Supporting Information

Primary Structure Control of Oligomers Based on Natural and Synthetic Building Blocks

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Experimental section

1. Materials

Chlorotrityl chloride resin (1.6 $mmol.g^{-1}$, 100-200 mesh), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, 99%), Fmoc-L-Lys(Boc)-OH (>99%), Fmoc-Gly-OH (>98%), Fmoc-L-Val-OH (>98%) and Fmoc-L-Cys(Trt)-OH (99%) were purchased from Iris Biotech GmbH. Sodium azide (NaN_3, 99%), 1-hydroxybenzotriazole hydrate (HOBt, >97%), Fmoc-L-Leu-OH (>97%), piperidine (99%), copper(I) bromide (CuBr, 98%), acetic acid (99.8%), *N*,*N*,*N*′,*N*′,*N*″-pentamethyldiethylenetriamine (PMDETA, 99%), triisopropylsilane (TIPS, 99%), hydrochloric acid (concentrated HCl, 37% in water), 2,4,6-trihydroxyacetophenone (THAP, matrix substance for MALDI-MS, ≥99.5%), cesium chloride (CsCl, 99.9%), anhydrous dichloromethane (CH_2Cl_2, anhydrous, >99.8%), anhydrous dimethylformamide (DMF, anhydrous, 99.8%) and dimethylformamide (DMF, >99%) were purchased from Aldrich. Bromoacetic acid (98+%), 6-bromohexanoic acid (98+%), 2-bromoisobutyric acid (98%), *N*-ethyldiisopropylamine (DIPEA, 99%), propargylamine (>95%), trifluoroacetic acid (TFA, 99%), and 2,2,2-trifluoroethanol (TFE, 99+%) were purchased from Alfa Aesar. 3-Bromoisobutyric acid (>97%) was purchased from TCI. Fmoc-L-Pro-OH was purchased from Novabiochem. Dichloromethane (CH_2Cl_2, RE, pure stabilized with amylene), methanol (RPE, for analysis, ACS reagent), diethyl ether (RE, pure stabilized with BHT) and acetone (RE, pure) were purchased from Carlo Erba. All the reagents were used as received except if otherwise noted. Copper(I) bromide was purified by stirring in acetic acid overnight, washing with methanol, and drying under vacuum at room temperature. All the syntheses on solid support were performed in solid phase extraction (SPE) tubes (12 mL polypropylene SPE tubes with polyethylene frits, 20 µm porosity purchased from SUPELCO®) and shaken using a modified IKA KS 130 basic shaker.

2. Characterization

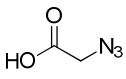
Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$, DMSO-d_6 or D_2O on a Bruker Avance 400 MHz spectrometer equipped with Ultrashield magnets.

Fourier transform infrared (FTIR) spectra were recorded on a Bruker Vertex 70 spectrometer using the attenuated total reflectance (ATR) technique.

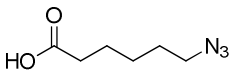
Electrospray ionization mass spectrometry (ESI-MS) experiments were performed on a Bruker Daltonics microTOF spectrometer equipped with an orthogonal electrospray (ESI) interface. Calibration was performed using a solution of 10 mM sodium formiate. Sample solutions were introduced into the spectrometer source with a syringe pump (Harvard type 55 1111: Harvard Apparatus Inc., South Natick, MA, USA) with a flow rate of 4 $\mu L.min^{-1}$.

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF) mass spectrometry experiments were performed on a Bruker Daltonics Autoflex spectrometer. Sample preparation was performed using the dried droplet method using a mixture of 0.5 µL of sample solution (2 mg of sample in 1 mL of 1/1 acetonitrile/water) with 1 µL of matrix solution (10 mg of 2,4,6-trihydroxyacetophenone and 10 mg of CsCl in 1 mL of 1/1 acetonitrile/water) dried at room temperature.

3. Synthesis of azidocarboxylic acid



azidoacetic acid



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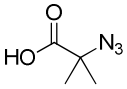
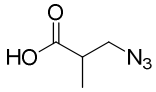


2-azidoisobutyric acid



3-azido-2-methylpropionic acid

Typical syntheses of azidocarboxylic acid were conducted by combining bromocarboxylic acid (54 mmol), NaN_3 (8.8 g, 135 mmol), water (100 mL) and acetone (20 mL) in a round bottom flask, which was then placed in an oil bath thermostated at 60 °C. The reaction mixture was stirred at this temperature for 6 h. The reaction mixture was then acidified by addition of concentrated HCl until pH 2 was reached and extracted 3 times with ethyl acetate. The organic phase was washed with Brine, dried over Na_2SO_4, concentrated by rotary evaporation and dried under vacuum at room temperature overnight.

Caution: organic azides are potentially-explosive substances. Routine precautions were taken to minimize the effect of possible explosions at all stages in the preparation and handling of azidocarboxylic acids.

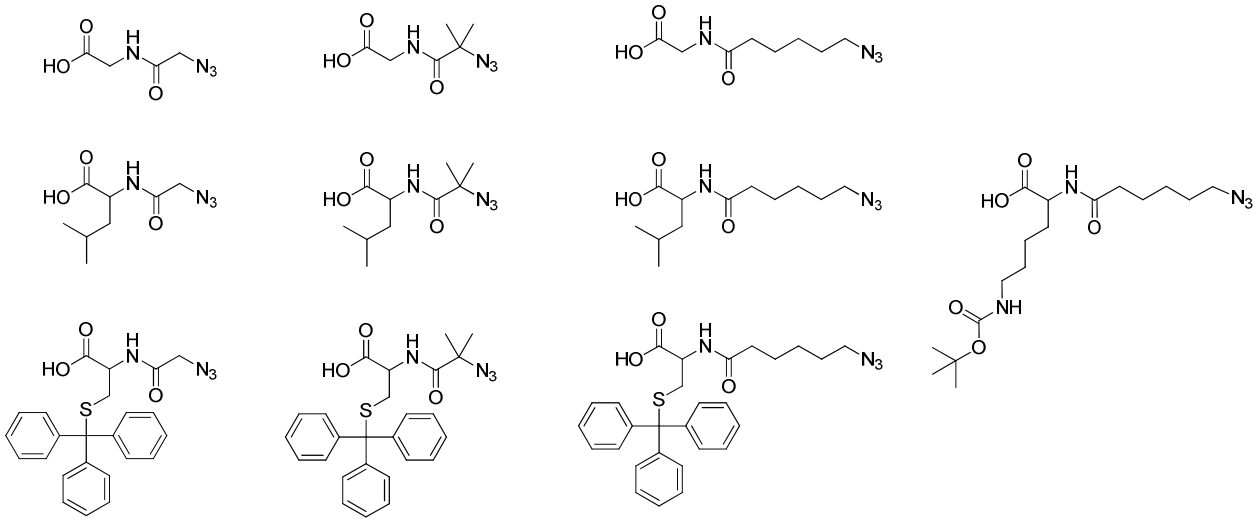
3.1. Azidoacetic acid. Synthesized from bromoacetic acid and stored as a solution in DMF. 92% yield. ^{1}H NMR: δ ($CDCl_3$, 7.26 ppm) 9.28 (s, 1H), 3.96 (s, 2H). ^{13}C NMR: δ ($CDCl_3$, 77.16 ppm) 174.02, 50.13. FTIR: 2106 cm^{-1}.

3.2. 6-Azidohexanoic acid. Synthesized from 6-bromohexanoic acid. 96% yield. ^{1}H NMR: δ ($CDCl_3$, 7.26 ppm) 11.02 (s, 1H), 3.26 (t, 2H), 2.35 (t, 2H), 1.54-1.71 (m, 4H), 1.35-1.47 (m, 2H). ^{13}C NMR: δ ($CDCl_3$, 77.16 ppm) 180.07, 51.24, 33.90, 28.87, 26.27, 24.19. FTIR: 2093 cm^{-1}.

