Supporting Information for:

Macrocyclic Peptides as Allosteric Inhibitors of Nicotinamide N-Methyltransferase (NNMT)

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Reprogrammed mRNA display protocol

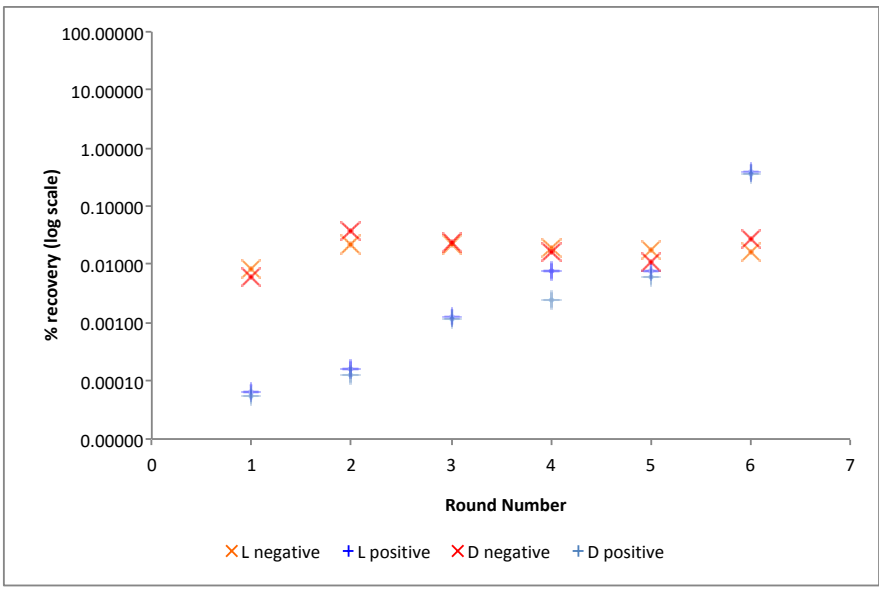


Figure S1. *Library enrichment by binding to NNMT plotted across all selection rounds (log scale on Y-axis), showing binding against both immobilised NNMT ('positive', blue/light blue plus symbol) and against the immobilisation medium alone ('negative', orange/red cross symbol) for both the L- and D-tyrosine initiated libraries (respectively).*

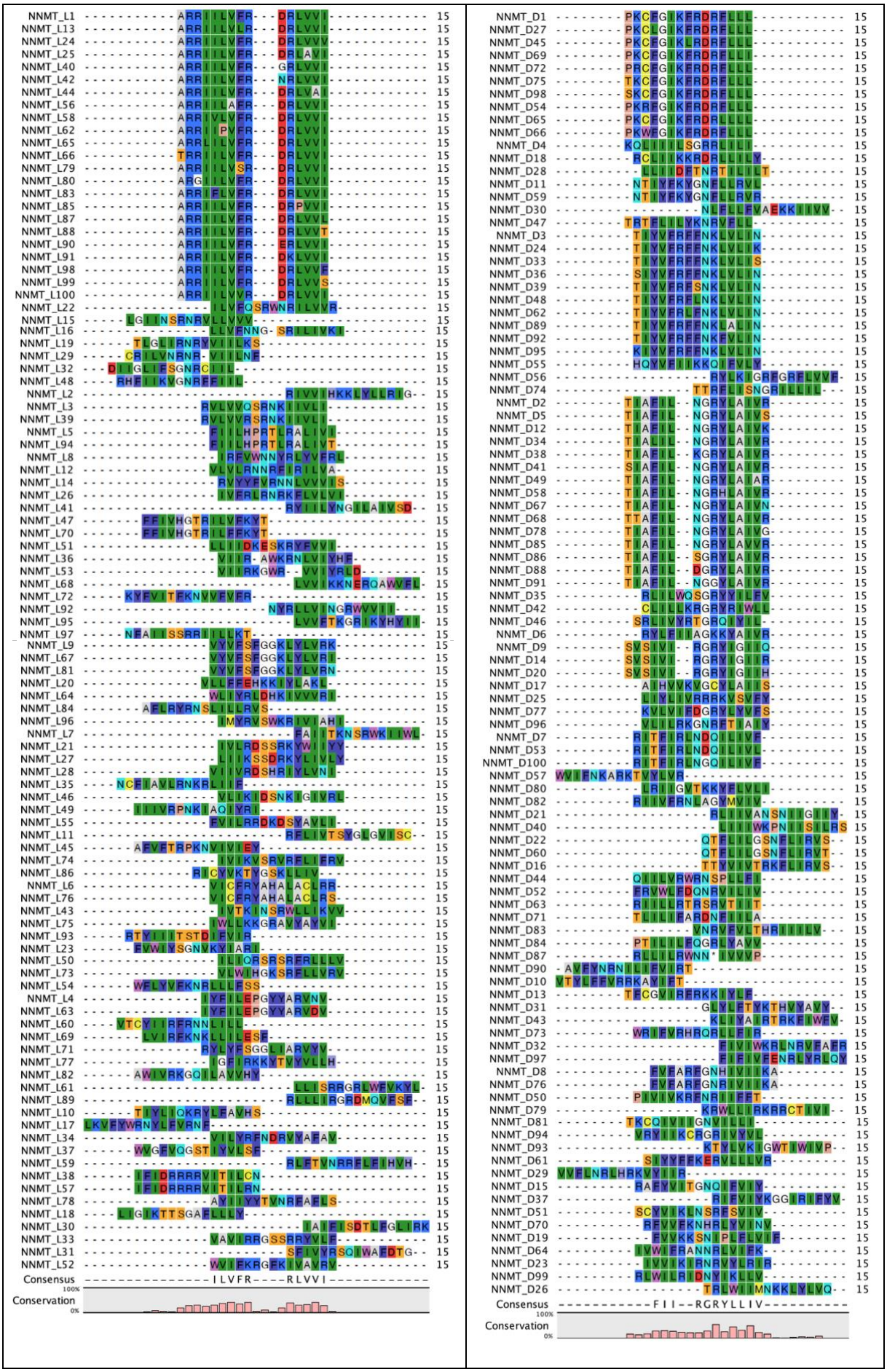
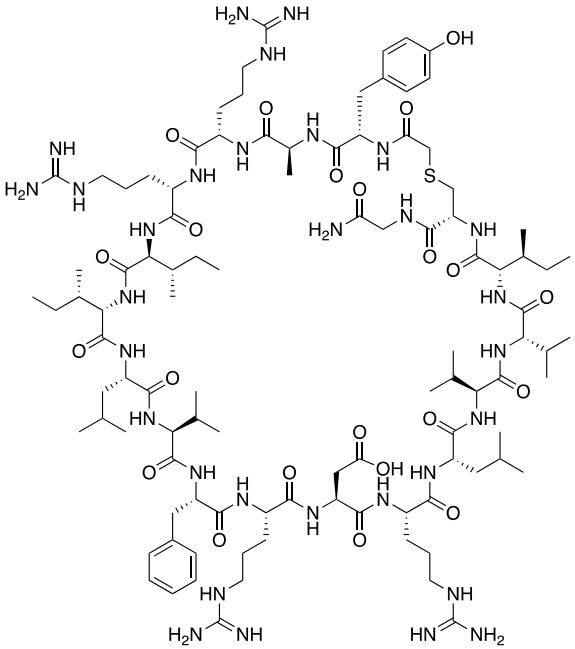
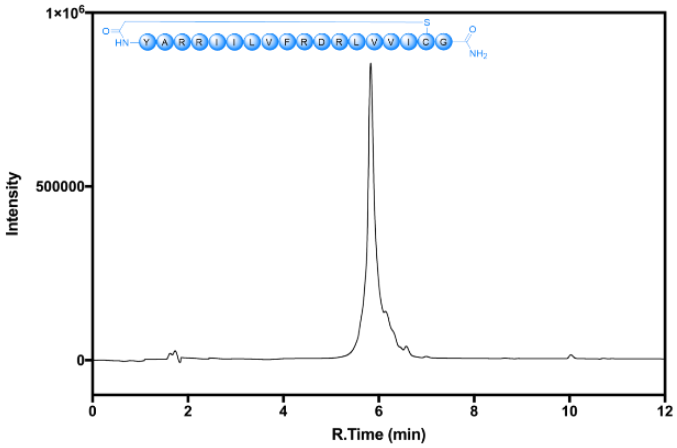


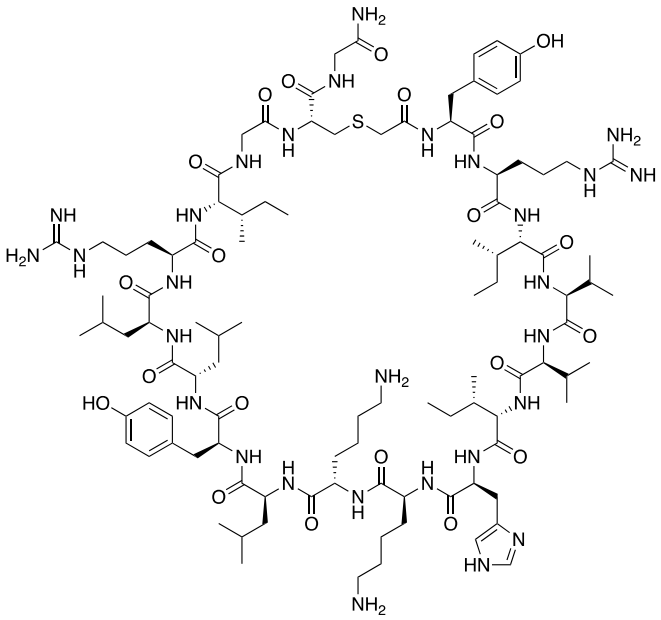
Figure S2. *Sequence alignment of the L-tyrosine library (left) and D-tyrosine library (right). Colors indicate the properties of the respective amino acids. In both libraries hydrophobic (green) and positively charged (blue) amino acids are enriched.*

