Determination of the Chlorine Left on the Resin. Resin samples (0.1–0.2 g) withdrawn at 1, 2, 4, 8, and 12 h and at the end of the reaction were washed, dried, and heated with pyridine (2 mL) in boiling water for 60 min. The solution with the resin was transferred to an Erlenmeyer flask with 50 mL of 20% acetic acid. A Volhard titration for chloride was carried out by addition of saturated ferric ammonium sulfate indicator (3 drops), concentrated nitric acid (5 mL), 0.1 N $AgNO_3$ (5 mL), and toluene (3 mL), followed by back-titration with 0.1 N KSCN. In the absence of the quaternization step with pyridine, no Cl was found.

Determination of the Extent of Polymerized 4-HMP on the Resin. A solution of 2 mL of 30% HBr in HOAc was added to 100 mg of the alkoxybenzyl alcohol resin (A and C) prepared by different procedures and to an unsubstituted divinylbenzene polystyrene resin control. After 1 h, the resin was filtered and washed with 1% HOAc. The filtrate was collected and diluted in 1% HOAc to 100 mL. Solid potassium bisulfite (20 mg) was added to the solution to reduce bromine to bromide. This solution absorbed strongly with maxima at 254 and 270 nm. The 270-nm absorption shifted to >330 nm when the solution was made strongly basic, indicative of conversion of the bromomethylphenol derivative to quinone methide and its products. Quantitation of bromomethylphenol was made at 270 nm ($\epsilon = 1.12 \times 10^3$ M^{-1} cm^{-1}).

Esterification of Bpoc-Val to 4-Alkoxybenzyl Alcohol Resin. 4-alkoxybenzyl alcohol resin (100 mg) was treated with Bpoc-Val (34.1 mg, 0.096 mmol), dicyclohexylcarbodiimide (19.8 mg, 0.096 mmol), and 4-(dimethylamino)pyridine (1.95 mg, 0.016 mmol) in 2.2 mL of CH_2Cl_2 in a small, screw-capped reaction vessel on a shaker for 3 h. The resin was washed with dichloromethane (6 times, 5 mL). The esterification was repeated one more time with the same excess of reagents.

Stepwise Solid-Phase Synthesis of Leucylalanylglycylvaline on 4-Alkoxybenzyl Alcohol Resin Prepared via Sodium Methoxide under Improved Conditions. The synthesis began with Bpoc-valyloxymethylphenoxymethyl resin (400 mg, 0.13 mmol). One cycle of the synthesis consisted of (1) deprotection with 0.5% TFA in CH_2Cl_2 (3 × 1 min, 1 × 20 min), (2) neutralization with 5% diisopropylethylamine in CH_2Cl_2 (3 × 2 min), (3) equilibration with Bpoc-amino acid (3 equiv, 5 min) in CH_2Cl_2, (4) coupling by addition of dicyclohexylcarbodiimide (3 equiv, 120 min). All intermediate washes were with dichloromethane. Steps 2, 3 and 4 were repeated for the second coupling. All wash and reaction volumes were 5 mL.

Protected-peptide resin (55.2 mg) was cleaved by treatment with 4 mL of 50% TFA in dichloromethane for 2 h. The resulting peptide mixture was analyzed chromatographically, before purification, for peptide composition as described previously.[15]

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Registry No. 2, 623-05-2; styrene-divinylbenzene copolymer, 9003-70-7; Bpoc-valine, 78004-68-9; leucylalanylglycylvaline, 17195-26-5; KF, 7789-23-3; $KHCO_3$, 17353-70-7; CsF, 13400-13-0; $CsHCO_3$, 15519-28-5; Et_4NF, 665-46-3; NaOMe, 124-41-4.

Synthesis of (MeAla)TANDEM, the Bis(*N*-methylalanine) Analogue of Des-*N*-tetramethyltriostin A

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[MeAla^2,MeAla^6]des-*N*-tetramethyltriostin A (**3**), an analogue of the bicyclic octadepsipeptide antibiotic triostin A (**1**), has been prepared. Analogue **3** is of interest in studies relating to the mode of binding of the triostin antibiotics to nucleic acids. The analogue contains *N*-methyl-L-alanine units in place of the two normal L-alanines and lacks the four *N*-methyl groups common to the two pair of cysteine and valine residues. Coupling *N*-methyl-L-alanine 2,2,2-trichloroethyl ester with the 2,4-dinitrophenyl ester of Z-D-serine gave dipeptide Z-D-Ser-MeAla-OTce (**6**). Depsipeptide **7** was prepared by esterification of dipeptide **6** with Boc-Val-OH by using a carbodiimide procedure catalyzed by 4-(dimethylamino)pyridine. Deprotection of **7**, followed by its coupling with Boc-Cys(Acm)-OH, gave tetradepsipeptide **8**, which, by appropriate deprotection, was converted into the respective tetradepsipeptides **9** and **10**. Fragment coupling of **9** and **10** furnished linear octadepsipeptide **11**, which upon subsequent transformation involving deprotection, cyclization, and disulfide formation gave cyclic product **13**. Removal of the *N*-[(benzyloxy)carbonyl] groups from the two D-serine residues in **13**, followed by acylation with 2-quinoxalinecarbonyl chloride, provided analogue **3**.

