# Development of a Solution-Phase Synthesis of Minor Groove Binding Bis-Intercalators Based on Triostin A Suitable for Combinatorial Synthesis

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*Received March 16, 2000*

The development of a solution phase synthesis of azatriostin A (**2**) suitable for the preparation of combinatorial libraries enlisting only liquid−liquid acid/base extractions for the isolation and purification of all intermediates and the final product is disclosed.

Triostin A (**1a**)[1] is a member of the quinoxaline family of antitumor antibiotics that bind to DNA by bisintercalation.[2,3] Triostin A binding in the minor groove exhibits a sequence preference for GC and positions the chromophores across a two base-pair site. The cyclic depsipeptide of triostin A is composed of two identical subunits each containing 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, and a disulfide bond bridges the two *N*-methyl-L-cysteines. A quinoxaline-2-carboxylic acid (Qxc) is attached to the amino group of each D-serine subunit. The GC selectivity of triostin A is disrupted by removal of the *N*-methyl amino acids and replacement with the natural unmethylated amino acids providing the synthetic bisintercalator TANDEM (**1b**) which binds selectively to AT sequences.[4,5] This change in sequence selectivity is thought to be derived from a difference in hydrogen bonding capabilities of the amide backbone. Thus, the *N*-methylated amides cannot form intramolecular $\beta$-sheetlike hydrogen bonds, and bind instead with hydrogen bonding to base residues in DNA.[6]

The development of related compounds that recognize and bind defined sequences and the identification of key structural features governing the binding affinity and selectivity have been hampered by the difficulty in generating and characterizing a large number of analogues. Adopting a technically nondemanding multistep, solution-phase strategy for the preparation of chemical libraries which relies on the removal of excess reactants and reagents by acid/base liquid−liquid or liquid−solid extractions,[7,8] a library of triostin A analogues could be formed with variations in the cyclic depsipeptide amino acids and chromophore. Herein, we report the first stage of this work with the development of a solution-phase synthesis of the triostin A analogue **2** enlisting only liquid−liquid acid/base extractions in the isolation and purification of the synthetic intermediates. Compound **2** differs from the natural antibiotic **1a**, by replacing the D-serine amino acid with D-$\beta$-aminoalanine providing an amide versus ester linkage in the cyclic peptide backbone. Provided **2** maintains DNA bisintercalation binding properties, this permits the use of a purification by acid/base liquid−liquid extractions at each step (Figure 1). These structures and approach complement the directed biosynthetic efforts of Waring[9−11] and the synthetic work of Shin,[5] Helbecque,[12] and Olsen,[4,13] which have provided a variety of analogues.

The plan for the generation of **2** and its subsequent libraries involved the preparation of tetrapeptide **7** as a key intermediate. Tetrapeptide **7** represents one-half of

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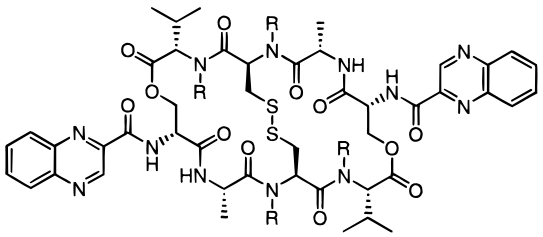
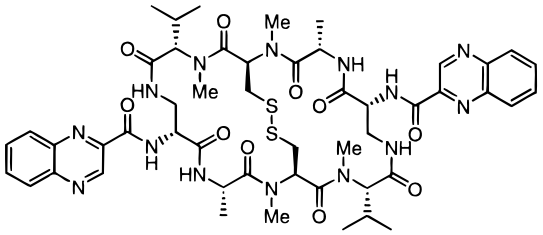
10.1021/jo000382r CCC: $19.00 
Published on Web 08/24/2000



Triostin A (**1a**): R = Me
TANDEM (**1b**): R = H



Azatriostin A (**2**)

**Figure 1.**

the symmetrical octapeptide portion of azatriostin A. Coupling of tetrapeptides **8** and **9**, each prepared from **7** by removal of appropriate protecting group, would give octapeptide **10** possessing the complete amino acid sequence of **2**. Further transformations involving cyclization, disulfide formation, and introduction of quinoxaline chromophore would provide azatriostin A **2** (Scheme 1). An important consideration in the design of intermediate tetrapeptide **7** is the orthogonal protection of the two amino groups and an ester. In particular, it was necessary to selectively deprotect the $\beta$ amino group of the $\beta$-aminoalanine residue without simultaneously cleaving the *N*-Boc protecting group and the C-terminal methyl ester. The $\beta$-(trimethylsilyl)ethoxycarbonyl (Teoc) group is ideally suited for this purpose and can be removed selectively ($Bu_4NF$) with the formation of easily removable byproducts.[14] The cysteine thiol group was protected with the acetamidomethyl (Acm)[15] group, which can be removed with concurrent disulfide formation using iodine in methanol.[16]

