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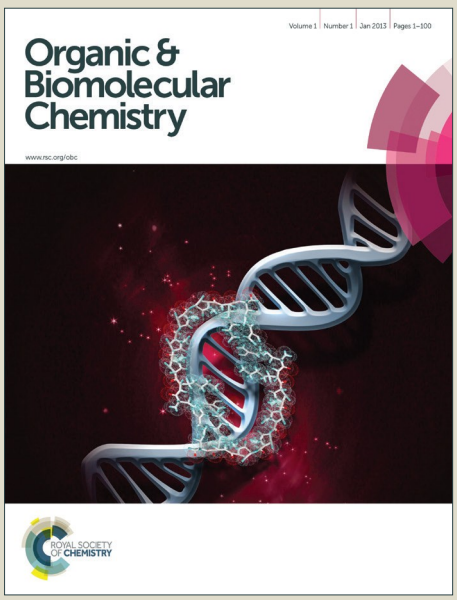

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Solution-Phase Synthesis and Biological Evaluation of Triostin A and its Analogues

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The authors declare no competing financial interest

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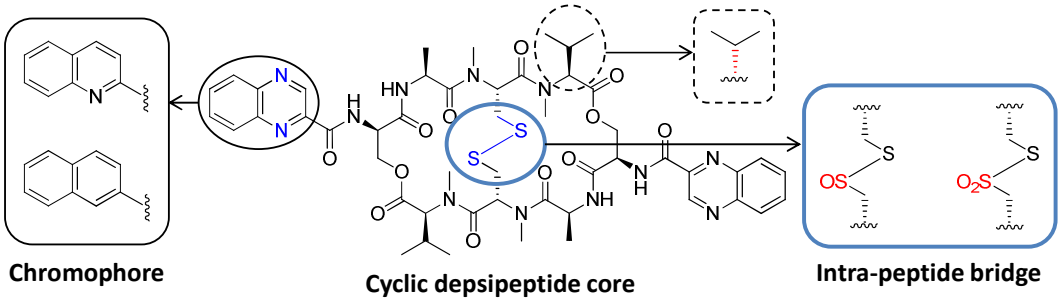


Triostin A: $IC_{50\ (HIF\text{-}1\ inhibition)}$; 26.9 nM, $IC_{50\ (cytotoxicity)}$; 4.1 μM

We have accomplished preparative solution-phase total syntheses of triostin A (17.5% for 13 steps) and its analogues with high yields to demonstrate their significant inhibitory activities on HIF-1 activation and cell proliferation.

Abstract

Triostin A is a biosynthetic precursor of echinomycin which is a one of the most potent hypoxia inducible factor 1 (HIF-1) inhibitors. An improved solution-phase synthesis of triostin A on a preparative scale has been achieved in 17.5% total yield for 13 steps. New analogues of triostin A with various aromatic chromophores, oxidized intra-peptide disulfide bridges and diastereoisomeric cyclic depsipeptide cores were also successfully synthesized. All analogues had a significant inhibitory effect on HIF-1 transcriptional activation in hypoxia and cytotoxicity on MCF-7 cells, with the exception of the derivatives containing a naphthalene chromophore or a thiosulfonate bridge. For the first time, triostin A, echinomycin and the thiosulfinate analogue of triostin A have been revealed to inhibit not only DNA binding of HIF-1 but also HIF-1α protein accumulation in MCF-7 cells. Furthermore, the thiosulfinate analogue and triostin A exhibited a hypoxia-selective cytotoxicity on MCF-7. The improved solution-phase synthetic procedure described herein will contribute to the development of diverse bicyclic depsipeptide drug candidates with the potential to act as novel anti-cancer agents targeting hypoxic tumor microenvironments.

Keywords:
Triostin A
Cyclic peptides
Natural products
Antitumor reagents
HIF-1 inhibitors

Introduction

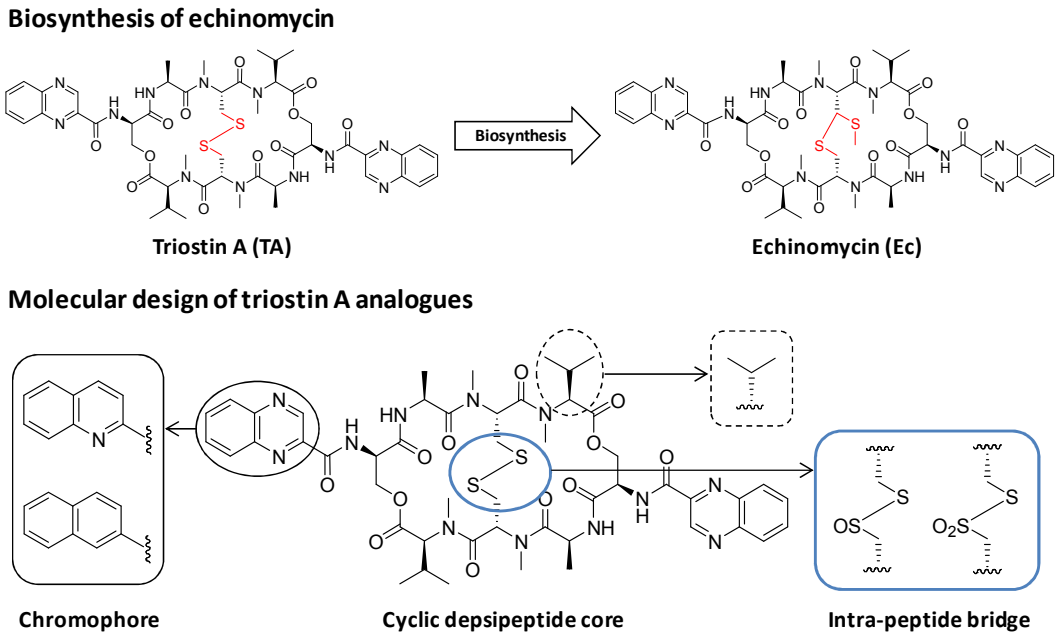


Figure 1. Chemical structures of triostin A and echinomycin and its analogues.

Triostin A (TA) is a member of the quinoxaline family of antibiotics, which is isolated from *Streptomyces aureus*.[1, 2] TA has been identified as a precursor of echinomycin during the biosynthesis.[3-5] Quinoxaline antibiotics are characterized by a pair of quinoxaline chromophores which are attached to a C_2-symmetric bicyclic depsipeptide (Figure 1).[5] They exhibit significant antibacterial and antitumor activities due to their bis-intercalation of the two quinoxaline rings into DNA.[6-8] Echinomycin (Ec) was recently found to show potent inhibition of the DNA-binding activity of hypoxia-inducible factor-1 (HIF-1); specifically, to a hypoxia response element (HRE) sequence.[7, 9] Since HIF-1α was recognized as a master regulator of the cellular response to hypoxia, it has emerged as an attractive molecular target for cancer therapy.[10, 11] Although Ec failed in clinical trials for cancer treatment,[12, 13] it has still been investigated to explore its potential therapeutic applications in the modulation of the HIF-1 pathway.[14, 15]

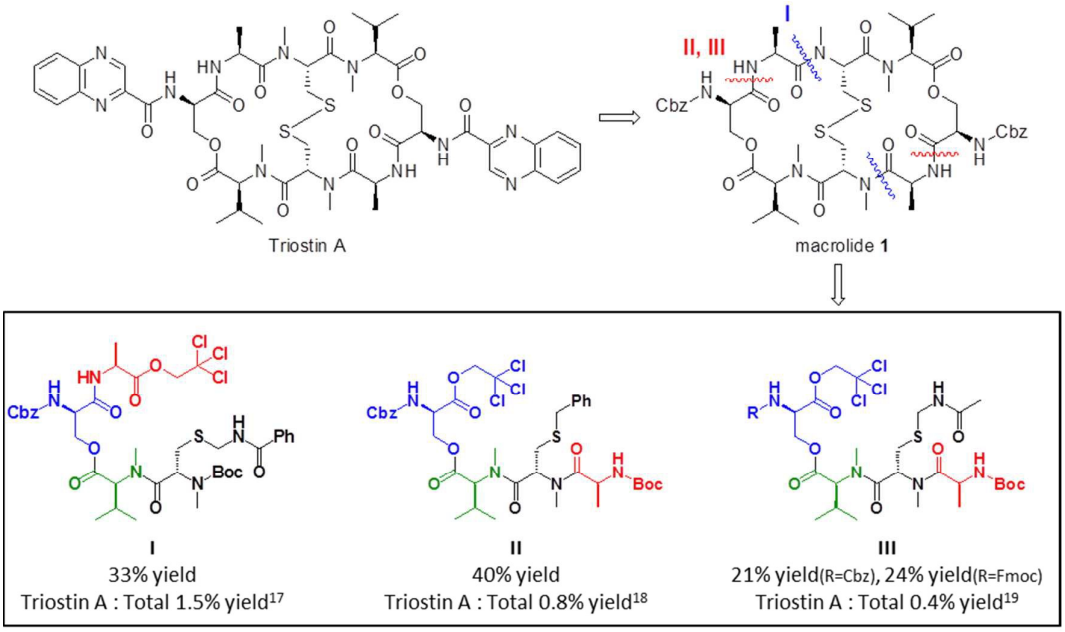


Figure 2. Tetradepsipeptides as key intermediates in previous methods of the total synthesis of triostin A.

TA and Ec have a similar cyclic octadepsipeptide scaffold that contains either an intra-peptide disulfide bridge or a thioacetal bridge between two cysteine residues, respectively. The effect on HIF-1 pathway of TA has not been examined so far. The total biosynthesis of Ec has been reported using an engineered *Escherichia coli* strain; TA acts as a precursor to Ec in the biosynthetic pathway.[3, 16] However, a total chemical synthesis has yet to be reported.

There have been few reports detailing the total chemical synthesis of TA by solution-phase procedures, and total yields were generally less than 1.5% (Figure 2).[17-19] Furthermore, various analogues of the quinoxaline antibiotics have been prepared, varying the aromatic rings or the cyclic depsipeptide backbone.[19-27] For many years, we have been interested in developing drugs that target tumor microenvironments such as hypoxia.[11, 28, 29] Our recent finding that Ec extremely reduced protein expression of HIF-1α as will be shown later encouraged us to investigate the synthesis of TA and its new analogues, and to evaluate their efficacy in HIF-1 inhibition; we predict that the cyclic octadepsipeptide scaffold may be functional to recognize not only DNA but also some upstream signal molecules for HIF-1 expression.

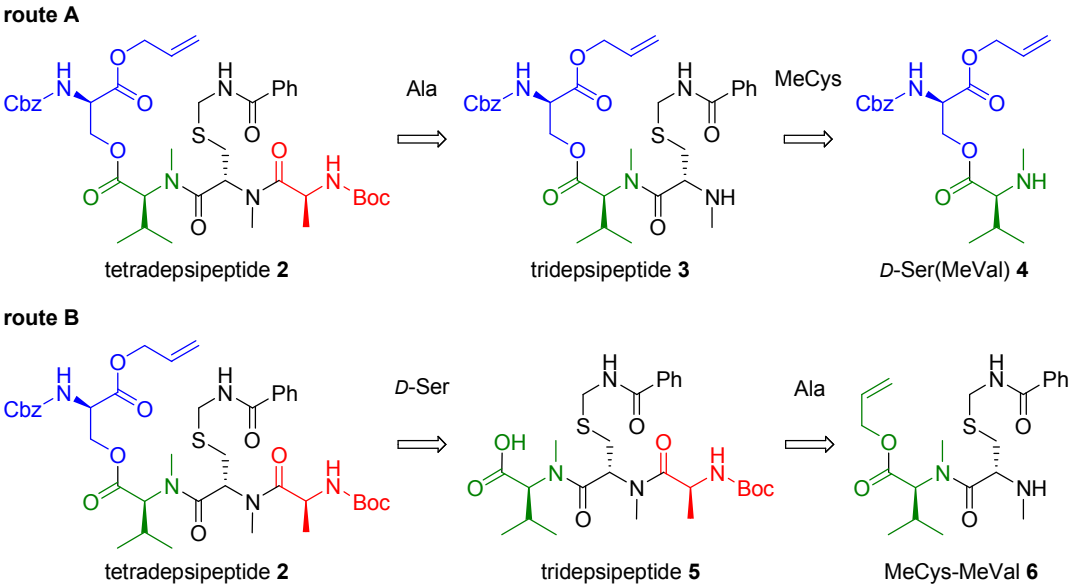
Accordingly, we develop here a preparative solution-phase synthetic method for construction of the cyclic octadepsipeptide found in both TA and Ec. This method allows practical diversity-oriented synthesis and biological evaluation of a suite of compounds

that may influence the HIF-1 signaling pathway in cancer cells. To utilize the solution-phase procedure, most steps were conducted on a preparative scale (several hundred milligrams to grams). From the crystal structures of Ec and TA, an intra-peptide bridge seems to be influential in restricting the conformation of the cyclic depsipeptide scaffold and controlling the distance between the two quinoxaline moieties, which may alter the cytotoxic activity (Figure 1).[3, 30, 31]

A sulfoxide analogue of the thioacetal bridge of Ec, which is derived from the culture broth of a marine streptomycete showed considerably lower cytotoxic activity.[32] Therefore, the oxidative modification of the disulfide bridge of TA was also examined to evaluate the structure-activity relationship on the basis of the configuration of cyclic depsipeptide core and the variety of aromatic rings (Figure 1).

Results and discussion

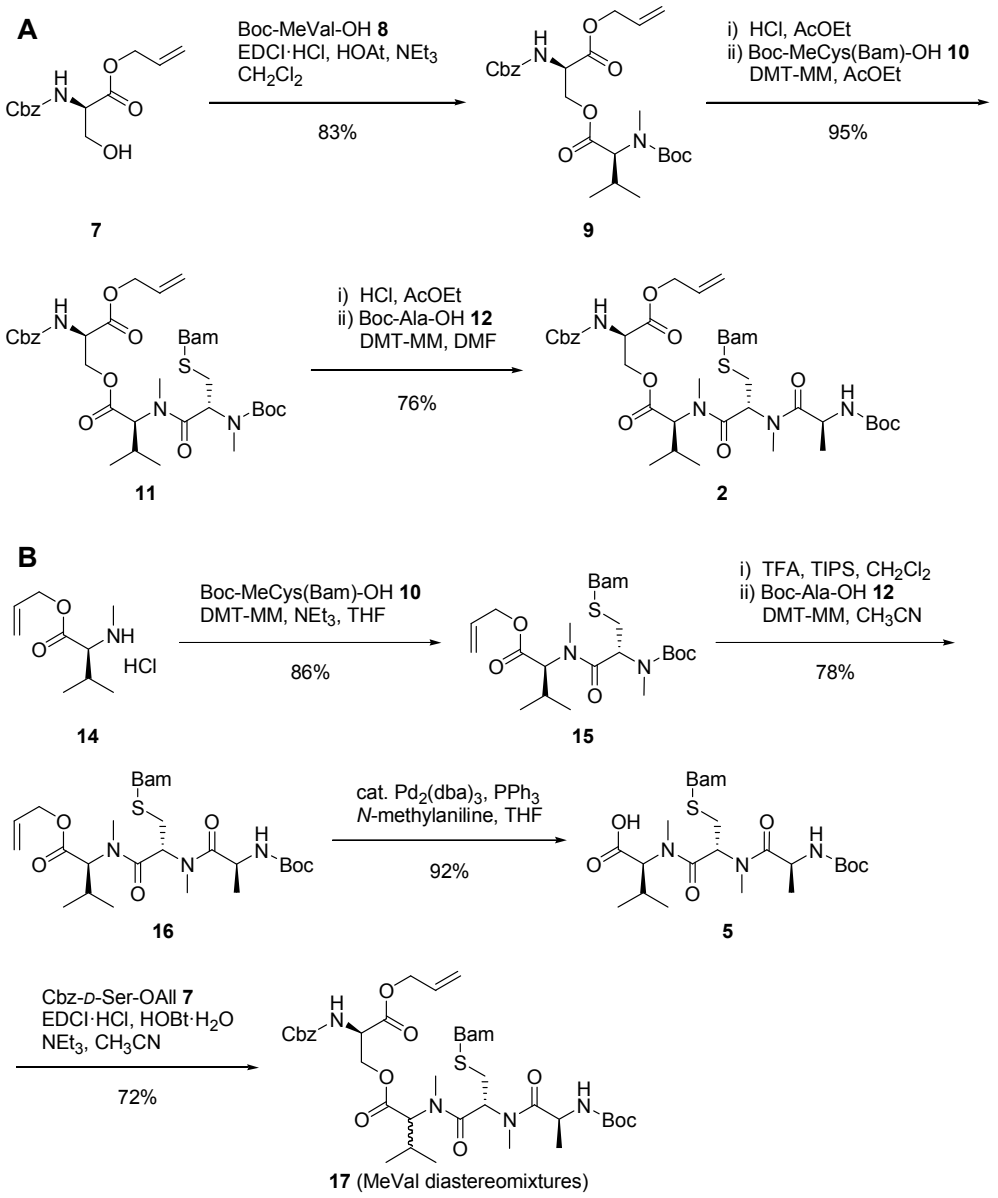
Chemistry



Scheme 1. The retrosynthetic strategy of tetradepsipeptide **2**.

All previous solution-phase procedures for the total synthesis of TA involved fragment coupling of tetradepsipeptide half-segments to yield macrolide **1** as a key intermediate shown in Figure 2.[17-19] During the fragment coupling of the tetradepsipeptide in the synthesis of des-*N*-tetramethyl analogue of triostin A reported by Chakravarty and Olsen, measurable racemization was observed at the Ala residue.[33] Although they did

not mention about racemization in their synthesis of triostin A using the tetradepsipeptide fragment I of similar sequence,[17] Shin *et al.* proposed tetradepsipeptide II to exclude the risk of racemization by the use of I (Fig. 2).[18] According to those studies, we chose tetradepsipeptide **2** as a key intermediate to prevent racemization. Initially, the appropriate protected amino acids and *N*-methyl amino acid derivatives **7**, **8**, **10** and **12** were synthesized. Acetamidomethyl (Acm)[19, 23, 34] and benzamidomethyl (Bam)[17, 35] protection of the cysteine thiol was generally suited to the disulfide bridge formation, as deprotection and oxidation can be accomplished in a single step. A comparative synthesis of protected *N*-methyl cysteine using either Acm or Bam revealed the latter to be more effective and reproducible in yielding the desired derivative. Although the 2,2,2-trichloroethyl (Tce) group is commonly used for protection of C-terminal carboxylic acids, the isolated yields for the protection and deprotection processes were not satisfactory in our experiment. On the basis of these considerations, we designed the tetradepsipeptide intermediate **2** with an allyl protecting group for the C-terminal carboxylic acid, a *t*-butoxycarbonyl (Boc) protecting group for the N-terminal amine, and a Bam protecting group for thiol protection, as shown in Scheme 1. Next, we tried to synthesize the tetradepsipeptide **2** using two different routes: 1) route A, wherein the amino acids were added sequentially from the C-terminal to the N-terminal and 2) route B, which involved conjugation of *D*-Ser to tridepsipeptide **5** in the last step (Scheme 1).

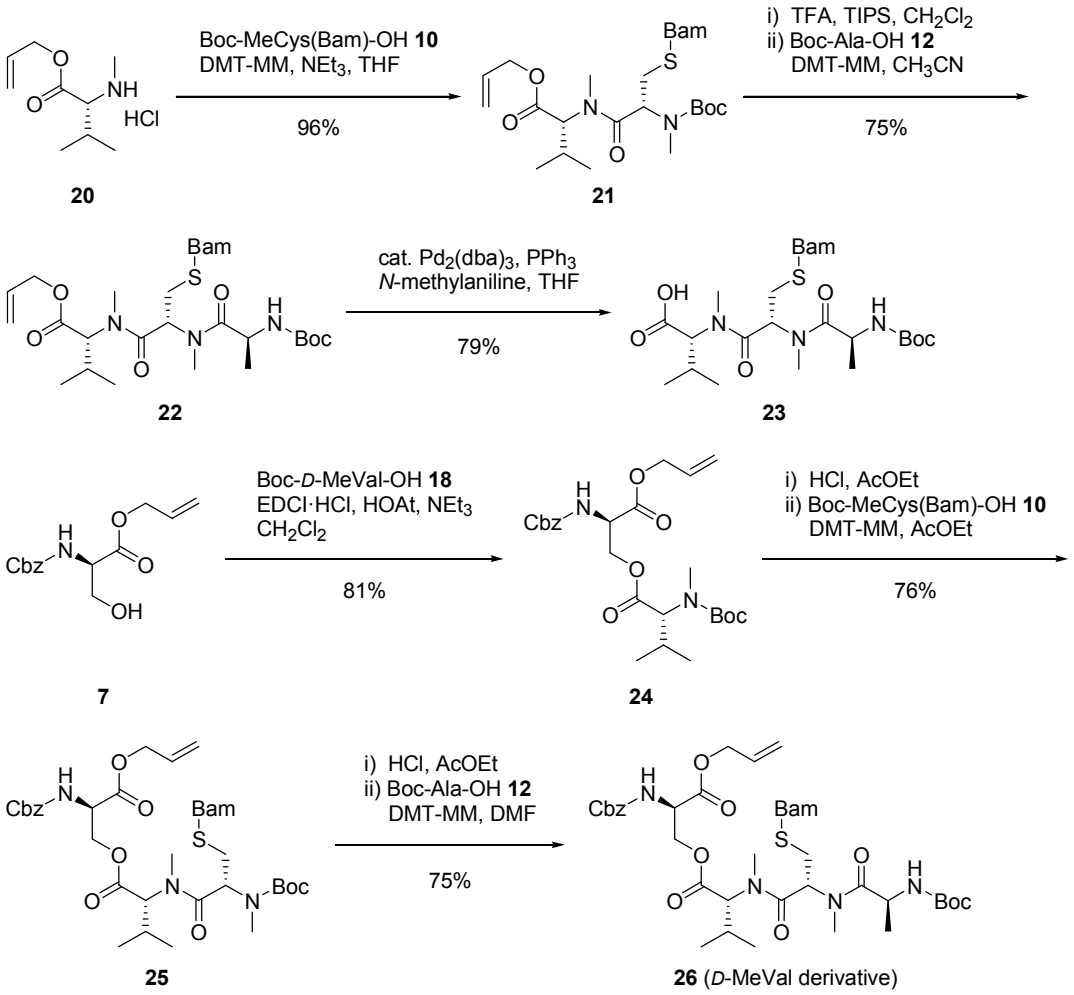


Scheme 2. Synthesis of tetradepsipeptide **2** *via* route A (A) and route B (B).

