SUPPORTING INFORMATION

Uncovering the Selection Criteria for the Emergence of Multi-Building-Block Replicators from Dynamic Combinatorial Libraries

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HPLC methods

Solutions containing peptides **2**-**7** and their oxidation products were analyzed using the following methods (all gradients are linear). For peptides **2**, **3**, **4** and **7**, Solvent A: double distilled water (0.1 v% trifluoroacetic acid), Solvent B: acetonitrile (0.1 v% trifluoroacetic acid). For peptides **5** and **6**, Solvent A: double distilled water (0.2 v% heptafluorobutyric acid), Solvent B: acetonitrile (0.2 v% heptafluorobutyric acid).

Peptide **2** (phenyl hexyl column)

Time (min)	A%	B%
0	75	25
30	55	45

Peptide **3** (phenyl hexyl column)

Time (min)	A%	B%
0	75	25
60	60	40

Peptide **4** (phenyl hexyl column)

Time (min)	A%	B%
0	82	18
60	62	38

Peptide **5** (Prodigy column)

Time (min)	A%	B%
0	68	32
1.5	68	32
5	61.5	38.5
7	55	45
8	5	95
9	5	95

Peptide **6** (Prodigy column)

Time (min)	A%	B%
0	62	38
6.5	60	40
8	55	45
9	5	95
10	5	95

Peptide **7** (phenyl hexyl column)

Time (min)	A%	B%
0	81	19
40	74	26

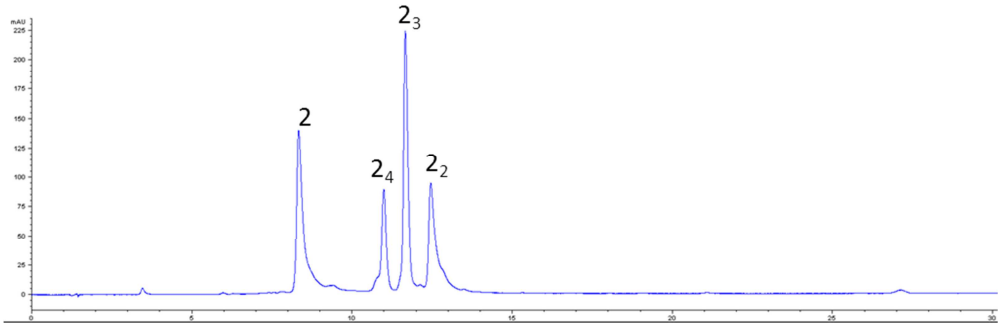

Figure S1. HPLC trace (monitored at 254 nm) of the product mixture obtained by oxidation of peptide **2** (3.8 mM) after stirring at 1200 rpm for 23 days.

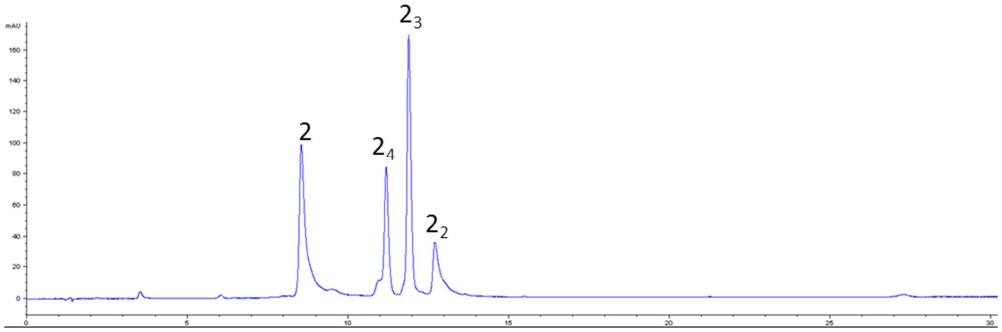

Figure S2. HPLC trace (monitored at 254 nm) of the product mixture obtained by oxidation of peptide **2** (3.8 mM) after shaking at 1200 rpm for 12 days.

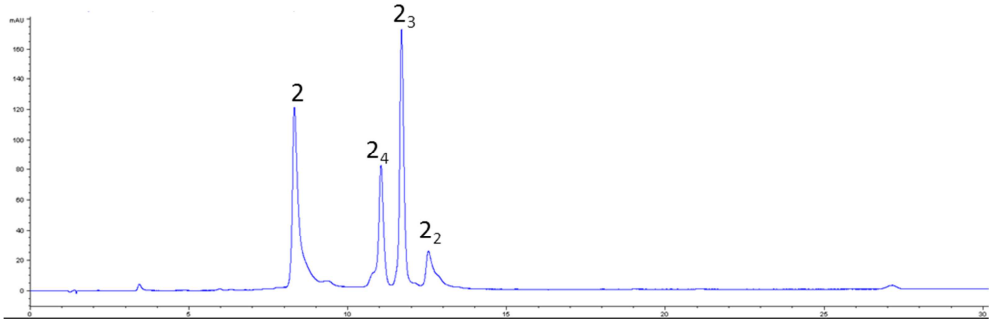

Figure S3. HPLC trace (monitored at 254 nm) of the product mixture obtained by oxidation of peptide **2** (3.8 mM) after 23 days with no agitation.

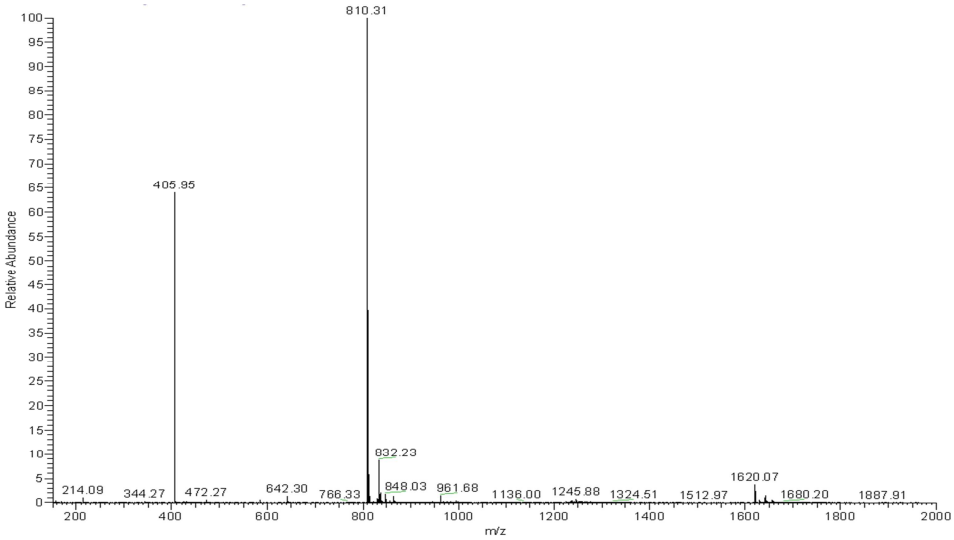

Figure S4. Mass spectrum of building block **2** from the LC-MS analysis of a stirred library made from peptide **2** (corresponding to Figure S1). Calculated isotopic profile (species, abundance): 809.36 (M, 100%), 810.36 (M+1, 47.7%), 811.36 (M+2, 12.4%); m/z calculated: 810.36 $[M+H]^{1+}$, 405.68 $[M+2H]^{2+}$; m/z observed: 810.31 $[M+H]^{1+}$, 405.95 $[M+2H]^{2+}$.

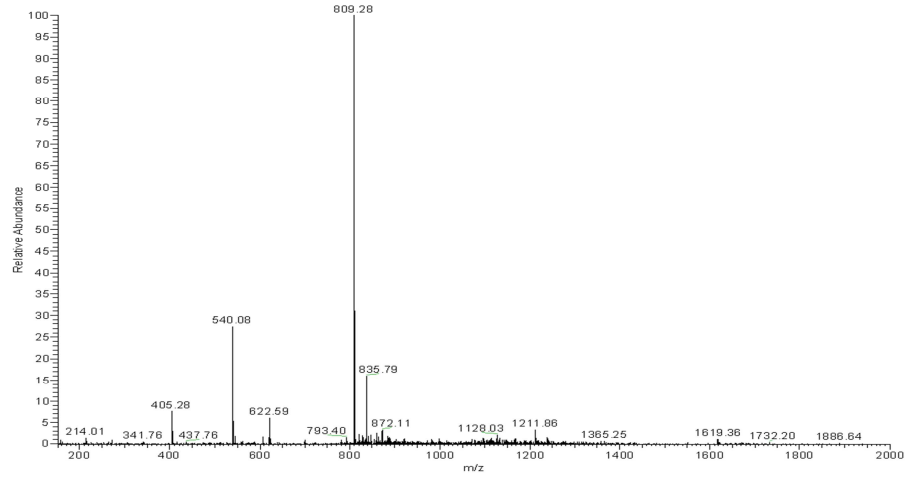

Figure S5. Mass spectrum of linear dimer $\mathbf{2}_2$ from the LC-MS analysis of a stirred library made from peptide **2** (corresponding to Figure S1). Calculated isotopic profile (species, abundance): 1616.71 (M, 100%), 1617.71 (M+1, 88.3%), 1618.71 (M+2, 48.8%); m/z calculated: 809.36 $[M+2H]^{2+}$, 539.9 $[M+3H]^{3+}$, 405.16 $[M+4H]^{4+}$; m/z observed: 809.28 $[M+2H]^{2+}$, 540.08 $[M+3H]^{3+}$, 405.28 $[M+4H]^{4+}$.

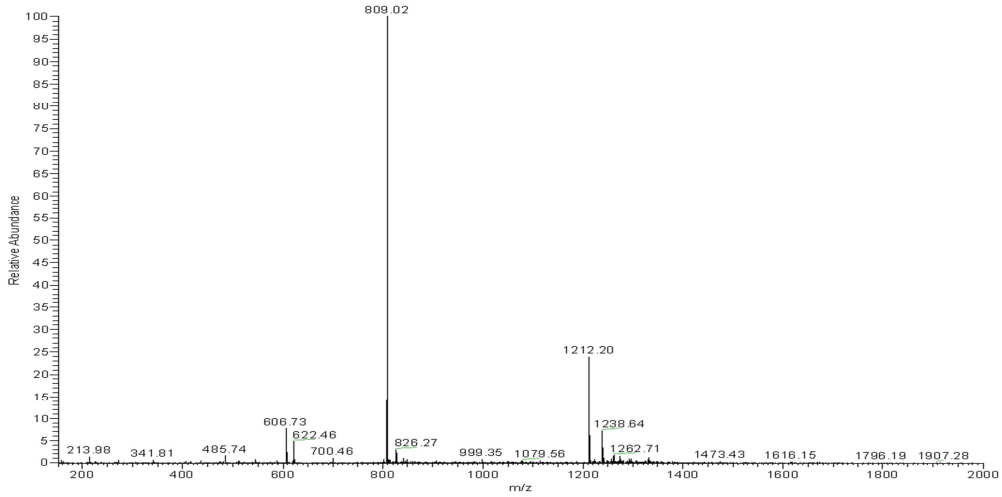

Figure S6. Mass spectrum of cyclic trimer $\mathbf{2}_3$ from the LC-MS analysis of a stirred library made from peptide **2** (corresponding to Figure S1). Calculated isotopic profile (species, abundance): 2423.04 (M+1, 100%), 2422.03 (M, 75.5%), 2424.04 (M+2, 73.9%); m/z calculated: 1212.52 $[(M+1)+2H]^{2+}$, 808.68 $[(M+1)+3H]^{3+}$, 606.76 $[(M+1)+4H]^{4+}$, 485.61 $[(M+1)+5H]^{5+}$; m/z observed: 1212.20 $[(M+1)+2H]^{2+}$, 808.02 $[(M+1)+3H]^{3+}$, 606.73 $[(M+1)+4H]^{4+}$, 485.74 $[(M+1)+5H]^{5+}$.

