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SYNTHESIS OF DES-N-TETRAMETHYLTRIOSTIN A FROM C-TERMINAL *Z*-D-SERINE TETRA- AND OCTADEPSIPEPTIDE INTERMEDIATES

MADHUP K. DHAON, JOSEPH H. GARDNER and RICHARD K. OLSEN*
Department of Chemistry and Biochemistry, Utah State University, Logan, UT 84322, U.S.A.

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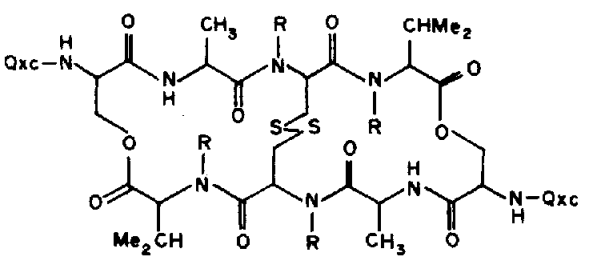
Abstract—Des-N-tetramethyltriostin A (**1**), a known DNA-intercalation agent, has been synthesized from tetra- and octapeptide intermediates that have *Z*-D-serine at the C-terminal position. The procedure thus allows the fragment coupling and cyclization reactions leading to the synthesis of the title compound to occur without racemization at the C-terminal amino acid. Esterification of Boc-Val-OH with the *p*-bromophenacyl ester of *Z*-D-serine provided didepsipeptide *Z*-D-Ser(Boc-Val)-OBpa (**4**). Stepwise addition of the requisite amino acids provided tetradepsipeptide *Z*-D-Ser[Boc-Ala-Cys(Acm)-Val]-OBpa (**6**). Fragment coupling of the respective C- and N-deprotected tetradepsipeptides **7** and **8**, derived from **6**, furnished linear octadepsipeptide **9**, which upon cyclization and disulfide formation gave the bicyclic octadepsipeptide **11**, a known synthetic precursor to **1**. The degree of racemization incurred in the alanine and valine residues of selected depsipeptides was measured and the results compared with those obtained in previous studies. It was concluded that alanine, perhaps because of sequence effects, undergoes a degree of racemization (4–10%) during hydrolysis of tetradepsipeptide **6** and octadepsipeptide **9**.

Des-N-tetramethyltriostin A (**1**) is a symmetrical, hetrodetic, bicyclic peptide belonging to the triostin family of the quinoxaline antibiotics[1] and represents the first example of a synthetic analogue reported[2] for this class of depsipeptide antibiotics. TANDEM, an acronym for **1**, is composed of two units each of D-serine, L-alanine, L-cysteine, and L-valine and differs from the natural antibiotic triostin A (**2**) by lack of N-Me groups on the valine and cysteine residues. The structure of the antibiotic is characterized by the presence of a disulfide bridge between the two cysteine units, a depsipeptide bond between each serine OH and valine carboxyl, and a 2-quinoxalinecarbonyl chromophore attached to each serine amino group. In common with the natural triostins, TANDEM is known[3] to bind to deoxyribonucleic acids by a mechanism involving bifunctional intercalation of both quinoxaline chromophores and, of special interest, to show high specificity in binding to the synthetic DNA, poly(dA-dT). The structure of TANDEM was recently determined by X-ray diffraction studies[4] and the results have led to a proposed model for its specificity in binding to poly(dA-dT).[5]

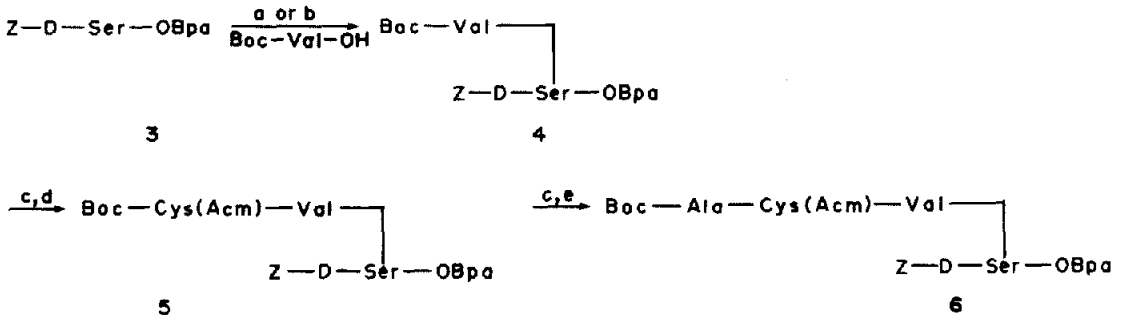
In an earlier reported[2] synthesis of TANDEM, a problem evolved due to the degree of racemization at the alanine residues in the fragment coupling and cyclization reactions owing to the presence of alanine at the C-terminal position in the tetradepsipeptide and octadepsipeptide undergoing the above reactions. In order to circumvent racemization, we have studied an approach in which *Z*-D-serine is placed at the C-terminal position of the depsipeptide intermediates employed in the synthesis, which by nature of the urethane protecting group at the serine amino function, would not be expected to undergo racemization at the serine residue in the fragment coupling and cyclization processes.[6]

