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Solid-Phase Synthesis of Triostin A Using a Symmetrical Bis(diphenylmethyl) Linker System

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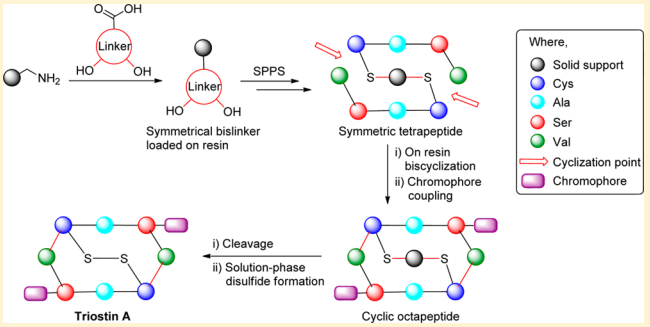
Supporting Information

ABSTRACT: Triostin A is a symmetric bicyclic depsipeptide with very potent antitumoral activity because of its bisintercalation into DNA. In this study, we report a new synthetic strategy that exploits a structural symmetry of triostin A. First, we prepared a novel symmetric linker molecule that is labile under mildly acidic conditions and suitable for a solid-phase synthesis procedure. Two Cys units were attached to a linker-resin conjugate via their free thiol groups, and double deprotection and double coupling reactions were then applied to synthesize linear tetradepsipeptides. Subsequently, the key biscyclization of the tetradepsipeptides was performed on the resin, and the resulting cyclic octapeptide was detached from the linker-resin conjugate to give a peptide with two free thiols. Finally, triostin A was obtained by oxidizing the free thiols in solution to produce a disulfide. The yield was improved through exploration of two different solid-phase synthetic approaches under similar strategy. Mainly, this strategy was developed to enable the ease and rapid preparation of libraries of symmetric bicyclic depsipeptides. It also addresses several synthetic problems with our synthesis, including diketopiperazine (DKP) formation, poor cyclization yields and preparation of noncommercial *N*-methyl amino acids in good yields.



INTRODUCTION

Triostin A (**1**),[1,2] echinomycin (**3**),[3] BE-22179 (**4**)[4] and thiocoraline (**5**)[5,6] (Figure 1) are quinoxaline antitumor antibiotics produced by marine bacteria. In 1961, triostin A was isolated from a strain of *Streptomyces aureus*.[1,7,8] Initial studies showed that triostin A and echinomycin exhibit significant antibacterial and antitumor activities because of their bifunctional intercalation of two aromatic rings into DNA.[9−13] Triostin A is well-known to exhibit CpG selectivity for bisintercalation into DNA, whereas it's *N*-demethylated derivative TANDEM (**2**) (Triostin A *N*-DEMethylated)[14,15] is known to be selective for TpA sequences.[16−19] Initially, this change in selectivity was attributed to differences in the hydrogen-bonding abilities of these compounds; however, their binding to DNA base pairs was later demonstrated to be driven by both hydrogen-bonding and stacking interactions.[10,20]

In recent reports, triostin A activity against three human cancer cell lines (breast MDA-MB-231, lung NSCLC A549, colon HT-29) was tested. It exhibited cell-growth inhibitory activities (GI_{50}) as high as 10^{-7} M.[21−23] Because of this activity profile, many researchers have focused on developing various synthesis methods for these types of peptides and on understanding their mode of action and potential applications in cancer therapy. To date, triostin A has been synthesized via two solution-phase methods[24−26] and one solid-phase method.[22] Generally, the solid-phase synthesis of these cyclic depsipeptides is preferred over tedious solution-phase methods because it allows various peptide analogues to be rapidly synthesized by changing the amino acid at each position.

Because of the therapeutic significance of quinoxaline antibiotics, we utilized our experience in peptide synthesis[27−31] to develop an efficient solid-phase synthetic strategy for this class of symmetric antibiotics, using triostin A synthesis as a model. In developing our synthetic strategy, we considered the following important points: (i) utilization of the structural C_2 symmetry of this family of molecules; (ii) minimization of the required number of synthetic steps while maximizing the use of a solid support; and (iii) straightforward synthesis of libraries of various bisintercalators by varying the amino acids and chromophores.

Structurally, triostin A is a symmetric bicyclic octadepsipeptide composed of four *N*-Me amino acids, two ester linkages, two chromophores and a disulfide backbone. These structural elements make it sterically hindered or conformationally rigid and therefore the use of a suitable linker system was necessary to minimize the difficulties encountered in the solid-phase synthesis of triostin A. Overall, the optimum synthesis procedure includes preparing the appropriate linker, synthesizing commercially unavailable amino acids with suitable protecting groups, choosing the first amino acid to couple to the linker and utilizing the structural symmetry of triostin A to reduce the number of synthetic steps. However, in the presence

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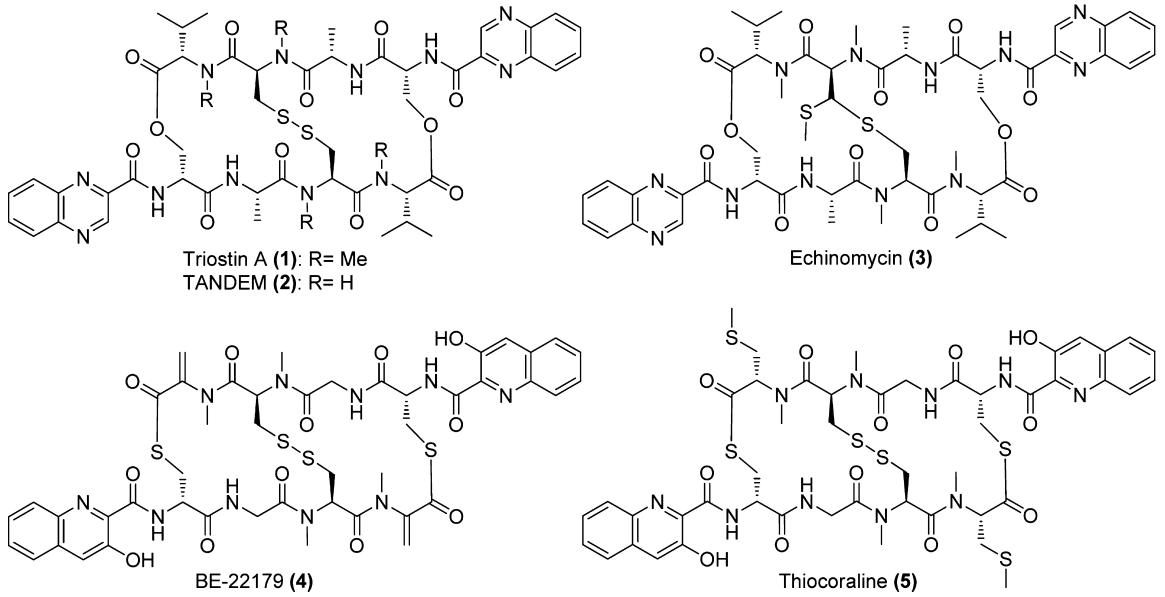
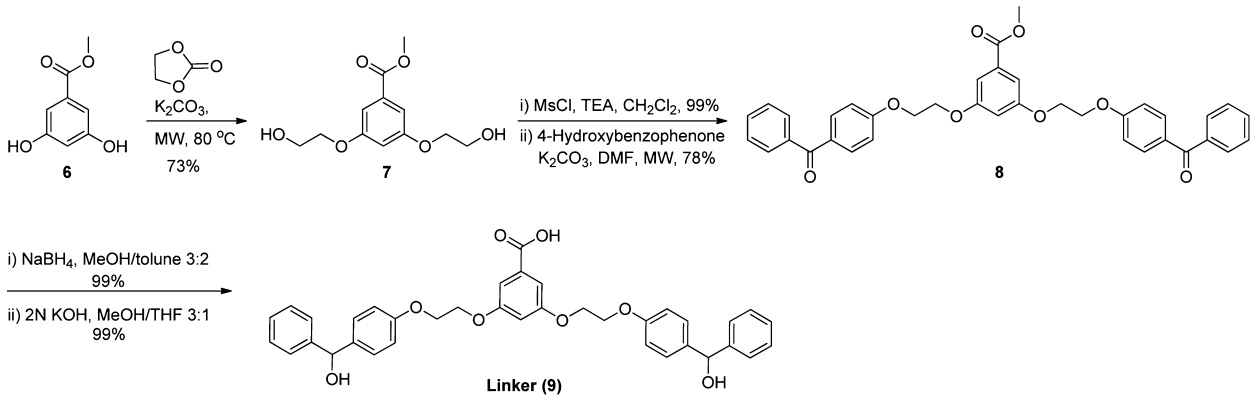


Figure 1. Structures of bicyclic bisintercalators **1**−**5**.

