Preparation and conformational analysis of polyproline tri-helix macrocycle nanoscaffolds of varied sizes

Electronic Supplementary Information

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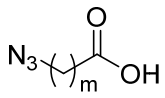
1. General methods for synthesis and characterization

All reagents and solvents were purchased from commercial source and without further purification. The composition of mixed solvents was given by volume ratio. Thin-layer chromatography (TLC) was performed on Merck Glass Plate TLC Silica gel 60 F_{254}. The spots were visualized by UV light and cerium ammonium molybdate solution or aqueous $KMnO_4$ solution. Column chromatography was performed on Merck Geduran Si 60 Silica gel (40-63 um). Nuclear magnetic resonance (NMR) spectroscopy was performed on Bruker AV-400 at 400 MHz (^{1}H). The signals are presented in parts per million (ppm, δ scale) unit. For ^{1}H NMR spectra, chemical shifts are expressed in ppm with residual proton signals in $CDCl_3$ (7.24 ppm) as standards. Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, tt = triplets of triplets, m = multiplet, br = broad), coupling constant (*J*) in Hertz (Hz) and integration.

2. Synthesis of connectors

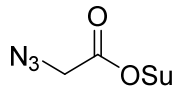


S1, m=1
S2, m=3

Azidoacetic acid (S1) and 4-azidobutanoic acid (S2)

To a solution of ethyl bromoacetate or ethyl 4-bromobutyrate (120 mmol) in DMF (60 mL) was added NaN_3 (23.4 g, 360 mmol) and stirred at 70 °C for overnight. The reaction mixture was added water (30 mL) before extraction with ethyl acetate (5 × 30 mL) and the combined organic layer was washed with brine (2 × 50 mL), dried over Na_2SO_4 and concentrated by rotary evaporation under a reduced pressure. The residue was purified by column chromatography (EtOAc/hexane = 1:10) to give ethyl azidoacetate or ethyl 4-azidobutyrate as a colorless oil, which was directly used in the next step.

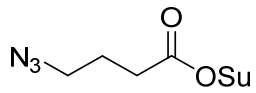
To a solution of ethyl azidooacetate or ethyl 4-azidobutyrate (10 mmol) in MeOH/H_2O = 1:1 (50 mL) was added $LiOH·H_2O$ (0.42 g, 10 mmol) and allowed to stir at rt for 1 h. The reaction mixture was added 1 N HCl (100 mL), extracted with ethyl acetate (3 × 100 mL), dried over Na_2SO_4 and concentrated by rotary evaporation under reduced pressure to give **S1** (1.0 g, 71%) or **S2** (1.2 g, 76%) as a light yellow oil, which was directly used without further purification. **S1**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (br s, 1H, COOH), 3.96 (s, 2H, CH_2). Proton NMR was consistent with literature data.[1] **S2**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.36 (t, J = 6.7 Hz, 2H), 2.45 (t, J = 7.2 Hz, 2H), 1.90 (tt, J = 6.9, 6.9 Hz, 2H). Proton NMR was consistent with literature data.[2]



20

Azidoacetic acid succinimidyl ester (20)

To a solution of azidoacetic acid **S1** (0.40 g, 4.0 mmol) and *N*-Hydroxysuccinimide (NHS) (0.55 g, 4.8 mmol) in CH_2Cl_2 (20 mL) was added 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (1.15 g, 6.0 mmol) and allowed to stir at rt for overnight. The reaction mixture was washed with 2.5% $NaHSO_4$(aq) and brine, dried over Na_2SO_4, concentrated by rotary evaporation under reduced pressure and without further purification to give **20** (0.42 g, 54%) as a white solid, which was directly used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.22 (s, 2H, CH_2N_3), 2.86 (s, 4H, OSu). Proton NMR was consistent with literature data.[3]



21

4-azidobutanoic acid succinimidyl ester (21)

To a solution of 4-azidobutanoic acid **S2** (0.69 g, 5.4 mmol) and NHS (0.93 g, 8.0 mmol) dissolved in CH_2Cl_2 (27 mL) was added EDC (1.85 g, 9.7 mmol) and allowed to stir at rt for overnight. The reaction mixture was washed with 2.5% $NaHSO_4$(aq) and brine, dried over Na_2SO_4 and concentrated by rotary evaporation under reduced pressure to give **21** (0.89 g, 73%) as a white solid, which was directly used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.43 (t, J = 6.6 Hz, 2H), 2.83 (br s, 4H, OSu), 2.71 (t, J = 7.2 Hz, 2H), 1.99 (tt, J = 6.9, 6.9 Hz, 2H). Proton NMR was consistent with literature data.[4]

3. Synthesis of peptide scaffolds

3-1. Solid phase peptide synthesis:

The peptides were prepared by manual solid phase peptide synthesis on 2-chlorotrityl chloride resins from Merck (Product No.855017). A solution of Fmoc-protected proline (Fmoc-Pro-OH, 4.0 equiv.), and *i*-Pr_2NEt (6.0 equiv.) in 1:1 v/v DMF/ CH_2Cl_2 (final concentration 0.1 M) was added to the resins and gently shaken at rt for overnight. After the reaction was finished, the resins were washed with DMF and CH_2Cl_2. A solution of CH_2Cl_2/MeOH/*i*-Pr_2NEt (17:2:1) was added and shaken for 1 h to block the unreacted sites on the resins. After washed with DMF and CH_2Cl_2, the amino acid loading was determined with a quantitative Fmoc test.

The resins were further used in iterative peptide synthesis. 10% piperidine in DMF was added to the resins to deprotect the Fmoc group. The reaction vessel was shaken for 10 min and washed with DMF and CH_2Cl_2. A solution of Fmoc-Pro-OH (4.0 equiv.), PyBOP (4.0 equiv., for Fmoc-Pro-OH) were dissolved in DMF and followed by the addition of NMM (4.0 equiv.) to give a final concentration of 0.05 M. The mixture was added to resins and gently shaken at rt for 1 h then washed with DMF and CH_2Cl_2.

After each coupling step, the resins were treated with Ac_2O (10 vol % in pyridine, 5.0 mL) for 10 min to cap the unreacted amino groups and then washed with DMF and CH_2Cl_2.

These procedures were repeated until the desired sequence was synthesized. To cleave the product peptide, the resins **8**-**13** were washed with CH_2Cl_2, treated with CH_2Cl_2/TFA/ (*i*-Pr)$_3$SiH (90:5:5) under shaking at rt for 1 h and repeated for a second time with 30 min shaking.

The filtrates were collected and all of the volatiles were removed under reduced pressure. The residues were dissolved in water (1-2 mL) and centrifuged before the supernatant was further purified by HPLC (Agilent Technology, 1260 Infinity) with a Vydac C18 column (218TP510 10 mm × 250 mm). 0.1% TFA in water (solvent A) and Acetonitrile (solvent B) served as the mobile phase for peptide chromatography.

3-2. Polyproline N-terminus azido modification:

A solution of azidoacetic acid succinimidyl ester **20** or 4-azidobutanoic acid succinimidyl ester **21** (8.0 equiv.) and *i*-Pr_2NEt (16.0 equiv.) in DMF was added to the resins carrying N-terminus deprotected peptide in DMF (final concentration of **20** or **21** at 0.05 M) under shaking at rt for 1 h. Then the resins were washed with DMF and CH_2Cl_2 and directly used for the next step.

3-3. Polyproline C-terminus alkyne modification:

A solution of the peptide acid obtained from resins **8**-**13** and hexafluorophosphate azabenzotriazole tetramethyl uronium (HATU) (4.0 equiv.) dissolved in DMF/CH_2Cl_2 (1:1, peptide concentration 20 mM) was added propargylamine (3.0 equiv.) and Et_3N (5.0 equiv.). After stirring for 1 h, CH_2Cl_2 was removed from the reaction mixture under reduced pressure. The remained mixture was purified by HPLC with a Vydac C18 column.

3-4. Peptide ligation by CuAAC reaction on resins:

The N-terminus azido-functionalized peptide on resins **22**-**27** were each treated

with a solution of corresponding alkynyl peptide **14**-**19** (1.5 equiv.), $CuSO_4$(aq) (0.13 equiv., 40 mM), tris(triazolyl)amine ligand[5] (0.13 equiv., 40 mM in DMSO), sodium ascorbate (aq) (2.6 equiv., 800 mM), and *i*-Pr_2NEt (4.0 equiv.) in THF (final copper concentration is 2 mM) to react at 25 °C for 16 h. The resulted resins were washed with sodium diethyldithiocarbamate solution (25 mg in 5 mL DMF with 25 μL *i*-Pr_2NEt), DMF and CH_2Cl_2.

3-5. Peptide cyclization:

A solution of peptide **34**-**40** dissolved in water at 2 mM, was added $CuSO_4$(aq) (4.2 equiv., 40 mM), tris(triazolyl)amine ligand (4.2 equiv., 40 mM in DMSO), sodium ascorbate (aq) (84 equiv., 800 mM), and *i*-Pr_2NEt (4.0 equiv.) to react at rt for 1 h. The resulted reaction mixture was purified by HPLC with a Vydac C18 column to give cyclized scaffold **1**-**7**.

4. Analytical data of peptide scaffolds

4-1. Synthesis of peptide monomers: peptide alkynes

Peptide **14-19** were prepared according to the methods for solid phase peptide synthesis and polyproline C-terminus alkyne modification. Yields are based on quantitative Fmoc test and the isolated weight after lyophilization.

Peptide **14**

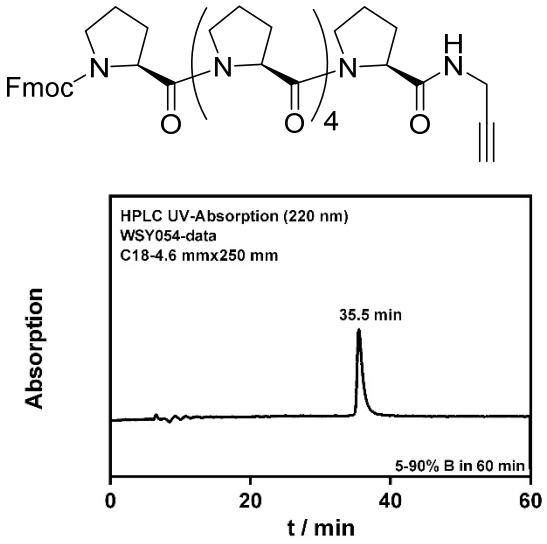


Figure S1. HPLC chromatogram of **14**

Yield: 26.9 mg, 53% (include SPPS)

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 35.5 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{48}H_{57}N_7NaO_8$: 882.416, found: 882.100.

Peptide **15**

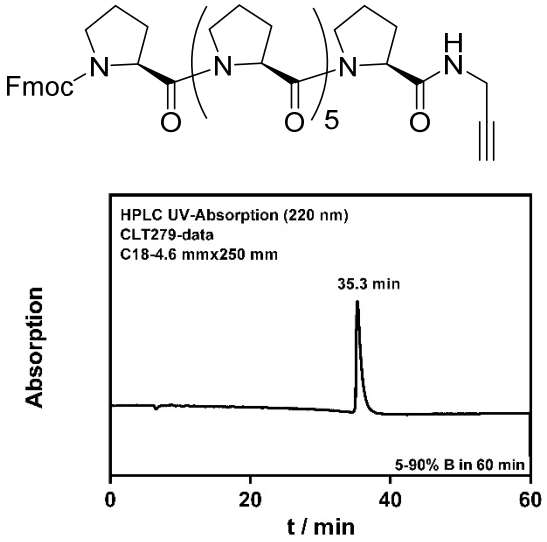


Figure S2. HPLC chromatogram of **15**

Yield: 143.5 mg, 84% (include SPPS)

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 35.3 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{53}H_{64}N_8NaO_9$: 979.469, found: 979.476.

Peptide **16**

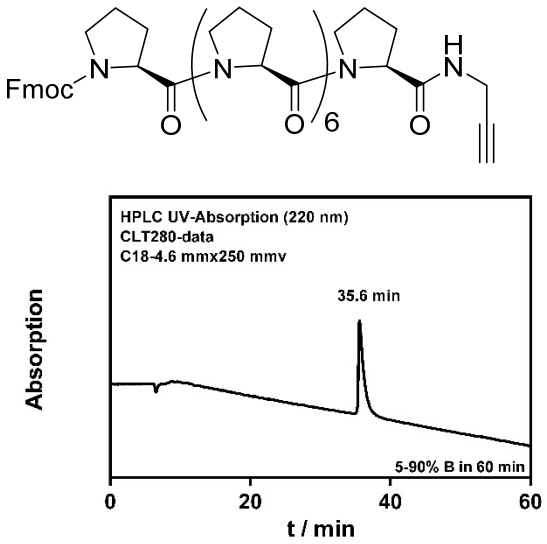


Figure S3. HPLC chromatogram of **16**

Yield: 120.9 mg, 65% (include SPPS)

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 35.6 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{58}H_{71}N_9NaO_{10}$: 1076.522, found: 1076.795.

Peptide **17**

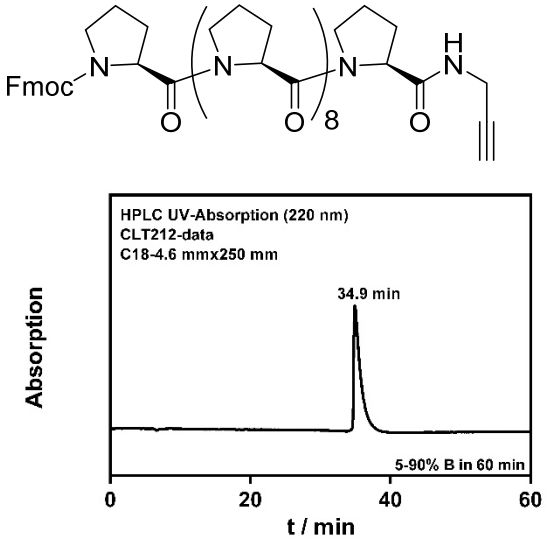


Figure S4. HPLC chromatogram of **17**

Yield: 80.8 mg, 51% (include SPPS)

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 34.9 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{68}H_{85}N_{11}NaO_{12}$: 1270.627, found: 1270.630.

Peptide **18**

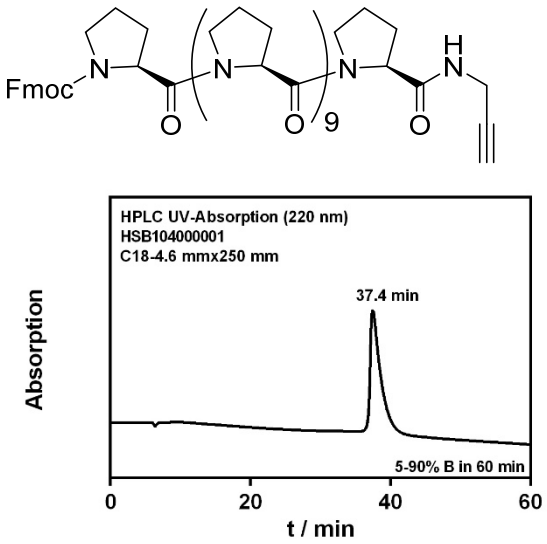


Figure S5. HPLC chromatogram of **18**

Yield: 97.7 mg, 79% (include SPPS)

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 37.4 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{73}H_{92}N_{12}NaO_{13}$: 1367.680, found: 1367.533.

