(70%) of compound **32**: NMR δ 6.87 (br s, 1 H, NH), 4.10 (dq, 1 H, C(5)H, irradiation on the NH proton leaves only one quartet), 3.00 (s, 3 H, NMe), 1.47 (d, 3 H, C(5)Me); mass spectrum, *m/e* 128 (M^+); mp (hexane/CH_2Cl_2) 110–112 °C.

Acknowledgment. This work was supported in part by the Koningin Wilhelmina Fonds.

Registry No. 6a, 70771-85-6; **6b**, 70771-86-7; **6c**, 73198-47-7; **6d**, 70771-87-8; **6e**, 73198-48-8; **8a**, 70771-89-0; DL-**8b**, 21653-11-2; **8c**, 73198-49-9; DL-**8d**, 70771-90-3; DL-**8e**, 73198-50-2; **10a**, 73198-51-3; DL-**10b**, 73198-52-4; **10c**, 73198-53-5; DL-**10d**, 73198-54-6; DL-**10e**, 73198-55-7; **11a**, 73198-56-8; **12b**, 41839-79-6; **12c**, 57294-55-0; **12d**, 62927-27-9; **12e**, 73198-57-9; **15a**, 73198-58-0; **15b**, 73198-59-1; **15c**, 73198-60-4; **18a**, 73198-61-5; **18b**, 73198-62-6; **18c**, 73198-63-7; **19a**, 73198-64-8; **19b**, 73198-65-9; *cis*-**19c**, 73198-66-0; **20a**, 73198-67-1; **21a**, 73198-68-2; **21b**, 73198-69-3; *cis*-**24a**, 73198-70-6; *trans*-**24a**, 73198-71-7; **26**, 73198-72-8; **27**, 52785-16-7; **28a**, 73198-73-9; **28b**, 73198-74-0; **30a**, 50627-39-9; **32**, 6851-79-2; pyruvoyl chloride, 5704-66-5.

Biosynthesis of Gliotoxin. Synthesis of Sulfur-Bridged Dioxopiperazines from *N*-Hydroxyamino Acids

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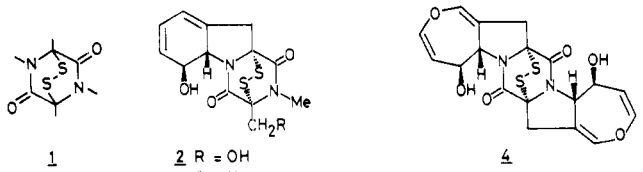
A biomimetic approach to the synthesis of sulfur-bridged dioxopiperazines **1** is described. It is postulated that the biosynthesis of this type of compound progresses by oxidation of dioxopiperazines **12** to the corresponding di-*N*-hydroxy compounds **13**, which might be converted into acylimmonium ions of type **14**. These could react with sulfur nucleophiles to give the sulfur-bridged compounds **15** (Scheme II). The biosynthesis of gliotoxin (**2**) might thus proceed as depicted in Scheme III, which features the stereochemically controlled cis addition of a disulfide. Support of this biosynthetically hypothetic scheme was come by as follows: the *N*-hydroxydioxopiperazines **23** and **24** could be converted efficiently into the sulfur-bridged dioxopiperazines **30** and **31** (Scheme IV). The key step in this scheme is the migration of the N functionality in **23** to a C(3)-position in **27**, a reaction that proceeds through the acylimine **26**. N-Methylation of **27a** followed by treatment with liquid H_2S in the presence of $ZnCl_2$ gave the dithiol **29a**. Surprisingly, under these reaction conditions **27b** afforded a mixture of dithiol **29b** and the monosulfide **31b**, whereas **27c** gave only the monosulfide **31c**. It is proposed (Scheme V) that from **28** a mixture of cis and trans dithiol **29** is formed, the ratio of which depends upon the ring substituent R. The trans dithiol is, in contradistinction to the cis dithiol, not stable under the reaction conditions and is converted by a transanular thiol attack into the monosulfide **31**.

