workup with ether and water the ether phase was dried with $MgSO_4$ and evaporated. The residue was dissolved in 25 mL of dioxane, 5 mL of water and 20 mg of *p*-toluenesulfonic acid were added, and the mixture was heated under reflux for 1 h. Upon cooling colorless crystals appeared which were filtered and washed with water, yield 27 mg (77%). The nearly pure compound was purified further by LC (column B, absolute MeOH), the main peak (80%) yielding isostelliferasterol (**2**): mp 119–120 °C after recrystallization from methanol–water; NMR (360 MHz) 5.36 (1, m), 5.205 (1, q, J = 6 Hz), 3.53 (1, m), 2.56 (1, sextet, J = 6 Hz), 1.585 (3, d, J = 6 Hz), 1.011 (3, s), 0.960 (3, d, J = 6 Hz), 0.941 (3, d, J = 6 Hz), 0.816 (3, t, J = 6.5 Hz), 0.683 ppm (3, s); MS (MS-9) *m/e* 426 (M^+, 4), 361 (2), 343 (2), 314 (100), 299 (21), 281 (25), 271 (10), 255 (5), 229 (22), 213 (12), 55 (50); MS (MAT 711) *m/e* 426.3834 (M^+, calcd 426.3861); $[\alpha]^{20}_D$ −27° ($CHCl_3$).

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Des-*N*-tetramethyltriostin A and Bis-L-seryldes-*N*-tetramethyltriostin A, Synthetic Analogues of the Quinoxaline Antibiotics[1]

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Abstract: The synthesis of the des-*N*-tetramethyl analogue of the quinoxaline depsipeptide antibiotic triostin A has been accomplished, as also has the synthesis of the corresponding bis-L-serine analogue. The synthesis of des-*N*-tetramethyltriostin A (**2**) proceeded by coupling L-alanine β,β,β-trichloroethyl ester with *N*-benzyloxycarbonyl-D-serine to give dipeptide Z-D-Ser-Ala-OTce. Depsipeptide bond formation was effected using *N*,*N*′-dicyclohexylcarbodiimide in pyridine to provide tridepsipeptide **3**. Coupling of **3** with Boc-Cys(Acm)-OH gave tetradepsipeptide **4**, which, by removal of appropriate protective groups, was converted to tetradepsipeptides **5** and **6**. Fragment coupling of **5** and **6** furnished the octadepsipeptide **7** containing the amino acid sequence of the antibiotic. A sequence of deprotection and cyclization converted **7** to the cyclic octadepsipeptide **8**. Treatment of **8** with iodine in methanol effected formation of disulfide **9**. Removal of the *N*-benzyloxycarbonyl groups in **9** followed by N-acylation with 2-quinoxalinecarbonyl chloride gave des-*N*-tetramethyltriostin A (**2**). Analogue **2** was found to bind to DNA and, in common with the natural quinoxaline antibiotics, to do so as a bifunctional intercalating agent. The bis-L-serine analogue of **2** was prepared following the above procedure to furnish a quinoxaline antibiotic that showed no appreciable binding to DNA.

The quinoxaline antibiotics are a group of bycyclic depsipeptide antibiotics. Two families of these antibiotics, the quinomycins[2,3a] and the triostins,[3] are known. The structural features of the triostins are represented by triostin A (**1**). Triostin A is a symmetrical bicyclic octadepsipeptide composed of two units each of D-serine, L-alanine, *N*-methyl-L-cysteine, and *N*-methyl-L-valine. The depsipeptide bond occurs between the hydroxyl group of D-serine and the carboxyl group of *N*-methyl-L-valine, while a disulfide bridge exists between the two *N*-methyl-L-cysteine residues. A 2-quinoxalinecarbonyl (Qxc) moiety is attached to the amino group of each D-serine unit.

The quinoxaline antibiotics are active against gram-positive bacteria[4,5b] and against certain animal tumors.[5] It is known that the antibiotics bind to DNA and thereby function as potent inhibitors of RNA synthesis.[6] Of current interest, the

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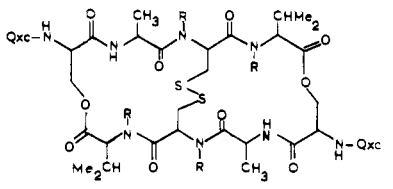

1 R = Me
2 R = H

Qxc = 2-Quinoxalinecarbonyl

quinoxaline antibiotics have been shown[7] to be novel bifunctional intercalating agents in their binding to DNA, in which both of the quinoxaline rings have undergone intercalation. A model, based upon conformational energy calculations, has been proposed[8a] for the binding of echinomycin (quinomycin A) to DNA, while NMR studies on the solution conformation of echinomycin have been reported.[8b]

Few reports[9,10] have appeared related to synthetic studies on the quinoxaline antibiotics. We wish to describe herein our studies that have resulted in the first total synthesis of an analogue of the quinoxaline antibiotic triostin A. Our synthetic objective was the preparation of des-*N*-tetramethyltriostin A (**2**). Compound **2** differs from the natural antibiotic, triostin A (**1**), by lack of *N*-methyl groups on the L-cysteine and L-valine residues. The decision to initially study the synthesis of the des-*N*-methyl analogue **2** was dictated by the synthetic convenience to be expected by use of the normal amino acids rather than their *N*-methyl derivatives.[11]

Our plan for the synthesis of **2**, as outlined in Figure 1, involved the preparation of tetradepsipeptide **4** as a key intermediate. Depsipeptide **4** represents one-half of the symmetrical octadepsipeptide portion of analogue **2**. Fragment coupling of tetradepsipeptides **5** and **6**, each prepared from **4** by removal of appropriate blocking groups, would give octadepsipeptide **7** possessing the complete amino acid sequence of the antibiotic. Further transformations involving cyclization, disulfide formation, and quinoxaloylation would provide des-*N*-tetramethyltriostin A (**2**). The successful realization of this approach is described herein.

Certain aspects that are of critical importance in the design of a synthesis of a depsipeptide antibiotic such as **2** can be illustrated by a consideration of the functional components incorporated into tetradepsipeptide **4**. An important consideration in the design of depsipeptide **4** is that two ester functions are present in the molecule and it must be possible, in the subsequent transformations of **4**, to deprotect the C-terminal alanine residue without simultaneously cleaving the depsipeptide ester function. Woodward's protective ester function, the β,β,β-trichloroethyl ester,[12] is ideally suited for this purpose and can be removed selectively with zinc in acetic acid without affecting the depsipeptide bond or, for that matter, other protective groups in the molecule.[13] Depsipeptide **4** also contains amino groups associated with the L-cysteine and D-serine residues that need to be blocked with groups capable of selective deprotection. This was accomplished by protection of the L-cysteine residue with the *tert*-butyloxycarbonyl (Boc) group,[14] which group can be selectively cleaved[15] in the presence of the benzyloxycarbonyl (Z) group used to protect D-serine. The thiol function of L-cysteine was protected with the known acetamidomethyl (Acm) group,[16] a thiol protecting group that can be removed with concurrent disulfide formation using iodine in methanol.[17a]

The synthesis of depsipeptide **4** was accomplished by coupling Z-D-Ser-OH with the β,β,β-trichloroethyl ester[13] of L-alanine using *N,N'*-dicyclohexylcarbodiimide (DCC) in methylene chloride to yield (71%) Z-D-Ser-Ala-OTce. The above dipeptide was treated with 1.5 equiv of Boc-Val-OH and DCC in pyridine[9,18] to furnish the tridepsipeptide **3** in 76%

