Electronic Supplementary Information for

Facile Synthesis of Stapled, Structurally Reinforced Peptide Helices via A Photoinduced Intramolecular 1,3-Dipolar Cycloaddition Reaction

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Materials and General Procedures

General Methods: ^{1}H-NMR spectra were recorded on either an Inova 500 MHz NMR instrument or an Inova 400 MHz NMR instrument. Chemical shifts were reported in parts per million (ppm) relative to internal solvent standards. Multiplicities were reported as follows: singlet (s), doublet (d), triplet (t), doublet of doublet (dd), quintet (q) or multiplet (m). UV-Vis Data were recorded using a HP 8452 Diode Array Spectrophotometer monitoring from 195-825 nm. Fluorescence measurements were conducted using 1-cm pathlength cuvettes on JY Fluorolog spectrofluorimeter at 20 °C. Fluorescence emission was scanned in the range of 400 to 700 nm through a 2-nm slit. Low-resolution LC-MS spectra were recorded using a Thermo Finnigan LCQ Advantage instrument operating in the positive ion mode. High-resolution ESI-MS analysis was performed by SUNY Buffalo Instrument Center.

HPLC Purification and Analysis: Purification of linear peptides was conducted using silica gel flash chromatography. Cyclic peptides were purified using a Gilson reverse-phase HPLC system and a semi-prep Phenomenex C_{18} column with a flow rate set to 5 mL/min and a gradient of 10-90% ACN/H_2O while monitoring at 220 nm and 370 nm. HPLC analyses were carried out using an analytical Gilson RP-HPLC system and a Keystone Scientific C_{18} column monitoring at 254 nm.

Circular Dichroism: CD spectra were obtained using 1-mm pathlength cuvettes on a JASCO J-715 instrument at 25 °C. The instrument scan range was set at 190 to 250 nm. The scan rate was set at 50 nm/min, the response time was set at 2 sec, and the bandwidth was set at 1 nm. The spectra were the averages of two scans. For the measurement of percent helicity, peptides were dissolved in 100% trifluoroethanol (TFE); for the *T*m determination, 20% TFE/H_2O were used as the solvent. The percent helicity was calculated by using the following equation[1,2]: % helicity = $\frac{[\theta]_{208MRE} - 4000}{-29000} \times 100$. The

melting temperature (*T*m) was determined by following a reported procedure[3]. Briefly, a sample of 100 μM peptide in 20% TFE/H_2O in an 1-mm pathlength cuvette was scanned at various temperatures from 20 °C to 85 °C (5-degree intervals) with an equilibration time of 3 min and a heating rate of 60 °C/hr. The $\theta_{222\ MRE}$ data was normalized by setting the maximum $\theta_{222\ MRE}$ to 100% and the minimum $\theta_{222\ MRE}$ to 0.

Cellular Uptake Study: HeLa cells cultured on cover slips were treated with 100 μM of peptide **13** or control peptide **18** in 2% DMSO serum-free DMEM medium for 4 hr in a 37 °C, 5% CO_2 incubator. In preparation for fixation, medium was aspirated and the cells were washed twice with PBS. The cover slips were treated to a freshly prepared 4% paraformaldehyde/PBS solution for 10 ~ 15 min, washed twice with PBS, and quenched with 50 mM NH_4Cl for 10 min. After washing with PBS twice, the cover-slip was flipped and placed on top of microscopy glass containing in situ-frame and PBS buffer to make a sealed sample chamber. The images were acquired on a Zeiss Axioimager motorized fluorescence microscope. DAPI filter (ex 365 nm, em 445 ± 25 nm) was used in acquiring fluorescence images. All image acquisitions and processing were performed under identical conditions.

Table S1. Solvent tolerability study of the photoinduced stapling reaction[a]

linear peptide	*solvent*	*product*	*yield*
8	MeCN	**16**	87
8	DCM	**16**	43
8	EtOAc	**16**	86
8	EtOH	**16**	92
8	^{i}PrOH	**16**	88
8	EtOH/H_2O (1:1)	**16**	88

[a] The reaction was performed by irradiating 1.2 mL of 150 μM of peptide **8** in a quartz test tube with a handheld UV lamp at 302 nm for 10 min. The solvent was removed under the reduced pressure and the residue was subjected to the reverse-phase HPLC analysis equipped with a C_{18} column. The yields were calculated based on the absorption at 254 nm in the HPLC traces.

