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Electronic Supplementary Information

Effect of the Amino Acid Composition of Cyclic Peptides on Their Self-Assembly in Lipid Bilayers

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Table of contents

Peptide synthesis protocol

Under an atmosphere of nitrogen, 2-chlorotrityl chloride resin (400 mg, 1.01 mmol/g) was swollen in a sinter-fitted syringe in anhydrous dichloromethane for 30 minutes. The resin was drained and treated with a solution of Fmoc protected amino acid (2.2 equiv. 0.8 mmol) and diisopropylethylamine (8 equiv, 3.55 mmol) in anhydrous dichloromethane. The resin suspension was agitated at ambient temperature for 2 hours. The resin was drained and subsequently treated with a solution of dichloromethane / diisopropylethylamine / methanol (17 : 1 : 2 v/v/v, 3 x 8 mL x 5 min) to cap any unreacted sites. The resin was washed with dichloromethane (5 x 8 mL), dimethylformamide (DMF, 5 x 8 mL) and dichloromethane (5 x 8 mL) and dried under reduced pressure. A small portion of the dried resin (~7.0 mg) was treated with 20 % piperidine in DMF to determine the degree of Fmoc-D-Leu-OH coupling. The supernatant was analyzed by UV-Vis using ε_{300} = 7800 $M^{-1}cm^{-1}$ corresponding to the dibenzofulvene-piperidine adduct from which the degree of substitution of the resin was determined.

N-terminal Fmoc deprotection was achieved by agitation of the resin bound Fmoc-D-Leu in a solution of 20 % piperidine in DMF (3 x 8 mL x 3 minutes). The resin was washed with DMF (5 x 8 mL), dichloromethane (5 x 8 mL) and DMF (5 x 8 mL) after which the subsequent coupling was performed. Under an atmosphere of nitrogen, a solution of Fmoc-protected amino acid (2.5 equiv relative to loading), *O*-(benzotriazole-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU, 2.5 equiv relative to loading) and diisopropylethylamine (8 equiv relative to loading) in anhydrous DMF (8 mL) was combined with the D-Leu bound resin. The suspension was agitated at ambient temperature for 3 hours. Alternatively an overnight coupling reaction could also be performed using a solution of Fmoc-protected amino acid (1.2 equiv

relative to loading), HBTU (1.2 equiv relative to loading) and diisopropylethylamine (2.4 equiv relative to loading). The resin was then drained and washed with DMF (5 x 8 mL) prior to the subsequent Fmoc deprotection.

After the removal of the final Fmoc group, the linear peptide was cleaved off the resin through agitation in a solution containing hexafluoroisopropanol / dichloromethane (1 : 4 v/v, 3 x 8 mL x 10 minutes) at ambient temperature. The drained solution was collected and the resin was washed with dichloromethane (3 x 8 mL). The solutions were pooled and dried via reduced pressure to obtain (Boc-protected) linear peptide. The linear peptide was assessed for purity by using liquid chromatography – mass spectrometry (LC-MS).

Characterization of the linear peptides 1 – 6

Linear peptide **1**.

H_2N-[L-Lys(Boc)-d-Leu-D-Trp(Boc)-D-Leu]$_2$-OH.

^{1}H-NMR (500 MHz, TFA-*d*) δ ppm: 8.12 – 8.10 (dd, *J* = 5.6 Hz, 2H), 7.55 – 7.53 (m, 2H), 7.49-7.45 (d, *J* = 21.6 Hz, 2H), 7.38 – 7.34 (m, 2H), 7.31 – 7.27 (m, 2H), 5.15 (m, 2H), 4.67 – 4.54 (m, 5H), 4.25 (t, *J* = 6.5 Hz, 1H), 3.34 – 3.12 (overlapping m, 9H), 2.09 – 1.11 (overlapping m, 59H), 1.11 – 0.74 (overlapping m, 25H).

^{13}C-NMR (125 MHz, TFA-*d*) δ ppm: 177.6, 174.3, 174.2, 173.5, 172.9, 172.5, 169.0, 159.4, 154.7, 135.4, 135.3, 129.8, 129.7, 125.9, 124.9, 124.3, 124.2, 123.9, 122.2, 120.0, 118.5, 118.4, 118.0, 90.3, 90.2, 90.1, 69.9, 69.3, 69.1, 68.8, 54.5, 53.8, 53.6, 52.7, 51.4, 41.1, 40.9, 40.8, 40.3, 39.8, 39.0, 33.3, 31.7, 30.4, 27.8, 25.9, 24.5, 24.3, 22.0, 21.4, 21.0, 20.9, 19.8, 19.7, 19.4.

LC-MS (ESI+) m/z $[M+H]^+$ calculated: 1500.9, found: 1500.9.

Linear peptide **2**

H_2N-L-Lys(Alloc)-D-Leu-L-Trp(Boc)-D-Leu-L-Lys(Boc)-D-Leu-L-Trp(Boc)-D-Leu-OH.