The present synthesis of TANDEM followed the general synthetic plan used in our earlier synthesis.[2] An initial objective was preparation of tetradepsipeptide **6**, representing one-half of the symmetrical octadepsipeptide moiety of TANDEM (Scheme 1). Combination of appropriately deprotected tetradepsipeptide fragments would provide the linear octadepsipeptide **9**, which would be caused to undergo cyclization, disulfide formation, and quinoxaloylation to furnish TANDEM (**1**) (Scheme 2).

Condensation of *Z*-D-Ser-OH with α,*p*-dibromoacetophenone in refluxing potassium bicarbonate-acetone gave the known[7] *Z*-D-Ser-OBpa ester **3**; the Bpa ester is known

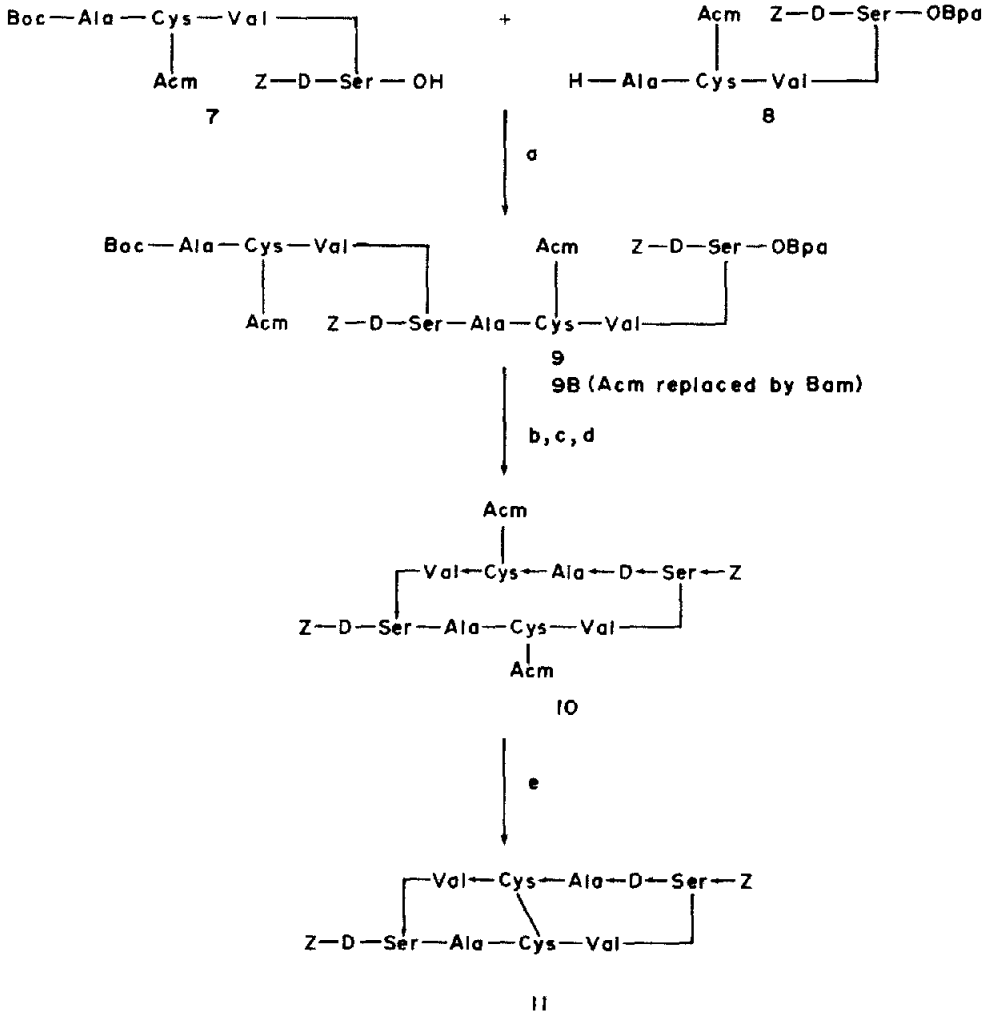


1: R = H
2: R = Me
Qxc = – 2-Quinoxalinecarbonyl



(a) pyridine, DCC, (b) EDC, DMAP, CH_2CH_2, (c) TFA, CH_2Cl_2, then $NaHCO_3$, (d) Boc-Cys(Acm)-OH, EDC, HOBT, THF, (e) Boc-Ala-OH, EDC, HOBT, THF. Abbreviations used in this paper are: DCC = N,N' - dicyclohexylcarbodiimide; DMAP = 4 - (N,N - dimethylamino)pyridine; EDC = 1 - ethyl - 3 - (3 - dimethylaminopropyl) - carbodiimide · HCl; TFA = trifluoroacetic acid; HOBT = 1 - hydroxybenzotriazole; Z = benzyloxycarbonyl; Boc = *tert* - butyloxycarbonyl; Bpa = *p* - bromophenacyl; Acm = acetamidomethyl.

Scheme 1.



(a) isobutyl chloroformate, N-methylmorpholine, THF, (b) zinc, 90% acetic acid, 0°, (c) TFA, CH_2Cl_2, (d) EDC, N-methylmorpholine. CH_2Cl_2–DMF, (e) I_2, MeOH.

Scheme 2.

to be removed under reductive conditions. It should be noted that attempts to prepare the related 2,2,2-trichloroethyl(Tce) ester of *Z*-D-Ser-OH by carbodiimide coupling with 2,2,2-trichloroethanol were not successful. Acylation of **3** with Boc-Val-OH to furnish didepsipeptide **4** was effected either by use of DCC in pyridine[2] or EDC-DMAP[8] in methylene chloride; see Scheme 1 for abbreviations used in this paper. Formation of the depsipeptide bond by the first procedure in the presence of pyridine gave the required product **4** in 65–87% yield along with 10–12% of N-acylurea by-product and a small amount of unconsumed reactants. During condensation of **3** with Boc-Val-OH in presence of EDC-DMAP in methylene chloride, no starting material or N-acylurea were found after completion of the reaction; however, the dehydroalanine elimination product *Z*-ΔAla-OBpa was formed in 5–6% along with required product **4** in 68% yield.