3.3. 2-Azidoisobutyric acid. Synthesized from 2-bromoisobutyric acid. 59% yield. ^{1}H NMR: δ ($CDCl_3$, 7.26 ppm) 9.56 (s, 1H), 1.52 (s, 6H). ^{13}C NMR: δ ($CDCl_3$, 77.16 ppm) 179.17, 62.97, 24.38. FTIR: 2093 cm^{-1}

3.4. 3-Azido-2-methylpropionic acid. Synthesized from 3-bromoisobutyric acid. 91% yield. ^{1}H NMR: δ ($CDCl_3$, 7.26 ppm) 11.09 (s, 1H), 3.35-3.59 (m, 2H), 2.63-2.77 (m, 1H), 1.24 (s, 3H). ^{13}C NMR: δ ($CDCl_3$, 77.16 ppm) 180.45, 53.40, 39.67, 14.49. FTIR: 2098 cm^{-1}

4. Synthesis of azidoamino acids



1.28 g of chlorotrityl chloride resin (2.0 mmol of functional groups) was weighted in a SPE vessel, swollen in CH_2Cl_2 for 10 min and washed 3 times with CH_2Cl_2. Fmoc-amino acid-OH (6.1 mmol) was added to the vessel, which was then degased by performing 3 vacuum/argon cycles. 7 mL of anhydrous CH_2Cl_2 was added to the vessel followed by 2.1 mL of DIPEA (12.1 mmol). The solution was

agitated for 1 h under argon. The solution was filtered and the resin was washed 6 times with DMF. 7 mL of a 80/15/5 CH_2Cl_2/methanol/DIPEA solution was agitated for 10 min (twice), and the resin was subsequently washed with DMF 6 times. The Fmoc-protecting groups were removed by agitation for 3 min with 7 mL of a 25% piperidine cleavage solution in DMF followed by filtration and agitation with a fresh cleavage solution for 20 min. The resin was then washed 6 times with DMF, 6 times with CH_2Cl_2, 3 times with methanol and 3 times with CH_2Cl_2. The resin was dried overnight under vacuum at room temperature. The loading density of amino acid grafted on the resin was determined by gravimetric analysis (typically 1.0 to 1.4 mmol.g^{-1}).

Azidocarboxylic acid (5.0 mmol, 3 eq. relative to the loading determined), HBTU (1.9 g, 5.0 mmol), HOBt (0.7 g, 5.0 mmol), DIPEA (1.8 mL, 10.3 mmol) and anhydrous DMF (7 mL) were added to the peptide vessel (1.7 mmol loading density), and agitated for 1 h. After filtration, the resin was washed 6 times with DMF and 6 times with CH_2Cl_2.

The azidoamino acid was cleaved from the resin by adding a 4/1 CH_2Cl_2/TFE solution to the peptide vessel for 45 min (twice). The resin was filtered, washed three times with CH_2Cl_2, and the filtrates were collected in a clean round-bottom flask. The solution was concentrated by rotary evaporation.

4.1. Gly-acetyl-N_3. Synthesized from Fmoc-Gly-OH and azidoacetic acid. 59% yield. ^{1}H NMR: δ (DMSO-d_6, 2.50 ppm) 9.04 (bs, 1H), 8.38 (t, 1H), 3.88 (s, 2H), 3.79 (d, 2H). ^{13}C NMR: δ (DMSO-d_6, 39.52 ppm) 171.02, 167.81, 50.77, 40.85. FTIR: 2108 cm^{-1}. ESI (m/z): $[M-H]^-$ calculated from C4H5N4O3, 157.04; found, 157.04.

4.2. Gly-isobutyryl-N_3. Synthesized from Fmoc-Gly-OH and 2-azidoisobutyric acid. 75% yield. ^{1}H NMR: δ (DMSO-d_6, 2.50 ppm) 10.51 (bs, 1H), 8.22 (t, 1H), 3.73 (d, 2H), 1.40 (s, 6H). ^{13}C NMR: δ (DMSO-d_6, 39.52 ppm) 172.25, 170.84, 63.66, 41.04, 24.24. FTIR: 2111 cm^{-1}. ESI (m/z): $[M+Na]^+$ calculated from C6H10N4O3Na, 209.07; found, 209.06.

4.3. Gly-hexyl-N_3. Synthesized from Fmoc-Gly-OH and 6-azidohexanoic acid. 67% yield. ^{1}H NMR: δ (DMSO-d_6, 2.50 ppm) 12.29 (bs, 1H), 8.08 (t, 1H), 3.72 (d, 2H), 3.30 (t, 2H), 2.12 (t, 2H), 1.44-1.60 (m, 4H), 1.24-1.38 (m, 2H). ^{13}C NMR: δ (DMSO-d_6, 39.52 ppm) 172.39, 171.41, 50.57, 40.56, 34.86,

27.98, 25.72, 24.67. FTIR: 2100 cm^{-1}. ESI (m/z): $[M-H]^-$ calculated from C8H13N4O3, 213.10; found, 213.09.

4.4. Leu-acetyl-N_3. Synthesized from Fmoc-Leu-OH and azidoacetic acid. 50% yield. ^1H NMR: δ (DMSO-d_6, 2.50 ppm) 12.40 (bs, 1H), 8.35 (d, 1H), 4.20-4.30 (m, 1H), 3.86 (s, 2H), 1.57-1.70 (m, 1H), 1.46-1.57 (m, 2H), 0.90 (d, 3H), 0.86 (d, 3H). ^{13}C NMR: δ (DMSO-d_6, 39.52 ppm) 173.68, 167.43, 50.49, 50.36, 24.30, 22.79, 21.29. FTIR: 2113 cm^{-1}. ESI (m/z): $[M-H]^-$ calculated from C8H13N4O3, 213.10; found, 213.08.

4.5. Leu-isobutyryl-N_3. Synthesized from Fmoc-Leu-OH and 2-azidoisobutyric acid. 57% yield. ^1H NMR: δ (DMSO-d_6, 2.50 ppm) 12.05 (bs, 1H), 8.00 (d, 1H), 4.16-4.33 (m, 1H), 1.45-1.76 (m, 3H), 1.41 (s, 3H), 1.39(s, 3H), 0.88 (d, 3H), 0.83 (d, 3H). ^{13}C NMR: δ (DMSO-d_6, 39.52 ppm) 173.71, 171.93, 63.84, 50.57, 24.45, 24.22, 22.93, 21.08. FTIR: 2103 cm^{-1}. ESI (m/z): $[M-H]^-$ calculated from C10H17N4O3, 241.13; found, 241.13.

4.6. Leu-hexyl-N_3. Synthesized from Fmoc-Leu-OH and 6-azidohexanoic acid. 64% yield. ^1H NMR: δ (DMSO-d_6, 2.50 ppm) 12.40 (bs, 1H), 8.00(d, 1H), 4.14-4.28 (m, 1H), 3.23-3.35 (t, 2H), 2.04-2.19 (m, 2H), 1.57-1.69 (m, 1H), 1.41-1.57 (m, 6H), 1.21-1.38 (m, 2H), 0.88 (d, 3H), 0.83 (d, 3H). ^{13}C NMR: δ (DMSO-d_6, 39.52 ppm) 174.27, 172.07, 50.62, 50.05, 34.84, 27.96, 25.67, 24.75, 24.34, 22.85, 21.20. FTIR: 2091 cm^{-1}. ESI (m/z): $[M-H]^-$ calculated from C12H21N4O3, 269.16; found, 269.17.