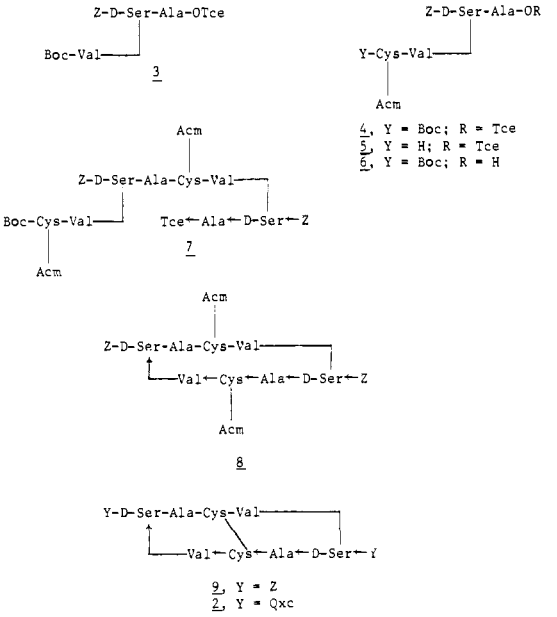

Figure 1.

yield. Deprotection of **3** with trifluoroacetic acid (TFA) followed by neutralization and coupling with Boc-Cys(Acm)-OH using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride[19] and hydroxybenztriazole[20] in combination as coupling reagents gave, in 69% yield, the tetradepsipeptide **4**.

Removal of the Boc function in **4** by use of TFA gave, in 91% yield, tetradepsipeptide **5**. Treatment of **4** with zinc in acetic acid effected removal[12,13] of the Tce ester group to furnish **6** in 87% yield. Fragment coupling of **5** and **6** was accomplished by use of the 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide–hydroxybenztriazole method to provide the linear octadepsipeptide **7** in a yield of 93%; **7** also could be obtained in a 78% yield by coupling **5** and **6** via the mixed anhydride[21] of **6** prepared from isobutyl chloroformate.

Octadepsipeptide **7** was treated sequentially with zinc in acetic acid to remove the Tce ester group and with TFA to remove the Boc group, followed by neutralization and cyclization under conditions of high dilution using *N*-hydroxysuccinimide–DCC[22] in tetrahydrofuran–dimethylformamide. The cyclic octadepsipeptide **8**, formed in the above sequence, was isolated in a yield of 26–43% from **7** by column chromatography on silica gel followed by recrystallization. Treatment of **8** with iodine in methanol[17] effected conversion (89%) to the disulfide **9**, which upon acidolysis of the benzyloxycarbonyl group using HBr in acetic acid and acylation[10] with 2-quinoxaloyl chloride in dimethylformamide gave, following column chromatography on silica gel and recrystallization, des-*N*-tetramethyltriostin A (**2**) in a yield of 35–57%.

The ^{1}H NMR spectrum of analogue **2**, when analyzed with reference to the published[3b] spectrum of the natural antibiotic triostin A (**1**), was consistent with the proposed structure. The field desorption spectrum of **2** possessed a molecular ion peak at 1031 mass units. The electron impact spectrum of **2** was most definitive in the low mass region, having peaks at *m/e* 102, 129, 157, 173, 226, 297, 366, and 482. The same fragmentation pattern was observed in the low mass region of the reported[2b] spectrum of the quinoxaline antibiotic echinomycin, except that the peaks at *m/e* 366 and 482 in the spectrum of **2** are shifted to *m/e* 380 and 509 in that of echinomycin because of the presence of *N*-methyl groups in the natural antibiotic. Apparently, the peak at *m/e* 482 in the spectrum of **2**, which differs by 27 rather than 28 mass units from the corresponding peak at *m/e* 509 in echinomycin, is due to the expected ion of

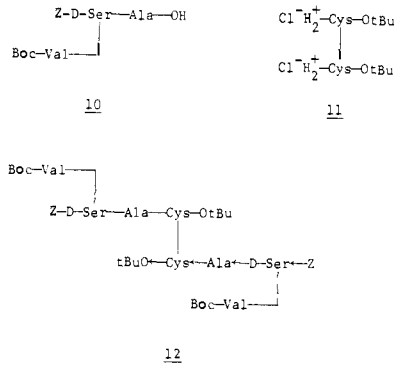

Figure 2.

mass 481 plus a proton. The observed low-field fragmentation pattern as shown by Dell et al.[2b] is due to the quinoxaloyl tetrapeptide sequence common to the quinoxaline antibiotics.

A second, but unsuccessful, approach investigated for the synthesis of analogue **2** involved coupling of tridepsipeptide **10**, prepared from **3** by removal of the β,β,β-trichloroethyl ester group, with L-cystine di-*tert*-butyl ester dihydrochloride (**11**) to furnish octadepsipeptide **12**. Attempts to convert **12** to the cyclic depsipeptide **8** by deprotection and cyclization resulted, in the cyclization step, in formation of complex mixtures that could not be separated to afford desired **8** (Figure 2).

Fragment coupling reactions are known to be susceptible to racemization of the carboxyl terminal amino acid involved in the coupling process.[23] In the synthesis of **2**, racemization could occur at the alanine residue during formation of **7** by the fragment coupling of tetradepsipeptides **5** and **6**, and also in the cyclization reaction leading to cyclic octadepsipeptide **8**. The degree of racemization of the alanine residues in purified samples of **7** and of des-*N*-tetramethyltriostin A (**2**) was determined by standard acid hydrolysis, followed by esterification, N-trifluoroacetylation, and analysis by VPC on a capillary column coated with an optically active stationary phase (*N*-lauroyl-L-valyl-*tert*-butylamide)[24] that clearly separates the D- and L-alanine derivatives. That racemization is a problem in the above synthesis was apparent in that 6.4% of the alanine units in **7** were of D configuration representing 6.4% diastereomeric impurity or 12.8% racemization of the alanine residues involved in the coupling process. The above figure would represent a minimum value as some diastereomeric separation could have occurred during purification of **7**. That diasteromeric purification has occurred in the steps leading to **2** was shown by the fact that only 1% of the alanine residues in **2** were of the D configuration. Since **2** contains two alanine residues that could have undergone racemization, this value (1% D-Ala) would correspond to the presence of 2% diastereomeric impurities in **2**.

Des-*N*-tetramethyltriostin A (**2**) has been reported by Lee and Waring[25] to bind as a bifunctional intercalating[7] agent to DNA. In its binding to DNA, analogue **2** is remarkable in that **2** shows a high specificity for A-T bases rather than G-C bases. In contrast to triostin A (**1**), **2** was inactive toward *Staphalococcus aureus*.

The bis-L-serine analogue of **2** was prepared following the same procedure as used in the synthesis of **2**, but with the incorporation of L-serine in place of D-serine. As might be expected, based upon the model proposed[8] for the binding of the quinoxaline antibiotic echinomycin to DNA, the L-serine analogue showed[25] no appreciable binding to DNA.

Studies on the synthesis of the triostin antibiotics have continued in our laboratories, and we recently have accomplished[26] the total synthesis of the natural antibiotic, triostin A (**1**).

