CHEMBIOCHEM

Supporting Information

CHEMBIOCHEM

Supporting Information

for

Peptide Tertiary Structure Nucleation by Sidechain Crosslinking with Metal Complexation and Double "Click" Cycloaddition

Oscar Torres, Deniz Yüksel, Matt Bernardina, Krishna Kumar, and Dennis Bong*

A) Synthesis

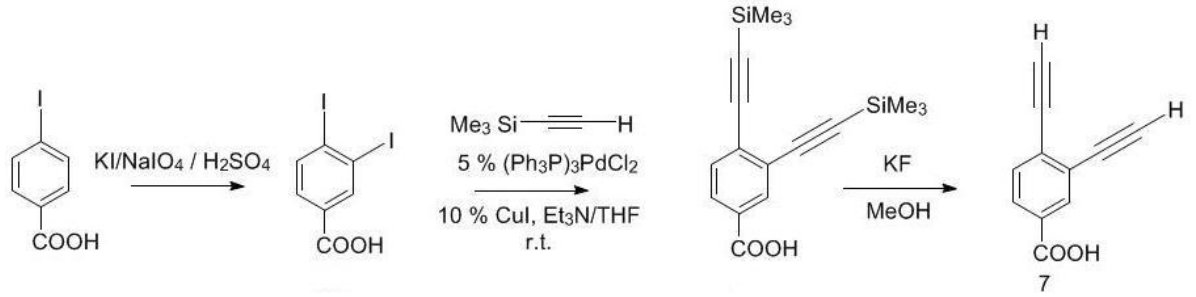
A.1 Peptide Synthesis and Purification

Peptides were synthesized on an Advanced ChemTech Apex SPPS synthesizer using Fmoc strategy. The synthesis was performed on 0.34 or 0.7mmol/g Rink amide resin. Crude peptides were purified via reversed-phase HPLC by using C18 preparative columns (Higgins Analytical and Phenomenex). Stock solutions of peptides were prepared in deionized water. Peptide concentration was obtained in pure water using UV absorbance of ABA (4-acetamidobenzoic acid) at 260 nm (ε_{260} = 18 000 M^{-1} cm^{-1}).

Histidine Peptides	Sequence	MW (g/mol)
(**1**)	ABA-RIKQLEEK-I**HGLGH**K-IEELEKK-NH_2	2816
(**4**, X=His)	ABA-R**KI**Q**EL**EK-I**HGLGHE**-**EKIEK**K**L**-NH_2	2817
(**5**, X=His)	ABA-RIKQ**K**EEK-I**HGAGH**K-**E**EELEKK-NH_2	2804
Azido Peptides		
(**2**)	ABA-RIKQLEEK-I**AzGLGAz**K-IEELEKK-NH_2	2766
(**3**)	ABA-RIKQLEEK-I**AzGLGS**K-IEELEKK-NH_2	2741
(**4**, X=Az)	ABA-R**KI**Q**EL**EK-I**AzGLGAzE**-**EKIEK**K**L**-NH_2	2766
(**5**, X=Az)	ABA-RIKQ**K**EEK-I**AzGAGAz**K-**E**EELEKK-NH_2	2754

Az = azidoalanine

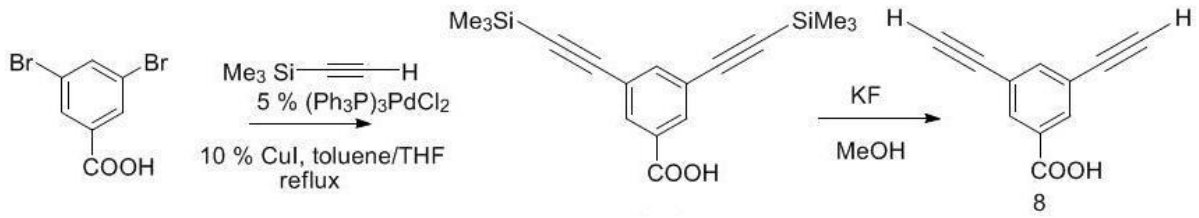
A.2 Synthesis of linkers and Fmoc-azidoalanine



Scheme S1. Synthesis of 3,4- Diethynylbenzoic acid, **7**. (3,4- diiodobenzoic acid, synthesis from: Kraszkiewicz, L., Sosnowski, M., Skulski, L., Tetrahedron **2004,** 60, 9113-9119.)