4.7. Cys(Trt)-acetyl-N_3. Synthesized from Fmoc-Cys(Trt)-OH and azidoacetic acid. 69% yield. ^1H NMR: δ (DMSO-d_6, 2.50 ppm) 10.90 (bs, 1H), 8.49 (d, 1H), 7.18-7.46 (m, 15H), 4.17-4.26 (m, 1H), 3.88 (s, 2H), 2.39-2.62 (m, 2H). ^{13}C NMR: δ (DMSO-d_6, 39.52 ppm) 171.20, 167.31, 144.15, 129.06, 128.06, 126.82, 66.16, 51.42, 50.46, 33.06. FTIR: 2113 cm^{-1}. ESI (m/z): $[M-H]^-$ calculated from C24H21N4O3S, 445.13; found, 445.13.

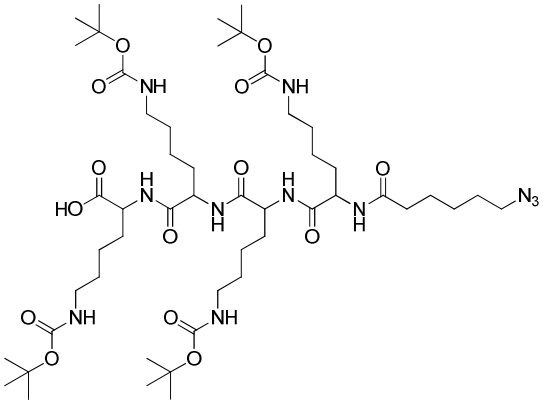
4.8. Cys(Trt)-isobutyryl-N_3. Synthesized from Fmoc-Cys(Trt)-OH and 2-azidoisobutyric acid. 90% yield. ^1H NMR: δ (DMSO-d_6, 2.50 ppm) 12.07 (bs, 1H), 8.17 (d, 1H), 7.19-7.40 (m, 15H), 4.08-4.17 (m, 1H), 2.41-2.76 (m, 2H), 1.42 (s, 3H), 1.41 (s, 3H). ^{13}C NMR: δ (DMSO-d_6, 39.52 ppm) 171.76, 171.28,

144.28, 129.08, 128.04, 126.79, 66.10, 63.79, 51.71, 32.67, 24.22, 24.14. FTIR: 2114 cm^{-1}. ESI (m/z): $[M-H]^-$ calculated from C26H25N4O3S, 473.16; found, 473.16.

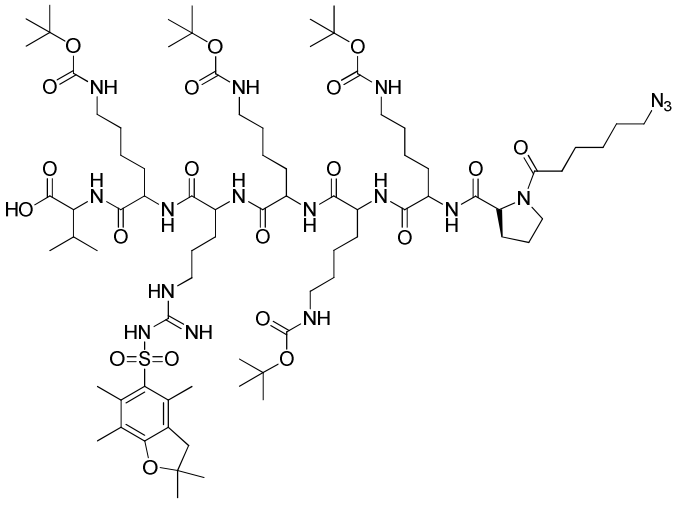
4.9. Cys(Trt)-hexyl-N_3. Synthesized from Fmoc-Cys(Trt)-OH and 6-azidohexanoic acid. 74% yield. ^{1}H NMR: δ (DMSO-d_6, 2.50 ppm) 10.55 (bs, 1H), 8.17 (d, 1H), 7.17-7.46 (m, 15H), 4.14-4.24 (m, 1H), 3.25 (t, 2H), 2.36-2.55 (m, 2H), 2.08-2.17 (m, 2H), 1.44-1.57 (m, 4H), 1.24-1.36 (m, 2H). ^{13}C NMR: δ (DMSO-d_6, 39.52 ppm) 171.96, 171.78, 144.24, 129.05, 128.02, 126.77, 66.02, 51.16, 50.55, 34.77, 27.94, 25.59, 24.69. FTIR: 2093 cm^{-1}. ESI (m/z): $[M-H]^-$ calculated from C28H29N4O3S, 501.20; found, 501.17.

4.10. Lys(Boc)-hexyl-N_3. Synthesized from Fmoc-Lys(Boc)-OH and 6-azidohexanoic acid. 88% yield. ^{1}H NMR: δ (DMSO-d_6, 2.50 ppm) 12.20 (bs, 1H), 7.99 (d, 1H), 6.27-6.89 (m, 1H), 4.06-4.22 (m, 1H), 3.30 (t, 2H), 2.82-2.95 (m, 2H), 2.05-2.17 (m, 2H), 1.60-1.73 (m, 2H), 1.44-1.74 (m, 6H), 1.37 (s, 9H), 1.18-1.34 (m, 6H). ^{13}C NMR: δ (DMSO-d_6, 39.52 ppm) 173.83, 172.13, 155.57, 77.33, 51.70, 50.58, 34.83, 30.76, 29.10, 28.26, 27.97, 25.70, 24.75, 22.82. FTIR: 2094 cm^{-1}. ESI (m/z): $[M+Na]^+$ calculated from C17H31N5O5Na, 408.22; found, 408.22.

5. Synthesis of azidopeptides



Lys(Boc)-Lys(Boc)-Lys(Boc)-Lys(Boc)-hexyl-N_3



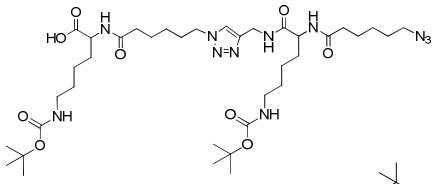
Val-Lys(Boc)-Arg(Pbf)-Lys(Boc)-Lys(Boc)-Lys(Boc)-Pro-hexyl-N_3

Azidopeptides were prepared similarly to azidoamino acids by solid-phase peptide synthesis through successive additions of amino acid in the desired order followed by end-capping with 6-azidohexanoic acid and cleavage from the resin.

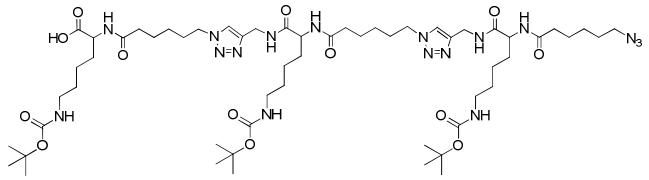
5.1. Lys(Boc)-Lys(Boc)-Lys(Boc)-Lys(Boc)-hexyl-N_3. The chlorotrityl chloride resin was loaded with Fmoc-Lys(Boc)-OH. The remaining lysine residues were successively coupled to the lysine functionalized resin using HBTU and HOBt as coupling agent. The peptide was end-functionalized with 6-azidohexanoic acid and cleaved from the resin using a CH_2Cl_2/TFE (4/1) solution. 70% yield. ^{1}H NMR: δ (DMSO-d_6, 2.50 ppm) 12.42 (bs, 1H), 7.73-8.05 (m, 4H), 6.25-6.85 (m, 4H), 4.07-4.32 (m, 4H), 3.31 (t, 2H), 2.80-2.95 (m, 8H), 2.06-2.21 (m, 2H), 1.13-1.79 (m, 66H). ^{13}C NMR: δ (DMSO-d_6, 39.52 ppm) 173.39, 172.16, 171.81, 171.42, 171.24, 155.52, 77.28, 52.53, 52.36, 52.19, 51.82, 50.54, 34.95, 31.79, 31.58, 30.77, 29.23, 28.24, 27.97, 25.73, 24.74, 22.75, 22.65, 22.50. FTIR: 2095 cm^{-1}. ESI (m/z): $[M+Na]^+$ calculated from C50H91N11O14Na, 1092.66; found, 1092.66.