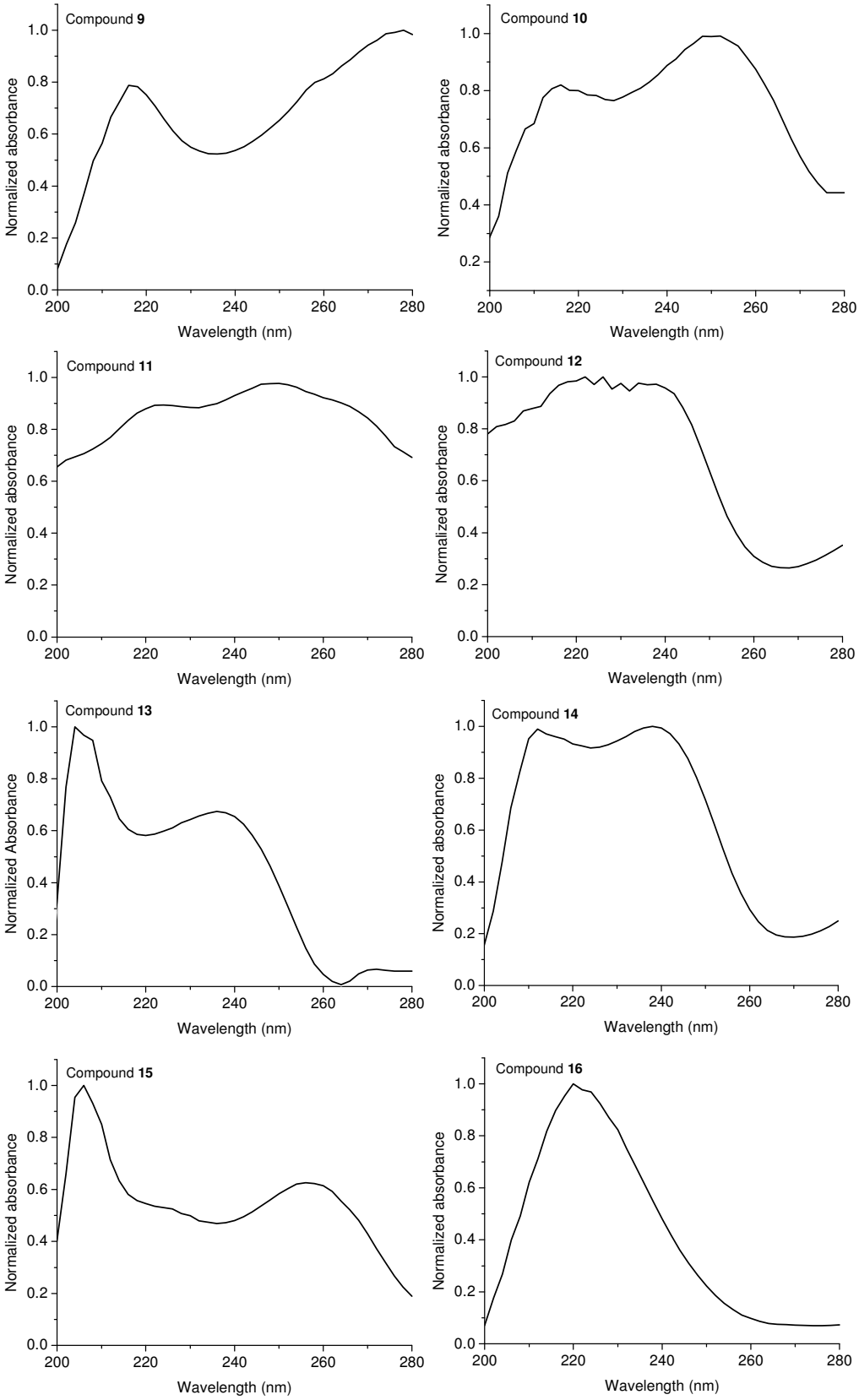


Figure S1. UV-Vis spectra of peptides **9-16** in the range of 220-280 nm.

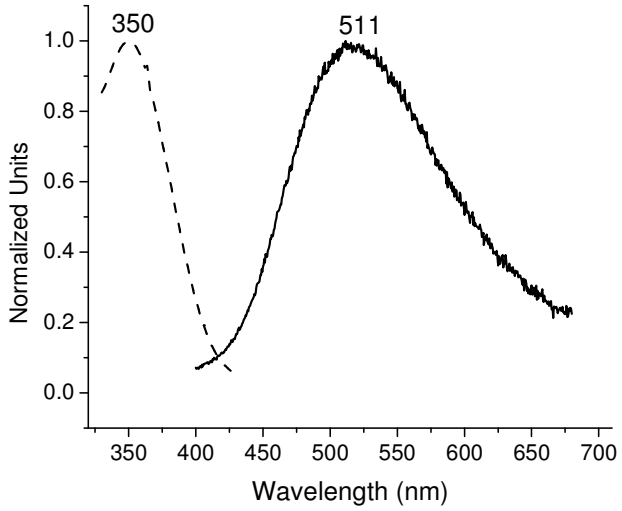
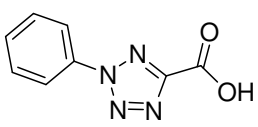


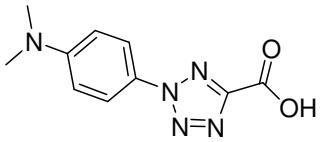
Figure S2. UV-Vis and fluorescence spectra of the linear control peptide **18**. Dashed lines represent the UV absorbance spectra while solid lines represent the fluorescence emission spectra. The absorption and emission maxima were marked on top of the spectra.

Synthesis and Characterization of Linear and Cyclic Peptides:

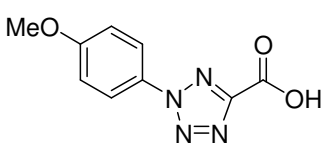


2-Phenyl-2*H*-tetrazole-5-carboxylic acid: To 0.8 mL ethyl glyoxylate in toluene (50%) was added 130 mL ethanol solution of sulfonyl hydrazide (0.69 g, 4.0 mmol). The resulting mixture was stirred for 1 hr at room temperature in an open flask before 130 mL of chilled distilled water was added. A white precipitate (sulfonylhydrazone) was generated, which was collected by filtration. The filtrate was subsequently dissolved in 50 ml pyridine to derive solution A. Separately, to a 10 mmol solution of aniline in a 1:1 mixture of ethanol and water (20 mL cooled to -5°C) was added 3.0 mL of concentrated HCl. To the resulting acidic aniline solution, 4 mL solution of sodium nitrate (0.690 g, 10 mmol) was added drop-wise generating the phenyl-diazonium salt solution (solution B). Both solutions were cooled to -5°C and solution B was added drop-wise to solution A over a period of 30 minutes. Following addition, the reaction mixture was allowed to warm up to room temperature over 45 min and subsequently extracted with ethyl acetate three times. The organic layer was subsequently washed with a 0.1 N HCl solution, dried over sodium sulfate, and concentrated. The crude mixture was purified via a silica gel chromatography using a stepwise gradient of ethyl acetate/hexane to afford ethyl 2-phenyl-2H-tetrazole-5-carboxylate as a pink-purple powder. Following purification, sodium hydroxide (7.5 mmol; 0.6 g dissolved in 2 mL of water) was added to a 50 mL solution of ethyl 2-phenyl-2*H*-tetrazole-5-carboxylate in ethanol. The reaction mixture was refluxed overnight and allowed to cool to -5°C. The mixture was acidified with 1.2 mL of dilute HCl solution.

The resulting solution was extracted five times with ethyl acetate to yield the titled compound (0.37 g, 49%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (d, *J* = 7.5 Hz, 2H) 7.60 (m, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 158.6, 158.3, 135.9, 130.7, 130.2, 120.3; HRMS (EI) calcd for $C_8H_6N_2O_2$ 162.0424 $[M-N_2]^+$, found 162.0423.



2-(4-Dimethylamino-phenyl)-2H-tetrazole-5-carboxylic acid: To 1.6 mL of ethyl glyoxilate in toluene was added 130 mL ethanol solution of sulfonyl hydrazide (1.38 g, 8.0 mmol). The resulting mixture was stirred for 1 hr at room temperature in an open flask before being quenched by addition of ice. A white precipitate (sulfonylhydrazone) was formed, and collected by filtration. The filtrate was subsequently dissolved in 50 ml of pyridine to derive solution A. Separately, to a 7.2 mmol solution of *N*, *N*-dimethylamino aniline in a 3:1 mixture of ethanol and water (20 mL, cooled to -5 °C) was added 3.0 mL of concentrated HCl. To the resulting acidic aniline solution, 4 mL solution of sodium nitrite (0.690 g, 10 mmol) was added dropwise to derive the phenyl diazonium salt solution (solution B). Solution A was cooled to -20 °C and solution B (kept at -5 °C) was added dropwise to solution A over a period of 30 minutes. Following addition, the reaction mixture was allowed to warm up to room temperature over 60 min and subsequently extracted with ethyl acetate three times. The organic layer was washed with 0.1 N HCl, dried over sodium sulfate, and concentrated. The crude mixture was purified by silica gel chromatography using a stepwise gradient of ethyl acetate/hexane to afford 2-(4-dimethylamino-phenyl)-2*H*-tetrazole-5-ethyl ester as a blue green powder. Following purification, sodium hydroxide (0.6 g, 7.5 mmol, dissolved in 2 mL water) was added to a 50 mL solution of 2-(4-dimethylamino-phenyl)-2*H*-tetrazole-5-ethyl ester in ethanol. The mixture was refluxed for 2 hrs and allowed to cool to room temperature. The mixture was acidified with a dilute HCl solution, and the solution was extracted three times with ethyl acetate to yield the titled compound (0.39 g, 23%): ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.88 (d, *J* = 9.0 Hz, 2H), 6.89 (d, *J* = 9.0 Hz, 2H), 3.01 (s, 6H); ^{13}C NMR (75.4 MHz, DMSO-d_6) δ 158.8, 157.7, 151.4, 125.0, 121.4, 121.3, 112.0, 39.8; HRMS (ESI) calcd for $C_{10}H_{12}N_5O_2$ 234.0986 $[M + H^+]$, found 234.0987.