3,4-Diethynylbenzoic acid, 7. In a round bottom flask, 50 mL of 20% Et_3N in THF was degassed vigorously for 30 minutes. The following compounds were then added in order: 3,4-diiodobenzoic acid (0.45 g, 1.20 mmol), trimethylsilylacetylene (0.471 g, 4.80 mmol), dichlorobis(triphenylphosphine) palladium(II) (0.042 g, 0.06 mmol) and Cu(I)I (0.023 g, 0.12 mmol). The reaction mixture was stirred at room temperature overnight. The organic solvents were removed under reduced pressure and the resulting residue was taken up in CH_2Cl_2 (20 mL) and washed with 1 N HCl (3 x 20mL). The organic layer was dried with $MgSO_4$ and concentrated to dryness. The brown residue was purified by flash column chromatography (EtOAc in hexanes with 1% CH_3COOH) to give the bis-(TMS) protected intermediate (60%).^{1}H NMR (250 MHz, $CDCl_3$): 8.1 (1H, s), 7.95 (1H, s), 7.6 (1H, s) 0.3 (18H, s). MS (negative ion mode) calcd for $C_{17}H_{22}O_2Si_2$: 314 $[M\text{-}H]^+$; found 313 .

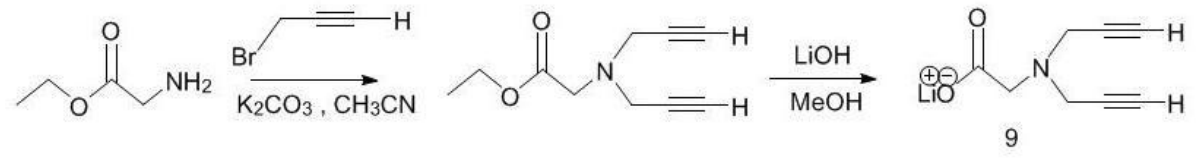
The bis-(TMS) protected intermediate (0.5 g, 1.59 mmol) was dissolved in neat MeOH and KF (2.31 g, 39.8 mmol) was added. The reaction mixture was stirred vigorously at room temperature for 3 h The solvent was removed under vacuo and the residue was taken up in EtOAc (20 mL). The organic layer was washed with 1N HCl (3 x 10 mL) , dried with $MgSO_4$ and concentrated in vacuo. The light brown solid was purified by flash column chromatography (EtOAc in Hexanes with 1% CH_3COOH) to give **7** (57%). ^{1}H NMR (250 MHz, MeOH): 8.2 (1H, d) 8.05 (1H, dd), 7.7 (1H, d), 4.1 (1H, s), 3.9 (1H, s). MS (negative ion mode) calcd for $C_{11}H_6O_2$: 170 $[M\text{-}H]^+$; found 169.



Scheme S2. Synthesis of 3,5- Diethynylbenzoic acid, **8**

3,5- Diethynylbenzoic acid, 8. In a sealed tube, 25 mL of 20% Et_3N in toluene was degassed vigorously for 30 minutes. The following compounds were then added in order: 3,5-dibromobenzoic acid (1.46 g, 5.36 mmol), trimethylsilylacetylene (4.21 g, 42.88 mmol), dichlorobis(triphenylphosphine) palladium(II) (0.188g, 0.268 mmol) and Cu(I)I (0.102g, 0.536 mmol). The dark mixture was refluxed overnight. The organic solvents were removed under reduced pressure and the resulting residue was taken up in CH_2Cl_2 (20 mL) and washed with 1 N HCl (3 x 20mL). The organic layer was dried with $MgSO_4$ and concentrated to dryness. The dark brown residue was purified by flash column chromatography (EtOAc in hexanes with 1% CH_3COOH) to give the bis(TMS)-protected intermediate (60%). ^{1}H NMR (250 MHz, $CDCl_3$): 7.95 (2H, s), 7.6 (1H, s), 0.3 (18H, s). MS (negative ion mode) calcd for $C_{17}H_{22}O_2Si_2$: 314 $[M\text{-}H]^+$; found 313.

The bis-(TMS) protected intermediate (0.4319 g, 1.38 mmol) was dissolved in neat MeOH and KF (2.00g, 34.42 mmol) was added. The reaction mixture was stirred vigorously at room temperature for 3 hours. Methanol was removed under low pressure and the residue was taken up in EtOAc (20 mL). The organic layer was washed with 1N HCl (3 x 10 mL) , dried with $MgSO_4$ and concentrated in vacuo. The light brown solid was purified by flash column chromatography (EtOAc in Hexanes with 1% CH_3COOH) to give **8** (55%). ^{1}H NMR (250 MHz, MeOH): 8.0 (2H, d), 7.7 (1H, t), 3.65 (2H, s). MS (negative ion mode) calcd for $C_{11}H_6O_2$, 170 $[M\text{-}H]^+$; found 169.