Scheme 1. Synthesis of the Linker (9)



of two ester linkages and adjacent *N*-Me amino acids, peptide syntheses are known to be challenging for several reasons, including DKP formation,[32,33] slow coupling reactions,[23,34] difficult cyclization,[35−37] and the risk of elimination reactions at the Cys residue.[32,38,39] Thus, in the present work, two different approaches were explored to overcome or minimize these problems, and triostin A was synthesized in a yield comparable to those obtained by solid-phase synthesis reported to date.[22]

RESULTS AND DISCUSSION

Linker Synthesis. To develop a suitable linker system, the following solid-phase synthesis requirements were considered: (i) appropriate length of the spacer between the peptide and polymer support, (ii) structural symmetry, (iii) lability under mildly acidic conditions and stability under Fmoc deprotection conditions, and (iv) ease of loading of the first amino acid to the linker. On the basis of these requirements, a bisdiphenylmethyl linker system was synthesized from the compound **6** (Scheme 1), which has C_2 symmetry and two reactive functional groups. The neat reaction of the ethylene carbonatespacer with the phenolic hydroxyl of **6** under microwave irradiation gave the symmetric disubstituted product **7** in 73% yield.[40] The reaction was monitored at short time intervals because longer treatments initiated byproducts formation through a decomposition of the desired product (byproducts include traces of the hydrolyzed product of **7** in the presence of moisture trapped by reagents). Initially, this reaction was performed in DMF under reflux; however, it was too slow to achieve complete conversion of the starting material. Next, the compound **8** was obtained by simple mesylation[41] of **7**, followed by nucleophilic substitution[42] with 4-hydroxybenzophenone under MW irradiation. Finally, the desired linker **9** was obtained from **8** by selectively reducing two keto groups in the presence of $NaBH_4$[42] and then performing methyl ester hydrolysis.

SPPS Synthesis. Because the *N*-Me-Cys residue was chosen to be coupled to the linker-resin conjugate, Fmoc-*N*-Me-Cys-OAllyl was synthesized from Alloc-*N*-Me-Cys(Trt)-OH[43] by sequentially performing Alloc deprotection, Fmoc protection, allyl esterification and trityl deprotection reactions (Experimental Section). The Trityl deprotected Fmoc-*N*-Me-Cys-OAllyl was unstable for a longer storage as it undergoes S−S dimerization; therefore, it was immediately utilized for the SPPS synthesis without purification. The synthesis of Fmoc-protected *N*-Me-Cys-OH was previously reported on the solid support for rapid and diverse synthesis;[43] however, we could prepare it on a multigram scale via a solution-phase reactions with high yield and purity.

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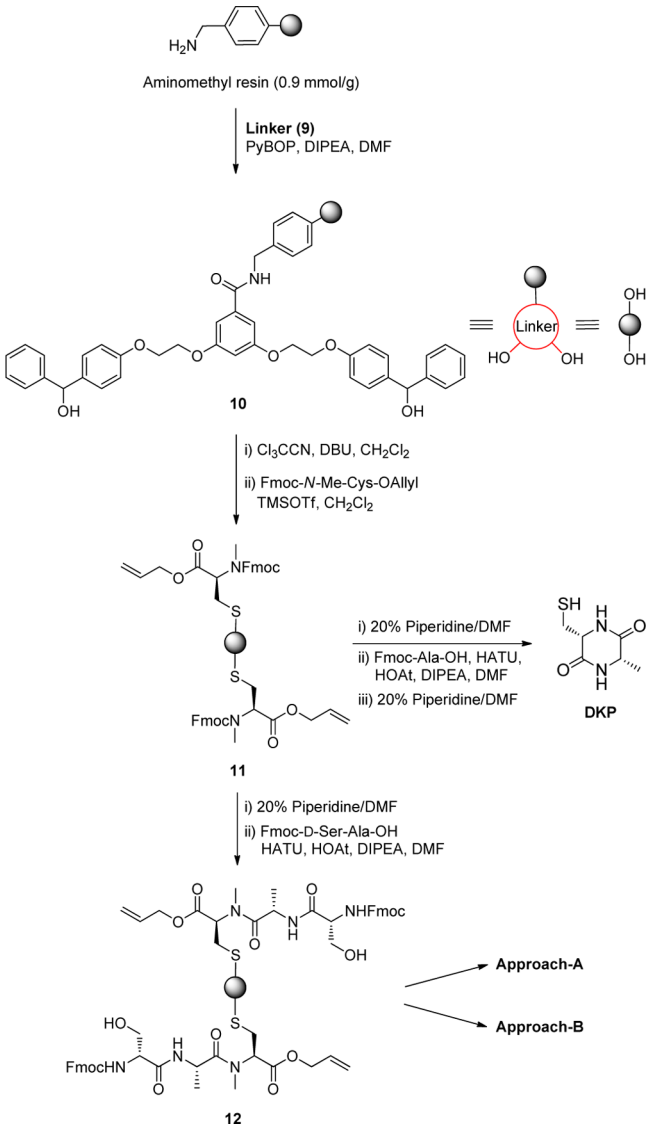
After the desired linker and Fmoc-*N*-Me-Cys-OAllyl were prepared, triostin A was synthesized on the amino methyl resin (Scheme 2). The symmetric linker **9** was first coupled to the amino methyl resin in the presence of (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP)/*N*,*N*-Diisopropylethylamine (DIPEA) to obtain the linker resin **10**. Complete conversion was confirmed by a negative Kaiser test,[44] and the loading was determined to be 90% on a w/w basis.

Scheme 2. Preparation of Resin-Bound Intermediate 12



For the peptide synthesis, the orthogonally protected Fmoc-*N*-Me-Cys-OAllyl was immobilized on the linker resin **10** in two steps; the activation of hydroxyl group of **10** to its trichloroacetimidate using Cl_3CCN/1,8-Diazabicyclo[5.4.0]-undec-7-ene (DBU) followed by the substitution with the free thiol of Fmoc-*N*-Me-Cys-OAllyl to obtain **11**.[45,46] The immobilization of Fmoc-*N*-Me-Cys-OAllyl was confirmed by FTIR spectroscopy, where the C═N stretching band (1663 cm^{-1}) of an intermediate trichloroacetimidate of **10** was replaced by C═O stretching band (1721 cm^{-1}) of Fmoc-*N*-Me-Cys-OAllyl. The loading level was determined to be approximately 82% based on the weight increase of the dried resin after the coupling of Fmoc-*N*-Me-Cys-OAllyl. The resin was subsequently treated with acetic anhydride/DIPEA to cap the unreacted sites.[47,48]

In the first synthesis attempt (Scheme 2), the Fmoc group of **11** was removed using 20% piperidine/DMF[31] and Fmoc-Ala-OH was coupled to the Cys residue using 7-azabenzotriazol-1-yl-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU)/1-hydroxy-7-azabenzotriazole (HOAt). However, during the next Fmoc deprotection reaction, the resulting dipeptide, as expected, underwent complete DKP formation in the presence of the allyl ester of *N*-Me-Cys.

To prevent DKP formation, a dipeptide was prepared separately in solution and then used in the solid-phase synthesis. Specifically, the dipeptide Fmoc-D-Ser-Ala-OH was prepared in two steps (Experimental Section).[49,50] The Fmoc group of **11** was then removed, and Fmoc-D-Ser-Ala-OH was coupled to the Cys residue using HATU/HOAt to give resin-bound peptide **12**. To prevent the free hydroxyl group of the Ser residue from reacting to form byproducts, the reaction was stopped immediately after a negative chloranil test result. After the resin-bound peptide **12** was obtained, two different approaches were tested to complete the synthesis of triostin A.

