Stereochemistry Rules: a Single Stereocenter Changes the Conformation of a Cyclic Tetrapeptide

Supporting Information

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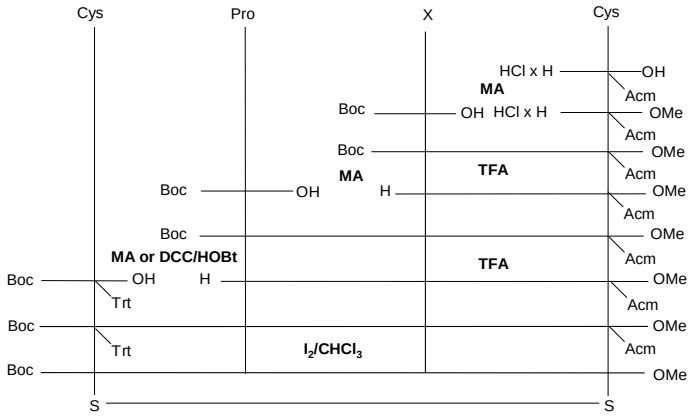
Peptide Synthesis Strategy



Scheme 1. Synthesis plan of cyclo(Boc-Cys-Pro-X-Cys-OMe) [X = D-Leu (**1**) and L-Leu (**2**)]

Synthesis Details: The cyclic tetrapeptides cyclo(Boc-Cys-Pro-X-Cys-OMe) [X = D-Leu (**1**) and L-Leu (**2**)] were prepared using as synthesis strategy a stepwise elongation, in which one residue is added to another one at a time. Starting from the protected H-Cys(Acm)-OMe*HCl the coupling with Boc-X-OH was carried out using the mixed anhydride method to obtain the dipeptide Boc-X-Cys(Acm)-OMe. Successively, after deprotection of the Boc group of the dipeptide by treatment with TFA, the elongation to the tripeptide was done by IBCF/NMM. The linear tetrapeptide was obtained by coupling of the Boc-deprotected tripeptide with the residue Boc-Cys(Trt)-OH using DCCI/HOBt.[1] The combination of both cysteine protecting groups Trt and Acm allows the cleavage and oxidation to the disulfide-bridged cyclic tetrapeptides (Boc-Cys-Pro-X-Cys-OMe) (X = **1** or **2**) in one synthesis step.[2] This was carried out by iodine/chloroform under high dilution conditions. Depending on the amino acid in the third position of the peptide system, yields between 67 - 65% could be obtained.

H-Cys(Acm)-OH*HCl (11): Boc-Cys(Acm)-OH*HCl (9.36 g, 0.032 mol) was dissolved in hydrochloric acid (1.2 N, 30 mL)/acetic acid (30 mL) and the reaction mixture was stirred for 20 minutes at 20°C. The solvent was evaporated to give colorless oil. 100 mL of dry ether were added to the compound, and the flask was placed in an ultrasonic bath for one hour. The precipitated colorless solid was obtained after extraction and was dried under high vacuum conditions (13.85 g, 94.6%). The product is hygroscopic and was kept under argon. ^{1}H NMR (400 MHz, (d_6)DMSO): δ = 8.76 (t, J = 6.3 Hz, Acm NH), 4.35-4.15 (m, Acm CH_2, Cys αCH), 3.74 (s, OCH_3), 3.58 (s, NH_3^+), 3.18-3.03 (Cys βCH_2), 1.85 (s, Acm CH_3) ppm. ^{13}C NMR (101 MHz, (d_6)DMSO): δ = 169.67 (Cys CO), 168.60 (Acm CO), 52.15 (Cys αCH), 40.63 (Acm CH_2), 30.28 (Cys βCH_2), 22.63 (Acm CH_3) ppm. FAB *m/z*: 193.1 (100) $[M - HCl + H]^+$.

H-Cys(Acm)-OMe*HCl (10): Methanol (30 mL) was placed in a double-walled reaction flask and cooled to -15°C. Thionyl chloride (5.2 mL, 0.073 mol) was added drop wise to the stirred methanol. Thereby the temperature should not increase beyond -5°C. H-Cys(Acm)-OH*HCl was added to the reaction mixture at once and stirred for 30 minutes at -5°C and then for five hours at 50°C. After removal of the solvent, the crude was carefully co-evaporated (five times) using methanol to give a white foam as product (13.76 g, 93%). M. p. 130 - 132°C. ^{1}H NMR (400 MHz, (d_6)DMSO): δ = 8.82 (br., Acm NH, NH_3^+), 4.33-4.18 (m, Acm CH_2, Cys αCH), 3.74 (s, OCH_3), 3.61 (br., NH_3^+), 3.41-3.25 (Cys βCH_2), 1.85 (s, Acm CH_3) ppm. ^{13}C NMR (101 MHz, (d_6)DMSO): δ = 169.67 (Cys CO), 168.60 (Acm CO), 52.94 (OCH_3), 52.15 (Cys αCH), 40.63 (Acm CH_2), 30.28 (Cys βCH_2), 22.63 (Acm CH_3) ppm. FAB *m/z*: 207.1 (100) $[M - HCl + H]^+$.

Stepwise elongation procedure using IBCF/NMM - Synthesis of dipeptide Boc-X-Cys(Acm)-OMe: Boc-X-OH (0.064 mol) was stirred in a mixture of DCM/DMF (1:1) and the solution was cooled to -20°C. NMM (0.13 mol) and IBCF (0.064 mol) were added drop wise using a syringe. After ten minutes a cooled suspension of H-Cys(Acm)-OMe*HCl (X) (0.064 mol) and NMM (0.13 mol) in DCM/DMF (1:1) was added to the reaction mixture at once at -15°C. The suspension was stirred for one hour at -15°C and afterwards for 90 minutes at -5°C. The reaction mixture was raised to 20°C and the reaction was stopped by adding 5% $KHCO_3$ (5 mL). The solvent was removed carefully in vacuum to give a yellow solid. The residue was dissolved in ethyl acetate/5% $KHSO_4$ (200 mL) and washed with 5% $KHSO_4$ (40 mL, four times), 5% $KHCO_3$ (40 mL, two times) and brine (20 mL). The organic layer was dried and the solvent was removed. The obtained crude compound was purified by column chromatography. Elution with DCM/Et_2O (7:3 and 1:1) and ethyl acetate afforded pure colorless dipeptide.

X = D-Leu (**8**, 14.6 g, 54.4%). M. p. 114°C. ^{1}H NMR (400 MHz, (d_6)DMSO): δ = 8.5 Hz, D-Leu NH), 4.49 (td, J = 8.2, 5.6 Hz, Cys αCH), 4.25-4.21 (m, Acm CH_2), 4.02 (dd, J = 15.1, 8.0 Hz, D-Leu αCH), 3.64 (s, OCH_3), 2.91 (ddd, J = 22.4, 13.8, 7.0 Hz, Cys βCH_2), 1.83 (s, Acm CH_3), 1.65-1.55 (m, D-Leu βCH_2), 1.44-1.40 (m, D-Leu γCH), (0.86 t, J = 6.5 Hz, D-Leu CH_3) ppm. ^{13}C NMR (101 MHz, (d_6)DMSO): δ = 172.64 (Leu CO, d), 170.95 (OMe CO), 169.31 (Acm CO), 155.18 (Boc CO), 77.94 (Boc Cq), 52.55 (D-Leu αCH, e), 52.05 (Cys αCH), 51.95 (OCH_3), 40.83 (D-Leu βCH_2), 40.40 (Acm CH_2), 31.71 (Cys βCH_2), 28.13 (Boc CH_3), 24.17 (D-Leu γCH), 22.89, 21.49 (D-Leu CH_3), 22.48 (Acm CH_3) ppm. IR: $\tilde{\nu}$ = 3365, 3280, 3082, 2954, 1738, 1707, 1650, 1559, 1545, 1527, 1472, 1457, 1436, 1418, 1370, 1328, 1279, 1255, 1243, 1176, 1113, 1046, 1012, 705 cm^{-1}. FAB-MS *m/z*: 442.2 (100) $[M + Na]^+$, 320.2 (26) $[M - Boc + H]^+$, 420.2 (43) $[M + H]^+$. $C_{18}H_{33}N_3O_6S$ (419.54): calcd. C 51.53, H 7.93, N 10.02, S 7.64; found C 51.53, H 8.13, N 10.38, S 7.80.

