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TOTAL SYNTHESIS OF GLIOTOXIN, DEHYDROGLIOTOXIN AND HYALODENDRIN†

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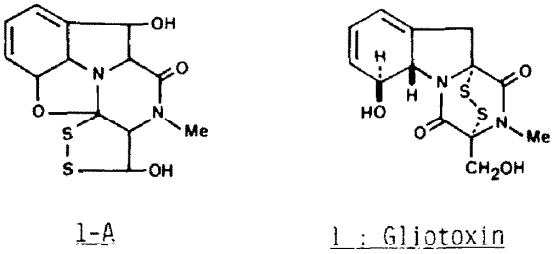
Abstract—Two general methods, Method A and Method B in Scheme 19, to synthesize epidithiapiperazinediones, are described. A total synthesis of racemic and optically active gliotoxin (**1**) and of racemic dehydrogliotoxin (**53**) was achieved by using Method A, whereas a total synthesis of racemic hyalodendrin (**52**) was completed by using Method B.

Gliotoxin was first isolated from cultures of the wood fungus *Gliocladium fimbriatum* by Weindling and Emerson in 1936.[1] This substance was since isolated as the metabolite of a variety of microorganisms, including *Aspergillus fumigatus* and *Penicillium terlikowskii*.[2,3] Gliotoxin was found to be a powerful bacteriostatic agent and to exhibit remarkable antifungal and antiviral activity.[4-6] However, the toxicity of gliotoxin observed with test animals precluded its therapeutic use.[7] The structure determination of gliotoxin was undertaken by Johnson *et al.* in the early 1940s. In 1953 they proposed structure **1-A** for gliotoxin on the basis of extensive degradation studies.[8] Since this formula could not account for all of the experimental observations, Bell, Johnson, Wildi and Woodward proposed structure **1** for gliotoxin in 1958.[9] Later, an X-ray crystallographic analysis of gliotoxin confirmed the proposed structure and established at the same time the stereochemistry including its absolute configuration.[10]



1-A 1 : Gliotoxin

Scheme 1.

Since the discovery of gliotoxin, a number of antibiotics containing the epidithiapiperazinedione system, including sporidesmins,[11] aranotins,[12] dehydrogliotoxin,[13] chaetocins[14] (verticillins[15]), chetomin,[16] hyalodendrin,[17] melacidins[18] and sirodesmins,[19] have been isolated as metabolites of various microorganisms. These natural products, like gliotoxin, exhibit remarkable antiviral, antifungal and bacteriostatic activity.[20] Apparently the biological activity is associated with the epidithiapiperazinedione moiety, since removal of the disulfide group results in loss of activity. For example, desulfurized products of gliotoxin and sporidesmin A show no antibacterial activity.[21] With this fact in mind, Trown first synthesized the simple epidithiapiperazinedione **5**, which did indeed exhibit strong antiviral activity.[22]

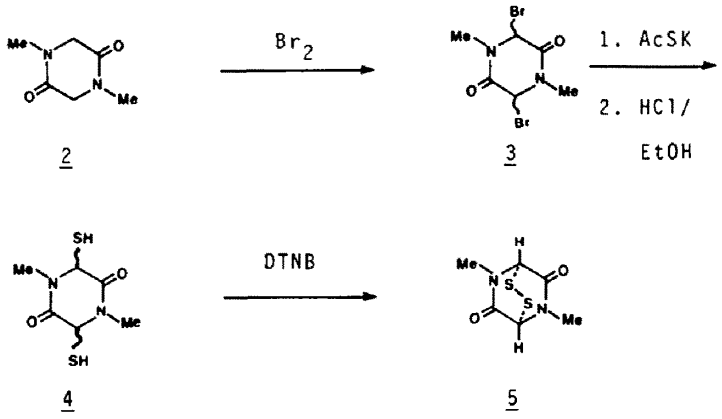
EPIDITHIAPIPERAZINEDIONE SYNTHESES: METHOD A (BICYCLIC ROUTE)

The unique structural features of this class of natural products have attracted considerable interest of many synthetic organic chemists. Apparently, the central problem in synthesizing these natural products was construction of the epidithiapiperazinedione system. In the fall of 1972 when we undertook this synthesis, there were only three epidithiapiperazinediones synthesized. Perhaps the most historical synthesis is the one achieved by Trown in 1968.[22] Hino *et al.* introduced the disulfide bridge into **6** by using carbanion chemistry.[23] Schmidt *et al.* succeeded in the synthesis of epidithiaproline anhydride **11** by a method somewhat similar to Hino's.[24,25]

We started this project with a brief examination of the chemical properties of the epidithiapiperazinediones and realized that they are extremely labile under oxidative (N-bromosuccinimide, bromine, *m*-chloroperbenzoic acid), reductive (lithium aluminum hydride, sodium borohydride),[26] and basic (sodium hydroxide, sodium methoxide, triethylamine) conditions,[27] but are acceptably stable under acidic (acetic acid, trifluoroacetic acid, boron trichloride) conditions. Accordingly, it seemed wise to introduce this system to a synthetic intermediate at as late a stage as possible. With this reasoning in mind, two extreme strategies were considered for the construction of the disulfide bridge on a synthetic intermediate; one to introduce the disulfide bridge at a late stage in the synthesis, and the other to introduce the protected disulfide bridge at an early stage, with the hope that the chemical properties of the protected disulfide group would be improved and consequently cause no technical problems in carrying out the synthesis, and to regenerate the disulfide bridge at a later stage. Recognizing the vigorous conditions required for introduction of the disulfide or thiol groups to a piperazinedione by any of the three known methods, we felt that the chances for suc-

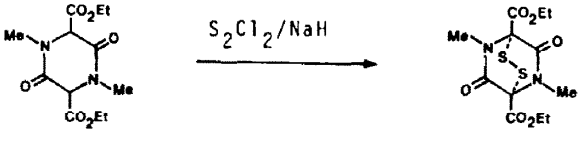
†Dedicated to the memory of Robert Burns Woodward, this paper will be included in the final book version of the Woodward revised supplement.

Trown Synthesis[22]



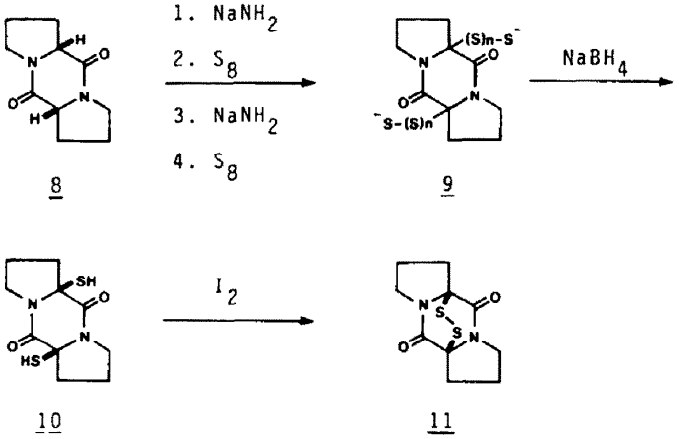
2 3 4 5

Hino Synthesis[23]



6 7

Schmidt Synthesis[24]



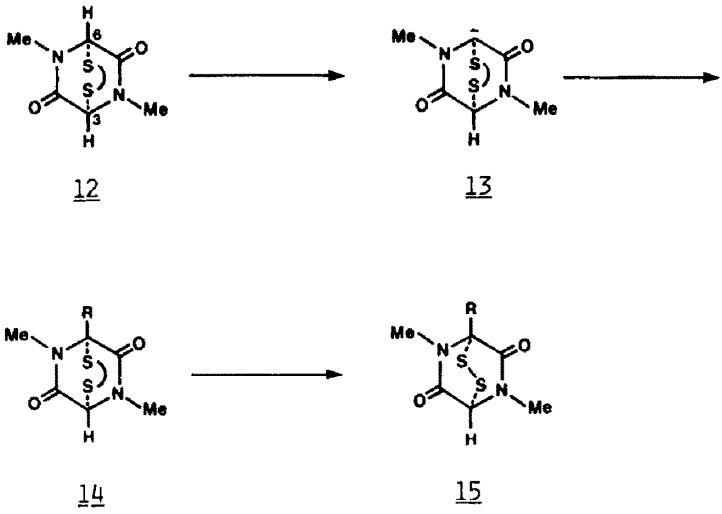
8 9 10 11

Scheme 2.

cess in the former approach would be extremely small, unless a new method were to be developed which allowed introduction of the disulfide bridge to a piperazinedione under conditions mild enough not to disturb other delicate functionalities existing in the target molecule such as a hydrated benzene ring. Based on this analysis, our decision was to pursue the latter approach.

The basic plan is depicted in Scheme 3, involving three major aspects, i.e. protection of the disulfide bridge as a bicyclic system such as **12**, functionalization of the C-6 (and C-3) position by using carbanion chemistry and regeneration of the disulfide bridge. A bycyclic system **12** was considered for controlling the stereochemistry of the C-3 and C-6 positions on the functionalization steps. The proposed carbanion formation at the bridgehead position might be difficult, but would not be necessarily unreasonable to propose, knowing of a facile deuterium–hydrogen exchange at the bridgehead position of a sulfur-containing bicyclic system as observed by Oae *et al.*[28]

Attempted preparation of the formaldehyde thioacetal **16**, with paraformaldehyde and *cis*-



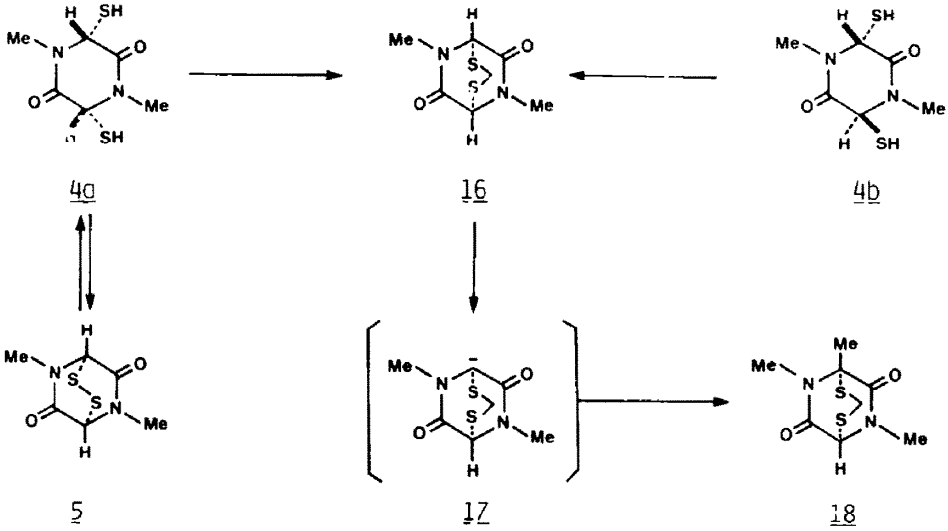
Scheme 3.

dithiopiperazinedione **4a**[22] under acidic conditions was fruitless. However, treatment of **4a** with diiodomethane and pyridine at room temperature yielded the thioacetal **16** in 51% yield. The thioacetal **16** was obtained also from *trans*-dithiopiperazinedione **4b**[22] under the same conditions in comparable yield, which allowed the utilization of the *cis*- and the *trans*-dithiopiperazinediones **4a** and **4b** for the following synthesis. Although at least three different mechanistic explanations for epimerization at the C-6 position in this thioacetalization could be postulated,[29] no experimental support is available to differentiate them at this time.

The crucial carbanion formation at the bridgehead position of **16** was realized by using a strong base such as *n*-butyllithium or lithium diisopropylamide (LDA) in tetrahydrofuran at −78°. This carbanion **17** was subsequently alkylated at −78° with methyl iodide to give the monomethylthioacetal **18** in 54% yield. There was no indication of carbanion formation at the methylene carbon of the thioacetal moiety, structurally somewhat similar to dithianes.

The next stage of the investigation was to establish a method to generate the disulfide bridge from the bicyclic thioacetal. Thioacetals and thioketals are often used for protection of aldehydes and ketones and a number of methods for their deprotection have been known.[30] However, most of them have focused on the regeneration of aldehydes or ketones rather than on the recovery of the sulfur-containing counterpart. Attempts to deprotect the thioacetal **16** under a variety of conditions including concentrated hydrochloric acid, sulfuric acid, hydrobromic acid, mercuric chloride, mercuric acetate-formic acid, silver nitrate and silver chloride–hydrochloric acid, were fruitless; in each case the starting material was recovered.

Since the deprotection of the thioacetal **16** to the *cis*-dithiol **4a** proved difficult, we turned our attention to the direct conversion of the thioacetal **16** into the epidithiapiperazinedione **5**. Related to this, we observed a clean formation of the epidithiapiperazinedione **5** from the *cis*-ditetrahydropyranyl derivative **19**, readily prepared from the *cis*-dithiol **4a**, upon treatment with iodine in



Scheme 4.

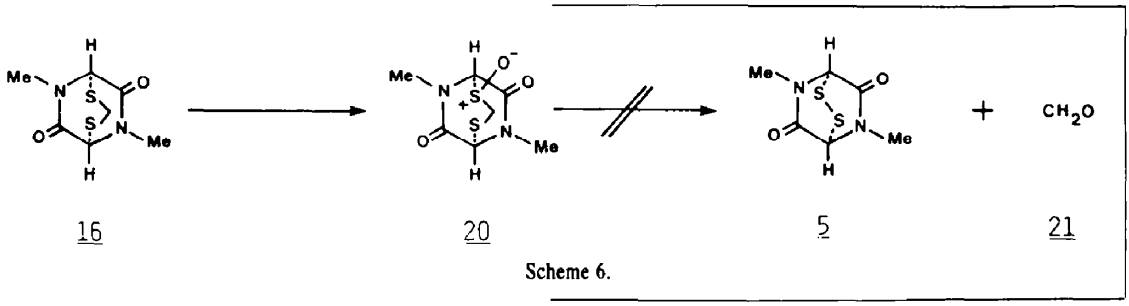
methylene chloride at room temperature. However, the thioacetal **16** was recovered unchanged under the same conditions.[31]

19 5

Scheme 5.

Under these circumstances, an alternative possibility depicted in Scheme 6 was investigated. Some literature precedents for this type of reaction are known in the carbohydrate field.[32] Treatment of the thioacetal **16** with 1 equivalent of *m*-chloroperbenzoic acid (MCPBA) at 0° in methylene chloride yielded the monosulfoxide **20** in quantitative yield. Attempts to convert the monosulfoxide into the epidithiapiperazinedione **5** under a variety of acidic conditions including perchloric acid, hydrobromic acid, boron trifluoride etherate and boron trichloride, were fruitless; in each case the starting material was recovered.[31] One of the reasons for the failure of these experiments could be attributed to the carbon-sulfur bond strength; namely, the formation of the disulfide bridge on the iodine oxidation of **19** might be facilitated by the two weak carbon-sulfur bonds due to the resonance stabilization for the fragment **24**, but there was no such assist in the case of the protonated form of **20**.

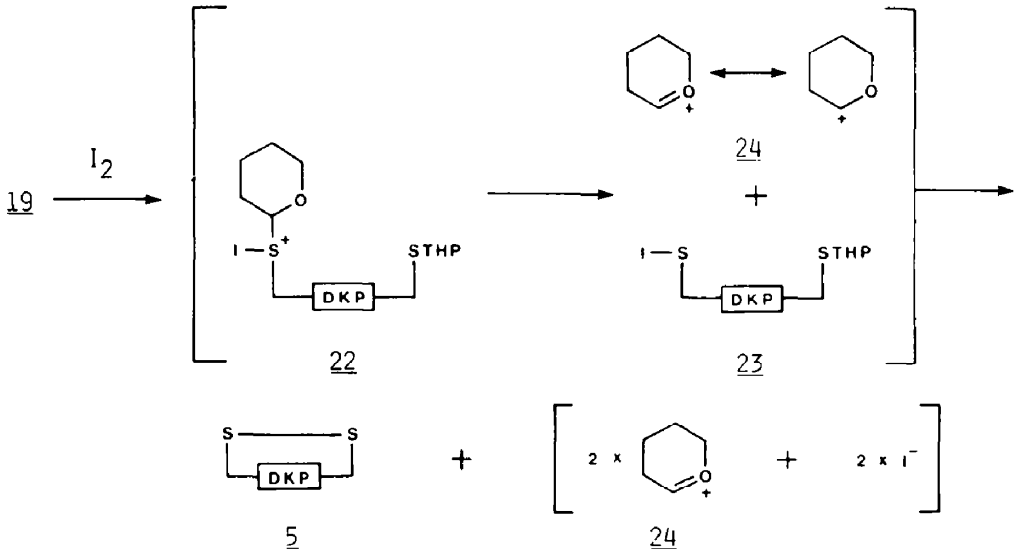
With this reasoning in mind, similar experiments using the thioacetals **25a–c** were investigated. Upon treatment with an aldehyde (acetaldehyde, benzaldehyde or *p*-anisaldehyde) and boron trifluoride etherate, the thioacetals **25a–c** were obtained from a mixture of the *cis*- and *trans*-dithiopiperazinediones **4a,b**, in yields of 73, 92 and 81%, respectively (see above). Since these thioacetals **25a–c** were again found to be inert against iodine,[31] they were subsequently converted to the monosulfoxides **26a–c** with *m*-chloroperbenzoic acid in quantitative yield. NMR and tlc analyses showed that the monosulfoxides **26a–c** were stereochemically homogeneous but their stereochemistry still remained unknown. Under a variety of acidic conditions such as perchloric acid, boron trichloride, boron trifluoride etherate and sulfuric acid, the monosulfides **26a** and **b**, derived from acetaldehyde and benzaldehyde, did not undergo the acid-cleavage reaction. However, under the same conditions, the sulfoxide **26c**, derived from *p*-anisaldehyde, clearly yielded the desired epidithiapiperazinedione **5** along with *p*-anisaldehyde!



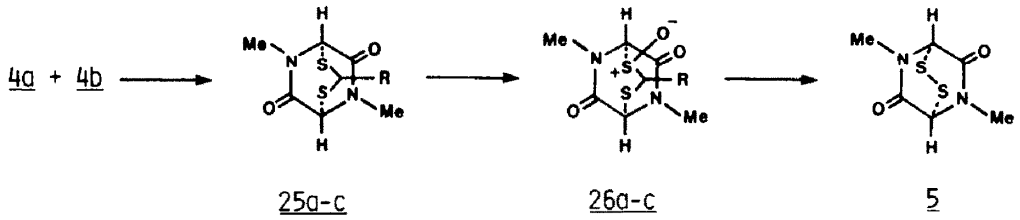
16 20 5 21

Scheme 6.

The reaction conditions used for generation of



Scheme 7.



25a-c 26a-c 5

a = CH_3

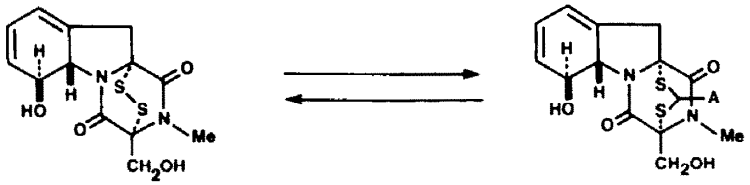
b = C_6H_5

c = $C_6H_4OCH_3$(p)

Scheme 8.

the epidithiapiperazinedione **5** from the thioacetal **25c** were confirmed mild enough to apply this method to the gliotoxin synthesis. Namely, treatment of natural gliotoxin (**1**)[33] with sodium borohydride and then with *p*-anisaldehyde and boron trifluoride etherate yielded a diastereomeric mixture of the thioacetals **27a,b**, which were separated by preparative thin layer chromatography. From either of these diastereomers **27a** and **b**, gliotoxin (**1**) was successfully generated in 65% yield upon treatment with 1.2 equivalents of *m*-chloroperbenzoic acid and then with a trace amount of perchloric acid at room temperature.

with *n*-butyllithium or lithium diisopropylamide (LDA) in tetrahydrofuran, followed by treatment with alkyl halides, aldehydes, or acyl halides. The mono-substituted thioacetals **28** were further alkylated or acylated to yield the di-substituted acetals **29** in the same manner. Since the half-life of the bridgehead carbanions was about 10 min at −78°, better yield was often realized by addition of the base to the mixture of the thioacetal and alkylating reagent. However, this procedure was limited to preparation of the symmetrically di-substituted thioacetals. Conversion of the substituted thioacetals into the corresponding epidi-



1 27a,b

A = C_6H_4OMe(p)

Scheme 9.

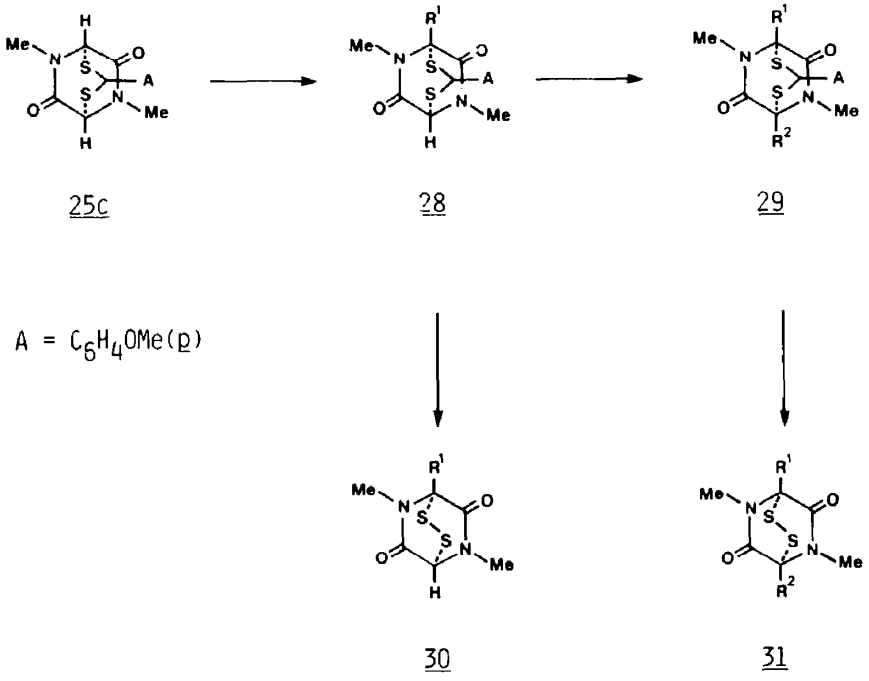
The remarkable stability of the thioacetal **25c** under a variety of conditions such as strongly acidic (concentrated hydrochloric acid at 100°), strongly basic (6 N sodium hydroxide at 50°), reductive (sodium borohydride, lithium borohydride), and certain oxidative (Collins reagent) conditions made this method even more attractive. However, one conceivable disadvantage of this system was that, because of introduction of a chiral center in the protecting group, a diastereomeric mixture might result upon alkylation at the bridgehead position, which fortunately turned out *not* to be the case (*vide infra*). Related to this, it should be mentioned that attempts to prepare the thioketal of the *cis*-dithiopiperazinedione **4a** with acetone or benzophenone were unsuccessful.

Monoalkylation or monoacylation of **25c** was successfully achieved by forming the carbanion

thiapiperazinediones was realized in a manner similar to that described for the thioacetal **25c**.

By this method, it was possible to synthesize a variety of epidithiapiperazinediones **30** and **31**, formerly derived from various amino-acids, from sarcosine anhydride (**2**).[33]

In the course of the alkylation or acylation studies of the thioacetal **25c**, an extremely interesting phenomenon was observed. Assuming that the acidity of the two bridgehead protons H_A and H_B is of the same order, a diastereomeric mixture should result; however, a *single* product was produced in each alkylation or acylation reaction. The following experiments clearly demonstrated this: the benzyl methyl thioacetals **29a** and **29b**, prepared from **25c** by methylation followed by benzylation or benzylation followed by methylation, were found to be distinctly different, judging

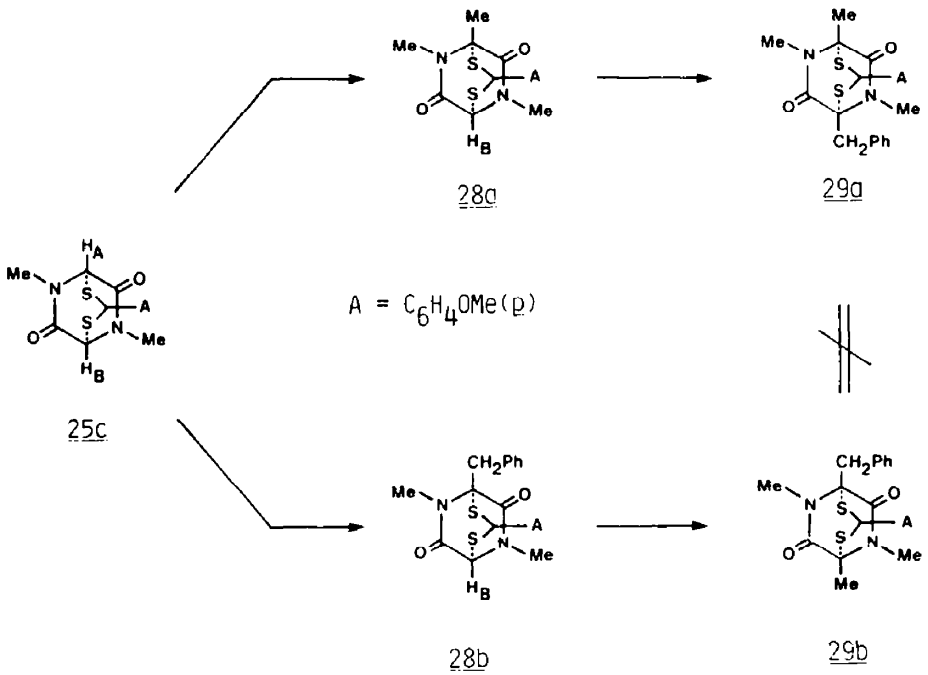


Scheme 10.

