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Solution-Phase Synthesis of Nucleobase-Substituted Analogues of Triostin A†

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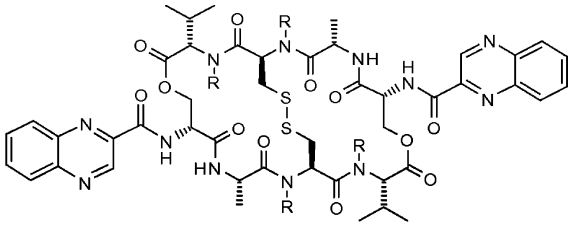
A synthesis of novel analogues of triostin A presenting two identical or different nucleobases instead of the original quinoxaline substituents has been developed. The DNA bisintercalator triostin A (**1**) with its rigid backbone provides an optimal scaffold for a parallel preorganization of the intercalating moieties. The bicyclic octadepsipeptide is built up stepwise in solution and modified with various nucleobase-substituted acetic acids at a late stage. The choice of orthogonal protecting groups allows for the synthesis of triostin analogues bearing two different substituents.

Introduction

Triostin A (**1**),[1] a cyclic octadepsipeptide containing two planar quinoxaline rings, is one of the most widely studied members of the naturally occurring quinoxaline antibiotics (Figure 1).[2] These bicyclic natural products, including the well-known echinomycin[3] and thiocoraline[4] families, efficiently block both transcription and replication, and therefore possess antibiotic and cytotoxic activity.

Their cytotoxic effects originate from their sequence-specific binding to double-stranded DNA via bisintercalation into the minor groove.[5] The rigid, bicyclic backbone composed of two identical peptide subunits provides an optimal scaffold for a parallel preorganization of the quinoxaline intercalators in a distance of approximately 10.5 Å. On the basis of a DNA base pair distance of 3.4 Å, exactly one dinucleotide can be spanned. Upon binding the bisintercalator, the DNA helix is significantly unwound and the binding mode of the adjacent nucleobase pairs can be altered from Watson−Crick to Hoogsteen pairing, depending on the DNA sequence.[6] The interaction is controlled by DNA−amino acid hydrogen-bonding and chromophore−base pair stacking, but the hydrophobic character of the inner surface of the depsipeptide backbone is decisive. The sequence specificity of the intercalation is remarkable: the amino acids of the backbone form hydrogen bonds to specific nucleobases in the DNA double strand and cause a CpG selectivity in the case of triostin A.[7]



Triostin A (**1**): R = CH_3
TANDEM (**2**): R = H

FIGURE 1. Triostin A (**1**) and TANDEM (**2**).

Many analogues of the quinoxaline antibiotics have been prepared so far, varying the intercalating chromophores or the backbone.[8] Various heteroaromatic chromophores have been incorporated and proven to be capable of intercalation.[8a−c] Backbone analogues also exhibit significant biological activity, as long as the hydrogen bonds to the DNA can be formed and permit the binding of the depsipeptide bicycle.[8d−f]

In this paper, we report the synthesis of triostin A analogues bearing two identical or different nucleobases instead of the natural quinoxaline substituents. As planar heteroaromatic compounds, the nucleobases can still act as intercalators (Figure 2A)[9] but could also form additional hydrogen bonds to the base pairs in the DNA double strand (Figure 2B). Thus, recognition of the Hoogsteen site of DNA base pairs in the major groove similar to triple strand formation could be accom-

† Dedicated to Prof. Dr. Axel Zeeck on the occasion of his 65th birthday.

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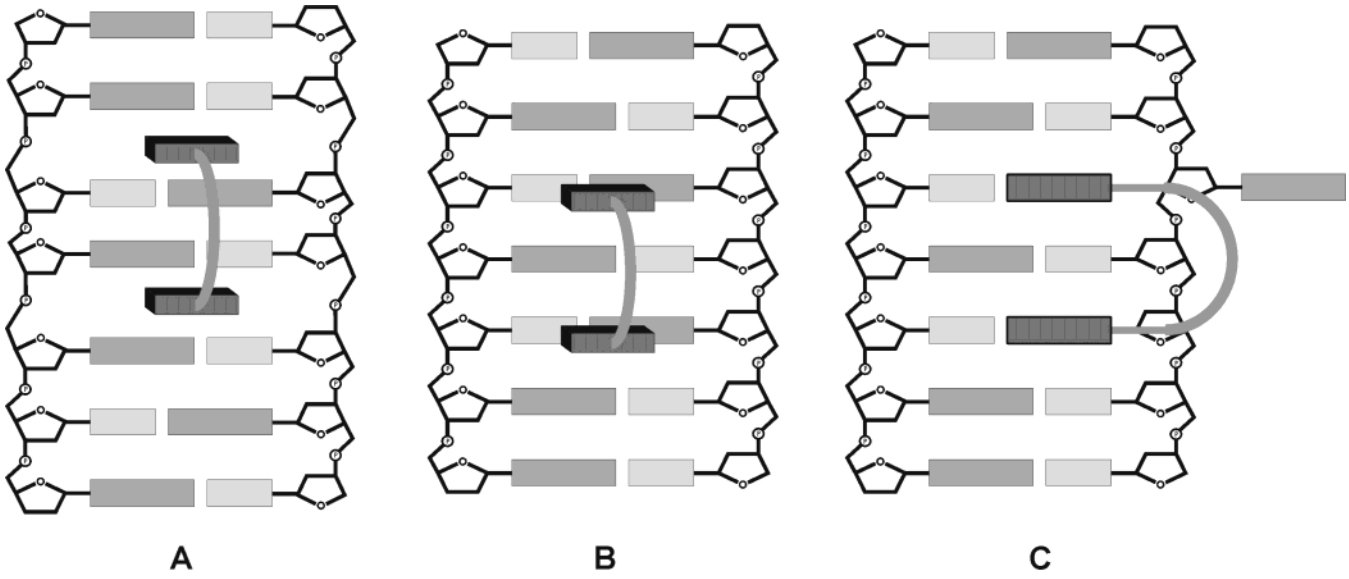


FIGURE 2. Possible modes of interaction with double-stranded DNA.

plished.[10] Additionally, the rigid template with its nucleobases oriented in parallel might serve as a probe for abasic sites in DNA, a mismatch position that lacks the nucleobase usually attached to the DNA backbone (Figure 2C). In this case, one nucleobase of the triostin A analogue might occupy the free abasic position, and the second could displace an additional nucleobase, flipping the original pairing base out of the double strand. The abasic position would be marked and could be easily detected.

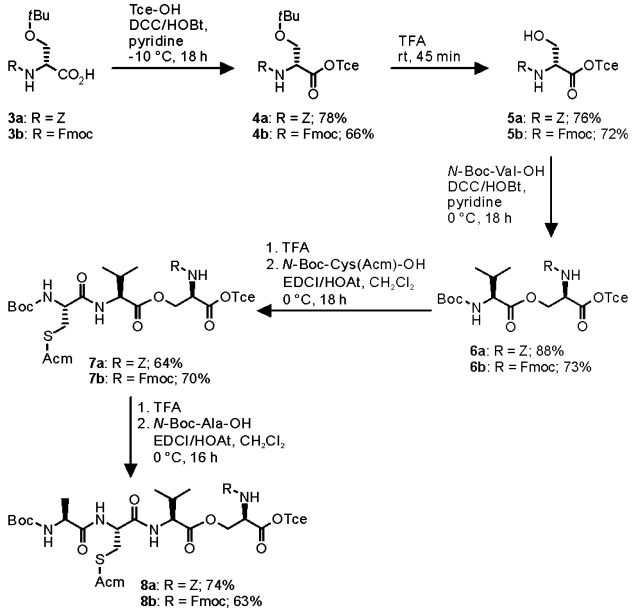
For these possible applications, nonsymmetrical functionalization of the triostin backbone is essential. Therefore, the synthesis of a triostin A analogue depsipeptide with two orthogonally protected positions was developed in order to link two different chromophores.

TANDEM (des-*N*-tetramethyltriostin A, **2**), a triostin A analogue consisting of unmethylated amino acids (Figure 1), can form additional β-sheetlike intramolecular hydrogen bonds and, therefore, shows slightly weaker binding constants and a different sequence selectivity in its binding to double-stranded DNA (TpA).[11] The rigid, C_2-symmetrical bicycle can serve as an optimal scaffold for the parallel orientation of two intercalating moieties and was chosen as the backbone for the first generation of nucleobase-substituted derivatives.

Results and Discussion

The TANDEM backbone is composed of two identical peptide sequences of D-serine, L-alanine, L-cysteine, and L-valine, which are linked with an ester bond.[12] The tetradepsipeptide **8** was built up stepwise in solution

SCHEME 1. Synthesis of the Tetradepsipeptide 8



(Scheme 1). The linkage of two suitably protected tetradepsipeptide units **9** and **10** by modern peptide synthesis protocols furnished the linear octadepsipeptide **11**. Two intramolecular cyclizations provided the key compound of the synthesis, the bicyclic depsipeptide scaffold **14** (Scheme 2). The choice of orthogonal protecting groups allowed for the synthesis of substrates presenting two

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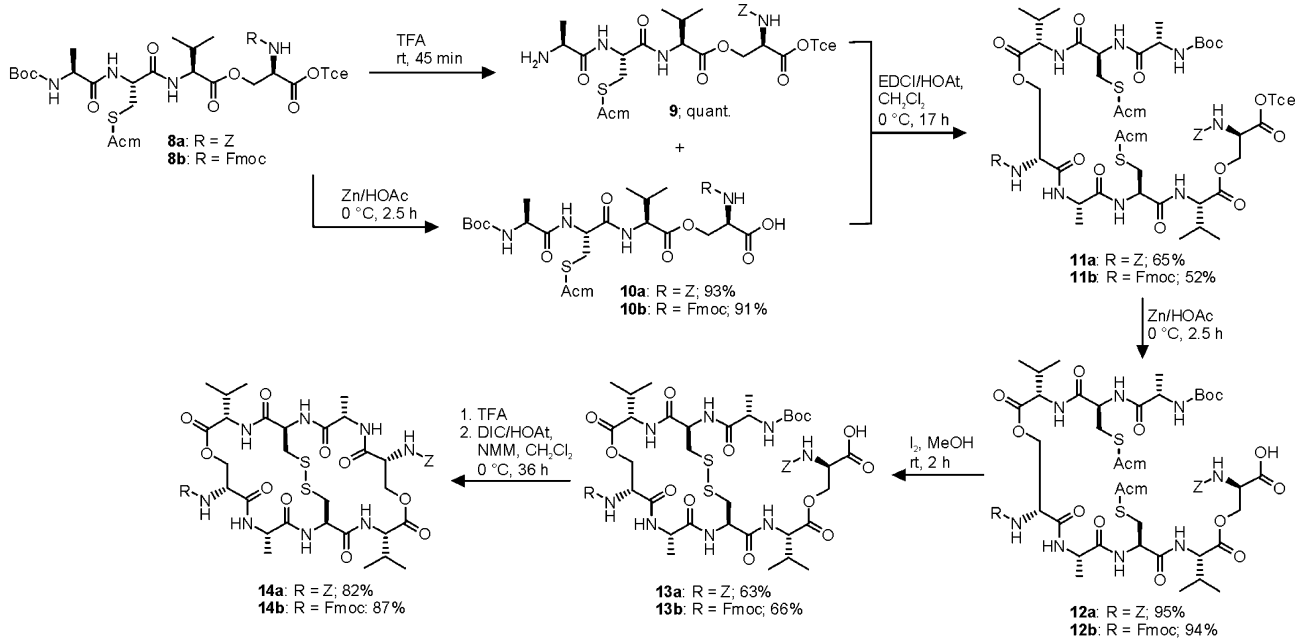
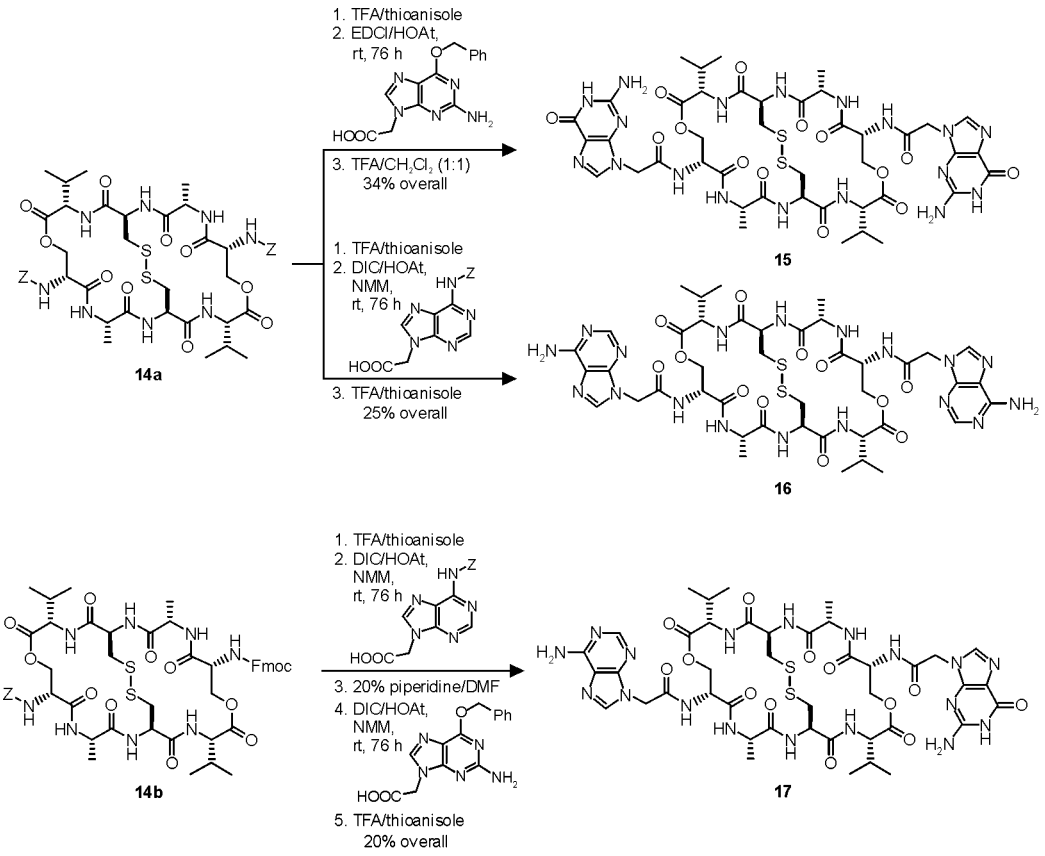
SCHEME 2. Synthesis of the TANDEM Backbone 14



SCHEME 3. Synthesis of the Nucleobase-Modified TANDEM Analogues



different substituents. After selective removal of the protecting groups, various nucleobase-substituted building blocks can be attached to the bicyclic scaffold (Scheme 3).