X = L-Leu (**9**, 20%). M. p. 85°C. ^{1}H NMR (400 MHz, (d_6)DMSO): δ = 8.48 (t, J = 6.1 Hz, Acm-NH), 8.22 (d, J = 7.7 Hz, Cys NH), 6.81 (d, J = 8.5 Hz, L-Leu NH), 4.48 (Hc, Cys αCH), 4.29-4.15 (m, Acm CH_2), 4.01 (dd, J = 15.6, 7.7 Hz, L-Leu αCH), 3.62 (s, OCH_3), 2.97, 2.85 (Ha, Hb, Cys βCH_2), 1.84 (s, 3H, Acm CH_3), 1.58 (tdd, J = 30.3, 19.1, 10.9 Hz, 1H, L-Leu γCH), 1.52-1.39 (m, L-Leu βCH_2), 1.37 (s, Boc CH_3), 0.93-0.81 (m, L-Leu-CH_3) ppm. ^{13}C NMR (101 MHz, (d_6)DMSO): δ = 172.67 (L-Leu CO), 170.97 (Acm CO), 169.40 (OMe CO), 155.18 (Boc CO), 77.96 (Boc Cq), 52.54 (L-Leu αCH), 52.26 (Cys αCH), 51.94 (OCH_3), 40.75 (L-Leu βCH_2), 40.48 (Acm CH_2), 31.49 (Cys βCH_2), 28.13 (Boc CH_3), 24.14 (L-Leu γCH), 22.50 (Acm CH_3), 22.89,

21.52 (L-Leu CH_3) ppm. IR: $\tilde{\nu}$ = 3334, 3245, 3051, 2956, 2871, 1730, 1690, 1652, 1527, 1448, 1437, 1417, 1374, 1367, 1325, 1274, 1248, 1235, 1210, 1167, 1114, 1093, 1056, 1029, 1012, 999, 985, 954, 920, 902, 875, 859, 800, 739, 710, 700, 635 cm^{-1}. FAB *m/z*: 442.2 (100) $[M + Na]^+$, 420.3 (53) $[M + H]^+$, 320.2 (78) $[M - Boc + H]^+$. $C_{18}H_{33}N_3O_6S$ (419.54): calcd. C 51.53, H 7.93, N 10.02, S 7.64; found: C 51.53, H 7.72, N 9.93, S 7.40.

Standard procedure for removing Boc protecting groups: A solution of Boc protected peptide (0.014 mol) was dissolved in TFA (0.77 mol) and stirred for 100 minutes. The solvent was removed in vacuo and the obtained oil was co-evaporated with hexane (five times). Subsequently, dry ether was added and then placed in a supersonic bath. The deprotected peptide was obtained as a colorless powder (92 - 94%). The absence of signals of Boc was controlled by ^1H NMR spectroscopy.

Stepwise elongation procedure using IBCF/NMM - Synthesis of tripeptide Boc-Pro-X-Cys(Acm)-OMe: The synthesis of the tripeptides were carried out according to the stepwise elongation procedure.

X = D-Leu (**6**, 40.8%). M.p. 70°C. ^1H NMR (400 MHz, (d_6)DMSO): δ = 8.49-8.45 (m, 1H, Acm NH), 8.19 (d, J = 6.8 Hz, 1H, Cys(4) NH), 7.89-7.83 (m, D-Leu NH), 4.53 (Hc, Cys(4) αCH), 4.39-4.32 (m, D-Leu αCH), 4.20 (ddd, J = 32.6, 13.6, 6.3 Hz, Acm CH_2), 4.10 (dd, J = 8.3, 3.3 Hz, Pro αCH), 3.63 (s, 3H, OCH_3), 3.39-3.24 (m, Pro δCH_2), 2.89 (Ha, Hb, Cys(4) βCH_2), 2.13-2.0, 1.80-1.68 (m, Pro $\gamma,\beta CH_2$), 1.83 (s, Acm CH_3), 1.63-1.54 (m, D-Leu γCH), 1.51-1.47 (m, D-Leu βCH_2), 1.39, 1.30 (s, 9H, Boc CH_3), 0.86 (dd, J = 11.5, 6.3 Hz, D-Leu CH_3) ppm. ^{13}C NMR (101 MHz, (d_6)DMSO): δ = 172.08 (D-Leu CO), 171.83 (Pro αCH), 170.94 (OMe CO), 169.27 (Acm CO), 153.45 (Boc CO), 78.42 (Boc Cq), 59.77 (Pro αCH), 51.95 (OCH_3, a), 51.85 (Cys αCH), 50.46 (D-Leu αCH), 46.51 (Pro δCH_2), 41.54 (D-Leu βCH_2), 40.54 (Acm CH_2), 31.93 (Cys(4) βCH_2), 31.19 (Pro βCH_2), 28.02, 27.88 (Boc CH_3), 24.04 (D-Leu γCH), 23.05 (Pro γCH_2), 22.49 (Acm CH_3), 24.05, 21.43 (D-Leu CH_3) ppm. IR: $\tilde{\nu}$ = 3421, 3067, 2957, 2872, 1748, 1654, 1540, 1391, 1367, 1258, 1209, 1164, 1125, 1092, 1092, 1032, 1001, 922, 889, 856, 774 cm^{-1}. FAB *m/z*: 441.2 (100) $[M + Na]^+$, 320.2 (0.1) $[M - Boc + H]^+$, 420.2 (43), $[M + H]^+$. $C_{23}H_4ON_4O_7S$ (516.66): calcd. C 53.47, H 7.80, N 10.84, S 6.21; found C 53.24, H 7.85, N 10.24, S 5.63.

X = L-Leu (**7**, 72%). M. p. 132°C. ^1H NMR (400 MHz, (d_6)DMSO): δ = 8.48 (t, J = 6.2 Hz, Acm NH), 8.32 (dd, J = 31.5, 7.2 Hz, Cys NH), 7.87 (d, J = 8.1 Hz, L-Leu NH), 4.47 (Hc, Cys αCH_2), 4.40-4.32 (m, L-Leu αCH), 4.27-4.13 (m, Acm CH_2, Pro αCH), 3.62 (s, OCH_3), 3.38-3.22 (m, Pro δCH_2), 2.99-2.81 (m, Cys βCH_2), 2.15-2.05/1.84-1.59 (m, Pro $\gamma,\beta CH_2$), 1.84 (s, Acm CH_3), 1.47-1.44 (m, L-Leu βCH), 1.39, 1.32 (m, Boc CH_3), 0.87 (dd, J = 16.1, 6.8 Hz, L-Leu CH_3) ppm. ^{13}C NMR (101 MHz, (d_6)DMSO): δ = 172.18 (CO), 170.88 (CO), 169.40 (CO), 153.29 (Boc CO), 78.25 (Boc qC), 59.12 (Pro αCH), 59.30 (Cys αCH), 51.94 (OCH_3), 50.58 (L-Leu αCH), 46.43 (Pro δCH_2), 40.95 (L-Leu βCH), 40.42 (Acm CH_2), 31.22 (Cys βCH_2), 30.62 (Pro $\beta/\gamma CH_2$), 27.89 (Boc CH_3) ppm. IR: $\tilde{\nu}$ = 3300, 3060, 2956, 2931, 2872, 1750, 1690, 1645, 1540, 1480, 1438, 1405, 1366, 1345, 1317, 1270, 1244, 1225, 1165, 1125, 1096, 1026, 998, 976, 927, 862, 774, 709, 669 cm^{-1}. FAB *m/z*: 539.3 (68) $[M + Na]^+$, 517.3 (39) $[M + H]^+$, 417.3 (39) $[M - Boc + H]^+$. $C_{23}H_{40}N_4O_7S$ (516.66): calcd. C 53.47, H 7.80, N 10.84, S 6.21; found C 53.03, H 8.13, N 10.66, S 5.85.

