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Supporting Information

Solid-Phase Peptide Macrocyclization and Multifunctionalization via Dipyrrin Construction

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Chemical Syntheses

Reagents. All amino acid building blocks for Fmoc-based SPPS were purchased from Bidepharm, except Fmoc-Orn(Mtt)-OH and Fmoc-Dap(Mtt)-OH that were purchased from ChemPep. The Rink Amide resin (100-200 mesh) and coupling reagent PyBOP were purchased from Sigma-Aldrich. The pyrrole building block 3-(2,4-dimethyl-1*H*-pyrrol-3-yl)propanoic acid was purchased from Bidepharm. All solvents and other reagents were purchased and used without further purification.

Analytical HPLC. Analytical HPLC was performed on an Agilent 1100 series HPLC system (Agilent Technologies, Stockport, UK) equipped with a diode-array detection (DAD) detector and Agilent C18 column (250 mm x 4.6 mm) at the gradients A and gradients B on **Table S1** and **Table S2**, respectively.

Table S1. The timetable of gradients A.

Time (min)	**A % (H_2O + 0.1 % TFA)**	**B % (MeCN + 0.1 % TFA)**	**Flow (mL/min)**
0	95	5	0.5
40	50	50	0.5
41	0	100	0.5
55	0	100	0.5

Table S2. The timetable of gradients B.

Time (min)	**A % (H_2O + 0.1 % TFA)**	**B % (MeCN + 0.1 % TFA)**	**Flow (mL/min)**
0	80	20	0.5
40	20	80	0.5
41	0	100	0.5
55	0	100	0.5

Preparative HPLC. The purifications of crude products were carried out on Waters semi-preparative system with Waters 2707 Autosampler, Water 1525 Binary HPLC Pump, Waters 2998 Photodiode Array Detector and Waters Fraction Collector III and Atlantis® T3 Prep OBD^{TM} column (C18, 5 μm, 19×250 mm). The Gradient usually refer to the analytical HPLC but flow rate is 5 mL/min. The fractions were collected and verified by ESI-MS.

Mass spectrometry. High-resolution mass spectra, reported as m/z, were obtained from Bruker Autoflex MALDI-TOF mass spectrometer. Low-resolution mass spectra were conducted by SCIEX 3200Q ESI mass spectrometer was also used for monitoring reaction and determining collected fraction during purification of product.

Nuclear magnetic resonance spectroscopy. NMR spectra were recorded on a Bruker Ultrashield 400 Plus NMR spectrometer (^{1}H NMR on 400 MHz, ^{13}C NMR on 101 MHz. The ^{1}H NMR chemical shifts were referenced to corresponding solvent peak (2.50 for DMSO-d_6). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet, br = broad.

General procedure of solid phase peptide synthesis. Standard Fmoc-based SPPS procedure was carried out manually. The empty SPE filtration tube with frits were used for most procedures except Alloc deprotection. To load first amino acid onto Rink Amide resin, Fmoc-deprotected resin was shaken with amino acid building block (4 eq.), PyBOP (4 eq.) and DIPEA (8 eq.) in DMF (4 mL/0.1 mmol) overnight. After loading the first amino acid, capping (the procedure to consume unreacted amine group on the surface of resin with excess acetic anhydride) was NOT conducted as the Mtt-protected amine on side chain of the first loaded amino acid may undergo acetylation. During peptide elongation, the resin was shaken with the amino acid building block (4 eq.), PyBOP (4 eq.) and DIPEA (8 eq.) in DMF (4 mL/0.1 mmol) for 2 h. To remove Fmoc protecting group during SPPS, the resin was shaken with alternative Fmoc deprotection cocktail[1] (5% w/v pyrazine, 1 % v/v DBU, 1 % v/v formic acid in DMF , 4 mL/0.1 mmol) for 25 min. For removing Mtt protecting group, the resin was mixed with 1 % v/v TFA in DCM (4 mL/0.1 mmol) and shaken for around 2 min before removing the solution phase; after repeating this operation for around 15 times, the change of color of resulting solution phase (colorless to yellow to colorless) indicated the completion of Mtt deprotection. To remove Alloc protecting group, the resin was transferred into a thin flash chromatography column with frits on its bottom, a solution of $Pd(PPh_3)_4$ (0.1 eq.) and $PhSiH_3$ (20 eq.) in DCM (8 mL/ 0.1 mmol) was mixed with resin, and the N_2 was bubbled from the bottom of chromatography column to agitate the resin and to keep the reaction under inert atmosphere. The reaction time of Alloc deprotection is around 4 h. To monitor the progress of Alloc deprotection, part of resin could be taken, wash, cleaved, precipitated and then verified by ESI-MS. The resin was fully washed with DMF and DCM after each single step, and washed two more times with DMF or DCM according to the solvent used for the coming steps. Before removing last Fmoc protecting group, the substitute value of resin bound peptide was calculated by the method from literature[2]. Global cleavage and deprotection was carried out with fresh-prepared cleavage cocktail (TFA/TIPS/H_2O, v/v/v, 95/2.5/2.5), the reaction time is 2.5 h for most products except **1i** and **6** which were cleaved overnight. Upon reaction completion, the post-cleavage solution phase was collected, and the resin was washed several more times with fresh prepared cleavage cocktail. The combined post-cleavage solution phase was concentrated by nitrogen-blow, then diethyl ester (over 5/1, v/v) was added, and formed precipitate was collected by centrifugation (10000 rpm, 8 min). The precipitate was re-dissolved by methanol, then repeat precipitation and centrifugation again. The crude product was re-dissolved in water/MeCN, and purified by preparative HPLC.

Synthesis of GHK dipyrrin-cyclopeptide. As shown on **Figure S1**, the Fmoc-Lys(Mtt)-OH, Fmoc-His(Trt)-OH and Fmoc-Gly-OH were coupled during SPPS. Then, Fmoc deprotection and Mtt deprotection was carried out to give a two-amine-containing resin-bound GHK peptide. The resulting resin was shaken with a solution of 3-(2,4-dimethyl-1*H*-pyrrol-3-yl)propanoic acid (5 eq.), PyBOP (5 eq.) and DIPEA (10 eq.) in DMF (4 mL/0.1 mmol) in dark overnight. Then the solution phase was fully washed, and the dipyrrin-cyclopeptide can be formation by following methods. Method A: the resin was shaken with a solution of orthoester (10 eq.) and $POCl_3$ (5 eq.) in DCM (4 mL/0.1 mmol) in dark overnight; Method B: the resin was shaken with a solution of aldehyde (5 eq.) and $BF_3{\cdot}OEt_2$ (1 eq.) in DCM (4 mL/0.1 mmol) in dark overnight, then fully washed, and shaken with a suspension of DDQ (5 eq.) in DCM (4 mL/0.1 mmol) for 90 min; Method C: the resin was shaken with a solution of acyl chloride (10 eq.) in DCM (4 mL/0.1 mmol) in dark overnight. After above-mentioned treatments, the resin was then fully washed, cleaved, precipitated and purified to give the following product **1a**–**1h**. Specially, for **1i**, additional steps were needed after formation of dipyrrin.

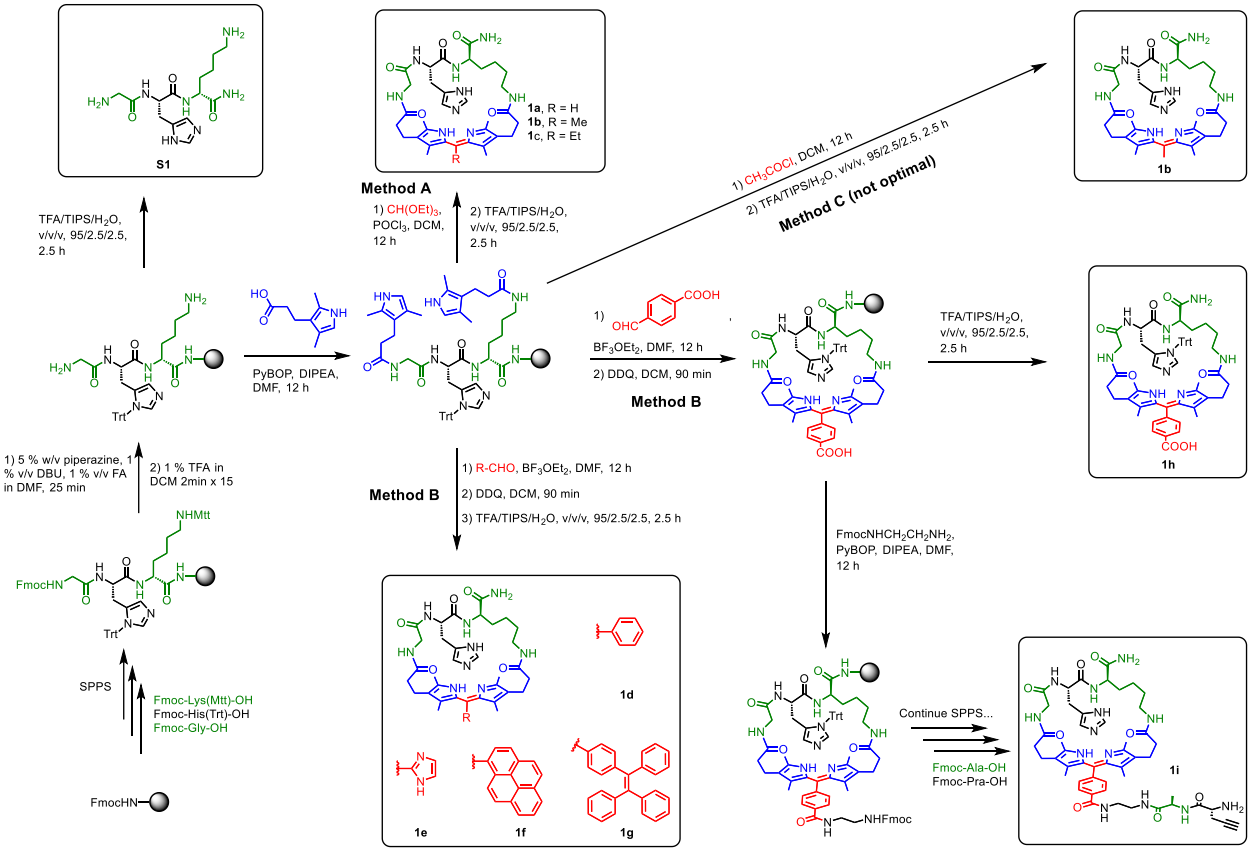

Figure S1. The synthetic route for **S1**, **1a**–**1i**.

*Gly-His-Lys (GHK peptide, **S1**).* The Fmoc-Lys(Mtt)-OH, Fmoc-His(Trt)-OH and Fmoc-Gly-OH were coupled during SPPS. The desired product was obtained after Fmoc deprotection, global cleavage and deprotection, precipitated and purification. Yield: 70 %. White powder. Analytic HPLC: Gradient A, retention time: 7.1 min, purity: 96.8 %. HRMS(MALDI-TOF): calc. for $C_{14}H_{26}N_7O_3^+$ $[M+H]^+$ 340.2092, found 340.2039; calc. for $C_{14}H_{25}N_7NaO_3^+$ $[M+Na]^+$ 362.1911, found 362.1856. ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.51 (s, 2H), 8.97 (t, J = 1.1 Hz, 1H), 8.68 (d, J = 8.0 Hz, 1H), 8.27 (d, J = 7.5 Hz, 1H), 8.07 (t, J = 6.0 Hz, 3H), 7.82 (s, 3H), 7.55 (s, 1H), 7.37 (s, 1H), 7.19 (s, 1H), 4.71 (q, J = 6.8 Hz, 1H), 4.14 (td, J = 8.3, 5.0 Hz, 1H), 3.59 (d, J = 5.6 Hz, 2H), 3.04 (qd, J = 15.3, 6.2 Hz, 2H), 2.75 (q, J = 6.5 Hz, 2H), 1.74 – 1.41 (m, 4H), 1.30 (q, J = 9.5, 8.1 Hz, 2H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 173.7, 169.2, 165.9, 133.8, 128.6, 117.1, 52.5, 51.5, 38.5, 31.1, 27.4, 26.6, 22.3.

1a. Dipyrrin was formed by Method A with triethyl orthoformate. Yield: 31 %. Yellow powder. Analytic HPLC: Gradient A, retention time: 29.5 min, purity: 97.1 %. HRMS(MALDI-TOF): calc. for $C_{33}H_{46}N_9O_5^+$ $[M+H]^+$ 648.3616, found 648.3406; calc. for $C_{33}H_{45}N_9NaO_5^+$ $[M+Na]^+$ 670.3436, found 670.3203. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.66 (d, J = 7.5 Hz, 1H), 8.35 (t, J = 5.7 Hz, 1H), 8.28 (d, J = 7.6 Hz, 1H), 8.22 (d, J = 5.3 Hz, 3H), 7.95 – 7.83 (m, 8H), 7.45 – 7.03 (m, 6H), 4.56 (td, J = 7.5, 5.7 Hz, 1H), 4.33 (q, J = 7.2 Hz, 1H), 4.11 (td, J = 8.6, 4.8 Hz, 1H), 3.83 (q, J = 5.8 Hz, 1H), 3.76 (d, J = 5.5 Hz, 2H), 3.11 (q, J = 6.5 Hz, 2H), 2.76 (q, J = 6.1, 5.6 Hz, 4H), 2.72 – 2.53 (m, 2H), 1.74 – 1.66 (m, 4H), 1.59 – 1.48 (m, 8H), 1.39 – 1.27 (m, 4H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.6, 172.0, 170.8, 169.5, 169.2, 153.9, 153.2, 143.8, 143.5, 133.7, 129.2, 127.4, 126.9, 126.4, 126.2, 119.9, 116.9, 52.2, 51.7, 42.3, 37.9, 34.7, 33.9, 31.1, 28.2, 26.7, 22.4, 19.6, 19.3, 12.6, 12.5, 9.9, 9.8.

1b. Dipyrrin was formed by either Method A with triethyl orthoacetate (Yield: 24 %) or Method C with acetyl chloride (Yield: 10 %). Orange powder. Analytic HPLC: Gradient A, retention time: 25.9 min, purity: 97.0 %. HRMS(MALDI-TOF): calc. for $C_{34}H_{48}N_9O_5^+$ $[M+H]^+$ 662.3773, found 662.4363; calc. for $C_{34}H_{47}N_9NaO_5^+$ $[M+Na]^+$ 684.3592, found 684.4176.

1c. Dipyrrin was formed by Method A with triethyl orthopropionate. Yield: 21 %. Orange powder. Analytic HPLC: Gradient A, retention time: 27.2 min, purity: 97.8 %. HRMS(MALDI-TOF): calc. for $C_{35}H_{50}N_9O_5^+$ $[M+H]^+$ 676.3929, found 676.3767; calc. for $C_{35}H_{49}N_9NaO_5^+$ $[M+Na]^+$ 698.3749, found 698.3571.

1d. Dipyrrin was formed by Method B with benzaldehyde. Yield: 34 %. Red powder. Analytic HPLC: Gradient A, retention time: 36.9 min, purity: 96.3 %. HRMS(MALDI-TOF): calc. for $C_{39}H_{50}N_9O_5^+$ $[M+H]^+$ 724.3929, found 724.3917.

1e. Dipyrrin was formed by Method B with 1*H*-imidazole-2-carbaldehyde. Yield: 45 %. Red powder. Analytic HPLC: Gradient A, retention time: 19.4 min, purity: 96.1 %. HRMS(MALDI-TOF): calc. for $C_{36}H_{48}N_{11}O_5^+$ $[M+H]^+$ 714.3834, found 714.4103; calc. for $C_{36}H_{47}N_{11}NaO_5^+$ $[M+Na]^+$ 736.3654, found 736.3939.

1f. Dipyrrin was formed by Method B with pyrene-1-carbaldehyde. Yield: 42 %. Red powder. Analytic HPLC: Gradient B, retention time: 26.2 min, purity: 97.2 %. HRMS(MALDI-TOF): calc. for $C_{49}H_{54}N_9O_5^+$ $[M+H]^+$ 848.4242, found 848.4385; calc. for $C_{49}H_{53}N_9NaO_5^+$ $[M+Na]^+$ 870.4062, found 870.4309. ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.26 (s, 2H), 11.58 (s, 2H), 8.99 (m, 1H), 8.53 – 8.29 (m, 7H), 8.26 – 8.02 (m, 3H), 7.87 (m, 3H), 7.38 (s, 1H), 7.15 (s, 2H), 4.57 (m, 1H), 4.11 (m, 1H), 3.71 (m, 2H), 3.19 (m, 2H), 2.99 (m, 2H), 2.72 (m, 4H), 2.43 – 2.20 (m, 10H), 2.04 – 1.93 (m, 2H), 1.74 – 1.24 (m, 10H).

1g. Dipyrrin was formed by Method B with 4-(1,2,2-triphenylvinyl)benzaldehyde. Yield: 39 %. Red powder. Analytic HPLC: Gradient B, retention time: 31.4 min, purity: 97.7 %. HRMS(MALDI-TOF): calc. for $C_{59}H_{64}N_9O_5^+$ $[M+H]^+$ 978.5025, found 978.6518.

1h. Dipyrrin was formed by Method B with 4-formylbenzoic acid. Yield: 36 %. Red powder. Analytic HPLC: Gradient A, retention time: 32.0 min, purity: 97.5 %. HRMS(MALDI-TOF): calc. for $C_{40}H_{50}N_9O_7^+$ $[M+H]^+$ 768.3828, found 768.4274. ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.24 (d, J = 21.2 Hz, 2H), 11.92 (s, 2H), 8.98 (s, 1H), 8.40 (m, 2H), 8.12 (d, J = 8.5 Hz, 2H), 7.82 (m, 2H), 7.44 (s, 2H), 7.38 – 7.34 (m, 1H), 7.17 (m, 2H), 4.52 (dd, J = 9.3, 3.4 Hz, 1H), 4.08 (td, J = 9.0, 5.2 Hz, 1H), 3.63 (s, 2H), 3.21 – 2.98 (m, 2H), 2.95 (m, 2H), 2.69 (dt, J = 12.9, 7.6 Hz, 4H), 2.37 (d, J = 4.8 Hz, 6H), 2.35 – 2.15 (m, 4H), 1.99 (m, 2H), 1.68 (m, 6H), 1.59 – 1.31 (m, 4H).

1i. The formation of dipyrrin was same as **1h**. After formation of dipyrrin (sample was taken, cleaved, precipitated and analyzed by the HPLC and ESI-MS, sample 1 in **Figure S2**), the resin was mixed with PyBOP (4 eq.), DIPEA (8 eq.) in DMF (4 mL/0.1mmol) for 5 min, then (9*H*-fluoren-9-yl)methyl (2-aminoethyl)carbamate ($FmocHNCH_2CH_2NH_2$, 4 eq.) was added. The reaction was shaken for 3 h (Sample 2 in **Figure S2**), then Fmoc deprotection was carried out after fully washing. The amino building block Fmoc-Ala-OH and Fmoc-Pra-OH were used to continue SPPS. The desired product was obtained after Fmoc deprotection, global cleavage and deprotection, precipitated (Sample 3 in **Figure S2**) and purification. Yield: 21 %. Red powder. Analytic HPLC: Gradient A, retention time: 24.8 min, purity: 98.9 %.

HRMS(MALDI-TOF): calc. for $C_{50}H_{66}N_{13}O_8^+$ $[M+H]^+$ 976.5152, found 976.5419; calc. for $C_{50}H_{65}N_{13}NaO_8^+$ $[M+Na]^+$ 998.4971, found 998.5286.

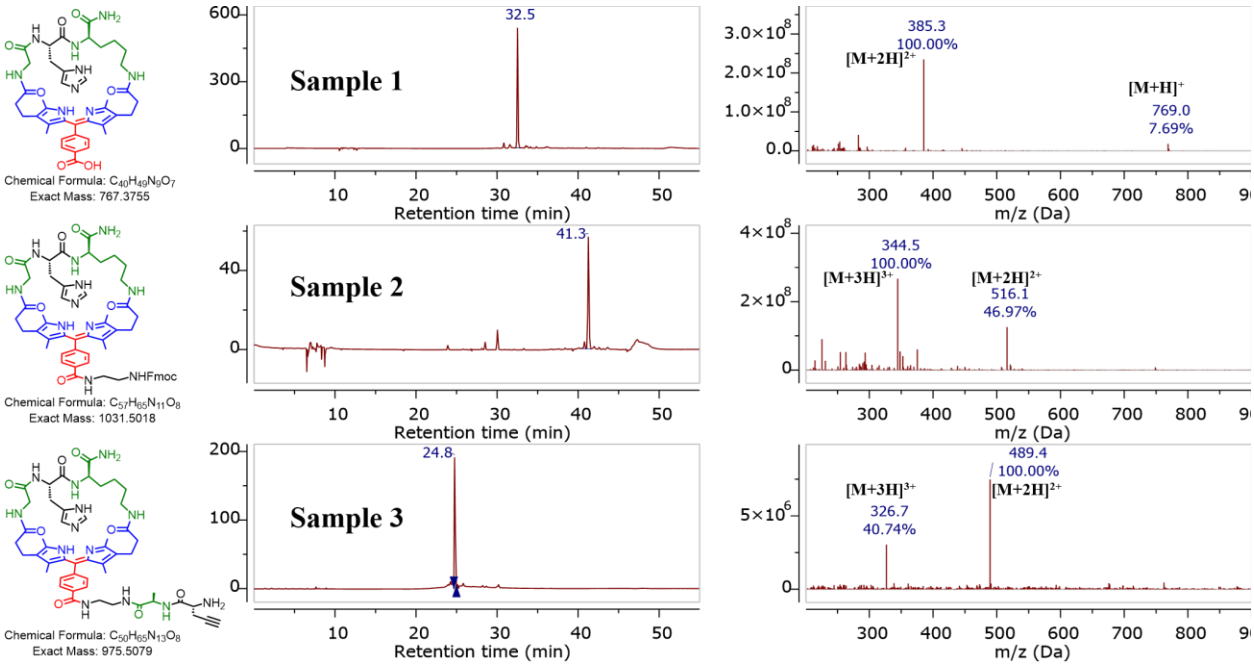

Figure S2. The crude products of intermediate steps for synthesizing **1i** were cleaved, precipitated and analyzed by HPLC and ESI-MS.

Synthesis of GAK dipyrrin-cyclopeptide (2). The Fmoc-Lys(Mtt)-OH, Fmoc-Ala-OH and Fmoc-Gly-OH were coupled during SPPS. Then, the Fmoc and Mtt protecting groups were removed, and the dipyrrin was form by Method A with triethyl orthoformate. The desired product was obtained after global cleavage and deprotection, precipitated and purification. Yield: 23 %. Yellow powder. Analytic HPLC: Gradient A, retention time: 30.0 min, purity: 95.7 %. HRMS(MALDI-TOF): calc. for $C_{30}H_{44}N_7O_5^+$ $[M+H]^+$ 582.3398, found 582.3429; calc. for $C_{30}H_{43}N_7NaO_5^+$ $[M+Na]^+$ 604.3218, found 604.3245. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.97 (S, 2H), 8.30 – 8.19 (m, 2H), 7.81 (t, J = 5.8 Hz, 1H), 7.76 (d, J = 8.5 Hz, 1H), 7.20 (s, 1H), 7.10 (m, 1H), 4.26 – 4.19 (m, 1H), 4.11 – 4.05 (m, 1H), 3.74 – 3.62 (m, 2H), 3.01 – 2.94 (m, 2H), 2.54 (t, J = 5.6 Hz, 4H), 2.40 (s, 6H), 2.32 – 2.13 (m, 10H), 1.99 (d, J = 7.5 Hz, 2H), 1.58 – 1.40 (m, 4H), 1.20 (d, J = 7.1 Hz, 3H).

Synthesis of XRGDX and corresponding dipyrrin-cyclopeptides with Fmoc-X(Mtt)-OH (X = Lys, Orn, Dap). As shown on **Figure S3**, the Fmoc-X(Mtt)-OH, Fmoc-Asp(^{t}Bu)-OH, Fmoc-Gly-OH, Fmoc-Arg(Pbf)-OH and Fmoc-X(Mtt)-OH were coupled during SPPS. The linear peptides (**3a**, **3b** and **3d**) were obtained after Fmoc deprotection, global cleavage and deprotection, precipitated and purification. And the corresponding dipyrrin-cyclopeptide (**4a**, **4b** and **4d**) were obtained after Mtt deprotection, dipyrrin formation (Method A with triethyl orthoformate), Fmoc deprotection, global cleavage and deprotection, precipitated and purification.

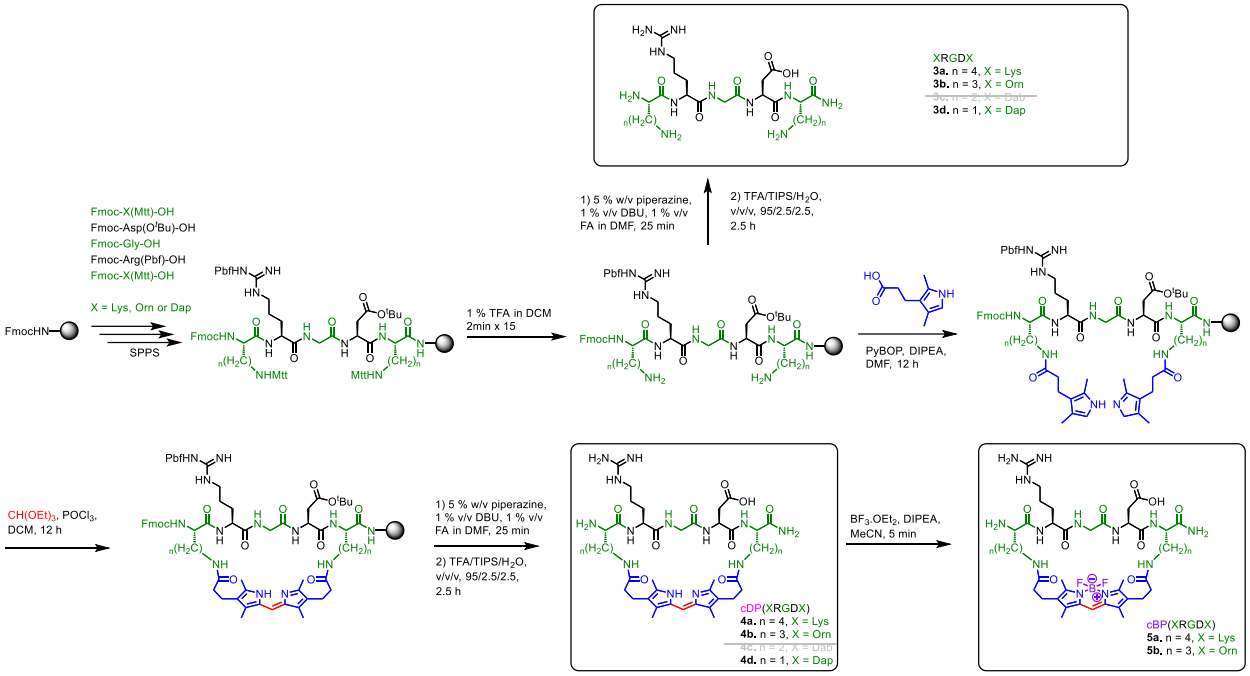

Figure S3. The synthetic route for **3a**, **3b**, **3d**, **4a**, **4b**, **4d**, **5a** and **5b**.

*Lys-Arg-Gly-Asp-Lys (**3a**)*. Yield: 68 %. White powder. Analytic HPLC: Gradient A, retention time: 12.6 min, purity: 98.5 %. HRMS(MALDI-TOF): calc. for $C_{24}H_{48}N_{11}O_7^+$ $[M+H]^+$ 602.3733, found 602.3129; calc. for $C_{24}H_{47}N_{11}NaO_7^+$ $[M+Na]^+$ 624.3552, found 624.2903. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.66 (d, J = 7.5 Hz, 1H), 8.35 (t, J = 5.7 Hz, 1H), 8.28 (d, J = 7.6 Hz, 1H), 8.22 (d, J = 5.3 Hz, 3H), 7.95 – 7.83 (m, 8H), 7.45 – 7.03 (m, 6H), 4.56 (td, J = 7.5, 5.7 Hz, 1H), 4.33 (q, J = 7.4, 6.9 Hz, 1H), 4.11 (td, J = 8.6, 4.8 Hz, 1H), 3.83 (q, J = 5.8 Hz, 1H), 3.76 (d, J = 5.5 Hz, 2H), 3.11 (q, J = 6.5 Hz, 2H), 2.76 (q, J = 6.1, 5.6 Hz, 4H), 2.72 – 2.53 (m, 2H), 1.74 – 1.66 (m, 4H), 1.59 – 1.48 (m, 8H), 1.39 – 1.27 (m, 4H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.4, 171.8, 171.3, 170.4, 168.7, 168.5, 156.9, 52.4, 52.3, 51.8, 49.5, 41.7, 40.3, 38.6, 38.4, 36.1, 30.9, 30.4, 29.0, 26.5, 26.3, 24.9, 22.1, 20.9.

*Orn-Arg-Gly-Asp-Orn (**3b**)*. Yield: 66 %. White powder. Analytic HPLC: Gradient A, retention time: 8.0 min, purity: 95.0 %. HRMS(MALDI-TOF): calc. for $C_{22}H_{44}N_{11}O_7^+$ $[M+H]^+$ 574.3420, found 574.3241; calc. for $C_{22}H_{43}N_{11}NaO_7^+$ $[M+Na]^+$ 596.3239, found 596.3008. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.68 (d, J = 7.7 Hz, 1H), 8.34 (m, 2H), 8.26 (d, J = 5.5 Hz, 3H), 7.97 (d, J = 8.1 Hz, 1H), 7.83 (dt, J = 18.7, 6.4 Hz, 7H), 7.50 – 7.03 (m, 6H), 4.57 (td, J = 7.8, 5.5 Hz, 1H), 4.36 (q, J = 7.4, 6.9 Hz, 1H), 4.15 (td, J = 8.0, 4.9 Hz, 1H), 3.88 (d, J = 5.1 Hz, 1H), 3.77 (d, J = 5.9 Hz, 2H), 3.11 (m, 2H), 2.79 (m, 4H), 2.73 – 2.51 (m, 2H), 1.77 – 1.49 (m, 12H).

*Dap-Arg-Gly-Asp-Dap (**3d**)*. Yield: 66 %. White powder. Analytic HPLC: Gradient A, retention time: 8.5 min, purity: 97.4 %. HRMS(MALDI-TOF): calc. for $C_{18}H_{36}N_{11}O_7^+$ $[M+H]^+$ 518.2794, found 518.3320; calc. for $C_{18}H_{35}N_{11}NaO_7^+$ $[M+Na]^+$ 540.2613, found 540.2977. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.86 (d, J = 7.3 Hz, 1H), 8.58 – 8.23 (m, 9H), 7.91 (s, 3H), 7.80 (s, 1H), 7.40 (m, 6H), 4.55 (d, J = 5.9 Hz, 1H), 4.46 (d, J = 6.0 Hz, 1H), 4.36 (m, 1H), 4.24 (m, 1H), 3.83 (m, 2H), 3.27 (m, 3H), 3.10 (m, 2H), 2.96 (m, 1H), 2.79 – 2.58 (m, 2H), 1.65 (m, 4H).

4a. Yield: 24 %. Yellow powder. Analytic HPLC: Gradient A, retention time: 27.2 min, purity: 95.6 %. HRMS(MALDI-TOF): calc. for $C_{43}H_{68}N_{13}O_9^+$ $[M+H]^+$ 910.5258, found 910.4834. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.21 (s, 2H), 8.57 (d, J = 8.0 Hz, 1H), 8.30 (t, J = 5.6 Hz, 1H), 8.25 (d, J = 7.7 Hz, 1H), 8.13

(d, *J* = 5.4 Hz, 3H), 7.81 – 7.65 (m, 4H), 7.36 – 7.00 (m, 7H), 4.54 (q, *J* = 7.0 Hz, 1H), 4.38 (q, *J* = 7.5, 7.0 Hz, 1H), 4.04 (td, *J* = 8.7, 4.5 Hz, 1H), 3.79 (s, 1H), 3.73 (m, 2H), 3.09 (q, *J* = 6.4 Hz, 2H), 2.90 (dh, *J* = 12.9, 6.6 Hz, 4H), 2.69 (dt, *J* = 10.1, 6.1 Hz, 4H), 2.53 (s, 2H), 2.44 (d, *J* = 4.5 Hz, 6H), 2.27 (m, 10H), 1.73 – 1.23 (m, 16H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.5, 171.9, 171.1, 171.0, 170.1, 168.6, 168.3, 156.8, 153.5, 143.5, 127.5, 127.4, 126.4, 126.4, 120.1, 52.3, 52.1, 51.9, 49.4, 41.7, 40.3, 38.2, 38.2, 36.0, 35.03, 34.9, 34.8, 31.2, 31.1, 30.7, 29.3, 29.0, 28.9, 28.9, 28.8, 28.7, 28.7, 28.6, 28.5, 26.5, 25.0, 24.8, 22.5, 22.0, 21.3, 19.5, 13.9, 12.5, 12.5, 9.8.

4b. Yield: 27 %. Yellow powder. Analytic HPLC: Gradient A, retention time: 29.2 min, purity: 97.4 %. HRMS(MALDI-TOF): calc. for $C_{41}H_{64}N_{13}O_9^+$ $[M+H]^+$ 882.4945, found 882.5381. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.11 (d, *J* = 7.7 Hz, 2H), 8.56 (d, *J* = 8.1 Hz, 1H), 8.27 (m, 2H), 8.13 (d, *J* = 5.3 Hz, 3H), 7.83 (d, *J* = 7.9 Hz, 1H), 7.77 (t, *J* = 5.8 Hz, 1H), 7.70 (dt, *J* = 12.2, 5.6 Hz, 2H), 7.34 – 6.98 (m, 7H), 4.51 (td, *J* = 7.7, 5.4 Hz, 1H), 4.42 – 4.36 (m, 1H), 4.04 – 3.95 (m, 1H), 3.87 – 3.65 (m, 3H), 3.07 (d, *J* = 6.4 Hz, 2H), 2.96 (t, *J* = 6.6 Hz, 2H), 2.91 – 2.83 (m, 2H), 2.71 – 2.64 (m, 4H), 2.57 (m, 2H), 2.43 (d, *J* = 2.4 Hz, 6H), 2.30 – 2.22 (m, 10H), 1.62 (m, 4H), 1.52 – 1.33 (m, 8H).

4d. Yield: 27 %. Yellow powder. Analytic HPLC: Gradient A, retention time: 30.1 min, purity: 96.7 %. HRMS(MALDI-TOF): calc. for $C_{37}H_{56}N_{13}O_9^+$ $[M+H]^+$ 826.4319, found 826.4820. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.15 (s, 2H), 8.74 (d, *J* = 7.7 Hz, 1H), 8.31 (t, *J* = 5.6 Hz, 1H), 8.29 – 8.10 (m, 3H), 8.02 (d, *J* = 7.8 Hz, 1H), 7.93 (t, *J* = 6.0 Hz, 1H), 7.81 (d, *J* = 7.9 Hz, 1H), 7.72 (td, *J* = 5.9, 2.3 Hz, 2H), 7.52 – 6.87 (m, 7H), 4.36 (dq, *J* = 10.1, 7.1 Hz, 2H), 4.06 (q, *J* = 6.7 Hz, 1H), 3.89 (s, 1H), 3.69 (ddd, *J* = 59.7, 16.9, 5.4 Hz, 2H), 3.46 – 3.27 (m, 2H), 3.24 – 3.12 (m, 2H), 3.08 (q, *J* = 6.5 Hz, 2H), 2.75 – 2.51 (m, 6H), 2.43 (d, *J* = 3.6 Hz, 6H), 2.38 – 2.26 (m, 10H), 1.70 – 1.42 (m, 4H).

Synthesis of XRGDX and corresponding dipyrrin-cyclopeptides with alternative strategy (X = Dab). As shown on **Figure S4**, the Fmoc-Dab(Mtt)-OH failed to couple during SPPS, alternative strategy was applied. Fmoc-Dab(Alloc)-OH, Fmoc-Asp(^{t}Bu)-OH, Fmoc-Gly-OH, Fmoc-Arg(Pbf)-OH and Boc-Dab(Fmoc)-OH were coupled during SPPS. The linear peptides (**3c**) were obtained after Alloc deprotection, Fmoc deprotection and global cleavage and deprotection, precipitated and purification. And the corresponding dipyrrin-cyclopeptide (**4c**) were obtained after Fmoc deprotection, Alloc deprotection, dipyrrin formation (Method A with triethyl orthoformate) and global cleavage and deprotection, precipitated and purification.

*Dab-Arg-Gly-Asp-Dab (**3c**).* Yield: 65 %. White powder. Analytic HPLC: Gradient A, retention time: 8.2 min, purity: 96.0 %. HRMS(MALDI-TOF): calc. for $C_{20}H_{40}N_{11}O_7^+$ $[M+H]^+$ 546.3107, found 546.3308; calc. for $C_{20}H_{39}N_{11}NaO_7^+$ $[M+Na]^+$ 568.2926, found 568.3089. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.72 (d, *J* = 7.0 Hz, 1H), 8.35 (m, 5H), 8.17 (d, *J* = 7.8 Hz, 1H), 8.05 – 7.76 (m, 7H), 7.31 (m, 6H), 4.56 (q, *J* = 7.0 Hz, 1H), 4.34 (q, *J* = 7.1, 6.6 Hz, 1H), 4.23 (td, *J* = 8.0, 5.3 Hz, 1H), 3.94 (s, 1H), 3.77 (qd, *J* = 16.8, 5.7 Hz, 2H), 3.11 (m, 2H), 2.94 (s, 2H), 2.80 (q, *J* = 7.3, 6.7 Hz, 2H), 2.74 – 2.53 (m, 2H), 2.05 – 1.50 (m, 8H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.4, 171.9, 171.2, 170.6, 168.6, 167.5, 156.8, 52.5, 50.3, 49.7, 49.5, 41.6, 40.3, 36.1, 34.8, 29.5, 29.1, 28.9, 24.9.

4c. Yield: 25 %. Yellow powder. Analytic HPLC: Gradient A, retention time: 26.4 min, purity: 97.0 %. HRMS(MALDI-TOF): calc. for $C_{39}H_{60}N_{13}O_9^+$ $[M+H]^+$ 854.4632, found 854.5409. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.18 (d, *J* = 9.7 Hz, 2H), 8.68 (d, *J* = 7.8 Hz, 1H), 8.32 (t, *J* = 5.5 Hz, 1H), 8.19 (m, 4H), 8.01 (d, *J* = 8.1 Hz, 1H), 7.91 (t, *J* = 5.8 Hz, 1H), 7.69 (dt, *J* = 10.4, 5.6 Hz, 2H), 7.48 – 6.92 (m, 7H), 4.53 (q, *J* = 6.9 Hz, 1H), 4.38 (q, *J* = 7.8, 7.2 Hz, 1H), 4.04 (td, *J* = 8.5, 5.2 Hz, 1H), 3.77 (d, *J* = 6.3 Hz, 1H), 3.72 (d, *J* = 5.3 Hz, 2H), 3.22 – 2.83 (m, 6H), 2.67 (dt, *J* = 16.5, 8.8 Hz, 4H), 2.57 (m, 2H), 2.45 (s, 6H), 2.28 (m, 10H), 1.77 – 1.40 (m, 8H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.8, 171.9, 171.6, 171.2, 171.1, 170.0,

168.5, 167.9, 156.7, 153.7, 153.2, 143.6, 143.3, 127.5, 127.4, 126.5, 126.3, 120.2, 52.0, 50.6, 50.3, 49.4, 41.8, 40.3, 36.1, 35.7, 35.6, 35.0, 34.8, 34.7, 34.6, 31.4, 31.2, 29.4, 26.5, 24.8, 19.5, 19.1, 12.6, 12.5, 9.8, 9.8.

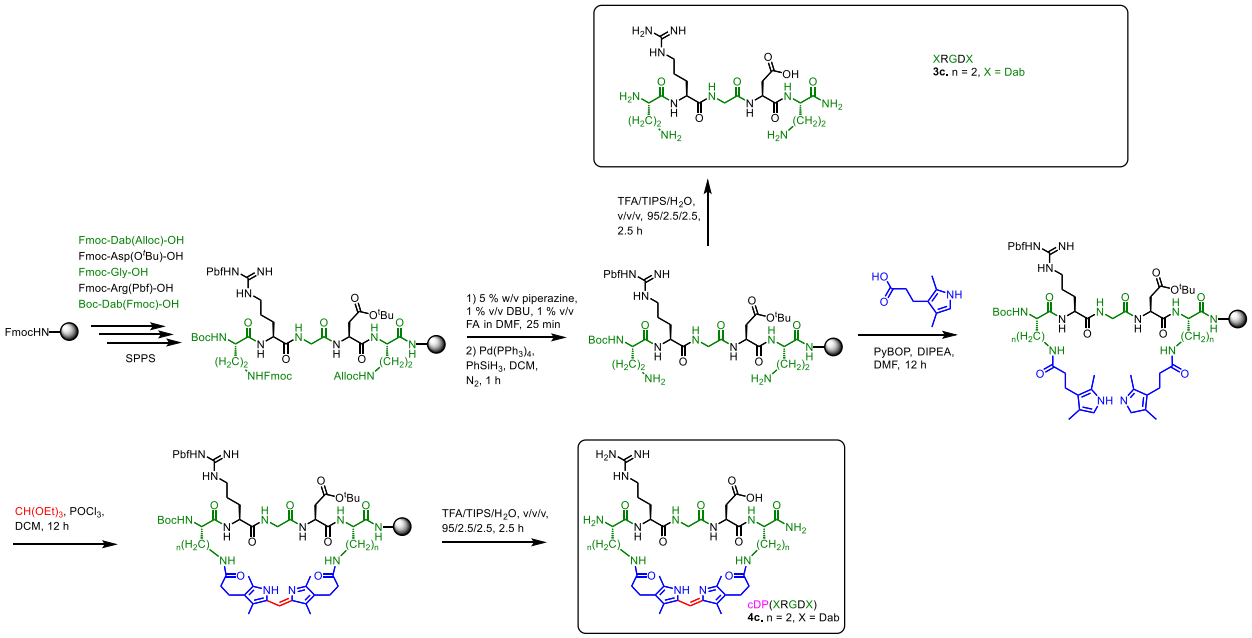

Figure S4. The synthetic route for **3c** and **4c**.

Synthesis of XRGDX BODIPY-cyclopeptide (4a-BODIPY and 4b-BODIPY). To a suspension of dipyrrin cyclopeptide (**4a**/**4b**, around 3 mg) in 500 µL MeCN, the DIPEA (85 µL) was added. The resulting mixture was sonicated for 5 min, and the $BF_3{\cdot}OEt_2$ (140 µL) was added in one portion. The resulting mixture was further sonicated for 5 min, then around 500 µL H_2O was added for quenching the excess $BF_3{\cdot}OEt_2$ (the resulting mixture showed bright yellow-to-green fluorescence). The crude product was purified by preparative HPLC immediately.

4a-BODIPY. Yield: 47 %. Orange powder. Analytic HPLC: Gradient A, retention time: 31.0 min, purity: 97.8 %. HRMS(MALDI-TOF): calc. for $C_{43}H_{67}BF_2N_{13}O_9^+$ $[M+H]^+$ 958.5240, found 958.6489; calc. for $C_{43}H_{68}BFN_{13}O_9^+$ $[M-F+2H]^+$ 940.5335, found 940.5219.

4b-BODIPY. Yield: 52 %. Orange powder. Analytic HPLC: Gradient A, retention time: 30.5 min, purity: 95.7 %. HRMS(MALDI-TOF): calc. for $C_{41}H_{63}BF_2N_{13}O_9^+$ $[M+H]^+$ 930.4927, found 930.6394; calc. for $C_{41}H_{64}BFN_{13}O_9^+$ $[M-F+2H]^+$ 912.5022, found 912.5293.

Synthesis of bremelanotide-derived (Nva-Lys-His-Phe-Arg-Trp-Lys) dipyrrin-cyclopeptide (5). The Fmoc-Lys(Mtt)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Phe-OH, Fmoc-His(Trt)-OH, Fmoc-Lys(Mtt)-OH and Fmoc-Nva-OH were coupled during SPPS. The corresponding dipyrrin-cyclopeptide (**5a** and **5b**) were obtained after Mtt deprotection, dipyrrin formation, Fmoc deprotection, global cleavage and deprotection, precipitated and purification.

5a. Dipyrrin formation was conducted by Method A with triethyl orthoformate. Yield: 22 %. Yellow powder. Analytic HPLC: Gradient B, retention time: 16.7 min, purity: 95.1 %. HRMS(MALDI-TOF): calc.

for $C_{68}H_{95}N_{18}O_9^+$ $[M+H]^+$ 1307.7524, found 1307.7774; calc. for $C_{68}H_{94}N_{18}NaO_9^+$ $[M+Na]^+$ 1329.7343, found 1329.7705. ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.2 (s, 2H), 12.0 (d, J = 23.8 Hz, 2H), 10.8 (s, 1H), 8.9 (s, 1H), 8.5 (d, J = 7.4 Hz, 1H), 8.3 (d, J = 8.2 Hz, 1H), 8.1 (s, 5H), 7.9 – 7.8 (m, 3H), 7.7 – 7.5 (m, 3H), 7.3 (d, J = 8.8 Hz, 2H), 7.2 – 7.1 (m, 6H), 7.1 – 6.9 (m, 9H), 4.6 – 4.5 (m, 3H), 4.3 – 4.1 (m, 3H), 3.8 – 3.7 (m, 1H), 3.1 – 3.1 (m, 2H), 3.0 – 2.9 (m, 10H), 2.7 – 2.6 (m, 4H), 2.5 – 2.3 (m, 12H), 2.3 – 2.3 (m, 4H), 1.7 – 1.3 (m, 20H), 0.9 – 0.8 (m, 3H).

5b. Dipyrrin formation was conducted by Method B with 9-anthracenecarboxaldehyde. Yield: 32 %. Red powder. Analytic HPLC: Gradient B, retention time: 21.5 min, purity: 96.5 %. HRMS(MALDI-TOF): calc. for $C_{82}H_{103}N_{18}O_9^+$ $[M+H]^+$ 1483.8150, found 1483.8150; calc. for $C_{82}H_{102}N_{18}NaO_9^+$ $[M+Na]^+$ 1505.7969, found 1505.8585.

Synthesis of KNKMKIK dipyrrin-cyclopeptides (6). The Fmoc-Lys(Mtt)-OH, Fmoc-Ile-OH, Fmoc-Lys(Alloc)-OH, Fmoc-Met-OH, Fmoc-Lys(Mtt)-OH, Fmoc-Asn(Trt)-OH and Boc-Lys(Fmoc)-OH were coupled during SPPS. The corresponding dipyrrin-cyclopeptide (**6a** and **6b**) were obtained after Mtt deprotection, dipyrrin formation, Fmoc deprotection, global cleavage and deprotection, precipitated and purification.

6a. Dipyrrin formation was conducted by Method A with triethyl orthoformate. Yield: 28 %. Yellow powder. Analytic HPLC: Gradient B, retention time: 17.1 min, purity: 95.3 %. HRMS(MALDI-TOF): calc. for $C_{62}H_{102}N_{15}O_{12}S^+$ $[M+H]^+$ 1280.7548, found 1280.7967; calc. for $C_{62}H_{101}N_{15}NaO_{12}S^+$ $[M+Na]^+$ 1302.7367, found 1302.8008. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.1 (d, J = 22.7 Hz, 2H), 8.6 (d, J = 8.0 Hz, 1H), 8.2 – 8.1 (m, 6H), 7.8 – 7.7 (m, 7H), 7.6 (d, J = 8.4 Hz, 1H), 7.3 (s, 1H), 7.2 (s, 2H), 7.0 (s, 2H), 5.9 (ddt, J = 15.7, 10.6, 5.4 Hz, 1H), 5.3 (d, J = 17.1 Hz, 1H), 5.2 (d, J = 10.3 Hz, 1H), 4.6 (q, J = 7.2 Hz, 1H), 4.4 (d, J = 5.4 Hz, 2H), 4.2 – 4.1 (m, 5H), 3.7 – 3.7 (m, 1H), 3.0 – 2.9 (m, 6H), 2.7 – 2.7 (m, 8H), 2.5 (s, 2H), 2.4 (s, 6H), 2.3 – 2.3 (m, 10H), 2.0 (s, 3H), 1.7 – 1.6 (m, 9H), 1.6 – 1.5 (m, 10H), 1.4 – 1.3 (m, 10H), 0.8 – 0.8 (m, 6H).

6b. Dipyrrin formation was conducted by Method B with 4-bromobenzaldehyde. Yield: 37 %. Red powder. Analytic HPLC: Gradient B, retention time: 21.1 min, purity: 95.7 %. HRMS(MALDI-TOF): calc. for $C_{68}H_{105}BrN_{15}O_{12}S^+$ $[M+H]^+$ 1434.6966, found 1434.8219.

Synthesis of RVCRKP dipyrrin-cyclopeptides (7). The Fmoc-Pro-OH, Fmoc-Lys(Mtt)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Val-OH and Fmoc-Arg(Pbf)-OH were coupled during SPPS. The corresponding dipyrrin-cyclopeptide (**7a** and **7b**) were obtained after Mtt deprotection, Fmoc-deprotection, dipyrrin formation, global cleavage and deprotection, precipitated and purification.

7a. Dipyrrin formation was conducted by Method A with triethyl orthoformate. Yield: 28 %. Yellow powder. Analytic HPLC: Gradient A, retention time: 28.6 min, purity: 98.6 %. HRMS(MALDI-TOF): calc. for $C_{50}H_{81}N_{16}O_8S^+$ $[M+H]^+$ 1065.6139, found 1065.6181. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.2 (s, 2H), 8.2 – 6.8 (m, 19H), 4.6 – 4.1 (m, 6H), 3.5 – 3.5 (m, 2H), 3.3 – 2.7 (m, 10H), 2.7 – 2.5 (m, 4H), 2.5 – 2.1 (m, 14H), 2.0 – 1.3 (m, 19H), 0.9 – 0.6 (m, 6H).

7b. Dipyrrin formation was conducted by Method B with 4-(trifluoromethyl)benzaldehyde. Yield: 41 %. Red powder. Analytic HPLC: Gradient A, retention time: 20.0 min, purity: 94.0 %. HRMS(MALDI-TOF): calc. for $C_{57}F_3H_{84}N_{16}O_8S^+$ $[M+H]^+$ 1209.6325, found 1209.6384.

Synthesis of ATSP-6935 derived (LTFKEYWAQLKS) peptide and its dipyrrin-cyclopeptides (8). The Fmoc-Ser(^{t}Bu)-OH, Fmoc-Lys(Mtt)-OH, Fmoc-Leu-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ala-OH, Fmoc-Trp(Boc)-OH, Fmoc-Tyr(^{t}Bu)-OH, Fmoc-Glu(^{t}Bu)-OH, Fmoc-Lys(Mtt)-OH, Fmoc-Phe-OH, Fmoc-Thr(^{t}Bu)-OH and Fmoc-Leu-OH were coupled during SPPS. The linear peptides (**S2**) were obtained after Fmoc deprotection, global cleavage and deprotection, precipitated and purification. And the corresponding dipyrrin-cyclopeptide (**8a** and **8b**) were obtained after Mtt deprotection, dipyrrin formation, Fmoc-deprotection, global cleavage and deprotection, precipitated and purification.

S2. Analytic HPLC: Gradient B, retention time: 17.6 min, purity: 98.4 %. HRMS(MALDI-TOF): calc. for $C_{73}H_{109}N_{17}NaO_{18}^+$ $[M+Na]^+$ 1534.8029, found 1534.7627. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.79 (s, 1H), 8.45 (d, *J* = 8.4 Hz, 1H), 8.22 (t, *J* = 8.8 Hz, 2H), 8.17 – 8.07 (m, 5H), 8.01 – 7.89 (m, 5H), 7.82 – 7.73 (m, 7H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 2H), 7.21 (d, *J* = 4.4 Hz, 6H), 7.17 – 7.11 (m, 3H), 7.07 – 7.03 (m, 1H), 6.99 – 6.93 (m, 3H), 6.85 (s, 1H), 6.58 (d, *J* = 8.2 Hz, 2H), 4.56 – 4.50 (m, 2H), 4.40 – 4.37 (m, 1H), 4.31 (d, *J* = 3.8 Hz, 1H), 4.27 – 4.19 (m, 6H), 4.17 – 4.14 (m, 1H), 3.97 (dd, *J* = 6.5, 4.9 Hz, 1H), 3.91 (q, *J* = 6.4 Hz, 1H), 3.58 (dt, *J* = 21.0, 5.5 Hz, 2H), 3.10 – 2.66 (m, 10H), 2.21 (t, *J* = 8.4 Hz, 2H), 2.16 – 2.10 (m, 2H), 1.91 – 1.82 (m, 2H), 1.78 – 1.43 (m, 18H), 1.35 – 1.25 (m, 4H), 1.20 (d, *J* = 6.9 Hz, 3H), 1.05 (d, *J* = 6.2 Hz, 3H), 0.86 – 0.80 (m, 12H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.0, 173.9, 172.2, 172.2, 171.7, 171.3, 171.2, 171.2, 171.2, 170.9, 169.0, 155.7, 137.4, 135.9, 130.0, 129.1, 127.9, 127.3, 127.2, 126.1, 123.5, 120.8, 118.3, 118.2, 114.7, 111.2, 109.7, 66.8, 61.5, 57.9, 55.0, 54.2, 53.7, 53.5, 52.5, 52.4, 52.3, 51.8, 51.2, 50.6, 48.5, 38.6, 38.6, 37.1, 36.5, 31.5, 31.1, 30.9, 30.1, 27.7, 27.5, 27.3, 26.5, 26.5, 24.0, 23.4, 23.0, 22.6, 22.2, 22.0, 21.8, 21.4, 19.2, 17.9.

8a. Dipyrrin formation was conducted by Method A with triethyl orthoformate. Yield: 19 %. Yellow powder. Analytic HPLC: Gradient B, retention time: 21.8 min, purity: 95.0 %. HRMS(MALDI-TOF): calc. for $C_{92}H_{130}N_{19}O_{20}^+$ $[M+H]^+$ 1820.9734, found 1821.0791. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.0 (s, 2H), 10.7 (s, 1H), 8.4 (d, *J* = 8.6 Hz, 1H), 8.2 (dd, *J* = 15.3, 7.8 Hz, 2H), 8.1 – 8.0 (m, 6H), 8.0 (d, *J* = 7.5 Hz, 2H), 7.9 (d, *J* = 7.4 Hz, 1H), 7.8 – 7.7 (m, 2H), 7.7 – 7.6 (m, 2H), 7.5 (d, *J* = 7.9 Hz, 1H), 7.3 – 7.2 (m, 3H), 7.2 – 7.1 (m, 9H), 7.0 (d, *J* = 7.1 Hz, 1H), 6.9 (d, *J* = 7.4 Hz, 1H), 6.9 (d, *J* = 8.5 Hz, 2H), 6.8 (s, 1H), 6.5 (d, *J* = 8.4 Hz, 2H), 4.6 – 4.5 (m, 1H), 4.5 – 4.4 (m, 1H), 4.4 – 4.3 (m, 1H), 4.3 – 4.3 (m, 1H), 4.3 – 4.2 (m, 1H), 4.2 – 4.1 (m, 5H), 4.0 – 3.9 (m, 1H), 3.9 – 3.9 (m, 1H), 3.6 – 3.6 (m, 2H), 3.1 – 2.8 (m, 10H), 2.7 (d, *J* = 6.5 Hz, 4H), 2.4 (s, 6H), 2.3 – 2.1 (m, 14H), 2.0 – 1.9 (m, 2H), 1.9 – 1.4 (m, 18H), 1.1 (d, *J* = 6.9 Hz, 3H), 1.1 (d, *J* = 6.2 Hz, 3H), 0.9 – 0.8 (m, 12H).

8b. Dipyrrin formation was conducted by Method B with 4-(trifluoromethyl)benzaldehyde. Yield: 24 %. Red powder. Analytic HPLC: Gradient B, retention time: 28.0 min, purity: 95.3 %. HRMS(MALDI-TOF): calc. for $C_{106}H_{141}N_{20}O_{20}^+$ $[M+H]^+$ 2014.0626, found 2014.2106. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.8 (s, 2H), 10.8 (s, 1H), 8.3 – 7.7 (m, 20H), 7.6 – 7.5 (m, 2H), 7.3 – 6.8 (m, 18H), 6.6 – 6.5 (m, 2H), 4.5 (s, 3H), 4.4 – 4.1 (m, 12H), 3.9 (s, 2H), 3.2 – 2.5 (m, 14H), 2.4 – 1.3 (m, 46H), 1.2 – 1.0 (m, 6H), 0.9 – 0.7 (m, 15H).