In route A (Scheme 2A), coupling of Cbz-*D*-Ser-OAll (**7**) and Boc-MeVal-OH (**8**)[36] with EDCI/HOAt in dichloromethane afforded Cbz-*D*-Ser(Boc-MeVal)-OAll (**9**) in 83% yield. After removal of the Boc group of ester **9** with HCl/AcOEt, the resultant amine derivative was conjugated with Boc-MeCys(Bam)-OH (**10**)[35] using DMT-MM[37] to obtain tridepsipeptide **11** in 95% yield. Finally, deprotection of **11** with HCl/AcOEt, followed by coupling with Boc-Ala-OH (**12**) in a similar manner, gave tetradepsipeptide **2** in 76% yield. The conditions of each process were optimized, as shown in the supplementary

material (Tables S1-3).

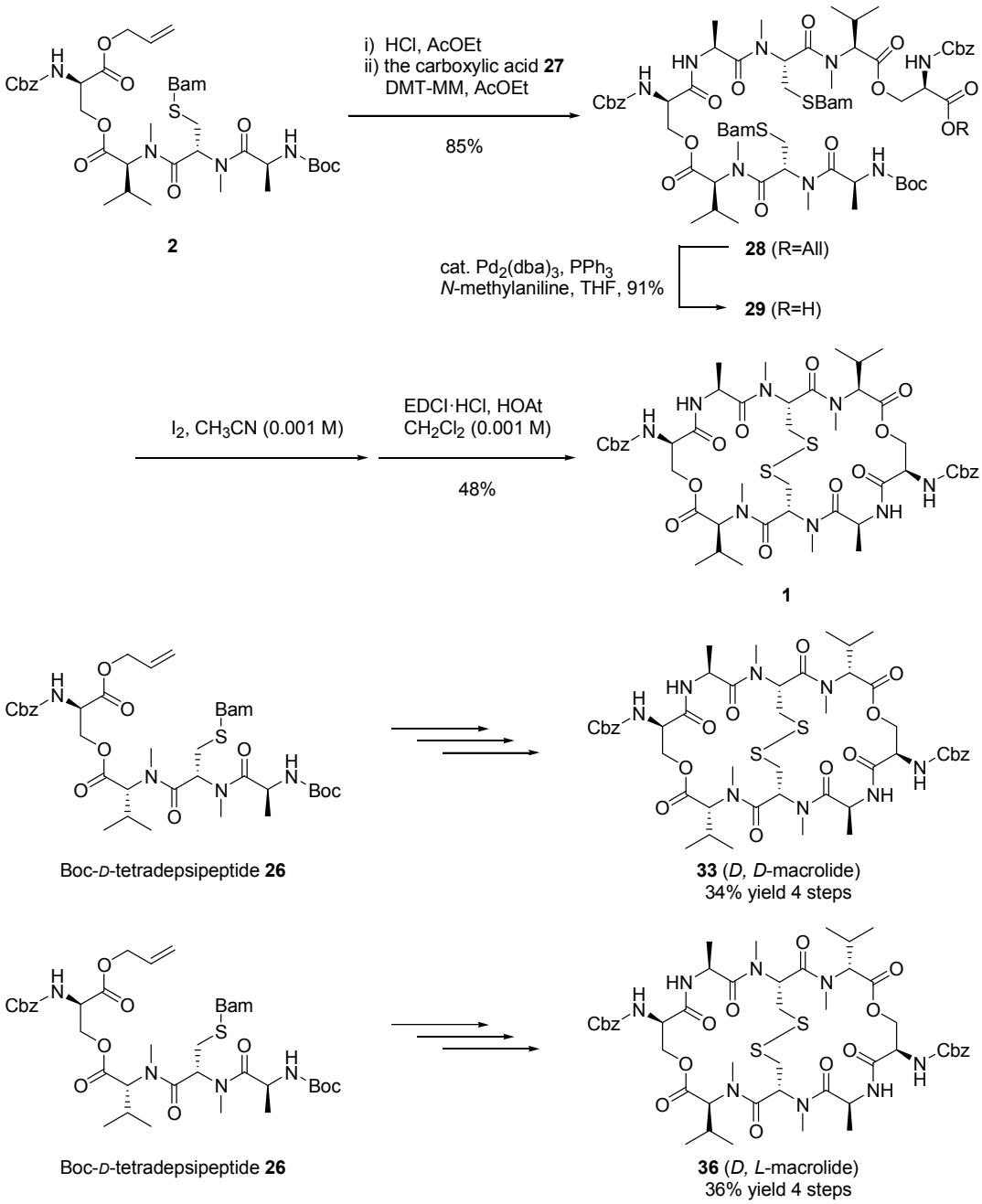
To reduce the overall cost, we evaluated route B, wherein expensive *D*-Ser was introduced in the last step (Scheme 2B). Coupling of MeVal-OAll (**14**) (Scheme S1) with Boc-MeCys(Bam)-OH (**10**) gave the peptide **15** in 86% yield. The cleavage of the Boc group of **15** with TFA/TIPS, followed by coupling with Boc-Ala-OH (**12**) gave tripeptide **16** in 78% yield. The allyl group of tripeptide **16** was removed using a palladium catalyst to obtain the carboxylic acid derivative **5** in 92% yield. Then, condensation of the tripeptide **5** with Cbz-*D*-Ser-OAll (**7**) afforded epimeric tetradepsipeptide **17** in 72% yield. During the final condensation step, epimerization at the α-carbon of MeVal occurred, giving a mixture of diastereoisomers that were identified by comparative LC/MS and NMR spectroscopic analyses against standard compounds prepared using the corresponding *D*-Val derivatives in the same manner as that described below.



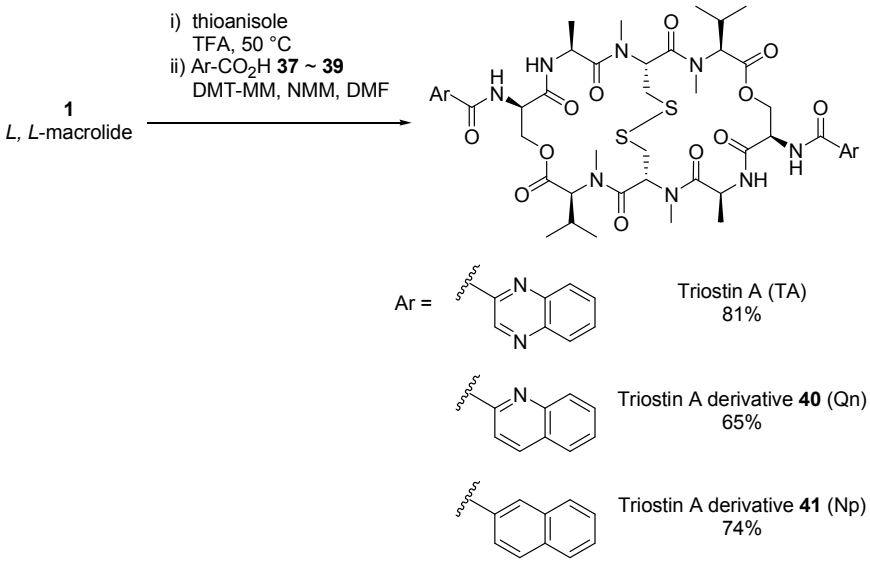
Scheme 3. Synthesis of *D*-MeVal derivatives **21**, **23** and **26**.

The coupling of **20**, derived from Boc-*D*-MeVal-OH (**18**) (Scheme S2), with Boc-MeCys(Bam)-OH (**10**) afforded **21** in 96% yield (Scheme 3). Removal of the Boc group of **21** with TFA/TIPS, followed by coupling with Boc-Ala-OH (**12**) gave **22** in 75% yield. Then, the allyl group of tripeptide **22** was removed to give the corresponding carboxylic acid derivative **23** in 79% yield. Tetradespipeptide **26** was then prepared from **18** in a manner similar to that of route A (Scheme 2A). A careful comparison of the ^{1}H NMR spectroscopic data of **15** and **5** with those of the corresponding *D*-MeVal isomers **21** and **23** (Figures S1 and 2) revealed that the coupling steps did not involve racemization. In contrast, the ^{1}H NMR spectrum of the tetradepsipeptide **17** prepared via route B was identical to the merged spectra of those of tetradepsipeptides **26** and **2** (Figure S3), which suggests that the coupling of tripeptide **5** and compound **7** in scheme 2B involved racemization of the MeVal residue. As shown in Figure S4, **17a** was also found to be a diastereomeric mixture of **2a** and **26a** by LC/MS analysis of the corresponding amine derivatives **2a**, **26a** and **17a**, prepared from the tetradepsipeptides **2**, **26** and **17**, respectively.

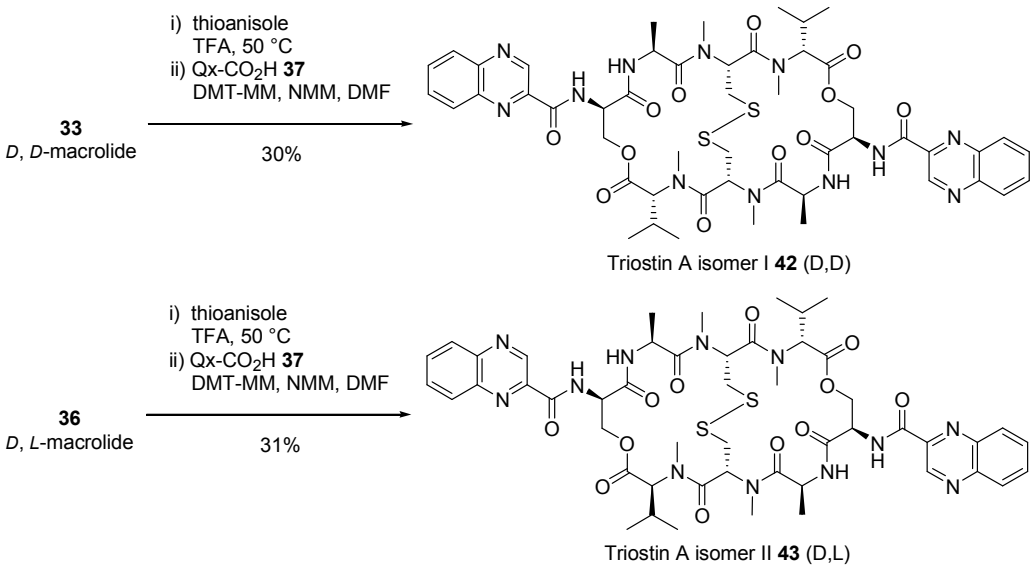
For the synthesis of macrolide precursor **1** (Scheme 4), the removal of the allyl group of the key intermediate **2** by a palladium catalyst system gave carboxylic acid **27.** Then, *N*-Boc deprotection of **2** and coupling with **27** (Scheme S3) were sequentially performed to afford the linear octadepsipeptide **28** in 85% yield (see Table S4 for optimization of the reaction conditions). Following this, allyl deprotection of **28** gave the corresponding carboxylic acid **29** in 91% yield. Oxidative deprotection of the Bam groups of **29** with iodine to form a disulfide linkage, and subsequent macrocyclization with EDCI/HOAt under high dilution conditions (0.001 M) gave the desired macrolide **1** in 48% yield.



Scheme 4. Synthesis of macrolide **1** and its diastereomers **33** and **36**.



Scheme 5. Synthesis of triostin A (TA) and its derivatives **40** (Qn) and **41** (Np).



Scheme 6. Synthesis of triostin A derivatives **42** (D,D) and **43** (D,L).

We also synthesized the diastereomeric macrolide isomers **33** (*D, D*-macrolide) and **36** (*D, L*-macrolide) by coupling the *D*-MeVal-containing tetradepsipeptide **26** with **2** or **26** over four steps using an identical procedure (Schemes 4, S4 and S5). A crystal of *D, D*-macrolide **33** was obtained and X-ray crystallography was used to confirm the absolute configurations at the α-carbons of the two MeVal residues as *R* (Figure S5).

The synthesis of TA was completed by deprotection of a pair of Cbz groups of **1** with

thioanisole/TFA, followed by conjugation with two 2-quinoxaline carboxylic acid residues, which gave TA in 81% yield (total 17.5% yield over 13 steps). The ^{1}H and ^{13}C NMR spectroscopic data of TA exactly matched that reported previously.[2, 31] To introduce variations in the nature of the aromatic ring, 2-quinoline and 2-naphthalene were introduced to produce **40** (Qn) in 65% yield and **41** (Np) in 74% yield (Scheme 5).

Diastereomeric isomers of TA were also synthesized from the corresponding Cbz derivatives **33** and **36** to give isomer I (**42**: *D,D*) and II (**43**: *D,L*) in moderate yields (Scheme 6). NMR spectroscopic data of **42** indicated that it exists as a single conformer with C_2-symmetry in $CDCl_3$, while **43** and TA exist as a mixture of two conformers in $CDCl_3$ (Figure 3A, see also page S96, S98 and S103-S106 in Supplementary Information).[2, 31, 38] Furthermore, the *D,D*-isomer **42** was crystallized and analyzed through X-ray crystallography (Figure 3B). Intriguingly, the crystal structure shows an unsymmetrical conformation bearing one *cis*-amide bond between MeCys and *D*-MeVal.

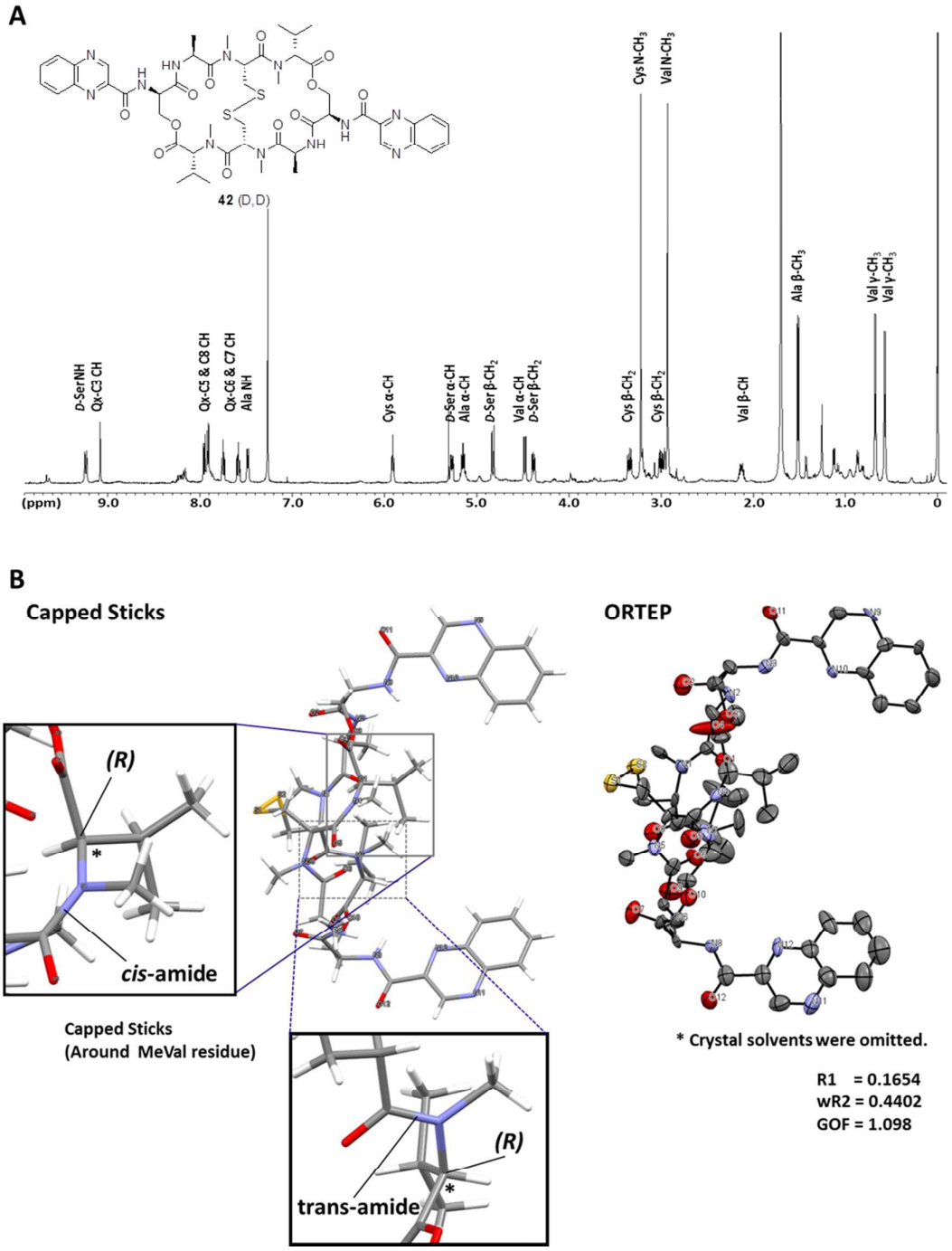
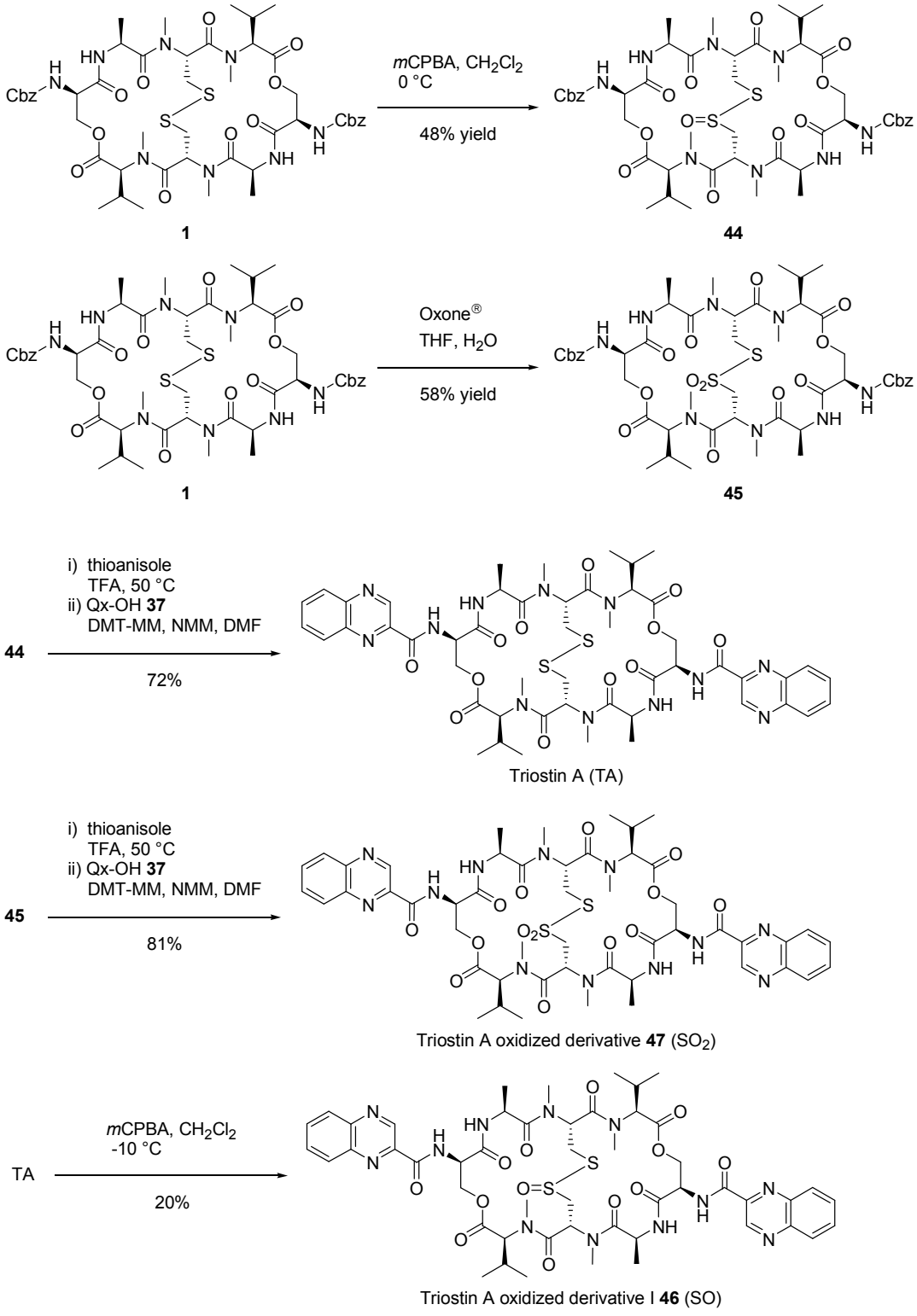


Figure 3. ^{1}H NMR spectrum(500 MHz, CDCl3) (A) and X ray crystal structural analysis (B) of isomer **42** (*D,D*).

The disulfide bridge was subjected to oxidative modifications (Scheme 7). Cbz-macrolide **1** was oxidized with *m*CPBA to give thiosulfinate derivative **44** in 48% yield, and with Oxone® to afford the thiosulfonate derivative **45** in 58% yield. We then

conducted deprotection of the Cbz groups, followed by introduction of the quinoxaline moieties. In the case of the thiosulfinate **44**, TA was the sole product of these treatments, isolated in 72% yield. Contrarily the thiosulfonate **45** could be converted to the corresponding thiosulfonate derivative **47** (SO_2) in 81% yield. To obtain the thiosulfinate derivative, TA was subjected to oxidation by using *m*CPBA to give the desired compound **46** (SO) in 20% yield with starting material, sulfone and undetermined substance. It was difficult to control overoxidation to sulfone. The structures of all the oxidized compounds were determined through ESI mass spectrometry and IR spectroscopy (Figures S6 and 7).



Scheme 7. Synthesis of oxidized derivatives of triostin A, **46** (SO) and **47** (SO_2).

