Synthesis and DNA-Binding Studies of [Lac[2],Lac[6]]TANDEM, an Analogue of Des-*N*-tetramethyltriostin A (TANDEM) Having L-Lactic Acid Substituted for Each L-Alanine Residue

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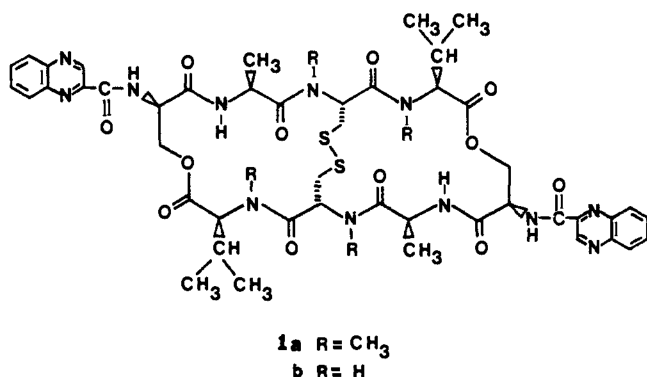
Abstract: The cyclic octadepsipeptide [Lac[2],Lac[6]]TANDEM (**2**) has been prepared by total synthesis in which L-lactic acid units are incorporated in place of each of the two L-alanine residues common to the triostin depsipeptide antibiotics. Analogue **2** is an isosteric analogue of des-*N*-tetramethyltriostin A (TANDEM) (**1b**) but lacks the structural features necessary for the formation of hydrogen bonds involving the alanine NH groups with a thymidine C-2 carbonyl or adenine N-3 postulated to be formed upon binding of the antibiotic to DNA. L-Lactic acid *p*-bromophenacyl ester was condensed with *N*-Cbz-D-serine tetrahydropyranyl ether (**4**) to furnish didepsipeptide **5** and provide the requisite ester bond present in **2**. Removal of the tetrahydropyranyl group followed by a sequence of depsipeptide bond formation by condensation with *N*-Boc-L-valine, deprotection of the resulting tridepsipeptide **6**, and amide bond formation by reaction with *N*-Boc-*S*-(acetamidomethyl)-L-cysteine provided tetradepsipeptide **7**. Fragment coupling between the respective N-terminal and C-terminal deprotected tetradepsipeptides **9** and **10** yielded octadepsipeptide **11**, which was converted by deprotection and cyclization to cyclic intermediate **12**. Oxidation of **12** to the bicyclic disulfide **13**, removal of the Cbz groups at each D-serine residue, and acylation with 2-quinoxalinecarbonyl chloride gave [Lac[2],Lac[6]]TANDEM (**2**). ^{1}H NMR spectral data of [Lac[2],Lac[6]]TANDEM were in good agreement with similar data for TANDEM, thereby providing evidence for similar conformations for these compounds. Analogue **2** failed to yield a footprinting pattern upon interaction with a 160-base-pair DNA restriction fragment under the same conditions where TANDEM shows a highly specific footprinting pattern. These results provide further evidence of the crucial role of hydrogen bonds involving the alanine NH groups in the mechanism of binding of the triostin antibiotics at specific sites on a DNA fragment.

Triostin A (**1a**), a member of the triostin depsipeptides,[1] is



known[2] to bind to deoxyribonucleic acids by the simultaneous intercalation of both quinoxaline chromophores attached to the depsipeptide ring system; interactions between functional groups of the depsipeptide and corresponding moieties in the double-stranded DNA segment are also considered to be important in the binding process. Studies to elucidate the structural parameters critical to the binding of the antibiotic to the DNA molecule have been carried out and are of continuing interest.

A synthetic analogue, des-*N*-tetramethyltriostin A (**1b**),[3] also known by the acronym TANDEM,[4] has shown very high specificity in binding to DNA. Footprinting studies on a 160-base-pair DNA restriction fragment established that TANDEM recognized only two binding sites involving ATA or TAT nucleotide sequences.[5] Earlier work had established that TANDEM showed high affinity for binding to poly(dA·dT)[4] and was effective in inhibiting the production of poly(dA·dT) during the de novo synthesis of other synthetic DNA polymers as catalyzed by *Escherichia coli* DNA polymerase I.[6] The crystal structure of TANDEM has been determined.[7] From an analysis of the crystal structure, a model was proposed for the binding of TANDEM to DNA in which a key feature was the formation of hydrogen bonds between each of the two alanine NH groups and a thymine C-2 carbonyl or adenine N-3 of the DNA base pairs.[8] More recently, the molecular structure of a DNA–triostin A complex has been reported.[9] In the complex, hydrogen bonds are present between the alanine NH groups and N-3 of two guanine residues present at the site of binding. These hydrogen-bonding interactions are considered to be very important in the binding process.

We report in this paper the synthesis and binding studies of [Lac[2],Lac[6]]-des-*N*-tetramethyltriostin A (**2**), i.e., [Lac[2],Lac[6]]-TANDEM. This molecule is an oxygen isosteric analogue of TANDEM, having the two L-alanine units replaced by L-lactic acid residues, and structurally lacks the possibility of formation of hydrogen bonds as proposed for the TANDEM complex with DNA. Analogue **2** should serve as a model to probe how critical the hydrogen bonds involving the alanine units are in the TANDEM and triostin A complexes with DNA.