Standard procedure for synthesis of tetrapeptide Boc-Cys(Trt)-Pro-X-Cys(Acm)-OMe using DCCI/HOBt: Boc-Cys(Trt)-OH (9.4 mmol) was dissolved in DMF (25 mL) and cooled to -18°C. Whilst stirring, DCCI (0.012 mol) and HOBt (0.012 mol) were dissolved in DMF (60 mL) and added one after another to the solution. The reaction mixture was stirred for 30 minutes at -20°C. A cooled solution of deprotected compound H-Pro-X-Cys(Acm)-OMe*CF_3COOH (9.4 mmol) and NMM (0.04 mol) dissolved in DMF was added at once to the reaction mixture. Afterwards the reaction mixture was stirred for two hours at -20°C and stirred for additional 20 hours at 20°C. The reaction mixture was allowed to stand for one hour and the precipitated dicyclohexylurea was filtered off. The solvent was evaporated, the residue was dissolved in ethyl acetate/5% $KHCO_3$ and additional dicyclohexylurea was filtered off. The filtrate was washed with 5% $KHCO_3$ (80 mL, four times), 5% $KHSO_4$ (80 mL, three times) and brine (80 mL). The collected organic layers were dried over Na_2SO_4 and the solvent was evaporated. As raw product a yellow foam was obtained which was purified using column chromatography. Elution with DCM/Et_2O (1:1), DCM/EtOAc (2:3) and ethyl acetate afforded the pure product as colorless foam.

X = D-Leu (**4**, 67%). M.p. 99°C. ^{1}H NMR (400 MHz, (d_6)DMSO): 8.47 (t, J = 6.2 Hz, 1H, Acm NH), 8.20 (d, J = 7.8 Hz, Cys(4) NH), 7.87 (d, J = 8.7 Hz, D-Leu NH), 7.41-7.19 (m, Ar-H), 7.05 (d, J = 8.5 Hz, 1H, Cys(1) NH), 4.45 (Hc, Cys(4) αCH), 4.32-4.13 (m, D-Leu αCH, Acm CH_2, Pro αCH), 4.03 (Hc, Cys(1) αCH), 3.62 (s, OCH_3), 3.27-3.20, 2.86-2.81 (m, Pro δCH_2), 2.92 (Ha, Hb, Cys(4) βCH_2), 2.53, 2.32 (Ha, Hb, Cys(1) βCH_2), 1.85 (s, Acm CH_3), 1.99-1.65 (m, Pro βCH_2, Pro γCH_2), 1.60-1.38 (m, D-Leu βCH_2, Leu γCH), 1.35 (s, Boc CH_3), 170.80 (dd, J = 11.1, 6.1 Hz, D-Leu CH_3,) ppm. ^{13}C NMR (101 MHz, (d_6)DMSO): δ =171.85 (D-Leu CO), 170.95 (OMe CO), 170.87 (Pro CO), 169.39 (Acm CO), 168.66 (Cys(1) CO), 155.06 (Boc CO), 144.32 (Ar C), 129.17 (Ar C), 127.97 (Ar C), 126.69 (Ar C), 78.20 (Boc qC), 66.39 (Ar qC), 59.83 (Pro αCH), 52.16 (Cys(1) αCH), 51.93 (Cys(4) αCH), 51.93 (OCH_3), 50.43 (D-Leu αCH), 46.43 (Pro δCH_2), 40.66 (D-Leu βCH_2), 40.47 (Acm CH_2), 32.53 (Cys(1) βCH_2), 31.88 (Cys(4) βCH_2), 29.07 (Pro βCH_2), 28.08 (Boc CH_3, s), 24.37 (Pro γCH_2), 24.11 (D-Leu γCH), 22.99 (Acm CH_3), 22.51, 21.32 (D-Leu CH_3) ppm. IR: $\tilde{\nu}$ = 3365, 3280, 3082, 2954, 1738, 1707, 1650, 1559, 1545, 1527, 1472, 1457, 1436, 1418, 1370, 1328, 1279, 1255, 1243, 1176, 1113, 146, 1012, 705 cm^{-1}. FAB *m/z*: 243.0 (100) [Trt + H]$^+$, 884.1 (15) [M + Na]$^+$. $C_{45}H_{59}N_5O_8S_2$ (862.13): calcd. C 62.69, H 6.90, N 8.16, S 7.44; found C 62.44, H 7.07, N 7.96, S 7.44.

X = L-Leu (**5**, 65%). M. p. 93°C. ^{1}H NMR (400 MHz, (d_6)DMSO): 8.47 (t, J = 6.2 Hz, Acm NH), 8.25 (d, J = 7.6 Hz, Cys(4) NH), 7.74 (d, J = 8.2 Hz, L-Leu NH), 7.40-7.21 (m, Ar H), 7.16 (d, J = 8.5 Hz, Cys(1) NH), 4.45 (Hc, Cys(4) αCH), 4.32-4.14 (m, Pro αCH, L-Leu αCH, Acm CH_2), 3.99 (Hc, Cys(1) αCH), 3.61 (s, OCH_3), 3.17, 2.68 (m, Pro δCH_2), 2.96, 2.82 (Ha, Hb, Cys(4) βCH_2), 2.54, 2.27 (Hc, Cys(1) βCH_2), 2.02-1.66 (m, Pro βCH_2, Acm CH_3, Pro γCH_2), 1.64-1.47 (m, L-Leu γCH), 1.47-1.46 (m, L-Leu βCH_2), 0.79 (dd, J = 23.3, 6.5 Hz, L-Leu CH_3) ppm. ^{13}C NMR (101 MHz, (d_6)DMSO): δ = 172.00 (L-Leu CO), 170.84 (OMe CO), 170.61 (Pro CO), 169.40 (Acm CO), 168.70 (Cys(1) CO), 155.09 (Boc CO), 144.31 (Ar C), 129.16 (Ar CO), 127.95 (Ar C), 126.68 (Ar C), 78.13 (Boc qC), 66.39 (Ar qC), 59.07 (Pro αCH), 52.33 (Cys(1) αCH), 52.26 (Cys(4) αCH), 51.93 (OMe), 50.74 (L-Leu αCH), 46.21 (Pro-δCH_2), 40.82 (L-Leu βCH_2), 40.43 (Acm CH_2), 32.43 (Cys(1) βCH_2) 31.38 (Cys(4) βCH_2), 28.39 (Pro βCH_2), 28.11

(Boc CH_3), 24.28 (Pro γCH_2), 23.96 (L-Leu γCH), 22.88, 21.69 (L-Leu CH_3), 22.49 (Acm CH_3) ppm. IR: $\tilde{\nu}$ = 3309, 3057, 2956, 2930, 2871, 1745, 1702, 1691, 1658, 1635, 1596, 1527, 1443, 1391, 1367, 1251, 1168, 1095, 1034, 859, 744, 702, 676, 622, 506 cm^{-1}. FAB *m/z*: 884.3 (10) $[M + Na]^+$, 243.0 (100), $[Trt + H]^+$. $C_{45}H_{59}N_5O_8S_2$ (862.13): calcd. C 62.69, H 6.90, N 8.16, S 7.44; found C 59.97, H 6.83, N 7.23, S 6.15.