^{1}H-NMR (500 MHz, TFA-*d*) δ ppm: 8.36 – 8.34 (overlapping m, 2H), 7.75 – 7.73 (m, 2H), 7.71 – 7.66 (overlapping d, 2H), 7.59 – 7.49 (overlapping m, 4H), 6.06 (m, *J* = 5.3 Hz, 1H), 5.52 – 5.30 (overlapping m, 3H), 4.90 – 4.70 (overlapping m, 7H), 4.60 – 4.43 (m, 1H), 3.56 – 3.32 (overlapping m, 8H), 2.40 – 1.30 (overlapping m, 51H), 1.30 – 0.85 (overlapping m, 24H).

^{13}C-NMR (125 MHz, TFA-*d*) δ ppm: 179.4, 176.0, 175.8, 175.2, 174.2, 174.1, 170.7, 161.5, 160.8, 156.4, 137.1, 137.0, 132.1, 131.6, 131.5, 127.6, 126.6, 126.0, 125.9, 125.6, 123.9, 121.7, 92.0, 91.9, 71.1, 56.2, 55.7, 55.5, 55.3, 54.5, 54.4, 54.3, 53.2, 42.4, 42.0, 32.1, 30.0, 27.6, 26.3, 26.2, 24.0, 22.8, 22.7, 22.6, 21.7, 21.4, 21.2.

LC-MS (ESI+) m/z $[M+H]^+$ calculated: 1484.8, found: 1484.8.

Linear peptide **3**

H_2N-L-Trp(Boc)-D-Leu-L-Lys(N_3)-D-Leu-L-Trp(Boc)-D-Leu-L-Lys(Alloc)-D-Leu-OH.

^{1}H-NMR (400 MHz, DMSO-*d6*) δ ppm: 8.8 (d, *J* = 8.6 Hz, 1H), 8.31 (app d, *J* = 8.1 Hz, 2H), 8.25 (d, *J* = 8.2 Hz, 1H), 8.23-8.15 (m, 4H), 8.12 (d, *J* = 8.3 Hz, 1H), 8.08 (d, *J* = 7.8 Hz, 1H), 8.03 (d, *J* = 8.2 Hz, 1H), 7.99 (d, *J* = 8.1 Hz, 1H), 7.79-7.72 (overlapping d, 2H), 7.62 (s, 1H), 7.50 (s, 1H), 7.34 (t, *J* = 7.4 Hz, 1H), 7.32-7.18 (overlapping m, 3H), 7.13 (t, *J* = 5.4 Hz, 1H), 5.94-5.82 (m, 1H), 5.25 (d, *J* = 17.2 Hz, 1H), 5.01 (d, *J* = 10.5 Hz, 1H), 4.72-4.64 (m, 1H), 4.50-4.41 (overlapping m, 3H), 4.41-4.19 (overlapping m, 5H), 4.17-4.08 (br m, 1H), 3.25 (dt, *J* = 2.4, 6.7 Hz, 2H), 3.21-3.13 (m, 1H), 3.11-2.98 (overlapping m, 24H), 2.97-2.81 (overlapping m, 3H), 1.62 (s, 9H), 1.61 (s, 9H), 1.59-0.99 (overlapping m, 24H), 0.87 (d, *J* =6.3 Hz, 3H), 0.81 (d, *J* =

6.3 Hz, 3H), 0.78 (d, *J* = 6.4 Hz, 3H), 0.73 (app d, *J* = 6.4 Hz, 6H), 0.70-0.63 (overlapping m, 9H).

^{13}C-NMR (100 MHz, DMSO-*d6*) δ ppm: 174.0, 171.8, 171.6, 171.5, 171.3, 171.1, 170.6, 167.7, 158.1, 157.8, 155.9, 149.0, 133.8, 130.1, 129.9, 125.4, 124.5, 124.4, 124.2, 122.4, 122.3, 119.6, 119.5, 116.8, 116.2, 114.7, 114.5, 113.5, 83.4, 64.1, 52.4, 52.1, 51.8, 51.7, 51.1, 51.0, 50.8, 50.6, 50.1, 41.7, 41.3, 41.1, 32.2, 32.0, 29.0, 27.7, 26.9, 24.3, 24.1, 24.0, 23.96, 22.93, 22.90, 22.83, 22.77, 22.4, 22.3, 21.5, 21.3, 21.2.

HRMS (ESI+) m/z $[M+H]^+$ calculated: 1409.8191, found: 1409.8171.

Linear peptide **4**

H_2N-[L-Trp(Boc)-D-Leu-L-Lys(N_3)-D-Leu]$_2$-OH.

Synthesis and characterization of linear peptide **4** has been described in Chapman et al.[1]

Linear peptide **5**

H_2N-[L-Trp(Boc)-D-Leu-L-Lys(Alloc)-D-Leu]$_2$-OH.