5.2. Val-Lys(Boc)-Arg(Pbf)-Lys(Boc)-Lys(Boc)-Lys(Boc)-Pro-hexyl-N_3. Val-Lys(Boc)-Arg(Pbf)-Lys(Boc)-Lys(Boc)-Lys(Boc)-Pro-hexyl-N_3 was prepared similarly to azidoamino acids by solid-phase peptide synthesis. The chlorotrityl chloride resin was loaded with Fmoc-Val-OH. The remaining amino acids were successively coupled to the valine functionalized resin using HBTU and HOBt as coupling agent in the following order: Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH and Fmoc-Pro-OH. The peptide was end-functionalized with 6-azidohexanoic acid and cleaved from the resin using a CH_2Cl_2/TFE (4/1) solution. 81% yield. ^{1}H NMR: δ (DMSO-d_6, 2.50 ppm) 12.52 (bs, 1H), 7.58-8.28 (m, 6H), 5.86-7.02 (m, 7H), 4.02-4.46 (m, 7H), 3.40-3.62 (m, 2H), 3.20-3.39 (m, 4H), 2.98-3.09 (m, 2H), 2.78-2.93 (m, 8H), 2.48 (s, 3H), 2.42 (s, 3H), 2.25-2.35 (m, 2H), 1.12-1.94 (m, 84H), 0.80-0.90 (m, 6H). ^{13}C NMR: δ (DMSO-d_6, 39.52 ppm) 172.83, 172.20, 171.96, 171.79, 171.65, 171.59, 171.41, 171.08, 170.65, 157.44, 156.09, 155.51, 137.25, 134.25, 131.42, 124.26, 116.22, 86.22, 77.29, 52.69, 52.62, 52.45, 52.36, 52.07, 51.97, 50.60, 42.49, 33.56, 31.72, 30.92, 29.92, 29.23, 28.23, 28.13, 25.93, 25.88, 24.32, 23.86, 23.68, 22.74, 22.57, 18.99, 18.89, 17.91, 17.54, 12.22. FTIR: 2095 cm^{-1}. ESI (m/z): $[M-H]^-$ calculated from C79H134N17O20S, 1672.97; found, 1672.96.

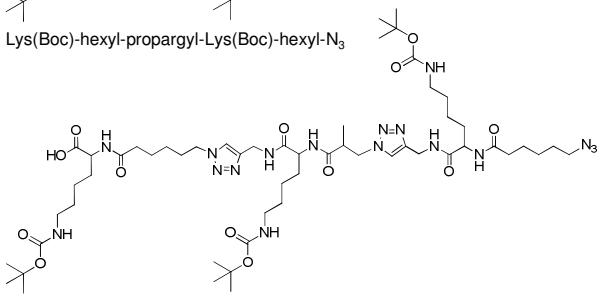
6. Synthesis of sequence-defined oligomers based on natural and synthetic building blocks



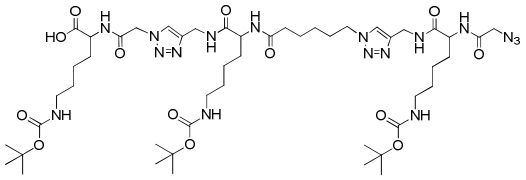
Lys(Boc)-hexyl-propargyl-Lys(Boc)-hexyl-N_3



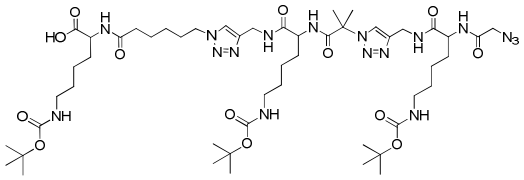
Lys(Boc)-hexyl-propargyl-Lys(Boc)-hexyl-propargyl-Lys(Boc)-hexyl-N_3



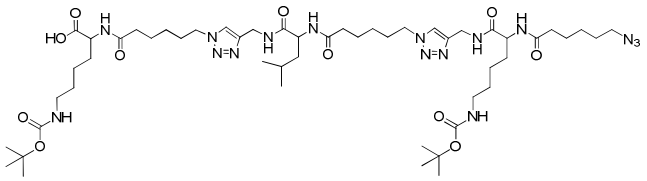
Lys(Boc)-hexyl-propargyl-Lys(Boc)-methylpropionyl-propargyl-Lys(Boc)-hexyl-N_3



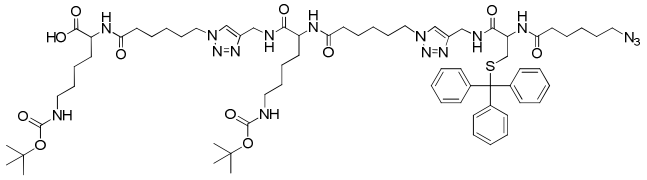
Lys(Boc)-acetyl-propargyl-Lys(Boc)-hexyl-propargyl-Lys(Boc)-acetyl-N_3



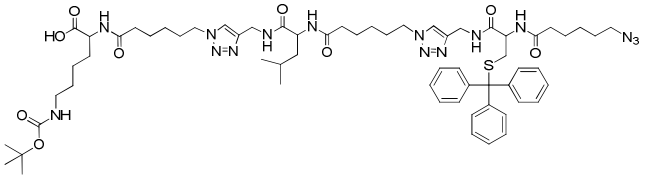
Lys(Boc)-hexyl-propargyl-Lys(Boc)-isobutyryl-propargyl-Lys(Boc)-acetyl-N_3



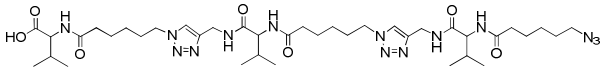
Lys(Boc)-hexyl-propargyl-Leu-hexyl-propargyl-Lys(Boc)-hexyl-N_3



Lys(Boc)-hexyl-propargyl-Lys(Boc)-hexyl-propargyl-Cys(Trt)-hexyl-N_3



Lys(Boc)-hexyl-propargyl-Leu-hexyl-propargyl-Cys(Trt)-hexyl-N_3



Val-hexyl-propargyl-Val-hexyl-propargyl-Val-hexyl-N_3

The chlorotrityl chloride resin was loaded with the desired Fmoc-amino acid-OH as described in Section 4. The oligomers were synthesized from successive additions of azidocarboxylic acid, propargylamine and Fmoc-amino acid-OH following the procedure described below until the desired oligomer ending with an azide was obtained.

i) Addition of azidocarboxylic acid. Azidocarboxylic acid (3 eq. relative to the loading), HBTU (3 eq. relative to the loading), HOBt (3 eq. relative to the loading), DIPEA (6 eq. relative to the loading) and

anhydrous DMF (5 mL per mmol of functional groups on the resin) were added to the peptide vessel, and agitated for 1 h. After filtration, the resin was washed 6 times with DMF and 6 times with CH_2Cl_2.

ii) Copper-assisted alkyne-azide cycloaddition with propargylamine. CuBr (3 eq. relative to the loading) was added to the peptide vessel, which was then degased through 3 vacuum/argon cycles. Anhydrous CH_2Cl_2 (7 mL per mmol of functional groups on the resin) were added to the peptide vessel, followed by PMDETA (6 eq. relative to the loading) and propargylamine (3 eq. relative to the loading). The solution was agitated under argon for 16 h. After filtration, the resin was washed 6 times with DMF and 6 times with CH_2Cl_2.

iii) Addition of Fmoc-amino acid-OH. Fmoc-amino acid-OH (3 eq. relative to the loading), HBTU (3 eq. relative to the loading), HOBt (3 eq. relative to the loading), DIPEA (6 eq. relative to the loading) and anhydrous DMF (5 mL per mmol of functional groups on the resin) were added to the peptide vessel, and agitated for 1 h. After filtration, the resin was washed 6 times with DMF. The Fmoc-protecting group was removed by agitation for 3 min with a 25% piperidine cleavage solution in DMF followed by filtration and agitation with a fresh cleavage solution for 20 min. The resin was then washed 6 times with DMF and 6 times with CH_2Cl_2.