X-Ray structural details

Comparison with cyclo(Boc-Pro-Gly-Cys-OMe) (3): Unlike in the peptide with X = Gly (**3**) previously reported by us,[3] D-Leucine and L-Leucine are chiral amino acids. However, the crystal structures of **1** and **3** are very similar with a RMSD value of 0.0743 Å for the peptide backbone. **1** and **3** show similar dihedral angles except for Φ_X and Ψ_X. In contrast, the dihedral angles Φ_X and Ψ_X of **2a** and **2b** are closer to the values of **3** (Table S2). Compared to **3**, **2a** and **2b** the dihedral angles of **1** are closer to the values of an ideal β-turn II structure. Like **3**, the disulfide bridge of **1**, **2a** and **2b** show a left-handed orientation evidenced by the χ_{ss} values in the range of 79.5 - 86.3 °, which are close to the ideal strain free value of 90°. The intermolecular interactions of **1** are similar to **3**, since the amide hydrogen atom (see N2, Figure 2 main text) is forming a hydrogen bond with the carbonyl oxygen (O1) of the Pro residue of another peptide molecule of **1**. Also, the Cys1 amide hydrogen proton (N4) interacts with the carbonyl oxygen (O6) of the neighbor molecule. **2a** shows similar intermolecular interactions as **1** and **3**: the amide hydrogen proton $HN_{L\text{-}Leu}$ (N2) of **2a** is involved in a hydrogen bond with the carbonyl oxygen $C{=}O_{Pro}$ (O9) of **2b** and HN_{Cys1} interacts with the oxygen of $C{=}O_{Boc}$ of **2b** (Figure 2, right). In contrast, the amide proton $HN_{L\text{-}Leu}$ (N6) of conformer 2b forms an hydrogen bond with the oxygen (O2) of $C{=}O_{L\text{-}leu}$ of **2a**. Additionally, the amide proton of Cys1 (N8) interacts with the carbonyl oxygen (O10) of $C{=}O_{L\text{-}Leu}$ of another molecule of **2b**.

Table S1. Crystallographic data obtained for **1** and **2**

Empirical formula	$C_{23}H_{38}N_4O_7S_2$ (**1**)	$C_{23}H_{38}N_4O_7S_2$ (**2**)
M_r (g mol^{-1})	546.69	546.69
λ (Å)	0.71073	1.54178
crystal size (mm^3)	0.20 x 0.10 x 0.10	0.18 x 0.06 x 0.02
crystal system	monoclinic	orthorhombic
space group	$P2_1$	$P2_1\,2_1\,2_1$
T (K)	213(2)	100
a (Å)	5.067(2)	16.1483(11)
b (Å)	18.451(8)	18.7091(12)
c (Å)	14.842(7)	18.8204(12)
β (°)	95.254(9)	
V ($Å^3$)	1381.8(10)	5686.0(6)
Z	2	8
ρ_{calc} (g cm^{-3})	1.314	1.277

μ (mm^{-1})	0.240	2.090
$2\theta_{max}$ (°)	50.00	100
data collected / unique	7437/4686	64928/6939
R_{int}	0.0674	0.1092
observed data ($I > 2\sigma(I)$)	4686	5623
Goodness-of-fit on F^2	1.031	1.018
refined parameters	334	662
R_1 ($I > 2\sigma(I)$)	0.0743	0.058
wR_2 (all data)	0.1879	0.146
residuals (e Å^{-3})	0.932 / -0.619	0.5 / -0.3

Table S2. Dihedral angles of peptide backbone of **1** and **2** in comparison to **3**

	1	**2a**	**2b**	**3**			β-turns[4]
					type I	type II	type III
Φ_{Cys1}	-133.5	-133.2	-127.5	-140.1			
Ψ_{Cys1}	47.7	55.2	57.8	52.2			
ω_{Cys1}	172.6	175.1	-175.3	176.9			
Φ_{Pro2}	-62.5	-60.3	-68.2	-62.2	-60	-60	-60
Ψ_{Pro2}	139.0	142.6	133.6	135.6	-30	120	-30
ω_{Pro2}	179.4	177.3	179.6	178.4			
Φ_X	83.5	64.3	65.5	60.4	-90	80	-60
Ψ_X	0.03	12.5	20.9	29.4	0	0	-30
ω_X	177.5	179.1	168.6	174.1			
Φ_{Cys4}	-80.5	-77.7	-72.9	-78.8			
χ^1_{Cys1}	-137.7	-138.5	-149.0	- 135.5			
χ^2_{Cys1}	-53.05	-55.0	-51.1	-52.9			
χ^1_{Cys4}	-66.2	-72.1	-69.6	-75.6			
χ^2_{Cys4}	174.6	178.1	175.4	174.9			
χ_{ss}	-82.1	-79.5	-86.3	-85.2			
χ^1_{Pro2}	32.4	26.7	25.7	28.1			
χ^2_{Pro2}	-39.6	-37.2	-37.6	-38.5			
χ^3_{Pro2}	30.5	31.6	34.3	33.4			
χ^4_{Pro2}	-10.1	-15.4	-18.9	-16.7			
θ_{Pro2}	-13.7	-7.0	-3.8	-6.9			

ϕ = C-N-Cα-C; ψ = N-Cα-C-N; ω = Cα-C-N-Cα; χ_{Cys}^1 = N-Cα-Cβ-S; χ_{Cys}^2 = Cα-Cβ-S-S; χ^1_{Pro} = N-Cα-Cβ-Cγ; χ^2_{Pro}= Cα-Cβ-Cγ-Cδ; χ^3_{Pro} = Cβ-Cγ-Cδ-N; χ^4_{Pro} = Cγ-Cδ-N-Cα, θ_{Pro} = Cδ-N-Cα-Cβ, χ_{ss} = Cβ-S-S-Cβ.

