Supporting Information

Triostin A derived hybrid for simultaneous DNA binding and metal coordination

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Content:

Peptide Synthesis

Z-D-Dap-L-Ala-L-Cys(Acm)-L-propargyl-Gly-β-D-Dap(Boc)-L-Ala-L-Cys(Acm)-L-Val-OH (7)

Solid phase peptide synthesis was performed on a 2-chlorotrityl resin preloaded with valine (339 mg, 312 μmol). (Benzotriazol-1-yl)-tetramethyluronium hexafluorophosphate (HBTU) and HBTU (5 eq) dissolved in NMP (5 mL) were used for coupling of Fmoc-amino acids (5 eq) (Fmoc-L-Ala-OH, Boc-D-Dap(Fmoc)-OH and Z-D-Dap(Fmoc)-OH). Diisopropylethylamine (DIEA) (5 eq) was added and the solution added to the deprotected and NMP-swollen peptidyl resin and shaken for 1 h. After coupling, the resin was washed with NMP (5 x 10 mL). DIC coupling: Fmoc-L-Cys(Acm)-OH (5 eq) was dissolved in DCM/NMP = 4/1 (5 mL). DIC (10 eq) was added and the resulting solution immediately added to the deprotected and DCM/NMP = 4/1 swollen peptidyl resin and shaken for 1 h. After coupling, the resin was washed with NMP (5 x 10 mL) and DCM (5 x 10 mL). HATU coupling: Fmoc-L-propargyl-Gly-OH (2 eq) was dissolved in NMP (2 mL) with HATU (1.9 eq) and HOAt (2.0 eq). DIEA (10 eq) was added and the solution added to the deprotected and NMP-swollen peptidyl resin and shaken for 1 h. After coupling, the resin was washed with NMP (5 x 10 mL). Deprotection: A solution of DMF/piperidine = 4/1 (5 mL) was added to the Fmoc-protected peptidyl resin and shaken for 15 min. After deprotection the resin was washed with NMP (5 x 10 mL). Cleavage: After Fmoc-deprotection the peptidyl resin was washed with MeOH and DCM (5 x 10 mL each) and dried over KOH for 12 h. The unprotected peptidyl resin was shaken with DCM/hexafluoroisopropanole = 4/1 (7 mL) for 45 min and then washed with the same solution (2 x 5 mL). The combined filtrates were concentrated to dryness and the resulting residue was precipitated with methyl-*tert*-butyl-ether (3 x 10 mL). The colorless precipitate was dissolved in water (50 mL) and lyophilized to give the linear octapeptide **7** (476 mg, 87%) as a white solid with a purity of >98% as indicated by HPLC (preparative, 10-70% B in 30 min, R_t = 25.3 min). ^{1}H-NMR (600 MHz, [D$_6$]DMSO): δ = 0.88 (d, $^3J_{H,H}$ = 6.7 Hz, 3 H, Val-CH_3), 0.98 (d, $^3J_{H,H}$ = 6.7 Hz, 3 H, Val-CH_3), 1.20 (d, $^3J_{H,H}$ = 7.0 Hz, 3 H, Ala-CH_3), 1.26 (d, $^3J_{H,H}$ = 7.0 Hz, 3 H, Ala-CH_3), 1.37 (s, 9 H, Boc-CH_3), 1.86 (s, 3 H, Acm-CH_3), 1.87 (s, 3 H, Acm-CH_3), 2.06 (m, 1 H, Val-CHβ), 2.41-2.47 (m, 1 H, propargyl-Gly-CH_2β), 2.55 (ddd, $^2J_{H,H}$ = 16.7 Hz, $^3J_{H,H}$ = 5.0 Hz, $^4J_{H,H}$ = 2.5 Hz, 1 H, propargyl-Gly-CH_2β), 2.64-2.70 (m, 2 H, Cys-

