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Synthetic Studies on Quinoxaline Antibiotics. II.[1] Synthesis of Triostin A[2]

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Triostin A, a cyclic octadepsipeptide, was synthesized with Z-D-Ser[Boc-Ala-MeCys(Bzl)-MeVal]-OH and Z-D-Ser[H-Ala-MeCys(Bzl)-MeVal]-OTce as key intermediates.† The synthetic antibiotic was compared with natural triostin A in terms of chromatographic behaviors, NMR spectra, and antimicrobial activity to establish their identity. The NMR data on *S,S'*-dibenzyldihydrotriostin A showed that this intermediate lacking the disulfide linkage also existed as two conformers in chloroform. This observation excludes the possibility that the conformer equilibrium known to occur with triostin A is a consequence of the reversed chirality of the disulfide bond.

Triostin A[3] is a member of the triostin family of the quinoxaline antibiotics.[4] The triostins were isolated and structure-elucidated in these Laboratories.[3] The quinomycins[5] which constitute another family of quinoxaline antibiotics are represented by echinomycin[6] (or quinomycin A[5]). In contrast to the quinomycins which are produced by widely distributed streptomycetes, the triostins have ever been found only in a strain of *Streptomyces aureus*.[7] The quinoxaline antibiotics possess antibacterial[5] and cytotoxic[4,8] activities. They are known to bind DNA as bifunctional intercalating agents[9] and thereby inhibit RNA synthesis.[10] Triostin A is characterized by its cyclic octadepsipeptide structure with a cross-bridged disulfide linkage (Fig. 1).[3b,11] It contains an *N,N'*-dimethyl-L-cystine residue and two sets of D-serine, L-alanine, and *N*-methyl-L-valine residues. The 2-quinoxalinylcarbonyl (Qxc) moiety is attached to the amino group of the D-serine residues. Recent NMR studies have shown that triostin A exists as a mixture of two conformers in deuteriochloroform.[11,12] Kalman *et al.*[13] have postulated that occurrence of the two conformers is a consequence of the reversal of chirality of the disulfide bond, while Kyogoku *et al.*[14] attributed it to the *cis-trans* isomerization of the *N*-methyl peptide bonds. The former possibility has recently been ruled out because it was found that the *S,S'*-dibenzyl derivative of dihydrotriostin A (reduced form of triostin A) also had two conformers.[15]

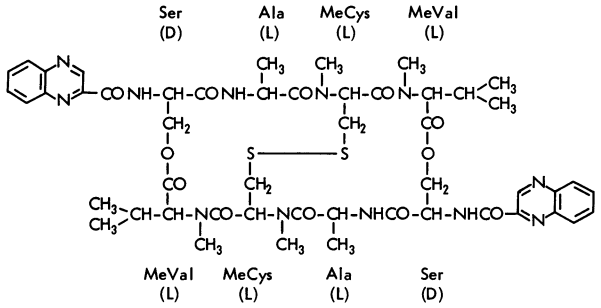

Fig. 1. Structure of Triostin A.

The synthesis of triostin A[16] and of some analogs[17,18] has been reported by Olsen *et al.* In a previous paper[1] we reported the synthesis of 2-quinoxalinylcarbonyl tetradepsipeptide derivatives of the types, Qxc-D-Ser(R^1-MeVal)-Ala-MeCys(Bzl)-OR^2 and Qxc-D-Ser[R^1-MeCys(Bzl)-MeVal]-Ala-OR^2 (R^1=Z, Bpoc, or H; R^2=Bu^t or H), but in the subsequent studies these derivatives proved to be poor intermediates for the synthesis of triostin A. The present paper describes our total synthesis of triostin A, performed with Z-D-Ser[Boc-Ala-MeCys(Bzl)-MeVal]-OH (**1**) and Z-D-Ser[H-Ala-MeCys(Bzl)-MeVal]-OTce (**2**) as key intermediates.

Figure 2 illustrates the procedure for the synthesis of the two tetradepsipeptide derivatives **1** and **2**. The starting material Z-D-Ser(Bu^t)-OH (**3**) was prepared as described for the corresponding L-isomer.[19] Com-

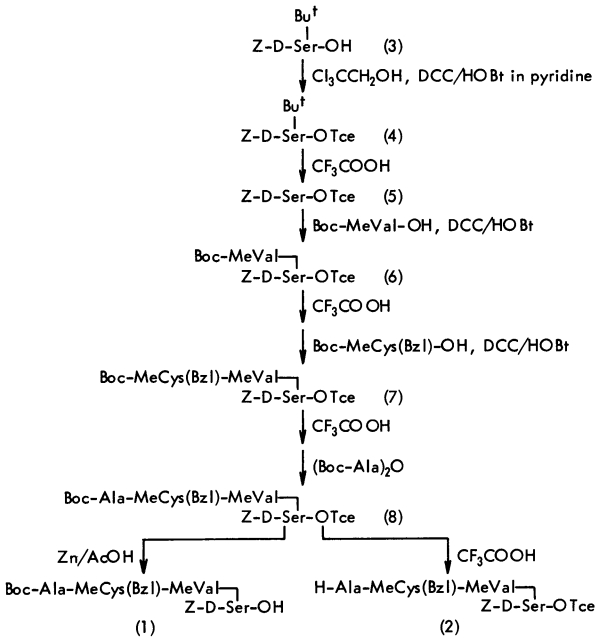

Fig. 2. Synthesis of tetradepsipeptide intermediates.

†Abbreviations used are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature [*Biochemistry*, **11**, 1726 (1972)] and include: MeCys, N-methyl-L-cysteine, MeVal; *N*-methyl-L-valine; Boc, *t*-butoxycarbonyl; Boc-SDP, *O-t*-butyl *S*-(4,6-dimethyl-2-pyrimidinyl) thiocarbonate; Bpoc, 1-(4-biphenylyl)-1-methylethoxycarbonyl; Qxc, 2-quinoxalinylcarbonyl; Z, benzyloxycarbonyl; Z(OMe), 4-methoxybenzyloxycarbonyl; Z(OMe)-SDP, *O-p*-methoxybenzyl *S*-(4,6-dimethyl-2-pyrimidinyl)thiocarbonate; Bu^t, *t*-butyl; Bzl, benzyl; Tce, 2,2,2-trichloroethyl; HOBt, 1-hydroxybenzotriazole; HONp, 4-nitrophenol; HOSu, *N*-hydroxysuccinimide; CDI, *N,N'*-carbonyldiimidazole; DCC, dicyclohexylcarbodiimide; DCU, *N,N'*-dicyclohexylurea; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; DPPA, diphenylphosphorazidate; AcOH, acetic acid; TFA, trifluoroacetic acid; DCHA, dicyclohexylamine; DIEA, *N,N*-diisopropylethylamine; TEA, triethylamine; DMF, *N,N*-dimethylformamide; and THF, tetrahydrofuran.

