18). Compound 18 was obtained according to the procedure described earlier[25] in 95% yield. R_f 0.90 (eluent ethylacetate/hexane, 1/1 v/v); NMR($CDCl_3$) δ 1.45 (s, 9H, t-Bu), 2.8 (d. 2H, $CHCH_2$), 3.7 (s, 2H, $C_6H_5CH_2S$), 4.15-4.7 (m, 1H, $CHCH_2S$), 5.1 (s, 2H, $C_6H_5CH_2O$), 5.5 (br. d, 1H, NH), 7.2 (s, 5H, $C_6H_5CH_2O$), 7.25 (s, 5H, C_6H_5S).

N-[(tert-butyloxy)carbonyl]-L- cystine(2- trimethylsilyl)ethyl ester(19a)

Compound 19a was prepared from cystine by subsequent introduction of the BOC-group and Tmse-group: N-BOC-cystine was prepared from cystine (9.61 g, 40 mmol) according to the procedure described earlier[29] with di-tert-butylpyrocarbonate. However, the reaction period was adapted in order to increase the yield. The yield is 70% after a reaction period of four days. The Tmse protecting group was introduced analogous to the procedure of Sieber a solution of N-BOC-cystine (8.79 g, 20 mmol) in 40 mL acetonitrile and 40 mL DMF, was added 6.4 mL pyridine followed by the addition of 6.8 mL of 2-trimethylsilylethanol. Subsequently the mixture was cooled (0 °C) and after 10 min. 9 g of DCC was added. The reaction and work-up procedure were carried out as described[28a]. The crude product was chromatographed over silica gel 60 H (eluent ethylacetate/hexane, 1/4 v/v) and obtained in 67% yield. R_f 0.57 (eluent ethylacetate/hexane, 1/3 v/v); NMR($CDCl_3$) δ 0.04 (s, 9H, $SiMe_3$), 0.82-1.16 (m of t, 2H, CH_2SiMe_3), 1.43 (s, 9H, t-Bu), 3.16 (d, 2H, $CHCH_2S$), 4.04-4.38 (m of t, 2H, OCH_2CH_2Si), 4.38-4.70 (m, 1H, $CHCH_2S$), 5.37 (br. d, 1H, NH).

N-[(tert-butyloxy)carbonyl]-S-oxo-S(chloromethyl)-L-cysteine(2- trimethylsilyl)ethyl ester(21a)

Compound 21a was prepared from 19a (4.48 g, 7 mmol) according to the procedure described earlier (Chapter III[17]) for the preparation of N-, O-protected cysteine α-chlorosulfoxides. The product was purified by HPLC (silica gel 60 H, eluent ethylacetate/hexane, 2/3 v/v) and obtained as a colourless semi-solid oil in 48% yield. NMR($CDCl_3$) δ 0.04 (s, 9H, $SiMe_3$), 0.84-1.18 (m of t, 2H, CH_2SiMe_3), 1.44 (s, 9H, t-Bu), 3.29 and 3.57 (AB part of ABX spectrum, J_{AX} = 5.1 Hz, J_{BX} = 6.3 Hz, J_{AB} = 13.2 Hz, 2H, $CHCH_2S(O)$), 4.07-4.42 (m of t, 2H, OCH_2CH_2Si), 4.42-4.73 (m, 3H, $CHCH_2S(O)$ and $S(O)CH_2Cl$), 4.59 (part of AB spectrum of $S(O)CH_2Cl$), 5.56 (br. d, 1H, NH).

Ethyl-N-[(tert-butyloxy)carbonyl]-2-aminoacrylate(24)

Treatment of compound 21a (386 mg, 1 mmol) with sodium methylmercaptide, analogous to the procedure described earlier for substitution of cysteinol α-halosulfoxides (Chapter III[17]), afforded 24 in quantitative yield. R_f 0.92 (eluent ethylacetate/hexane, 1/1 v/v); NMR($CDCl_3$) δ 1.3 (t, 3H, CH_2CH_3), 1.45 (s, 9H, t-Bu), 4.25 (q, 2H, CH_2CH_3), 5.65 (d, 1H, C=CH), 6.05 (s, 1H, C=CH), 7.0 (br, 1H, NH).