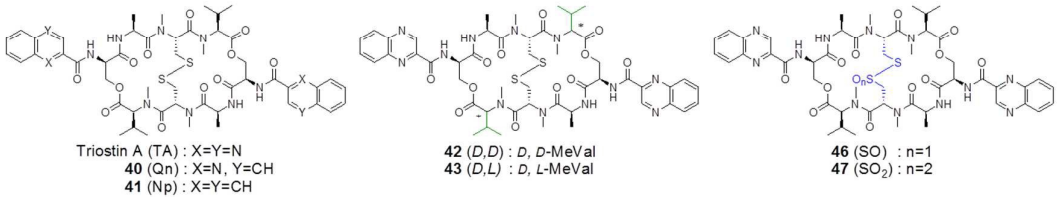
Biological evaluation

Inhibitory effect on HIF-1α transcriptional activation and cytotoxicity against MCF-7

Table 1. Half maximal inhibitory concentration (IC_{50}) on hypoxia-induced HIF-1 activation and cytotoxicity against MCF-7 treated with triostin A and its analogues.

IC_{50}	Ec	TA	40 (Qn)	41 (Np)	42 (*D,D*)	43 (*D,L*)	46 (SO)	47 (SO_2)
HIF-1[a]	0.35 ± 0.03 nM	26.9 ± 1.3 nM	3.0 ± 0.6 μM	> 100 μM	0.59 ± 0.12 μM	2.0 ± 0.8 μM	55.6 ± 3.3 nM	> 100 μM
MCF-7[b]	5.5 ± 0.2 nM	4.1 ± 0.3 μM	9.0 ± 0.2 μM	> 100 μM	5.5 ± 0.2 μM	14.6 ± 1.2 μM	41.7 ± 2.6 μM	> 100 μM
R_m[c]	0.25	0.13	0.25	0.18	0.23	0.21	0.14	0.08

[a] IC_{50} values for inhibition of HIF-1 activity were obtained by HIF-1 dependent luciferase assay using HEK293 p2.1 #3 cells under hypoxia (1% O_2) for 24 h with test compounds.
[b] IC_{50} values for cytotoxicity were obtained by MTT assay using MCF-7 treated with test compounds under aerobic condition for 24 h.
[c] R_m values were calculated as shown in experimental section.



In an HIF-1-targeted cell-based high-throughput screen of a small-molecule library at the National Cancer Institute, echinomycin was identified as the most potent inhibitor of HIF-1-DNA binding activity and HIF-1-dependent luciferase expression.[9] Therefore, we performed the luciferase reporter assay to examine the transcriptional activation of HIF-1 under the hypoxic conditions (1% O_2) that are commonly used for the screening of HIF-1 inhibitory compounds. To evaluate the HIF-1 inhibitory effects of our cyclic depsipeptides, we used stable transformants of HEK293 cells expressing reporter plasmid p2.1 (a kind gift from Dr. Gregg L. Semenza, Johns Hopkins University), which have been described in a previous report.[28] The cells were incubated with various concentrations of the test compounds under normoxic or hypoxic (1% O_2) conditions for 24 h. Ec was also examined using our luciferase assay system for comparison. Cytotoxic effects were evaluated by an MTT assay using MCF-7 cells treated with the test compounds for 24 h.

The IC_{50} values are summarized in Table 1 (individual data for all compounds are shown in Figures S8 and 9). Echinomycin exhibited extremely potent inhibitory activity (IC_{50} = 0.35 ± 0.03 nM) and cytotoxic effects (IC_{50} = 5.5 ± 0.2 nM). Most of the compounds synthesized here showed significant inhibitory effects on hypoxia-induced HIF-1 activation and cytotoxic activity against MCF-7 cells in a dose-dependent manner, respectively (Figures S8 and 9). Compared to Ec, TA was shown to have inhibitory

activity on HIF-1 activation (IC_{50} = 26.9 ± 1.3 nM) as low as two orders of magnitude, and cytotoxicity (IC_{50} = 4.1 ± 0.3 μM) showed magnitude as low as three orders. However, the HIF-1 inhibitory activity of TA was still fairly potent, though its sequence preference in DNA binding was different from that of Ec.[5, 8]

The structure-activity relationships of the octadepsipeptide analogues on HIF-1 inhibition and cytotoxicity displayed almost identical trends. Replacement of the aromatic chromophores had a detrimental effect on HIF-inhibitory activity and cytotoxicity. This may be because the DNA binding affinity and sequence preference of quinomycin bis-intercalators are affected by the charge and lipophilicity of the chromophore moiety.[39] The diastereoisomers consisting of *D*- or *L*-MeVal residues, **42** (*D,D*) and **43** (*D,L*), respectively, display lower activities than those of TA (*L,L*). By comparing of the X-ray crystal structure of **42** (*D,D*) with those of TA and Ec,[30] it was found that the conformation of the octadepsipeptide scaffold varied the distance between the pair of carbonyl carbon atoms of the quinoxaline-2-carboxamide moieties[3] (Figures 1 and S10). As the distance increased (Ec: 10.1 Å, TA: 13.6 Å, **42**: 14.2 Å), both biological activities were diminished. Interestingly, the HIF-1 inhibitory activity of thiosulfinate **46** (SO) was almost identical compared to that of TA, while its cytotoxic effect was one-tenth that of TA. Thiosulfonate derivative **47** (SO_2) had neither an HIF-1 inhibitory effect nor a cytotoxic effect; it is assumed that the hydrophobicity may be extremely low to be incorporated into cell membranes (R_m = 0.08).

To examine whether inhibition of hypoxia-induced HIF-1 activation contributes to the cytotoxicity of the compounds studied, we also performed an MTT assay against MCF-7 under hypoxic conditions (Figure 4). Upon treatment with Ec, there were no differences between the cytotoxic effects under aerobic and hypoxic conditions. However, TA and **46** (SO) did show a slight, but significant, hypoxic selectivity. The hypoxic-selective cytotoxicity can be attributed to the substantially large difference between the IC_{50} for HIF-1 inhibition and the IC_{50} for cytotoxicity of TA and **46**; $IC_{50\ (cytotoxicity)}/IC_{50\ (HIF\text{-}1\ inhibition)}$ = 152 (TA), 745 (**46**), 15.7 (Ec). In either case, the HIF-1 transcriptional activation may not be a critical factor for tumor cell survival.

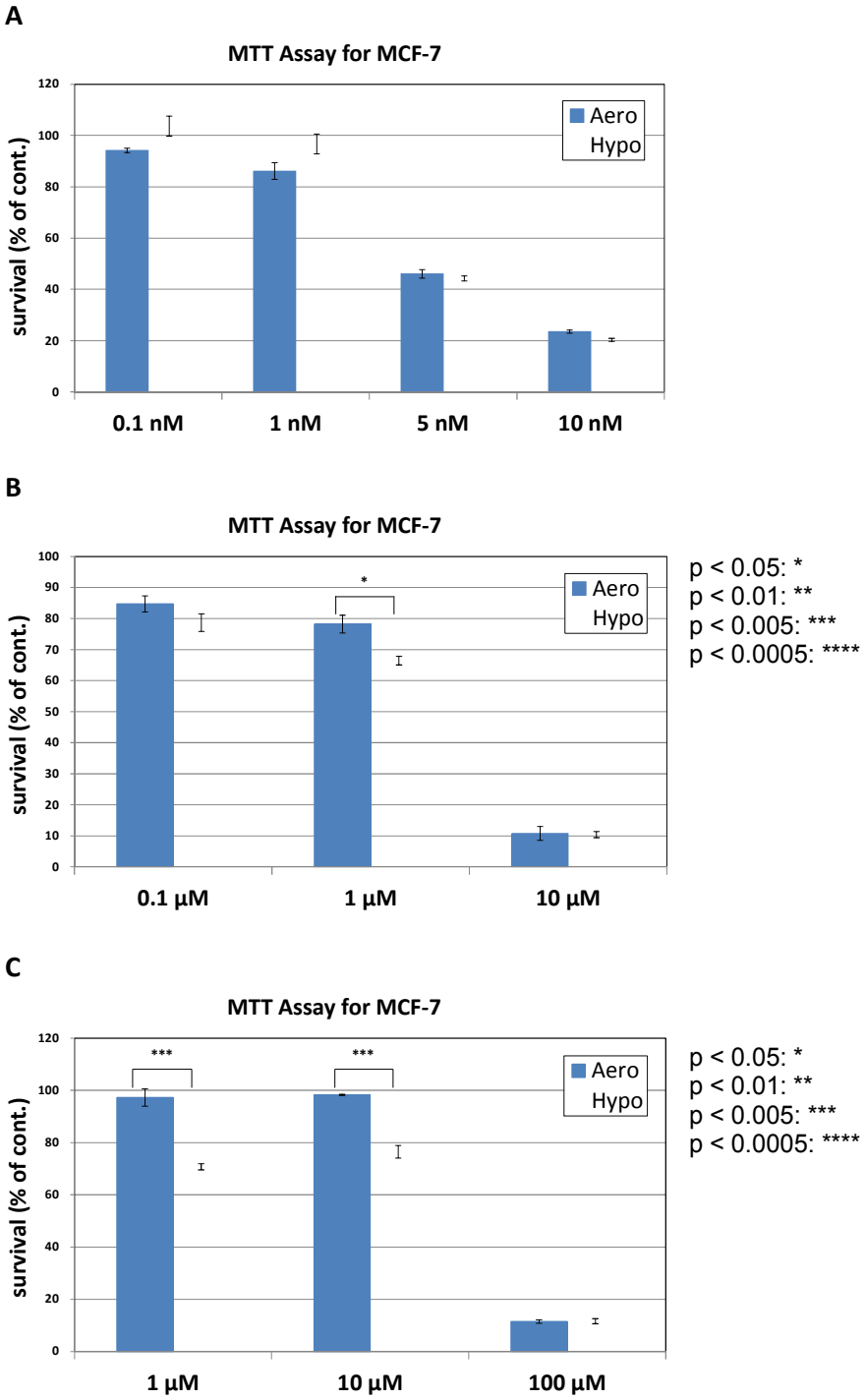


Figure 4. Hypoxic-selective cytotoxicity on MCF-7 treated with Ec (A), TA (B), and oxidized derivative **46** (C). MCF-7 cells were incubated with Ec, TA and **46** (SO) for 24 h under aerobic (20% O_2) or hypoxic (1% O_2) condition.

Effect on protein accumulation of HIF-1α induced by hypoxia

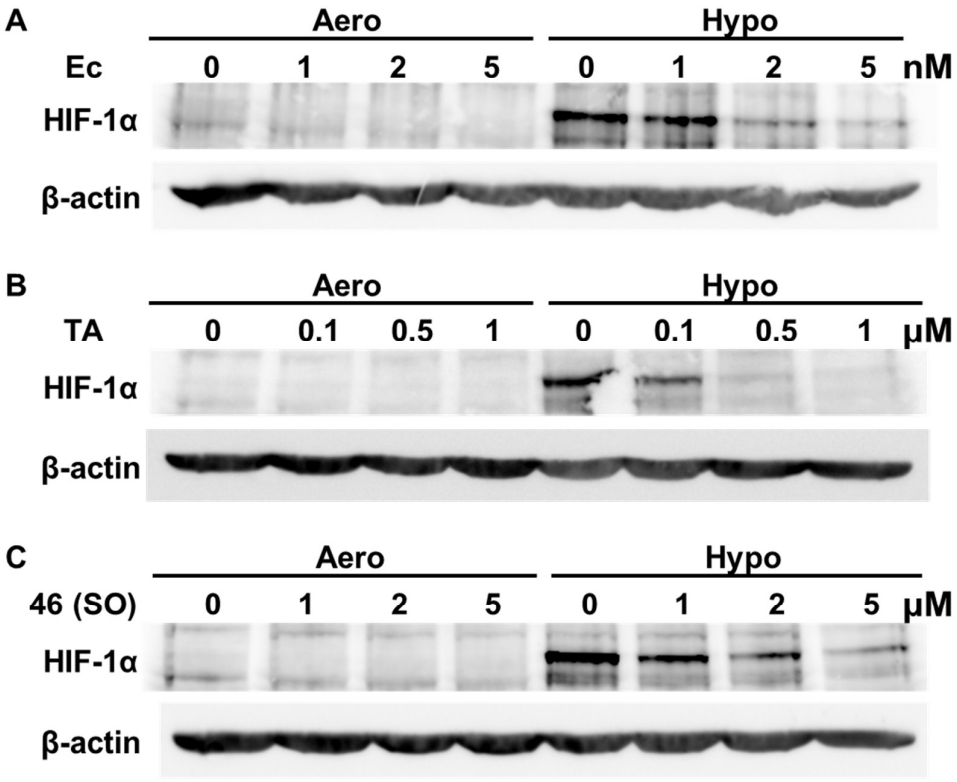


Figure 5. Western blot analyses of HIF-1α to evaluate inhibition of Ec (A), TA (B) and **46** (C) on HIF-1α protein expression of MCF-7 cells induced by hypoxia. MCF-7 cells were incubated with several concentrations of Ec, TA and **46** for 16 h under aerobic (20% O_2) or hypoxic (1% O_2) condition.

Hypoxia-induced HIF-1α accumulation occurs by suppression of its degradation by the ubiquitin-proteasome pathway. Echinomycin was previously assumed not to inhibit HIF-1α protein accumulation but DNA binding of HIF-1 to HRE.[9, 40] We examined the effects of TA, Ec and **46** (SO) on HIF-1α accumulation in MCF-7 cells under hypoxic conditions using western blot analysis. As shown in Figure 5, after incubation for 16 h under hypoxic condition (1% O_2), HIF-1α protein was accumulated, and all of the tested compounds clearly reduced the protein expression in a dose-dependent manner at concentrations lower than $IC_{50(cytotoxicity)}$ values of each compound.

In previous reports, echinomycin slightly increased, or did not affect, HIF-1α protein expression under hypoxic conditions in U251, HepG2 and HeLa cells.[9, 40] Collaboratively, these findings suggest that the mode of effect of the depsipeptides on HIF-1 inhibition

may vary with the kind of tumor cell line and that a target molecule other than HRE seems to be found upstream of the signal pathway of HIF-1α gene expression in MCF-7 cells.

Conclusions

We have successfully achieved the solution-phase synthesis of triostin A on a preparative scale with high total yield (17.5%, 13 steps). The optimization involved careful selection of the protecting groups; specifically, the *S*-benzamidomethyl (Bam) group for cysteine and the allyl group for C-terminal carboxylic acid and manipulation of the sequence of amino acid coupling for the synthesis of the key tetradepsipeptide without racemization. Using the improved procedure, new triostin A analogues with quinoline (**40**) and naphthalene (**41**) chromophores, diastereoisomeric cyclic depsipeptide cores (**42**, **43**), and oxidized intra-peptide disulfide bridges (**46**, **47**) were synthesized and evaluated for effects on the HIF-1 transcriptional activation under hypoxic conditions and cytotoxicity on MCF-7 cells. All analogues except **41** (Np) and thiosulfonate **47** (SO_2) exhibited significant inhibitory effects on HIF-1 transcriptional activation, as well as high cytotoxicity. Triostin A, echinomycin and thiosulfinate **46** were shown to inhibit not only DNA binding of HIF-1 but, for the first time, also to reduce HIF-1α protein accumulation in MCF-7 cells. These data indicated that the potent cytotoxic effects of the compounds were not to be attributed to their HIF-1 inhibitory effects. Furthermore, thiosulfinate **46** exhibited hypoxia-selective cytotoxicity, which suggests that the cyclic depsipeptide core may be an attractive and premising scaffold to develop novel tumor-selective agents targeting the hypoxic tumor microenvironment. The present method of solution phase synthesis of the cyclic depsipeptides will contribute to antitumor drug development on the basis of such a novel strategy.

Experimental Section

Synthesis

General experimental conditions

All commercially available reagents and solvents were used without further purification. These reactions were carried out under the nitrogen atmosphere. Normal-phase TLC was carried out on Silica gel 60 F_{254} (Merck, 1.05715.0009) using

reagent grade solvents. TLC was detected by the absorption of UV light (254 nm) or visualization reagent (molybdophosphoric acid). Column chromatography was performed on silica gel (AP-300S Taiko-shoji) with mixed solvents as described. ^{1}H and ^{13}C NMR spectra were obtained for samples in indicated solution at 25 ˚C on a JEOL JNM-ECA500 spectrometer at 500 MHz frequency or JNM-AL400 spectrometer at 400 MHz frequency in $CDCl_3$ with tetramethylsilane as an internal standard. ^{1}H NMR chemical shifts are reported in terms of chemical shift (δ, ppm) relative to the singlet at 0 ppm for tetramethylsilane. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; dd, doubledoublet; td, tripledoublet; q, quartet; ddd, doubledoubledoublet; ddt, doubledoubletriplet; m, multiplet; br, broad. Coupling constants are reported in Hz. ^{13}C NMR spectra were fully decoupled and are reported in terms of chemical shift (δ, ppm) relative to the triplet at δ = 77.0 ppm for $CDCl_3$. Melting points were obtained on cover glasses and were uncorrected. The direct analysis in real time ESI-MS or DART-MS measurements were carried out on a JEOL JMS-T100TD. Optical rotations were measured on JASCO P-1020. Infrared Spectroscopy (IR) was recorded on a JASCO FT/IR-230 spectrometer.

***N*-Cbz-*D*-Ser(*N*-Boc-*N*-Me-*L*-Val)-OAll (9).** To a solution of *N*-Cbz-*D*-Ser-OAll **7** 5 g (17.9 mmol), *N*-Boc-*N*-Me-*L*-Val-OH **8** 4.97 g (21.5 mmol, 1.2 equiv.), HOAt 3.66 g (26.9 mmol, 1.5 equiv.) and NEt_3 3.7 mL (26.9 mmol, 1.5 equiv.) in CH_2Cl_2 (100 mL) was added EDCI·HCl 5.15 g (26.9 mmol, 1.5 equiv.), and the resulting solution was stirred at room temperature for overnight (21 h). The mixture was then extracted with AcOEt (300 mL × 2) and water (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (200 g) column chromatography eluted with *n*-hexane/AcOEt (10:0, 10:1, 9:1, 8:2) to afford the target compound **9** 7.32 g (14.9 mmol, 83% yield) as pale yellow oil: R_f = 0.5 (*n*-hexane:AcOEt = 7:3); ^{1}H NMR (mixture of rotamers, 400 MHz, $CDCl_3$) δ 7.40-7.31 (m, 5H, Cbz-Ar), 6.09-5.79 (m, 1H, All-CH_2), 5.73 (br, 0.54H, Ser-NH), 5.53 (br, 0.46H, Ser-NH), 5.33 (d, *J* = 16.9 Hz, 1H, All-CH_2 (*E*)), 5.26 (d, *J* = 10.1 Hz, 1H, All-CH_2 (*Z*)), 5.11 (s, 2H, Cbz-CH_2), 4.78-4.00 (m, 6H, All-OCH_2, Ser-β-CH_2, Ser-α-CH, Val-α-CH), 2.89 (s, 0.35H, Val-*N*-Me), 2.80 (s, 2.65H, Val-*N*-Me), 2.16 (br, 1H, Val-β-CH), 1.48 (s, 1.46H, Boc-Me), 1.43 (s, 7.54H, Boc-Me), 1.02 (d, *J* = 6.3 Hz, 0.32H, Val-γ-CH_3), 0.93 (d, *J* = 6.8 Hz, 3.21H, Val-γ-CH_3), 0.88 (d, *J* = 6.8 Hz, 2.47H, Val-γ-CH_3); ^{13}C NMR (mixture of rotamers, 100 MHz, $CDCl_3$) δ 170.6 (Val-CO), 168.9 (Ser-CO), 156.2 155.8, 155.6, 155.4 (Cbz-CO, Boc-CO), 136.1, 135.4 (All-CH), 131.2, 131.1, 128.7, 128.1 (Cbz-Ar), 119.3, 119.1 (All-CH_2), 80.4, 80.2 (Boc-C), 67.1, 66.5, 64.9,

64.2, 63.4 (Cbz-CH_2, All-OCH_2, Ser-β-CH_2, Ser-α-CH), 53.4 (Val-α-CH), 30.9, 30.6 (Val-*N*-Me), 28.3 (Boc-Me), 27.7 (Val-β-CH), 19.8, 19.6, 18.9 (Val-γ-CH_3); HRMS (DART) calcd for $C_{25}H_{37}N_2O_8^+$ $[M+H]^+$ 493.2544, found:493.2547.