Removal of the Boc group from **4** by TFA-CH_2Cl_2 followed by coupling to Boc-Cys(Acm)OH[9] using EDC-HOBT[10] as coupling agents in THF gave tridepsipeptide **5**. By a similar sequence of reactions, **5** was converted in good yield to tetradepsipeptide **6**. Treatment of **6** with zinc in 90% aqueous acetic acid effected reductive cleavage[7,11] of the Bpa ester function to provide tetradepsipeptide **7** having a free C-terminal carboxyl group.

Tetradepsipeptide **8**, required for fragment coupling with **7**, was prepared by treatment of **6** with TFA-CH_2Cl_2 and was isolated as the trifluoroacetate salt. Neutralization of the trifluoroacetate salt of **8** with $NaHCO_3$ and isolation of the free amine also gave some dehydroalanine elimination product *Z*-ΔAla-OBpa. This represents yet another instance, as observed above, of the formation of dehydroalanine side-product and likely reflects the enhanced acidity of the serine α-hydrogen by the Bpa ester. In our prior synthesis[2] of TANDEM, an amide bond existed between serine and alanine, and the formation of dehydroalanine was not observed.

Fragment coupling of **7** and **8** was accomplished by conversion of **7** to the mixed anhydride[12] by reaction with isobutyl chloroformate in THF, followed by the addition of the TFA salt of **8** to the mixed anhydride containing an added equivalent of N-methylmorpholine to neutralize and liberate the free amine **8**. The linear octadepsipeptide **9** was obtained in 78% yields and without any observed formation of dehydroalanine.

The 360 MHz ^{1}H NMR data for tetradepsipeptide **6** and octadepsipeptide **9** were in complete accord for the assigned structures for these compounds. Of particular interest, the Bpa methylene and the *Z* methylene protons each appeared as AB quartets in the spectrum of tetradepsipeptide **6**. A similar result occurred for octadepsipeptide **9** in which the *Z* methylene protons of the C-terminal *Z*-D-Ser-OBpa unit appear as an AB quartet (δ5.04) with the internal *Z*-D-Ser benzyl methylene protons being a normal singlet at δ5.08 superimposed upon the above quartet; the Bpa methylene protons occur as an AB pattern at δ5.57.