Peptide **19**

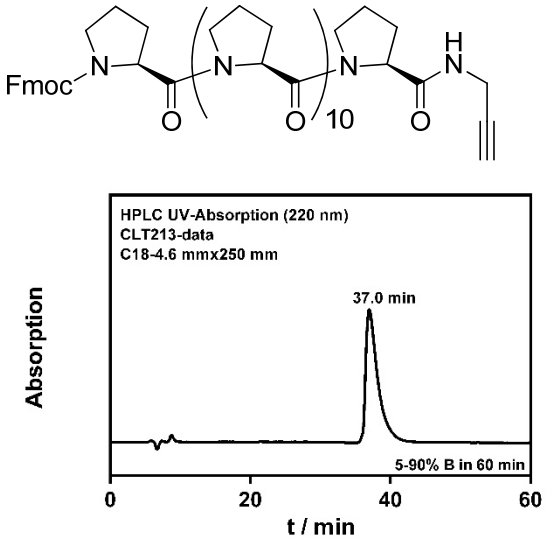


Figure S6. HPLC chromatogram of **19**

Yield: 103.3 mg, 56% (include SPPS)

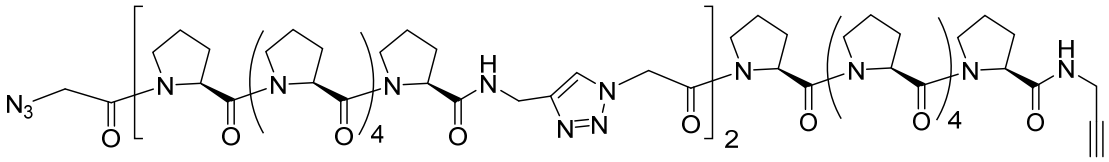
Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 37.0 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{78}H_{99}N_{13}NaO_{14}$: 1464.733, found: 1463.660.

4-2. Synthesis of linear peptide trimers: peptide alkynes

Peptide **34**-**36** and **38-40** were prepared according to the methods for solid phase peptide synthesis, polyproline N-terminus azido modification, peptide assembly by CuAAc reaction on resins and polyproline C-terminus alkyne modification. Yields are based on quantitative Fmoc test and the isolated weight after lyophilization.

Peptide **34a**



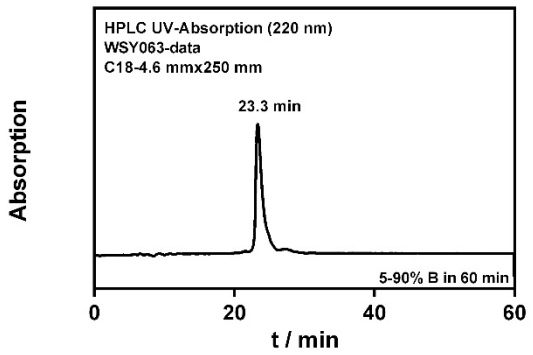


Figure S7. HPLC chromatogram of **34a**

Yield: 4.13 mg, 33% (include SPPS)

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 23.3 min.

MS(MALDI): $[M+H]^+$ calcd. For $C_{105}H_{145}N_{30}O_{21}$: 2162.120, found: 2163.269.

Peptide **34b**

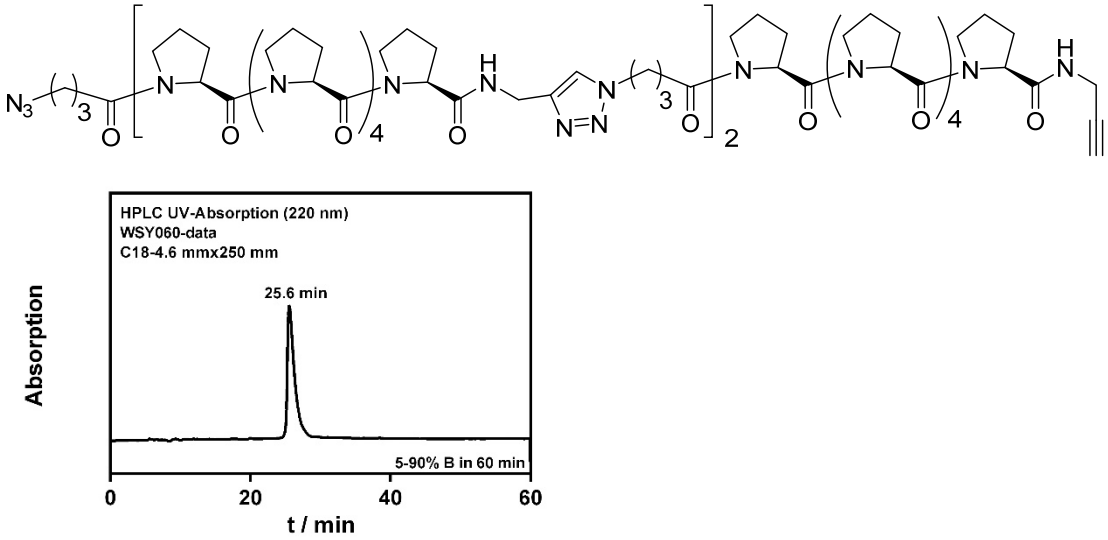


Figure S8. HPLC chromatogram of **34b**

Yield: 8.44 mg, 64% (include SPPS)

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 25.6 min.

MS(MALDI): $[M+H]^+$ calcd. For $C_{111}H_{157}N_{30}O_{21}$: 2246.213, found: 2248.461.

Peptide **35a**

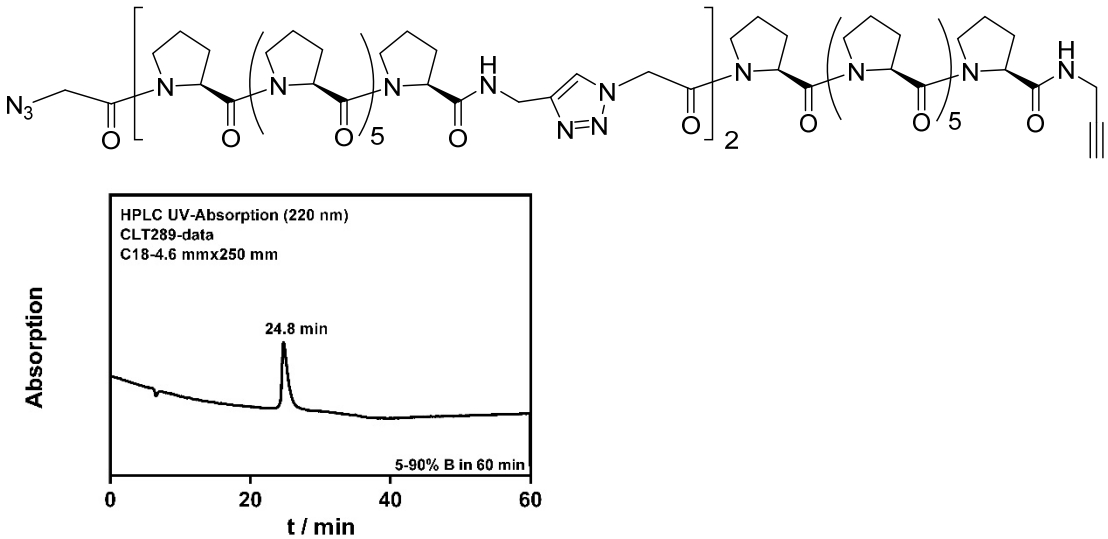


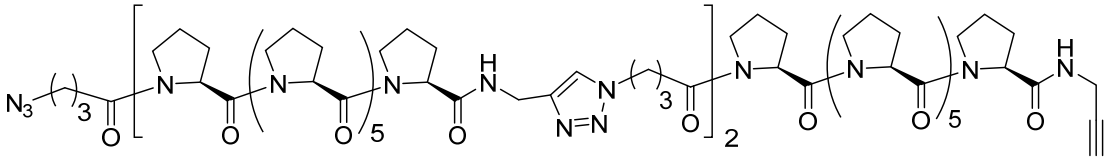
Figure S9. HPLC chromatogram of **35a**

Yield: 15.1 mg, 52% (include SPPS)

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 24.8 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{120}H_{165}N_{33}NaO_{24}$: 2475.260, found: 2476.361.

Peptide **35b**



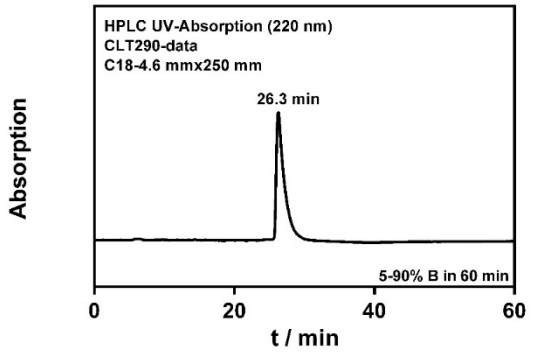


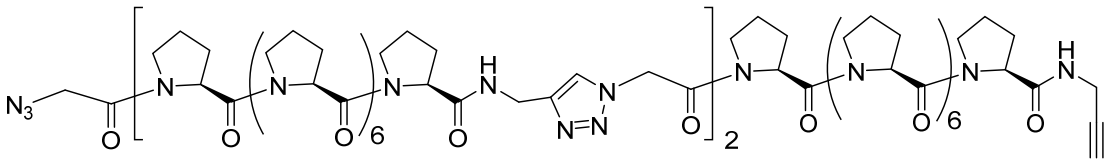
Figure S10. HPLC chromatogram of **35b**

Yield: 17.8 mg, 69% (include SPPS)

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 26.3 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{126}H_{177}N_{33}NaO_{24}$: 2559.354, found: 2559.550.

Peptide **36a**



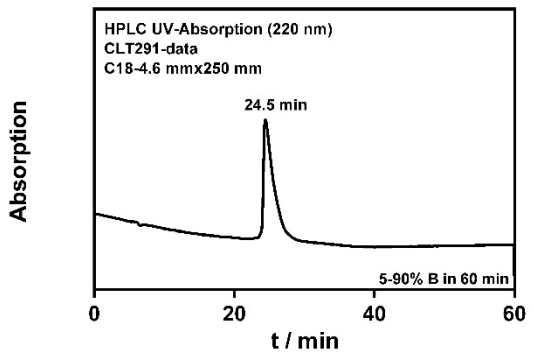


Figure S11. HPLC chromatogram of **36a**

Yield: 13.8 mg, 57% (include SPPS)

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 24.5 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{135}H_{186}N_{36}NaO_{27}$: 2766.418, found: 2767.095.

Peptide **36b**

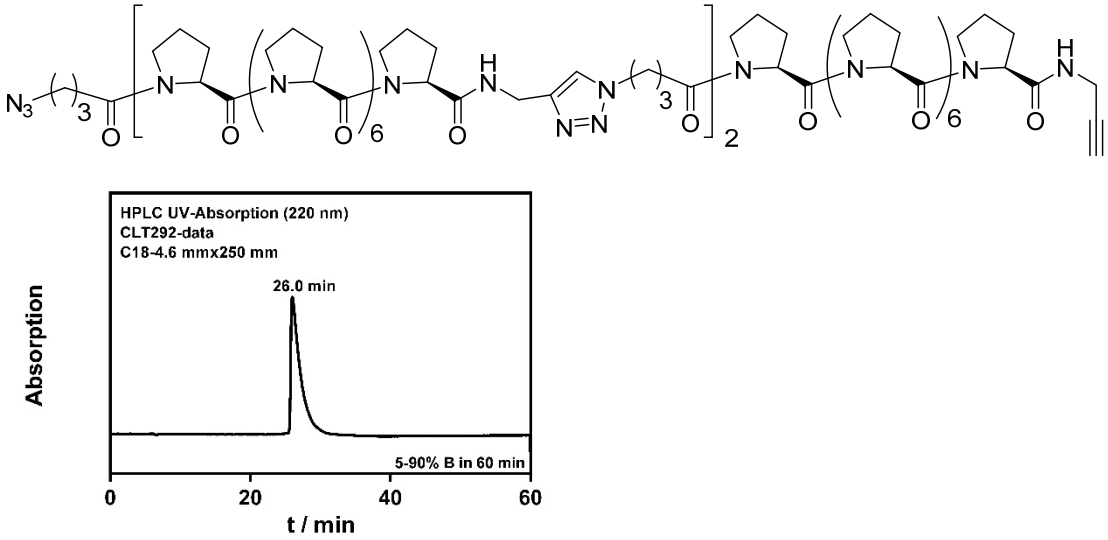


Figure S12. HPLC chromatogram of **36b**

Yield: 15.4 mg, 63% (include SPPS)

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 26.0 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{141}H_{198}N_{36}NaO_{27}$: 2850.512, found: 2851.875.

Peptide **38a**

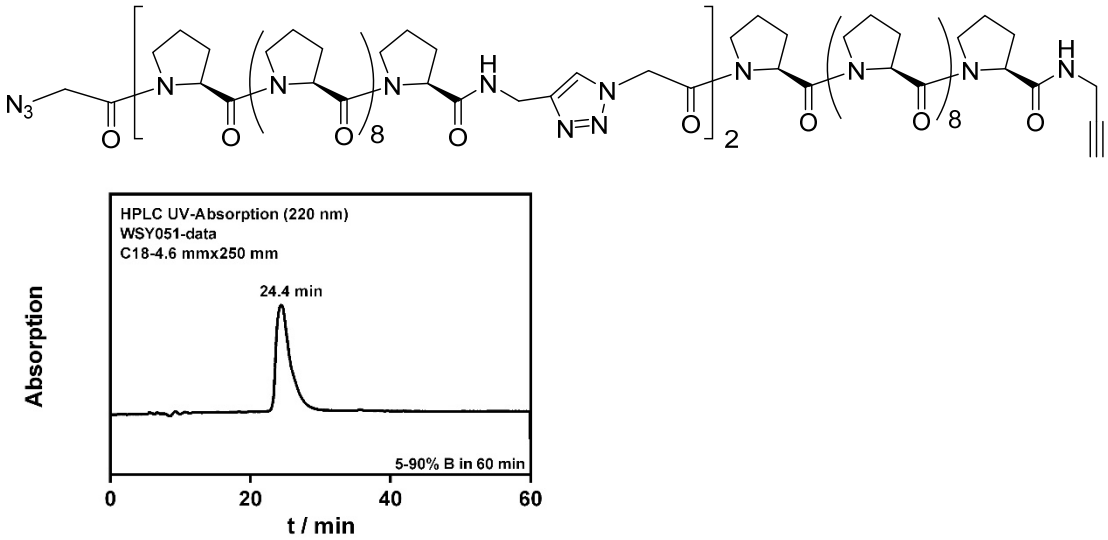


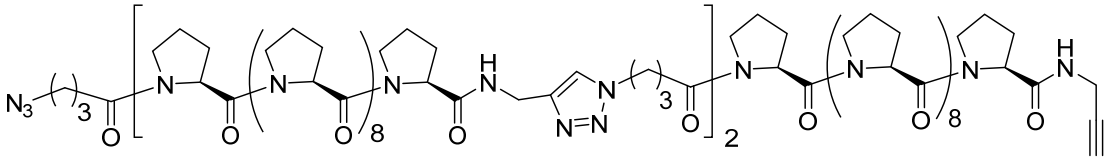
Figure S13. HPLC chromatogram of **38a**

Yield: 1.72 mg, 31% (include SPPS)

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 24.4 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{165}H_{228}N_{42}NaO_{33}$: 3348.735, found: 3350.018.