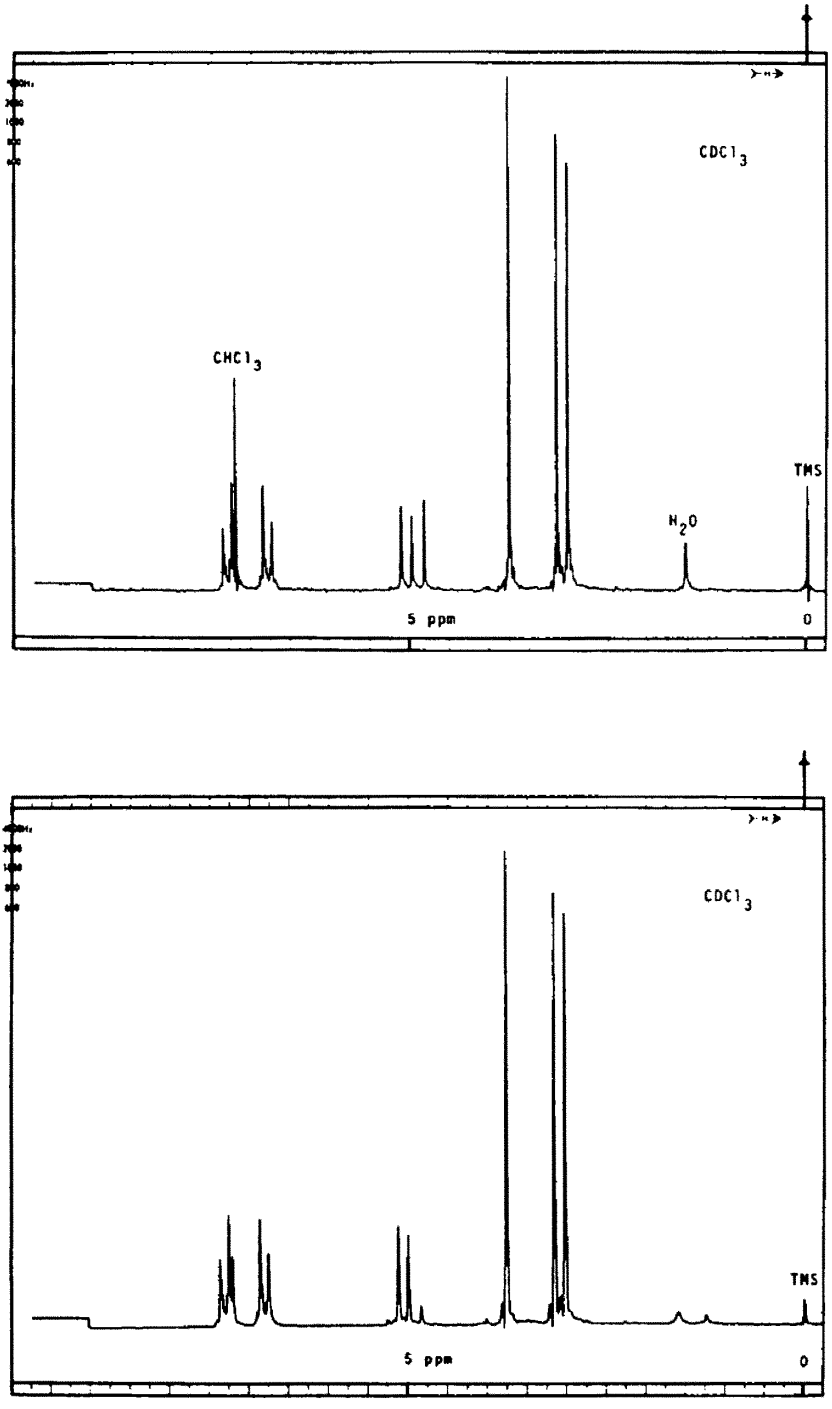
from NMR and IR spectra and also from the mixed m.p. determination. The only explanation for this observation was that the formation of the carbanion on **25c** took place in a *regiospecific* manner.

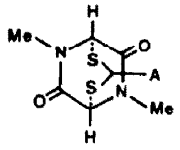
A deuterium exchange experiment also produced the same conclusion. Mono-deuterated thioacetal **25c** was obtained upon treatment with 1 equivalent of *n*-butyllithium at −78° in tetrahydrofuran, followed by addition of deuterium chloride in deuterium oxide. As shown in Fig. 1, only a singlet at δ 4.84 ppm was exchanged with deuterium (80% deuterium incorporation estimated from mass and NMR spectra).

Further evidence for the regiospecific carbanion formation was obtained in the course of the total synthesis of dehydrogliotoxin. The stereochemistry of the chlorides **32** and **35** was assigned as shown, based on the NMR spectrum (*vide infra*). Treatment of **32** with 1.2 equivalents of phenyl-

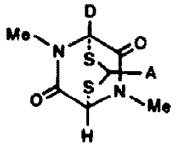


Scheme 11.





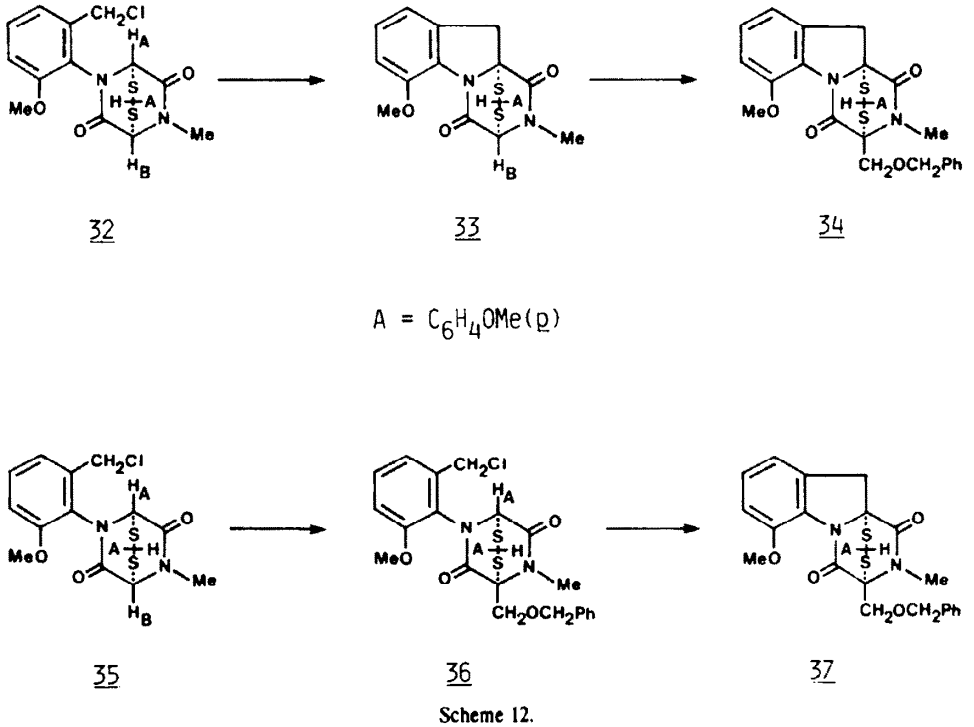
25c : A = $C_6H_4OMe(p)$



25c (monodeuterated)

Fig. 1.

lithium at −78° in tetrahydrofuran effected the cyclization to give **33** in 79% yield. Subsequent alkylation of **33** with phenyllithium and benzyl chloromethyl ether yielded the protected dehydrogliotoxin thioacetal **34** in 71% yield. On the other hand, the chloride **35**, a stereoisomer of **32** with respect to the anisaldehyde residue, did not undergo cyclization on treatment with 1.2 equivalents of phenyllithium at −78° in tetrahydrofuran, but instead on addition of benzyl chloromethyl ether gave the alkylated product **36** in 72% yield. The alkylated product **36** was successfully cyclized upon treatment with 1.2 equivalents of phenyllithium to give the protected dehydrogliotoxin thioacetal **37** in 93% yield. These experiments clearly demonstrated that: (a) H_A is more acidic than H_B in the thioacetal **32**, whereas H_B is more acidic than H_A in **35**. (b) A difference in acidity of the bridgehead protons is associated with the stereochemistry of the anisaldehyde residue. (c) The carbanion at the bridgehead is kinetically formed and no appreciable scrambling of the carbanion takes place.

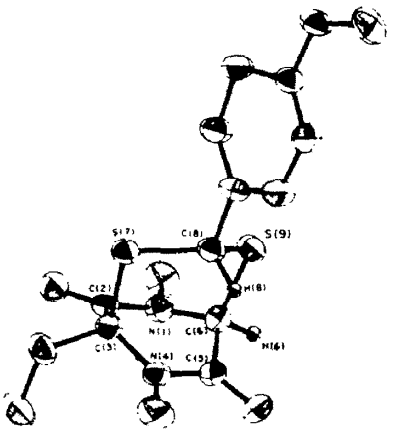


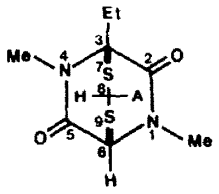
Scheme 12.

In order to bring to light the reason(s) for this regiospecific carbanion formation, more conclusive structural information on the alkylated or acylated thioacetals was desired. Accordingly, an X-ray crystallographic analysis of the ethyl thioacetal **28c** (racemic), prepared from **25c** in the usual manner, was carried out.[35] The structure was consistent with that anticipated from results obtained in the dehydrogliotoxin synthesis. X-ray analysis showed that the dihedral angle of C(8)–S(9)–C(6)–H(154°) was close to that for C(8)–S(7)–C(3)–Et(155.7°), making it difficult to attribute the regiospecificity to the difference in the efficiency in overlap of the 3d orbitals of sulfur and the orbital of the bridgehead carbanion.[39] One possible explanation might be that the regiospecificity could arise as a result of the interaction between the lone pair of the sulfur atom and the carbonyl group. Examination of Dreiding molecular models revealed that one of the lone pairs on S(7) is sterically close to C(2), whereas no such interaction is expected for the lone pair on S(9) with C(5). Assuming such an interaction as represented by the canonical structure **25c–B**, the proton at C(3) could be more acidic than that at C(6).

EPIDITHIAPIPERAZINEDIONE SYNTHESES: METHOD B (MONOCYCLIC ROUTE)

Although the synthetic method of epidithiapiperazinediones using a bicyclic thioacetal proved to be useful for a variety of compounds, there still remained two problems which caused technical trouble in some cases; one was the short half-life (about 10 min at −78° in tetrahydrofuran) of the bridgehead carbanion, and the other was the subtle reaction conditions required for acid cleavage of the thioacetal monosulfoxide. To overcome these potential problems, a different route was investigated. The key question in this approach was the stereochemical outcome of an alkylation reaction on a protected dithiopiperazinedione like **19**. This was first studied by using the *cis*-dimethylthio compound **38**.[24] Upon treatment with 2.2 equivalents of *n*-butyllithium and excess methyl iodine, the dimethylation of **38** yielded a *single* product in 84% yield. This product was found to be identical (NMR, IR, MS, tlc) with dimethyl dimethylthiopiperazinedione **40**, prepared from the dimethyl epidithiapiperazinedione **31c** by

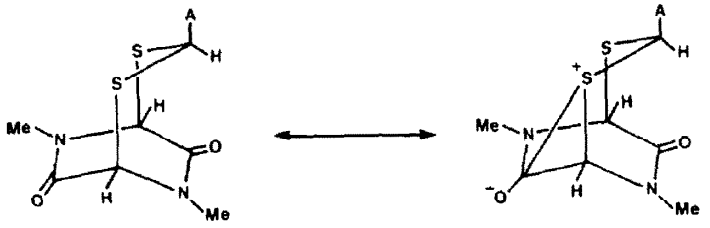




28c

A = $C_6H_4OMe(p)$

Fig. 2.

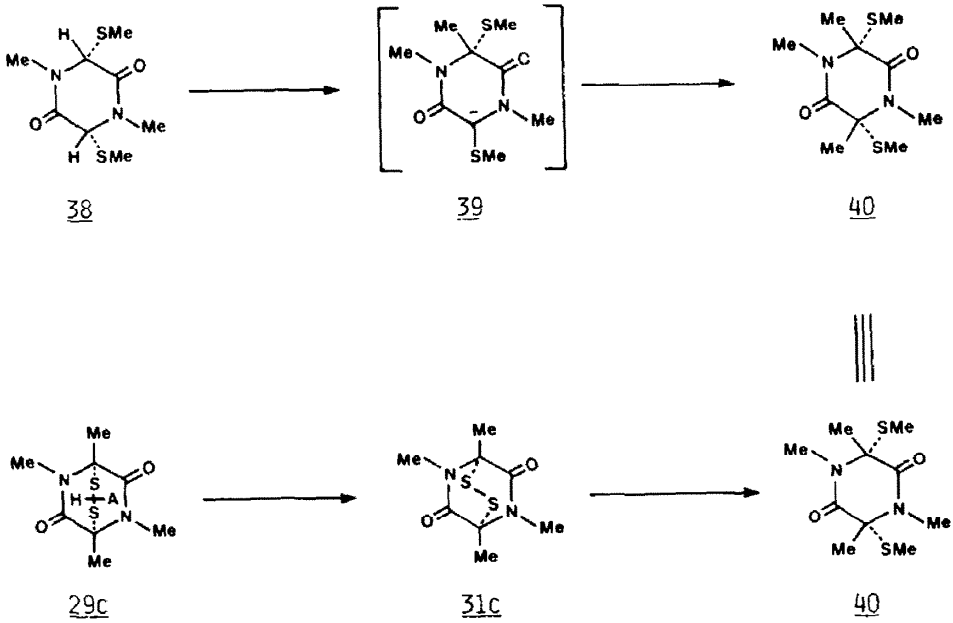


25c-A 25c-B

Fig. 3.

sodium borohydride reduction, followed by methyl iodide and pyridine treatment. Taking into account the easy epimerization observed before in a similar system (see above), this experiment did not necessarily establish but strongly suggested that the relative stereochemistry at the C-3 and C-6 positions of the alkylation product was *cis*.[36]

The carbanion in these experiments was shown to be generated under thermodynamic conditions. Namely, when the carbanion generated from **38**



38 39 40

29c 31c 40

A = $C_6H_4OMe(p)$

Scheme 13.

with 1.0 equivalents of *n*-butyllithium in tetrahydrofuran at −78° was quenched with deuterium chloride, net 41% deuterium incorporation, due to 10% of the di-, 60% of the mono- and 30% of the non-deuterated **38**, was observed. Almost identical deuterium distribution, i.e. 9% of the di-, 60% of the mono- and 31% of the non-deuterated **38**, was found when the dicarbanion generated from **38** with 2.0 equivalents of *n*-butyllithium was mixed with 1.0 equivalent of **38** at −78° and immediately quenched with deuterium chloride. Thus, it was evident that rapid scrambling of the carbanions took place under the conditions employed for the alkylation reaction.

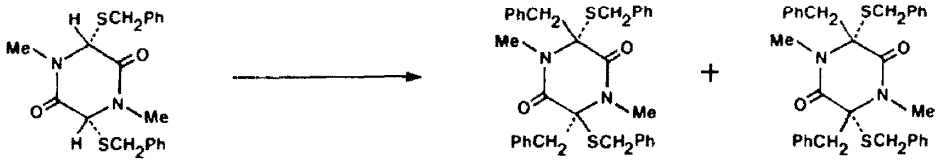
The stereochemical course of this reaction could be analyzed as follows. Two extreme conformations **39-A** and **39-B** would be considered for the carbanion of the monoalkylated product **39**. Conformation **39-A** might be preferred to conformation **39-B** which is destabilized due to steric compression between the two methyl groups (see the double arrow in **39-B**). The β-face of conformation **39-A** would be more sterically crowded (see the shadow in **39-A**), allowing the alkylating agent access from the α-face.

To demonstrate that the primary factor controlling the *cis*-alkylation was steric, dibenzylation of the *cis*-dibenzylthiopiperazinedione **41** was studied. Upon treatment with 2.1 equivalents of *n*-butyllithium and excess benzyl bromide, **41** yielded a 5:4 mixture of two stereoisomers **43** and **42** in 64% yield. The *cis*-configuration was assigned to the major product **43** by comparison with the authentic sample, prepared from the dibenzyl epidithiapiperazinedione **31e**. Four extreme conformations, *A*, *B*, *C* and *D*, would be considered for this case. Among them, conformations *C* and *D* would be less important for the analysis of the stereochemical course of the alkylation reaction. The benzylthio group would cover the β-face of conformation *A*, whereas the benzyl group would cover the α-face of conformation *B*, resulting in *cis* and *trans* isomers in almost equal amounts.

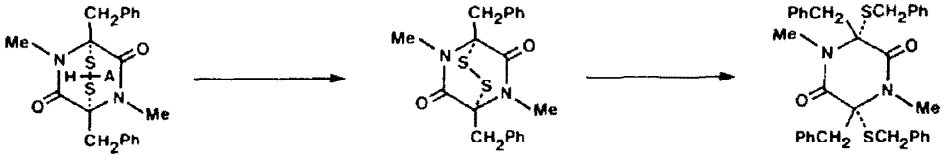
In order to apply this stereospecific alkylation reaction to a general synthesis of epidithiapiperazinediones, a suitable protecting group for the thiol moieties was needed. As described before, the ditetrahydropyranyl derivative **19** was cleanly oxidized to **5** with iodine; unfortunately, attempted alkylation of **19** with 2 equivalents of *n*-butyl-

39-A 39-B

Fig. 4.



41 42 43



29e 31e 43

A = $C_6H_4OMe(p)$

Scheme 14.

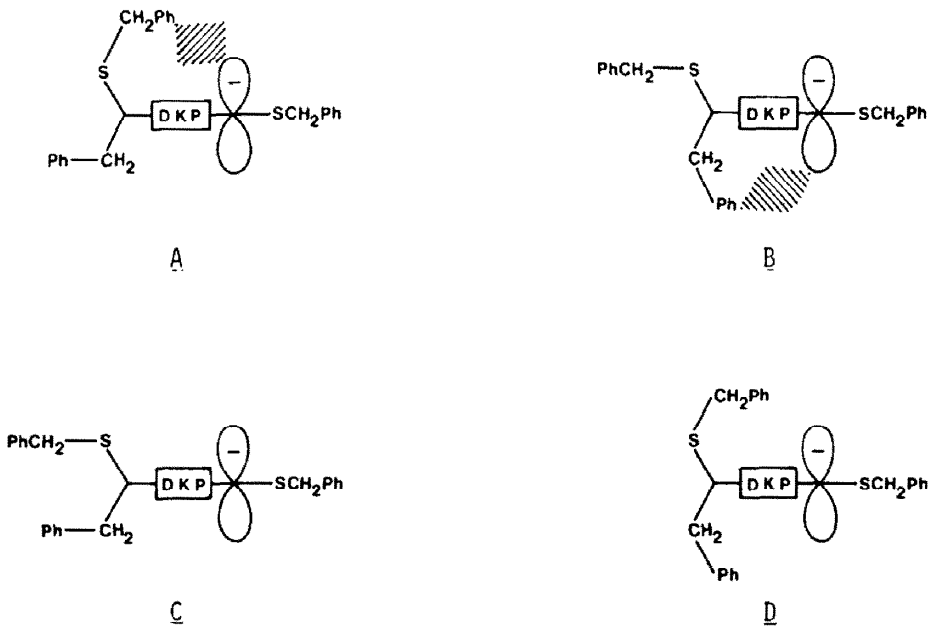
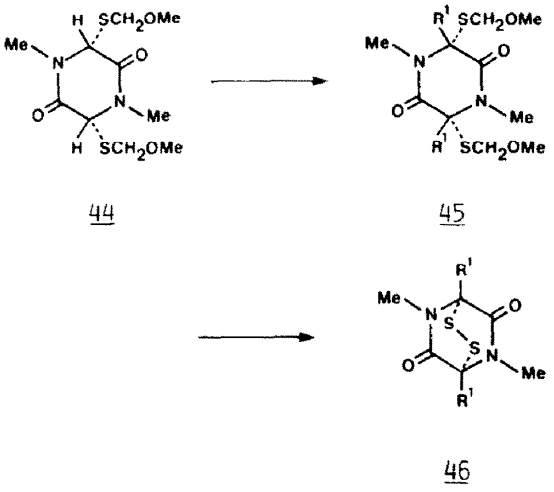


Fig. 5.

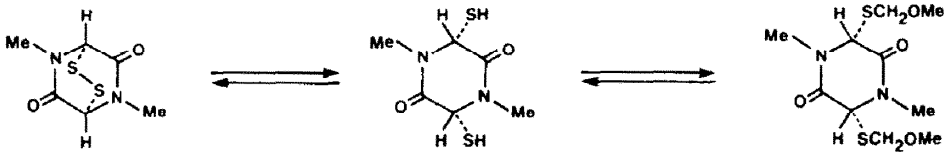
lithium and excess methyl iodide resulted in a complex mixture of products. This complexity was, at least partially, attributable to the two chiral centers in the protecting groups. For this reason, the dimethoxymethylthio compound **44** was prepared from **4** by treatment with 2.1 equivalents of potassium *t*-butoxide and chloromethyl methyl ether in 80% yield. The *cis*-configuration was tentatively assigned to **44**, based on the data of thermodynamic stability of the corresponding dimethylthio compound **38**.[24] Based on the previous observations (see above), it was not surprising to find that **44** was stable against iodine in methylene chloride at room temperature. Oxidation of **44** with *m*-chloroperbenzoic acid did not give encouraging results. Attempted hydrolysis to the *cis*-dithiol **4a** with protic acids such as sulfuric, hydrochloric, hydrobromic, perchloric, or trifluoroacetic acid under a variety of conditions were also uniformly fruitless. However, boron trichloride cleanly attacked the methoxymethyl group of **44** at 0° in methylene chloride, to give the *cis*-dithiol **4a**, after methanol workup. This facile deprotection could be attributed to the complex formation of boron trichloride and the carbonyl group. The *cis*-dithiol **4a** was then oxidized with iodine to yield the epidithiapiperazinedione **5**.

The dianion, generated from **44** using 2.3 equivalents of lithium diisopropylamide (LDA) in tetrahydrofuran at −78°, reacted with a variety of alkylating agents to yield *exclusively* one product **45**. The *cis*-configuration of the product was supported by the fact that **45** yielded cleanly the corresponding epidithiapiperazinedione **46** upon treatment with boron trichloride, followed by iodine oxidation.



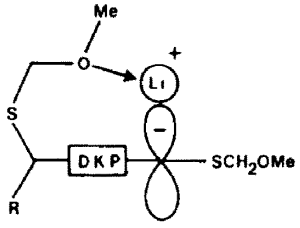
Scheme 16.

The remarkable stereospecificity observed for various alkylation reagents, even for benzyl bromide (compare the results discussed previously), could be explained in the following manner: coordination of the oxygen lone pairs towards



Scheme 15.

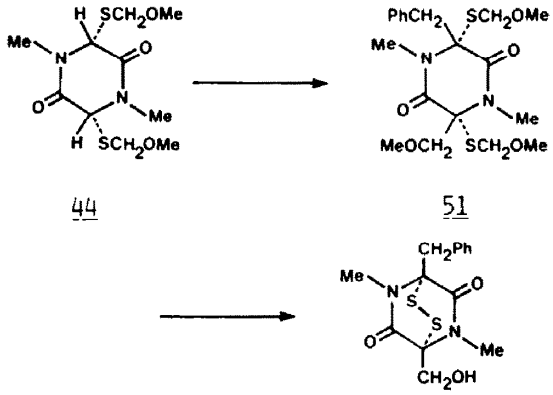
the lithium cation might stabilize the conformation of the monoalkylated carbanion shown in *E*, making the β-face sterically more crowded for the incoming alkylating reagent than the α-face.



E

Fig. 6.

During the alkylation studies, a remarkable difference in reactivity between the dicarbanion and the carbanion of the monoalkylated derivative was recognized. When the dicarbanion of **44** was treated with excess alkyl halide and then worked up with acetic acid *at* −78°, the *cis*-mono-alkylated piperazinedione **47** was obtained in high yield. The mono-alkylated piperazinedione was subsequently converted to the corresponding epidithiapiperazinedione **48** in the same manner as described for the dialkylated compounds. Furthermore, the unsymmetrically di-substituted epidithiapiperazinedione **50** was readily available by alkylation of the mono-substituted derivative **47**, followed by the usual deprotection–oxidation procedure.