Synthesis and characterization of peptides

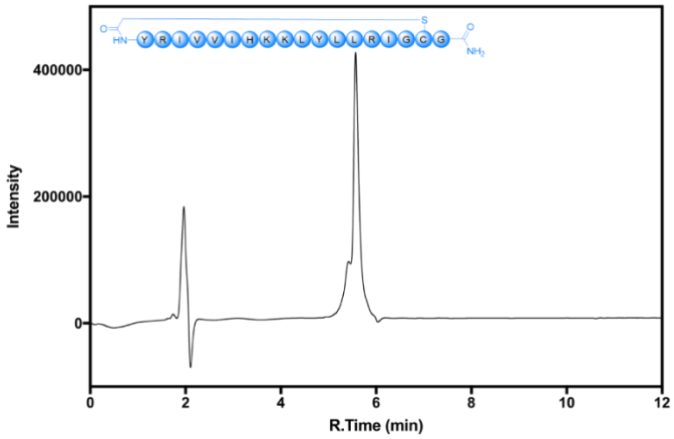


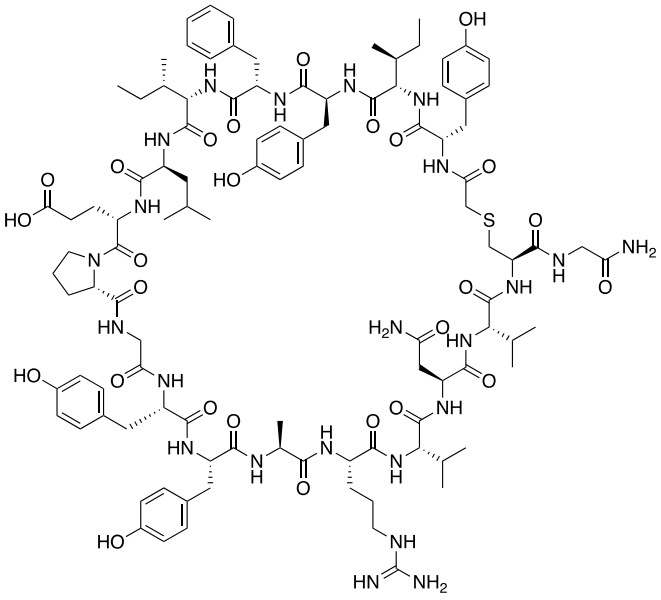
Peptide 1: Rink Amide AM resin (146 mg, 100 µmol, 0.684 mmol/g) was used for Fmoc solid phase peptide synthesis (Fmoc SPPS) according to general procedure **A**. After checking the crude peptide by LC-MS, a portion (25 µmol) of the peptide was capped with chloroacetyl chloride following general procedure **B**. The peptide was deprotected and cleaved from the resin according to general procedure **C**. Subsequently, the peptide was cyclized following general procedure **D** and purified by preparative HPLC (0-100%, buffer B) affording cyclic peptide **1** as a white solid (1.6 mg, 2.9%). HRMS (m/z): $[M+2H]^{2+}$ calculated for $C_{103}H_{173}N_{29}O_{21}S^{2+}$, 1101.1468, found 1101.1462. LC-MS R_t 6.07 min (0 to 100 % B over 12 min, 0.1% FA, λ = 214 nm).



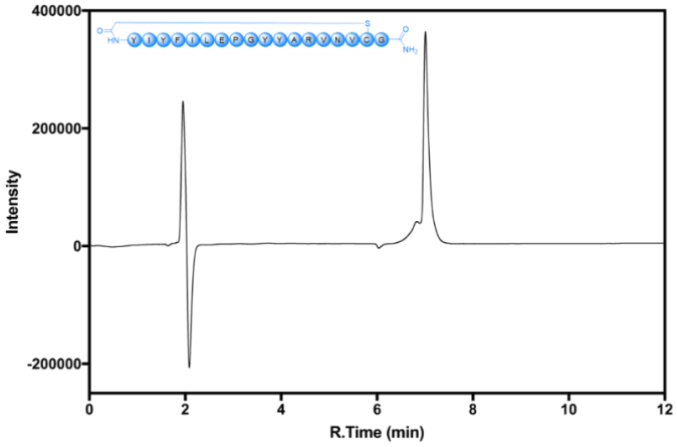


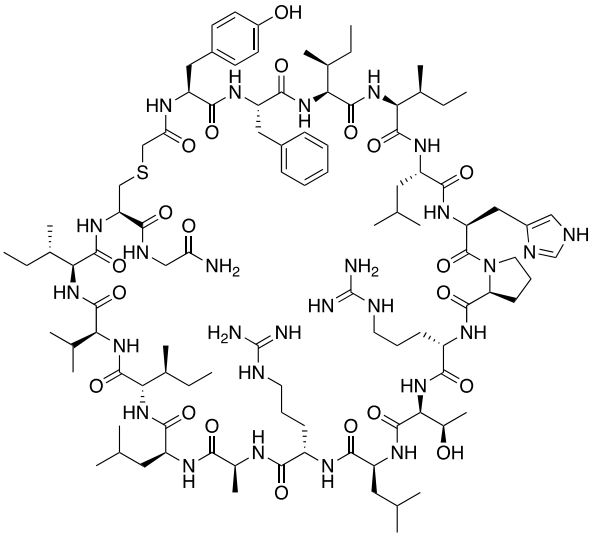
Peptide 2: Rink Amide AM resin (146 mg, 100 µmol, 0.684 mmol/g) was used for Fmoc solid phase peptide synthesis (Fmoc SPPS) according to general procedure **A**. After checking the crude peptide by LC-MS, a portion (25 µmol) of the peptide was capped with chloroacetyl chloride following general procedure **B**. The peptide was deprotected and cleaved from the resin according to general procedure **C**. Subsequently, the peptide was cyclized following general procedure **D** and purified by preparative HPLC (0-100%, buffer B) affording cyclic peptide **2** as a white solid (3.5 mg, 6.5%). HRMS (m/z): $[M+2H]^{2+}$ calculated for $C_{101}H_{171}N_{31}O_{22}S^{2+}$,1092.1541, found 1092,1534. LC-MS R_t 5.57 min (0 to 100 % B over 12 min, 0.1% FA, λ = 214 nm).



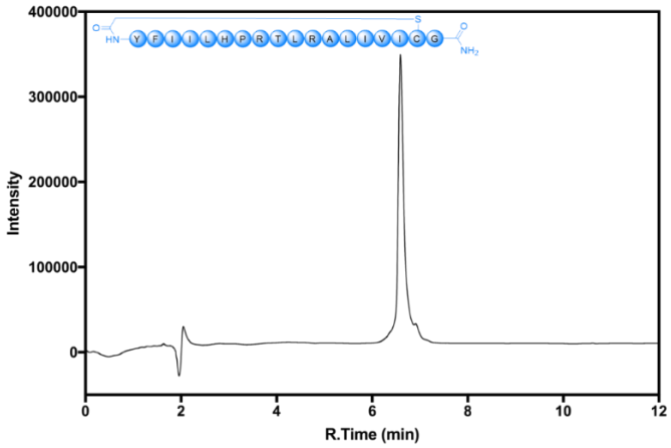


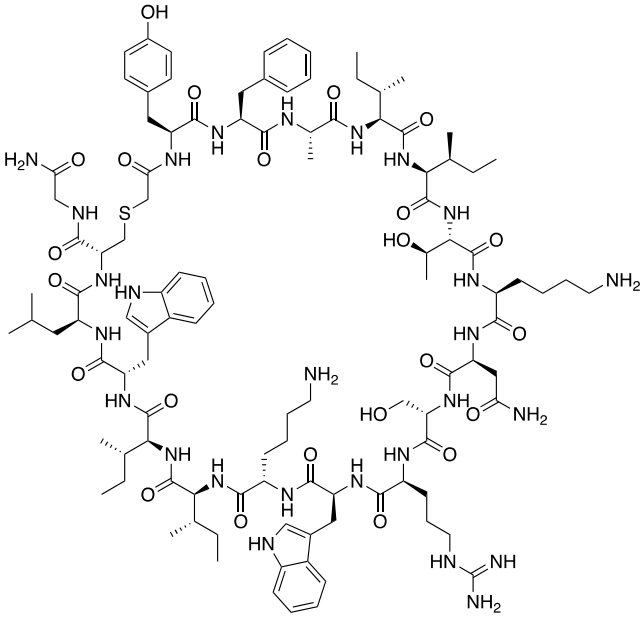
Peptide 3: Rink Amide AM resin (146 mg, 100 µmol, 0.684 mmol/g) was used for Fmoc solid phase peptide synthesis (Fmoc SPPS) according to general procedure **A**. After checking the crude peptide by LC-MS, a portion (25 µmol) of the peptide was capped with chloroacetyl chloride following general procedure **B**. The peptide was deprotected and cleaved from the resin according to general procedure **C**. Subsequently, the peptide was cyclized following general procedure **D** and purified by preparative HPLC (0-100%, buffer B) affording cyclic peptide **3** as a white solid (4.2 mg, 7.8%). HRMS (m/z): $[M+2H]^{2+}$ calculated for $C_{105}H_{149}N_{23}O_{26}S^{2+}$, 1090.0382, found 1090.0371. LC-MS R_t 7.01 min (0 to 100 % B over 12 min, 0.1% FA, λ = 214 nm).



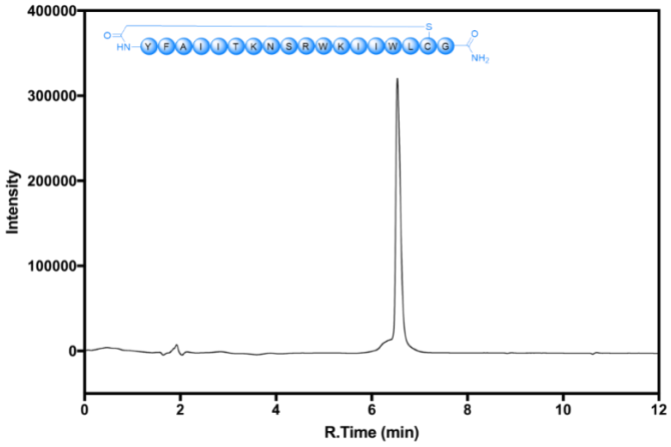


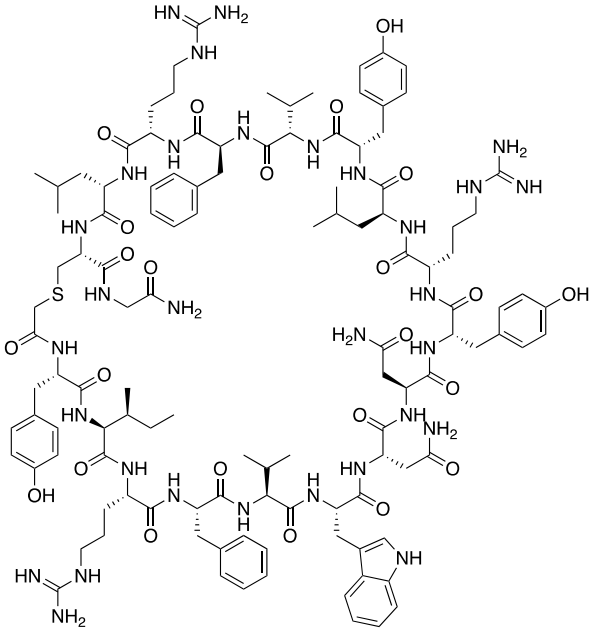
Peptide 4: Rink Amide AM resin (146 mg, 100 µmol, 0.684 mmol/g) was used for Fmoc solid phase peptide synthesis (Fmoc SPPS) according to general procedure **A**. After checking the crude peptide by LC-MS, a portion (25 µmol) of the peptide was capped with chloroacetyl chloride following general procedure **B**. The peptide was deprotected and cleaved from the resin according to general procedure **C**. Subsequently, the peptide was cyclized following general procedure **D** and purified by preparative HPLC (0-100%, buffer B) affording cyclic peptide **4** as a white solid (3.6 mg, 6.7%). HRMS (m/z): $[M+2H]^{2+}$ calculated for $C_{107}H_{167}N_{27}O_{21}S^{2+}$, 1069.1275, found 1069.1274. LC-MS R_t 7.01 min (0 to 100 % B over 12 min, 0.1% FA, λ = 214 nm).