***N*-Cbz-*D*-Ser[*N*-Boc-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val]-OAll (11).** To a solution of *N*-Cbz-*D*-Ser(*N*-Boc-*N*-Me-*L*-Val)-OAll **9** 7 g (14.2 mmol) in AcOEt (40 mL) was added 4 M HCl in AcOEt 50 mL (200 mmol) at 0 ˚C. Then the resulting solution was warmed to room temperature and stirred at the temperature for overnight (16 h). The mixture was concentrated under the reduced pressure, and the residue was extracted with AcOEt (300 mL × 2) and sat. $NaHCO_3$ aq. (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. Then a solution of the residue (pale yellow oil, 7.40 g), *N*-Boc-*N*-Me-*L*-Cys(Bam)-OH **10** 6.26 g (17.0 mmol, 1.2 equiv.) and DMT-MM 5.89 g (21.3 mmol, 1.5 equiv.) in AcOEt (70 mL) was stirred at room temperature for overnight (23 h). The mixture was filtrated, and the filtrate was extracted with AcOEt (300 mL × 2) and water (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (200 g) column chromatography eluted with *n*-hexane/AcOEt (10:0, 9:1, 8:2, 7:3) to afford the target compound **11** 10.05 g (13.5 mmol, 95% yield on 2 steps) as colorless amorphous solid: R_f = 0.5 (*n*-hexane:AcOEt = 5:5); ^{1}H NMR (mixture of rotamers, 500 MHz, $CDCl_3$) δ 8.16-7.70, 7.56-7.29 (m, 11H, Bam-NH, Ar, Cbz-Ar), 5.85 (ddt, *J* = 22.6, 10.9 Hz, 1H, All-CH), 5.76 (d, *J* = 7.4 Hz, 0.76H, Ser-NH), 5.60-5.53 (br, 0.24H, Ser-NH), 5.42-4.99 (m, 5H, All-CH_2, Cbz-CH_2, Cys-α-CH), 4.93-4.03 (m, 8H, Bam-CH_2, All-OCH_2, Ser-β-CH_2, Ser-α-CH, Val-α-CH), 3.31-2.57 (m, 8H, Cys-β-CH_2, Cys-*N*-Me, Val-*N*-Me), 2.27-2.03 (m, 1H, Val-β-CH), 1.48 (s, 2.43H, Boc-Me), 1.46 (s, 1.61H, Boc-Me), 1.45 (s, 4.96H, Boc-Me), 0.97 (d, *J* = 6.9 Hz, 2.11H, Val-γ-CH_3), 0.93 (d, *J* = 6.9 Hz, 0.47H, Val-γ-CH_3), 0.87 (d, *J* = 6.3 Hz, 0.51H, Val-γ-CH_3), 0.81 (d, *J* = 6.9 Hz, 2.16H, Val-γ-CH_3), 0.75 (d, *J* = 6.3 Hz, 0.39H, Val-γ-CH_3), 0.69 (d, *J* = 6.9 Hz, 0.36H, Val-γ-CH_3); ^{13}C NMR (mixture of rotamers, 125 MHz, $CDCl_3$) δ 170.5, 169.9, 169.5, 169.4, 169.2, 168.8, 168.6, 168.5, 167.2, 167.1, 166.9, 166.8 (Val-CO, Cys-CO, Ser-CO, Bam-CO), 156.4, 156.2, 155.74, 155.66, 155.5, 154.6, 154.5 (Cbz-CO, Boc-CO), 136.4, 136.0, 135.7, 133.8, 133.7, 133.6, 128.3, 128.2, 128.1, 128.0, 127.8, 127.6, 127.3, 127.2, 127.0 (Cbz-Ar, Bam-Ar), 131.5, 131.4, 131.3, 131.0, 130.8 (All-CH), 119.3, 119.0 (All-CH_2), 81.8, 81.1, 80.9, 80.7 (Boc-C), 67.3, 67.1, 66.9, 66.6, 66.4, 66.2, 65.0, 64.6, 64.3, 64.1, 64.0 (All-OCH_2, Ser-β-CH_2), 62.3, 55.6, 55.3, 54.6, 53.4, 53.1 (Ser-α-CH, Val-α-CH, Cys-α-CH), 43.8, 42.8, 42.3, 41.5 (Cys-β-CH_2), 32.8, 31.5 (Cys-β-CH_2), 30.9, 29.7, 29.4, 29.3, 29.2, 29.1

(Cys-*N*-Me, Val-*N*-Me), 28.3, 28.2, 28.1 (Boc-Me), 27.8, 26.9, 26.6 (Val-β-CH), 19.6, 19.2, 19.0, 18.73, 18.65 (Val-γ-CH_3); HRMS (ESI) calcd for $C_{37}H_{50}N_4NaO_{10}S^+$ $[M+Na]^+$ 765.3140, found: 765.3139.

***N*-Cbz-*D*-Ser[*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val]-OAll (2).** To a solution of *N*-Cbz-*D*-Ser[*N*-Boc-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val]-OAll **11** 5 g (6.7 mmol) in AcOEt (40 mL) was added 4 M HCl in AcOEt 10 mL (40 mmol) at 0 ˚C. Then the resulting solution was warmed to room temperature, and stirred at the temperature for overnight (14 h). The mixture was concentrated under the reduced pressure, and the residue was extracted with AcOEt (300 mL) and sat. $NaHCO_3$ aq. (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. Then a solution of the residue (colorless amorphous solid, 5.47 g), *N*-Boc-*L*-Ala-OH **12** 1.51 g (8.0 mmol, 1.2 equiv.) and DMT-MM 2.79 g (10.1 mmol, 1.5 equiv.) in DMF (50 mL) was stirred at room temperature for overnight (21 h). The mixture was extracted with AcOEt (300 mL) and water (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (200 g) column chromatography eluted with *n*-hexane/AcOEt (10:0, 8:2, 6:4, 4:6) to afford the target compound **2** 4.15 g (5.1 mmol, 76% yield on 2 steps) as colorless amorphous solid: R_f = 0.3 (*n*-hexane:AcOEt = 5:5); ^{1}H NMR (mixture of rotamers, 500 MHz, $CDCl_3$) δ 8.05-7.80, 7.65-7.28 (m, 11H, Bam-NH, Ar, Cbz-Ar), 6.01-5.78 (m, 1H, All-CH), 5.72 (t, *J* = 7.4 Hz, 0.25H, Cys-α-CH), 5.63 (t, *J* = 7.2 Hz, 0.75H, Cys-α-CH), 5.56-5.34 (m, 1H, Ala-NH or Ser-NH), 5.30 (d, *J* = 17.2 Hz, 1H, All-CH_2 (*E*)), 5.25 (d, *J* = 10.3 Hz, 1H, (*Z*)), 5.23-5.12 (m, 1H, Ala-NH or Ser-NH), 5.10 (s, 2H, Cbz-CH_2), 4.89-4.25 (m, 9H, Bam-CH_2, All-OCH_2, Ser-β-CH_2, Ala-α-CH, Val-α-CH, Ser-α-CH), 3.23 (dd, *J* = 14.6, 7.2 Hz, 0.15H, Cys-β-CH_2), 3.16 (dd, *J* = 14.3, 6.9 Hz, 0.85H, Cys-β-CH_2), 3.10-2.73 (m, 7H, Cys-*N*-Me, Val-*N*-Me), 2.33-2.08 (m, 1H, Val-β-CH), 1.44 (s, 2.92H, Boc-Me), 1.43 (s, 3.76H, Boc-Me), 1.41 (s, 1.48H, Boc-Me), 1.36-1.27 (m, 2.43H, Ala-β-CH_3), 1.24-1.13 (m, 0.57H, Ala-β-CH_3), 0.96 (d, *J* = 6.9 Hz, 2.43H), 0.85 (d, *J* = 6.3 Hz, 0.53H), 0.79 (d, *J* = 6.9 Hz, 0.79H), 0.77 (d, *J* = 6.9 Hz, 2.24H); ^{13}C NMR (mixture of rotamers, 125 MHz, $CDCl_3$) δ 174.0, 173.9, 173.7, 170.1, 169.9, 169.7, 169.4, 168.8, 168.7, 167.1, 166.8 (Ala-CO, Cys-CO, Val-CO, Ser-CO, Bam-CO), 155.8, 155.7, 155.1, 154.9 (Cbz-CO, Boc-CO), 135.9, 133.7, 133.5, 128.34, 128.27, 128.23, 128.0, 127.2, 127.1 (Cbz-Ar, Bam-CO), 131.5, 131.4, 131.0 (All-CH), 119.0 (All-CH_2), 79.6 (Boc-C), 66.9, 66.4, 64.7, 64.1 (Cbz-CH_2, All-OCH_2, Ser-β-CH_2), 62.3, 53.6, 53.4, 53.2, 53.0, 46.6, 46.4, 46.0 (Ala-α-CH, Ser-α-CH, Val-α-CH, Cys-α-CH), 42.6, 42.3 (Bam-CH_2), 31.5, 30.4 (Cys-β-CH_2), 31.2, 30.1, 29.9 (Cys-*N*-Me,

Val-*N*-Me), 28.2 (Boc-Me), 26.9 (Val-β-CH), 20.8, 19.7, 19.1, 18.9, 18.6, 17.9 (Val-γ-CH_3, Ala-β-CH_3); HRMS (ESI) calcd for $C_{40}H_{55}N_5NaO_{11}S^+$ $[M+Na]^+$ 836.3511, found: 836.3541.

***N*-Boc-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val-OAll (15).** To a solution of *N*-Me-*L*-Val-OAll·HCl **14** 3.39 g (16.3 mmol, 1.2 equiv.), DMT-MM 4.51 g (16.3 mmol, 1.2 equiv.) and NEt_3 2.3 mL (16.3 mmol, 1.2 equiv.) in THF (50 mL) was added *N*-Boc-*N*-Me-*L*-Cys(Bam)-OH **10** 5 g (13.6 mmol). The resulting solution was stirred at room temperature for overnight (23 h). The mixture was then filtrated and the filtrate was extracted with AcOEt (300 mL) and water (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (200 g) column chromatography eluted with *n*-hexane/AcOEt (10:0, 9:1, 8:2, 6:4) to afford the target compound **15** 6.10 g (11.7 mmol, 86% yield) as pale yellow oil: R_f = 0.5 (*n*-hexane:AcOEt = 6:4); ^{1}H NMR (mixture of rotamers, 500 MHz, $CDCl_3$) δ 8.17-7.79 (m, 3H, Bam-Ar, NH), 7.58-7.36 (m, 3H, Bam-Ar), 5.93-5.80 (m,1H, All-CH), 5.53-4.93 (m, 3H, All-CH_2, Cys-α-CH), 4.94-4.30 (m, 5H, Bam-CH_2, All-OCH_2, Val-α-CH), 3.29-2.61 (m, 8H, Cys-β-CH_2, Cys, Val-*N*-Me), 2.32-2.09 (m, 1H, Val-β-CH_2), 1.56-1.43 (m, 9H, Boc), 1.20-0.68 (m, 6H, Val-γ-CH_3); ^{13}C NMR (mixture of rotamers, 125 MHz, $CDCl_3$) δ 170.2, 169.9, 169.7, 169.4, 169.3 (Val-CO, Cys-CO), 167.0, 166.9, 166.8, 166.8 (Bam-CO), 156.1, 156.0, 154.6, 153.7 (Boc-CO), 133.7, 133.6, (Bam-Ar), 131.4, 131.3 (All-CH), 128.2, 128.1, 127.3, 127.2, 127.0 (Bam-Ar), 119.5, 119.4, 118.5 (All-CH_2), 80.9, 80.8, 80.7 (Boc-C), 66.4, 65.9, 65.7, 65.2 (All-OCH_2), 64.6, 64.4, 62.5, 62.4, 56.5, 55.8, 54.9, 54.3 (Val-α-CH, Cys-α-CH), 44.0, 43.1, 42.0, 41.5 (Bam-CH_2), 33.1, 31.9, 30.9, 30.2 (Cys-β-CH_2), 31.1, 29.4, 29.3, 29.1, 29.0, 28.8 (Val-*N*-Me, Cys-*N*-Me), 28.2, 28.0 (Boc-Me), 27.3, 27.2, 27.1 (Val-β-CH_2), 19.8, 19.6, 19.0, 18.9, 18.7, 18.3 (Val-γ-CH_3); HRMS (ESI) calcd for $C_{26}H_{39}N_3NaO_6S^+$ $[M+Na]^+$ 544.2452, found: 544.2449.

***N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val-OAll (16).** To a solution of *N*-Boc-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val-OAll **15** 6.1 g (11.7 mmol) and triisopropylsilane 2.4 mL (11.7 mmol, 1 equiv.) in CH_2Cl_2 (50 mL) was added TFA 8.7 mL (117 mmol, 10 equiv.) at 0 ˚C. Then the resulting solution was warmed to room temperature and stirred at the temperature for overnight (13 h). The mixture was then quenched with sat. $NaHCO_3$ aq. (300 mL), and extracted with AcOEt (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. Then a solution of the residue (colorless oil, 5.64 g), *N*-Boc-*L*-Ala-OH **12** 2.64 g (14.0 mmol, 1.2 equiv.) and DMT-MM 4.87 g (17.6 mmol, 1.5

equiv.) in CH_3CN (50 mL) was stirred at room temperature for overnight (20 h). The mixture was extracted with AcOEt (300 mL) and water (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (200 g) column chromatography eluted with *n*-hexane/AcOEt (10:0, 9:1, 8:2, 6:4) to afford the target compound **16** 5.37 g (9.1 mmol, 78% yield on 2 steps) as colorless amorphous solid: R_f = 0.3 (*n*-hexane:AcOEt = 5:5); ^{1}H NMR (mixture of rotamers, 500 MHz, $CDCl_3$) δ 8.02-7.36 (m, 6H, Bam- Ar, NH), 5.96-5.74 (m, 1H, All-CH), 5.66 (t, *J* = 7.2 Hz, 0.68H, Cys-α-CH), 5.54 (t, *J* = 7.2 Hz, 0.32H, Cys-α-CH), 5.47-5.09 (m, 3H, Ala-NH, All-CH_2), 4.90-4.36 (m, 6H, Bam-CH_2, All-OCH_2, Ala-α-CH, Val-α-CH), 3.29 (dd, *J* = 14.3, 8.0 Hz, 0.33H, Cys-β-CH_2), 3.18 (dd, *J* = 14.3, 7.4 Hz, 0.67H, Cys-β-CH_2), 3.01-2.73 (m, 7H, Val-*N*-Me, Cys-*N*-Me, Cys-β-CH_2), 2.35-2.06 (m, 1H, Val-β-CH_2), 1.43 (s, 9H, Boc), 1.33-1.17 (m, 3H, Ala-β-CH_3), 1.01 (d, *J* = 6.3 Hz, 2.1H, Val-γ-CH_3), 0.82 (d, *J* = 6.3 Hz, 0.9H, Val-γ-CH_3), 0.80 (d, *J* = 6.9 Hz, 2.0H, Val-γ-CH_3), 0.77 (d, *J* = 6.3 Hz, 1.0H, Val-γ-CH_3); ^{13}C NMR (mixture of rotamers, 125 MHz, $CDCl_3$) δ 173.9, 173.6 (Val-CO), 169.8, 169.7, 169.24, 169.17 (Cys-CO, Ala-CO), 166.9, 166.7 (Bam-CO), 155.1, 155.0 (Boc-CO), 133.7, 131.1 (All-CH), 131.6, 131.5, 128.4, 127.3 (Bam-Ar), 120.0, 118.9 (All-CH_2), 79.9, 79.8 (Boc-C), 66.0, 65.5, 64.9, 62.8, 54.5, 53.6, 46.7, 46.4 (All-OCH_2, Val-α-CH, Cys-α-CH, Ala-α-CH), 43.7, 42.8 (Bam-CH_2), 32.4, 31.5, 31.0, 30.4, 30.3, 30.0 (Cys-*N*-Me, Val-*N*-Me, Cys-β-CH_2), 28.3 (Boc-Me), 27.6, 27.2 (Val-β-CH), 20.0, 19.4, 18.8, 18.7 (Val-γ-CH_3), 18.6, 18.2 (Ala-β-CH_3); HRMS (ESI) calcd for $C_{29}H_{44}N_4NaO_7S^+$ $[M+Na]^+$ 615.2823, found: 615.2814.

***N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val-OH (5).** To a solution of *N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val-OAll **16** 5.37 g (9.1 mmol), PPh_3 191.5 mg (0.73 mmol, 8 mol%), *N*-methylaniline 1.2 mL (10.9 mmol, 1.2 equiv.) in THF (50 mL) was added $Pd_2(dba)_3$ 164.8 mg (0.18 mmol, 2 mol%). The resulting solution was stirred at room temperature for overnight (12 h) in the dark. Then the mixture extracted with AcOEt (200 mL) and sat. NH_4Cl aq. (200 mL). The organic layer was washed with sat. NaCl aq. (200 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (200 g) column chromatography eluted with CH_2Cl_2/MeOH (10:0, 100:1, 50:1) to afford the target compound **5** 4.66 g (8.4 mmol, 92% yield) as pale yellow amorphous solid: R_f = 0.3 (CH_2Cl_2:MeOH = 10:1); ^{1}H NMR (mixture of rotamers, 500 MHz, $CDCl_3$) δ 8.01-7.81, 7.54-7.34 (m, 6H, Bam-NH, Ar), 5.75-5.46 (m, 2H, Ala-NH, Cys-α-CH), 4.84-4.34 (m, 4H, Bam-CH_2, Ala-α-CH, Val-α-CH), 3.28 (dd, *J* = 14.3, 8.0 Hz, 0.38H, Cys-β-CH_2), 3.19 (dd, *J* = 14.3, 7.4 Hz, 0.62H,

Cys-β-CH_2), 3.08-2.73 (m, 7H, Cys-*N*-Me, Val-*N*-Me, Cys-β-CH_2), 2.36-2.06 (m, 1H, Val-β-CH), 1.42 (s, 5.6H, Boc-Me), 1.41 (s, 3.4H, Boc-Me), 1.29 (d, J = 6.9 Hz, 1.92H, Ala-β-CH_3), 1.24 (d, J = 6.9 Hz, 1.08H, Ala-β-CH_3), 1.03 (d, J = 6.3 Hz, 1.88H, Val-γ-CH_3), 0.86 (d, J = 6.3 Hz, 1.09H, Val-γ-CH_3), 0.79 (d, J = 6.3 Hz, 1.89H, Val-γ-CH_3), 0.76 (d, J = 6.9 Hz, 1.13H, Val-γ-CH_3); ^{13}C NMR (mixture of rotamers, 125 MHz, $CDCl_3$) δ 174.2, 173.7 (Val-CO), 172.6, 171.2, 170.0, 169.7 (Ala-CO, Cys-CO), 168.1, 167.2 (Bam-CO), 155.2, 155.1 (Boc-CO), 133.5, 133.1, 131.8, 131.7, 128.4, 127.4, 127.3 (Bam-Ar), 80.1, 80.0 (Boc-C), 64.8, 63.5, 54.4, 53.7, 53.4, 46.7 (Cys-α-CH, Val-α-CH, Ala-α-CH), 43.5, 42.8 (Bam-CH_2), 32.2, 30.8, 30.5, 30.4, 30.0 (Cys-*N*-Me, Val-*N*-Me, Cys-β-CH_2), 28.3 (Boc-Me), 27.4, 27.0 (Val-β-CH), 20.1, 19.5, 18.8, 18.6, 17.7 (Val-γ-CH_3, Ala-β-CH_3); HRMS (ESI) calcd for $C_{26}H_{39}N_4O_7S^-$ $[M-H]^-$ 551.2545, found: 551.2544.

***N*-Cbz-*D*-Ser[*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*D*, *L*-Val]-OAll (17).** To a solution of *N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val-OH **5** 3.32 g (6.0 mmol), *N*-Cbz-*D*-Ser-OAll **7** 2.01 g (7.2 mmol, 1.2 equiv.), HOBt·H_2O 1.38 g (9.0 mmol, 1.5 equiv.) and NEt_3 1.2 mL (9.0 mmol, 1.5 equiv.) in CH_3CN (30 mL) was added EDCI·HCl 1.73 g (9.0 mmol, 1.5 equiv.). The resulting solution was stirred at room temperature for overnight (15 h). The mixture was then extracted with AcOEt (200 mL) and sat. $NaHCO_3$ aq. (200 mL). The organic layer was washed with sat. NaCl aq. (200 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (200 g) column chromatography eluted with *n*-hexane/AcOEt (10:0, 9:1, 8:2, 6:4) to afford the target compound **17** 3.52 g (4.3 mmol, 72% yield) as colorless amorphous solid: R_f = 0.5 (*n*-hexane:AcOEt = 5:5); HRMS (ESI) calcd for $C_{40}H_{55}N_5NaO_{11}S^+$ $[M+Na]^+$ 836.3511, found: 836.3518; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) chart were shown in Supporting Information.

***N*-Boc-*N*-Me-*L*-Cys(Bam)-*N*-Me-*D*-Val-OAll (21).** To a solution of *N*-Me-*D*-Val-OAll·HCl **20** 0.68 g (3.3 mmol, 1.2 equiv.), DMT-MM 0.90 g (3.3 mmol, 1.2 equiv.) and NEt_3 0.45 mL (3.3 mmol, 1.2 equiv.) in THF (10 mL) was added *N*-Boc-*N*-Me-*L*-Cys(Bam)-OH **10** 1 g (2.7 mmol). The resulting solution was stirred at room temperature for overnight (16 h). The mixture was then filtrated and the filtrate was extracted with AcOEt (100 mL) and water (100 mL). The organic layer was washed with sat. NaCl aq. (100 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (100 g) column chromatography eluted with *n*-hexane/AcOEt (10:0, 9:1, 8:2, 7:3) to afford the target compound **21** 1.37 g (2.6 mmol, 96% yield) as colorless amorphous solid: R_f = 0.5 (*n*-hexane:AcOEt = 6:4); ^{1}H NMR (mixture of rotamers, 500

MHz, $CDCl_3$) δ 8.10-7.77 (m, 3H, Bam-Ar, NH), 7.54-7.30 (m, 3H, Bam-Ar), 6.00-5.65 (m, 1H, All-CH), 5.59-4.08 (m, 8H, Bam-CH_2, All-CH_2, OCH_2, Cys-α-CH, Val-α-CH), 3.31-2.63 (m, 8H, Cys-β-CH_2, Cys, Val-*N*-Me), 2.34-2.07 (m, 1H, Val-β-CH_2), 1.62-1.37 (m, 9H, Boc), 1.08-0.88 (m, 3H, Val-γ-CH_3), 0.88-0.65 (m, 3H, Val-γ-CH_3); ^{13}C NMR (mixture of rotamers, 125 MHz, $CDCl_3$) δ 170.4, 170.3, 170.12, 170.05, 170.0, 169.4, 169.3 (Val-CO, Cys-CO), 167.0, 166.9, 166.8 (Bam-CO), 156.2, 156.1, 154.6, 154.1 (Boc-CO), 133.8, 133.7, (Bam-Ar), 131.61, 131.55, 131.4, 131.3, 131.1 (All-CH, Bam-Ar), 128.4, 128.2, 128.1, 127.4, 127.3, 127.1 (Bam-Ar), 119.5, 119.2, 118.9, 118.7 (All-CH_2), 81.4, 81.2, 81.0, 80.8 (Boc-C), 66.5, 65.9, 65.7, 65.6, 65.4, 65.3, 64.6, 64.5, 62.0, 56.1, 55.3, 54.4, 53.9 (All-OCH_2, Val-α-CH, Cys-α-CH), 43.9, 43.6, 43.3, 42.3, 41.8 (Bam-CH_2), 32.7, 32.1, 31.1, 30.7, 30.4, 29.3, 29.1, 28.9, 28.6, 28.34, 28.30, 28.23, 28.15 (Cys-β-CH_2, Val-*N*-Me, Cys-*N*-Me, Boc-Me), 27.2, 26.8, 26.7 (Val-β-CH_2), 19.64, 19.55,, 19.3, 18.7, 18.5, 17.9, 15.2 (Val-γ-CH_3); HRMS (ESI) calcd for $C_{26}H_{39}N_3NaO_6S^+$ $[M+Na]^+$ 544.2452, found: 544.2477.