Synthesis of the Symmetrically Protected TANDEM Backbone. Amino acid (*Z*)-D-Ser-OTce (**5a**) was prepared according to the literature[13] starting from (*Z*)-D-Ser(*t*Bu)-OH (**3a**) by esterification with trichloroethanol and subsequent deprotection with TFA (Scheme 1). The conversion to the depsipeptide **6a** occurred without significant racemization (<4%) in good yields of about 90% and could not be increased by prior formation of the symmetrical anhydride $(Boc\text{-}Val)_2O$.[12a]

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Depsipeptide **6a** was deprotected with neat TFA, and the free amine was obtained by washing with 5% aqueous $NaHCO_3$. The trichloroethyl (Tce)-protected C-terminus increased the solubility of all depsipeptides in organic solvents and enabled for the isolation of the free amines by extraction with ethyl acetate. Thus, higher coupling yields have been reached, since the TFA salts of amino acids often proved to react slowly and insufficiently in amide bond formation. Coupling with Boc-L-Cys(Acm)-OH was achieved by the use of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI·HCl) as a water-soluble activation reagent and 1-hydroxy-7-azabenzotriazole (HOAt) as a coupling additive in dichloromethane at 0 °C.[14] The additive served to inhibit side reactions and reduced the risk of racemization.[12c,d] Slightly lower conversions were obtained using 1-hydroxybenzotriazole (HOBt) instead of HOAt. The acetamidomethyl (Acm) protection of the cysteine thiol offers two advantages: side reactions are rare and, on formation of the disulfide bridge, deprotection and oxidation can be accomplished in a single step. High yields can be achieved, and no intermolecular reactions are observed.[15] Tridepsipeptide **7a** was then converted to the tetradepsipeptide **8a** by TFA deprotection followed by EDCI/HOAt-mediated coupling with Boc-L-Ala-OH.

Following the convergent approach, tetradepsipeptide **8a** had to be selectively deprotected for the assembly of the linear octadepsipeptide **11a** (Scheme 2). The free amine **9** was obtained by treatment of **8a** with TFA, neutralization, and subsequent extraction in ethyl acetate. To reveal the free carboxylic acid, **8a** was treated with zinc powder in 90% acetic acid to remove the Tce ester selectively. No loss of the acid-labile Boc protecting group or cleavage of the intramolecular ester was observed under the conditions applied. Compound **10a** was previously prepared and employed without purification and characterization as an intermediate in the synthesis of Olsen et al.[12b]

Coupling of fragments **9** and **10a** to the linear octadepsipeptide **11a** was accomplished by standard peptide coupling protocols (EDCI/HOAt in CH_2Cl_2) in good yields. In the next step, the Tce ester had to be removed by reductive cleavage prior to the disulfide bond formation. Selective intramolecular formation of the disulfide bond occurred spontaneously after iodine-mediated deprotection of the Acm groups under conditions of high dilution.

The C-terminus and the TFA-liberated N-terminal end were now orientated ideally in close proximity, and the macrocyclization was favored by the structural rigidness. Thus, the diisopropylcarbodiimide (DIC)-activated and HOAt-assisted ring closure to the final bicycle **14a** proceeded with respectable yields (typically 60−85%).[12b−d] To deprotect the amine functionalities for coupling with the nucleobase moieties, the benzyloxycarbonyl (Z) group could be removed by a TFA/thioanisole-mediated push−pull mechanism.[16]

Synthesis of the Orthogonally Protected TANDEM Backbone. For the synthesis of TANDEM analogues presenting two different nucleobases, the key compound of the synthesis, the bicyclic core **14**, had to be furnished with two orthogonal protecting groups at the amine functionalities (Scheme 2). The 9-fluorenylmethyloxycarbonyl (Fmoc) group seemed to be ideally suited to be selectively removed by treatment with base (typically 20% piperidine in DMF) in contrast to the acid labile Z group, which can be detached with 10% thioanisole in TFA.

The Fmoc-protected tetradepsipeptide **8b** was prepared in analogy to the procedure described above starting from Fmoc-D-Ser(*t*Bu)-OH (**3b**)[17] in a slightly lower overall yield, which may be due to the higher steric hindrance of the large Fmoc group in the coupling reactions (Scheme 1). Addition of $NaHCO_3$ as a base showed no effect. Extension of the reaction times or conversion at slightly higher temperatures resulted in significant racemization. The C-terminus was deprotected by zinc powder in 90% acetic acid, and the resulting compound **10b** was coupled to the Z-protected tetradepsipeptide **9** by activation with EDCI and HOAt. The use of DIC and HOAt in this coupling reaction, assisted by *N*-methylmorpholine (NMM) as a base, showed no improvement but required a much more problematic purification procedure due to the higher number of side products. Deprotection of the terminal ester, followed by oxidative disulfide bond formation with iodine, provided compound **13b**, which was deprotected at the N-terminus and cyclized by DIC/HOAt activation to furnish the orthogonally protected bicyclic core **14b** (Scheme 2). Here, the addition of NMM as a base proved to be favorable. Higher yields could be obtained, and separation from unwanted side products was easily feasible by flash chromatography due to the different elution behavior of the bicycle. Similar conversions were observed using diphenyl phosphorazidate (DPPA) as an activating reagent, but purification of the crude product could only be achieved by HPLC separation, which resulted in a lower overall yield.

Nucleobase Modification of the TANDEM Backbone. The nucleobase-substituted acetic acids were synthesized and furnished with suitable protecting groups according to the literature[18] in order to increase their solubility in organic solvents and to prevent coupling to reactive amine groups of the nucleobases.

Coupling of the respective nucleobase moieties [2-amino-6-(benzyloxy)purin-9-yl]acetic acid[18] or (N^6-(*Z*)-adenin-9-yl)acetic acid[18] to the deprotected symmetrical TANDEM backbone was accomplished using HOAt as an additive, either in combination with EDCI or DIC as a coupling reagent (Scheme 3). The final compounds bearing the respective nucleobases in the side chain (guanine **15** or adenine **16**) were suitably deprotected, each purified by HPLC, and characterized. ^1H and ^{13}C NMR data proved the C_2-symmetry of the triostin A analogues.

For the synthesis of the analogues presenting two different nucleobases, the Z protecting group of compound **14b** was removed and the first nucleobase moiety (N^6-(*Z*)-adenin-9-yl)acetic acid[18] was attached to the free amine group by treatment with DIC and HOAt (Scheme 3). The Fmoc group was removed subsequently by addi-

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tion of 20% piperidine in DMF. After coevaporation with DMF and toluene and additional washing with diethyl ether to remove the typical polymeric side products of the Fmoc group, the second nucleobase-substituted amino acid [2-amino-6-(benzyloxy)purin-9-yl]acetic acid[18] was coupled to the TANDEM backbone. The resulting analogue **17** was deprotected with a mixture of thioanisole and TFA to remove the Z and the OPh group and purified by HPLC. The spectroscopic data confirmed the connectivity of the triostin A analogue.

Sufficient synthetic material has been assembled by these solution-phase syntheses to permit initial studies on the binding of the modified triostin analogues to double-stranded DNA by gel electrophoresis and UV and CD spectroscopy. Additionally, cocrystallization experiments with DNA will provide further insight into the exact binding behavior. These studies are currently underway, and their results will be disclosed in due course.

Conclusion

A synthesis of novel analogues of the DNA bisintercalator triostin A presenting identical or two different nucleobases instead of the natural quinoxaline substituents has been developed. In particular, the synthesis furnishing the orthogonally protected TANDEM backbone will widen the scope of synthetic analogues and allow further investigation of the influence of the intercalating moieties on the binding mode of triostin analogues. The modification of the intercalators might alter the binding mode of the triostin A analogues to double-stranded DNA. X-ray crystallographic studies of triostin A analogue−DNA complexes as well as DNA binding studies via gel electrophoresis and UV and CD spectroscopy are in progress.

Experimental Section

(*Z*)-D-Ser[*N*-Boc-L-Cys(Acm)-L-Val]-OTce 7a. Compound **6a** (4.54 g, 7.97 mmol) was treated with TFA (50 mL) at 25 °C for 30 min, followed by evaporation in vacuo. The residue was dissolved in ethyl acetate (100 mL), washed with 5% aqueous $NaHCO_3$ (80 mL) and saturated aqueous NaCl (50 mL), and dried ($MgSO_4$). Filtration and evaporation in vacuo afforded a white foam, which was dissolved in CH_2Cl_2 (30 mL) and directly used in the next step.

A solution of Boc-L-Cys(Acm)-OH (3.49 g, 11.9 mmol, 1.5 equiv) in CH_2Cl_2 (70 mL) was cooled to −10 °C and treated sequentially with HOAt (1.63 g, 11.9 mmol, 1.5 equiv) and EDCI (2.29 g, 11.9 mmol, 1.5 equiv). After 10 min, the solution of (*Z*)-D-Ser(l-Val)-OTce in CH_2Cl_2 was added, and the mixture was allowed to stir at 0 °C for 18 h. The reaction mixture was poured onto cold 1 N aqueous HCl (100 mL); the phases were separated, and the aqueous phase was extracted with ethyl acetate (2 × 100 mL). The combined organic phases were washed with 5% aqueous $NaHCO_3$ (80 mL) and saturated aqueous NaCl (50 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 6 × 17 cm, 50% ethyl acetate−hexane) yielded **7a** (3.80 g, 5.11 mmol, 64%) as a pale yellow foam: TLC (50% ethyl acetate/hexane) R_f 0.30; $[\alpha]^{25}_D$ −15 (*c* 0.07, MeOH); mp 61−64 °C; UV (MeOH) $\lambda_{max}(\epsilon)$ 257 nm; IR (KBr) ν_{max} 3320, 2969, 1716, 1538, 1370, 1254, 1167, 1058, 719 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.90 (d, 3H, ^3J = 7 Hz, Val-CH_3), 0.92 (d, 3H, ^3J = 7 Hz, Val-CH_3), 1.41 (s, 9H, *t*Bu-CH_3), 1.97 (s, 3H, Acm-CH_3), 2.11−2.18 (m, 1H, Val-Hβ), 2.56 (dd, 1H, ^3J = 8 Hz, ^2J = 15 Hz, Cys-Hβ), 2.89 (dd, 1H, ^3J = 4 Hz, ^2J = 15 Hz, Cys-Hβ), 4.31−4.34 (m, 2H, Val-Hα, Acm-CH_2), 4.42 (ddd, 1H, ^3J = 4 Hz, ^3J = 8 Hz, ^3J = 8 Hz, Cys-Hα), 4.47−4.50 (m, 2H, Ser-Hβ, Acm-CH_2), 4.63−4.64 (m, 1H, Ser-Hβ), 4.68 (d, 1H, ^2J = 12 Hz, Tce-CH_2), 4.82 (ddd, 1H, ^3J = 3 Hz, ^3J = 3 Hz, ^3J = 9 Hz, Ser-Hα), 4.88 (d, 1H, ^2J = 12 Hz, Tce-CH_2), 5.06−5.12 (m, 2H, Z-CH_2), 5.41 (d, 1H, ^3J = 8 Hz, Cys-NH), 6.47 (d, 1H, ^3J = 9 Hz, Ser-NH), 6.76−6.78 (m, 1H, Acm-NH), 7.28−7.36 (m, 5H, Z-aromat CH), 7.43 (d, 1H, ^3J = 8 Hz, Val-NH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 17.6, 18.9 (Val-CH_3), 23.1 (Acm-CH_3), 28.2 (*t*Bu-CH_3), 30.4 (Val-CHβ), 35.0 (Cys-$CH_2\beta$), 41.2 (Acm-CH_2), 53.2 (Ser-CHα, Cys-CHα), 57.8 (Val-CHα), 64.4 (Ser-$CH_2\beta$), 67.2 (Z-CH_2), 74.6 (Tce-CH_2), 80.1 (*t*Bu-quart C), 94.2 (Tce-CCl_3), 128.1, 128.2, 128.5 (Z-aromat CH), 135.9 (Z-quart C), 155.95, 156.01 (Boc-CO, Z-CO), 168.1, 170.9, 171.4, 171.6 (Ser-CO, Val-CO, Cys-CO, Acm-CO); HRMS (ESI) calcd for $C_{29}H_{41}Cl_3N_4O_{10}SNa$ ([M + Na]$^+$) 765.1501, found 765.1504.

(*Z*)-D-Ser[*N*-Boc-L-Ala-L-Cys(Acm)-L-Val]-OTce 8a. Compound **7a** (3.93 g, 5.28 mmol) was treated with TFA (40 mL), and the mixture was stirred at 25 °C for 30 min before the TFA was removed by evaporation in vacuo. The residue was taken up in ethyl acetate (100 mL), washed with 5% aqueous $NaHCO_3$ (80 mL) and saturated aqueous NaCl (50 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo. The resulting yellow foam was dissolved in CH_2Cl_2 (20 mL) and directly used in the next step.