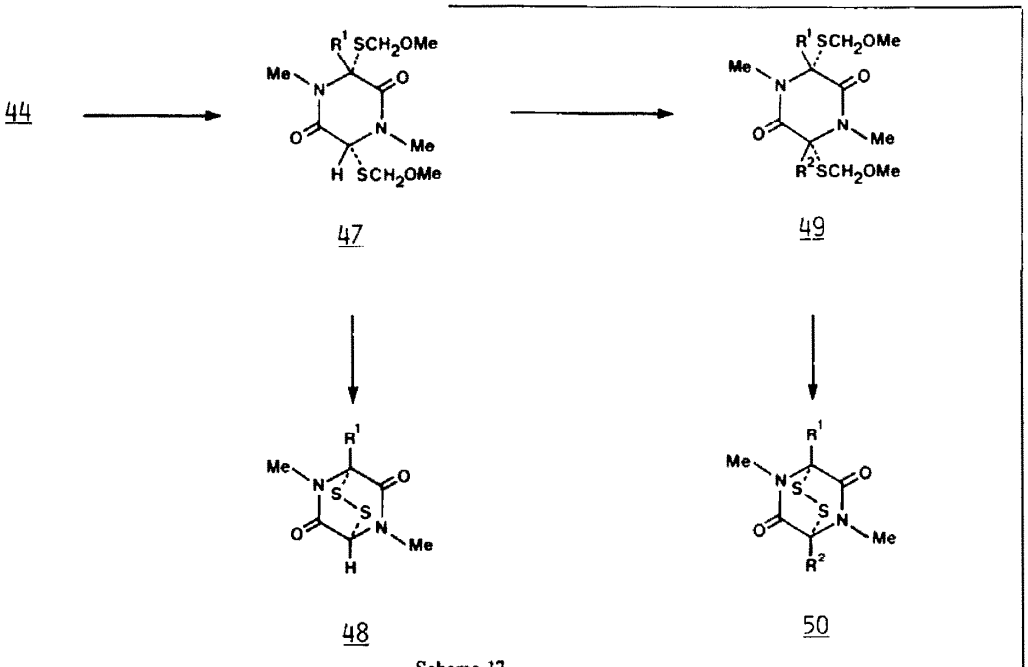
44 51

52 : Hyalodendrin

Scheme 18.

benzyl bromide for 20 min at −78° and then with 4 equivalents of bromoethyl methyl ether for 10 min at −78°, giving the benzyl methoxymethyl derivative **51** in 63% yield, which was converted to *dl*-hyalodendrin (**52**) in the usual manner in 28% overall yield.[34] The synthetic compound was found to be identical with natural hyalodendrin on comparison of spectroscopic (NMR, MS) and tlc data.[38] The NMR spectra of synthetic and natural hyalodendrin are illustrated in Fig. 7.

We have now established two general methods for the synthesis of epidithiapiperazinediones.



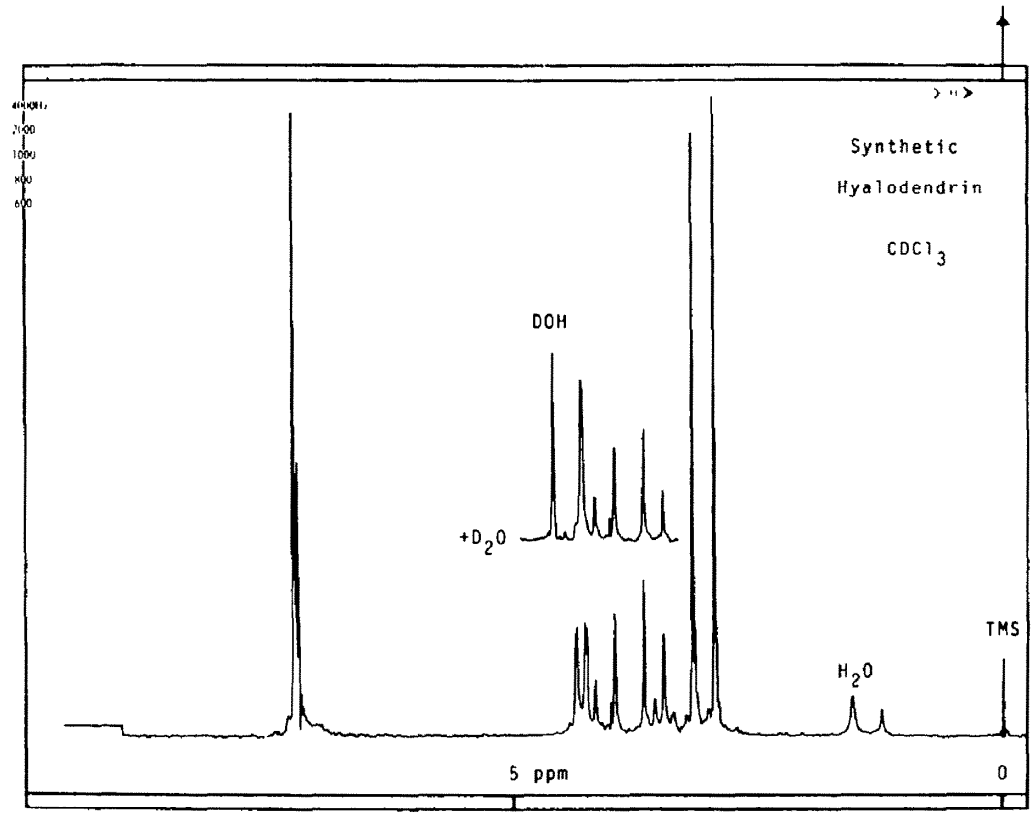
47 49

48 50

Scheme 17.

By utilizing the reactivity difference of alkylating agents, one-pot synthesis of the unsymmetrically di-substituted compounds was also possible. This procedure was demonstrated in a total synthesis of hyalodendrin **52**.[17] The dicarbanion generated from **44** with 2.3 equivalents of lithium diisopropylamide was treated with 5 equivalents of

Comparison between these methods reveals that the first (Route A)[39] is more suitable for multistage synthesis of complex molecules because of the stability of the thioacetals under various conditions as demonstrated in the total syntheses of dehydrogliotoxin,[40] sporidesmin A,[41] sporidesmin B,[42] hyalodendrin[43] and gliotoxin.[44] On the other


Synthetic
Hyalodendrin
CDCl3
DOH
+D2O
H2O
TMS
5 ppm
0

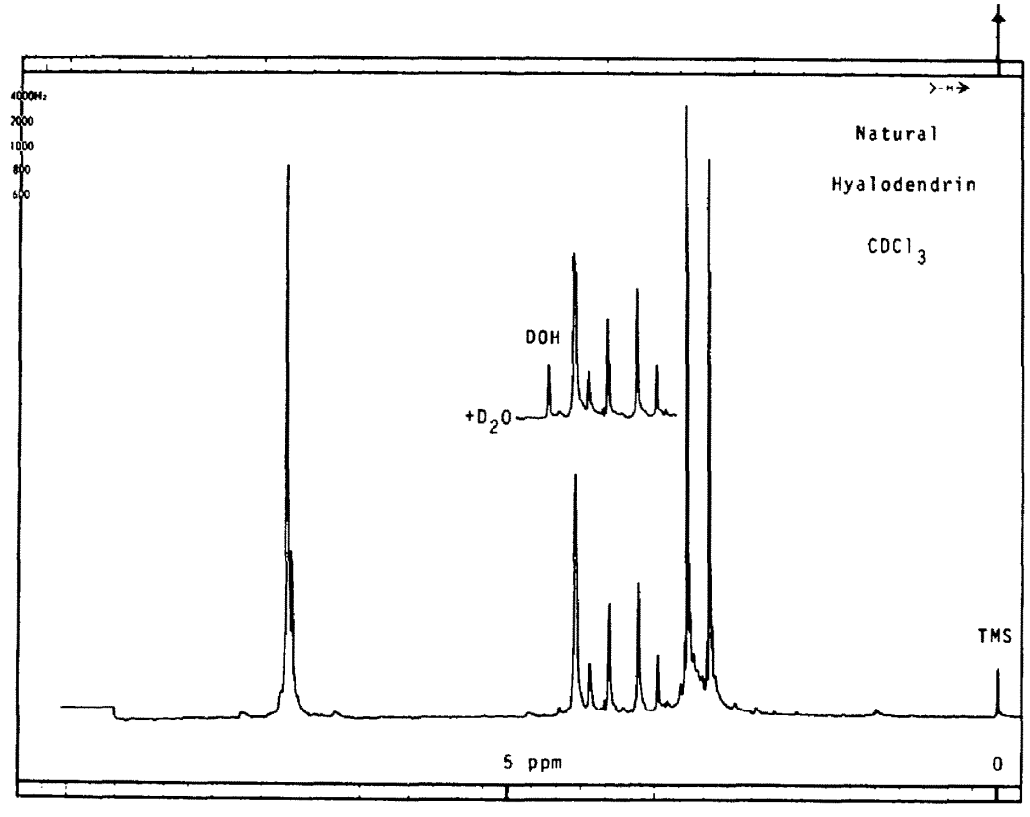
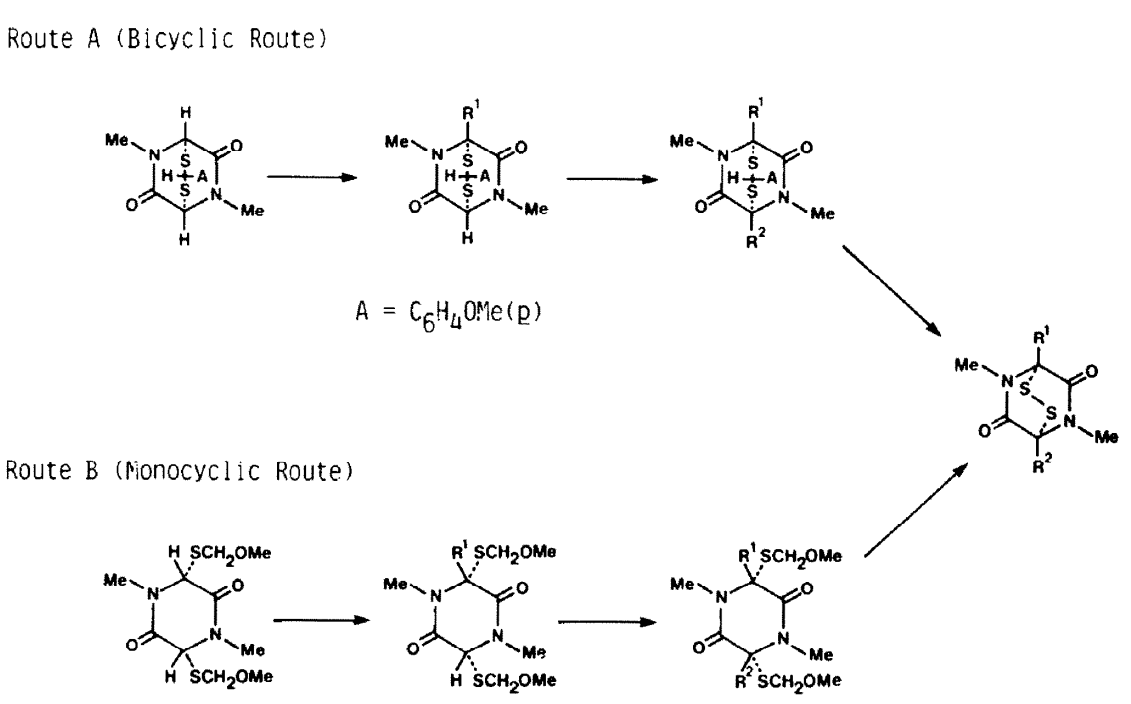

Natural
Hyalodendrin
CDCl3
DOH
+D2O
TMS
5 ppm
0

Fig. 7.



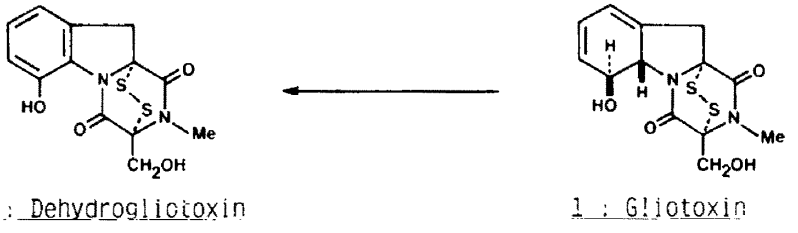
Scheme 19.

hand, the second method (Route B)[45] is more suitable for large-scale synthesis of simple epidithiapiperazinediones because of the simplicity of procedures and high yields.

TOTAL SYNTHESIS OF DEHYDROGLIOTOXIN

Dehydrogliotoxin was isolated by Taylor *et al.* from surface cultures of *Penicillium terlikowskii*.[13] The structure determination of dehydrogliotoxin (**53**) was carried out primarily by degradation and NMR studies. The most conclusive evidence for the proposed structure was that dehydration of gliotoxin (**1**) with *o*-chloranil yielded a product which was found to be identical with dehydrogliotoxin (**1**).

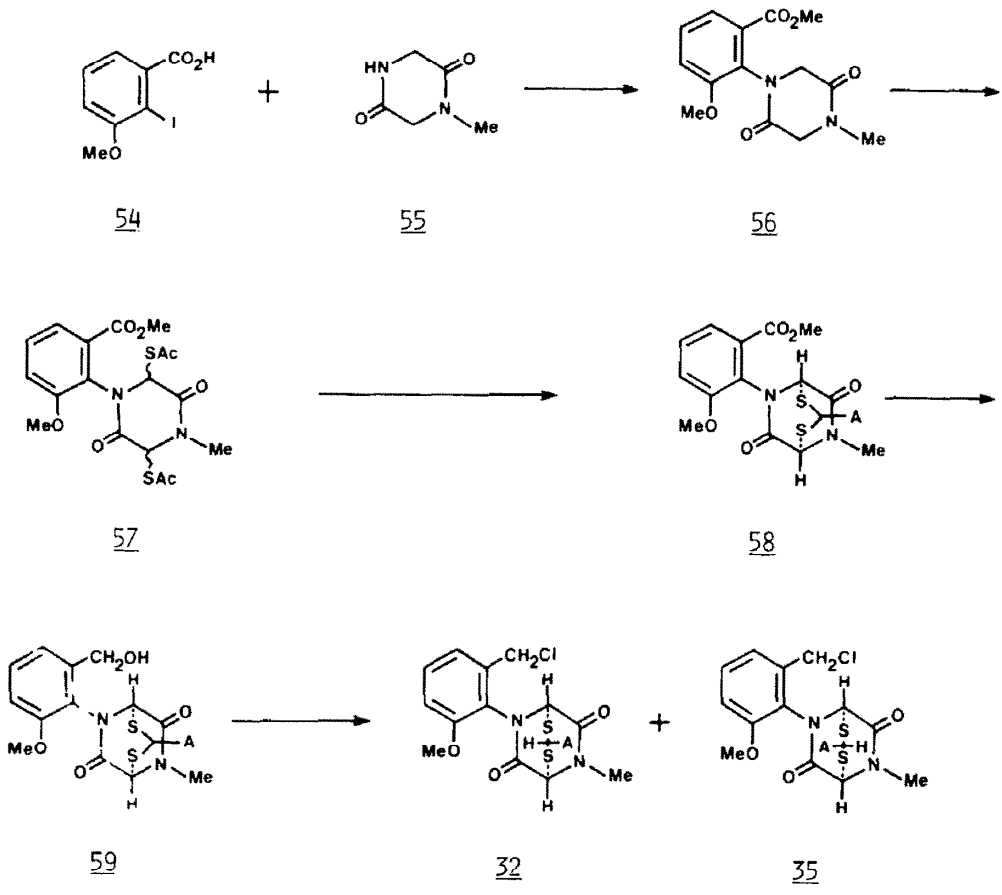
Ullmann-type reaction of 2-iodo-3methoxybenzoic acid (**54**)[46] and glysine–sarcosine anhydride (**55**)[47] in the presence of cuprous iodide and potassium carbonate in nitrobenzene at 170° for 40 min gave, after esterification with diazomethane, the sterically crowded piperazinedione **56** in 35% yield (based on **54**). A 1:1 mixture of *cis*- and *trans*-dithioacetates **57** was obtained from **56** in 65% overall yield by refluxing 2.8 equivalents of N-bromosuccinimide in the presence of a catalytic amount of benzoyl peroxide in carbon tetrachloride for 90 min, followed by treatment with potassium thioacetate in methylene chloride at room temperature. Methanolysis of the thioacetates **57** with methanolic hydrogen chloride



Scheme 20.

Dehydrogliotoxin seemed to be a good target molecule to test the applicability of the synthetic methods developed in the previous section. For the reasons summarized above, the synthesis of dehydrogliotoxin was planned via the thioacetals **32** and **35**; namely, formation of the two carbon–carbon bonds was envisioned by using the carbanion chemistry discussed before.

The hitherto unknown piperazinedione **56** was chosen as starting material for this synthesis. The at 50° for 40 min, followed by treatment with *p*-anisaldehyde and boron trifluoride etherate in methylene chloride at room temperature overnight, gave an approximately 1:1 diastereomeric mixture of the thioacetals **58** in 72% overall yield. Conversion of the ester **58** to the alcohol **59** was realized via the mixed anhydride; reduction of the mixed anhydride, prepared from **58** by a routine method, was most conveniently achieved by direct addition of a chilled methanolic solution of excess



Scheme 21.

sodium borohydride to an ice-cooled solution of the mixed anhydride in methylene chloride. Overall yield of **59** from **58** was 69%. Subsequent treatment of the alcohol with methanesulfonyl chloride and triethylamine at room temperature gave the mesylate, which was converted into an about 1:1 diastereomeric mixture of the chlorides **32** and **35** with lithium chloride in dimethylformamide at room temperature in 89% overall yield.

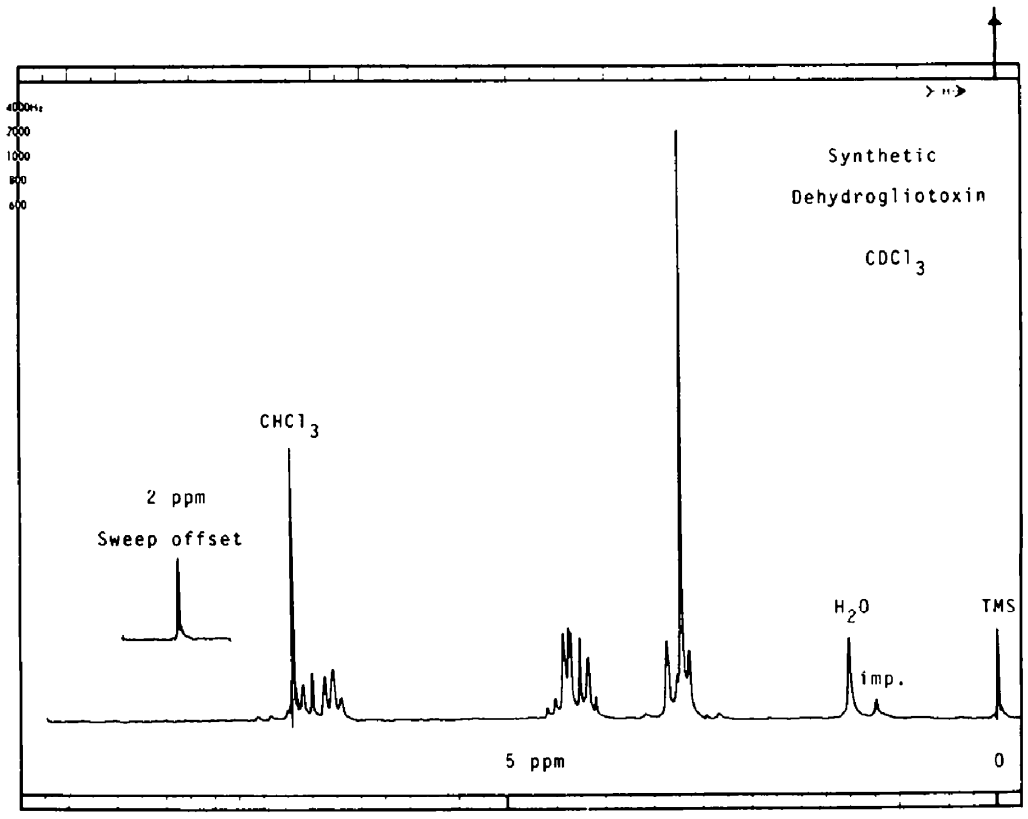
The diastereomeric chlorides **32** and **35** could not be separated chromatographically, but one of the isomers was highly crystalline, allowing their separation by fractional crystallization from ethyl acetate. The stereochemistry of the diastereomers **32** and **35** was assigned, based on the NMR spectra. The signal of the proton on the thioacetal bridge of **32** appeared at δ 6.32 ppm, whereas that of **35** was at δ 5.37 ppm, which is close to the chemical shift, δ 5.13 ppm, of the bridgehead proton of the simple thioacetal **25c**. The unusual downfield shift observed for one of the diastereomers could be attributed to the deshielding effect of the aromatic ring, establishing the stereochemistry of the diastereomeric chlorides **32** and **35**.

The regiospecific metallation at the bridgehead positions of **32** and **35** was cleanly realized as discussed previously. Thus, the acetals **34** and **37** were obtained from **32** and **35**, respectively, in good yield (see above). A more convenient one-pot synthesis of **34** from **32** was also realized by careful addition of 2.5 equivalents of phenyllithium to a solution of **32** and benzyl chloromethyl ether in tetrahydrofuran at −78°, affording **34** in 69% yield. Following exactly the same procedure, **35** gave **37** in 61% yield.

Cleavage of both the benzyl and methyl ethers of **34** and **37** with boron trichloride in methylene chloride at 0° smoothly yielded the dehydrogliotoxin thioacetals **60** and **61** in 67 and 66% yield, respectively. Finally, *dl*-dehydrogliotoxin **53** (m.p. 177–178°) was obtained from **60** and **61** in 72% yield, using the conditions established for the conversion of the natural gliotoxin thioacetals into gliotoxin (see earlier). The synthetic substance was found to be identical with natural dehydrogliotoxin[13,33] on comparison of spectroscopic (NMR, IR, UV, MS) and tlc data. The NMR spectra of synthetic and natural dehydrogliotoxin are illustrated in Fig. 8.