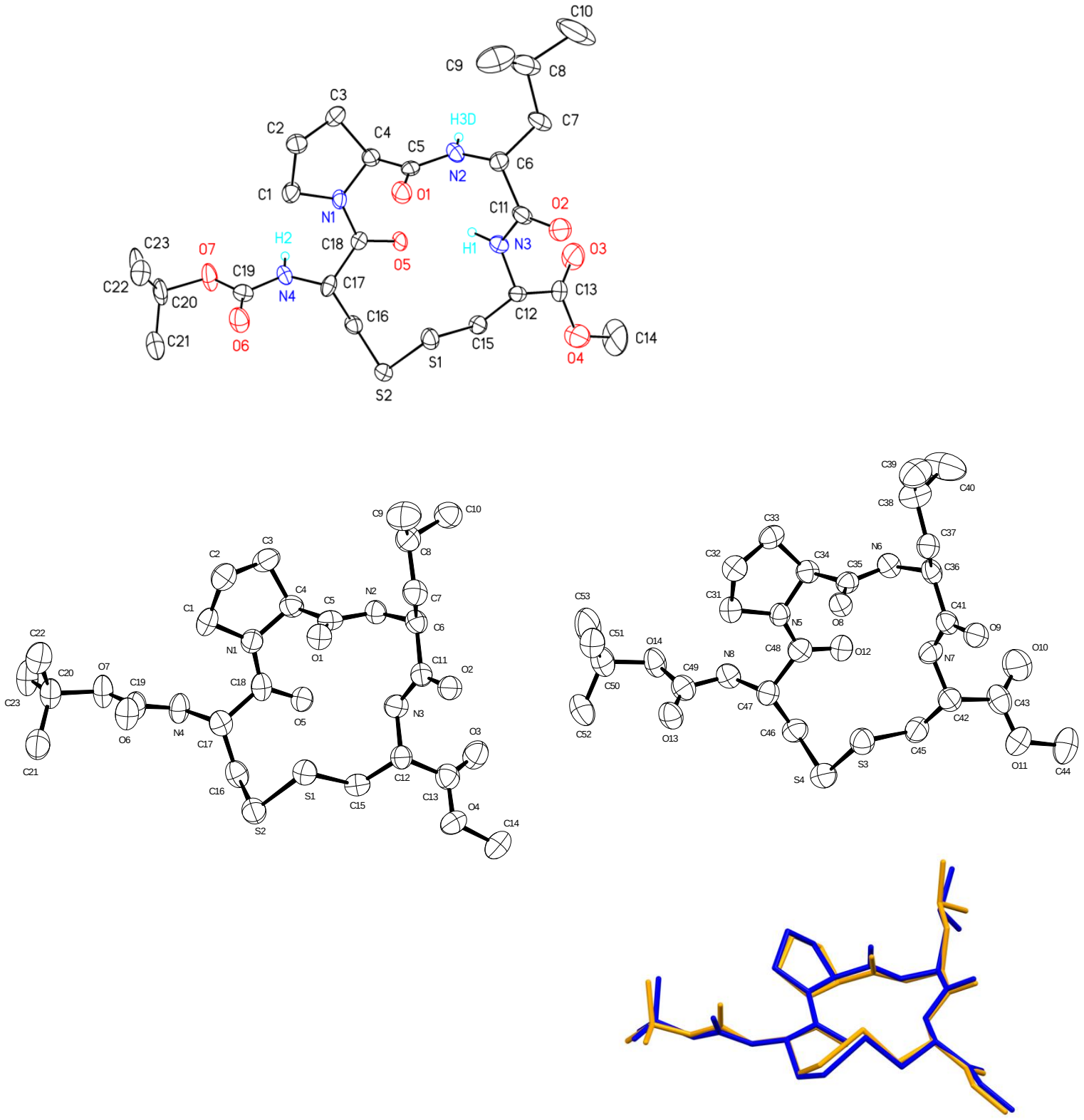


Figure S1. Crystal structures of **1** (top), **2a** (middle left) and **2b** (middle right) and overlay of the two crystallographically independent molecules of **2** (bottom)

NMR studies

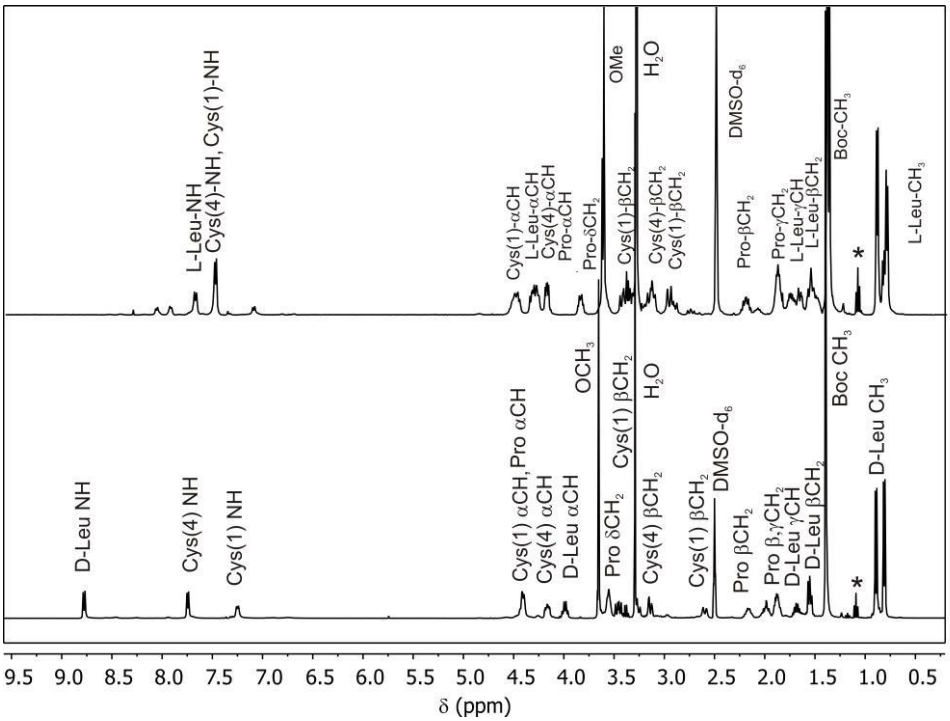


Figure S2. 400 MHz ^{1}H NMR spectra of cyclo(Boc-Cys-Pro-X-Cys-OMe) in DMSO-d_6, X = D-Leu (**1**, bottom) and L-Leu (**2**, top). Signals marked with a star belong to diethyl ether

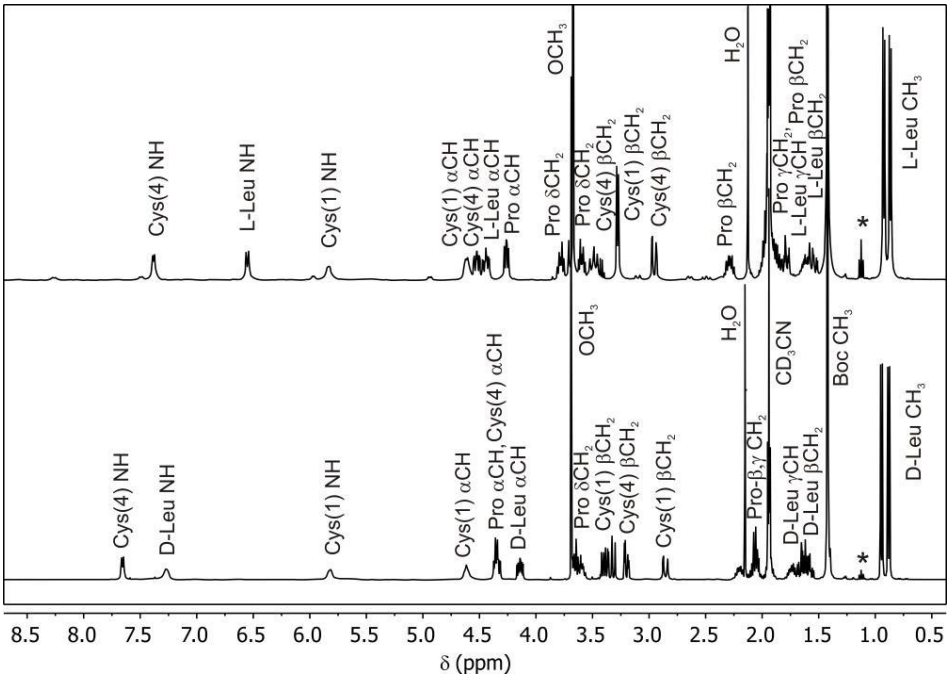


Figure S3. 400 MHz ^{1}H NMR spectra of cyclo(Boc-Cys-Pro-X-Cys-OMe) in CD_3CN, X = D-Leu (**1**, bottom) and L-Leu (**2**, top). Signals marked with a star belong to diethyl ether

