Supporting Information

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SI Text

Synthesis of Fmoc-Protected Hot=Tap Dipeptide Mimic. Compound **1**: Synthesis of **1** is described in ref. 1. Analytical data are according to the literature. Mp.: 135 °C.

^{1}H NMR (300 MHz, $DMSO_{d6}$): δ(ppm) = 5.31 (t, J = 5.0 Hz, 1 H), 4.77 (s, 1 H), 4.60 (t, J = 2.1 Hz, 1 H), 3.64–3.60 (m, 2 H), 1.35 (s, 3 H), 1.31 ppm (s, 3 H). **^{13}C NMR** (75 MHz, $DMSO_{d6}$): δ(ppm) = 174.3, 111.6, 82.3, 78.1, 75.0, 60.4, 26.5, 25.1.

HRMS (ESI): $C_8H_{12}O_5$ (M^+Na^+), calc.: 211.0577, found: 211.0578.

Compound **2**: IBX (23.0 g, 82 mmol) was added to a solution of **1** (11.9 g, 63.3 mmol) in acetonitrile (100 mL). The reaction mixture was refluxed for 2 h and then cooled with an ice bath for 1 h. A precipitate formed, which was separated and washed twice with acetonitrile (50 mL). The combined organic phases were concentrated in vacuo, and the residue dissolved in MeOH/pyridine (170 mL, 7.5 : 1). L-cysteinemethylester·HCl (81.9 mmol) was added under exclusion of oxygen and the reaction mixture was stirred at room temperature for two weeks. Another portion of L-cysteinemethylester·HCl (2.8 g, 16.4 mmol) was added and the reaction mixture kept at 50 °C for another two days. The solvent was removed and the residue dissolved in ethyl acetate (100 mL) and washed first with water and then with a saturated solution of $NaHCO_3$. The organic phase was dried ($MgSO_4$) and the solvent removed in vacuo. The product crystallized as a yellow solid (15.6 g, 51.5 mmol, 82%). Mp.: 159.5 °C.

^{1}H NMR (300 MHz, $DMSO_{d6}$): δ(ppm) = 5.67 (dd, $^3J_{3\text{-H,2-HproR}}$ = 6.0 Hz, $^3J_{3\text{-H,2-HproS}}$ = 1.9 Hz, 1 H, 3-H), 4.86 (s, 1 H, 8a-H), 4.66 (d, $^3J_{6\text{-H,7-H}}$ = 8.7 Hz, 1 H, 6-H), 4.55 (dd, $^3J_{7\text{-H,6-H}}$ = 8.7 Hz, $^3J_{7\text{-H,8-H}}$ = 3.9 Hz, 1 H, 7-H), 4.17 (d, $^3J_{8\text{-H,7-H}}$ = 3.9 Hz, 1 H, 8-H), 3.76 (s, 3 H, CO_2CH_3), 3.26 (dd, $^2J_{2\text{-HproR,2-HproS}}$ = 11.1 Hz, $^3J_{2\text{-HproR,3-H}}$ = 6.1 Hz, 1 H, 2-H^{proR}), 3.20 (dd, $^2J_{2\text{-HproS,2-HproR}}$ = 11.0 Hz, $^3J_{2\text{-HproS,3-H}}$ = 1.9 Hz, 1 H, 2-H^{proS}), 2.51 (bs, 1 H, 8-OH), 1.57, 1.45 (s, 3 H, isopr.CH_3). **^{13}C NMR** (125 MHz, $DMSO_{d6}$): δ(ppm) = 170.0 (CO_2CH_3), 166.6 (5-C), 111.8 (isopr.quart.), 74.3 (7-C), 71.9 (6-C), 66.9 (8-C), 61.9, 61.8 (8-a/3-C), 53.1 (CO_2CH_3), 31.7 (2-C), 26.1, 24.3 (isopr.CH_3).

HRMS (ESI): $C_{12}H_{17}NO_6SNa^+$ (M^+Na^+), calc.: 326.0669, found: 326.0672.

CHN analysis: calc: C: 47.52% H: 5.65% N: 4.62% found: C: 46.51% H: 5.57% N: 4.36%.

IR(KBr):=3581 w, 3531 w, 2990 w, 2942 w, 1761 s, 1750 s, 1664 s, 1417 s, 1375 m, 1321 w, 1262 s, 1176 m, 1109 w, 1089 w, 1057 w.

$[\alpha]^{20}_D$ = −89.8 (c = 1.00 g/100 mL, MeOH).

Compound **3**: In the first step, compound **2** (5.0 g, 16.5 mmol) was dissolved in DCM (50 mL) and TFA (5 mL) was added drop wise under stirring. After three hours at room temperature the solvent was extracted with water (100 mL). The organic phase was dried with $MgSO_4$ and the solvent removed in vacuo to obtain 4.73 g (15.6 mmol, 95%) of a colourless powder (mp.: 157 °C). This compound was dissolved in dry DCM (50 mL) together with dry pyridine (2 mL) and then cooled with an ice bath. Trifluoromethane sulfonic acid anhydride (3.9 mL, 23.4 mmol) in 5 mL dry DCM was added slowly. The reaction mixture was kept at 0 °C for 15 min and then at room temperature for another 30 min. The reaction was stopped by the addition of 10 g ice. The organic phases were separated and dried with $MgSO_4$ and then under high vacuum. 6.24 g (14.3 mmol, 92%) **3** were obtained as a brownish powder. Mp.: 201.5 °C.