TOTAL SYNTHESIS OF GLIOTOXIN

At this stage in the study, the most difficult problem remaining unsolved for the synthesis of gliotoxin was apparently the method to incorporate the dihydrobenzene ring system to a synthetic intermediate, since construction of the rest of the molecule was expected to be achieved by using the method tested in the synthesis of dehydrogliotoxin. Berchtold *et al.* reported a synthesis of 4-carbo-*t*-butoxyoxepin, which exists primarily as the tautomer **62-A** but reacts with a nucleophile


Synthetic
Dehydrogliotoxin
$CDCl_3$
$CHCl_3$
2 ppm
Sweep offset
H_2O
imp.
TMS
5 ppm
0

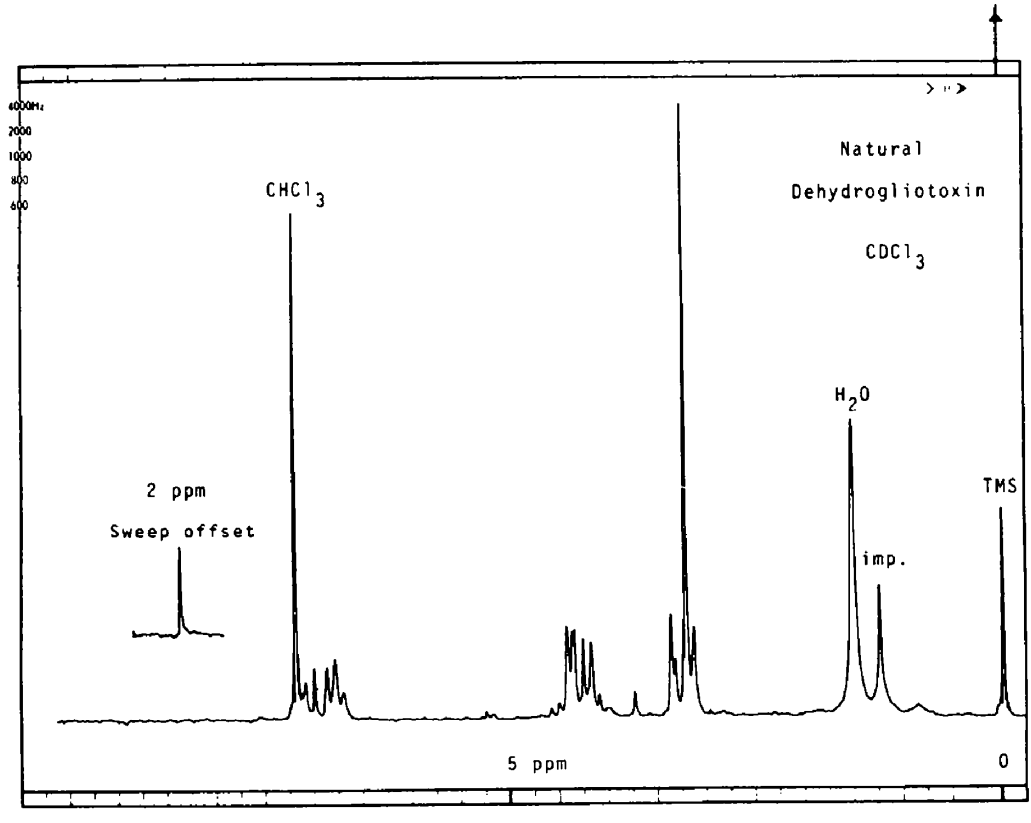
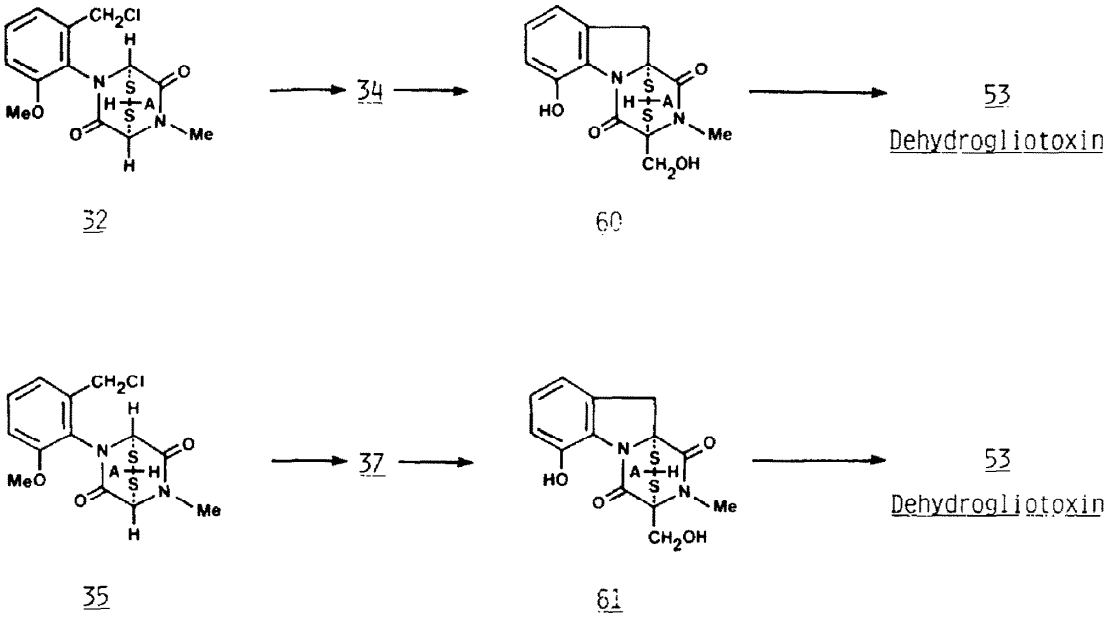
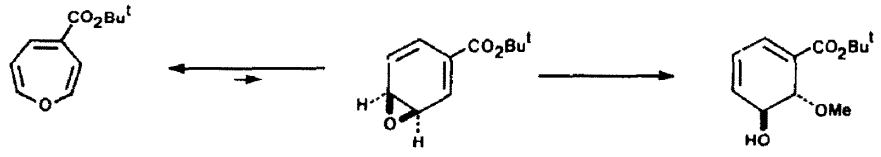

Natural
Dehydrogliotoxin
$CDCl_3$
$CHCl_3$
2 ppm
Sweep offset
H_2O
imp.
TMS
5 ppm
0

Fig. 8.



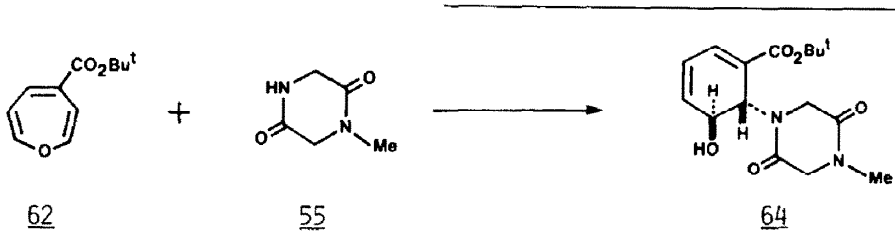
Scheme 22.

via the tautomer **62-B**; thus **62** yields exclusively the *trans*-adduct **63** upon treatment with lithium hydroxide in refluxing methanol.[48] This reaction seemed to suggest an interesting possibility for solving the present problem, which was first verified by using glycine–sarcosine anhydride (**55**). In fact, **55** was found to react smoothly with **62** in methylene chloride containing benzyltrimethylammonium methoxide (Triton B) at room temperature, to yield the expected product **64** in 36% yield. Encouraged by these results, we began the synthesis of the thioacetal **68**.



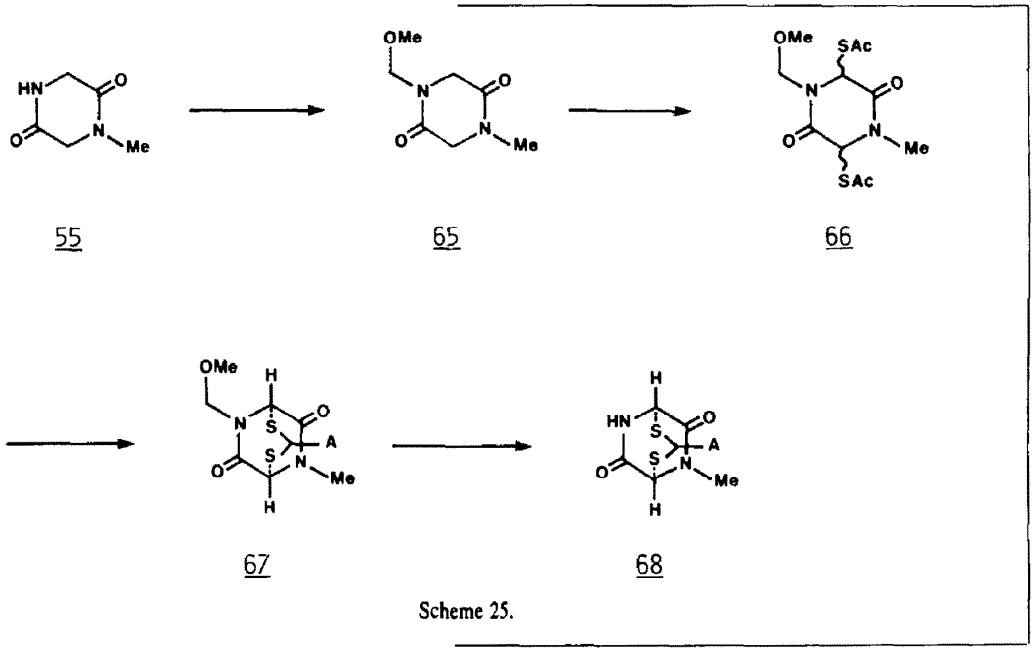
Scheme 23.

The N–H group of glycine–sarcosine anhydride (**55**) was protected as a methoxymethyl group, using potassium *t*-butoxide and chloromethyl methyl ether at 0° in 75% yield. The methoxymethyl derivative **65** was converted to a *cis* and *trans* mixture of the dithioacetates **66** upon treatment with 2.5 equivalents of N-bromosuccinimide and catalytic amounts of benzoyl peroxide in refluxing carbon tetrachloride, followed by potassium thioacetate in methylene chloride at room temperature. The resultant mixture of *cis*- and *trans*-dithioacetates **66** was subjected to methanolysis with methanolic hydrogen chloride at 50° to give the dithiols, which were then treated with *p*-anisaldehyde and boron trifluoride etherate in methylene chloride at room temperature to yield the methoxymethyl thioacetal **67**. Removal of the methoxymethyl group of **67** was most efficiently



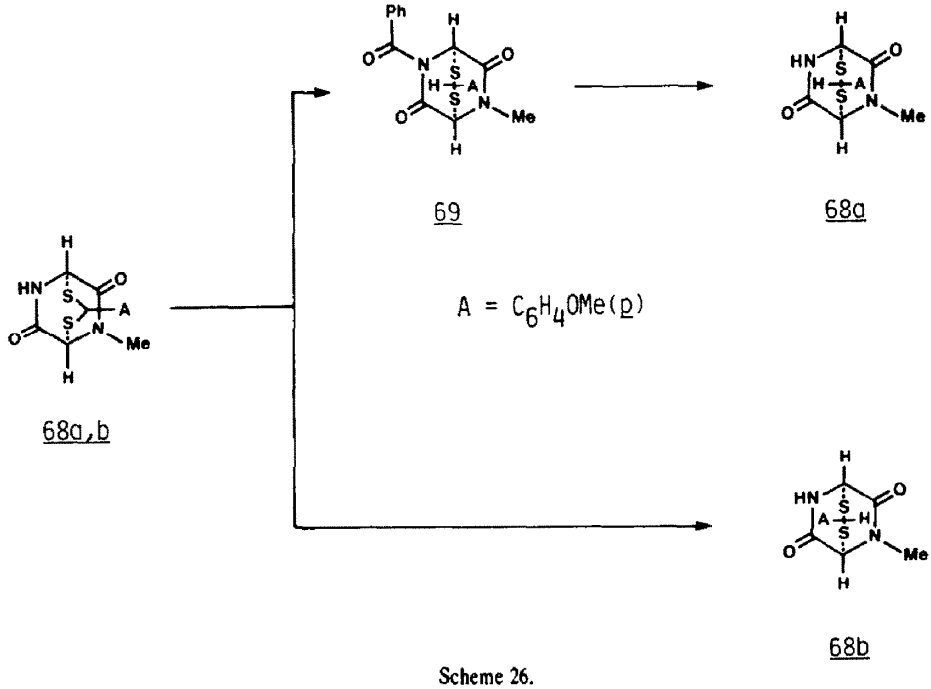
Scheme 24.

achieved in a refluxing solution of concentrated hydrochloric acid–ethanol (1:1). Since a diastereomeric mixture of the thioacetal **68** crystallized out from the crude reaction mixture, no chromatographic separation of the intermediates was necessary from **65** to **68**. The overall yield from **65** to **68** by this procedure was 30%.



Scheme 25.

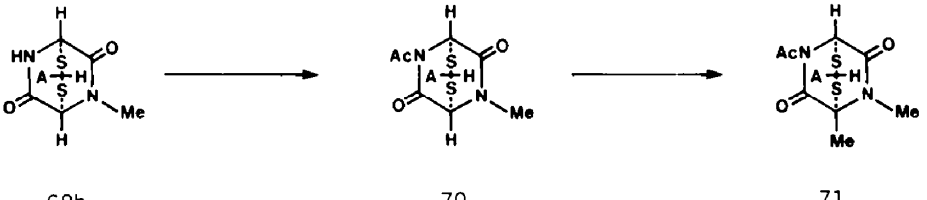
Attempted separation of the diastereomeric mixture of the thioacetals **68** by chromatography or fractional crystallization was fruitless, but it was realized by using the remarkable reactivity difference between these two diastereomers for N-benzoylation. A solution of a 1:1 diastereomeric mixture of the thioacetals **68a,b** in methylene chloride was treated with benzoyl chloride and triethylamine at room temperature, and the reaction was intercepted when about half of the starting material had been consumed. The mixture was then separated on silica gel column chromatography. Analysis of the NMR spectra showed that the N-benzoyl thioacetal **69** was an about 10:1 mixture favoring the one diastereomer, whereas the recovered thioacetal **68** was an about 10:1 mixture favoring the other diastereomer. The thioacetal **68a** was readily recovered by passing ammonia through a methylene chloride solution of



Scheme 26.

the N-benzoyl thioacetal **69**. Recrystallization of the thioacetals **68a** and **68b**, thus obtained, from hot ethanol, allowed isolation of the pure diastereomers, **68a** and **68b**, respectively.

The stereochemistry of the thioacetals **68a** and **68b** was established by chemical transformation. After N-acetylation with acetic anhydride at 130°, **68b** was alkylated on treatment with methyl iodide and *n*-butyllithium in tetrahydrofuran at −78°, to give the N-acetyl-C-methyl thioacetal **71**. Ammonolysis of **71** yielded exclusively the monomethyl thioacetal **72**. The structure of **72**, except the stereochemistry of the anisaldehyde residue, was evidenced by the NMR spectrum; the signal of the bridgehead proton appeared as a doublet at δ 5.37 ppm (J = 6 Hz), which became a singlet on addition of deuterium oxide. Upon treatment with potassium *t*-butoxide and methyl iodide in tetrahydrofuran, **72** yielded the N-methyl thioacetal **28a**. The substance was found to be identical with the authentic thioacetal **28a**, derived from **25c** by treatment with *n*-butyllithium and methyl iodide (see earlier), on comparison of spectroscopic data (NMR, IR, MS) and mixed m.p. determination.



68b 70 71

72 28a

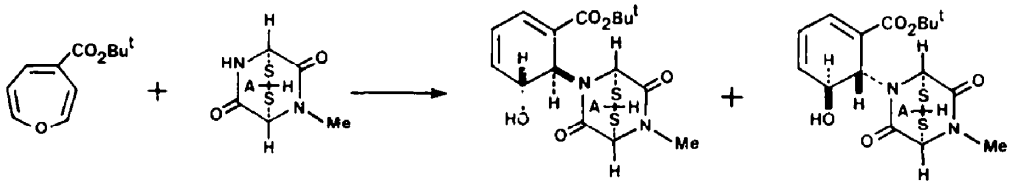
Scheme 27.

The pronounced reactivity difference in the N-benzoylation of the two diastereomers **68a** and **68b** allowed the establishment of a practical method for their separation, however the reason for the origin of the reactivity difference remained obscure. From analysis of the molecular models, the steric effect did not seem to provide a reasonable explanation for the observed phenomena. Some electronic effect like that discussed earlier might be important to consider.

Treatment of a methylene chloride solution of **68b** and 4-carbo-*t*-butoxyoxepin (**62**) with Triton B at room temperature for 30 min yielded a 3:1 mixture of the Michael adducts **73** and **74** in 60% yield. The ratio of the Michael adducts in this reaction was found to be dependent on the solvent and the reaction time. Thus, a 3:1 ratio favoring the adduct **74**, the minor product in Triton B-methylene chloride, was realized when the reaction was conducted in dimethyl sulfoxide at room temperature for 30 min. A slow elimination reaction, regenerating the thioacetal **68b** from the adducts **73** and **74**, was observed at room temperature either in methylene chloride or dimethyl sulfoxide in the presence of Triton B. Thus, an approximately 1:1 mixture of the adducts **73** and **74** resulted from either **68b**, **73** or **74** on Triton B treatment in methylene chloride or dimethyl sulfoxide in the presence of excess **62** at room temperature overnight.

Since the overall *trans* ring opening was expected for **62**, the adducts **73** and **74** should be the epimers with respect to the relative configuration of the thioacetal bridge and the alcoholic group. Two probable orientations *F* and *G* were considered for the transition state of the Michael reaction when **62** and **68b** approached each other in such a way as to cause the least steric hindrance. It was anticipated that the favorable dipole interaction involved in *F* might be favored over *G* in a non-polar solvent such as methylene chloride. Thus, the undesired stereochemistry was tentatively assigned to the adduct **73** and the desired stereochemistry to **74**. This assignment was later confirmed by the fact that the adduct **74** yielded gliotoxin, whereas the adduct **73** gave the epimer of gliotoxin at the sulfur bridge.

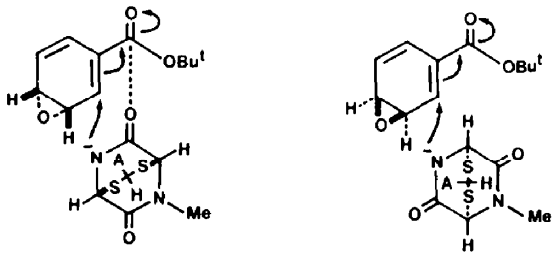
The adduct **74** was treated with acetic anhydride and pyridine at room temperature to yield the acetate **75** in 97% yield. The carboxylic acid **76** was obtained upon treatment of **75** with trifluoroacetic acid at room temperature for 8 min. The acid **76** was then converted to the mixed



62 68b 73 74

A = $C_6H_4OMe(p)$

Scheme 28.



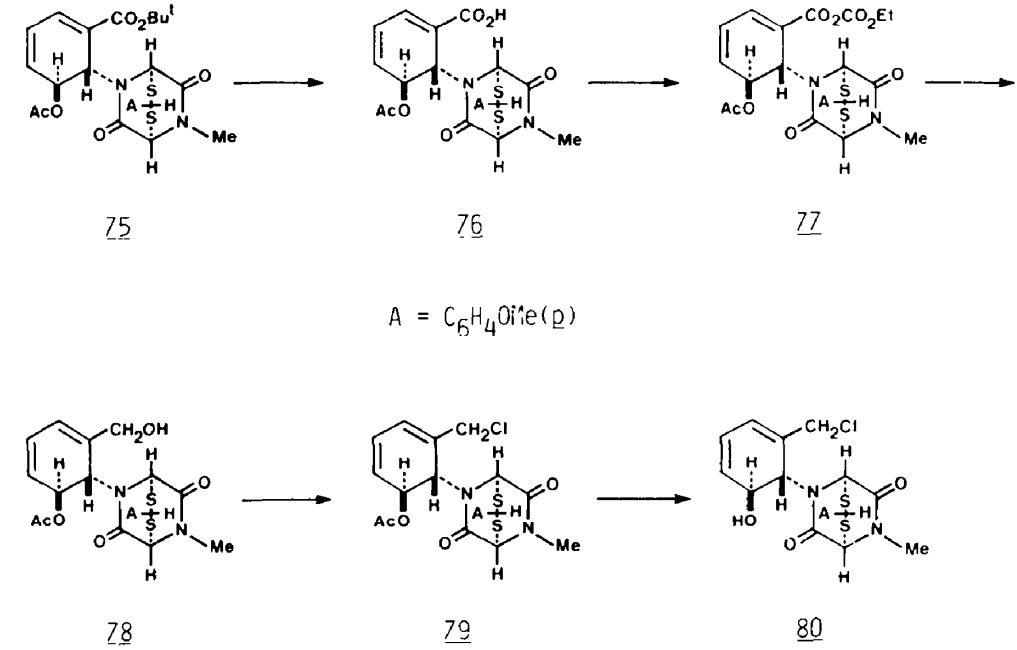
F G

Fig. 9.

anhydride **77** by adding triethylamine to a solution of **76** and ethyl chloroformate in methylene chloride at room temperature. The reduction of the mixed anhydride **77** to the alcohol **78** was most efficiently achieved by the direct addition of a chilled solution of excess sodium borohydride in methanol to an ice-cooled solution of **77** in methylene chloride. The overall yield of **78** from **75** was 79%. The alcohol **78** was treated with methanesulfonyl chloride and triethylamine in methylene chloride and then with lithium chloride in dimethylformamide at room temperature to afford the chloride acetate **79** in 96% overall yield. Methanolysis of **79** with 0.5 equivalent of sodium methoxide in 10% methanol in methylene chloride at room temperature gave the chloride alcohol **80** in 94% yield.

The crucial cyclization–alkylation reaction was accomplished by careful addition of 3.2 equivalents of phenyllithium to a solution of **80** and benzyl chloromethyl ether in tetrahydrofuran at −78°, to yield the desired cyclized compound **83** in 52% yield. Apparently the presence of the free alcoholic group of **80** was essential to prevent aromatization under these conditions.[49]

Cleavage of the benzyl ether of **83** was achieved upon treatment with boron trichloride in methylene chloride at 0° to give the gliotoxin thioacetal **27b** in 60% yield. The unusual stability of the cyclohexadienol system under such strongly acidic conditions could be explained in terms of the complex formation with boron trichloride, which might hold the alcohol group equatorial, hence preventing the elimination of water. The *dl*-gliotoxin thioacetal **27b** was identical with an authen-

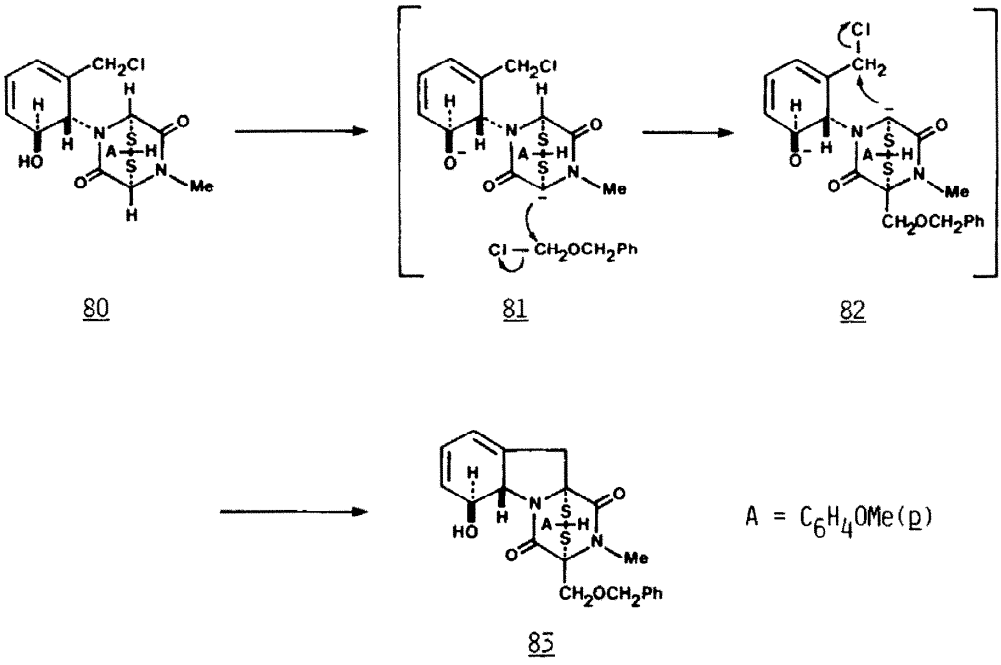


75 76 77

A = $C_6H_4OMe(p)$

78 79 80

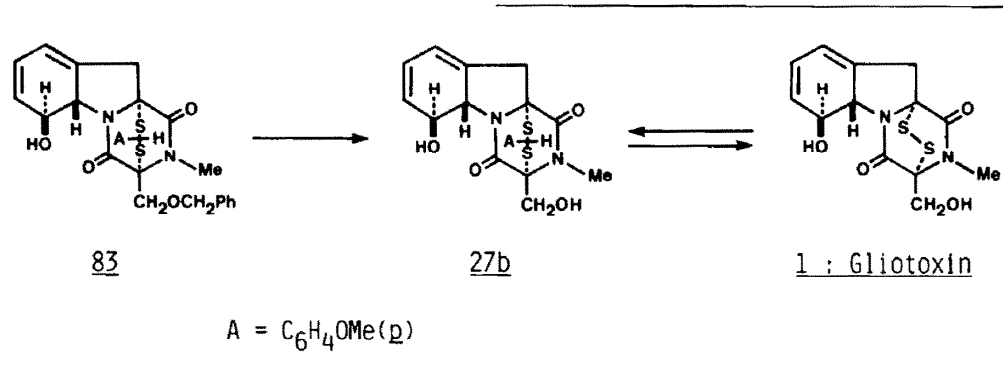
Scheme 29.



Scheme 30.

tic sample, prepared by treating natural gliotoxin[33] with sodium borohydride and then with *p*-anisaldehyde and boron trifluoride etherate, by comparison of spectroscopic (NMR, IR, UV, MS) and tlc data.

was studied, resulting in the efficient method shown in Scheme 32. Treatment of **68a** with (−)-α-methylbenzyl isocyanate[50] and triethylamine in methylene chloride at room temperature yielded a diastereomeric mixture of the ureas **84** and **85** in



Scheme 31.

Finally the *dl*-gliotoxin thioacetal was treated with 1.2 equivalents of *m*-chloroperbenzoic acid in methylene chloride at 0° and then with a catalytic amount of perchloric acid at room temperature to afford *dl*-gliotoxin (**1**) (m.p. 165–166°, capillary) in 58% yield. The synthetic substance was found to be identical with natural gliotoxin[33] on comparison of spectroscopic (NMR, IR, UV, MS) and tlc data.