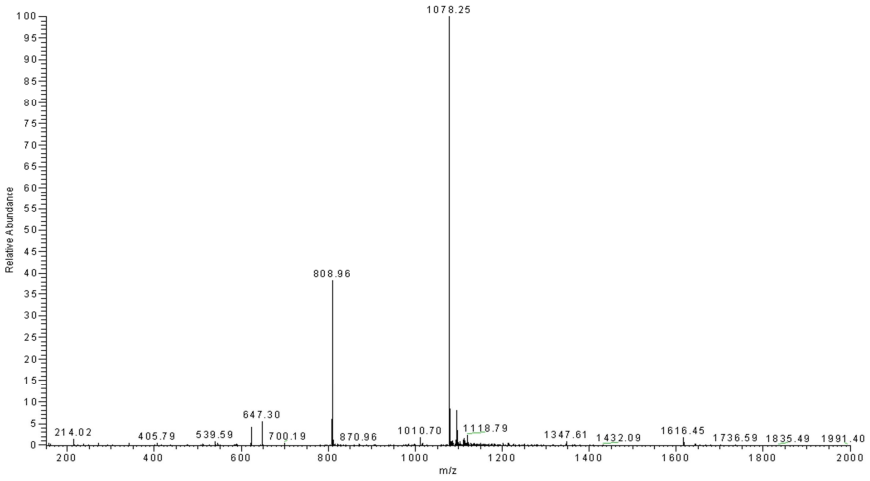

Figure S7. Mass spectrum of cyclic tetramer $\mathbf{2}_4$ from the LC-MS analysis of a stirred library made from peptide **2** (corresponding to Figure S1). Calculated isotopic profile (species, abundance): 3230.38 (M+1, 100%), 3231.39 (M+2, 81.1%), 3232.39 (M+3, 57.9%); m/z calculated: 1616.14 $[(M+1)+2H]^{2+}$, 1077.80 $[(M+1)+3H]^{3+}$, 808.59 $[(M+1)+4H]^{4+}$, 647.05 $[(M+1)+5H]^{5+}$; m/z observed: 1616.45 $[(M+1)+2H]^{2+}$, 1078.25 $[(M+1)+3H]^{3+}$, 808.96 $[(M+1)+4H]^{4+}$, 647.30 $[(M+1)+5H]^{5+}$.

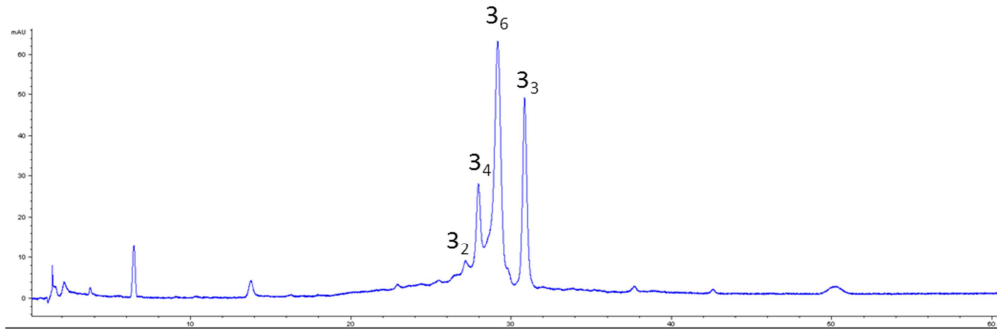

Figure S8. HPLC trace (monitored at 254 nm) of the product mixture obtained by oxidation of peptide **3** (3.8 mM) after stirring at 1200 rpm for 7 days.

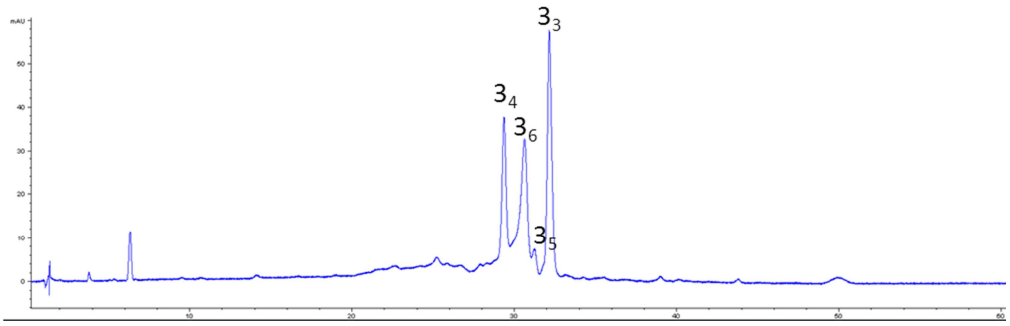

Figure S9. HPLC trace (monitored at 254 nm) of the product mixture obtained by oxidation of peptide **3** (3.8 mM) after shaking at 1200 rpm for 14 days.

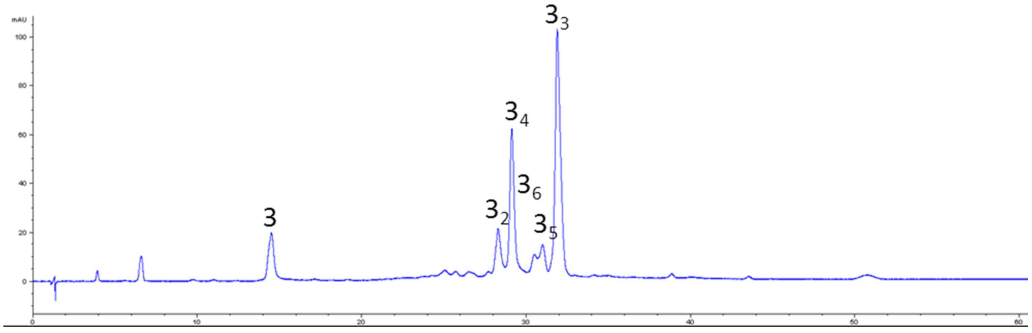

Figure S10. HPLC trace (monitored at 254 nm) of the product mixture obtained by oxidation of peptide **3** (3.8 mM) after 7 days with no agitation.

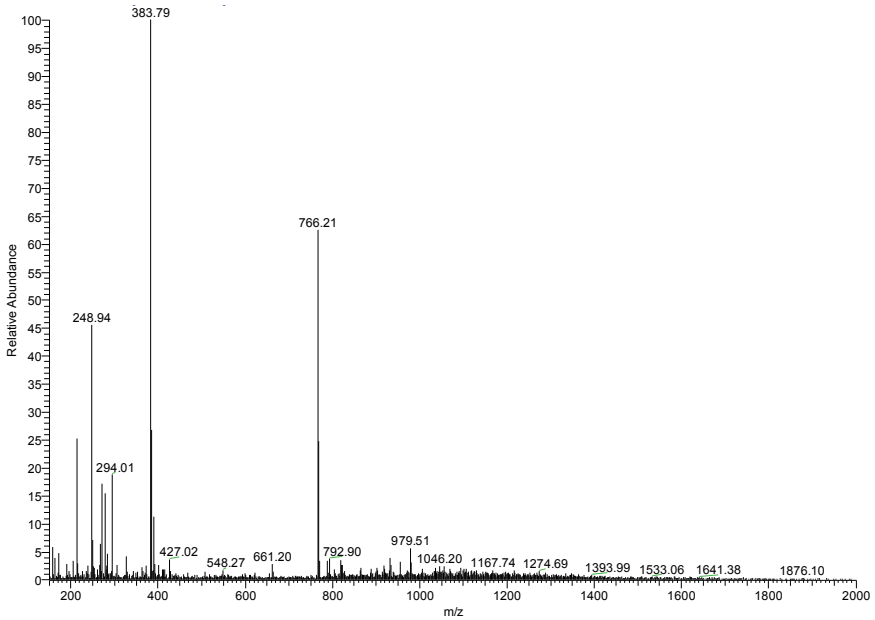

Figure S11. Mass spectrum of dithiol **3** from the LC-MS analysis of a stirred library made from peptide **3** (corresponding to Figure S8). Calculated isotopic profile (species, abundance): 765.39 (M, 100%), 766.4 (M+1, 39.9%), 767.39 (M+2, 10.8%); m/z calculated: 766.39 $[M+H]^{1+}$, 383.69 $[M+2H]^{2+}$; m/z observed: 766.21 $[M+H]^{1+}$, 383.79 $[M+2H]^{2+}$.

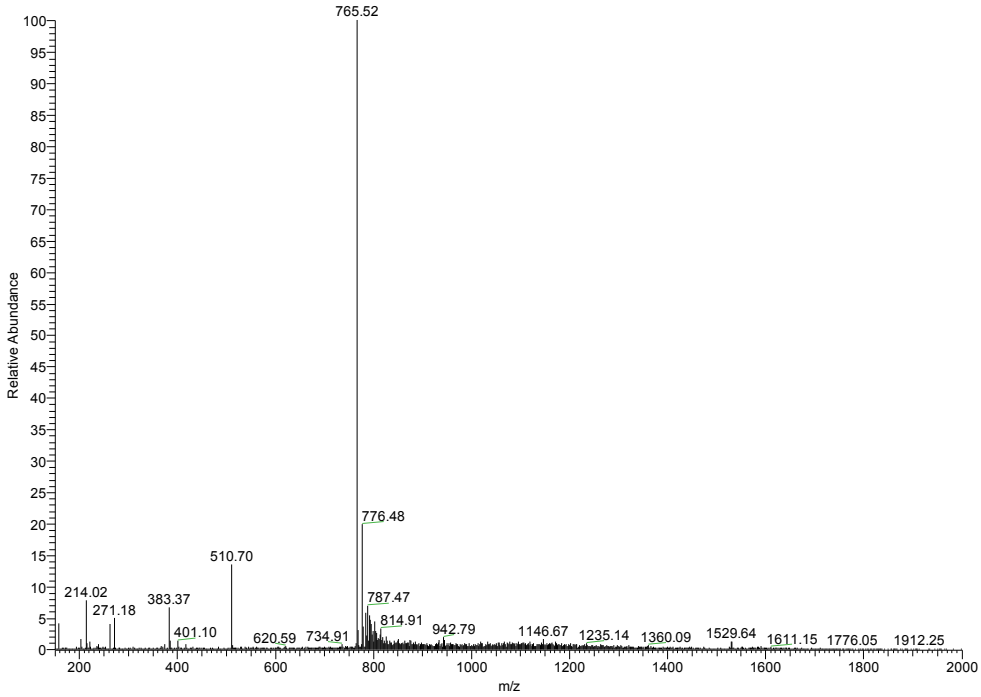

Figure S12. Mass spectrum of linear dimer $\mathbf{3}_2$ from the LC-MS analysis of a stirred library made from peptide **3** (corresponding to Figure S8). Calculated isotopic profile (species, abundance): 1528.77 (M, 100%), 1539.77 (M+1, 82.9%), 1530.77 (M+2, 39.5%); m/z calculated: 1528.77 $[M+H]^{1+}$, 765.38 $[M+2H]^{2+}$; 510.59 $[M+3H]^{3+}$; m/z observed: 1529.64 $[M+H]^{1+}$, 765.52 $[M+2H]^{2+}$, 510.70 $[M+3H]^{3+}$.

