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Syntheses of Triostin A Antibiotic and Nucleobase-Functionalized Analogs as New DNA Binders

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A total synthesis of the natural product triostin A, wherein the *N*-methylated depsipeptide scaffold is constructed by solution-phase peptide chemistry followed by disulfide formation and macrocyclization, is described. Finally, the quinoxalines were attached to provide the DNA bisintercalator. Analogs of triostin A were obtained by the successive functionalization of the cyclic depsipeptide with pyrimidine or purine recognition units. The attachment of functional units was achieved by the orthogonal protection of the respective side chain amino functionalities. The nucleobase-functionalized triostin analogs have the potential to recognize double-stranded DNA by hydrogen bonding. The interaction with DNA was investigated by UV spectroscopy and fluorescence intercalator displacement.

Introduction

The natural product triostin A isolated from *Streptomyces aureus* belongs to the family of quinoxaline antibiotics.[1–4] They are potent antitumor agents inhibiting RNA synthesis by specific binding of double-stranded DNA (ds-DNA) through bisintercalation. A structural characteristic of triostin A (**1**) is a rigid, disulfide-bridged, bicyclic, depsipeptide scaffold, which preorganizes two quinoxaline intercalating units (Figure 1).[5,6] The aromatic groups are oriented in parallel at a distance of 10.5 Å. This is a perfect orientation of two intercalators to interact with two adjacent DNA base pairs. Indeed, the specificity of triostin A for a C-G sequence of DNA is based on the defined orientation of the quinoxalines – which interact by π-stacking with the DNA bases – and the hydrogen bonding between the peptide scaffold and ds-DNA.[7] Our interest in triostin A is based on the unique characteristic of the depsipeptide scaffold to provide recognition units in a preorganized orientation.[8–10] Besides bisintercalation, the distance of 10.5 Å for the parallel-oriented recognition units generally allows for DNA recognition by hydrogen bonding with the nucleobases in the major or minor groove. The major groove – presenting the Hoogsteen base-pair site – should especially be a target for specific differentiation between the canonical nucleobase pairs. Considering the rigid backbone of triostin A, every third DNA base pair would be addressed provided that the recognition unit has the required hydrogen-bonding pattern. The triostin A backbone organization is further advantageous as an abasic site recognition motif. Abasic sites are DNA lesions obtained by depurination and are also known intermediates in the DNA repair process.[11–14] A nucleobase attached to the triostin depsipeptide scaffold could replace the lost nucleobase, whereas the other recognition unit might function as an efficient intercalator.

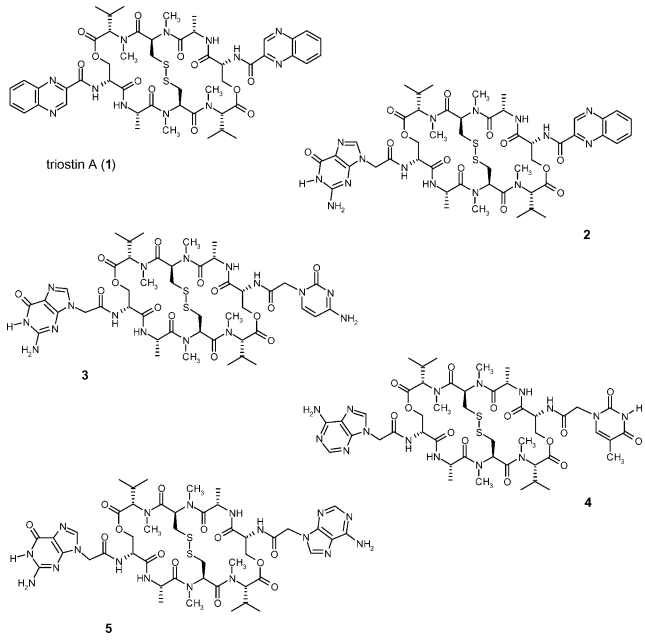

Figure 1. Structure of triostin A (**1**) and nucleobase-substituted analogs **2**–**5** based on the triostin A backbone.

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Synthetic access to the bicyclic triostin A depsipeptide backbone opens the possibility of specifically attaching various recognition units and subsequently making various backbone modifications. A key intermediate would be an orthogonally amine-protected bicyclic depsipeptide, which could be successively functionalized using amide formation. Since larger quantities of triostin A analogs were required for cocrystallization experiments with ds-DNA, the synthesis of triostin A and nucleobase analogs was approached by solution-phase peptide synthesis.

Triostin A (**1**) is an octadepsipeptide symmetrically composed of two tetrapeptides containing amino acids *N*-methyl-L-cysteine, D-serine, L-alanine, and *N*-methyl-L-valine. The tetrapeptides are macrocyclized forming two ester linkages between the serine side chain and the *C*-terminus of the opposite tetrapeptide. The disulfide-bridged depsipeptide is provided by oxidation; serine amino groups are orthogonally protected to allow for attachment of different recognition units. The *N*-methylation of four amino acids renders amide formation more difficult. Two solution-phase syntheses of triostin A by Olsen et al. and Shin et al. are known to date, both following a strategy of linking the tetrapeptides followed by macrocyclization and oxidation or vice versa.[15,16] A differentiation of the amino groups for functionalization was not required in these triostin A syntheses as triostin A is symmetrical. In addition, an elegant solid-phase protocol was recently provided.[17] Our approach to triostin A is driven by the desire to access derivatives with two different DNA recognition functionalities. This approach requires orthogonal protection of the amino groups of the triostin A scaffold and was used previously to synthesize triostin A backbone analogs, like TANDEM (des-*N*-tetramethyltriostin) and des-*N*-(tetramethyl)azatriostin.[8–10,18,19]

The solution-phase peptide synthesis of triostin A is described. Furthermore, four triostin derivatives **2**–**5** were prepared containing nucleobases as recognition units in different combinations. Their interaction with ds-DNA was evaluated by spectroscopic methods.

Results and Discussion

We obtained the disulfide-bridged depsipeptide scaffold of triostin A by the fusion of two tetrapeptides, disulfide formation, and macrocyclization. Since the tetrapeptides were identical in sequence but differed in their serine protecting groups, we decided to include the ester linkage in the tetrapeptide and use amide bond formation for linkage and macrocyclization.

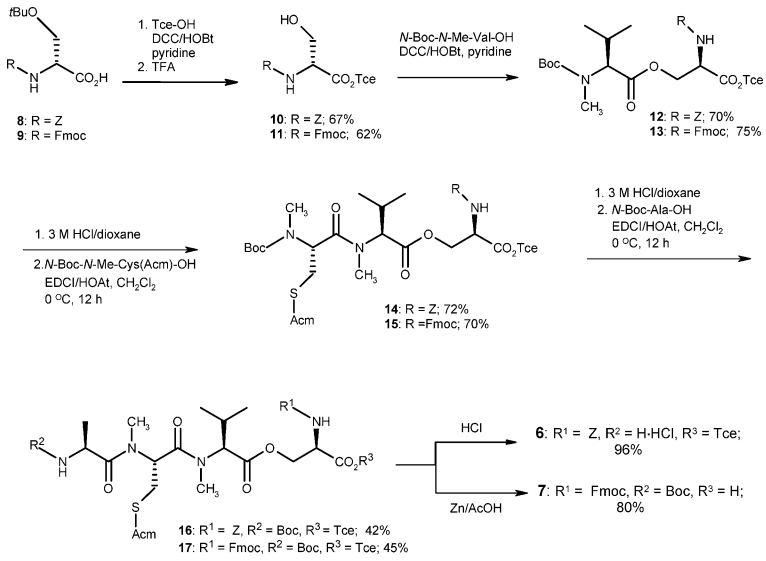
Synthesis of Orthogonally Protected Triostin A Backbone and Functionalization with Various Recognition Units

We built suitably protected tetradepsipeptides **6** and **7** stepwise in solution. Starting with Z-D-Ser(*t*Bu)-OH (**8**) and Fmoc-D-Ser(*t*Bu)-OH (**9**),[19–21] we prepared the trichloroethanol (Tce) esters and subsequently deprotected the side chains (Scheme 1). The Tce group increased the solubility of all the depsipeptides in organic solvents and enabled the isolation of the free amines by extraction with ethyl acetate. We coupled amino acids Z-D-Ser-OTce (**10**) and Fmoc-D-Ser-OTce (**11**) with *N*-Boc-*N*-Me-Val-OH to yield the esters **12** and **13**, respectively.[16] We deprotected these depsipeptides using 4 M HCl in dioxane and then coupled them with *N*–Me–Boc-L-Cys(Acm)-OH.[22,23] We obtained tridepsipeptides **14** and **15**, respectively, by activation with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI·HCl) and 1-hydroxy-7-azabenzotriazole (HOAt).[24] We used acetamidomethyl (Acm) side chain protection for cysteine to provide deprotection and oxidation in a single step at a later stage. We obtained tetradepsipeptides **16** and **17** after the Boc-deprotection of the respective tridepsipeptides with 4 M HCl in dioxane followed by EDCI/HOAt-mediated coupling with Boc-L-Ala-OH. We selectively deprotected tetradepsipeptide **16** to hydrochloride **6** and oligomer **17** to the free carboxylic acid **7** using 4 M HCl in dioxane and zinc powder in 90% HOAc, respectively.