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.77 (d, *J* = 8.3 Hz, 1 H), 8.35-8.23 (overlapping m, 3H), 8.23-8.16 (m, 4H), 8.14 (d, *J* = 8.1 Hz, 1H), 8.09-8.01 (overlapping d, 2H), 7.79 (d, *J* = 7.6 Hz, 1H), 7.77 (d, *J* = 7.8 Hz, 1H), 7.74 (d, *J* = 7.8, 1H), 7.62 (s, 1H), 7.51 (s, 1H), 7.34 (t, *J* = 7.7 Hz, 1H), 7.32-7.24 (m, 2 H), 7.22 (t, *J* = 7.3 Hz, 1H), 7.17-7.09 (m, 2H), 5.94-5.83 (m, 2H), 5.25 (d, *J* = 17.2 Hz, 2H), 5.15 (d, *J* = 10.5 Hz, 2H), 4.72-4.64 (m, 1H), 4.93-4.40 (overlapping m, 5H), 4.40-4.20 (overlapping m, 5H), 4.17-4.08 (br m, 1H), 3.20-3.13 (m, 1H), 3.11-2.99 (overlapping m, 2H), 2.97-2.81 (overlapping m, 5H), 1.62 (s, 9H), 1.61 (s, 9H), 1.60-0.98

(overlapping m, 24H), 0.86 (d, J = 6.4 Hz, 3H), 0.81 (d, J = 6.4 Hz), 0.78 (d, J = 6.5 Hz, 3H), 0.75-0.70 (overlapping m, 6H), 0.70 (overlapping m, 6H).

^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 174.0, 171.8, 171.6, 171.5, 171.22, 171.20, 170.6, 167.7, 155.89, 155.86, 149.0, 133.8, 130.1, 129.9, 125.4, 124.5, 124.4, 124.2, 122.4, 122.3, 119.6, 119.5, 116.8, 116.2, 114.7, 114.5, 113.5, 83.7, 83.4, 64.1, 52.4, 52.1, 51.9, 51.1, 51.0, 50.8, 50.1, 41.7, 41.3, 41.1, 32.2, 29.0, 27.72, 27.71, 26.9, 24.3, 24.1, 24.0, 23.9, 22.94, 22.89, 22.84, 22.77, 22.4, 21.5, 21.30, 21.25, 21.2 (14 carbon signals obscured or overlapping).

HRMS (ESI+) m/z $[M+H]^+$: 1467.8498, found: 1467. 8521.

Linear peptide **6**

H_2N-[L-Lys(N_3)-D-Leu]$_4$.

Synthesis and characterization of linear peptide **6** has been described in Chapman et al.[2]