The fully protected octadepsipeptide **9** was treated with Zn in acetic acid as in the case of tetradepsipeptide **6** to remove the *p*-bromophenacyl group, followed by treatment with TFA to effect removal of the Boc function. Cyclization of deprotected octadepsipeptide was caused to occur under high dilution conditions using EDC-HOBT in DMF-CH_2Cl_2 to give the known[2] cyclic depsipeptide **10**, which was converted by reaction with iodine[13] in methanol to the disulfide **11** in an overall yield of 50%. Disulfide **11** was shown to be identical with an authentic sample by comparison of mp, tlc, ^{1}H NMR, and optical rotation data. As disulfide **11** has previously[2] been converted to TANDEM, this represents a second procedure for the total synthesis of this novel depsipeptide antibiotic.

Racemization studies on tetradepsipeptide **6** and octadepsipeptide **9** were carried out, following standard acid hydrolysis, by analysis of the N-trifluoroacetyl isopropyl esters of the hydrolyzed amino acids by vapor phase chromatography on a capillary column having an optically active stationary phase that clearly separated the enantiomeric amino acid derivatives.[14] Because of the observed formation of *Z*-ΔAla-OBpa from depsipeptides **4** and **8** as described above, it is possible that the serine residue is not free of racemization. Thus, if the elimination reaction leading to the dehydroamino acid should occur by an E_1CB mechanism rather than the more common E_2 elimination, some racemization of the serine residue could result by reversible ionization of the α-hydrogen. Racemization of serine residues in peptides by bases has been observed.[15] Unfortunately, we were not able to analyze for serine, which apparently decomposed on the column during the course of the GC analysis. The degree of racemization of alanine and valine were determined. The valine residues in both **6** and **9** did not show any racemization; however, analysis of **6** showed the presence of 1.9% of D-alanine, while 4.9% of D-alanine was observed for octadepsipeptide **9**. The latter value is similar to the 6.4% D-alanine observed in our earlier synthetic octadepsipeptide in which alanine was at the C-terminal position.[2] Racemization of alanine is not to be expected as alanine was introduced as the N-alkoxycarbonyl-protected derivative or was at the N-terminal position, and under coupling conditions known[6,10] to result in negligible racemization. It is likely that racemization is occurring in the intact depsipeptide or peptide fragments during acid hydrolysis of these depsipeptides; racemization during hydrolysis of certain peptide sequences has been observed.[16] Control studies have shown a small amount (0.61%) of D-alanine is formed when the free amino acid L-alanine is treated to the conditions of acid hydrolysis and we have corrected for this in reporting the above values for D-alanine.

Disulfide **11** did not show, upon analysis, any diasteromeric impurity with either the analine or valine residues; a similar lack of racemization of these residues was observed previously[2] in the earlier synthesis of TANDEM.

We also prepared, by a similar procedure as for **9**, octadepsipeptide **9B** in which the cysteine sulfur functions were protected with the S-benzamidomethyl (Bam) group[17] in place of Acm as in **9**. However, attempts to effect cyclization of this octadepsipeptide using the same procedure as was successful for cyclization of **9**, did not appear to yield any of the desired cyclic product. A single attempt to effect cyclization of **9B** *via* the mixed anhydride gave isolated product in rather low yield having an R_f value similar to that of the related cyclic product **10**; however, this approach was not pursued as further studies in the Bam series were discontinued. It should be noted that in the Acm series of compounds, most intermediate were crystalline solids, while for the Bam series most often oils or amphorous solids were obtained. Thus, the use of the S-acetamidomethyl group is the most suitable protecting group for use in this approach to the synthesis of TANDEM.

EXPERIMENTAL

Mps were measured on a Thomas Hoover capillary mp apparatus and are uncorrected. ^{1}H NMR spectra were recorded for all compounds reported using a Varian EM-360, XL-100-12, or a Nicolet NT-360 spectrometer; satisfactory NMR data were obtained for all compounds and data for selected intermediates are reported. Optical rotations were recorded on a Perkin–Elmer 241 automatic polarimeter. Tlc was performed on commercially prepared silica gel on glass plates using the following solvent systems: A, hexane–acetone, 6:4; B, n-BuOH–AcOH, -H_2O 10:2:3; C, $CHCl_3$–MeOH–AcOH, 90:8:2; D, $CHCl_3$–MeOH, 9:1. Medium pressure liquid chromatography[18] (mplc) was performed on columns packed with silica gel 60 (0.040–0.064 mm).