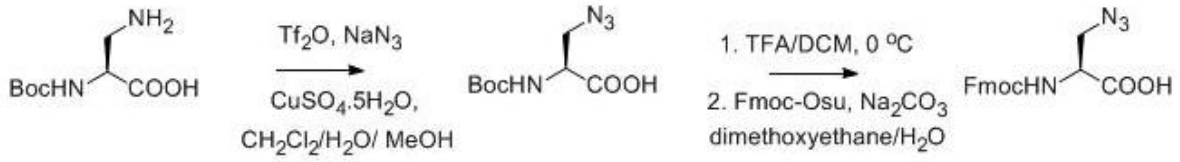


Scheme S3. Synthesis of N,N- dipropargylglycine, **9**

Dipropargyl glycine, 9. Glycine ethyl ester hydrochloride (1.51 g, 10.87 mmol) was dissolved in 25 mL aqueous solution of K_2CO_3 (5.00 g). The resulting solution was extracted with CH_2Cl_2 (3 x 25mL). The combined organic layers were concentrated in vacuo and were subsequently taken up in 25 mL suspension of K_2CO_3 (3.01 g, 21.78 mmol) in CH_3CN. Propargyl bromide (3.23 g, 27.15 mmol) was added to the mixture and stirred overnight. Aceto-

nitrile was removed under reduced pressure and water was added to the slurry. The aqueous layer was extracted with dichloromethane (3 x 20 mL). The combined organic layers were washed with brine, dried with $MgSO_4$ and concentrated in vacuo. The residue was purified with flash chromatography (5% MeOH in CH_2Cl_2) to give light brown liquid (86 %). ^{1}H NMR (250 MHz, $CDCl_3$): 4.2 (2H, q), 3.6 (4H, d), 3.4 (2H, s), 2.3 (2H, t), 1.3 (3H, t). MS (positive ion mode) calcd for $C_{10}H_{13}NO_2$: 179 $[M+H]^+$; found 180.

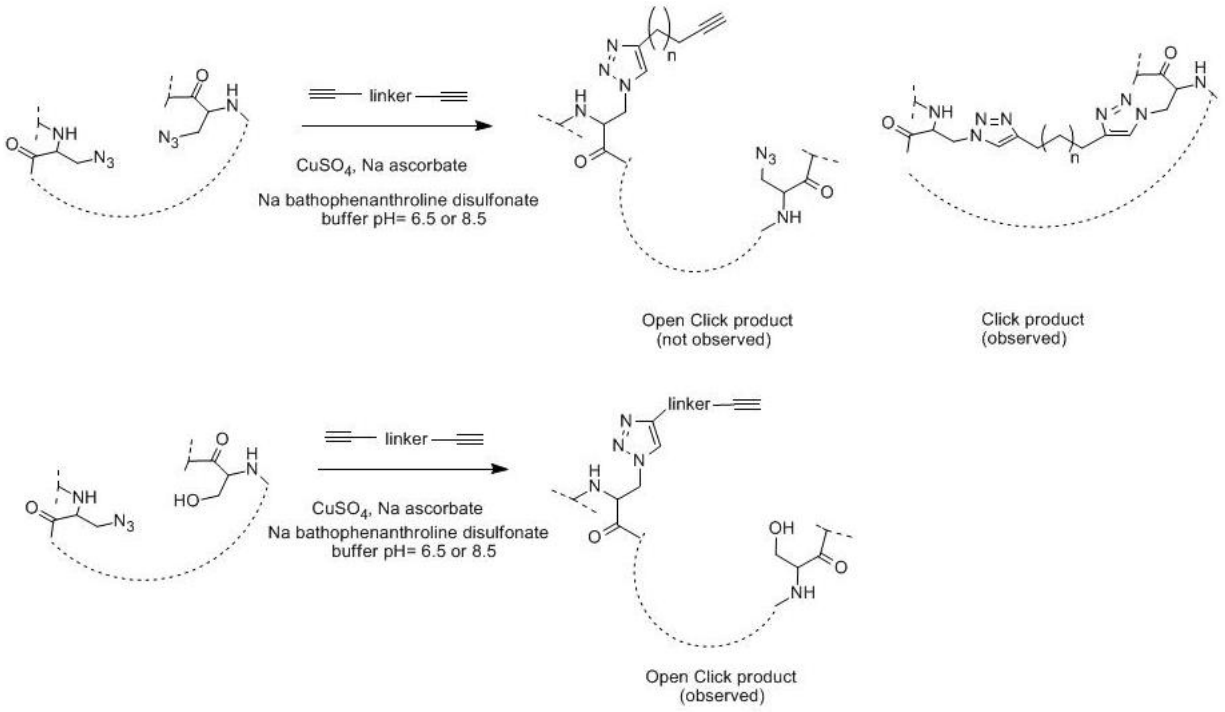
Ethyl ester (0.16 g, 0.894 mmol) was dissolved in neat MeOH and LiOH (0.21 g, 8.77 mmol) was added. The turbid mixture was sonicated for 5 min followed by stirring for 1 h. Methanol was removed in vacuo and the resulting residue was triturated with Et_2O and dried. Yield for **9** was quantitative. ^{1}H NMR (250 MHz, D_2O): 3.6 (4H, d), 3.4 (2H, s), 2.3 (2H, t). MS (positive ion mode) calcd for $C_8H_9NO_2$: 151 $[M+Li]^+$; found 158.