^{1}H NMR (300 MHz, $DMSO_{d6}$): δ(ppm) = 6.02 (d, $^3J_{6\text{-H,7-H}}$ = 3.4 Hz, 1 H, 6-H), 5.34–5.32 (m, 1 H, 3-H), 5.22 (d, $^3J_{8a\text{-H,8-H}}$ = 2.0 Hz, 1 H, 8a-H), 4.92 (dd, $^3J_{7\text{-H,8-H}}$ = 7.6 Hz, $^3J_{7\text{-H,6-H}}$ = 3.6 Hz, 1 H, 7-H), 4.60 (dd, $^3J_{8\text{-H,7-H}}$ = 7.6 Hz, $^3J_{8\text{-H8a3-H}}$ = 2.0 Hz, 1 H, 8-H), 3.70 (s, 3 H, CO_2CH_3), 3.21 (dd, $^2J_{2\text{-HproR,2-HproS}}$ = 11.3 Hz, $^3J_{2\text{-HproR,3-H}}$ = 6.6 Hz, 1 H, 2-H^{proR}), 3.20 (dd, $^3J_{2\text{-HproS,2-HproR}}$ = 11.3 Hz, $^3J_{2\text{-HproS,3-H}}$ = 1.3 Hz, 1 H, 2-H^{proS}), 1.32, 1.30 (s, 3 H, isopr. CH_3). **^{13}C NMR** (125 MHz, $DMSO_{d6}$): δ(ppm) = 169.2 (CO_2CH_3), 160.6 (5-C), 110.1 (isopr.quart.), 81.9 (6-C), 76.7 (8-C), 73.6 (7-C), 61.7 (3-C), 59.2 (8a-C), 52.7 (CO_2CH_3), 32.4 (2-C), 25.9, 24.0 (isopr.CH_3).

HRMS (ESI): $C_{13}H_{16}F_3NO_8S_2Na^+$ (M^+Na^+), calc.: 436.0342, found: 436.0339.

CHN analysis: calc.: C: 35.86% H: 3.70% N: 3.22% found: C: 30.83% H: 5.75% N: 1.24%.

IR(KBr):=3435 m, 2964 m, 1749 s, 1699 s, 1420 s, 1372 w, 1260 m, 1215 s, 1144 m, 1092 m, 1007 m, 806 m.

$[\alpha]^{20}_D$ = −77.7 (c = 1.10 g/100 mL, $CHCl_3$).

Compound **4**: Compound **3** (6.24 g, 14.3 mmol) was dissolved in DMF (20 mL) together with sodium azide (4.65 g, 71.5 mmol) and stirred at room temperature for 36 h. The solvent was removed in vacuo and the residue dissolved in water (100 mL) and extracted three times with ethyl acetate (50 mL). The combined organic phases were dried with $MgSO_4$ and the solvent removed in vacuo. The azide **4** crystallized within two days as colorless crystals 4.46 g (13.6 mmol, 95%). Mp.: 105.5 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ(ppm) = 5.39 (dd, $^3J_{3\text{-H,2-HproR}}$ = 5.7 Hz, $^3J_{3\text{-H,2-HproS}}$ = 1.6 Hz, 1 H, 3-H), 5.19 (d, $^3J_{8a\text{-H,8-H}}$ = 2.0 Hz, 1 H, 8^a-H), 4.49 (dd, $^3J_{8\text{-H,7-H}}$ = 7.2 Hz, $^3J_{8\text{-H,8a-H}}$ = 2.0 Hz, 1 H, 8-H), 4.40 (dd, $^3J_{7\text{-H,8-H}}$ = 7.2 Hz, $^3J_{7\text{-H,6-H}}$ = 2.3 Hz, 1 H, 7-H), 4.30 (d, $^3J_{6\text{-H,7-H}}$ = 2.3 Hz, 1 H, 6-H), 3.79 (s, 3 H, CO_2CH_3), 3.26 (dd, $^2J_{2\text{-HproS,2-HproR}}$ = 11.1 Hz, $^2J_{2\text{-HproS,3-H}}$ = 1.1 Hz, 1 H, 2-H^{proS}), 3.22 (dd, $^2J_{2\text{-HproS,2-HproR}}$ = 11.1 Hz, $^2J_{2\text{-HproR,3-H}}$ = 5.7 Hz, 1 H, 2-H^{proR}), 1.37 (s, 3 H, isopr.CH_3), 1.35 (s, 3 H, isopr.CH_3). **^{13}C NMR** (125 MHz, $CDCl_3$): δ(ppm) = 169.4 (CO_2CH_3), 164.0 (5-C), 110.3 (isopr.quart.), 76.1 (8-C), 75.0 (7-C), 62.2 (6-C), 61.8 (3-C), 59.9 (8a-C), 53.3 (CO_2CH_3), 31.8 (2-C), 26.4, 24.2 (isopr.CH_3).

HRMS (ESI): $C_{12}H_{16}N_4O_5SNa^+$ (M^+Na^+), calc.: 329.0914, found: 329.0911.

CHN analysis: calc.: C: 43.90% H: 4.91% N: 17.06% found: C: 43.19% H: 5.59% N: 16.02%.

IR (KBr): ν = 3437 s, 2996 w, 2964 w, 2924 w, 2116 s, 1729 s, 1682 s, 1422 m, 1379 m, 1317 w, 1286 m, 1262 m, 1243 m, 1213 m, 1061 m.