Boc-MeCys(Acm)-OH (**3**)[5,17] was coupled with MeVal-OMe hydrochloride (EDCI−HOAt) to give the dipeptide **4** in 75%. Sequentially washing the crude product diluted in EtOAc with 10% aqueous HCl and saturated aqueous $NaHCO_3$ served to remove unreacted amine, carboxylic acid, EDCI, and its reaction byproducts providing pure **4** (≥90% pure). Dipeptide **4** was treated with HCl in EtOAc to remove the Boc group and then coupled with Boc-Ala-OH (EDCI−HOAt) to give tripeptide **5** in 84% yield and in superb purity following purification by acid/base extractions. Tripeptide **5** was converted to tetrapeptide **7** by deprotection of Boc group followed by coupling with $N^{\alpha}$-Boc-$N^{\beta}$-Teoc-D-$\beta$-aminoalanine (**6**).[14] Treatment of **7** with LiOH in THF−MeOH−$H_2O$ (3/3/1) afforded acid **8** in 95% yield, while removal of the Teoc group in **7** by use of a 1 M solution of $Bu_4NF$ (5−10 equiv) in THF in the presence of 4 Å molecular sieves gave **9** in 76% yield. The coupling of segments **8** and **9** was accomplished by use of EDCI−HOAt to provide the linear octapeptide **10** in 79% yield. Octapeptide **10** was treated with a 1 M solution of $Bu_4NF$ (10 equiv) in THF in the presence of 4 Å molecular sieves to provide **11** in 71% yield and then converted to the its corresponding carboxylic acid **12** by LiOH ester hydrolysis. Intramolecular disulfide formation with **12** was achieved by treatment with iodine in $CH_2Cl_2$/MeOH (5/1, 1 mM concentration).[16] Without further purification, the organic extract containing the compound **13** ($CH_2Cl_2$) was subjected to cyclization under conditions of high dilution using EDCI−HOBt in $CH_2Cl_2$/DMF (5/1). The resulting bicyclic peptide was treated with HCl in EtOAc to remove Boc group and acylated with quinoxaline-2-carboxylic acid (EDCI−HOAt) providing the desired azatriostin A (**2**). This completed a 14-step synthesis of **2** in which only liquid−liquid acid/base extractions were employed for purifications of each of the synthetic intermediates. In many instances, especially those involving the removal of protecting groups, no purification was required. The final product was purified by column chromatography to ensure the integrity of the evaluations and conclusions drawn from them. The alternative sequence of amide macrocyclization of **12** (EDCI−HOAt, 20% DMF−$CH_2Cl_2$, 5 mM, −5 °C, 20 h, ca. 20−30%) followed by thiol deprotection ($I_2$, MeOH, 5 mM, 50−100%) also provided the bicyclic peptide precursor to **2** in comparable conversions. However, the amide cyclized product either was unstable to storage and purification or suffered from time-dependent conformational changes that made its characterization and use as an intermediate less satisfactory. In addition, the sequence involving thiol deprotection of **7**, dimerization with intermolecular disulfide bond formation, and subsequent one-step amide bond formation and macrocyclization was also briefly investigated and found to provide the desired bycyclic peptide in a sequence that may prove especially useful in the formation of combinatorial libraries.

The conversions appear comparable to those observed by Olsen[4] with macrocyclization conducted at the Ala-MeCys site but lower than those of Shin[5] observed with closure at the D-Ser-Ala site. Like Triostin A and echinomycin, **2** was found to exist in multiple distinguishable conformations which varied in stability depending on the solvent conditions.[18] At least two conformations were detected by $^1H$ NMR ($CDCl_3$), and two separable and slowly interconverting conformations (1:1) were observed by HPLC (C-18 reverse phase, 3.9 × 300 mm, 10% $H_2O$−$CH_3CN$, 0.5 mL/min, $t_R$ = 3.76 and 4.12 min). Similar observations have been previously detailed for triostin A and echinomycin.[18] This conformational heterogeneity complicated the intermediate purification and characterization of **13** and the subsequent bicyclic dectapdeptide obtained by cyclization of **13**. Consequently, the conversion of **12** to **2** was carried out without characterization of the intermediates.