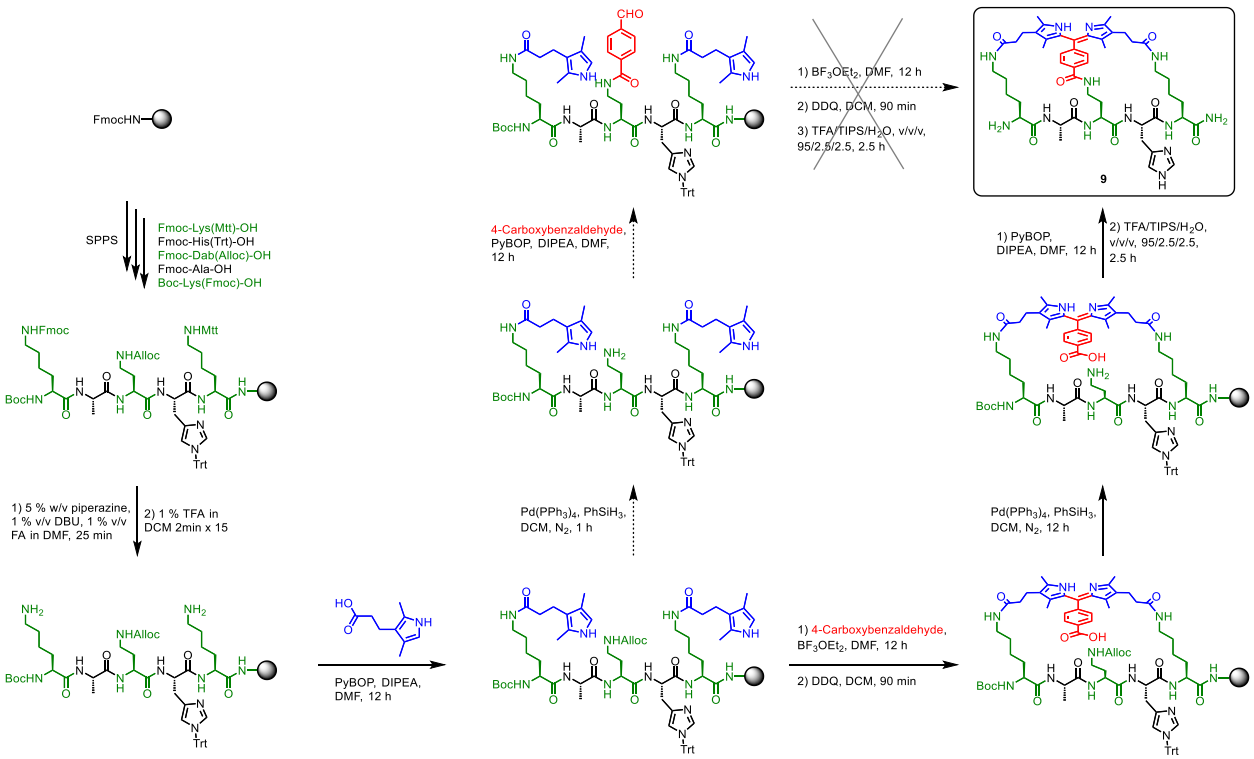

Figure S5. The synthetic route for **9**.

Synthesis of dipyrrin-bicyclopeptide (9). As shown on **Figure S5**, the Fmoc-Lys(Mtt)-OH, Fmoc-His(Trt)-OH, Fmoc-Dab(Alloc)-OH, Fmoc-Ala-OH and Boc-Lys(Fmoc)-OH were coupled during SPPS (sample was taken, cleaved, precipitated and analyzed by the HPLC and ESI-MS, sample 1 in **Figure S6**). The Fmoc and Mtt protecting groups were removed (sample 2 in **Figure S6**), and 3-(2,4-dimethyl-1*H*-pyrrol-3-yl)propanoic acid was coupled onto two unprotected amine groups. We tried two different way to desired product here: 1. Removing Alloc protecting groups and coupling 4-formylbenzoic acid on the side chain of Dab, then tried intramolecular dipyrrin formation between two pyrroles and one aldehyde on resin; 2. To form dipyrrin with two coupled pyrroles on resin and 4-formylbenzoic acid in solution, then removing Alloc protecting groups and doing intramolecular amidation between -COOH on dipyrrin and -NH_2 on the side chain of Dab. We fail to obtain desired product by first way, so second way was used. Dipyrrin formation was conducted by Method B with 4-formylbenzoic acid (sample 3 in **Figure S6**), then the Alloc protecting groups was removed (sample 4 in **Figure S6**). After fully washed, the resin was mixed with PyBOP (4 eq.) and DIPEA (8 eq.) in DMF (4 mL/0.1 mmol) for 12 h. The desired product was obtained after global cleavage and deprotection, precipitated (sample 5 in **Figure S6**) and purification. Yield: 19 %. Red powder. Analytic HPLC: Gradient A, retention time: 22.6 min, purity: 95.3 %. HRMS(MALDI-TOF): calc. for $C_{51}H_{70}N_{13}O_8^+$ $[M+H]^+$ 992.5465, found 992.5787; calc. for $C_{51}H_{69}N_{13}NaO_8^+$ $[M+Na]^+$ 1014.5284, found 1014.5633.

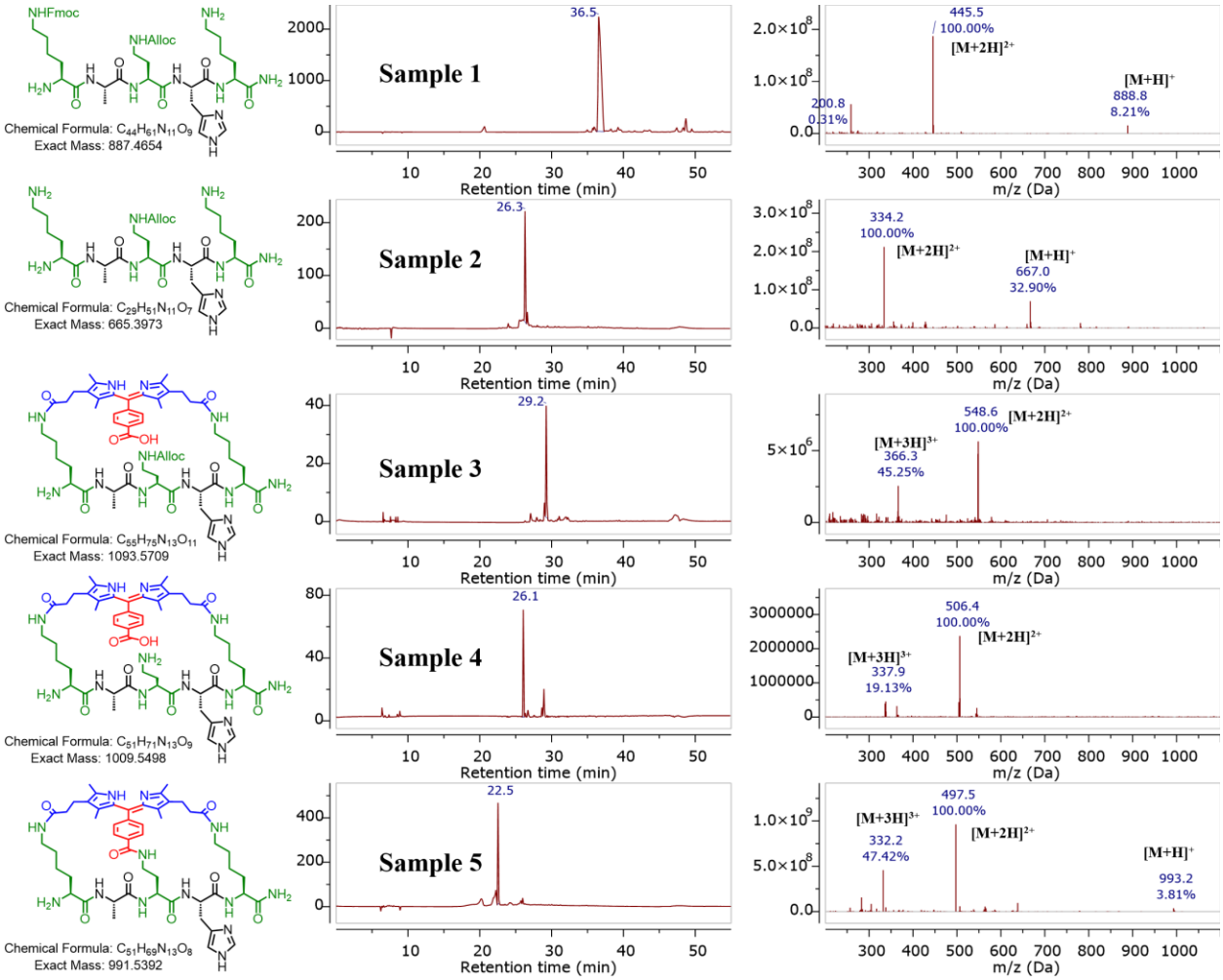

Figure S6. The crude products of intermediate steps for synthesizing **9** were cleaved, precipitated and analyzed by HPLC and ESI-MS.

Synthesis of RGD peptide (S3) and Bis-RGD dipyrrin-peptide (10). As shown on **Figure S7**, the Fmoc-Asp(ᵗBu)-OH, Fmoc-Gly-OH and Fmoc-Arg(Pbf)-OH were coupled onto resin. After removing N-terminal Fmoc, **S3** can be obtained by global cleavage and deprotection, precipitated and purification. For synthesis **7**, 3-(2,4-dimethyl-1*H*-pyrrol-3-yl)propanoic acid was coupled, and the dipyrrin was formed between two peptide chains by Method A with triethyl orthoformate. The desired product was obtained after global cleavage and deprotection, precipitated and purification.

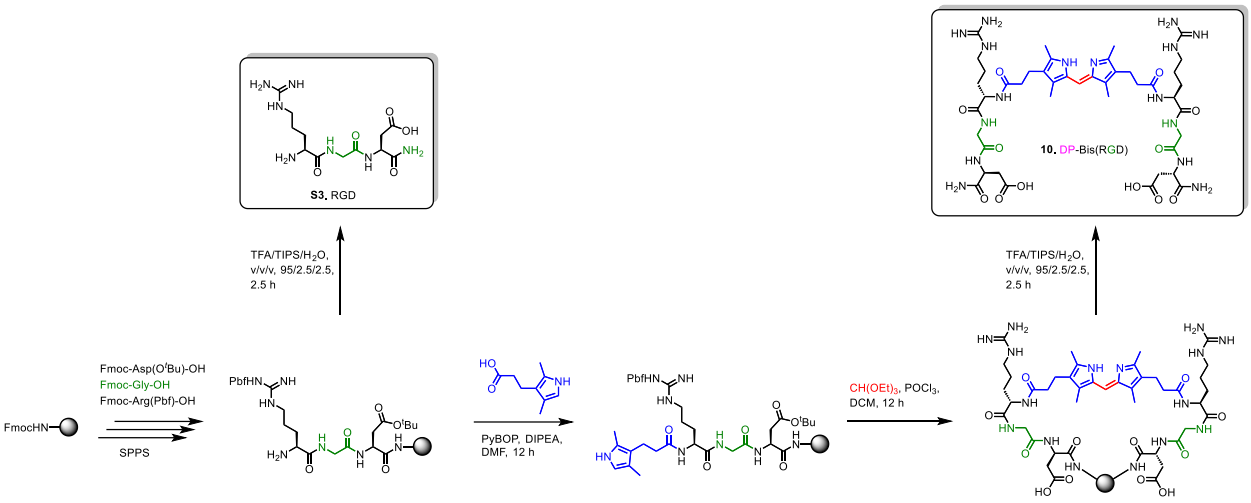

Figure S7. The synthetic route for **S3** and **10.**

*Arg-Gly-Asp (RGD peptide, **S3**).* Yield: 68 %. White powder. Analytic HPLC: Gradient A, retention time: 4.9 min, purity: 95.6 %. HRMS(MALDI-TOF): calc. for $C_{12}H_{24}N_7O_5^+$ $[M+H]^+$ 346.1833, found 346.1748; calc. for $C_{12}H_{23}N_7NaO_5^+$ $[M+Na]^+$ 368.1653, found 368.1553. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (t, J = 5.5 Hz, 1H), 8.29 (d, J = 8.2 Hz, 1H), 8.22 (s, 3H), 7.86 (s, 1H), 7.23 (m, 6H), 4.50 (td, J = 7.8, 5.6 Hz, 1H), 3.92 – 3.77 (m, 3H), 3.11 (q, J = 6.7 Hz, 2H), 2.67 – 2.48 (m, 2H), 1.72 m, 2H), 1.55 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.4, 171.7, 168.7, 168.0, 156.9, 51.7, 49.4, 41.8, 40.1, 36.2, 28.3, 24.0.

10. Yield: 39 %. Yellow powder. Analytic HPLC: Gradient A, retention time: 25.6 min, purity: 97.0 %. HRMS(MALDI-TOF): calc. for $C_{43}H_{67}N_{16}O_{12}^+$ $[M+H]^+$ 999.5119, found 999.4611. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.32 (s, 2H), 8.30 (t, J = 5.7 Hz, 2H), 8.21 (d, J = 7.2 Hz, 2H), 8.08 (d, J = 8.1 Hz, 2H), 7.74 (t, J = 5.6 Hz, 2H), 7.53 – 6.94 (m, 13H), 4.49 (td, J = 8.0, 5.3 Hz, 2H), 4.20 (q, J = 7.0 Hz, 2H), 3.82 – 3.62 (m, 4H), 3.06 (q, J = 6.5 Hz, 4H), 2.74 – 2.51 (m, 8H), 2.45 (s, 6H), 2.31 (m, 10H), 1.71 – 1.60 (m, 2H), 1.55 – 1.39 (m, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.5, 172.0, 171.8, 171.7, 168.6, 156.8, 153.3, 143.2, 127.6, 126.5, 120.4, 52.5, 49.3, 42.0, 40.3, 36.0, 34.8, 28.8, 24.8, 19.5, 12.4, 9.7.

Synthesis of cyclopeptide with double dipyrrin cyclic linker (11). As shown on **Figure S8**, the Fmoc-Asn(Trt)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Lys(Alloc)-OH, Fmoc-His(Trt)-OH, Fmoc-Phe-OH, Fmoc-Ile-OH, Fmoc-Lys(Alloc)-OH, Fmoc-His(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Lys(Mtt)-OH, Fmoc-His(Trt)-OH and Fmoc-Phe-OH were coupled during SPPS (sample was taken, cleaved, precipitated and analyzed by the HPLC and ESI-MS, sample 1 in **Figure S9**). The Fmoc and Mtt protecting groups were removed, and 3-(2,4-dimethyl-1*H*-pyrrol-3-yl)propanoic acid was coupled onto two unprotected amine groups. The first dipyrrin formation was conducted by Method B with 4-(trifluoromethyl)benzaldehyde (Sample 2 in **Figure S9**).The two Alloc protecting groups were then removed (Sample 3 in **Figure S9**), and 3-(2,4-dimethyl-1*H*-pyrrol-3-yl)propanoic acid was coupled onto another two unprotected amine groups. The second dipyrrin formation was conducted by Method B with 4-nitrobenzaldehyde. The desired product was obtained after global cleavage and deprotection, precipitated (Sample 4 in **Figure S9**) and purification. Yield: 11 %. Red powder. Analytic HPLC: Gradient B, retention time: 24.9 min, purity: 97.3 %. HRMS(MALDI-TOF): calc. for $C_{131}H_{172}F_3N_{36}O_{21}^+$ $[M+H]^+$ 2642.3444, found

2642.3737; calc. for $C_{131}H_{171}F_3N_{36}NaO_{21}^+$ $[M+Na]^+$ 2664.3264, found 2664.3598; calc. for $C_{131}H_{171}F_3KN_{36}O_{21}^+$ $[M+K]^+$ 2680.3003, found 2680.3379.

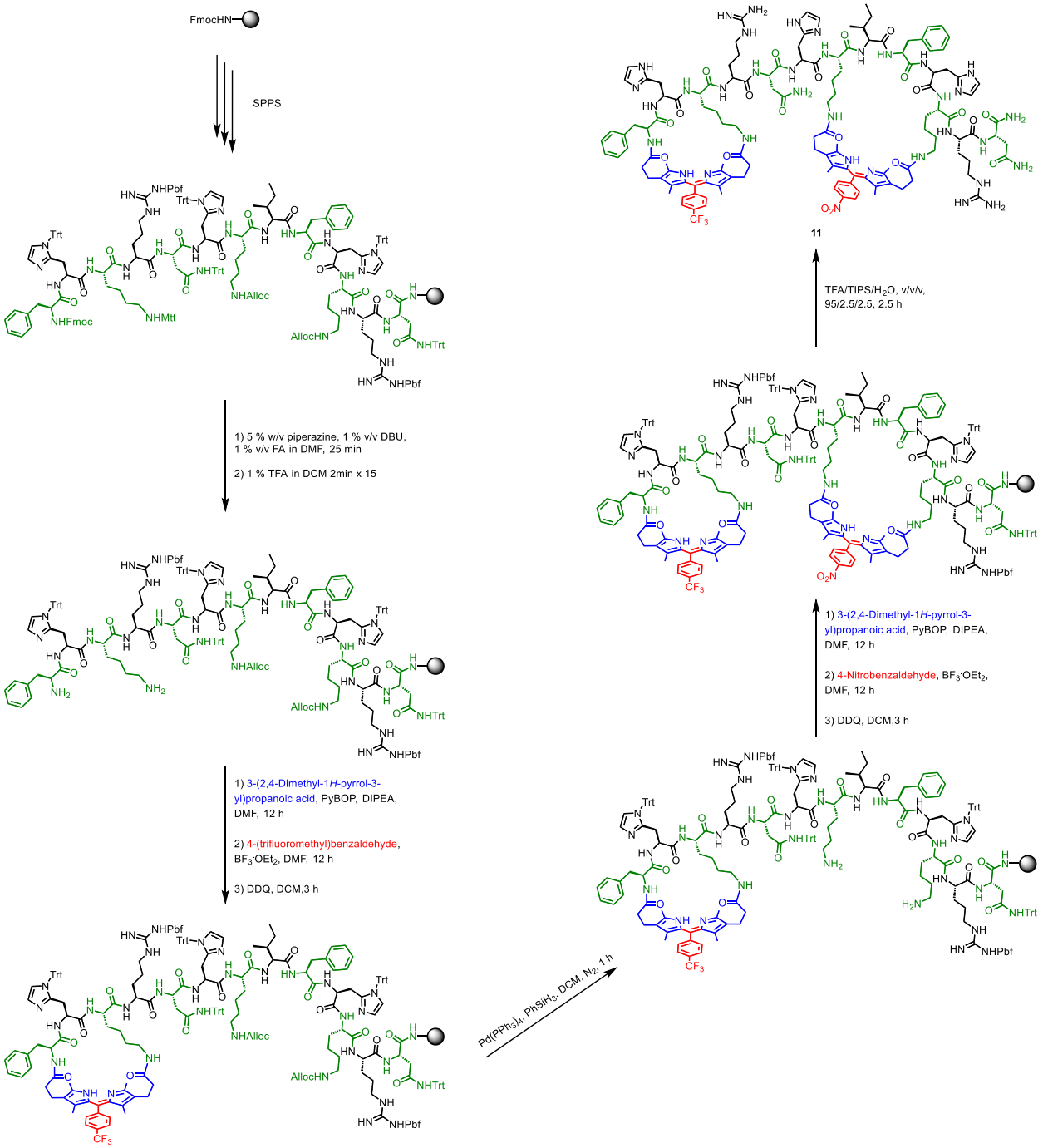

Figure S8. The synthetic route for **11.**

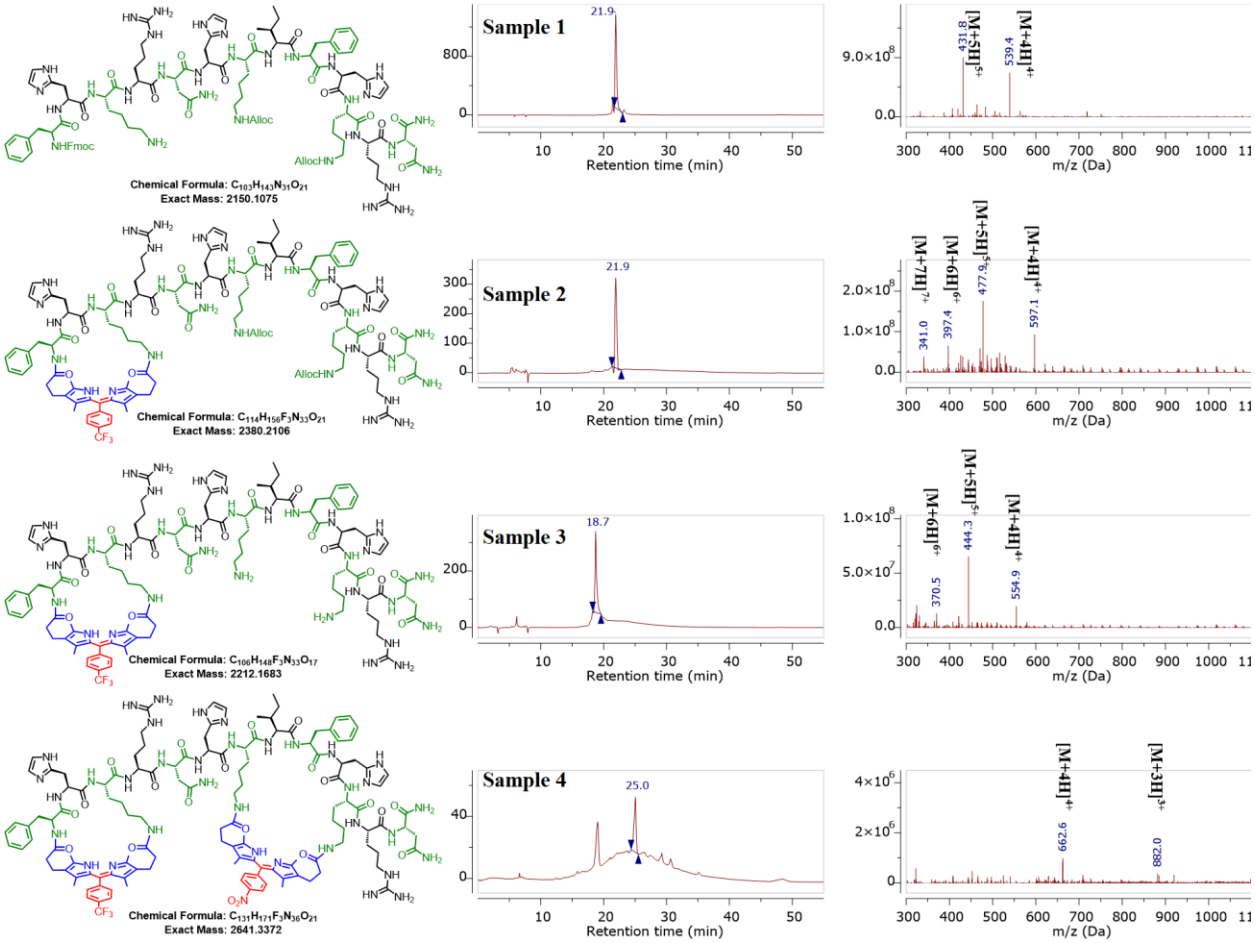

Figure S9. The crude products of intermediate steps for synthesizing **11** were cleaved, precipitated and analyzed by HPLC and ESI-MS.

Synthesis of linear GHK dipyrrin-peptide (S6). As shown on **Figure S10**, the dipyrrin-peptide conjugate **S6** was synthesized by routine SPPS with a prepared dipyrrin building block **S5**.

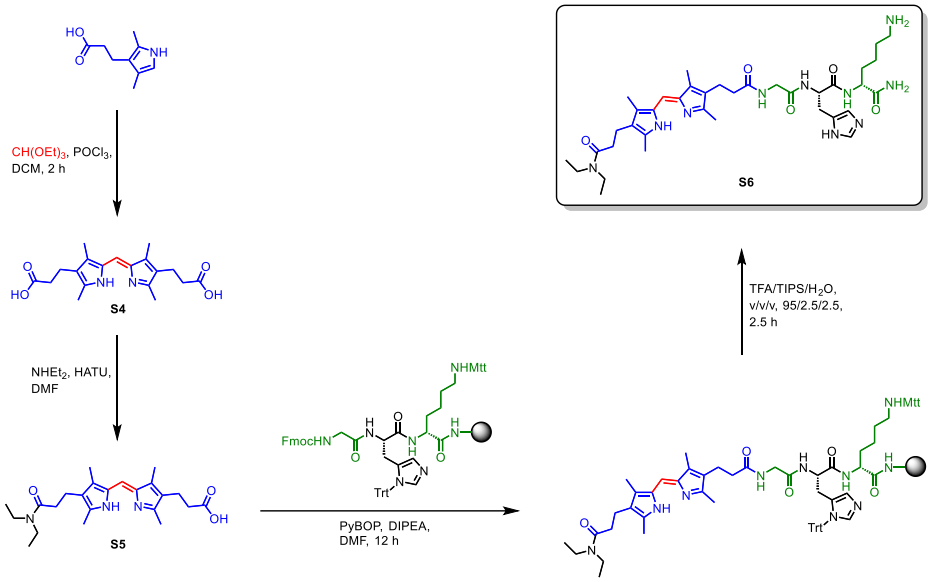

Figure S10. The synthetic route for **S6**.

S4. To a solution of 3-(2,4-dimethyl-1*H*-pyrrol-3-yl)propanoic acid (2 mmol) in DCM (15 ml) , triethyl orthoformate (1.1 mmol) and $POCl_3$ (1.2 mmol) were added at 0 ℃. The reaction was stirred at r.t. for 3 h before it quenched by water. The solvent was evaporated and the resulting residue was purified by column chromatography on silica gel (mobile phase: DCM/MeOH, v/v, 100/0 to 90/10). Yield: 56 %. Yellow powder. Analytic HPLC: Gradient A, retention time: 18.5 min, purity: 96.6 %. HRMS(MALDI-TOF): calc. for $C_{19}H_{25}N_2O_4^+$ $[M+H]^+$ 345.1809, found 345.1154. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.78 (s, 2H), 7.34 (s, 1H), 2.65 (t, J = 7.5 Hz, 4H), 2.49 (s, 7H), 2.40 (t, J = 7.5 Hz, 4H), 2.32 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.6, 153.4, 143.1, 127.2, 126.3, 120.5, 33.5, 18.8, 12.4, 9.8.

S5. The solution of **S4** (1 mmol), HATU (1 mmol), diethylamine (1 mmol) and DIPEA (2 mmol) in DMF (2 mL) was stirred at r.t. overnight. The reaction mixture was monitored by analytic HPLC, both mono-amidated and di-amidated, as well as unreacted **S4**, were detected. The mono-amidated product was separated selectively by preparative HPLC. Yield: 38 %. Yellow powder. Analytic HPLC: Gradient A, retention time: 22.1 min, purity: 96.2 %. HRMS(MALDI-TOF): calc. for $C_{23}H_{34}N_3O_3^+$ $[M+H]^+$ 400.2595, found 400.2149. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.12 (d, J = 2.9 Hz, 2H), 7.40 (s, 1H), 3.25 (q, J = 7.0 Hz, 4H), 2.67 (t, J = 7.5 Hz, 4H), 2.50 (s, 6H), 2.42 (dt, J = 15.0, 7.5 Hz, 7H), 2.33 (d, J = 2.5 Hz, 6H), 1.02 (dt, J = 18.3, 7.1 Hz, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.6, 170.0 153.8, 153.0, 143.3, 143.1, 128.2, 127.3, 126.8, 126.6, 120.8, 41.2, 33.5, 31.8, 19.3, 18. 9, 14.2, 13.1, 12.8, 12.7, 9.9, 9.8.

S6. **S5** was coupled on resin-bound GHK peptide as routine SPPS procedure, and the desired product was obtained after global cleavage and deprotection, precipitated and purification. Yield: 48 %. Yellow powder. Analytic HPLC: Gradient A, retention time: 29.7 min, purity: 98.9 %. HRMS(MALDI-TOF): calc. for $C_{37}H_{57}N_{10}O_5^+$ $[M+H]^+$ 721.4508, found 721.5026; calc. for $C_{37}H_{56}N_{10}NaO_5^+$ $[M+Na]^+$ 743.4327, found 743.4814.

Table S3. The chemical shift of αH/NH and corresponding coupling constant of RGD motif of **3a-3b**, **4a-4b**

	J^3 (αH-NH)			Chemical shift (αH, ppm)			Chemical shift (NH, ppm)		
	Arg (R)	**Gly (G)**	**Asp (D)**	**Arg (R)**	**Gly (G)**	**Asp (D)**	**Arg (R)**	**Gly (G)**	**Asp (D)**
3a	7.5	5.7	7.6	4.11	3.76	4.56	7.89	8.35	8.28
3b	7.7	5.3	6.9	4.36	3.77	4.57	8.69	8.34	8.34
3c	7.0	5.5	7.4	4.34	3.77	4.56	8.72	8.35	8.35
3d	7.3	5.6	7.2	4.36	3.83	4.55	8.86	8.55	8.46
4a	8.0	5.6	7.7	4.38	3.73	4.55	8.57	8.3	8.25
4b	8.1	5.1	7.6	4.39	3.78	4.52	8.56	8.27	8.27
4c	7.8	5.5	7.6	4.38	3.72	4.53	8.68	8.32	8.21
4d	7.7	5.6	7.8	4.36	3.69	4.37	8.74	8.31	8.02

Photophysical experiments

Table S4. The photophysical properties of **5a** and **5b** in HEPES buffer.

	λ_{abs} (nm)	ε (× 10^4 M^{-1} cm^{-1})	λ_{exc} (nm)	λ_{em} (nm)	τ (ns)	Φ
4a-BODIPY	525	2.80	522	533	7.2	0.92
4b-BODIPY	525	2.76	522	533	7.1	0.93

Table S5. The photophysical properties of **1a**, **2** and **8** in HEPES buffer with saturated zinc ion.

	λ_{abs} (nm)	ε (× 10^4 M^{-1} cm^{-1})	λ_{exc} (nm)	λ_{em} (nm)	τ (ns)	Φ
1a	496	2.66	498	503	2.9	0.31
2	497	2.79	498	503	2.0	0.22
S6	496	2.33	498	503	1.7	0.13

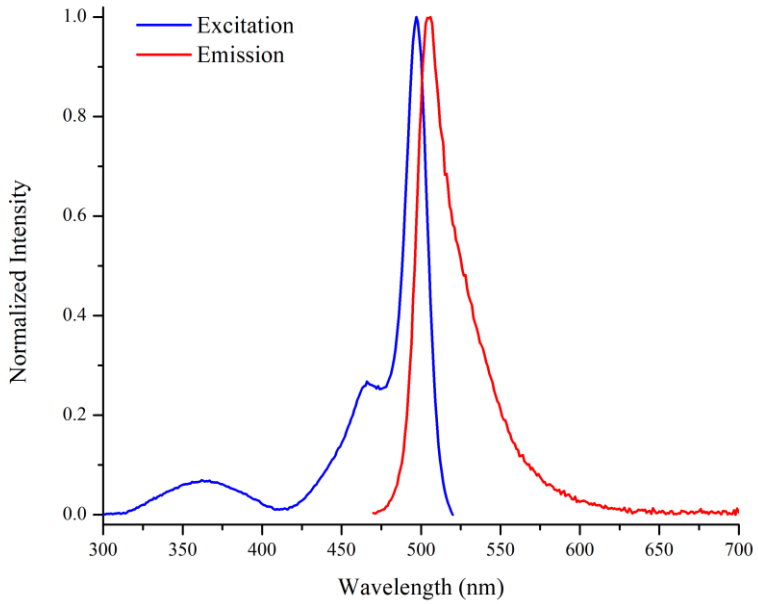

Figure S11. Normalized excitation/emission spectra of **1a** (1 μM) in HEPES buffer with $ZnCl_2$ (5 μM)

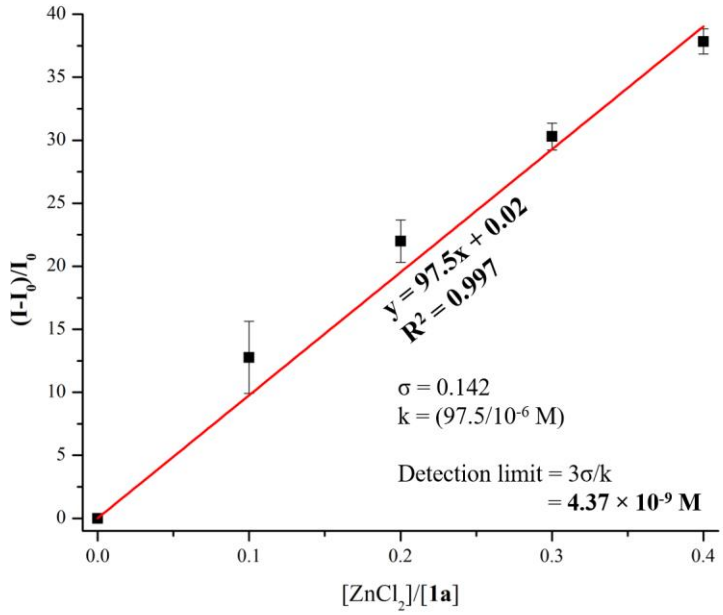

Figure S12. Determination of detection limit of **1a** from first five points of fluorescent titration curve.

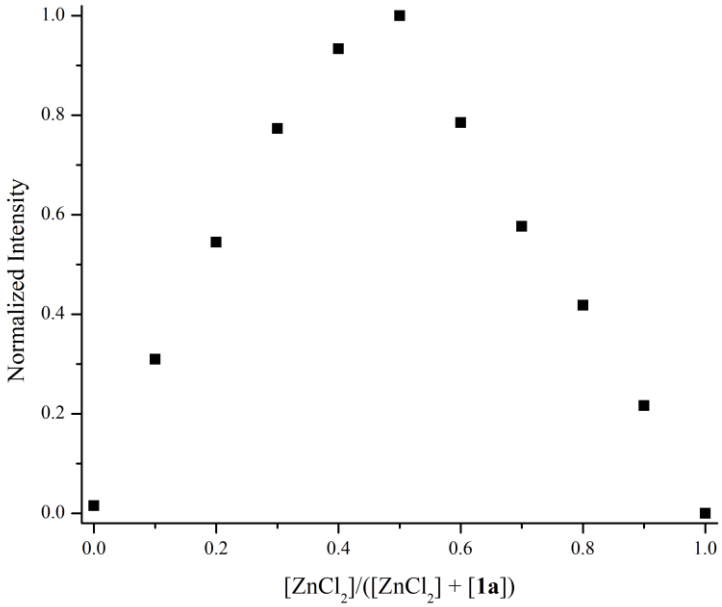

Figure S13. Determination of stoichiometry between **1a** and zinc ion via Jobs' plot. The total concentration of **1a** and $ZnCl_2$ is 1 μM.

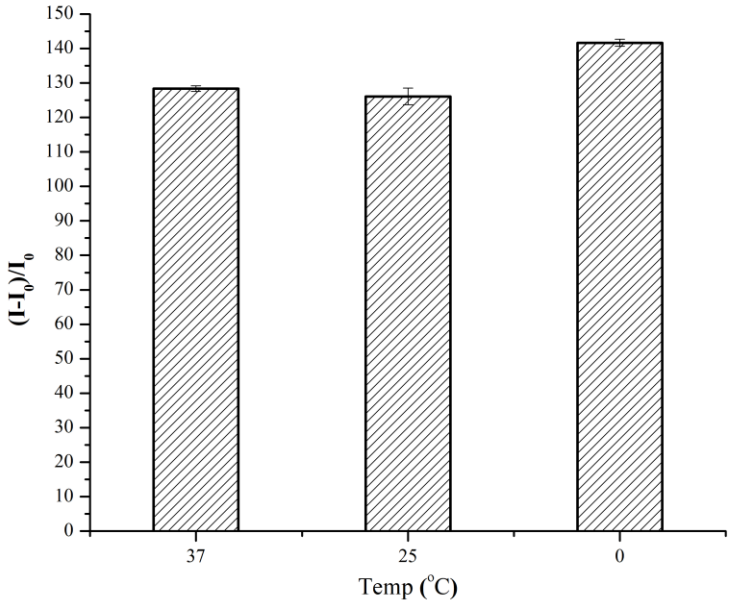

Figure S14. Influence of temperature toward the emission intensity of **1a** (1 μM) in HEPES buffer with $ZnCl_2$ (5 μM)

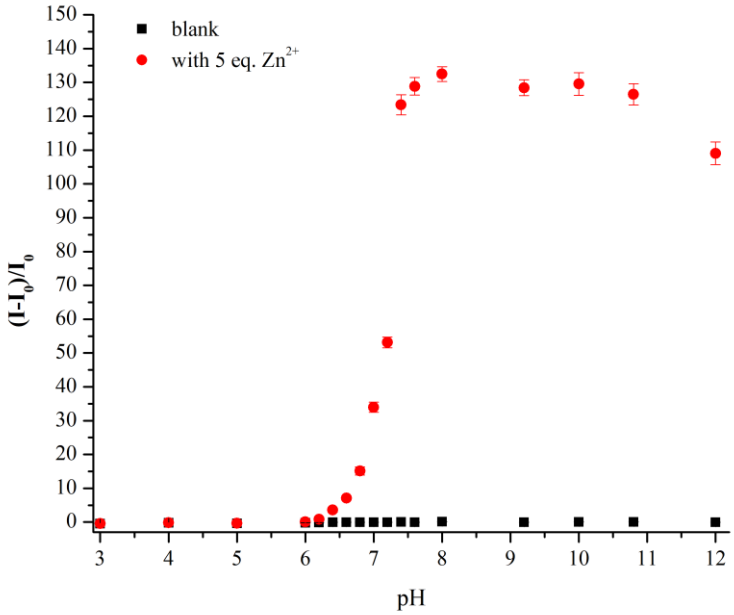

Figure S15. Influence of pH toward the emission intensity of **1a** (1 μM) in different buffer with/without $ZnCl_2$ (5 μM)

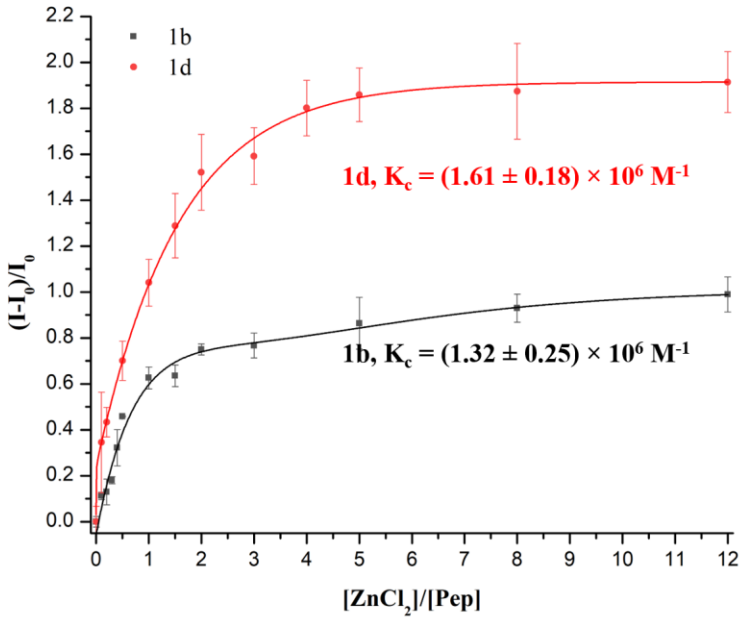

Figure S16. Fluorescent titration curves (emission intensity at wavelength 505 nm) of **1b** and **1d** in HEPES buffer with $ZnCl_2$.

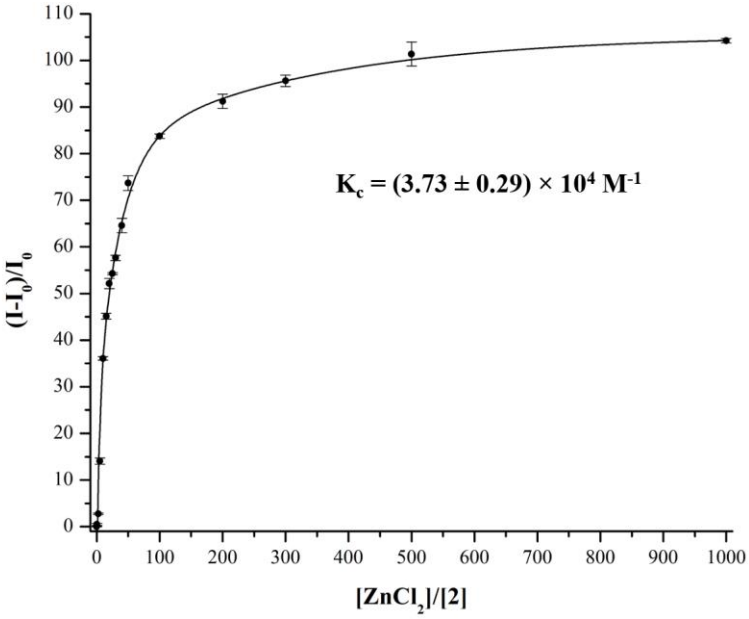

Figure S17. Fluorescent titration curve (emission intensity at wavelength 503 nm) of **2** in HEPES buffer with $ZnCl_2$.

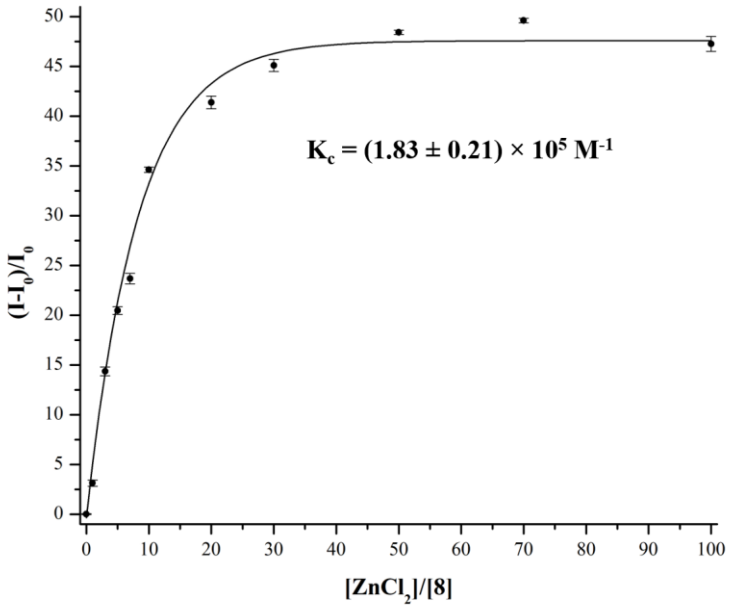

Figure S18. Fluorescent titration curve of **8** (emission intensity at wavelength 503 nm) in HEPES buffer with $ZnCl_2$.

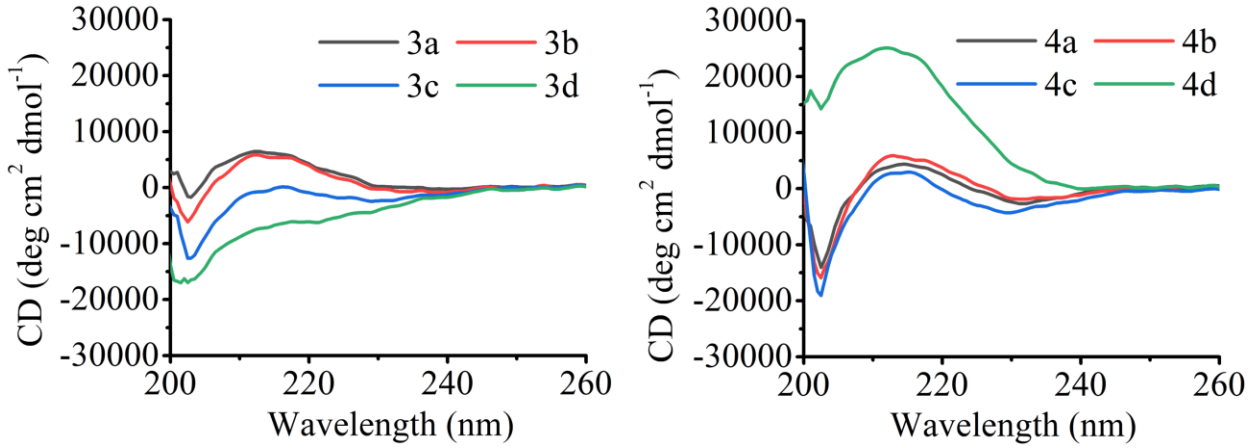

Figure S19. CD spectra of 50 µM **3a**–**3d** and **4a**–**4d**.

Biological experiments

Protease stability assays. Peptide protease degradation kinetics was determined with Thermo Pierce™ MS-Grade Trypsin Protease. The solution of trypsin (10 μg/mL) in PBS with 5 mM DTT and 2 mM $CaCl_2$ was prepared and divided into 10 identical parts (200 μL for each part). The peptides (2000 eq. compare with trypsin in 200 μL above buffer) were added to form 5 identical reaction mixtures for both **3a** and **4a**. Samples were then incubated at 37°C, 500 rpm. At 5 min, 15 min, 30 min, 120 min, and 240 min, a sample for both **3a** and **4a** were taken out and quenched in a 600 μL of 2% TFA/H_2O on ice. The samples were centrifuged for 3 min at 10,000 rpm to precipitate quenched trypsin. Supernatant solutions were then analyzed and intact peptide quantified by analytical HPLC.

Cell line and culture. T24 cells were grown in RPMI-1640 medium. HeLa cells were cultivated in DMEM medium and MRC5 cells are cultivated in MEM medium (Gibco, USA). All media are supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin antibiotics. All the cells are cultivated in a humidified incubator with 5% CO_2 at 37°C.

MTT cell viability assay. Two cell lines (HeLa and MRC-5) (1 x 104 cells/mL) were seeded on a 96-well plate overnight and different concentrations of **1a** was added into the cells on the next day. After 24 hours incubation, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) (conc. = 0.5 mg/mL) was added and they were incubated at 37 ℃ for 3 hours. The formazan formed were dissolved in dimethyl sulfoxide (DMSO) and the absorbance of the solution was measured in a microplate reader at 540 nm wavelength (reference wavelength = 690 nm). Quadruplicates were performed to obtain the data.

Integrin binding affinity assay. 0.4 μg/mL of purified recombinant human $\alpha_v\beta_3$ integrin (derived from CHO cells, R&D Systems) was adsorbed onto a 96-well ELISA plate (Nunc, Roskilde, Denmark) at 4 ℃ overnight. After blocking the integrin-coated wells, **3b**, **4a**–**4d** and Cilengitide of different concentrations (from 2.5 to 80 μM) were added to compete with 0.25 μg/mL biotinylated Vitronectin (Abcam, Cambridge, UK) to bind with the recombinant $\alpha_v\beta_3$ integrin. After three hours incubation, peroxidase reagent was added to the wells followed by ABTS (2,2'azino-bis(3-ethyl benzothiazoline-6-sulphonic acid) reagent. The absorbance of the solution was measured in a microplate reader at 405 nm wavelength. The result was expressed as the percentage displacement of Vitronectin by **3b**, **4a**–**4d** and Cilengitide.

Immunoluminescence assay of 4b-BODIPY in various cell lines. These assays were prepared and conducted at room temperature, unless otherwise stated. Cells after treatment of 10 μM **4b-BODIPY** for 4 h were fixed by formalin for 15 min. The samples were then washed with PBS. 0.2% Triton-X buffer was then added onto the samples for 15 min. 3% BSA in PBS was served as the blocking buffer and applied onto the sample for 30 min at room temperature. The primary antibody diluted in blocking buffer was added onto the sample at 4°C overnight. The samples were washed with PBS and corresponding secondary antibodies were added over 1 h. Samples were washed with PBS and mountant was applied to each sample. Confocal images were acquired by the Nikon Eclipse Ti2 Confocal Microscope. Signal from **4b-BODIPY** was obtained upon the excitation at 488 nm and the signal of secondary antibody was obtained upon the excitation at 561 nm.

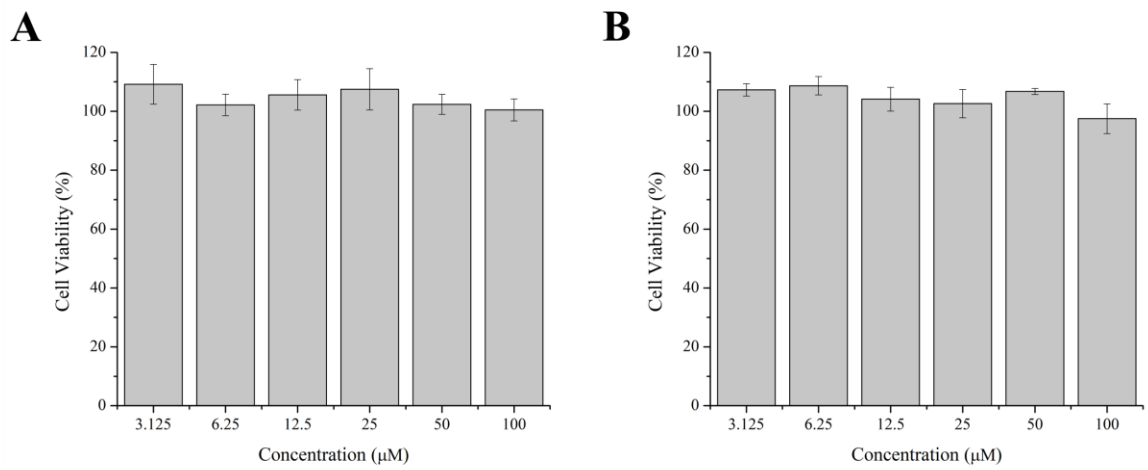

Figure S20. MTT assay of **1a** toward (A) HeLa and (B) MRC5 cell lines.

Computations

The initial structures were constructed using Avogadro (version 2.0).[3] The partial charges of unusual amino acid residues were obtained by using R.E.D. Development Server.[4] The clustered conformation of each compound was docked into the $\alpha_v\beta_3$ crystal structure (PDB: 4MMY) using AutoDock Vina,[5] which the lattice box of size 30 Å × 30 Å × 30 Å. The docking parameters were set at the default values, except the "exhaustiveness" was set to 64 for more comprehensive searching on the docking conformations. Docked structures of peptide facing the $\alpha_v\beta_3$ side with lowest energies were chosen for further analysis.

Table S6. The estimated docking energies of **3a**~**3d** and **4a**~**4d** with $\alpha_v\beta_3$ protein (PDB: 4MMY) with the residues that exist H-bond interactions with the docked position of respective compound.

Compound	Δ*G* (kcal/mol)	H-bond interaction residues	Compound	Δ*G* (kcal/mol)	H-bond interaction residues
3a	−6.1	–	**4a**	−8.0	TYR 178, SER 1047
3b	−5.5	ASP 148, TYR 1090	**4b**	−8.8	ASP218, TYR 1090
3c	−5.2	GLU 1144	**4c**	−8.0	ALA 215
3d	−5.2	ALA 1142	**4d**	−7.2	ALA 215

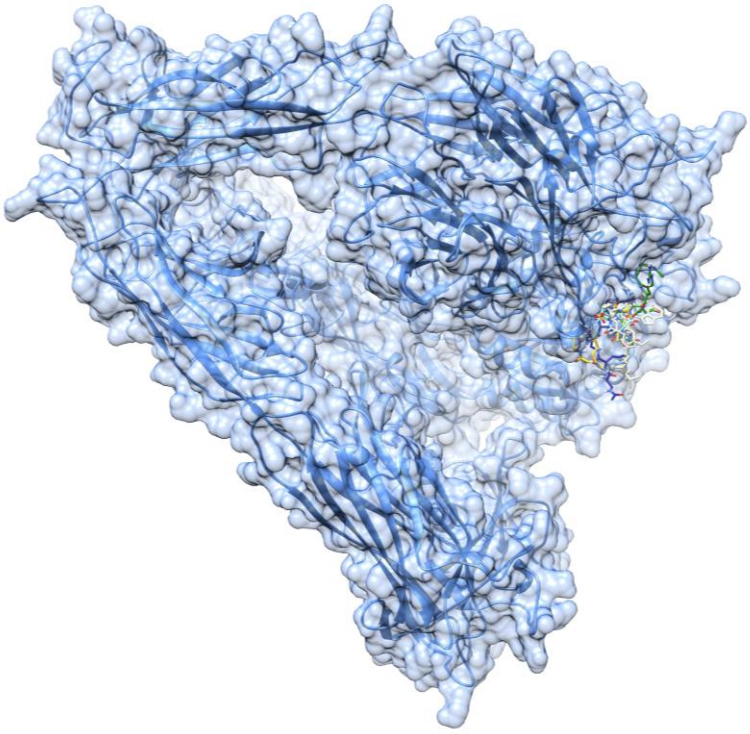

Figure S21. The overlay structure of **3a**–**3d** with $\alpha_v\beta_3$ crystal structure with fibronectin (PDB: 4MMY).

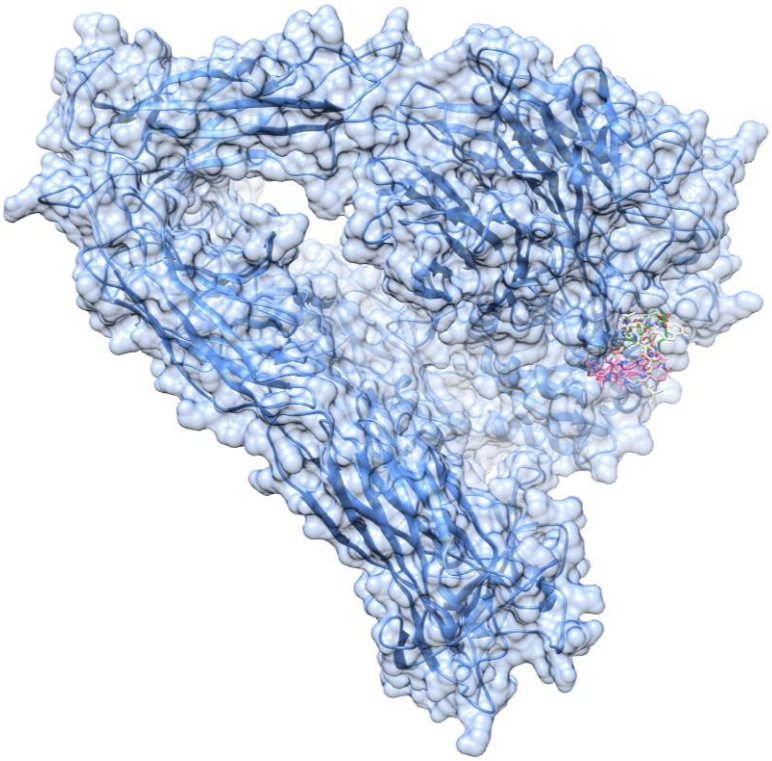

Figure S22. The overlay structure of **4a**–**4d** with $\alpha_v\beta_3$ crystal structure with fibronectin (PDB: 4MMY).

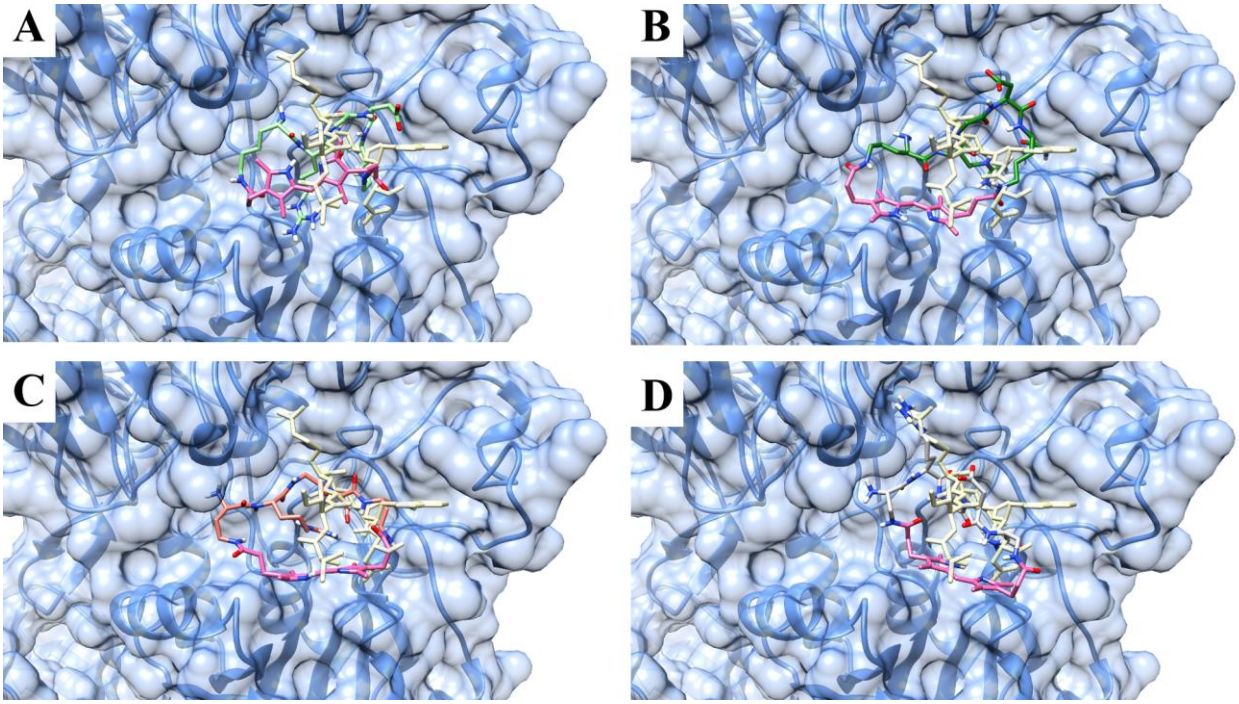

Figure S23. The docked structure of (A) **4a**, (B) **4b**, (C) **4c** and (D) **4d** with $\alpha_v\beta_3$ protein structure (PDB: 4MMY). The dipyrrin connector are shown in magenta, while the fibronectin was shown in yellowish white with transparency.