CH_2β), 2.75 (t, $^4J_{H,H}$ = 2.5 Hz, 1 H, propargyl-Gly-CHδ), 2.90-2.96 (m, 2 H, Cys-CH_2β), 2.96-3.01 (m, 1 H, Dap-CH_2β), 3.16-3.27 (m, 2 H, Dap-CH_2β), 3.41-3.48 (m, 1 H, Dap-CH_2β), 4.04-4.09 (m, 1 H, Dap-CHα), 4.12-4.21 (m, 3 H, Acm-CH_2, Val-CHα), 4.27-4.40 (m, 6 H, Acm-CH_2, Ala-CHα, Dap-CHα, propargyl-Gly-CHα), 4.52-4.61 (m, 2 H, Cys-CHα), 5.07 (s, 2 H, Z-CH_2), 6.71 (d, $^3J_{H,H}$ = 7.7 Hz, 1 H, Dap-NHα), 7.30-7.40 (m, 5 H, Z-CH), 7.60 (d, $^3J_{H,H}$ = 8.0 Hz, 1 H, Ala-NH), 7.69 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, Val-NH), 7.88-7.93 (m, 2 H, Dap-NHβ), 7.95 (d, $^3J_{H,H}$ = 7.9 Hz, 1 H, Dap-NHα), 7.96-8.02 (m, 2 H, Dap-NHβ, propargyl-Gly-NH), 8.11 (d, $^3J_{H,H}$ = 7.7 Hz, 1 H, Cys-NH), 8.18 (d, $^3J_{H,H}$ = 7.1 Hz, 1 H, Ala-NH), 8.22 (d, $^3J_{H,H}$ = 7.9 Hz, 1 H, Cys-NH), 8.49 (t, $^3J_{H,H}$ = 6.3 Hz, 1 H, Acm-NH), 8.54 (t, $^3J_{H,H}$ = 6.3 Hz, 1 H, Acm-NH) ppm. ^{13}C-NMR (125 MHz, [D$_6$]DMSO): δ = 17.7 (Val-CH$_3$), 18.3, 18.4 (Ala-CH$_3$), 18.9 (Val-CH$_3$), 22.0 (propargyl-Gly-CH$_2$β), 22.4, 22.5 (Acm-CH$_3$), 28.1 (Boc-CH$_3$), 29.8 (Val-CHβ), 32.0, 23.2 (Cys-CH$_2$β), 39.0-40.0 (Acm-CH$_2$, DMSO), 40.2, 40.6 (Dap-CH$_2$β), 48.2, 48.3 (Ala-CHα), 51.5 (propargyl-Gly-CHα), 52.2 (Cys-CHα), 52.4 (Dap-CHα), 52.5 (Cys-CHα), 54.1 (Dap-CHα), 57.1 (Val-CHα), 66.0 (Z-CH$_2$), 72.9 (propargyl-Gly-CHδ), 78.5 (Boc-C), 80.2 (propargyl-Gly-Cγ), 127.8, 127.9, 128.3 (Z-CH), 136.5 (Z-C), 155.0 (Boc-CO), 155.9 (Z-CO), 168.3 (Dap-CO), 169.5, 169.9, 170.0, 170.1, 170.2 (Acm-CO, Cys-CO, Dap-CO, propargyl-Gly-CO), 171.9, 172.2 (Ala-CO), 172.6 (Val-CO) ppm. MS (ESI, MeOH): *m/z* (%) = 1109 (100) [M+H]$^+$. HRMS (ESI): calcd. for $C_{47}H_{73}N_{12}O_{15}S_2$ ([M+H]$^+$): 1109.47543, found: 1109.47456.