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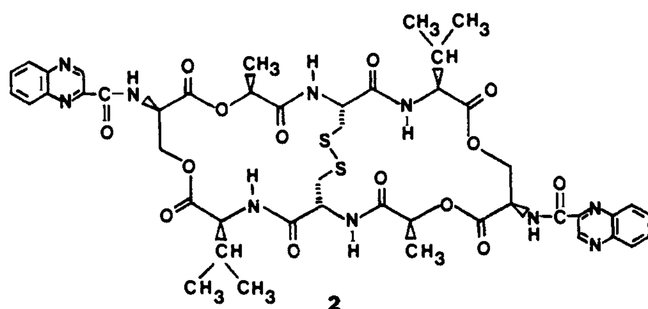
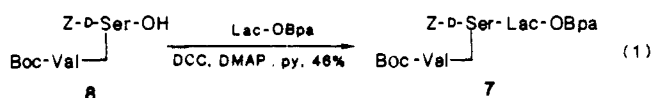


The synthesis of **2** was patterned after synthetic routes used for the preparation of TANDEM (**1b**).[3] Analogue **2** does contain two additional depsipeptide (ester) bonds resulting from the incorporation of the two L-lactic acid residues. As previously,[3] our approach was to prepare the tetradepsipeptide **7**, effect fragment coupling of the respective C- and N-deprotected units derived from **7** to provide the linear octadepsipeptide **11**, and transform **11** to the desired cyclic product. The preparation of the requisite tetradepsipeptide **7** is given in Scheme I.

N-(Benzyloxycarbonyl)-D-serine (**3**) was protected at its alcohol function as the tetrahydropyranol ether **4**. L-Lactic acid *p*-bromophenacyl ester was prepared by alkylation of the carboxylate anion with α,4-dibromoacetophenone.[10] Ester bond formation between **4** and the above lactate using the carbodiimide/4-(dimethylamino)pyridine procedure[11] in pyridine furnished didepsipeptide **5** in a yield of 54%. Removal of the THP group (methanol, TsOH), and coupling to *N*-(*tert*-butyloxycarbonyl)-L-valine gave tridepsipeptide **6** in 85% yield from **5**. Treatment of **6** with trifluoroacetic acid (TFA) in CH_2Cl_2 caused removal of the *tert*-butyloxycarbonyl (Boc) group; neutralization of the resulting TFA salt and condensation with *N*-Boc-*S*-(acetamidomethyl)-L-cysteine provided tetradesipeptide **7** in a yield of 88%.

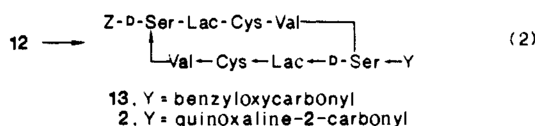
We initially prepared the tridepsipeptide **6** by esterification of *Z*-*O*-(Boc-L-valyl)-D-serine (**8**) (*Z* = benzyloxycarbonyl) with *p*-bromophenacyl L-lactate (eq 1); compound **8** was prepared[3b]



in three steps from Z-D-serine. This reaction, using various coupling methods, gave **6** in yields of 25–40%, with the best yield (46%) being obtained with *N*,*N*′-dicyclohexylcarbodiimide/4-(dimethylamino)pyridine in pyridine. Purification of **6** obtained by this route was difficult, due primarily to the coelution of unreacted lactate *p*-bromophenacyl ester, and often required two different chromatographic separations using medium-pressure liquid chromatography. The overall yield of **6** from *Z*-D-serine by this route was 34% as compared to an overall yield of 46% by the procedure outlined in Scheme I.

Tetradepsipeptide **7** was transformed to cyclic product **12** following our previously developed procedures[3a] (Scheme II). Batchwise deprotection of **7** provided **9** and **10**; fragment coupling of these components gave the linear octadepsipeptide **11** in 72% yield. Deprotection at the termini of **11** and cyclization gave cyclic product **12**. The cyclization reaction was carried out in CH_2Cl_2/DMF at high dilution (5.8×10^{-4} M in peptide) using 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride and 1-hydroxybenzotriazole to furnish **12** in a yield of 56%.

The final steps of the synthesis of **2** involved formation of the disulfide cross-bridge by oxidation of **12** (eq 2) with iodine in