The amino acids, their derivatives, and the coupling reagents used in this study were obtained from appropriate commercial sources. THF was distilled prior to use from sodium benzophenone ketyl. CH_2Cl_2 was distilled from P_2O_5 and stored over Linde 3A molecular sieves. DMF was refluxed and distilled over CaH_2 and stored over appropriate molecular sieves.

N - *Benzyloxcarbonyl* - O - (N - *t* - *butyloxycarbonyl* - L - *valyl* - D - *serine* p - *bromophenacyl ester* (4)

Method A. Compound **3**[7] (7.5 g, 17.2 mmol) and N-t-butyloxycarbonyl-L-valine (5.4 g, 25.0 mmol) were dissolved in 100 ml pyridine. The soln was chilled to 0° followed by the addition of N,N'-dicyclohexylcarbodiimide (5.25 g, 25 mmol). The reaction was maintained at 0° for 4 hr followed by 16 hr at room temp. The mixture was filtered and the filtrate was concentrated to a yellow oil. This material was dissolved in EtOAc and washed with H_2O, sat $NaHCO_3$ aq, 10% citric acid, and H_2O (75 ml each). The EtOAc layer was dried over $MgSO_4$ and evaporated to dryness. The resulting oil crystallized, after being refrigerated overnight, from petroleum ether (b.p. 30–60°). The white solid was recrystallized from EtOAc-diethyl ether, 25:75, followed by the addition of a small amount of petroleum ether (b.p. 30–60°) to yield 9.5 g (87%) of **4**, m.p. 95.5–96°, R_f 0.69 (solvent A), $[\alpha]_D^{25}$ + 2.75° (*c* 2, $CHCl_3$). (Found: C, 55.01; H, 5.63; N, 4.35. $C_{29}H_{35}N_2O_9Br$ requires: C, 54.81; H, 5.55; N, 4.41%).

In a separate case, the reaction (2.3 mmol scale) product was purified by MPLC using 80:20 n-hexane–acetone as eluant. The fractions eluted from the column prior to **4** were pooled and concentrated to a white solid (0.20 g, 15%); m.p. 163–164°, tlc (solvent A) R_f 0.76. The ^{1}H NMR spectrum of this material appeared consistent for the N-acylurea by-product.

Method B. A stirred suspension of compound **3** (1.0 g, 2.29 mmol), N-t-butyloxycarbonyl-L-valine (0.5 g, 2.31 mmol), 4-(N,N-dimethylamino)pyridine (0.14 g, 1.15 mmol) in CH_2Cl_2 (20 ml) was cooled to 0° in an ice bath. EDC (0.46 g, 2.4 mmol) was added and the mixture was stirred for 1 hr at 0° and overnight at room temp. The mixture was concentrated and the residue was taken up in EtOAc (40 ml) and washed with water (2 × 20 ml), sat $NaHCO_3$ aq (2 × 20 ml), and water (25 ml). The organic phase was dried over Na_2SO_4 and concentrated to a yellow viscous oil. The oil was chromatographed on a silica gel mplc column using 80:20 n-hexane–acetone as eluant. The fractions having R_f 0.65 (solvent A) were pooled and concentrated to a solid which was recrystallized from EtOAc-petroleum ether (b.p. 30–60°) to give 1.0 g (68%) of **4**, m.p. 94–95%, $[\alpha]_D^{25}$ + 2.75° (c 2, $CHCl_3$).

The fractions having R_f 0.68 (solvent A) were pooled and concentrated to give a white solid which was recrystallized from diethyl ether–hexane, m.p. 97–98°, 55 mg (6%). This solid was characterized by ^{1}H NMR to be the corresponding dehydroamino acid of **3**.

N-*Benzyloxcarbonyl*-O-(N-t-*butyloxycarbonyl*-S-*acetamidomethyl*-L-*cysteinyl*-L-*valyl*)-D-*serine* p-*bromophenacyl ester* (5)

Didepsipeptide **4** (4.0 g, 6.29 mmol) was stirred in a soln of anhyd trifluoroacetic acid (14 ml) and CH_2Cl_2 (10 ml) for 30 min at room temp. The soln was concentrated to an oily residue, which was dissolved in EtOAc (60 ml) and the organic phase was washed with sat $NaHCO_3$ aq (2 × 25 ml), water (2 × 20 ml), dried over $NaSO_4$, and concentrated to a white solid (3.15 g, 96%).