To a solution of Boc-L-Ala-OH (1.44 g, 7.92 mmol, 1.5 equiv) in CH_2Cl_2 (60 mL) at −10 °C were added sequentially HOAt (1.08 g, 7.92 mmol, 1.5 equiv), EDCI (1.52 g, 7.92 mmol, 1.5 equiv), and after 10 min the solution of (*Z*)-D-Ser[l-Cys(Acm)-L-Val]-OTce in CH_2Cl_2. The mixture was allowed to react at 0 °C for 16 h before being poured onto cold 1 N aqueous HCl (100 mL). The phases were separated, and the aqueous phase was extracted with ethyl acetate (2 × 100 mL). The combined organic phases were washed with 5% aqueous $NaHCO_3$ (80 mL) and saturated aqueous NaCl (50 mL) and dried ($MgSO_4$). Filtration and concentration in vacuo were followed by separation by flash chromatography (SiO_2, 6 × 15 cm, 25% hexanes−ethyl acetate) to afford **8a** (3.18 g, 3.90 mmol, 74%) as a pale yellow foam: TLC (25% hexane/ethyl acetate) R_f 0.47; $[\alpha]^{25}_D$ −32 (*c* 0.12, MeOH); mp 64−66 °C; UV (MeOH) $\lambda_{max}(\epsilon)$ 264 nm; IR (KBr) ν_{max} 3313, 2973, 1743, 1657, 1532, 1250, 1166, 1063, 720 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 0.88 (d, 3H, ^3J = 6 Hz, Val-CH_3), 0.89 (d, 3H, ^3J = 6 Hz, Val-CH_3), 1.28 (d, 3H, ^3J = 7 Hz, Ala-CH_3), 1.40 (s, 9H, *t*Bu-CH_3), 1.96 (s, 3H, Acm-CH_3), 2.12−2.19 (m, 1H, Val-Hβ), 2.73 (dd, 1H, ^3J = 8 Hz, ^2J = 13 Hz, Cys-Hβ), 2.91−2.97 (m, 1H, Cys-Hβ), 4.11−4.17 (m, 1H, Ala-Hα), 4.29−4.37 (m, 2H, Val-Hα, Acm-CH_2), 4.38−4.45 (m, 2H, Ser-Hβ, Acm-CH_2), 4.64−4.69 (m, 2H, Cys-Hα, Tce-CH_2), 4.73 (dd, 1H, ^3J = 3 Hz, ^2J = 11 Hz, Ser-Hβ), 4.82 (ddd, 1H, ^3J = 3 Hz, ^3J = 3 Hz, ^3J = 9 Hz, Ser-Hα), 4.89 (d, 1H, ^2J = 12 Hz, Tce-CH_2), 5.05 (d, 1H, ^3J = 7 Hz, Ala-NH), 5.06−5.12 (m, 2H, Z-CH_2), 6.72 (d, 1H, ^3J = 9 Hz, Ser-NH), 6.77 (t, 1H, ^3J = 6 Hz, Acm-NH), 7.20 (d, 1H, ^3J = 7 Hz, Cys-NH), 7.28−7.34 (m, 5H, Z-aromat CH), 7.41 (d, 1H, ^3J = 8 Hz, Val-NH); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 17.6 (Ala-CH_3), 18.2, 18.9 (Val-CH_3), 23.3 (Acm-CH_3), 28.2 (*t*Bu-CH_3), 30.6 (Val-CHβ), 34.0 (Cys-$CH_2\beta$), 41.5 (Acm-CH_2), 50.4 (Ala-CHα), 52.6 (Cys-CHα), 53.3 (Ser-CHα), 57.8 (Val-CHα), 64.6 (Ser-$CH_2\beta$), 67.2 (Z-CH_2), 74.6 (Tce-CH_2), 80.3 (*t*Bu-quart C), 94.2 (Tce-CCl_3), 128.1, 128.3, 128.5 (Z-aromat CH), 136.0 (Z-quart C), 155.5, 156.2 (Boc-CO, Z-CO), 168.2, 170.5, 171.7, 173.4 (Ser-CO, Val-CO, Cys-CO, Ala-CO, Acm-CO); HRMS (ESI) calcd for $C_{32}H_{46}Cl_3N_5O_{11}S$ ([M + H]$^+$) 814.2053, found 814.2055.

(*Z*)-D-Ser[L-Ala-L-Cys(Acm)-L-Val]-OTce 9. Compound **8a** (700 mg, 0.86 mmol) was treated with TFA (15 mL) at 25 °C for 30 min. After the TFA was removed by evaporation in vacuo, the residue was dissolved in ethyl acetate (50 mL), washed with 5% aqueous $NaHCO_3$ (50 mL) and saturated aqueous NaCl (40 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo. The resulting yellow foam was directly used in the next step without further purification: TLC ($CHCl_3$/MeOH/

H_2O/HOAc = 70:30:3:0.35) R_f 0.76; ESI-MS (MeOH) *m/z* 716 ($[M + H]^+$, 32), 738 ($[M + Na]^+$, 100), 1452 ($[2M - H + Na]^+$, 20).

(*Z*)-D-Ser[*N*-Boc-L-Ala-L-Cys(Acm)-L-Val]-OH 10a. A solution of **8a** (800 mg, 0.98 mmol) in 90% aqueous acetic acid (30 mL) was cooled to 0 °C, and zinc powder (3.21 g, 49.0 mmol, 50 equiv) was added. The suspension was stirred for 2.5 h at 0 °C, filtered, and concentrated in vacuo. The residue was treated with cold 1 N aqueous HCl (50 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic phases were washed with saturated aqueous NaCl (50 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (SiO_2, 3 × 17 cm, 20% methanol−ethyl acetate, 0.5% HOAc) to provide **10a** (623 mg, 0.91 mmol, 93%) after coevaporation with toluene (3 × 5 mL) as an amorphous white solid: TLC (20% methanol/ethyl acetate, 0.5% HOAc) R_f 0.42; $[\alpha]^{25}_D$ −32 (*c* 0.05, DMSO); mp 165−168 °C; UV (DMSO) $\lambda_{max}(\epsilon)$ 278 nm; IR (KBr) ν_{max} 3425, 2973, 2362, 1652, 1542, 1527, 1370, 1252, 1166, 1064 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 0.81 (d, 3H, ^3J = 7 Hz, Val-CH_3), 0.82 (d, 3H, ^3J = 7 Hz, Val-CH_3), 1.16 (d, 3H, ^3J = 7 Hz, Ala-CH_3), 1.36 (s, 9H, *t*Bu-CH_3), 1.81 (s, acetate-CH_3), 1.83 (s, 3H, Acm-CH_3), 1.99−2.06 (m, 1H, Val-Hβ), 2.64 (dd, 1 H, ^3J = 11 Hz, ^2J = 14 Hz, Cys-Hβ), 3.01 (dd, 1 H, ^3J = 4 Hz, ^2J = 13 Hz, Cys-Hβ), 3.95−4.04 (m, 2H, Ser-Hα, Ala-Hα), 4.14−4.20 (m, 2H, Val-Hα, Acm-CH_2), 4.26−4.36 (m, 3H, Ser-Hβ, Acm-CH_2), 4.53−4.60 (m, 1H, Cys-Hα), 4.94−5.02 (m, 2H, Z-CH_2), 6.76 (d, 1H, ^3J = 4 Hz, Ser-NH/Ala-NH), 6.83 (d, 1H, ^3J = 7 Hz, Ser-NH/Ala-NH), 7.28−7.36 (m, 5H, Z-aromat CH), 7.86 (d, 1H, ^3J = 8 Hz, Cys-NH), 8.05 (d, 1H, ^3J = 8 Hz, Val-NH), 9.41 (s, br, 1H, Ser-CO_2H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 17.6 (Val-CH_3), 18.1 (Ala-CH_3), 18.6 (Val-CH_3), 20.9 (acetate-CH_3), 22.3 (Acm-CH_3), 27.9 (*t*Bu-CH_3), 29.8 (Val-CHβ), 32.6 (Cys-$CH_2\beta$), 40.0 (Acm-CH_2), 49.5 (Ala-CHα), 51.8 (Cys-CHα), 54.7 (Ser-CHα), 57.4 (Val-CHα), 65.2 (Z-CH_2), 65.6 (Ser-$CH_2\beta$), 77.8 (*t*Bu-quart C), 127.6, 127.9, 128.2 (Z-aromat CH), 137.1 (Z-quart C), 154.8, 155.3 (Boc-CO, Z-CO), 169.5, 170.4, 170.8, 170.9, 172.6 (Ser-CO, Val-CO, Cys-CO, Ala-CO, Acm-CO), 176.3 (acetate-CO); HRMS (ESI) calcd for $C_{30}H_{45}N_5O_{11}S$ ($[M + H]^+$) 684.2909, found 684.2907.

(*Z*)-D-Ser{(*Z*)-D-Ser[Boc-L-Ala-L-Cys(Acm)-L-Val]-L-Ala-L-Cys(Acm)-L-Val}-OTce 11a. To a solution of **10a** (586 mg, 0.86 mmol) in CH_2Cl_2 (30 mL) at −10 °C were added subsequently HOAt (140 mg, 1.03 mmol, 1.2 equiv), EDCI (197 mg, 1.03 mmol, 1.2 equiv), and after 5 min a solution of **9** (0.86 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred at 0 °C for 17 h, before being poured onto cold 1 N aqueous HCl (100 mL). The phases were separated, and the aqueous phase was extracted with ethyl acetate (2 × 100 mL). The combined organic phases were washed with 5% aqueous $NaHCO_3$ (80 mL) and saturated aqueous NaCl (50 mL). Drying ($MgSO_4$) was followed by filtration and concentration in vacuo. Flash chromatography (SiO_2, 3 × 14 cm, 10% methanol−ethyl acetate) yielded **11a** (773 mg, 0.56 mmol, 65%) as a white foam: TLC (10% methanol/ethyl acetate) R_f 0.70; $[\alpha]^{25}_D$ −46 (*c* 0.08, MeOH); mp 141−144 °C; UV (MeOH) $\lambda_{max}(\epsilon)$ 258 nm; IR (KBr) ν_{max} 3300, 2969, 1693, 1650, 1540, 1452, 1371, 1265, 1169, 1061, 720, 698 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 0.81 (d, 6H, ^3J = 6 Hz, Val-CH_3), 0.82 (d, 6H, ^3J = 6 Hz, Val-CH_3), 1.17 (d, 3H, ^3J = 8 Hz, Ala-CH_3), 1.18 (d, 3H, ^3J = 8 Hz, Ala-CH_3), 1.36 (s, 9H, *t*Bu-CH_3), 1.84 (s, 3H, Acm-CH_3), 1.85 (s, 3H, Acm-CH_3), 2.01−2.10 (m, 2H, Val-Hβ), 2.64−2.71 (m, 2H, Cys-Hβ), 2.88−2.94 (m, 2H, Cys-Hβ), 3.96−4.04 (m, 1H, Ala-Hα), 4.12−4.22 (m, 3H, Ser-Hβ, Acm-CH_2), 4.22−4.26 (m, 1H, Val-Hα), 4.29−4.36 (m, 6H, Acm-CH_2, Ala-Hα, Val-Hα, Ser-Hβ), 4.40−4.45 (m, 2H, Ser-Hα, Ser-Hβ), 4.55 (ddd, 1H, ^3J = 4 Hz, ^3J = 9 Hz, ^3J = 9 Hz, Cys-Hα), 4.57−4.63 (m, br, 1H, Cys-Hα), 4.65 (ddd, 1H, ^3J = 4 Hz, ^3J = 6 Hz, ^3J = 10 Hz, Ser-Hα), 4.88 (d, 1H, ^2J = 12 Hz, Tce-CH_2), 4.96 (d, 1H, ^2J = 12 Hz, Tce-CH_2), 4.99−5.07 (m, 4H, Z-CH_2), 6.85 (d, 1H, ^3J = 7 Hz, Ala-NH), 7.27−7.38 (m, 10H, Z-aromat CH), 7.54 (d, 1H, ^3J = 8 Hz, Ser-NH), 7.86 (d, 1H, ^3J = 9 Hz, Val-NH), 7.88−7.93 (m, 2H, Val-NH, Cys-NH), 7.99 (d, 1H, ^3J = 9 Hz, Ser-NH), 8.20 (d, 1H, ^3J = 8 Hz, Cys-NH), 8.22 (d, 1H, ^3J = 8 Hz, Ala-NH), 8.48 (t, 1H, ^3J = 6 Hz, Acm-NH), 8.51 (t, 1H, ^3J = 6 Hz, Acm-NH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 17.4, 17.5, 18.2, 18.4, 18.7 (Ala-CH_3, Val-CH_3), 22.4 (Acm-CH_3), 22.5 (Acm-CH_3), 28.1 (*t*Bu-CH_3), 29.9 (Val-CHβ), 30.0 (Val-CHβ), 32.0 (Cys-$CH_2\beta$), 32.6 (Cys-$CH_2\beta$), 40.0 (Acm-CH_2), 40.1 (Acm-CH_2), 48.1 (Ala-CHα), 49.7 (Ala-CHα), 52.0 (Cys-CHα), 52.6 (Cys-CHα), 52.8 (Ser-CHα), 53.4 (Ser-CHα), 56.7 (Val-CHα), 56.8 (Val-CHα), 63.2 (Ser-$CH_2\beta$), 64.4 (Ser-$CH_2\beta$), 65.7 (Z-CH_2), 65.8 (Z-CH_2), 73.7 (Tce-CH_2), 78.0 (*t*Bu-quart C), 94.7 (Tce-CCl_3), 127.7, 127.8, 128.2, 128.3 (Z-aromat CH), 136.5, 136.6 (Z-quart C), 154.9, 155.8, 155.9 (Boc-CO, Z-CO), 168.0, 168.1, 169.9, 170.1, 170.2, 170.3, 170.6, 170.7, 172.0, 172.8 (Ser-CO, Val-CO, Cys-CO, Ala-CO, Acm-CO); HRMS (ESI) calcd for $C_{57}H_{82}Cl_3N_{10}O_{19}S_2$ ($[M + H]^+$) 1379.4259, found 1379.4261.