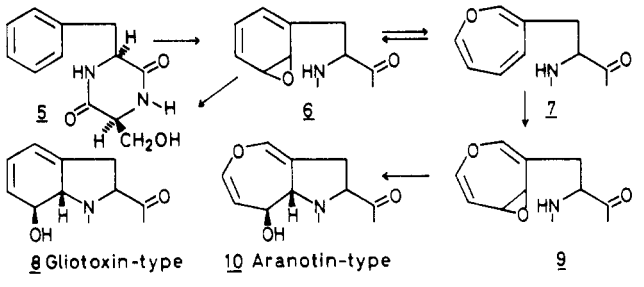
The epidithiodioxopiperazine moiety **1** is a common feature of a substantial number of natural products, exhibiting antiviral, antifungal, or antibacterial activity.[1] The best known representative of this class of compounds is gliotoxin (**2**), a metabolite of various *Fungi imperfecti*.



1 **2** R = OH; **3** R = H **4**

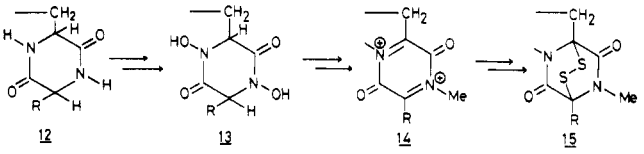
During the last decades several new specimens of this class have been isolated, one of which is aranotin (**4**). The structural resemblance of **4** to **2** has led to considerable speculation on their biosynthesis. Cyclo-L-Phe-L-Ser (**5**)[2] has been shown to be an efficient precursor of gliotoxin (**2**), and further labeling studies have demonstrated that the *N*-methyl group is derived from methionine,[3] whereas the sulfur atoms are delivered by cystine.[4] The most likely explanation for the formation of the dihydro aromatic systems has been provided by Neuss et al.,[5] who invoked the intermediacy of benzene oxides **6**. This system is in equilibrium with the isomeric oxepin **7** (Scheme I). Nucleophilic attack by the dioxopiperazine amide group of **6** would produce a substituted cyclohexadienol **8** of the

Scheme I



8 Gliotoxin-type **10** Aranotin-type **9**

Scheme II



12 **13** **14** **15**

(1) For reviews on epipolythiodioxopiperazines see: (a) A. Taylor in "Microbial Toxins", Vol. VII, S. Kadis, A. Ciegler, and S. J. Ajl, Eds., Academic Press, New York, 1971, p 337; (b) P. G. Sammes, *Fortschr. Chem. Org. Naturst.*, **32**, 87 (1975); (c) C. Leigh and A. Taylor, *Adv. Chem. Ser.*, **No. 133**, 228 (1976); (d) S. Johne and D. Gröger, *Pharmazie*, **32**, 1 (1977); (e) B. Ganem, *Tetrahedron*, **34**, 3375 (1978).

(2) (a) J. D. Bu'Lock and C. Leigh, *J. Chem. Soc., Chem. Commun.*, 628 (1975); (b) G. W. Kirby, G. L. Patrick, and D. J. Robins, *J. Chem. Soc., Perkin Trans. 1*, 1336 (1978); (c) G. W. Kirby, *Pure Appl. Chem.*, **51**, 705 (1979).

(3) J. A. Winstead and R. J. Suhaldonik, *J. Am. Chem. Soc.*, **82**, 1644 (1960).

(4) N. Neuss, L. D. Boeck, D. R. Brannon, J. C. Cline, D. C. DeLong, M. Gorman, L. L. Huckstep, D. H. Lively, J. Mabe, M. M. Marsh, B. B. Molloy, R. Nagarajan, J. D. Nelson, and W. M. Stark, *Antimicrob. Agents Chemother.*, 213 (1968).

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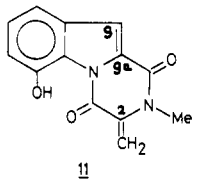
type found in gliotoxin. Alternatively, further oxidation of the oxepin **7** to **9**, followed by a similar nucleophilic process, might yield the aranotin-type system **10**. However, so far no support for the nucleophilic ring opening (**6** → **8**) has been obtained: in 3-(β-aminoethyl)benzene oxide, a model for the putative gliotoxin precursor, the epoxide could not be opened by intramolecular amine attack.[6]

Details concerning the introduction of the sulfur bridge remain obscure. An earlier suggestion[7] that the dethiodehydropiperazine **11** co-occurring in the fungus is an in-