There is considerable interest[1] in the mode of binding of the triostin[2] and quinomycin[3] quinoxaline depsipeptide antibiotics to nucleic acids. Waring and co-workers[4] have shown these antibiotics to bind to various natural and synthetic DNA molecules by a mechanism involving bifunctional intercalation of both quinoxaline chromophores common to the antibiotics. Solution NMR conformational studies of echinomycin (quinomycin A) and of triostin A have been reported by Williams and co-workers.[5,6] A model, based on conformational energy calculations, has been proposed by Ughetto and Waring for the binding of echinomycin to DNA.[7]

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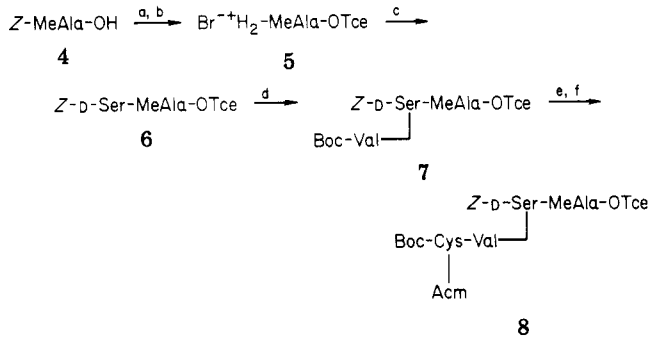
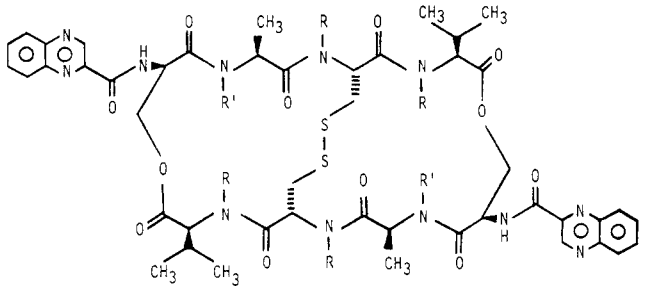
0022-3263/81/1946-3436$01.25/0

Scheme I[a]



[a] (a) CCl_3CH_2OH, DCC, DMAP, CH_2Cl_2; (b) HBr, AcOH; (c) (*Z*)-D-Ser-ODNP, TEA, CH_2Cl_2; (d) Boc-Val-OH, WSC, DMAP, CH_2Cl_2; (e) TFA; (f) Boc-Cys(Acm)-OH, HOBT, WSC, THF. Abbreviations used in this paper are as follows: DCC, *N,N'*-dicyclohexylcarbodiimide; DMAP, 4-(dimethylamino)pyridine; DNP, 2,4-dinitrophenyl; TEA; triethylamine; TFA, trifluoroacetic acid; Boc, *N*-[(*tert*-butyloxy)carbonyl]; Acm, *S*-(acetamidomethyl); HOBT, 1-hydroxybenzotriazole; WSC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; Z, (benzyloxy)carbonyl.

We recently have accomplished the total synthesis of triostin A (1) and of several analogues.[8-10] Des-*N*-tetramethyltriostin A (2),[9] given the acronym TANDEM by Lee