Experimental Section

All melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra (NMR) were obtained for all compounds reported in this study using a Varian EM-360, EM-390, or XL-100-12 spectrometer; NMR spectral data are reported for selected intermediates. Optical rotations were recorded using a Rudolf and Sons Model 80 polarimeter. Thin layer chromatography (TLC) was performed on commercially prepared silica gel on glass plates using the following solvent systems: A, chloroform–methanol–acetic acid (85:10:5); B, chloroform–acetic acid (95:5); C, chloroform–acetone (80:20); D, chloroform–absolute ethanol (80:20); E, *n*-BuOH–acetic acid–water (10:2:3); F, chloroform–absolute ethanol (90:10). All elemental analyses were performed by H-M-W Laboratories, Phoenix, Ariz.

The L-amino acid derivatives used in this study were commercially available. The D-serine and 2-qunioxalinecarbonyl chloride used were purchased from Sigma and from Aldrich, respectively. All coupling reagents employed were obtained from commercial sources.

The preparation of the L-serine intermediates leading to the L-serine analogue of **2** is given immediately after that of the corresponding D-serine derivative.

L-Alanine β,β,β-Trichloroethyl Ester. A stirred solution of *N*-benzyloxycarbonyl-L-alanine (3.4 g, 15.2 mmol) in anhydrous pyridine (60 mL) was cooled to 0 °C in an ice bath. *N,N'*-Dicyclohexylcarbodiimide (3.3 g, 16.0 mmol) was then added followed by β,β,β-trichloroethanol (2.98 g, 20 mmol). The mixture was stirred at 0 °C for 4 h and then at room temperature overnight. The reaction mixture was filtered and the residue was washed well with pyridine. The filtrate and washings were combined and concentrated to a viscous oil. The oil was dissolved in ethyl acetate (150 mL) and filtered. The filtrate was washed successively with H_2O (2 × 75 mL), 1 N aqueous HCl (75 mL), saturated sodium bicarbonate solution (2 × 75 mL), and H_2O (75 mL). After drying ($MgSO_4$) the solution was concentrated to a viscous, yellow oil which was dried in vacuo over P_2O_5. The oil was then treated with a saturated solution of hydrogen bromide in acetic acid (15 mL) for 1 h at room temperature. Anhydrous diethyl ether (250 mL) was added to the solution and the resulting mixture was stored at −15 °C for 3 h. The precipitated solid was filtered, washed well with anhydrous diethyl ether, and dried. After recrystallization from ethanol–diethyl ether, 3.03 g (66%) of a white, crystalline solid was obtained, mp 239–241 °C, $[\alpha]_D^{25}$ −10.2° (*c* 5, DMF) [lit.[13] mp 240–243 °C, $[\alpha]_D^{25}$ −1.8° (*c* 5.73, DMF)].

It should be noted that all attempts to prepare the trichloroethyl ester by the published procedures using phosphoryl chloride or phosphorus pentachloride failed.[13] The difference in literature and observed optical rotation values may be significant. Later GLC racemization studies showed our product to be only L enantiomer. The product obtained in ref 13 may have been partially racemized.

***N*-Benzyloxycarbonyl-D-seryl-L-alanine β,β,β-Trichloroethyl Ester.** A stirred mixture of L-alanine β,β,β-trichloroethyl ester hydrobromide (6.0 g, 19.7 mmol) and benzyloxycarbonyl-D-serine[27] (4.8 g, 20.0 mmol) in dry methylene chloride (180 mL) was cooled to 0 °C in an ice bath. *N,N'*-Dicyclohexylcarbodiimide (4.3 g, 21.0 mmol) was added followed by triethylamine (2.02 g, 20.0 mmol). The mixture was stirred at 0 °C for 3 h and then overnight at room temperature. The reaction mixture was filtered and the residue washed well with methylene chloride. The filtrate and washings were combined and concentrated to an oily residue. The oil was dissolved in ethyl acetate (50 mL) and filtered, and the filtrate was washed successively with saturated sodium bicarbonate solution (25 mL), 1 N aqueous HCl (25 mL), saturated sodium bicarbonate solution (25 mL), and H_2O (25 mL). After drying ($MgSO_4$), the ethyl acetate solution was concentrated to a solid residue which was dissolved in diethyl ether (200 mL) and stored overnight in the freezer at −15 °C. The resulting precipitate was collected by filtration, dried, and recrystallized twice from diethyl ether to yield 6.2 g (71%) of a white solid: mp 75–77 °C; $[\alpha]_D^{25}$ 1.40 (*c* 2, $CHCl_3$); TLC (solvent A) R_f 0.68. Anal. ($C_{16}H_{19}N_2O_6Cl_3$) C, H, N.

***N*-Benzyloxycarbonyl-L-seryl-L-alanine β,β,β-Trichloroethyl Ester.** Z-L-Ser-Ala-OTce was prepared in a similar manner by condensation of L-alanine β,β,β-trichloroethyl ester hydrobromide (6.95 g, 22.8 mmol) with *N*-benzyloxycarbonyl-L-serine (5.56 g, 23.1 mmol) at 0 °C in methylene chloride (110 mL) containing *N*-methylmorpholine (2.31 g, 22.8 mmol) and *N,N'*-dicyclohexylcarbodiimide (4.97 g, 24.1 mmol). The crude product was recrystallized from diethyl ether in

a yield of 7.6 g (75%): mp 101–102 °C; $[\alpha]_D^{25}$ −50.6° (*c* 1, MeOH); TLC (solvent C) R_f 0.28. Anal. ($C_{16}H_{19}N_2O_6Cl_3$) C, H, N.

***N*-Benzyloxycarbonyl-*O*-(*tert*-butyloxycarbonyl-L-valyl)-D-seryl-L-alanine β,β,β-Trichloroethyl Ester (3).** A solution of *N*-benzyloxycarbonyl-D-seryl-L-alanine β,β,β-trichloroethyl ester (4.5 g, 10.5 mmol) and *N*-*tert*-butyloxycarbonyl-L-valine (3.4 g, 15.6 mmol) in anhydrous pyridine (50 mL) was cooled to 0 °C in an ice bath. *N*,*N*′-Dicyclohexylcarbodiimide (3.3 g, 16 mmol) was added and the mixture was stirred at 0 °C for 4 h and then overnight at room temperature. The reaction mixture was filtered and the residue was washed well with pyridine. The filtrate and washings were combined and concentrated to a yellow oil. The oil was dissolved in ethyl acetate (250 mL), filtered to remove any solid, and washed successively with H_2O (75 mL), saturated sodium bicarbonate solution (75 mL), 10% citric acid solution (2 × 25 mL), saturated sodium bicarbonate solution (75 mL), and H_2O (75 mL). The ethyl acetate solution was dried ($MgSO_4$) and concentrated to a sticky residue. The residue was dissolved in ethyl acetate and crystallized by the addition of petroleum ether (bp 30–60 °C) to give 5.1 g (76%) of a white solid: mp 96–97 °C; $[\alpha]_D^{25}$ 3° (*c* 2, $CHCl_3$); TLC (solvent C) R_f 0.60. Anal. ($C_{26}H_{36}N_3O_9Cl_3$) C, H, N.

***N*-Benzyloxycarbonyl-*O*-(*tert*-butyloxycarbonyl-L-valyl)-L-seryl-L-alanine β,β,β-Trichloroethyl Ester (3-L-Ser).** Tridepsipeptide **3**-L-Ser was prepared as above on a 20-mmol scale of dipeptide to give 10.5 g (82%) of product: mp 105–106°C; $[\alpha]_D^{25}$ −27.3° (*c*, 1, MeOH); TLC (solvent C) R_f 0.68. Anal. ($C_{26}H_{36}N_3O_9Cl_3$) C, H, N.