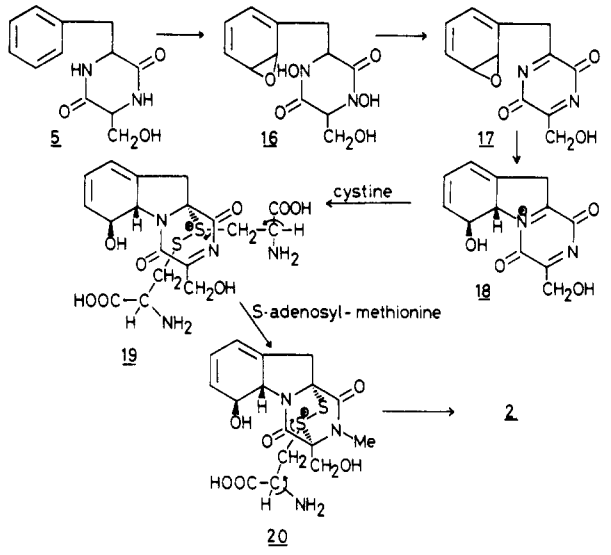
11

termediate has been ruled out because of the following findings. Phenylalanine is incorporated into the metabolites without loss of either of the benzylic hydrogens,[8] making an intermediate with a C_9–C_{9a} double bond unlikely. The in vivo conversion of phenylalanine–alanine anhydride into C_{11}-deoxygliotoxin (**3**) suggests that an *exo*-methylene bond at C_2 does not occur either during the biosynthesis.[9] In addition, we recently found that addition of H_2S to a conjugated double bond in dehydrocyclodipeptides is hard to achieve.[10]

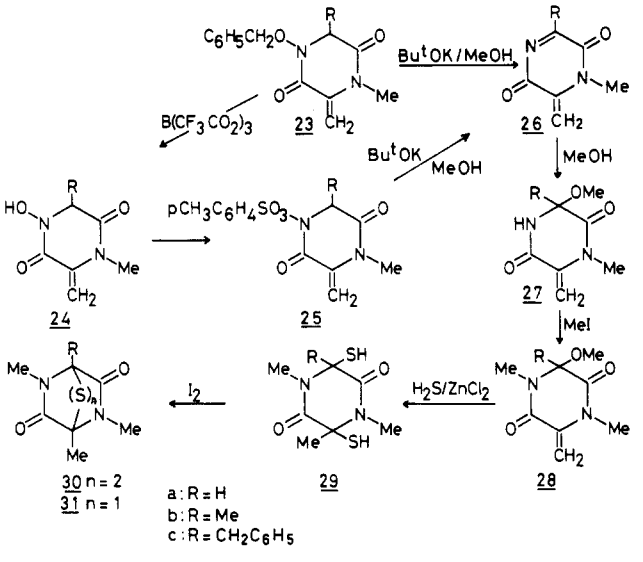
A reasonable mechanism for the incorporation of sulfur has been suggested by Sammes,[1b] who supposed that an acylimine intermediate of type **14** (Scheme II), similar to that proposed for the biosynthesis of brevianamide A[11] or the ergot peptides,[12] might be involved. This leaves us with the intriguing question of how acylimines are formed biosynthetically. Bycroft[12] has proposed that, in general, they might arise by dehydrogenation of corresponding acylamino acid derivatives, e.g., **12**. However, we feel that *oxidation of the amide nitrogen in* ***12*** *to form hydroxamic acids of type* ***13****, followed by dehydration to the acylimines* ***14****, is more likely to occur.*

Our proposal that in biosyntheses α-functionalization of dioxopiperazines might be achieved by transposition of an N functionality by an elimination–addition mechanism is based on the following arguments. During the last decades a substantial number of natural products containing one or more oxidized peptide bonds C(O)–N(OH) have been isolated.[13] In addition, in the preceding article[14] we have shown that *N*-hydroxy-*N*-acyl-α-amino acid derivatives are easily converted into the corresponding α-substituted acylamino acids. With this hypothesis in mind, the biosynthesis of gliotoxin (**2**) might be as depicted in Scheme III. Phenylalanine-serine anhydride (**5**) is oxidized to the di-*N*-hydroxybenzene oxide **16**, which after

Scheme III



Scheme IV



dehydration gives the acylimine **17**. Then the benzene oxide might be attacked by the N atom of the phenylalanine residue. Simultaneously with or subsequent to this ring opening, sulfur is introduced by reaction of cystine with the acylimmonium ion to give the sulfonium ion **19**. β-Elimination in the cystine fragment and N-methylation give a second acylimmonium ion, which in turn might react with the disulfide to give **20**. Finally, a second β-elimination reaction might lead to gliotoxin (**2**).