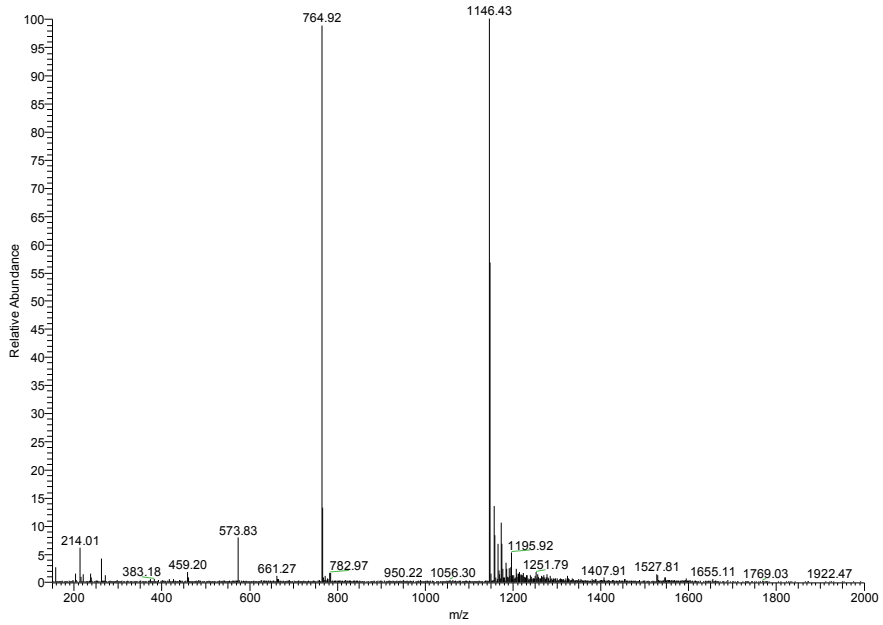

Figure S13. Mass spectrum of cyclic trimer $\mathbf{3}_3$ from the LC-MS analysis of a stirred library made from peptide **3** (corresponding to Figure S8). Calculated isotopic profile (species, abundance): 2291.13 (M+1, 100%), 2290.13 (M, 75.7%), 2292.13 (M+2, 65.9%); m/z calculated: 1146.56 $[(M+1)+2H]^{2+}$, 764.71 $[(M+1)+3H]^{3+}$; 573.78 $[(M+1)+4H]^{4+}$; m/z observed: 1146.43 $[(M+1)+2H]^{2+}$, 764.92 $[(M+1)+3H]^{3+}$; 573.83 $[(M+1)+4H]^{4+}$.

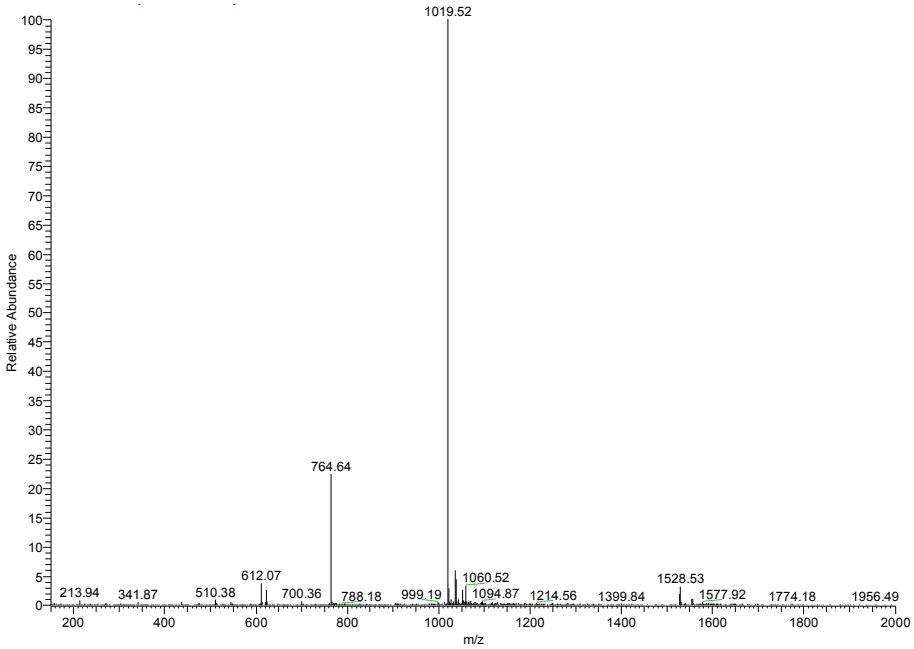

Figure S14. Mass spectrum of cyclic tetramer $\mathbf{3}_4$ from the LC-MS analysis of a stirred library made from peptide **3** (corresponding to Figure S8). Calculated isotopic profile (species, abundance): 3054.51 (M+1, 100%), 3055.51 (M+2, 89.4%), 3053.50 (M, 62.7%); m/z calculated: 1528.25 $[(M+1)+2H]^{2+}$, 1019.17 $[(M+1)+3H]^{3+}$; 764.62 $[(M+1)+4H]^{4+}$; 611.9 $[(M+1)+5H]^{5+}$, m/z observed: 1528.53 $[(M+1)+2H]^{2+}$, 1019.52 $[(M+1)+3H]^{3+}$; 764.64 $[(M+1)+4H]^{4+}$; 612.07 $[(M+1)+5H]^{5+}$.

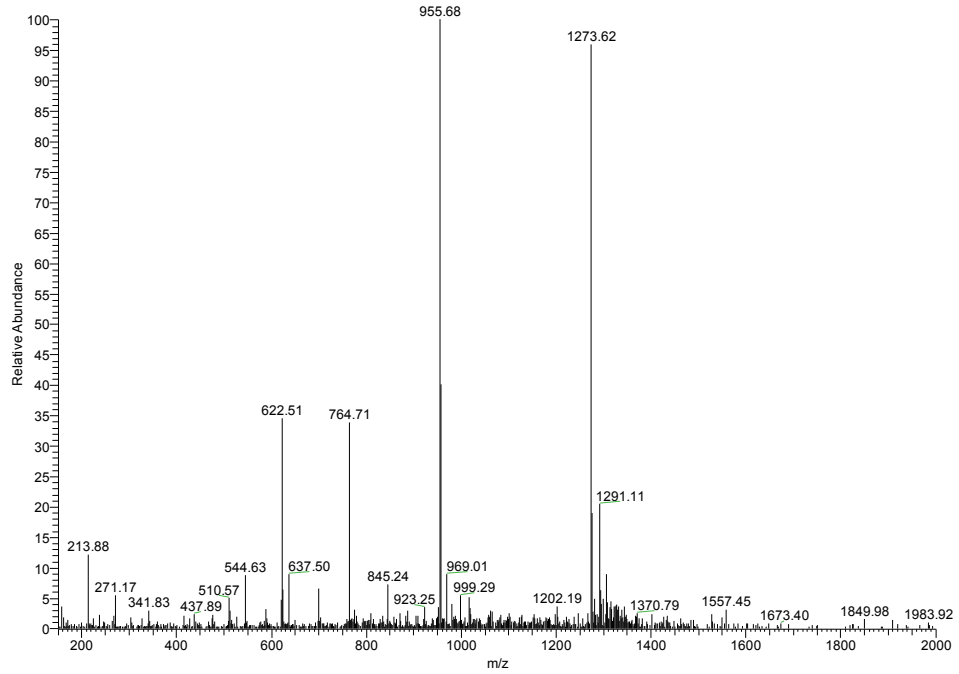

Figure S15. Mass spectrum of cyclic pentamer $\mathbf{3}_5$ from the LC-MS analysis of a stirred library made from peptide **3** (corresponding to Figure S8). Calculated isotopic profile (species, abundance): 3817.88 (M+1, 100%), 3818.89 (M+2, 87.4%), 3819.89 (M+3, 69.5%); m/z calculated: 1273.63 $[(M+1)+3H]^{3+}$, 955.47 $[(M+1)+4H]^{4+}$; 764.58 $[(M+1)+5H]^{5+}$; 637.31 $[(M+1)+6H]^{6+}$, m/z observed: 1273.62 $[(M+1)+3H]^{3+}$, 955.68 $[(M+1)+4H]^{4+}$; 764.71 $[(M+1)+5H]^{5+}$; 637.5 $[(M+1)+6H]^{6+}$.

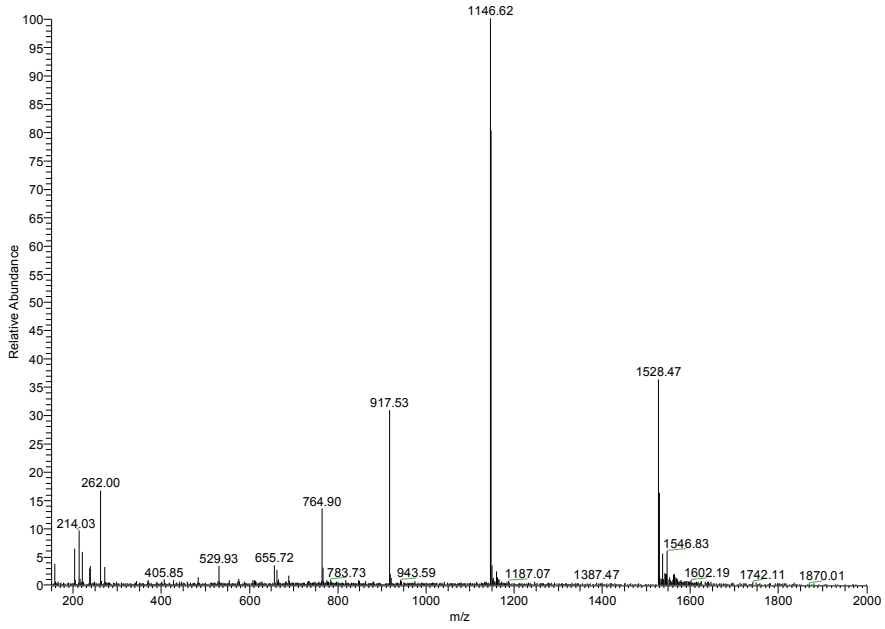

Figure S16. Mass spectrum of cyclic hexamer $\mathbf{3}_6$ from the LC-MS analysis of a stirred library made from peptide **3** (corresponding to Figure S8). Calculated isotopic profile (species, abundance): 4582.26 (M+2, 100%), 4581.26 (M+1, 71.8%), 4583.26 (M+3, 64.5%); m/z calculated: 1528.42 $[(M+2)+3H]^{3+}$, 1146.56 $[(M+2)+4H]^{4+}$; 917.45 $[(M+2)+5H]^{5+}$; 764.71 $[(M+2)+6H]^{6+}$, m/z observed: 1528.47 $[(M+2)+3H]^{3+}$, 1146.62 $[(M+2)+4H]^{4+}$; 917.53 $[(M+2)+5H]^{5+}$; 764.9 $[(M+2)+6H]^{6+}$.

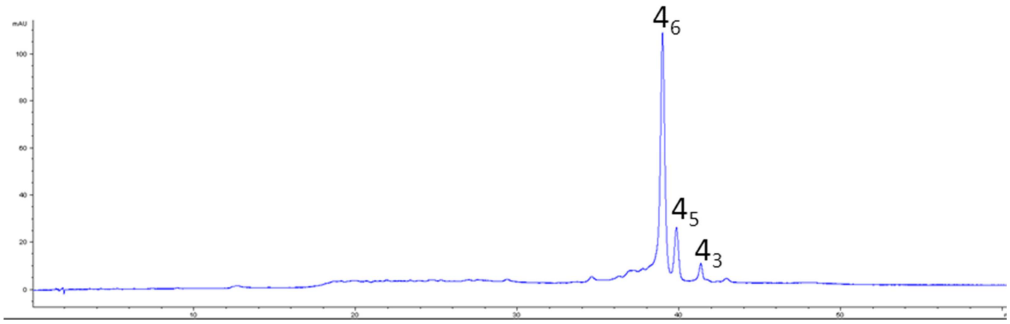

Figure S17. HPLC trace (monitored at 254 nm) of the product mixture obtained by oxidation of peptide **4** (3.8 mM) after stirring at 1200 rpm for 18 days.