[Z-D-Dap-L-Ala-L-Cys-L-propargyl-Gly-β-D-Dap(Boc)-L-Ala-L-Cys-L-Val-OH]-disulfide

A solution of **7** in AcOH/H$_2$O = 4/1 (50 mL) was saturated with argon. Iodine (91 mg, 0.36 mmol) was added and the solution stirred for 1 h at rt. The reaction was quenched with water (50 mL) and extracted with CCl_4 (6 x 15 mL). The aqueous layer was evaporated and dried in vacuo to give a brown solid which was purified by HPLC (preparative, 30-80% B in 30 min, R_t = 19.2 min) to give a colorless solid (9.0 mg, 26%). ^{1}H-NMR (600 MHz, [D$_6$]DMSO): δ = 0.88 (d, $^3J_{H,H}$ = 6.4 Hz, 3 H, Val-CH_3), 0.89 (d, $^3J_{H,H}$ = 6.4 Hz, 3 H, Val-CH_3), 1.18-1.29 (m, 6 H, Ala-CH_3), 1.37 (s, 3 H, Boc-CH_3), 1.40 (s, 6 H, Boc-CH_3), 2.15-2.12 (m, 1 H, Val-CHβ), 2.56 (ddd, $^2J_{H,H}$ = 17.0 Hz, $^3J_{H,H}$ = 7.5 Hz, $^4J_{H,H}$ = 2.3 Hz, 1 H, propargyl-Gly-CH_2β), 2.64 (ddd, $^2J_{H,H}$ = 17.0 Hz, $^3J_{H,H}$ = 6.0 Hz, $^4J_{H,H}$ = 2.3 Hz, 1 H, propargyl-Gly-CH_2β), 2.74 (t, $^4J_{H,H}$ = 2.3 Hz, 1 H, propargyl-Gly-CHδ), 2.89 (dd, $^2J_{H,H}$ = 13.9 Hz,

$^{3}J_{H,H}$ = 10.5 Hz, 1 H, Cys-CH_2β), 2.94-3.02 (m, 2 H, Cys-CH_2β, Dap-CH_2β), 3.09 (dd, $^{2}J_{H,H}$ = 13.9 Hz, $^{3}J_{H,H}$ = 2.4 Hz, 1 H, Cys-CH_2β), 3.15-3.20 (m, 1 H, Dap-CH_2β), 3.22 (dd, $^{2}J_{H,H}$ = 13.6 Hz, $^{3}J_{H,H}$ = 3.9 Hz, 1 H, Cys-CH_2β), 3.27-3.33 (m, 1 H, Dap-CH_2β), 3.48-3.55 (m, 1 H, Dap-CH_2β), 4.00-4.08 (m, 1 H, Dap-C*H*α), 4.18 (dd, $^{3}J_{H,H}$ = 6.4, 8.6 Hz, 1 H, Val-C*H*α), 4.25-4.37 (m, 4 H, Ala-C*H*α, Dap-C*H*α, propargyl-Gly-C*H*α), 4.68-4.74 (m, 1 H, Cys-C*H*α), 4.80-4.87 (m, 1 H, Cys-C*H*α), 5.07 (d, $^{2}J_{H,H}$ = 14.2 Hz, 1 H, Z-CH_2), 5.09 (d, $^{2}J_{H,H}$ = 14.2 Hz, 1 H, Z-CH_2), 6.85 (d, $^{3}J_{H,H}$ = 7.5 Hz, 1 H, Dap-N*H*α), 7.26-7.41 (m, 5 H, Z-C*H*), 7.60 (d, $^{3}J_{H,H}$ = 8.0 Hz, 1 H, Ala-N*H*), 7.63-7.69 (m, 2 H, Ala-N*H*, Dap-N*H*β), 7.90 (d, $^{3}J_{H,H}$ = 8.6 Hz, 1 H, Val-N*H*), 7.91-7.97 (m, 2 H, Dap-NH_2β), 8.17-8.26 (m, 3 H, Cys-N*H*, Dap-N*H*α, propargyl-Gly-N*H*), 8.47 (d, $^{3}J_{H,H}$ = 8.6 Hz, 1 H, Cys-N*H*), 12.70 (s_{br}, 1 H, COO*H*) ppm. ^{13}C-NMR (125 MHz, [D_6]DMSO): δ = 17.7 (Val-*C*H_3), 18.1 (Ala-*C*H_3), 18.9 (Val-*C*H_3), 21.1 (propargyl-Gly-*C*H_2β), 28.0 (Boc-*C*H_3), 30.0 (Val-*C*Hβ), 40.1, 40.6 (Dap-*C*H_2β), 42.1, 42.3 (Cys-*C*H_2β), 48.2, 48.6 (Ala-*C*Hα), 52.2, 52.2 (Cys-*C*Hα), 52.3 (propargyl-Gly-*C*Hα), 52.4 (Dap-*C*Hα), 57.1 (Val-*C*Hα), 66.1 (Z-*C*H_2), 72.7 (propargyl-Gly-*C*γ), 78.8 (Boc-*C*), 80.5 (propargyl-Gly-*C*Hδ), 172.8, 172.9, 128.3 (Z-*C*H), 136.5 (Z-*C*), 154.7 (Boc-*C*O), 155.9 (Z-*C*O), 168.4, 169.5, 169.7, 169.9 (Cys-*C*O, Dap-*C*O, propargyl-Gly-*C*O), 172.1, 172.5 (Ala-*C*O), 172.6 (Val-*C*O) ppm. MS (ESI, MeOH): *m/z* (%) = 965 (100) $[M+H]^+$. HRMS (ESI): calcd. for $C_{41}H_{61}N_{10}O_{13}S_2$ ($[M+H]^+$): 965.38555, found: 965.38527.