One of the features of this scheme is the conversion of **16** into **2** by a stereochemically controlled disulfide addition reaction.

We argued that the proposed role of *N*,*N*′-dihydroxydioxopiperazines **13** in the biosynthetic conversion of dioxopiperazines **12** into the sulfur-bridged compounds **15** might gain in probability if the latter could be obtained chemosynthetically by starting from **13**. Herein we wish to report our first results toward this directive, which lends tentative support to our hypothesis.

As the reported syntheses[15] of di-*N*-hydroxydioxopiperazines **13** have the disadvantage of limited applicability, we decided to study the conversion of **13** into **15** with a model reaction. In the foregoing article[14] we showed that

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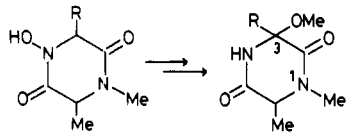
N-hydroxydioxopiperazines of type **21** can be converted



21 **22**

a: R = H, b: R = Me, c: R = $CH_2C_6H_5$

efficiently into the corresponding *C*(3)-methoxy derivatives **22** via an acylimine. In addition, an efficient method was described for the preparation of *N*-hydroxy-3-methylenedioxopiperazines **23** and **24a** (Scheme IV). As we found earlier that a Markownikoff-type H_2S addition to an *exo*-methylene bond can be performed by using $ZnCl_2$ as a catalyst,[10,16] it was self-evident to study the conversion of **23** or **24** into the corresponding 3,6-dimercaptodioxopiperazines **29**. Oxidation of this compound was expected to give the epidithio compounds **30**.

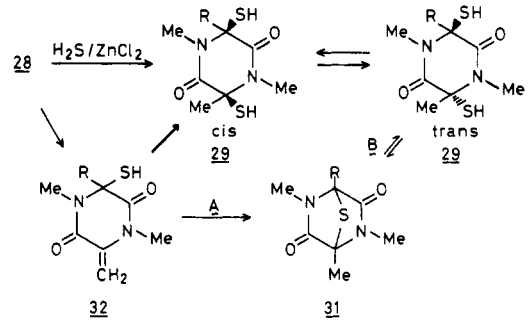
Results and Discussion

For facilitation of the formation of the intermediate acylimine **26a**, the *N*-hydroxyamide **24a** was tosylated to give **25a**. Subsequent treatment of **25a** with 1 equiv of *t*-BuOK in MeOH afforded the 3-methoxy-6-methylenedioxopiperazine **27a** in 23% overall yield (Scheme IV). It must have been formed by addition of methanol to the C=N bond of **26a**. As we had found earlier that epidithiodioxopiperazines with a secondary amide function are not stable,[17] compound **27a** was converted into **28a** (92%) by treatment with CH_3I and K_2CO_3 in dimethylformamide.[18] Reaction of **27a** with liquid H_2S and $ZnCl_2$ as a catalyst yielded the dithiol **29a**, which appeared to be only one stereoisomer by ^1H NMR spectroscopy. Oxidation with I_2/pyridine gave the epidithiodioxopiperazine **30a** (5% overall yield from **24a**). Optimization of the reaction sequence **24a** → **30a** could be achieved by omitting several purification steps. Furthermore, it was argued that the overall yield might be improved by starting from the precursor of **24**, namely, **23**, and treating this compound directly with the base. Indeed, reaction of **23a** with 1 equiv of *t*-BuOK in MeOH afforded, according to ^1H NMR, **27a** almost quantitatively. This reaction mixture was used without purification for the preparation of the epidithiodioxopiperazine **30a** as described above. By this procedure the overall yield of **23a** → **30a** was raised to 46%.