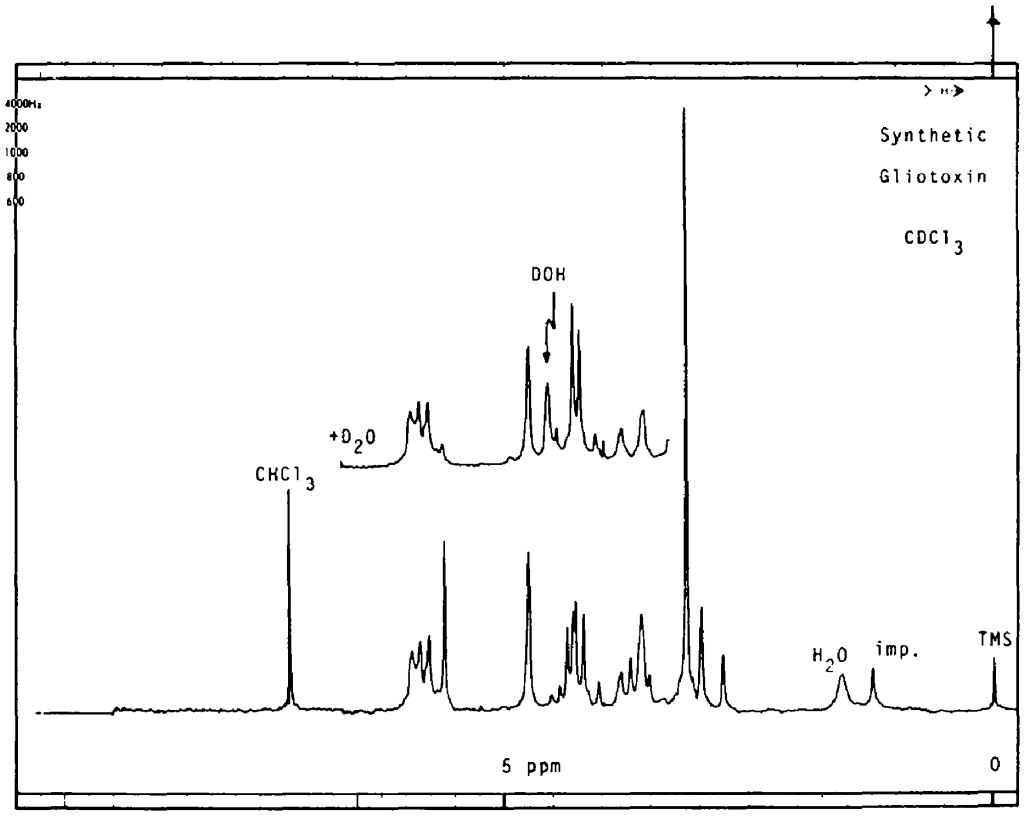
Exactly parallel results were obtained for the thioacetal **68a**, yielding totally synthetic *dl*-gliotoxin.

The NMR spectra of synthetic and natural gliotoxin are shown in Fig. 10.

SYNTHESIS OF OPTICALLY ACTIVE GLIOTOXIN

Having completed the synthesis of racemic gliotoxin, optical resolution of the thioacetal **68a** 71% yield. The diastereomers **84** and **85** could be readily separated by silica gel chromatography. Removal of the resolving agent was effected by pyrolysis of 230° under an argon atmosphere. Thus, optically active thioacetals **86** and **87** were obtained from the racemic thioacetal **68a** in 64% and 66% yields (based on each enantiomer in racemic **68a**), respectively.[51]

The absolute configurations of the thioacetals **86** and **87** were tentatively assigned by comparison of the circular dichroism (CD) spectra with that of the gliotoxin thioacetal **27a**, whose absolute configuration was known from an X-ray analysis of gliotoxin.[10] Since the CD band at the 230 nm region of gliotoxin was assigned as peptide n-π^* transitions,[52] the close resemblance of the CD band of **86** and **27a** would suggest that the


Synthetic
Gliotoxin
CDCl3
DOH
+D2O
CHCl3
H2O
imp.
TMS
5 ppm
0

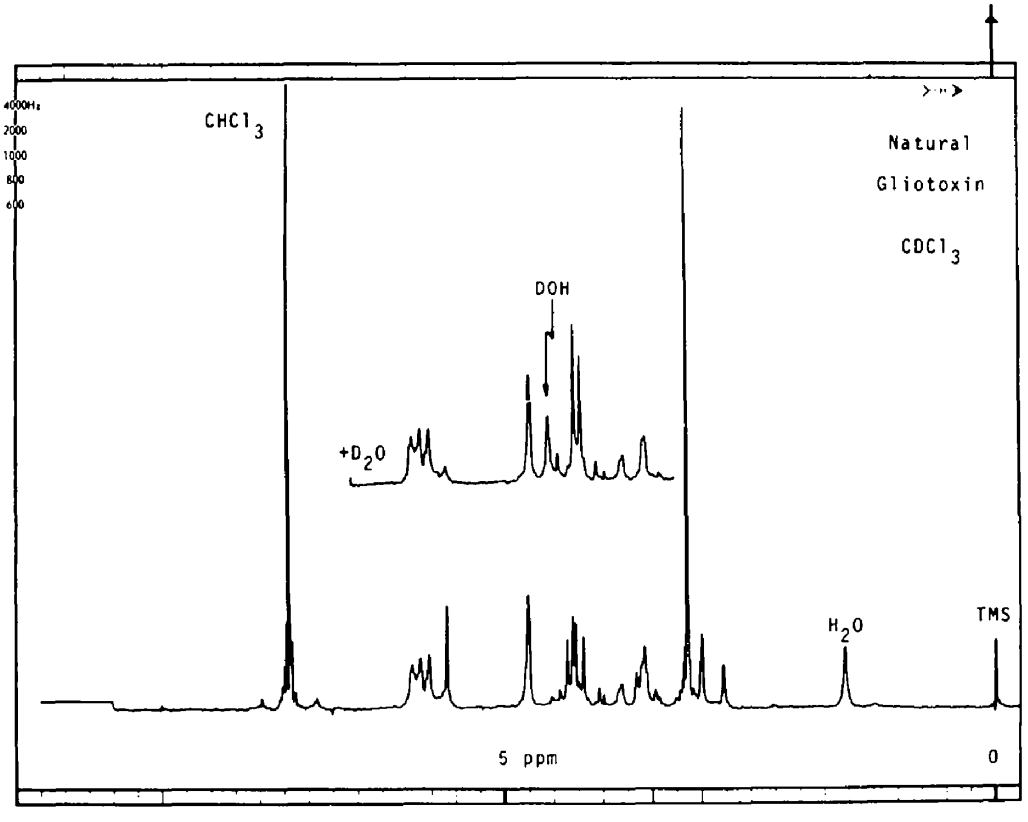
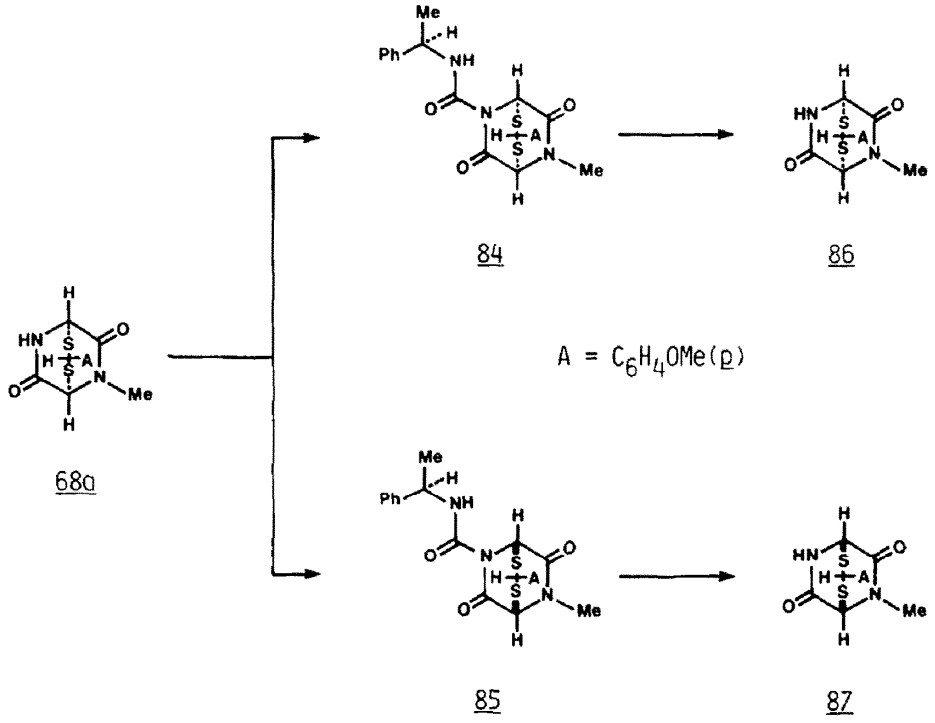

CHCl3
Natural
Gliotoxin
CDCl3
DOH
+D2O
H2O
TMS
5 ppm
0

Fig. 10.

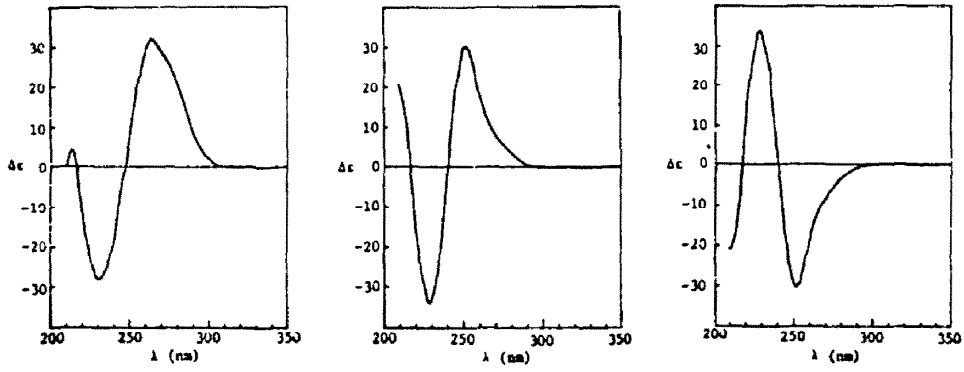


68a 84 85 86 87

Scheme 32.

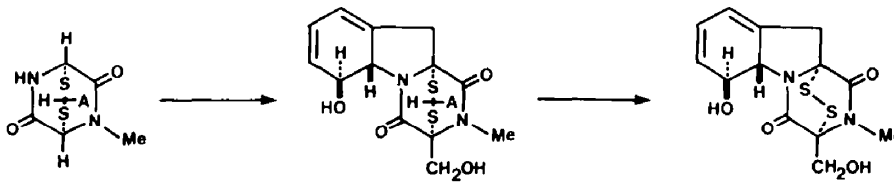
thioacetal **86** had the absolute configuration desired for the gliotoxin synthesis.

Using the optically active thioacetal **86** as starting material, the optically active gliotoxin thioacetal **27a** was prepared by following exactly the same procedure established for the total synthesis of *dl*-gliotoxin. The assignment of the absolute configuration for **86** was confirmed by the fact that both synthetic and natural gliotoxin thioacetal showed the same optical rotation. Conversion of the gliotoxin thioacetal **27a** into gliotoxin **1** was performed in the same manner as before. The



27a 86 87

Fig. 11.



Scheme 33.

totally synthetic substance was found to be identical with natural gliotoxin[33] on comparison of spectra (NMR, IR, UV, MS, α_D) and tlc data.

Acknowledgements—Financial support from the National Institutes of Health (NS 12108), National Science Foundation (CHE 78-06296) and the Hoffmann–La Roche Company is gratefully acknowledged.

EXPERIMENTAL

Melting points (m.p.), determined on a Kofler hot stage and boiling points (b.p.) are uncorrected. Infrared (IR) spectra were recorded on a Perkin–Elmer Model 727 spectrophotometer and are reported in wave numbers (cm^{-1}). Nuclear magnetic resonance (NMR) spectra were determined on a Varian HFT-80 instrument in the Fourier Transform mode. Chemical shifts are reported in parts per million downfield from tetramethylsilane (δ) as internal standard. Following abbreviations are used for spin multiplicity; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Mass spectra (MS) and high resolution mass spectra (Exact Mass) were determined on an AEI MS-9 double-focusing instrument at 70 eV using direct probe insertion at a temperature of 150–200°. Optical rotations were measured on a Perkin–Elmer Model 241 polarimeter at ambient temperature. Circular dichroism (CD) spectra were determined on a Cary Model 60 spectropolarimeter. Elemental analyses were performed by Analytical Laboratories, Meijo University, Nagoya, Japan. Analytical thin layer chromatography (tlc) was performed on either 0.25 mm pre-coated silica gel or aluminum oxide Type T plates supplied by E. Merck. Preparative TLC separations were made on plates (20 × 20 cm) prepared with a 2-mm layer of silica gel PF_{254} or aluminum oxide PF_{254} Type T both obtained from E. Merck. Column chromatography was performed on silica gel obtained from Kanto Chemical Co., Japan. Reagents and solvents were commercial grades and were used as supplied with the following exceptions: Methylene chloride: distilled. Ether (dry): distilled from sodium benzophenone ketyl. Tetrahydrofuran (dry): distilled from sodium benzophenone ketyl. *t*-Butanol (dry): distilled from sodium. Pyridine: dried over potassium hydroxide. Diisopropyl amine: distilled from sodium hydride. Dihydropyran: distilled. All alkylating or acylating agents were passed through a short aluminum oxide column prior to use. Lithium diisopropylamide (LDA) was prepared by dropwise addition of *n*-butyllithium in hexane to a stirred solution of diisopropylamine in tetrahydrofuran at 0° and was used after stirring for 5 min. All reactions sensitive to oxygen or moisture were conducted under an argon atmosphere.

Epidithiapiperazinedione syntheses (Method A)

Formaldehyde thioacetal **16**. A mixture of 200 mg (0.971 mmol) of dithiopiperazinedione **4**[22] (*cis* and *trans* mixture) and 1 ml of diiodomethane in 4 ml of pyridine was stirred at room temperature for 15 hr. The mixture was evaporated to a small volume under reduced pressure, diluted with methylene chloride and poured into a mixture of ice and concentrated hydrochloric acid. The aqueous layer was thoroughly extracted with methylene chloride. The combined extracts were washed with 10% hydrochloric acid, dried over anhydrous sodium sulfate, filtered, evaporated and chromatographed on a silica gel column (eluted with 1% methanol in methylene chloride) to give 109 mg (51.5%) of **16** as white crystals, m.p. 236–237° (methanol–ethyl acetate). IR (KBr): 1681, 1664. NMR ($CDCl_3$): 3.08 (6H, s), 3.90 (2H, s), 4.82 (2H, s). MS: 218 (43, M), 140 (100), 112 (38). Exact Mass: Found: 218.0184. Calc. for $C_7H_{10}N_2O_2S_2$: 218.0184. Analysis (recrystallized from methanol–ethyl acetate): Found: C, 38.45; H, 4.58; N, 12.86. Calc. for $C_7H_{10}N_2O_2S_2$: C, 38.52; H, 4.62; N, 12.83%.

By using the same procedure, the formaldehyde thioacetal **16** was obtained from pure *cis*-dithiopiperazinedione **4a**[22] or *trans*-dithiopiperazinedione **4b**[22] in comparable yield.

Monomethyl thioacetal **18**. A solution of 57.0 mg (0.261 mmol) of the thioacetal **16** in 10 ml of dry tetrahydrofuran was cooled in a dry ice–acetone bath. To the stirred solution was added dropwise 0.150 ml (1.2 eq) of 2.10 N *n*-butyllithium in hexane over a period of 1 min. After 30 sec, 85 μl (5 eq) of methyl iodide was added and stirring was continued for an additional 30 min. The solution was allowed to warm to room temperature and poured into a saturated sodium chloride solution. The aqueous layer was thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and separated on preparative silica gel tlc [eluted with ethyl acetate–hexane (7:3)] to give 33.1 mg (54.5%) of **18** as white crystals, m.p. 175–176° (ethyl acetate). IR (KBr): 1690, 1660. NMR ($CDCl_3$): 1.85 (3H, s), 3.09 (6H, s), 3.86 (2H, s), 4.91 (1H, s). MS: 232 (1, M), 186 (5), 154 (100), 126 (56). Exact Mass: Found: 232.0342. Calc. for $C_8H_{12}N_2O_2S_2$: 232.0340.

Ditetrahydropyranyl derivative **19**. A mixture of 200 mg (0.971 mmol) of *cis*-dithiopiperazinedione **4a**,[22] 3 ml of dihydropyran and 50 mg of *dl*-camphorsulfonic acid in 50 ml of benzene was heated under reflux for 2 h. After cooling, the mixture was washed with a saturated sodium bicarbonate solution. The aqueous layer was thoroughly extracted with methylene chloride. The combined organic extracts were dried over anhydrous sodium sulfate, filtered, evaporated and chromatographed on a silica gel column (eluted with 0.5% methanol in methylene chloride) to afford 188 mg (51.7%) of **19** as white crystals, m.p. 163–165° (ethyl acetate). IR (KBr): 1680. MS: 374 (<1, M), 290 (10), 174 (86), 141 (90), 140 (58), 85 (100). Exact Mass: Found: 374.1343. Calc. for $C_{16}H_{26}N_2O_4S_2$: 374.1334.

Iodine oxidation of **19**. To a stirred solution of 100 mg (0.267 mmol) of ditetrahydropyranyl derivative **19** in 10 ml of methylene chloride was added dropwise a methylene chloride solution of iodine (1 g in 20 ml) at

room temperature until **19** completely disappeared (monitored by silica gel tlc by using 5% methanol in methylene chloride as the eluent). The reaction mixture was evaporated to dryness under reduced pressure. The crystalline residue was crystallized from methanol to afford 39.2 mg (72%) of **5**, m.p. 180–183° (lit.[22] 185° dec.), as pale yellow crystals.

p-Anisaldehyde thioacetal **25c**. To a stirred solution of 100 mg (0.486 mmol) of dithiopiperazinedione **4**[22] (*cis* and *trans* mixture) and 0.3 ml of *p*-anisaldehyde in 10 ml of methylene chloride was added 50 μl of boron trifluoride etherate. After stirring at room temperature for 12 hr, the solution was poured into a saturated sodium bicarbonate solution. The aqueous layer was thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered and evaporated to give crystals which were collected with ether to yield 130 mg (82.6%) of **25c**, m.p. 267–268° (ethyl acetate).
IR (KBr): 1680, 1608, 1510. NMR ($CDCl_3$): 3.05 (3H, s), 3.18 (3H, s), 3.79 (3H, s), 4.84 (1H, s), 5.00 (1H, s), 5.13 (1H, s), 6.82 (2H, d, J = 9 Hz), 7.31 (2H, d, J = 9 Hz). MS: 324 (2, M), 260 (3), 140 (100), 112 (13). Exact Mass: Found: 324.0593. Calc. for $C_{14}H_{16}N_2O_3S_2$: 324.0602. Analysis (recrystallized from methylene chloride–ether): Found: C, 51.64; H, 4.86; N, 8.53. Calc. for $C_{14}H_{16}N_2O_3S_2$: C, 51.83; H, 4.97; N, 8.64%.

By using the same procedure, the *p*-anisaldehyde thioacetal **25c** was obtained from pure *cis*-dithiopiperazinedione **4a**[22] or *trans*-dithiopiperazinedione **4b**[22] in comparable yield.

By using the same procedure, the thioacetals **25a** and **25b** were synthesized.

Acetaldehyde thioacetal **25a**. Yield: 73.2%. M.P. 186–188° (capillary, methanol). IR (KBr): 1670. NMR ($CDCl_3$): 1.55 (3H, d, J = 7.5 Hz), 3.01 (3H, s), 3.11 (3H, s), 4.26 (1H, q, J = 7.5 Hz), 4.77 (1H, s), 4.94 (1H, s). MS: 232 (15, M), 140 (100), 112 (22). Exact Mass: Found: 232.0338. Calc. for $C_8H_{12}N_2O_2S_2$: 232.0340. Analysis (recrystallized from methanol): Found: C, 41.22; H, 5.14; N, 12.08. Calc. for $C_8H_{12}N_2O_2S_2$: C, 41.36; H, 5.21; N, 12.06%.

Benzaldehyde thioacetal **25b**. Yield: 92.4%. M.P. 272–273° (capillary, ethyl acetate). IR (KBr): 1680. NMR ($CDCl_3$): 3.05 (3H, s), 3.19 (3H, s), 4.85 (1H, s), 5.01 (1H, s), 5.16 (1H, s), 7.35 (5H, m). MS: 294 (1, M), 230 (17), 140 (100), 112 (14). Exact Mass: Found: 294.0492. Calc. for $C_{13}H_{14}N_2O_2S_2$: 294.0497. Analysis (recrystallized from methylene chloride–ether): Found: C, 52.79; H, 4.66; N, 9.39. Calc. for $C_{13}H_{14}N_2O_2S_2$: C, 53.04; H, 4.79; N, 9.52%.

Monomethyl thioacetal **28a**. A solution of 300 mg (0.926 mmol) of the thioacetal **25c** in 36 ml of dry tetrahydrofuran was cooled in a dry ice–acetone bath. To the stirred solution was added dropwise 0.463 ml (1.1 eq) of 2.20 N *n*-butyllithium in hexane over a period of 1 min. After 30 sec, 0.276 ml (5 eq) of methyl iodide was added and stirring was continued for an additional 5 min. The cooling bath was removed and the solution was allowed to warm to room temperature. The mixture was poured into a saturated sodium chloride solution and the aqueous layer was thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and separated on preparative silica gel tlc [eluted with ethyl acetate–hexane (1:1)] to give 196 mg (62.6%) of **28a** as white crystals, m.p. 205–206° (ethyl acetate). IR (KBr): 1690, 1679, 1603, 1509. NMR ($CDCl_3$): 1.87 (3H, s), 3.07 (3H, s), 3.18 (3H, s), 3.79 (3H, s), 5.07 (1H, s), 5.10 (1H, s), 6.81 (2H, d, J = 9 Hz), 7.28 (2H, d, J = 9 Hz). MS: 338 (5, M), 154 (100), 126 (26). Exact Mass: Found: 338.0753. Calc. for $C_{15}H_{18}N_2O_2S_2$: 338.0759. Analysis (recrystallized from methanol): Found: C, 53.17; H, 5.32; N, 8.22. Calc. for $C_{15}H_{18}N_2O_3S_2$: C, 53.23; H, 5.36; N, 8.28%.

The mono-substituted thioacetals **28** were synthesized by using the same procedure as the one given for **28a**.[39]

Monobenzyl thioacetal **28b**. Reagent: $C_6H_5CH_2Br$ (3 eq). Yield: 65.5%. M.P.: 268–270° (chloroform–methanol). IR (KBr): 1680, 1603, 1510. NMR ($CDCl_3$): 3.10 (3H, s), 3.13 (3H, s), 3.22 (1H, AB, J = 17 Hz), 3.80 (3H, s), 4.10 (1H, AB, J = 17 Hz), 5.08 (1H, s), 5.15 (1H, s), 6.83 (2H, d, J = 9 Hz), 7.15 (5H, m), 7.30 (2H, d, J = 9 Hz). MS: 414 (4, M), 230 (100), 202 (9), 91 (26). Exact Mass: Found: 414.1070. Calc. for $C_{21}H_{22}N_2O_3S_2$: 414.1072. Analysis (recrystallized from chloroform–methanol): Found: C, 58.27; H, 5.14; N, 6.37. Calc. for $C_{21}H_{22}N_2O_3S_2 \cdot H_2O$: C, 58.31; H, 5.59; N, 6.48%.

Monoethyl thioacetal **28c**. Reagent: C_2H_5I (5 eq). Yield: 60.2% M.P.: 202–203° (ethyl acetate). IR (KBr): 1680, 1602, 1503. NMR ($CDCl_3$): 1.00 (3H, t, J = 7 Hz), 1.90 (1H, m), 2.50 (1H, m), 3.07 (3H, s), 3.19 (3H, s), 3.79 (3H, s), 5.01 (1H, s), 5.08 (1H, s), 6.81 (2H, d, J = 9 Hz), 7.27 (2H, d, J = 9 Hz). MS: 352 (5, M), 168 (100), 140 (8). Exact Mass: Found: 352.0909. Calc. for $C_{16}H_{20}N_2O_3S_2$: 352.0915. Analysis (recrystallized from methanol): Found: C, 54.29; H, 5.80; N, 7.83. Calc. for $C_{16}H_{20}N_2O_3S_2$: C, 54.52; H, 5.72; N, 7.95%.

Benzyl methyl thioacetal **29a** *from* **28a**. A solution of 108 mg (0.319 mmol) of the monomethyl thioacetal **28a** and 0.114 ml (3 eq) of benzyl bromide in 11 ml of dry tetrahydrofuran was cooled in a dry ice–acetone bath. To the stirred solution was added dropwise 0.198 ml (1.3 e) of 2.10 N *n*-butyllithium in hexane over a period of 15 min. Stirring was continued for an additional 5 min and the solution was allowed to warm to room temperature. The mixture was poured into a saturated sodium chloride solution and thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and separated on preparative silica gel tlc [eluted with ethyl acetate–hexane (2:3)] to give 107 mg (78.3%) of **29a** as white crystals, m.p. 219–110° (methanol–ethyl acetate). IR (KBr): 1679, 1604, 1511. NMR ($CDCl_3$): 1.94 (3H, s), 2.94 (3H, s), 3.10 (1H, AB, J = 14 Hz), 3.31 (3H, s), 3.80 (3H, s), 4.36 (1H, AB, J = 14 Hz), 5.10 (1H, s), 6.81 (2H, d, J = 9 Hz), 6.95–7.35 (7H, m). MS: 428 (<1, M), 244 (100), 216 (14), 91 (23). Exact Mass: Found: 428.1223. Calc. for $C_{22}H_{24}N_2O_3S_2$: 428.1228. Analysis (recrystallized from methanol–ethyl acetate): Found: C, 61.73; H, 5.54; N, 6.51. Calc. for $C_{22}H_{24}N_2O_3S_2$: C, 61.66; H, 5.64; N, 6.54%.