$[\alpha]^{20}_D$ = −256.9 (c = 1.10 g/100 mL, $CHCl_3$).

Fmoc-protected Hot=Tap dipeptide mimic (compound **5**): Compound **4** (16.1 g, 49.1 mmol) was dissolved in DCM/MeOH (80 mL, 1 : 1). 1.6 g Pd/C was added and the reaction mixture stirred for three days under hydrogen atmosphere (10 bar). The catalyst was separated by filtration and the solvent was removed in vacuo. The residue was dissolved in wet dioxane (200 mL) together with LiOH (50 mL, 1 mol/L). After 15 min the reaction mixture was neutralized and the solvent removed *in vacuo*. The dipeptide was dissolved in dioxane/water (200 mL, 4 : 1) and cooled with an ice bath followed by slow addition of Fmoc-OSu (21.5 g, 63.8 mmol) in dioxane (50 mL). The

pH was adjusted to 9 with DIPEA. After 3 h at room temperature thin layer chromatography (DCM/MeOH 5:1; Rf = 0.43) showed complete transformation of the educt and the solvent was removed in vacuo. The residue was taken up in ethyl acetate (200 mL) and water (50 mL) and the aqueous phase adjusted to pH 2 with HCl (c = 1 mol/L). The aqueous phase was extracted three times with ethyl acetate and the combined organic phases dried with $MgSO_4$. After removal of the solvent, compound **5** was obtained as a colourless powder (21.3 g, 42.0 mmol, 86%) and used in subsequent polypeptide syntheses. Mp: Decomposition >270 °C.

^{1}H NMR (300 MHz, $DMSO_{d6}$): δ(ppm) = 7.88 (d, J = 7.6 Hz, 2 H, Fmoc arom.), 7.72 (t, J = 7.1 Hz, 2 H, Fmoc arom.), 7.42 (t, J = 7.5 Hz, 2 H, Fmoc arom.), 7.34 (t, J = 7.6 Hz, 2 H, Fmoc arom.), 5.28 (s, 1 H, 8a-H), 4.84 (d, $^3J_{3\text{-H},2\text{-H}proR}$ = 6.2 Hz, 1 H, 3-H), 4.47 (dd, $^3J_{8\text{-H},7\text{-H}}$ = 7.2 Hz, $^3J_{8\text{-H}8a\text{-H}}$ = 2.2 Hz, 1 H, 8-H), 4.41 (dd, $^3J_{7\text{-H},8\text{-H}}$ = 7.2 Hz, $^3J_{7\text{-H},6\text{-H}}$ = 1.8 Hz, 1 H, 7-H), 4.38–4.30 (m, 2 H, Fmoc-CH_2), 4.24–4.23 (m, 1 H, 6-H), 4.12 (d, J = 7.6 Hz, 1 H, Fmoc-CH), 3.19 (d, $^3J_{2\text{-H}proS,2\text{-H}proR}$ = 10.2 Hz, 1 H, 2-H^{proS}), 2.99 (dd, $^3J_{2\text{-H}proS,2\text{-H}proR}$ = 10.3 Hz, $^3J_{2\text{-H}proR,3\text{-H}}$ = 6.2 Hz, 1 H, 2-H^{proR}), 1.27, 1.26 (s, 3 H, isopr. CH_3). **^{13}C NMR** (125 MHz, $DMSO_{d6}$): δ(ppm) = 172.8 (CO_2H), 164.3 (5-C), 156.0 (Fmoc-CO), 143.8, 140.7, 127.6, 127.1, 125.2, 120.1 (Fmoc arom.), 108.2 (isopr.quart.), 75.6 (7-C), 75.2 (8-C), 66.1 (Fmoc-CH_2), 63.7 (3-C), 59.0 (8a-C), 55.0 (Fmoc-CH), 46.6 (6-C), 31.7 (2-C), 26.3, 24.1 (isopr.CH_3).

HRMS (ESI): $C_{26}H_{26}N_2O_7SNa^+$ (M^+Na^+), calc.: 533.1353, found: 533.1371.

CHN analysis: calc.: C: 61.16% H: 5.13% N: 5.49% found: C: 53.45% H: 5.51% N: 4.72%.

IR (KBr): ν = 3414 br, 2986 m, 1723 s, 1654 s, 1450 m, 1406 m, 1265 m, 1213 m, 1163 s, 1120 s, 1066 m, 888 m, 872 m, 760 m, 741 m.

$[\alpha]^{20}_D$ = −61.3 (c = 0.98 g/100 mL, MeOH).

Polypeptide synthesis. Solid phase synthesis of the fibritin-foldon was done in a 0.1 mM scale on 2-chloro-2-trityl-polystyrene-resin. After preloading the resin with the first Fmoc-amino acid the synthesis was performed according to the following protocol. a) swelling in 2.5 mL DMF for 15 min; b) 2× deprotection with 2.0 mL 20% piperidine solution in DMF for 10 min; c) 5× washing with 2.0 mL DMF; d) 5× washing with 2.0 mL DCM; e) swelling in 2.5 mL DMF for 15 min; f) coupling with 3.0 eq HBTU/HOBt/Fmoc-amino acid for 60 min; g) 2× washing with 0.4 mL DMF; h) coupling with 1.0 eq HBTU/HOBt/Fmoc-amino acid for 60 min; i) 2× washing with 0.4 mL DMF.