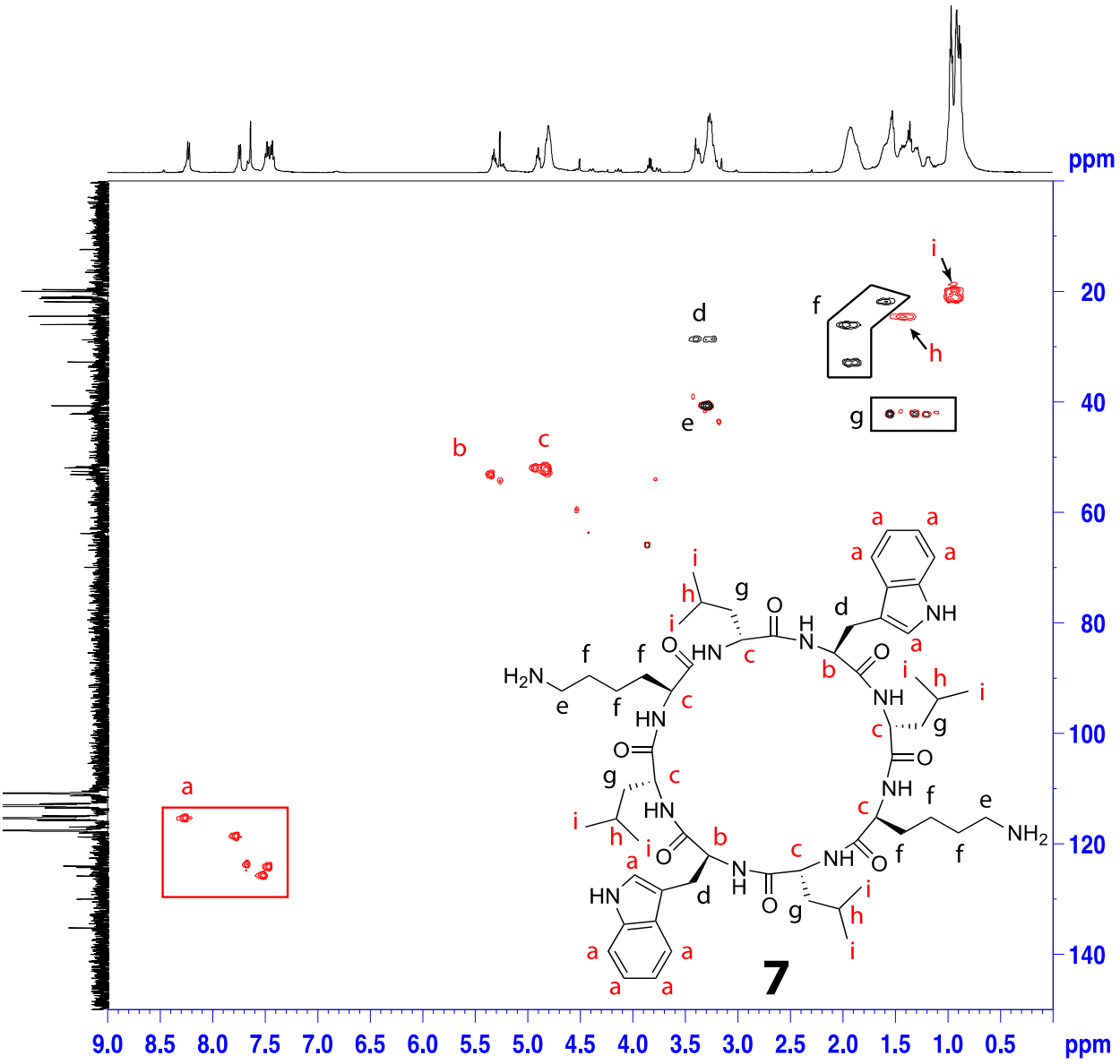

Figure S1. HSQC NMR of cyclic peptide **7.**

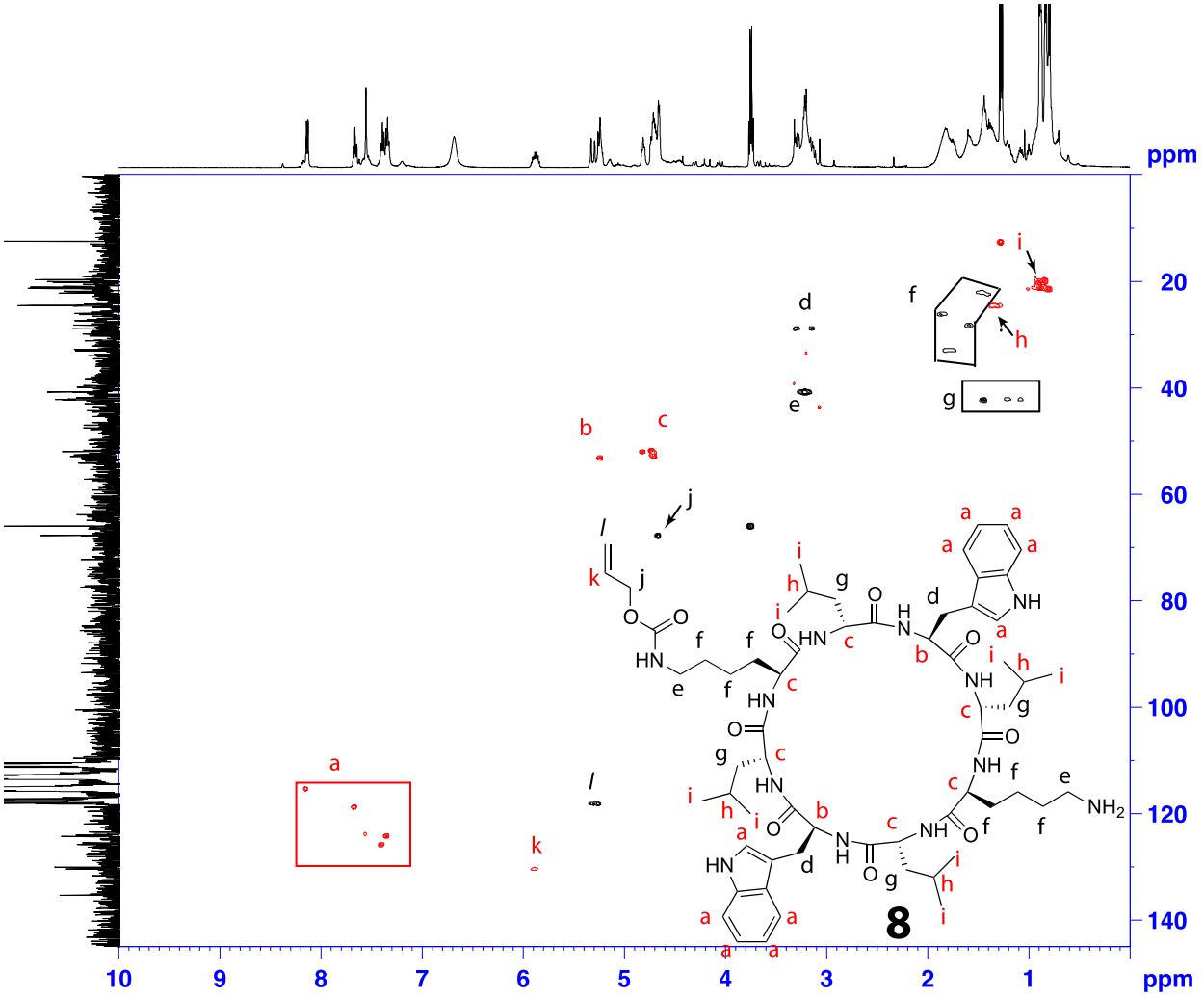

Figure S2. HSQC NMR of cyclic peptide **8.**