S-oxo-S-(chloromethyl)-L-cysteine(2- trimethylsilyl)ethyl ester hydrochloride(26)

The BOC-group was removed from 21a (256 mg, 0.66 mmol) with HCl in trifluoro ethanol analogous to the procedure of Sieber[28a]. The product thus obtained was used without purification for the next experiment. R_f 0.66 (eluent acetonitrile/H_2O, 9/1 v/v); NMR($CDCl_3$), δ 0.05 (s, 9H, $SiMe_3$), 0.8-1.35 (br, 2H, CH_2SiMe_3), 3.05-4.05 (br, 2H, $CHCH_2S(O)$), 4.05-4.65 (br, 2H, OCH_2CH_2Si), 4.65-5.15 (br, 3H, $S(O)CH_2Cl$ and $CHCH_2S(O)$).

N-[(benzyloxy)carbonyl]-S-oxo-S-(chloromethyl)-L-cysteine(2-trimethylsilyl) ethyl ester(27)

Starting from 26, we introduced the (benzyloxy)carbonyl group using its N-hydroxyphthalimide derivative[31]. Compound 27 was obtained after HPLC purification (eluent, ethylacetate/hexane, 2/3 v/v) in 60% yield. R_f 0.37 (eluent ethylacetate/hexane, 1/1 v/v); NMR($CDCl_3$) δ 0.04 (s, 9H, $SiMe_3$), 0.74-1.15 (m of t, 2H, $CHCH_2Si$), 3.29 and 3.51 (AB part of ABX spectrum, J_{AX} = 5.1 Hz, J_{BX} = 6.3 Hz, J_{AB} = 13.2 Hz, 2H, $CHCH_2S(O)$), 4.04-4.36 (m of t, 2H, OCH_2CH_2Si),4.36-4.49 (m, 2H, $S(O)CH_2Cl$), 4.49-4.88 (m, 1H, $CHCH_2S(O)$), 5.07 (s, 2H, $C_6H_5CH_2$), 5.83 (br. d, 1H, NH), 7.28 (s, 5H, C_6H_5).

N-[(tert-butyloxy)carbonyl]-S-oxo-S-methoxy-L-cysteine(2- trimethylsilyl) ethyl ester(31)

The aforecited procedure, used for the preparation of 21a was adapted in order to prepare the sulfinate ester 31. Wet chlorine instead of dry chlorine was used. The sulfinate ester 31 was obtained after treatment of 30 with diazomethane and subsequent chromatography (eluent, ethylacetate/hexane, 1/3 v/v) in 56% yield. R_f 0.77 (eluent, ethylacetate/hexane, 1/1 v/v), NMR($CDCl_3$) δ 0.05 (s, 9H, $SiMe_3$), 0.7 -0.8 (m of t, 2H, CH_2CH_2Si), 1.4 (s, 9H, t-Bu), 2.95-3.35 (m, 2H, $CHCH_2S(O)$), 3.75 (s, 3H, $S(O)OCH_3$), 3.8 (s, 3H, $C(O)OCH_3$) 4.0 -4.4 (m of t, 2H, OCH_2CH_2Si), 4.40-4.85 (m, 1H, $CHCH_2S(O)$), 5.4 (br. d, 1H, NH).

N-[(benzyloxy)carbonyl]-S-oxo-S-(chloromethyl)-L-cysteine(28)

A solution of 28 (63 mg, 0.15 mmol) in 2 mL of trifluoroacetic acid was stirred for 2 h at room temperature. After removal of trifluoroacetic acid *in vacuo* compound 28 was obtained. R_f 0.27 (eluent, toluene/ethylformiate/formic acid, 10/7/3 v/v); NMR($CDCl_3$) δ 3.35-3.85 (m, 2H, $CHCH_2S(O)$), 4.4-5.1 (m, 5H, $CHCH_2S(O)$, $S(O)CH_2Cl$), 5.2 (s, 2H, $C_6H_5CH_2$), 7.4 (s, 5H, C_6H_5).