The di-substituted thioacetals **29** were synthesized by using the same procedure as the one given for **29a**.[39]

Benzyl methyl thioacetal **29b** from **28b**. Reagent: CH_3I (5 eq). Yield: 76.0%. M.P.: 231–232° (methylene chloride–ether). IR (KBr): 1681, 1670, 1602, 1510. NMR ($CDCl_3$): 1.97 (3H, s), 3.15 (6H, s), 3.24 (1H, AB, J = 14 Hz), 3.78 (3H, s), 4.16 (1H, AB, J = 14 Hz), 5.04 (1H, s), 6.81 (2H, d, J = 9 Hz), 6.95–7.35 (7H, m). MS: 428 (<1, M), 244 (100), 216 (13), 91 (21). Exact Mass: Found: 428.1223. Calc. for $C_{22}H_{24}N_2O_3S_2$: 428.1228. Analysis (recrystallized from methylene chloride–ether): Found: C, 61.53; H, 5.52; N, 6.43. Calc. for $C_{22}H_{24}N_2O_3S_2$: C, 61.66; H, 5.64; N, 6.54%.

Dimethyl thioacetal **29c** (*one-pot procedure*). A solution of 100 mg (0.309 mmol) of the thioacetal **25c** and 0.184 ml (10 eq) of methyl iodide in 12 ml of dry tetrahydrofuran was cooled in a dry ice–acetone bath. To the stirred solution was added dropwise 0.338 ml (2.3 eq) of 2.10 N *n*-butyllithium in hexane over a period of 10 min. The progress of the reaction was carefully monitored by tlc. After stirring for an additional 5 min, the cooling bath was removed and the solution was allowed to warm to room temperature. The mixture was then poured into a saturated sodium chloride solution and the aqueous layer was thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and separated on preparative silica gel tlc [eluted with ethyl acetate–hexane (1:1)] to give 88.0 mg (81.0%) of **29c** as white crystals, m.p. 190–191° (methanol). IR (KBr): 1679, 1606, 1510. NMR ($CDCl_3$):

1.89 (3H, s), 1.91 (3H, s), 3.09 (3H, s), 3.11 (3H, s), 3.76 (3H, s), 5.01 (1H, s), 6.78 (2H, d, J = 9 Hz), 7.24 (2H, d, J = 9 Hz). MS: 352 (4, M), 168 (100), 140 (17). Exact Mass: Found: 352.0906. Calc. for $C_{16}H_{20}N_2O_3S_2$: 352.0915. Analysis (recrystallized from methanol): Found: C, 54.34; H, 5.73; N, 7.83. Calc. for $C_{16}H_{20}N_2O_3S_2$: C, 54.52; H, 5.72; N, 7.95%.

Monomethyl epidithiapiperazinedione **30a**. To an ice-cooled stirred solution of 50 mg (0.148 mmol) of the monomethyl thioacetal **28a** in 5 ml of methylene chloride was added 36 mg (85%, 1.2 eq) of *m*-chloroperbenzoic acid in one portion. After stirring for 10 min, the solution was poured into a saturated sodium bicarbonate solution containing sodium thiosulfate and thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered and evaporated.

The resulting monosulfoxide was dissolved in 10 ml of methylene chloride and treated with a solution of 12.5 μl (1 eq) of 70% perchloric acid in 0.5 ml of tetrahydrofuran. The solution was stirred at room temperature for 4 hr and then poured into a saturated sodium bicarbonate solution. The aqueous layer was thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and chromatographed on a silica gel column (eluted with 1% methanol in methylene chloride) to give 18.0 mg (55.8%) of **30a** as pale yellow crystals, m.p. 127–128° (methanol). IR (KBr): 1688, 1671. NMR ($CDCl_3$): 1.98 (3H, s), 3.03 (3H, s), 3.13 (3H, s), 5.26 (1H, s). MS: 218 (9, M), 154 (100), 126 (47). Exact Mass: Found: 218.0189. Calc. for $C_7H_{10}N_2O_2S_2$: 218.0184. Analysis (recrystallized from methanol): Found: C, 38.47; H, 4.67; N, 12.85. Calc. for $C_7H_{10}N_2O_2S_2$: C, 38.52; H, 4.62; N, 12.83%.

The mono-substituted and di-substituted epidithiapiperazinediones **30** and **31** were synthesized by using the same procedure as the one given for **30a**.[39] For the m.ps of some of the compounds (see Table 1).

Epidithiapiperazinedions syntheses (*Method* B)

cis-*Dimethyl dimethylthiopiperazinedione* **40**. A solution of 292 mg (1.25 mmol) of dimethylthiopiperazinedione **38**[24] in 20 ml of dry tetrahydrofuran was cooled in a dry ice–acetone bath. To the stirred solution was added dropwise 1.307 ml (2.2 eq) of 2.10 N *n*-butyllithium in hexane over a period of 1 min. After 1 min, 0.777 ml (10 eq) of methyl iodide was added. The solution was stirred for an additional 5 min and allowed to warm to room temperature. The mixture was poured into a saturated sodium chloride solution and the aqueous layer was thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and separated on preparative silica gel tlc [eluted with ethyl acetate–hexane (1:1)] to give 275 mg (84.1%) of **40** as a colorless oil. IR (CH_2Cl_2): 1658, 1375. NMR ($CDCl_3$): 1.85 (6H, s), 2.21 (6H, s), 3.12 (6H, s). MS: 262 (1, M), 215 (86), 168 (100), 140 (86). Exact Mass: Found: 215.0853. Calc. for $C_9H_{15}N_2O_2S$ (M–SCH_3): 215.0854.

Dimethoxymethylthiopiperazinedione **44**. To an ice-cold stirred solution of 500 mg (2.43 mmol) of *cis*-dithiopiperazinedione **4a**[22] in 100 ml of dry tetrahydrofuran was added 4.64 ml (2.1 eq) of 1.10 N potassium *t*-butoxide in *t*-butanol over a 1 min period. After 1 min, 2.3 ml of chloromethyl methyl ether was added and stirring was continued for an additional 40 min. The mixture was poured into water and the aqueous layer was thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered and evaporated to give white crystals which were collected with chilled ether to yield 574 mg (80.4%) of **44**, m.p. 141–142° (ethyl acetate). IR (KBr): 1678, 1075. NMR ($CDCl_3$): 3.04 (6H, s), 3.44 (6H, s), 4.50 (2H, AB, J = 12 Hz), 4.83 (2H, s), 5.22 (2H, AB, J = 12 Hz). MS: 294 (1, M), 218 (46), 217 (54), 171 (100). Exact Mass: Found: 294.0705. Calc. for $C_{10}H_{18}N_2O_4S_2$: 294.0708. Analysis (recrystallized from methanol): Found: C, 40.76; H, 6.37; N, 9.45. Calc. for $C_{10}H_{18}N_2O_4S_2$: C, 40.80; H, 6.16; N, 9.52%.

Dibenzylthiopiperazinedione **41**. This substance was prepared from **4a** by using the same procedure as the one given for **44**. Reagent: $C_6H_5CH_2Br$. Yield: 63.6%. M.P.: 134–135° (methanol). IR (KBr): 1679. NMR ($CDCl_3$): 2.65 (6H, s), 3.90 (2H, AB, J = 14 Hz), 3.98 (2H, AB, J = 14 Hz), 4.33 (2H, s), 7.15–7.5 (10H, m). MS: 386 (<1, M), 264 (11), 141 (53), 91 (100). Exact Mass: Found: 386.1123. Calc. for $C_{20}H_{22}N_2O_2S_2$: 386.1123. Analysis (recrystallized from methanol): Found: C, 62.02; H, 5.79; N, 7.26. Calc. for $C_{20}H_{22}N_2O_2O_2$: C, 62.15; H, 5.74; N, 7.25%.

Dibenzyl dibenzylthiopiperazinediones **42** *and* **43**. A solution of 33.7 mg (0.087 mmol) of dibenzylthiopiperazinedione **41** in 3.4 ml of dry tetrahydrofuran was cooled in dry ice–acetone bath. To the stirred solution was added dropwise 0.109 ml (2.1 eq) of 1.68 N *n*-butyllithium in hexane over a period of 1 min. After 1 min, 55 μl of benzyl bromide was added and stirring was continued for 5 min. The solution was allowed to warm to room temperature and poured into water. The aqueous layer was thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and separated on preparative alumina tlc [developed twice with hexane–methylene chloride (3:2)] to give 17.5 mg (35.4%) of the *cis* product **43**, m.p. 122–123° (methanol) and 14.1 mg (28.5%) of the *trans* product **42**, m.p. 165–166° (ethyl acetate–methanol).

cis-*Dibenzyl dibenzylthiopiperazinedione* **43**. IR (KBr): 1661, 1642, 1602, 1581, 1496. NMR ($CDCl_3$): 3.00 (2H, AB, J = 14.5 Hz), 3.16 (6H, s), 3.47 (2H, AB, J = 14.5 Hz), 3.98 (4H, s), 6.7–7.35 (20H, m). Analysis (recrystallized from methanol): Found: C, 71.96; H, 6.08; N, 4.86. Calc. for $C_{34}H_{34}N_2O_2S_2$: C, 72.05; H, 6.05; N, 4.94%.

trans-*Dibenzyl dibenzylthiopiperazinedione* **42**. IR (KBr): 1655, 1602, 1495. NMR ($CDCl_3$): 2.95 (2H, AB, J = 12 Hz), 3.03 (2H, AB, J = 14 Hz), 3.10 (2H, AB, J = 12 Hz), 3.27 (6H, s), 3.62 (2H, AB, J = 14 Hz), 6.75–7.30 (20H, m). Analysis (recrystallized from ethyl acetate–methanol): Found: C, 71.95; H, 6.14; N, 4.86. Calc. for $C_{34}H_{34}N_2O_2S_2$: C, 72.05; H, 6.05; N, 4.94%.

Dibenzyl dimethoxymethylthiopiperazinedione **45a**. A solution of 300 mg (1.02 mmol) of dimethoxymethylthiopiperazinedione **44** in 21 ml of dry tetrahydrofuran was cooled in a dry ice–acetone bath. A solution of 2.34 mmol (2.3 eq) of lithium diisopropylamide in 1.1 ml of tetrahydrofuran was added dropwise with stirring over a 2 min period. After 1 min, 1.21 ml (10 eq) of benzyl bromide was added and stirring was continued for 1 hr. The solution was then allowed to warm to room temperature and poured into a saturated sodium chloride solution. The aqueous layer was thoroughly extracted with chloroform. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and chromatographed on a silica gel column (eluted with 0.5% methanol in methylene chloride) to give 406 mg (83.9%) of **45a** as white crystals, m.p. 106–107° (methylene chloride–hexane). IR (KBr): 1661, 1080. NMR ($CDCl_3$): 2.97 (2H, AB, J = 14 Hz), 3.06 (6H, s), 3.12 (2H, AB, J = 14 Hz), 3.34 (6H, s), 4.45 (2H, AB, J = 12 Hz), 5.20 (2H, AB, J = 12 Hz), 6.80–7.25 (10H, m). MS: 397 (89, M–SCH_2OCH_3), 320 (68), 91 (100). Exact Mass: Found: 397.1598. Calc. for $C_{22}H_{25}N_2O_3S$ (M–SCH_2OCH_3): 397.1586. Analysis (recrystallized from methylene chloride–hexane): Found: C, 60.51; H, 6.31; N, 5.78. Calc. for $C_{24}H_{30}N_2O_4S_2$: C, 60.73; H, 6.37; N, 5.90%.

The symmetrically di-substituted piperazinediones were synthesized by using the same procedure as the one given for **45a**. Some of them are listed in Table 1.

Monomethyl dimethoxymethylthiopiperazinedione **47a**. A solution of 200 mg (0.680 mmol) of dimethoxymethylthiopiperazinedione **44** in 14 ml of dry tetrahydro-

furan was cooled in a dry ice–acetone bath. A solution of 1.56 mmol (2.3 eq) of lithium diisopropylamide in 1 ml of tetrahydrofuran was added dropwise with stirring over a period of 2 min. After 1 min, 0.6 ml (6.5 mmol) of methyl iodide was added and stirring was continued for 2 hr. The solution was treated with 0.1 ml of acetic acid and then allowed to warm to room temperature. The mixture was poured into a saturated sodium bicarbonate solution and the aqueous layer was thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and chromatographed on a silica gel column (eluted with 2% methanol in methylene chloride) to give 200 mg (95.4%) of **47a** as white crystals, m.p. 78–79° (methylene chloride–hexane). IR (KBr): 1660, 1081. NMR ($CDCl_3$): 1.94 (3H, s), 3.05 (3H, s), 3.10 (3H, s), 3.34 (3H, s), 3.45 (3H, s), 4.48 (2H, AB, J = 12 Hz), 4.91 (1H, s), 5.06 (1H, AB, J = 12 Hz), 5.29 (1H, AB, J = 12 Hz). MS: 308 (<1, M), 231 (72), 154 (49), 153 (84), 56 (100). Exact Mass: Found: 231.0803. Calc. for $C_9H_{15}N_2O_3S$ ($M-SCH_2OCH_3$): 231.0803. Analysis (recrystallized from methylene chloride–hexane): Found: C, 42.85; H, 6.66; N, 9.20. Calc. for $C_{11}H_{20}N_2O_4S_2$: C, 42.84; H, 6.54; N, 9.08%.

The mono-substituted piperazinediones **47** were synthesized by using the same procedure as the one given for **47a**. Some of them are listed in Table 1.

Benzyl methyl dimethoxymethylthiopiperazinedione **49a**. A solution of 200 mg (0.649 mmol) of monomethyl dimethoxymethylthiopiperazinedione **47a** in 10 ml of dry tetrahydrofuran was cooled in a dry ice–acetone bath. A solution of 0.779 mmol (1.2 eq) of lithium di-isopropylamide in 0.4 ml of tetrahydrofuran was added dropwise with stirring over a 2 min period. After 1 min, 0.39 ml (5 eq) of benzyl bromide was added and stirring was continued for 2 hr. The solution was then allowed to warm to room temperature and poured into a saturated sodium chloride solution. The aqueous layer was thoroughly extracted with chloroform. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and separated on preparative silica gel tlc (eluted with 1.5% methanol in methylene chloride) to give 194 mg (75.0%) of **49a** as white crystals, m.p. 117–118° (methylene chloride–hexane). IR (KBr): 1660, 1648, 1080. NMR ($CDCl_3$): 0.80 (3H, s), 2.79 (3H, s), 3.14 (1H, AB, J = 13.5 Hz), 3.27 (6H, s), 3.36 (3H, s), 3.49 (1H, AB, J = 13.5 Hz), 4.33 (1 H, AB, J = 12 Hz), 4.49 (1H, AB, J = 12 Hz), 5.05 (1H, AB, J = 12 Hz), 5.23 (1H, AB, J = 12 Hz), 6.90–7.30 (5H, m). MS: 321 (34, $M-SCH_2OCH_3$), 244 (29), 91 (85), 56 (100). Exact Mass: Found: 321.1273. Calc. for $C_{16}H_{21}N_2O_3S$ ($M-SCH_2OCH_3$): 321.1273. Analysis (recrystallized from methylene chloride–hexane): Found: C, 54.20; H, 6.56; N, 6.97. Calc. for $C_{18}H_{26}N_2O_4S_2$: C, 54.25; H, 6.58; N, 7.03%.

The unsymmetrically di-substituted piperazinediones were synthesized by using the same procedure as the one given for **49a**. Some of them are listed in Table 1.

Dimethyl epidithiapiperazinedione **46b**. To an ice-cooled solution of 250 mg (0.776 mmol) of dimethyl dimethoxymethylthiopiperazinedione **45b** in 12.5 ml of methylene chloride was added 1.25 ml of boron trichloride with stirring. After 5 min, the solution was evaporated to dryness. The residue was dissolved in 25 ml of 10% methanol in methylene chloride and treated with a solution of 197 mg (1 eq) of iodine in 4 ml of chloroform. The solution was allowed to stand at room temperature for 5 min and then poured into a saturated sodium bicarbonate solution containing sodium thiosulfate. The aqueous layer was thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and chromatographed on a silica gel column (eluted with 0.5% methanol in methylene chloride) to give 159 mg (88.2%) of **46b** as pale yellow crystals, m.p. 143–145° (methanol). IR (KBr): 1680. NMR ($CDCl_3$): 2.00 (6H, s), 3.06 (6H, s). MS: 232 (1, M), 168 (77), 56 (100). Exact Mass: Found: 232.0342. Calc. for $C_8H_{12}N_2O_2S_2$: 232.0340. Analysis (recrystallized from methanol): Found: C, 41.30; H, 5.29; N, 12.18. Calc. for $C_8H_{12}N_2O_2S_2$: C, 41.36; H, 5.21; N, 12.06%.

The synthesis of the symmetrically di-substituted, unsymmetrically di-substituted and mono-substituted dithiopiperazinediones **46**, **48** and **50** were synthesized by using the same procedure as the one given for **46b**. Some of them are listed in Table 1.

Benzyl methoxymethyl dimethoxymethylthiopiperazinedione **51** (*one-pot procedure*). A solution of 300 mg (1.02 mmol) of dimethoxymethylthiopiperazinedione **44** in 21 ml of dry tetrahydrofuran was cooled in a dry ice–acetone bath. A solution of 2.34 mmol (2.3 eq) of lithium diisopropylamide in 1.1 ml of tetrahydrofuran

Table 1.

	44 → **45**, **47**, or **49**			→ **46**, **48**, or **50**	
compound	alkyl halide	yield	mp	yield	mp
46a. $R^1=R^2=CH_2C_6H_5$	$C_6H_5CH_2Br$	84%	106–107°	82%	152–153°
46b: $R^1=R^2=CH_3$	CH_3I	41%	92–93°	88%	143–145°
46c. $R^1=R^2=CH_2CH_3$	CH_3CH_2I	31%	95–96°	75%	113–114°
46d: $R^1=R^2=CH_2CO_2Et$	EtO_2CCH_2Br	65%	85–86°	51%	179–180°
48a: $R^1=CH_3$	1. CH_3I				
$R^2=H$	2. (AcOH)	95%	78–79°	96%	127–128°
49b $R^1=CH_2C_6H_5$	1. $C_6H_5CH_2Br$				
$R^2=H$	2. (AcOH)	59%	86–87°	89%	175–176°
50a: $R^1=CH_3$	1. CH_3I				
$R^2=CH_2C_6H_5$	2. $C_6H_5CH_2Br$	75%	117–118°	91%	129–130°
50b: $R^1=CH_2C_6H_5$	1. $C_6H_5CH_2Br$				
$R^2=CH_2OH$	2. CH_3OCH_2Br	63%	oil	28%[34]	101–102°

was added dropwise with stirring over a 2 min period. After 1 min, 0.61 ml (5 eq) of benzyl bromide was added and stirring was continued for 20 min. The solution was then treated with 0.33 ml (4 eq) of bromomethyl methyl ether and stirring was continued for an additional 10 min. The mixture was allowed to warm to room temperature and poured into a saturated sodium chloride solution. The aqueous layer was thoroughly extracted with chloroform. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and separated on preparative silica gel tlc [eluted with ethyl acetate–hexane (2:3)] to give 275 mg (62.9%) of **51** as an oil. IR (CH_2Cl_2): 1660, 1080. NMR ($CDCl_3$): 2.90 (3H, s), 3.04 (3H, s), 3.19 (3H, s), 3.19 (1H, AB, J = 14 Hz), 3.31 (3H, s), 3.39 (3H, s), 3.49 (1H, AB, J = 10 Hz), 3.53 (1H, AB, J = 10 Hz), 3.84 (1H, AB, J = 14 Hz), 4.40 (1H, AB, J = 12 Hz), 4.55 (1H, AB, J = 12 Hz), 5.34 (2H, AB, J = 12 Hz), 6.9–7.35 (5H, m). MS: 351 (100, M–SCH_2OCH_3), 274 (28), 273 (43), 91 (56). Exact Mass: Found: 351.1382. Calc. for $C_{17}H_{23}N_2O_4S$ (M–SCH_2OCH_3): 351.1378.

dl-*Hyalodendrin* **52**. A solution of 430 mg (1.00 mmol) of benzyl methoxymethyl dimethoxymethylthiopiperazinedione **51** in 15 ml of methylene chloride was cooled in a dry ice–acetone bath. To the stirred solution was added 0.5 ml of boron trichloride. After stirring at −78° for 20 min, the solution was allowed to warm to room temperature and evaporated to dryness. The residue was dissolved in 43 ml of 10% methanol in methylene chloride. The solution was treated with a solution of 255 mg (1 eq) of iodine in 5 ml of chloroform and allowed to stand at room temperature for 5 min. The mixture was poured into a saturated sodium bicarbonate solution containing sodium thiosulfate and thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered and evaporated.

The residue was dissolved in 15 ml of methylene chloride and cooled in an ice bath. The solution was treated with 0.1 ml of boron trichloride and allowed to stand at 0° for 15 min. The solution was evaporated and separated on preparative silica gel tlc (developed twice with 2% methanol in methylene chloride) to give 91.0 mg (27.9%) of **52** as pale yellow crystals, m.p. 101–102° (ethanol). IR (KBr): 3350, 1680, 1648. NMR ($CDCl_3$): 2.98 (3H, s), 3.20 (3H, s), 3.48 (1H, t, J = 7 Hz), 3.63 (1H, AB, J = 14 Hz), 4.05 (1H, AB, J = 14 Hz), 4.32 (2H, d, J = 7 Hz), 7.27 (5H, s). MS: 324 (2, M), 260 (100), 91 (29). Exact Mass: Found: 324.0593. Calc. for $C_{14}H_{16}N_2O_3S_2$: 324.0602. Analysis (recrystallized from ethanol): Found: C, 51.78; H, 4.81; N, 8.74. Calc. for $C_{14}H_{16}N_2O_3S_2$: C, 51.83; H, 4.97; N, 8.64%.

Synthesis of dehydrogliotoxin

Methyl ester piperazinedione **56**. A mixture of 6.275 g (22.6 mmol) of 2-iodo-3-methoxybenzoic acid (**54**),[46] 5.78 g (45.1 mmol) of glycine–sarcosine anhydride (**55**),[47] 4.30 g (22.6 mmol) of cuprous iodide and 6.23 g (45.1 mmol) of anhydrous potassium carbonate in 63 ml of nitrobenzene was heated at 170° for 40 min with vigorous stirring. After cooling, the solvent was removed by decantation. The residue was dissolved in methanol and the pH of the solution was adjusted to 3 with concentrated hydrochloric acid. The solution was filtered and evaporated to dryness. The residue was then dissolved in 300 ml of methanol. A solution of diazomethane in ether was added at room temperature until the esterification had been complete. The mixture was concentrated and chromatographed on a silica gel column (eluted with 2% methanol in methylene chloride) to give 2.34 g (35.5%, based on **54**) of **56** as white crystals, m.p. 140–141° (ethyl acetate). IR (KBr): 1718, 1679, 1662, 1581. NMR ($CDCl_3$): 3.04 (3H, s), 3.85 (6H, s), 4.06 (2H, s), 4.22 (2H, s), 7.00–7.65 (3H, m). MS: 292 (100, M), 261 (16). Exact Mass: Found: 292.1070. Calc. for $C_{14}H_{16}N_2O_5$: 292.1059.

Dithioacetate **57**

A mixture of 2.278 g (7.80 mmol) of the piperazinedione **56**, 3.88 g (2.8 eq) of N-bromosuccinimide and 116 mg of benzoyl peroxide in 230 ml of carbon tetrachloride was heated under reflux for 90 min. After cooling, succinimide was filtered and washed with carbon tetrachloride. The filtrate and the washings were combined and the solvent was removed.

The residue was dissolved in 140 ml of methylene chloride and treated with 6.8 g of potassium thioacetate. The mixture was stirred overnight at room temperature. The salts were filtered and washed with methylene chloride. The filtrate and the washings were combined, concentrated and chromatographed on a silica gel column eluted with 1% methanol in methylene chloride) to give 2.23 g (65.0%) of **57** as a 1:1 *cis* and *trans* mixture, m.p. 168–175° (benzene). IR (KBr): 1698, 1580. MS: 440 (3, M), 397 (24), 365 (100), 323 (77), 295 (79), 291 (54). Exact Mass: Found: 440.0705. Calc. for $C_{18}H_{20}N_2O_7S_2$: 440.0712.

Thioacetal ester **58**. To a solution of 2.20 g (5 mmol) of the dithioacetate **57** in 200 ml of methanol was added 20 ml of HCl in methanol (saturated at 0°). The mixture was heated at 50° for 40 min and then the solvent was removed. The residue was dissolved in 110 ml of methylene chloride and treated with 3.3 ml of *p*-anisaldehyde and 0.22 ml of boron trifluoride etherate. The mixture was allowed to stand overnight at room temperature and then poured into a saturated sodium bicarbonate solution. The aqueous layer was thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and chromatographed on a silica gel column (eluted with 0.5% methanol in methylene chloride) to give 1.71 g (72.0%) of **58** as an about 1:1 diastereomeric mixture, m.p. 222–225° (benzene). IR (KBr): 1719, 1685, 1602, 1580, 1508. MS: 474 (<1, M), 410 (14), 290 (100), 262 (17). Exact Mass: Found: 474.0927. Calc. for $C_{22}H_{22}N_2O_6S_2$: 474.0919.