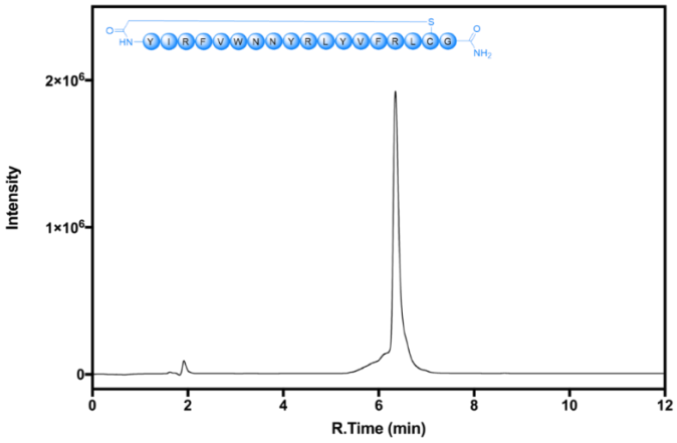


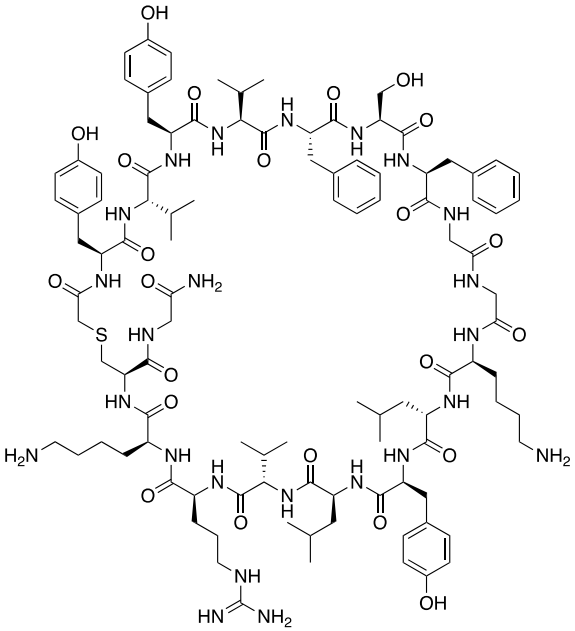
Peptide 5: Rink Amide AM resin (146 mg, 100 µmol, 0.684 mmol/g) was used for Fmoc solid phase peptide synthesis (Fmoc SPPS) according to general procedure **A**. After checking the crude peptide by LC-MS, a portion (25 µmol) of the peptide was capped with chloroacetyl chloride following general procedure **B**. The peptide was deprotected and cleaved from the resin according to general procedure **C**. Subsequently, the peptide was cyclized following general procedure **D** and purified by preparative HPLC (0-100%, buffer B) affording cyclic peptide **5** as a white solid (5.1 mg, 9.1%). HRMS (m/z): $[M+2H]^{2+}$ calculated for $C_{109}H_{165}N_{27}O_{23}S^{2+}$,1126.1146, found 1126.1140. LC-MS R_t 6.54 min (0 to 100 % B over 12 min, 0.1% FA, λ = 214 nm).



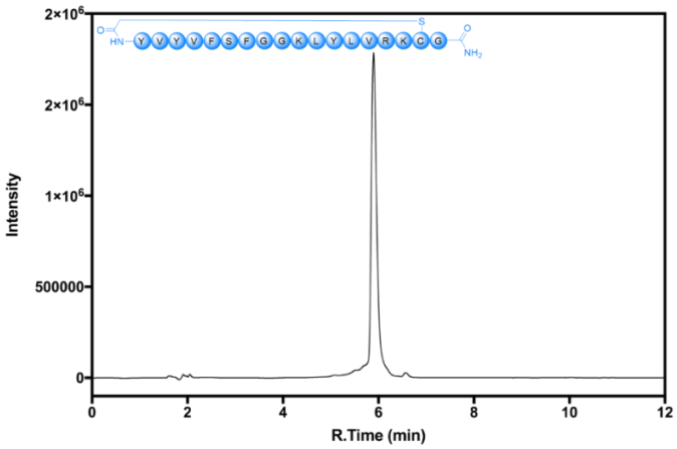


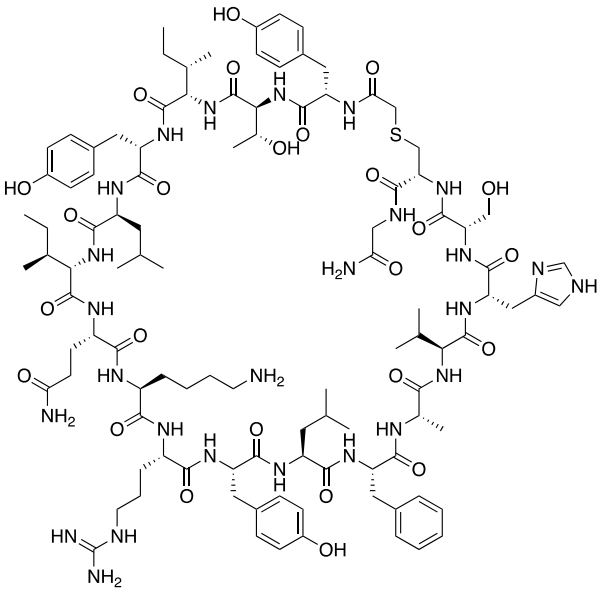
Peptide 6: Rink Amide AM resin (146 mg, 100 µmol, 0.684 mmol/g) was used for Fmoc solid phase peptide synthesis (Fmoc SPPS) according to general procedure **A**. After checking the crude peptide by LC-MS, a portion (25 µmol) of the peptide was capped with chloroacetyl chloride following general procedure **B**. The peptide was deprotected and cleaved from the resin according to general procedure **C**. Subsequently, the peptide was cyclized following general procedure **D** and purified by preparative HPLC (0-100%, buffer B) affording cyclic peptide **6** as a white solid (4.7 mg, 7.8%). HRMS (m/z): $[M+2H]^{2+}$ calculated for $C_{117}H_{167}N_{31}O_{24}S^{2+}$,1211.1260, found 1211.1253. LC-MS R_t 6.35 min (0 to 100 % B over 12 min, 0.1% FA, λ = 214 nm).



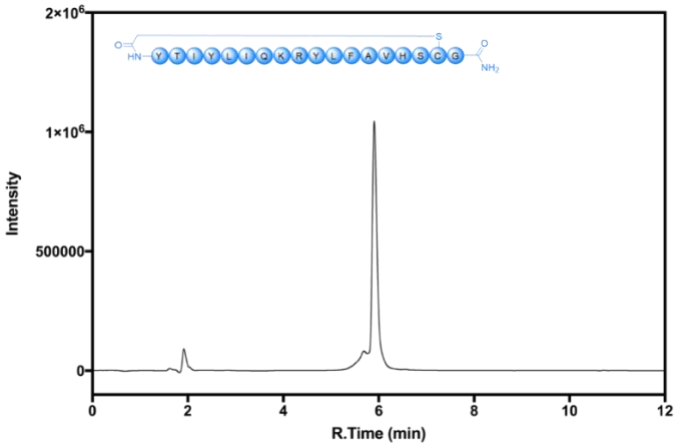


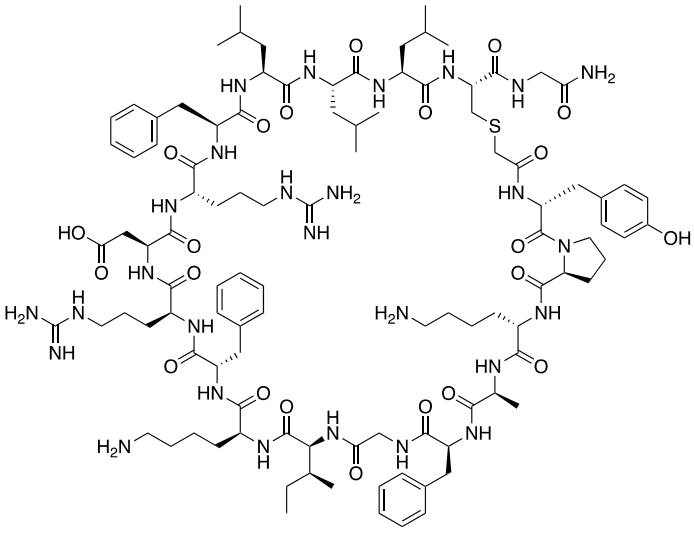
Peptide 7: Rink Amide AM resin (146 mg, 100 µmol, 0.684 mmol/g) was used for Fmoc solid phase peptide synthesis (Fmoc SPPS) according to general procedure **A**. After checking the crude peptide by LC-MS, a portion (25 µmol) of the peptide was capped with chloroacetyl chloride following general procedure **B**. The peptide was deprotected and cleaved from the resin according to general procedure **C**. Subsequently, the peptide was cyclized following general procedure **D** and purified by preparative HPLC (0-100%, buffer B) affording cyclic peptide **7** as a white solid (6.5 mg, 12.2%). HRMS (m/z): $[M+2H]^{2+}$ calculated for $C_{104}H_{154}N_{24}O_{23}S^{2+}$, 1069.5670, found 1069.5665. LC-MS R_t 5,90 min (0 to 100 % B over 12 min, 0.1% FA, λ = 214 nm).