In **Approach A** (Scheme 2), the Fmoc group of tripeptide **12** was removed and 2-quinoxaline carboxylic (Qxc) acid was coupled to the peptide by activation with HATU/HOAt. However, **13a** was obtained as a major byproduct. We attempted to control this reaction stoichiometrically; however, the second coupling reaction to the Ser free hydroxyl group occurred too quickly because the conformation of the desired product **13** imposed by the attached Qxc group might be favorable for this side reaction. The unwanted product **13a** was then converted back to the desired product **13** by alcoholysis on the resin in the presence of K_2CO_3 and allyl alcohol.[51] Allyl alcohol was used as a solvent to prevent the hydrolysis of the allyl ester of the *N*-Me-Cys residue. This reaction was effectively completed, but washing the resin with water to remove K_2CO_3 led to the loss of some of the peptide from the resin, as indicated by HPLC analysis. Next, Alloc-*N*-Me-Val-OH was coupled to the growing peptide to obtain the resin-bound peptide **14**, and the Alloc and allyl protecting groups were removed using $[Pd(PPh_3)_4]$ and $PhSiH_3$ in CH_2Cl_2. For the biscyclization step, several reagents, including (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP), HATU, 1-Ethyl-3-(3-(dimethylamino)propyl)-carbodiimide (EDC), diphenylphosphoryl azide (DPPA), *N*,*N*′-bis(2-oxo-3-oxazolidinyl) phosphinic chloride (BOP-Cl) and Tetramethylfluoroformamidinium Hexafluorophosphate (TFFH) were tested to obtain the resin-bound cyclic octapeptide **15**; however, in all cases, the monocyclic tetradepsipeptide **15a** and/or unidentified byproducts were formed. Comparatively, the best biscyclization results were obtained using *N*,*N*′-diisopropylcarbodiimide (DIC)/HOAt with a DMSO/DMF (3:2) solvent mixture.[22,38,52,53] The peptide was cleaved from the resin by treatment with TFA/TIS/CH_2Cl_2 (10:5:85), and the crude product was purified by semipreparative HPLC to give the desired product **15** (7.1 mg, 4.0% overall yield from the Cys-loaded linker resin).

During the cyclization step, in addition to the desired product **15**, the cyclic tetradepsipeptide **15a** was obtained as a major byproduct. The structure of **15a** was analyzed by NMR and subsequently confirmed by mass analysis of its *N*-ethyl maleimide (NEM) adduct (Supporting Information).[54] With this approach, the desired product was obtained in low yield;

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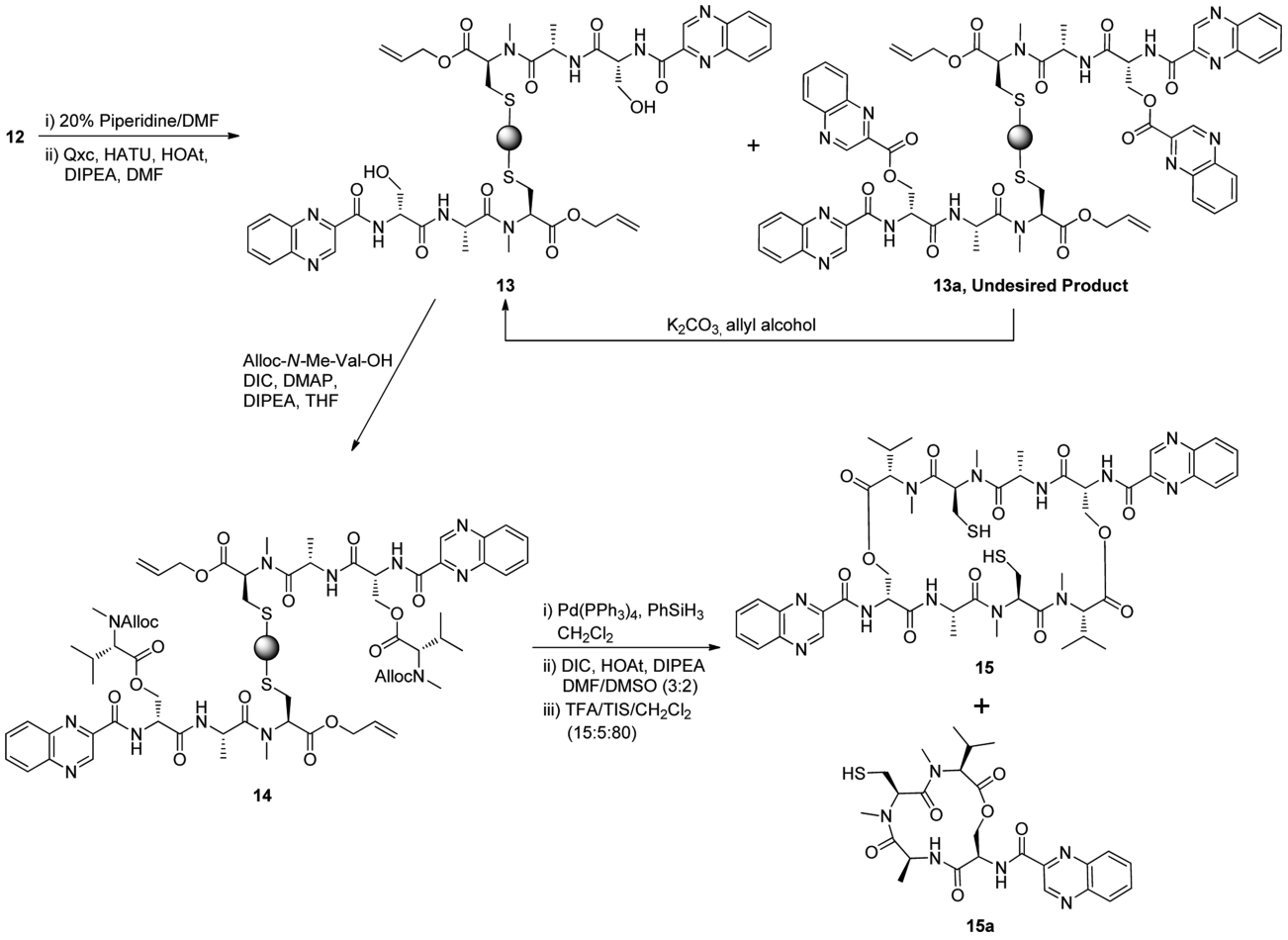
Scheme 3. Solid-Phase Synthesis of 15 (Approach A)



therefore, the synthesis approach was revised. The major drawbacks of Approach A were as follows: (i) side-product formation during the Qxc acid coupling; (ii) peptide loss from the resin when it was washed with water to remove K_2CO_3 after the alcoholysis reaction; and (iii) a relatively low biscyclization yield along with the formation of the undesired monocyclic tetradepsipeptide as a major byproduct. To address these problems, the sequence of reactions in Approach A was changed.

In **Approach B** (Scheme 4), the free hydroxyl group of the Ser residue in **12** was blocked from reacting to form side product **13a** by coupling Alloc-*N*-Me-Val-OH to the peptide before the Fmoc deprotection and Qxc acid coupling reactions. The cyclization yield was assumed to be affected by the presence of the Fmoc group and by the absence of Qxc acid because the steric environment would otherwise be different. Therefore, Alloc-*N*-Me-Val-OH was coupled to the free hydroxyl group of **12** to obtain Fmoc-protected peptide **16**. Then, as in Approach A, the Alloc and allyl groups were removed and biscyclization was performed to obtain the resin-bound cyclic peptide **17**. The HPLC yield of this cyclization step was nearly 2.5 times larger than that of the cyclization step in Approach A (Supporting Information).

Next, the Fmoc group was removed and Qxc acid was coupled to the peptide, which was then cleaved from the resin. The crude product was purified by semipreparative HPLC to give the desired product **15** (6.5 mg) in 9.1% overall yield.

Finally, the pure product **15** was oxidized to form triostin A (**1**). The aerial oxidation[55] of **15** in acetonitrile (ACN) or in DMSO was slow. Therefore, the dithiol **15** was oxidized using I_2 in ACN/DMSO (2:1).[56] The crude reaction mixture was purified by semipreparative HPLC to obtain triostin A (**1**) in 80% yield. Similarly, the byproduct **15a** was also oxidized to obtain the dimer **15b**, and its NMR spectrum was compared to that of triostin A (Supporting Information).