References

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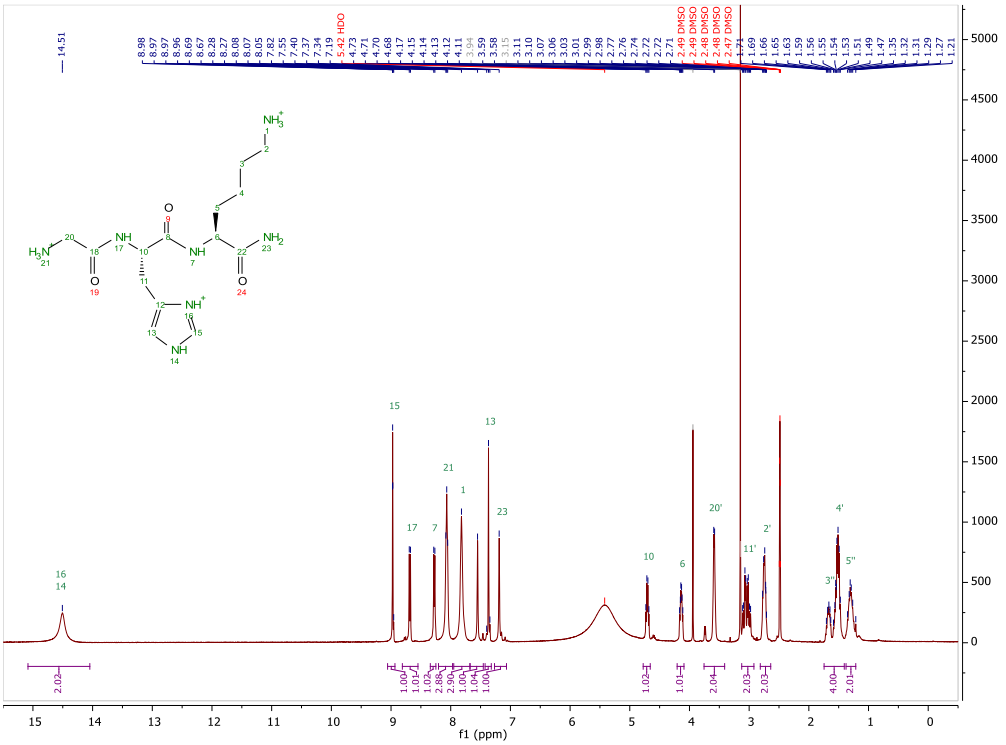
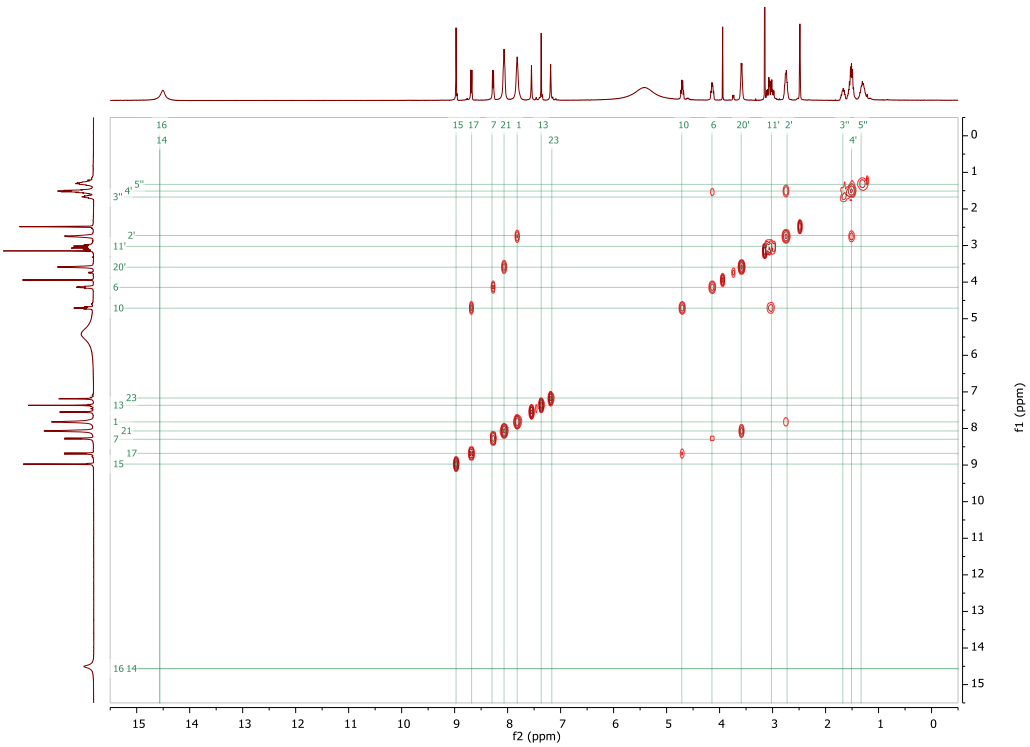
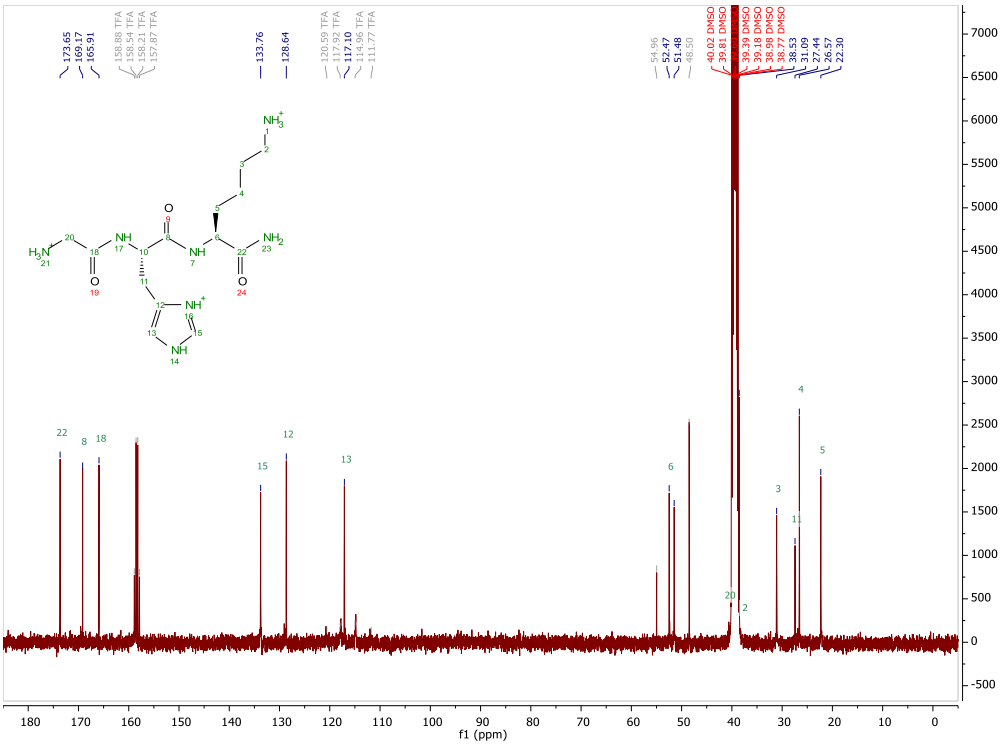
NMR spectra of products



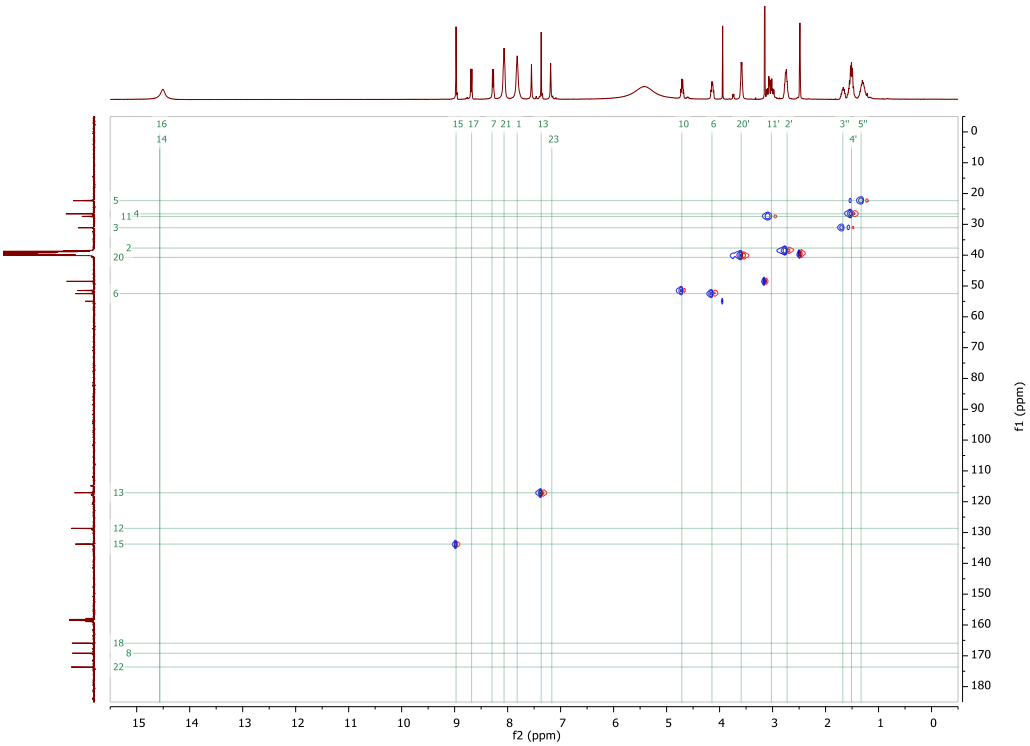
^{1}H-NMR spectrum of GHK (**S1**) peptide in DMSO-d_6**.**



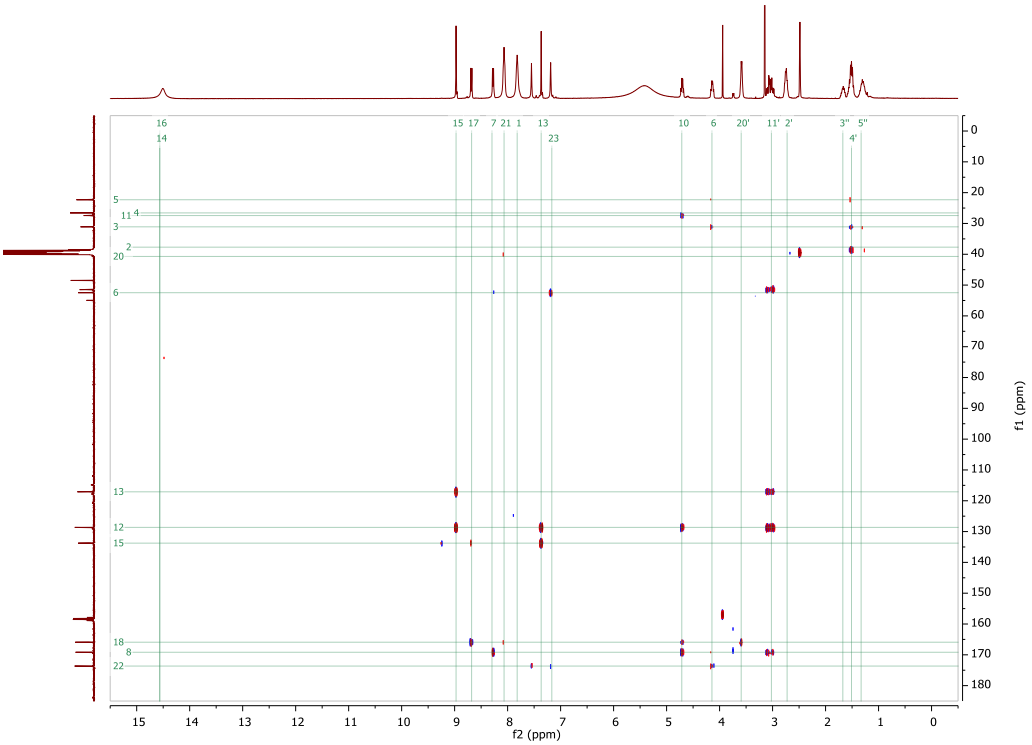
COSY spectrum of GHK peptide (**S1**) in DMSO-d_6**.**



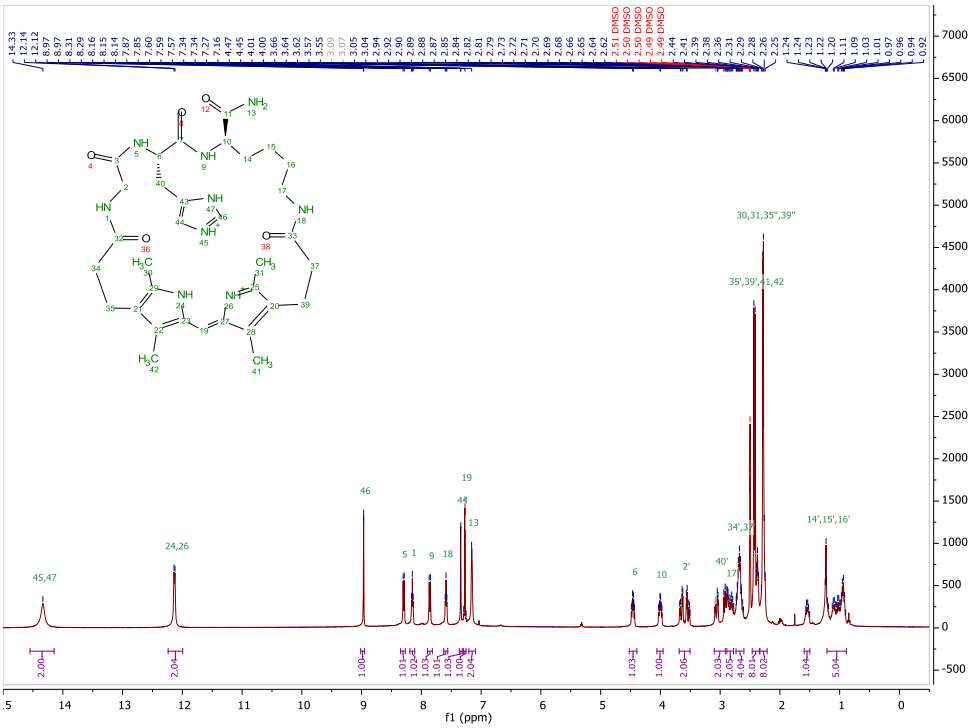
^{13}C-NMR spectrum of GHK peptide (**S1**) in DMSO-d_6**.**



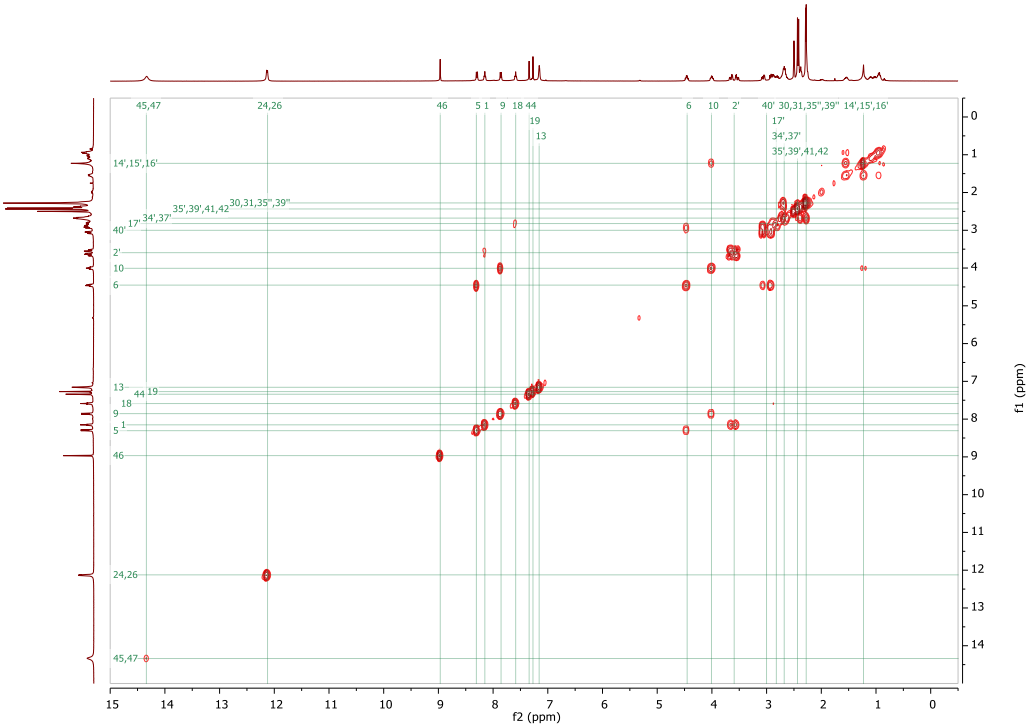
HSQC spectrum of GHK peptide (**S1**) in DMSO-d_6**.**



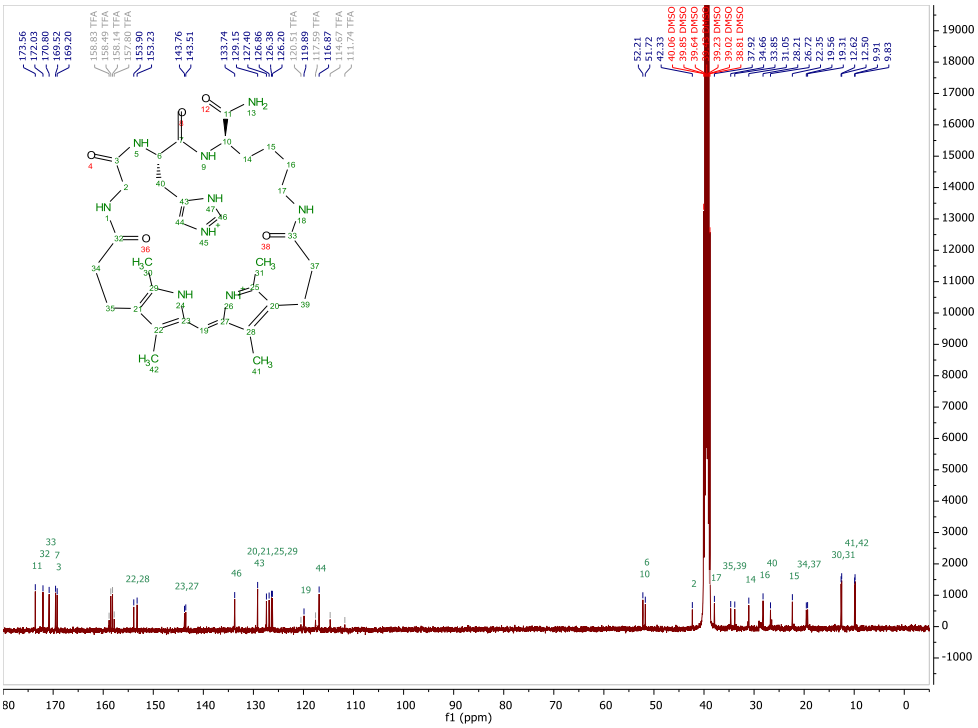
HMBC spectrum of GHK peptide (**S1**) in DMSO-d_6**.**



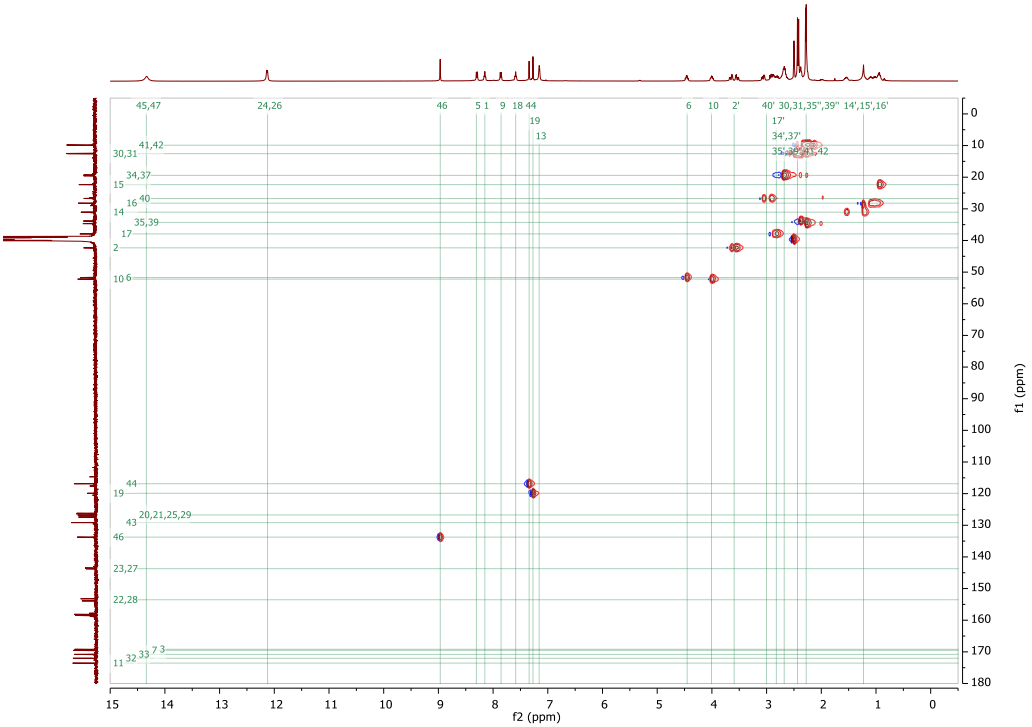
^{1}H-NMR spectrum of **1a** in DMSO-d_6.



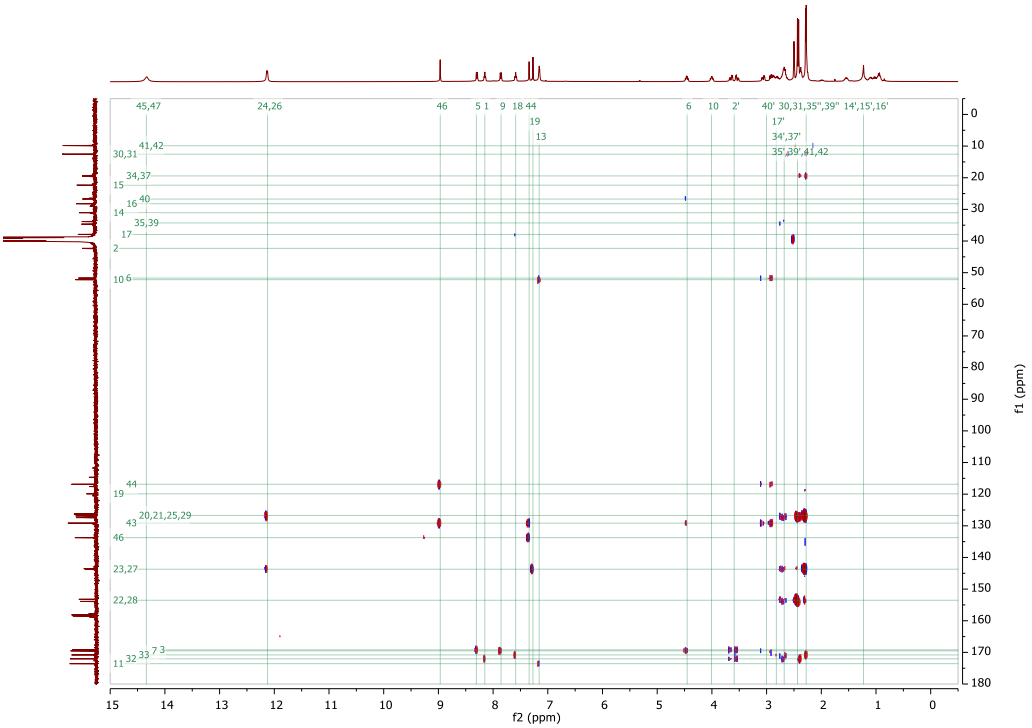
COSY spectrum of **1a** in DMSO-d_6.



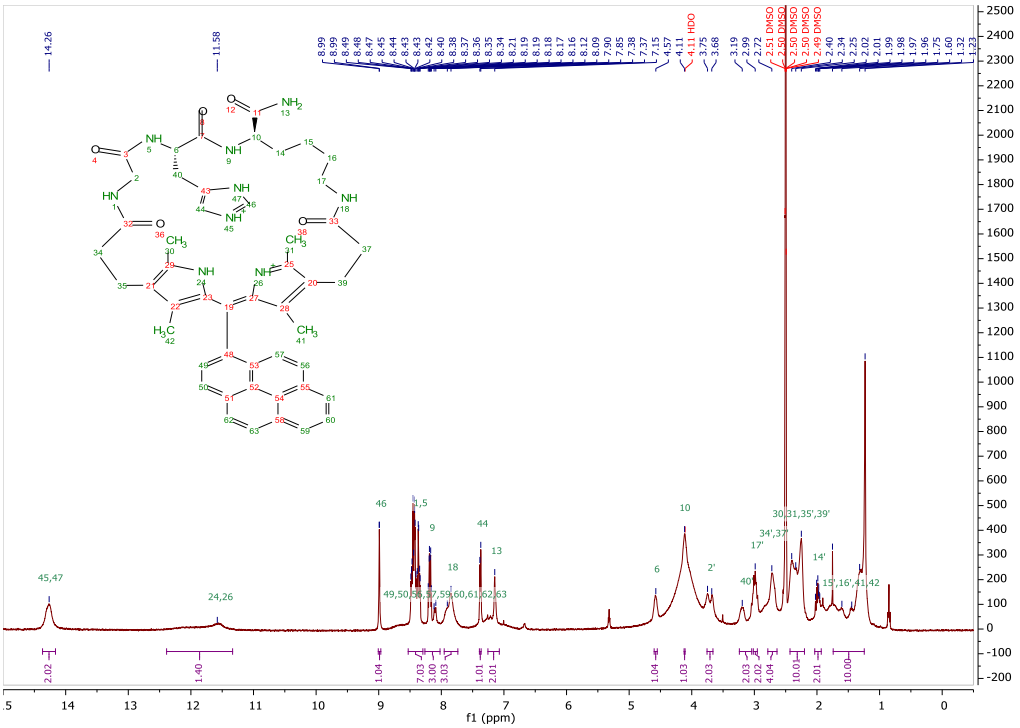
^{13}C-NMR spectrum of **1a** in DMSO-d_6.



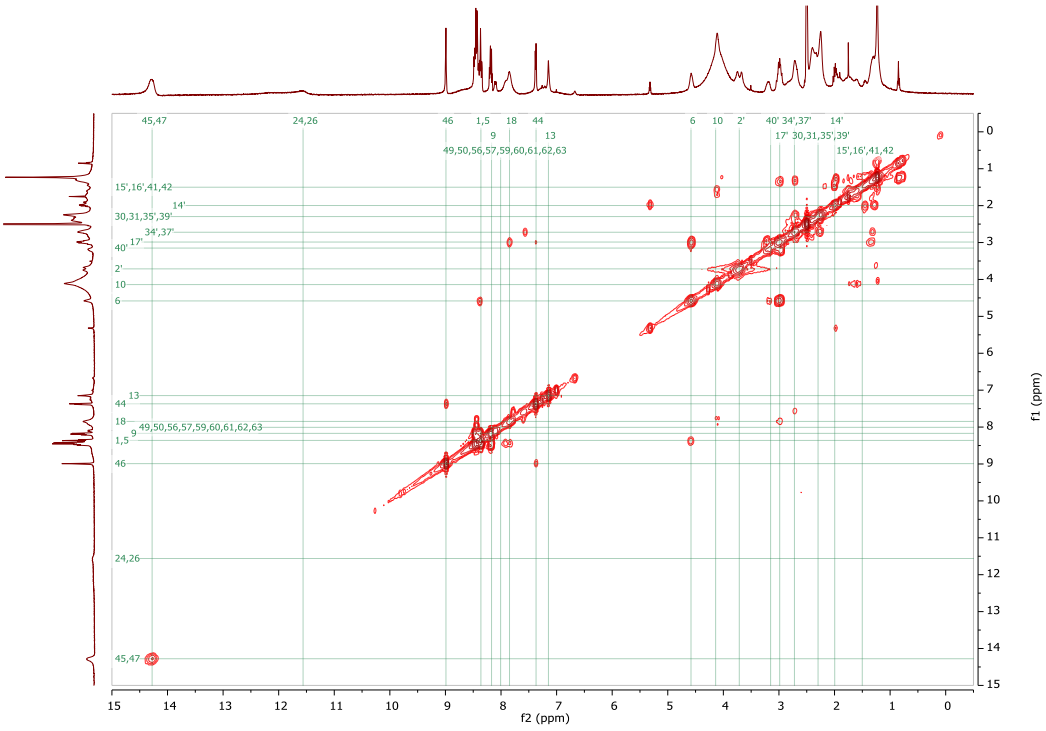
HSQC spectrum of **1a** in DMSO-d_6.



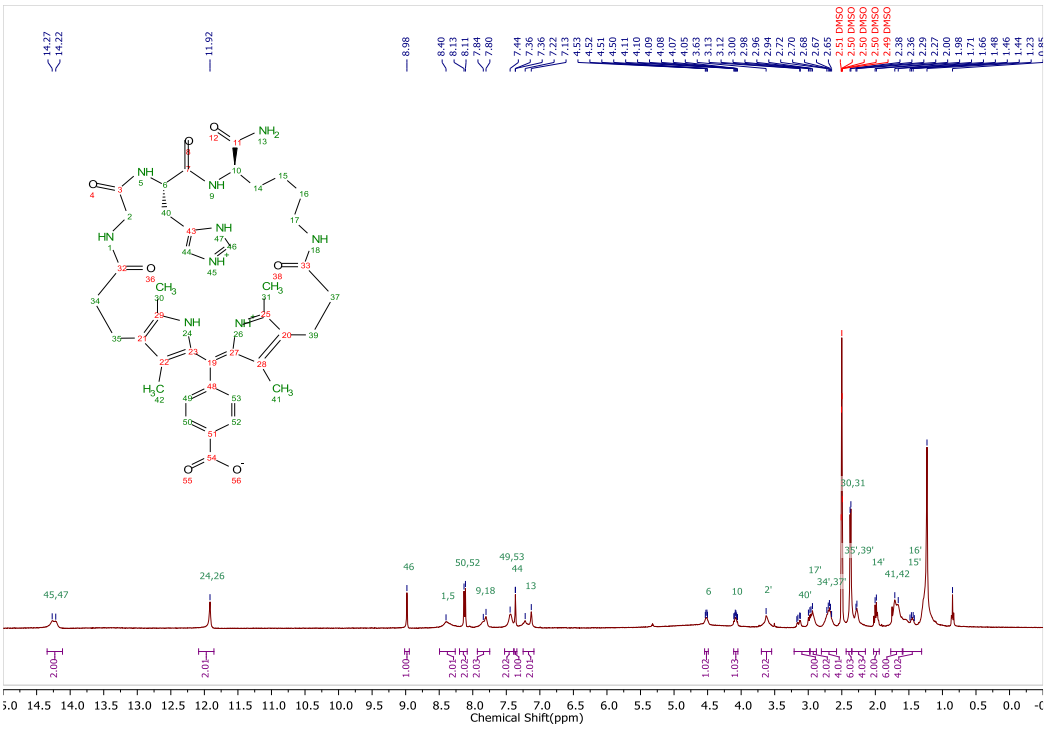
HMBC spectrum of **1a** in DMSO-d_6.



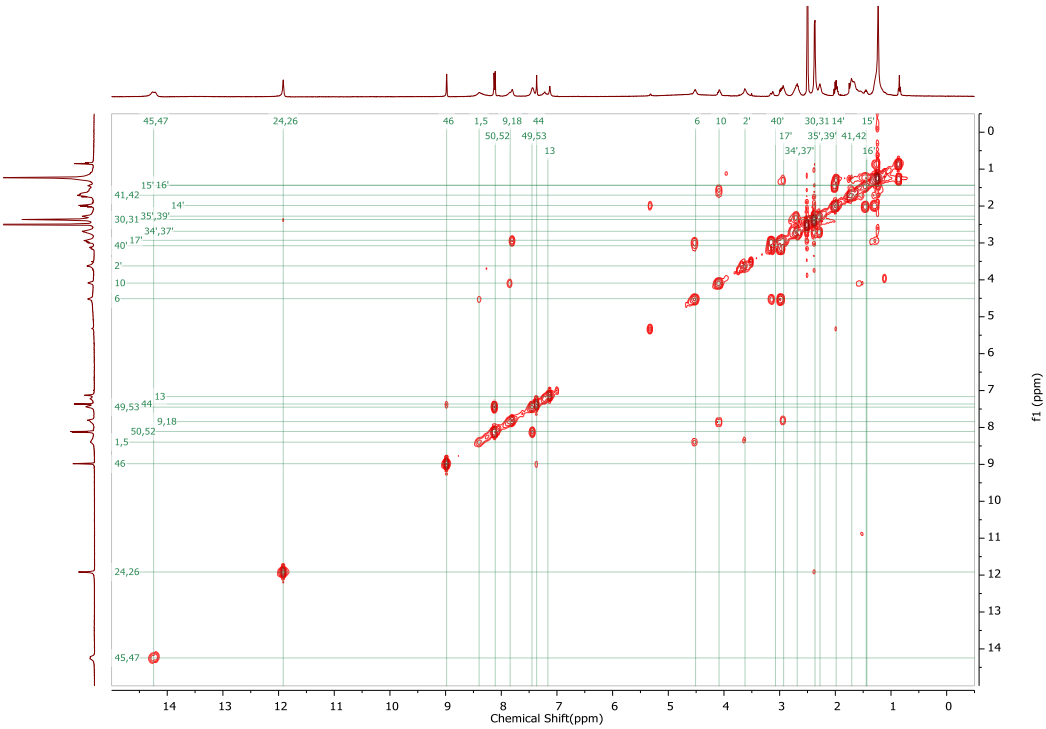
^{1}H-NMR spectrum of **1f** in DMSO-*d*$_6$**.**



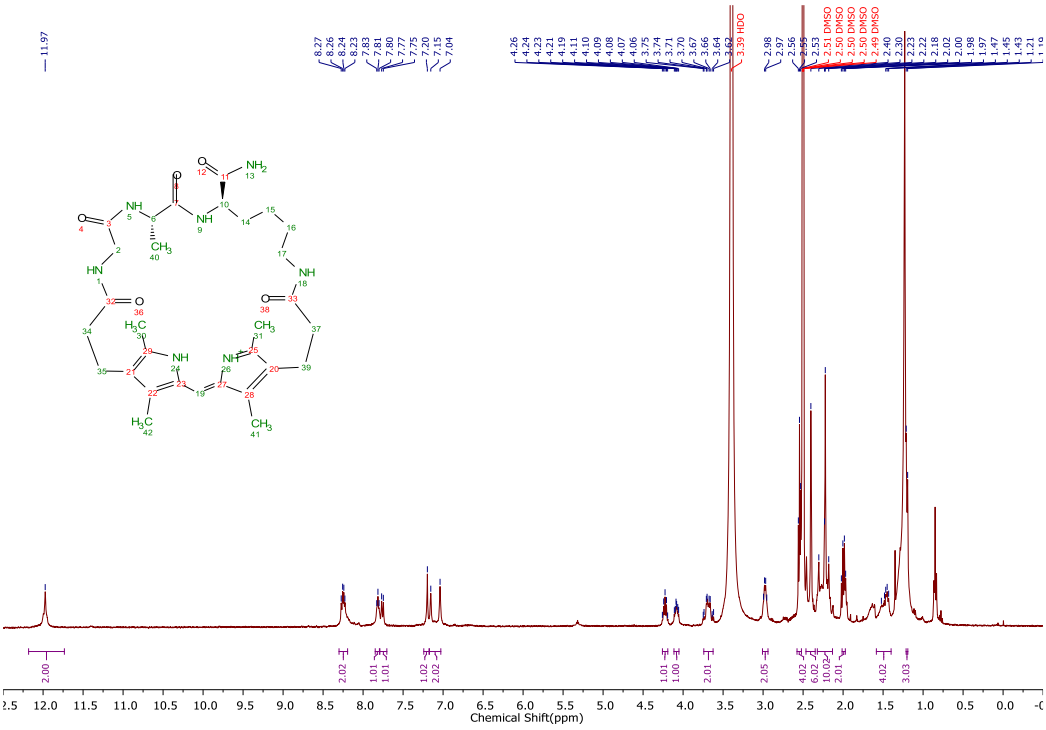
COSY spectrum of **1f** in DMSO-*d*$_6$**.**



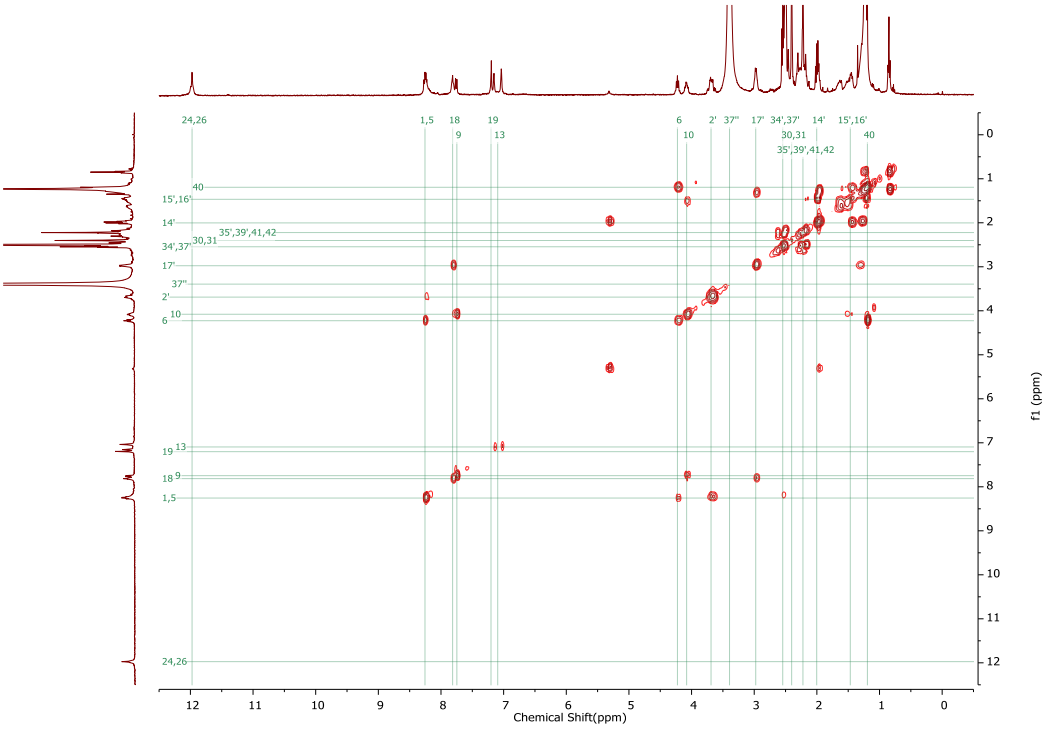
^{1}H-NMR spectrum of **1h** in DMSO-d_6.



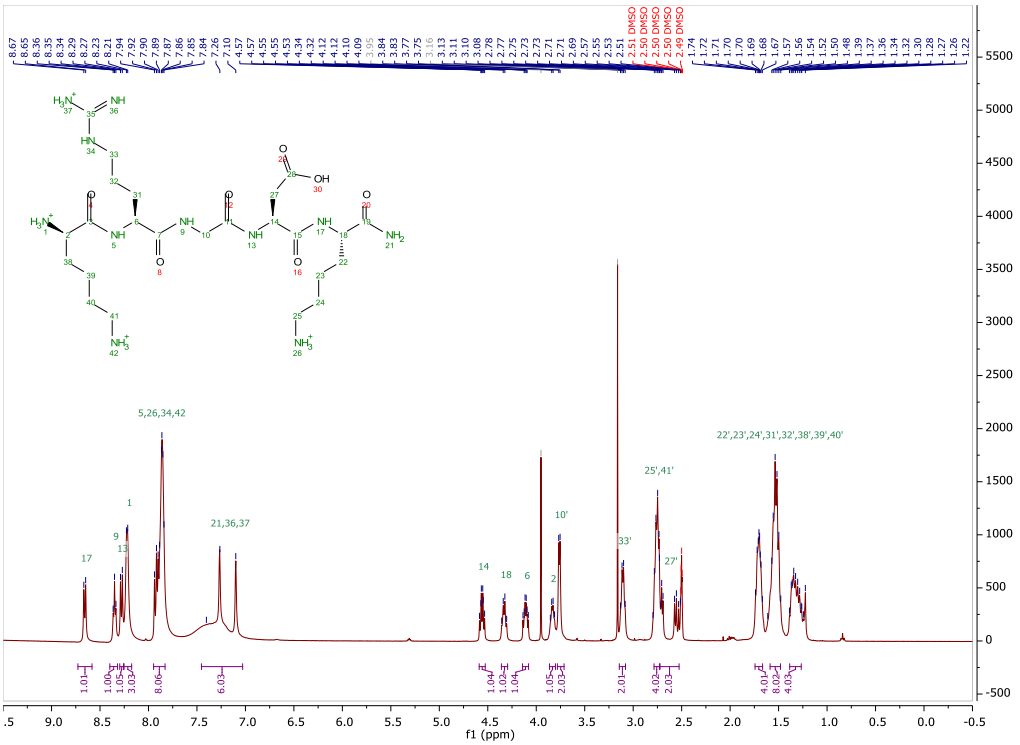
COSY spectrum of **1h** in DMSO-d_6.



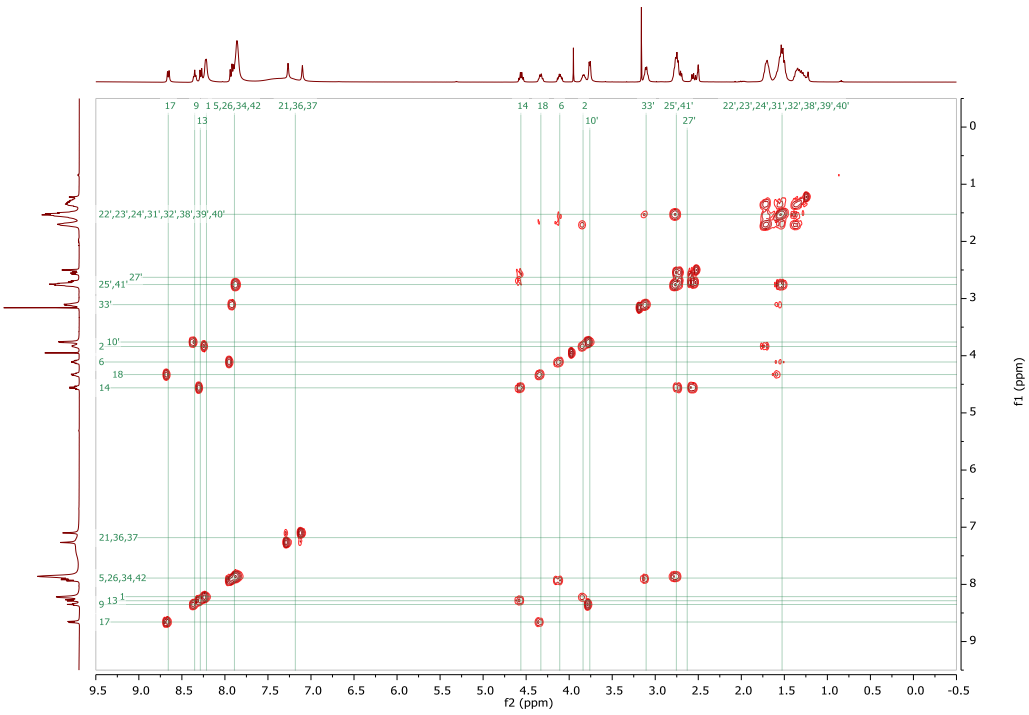
^{1}H-NMR spectrum of **2** in DMSO-d_6.



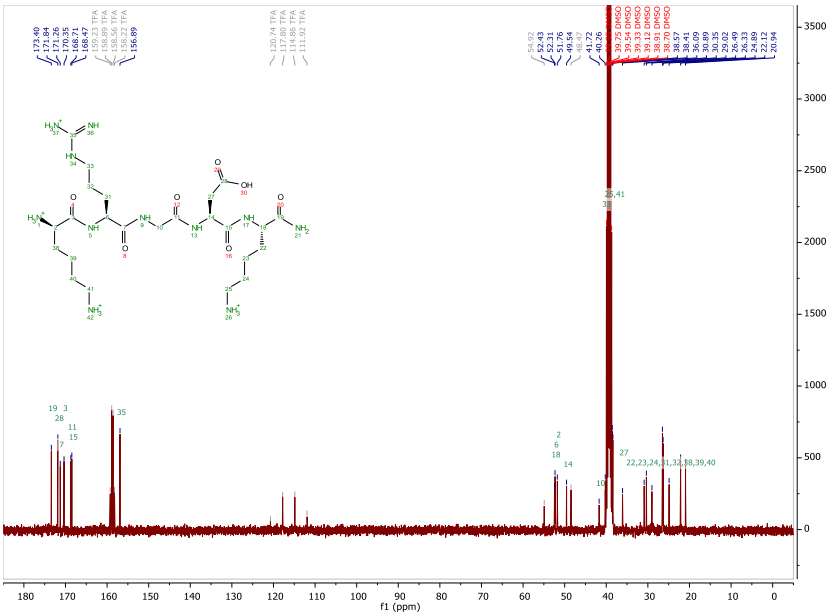
COSY spectrum of **2** in DMSO-d_6.



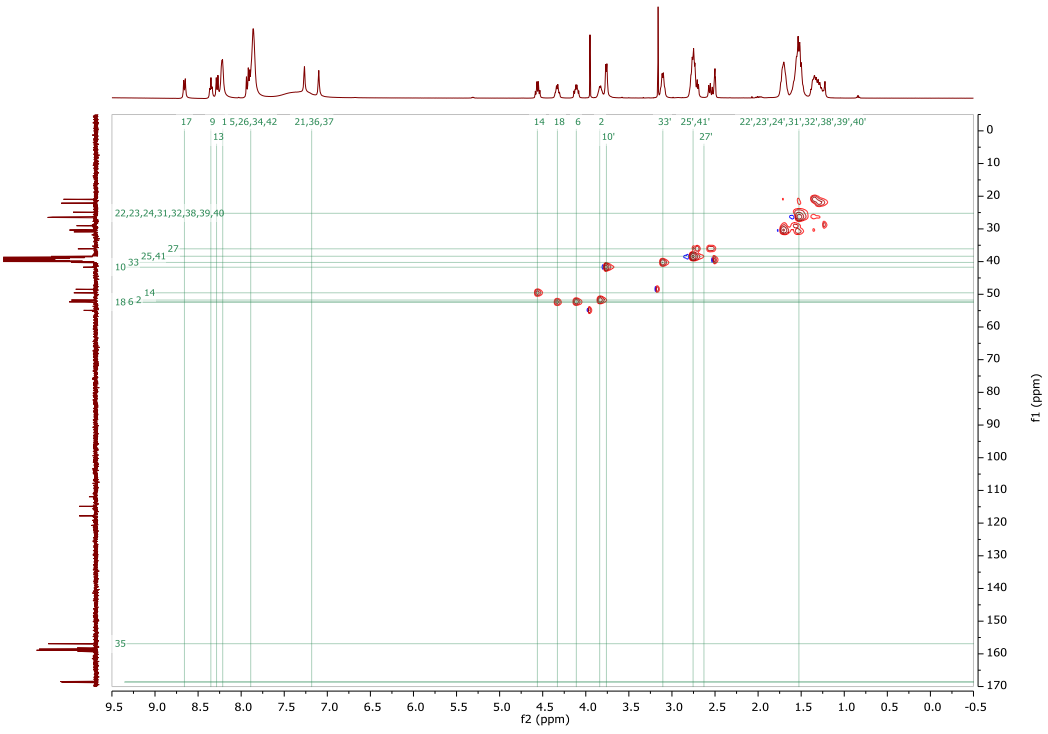
^{1}H-NMR spectrum of **3a** in DMSO-d_6.



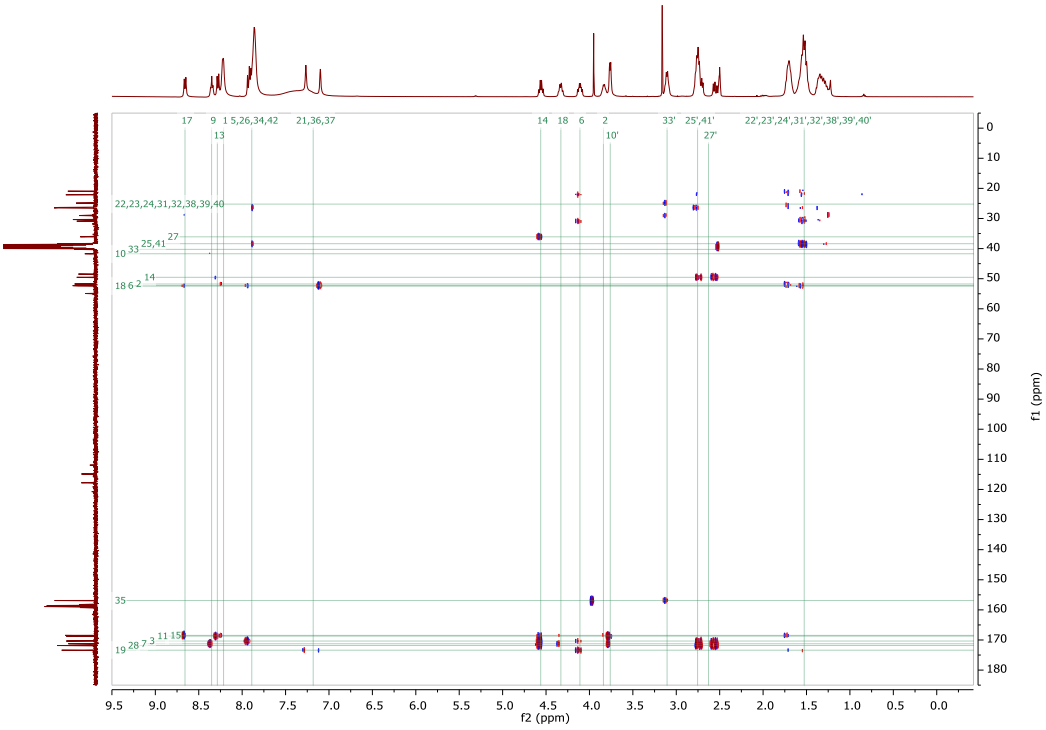
COSY spectrum of **3a** in DMSO-d_6.



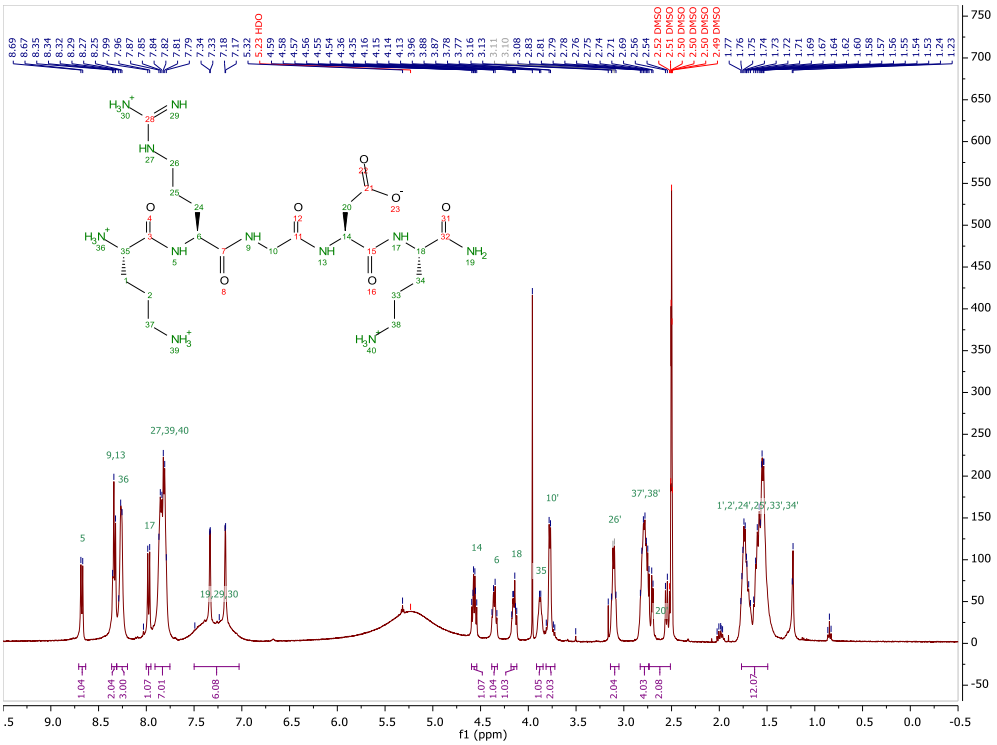
^{13}C-NMR spectrum of **3a** in DMSO-d_6.



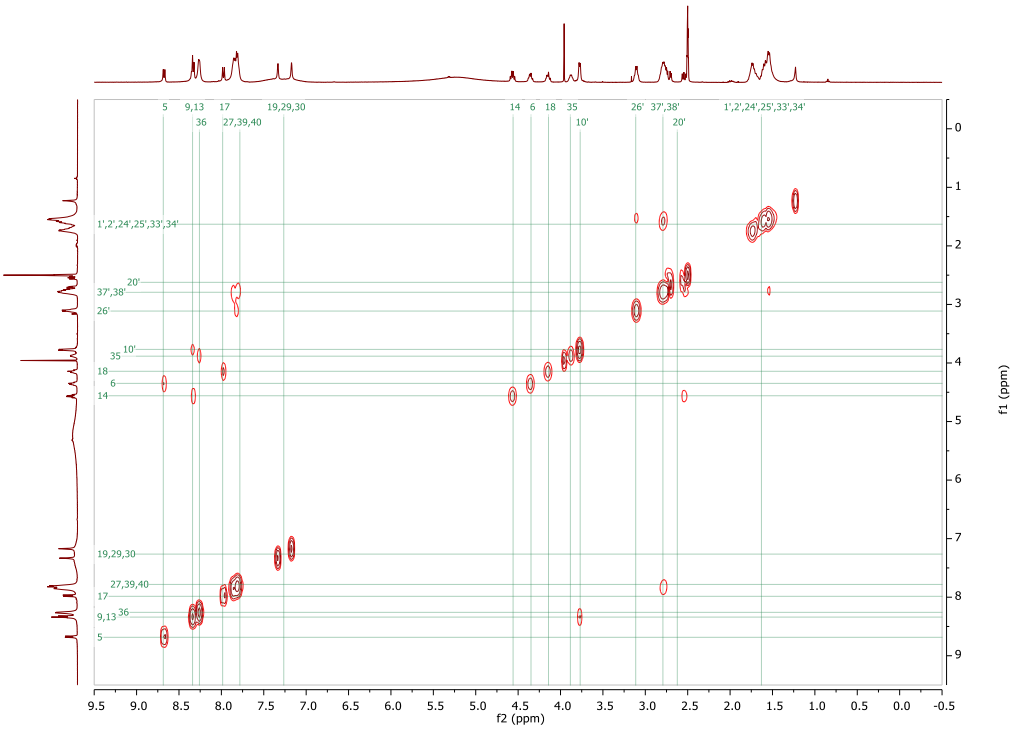
HSQC spectrum of **3a** in DMSO-d_6.