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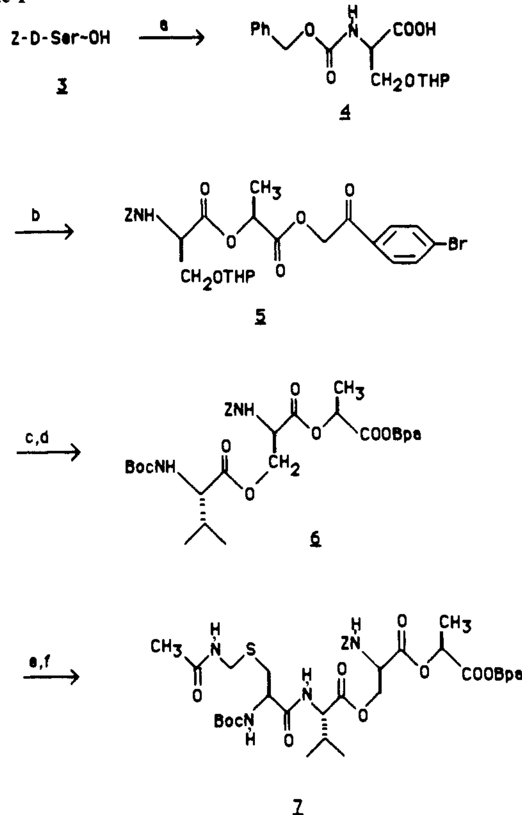
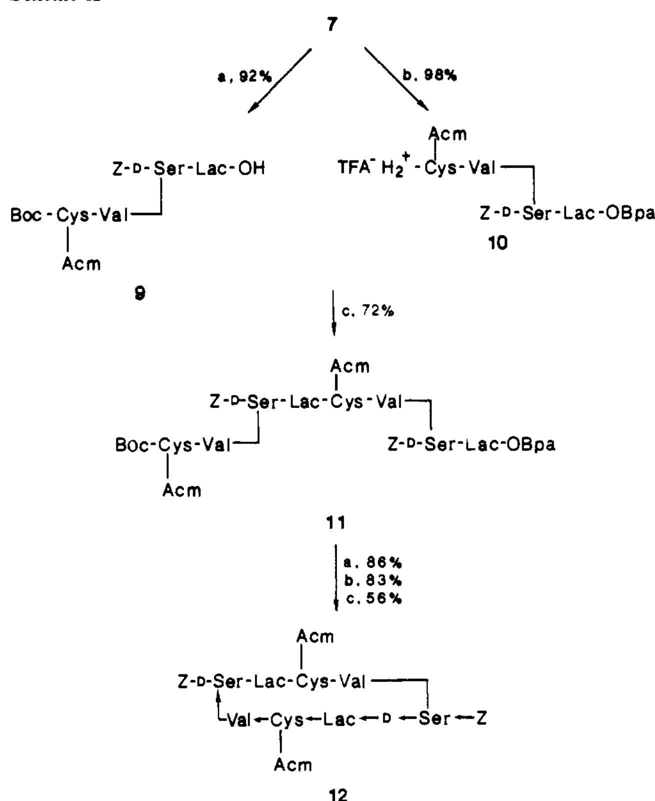
Scheme I[a]



[a]Key: (a) Dihydropyran, PPTS, CH_2Cl_2, 92%; (b) L-lactic acid Bpa ester, EDC, DMAP, pyridine, 54%; (c) CH_3OH, TsOH, 91%, (d) Boc-Val-OH, EDC, DMAP, CH_2Cl_2, 93%; (e) TFA, CH_2Cl_2, 98%; (f) Boc-Cys(Acm)-OH, Et_3N, EDC, HOBT, THF, 90%. Compounds: EDC = 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride; DMAP = 4-(dimethylamino)pyridine; HOBT = 1-hydroxybenzotriazole.

Scheme II[a]



[a]Key: (a) Zn, 90% AcOH; (b) TFA, CH_2Cl2; (c) EDC, HOBT, THF; (d) HOBT, EDC, NMN, DMF/CH_2Cl_2.

Table I. ^1H NMR Chemical Shift Values (ppm) for TANDEM (**1b**)[a] and [Lac[2],Lac[6]]TANDEM (**2**)

		in $CDCl_3$		in Me_2SO-d_6	
		1b	**2**	**1b**	**2**
Ser	β_1	4.66	4.47	4.13	4.36
	β_2	5.00	5.28	4.64	4.52
	α	4.87	5.14	4.76	5.03
	NH	8.81	8.78	8.66	8.96
Ala or	β_1	1.38	1.50	1.24	1.36
Lac	α	4.45	5.14	4.28	5.21
	NH	6.30		8.13	
Cys	β_1	2.92	2.94	2.91	2.97
	β_2	2.95	2.96	2.69	2.75
	α	5.68	5.64	5.28	5.43
	NH	6.57	7.01	8.71	9.13
Val	γ	1.13, 1.17	1.07, 1.13	0.93, 1.08	0.90, 0.96
	β	2.54	2.38	2.30	2.20
	α	4.84	4.76	4.13	5.03
	NH	8.43	8.67	8.20	8.47
Qxc	H-3	9.68	9.67	9.50	9.53
	H-6,7	7.90	7.90	8.03	8.04
	H-5	8.10	8.04	8.13	8.12
	H-8	8.23	8.23	8.23	8.24

[a] Data for **1b** taken from ref 12.

Table II. ^1H–^1H Coupling Constants, J, for TANDEM (**1b**)[a] and [Lac[2],Lac[6]]TANDEM (**2**)

		in $CDCl_3$		in Me_2SO-d_6	
		1b	**2**	**1b**	**2**
Ser	β_1,β_2	11.0	11.2	11.2	10.0
	α,β_1	2.0	2.7	2.7	1.0
	α,β_2	2.5	1.8	3.3	4.5
	α,NH	6.7	7.9	8.4	7.1
Ala or	α,β	7.1	6.9	7.4	6.8
Lac	α,NH	6.0		6.6	
Cys	β_1,β_2	15.0	14.6	14.0	14.7
	α,β_1	12.8	10.4	11.9	12.0
	α,β_2	2.8	4.0	3.6	3.2
	α,NH	10.0	9.8	9.9	10.2
Val	β,γ	6.6,6.9	6.7,6.8	6.5	6.8
	α,β	4.5	4.5	7.8	6.5
	α,NH	9.6	9.9	9.3	10.2

[a] Data for **1b** taken from ref 12.