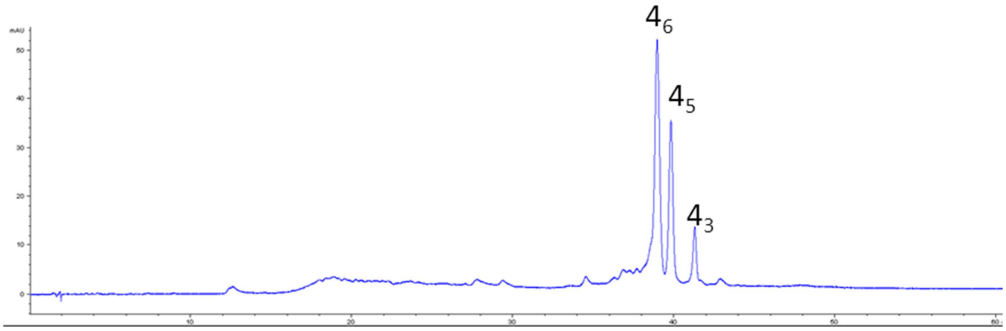

Figure S18. HPLC trace (monitored at 254 nm) of the product mixture obtained by oxidation of peptide **4** (3.8 mM) after shaking at 1200 rpm for 18 days.

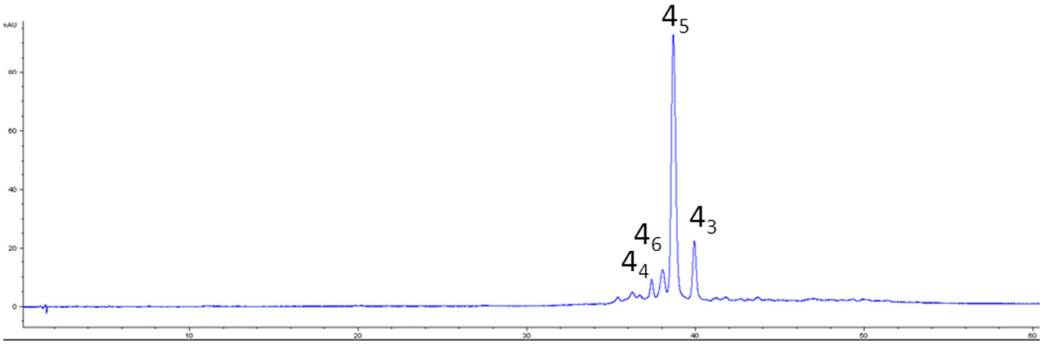

Figure S19. HPLC trace (monitored at 254 nm) of the product mixture obtained by oxidation of peptide **4** (3.8 mM) after 9 days with no agitation.

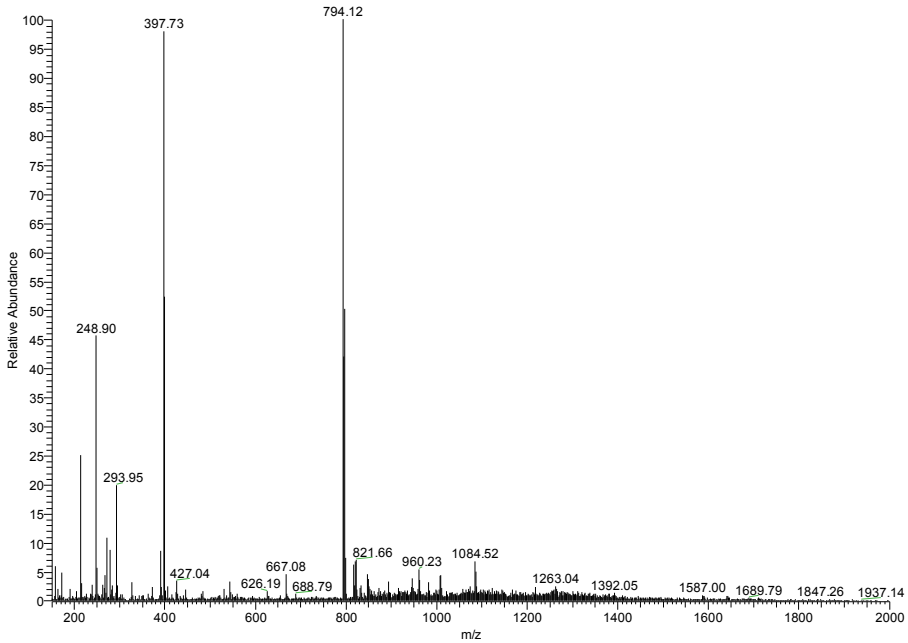

Figure S20. Mass spectrum of dithiol **4** from the LC-MS analysis of a stirred library made from peptide **4** (corresponding to Figure S17). Calculated isotopic profile (species, abundance): 793.31 (M, 100%), 794.3 (M+1, 41%), 795.3 (M+2, 16.7%); m/z calculated: 794.31 $[M+H]^{1+}$, 397.65 $[M+2H]^{2+}$; m/z observed: 794.12 $[M+H]^{1+}$, 379.73 $[M+2H]^{2+}$.

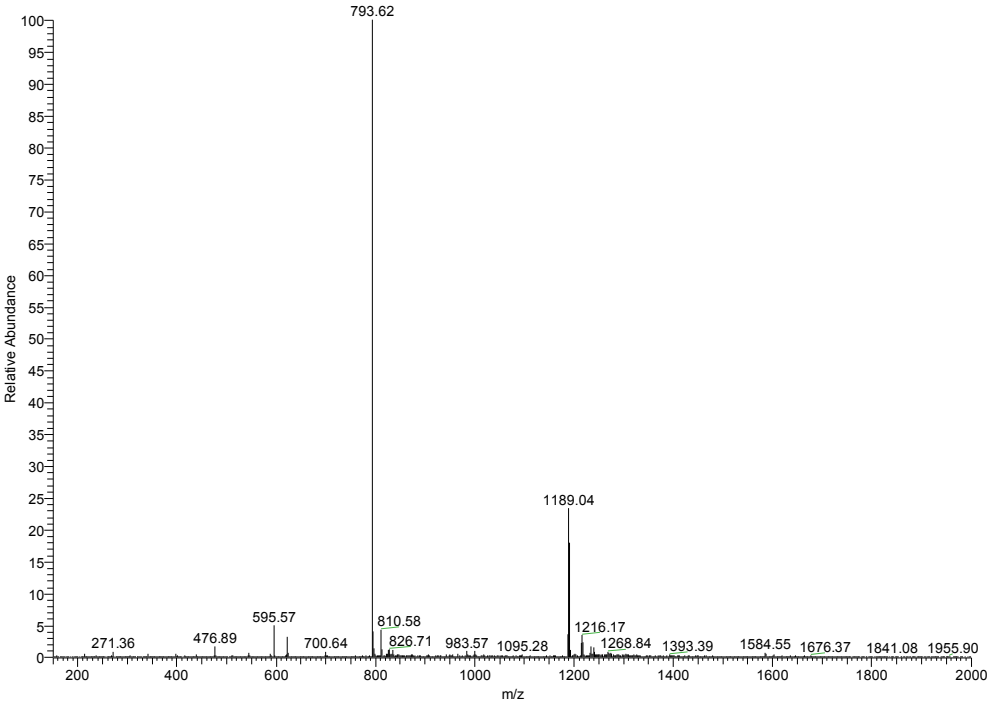

Figure S21. Mass spectrum of cyclic trimer $\mathbf{4}_3$ from the LC-MS analysis of a stirred library made from peptide **4** (corresponding to Figure S17). Calculated isotopic profile (species, abundance): 2376.87 (M+3, 100%), 2375.87 (M+2, 89.7%), 2374.87 (M+1, 81.8%); m/z calculated: 1189.43 $[(M+3)+2H]^{2+}$, 793.29 $[(M+3)+3H]^{3+}$; 595.22 $[(M+3)+4H]^{4+}$; 476.37 $[(M+3)+5H]^{5+}$, m/z observed: 1189.04 $[(M+3)+2H]^{2+}$, 793.62 $[(M+3)+3H]^{3+}$; 595.57 $[(M+3)+4H]^{4+}$; 476.89 $[(M+3)+5H]^{5+}$.

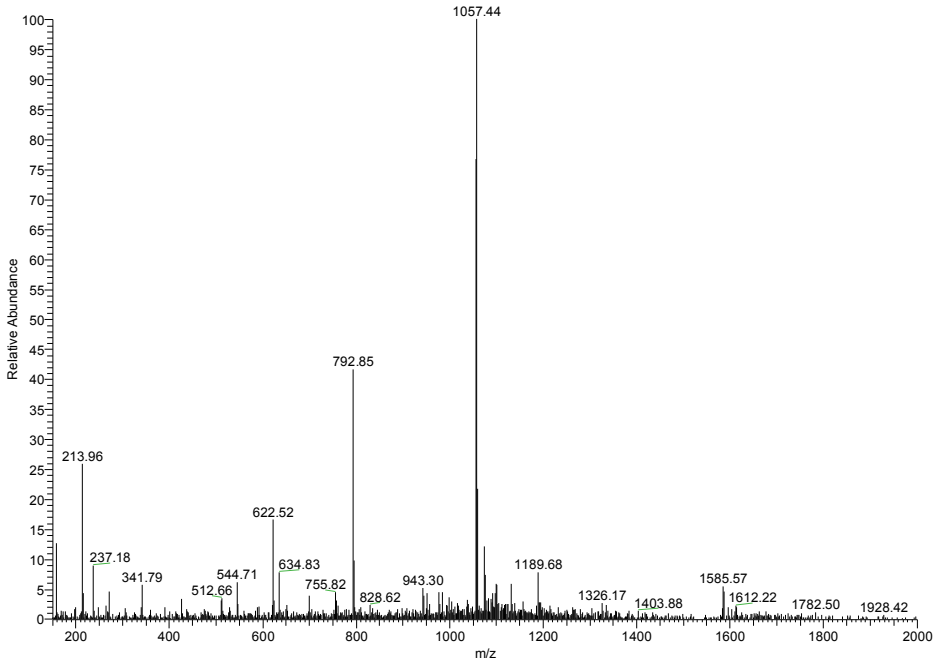

Figure S22. Mass spectrum of cyclic tetramer $\mathbf{4}_4$ from the LC-MS analysis of a stirred library made from peptide **4** (corresponding to Figure S17). Calculated isotopic profile (species, abundance): 3168.16 (M+3, 100%), 3169.16 (M+4, 88.1%), 3170.16 (M+4, 77.6%); m/z calculated: 1585.18 $[(M+3)+2H]^{2+}$, 1057.05 $[(M+3)+3H]^{3+}$; 793.04 $[(M+3)+4H]^{4+}$; 634.63 $[(M+3)+5H]^{5+}$, m/z observed: 1585.57 $[(M+3)+2H]^{2+}$, 1057.44 $[(M+3)+3H]^{3+}$; 793.85 $[(M+3)+4H]^{4+}$; 634.83 $[(M+3)+5H]^{5+}$.

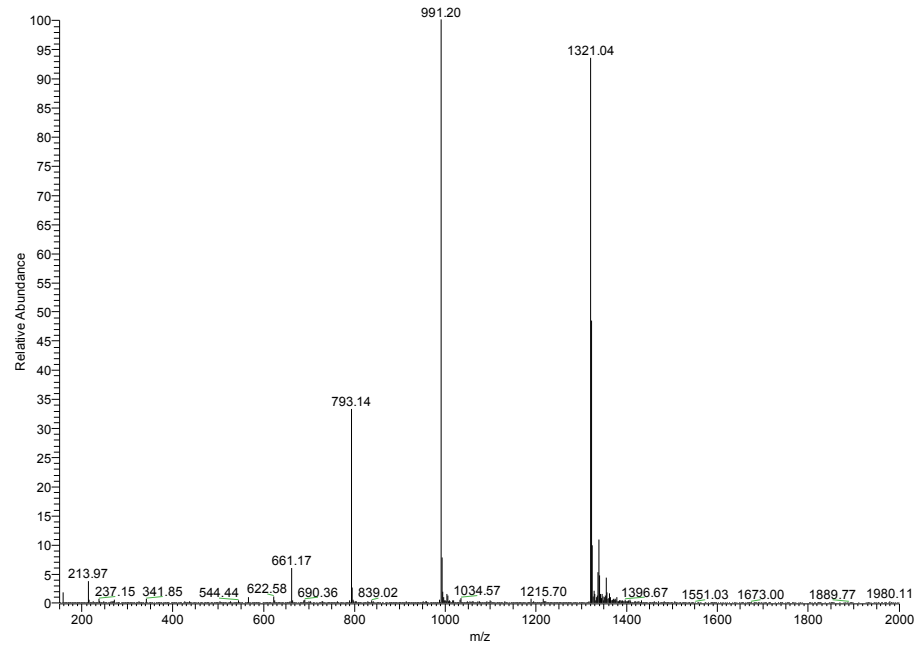

Figure S23. Mass spectrum of cyclic pentamer $\mathbf{4}_5$ from the LC-MS analysis of a stirred library made from peptide **4** (corresponding to Figure S17). Calculated isotopic profile (species, abundance): 3960.45 (M+4, 100%), 3961.45 (M+5, 94.2%), 3959.45 (M+3, 93.7%); m/z calculated: 1321.15 $[(M+4)+3H]^{3+}$, 991.11 $[(M+4)+4H]^{4+}$; 793.09 $[(M+4)+5H]^{5+}$; 661.07 $[(M+4)+6H]^{6+}$, m/z observed: 1321.04 $[(M+4)+3H]^{3+}$, 991.2 $[(M+4)+4H]^{4+}$; 793.14 $[(M+4)+5H]^{5+}$; 661.17 $[(M+4)+6H]^{6+}$.