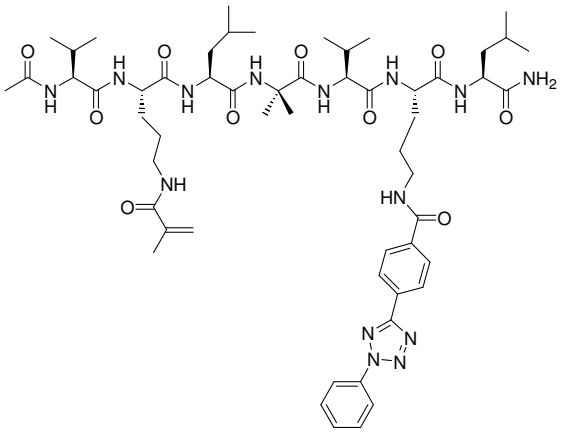
2-(4-Methoxy-phenyl)-2H-tetrazole-5-carboxylic acid: To 1.6 mL of ethyl glyoxilate in toluene was added 130 mL ethanol solution of sulfonyl hydrazide (1.38 g, 8.0 mmol). The resulting mixture was stirred for 1 hr at room temperature in an open flask before being pored over 100 g of ice. A white precipitate (sulfonylhydrazone) was generated, which was collected by filtration. The filtrate was subsequently dissolved in 50 mL pyridine to derive solution A. Separately, to a 7.2 mmol solution of 4-methoxy-aniline in a 3:1 mixture of ethanol and water (20 mL cooled to -5 °C) was added 3.0 mL of concentrated HCl. To the resulting acidic aniline solution, 4 mL solution of sodium nitrite (0.690 g, 10 mmol) was

added dropwise to generate the phenyldiazonium salt (solution B). Solution A was cooled to -20 °C and solution B (kept at -5 °C) was added dropwise to solution A over a period of 30 minutes. Following addition, the reaction mixture was allowed to warm up to room temperature over 60 minutes and subsequently extracted with ethyl acetate three times. The organic layer was then washed with 0.1 N HCl, dried over sodium sulfate, and concentrated. The crude mixture was purified via a silica gel chromatography using a stepwise gradient of ethyl acetate/hexane to afford 2-(4-methoxy-phenyl)-2*H*-tetrazole-5-ethyl ester as a light yellow-brown powder. Following purification, sodium hydroxide (7.5 mmol; 0.6 g dissolved in 2 mL of water) was added to a 50 mL solution of 2-(4-methoxy-phenyl)-2*H*-tetrazole-5-ethyl ester in ethanol. The mixture was refluxed for 2 hrs before cooling down to room temperature. The mixture was acidified with a dilute HCl solution. The resulting 2-(4-methoxy-phenyl)-2*H*-tetrazole-5-carboxylic acid was extracted three times with ethyl acetate to yield the titled compound (0.55 g, 35%): ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.02 (d, J = 9.5 Hz, 2H), 7.20 (d, J = 8.5 Hz, 2H), 3.85 (s, 3H); ^{13}C NMR (75.4 MHz, DMSO-d_6) δ 158.6, 160.4, 159.1, 129.7, 121.8, 115.3, 115.0, 55.8, 55.6; HRMS (EI) calcd for $C_9H_8N_2O_3$ 192.0529 $[M-N_2]^+$, found 192.0531.

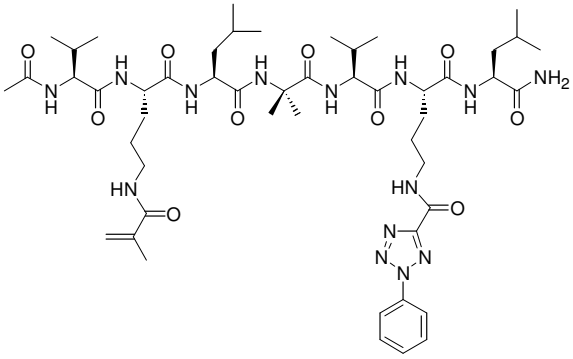
Solid Phase Peptide Synthesis: Linear peptides were synthesized by following standard 9*H*-fluoren-9-yl-methoxycarbonyl (Fmoc) solid phase peptide synthesis protocol on the Rink-amide resin. Briefly, three equiv of HBTU/HOBt, three equiv of amino acids, and six equiv of DIEA in DMF were used in each coupling reaction. The coupling reaction was allowed to proceed for 40 minutes. The Fmoc deprotection was accomplished by treating the resin-bound peptides with 20% piperidine/DMF (2×, 10 min each). Fmoc-Lys(Mtt)-OH and Fmoc-Orn(Mtt) were used as in the assembly of the peptides. The selective removal of Mtt was accomplished by treating the resin with 1%TFA/DCM (5×, 10 min each). The coupling of the tetrazole to the deprotected Lys/Orn sidechain was achieved by treating the resin with 3 equiv of HBTU/HOBT, 3 equiv of tetrazole, and 6 equiv of DIEA in DCM/DMF (1:1) for 1 hour. The coupling of methacrylic acid to the deprotected Lys/Orn sidechain was carried out by treating the resin with 20 equiv of methacrylic anhydride and 30 equiv of DIEA in DCM for 1 hour. The peptides were cleaved from the Rink amide resin by treating the resin with a cleavage cocktail containing 95% TFA, 2.5% triisopropylsilane, and 2.5% water for 1 hour. The filtrates were concentrated and then extracted with DCM. The organic layer was washed 3× with saturated sodium bicarbonate solution, concentrated, and dried under high vacuum. The crude peptides were purified by either silica gel flash chromatography or preparative HPLC.

General Procedure for Photoinduced Peptide Stapling: A stirred solution of linear peptide (150 μM, dissolved in HPLC-grade acetonitrile) in a quartz round-bottom flask was irradiated with a 302-nm UV lamp (UVM-57, 302 nm, 115V, 0.16 AMPS) under argon for 2 hours. The solution was concentrated and

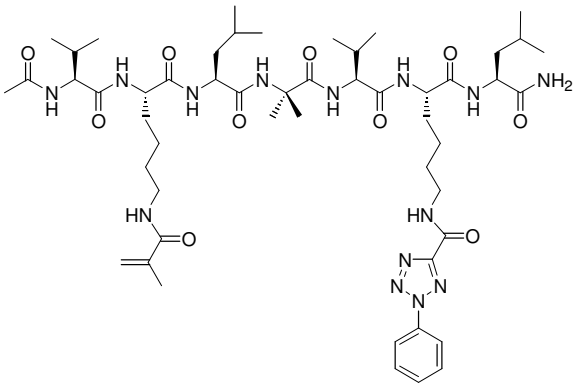
the crude product was purified by preparative RP-HPLC. The fractions that showed greater than 95% purity with the correct masses were pooled and lyophilized to give the stapled peptide as a fluffy white powder.