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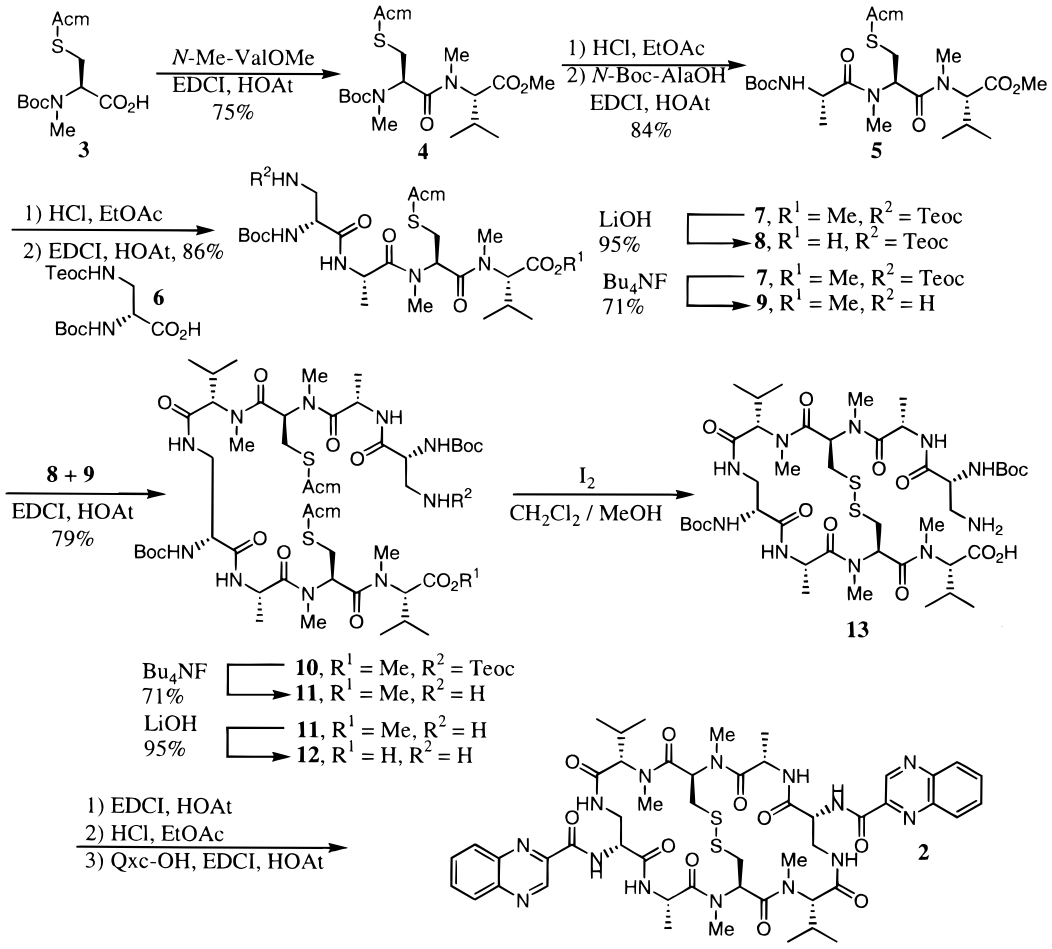
**Scheme 1**



Extensions of these studies to the preparation of a library of triostin A analogues and their evaluation are in progress and will be disclosed in due course.[19]