Finally, the oligomer was cleaved from the resin by adding a 4/1 CH_2Cl_2/TFE solution to the peptide vessel for 45 min (twice). The resin was filtered, washed three times with CH_2Cl_2, and the filtrates were collected in a clean round-bottom flask. The solution was concentrated by rotary evaporation.

6.1. Lys(Boc)-hexyl-propargyl-Lys(Boc)-hexyl-N_3. Synthesized from Fmoc-Lys(Boc)-OH and 6-azidohexanoic acid. 76% yield. ^{1}H NMR: δ (DMSO-d_6, 2.50 ppm) 12.20 (bs, 1H), 7.69-8.48 (m, 4H), 6.24-6.86 (m, 2H), 4.07-4.37 (m, 6H), 3.29 (t, 2H), 2.78-2.97 (m, 4H), 1.98-2.22 (m, 4H), 1.06-1.90 (m, 42H). ^{13}C NMR: δ (DMSO-d_6, 39.52 ppm) 172.16, 171.97, 155.61, 122.52, 77.36, 52.51, 50.58, 49.20, 48.62, 34.99, 34.88, 34.33, 31.70, 29.52, 29.20, 28.28, 28.00, 25.78, 25.46, 24.75, 24.65, 22.90, 22.80. ESI (m/z): $[M+Na]^+$ calculated from C37H65N11O9Na, 830.49; found, 830.49.

6.2. Lys(Boc)-hexyl-propargyl-Lys(Boc)-hexyl-propargyl-Lys(Boc)-hexyl-N_3. Synthesized from Fmoc-Lys(Boc)-OH and 6-azidohexanoic acid. 60% yield. ^{1}H NMR: δ (DMSO-d_6, 2.50 ppm) 11.50 (bs, 1H), 7.67-8.74 (m, 7H), 6.22-6.84 (m, 3H), 3.97-4.53 (m, 11H), 3.27 (t, 2H), 2.73-2.97 (m, 6H), 1.94-

2.20 (m, 6H), 0.96-1.90 (m, 63H). ^{13}C NMR: δ (DMSO-d_6, 39.52 ppm) 172.35, 172.16, 172.06, 155.71, 144.91, 122.64, 77.45, 52.74, 50.66, 49.29, 48.66, 35.06, 34.41, 31.68, 31.26, 29.56, 29.22, 28.33, 28.05, 25.82, 25.51, 24.74, 24.66, 22.95, 22.84. ESI (m/z): $[M+Na]^+$ calculated from C57H99N17O13Na, 1252.75; found, 1252.74.

6.3. Lys(Boc)-hexyl-propargyl-Lys(Boc)-methylpropionyl-propargyl-Lys(Boc)-hexyl-N_3. Synthesized from Fmoc-Lys(Boc)-OH, 6-azidohexanoic acid, and 3-azido-2-methylpropionic acid. 75% yield. ^{1}H NMR: δ (DMSO-d_6, 2.50 ppm) 10.90 (bs, 1H), 7.69-8.65 (m, 7H), 6.24-6.86 (m, 3H), 4.41-4.57 (m, 2H), 4.10-4.39 (m, 9H), 3.29 (t, 2H), 2.95-3.06 (m, 1H), 2.78-2.95 (m, 6H), 1.97-2.22 (m, 4H), 1.04-1.88 (m, 57H), 0.89-1.03 (m, 3H). ^{13}C NMR: δ (DMSO-d_6, 39.52 ppm) 172.92, 172.66, 172.09, 171.88, 171.63, 155.58, 144.55, 122.54, 77.34, 52.60, 52.57, 52.42, 52.38, 52.06, 51.84, 50.56, 49.20, 48.60, 35.76, 34.99, 34.33, 34.21, 31.79, 30.79, 29.49, 29.20, 28.26, 27.99, 25.77, 25.44, 24.73, 24.65, 22.78, 15.54, 15.17. ESI (m/z): $[M+Na]^+$ calculated from C55H95N17O13Na, 1224.72; found, 1224.71.

6.4. Lys(Boc)-acetyl-propargyl-Lys(Boc)-hexyl-propargyl-Lys(Boc)-acetyl-N_3. Synthesized from Fmoc-Lys(Boc)-OH, 6-azidohexanoic acid, and azidoacetic acid. 69% yield. ^{1}H NMR: δ (DMSO-d_6, 2.50 ppm) 12.10 (bs, 1H), 7.68-8.91 (m, 7H), 6.18-6.90 (m, 3H), 5.10-5.33 (m, 2H), 4.12-4.45 (m, 11H), 2.77-3.01 (m, 6H), 2.05-2.18 (m, 2H), 1.03-1.91 (m, 51H). ^{13}C NMR: δ (DMSO-d_6, 39.52 ppm) 172.11, 171.94, 171.34, 167.40, 155.62, 144.59, 122.56, 77.39, 52.79, 52.42, 51.57, 50.60, 49.21, 34.97, 34.27, 31.78, 29.55, 29.20, 28.28, 25.55, 24.60, 22.80, 22.68. ESI (m/z): $[M-H]^-$ calculated from C49H82N17O13, 1116.63; found, 1116.63.

6.5. Lys(Boc)-hexyl-propargyl-Lys(Boc)-isobutyryl-propargyl-Lys(Boc)-acetyl-N_3. Synthesized from Fmoc-Lys(Boc)-OH, 6-azidohexanoic acid, 2-azidoisobutyric acid, and azidoacetic acid. 60% yield. ^{1}H NMR: δ (DMSO-d_6, 2.50 ppm) 11.60 (bs, 1H), 7.68-8.65 (m, 7H), 6.18-6.89 (m, 3H), 4.16-4.45 (m, 9H), 3.22-3.37 (m, 2H), 2.78-2.97 (m, 6H), 2.02-2.20 (m, 2H), 0.94-1.92 (m, 57H). ^{13}C NMR: δ (DMSO-d_6, 39.52 ppm) 171.96, 171.36, 170.57, 167.33, 155.56, 144.49, 122.44, 77.31, 64.64, 53.16, 52.76, 50.51, 49.18, 34.89, 34.36, 29.49, 29.19, 28.24, 25.59, 25.44, 24.62, 22.65. ESI (m/z): $[M-H]^-$ calculated from C51H86N17O13, 1244.66; found, 1244.67.

6.6. Lys(Boc)-hexyl-propargyl-Leu-hexyl-propargyl-Lys(Boc)-hexyl-N_3. Synthesized from Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, and 6-azidohexanoic acid. 80% yield. ^{1}H NMR: δ (DMSO-d_6, 2.50 ppm) 11.30 (bs, 1H), 7.66-8.60 (m, 7H), 6.22-6.84 (m, 2H), 4.13-4.50 (m, 11H), 3.29 (t, 2H), 2.78-2.95 (m, 4H), 1.99-2.21 (m, 6H), 1.07-1.88 (m, 51H), 0.86 (d, 3H), 0.81 (d, 3H). ^{13}C NMR: δ (DMSO-d_6, 39.52 ppm) 172.32, 172.12, 172.01, 171.91, 155.57, 144.74, 122.47, 77.32, 52.47, 50.97, 50.56, 49.17, 48.58, 40.84, 34.96, 34.91, 34.32, 31.66, 29.46, 29.23, 28.25, 27.96, 25.75, 25.44, 24.71, 24.64, 24.57, 24.25, 22.97, 21.46. ESI (m/z): $[M+H]^+$ calculated from C52H91N16O11, 1115.71; found, 1115.69.