To a soln of the above solid in 40 ml of THF was added N-t-butyloxycarbonyl-S-acetamidomethyl-L-cysteine[9] (1.75 g, 6.0 mmol) and 1-hydroxybenzotriazole (1.62 g, 12.0 mmol). The soln was stirred and cooled to 0° and EDC (1.18 g, 6.2 mmol) was added. The mixture was stirred at 0° for 1 hr and for 5 hr at room temp. The mixture was concentrated to a viscous oil, which was dissolved in EtOAc (100 ml) and washed with water (1 × 20 ml), 1 N HCl (2 × 25 ml), sat $NaHCO_3$ aq (2 × 25 ml), and water (2 × 20 ml). After drying (Na_2SO_4), the soln was concentrated to a yellow oil, which was recrystallized from EtOAc-petroleum ether (b.p. 30–60°) to give a white solid, (4.1 g, 87%); m.p. 125–126°; tlc (solvent A) R_f 0.50; $[\alpha]_D^{25}$ – 2.5° (*c*2, $CHCl_3$); ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.05 (t, 6H, Val Me's), 1.48 (s, 9H, Boc), 2.02 (s, 3H, Acm methyl), 2.20–2.40 (m, 1H, Val methine), 2.55–3.00 (m, 2H, Cys β H), 4.0–5.0 (m, 7H, three α H, Acm and Ser CH_2), 5.12 (s, 2H, benzyl), 5.40 (s, 3H, Bpa CH_2 and NH), 6.30–7.05 (m, 3H, NH), 7.48 (s, 5H, benzyl aromatic), 7.82 (A_2B_2, 4H, Bpa aromatic), (Found: C, 52.16; H, 5.85; N, 7.04. $C_{35}H_{45}N_4BrO_{11}S$ requires: C, 51.91; H, 5.56; N, 6.92).

N-*Benzyloxcarbonyl*-O-(N-t-*butyloxycarbonyl*-L-*alanyl*-S-*acetamidomethyl*-L-*cysteinyl*-L-*valyl*)-D-*serine* p-*bromophenacyl ester* (6)

A soln of **5** (3.1 g, 3.8 mmol) in anhyd trifluoroacetic acid (12 ml) and CH_2Cl_2 (10 ml) was stirred at room temp for 30 min. The mixture was concentrated, taken up in EtOAc (60 ml) and washed with sat $NaHCO_3$ aq (2 × 30 ml), and water (2 × 20 ml). After drying (Na_2SO_4), the soln was concentrated to yield 2.65 g (98%) of an oil. A soln of this residue (2.82 g, 3.97 mmol), N-t-butyloxycarbonyl-L-alanine (0.79 g, 4.2 mmol) and 1-hydroxybenzotriazole (1.0 g, 8 mmol) in anhyd THF (25 ml) was cooled to 0° and EDC (0.84 g, 4.4 mmol) was added. The mixture was stirred at 0° for 1 hr, then for 5 hr at room temp. The mixture was concentrated to a viscous yellow oil, which was dissolved in EtOAc (80 ml) and the organic layer was washed with water (2 × 25 ml), 1 N HCl (2 × 20 ml), sat $NaHCO_3$ aq (2 × 20 ml) water (2 × 15 ml), and dried (Na_2SO_4). The soln was concentrated to a viscous oil, which was purified on MPLC using 65:35 followed by 60:40 n-hexane–acetone as eluant. The product was recrystallized from EtOAc-petroleum ether (b.p. 30–60°) to yield 3.0 g (94%) of **6**; m.p. 107–108°; tlc (solvent A) R_f 0.33; $[\alpha]_D^{25}$ – 23.5° (*c* 2, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$) δ 0.96 (t, 6H, Val methyls), 1.32 (d, 3H, Ala Me), 1.44 (s, 9H, Boc), 1.98 (s, 3H, Acm Me), 2.24 (m, 1 H, Val methine), 2.68 (m, 1 H, Cys-β_1), 2.96 (m, 1 H, Cys-β_2), 4.15–4.93 (set of multiplets, 7 H, Acm, Ser methylenes, α-hydrogens), 4.97 (m, 1 H, α-H), 5.16 (q, 2 H, benzyl), 5.36 (q, 2 H, phenacyl methylene), 6.50–6.68 (m, 2 H, NH), 7.13 (m, 1 H, NH), 7.38 (s, 6 H, benzyl aromatic and NH), 7.64 (d, 2 H, phenacyl aromatic), 7.76 (d, 3 H, phenacyl aromatic and NH). (Found: C, 52.02; H, 5.67; H, 8.51. $C_{38}H_{50}N_5BrO_{12}S$ requires: C, 51.81; H, 5.68; N, 8.55%).