For the investigation of the general applicability of this method, **23b** and **23c** were also subjected to the same reaction conditions.[19] The first two steps proceeded well, but the reaction of **28b** and **28c** with liquid H_2S in the presence of $ZnCl_2$ gave a surprising result. Treatment of **28b** yielded, besides dithiol **29b**, the monosulfide **31b** (Scheme IV), so that on oxidation a mixture of **30b** and **31b** was obtained in a ratio of 1:3. Treatment of this mixture with Ph_3P in dioxane[16] afforded, after column chromatography, the pure monosulfide **31b** in an overall yield of 57% from **23b**.

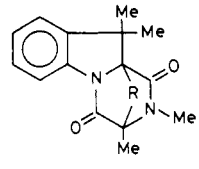
In contradistinction to **28b**, **28c** yielded on treatment with $H_2S/ZnCl_2$ only the monosulfide **31c** (22% overall yield from **23c**). The formation of **31**, besides **29**, may be explained in the following way (Scheme V). Replacement

Scheme V



32 **31**

of the COMe group in **28** by a mercapto group leads to the mercaptoalkene **32**. Protonation of the double bond might be followed either by the introduction of a second mercapto function to give **29** or by an intramolecular S_N1 attack of the thiol function to yield **31** (pathway A). This mechanism has been proposed by Yoshimura[20] for a similar reaction. Another possible interpretation is that **31** is formed by a transanular thiol attack on the trans dithiol **29** (pathway B), which is formed, besides the cis dithiol **29**, when **32** is treated with liquid H_2S and $ZnCl_2$. Although there is no definite evidence permitting a choice between these two explanations, we were able to show that pathway B can certainly play a role in the formation of **31**. Monosulfide **31b** was found to be unstable under the reaction conditions used: treatment with liquid H_2S and $ZnCl_2$ gave, besides recovered starting material (75%), the cis dithiol **29b** in 25% yield. As $ZnCl_2$-catalyzed decomposition of **31** into **32** can be ruled out,[21] this establishes equilibration between *cis*-**29**, *trans*-**29**, and **31**. One might argue that **31** formed in this experiment might have arisen through pathway A by decomposition of *cis*-**29** into **32**. However, this is not likely as *cis*-**29a** and **33** were found to be stable compounds.[22] As additional evidence for the cis–trans equilibration, we found that the optically active cis dithiol[23] **33** racemizes when treated with liquid H_2S and



33 R = -SH,HS-
34 R = -S-S-
35 R = -S-

$ZnCl_2$. Finally, the formation of **31** from the trans dithiol **29** by a transanular attack is related to the internal S_N2 reaction by which the monosulfide **35** is formed from **34** on treatment with triphenylphosphine.[24] The synthesis of sulfur-bridged dioxopiperazines from **24** lends support to the hypothesis that the same kind of compounds might be obtained from di-*N*-hydroxydioxopiperazines. Presently, this conversion is being studied. In addition, work is in progress to use *N*-hydroxy-α-amino acid derivatives as synthons for other natural products that contain α-substituted amino acids.

Experimental Section

Infrared spectra were measured with a Perkin-Elmer spectrophotometer, Model 397. Proton magnetic resonance spectra