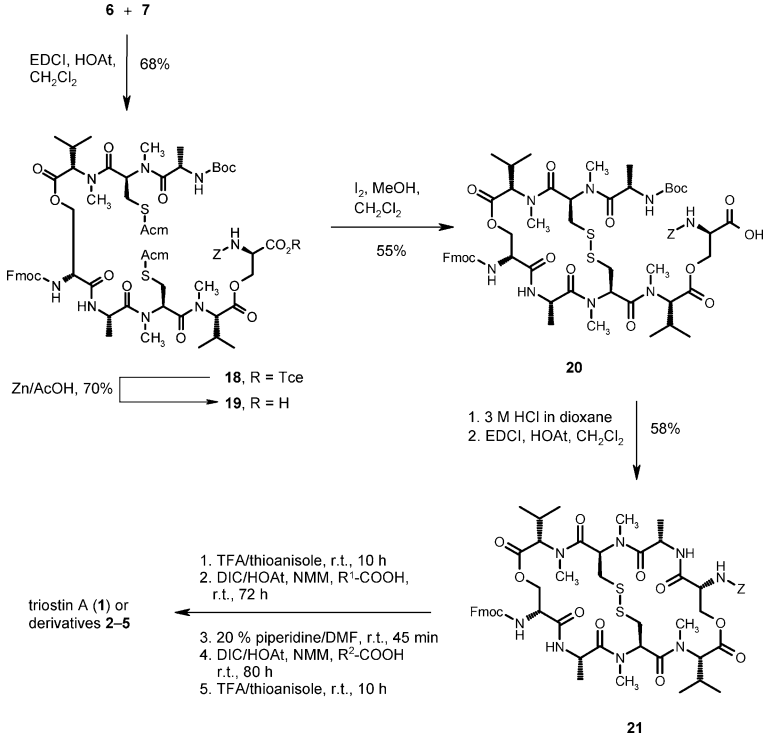
We accomplished the coupling of fragments **6** and **7** to yield the linear octadepsipeptide **18** by standard peptide coupling with EDCI/HOAt in DCM in good yield (Scheme 2). The reductive cleavage of the Tce ester **18** was followed by the iodine-mediated deprotection of the Acm groups and spontaneous disulfide bond formation under high dilution. The terminal ends of resulting depsipeptide **20** were sufficiently preorganized for macrocyclization. We obtained the key **21** by acidic *N*-terminal deprotection followed by ring closure yielding the bicyclic octadepsipeptide in a respectable 58% yield using diisopropylcarbodiimide (DIC)/HOAt activation. The orthogonal protecting groups at the amino groups allowed the synthesis of substrates presenting two different recognition units. Therefore, besides triostin A, various nucleobase-substituted building blocks were accessible based on the bicyclic scaffold **21** after selective Fmoc and Z deprotection.

We accomplished the synthesis of the natural product triostin A (**1**) with two quinoxaline moieties by Z-group deprotection followed by coupling of 2-quinoxaline carboxylic acid mediated by DIC/HOAt activation, Fmoc-deprotection, and coupling of the second quinoxaline unit under identical conditions. The successive deprotection and coupling procedure is not the most beneficial strategy for triostin A itself, as indicated by a 15% yield over the last four steps. Nevertheless, the main focus of this study was set on the triostin A derivatives with mixed functionalities.

We performed the functionalization of the triostin A scaffold **21** with two different recognition units by subsequent deprotection and coupling. We prepared the required nucleobase-substituted acetic acids with suitable protecting groups accordingly to the synthesis of nucleo amino acids used for PNA oligomers.[25] We selected benzyloxycarbonyl (Z) and phenoxy (PhO) protecting groups to increase the solubility in organic solvents and to prevent nucleophilic reactivity of the nucleobase amino groups. We obtained the first triostin A derivative **2** by coupling methylene guanine instead of the second quinoxaline unit. We obtained further



Scheme 1. Synthesis of tetradepsipeptides **6** and **7**.



Scheme 2. Macrocyclization, oxidation, and functionalization to yield the natural product triostin A (**1**) and its nucleobase analogs **2**–**5**.

derivatives by the linkage of two nucleobase recognition units. For the synthesis of triostin A analogs **3**–**5** presenting two different recognition units, we removed the Z protecting group of **21** with TFA/thioanisole and attached the first nucleobase moiety to the free amino group by treatment with DIC and HOAt (Scheme 2). We removed the Fmoc group subsequently by addition of 20% piperidine in DMF. We coupled the second nucleobase moiety furnishing triostin A analogs **3**–**5**. We deprotected these analogs with a mixture of thioanisole and TFA in order to remove the nucleobase protecting groups and purified them by HPLC. We confirmed the integrity of the respective derivatives by mass spectrometry and NMR spectroscopy. The NMR spectroscopic data of the synthesized triostin A (**1**) were identical with the data previously reported.[26]

DNA Interaction of Nucleobase-Containing Triostin A Analogs

We investigated the DNA-binding potential of the new triostin A derivatives using a well-defined ds-DNA decamer

with an adeninyl-hairpin structure given by the sequence $^{5'}$CGTAGCGTAC AAAAA GTACGCTACG$^{3'}$ (**22**). In a second DNA hairpin structure ($^{5'}$CGTA**X**CGTAC AAAAA GTACGCTACG$^{3'}$, **23**) we exchanged one guanosine nucleotide for an abasic site (**X**) in order to also investigate the abasic site binding potential of the synthetic triostin analogs **2**–**5**.

Temperature-Dependent UV Spectroscopy

We determined the double strand stability of hairpin DNA **22** by temperature-dependent UV spectroscopy indicating the cooperative destacking of nucleobase pairs with increasing temperature. For the double strand **22**, we determined a stability of T_m = 64 °C (1.5 μM) (Figure 2). Adding five equivalents of triostin A significantly increased the stability to T_m = 72 °C indicating the contribution of two intercalating quinoxalines and stabilization based on backbone recognition. The influence of the triostin analogs **2**–**5** on the stability of hairpin DNA **22** was rather weak. We found a slight increase in DNA duplex stability in the presence of the Q-G analog **2** (T_m = 65 °C) or the A-G analog **5** (T_m = 65 °C). We detected stabilities comparable to or slightly lower than the self association of DNA **22** in case the C-G analog **3** (T_m = 62 °C) or the T-A analog **4** (T_m = 63 °C). From the thermal UV profiles of analogs **2**–**5** with DNA **22**, we could draw no conclusions about the possible interacting modes except that they differ from triostin A bisintercalation. The interaction of triostin A and analogs **2**–**5** with DNA **23** containing the abasic site provided a similar result (Supporting Information). Triostin A stabilized the hairpin DNA **23** (T_m = 40 °C, 1.5 μM to T_m = 50 °C), whereas the derivatives **2** (T_m = 41 °C), **3** (T_m = 43 °C), **4** (T_m = 43 °C), and **5** (T_m = 43 °C) only provided minor duplex stabilization. None of the triostin analogs displayed especially favored recognition of an abasic site.

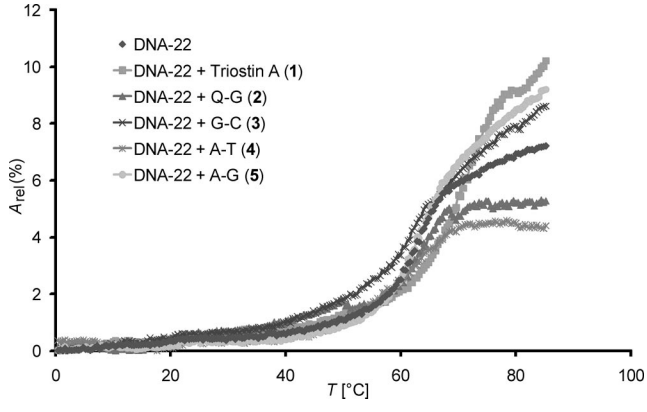


Figure 2. Temperature-dependent UV spectra of DNA hairpin $^{5'}$CGTAGCGTAC AAAAA GTACGCTACG$^{3'}$ (**22**) and **22** (2 mM, 1.5 μM, HEPES, NaCl 10 mM, pH 7.0) with triostin A (**1**) and respective nucleobase analogs **2**–**5** (7.5 μM).

Fluorescence Intercalator Displacement (FID)

In a FID assay, the fluorescence of DNA-bound ethidium bromide is detected.[27,28] Starting with ds-DNA saturated with intercalating ethidium bromide, the addition of competing intercalators leads to a decrease of fluorescence intensity. Since there is a CG specificity of bisintercalating triostin A and considering nearest neighbor exclusion of intercalator positions, we expected a substitution of about 50% of the ethidium bromide molecules. Indeed, by the addition of successive amounts of triostin A to DNA **22** saturated with ethidium bromide we obtained a value of about 50% fluorescence intensity (Figure 3). The A-T triostin analog **4** provided similar ethidium displacement results, whereas the other nucleobase-modified triostin analogs **2**, **3**, and **5** showed significantly lower ethidium displacement pointing to a binding mode that differs from bisintercalation.

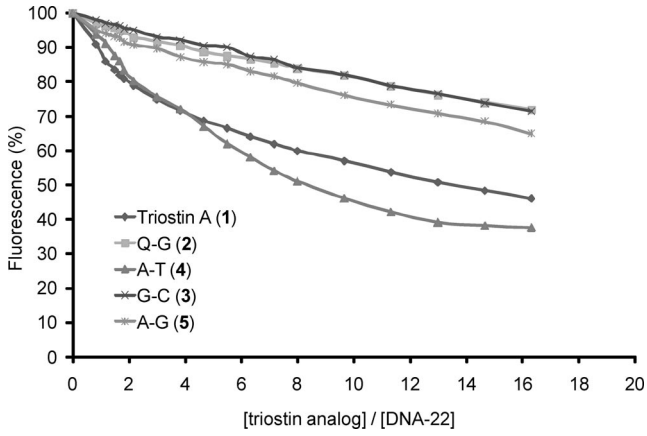


Figure 3. Ethidium bromide fluorescence intercalator displacement with DNA hairpin sequence $^{5'}$CGTAGCGTAC AAAAA GTACGCTACG$^{3'}$ (**22**) and triostin A (**1**) and **22** with the respective nucleobase analogs **2**–**5**.