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26. Interestingly, an unprotected carboxylic acid function may give rise to a carboxylic-sulfinic anhydride[27] (route D, Scheme IV). This mixed anhydride might also be used as a synthetic intermediate for the synthesis of marasmin[3] *(vide infra)*.

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SAMENVATTING

Syntheses en biologische activiteit van sparsomycine en analoga. De chemie van chirale gefunctionaliseerde sulfoxides en sultines afgeleid van cysteine.

In hoofdstuk I werd een kort overzicht gegeven van het tot nu toe bekende over de biologische activiteit van sparsomycine. Tevens werden de motieven uiteengezet die geleid hebben tot onderzoek aan sparsomycine en analoge verbindingen. Deze algemene inleiding werd gevolgd door een inleiding op de hoofdstukken.

In hoofdstuk II werd de absolute configuratie van het sulfoxide-zwavel atoom beschreven. Immers de structuur van sparsomycine werd in 1970 door Wiley en MacKellar opgehelderd met uitzondering van de absolute configuratie van het sulfoxide-zwavel atoom. Deze kon door ons opgehelderd worden met behulp van CD spectroscopie aan de intermediairen die gebruikt werden in de synthese van het enantiomeer van sparsomycine (verb. 1 pg **9**): door uitbreiding van de Mislow-Snatzke regel kon uit het teken van het Cotton effect in de verschillende CD spectra de absolute configuratie van het sulfoxide vastgesteld worden. Dit sulfoxide bleek in sparsomycine de R configuratie te bezitten. Hetgeen bevestigd werd door een röntgenstructuuranalyse van een (verb. 5 pg **9**) van de bij de synthese van het enantiomeer van sparsomycine gebruikte intermediairen.

In hoofdstuk III werd de eerste totaal synthese van sparsomycine en de drie stereomeren beschreven. Men kan sparsomycine beschouwen als een amide dat gevormd wordt door koppeling van het β-6(methyl-uracilyl)acrylzuur (verb. 2 pg **13**) en het cysteinol mono-oxodithioacetaal (verb. 3 pg **13**). Beide synthons werden gesynthetiseerd.
Uitgangsstof voor het 6-methyl-uracil acrylzuur was het 6-methyl-uracil (verb. 4 pg **13**). Hieruit werd het 5-formyl uracil (verb. 19 pg **14**) bereid, dat na koppeling met het ylide van de α-broomazijnzure ethyl ester (verb. 20 pg **14**) de uracil acrylzure ethyl ester (Schema III pg **14**) leverde. Door verzeping van de ester (verb. 18 pg **14**) werd vervolgens het uracil acrylzuur verkregen.
De synthese van het cysteine derivaat werd als een bijzondere uitdaging gezien vanwege de aanwezigheid van een mono-oxodithioacetaal functie. De voor de hand liggende mogelijkheid (n.l. oxidatie van het dithioacetaal (route A, Schema II pg **13** verb. 23-26 pg **14**)) leidde tot de vorming van het niet gewenste regio isomeer (verb. 27-30, Tabel I pg **14**). Het bleek echter wel mogelijk door reactie van een α-halogeen sulfoxide (route B, Schema II pg **13** verb. 36-41 pg **15** , 53, 54 pg **16**) de mono-oxodithioacetaal functie te synthetiseren. De α-halogeen sulfoxides werden bereid met als uitgangsstof een cysteine derivaat waarvan zowel de amino groep als de carboxyl groep beschermd was. Uitgaande van een beschermd D-cysteine derivaat kon het cysteinol mono-oxodithioacetaal fragment van sparsomycine (verb. 3 pg **13**) naast het diastereoisomere cysteinol mono-oxodithioacetaal fragment (verb. 60 pg **17**) verkregen worden, in een opbrengst van 40% (over 5 stappen, Schema VII pg **17**). Hieruit konden door koppeling met het uracil acrylzuur via de gemengd anhydride methode sparsomycine en het S_C S_S diastereoisomeer (verb. 67 pg **17**) bereid worden. Uitgaande van een beschermd L-cysteine derivaat werden op analoge wijze beie andere stereoisomeren van sparsomycine verkregen (verb. 65 en 66 pg **17**).
In de CD spectra (Fig. 1 pg **18**) van sparsomycine en de stereoisomeren kon het Cotton effect, gerelateerd aan het chirale koolstof atoom, en het Cotton effect van het sulfoxide afzonderlijk waargenomen worden.