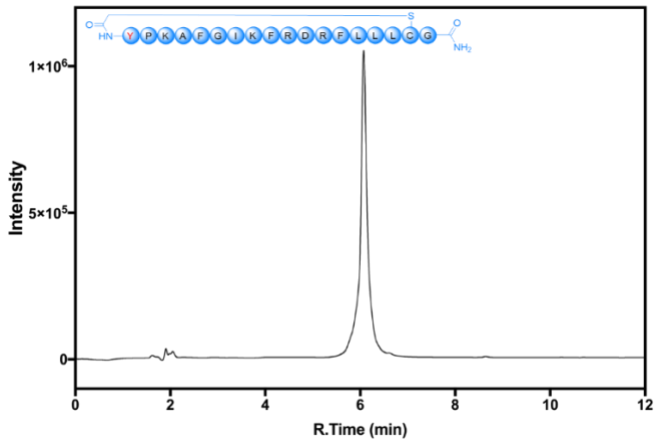


Peptide 8: Rink Amide AM resin (146 mg, 100 µmol, 0.684 mmol/g) was used for Fmoc solid phase peptide synthesis (Fmoc SPPS) according to general procedure **A**. After checking the crude peptide by LC-MS, a portion (25 µmol) of the peptide was capped with chloroacetyl chloride following general procedure **B**. The peptide was deprotected and cleaved from the resin according to general procedure **C**. Subsequently, the peptide was cyclized following general procedure **D** and purified by preparative HPLC (0-100%, buffer B) affording cyclic peptide **8** as a white solid (5.7 mg, 10.4%). HRMS (m/z): $[M+2H]^{2+}$ calculated for $C_{105}H_{158}N_{26}O_{25}S^{2+}$, 1107.5806, found 1107.5807. LC-MS R_t 5,91 min (0 to 100 % B over 12 min, 0.1% FA, λ = 214 nm).

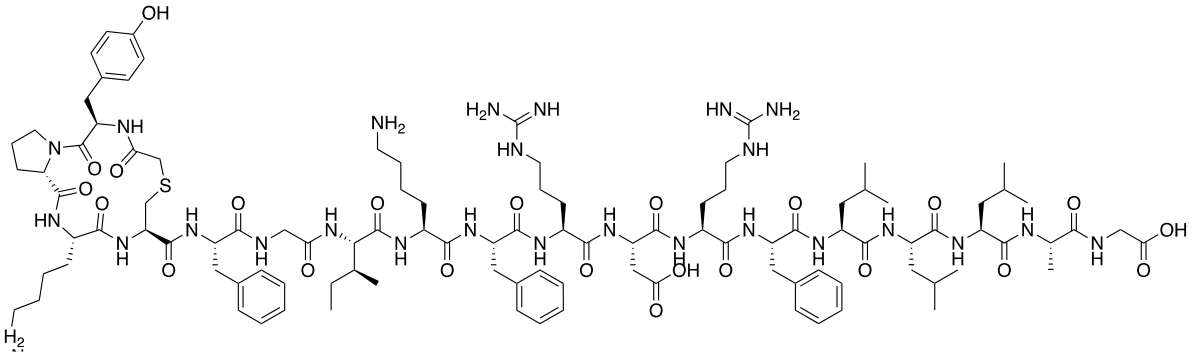




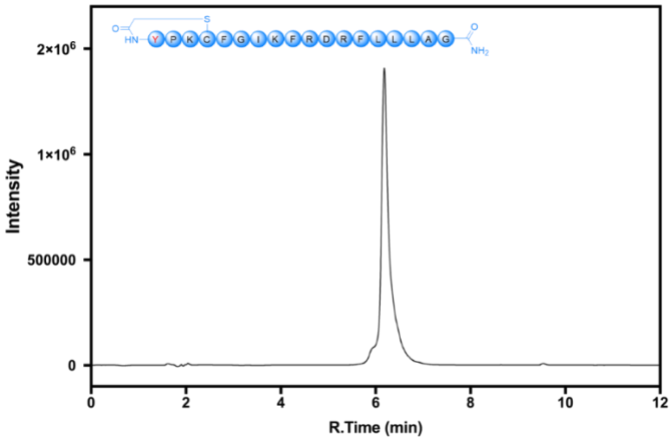
Peptide 9: Rink Amide AM resin (146 mg, 100 µmol, 0.684 mmol/g) was used for Fmoc solid phase peptide synthesis (Fmoc SPPS) according to general procedure **A**. After checking the crude peptide by LC-MS, a portion (25 µmol) of the peptide was capped with chloroacetyl chloride following general procedure **B**. The peptide was deprotected and cleaved from the resin according to general procedure **C**. Subsequently, the peptide was cyclized following general procedure **D** and purified by preparative HPLC (0-100%, buffer B) affording cyclic peptide **9** as a white solid (4.9 mg, 9.1%). HRMS (m/z): $[M+2H]^{2+}$ calculated for $C_{105}H_{161}N_{27}O_{22}S^{2+}$, 1092.1015, found 1092.1008. LC-MS R_t 6.07 min (0 to 100 % B over 12 min, 0.1% FA, λ = 214 nm).

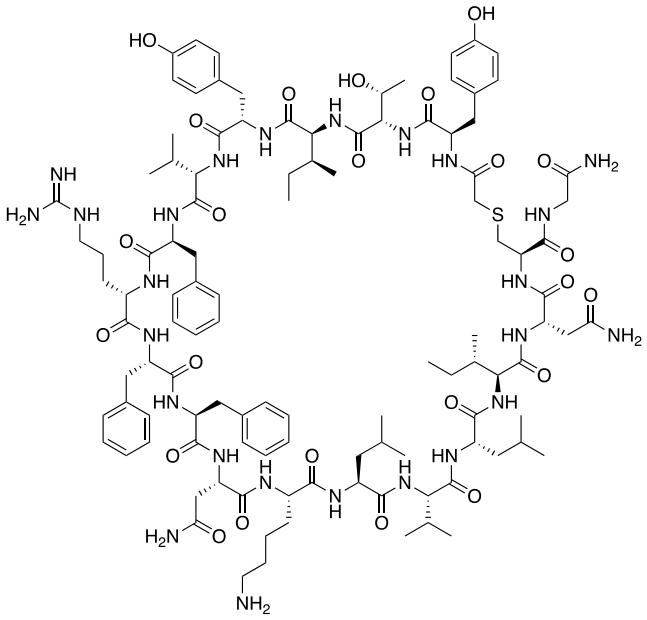


Peptide 10:

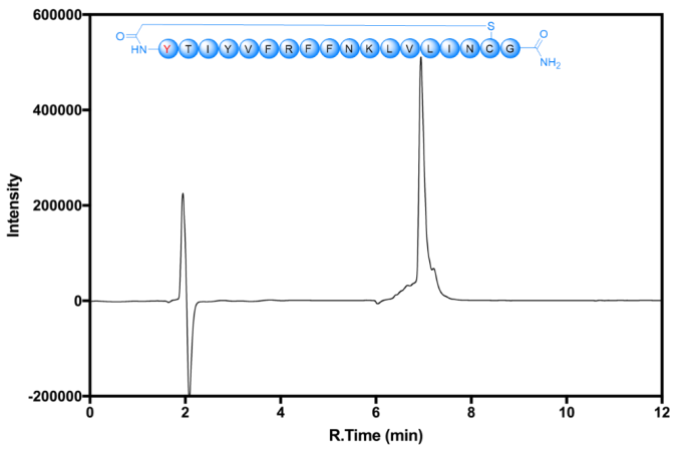


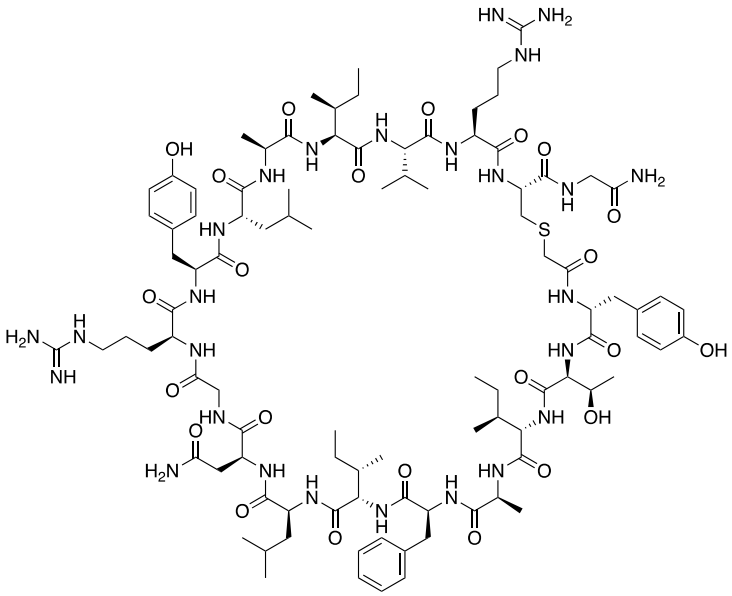
Rink Amide AM resin (146 mg, 100 µmol, 0.684 mmol/g) was used for Fmoc solid phase peptide synthesis (Fmoc SPPS) according to general procedure **A**. After checking the crude peptide by LC-MS, a portion (25 µmol) of the peptide was capped with chloroacetyl chloride following general procedure **B**. The peptide was deprotected and cleaved from the resin according to general procedure **C**. Subsequently, the peptide was cyclized following general procedure **D** and purified by preparative HPLC (0-100%, buffer B) affording cyclic peptide **10** as a white solid (5.4 mg, 10.2%). HRMS (m/z): $[M+2H]^{2+}$ calculated for $C_{105}H_{161}N_{27}O_{22}S^{2+}$, 1092.1015, found 1092.1006. LC-MS R_t 6.18 min (0 to 100 % B over 12 min, 0.1% FA, λ = 214 nm).