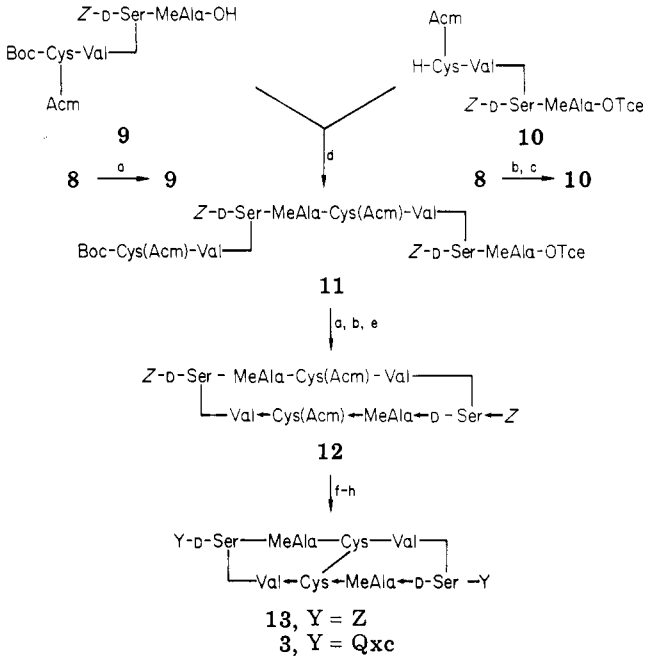
1 R = Me, R' = H

2 R = R' = H

3 R = H, R' = Me

and Waring,[11] was shown to bind to DNA as a bifunctional intercalating agent and to possess a remarkedly high specificity for binding to poly(dA-dT). The crystal structure of TANDEM has recently been determined,[12] which represents the first successful crystallographic studies of a triostin or a quinomycin antibiotic. The X-ray crystallographic results have led to a proposed model for TANDEM's specificity in binding to poly(dA-dT).[13] A key element in this model involves hydrogen bonds between the alanyl NH and a carbonyl oxygen of thymine. As a probe of this model, it would be of interest to study the bis(*N*-methylalanine) analogue of TANDEM, which would lack the potential of hydrogen bond formation. We report in this paper the synthesis of this analogue [MeAla^2,MeAla^6]-des-*N*-tetramethyltriostin A (3, or (MeAla)TANDEM).

Scheme II



[a] (a) Zn, 90% aqueous AcOH, room temperature; (b) TFA, CH_2Cl_2, room temperature; (c) $NaHCO_3$; (d) WSC, HOBT, THF; (e) WSC, HOBT, CH_2Cl_2, high dilution; (f) I_2, MeOH, (g) HBr, AcOH; (h) Qxc-Cl, TEA, DMF. For abbreviations used, see Scheme I; Qxc, 2-quinoxalinecarbonyl.

The synthesis of (MeAla)TANDEM followed the procedures as are outlined in Schemes I and II. The initial objective was preparation of tetradepsipeptide 8, as is given in Scheme I. *N*-[(Benzyloxy)carbonyl]-*N*-methyl-L-alanine (4), prepared by following the procedure of Benoiton,[14] was converted to the 2,2,2-trichloroethyl ester by esterification with 2,2,2-trichloroethanol using Hassner's method[15] involving *N,N'*-dicyclohexylcarbodiimide (DCC) and catalyzed by 4-(dimethylamino)pyridine (DMAP). Subsequent removal of the *N*-[(benzyloxy)carbonyl] group with HBr in acetic acid gave hydrobromide 5 in 87% overall yield.

Neutralization of 5 and carbodiimide-mediated coupling with Z-D-Ser-OH gave dipeptide 6 in only moderate yield along with other products. A successful preparation of dipeptide 6 was achieved in 88% yield by the active ester method involving reaction of the free amine generated from 5 with the 2,4-dinitrophenyl ester[16] of Z-D-serine.

Depsipeptide bond formation was effected by the WSC–DMAP method[15] in which 6 was caused to react with Boc-Val-OH to give (87%) tridepsipeptide 7. The preparation of tetradepsipeptide 8 from 7 proceeded in good yield by N-deprotection with trifluoroacetic acid and coupling to Boc-Cys(Acm)-OH by using 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (WSC) with 1-hydroxybenzotriazole (HOBT) as an additive.[17]

The synthesis of (MeAla)TANDEM was completed (Scheme II) by the deprotective conversion of 8 to tetradepsipeptides 9 and 10, respectively. Depsipeptide 9 was

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Table I. Analysis of Hydrolysates of Depsipeptides for Percent of D-Amino Acids[a]

compd	% of D-MeAla[b]	% of D-Val[c]
7	0	0
8	1.35	0.60
11	2.50	2.00
13	0	1.10
3	0	0

[a] Hydrolysis carried out under standard conditions in 6 N HCl at 100 °C for 22 h. The amino acids in the hydrolysates were converted to their respective *N*-trifluoroacetyl isopropyl esters and analyzed by gas chromatography on a capillary column coated with a chiral phase as described in ref 9 and 20. [b] Percent given refers to value above that of a control sample of *N*-methyl-L-alanine, which gave 1.2% *N*-methyl-D-alanine. [c] As for footnote *b* above with 0.95% D-valine observed.

prepared by removal[18] of the 2,2,2-trichloroethyl ester with zinc in 90% aqueous acetic acid, which conditions leave the ester depsipeptide bond intact; **10** was prepared by removal of the Boc group with trifluoroacetic acid. Fragment coupling of **9** and **10** was effected by the above WSC–HOBT method to give linear octadepsipeptide **11** in 88% yield. Sequential deprotection of the amino and carboxyl termini of **11** followed by cyclization under high dilution by the slow addition of deprotected **11** to a methylene chloride solution of WSC–HOBT furnished cyclic product **12** in 55% yield. Oxidative removal[19] of the *S*-(acetamidomethyl) (Acm) groups gave (87%) disulfide **13**, which upon removal of the (benzyloxy)carbonyl functions on the serine units and subsequent condensation with 2-quinoxalinecarbonyl chloride gave (MeAla)TANDEM (**3**) in 61% overall yield from disulfide **13**.