Comparable FID experiments with DNA **23** provided a lower level of displacement for triostin A of about 25%, indicating the influence of the abasic site (Figure 4). As for DNA analog **23**, the A-T triostin analog **4** showed a similar displacement. Nevertheless, at higher concentrations, we reached further displacement of approximately 40%. The A-G analog **5** also attained a level of displacement comparable to triostin A, at least at higher concentrations. The

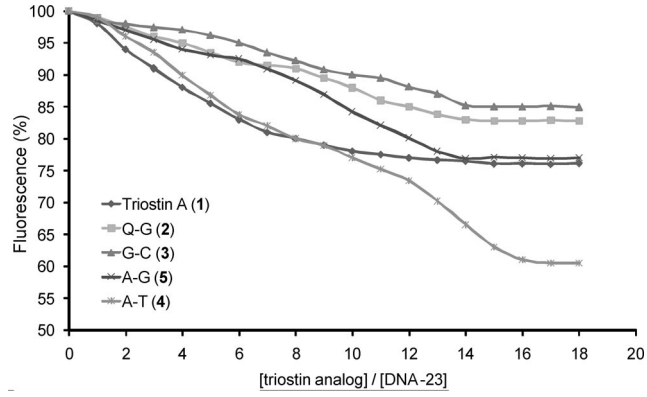


Figure 4. Ethidium bromide fluorescence intercalator displacement with DNA hairpin sequence $^{5'}$CGTAXCGTAC AAAAA GTACGCTACG$^{3'}$ (**23**) and triostin A (**1**) and **23** with the respective nucleobase analogs **2**–**5**.

FID of the other derivatives was negligible. Overall, the FID experiments indicated differences in binding of triostin A and its analogs but did not provide conclusions on the mode of interaction. Cocrystallization of the triostin analogs with ds-DNA is currently being attempted.

Conclusions

A total synthesis of the natural product triostin A and analogs carrying nucleobases instead of the quinoxalines as recognition units is presented. The key intermediate allowing variable functionalization of the triostin backbone is the orthogonally protected, disulfide-bridged cyclodepsipeptide. This derivative is used as a template, allowing the attachment of all kinds of functionalities to be organized at a 10.5 Å distance. The nucleobase-containing triostin analogs were prepared with the intention to create new ds-DNA binding motifs. UV and FID experiments provide evidence for the recognition that differs from that of triostin A bisintercalation.

Experimental Section

General Remarks: Solvents were used in the highest grade available. DCM was distilled from calcium hydride prior to use. DMF was purchased dry and stored over molecular sieves (4 Å). Commercially available reagents were of analytical grade and used without further purification. Melting points were obtained with a Büchi 501 Dr. Tottoli apparatus. Optical rotations were determined with a Perkin–Elmer 241 polarimeter. IR spectra were recorded with a Perkin–Elmer 1600 Series FT-IR spectrometer using KBr pellets. NMR spectra were recorded with Varian INOVA-600 and INOVA-300 instruments. Chemical shifts are referenced to the residual solvent peaks. ESI-MS data were measured with a LCQ Finnigan spectrometer. HRMS data were determined with a Bruker APEX-Q IV 7T spectrometer. HPLC was performed with a Pharmacia Äkta basic system using YMC J′sphere ODS-H80, RP-C18 columns for both analytical samples (250 × 4.6 mm, 5 μM, 120 Å, 1 mL min^{-1}) and preparative runs (250 × 20 mm, 5 μM, 120 Å, 10 mL min^{-1}); eluent A: water/TFA (0.1%); eluent B: acetonitrile/water, 4:1, TFA (0.1%). Analytical thin-layer chromatography was performed by using Merck silica gel 60, F_{254}-precoated aluminum plates. UV light (254 nm) or dyeing with ninhydrin (5% in ethanol) was used for detection.

UV Melting Experiment: To ds-DNA $^{5'}$CGTAGCGTAC AAAAA GTACGCTACG$^{3'}$ (**22**) or $^{5'}$CGTA**X**CGTAC AAAAA GTACGCTACG$^{3'}$ (**23**) at 1.5 μM in buffer (500 μL of 2 mM HEPES buffer in 10 mM aq. NaCl at pH 7.0 in a 10 mm cuvette) triostin or a triostin analog (7.5 μM, 5 equiv. in DMSO added in 2 μL from a stock solution) was added. Measurements of temperature-dependent UV spectra were performed with a JASCO V-550 UV/Vis spectrometer equipped with JASCO ETC-505S/ETC-505T temperature controller. The determination of the oligomer concentration was based on the absorption at 260 nm measured at 20 °C. Samples were placed in a quartz cell of 1 cm path length, and the data were collected at eight different wavelengths (240–300 nm) according to the following temperature protocol with a regular flow of nitrogen: 20 °C → 80 °C (10 min) → 80 °C (3 min) → 0 °C (26 min) → 0 °C (10 min) → 80 °C (180 min) → 0 °C (180 min) → 28 °C (5 min).

FID Experiment: ds-DNA **22** or **23** was added to ethidium bromide (5 equiv., one equiv. per two base pairs of the hairpin loop) in 2 mM HEPES buffer (10 mM aq. NaCl at pH 7.0, 10 mm cuvette, 500 μL of total volume). Triostin A or an analog was added in portions (2 μL of 0.8 mM solution in DMSO), and the sample was incubated at 25 °C for 30 min before the emission spectrum was measured ($\lambda_{excitation}$ = 545 nm, $\lambda_{emission}$ = 595 nm).

***N*-Z-D-Ser{*N*-Fmoc-D-Ser[*N*-Boc-L-Ala-*N*-Me-L-Cys(Acm)-*N*-Me-L-Val]-L-Ala-*N*-Me-L-Cys(Acm)-*N*-Me-L-Val}-OTce (18):** To a solution of **7** (1.5 g, 1.88 mmol) in DCM (5 mL) at 0 °C, HOAt (308 mg, 2.26 mmol, 1.2 equiv.) and EDCI (434 mg, 2.26 mmol, 1.2 equiv.) were added sequentially. The mixture was stirred at 0 °C for 30 min followed by the addition of a solution of **6** (1.5 g, 2.02 mmol, 1.07 equiv.) in DCM (12 mL). The reaction mixture was stirred at 0 °C for 9 h under argon before being poured into cold aq. HCl (1 N, 200 mL). The aq. phase was extracted with ethyl acetate (2 × 200 mL). The combined organic phases were washed with $NaHCO_3$ (5%, 200 mL) and saturated NaCl (120 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (SiO_2, 4 × 15 cm, MeOH/ethyl acetate, 1:19) yielded **18** (1.95 g, 1.28 mmol, 68%) as a white foam. R_f (ethyl acetate/MeOH, 20:1) = 0.62. M.p. 104 °C. $[\alpha]_D^{20}$ = –108 (MeOH, *c* = 0.08). UV (MeOH): λ_{max} = 266 nm. IR (KBr): $\tilde{\nu}$ = 3417, 2967, 2953, 1727, 1648, 1526, 1437, 1377, 1255, 1170, 1058, 739, 575 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C): δ = 0.73–0.78 (m, 6 H, Val-CH_3), 0.95–0.99 (m, 6 H, Val-CH_3), 1.27–1.30 (m, 6 H, Ala-CH_3), 1.41 (s, 6.5 H, *t*Bu-rot), 1.42 (s, 2.5 H, *t*Bu-rot), 1.95 (s, 3 H, Acm-CH_3-rot), 2.05 (s, 3 H, Acm-CH_3-rot), 2.13–2.20 (m, 2 H, Val-Hβ), 2.79–2.83 (m, 2 H, Cys-Hβ), 2.87 (s, 3 H, Val-NCH_3/Cys-NCH_3), 2.88 (s, 3 H, Val-NCH_3/Cys-NCH_3), 2.91 (s, 3 H, Val-NCH_3/Cys-NCH_3), 2.92 (s, 3 H, Val-NCH_3/Cys-NCH_3), 3.02–3.14 (m, 2 H, Cys-Hβ), 4.19–4.23 (m, 2 H, Fmoc-CH), 4.33–4.36 (m, 4 H, Acm-CH_2), 4.44–4.92 (m, 13 H, Tce-CH_2, Ala-Hα, Fmoc-CH_2, Val-Hα, Ser-Hα, Ser-Hβ), 5.09–5.12 (m, 2 H, Z-CH_2), 5.35–5.38 (m, 1 H, NH), 5.56–5.70 (m, 2 H, Cys-Hα), 6.52–6.59 (m, 1 H, NH), 6.71–6.78 (m, 1 H, NH), 7.26–7.38 (m, 9 H, Fmoc-H3, Fmoc-H4, Z-Ph), 7.57–7.61 (m, 2 H, Fmoc-H2), 7.73–7.74 (m, 2 H, Fmoc-H5) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ = 18.2 (Ala-CH_3), 18.6, 18.7, 18.9, 19.8, 19.9, 20.0 (Val-CH_3, Ala-CH_3), 23.0, 23.1 (Acm-CH_3), 26.9 (Val-CHβ), 28.3 [C(*C*H$_3$)$_3$], 30.0, 30.3, 30.4, 30.5 (Val-NCH_3, Cys-NCH_3, Cys-CH_2β), 41.8 (Acm-CH_2), 46.7 (Ala-CHα), 47.0 (Ala-CHα), 52.6 (Fmoc-CH), 53.4, 53.5 (Ser-CHα, Cys-CHα), 61.9 (Val-CHα), 64.4 (Ser-CH_2β), 67.2 (Fmoc-CH_2), 67.9 (Z-CH_2), 74.6 (Tce-CH_2), 79.9 [*C*(CH$_3$)$_3$], 94.2 (Tce-CCl_3), 120.0 (Fmoc-C5), 125.1, 125.2 (Fmoc-C2), 127.1, 127.7, 128.0 (Fmoc-C3/Z-Ph), 128.2 (Fmoc-C4), 128.6 (Fmoc-C3/Z-Ph), 136.1 (Z-Ph$_{ipso}$), 141.3 (Fmoc-C6), 143.7 (Fmoc-C1), 155.1, 156.1, 156.6 (Boc-CO, Fmoc-CO, Z-CO), 167.8, 170.1, 170.6 (Ser-CO, Val-CO, Cys-CO, Ala-CO, Acm-CO) ppm. HRMS (ESI): calcd. for $C_{68}H_{94}Cl_3N_{10}O_{19}S_2$ $[M + H]^+$ 1523.5198; found 1523.5207.