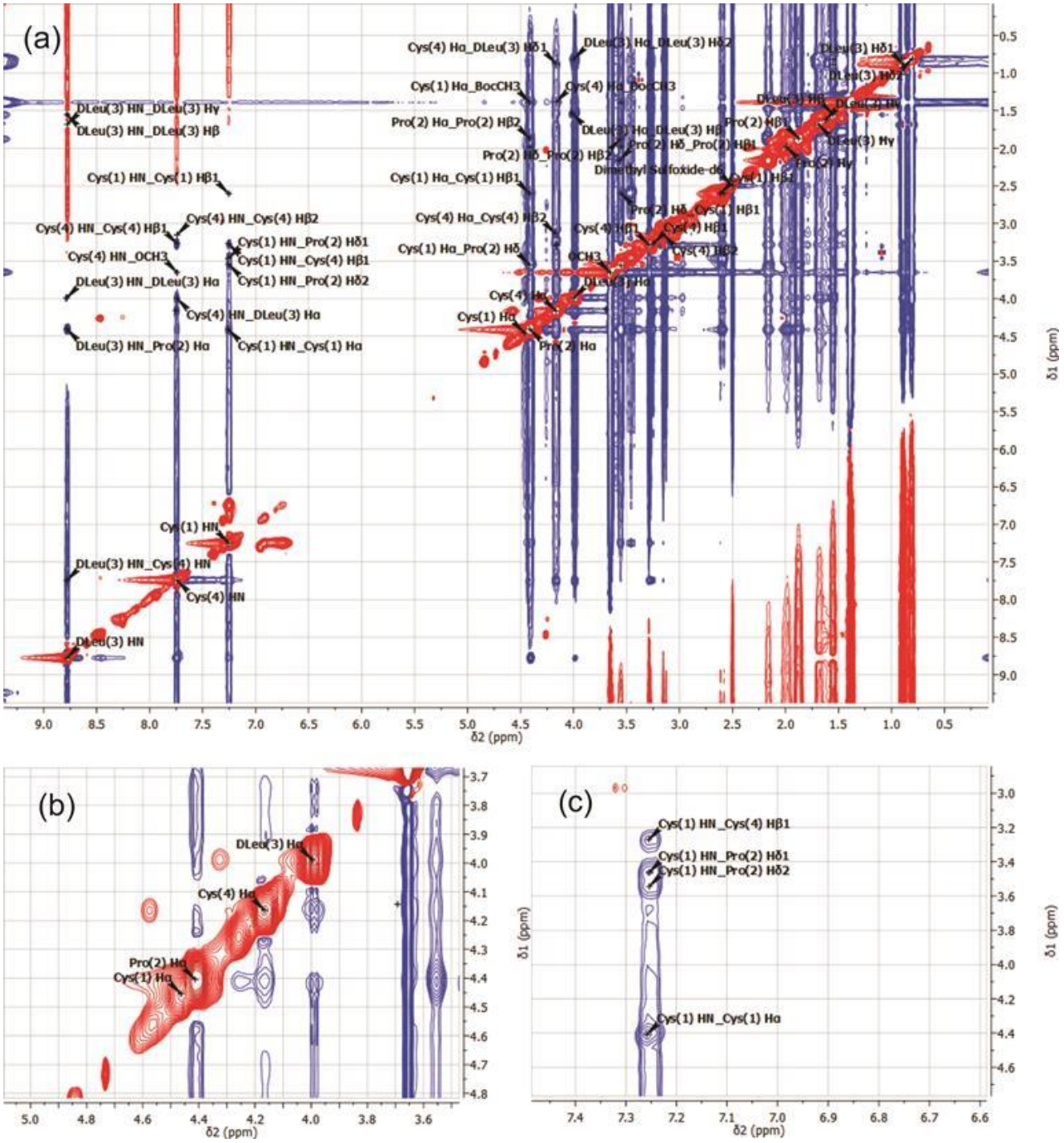


Figure S4. 400 MHz NOESY spectrum of **1** in DMSO-d_6 (a) overview (b) section showing the absences of a Pro(2) αCH/Cys(1) αCH NOE cross peak and (c) section showing a NOE cross peak of Cys(1) NH and Pro(2) δCH as a direct evidence of a *trans* Cys-Pro isomer

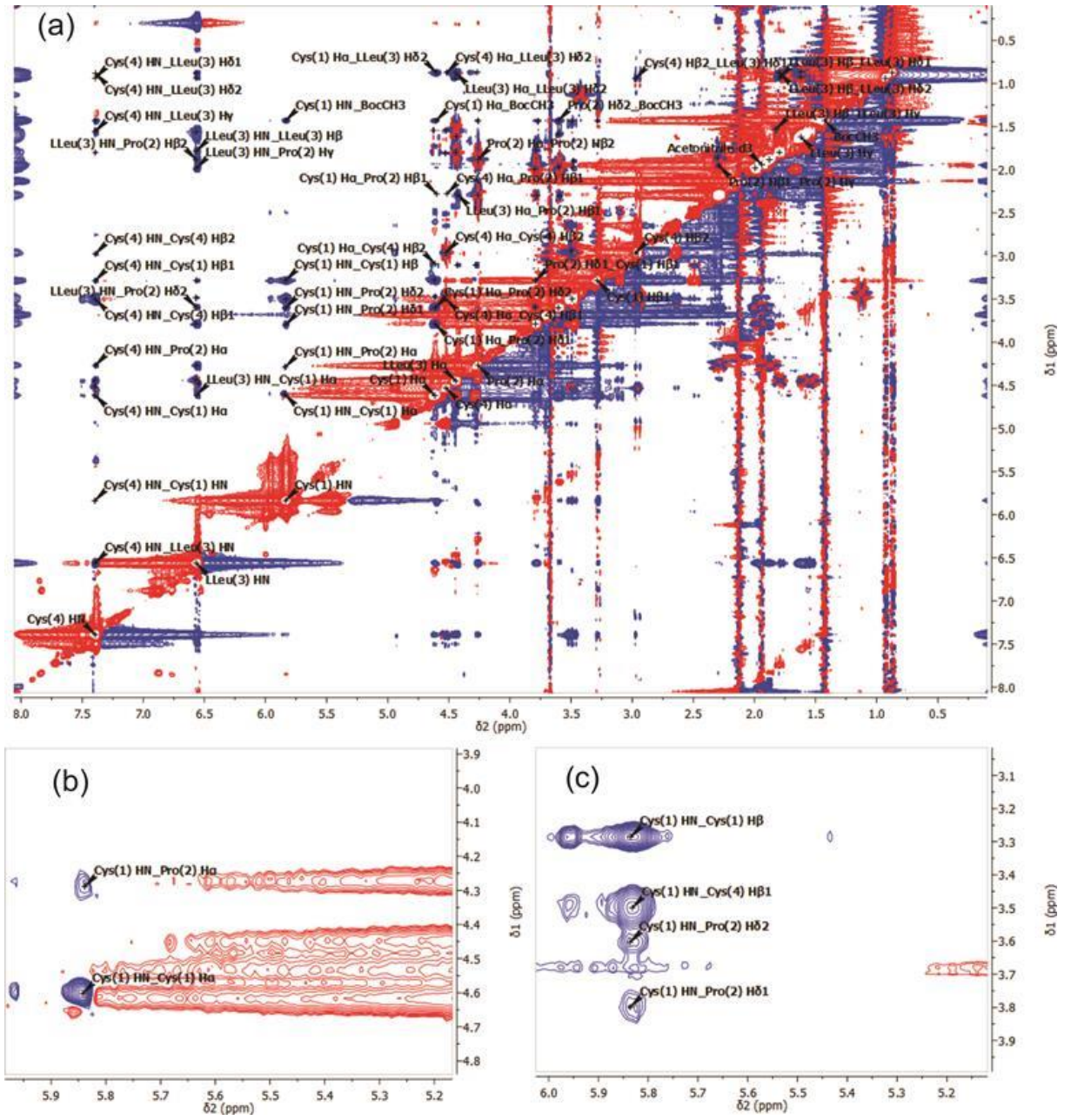


Figure S5. 400 MHz NOESY spectrum of **2** in CD_3CN (a) overview (b) a cross peak between Pro(2) αCH and Cys(1) NH could be related to the presence of very small amounts of the *cis* Cys-Pro isomer and (c) section showing a NOE cross peak of Cys(1) NH and Pro(2) δCH as a direct evidence of the existence of a *trans* Cys-Pro isomer

Table S3. Structural statistics of **1** and **2** in CD_3CN

	1	**2**
Distance constraints		
Total NOEs	59	63
Intra-residual NOEs	19	29
NOE violations (> 0.5 Å)	1	0
RMSD		
Backbone atoms (Å)	0.15 ± 0.07	0.04 ± 0.01
Heavy atoms (Å)	0.70 ± 0.32	0.52 ± 0.21