N-*Benzyloxcarbonol*-O-(N-t-*butyloxycarbonyl*-L-*alanyl*-S-*acetamidomethyl*-L-*cysteinyl*-L-*valyl*)-D-*serine* (7)

Zn powder (4.7 g, 73 mmol) was added in portions to a vigorously stirred ice cold soln of **6** (1.3 g, 1.47 mmol) in 90% AcOH (40 ml). The mixture was stirred at 0° for 2 hr and at room temp for 3 hr. The mixture was filtered and the residue was washed with 90% AcOH and the filtrate was concentrated to a white solid. The solid was shaken with a mixture of EtOAc (40 ml) and 1 N HCl (15 ml). The EtOAc phase was separated, washed with water (2 × 15 ml) and extracted with sat $NaHCO_3$ aq (4 × 20 ml). The combined bicarbonate extracts were washed with EtOAc (1 × 25 ml), cooled in an ice bath and acidified to pH 4 with 6 N HCl. The acidified soln was saturated with NaCl and extracted with EtOAc (3 × 25 ml). The EtOAc extracts were combined, dried (Na_2SO_4) and concentrated to a white solid, 0.90 g (90%); m.p. 85–88°; tlc (solvent C) R_f 0.35. This product, **7**, was used without further purification.

N-*Benzyloxycarbonyl*-O-(L-*alanyl*-S-*acetamidomethyl*-L-*cysteinyl*-L-*valyl*)-D-*serine* p-*bromophenacyl ester trifluoroacetate* (8)

Tetradepsipeptide **6** (1.0 g, 1.13 mmol) was dissolved in anhyd trifluoroacetic acid (3 ml) and CH_2Cl_2 (2 ml). The mixture was

stirred for 30 min at room temp. The solvent was removed and the residue was triturated with anhyd diethyl ether to give **8** as a white solid that was filtered and dried over P_2O_5; yield 1.0 g (99%), m.p. 103–106°, tlc (solvent B) R_f 0.44.

N-*Benzyloxycarbonyl*-O-[N-*benzyloxycarbonyl*-O-(N-t-*butyloxycarbonyl*-L-*alanyl*-S-*acetamidomethyl*-L-*cysteinyl*-L-*valyl*)-D-*seryl*-L-*analyl*-S-*acetamidomethyl*-L-*cysteinyl*-L-*valyl*]-D-*serine* p-*bromophenacyl ester* (**9**)

A soln of **7** (0.20 g, 0.29 mmol) in anhyd THF (5 ml) was cooled to −20° in a CCl_4-dry ice bath. N-Methylmorpholine (0.03 g, 0.30 mmol) was added, followed by isobutyl chloroformate (0.042 g, 0.30 mmol), and the mixture was stirred at −20° for 10 min. After the formation of the mixed anhyd, N-methyl morpholine (0.03 g, 0.30 mmol) was further added, followed by dropwise addition of **8** (0.26 g, 0.30 mmol) in THF (5 ml). The mixture was stirred at −20° for 15 min and then for 1 hr at room temp. The mixture was concentrated to dryness and the residue was triturated with water, filtered, washed successively with several portions of water, ice cold 1 N HCl, sat $NaHCO_3$ aq, and water. After drying *in vacuo* over P_2O_5, the solid was taken up in MeOH-diethyl ether and the resulting soln was cooled overnight in a refrigerator. The resulting gel that separated was filtered and recrystallized from MeOH-diethyl ether to give **9**, 0.32 g (78%); m.p. 165–167°; $[\alpha]_D^{25}$-18.7° (*c* 1, DMF), tlc (solvent D) R_f 0.58; ^{1}H NMR (360 MHz, DMSO-d_6) δ 0.85 (s, 12 H, Val Me's), 1.18 (d, 6 H, Ala Me's), 1.36 (s, 9 H, Boc), 1.85 (s, 6 H, Acm Me's), 2.00–2.16 (m, 2 H, Val methine), 2.60–2.70 (m, 2 H, Cys β methylene), 2.85–2.96 (m, 2 H, Cys β methylene), 3.94–4.72 (set of multiplets, 16 H, Acm, Ser methylenes, α-H's), 5.04 (AB q, 2 H, benzyl), 5.08 (s, 2 H, benzyl), 5.57 (AB q, 2 H, phenacyl methylenes), 6.92 (d, 1 H, NH), 7.35 (d, 10 H, benzyl aromatic), 7.62 (d, 1 H, NH), 7.84 (A_2B_2 q, 4 H, phenacyl aromatic), 7.93–8.05 (m, 4 H, NH), 8.26 (t, 2 H, NH), 8.55 (m, 2 H, NH). (Found: C, 51.38; H, 5.87; N, 9.52. $C_{63}H_{85}N_{10}BrO_{20}S_2 \cdot H_2O$ requires: C, 51.67; H, 5.80; N, 9.56%).