Bij de in hoofdstuk IV beschreven tweede benadering (route C, Schema II pg **24**) van de synthese van het cysteinol mono-oxodithioacetaal gedeelte van sparsomycine en de stereoisomeren, werd gebruik gemaakt van het sultine als synthon. Daartoe werd eerst voor de benodigde sultines een synthese ontwikkeld.

Het bleek mogelijk deze verbindingen te bereiden uit een N-beschermd cystinol derivaat (verb. 11 en 20 pg **25**) met N-chloor succinimide en azijnzuur (Schema III en IV pg **25**). Vervolgens leidde nucleofiele ring opening van de sultines door het anion van de dimethyl sulfide tot de vorming van de gewenste cysteinol mono-oxodithioacetalen (Schema V pg **26**). Deze ring opening reactie, waarbij de S-O band verbroken werd kon ook door andere anionen bewerkstelligd worden. (n-BuLi, $C_6H_5C(Li)(H)CN$ (Schema V pg **26**)). De ring opening is stereospecifiek en verloopt met inversie van configuratie van het zwavelatoom.
De sultine ring kan ook geopend worden door verbreking van de C-O band. Deze ring opening leidt, als ze door chloor teweeg gebracht wordt tot het sulfonyl chloride (verb. 15, Schema III pg **25**). Naast deze intermoleculaire opening van de sultine ring door splitsing van de C-O band is er ook een intermoleculaire ring opening zoals beschreven in hoofdstuk VII mogelijk.
De sultine route bleek aantrekkelijk te zijn vanwege de hoge opbrengsten en omdat via deze route analoga van sparsomycine bereid kunnen worden, die op een andere wijze moeilijk toegankelijk zijn.
CD metingen kunnen ook bij sultines gebruikt worden om de absolute configuratie van het sulfoxide-zwavel atoom vast te stellen (Fig **1** pg **26**).