## Experimental Section

**Boc-MeCys(Acm)-MeVal-OMe (4).** A solution of Boc-MeCys(Acm)-OH (**3,** 1.24 g, 4.4 mmol), MeVal-OMe hydrochloride (0.6 g, 3.1 mmol), and $NaHCO_3$ (260 mg, 3.1 mmol) in $CH_2Cl_2$−DMF (5:1, 12 mL) was treated with HOAt (0.56 g 4.1 mmol) at 0 °C, and the mixture was stirred for 30 min at 0 °C. EDCI (0.78 g, 4.1 mmol) was added at 0 °C, and the mixture was stirred for an additional 15 h at 25 °C. The reaction mixture was transferred into 50 mL of 10% aqueous HCl in a separatory funnel. The product was extracted into EtOAc (70 mL); the organic layer was successively washed with 10% aqueous HCl (2 × 50 mL), saturated aqueous $NaHCO_3$ (2 × 50 mL), and saturated aqueous NaCl and dried over $Na_2SO_4$; and the solvent was removed in vacuo to afford 1.01 g (75%) of Boc-MeCys(Acm)-MeVal-OMe (4) as a white foam: $^1$H NMR ($CDCl_3$, 400 MHz, mixture of rotamers) $\delta$ 7.20 (br s, 1H), 4.94−5.15 (m, 1H), 4.78 (d, $J$ = 10.5 Hz, 2H), 4.10−4.51 (m, 2H), 3.71 (s, 3H), 2.76−3.08 (m, 2H), 2.98, 2.86, 2.66 and 2.60 (four s, 6H), 2.23 (m, 1H), 2.01 (s, 3H), 1.51 (s, 9H), 0.99, 0.98, 0.90, and 0.82 (four d, $J$ = 6.7 Hz, 6H); IR (film) $\nu_{max}$ 3321, 2960, 1717, 1641, 1523 cm$^{-1}$; FABHRMS (NBA-NaI) $m/z$ 456.2154 (M + Na$^+$, $C_{19}H_{35}N_3O_7S$ requires 456.2144).

**Boc-Ala-MeCys(Acm)-MeVal-OMe (5).** A solution of **4** (470 mg, 1.1 mmol) in 2 mL of EtOAc was treated with 2 mL of a 4 M solution of HCl in EtOAc for 1 h at 25 °C before the solvent was removed with a $N_2$ stream to give a white solid. The resulting white solid was dissolved in $CH_2Cl_2$−DMF (5:1, 6 mL) and treated with Boc-Ala-OH (227 mg, 1.2 mmol) and $NaHCO_3$ (240 mg, 2.9 mmol). The mixture was treated with HOAt (165 mg, 1.2 mmol) at 0 °C and was stirred for 30 min. EDCI (230 mg, 1.2 mmol) was added at 0 °C, and the mixture was stirred for an additional 15 h at 25 °C. The reaction mixture was transferred to 30 mL of 10% aqueous HCl in a separatory funnel. The product was extracted into EtOAc (50 mL); the organic layer was successively washed with 10% aqueous HCl (2 × 30 mL), saturated aqueous $NaHCO_3$ (2 × 50 mL), and saturated aqueous NaCl and dried over $Na_2SO_4$; and the solvent was removed in vacuo to afford 547 mg (84%) of the tripeptide **5** as a white foam: $^1$H NMR ($CD_3OD$, 400 MHz, mixture of rotamers) $\delta$ 5.65 and 5.58 (two t, $J$ = 6.7 Hz, 1H), 4.40−4.61 (m, 3H), 4.05−4.15 (m, 1H), 3.72 and 3.69 (two s, 3H), 3.01, 2.98, 2.89, and 2.83 (four s, 6H), 2.70−3.26 (m, 2H), 2.29 (m, 1H), 1.96 (s, 3H), 1.42 (s, 9H), 1.27 and 1.25 (two d, $J$ = 7.0 Hz, 3H), 1.05 (d, $J$ = 6.4 Hz, 3H), 0.95 and 0.83 (two d, $J$ = 6.7 Hz, 3H); IR (film) $\nu_{max}$ 3343, 2954, 1739, 1704, 1643, 1402 cm$^{-1}$; FABHRMS (NBA-NaI) $m/z$ 505.2696 (M + H$^+$, $C_{22}H_{40}N_4O_7S$ requires 505.2710).

***N*$^{\alpha}$-Boc-*N*$^{\beta}$-Teoc-D-3-Aminoalanine (6).** A solution of $Ph_3P$ (4.85 g, 18.5 mmol) in THF (50 mL) was cooled to −78 °C and treated with DEAD (3.39 g, 18.5 mmol), $HN_3$−benzene (18 mL, 1.03 M, 18.5 mmol), and a solution of *N*-(*tert*-butyloxy)-carbonyl-D-serine methyl ester in THF (10 mL). The reaction mixture was stirred at 25 °C for 16 h before the solvent was removed in vacuo. Chromatography ($SiO_2$, 20% $Et_2O$−hexane) afforded 2.73 g (91%) of methyl (*R*)-3-azido-2-[(*tert*-butyloxycarbonyl)amino]propionate as a colorless oil. A solution of (*R*)-3-azido-2-[(*tert*-butyloxycarbonyl)amino]propionate (2.73 g, 11.2 mmol) in MeOH (80 mL) was treated with 10% Pd−C (270