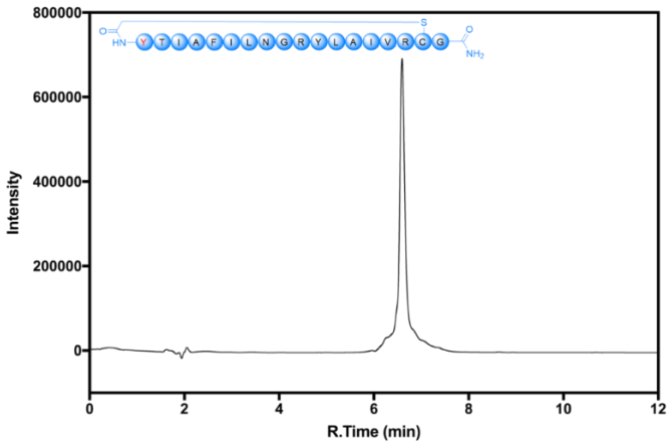


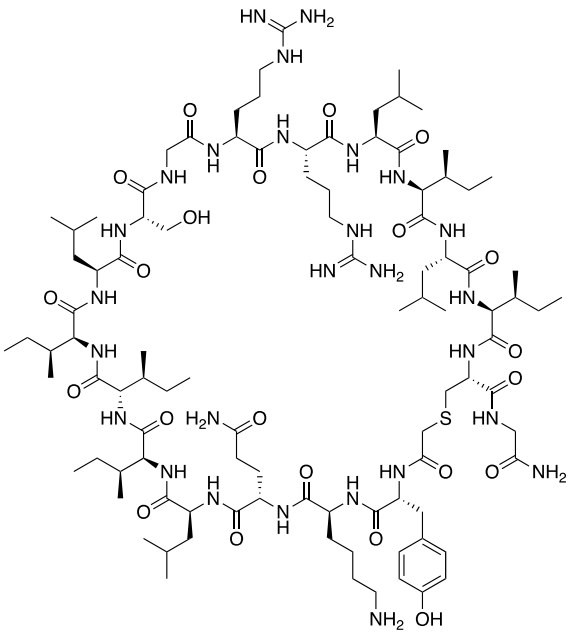
Peptide 11: Rink Amide AM resin (146 mg, 100 µmol, 0.684 mmol/g) was used for Fmoc solid phase peptide synthesis (Fmoc SPPS) according to general procedure **A**. After checking the crude peptide by LC-MS, a portion (25 µmol) of the peptide was capped with chloroacetyl chloride following general procedure **B**. The peptide was deprotected and cleaved from the resin according to general procedure **C**. Subsequently, the peptide was cyclized following general procedure **D** and purified by preparative HPLC (0-100%, buffer B) affording cyclic peptide **11** as a white solid (6.1 mg, 10.8%). HRMS (m/z): $[M+2H]^{2+}$ calculated for $C_{110}H_{163}N_{25}O_{24}S^{2+}$, 1125,1012, found 1125.1005. LC-MS R_t 6.67 min (0 to 100 % B over 12 min, 0.1% FA, λ = 214 nm).



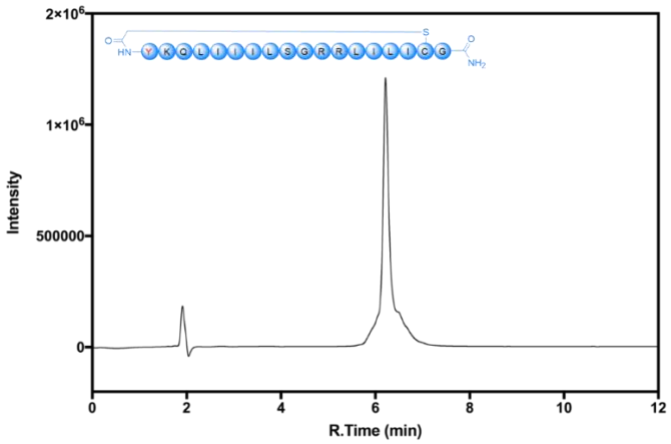


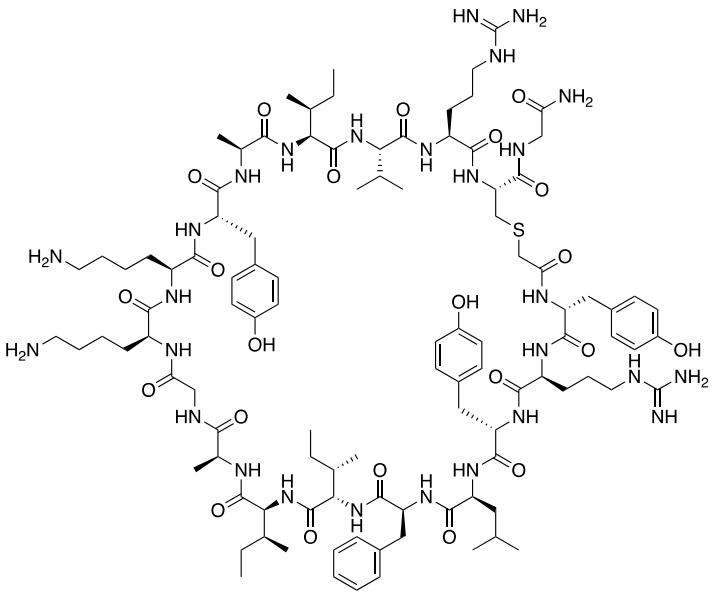
Peptide 12: Rink Amide AM resin (146 mg, 100 µmol, 0.684 mmol/g) was used for Fmoc solid phase peptide synthesis (Fmoc SPPS) according to general procedure **A**. After checking the crude peptide by LC-MS, a portion (25 µmol) of the peptide was capped with chloroacetyl chloride following general procedure **B**. The peptide was deprotected and cleaved from the resin according to general procedure **C**. Subsequently, the peptide was cyclized following general procedure **D** and purified by preparative HPLC (0-100%, buffer B) affording cyclic peptide **12** as a white solid (4.4 mg, 8.5%). HRMS (m/z): $[M+2H]^{2+}$ calculated for $C_{97}H_{154}N_{26}O_{23}S^{2+}$, 1041.5700, found 1041.5696. LC-MS R_t 6.59 min (0 to 100 % B over 12 min, 0.1% FA, λ = 214 nm).



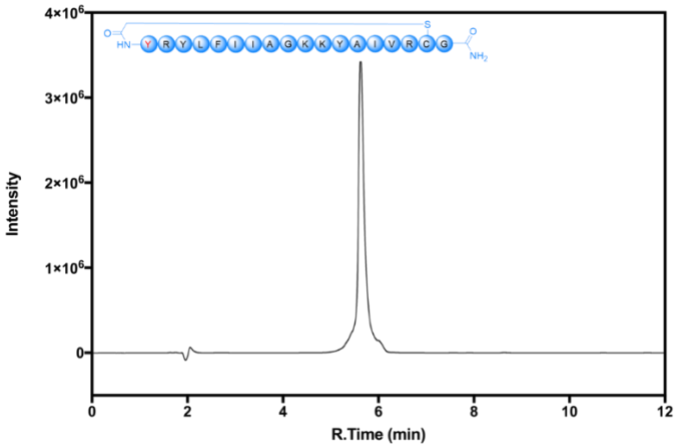


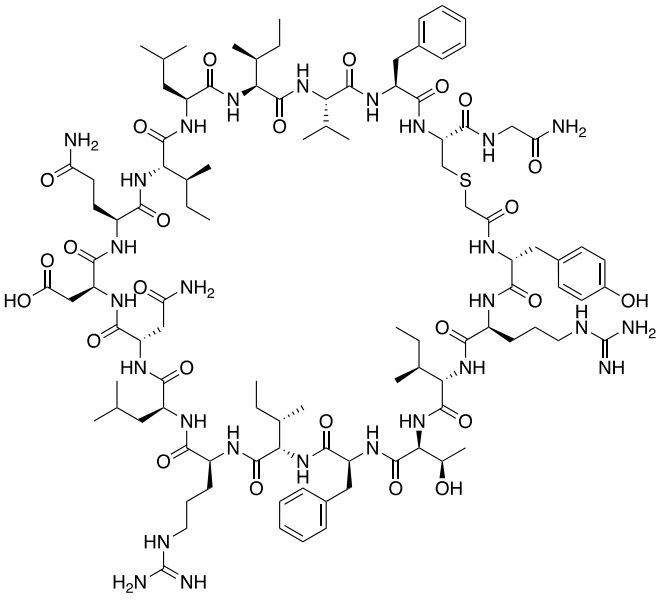
Peptide 13: Rink Amide AM resin (146 mg, 100 µmol, 0.684 mmol/g) was used for Fmoc solid phase peptide synthesis (Fmoc SPPS) according to general procedure **A**. After checking the crude peptide by LC-MS, a portion (25 µmol) of the peptide was capped with chloroacetyl chloride following general procedure **B**. The peptide was deprotected and cleaved from the resin according to general procedure **C**. Subsequently, the peptide was cyclized following general procedure **D** and purified by preparative HPLC (0-100%, buffer B) affording cyclic peptide **13** as a white solid (5.2 mg, 10.0%). HRMS (m/z): $[M+2H]^{2+}$ calculated for $C_{98}H_{173}N_{27}O_{22}S^{2+}$, 1056.1485, found 1056.1474. LC-MS R_t 6.21 min (0 to 100 % B over 12 min, 0.1% FA, λ = 214 nm).



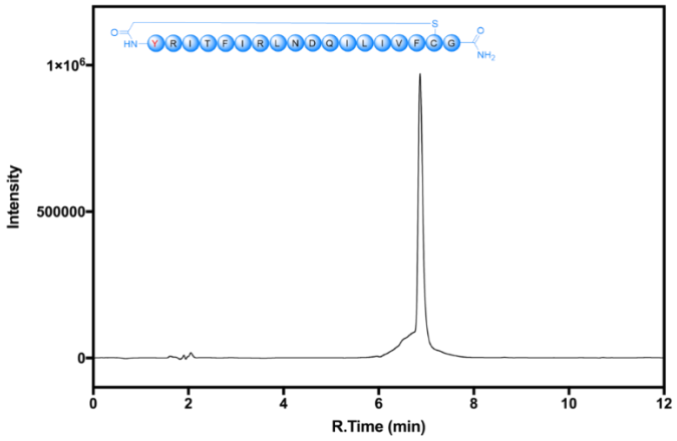


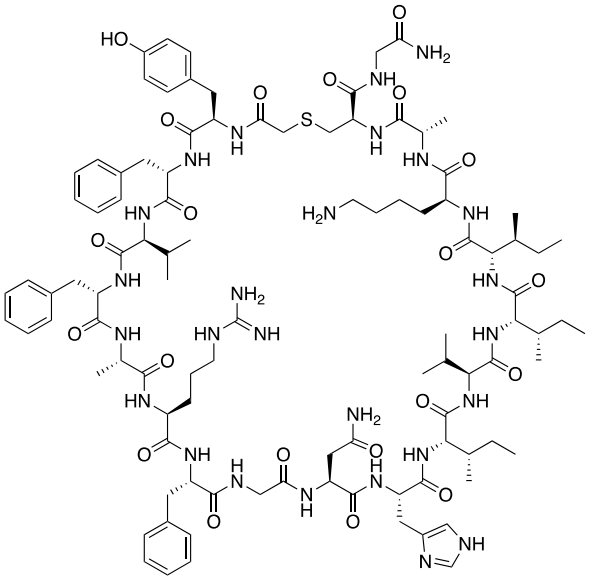
Peptide 14: Rink Amide AM resin (146 mg, 100 µmol, 0.684 mmol/g) was used for Fmoc solid phase peptide synthesis (Fmoc SPPS) according to general procedure **A**. After checking the crude peptide by LC-MS, a portion (25 µmol) of the peptide was capped with chloroacetyl chloride following general procedure **B**. The peptide was deprotected and cleaved from the resin according to general procedure **C**. Subsequently, the peptide was cyclized following general procedure **D** and purified by preparative HPLC (0-100%, buffer B) affording cyclic peptide **14** as a white solid (4.7 mg, 8.7%). HRMS (m/z): $[M+2H]^{2+}$ calculated for $C_{104}H_{163}N_{27}O_{22}S^{2+}$, 1087.1093, found 1087.1088. LC-MS R_t 5.64 min (0 to 100 % B over 12 min, 0.1% FA, λ = 214 nm).