Peptide **38b**



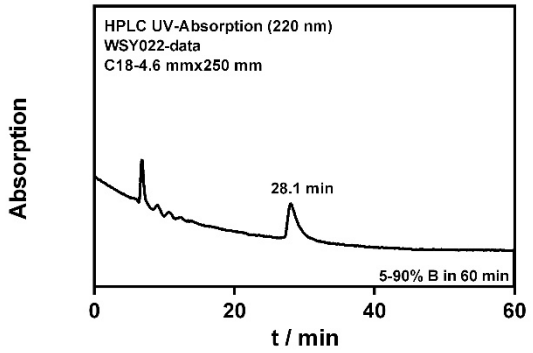


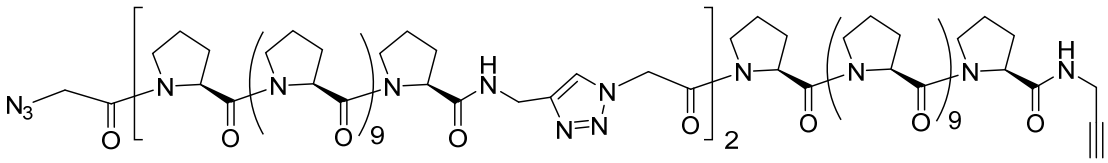
Figure S14. HPLC chromatogram of **38b**

Yield: 0.14 mg, 36% (include SPPS)

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 28.1 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{171}H_{240}N_{42}NaO_{33}$: 3432.829, found: 3433.745.

Peptide **39a**



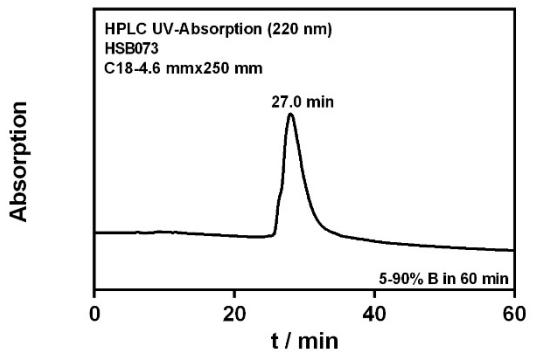


Figure S15. HPLC chromatogram of **39a**

Yield: 4.10 mg, 37% (include SPPS)

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 27.0 min.

MS(MALDI): $[M+H]^+$ calcd. for $C_{180}H_{250}N_{45}O_{36}$: 3617.911, found: 3615.941.

Peptide **39b**

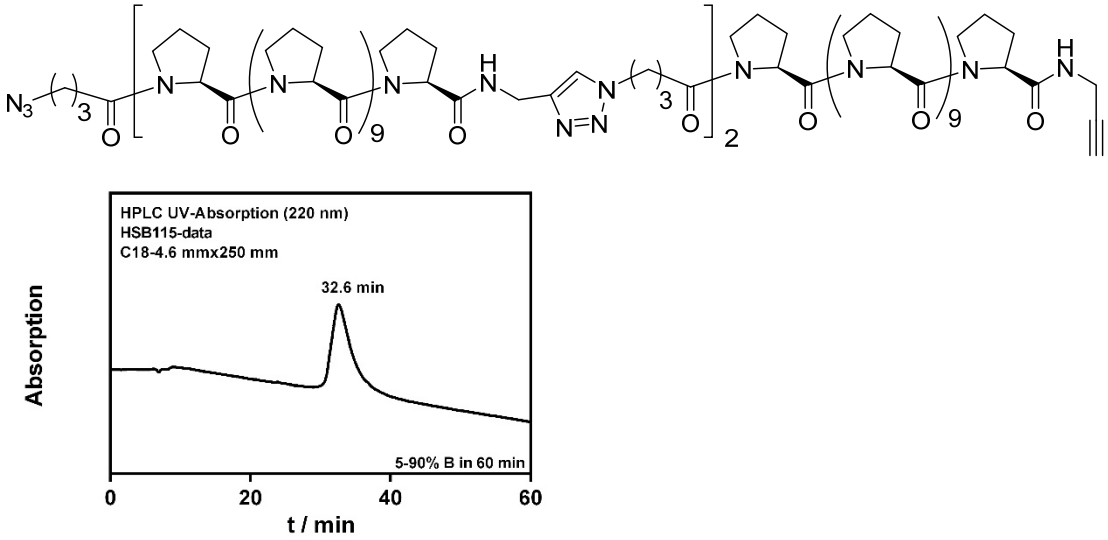


Figure S16. HPLC chromatogram of **39b**

Yield: 3.20 mg, 37% (include SPPS)

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 32.6 min.

MS(MALDI): $[M+Na]^+$ calcd. for $C_{186}H_{261}N_{45}NaO_{36}$: 3723.987 found: 3723.433.

Peptide **40a**

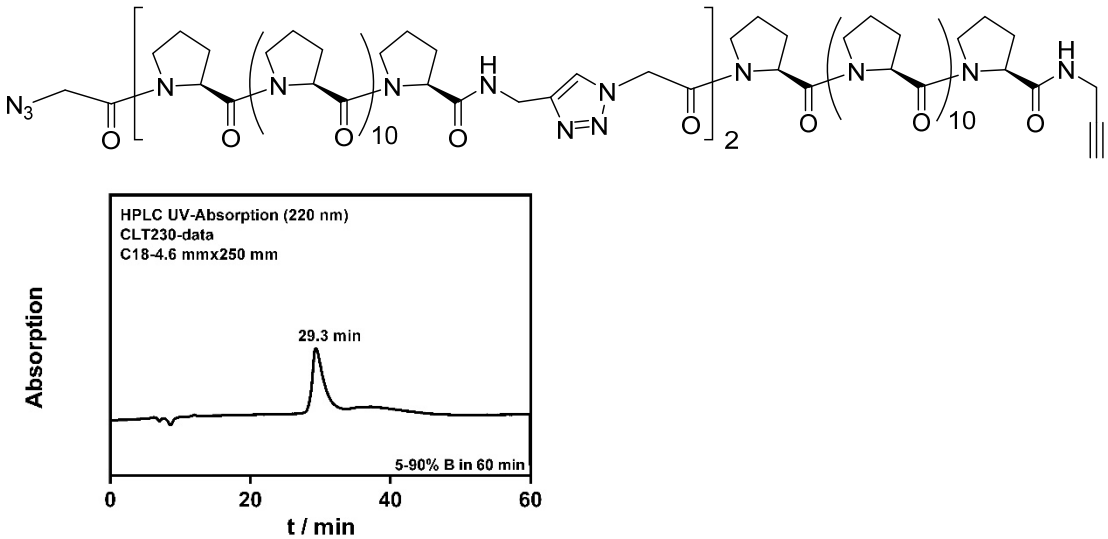


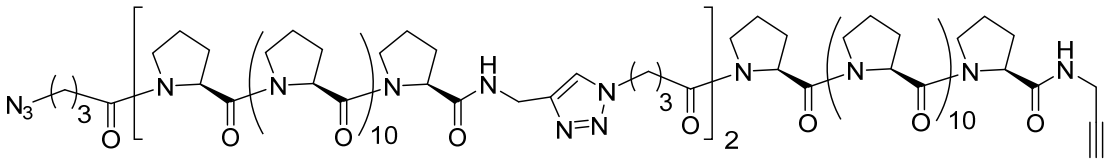
Figure S17. HPLC chromatogram of **40a**

Yield: 4.59 mg, 36% (include SPPS)

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 29.3 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{195}H_{270}N_{48}NaO_{39}$: 3931.051, found: 3930.834.

Peptide **40b**



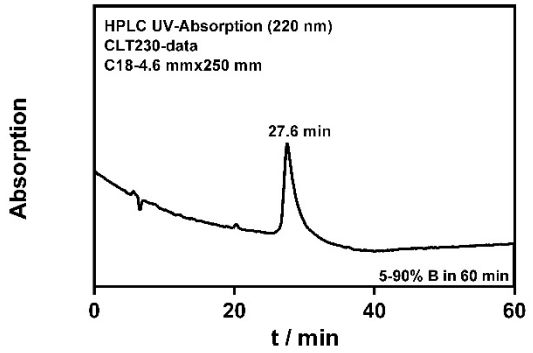


Figure S18. HPLC chromatogram of **40b**

Yield: 4.18 mg, 26% (include SPPS)

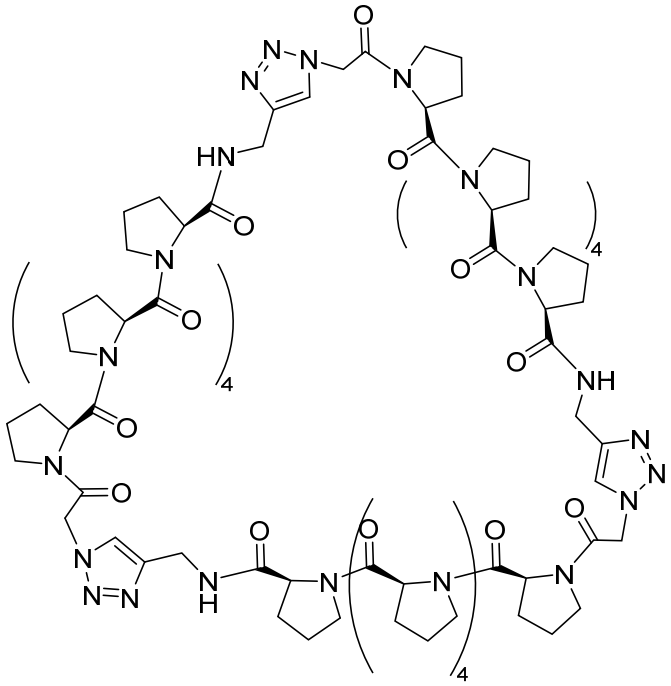
Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 27.6 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{201}H_{282}N_{48}NaO_{39}$: 4015.145, found: 4014.469.

4-3. Synthesis of cyclic peptide trimers

Peptide **1**-**7** were prepared according to the methods for peptide cyclization. Yields are based on the isolated weight after lyophilization.

Peptide **1a**



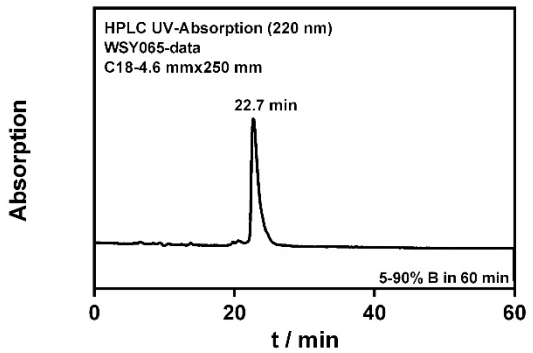


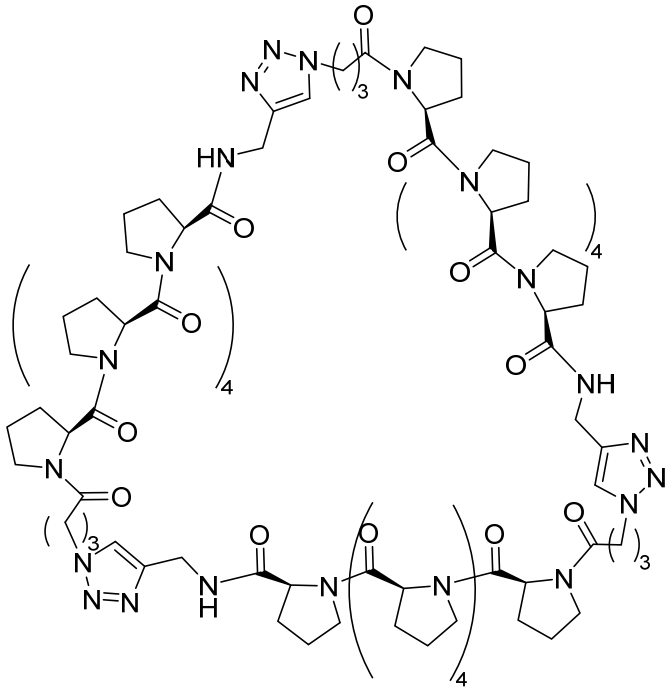
Figure S19. HPLC chromatogram of **1a**

Yield: 1.54 mg, 60%

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 22.7 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{105}H_{144}N_{30}NaO_{21}$: 2184.101, found: 2185.917.

Peptide **1b**



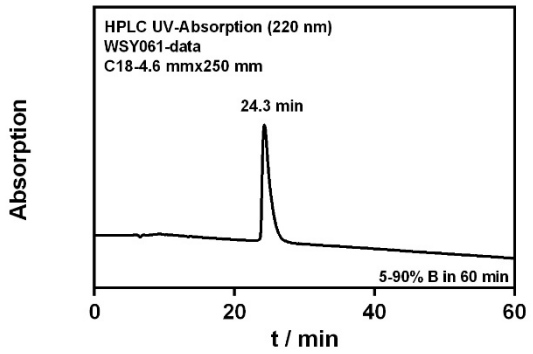


Figure S20. HPLC chromatogram of **1b**

Yield: 3.34 mg, 55%

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 24.3 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{111}H_{156}N_{30}NaO_{21}$: 2268.195, found: 2269.628.