Hoofdstuk V is gewijd aan de biologische activiteit van sparsomycine en analoga. Voor een goed begrip hiervan werd in de eerste paragraaf van dit hoofdstuk achtergrond informatie gegeven over het aangrijpingspunt van sparsomycine: het peptidyltransferase centrum van het ribosoom. Interactie met dit centrum leidt tot remming van de eiwit synthese. Tevens werden enkele methoden beschreven die gebruikt worden om de peptidyltransferase activiteit van het ribosoom te meten.
In de tweede paragraaf werd een van deze methoden gebruikt om de remming van de eiwit synthese van sparsomycine en analoga (verb. 1-4, 12, 13, 14, 16, Tabel I pg **39**) in een cel-vrij systeem van gist te bestuderen. Ook werd de remming in een polyfenylalanine (Tabel I pg **39**) synthetiserend cel-vrij systeem van gist bepaald.
In de derde paragraaf werd vervolgens de activiteit van deze verbindingen tegen intacte leukemie L1210 cellen beschreven. Naast deze sparsomycine analoga zijn ook andere analoga (verb. 17 en 20 Tabel I pg **46**) onderzocht op hyn cytostatische werking tegen leukemie L1210 cellen *in vitro* door de mate van remming van de vorming van celkolonies te bestuderen. De activiteit van een analogon werd uitgedrukt in de ED_{50} (§ 2 Tabel I pg **39**) respectievelijk de ID_{50} (§ 3 Tabel I pg **46**) waarde en werd vergeleken met die van sparsomycine. Aangezien ieder analogon niet meer dan twee structuurwijzigingen ten opzichte van sparsomycine bevat, was het mogelijk om eenduidig de structuur en stereochemische kenmerken te bepalen die vereist zijn voor een optimale biologische activiteit. De resultaten verkregen uit experimenten met een cel-vrij systeem, waarin de fragment reactie gebruikt werd, lopen bijna parallel met die verkregen uit de experimenten met L1210 cellen. Voor beide systemen geldt dat de S configuratie van het chirale koolstofatoom evenals de aanwezigheid van een zuurstofatoom op het α-zwavelatoom essentieel zijn voor de optimale activiteit. Daarentegen is de R configuratie van het zwavelatoom, hoewel van belang, niet essentieel.
Het geïsomeriseerde sparsomycine (isosparsomycine (verb. 16 Tabel I pg **39** en Tabel I pg **46**)) bezit in een van beide cel-vrije systemen een aanzienlijke activiteit, terwijl deze in het L1210 systeem vrijwel afwezig is.
Verder werd de rol van de hydroxy functie van sparsomycine in het L1210 cel systeem bestudeerd. Naar het schijnt is deze functie niet betrokken bij de werking van sparsomycine op moleculair niveau op een vergelijkbare wijze als de aminofunctie van puromycine (verb. 18 pg **44**), aangezien acylering van de hydroxy functie de biologische activiteit niet beïnvloedt.
De cystostatische activiteit in dit systeem neemt sterk toe wanneer de lipofiliteit van het effector molecuul toeneemt: octyl sparsomycine is 3-4 maal zo actief als sparsomycine zelf (Tabel I pg **46**).

Dit analogon heeft een vergelijkbare activiteit als de in de kliniek gebruikte cytostatica fluorouracil en adriamycine.
In de vierde paragraaf werd de cytostatische activiteit van sparsomycine beschreven. Deze is waarschijnlijk grotendeels het gevolg van de remming van de eiwit biosynthese. Verder werd het huidig en toekomstige onderzoek aan sparsomycine en analoga met betrekking tot de cytostatische activiteit nader toegelicht.