Octadepsipeptide **11** and cyclic product **13** were analyzed[20] for the degree of racemization that may have occurred during the fragment coupling reactions[21] leading to their formation. The results obtained, along with data for other peptide intermediates prepared in this study, are given in Table I. Octadepsipeptide **11** showed 2.5% *N*-methyl-D-alanine (D-MeAla) to be present after hydrolysis. This value should be compared to the value of 1.35% of D-MeAla observed for tetradepsipeptide **8**, which should not contain any racemized MeAla units. We interpret the small amount of D-MeAla obtained upon analysis of tetradepsipeptide **8** as arising from racemization during hydrolysis of the peptide. Small percentages of D-valine also were observed for depsipeptides **8**, **11**, and **13**, which likely arise in a similar manner. The 2.5% diasteromeric impurity indicated from the analysis of octadepsipeptide **8** is an upper limit for racemization, which likely is lower than what would be represented by these values. After cyclization, cyclic depsipeptides **13** and **3** were shown to contain no D-MeAla. The small amounts of diastereomeric impurities present are either removed upon purification of cyclized product or do not undergo favorable cyclization processes and are removed in the acid and base washes during workup of the reaction. Similar results have been observed[9] in the synthesis of TANDEM.

(MeAla)TANDEM (**3**) was evaluated, in collaboration with Professor M. J. Waring of the University of Cambridge, with regard to its binding to DNA and any specificity that may be observed in binding to poly(dA-dT). Unfortunately, because of the extreme insolubility of **3** in aqueous media, binding data for the analogue could not be measured.

Experimental Section

The L amino acid derivatives used in this study were, in most cases, commercially available. D-Serine and 2-quinoxalinecarbonyl chloride were purchased from Sigma and from Aldrich, respectively. All coupling reagents employed were obtained from commercial sources. Tetrahydrofuran was distilled prior to use from sodium benzophenone ketyl. Dichloromethane was distilled from phosphorus pentoxide and stored over Linde 3A molecular sieves.

Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded for all compounds reported by using a Varian EM-360 or XL-100-12 or a Nicolet NT-360 spectrometer; satisfactory NMR spectral data were obtained for all compounds, and data for selected intermediates are reported. Optical rotations were recorded on a Perkin-Elmer 241 automatic polarimeter. Thin-layer chromatography was performed on commercially prepared silica gel on glass plates by using the following solvent systems: A, hexane–acetone, 70:30; B, $CHCl_3$–MeOH–AcOH, 90:8:2; C, hexane–acetone, 60:40; D, *n*-BuOH–AcOH–H_2O, 10:2:3; E, $CHCl_3$–MeOH, 95:5. The majority of compounds were purified by medium-pressure liquid chromatography (MPLC) using columns packed with silica gel 60 (0.040–0.063 mm) and elution with specified solvent.[22]

***N*-Methyl-L-alanine 2,2,2-Trichloroethyl Ester (5).** A stirred solution of *N*-[(benzyloxy)carbonyl]-*N*-methyl-L-alanine[14] (9.0 g, 37.9 mmol), 2,2,2-trichloroethanol (7.45 g, 49.8 mmol), and 4-(dimethylamino)pyridine (0.46 g, 3.79 mmol) in dry methylene chloride (100 mL) was cooled to 0 °C in an ice bath. *N,N'*-Dicyclohexylcarbodiimide (8.25 g, 40 mmol) was added, and the mixture was stirred at 0 °C for 4 h and overnight at room temperature. The urea that separated was filtered and washed with methylene chloride. The filtrate and washings were combined and concentrated to a viscous oil. The oil was dissolved in ethyl acetate (250 mL) and filtered, and the filtrate was washed successively with H_2O (2 × 75 mL), saturated sodium bicarbonate solution (2 × 75 mL), and H_2O (75 mL), dried (Na_2SO_4), and concentrated to a yellow oil, which was dried in vacuo over P_2O_5; TLC (solvent A) R_f 0.63.

The oil was stirred with a solution of hydrogen bromide in acetic acid (32%, 50 mL) for 1 h at room temperature. Anhydrous diethyl ether (400 mL) was added, and the solution was stored at 0 °C overnight. The solid was filtered, washed well with anhydrous diethyl ether, and dried. After recrystallization from absolute ethanol–diethyl ether, 10.6 g (87%) of a white crystalline solid was obtained: mp 189–190 °C; $[\alpha]^{25}_D$ −12.58° (*c* 2, DMF). Anal. ($C_6H_{11}BrCl_3NO_2$) C, H, N.