Cyclo[β-D-Dap(Z)-L-Ala-L-Cys-L-propargyl-Gly-β-D-Dap(Boc)-L-Ala-L-Cys-L-Val-OH]-disulfide (8)

Peptide [Z-D-Dap-L-Ala-L-Cys-L-propargyl-Gly-β-D-Dap(Boc)-L-Ala-L-Cys-L-Val-OH]-disulfide (7.5 mg, 7.8 µmol) was dissolved in dry DCM/DMF = 9/1 (25 mL) and cooled to 0 °C. HOAt (1.1 mg, 7.8 µmol), NMM (2.6 µL, 23.3 µmol) and DIC (12 µL, 78 µmol) were added and the solution stirred for 1 h at 0 °C. The mixture was allowed to warm to rt and was stirred for further 48 h. The resulting solution was evaporated to dryness and the residue purified by HPLC (preparative, 50-100% B in 30 min, R_t = 16.5 min) to give a white solid (2.2 mg, 30%). ^{1}H-NMR (600 MHz, [D_6]DMSO): δ = 0.79 (d, $^{3}J_{H,H}$ = 6.8 Hz, 3 H, Val-CH_3), 0.88 (d, $^{3}J_{H,H}$ = 6.6 Hz, 3 H, Val-CH_3), 1.19 (d, $^{3}J_{H,H}$ = 7.0 Hz, 3 H, Ala-CH_3), 1.22-1.29 (m, 3 H, Ala-CH_3), 1.38 (s, 6 H, Boc-CH_3), 1.40 (s, 3 H, Boc-CH_3), 2.32-2.42 (m,