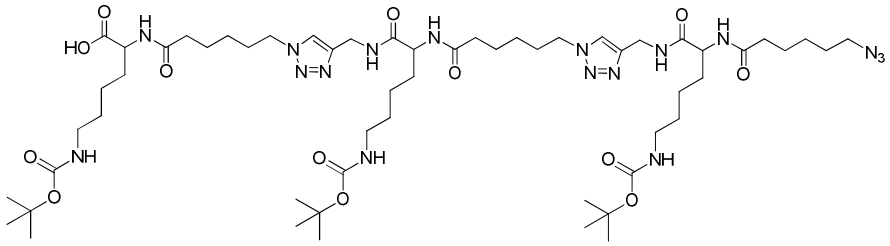
6.7. Lys(Boc)-hexyl-propargyl-Lys(Boc)-hexyl-propargyl-Cys(Trt)-hexyl-N_3. Synthesized from Fmoc-Lys(Boc)-OH, Fmoc-Cys(Trt)-OH, and 6-azidohexanoic acid. 57% yield. ^{1}H NMR: δ (DMSO-d_6, 2.50 ppm) 12.20 (bs, 1H), 7.64-8.63 (m, 7H), 7.10-7.42 (m, 15H), 6.20-6.88 (m, 2H), 4.09-4.50 (m, 11H), 3.23 (t, 2H), 2.79-2.92 (m, 4H), 1.98-2.21 (m, 6H), 1.01-1.91 (m, 50H). ^{13}C NMR: δ (DMSO-d_6, 39.52 ppm) 171.99, 155.50, 144.21, 129.01, 127.99, 127.76, 127.51, 122.44, 77.32, 65.71, 52.46, 50.55, 49.13, 34.89, 34.31, 31.72, 29.48, 29.25, 25.45, 24.64, 22.76. ESI (m/z): $[M-H]^-$ calculated from C68H97N16O11S, 1345.72; found, 1345.72.

6.8. Lys(Boc)-hexyl-propargyl-Leu-hexyl-propargyl-Cys(Trt)-hexyl-N_3. Synthesized from Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Cys(Trt)-OH, and 6-azidohexanoic acid. 55% yield. ^{1}H NMR: δ (DMSO-d_6, 2.50 ppm) 11.80 (bs, 1H), 7.64-8.65 (m, 7H), 7.08-7.62 (m, 15H), 6.20-6.87 (m, 1H), 4.09-4.71 (m, 11H), 3.29 (t, 2H), 2.78-2.92 (m, 2H), 1.99-2.21 (m, 6H), 0.97-1.90 (m, 38H), 0.85 (d, 3H), 0.80 (d, 3H). ^{13}C NMR: δ (DMSO-d_6, 39.52 ppm) 172.36, 172.07,155.61, 144.31, 129.08, 127.79, 126.77, 122.57, 77.36, 65.81, 50.98, 50.57, 49.20, 40.88, 34.93, 34.35, 29.27, 28.28, 25.45, 24.68, 24.28, 23.00, 21.48. ESI (m/z): $[M-H]^-$ calculated from C63H88N15O9S, 1230.66; found, 1230.66.

6.9. Val-hexyl-propargyl-Val-hexyl-propargyl-Val-hexyl-N_3. Synthesized from Fmoc-Val-OH, and 6-azidohexanoic acid. 66% yield. ^{1}H NMR: δ (DMSO-d_6, 2.50 ppm) 11.40 (bs, 1H), 7.66-8.69 (m, 7H), 4.08-4.43 (m, 11H), 3.290 (t, 2H), 2.02-2.29 (m, 6H), 1.09-1.84 (m, 21H), 0.66-0.99 (m, 18H). ^{13}C NMR: δ (DMSO-d_6, 39.52 ppm) 172.20, 171.21, 144.72, 122.60, 77.35, 57.80, 50.60, 40.18, 35.78, 34.96, 34.90, 34.19, 30.78, 30.38, 29.51, 28.26, 27.99, 25.79, 25.44, 24.87, 24.76, 19.21, 18.22. ESI (m/z): $[M-H]^-$ calculated from C39H65N14O7, 841.52; found, 841.52.

7. Optimized reaction conditions for the synthesis of sequence-defined oligomers based on natural and synthetic building blocks

The previous reaction conditions for the synthesis of sequence-defined oligomers were optimized for the model oligomer Kh▪Kh▪Kh by reducing the reaction times of each step and the amount of copper used for the cycloaddition reaction while obtaining successfully the desired oligomers.



As previously the chlorotrityl chloride resin was loaded with Fmoc-Lys(Boc)-OH as described in Section 4 and the oligomers were synthesized from successive additions of 6-azidohexanoic acid, propargylamine and Fmoc-Lys(Boc)-OH following the procedure described below until the sequence was obtained.

i) Addition of 6-azidohexanoic acid. 6-Azidohexanoic acid (3 eq. relative to the loading), HBTU (3 eq. relative to the loading), HOBt (3 eq. relative to the loading), DIPEA (6 eq. relative to the loading) and anhydrous DMF (5 mL per mmol of functional groups on the resin) were added to the peptide vessel, and agitated for 30 min. After filtration, the resin was washed 6 times with DMF and 6 times with CH_2Cl_2.

ii) Copper-assisted alkyne-azide cycloaddition with propargylamine. CuBr (0.2 eq. relative to the loading) was added to the peptide vessel, which was then degased through 3 vacuum/argon cycles. Anhydrous CH_2Cl_2 (7 mL per mmol of functional groups on the resin) were added to the peptide vessel, followed by PMDETA (0.4 eq. relative to the loading) and propargylamine (3 eq. relative to the loading). The solution was agitated under argon for 1 h. After filtration, the resin was washed 6 times with DMF and 6 times with CH_2Cl_2.

iii) Addition of Fmoc-Lys(Boc)-OH. Fmoc-Lys(Boc)-OH (3 eq. relative to the loading), HBTU (3 eq. relative to the loading), HOBt (3 eq. relative to the loading), DIPEA (6 eq. relative to the loading) and anhydrous DMF (5 mL per mmol of functional groups on the resin) were added to the peptide vessel, and agitated for 30 min. After filtration, the resin was washed 6 times with DMF. The Fmoc-protecting group was removed by agitation for 3 min with a 25% piperidine cleavage solution in DMF followed by filtration and agitation with a fresh cleavage solution for 20 min. The resin was then washed 6 times with DMF and 6 times with CH_2Cl_2.

The oligomer was cleaved from the resin by adding a 4/1 CH_2Cl_2/TFE solution to the peptide vessel for 45 min (twice). The resin was filtered, washed three times with CH_2Cl_2, and the filtrates were collected in a clean round-bottom flask. The solution was concentrated by rotary evaporation.

Lys(Boc)-hexyl-propargyl-Lys(Boc)-hexyl-propargyl-Lys(Boc)-hexyl-N_3. 3 cycles (Kh▪Kh▪Kh) 75% yield. ^{1}H NMR: δ (DMSO-d_6, 2.50 ppm) 11.50 (bs, 1H), 7.67-8.74 (m, 7H), 6.22-6.84 (m, 3H), 3.97-4.53 (m, 11H), 3.27 (t, 2H), 2.73-2.97 (m, 6H), 1.94-2.20 (m, 6H), 0.96-1.90 (m, 63H). ^{13}C NMR: δ (DMSO-d_6, 39.52 ppm) 172.35, 172.16, 172.06, 155.71, 144.91, 122.64, 77.45, 52.74, 50.66, 49.29, 48.66, 35.06, 34.41, 31.68, 31.26, 29.56, 29.22, 28.33, 28.05, 25.82, 25.51, 24.74, 24.66, 22.95, 22.84. ESI (m/z): $[M-H]^-$ calculated from C57H98N17O13, 1228.75; found, 1228.74.

8. Removal of the protecting groups of the model sequence-defined oligomer

The Boc protecting groups of the model sequence-defined oligomer Kh▪Kh▪Kh were removed by combining the oligomer (0.2 g, 0.16 mmol) with 1 mL of a solution of TFA/TIPS/water (95/2.5/2.5) in a round-bottom flask. The mixture was stirred at room temperature for 90 min and then precipitated into diethyl ether. The precipitate was isolated by filtration and dried under vacuum at room temperature.