***N*-Benzyloxycarbonyl-*O*-(*N*-*tert*-butyloxycarbonyl-*S*-acetamidomethyl-L-cysteinyl-L-valyl)-D-seryl-L-alanine β,β,β-Trichloroethyl Ester (4).** *N*-Benzyloxycarbonyl-*O*-(*N*-*tert*-butyloxycarbonyl-L-valyl)-D-seryl-L-alanine β,β,β-trichloroethyl ester (**3**, 3.80 g, 5.94 mmol) was dissolved in anhydrous trifluoroacetic acid (6.0 mL) and stirred for 30 min at room temperature. The solution was concentrated to a sticky residue, which was dissolved in diethyl ether (100 mL). The ethereal solution was washed with saturated sodium bicarbonate solution (2 × 50 mL) followed by H_2O (50 mL). After drying ($MgSO_4$) the solution was concentrated to a foam. A stirred solution of this foam, *N*-*tert*-butyloxycarbonyl-*S*-acetamidomethyl-L-cysteine[16] (1.75 g, 5.94 mmol), and 1-hydroxybenzotriazole monohydrate (1.85 g, 12.0 mmol) in tetrahydrofuran (50 mL) was cooled to 0 °C in an ice bath. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.22 g, 6.5 mmol) was added and the resulting mixture was stirred for 1 h at 0 °C and then at room temperature for 4 h.

The reaction mixture was concentrated to a viscous oil, which was dissolved in ethyl acetate (100 mL) and washed successively with H_2O (75 mL), 2 N aqueous HCl (2 × 75 mL), saturated sodium bicarbonate solution (2 × 75 mL), H_2O (75 mL), and saturated sodium chloride solution (50 mL). The ethyl acetate phase was dried ($MgSO_4$) and concentrated to a foam. The foam was dissolved in ethyl acetate (6 mL) and filtered through a column packed with basic alumina (4 × 10 cm) over silica gel (4 × 5 cm) using ethyl acetate as the eluant (400 mL collected). The eluate was concentrated to a sticky residue which was dissolved with warming in diethyl ether (150 mL). The ethereal solution was placed in the freezer overnight and the resulting gel was filtered and dried in vacuo to give a white solid (2.76 g). A second crop was obtained from the mother liquors (0.53 g): total yield 3.29 g (69%); mp 89–92 °C; $[\alpha]_D^{25}$ 6.8° (*c* 4, $CHCl_3$); TLC (solvent B) R_f 0.16; NMR ($CDCl_3$) δ 0.9 (d, 6 H, Val methyl), 1.2–1.7 (m, 12 H, Boc and Ala methyls), 2.0–2.9 (m, 6 H, Acm methyl, Cys methylene, Val methyne), 4.0–4.9 (m, 10 H, Acm, Ser, and Tce methylenes, α hydrogens), 5.1 (s, 2 H, benzyl), 5.6 (d, 1 H, NH), 6.4 (d, 1 H, NH), 7.0–7.9 (m, 8 H, benzyl aromatic, 3 NH). Anal. ($C_{32}H_{46}N_5O_{11}SCl_3$) C, H, N, S.

Preparation of L-Serine Analogue 4-L-Ser. Six grams (9.4 mmol) of tridepsipeptide was treated as above, except that the coupling reaction was allowed to proceed for 4 h at 0 °C and overnight at room temperature. The product, obtained as a foam, was not chromatographed, but was crystallized directly from ethyl acetate to yield 6.8 g (90%) of crystalline material: mp 106–108 °C; $[\alpha]_D^{25}$ −63° (*c* 1, MeOH); TLC (solvent C) R_f 0.70. Anal. ($C_{32}H_{46}N_5O_{11}SCl_3$) C, H, N.

***N*-Benzyloxycarbonyl-*O*-(*S*-acetamidomethyl-L-cysteinyl-L-valyl)-D-seryl-L-alanine β,β,β-Trichloroethyl Ester (5).** *N*-Benzyloxycarbonyl-D-seryl-*O*-(*N*-*tert*-butyloxycarbonyl-*S*-acetamidomethyl-L-cysteinyl-L-valyl)-L-alanine β,β,β-trichloroethyl ester (**4**, 2.0 g, 2.46 mmol) was dissolved in anhydrous trifluoroacetic acid (4.0 mL) and stirred for 30 min at room temperature. The solution was then concentrated and the resulting oily residue dissolved in ethyl acetate (100 mL). The ethyl acetate solution was washed with saturated sodium bicarbonate solution (2 × 25 mL) and H_2O (25 mL). After drying ($MgSO_4$), the solution was concentrated to a white solid which was recrystallized from chloroform–diethyl ether to give a crystalline solid, 1.60 g (91%): mp 99–100.5 °C; $[\alpha]_D^{25}$ 6.0° (*c* 0.5, $CHCl_3$); TLC (solvent D) R_f 0.61. Anal. ($C_{27}H_{38}N_5O_9SCl_3$) C, H, N, S.

***N*-Benzyloxycarbonyl-*O*-(*S*-acetamidomethyl-L-cysteinyl-L-valyl)-L-seryl-L-alanine β,β,β-Trichloroethyl Ester (5-L-Ser).** Tetradepsipeptide **5**-L-Ser was prepared by treatment of **4**-L-Ser (0.50 g, 0.61 mmol) with 1.5 mL of anhydrous trifluoroacetic acid. The product, after workup as described above, was obtained as an oil (TLC (solvent E) R_f 0.50) that was used directly in the subsequent coupling procedure.

***N*-Benzyloxycarbonyl-*O*-[*N*-benzyloxycarbonyl-*O*-(*N*-*tert*-butyloxycarbonyl-*S*-acetamidomethyl-L-cysteinyl-L-valyl)-D-seryl-L-alanyl-*S*-acetamidomethyl-L-cysteinyl-L-valyl]-D-seryl-L-alanine β,β,β-Trichloroethyl Ester (7).** Zinc powder (18 g) was added to a vigorously stirred, ice-cold solution of *N*-benzyloxycarbonyl-D-seryl-*O*-(*N*-*tert*-butyloxycarbonyl-*S*-acetamidomethyl-L-cysteinyl-L-valyl)-L-alanine β,β,β-trichloroethyl ester (**4**, 4.5 g, 5.5 mmol) in 90% aqueous acetic acid (180 mL). After stirring at 0 °C for 1.5 h the mixture was filtered and the residue was washed well with 90% aqueous acetic acid. The filtrate and washings were combined and concentrated to a solid residue. The residue was shaken with a mixture of ethyl acetate (200 mL) and 1 N aqueous HCl (75 mL) until all the solid dissolved. The ethyl acetate phase was separated, washed repeatedly with H_2O, and then extracted with saturated sodium bicarbonate solution (4 × 75 mL). The bicarbonate extracts were combined, washed with ethyl acetate (75 mL), and carefully acidified to pH 4–5 with 6 N aqueous HCl. After the acidified solution was saturated with sodium chloride, the resulting mixture was extracted with ethyl acetate (3 × 100 mL). The ethyl acetate extracts were combined, dried ($MgSO_4$), and concentrated to a solid residue which was recrystallized from ethyl acetate–petroleum ether (bp 30–60 °C) to give a white solid, 3.21 g (87%): mp 95–97 °C; TLC (solvent D) R_f 0.15. This product, tetradepsipeptide **6**, was used without purification for the following fragment couplings.