(16) H. C. J. Ottenheijm, J. D. M. Herscheid, G. P. C. Kerkhoff, and T. F. Spande, *J. Org. Chem.*, 41, 3433 (1976).
(17) H. C. J. Ottenheijm, J. D. M. Herscheid, M. W. Tijhuis, M. Oosterbaan, and E. DeClercq, *J. Med. Chem.*, 21, 796 (1978).
(18) D. H. Rich, J. Tam, P. Mathiaparanam, and J. Grant, *Synthesis*, 402 (1975).
(19) Earlier we had found (see ref 14) that the conversion of **23c** into **24c** did not proceed satisfactorily. Therefore, the planned synthesis of the hyalodendrin analogue **30c** had to start with **23c**.
(20) J. Yoshimura, H. Nakamura, and K. Matsanari, *Bull. Chem. Soc. Jpn.*, 48, 605 (1975).
(21) During the workup excess H_2S is removed, leaving the reaction products in a suspension of $ZnCl_2$ in CH_2Cl_2.
(22) The stability of *cis*-dimercaptodioxopiperazines in the presence of a Lewis acid has also been shown by T. Fukuyama, S. Nakatsuka, and Y. Kishi, *Tetrahedron Lett.*, 3393 (1976).
(23) H. C. J. Ottenheijm, J. D. M. Herscheid, M. W. Tijhuis, R. J. F. Nivard, E. DeClercq, and P. A. J. Prick, *J. Med. Chem.*, 21, 799 (1978).
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were measured on a Varian Associates Model T-60 or a Bruker WH-90 spectrometer. Chemical shifts are reported as δ values (parts per million relative to tetramethylsilane as an internal standard); deuteriochloroform was used as solvent. Mass spectra were obtained with a double-focusing Varian Associates SMI-B spectrometer. Thin-layer chromatography (TLC) was carried out by using Merck precoated silica gel F-254 plates (thickness 0.25 mm). Spots were visualized with a UV hand lamp, with iodine vapor, and, in the case of sulfur-containing products, by spraying with 2% aqueous $AgNO_3$.[25] The reaction with liquid H_2S was carried out in a commercial flask [Lab Crest aerosol reaction vessel (Fischer and Porter)] which was protected by a cylindrical plexiglass shield.

1-(Tosyloxy)-3-methylene-4-methyl-2,5-dioxopiperazine (25a). A stirred solution of 781 mg (5 mmol) of **24a**[14] in 30 mL of dry acetonitrile was treated at room temperature with 953 mg (5 mmol) of tosyl chloride and 506 mg (5 mmol) of triethylamine. The reaction mixture was stirred at room temperature overnight, after which the solvent was evaporated. The residue was dissolved in CH_2Cl_2, and this solution was washed with water. Drying (Na_2SO_4) followed by evaporation of the solvent gave the tosylate **25a** in quantitative yield, which was homogeneous on TLC (3% MeOH/CH_2Cl_2): ^1H NMR δ 8.00–7.21 (AB spectrum, 4 H, C_6H_4), 5.70 (d, 1 H, C=CH_α), 4.98 (d, 1 H, C=CH_β), 4.52 (s, 3 H, C(6)H_2), 3.20 (s, 3 H, NCH_3), 2.47 (s, 3 H, CCH_3).

1-Methyl-3-methoxy-6-methylene-2,5-dioxopiperazine (27a). From 25a. A stirred solution of 310 mg (1 mmol) of **25a** in 100 mL of MeOH was treated at room temperature with 112 mg (1 mmol) of $(CH_3)_3COK$. Stirring was continued at room temperature for 15 min. After evaporation of the solvent CH_2Cl_2 was added. The salts were removed by filtration, after which the solvent was evaporated. Column chromatography on 40 g of Merck 60 silica gel H in CH_2Cl_2/2% MeOH under slightly increased pressure (about 10 cmHg) afforded 40 mg (23%) of **27a** which was homogeneous by TLC (CH_2Cl_2/8% MeOH): ^1H NMR δ 8.0 (br s, 1 H, NH), 5.73 (d, 1 H, C=CH_α), 4.97 (d, 1 H, C=CH_β), 4.83 (br s, 1 H, C(3)H), 3.36 (s, 3 H, OCH_3), 3.13 (s, 3 H, NCH_3).

From 23a. Compound **23a**[14] (246 mg, 1 mmol) was treated as described for **25a**, with the exception that the reaction mixture was stirred for 16 h instead of 15 min. Before evaporation of the MeOH, the reaction mixture was neutralized with NH_4Cl, after which the procedure described above was used. According to TLC (CH_2Cl_2/8% MeOH) and ^1H NMR spectroscopy, the reaction mixture consisted of **27a** and benzyl alcohol; it was used without further purification for the synthesis of **28a**.