CH_3OH to furnish **13** (89%). Subsequent removal of the *N*-benzyloxycarbonyl groups (HBr in acetic acid), neutralization, and acylation with 2-quinoxalinecarbonyl chloride gave [Lac[2],Lac[6]]TANDEM (56% yield).

For [Lac[2],Lac[6]]TANDEM (**2**) to serve as a valid molecule to test the importance of hydrogen bonding of the alanyl NH groups in the DNA complex, it is necessary that its conformation be similar to that of TANDEM, thereby making it unlikely that any lack of specificity or binding by [Lac[2],Lac[6]]TANDEM is not due to a significant change in the conformation of the depsipeptide ring.

The solution conformation[12] and crystal structure[7] of TANDEM have been determined. The bicyclic depsipeptide ring has been found to be a rather rigid disklike structure, with the two quinoxaline rings oriented at right angles to and on the same face of the peptide ring. Factors contributing to the rigidity of the depsipeptide ring are due in part to the constraints imposed by the disulfide cross-bridge and the presence of the two hydrogen bonds between the valine NH and alanine carbonyl. ^1H NMR solution conformational studies have shown the cysteinyl and valyl units to be quite conformationally rigid, but the seryl C^α–NH and C^α–carbonyl bonds as also the alanyl C^α–NH bond to possess some conformational mobility.[12]

The coupling interactions and coupling constants, J, for analogue **2** were obtained by HCOSY and selected decoupling experiments. Comparison of the chemical shift values and coupling constants of TANDEM and [Lac[2],Lac[6]]TANDEM (Tables I and II) provides evidence that both molecules have similar conformations. The low-field positions of the serine and valine NH groups are similar to those observed for TANDEM; the latter shift value is indicative of hydrogen-bond formation involving the valine NH. The agreement of the coupling constants, J, for the valyl α,β and α,NH protons in **2** is the same as for the corresponding units in TANDEM. There are some differences observed for the coupling constants for the seryl and cysteinyl units. These differences are small, and considering the known rigidity of these depsipeptide ring systems, it seems likely that no significant conformational changes have occurred in analogue **2** when compared to TANDEM. We conclude, therefore, that analogue **2** should serve as a suitable model of TANDEM, but one, because of the inclusion of lactic acid, that will lack the ability to form the critical hydrogen bonds upon complexation to DNA.

Studies on the possible interaction between [Lac[2],Lac[6]]TANDEM and DNA were carried out by the method of DNase I footprinting using the 160-base-pair *Tyr*T restriction fragment of DNA previously employed for experiments with triostin A and other quinoxalines.[5,12] The natural antibiotics triostin A and echinomycin bind to six or seven sites in this DNA, each containing one or more of the recognition sequences CpG.[5,13] TANDEM, on the other hand, protects only two sites from digestion by the enzyme, and they share the consensus sequence TpA (or ApT);[5] recent evidence with a partially methylated TANDEM derivative [*N*-MeCys,[3]*N*-MeCys[7]]TANDEM strongly suggests that the requisite sequence is in fact TpA and that recognition demands hydrogen-bond formation with the alanine NH functionalities.[14] Tested under identical conditions, [Lac[2],Lac[6]]TANDEM failed to produce detectable protection of any sequences in either DNA strand from nuclease attack, even when the depsipeptide concentration was raised from the standard value of 15 μM to as much as 40 μM (close to the limits imposed by the solubility of the new analogue). Thus, there is no evidence of binding of [Lac[2],Lac[6]]TANDEM to DNA under conditions that are highly suitable for the parent compound as well as for the natural antibiotics. In the crystal structure of TANDEM,[7] the depsipeptide ring is held rigid by internal hydrogen bonds between the carbonyl groups of L-alanine and amino groups of L-valine. Substitution of L-lactic acid for L-alanine should not affect the ability to form these internal hydrogen bonds but does remove the amino groups of the alanine residues that, it is proposed, function as hydrogen-bond donors to interact with acceptors on the DNA helix.[8,14] The retention of the internal hydrogen bonds, as is evident from the ^1H NMR data of **2**, ensures that the carbonyl groups are not available for interaction with the 2-amino groups of guanine nucleotides, as proposed for the parent antibiotic triostin A. We are left with the presumption that the evident failure of [Lac[2],Lac[6]]TANDEM to bind to DNA at all results from lack of the L-alanine NH groups as hydrogen bond donors.

Experimental Section

The amino acids and coupling reagents used were obtained from commercial sources. All solvents were distilled in glass. Tetrahydrofuran was distilled from $LiAlH_4$ and stored over 3A molecular sieves. Methylene chloride was distilled from P_2O_5 and stored over molecular sieves. Dimethylformamide was distilled from CaH. Melting points were determined by capillary melting points and are uncorrected. TLC was performed on commercially prepared silica gel on 1 × 3 in. glass plates, and spots were detected by ultraviolet light or iodine vapor. MPLC[15] and flash chromatography[16] were carried out on columns packed with silica gel 60 (0.040–0.064 mm). ^1H NMR spectra were recorded at 60, at 90, or, as indicated in the Experimental Section, at 360 MHz. Optical rotations were recorded on an automatic polarimeter.