During the synthesis, only one triostin A conformer of the two possible conformers reported by Tulla-Puche, Albericio and co-workers[22] was obtained. Based on the characteristic peaks of Cys-α (δ 5.79) and Cys-β (δ 3.28), the ^{1}H NMR spectrum of our product was consistent with that of the least-polar conformer that they reported.[22] The Ala *N*-H signal of our synthetic sample (δ 6.74) is significantly different from the previously reported (δ 6.45) data of triostin A.[22] We observed that the chemical shift of Ala *N*-H is dependent on the sample concentration which indicates the Ala *N*-H is solvent exposed (Supporting Information). It was known that the Ala *N*-H group forms hydrogen bonds to the nucleic acid in the crystal structure of a DNA-triostin A complex.[12]

■ CONCLUSIONS

Triostin A was synthesized via two different approaches using a novel solid-phase synthesis strategy. The yields obtained were comparable to those obtained via other solid-phase synthesis procedures reported in the literature.[57] To demonstrate the synthesis of C_2-symmetric antibiotics, a novel C_2-symmetric

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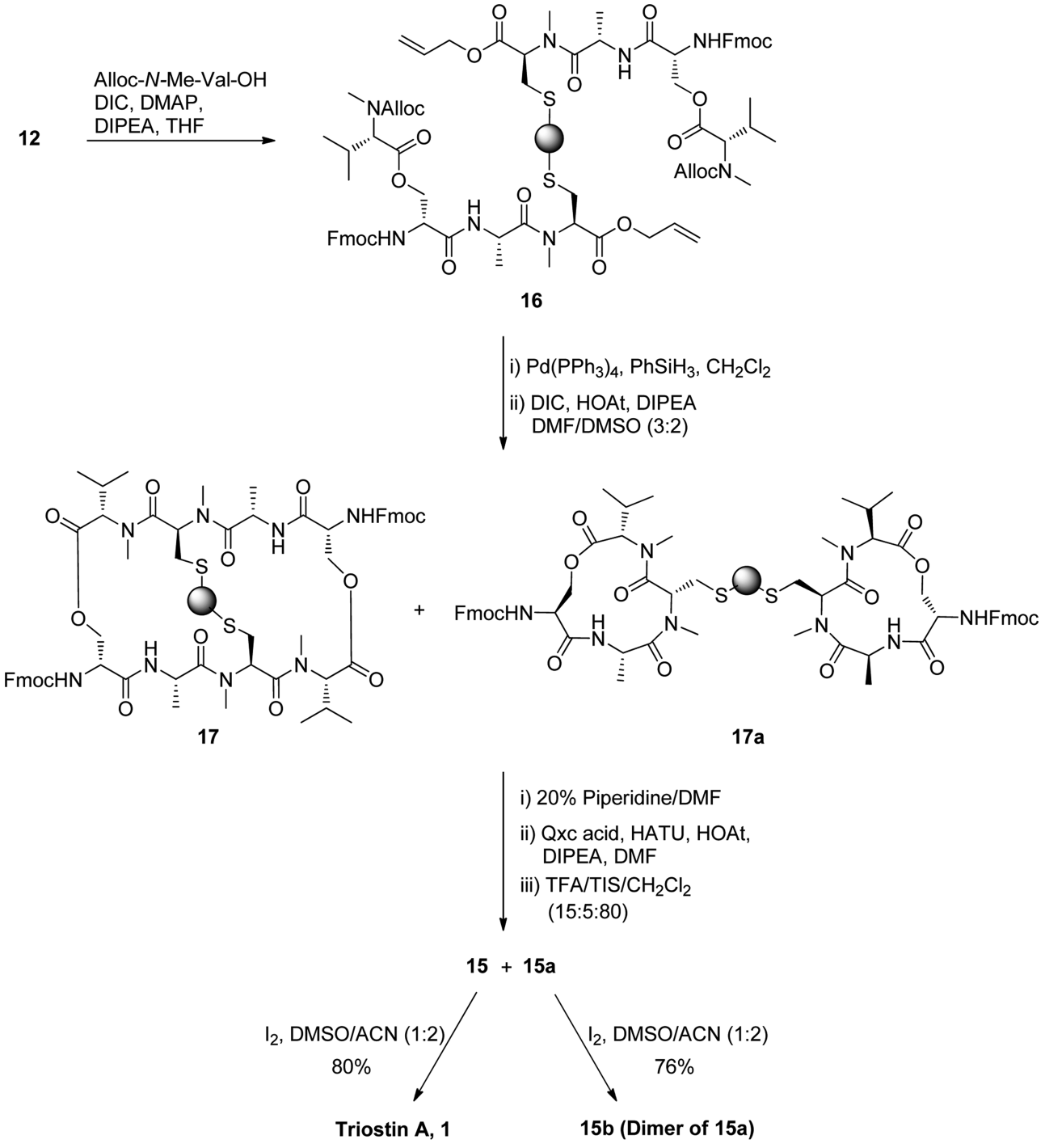
Scheme 4. Solid-Phase Synthesis of 15 (Approach B)



linker was prepared and employed in the synthesis of triostin A. In this synthesis, each coupling, deprotection and cyclization reaction on the resin was performed successfully in a symmetrical manner, which considerably reduced the number of synthetic steps. The biscyclization yields were improved through the exploration of two different approaches. The solid-phase strategy of using a C_2-symmetric linker can be exploited to prepare symmetric bicyclic depsipeptides and their libraries. The syntheses of new analogues are underway in our laboratory and will be presented in future reports.

EXPERIMENTAL SECTION

General Experimental. All reagents were obtained from commercial suppliers and used without further purification, unless specified. The aminomethyl resin (100−200 mesh, 1% DVB) was purchased from Advanced ChemTech. Tetrahydrofuran (THF) and dichloromethane (CH_2Cl_2) solvents were distilled over sodium and benzophenone. ^{1}H and ^{13}C NMR spectra were collected at resonance frequencies of 500.1 and 125.7 MHz, respectively. The solvents used for NMR were DMSO-d_6 or $CDCl_3$ as indicated. The chemical shifts for ^{1}H NMR are reported in ppm from tetramethylsilane (0 ppm) or referenced to the solvent (chloroform 7.26 ppm; DMSO-d_6 2.49 ppm) on the δ scale. Chemical shifts (δ) for ^{13}C NMR spectra are referenced to signals for residual deuterated solvents (chloroform-d 77.16 ppm,

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DMSO-d_6 39.5 ppm). Multiplicities are reported by the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (double doublet), brs (broad singlet), J (coupling constants in hertz). Analytical reverse-phase high performance liquid chromatography (RP-HPLC) was carried out using a C18 (4.6 × 150 mm) reverse-phase column at a flow rate of 1 mL/min with UV detection at 214 and 254 nm. Linear gradients of CH_3CN/H_2O solvents, each containing 0.1% TFA were used as follows: condition A (50 to 100% CH_3CN gradient over 20 min), condition B (30 to 80% CH_3CN gradient over 20 min), condition C (36 to 62% CH_3CN gradient over 25 min) and condition D (40 to 64% CH_3CN gradient over 25 min). For preparative HPLC, a C18 column (5 μm, 10 × 150 mm) was employed at a flow rate of 4 mL/min using the gradient condition C and D. High resolution mass spectra (HRMS) were recorded using two different instruments: (i) fast atom bombardment ionization using a double-focusing magnetic sector mass analyzer (for compounds **1**, **15**, **15a**, NEM adduct of **15a** and **15b**), (ii) electrospray ionization using an ion trap analyzer (for all other compounds). The microwave heated reactions were performed in a CEM Discover reactor. Only the strongest and/or structurally important absorptions of IR spectra were reported in wavenumbers (cm^{-1}). Reactions in solution-phase were monitored by thin-layer chromatography (TLC) performed on glass packed silica gel plates (60F-254) with UV light and visualized with ninhydrin, *p*-anisaldehyde, phosphomolybdic acid or $KMnO_4$ solution stains. Flash column chromatography was performed with silica gel (100−200 mesh) with the indicated solvent system.

Linker Synthesis. *Methyl 3,5-bis(2-hydroxyethoxy)benzoate (**7**).* In a round-bottom flask, a neat mixture of **6** (3.0 g, 17.85 mmol), ethylene carbonate (5.72 g, 178.5 mmol) and K_2CO_3 (6.16 g, 44.64 mmol) were set to treat on Microwave reactor using open vessel method. The mixture was stirred at 80 °C under a microwave (25 W) irradiation for 30 to 40 min (20 min × 1, 10 min × 1 or 2 times) until the disappearance of the starting material was confirmed by TLC. The reaction mixture was diluted with water and extracted with ethyl acetate (3 × 100 mL). The organic layers were combined and washed with brine and then dried over anhydrous $MgSO_4$. The solvent was removed under a vacuum, and the residue was purified by flash column chromatography on silica gel using ethyl acetate/hexanes as the eluent to afford the desired product 7 (3.3 g, 73% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21 (d, J = 2.3 Hz, 2H), 6.69 (t, J = 2.3 Hz, 1H), 4.10 (t, J = 4.2 Hz, 4H), 3.97 (dd, J = 5.1 Hz, 9.2 Hz, 4H), 3.90 (s, 3H), 2.12 (t, J = 6.0 Hz, 2H). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 166.8, 159.8, 132.3, 108.3, 107.1, 69.8, 61.4, 52.4. HRMS m/z calcd for $C_{12}H_{16}O_6Na$ $[M + Na]^+$ 279.0845, found $[M + Na]^+$ 279.0839.