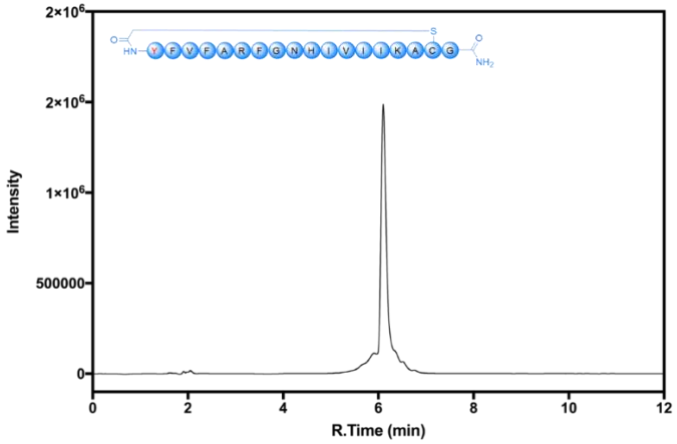


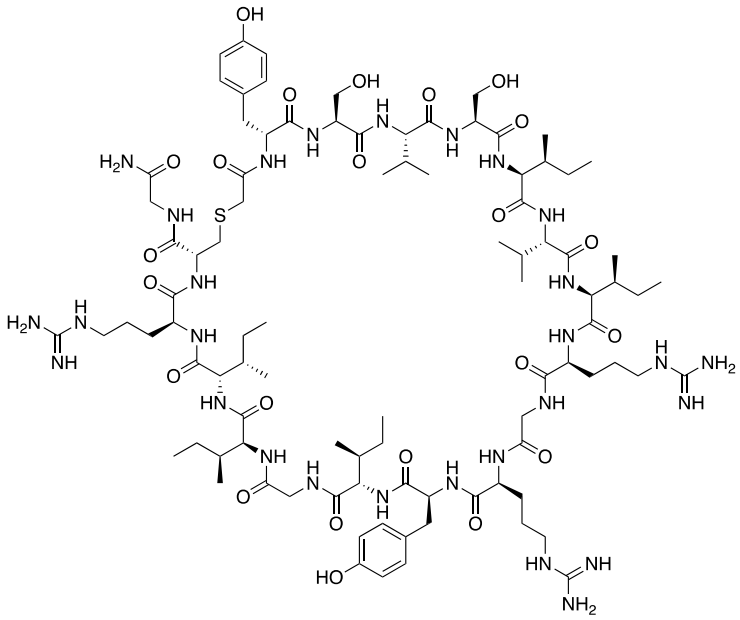
Peptide 15: Rink Amide AM resin (146 mg, 100 µmol, 0.684 mmol/g) was used for Fmoc solid phase peptide synthesis (Fmoc SPPS) according to general procedure **A**. After checking the crude peptide by LC-MS, a portion (25 µmol) of the peptide was capped with chloroacetyl chloride following general procedure **B**. The peptide was deprotected and cleaved from the resin according to general procedure **C**. Subsequently, the peptide was cyclized following general procedure **D** and purified by preparative HPLC (0-100%, buffer B) affording cyclic peptide **15** as a white solid (5.9 mg, 10.6%). HRMS (m/z): $[M+2H]^{2+}$ calculated for $C_{104}H_{165}N_{27}O_{25}S^{2+}$, 1112.1095, found 1112.1095. LC-MS R_t 6.87 min (0 to 100 % B over 12 min, 0.1% FA, λ = 214 nm).



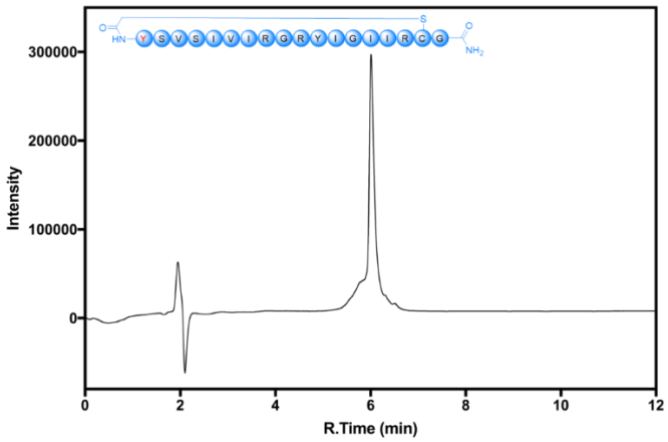


Peptide 16: Rink Amide AM resin (146 mg, 100 µmol, 0.684 mmol/g) was used for Fmoc solid phase peptide synthesis (Fmoc SPPS) according to general procedure **A**. After checking the crude peptide by LC-MS, a portion (25 µmol) of the peptide was capped with chloroacetyl chloride following general procedure **B**. The peptide was deprotected and cleaved from the resin according to general procedure **C**. Subsequently, the peptide was cyclized following general procedure **D** and purified by preparative HPLC (0-100%, buffer B) affording cyclic peptide **16** as a white solid (4.7 mg, 9.0%). HRMS (m/z): $[M+2H]^{2+}$ calculated for $C_{101}H_{150}N_{26}O_{21}S^{2+}$, 1047.5595, found 1047.5594. LC-MS R_t 6.10 min (0 to 100 % B over 12 min, 0.1% FA, λ = 214 nm).





Peptide 17: Rink Amide AM resin (146 mg, 100 µmol, 0.684 mmol/g) was used for Fmoc solid phase peptide synthesis (Fmoc SPPS) according to general procedure **A**. After checking the crude peptide by LC-MS, a portion (25 µmol) of the peptide was capped with chloroacetyl chloride following general procedure **B**. The peptide was deprotected and cleaved from the resin according to general procedure **C**. Subsequently, the peptide was cyclized following general procedure **D** and purified by preparative HPLC (0-100%, buffer B) affording cyclic peptide **17** as a white solid (5.6 mg, 10.9%). HRMS (m/z): $[M+2H]^{2+}$ calculated for $C_{93}H_{156}N_{28}O_{23}S^{2+}$, 1032.5809, found 1032.5797. LC-MS R_t 6.01 min (0 to 100 % B over 12 min, 0.1% FA, λ = 214 nm).



IC_{50} curves

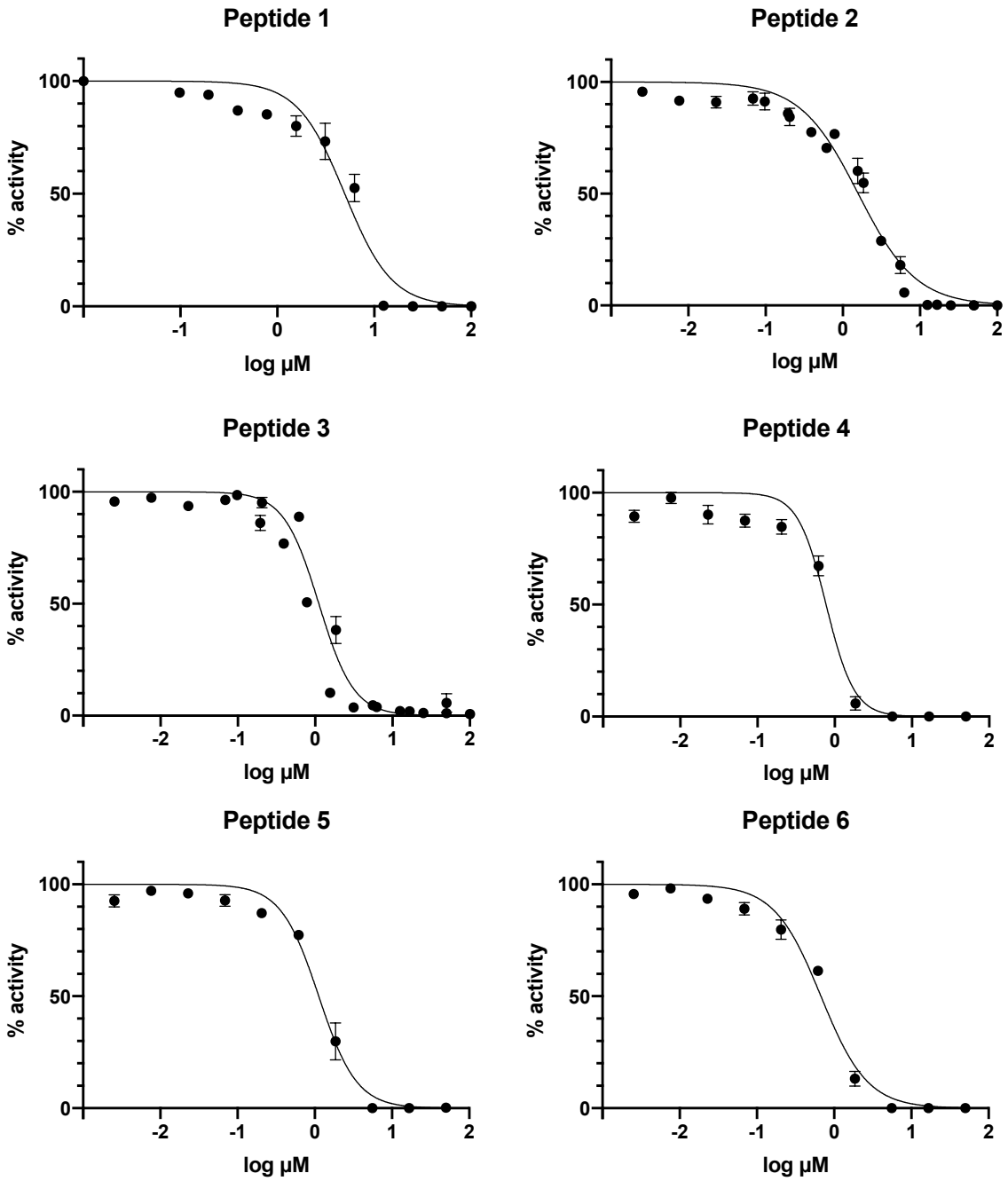


Figure S3. *IC_{50} curves for peptides **1**-**6** against hNNMT. Data is based on triplicate data of at least 10 different concentrations*