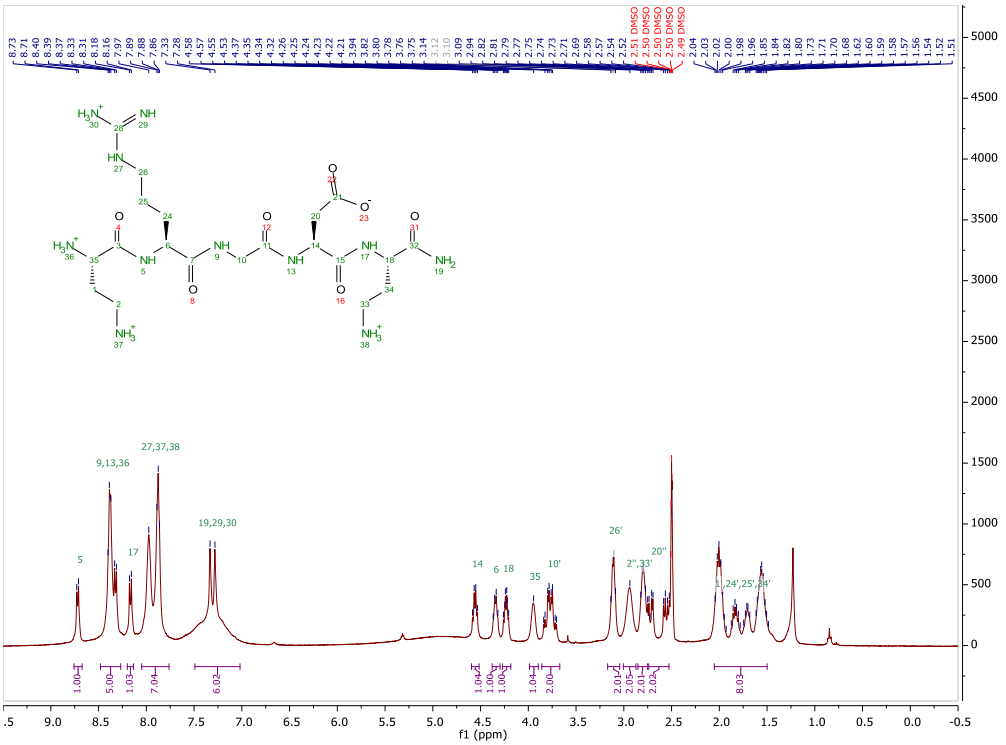
HMBC spectrum of **3a** in DMSO-d_6.



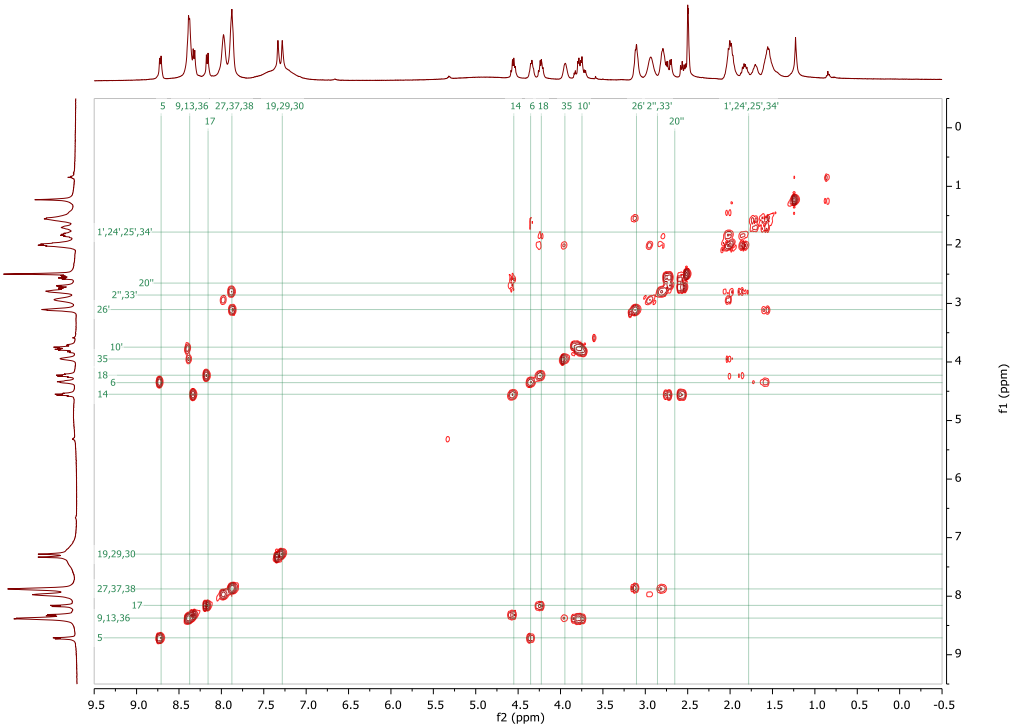
^{1}H-NMR spectrum of **3b** in DMSO-d_6**.**



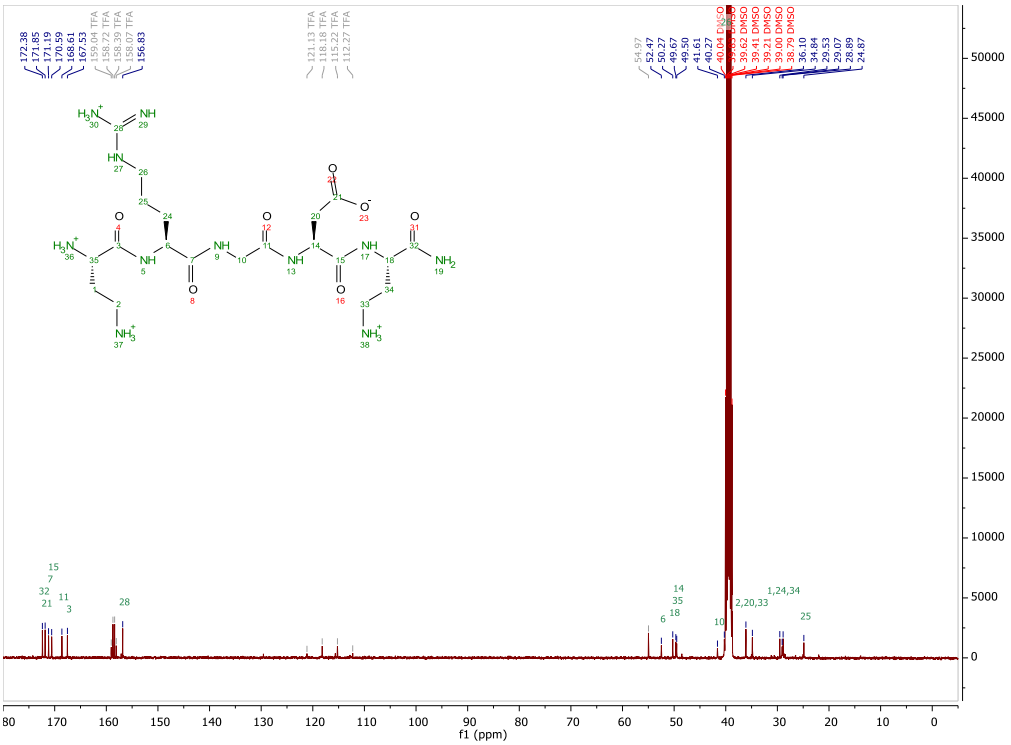
COSY spectrum of **3b** in DMSO-d_6**.**



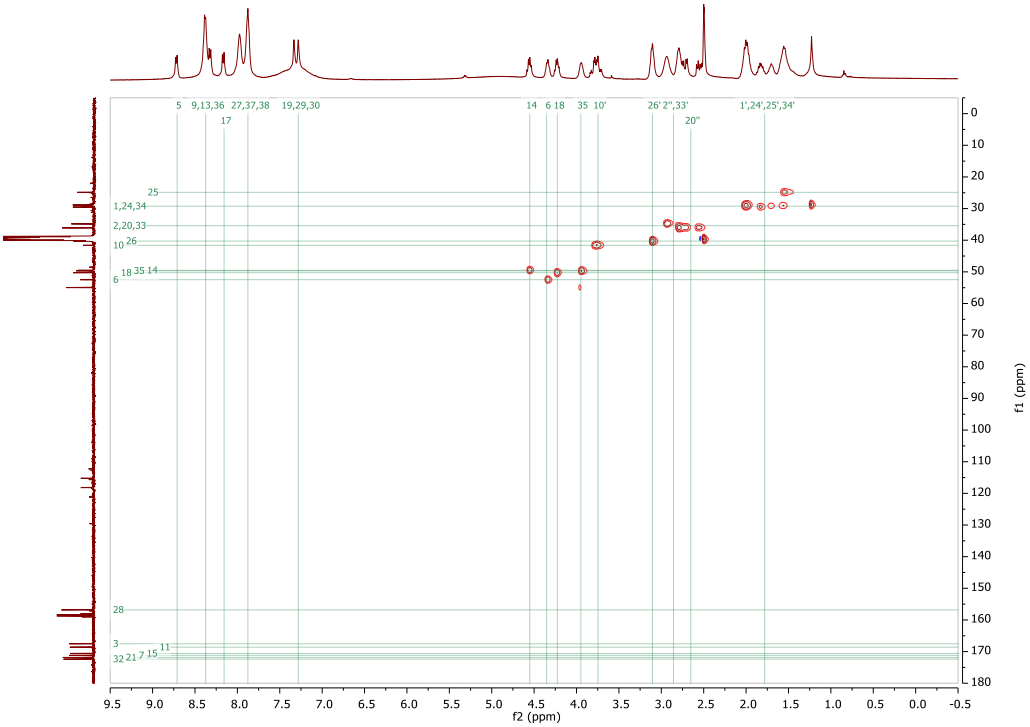
^{1}H-NMR spectrum of **3c** in DMSO-*d*$_6$**.**



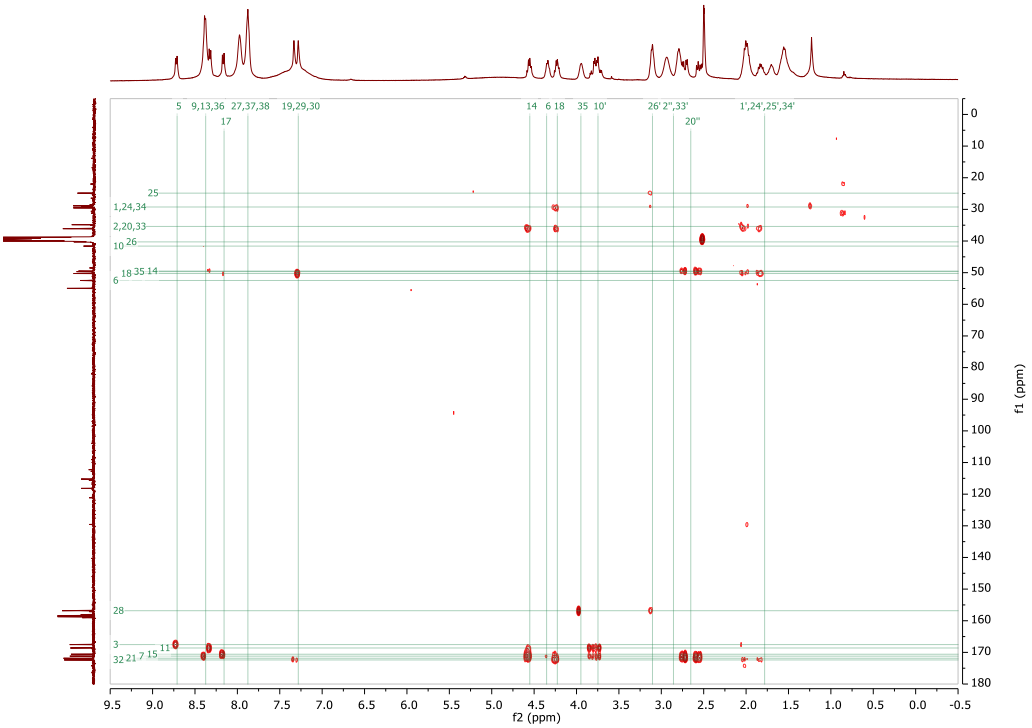
COSY spectrum of **3c** in DMSO-*d*$_6$**.**



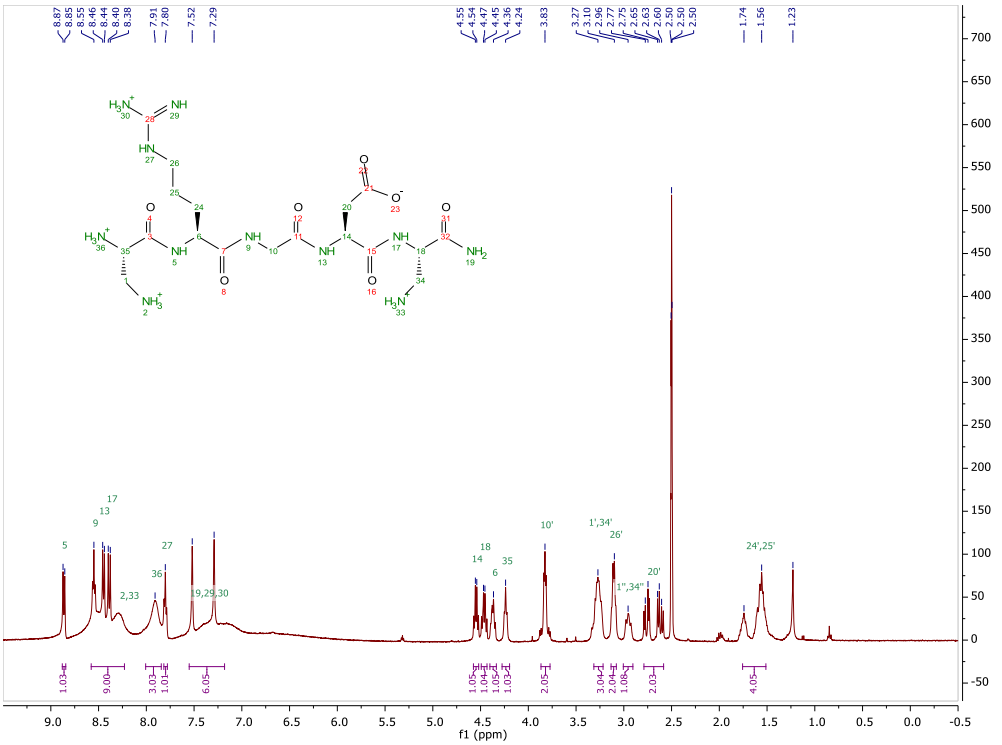
^{13}C-NMR spectrum of **3c** in DMSO-*d*$_6$**.**



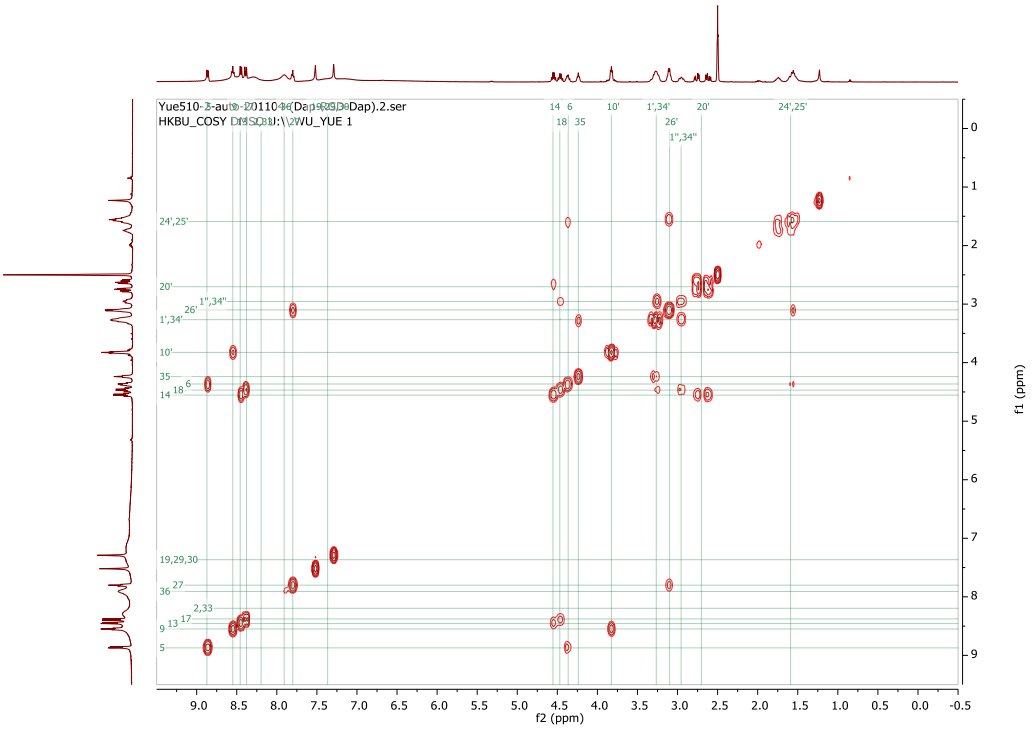
HSQC spectrum of **3c** in DMSO-*d*$_6$**.**



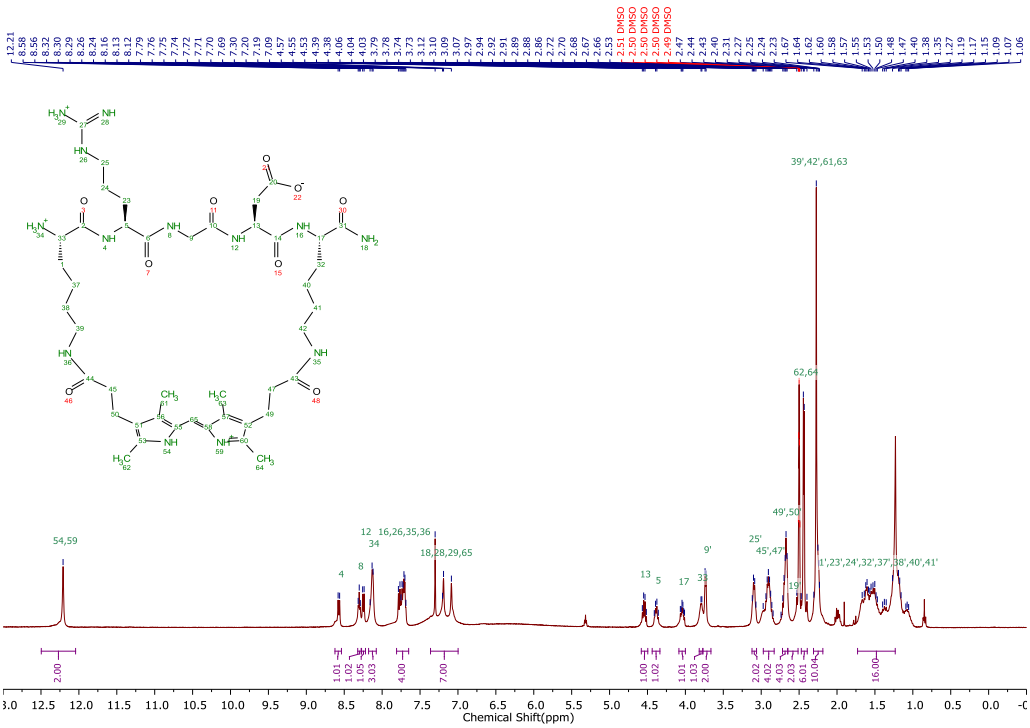
HMBC spectrum of **3c** in DMSO-d_6.



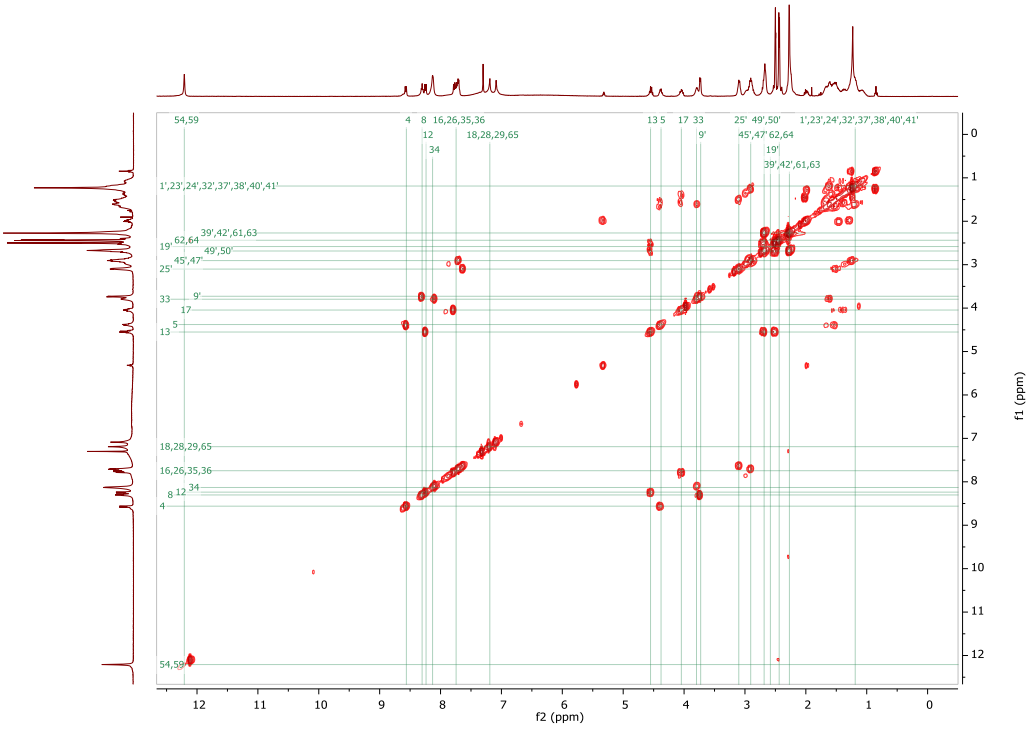
^{1}H-NMR spectrum of **3d** in DMSO-*d*$_6$**.**



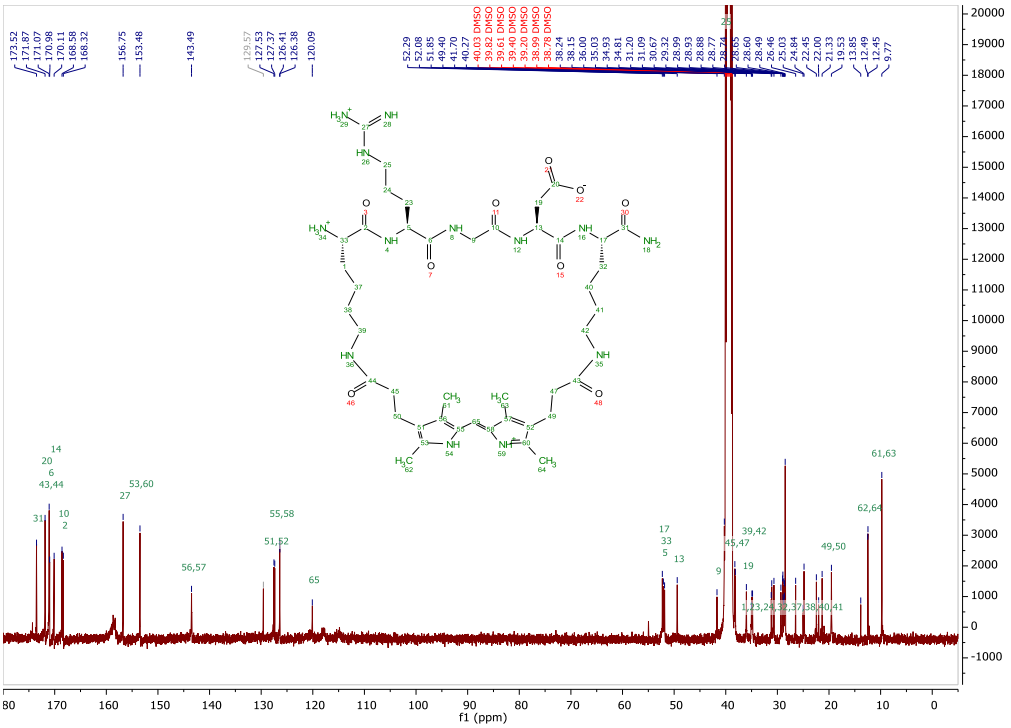
COSY spectrum of **3d** in DMSO-*d*$_6$**.**



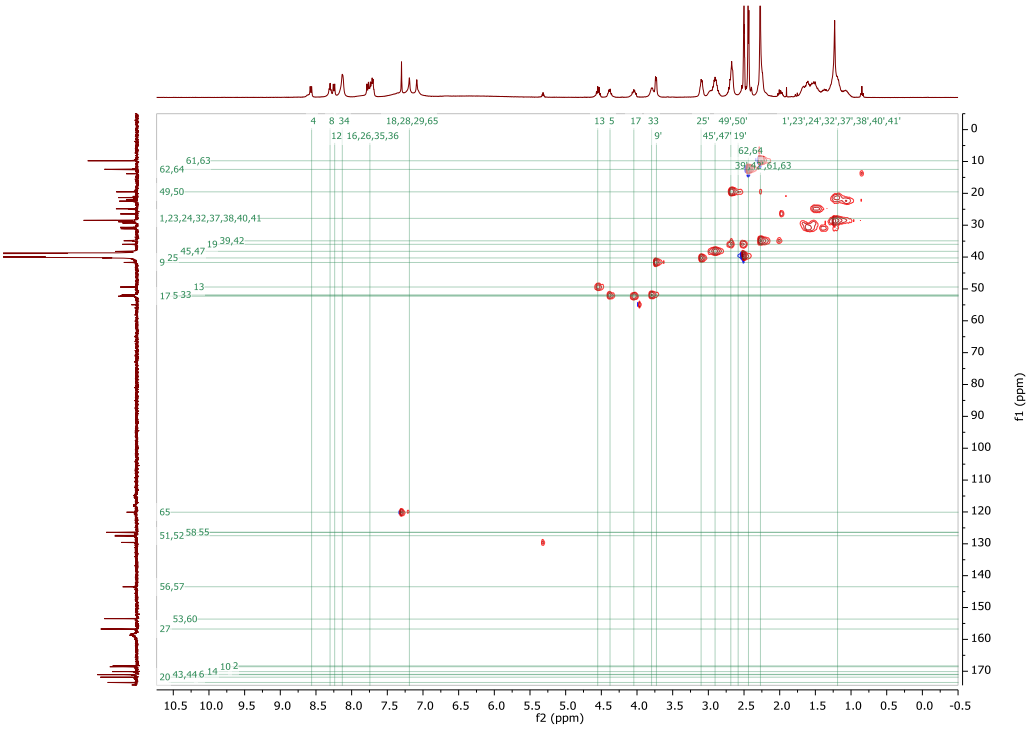
^{1}H-NMR spectrum of **4a** in DMSO-*d*$_6$**.**



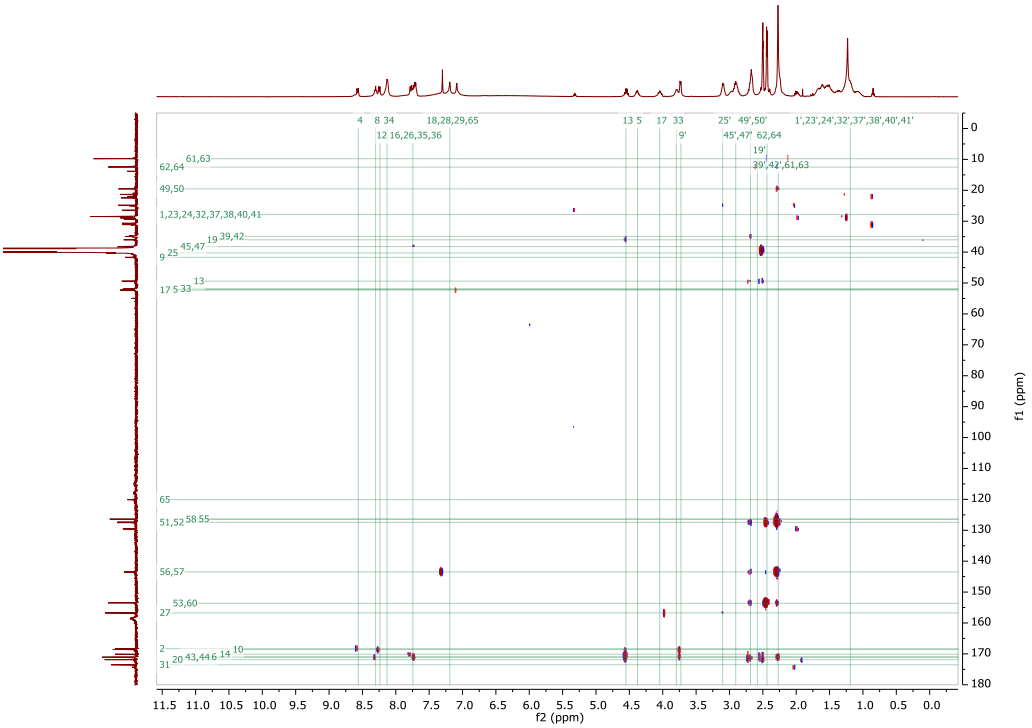
COSY spectrum of **4a** in DMSO-*d*$_6$**.**



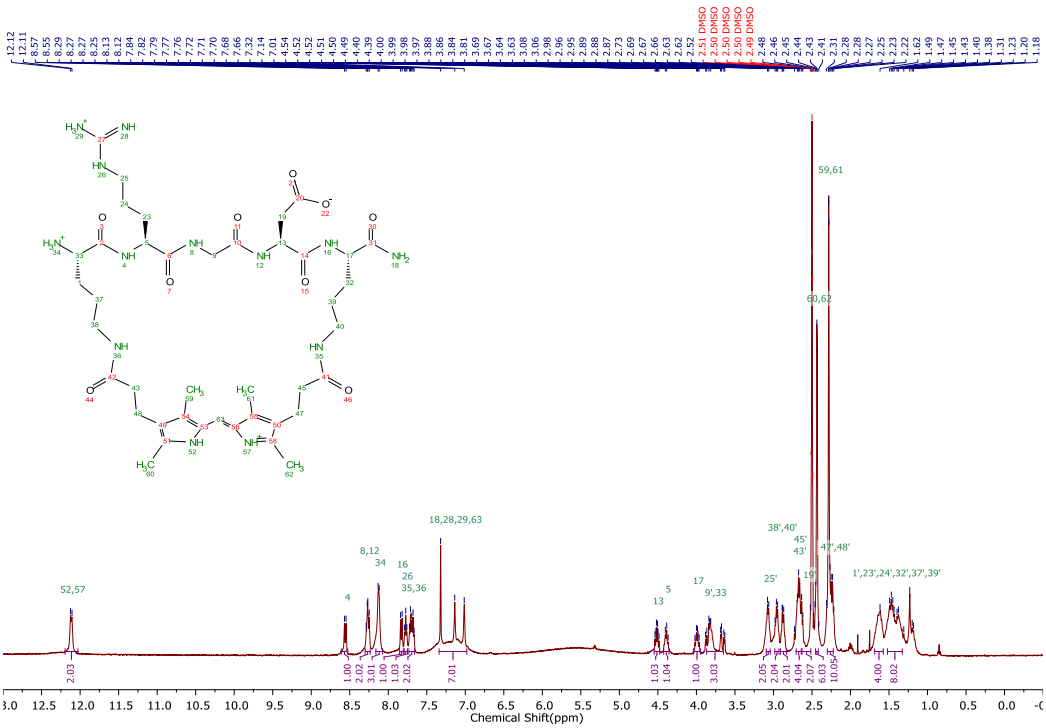
^{13}C-NMR spectrum of **4a** in DMSO-*d*$_6$**.**



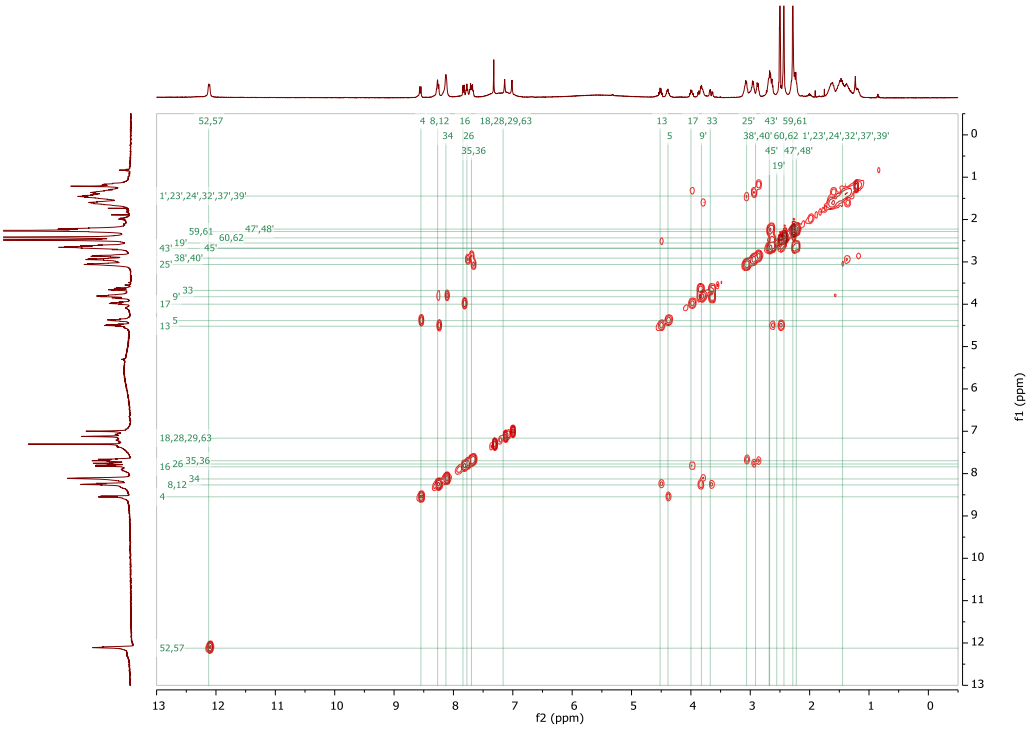
HSQC spectrum of **4a** in DMSO-*d*$_6$**.**



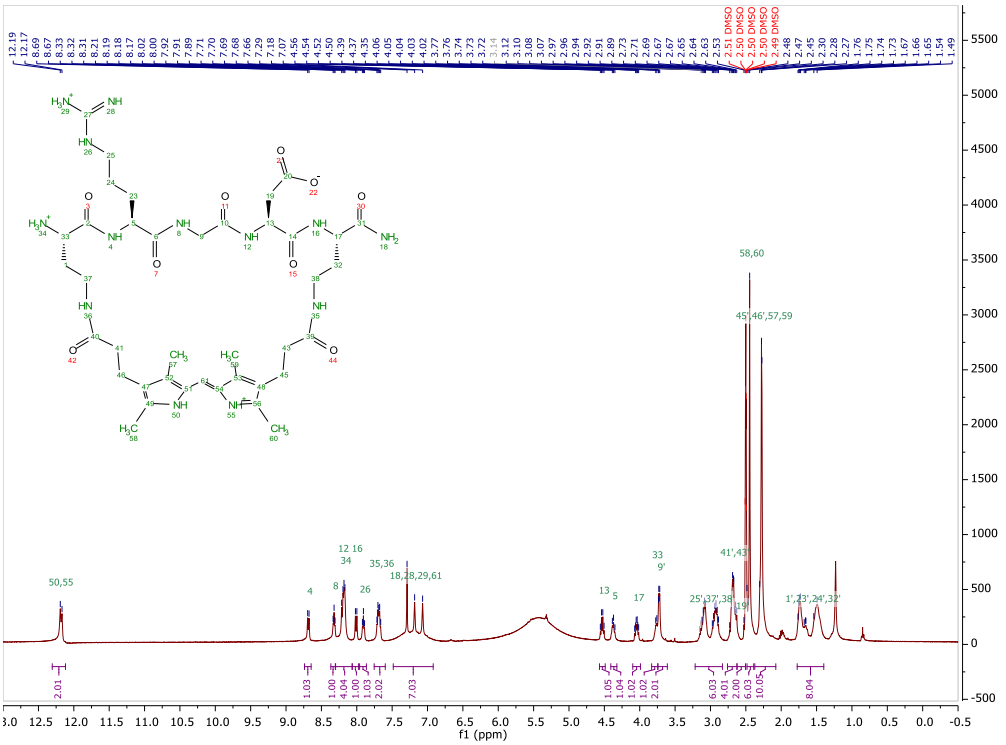
HMBC spectrum of **4a** in DMSO-d_6.



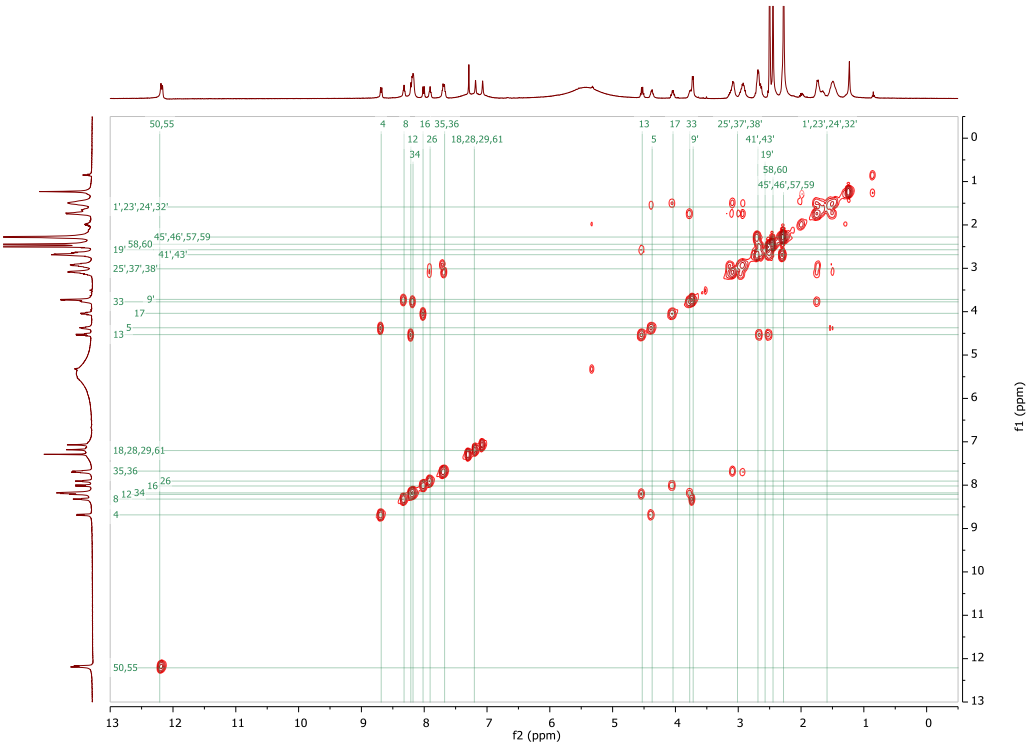
^{1}H-NMR spectrum of **4b** in DMSO-*d*$_6$**.**



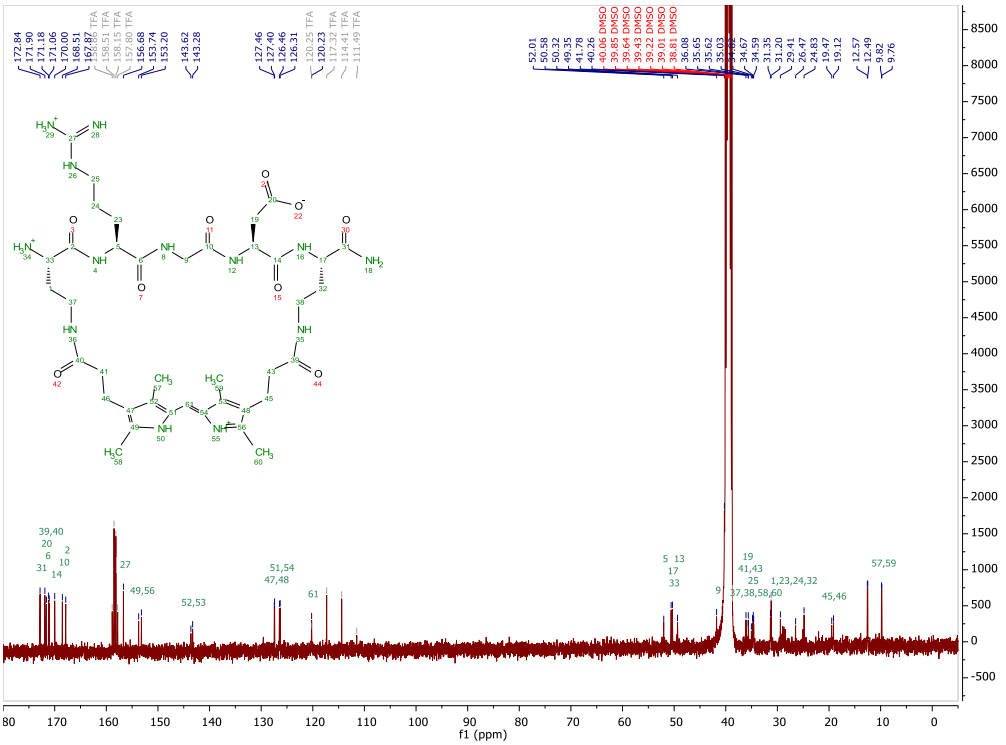
COSY spectrum of **4b** in DMSO-*d*$_6$**.**



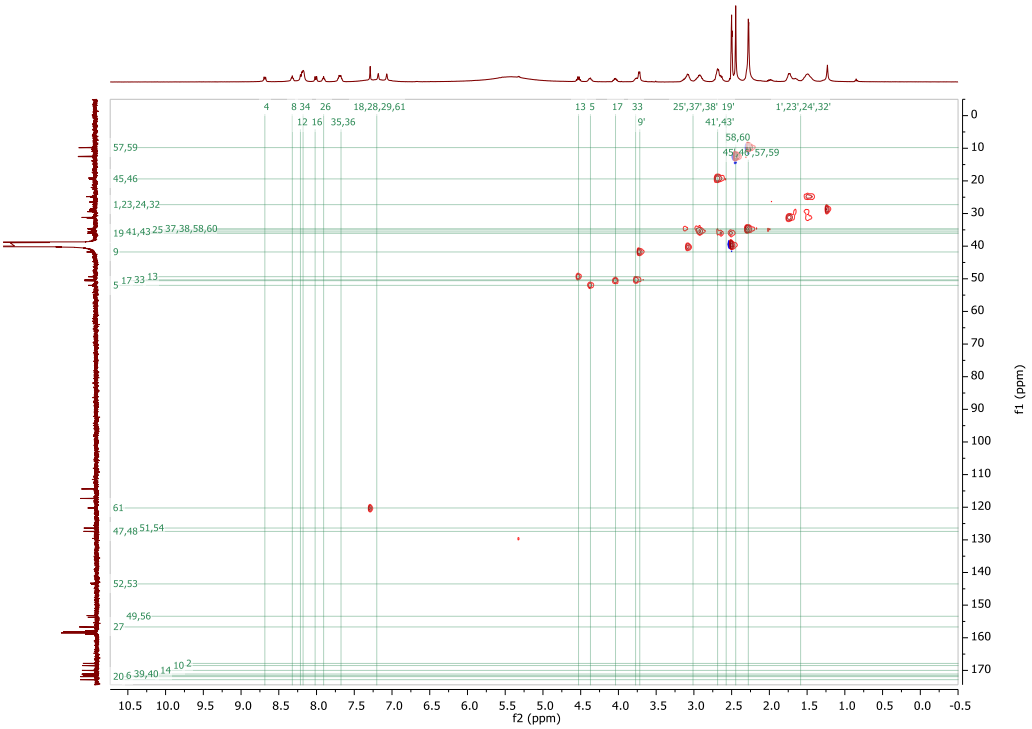
^{1}H-NMR spectrum of **4c** in DMSO-d_6**.**



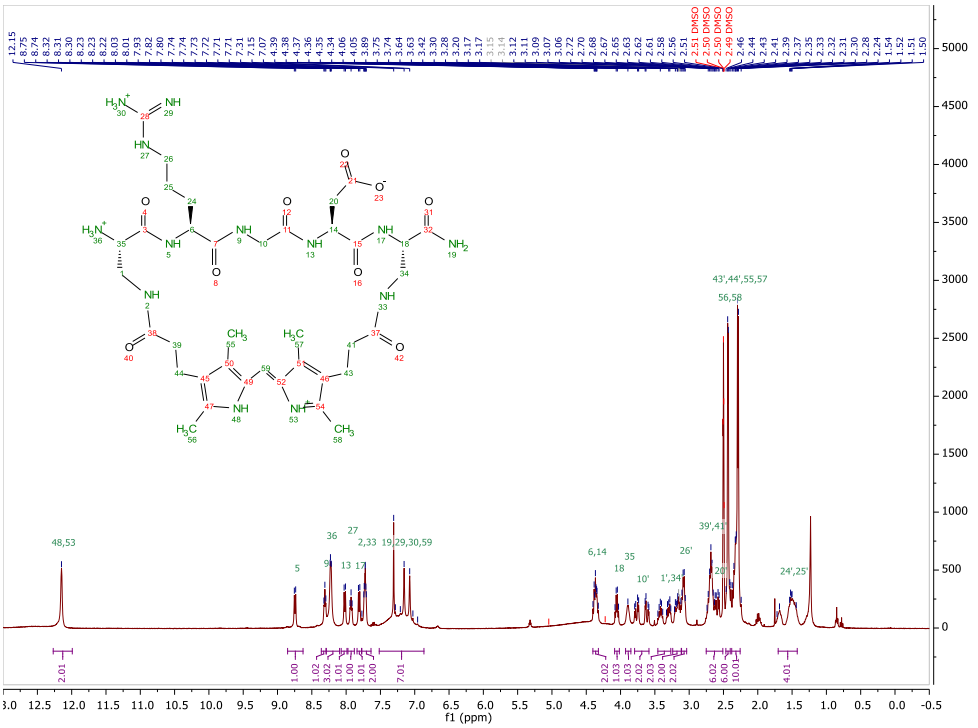
COSY spectrum of **4c** in DMSO-d_6**.**



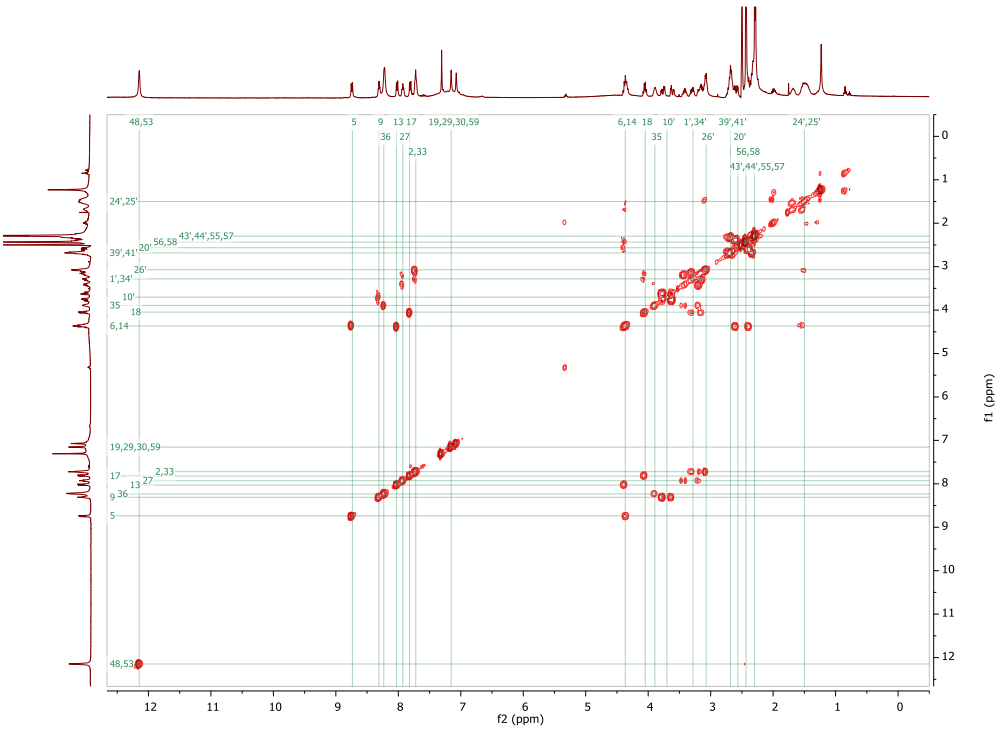
^{13}C-NMR spectrum of **4c** in DMSO-*d*$_6$**.**



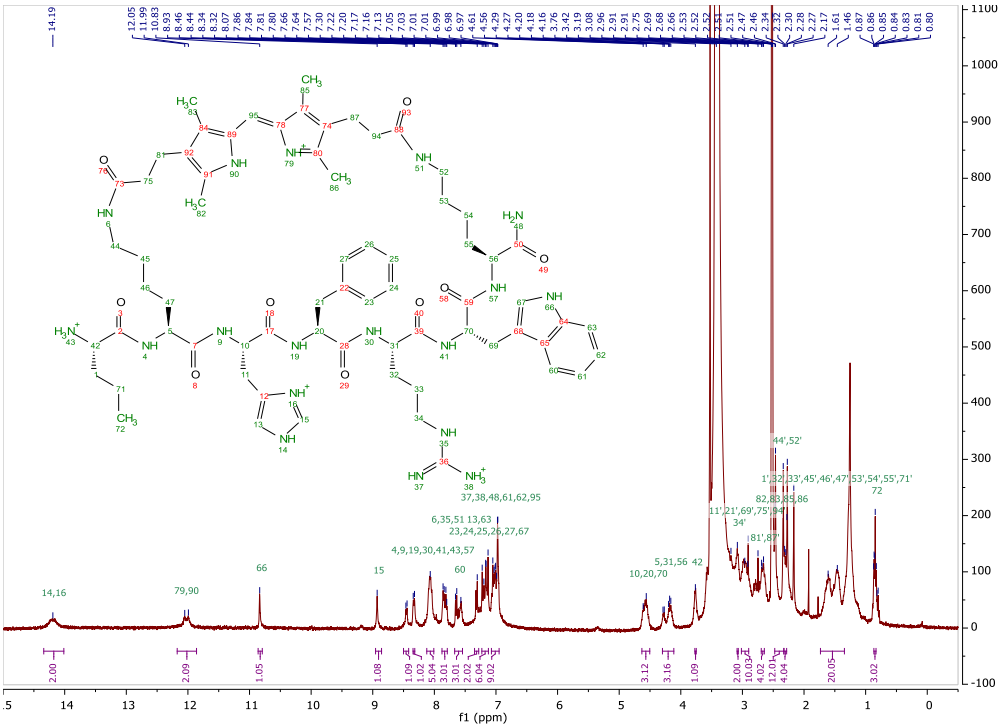
HSQC spectrum of **4c** in DMSO-*d*$_6$**.**



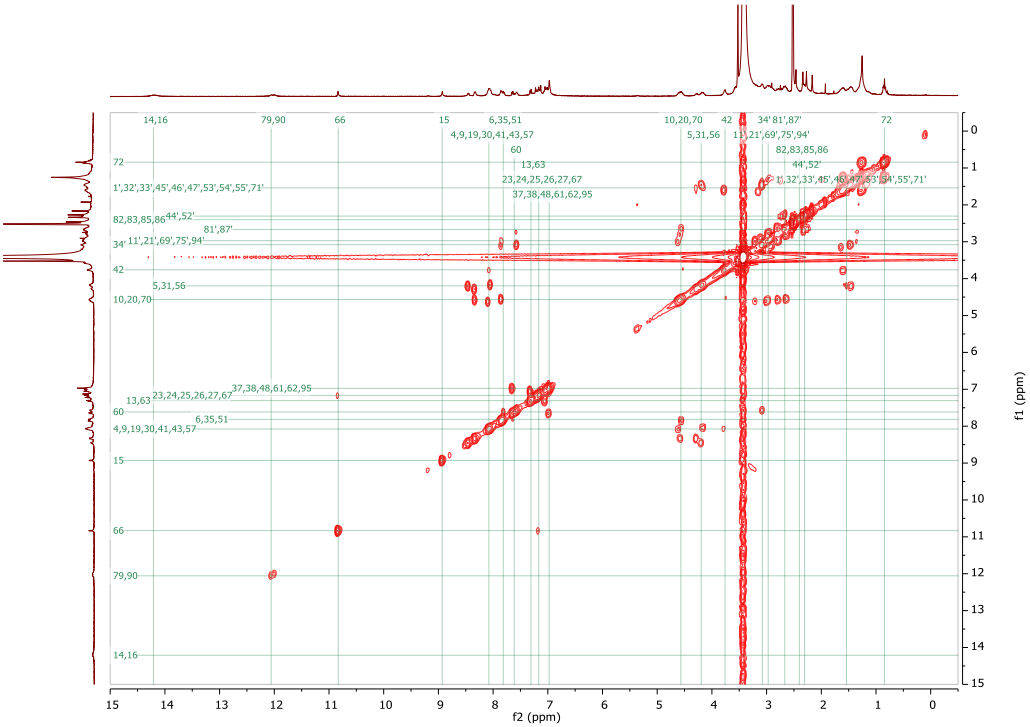
^{1}H-NMR spectrum of **4d** in DMSO-d_6.



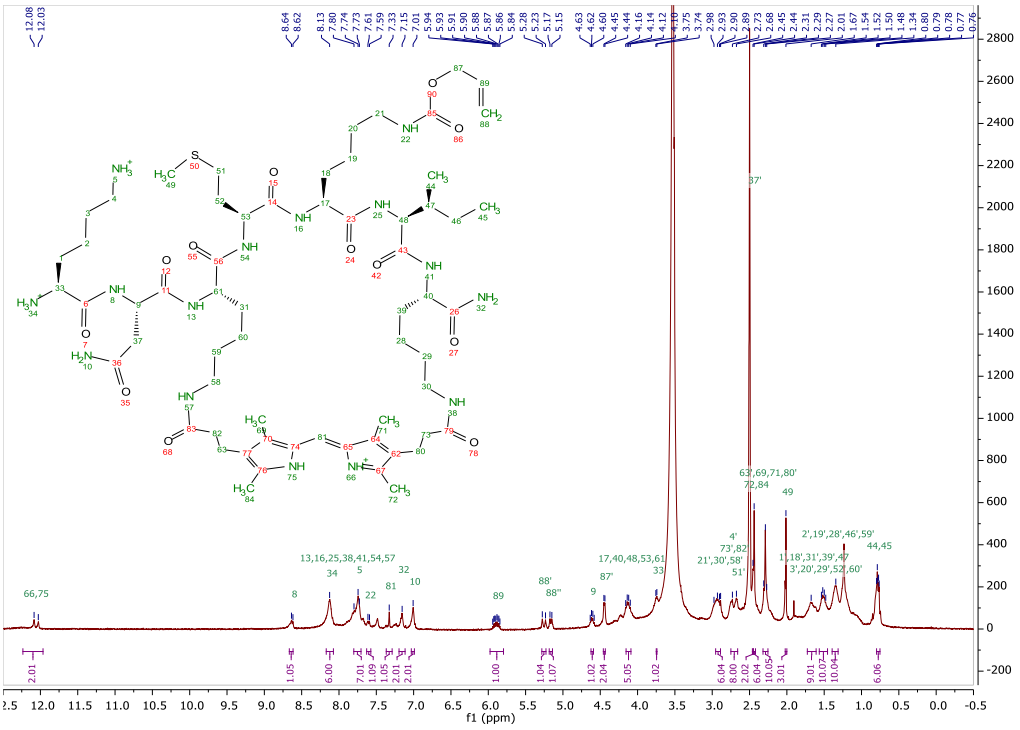
COSY spectrum of **4d** in DMSO-d_6.



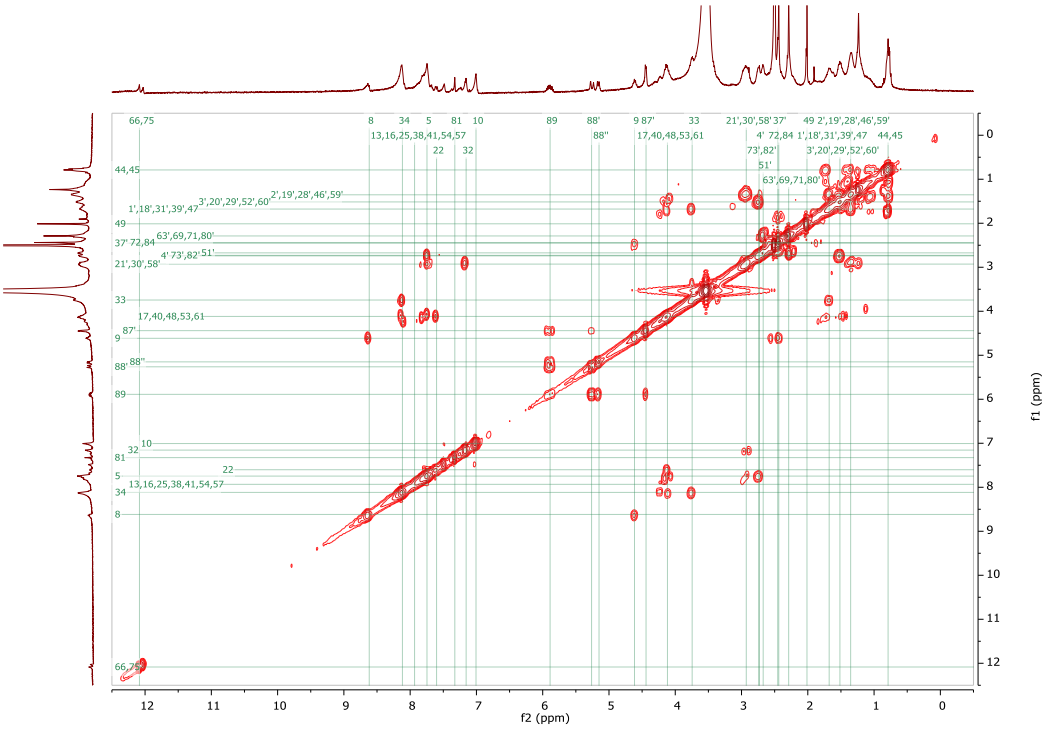
^{1}H-NMR spectrum of **5a** in DMSO-d_6.