(*Z*)-D-Ser{(*Z*)-D-Ser[Boc-L-Ala-L-Cys(Acm)-L-Val]-L-Ala-L-Cys(Acm)-L-Val}-OH 12a. Compound **11a** (300 mg, 0.22 mmol) was dissolved in 90% aqueous acetic acid (7.5 mL) and cooled to 0 °C, and zinc powder (710 mg, 10.9 mmol, 50 equiv) was added. The suspension was stirred for 2.5 h at 0 °C; the zinc was filtered off, and the filtrate was concentrated in vacuo. The residue was treated with cold 1 N aqueous HCl (15 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic phases were washed with saturated aqueous NaCl (10 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. After purification by flash chromatography (SiO_2, 2 × 16 cm, 20% methanol−ethyl acetate, 0.5% HOAc) and coevaporation with toluene (3 × 5 mL), **12a** (262 mg, 0.21 mmol, 95%) could be obtained as an amorphous white solid: TLC (20% methanol/ethyl acetate, 0.5% HOAc) R_f 0.31; $[\alpha]^{25}_D$ −48 (*c* 0.10, MeOH); mp 150−152 °C; UV (MeOH) $\lambda_{max}(\epsilon)$ 258 nm; IR (KBr) ν_{max} 3301, 2968, 2929, 1731, 1687, 1647, 1541, 1453, 1371, 1252, 1063, 689 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 0.79−0.82 (m, 12H, Val-CH_3), 1.15−1.18 (m, 6H, Ala-CH_3), 1.36 (s, 9H, *t*Bu-CH_3), 1.76 (s, 3H, acetate-CH_3), 1.82 (s, 3H, Acm-CH_3), 1.83 (s, 3H, Acm-CH_3), 2.02−2.04 (m, 2H, Val-Hβ), 2.62−2.68 (m, 2H, Cys-Hβ), 2.91 (dd, 1H, ^3J = 4 Hz, ^2J = 14 Hz, Cys-Hβ), 3.06 (dd, 1H, ^3J = 4 Hz, ^2J = 14 Hz, Cys-Hβ), 3.89 (ddd, 1H, ^3J = 3 Hz, ^3J = 3 Hz, ^3J = 7 Hz, Ser-Hα), 3.98−4.01 (m, 1H, Ala-Hα), 4.10 (dd, 1H, ^3J = 6 Hz, ^3J = 8 Hz, Val-Hα), 4.12−4.20 (m, 3H, Acm-CH_2, Ser-Hβ), 4.22−4.25 (m, 2H, Val-Hα, Ser-Hβ), 4.29−4.38 (m, 5H, Acm-CH_2, Ala-Hα, Ser-Hβ), 4.40−4.43 (m, 1H, Ser-Hα), 4.48−4.52 (m, 1H, Cys-Hα), 4.58−4.60 (m, 1H, Cys-Hα), 4.96−5.05 (m, 4H, Z-CH_2), 6.62 (d, 1H, ^3J = 6 Hz, Ser-NH), 6.86 (d, 1H, ^3J = 6 Hz, Ala-NH), 7.28−7.35 (m, 10H, Z-aromat CH), 7.65 (d, 1H, ^3J = 7 Hz, Ser-NH), 7.93 (d, 2H, ^3J = 7 Hz, Cys-NH, Val-NH), 8.06 (d, 1H, ^3J = 8 Hz, Val-NH), 8.17 (d, 1H, ^3J = 6 Hz, Cys-NH), 8.23 (d, 1H, ^3J = 7 Hz, Ala-NH), 8.53 (m, br, 1H, Acm-NH), 9.84 (s, br, 1H, COOH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 17.5 (Val-CH_3), 17.8 (Val-CH_3), 18.2 (Ala-CH_3), 18.5 (Ala-CH_3), 18.8 (acetate-CH_3, Val-CH_3), 22.5 (Acm-CH_3), 23.4 (Acm-CH_3), 28.1 (*t*Bu-CH_3), 29.8 (Val-CHβ), 29.9 (Val-CHβ), 32.3 (Cys-$CH_2\beta$), 32.7 (Cys-$CH_2\beta$), 40.0 (Acm-CH_2), 48.1 (Ala-CHα), 49.7 (Ala-CHα), 52.0 (Cys-CHα), 52.3 (Cys-CHα), 53.7 (Ser-CHα), 54.7 (Ser-CHα), 57.0 (Val-CHα), 57.6 (Val-CHα), 64.4 (Ser-$CH_2\beta$), 65.2 (Z-CH_2), 65.5 (Ser-$CH_2\beta$), 65.9 (Z-CH_2), 78.3 (*t*Bu-quart C), 127.6, 127.7, 128.2 (Z-aromat CH), 136.7, 137.1 (Z-quart C), 155.0, 155.4, 156.0 (Boc-CO, Z-CO), 168.0, 169.5, 169.9, 170.4, 170.7, 171.9, 172.8 (Ser-CO, Val-CO, Cys-CO, Ala-CO, Acm-CO), 176.4 (acetate-CO); HRMS (ESI) calcd for $C_{55}H_{81}N_{10}O_{19}S_2$ ($[M + H]^+$) 1249.5115, found 1249.5112.

(*Z*)-D-Ser[(*Z*)-D-Ser(Boc-L-Ala-L-Cys-L-Val)-L-Ala-L-Cys-L-Val]-OH Disulfide 13a. To a solution of iodine (203 mg, 800 μmol, 10 equiv) in 90 mL of CH_2Cl_2−MeOH (10:1) was added dropwise a solution of **12a** (100 mg, 80.0 μmol) in 30 mL of CH_2Cl_2−MeOH (15:1), and the mixture was stirred at 25 °C for 2.5 h. The reaction mixture was cooled to 0 °C, and 5% aqueous $Na_2S_2O_3$ was added slowly until the excess iodine was discharged and the color disappeared. The phases were separated, and the organic phase was washed with 1 N

aqueous HCl (80 mL) and saturated aqueous NaCl (50 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 2 × 18 cm, 15% methanol−ethyl acetate, 0.3% HOAc) furnished **13a** (57.5 mg, 52.0 μmol, 63%) after coevaporation with toluene (3 × 5 mL) as an amorphous white solid: TLC (20% methanol/ethyl acetate, 0.5% HOAc) R_f 0.58; $[\alpha]^{25}_D$ −44 (*c* 0.03, MeOH); mp 189−193 °C; UV (MeOH) $\lambda_{max}(\epsilon)$ 264 nm; IR (KBr) ν_{max} 3421, 2966, 2363, 1655, 1526, 1454, 1401, 1254, 1061, 583 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 0.81 (d, 3H, ^3J = 6 Hz, Val-CH_3), 0.82 (d, 3H, ^3J = 6 Hz, Val-CH_3), 0.84 (d, 3H, ^3J = 7 Hz, Val-CH_3), 0.85 (d, 3H, ^3J = 7 Hz, Val-CH_3), 1.19 (d, 3H, ^3J = 7 Hz, Ala-CH_3), 1.26 (d, 3H, ^3J = 7 Hz, Ala-CH_3), 1.35 (s, 9H, *t*Bu-CH_3), 1.77 (s, 3H, acetate-CH_3), 2.01−2.14 (m, 2H, Val-Hβ), 2.89 (dd, 1H, ^3J = 11 Hz, ^2J = 14 Hz, Cys-Hβ), 2.92−2.99 (m, 2H, Cys-Hβ), 3.00−3.08 (m, 1H, Cys-Hβ), 3.14−3.23 (m, 4H, Val-Hα, Ser-Hβ, revealed by two-dimensional spectroscopy), 3.89−3.95 (m, 1H, Ser-Hα), 3.96−4.03 (m, 1H, Ala-Hα), 4.26−4.33 (m, 2H, Ala-Hα, Ser-Hβ), 4.34−4.39 (m, 2H, Ser-Hα, Ser-Hβ), 4.86−4.97 (m, 2H, Cys-Hα), 4.99 (s, 2H, Z-CH_2), 5.05−5.13 (m, 2H, Z-CH_2), 6.57−6.59 (m, br, 1H, Ser-NH), 6.89 (d, 1H, ^3J = 7 Hz, Ala-NH), 7.27−7.38 (m, 10H, Z-aromat CH), 7.71 (d, 1H, ^3J = 7 Hz, Ser-NH), 7.89 (d, 1H, ^3J = 6 Hz, Ala-NH), 7.99−8.03 (m, 2H, Cys-NH, Val-NH), 8.17−8.22 (m, 1H, Val-NH), 8.52 (d, 1H, ^3J = 9 Hz, Cys-NH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 17.6, 18.0, 18.4, 18.8 (Val-CH_3, Ala-CH_3), 23.8 (acetate-CH_3), 28.2 (*t*Bu-CH_3), 29.7 (Val-CHβ), 30.1 (Val-CHβ), 43.2 (Cys-CH_2β), 43.5 (Cys-CH_2β), 48.5 (Ala-CHα), 50.3 (Ala-CHα), 51.7 (Cys-CHα), 52.3 (Cys-CHα), 53.9 (Ser-CHα), 55.2 (Ser-CHα), 57.3 (Val-CHα), 58.1 (Val-CHα), 64.6 (Ser-CH_2β), 65.2 (Z-CH_2), 65.9 (Z-CH_2, Ser-CH_2β), 78.2 (*t*Bu-quart C), 127.5, 127.7, 128.0, 128.2, 128.3 (Z-aromat CH), 136.6 (Z-quart C), 137.1 (Z-quart C), 154.8, 155.5, 155.6 (Boc-CO, Z-CO), 169.6, 169.6, 169.9, 170.3, 170.9, 172.7 (Ser-CO, Val-CO, Cys-CO, Ala-CO, acetate-CO); HRMS (ESI) calcd for $C_{49}H_{68}N_8O_{17}S_2$ ([M + H]$^+$) 1105.4217, found 1105.4224.

((*Z*)-D-Ser-L-Ala-L-Cys-L-Val)$_2$ (Serine Hydroxyl) Dilactone Disulfide 14a. A sample of **13a** (35.0 mg, 31.7 μmol) was treated with TFA (2 mL), and the mixture was stirred at 25 °C for 30 min before the TFA was removed by evaporation in vacuo. The residue was diluted with 1 N aqueous HCl (10 mL), extracted with ethyl acetate (3 × 10 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo to give a yellow oil, which was dissolved in 20 mL of CH_2Cl_2−DMF (9:1) and directly used in the next step.

The solution was cooled to 0 °C and treated under vigorous stirring sequentially with HOAt (4.05 mg, 31.7 μmol, 1 equiv), NMM (9.81 μL, 95.1 μmol, 3 equiv), and DIC (47.0 μL, 317 μmol, 10 equiv). The mixture was allowed to react at 4 °C for 36 h before being poured onto cold 1 N aqueous HCl (10 mL). The phases were separated, and the aqueous phase was extracted with ethyl acetate (2 × 10 mL). The combined organic phases were washed with 5% aqueous $NaHCO_3$ (10 mL) and saturated aqueous NaCl (5 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 1 × 12 cm, ethyl acetate) afforded **14a** (24.4 mg, 24.7 μmol, 82%) as an amorphous pale yellow solid. The characteristic data was identical to the literature.[12b−d]

[(Guanin-9-yl acetyl)-D-Ser-L-Ala-L-Cys-L-Val]$_2$ (Serine Hydroxyl) Dilactone Disulfide 15. A sample of **14a** (11.1 mg, 11.3 μmol) was treated with thioanisole (190 μL) and TFA (1.90 mL), and the mixture was stirred for 10 h at 20 °C before being evaporated. The hydrochloride salt was formed by repeated addition of 2 N aqueous HCl (3 × 2 mL) and subsequent evaporation.

[2-Amino-6-(benzyloxy)purin-9-yl]acetic acid[18] (13.5 mg, 45.2 μmol, 4 equiv) was suspended in CH_2Cl_2 (4 mL) and DMF was added at 0 °C dropwise until a clear solution was formed. To this solution were added subsequently $NaHCO_3$ (9.49 mg, 113 μmol, 10 equiv), HOAt (9.23 mg, 67.8 μmol, 6 equiv), and EDCI (8.66 mg, 45.2 μmol, 4 equiv). After 10 min, the hydrochloride salt was dissolved in DMF (1 mL) and added to the reaction mixture. The mixture was stirred for 3 days at 25 °C, diluted with ethyl acetate (10 mL), washed with water (5 mL), and concentrated in vacuo. The residue was dissolved in 1 mL of CH_2Cl_2−TFA (1:1) and stirred for 2.5 h at 25 °C before being evaporated in vacuo. HPLC separation (RP-C18, gradient: 15−25% B [B = ACN/H_2O 9:1 + 0.1% TFA] in 20 min) afforded **15** (4.24 mg, 3.85 μmol, 34%) as a white solid: analytic HPLC (RP-C18, gradient: 15−25% B [B = ACN/H_2O 9:1 + 0.1% TFA] in 20 min) 13.7 min; UV (H_2O) $\lambda_{max}(\epsilon)$ 250 nm; ^1H NMR (DMSO-d_6, 600 MHz) δ 0.89 (d, 6H, ^3J = 7 Hz, Val-CH_3), 0.93 (d, 6H, ^3J = 7 Hz, Val-CH_3), 1.30 (d, 6H, ^3J = 7 Hz, Ala-CH_3), 2.16−2.22 (m, 2H, Val-Hβ), 2.60−2.63 (m, 2H, Cys-Hβ), 2.87 (dd, 2H, ^3J = 12 Hz, ^2J = 14 Hz, Cys-Hβ), 4.19 (dd, 2H, ^3J = 3 Hz, ^2J = 11 Hz, Ser-Hβ), 4.25−4.31 (m, 4H, Val-Hα, Ser-Hβ), 4.37 (dq, 2H, ^3J = 7 Hz, ^3J = 7 Hz, Ala-Hα), 4.47 (ddd, 2H, ^3J = 4 Hz, ^3J = 4 Hz, ^3J = 7 Hz, Ser-Hα), 4.70 (d, 2H, ^2J = 17 Hz, acetyl-CH_2), 4.79 (d, 2H, ^2J = 17 Hz, acetyl-CH_2), 5.24 (ddd, 2H, ^3J = 4 Hz, ^3J = 11 Hz, ^3J = 11 Hz, Cys-Hα), 6.28 (s, 4H, guanine-NH_2), 7.59 (s, 2H, guanine-CH8), 7.65 (d, 2H, ^3J = 7 Hz, Ala-NH), 8.10 (d, 2H, ^3J = 10 Hz, Val-NH), 8.26 (d, 2H, ^3J = 7 Hz, Ser-NH), 8.70 (d, 2H, ^3J = 10 Hz, Cys-NH), 10.51 (s, br, 2H, guanine-NH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 17.9 (Val-CH_3), 18.1 (Ala-CH_3), 19.0 (Val-CH_3), 30.3 (Val-CHβ), 39.9 (Cys-CH_2β), 44.8 (acetyl-CH_2), 48.1 (Ala-CHα), 52.7 (Cys-CHα, Ser-CHα), 58.2 (Val-CHα), 64.8 (Ser-CH_2β), 116.1 (guanine-C5), 138.0 (guanine-C8), 141.6 (guanine-C4), 151.4, 153.4 (guanine-C2, guanine-C6), 156.4, 166.9, 169.6, 173.2 (Ser-CO, Val-CO, Cys-CO, Ala-CO, acetyl-CO); HRMS (ESI) calcd for $C_{42}H_{57}N_{18}O_{14}S_2$ ([M + H]$^+$) 1101.3738, found 1101.3735.