[*N*-[(Benzyloxy)carbonyl]-D-seryl]-*N*-methyl-L-alanine 2,2,2-Trichloroethyl Ester (6). A stirred suspension of **5** (3.2 g, 10.1 mmol) in 35 mL methylene chloride was cooled to 0 °C in an ice bath and triethylamine (1.04 g, 10.3 mmol) was added. The reaction mixture was stirred for 5 min and then *N*-[(benzyloxy)carbonyl]-D-serine 2,4-dinitrophenyl ester[16] (4.0 g, 9.87 mmol) was added. The ice bath was removed, and the reaction mixture was stirred at room temperature for 2.5 days and concentrated to a yellow viscous oil. The oil was taken up in ethyl acetate (150 mL) and washed with 1 N HCl (2 × 50 mL), saturated sodium bicarbonate solution (8 × 40 mL), and H_2O (2 × 50 mL). After being dried (Na_2SO_4), the ethyl acetate solution was concentrated to a viscous oil which was chromatographed by MPLC with 7.5:2.5 hexane–acetone as the eluant: yield 3.9 g (88%); $[\alpha]^{25}_D$ −9.15° (*c* 2, MeOH); TLC (solvent C) R_f 0.38. Anal. ($C_{17}H_{21}Cl_3N_2O_6$) C, H, N.

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[*N*-[(Benzyloxy)carbonyl]-*O*-[[(*tert*-butyloxy)carbonyl]-*S*-(acetamidomethyl)-L-cysteinyl-L-valyl]-D-seryl]-*N*-methyl-L-alanine 2,2,2-Trichloroethyl Ester (8). A solution of **7** (2.3 g, 3.5 mmol) in anhydrous trifluoroacetic acid (4 mL) was stirred for 30 min at room temperature. The solution was concentrated to an oily residue, which was dissolved in ethyl acetate (40 mL), and washed with saturated sodium bicarbonate solution (2 × 25 mL) and H_2O (2 × 20 mL), dried (Na_2SO_4), and concentrated to a foam to give deprotected **7**.

A stirred solution of the above compound, *N*-[(*tert*-butyloxy)carbonyl]-*S*-(acetamidomethyl)-L-cysteine[23] (1.05 g, 3.6 mmol), and 1-hydroxybenzotriazole (0.95 g, 7 mmol) in dry THF (40 mL) was cooled to 0 °C in an ice bath. 1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (0.76 g, 4 mmol) was added, and the reaction mixture was stirred for 1 h at 0 °C and 5 h at room temperature. The reaction mixture was concentrated to a viscous yellow oil, which was dissolved in ethyl acetate (80 mL) and washed successively with 1 N HCl (2 × 30 mL), saturated sodium bicarbonate (2 × 30 mL), and H_2O (2 × 30 mL). The organic phase was dried (Na_2SO_4) and concentrated to an oil. The oil was purified by MPLC with 7:3 hexane–acetone as the eluant, and the eluate was concentrated to a white crystalline solid: 2.5 g (87%); mp 75–77 °C; $[\alpha]^{25}_D$ −20.3° (*c* 1, MeOH); TLC (solvent C) R_f 0.29; NMR ($CDCl_3$, 360 MHz) δ 0.92 (dd, 6 H, Val methyl), 1.44 (s, 9 H, Boc), 1.50 (d, 3 H, Ala methyl), 2.03 (s, 3 H, Acm), 2.12–2.24 (m, 1 H, Val methine), 2.66–2.75 (dd, 1 H, Cys β_1), 2.92–3.00 (dd, 1 H, Cys β_2), 3.14 (s, 3 H, *N*-methyl of Ala), 4.20–4.70 (set of multiplets, 5 H, Acm, Ser methylene, and α-hydrogen), 4.74 (q, 2 H, Tce), 5.00–5.20 (set of multiplets, 3 H, α-hydrogens), 5.11 (s, 2 H, benzyl), 5.53 (d, 1 H, NH), 5.93 (d, 1 H, NH), 6.84 (m, 1 H, NH), 7.20 (m, 1 H, NH) 7.18–7.4 (m, 5 H, benzyl aromatic). Anal. ($C_{33}H_{48}Cl_3N_5O_{11}S$) C, H, N.