1 H, Val-C*H*β), 2.56-2.68 (m, 2 H, propargyl-Gly-CH_2β, -C*H*δ), 2.69-2.76 (m, 1 H, propargyl-Gly-CH_2β), 2.79-2.88 (m, 1 H, Cys-CH_2β), 2.91-3.00 (m, 1 H, Cys-CH_2β), 3.03-3.11 (m, 1 H, Cys-CH_2β), 3.11-3.18 (m, 1 H, Cys-CH_2β), 3.18-3.24 (m, 1 H, Dap-CH_2β), 3.25-3.45 (m, Dap-CH_2β, H_2O), 3.75-3.90 (m, 2 H, Dap-CH_2β), 3.93-3.99 (m, 0.5 H, Dap-C*H*α), 3.99-4.08 (m, 1 H, Dap-C*H*α), 4.08-4.15 (m, 0.5 H, Dap-C*H*α), 4.25-4.36 (m, 2 H, Val-C*H*α, propargyl-Gly-C*H*α), 4.42-4.49 (m, 1 H, Ala-C*H*α), 4.49-4.57 (m, 1 H, Ala-C*H*α), 4.97-5.10 (m, 3 H, Cys-C*H*α, Z-CH_2), 5.21-5.28 (m, 1 H, Cys-C*H*α), 6.71 (d, $^3J_{H,H}$ = 6.4 Hz, 1 H, Dap-N*H*α), 6.83 (d, $^3J_{H,H}$ = 6.7 Hz, 0.3 H, Dap-N*H*α), 7.01-7.06 (m, 0.7 H, Dap-N*H*α), 7.24 (d, $^3J_{H,H}$ = 7.5 Hz, 1 H, Ala-N*H*), 7.27-7.38 (m, 5 H, Z-C*H*), 7.40 (d, $^3J_{H,H}$ = 7.5 Hz, 1 H, Ala-N*H*), 7.59-7.67 (m, 1 H, Dap-N*H*β), 7.81-7.87 (m, 1 H, Dap-N*H*β), 7.90 (d, $^3J_{H,H}$ = 9.8 Hz, Val-N*H*), 7.94 (d, $^3J_{H,H}$ = 9.3 Hz, propargyl-Gly-N*H*), 8.77 (d, $^3J_{H,H}$ = 9.6 Hz, 1 H, Cys-N*H*), 8.83 (d, $^3J_{H,H}$ = 8.9 Hz, 1 H, Cys-N*H*) ppm. ^{13}C-NMR (125 MHz, [D$_6$]DMSO): δ = 16.7 (Val-*C*H$_3$), 17.9, 18.8 (Ala-*C*H$_3$), 19.1 (Val-*C*H$_3$), 22.0 (propargyl-Gly-*C*H$_2$β), 28.0 (Boc-*C*H$_3$), 29.7 (Val-*C*Hβ), 40.1, 40.4 (Dap-*C*H$_2$β), 41.2, 42.9 (Cys-*C*H$_2$β), 50.9 (propargyl-Gly-*C*Hα), 53.0, 53.3 (Cys-*C*Hα), 54.9, 57.2 (Dap-*C*Hα), 65.8 (Z-*C*H$_2$), 72.1 (propargyl-Gly-*C*γ), 78.7 (Boc-*C*), 80.4 (propargyl-Gly-*C*Hδ), 127.6, 127.6, 127.8, 128.3 (Z-*C*H), 136.7 (Z-*C*), 154.7 (Boc-*C*O), 155.7 (Z-*C*O), 168.4, 168.9 (Dap-*C*O), 169.7, 170.0 (Cys-*C*O), 170.7 (Val-*C*O), 172.2 (propargyl-Gly-*C*O), 173.1, 173.2 (Ala-*C*O) ppm. MS (ESI, MeOH): *m/z* (%) = 969 (100) [M+Na]$^+$, 945 (100) [M-H]$^-$. HRMS (ESI): calcd. for $C_{41}H_{59}N_{10}O_{12}S_2$ ([M+H]$^+$): 947.37499, found: 947.37495.

Cyclo[β-D-Dap(Z)-L-Ala-L-Cys-L-propargyl-Gly-β-D-Dap(thymine-1-yl-acetyl)-L-Ala-L-Cys-L-Val]-disulfide (11)

A solution of peptide **8** (6.6 mg, 7.0 μmol) in TFA/DCM = 1/1 (8 mL) was stirred for 15 min at rt. The mixture was evaporated to dryness and the residue was dissolved in dry DCM/DMF = 9/1 (10 mL). (thymine-1-yl)-acetic acid (**9**) (5.1 mg, 27.9 mmol) was added and the solution cooled to 0 °C. HOAt (3.8 mg, 28 μmol), NMM (9.2 μL, 84 μmol) and DIC (44 μL, 0.28 mmol) were added and the solution stirred for 1 h at 0 °C, the solution was allowed to warm to rt and stirred for further 48 h. The mixture was evaporated to dryness in vacuo and the residue purified by HPLC (preparative, 40-80% B in 30 min, R_t = 14.5 min) to yield a white solid (3.3 mg, 47%). ^{1}H-NMR (600 MHz, [D$_6$]DMSO): δ = 0.81