pound **3** was esterified with 2,2,2-trichloroethanol (HOTce)[20] by the action of DCC in the presence of HOBt to give **4**. The yield of the Tce ester was as high as 97% when pyridine was used as a solvent. To remove the Bu^t group compound **4** was treated with TFA and the resulting Z-D-Ser-OTce (**5**) was coupled with Boc-MeVal-OH by the use of DCC-HOBt in pyridine[16] to give depsipeptide **6** in 60—70% yields. In the absence of HOBt the yield of **6** was 35% and the formation of *N*-acylurea was predominant (62%). Compound **6** was, after removal of the Boc group with TFA, coupled with Boc-MeCys(Bzl)-OH by the DCC-HOBt method[21] to give **7** in 70—80% yields. Compound **7** was also synthesized using Z(OMe)-MeVal-OH in place of Boc-MeVal-OH. Removal of the Boc group from **7** was followed by the incorporation of Boc-Ala to give **8**, in which the symmetrical anhydride $(Boc\text{-}Ala)_2O$ proved to be a good acylating agent, whereas the usual DCC-mediated coupling with Boc-Ala-OH failed to furnish the desired compound. When acylated with the mixed anhydride derived from Boc-Ala-OH and isobutyl chloroformate, Z-D-Ser[isobutoxycarbonyl-MeCys(Bzl)-MeVal]-OTce was the sole product (90%). The protected tetradepsipeptide (**8**) thus obtained in a 70% yield was treated with either Zn in 90% AcOH[22] to remove the Tce group or TFA to remove the Boc group, giving the key compounds **1** and **2**, respectively. The removal of the Tce group was complete within 3 h and compound **1** was obtained in a 90% yield. Further steps leading to the formation of triostin A are shown in Fig. 3.

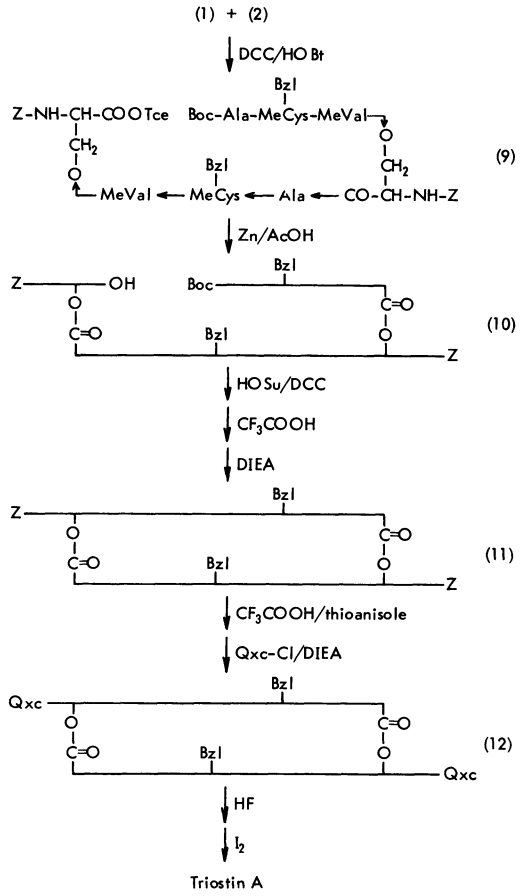

Fig. 3. Synthesis of triostin A.

The coupling of **1** and **2** with DCC-HOBt in THF afforded the linear octadepsipeptide (**9**) in 82—92% yields. Compound **9** was then treated with Zn in 90% AcOH to effect the complete removal of the Tce group giving **10** in yields higher than 90%. For the subsequent cyclization compound **10** was converted into the *N*-hydroxysuccinimide ester in the usual manner except for using HOSu and DCC in large excess and, after removal of the Boc group with TFA, the active ester was dissolved in a large volume of ethyl acetate. The cyclization was then initiated by the addition of DIEA under conditions of high dilution (0.2 mmol peptide/l). The progress of the reaction was monitored by high-performance liquid chromatography (HPLC) on a μ-Porasil column with 2-propanol-hexane (5:95) as solvent to show that the reaction was complete within 3 h. Cyclic octadepsipeptide **11** was isolated in pure form in 58% yield from **10** (mean for seven trials). The ^{1}H-NMR spectrum and the field desorption mass spectrum of **11** were consistent with the proposed structure. Conversion of **11** to *S,S'*-dibenzyl-dihydrotriostin A (**12**) was effected by selective removal of the Z group with TFA-thioanisole,[23] and the subsequent acylation with Qxc-Cl[24] or Qxc-ONp[1] in the presence of triethylamine. The yield of **12** was 28—37% as estimated by HPLC. The crude product was purified on silica-gel columns to give a pure preparation of **12** in an over-all yield of 13—22% from **11**. Compound **12** was treated with HF-anisole at 25 °C for 30 min to remove the benzyl groups, followed by treatment with iodine in methanol[25] to form the disulfide bond. The crude product was purified by silica-gel column chromatography and by recrystallization affording triostin A in a 20% yield from **12**. The synthetic antibiotic thus obtained was indistinguishable from natural triostin A in TLC and in HPLC (Fig. 5). The identity of natural and synthetic preparations was also confirmed by their superimposable 360 MHz ^{1}H-NMR spectra (Fig. 4). Synthetic triostin A was as active as natural triostin A when assayed for antimicrobial activity against *Bacillus subtilis* PCI-219. The minimum effective concentration was 0.5 μg/ml.

In 1978 Chakravarty and Olsen[16] briefly reported the first synthesis of triostin A, in which they used tetradepsipeptide fragments, Z-D-Ser[R-MeCys(Bam)-MeVal]-Ala-OH (I: R=Boc, II: R=H; Bam=benzamidomethyl), as intermediates to prepare their cyclic octadepsipeptide (III). Fragment couplings are usually susceptible to racemization at the *C*-terminal amino acid of the carboxyl component. In this case, therefore, racemization could occur at the Ala residue during the formation of a linear octadepsipeptide from I and II, and also in the subsequent cyclization leading to III. In fact, Olsen and collaborators[17] observed that a linear octadepsipeptide, an intermediate to des-*N*-tetramethyltriostin A, derived from Z-D-Ser[Boc-Cys(Acm)-Val]-Ala-OH and Z-D-Ser[H-Cys(Acm)-Val]-Ala-OTce (Acm=acetamidomethyl) by the EDC-HOBt method contained 6.4% of D-alanine. This means that 25.6% racemization had occurred to the Ala residue involved in the coupling reaction. This racemization was a problem in their synthesis of triostin A and analogs, although they suggested that the diaste-