_N_-Boc-_L_-Ala-_N_-Me-_L_-Cys(Bam)-_N_-Me-_D_-Val-OAll (22). To a solution of *N*-Boc-*N*-Me-*L*-Cys(Bam)-*D*-MeVal-OAll **21** 1.37 g (2.6 mmol) and triisopropylsilane 0.54 mL (2.6 mmol, 1 equiv.) in CH_2Cl_2 (10 mL) was added TFA 2.0 mL (26.0 mmol, 10 equiv.) at 0 ˚C. Then the resulting solution was warmed to room temperature, and stirred at the temperature for overnight (17 h). The mixture was then quenched with sat. $NaHCO_3$ aq. (100 mL), and extracted with AcOEt (100 mL). The organic layer was washed with sat. NaCl aq. (100 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. Then a solution of the residue (colorless oil, 1.12 g), *N*-Boc-*L*-Ala-OH **12** 0.60 g (3.2 mmol, 1.2 equiv.) and DMT-MM 0.87 g (3.2 mmol, 1.5 equiv.) in CH_3CN (10 mL) was stirred at room temperature for overnight (11 h). The mixture was extracted with AcOEt (100 mL) and water (100 mL). The organic layer was washed with sat. NaCl aq. (100 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (100 g) column chromatography eluted with *n*-hexane/AcOEt (10:0, 8:2, 7:3, 6:4) to afford the target compound **22** 1.17 g (2.0 mmol, 75% yield on 2 steps) as colorless amorphous solid: R_f = 0.4 (*n*-hexane:AcOEt = 6:4); ^1H NMR (mixture of rotamers, 500 MHz, $CDCl_3$) δ 8.07-7.31 (m, 6H, Bam-NH, Ar), 6.06-5.75 (m, 1H, All-CH), 5.60 (t, *J* = 7.2 Hz, 1H, Cys-α-CH), 5.44-5.10 (m, 3H, Ala-NH, All-CH_2), 5.05-4.15 (m, 6H, Bam-CH_2, All-OCH_2, Ala-α-CH, Cys-α-CH), 3.33-3.11 (m, 1H, Cys-β-CH_2), 3.10-2.65 (m, 7H, Cys-*N*-Me, Val-*N*-Me, Cys-α-CH), 2.36-2.10 (m, 1H, Val-β-CH_2), 1.47 (s, 7.1H, Boc-Me), 1.41 (s, 1.9H, Boc-Me), 1.36-1.27 (m, 3H, Ala-β-CH_3), 0.97 (d, *J* = 6.9 Hz, 2.1H, Val-γ-CH_3), 0.95 (d, *J* =

6.9 Hz, 0.7H, Val-γ-CH_3), 0.88-0.67 (m, 3.2H, Val-γ-CH_3); ^{13}C NMR (mixture of rotamers, 125 MHz, $CDCl_3$) δ 173.9, 173.7 (Val-CO), 170.2, 170.1, 169.5 (Cys-CO, Ala-CO), 166.8 (Bam-CO), 155.0 (Boc-CO), 133.7, 131.1 (All-CH), 131.6, 131.5, 128.4, 128.3, 127.31, 127.25 (Bam-Ar), 119.4, 119.2 (All-CH_2), 79.8 (Boc-C), 65.9, 65.8, 65.6, 64.9, 62.1, 54.1, 52.2, 46.7, 46.5 (All-OCH_2, Val-α-CH, Cys-α-CH, Ala-α-CH), 43.7, 42.8 (Bam-CH_2), 31.5, 31.1, 30.5, 30.3, 28.8, 28.7 (Cys-*N*-Me, Val-*N*-Me, Cys-β-CH_2), 28.3 (Boc-Me), 27.0, 26.8 (Val-β-CH), 19.7, 19.4, 18.9, 18.8 (Val-γ-CH_3), 18.3, 17.6 (Ala-β-CH_3); HRMS (ESI) calcd for $C_{29}H_{44}N_4NaO_7S^+$ $[M+Na]^+$ 615.2823, found: 544.2844.

***N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*D*-MeVal-OH (23).** To a solution of *N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*D*-MeVal-OAll **22** 1.08 g (1.8 mmol), PPh_3 19.1 mg (0.073 mmol, 8 mol%), *N*-methylaniline 0.30 mL (2.7 mmol, 1.5 equiv.) in THF (10 mL) was added $Pd_2(dba)_3$ 16.7 mg (0.018 mmol, 2 mol%). The resulting solution was stirred at room temperature for overnight (12 h) in the dark. Then the mixture was extracted with AcOEt (100 mL) and sat. NH_4Cl aq. (100 mL). The organic layer was washed with sat. NaCl aq. (100 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (100 g) column chromatography eluted with CH_2Cl_2/MeOH (10:0, 100:1, 50:1) to afford the target compound **23** 792.5 mg (1.4 mmol, 79% yield) as pale brown amorphous solid: R_f = 0.3 (CH_2Cl_2:MeOH = 9:1); ^1H NMR (mixture of rotamers, 500 MHz, $CDCl_3$) δ 8.30-7.31 (m, 6H, Bam-NH, Ar), 6.01 (t, *J* = 7.2 Hz, 0.57H, Cys-α-CH), 5.73-5.46 (m, 1.43H, Ala-NH, Cys-α-CH), 4.96-4.73 (m, 2H, Bam-CH_2, Ala-α-CH), 4.70-4.61 (m, 1H, Bam-CH_2), 4.44 (dd, *J* = 13.7, 4.6 Hz, 0.74H, Val-α-CH), 4.33 (dd, *J* = 14.0, 4.3 Hz, 0.26H, Val-α-CH), 3.19 (dd, *J* = 14.0, 8.3 Hz, 0.68H, Cys-β-CH_2), 3.12 (dd, *J* = 14.6, 6.6 Hz, 0.32H, Cys-β-CH_2), 3.02 (s, 0.94H, Cys-*N*-Me), 2.97-2.73 (m, 6.06H, Cys-*N*-Me, Val-*N*-Me, Cys-β-CH_2), 2.32-2.08 (m, 1H, Val-β-CH), 1.42 (s, 6.86H, Boc-Me), 1.41 (s, 2.14H, Boc-Me), 1.31 (d, *J* = 6.9 Hz, 1.14H, Ala-β-CH_3), 1.27 (d, *J* = 6.9 Hz, 1.86H, Ala-β-CH_3), 1.00 (d, *J* = 6.9 Hz, 2.05H, Val-γ-CH_3), 0.94 (d, *J* = 5.7 Hz, 0.86H, Val-γ-CH_3), 0.76 (d, *J* = 6.9 Hz, 2.17H, Val-γ-CH_3), 0.74 (d, *J* = 6.9 Hz, 0.93H, Val-γ-CH_3); ^{13}C NMR (mixture of rotamers, 125 MHz, $CDCl_3$) δ 174.2, 173.7 (Val-CO), 172.5, 171.2, 170.0, 169.7 (Ala-CO, Cys-CO), 168.1, 167.2 (Bam-CO), 155.2, 155.1 (Boc-CO), 133.5, 133.1, 131.8, 131.7, 128.4, 127.4, 127.3 (Bam-Ar), 80.1, 79.9 (Boc-C), 65.1, 62.5, 54.2, 51.8, 46.7, 46.5 (Cys-α-CH, Val-α-CH, Ala-α-CH), 43.0, 42.2 (Bam-CH_2), 31.2, 30.9, 30.3, 30.2, 28.9 (Cys-*N*-Me, Val-*N*-Me, Cys-β-CH_2), 28.2 (Boc-Me), 26.9, 26.6 (Val-β-CH), 19.9, 19.4, 19.0, 18.7, 18.4, 18.2 (Val-γ-CH_3, Ala-β-CH_3); HRMS (ESI) calcd for $C_{26}H_{39}N_4O_7S^-$ $[M-H]^-$ 551.2545, found: 551.2528.

***N*-Cbz-*D*-Ser(*N*-Boc-*D*-MeVal)-OAll (24).** To a solution of *N*-Cbz-*D*-Ser-OAll **7** 5 g (17.9 mmol), *N*-Boc-*N*-Me-*D*-Val-OH **18** 4.97 g (21.5 mmol, 1.2 equiv.), HOAt 3.66 g (26.9 mmol, 1.5 equiv.) and NEt_3 3.7 mL (26.9 mmol, 1.5 equiv.) in CH_2Cl_2 (100 mL) was added EDCI·HCl 5.15 g (26.9 mmol, 1.5 equiv.), and the resulting solution was stirred at room temperature for overnight (17 h). The mixture was then extracted with AcOEt (300 mL × 2) and water (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (200 g) column chromatography eluted with *n*-hexane/AcOEt (10:0, 10:1, 9:1, 8:2) to afford the target compound **24** 7.12 g (14.5 mmol, 81% yield) as yellow oil: R_f = 0.7 (*n*-hexane:AcOEt = 7:3); ^{1}H NMR (mixture of rotamers, 500 MHz, $CDCl_3$) δ 7.44-7.28 (m, 5H, Cbz-Ar), 5.88 (br, 1H, All-CH), 5.72 (br, 0.59H, Ser-NH), 5.54 (br, 0.41H, Ser-NH), 5.32 (d, *J* = 16.6 Hz, 1H, All-CH_2 (*E*)), 5.26 (d, *J* = 10.3 Hz, 1H, All-CH_2 (*E*)), 5.12 (s, 2H, Cbz-CH_2), 4.80-4.01 (m, 6H, All-OCH_2, Ser-β-CH_2, Ser-α-CH, Val-α-CH), 2.78 (s, 1.63H, Val-*N*-Me), 2.76 (s, 1.37H, Val-*N*-Me), 2.15 (br, 1H, Val-β-CH), 1.44 (s, 9H), 0.95 (d, *J* = 5.2 Hz, 3H, Val-γ-CH_3), 0.87 (d, *J* = 6.3 Hz, 3H, Val-γ-CH_3); ^{13}C NMR (mixture of rotamers, 125 MHz, $CDCl_3$) δ 171.0, 170.6 (Val-CO), 169.0, 168.9 (Ser-CO), 156.2, 155.9, 155.7, 155.4 (Cbz-CO, Boc-CO), 136.2, 136.1 (All-CH), 131.4, 131.2, 128.6, 128.2 (Cbz-Ar), 119.4, 119.2 (All-CH_2), 80.4, 80.3 (Boc-C), 67.2, 66.7, 66.6, 65.1, 64.4, 64.1, 63.6 (Cbz-CH_2, All-OCH_2, Ser-β-CH_2, Ser-α-CH), 53.5 (Val-α-CH), 30.9, 30.7 (Val-*N*-Me), 28.4 (Boc-Me), 27.7, 27.5 (Val-β-CH), 20.0, 19.9, 18.9 (Val-γ-CH_3); HRMS (ESI) calcd for $C_{25}H_{36}N_2NaO_8^+$ $[M+Na]^+$ 515.2364, found: 515.2372.

***N*-Cbz-*D*-Ser[*N*-Boc-*N*-Me-*L*-Cys(Bam)-*N*-Me-*D*-Val]-OAll (25).** To a solution of *N*-Cbz-*D*-Ser(*N*-Boc-*N*-Me-*D*-Val)-OAll **24** 7.12 g (14.5 mmol) in AcOEt (30 mL) was added 4 M HCl in AcOEt 20 mL (80 mmol) at 0 ˚C. Then the resulting solution was warmed to room temperature, and stirred at the temperature for overnight (16 h). The mixture was concentrated under the reduced pressure, and the residue was extracted with AcOEt (300 mL × 2) and sat. $NaHCO_3$ aq. (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. Then a solution of the residue (pale yellow oil, 5.66 g), *N*-Boc-*N*-Me-*L*-Cys(Bam)-OH **10** 6.39 g (17.3 mmol, 1.2 equiv.) and DMT-MM 6.00 g (21.7 mmol, 1.5 equiv.) in AcOEt (50 mL) was stirred at room temperature for overnight (14 h). The mixture was extracted with AcOEt (300 mL × 2) and water (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (200 g) column chromatography eluted with *n*-hexane/AcOEt (10:0, 8:2, 7:3, 6:4) to afford the

target compound **25** 8.19 g (11.0 mmol, 76% yield on 2 steps) as pale yellow amorphous solid: R_f = 0.5 (*n*-hexane:AcOEt = 5:5); ^{1}H NMR (mixture of rotamers, 500 MHz, $CDCl_3$) δ 8.00-7.72, 7.56-7.27 (m, 11H, Bam-NH, Ar, Cbz-Ar), 6.29 (d, *J* = 8.6 Hz, 0.83H, Ser-NH), 5.95-5.75 (m, 1H, All-CH), 5.63 (d, *J* = 7.4 Hz, 0.17H, Ser-NH), 5.42-5.20 (m, 2H, All-CH_2), 5.20-4.04 (m, 11H, Cbz-CH_2, All-OCH_2, Cys-α-CH, Bam-CH_2, Ser-α-CH, Ser-β-CH_2, Ala-α-CH), 3.24-2.53 (m, 8H, Cys-β-CH_2, Cys-*N*-Me, Val-*N*-Me), 2.36-2.08 (m, 1H, Val-β-CH), 1.50 (s, 1.21H, Boc-Me), 1.46 (s, 2.32H, Boc-Me), 1.44 (s, 5.47H, Boc-Me), 1.03 (d, *J* = 6.3 Hz, 0.13H, Val-γ-CH_3), 0.98 (d, *J* = 6.3 Hz, 0.37H, Val-γ-CH_3), 0.90 (d, *J* = 6.9 Hz, 2.45H, Val-γ-CH_3), 0.86 (d, *J* = 6.3 Hz, 0.27H, Val-γ-CH_3), 0.80 (d, *J* = 6.9 Hz, 0.30H, Val-γ-CH_3), 0.76 (d, *J* = 6.3 Hz, 2.49H, Val-γ-CH_3); ^{13}C NMR (mixture of rotamers, 125 MHz, $CDCl_3$) δ 170.5, 170.2, 170.1, 169.8, 169.4, 168.9, 168.7, 167.4, 167.2, 166.8 (Val-CO, Cys-CO, Ser-CO, Bam-CO), 156.25, 156.16, 156.0, 155.6 (Cbz-CO, Boc-CO), 135.9, 135.8, 135.7, 134.1, 133.8, 128.5, 128.4, 128.3, 128.2, 128.1, 127.3, 127.1 (Cbz-Ar, Bam-Ar), 131.5, 131.3, 131.1, 130.8 (All-CH), 119.4, 119.2 (All-CH_2), 81.3, 80.9 (Boc-C), 67.3, 67.1, 66.9, 66.8, 66.53, 66.48, 65.8, 64.5, 64.1, 63.9, 62.7, 58.2, 55.2, 54.5, 53.7, 53.4, 53.1 (All-OCH_2, Ser-β-CH_2, Ser-α-CH, Val-α-CH, Cys-α-CH), 43.9, 43.3, 41.4, 41.0 (Bam-CH_2), 32.1, 31.4, 30.6, 30.2, 29.6, 29.4, 29.1, 28.9 (Cys-β-CH_2, Cys-*N*-Me, Val-*N*-Me), 28.5, 28.4, 28.2 (Boc-Me), 27.1, 26.7 (Val-β-CH), 19.7, 19.3, 18.9, 18.3, 18.2, 17.8 (Val-γ-CH_3); HRMS (ESI) calcd for $C_{37}H_{50}N_4NaO_{10}S^+$ $[M+Na]^+$ 765.3140, found: 765.3112.

***N*-Cbz-*D*-Ser[*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*D*-Val]-OAll (26).** To a solution of *N*-Cbz-*D*-Ser[*N*-Boc-*N*-Me-*L*-Cys(Bam)-*N*-Me-*D*-Val]-OAll **25** 8.19 g (11.0 mmol) in AcOEt (20 mL) was added 4 M HCl in AcOEt 20 mL (80 mmol) at 0 ˚C. Then the resulting solution was warmed to room temperature, and stirred at the temperature for overnight (9 h). The mixture was concentrated under the reduced pressure, and the residue was extracted with AcOEt (300 mL × 2) and sat. $NaHCO_3$ aq. (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. Then a solution of the residue (pale yellow amorphous solid), *N*-Boc-*L*-Ala-OH **12** 2.50 g (13.2 mmol, 1.2 equiv.) and DMT-MM 4.58 g (16.5 mmol, 1.5 equiv.) in DMF (40 mL) was stirred at room temperature for overnight (17 h). The mixture was extracted with AcOEt (300 mL × 2) and water (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (200 g) column chromatography eluted with *n*-hexane/AcOEt (10:0, 8:2, 7:3, 6:4, 5:5) to afford the target compound **26** 6.72 g (8.3 mmol, 75% yield on 2 steps) as pale yellow

amorphous solid: R_f = 0.4 (*n*-hexane:AcOEt = 6:4); ^{1}H NMR (mixture of rotamers, 500 MHz, $CDCl_3$) δ 8.00-7.60, 7.57-7.25 (m, 11H, Bam-NH, Ar, Cbz-Ar), 6.46 (d, *J* = 6.9 Hz, 0.39H, Ser-NH or Ala-NH), 5.94-5.80 (m, 1H, All-CH), 5.80-5.72 (m, 0.42H, Cys-α-CH), 5.54 (t, *J* = 7.2 Hz, 0.58H, Cys-α-CH), 5.42 (d, *J* = 8.0 Hz, 0.61H, Ser-NH or Ala-NH), 5.36-5.19 (m, 2H, All-CH_2), 5.11 (s, 2H, Cbz-CH_2), 5.06 (d, *J* = 12.0 Hz, 0.58H, Ala-α-CH or Ser-α-CH), 5.00 (d, *J* = 12.0 Hz, 0.42H, Ala-α-CH or Ser-α-CH), 4.79 (dd, *J* = 14.0, 7.2 Hz, 1H, Bam-CH_2), 4.73-4.31 (m, 7H, Bam-CH_2, All-OCH_2, Ser-β-CH_2, Ala-α-CH or Ser-α-CH, Ser-NH or Ala-NH), 4.28 (d, *J* = 8.6 Hz, 0.53H, Val-α-CH), 4.20 (d, *J* = 10.9 Hz, 0.47H, Val-α-CH), 3.32-2.68 (m, 8H, Cys-β-CH_2, Cys-*N*-Me, Val-*N*-Me), 2.28-2.07 (m, 1H, Val-β-CH), 1.44 (s, 4.71H, Boc-Me), 1.43 (s, 4.29H, Boc-Me), 1.35-1.12 (m, 3H, Ala-β-CH_3), 0.91 (d, *J* = 6.3 Hz, 1.76H), 0.87 (d, *J* = 6.3 Hz, 1.19H), 0.76 (d, *J* = 6.9 Hz, 1.71H), 0.71 (d, *J* = 6.3 Hz, 1.20H), 0.66 (d, *J* = 6.3 Hz, 0.08H), 0.58 (d, *J* = 6.9 Hz, 0.06H); ^{13}C NMR (mixture of rotamers, 125 MHz, $CDCl_3$) δ 173.9, 173.6, 170.1, 169.9, 169.8, 169.5, 169.3, 168.9, 167.1, 166.8 (Ala-CO, Cys-CO, Ser-CO, Val-CO, Bam-CO), 156.1, 155.7, 155.0, 154.9 (Cbz-CO, Boc-CO), 135.9, 133.7, 133.6, 128.5, 128.4, 128.24, 128.23, 128.10, 128.05, 127.8, 127.2 (Cbz-Ar, Bam-Ar), 133.7, 133.6, 131.0, 130.9 (All-CH), 119.3, 119.1 (All-CH_2), 79.8, 79.7 (Boc-C), 67.3, 67.1, 66.7, 66.5, 64.5, 64.0 (Cbz-CH_2, All-OCH_2, Ser-β-CH_2), 62.6, 55.8, 54.0, 53.3, 51.9, 46.7, 46.5 (Ala-α-CH, Cys-α-CH, Ser-α-CH, Val-α-CH), 42.8, 41.7 (Bam-CH_2), 31.2, 31.1, 30.3, 30.0 (Cys-β-CH_2, *N*-Me, Val-*N*-Me), 28.3 (Val-β-CH), 19.7, 19.4, 19.0, 18.8, 18.5, 18.1 (Val-γ-CH_3, Ala-β-CH_3); HRMS (ESI) calcd for $C_{40}H_{55}N_5NaO_{11}S^+$ $[M+Na]^+$ 836.3511, found: 836.3486.

_N_-Cbz-_D_-Ser[_N_-Boc-_L_-Ala-_N_-Me-_L_-Cys(Bam)-_N_-Me-_L_-Val]-OH (27). To a solution of *N*-Cbz-*D*-Ser[*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val]-OAll **2** 3 g (3.7 mmol), PPh_3 78.7 mg (0.30 mmol, 8 mol%), *N*-methylaniline 0.48 mL (4.4 mmol, 1.2 equiv.) in THF (40 mL) was added $Pd_2(dba)_3$ 67.8 mg (0.074 mmol, 2 mol%). The resulting solution was stirred at room temperature for overnight (17 h) in the dark. Then the mixture extracted with AcOEt (300 mL) and sat. NH_4Cl aq. (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (200 g) column chromatography eluted with CH_2Cl_2/MeOH (10:0, 100:1, 50:1) to afford the target compound **27** 2.80 g (3.6 mmol, 97% yield) as yellow amorphous solid: R_f = 0.3 (CH_2Cl_2:MeOH = 10:1); ^{1}H NMR (mixture of rotamers, 500 MHz, $CDCl_3$) δ 7.95-7.71, 7.67-7.20 (m, 11H, Bam-NH, Ar, Cbz-Ar), 5.80 (dd, *J* = 10.0, 4.9 Hz, 1H, Cys-α-CH), 5.74 (d, *J* = 6.3 Hz, 1H, Ser-NH), 5.48 (d, *J* = 8.0 Hz, 1H, Ala-NH), 5.19 (dd, *J* = 14.0, 8.3 Hz,

1H, Bam-CH_2), 5.15-5.09 (m, 2H, Cbz-CH_2), 4.84 (d, J = 10.9 Hz, 1H, Val-α-CH), 4.71 (dd, J = 11.2, 2.6 Hz, 1H, Ser-β-CH_2), 4.68-4.59 (m, 1H, Ala-α-CH), 4.59-4.48 (m, 1H, Ser-α-CH), 4.32 (dd, J = 11.5, 2.9 Hz, 1H, Ser-β-CH_2), 4.13 (dd, J = 14.3, 4.0 Hz, 1H, Bam-CH_2), 3.19 (dd, J = 14.9, 4.6 Hz, 1H, Cys-β-CH_2), 3.10-2.68 (m, 7H, Cys-β-CH_2, Cys-*N*-Me, Val-*N*-Me), 2.31-2.03 (m, 1H, Val-β-CH), 1.48 (s, 0.89H, Boc-CH_3), 1.45 (s, 7.40H, Boc-CH_3), 1.42 (s, 0.72H, Boc-CH_3), 1.31 (d, J = 6.9 Hz, 3H, Ala-β-CH_3), 1.02 (d, J = 6.9 Hz, 3H, Val-γ-CH_3), 0.78 (d, J = 6.9 Hz, 3H, Val-γ-CH_3); ^{13}C NMR (mixture of rotamers, 125 MHz, $CDCl_3$) δ 173.8, 170.5, 170.1, 169.6, 169.0 (Ser-CO, Ala-CO, Cys-CO, Val-CO, Bam-CO), 155.6, 155.0 (Cbz-CO, Boc-CO), 136.0, 133.0, 132.0, 128.5, 128.4, 128.0, 128.0, 127.3, 127.2 (Cbz-Ar, Bam-Ar), 79.7 (Boc-C), 66.9, 64.2 (Cbz-CH_2, Ser-β-CH_2), 62.2, 53.4, 53.3, 52.7 46.7 (Ser-α-CH, Ala-α-CH, Cys-α-CH, Val-α-CH), 41.8 (Bam-CH_2), 31.1, 30.1 (Cys-*N*-Me, Val-*N*-Me), 30.0 (Cys-β-CH_2), 28.2 (Boc-CH_3), 26.7 (Val-β-CH), 19.7, 18.7, 18.6 (Val-γ-CH_3, Ala-β-CH_3); HRMS (ESI) calcd for $C_{37}H_{50}N_5O_{11}S^-$ $[M-H]^-$ 772.3233, found: 772.3233.