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***N*-(Benzyloxycarbonyl)-*O*-(tetrahydropyranyl)-D-serine (4).** Z-D-Serine (2.39 g, 10.0 mmol) was suspended in dry CH_2Cl_2 (100 mL) and treated with dihydropyran (0.92 g, 11.0 mmol) and pyridinium *p*-toluenesulfonate (PPTS, 300 mg, 1.2 mmol). The reaction mixture was stirred at room temperature for 12 h. Dichloromethane (50 mL) was added, and the organic phase was washed with half-saturated brine (30 mL), dried, and concentrated. The crude product was purified by MPLC, eluting with $CHCl_3$/acetone (9:1) and $CHCl_3$/MeOH (9:1), respectively, to yield 3 g (92%) of a clear oil.

***Z*-D-Ser(THP)-Lac-OBpa (5).** A solution of **4** (3.0 g, 9.3 mmol) and *p*-bromophenacyl L-lactate (2.6 g, 9.1 mmol; *p*-bromophenacyl = Bpa) in pyridine (75 mL) was cooled to 0 °C. To this cold, stirred solution were added 4-(dimethylamino)pyridine (0.3 g, 2.5 mmol) and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (1.9 g, 10 mmol). The reaction mixture was stirred at 0 °C for 2 h and at room temperature for 1 day. The solution was evaporated to dryness, and the residue was suspended in water (50 mL) and extracted with ethyl acetate (2 × 70 mL). The EtOAc extract was washed with 1 N HCl (2 × 30 mL), saturated $NaHCO_3$ (2 × 20 mL), H_2O (30 mL), and brine (20 mL) and dried (Na_2SO_4). The product was dissolved in a minimum amount of CCl_4 and loaded onto an MPLC column. The column was eluted with $CHCl_3$/acetone (97.5:2.5) to give pure product: yield, 2.9 g (54%); ^{1}H NMR ($CDCl_3$) δ 1.58 (m, 9 H), 3.25–5.60 (m, 11 H), 5.77 (m, 1 H, NH), 7.20–7.80 (m, 9 H, phenyl).

***p*-Bromophenacyl L-Lactate.** A solution containing L-lactic acid (2.0 g, 22.2 mmol), α,4-dibromoacetophenone (6.18 g, 22.2 mmol), and $KHCO_3$ (26 mmol) in 80 mL of acetone was heated at reflux for 5 h. The cooled mixture was filtered, and the filtrate was concentrated in vacuo. The product was recrystallized from acetone/hexane to furnish 4.14 g (65%): mp 103–104 °C; $[\alpha]^{23}{}_D$ +1.99° (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.60 (d, 3 H, CH_3), 2.83 (d, 1 H, OH), 4.51 (m, 1 H, α-H), 5.39 (AB q, 2 H, Bpa CH_2), 7.74 (AB q, 4 H, aromatic).

Z-D-Ser-Lac-OBpa. Dipeptide **5** (2.7 g, 4.6 mmol) in methanol (50 mL) was treated with *p*-toluenesulfonic acid (86 mg, 0.5 mmol), and the reaction mixture was stirred at room temperature for 3 h. Methanol was removed to give a solid, which was partitioned between EtOAc (150 mL) and water (70 mL). The EtOAc extract was washed with water (30 mL) and brine (20 mL), dried, and evaporated to dryness to give 2.1 g (91%) of a white powder: mp 122–125 °C; ^{1}H NMR ($CDCl_3$) δ 1.58 (d, 3 H), 3.40–5.40 (m, 8 H), 6.79 (d, 1 H, NH), 7.32 (s, 5 H, phenyl), 7.67 (q, 4 H, aromatic).

Z-D-Ser(Boc-Val)-Lac-OBpa (6). Z-D-Ser-Lac-OBpa (2.0 g, 3.9 mmol), Boc-Val-OH (0.87 g, 4 mmol), 4-(dimethylamino)pyridine (60 mg, 0.5 mmol), and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (0.76 g, 4 mmol) in dry CH_2Cl_2 (50 mL) was stirred at 0 °C for 3 h and at room temperature for 12 h. The reaction mixture was evaporated to dryness; the residue was suspended in water (100 mL) and extracted with ethyl acetate (2 × 60 mL). The EtOAc extract was washed with 1 N HCl (50 mL), saturated $NaHCO_3$ (2 × 20 mL), H_2O (30 mL), and brine. The organic phase, after drying (Na_2SO_4), was evaporated to dryness. The crude product was purified by chromatography (MPLC) using $CHCl_3$ as eluant to give **6**: 2.6 g (93%); mp 80–85 °C; $[\alpha]^{25}{}_D$–25° (*c* 1.14, $CHCl_3$); ^{1}H (NMR ($CDCl_3$) δ 0.88 (t, 6 H, Val, methyl), 1.41 (s, 9 H, Boc), 1.67 (d, 3 H, Lac methyl), 2.10 (m, 1 H, Val methine), 4.00–5.50 (m, 9 H, Bpa, Ser and benzyl methylenes, α-H), 5.93 (d, 1 H, NH), 7.32 (d, 6 H, phenyl, NH), 7.64 (q, 4 H, aromatic). Anal. Calcd for $C_{32}H_{39}N_2O_{11}Br$: C, 54.32; H, 5.55; N, 3.96. Found: C, 54.23; H, 5.65; N, 3.90.