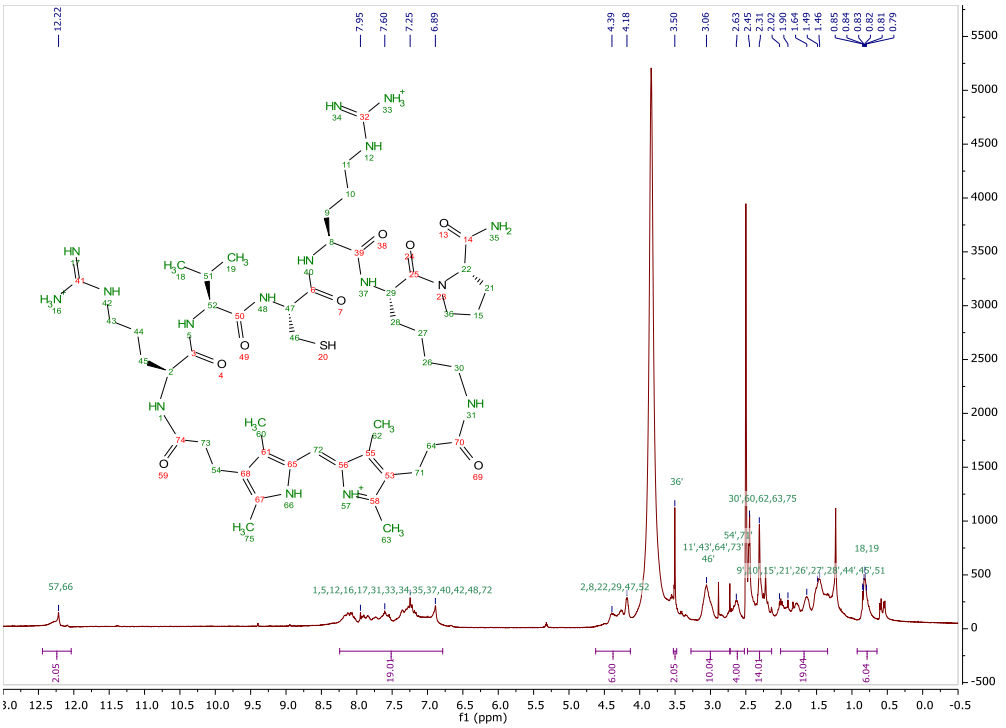
COSY spectrum of **5a** in DMSO-d_6.



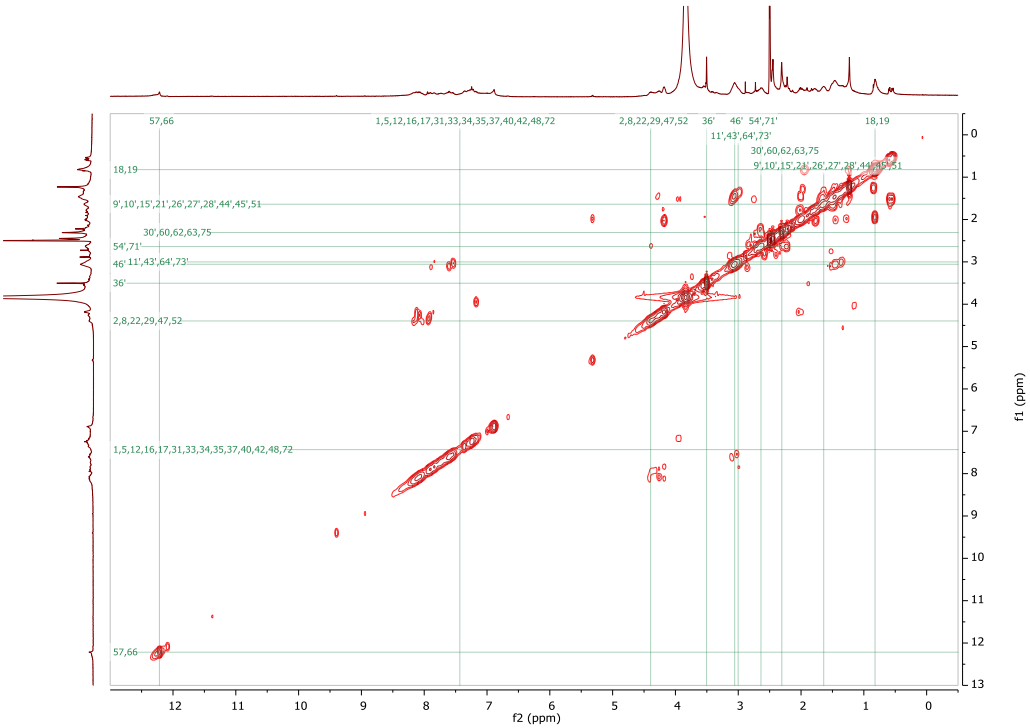
^{1}H-NMR spectrum of **6a** in DMSO-d_6.



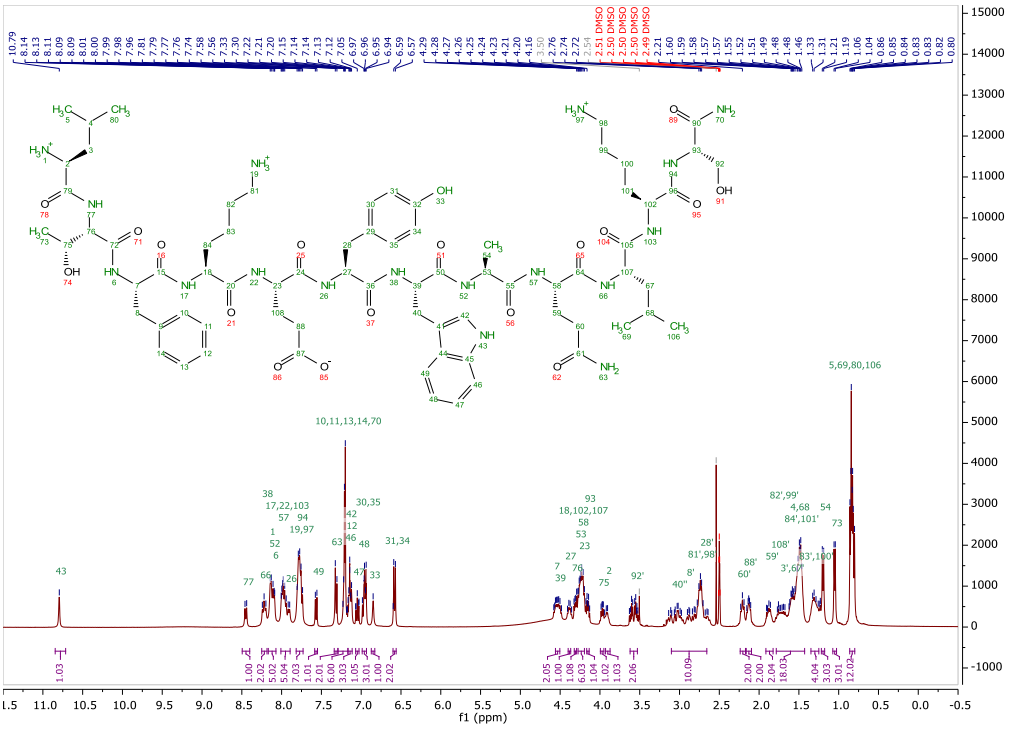
COSY spectrum of **6a** in DMSO-d_6.



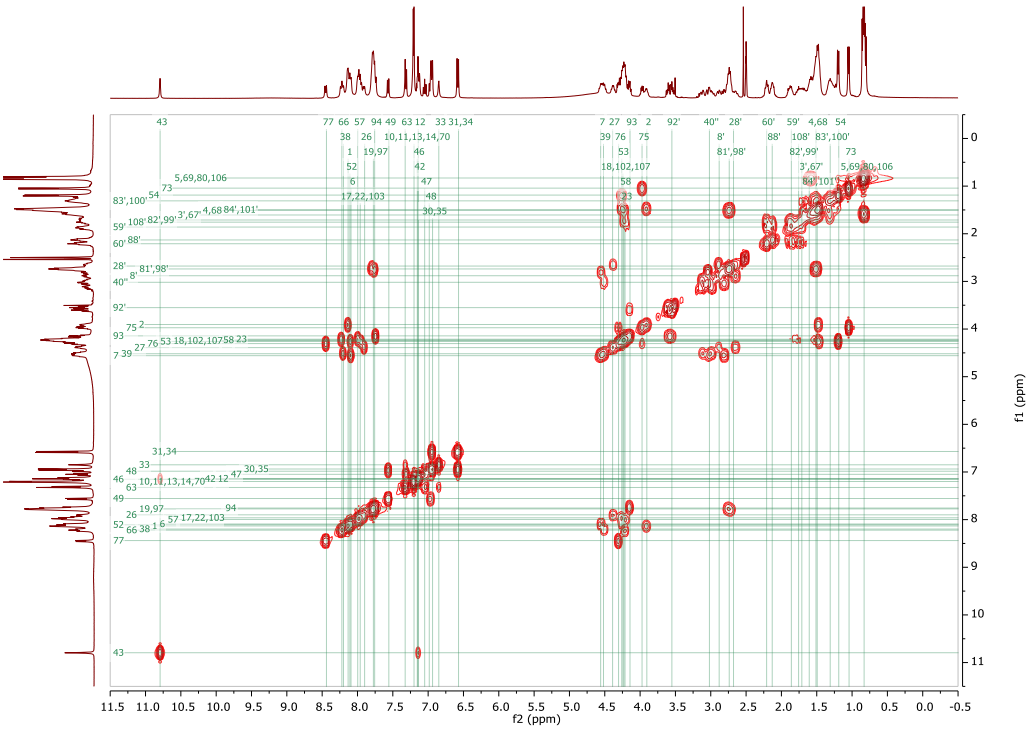
^{1}H-NMR spectrum of **7a** in DMSO-*d*$_6$.



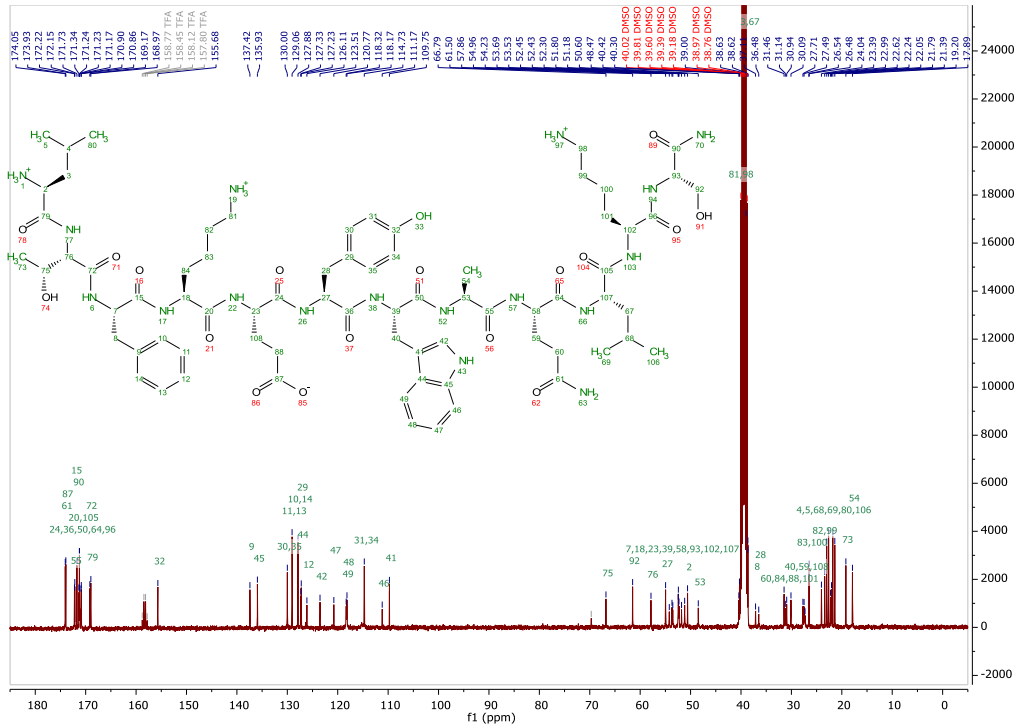
COSY spectrum of **7a** in DMSO-*d*$_6$.



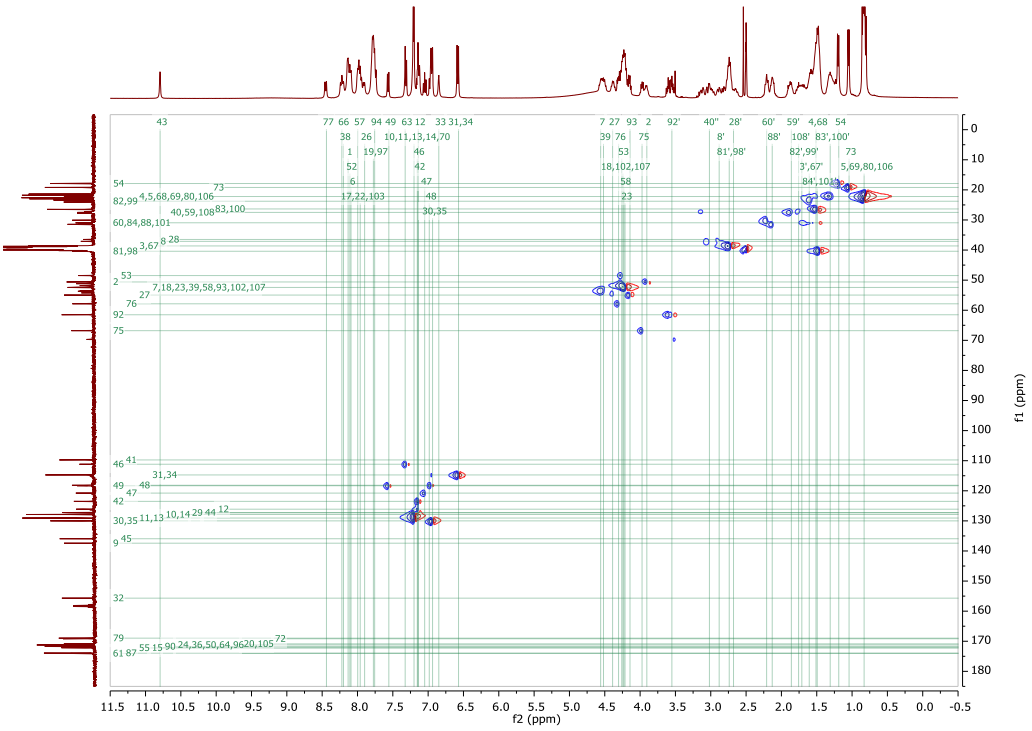
^{1}H-NMR spectrum of **S2** in DMSO-d_6.



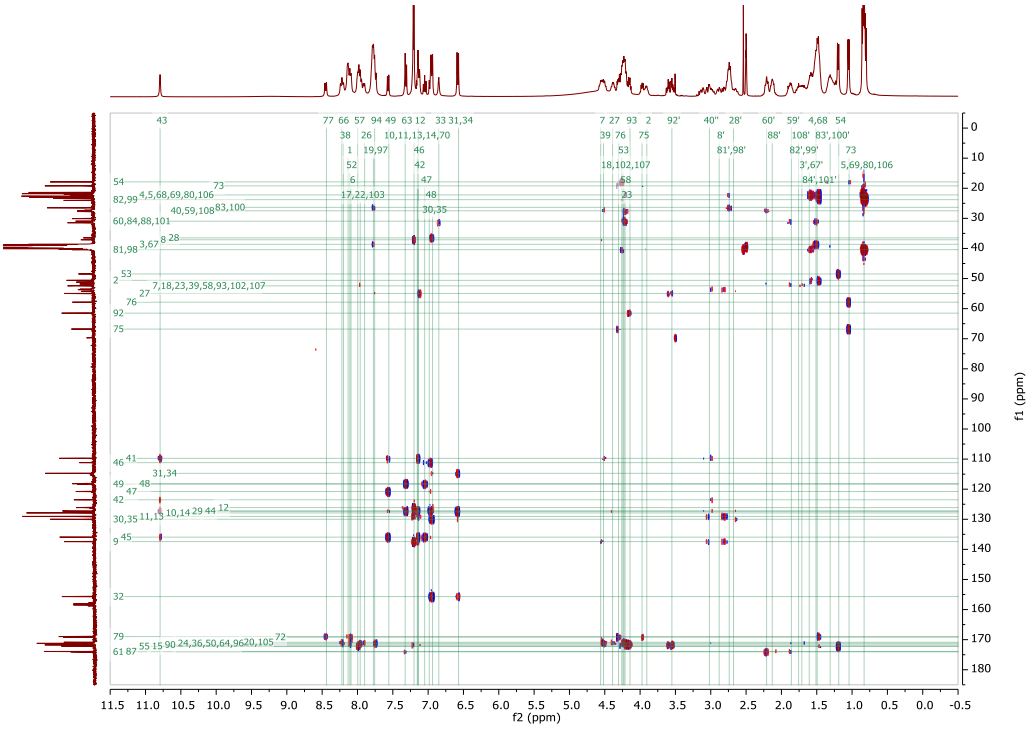
COSY spectrum of **S2** in DMSO-d_6.



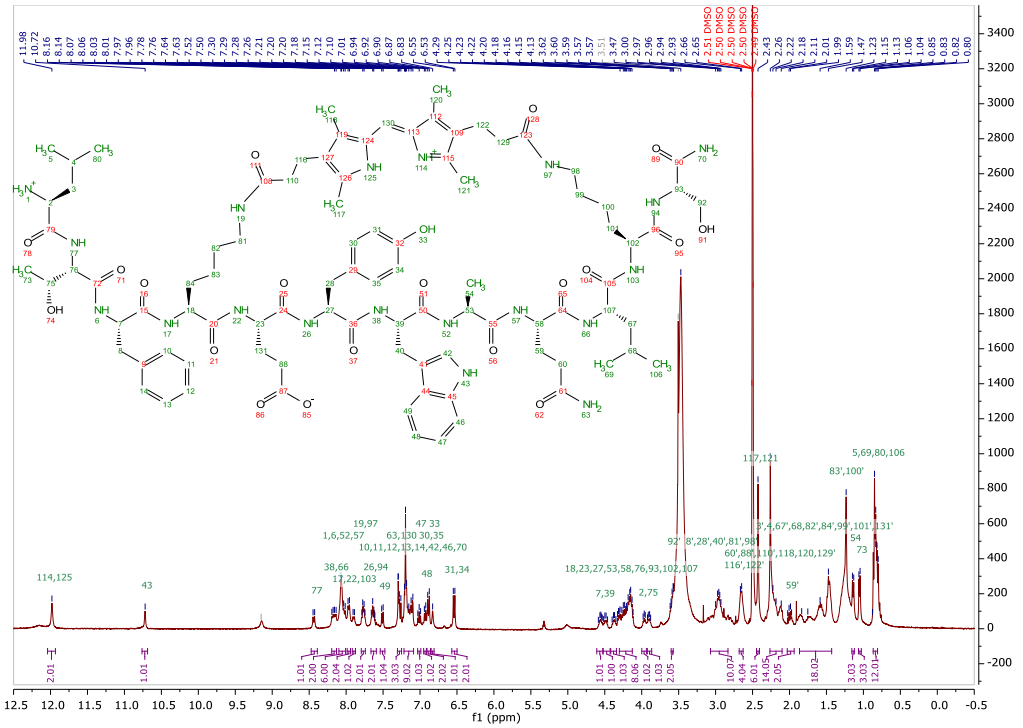
^{13}C-NMR spectrum of **S2** in DMSO-d_6**.**



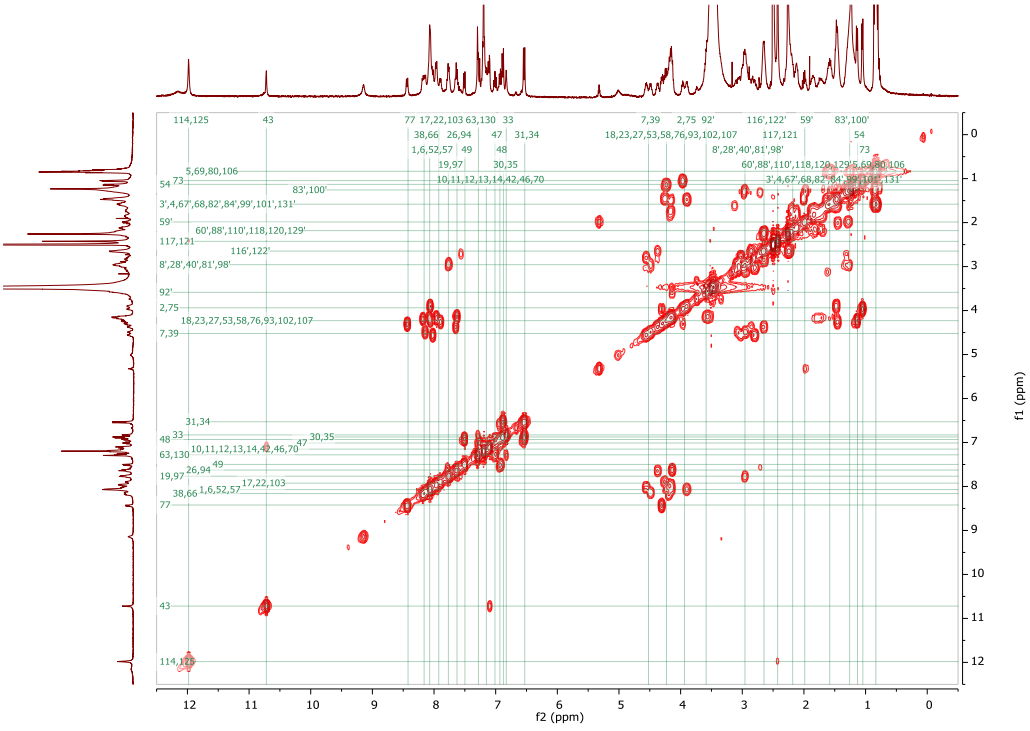
HSQC spectrum of **S2** in DMSO-d_6**.**



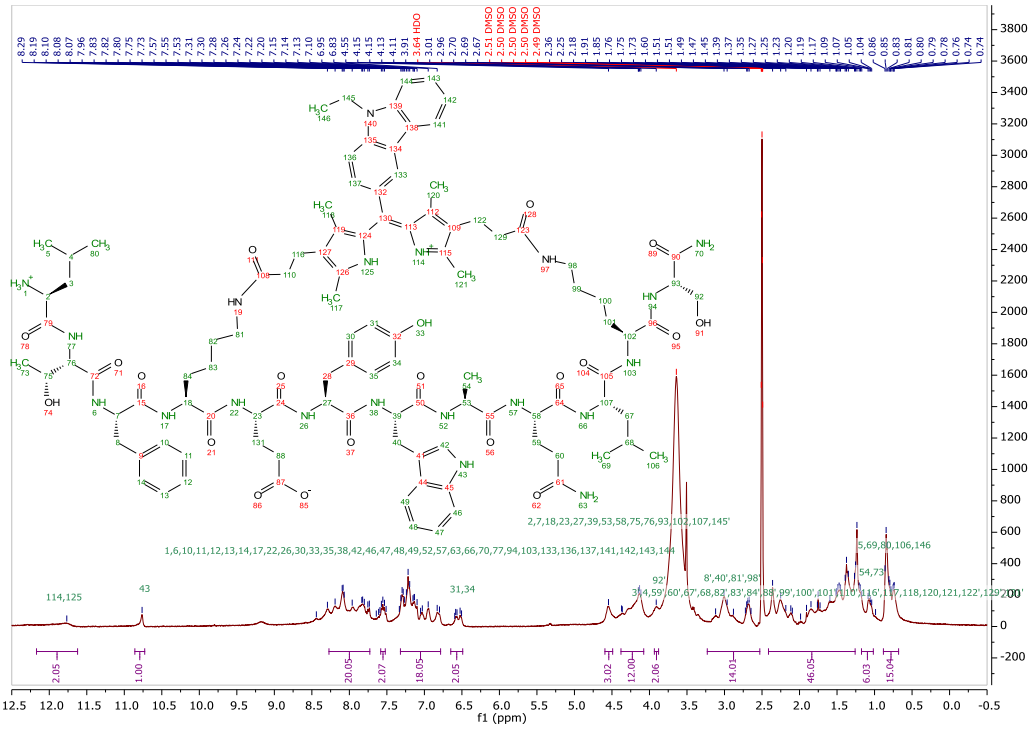
HMBC spectrum of **S2** in DMSO-d_6**.**



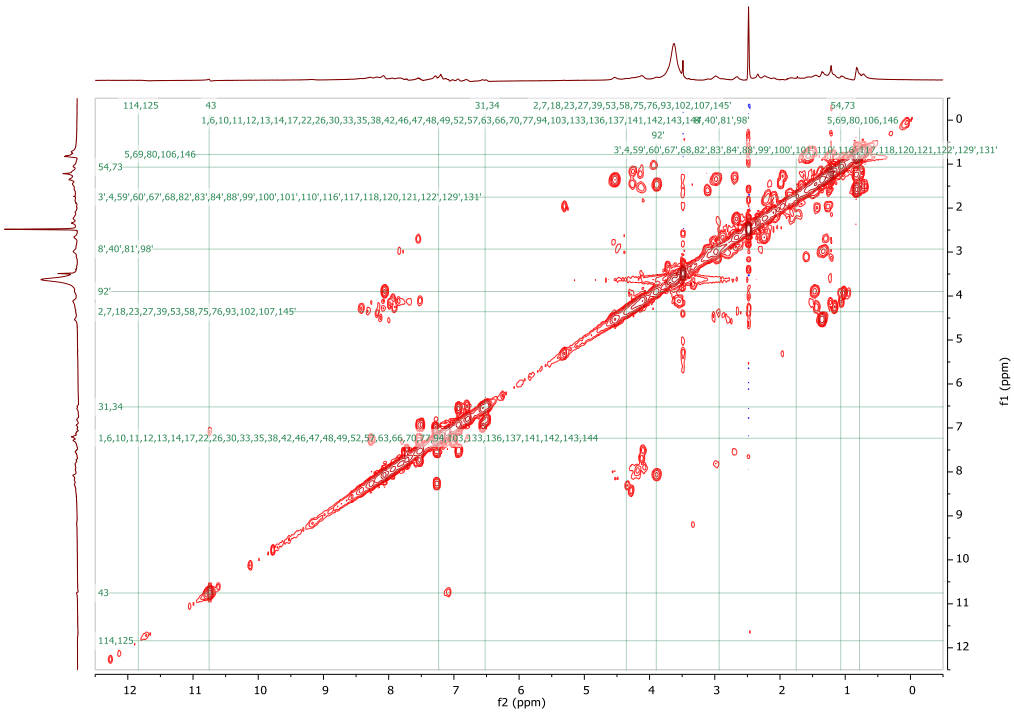
^{1}H-NMR spectrum of **8a** in DMSO-d_6.



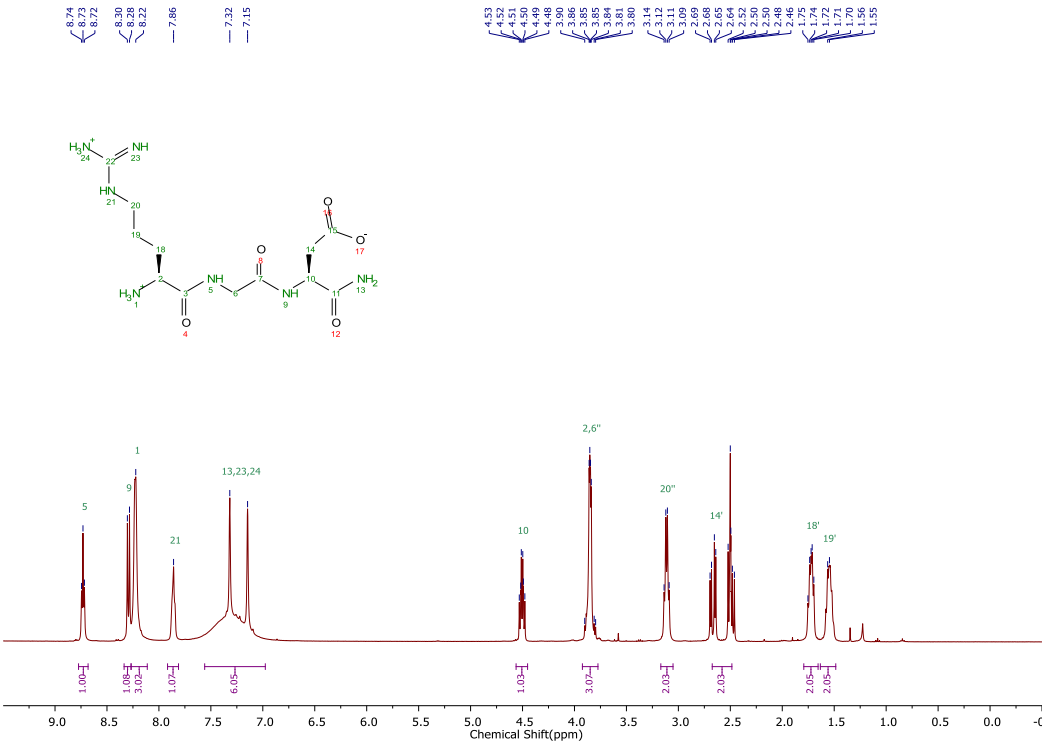
COSY spectrum of **8a** in DMSO-d_6.



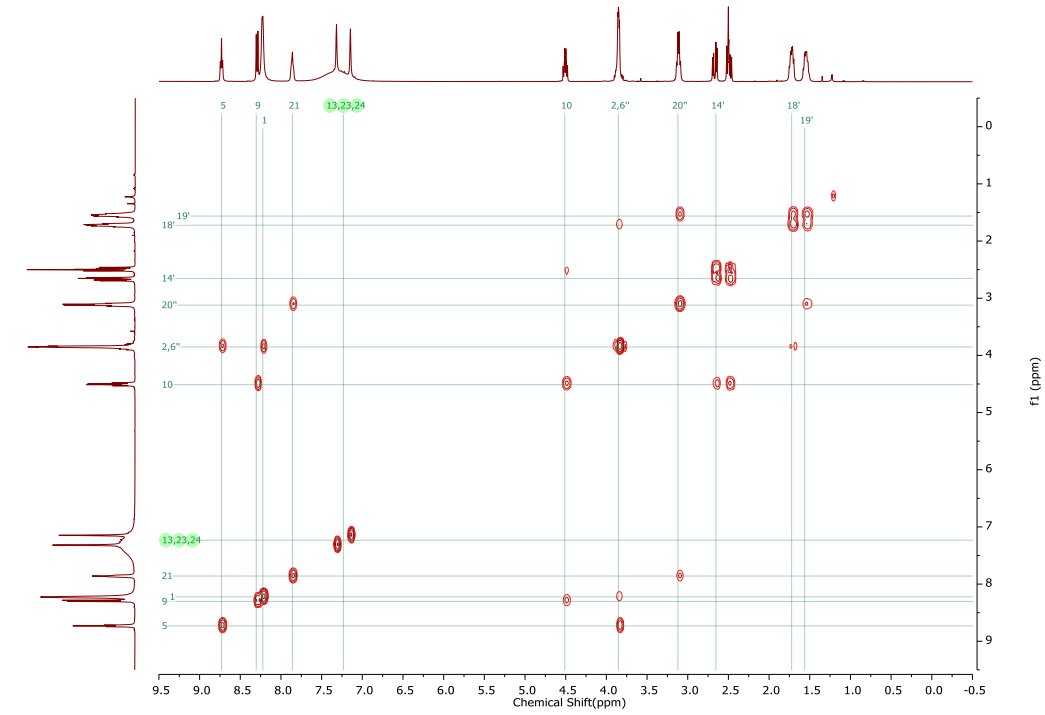
^{1}H-NMR spectrum of **8b** in DMSO-d_6**.**



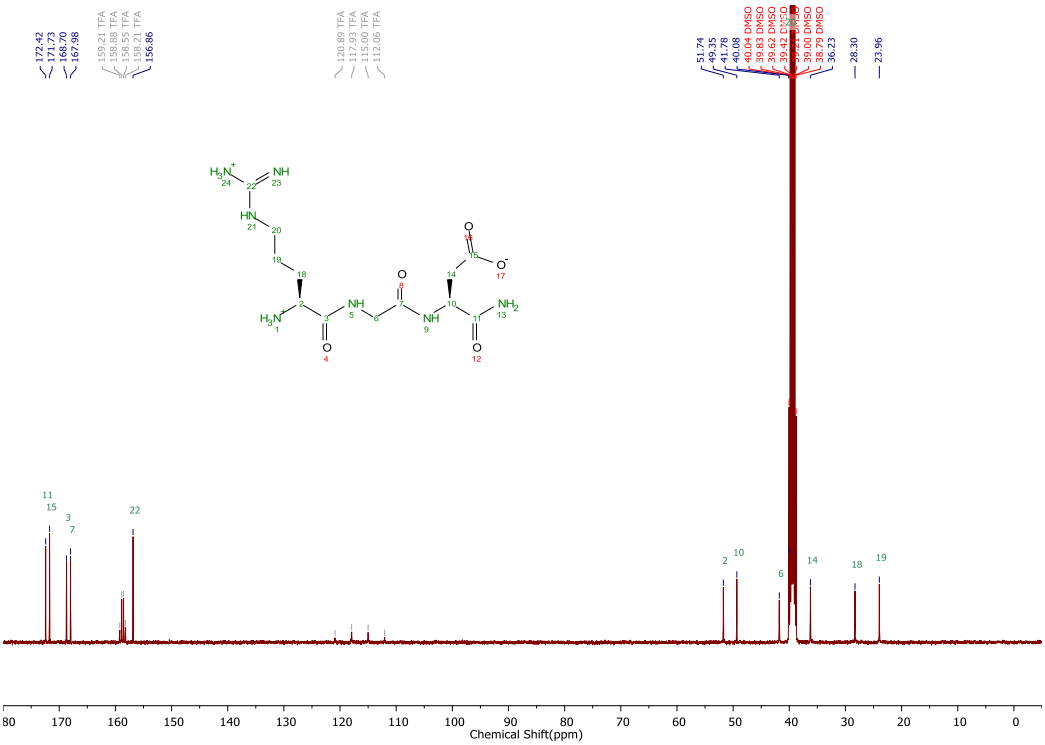
COSY spectrum of **8b** in DMSO-d_6**.**



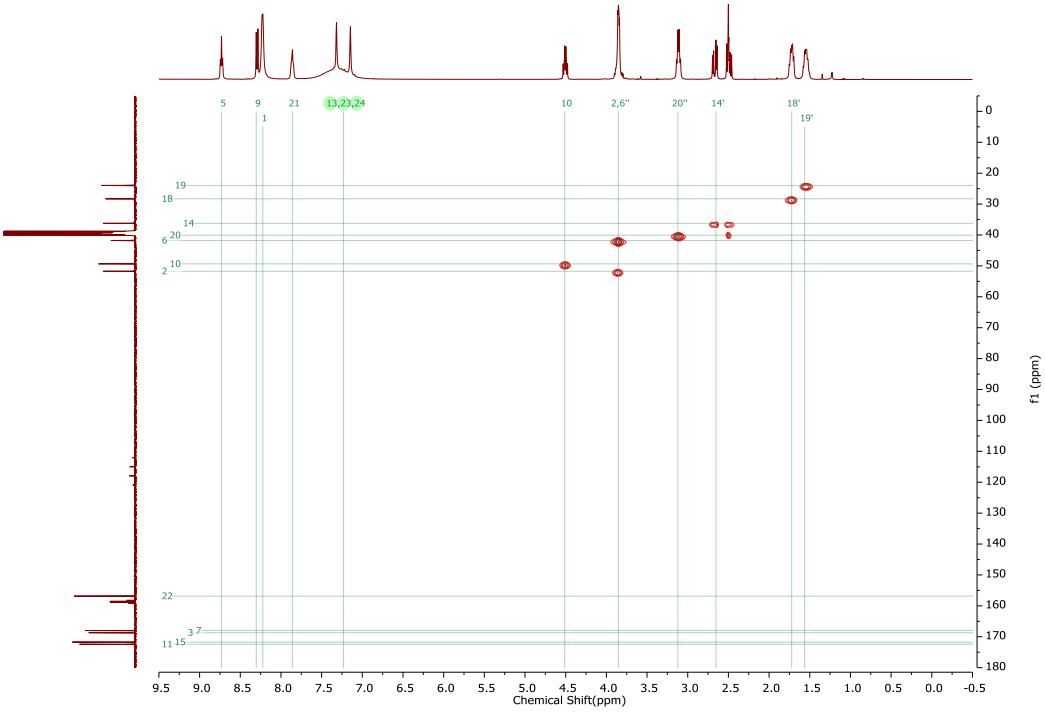
^{1}H-NMR spectrum of RGD peptide (**S3**) in DMSO-d_6**.**



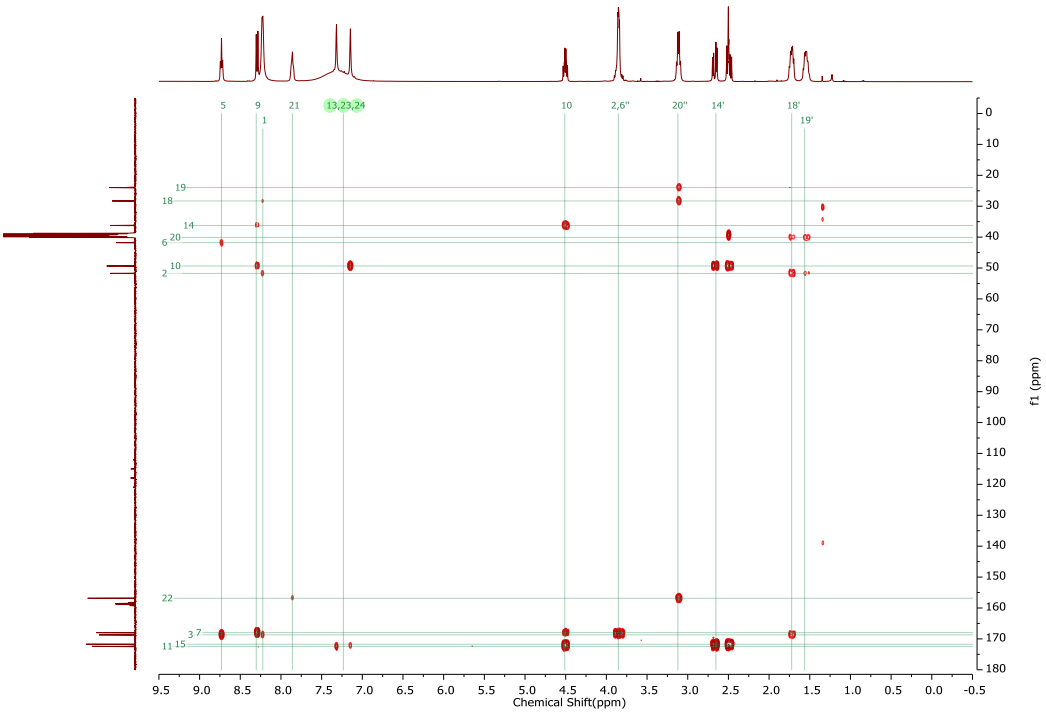
COSY spectrum of RGD peptide (**S3**) in DMSO-d_6**.**



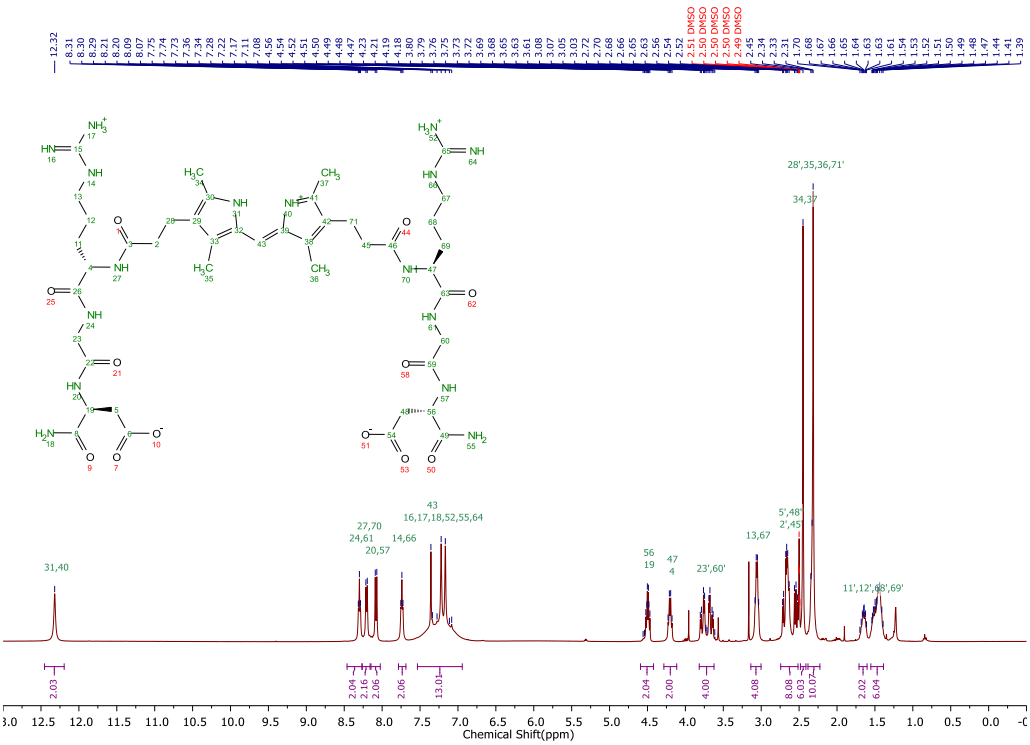
^{13}C-NMR spectrum of RGD peptide (**S3**) in DMSO-*d*$_6$**.**



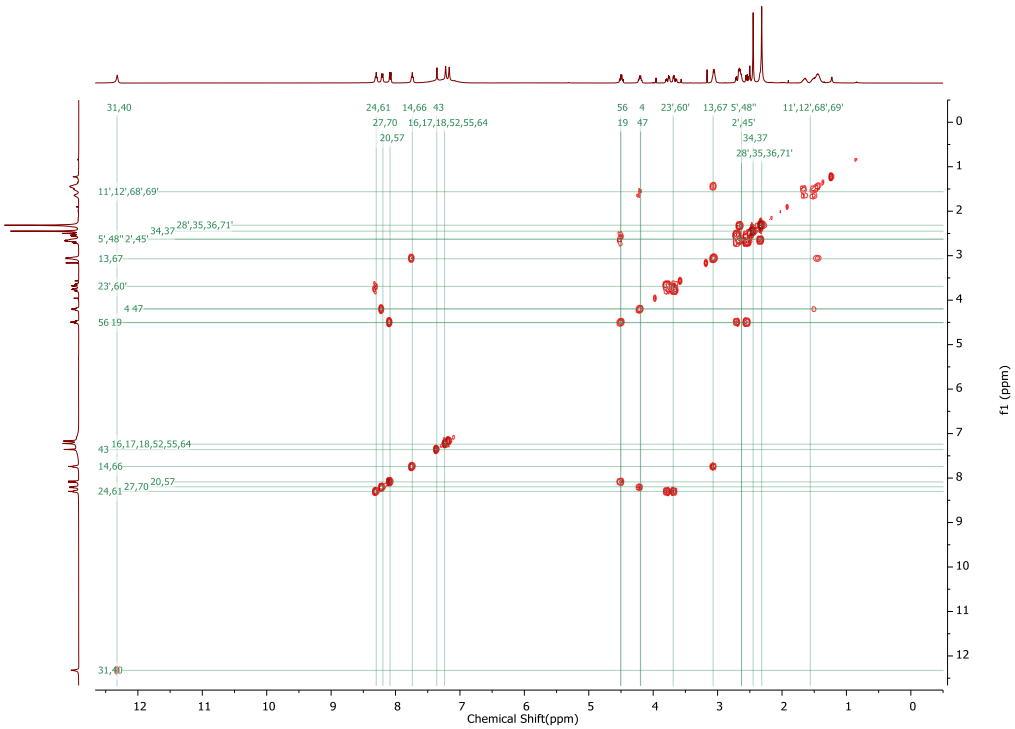
HSQC spectrum of RGD peptide (**S3**) in DMSO-*d*$_6$**.**



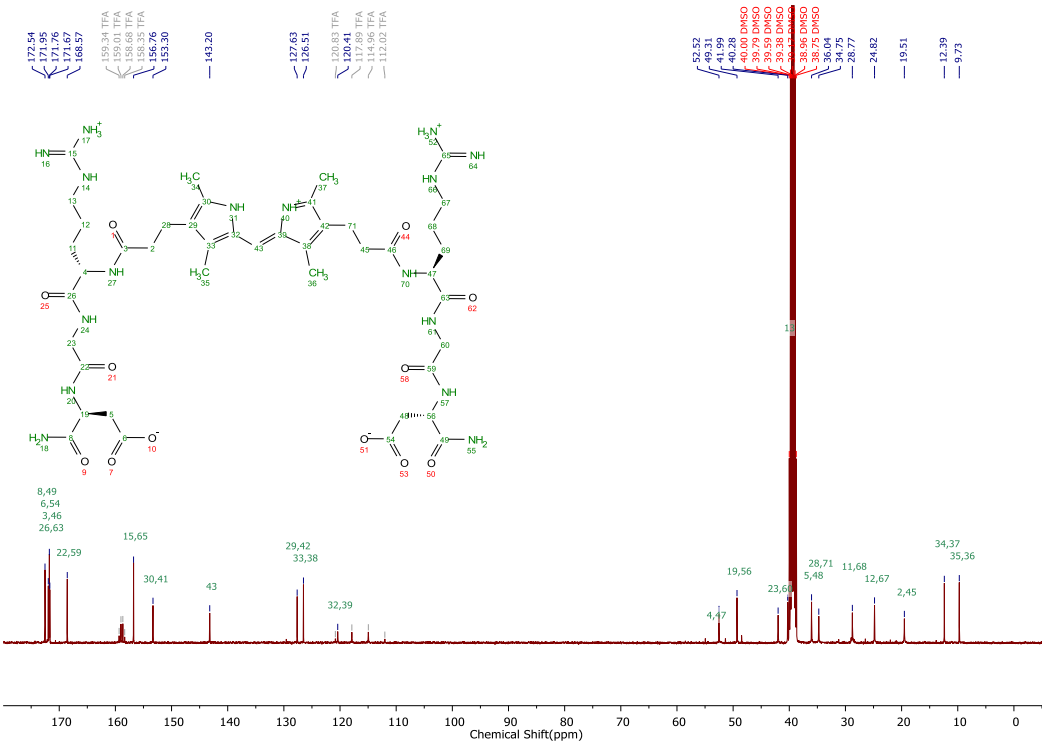
HMBC spectrum of RGD peptide (**S3**) in DMSO-d_6**.**



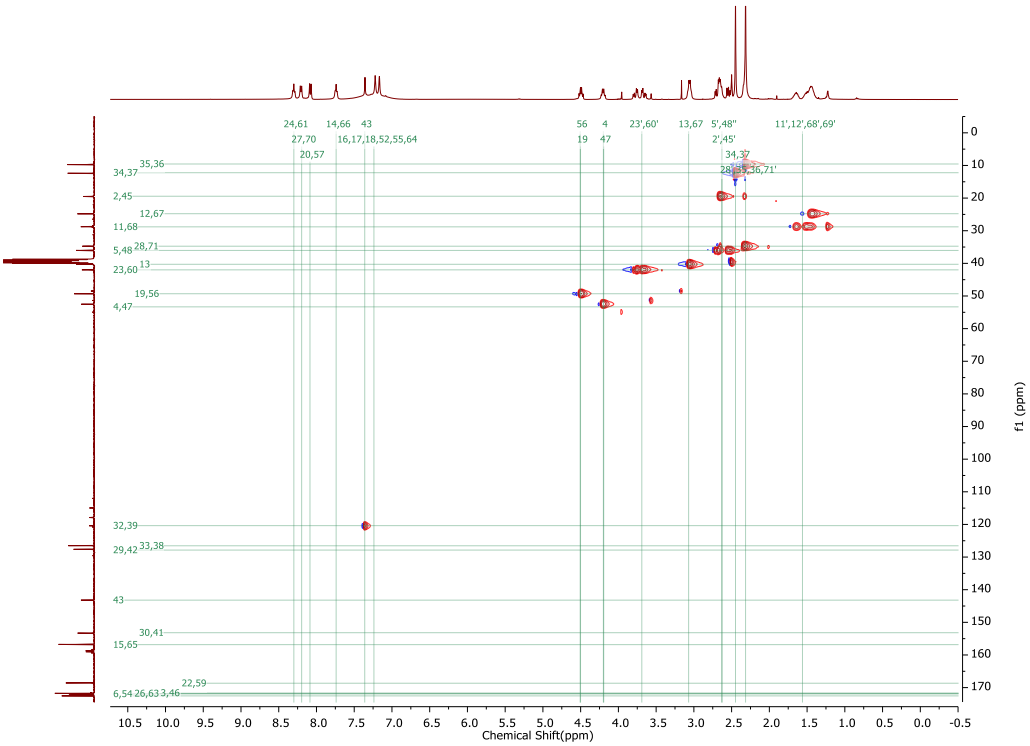
^{1}H-NMR spectrum of **10** in DMSO-*d*$_6$**.**



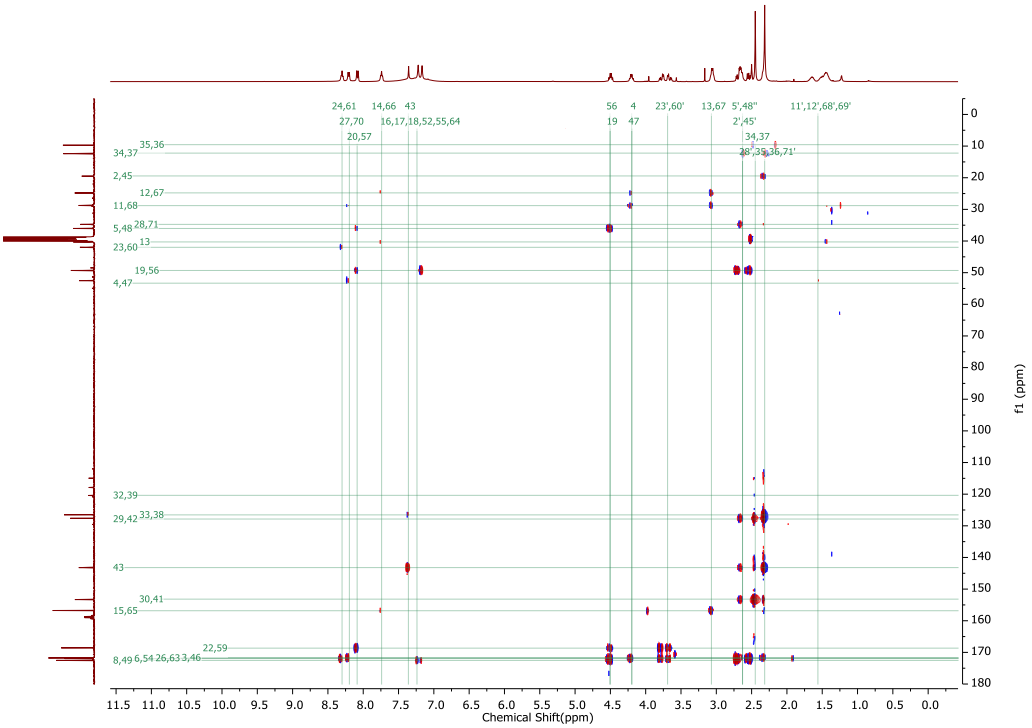
COSY spectrum of **10** in DMSO-*d*$_6$**.**



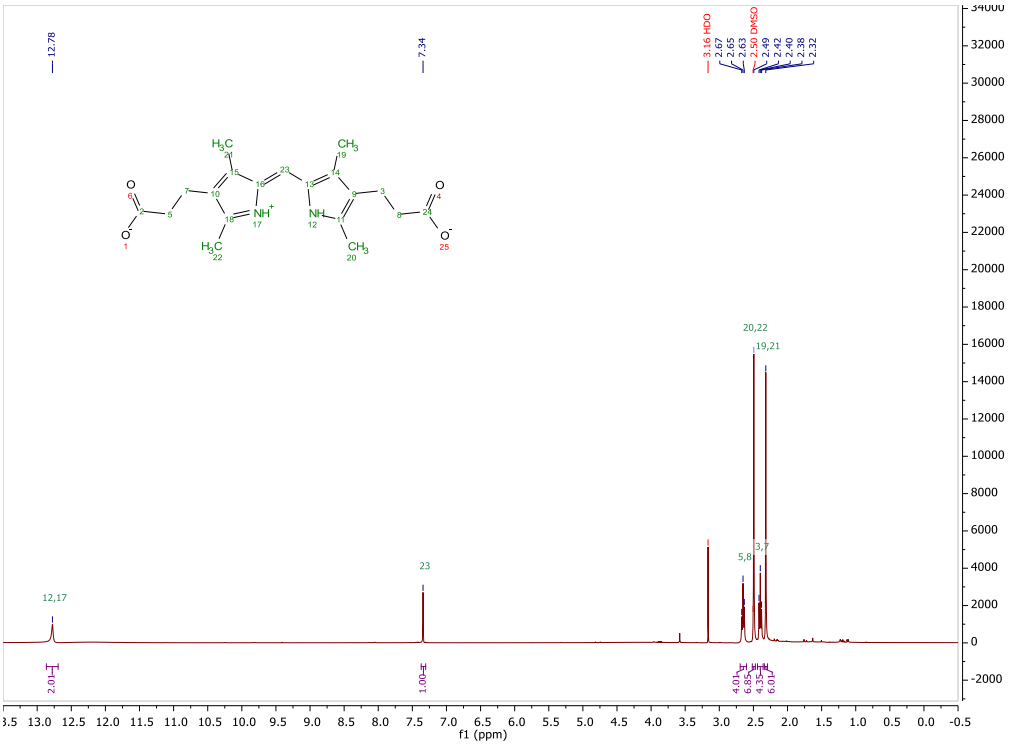
^{13}C-NMR spectrum of **10** in DMSO-d_6**.**



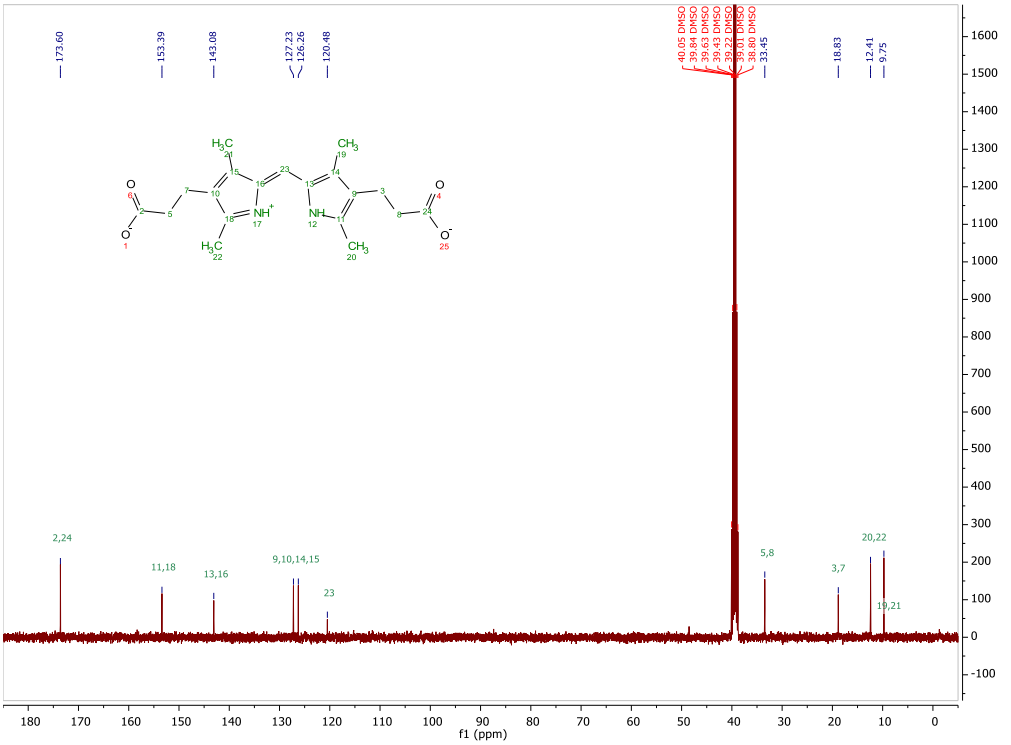
HSQC spectrum of **10** in DMSO-d_6**.**



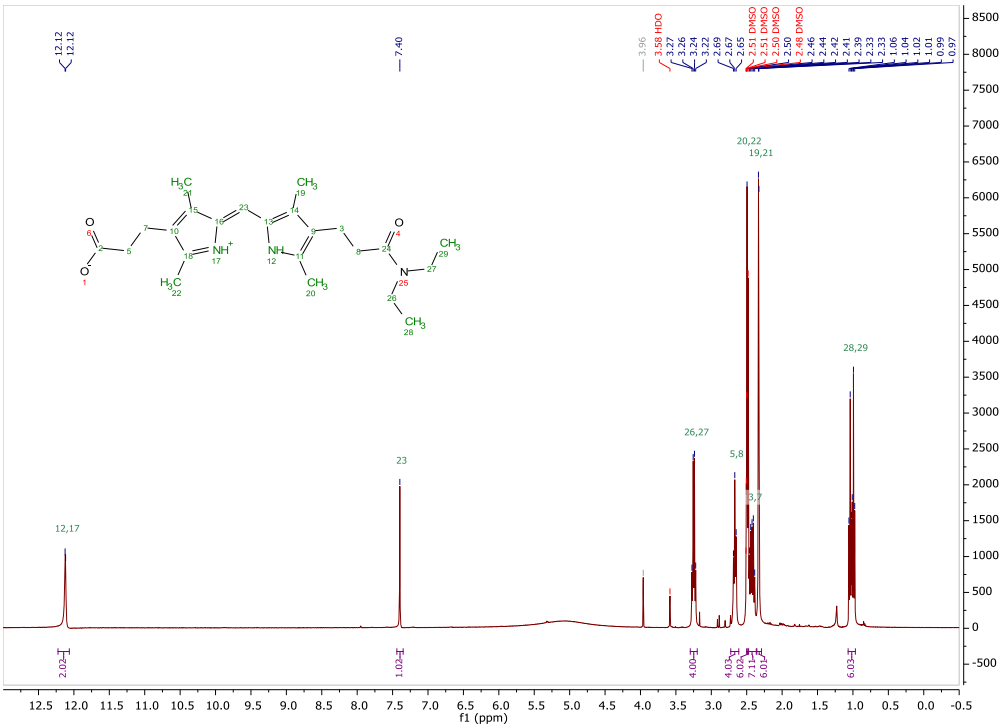
HMBC spectrum of **10** in DMSO-d_6.