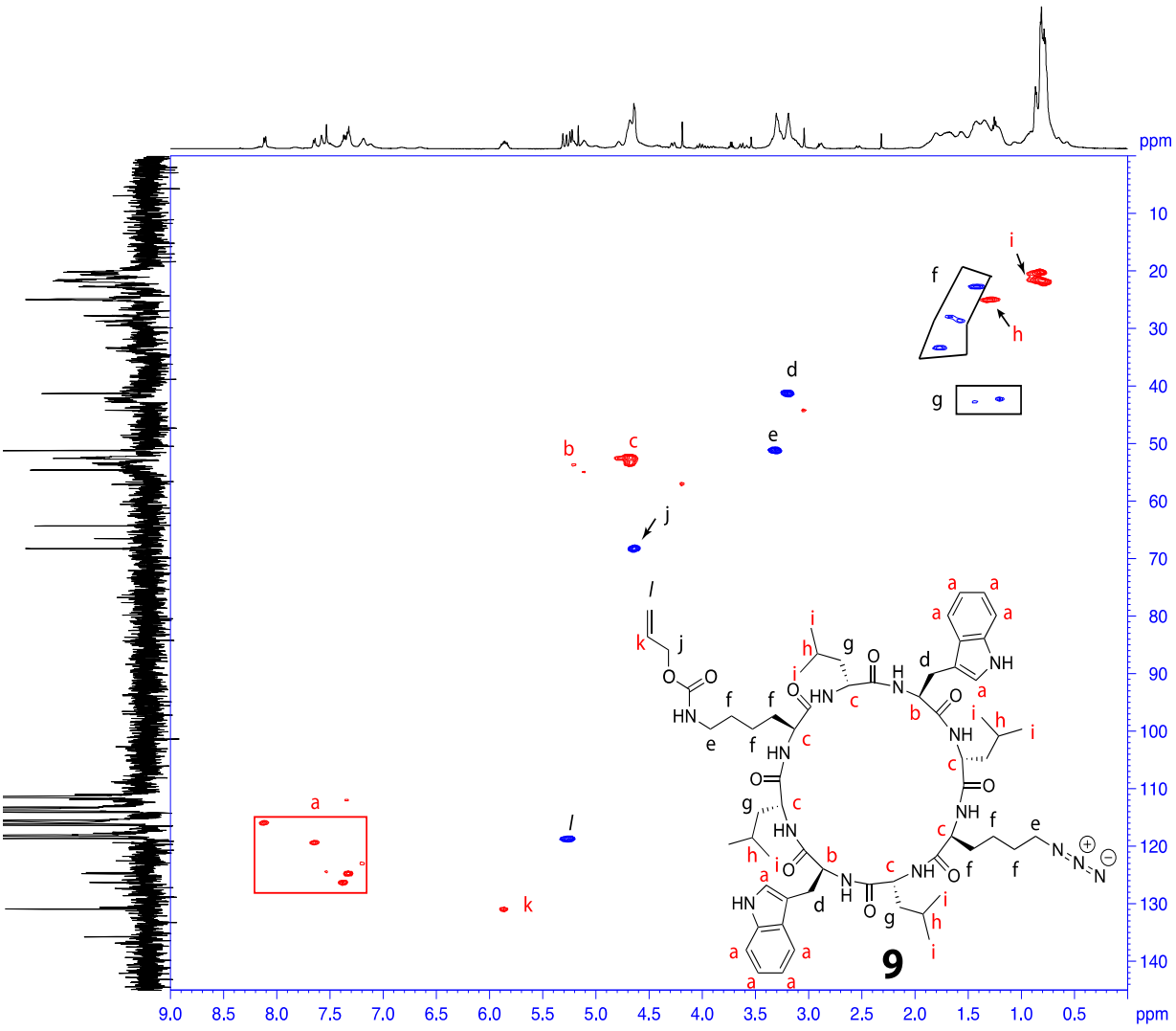

Figure S3. HSQC NMR of cyclic peptide **9.**

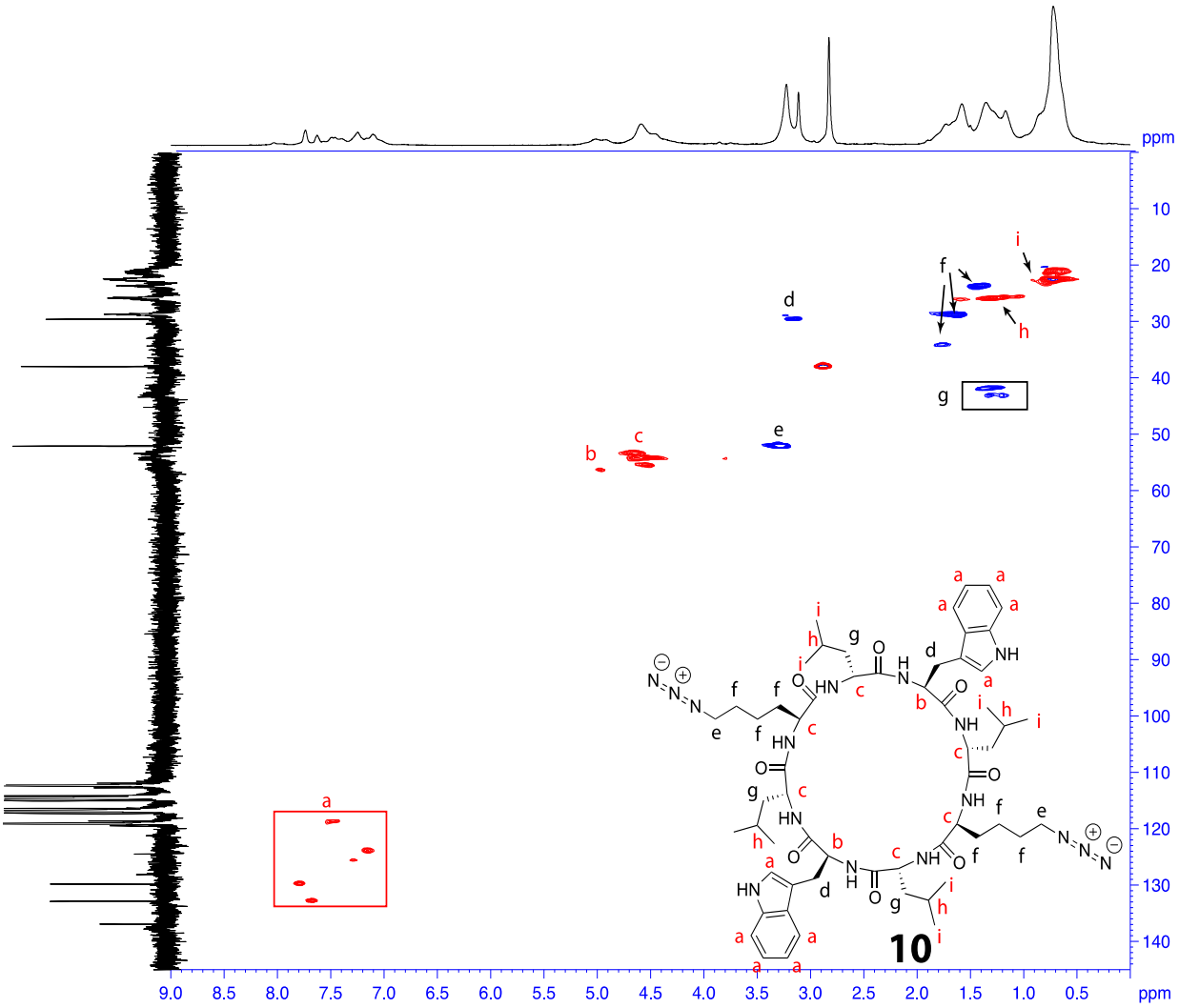

Figure S4. HSQC NMR of cyclic peptide **10.**