(19) L1210 $IC_{50}$: echinomycin = 0.003 $\mu$M, triostin A = 0.08 $\mu$M, and azatriostin A (**2**) = 7 $\mu$M. HIV-1 reverse transciptase inhibition $IC_{50}$: echinomycin = 700 $\mu$M, azatriostin A (**2**) = 50 $\mu$M, sandramycin = 0.3 $\mu$M.

mg, 0.1 wt equiv) under $H_2$ for 5 h. The reaction mixture was filtered through Celite (MeOH, 3 × 20 mL), and solvent was removed in vacuo to afford 2.40 g (98%) of $N^{\alpha}$-Boc-D-3-aminoalanine methyl ester as a viscous oil that was used directly in the next step. A solution of the methyl ester (0.93 g, 4.3 mmol) in dioxane−$H_2O$ (1:1, 10 mL) and $Et_3N$ (0.8 mL, 5.8 mmol) was treated with 1-[2-(trimethylsilyl)ethoxycarbonyl]benzotriazole (Teoc-OBt, 1.14 g, 4.0 mmol) for 16 h at 25 °C. The product was extracted into ether (50 mL), the organic layer was successively washed with 10% aqueous HCl (2 × 30 mL) and saturated aqueous NaCl and dried over $Na_2SO_4$; and the solvent was removed in vacuo to afford 1.28 g (85%) of **6** methyl ester as a colorless oil. A solution of the resulting ester (1.28 g 3.40 mmol) in THF−MeOH−$H_2O$ (3:1:1, 8 mL) was treated with LiOH hydrate (430 mg, 10.2 mmol) at 0 °C for 2 h. The reaction mixture was acidified with 10% aqueous HCl, extracted twice with EtOAc, washed with saturated aqueous NaC1, dried over $Na_2SO_4$, and concentrated to afford 297 mg (95%) of **6** as a white solid: $^1$H NMR ($CD_3OD$, 400 MHz) $\delta$ 4.22 (m, 1H), 4.13 (t, $J$ = 8.2 Hz, 2H), 3.52 (dd, $J$ = 14.1, 4.1 Hz, 2H), 3.36 (dd, $J$ = 14.1, 7.6 Hz, 2H), 1.44 (s, 9H), 0.98 (t, $J$ = 8.2 Hz, 2H), 0.04 (s, 9H); IR (film) $\nu_{max}$ 3348, 2954, 1704, 1525, 1250 cm$^{-1}$; MALDIHRMS (DHB) $m/z$ 371.1629 (M + $Na^+$, $C_{14}H_{28}N_2O_{6Si}$ requires 371.1614).

**Boc-D-Ala(NHTeoc)-Ala-MeCys(Acm)-MeVal-OMe (7).** A solution of **5** (540 mg, 1.1 mmol) in 2 mL of EtOAc was treated with 2 mL of a 4 M solution of HCl in EtOAc for 1 h at 25 °C before the solvent was removed with a $N_2$ stream to give a white solid. The resulting Ala-MeCys(Acm)-MeVal-OMe was dissolved in $CH_2Cl_2$−DMF (5:1, 6 mL). To this were added **6** (453 mg, 1.2 mmol), and $NaHCO_3$ (270 mg, 3.3 mmol). The mixture was treated with HOAt (160 mg, 1.2 mmol) at 0 °C, and it was stirred for 30 min. EDCI (230 mg, 1.2 mmol) was added at 0 °C, and the mixture was stirred for an additional 15 h at 25 °C. The reaction mixture was transferred into 30 mL of 10% aqueous HCl in a separatory funnel. The product was extracted into EtOAc (50 mL); the organic layer was successively washed with 10% aqueous HCl (2 × 30 mL), saturated aqueous $NaHCO_3$ (2 × 50 mL), and saturated aqueous NaCl and dried over $Na_2SO_4$; and the solvent was removed in vacuo to afford 677 mg (86%) of **7** as a white foam: $^1$H NMR ($CD_3OD$, 400 MHz, mixture of rotamers) $\delta$ 5.65 and 5.58 (two t, $J$ = 6.7 Hz, 1H), 4.40−4.61 (m, 3H), 4.05−4.15 (m, 1H), 3.72 and 3.69 (two s, 3H), 3.01, 2.98, 2.89, and 2.83 (four s, 6H), 2.70−3.26 (m, 2H), 2.29 (m, 1H), 1.96 (s, 3H), 1.42 (s, 9H), 1.27 and 1.25 (two d, $J$ = 7.0 Hz, 3H), 1.05 (d, $J$ = 6.4 Hz, 3H), 0.95 and 0.83 (two d, $J$ = 6.7 Hz, 3H); IR (film) $\nu_{max}$ 3343, 2954, 1739, 1704, 1643, 1402 cm$^{-1}$; MALDIHRMS (DHB) $m/z$ 757.3583 (M + $Na^+$, $C_{31}H_{58}N_6O_{10}OSi$ requires 757.3602).