Omdat de chemie van sultines, en met name van de gefunctionaliseerde sultines een nagenoeg ongeëxploreerd terrein is, hebben we besloten meer aandacht aan deze verbindingen te schenken. Een eerste aanzet hiertoe werd beschreven in hoofdstuk VI. De aanleiding tot dit onderzoek was dat bij ring opening van het sultine door een prochiraal nucleofiel chirale inductie plaats vond (hoofdstuk IV, pg 26). Dit vormde o.a. voor ons een reden om de conformatie(s) van deze verbindingen in oplossing te gaan onderzoeken. Van twee sultines (verb. 1 en 2 pg 57) is de conformatie analyse beschreven. In deze conformatie analyse werd gebruik gemaakt van een nieuwe empirische generalisering van de klassieke Karplus vergelijking (vergelijking 4,pg 58). Door toepassing van deze vergelijking en vergelijkingen die gebruikt worden voor de beschrijving van 5-ringen (vergelijking 1 en 2, pg 57,58) kunnen vicinale koppelingsconstanten, verkregen met behulp van 500 MHz proton NMR spectroscopie, vertaald worden in proton-proton torsiehoeken. Met behulp van het pseudorotatie concept kon de conformatie en de 'puckering' van de 5-ring quantitatief beschreven worden.
Aangetoond kon worden dat het BOC-sultine (verb. 1 pg) waarin het zuurstofatoom een syn positie inneemt ten opzichte van de C-N band, in chloroform bij -40 °C en bij kamertemperatuur aanwezig is als een 'twist-chair' conformeer ($^4T_5$, 1a-c pg 65). In DMSO is de conformeer van deze verbindingen in evenwicht met een andere 'twist-chair' conformeer(^3_2T, 1d pg 65). Het sultine (verb. 2 pg 60), waarin de sulfoxide functie een anti positie inneemt ten opzichte van de C-N band, verkeert in chloroform bij -40 °C in een op de NMR tijdschaal langzaam en snel conformatie evenwicht. Het langzame evenwicht behelst een evenwicht tussen een component die in grote overmaat (major component) aanwezig is en een andere component die in ondermaat aanwezig is (minor component). Dit evenwicht is een gevolg van een belemmerende rotatie van de urethaan binding. In het 'snelle' evenwicht zijn een 'twisted-chair' conformatie (^1_3T, 2a, 2c, 2e, 2g pg 66) en een 'envelop' achtig conformeer ($_1E$, 2b, 2d, 2f, 2h pg 66) met elkaar in evenwicht. Het 'langzame' evenwicht wordt niet waargenomen in DMSO bij kamertemperatuur en in tetrachloorethaan bij 110 °C.
De effecten die een rol zouden kunnen spelen bij het bepalen van de conformaties van 1 en 2 zijn het gauche effect, het anomeer effect en de waterstof brugvorming. Dit laatste effect bepaalt waarschijnlijk de waargenomen vaste stof conformatie die ook in dit hoofdstuk gerapporteerd werd. Vergelijking van deze vaste stof conformatie met de conformatie aanwezig in oplossing laat duidelijke verschillen zien.
Naast de conformatie van de sultine ring werd met behulp van röntgenanalyse ook de absolute configuratie van het sulfoxide-zwavelatoom bepaald. Dit in combinatie met de bovenbeschreven absolute configuratie van de producten die ontstaan bij ring opening (hoofdstuk II en IV) leidde tot de conclusie dat ring opening reacties van sultine (hoofdstuk IV) stereospecifiek zijn en gepaard gaan met inversie van configuratie.

Een ander facet van de chemie van (gefunctionaliseerde) sultines is de in hoofdstuk VII beschreven flits vacuum thermolyse reactie. Flits vacuum thermolyse van de 4-(benzamido)-γ-sultines (verb. 5a en 5b pg 77) leidde tot een mengsel van producten met als hoofdproduct het allylamide (verb. 6 pg 77) en verder het Z- en E-enamide verb. 7 en 8 pg 77) en het benzamide. In deze reactie verhuist de benzamide groep mogelijk via een mechanisme waarin de C-O binding van het sultine heterolytisch gesplitst wordt door een nucleofiele aanval van het amide zuurstof atoom in een nabuurparticipatie reactie.

Dit mechanisme kon onderbouwd worden door de flits vacuum thermolyse van een gedeutereerde sultine (verb. 5a-d_2 pg 78) uit te voeren.

In het laatste hoofdstuk van dit proefschrift werden enige voorlopige resultaten van het onderzoek naar de synthese van het marasmine gedeelte van γ-glutamyl marasmine behandeld.
γ-Glutamyl marasmine is het enige ander bekende molecuul dat ook een monooxodithioacetaal functie bevat. Vier mogelijke routes die echter tot op heden nog niet tot een afgeronde synthese van marasmine hebben geleid, werden beschreven. De routes waaraan het meeste onderzoek is verricht zijn die waarin een sulfilimide als synthetisch intermediair gebruikt werd, en een route die berust op toepassing van de α-chloorsulfoxide benadering van sparsomycine. Omdat deze benadering (Schema X pg 91), die voor de synthese van de monooxodithioacetaal functie gebruik maakt van een α-chloor sulfoxide, als synthetisch intermediair, niet zonder meer gebruikt kon worden voor de synthese van het overeenkomstig fragment in marasmine, dient de laatste stap van de synthese van marasmine n.l. substitutie van het chlooratoom door het methylthiolaat anion nog verwezenlijkt te worden.