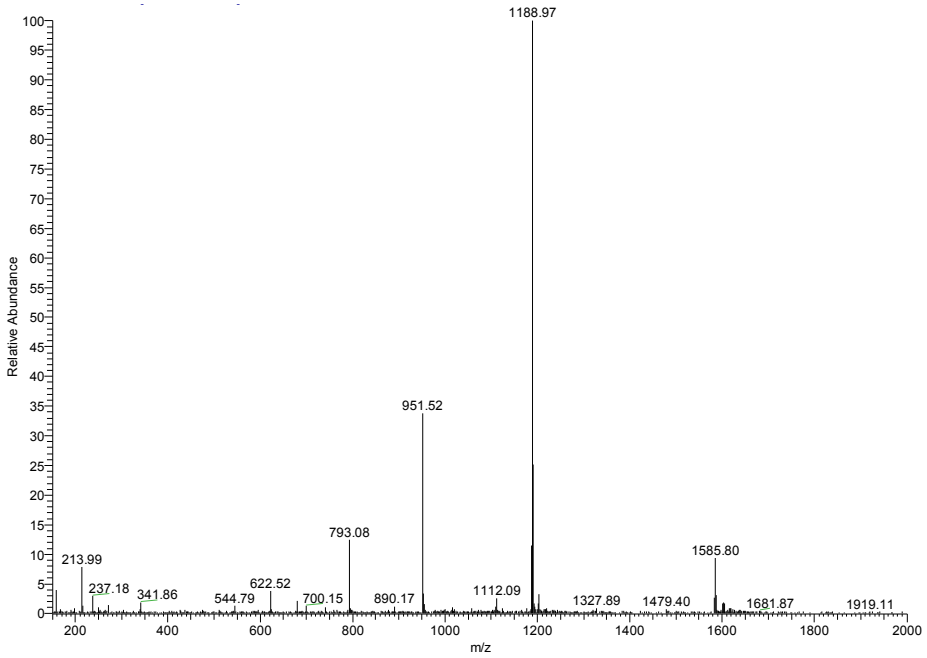

Figure S24. Mass spectrum of cyclic hexamer $\mathbf{4}_6$ from the LC-MS analysis of a stirred library made from peptide **4** (corresponding to Figure S17). Calculated isotopic profile (species, abundance): 4751.74 (M+4, 100%), 4753.74 (M+6, 88.0%), 4752.74 (M+5, 82.4%); m/z calculated: 1584.91 $[(M+4)+3H]^{3+}$, 1188.93 $[(M+4)+4H]^{4+}$; 951.35 $[(M+4)+5H]^{5+}$; 792.95 $[(M+4)+6H]^{6+}$, m/z observed: 1585.8 $[(M+4)+3H]^{3+}$, 1188.97 $[(M+4)+4H]^{4+}$; 951.52 $[(M+4)+5H]^{5+}$; 793.08 $[(M+4)+6H]^{6+}$.

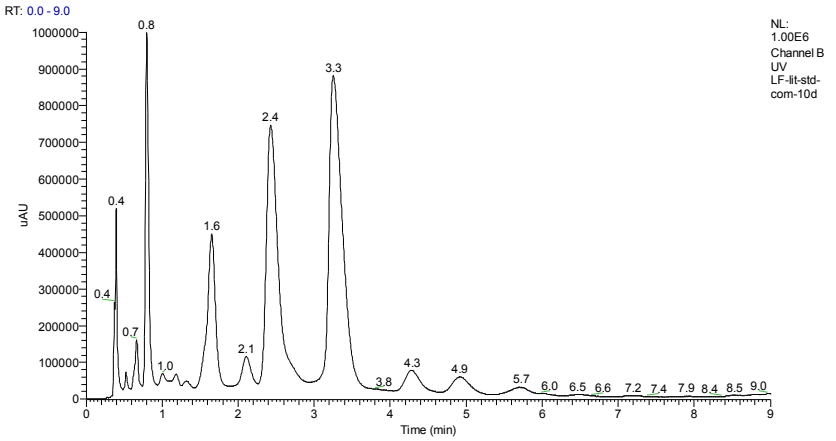

Figure S25. HPLC trace (monitored at 254 nm) of the product mixture obtained by oxidation of peptide **5** (3.8 mM) after 10 days with no agitation.

Retention time (min)	0.8	1.6	2.4	3.3	4.9
Species	**5**	$\mathbf{5}_2$	$\mathbf{5}_4$	$\mathbf{5}_3$	$\mathbf{5}_6$

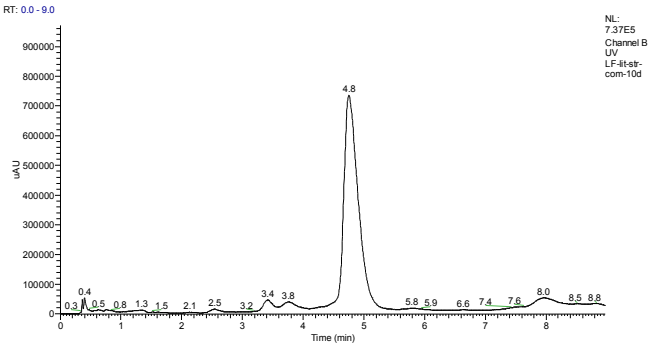

Figure S26. HPLC trace (monitored at 254 nm) of product mixtures obtained by oxidation of peptide **5** (3.8 mM) after stirring at 1200 rpm for 10 days.

Retention time (min)	0.8	1.5	2.5	3.4	4.8
Species	**5**	$\mathbf{5}_2$	$\mathbf{5}_4$	$\mathbf{5}_3$	$\mathbf{5}_6$

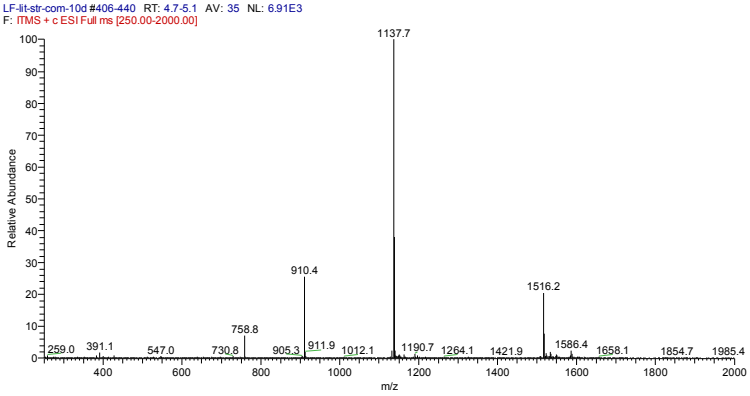

Figure S27. Mass spectrum of cyclic hexamer $\mathbf{5}_6$ from the LC-MS analysis of a stirred library made from peptide **5** (corresponding to Figure S26). Calculated isotopic profile (species, abundance): 4546 (M+2, 100), 4547 (M+3, 87.9), 4545 (M+1, 68.9); *m/z* calculated: 1516.3 $[(M+2)+3H]^{3+}$, 1137.5 $[(M+2)+4H]^{4+}$, 910.2 $[(M+2)+5H]^{5+}$, 758.7 $[(M+2)+6H]^{6+}$; *m/z* observed : 1516.2 $[(M+2)+3H]^{3+}$, 1137.2 $[(M+2)+4H]^{4+}$, 910.4 $[(M+2)+5H]^{5+}$, 758.8 $[(M+2)+6H]^{6+}$.

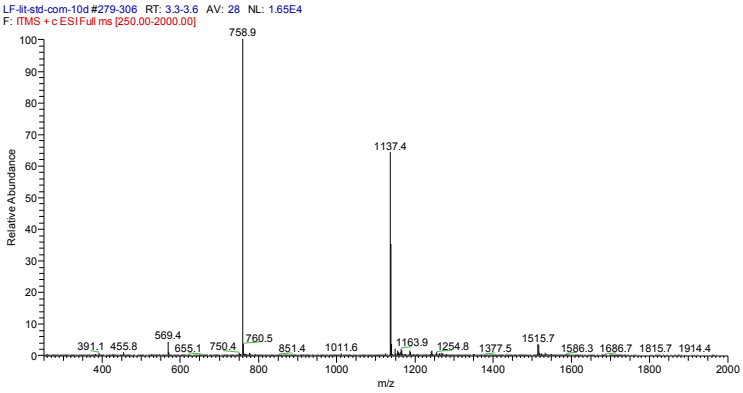

Figure S28. Mass spectrum of cyclic tetramer $\mathbf{5}_4$ from the LC-MS analysis of a non-agitated library made from peptide **5** (corresponding to Figure S25). Calculated isotopic profile (species, abundance): 2273.0 (M+1, 100), 2272.0 (M, 80.5), 2274.0 (M+2, 70.8); *m/z* calculated: 1137.5 $[(M+1)+2H]^{2+}$, 758.7 $[(M+1)+3H]^{3+}$, 569.3 $[(M+1)+4H]^{4+}$; *m/z* observed: 1137.4 $[(M+1)+2H]^{2+}$, 758.9 $[(M+1)+3H]^{3+}$, 569.4 $[(M+1)+4H]^{4+}$.

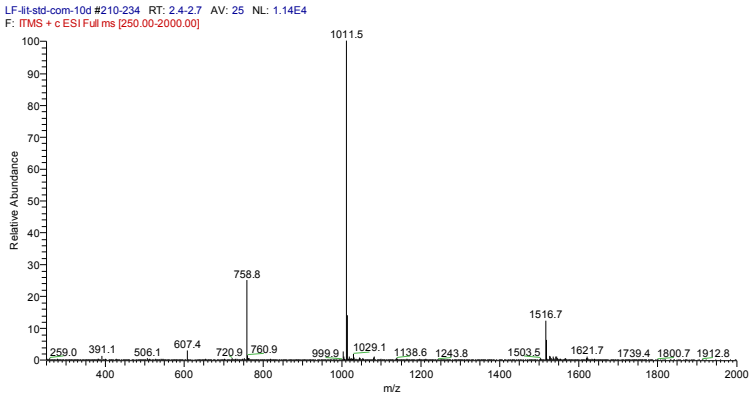

Figure S29. Mass spectrum of cyclic trimer $\mathbf{5}_3$ from the LC-MS analysis of a non-agitated library made from peptide **5** (corresponding to Figure S25). Calculated isotopic profile (species, abundance): 3030.3 (M+1, 100), 3031.3 (M+2, 93.4), 3029.3 (M, 60.4); *m/z* calculated: 1516.2 $[(M+1)+2H]^{2+}$, 1011.1 $[(M+1)+3H]^{3+}$, 758.6 $[(M+1)+4H]^{4+}$, 607.1 $[(M+1)+5H]^{5+}$; *m/z* observed: 1516.7 $[(M+1)+2H]^{2+}$, 1011.5 $[(M+1)+3H]^{3+}$, 758.8 $[(M+1)+4H]^{4+}$, 607.4 $[(M+1)+5H]^{5+}$.