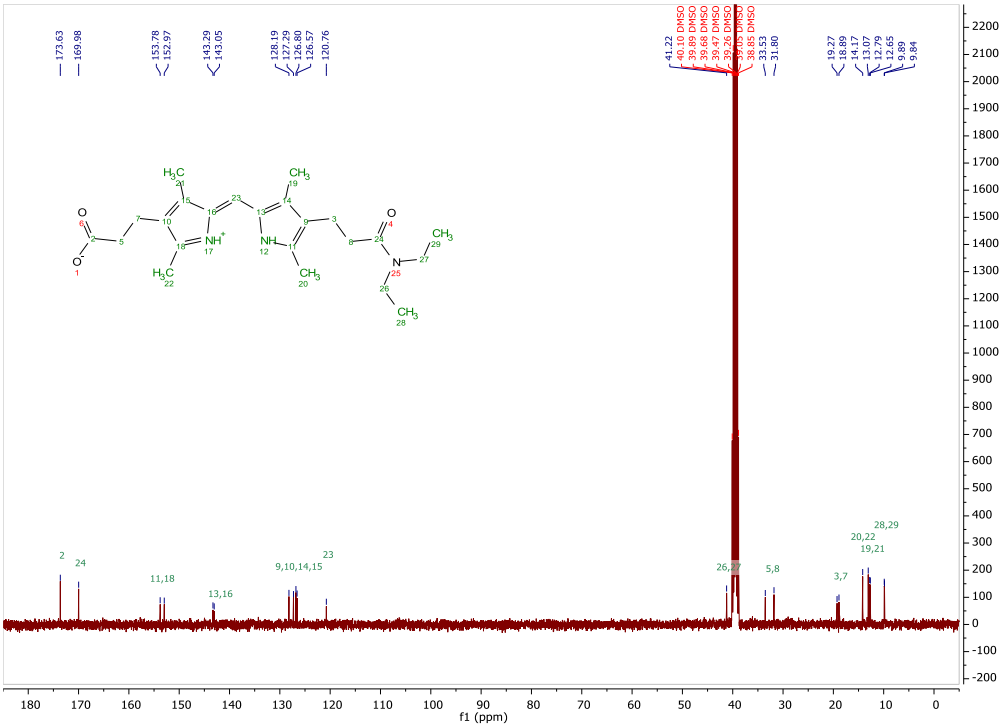
^{1}H-NMR spectrum of **S4** in DMSO-*d*$_6$**.**



^{13}C-NMR spectrum of **S4** in DMSO-*d*$_6$**.**

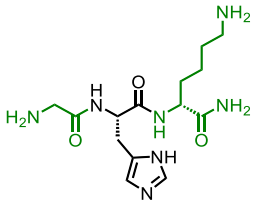


^{1}H-NMR spectrum of **S5** in DMSO-d_6**.**



^{13}C-NMR spectrum of **S5** in DMSO-d_6**.**

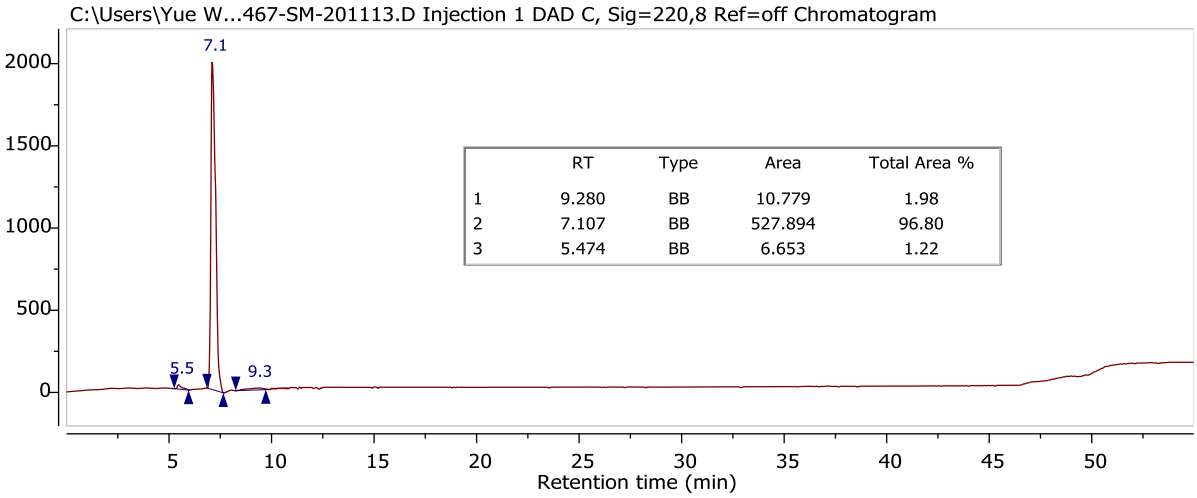
HPLC chromatograms and MALID-TOF HRMS spectra of products



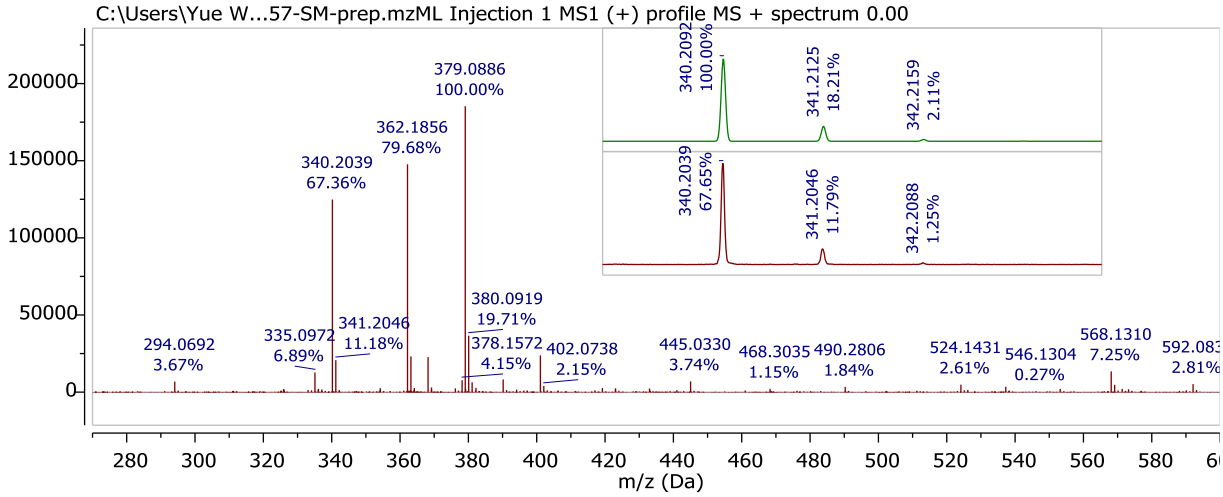
S1

Chemical Formula: $C_{14}H_{25}N_7O_3$

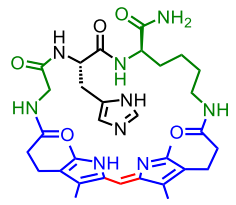
Exact Mass: 339.2019



Analytic HPLC of GHK peptide, **S1 (**Gradient A, 220 nm).



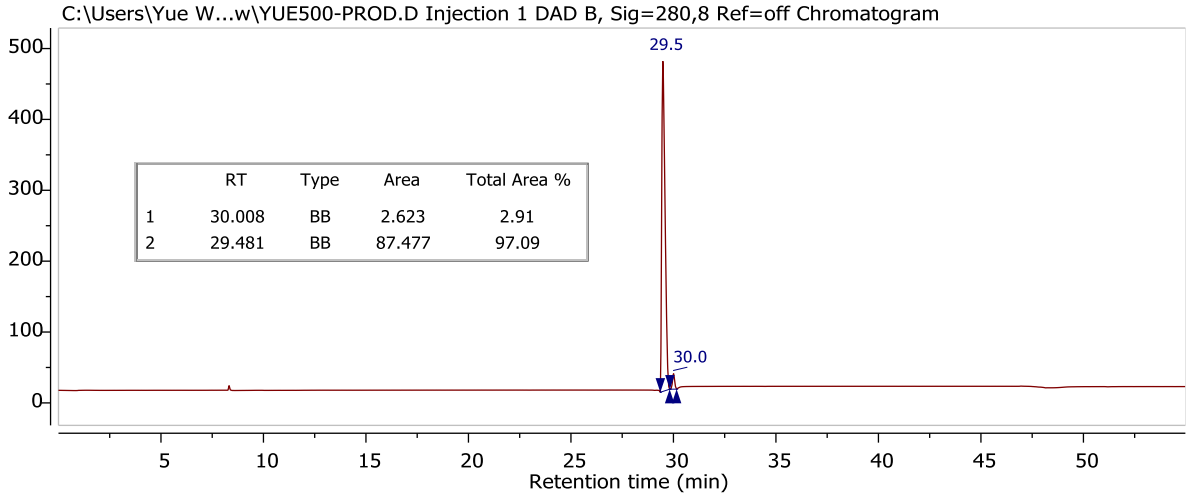
HRMS(MAIDL-TOF) of GHK peptide, **S1**.



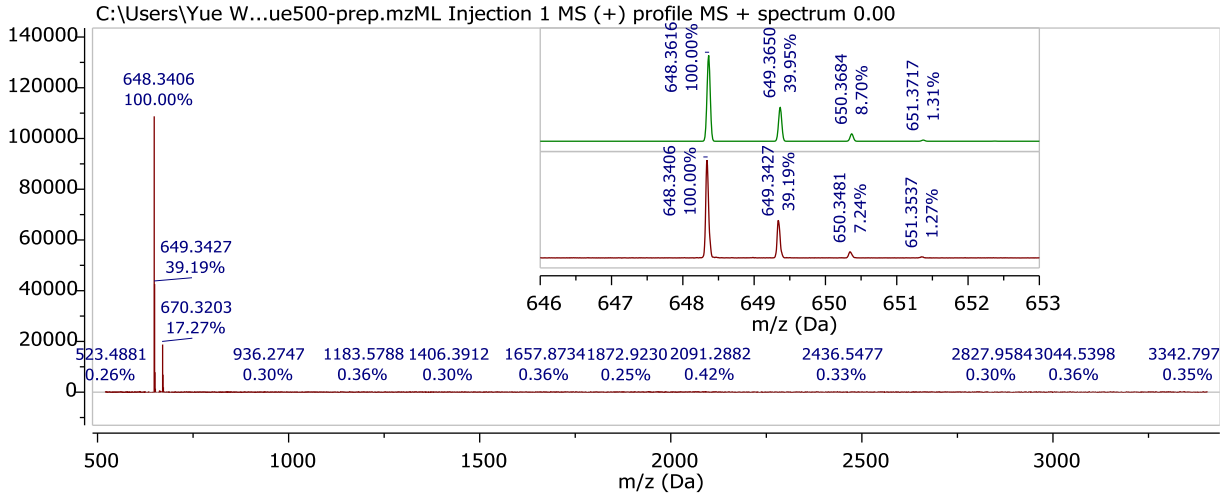
1a

Chemical Formula: $C_{33}H_{45}N_9O_5$

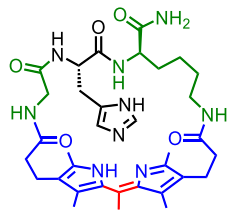
Exact Mass: 647.3544



Analytic HPLC of **1a (**Gradient A, 280 nm).



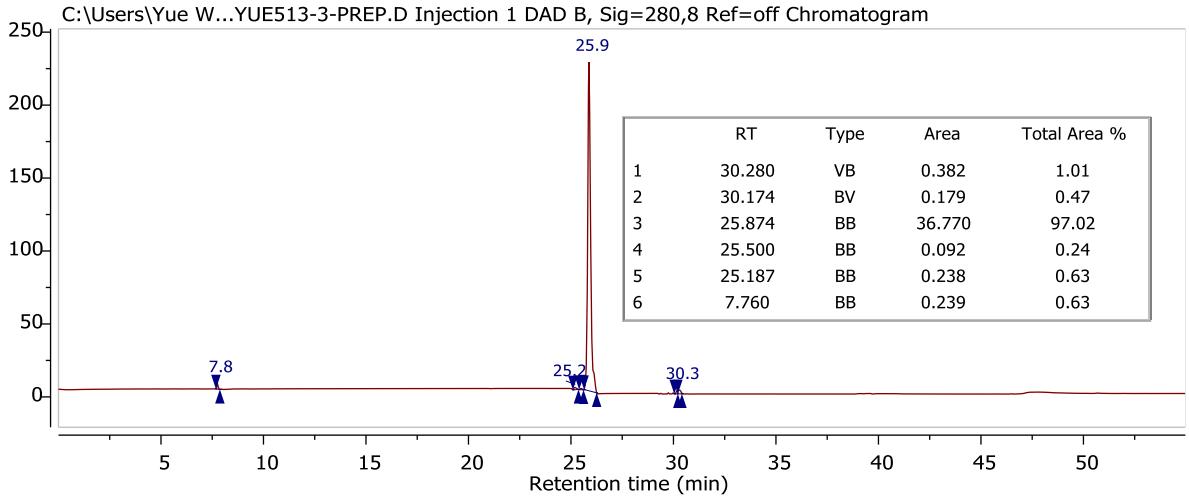
HRMS(MAIDL-TOF) of **1a**.



1b

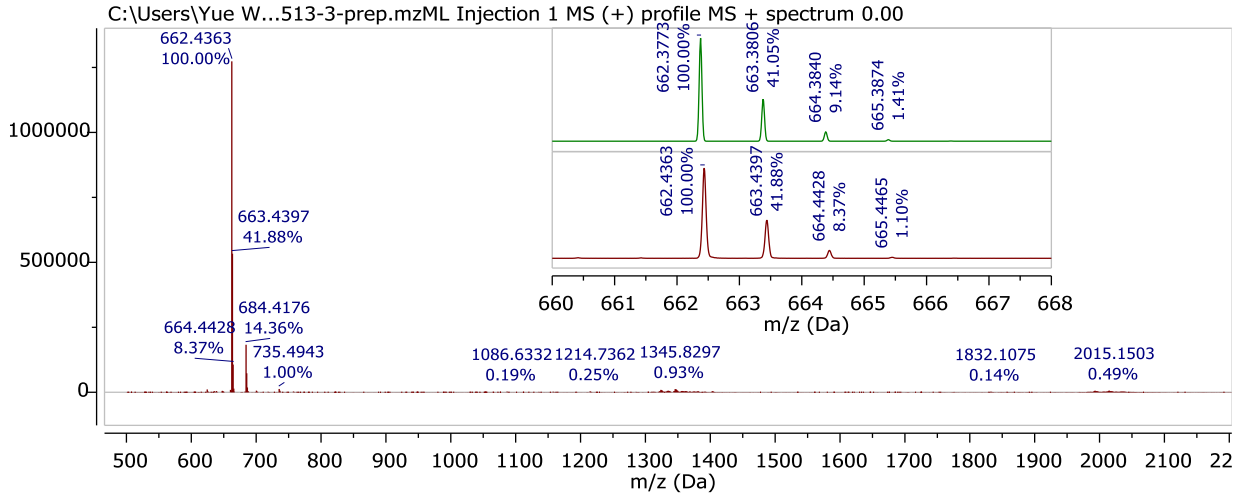
Chemical Formula: $C_{34}H_{47}N_9O_5$

Exact Mass: 661.3700

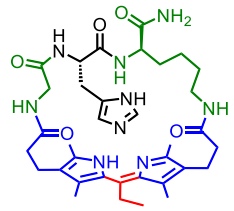


	RT	Type	Area	Total Area %
1	30.280	VB	0.382	1.01
2	30.174	BV	0.179	0.47
3	25.874	BB	36.770	97.02
4	25.500	BB	0.092	0.24
5	25.187	BB	0.238	0.63
6	7.760	BB	0.239	0.63

Analytic HPLC of **1b (**Gradient A, 280 nm).



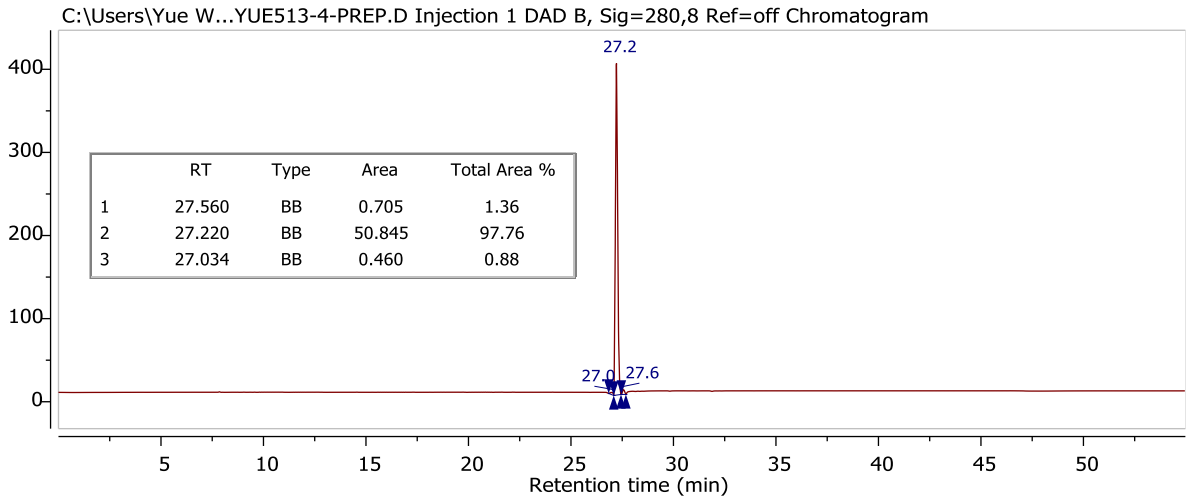
HRMS(MAIDL-TOF) of **1b**.



1c

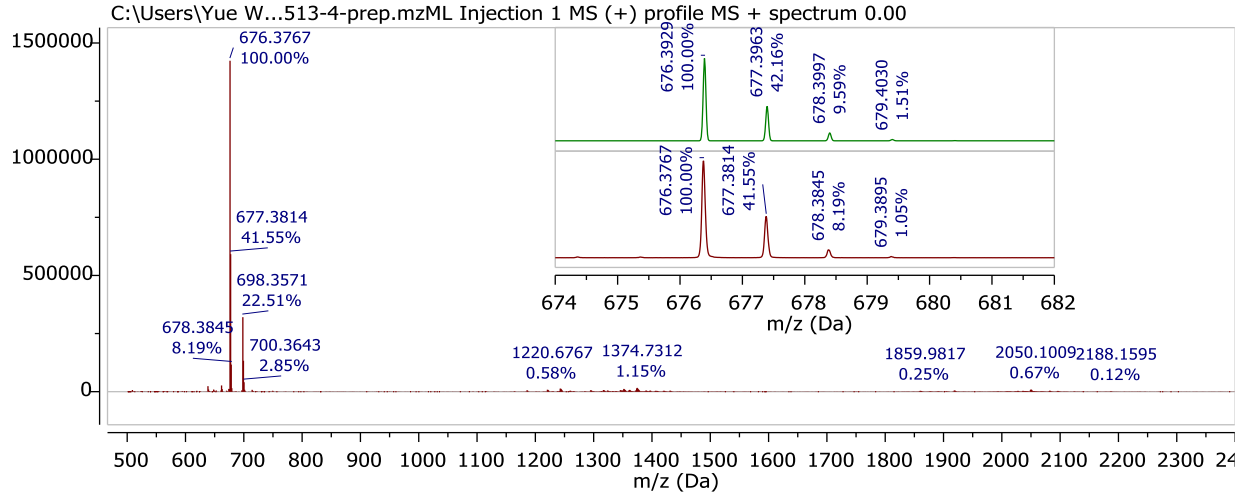
Chemical Formula: $C_{35}H_{49}N_9O_5$

Exact Mass: 675.3857

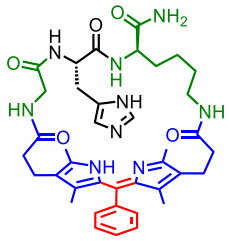


	RT	Type	Area	Total Area %
1	27.560	BB	0.705	1.36
2	27.220	BB	50.845	97.76
3	27.034	BB	0.460	0.88

Analytic HPLC of **1c (**Gradient A, 280 nm).



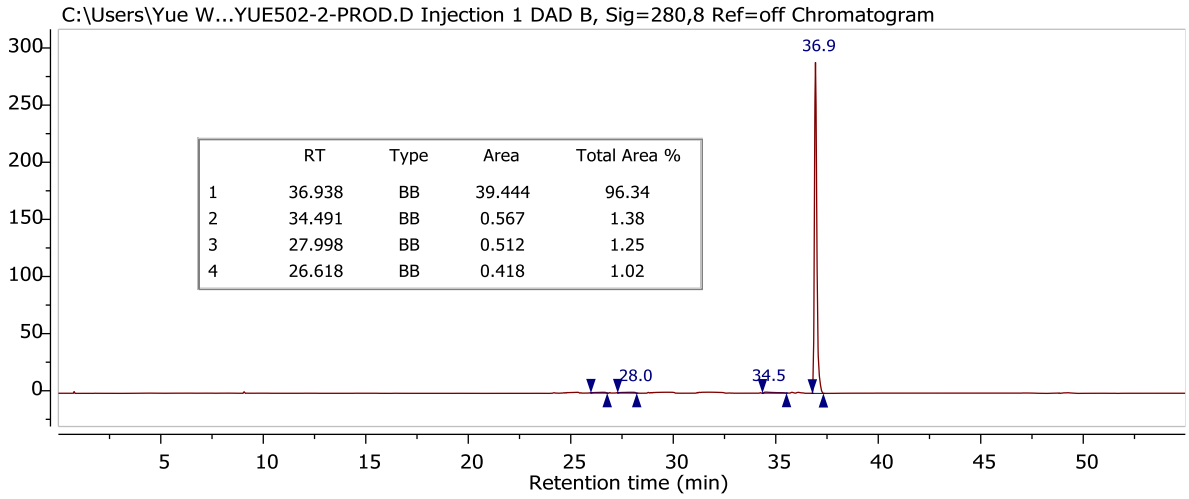
HRMS(MAIDL-TOF) of **1c**.



1d

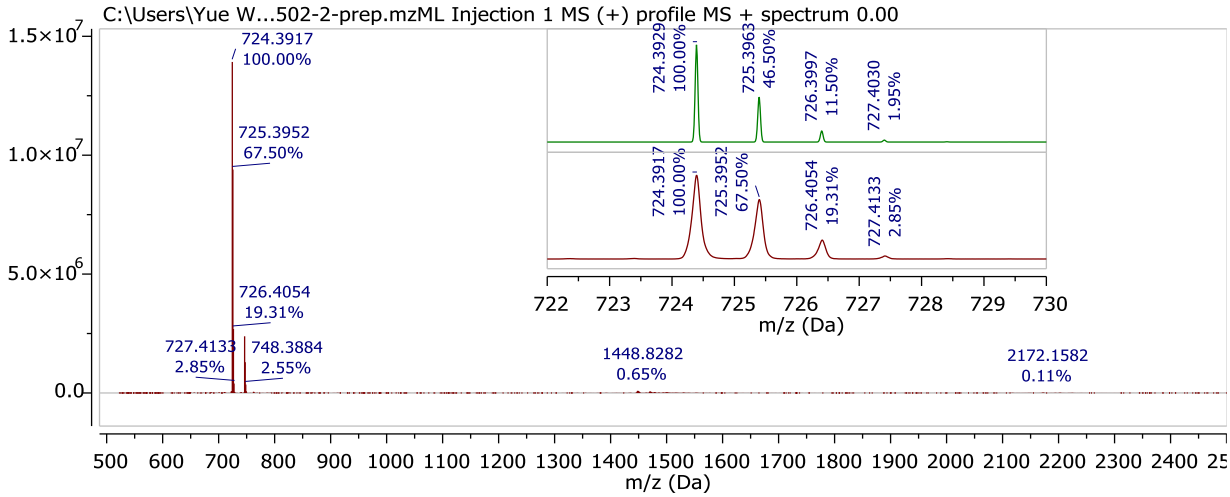
Chemical Formula: $C_{39}H_{49}N_9O_5$

Exact Mass: 723.3857

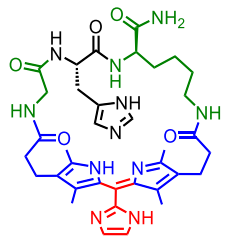


	RT	Type	Area	Total Area %
1	36.938	BB	39.444	96.34
2	34.491	BB	0.567	1.38
3	27.998	BB	0.512	1.25
4	26.618	BB	0.418	1.02

Analytic HPLC of **1d** (Gradient A, 280 nm).



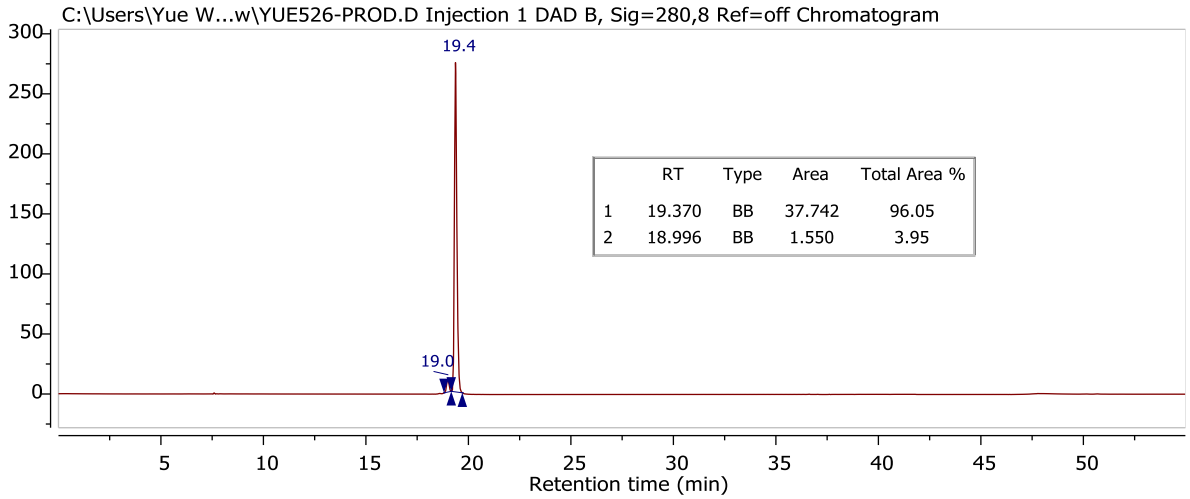
HRMS(MAIDL-TOF) of **1d**.



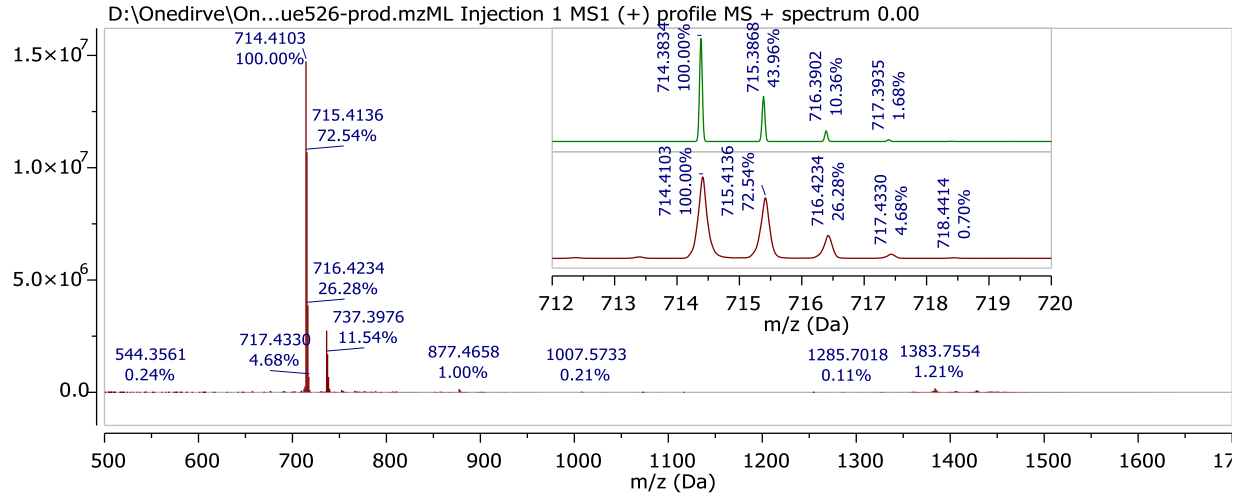
1e

Chemical Formula: $C_{36}H_{47}N_{11}O_5$

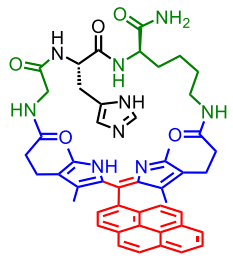
Exact Mass: 713.3762



Analytic HPLC of **1e** (Gradient A, 280 nm).



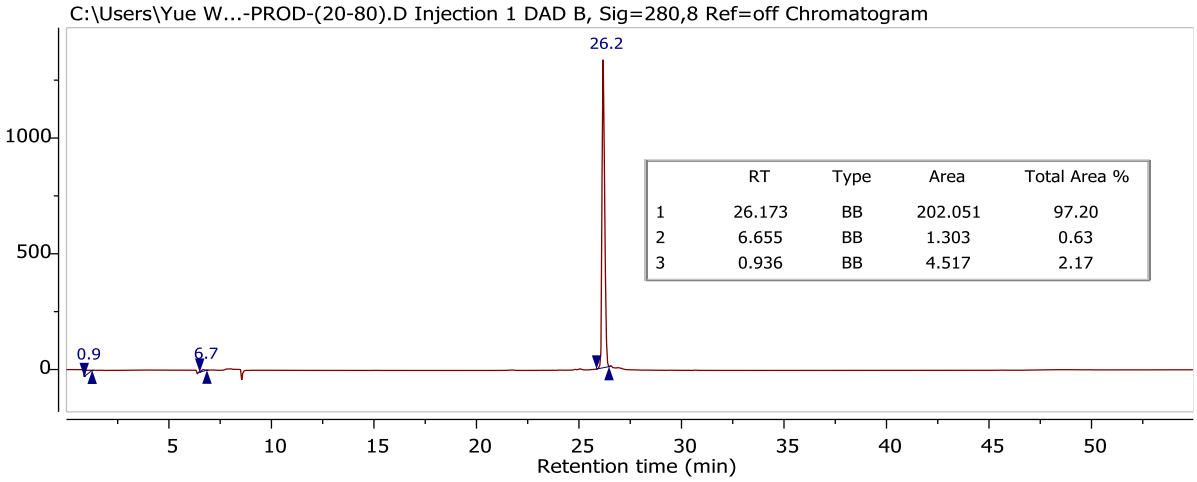
HRMS(MAIDL-TOF) of **1e**.



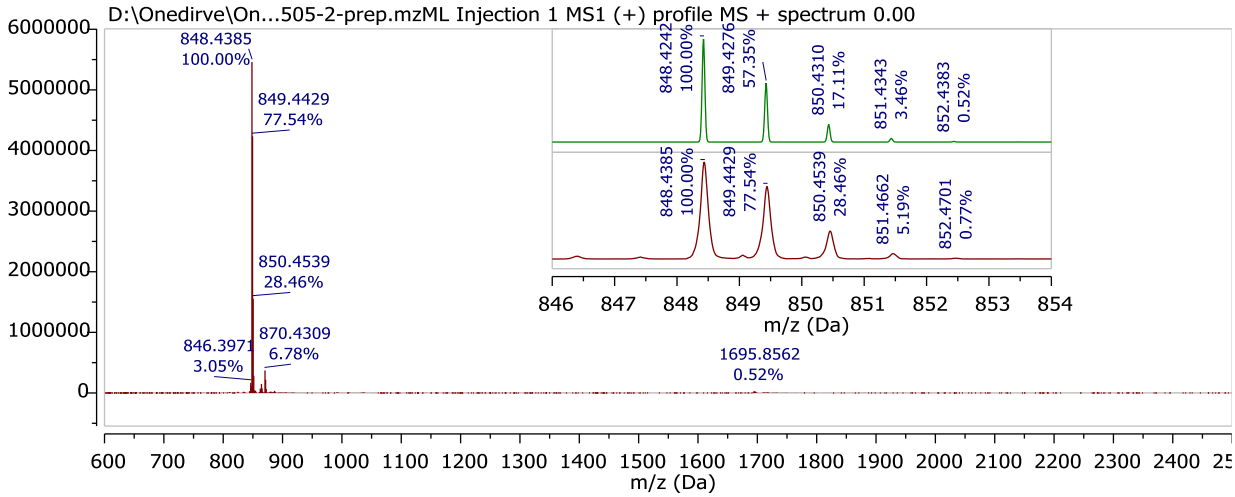
1f

Chemical Formula: $C_{49}H_{53}N_9O_5$

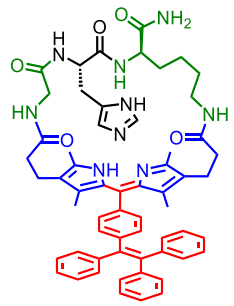
Exact Mass: 847.4170



Analytic HPLC of **1f (**Gradient B, 280 nm).



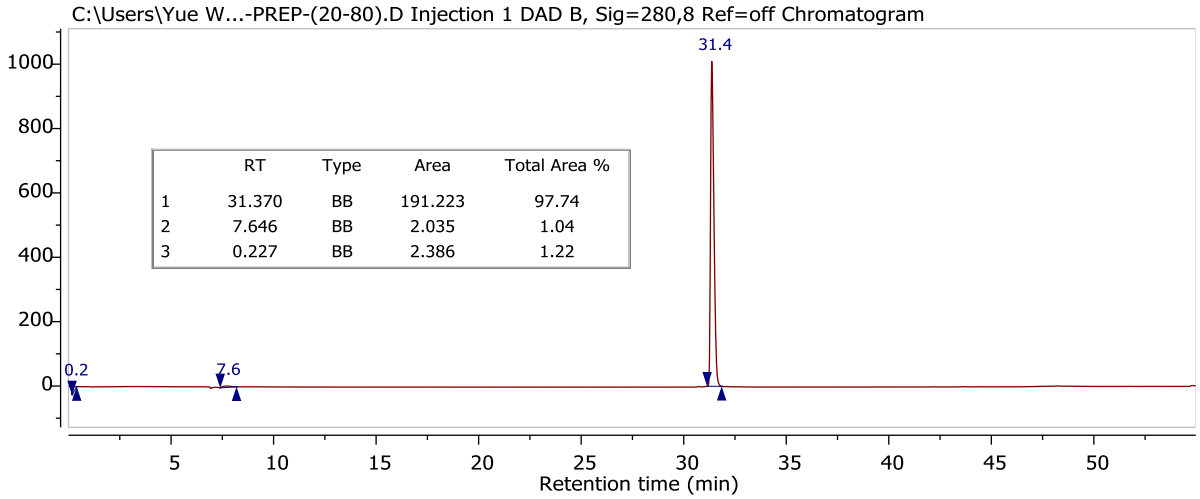
HRMS(MAIDL-TOF) of **1f**.



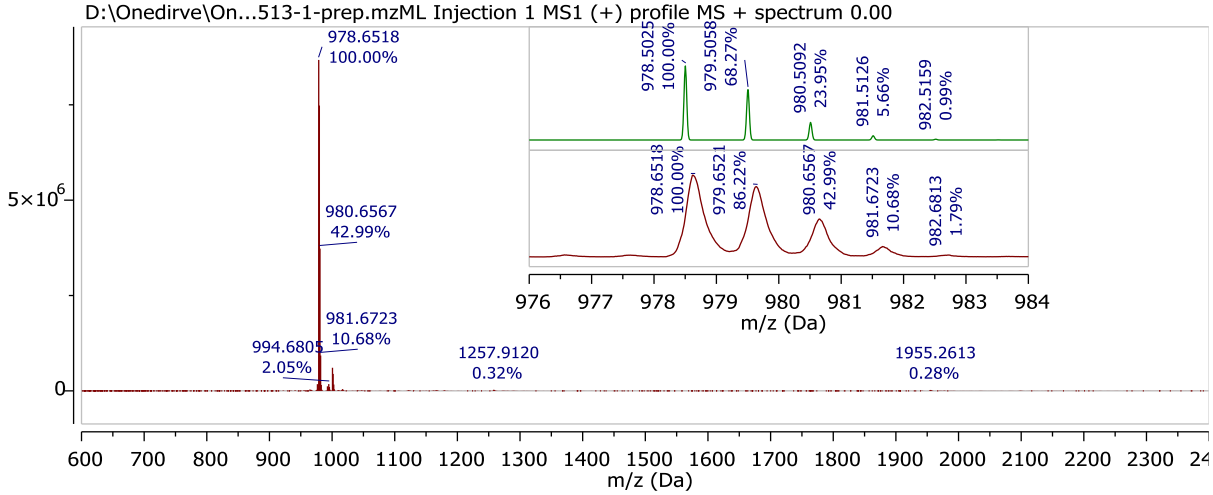
1g

Chemical Formula: $C_{59}H_{63}N_9O_5$

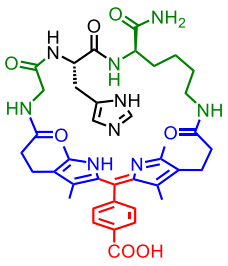
Exact Mass: 977.4952



Analytic HPLC of **1g (**Gradient B, 280 nm).



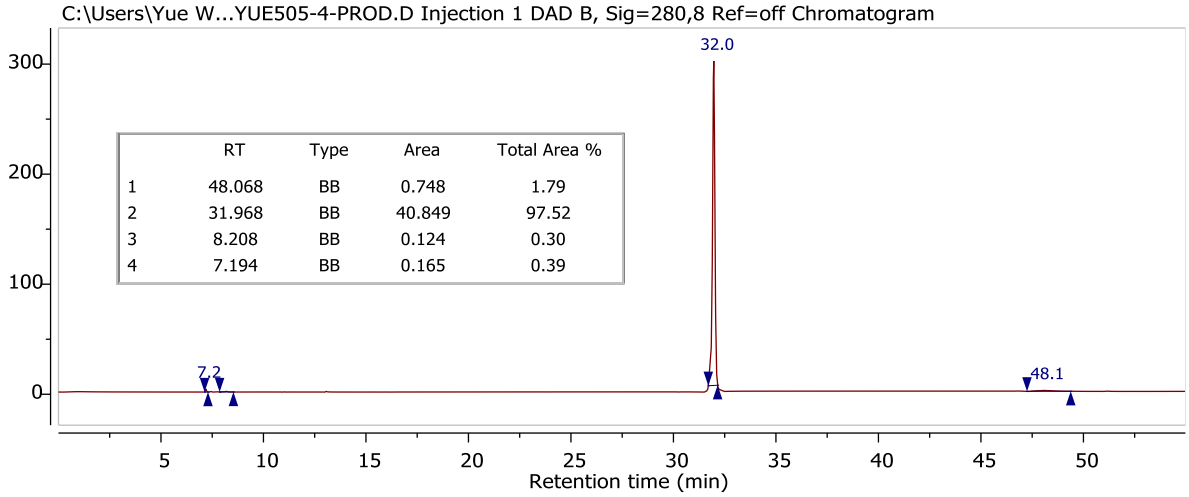
HRMS(MAIDL-TOF) of **1g**.



1h

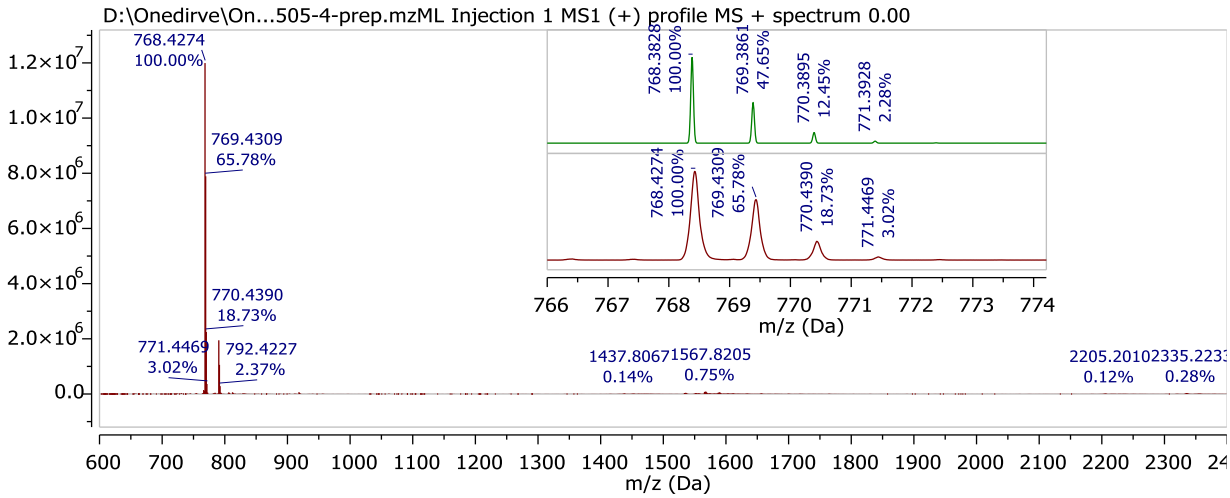
Chemical Formula: $C_{40}H_{49}N_9O_7$

Exact Mass: 767.3755

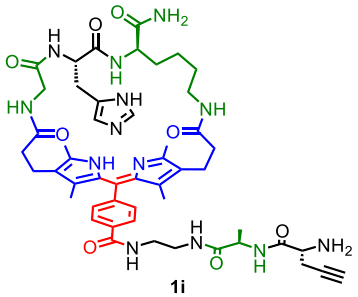


	RT	Type	Area	Total Area %
1	48.068	BB	0.748	1.79
2	31.968	BB	40.849	97.52
3	8.208	BB	0.124	0.30
4	7.194	BB	0.165	0.39

Analytic HPLC of **1h** (Gradient A, 280 nm).



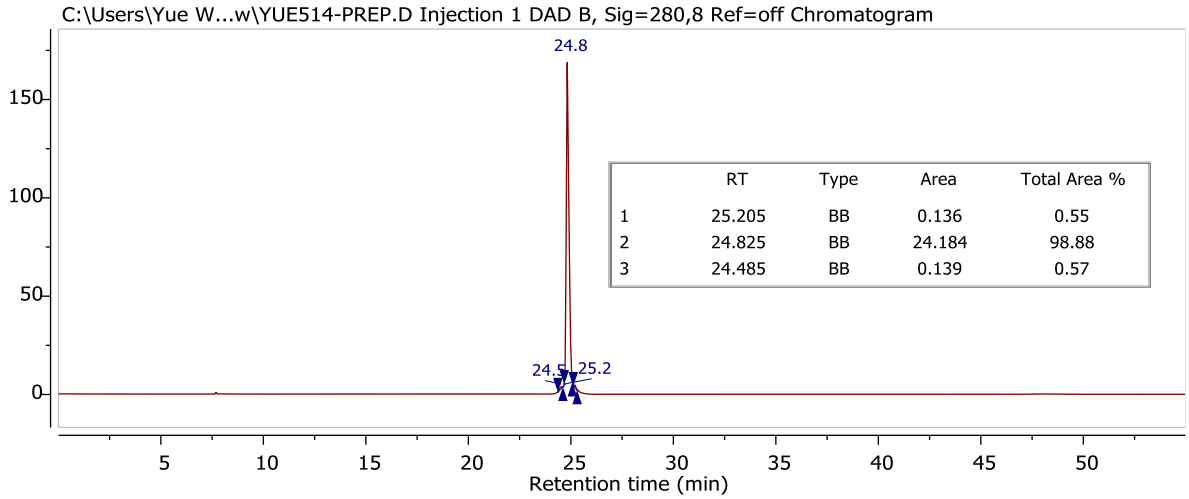
HRMS(MAIDL-TOF) of **1h**.



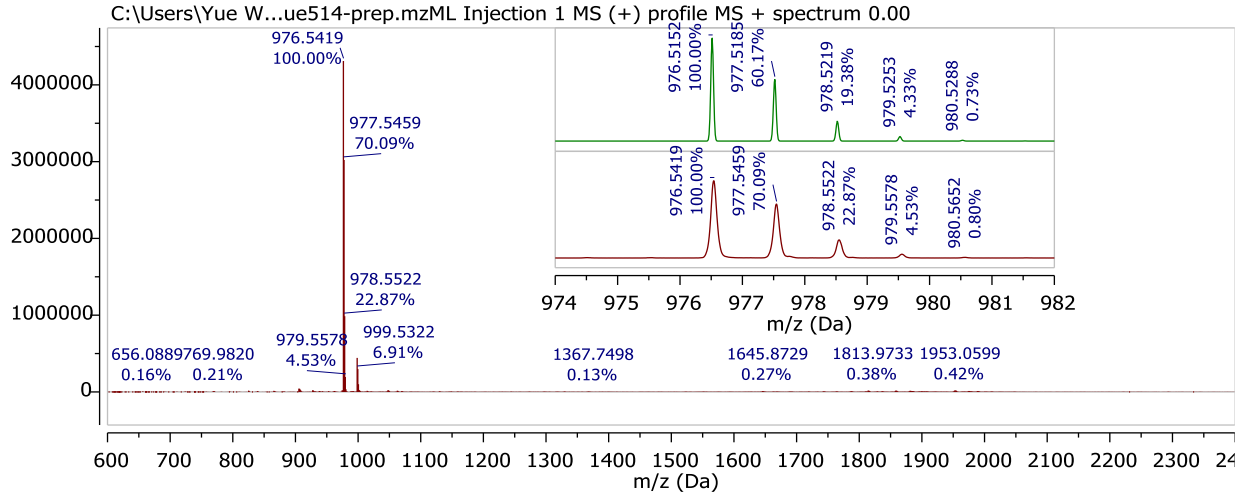
1i

Chemical Formula: $C_{50}H_{65}N_{13}O_8$

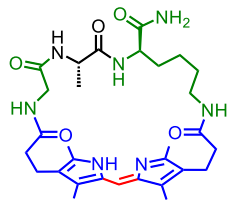
Exact Mass: 975.5079



Analytic HPLC of **1i** (Gradient A, 280 nm).

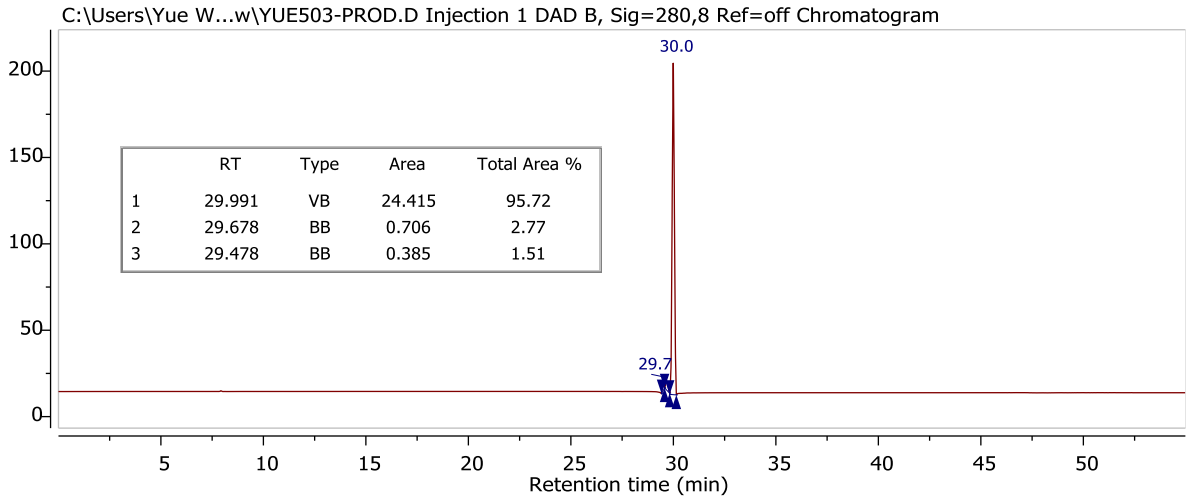


HRMS(MAIDL-TOF) of **1i**.

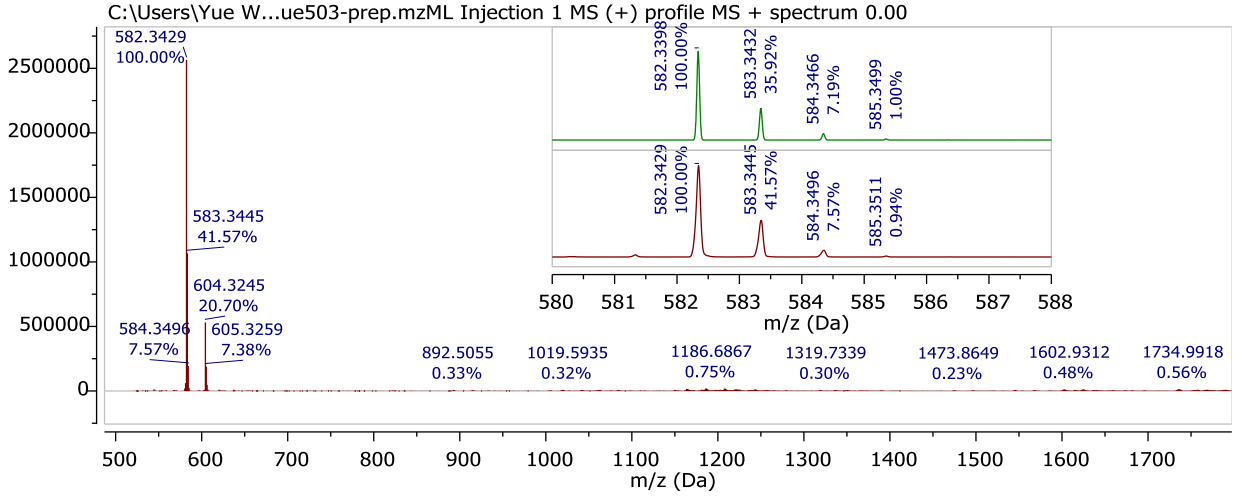


2

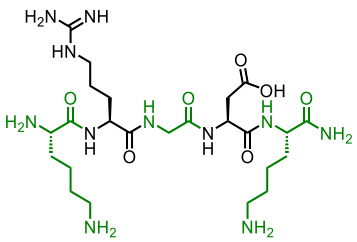
Chemical Formula: $C_{30}H_{43}N_7O_5$
Exact Mass: 581.3326



Analytic HPLC of **2** (Gradient A, 280 nm).



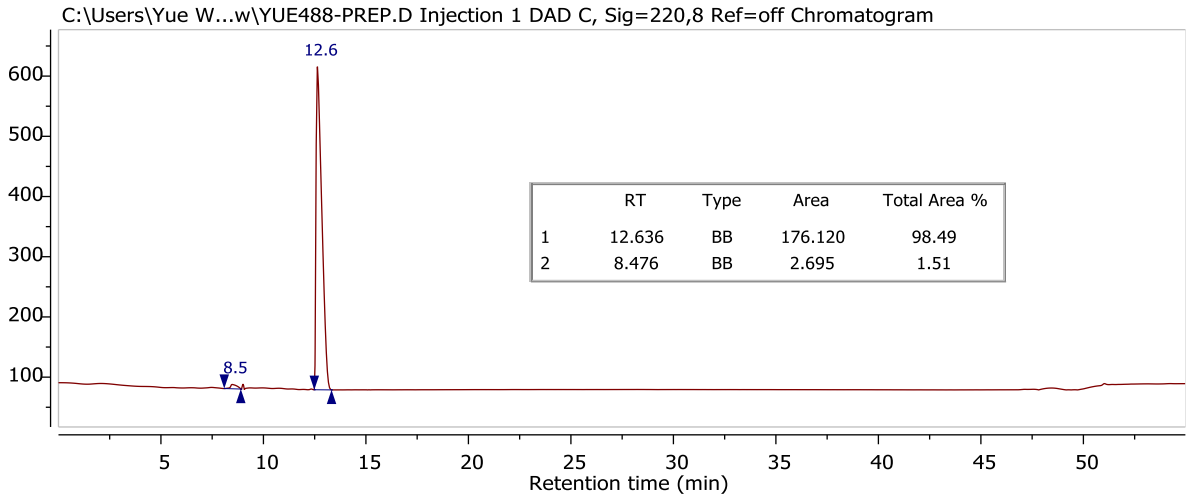
HRMS(MAIDL-TOF) of **2**.



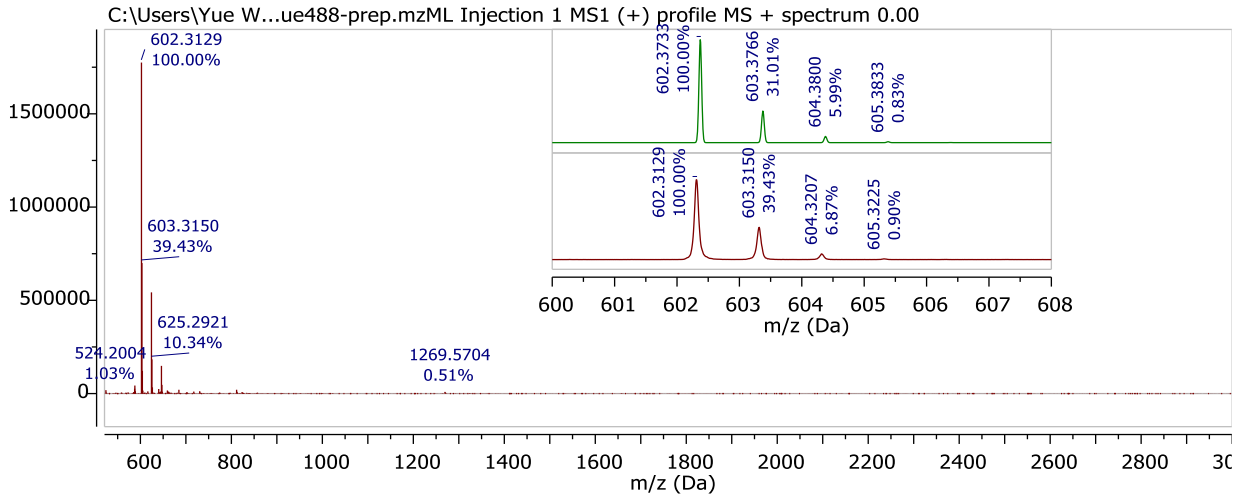
3a

Chemical Formula: $C_{24}H_{47}N_{11}O_7$

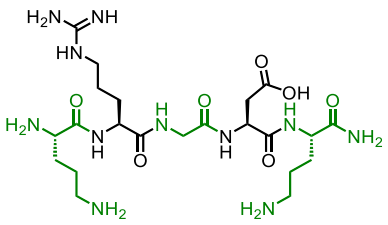
Exact Mass: 601.3660



Analytic HPLC of **3a (**Gradient A, 220 nm).