Lys-hexyl-propargyl-Lys-hexyl-propargyl-Lys-hexyl-N_3. 83% yield. ^{1}H NMR: δ (D_2O, 4.79 ppm) 7.43-8.21 (m, 2H), 4.11-4.64 (m, 11H), 3.28 (t, 2H), 2.87-3.10 (m, 6H), 2.18-2.40 (m, 6H), 1.11-2.01 (m, 36H). ^{13}C NMR: δ (D_2O) 176.88, 176.77, 174.10, 53.90, 52.62, 50.95, 50.37, 39.17, 35.06, 30.39, 29.96, 28.98, 27.72, 26.27, 26.23, 25.45, 25.09, 24.99, 24.77, 24.56, 22.16.

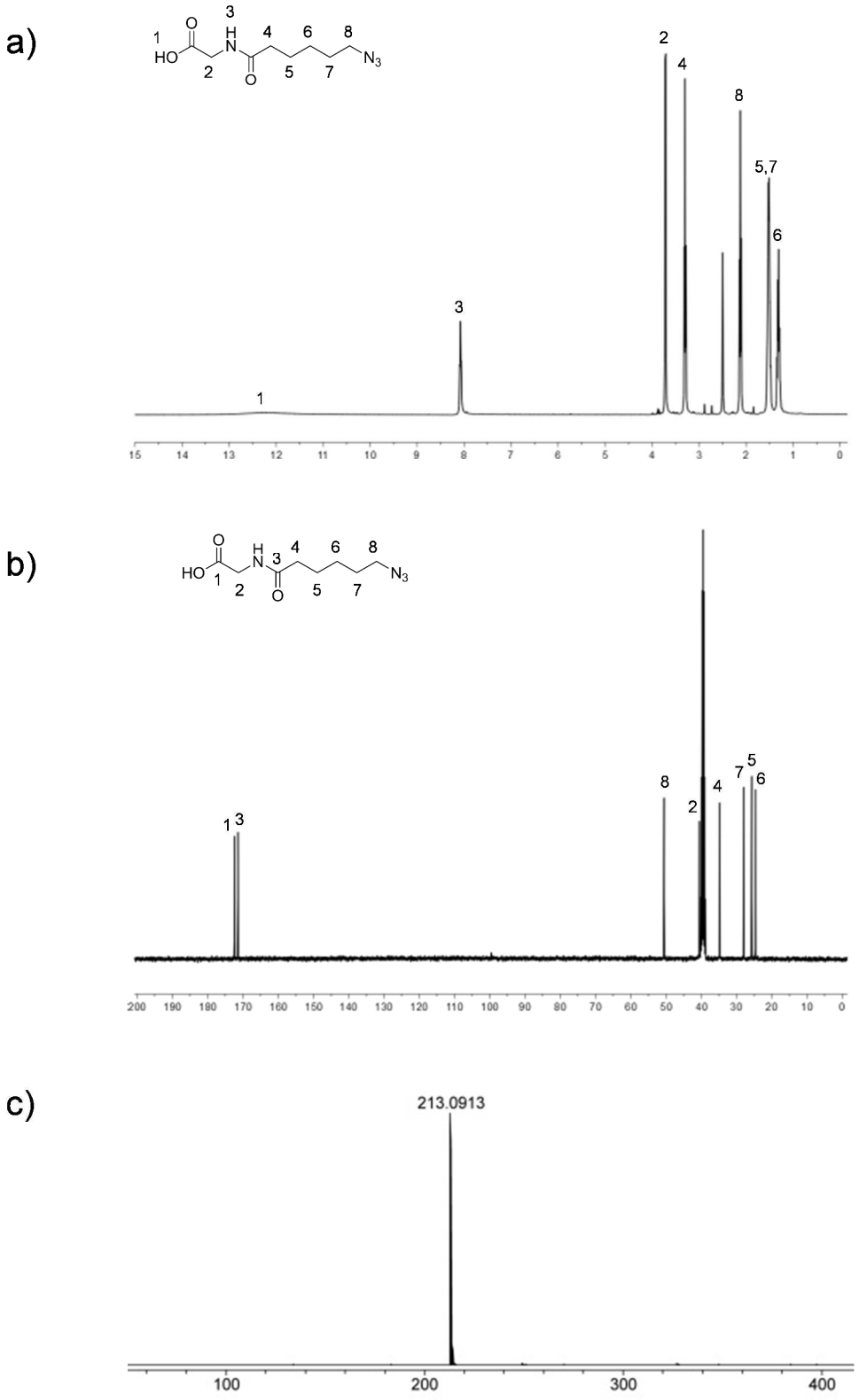


Figure S1. Characterization of Gly-hexyl-N_3 (Gh) by a) ^{1}H and b) ^{13}C NMR in DMSO-d_6, and c) ESI-MS.