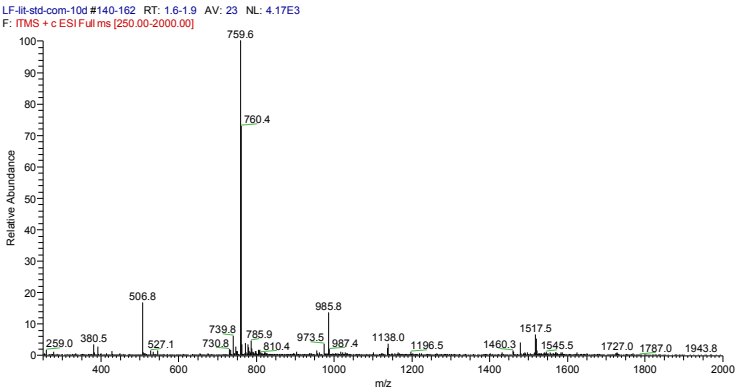

Figure S30. Mass spectrum of linear dimer $\mathbf{5}_2$ from the LC-MS analysis of a non-agitated library made from peptide **5** (corresponding to Figure S25). Calculated isotopic profile (species, abundance): 1516.7 (M, 100), 1517.7 (M+1, 88), 1518.6 (M+2, 39.5); *m/z* calculated: 1517.7 $[M+H]^{+}$, 759.4 $[M+2H]^{2+}$, 506.6 $[M+3H]^{3+}$, 380.2 $[M+5H]^{3+}$; *m/z* observed: 1517.5 $[M+H]^{+}$, 759.6 $[M+2H]^{2+}$, 506.8 $[M+3H]^{3+}$, 380.5 $[M+5H]^{3+}$.

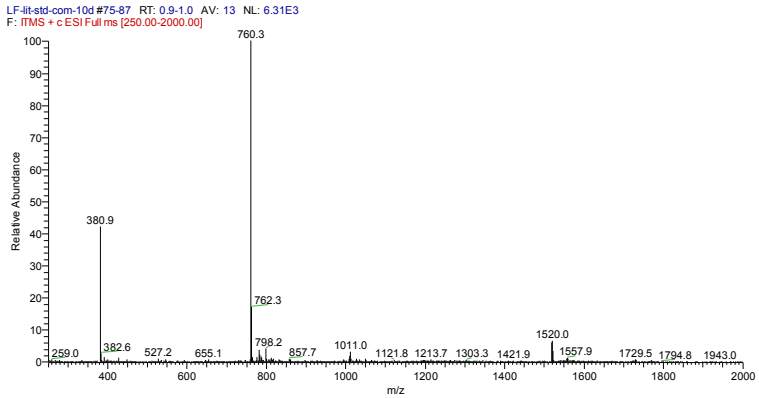

Figure S31. Mass spectrum of dithiol **5** from the LC-MS analysis of a non-agitated library made from peptide **5** (corresponding to Figure S25). Calculated isotopic profile (species, abundance): 759.3 (M, 100.0%), 760.4 (M+1, 39.8%), 761.4 (M+2, 10.8%); *m/z* calculated: 760.3 $[M+H]^{+}$, 380.7 $[M+2H]^{2+}$; *m/z* observed: 760.3 $[M+H]^{+}$, 380.9 $[M+2H]^{2+}$.

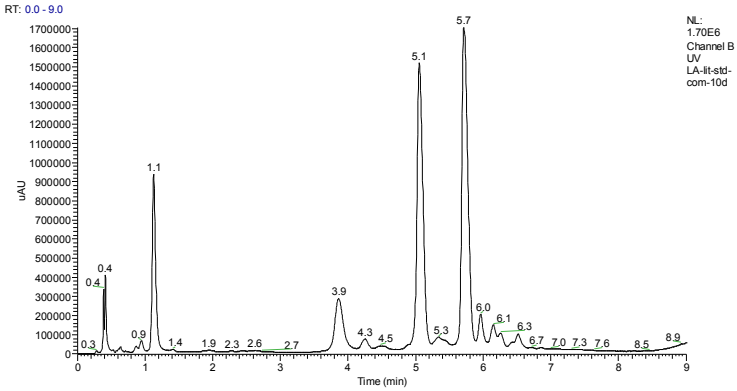

Figure S32. HPLC trace (monitored at 254 nm) of the product mixture obtained by oxidation of peptide **6** (3.8 mM) after 10 days with no agitation.

Retention time (min)	1.1	3.9	5.1	5.7	6.1
Species	**6**	$\mathbf{6}_2$	$\mathbf{6}_4$	$\mathbf{6}_3$	$\mathbf{6}_8$

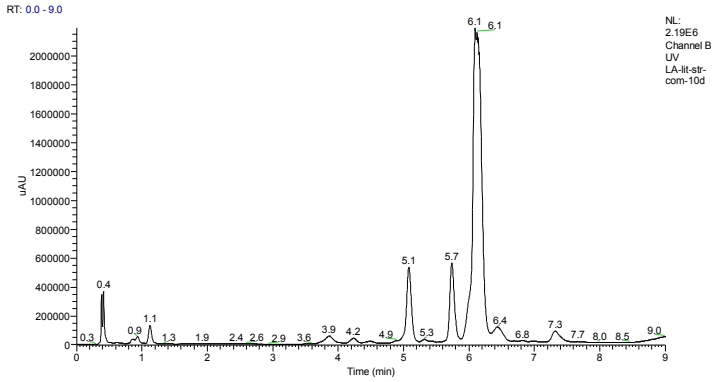

Figure S33. HPLC trace (monitored at 254 nm) of the product mixture obtained by oxidation of peptide **6** (3.8 mM) after stirring at 1200 rpm for 10 days.

Retention time (min)	1.1	3.9	5.1	5.7	6.1
Species	**6**	$\mathbf{6}_2$	$\mathbf{6}_4$	$\mathbf{6}_3$	$\mathbf{6}_8$

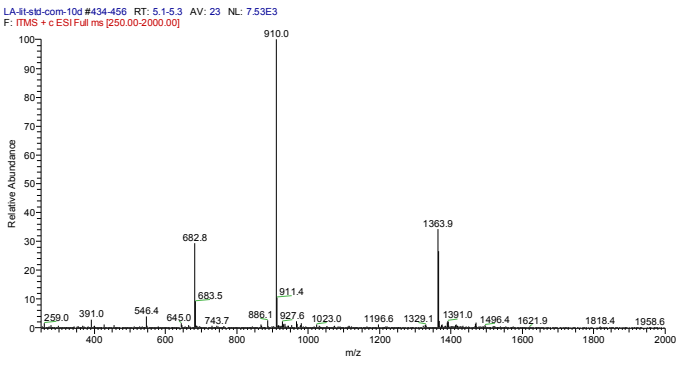

Figure S34. Mass spectrum of cyclic tetramer $\mathbf{6}_4$ from the LC-MS analysis of a non-agitated library made from peptide **6** (corresponding to Figure S32). Calculated isotopic profile (species, abundance): 2726.2 (M+1, 100), 2725.2 (M, 68.2), 2728.2 (M+2, 63.9); *m/z* calculated: 1364.1 $[M+2H]^{2+}$, 909.7 $[M+3H]^{3+}$, 682.6 $[M+4H]^{4+}$, 546.2 $[M+5H]^{5}$; *m/z* observed: 1363.9 $[M+2H]^{2+}$, 910.0 $[M+3H]^{3+}$, 682.8 $[M+4H]^{4+}$, 546.4 $[M+5H]^{5+}$.

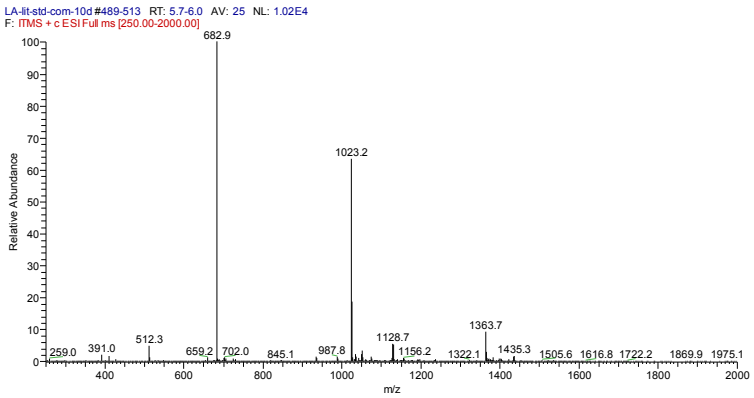

Figure S35. Mass spectrum of cyclic trimer $\mathbf{6}_3$ from the LC-MS analysis of a non-agitated library made from peptide **6** (corresponding to Figure S32). Calculated isotopic profile (species, abundance): 2043.9 (M, 100), 2044.9 (M+1, 99.8), 2045.9 (M+2, 58.5); *m/z* calculated: 1023.0 $[M+2H]^{2+}$, 682.3 $[M+3H]^{3+}$, 512.0 $[M+4H]^{4+}$; *m/z* observed: 1023.2 $[M+2H]^{2+}$, 682.9 $[M+3H]^{3+}$, 512.3 $[M+4H]^{4+}$.

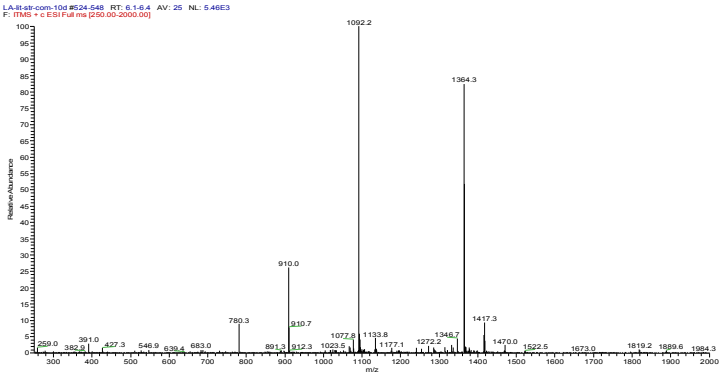

Figure S36. Mass spectrum of cyclic octamer $\mathbf{6}_8$ from the LC-MS analysis of a stirred library made from peptide **6** (corresponding to Figure S33). Calculated isotopic profile (species, abundance): 5453.4 (M+3, 100), 5454.4 (M+4, 90), 5452.4 (M+1, 88.4); *m/z* calculated: 1364.4 $[(M+3)+4H]^{4+}$, 1091.7 $[(M+3)+5H]^{5+}$, 909.9 $[(M+3)+6H]^{6+}$, 780.1 $[(M+3)+7H]^{7+}$; *m/z* observed: 1364.3 $[(M+3)+4H]^{4+}$, 1092.2 $[(M+3)+5H]^{5+}$, 910.0 $[(M+3)+6H]^{6+}$, 780.3 $[(M+3)+7H]^{7+}$.