[(Adenin-9-yl Acetyl)-D-Ser-L-Ala-L-Cys-L-Val]$_2$ (Serine Hydroxyl) Dilactone Disulfide 16. A sample of **14a** (15.0 mg, 15.2 μmol) was treated with thioanisole (200 μL) and TFA (2 mL), and the mixture was stirred for 10 h at 20 °C before being evaporated. The hydrochloride salt was formed by repeated addition of 2 N aqueous HCl (3 × 2 mL) and subsequent evaporation.

(N^6-(*Z*)-Adenin-9-yl)acetic acid[18] (19.9 mg, 60.8 μmol, 4 equiv) was suspended in CH_2Cl_2 (5 mL) and cooled to 0 °C, and DMF was added dropwise until a clear solution was formed. To this solution were added subsequently HOAt (4.14 mg, 30.4 μmol, 2 equiv), NMM (10.0 μL, 91.2 μmol, 6 equiv), and DIC (47.4 μL, 304 μmol, 20 equiv). After 5 min, the hydrochloride salt was dissolved in DMF (1 mL) and added to the reaction mixture. The mixture was stirred for 3 days at 4 °C, diluted with ethyl acetate (10 mL), washed with water (5 mL), and concentrated in vacuo. The residue was treated with thioanisole (200 μL) and TFA (2 mL) and stirred for 10 h at 25 °C before being evaporated in vacuo. **16** (4.02 mg, 3.74 μmol, 25%) could be obtained by HPLC separation (RP-C18, gradient: 15−25% B [B = ACN/H_2O 9:1 + 0.1% TFA] in 20 min) as a white solid: analytic HPLC (RP-C18, gradient: 10−35% B [B = ACN/H_2O 9:1 + 0.1% TFA] in 25 min) 20.0 min; UV (MeOH) $\lambda_{max}(\epsilon)$ 260 nm; ^1H NMR (DMSO-d_6, 600 MHz) δ 0.90 (d, 6H, ^3J = 7 Hz, Val-CH_3), 0.91 (d, 6H, ^3J = 7 Hz, Val-CH_3), 1.30 (d, 6H, ^3J = 7 Hz, Ala-CH_3), 2.07−2.15 (m, 2H, Val-Hβ), 2.57−2.63 (m, 2H, Cys-Hβ), 2.87 (dd, 2H, ^3J = 12 Hz, ^2J = 14 Hz, Cys-Hβ), 4.18 (dd, 2H, ^3J = 3 Hz, ^2J = 11 Hz, Ser-Hβ), 4.23 (dd, 2H, ^3J = 9 Hz, ^3J = 9 Hz, Val-Hα), 4.31 (dd, 2H, ^3J = 4 Hz, ^2J = 11 Hz, Ser-Hβ), 4.37−4.43 (m, 2H, Ala-Hα), 4.45 (ddd, 2H, ^3J = 3 Hz, ^3J = 3 Hz, ^3J = 7 Hz, Ser-Hα), 4.96 (d, 2H, ^2J = 17 Hz, acetyl-CH_2), 5.10 (d, 2H, ^2J = 17 Hz, acetyl-CH_2), 5.26 (ddd, 2H, ^3J = 4 Hz, ^3J = 10 Hz, ^3J = 10 Hz, Cys-Hα), 7.74 (d, 2H, ^3J = 7 Hz, Ala-NH), 8.07−8.19 (m, br, 6H, adenine-NH_2, Val-NH), 8.24 (s, br, 2H, adenine-H2/H8), 8.26 (s, br, 2H, adenine-H2/H8), 8.43−8.46 (m, 2H, Ser-NH), 8.69 (d, 2H, ^3J = 9 Hz, Cys-NH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 18.0 (Val-CH_3, Ala-CH_3), 18.2 (Ala-CH_3), 19.0 (Val-CH_3), 30.3 (Val-CHβ), 43.9 (Cys-CH_2β), 45.5 (acetyl-CH_2), 48.0 (Ala-CHα), 52.7 (Cys-CHα), 52.9 (Ser-CHα), 58.3 (Val-CHα), 64.9 (Ser-CH_2β), 118.0 (adenine-C5), 143.3 (adenine-C8), 149.0, 149.4 (adenine-C4), 153.1 (adenine-C2), 158.1 (adenine-C6), 166.8,

167.3, 169.4, 169.8, 173.5 (Ser-CO, Val-CO, Cys-CO, Ala-CO, acetyl-CO); HRMS (ESI) calcd for $C_{42}H_{57}N_{18}O_{12}S_2$ ([M + H]$^+$) 1069.3839, found 1069.3838.

Fmoc-D-Ser(*t*Bu)-OTce 4b. To a solution of Fmoc-D-Ser(*t*Bu)-OH (**3b**; 10.0 g, 26.1 mmol) in pyridine (80 mL) at −10 °C were added sequentially HOBt (5.99 g, 39.1 mmol, 1.5 equiv) and Tce-OH (3.26 mL, 33.9 mmol, 1.3 equiv). After the mixture was stirred for 5 min, a solution of DCC (8.07 g, 39.1 mmol, 1.5 equiv) in pyridine (20 mL) was added dropwise and the mixture was allowed to stir for 18 h at 0 °C. The precipitated DCU was filtered off; the filtrate was concentrated in vacuo, and the residue was diluted with ethyl acetate (200 mL). The solution was washed consecutively with cold 1 N aqueous HCl (2 × 200 mL), 5% aqueous $NaHCO_3$ (200 mL), and saturated aqueous NaCl (150 mL). Drying ($MgSO_4$), filtration and concentration in vacuo afforded the crude product, which was purified by flash chromatography (SiO_2, 8 × 18 cm, 35% ethyl acetate−hexane) to obtain **4b** (8.84 g, 17.2 mmol, 66%) as syrupy residue, which solidified upon prolonged standing: TLC (20% ethyl acetate/hexane) R_f 0.65; $[\alpha]^{25}_D$ +26 (*c* 0.05, MeOH); mp 105−108 °C; UV (MeOH) λ_{max}(ϵ) 265 nm; IR (KBr) ν_{max} 3441, 2970, 1768, 1723, 1517, 1450, 1287, 1338, 1262, 1219, 1193, 1163, 1111, 1069, 853, 820, 760, 738, 710 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.11 (s, 1H, *t*Bu-CH_3-rotamer), 1.17 (s, 8H, *t*Bu-CH_3-rotamer), 3.65 (dd, 1H, ^3J = 3 Hz, ^2J = 9 Hz, Ser-Hβ), 3.95 (dd, 1H, ^3J = 3 Hz, ^2J = 9 Hz, Ser-Hβ), 4.25 (t, 1H, ^3J = 7 Hz, Fmoc-CH), 4.33−4.47 (m, 2H, Fmoc-CH_2), 4.64 (ddd, 1H, ^3J = 3 Hz, ^3J = 3 Hz, ^3J = 9 Hz, Ser-Hα), 4.73 (d, 1H, ^2J = 12 Hz, Tce-CH_2), 4.84 (d, 1H, ^2J = 12 Hz, Tce-CH_2), 5.70 (d, 1H, ^3J = 9 Hz, NH), 7.36−7.43 (m, 4H, Fmoc-H3, Fmoc-H4), 7.60 (d, 1H, ^3J = 4 Hz, Fmoc-H2), 7.63 (d, 1H, ^3J = 4 Hz, Fmoc-H2), 7.76 (d, 2H, J = 8 Hz, Fmoc-H5); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 27.3 (*t*Bu-CH_3), 47.0 (Fmoc-CH), 54.5 (Ser-CHα), 61.8 (Ser-$CH_2\beta$), 67.3 (Fmoc-CH_2), 73.7 (*t*Bu-quart C), 74.6 (Tce-CH_2), 94.4 (Tce-CCl_3), 120.0 (Fmoc-C5), 125.07 (Fmoc-C2), 125.11 (Fmoc-C2), 127.02 (Fmoc-C3/C4), 127.04 (Fmoc-C3/C4), 127.7 (Fmoc-C3/C4), 141.2 (Fmoc-C6), 143.7 (Fmoc-C1), 143.8 (Fmoc-C1), 156.1 (Fmoc-CO), 169.3 (CO); HRMS (ESI) calcd for $C_{24}H_{26}Cl_3NO_5Na$ ([M + Na]$^+$) 536.0769, found 536.0772.

Fmoc-D-Ser-OTce 5b. Compound **4b** (8.16 g, 15.8 mmol) was treated with TFA (40 mL), and the reaction mixture was stirred at 10 °C for 45 min. The TFA was removed in vacuo; the residue was dissolved in ethyl acetate (150 mL), washed with 5% aqueous $NaHCO_3$ (150 mL), and saturated aqueous NaCl (100 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 6 × 16 cm, 33% ethyl acetate−hexane) afforded **5b** (5.19 g, 11.3 mmol, 72%) as a syrupy residue, which crystallized upon prolonged standing: TLC (33% ethyl acetate/hexane) R_f 0.35; [a]$^{25}_D$ +15 (*c* 0.13, MeOH); mp 107−109 °C; UV (MeOH) λ_{max}(ϵ) 255, 265 nm; IR (KBr) ν_{max} 3454, 3365, 2941, 1768, 1677, 1538, 1450, 1397, 1253, 1341, 1204, 1088, 1057, 764, 740, 723, 571, 424 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.96 (dd, 1H, ^3J = 2 Hz, ^2J = 11 Hz, Ser-Hβ), 4.09−4.13 (m, 1H, Ser-Hβ), 4.21 (t, 1H, ^3J = 6 Hz, Fmoc-CH), 4.37−4.46 (m, 2H, Fmoc-CH_2), 4.58 (ddd, 1H, ^3J = 4 Hz, ^3J = 4 Hz, ^3J = 8 Hz, Ser-Hα), 4.71 (d, 1H, ^2J = 12 Hz, Tce-CH_2), 4.90 (d, 1H, ^2J = 12 Hz, Tce-CH_2), 5.80 (d, 1H, ^3J = 8 Hz, NH), 7.27−7.31 (m, 2H, Fmoc-H3), 7.386 (dd, 1H, ^3J = 8 Hz, ^3J = 8 Hz, Fmoc-H4), 7.387 (dd, 1H, ^3J = 8 Hz, ^3J = 8 Hz, Fmoc-H4), 7.57−7.60 (m, 2H, Fmoc-H2), 7.75 (d, 2H, ^3J = 7 Hz, Fmoc-H5); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 47.0 (Fmoc-CH), 55.9 (Ser-CHα), 62.9 (Ser-$CH_2\beta$), 67.3 (Fmoc-CH_2), 74.5 (Tce-CH_2), 94.3 (Tce-CCl_3), 120.0 (Fmoc-C5), 125.0 (Fmoc-C2), 127.1 (Fmoc-C3/C4), 127.8 (Fmoc-C3/C4), 141.3 (Fmoc-C6), 143.6 (Fmoc-C1), 143.7 (Fmoc-C1), 156.2 (Fmoc-CO), 169.1 (CO); HRMS (ESI) calcd for $C_{20}H_{18}Cl_3NO_5$ ([M + H]$^+$) 458.0323, found 458.0319.

Fmoc-D-Ser(*N*-Boc-L-Val)-OTce 6b. A solution of Boc-L-Val-OH (3.67 g, 16.9 mmol, 1.5 equiv) in pyridine (40 mL) at −10 °C was treated sequentially with HOBt (2.59 g, 16.9 mmol, 1.5 equiv) and a solution of **5b** (5.19 g, 11.3 mmol) in 10 mL of pyridine. After 5 min, a solution of DCC (3.49 g, 16.9 mmol, 1.5 equiv) in pyridine (10 mL) was added and the mixture was stirred for 18 h at 0 °C. The precipitate was removed by filtration and the solvent evaporated in vacuo. The residue was taken up in ethyl acetate (100 mL), washed consecutively with cold 1 N aqueous HCl (100 mL), 5% aqueous $NaHCO_3$ (100 mL), and saturated aqueous NaCl (80 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 5 × 16 cm, 20% ethyl acetate−hexane) furnished **6b** (4.68 g, 7.12 mmol, 63%) and **5b** (1.49 g, 3.25 mmol), which was reisolated and subjected to the same procedure, resulting in an overall yield of 73% **6b** (5.43 g, 8.25 mmol) as a white foam: TLC (20% ethyl acetate/hexane) R_f 0.28; $[\alpha]^{25}_D$ +3 (*c* 0.15, MeOH); mp 98−102 °C; UV (MeOH) λ_{max}(ϵ) 256, 264 nm; IR (KBr) ν_{max} 3363, 2967, 1745, 1695, 1518, 1451, 1367, 1285, 1246, 1172, 1153, 1088, 1055, 739, 572, 425 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.87 (d, 3H, ^3J = 7 Hz, Val-CH_3), 0.94 (d, 3H, ^3J = 7 Hz, Val-CH_3), 1.42 (s, 9H, *t*Bu-CH_3), 1.98−2.08 (m, 1H, Val-Hβ), 4.09−4.15 (m, 1H, Val-Hα), 4.23 (t, 1H, ^3J = 8 Hz, Fmoc-CH), 4.33−4.43 (m, 2H, Fmoc-CH_2), 4.58 (dd, 1H, ^3J = 3 Hz, ^2J = 12 Hz, Ser-Hβ), 4.65 (dd, 1H, ^3J = 3 Hz, ^2J = 12 Hz, Ser-Hβ), 4.75 (d, 1H, ^2J = 12 Hz, Tce-CH_2), 4.80−4.87 (m, 2H, Ser-Hα, Tce-CH_2), 4.95 (d, 1H, ^3J = 8 Hz, Val-NH), 5.92 (d, 1H, ^3J = 9 Hz, Ser-NH), 7.28−7.33 (m, 2H, Fmoc-H3), 7.39 (dd, 2H, ^3J = 8 Hz, ^3J = 8 Hz, Fmoc-H4), 7.60 (d, 1H, ^3J = 7 Hz, Fmoc-H2), 7.61 (d, 1H, ^3J = 7 Hz, Fmoc-H2), 7.75 (d, 2H, ^3J = 8 Hz, Fmoc-H5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 17.7 (Val-CH_3), 19.0 (Val-CH_3), 28.2 (*t*Bu-CH_3), 30.7 (Val-CHβ), 47.0 (Fmoc-CH), 53.5 (Ser-CHα), 58.8 (Val-CHα), 64.1 (Ser-$CH_2\beta$), 67.6 (Fmoc-CH_2), 74.8 (Tce-CH_2), 80.2 (*t*Bu-quart C), 94.1 (Tce-CCl_3), 119.9 (Fmoc-C5), 125.1 (Fmoc-C2), 127.1 (Fmoc-C3/C4), 127.7 (Fmoc-C3/C4), 141.2 (Fmoc-C6), 143.6 (Fmoc-C1), 155.7, 155.9 (Boc-CO, Fmoc-CO), 168.0, 172.1 (Ser-CO, Val-CO); HRMS (ESI) calcd for $C_{30}H_{35}Cl_3N_2O_8Na$ ([M + Na]$^+$) 679.1351, found 679.1349.