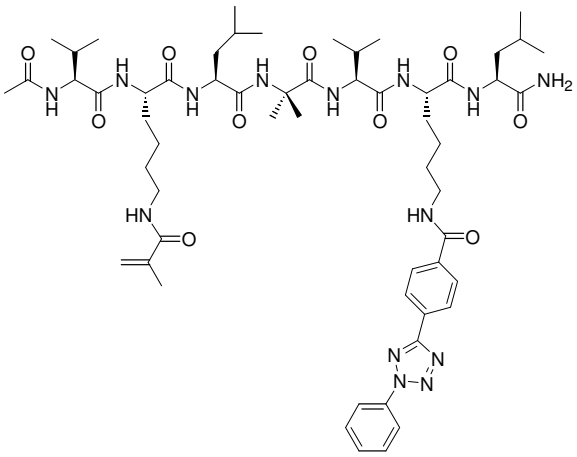
Linear Peptide (**1**): Heptapeptide **1** was prepared according to the general procedure. After purification, a yellow-white crystalline solid was obtained in 7% yield (based on resin substitution): ^{1}H-NMR (CD_3OD, 500 MHz) δ 8.18 (d, *J* = 7.8 Hz, 2H), 7.65 (t, *J* = 8.0 Hz, 2H), 7.62 (d, *J* = 7.2 Hz, 1H), 5.69 (d, *J* = 14.0 Hz, 1H), 5.37 (d, *J* = 16.8 Hz, 1H), 4.37 (d, *J* = 7.7 Hz, 1H), 4.18-4.08 (br, m, 3H), 3.98 (t, *J* = 6.6 Hz, 1H), 3.89 (d, *J* = 6.8 Hz, 1H), 3.81 (d, *J* = 7.3 Hz, 1H), 3.63 (t, *J* = 5.9 Hz, 1H), 3.56-3.45 (br, m, 1H), 3.23 (br, m, 1H), 2.23 (br, m, 1H), 2.09 (s, 3H), 2.05 (s, 1H), 2.01-1.96 (br, m, 1H), 1.93 (s, 1H), 1.89 (s, 1H), 1.79-1.59 (br, m, 10H), 1.50 (s, 3H), 1.47 (s, 2H), 1.44 (s, 3H), 1.37-1.28 (br, m, 2H), 1.07-0.85 (br, m, 24H); HRMS (ESI) calcd for $C_{50}H_{80}N_{14}O_{10}Na$ 1059.6074 [M + Na^+], found 1059.6069.



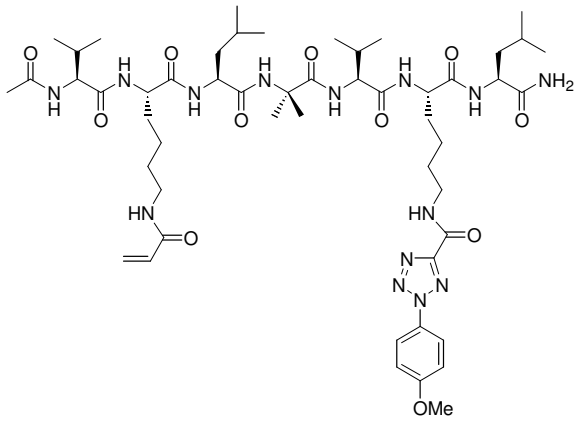
Linear Peptide (**2**): Heptapeptide **2** was prepared according to the general procedure. After purification, a yellow-white crystalline solid was obtained in 10% yield (based on resin substitution): ^{1}H-NMR (CD_3OD, 400 MHz) δ 8.31 (d, *J* = 8.2 Hz, 2H), 8.22 (d, *J* = 7.8 Hz, 2H), 7.66 (t, *J* = 7.8 Hz, 2H),7.59 (t, *J* = 7.3 Hz, 1H), 5.66 (s, 1H), 5.32 (s, 1H), 4.37 (dd, *J* = 7.5, 4.0 Hz, 1H), 4.17 (t, *J* = 5.0 Hz, 2H), 4.11 (t, *J* = 7.9 Hz, 1H), 3.93 (d, *J* = 6.6 Hz, 1H), 3.83 (d, *J* = 6.7 Hz, 1H), 3.48 (br, m, 2H), 3.24 (m, 2H), 2.23 (m, 2H), 2.07 (s, 3H), 1.99 (br, m, 3H), 1.89 (s, 3H), 1.77 (br, m, 4H), 1.63 (br, m, 6H), 1.49 (s, 3H), 1.45 (s, 3H), 1.19 (br, m, 1H), 1.07-0.99 (br, m, 13H), 0.96-0.90 (br, m, 13 H); HRMS (ESI) calcd for $C_{56}H_{84}N_{14}O_{10}Na$ 1135.6387 [M + Na^+], found 1135.6375.



Linear Peptide (**3**): Heptapeptide **3** was prepared according to the general procedure described above. After purification, a yellow-white crystalline solid was obtained in 25% yield (based on resin substitution): ^{1}H-NMR (CD_3OD, 500 MHz) δ 8.17 (d, *J* = 7.3 Hz, 2H), 7.65 (t, *J* = 7.0 Hz, 2H), 7.61 (d, *J* = 7.1 Hz, 1H), 5.67 (d, *J* = 11.3 Hz, 1H), 5.33 (d, *J* = 8.8 Hz, 1H), 4.38 (m, 1H), 4.12 (m, 3H), 3.92 (d, *J* = 6.7 Hz, 1H), 3.80 (d, *J* = 7.2 Hz,1H), 3.48 (m, 2H), 3.22 (t, *J* = 6.7 Hz, 2H), 2.21 (m, 1H), 2.14 (s, 1H), 2.07 (s, 3H), 1.89 (br, m, 5H), 1.86-1.63 (br, m, 11H), 1.55 (br, m,2H), 1.49 (s, 3H), 1.44 (s, 3H), 1.24-1.18 (br, m, 1H), 1.06-0.84 (br, m, 26H); HRMS (ESI) for $C_{52}H_{84}N_{12}O_{10}Na$ 1087.6387 [M + Na^+], found 1087.6417.