[*N*-[(Benzyloxy)carbonyl]-*O*-[[(*tert*-butyloxy)carbonyl]-*S*-(acetamidomethyl)-L-cysteinyl]-L-valyl]-D-seryl]-*N*-methyl-L-alanine (9). To a vigorously stirred ice-cold solution of **8** (2.7 g, 3.2 mmol) in 90% aqueous acetic acid (70 mL) was added in portions Zn powder (10.4 g, 160 mmol). After being stirred at 0 °C for 2 h, the reaction mixture was stirred for 3 h at room temperature and filtered, and the residue was washed well with 90% AcOH. The filtrate was concentrated to a solid residue, which was shaken with a mixture of ethyl acetate (60 mL) and 1 N HCl (15 mL). The acid layer was separated and extracted with ethyl acetate (20 mL), and the organic layers were combined, washed with water (3 × 20 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed by MPLC with 90% chloroform–methanol as the eluant: yield 1.63 g (72%); $[\alpha]^{25}_D$ −22.6° (*c* 2, MeOH); TLC (solvent B) R_f 0.47. Anal. ($C_{31}H_{47}N_5O_{11}S$) C, H, N.

[*N*-[(Benzyloxy)carbonyl]-*O*-[[*S*-(acetamidomethyl)-L-cysteinyl]-L-valyl]-D-seryl]-*N*-methyl-L-alanine 2,2,2-Trichloroethyl Ester (10). A solution of **8** (2.0 g, 2.4 mmol) in anhydrous TFA (4.5 mL) and methylene chloride (2 mL) was stirred for 30 min at room temperature.

The solution was concentrated, and the residue was taken up in ethyl acetate (40 mL), washed successively with saturated sodium bicarbonate solution (3 × 20 mL) and water (2 × 20 mL), dried (Na_2SO_4), and concentrated to an oily residue: yield 1.6 g (93%); $[\alpha]^{25}_D$ +2.46° (*c* 3, MeOH).

[*N*-[(Benzyloxy)carbonyl]-*O*-[[[[*N*-[(benzyloxy)carbonyl]-*O*-[[*N*-[(*tert*-butyloxy)carbonyl]-*S*-(acetamidomethyl)-L-cysteinyl]-L-valyl]-D-seryl]-*N*-methyl-L-alanyl]-*S*-(acetamidomethyl)-L-cysteinyl]-L-valyl]-D-seryl]-*N*-methyl-L-alanine 2,2,2-Trichloroethyl Ester (11). A stirred solution of **9** (2.02 g, 2.9 mmol), **10** (2.1 g, 2.88 mmol), and 1-hydroxybenzotriazole (0.79 g, 5.8 mmol) in dry THF (65 mL) was cooled to 0 °C in an ice bath. 1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride was added, and the reaction mixture was stirred at 0 °C for 1 h and at room temperature for 6 h. The reaction mixture was concentrated, taken up in ethyl acetate (60 mL), and washed successively with H_2O (25 mL), 1 N HCl (2 × 20 mL), saturated $NaHCO_3$ solution (2 × 20 mL), and H_2O (2 × 20 mL). The ethyl acetate layer was dried (Na_2SO_4) and concentrated to dryness. The crude product was purified by MPLC with 95:5 chloroform–methanol as the eluant. The fractions were concentrated to give a white crystalline product: yield 3.5 g (87.5%); mp 89–91 °C; $[\alpha]^{25}_D$ −36.5° (*c* 2, MeOH); NMR ($CDCl_3$, 360 MHz) δ 0.92 (sextet, 12 H, Val methyl), 1.34 (d, 3 H, Ala methyl), 1.46 (s, 9 H, Boc), 1.51 (d, 3 H, Ala methyl), 1.97 (m, 3 H, Acm methyl), 2.13 (s, 3 H, Acm methyl), 2.12–2.26 (m, 2 H, Val methine), 2.57–3.0 (m, 4 H, Cys β-methylene), 3.06 (s, 3 H, *N*-methyl on Ala), 3.12 (s, 3 H, *N*-methyl on Ala), 4.08–5.06 (set of multiplets, 18 H, Acm, Ser, Tce methylene, and α-hydrogens), 5.10 (s, 4 H, benzyl), 5.55 (d, 1 H, NH), 6.06 (m, 2 H, NH), 6.63 (m, 1 H, NH), 6.93 (m, 1 H, NH), 7.06 (d, 1 H, NH), 7.2–7.4 (m, 12 H, benzyl aromatic and NH). Anal. ($C_{59}H_{85}Cl_3N_{10}O_{19}S_2$) C, H, N.