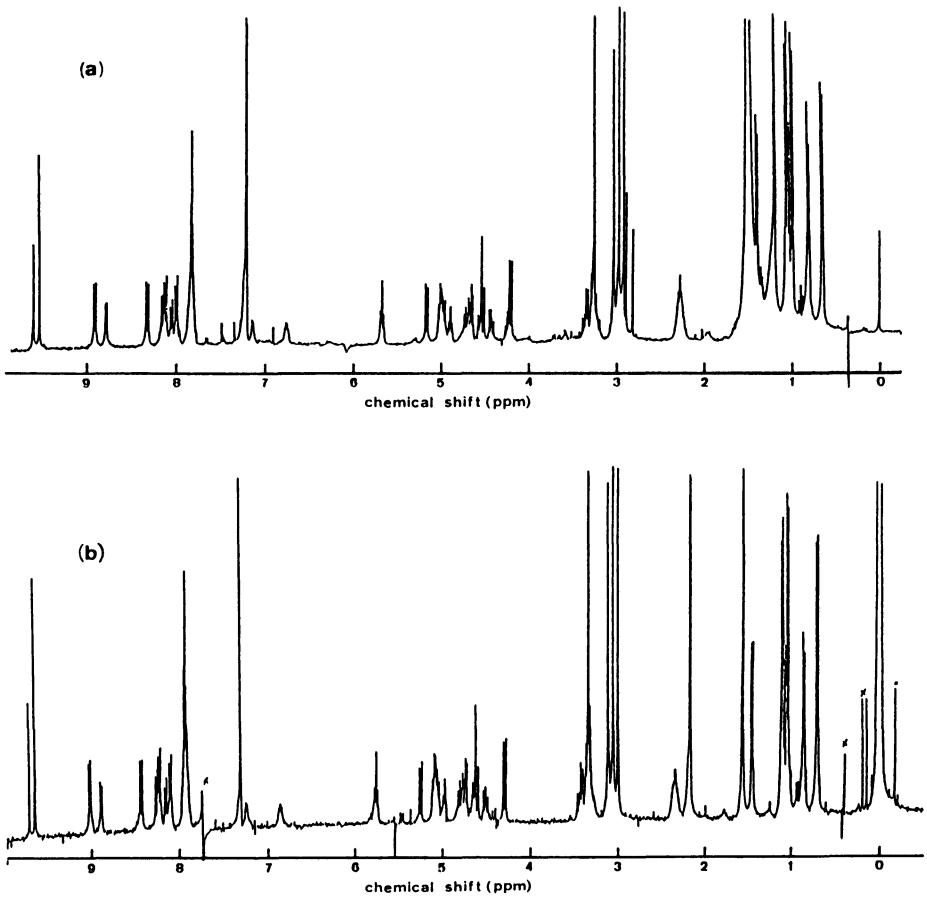

Fig. 4. 360 MHz ^{1}H-NMR spectra of (a) synthetic and (b) natural preparations of triostin A in $CDCl_3$.

reoisomeric impurity could largely be removed during purification. In our present synthesis of triostin A, the coupling of two tetradepsipeptides **1** and **2**, and the subsequent cyslization of the linear octadepsipeptide (**10**) were both performed by activation of the carboxyl function of the *O*-acylated Z-D-serine, Z-D-Ser(R)-OH (R=Boc-peptide group), to form a normal Ser-Ala bond, as shown in Fig. 2 and Fig. 3. This new approach should not only eliminate the danger of racemization, but also make the coupling reaction easier to proceed than what involves the *N*-methylamino acid.

Chakravarty and Olsen[16] stated that the removal of the Tce group from a depsipeptide of the type Z-D-Ser(R)-Ala-OTce (R=Boc-peptide) with zinc in acetic acid was incomplete (65%) in the case of tetradepsipeptide and it was practically impossible in the case of octadepsipeptide. In our synthesis (Fig. 2 and Fig. 3), on the other hand, the complete removal of the Tce group from a depsideptide of the type Z-D-Ser(R)-OTce (R=Boc-peptide) was readily achieved in both tetradepsipeptide **8** and octadepsipeptide **9** by the treatment with zinc in 90% acetic acid, suggesting that the Tce ester of the D-serine in **8** and **9** is exposed to the molecular surface, whereas the alanine ester in Olsen's intermediates is rather buried, especially in the case of the octadepsipeptide.

The cyclization of linear octadepsipeptide **10** was performed by the *N*-hydroxysuccinimide ester method as described above. Table 1 represents the results of the test experiment, in which the data show that the formation of cyclic peptide **11** from **10** is remarkably increased as the peptide concentration is decreased. This is naturally because the undesired formation of oligomers should be suppressed by lowering the concentration, while the intramolecular cyclization is independent of concentration. As is seen in Table 1, the yield of cyclic peptide **11** does not depend on the solvent used, we therefore employed ethyl acetate as solvent in the cyclization reaction of a preparative scale. Chakravarty and Olsen[16] synthesized a cyclic octadepsipeptide as intermediate to triostin A by one-step cyclization of the corresponding linear peptide with both *C*- and *N*-terminals unprotected, in which the yield of the cyclic peptide was 22% when the EDC-HOSu method and a peptide concentration of *ca.* 2.5 mmol/l were employed. In the case of cyclization leading to des-*N*-tetramethyltriostin A (nortriostin A) analogs,[17,18] however, higher yields up to 55% have been reported. In the present work we also tried this one-step method. Compound **10** was treated with TFA to remove the Boc group and the resulting material (2 mmol/l in THE or DMF) was treated with 5 or 10 equiv of DCC-HOBt, EDC-HOBt, or CDI at 25 °C, or with 5 equiv of DPPA[26] at 5 °C in the presence of DIEA (1 equiv). Among these the DPPA method gave the best result, producing cyclic peptide **11** in 24% yield as estimated by HPLC. The DCC-HOBt method pro-

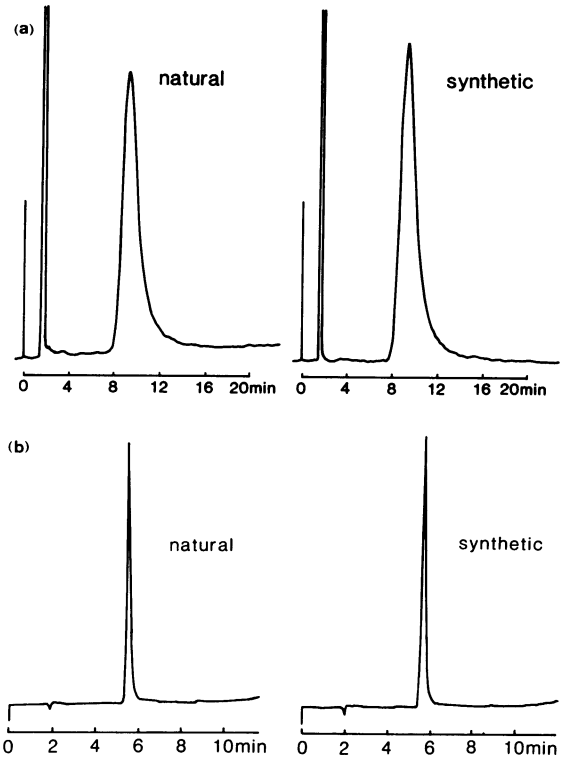

Fig. 5. HPLC profiles of synthetic and natural preparations of triostin A. (a) Column: m-Porasil, 30 cm×3.9 mm ID; solvent: 2-propanol-hexane (27.5:72.5); flow rate: 2 ml/min; detection: at 220 nm. (b) Column: Hypersil $3C_{18}$, 10 cm×6.0 mm ID; solvent: a linear gradient (20 min) of 50—90% CH_3CN in 0.1 M (1 M=1 mol dm^{-3}) triethylammonium acetate (pH 7.0); flow rate: 1 ml/min; detection: at 243 nm.