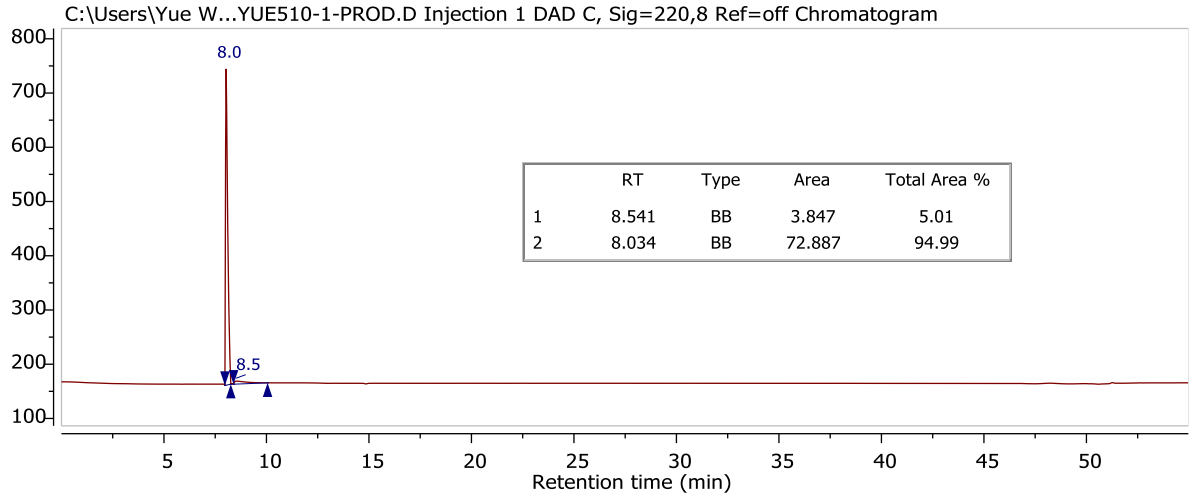
HRMS(MAIDL-TOF) of **3a**.



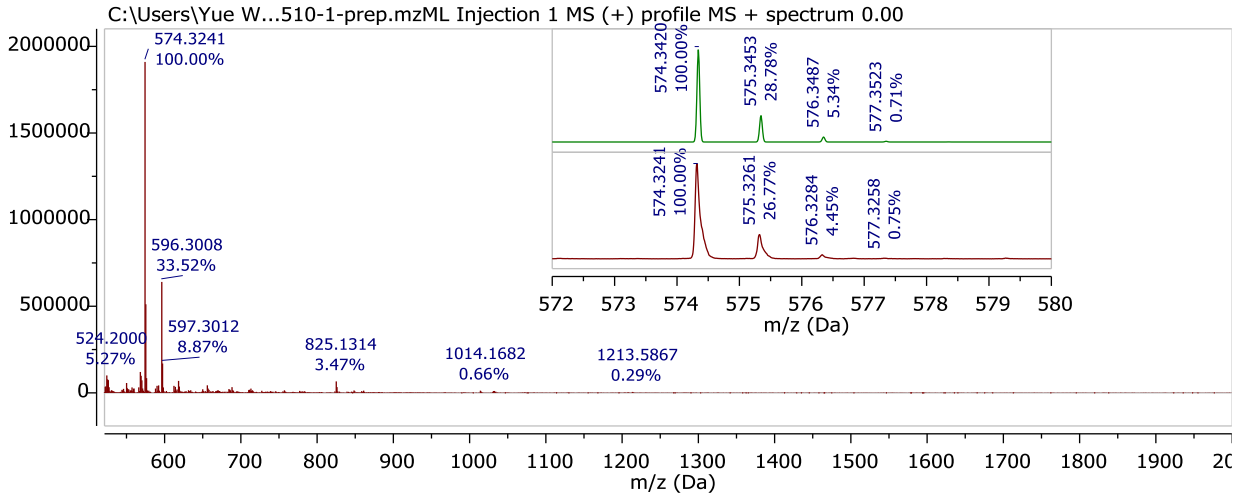
3b

Chemical Formula: $C_{22}H_{43}N_{11}O_7$

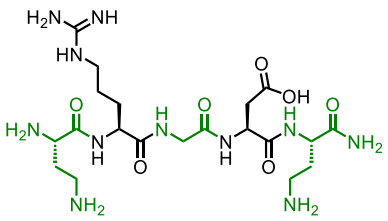
Exact Mass: 573.3347



Analytic HPLC of **3b (**Gradient A, 220 nm).



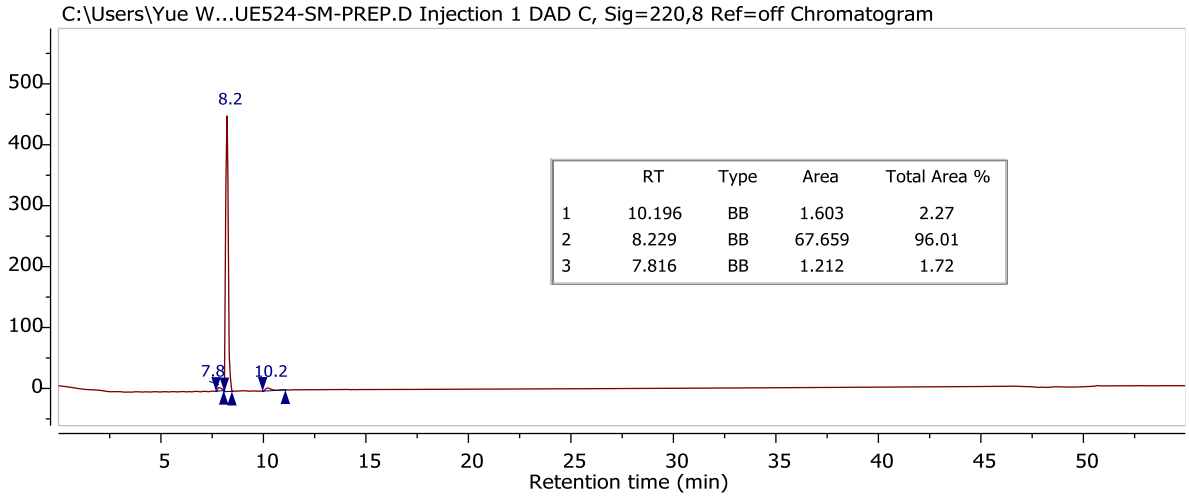
HRMS(MAIDL-TOF) of **3b**.



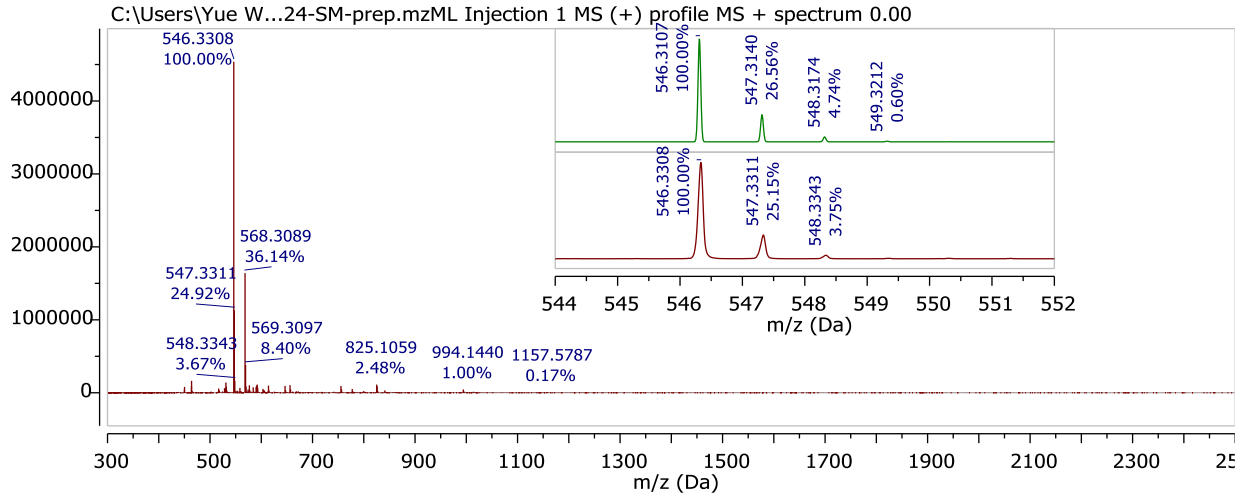
3c

Chemical Formula: $C_{20}H_{39}N_{11}O_7$

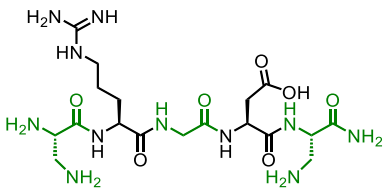
Exact Mass: 545.3034



Analytic HPLC of **3c (**Gradient A, 220 nm).



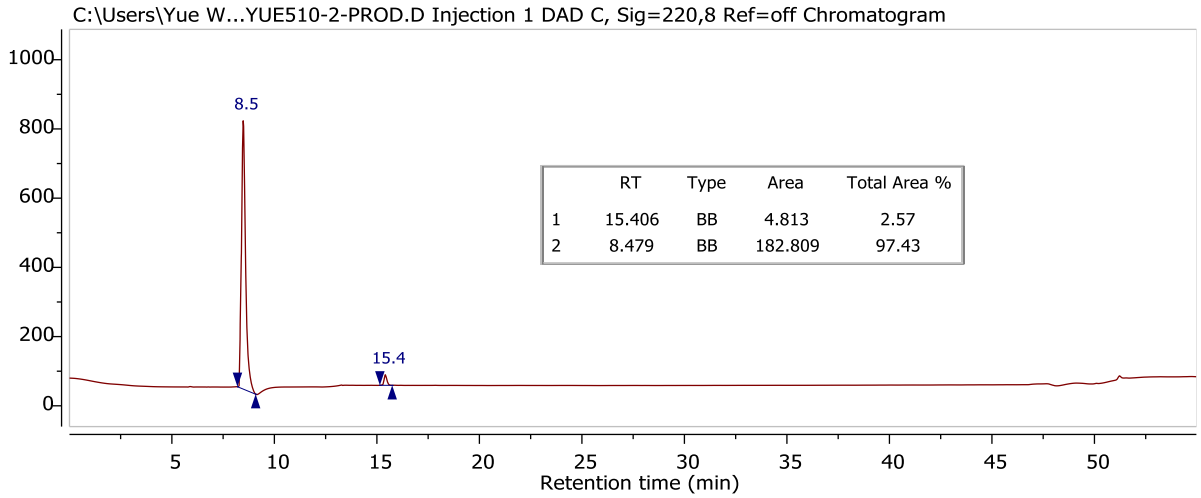
HRMS(MAIDL-TOF) of **3c**.



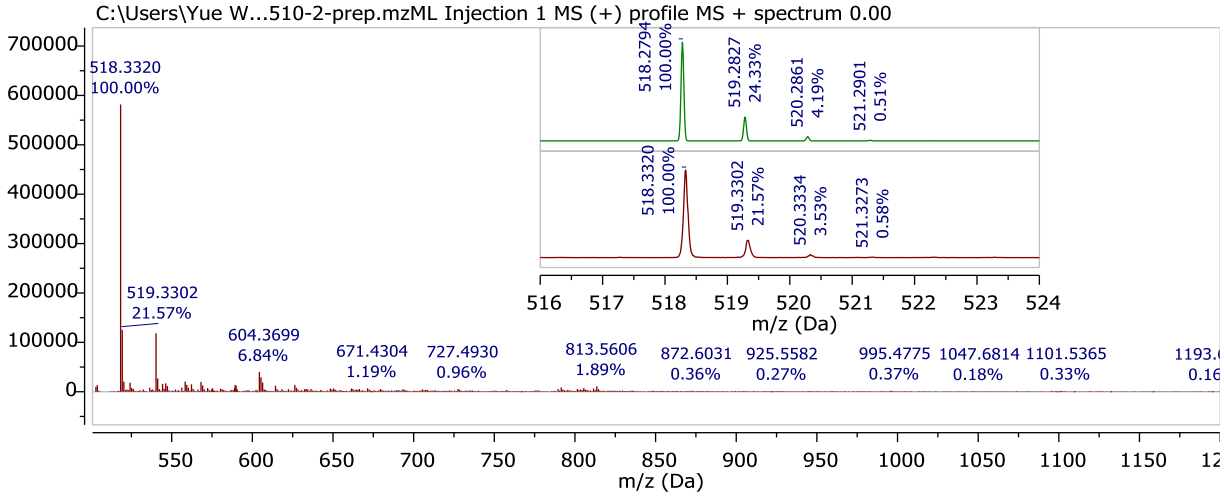
3d

Chemical Formula: $C_{18}H_{35}N_{11}O_7$

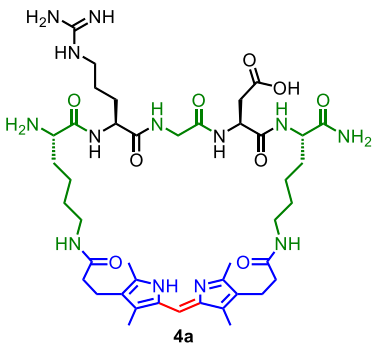
Exact Mass: 517.2721



Analytic HPLC of **3d (**Gradient A, 220 nm).

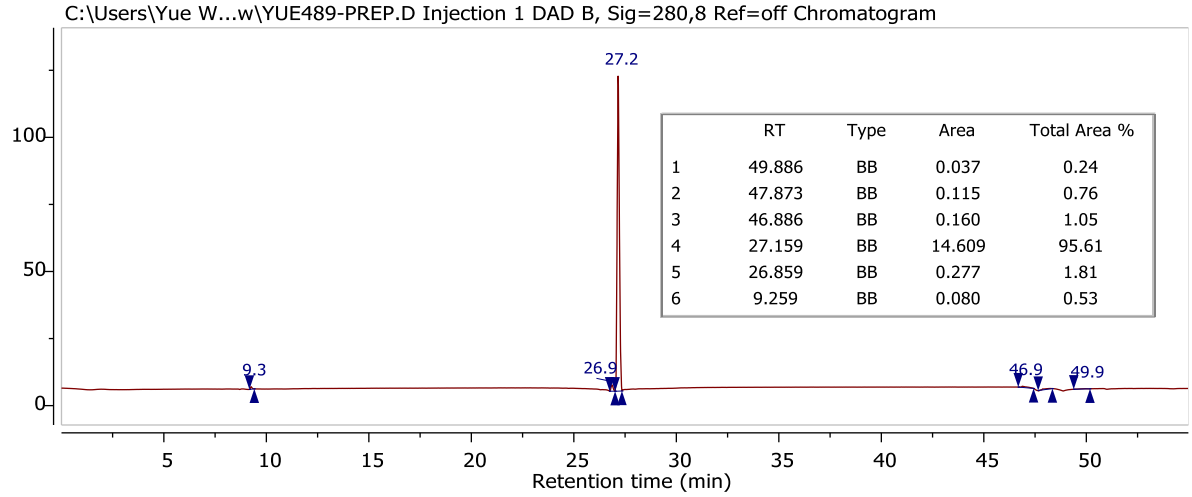


HRMS(MAIDL-TOF) of **3d**.



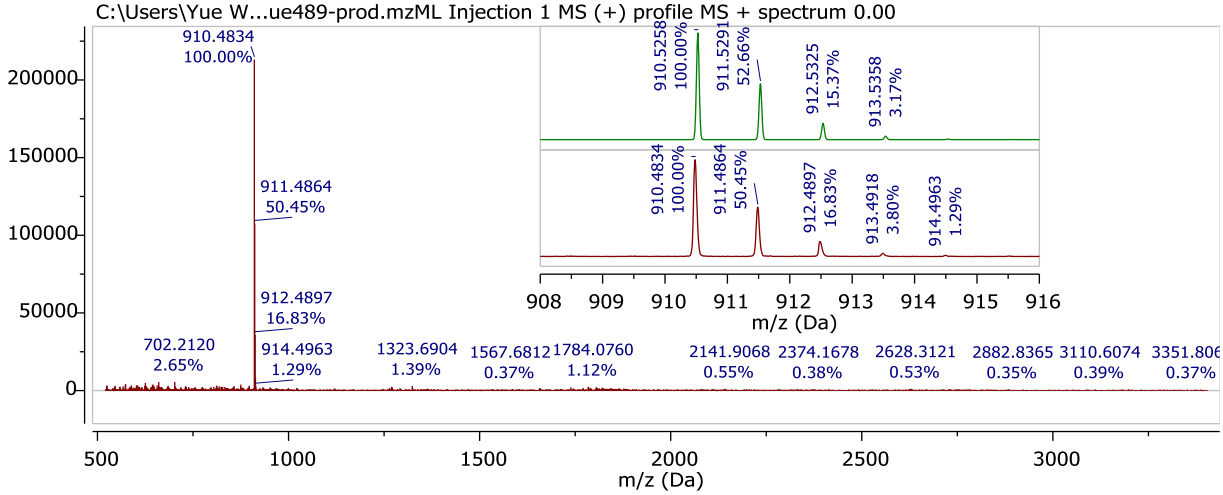
4a

Chemical Formula: $C_{43}H_{67}N_{13}O_9$
Exact Mass: 909.5185

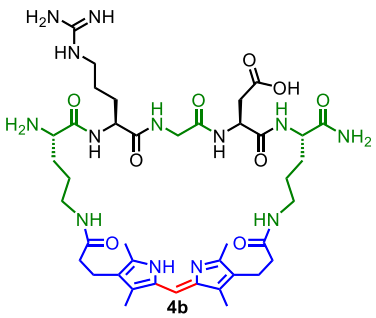


	RT	Type	Area	Total Area %
1	49.886	BB	0.037	0.24
2	47.873	BB	0.115	0.76
3	46.886	BB	0.160	1.05
4	27.159	BB	14.609	95.61
5	26.859	BB	0.277	1.81
6	9.259	BB	0.080	0.53

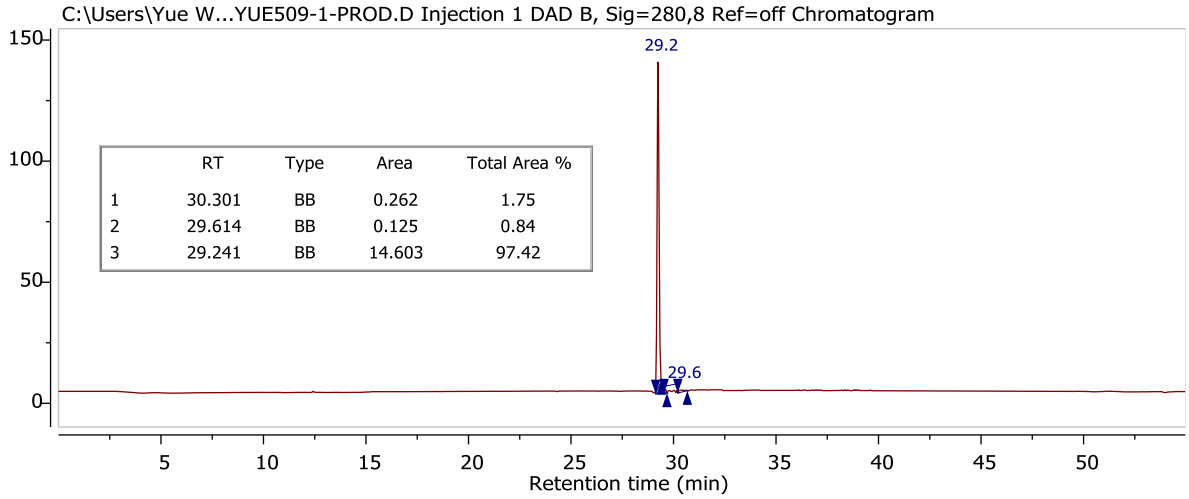
Analytic HPLC of **4a** (Gradient A, 280 nm).



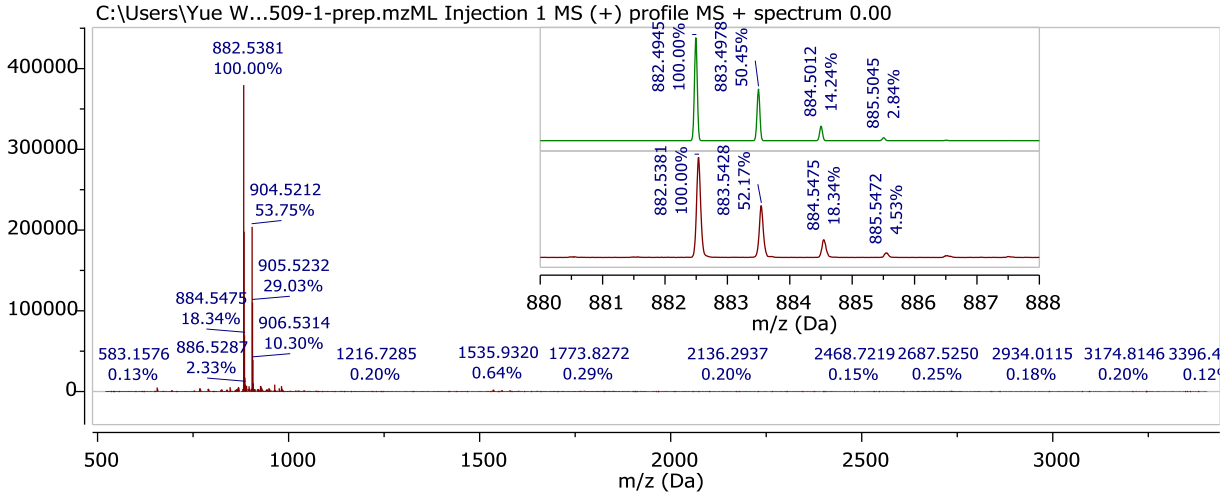
HRMS(MAIDL-TOF) of **4a**.



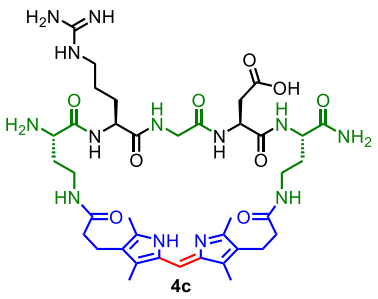
Chemical Formula: $C_{41}H_{63}N_{13}O_9$
Exact Mass: 881.4872



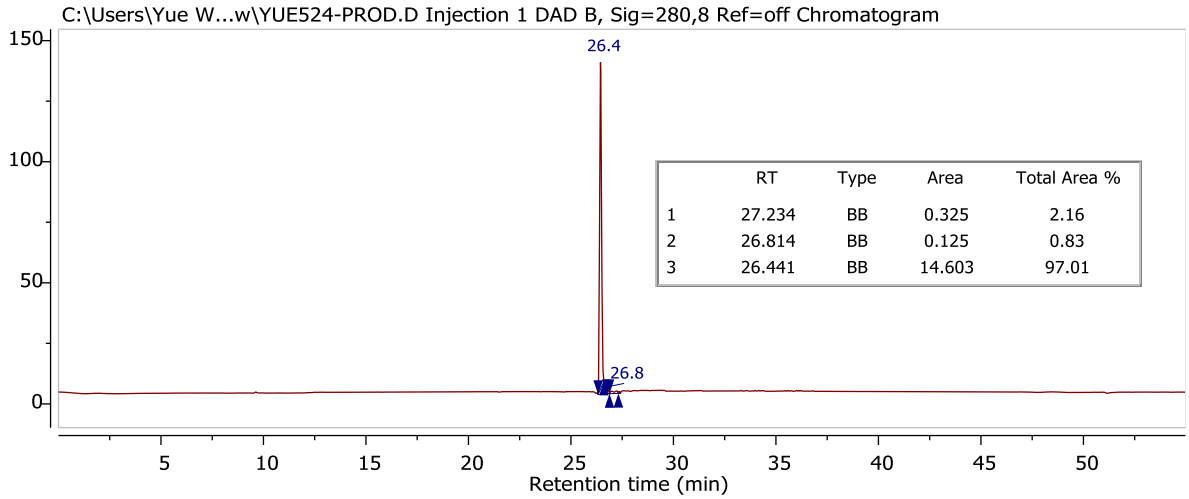
Analytic HPLC of **4b** (Gradient A, 280 nm).



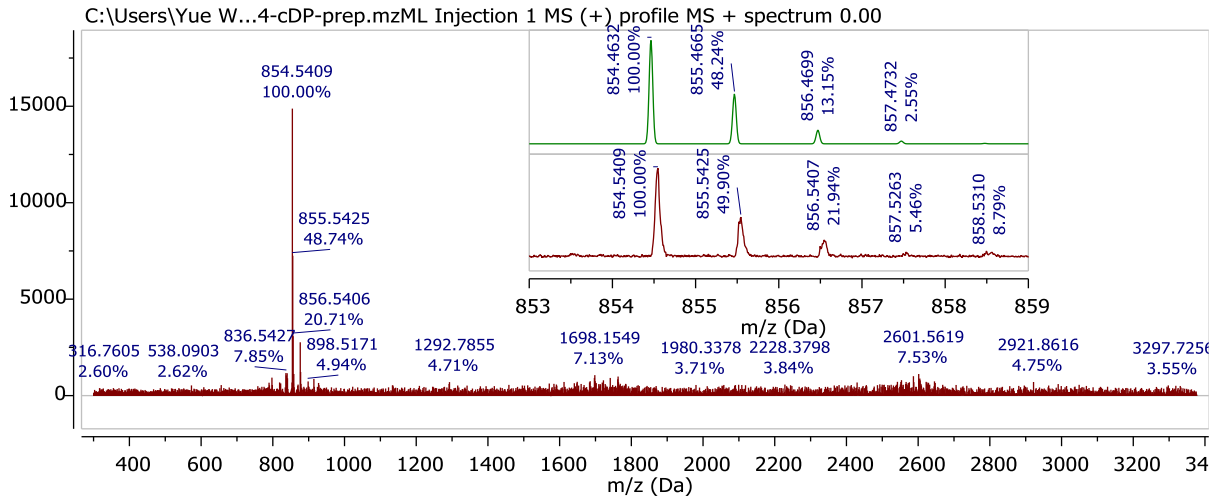
HRMS(MAIDL-TOF) of **4b**.



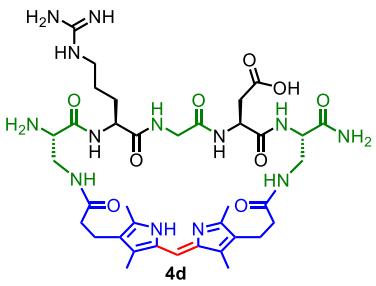
Chemical Formula: $C_{39}H_{59}N_{13}O_9$
Exact Mass: 853.4559



Analytic HPLC of **4c** (Gradient A, 280 nm).



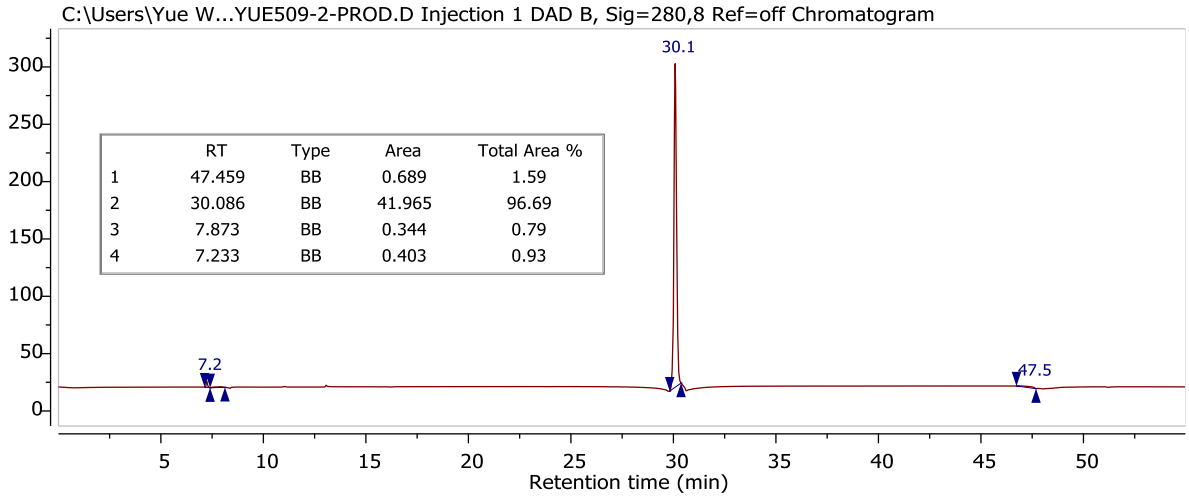
HRMS(MAIDL-TOF) of **4c**.



4d

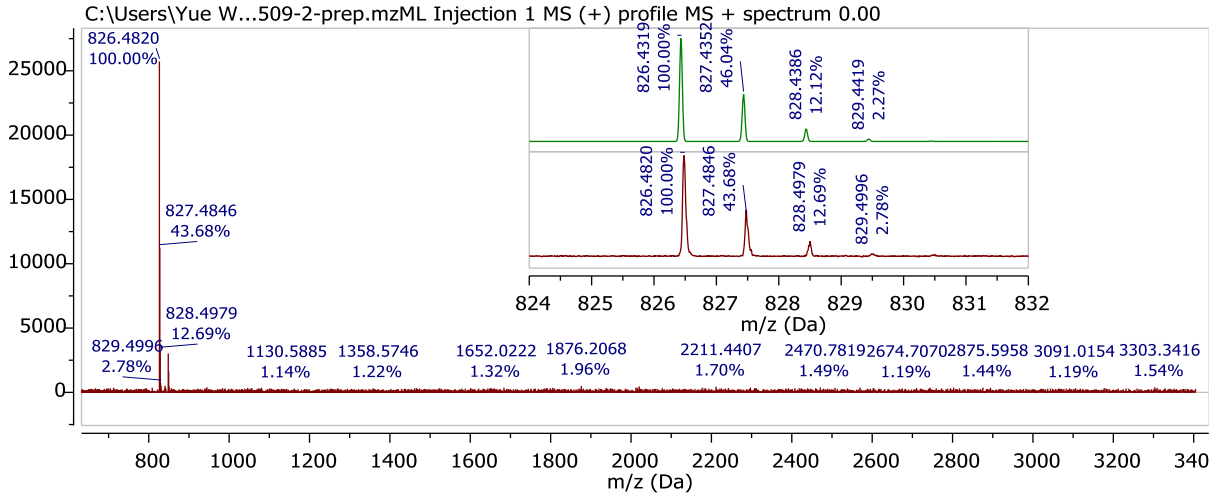
Chemical Formula: $C_{37}H_{55}N_{13}O_9$

Exact Mass: 825.4246

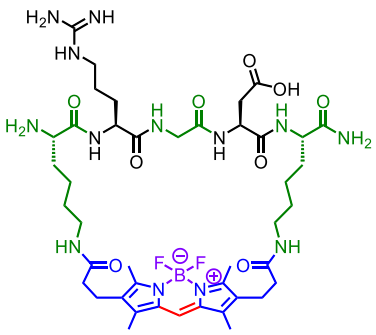


	RT	Type	Area	Total Area %
1	47.459	BB	0.689	1.59
2	30.086	BB	41.965	96.69
3	7.873	BB	0.344	0.79
4	7.233	BB	0.403	0.93

Analytic HPLC of **4d** (Gradient A, 280 nm).



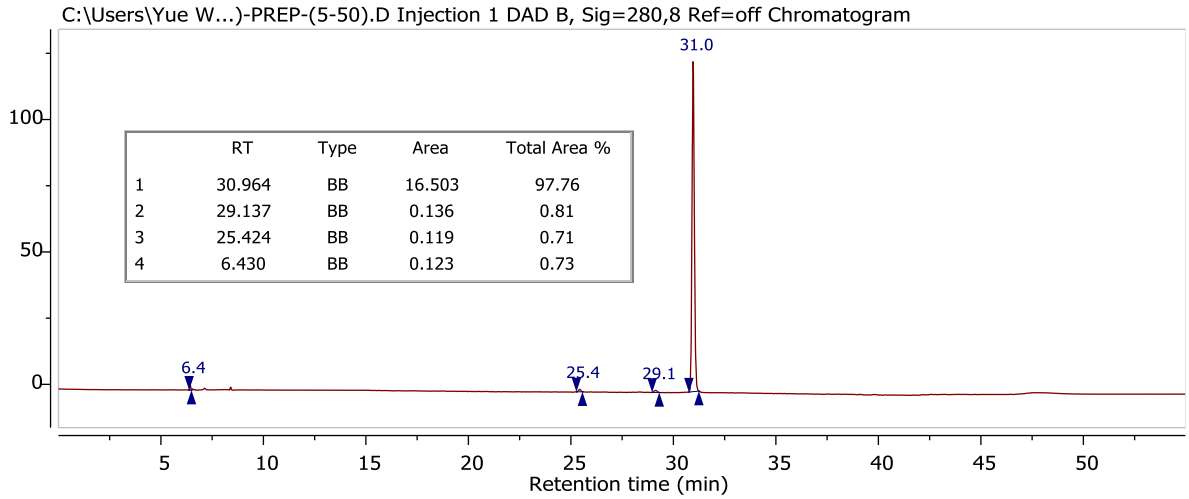
HRMS(MAIDL-TOF) of **4d**.



4a-BODIPY

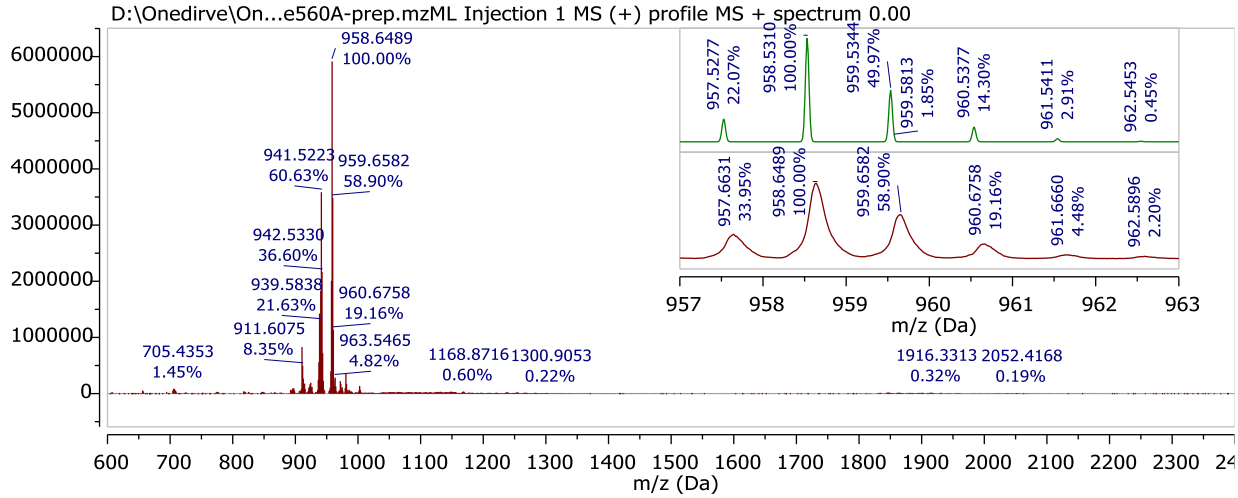
Chemical Formula: $C_{43}H_{66}BF_2N_{13}O_9$

Exact Mass: 957.5168

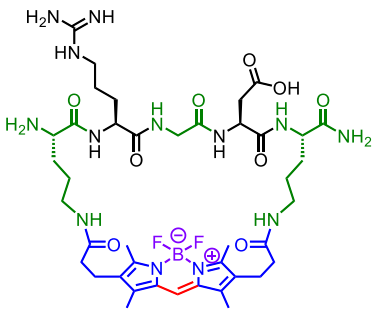


	RT	Type	Area	Total Area %
1	30.964	BB	16.503	97.76
2	29.137	BB	0.136	0.81
3	25.424	BB	0.119	0.71
4	6.430	BB	0.123	0.73

Analytic HPLC of **4a-BODIPY** (Gradient A, 280 nm).



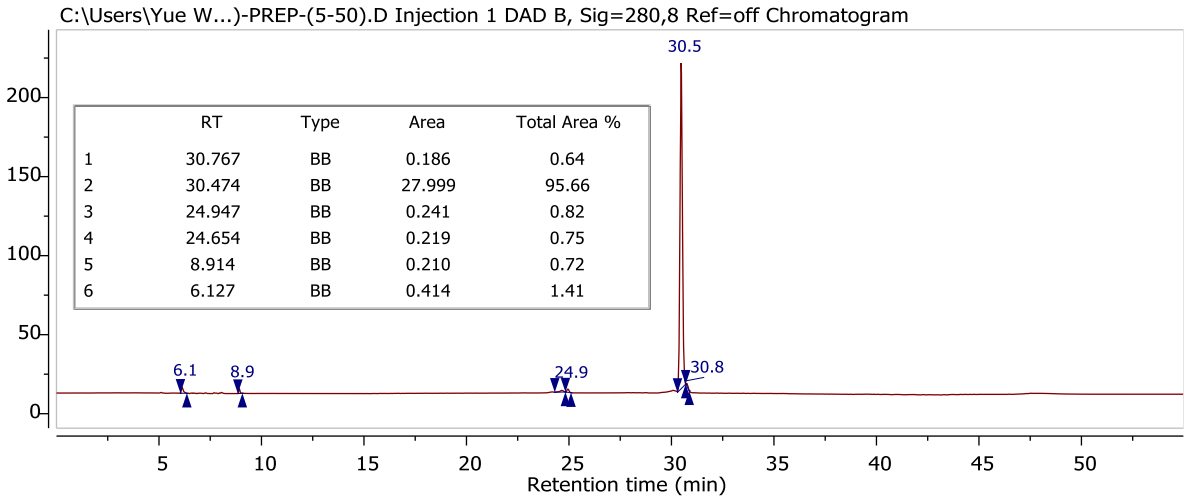
HRMS(MAIDL-TOF) of **4a-BODIPY**.



4b-BODIPY

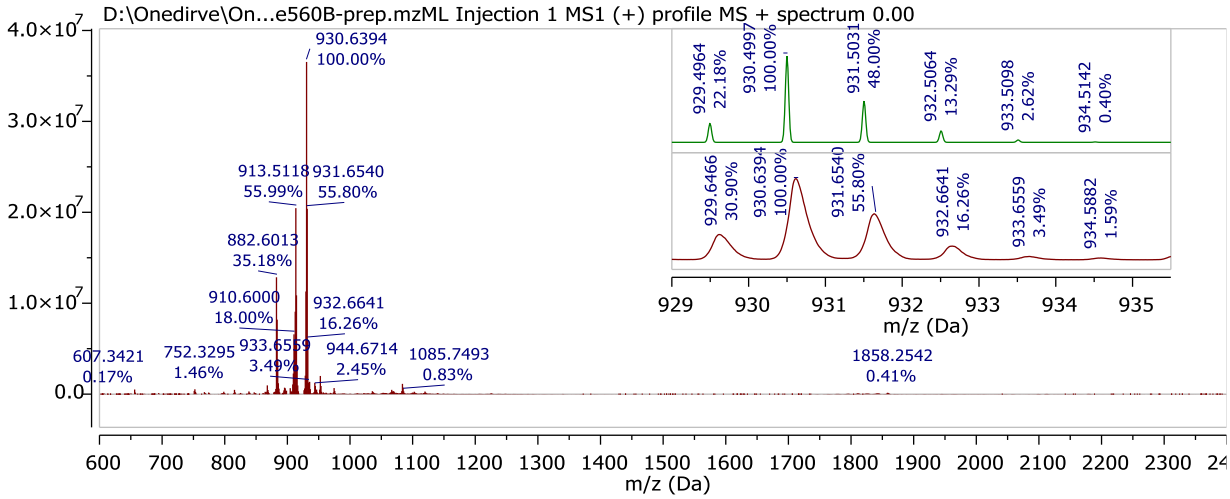
Chemical Formula: $C_{41}H_{62}BF_2N_{13}O_9$

Exact Mass: 929.4855

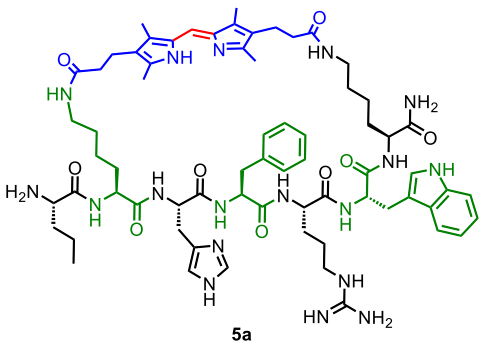


	RT	Type	Area	Total Area %
1	30.767	BB	0.186	0.64
2	30.474	BB	27.999	95.66
3	24.947	BB	0.241	0.82
4	24.654	BB	0.219	0.75
5	8.914	BB	0.210	0.72
6	6.127	BB	0.414	1.41

Analytic HPLC of **4b-BODIPY** (Gradient A, 280 nm).

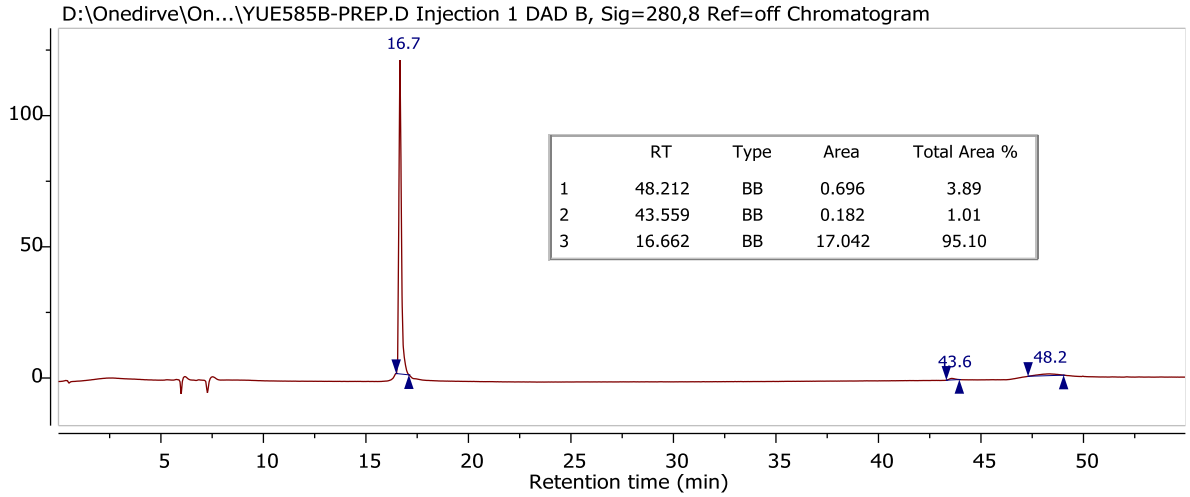


HRMS(MAIDL-TOF) of **4b-BODIPY**.

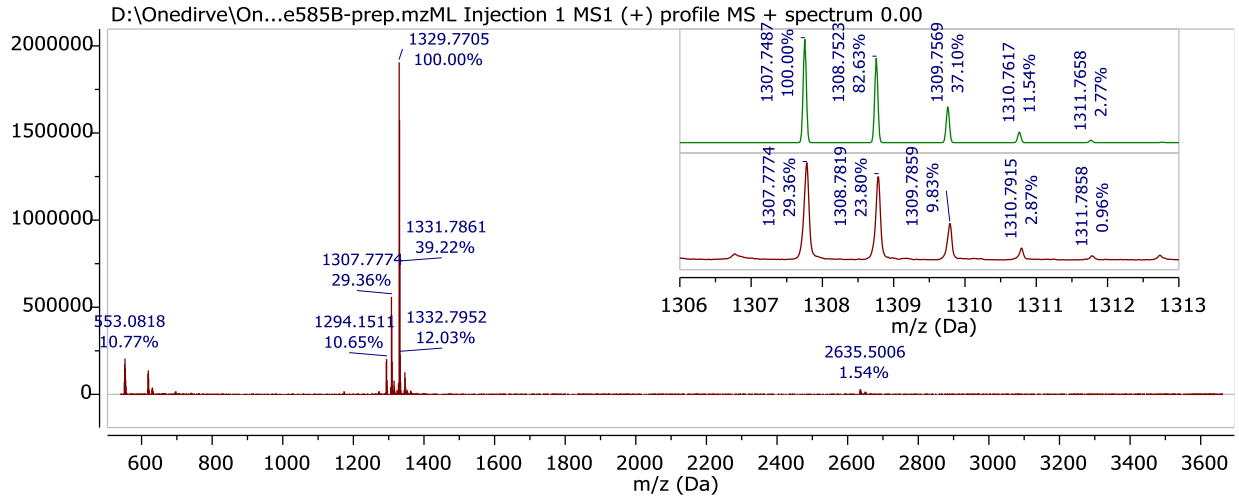


5a

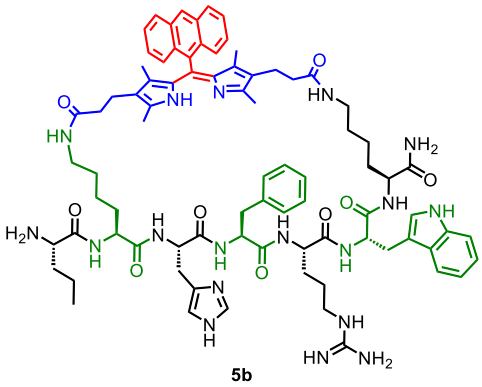
Chemical Formula: $C_{68}H_{94}N_{18}O_9$
Exact Mass: 1306.7451



Analytic HPLC of **5a** (Gradient B, 280 nm).



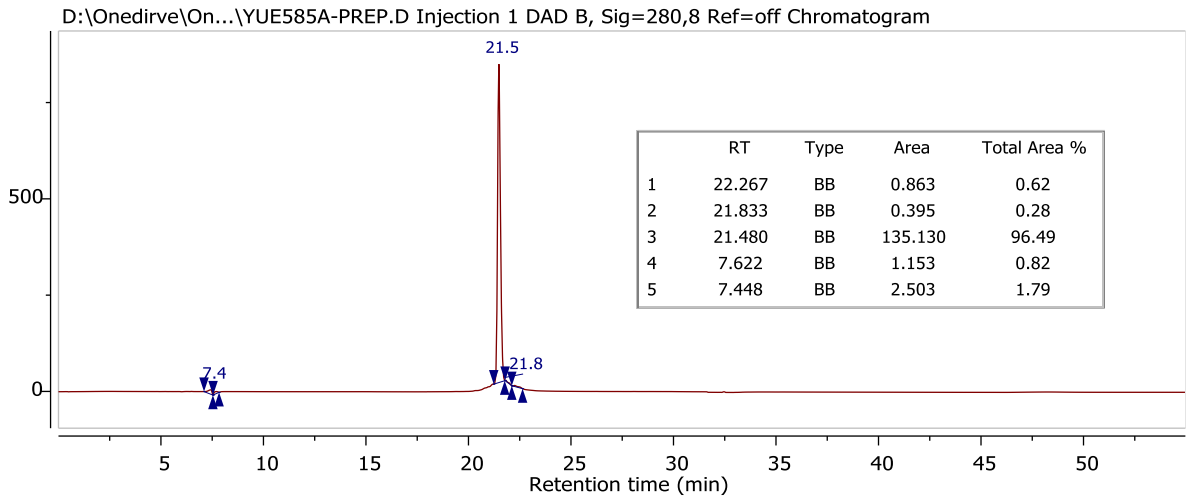
HRMS(MAIDL-TOF) of **5a**.



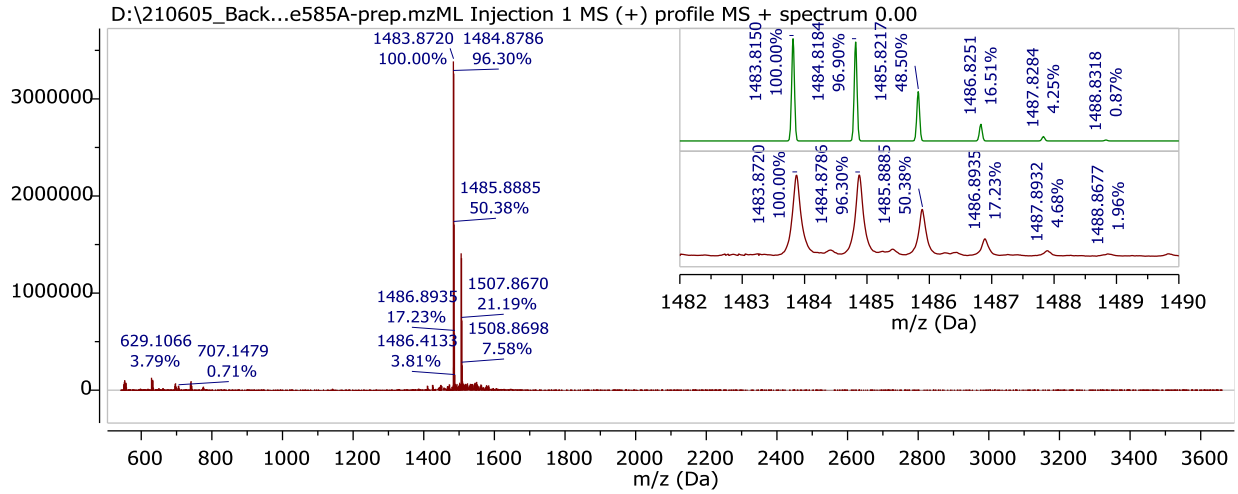
5b

Chemical Formula: $C_{82}H_{102}N_{18}O_9$

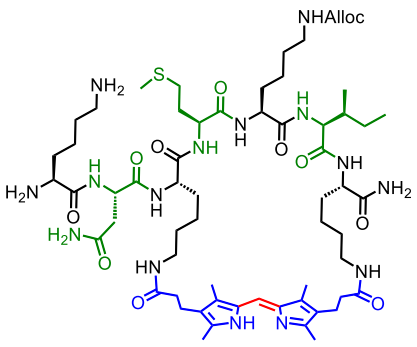
Exact Mass: 1482.8077



Analytic HPLC of **5b** (Gradient B, 280 nm).



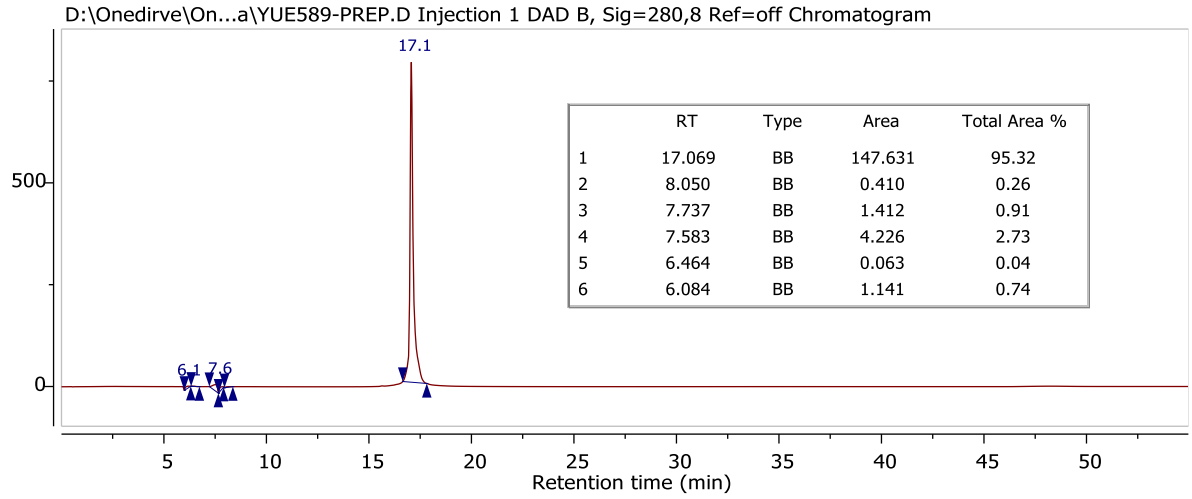
HRMS(MAIDL-TOF) of **5b**.



6a

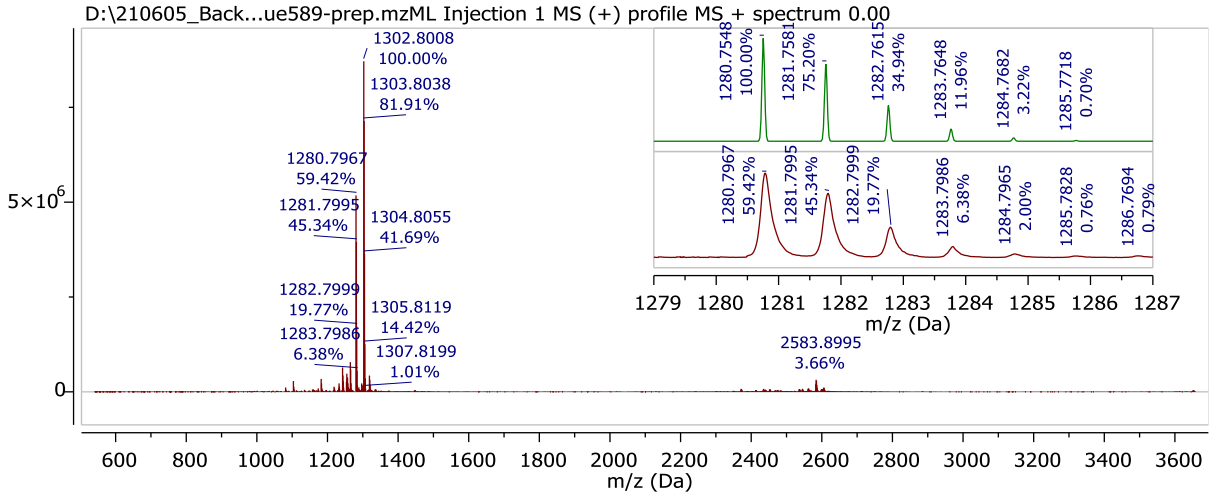
Chemical Formula: $C_{62}H_{101}N_{15}O_{12}S$

Exact Mass: 1279.7475

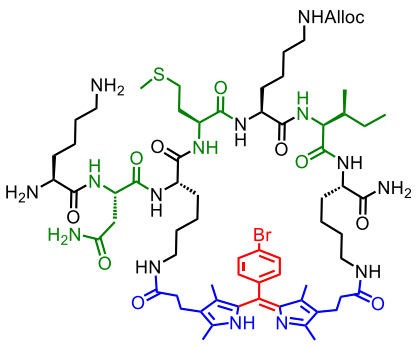


	RT	Type	Area	Total Area %
1	17.069	BB	147.631	95.32
2	8.050	BB	0.410	0.26
3	7.737	BB	1.412	0.91
4	7.583	BB	4.226	2.73
5	6.464	BB	0.063	0.04
6	6.084	BB	1.141	0.74

Analytic HPLC of **6a** (Gradient B, 280 nm).



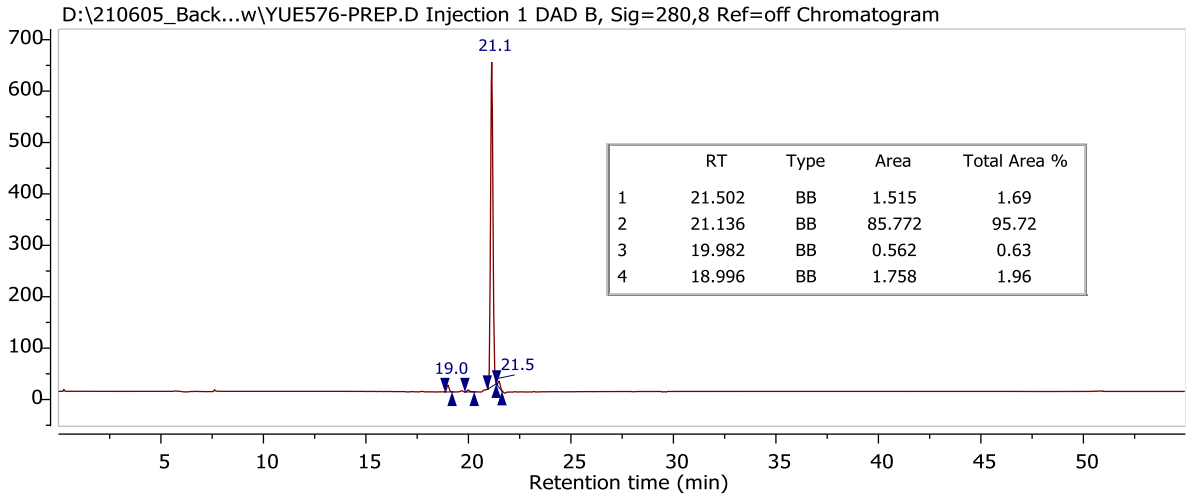
HRMS(MAIDL-TOF) of **6a**.