Repeating the steps b)–i) referring to the foldon sequence finally yielded the desired resin-bound and side-chain protected peptide: Boc-Nal-Ile-Pro-Glu(OtBu)-Ala-Pro-Arg(Pbf)-Asp(OtBu)-D-Ala-Gln(Trt)-Ala-Tyr(tBu)-Val-Arg(Pbf)-Lys(Boc)-Hot=Tap-Glu(OtBu)-Trp(Boc)-Val-Leu-Leu-Ser(tBu)-Thr(tBu)-Phe-Leu-[Resin]. Removal of all side-chain protecting groups and cleavage from the resin was performed by shaking for 90 min in TFA/H_2O/TIPS/phenol 95:2:2:1. The crude peptide was precipitated in ether and washed several times. The final purification of this and other fibritin-foldon variants was done by RP-HPLC in a 200 mg scale; after lyophilization 13% of the peptide was yielded as a colorless solid. Other foldon variants listed in Table 1 were likewise prepared.

Ring Puckering of Hot=Tap. The $^3J_{H,H}$ coupling constants of Hot=Tap in different chemical environments are listed in Table S1. $^3J_{H,H}$ coupling constants of less than 3 Hz identify *gauche* relative orientations of the two involved hydrogens. A single intermediate $^3J_{H,H}$ coupling constant of approx. 7 Hz cannot be caused by conformational averaging because flexibility cannot affect only a single bond within a ring. Therefore, each of the three thiaindolizidinone ring systems populates a single main conformation in solution.

The δ-valerolactam of the assumes a boat conformation (^{6,8a}B) in the crystal of azide **4** (CCDC 780552 Cambridge Structural Database) and in solution (Table S1). The $^3J_{H,H}$ coupling constants of **4** and of the cyclic hexapeptide 7,8-isopropylidene-cyclo [Hot=Tap-Gly]$_2$ are nearly identical, proving the ^{6,8a}B boat conformation also for the isopropylidene-protected cyclic hexapeptide. Deprotection of the 7,8-isopropylidene protecting group is accompanied by relaxation of the flag pole position C6 to the envelope E_8 conformation in the cyclic hexapeptide **6** (Fig. 2). This ring conformation is also observed in the crystal structure of FV-1.

Spectroscopic Data of cyclo[Hot=Tap-Gly]$_2$ (6). The NOE contact between Gly-NH and the bridge head proton (8aH) in the ROESY spectrum verifies the internal orientation of Gly-NH. The J-coupling data are also consistent with the assumed hexapeptide conformations.

^{1}H NMR (600 MHz, $DMSO_{d6}$): δ(ppm) = 9.25 (d, $^3J_{6\text{-NH},6\text{-H}}$ = 6.7 Hz, 1 H, 6-NH), 7.46 (t, $^3J_{\text{Gly-NH},\alpha\text{-H}}$ = 2.6 Hz, 1 H, Gly-NH), 5.50 (d, $^3J_{8\text{-OH},8\text{-H}}$ = 4.7 Hz, 1 H, 8-OH), 5.32 (d, $^3J_{7\text{-OH},7\text{-H}}$ = 4.7 Hz, 1 H, 7-OH), 5.11 (dd, $^3J_{3\text{-H},2\text{-H}^{proR}}$ = 6.4 Hz, $3J_{3\text{-H},2\text{-H}^{proS}}$ = 1.9 Hz, 1 H, 3-H), 4.98 (d, $^3J_{8a\text{-H},8\text{-H}}$ = 1.5 Hz, 1 H, 8a-H), 3.94 (ddd, $^3J_{7\text{-H},6\text{-H}}$ = 8.7 Hz, $^3J_{7\text{-H},7\text{-OH}}$ = 6.8 Hz, $^3J_{7\text{-H},8\text{-H}}$ = 2.3 Hz, 1 H, 7-H), 3.90 (dd, $^2J_{\alpha\text{-H},\alpha\text{-H}}$ = 17.6 Hz, $^3J_{\alpha\text{-H,Gly-NH}}$ = 4.0 Hz, 1 H, α^T-H), 3.85 (ddd, $^3J_{8\text{-H},8\text{-OH}}$ = 4.7 Hz, $^3J_{8\text{-H},7\text{-H}}$ = 2.3 Hz, $^3J_{8\text{-H},8a\text{-H}}$ = 1.5 Hz, 1 H, 8-H), 3.74 (dd, $^2J_{\alpha\text{-H},\alpha\text{-H}}$ = 17.6 Hz, $^3J_{\alpha\text{-H,Gly-NH}}$ = 3.1 Hz, 1 H, α^H-H), 3.33 (dd, $^3J_{6\text{-H},7\text{-H}}$ = 8.5 Hz, $^3J_{6\text{-H},6\text{-NH}}$ = 6.6 Hz, 1 H, 6-H), 3.20 (d, $^2J_{2\text{-H}^{proS},2\text{-H}^{proR}}$ = 10.8 Hz, $^3J_{2\text{-H}^{proR},3\text{-H}}$ = 2.3 Hz, 1 H, 2-H^{proS}), 2.98 (dd, $^2J_{2\text{-H}^{proR},2\text{-H}^{proS}}$ = 10.8 Hz, $^3J_{2\text{-H}^{proR},3\text{-H}}$ = 6.6 Hz, 1 H, 2-H^{proR}).