TABLE 1. CONCENTRATION DEPENDECY OF CYCLIZATION REACTION IN THE CONVERSION OF **10** TO **11**[a]

Concentration of peptide (mmol/l)	Solvent	Yield[b] (%)
20	EtOAc	13
2	EtOAc	39
0.2	EtOAc	56
20	DMF	12
2	DMF	42(31)[c]
0.2	DMF	58

a) The cyclization was carried out at 25 °C for 3 h and monitored by HPLC under the following conditions: column: μ-Porasil (Waters), 30 cm×3.9 mm ID; solvent: 2-propanol-hexane (5:95, v/v); flow rate: 2 ml/min; detection: at 220 nm and 243 nm. b) Overall yield of **11** from **10** (see Fig. 3). c) In the presence of HOBt (20 mmol/l).

duced **11** in 12, 19, and 14% yields when the peptide concentrations were 10, 2, and 0.4 mmol/l, respectively. These low yields may be ascribed to the peptide concentrations employed, which must have been lower than adequate for the activation of the linear peptide and, at the same time, too high to prevent the undesired intermolecular reaction of the activated peptide. In fact, the occurrence of a larger amount of by-products was observed in the one-step method than in the two-step cyclization by the active ester method.

The selective removal of the Z groups from **11** by the treatment with TFA-thioanisole was based on the "push-pull" mechanism described by Kiso *et al.*[23] Preliminary tests were performed with Z-Cys(Bzl)-OH, Z-MeCys(Bzl)-OH, Z-D-Ser[isobutoxycarbonyl-MeCys-(Bzl)-MeVal]-OTce, and Qxc-D-Ser(Z-MeVal)-Ala-MeCys(Bzl)-OH[1] as model compounds to show that in every case the Z group was completely removed in 4—6 h at 25 °C without appreciable cleavage of the *S*-benzyl group. The similar conditions (25 °C, 6 h) could be applied to compound **11** to give the N^{α}-free intermediate (*S,S'*-dibenzyldihydroapotriostin A) without any difficulties. The subsequent quinoxalylcarbonylation leading to **12** was carried out with 50—100% excess of Qxc-Cl and a minimum amount of TEA to minimize the O→N acyl shift at the D-Ser residue.

Compound **12** obtained above is the *S,S'*-dibenzyl derivative of dihydrotriostin A and lacks the disulfide bond. The ^{1}H-NMR data for **12** clearly show, however, that this compound still has two conformations in $CDCl_3$ as does triostin A itself. This evidence now excludes the possibility, postulated by Kalman *et al.*,[13] that the conformer isomerism of triostin A is resulted from the reversal of chirality of the disulfide bond.

Experimental

Thin-layer chromatography (TLC) was performed on silica-gel plates (precoated Kieselgel 60 F_{254}, Merck) with the following solvent systems (ratios by volume): A, chloroform-methanol (95:5); B, chloroform-methanol-acetic acid (95:5:3); C, benzene-ethyl acetate (90:10); D, benzene-ethyl acetate (70:30); E, benzene-ethyl acetate-acetic acid (70:30:3). Spots were revealed by UV, ninhydrin staining or by charring with sulfuric acid. For column chromatography, prepacked silica-gel columns (Lobar, size B or C, Merck) were mostly used. High-performance liquid chromatography (HPLC) was carried out on a Waters Associates Model 6000A solvent delivery system, equipped with a Waters U6K injector and a Japan Spectroscopic UVIDEC 100-II variable wavelength UV detector. Proton NMR spectra were recorded on a Varian T-60 NMR spectrometer operated at 60 MHz and/or a Bruker WM 360 wb NMR spectrometer at 360 MHz.

D-Serine was purchased from Fluka AG, Buchs, Switzerland, 2-methylquinoxaline from Aldrich Chemical Co., Inc., Milwaukee, WI, U.S.A., Boc-SDP and Z(OMe)-SDP from Protein Research Foundation, Osaka, diphenylphosphorazidate (DPPA) from Nakarai Chemicals, Ltd., Kyoto, and 2,2,2-trichloroethanol (TceOH) from Tokyo Kasei Kogyo Co., Ltd., Tokyo. *N*-Methyl-L-valine,[27] *N*-methyl-*S*-benzyl-L-cysteine,[28] quinoxaline-2-carboxylic acid (Qxc-OH),[6b] and 2-quinoxalinylcarbonyl chloride (Qxc-Cl)[24] were synthesized according to the literature. 4-Nitrophenyl quinoxaline-2-carboxylate (Qxc-ONp) was prepared as described previously.[1]

Z-D-Ser(Bu^t)-OH (3). This compound was prepared basically following the procedure described for the L-isomer;[19] mp 81—84 °C, $[\alpha]_D^{24}$ −18.5±0.6° (*c* 1.0, ethanol). Lit, for the L-isomer:[20] mp 83.5—85 °C, $[\alpha]_D^{23}$ +22.0° (*c* 1, ethanol). Found: C, 61.19; H, 7.19; N, 4.73%. Calcd for $C_{15}H_{21}NO_5$: C, 61.00; H, 7.17; N, 4.74%.