Bis[[[[*N*-[(benzyloxy)carbonyl]-D-seryl]-*N*-methyl-L-alanyl]-*S*-(acetamidomethyl)-L-cysteinyl]-L-valie] Dilactone (12). Zinc powder (5.5 g, 85 mmol) was added in portions to an ice-cold solution of **11** (2.4 g, 1.7 mmol) in 90% aqueous acetic acid (60 mL); stirring was continued at 0 °C for 2 h and then at room temperature for 3 h. The reaction mixture was filtered and washed with 90% aqueous AcOH. The filtrate and washing were combined and concentrated to a solid residue. The residue was shaken with a mixture of 1 N aqueous HCl (25 mL) and ethyl acetate (70 mL). The acid layer was separated and extracted once with ethyl acetate (25 mL), and the organic layers were combined, washed with H_2O (2 × 25 mL), and dried (Na_2SO_4). After the solution was concentrated, the residue was chromatographed by MPLC with 90:10 $CHCl_3$/MeOH as the eluant. The earlier washings gave a little starting material and pure product was obtained from later fractions, which on concentration gave a white crystalline solid: yield 1.6 g (76%); mp 113–115 °C; $[\alpha]^{25}_D$ −36.4° (*c* 2, MeOH); TLC (solvent B) R_f 0.40.

The solution of the above compound (0.35 g, 0.27 mmol) in anhydrous TFA (3 mL) was stirred at room temperature for 30 min. The solution was concentrated to a viscous oil, which was triturated well with anhydrous diethyl ether, filtered, and washed with anhydrous diethyl ether. The white solid obtained was dried in vacuo over P_2O_5: yield 0.35 g; mp 120–123 °C.

A solution of the above solid (0.35 g, 0.27 mmol) in dry DMF (5 mL) was treated with *N*-methylmorpholine (0.028 g, 0.28 mmol) and diluted with dry methylene chloride (150 mL). This solution was added in 6 h to an ice-cold stirred solution of 1-hydroxybenzotriazole (0.145 g, 1.08 mmol) and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (0.15 g, 0.81 mmol) dissolved in dry DMF (5 mL) and diluted with methylene chloride (400 mL). After the completion of the addition, the reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 4 days. The reaction mixture was concentrated to dryness, and the residue was taken up in ethyl acetate (80 mL) and shaken with water (30 mL). The aqueous layer was extracted with ethyl acetate (25 mL), and the combined organic layers were washed successively with 1 N HCl (2 × 20 mL), saturated sodium bicarbonate solution (2 × 25 mL), and water (2 × 25 mL). After being dried (Na_2SO_4), the solution was concentrated and the residue was purified on a gravity column of silica gel (24 cm ×

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2.5 cm; 230-400 mesh) with 95:5 chloroform-methanol as the eluant. The fractions containing the product [TLC (solvent E) R_f 0.42-0.44] were pooled and concentrated to a solid residue. The residue was recrystallized from chloroform-diethyl ether to give a white solid: 0.17 g (55%); mp 89-91 °C; $[\alpha]^{25}_d$ -57.7° (c 1, MeOH); TLC (solvent E) R_f 0.42 (ninhydrin negative). Anal. ($C_{52}H_{74}N_{10}O_{16}S_2$) C, H, N.

Bis[[[[*N*-[(benzyloxy)carbonyl]-D-seryl]-*N*-methyl-L-alanyl]-L-cysteinyl]-L-valine] Serine Hydroxyl Dilactone Disulfide (13). To a stirred solution of **12** (0.28 g, 0.24 mmol) in methanol (70 mL) was added dropwise a solution of iodine (0.6 g, 4.8 mmol) in methanol (110 mL) in 2 h. The solution was stirred for an additional 4 h and cooled in an ice bath, and 1 N aqueous sodium thiosulfate was added dropwise until the solution became colorless. The reaction mixture was concentrated, and the residue was triturated well with water. The resulting solid was filtered, and the residue was washed with several portions of water and dried in vacuo over P_2O_5. The product was purified on a short column of silica gel (230-400 mesh) with chloroform and chloroform-methanol (97:3 v/v) as the eluant. The fractions containing product [TLC (solvent E) R_f 0.65] were pooled and concentrated to a solid residue. The solid was recrystallized from chloroform-diethyl ether to give a white solid: yield 0.21 g (87.5%); mp 251-253 °C dec; TLC (solvent E) R_f 0.65; NMR ($CDCl_3$, 360 MHz) δ 0.9 (t, 12 H, Val methyl), 1.65 (d, 6 H, Ala methyl), 2.12-2.25 (m, 2 H, Val methine), 2.60-2.65 (m, 2 H, Cys β-methylene), 2.75-2.89 (m, 2 H, Cys β-methylene), 3.2 (s, 3 H, *N*-methyl on Ala), 3.23 (s, 3 H, *N*-methyl on Ala), 3.80 (m, 2 H, α-hydrogen), 4.58 (m, 2 H, Ser β-methylene), 5.85 (t, 2 H, Ser β-methylene), 5.98 (m, 2 H, α-hydrogen), 5.07 (s, 2 H, benzyl), 5.08 (s, 2 H, benzyl), 5.15 (m, 2 H, α-hydrogen), 5.26 (m, 2 H, α-H), 7.35 (s, 10 H, benzyl aromatic), 7.70 (d, 1 H, NH), 7.80 (s, 1 H, NH), 7.95 (d, 2 H, NH), 8.30 (d, 2 H, NH). Anal. ($C_{46}H_{62}N_8O_{14}S_2$) C, H, N.