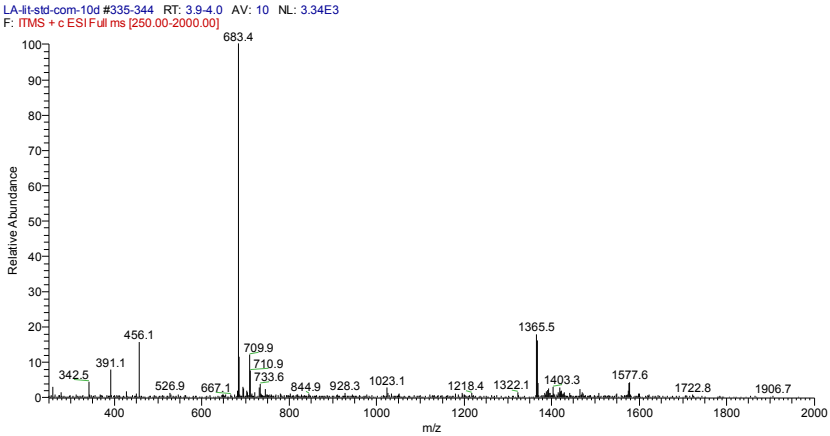

Figure S37. Mass spectrum of linear dimer $\mathbf{6}_2$ from the LC-MS analysis of a non-agitated library made from peptide **6** (corresponding to Figure S32). Calculated isotopic profile (species, abundance): 1364.6 (M, 100), 1365.6.2 (M+1, 73.3), 1366.6 (M+2, 24.7); *m/z* calculated: 1365.6 $[M+H]^{+}$, 683.3 $[M+2H]^{2+}$, 455.9 $[M+3H]^{3+}$, 342.2 $[M+4H]^{4+}$; *m/z* observed: 1365.6 $[M+H]^{+}$, 683.4 $[M+2H]^{2+}$, 456.1 $[M+3H]^{3+}$, 342.5 $[M+4H]^{4+}$.

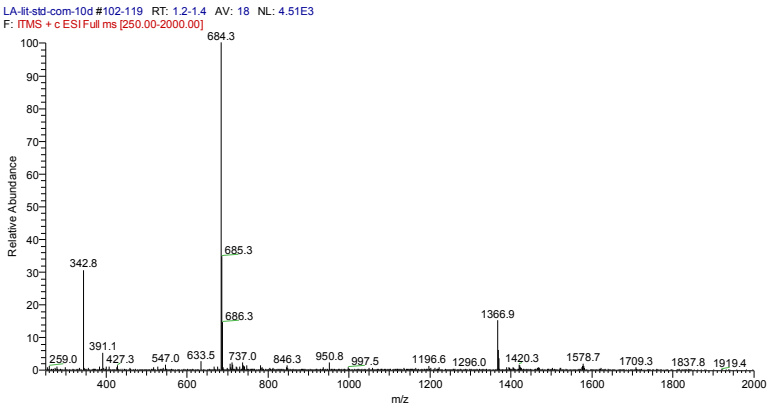

Figure S38. Mass spectrum of dithiol **6** from the LC-MS analysis of a non-agitated library made from peptide **6** (corresponding to Figure S32). Calculated isotopic profile (species, abundance): 683.3 (M, 100), 684.3 (M+1, 33.3), 685.3 (M+2, 10.0); *m/z* calculated : 684.4 $[M+H]^+$, 342.5 $[M+2H]^{2+}$; *m/z* observed : 684.3 $[M+H]^+$, 342.7 $[M+2H]^{2+}$.

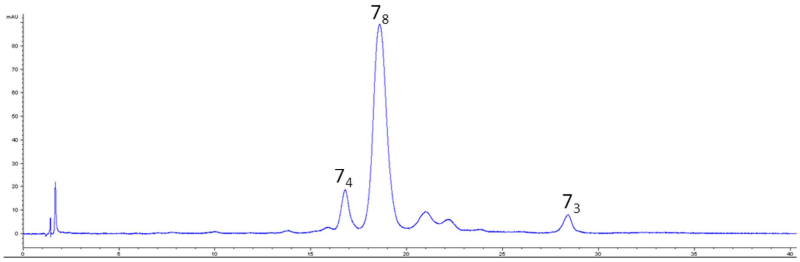

Figure S39. HPLC trace (monitored at 254 nm) of the product mixture obtained by oxidation of peptide **7** (3.8 mM) after stirring at 1200 rpm for 10 days.

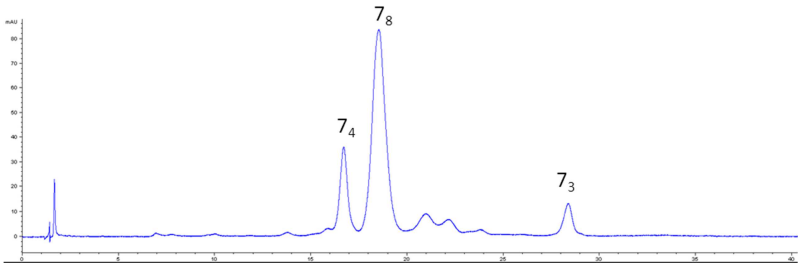

Figure S40. HPLC trace (monitored at 254 nm) of the product mixture obtained by oxidation of peptide **7** (3.8 mM) after shaking at 1200 rpm for 18 days.

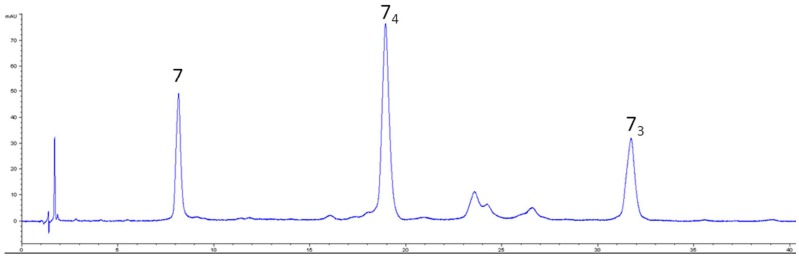

Figure S41. HPLC trace (monitored at 254 nm) of the product mixture obtained by oxidation of peptide **7** (3.8 mM) 7 days with no agitation.

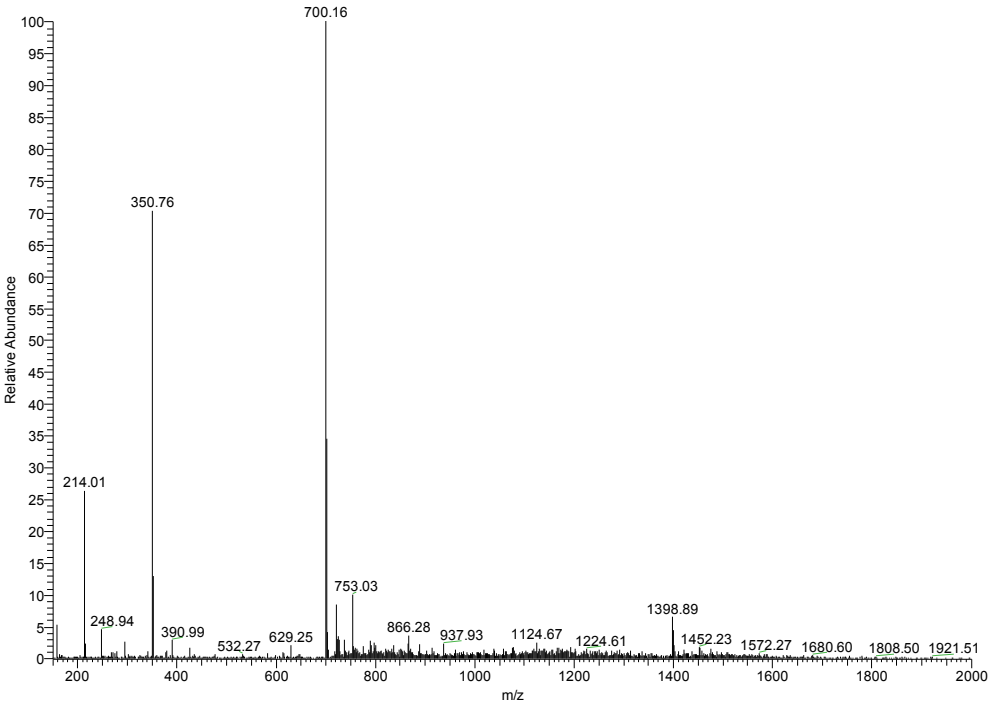

Figure S42. Mass spectrum of dithiol **7** from the LC-MS analysis of a stirred library made from peptide **7** (corresponding to Figure S39). Calculated isotopic profile (species, abundance): 699.31 (M, 100%), 700.31 (M+1, 37.5%), 701.3 (M+2, 9.1%); m/z calculated: 700.31 $[M+H]^{1+}$, 350.65 $[M+2H]^{2+}$; m/z observed: 700.16 $[M+H]^{1+}$, 350.76 $[M+2H]^{2+}$.

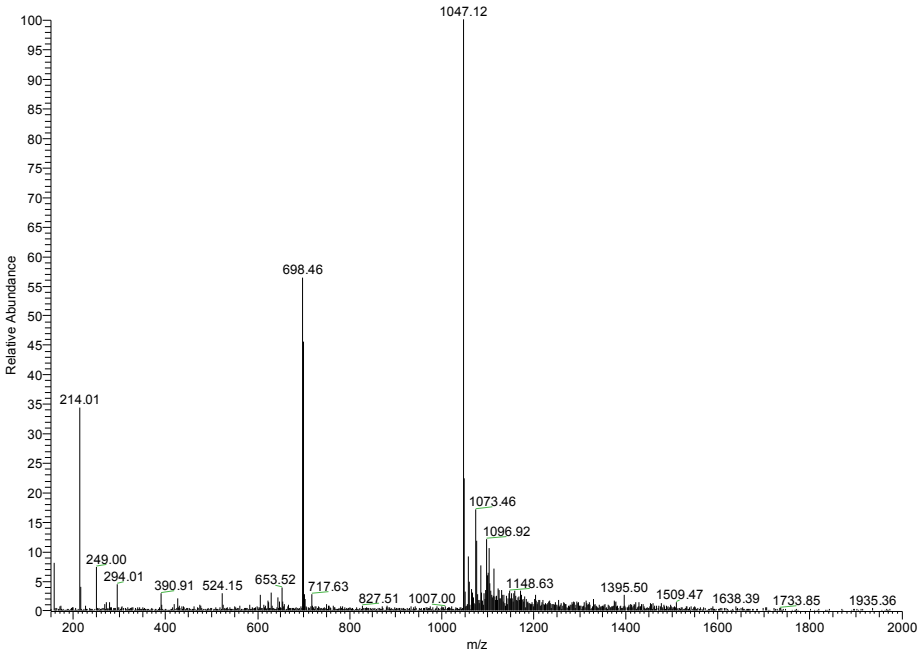

Figure S43. Mass spectrum of cyclic trimer $\mathbf{7}_3$ from the LC-MS analysis of a stirred library made from peptide **7** (corresponding to Figure S39). Calculated isotopic profile (species, abundance): 2092.88 (M+1, 100%), 2091.88 (M, 88.9%), 2093.88 (M+2, 30.7%); m/z calculated: 1047.44 $[(M+1)+2H]^{2+}$, 698.62 $[(M+1)+3H]^{3+}$; 524.22 $[(M+1)+4H]^{4+}$; m/z observed: 1047.12 $[(M+1)+2H]^{2+}$, 698.46 $[(M+1)+3H]^{3+}$; 524.15 $[(M+1)+4H]^{4+}$.