Z-D-Ser[Boc-Cys(Acm)-Val]-Lac-OBpa (7). Tripeptide **6** (10 g) in dry CH_2Cl_2 (30 mL) was treated with TFA (30 mL), and the reaction mixture was stirred at room temperature for 30 min. The solution was evaporated to dryness, and the residue was dissolved in dry CH_3OH (20 mL) and evaporated to dryness. This procedure was repeated three times, after which the residue was triturated well with ether (dry) to give a solid. The solid was filtered, washed with ether, and dried in vacuo over P_2O_5 to yield 10 g (98%) of a foam.

The above material (10 g, 14 mmol) and *N*-Boc-*S*-(acetamidomethyl)-L-cysteine (4.4 g, 15 mmol, acetamidomethyl = Acm) were dissolved in dry THF (100 mL), and the resultant mixture was cooled to 0 °C. To this solution were added 1-hydroxybenzotriazole (2 g, 15 mmol), triethylamine (1.5 g, 15 mmol), and 1-ethyl-3-3-(dimethylamino)propyl]carbodiimide hydrochloride (2.85 g, 15 mmol), and the mixture was stirred at 0 °C for 3 h and at room temperature overnight. The solution was evaporated to dryness, and the residue was extracted with ethyl acetate (2 × 75 mL). The EtOAc extract was washed with 5% HCl, saturated $NaHCO_3$, H_2O, and brine, dried, and concentrated. The crude product was purified by MPLC using $CHCl_3$/acetone (9:1) as eluant: yield, 11 g (90%); $[\alpha]^{25}{}_D$ –19.1° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.94 (t, 6 H, Val methyl), 1.43 (s, 9 H, Boc), 1.67 (d, 3 H, Lac methyl), 2.01 (s, 3 H, Acm methyl), 2.19 (m, 1 H, Val methine), 2.65–2.9 (m, 2 H, Cys methylene), 4.20–4.32 (m, 1 H), 4.35–4.52 (m, 3 H), 4.55–4.72 (m, 2 H), 4.77–4.84 (m, 1 H), 5.12 (s, 2 H, benzyl), 5.30 (AB pattern, 2 H, Bpa), 5.42 (d, 1 H, NH), 6.2 (d, 1 H, NH), 6.7 (d, 1 H, NH), 7.25–7.40 (m, 5 H, phenyl), 7.70 (dd, 4 H, aromatic). Anal. Calcd for $C_{38}H_{49}N_4O_{13}SBr$: C, 51.76; H, 5.60; N, 6.35. Found: C, 51.57; H, 5.51; N, 6.13.

Z-D-Ser[Boc-Cys(Acm)-Val]-Lac-OH (9). Zn powder (23.4 g, 360 mmol) was added, in small portions, to a stirred solution of **7** (6.3 g, 7.15 mmol) in 90% AcOH (200 mL) at 0 °C over a period of 1 h. After the addition, the reaction mixture was stirred at 0 °C for 2 h and at room temperature for 3 h. The reaction mixture was filtered and washed well with 90% AcOH, and the filtrate was evaporated to dryness. The residue was shaken with a mixture of EtOAc (200 mL) and 1 N HCl (150 mL). The EtOAc extract was washed with 1 N HCl (50 mL), H_2O (50 mL), and brine (30 mL), dried (Na_2SO_4), and concentrated. The oily residue was chromatographed on a gravity column of silica gel using $CHCl_3$, $CHCl_3$/acetone (9:1), $CHCl_3$/acetone (7:3), and $CHCl_3$/MeOH (85:15), respectively, as eluant: yield, 4.5 g (92%); $[\alpha]^{25}{}_D$ –15.8° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.9 (br s, Val methyl), 1.30 (d, Lac methyl), 1.42 (s, 9 H, Boc), 2.03 (s, 3 H, Acm methyl), 2.16 (m, 1 H, Val methine), 2.60–3.10 (m, 2 H, Cys methylene), 4.22–4.89 (m, 8 H, Acm and Ser methylenes, α-H), 5.11 (s, 2 H, benzyl), 5.91 (d, 1 H, NH), 6.30 (d, 1 H, NH), 7.32 (m, 7 H, phenyl and NH), 10.9 (br s, 1 H, COOH).

Z-D-Ser[TFA⁻H₂⁺-Cys(Acm)-Val]-Lac-OBpa (10). Compound **10** was prepared by using the same procedure employed for the removal of the Boc group in **6** during the preparation of **7**. Materials used were the following: tetradepsipeptide **7** (5.2 g); dry CH_2Cl_2 (10 mL); TFA (10 mL). Yield: 5.2 g (98%) as a foam.