Z-D-Ser(Bu^t)-OTce (4). To a solution of **3** (14.8 g, 50 mmol), Tce-OH (8.22 g, 55 mmol), and HOBt (7.43 g, 55 mmol) in pyridine (100 ml) was added DCC (11.4 g,

55 mmol) and the mixture was stirred at 0—5 °C overnight. After removal of the separated DCU by filtration, the filtrate was evaporated *in vacuo*. The resulting material was dissolved in ethyl acetate and the solution was washed consecutively with ice-cold M hydrochloric acid, water, 5% sodium hydrogencarbonate, and water, dried over magnesium sulfate, and evaporated *in vacuo*. The residue (23 g) was subjected to chromatography on a column (6.0×28 cm) of silicagel (350 g, Kieselgel 60, Merck) with chloroform as solvent. The fractions containing the desired material, as monitored by TLC with benzene-ethyl acetate (90:10) as solvent, were collected and evaporated *in vacuo* to give a sirupy residue, which crystallized upon prolonged standing *in vacuo*; 20.8 g (97%), mp 67—70 °C, $[\alpha]_D^{25}$ +8.4±0.5 ° (*c* 1.0, methanol). TLC: homogeneous in system D. Found: C, 47.79; H, 5.23; N, 3.27; Cl, 24.90%. Calcd for $C_{17}H_{22}NO_5Cl_3$: C, 47.85; H, 5.20; N, 3.28; Cl, 24.92%.

Z-D-Ser-OTce (5). Compound **4** (4.27 g, 10 mmol) was treated with TFA (20 ml) at 25 °C for 30 min. After evaporation of the TFA *in vacuo* the residue was dissolved in ethyl acetate and the solution was washed with 5% sodium hydrogencarbonate and water, dried over magnesium sulfate, and evaporated *in vacuo*. The oily residue was subjected to a Lobar column (size C) with chloroform-methanol (95:5) as solvent. The chromatography was repeated twice and the fractions containing the desired compound as a single component were collected. Removal of the solvent *in vacuo* yielded **5** as a sirupy residue; 2.97 g (80%), $[\alpha]_D^{25}$ +14.9±0.5 ° (*c* 1.2, methanol). TLC: homogeneous in system A. Found: C, 42.31; H, 3.78; N, 3.75; Cl, 28.44%. Calcd for $C_{13}H_{14}NO_5Cl_3$: C, 42.13; H, 3.81; N, 3.78; Cl, 28.70%.

Boc-MeVal-OH Piperidine Salt. To a suspension of *N*-methyl-L-valine (9.18 g, 70 mmol) in water (40 ml) containing TEA (22.3 ml, 160 mmol) was added a solution of Boc-SDP (25.2 g, 105 mmol) in dioxane (40 ml) and the mixture was stirred at 25 °C overnight. After addition of ice-cold M sodium hydroxide (100 ml) and washing with ether, the aqueous solution was then acidified with ice-cold M hydrochloric acid. The product separated was taken into ethyl acetate and the organic solution was washed with water, dried over magnesium sulfate and evaporated *in vacuo*. The resulting oil was dissolved in ether and to this was added piperidine (5.02 g, 59 mmol). After evaporation of the ether the residue was crystallized from petroleum ether (16.4 g, 74%). Recrystallization from ethyl acetate-petroleum ether afforded a pure preparation of the desired compound; 13.0 g (59%), mp 97—99 °C, $[\alpha]_D^{25}$ −69.9±1.1 ° (*c* 1.0, methanol). Found: C, 60.24; H, 10.08; N, 8.52%. Calcd for $C_{11}H_{21}NO_4 \cdot C_5H_{11}N$: C, 60.73; H, 10.19; N, 8.85%.

The same compound was obtained in 54% yield with Boc-N_3 as acylating agent.[29]

Z(OMe)-MeVal-OH Piperidine Salt. *N*-Methyl-L-valine (2.62 g, 20 mmol) was acylated with Z(OMe)-SDP (7.61 g, 25 mmol) as in the corresponding Boc-derivative to give the desired compound; 3.98 g (52%), mp 92—93 °C, $[\alpha]_D^{22}$ −62.7±1.0 ° (*c* 1.0, methanol). Found: C, 63.36; H, 8.65; N, 7.20%. Calcd. for $C_{15}H_{21}NO_5 \cdot C_5H_{11}N$: C, 63.14; H, 8.48; N, 7.36%.

Boc-MeCys(Bzl)-OH DCHA Salt. *N*-Methyl-*S*-benzyl-L-cysteine (13.5 g, 60 mmol) was acylated with Boc-SDP in water-dioxane (1:2) in a manner similar to that described for Boc-MeVal-OH to give the desired compound as the DCHA salt (28.5 g). The crude product was purified by repeated recrystallization from ethyl acetate-petroleum ether; 24.6 g (81%), mp 104—105 °C, $[\alpha]_D^{24.5}$ −56.0±1.0 ° (*c* 1, methanol). Found: C, 66.27; H, 9.27; N, 5.39; S, 6.47%. Calcd for $C_{16}H_{23}NO_4S \cdot C_{12}H_{23}N$: C, 66.37; H, 9.15; N, 5.53; S, 6.33%.

Z-D-Ser(Boc-MeVal)-OTce (6). The piperidine salt of Boc-MeVal-OH (2.78 g, 8.8 mmol) was converted into the free acid by the treatment with Dowex 50W×8 (H^+ form) in 50% ethanol at 25 °C for 30 min. Boc-MeVal-OH obtained was dissolved in pyridine (25 ml) together with **5** (2.96 g, 8 mmol) and HOBt (1.19 g, 8.8 mmol), and to this was added a solution of DCC (1.82 g, 8.8 mmol) in pyridine (2 ml) at 0 °C. The mixture was stirred at 4 °C overnight, followed by evaporation *in vacuo*. The residue was dissolved in ethyl acetate and, after removal of precipitates by filtration, the solution was washed consecutively with ice-cold M hydrochloric acid, water, 5% sodium hydrogencarbonate and water, dried over magnesium sulfate and evaporated *in vacuo* to give an oil (5.1 g). The crude product was subjected to chromatography on a Lobar column (size C) with chloroform-methanol (99:1) as solvent. The fractions containing the desired material, as monitored by TLC with system C, were collected and evaporated *in vacuo*. The partially purified material was repeatedly chromatographed on Lobar columns with benzene-ethyl acetate systems as solvent to give **6** as an oil; 3.21 g (69%), $[\alpha]_D^{25}$ −28.3±0.5 ° (*c* 1.4, methanol). TLC: homogeneous in systems A and C. Found: C, 49.40; H, 5.82; N, 4.72; Cl, 18.19%. Calcd for $C_{24}H_{33}N_2O_8Cl_3$: C, 49.37; H, 5.70; N, 4.80; Cl, 18.22%.

Z-D-Ser[Z(OMe)-MeVal]-OTce (6a). Compound **6a** was synthesized as described for **6** except for the use of Z(OMe)-MeVal-OH in place of Boc-MeVal-OH; yield 74% (oil), $[\alpha]_D^{24}$ −24.1±0.6 ° (*c* 1.0, methanol). TLC: homogeneous in systems A and D. Found: C, 51.64; H, 5.19; N, 4.27; Cl, 16.16%. Calcd for $C_{28}H_{33}N_2O_9Cl_3$: C, 51.90; H, 5.13; N, 4.32; Cl, 16.42%.