*Methyl 3,5-bis[2-(4-benzoylphenoxy)ethoxy]benzoate (**8**).* To a solution of 7 (3.3 g, 12.89 mmol) in anhydrous CH_2Cl_2 (90 mL) at 0 °C, was added triethylamine (8.77 mL, 64.45 mmol) followed by mesyl chloride (2.99 mL, 38.67 mmol). The reaction temperature was maintained at 0 °C during the first hour, followed by warming to room temperature for another 2 h under N_2 atmosphere with vigorous stirring. At the end of reaction, the mixture was diluted with water and extracted with dichloromethane. The combined organic layers were washed with a 1 N aqueous hydrochloric acid solution, brine, dried over $MgSO_4$ and evaporated to afford the intermediate methyl 3,5-bis(2-((methylsulfonyl)oxy)ethoxy)benzoate as a faint brown solid (5.25 g, 99% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21 (d, J = 2.4 Hz, 2H), 6.68 (t, J = 2.3 Hz, 1H), 4.58 (m, 4H), 4.28 (m, 4H), 3.90 (s, 3H), 3.10 (s, 6H); HRMS m/z calcd for $C_{14}H_{20}O_{10}S_2Na$ $[M + Na]^+$ 435.0396, found $[M + Na]^+$ 435.0391. Next, to a solution of methyl 3,5-bis(2-((methylsulfonyl)oxy)ethoxy)benzoate (5.2 g, 12.62 mmol) in DMF were added K_2CO_3 (6.09 g, 44.17 mmol) followed by 4-hydroxybenzophenone (5.37 g, 27.13 mmol) and stirred at room temperature for 5 min under N_2 atmosphere. Then the reaction mixture was stirred at 80 °C under a microwave (25 W) irradiation for 60 min (30 min × 2 times) until the disappearance of the starting material was confirmed by TLC. The reaction mixture was diluted with water and extracted with ethyl acetate (3 × 100 mL). The organic layers were combined and washed with brine and then dried over anhydrous $MgSO_4$. The solvent was removed under a vacuum, and the residue was purified by flash column chromatography on silica gel using ethyl acetate/hexane as the eluent to afford the desired product **8** (6.1 g, 78% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (dd, J = 1.9, 7.1 Hz, 4H), 7.76 (m, 4H), 7.57 (m, 2H), 7.48 (t, J = 7.8 Hz, 4H), 7.28 (d, J = 2.3 Hz, 2H), 7.02 (m, 4H), 6.78 (t, J = 2.4 Hz, 1H), 4.42 (m, 4H), 4.40 (m, 4H), 3.92 (s, 3H). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 195.6, 166.7, 162.3, 159.7, 138.4, 132.7, 132.4, 132.1, 130.8, 129.9, 128.4, 114.3, 108.5, 107.4, 66.9, 66.7, 52.5. HRMS m/z calcd for $C_{38}H_{32}O_8Na$ $[M + Na]^+$ 639.1995, found $[M + Na]^+$ 639.1990.

*3,5-Bis(2-(4-(hydroxy(phenyl)methyl)phenoxy)ethoxy)benzoic acid (**9**).* The compound **8** (6.0 g, 9.74 mmol) was dissolved in methanol/toluene (2:1) and cooled to 0 °C. Into the reaction mixture $NaBH_4$ (3.11 g, 77.92 mmol) was added in portions and the mixture was left stirred for 3 h at room temperature. The reaction mixture was quenched by aqueous NH_4Cl and organic solvents were removed on rotary evaporator. The crude aqueous mixture was extracted with EtOAc and the combined organic layers were washed with brine, dried over anhydrous $MgSO_4$ and the solvents were removed under a vacuum. The crude product was precipitated using ether/hexane (1:3) to obtain the desired methyl 3,5-bis(2-(4-(hydroxy(phenyl)methyl)-phenoxy)ethoxy)benzoate as a white solid (5.95 g, 99% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38−7.24 (m, 16H, overlapping with solvent residual peak), 6.91 (d, J = 8.6 Hz, 4H), 6.74 (s, 1H), 5.82 (s, 2H), 4.32 (d, J = 4.1 Hz, 8H), 3.90 (s, 3H), 2.16 (bs, 2H). HRMS m/z calcd for $C_{38}H_{36}O_8Na$ $[M + Na]^+$ 643.2308, found $[M + Na]^+$ 643.2302. The obtained methyl 3,5-bis(2-(4-(hydroxy(phenyl)-methyl)phenoxy)ethoxy)benzoate (5.9 g, 9.52 mmol) was dissolved in MeOH/THF (3:1) and 2 N aqueous KOH solution was slowly added to it. After 6 h stirring at room temperature, the reaction mixture was acidified with 2 N HCl solution and volatiles were removed on a rotary evaporator. The crude aqueous mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$ and the solvents were removed under a vacuum to obtain the desired product **9** as a white solid (5.7 g, 99% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38−7.27 (m, 16H, overlapping with solvent residual peak), 6.91 (m, 4H), 6.79 (t, J = 2.3 Hz 1H), 5.82 (s, 2H), 4.34−4.31 (m, 8H). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.8, 159.9, 158.1, 144.1, 136.9, 131.3, 128.6, 128.1, 127.6, 126.6, 114.9, 114.1, 108.9, 108.2, 76.0, 67.1, 67.0. IR (neat) 3388, 2981, 1690, 1595, 1509, 1445, 1244, 1164, 1069, 1008, 698 cm^{-1}; HRMS m/z calcd. for $C_{37}H_{34}O_8Na$ $[M + Na]^+$ 629.2151, found $[M + Na]^+$ 629.2147.

Fmoc-N-Me-Cys(Trt)-OH. The Alloc protecting group of Alloc-*N*-Me-Cys(Trt)-OH (3.6 g, 7.81 mmol) was cleaved by treatment of $Pd(PPh_3)_4$ (0.9 g, 0.781 mmol) and $PhSiH_3$ (5.7 mL, 46.85 mmol) in CH_2Cl_2 (180 mL) for 30 min at room temperature. The reaction mixture was concentrated on a rotary evaporator and the crude product was used for the next step without further purification. *N*-Me-Cys(Trt)-OH (2.94 g, 7.79 mmol) was dissolved in dioxane/H_2O (1:1) (50 mL) and 2% aqueous Na_2CO_3 (50 mL) and the solution was cooled to 4 °C. The solution of Fmoc-OSu (2.9 g, 8.57 mmol) in dioxane (25 mL) was added slowly to the amino acid solution. The mixture was stirred for 2 h at 4 °C and for further 16 h at 25 °C, with the pH maintained between 9 and 10. The dioxane was removed and the aqueous solution was acidified with 1 N HCl to pH 3. The crude product was extracted with EtOAc (3 × 80 mL) and the combined organic layers were washed with saturated aqueous NaCl (100 mL), dried over ($MgSO_4$), filtered, and concentrated under a vacuum. Column chromatography (eluent, 40% ethyl acetate/hexane +1% acetic acid) yielded Fmoc-*N*-Me-Cys(Trt)-OH (4.15 g, 89%) as a white solid. ^{1}H NMR data were identical to the data reported previously.[43]

Fmoc-N-Me-Cys(Trt)-OAllyl. To a solution of Fmoc-*N*-Me-Cys-(Trt)-OH (4.1 g, 6.84 mmol) in ACN (30 mL) at 0 °C was added slowly *N*,*N*-diisopropylethylamine (1.78 mL, 10.25 mmol) followed by allyl bromide (11.83 mL, 136.74 mmol). The mixture was stirred at ambient temperature for 16 h until complete conversion was observed on TLC. The reaction mixture was concentrated to remove ACN and diluted with EtOAc (100 mL). The solution was subsequently washed with water, 2.5% aqueous $NaHCO_3$ and brine. The combined organic