A. A solution of **6** (0.75 g, 1.10 mmol) in anhydrous tetrahydrofuran (20 mL) was cooled to −10 °C in an ice–salt bath. Isobutyl chloroformate (0.157 g, 1.15 mmol) was added followed by *N*-methylmorpholine (0.111 g, 1.10 mmol) and the mixture was stirred for 5 min at −10 °C. *N*-Benzyloxycarbonyl-D-seryl-*O*-(*S*-acetamidomethyl-L-cysteinyl-L-valyl)-L-alanine β,β,β-trichloroethyl ester (**5**, 0.79 g, 1.10 mmol) was added and the mixture was stirred at −10 °C for 5 min and then at room temperature for 45 min. The resulting solid gelatinous mass was triturated with chloroform (75 mL) and filtered. The residue was washed well with H_2O, dried in vacuo, and reprecipitated from a minimum volume of dimethylformamide by the addition of chloroform (200 mL) and cooling in the freezer overnight. The precipitated gel was collected by filtration and dried to give a white solid, 1.20 g (78%): mp 177–179 °C; $[\alpha]_D^{25}$ −29.3° (*c* 1.8, DMF); TLC (solvent D) R_f 0.79; NMR (Me_2SO-d_6) δ 0.9 (d, 12 H, Val methyl), 1.1 (d, 6 H, Ala methyl), 1.4 (s, 9 H, Boc methyl), 1.8 (s, 6 H, Acm methyl), 2.1 (m, 2 H, Val methyne), 2.5–3.0 (m, 4 H, Cys methylene), 4.0–4.6 (m, 16 H, Ser and Acm methylenes, α hydrogens), 4.8 (AB q, 2 H, Tce methylene), 5.0 (s, 4 H, benzyl), 6.8 (m, 2 H, NH), 7.3 (s, 10 H, benzyl aromatic), 7.5, 7.8, 8.2, 8.6 (m, 8 H, NH). Anal. ($C_{57}H_{81}N_{10}O_{19}S_2Cl_3$) C, H, N, S.

B. A solution of **6** (2.26 g, 3.30 mmol), *N*-benzyloxycarbonyl-D-seryl-*O*-(*S*-acetamidomethyl-L-cysteinyl-L-valyl)-L-alanine β,β,β-trichloroethyl ester (**5**, 2.34 g, 3.27 mmol), and 1-hydroxybenzotriazole monohydrate (1.01 g, 6.60 mmol) in tetrahydrofuran (75 mL) was cooled to 0 °C in an ice bath. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.66 g, 3.5 mmol) was added and the mixture was stirred at 0 °C for 45 min and then at room temperature for 4 h. After the reaction mixture was concentrated to dryness, the residue was triturated well with H_2O. The solid was collected by filtration and washed successively with several portions of H_2O, ice-cold 1 N aqueous HCl, saturated sodium bicarbonate solution, and H_2O. After drying in vacuo, the solid was stirred well with hot chloroform (100 mL). Diethyl ether (50 mL) was added to the mixture and the resulting gel was filtered and dried in vacuo. The solid was reprecipitated as a gel from dimethylformamide–chloroform as in A,

collected, and dried to give 4.23 g (93%) of a white solid: mp 175–178 °C; $[\alpha]^{25}_D$ − 29.7° (*c* 2.0, DMF); TLC (solvent D) R_f 0.75; the NMR spectrum (Me_2SO-d_6) was identical with the spectrum of the product obtained by method A.

Preparation of Linear Octadepsipeptide L-Serine Analogue, 7-L-Ser. Treatment of tetradepsipeptide **4**-L-Ser (2.0 g, 2.46 mmol) with zinc powder (6.0 g) in 80 mL of 80% acetic acid and workup of the reaction mixture as above gave the acid Z-Ser[Boc-Cys(Acm)-Val]-Ala-OH (**6**-L-Ser) as a solid, which was recrystallized from ether–petroleum ether (bp 30–60 °C) to yield 1.55 g (92%) of product: mp 148–150 °C; $[\alpha]^{25}_D$ −59.5° (*c* 1, MeOH); TLC (solvent D) R_f 0.21. Anal. ($C_{30}H_{45}N_5O_{11}S$) C, H, N.

Coupling of **6**-L-Ser (0.42 g, 0.61 mmol) with tetradepsipeptide **5**-L-Ser (0.61 mmol) was accomplished, as for preparation of **7**, by use of 1-hydroxybenzotriazole monohydrate (0.19 g, 1.24 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.14 g, 0.74 mmol) in 10 mL of tetrahydrofuran at 0° C for 4 h and at room temperature overnight. After workup, the product was precipitated from methanol–ethyl acetate as a gel, which upon drying gave 0.65 g (77%) of solid product: mp 180–182°C; $[\alpha]^{25}_D$ −46° (*c* 1, MeOH); TLC (solvent D) R_f 0.80. Anal. ($C_{57}H_{81}N_{10}O_{19}S_2Cl_3$) C, H, N.

(*N*-Benzyloxycarbonyl-D-seryl-L-alanyl-*S*-acetamidomethyl-L-cystelnyl-L-valine)$_2$ (Serine Hydroxyl) Dilactone (8). Zinc powder (9.2 g) was added in portions to an ice-cold, stirred solution of **7** (4.1 g, 2.96 mmol) in 90% aqueous acetic acid (75 mL). Stirring was continued at 0°C for 1.5 h. The mixture was then filtered and the residue was washed well with 90% aqueous acetic acid. The filtrate and washings were combined and concentrated to dryness. The residue was shaken with a mixture of 1 N aqueous HCl (150 mL) and ethyl acetate–methanol (200 mL, 4/1 v/v) until all the solid dissolved. The organic phase was separated and the aqueous phase was saturated with sodium chloride and extracted with ethyl acetate (2 × 100 mL). The organic phase and ethyl acetate extracts were combined, washed several times with H_2O, and dried ($MgSO_4$). After the solution was concentrated, the resulting solid was reprecipitated as a gel from methanol by the addition of diethyl ether with cooling. The product was collected by filtration and dried to give 3.50 g (95%) of a white solid, mp 174–176 °C.

A solution of this solid (3.50 g, 2.80 mmol) in anhydrous trifluoroacetic acid (10 mL) was stirred at room temperature for 30 min. The solution was then concentrated to a sticky residue which was triturated well with anhydrous diethyl ether. The resulting white solid was collected by filtration, washed with anhydrous diethyl ether, and dried in vacuo over KOH pellets.

A stirred solution of the above solid (3.50 g, 2.76 mmol) in dimethylformamide (80 mL) was cooled to 0 °C in an ice bath. *N*-Methylmorpholine (0.276 g, 2.76 mmol) and *N*-hydroxysuccinimide (1.30 g, 11.3 mmol) were added and the solution was diluted with anhydrous tetrahydrofuran (1000 mL). After the stirred solution was allowed to cool to 0°C, *N*,*N*′-dicyclohexylcarbodiimide (1.54 g, 7.45 mmol) was added and the mixture was stirred at 0 °C for 30 min and then at room temperature for 4 days.

The reaction mixture was concentrated to dryness and the residue was taken up in ethyl acetate (150 mL). The insoluble material was removed by filtration and washed well with ethyl acetate. The filtrate and washings were combined and washed successively with 1 N aqueous HCl (2 × 50 mL), saturated sodium bicarbonate solution (50 mL), and H_2O (50 mL). After drying ($MgSO_4$), the solution was concentrated and the residue was taken up again in ethyl acetate (50 mL). The insoluble material was removed by filtration and the filtrate was concentrated to a sticky oil.