Z-D-Ser[Boc-MeCys(Bzl)-MeVal]-OTce (7). *a) From 6.* Compound **6** (7.30 g, 12.5 mmol) was treated with TFA (30 ml) at 25 °C for 30 min, followed by evaporation *in vacuo*. The resulting oil was dissolved in ethyl acetate and the solution was washed with 5% sodium hydrogencarbonate, dried over magnesium sulfate, and evaporated *in vacuo*. The residue was dissolved in THF (25 ml) together with Boc-MeCys(Bzl)-OH, derived from the DCHA salt (6.97 g, 13.8 mmol) by the treatment with Dowex 50W×8 (H^+ form) in 50% ethanol, and HOBt (1.86 g, 13.8 mmol), and after addition of DCC (2.84 g, 13.8 mmol) the mixture was stirred at 4 °C overnight. The reaction mixture was then worked up in the usual manner to give a crude product (10.5 g) which was repeatedly chromatographed on a Lobar column (size C) with chloroform-methanol (95:5) and benzene-ethyl acetate (9:1) as solvent. The fractions containing the desired product as a single component were collected and evaporated *in vacuo* to give **7** as an oil; 7.47 g (76%), $[\alpha]_D^{27}$ −81.8±1.1 ° (*c* 1.1, methanol). TLC: homogeneous in systems A and D. Found: C, 52.95; H, 5.99; N, 5.22; S, 4.27; Cl, 13.33%. Calcd for $C_{35}H_{46}N_3O_9SCl_3$: C, 53.13; H, 5.86; N, 5.31; S, 4.05; Cl, 13.44%.

b) From 6a. Compound **7** was also synthesized from **6a** in basically the same manner as described in a); 74%(oil), $[\alpha]_D^{25.5}$ −81.1±1.1 ° (*c* 1.1, methanol). Found: C, 52.76; H, 5.86; N, 5.14; S, 4.41; Cl, 13.37%.

Z-D-Ser[Boc-Ala-MeCys(Bzl)-MeVal]-OTce (8). Boc-Ala-OH (1.70 g, 9 mmol) was treated with DCC (0.93 g, 4.5 mmol) in THF at 0—5 °C for 2 h. Removal of the separated DCU by filtration afforded a solution of $(Boc\text{-}Ala)_2O$. On the other hand, compound **7** (2.37 g, 3.0 mmol) was treated with TFA (12 ml) at 25 °C for 30 min, followed by evaporation *in vacuo*. The residue was, after being dried over potassium hydroxide pellets under reduced pressure, dissolved in THF and the solution was chilled at 0 °C. To this were then added DIEA (2.5 ml) and the solution of $(Boc\text{-}Ala)_2O$ obtained above. The mixture was stirred at 0—5 °C overnight and then worked up in the usual manner. The crude preparation of **8** was chromatographed on a Lobar column

(size C) with benzene-ethyl acetate (8:2) as solvent. The fractions containing the desired material were pooled and evaporated *in vacuo* to give **8** as amorphous solid; 2.00 g (77%), $[\alpha]_D^{25.5}$ −96.5±1.4° (*c* 1.0, methanol). TLC: homogeneous in systems A and D. Found: C, 52.81; H, 6.02; N, 6.35; S, 3.95; Cl, 12.55%. Calcd for $C_{38}H_{51}N_4O_{10}SCl_3$: C, 52.93; H, 5.96; N, 6.50; S, 3.72; Cl, 12.33%.

Unexpected Formation of Z-D-Ser[isobutoxycarbonyl-MeCys-(Bzl)-MeVal]-OTce. Boc-Ala-OH (0.23 g, 1.2 mmol) was dissolved in anhydrous THF (3 ml) and DIEA (0.21 ml, 1.2 mmol) was added. The solution was chilled to −10 °C and to this was added isobutyl chloroformate (0.16 ml, 1.2 mmol). The mixture was stirred for 1 min, after which there was added a solution of Z-D-Ser[H-MeCys(Bzl)-MeVal]-OTce TFA salt derived from **7** by the treatment with TFA as described in the prepation of **8**. The reaction mixture was stirred at 5 °C overnight and then worked up as usual. The crude product was chromatographed on a Lobar column (size B) with benzene-ethyl acetate (9:1) as solvent. The fractions containing the major product as a single component were collected, washed with water, and evaporated *in vacuo* to give an oily material; 0.71 g (90% as Z-D-Ser[isobutoxycarbonyl-MeCys(Bzl)-MeVal]-OTce), $[\alpha]_D^{25.5}$ −84.6±1.2° (*c* 1.0, methanol). TLC: homogeneous in systems A and D. Found: C, 52.83; H, 5.99; N, 5.20; S, 4.44; Cl, 13.34%. Calcd for $C_{35}H_{46}N_3O_9SCl_3$: C, 53.13; H, 5.86; N, 5.31; S, 4.05; Cl, 13.44%.

Z-D-Ser[Boc-Ala-MeCys(Bzl)-MeVal]-OH (1). Compound **8** (2.59 g, 3.0 mmol) was dissolved in 90% acetic acid (66 ml) and chilled in an ice-bath. To this was added zinc powder (9.8 g) and the mixture was stirred at 0 °C for 3 h. After the excess zinc had been filtered off, the filtrate was evaporated *in vacuo*. To the residue was added ethyl acetate and the mixture was washed with M hydrochloric acid and with water, dried over magnesium sulfate, and evaporated *in vacuo* to give a solid residue (1.6 g). The crude product was purified on a column of silica gel (110 g, Kieselgel H, Merck) with chloroform-methanol-acetic acid (99:1:1) as solvent to give compound **1** as amorphous solid; 1.97 g (90%), $[\alpha]_D^{25}$ −124.9±0.6° (*c* 1.0, methanol). TLC: homogeneous in systems B and E. Found: C, 59.16; H, 6.90; N, 7.67; S, 4.39%. Calcd for $C_{36}H_{50}N_4O_{10}S$: C, 59.42; H, 7.21; N, 7.68; S, 4.42%.

Z-D-Ser{Z-D-Ser[Boc-Ala-MeCys(Bzl)-MeVal]-Ala-MeCys-(Bzl)-MeVal}-OTce (9). Compound **8** (2.16 g, 2.5 mmol) was treated with TFA (25 ml) at 25 °C for 30 min, followed by evaporation *in vacuo*. The oily residue was dissolved in ethyl acetate and the solution was shaken with ice-cold 50% potassium carbonate, dried over magnesium sulfate and evaporated *in vacuo* to give Z-D-Ser[H-Ala-MeCys(Bzl)-MeVal]-OTce (**2**) as an oil. Compound **2** was dissolved in THF (50 ml) together with compound **1** (1.83 g, 2.5 mmol) and HOBt (0.51 g, 3.75 mmol) and to this solution was added DCC (0.77 g, 3.75 mmol) at 0 °C. The mixture was stirred at 5 °C overnight and then worked up in the usual manner. The crude product was chromatographed on a silica-gel column (Kieselgel 60, 5×16 cm) with chloroform-methanol (99:1) and then on a Lobar column (size C) with benzene-ethyl acetate (9:1) as solvent to give the linear octadepsipeptide (**9**) as amorphous solid; 3.21 g (87%), $[\alpha]_D^{25}$ −115.0±1.5° (*c* 1.0, methanol). TLC: homogeneous in systems A, B, D, and E. Found: C, 55.97; H, 6.25; N, 7.52; S, 4.65; Cl, 7.40%. Calcd for $C_{69}H_{91}N_8O_{17}S_2Cl_3$: C, 56.19; H, 6.22; N, 7.60; S, 4.35; Cl, 7.21%.