Fmoc-D-Ser[*N*-Boc-L-Cys(Acm)-L-Val]-OTce 7b. Compound **6b** (2.32 g, 3.53 mmol) was treated with TFA (25 mL) at 25 °C for 30 min, followed by evaporation in vacuo. The oily residue was diluted with ethyl acetate (100 mL), washed with 5% aqueous $NaHCO_3$ (80 mL) and saturated aqueous NaCl (50 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo. The resulting white foam was dissolved in CH_2Cl_2 (10 mL) and directly used in the next step.

A solution of Boc-L-Cys(Acm)-OH (1.55 g, 5.30 mmol, 1.5 equiv) in CH_2Cl_2 (30 mL) was cooled to −10 °C and treated sequentially with HOAt (721 mg, 5.30 mmol, 1.5 equiv), EDCI (1.02 g, 5.30 mmol, 1.5 equiv), and after 10 min the solution of Fmoc-D-Ser(l-Val)-OTce in CH_2Cl_2. The mixture was allowed to stir at 0 °C for 18 h; before being poured onto cold 1 N aqueous HCl (80 mL), the phases were separated and the aqueous phase was extracted with ethyl acetate (2 × 80 mL). The combined organic phases were washed with 5% aqueous $NaHCO_3$ (60 mL) and saturated aqueous NaCl (40 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (SiO_2, 4 × 17 cm, 33% ethyl acetate−hexane) to afford **7b** (2.06 g, 2.48 mmol, 70%) as a pale yellow foam: TLC (33% hexane/ethyl acetate) R_f 0.47; $[\alpha]^{25}_D$ −9 (*c* 0.10, MeOH); mp 73−76 °C; UV (MeOH) λ_{max}(ϵ) 264 nm; IR (KBr) ν_{max} 3314, 3067, 2970, 2363, 1720, 1665, 1530, 1450, 1370, 1250, 1167, 1087, 1054, 762, 740, 721, 571 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.93 (d, 3H, ^3J = 7 Hz, Val-CH_3), 0.95 (d, 3H, ^3J = 7 Hz, Val-CH_3), 1.37 (s, 9H, *t*Bu-CH_3), 1.96 (s, 3H, Acm-CH_3), 2.13−2.21 (m, 1H, Val-Hβ), 2.64 (dd, 1H, ^3J = 8 Hz, ^2J = 14 Hz, Cys-Hβ), 2.94 (dd, 1H, ^3J = 4 Hz, ^2J = 15 Hz, Cys-Hβ), 4.21 (t, 1H, ^3J = 7 Hz, Fmoc-CH), 4.31−4.55 (m, 7H, Fmoc-CH_2, Acm-CH_2, Val-Hα, Cys-Hα, Ser-Hβ), 4.66 (dd, 1H, ^3J = 3 Hz, ^2J = 11 Hz, Ser-Hβ), 4.69 (d, 1H, ^2J = 12 Hz, Tce-CH_2), 4.83 (ddd, 1H, ^3J = 3 Hz, ^3J = 3 Hz, ^3J = 9 Hz, Ser-Hα), 4.88 (d, 1H, ^2J = 12 Hz, Tce-CH_2), 5.48 (d, 1H, ^3J = 8 Hz, NH), 6.49 (d, 1H, ^3J = 8 Hz, NH), 6.70−6.76 (m, br, 1H, Acm-NH), 7.27−7.32 (m, 2H, Fmoc-H3), 7.36−7.41 (m, 2H, Fmoc-H4), 7.43 (d, 1H, ^3J = 8 Hz, NH),

7.598 (d, 1H, ^3J = 7 Hz, Fmoc-H2), 7.599 (d, 1H, ^3J = 7 Hz, Fmoc-H2), 7.74 (d, 2H, ^3J = 8 Hz, Fmoc-H5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 17.7 (Val-CH_3), 19.0 (Val-CH_3), 23.2 (Acm-CH_3), 28.3 (*t*Bu-CH_3), 30.5 (Val-C*H*β), 35.0 (Cys-$CH_2\beta$), 41.2 (Acm-CH_2), 47.1 (Fmoc-CH), 53.2 (Ser-C*H*α/Cys-C*H*α), 53.3 (Ser-C*H*α/Cys-C*H*α), 57.9 (Val-C*H*α), 64.5 (Ser-$CH_2\beta$), 67.3 (Fmoc-CH_2), 74.7 (Tce-CH_2), 80.2 (*t*Bu-quart C), 94.2 (Tce-CCl_3), 120.0 (Fmoc-C5), 125.1 (Fmoc-C2), 127.1 (Fmoc-C3/C4), 127.8 (Fmoc-C3/C4), 141.2 (Fmoc-C6), 143.6, 143.7 (Fmoc-C1), 156.0, 156.1 (Boc-CO, Fmoc-CO), 168.2, 170.9, 171.3, 171.5 (Ser-CO, Val-CO, Cys-CO, Acm-CO); HRMS (ESI) calcd for $C_{36}H_{46}Cl_3N_4O_{10}S$ ([M + H]$^+$) 831.1995, found 831.1998.

Fmoc-D-Ser[*N*-Boc-L-Ala-L-Cys(Acm)-L-Val]-OTce 8b. Compound **7b** (2.06 g, 2.48 mmol) was treated with TFA (30 mL), and the mixture was stirred at 25 °C for 30 min before the TFA was removed by evaporation in vacuo. The residue was dissolved in ethyl acetate (100 mL), washed with 5% aqueous $NaHCO_3$ (80 mL), and saturated aqueous NaCl (50 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo to give a yellow foam, which was dissolved in CH_2Cl_2 (10 mL) and used in the next step without further purification.

To a solution of Boc-L-Ala-OH (678 mg, 3.72 mmol, 1.5 equiv) in CH_2Cl_2 (25 mL) at −10 °C were added sequentially HOAt (506 mg, 3.72 mmol, 1.5 equiv) and EDCI (713 mg, 3.72 mmol, 1.5 equiv). After 10 min, the solution of Fmoc-D-Ser[L-Cys(Acm)-L-Val]-OTce in CH_2Cl_2 was added. The mixture was allowed to react at 0 °C for 16 h before being poured onto cold 1 N aqueous HCl (100 mL). The phases were separated, and the aqueous phase was extracted with ethyl acetate (2 × 100 mL). The combined organic phases were washed with 5% aqueous $NaHCO_3$ (80 mL) and saturated aqueous NaCl (50 mL) and dried ($MgSO_4$). Filtration and concentration in vacuo were followed by flash chromatography (SiO_2, 4 × 18 cm, 25% hexanes−ethyl acetate) to provide **8b** (1.41 g, 1.56 mmol, 63%) as a pale yellow foam: TLC (25% hexane/ethyl acetate) R_f 0.39; $[\alpha]^{25}_D$ −24 (*c* 0.05, MeOH); mp 79−80 °C; UV (MeOH) $\lambda_{max}(\epsilon)$ 255, 264 nm; IR (KBr) ν_{max} 3411, 2975, 1723, 1653, 1542, 1527, 1370, 1270, 1196, 1164, 1088, 569 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.90−0.93 (m, 6H, Val-CH_3), 1.31 (d, 3H, ^3J = 7 Hz, Ala-CH_3), 1.39 (s, 9H, *t*Bu-CH_3), 1.97 (s, 3H, Acm-CH_3), 2.16−2.22 (m, 1H, Val-Hβ), 2.79 (dd, 1H, ^3J = 8 Hz, ^2J = 14 Hz, Cys-Hβ), 2.98 (dd, 1H, ^3J = 4 Hz, ^2J = 15 Hz, Cys-Hβ), 4.12−4.18 (m, 1H, Ala-Hα), 4.21 (t, 1H, ^3J = 7 Hz, Fmoc-CH), 4.32−4.45 (m, 6H, Fmoc-CH_2, Acm-CH_2, Val-Hα, Ser-Hβ), 4.66−4.77 (m, 3H, Tce-CH_2, Ser-Hβ, Cys-Hα), 4.85 (ddd, 1H, ^3J = 3 Hz, ^3J = 3 Hz, ^2J = 9 Hz, Ser-Hα), 4.89 (d, 1H, ^2J = 12 Hz, Tce-CH_2), 5.08 (d, 1H, ^3J = 5 Hz, Ala-NH), 6.79 (d, 1H, ^3J = 6 Hz, Ser-NH), 6.81−6.85 (m, br, 1H, Acm-NH), 7.27−7.32 (m, 3H, Fmoc-H3, Cys-NH), 7.35−7.39 (m, 2H, Fmoc-H4), 7.43 (d, 1H, ^3J = 8 Hz, Val-NH), 7.60 (d, 1H, ^3J = 8 Hz, Fmoc-H2), 7.61 (d, 1H, ^3J = 8 Hz, Fmoc-H2), 7.74 (d, 2H, ^3J = 8 Hz, Fmoc-H5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 17.6 (Val-CH_3), 18.2 (Ala-CH_3), 19.0 (Val-CH_3), 23.2 (Acm-CH_3), 28.2 (*t*Bu-CH_3), 30.5 (Val-C*H*β), 33.9 (Cys-$CH_2\beta$), 41.5 (Acm-CH_2), 47.0 (Fmoc-CH), 50.5 (Ala-C*H*α), 52.6 (Cys-C*H*α), 53.3 (Ser-C*H*α), 57.9 (Val-C*H*α), 64.7 (Ser-$CH_2\beta$), 67.2 (Fmoc-CH_2), 74.7 (Tce-CH_2), 80.3 (*t*Bu-quart C), 94.2 (Tce-CCl_3), 120.0 (Fmoc-C5), 124.6 (Fmoc-C2), 125.1 (Fmoc-C2), 127.1 (Fmoc-C3), 127.7 (Fmoc-C4), 141.2 (Fmoc-C6), 143.6 (Fmoc-C1), 155.4, 156.3 (Boc-CO, Fmoc-CO), 168.2, 170.5, 171.2, 171.7, 173.5 (Ser-CO, Val-CO, Cys-CO, Ala-CO, Acm-CO); HRMS (ESI) calcd for $C_{39}H_{50}Cl_3N_5O_{11}S$ ([M + H]$^+$) 902.2366, found 902.2368.

Fmoc-D-Ser[*N*-Boc-L-Ala-L-Cys(Acm)-L-Val]-OH 10b. A solution of **8b** (900 mg, 1.00 mmol) in 90% aqueous acetic acid (30 mL) was cooled to 0 °C, and zinc powder (3.26 g, 49.8 mmol, 50 equiv) was added. The suspension was stirred for 2.5 h at 0 °C, filtered, and concentrated in vacuo. The residue was treated with cold 1 N aqueous HCl (50 mL), and the resulting suspension was extracted with ethyl acetate (3 × 50 mL). The combined organic phases were washed with saturated aqueous NaCl (50 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 3 × 18 cm, 20% methanol−ethyl acetate, 0.5% HOAc) yielded **10b** (702 mg, 0.91 mmol, 91%) after coevaporation with toluene (3 × 5 mL) as an amorphous white solid: TLC (20% methanol/ethyl acetate, 0.5% HOAc) R_f 0.47; $[\alpha]^{25}_D$ −62 (*c* 0.05, MeOH); mp 121−124 °C; UV (MeOH) $\lambda_{max}(\epsilon)$ 265 nm; IR (KBr) ν_{max} 3405, 3315, 2973, 1662, 1529, 1450, 1395, 1369, 1252, 1166, 1057, 760, 739 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 0.80 (d, 3H, ^3J = 7 Hz, Val-CH_3), 0.84 (d, 3H, ^3J = 7 Hz, Val-CH_3), 1.16 (d, 3H, ^3J = 7 Hz, Ala-CH_3), 1.36 (s, 9H, *t*Bu-CH_3), 1.825 (s, 3H, Acm-CH_3/acetate-CH_3), 1.824 (s, 3H, Acm-CH_3/acetate-CH_3), 1.98−2.05 (m, 1H, Val-Hβ), 2.65 (dd, 1H, ^3J = 11 Hz, ^2J = 13 Hz, Cys-Hβ), 3.05 (dd, 1H, ^3J = 4 Hz, ^2J = 14 Hz, Cys-Hβ), 3.38−3.47 (m, 1H, Ser-Hβ, revealed by two-dimensional spectroscopy), 3.58−3.65 (m, 1H, Ser-Hβ, revealed by two-dimensional spectroscopy), 3.86−3.90 (m, 1H, Ser-Hα), 3.95−4.02 (m, 1H, Ala-Hα), 4.09−4.37 (m, 6H, Fmoc-CH, Fmoc-CH_2, Acm-CH_2, Val-Hα), 4.55−4.58 (m, 1H, Cys-Hα), 6.63 (d, 1H, ^3J = 5 Hz, NH), 6.83 (d, 1H, ^3J = 7 Hz, NH), 7.28−7.35 (m, 2H, Fmoc-H3), 7.38−7.42 (m, 2H, Fmoc-H4), 7.66 (d, 2H, ^3J = 7 Hz, Fmoc-H2), 7.80−7.83 (m, 2H, NH), 7.87 (d, 2H, ^3J = 8 Hz, Fmoc-H5), 8.07 (d, 1H, ^3J = 8 Hz, NH), 9.83 (s, br, 1H, COOH); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 17.9, 18.3, 18.8 (Val-CH_3, Ala-CH_3), 22.5 (acetate-CH_3), 23.0 (Acm-CH_3), 28.1 (*t*Bu-CH_3), 29.8 (Val-C*H*β), 32.8 (Cys-$CH_2\beta$), 40.1 (Acm-CH_2, revealed by two-dimensional spectroscopy), 46.7 (Fmoc-CH), 49.7 (Ala-C*H*α), 51.8 (Ser-C*H*α/Cys-C*H*α), 54.7 (Ser-C*H*α/Cys-C*H*α), 57.5 (Val-C*H*α), 60.4 (Ser-$CH_2\beta$), 65.5 (Fmoc-CH_2), 78.0 (*t*Bu-quart C), 120.0 (Fmoc-C5), 121.3 (Fmoc-C5), 125.1 (Fmoc-C2), 127.0 (Fmoc-C3/C4), 127.2 (Fmoc-C3/C4), 127.5 (Fmoc-C3/C4), 128.9 (Fmoc-C3/C4), 140.7 (Fmoc-C6), 143.9 (Fmoc-C1), 154.9, 155.4 (Boc-CO, Fmoc-CO), 169.6, 170.5, 170.9, 171.1, 172.7 (Ser-CO, Val-CO, Cys-CO, Ala-CO, Acm-CO, acetate-CO); HRMS (ESI) calcd for $C_{37}H_{49}N_5O_{11}SNa$ ([M + Na]$^+$) 794.3042, found 794.3039.