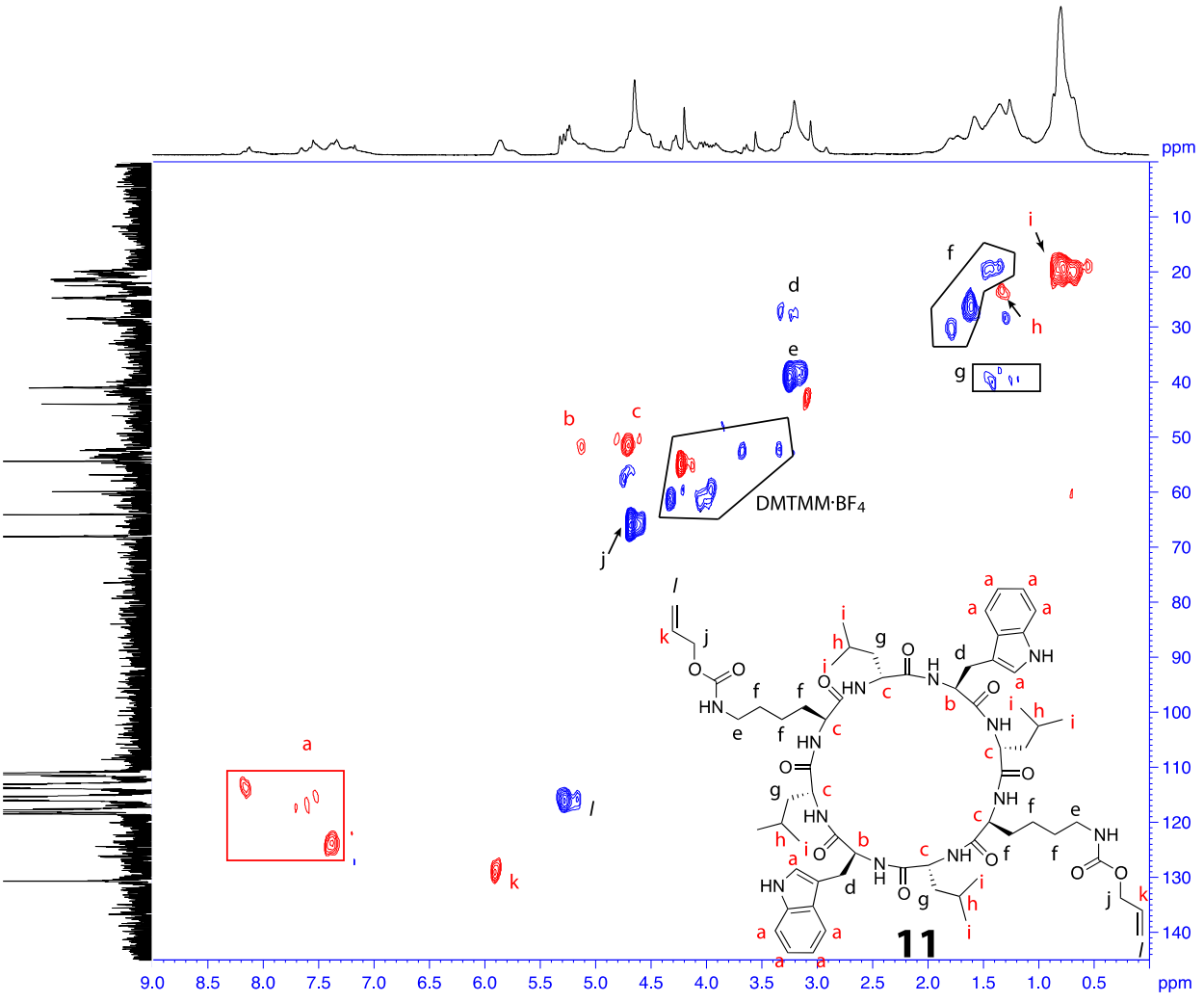

Figure S5. HSQC NMR of cyclic peptide **11.**

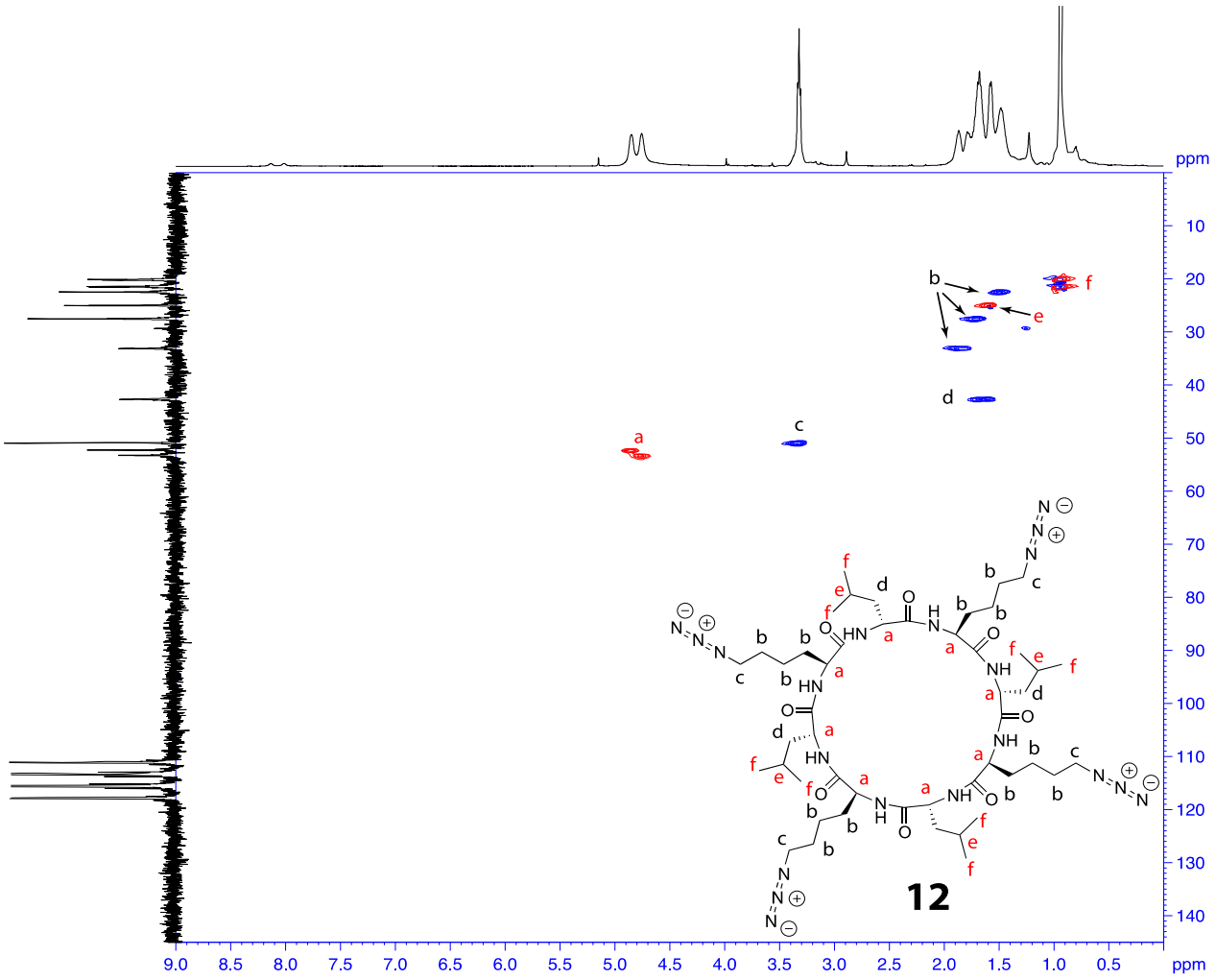

Figure S6. HSQC NMR of cyclic peptide **12.**