Temperature-dependent ^{1}H NMR experiments

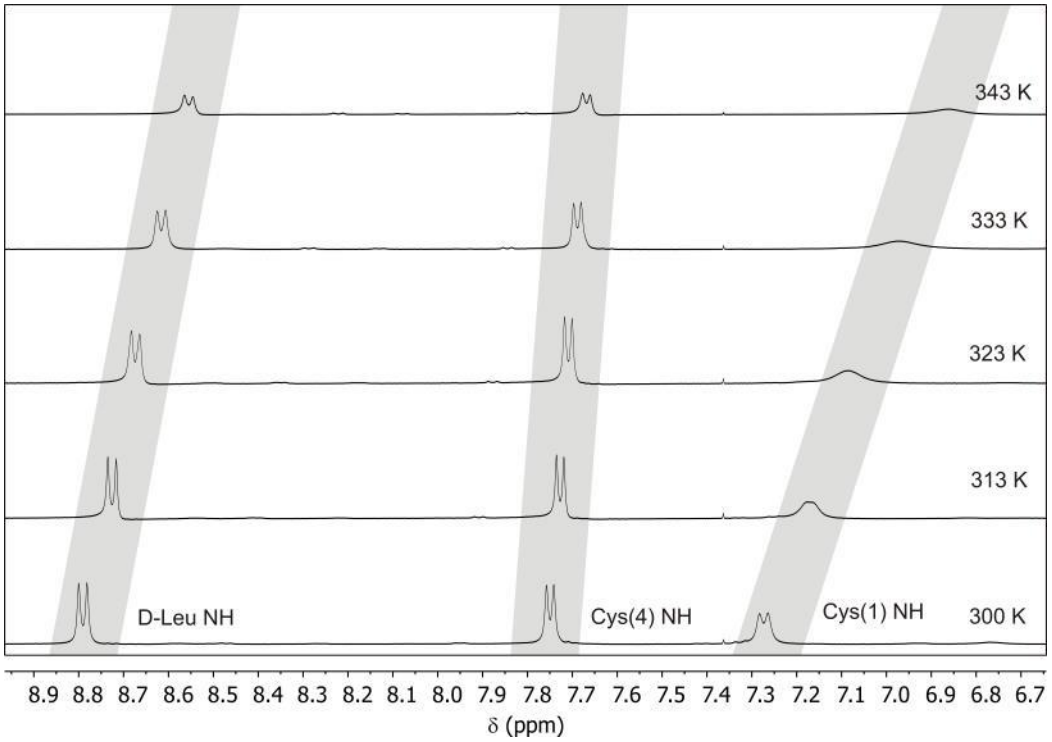


Figure S6. Temperature-dependent ^{1}H NMR spectra of cyclo(Boc-Cys-Pro-D-Leu-Cys-OMe) (**1**) in DMSO-d_6

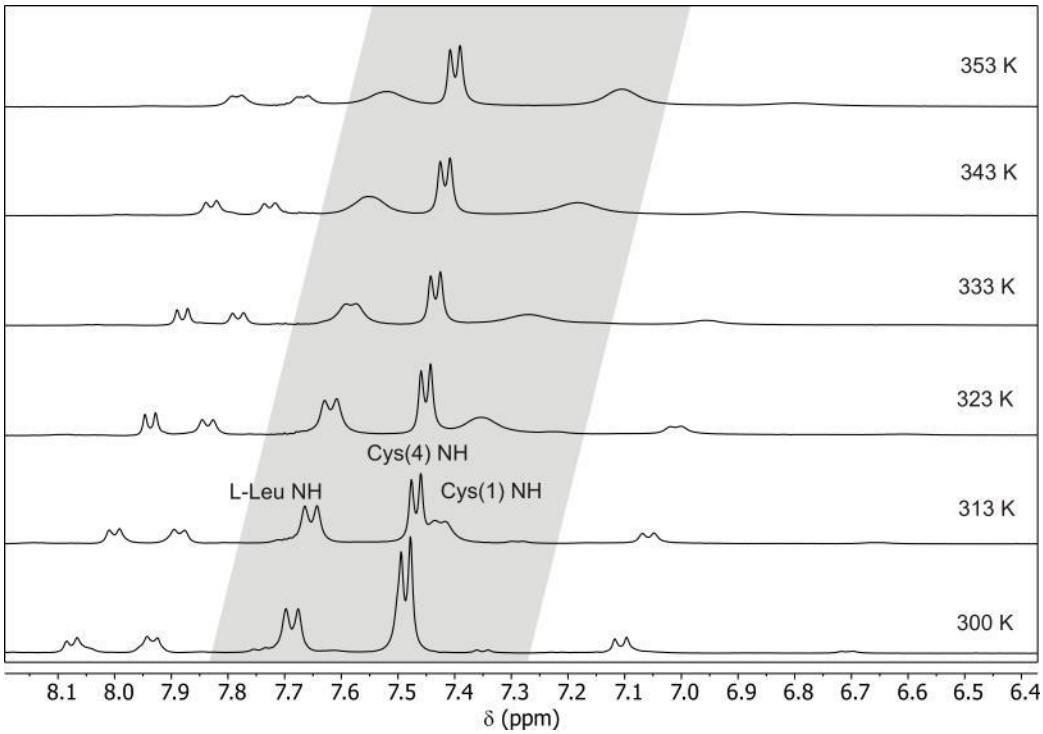


Figure S7. Temperature-dependent ^{1}H NMR spectra of cyclo(Boc-Cys-Pro-L-Leu-Cys-OMe) (**2**) in DMSO-d_6. The assigned signals belong to the major conformation of **2**

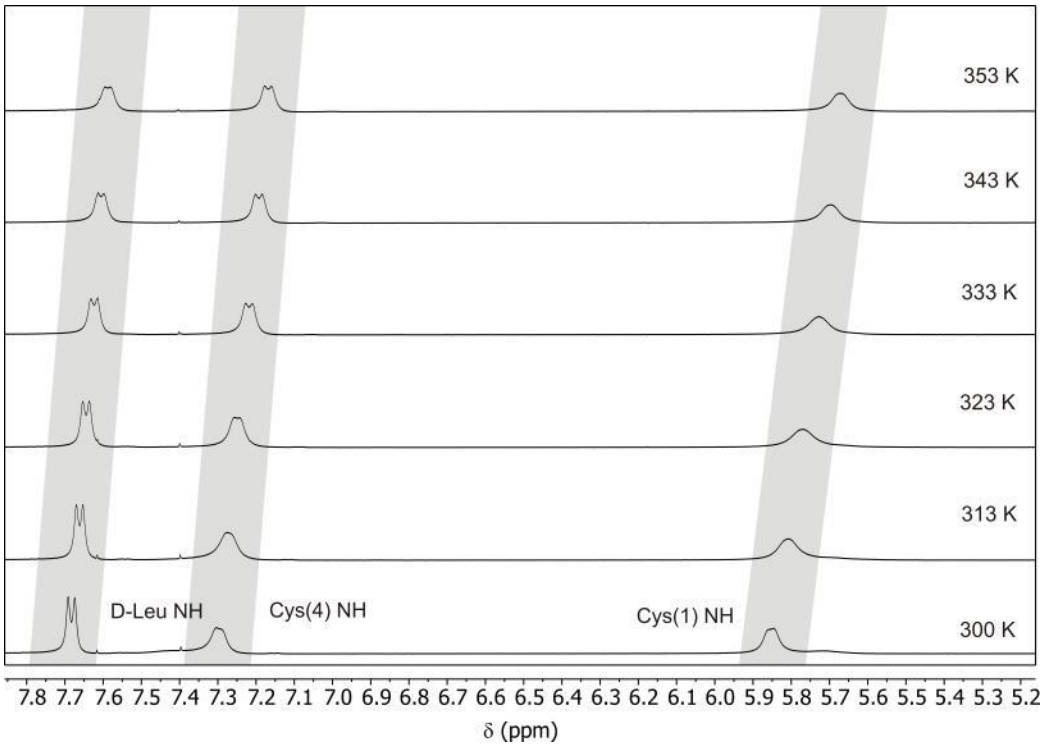


Figure S8. Temperature-dependent ^{1}H NMR spectra of cyclo(Boc-Cys-Pro-D-Leu-Cys-OMe) (**1**) in DMSO-d_6