Z-D-Ser{Z-D-Ser[Boc-Ala-MeCys(Bzl)-MeVal]-Ala-MeCys-(Bzl)-MeVal}-OH (10). To a solution of **9** (3.54 g, 2.4 mmol) in 90% acetic acid (120 ml) was added zinc powder (7.85 g) at 0 °C and the mixture was stirred at 0 °C for 3 h. After removal of the excess zinc by filtration the solvent was evaporated *in vacuo*. The resulting oily residue was dissolved in ethyl acetate, washed with ice-cold M hydrochloric acid and water, dried over magnesium sulfate, and evaporated *in vacuo* to give a solid residue. This crude product was chromatographed on a silica-gel column (Kieselgel H, 6×20 cm) with chloroform-methanol-acetic acid (98:2:0.5) as solvent. The fractions containing the desired material were pooled, washed with water, dried over magnesium sulfate, and evaporated *in vacuo* to give **10** as amorphous solid; 3.03 g (94%), $[\alpha]_D^{25}$ −132.2±1.8° (*c* 1.0, methanol). TLC: homogeneous in systems B and E. Found: C, 59.88; H, 6.87; N, 8.11; S, 4.78%. Calcd for $C_{67}H_{90}N_8O_{17}S_2$: C, 59.89; H, 6.75; N, 8.34; S, 4.64%.

{Z-D-Ser[Z-D-Ser-Ala-MeCys(Bzl)-MeVal]-Ala-MeCys(Bzl)-MeVal}Dilactone, N,N′-Z_2-S,S′-Bzl_2-*Dihydroapotriostin A (11).*

a) By the Active Ester Method. Compound **10** (0.269 g, 0.2 mmol) and HOSu (0.230 g, 2 mmol) were dissolved in THF (6 ml) and chilled in an ice-bath. To this was added DCC (0.413 g, 2 mmol) and the mixture was stirred at 0—5 °C for 3 h and at 25 °C for 2 h. The DCU which had formed was filtered off and the filtrate was evaporated *in vacuo* to give a residue which was then treated with TFA (10 ml) at 25 °C for 30 min, followed by evaporation *in vacuo*. The residue was, after being dried over sodium hydroxide pellets *in vacuo*, dissolved in ethyl acetate (1000 ml) and to this was added DIEA until the solution became neutral as checked with a piece of wetted pH indicator paper (Whatman). The reaction mixture was stirred at 25 °C for 3 h and, after concentration to a small volume, washed with M hydrochloric acid, water, and 5% sodium hydrogencarbonate, dried over magnesium sulfate, and evaporated *in vacuo*. The same cyclization reaction was carried out six times with 0.285 mmol each of compound **10** as starting material. The resulting crude product combined (4.4 g from 1.91 mmol of **10**) was chromatographed on a column (4×49 cm) of silica-gel (270 g, Kieselgel 60) with chloroform-methanol (98:2) as solvent. The fractions containing the desired material were combined and evaporated *in vacuo*. The residue was rechromatographed on a Lobar column (size C) with benzene-ethyl acetate (1:1) to give the title compound as amorphous solid; 1.36 g (58%), $[\alpha]_D^{23}$ −124.8±3.1° (*c* 0.5, methanol). NMR ($CDCl_3$, 360 MHz) δ=0.51 (d, 6H, MeVal γ- or γ′-CH_3), 0.71 (d, 6H, MeVal γ- or γ′-CH_3), 0.74 (d, 6H, Ala β-CH_3), 2.18 (m, 2H, MeVal β-CH), 2.47, 2.53 (m, 4H, MeCys β- and β′-CH_2), 2.82 (s, 6H, N-CH_3), 2.88 (s, 6H, N-CH_3), 3.42 (d, 2H, Bzl S-CH), 3.50 (d, 2H, MeVal α-CH), 3.67 (d, 2H, Bzl S-CH), 4.59 (m, 2H, Ser β-CH_2), 4.74 (m, 2H, Ala α-CH), 4.75 (m, 2H, Ser β-CH_2), 4.87 (m, 2H, Ser α-CH), 4.91 (d, 2H, Bzl O-CH_2), 5.40 (d, 2H, Bzl O-CH_2), 5.57 (m, 2H, MeCys α-CH), 7.2—7.4 (m, 20H, Bzl aromatic), 7.71 (d, 2H, Ala NH), and 8.22 (d, 2H, Ser NH). FD-MS: M^+ 1224. TLC: homogeneous in systems A and D. Found: C, 60.09; H, 6.57; N, 9.00; S, 4.85%. Calcd for $C_{62}H_{80}N_8O_{14}S_2$: C, 60.77; H, 6.58; N, 9.14; S, 5.23%.

b) By the DCC-HOBt Method. To a solution of **10** (0.336 g, 0.25 mmol) in dioxane (2.5 ml) was added 4 M hydrogen chloride in dioxane (10 ml) and the mixture was kept at 25 °C for 60 min, followed by evaporation *in vacuo*. The oily residue was dissolved in THF (125 ml) and to this were added HOBt (0.338 g, 2.5 mmol) and DIEA (0.09 ml, 0.5 mmol). After the subsequent addition of DCC (0.516 g, 2.5 mmol), the mixture was stirred at 25 °C for 44 h and then evaporated *in vacuo*. The residue was dissolved in ethyl acetate and the solution was filtered off and evaporated. The crude product was purified on a column (3.3×16 cm) of silica gel (50 g, Kieselgel H) with chloroform-methanol (95:5) as solvent. Rechromatography on a Lobar column (size B) in benzene-ethyl acetate (1:1) yielded **11** as amorphous solid; 0.064 g (21%), mp 100—102 °C, $[\alpha]_D^{25}$ −123.9±3.1° (*c* 0.5, methanol). Found: C, 60.73; H, 6.29; N, 8.84; S, 5.37%.

c) By the DPPA Method. Compound **10** (0.269 g, 0.2 mmol) was treated with hydrogen chloride in dioxane in the same manner as described above. The product was dissolved in DMF (100 ml) and chilled in ice. To this were then added DIEA (0.24 ml, 1.4 mmol) and DPPA (0.275 g, 1.0 mmol) and the mixture was stirred at 0 °C for 6 h. The crude product was purified on silica-gel columns in almost the same manner as described in b) to give purified **11** as amorphous solid; 0.053 g (22%), $[\alpha]_D^{22}$ −125.7±3.3° (*c* 0.5, methanol). Found: C, 60.28; H, 6.73; N, 8.66; S, 5.57%.