The oil was purified by chromatography on a column of silica gel (Sigma type IV, 325 mesh and finer) using chloroform–absolute ethanol (80/20 v/v) as the eluant. The fractions containing the product (TLC (solvent D) R_f 0.48–0.50) were pooled and concentrated to a solid residue. The residue was recrystallized from ethyl acetate–petroleum ether (bp 30–60 °C) to give 0.82 g (26%) of a white solid. The same procedure, carried out on a reduced scale (0.5–0.7 mmol), gave **8** in yields of 30–43%: mp 134–140 °C; $[\alpha]^{25}_D$ −67.3° (*c* 2, $CHCl_3$); TLC (solvent D) R_f 0.50 (ninhydrin negative); NMR ($CDCl_3$) δ 0.9 (m, 12 H, Val methyl), 1.2 (m, 6 H, Ala methyl), 1.8–2.3 (m, 8 H, Acm methyl, Val methyne), 2.9 (m, 4 H, Cys methylenes), 3.8–5.0 (m, 16 H, Acm and Ser methylenes, α hydrogens), 5.2 (AB q, 4 H, benzyl), 7.0 (m, 2 H, NH), 7.4 (s, 10 H, benzyl aromatic), 7.7, 8.3, 8.7 (m, 6 H, NH) (two exchangeable protons not accounted for). Anal. ($C_{50}H_{70}N_{10}O_{16}S_2$) C, H, N, S.

Preparation of Cyclic Octadepsipeptide 8-L-Ser. Linear octadepsipeptide **7**-L-Ser (1.80 g, 1.30 mmol) in 90% aqueous acetic acid (36 mL) was treated with zinc powder (4.1 g) as described above. The product obtained upon removal of the Tce ester groups was recrystallized from methanol to yield 1.48 g (91%) of material, mp 182–184 °C dec. This octadepsipeptide diacid was treated with 10 mL of anhydrous trifluoroacetic acid as described above. The solid product obtained was dissolved in anhydrous dimethylformamide (36 mL) and cooled to 0 °C. *N*-Methylmorpholine (0.13 mL, 1.19 mmol) and *N*-hydroxysuccinimide (0.61 g, 5.3 mmol) were added followed by dilution with 400 mL of tetrahydrofuran and the addition of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.70 g, 3.64 mmol). The reaction mixture was stirred for 1 h at 0 °C and at room temperature for 4 days. Workup and chromatography of the reaction mixture as described above for **8** gave cyclic product: 0.60 g, 45%; mp 144–148 °C from ethyl acetate–petroleum ether (bp 30–60 °C); $[\alpha]^{25}_D$ −113° (*c* 1, MeOH); TLC (solvent D) R_f 0.71. Anal. ($C_{50}H_{70}N_{10}O_{16}S_2$) C, H, N.

(*N*-Benzyloxycarbonyl-D-seryl-L-alanyl-L-cysteinyl-L-valine)$_2$ (Serine Hydroxyl) Dilactone Disulfide (9). To a stirred solution of **8** (680 mg, 0.60 mmol) in methanol (100 mL) was added dropwise a solution of iodine (745 mg, 6.0 mmol) in methanol (170 mL) over a period of 1.5 h.[17b] The solution was then stirred for an additional 3 h. After the solution was cooled to 0°C in an ice bath, 1 N aqueous sodium thiosulfate was added, dropwise, until the solution became colorless. The methanol was removed by concentration (below 40 °C) and the residue was triturated well with H_2O. The resulting solid was collected by filtration, washed well with several portions of H_2O, and dried in vacuo over P_2O_5. The product was purified by chromatography on a column of silica gel (Sigma type IV, 325 mesh and finer) using chloroform–absolute ethanol (80/20 v/v) as the eluant. The fractions containing the product (TLC (solvent D) R_f 0.85) were pooled and concentrated to a solid residue. The solid was recrystallized from chloroform–diethyl ether to give a white solid, 530 mg (90%): mp 166–169 °C; $[\alpha]^{25}_D$ −2.5° (*c* 2, $CHCl_3$); TLC (solvent D) R_f 0.85; NMR ($CDCl_3$) δ 1.0 (m, 12 H, Ala and Val methyl), 2.0–3.0 (m, 6 H, Cys methylenes, Val methyne), 4.5 (m, 12 H, Ser methylene, α hydrogens), 5.2 (AB q, 4 H, benzyl), 6.4, 6.8 (m, 4 H, NH), 7.5 (s, 10 H, benzyl aromatic), 8.5 (m, 4 H, NH). Anal. ($C_{44}H_{58}N_8O_{14}S_2$) C, H, N, S.

(*N*-Benzyloxycarbonyl-L-seryl-L-alanyl-L-cysteinyl-L-valine)$_2$ (Serine Hydroxyl) Dilactone Disulfide (9-L-Ser). Disulfide **9**-L-Ser was obtained by treatment of **8**-L-Ser (0.30 g, 0.26 mmol) in 60 mL of methanol with a solution of iodine (0.34 g, 1.34 mmol) in 80 mL of methanol. Workup of the reaction mixture as above gave solid material, which, without chromatography, was crystallized from acetone–petroleum ether (bp 30–60 °C) to yield 0.25 g (96%) of product: mp 188–190 °C; $[\alpha]^{25}_D$ −59° (*c* 0.5, MeOH); TLC (solvent F) R_f 0.62. Anal. ($C_{44}H_{58}N_8O_{14}S_2$) C, H, N.

Des-*N*-tetramethyltriostin A (2). Compound **9** (430 mg, 0.435 mmol) was stirred with a solution of 22% hydrogen bromide in glacial acetic acid (20 mL) for 45 min at room temperature. Anhydrous diethyl ether (125 mL) was then added and the precipitated solid was collected by filtration and washed well with anhydrous diethyl ether. After recrystallization from methanol–diethyl ether, the solid product, mp 275–278 °C dec, was dried in vacuo over P_2O_5.

Triethylamine (69 mg, 0.69 mmol) was added to a stirred, ice-cold solution of this solid (300 mg, 0.34 mmol) in dimethylformamide (20 mL). Quinoxaloyl chloride (154 mg, 0.80 mmol) was then added simultaneously with triethylamine (80 mg, 0.80 mmol) in three equal portions over 15 min. After addition, the mixture was stirred at 0 °C for 30 min and then at room temperature overnight. The reaction mixture was concentrated to dryness and the residue was triturated well with diethyl ether. The resulting solid was collected by filtration and washed successively with several portions of diethyl ether and H_2O. After drying in vacuo over P_2O_5, the straw-colored solid was purified by chromatography on a 1.5 × 10 cm column of silica gel (Sigma type IV, 325 mesh and finer) using chloroform–absolute ethanol (80/20 v/v) as the eluant. The fractions containing the product (TLC (solvent D) R_f 0.70) were pooled and concentrated to a solid residue. After recrystallization from chloroform–diethyl ether and drying in vacuo, 123 mg (35%) of a white solid was obtained. The above procedure, when carried out on a 68-μmol scale, gave 40 mg (57%) of **2**: mp 226–229 °C; $[\alpha]^{25}_D$ −43° (*c* 1.5, $CHCl_3$); TLC (solvent D) R_f 0.69, (solvent A) R_f 0.60; NMR ($CDCl_3$) δ 1.1 (q, 12 H, Val methyl), 1.3 (d, 6 H, Ala methyl), 2.5 (m, 2 H, Val methyne),