1,4-Dimethyl-3-methylene-6-methoxy-2,5-dioxopiperazine (28a). To a stirred solution of the crude product **27a**, obtained from **23a** (1 mmol), in 6 mL of DMF (distilled and stored over 4-Å molecular sieves) were added at room temperature 790 mg (5 mmol) of CH_3I (eluted over neutral aluminum oxide) and 475 mg (5 mmol) of K_2CO_3 (dried at 200 °C). The reaction mixture was stirred for 2 days at room temperature, after which solvent and excess reagent were removed in vacuo (1 mmHg, bath temperature 40 °C). Then CH_2Cl_2 and water were added. The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo to give **28a**, which was homogeneous by TLC (CH_2Cl_2/6% MeOH). This product was converted into **29a** without further purification. For **28a**: ^1H NMR δ 5.77 (d, 1 H, C=CH_α), 4.94 (d, 1 H, C=CH_β), 4.80 (s, 1 H, C(3)H), 3.36 (s, 3 H, OCH_3), 3.17 (s, 3 H, N(1)CH_3), 3.03 (s, 3 H, N(4)CH_3).

1,3,4-Trimethyl-3,6-dimercapto-2,5-dioxopiperazine (29a). The crude product **28a** (1 mmol) was converted into **29a** as has been described[16] by treatment with liquid H_2S in the presence of $ZnCl_2$: ^1H NMR δ 5.30 (s, 1 H, C(3)H), 3.13 (s, 3 H, N(1)CH_3), 3.10 (s, 3 H, N(4)CH_3), 2.0 (s, 3 H, C(6)CH_3).

1,3,4-Trimethyl-3,6-epidithio-2,5-dioxopiperazine (30a). The crude dithiol **29a** (1 mmol) was oxidized to the corresponding disulfide **30a** with I_2 in pyridine and CH_2Cl_2 as has been described.[16] Column chromatography (column 25 × 2.5 cm) on 30 g of silica gel H (Merck 60) with 2% MeOH/CH_2Cl_2 under slightly increased pressure (10 cmHg) afforded 100 mg (0.46 mmol, 46% overall yield from **23a**) of **30a**, which was homogeneous on TLC (3% MeOH/CH_2Cl_2) and $AgNO_3$ positive: IR ($CHCl_3$) 1694 cm^{-1} (C=O); ^1H NMR δ 5.33 (s, 1 H, C(3)H), 3.13 (s, 3 H, N(1)CH_3), 3.06 (s, 3 H, N(4)CH_3), 2.0 (s, 3 H, C(6)CH_3). Accurate mass determination (chemical ionization) calcd for $C_7H_{10}N_2O_2S_2$: mol wt 218.1994. Found: mol wt 218.1993.

1,3-Dimethyl-3-methoxy-6-methylene-2,5-dioxopiperazine (27b). Compound **27b** was prepared from **23b**[14] (520 mg, 2 mmol) as has been described for the synthesis of **27a** from **23a**. The crude reaction mixture was used without further purification in the next step. For **27b**: ^1H NMR δ 5.79 (d, 1 H, C=CH_α), 4.91 (d, 1 H, C=CH_β), 3.18 (s, 3 H, OCH_3), 3.12 (s, 3 H, NCH_3), 1.67 (s, 3 H, C(3)CH_3).

1-Methyl-3-benzyl-3-methoxy-6-methylene-2,5-dioxopiperazine (27c). This compound was prepared from **23c**[14] (168 mg, 0.5 mmol) as has been described for the preparation of **27a** from **23a**. The reaction product was used without further purification in the next reaction. For **27c**: ^1H NMR δ 7.13 (m, 5 H, C_6H_5), 5.60 (d, 1 H, C=CH_α), 4.70 (d, 1 H, C=CH_β), 3.30 (s, 3 H, OCH_3), 3.23 (m, 2 H, CH_2), 3.13 (s, 3 H, NCH_3).

1,4,6-Trimethyl-3-methylene-6-methoxy-2,5-dioxopiperazine (28b). Compound **28b** was prepared from **27b** as has been described for the synthesis of **28a**. Methylation was complete according to TLC (3% MeOH/CH_2Cl_2) and ^1H NMR spectroscopy: ^1H NMR δ 5.84 (d, 1 H, C=CH_α), 4.97 (d, 1 H, C=CH_β), 3.23 (s, 3 H, OCH_3), 3.07, 3.00, and 1.66 (3 s, 3 H each, N(1)CH_3, N(4)CH_3, and C(6)CH_3, respectively).