[MeAla²,MeAla⁶]-Des-*N*-tetramethyltriostin A (3). A solution of **13** (0.11 g, 0.1 mmol) in 32% hydrogen bromide in glacial acetic acid (4 mL) was stirred for 45 min at room temperature. Anhydrous diethyl ether was added, and the precipitated solid was kept in a refrigerator overnight. The solid was filtered in a stream of N_2 and washed well with anhydrous diethyl ether and dried in vacuo over P_2O_5. The solid was recrystallized from methanol-diethyl ether: yield 0.085 g (97.7%); mp 272-276 °C dec.

An ice-cold stirred solution of the above solid (0.085 g, 0.09 mmol) in dry DMF (4 mL) was treated with triethylamine (0.022 g, 0.22 mmol), and the reaction mixture was stirred for 10 min. Another aliquot of triethylamine (0.022 g, 0.22 mmol) was added followed by the dropwise addition of quinoxalyl chloride (0.045 g, 0.23 mmol) in DMF (4 mL). The reaction mixture was stirred at 0 °C for 30 min and at room temperature for 22 h and then treated on a water bath at 50 °C for 2 h. The reaction mixture was concentrated, and the residue was triturated well with ether and filtered. The solid was washed successively with saturated sodium bicarbonate solution, water, ether, and dried in vacuo over P_2O_5. The straw-colored solid was purified by chromatography on a 2 × 25 cm column of silica gel (230-400 mesh) with chloroform-methanol (97:3) as the eluant. The fractions containing the product [TLC (solvent E) R_f 0.62] were pooled and concentrated to a solid residue. After crystallization from chloroform-diethyl ether and drying in vacuo, 0.06 g (61%) of a white solid was obtained: mp 225-228 °C; $[\alpha]^{25}_D$ -3.5° (c 1, $CHCl_3$); TLC (solvent E) R_f 0.62; quinoxaline ($CDCl_3$, 360 MHz) δ 0.96 (t, 12 H, Val methyl), 1.70 (d, 6 H, Ala methyl), 2.14 (m, 2 H, Val methine), 2.80 (s, 6 H, *N*-methyl on Ala), 2.91-3.07 (m, 4 H, Cys β-methylene), 4.09 (m, 2 H, α-hydrogen), 4.72 (m, 2 H, Ser β-methylene), 5.06 (t, 2 H, Ser β-methylene), 5.52 (d, 2 H, α-hydrogen), 5.72 (m, 4 H, α-hydrogen), 7.01 (d, 2 H, NH), 7.92 (m, 4 H, quinoxaline 6-H and 7-H), 8.14 (d, 2 H, quinoxaline), 8.23 (d, 2 H, quinoxaline), 8.38 (d, 2 H, NH), 8.50 (d, 2 H, NH), 9.68 (s, 2 H, qui noxaline 3-H). Anal. ($C_{48}H_{58}N_{12}O_{12}S_2 \cdot H_2O$) C, H, N.

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Marine Alkaloids. 3.[1] Bromo-Substituted Alkaloids from the Marine Bryozoan *Flustra foliacea*, Flustramine C and Flustraminol A and B

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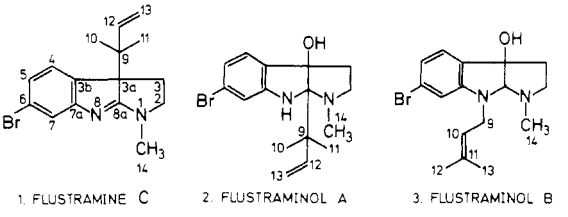
Three new bromo-substituted alkaloids, flustramine C and flustraminol A and B, have been isolated from the marine bryozoan *Flustra foliacea* (L.). The structures have been elucidated by using spectroscopic methods.

In continuation of a study of brominated alkaloids from the marine bryozoan *Flustra foliacea* (L.),[1] we report the isolation and structure elucidation of three new alkaloids, flustramine C (**1**), flustraminol A (**2**), and flustraminol B (**3**). These structures seem, at least formally, closely related to flustramine A (**4**) and flustramine B (**5**). Like flustramine A (**4**) and B (**5**), the structures encompass the basic 6-bromo-substituted physostigmine skeleton; however, in contrast to **4** and **5** they only have one isoprene



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