Peptide **2a**

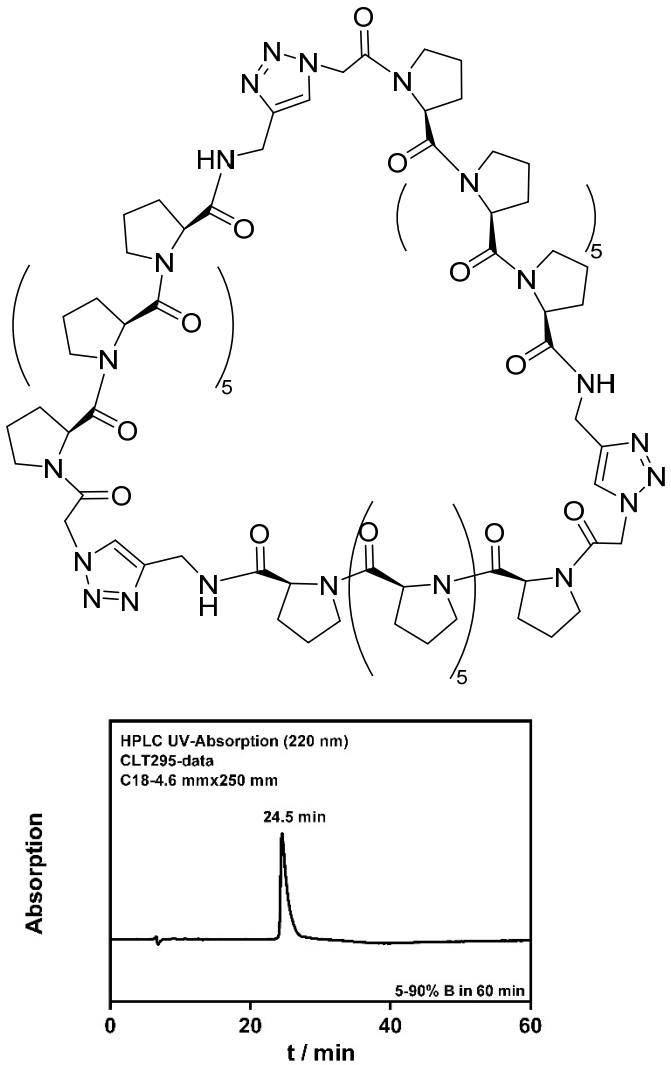


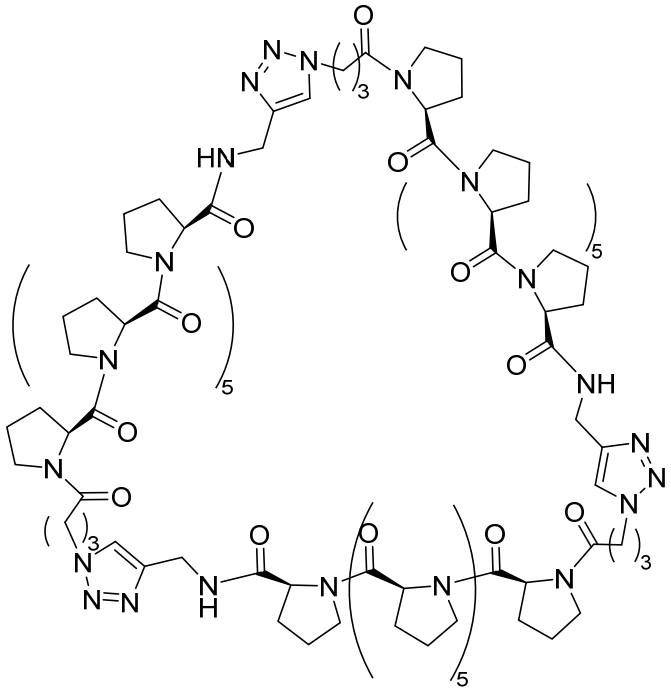
Figure S21. HPLC chromatogram of **2a**

Yield: 4.06 mg, 58%

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 24.5 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{120}H_{165}N_{33}NaO_{24}$: 2475.260, found: 2476.730.

Peptide **2b**



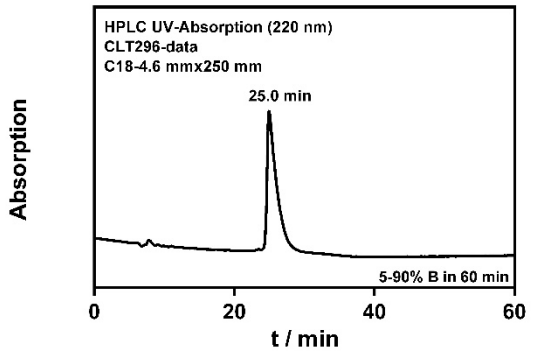


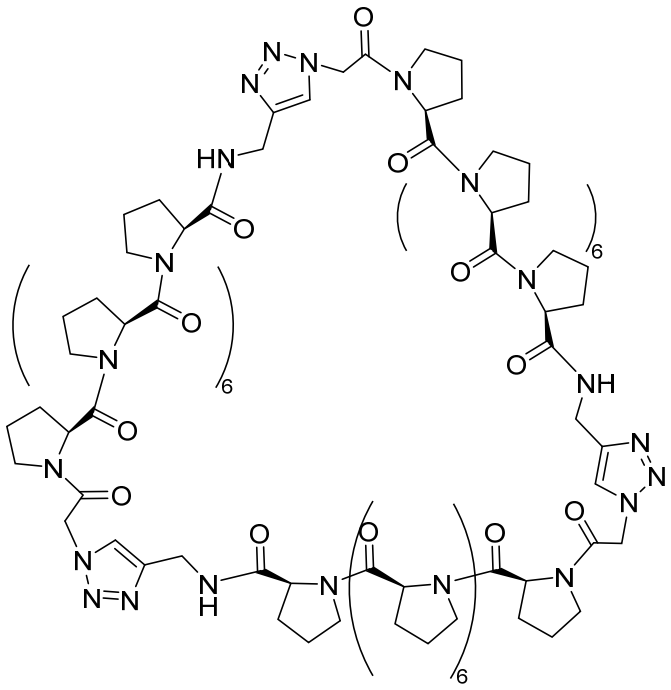
Figure S22. HPLC chromatogram of **2b**

Yield: 2.73 mg, 39%

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 25.0 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{126}H_{177}N_{33}NaO_{24}$: 2559.354, found: 2560.927.

Peptide **3a**



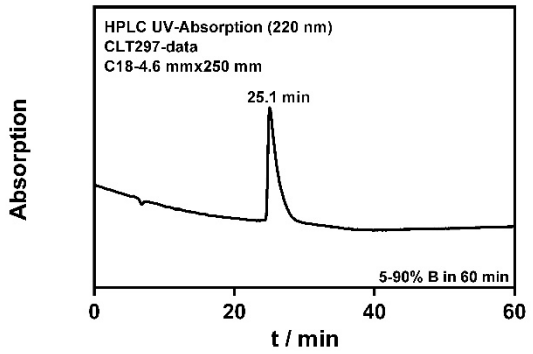


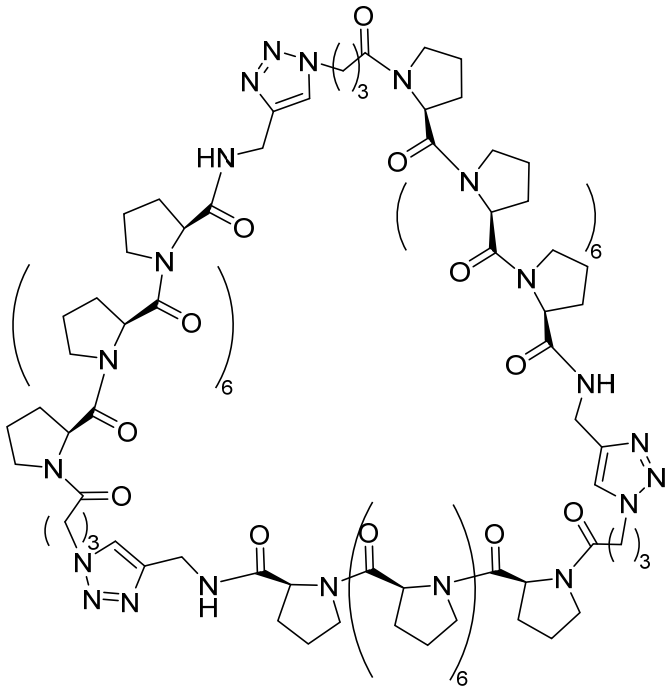
Figure S23. HPLC chromatogram of **3a**

Yield: 2.71 mg, 39%

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 25.1 min.

MS(MALDI): $[M+H]^+$ calcd. For $C_{135}H_{187}N_{36}O_{27}$: 2744.436, found: 2744.941.

Peptide **3b**



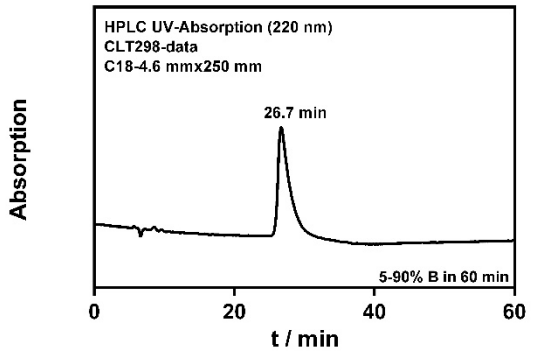


Figure S24. HPLC chromatogram of **3b**

Yield: 2.86 mg, 41%

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 26.7 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{141}H_{198}N_{36}NaO_{27}$: 2850.512, found: 2850.286.

Peptide **5a**

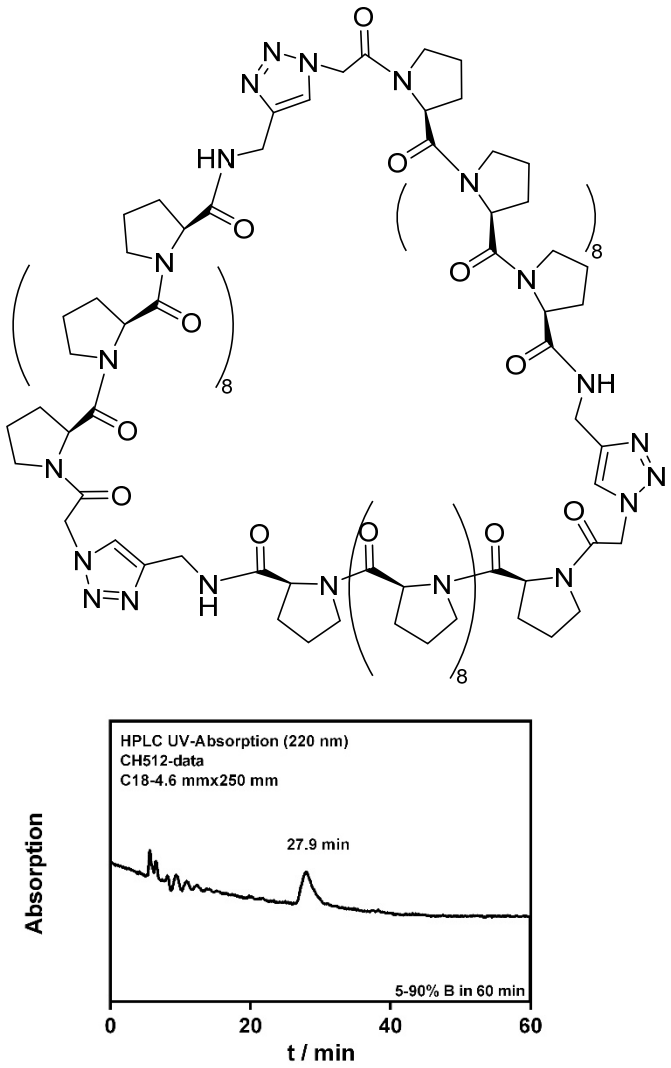


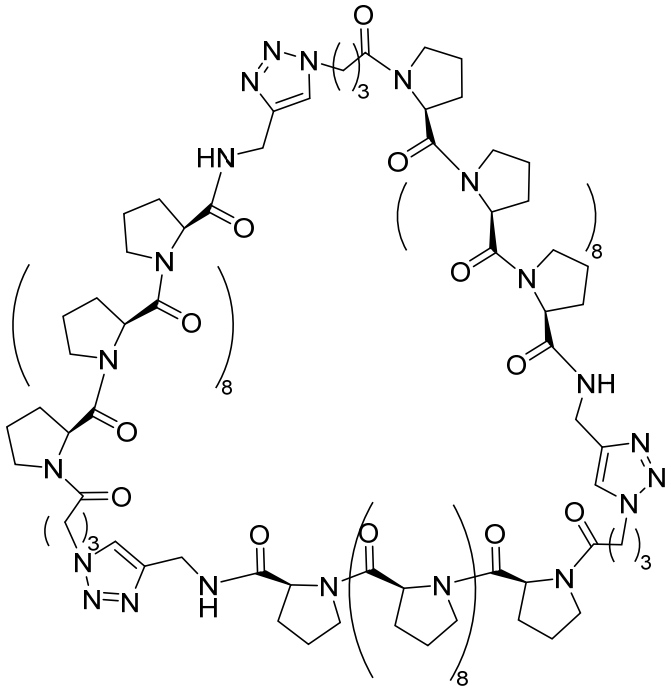
Figure S25. HPLC chromatogram of **5a**

Yield: 0.27 mg, 56%

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 27.9 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{165}H_{228}N_{42}NaO_{33}$: 3348.735, found: 3349.855.

Peptide **5b**



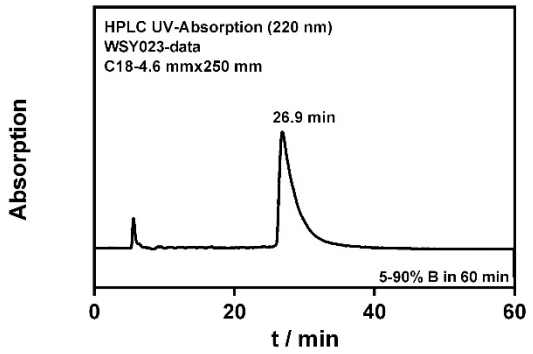


Figure S26. HPLC chromatogram of **5b**

Yield: 0.30 mg, 34%

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 26.9 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{171}H_{240}N_{42}NaO_{33}$: 3432.829, found: 3431.031.