(*Z*)-D-Ser{Fmoc-D-Ser[Boc-L-Ala-L-Cys(Acm)-L-Val]-L-Ala-L-Cys(Acm)-L-Val}-OTce 11b. To a solution of **10b** (500 mg, 0.65 mmol) in CH_2Cl_2 (25 mL) at −10 °C were added subsequently HOAt (106 mg, 0.78 mmol, 1.2 equiv), EDCI (149 mg, 0.78 mmol, 1.2 equiv), and after 5 min a solution of **9** (463 mg, 0.65 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred at 0 °C for 18 h before being poured onto cold 1 N aqueous HCl (50 mL). The phases were separated, and the aqueous phase was extracted with ethyl acetate (2 × 50 mL); the combined organic phases were washed with 5% aqueous $NaHCO_3$ (50 mL) and saturated aqueous NaCl (40 mL), dried ($MgSO_4$), and filtered. Concentration in vacuo afforded the crude product, which was purified by flash chromatography (SiO_2, 2 × 19 cm, 10% methanol-ethyl acetate) to provide **11b** (499 mg, 0.34 mmol, 52%) as a white solid: TLC (10% methanol/ethyl acetate) R_f 0.52; $[\alpha]^{25}_D$ −22 (*c* 0.06, MeOH); mp 122−124 °C; UV (MeOH) $\lambda_{max}(\epsilon)$ 256, 265 nm; IR (KBr) ν_{max} 3422, 2925, 2361, 1700, 1646, 1542, 1450, 1369, 1262, 1164, 1055, 740, 668, 576 cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 0.80−0.83 (m, 12H, Val-CH_3), 1.17 (d, 3H, ^3J = 8 Hz, Ala-CH_3), 1.20 (d, 3H, ^3J = 7 Hz, Ala-CH_3), 1.36 (s, 9H, *t*Bu-CH_3), 1.84 (s, 3H, Acm-CH_3), 1.85 (s, 3H, Acm-CH_3), 2.02−2.10 (m, 2H, Val-Hβ), 2.64−2.71 (m, 2H, Cys-Hβ), 2.88−2.94 (m, 2H, Cys-Hβ), 3.96−4.00 (m, 1H, Ala-Hα), 4.12−4.38 (m, 13H, Fmoc-CH, Fmoc-CH_2, Acm-CH_2, Val-Hα, Ala-Hα, Ser-Hβ), 4.40−4.45 (m, 2H, Ser-Hα, Ser-Hβ), 4.55 (ddd, 1H, ^3J = 4 Hz, ^3J = 9 Hz, ^3J = 9 Hz, Cys-Hα), 4.58−4.63 (m, 1H, Ser-Hα), 4.63−4.67 (m, 1H, Cys-Hα), 4.88 (d, 1H, ^2J = 12 Hz, Tce-CH_2), 4.95 (d, 1H, ^2J = 12 Hz, Tce-CH_2), 5.03−5.08 (m, br, 2H, Z-CH_2), 6.85 (d, 1H, ^3J = 7 Hz, Ala-NH), 7.28−7.37 (m, 7H, Fmoc-H3, Z-aromat CH), 7.40 (dd, 2H, ^3J = 7 Hz, ^3J = 7 Hz, Fmoc-H4), 7.65 (d, 1H, ^3J = 8 Hz, Ser-NH), 7.70 (d, 1H, ^3J = 6 Hz, Fmoc-H2), 7.71 (d, 1H, ^3J = 6 Hz, Fmoc-H2), 7.87 (d, 2H, ^3J = 7 Hz, Fmoc-H5), 7.89−7.93 (m, 3H, Val-NH, Ser-NH), 7.99 (d, 1H, ^3J = 8 Hz, Cys-NH), 8.20 (d, 1H, ^3J = 8 Hz, Cys-NH), 8.23 (d, 1H, ^3J = 7 Hz, Ala-NH), 8.49 (t, ^3J = 6 Hz, Acm-NH), 8.51 (t, 1H, ^3J = 6 Hz, Acm-NH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 17.4, 17.6, 18.2, 18.4, 18.7 (Val-CH_3, Ala-CH_3), 22.5 (Acm-CH_3), 28.1 (*t*Bu-CH_3), 29.9 (Val-C*H*β), 32.0 (Cys-

$CH_2\beta$), 32.6 (Cys-$CH_2\beta$), 40.1 (Acm-CH_2), 46.5 (Fmoc-CH), 48.1 (Ala-CHα), 49.7 (Ala-CHα), 52.0, 52.6, 52.8, 53.4 (Ser-CHα, Cys-CHα), 56.8 (Val-CHα), 56.9 (Val-CHα), 63.2 (Ser-$CH_2\beta$), 64.3 (Ser-$CH_2\beta$), 65.9 (Fmoc-CH_2, Z-CH_2), 73.7 (Tce-CH_2), 78.0 (*t*Bu-quart C), 94.7 (Tce-CCl_3), 120.0 (Fmoc-C5), 125.2 (Fmoc-C2), 127.0, 127.6, 127.8, 128.3 (Fmoc-C3, Fmoc-C4, Z-aromat CH), 136.6 (Z-quart C), 140.6 (Fmoc-C6), 143.7 (Fmoc-C1), 154.9, 155.9, 156.0 (Boc-CO, Fmoc-CO, Z-CO), 168.0, 168.1, 169.9, 170.1, 170.3, 170.4, 170.7, 170.8, 172.0, 172.8 (Ser-CO, Val-CO, Cys-CO, Ala-CO, Acm-CO); HRMS (ESI) calcd for $C_{64}H_{85}Cl_3N_{10}O_{19}S_2Na$ ($[M + Na]^+$) 1489.4392, found 1489.4415.

(*Z*)-D-Ser{Fmoc-D-Ser[Boc-L-Ala-L-Cys(Acm)-L-Val]-L-Ala-L-Cys(Acm)-L-Val}-OH 12b. Compound **11b** (230 mg, 0.16 mmol) was dissolved in 90% aqueous acetic acid (6 mL) and cooled to 0 °C, and zinc powder (512 mg, 7.83 mmol, 50 equiv) was added. The resulting suspension was stirred for 2.5 h at 0 °C; the zinc was filtered off, and the filtrate was concentrated in vacuo. The residue was treated with cold 1 N aqueous HCl (15 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic phases were washed with saturated aqueous NaCl (10 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 2 × 18 cm, 20% methanol−ethyl acetate, 0.5% HOAc) furnished **12b** (198 mg, 0.15 mmol, 94%) after coevaporation with toluene (3 × 5 mL) as an amorphous white solid: TLC (20% methanol/ethyl acetate, 0.5% HOAc) R_f 0.14; $[\alpha]^{25}_D$ −45 (*c* 0.06, MeOH); mp 124−128 °C; UV (MeOH) $\lambda_{max}(\epsilon)$ 265 nm; IR (KBr) ν_{max} 3420, 2967, 1654, 1541, 1449, 1420, 1254, 1157, 742, 620 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 0.78−0.85 (m, 12H, Val-CH_3), 1.17 (d, 3H, ^3J = 7 Hz, Ala-CH_3), 1.18 (d, 3H, ^3J = 7 Hz, Ala-CH_3), 1.36 (s, 9H, *t*Bu-CH_3), 1.76 (s, acetate-CH_3), 1.83 (s, 3H, Acm-CH_3), 1.85 (s, 3H, Acm-CH_3), 1.99−2.08 (m, 2H, Val-Hβ), 2.57−2.70 (m, 2H, Cys-Hβ), 2.87−2.94 (m, 1H, Cys-Hβ), 3.05−3.10 (m, 1H, Cys-Hβ), 3.44−3.52 (m, 1H, Ser-Hβ, revealed by two-dimensional spectroscopy), 3.85−3.90 (m, 1H, Ser-Hα), 3.96−4.03 (m, 1H, Ala-Hα), 4.07−4.12 (m, 1H, Val-Hα), 4.13−4.39 (m, 11H, Fmoc-CH, Fmoc-CH_2, Acm-CH_2, Ser-Hβ, Ala-Hα), 4.40−4.45 (m, 1H, Ser-Hα), 4.48−4.54 (m, 1H, Cys-Hα), 4.57−4.64 (m, 1H, Cys-Hα), 4.95 (d, 1H, ^2J = 12 Hz, Z-CH_2), 5.01 (d, 1H, ^2J = 12 Hz, Z-CH_2), 6.60 (d, 1H, ^3J = 6 Hz, Ser-NH), 6.86 (d, 1H, ^3J = 8 Hz, Ala-NH), 7.26−7.36 (m, 7H, Fmoc-H3, Z-aromat CH), 7.37−7.43 (m, 2H, Fmoc-H4), 7.70 (d, 1H, ^3J = 8 Hz, Fmoc-H2), 7.72 (d, 1H, ^3J = 8 Hz, Fmoc-H2), 7.79 (d, 1H, ^3J = 6 Hz, Ser-NH), 7.87 (d, 2H, ^3J = 8 Hz, Fmoc-H5), 7.91−7.97 (m, 2H, Cys-NH, Val-NH), 8.08 (d, 1H, ^3J = 8 Hz, Val-NH), 8.16−8.21 (m, 1H, Cys-NH), 8.26 (d, 1H, ^3J = 7 Hz, Ala-NH), 8.54 (t, 1H, ^3J = 8 Hz, Acm-NH), 9.94 (s, br, 1H, COOH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 17.9, 18.2, 18.8, 19.1 (Val-CH_3, Ala-CH_3), 22.5 (Acm-CH_3), 22.9 (acetate-CH_3), 28.1 (*t*Bu-CH_3), 29.8 (Val-CHβ), 30.4 (Val-CHβ), 32.3 (Cys-$CH_2\beta$), 32.6 (Cys-$CH_2\beta$), 40.3 (Acm-CH_2, revealed by two-dimensional spectroscopy), 47.7 (Ala-CHα/Fmoc-CH), 48.2 (Ala-CHα/Fmoc-CH), 49.7 (Ala-CHα), 52.2 (Cys-CHα), 53.5 (Cys-CHα), 54.7 (Ser-CHα), 55.2 (Ser-CHα), 57.2 (Val-CHα), 57.8 (Val-CHα), 61.8 (Ser-$CH_2\beta$), 65.1 (Fmoc-CH_2/Z-CH_2), 65.4 (Fmoc-CH_2/Z-CH_2), 78.0 (*t*Bu-quart C), 119.9 (Fmoc-C5), 121.3 (Fmoc-C5), 124.1 (Fmoc-C2), 127.2, 127.6, 128.2, 128.8 (Fmoc-C3, Fmoc-C4, Z-aromat CH), 137.4 (Z-quart C), 139.3 (Fmoc-C6), 142.5 (Fmoc-C1), 154.9, 155.2 (Boc-CO, Fmoc-CO, Z-CO), 169.4, 169.9, 170.4, 170.7, 172.0, 172.7 (Ser-CO, Val-CO, Cys-CO, Ala-CO, Acm-CO, acetate-CO); HRMS (ESI) calcd for $C_{62}H_{85}N_{10}O_{19}S_2$ ($[M + H]^+$) 1337.5428, found 1337.5422.