Z-D-Ser{*N*-Fmoc-D-Ser[*N*-Boc-L-Ala-*N*-Me-L-Cys(Acm)-*N*-Me-L-Val]-L-Ala-*N*-Me-L-Cys(Acm)-*N*-Me-L-Val}-OH (19): A solution of **18** (1.9 g, 1.25 mmol) in 90% aq. HOAc (65 mL) was cooled to 0 °C followed by the addition of zinc powder (4.09 g, 62.5 mmol, 50 equiv.). The suspension was stirred at 0 °C for 4 h, filtered, and the filtrate was concentrated in vacuo. The residue was treated with cold HCl (1 N, 150 mL) and extracted with $CHCl_3$ (3 × 150 mL). The combined organic phases were washed with saturated aq. NaCl (150 mL), dried (Na_2SO_4), and concentrated in vacuo. After purification by flash chromatography (SiO_2, 2 × 16 cm, MeOH/ethyl acetate/HOAc, 3:16:1) and coevaporation with toluene (3 × 25 mL), **19** (1.23 g, 0.88 mmol, 70%) was obtained as a white foam. R_f (ethyl acetate/MeOH/HOAc, 10:2:1) = 0.30. M.p. 209 °C. $[\alpha]_D^{20}$ = –97

(MeOH, c = 0.04). UV (MeOH): λ_{max} = 261 nm. IR (KBr): $\tilde{\nu}$ = 3440, 2925, 2362, 1646, 1464, 1381, 1262, 1060, 670 cm^{-1}. ^1H NMR (600 MHz, [D$_6$]DMSO, 25 °C): δ = 0.75 (d, $^3J_{H,H}$ = 7 Hz, 4.5 H, Val-CH$_3$), 0.84–0.86 (m, 1.5 H, Val-CH$_3$), 0.89–0.91 (m, 4.5 H, Val-CH$_3$), 0.94 (d, $^3J_{H,H}$ = 7 Hz, 1.5 H, Val-CH$_3$), 1.11 (d, $^3J_{H,H}$ = 7 Hz, 0.7 H, Ala-CH$_3$), 1.14 (d, $^3J_{H,H}$ = 7 Hz, 3 H, Ala-CH$_3$), 1.19 (d, $^3J_{H,H}$ = 7 Hz, 2.3 H, Ala-CH$_3$), 1.35 (s, 2 H, *t*Bu-rot), 1.37 (s, 7 H, *t*Bu-rot), 1.82 (s, 6 H, Acm-CH$_3$), 2.14–2.19 (m, 2 H, Val-Hβ), 2.57–2.63 (m, 0.5 H, Cys-Hβ), 2.69–2.71 (m, 1.5 H, Cys-Hβ), 2.72–2.75 (m, 3 H, Val-NCH$_3$/Cys-NCH$_3$), 2.85–2.88 (m, 9 H, Val-NCH$_3$/Cys-NCH$_3$), 2.96–3.02 (m, 1.5 H, Cys-Hβ), 3.06–3.10 (m, 0.5 H, Cys-Hβ), 3.98–4.01 (m, 1 H, Ser-Hα), 4.02–4.06 (m, 2 H, Acm-CH$_2$, Ser-Hβ), 4.15–4.24 (m, 3 H, Acm-CH$_2$, Ser-Hβ), 4.25–4.33 (m, 5 H, Fmoc-CH, Fmoc-CH$_2$, Acm-CH$_2$, Ser-Hβ), 4.34–4.37 (m, 3 H, Ala-Hα, Ser-Hα, Val-Hα), 4.42–4.45 (m, 2 H, Val-Hα, Ser-Hβ), 4.52 (d, $^3J_{H,H}$ = 10 Hz, 1 H, Ser-NH), 4.56 (d, $^3J_{H,H}$ = 10 Hz, 1 H, Ser-NH), 4.68–4.71 (m, 0.2 H, Ala-Hα), 4.74–4.79 (m, 0.8 H, Ala-Hα), 5.03 (s, 0.5 H, Z-CH$_2$), 5.05 (s, 1.5 H, Z-CH$_2$), 5.39–5.44 (m, 0.5 H, Cys-Hα), 5.46–5.51 (m, 1.5 H, Cys-Hα), 6.49–6.62 (m, 1 H, Ser-NH), 6.80–6.89 (m, 1 H, Ser-NH), 7.31–7.36 (m, 7 H, Fmoc, Z-Ph), 7.40–7.42 (m, 2 H, Fmoc), 7.63–7.72 (m, 3 H, Fmoc, Ala-NH), 7.87–7.89 (d, $^3J_{H,H}$ = 7 Hz, 2 H, Fmoc), 8.27–8.39 (m, 3 H, Ala-NH, Acm-NH) ppm. ^{13}C NMR (150 MHz, [D$_6$]-DMSO, 25 °C): δ = 17.3 (Ala-CH$_3$), 17.7 (Ala-CH$_3$), 18.7 (Val-CH$_3$), 19.7 (Val-CH$_3$), 19.8 (Val-CH$_3$), 22.5 (Acm-CH$_3$), 26.9 (Val-CHβ), 27.0 (Val-CHβ), 28.1 (Cys-CH$_2$β), 29.0 [C(*C*H$_3$)$_3$], 29.1 (Cys-CH$_2$β), 30.0, 30.1, 31.5, 31.7 (Val-NCH$_3$/Cys-NCH$_3$), 40.1 (Acm-CH$_2$), 45.2 (Ala-CHα), 46.4 (Ala-CHα), 46.5 (Fmoc-CH), 52.5 (Cys-CHα), 52.7 (Cys-CHα), 52.8 (Ser-CHα), 53.4 (Ser-CHα), 62.3 (Val-CHα), 62.5 (Val-CHα), 63.5 (Ser-CH$_2$β), 64.0 (Ser-CH$_2$β), 65.6 (Z-CH$_2$), 66.0 (Fmoc-CH$_2$), 78.0 [*C*(CH$_3$)$_3$], 120.0 (Fmoc-C5), 125.2 (Fmoc-C2), 127.0, 127.6, 127.7, 127.8, 128.3, (Fmoc, Z-Ph), 136.8 (Z-Ph$_{ipso}$), 140.6 (Fmoc), 143.7 (Fmoc), 154.8, 155.8, 155.9 (Boc-CO, Fmoc-CO, Z-CO), 167.6, 169.3, 169.5, 169.6, 169.7, 170.6, 172.0 (Ser-CO, Val-CO, Cys-CO, Ala-CO, Acm-CO) ppm. HRMS (ESI): calcd. for $C_{66}H_{93}N_{10}O_{19}S_2$ [M + H]$^+$ 1393.6054; found 1393.6055.