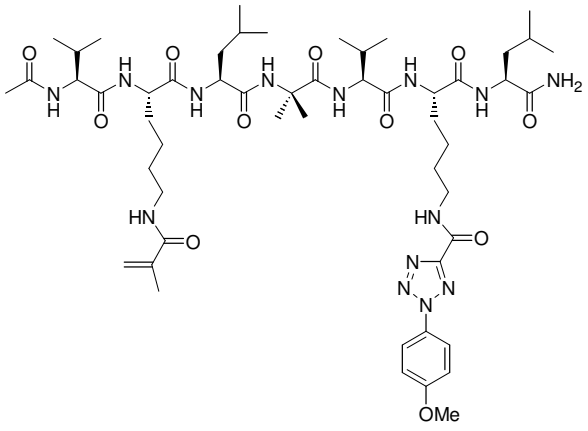


Linear peptide (**4**): Heptapeptide **4** was prepared according to the general procedure described above. After purification, a yellow-white crystalline solid was obtained in 11% yield (based on resin substitution): ^{1}H-NMR (CD_3OD, 500 MHz) δ 8.34 (d, *J* = 8.3 Hz, 2H), 8.25 (d, *J* = 7.4 Hz, 2H), 8.04 (d, *J* = 8.7 Hz 2H), 7.69 (t, *J* = 8.1 Hz, 2H), 7.62 (t, *J* = 7.2 Hz, 1H), 5.67 (s, 1H), 5.34 (s,1H), 4.40 (dd, *J* = 7.6, 3.7 Hz,1H), 4.15 (m, 3H), 3.97 (d, *J* = 6.7 Hz, 1H), 3.85 (d, *J* = 7.0 Hz, 1H), 3.44 (br, m, 2H), 3.22 (br, m, 2H), 2.26 (br, m, 1H), 2.10 (s, 1H), 2.08-1.95 (br, m, 3H), 1.92 (s,3H), 1.84-1.65 (br, m, 12H), 1.55 (m, 2H), 1.52 (s, 3H), 1.48 (s, 3H), 1.40-1.28 (br, m, 2H), 1.10-0.99 (br, m, 26H); HRMS (ESI) calcd for $C_{58}H_{88}N_{14}O_{10}Na$ 1163.6700 [M + Na^+], found 1163.6722.

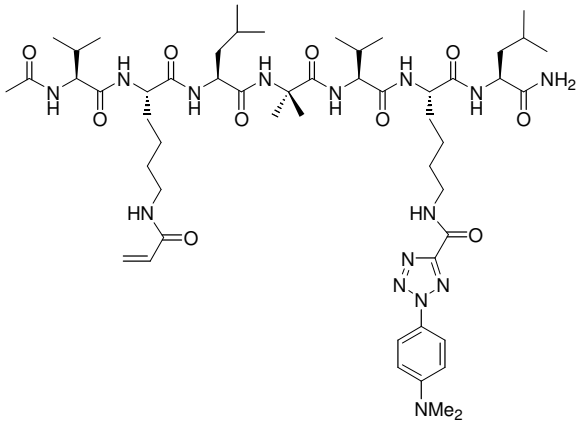


Linear Peptide (**5**): Heptapeptide **5** was prepared according to the general procedure. After RP-HPLC a yellowish white powder was obtained in 18% yield based on resin substitution: ^{1}H-NMR (CD_3OD, 500 MHz) δ 8.09 (d, *J* = 7.0 Hz, 2H), 7.17 (d, *J* = 7.1Hz, 2H), 6.20 (d, *J* = 8.6 Hz, 2H), 5.62 (dd, *J* = 8.6, 3.4 Hz, 1H), 4.37 (dd, *J* = 11.3, 3.7 Hz 1H), 4.14 (m,3H), 3.94 (d, *J* = 6.7 Hz, 1H), 3.83 (s, 3H), 3.61 (m, 2H), 3.81 (d, *J* = 7.1 Hz, 1H) 3.44 (m, 4H), 3.22 (m, 2H), 3.16 (m,

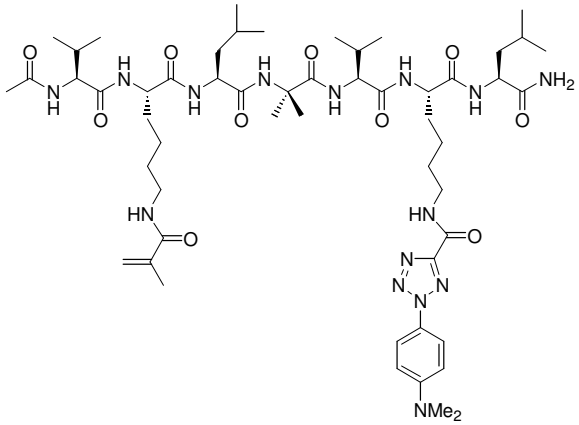
2H), 2.05 (s, 3H), 1.93 (m, 1H), 1.78-1.60 (br, m, 10H), 1.55-1.44 (br, m, 10H), 1.02-0.84 (br, m, 25H); HRMS (ESI) calcd for $C_{52}H_{84}N_{14}O_{11}Na$ 1103.6342 [M + Na^+], found 1103.6351.



Linear Peptide (**6**): Heptapeptide **6** was prepared according to the general procedure. After RP-HPLC a yellowish white powder was obtained in 7% yield based on resin substitution: ^{1}H-NMR (CD_3OD, 500 MHz) δ 8.10 (d, *J* = 9.0 Hz, 2H), 7.18 (d, *J* = 9.0 Hz, 2H), 5.65 (s, 1H), 5.32 (s,1H), 4.37 (dd, *J* = 11.3, 3.4 Hz, 1H), 4.13 (m,3H), 3.94 (d, *J* = 6.7 Hz, 1H), 3.90 (s, 3H), 3.81 (d, *J* = 6.7 Hz, 1H), 3.44 (m, 4H), 3.22 (m, 2H), 3.16 (m, 2H), 2.05 (s, 3H), 1.93 (m, 1H), 1.78-1.60 (br, m, 10H), 1.55-1.44 (br, m, 13H), 1.02-0.84 (br, m, 25H); HRMS (ESI) calcd for $C_{53}H_{86}N_{14}O_{11}Na$ 1117.6498 [M + Na^+], found 1117.6501.

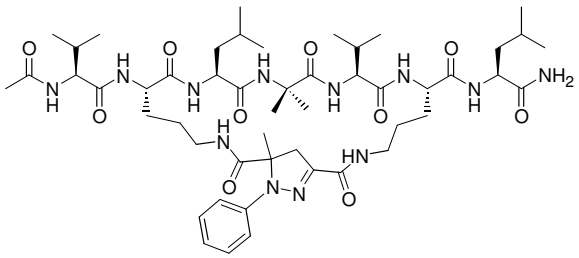


Linear Peptide (**7**): Heptapeptide **7** was prepared according to the general procedure. After RP-HPLC a yellowish white powder was obtained in 4% yield based on resin substitution: ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.97 (d, *J* = 9.1 Hz, 2H), 6.93 (d, *J* = 9.2 Hz, 2H), 6.23 (d, *J* = 8.5 Hz, 2H), 5.65 (dd, *J* = 8.7, 3.8 Hz, 1H), 4.41 (dd, *J* = 10.9, 4.9 Hz, 1H), 4.14 (m, 3H), 3.97 (d, *J* = 7.0 Hz, 1H), 3.47 (m, 4H), 3.25 (m, 4H), 3.09 (s, 6H) 2.05 (s, 3H), 1.93 (m, 1H), 1.78-1.60 (br, m, 10H), 1.55-1.44 (br, m, 10H), 1.02-0.84 (br, m, 25H); HRMS (ESI) calcd for $C_{53}H_{87}N_{15}O_{10}Na$ 1116.6658 [M + Na^+], found 1116.6680.