(*Z*)-D-Ser[Fmoc-D-Ser(Boc-L-Ala-L-Cys-L-Val)-L-Ala-L-Cys-L-Val]-OH Disulfide 13b. A solution of **12b** (90.0 mg, 67.3 μmol) in 30 mL of CH_2Cl_2−MeOH (15:1) was added dropwise to a solution of iodine (171 mg, 673 μmol, 10 equiv) in 80 mL of CH_2Cl_2−MeOH (10:1), and the mixture was stirred at 25 °C for 2.5 h. The mixture was cooled to 0 °C, and 5% aqueous $Na_2S_2O_3$ was added slowly until the excess iodine was discharged and the color disappeared. The phases were separated, and the organic phase was washed with 1 N aqueous HCl (80 mL) and saturated aqueous NaCl (50 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 2 × 15 cm, 15% methanol−ethyl acetate, 0.3% HOAc) afforded **13b** (52.7 mg, 44.2 μmol, 66%) after coevaporation with toluene (3 × 5 mL) as an amorphous white solid: TLC (20% methanol/ethyl acetate, 0.5% HOAc) R_f 0.33; $[\alpha]^{25}_D$ −36 (*c* 0.05, MeOH); mp 165−168 °C; UV (MeOH) $\lambda_{max}(\epsilon)$ 264 nm; IR (KBr) ν_{max} 3420, 2926, 1684, 1654, 1560, 1541, 1458, 1399, 1249, 1168, 1062, 742 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 0.82−0.88 (m, 12H, Val-CH_3), 1.20 (d, 3H, ^3J = 7 Hz, Ala-CH_3), 1.26 (d, 3H, ^3J = 7 Hz, Ala-CH_3), 1.36 (s, 9H, *t*Bu-CH_3), 2.05−2.16 (m, 2H, Val-Hβ), 2.84 (dd, 1H, ^3J = 11 Hz, ^2J = 13 Hz, Cys-Hβ), 2.90−3.05 (m, 3H, Cys-Hβ), 3.42−3.54 (m, Ser-Hβ, revealed by two-dimensional spectroscopy), 3.97−4.03 (m, 1H, Ser-Hα), 4.09−4.15 (m, 1H, Ala-Hα), 4.21 (t, 1H, ^3J = 8 Hz, Fmoc-CH), 4.24−4.41 (m, 8H, Fmoc-CH_2, Val-Hα, Ser-Hα, Ser-Hβ), 4.42−4.47 (m, 1H, Ala-Hα), 4.91−4.97 (m, 1H, Cys-Hα), 4.99−5.04 (m, 3H, Z-CH_2, Cys-Hα), 6.86 (d, 1H, ^3J = 8 Hz, NH), 7.28−7.36 (m, 7H, Fmoc-H3, Z-aromat CH), 7.38−7.43 (m, 2H, Fmoc-H4), 7.53−7.59 (m, br, 1H, NH), 7.62 (d, 1H, ^3J = 6 Hz, NH), 7.67 (d, 2H, ^3J = 7 Hz, Fmoc-H2), 7.77 (d, 1H, ^3J = 6 Hz, NH), 7.87 (d, 1H, ^3J = 8 Hz, Fmoc-H5), 7.88 (d, 1H, ^3J = 8 Hz, Fmoc-H5), 8.00 (d, 1H, ^3J = 5 Hz, NH), 8.10−8.21 (m, 2H, NH), 8.54 (d, 1H, ^3J = 9 Hz, Cys-NH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 17.7, 17.9, 18.3, 18.8 (Val-CH_3, Ala-CH_3), 28.1 (*t*Bu-CH_3), 28.9 (Val-CHβ), 30.6 (Val-CHβ), 43.4 (Cys-$CH_2\beta$), 47.7 (Fmoc-CH), 48.1 (Ala-CHα), 48.5 (Ala-CHα), 51.8 (Cys-CHα), 52.3 (Cys-CHα), 54.3 (Ser-CHα), 55.0 (Ser-CHα), 57.4 (Val-CHα), 57.7 (Val-CHα), 65.1 (Fmoc-CH_2/Z-CH_2), 65.9 (Ser-$CH_2\beta$), 66.7 (Fmoc-CH_2/Z-CH_2), 78.3 (*t*Bu-quart C), 119.9 (Fmoc-C5), 121.3 (Fmoc-C2), 127.2, 127.6, 128.2, 128.9 (Fmoc-C3, Fmoc-C4, Z-aromat CH), 137.4 (Z-quart C), 139.4 (Fmoc-C6), 142.5 (Fmoc-C1), 154.8, 155.4 (Boc-CO, Fmoc-CO, Z-CO), 169.2, 169.2, 170.0, 170.1, 171.0, 171.2 (Ser-CO, Val-CO, Cys-CO, Ala-CO); HRMS (ESI) calcd for $C_{56}H_{72}N_8O_{17}S_2$ ($[M + H]^+$) 1193.4530, found 1193.4529.

((*Z*)-D-Ser-L-Ala-L-Cys-L-Val-Fmoc-D-Ser-L-Ala-L-Cys-L-Val) (Serine Hydroxyl) Dilactone Disulfide 14b. A sample of **13b** (23.8 mg, 19.9 μmol) was treated with TFA (1 mL), and the mixture was stirred at 25 °C for 30 min. The TFA was removed by evaporation in vacuo; the residue was treated with 1 N aqueous HCl (10 mL), extracted with ethyl acetate (3 × 10 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo. The resulting white solid was dissolved in 15 mL of CH_2Cl_2−DMF (9:1) and directly used in the next step.

The solution was cooled to 0 °C and under vigorous stirring were added sequentially HOAt (2.71 mg, 19.9 μmol, 1 equiv), NMM (6.58 μL, 59.8 μmol, 3 equiv) and DIC (31.2 μL, 199 μmol, 10 equiv). The mixture stirred at 4 °C for 36 h before being poured onto cold 1n aqueous HCl (10 mL). The phases were separated and the aqueous phase extracted with ethyl acetate (2 × 10 mL). The combined organic phases were washed with 5% aqueous $NaHCO_3$ (10 mL) and saturated aqueous NaCl (5 mL), dried ($MgSO_4$), filtered and concentrated in vacuo. Purification by flash chromatography (SiO_2, 1 × 14 cm, ethyl acetate) provided **14b** (19.1 mg, 17.4 μmol, 87%) as amorphous pale yellow solid: TLC (20% hexane/ethyl acetate) R_f 0.43; $[\alpha]^{25}_D$ −64 (*c* 0.03, MeOH); mp 165−168 °C; UV (MeOH) $\lambda_{max}(\epsilon)$ 265 nm; IR (KBr) ν_{max} 3422, 2961, 2924, 2364, 1617, 1576, 1458, 1385, 1249, 1169 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 0.86 (d, 6H, ^3J = 7 Hz, Val-CH_3), 0.88 (d, 6H, ^3J = 7 Hz, Val-CH_3), 1.22−1.27 (m, 6H, Ala-CH_3), 2.17−2.28 (m, br, 2H, Val-Hβ), 2.61−2.68 (m, br, 2H, Cys-Hβ), 2.81 (dd, 2H, ^3J = 11 Hz, ^2J = 14 Hz, Cys-Hβ), 4.08−4.43 (m, 13H, Fmoc-CH, Fmoc-CH_2, Ala-Hα, Ser-Hα, Ser-Hβ, Val-Hα), 5.06−5.11 (m, br, 2H, Z-CH_2), 5.21−5.28 (m, br, 2H, Cys-Hα), 7.28−7.34 (m, 2H, Fmoc-H3), 7.34−7.38 (m, 5H, Z-aromat CH), 7.39−7.43 (m, 2H, Fmoc-H4), 7.50−7.57 (m, br, 2H, NH), 7.66 (d, 2H, ^3J = 8 Hz, Fmoc-H2), 7.74 (d, 2H, ^3J = 7 Hz, Ala-NH), 7.87 (d, 1H, ^3J = 7 Hz, Fmoc-H5), 7.88 (d, 1H, ^3J = 7 Hz, Fmoc-H5), 8.12−8.20 (m, 2H, Val-NH), 8.65−8.71 (m, 2H, Cys-NH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 18.1 (Ala-CH_3), 18.2 (Val-CH_3, Ala-CH_3), 18.89 (Val-CH_3), 18.94 (Val-CH_3), 30.0 (Val-CHβ), 30.2

(Val-CHβ), 44.0 (Cys-CH$_2\beta$), 46.6 (Fmoc-CH), 48.0 (Ala-CHα), 52.7 (Cys-CHα), 54.6 (Ser-CHα), 58.3 (Val-CHα), 58.4 (Val-CHα), 65.8 (Fmoc-CH$_2$, Z-CH$_2$, Ser-CH$_2\beta$), 65.9 (Fmoc-CH$_2$, Z-CH$_2$), 120.1 (Fmoc-C5), 124.87 (Fmoc-C2), 124.94 (Fmoc-C2), 126.87 (Fmoc-C3), 126.92 (Fmoc-C3), 127.6 (Fmoc-C4), 127.7, 127.8, 128.3, 129.3 (Z-aromat CH), 136.6 (Z-quart C), 140.6 (Fmoc-C6), 143.7 (Fmoc-C1), 169.28, 169.34, 169.5, 173.1 (Ser-CO, Val-CO, Cys-CO, Ala-CO); HRMS (ESI) calcd for $C_{51}H_{62}N_8O_{14}S_2$ ([M + H]$^+$) 1075.3900, found 1075.3903.

[(Adenin-9-yl acetyl)-D-Ser-L-Ala-L-Cys-L-Val]-[(guanin-9-yl acetyl)-D-Ser-L-Ala-L-Cys-L-Val] (Serine Hydroxyl) Dilactone Disulfide 17. A sample of **14b** (15.0 mg, 13.7 μmol) was treated with thioanisole (200 μL) and TFA (2 mL), and the mixture was stirred for 10 h at 20 °C before being evaporated. The hydrochloride salt was formed by repeated addition of 2 N aqueous HCl (3 × 2 mL) and subsequent evaporation.

(N^6-(*Z*)-Adenin-9-yl)acetic acid[18] (8.99 mg, 27.4 μmol, 2 equiv) was suspended in CH_2Cl_2 (3 mL) and cooled to 0 °C, and DMF was added dropwise until a clear solution was formed. To this solution were added subsequently HOAt (1.86 mg, 13.7 μmol, 1 equiv), NMM (4.52 μL, 41.1 μmol, 3 equiv), and DIC (21.5 μL, 137 μmol, 10 equiv). After 5 min, the hydrochloride salt was dissolved in DMF (1 mL) and added to the reaction mixture. The mixture was stirred for 4 days at 4 °C, diluted with ethyl acetate (10 mL), washed with water (5 mL), and concentrated in vacuo.

To the resulting residue was added 20% piperidine in DMF (1 mL), and the mixture was stirred for 20 min at 25 °C before the volatiles were removed in vacuo. Once again, 20% piperidine in DMF (1 mL) was added; the mixture was stirred for another 15 min, and the solvents were evaporated before the residue was coevaporated subsequently with DMF (3 × 2 mL) and toluene (2 × 2 mL). The resultant solid was washed with diethyl ether (2 × 2 mL) and dried thoroughly.

[2-Amino-6-(benzyloxy)purin-9-yl]acetic acid[18] (8.20 mg, 27.4 μmol, 2 equiv) was suspended in CH_2Cl_2 (3 mL) and cooled to 0 °C, and DMF was added dropwise until a clear solution was formed. To this solution were added subsequently HOAt (1.86 mg, 13.7 μmol, 1 equiv), NMM (4.52 μL, 41.1 μmol, 3 equiv), and DIC (21.5 μL, 137 μmol, 10 equiv). After 5 min, the deprotected bicyclus was dissolved in DMF (1 mL) and added to the reaction mixture. The mixture was stirred for 3 days at 4 °C, diluted with ethyl acetate (10 mL), washed with water (5 mL), and concentrated in vacuo.

The residue was treated with thioanisole (150 μL) and TFA (1.5 mL) and stirred for 10 h at 25 °C, before being evaporated in vacuo. HPLC separation (RP-C18, gradient: 10−35% B [B = ACN/H_2O 9:1 + 0.1% TFA] in 25 min) afforded **17** (3.02 mg, 2.77 μmol, 20%) as a white solid: analytic HPLC (RP-C18, gradient: 10−35% B [B = ACN/H_2O 9:1 + 0.1% TFA] in 25 min) 19.8 min; UV (MeOH) $\lambda_{max}(\epsilon)$ 258; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 0.88 (d, 3H, ^3J = 7 Hz, Val-CH$_3$), 0.89 (d, 3H, ^3J = 7 Hz, Val-CH$_3$), 0.90 (d, 3H, ^3J = 7 Hz, Val-CH$_3$), 0.93 (d, 3H, ^3J = 7 Hz, Val-CH$_3$), 1.30 (d, 6H, ^3J = 7 Hz, Ala-CH$_3$), 2.06−2.12 (m, 1H, Val-Hβ), 2.17−2.23 (m, 1H, Val-Hβ), 2.56−2.65 (m, 2H, Cys-Hβ), 2.86 (dd, 2H, ^3J = 12 Hz, ^2J = 14 Hz, Cys-Hβ), 4.17 (dd, 1H, ^3J = 3 Hz, ^3J = 11 Hz, Ser-Hβ), 4.18−4.32 (m, 5H, Ser-Hβ, Val-Hα), 4.36−4.41 (m, 2H, Ala-Hα), 4.44 (ddd, 1H, ^3J = 3 Hz, ^3J = 3 Hz, ^3J = 7 Hz, Ser-Hα), 4.47 (ddd, 1H, ^3J = 3 Hz, ^3J = 3 Hz, ^3J = 7 Hz, Ser-Hα), 4.72 (d, 1H, ^2J = 17 Hz, acetyl-CH$_2$), 4.82 (d, 1H, ^2J = 17 Hz, acetyl-CH$_2$), 4.94 (d, 1H, ^2J = 17 Hz, acetyl-CH$_2$), 5.08 (d, 1H, ^2J = 17 Hz, acetyl-CH$_2$), 5.22−5.27 (m, 2H, Cys-Hα), 6.37 (s, br, 2H, guanine-NH$_2$), 7.68 (d, 1H, ^3J = 7 Hz, Ala-NH), 7.73 (d, 1H, ^3J = 7 Hz, Ala-NH), 7.75 (s, br, 1H, guanine-CH8), 7.89 (s, br, 2H, adenine-NH$_2$), 8.08−8.13 (m, 2H, Val-NH), 8.20 (s, 1H, adenine-H2/H8), 8.21 (s, 1H, adenine-H2/H8), 8.32 (d, 1H, ^3J = 7 Hz, Ser-NH), 8.40 (d, 1H, ^3J = 7 Hz, Ser-NH), 8.69 (d, 2H, ^3J = 10 Hz, Cys-NH), 10.63 (s, br, 1H, guanine-NH); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 18.0, 19.0 (Val-CH$_3$, Ala-CH$_3$), 30.3 (Val-CHβ), 43.8 (Cys-CH$_2\beta$), 44.9 (acetyl-CH$_2$), 45.5 (acetyl-CH$_2$), 48.0 (Ala-CHα), 52.7 (Cys-CHα, Ser-CHα), 58.3 (Val-CHα), 64.8 (Ser-CH$_2\beta$), 118.1 (adenine-C5, guanine-C5), 138.1 (guanine-C8), 150.0 (adenine-C4, adenine-C8, guanine-C4), 167.5, 169.7, 173.3 (Ser-CO, Val-CO, Cys-CO, Ala-CO, acetyl-CO); HRMS (ESI) calcd for $C_{42}H_{56}N_{18}O_{13}S_2$ ([M + H]$^+$) 1085.3788, found 1085.3788.

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Supporting Information Available: General experimental data and experimental details for compounds **4a**, **5a**, **6a**, and **14a** and appropriate spectral data for all compounds [^{1}H and ^{13}C spectra, including two-dimensional spectra for compounds **7a**, **8a**, **10a**, **11a**, **12a**, **13a**, **14a**, **15**, **16**, **8b**, **10b**, **11b**, **12b**, **13b**, **14b**, and **17**]. This material is available free of charge via the Internet at http://pubs.acs.org.

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