CURRICULUM VITAE

Rob M.J. Liskamp was born on June 21, 1955 at Nijmegen, The Netherlands.
In 1972 he graduated from the 'Canisius College' at Nijmegen.
In September of the same year he started his studies in chemistry at the University of Nijmegen, Nijmegen, The Netherlands. He obtained his bachelor's degree in September 1975. In November 1978 he obtained his doctoral degree in organic chemistry (Prof.Dr. R.J.F. Nivard, Dr. H.C.J. Ottenheijm), biochemistry (Prof.Dr. H.P.J. Bloemers) and farmacochemistry (Prof.Dr. J.M. van Rossum, Dr. F. Seutter-Berlage).
From August 1976 to August 1977 he was part-time chemistry teacher at the 'Nijmeegse Scholengemeenschap'.
From 1975 to 1977 he was an elected member of the Department Council and the Faculty Council (student seat).
In December 1978 he was appointed as a research fellow by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO), in the Department of Organic Chemistry (head: Prof.Dr. R.J.F. Nivard), bio-organic chemistry group (Dr. H.C.J. Ottenheijm), at the University of Nijmegen. The research, of which the results are described in this thesis, were performed in this group.
During his studies he participated in teaching undergraduate and graduate students.
In February 1979 he was awarded the University Award for research in chemistry.
In 1980 he received a travel grant from the Faculty of Science for visiting the Third IUPAC Symposium on Organic Synthesis in Madison (Wisconsin), the National Institutes of Health/National Cancer Institute, Bethesda (Maryland) and the State University of New York at Stony Brook (New York).
In 1982 he received a grant for participation of the Bürgenstock Conference on Stereochemistry (Bürgenstock, Switzerland).

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CURRICULUM VITAE

Rob Liskamp werd op 21 juni 1955 te Nijmegen geboren.
In 1972 behaalde hij aan het Canisius College te Nijmegen het diploma HBS-B.
In september van datzelfde jaar begon hij zijn studie scheikunde aan de Katholieke Universiteit te Nijmegen. Het kandidaatsexamen (S_2) werd afgelegd in september 1975. In november 1978 werd het doctoral examen scheikunde afgelegd met als hoofdvak organische chemie (Prof.Dr. R.J.F. Nivard, Dr. H.C.J. Ottenheijm) en bijvakken biochemie (Prof.Dr. H.P.J. Bloemers) en farmacochemie (Prof.Dr. J.M. van Rossum, Dr. F. Seutter-Berlage). Tevens werd hierbij onderwijsbevoegdheid verkregen in de scheikunde.
Van 1 augustus 1976 tot 1 augustus 1977 was hij verbonden als docent scheikunde aan de Nijmeegse Scholengemeenschap.
Vanaf december 1978 was hij als wetenschappelijk ambtenaar in dienst van de Nederlandse Organisatie voor Zuiver Wetenschappelijk Onderzoek (ZWO), werkzaam op het Laboratorium voor Organische Chemie (Hoofd: Prof.Dr. R.J.F. Nivard) in de werkgroep Bio-organische Chemie (Dr. H.C.J. Ottenheijm). Daar werd het in dit proefschrift beschreven onderzoek verricht.
Gedurende zijn studie en promotie-onderzoek was hij betrokken bij het onderwijs aan studenten, als assistent bij practica en werkkolleges.
In februari 1979 werd hem de faculteitsprijs toegekend voor onderzoek verricht tijdens zijn doctoraalstudie.
In 1980 werd hij door financiële steun van de Faculteit der Wiskunde en Natuurwetenschappen in de gelegenheid gesteld om het 'Third IUPAC Symposium on Organic Synthesis' in Madison (Wisconsin) bij te wonen en de National Institutes of Health/National Cancer Institute (Bethesda, Maryland) evenals de State University of New York te Stony Brook (New York) te bezoeken.
In 1982 bezocht hij de 'Bürgenstock Conference on Stereochemistry', daartoe in de gelegenheid gesteld door de Stichting Scheikundig Onderzoek Nederland (SON) met financiële steun van het ZWO.

STELLINGEN

1.