Z-D-Ser[Boc-Cys(Acm)-Val]-Lac-Cys(Acm)-Val Z-D-Ser-Lac-OBpa (11). Linear octadepsipeptide **11** was prepared by using the same procedure employed for the preparation of **7**. Materials used were the following: tetradepsipeptide free acid **9** (4.0 g, 5.8 mmol); TFA salt **10** (5.2 g, 5.8 mmol); 1-hydroxybenzotriazole (0.8 g, 6.0 mmol); *N*-methylmorpholine (0.74 mL, 7.0 mmol); 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide hydrochloride (1.2 g, 6.0 mmol); dry THF (150 mL). The product was purified on MPLC using $CHCl_3$/acetone (7:3) as eluant: yield, 5.8 g (72%); $[\alpha]^{25}{}_D$ –28.5° (*c* 2, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$) δ 0.82 (d, 12 H, Val methyl), 1.32 (d, 3 H, Lac methyl), 1.35 (s, 9 H, Boc methyl), 1.54 (d, 3 H, Lac methyl), 1.85 (s, 6 H, Acm methyl), 2.00–2.10 (m, 2 H, Val methine), 2.50 (s, H_2O), 2.55–2.95 (m, 4 H, Cys methylene), 4.05–4.60 (m, 14 H, Acm and Ser methylenes, α-H), 5.05 (s, 4 H, benzyl), 5.08 (q, 1 H, Lac α-H) 5.23 (q, 1 H, Lac α-H), 5.58 (dd, 2 H, Bpa methylene), 7.02 (d, 1 H, NH), 7.35 (m, 10 H, phenyl), 7.75–8.00 (m, 7 H, Bpa aromatic and NH), 8.30 (d, 1 H, NH), 8.58 (m, 2 H, NH). Anal. Calcd for $C_{63}H_{83}N_8O_{22}S_2Br$: C, 52.25; H, 5.78; N, 7.73. Found: C, 52.11; H, 5.64; N, 7.44.

Z-D-Ser[Boc-Cys(Acm)-Val]-Lac-Cys(Acm)-Val Z-D-Ser-Lac-OH. This compound was prepared by the same procedure employed for the preparation of acid **9**. Materials used were the following: compound **11** (5.5 g, 3.8 mmol); Zn powder (12.35 g, 190 mmol); 90% AcOH (100 mL). Data: yield 4.1 g (86%); $[\alpha]^{25}{}_D$ –39° (*c* 0.5, MeOH).

Preparation of 12. The above octadepsipeptide acid (2.5 g, 2.26 mmol) in dry CH_2Cl_2 (7 mL) was treated with TFA (7 mL), and the mixture was stirred at room temperature for 0.5 h. The solution was evaporated to dryness in vacuo. The residue was dissolved in dry CH_3OH and evaporated to dryness. This was repeated three times, and the residue was triturated well with ether to yield a solid, which was filtered, washed with ether, and dried in vacuo over P_2O_5; yield 2.1 g (83%).

The above TFA salt (400 mg, 0.32 mmol) and 1-hydroxybenzotriazole (200 mg, 1.5 mmol) were dissolved in 50 mL of dry DMF, and the solution was diluted with dry CH_2Cl_2 (300 mL) and cooled to 0 °C. To this cold solution was added 1-ethyl-3-[3-(dimethylaminopropyl]carbodiimide hydrochloride (150 mg, 0.8 mmol), and the mixture was stirred for 15 min. *N*-Methylmorpholine (0.05 mL, 0.5 mmol) in dry CH_2Cl_2 (200 mL) was added during a 6-h period. After addition, the reaction mixture was stirred at 0 °C for 2 h and at room temperature for 4 days. The solution was evaporated to dryness and the residue was suspended in H_2O (50 mL) and extracted with ethyl acetate (2 × 75 mL). The EtOAc extract was washed with 1 N HCl (2 × 30 mL), saturated $NaHCO_3$ (2 × 30 mL), H_2O (40 mL), and brine (30 mL), dried (Na_2SO_4), and concentrated to a foam. The product was purified on MPLC using $CHCl_3$/acetone (1:1) as eluant. The fractions having R_f 0.40 [$CHCl_3$/acetone (1:1)] were collected and evaporated to a foam: yield 200 mg (56%); $[\alpha]^{25}{}_D$ +52.6° (*c* 0.5, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$) δ 0.88 (m, 12 H, Val methyl), 1.48 (d, 6 H, Lac methyl), 2.05 (s, 6 H, Acm methyl), 2.06–2.08 (m, 2 H, Val methine), 2.20 (s, H_2O), 2.35–2.50 (m, 2 H, Cys methylene), 2.85–3.05 (m, 2 H, Cys methylene), 4.00–5.30 (series of m, 20 H, Acm, Ser and benzyl methylenes, α-H), 6.62 (m, 2 H, NH), 6.97 (m, 2 H, NH), 7.30–7.50 (m, 12 H, phenyl and

NH), 7.74 (m, 2 H, NH). Anal. Calcd for $C_{50}H_{68}N_8O_{18}S_2$: C, 52.99; H, 6.05; N, 9.88. Found: C, 52.72; H, 6.04; N, 10.04.

Preparation of 13. Iodine (0.99 g, 8.0 mmol) in methanol (150 mL) was added to a solution of **12** (900 mg, 0.80 mmol) in methanol (200 mL) during a 2.5-h period at room temperature. The reaction mixture was stirred at room temperature for 4 h and cooled to 0 °C. To this cold solution was added 1 N aqueous $Na_2S_2O_3$ until the solution became colorless. The solution was evaporated to dryness under reduced pressure below 35 °C, and the residue was triturated with water (15 mL), filtered, washed with water, and dried in vacuo over P_2O_5. The crude product, upon chromatography using $CHCl_3$/acetone (8:2) as eluant, gave **13**: 700 mg (89%); $[\alpha]^{25}_D$ +58° (*c* 0.5, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$) δ 0.95 (m, 12 H, Val methyl), 1.42 (d, 6 H, Lac methyl), 2.20-2.30 (m, 2 H, Val methine), 2.75-2.90 (m, 4 H, Cys methylene), 4.25 (d, 2 H), 4.50-4.70 (m, 4 H), 4.90-5.40 (m, 8 H), 5.45-5.60 (m, 4 H), 6.85 (d, 2 H, NH), 7.35 (s, 10 H, phenyl), 8.48 (d, 2 H, NH). Anal. Calcd for $C_{44}H_{56}N_6O_{12}S_2$: C, 53.42; H, 5.71; N, 8.49. Found: C, 53.25; H, 5.88; N, 8.47.