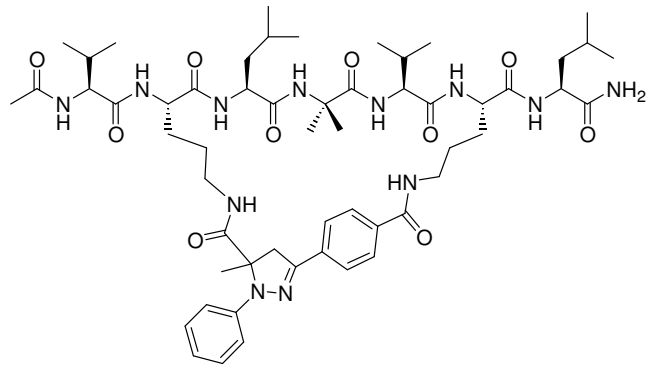


Linear Peptide (**8**): Heptapeptide **8** was prepared according to the general procedure. After RP-HPLC a yellowish white powder was obtained in 2% yield based on resin substitution: ^{1}H-NMR (CD_3OD, 400 MHz) δ 7.97 (d, *J* = 9.2 Hz, 2H), 6.92 (d, *J* = 9.1Hz, 2H), 5.65 (s, 1H), 5.32 (s, 1H), 4.38 (dd, *J* = 8.1, 2.9 Hz, 1H), 4.14 (m,3H), 3.94 (d, *J* = 6.6 Hz, 1H), 3.81 (d, *J* = 8.9 Hz, 1H) 3.44 (m, 3H), 3.16 (m, 4H), 3.06 (s, 6H),

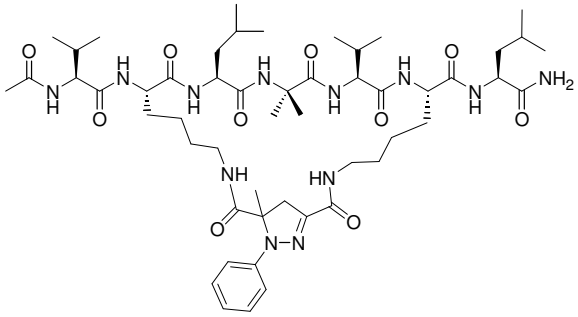
2.05 (s, 3H), 1.93 (m, 1H), 1.78-1.60 (br, m, 10H), 1.55-1.44 (br, m, 13H), 1.02-0.84 (br m, 25H). HRMS (ESI) calcd for $C_{54}H_{89}N_{15}O_{10}Na$ 1130.6815 [M + Na^+], found 1130.6799.



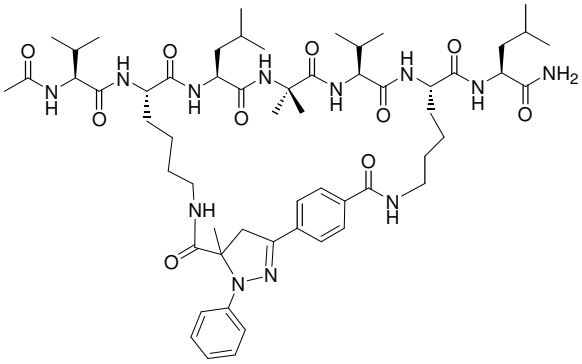
Cyclic Peptide (**9**): 15 milligrams of **1** was used in the photoactivated cycloaddition reaction to yield 2.2 mg cyclic peptide as a fluffy white powder (15%): ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.25 (t, *J* = 7.5 Hz, 2H), 7.11 (d, *J* = 7.8 Hz, 2H), 6.94 (t, *J* = 7.4 Hz, 1H), 4.32 (br, m, 2H), 4.00 (t, *J* = 7.3 Hz, 1H), 3.90 (m, 1H), 3.81 (d, *J* = 6.5 Hz, 1H), 3.79-3.68 (br, m, 2H), 3.16-3.08 (br, m, 2H), 2.83 (m, 1H), 2.17 (m, 1H), 2.09 (s, 3H), 2.06 (s, 1H), 1.95-1.60 (br, m, 13H), 1.52 (d, *J* = 6.4 Hz, 5H), 1.44 (s, 3 H), 1.20 (m, 1H), 1.07-0.86 (br, m, 24H); HRMS (ESI) calcd for $C_{50}H_{80}N_{12}O_{10}Na$ 1031.6013 [M + Na^+], found 1031.6028.



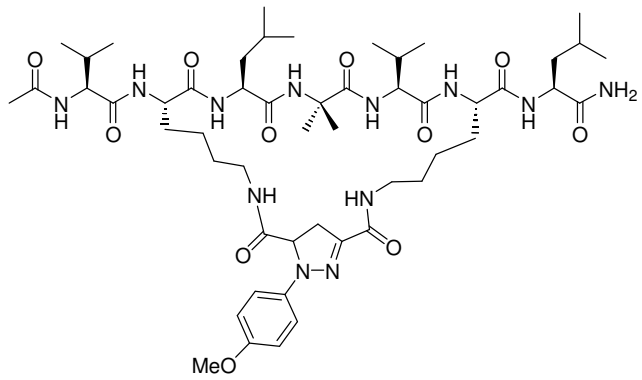
Cyclic Peptide (**10**): 18.5 milligrams of **2** was used in the photoactivated cycloaddition reaction to yield 2.8 mg cyclic peptide as a fluffy white powder (15%): ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.77 (d, *J* = 8.7 Hz, 2H), 7.65 (d, *J* = 8.6 Hz, 2H), 7.29 (t, *J* = 8.1 Hz, 2H), 7.21 (d, *J* = 7.7 Hz, 2H), 6.88 (t, *J* = 7.3 Hz, 1H), 4.49 (m, 1H), 4.35 (m, 1H), 4.07 (m, 3H), 3.85 (m, 2H), 3.71 (br, m, 2H), 3.44 (q, 1H), 3.16 (q, 1H), 3.04 (br, m, 1H), 2.18 (s, 3H), 2.10 (br, m, 3H), 1.75 (br, m, 9H), 1.59 (s, 3H), 1.48 (s, 6H), 1.44 (s, 3H), 1.42 (s, 1H), 1.09-0.88 (br, m, 24H); HRMS (ESI) calcd for $C_{56}H_{84}N_{12}O_{10}Na$ 1107.6326 [M + Na^+], found 1107.6368.