{Qxc-D-Ser[QxC-D-Ser-Ala-MeCys(Bzl)-MeVal]-Ala-MeCys-(Bzl)-MeVal}Dilactone, S,S'-Bzl$_2$-Dihydrotriostin A (12). Compound **11** (123 mg, 0.1 mmol) and thioanisole (1.2 ml) were dissolved in TFA (12 ml) and the mixture was kept at 25 °C for 6 h. Evaporation of the TFA *in vacuo* afforded an oily residue, which was dried over sodium hydroxide pellets *in vacuo*. The residue was then precipitated from ether-petroleum ether to give the TFA salt of *S,S'*-dibenzyldihydroapotriostin A as amorphous solid. TEA (0.028 ml, 0.2 mmol) was added to a stirred ice-cold solution of this solid in DMF (1 ml) and to this were then added Qxc-Cl (58 mg, 0.3 mmol) and TEA (0.056 ml, 0.4 mmol) simultaneously in three equal portions over 30 min. The reaction mixture was stirred at 0 °C for 6 h. The formation of **12** was 27, 30, 34, 36, and 37% at reaction time of 1, 2, 3, 4, and 6 h, respectively, as monitored by HPLC (Waters μ-Porasil, 3.9×300 mm; 2-propanol-hexane (27.5:72.5)). After dilution with ethyl acetate the mixture was successively washed with M hydrochloric acid, water, and 5% sodium hydrogencarbonate, dried over magnesium sulfate, and evaporated *in vacuo*. The residue was chromatographed on a column (1.7×22 cm) of silica-gel (20 g, Kieselgel H) with chloroform-methanol (99:1) and repeatedly rechromatographed on a Lobar column (size A) with ethyl acetate and with chloroform-methanol (99:1) as solvent. The resulting material was dissolved in ethyl acetate and the solution was washed with water, dried over magnesium sulfate and evaporated *in vacuo* to afford a pure preparation of **12**; 0.028 g (22%), $[\alpha]_D^{22.5}$ −152.6±1.9° (*c* 1.0, chloroform). NMR ($CDCl_3$, 360 MHz; I=conformer I, II=conformer II); δ=0.67 (d, 6H, MeVal γ- or γ'-CH_3, I), 0.78 (d, 6H, MeVal γ- or γ'-CH_3, I), 0.83 (d, 6H, MeVal γ- or γ'-CH_3, II), 1.09 (d, 6H, MeVal γ- or γ'-CH_3, II), 1.28 (d, 6H, Ala β-CH_3, I), 1.38 (d, 6H, Ala β-CH_3, II), 2.19 (m, 2H, MeVal β-CH, I), 2.36 (m, 2H, MeVal β-CH, II), 2.66, 2.67 (m, 4H, MeCys β- and β'-CH_2, I), 2.92, 2.99 (m, 4H, MeCys β- and β'-CH_2, II), 2.81, 2.89, 3.03, 3.12 (s, 12H, N-CH_3, I and II), 3.54, 3.80 (m, 8H, Bzl S-CH_2, I and II), 3.92 (m, 2H, MeVal α-CH, I), 4.38 (m, 2H, Ser β-CH_2, II), 4.41 (m, 2H, Ser β-CH_2, I), 4.59 (m, 2H, Ser β'-CH_2, II), 4.82 (m, 2H, Ala α-CH, II), 4.85 (m, 2H, Ser β'-CH_2, I), 5.02 (m, 2H, Ala α-CH, I), 5.13 (m, 2H, MeVal α-CH, II), 5.25 (m, 2H, Ser α-CH, I), 5.39 (m, 2H, MeCys α-CH, I), 7.07 (d, 2H, Ala NH, II), 7.2—7.4 (m, 10H, Bzl aromatic, I and II), 7.81, 7.89, 8.11, 8.22 (m, 8H, Qxc, I and II), 8.46 (d, 2H, Ala NH, I), 8.77 (d, 2H, Ser NH, II), 8.80 (d, 2H, Ser NH, I), 9.62 (s, 2H, Qxc 3-CH, I), and 9.67 (s, 2H, Qxc 3-CH, II). TLC: homogeneous in system A. Found: C, 59.43; H, 6.01; N, 12.82; S, 5.32%. Calcd for $C_{64}H_{76}N_{12}O_{12}S_2 \cdot H_2O$: C, 59.70; H, 6.11; N, 13.05; S, 4.98%.

Triostin A. Compound **12** (38.1 mg, 0.03 mmol) was treated with HF (6 ml) in the presence of anisole (0.3 ml) at 25 °C for 60 min. After the HF had been removed by evaporation, the residue was dissolved in methanol (30 ml) and the solution was neutralized with TEA. To this was added a solution of iodine (39 mg, 0.15 mmol) in methanol (2 ml) and the mixture was stirred at 25 °C for 2 h. The excess iodine was discharged with M sodium thiosulfate, in which the pH was kept neutral by the addition of 10% ascorbic acid. After removal of the solvent by evaporation *in vacuo*, the residue was purified on a silica-gel column (Kieselgel H, 1.7×23.5 cm) with chloroform-methanol (98:2) as solvent. The fractions containing the desired product were pooled and evaporated *in vacuo*. The resulting residue (9.8 mg) was crystallized from carbon tetrachloride-ethanol and recrystallized twice from dichloromethane-ethanol to afford pure triostin A; 7.0 mg (20%), mp 222—223 °C, $[\alpha]_D^{21.5}$ −151.9±19.8° (*c* 0.1, chloroform). The authentic sample of natural triostin A showed mp 220—223 °C. Lit. for natural triostin A:[3b,11] mp 245—248 °C dec, $[\alpha]_D^{23.5}$ −157±2° (*c* 0.97, chloroform). Lit, for synthetic triostin A:[16] mp 239—243 °C dec, $[\alpha]_D^{25}$ −154° (*c* 1.0, chlorofororm). λ_{max}^{MeOH} 243 nm (log ε 4.92), 317 nm (4.15), 325 nm (4.16). NMR: see Fig. 4. TLC: homogeneous in system A. HPLC: see Fig. 5. Found: C, 51.99; H, 5.89; N, 14.36; S, 6.05%. Calcd for $C_{50}H_{62}N_{12}O_{12}S_2 \cdot 3.5H_2O$: C, 52.21; H, 6.05; N, 14.61; S, 5.57%.

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