^{13}C NMR (125 MHz, $DMSO_{d6}$): δ(ppm) = 168.5 (3-CO), 167.7 (Gly-CO), 167.6 (5-CO), 70.2 (8-C/7-C), 63.3 (8a-C), 63.2 (3-C), 56.3 (6-C), 42.9 (α-C), 30.4 (2-C).

HRMS (ESI): $C_{20}H_{26}N_6O_{10}S_2Na^+$ (M^+Na^+), calculated: 597.1044, found: 597.1043.

1. Williams JD, Kamath VP, Morris PE, Townsend LB (2005) D-Ribolactone and 2, 3-Isopropylidene(D-Ribolactone). *Org Synth* 82:75–79.

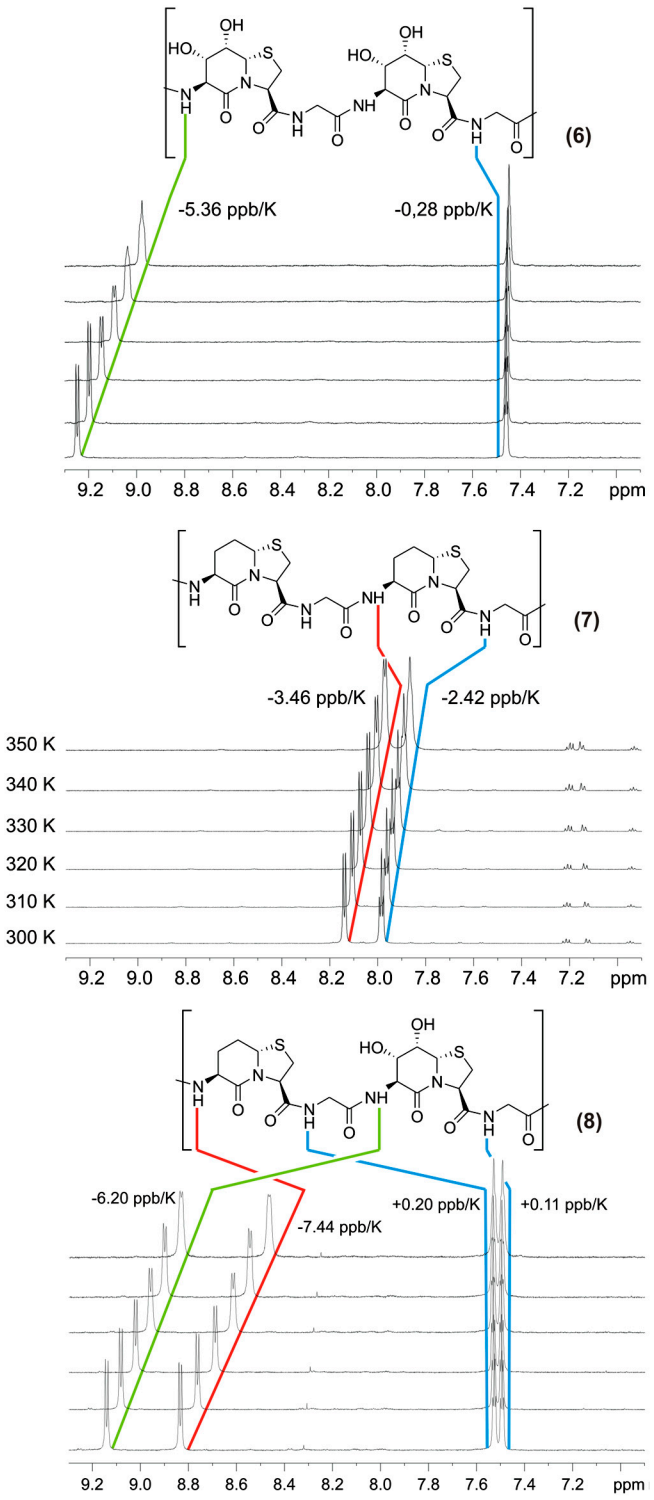


Fig. S1. Overlay of expansions (6.90–9.30 ppm) from ^{1}H NMR spectra measured between 300 and 350 K in 10 K steps showing the amide region (600 MHz, $DMSO_{d6}$). Amide proton temperature gradients as discussed in the main text were determined from these one-dimensional spectra. Cyclo[Hot=Tap-Gly-]$_2$ (**6**) and cyclo[Hot=Tap-BTD-Gly-] (**8**) show a strong dispersion of the amide protons. Cyclo[BTD-Gly-]$_2$ (**7**) peptide shows an intermediate temperature dependency.