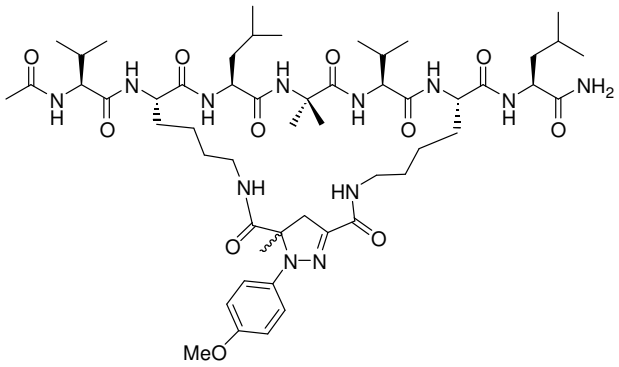
Cyclic Peptide (**11**): 20 milligrams of **3** was used in the photoactivated cycloaddition reaction to yield 8.2 mg cyclic peptide as a fluffy white powder (41%): ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.25 (t, *J* = 7.3 Hz, 2H), 7.15 (m, 2H), 6.94 (t, *J* = 7.3 Hz, 1H), 4.36 (m, 1H), 4.12 (m, 1H), 4.03 (t, *J* = 7.0 Hz, 1H), 3.90 (dd, *J* = 8.8, ?? Hz, 2H), 3.72 (m, 1H), 3.52 (m, 1H), 3.25 (m, 3H), 3.08 (m, 2H), 2.18 (m, 1H), 2.06 (s, 3H), 2.03 (s, 1H), 1.91 (br, m, 4H), 1.75-1.53 (br, m, 14H), 1.48 (s, 3H), 1.44 (d, *J* = 1.7 Hz, 3H), 1.42 (s, 1H), 1.24 (m, 3H), 1.14 (t, *J* = 7.1 Hz, 1H), 0.95-0.87 (br, m, 13H), 0.83-0.78 (br, m, 9H); HRMS (ESI) for $C_{52}H_{84}N_{12}O_{10}Na$ 1059.6326 [M + Na^+], found 1059.6356.



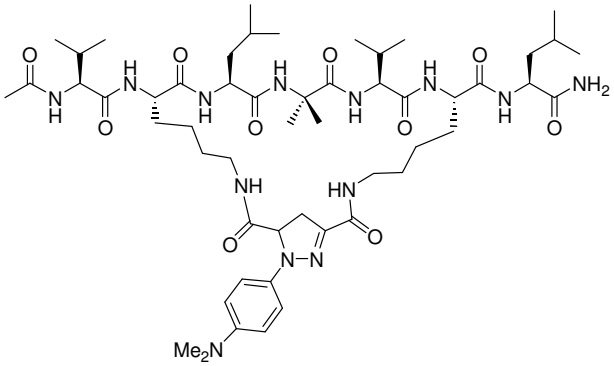
Cyclic Peptide (**12**): 20 milligrams of **4** was used in the photoinduced cycloaddition reaction to yield 7.5 mg cyclic peptide as a fluffy white powder (38%): ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.83 (d, *J* = 8.5 Hz, 2H), 7.73 (d, *J* = 8.5 Hz, 2H), 7.29 (t, *J* = 8.5 Hz, 2H), 7.20 (d, *J* = 7.5 Hz, 2H), 6.88 (t, *J* = 7.5 Hz, 1H), 4.40 (m, 1H), 4.29 (t, *J* = 8.5 Hz, 1H), 4.18 (m, 2H), 4.05 (d, *J* = 10.5 Hz, 1H), 3.88 (d, *J* = 5.0 Hz, 1H), 3.82 (d, *J* = 5.5 Hz, 1H), 3.74 (d, *J* = 12.5 Hz, 1H), 3.66 (m, 1H), 3.55 (m, 1H), 3.47 (m, 2H), 3.16 (m, 1H), 2.06 (s, 3H), 2.03 (s, 1H), 1.91 (br, m, 4H), 1.75-1.53 (br, m, 14H), 1.48 (s, 3H), 1.44 (d, *J* = 1.7 Hz, 3H), 1.24 (m, 3H), 0.95-0.78 (br, m, 23H); HRMS (ESI) calcd for $C_{58}H_{88}N_{12}O_{10}Na$ 1135.6639 [M + Na^+], found 1135.6650.



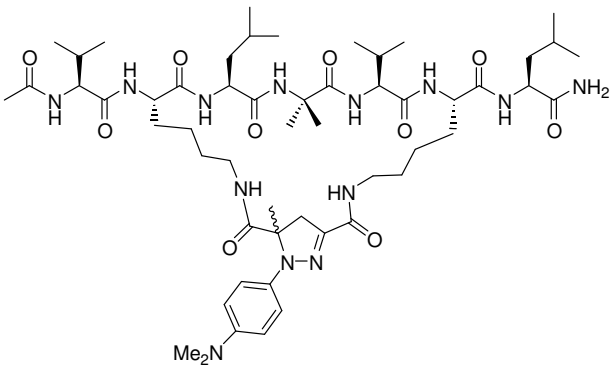
Cyclic Peptide (**13**): 15.0 mg of **5** was used in the photoinduced cycloaddition reaction to yield 9.1 mg cyclic peptide as a fluffy white powder in 64% yield: ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.05 (d, *J* = 9.0 Hz, 2H), 6.87 (d, *J* = 9.1 Hz, 2H), 4.68 (m, 1H), 4.36 (m, 2H), 4.17 (m, 1H), 4.13 (m, 2H), 3.98 (m, 1H), 3.89 (d, *J* = 5.7 Hz, 1H), 3.74 (s, 3H) 3.61 (m,2H), 3.44 (s, 1H), 2.18 (m, 1H), 2.05 (s, 3H), 1.72-1.56 (br, m, 12H), 1.48-1.28 (br, m, 12H), 1.05-0.87 (br, m, 25H); HRMS (ESI) calcd for $C_{52}H_{84}N_{12}O_{11}Na$ 1075.6280 [M + Na^+], found 1075.6279.



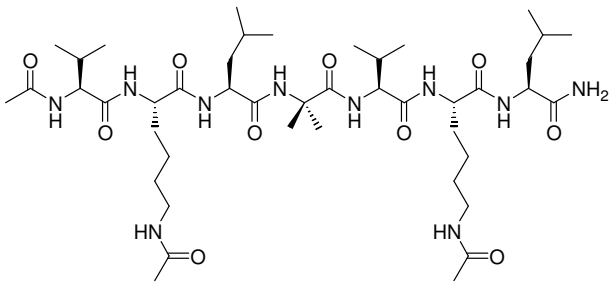
Cyclic Peptide (**14**): 10.1 milligrams of **6** was used in the photoinduced cycloaddition reaction to yield 5.3 mg cyclic peptide as a fluffy white powder in 53% yield: ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.09 (d, *J* = 9.1 Hz, 2H), 6.85 (d, *J* = 9.1 Hz, 2H), 6.20 (d, *J* = 8.6 Hz, 2H), 5.62 (dd, *J* = 8.6, 3.4 Hz, 1H), 4.37 (dd, *J* = 11.3, 3.7 Hz, 1H), 4.14 (m, 3H), 3.94 (d, *J* = 6.7 Hz, 1H), 3.83 (s, 3H), 3.61 (m, 2H), 3.81 (d, *J* = 7.1 Hz, 1H), 3.44 (m, 4H), 3.16 (m, 2H), 2.05 (s, 3H), 1.93 (m, 1H), 1.78-1.60 (br, m, 10H), 1.55-1.44 (br, m, 10H), 1.02-0.84 (br, m, 25H); HRMS (ESI) calcd for $C_{53}H_{86}N_{12}O_{11}Na$ 1089.6437 [M + Na^+], found 1089.6428.