**Boc-D-Ala(NHTeoc)-Ala-MeCys(Acm)-MeVal-OH (8).** A solution of **7** (310 mg, 0.42 mmol) in THF−MeOH−$H_2O$ (3:1:1, 5 mL) was treated with LiOH hydrate (53 mg, 1.3 mmol) at 0 °C for 2 h. The reaction mixture was acidified with 10% aqueous HCl, extracted twice with EtOAc, washed with saturated aqueous NaCl, dried over $Na_2SO_4$, and concentrated to afford 297 mg (98%) of **8** as a white solid: $^1$H NMR ($CD_3OD$, 400 MHz, mixture of rotamers) $\delta$ 5.67 and 5.60 (two t, $J$ = 6.5 Hz, 1H), 4.30−4.62 (m, 3H), 3.90−4.25 (m, 4H), 3.43 (m, 1H), 3.03, 2.98, and 2.85 (three s, 6H), 2.70−3.21 (m, 2H), 2.25 (m, 1H), 1.98 (s, 3H), 1.43 (s, 9H), 1.31 and 1.29 (two d, $J$ = 7.0 Hz, 3H), 1.05 (d, $J$ = 6.4 Hz, 3H), 0.99 (d, $J$ = 6.8 Hz, 2H), 0.95 and 0.83 (two d, $J$ = 6.7 Hz, 3H), 0.41 (s, 9H); IR (film) $\nu_{max}$ 3343, 2954, 1739, 1704, 1643, 1402 cm$^{-1}$; MALDIHRMS (DHB) $m/z$ 743.3450 (M + $Na^+$, $C_{30}H_{56}N_6O_{10}SSi$ requires 743.3446).

**Boc-D-Ala($NH_2$)-Ala-MeCys(Acm)-MeVal-OMe (9).** A solution of **7** (330 mg, 0.45 mmol) was treated with a 1 M solution of $Bu_4NF$ (2.7 mL, 2.7 mmol) in THF and 4 Å molecular sieves for 3−5 h at 25 °C. After removal of molecular sieves by filtration, the filtrate was extracted with EtOAc (5 × 50 mL), washed with saturated aqueous NaCl, dried over $Na_2SO_4$, and then concentrated to afford 210 mg (76%) of **9** as a white solid: $^1$H NMR ($CD_3OD$, 400 MHz, mixture of rotamers) $\delta$ 5.64 and 5.56 (two t, $J$ = 6.5 Hz, 1H), 4.79−4.81 (m, 1H), 4.38−4.52 (m, 1H), 4.05−4.15 (m, 3H), 3.70 and 3.67 (two s, 3H), 3.01, 2.97, 2.87 and 2.82 (four s, 6H), 2.70−3.23 (m, 2H), 2.29 (m, 1H), 1.98 and 1.95 (two s, 3H), 1.44 (s, 9H), 1.30 (d, $J$ = 7.0 Hz, 3H), 0.99 and 0.82 (d, $J$ = 6.4 Hz, 6H); IR (film) $\nu_{max}$ 3343, 2954, 1739, 1704, 1643, 1402 cm$^{-1}$; MALDIHRMS (DHB) $m/z$ 613.3016 (M + $Na^+$, $C_{25}H_{46}N_6O_8S$ requires 613.2995).