Thioacetal alcohol **59**. To a solution of 1.68 g (3.54 mmol) of the ester **58** in 84 ml of dioxane was added 35.4 ml (10 eq) of 1 N sodium hydroxide with vigorous stirring at room temperature. The mixture was stirred for 3 hr and then 3.3 ml of concentrated hydrochloric acid was added. The mixture was evaporated to a small volume and thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered and evaporated.

The residue was dissolved in 80 ml of methylene chloride. To the stirred solution was added dropwise 1.04 ml of triethylamine at room temperature, followed by 1.6 ml of ethyl chloroformate. After stirring at room temperature for 10 min, the solution was evaporated to dryness.

The residue was dissolved in 130 ml of methylene chloride and cooled in an ice bath. A chilled solution of 1.6 g of sodium borohydride in 16 ml of methanol was added in one portion with stirring. After 5 min, the mixture was poured into a saturated sodium chloride solution containing dilute hydrochloric acid and thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and chromatographed on a silica gel column (eluted with 1.5% methanol in methylene chloride) to give 1.09 g (69.2%) of **59** as an about 1:1 diastereomeric mixture m.p. 146–150° (benzene). IR (KBr): 3370, 1685, 1603, 1582, 1507. MS: 446 (1, M), 382 (7), 262 (100). Exact Mass: Found: 446.0981. Calc. for $C_{21}H_{22}N_2O_5S_2$: 446.0970.

Thioacetal chlorides **32** *and* **35**. To a stirred solution of 16.0 g (2.38 mmol) of the alcohol **59** and 1 ml of triethylamine in 50 ml of methylene chloride was added dropwise 0.6 ml of methanesulfonyl chloride at room temperature. The solution was stirred at room temperature for 10 min and evaporated to dryness.

The residue was dissolved in 30 ml of dimethylformamide and treated with 2.1 g of lithium chloride. The mixture was stirred at room temperature for 15 min and then poured into ice water. The aqueous layer was thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and chromatographed on a silica gel column (eluted with 0.5% methanol in methylene chloride) to give a 960 mg (86.9%) of amorphous solid which was fractionally crystallized from ethyl acetate to yield 338 mg (30.6%) of **32**, m.p. 212–213° and 242 mg (21.9%) of **35**, m.p. 151–153°.

Thioacetal chloride **32**. IR (KBr): 1683, 1603, 1580, 1507. NMR ($CDCl_3$): 3.15 (3H, s), 3.81 (3H, s), 3.94 (3H, s), 4.39 (2H, s), 5.10 (1H, s), 5.15 (1H, s), 6.32 (1H, s), 6.65–7.50 (7H, m). MS: 464 (1, M), 282 (33), 280 (100). Exact Mass: Found: 464.0629. Calc. for $C_{21}H_{21}N_2O_4S_2{}^{35}Cl$: 464.0631.

Thioacetal chloride **35**. IR (KBr): 1686, 1605, 1583, 1505. NMR ($CDCl_3$): 3.29 (3H, s), 3.81 (3H, s), 3.95 (3H, s), 4.43 (2H, s), 4.99 (1H, s), 5.21 (1H, s), 5.37 (1H, s), 6.70–7.50 (7H, m). MS: 464 (1, M), 282 (31), 280 (100). Exact Mass: Found: 464.0620. Calc. for $C_{21}H_{21}N_2O_4S_2{}^{35}Cl$: 464.0631.

Cyclized thioacetal **33**. A solution of 30.0 mg (0.0646 mmol) of the chloride **32** in 6 ml of dry tetrahydrofuran was cooled in a dry ice–acetone bath. To the stirred solution was added dropwise 66 μl (1.2 eq) of 1.17 N phenyllithium in benzene–ether (7:3) over a 2 min period. After the solution had been stirred for 10 min, 50 μl of acetic acid was added. The solution was allowed to warm to room temperature and poured into a saturated sodium bicarbonate solution. The aqueous layer was thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and separated on preparative silica gel tlc [eluted with ethyl acetate–hexane (1:1)] to give 22.0 mg (79.5%) of **33** as white crystals, m.p. 223–224° (benzene). IR (KBr): 1689, 1608, 1510. NMR ($CDCl_3$): 3.16 (3H, s), 3.16 (1H, AB, J = 18 Hz), 3.77 (3H, s), 3.96 (1H, AB, J = 18 Hz), 4.01 (3H, s), 5.04 (1H, s), 5.47 (1H, s), 6.65–7.40 (7H, m). MS: 428 (1, M), 224 (100). Exact Mass: Found: 428.0878. Calc. for $C_{21}H_{20}N_2O_4S_2$: 428.0864.

Protected dehydrogliotoxin thioacetal **34**. A solution of 32.0 mg (0.0747 mmol) of the thioacetal **33** in 6.4 ml of dry tetrahydrofuran was cooled in a dry ice-acetone bath. To the stirred solution was added dropwise 77 μl (1.2 eq) of 1.17 N phenyllithium in benzene–ether (7:3) over a 1 min period. After 30 sec, 32 μl of benzyl chloromethyl ether was added. The solution was stirred at −78° for 10 min and then poured into a saturated sodium chloride solution and thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and separated on preparative silica gel tlc [eluted with ethyl acetate–hexane (1:1)] to afford 29.0 mg (71.0%) of **34** as white crystals, m.p. 144–145° (ethyl acetate). IR (KBr): 1700, 1683, 1604, 1508. NMR ($CDCl_3$): 3.15 (1H, AB, J = 18 Hz), 3.22 (3H, s), 3.76 (3H, s), 3.85 (1H, AB, J = 11 Hz), 3.98 (1H, AB, J = 18 Hz), 4.02 (3H, s), 4.40 (1H, AB, J = 11 Hz), 4.62 (1H, AB, J = 11 Hz), 4.71 (1H, AB, J = 11 Hz), 5.40 (1H, s), 6.60–7.40 (12H, m). MS: 548 (<1, M), 364 (86), 257 (48), 91 (100). Exact Mass: Found: 548.1452. Calc. for $C_{29}H_{28}N_2O_5S_2$: 548.1440.

Protected dehydrogliotoxin thioacetal **34** (*one-pot procedure*). A solution of 50.0 mg (0.108 mmol) of the chloride **32** and 0.15 ml of benzyl chloromethyl ether in 10 ml of dry tetrahydrofuran was cooled in a dry ice–acetone bath. To the stirred solution was added dropwise 0.228 ml (2.5 eq) of 1.17 N phenyllithium in benzene–ether (7:3) over a 15 min period. The progress of the reaction was carefully monitored by tlc. After stirring at −78° for an additional 5 min, the solution was allowed to warm to room temperature. The solution was poured into a saturated sodium chloride solution and thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and separated on preparative silica gel tlc [eluted with ethyl acetate–hexane (1:1)] to give 41.0 mg (69.5%) of **34** as white crystals. This was identical with the compound prepared by a stepwise procedure.

Benzyloxymethyl chloride **36**. A solution of 30.0 mg (0.646 mmol) of the chloride **35** in 6 ml of dry tetrahydrofuran was cooled in a dry ice–acetone bath. To the stirred solution was added dropwise 66 μl (1.2 eq) of 1.17 N phenyllithium in benzene–ether (7:3) over a 1 min period. After 30 sec, 30 μl of benzyl chloromethyl ether was added. The solution was stirred at −78° for 10 min and then poured into a saturated sodium chloride solution and thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and separated on preparative silica gel tlc [eluted with ethyl acetate–hexane (1:1)] to afford 27.4 mg (72.5%) of **36** as white crystals, m.p. 153–154° (ethyl acetate). IR (KBr): 1687, 1603, 1582, 1506. NMR ($CDCl_3$): 3.34 (3H, s), 3.80 (3H, s), 3.93 (1H, AB, J = 11 Hz), 3.95 (3H, s), 4.21 (1H, AB, J = 11 Hz), 4.30 (1H, AB, J = 11 Hz), 4.39 (1H, AB, J = 11 Hz), 4.55 (1H, AB, J = 11 Hz), 4.65 (1H, AB, J = 10 Hz), 5.10 (1H, s), 5.42 (1H, s), 6.70–7.45 (12H, m). MS: 584 (1, M), 402 (18), 400 (53), 294 (16), 91 (100). Exact Mass: Found: 584.1219. Calc. for $C_{29}H_{29}N_2O_5S_2{}^{35}Cl$: 584.1206.

Protected dehydrogliotoxin thioacetal **37**. A solution of 25.0 mg (0.0427 mmol) of the chloride **36** in 5 ml of dry tetrahydrofuran was cooled in a dry ice–acetone bath. To the stirred solution was added dropwise 44 μl (1.2 eq) of 1.17 N phenyllithium in benzene–ether (7:3) over a 1 min period. After stirring for 10 min at −78°, the solution was allowed to warm to room temperature and poured into a saturated sodium chloride solution. The aqueous layer was thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and separated on preparative silica gel tlc [eluted with ethyl acetate–hexane (1:1)] to give 22.0 mg (93.8%) of **37** as white crystals, m.p. 149–151° (ethyl acetate). IR (KBr): 1701, 1682, 1603, 1510. NMR ($CDCl_3$): 3.17 (1H, AB, J = 18 Hz), 3.35 (3H, s), 3.75 (3H, s), 3.88 (1H, AB, J = 11 Hz), 3.95 (3H, s), 4.08 (1H, AB, J = 18 Hz), 4.25 (1H, AB, J = 11 Hz), 4.58 (1H, AB, J = 12 Hz), 4.68 (1H, AB, J = 12 Hz), 5.25 (1H, s), 6.60–7.35 (12H, m). MS: 548 (3, M), 364 (100), 257 (58), 91 (90). Exact Mass: Found: 548.1452. Calc. for $C_{29}H_{28}N_2O_5S_2$: 548.1440.

Protected dehydrogliotoxin thioacetal **37** (*one-pot procedure*). A solution of 50.0 mg (0.108 mmol) of the chloride **35** and 0.15 ml of benzyl chloromethyl ether in 10 ml of dry tetrahydrofuran was cooled in a dry ice–acetone bath. To the stirred solution was added dropwise 0.230 ml (2.5 eq) of 1.17 N phenyllithium in benzene–ether (7:3) over a 15 min period. The progress of the reaction was carefully monitored by tlc. After stirring at −78° for an additional 5 min, the solution was allowed to warm to room temperature. The solution was poured into a saturated sodium chloride solution and thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and separated on preparative silica gel tlc [eluted with ethyl acetate–hexane (1:1)] to give 36.2 mg (61.3%) of **37** as white crystals. This substance was identical with the compound prepared by a stepwise procedure.

Dehydrogliotoxin thioacetal **60**. To a solution of 40.0 mg (0.0730 mmol) of the thioacetal **34** in 8 ml of methylene chloride was added 0.13 ml of boron trichloride at room temperature. The solution was allowed to stand at room temperature for 20 min and then poured into ice water. The aqueous layer was thoroughly extracted with methylene chloride. The extracts were

dried over anhydrous sodium sulfate, filtered and evaporated to give white crystals which were collected with ethyl acetate–ether to yield 21.7 mg (66.9%) of **60**, m.p. 275–276° (capillary, methylene chloride). IR (KBr): 3420, 1678, 1650, 1602, 1505. NMR ($CDCl_3$): 2.48 (1H, d of d, J = 6, 10 Hz), 3.21 (1H, AB, J = 18 Hz), 3.24 (3H, s), 3.76 (3H, s), 3.91 (1H, d of d, J = 10, 13 Hz), 3.93 (1H, AB, J = 18 Hz), 4.63 (1H, d of d, J = 6, 13 Hz), 5.31 (1H, s), 6.60–7.35 (7H, m), 10.48 (1H, s). MS: 444 (1, M), 260 (35), 243 (18), 185 (100). Exact Mass: Found: 444.0816. Calc. for $C_{21}H_{20}N_2O_5S_2$: 444.0814.

Dehydrogliotoxin thioacetal **61**. This substance [m.p. 249–250° (capillary, methylene chloride–ether)] was synthesized from **37** in 66.2% yield by using the same procedure as the one given for **60**. IR (KBr): 3420, 1679, 1643, 1603. NMR ($CDCl_3$): 2.64 (1H, d of d, J = 6, 10 Hz), 3.25 (1H, AB, J = 18 Hz), 3.39 (3H, s), 3.76 (3H, s), 4.11 (1H, AB, J = 18 Hz), 3.95–4.45 (2H, m), 5.20 (1H, s), 6.60–7.35 (7H, m), 10.67 (1H, s). MS: 444 (7), 260 (44), 185 (100). Exact Mass: Found: 444.0824. Calc. for $C_{21}H_{20}N_2O_5S_2$: 444.0814.

dl-*Dehydrogliotoxin* **53**. To a stirred solution of 10.0 mg (0.0225 mmol) of the dehydrogliotoxin thioacetal **60** in 5 ml of methylene chloride was added 5.5 mg (85%, 1.2 eq) of *m*-chloroperbenzoic acid at room temp. After 5 min of stirring at room temperature, 10 μl of dimethyl sulfide was added. The solution was then treated with 20 μl of a solution of 70% perchloric acid in methanol (1:5). The solution was allowed to stand at room temperature for 7 hr and then poured into a saturated sodium bicarbonate solution. The aqueous layer was thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and chromatographed on a silica gel column (eluted with 0.5% methanol in methylene chloride) to afford 5.3 mg (72%) of **53** as pale yellow crystals, m.p. 177–178° (methanol). IR (KBr): 3460, 1678, 1663, 1603, 1480. NMR ($CDCl_3$): 3.25 (3H, s), 3.28 (1H, AB, J = 18 Hz), 4.28 (1H, AB, J = 18 Hz), 4.10–4.60 (2H, m), 6.65–7.30 (3H, m), 10.39 (1H, s). MS: 324 (3, M), 260 (100), 243 (33), 242 (30). Exact Mass: Found: 324.0238. Calc. for $C_{13}H_{12}N_2O_4S_2$: 324.0238.

dl-Dehydrogliotoxin (**53**), was synthesized also from **61** in 72% yield by using the same procedure as the one given for **60**.

Synthesis of Gliotoxin

Methoxymethyl piperazinedione **65**. To a stirred solution of 9.80 g (76.5 mmol) of glycine–sarcosine anhydride (**55**)[47] in 135 ml of *t*-butanol was added 110 ml (1.3 eq) of 0.904 N potassium *t*-butoxide in *t*-butanol over a period of 2 min at room temperature. After 1 min, 7.6 ml (1.3 eq) of chloromethyl methyl ether was added and stirring was continued for 10 min. The solution was treated with 2 ml of acetic acid and the solvent was removed. The residue was taken up in a dilute sodium chloride solution and thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered and evaporated to give an oil which was short-path distilled under reduced pressure to yield 9.83 g (74.6%) of **65** b.p. 142–144° (0.3 torr). IR (CH_2Cl_2): 1676. NMR ($CDCl_3$): 2.98 (3H, s), 3.33 (3H, s), 4.03 (4H, s), 4.81 (2H, s). MS: 172 (25, M), 157 (100). Exact Mass: Found: 172.0846. Calc. for $C_7H_{12}N_2O_3$: 172.0848.

Unprotected thioacetal **68**

A mixture of 9.83 g (57 mmol) of the piperazinedione **65**, 25.4 g (2.5 eq) of N-bromosuccinimide and 760 mg of benzoyl peroxide in 1180 ml of carbon tetrachloride was heated under reflux for 1 hr. The succinimide was removed by filtration and washed with carbon tetrachloride. The filtrate and the washings were combined and evaporated to dryness.

The residue was dissolved in 500 ml of methylene chloride and treated with 25 g of potassium thioacetate. The mixture was stirred overnight at room temperature. The salts were removed by filtration and washed with methylene chloride. The filtrate and the washings were combined and evaporated.

The residue was dissolved in 900 ml of methanol and treated with 100 ml of a saturated hydrogen chloride solution in methanol (saturated at 0°). The solution was heated at 50° for 40 min with stirring and the solvent was removed.

The residue was taken up in 300 ml of methylene chloride and treated with 15 ml of *p*-anisaldehyde and 1.25 ml of boron trifluoride etherate. The solution was allowed to stand overnight at room temperature and then poured into a saturated sodium bicarbonate solution. The aqueous layer was thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered and evaporated.

The residue was dissolved in 1000 ml of concentrated hydrochloric acid–ethanol (1:1). The solution was heated under reflux for 45 min. Evaporation of the solvent gave off-white crystals which were collected with methanol–ether to yield 5.26 g (29.6% from **65**) of **68** as a 1:1 diastereomeric mixture.

Separation of the diastereomers **68a** *and* **68b**. A mixture of 500 mg (1.61 mmol) of the diastereomers **68**, 0.25 ml of benzoyl chloride and 1 ml of triethylamine in 50 ml of methylene chloride was stirred at room temperature for 1 hr. The solution was poured into a saturated sodium chloride solution containing dilute hydrochloric acid. The aqueous layer was thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and chromatographed on a silica gel column to give 264 mg (39.5%) of the N-benzoyl thioacetal **69**, m.p. 197–198° (methylene chloride–ether) and 178 mg (35.6%) of the unreacted diastereomer **68b**, m.p. 249–251° (ethanol).

Ammonia was passed through a solution of 264 mg of the N-benzoyl thioacetal **69** in 20 ml of methylene chloride for 5 min at room temp. Removal of the solvent afforded off-white crystals which were collected with ethyl acetate yielding 192 mg (38.4%) of **68a**, m.p. 250–252° (ethanol).

N-Benzoyl thioacetal **69**. IR (KBr): 1721, 1685, 1602, 1508. NMR ($CDCl_3$): 3.13 (3H, s), 3.80 (3H, s), 5.13 (1H, s), 5.53 (1H, s), 5.81 (1H, s), 6.70–7.75 (9H, m). MS: 414 (<1, M), 350 (6), 105 (100). Exact Mass: Found: 350.1270. Calc. for $C_{20}H_{18}N_2O_4$ ($M-S_2$): 350.1267. Analysis (recrystallized from benzene): Found: C, 57.96; H, 4.23; N, 6.68. Calc. for $C_{20}H_{18}N_2O_4S_2$: C, 57.96; H, 4.38; N, 6.76%.

Unprotected thioacetal **68a**. IR (KBr): 3190, 1673, 1605, 1510. NMR ($CDCl_3$): 3.07 (3H, s), 3.79 (3H, s), 4.96 (1H, d, J = 2 Hz), 5.01 (1H, d, J = 6 Hz), 5.53 (1H, s), 6.46 (1H, m), 6.84 (2H, d, J = 9 Hz), 7.34 (2H, d, J = 9 Hz). MS: 310 (3, M), 185 (100), 126 (31). Exact Mass: Found: 310.0436. Calc. for $C_{13}H_{14}N_2O_3S_2$: 310.0446. Analysis (recrystallized from ethanol): Found: C, 50.20; H, 4.62; N, 8.92. Calc. for $C_{13}H_{14}N_2O_3S_2$: C, 50.31; H, 4.55; N, 9.03%.

Unprotected thioacetal **68b**. IR (KBr): 3210, 1669, 1605, 1510. NMR ($CDCl_3$): 3.20 (3H, s), 3.79 (3H, s), 4.78 (1H, d, J = 2 Hz), 5.13 (1H, s), 5.18 (1H, d, J = 6 Hz), 6.34 (1H, m), 6.84 (2H, d, J = 9 Hz), 7.33 (2H, d, J = 9 Hz). MS: 310 (2, M), 185 (100), 126 (27). Exact Mass: Found: 310.0442. Calc. for $C_{13}H_{14}N_2O_3S_2$: 310.0446. Analysis (recrystallized from ethanol): Found: C, 50.28; H, 4.54; N, 8.83. Calc. for $C_{13}H_{14}N_2O_3S_2$: C, 50.31; H, 4.55; N, 9.03%.

Michael adducts **73** *and* **74**. To a solution of 100 mg (0.322 mmol) of the thioacetal **68b** and 200 mg (1.03 mmol) of 4-carbo-*t*-butoxyoxepin **62**[48] in 4 ml of dimethyl sulfoxide was added 60 μl of a 40% methanolic solution of benzyltrimethylammonium methoxide (Triton

B) with stirring at room temperature. After 30 min, the solution was poured into a mixture of ice and a saturated sodium chloride solution. The aqueous layer was thoroughly extracted with ethyl acetate. The extracts were washed with a saturated sodium chloride solution, dried over anhydrous magnesium sulfate, filtered, evaporated and separated on preparative silica gel tlc [eluted with ethyl acetate–hexane (2:1)] to give 52.0 mg (32.0%) of **73**, m.p. 225–227° (dec., ethyl acetate) and 108 mg (66.4%) of **74**, m.p. 205–207° (dec., ethyl acetate).

Michael adduct **73**. IR (KBR): 3350, 1699, 1680, 1668, 1603, 1507. NMR ($CDCl_3$): 1.55 (9H, s), 3.15 (3H, s), 3.77 (3H, s), 4.15 (1H, br. s), 4.79 (1H, s), 4.92 (1H, s), 5.01 (1H, s), 5.56 (1H, br. s), 6.40 (2H, m), 6.77 (2H, d, J = 9 Hz), 7.25 (1H), 7.27 (2H, d, J = 9 Hz). Analysis (recrystallized from methanol): Found: C, 57.12; H, 5.57; N, 5.37. Calc. for $C_{24}H_{28}N_2O_6S_2$: C, 57.13; H, 5.59; N, 5.55%.

Michael adduct **74**. IR (KBr): 3370, 1680, 1602, 1508. NMR ($CDCl_3$): 1.45 (9H, s), 2.25 (1H, d, J = 6 Hz), 3.15 (3H, s), 3.78 (3H, s), 4.60 (1H, m), 4.68 (1H, s), 4.91 (1H, s), 5.02 (1H, s), 5.54 (1H, br. s), 6.10–6.55 (2H, m), 6.80 (2H, d, J = 9 Hz), 7.27 (2H, d, J = 9 Hz), 7.40 (1H). Analysis (recrystallized from methanol): Found: C, 56.11; H, 5.69; N, 5.19. Calc. for $C_{24}H_{28}N_2O_6S_2 \cdot 1/2\,H_2O$: C, 56.12; H, 5.69; N, 5.45%.

A 1:3 ratio of **73** and **74** was realized in small scale experiments (1 ~ 5 mg scale) in DMSO.

When the reaction was run in methylene chloride using the same ratio of the reactants and the base, a 3:1 mixture of **73** and **74** was obtained in comparable yield.

Thioacetal acetate **75**. A mixture of 108 mg (0.214 mmol) of the alcohol **74**, 1 ml of acetic anhydride and 1 ml of pyridine was allowed to stand at room temperature for 1 hr. The solution was evaporated under reduced pressure to give white crystals which were collected with hexane–ether to yield 114 mg (97.4%) of **75**, m.p. 211–212° (ethyl acetate). IR (KBr): 1737, 1709, 1685, 1603, 1509. NMR ($CDCl_3$): 1.46 (9H, s), 2.05 (3H, s), 3.15 (3H, s), 3.79 (3H, s), 4.68 (1H, s), 4.90 (1H, s), 5.04 (1H, s), 5.53 (1H, d, J = 6 Hz), 5.56 (1H, br. s), 6.17–6.62 (2H, m), 6.84 (2H, d, J = 9 Hz), 7.31 (2H, d, J = 9 Hz), 7.40 (1H). MS: 546 (<1, M), 490 (3), 362 (2), 306 (11), 126 (100). Exact Mass: Found: 546.1499. Calc. for $C_{26}H_{30}N_2O_7S_2$: 546.1494. Analysis (recrystallized from methanol–ethyl acetate): Found: C, 56.78; H, 5.80; N, 4.92. Calc. for $C_{26}H_{30}N_2O_7S_2$: C, 57.13; H, 5.53; N, 5.12%.

Thioacetal alcohol **78**. A solution of 100 mg (0.183 mmol) of the ester **75** in 1 ml of trifluoroacetic acid was allowed to stand at room temperature for 8 min. Trifluoroacetic acid was removed under reduced pressure. To a stirred mixture of the resulting acid and 0.1 ml of ethyl chloroformate in 10 ml of methylene chloride was added 45 μl of triethylamine. After stirring at room temperature for 10 min, the solution was evaporated to dryness. The residue was dissolved in 20 ml of methylene chloride and cooled in an ice bath. An ice-cooled solution of 100 mg of sodium borohydride in 1 ml of methanol was added in one portion with stirring. After 10 min, the mixture was poured into a saturated sodium chloride solution and thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and separated on preparative silica gel tlc [eluted with ethyl acetate–hexane (3:1)] to give 69.0 mg (79.1%) of **78**, m.p. 174–175° (ethyl acetate). IR (KBr): 3410, 1729, 1682, 1666, 1603, 1510. NMR ($CDCl_3$): 2.06 (3H, s), 3.18 (3H, s), 3.80 (3H, s), 4.15 (2H, s), 4.86 (1H, s), 4.90 (1H, s), 5.04 (1H, s), 5.19 (1H, br. s), 5.57 (1H, m), 6.18 (2H, m), 6.41 (1H, br. s), 6.85 (2H, d, J = 9 Hz), 7.31 (2H, d, J = 9 Hz). MS: 476 (<1, M), 292 (1), 232 (100). Exact Mass: Found: 476.1068. Calc. for $C_{22}H_{24}N_2O_6S_2$: 476.1076. Analysis (recrystallized from methanol): Found: C, 55.22; H, 5.21; N, 5.67. Calc. for $C_{22}H_{24}N_2O_6S_2$: C, 55.45; H, 5.08; N, 5.88%.