Z-D-Ser[*N*-Fmoc-D-Ser(*N*-Boc-L-Ala-*N*-Me-L-Cys-*N*-Me-L-Val)-L-Ala-*N*-Me-L-Cys-*N*-Me-L-Val]-OH Disulfide (20): To a solution of iodine (2.17 g, 8.5 mmol, 10 equiv.) in DCM/MeOH (770 mL, 9:1) a solution of **19** (1.18 g, 0.85 mmol) in DCM (350 mL) was added dropwise, and the mixture was stirred at 25 °C for 6 h. The reaction mixture was cooled to 0 °C, and $Na_2S_2O_3$ (5%) was added slowly until the excess iodine was discharged, and the color disappeared. The organic phase was washed with aq. HCl (1 N, 120 mL) and saturated aq. NaCl (100 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 2 × 18 cm, MeOH/ethyl acetate, 3:17, 0.3% HOAc) followed by coevaporation with toluene (3 × 25 mL) furnished **20** (590 mg, 470 μmol, 55%) as a pale yellow solid. R_f (ethyl acetate/MeOH, 5:1, 0.5% HOAc) = 0.34. M.p. 281–283 °C. $[\alpha]_D^{20}$ = –119 (MeOH, c = 0.05). UV (MeOH): λ_{max} = 257, 264 nm. IR (KBr): $\tilde{\nu}$ = 3423, 2926, 2371, 1956, 1647, 1519, 1463, 1402, 1256, 1172, 1054, 741, 523, 416 cm^{-1}. ^1H NMR (600 MHz, [D$_6$]DMSO, 25 °C): δ = 0.75–0.76 (m, 6 H, Val-CH$_3$), 0.91–0.93 (m, 6 H, Val-CH$_3$), 1.15–1.16 (m, 3 H, Ala-CH$_3$), 1.24 (d, $^3J_{H,H}$ = 7 Hz, 3 H, Ala-CH$_3$), 1.37 (s, 9 H, *t*Bu), 2.17–2.23 (m, 2 H, Val-Hβ), 2.82 (m, 2 H, Cys-Hβ), 2.87–2.97 (m, 12 H, Val-NCH$_3$/Cys-NCH$_3$), 3.18–3.21 (m, 2 H, Cys-Hβ), 4.25–4.52 (m, 13 H, Ala-Hα, Val-Hα, Fmoc-CH, Fmoc-CH$_2$, Ser-Hα, Ser-Hβ), 5.03–5.04 (m, 2 H, Z-CH$_2$), 5.54–5.62 (m, 0.7 H, Cys-Hα), 5.68–5.71 (m, 1.3 H, Cys-Hα), 7.32–7.35 (m, 8 H, Fmoc, Z-Ph, Ser-NH), 7.40–7.43 (m, 3 H, Fmoc, Ser-NH), 7.69–7.70 (m, 2 H, Fmoc), 7.83–7.90 (m, 3 H, Fmoc/NH) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): δ = 16.5, 17.2, 17.3, 18.5, 18.8, 19.0, 19.5, 19.6, 19.7, 19.8 (Val-CH$_3$, Ala-CH$_3$), 26.6 (Val-CHβ), 26.9 (Val-CHβ), 28.1 [C(*C*H$_3$)$_3$], 30.2, 30.7, 31.0 (Val-NCH$_3$/Cys-NCH$_3$, Cys-CH$_2$β), 46.1, 46.4, 46.6 (Fmoc, Ala-CHα), 53.8 (Cys-CHα), 62.3 (Ser-CHα), 63.0 (Ser-CHα), 63.4 (Val-CHα), 65.6 (Ser-CH$_2$β), 65.7 (Z-CH$_2$-rot), 66.1 (Fmoc-CH$_2$), 78.0 [*C*(CH$_3$)$_3$], 120.1 (Fmoc), 125.1 (Fmoc-C2), 127.0 (Fmoc), 127.6, 127.7, 127.8, 128.3 (Fmoc-C3, Fmoc, Z-Ph), 136.6 (Z-Ph$_{ipso}$), 136.7 (Fmoc), 140.7 (Fmoc), 143.6 (Fmoc), 143.7 (Fmoc), 154.8, 155.9 (Boc-CO, Fmoc-CO, Z-CO), 169.2, 169.3, 169.4, 169.6, 170.6, 172.0 (Ser-CO, Val-CO, Cys-CO, Ala-CO) ppm. HRMS (ESI): calcd. for $C_{60}H_{81}N_5O_{17}S_2$ [M + Na]$^+$ 1249.5155; found 1249.5149.

***N*-Z-D-Ser-L-Ala-*N*-Me-L-Cys-*N*-Me-L-Val-*N*-Fmoc-D-Ser-L-Ala-*N*-Me-L-Cys-*N*-Me-L-Val) (Serine-hydroxy)-Dilactone Disulfide (21):** A solution of **20** (540 mg, 430 μmol) in dioxane (1.1 mL) was treated with HCl (4 M, 3.2 mL) in dioxane at 0 °C. The mixture was stirred at 0 °C for 30 min and at 25 °C for 30 min before the volatiles were removed in vacuo. The residual HCl was removed by adding Et_2O (30 mL) to the hydrochloride salt followed by solvent removal in vacuo. The residue was dissolved in DCM (400 mL), and the resulting solution was treated sequentially with HOAt (350 mg, 2.58 mmol) and EDCI (495 mg, 2.58 mmol). The reaction mixture was stirred at 0 °C for 12 h before being poured into HCl (1 N, 117 mL). The organic phase was extracted with ethyl acetate (2 × 234 mL), and the combined organic phases were washed with $NaHCO_3$ (5%, 234 mL) and saturated aq. NaCl (70 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, 4 × 15 cm, ethyl acetate) afforded **21** (282 mg, 250 μmol, 58%) as a white solid. R_f (ethyl acetate) = 0.5. $[\alpha]_D^{20}$ = –119 (MeOH, c = 0.05). UV (MeOH): λ_{max} = 257, 264 nm. IR (KBr): $\tilde{\nu}$ = 3423, 2926, 2371, 1956, 1647, 1519, 1463, 1402, 1256, 1172, 1054, 741, 523, 416 cm^{-1}. ^1H NMR (600 MHz, [D$_6$]DMSO, 25 °C): δ = 0.85 (d, $^3J_{H,H}$ = 7 Hz, 3 H, Val-CH$_3$), 0.88 (d, $^3J_{H,H}$ = 6 Hz, 3 H, Val-CH$_3$), 0.94 (d, $^3J_{H,H}$ = 7 Hz, 6 H, Val-CH$_3$), 1.24–1.29 (m, 6 H, Ala-CH$_3$), 2.23–2.35 (br. m, 4 H, Val-Hβ, Cys-Hβ), 2.78 (s, 3 H, Val-NCH$_3$/Cys-NCH$_3$), 2.79 (s, 3 H, Val-NCH$_3$/Cys-NCH$_3$), 3.14 (s, 3 H, Val-NCH$_3$/Cys-NCH$_3$), 3.16 (s, 3 H, Val-NCH$_3$/Cys-NCH$_3$), 3.48–3.52 (m, 2 H, Cys-Hβ), 4.17–4.24 (m, 3 H, Ser-Hβ), 4.25–4.30 (m, 2 H, Fmoc-CH, Ser-Hβ), 4.30–4.38 (m, 4 H, Fmoc-CH$_2$, Ser-Hα), 4.39–4.46 (m, 2 H, Ala-Hα), 4.66–4.70 (m, 2 H, Val-Hα), 5.06–5.15 (m, 2 H, Z-CH$_2$), 6.08–6.15 (br. m, 2 H, Cys-Hα), 7.31–7.44 (m, 9 H, Z-Ph, Fmoc), 7.59–7.62 (s, Ala-NH, 1 H, br.), 7.66–7.73 (m, 5 H, Fmoc, Ala-NH, Ser-NH), 7.88 (d, $^3J_{H,H}$ = 8 Hz, 2 H, Fmoc-H5) ppm. ^{13}C NMR (150 MHz, [D$_6$]DMSO, 25 °C): δ = 15.7 (Ala-CH$_3$), 15.8 (Ala-CH$_3$), 19.5 (Val-CH$_3$), 19.6 (Val-CH$_3$), 19.7 (Val-CH$_3$), 19.8 (Val-CH$_3$), 26.6 (Val-CHβ), 30.1 (Val-NCH$_3$/Cys-NCH$_3$), 31.0 (Val-NCH$_3$/Cys-NCH$_3$), 31.1 (Val-NCH$_3$/Cys-NCH$_3$), 42.8 (Cys-CH$_2$β), 46.4 (Ala-CHα), 46.6 (Fmoc-CH), 53.7 (Ser-CHα), 56.4 (Cys-CHα), 63.0 (Val-CHα), 64.9 (Ser-CH$_2$β, Fmoc-CH$_2$), 66.0 (Z-CH$_2$), 119.9 (Fmoc), 120.1 (Fmoc), 121.2 (Fmoc), 124.9 (Fmoc), 126.9, 126.9, 127.2, 127.6, 127.6, 127.8, 128.2, 128.8 (Fmoc, Z-Ph), 136.6 (Z-Ph$_{ipso}$), 140.7 (Fmoc), 143.5, 143.6 (Fmoc), 168.5 (Z-CO), 169.5 (Fmoc-CO), 169.6, 169.8, 169.9 (Ser-CO, Val-CO, Cys-CO), 171.0 (Ala-CO) ppm. HRMS (ESI): calcd. for $C_{55}H_{71}N_8O_{14}S_2$ [M + H]$^+$ 1131.4525; found 1131.4529.

General Method of Preparation of Nucleobase-Substituted Triostin A Analogs. Step 1: Depsipeptide **21** was treated with thioanisole (14.6 μL per μmol of **21**) and TFA (146 μL per μmol of **21**). The mixture was stirred for 10 h at 20 °C before being evaporated. The hydrochloride salt was formed by the repeated addition of HCl in dioxane (2 M, 3 × 146 μL per μmol of **21**) and subsequent evaporation. **Step 2:** The resulting residue was dissolved in DCM/DMF

(9:1, 200 μL per μmol of **21**) at 0 °C and, whilst stirring, was treated sequentially with HOAt (2 equiv.), NMM (3 equiv.), DIC (10 equiv.), and the nucleobase acetic acid derivatives R^1-COOH (2 equiv.) under argon. After being stirred for 1 h, the mixture was reacted at room temperature for 72 h, diluted with ethyl acetate (700 μL per μmol of **21**), washed with water (350 μL per μmol of **21**), and concentrated in vacuo. **Step 3:** To the resulting residue, 20% piperidine in DMF (75 μL per μmol of **21**) was added, and the mixture was stirred for 20 min at 25 °C before the volatiles were removed in vacuo. Again, 20% piperidine in DMF (75 μL per μmol of **21**) was added, the mixture was stirred for another 15 min, and the solvents were evaporated before the residue was coevaporated subsequently with DMF (3 × 146 μL per μmol of **21**) and toluene (2 × 146 μL per μmol of **21**). The resulting solid was washed with Et_2O (2 × 146 μL per μmol of **21**) and dried thoroughly. **Step 4:** The resulting residue was dissolved in DCM/DMF (9:1, 200 μL per μmol of **21**) at 0 °C and, whilst stirring, was treated sequentially with HOAt (2 equiv.), NMM (3 equiv.), DIC (10 equiv.), and the nucleobase amino acid derivative R^2-COOH (2 equiv.) under argon. After being stirred for 1 h, the mixture was reacted at room temperature for 72 h, diluted with ethyl acetate (700 μL per μmol of **21**), washed with water (350 μL per μmol of **21**), and concentrated in vacuo. **Step 5:** The residue was treated with thioanisole (11 μL per μmol of **21**) and TFA (110 μL per μmol of **21**) and stirred for 10 h at 25 °C, before being concentrated in vacuo.