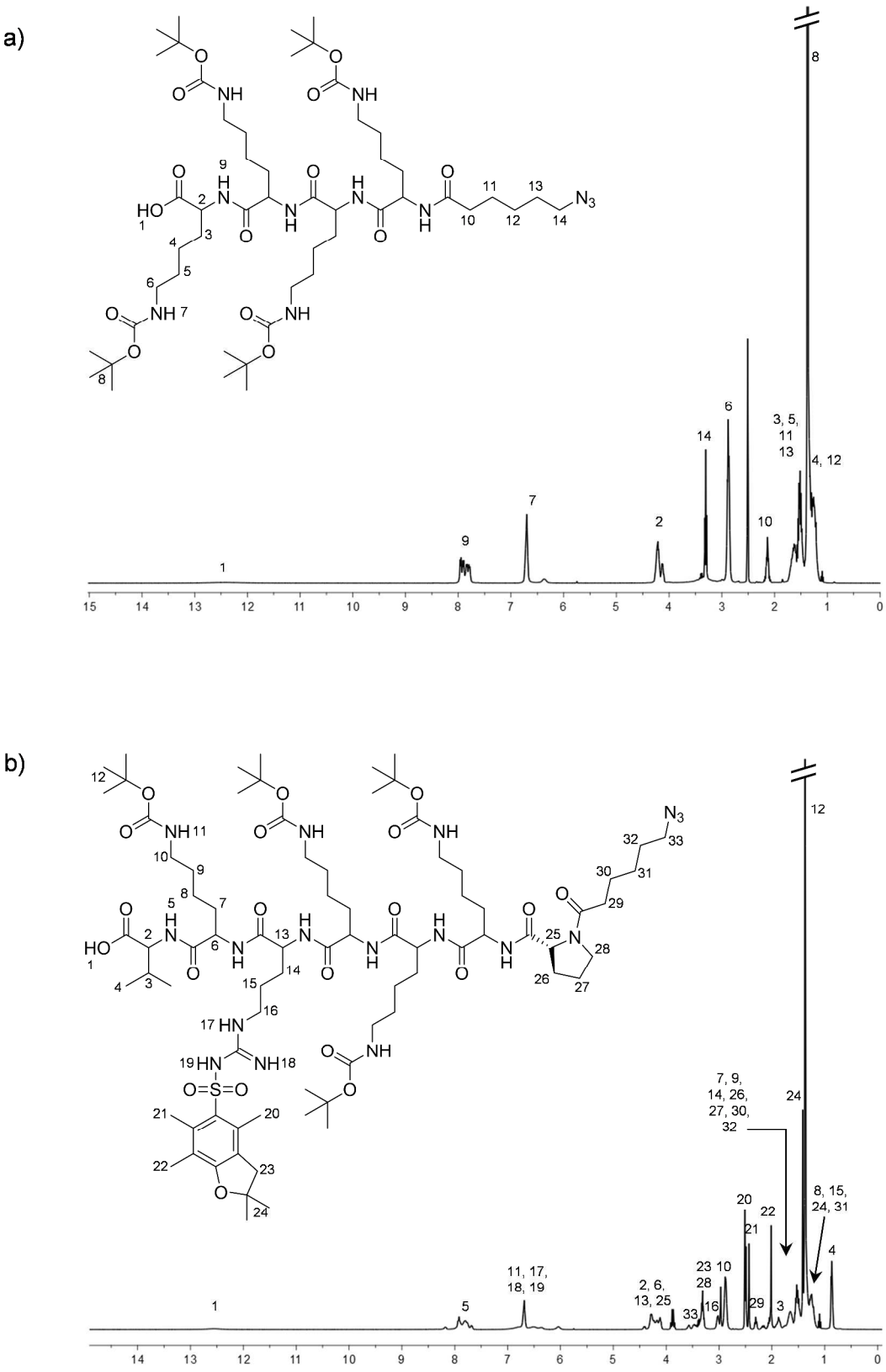


Figure S2. ^{1}H NMR spectra in DMSO-d_6 of a) Lys(Boc)-Lys(Boc)-Lys(Boc)-Lys(Boc)-hexyl-N_3 (KKKKh) and b) Val-Lys(Boc)-Arg(Pbf)-Lys(Boc)-Lys(Boc)-Lys(Boc)-Pro-hexyl-N_3 (VKRKKKPh).

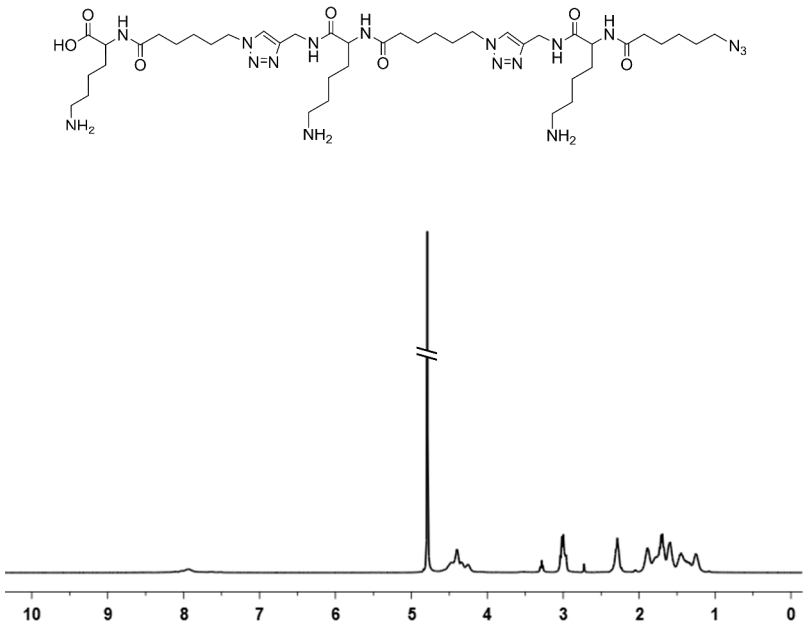


Figure S3. ^{1}H NMR spectrum of Lys-hexyl-propargyl-Lys-hexyl-propargyl-Lys-hexyl-N_3 in D_2O.

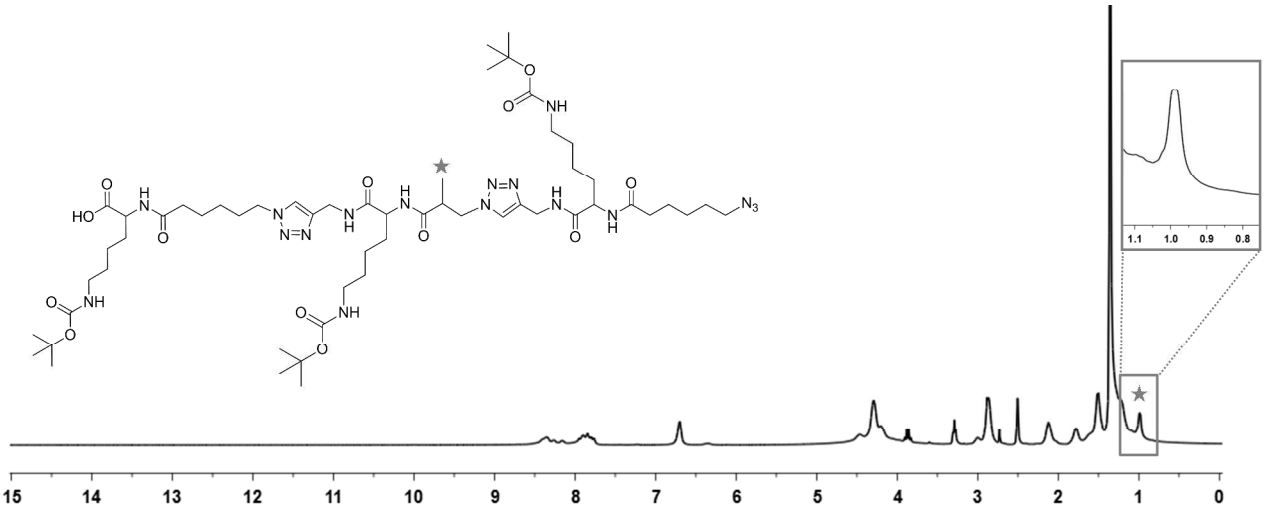


Figure S4. ^{1}H NMR spectrum in DMSO-d_6 of Lys(Boc)-hexyl-propargyl-Lys(Boc)-methylpropionyl-propargyl-Lys(Boc)-hexyl-N_3.

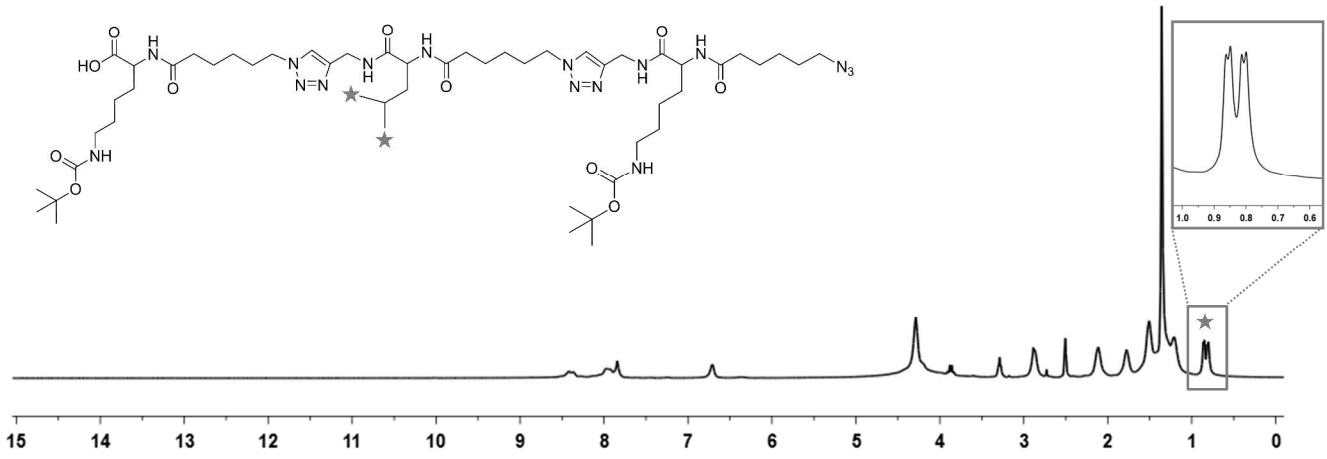


Figure S5. ^{1}H NMR spectrum in DMSO-d_6 of Lys(Boc)-hexyl-propargyl-Leu-hexyl-propargyl-Lys(Boc)-hexyl-N_3.