Thioacetal chloride **79**. To a solution of 130 mg (0.273 mmol) of the alcohol **78** and 0.130 ml of triethylamine in 13 ml of methylene chloride was added 65 μl of methanesulfonyl chloride with stirring at room temperature. After 10 min, the solvent was removed under reduced pressure. A solution of the resulting mesylate and 650 mg of lithium chloride in 13 ml of dimethylformamide was stirred at room temperature for 15 min. The solution was then poured into ice–water. The aqueous layer was thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and separated on preparative silica gel tlc [eluted with ethyl acetate–hexane (3:2)] to afford 130 mg (96.2%) of **79** as white crystals, m.p. 159–160° (ethyl acetate). IR (KBr): 1727, 1689, 1603, 1515. NMR ($CDCl_3$): 2.05 (3H, s), 3.16 (3H, s), 3.79 (3H, s), 4.09 (2H, s), 4.80 (1H, s), 4.90 (1H, s), 5.03 (1H, s), 5.26 (1H, br. s), 5.54 (1H, m), 6.20 (2H, m), 6.51 (1H, m), 6.84 (2H, d, J = 9 Hz), 7.30 (2H, d, J = 9 Hz). MS: 494 (1, M), 432 (5), 430 (14), 312 (3), 310 (7), 252 (16), 250 (46), 126 (100). Exact Mass: Found: 494.0746. Calc. for $C_{22}H_{23}N_2O_5S_2{}^{35}Cl$: 494.0737. Analysis (recrystallized from methanol): Found: C, 53.30; H, 4.80; N, 5.48. Calc. for $C_{22}H_{23}N_2O_5S_2Cl$: C, 3.38; H, 4.68; N, 5.66%.

Thioacetal alcohol **80**. To a solution of 250 mg (0.505 mmol) of the acetate **79** in 12.5 ml of 10% methanol in methylene chloride was added 79 μl (0.5 eq) of 3.20 N sodium methoxide in methanol with stirring at room temperature. After 5 min, the solution was poured into a saturated sodium chloride solution containing dilute hydrochloric acid. The aqueous layer was thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered and evaporated to give white crystals which were collected with ether–hexane to yield 215 mg (94.0%) of **80**, m.p. 145–146° (ethyl acetate). IR (KBr): 3410, 1681, 1605, 1509. NMR ($CDCl_3$): 3.17 (3H, s), 3.79 (3H, s), 4.08 (2H, s), 4.59 (1H, m), 4.83 (1H, s), 4.93 (1H, s), 5.04 (1H, s), 5.28 (1H, m), 6.16 (2H, m), 6.51 (1H, m), 6.83 (2H, d, J = 9 Hz), 7.29 (2H, d, J = 9 Hz). MS: 434 (2, M–H_2O), 252 (35), 250 (100). Exact Mass: Found: 434.0532. Calc. for $C_{20}H_{19}N_2O_3S_2{}^{35}Cl$ (M–H_2O): 434.0526. Analysis (recrystallized from ethyl acetate): Found: C, 52.64; H, 4.49; N, 6.01. Calc. for $C_{20}H_{21}N_2O_4S_2Cl$: C, 53.03; H, 4.67; N, 6.18%.

Benzylgliotoxin thioacetal **83**. A solution of 179 mg (0.395 mmol) of the chloride **80** and 0.54 ml of benzyl chloromethyl ether in 36 ml of dry tetrahydrofuran was cooled in a dry ice–acetone bath. To the stirred solution was added dropwise 1.08 ml (3.2 eq) of 1.17 N phenyllithium in benzene–ether (7:3) over a 15 min period. The progress of the reaction was carefully monitored by tlc. After stirring at −78° for an additional 5 min, 0.18 ml of acetic acid was added and the solution was allowed to warm to room temperature. The solution was poured into a saturated sodium bicarbonate solution and thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and separated on preparative silica gel tlc [eluted with ethyl acetate–hexane (1:1)] to give 111 mg (52.3%) of **83** as white crystals, m.p. 173–175° (methanol). IR (KBr): 3320, 1683, 1658, 1602, 1505. NMR ($CDCl_3$): 2.92 (1H, AB, J = 18 Hz), 3.24 (3H, S), 3.37 (1H, AB, J = 18 Hz), 3.77 (3H, s), 3.80 (1H, AB, J = 11 Hz), 4.17 (1H, AB, J = 11 Hz), 4.55 (1H, AB, J = 12 Hz), 4.68 (1H, AB, J = 12 Hz), 4.95 (1H, br. s), 5.02 (1H, br. s), 5.08 (1H, S), 5.80 (3H, m), 6.16 (1H, s), 6.80 (2H, d, J = 9 Hz), 7.24 (2H, d, J = 9 Hz), 7.30 (5H, s). MS: 536 (7, M), 352 (26), 334 (36), 91 (100). Exact Mass: Found: 536.1428. Calc. for $C_{28}H_{28}N_2O_5S_2$: 536.1440. Analysis (recrystallized from methanol): Found: C, 61.95; H, 5.28; N, 5.00. Calc. for $C_{28}H_{28}N_2O_5S_2 \cdot 1/2H_2O$: C, 61.93; H, 5.47; N, 5.07%.

Gliotoxin thioacetal **27b**. To an ice-cooled solution of 100 mg (0.186 mmol) of the benzylgliotoxin thioacetal **83**

in 20 ml of methylene chloride was added 0.2 ml of boron trichloride. The solution was allowed to stand at 0° for 10 min and then poured into ice water. The aqueous layer was thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and separated on preparative silica gel tlc (developed twice with 2% methanol in methylene chloride) to give 49.8 mg (59.8%) of **27b** as white crystals, m.p. 155–157° (ethyl acetate–methanol). IR (KBr): 3400, 1662, 1651, 1603, 1515. NMR ($CDCl_3$): 2.85 (1H, m), 2.91 (1H, AB, J = 18 Hz), 3.30 (3H, s), 3.35 (1H, AB, J = 18 Hz), 3.76 (3H, s), 3.75–4.40 (2H, m), 4.90 (1H, br. s), 4.97 (1H, br. s), 5.09 (1H, s), 5.65–5.95 (4H, m), 6.79 (2H, d, J = 9 Hz), 7.24 (2H, d, J = 9 Hz). MS: 446 (21, M), 294 (24), 262 (100), 245 (11), 244 (29), 185 (78). Exact Mass: Found: 446.0973. Calc. for $C_{21}H_{22}N_2O_5S_2$: 446.0970. Analysis (recrystallized from ethyl acetate–methanol): Found: C, 56.51; H, 5.02; N, 6.11. Calc. for $C_{21}H_{22}N_2O_5S_2$: C, 56.49; H, 4.97; N, 6.27%.

dl-*Gliotoxin* (**1**). To an ice-cooled solution of 40.0 mg (0.0896 mmol) of the *dl*-gliotoxin thioacetal **27b** in 20 ml of methylene chloride was added 21.8 mg (85%, 1.2 eq) of *m*-chloroperbenzoic acid with stirring. After 10 min of stirring at 0°, 20 μl of dimethyl sulfide was added. The solution was then treated with 40 μl of a solution of 70% perchloric acid in methanol (1:5). The solution was allowed to stand at room temperature for 9 hr and then poured into a saturated sodium bicarbonate solution. The aqueous layer was thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and chromatographed on a silica gel column (eluted with 1% methanol in methylene chloride) to afford 17.0 mg (58.1%) of **1** as pale yellow crystals, m.p. 165–166° (capillary, methanol). IR (KBr): 3350, 1683, 1663. NMR ($CDCl_3$): 2.93 (1H, AB, J = 18 Hz), 3.17 (3H, s), 3.65 (1H, m), 3.69 (1H, AB, J = 18 Hz), 4.00–4.55 (2H, m), 4.76 (2H, br. s), 5.62 (1H, s), 5.55–6.05 (3H, m). MS: 326 (3, M), 262 (100), 245 (6), 244 (31). Exact Mass: Found: 326.0399. Calc. for $C_{13}H_{14}N_2O_4S_2$: 326.0395. Analysis (recrystallized from methanol): Found: C, 47.56; H, 4.40; N, 8.37. Calc. for $C_{13}H_{14}N_2O_4S_2$: C, 47.84; H, 4.32; N, 8.58%.

Gliotoxin thioacetal **27a** *and* **27b** *from natural gliotoxin*. A solution of 100 mg (0.307 mmol) of natural gliotoxin (**1**)[33] in 30 ml of methylene chloride was cooled in an ice bath. To the stirred solution was added an ice-cooled solution of sodium borohydride in methanol. After the reduction had been complete, the mixture was treated with 1 ml of *p*-anisaldehyde and 1 ml of boron trifluoride etherate and allowed to warm to room temperature. The solution was stirred at room temperature for 4 hr and then poured into a saturated sodium bicarbonate solution. The aqueous layer was thoroughly extracted with methylene chloride. The extracts were dried over anhydrous sodium sulfate, filtered, evaporated and separated on preparative silica gel tlc (developed three times with 2% methanol in methylene chloride) to afford 30.8 mg (22.5%) of **27a**, m.p. 228–230° (ethyl acetate) and 66.1 mg (48.3%) of **27b**, m.p. 204–206° (methanol).

Gliotoxin thioacetal **27a**. IR (KBr): 3300, 1678, 1659, 1603, 1510. NMR ($CDCl_3$): 2.88 (1H, AB, J = 18 Hz), 3.17 (3H, s), 3.21 (1H, AB, J = 18 Hz), 3.76 (3H, s), 3.79 (1H, AB, J = 12 Hz), 4.56 (1H, AB, J = 12 Hz), 4.85 (2H, br. s), 5.20 (1H, s), 5.60–6.05 (4H, m), 6.81 (2H, d, J = 9 Hz), 7.27 (2H, d, J = 9 Hz). MS: 446 (3, M), 294 (3), 262 (77), 245 (13), 244 (44), 185 (100). Exact Mass: Found: 446.0960. Calc. for $C_{21}H_{22}N_2O_5S_2$: 446.0970. $[\alpha]_D^{23}$ + 15.7° (c = 0.0065, 10% methanol in chloroform) CD (ethanol): 230 ($\Delta\epsilon$ −28), 264 nm ($\Delta\epsilon$ +32).

Gliotoxin thioacetal **27b**. IR (KBr): 3350, 1680, 1655, 1603, 1509. NMR ($CDCl_3$): 2.91 (1H, AB, J = 18 Hz), 3.30 (3H, s), 3.35 (1H, AB, J = 18 Hz), 3.77 (3H, s), 3.75–4.40 (2H, m), 4.91 (1H, br. s), 4.98 (1H, br. s), 5.09 (1H, s), 5.65–5.95 (4H, m), 6.80 (2H, d, J = 9 Hz), 7.25 (2H, d, J = 9 Hz). MS: 446 (4, M), 294 (6), 262 (34), 245 (11), 244 (46), 185 (100). Exact Mass: Found: 446.0969. Calc. for $C_{21}H_{22}N_2O_5S_2$: 446.0970. $[\alpha]_D^{23}$ + 30° (c = 0.0049, 10% methanol in chloroform).

The gliotoxin thioacetals **27a** and **27b** were converted back to gliotoxin (**1**) by using the same procedure as the one given in the totally synthetic series.

Synthesis of optically active gliotoxin

Thioacetal ureas **84** *and* **85**. A solution of 500 mg (1.61 mmol) of the thioacetal **68a**, 1 ml of α-(−)-methylbenzyl isocyanate,[50] and 2 ml of triethylamine in 100 ml of methylene chloride was stirred at room temperature for 3 hr. The solution was then concentrated under reduced pressure and separated on preparative silica gel tlc (developed three times with 0.6% methanol in methylene chloride) to give 256 mg (34.7%, R_f 0.22) of **84**, m.p. 203–220° (dec., ethyl acetate) and 271 mg (36.7%, R_f 0.20) of **85**, m.p. 190–198° (dec., ethyl acetate).

Thioacetal urea **84**. IR (KBr): 3270, 1719, 1693, 1603, 1525, 1510. NMR ($CDCl_3$): 1.62 (3H, d, J = 7 Hz), 3.03 (3H, s), 3.79 (3H, s), 5.06 (1H, s), 5.12 (1H, q, J = 7 Hz), 5.35 (1H, s), 6.17 (1H, s), 7.81 (2H, d, J = 9 Hz), 7.30 (5H, s), 7.33 (2H, d, J = 9 Hz), 8.94 (1H, br. s). MS: 393 (26, M–S_2), 185 (100), 105 (98). Exact Mass: Found: 393.1679. Calc. for $C_{22}H_{23}N_3O_4$ (M–S_2): 393.1688. $[\alpha]_D^{23}$ − 18.5° (c = 0.0338, 10% methanol in chloroform).

Thioacetal urea **85**. IR (KBr): 3280, 1710, 1685, 1665, 1603, 1506. NMR ($CDCl_3$): 1.58 (3H, d, J = 7 Hz), 3.05 (3H, s), 3.76 (3H, s), 5.07 (1H, s), 5.14 (1H, q, J = 7 Hz), 5.23 (1H, s), 6.20 (1H, s), 6.79 (2H, d, J = 9 Hz), 7.25 (2H, d, J = 9 Hz), 7.33 (5H, s), 8.95 (1H, br. s). MS: 393 (26, M–S_2), 185 (91), 105 (100). Exact Mass: Found: 393.1690. Calc. for $C_{22}H_{23}N_3O_4$(M–S_2): 393.1688. $[\alpha]_D^{23}$ − 3.1° (c = 0.032, 10% methanol in chloroform).

Optically active thioacetal **86** (*optically active form of* **68a**). In a 25 ml round-bottomed flask was placed 230 mg (0.503 mmol) of the crystalline urea **84**. The crystals were heated at 230° in an oil bath under slow stream of argon for 10 min. After cooling, the resulting crystals were triturated with ether to yield 145 mg (92.9%) of **86**, m.p. 251–253° (ethyl acetate). $[\alpha]_D^{23}$ + 73° (c = 0.0056, 10% methanol in chloroform) CD (ethanol): 228 ($\Delta\epsilon$ −34), 251 nm ($\Delta\epsilon$ +30).

Following the sequence of reactions from **68b** to **1**, the totally synthetic gliotoxin (**1**) was obtained in racemic and optically active forms from the racemic thioacetal **68a** and its optically active form **86**. Since the experimental procedures for each step were identical to those described for the sequence from **68b** to **1**, only the yields and physical data for the intermediates are listed below.

Michael adduct corresponding to **73**. *Racemic form*. Yield: 34.4%. M.P.: 217–219° (dec., ethyl acetate). IR (KBr): 3440, 1691, 1679, 1657, 1604, 1509. NMR ($CDCl_3$): 1.35 (9H, s), 2.42 (1H, d, J = 6 Hz), 3.00 (3H, s), 3.77 (3H, s), 4.17 (1H, br. s), 4.58 (1H, s), 5.02 (1H, s), 5.55 (1H, s), 5.64 (1H, br. s), 6.37 (2H, m), 6.78 (2H, d, J = 9 Hz), 7.35 (2H, d, J = 9 Hz), 7.38 (1H). *Optically active form*. Yield: 28.0%. M.P.: 227–229° (dec., ethyl acetate). $[\alpha]_D^{23}$ − 463° (c = 0.0094, 10% methanol in chloroform).

Michael adduct corresponding to **74**. *Racemic form*. Yield: 62.1%. M.P.: 225–227° (dec., ethyl acetate). IR (KBr): 3390, 1702, 1690, 1604, 1510. NMR ($CDCl_3$): 1.44 (9H, s), 2.15 (1H, d, J = 8 Hz), 3.01 (3H, s), 3.77 (3H, s), 4.62 (1H, br. s), 4.66 (1H, s), 5.06 (1H, s), 5.53 (1H, s), 5.77 (1H, d of d, J = 1, 6 Hz), 6.0–6.5 (2H, m), 6.81 (2H, d, J = 9 Hz), 7.29 (2H, d, J = 9 Hz), 7.3 (1H). *Optically active form*. Yield: 59.0%. M.P.: 266–268° (dec., ether). $[\alpha]_D^{23}$ + 108° (c = 0.00846, 10% methanol in chloroform).

Thioacetal acetate corresponding to **75**. *Racemic form*. Yield: 96.8%. M.P.: 195–196° (ethyl acetate). IR (KBr): 1734, 1690, 1603, 1508. NMR ($CDCl_3$): 1.44 (9H, s), 2.12

(3H, s), 3.00 (3H, s), 3.78 (3H, s), 4.59 (1H, s), 5.05 (1H, s), 5.57 (1H, s), 5.65 (1H, m), 5.75 (1H, br. s), 6.10–6.45 (2H, m), 6.83 (2H, d, J = 9 Hz), 7.36 (2H, d, J = 9 Hz), 7.36 (1H). MS: 546 (1, M), 490 (4), 362 (2), 306 (13), 126 (100). Exact Mass: Found: 546.1499. Calc. for $C_{26}H_{30}N_2O_7S_2$: 546.1494. *Optically active form.* Yield: 92.0%. M.P.: 187–188° (ethyl acetate). $[\alpha]_D^{23} + 241°$ (c = 0.00979, 10% methanol in chloroform).

Thioacetal alcohol corresponding to **78**. *Racemic form.* Yield: 68.8%. M.P.: 181–182° (ethyl acetate). IR (KBr): 3400, 1720, 1695, 1675, 1603, 1508. NMR ($CDCl_3$): 1.85 (1H, t, J = 5 Hz), 2.14 (3H, s), 3.04 (3H, s), 3.80 (3H, s), 4.08 (2H, d, J = 5 Hz), 4.70 (1H, s), 5.04 (1H, s), 5.44 (1H, br, s), 5.71 (1H, s), 5.75–6.45 (4H, m), 6.84 (2H, d, J = 9 Hz), 7.39 (2H, d, J = 9 Hz). MS: 476 (<1, M), 292 (4), 232 (100), 126 (61). Exact Mass: Found: 476.1072. Calc. for $C_{22}H_{24}N_2O_6S_2$: 476.1076. *Optically active form.* Yield: 71.5%. M.P.: 180–181° (ethyl acetate). $[\alpha]_D^{23} + 258°$ (c = 0.0104, 10% methanol in chloroform).

Thioacetal chloride corresponding to **79**. *Racemic form.* Yield: 95.4%. M.P.: 179–180° (ethyl acetate). IR (KBr): 1730, 1682, 1673, 1608, 1515. NMR ($CDCl_3$): 2.12 (3H, s), 3.02 (3H, s), 3.79 (3H, s), 4.03 (2H, s), 4.65 (1H, s), 5.05 (1H, s), 5.52 (1H, br, s), 5.66 (1H, s), 5.70–6.55 (4H, m), 6.85 (2H, d, J = 9 Hz), 7.37 (2H, d, J = 9 Hz). MS: 494 (<1, M), 312 (2), 310 (6), 252 (7), 250 (20), 126 (100). Exact Mass: Found: 494.0737. Calc. for $C_{22}H_{23}N_2O_5S_2{}^{35}Cl$: 494.0737.

Optically active form. Yield: 92.1%. M.P.: 177–179° (ethyl acetate). $[\alpha]_D^{23} + 239°$ (c = 0.00955, 10% methanol in chloroform).

Thioacetal alcohol corresponding to **80**. *Racemic form.* Yield: 95.1%. M.P.: 200–201° (ethyl acetate). IR (KBr): 3410, 1692, 1603, 1512. NMR ($CDCl_3$): 3.04 (3H, s), 3.78 (3H, s), 4.02 (2H, s), 4.71 (1H, br. d, J = 6 Hz), 4.80 (1H, s), 5.09 (1H, s), 5.64 (1H, m), 5.65 (1H, s), 6.08 (2H, m), 6.36 (1H, m), 6.83 (2H, d, J = 9 Hz), 7.32 (2H, d, J = 9 Hz), MS: 436 (1, M–H_2O), 434 (3, M–H_2O), 252 (36), 250 (100). Exact Mass: Found: 434.0536. Calc. for $C_{20}H_{19}N_2O_3S_2{}^{35}Cl$ (M–H_2O): 434.0526. *Optically active form.* Yield: 97.4%. M.P.: 110–113° (ether). $[\alpha]_D^{23} + 67°$ (c = 0.0107, 10% methanol in chloroform).

Benzylgliotoxin thioacetal corresponding to **83**. *Racemic form.* Yield: 47.5%. M.P.: 210–212° (ethyl acetate). IR (KBr): 3350, 1676, 1668, 1603, 1513. NMR ($CDCl_3$): 2.85 (1H, AB, J = 18 Hz), 3.13 (3H, s), 3.24 (1H, AB, J = 18 Hz), 3.72 (1H, AB, J = 11 Hz), 3.78 (3H, s), 4.37 (1H, AB, J = 11 Hz), 4.57 (1H, AB, J = 12 Hz), 4.64 (1H, AB, J = 12 Hz), 4.88 (2H, m), 5.21 (1H, s), 5.86 (3H, m), 6.11 (1H, br. s), 6.79 (2H, d, J = 9 Hz), 7.26 (2H, d, J = 9 Hz), 7.31 (5H, s). MS: 536 (1, M), 352 (26), 334 (38), 91 (100). Exact Mass: Found: 536.1458. Calc. for $C_{28}H_{28}N_2O_5S_2$: 536.1440.

Gliotoxin thioacetal (**27a**). *Racemic form.* Yield: 55.0%. M.p.: 241–242° (methylene chloride). IR (KBr): 3350, 3280, 1670, 1602, 1512. NMR ($CDCl_3$): 2.65 (1H, m), 2.88 (1H, AB, J = 18 Hz), 3.17 (3H, s), 3.22 (1H, AB, J = 18 Hz), 3.77 (3H, s), 3.80 (1H, m), 4.60 (1H, m), 4.88 (2H, br. s), 5.23 (1H, s), 5.60–6.10 (4H, m), 6.81 (2H, d, J = 9 Hz), 7.28 (2H, d, J = 9 Hz). MS: 446 (4, M), 294 (5), 262 (100), 245 (11), 244 (38), 185 (84). Exact Mass: Found: 446.0981. Calc. for $C_{21}H_{22}N_2O_5S_2$: 446.0970. *Optically active form.* Yield: 52%. M.P. 228–230° (ethyl acetate). $[\alpha]_D^{23} + 15.5°$ (c = 0.00303, 10% methanol in chloroform).

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[26]Reduction of an epidithiapiperazinedione with sodium borohydride yields the corresponding dithiol, which can be oxidized back to the starting epidithiapiperazinedione. However, this is often complicated because of elimination of hydrogen sulfide from the dithiol.

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[30]For example, see I. T. Harrison and S. Harrison, *Compendium of Organic Synthetic Methods* (Wiley-Interscience), Vol. I, p. 451, Vol. II, p. 181, Vol. III, p. 291 and Vol. IV, p. 287.

[31]It was not clear whether the thioacetal **16** was not attacked by iodine at all or whether the initially formed intermediate was reduced back to **16** by iodide. Related to this, it is interesting to mention that upon treatment with concentrated sulfuric acid the monosulfoxide **20** was reduced to the thioacetal **16**.

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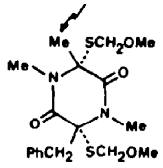
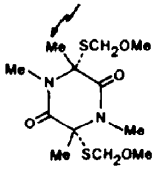
[36]In some cases, the *cis* configuration of the alkylated product was directly confirmed from NMR spectra. For example, the C-methyl signal of the benzyl methyl dimethoxymethylthiopiperazinedione **49a**, prepared by benzylation of **47b**, appears at δ 0.80 ppm, whereas that of the dimethyl dimethoxythiopiperazinedione **45b** appears at δ 1.89 ppm, a normal chemical shift for this type of methyl group. The unusual upfield shift (1.09 ppm) in **49a** can only be explained by the anisotropic effect of the benzene ring, concluding the *cis* configuration for **49**.

δ 0.80 ppm



49a

δ 1.89 ppm



45b

[37]The low yield of this reaction is probably due to a competitive demethylation and demethoxymethylation with boron trichloride.
[38]We are indebted to Dr. G. M. Strunz, Canadian Forestry Service, New Brunswick, Canada, for a sample of natural hyalodendrin.
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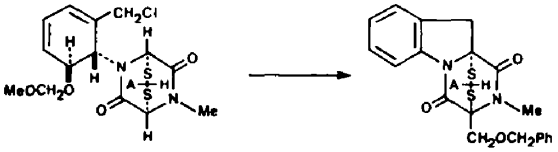

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