Triostin A (1): R^1-COOH = 2-quinoxalinecarboxylic acid, R^2-COOH = 2-quinoxalinecarboxylic acid. The resulting residue after step 4 of the general procedure, from the starting **21** (20 mg, 17.7 μmol) was dissolved in acetonitrile/water, filtered, and purified by HPLC (35–100% eluent B in 30 min, t_R = 17.4 min) to afford **1** (2.8 mg, 15%) as a pale yellow solid. ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C): δ = (major conformer) = 0.67 (d, $^3J_{H,H}$ = 7 Hz, 1 H, Ala-CH_3), 0.70 (d, $^3J_{H,H}$ = 7 Hz, 5 H, Ala-CH_3), 1.03 (d, $^3J_{H,H}$ = 7 Hz, 6 H, Val-CH_3), 1.1 (d, $^3J_{H,H}$ = 7 Hz, 6 H, Val-CH_3), 2.29–2.34 (m, 2 H, Val-Hβ), 3.02 (s, 6 H, Val-NCH_3/Cys-NCH_3), 3.30 (s, 6 H, Val-NCH_3/Cys-NCH_3), 3.31 (dd, $^3J_{H,H}$ = 7, $^3J_{H,H}$ = 16 Hz, 2 H, Cys-Hβ), 4.40 (dd, $^3J_{H,H}$ = 8, $^3J_{H,H}$ = 15 Hz, 2 H, Cys-Hβ), 4.24 (d, $^3J_{H,H}$ = 10 Hz, 2 H, Val-Hα), 4.56 (dd, $^3J_{H,H}$ = 1, $^3J_{H,H}$ = 11 Hz, 2 H, Ser-Hβ), 4.70 (dd, $^3J_{H,H}$ = 6, $^3J_{H,H}$ = 11 Hz, 2 H, Ser-Hβ), 5.00–5.02 (m, 2 H, Ala-Hα), 5.04–5.06 (m, 2 H, Ser-Hα), 5.70 (m, 2 H, Cys-Hα), 7.83–8.20 (m, 8 H, quin-H5, H6, H7, H8), 8.38 (d, $^3J_{H,H}$ = 9 Hz, 2 H, Ala-NH), 8.96 (d, $^3J_{H,H}$ = 9 Hz, 2 H, Ser-NH), 9.58 (s, 2 H, quin-H3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ = 17.4 (Ala-CH_3), 20.0 (Val-CH_3), 20.6 (Val-CH_3), 29.7 (Val-CHβ), 30.0 (Val-NCH_3/Cys-NCH_3), 32.4 (Val-NCH_3/Cys-NCH_3), 39.8 (Cys-CH_2β), 44.8 (Ala-CHα), 53.4 (Ser-CHα), 53.8 (Cys-CHα), 64.6 (Ser-CH_2β), 65.3 (Val-CHα), 129.5 (quin-C8), 129.7 (quin-C6), 131.4 (quin-C5), 132.1 (quin-C8), 140.2 (quin-C9), 142.6 (quin-C10), 142.7 (quin-C3), 143.7 (quin-C2), 163.9, 168.4, 170.2, 170.6, 172.9 (Val-CO, Ser-CO, Ala-CO, Cys-CO, quin-CO) ppm. ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C): δ (minor conformer) = 0.85 (d, $^3J_{H,H}$ = 7 Hz, 6 H, Val-CH_3), 1.07 (d, $^3J_{H,H}$ = 7 Hz, 6 H, Val-CH_3), 1.43 (d, $^3J_{H,H}$ = 7 Hz, 6 H, Ala-CH_3), 2.29–2.34 (m, 2 H, Val-Hβ), 2.96 (s, 6 H, Val-NCH_3/Cys-NCH_3), 3.07 (s, 6 H, Val-NCH_3/Cys-NCH_3), 3.27–3.28 (m, 4 H, Cys-Hβ), 4.46 (dd, $^3J_{H,H}$ = 8, $^3J_{H,H}$ = 11 Hz, 2 H, Ser-Hβ), 4.59 (dd, $^3J_{H,H}$ = 2, $^3J_{H,H}$ = 12 Hz, 2 H, Ser-Hβ), 4.74–4.77 (m, 2 H, Ala-Hα), 4.92–4.94 (m, 2 H, Ser-Hα), 5.20 (d, $^3J_{H,H}$ = 10 Hz, 2 H, Val-Hα), 6.79 (m, 2 H, Cys-Hα), 7.26 (d, $^3J_{H,H}$ = 1 Hz, 2 H, Ala-NH), 7.83–8.20 (m, 8 H, quin-H5, H6, H7, H8), 8.96 (d, $^3J_{H,H}$ = 7 Hz, 2 H, Ser-NH), 9.64 (s, 2 H, quin-H3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ = 17.8 (Ala-CH_3), 18.4 (Val-CH_3), 20.4 (Val-CH_3), 27.4 (Val-CHβ), 30.1 (Val-NCH_3/Cys-NCH_3), 31.1 (Val-NCH_3/Cys-NCH_3), 40.4 (Cys-CH_2β), 47.2 (Ala-CHα), 51.6 (Ser-CHα), 53.7 (Cys-CHα), 61.9 (Val-CHα), 64.8 (Ser-CH_2β), 129.5 (quin-C8), 129.6 (quin-C6), 131.1 (quin-C5), 132.0 (quin-C8), 140.3 (quin-C9), 142.6 (quin-C10), 143.6 (quin-C3), 143.9 (quin-C2), 163.7, 167.9, 169.3, 170.4, 172.8 (Val-CO, Ala-CO, Cys-CO, quin-CO) ppm. HRMS (ESI): calcd. for $C_{50}H_{63}N_{12}O_{12}S_2$ $[M + H]^+$ 1087.4124; found 1087.4123.

[*N*-(Quinoxaline-9-carbonyl)-D-Ser-L-Ala-*N*-Me-L-Cys-*N*-Me-L-Val]-[*N*-(guanin-9-yl acetyl)-D-Ser-L-Ala-*N*-Me-L-Cys-*N*-Me-L-Val] (Serine Hydroxy)-Dilactone Disulfide (2): R^1-COOH = 2-quinoxalinecarboxylic acid, R^2-COOH = [2-amino-6-(benzyloxy)purin-9-yl]acetic acid. The resulting residue obtained, after step 5 of the general method of preparation, from the starting **21** (20 mg, 17.7 μmol) was dissolved in acetonitrile/water, filtered, and purified by HPLC (20–90% eluent B in 30 min, t_R = 16.4 min) to afford the pale yellow solid **2** (3.1 mg, 16%). ^{1}H NMR (600 MHz, [D_6]-DMSO, 25 °C): δ = 0.84 (d, $^3J_{H,H}$ = 7 Hz, 3 H, Val-CH_3), 0.94 (d, $^3J_{H,H}$ = 7 Hz, 3 H, Val-CH_3), 0.96 (d, $^3J_{H,H}$ = 7 Hz, 3 H, Val-CH_3), 0.99 (d, $^3J_{H,H}$ = 7 Hz, 3 H, Val-CH_3), 1.27 (d, $^3J_{H,H}$ = 7 Hz, 3 H, Ala-CH_3), 1.37 (d, $^3J_{H,H}$ = 7 Hz, 3 H, Ala-CH_3), 2.19–2.25 (m, 1 H, Val-Hβ), 2.31 (d, $^2J_{H,H}$ = 14 Hz, 2 H, Cys-Hβ), 2.37–2.40 (m, 1 H, Val-Hβ), 2.80 (s, 3 H, Val-NCH_3/Cys-NCH_3), 2.84 (s, 3 H, Val-NCH_3/Cys-NCH_3), 3.1 (s, 3 H, Val-NCH_3/Cys-NCH_3), 3.33 (s, 3 H, Val-NCH_3/Cys-NCH_3), 3.52–3.58 (m, 2 H, Cys-Hβ), 4.09 (dd, $^3J_{H,H}$ = 4, $^2J_{H,H}$ = 11 Hz, 1 H, Ser-Hβ), 4.41–4.48 (m, 2 H, Ser-Hβ) 4.50–4.56 (m, 2 H, Ala-Hα), 4.61–4.63 (m, 1 H, Ser-Hα), 4.72 (d, $^3J_{H,H}$ = 11 Hz, 1 H, Val-Hα), 4.78 (d, $^2J_{H,H}$ = 17 Hz, 1 H, acetyl-CH_2), 4.84 (d, $^3J_{H,H}$ = 11 Hz, 1 H, Val-Hα), 4.85 (d, $^2J_{H,H}$ = 17 Hz, 1 H, acetyl-CH_2), 4.90–4.93 (m, 1 H, Ser-Hα), 6.13–6.16 (m, 2 H, Cys-Hα), 6.34 (br. s, 2 H, G-NH_2), 7.67 (br. s, 1 H, G-CH8), 7.74 (dd, $^2J_{H,H}$ = 13, $^3J_{H,H}$ = 6 Hz, Ser-Hβ), 7.94–8.07 (m, 4 H, quin-H5, H6, H7, H8), 8.12 (d, $^3J_{H,H}$ = 9 Hz, 1 H Ser-NH), 8.19–8.28 (m, 2 H, Ala-NH), 8.56 (d, $^3J_{H,H}$ = 9 Hz, 1 H, Ser-NH), 9.56 (s, 1 H, quin-H3), 10.58 (br. s, 1 H, G-NH) ppm. ^{13}C NMR (125 MHz, [D_6]DMSO, 25 °C): δ = 15.5 (Ala-CH_3), 15.8 (Ala-CH_3) 19.5 (Val-CH_3), 19.6 (Val-CH_3), 19.7 (Val-CH_3), 26.6 (Val-CHβ), 27.6 (Val-CHβ), 30.1 (Val-NCH_3/Cys-NCH_3), 30.2 (Val-NCH_3/Cys-NCH_3), 30.7 (Val-NCH_3/Cys-NCH_3), 31.2 (Val-NCH_3/Cys-NCH_3), 43.7 (Cys-CH_2β), 45.1 (acetyl-CH_2), 46.6 (Ser-CH_2β), 46.8 (Ala-CHα), 50.9 (Ser-CHα), 51.4 (Ser-CHα), 56.2 (Cys-CHα), 56.4 (Cys-CHα), 62.8 (Val-CHα), 62.9 (Val-CHα), 64.7 (Ser-CH_2β), 123.8 (G-C3), 126.0 (quin-C5, C6, C7, C8, minor conformer), 128.7 (quin-C5), 131.1 (quin-C7, C8), 131.7 (quin-C7, C8), 132.3 (quin-C6), 135.2 (quin-C9), 139.4 (quin-C10), 143.1 (quin-C2), 143.6 (quin-C3), 153.6 (G-C4, C6), 156.3 (G-C2), 162.8, 167.0, 167.8, 168.2, 169.5, 169.6, 169.7, 171.1, 171.2 (Ser-CO, Val-CO, Cys-CO, Ala-CO, acetyl-CO) ppm. HRMS (ESI): calcd. for $C_{48}H_{64}N_{15}O_{13}S_2$ $[M + H]^+$ 1122.2788; found 1122.2786.