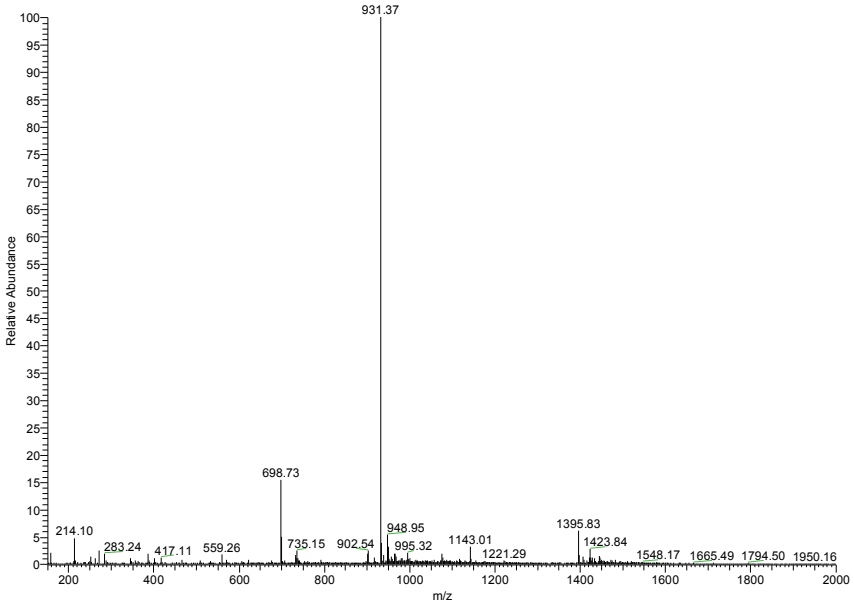

Figure S44. Mass spectrum of cyclic tetramer $\mathbf{7}_4$ from the LC-MS analysis of a stirred library made from peptide **7** (corresponding to Figure S39). Calculated isotopic profile (species, abundance): 2790.17 (M+1, 100%), 2789.17 (M, 68.2%), 2791.18 (M+2, 64.6%); m/z calculated: 1396.08 $[(M+1)+2H]^{2+}$, 931.74 $[(M+1)+3H]^{3+}$; 698.54 $[(M+1)+4H]^{4+}$; 559.03 $[(M+1)+5H]^{5+}$; m/z observed: 1395.83 $[(M+1)+2H]^{2+}$, 931.37 $[(M+1)+3H]^{3+}$; 698.73 $[(M+1)+4H]^{4+}$; 559.26 $[(M+1)+5H]^{5+}$.

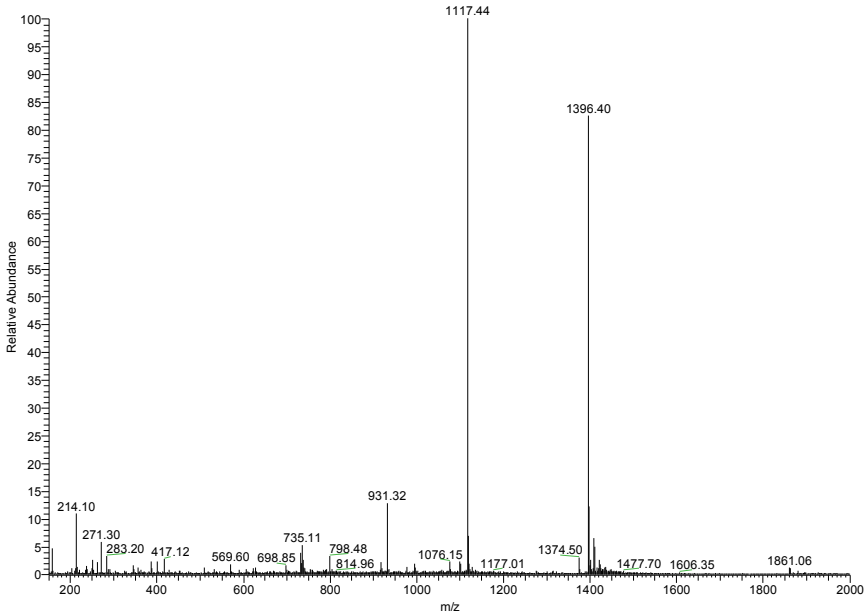

Figure S45. Mass spectrum of cyclic octamer $\mathbf{7}_8$ from the LC-MS analysis of a stirred library made from peptide **7** (corresponding to Figure S39). Calculated isotopic profile (species, abundance): 5581.35 (M+3, 100%), 5580.35 (M+2, 80.3%), 5582.34 (M+4, 73.0%); m/z calculated: 1861.45 $[(M+3)+3H]^{3+}$, 1396.34 $[(M+3)+4H]^{4+}$; 1117.27 $[(M+3)+5H]^{5+}$; 931.22 $[(M+3)+6H]^{6+}$; m/z observed: 1861.06 $[(M+3)+3H]^{3+}$, 1396.4 $[(M+3)+4H]^{4+}$; 1117.44 $[(M+3)+5H]^{5+}$; 931.32 $[(M+3)+6H]^{6+}$.

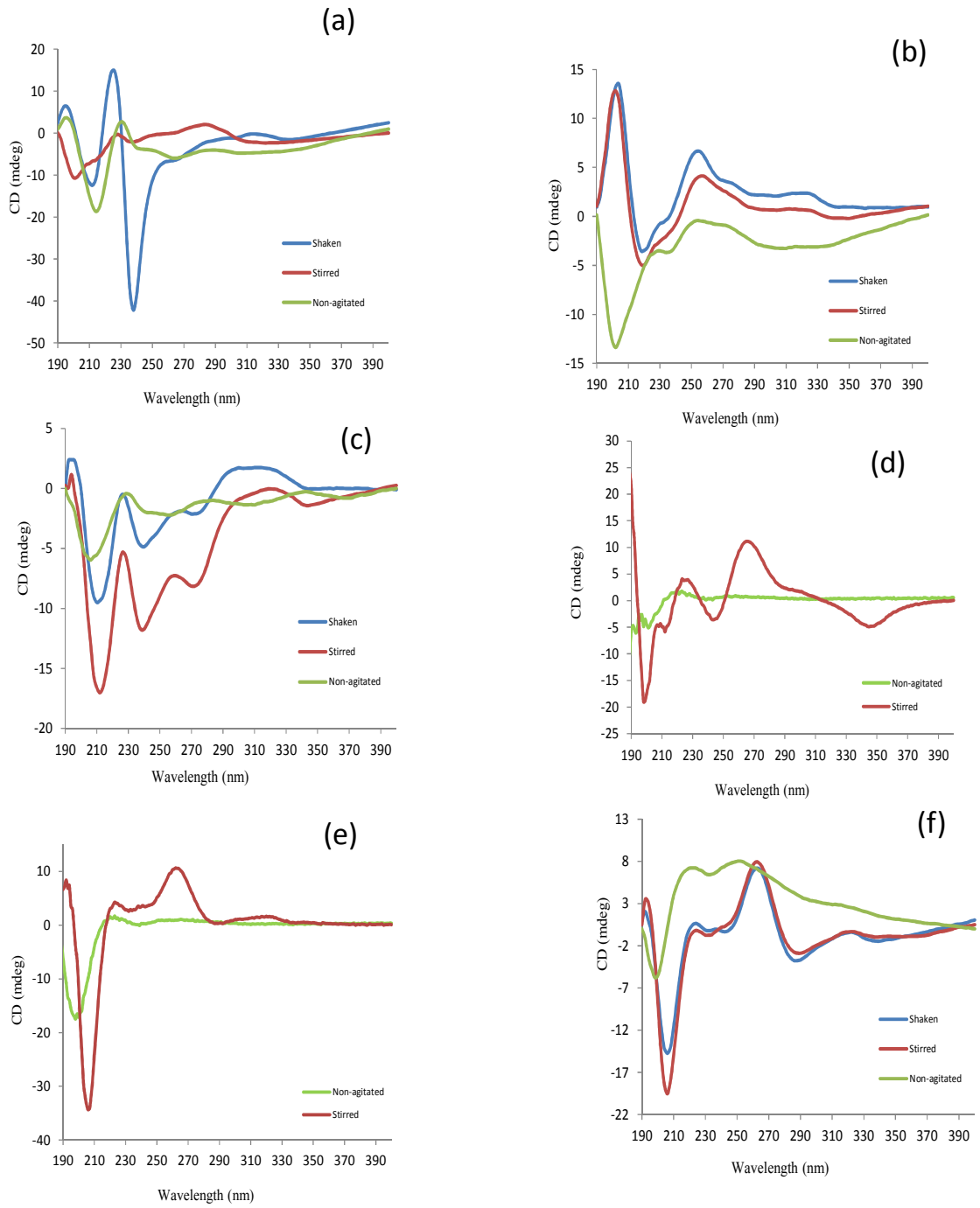

Figure S46. Extended CD spectra of DCLs made from peptides **2** (a), **3** (b), **4** (c), **5** (d), **6** (e) and **7** (f) under different agitation conditions.

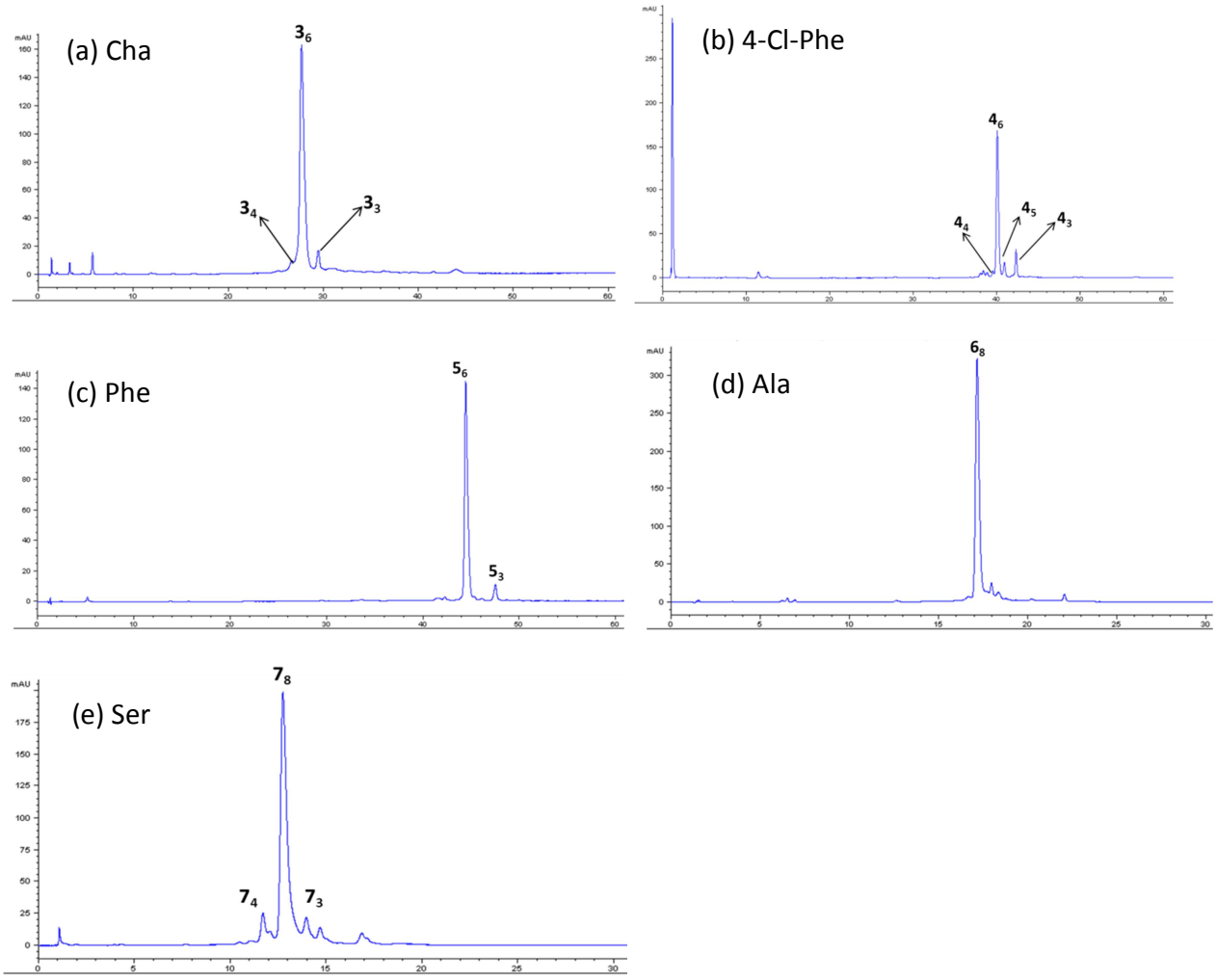

Figure S47. HPLC analyses of stirred DCLs made from peptides **3** (a), **4** (b), **5** (c), **6** (d) and **7** (e) after the replication process was complete, showing the replicating macrocycle as the dominant product.