(d, $^{3}J_{H,H}$ = 6.5 Hz, 3 H, Val-CH_3), 0.83-0.92 (m, 3 H, Val-CH_3), 1.15-1.30 (m, 6 H, Ala-CH_3), 1.77 (s, 3 H, Thymin-CH_3), 2.31-2.40 (m, 1 H, Val-CHβ), 2.55-2.61 (m, 1 H, propargyl-Gly-CHδ), 2.62-2.70 (m, 2 H, propargyl-Gly-CH_2β), 2.81-2.95 (m, 2 H, Cys-CH_2β), 3.01-3.11 (m, 1 H, Cys-CH_2β), 3.13-3.25 (m, 3 H, Cys-CH_2β, Dap-CH_2β), 3.68-3.73 (m, 1 H, Dap-CH_2β), 3.81-3.89 (m, 1 H, Dap-CH_2β), 4.01-4.08 (m, 1 H, Dap-CHα), 4.19-4.25 (m, 1 H, Dap-CHα), 4.24-4.28 (m, 1 H, Val-CHα), 4.27 (d, $^{2}J_{H,H}$ = 16.6 Hz, 1 H, acetyl-CH_2), 4.38 (d, $^{2}J_{H,H}$ = 16.6 Hz, 1 H, acetyl-CH_2), 4.43-4.52 (m, 2 H, Ala-CHα), 4.52-4.58 (m, 1 H, propargyl-Gly-CHα), 5.02-5.12 (m, 3 H, Cys-CHα, Z-CH_2), 5.23-5.29 (m, 1 H, Cys-CHα), 7.03-7.11 (m, 1 H, Dap-NHα), 7.25 (d, $^{3}J_{H,H}$ = 6.4 Hz, 1 H, Ala-NH), 7.29-7.41 (m, 6 H, Ala-NH, Z-CH), 7.44 (s, 1 H, thymine-CH), 7.58-7.67 (m, 1 H, Dap-NHβ), 7.87-7.93 (m, 1 H, Dap-NHβ), 7.95 (d, $^{3}J_{H,H}$ = 7.8 Hz, 1 H, Val-NH), 8.08 (d, $^{3}J_{H,H}$ = 8.08 Hz, 1 H, propargyl-Gly-NH), 8.14 (d, $^{3}J_{H,H}$ = 6.2 Hz, 1 H, Dap-NHα), 8.80 (d, $^{3}J_{H,H}$ = 7.2 Hz, 1 H, Cys-NH), 8.85 (d, $^{3}J_{H,H}$ = 7.6 Hz, 1 H, Cys-NH), 11.28 (s, 1 H, thymine-NH) ppm. ^{13}C-NMR (125 MHz, [D$_6$]DMSO): δ = 11.8 (thymine-CH$_3$), 16.8 (Val-CH$_3$), 18.8, 18.9 (Ala-CH$_3$), 19.2 (Val-CH$_3$), 21.9 (propargyl-Gly-CH$_2$β), 29.8 (Val-CHβ), 40.2, 40.5 (Dap-CH$_2$β), 41.9, 43.1 (Cys-CH$_2$β), 47.3, 47.4 (Ala-CHα), 50.1 (acetyl-CH$_2$), 50.9 (propargyl-Gly-CHα), 53.2, 53.4 (Cys-CHα), 55.8, 57.2 (Dap-CHα), 57.3 (Val-CHα), 65.8 (Z-CH$_2$), 72.3 (propargyl-Gly-Cγ), 80.4 (propargyl-Gly-CHδ), 108.4 (thymine-C5), 127.6, 127.8, 128.3 (Z-CH), 136.7 (Z-C), 141.9 (thymine-CH6), 151.1 (thymine-C2), 155.4 (Z-CO), 164.3 (thymine-C4), 167.0 (acetyl-CO), 167.9, 168.4 (Dap-CO), 169.9, 170.0 (Cys-CO), 171.3, 172.2 (Val-CO, propargyl-Gly-CO), 173.2, 173.3 (Ala-CO) ppm. MS (ESI, MeOH): *m/z* (%) = 1035 (100) [M+Na]$^+$, 1011 (100) [M-H]$^-$. HRMS (ESI): calcd. for $C_{43}H_{57}N_{12}O_{13}S_2$ ([M+H]$^+$): 1013.36040, found: 1013.36065.