[*N*-(Cytosin-1-ylacetyl)-D-Ser-L-Ala-*N*-Me-L-Cys-*N*-Me-L-Val]-[*N*-(guanin-9-yl acetyl)-D-Ser-L-Ala-*N*-Me-L-Cys-*N*-Me-L-Val] (Serine Hydroxy)-Dilactone Disulfide (3): R^1-COOH = [(4-*N*-benzyloxycarbonyl)cytosin-1-yl]acetic acid,[25] R^2-COOH = [2-amino-6-(benzyloxy)purin-9-yl]acetic acid. The resulting residue obtained, after step 5 of the general method of preparation, from the starting **21** (20 mg, 17.7 μmol) was dissolved in acetonitrile/water, filtered, and purified by HPLC (0–50% eluent B in 30 min, t_R = 20 min) to afford a white solid **3** (3.4 mg, 18%). ^{1}H NMR (600 MHz, [D_6]-DMSO, 25 °C): δ = 0.79 (d, $^3J_{H,H}$ = 7 Hz, 3 H, Val-CH_3), 0.85 (d, $^3J_{H,H}$ = 7 Hz, 3 H, Val-CH_3), 0.88 (d, $^3J_{H,H}$ = 7 Hz, 3 H, Val-CH_3), 0.97 (d, $^3J_{H,H}$ = 7 Hz, 3 H, Val-CH_3), 1.30 (d, $^3J_{H,H}$ = 7 Hz, 3 H, Ala-CH_3), 1.31 (d, $^3J_{H,H}$ = 7 Hz, 3 H, Ala-CH_3), 2.09 (br. s, 1 H, Val-Hβ), 2.27–2.32 (m, 2 H, Cys-Hβ), 2.35–2.41 (m, 1 H, Val-Hβ), 2.77 (s, 3 H, Val-NCH_3/Cys-NCH_3), 2.79 (s, 3 H, Val-NCH_3/Cys-NCH_3), 3.07 (s, 3 H, Val-NCH_3/Cys-NCH_3), 3.20 (s, 3 H, Val-

NCH_3/Cys-NCH_3), 3.51 (d, $^2J_{H,H}$ = 12 Hz, 1 H, Cys-Hβ), 3.53 (d, $^3J_{H,H}$ = 12 Hz, 1 H, Cys-Hβ), 4.09 (dd, ^3J = 4, $^2J_{H,H}$ = 11 Hz, 2 H, Ser-Hβ), 4.43–4.47 (m, 3 H, Ser-Hβ, acetyl-CH_2), 4.48–4.50 (m, 2 H, Ala-Hα), 4.58–4.59 (m, 1 H, Ser-Hα), 4.63–4.64 (m, 1 H, Ser-Hα), 4.68–4.70 (m, 1 H, acetyl-CH_2), 4.72 (d, $^3J_{H,H}$ = 11 Hz, 2 H, Val-Hα), 4.82 (d, $^2J_{H,H}$ = 17 Hz, 1 H, acetyl-CH_2), 4.87 (d, $^2J_{H,H}$ = 17 Hz, 1 H, acetyl-CH_2), 6.00 (d, $^3J_{H,H}$ = 7 Hz, 1 H, C-H5), 6.09 (dd, $^3J_{H,H}$ = 3, $^3J_{H,H}$ = 11 Hz, 1 H, Cys-Hα), 6.19 (dd, $^3J_{H,H}$ = 4, $^3J_{H,H}$ = 11 Hz, 1 H, Cys-Hα), 6.53 (br. s, 2 H, G-NH_2), 7.56 (d, $^3J_{H,H}$ = 6 Hz, 1 H, Ala-NH), 7.76 (br. s, 1 H, G-CH8), 7.82 (d, $^3J_{H,H}$ = 7 Hz, 1 H, Ala-NH), 7.86 (d, $^3J_{H,H}$ = 7 Hz, 1 H, C-H6), 8.26 (d, $^3J_{H,H}$ = 8 Hz, 1 H, Ser-NH), 8.50 (br. s, 1 H, Ser-NH), 11.18 (br. s, 1 H, G-NH) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): δ = 15.7 (Ala-CH_3), 15.9 (Ala-CH_3), 19.4 (Val-CH_3), 19.5 (Val-CH_3), 19.6 (Val-CH_3), 19.7 (Val-CH_3), 26.6 (Cys-CH_2β, Val-CHβ), 26.8 (Cys-CH_2β, Val-CHβ), 30.1 (Val-NCH_3/Cys-NCH_3), 30.8 (Val-NCH_3/Cys-NCH_3), 42.5 (Cys-CH_2β), 45.9 (acetyl-CH_2), 46.4 (Ala-CHα), 46.5 (Ala-CHα), 51.4 (Ser-CHα), 51.5 (Ser-CHα), 51.9, 55.8 (Cys-CHα), 56.0 (Cys-CHα), 62.8 (Val-CHα, Val-CHβ), 62.9 (Val-CHα, Val-CHβ), 64.3 (Ser-CH_2β), 64.4 (Ser-CH_2β), 93.5 (C-C5), 115.8 (G-C5), 138.3 (C-C6), 151.5 (G-C8), 153.8 (G-C6), 156.9 (G-C2), 157.9 (C-C2), 158.2 (G-C4), 167.0 (C-C4), 167.4, 167.5, 167.7, 169.4, 169.5, 169.6, 169.8, 170.9, 171.3 (Ser-CO, Val-CO, Cys-CO, Ala-CO, acetyl-CO) ppm. HRMS (ESI): calcd. for $C_{45}H_{65}N_{16}O_{14}S_2$ [M + H]$^+$ 1117.2387; found 1117.2385.