Uitvoering en publicatie van "dubbel" en dus overbodig onderzoek, zoals blijkt uit het herhaaldelijk aantreffen in de literatuur van reeds eerder beschreven syntheses en synthetische methoden, kan, bij de huidige sterk toenemende hoeveelheid literatuur, voorkomen worden door een beter gebruik van online literatuuronderzoek.

bijv. D.D. Sternbach, W.C.L. Jamison, Tetrahedron Lett. (1981), 22, 333
A. Ahmad, Bull. Chem. Soc. Japan, (1974), 47, 1819
B.L. Moller, I.J. McFarlane, E.E. Conn, Acta Chem. Scand. (1977), B31, 343

2.

De veronderstelling van Meskens dat de bij de bereiding van acetalen gebruikte ortho ester niet alleen een wateronttrekkende functie heeft, maar ook deelneemt aan de acetaalvorming zelf, berust op een verkeerde interpretatie van de gerefereerde literatuur.

F.A.J. Meskens, Synthesis, (1981), 505
J.W. Scheeren, J.E.W. van Melick, R.J.F. Nivard, J. Chem. Soc. Chem. Comm. (1969), 1175

3.

De waarneming van Yamashiro dat thiofenol in HF niet in staat is het methionine sulfoxide te reduceren, terwijl 2-mercaptopyridine deze reactie wel kan bewerkstelligen, verdient een betere toelichting dan de opmerking van de auteur:
"2-mercaptopyridine possesses structural features, which uniquely facilitate the reaction".

D. Yamashiro, Int. J. Pept. Prot. Res. (1982), 20, 63

4.

De methode van Drabowicz voor de bereiding van sulfinaat esters uit sulfinyl-chloriden is geen wezenlijk verbeterde versie van die van Harpp et al.

J. Drabowicz, Chem. Lett. (1981), 1753
D.N. Harpp, B.T. Friedlander, C. Larsen, K. Steliou, A. Stockton, J. Org. Chem. (1978), 43, 3481

5.

De karakterisering van het "red organic pigment" en de synthetische intermediairen, gebonden aan silicagel is onvoldoende voor de opgegeven structuren.

M. Suzuki, S. Itô, T. Kuwahara, Chem. Lett. (1981), 1785

6.

De door Lenz et al. gepubliceerde tertiaire structuur van het envelop precursor eiwit $gPr82^{env}$ draagt niet bij tot begrip van de interactie tussen gp70 en het membraan, aangezien deze precursor niet ongesplitst voorkomt op het membraan van muizecellen, geïnfecteerd met AKR virus.

J.W. Lenz, R. Crowther, A. Straceski, W. Haseltine, J. Virol. (1982), 42, 519

Voor een zinvolle vergelijking van de activiteit van verschillende cytostatica dient de werkzame dosis of concentratie niet in massa per massa of massa per volume eenheid (bijv. µg/kg resp. µg/ml) aangegeven te worden, maar in grammol per massa of grammol per volume eenheid (bijv. µmol/kg, µmol/ml).

bijv. P.-C. Wu, R.F. Ozols, M. Hatanaka, C.W. Boone, J. Natl. Cancer Inst. (1982), 68, 115

8.

Vanwege, de aan cytostatica, die aangrijpen op het DNA, inherente carcinogene eigenschappen, dient de ontwikkeling van cytostatica, die werken op een ander niveau bevorderd te worden.

9.

De aard van de structuren van recent als zodanig onderkende tumorpromotoren, rechtvaardigt de stellige verwachting dat in de nabije toekomst nieuwe modulerende factoren, die de carcinogenese bevorderen (co-carcinogenen, tumorpromotoren) of onderdrukken (anti-carcinogenen), geïdentificeerd zullen worden.

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H. Fujiki, M. Suganuma, M. Nakayasu, H. Hoshino, R.E. Moore, T. Sugimura, Gann. (1982), 73, 497
A.D. Horowitz, H. Fujiki, I.B. Weinstein, A. Jeffrey, E. Okin, R.E. Moore, T. Sugimura, in press

Nijmegen, 10 december 1982

Rob M.J. Liskamp