1,4-Dimethyl-3-methylene-6-benzyl-6-methoxy-2,5-dioxopiperazine (28c). Compound **27c** was converted into **28c** as has been described for the synthesis of **28a**. The methylation was complete according to TLC (3% MeOH/CH_2Cl_2) and ^1H NMR spectroscopy: ^1H NMR δ 7.50–6.90 (m, 5 H, C_6H_5), 5.40 (d, 1 H, C=CH_α), 4.47 (d, 1 H, C=CH_β), 3.40 (m, 2 H, CH_2), 3.20 (s, 3 H, OCH_3), 3.13 (s, 3 H, N(1)CH_3), 3.0 (s, 3 H, N(4)CH_3).

1,3,4,6-Tetramethyl-3,6-epithio-2,5-dioxopiperazine (31b). The crude product **28b** (2 mmol) was treated with liquid H_2S in the presence of $ZnCl_2$ as has been described.[16] By this treatment a mixture of the dithiol **29b** and the monosulfide **31b** (ratio 1:3) was formed. The dithiol in this reaction mixture could be converted into the disulfide **30b** on treatment with I_2 in pyridine as has been described;[16] partial desulfurization of the latter with 130 mg (0.5 mmol) of triphenylphosphine in dioxane as has been reported[24] gave **31b**. The reaction mixture was column chromatographed (column 21 × 3.5 cm) on 75 g of silica gel H (Merck 60) with CH_2Cl_2/1% MeOH as eluent to yield 227 mg of **31b** (1.14 mmol, 57% overall yield from **23b**). **31b**: ^1H NMR δ 2.80 (s, 6 H, 2 NCH_3), 1.77 (s, 6 H, 2 C(CH_3)); IR ($CHCl_3$) 1710 cm^{-1} (CO). Accurate mass determination (chemical ionization) calcd for $C_8H_{12}N_2O_2S$: mol wt 200.1525. Found: mol wt 200.1504. **29b**: ^1H NMR δ 3.20 (s, 6 H, 2 NCH_3), 2.03 (s, 6 H, 2 C(CH_3)). **30b**: ^1H NMR δ 3.07 (s, 6 H, 2 NCH_3), 2.01 (s, 6 H, 2 C(CH_3)); $AgNO_3$ positive.

1,3,4-Trimethyl-6-benzyl-3,6-epithio-2,5-dioxopiperazine (31c). The crude compound **28c** (0.5 mmol) was treated with liquid H_2S in the presence of $ZnCl_2$ as has been described.[16] According to TLC (2% MeOH/CH_2Cl_2) and ^1H NMR spectroscopy, the reaction mixture consisted mainly of **31c**. Accordingly, only a trace of I_2 was consumed when the crude product was treated with I_2 and pyridine.[16] Column chromatography (column 25 × 2.5 cm) on 35 g of silica gel H (Merck 60) with 1% MeOH/CH_2Cl_2 as eluent under increased pressure (10 cmHg) gave 30 mg of **31c** (22% overall yield from **23c**), which was homogeneous on TLC (2% MeOH/CH_2Cl_2) and $AgNO_3$ positive: ^1H NMR δ 7.33 (m, 5 H, C_6H_5), 3.83 and 3.33 (2 d, J = 13 Hz, $C_6H_5CH_\alpha$ and $C_6H_5CH_\beta$), 3.00, 2.87, and 1.83 (3 s, 3 H each, N(1)CH_3, N(4)CH_3, and C(6)CH_3, respectively); IR ($CHCl_3$) 1710 cm^{-1}. Accurate mass determination (chemical ionization) calcd for $C_{14}H_{16}N_2O_2S$: mol wt 276.1838. Found: mol wt 276.1861.

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Registry No. 23a, 73198-61-5; **23b**, 73333-68-3; **23c**, 73198-63-7; **24a**, 73198-67-1; **25a**, 73333-69-4; **27a**, 73333-70-7; **27b**, 73333-71-8; **27c**, 73333-72-9; **28a**, 73333-73-0; **28b**, 73333-74-1; **28c**, 73333-75-2; *cis*-**29a**, 73333-76-3; *cis*-**29b**, 73333-77-4; **30a**, 49593-06-8; **30b**, 53338-36-6; **31b**, 53338-32-2; **31c**, 73347-60-1; *trans*-**29b**, 73333-78-5; gliotoxin, 67-99-2.

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