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layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (eluent 20% EtOAc/hexane) to afford Fmoc-*N*-Me-Cys(Trt)-OAllyl (3.45 g, 80%) as a hygroscopic pale yellow solid. ^{1}H NMR (500 MHz, CDCl3) δ 7.77(m, 2H)*, 7.58 (d, J = 7.2 Hz, 2H), 7.45−7.36 (m, 9H), 7.30−7.21 (m, 10H, overlapping with solvent residual peak), 5.79−5.76 (m, 1H), 5.21−5.15 (m, 2H), 4.53−4.35 (m, 4H)*, 4.28 (t, J = 7.2 Hz 0.6H), 4.21−4.17 (m, 1H), 4.02 (bs, 0.4H), 2.86 (dd, J = 5.0, 13.2, Hz, 0.6H), 2.77 and 2.74* (s, 3H), 2.72−2.66 (m, 1H)*, 2.48* (m, 0.4H). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 169.7, 169.4*, 156.5, 156.0*, 144.6, 144.1, 141.5, 131.7, 131.6*, 129.8, 129.7*, 128.1, 127.8*, 127.2*, 126.9, 125.3, 125.2*, 120.2*, 120.1, 118.5*, 118.4, 67.9, 67.3, 65.9, 59.8, 59.3*, 47.3, 32.7, 31.3*, 31.2 (*minor rotamer). HRMS m/z calcd. for $C_{41}H_{37}NO_4SNa$ $[M + Na]^+$ 662.2341, found $[M + Na]^+$ 378.2336.

Fmoc-N-Me-Cys-OAllyl. To a round-bottom flask containing Fmoc-*N*-Me-Cys(Trt)-OH (1.0 g, 1.56 mmol) in CH_2Cl_2 (10 mL) at 0 °C, a solution of TFA and TIS in CH_2Cl_2 (20:5:75, 20 mL) was added slowly. The solution was stirred for 1 h at room temperature. On complete conversion of the starting material, the solvent was evaporated under a reduced pressure. The residue was diluted with water and extracted with EtOAc (3 × 60 mL). The combined organic layers were washed with saturated aqueous NaCl (100 mL), dried over ($MgSO_4$), filtered, and concentrated under a vacuum. The crude product was immediately used for the SPPS without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, J = 7.6 Hz, 2H), 7.62−7.58 (m, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.32 (t, J = 7.2 Hz, 2H), 5.90−5.86 (m, 1H), 5.33−5.23 (m, 2H), 4.73 (q, J = 5.4 Hz, 9.8 Hz, 0.6H), 4.65−4.57* (m, 1.4H), 4.52−4.43 (m, 3H), 4.30 (t, J = 6.8 Hz, 0.6H), 4.24* (t, J = 5.6 Hz, 0.4H), 3.14 (m, 0.6H), 2.94−2.86 (m, 4H, overlapping)*, 2.53 (m, 1H)*, 1.48 and 1.34* (t, J = 10.0 Hz, 1H). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 169.5, 169.2*, 156.9, 156.1*, 144.0, 141.5, 131.6, 131.5*, 127.9, 127.2, 125.2, 124.9, 120.1, 119.1, 119.1*, 68.0, 67.7*, 66.2, 62.4, 62.3*, 47.4, 32.2, 23.9 (*minor rotamer). HRMS m/z calcd. for $C_{22}H_{23}NO_4SNa$ $[M + Na]^+$ 420.1245, found $[M + Na]^+$ 378.1239.

Fmoc-D-Ser-Ala-OH. Dicyclohexylcarbodiimide (0.76 g, 3.66 mmol) was added to a solution of Fmoc-D-Ser-OH (1 g, 3.05 mmol) and *N*-hydroxysuccinimide (0.37 g, 3.21 mmol) in dry THF (20 mL) at 0 °C under N_2 atmosphere. The mixture was stirred at room temperature for 18 h and then cooled to 0 °C. The reaction mixture was concentrated under a vacuum to obtain the crude product which was used for next step without further purification. To a solution of H-Ala-OH (0.3 g, 3.36 mmol) and triethylamine (0.51 mL, 3.67 mmol) in ACN/H_2O (1:1, 16 mL) was added a solution of Fmoc-D-Ser-OSu (1.29 g, 3.05 mmol) in dry THF (8 mL) at 0 °C. The resulting mixture was stirred for 6 h at room temperature. On complete conversion, the solvent was removed under a vacuum and 10% citric acid solution was added to the mixture. The aqueous solution was extracted with EtOAc (3 × 60 mL). The combined organic layers were washed with brine (40 mL), dried over Mg_2SO_4, filtered and concentrated to obtain the crude product which was further purified by precipitation using a mixture of $CHCl_3$/hexane (1:9) to afford the desired product as a white solid (1.2 g, quantitative). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.14 (d, J = 7.3 Hz, 1H), 7.89 (d, J = 7.5 Hz, 2H), 7.74 (t, J = 6.4 Hz, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.33 (t, J = 7.5 Hz, 2H), 7.27 (d, J = 8.5 Hz, 1H), 4.88 (bs, 1H), 4.30−4.11 (m, 5H), 3.58−3.54 (m, 2H, overlapping with residual water), 1.26 (d, J = 7.3 Hz, 3H). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 174.0, 169.9, 155.9, 143.9, 140.8, 127.7, 127.1, 125.4, 125.3, 120.1, 65.8, 62.0, 56.8, 47.6, 46.7, 17.5 (minor aliphatic impurities are observed). HRMS m/z calcd for $C_{21}H_{22}N_2O_6Na$ $[M + Na]^+$ 421.1376, found $[M + Na]^+$ 421.1370.

Solid-Phase Peptide Synthesis. Solid-phase peptide synthesis was conducted in sintered glass vials using EYELA personal solid-phase synthesizer (CCS-600). Solvents and soluble reagents were removed by suction. Removal of the Fmoc group was carried out with 20% piperidine/DMF (10 mL/g, 2 × 8 min and 2 × 5 min). Washings between deprotection, couplings steps were carried out with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min) using 8 mL solvent/g of resin for each wash. Reaction completion was determined by qualitative Kaiser or chloranil tests and analyzed by RP-HPLC after cleavage of small sample of the peptidyl resin (∼1 mg) using TFA/TIS/CH_2Cl_2 (10:4:86) cocktail. The peptide synthesis transformations and the resin washings were performed at 25 °C.

*Preparation of the Linker Resin (**10**).* The amino methyl resin (1.0 g, 0.9 mmol/g) was swelled in DMF (10 mL) for 15 min. The linker (**9**) (654 mg, 1.08 mmol) was activated using PyBOP (702 mg, 1.35 mmol) and DIPEA (469 μL, 2.70 mmol) in DMF (10 mL) and added to the swelled resin. The progress of the reaction was monitored by Kaiser test. After 5 h, the solution was removed from the reaction vessel by filtration and the resin was washed with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min). The linker attachment was confirmed by IR analysis and the loading was determined to be 90% (0.81 mmol/g) on a w/w basis. IR (neat) 3657, 3339, 3025, 2980, 2924, 1663, 1593, 1509, 1492, 1451, 1380, 1246, 1165, 1071, 951, 757 cm^{-1}.

*Loading of Fmoc-N-Me-Cys-OAllyl on the Linker Resin to Produce **11**.* To the preswelled linker resin **10** (1.0 g, 0.81 mmol/g) in CH_2Cl_2 (10 mL) was added trichloroacetonitrile (2.45 mL, 24.3 mmol) and solution of DBU (72.4 μL, 0.49 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was shaken for 4 h at room temperature, filtered and washed with CH_2Cl_2 (2 × 1 min), DMF (2 × 0.5 min) and CH_2Cl_2 (3 × 1 min) sequentially. To the pre swelled trichloroacetimidate resin, Fmoc-*N*-Me-Cys-OAllyl (1.29 g, 3.24 mmol) in dry CH_2Cl_2 (8 mL) was added, followed by addition of TMSOTf (29.3 μL, 0.16 mmol) in dry CH_2Cl_2 (2 mL). The mixture was shaken for 12 h, filtered and the resin was washed with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min). The attachment of Fmoc-*N*-Me-Cys-OAllyl was confirmed by IR analysis and loading was determined to be 82% on w/w basis (1.33 mmol/g based on the loaded Cys). IR (neat) 3402, 3059, 3025, 2923, 1721, 1599, 1507, 1493, 1450, 1247, 1168, 1067, 1029, 758, 735 cm^{-1}.