**[Boc-D-Ala(NHTeoc)-Ala-MeCys(Acm)-MeVal]-Boc-D-Ala(NH)-Ala-MeCys(Acm)-MeVal-OMe (10).** A solution of the acid **8** (396 mg, 0.55 mmol), the amine **9** (250 mg, 0.42 mmol), and $NaHCO_3$(46 mg, 0.55 mmol) in $CH_2Cl_2$−DMF (5:1, 6 mL) was treated with HOAt (74 mg, 0.55 mmol) at 0 °C and was stirred for 30 min. EDCI (106 mg, 0.55 mmol) was added at 0 °C, and the mixture was stirred for an additional 15 h at 25 °C. The reaction mixture was transferred into 30 mL of 10% aqueous HCl in a separatory funnel. The product was extracted into EtOAc (50 mL); the organic layer was successively washed with 10% aqueous HCl (2 × 30 mL), saturated aqueous $NaHCO_3$ (2 × 50 mL), and saturated aqueous NaCl and dried over $Na_2SO_4$; and the solvent was removed in vacuo to afford 430 mg (79%) of **10** as a white foam: $^1$H NMR ($CDCl_3$, 400 MHz, mixture of rotamers) $\delta$ 6.85 and 6.71 (two br s), 5.66 and 5.58 (two d, $J$ = 6.7 Hz, 1H), 4.78−4.85 (m, 2H), 4.35−4.58 (m, 2H), 4.10−4.17 (m, 6H), 3.72 and 3.69 (two s, 3H), 3.47 (m, 2H), 3.04, 3.00, 2,98, and 2.83 (four s, 12H), 2.98−3.20 (m, 4H), 2.26 (m, 2H), 1.98 and 1.95 (two s, 6H), 1.42 (s, 18H), 1.32 (d, $J$ = 6.7 Hz, 6H), 1.01, 0.95, 0.84, and 0.80 (d, $J$ = 6.4 Hz, 14H), 0.04 (s, 9H); IR (film) $\nu_{max}$ 3312, 2970, 1707, 1643, 1521 cm$^{-1}$; MALDIHRMS (DHB) $m/z$ 1315.6471 (M + $Na^+$, $C_{55}H_{100}N_{12}O_{17}S_2Si$ requires 1315.6437).

**[Boc-D-Ala($NH_2$)-Ala-MeCys(Acm)-MeVal]-Boc-D-Ala-(NH)-Ala-MeCys (Acm)-MeVal-OMe (11).** A solution of **10** (198 mg, 0.15 mmol) was treated with a 1 M solution of $Bu_4NF$ (1.5 mL, 2.7 mmol) in THF and 4 Å molecular sieves (190 mg) for 3−5 h at 25 °C. After removal of molecular sieves by filtration, the filtrate was extracted with $CH_2Cl_2$ (5 × 50 mL), washed with saturated aqueous NaCl, dried over $Na_2SO_4$, and concentrated to afford 124 mg (71%) of **11** as a white solid: $^1$H NMR ($CD_3OD$, 400 MHz, mixture of rotamers) $\delta$ 5.67 and 5.60 (two m, 2H), 4.78−4.83 (m, 2H), 4.47−4.64 (m, 2H), 4.06−4.17 (m, 4H), 3.72 and 3.69 (two s, 3H), 3.48 (m, 2H), 3.03, 2.99, 2.98, and 2.90 (four s, 12H), 2.71−3.25 (m, 4H), 2.29 (m, 2H), 1.97 and 1.95 (two s, 6H), 1.45 (s, 18H), 1.32 (d, $J$ = 6.4 Hz, 6H), 1.12, 0.97, 0.84, and 0.80 (d, $J$ = 6.4 Hz, 12H); IR (film) $\nu_{max}$ 3313, 2975, 1707, 1639, 1522 cm$^{-1}$; MALDIHRMS (DHB) $m/z$ 1149.6012 (M + $H^+$, $C_{49}H_{88}N_{12}O_{15}S_2$ requires 1149.6011).

**[Boc-D-Ala($NH_2$)-Ala-MeCys(Acm)-MeVal]-Boc-D-Ala-(NH)-Ala-MeCys(Acm)-MeVal-OH (12).** A solution of **11** (120 mg, 0.10 mmol) in THF−MeOH−$H_2O$ (3:1:1, 4 mL) was treated with LiOH hydrate (12.6 mg, 0.3 mmol) at 0 °C and the mixture stirred for 2−3 h at 25 °C. The reaction mixture was acidified with 10% aqueous HCl, extracted twice with $CHCl_3$, washed with saturated aqueous NaCl, dried over $Na_2SO_4$, and concentrated to afford 119 mg (100%) of **12** as a white solid: $^1$H NMR ($CD_3OD$, 400 MHz, mixture of rotamers) $\delta$ 5.65 and 5.55 (two m, 2H), 4.78−4.85 (m, 2H), 4.36−4.58 (m, 2H), 4.15−4.24 (m, 4H), 3.45 (m, 2H), 2.98 and 2.87 (two s, 12H), 2.98−3.20 (m, 4H), 2.23 (m, 2H), 1.98 (s, 6H), 1.43 (s, 18H), 1.34 (d, $J$ = 6.4 Hz, 6H), 0.80−1.04 (three m, 12H); IR (film) $\nu_{max}$ 3306, 2906, 1643, 1528 cm$^{-1}$; MALDIHRMS (DHB) $m/z$ 1135.5836 (M + $H^+$, $C_{48}H_{86}N_{12}O_{15}S_2$ requires 1135.5855).