Peptide **6a**

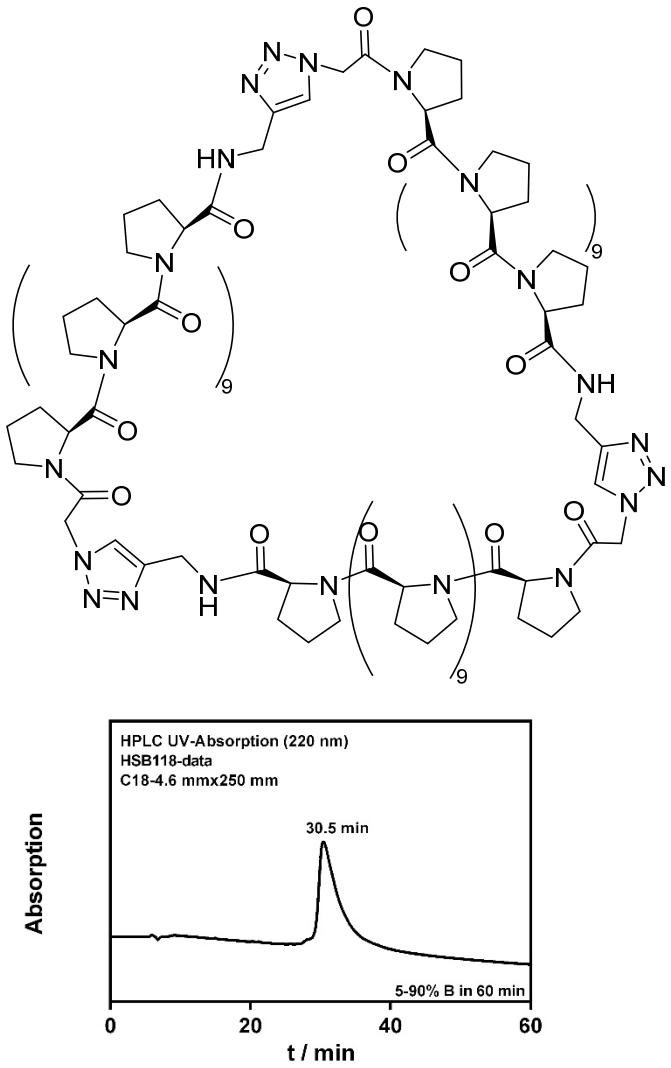


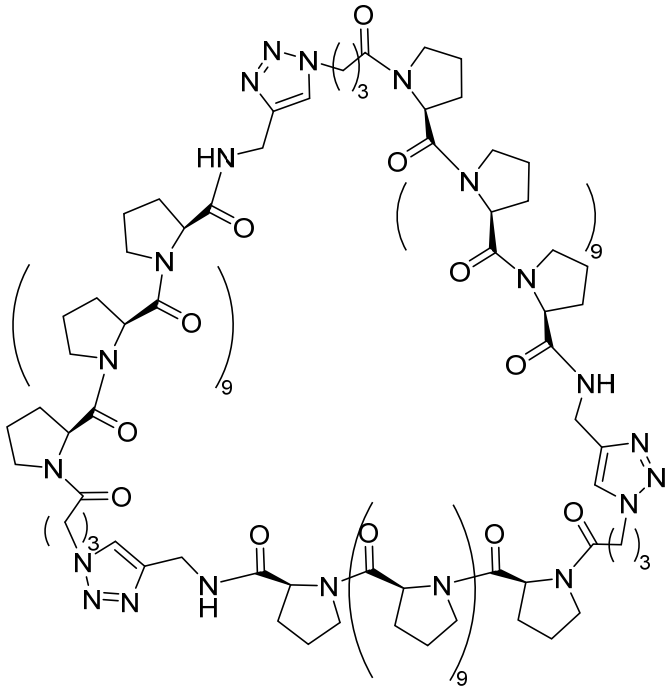
Figure S27. HPLC chromatogram of **6a**

Yield: 0.70 mg, 51%

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 30.5 min.

MS(MALDI): $[M+H]^+$ calcd. For $C_{180}H_{250}N_{45}O_{36}$: 3617.911, found: 3616.592.

Peptide **6b**



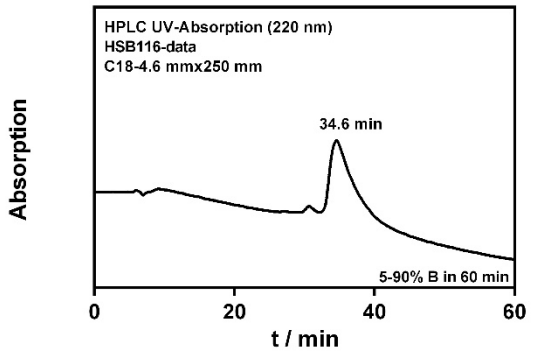


Figure S28. HPLC chromatogram of **6b**

Yield: 0.70 mg, 30%

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.5 mL/min; t_R = 34.6 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{186}H_{261}N_{45}NaO_{36}$: 3723.987, found: 3724.313.

Peptide **7a**

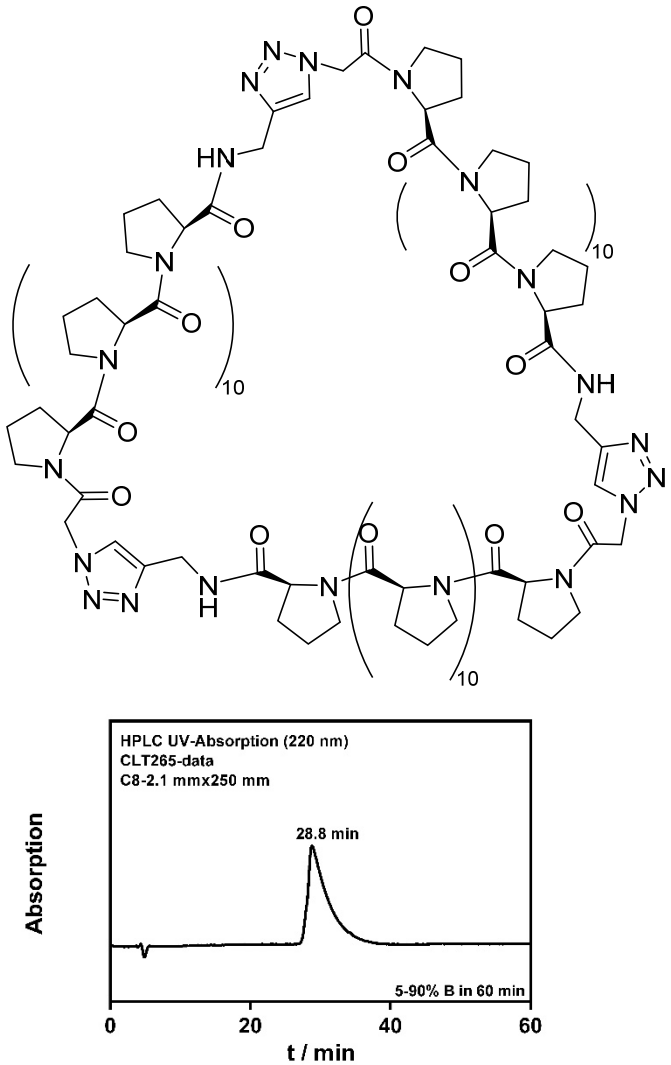


Figure S29. HPLC chromatogram of **7a**

Yield: 0.90 mg, 39%

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.2 mL/min; t_R = 28.8 min.

MS(MALDI): $[M+Na]^+$ calcd. For $C_{195}H_{270}N_{48}NaO_{39}$: 3931.051, found: 3930.732.

Peptide **7b**

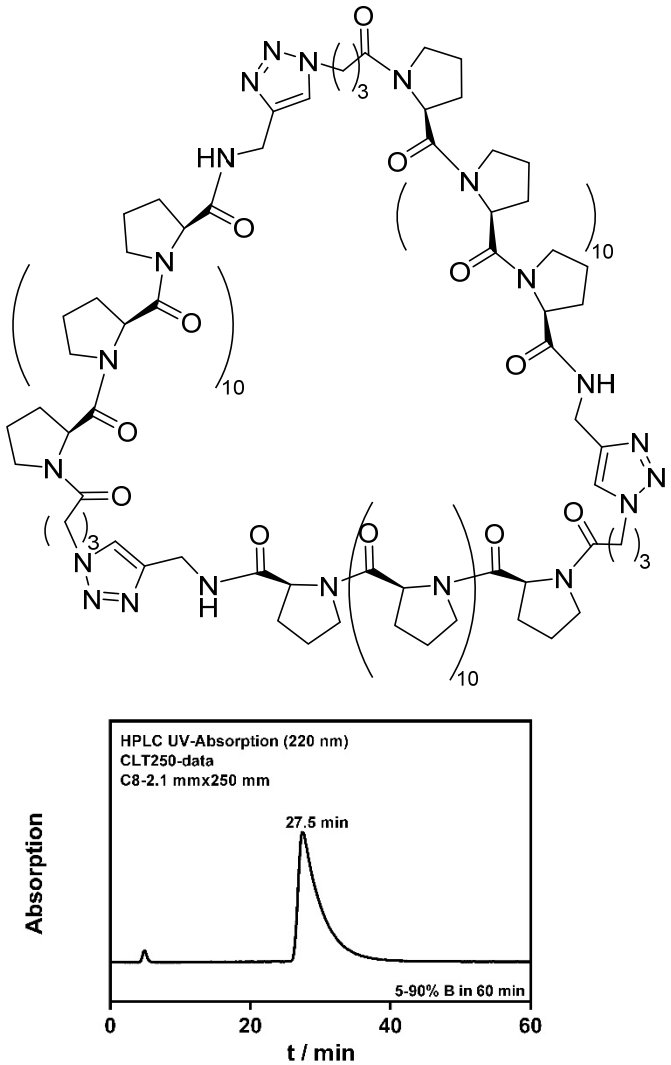


Figure S30. HPLC chromatogram of **7b**

Yield: 0.85 mg, 36%

Analytical HPLC: 5% to 90% B (ACN) over 60 min, 0.2 mL/min; t_R = 27.5 min.

MS(MALDI): $[M+H]^+$ calcd. For $C_{201}H_{283}N_{48}O_{39}$: 3993.163, found: 3993.958.

5. Circular dichroism spectroscopy

The CD spectrum measurements were conducted on an Aviv Model 410 circular dichroism spectrometer. For the measurements in aqueous conditions, the peptide (100 μM) was dissolved in H_2O and incubated for more than 72 h at rt. For measurement in *n*-propanol, the peptide (100 μM) was dissolved in 99.7 % *n*-propanol and incubated for more than 96 h at rt before measurement.

The CD spectrum was measured in the cuvette with a path length of 1.0 mm and contained peptide solution (300 μL) at 25°C. The measured wavelength ranged from 260 to 195 nm, and the absorbance at 10 s averaging was collected. The raw data were normalized according to the concentration of peptide, path length of cuvette and the number of proline residues. λ_{max} was identified after smoothing the CD spectrum with the Origin 8.5.

6. Ion mobility spectrometry–mass spectrometry

Electrospray ionization (ESI) mass spectrometry and traveling wave ion mobility (TWIM) experiments were conducted on a Waters Synapt HDMS G2 instrument with a LockSpray ESI source, following procedures described in the literature.[6] The used parameters are as follows: ESI capillary voltage, 3.0 kV; sample cone voltage, 35-85 V; extraction cone voltage, 0 V; desolvation gas flow, 500 L/h (N_2); trap collision energy (CE), 4 V; transfer CE, 0 V; trap gas flow, 2.0 mL/min (Ar); helium cell gas flow, 180.0 mL/min; ion mobility cell gas flow, 90.0 mL/min (N_2); sample flow rate, 5 μL/min; source temperature, 30-50 °C; desolvation temperature, 30-80 °C; IMS wave height, 26.3 V; and IMS wave velocity, 683 m/s. Data were collected and analyzed using MassLynx 4.1 and DriftScope 2.4 (Waters). The drift time were determined via Gaussian fitting of chromatogram using Origin 8.5.[7]

7. Molecular modeling

The PPII helix structure acquired from CCDC was arbitrarily organized into a triangular or propeller arrangement,[8] and then the helices were connected with connectors. A number of the arrangements were generated with alteration in the position (helices were moved in/out to the centroid), rotation angle and pitch (propeller) angle of the helices. After constraining the PPII structure, each of the arrangements was proceeded with Geometry Optimization in the Forcite module of Materials Studio version 7.0 program (Accelrys Software, Inc.) to optimize the structure of connectors and subjected to Anneal and Geometry Optimization. An initially energy-minimized structure was subjected to 300 annealing cycles with the initial and midcycle temperatures of 300 and 1400 K, respectively, twenty heating

ramps per cycle, one thousand dynamics steps per ramp, and one dynamics step per femtosecond. For each arrangement, 300 conformers after annealing were generated and converted to CCSs (*vide infra*).

8. Calculation of collision cross sections (CCSs)

The CCS calibration curve was established according to the reported protocol[9, 10] using published CCSs of cytochrome *c* (bovine), reserpine, and insulin (human).[11-15] A plot of corrected drift times versus CCSs of calibrants fitted with power functions was used as a calibration curve for experimental CCS measurements.

Molecular models of two possible helix arrangements for **1b** were created to obtain the corresponding theoretical CCSs. Each conformer was converted into the corresponding CCS using the trajectory method (TM) and projection approximation (PA) in MOBCAL.[16, 17]

9. IMS-MS data of peptide scaffolds

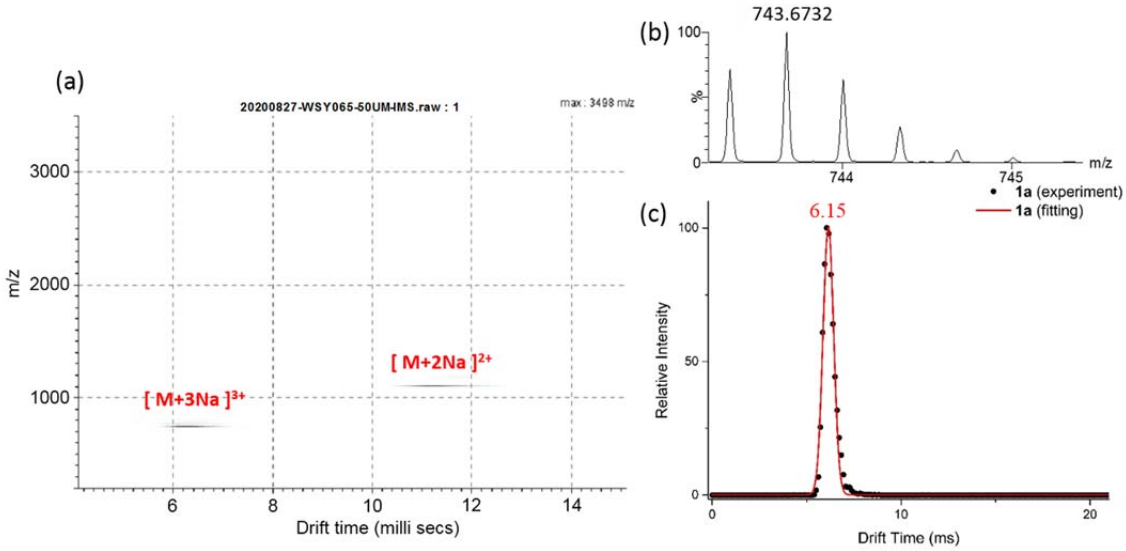


Figure S31. (a) IMS-MS spectrum; (b) isotope pattern and (c) drift-time distribution of [**1a** + 3Na]$^{3+}$. The drift-time distribution was fitted by Gaussian normal distribution (red line) from the experimental data (black dots).