Solid-Phase Synthesis of Dithiol Triostin A 15 (Approach-A). The Fmoc group of the resin **11** (0.33 mmol based on the loaded Cys) was removed by 20% piperidine/DMF and the resin was washed with DMF (4 × 1 min), CH_2Cl_2 (4 × 1 min). Fmoc-D-Ser-Ala (263 mg, 0.66 mmol) was coupled using HATU (251 mg, 0.66 mmol), HOAt (89.8 mg, 0.66 mmol) and DIPEA (229 μL, 1.32 mmol) in DMF (3 mL) and the mixture was stirred for 1 h until negative Kaiser test was observed. The mixture was filtered and the resin was washed with DMF (5 × 1 min), CH_2Cl_2 (5 × 1 min) to obtain resin-bound peptide **12**. A small portion of the resin was cleaved and the crude product was analyzed by RP-HPLC (t_R = 8.1 min, condition A). Next, the Fmoc group was removed and the resin was washed with DMF (4 × 1 min) and CH_2Cl_2 (4 × 1 min). The solution of 2-Quinoxaline carboxylic acid (115 mg, 0.66 mmol), HATU (251 mg, 0.66 mmol), HOAt (89.8 mg, 0.66 mmol) and DIPEA (229 μL, 1.32 mmol) in DMF (3 mL) was added to the resin and shaken for 50 min. Then the mixture was filtered and the resin was washed with DMF (5 × 1 min), CH_2Cl_2 (5 × 1 min) to obtain resin-bound peptide **13** (t_R = 3.0 min, condition A) along with **13a** (t_R = 6.5 min, condition A). Undesired resin-bound peptide **13a** was converted back to **13** by an alcoholysis reaction using K_2CO_3 (45.5 mg, 0.33 mmol) and allyl alcohol (2.5 mL) with 16 h shaking. Introduction of Alloc-*N*-Me-Val to the side chain of the Ser residue was achieved by mixing the resin with the anhydride solution, which was prepared in a separate flask by combining Alloc-*N*-Me-Val (426 mg, 1.98 mmol), DIC (137 μL, 0.99 mmol), DMAP (3.66 mg, 0.03 mmol) and DIPEA (115 μL, 0.66 mmol) in THF (3 mL) at 0 °C for 20 min. The mixture was shaken for 12 h, filtered and the resin was washed with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min) to obtain resin-bound peptide **14** (t_R = 18.8 min, condition B).

The Alloc and allyl protecting groups were removed by shaking the resin with $Pd(PPh_3)_4$ (57.2 mg, 0.05 mmol) and $PhSiH_3$ (406 μL, 3.30 mmol) in dry CH_2Cl_2 (3 mL) under nitrogen atmosphere for 30 min. The process was repeated twice. For the final cyclization, DIC (229 μL, 1.65 mmol) was added to the resin solution in DMF/DMSO (3:2, 1.5 mL), followed by a solution of HOAt (135 mg, 0.99 mmol) and DIPEA (57.4 μL, 0.33 mmol) in DMF/DMSO (3:2, 1 mL). The mixture was shaken for 12 h, filtered and the resin was washed with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min) to obtain resin-bound peptide **15** and **15a**. The peptides were cleaved from the resin by

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treatment of TFA/TIS/CH_2Cl_2 (10:4:86) (8 mL, 3 × 20 min) and the combined solution was evaporated under a vacuum. The crude sample was purified by semi preparative HPLC (condition C) to provide the desired dithiol triostin A (**15**) (7.1 mg; 4.0% yield from the Cys loaded resin) and the monocyclic tetradepsipeptide (**15a**) (21 mg; 12% yield from the Cys loaded resin).

15: (t_R= 12.5 min, condition B); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.75 (s, 1H, CH-Ar), 8.64 (d, 1H, J = 8.1 Hz, *N*H-Ser), 8.22 (d, 1H, J = 7.9 Hz, CH-Ar), 8.16 (d, 1H, J = 7.7 Hz, CH-Ar), 7.89 (m, 2H, CH-Ar), 6.84 (d, 1H, J = 9.6 Hz, *N*H-Ala), 5.53 (t, 1H, J = 7.2 Hz, CH$^{\alpha}$-Cys), 5.31 (m, 1H, CH$^{\alpha}$-Ala), 5.08 (dd, 1H, J = 10.2 Hz, 16.8 Hz, CH$^{\alpha}$-Ser), 4.60 (dd, 1H, 1H, J = 6.7 Hz, 9.7 Hz, CH$^{\beta}$-Ser), 4.23 (t, 1H, J = 10.7 Hz, CH$^{\beta}$-Ser), 4.12 (d, 1H, J = 9.8 Hz, CH$^{\alpha}$-Val), 3.19 (m, 1H, CH$^{\beta}$-Cys), 3.04 (s, 3H, *N*-Me-Cys), 2.94 (s, 3H, *N*-Me-Val), 2.63−2.58 (m, 1H, CH$^{\beta}$-Cys), 2.28−2.07 (m, 1H, CH$^{\beta}$-Val), 1.56−1.53 (m, 1H, SH-Cys), 1.43 (d, 3H, J = 6.5 Hz, CH_3-Ala), 1.02 (d, 3H, J = 6.3 Hz, CH_3-Val), 0.97 (d, 3H, J = 6.5 Hz, CH_3-Val). ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.5, 169.0, 168.9, 168.8, 163.4, 144.2, 143.9, 142.2, 140.4, 132.2, 131.2, 129.9, 129.5, 64.2, 63.7, 55.8, 50.9, 50.8, 50.8, 43.9, 30.8, 30.2, 28.5, 23.7, 19.7, 19.1 17.7. HRMS m/z calcd. for $C_{50}H_{64}N_{12}O_{12}S_2Na$, 1111.4106 $[M + Na]^+$, found 1111.4104 $[M + Na]^+$. The compound **15** was also analyzed by 2D NMR spectroscopy (COSY, HSQC, HMBC) for more structural confirmations (Supporting Information).

15a: (t_R= 11.6 min, condition B); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.64 (s, 1H, CH-Ar), 8.71 (d, 1H, J = 6.6 Hz, *N*H-Ser), 8.25 (d, 1H, J = 8.1 Hz, CH-Ar), 8.05 (d, 1H, J = 8.1 Hz, CH-Ar), 7.96−7.89 (m, 2H, CH-Ar), 6.80 (d, 1H, J = 9.8 Hz, *N*H-Ala), 5.70 (dd, 1H, J = 6.0 Hz, 8.8 Hz, CH$^{\alpha}$-Cys), 5.30 (q, 1H, J = 6.7 Hz, 9.5 Hz, CH$^{\alpha}$-Ala), 5.06 (d, 1H, J = 10.5 Hz CH$^{\alpha}$-Val), 4.96 (m, 1H, CH$^{\beta}$-Ser), 4.89 (m, 1H, CH$^{\alpha}$-Ser), 4.62 (dd, 1H, J = 1.9 Hz, 11.4 Hz, CH$^{\beta}$-Ser), 3.20−3.17 (m, 1H, CH$^{\beta}$-Cys), 3.05 (s, 3H, *N*-Me-Cys), 2.99 (s, 3H, *N*-Me-Val), 2.61 (m, 1H, CH$^{\beta}$-Cys), 2.37−2.35 (m, 1H, CH$^{\beta}$-Val), 1.52 (t, 1H, J = 9.3 Hz, SH-Cys), 1.34 (d, 3H, J = 6.6 Hz, CH_3-Ala), 1.14 (d, 3H, J = 6.2 Hz, CH_3-Val), 0.95 (d, 3H, J = 6.7 Hz, CH_3-Val). ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.9, 171.3, 169.3, 167.1, 164.4, 144.4, 143.6, 142.1, 140.3, 132.5, 131.5, 129.8, 65.7, 62.3, 55.3, 54.7, 45.3, 31.3, 30.9, 26.7, 22.5, 20.2, 18.7, 16.4. HRMS m/z calcd. for $C_{25}H_{33}N_6O_6S$, 545.2182 $[M + H]^+$, found 545.2182 $[M + H]^+$.