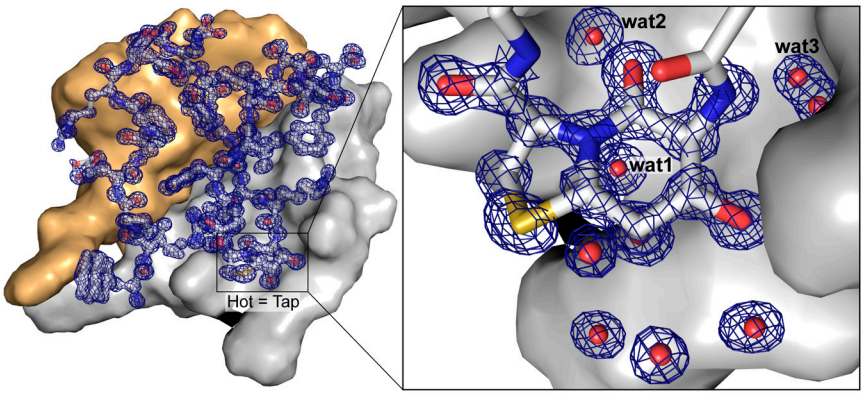


Fig. S2. SIGMAA-weighted $2F_{obs} - F_{calc}$ electron density for chain A of the FV-1 in front of chains B and C forming in combination the trimer and oriented as given in Fig. 4. Enlarged in black box SIGMAA-weighted OMIT electron density is given for Hot=Tap and surrounding waters as discussed in Fig. 5. Contouring-level for both maps calculated at 1.06 Å resolution was $1.2\sigma \equiv 0.88$ e/Å^3.

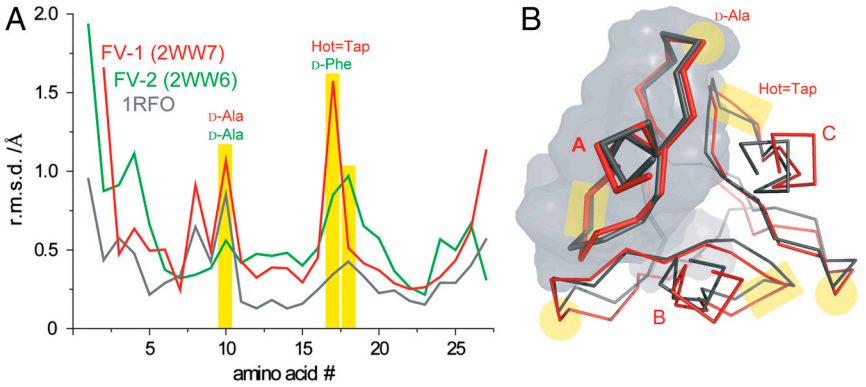


Fig. S3. Deviations of foldon C_αs calculated with the program SUPERPOSE (1). (*A*) C_α-plot of FV-1, FV-2 and NMR-structure (PDB code: 1RFO) (2) against high resolution crystal structure of foldon fusion protein (PDB code: 2IBL) (3). Deviations for all chains were calculated separately and averaged afterward, showing only small deviations for the termini and the Hot=Tap moiety inducing a βII′-turn. (*B*) Superposition of foldon trimers calculated for chain A from FV-1 upon chain A of NMR foldon structure (PDB code: 1RFO) (2) indicating no major disturbance of the trimer assembly.

1. CCP4 (1994) The CCP4 suite: programs for protein crystallography. *Acta Crystallogr D* 50:760–763.
2. Guthe S et al. (2004) Very fast folding and association of a trimerization domain from bacteriophage T4 fibritin. *J Mol Biol* 337:905–915.
3. Boudko SP, Kuhn RJ, Rossmann MG (2007) The coiled-coil domain structure of the sin nombre virus nucleocapsid protein. *J Mol Biol* 366:1538–1544.