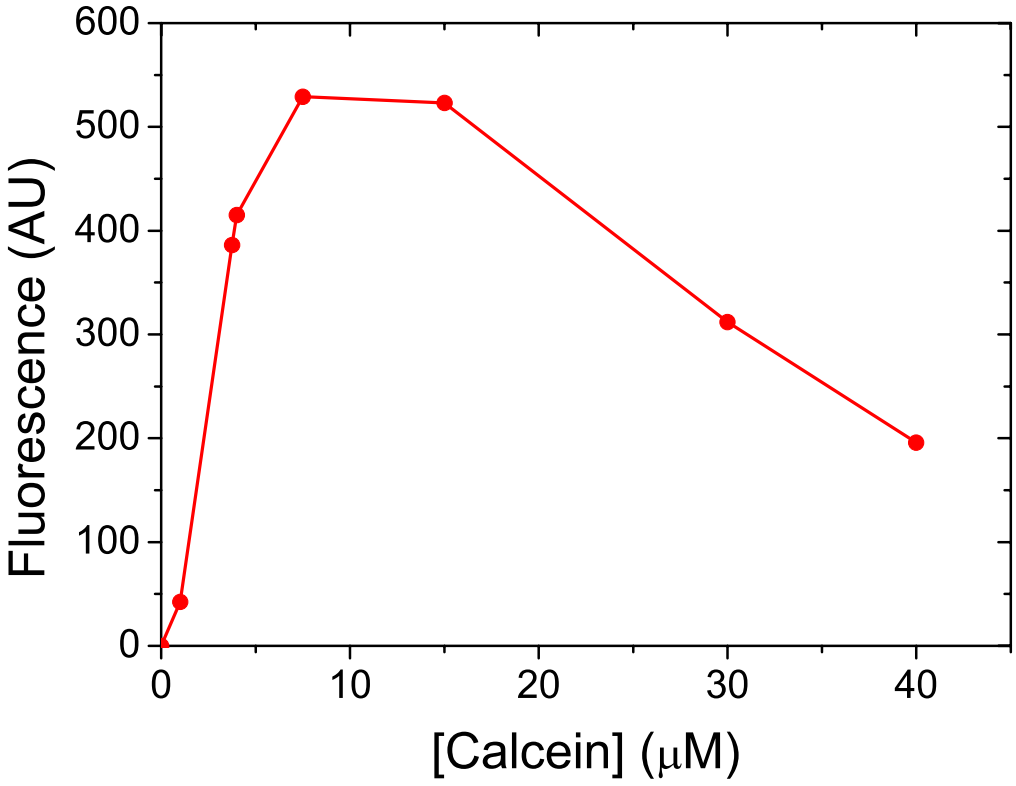

Figure S7. Calcein fluorescence measured at 505 nm as a function of concentration.

References

1. Chapman, R.; Jolliffe, K. A.; Perrier, S., *Adv. Mater.* **2013,** *25*, 1170-1172.

2. Chapman, R.; Jolliffe, K. A.; Perrier, S., *Aust. J. Chem.* **2010,** *63*, 1169-1172.