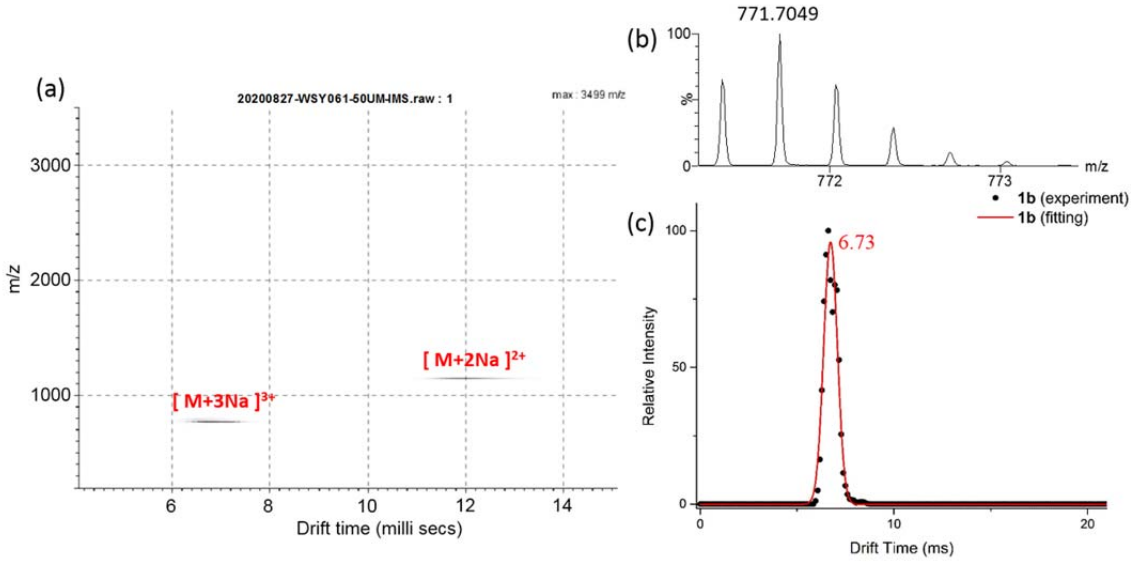


Figure S32. (a) IMS-MS spectrum; (b) isotope pattern and (c) drift-time distribution of [**1b** + 3Na]$^{3+}$. The drift-time distribution was fitted by Gaussian normal distribution (red line) from the experimental data (black dots).

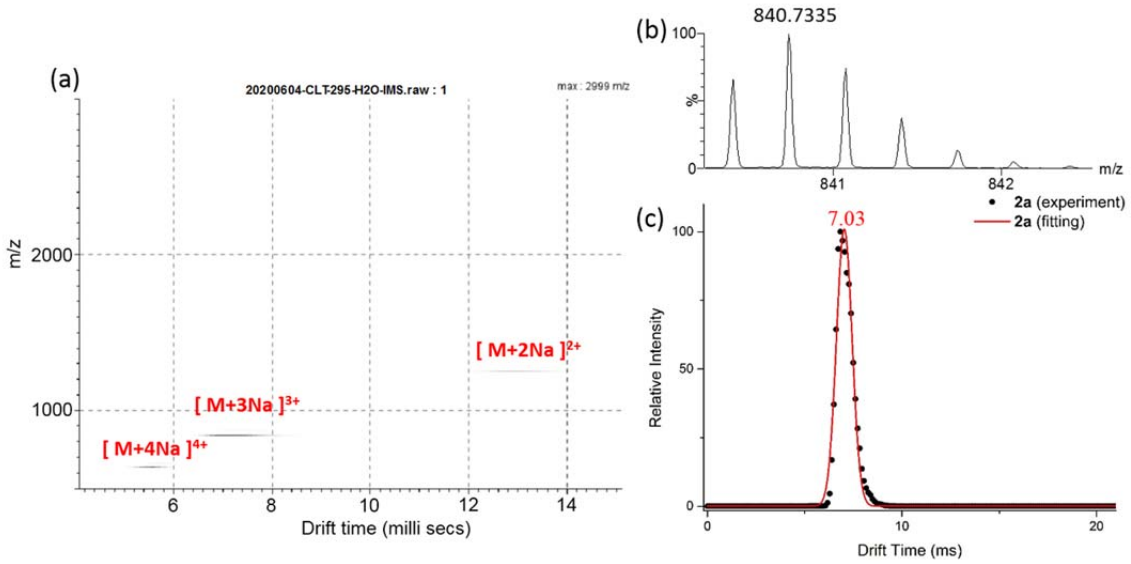


Figure S33. (a) IMS-MS spectrum; (b) isotope pattern and (c) drift-time distribution of [**2a** + 3Na]$^{3+}$. The drift-time distribution was fitted by Gaussian normal distribution (red line) from the experimental data (black dots).

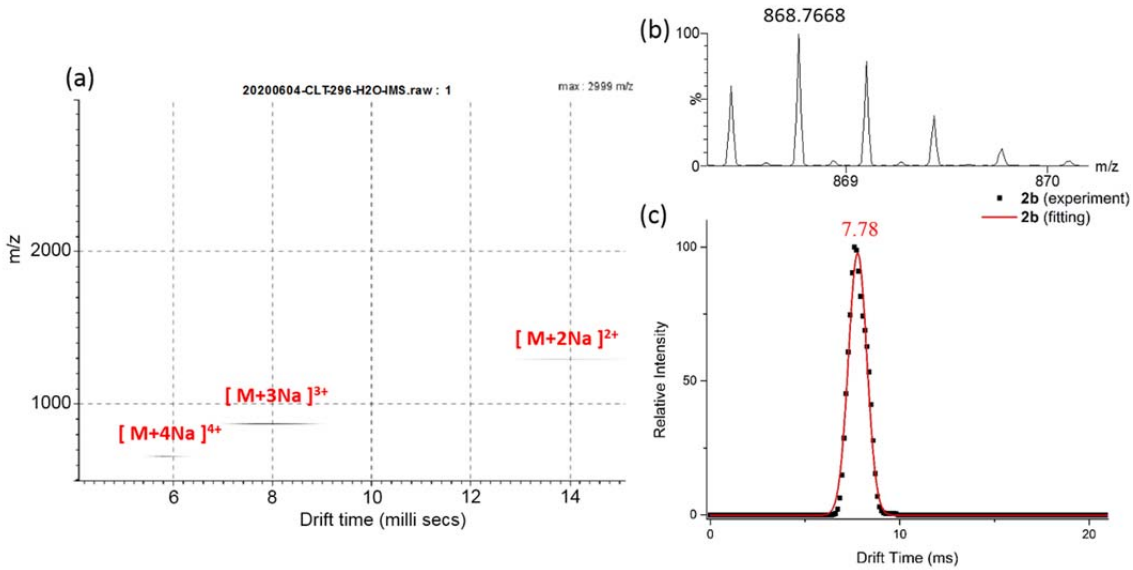


Figure S34. (a) IMS-MS spectrum; (b) isotope pattern and (c) drift-time distribution of [**2b** + 3Na]$^{3+}$. The drift-time distribution was fitted by Gaussian normal distribution (red line) from the experimental data (black dots).

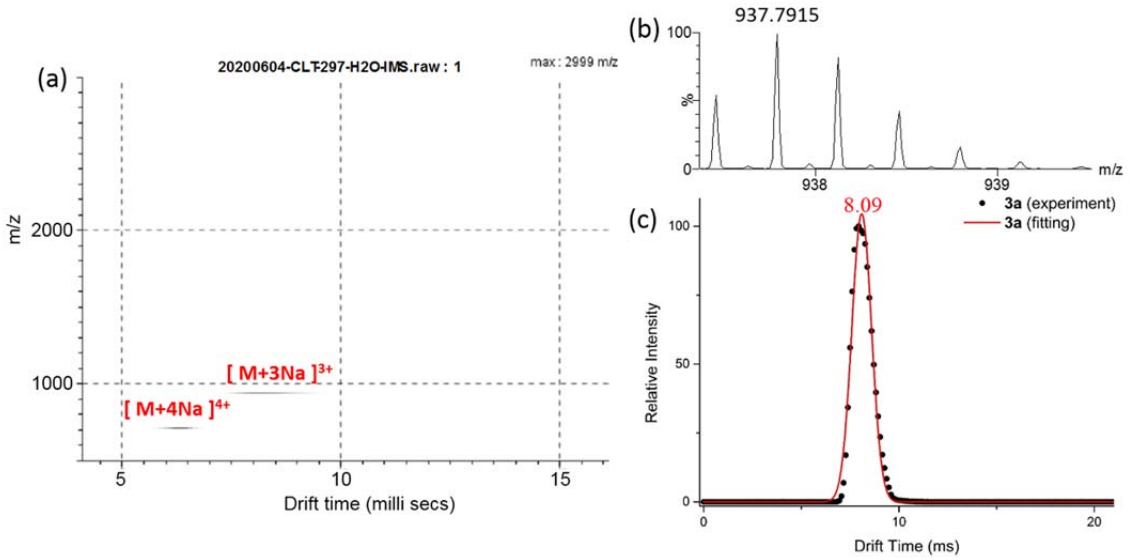


Figure S35. (a) IMS-MS spectrum; (b) isotope pattern and (c) drift-time distribution of [**3a** + 3Na]$^{3+}$. The drift-time distribution was fitted by Gaussian normal distribution (red line) from the experimental data (black dots).

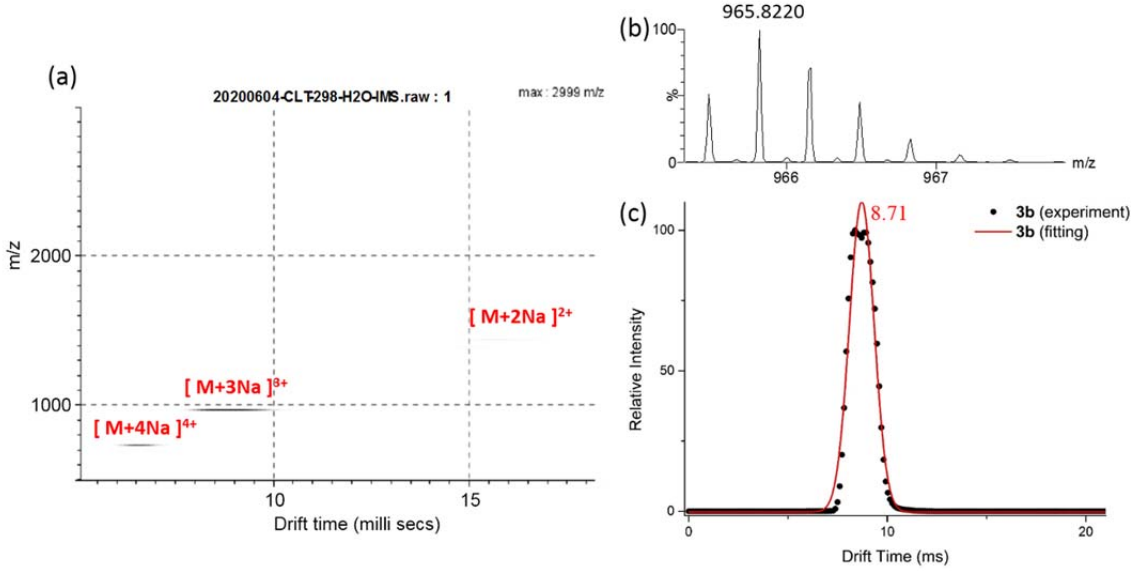


Figure S36. (a) IMS-MS spectrum; (b) isotope pattern and (c) drift-time distribution of [**3b** + 3Na]$^{3+}$. The drift-time distribution was fitted by Gaussian normal distribution (red line) from the experimental data (black dots).

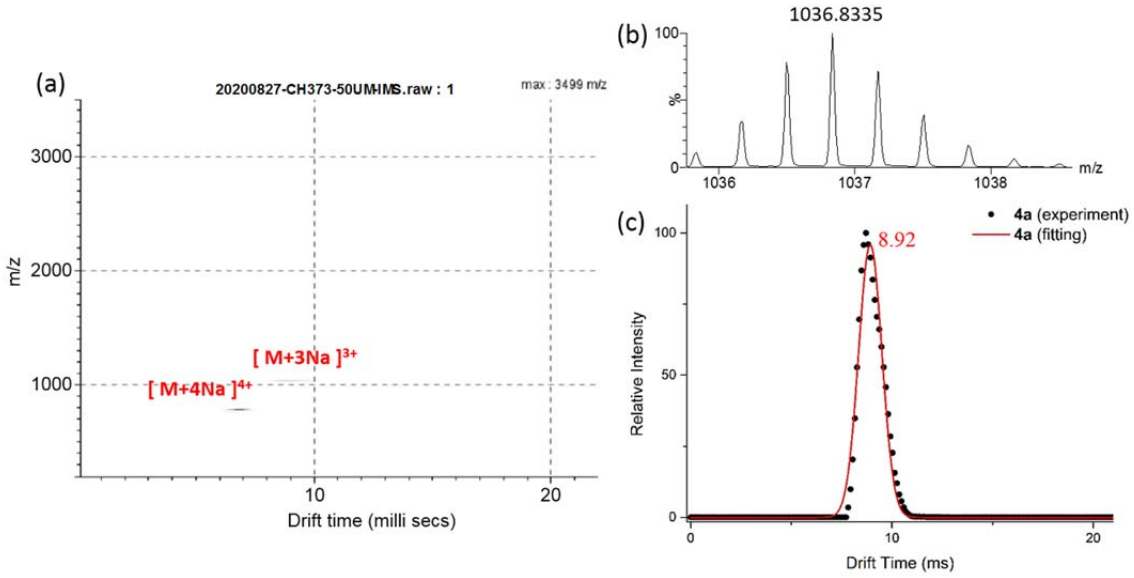


Figure S37. (a) IMS-MS spectrum; (b) isotope pattern and (c) drift-time distribution of [**4a** + 3Na]$^{3+}$. The drift-time distribution was fitted by Gaussian normal distribution (red line) from the experimental data (black dots).

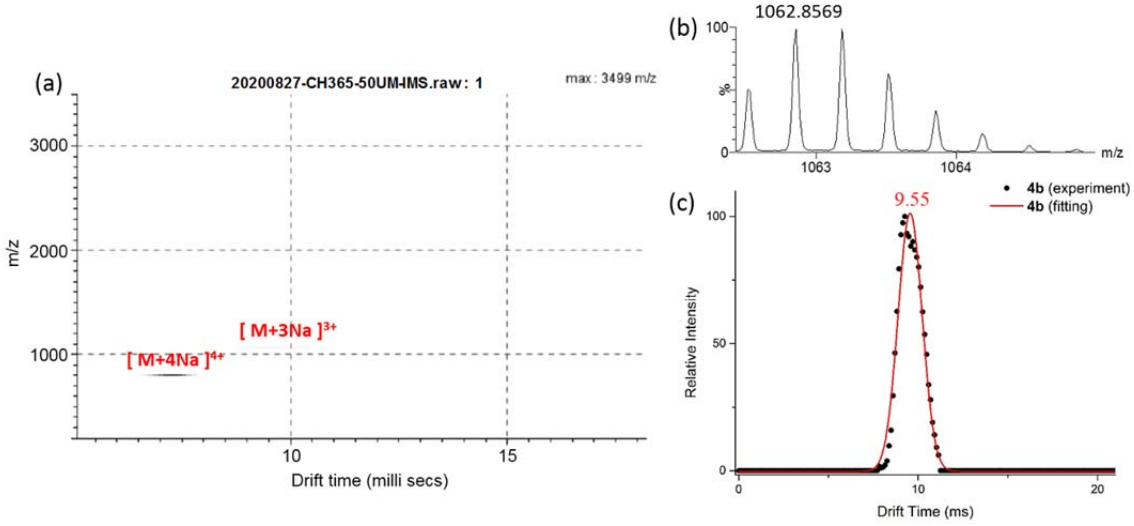


Figure S38. (a) IMS-MS spectrum; (b) isotope pattern and (c) drift-time distribution of [**4b** + 3Na]$^{3+}$. The drift-time distribution was fitted by Gaussian normal distribution (red line) from the experimental data (black dots).