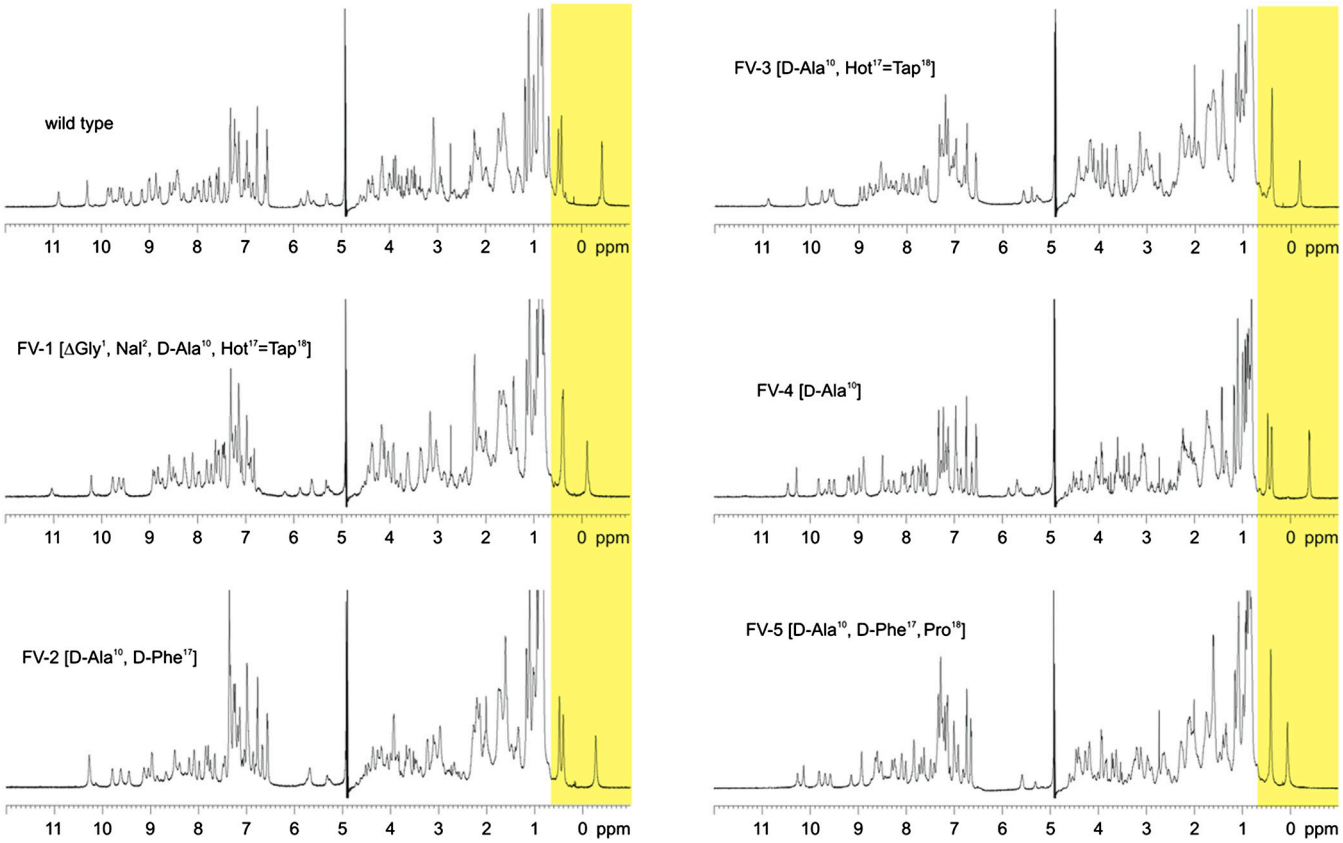


Fig. S4. ^{1}H NMR spectra of wild type and FV-1 to FV-5, 600 MHz, 9 mM potassium phosphate, pH 7.0, 10% (v/v) D_2O, 287 K. Methyl resonance signals highlighted by yellow region (<0.7 ppm) indicate proper folding and trimerization.

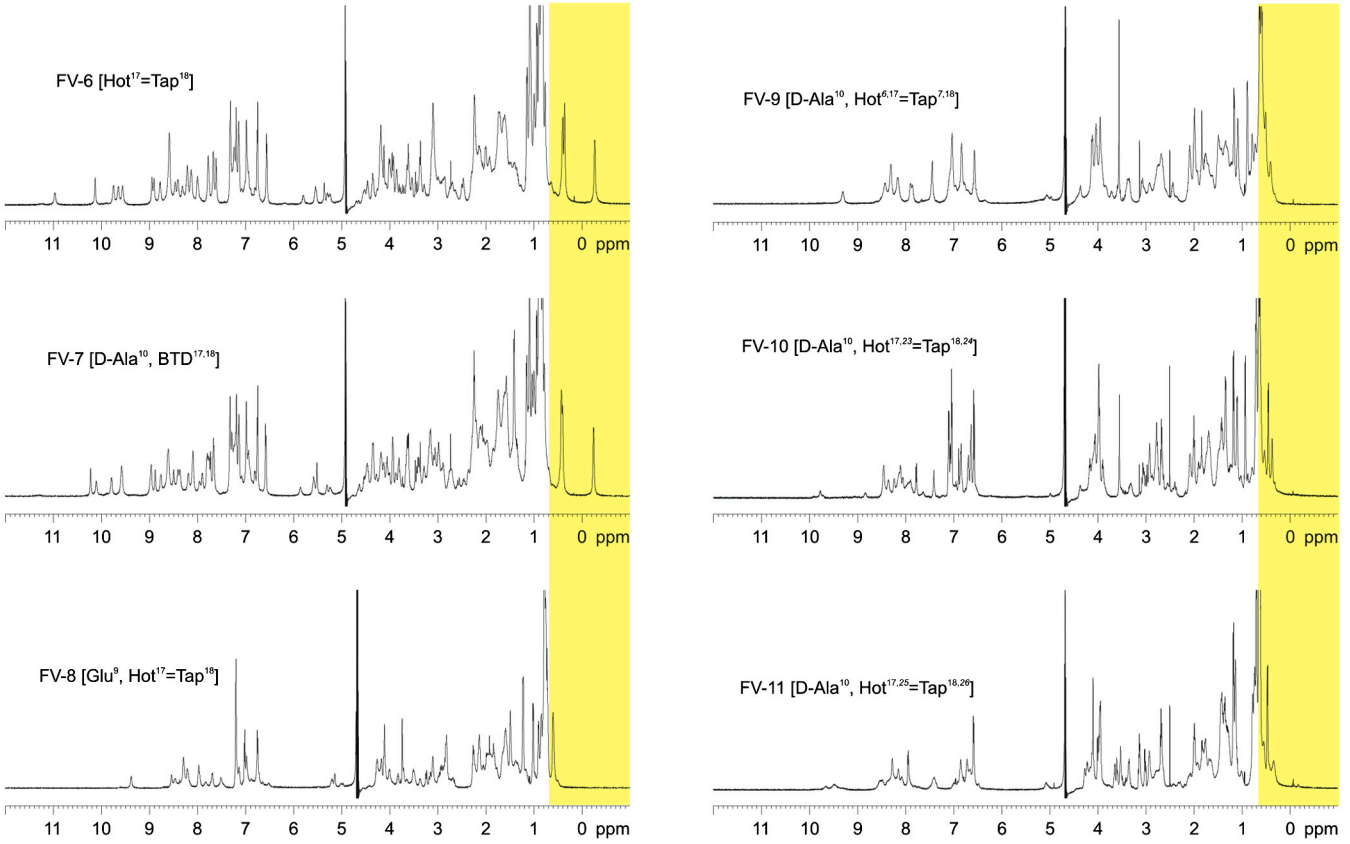


Fig. S5. ^{1}H NMR spectra of FV-6 to FV-11, 600 MHz, 9 mM potassium phosphate, pH 7.0, 10% (v/v) D_2O, 287 K. Methyl resonance signals highlighted by yellow region (<0.7 ppm) indicate proper folding and trimerization.