Cyclo[β-D-Dap(*N*^{6}-Z-adenine-9-yl-acetyl)-L-Ala-L-Cys-L-propargyl-Gly-β-D-Dap(thymine-1-yl-acetyl)-L-Ala-L-Cys-L-Val]-disulfide (5)

Peptide **11** was dissolved in TFA/thioanisole = 10/1 (5 mL) and stirred at rt for 48 h. The mixture was evaporated to dryness and the residue dissolved in dry DMF (50 µL). This solution was cooled to 0 °C and (*N*^{6}-Z-adenine-9-yl)-acetic acid (**10**) (8.5 mg, 26 µmol), PyBroP (12 mg, 26 µmol) and DIEA (13 µL, 78 µmol) were added. The resulting mixture was stirred at 0 °C for 1 h and then allowed to come

to rt followed by further stirring at rt for 48 h. The solution was evaporated to dryness and the residue purified by HPLC (preparative, 10-70% B in 30 min, R_t = 24.4 min) to yield a colorless solid (2.3 mg, 37%). ^{1}H-NMR (600 MHz, [D$_6$]DMSO): δ = 0.78 (d, $^3J_{H,H}$ = 6.9 Hz, 3 H, Val-CH_3), 0.80 (d, $^3J_{H,H}$ = 6.9 Hz, 3 H, Val-CH_3), 1.21 (d, $^3J_{H,H}$ = 7.4 Hz, 3 H, Ala-CH_3), 1.22 (d, $^3J_{H,H}$ = 7.3 Hz, 3 H, Ala-CH_3), 1.76-1.78 (m, 3 H, thymine-CH_3), 2.11-2.19 (m, 1 H, Val-CHβ), 2.60-2.68 (m, 1 H, propargyl-Gly-CH_2β), 2.68-2.71 (m, 1 H, propargyl-Gly-CHδ), 2.71-2.78 (m, 1 H, propargyl-Gly-CH_2β), 2.80-2.88 (m, 1 H, Cys-CH_2β), 2.88-2.99 (m, 1 H, Cys-CH_2β), 3.03-3.07 (m, 1 H, Cys-CH_2β), 3.14-3.20 (m, 1 H, Cys-CH_2β), 3.20-3.29 (m, 2 H, Dap-CH_2β), 3.68-3.78 (m, 2 H, Dap-CH_2β), 4.07-4.10 (m, 1 H, Dap-CHα), 4.18-4.22 (m, 1 H, Val-CHα), 4.22-4.25 (m, 1 H, Dap-CHα), 4.27 (d, $^2J_{H,H}$ = 16.4 Hz, 1 H, acetyl-CH_2), 4.38 (d, $^2J_{H,H}$ = 16.4 Hz, 1 H, acetyl-CH_2), 4.45-4.50 (m, 1 H, Ala-CHα), 4.50-4.54 (m, 1 H, Ala-CHα), 4.56-4.61 (propargyl-Gly-CHα), 4.98 (d, $^2J_{H,H}$ = 16.6 Hz, 1 H, acetyl-CH_2), 5.06 (d, $^2J_{H,H}$ = 16.6 Hz, 1 H, acetyl-CH_2), 5.14-5.20 (Cys-CHα), 5.22 (s, 2 H, Z-CH_2), 5.26-5.35 (m, 1 H, Cys-CHα), 7.32-7.39 (m, 2 H, Ala-NH), 7.39-7.46 (m, 5 H, Z-CH), 7.47 (s, 1 H, thymine-CH), 7.59-7.65 (m, 1 H, Dap-NHβ), 7.87-7.91 (m, 1 H, Dap-NHβ), 7.98 (d, $^3J_{H,H}$ = 9.7 Hz, 1 H, Val-NH), 8.05-8.10 (m, 1 H, propargyl-Gly-NH), 8.13 (d, $^3J_{H,H}$ = 6.8 Hz, 1 H, Dap-NHα), 8.18 (d, $^3J_{H,H}$ = 7.0 Hz, 1 H, Dap-NHα), 8.42 (s, 1 