***N*-Cbz-*D*-Ser[*N*-Cbz-*D*-Ser(*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val)-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val]-OAll (28).** To a solution of *N*-Cbz-*D*-Ser[*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val]-OAll **2** 2.52 g (3.1 mmol, 1.2 equiv.) in AcOEt (25 mL) was added 4 M HCl in AcOEt 5 mL (20 mmol) at 0 ˚C. Then the resulting solution was warmed to room temperature, and stirred at the temperature for overnight (17 h). The mixture was concentrated under the reduced pressure, and the residue was extracted with AcOEt (300 mL) and sat. $NaHCO_3$ aq. (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. Then a solution of the residue (colorless amorphous solid, 2.21 g), *N*-Cbz-*D*-Ser[*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val]-OH **27** 2 g (2.6 mmol) and DMT-MM 0.86 g (3.1 mmol, 1.2 equiv.) in AcOEt (30 mL) was stirred at room temperature for overnight (21 h). The mixture was then filtrated, and the filtrate was extracted with AcOEt (300 mL × 2) and water (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (200 g) column chromatography eluted with *n*-hexane/AcOEt (10:0, 6:4, 4:6, 2:8) to afford the target compound **28** 3.20 g (2.2 mmol, 85% yield on 2 steps) as pale yellow amorphous solid: R_f = 0.5 (*n*-hexane:AcOEt = 2:8); HRMS (ESI) calcd for $C_{72}H_{96}N_{10}NaO_{19}S_2^+$ $[M+Na]^+$ 1491.6187, found: 1491.6184; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) chart were shown on page S74, 75 in Supplementary Information.

***N*-Cbz-*D*-Ser[*N*-Cbz-*D*-Ser(*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val)-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val]-OH (29).** To a solution of *N*-Cbz-*D*-Ser[*N*-Cbz-*D*-Ser(*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val)-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val]-OAll **28** 1 g (0.68 mmol), PPh_3 14.2 mg (0.052 mmol, 8 mol%), *N*-methylaniline 0.09 mL (0.82 mmol, 1.2 equiv.) in THF (20 mL) was added $Pd_2(dba)_3$ 12.8 mg (0.014 mmol, 2 mol%). The resulting solution was stirred at room temperature for overnight (14 h) in the dark. Then the mixture was extracted with AcOEt (100 mL) and sat. NH_4Cl aq. (100 mL). The organic layer was washed with sat. NaCl aq. (100 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (100 g) column chromatography eluted with CH_2Cl_2/MeOH (10:0, 100:1, 50:1, 20:1) to afford the target compound **29** 888.4 mg (0.62 mmol, 91% yield) as yellow amorphous solid: R_f = 0.3 (CH_2Cl_2:MeOH = 10:1); HRMS (ESI) calcd for $C_{69}H_{91}N_{10}O_{19}S_2^-$ $[M-H]^-$ 1427.5909, found: 1427.5884; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) chart were shown in on page S76, 77 in Supplementary Information.

(*N*-Cbz-*D*-Ser-*L*-Ala-*N*-Me-*L*-Cys-*N*-Me-*L*-Val)$_2$ (Serine-hydroxy)-Dilactone Disulfide (1).[17] To a solution of Boc-*L, L*-octadepsipeptide-OH **29** 500 mg (0.35 mmol) in CH_3CN (300 mL, total 0.001 M) was added a solution of iodine 887.7 mg (3.5 mmol, 10 equiv.) in CH_3CN (50 mL). The resulting solution was stirred at room temperature for overnight (12 h). The reaction solution was then quenched with sat. $Na_2S_2O_3$ aq. (100 mL), and the mixture was concentrated under the reduced pressure to remove CH_3CN. Then the concentrated solution was extracted with AcOEt (200 mL). The organic layer was washed with sat. NaCl aq. (100 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. To a solution of the residue (pale yellow amorphous solid, 0.38 g) and HOAt 285.6 mg (2.1 mmol, 6 equiv.) in CH_2Cl_2 (350 mL, 0.001 M) was added EDCI·HCl 402.3 mg (2.1 mmol, 6 equiv.). The resulting solution was stirred at room temperature for overnight (24 h). The mixture was then washed with water (100 mL). The organic layer was washed with sat. NaCl aq. (100 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (30 g) column chromatography eluted with *n*-hexane/AcOEt (10:0, 6:4, 4:6, 3:7, 2:8) to afford the target compound **1** 176.1 mg (0.17 mmol, 48% yield on 2 steps) as colorless solid: R_f = 0.5 (*n*-hexane:AcOEt = 2:8); ^1H NMR (500 MHz, $CDCl_3$) δ 7.94 (d, *J* = 10.3 Hz, 2H、Ser-NH), 7.54 (d, *J* = 8.6 Hz, 2H, Ala-NH), 7.48-7.28 (m, 10H, Cbz-Ar), 5.84 (t, *J* = 7.7 Hz, 2H, Cys-α-CH), 5.53 (d, *J* = 12.0 Hz, 2H, Cbz-CH_2), 5.02-4.82 (m, 6H, Ser- α-CH, Cbz-CH_2, Ala-α-CH), 4.69 (d, *J* = 10.9 Hz, 2H, Ser-β-CH_2), 4.39 (d, *J* = 10.9 Hz, 2H,

Ser-β-CH_2), 3.94 (d, *J* = 10.3 Hz, 2H, Val-α-CH), 3.41-2.80 (m, 16H, Cys-β-CH_2, Cys-*N*-Me, Val-*N*-Me), 2.47-2.14 (m, 2H, Val-β-CH), 0.96 (d, *J* = 6.3 Hz, 6H, Val-γ-CH_3), 0.91 (d, *J* = 6.9 Hz, 6H, Val-γ-CH_3), 0.76 (d, *J* = 6.9 Hz, 6H, Val-γ-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3 (Ala-CO), 170.0 (Val-CO), 169.8 (Cys-CO), 167.7 (Ser-CO), 156.6 (Cbz-CO), 135.3, 129.7, 128.7, 128.6 (Cbz-Ar), 68.6 (Cbz-CH_2), 65.8 (Ser-β-CH_2), 65.2 (Val-α-CH), 53.8 (Ser-α-CH), 50.6 (Cys-α-CH), 44.8 (Al-α-CH), 40.5 (Cys-β-CH_2), 31.0 (Cys-*N*-Me), 29.4 (Val-*N*-Me), 20.0, 19.8 (Val-γ-CH_3), 16.9 (Ala-β-CH_3); HRMS (ESI) calcd for $C_{48}H_{66}N_8NaO_{14}S_2^+$ $[M+Na]^+$ 1065.4032, found: 1065.4058; m.p. 131.1-135.2 °C; $[\alpha]_D^{27.6}$ +48.4 ° (c 0.1, $CHCl_3$).

(*N*-Cbz-*D*-Ser-*L*-Ala-*N*-Me-*S*-monoxide-*L*-Cys-*N*-Me-*L*-Val)-(*N*-Cbz-*D*-Ser-*L*-Ala-*N*-Me-*L*-Cys-*N*-Me-*L*-Val) (Serine-hydroxy)-Dilactone Disulfide (44). To a solution of macrolide **1** 50 mg (0.048 mmol) in CH_2Cl_2 (5 mL) was added *m*CPBA 14.3 mg (0.058 mmol, 1.2 equiv.) at 0 ˚C, and the mixture was stirred at 0 ˚C for 8 h. The reaction mixture was quenched with sat. $NaHCO_3$ aq. (50 mL), and then extracted with AcOEt (50 mL). The organic layer was washed with sat. NaCl aq. (50 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (10 g) column chromatography eluted with *n*-hexane/AcOEt (10:0, 6:4, 4:6, 3:7, 2:8) to afford the target compound **44** 24.7 mg (0.023 mmol, 48% yield) as colorless solid: R_f = 0.15 (*n*-hexane:AcOEt = 2:8); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.16 (d, *J* = 10.3 Hz, 1H, Ser-NH), 7.78 (d, *J* = 9.7 Hz, 1H, Ser-NH), 7.65 (d, *J* = 8.6 Hz, 1H, Ala-NH), 7.61 (d, *J* = 8.0 Hz, 1H, Ala-NH), 7.54-7.10 (m, 10H, Cbz-Ar), 6.68 (d, *J* = 11.5 Hz, 1H, Cys-α-CH), 5.69 (dd, *J* = 12.3, 2.0 Hz, 1H, Cys-α-CH), 5.51 (d, *J* = 6.9 Hz, 1H, Cbz-CH_2), 5.49 (d, *J* = 6.9 Hz, 1H, Cbz-CH_2), 5.02-4.84 (m, 6H, Ser-α-CH, Cbz-CH_2, Ser-β-CH, Ala-α-CH), 4.75 (dd, *J* = 10.9, 2.3 Hz, 1H, Ser-β-CH_2), 4.66-4.54 (m, 1H, Ala-α-CH), 4.23-4.06 (m, 4H, Ser-β-CH_2, Val-α-CH), 3.87 (dd, *J* = 15.5, 12.6 Hz, 1H, Cys-α-CH_2), 3.78 (dd, *J* = 16.0, 12.6 Hz, 1H, Cys-β-CH_2), 3.23 (s, 6H, Cys-*N*-Me), 3.12 (d, *J* = 13.7 Hz, 1H, Cys-β-CH_2), 2.91 (s, 3H, Val-*N*-Me), 2.90 (s, 3H, Val-*N*-Me), 2.70 (d, *J* = 14.9 Hz, 1H, Cys-β-CH_2), 2.51-2.14 (m, 2H, Val-β-CH), 0.97 (d, *J* = 6.3 Hz, 3H, Val-γ-CH_3), 0.94-0.89 (m, 6H, Val-γ-CH_3), 0.87 (d, *J* = 6.9 Hz, 3H, Val-γ-CH_3), 0.83 (d, *J* = 6.9 Hz, 3H, Ala-β-CH_3), 0.80 (d, *J* = 6.9 Hz, 3H, Ala-β-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.1, 174.0 (Ala-CO), 170.1, 169.73 (Cys-CO), 169.70, 168.8 (Val-CO), 167.4, 167.2 (Ala-CO), 156.8, 156.6 (Cbz-CO), 135.5, 129.7, 129.6, 128.5 (Cbz-Ar), 68.4, 68.3 (Cbz-CH_2), 66.9 (Ser-β-CH_2), 65.3, 64.1 (Val-α-CH), 53.8, 53.7 (Ser-α-CH), 53.0, 47.5 (Cys-α-CH), 47.4 (Cys-β-CH_2), 45.9, 45.2 (Ala-α-CH), 33.3 (Cys-β-CH_2), 31.9, 31.5 (Cys-*N*-Me), 28.6 (Val-*N*-Me), 28.4, 28.3 (Val-β-CH), 19.4, 19.0, 18.9, 18.2 (Val-γ-CH_3), 17.34, 17.26 (Ala-β-CH_3); HRMS (ESI)

calcd for $C_{48}H_{66}N_8NaO_{15}S_2^+$ $[M+Na]^+$ 1081.3981, found: 1081.3954; m.p. 155.7-157.9 °C; $[\alpha]_D^{27.1}$ -26.0 ° (c 0.1, $CHCl_3$); IR (KBr) 1131 (SO) cm^{-1}.

(*N*-Cbz-*D*-Ser-*L*-Ala-*N*-Me-*S*-dioxide-*L*-Cys-*N*-Me-*L*-Val)-(*N*-Cbz-*D*-Ser-*L*-Ala-*N*-Me-*L*-Cys-*N*-Me-*L*-Val) (Serine-hydroxy)-Dilactone Disulfide (45). A solution of macrolide **1** 100 mg (0.096 mmol) and Oxone® 129.1 mg (0.21 mmol, 2.2 equiv.) in a mixture of THF (4 mL) and H_2O (1 mL) was stirred at room temperature for overnight (12 h). The mixture was then extracted with AcOEt (50 mL) and water (50 mL). The organic layer was washed with sat. NaCl aq. (50 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (10 g) column chromatography eluted with *n*-hexane/AcOEt (10:0, 6:4, 4:6, 3:7, 2:8) to afford the target compound **45** 60.5 mg (0.056 mmol, 58% yield) as colorless solid: R_f = 0.45 (*n*-hexane:AcOEt = 2:8); ^1H NMR (500 MHz, $CDCl_3$) δ 8.08 (d, *J* = 10.3 Hz, 1H, Ser-NH), 7.63 (d, *J* = 8.0 Hz, 1H, Ala-NH), 7.61 (d, *J* = 10.0 Hz, 1H, Ser-NH), 7.58 (d, *J* = 8.0 Hz, 1H, Ala-NH), 7.49-7.20 (m, 10H, Cbz-Ar), 6.35 (d, *J* = 12.0 Hz, 1H, Cys-α-CH), 5.95 (dd, *J* = 10.3, 5.7 Hz, 1H, Cys-α-CH), 5.50 (d, *J* = 10.9 Hz, 1H, Cbz-CH_2), 5.48 (d, *J* = 11.0 Hz, 1H, Cbz-CH_2), 5.08-4.83 (m, 6H, Ser-α-CH, Cbz-CH_2, , Ser-β-CH_2, Ala-α-CH), 4.80 (dd, *J* = 10.9, 2.3 Hz, 1H, Ser-β-CH_2), 4.77-4.57 (m, 1H, Ala-α-CH), 4.34-4.06 (m, 3H, Ser-β-CH_2, Val-α-CH), 4.06-3.90 (m, 2H, Cys-β-CH_2, Val-α-CH), 3.75-3.47 (m, 2H, Cys-β-CH_2), 3.29 (d, *J* = 15.5 Hz, 1H, Cys-β-CH_2), 3.24 (s, 3H, Cys-*N*-Me), 3.17 (s, 3H, Cys-*N*-Me), 2.91 (s, 6H, Val-*N*-Me), 2.48-2.24 (m, 2H, Val-β-CH), 1.10-0.95 (m, 6H, Val-γ-CH_3), 0.93 (d, *J* = 6.5 Hz, 3H, Val-γ-CH_3), 0.92 (d, *J* = 6.5 Hz, 3H, Val-γ-CH_3), 0.82 (d, *J* = 6.9 Hz, 3H, Ala-β-CH_3), 0.79 (d, *J* = 6.9 Hz, 3H, Val-β-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.6, 174.4 (Ala-CO), 169.7 (Cys-CO), 169.4, 168.5 (Val-CO), 168.2 (Cys-CO), 167.3, 167.3 (Ser-CO), 156.61, 156.58 (Cbz-CO), 135.41, 135.38, 129.7, 128.6, 128.5 (Cbz-Ar), 68.6, 68.4 (Cbz-CH_2), 66.9 (Ser-β-CH_2), 65.7, 64.3 (Val-α-CH), 56.4 (Cys-β-CH_2), 53.8, 53.7 (Ser-α-CH), 51.5, 45.8 (Cys-α-CH), 45.7, 45.2 (Ala-α-CH), 31.4, 31.3 (Cys-*N*-Me), 29.5 (Cys-β-CH_2), 28.8, 28.4 (Val-*N*-Me), 28.22, 28.19 (Val-β-CH), 19.4, 19.3, 18.2, 18.1 (Val-γ-CH_3), 17.4, 17.2 (Ala-β-CH_3); HRMS (ESI) calcd for $C_{48}H_{66}N_8NaO_{16}S_2^+$ $[M+Na]^+$ 1097.3930, found: 1097.3941, m.p. 153.6-156.9 °C, $[\alpha]_D^{27.1}$ -40.9 ° (c 0.1, $CHCl_3$) ; IR (KBr) 1315 (SO_2), 1123 (SO_2) cm^{-1}.

[*N*-(Quinoxaline-2-carbonyl)-*D*-Ser-*L*-Ala-*N*-Me-*L*-Cys-*N*-Me-*L*-Val]$_2$ (Serine-hydroxy)-Dilactone Disulfide (Triostin A, TA). A solution of macrolide **1** 50 mg (0.048 mmol) and thioanisole 0.06 mL (0.48 mmol, 10 equiv.) in TFA (5 mL) was stirred at 50 ˚C for overnight (14 h). Then the mixture was concentrated under the reduced

pressure. To solution of the residue (a mixture of solid and oil, 85.1 mg), *N*-methylmorpholine 0.03 mL (0.29 mmol, 6 equiv.), and 2-quinoxalinecarboxylic acid **37** 33.5 mg (0.19 mmol, 4 equiv.) in DMF (5 mL) was added DMT-MM 80.2 mg (0.29 mmol. 6 equiv.). The resulting solution was stirred at room temperature for overnight (21 h). The mixture was then extracted with AcOEt (50 mL) and water (50 mL). The organic layer was washed with sat. NaCl aq. (50 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (30 g) column chromatography eluted with CH_2Cl_2/MeOH (10:0, 200:1, 100:1, 50:1) to afford the target compound, TA 42.6 mg (0.039 mmol, 81% yield on 2 steps) as colorless solid. In the 200 mg (0.19 mmol) scale, the target compound, TA was obtained 154.7 mg (0.14 mmol, 75% yield on 2 steps): R_f = 0.1 (AcOEt only); ^{1}H NMR (mixture of conformers, 500 MHz, $CDCl_3$) δ 9.65 (s, 0.88H, Qx-C3), 9.59 (s, 1.12H, Qx-C3), 8.98 (d, *J* = 8.6 Hz, 1.12H, Ser-NH), 8.83 (d, *J* = 6.9 Hz, 1.12H, Ser-NH), 8.40 (d, *J* = 9.7 Hz, 1.27H, Ala-NH), 8.27-8.11, 8.11-7.99, 7.94-7.79 (m, 2H+2H+4H, Qx), 7.23 (d, *J* = 4.6 Hz, 0.73H, Ala-NH), 6.78 (br, 0.72H, Cys-α-CH), 5.73 (t, *J* = 7.4 Hz, 1.28H, Cys-α-CH), 5.22 (d, *J* = 10.3 Hz, 0.95H, Val-α-CH), 5.15-5.00 (m, 1.19H+1.16H, Ser-α-CH and Ala-α-CH), 4.97 (td, *J* = 7.0, 1.3 Hz, 0.81H, Ser-α-CH), 4.79 (q, *J* = 6.5 Hz, 0.84H, Ala-α-CH), 4.74 (dd, *J* = 11.5, 6.5 Hz, 1.23H, Ser-β-CH_2), 4.64 (d, *J* = 10.3 Hz, 0.84H, Ser-β-CH_2), 4.59 (dd, *J* = 11.2, 1.4 Hz, 1.16H, Ser-β-CH_2), 4.50 (dd, *J* = 11.2, 7.2 Hz, 0.77H, Ser-β-CH_2), 4.27 (d, *J* = 10.3 Hz, 1.05H, Val-α-CH), 3.45-3.25, 3.27-3.03 (m, 4H, major and minor Cys-β-CH_2), 3.32, 3.00 (s, 3H, major and minor Cys-*N*-Me), 3.13, 3.05 (s, 3H, minor and major Val-*N*-Me), 2.43-2.28 (m, 2H, Val-β-CH), 1.46 (d, *J* = 6.9 Hz, 2.48H, Ala-β-CH_3), 1.13 (d, *J* = 6.3 Hz, 3.47H, Val-γ-CH_3), 1.09 (d, *J* = 6.9 Hz, 2.68H, Val-γ-CH_3), 1.06 (d, *J* = 6.3 Hz, 3.41H, Val-γ-CH_3), 0.88 (d, *J* = 6.9 Hz, 2.44H, Val-γ-CH_3), 0.72 (d, *J* = 6.3 Hz, 3.52H, Ala-β-CH_3); ^{1}H NMR (single conformer, 500 MHz, DMSO-d_6) δ 9.53 (s, 2H, Qx-C3), 8.52 (d, J = 9.7 Hz, 2H, Ser-NH), 8.25-8.21 (m, 2H, Qx-C5 and C8), 8.19 (d, J = 5.7 Hz, 2H, Ala-NH), 8.03-7.92 (m, 6H, Qx-C6 and C7), 6.16 (dd, J = 10.9, 3.4 Hz, 2H, Cys-α-CH), 4.92 (dd, J = 9.5, 3.7 Hz, 2H, Ser-α-CH), 4.83 (d, J = 9.7 Hz, 2H, Val-α-CH), 4.66-4.49 (m, 4H, Ser-β-CH_2 and Ala-α-CH), 4.44 (dd, J = 10.9, 4.0 Hz, 2H, Ser-β-CH_2), 3.61 (t, J = 12.3 Hz, 2H, Cys-β-CH_2), 3.31 (s, 6H, Cys-*N*-Me), 2.86 (s, 6H, Val-*N*-Me), 2.43-2.33 (m, 2H, Val-β-CH), 2.31 (dd, J = 13.5, 3.2 Hz, 2H, Cys-β-CH_2), 1.30 (d, J = 7.4 Hz, 6H, Ala-β-CH_3), 0.99 (d, *J* = 7.0 Hz, 6H, Val-γ-CH_3), 0.95 (d, *J* = 7.5 Hz, 6H, Val-γ-CH_3); ^{13}C NMR (mixture of conformers, 125 MHz, $CDCl_3$) δ 172.9, 172.8 (major and minor Ala-CO), 170.6 (major Val-CO), 170.4 (major Cys-CO), 170.2 (minor Val-CO), 169.3 (minor Cys-CO), 168.4 (major Ser-CO), 167.9 (minor Ser-CO), 163.9, 163.7 (major and minor Qx-CO), 144.2, 143.9, 143.8, 143.6, 142.7, 142.6, 140.25, 142.22, 132.1, 132.0, 131.3,

131.0, 129.7, 129.61, 129.57, 129.48 (Qx), 65.6 (minor Ser-β-CH_2), 65.2 (major Val-α-CH), 64.6 (major Ser-β-CH_2), 61.9 (minor Val-α-CH), 53.8 (minor Cys-α-CH), 53.4 (major Ser-α-CH), 53.2 (major Cys-α-CH), 51.7 (minor Ser-α-CH), 47.2 (minor Ala-α-CH), 44.8 (major Ala-α-CH), 39.9 (major Cys-β-CH_2), 38.5 (minor Cys-β-CH_2), 32.3 (major Cys-*N*-Me), 31.2 (minor Val-*N*-Me), 30.04 (minor Cys-*N*-Me), 29.96 (major Val-*N*-Me), 29.71 (major Val-β-CH), 27.4 (minor Val-β-CH), 20.5 (major Val-γ-CH_3), 20.4 (minor Val-γ-CH_3), 20.2 (major Val-γ-CH_3), 18.4 (minor Val-γ-CH_3), 17.7 (minor Ala-β-CH_3), 17.3 (major Ala-β-CH_3); HRMS (ESI) calcd for $C_{50}H_{63}N_{12}O_{12}S_2^+$ $[M+Na]^+$ 1109.3944, found: 1109.3915; m.p. 220.4-222.9 °C (dec.) (recrystallization from AcOEt/MeOH) (lit. 245-248°C (dec.))[18],; $[\alpha]_D^{26.5}$ -142.1 ° (c 0.108, $CHCl_3$) (lit. $[\alpha]_D^{25}$ -154 ° (c 1.0, $CHCl_3$))[18].