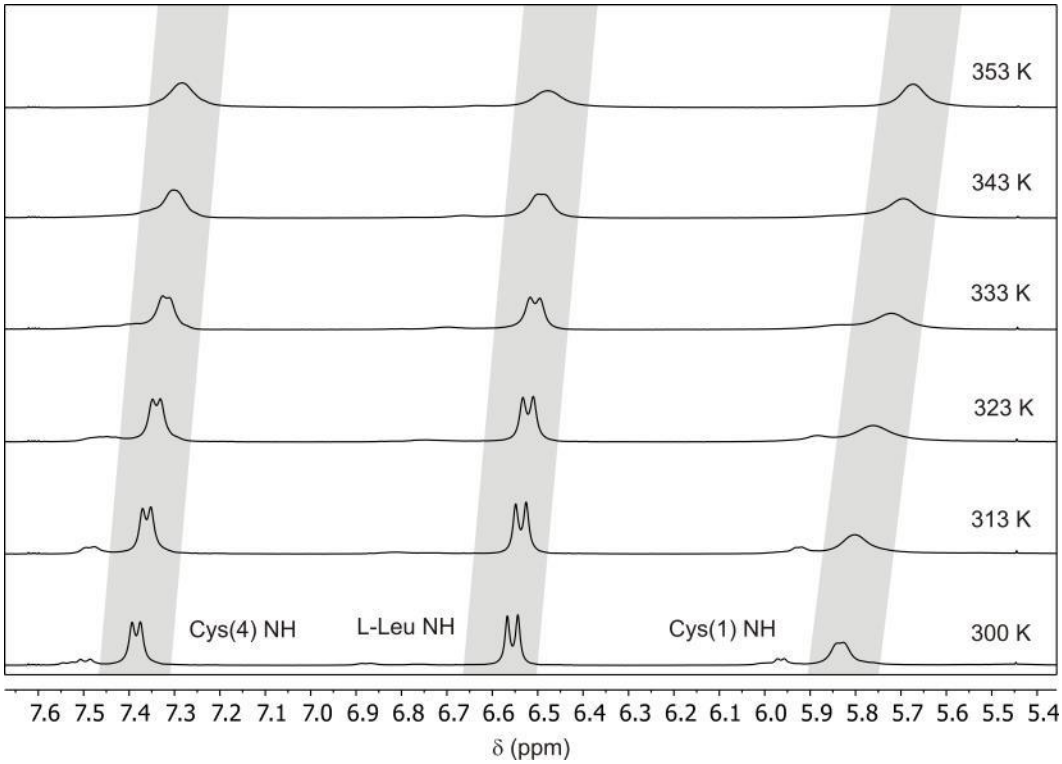


Figure S9. Temperature-dependent ^{1}H NMR spectra of cyclo(Boc-Cys-Pro-L-Leu-Cys-OMe) (**2**) in DMSO-d_6

Temperature-dependent FITR/ATR experiments

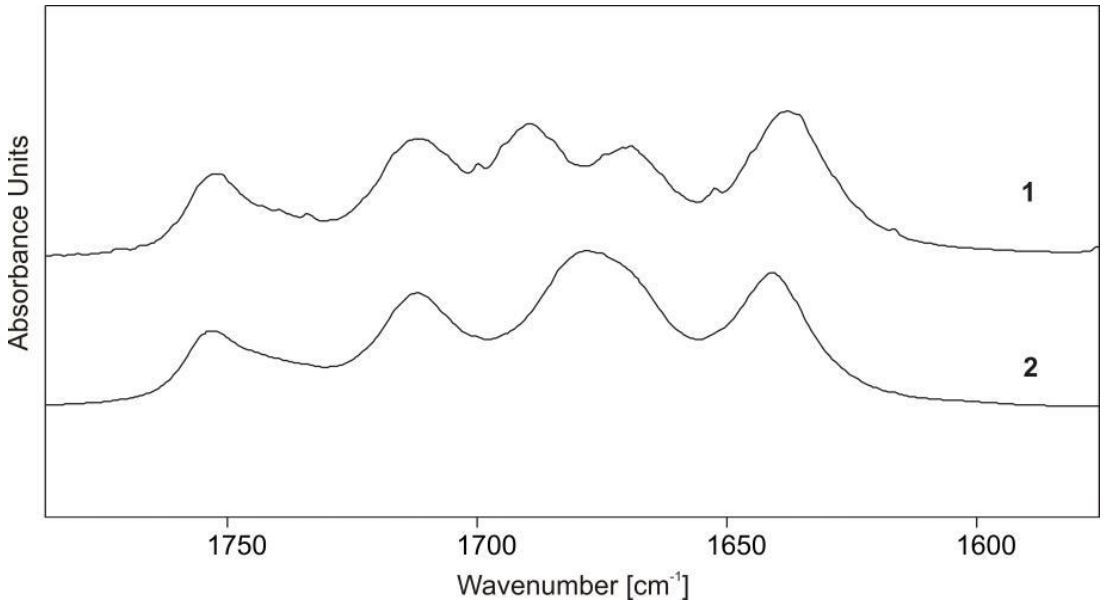


Figure S10. Static FTIR spectra of the cyclic tetrapeptides cyclo(Boc-Cys-Pro-X-Cys-OMe), X = D-Leu (**1**) or L-Leu (**2**) in CH_3CN, T ~ 22°C, spectral resolution is 1 cm^{-1}

Computational Details

REMD Results

Table S4. Values of the backbone angles Φ and Ψ during the REMD simulations (300 K trajectory) and for representative structures obtained with the cluster analysis. Angles are in degrees and distances in Å. Values in ***bold italic*** are the ideal values used for defining the types of β-turn.[4] The REMD simulations of D-Leu$_{I}$ (peptide **1** with β-I turn structure as initial geometry) rapidly converged during the equilibration phase to a β-II turn structure which was conserved for the rest of the simulation. Our second set of calculations starting with β-I turns corroborates that the REMD simulations are independent of the starting geometries. However, in cases like **2** in which both β-II and β-I conformers have very similar stabilities, larger simulation times are needed to converge the values of conformer populations obtained from each simulation. Our work nevertheless clearly shows that while in **2** both conformers are possible, in **1** only the β-II structure is found.

System		Φ_{Pro}	Ψ_{Pro}	Φ_{X}	Ψ_{X}	$C\alpha_{Cys1}$-$C\alpha_{Cys4}$	β-turn
D-Leu$_{II}$	Cluster 300 K	-76	110	70	31	5.2	II
	Cluster 400 K	-77	97	93	-14	5.5	II
	REMD	-75 ± 8	110 ± 17	80 ± 13	6 ± 28	5.4 ± 0.3	II
L-Leu$_{II}$	Cluster 300 K	-69	106	74	4	5.6	II
	Cluster 400 K	-83	106	81	-6	5.5	II
	REMD	-75 ± 7	-40 ± 15	-140 ± 25	11 ± 41	5.5 ± 0.3	I
			107 ± 14	77 ± 10			II
L-Leu$_{I}$	Cluster 300 K	-66	-14	-116	12	5.4	I
	Cluster 400 K	-64	-23	-112	14	5.3	I
	REMD	-65 ± 12	-21 ± 15	-108 ± 20	14 ± 33	5.4 ± 0.2	I
			119 ± 15	78 ± 11			II
Ideal values		***-60***	***-30***	***-90***	***0***	***< 7.0***	I
		-60	***120***	***80***	***0***	***< 7.0***	II

References

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(4) Venkatachalam, C. M. Stereochemical Criteria for Polypeptides and Proteins. V. Conformation of a System of Three Linked Peptide Units. *Biopolymers* **1968**, *6*, 1425–1436.