Solid-Phase Synthesis of Dithiol Triostin A 15 (Approach-B). Introduction of Alloc-*N*-Me-Val to the side chain of the Ser residue was achieved by mixing the peptidyl resin **12** (0.13 mmol based on the loaded Cys) with the anhydride solution, which was prepared in a separate flask by combining Alloc-*N*-Me-Val (168 mg, 0.78 mmol), DIC (54.1 μL, 0.39 mmol), DMAP (1.58 mg, 0.013 mmol) and DIPEA (22.6 μL, 0.13 mmol) in THF (1.5 mL) at 0 °C for 20 min. The mixture was shaken for 12 h, filtered and the resin was washed with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min) to obtain resin-bound peptide **16** (t_R = 16.2 min, condition A). The Alloc and allyl protecting groups were removed by shaking the resin with $Pd(PPh_3)_4$ (22.5 mg, 0.020 mmol) and $PhSiH_3$ (160 μL, 1.30 mmol) in dry CH_2Cl_2 (1.5 mL) under nitrogen atmosphere for 30 min. The process was repeated twice and the resin was washed with DMF (5 × 1 min), CH_2Cl_2 (5 × 1 min). For the cyclization step, DIC (90.1 μL, 0.65 mmol) was added to the resin solution in DMF/DMSO (3:2, 0.6 mL), followed by a solution of HOAt (53.0 mg, 0.39 mmol) and DIPEA (230 μL, 0.13 mmol) in DMF/DMSO (3:2, 0.4 mL). The mixture was shaken for 12 h, filtered and the resin was washed with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min) to obtain the resin-bound cyclic peptide **17** (t_R = 17.9 min, condition A) and **17a** (t_R = 18.6 min, condition A). For the final Qxc acid coupling, the Fmoc group of cyclized peptide was removed and the resin was washed with DMF (4 × 1 min) and CH_2Cl_2 (4 × 1 min). The solution of 2-Quinoxaline carboxylic acid (45.3 mg, 0.26 mmol), HATU (98.8 mg, 0.26 mmol), HOAt (35.4 mg, 0.26 mmol) and DIPEA (67.8 μL, 0.39 mmol) in DMF (1.5 mL) was added to the resin and shaken for 1 h. Then the mixture was filtered and the resin was washed with DMF (5 × 1 min), CH_2Cl_2 (5 × 1 min) to obtain resin-bound peptide **15** and **15a**.

Finally, the peptides were cleaved from the resin by treatment of TFA/TIS/CH_2Cl_2 (10:4:86) (4 mL, 3 × 20 min) and the combined solution was evaporated under a vacuum. The crude sample was purified by semi preparative HPLC (condition C) to provide the desired dithiol triostin A (**15**) (6.5 mg; 9.1% yield from the Cys loaded resin) and the monocyclic tetradepsipeptide (**15a**) (6.9 mg, 9.6% yield from the Cys loaded resin).

*Synthesis of Triostin A (**1**).* To a solution of the dithiol triostin A (**15**) (8.0 mg, 7.35 μmol) in dry ACN/DMSO (9:1, 1.6 mL) at 0 °C; a solution of I_2 (1.87 mg, 14.70 μmol) in dry ACN (0.8 mL) was added dropwise over 30 min. The reaction mixture was stirred for 30 min at ambient temperature to achieve complete conversion of the starting material into the desired disulfide product, monitored by RP-HPLC. The reaction mixture was lyophilized to remove organic solvents and the crude residue was purified by semi prep HPLC (condition D) to provide the desired triostin A (**1**) (6.3 mg, 80% yield). (RP-HPLC t_R= 17.2 min, condition B). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.72 (s, 1H, CH-Ar), 8.63 (d, 1H, J = 8.4 Hz, *N*H-Ser), 8.21 (m, 1H, CH-Ar), 8.16 (m, 1H, CH-Ar), 7.92−7.86 (m, 2H, CH-Ar), 6.74 (bs, 1H, *N*H-Ala), 5.79 (t, 1H, J = 7.3 Hz, CH$^{\alpha}$-Cys), 5.29 (m, 1H, CH$^{\alpha}$-Ala), 5.06 (m, 1H, CH$^{\alpha}$-Ser), 4.59 (m, 1H, CH$^{\beta}$-Ser), 4.23 (t, 1H, J = 10.6 Hz, CH$^{\beta}$-Ser), 4.18 (d, 1H, J = 9.8 Hz, CH$^{\alpha}$-Val), 3.28 (dd, 1H, J = 7.4 Hz, J = 13.8 Hz, CH$^{\beta}$-Cys), 3.05 (s, 3H, *N*-Me-Cys), 2.94 (s, 3H, *N*-Me-Val), 2.97−2.90 (m, 1H, CH$^{\beta}$-Cys, overlap with *N*-Me-Val), 2.30−2.26 (m, 1H, CH$^{\beta}$-Val), 1.41 (d, 3H, J = 6.6 Hz, CH_3-Ala), 1.03 (d, 3H, J = 6.3 Hz, CH_3-Val), 0.96 (d, 3H, J = 6.6 Hz, CH_3-Val). HRMS m/z for $C_{50}H_{62}N_{12}O_{12}S_2Na$ $[M + Na]^+$ calcd 1109.3949, found 1109.3947.

*Synthesis of Monocyclic Tetradepsipepide Dimer (**15b**).* Pure monocyclic tetradepsipeptide free thiol (**15a**) (16 mg, 0.029 mmol) was oxidized by following the same procedure explained above to obtain monocyclic tetradepsipeptide dimer (**15b**). The reaction mixture was lyophilized to remove organic solvent and obtained crude residue was redissolved in minimum amount of ACN/DMSO (3:1) to purify by semi prep HPLC (condition D). Purification provided monocyclic tetradepsipeptide dimer (**15b**) (12.1 mg) in 76% yield (RP-HPLC t_R= 16.3 min, condition B). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.65 (s, 1H, CH-Ar), 8.66 (d, 1H, J = 7.0 Hz, *N*H-Ser), 8.25 (m, 1H, CH-Ar), 8.04 (m, CH-Ar), 7.97−7.92 (m, 2H, CH-Ar), 6.38 (d, 1H, 10 Hz, NH-Ala), 6.07 (dd, 1H, J = 6 Hz, 9 Hz CH$^{\alpha}$-Cys), 5.22 (m, 1H, CH$^{\alpha}$-Ala), 5.04 (d, 1H, 10.5 Hz, CH$^{\alpha}$-Ser), 4.94−4.89 (m, 2H, CH_2^{β}-Ser, overlap), 4.60 (dd, 1H, J = 2.3, 11.2 Hz, CH$^{\alpha}$-Val), 3.27 (dd, 1H, J = 9 Hz, 13.3 Hz, CH$^{\beta}$-Cys), 3.06 (s, 3H, *N*-Me-Cys), 2.03 (s, 3H, *N*-Me-Val), 2.95 (dd, 1H, J = 6 Hz, 13.3 Hz, CH$^{\beta}$-Cys), 2.34−2.32 (m, 1H, CH$^{\beta}$-Val), 1.32 (d, 3H, J = 6.7 Hz, CH_3-Ala), 1.11 (d, 3H, J = 6.4 Hz, CH_3-Val), 0.93 (d, 3H, J = 6.7 Hz, CH_3-Val). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.5, 171.2, 169.1, 167.4, 164.4, 144.0, 143.3, 142.4, 140.5, 132.7, 131.7, 129.8, 129.4, 65.74, 62.42, 54.84, 52.35, 45.23, 36.09, 31.46, 31.20, 26.74, 20.17, 18.72, 16.24. HRMS m/z for $C_{50}H_{62}N_{12}O_{12}S_2Na$ $[M + Na]^+$ calcd 1109.3949, found 1109.3949.

*Preparation of NEM Adduct of **15a**.* To a pure sample of monocyclic tetradepsipeptide (**15a**) (0.4 mg, 0.74 μmol) in ACN (100 μL) was added *N*-ethylmaleimide (10 mM in DMF, 100 μL) and the mixture was stirred for 4 h at room temperature. The reaction was monitored by RP-HPLC. Starting was completely disappeared and new peaks were purified by analytical RP-HPLC. Desired NEM adduct of **15a** (t_R= 12.1 min, condition B) was confirmed by mass analysis (Supporting Information, Page no. S7). HRMS m/z for $C_{31}H_{40}N_7O_8S$ $[M + H]^+$ calcd 670.2659, found 672.2659.

■ ASSOCIATED CONTENT

Supporting Information

Supporting Figures S1−S32 including copies of ^{1}H, ^{13}C and 2D NMR, HPLC and, HRMS spectra for all required compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b01055.

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Notes

The authors declare no competing financial interest.

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