6b

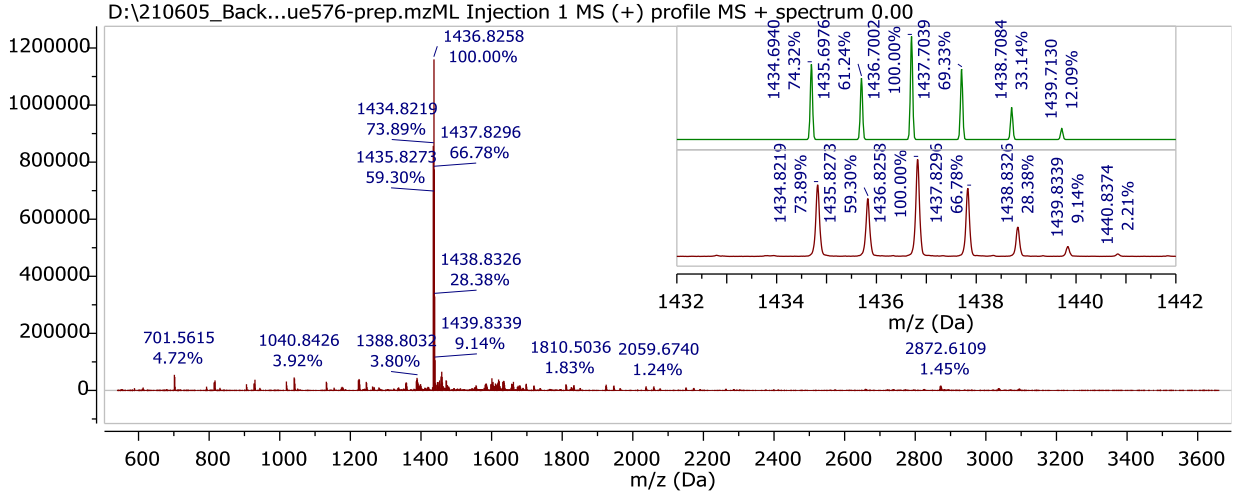
Chemical Formula: $C_{68}H_{104}BrN_{15}O_{12}S$

Exact Mass: 1433.6893

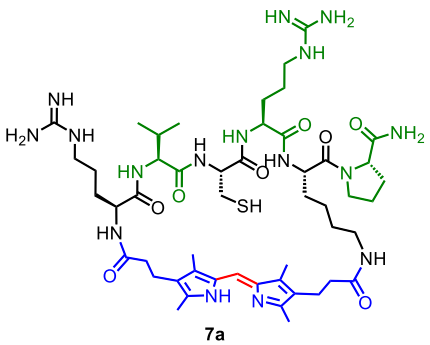


	RT	Type	Area	Total Area %
1	21.502	BB	1.515	1.69
2	21.136	BB	85.772	95.72
3	19.982	BB	0.562	0.63
4	18.996	BB	1.758	1.96

Analytic HPLC of **6b** (Gradient B, 280 nm).



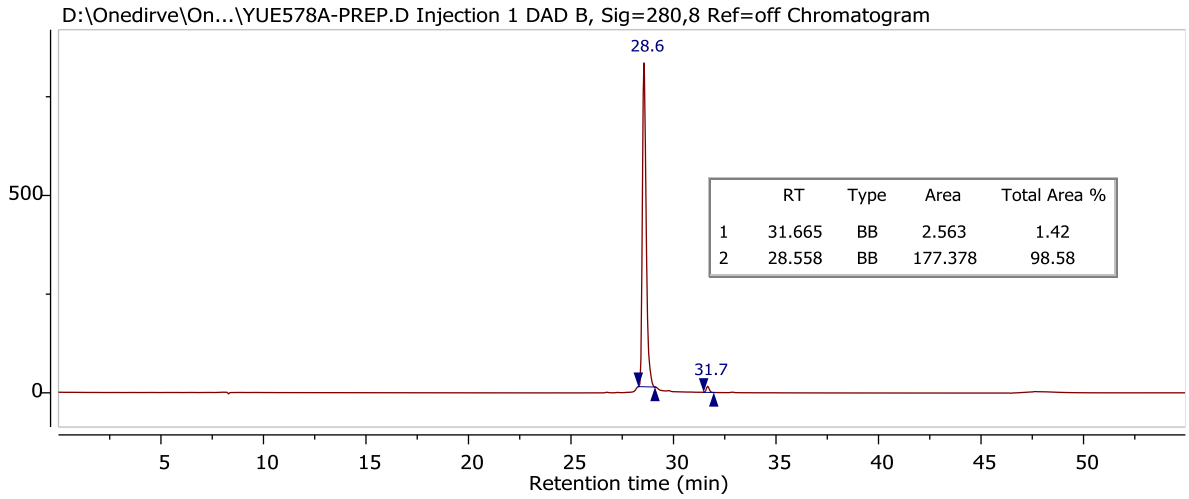
HRMS(MAIDL-TOF) of **6b**.



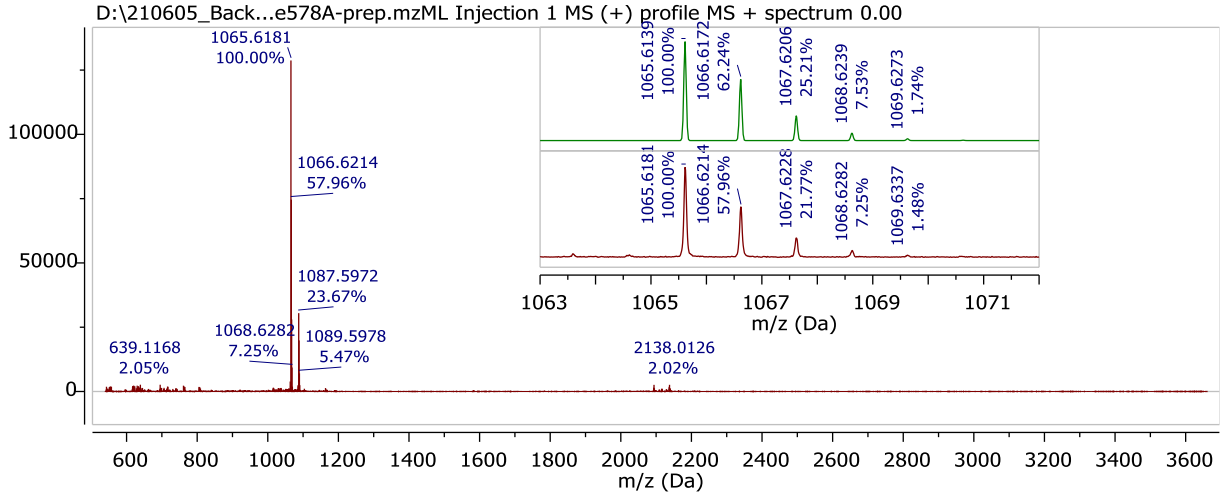
7a

Chemical Formula: $C_{50}H_{80}N_{16}O_8S$

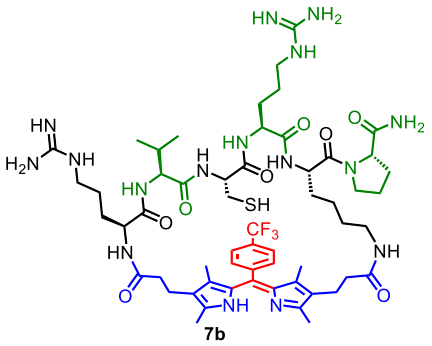
Exact Mass: 1064.6066



Analytic HPLC of **7a** (Gradient A, 280 nm).



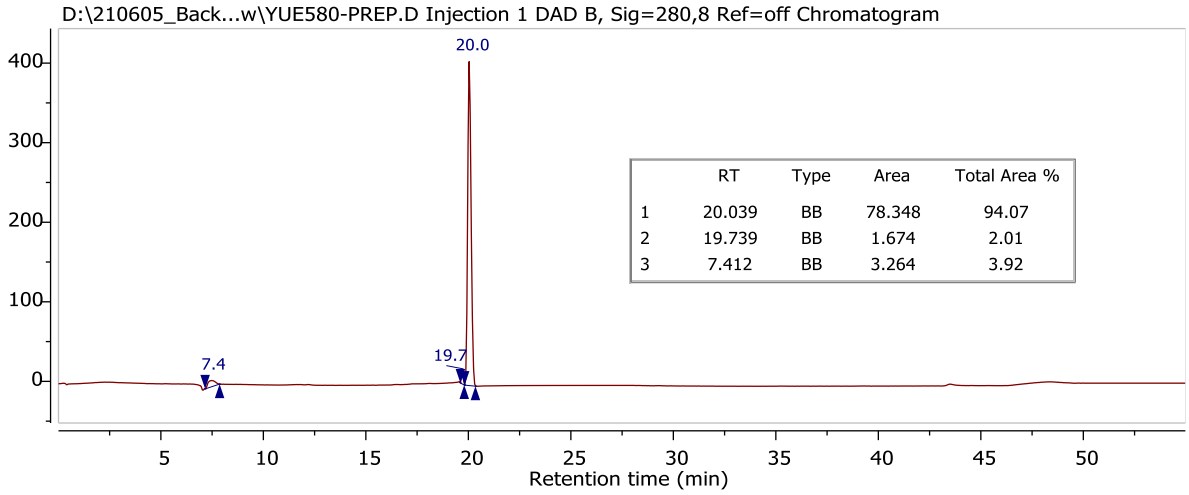
HRMS(MAIDL-TOF) of **7a**.



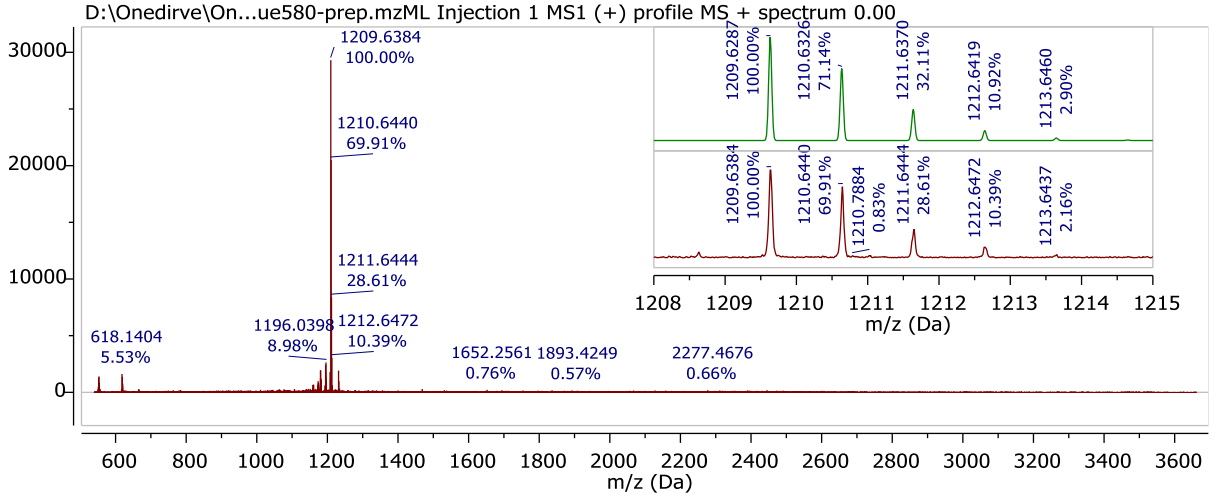
7b

Chemical Formula: $C_{57}H_{83}F_3N_{16}O_8S$

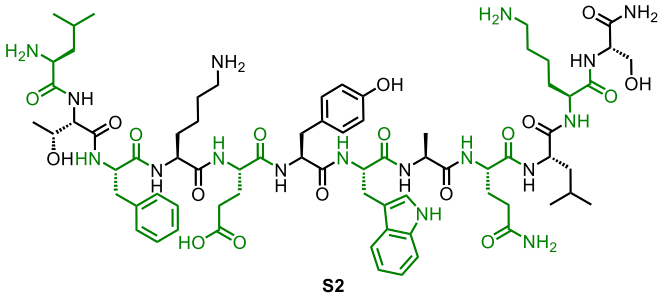
Exact Mass: 1208.6253



Analytic HPLC of **7b** (Gradient A, 280 nm).



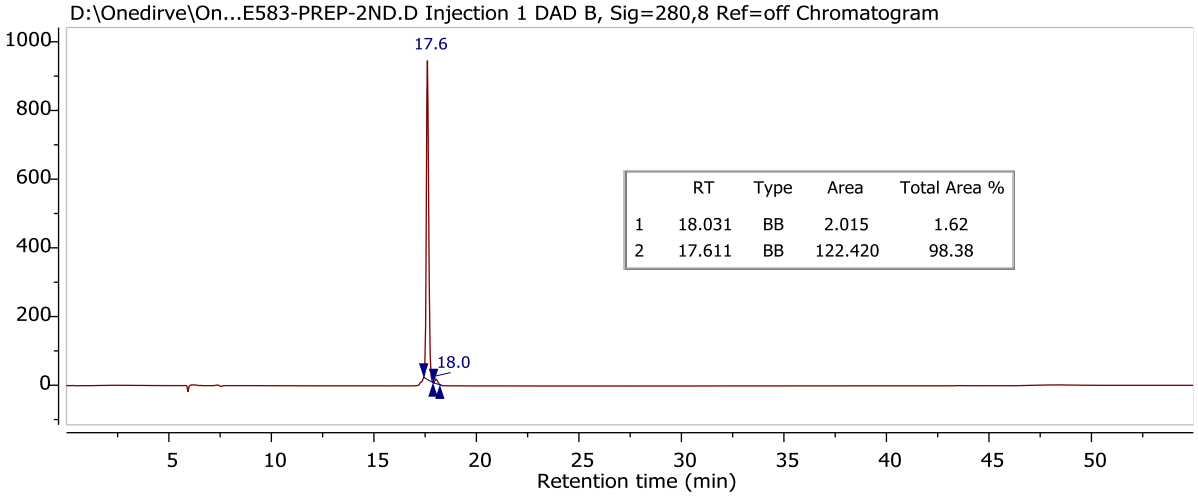
HRMS(MAIDL-TOF) of **7b**.



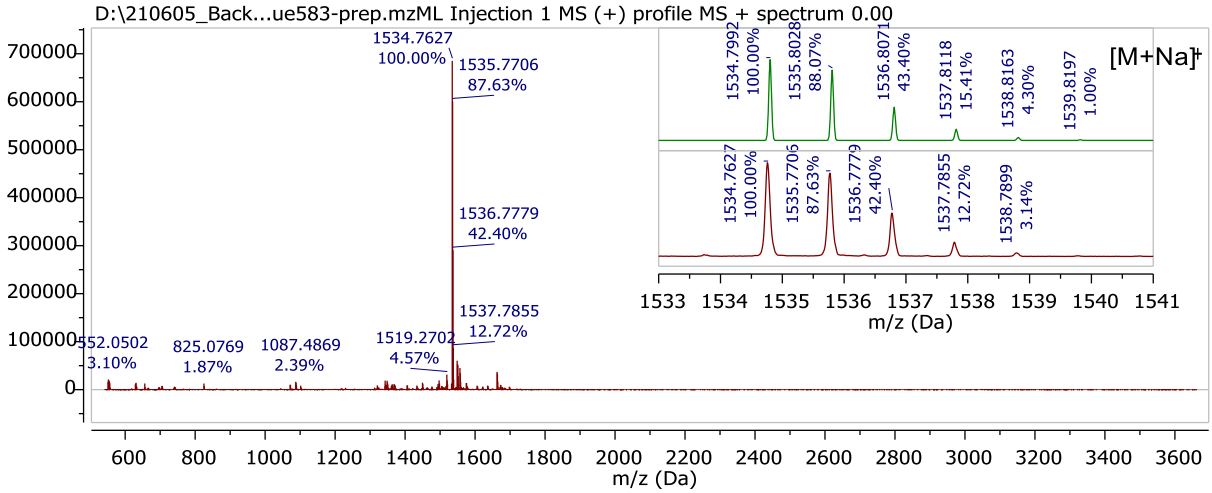
S2

Chemical Formula: $C_{73}H_{109}N_{17}O_{18}$

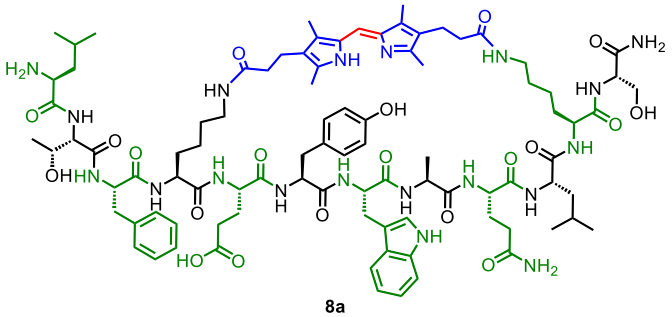
Exact Mass: 1511.8136



Analytic HPLC of **S2** (Gradient B, 280 nm).



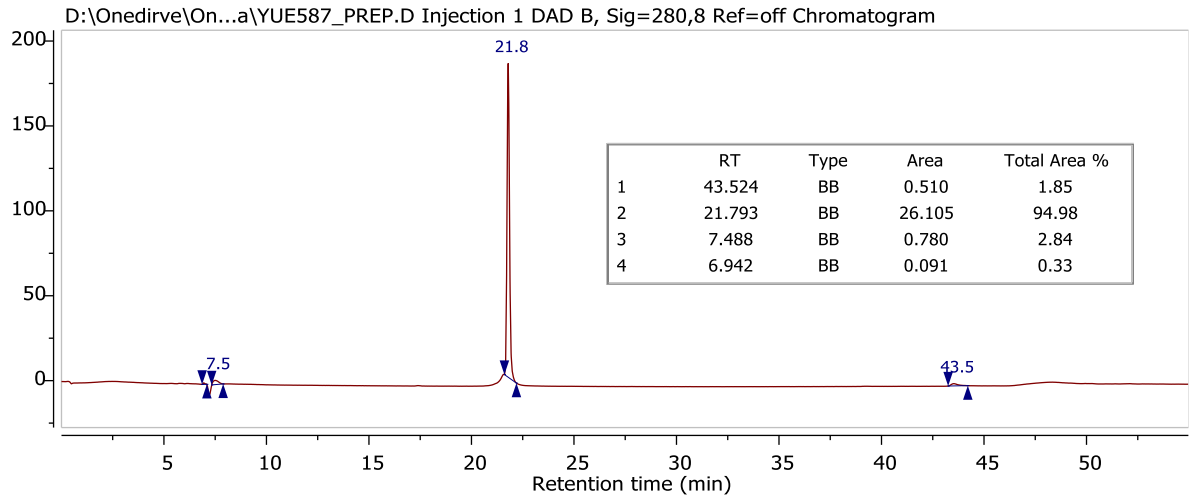
HRMS(MAIDL-TOF) of **S2**.



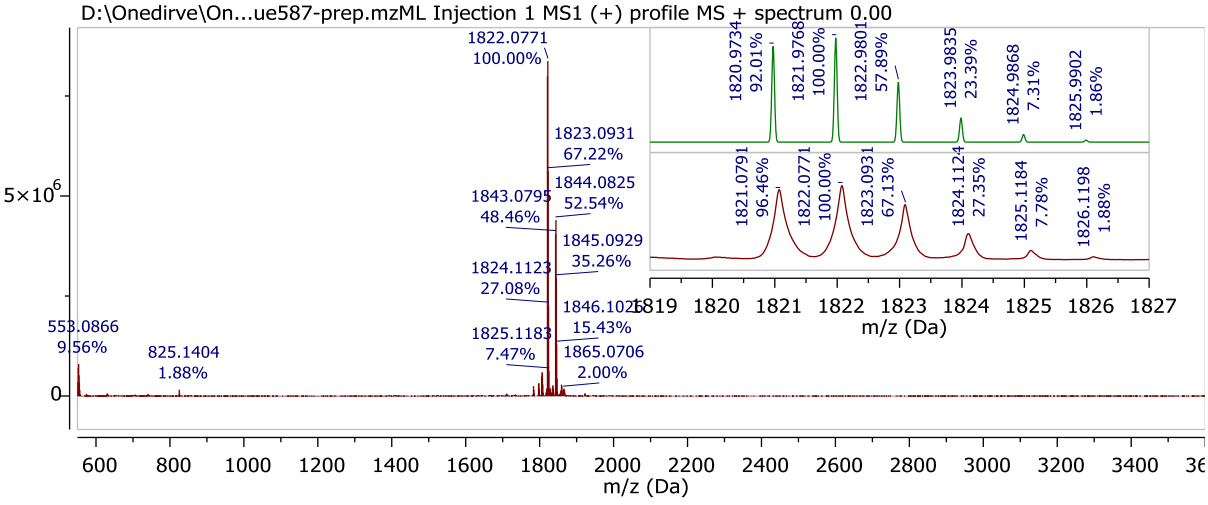
8a

Chemical Formula: $C_{92}H_{129}N_{19}O_{20}$

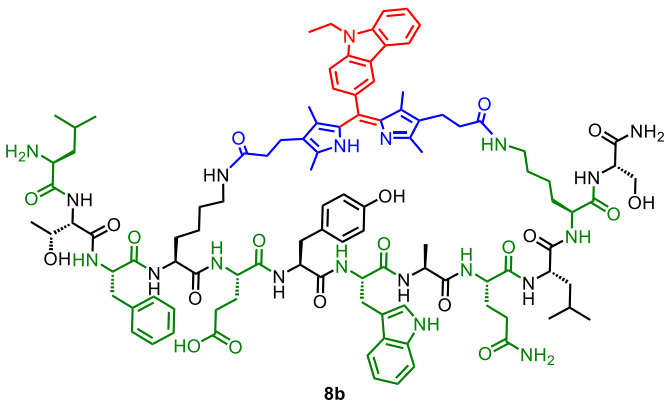
Exact Mass: 1819.9661



Analytic HPLC of **8a** (Gradient B, 280 nm).



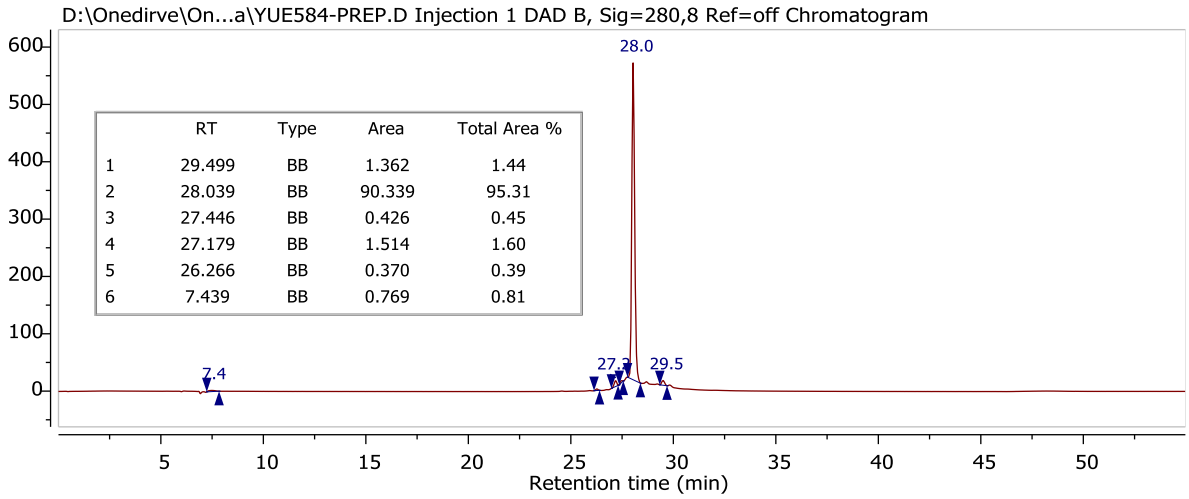
HRMS(MAIDL-TOF) of **8a**.



8b

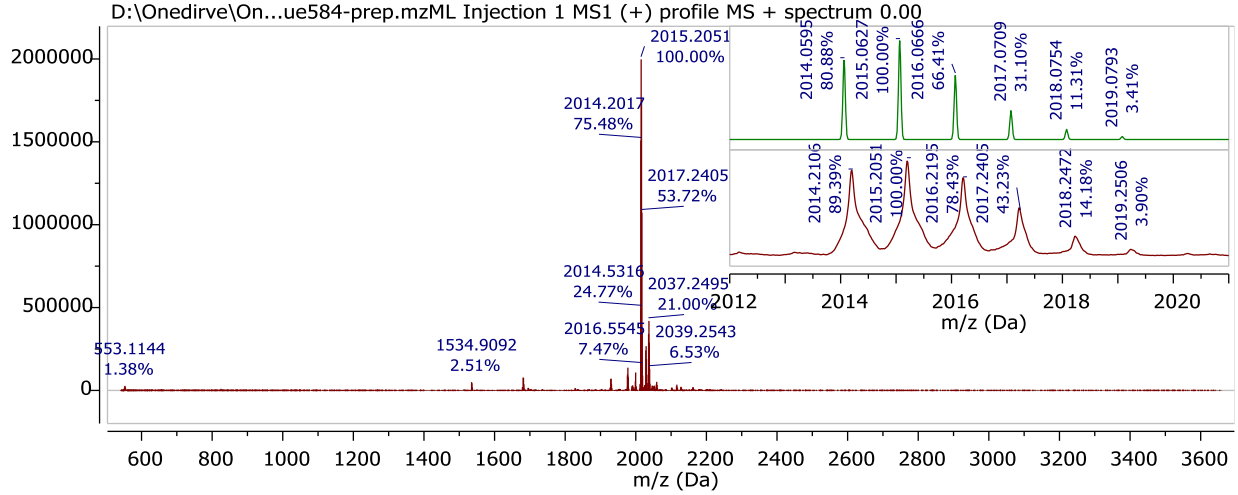
Chemical Formula: $C_{106}H_{140}N_{20}O_{20}$

Exact Mass: 2013.0553

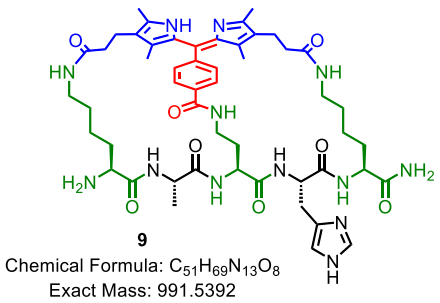


	RT	Type	Area	Total Area %
1	29.499	BB	1.362	1.44
2	28.039	BB	90.339	95.31
3	27.446	BB	0.426	0.45
4	27.179	BB	1.514	1.60
5	26.266	BB	0.370	0.39
6	7.439	BB	0.769	0.81

Analytic HPLC of **8b** (Gradient B, 280 nm).



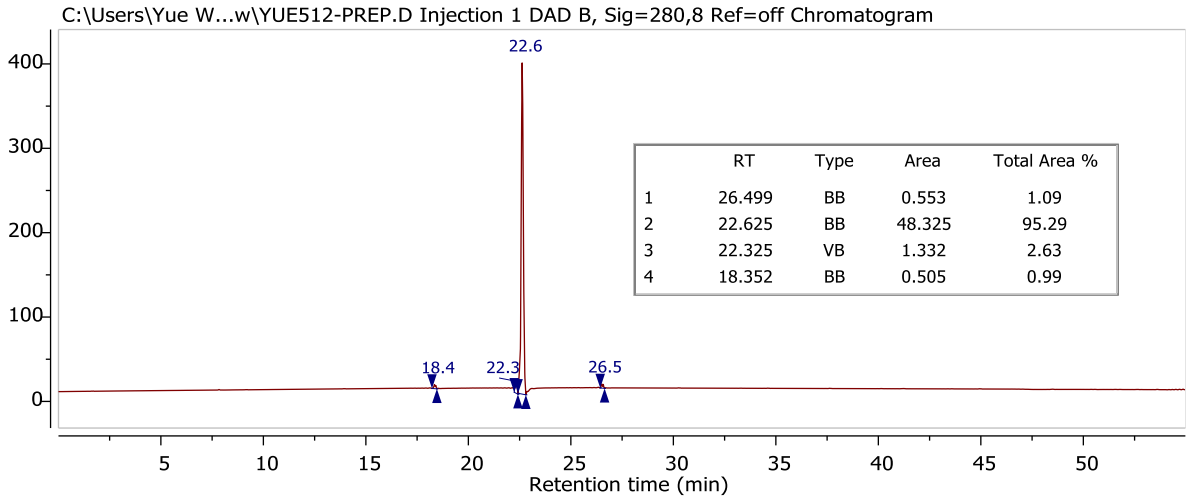
HRMS(MAIDL-TOF) of **8b**.



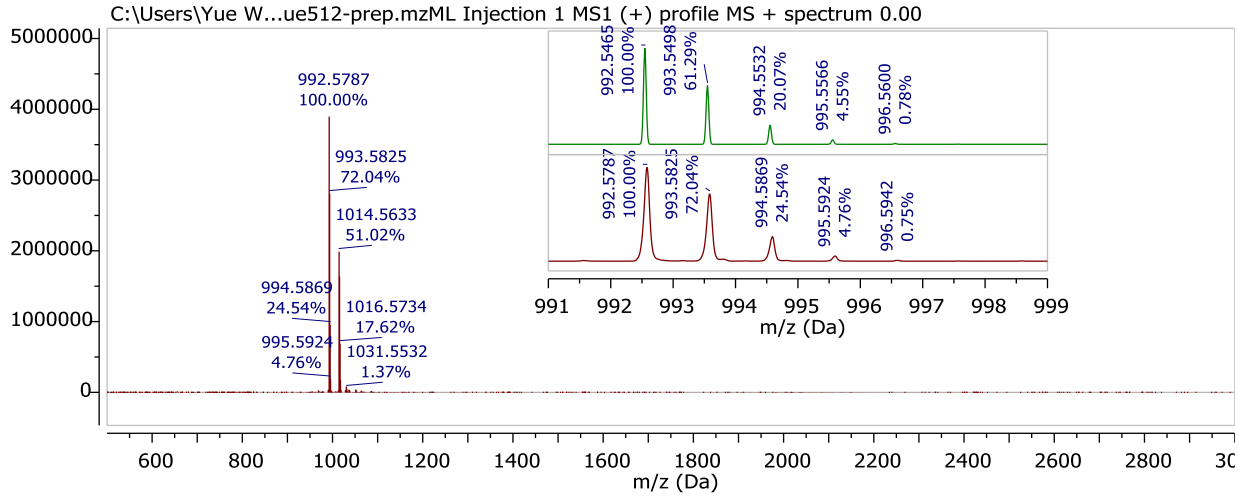
9

Chemical Formula: $C_{51}H_{69}N_{13}O_8$

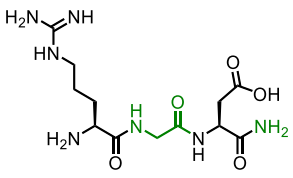
Exact Mass: 991.5392



Analytic HPLC of **9** (Gradient A, 280 nm).



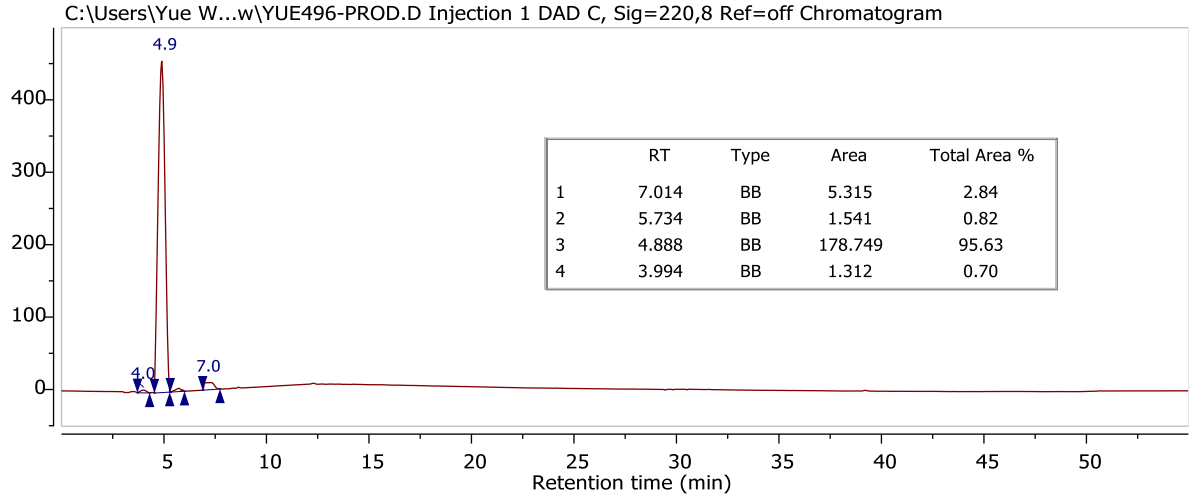
HRMS(MAIDL-TOF) of **9**.



S3

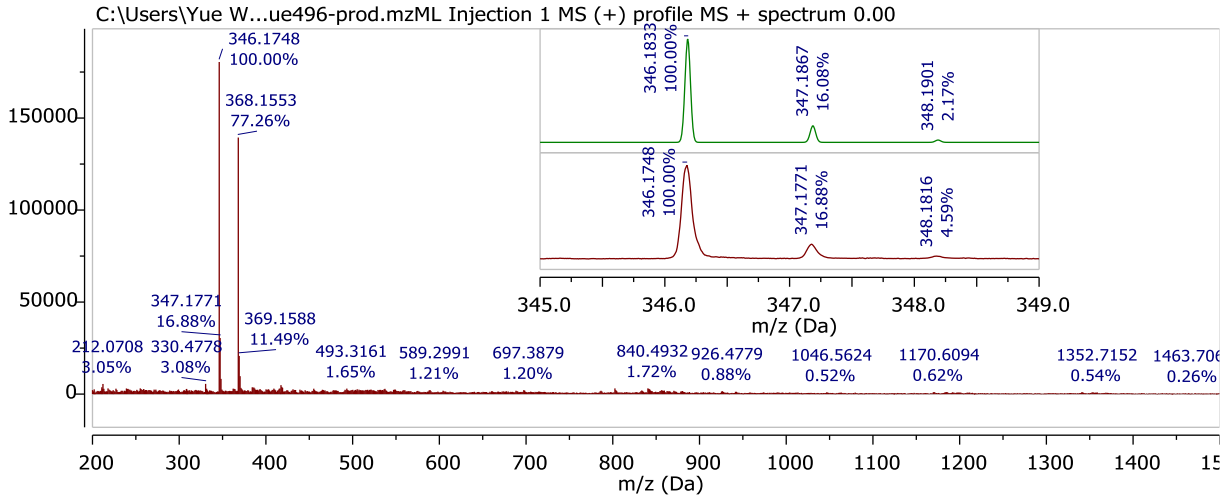
Chemical Formula: $C_{12}H_{23}N_7O_5$

Exact Mass: 345.1761

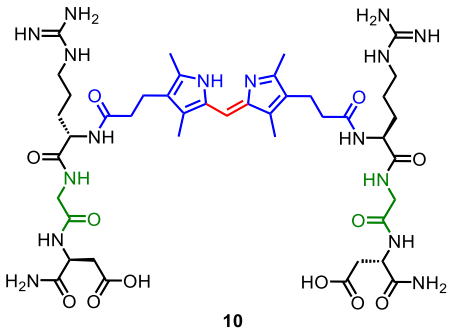


	RT	Type	Area	Total Area %
1	7.014	BB	5.315	2.84
2	5.734	BB	1.541	0.82
3	4.888	BB	178.749	95.63
4	3.994	BB	1.312	0.70

Analytic HPLC of RGD peptide, **S3 (**Gradient A, 220 nm).



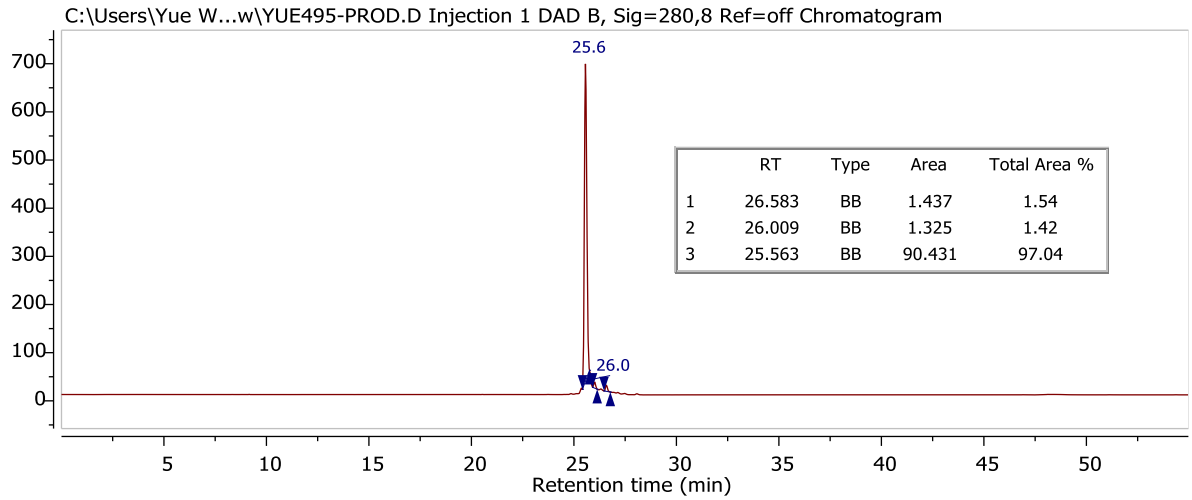
HRMS(MAIDL-TOF) of RGD peptide, **S3**.



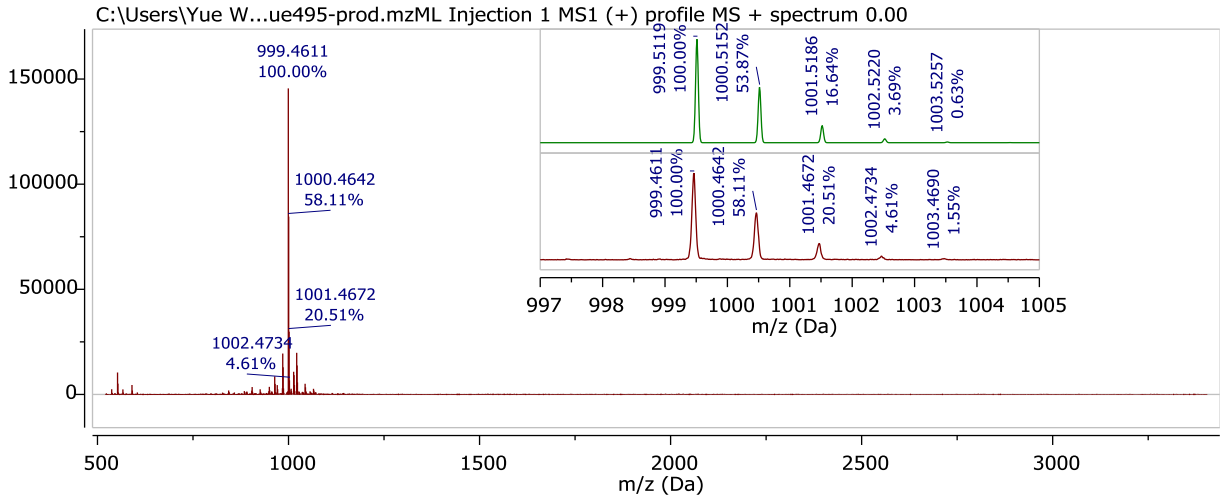
10

Chemical Formula: $C_{43}H_{66}N_{16}O_{12}$

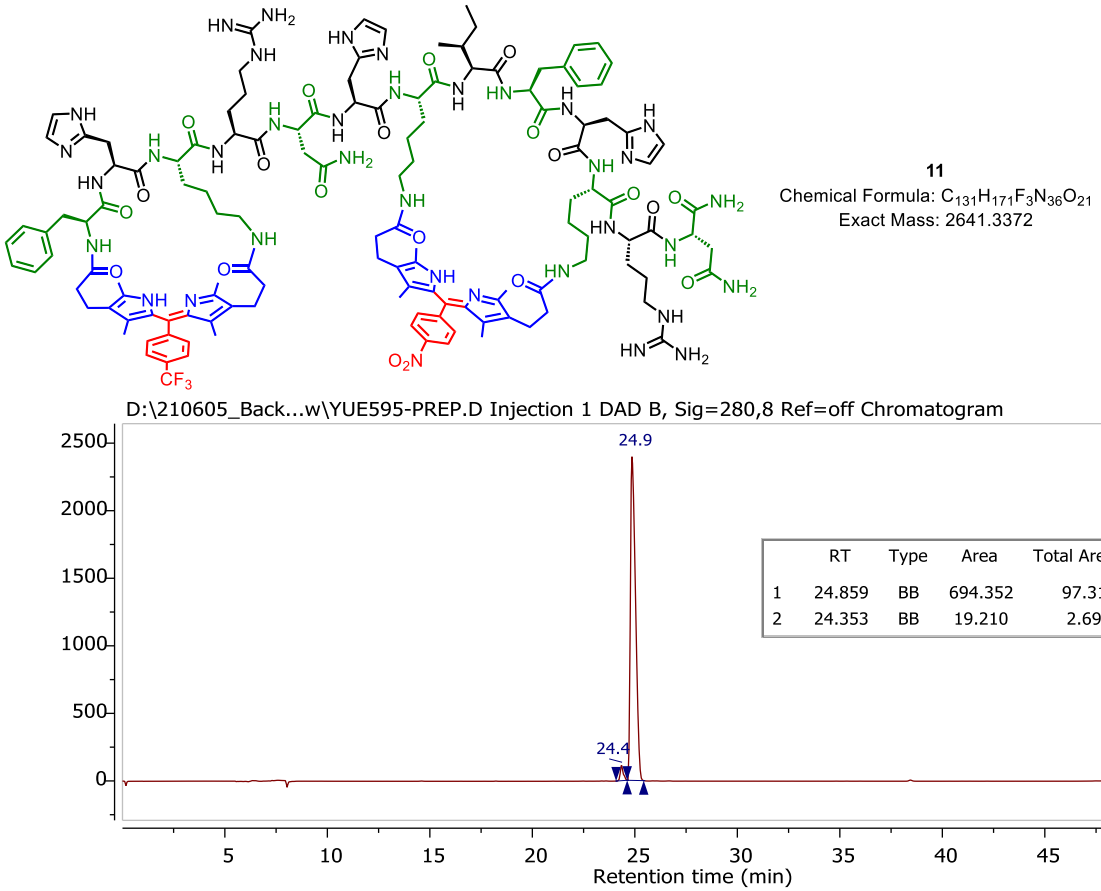
Exact Mass: 998.5046



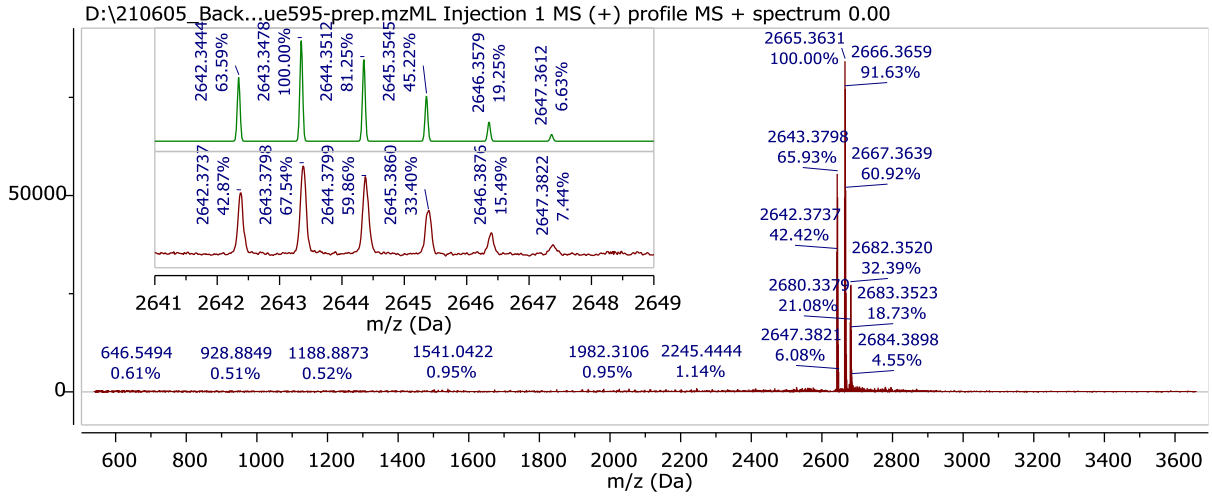
Analytic HPLC of **10** (Gradient A, 280 nm).



HRMS(MAIDL-TOF) of **10**.



Analytic HPLC of **11** (Gradient B, 280 nm).

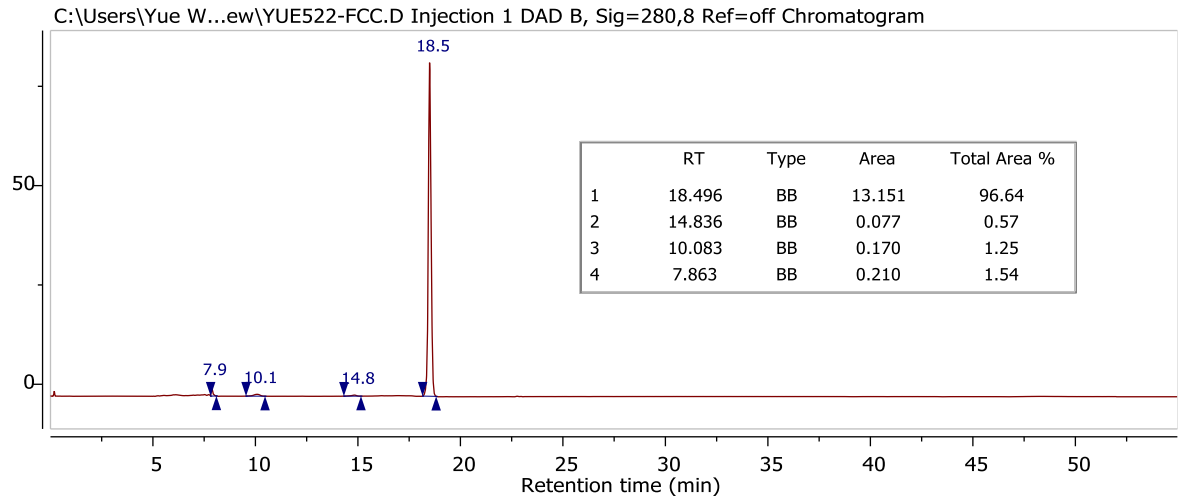


HRMS(MAIDL-TOF) of **11**.

S4

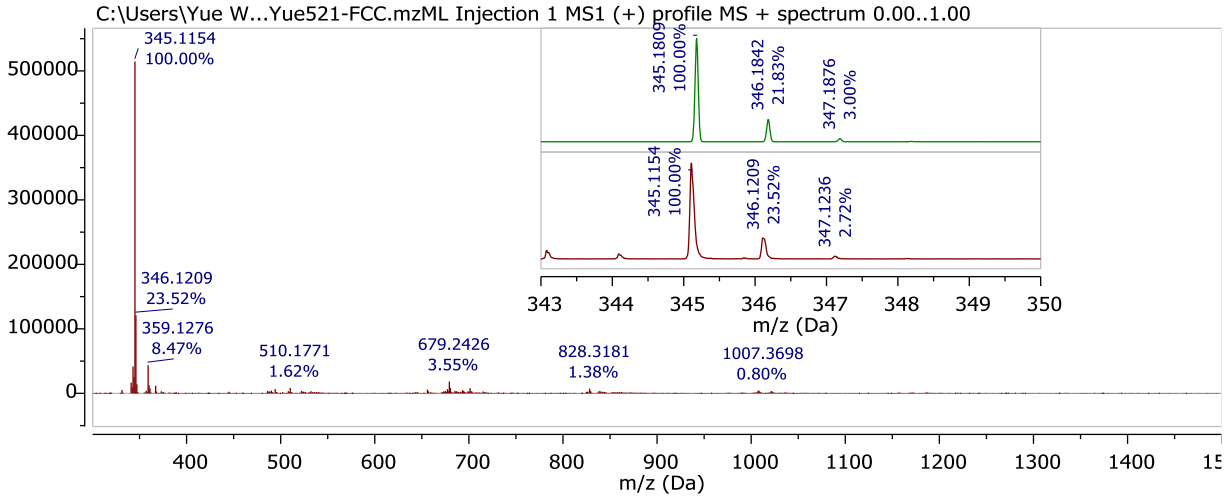
Chemical Formula: $C_{19}H_{24}N_2O_4$

Exact Mass: 344.1736

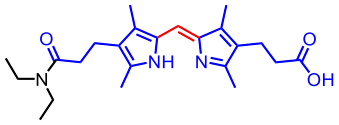


	RT	Type	Area	Total Area %
1	18.496	BB	13.151	96.64
2	14.836	BB	0.077	0.57
3	10.083	BB	0.170	1.25
4	7.863	BB	0.210	1.54

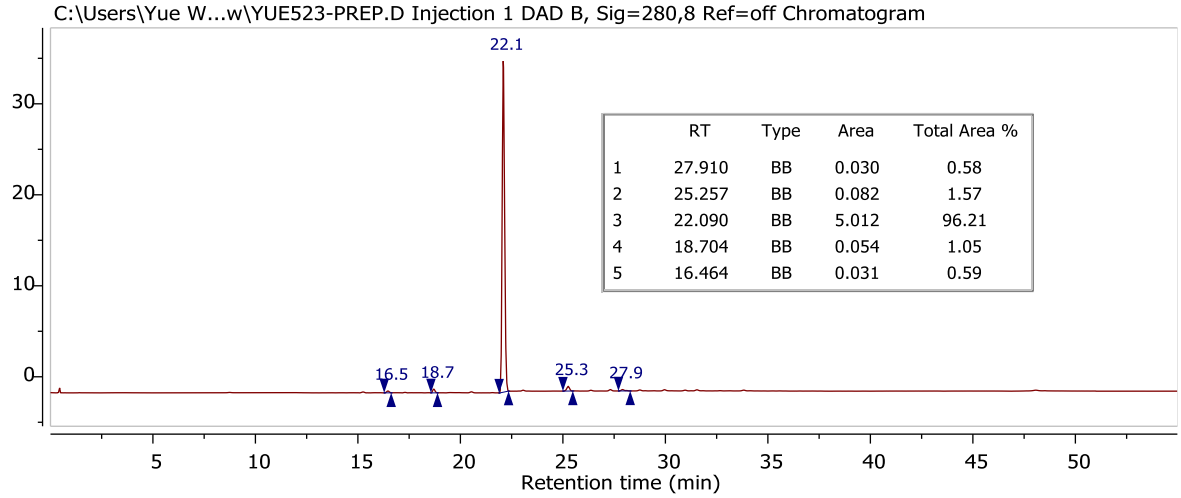
Analytic HPLC of **S4** (Gradient A, 280 nm).



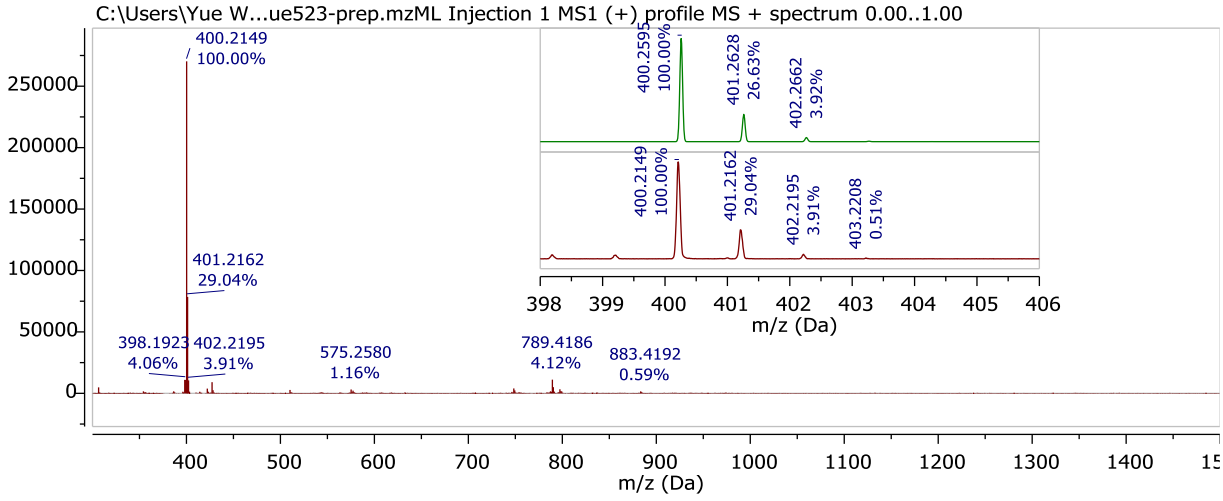
HRMS(MAIDL-TOF) of **S4**.



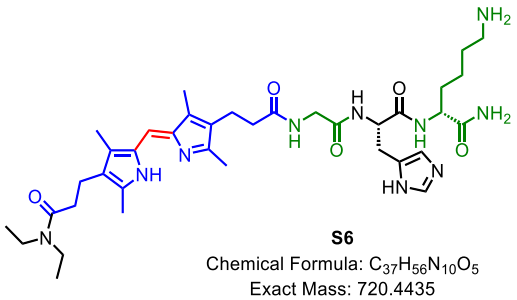
S5
Chemical Formula: $C_{23}H_{33}N_3O_3$
Exact Mass: 399.2522



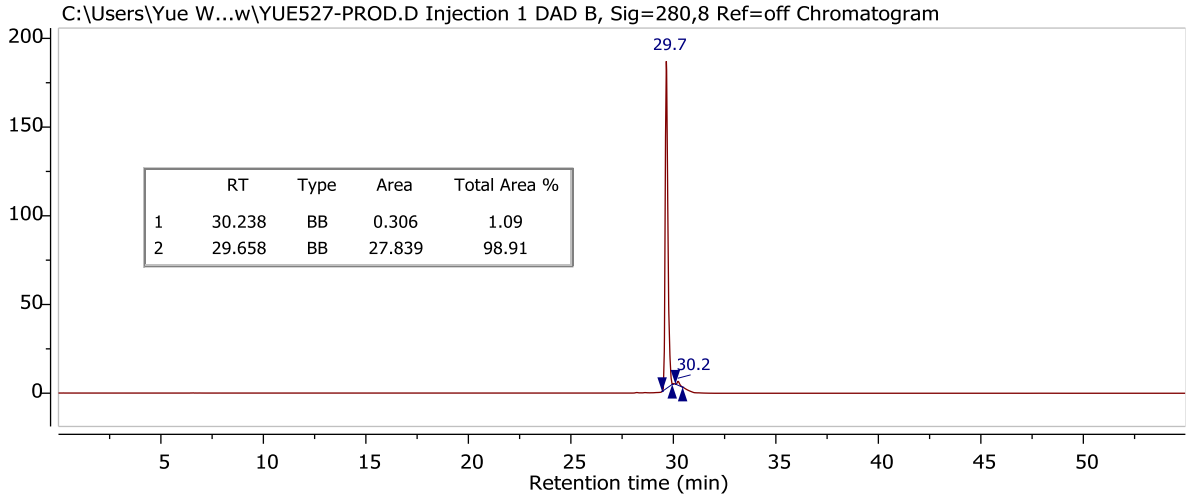
Analytic HPLC of **S5** (Gradient A, 280 nm).



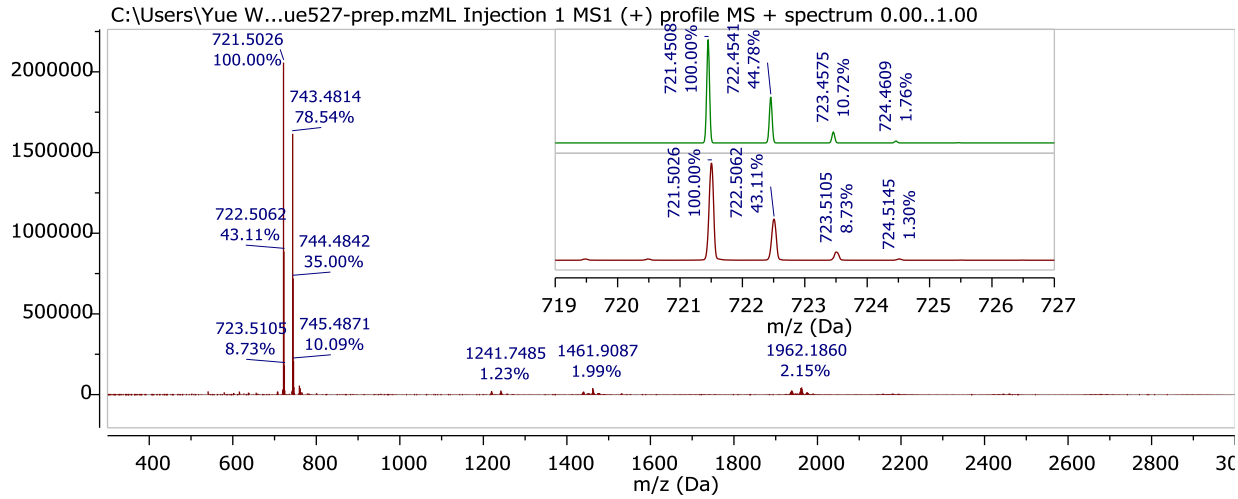
HRMS(MAIDL-TOF) of **S5**.



S6
Chemical Formula: $C_{37}H_{56}N_{10}O_5$
Exact Mass: 720.4435



Analytic HPLC of **S6** (Gradient A, 280 nm).



HRMS(MAIDL-TOF) of **S6**.