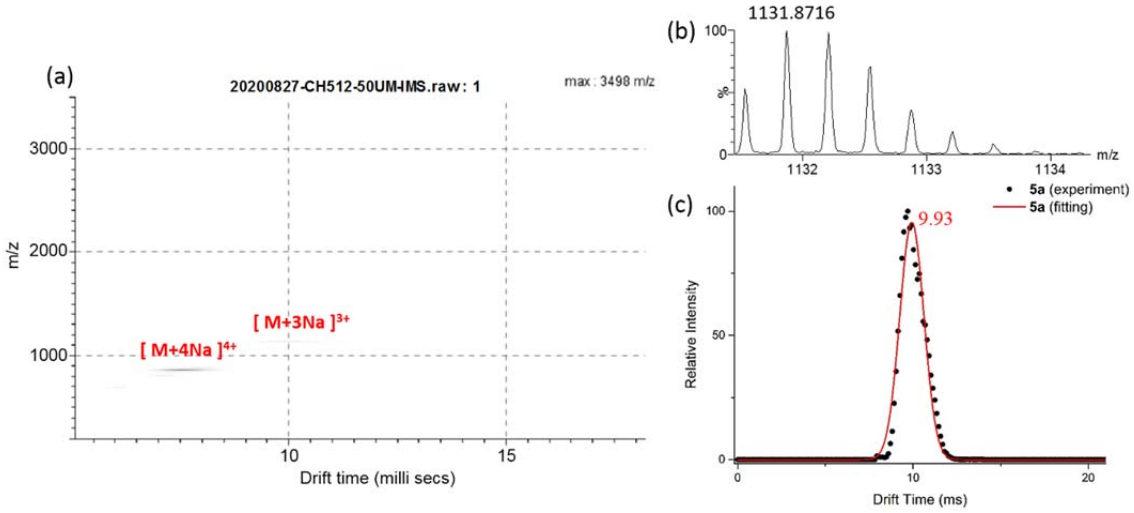


Figure S39. (a) IMS-MS spectrum; (b) isotope pattern and (c) drift-time distribution of [**5a** + 3Na]$^{3+}$. The drift-time distribution was fitted by Gaussian normal distribution (red line) from the experimental data (black dots).

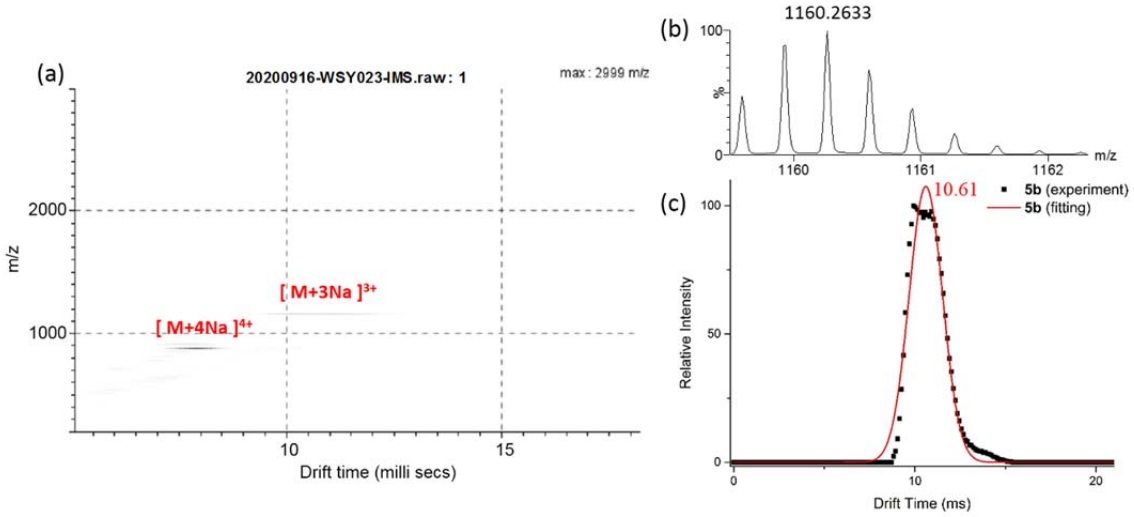


Figure S40. (a) IMS-MS spectrum; (b) isotope pattern and (c) drift-time distribution of [**5b** + 3Na]$^{3+}$. The drift-time distribution was fitted by Gaussian normal distribution (red line) from the experimental data (black dots).

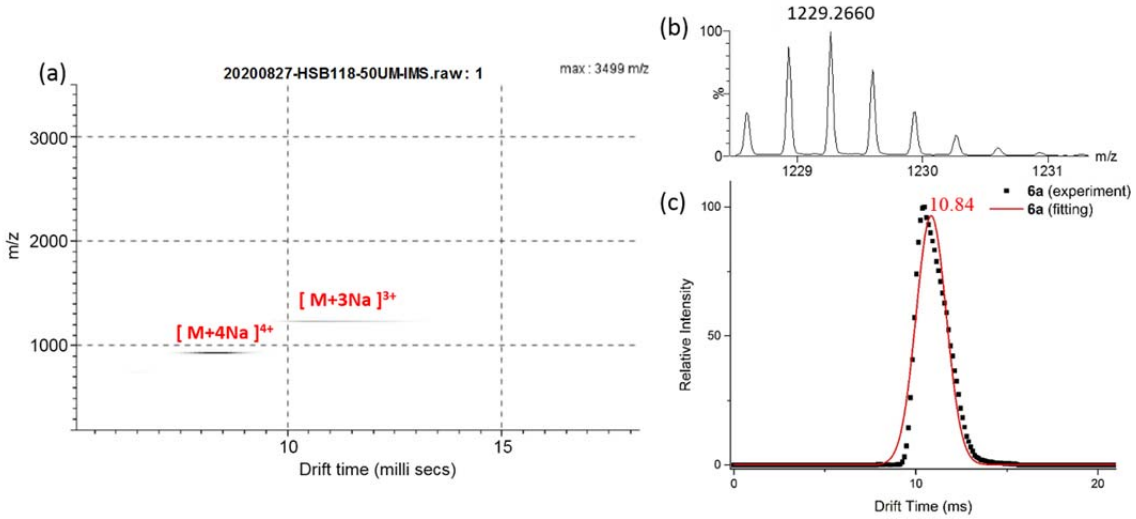


Figure S41. (a) IMS-MS spectrum; (b) isotope pattern and (c) drift-time distribution of [**6a** + 3Na]$^{3+}$. The drift-time distribution was fitted by Gaussian normal distribution (red line) from the experimental data (black dots).

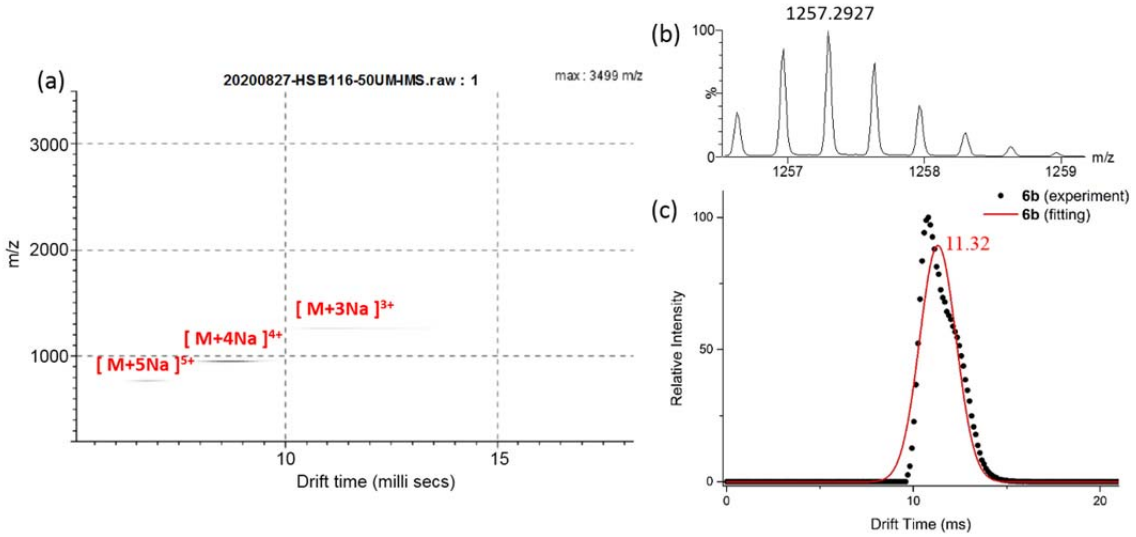


Figure S42. (a) IMS-MS spectrum; (b) isotope pattern and (c) drift-time distribution of [**6b** + 3Na]$^{3+}$. The drift-time distribution was fitted by Gaussian normal distribution (red line) from the experimental data (black dots).

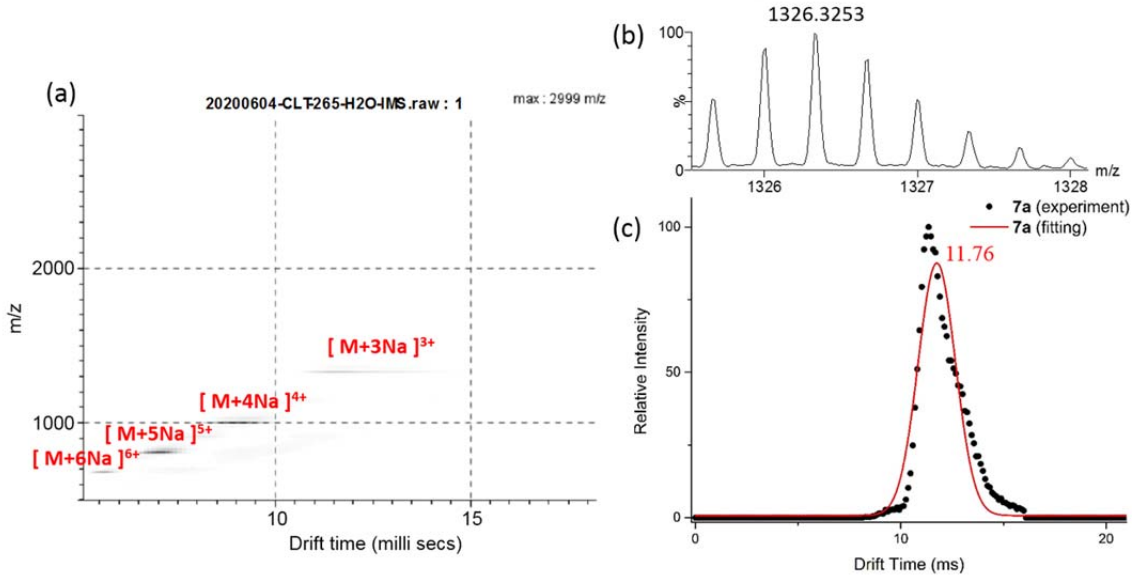


Figure S43. (a) IMS-MS spectrum; (b) isotope pattern and (c) drift-time distribution of [**7a** + 3Na]$^{3+}$. The drift-time distribution was fitted by Gaussian normal distribution (red line) from the experimental data (black dots).

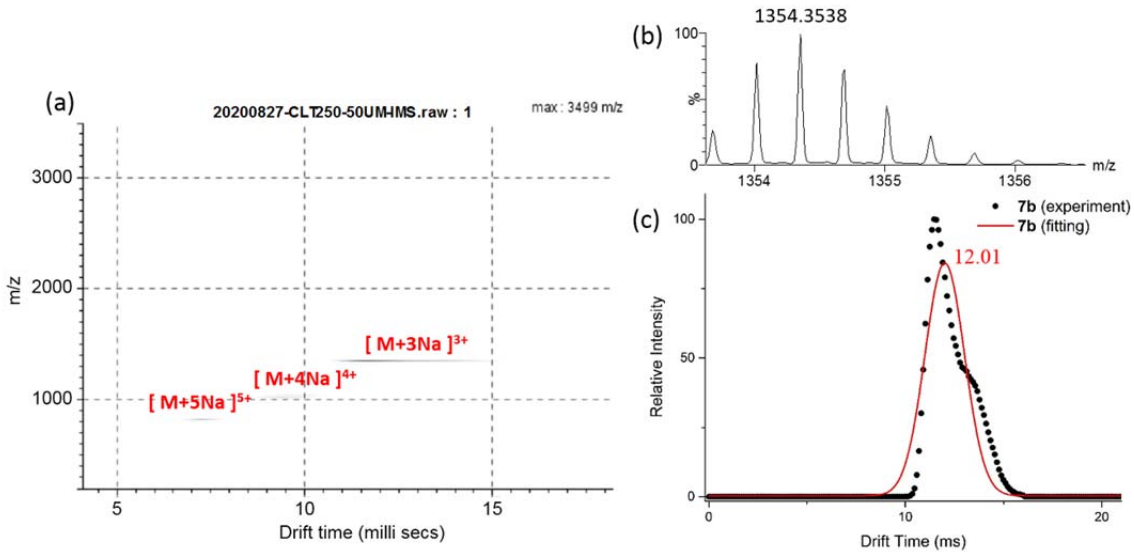


Figure S44. (a) IMS-MS spectrum; (b) isotope pattern and (c) drift-time distribution of [**7b** + 3Na]$^{3+}$. The drift-time distribution was fitted by Gaussian normal distribution (red line) from the experimental data (black dots).