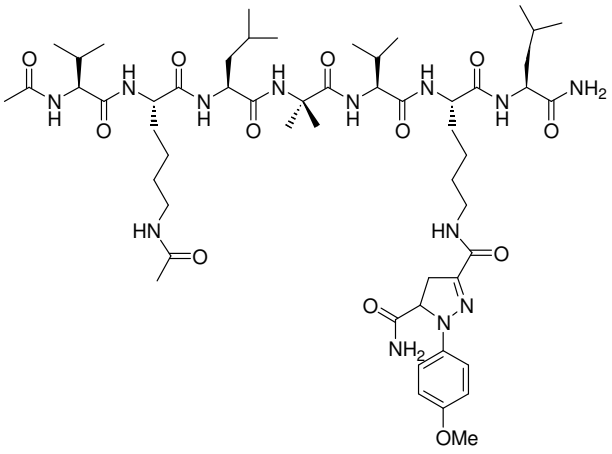
Cyclic Peptide (**15**): 7.7 milligrams of **7** was used in the photo activated cyclo addition reaction to yield 4.4 mg cyclic peptide as a fluffy white powder in 64% yield. ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.02 (d, *J*=9.0Hz, 2H), 6.83 (d, *J*=9.1Hz, 2H), 4.89 (m, 1H), 4.38 (m, 2H), 4.19 (m, 1H), 4.12 (m,2H), 3.98 (m, 1H), 3.90 (d, *J*=5.7hz, 1H), 3.59 (m,2H), 3.44 (s, 1H), 3.07 (s, 6H) 2.18 (m, 1H), 2.05 (s, 3H), 1.72-1.56 (br m, 12H), 1.48-1.28 (br m, 12H), 1.05-.87 (br m, 25H). HRMS (ESI) calcd for $C_{53}H_{87}N_{13}O_{10}Na$ 1088.6597 [M + Na^+], found 1088.6585.



Cyclic Peptide (**16**): 3.4 milligrams of **8** was used in the photoinduced cyclo-addition reaction to yield 3.2 mg cyclic peptide as a fluffy white powder in 94% yield: ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.04 (d, *J* = 9.1 Hz, 2H), 6.74 (d, *J* = 9.1 Hz, 2H), 4.31 (dd, *J* =11.2, 3.5 Hz,1H), 4.11 (m, 1H) 4.05 (dd, *J* =10.4, 3.6 Hz, 1H) 4.00 (t, *J* = 7.1 Hz 1H), 3.91(d, *J* = 6.8 Hz, 1H*),* 3.86 (d, *J* = 6.4 Hz, 1H), 4.32 (d, *J* = 5.8 Hz, 1H) 3.67 (m, 1H), 3.39 (d, *J* = 5.7 Hz, 1H), 3.18 (s, 2H), 3.84 (s, 6H), 2.18 (m, 1H), 2.05 (s, 3H), 1.72-1.56 (br, m, 12H), 1.48-1.28 (br, m, 12H), 1.05-0.87 (br, m, 25H); HRMS (ESI) calcd for $C_{54}H_{89}N_{13}O_{10}Na$ 1102.6753 [M + Na^+], found 1102.6773.



Linear Peptide (**17**)**:** Heptapeptide **17** was prepared according to the general procedure. After purification, a yellow-white crystalline solid was obtained in 5% yield (based on resin substitution): ^{1}H-NMR (CD_3OD, 500 MHz) δ 8.25 (d, *J* = 5.0 Hz, 1H), 8.20 (d, *J* = 5.0 Hz, 1H), 7.97 (s, 1H), 7.78 (d, *J* = 10.0 Hz, 1H), 7.69 (d, *J* = 10.0 Hz, 1H), 7.62 (d, *J* = 10.0 Hz, 1H), 7.55 (d, *J* = 5.0 Hz, 1H), 4.35 (m, 1H), 4.09 (m, 3H), 3.92 (t, *J* = 5.0 Hz, 1H), 3.81 (t, *J* = 10.0 Hz, 1H), 3.17 (m, 4H), 2.21 (m, 1H), 2.08 (s, 4H), 1.92 (d, *J* = 10.0 Hz, 8H), 1.82-1.60 (br, m, 9H), 1.49 (s, 7H), 1.44 (s, 7H), 1.06-0.86 (br, m, 24H); HRMS (ESI) calcd for $C_{44}H_{80}N_{10}O_{10}Na$ 931.5951 [M + Na^+], found 931.5941.



Linear Peptide (**18**)**:** Heptapeptide **18** was prepared according to the general procedure. The tetrazole containing precursor was obtained as a yellow-white powder in 23% yield (25 mg, 23 μmole) based on resin substitution. The linear peptide was subjected to photoactivated 1,3-dipolar cycloaddition in the presence of 20 equiv of acrylamide (33 mg, 0.46 mmole) in 50 mL acetonitrile for 4 hours at 302 nm. Following the cycloadditon reaction, the mixture was concentrated and purified by reverse phase HPLC according to the general methods to give a yellow-white solid in 16% yield (4.0 mg): ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.81 (d, *J* = 6.0 Hz, 1H), 7.72 (d, *J* = 8.5 Hz. 1H), 7.55 (t, *J* = 7.4, 2H), 7.42 (m, 1H), 7.40 (d, *J* = 9.0 Hz, 2H), 7.28 (s, 1H), 7.06 (d, *J* = 8.0 Hz, 1H), 7.01 (d, *J* = 9.0 Hz, 2H), 6.98 (m, 2H), 4.36 (m, 3H), 4.19 (m, 2H), 4.12 (m, 5H), 3.95 (m, 1H), 3.84 (s, 3H), 3.81 (d, *J* = 4.5 Hz, 1H), 3.65 (m,1H), 3.44 (t, *J* =1.0 Hz, 1H), 3.16 (m, 3H), 2.18 (m, 1H), 2.05 (s, 3H), 1.72-1.56 (br, m, 12H), 1.48-1.28 (br, m, 12H), 1.05-0.87 (br, m, 25H); ESI-MS calcd for $C_{54}H_{90}N_{13}O_{12}$ 1112.7 [M + H^+], found 1112.5.

References

1. Wallimann, P., Kennedy, R. J., Kemp, D. S. *Angew. Chem. Intl. Ed.* **1999,** *38*, 1290-1292.
2. Greenfield, N., Fasman, G. D. *Biochemistry* **1969,** *8*, 4108-4116.
3. Flint, D. G., Kumita, J. R., Smart, O. S., Woolley, G. A. *Chem. Biol.* **2002,** *9*, 391-397.