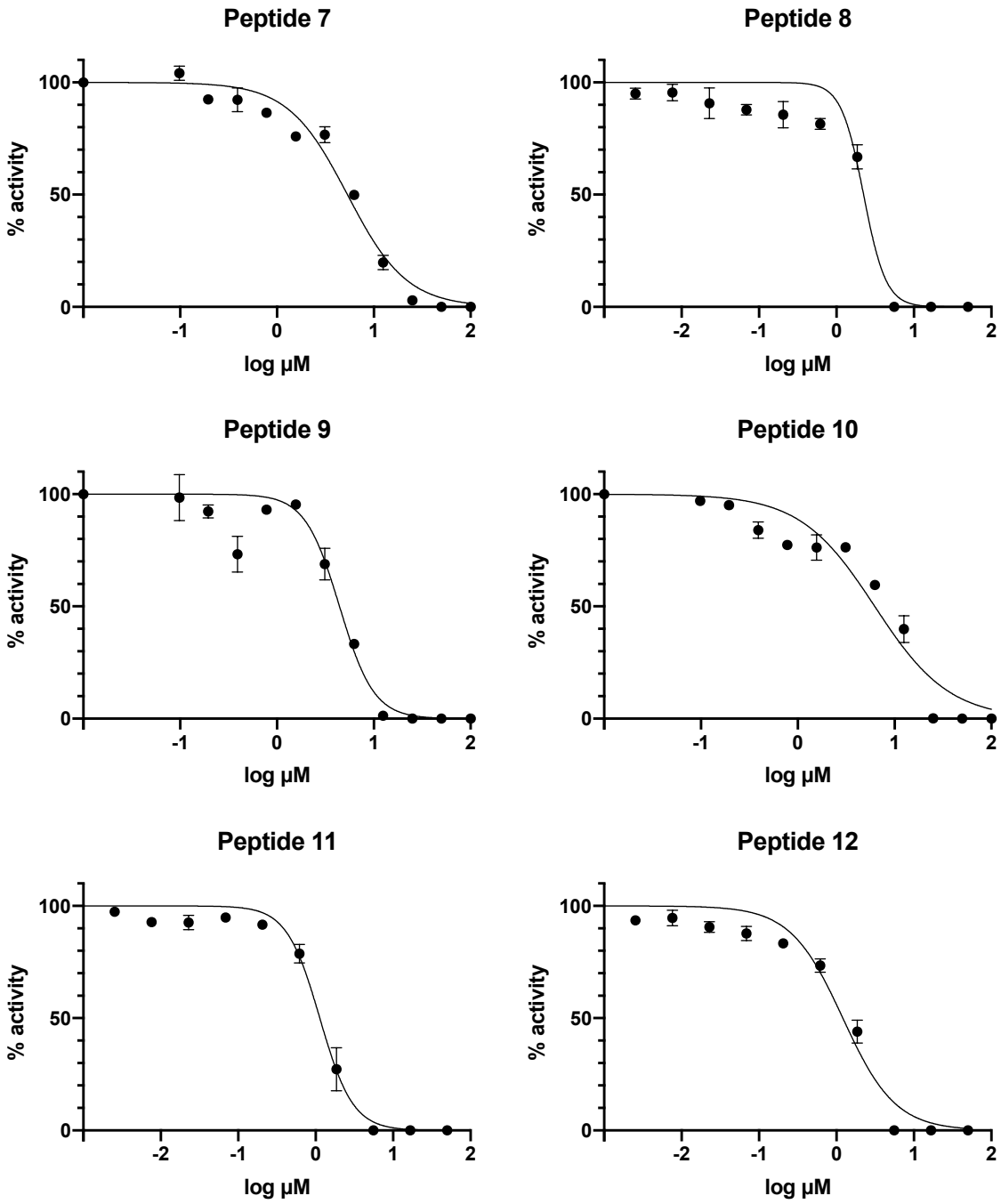


Figure S4. *IC_{50} curves for peptides **7**-**12** against hNNMT. Data is based on triplicate data of at least 10 different concentrations*

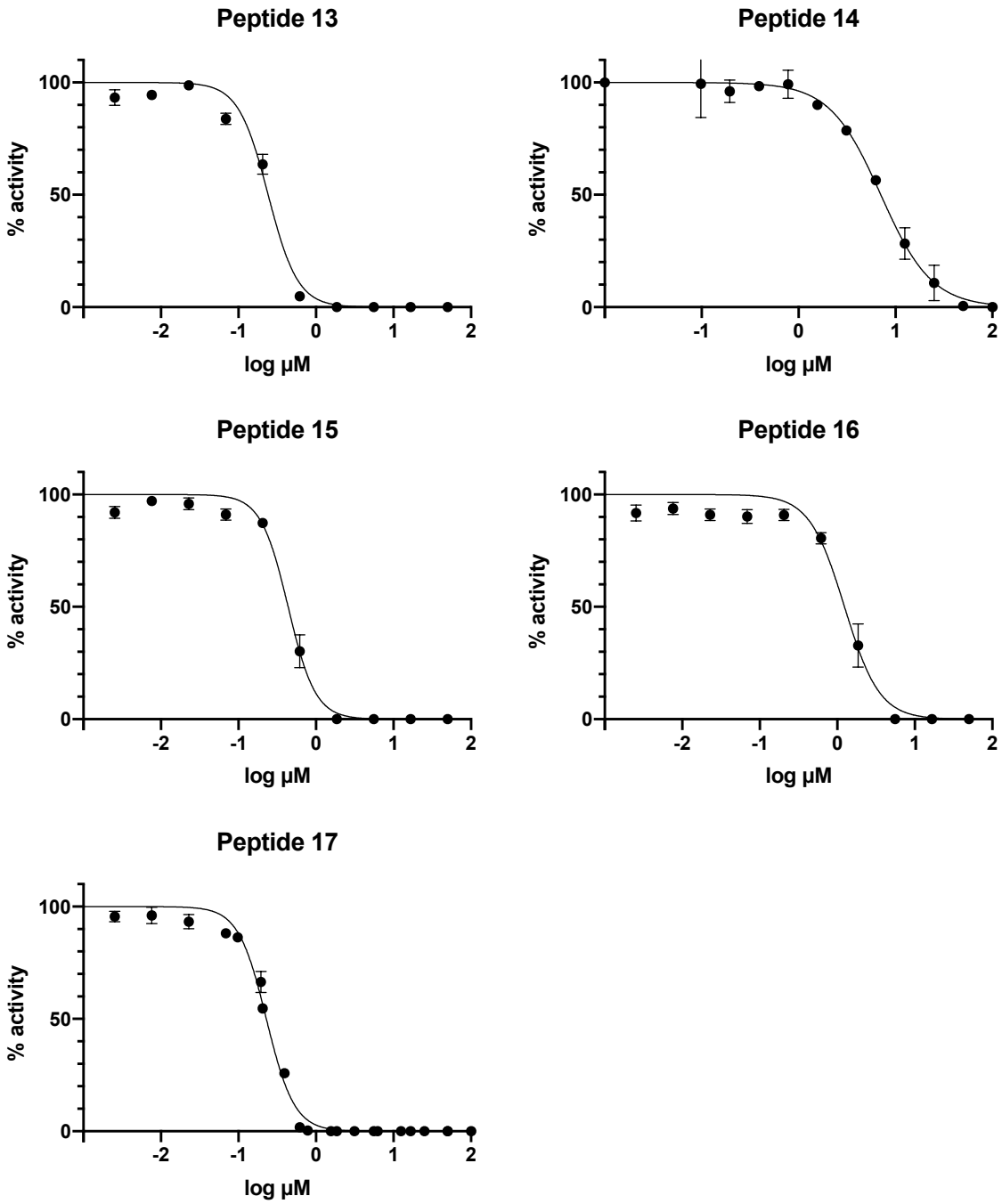


Figure S5. *IC_{50} curves for peptides **13-17** against hNNMT. Data is based on triplicate data of at least 10 different concentrations*