[Lac^2,Lac^6]Des-*N*-tetramethyltriostin A (2). Octadepsipeptide **13** (550 mg, 0.59 mmol) was stirred with 30-32% HBr in AcOH (20 mL) at room temperature for 1 h and diluted with ether (200 mL). This mixture was kept inside the refrigerator overnight, filtered in a stream of N_2, washed with ether, and dried in vacuo over P_2O_5: yield 460 mg (94%); mp 230 °C (dec).

The above HBr salt (100 mg, 0.11 mmol) was dissolved in dry DMF (10 mL) and the resultant solution cooled to 0 °C under N_2. To this cold, stirred solution was added triethylamine (24 mg, 0.24 mmol), followed by 2-quinoxalinecarbonyl chloride (30 mg). After 5 min, triethylamine (24 mg) and 2-quinoxalinecarbonyl chloride (30 mg) were added. Once again, triethylamine (24 mg) and 2-quinoxalinecarbonyl chloride (30 mg) were added after 5 min. The reaction mixture was stirred at 0 °C for 3 h and at room temperature overnight. The solution was evaporated to dryness and the residue triturated well with ether. The solid was filtered and washed with ether and water, respectively. The solid was dried in vacuo over P_2O_5 and purified by preparative circular TLC using $CHCl_3$/acetone (7:3) as eluant. Pure product, R_f 0.38 [$CHCl_3$/acetone (7:3)], was crystallized from $CHCl_3$/ethyl ether as a fine powder: yield 65 mg (56%); mp 263-265 °C; $[\alpha]^{15}_D$ -15.4° (*c* 0.5, MeOH); ^{1}H NMR (360 MHz) see Tables I and II. Anal. Calcd for $C_{46}H_{52}N_{10}O_{14}S_2$: C, 53.46; H, 5.07; N, 13.55. Found: C, 53.33; H, 5.17; N, 13.37.

Footprinting Experiments

Stock solutions of [Lac^2,Lac^6]TANDEM and deoxyribonuclease I (DNase I) were prepared as previously described.[5] Footprinting experiments were conducted with the 160-base pair duplex *Tyr*T DNA fragment labeled at one of its 3′ ends by incubation with reverse transcriptase and α-[^{32}P]dATP or α-[^{32}P]dCTP. Aliquots (3 μL) of the labeled DNA (9 pmol in base pairs) were incubated with 5 μL of the ligand (10-40 μM) at 37 °C for 30 min and then digested with 2 μL DNase I (final concentration 0.05 unit/mL). Samples (3 μL) were removed from the mixture after 1-, and 5-, and 30-min digestions, and the reaction was stopped by adding 2.5 μL of 80% formamide solution containing 0.1% bromophenol blue and 10 mM EDTA. These were heated at 100 °C for at least 2 min prior to electrophoresis. The products of digestion were fractionated on 8% (w/v) polyacrylamide gels (0.3 mm thick) containing Tris-borate-EDTA buffer and 7 M urea. Gels were fixed in 10% acetic acid, transferred to Whatman 3MM paper, dried under vacuum, and subjected to autoradiography at -70 °C with an intensifying screen. Final analysis of autoradiographs was accomplished using microdensitometer scans as previously described.[12]

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Communications to the Editor

Organoborane-Catalyzed Hydroalumination of Olefins

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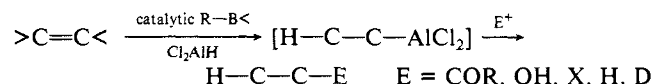
Among various hydrometalation reactions of olefins, hydroboration is far superior in view of the efficiency and versatility of the reaction.[1] However, the resulting organoboranes (C-B bonds) are relatively stable and not readily functionalized compared to the corresponding organoaluminums (C-Al bonds) that possess more carbanion character and hence undergo facile intermolecular transfer reactions with a variety of organic and inorganic electrophiles under milder conditions. Accordingly, considerable interest has been directed toward the synthetic potential of hydroalumination reactions, although the ionic Al-H bond has a weak affinity for olefins in nature. Several successful examples have been recently reported which utilize the transition-metal catalysts to effect the smooth hydroalumination,[2] but these systems are not always satisfactorily employable for subsequent functionalization.[3] In this context we have been intrigued for some time in the possibility of organoborane-catalyzed hydroalumination with consideration for the distinct advantage of hydroboration. Here we wish to disclose the realization of our expectation by combining use of catalytic organoborane and dichloroaluminum hydride (Cl_2AlH) as a hydrometalation agent.



First, we have examined various combinations of boron catalysts with aluminum hydride-type reagents in ether for effecting hydroalumination of 1-dodecene. Yields of dodecane by protonolysis of the hydroalumination product after 2 h at room temperature are as follows: $PhB(OH)_2/Cl_2AlH$, 94% (83% after 30 min); Et_3B/Cl_2AlH, 91%; 9-BBN/Cl_2AlH, 53%; $B(OH)_3/Cl_2AlH$, 19%;

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