(N-*Benzyloxycarbonyl*-D-*seryl*-L-*alanyl*-L-*cysteinyl*-L-*valine*)$_2$ (*serine hydroxyl*) *dilactone disulfide* (**11**)

Zn powder (0.32 g, 5 mmol) was added to an ice cold, vigorously stirred soln of **9** (0.15 g, 0.10 mmol) in 90% aqueous AcOH (10 ml). Stirring was continued at 0° for 2 hr and then at room temp for 3 hr. The mixture was filtered, and the residue was washed well with 90% aqueous AcOH. The filtrate was concentrated to a white solid, which was shaken with a mixture of EtOAc (25 ml) and 1 N HCl (7 ml). The organic layer was separated, washed with water, dried (Na_2SO_4), and concentrated to give 0.12 g (93%) of a solid; tlc (solvent D) R_f 0.32.

A soln of the above solid (0.12 g, 0.09 mmol) in anhyd trifluoroacetic acid (2 ml) and CH_2Cl_2 (2 ml) was stirred for 30 min at room temp. The soln was then concentrated to an oily residue, which upon trituration with anhyd ether gave a white solid that was dried, after filtration, *in vacuo* over P_2O_5.

A soln of the above solid (0.11 g, 0.87 mmol) and N-methylmorpholine (0.01 ml, 0.09 mmol) in dry DMF (2 ml) diluted with 50 ml of CH_2Cl_2, was added in 2.5 hr to an ice cold stirred soln of EDC (0.042 g, 0.22 mmol) and 1-hydroxybenzotriazole (0.047 g, 0.35 mmol) in DMF (2 ml) and CH_2Cl_2 (150 ml). After the completion of addition, the mixture was stirred for 1 hr at 0° and for 5 days at room temp. The solvent was removed *in vacuo* and the residue was taken up in EtOAc (30 ml). The organic phase was washed with water (1 × 15 ml), 1 N HCl (2 × 10 ml), sat $NaNCO_3$ aq (2 × 10 ml), and water (2 × 10 ml). The soln was dried (Na_2SO_4) and concentrated *in vacuo* to yield crude monocyclic product **10**.

To a stirred soln of crude **10** (70 mg, 0.06 mmol) in MeOH (25 ml) was added dropwise a soln of I_2 (0.15 g, 1.2 mmol) in MeOH (20 ml). The soln was stirred for an additional 4 hr, cooled, and excess iodine was decomposed by the dropwise addition of 1 N $Na_2S_2O_3$ aq. The soln was concentrated to a solid residue, which was triturated with water and filtered. The residue was washed well with water and dried *in vacuo* over P_2O_5. The crude compound was purified on a short column of silica gel (230–400 mesh) using $CHCl_3$ and $CHCl_3$–MeOH (97:3) as eluant. The fractions containing the product, tlc (solvent D) R_f 0.65, were pooled and concentrated to a solid residue. Recrystallization from $CHCl_3$–diethyl ether gave 42 mg (50%) of **11** as a white solid; m.p. 165–168°; $[\alpha]_D^{25}$ − 2.38° (c 2, $CHCl_3$); reported m.p. 166–169°, $[\alpha]_D^{25}$-2.5 (*c* 2, $CHCl_3$)[2]. Tlc, NMR, and analytical data were in accordance with that reported[2] for **11**.

Racemization studies on depsipeptides **6** *and* **9**

Hydrolysis of 5 mg samples of **6** and **9** were carried out under standard conditions in 6 N HCl at 100° for 22 hr. 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 N-lauryl-L-valyl-t-butylamide as a chiral phase as described in Refs 2 and 14. The following percentages, corrected for control values, of D-amino acids were obtained: for tetradepsipeptide **6**, D-alanine 1.9, D-valine 0.0; for octadepsipeptide **9**, D-alanine 4.9, D-valine 0.8; for bicyclic disulfide **11**, no D-alanine or D-valine was detected.

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