**Azatriostin A (2).** A solution of **12** (27 mg, 0.02 mmol) in $CH_2Cl_2$−MeOH (9:1, 20 mL) was added to a solution of $I_2$ (30.2 mg, 0.12 mmol) in $CH_2CI_2$−MeOH (9:1, 10 mL) over 30 min at 25 °C. After being stirred an additional 1 h at 25 °C, the reaction was quenched with a 1 N $Na_2S_2O_3$ aqueous solution at 0 °C. The organic layer was washed with saturated aqueous NaCl (3×), dried over $Na_2SO_4$, and then subjected to a cyclization. The $CH_2Cl_2$ layer (ca. 30 mL) was combined with 5 mL of DMF, the mixture was treated with HOAt (15.5 mg, 0.11 mmol) and EDCI (22.1 mg, 0.11 mmol) at 25 °C; and the mixture was stirred for 24 h at 25 °C. The product was extracted into EtOAc (50 mL); the organic layer was successively washed with 10% aqueous HCl (2 × 30 mL), saturated aqueous $NaHCO_3$ (2 × 30 mL), and saturated aqueous NaCl and dried over $Na_2SO_4$; and the solvent was removed in vacuo.

The resulting residue was treated with 1 mL of 4 M HCl in EtOAc for 1 h at 25 °C before the solvent was removed with a $N_2$ stream to give a yellow solid (8.2 mg, 34%). A solution of the solid in $CH_2Cl_2$−DMF (5:1, 2 mL) was treated with quinoxaline-2-carboxylic acid (14.6 mg, 0.08 mmol), HOAt (11.5 mg, 0.08 mmol), and EDCI (16.4 mg, 0.08 mmol), and the mixture was stirred at 25 °C for 24 h. The product was extracted into EtOAc (30 mL); the organic layer was successively washed with 10% aqueous HCl (2 × 30 mL), saturated aqueous $NaHCO_3$ (2 × 30 mL), and saturated aqueous NaCl and dried over $Na_2SO_4$; and the solvent was removed in vacuo. PTLC ($SiO_2$, the 5% MeOH−$CH_2Cl_2$) gave 2.5 mg of **2** as white solid: $^1$H NMR ($CDCD_3$, 500 MHz, mixture of conformational isomers) $\delta$ 9.67 and 9.60 (two s, 2H), 8.32 and 8.08 (two d, $J$ = 6.2 Hz, 2H), 8.20, 7.88, 7.70, and 7.52 (four m, 8H), 6.89 and 6.81 (two d, $J$ = 3.8 Hz, 2H), 6.53 and 6.43 (two m, 2H), 6.35 and 6.25 (two br s, 2H), 5.11 and 5.06 (two d, $J$ = 9.5 Hz, 2H), 4.93−4.72 (m, 2H), 4.55−4.46 (m, 2H), 4.22−4.10 (m, 4H), 3.40−3.65 (m, 2H), 3.15, 3.13, 3.04, and 2.96 (four s, 12H), 2.45−2.34 (m, 2H), 1.34 and 1.31 (two dd, $J$ = 8.2, 2.3 Hz, 6H), 1.17, 1.14, 1.10, 0.96, 0.93, and 0.80 (six d, $J$ = 6.3 Hz, 12H); IR (film) $\nu_{max}$ 3518, 3342, 2954, 2931, 1719, 1654, 1518, 1488 cm$^{-1}$; MALDIHRMS (DHB) $m/z$ 1107.4260 (M + H$^+$, $C_{50}H_{64}N_{14}O_{10}S_2$ requires 1107.4269).

**Acknowledgment.** We gratefully acknowledge the financial support of the National Institutes of Health (CA 78045), The Skaggs Institute for Chemical Biology, and Novartis.

**Supporting Information Available:** $^1$H NMR spectra of **2**−**12**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO000382R