2.8 (d, 4 H, Cys methylene), 4.4–5.1 (m, 10 H, Ser methylene, 6 α hydrogens), 5.6 (m, 2 H, α hydrogens), 6.4 (d, 2 H, NH), 7.3 (d, 2 H, NH), 7.7–8.2 (m, 8 H, quinoxaline 5, 6, 7, and 8), 8.5 (d, 2 H, NH), 8.7 (d, 2 H, NH), 9.6 (s, 2 H, quinoxaline 3 hydrogen); λ_{max} (CH_3CN) 325 nm (ϵ 1.11×10^4), 315 (1.13×10^4); mass spectrum, field desorption, M^+ 1031; electron impact (partial spectral data given) *m/e* (rel intensity) $(M^+ + 1)/2$ 516 (8), 482 (3.2), 366 (1.2), 297 (12), 226 (40), 173 (32), 157 (27), 130 (100), 129 (60), 102 (48). Amino acid analysis: Ala 1.08, Cys 0.87, Ser 1.06, Val 1.00. Anal. ($C_{46}H_{54}N_{12}O_{12}S_2 \cdot H_2O$) C, H, N, S.

Analysis for racemization was carried out as follows: compound **7** and compound **2** (5 mg) were each hydrolyzed (5 mL of 6 N HCl, 110 °C, 36 h) in a sealed tube under N_2 and the solvent was removed by evaporation under a stream of N_2. To the residue was added 3 mL of a saturated solution of HCl in 2-propanol and the tube was resealed and heated at 100 °C for 2 h. The solvent was removed under N_2 and the residue was treated at room temperature for 24 h with 0.5 mL of trifluoroacetic anhydride in 2 mL of methylene chloride. The solvent was evaporated and the residue dried overnight over P_2O_5 and NaOH. Methylene chloride (2 mL) was added to the residue and a 5-μL sample was injected into a gas chromatograph (Hewlett-Packard Model 5830A) and the alanine residues were analyzed on a capillary column coated with *N*-lauryl-L-valyl-*tert*-butylamide.[24] A parallel control experiment was conducted by treating L-alanine (5 mg) to the same sequence of reactions, followed by analysis. The D- and L-alanine derivatives were cleanly resolved on the above capillary column. Analysis gave values for D-alanine as follows: control, 2.4%; sample from **7**, 6.4% above control, representing 12.8% racemization; sample from **2**, 1.0% above control, representing 2% diastereomeric impurity (4% racemization) due to two racemizable Ala units in **2**.

L-Seryldes-*N*-tetramethyltriostin A (2-L-Ser). The des-*N*-tetramethyltriostin A analogue **2**-L-Ser was prepared from **9**-L-Ser (0.25 g, 0.24 mmol) by the same procedure as above. The column chromatography was carried out using E. Merck silica gel 60 (230–400 mesh) and by elution with chloroform–absolute ethanol (9:1 v/v). The material obtained from the column was recrystallized from chloroform–diethyl ether to yield 0.15 g (58%) of **2**-L-Ser: mp 242–246 °C; $[\alpha]^{25}_D$ −11.2° (*c* 1, DMF); TLC (solvent F) R_f 0.57; NMR ($CDCl_3$) δ 0.9 (d, 12 H, Val methyl), 1.35 (q, 6 H, Ala methyl), 2.1 (m, 2 H, Val methyne), 2.9 (m, 4 H, Cys methylene), 3.6–4.3 (m, 3 H, α hydrogens), 4.4–5.2 (m, 7 H, α hydrogens and Ser methylene), 5.7 (m, 2 H, α hydrogens), 7.3 (m, 4 H, NH), 7.6–8.3 (m, 8 H, quinoxaline 5, 6, 7, and 8), 8.4–9.0 (m, 4 H, NH), 9.6 (s, 2 H, quinoxaline 3 hydrogen). Anal. ($C_{46}H_{54}N_{12}O_{12}S_2$) C, H, N.

***N*-Benzyloxycarbonyl-*O*-(*tert*-butyloxycarbonyl-L-valyl)-L-alanine (10).** Zinc powder (4.0 g) was added to a vigorously stirred ice-cold solution of *N*-benzyloxycarbonyl-*O*-(*tert*-butyloxycarbonyl-L-valyl)-D-seryl-L-alanine β,β,β-trichloroethyl ester (**3**, 1.0 g, 1.59 mmol) in 90% aqueous acetic acid (50 mL). After stirring for 1 h at 0 °C, the mixture was filtered and the residue was washed well with 90% aqueous acetic acid. The filtrate and washings were combined and concentrated to a white solid. The solid was shaken with a mixture of diethyl ether (75 mL) and 0.5 N aqueous HCl (75 mL) until all the solid dissolved. The ethereal layer was separated, washed several times with H_2O, and dried ($MgSO_4$). After concentration, the resulting solid was recrystallized from diethyl ether–petroleum ether (bp 30–60 °C) to give a crystalline solid, 0.72 g (90%): mp 102–103.5 °C; $[\alpha]^{25}_D$ −11.5° (*c* 4, $CHCl_3$); TLC (solvent A) R_f 0.65. Anal. ($C_{24}H_{35}N_3O_9$) C, H, N.

***N*,*N*′-Di[*N*-benzyloxycarbonyl-*O*-(*tert*-butyloxycarbonyl-L-valyl)-D-seryl-L-alanyl]-L-cystine Di-*tert*-butyl Ester (12).** A mixture of cystine di-*tert*-butyl ester dihydrochloride (**11**,[28] 0.247 g, 0.58 mmol) and *N*-methylmorpholine (0.120 g, 1.18 mmol) in tetrahydrofuran (30 mL) was stirred vigorously at 0 °C for 20 min. To this stirred, cold mixture was added tridepsipeptide **10** (0.58 g, 1.16 mmol) and 1-hydroxybenzotriazole monohydrate (0.355 g, 2.32 mmol) followed by *N*,*N*′-dicyclohexylcarbodiimide (0.257 g, 1.25 mmol). The mixture was stirred at 0 °C for 1.5 h and then overnight at room temperature. The insoluble material was removed by filtration and washed well with tetrahydrofuran. The filtrate and washings were combined and concentrated in vacuo. The residue was dissolved in ethyl acetate (75 mL) and washed successively with H_2O (30 mL), 1 N aqueous HCl (2 × 30 mL), saturated sodium bicarbonate solution (2 × 30 mL), H_2O (30 mL), and saturated sodium chloride solution (25 mL). The ethyl acetate solution was dried ($MgSO_4$) and concentrated to a yellow solid. The solid was dissolved in a small volume of ethyl acetate and filtered through a 1.5 × 8 cm column of basic alumina using ethyl acetate as the eluant (100 mL collected). The eluate was concentrated to a solid residue which was crystallized from ethyl acetate–diethyl ether. The two crops obtained were combined and triturated with diethyl ether. The trituration mixture was cooled in the freezer overnight before filtering. After filtration and drying, 0.48 g (63%) of a white solid was obtained: mp 114–115 °C; $[\alpha]^{25}_D$ 5.1° (*c* 4, $CHCl_3$); TLC (solvent B) R_f 0.38. Anal. ($C_{62}H_{94}N_8O_{20}S_2$) C, H, N.