[*N*-(Thymin-1-ylacetyl)-D-Ser-L-Ala-*N*-Me-L-Cys-*N*-Me-L-Val]-[*N*-(adenin-9-yl acetyl)-D-Ser-L-Ala-*N*-Me-L-Cys-*N*-Me-L-Val] (Serine-Hydroxy)-Dilactone Disulfide (4): R^1-COOH = (thymin-1-yl)acetic acid, R^2-COOH = [N^6-(*Z*)-adenin-9-yl]acetic acid. The resulting residue obtained, after step 5 of the general method of preparation starting with **21** (20 mg, 17.7 μmol), was dissolved in acetonitrile/water, filtered, and purified by HPLC (0–70% eluent B in 30 min, t_R = 20 min) to afford the white solid **4** (4.12 mg, 21%). ^{1}H NMR (600 MHz, CD_3CN, 25 °C): δ = 0.83 (d, $^3J_{H,H}$ = 7 Hz, 3 H, Val-CH_3), 0.84 (d, $^3J_{H,H}$ = 7 Hz, 3 H, Val-CH_3), 0.95 (d, $^3J_{H,H}$ = 7 Hz, 3 H, Val-CH_3), 0.97 (d, $^3J_{H,H}$ = 7 Hz, 3 H, Val-CH_3), 1.22 (d, $^3J_{H,H}$ = 7 Hz, 3 H, Ala-CH_3), 1.38 (d, $^3J_{H,H}$ = 7 Hz, 3 H, Ala-CH_3), 1.89 (d, $^3J_{H,H}$ = 1 Hz, 3 H, Thy-CH_3), 1.98–2.02 (m, 1 H, Val-Hβ), 2.05–2.08 (m, 1 H, Val-Hβ), 2.23–2.27 (m, 2 H, Cys-Hβ), 2.77 (s, 3 H, Val-NCH_3/Cys-NCH_3), 2.79 (s, 3 H, Val-NCH_3/Cys-NCH_3), 2.87 (s, 3 H, Val-NCH_3/Cys-NCH_3), 3.09 (s, 3 H, Val-NCH_3/Cys-NCH_3), 3.47–3.53 (m, 2 H, Cys-Hβ), 4.11 (dd, $^3J_{H,H}$ = 4, $^2J_{H,H}$ = 12 Hz, 1 H, Ser-Hβ), 4.20 (dd, $^3J_{H,H}$ = 4, $^3J_{H,H}$ = 12 Hz, 1 H, Ser-Hβ), 4.36 (s, 2 H, T-CH_2), 4.44–4.49 (m, 2 H, Ala-Hα), 4.56–4.61 (m, 1 H, Ser-Hα), 4.56–4.61 (m, 2 H, Ser-Hβ), 4.67 (d, $^3J_{H,H}$ = 11 Hz, 1 H, Val-Hα), 4.68–4.69 (m, 1 H, Ser-Hα), 4.74 (d, $^3J_{H,H}$ = 11 Hz, 1 H, Val-Hα), 4.86 (d, $^2J_{H,H}$ = 17 Hz, 1 H, A-CH_2), 5.12 (d, $^2J_{H,H}$ = 17 Hz, 1 H, A-CH_2), 6.03 (dd, $^3J_{H,H}$ = 4, $^3J_{H,H}$ = 11 Hz, 1 H, Cys-Hα), 6.15 (dd, $^3J_{H,H}$ = 4, $^3J_{H,H}$ = 11 Hz, 1 H, Cys-Hα), 6.62 (br. s, 2 H, A-NH_2), 7.04 (d, $^3J_{H,H}$ = 7 Hz, 1 H, Ala-NH), 7.06 (d, $^3J_{H,H}$ = 7 Hz, 1 H, Ser-NH), 7.12–7.13 (br. s, 1 H, Ser-NH), 7.18 (d, $^3J_{H,H}$ = 6 Hz, 1 H, Ala-NH), 7.36 (d, $^4J_{H,H}$ = 1 Hz, 1 H, Thy-H6), 8.19 (s, 1 H, A-H2/H8), 8.33 (s, 1 H, A-H2/H8), 11.96 (br. s, 1 H, Thy-NH) ppm. ^{13}C NMR (125 MHz, CD_3CN, 25 °C): δ = 12.3 (Thy-CH_3), 16.4 (Ala-CH_3), 16.9 (Ala-CH_3), 20.0 (Val-CH_3), 20.1 (Val-CH_3), 20.3 (Val-CH_3), 28.1 (Val-CHβ), 28.3 (Val-CHβ), 30.9 (Val-NCH_3/Cys-NCH_3), 31.1 (Val-NCH_3/Cys-NCH_3), 31.5 (Val-NCH_3/Cys-NCH_3), 31.6 (Val-NCH_3/Cys-NCH_3), 47.7 (Ala-CHα), 47.8 (Ala-CHα), 48.3 (A-CH_2), 52.5 (Ser-CHα), 52.6 (Ser-CHα), 52.9 (Ser-CH_2β), 53.4, (T-CH_2), 57.0 (Cys-CHα), 57.2 (Cys-CHα), 64.3 (Val-CHα), 64.4 (Val-CHα), 65.0 (Ser-CH_2β), 66.2 (Ser-CH_2β), 111.9 (A-C3), 128.0 (T-C5), 129.1 (T-C6), 130.4 (A-C8), 131.2 (A-C4), 142.8 (A-C6), 150.9 (T-C2), 153.7 (A-C2), 156.0 (T-C4), 165.9, 167.3, 168.2, 168.6, 168.6, 168.8, 170.9, 170.9, 171.2, 172.1, 172.4 (Ser-CO, Val-CO, Cys-CO, Ala-CO, acetyl-CO, COOH) ppm. HRMS (ESI): calcd. for $C_{44}H_{61}N_{15}O_{14}S_2$ [M + H]$^+$ 1116.4352; found 1116.4351.

[*N*-(Adenin-9-ylacetyl)-D-Ser-L-Ala-*N*-Me-L-Cys-*N*-Me-L-Val]-[*N*-(guanin-9-yl acetyl)-D-Ser-L-Ala-*N*-Me-L-Cys-*N*-Me-L-Val] (Serine-Hydroxy)-Dilactone Disulfide (5): R^1-COOH = (N^6-(*Z*)-adenin-9-yl)acetic acid, R^2-COOH = [2-amino-6-(benzyloxy)purin-9-yl]acetic acid. The resulting residue obtained, after step 5 of the general procedure, starting with **21** (20 mg, 17.7 μmol), was dissolved in acetonitrile/water, filtered, and purified by HPLC (10–70% eluent B in 30 min, t_R = 13.2 min) to afford **5** as a white solid (3.86 mg, 19%). ^{1}H NMR (600 MHz, [D$_6$]DMSO, 25 °C): δ = 0.85 (d, $^3J_{H,H}$ = 6 Hz, 3 H, Val-CH_3), 0.86 (d, $^3J_{H,H}$ = 6 Hz, 3 H, Val-CH_3), 0.95 (d, $^3J_{H,H}$ = 3 Hz, 3 H, Val-CH_3), 0.96 (d, $^3J_{H,H}$ = 3 Hz, 3 H, Val-CH_3), 1.34 (d, $^3J_{H,H}$ = 7 Hz, 6 H, Ala-CH_3), 2.28–2.30 (m, 2 H, Val-Hβ), 2.79 (s, 6 H, Val-NCH_3/Cys-NCH_3), 3.12 (s, 3 H, Val-NCH_3/Cys-NCH_3), 3.16 (s, 3 H, Val-NCH_3/Cys-NCH_3), 3.50 (d, $^3J_{H,H}$ = 12 Hz, 2 H, Cys-Hβ), 3.52 (d, $^3J_{H,H}$ = 12 Hz, 2 H, Cys-Hβ), 4.11 (d, $^3J_{H,H}$ = 4, $^2J_{H,H}$ = 11 Hz, 2 H, Ser-Hβ), 4.41–4.45 (m, 2 H, Ala-Hα), 4.41–4.45 (m, 2 H, Ser-Hβ), 4.63–4.65 (m, 2 H, Ser-Hα), 4.71 (d, $^3J_{H,H}$ = 10 Hz, 1 H, Val-Hα), 4.73 (d, $^3J_{H,H}$ = 10 Hz, 1 H, Val-Hα), 4.83 (d, $^2J_{H,H}$ = 17 Hz, 1 H, acetyl-CH_2), 4.91 (d, $^2J_{H,H}$ = 17 Hz, 1 H, acetyl-CH_2), 5.08 (d, $^2J_{H,H}$ = 17 Hz, 1 H, acetyl-CH_2), 5.19 (d, $^2J_{H,H}$ = 17 Hz, 1 H, acetyl-CH_2), 6.10 (dd, $^3J_{H,H}$ = 3, $^2J_{H,H}$ = 11 Hz, 1 H, Cys-Hα), 6.14 (dd, $^3J_{H,H}$ = 3, $^2J_{H,H}$ = 11 Hz, 1 H, Cys-Hα), 6.46 (br. s, 2 H, G-NH_2), 7.78 (d, $^3J_{H,H}$ = 6 Hz, 1 H, Ala-NH), 7.79 (d, $^3J_{H,H}$ = 6 Hz, 1 H, Ala-NH), 7.86 (br. s, 1 H, G-CH8), 8.13–8.18 (br., 2 H, A-NH_2), 8.27 (s, 1 H, A-H2/H8), 8.28 (s, 1 H, A-H2/H8), 8.26–8.30 (1 H, Ser-NH), 8.56 (d, $^3J_{H,H}$ = 9 Hz, 1 H, Ser-NH), 10.73 (br. s, 1 H, G-NH) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): δ = 15.8 (Ala-CH_3), 19.4 (Val-CH_3), 19.5 (Val-CH_3), 19.6 (Val-CH_3), 23.2, 26.6 (Val-CHβ), 30.1 (Val-NCH_3/Cys-NCH_3), 30.8 (Val-NCH_3/Cys-NCH_3), 30.9 (Val-NCH_3/Cys-NCH_3), 42.6 (Cys-CH_2β), 45.2 (acetyl-CH_2), 45.5 (acetyl-CH_2), 46.6 (Ala-CHα), 51.4 (Ser-CHα), 51.6 (Ser-CHα), 56.2 (Cys-CHα), 62.9 (Val-CHα), 63.1 (Val-CHα), 64.6 (Ser-CH_2β), 64.7 (Ser-CH_2β), 117.9 (G-C5), 121.7 (A-C5), 138.0 (G-C8), 143.2 (A-C2/C8), 149.2 (A-C2/C8), 151.2 (A-C6, G-C6), 153.8 (G-C2), 156.2 (A-C4), 158.2 (G-C4), 166.8, 166.9, 167.8, 167.9, 169.5, 169.8, 169.8, 171.0 (Ser-CO, Val-CO, Cys-CO, Ala-CO, acetyl-CO) ppm. HRMS (ESI): calcd. for $C_{44}H_{61}N_{18}O_{13}S_2$ [M + H]$^+$ 1141.4416; found 1141.4414.

Supporting Information (see also the footnote on the first page of this article): Temperature-dependent UV data for the interaction of triostin A and respective analogs with DNA **23**. Experimental and analytical details for the synthesis of **10**–**17**, **6**, and **7**. ^{1}H NMR spectra for **18**–**21** and **1**–**5**.

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