[*N*-(Quinoline-2-carbonyl)-*D*-Ser-*L*-Ala-*N*-Me-*L*-Cys-*N*-Me-*L*-Val]$_2$ (Serine-hydroxy)-Dilactone Disulfide 40 (Qn).[41] The reaction was performed in almost the same way as the method of triostin A. 2-Quinolinecarboxylic acid **38** 32.9 mg (0.19 mmol, 4 equiv.) was used instead of 2-quinoxalinecarboxylic acid **37**. The target compound **40** was obtained 34.0 mg (0.031 mmol, 65% yield on 2 steps) as colorless solid: R_f = 0.1 (AcOEt only); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.16 (d, *J* = 8.6 Hz, 1H, Ser-NH), 9.01 (d, *J* = 8.0 Hz, 1H, Ser-NH), 8.39 (d, *J* = 9.2 Hz, 1H, Ala-NH), 8.35 (d, *J* = 8.0 Hz, 1H, quinoline), 8.29 (d, *J* = 5.2 Hz, 1H, quinoline), 8.28-8.24 (m, 1H, quinoline), 8.20 (d, *J* = 8.0 Hz, 1H, quinoline), 8.00 (d, *J* = 8.6 Hz, 1H, quinoline), 7.97 (d, *J* = 8.6 Hz, 1H, quinoline), 7.90 (d, *J* = 8.0 Hz, 1H, quinoline), 7.86 (d, *J* = 8.0 Hz, 1H, quinoline), 7.79-7.61 (m, 4H, quinoline), 6.94 (d, *J* = 4.6 Hz, 1H, Ala-NH) 6.60 (t, *J* = 6.6 Hz, 1H, Cys-α-CH), 5.74 (t, *J* = 7.4 Hz, 1H, Cys-α-CH), 5.18 (d, *J* = 10.3 Hz, 1H, Val-α-CH), 5.02 (t, *J* = 6.6 Hz, 1H, Ser-α-CH), 4.99-4.90 (m, 2H, Ser-α-CH, Ala-α-CH), 4.85-4.63 (m, 3H, Ala-α-CH, Ser-β-CH_2), 4.62-4.47 (m, 2H, Ser-β-CH_2), 4.29 (d, *J* = 10.3 Hz, 1H, Val-α-CH), 3.53-2.80 (m, 16H, Cys-β-CH_2, *N*-Me, Val-*N*-Me), 2.51-2.22 (m, 2H, Val-β-CH), 1.41 (d, *J* = 6.9 Hz, 3H, Ala-β-CH_3), 1.12 (d, *J* = 6.9 Hz, 3H, Val-γ-CH_3), 1.07 (d, *J* = 6.9 Hz, 3H, Val-γ-CH_3), 1.06 (d, *J* = 7.0 Hz, 3H, Val-γ-CH_3), 0.91 (d, *J* = 6.9 Hz, 3H, Val-γ-CH_3), 0.62 (d, *J* = 6.3 Hz, 3H, Ala-β-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.8, 172.6 (Ala-CO), 170.74 (Cys-CO), 170.66 (Ser-CO), 170.1 (Val-CO), 169.5 (Cys-CO), 168.7 (Ser-CO), 168.2 (Val-CO), 165.1, 165.0 (quinolinic acid CO), 148.8, 148.6, 146.4, 137.8, 137.5, 130.5, 130.3, 129.7, 129.6, 129.4, 129.3, 128.3, 127.9, 127.7, 118.9, 118.8 (quinoline), 65.2 (Val-α-CH), 64.9, 64.7 (Ser-β-CH_2), 62.2, 62.1 (Val-α-CH), 54.1 (Cys-α-CH), 53.74, 53.70 (Ser-α-CH), 52.9 (Cys-α-CH), 51.90, 51.87 (Ser-α-CH), 46.76, 46.74, 44.79, 44.74 (Ala-α-CH), 40.1, 38.3 (Cys-β-CH_2), 32.2, 31.6 (Cys-*N*-Me), 30.3, 30.0 (Val-*N*-Me), 29.8, 28.0 (Val-β-CH), 20.6, 20.2, 20.0, 18.9 (Val-γ-CH_3), 17.24, 17.20 (Ala-β-CH_3); HRMS (ESI)

calcd for $C_{52}H_{64}N_{10}NaO_{12}S_2^+$ $[M+Na]^+$ 1107.4039, found: 1107.4047; m.p. 227.8-231.6 °C (dec.)(recrystallization from AcOEt/MeOH); $[\alpha]_D^{28.0}$ -141.6 ° (c 0.1, $CHCl_3$)

[*N*-(Naphthalene-2-carbonyl)-*D*-Ser-*L*-Ala-*N*-Me-*L*-Cys-*N*-Me-*L*-Val]$_2$ (Serine-hydroxy)-Dilactone Disulfide 41 (Np). The reaction was performed in almost the same way as the method of triostin A. 2-Naphthoic acid **39** 33.0 mg (0.19 mmol, 4 equiv.) was used instead of 2-quinoxalinecarboxylic acid **37**. The target compound **41** was obtained 35.8 mg (0.033 mmol, 74% yield on 2 steps) as pale yellow solid: R_f = 0.5 (CH_2Cl_2:MeOH = 10:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 (s, 2H), 7.96-7.88 (m, 6H, Ser-NH, Ala-NH, naphthalene C5 or C8), 7.87 (d, *J* = 8.6 Hz, 2H, naphthalene C5 or C8), 7.85 (d, *J* = 8.5 Hz, 2H, naphthalene C4), 7.63 (dd, *J* = 8.6, 1.7 Hz, 2H, naphthalene C3), 7.62-7.52 (m, 4H, naphthalene C6, C7), 5.64 (t, *J* = 6.0 Hz, 2H, Ser-α-CH), 5.10 (dd, *J* = 8.0, 4.6 Hz, 2H, Cys-α-CH), 5.01-4.81 (m, 4H, Ser-β-CH_2, Ala-α-CH), 4.51 (d, *J* = 10.3 Hz, 2H, Ser-β-CH_2), 4.23 (d, *J* = 10.3 Hz, 2H, Val-α-CH), 3.41-3.30 (m, 4H, Cys-β-CH_2), 3.29 (s, 6H, Cys-*N*-Me), 3.12 (s, 6H, Val-*N*-Me), 2.56-2.33 (m, 2H, Val-α-CH), 1.10 (d, *J* = 6.3 Hz, 6H, Val-γ-CH_3), 1.07 (d, *J* = 6.9 Hz, 6H, Val-γ-CH_3), 0.35 (d, *J* = 6.3 Hz, 6H, Ala-β-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.6 (Ala-CO), 171.2 (Val-CO), 170.4 (Cys-CO), 168.4 (Ser-CO), 167.4 (naphthoic acid CO), 134.8, 132.4, 130.7 (naphthalene C2, C9, C10), 128.9 (naphthalene C8), 128.4 (naphthalene C4), 127.99 (naphthalene C6 or C7), 127.93 (naphthalene C1), 127.7 (naphthalene C5), 127.0 (naphthalene C6 or C7), 123.7 (naphthalene C3), 65.3 (Ser-β-CH_2), 64.9 (Val-α-CH), 53.8 (Ser-α-CH), 53.7, 53.6 (Cys-α-CH), 44.4 (Ala-α-CH), 39.7 (Cys-β-CH_2), 32.5 (Cys-*N*-Me), 30.0 (Val-*N*-Me), 29.7 (Val-β-CH), 20.4, 19.9 (Val-γ-CH_3), 17.1 (Ala-β-CH_3); HRMS (ESI) calcd for $C_{54}H_{66}N_8NaO_{12}S_2^+$ $[M+Na]^+$ 1105.4134 found: 1105.4105; m.p. 170.4-174.3 °C ; $[\alpha]_D^{23.7}$ -80.7 ° (c 0.1, $CHCl_3$).

[*N*-(Quinoxaline-2-carbonyl)-*D*-Ser-*L*-Ala-*N*-Me-*L*-Cys-*N*-Me-*D*-Val]$_2$ (Serine-hydroxy)-Dilactone Disulfide 42 (*D,D*). The reaction was performed in almost the same way as the method of triostin A. *D, D*-Macrolide **33** 50 mg (0.048 mmol) was used instead of *L, L*-macrolide **1**. The target compound **42** was obtained 15.3 mg (0.014 mmol, 30% yield on 2 steps) as colorless solid: R_f = 0.1 (AcOEt only); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.24 (d, *J* = 9.7 Hz, 2H, Ser-NH), 9.08 (s, 2H, Qx-C3), 7.95 (d, *J* = 8.0 Hz, 2H, Qx-C5 or C8), 7.91 (d, *J* = 8.0 Hz, 2H, Qx-C5 or C8), 7.75 (t, *J* = 7.4 Hz, 2H, Qx-C6 or C7), 7.58 (t, *J* = 7.7 Hz, 2H, Qx-C6 or C7), 7.48 (d, *J* = 8.0 Hz, 2H, Ala-NH), 5.91 (t, *J* = 6.6 Hz, 2H, Cys-α-CH), 5.27 (dd, *J* = 10.0, 4.3 Hz, 2H, Ser-α-CH), 5.22-5.09 (m, 2H, Ala-α-CH), 4.82 (d, *J* = 10.9 Hz, 2H, Val-α-CH), 4.48 (d, *J* = 10.9 Hz, 2H, Ser-β-CH_2), 4.38 (dd, *J* =

11.2, 4.9 Hz, 2H, Ser-β-CH_2), 3.34 (dd, *J* = 13.7, 6.9 Hz, 2H, Cys-β-CH_2), 3.22 (s, 6HCys-*N*-Me), 3.00 (dd, *J* = 13.7, 5.7 Hz, 2H, Cys-β-CH_2), 2.93 (s, 6H Val-*N*-Me), 2.20-2.05 (m, 2H, Val-β-CH), 1.51 (d, *J* = 6.9 Hz, 6H, Ala-β-CH_3), 0.68 (d, *J* = 6.9 Hz, 6H, Val-γ-CH_3), 0.57 (d, *J* = 6.3 Hz, 6H, Val-γ-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0 (Ala-CO), 169.9 (Val-CO), 169.1 (Cys-CO), 167.6 (Ser-CO), 163.6 (Qx-CO), 142.27 (Qx-C2), 143.23 (Qx-C3), 141.8, 139.5 (Qx-C9 and C10), 131.8, 130.7 (Qx-C5 and C8), 129.6, 128.8 (Qx-C6 and C7), 63.7 (Val-α-CH), 63.5 (Ser-β-CH_2), 53.4 (Cys-α-CH), 50.8 (Ser-α-CH), 45.8 (Ala-α-CH), 39.7 (Cys-β-CH_2), 31.3 (Cys-*N*-Me), 28.8 (Val-*N*-Me), 28.9 (Val-β-CH), 18.9, 18.40 (Val-γ-CH_3), 18.36 (Ala-β-CH_3); HRMS (ESI) calcd for $C_{50}H_{63}N_{12}O_{12}S_2^+$ $[M+Na]^+$ 1109.3944, found: 1109.3927; plates; m.p. 207.0-210.1 °C (dec.)(recrystallization from CH_2Cl_2/MeOH); $[\alpha]_D^{27.4}$ -43.9 ° (c 0.1, $CHCl_3$). Crystallographic data for the structure of **42** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 1441096.

[*N*-(Quinoxaline-2-carbonyl)-*D*-Ser-*L*-Ala-*N*-Me-*L*-Cys-*N*-Me-*D*-Val]-[*N*-(Quinoxaline-2-carbonyl)-*D*-Ser-*L*-Ala-*N*-Me-*L*-Cys-*N*-Me-*L*-Val) (Serine-hydroxy)-Dilactone Disulfide 43 (*D,L*). The reaction was performed in almost the same way as the method of triostin A. *D, L*-Macrolide **36** 50 mg (0.048 mmol) was used instead of *L, L*-macrolide **1**. The target compound **43** was obtained 16.4 mg (0.015 mmol, 31% yield on 2 steps) as colorless solid: R_f = 0.1 (AcOEt only); ^{1}H NMR (mixture of conformers, 500 MHz, $CDCl_3$) δ 9.69 (s, minor, Qx-C3), 9.65 (s, minor, Qx-C3), 9.63 (s, major, Qx-C3), 9.61 (s, major Qx-C3), 9.43 (d, *J* = 10.3 Hz, major Ser-NH), 9.29 (d, *J* = 6.9 Hz, minor Ser-NH), 8.85 (d, *J* = 5.7 Hz, minor Ser-NH), 8.64 (d, *J* = 7.5 Hz, major Ser-NH), 8.32-8.10, 8.04, 8.00-7.79 (m, major Ala-NH, Qx), 7.61 (d, *J* = 9.7 Hz, major Ala-NH), 7.21 (d, *J* = 6.3 Hz, minor Ala-NH), 6.50 (d, *J* = 8.0 Hz, minor Ala-NH), 6.45 (dd, *J* = 1.5, 4.6 Hz, minor Cys-α-CH), 6.38 (dd, *J* = 10.9, 5.2 Hz, minor Cys-α-CH), 5.73 (dd, *J* = 10.9, 2.3 Hz, major Cys-α-CH), 5.54 (dd, *J* = 13.5, 3.7 Hz, major Cys-α-CH), 5.47-5.38 (m, major Ala-α-CH), 5.36 (dd, *J* = 10.3, 2.9 Hz, major Ser-α-CH), 5.23 (d, *J* = 9.2 Hz, minor Val-α-CH), 5.18 (d, *J* = 10.3 Hz, minor Val-α-CH), 5.02-4.88 (m, minor Ser-α-CH, major Ser-α-CH and minor Ala-α-CH), 4.85 (dd, *J* = 11.5, 2.3 Hz, major Ser-β-CH_2), 4.83-4.74 (m, major Ser-β-CH_2, minor Ala-α-CH and minor Ser-α-CH), 4.74-4.60 (m, major Ala-α-CH, minor Ser-β-CH_2 and major Ser-β-CH_2), 4.51 (dd, *J* = 10.3, 8.0 Hz, minor Ser-β-CH_2), 4.24 (dd, *J* = 11.2, 8.3 Hz, major Ser-β-CH_2), 4.18 (d, *J* = 10.9 Hz, major Val-α-CH), 4.01 (d, *J* = 11.0 Hz, major Val-α-CH), 3.88 (dd, *J* = 14.3, 12.0 Hz, minor Cys-β-CH_2), 3.74 (dd, *J* = 15.8, 13.5 Hz, major Cys-β-CH_2), 3.57 (dd, *J* = 12.6, 11.5 Hz, major Cys-β-CH_2), 3.21 (s, minor Cys-*N*-Me), 3.17 (s, major Cys-*N*-Me), 3.16 (s, major Cys-*N*-Me), 3.15 (s, minor

Val-*N*-Me), 3.14-3.08 (m, major Cys-β-CH_2), 3.06 (s, major Val-*N*-Me), 3.06-2.95 (m, minor Cys-β-CH_2), 2.95 (s, minor Cys-*N*-Me), 2.92 (s, major Val-*N*-Me), 2.83 (s, minor Val-*N*-Me), 2.65-2.55 (m, minor Val-β-CH), 2.55-2.39 (m, major Cys-β-CH_2, major Val-β-CH and minor Cys-β-CH_2), 2.39-2.14 (m, minor Val-β-CH and major Val-β-CH), 1.41 (d, J = 6.3 Hz, minor Ala-β-CH_3), 1.39 (d, J = 6.9 Hz, major Ala-β-CH_3), 1.28 (d, J = 6.9 Hz, minor Ala-β-CH_3), 1.11-1.02 (m, major Val-γ-CH_3 and minor Val-γ-CH_3), 0.83 (d, J = 6.9 Hz, major Val-γ-CH_3 and minor Val-γ-CH_3), 0.76 (d, J = 6.3 Hz, major Val-γ-CH_3 and minor Val-γ-CH_3), 0.67 (d, J = 6.9 Hz, minor Ala-β-CH_3); ^{13}C NMR (mixture of conformers, 125 MHz, $CDCl_3$) δ 173.7 (major Ala-CO), 173.5, 173.0 (minor Ala-CO), 171.7 (major Cys-CO and minor Cys-CO), 170.9 (major Ala-CO), 170.5 (minor Val-CO), 170.0 (major Cys-CO), 169.5 (major Val-CO), 169.4 (minor Val-CO), 169.2 (major-Val-CO), 168.6 (major Ser-CO), 167.87, 167.84 (minor Ser-CO), 167.7 (major Ser-CO), 167.2 (major Cys-CO), 164.03 (minor Qx-CO), 163.99 (major Qx-CO), 163.8 (minor Qx-CO), 162.9 (major Qx-CO), 144.2, 144.1, 143.9, 143.8, 143.6, 142.9, 142.7, 142.5, 142.4, 140.32, 140.26, 140.1 (Qx-C2, C3, C9 and C10), 132.3, 132.2, 131.9, 131.8, 131.4, 131.1, 131.0, 130.9, 129.8, 129.75, 129.72, 129.6, 129.55, 129.47, 129.44 (Qx- C5, C6, C7 and C8), 66.5 (major Ser-β-CH_2), 66.4 (minor Ser-β-CH_2), 65.5 (major Val-α-CH), 64.7 (minor Ser-β-CH_2), 64.6 (minor Val-α-CH), 63.0 (major Val-α-CH), 62.1 (major Ser-β-CH_2), 61.1 (Val-α-CH), 57.1 (major Cys-α-CH), 54.4, 53.1 (minor Ser-α-CH), 52.9 (major Cys-α-CH), 52.4 (major Ser-α-CH), 52.2 (minor Cys-α-CH), 51.8 (major Ser-α-CH), 49.6 (minor Cys-α-CH), 47.4, 46.1 (minor Ala-α-CH), 45.7 (major Ala-α-CH), 45.3 (major Cys-β-CH_2), 43.5 (major Ala-α-CH), 41.7, 38.7 (minor Cys-β-CH_2), 38.3 (major Cys-β-CH_2), 33.0, 31.7 (major Cys-*N*-Me), 31.3 (minor Cys-*N*-Me), 30.7, 30.1 (minor Val-*N*-Me), 29.4 (minor Cys-*N*-Me), 29.2, 28.9 (major Val-*N*-Me), 28.5, 28.1 (major Val-β-CH), 28.0, 26.7 (minor Val-β-CH), 20.3 (minor Val-γ-CH_3), 20.1, 19.4, 19.2, 19.0 (major Val-γ-CH_3), 18.9 (minor Val-γ-CH_3), 18.5 (major Ala-β-CH_3), 18.3 (minor Val-γ-CH_3), 18.2 (minor Ala-β-CH_3), 17.8 (minor Val-γ-CH_3), 16.8 (minor Ala-β-CH_3), 15.9 (major Ala-β-CH_3); HRMS (ESI) calcd for $C_{50}H_{63}N_{12}O_{12}S_2^+$ $[M+Na]^+$ 1109.3944, found: 1109.3971; m.p. 214.6-216.0 °C (dec.)(recrystallization fromCH_2Cl_2/MeOH); $[\alpha]_D^{27.3}$ -87.6° (c 0.1, $CHCl_3$).