H, adenine-CH), 8.53 (s, 1 H, adenine-CH), 8.75-8.80 (m, 1 H, Cys-NH), 8.81-8.86 (m, 1 H, Cys-NH), 10.65 (s_{br}, 1 H, adenine-NH), 11.29 (s, 1 H, thymine-NH) ppm. ^{13}C-NMR (125 MHz, [D$_6$]DMSO): δ = 11.8 (thymine-CH$_3$), 17.2 (Val-CH$_3$), 18.6, 19.0 (Ala-CH$_3$), 19.5 (Val-CH$_3$), 22.0 (propargyl-Gly-CH$_2$β), 29.6 (Val-CHβ), 30.2, 30.3 (Dap-CH$_2$β), 42.0, 44.0 (Cys-CH$_2$β), 46.0 (acetyl-CH$_2$), 47.2, 47.5 (Ala-CHα), 50.0 (acetyl-CH$_2$), 50.8 (propargyl-Gly-CHα), 53.0, 53.3 (Cys-CHα), 55.8, 55.9 (Dap-CHα), 56.8 (Val-CHα), 66.1 (Z-CH$_2$), 72.4 (propargyl-Gly-Cγ), 80.6 (propargyl-Gly-CHδ), 108.3 (thymine-C5), 122.9 (adenine-C5), 127.7, 127.9, 128.3 (Z-CH), 136.3 (Z-C), 141.9 (thymine-CH6), 144.8 (adenine-CH8), 149.4 (Z-CO), 151.1 (thymine-C2), 151.4, 151.9, 152.1 (adenine-CH2, -C4, -C6), 164.3 (thymine-C4), 166.5, 167.0, 167.8, 169.9, 170.0, 170.1, 171.4, 172.3 (acetyl-CO, Cys-CO, Dap-CO, propargyl-Gly-CO, Val-CO), 173.1, 173.3 (Ala-CO) ppm. MS (ESI, MeOH): *m/z* (%) = 1188 (20) [M+H]$^+$. HRMS (ESI): calcd. for $C_{50}H_{62}N_{17}O_{14}S_2$ ([M+H]$^+$): 1188.4098, found: 1188.4066.

Temperature dependent UV spectra

Melting curves were recorded with a JASCO V550 UV/VIS-spectrophotometer. The temperature was regulated by a programmable JASCO ETC-50S/ETC-505T-peltier element, whereas the temperature was measured directly next to the cuvette. A blackened quartz cuvette (d = 1.0 cm) and detection at wavelengths 280, 260 and 255 nm were used. Concentrations were calculated from UV absorption at 20 °C. The following temperature program was employed for all measurements: 20 °C → 80 °C (10 min) → 80 °C (3 min) → -2 °C (26 min) → -2 °C (60 min) → 85 °C (120 min) → -2 °C (120 min) → -2 °C (60 min) → 85 °C (120 min) → -2 °C (120 min) → 28 °C (5 min). The hyperchromicity (A_{rel} / %) is defined as:

$$A_{rel}(T) = \frac{(A(T) - A_0) \cdot 100}{A_0}$$

Whereas $A(T)$ is the absorption at temperature T and A_0 the minimal absorption of a decoupling experiment. All UV measurements were done with 2 μM DNA solutions in (4-(2-hydroxyethyl)-1-peperazinethansulfonic acid (HEPES) buffer (2 mM HEPES, 10 mM NaCl, pH = 7.0).