Table S1. $^{3}J_{H,H}$ coupling constants of the thiaindolizidinone ring

	4	7,8-isopropylidene-cyclo[Hot=Tap-Gly]$_2$	6 cyclo[Hot=Tap-Gly]$_2$
$^{3}J_{6H,7H}$	2,3	2,1	8,4
$^{3}J_{7H,8H}$	7,2	6,8	2,3
$^{3}J_{8H,8aH}$	2,0	1,9	1,4
$^{3}J_{2H^{proS},3H}$	1,6	<1	2,2
$^{3}J_{2H^{proR},3H}$	5,7	6,3	6,3

Structure of 7,8-isopropylidene-cyclo[Hot=Tap-Gly]$_2$

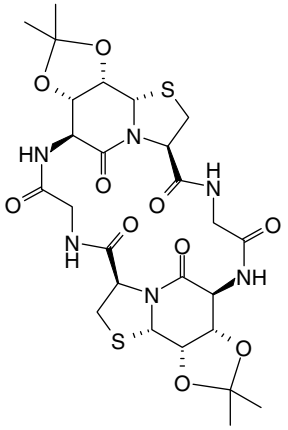


Table S2. Crystallographic table for FV-1 and FV-2

Data collection and processing	[ΔGly[1], Nal[2], D-Ala[10], Hot[17] = Tap[18]] foldon (FV-1, 2WW7)	[D-Ala[10], D-Phe[17]] foldon (FV-2, 2WW6)
X-ray source	ID23-1, ESRF	ID23-1, ESRF
Detector	ADSC Quantum Q315r	ADSC Quantum Q315r
Wavelength (Å)	0.76990	0.76990
Space group	P1	$P3_2$
Cell dimensions a,b,c (Å); α,β,γ (°)	27.73, 28.50, 48.76; 77.25, 88.00, 69.45	29.83, 29.83, 75.08; 90.00, 90.00, 120.00
Resolution (Å)	40–1.06	20–0.98
Temperature (K)	100	100
Observed reflections	115501	98885
Multiplicity	2.0 (1.9)	2.3 (2.2)
Unique reflections	58056	42623
R_{merge} *	0.029 (0.072)	0.052 (0.283)
Completeness (%) †	94.1 (86.8)	99.2 (98.4)
$I/\sigma(I)$ †	19.2 (9.5)	10.4 (2.8)
Mosaicity (°)	0.31	0.53
Wilson B-factor (Å^2)	4.41	7.14
Refinement statistics		
Resolution (Å)	26.02–1.06 (1.09–1.06)	17.98–0.98 (1.01–0.98)
R_{work} (%), R_{free} *, ‡, §	10.8, 14.0 (11.3, 15.9)	12.9, 14.7 (20.3, 22.5)
Reflections of working and test set	56886, 1170	41298, 1300
rmsd bond lengths from ideal (Å)	0.010	0.010
rmsd bond angles from ideal (°)	2.809	1.246
Total no. of atoms	2030	915
Foldon trimers per a.s.u.	2	1
Mean B value (Å^2)	8.44	7.61

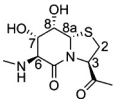
*$R_{merge} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl} \sum_i I_i(hkl)$

†Values in parentheses correspond to the highest resolution shell.

‡$R_{work} = \sum |F_{obs} - F_{calc}| / \sum (F_{obs})$

§R_{free} crystallographic *R*-factor based the data withheld from the refinement for cross-validation.

Table S3. Torsion angles of the bicyclic rings in FV-1 from the six independent peptide strands A-F allow a precise analysis of the ring pucker.



Torsion	Consistent foldons					Average	*Pucker* E_1	*Pucker* E_2
Foldon	A	B	D	E	F	A,B,D,E,F	C	
8a-3a-5-6	5.1	5.0	7.2	1.9	7.3	5.3	3.8	1.8
3a-5-6-7:	−0.8	−2.8	−4.4	−5.3	−6.2	−3.9	−0.6	−4.3
5-6-7-8:	28.6	31.2	31.4	37.8	33.5	32.5	29.3	34.6
6-7-8-8a:	−57.8	−60.7	−58.9	−64.6	−62	−60.8	−61.0	−91.3
7-8-8a-3a:	61.6	62.4	59.9	58.1	63.2	61.04	59.0	81.4
8-8a-3a-5	−36.4	−35.0	−35.9	−30.0	−35.8	−34.6	−33.4	−28.3
3-3a-8a-S	20.1	20.8	20.5	21.1	20.0	20.5	30.8	23.8
3a-8a-S-2:	7.3	7.1	7.6	8.3	8.7	7.8	11.9	−36.5
8a-S-2-3:	−30.3	−30.5	−31	−33.5	−32.6	−31.6	−4.0	37.8
S-2-3-3a:	45.1	46.1	45.9	49.5	46.2	46.6	63.7	−31.1
2-3-3a-8a	−43.4	−44.7	−44.3	−44.3	−44.0	−44.1	−64.6	3.9