Table S1. A list of ESI-MS *m/z* ratios for peptides

Peptide	$[M+2Na]^{2+}$	$[M+3Na]^{3+}$	$[M+4Na]^{4+}$
1a	1104.0082	743.6732	-
1b	1146.0594	771.7049	-
2a	1249.6108	840.7335	636.3007
2b	1291.6492	868.7668	657.3273
3a	1395.1923	937.7915	709.0923
3b	1437.2363	965.8220	730.1206
4a	1543.7589	1036.8335	783.3786
4b	1583.2837	1063.1851	803.1412
5a	1686.8044	1131.8716	854.9031
5b	1728.8926	1160.2633	875.6952
6a	1832.4023	1229.2660	927.6988
6b	1874.4564	1257.2927	948.7223
7a	1977.9946	1326.3253	1000.4958
7b	2020.0223	1354.3538	1021.5084

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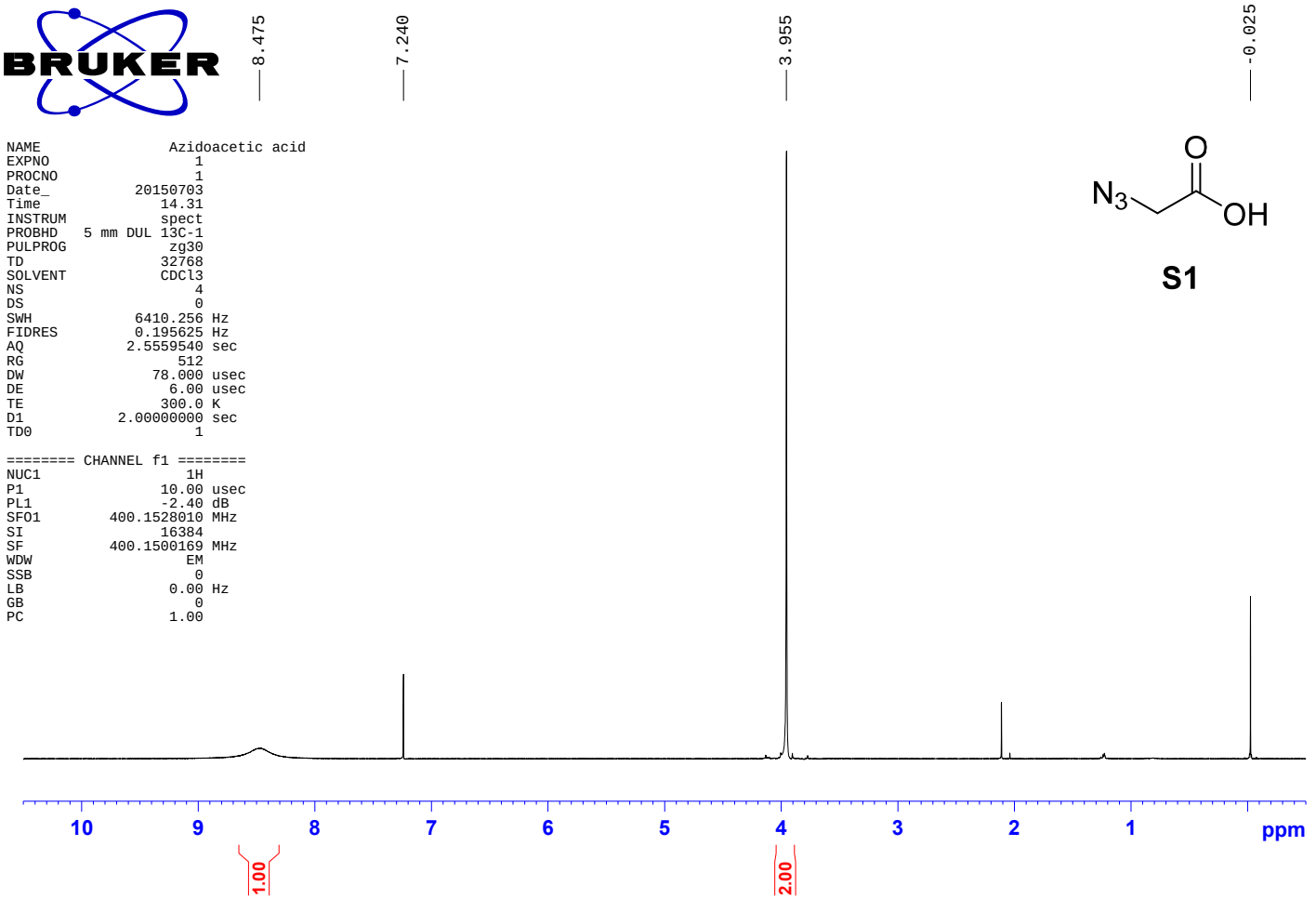
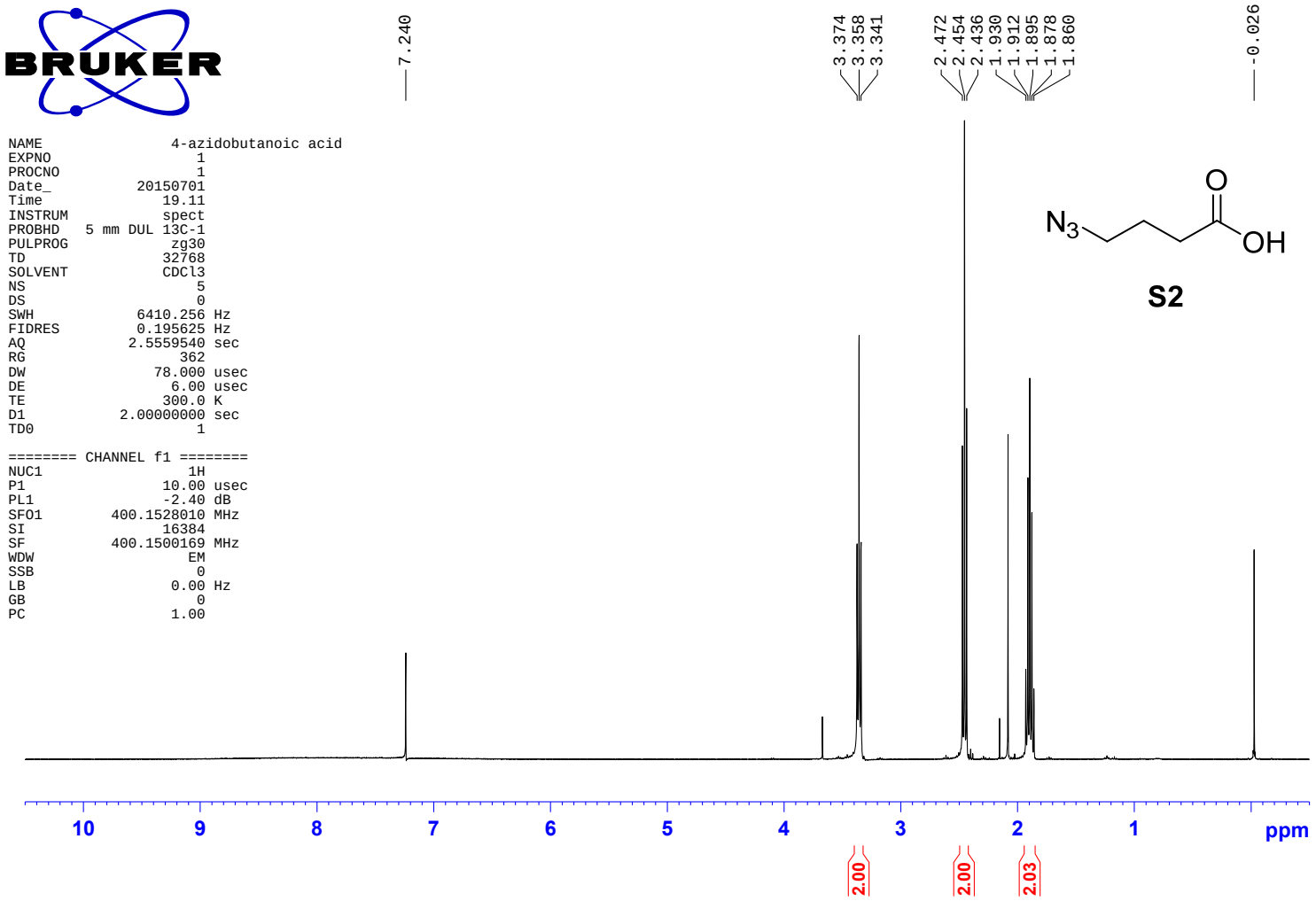
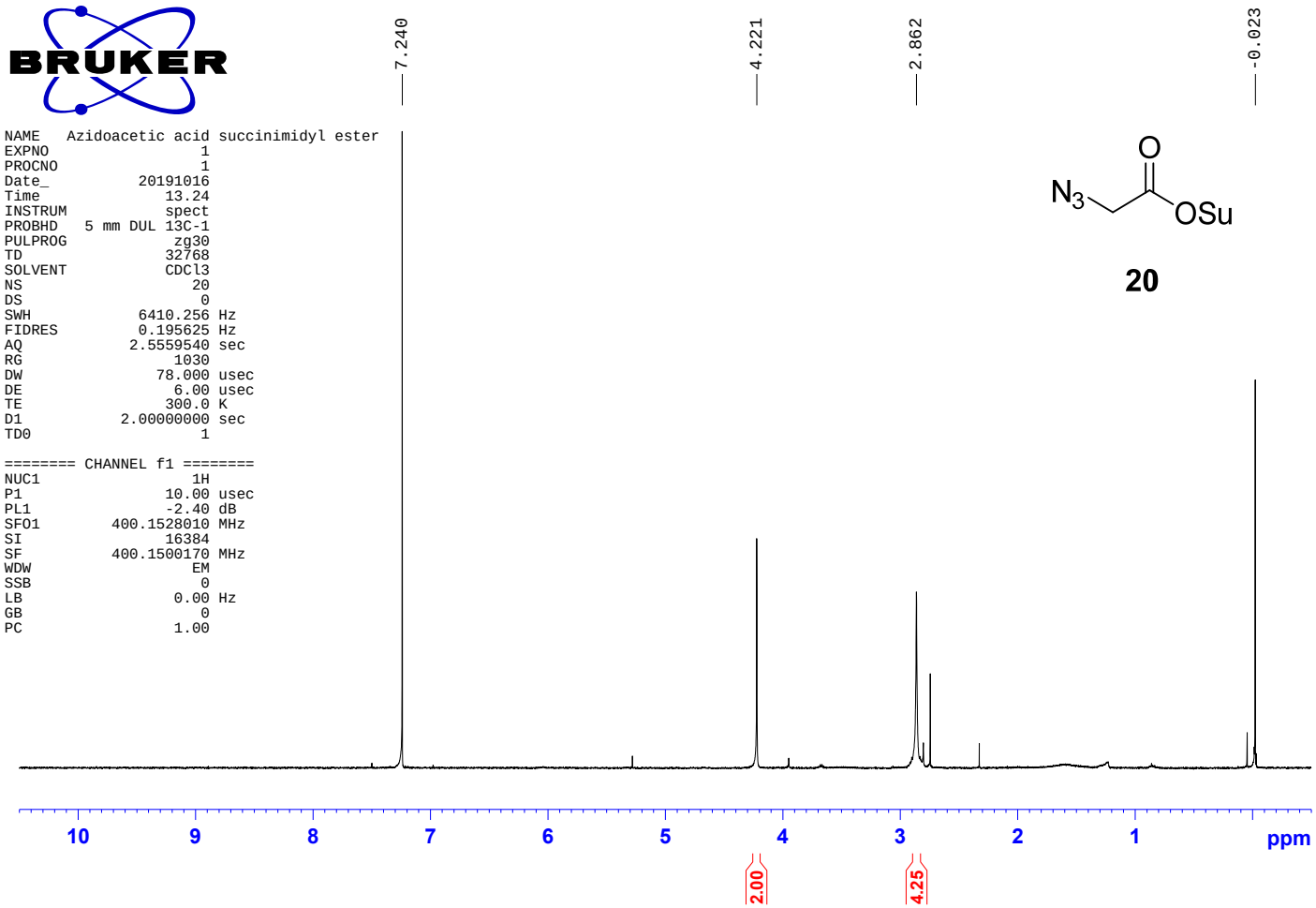


^{1}H NMR spectrum of **S1** (400 MHz, $CDCl_3$)



^{1}H NMR spectrum of **S2** (400 MHz, $CDCl_3$)



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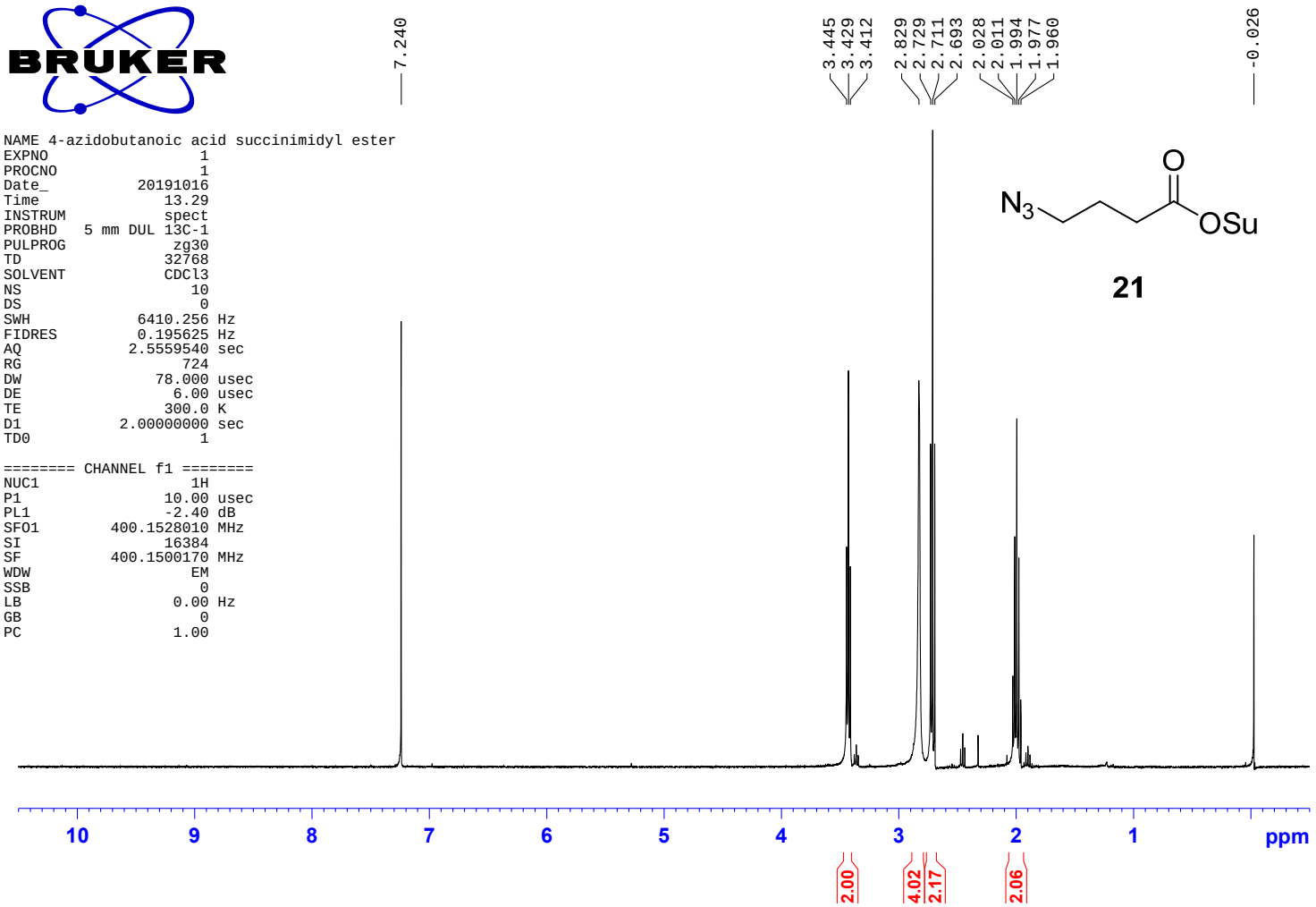

^{1}H NMR spectrum of **21** (400 MHz, $CDCl_3$)