Attempted Preparation of Cyclic Octadepsipeptide 8 from 12. Compound **12** (280 mg, 0.21 mmol) was dissolved in anhydrous trifluoroacetic acid (3.0 mL) and stirred at room temperature for 30 min. The solution was concentrated and the oily residue was triturated well with anhydrous diethyl ether. The resulting solid was collected by filtration, washed with diethyl ether, and dried in vacuo over KOH to give 220 mg (85%) of a white solid, mp 156–162 °C.

A stirred solution of this solid (250 mg, 0.2 mmol) and *N*-hydroxysuccinimide (190 mg, 1.64 mmol) in dimethylformamide (1.5 mL) was diluted with methylene chloride (150 mL). Triethylamine (40 mg, 0.40 mmol) was added and the solution was cooled to 0 °C in an ice bath. *N*,*N*′-Dicyclohexylcarbodiimide (228 mg, 1.11 mmol) was added and the mixture was stirred at 0 °C for 2 h and then at room temperature for 5 days.

The mixture was concentrated to dryness and the solid residue was taken up in ethyl acetate (75 mL). The insoluble material was removed by filtration and the filtrate was washed successively with H_2O (50 mL), saturated sodium bicarbonate solution (50 mL), 1 N aqueous HCl (50 mL), saturated sodium bicarbonate solution (50 mL), H_2O (50 mL), and saturated sodium chloride solution (50 mL). The ethyl acetate solution was dried ($MgSO_4$) and concentrated to a solid residue. TLC analysis (solvents B and C) showed the product to be a complex mixture containing considerable unreacted starting material. None of the desired compound **8** could be isolated by preparative TLC from this mixture.

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Synthesis and Characterization of Prostacyclin, 6-Ketoprostaglandin $F_1\alpha$, Prostaglandin I_1, and Prostaglandin I_3

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Abstract: The key intermediates, (5*S*,6*S*)-5-iodoprostaglandin I_1 methyl ester (**7**) and (5*R*,6*R*)-5-iodoprostaglandin I_1 methyl ester (**8**), have been prepared from the reaction of prostaglandin $F_2\alpha$ methyl ester (**6**) with iodine. The diastereomers, (5*R*,6*S*)-5-iodoprostaglandin I_1 methyl ester (**17**) and (5*S*,6*R*)-5-iodoprostaglandin I_1 methyl ester (**18**), have been prepared from the reaction of 5-*trans*-prostaglandin $F_2\alpha$ methyl ester (**16**) with iodine. Reductive removal of iodine from either **7** and **8** or from **17** and **18** gave (6*R*)-prostaglandin I_1 methyl ester (**15**) and (6*S*)-prostaglandin I_1 methyl ester (**12**), respectively. Compounds **15** and **12** have also been prepared from the reaction of **6** with mercuric acetate followed by reduction with sodium borohydride or from the reaction of 6-ketoprostaglandin $F_1\alpha$ methyl ester (**22**) with excess sodium cyanoborohydride. Catalytic reduction of **12** gave (6*S*)-13,14-dihydroprostaglandin I_1 methyl ester (**13**) and (6*S*)-13,14-dihydro-15-deoxyprostaglandin I_1 methyl ester (**14**). The parent PGI_1 structures, (6*R*)-PGI_1 (**23**, mp 97–99 °C) and (6*S*)-PGI_1 (**24**, mp 79–81 °C), have been prepared either by reaction of $PGF_2\alpha$ (**25**) with mercuric acetate followed by sodium borohydride or by saponification of the methyl esters **15** and **12**, respectively. Prostacyclin sodium salt (**32**, PGI_2 sodium salt) has been prepared from iodo ethers **7** or **8**. The reaction of **7** or **8** with 1,5-diazabicyclo[4.3.0]non-5-ene or with potassium superoxide gave PGI_2 methyl ester (**26**) together with traces of (4*E*,6*S*)-Δ^4-prostaglandin I_1 methyl ester (**27**) and (4*E*,6*R*)-Δ^4-prostaglandin I_1 methyl ester (**28**). Treatment of **26** with sodium hydroxide or sodium carbonate gave the desired sodium salt of PGI_2 (**32**). The isomeric (5*E*)-PGI_2 methyl ester (**31**) has been prepared from **18** by reaction with potassium superoxide and, upon saponification, gave (5*E*)-PGI_2 sodium salt (**34**). Δ^6-Prostaglandin I_1 methyl ester diacetate (**43**) together with PGI_2 methyl ester diacetate (**41**) and (5*E*)-PGI_2 methyl ester diacetate (**42**) have been prepared by dehydration of 6-ketoprostaglandin $F_1\alpha$ methyl 11,15-diacetate (**39**). Δ^6-PGI_1 sodium salt (**38**) has been prepared by hydrolysis and saponification of **43**. A radiolabeled sample of enzymatically produced PGI_2 did not contain significant amounts of either (5*E*)-PGI_2 or Δ^6-PGI_1. 6-Ketoprostaglandin $F_1\alpha$ methyl ester (**22**) has been prepared either from **8** via silver ion assisted elimination and hydrolysis or more directly from hydrolysis of **26**. 6-Ketoprostaglandin $F_1\alpha$ (**33**) has been prepared either by hydrolysis of **32** or by saponification of **22**. A convenient assay for the purity of prostacyclin samples has been developed. For this assay, prostacyclin is converted to the *p*-phenylphenacyl ester and analyzed by thin layer chromatography. This derivative of prostacyclin is stable to the chromatography conditions and is cleanly separated from impurities. (5*S*,6*S*)-5-Iodo-*cis*-Δ^{17}-prostaglandin I_1 methyl ester (**55**) and (5*R*,6*R*)-5-iodo-*cis*-Δ^{17}-prostaglandin I_1 methyl ester (**56**) have been prepared from the reaction of prostaglandin $F_3\alpha$ methyl ester (**54**) with iodine. From the major iodo ether **56**, prostaglandin I_3 methyl ester (**57**) was prepared and was converted to prostaglandin I_3 sodium salt (**52**). Hydrolysis of **52** and **57** gave respectively 6-keto-*cis*-Δ^{17}-prostaglandin $F_1\alpha$ (**53**) and 6-keto-*cis*-Δ^{17}-prostaglandin $F_1\alpha$ methyl ester (**58**).

The discovery of prostacyclin (originally called prostaglandin X) by Moncada, Gryglewski, Bunting, and Vane[1] has added an exciting new dimension to the role of arachidonic acid metabolism in biology.[2-7] Prostacyclin (**1**), acting via cyclic nucleotide mediation,[8,9] is the most potent inhibitor of platelet aggregation yet discovered as well as being a powerful vasodilator.[1-4] Prostacyclin is derived biosynthetically from arachidonic acid (**2**) by way of the intermediate prostaglandin endoperoxides, PGG_2 and PGH_2 (**3** and **4**).[1] The endoperoxides[10] also are precursors to thromboxane A_2 (**5**), a molecule having biological properties opposite those of prostacyclin.[11] Thromboxane A_2 is a potent inducer of platelet aggregation as well as being a powerful vasoconstrictor. The biosynthesis of prostacyclin in the circulatory system is concentrated within the vascular walls,[2] whereas biosynthesis of thromboxane A_2 is concentrated in the platelets.[11] The chemical properties of these two compounds are such that both are susceptible to rapid hydrolysis and, consequently, inactivation. Nature appears to have devised in this delicately balanced system a mechanism whereby blood vessels are protected under normal conditions from the harmful deposition of platelet aggregates, but, in case of injury, are able to promptly form such aggregates in the area of damage.

The structure of prostacyclin has been determined and is

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