IC_{50} curves substrate competition

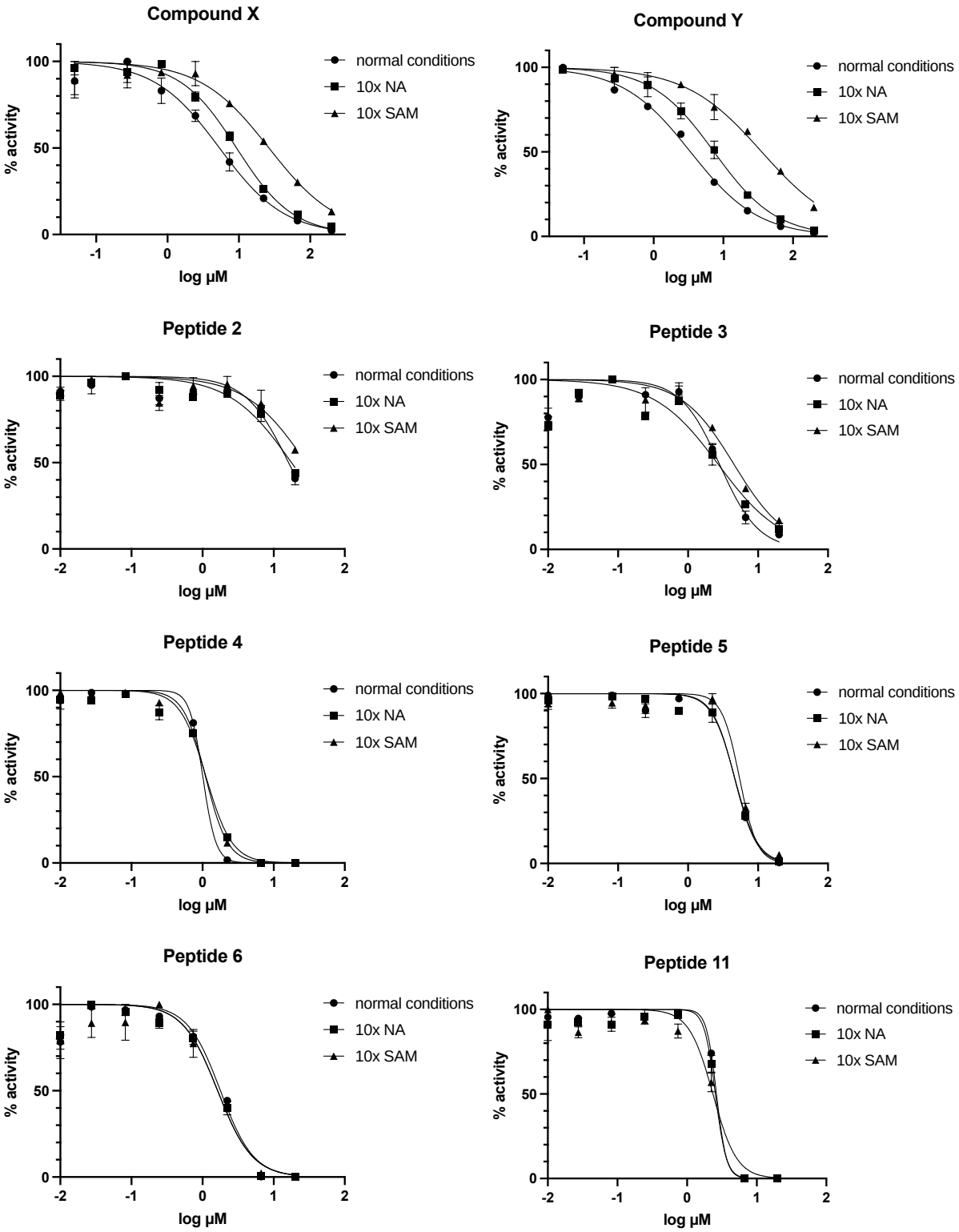


Figure S6. *IC_{50} curves for compounds **X** and **Y** and peptides **2**-**6** and **11** against hNNMT. Compounds were tested using normal conditions (substrates at their K_M value), or in the presence of 10-fold higher concentration of either nicotinamide (NA) or S-adenosyl-L-methionine (SAM). Data is based on duplicate data of at 8 different concentrations.*

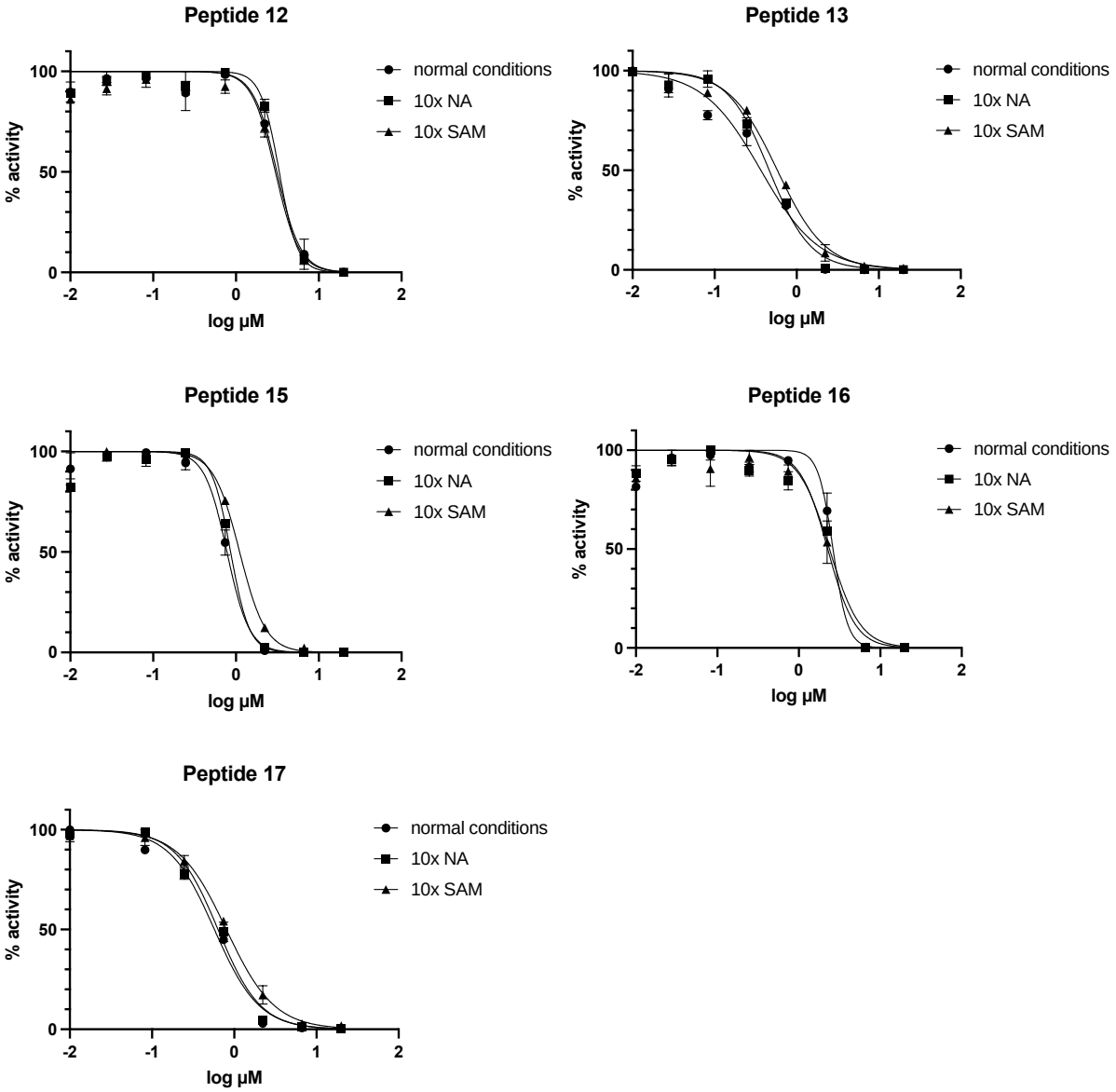
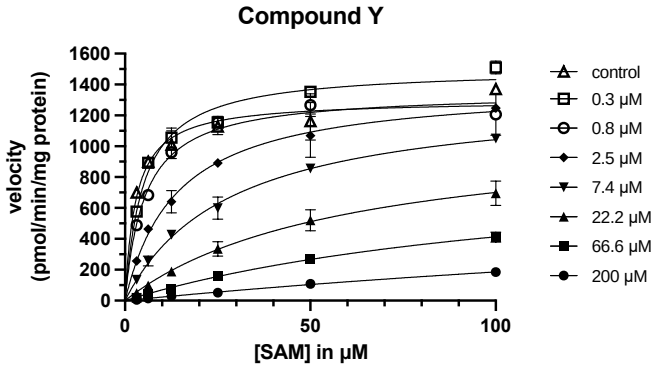
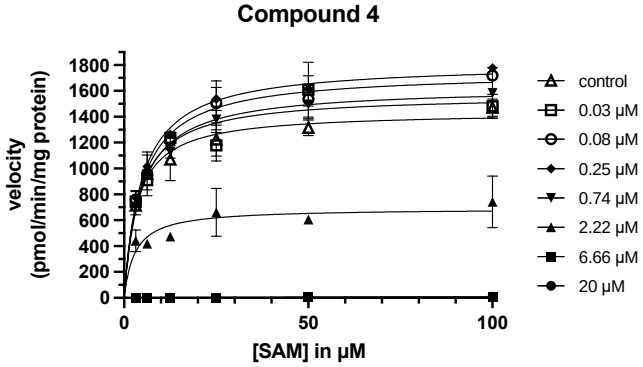


Figure S7. *IC_{50} curves for peptides **12**-**13** and **15-17** against hNNMT. Peptides were tested using normal conditions (substrates at their K_M value), or in the presence of 10-fold higher concentration of either nicotinamide (NA) or S-adenosyl-L-methionine (SAM). Data is based on duplicate data of at 8 different concentrations.*

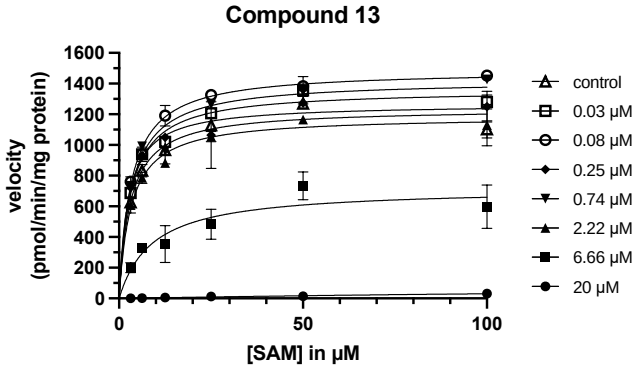
Kinetic analysis mode of inhibition



Compound **Y**	V_{max} (pmol/min/mg)	K_M (µM)
200 µM	853,66 ± 280,87	359,80 ± 144,56
66.6 µM	920,18 ± 95,95	122,58 ± 20,00
22.2 µM	1115,25 ± 107,57	59,12 ± 11,21
7.4 µM	1337,44 ± 47,71	28,24 ± 2,52
2.5 µM	1396,86 ± 50,82	14,00 ± 1,58
0.8 µM	1350,80 ± 43,44	5,42 ± 0,72
0.3 µM	1503,99 ± 40,47	5,03 ± 0,58
control	1299,61 ± 42,57	2,93 ± 0,50



Compound **4**	V_{max} (pmol/min/mg)	K_M (µM)
20 µM	-	-
6.66 µM	-	-
2.22 µM	690.79 ± 60.07	2.92 ± 1.34
0.74 µM	1627.80 ± 52.10	4.51 ± 0.64
0.25 µM	1811.27 ± 40.56	4.73 ± 0.46
0.08 µM	1744.78 ± 50.42	4.74 ± 0.59
0.03 µM	1568.55 ± 79.20	4.00 ± 0.93
control	1437.15 ± 55.94	3.41 ± 0.65



Compound **13**	V_{max} (pmol/min/mg)	K_M (µM)
20 µM	-	-
6.66 µM	719,53 ± 76,43	9,08 ± 3.39
2.22 µM	1186,86 ± 32,66	3,23 ± 0.45
0.74 µM	1421,84 ± 47,38	3,29 ± 0.55
0.25 µM	1266,71 ± 47,37	2,41 ± 0.52
0.08 µM	1487,49 ± 18,05	3,22 ± 0.20
0.03 µM	1360,22 ± 32,96	3,11 ± 0.39
control	1237,38 ± 37,19	3,03 ± 0.47

***Figure S8**. V_{max} and K_M values for NNMT and SAM respectively after treatment of varying concentrations of compound **Y, 4** or **13**. The change in K_M observed for SAM after treatment with compound **Y** supports competitive inhibition. The unchanged K_M and changing V_{max} observed for compounds **4** and **13** supports the non-competitive or allosteric mode of inhibition for the cyclic peptides.*