[*N*-(Quinoxaline-2-carbonyl)-*D*-Ser-*L*-Ala-*N*-Me-*S*-monoxide-*L*-Cys-*N*-Me-*D*-Val]-[*N*-(Quinoxaline-2-carbonyl)-*D*-Ser-*L*-Ala-*N*-Me-*L*-Cys-*N*-Me-*L*-Val) (Serine-hydroxy)-Dilactone Thiosulfinate 46 (SO). To a solution of TA 50 mg (0.046 mmol) in CH_2Cl_2 (5 mL) was added *m*CPBA 13.6 mg (0.055 mmol, 1.2 equiv.), and the resulting mixture was stirred at -10 ˚C for 12 h. The reaction mixture was quenched

with sat. $NaHCO_3$ aq. (50 mL), and then extracted with AcOEt (50 mL). The organic layer was washed with sat. NaCl aq. (50 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue (47.0 mg) was purified by liquid chromatography (GL Science Intersil WP300 C4, 5 μm×20×150 mm) eluted with H_2O/CH_3CN containing 0.1% TFA (70:30 ~ 50:50, 30 min, Folw: 8.8 mL/min, Detect: 220 nm) to afford the target compound **46** 10.1 mg (0.0092 mmol, 20% yield) as pale yellow solid: retention time 17.5 min (GL Science Intersil WP300 C4 5 μm 3×150 mm, Folw: 0.2 mL/min, Solvent: H_2O:CH_3CN containing 0.1% TFA = 70:30~45:55, 30 min, Detect: 220 nm); ^{1}H NMR (mixture of conformers, 500 MHz, $CDCl_3$) δ 9.68 (s, minor Qx-C3), 9.65 (s, minor Qx-C3), 9.59 (s, major Qx-C3), 9.56 (s, major Qx-C3), 9.07 (d, *J* = 10.3 Hz, major Ser-NH), 9.04 (d, *J* = 8.6 Hz, major Ser-NH), 8.84 (d, *J* = 6.9 Hz, minor Ser-NH), 8.67 (d, *J* = 8.6 Hz, minor Ser-NH), 8.46 (d, *J* = 9.2 Hz, major Ala-NH), 8.28-7.77 (m, Qx), 8.28-8.00 (m, Qx), 8.00-7.77 (m, major Ala-NH, Qx), 7.04 (br, minor Ala-NH), 6.98 (d, *J* = 10.9 Hz, major Cys-α-CH), 6.80 (dd, *J* = 11.5, 3.4 Hz, minor Cys-α-CH), 6.71 (d, *J* = 6.9 Hz, 1H, minor Ala-NH), 5.93 (d, *J* = 7.4 Hz, minor Cys-α-CH), 5.86 (dd, *J* = 12.6, 2.9 Hz, major Cys-α-CH), 5.25 (dd, *J* = 9.7, 4.0 Hz, major Ser-α-CH), 5.21 (d, *J* = 9.7 Hz, minor Val-α-CH), 5.19-5.13 (m, major Ala-α-CH), 5.11-4.96 (m, minor Val-α-CH, major Ser-β-CH_2, minor Ser-α-CH, major Ser-α-CH), 4.95-4.90 (m, minor Ser-α-CH), 4.89-4.72 (m, minor Ser-β-CH_2, major Ala-α-CH, minor Ala-α-CH), 4.72-4.50 (m, minor Ser-β-CH_2, major Val-α-CH, major Ser-β-CH_2), 4.25 (d, *J* = 10.3 Hz, major Val-α-CH), 4.03-3.91 (m, major Cys-β-CH_2, minor Cys-β-CH_2), 3.81 (dd, *J* = 16.3, 12.9 Hz, major Cys-β-CH_2), 3.66-3.46 (m, minor Cys-β-CH_2, major Cys-β-CH_2), 3.44 (s, major Cys-*N*-Me), 3.40 (s, major Cys-*N*-Me), 3.42-3.37 (m, minor Cys-β-CH_2), 3.21 (s, minor Val-*N*-Me), 3.09-2.99 (m, minor Cys-β-CH_2, major Cys-β-CH_2), 3.07 (s, minor Val-*N*-Me), 3.05 (s, minor Cys-*N*-Me), 3.03 (s, minor Cys-*N*-Me), 3.00 (s, major Val-*N*-Me), 2.97 (s, major Val-*N*-Me), 2.51-2.24 (m, major Val-β-CH, minor Val-β-CH), 1.45 (d, *J* = 6.9 Hz, minor Ala-β-CH_3), 1.36 (d, *J* = 7.4 Hz, minor Ala-β-CH_3), 1.13 (d, *J* = 6.9 Hz, major Val-γ-CH_3), 1.12-1.02 (m, minor Val-γ-CH_3, major Val-γ-CH_3), 0.99 (d, *J* = 6.9 Hz, minor Val-γ-CH_3), 0.97 (d, *J* = 6.9 Hz, major Val-γ-CH_3), 0.86 (d, *J* = 6.9 Hz, minor Val-γ-CH_3), 0.55 (br, major Ala-β-CH_3), 0.42 (d, *J* = 6.3 Hz, major Ala-β-CH_3); ^{13}C NMR (mixture of conformers, 125 MHz, $CDCl_3$) δ 173.6 (minor Ala-CO), 172.6 (major Ala-CO), 172.5 (minor Ala-CO), 172.1 (major Ala-CO), 170.7 (minor Val-CO), 170.44 (major Cys-CO), 170.36 (major Ser-CO), 170.0 (minor Val-CO), 169.8 (major Cys-CO), 169.7 (major Cys-CO), 169.4 (minor Cys-CO), 168.5 (major Ser-CO), 168.3 (major Cys-CO), 167.9, 167.5 (minor Ser-CO), 164.1, 163.9, 163.8, 163.7 (Qx-CO), 144.3, 144.1, 144.0, 143.8, 143.7, 143.6 (Qx-C2, C3), 143.0, 142.9, 142.5, 142.2, 140.32, 140.29, 140.20, 140.0

(Qx-C9, C10), 132.2, 132.1, 132.0, 131.9, 131.30, 131.26, 131.20, 131.1, 129.9, 129.8, 129.6, 129.5, 129.4, 129.3, 129.0 (Qx-C5, C6, C7, C8), 65.0 (minor Ser-β-CH_2, major Ser-β-CH_2), 64.7 (major Val-α-CH), 64.5 (minor Ser-β-CH_2), 64.2 (major Ser-β-CH_2), 63.7 (major Val-α-CH), 62.8, 61.9 (minor Val-α-CH), 54.46 (minor Cys-α-CH), 54.39 (major Cys-α-CH), 53.1 (major Ser-α-CH), 52.87 (minor Cys-β-CH_2), 52.79 (major Ser-α-CH), 52.2, 52.1 (Ser-α-CH), 50.6 (major Cys-α-CH), 48.6 (major Cys-β-CH_2), 47.0, 46.4 (minor Ala-α-CH), 45.0, 44.4 (major Ala-α-CH), 33.5, 33.0 (major Cys-*N*-Me), 32.6 (major Cys-β-CH_2), 31.5, 31.2 (minor Val-*N*-Me), 31.1, 29.7 (minor Cys-*N*-Me), 29.2 (major Val-*N*-Me), 28.95 (major Val-β-CH), 28.88 (major Val-*N*-Me), 28.85 (major Val-β-CH), 28.3, 27.7 (minor Val-β-CH), 25.1 (minor Cys-β-CH_2), 20.4, 20.1 (minor Val-γ-CH_3), 20.0 (major Val-γ-CH_3), 19.7 (minor Val-γ-CH_3), 19.6, 19.5, 19.2 (major Val-γ-CH_3), 18.4 (minor Val-γ-CH_3), 17.9 (minor Ala-β-CH_3, major Ala-β-CH_3), 17.3 (major Ala-β-CH_3), 16.5 (minor Ala-β-CH_3); HRMS (ESI) calcd for $C_{50}H_{62}N_{12}NaO_{13}S_2^+$ $[M+Na]^+$ 1125.3893, found: 1125.3878; m.p. 196.1-202.0 °C (dec.); $[\alpha]_D^{27.7}$ -182.8 ° (c 0.1, $CHCl_3$) ; IR (KBr) 1131 (SO) cm^{-1}.

[*N*-(Quinoxaline-2-carbonyl)-*D*-Ser-*L*-Ala-*N*-Me-*S*-dioxide-*L*-Cys-*N*-Me-*D*-Val]-[*N*-(Quinoxaline-2-carbonyl)-*D*-Ser-*L*-Ala-*N*-Me-*L*-Cys-*N*-Me-*L*-Val) (Serine-hydroxy)-Dilactone Thiosulfonate 47 (SO_2). The reaction was performed in almost the same way as the method of triostin A. SO_2-macrolide **45** 50 mg (0.047 mmol) was used instead of macrolide **1**. The target compound **47** was obtained 42.7 mg (0.038 mmol, 81% yield on 2 steps) as pale yellow solid: R_f = 0.1 (AcOEt only); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.66 (s, 2H, Qx-C3), 8.96 (d, *J* = 5.7 Hz, 1H, Ser-NH), 8.89 (d, *J* = 5.7 Hz, 1H, Ser-NH), 8.29-8.14 (m, 4H, Qx), 7.98-7.82 (m, 4H, Qx), 7.35 (d, *J* = 4.0 Hz, 1H, Ala-NH), 7.28 (d, *J* = 5.2 Hz, 1H, Ala-NH), 7.15 (dd, *J* = 10.9, 3.4 Hz, 1H, Cys-α-CH), 7.02 (d, *J* = 7.4 Hz, 1H, Cys-α-CH), 5.26 (d, *J* = 10.9 Hz, 1H, Val-α-CH), 5.20 (d, *J* = 10.9 Hz, 1H, Val-α-CH), 4.98-4.87 (m, 2H, Ser-α-CH), 4.87-4.70 (m, 2H, Ala-α-CH), 4.58-4.46 (m, 3H, Ser-β-CH_2), 4.38 (t, *J* = 10.0 Hz, 1H, Ser-β-CH_2), 4.05-3.79 (m, 3H, Cys-β-CH_2), 3.64 (dd, *J* = 16.0, 3.4 Hz, 1H, Cys-β-CH_2), 3.04 (s, 3H, Cys-*N*-Me or Val-*N*-Me), 3.02 (s, 3H, Cys-*N*-Me or Val-*N*-Me), 2.98 (s, 3H, Cys-*N*-Me or Val-*N*-Me), 2.97 (s, 3H, Cys-*N*-Me or Val-*N*-Me), 2.39-2.21 (m, 2H, Val-β-CH), 1.50 (d, *J* = 7.0 Hz, 3H, Ala-β-CH_3), 1.49 (d, *J* = 6.0 Hz, 3H, Ala-β-CH_3), 1.13 (d, *J* = 6.5 Hz, 3H, Val-γ-CH_3), 1.12 (d, *J* = 6.0 Hz, 3H, Val-γ-CH_3), 0.87 (d, *J* = 6.9 Hz, 3H, Val-γ-CH_3), 0.83 (d, *J* = 6.9 Hz, 3H, Val-γ-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.2, 172.9 (Ala-CO), 170.0, 169.8 (Val-CO), 168.7 (Cys-CO), 168.0 (Ser-CO), 167.7 (Cys-CO), 163.7, 163.4 (Qx-CO), 144.05, 143.99, 143.5, 142.8, 142.6, 140.3, 140.2, 132.0, 131.9, 131.8, 131.3, 131.0, 129.8, 129.6, 129.5, 129.4 (Qx),

66.3, 65.9 (Ser-β-CH_2), 62.3, 61.6 (Val-α-CH), 60.9 (Cys-β-CH_2), 54.1 (Cys-α-CH), 51.6, 51.3 (Ser-α-CH), 50.6 (Cys-α-CH), 47.5, 47.4 (Ala-α-CH), 35.2 (Cys-β-CH_2), 31.1 (Cys-*N*-Me), 30.6 (Val-*N*-Me), 30.1 (Cys-*N*-Me), 29.7 (Val-*N*-Me), 20.5, 20.3 (Val-γ-CH_3), 18.32, 18.30 (Val-γ-CH_3 and Ala-β-CH_3), 18.0 (Val-γ-CH_3), 17.8 (Ala-β-CH_3); HRMS (ESI) calcd for $C_{50}H_{62}N_{12}NaO_{14}S_2^+$ $[M+Na]^+$ 1141.3842, found: 1141.3866; m.p. 201.2-204.3 °C (dec.); $[\alpha]_D^{27.6}$ -187.9 ° (c 0.1, $CHCl_3$) ; IR (KBr) 1324 (SO_2), 1125 (SO_2) cm^{-1}.

Biological Evaluation

Preparation of Test Compounds Solution.

All compounds were prepared as stock solution in DMSO and stored in aliquots at -20 ˚C. The final concentration of DMSO was less than 1.0% (v/v) in the biological assays.

Cell Culture.

HEK293 clone cell line were maintained in Eagle's minimum essential medium (Wako) containg 1% (v/v) nonessential amino acids (GIBCO), supplemented with 10 % fetal bovine serum (FBS, Nichirei), 50 units/mL penicillin (Meiji), 50 μg/mL streptomycin (Meiji), 50 μg/mL kanamycin (Meiji). MCF-7 cell line were maintained in Eagle's minimum essential medium (Wako) containg 1% nonessential amino acids (GIBCO) and 1 mmol/L sodium pyruvate (Wako), supplemented with 10 % fetal bovine serum (FBS, Nichirei), 50 units/mL penicillin (Meiji), 50 μg/mL streptomycin (Meiji), 50 μg/mL kanamycin (Meiji). Cells were incubated in a standard tissue culture incubator in 95% air and 5% CO_2 for normoxic conditions, and in 1% O_2, 94% N_2 and 5% CO_2 for hypoxic conditions.

HIF-1 Dependent Luciferase Assay

We performed the luciferase assay according to the procedure in our previous report.[28] In brief, HEK293 clone cells were seeded on 24-well plate (8.0×10^4 cells/well), and then incubated for 24 h under the normoxic condition. The each medium was exchanged with a fresh medium containing 0.1% DMSO and several concentrations of test compound and incubated for 1 h, and then cells were treated under the normoxic or hypoxic condition (1% O_2) for 24 h. The luciferase assay was performed using the luciferase assay kit instructions (Roche Applied Science, Bavaria, Germany). The activity was measured using a luminometer (Sirius, Berthold Detection System,

Baden-Württemberg, Germany). The luciferase activity data was compensated by protein content.

MTT Assay

MCF-7 cells were plated at a density of 8.0×10^3 cells/well (96-well plate, TPP Techno Plastic Products AG) in 100 μL of the medium and treated with test compounds under normoxic or hypoxic (1% O_2) condition for 24 h. The each medium was then exchanged with a fresh medium and incubated under normoxic condition for 16 h. The MTT reagent (0.5 mg/mL, Sigma-Aldrich) was added to the media. After 4 h incubation at 37 ˚C, the media were removed, and the cells were lysed with DMSO. Absorbance at 570 nm was measured on a Multiskan JX plate reader (Thermo Fisher Scientific).

R_M Value

R_f values of tested compounds were measured by TLC (60 RP-8 F_{254S}, MERCK) analysis eluted with mixture of MeOH and 20 mM Sodium phosphate buffer (pH 7.2) (8:2). Then R_M values were calculated as following scheme: $R_M = \log(1/R_f - 1)$.

Western Blotting

Proteins were extracted from the MCF-7 cells (3×10^6 cells/dish, 100 mm petri dish) treated with test compounds under normoxic or hypoxic (1% O_2) condition for 16 h. Cell extracts were separated by SDS-PAGE (10% SDS) and transferred to a nitrocellulose membrane. Immunoblotting was performed as described in our previous repote.[28] Antibodies to HIF-1 alpha (a dilution of 1:3000, Novus) and β-actin (a dilution of 1:1000, Sigma-Aldrich) and horseradish peroxidase-conjugated goat anti-mouse IgG (Sigma-Aldrich) were used. The proteins were visualized by using chemiluminescence detection reagents (Pierce ® Western Blotting Substrate or Immobilon™ Western Chemiluminescent HRP substrate), and measured on a luminescent image analyzer (LAS3000, Fujifilm).

Statistical analysis

For the results of the reporter assays and MTT assay, statistical differences between the control and compound-treated samples were evaluated by Student's *t*-test, as

indicated in the figures (*P,0.05, **P,0.01 ***P,0.005 ****P,0.0005 versus control). The dose-response results of the reporter assays and cell viability assays were presented as the mean ± SD of three independent experiments, with each treatment performed in duplicate for the reporter assays and in triplicate for the cell viability assay.

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References

1. J. I. Shoji and K. Katagiri, *J. Antibiot.*, 1961, **14**, 335-339.
2. H. Otsuka, J. Shoji, K. Kawano and Y. Kyogoku, *J. Antibiot.*, 1976, **29**, 107-110.
3. K. Hotta, R. M. Keegan, S. Ranganathan, M. Fang, J. Bibby, M. D. Winn, M. Sato, M. Lian, K. Watanabe, D. J. Rigden and C. Y. Kim, *Angew. Chem. Int. Ed. Engl.*, 2014, **53**, 824-828.
4. K. Koketsu, H. Oguri, K. Watanabe and H. Oikawa, *Chem. Biol.*, 2008, **15**, 818-828.
5. O. E. Zolova, A. S. A. Mady and S. Garneau-Tsodikova, *Biopolymers*, 2010, **93**, 777-790.
6. M. J. Waring and L. P. G. Wakelin, *Nature*, 1974, **252**, 653-657.
7. M. M. Van Dyke and P. B. Dervan, *Science*, 1984, **225**, 1122-1127.
8. J. S. Lee and M. J. Waring, *Biochem. J*, 1978, **173**, 115-128.
9. D. Kong, E. J. Park, A. G. Stephen, M. Calvani, J. H. Cardellina, A. Monks, R. J. Fisher, R. H. Shoemaker and G. Melillo, *Cancer Res.*, 2005, **65**, 9047-9055.
10. G. L. Semenza, *Semin. Cancer Biol.*, 2009, **19**, 12-16.
11. H. Nagasawa, *J. Pharmacol. Sci.*, 2011, **115**, 446-452.
12. B. J. Foster, K. Clagett-Carr, D. D. Shoemaker, M. Suffness, J. Plowman, L. A. Trissel, C. K. Grieshaber and B. Leyland-Jones, *Invest. New Drugs*, 1985, **3**, 403-410.
13. A. Y. Chang, K. Kim, H. Boucher, P. Bonomi, J. A. Stewart, D. D. Karp and R. H. Blum, *Cancer*, 1998, **82**, 292-300.
14. S. Yonekura, M. Itoh, O. Yuki, T. Yusuke, A. Ono, N. Nara and S. Tohda, *Anticancer*

Res., 2013, **33**, 3099-3103.

15. Y. Wang, Y. Liu, F. Tang, K. M. Bernot, R. Schore, G. Marcucci, M. A. Caligiuri, P. Zheng and Y. Liu, *Blood*, 2014, **124**, 1127-1135.

16. K. Watanabe, K. Hotta, A. P. Praseuth, K. Koketsu, A. Migita, C. N. Boddy, C. C. Wang, H. Oguri and H. Oikawa, *Nat. Chem. Biol.*, 2006, **2**, 423-428.

17. P. K. Chakravarty and R. K. Olsen, *Tetrahedron Lett.*, 1978, **19**, 1613-1616.

18. M. Shin, K. Inouye and H. Otsuka, *Bull. Chem. Soc. Jpn.*, 1984, **57**, 2203-2210.

19. A. K. Ray and U. Diederichsen, *Eur. J. Org. Chem.*, 2009, 4801-4809.

20. D. L. Boger and K. W. Saionz, *Bioorg. Med. Chem.*, 1999, **7**, 315-321.

21. T. Yoshida, Y. Kimura and K. Katagiri, *J. Antibiot.*, 1968, **21**, 465-467.

22. D. L. Boger and J. K. Lee, *J. Org. Chem.*, 2000, **65**, 5996-6000.

23. D. L. Boger, S. Ichikawa, W. C. Tse, M. P. Hedrick and Q. Jin, *J. Am. Chem. Soc.*, 2001, **123**, 561-568.

24. K. B. Lorenz and U. Diederichsen, *J. Org. Chem.*, 2004, **69**, 3917-3927.

25. B. Dietrich and U. Diederichsen, *Eur. J. Org. Chem.*, 2005, 147-153.

26. U. M. Kotyrba, K. Propper, E. F. Sachs, A. Myanovska, T. Joppe, F. Lissy, G. M. Sheldrick, K. Koszinowski and U. Diederichsen, *ChemistryOpen*, 2014, **3**, 152-160.

27. Y. B. Kim, Y. H. Kim, J. Y. Park and S. K. Kim, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 541-544.

28. H. Hattori, K. Okuda, T. Murase, Y. Shigetsura, K. Narise, G. L. Semenza and H. Nagasawa, *Bioorg. Med. Chem.*, 2011, **19**, 5392-5401.

29. K. Narise, K. Okuda, Y. Enomoto, T. Hirayama and H. Nagasawa, *Drug. Des. Devel. Ther.*, 2014, **8**, 701-717.

30. G. M. Sheldrick, A. Heine, K. Schmidt-Bäse, E. Pohl, P. G. Jones, E. Paulus and M. J. Waring, *Acta Crystallogr. Sect. B: Struct. Sci.*, 1995, **51**, 987-999.

31. Y. Kyogoku, N. Higuchi, M. Watanabe and K. Kawano, *Biopolymers*, 1981, **20**, 1959-1970.

32. A. M. Socha, K. L. Laplante, D. J. Russell and D. C. Rowley, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 1504-1507.

33. T. L. Ciardelli, P. K. Chakravarty and R. K. Olsen, *J. Am. Chem. Soc.*, 1978, **100**, 7684-7690.

34. E. Marcucci, N. Bayó-Puxan, J. Tulla-Puche, J. Spengler and F. Albericio, *J. Comb. Chem.*, 2008, **10**, 69-78.

35. P. K. Chakravarty and R. K. Olsen, *J. Org. Chem.*, 1978, **43**, 1270-1271.

36. A. V. Malkov, K. Vrankova, M. Cerny and P. Kocovsky, *J. Org. Chem.*, 2009, **74**, 8425-8427.

37. M. Kunishima, C. Kawachi, F. Iwasaki, K. Terao and S. Tani, *Tetrahedron Lett.*, 1999, **40**, 5327-5330.

38. T. J. Blake, J. R. Kalman and D. H. Williams, *Tetrahedron Lett.*, 1977, 2621-2624.

39. A. Cornish, K. R. Fox and M. J. Waring, *Antimicrob. Agents Chemother.*, 1983, **23**, 221-231.

40. B. Vlaminck, S. Toffoli, B. Ghislain, C. Demazy, M. Raes and C. Michiels, *FEBS J.*, 2007, **274**, 5533-5542.

41. S. Santikarn, S. J. Hammond, D. H. Williams, A. Cornish and M. J. Waring, *J. Antibiot.*, 1983, **36**, 362-364.