Scheme S4. Synthesis of Fmoc-azidoalanine. (Boc-azidoalanine, synthesis from: Link, A. J., Vink, M.K.S., Tirrell, D.A., J. Am. Chem. Soc. **2004,** 126, 10598-10602.) Modification: Boc-azidoalanine was used in the next reaction without further purification.

Fmoc-azidoalanine. Boc-azidoalanine (1.31g, crude) was dissolved in 20 mL CH_2Cl_2 (0-5°C) and treated with 20 mL TFA. After 1.0 -1.5 hrs, the reaction mixture was concentrated to thick yellow liquid. Diethyl ether (100 mL, previously cooled at -20°C) was added. The resulting white solids were collected and dried. Azidoalanine-TFA salt (0.7930 g, 3.25 mmol) was dissolved in 10 % Na_2CO_3 (15 mL). The basic solution was added to Fmoc-Osu (1.65 g, 4.88 mmol) dissolved in 30 mL dimethoxyethane (stored at 4°C). The reaction mixture was stirred at 0-5°C for 30 minutes, filtered and filtrate acidified to pH 2-3 using concentrated HCl. Dimethoxyethane was removed under high vacuum and the residue was extracted with EtOAc (5 x 50 mL). The combined organic layers were washed with H_2O (50 mL), washed with brine (50 mL), dried with $MgSO_4$ and concentrated in vacuo. The oily residue (dry loading technique) was purified by flash column chromatography (95% $CHCl_3$, 4% MeOH, 1% CH_3CO_2H) to give Fmoc-azidoalanine (45-50%). IR: 2106.4, 1718.3 cm^{-1}.^{1}H NMR (250 MHz, DMSO): 7.9 (2H, d) 7.7 (2H, d), 7.4 (2H, t), 7.3 (2H, t), 4.35-4.15 (4H, m), 3.6 (2H, d). ^{13}C NMR (250 MHz, DMSO): 171.4, 156.2, 144, 141, 127.8, 127.2, 125.5, 120.2, 66.2, 54, 51, 46.8. MS (negative ion mode) calcd for $C_{18}H_{16}N_4O_4$: 352 $[M-H]^+$; found 351.

A.3 Synthesis of Click Peptides



Scheme S5. Click chemistry in Aqueous Solution. (Click chemistry, modified from: Lewis, W.G., Magallon F.G., Fokin, V.V., and Finn M. G J. Am. Chem. Soc**. 2004,** 9152-9153.)

Click chemistry in aqueous solution

*For peptides with linkers **6**-**8**.*

All solutions were degassed by argon sparge prior to use. Alkyne was dissolved in TBS buffer with the peptide. Separately, $CuSO_4$ was treated with sodium ascorbate followed by bathophenanthroline disulfonate ligand. The copper complex was vortexed and immediately added to the peptide solution. The red-brown complex was stirred under argon for 3-12 h (40-50% HPLC isolated yield). The formation of di-coupled product can be prevented by adjusting the stoichiometry of linker:peptide.

	Peptide (azido)	Linker (alkyne)	Na ascorbate	$CuSO_4$	Bathophenanthroline disulfonate
Equivalence	**1**	**1.5**	**50**	**4**	**7.2**
Stock Solution (mM)		57.6•	865.0?	75.8?	216.0?
Final Concentration (mM)	~3.62	5.41	173.0	15.2	26.4

Total Volume: 1000 µL ? - Stock solution in TBS buffer (10 mM Tris.Cl, 110 mM NaCl pH 8.5). ?- Stock solution in pure H_2O. •- Stock solution in DMSO

*For peptides with linker **9**.*

All solutions were degassed by argon sparge prior to use. Alkyne was dissolved in HEPES buffer with the peptide. Separately, $CuSO_4$ was treated with sodium ascorbate fol-

lowed by bathophenanthroline disulfonate ligand. The copper complex was vortexed and immediately added to the peptide solution. The blue-green complex was stirred under argon for 2.5-12 h (40-50% HPLC isolated yield).

	Peptide (azido)	Linker (alkyne)	Na ascorbate	$CuSO_4$	Bathophenanthroline disulfonate
Equivalence	**1**	**1.5**	**50**	**4**	**7.2**
Stock Solution (mM)		11.14*	865.0*	75.8?	216.0?
Final Concentration (mM)	~3.62	5.43	171.46	15.02	26.12

Total Volume: 1000 µL. *- Stock solution in HEPES buffer (10 mM HEPES pH 6.5). ?- Stock solution in pure H_2O

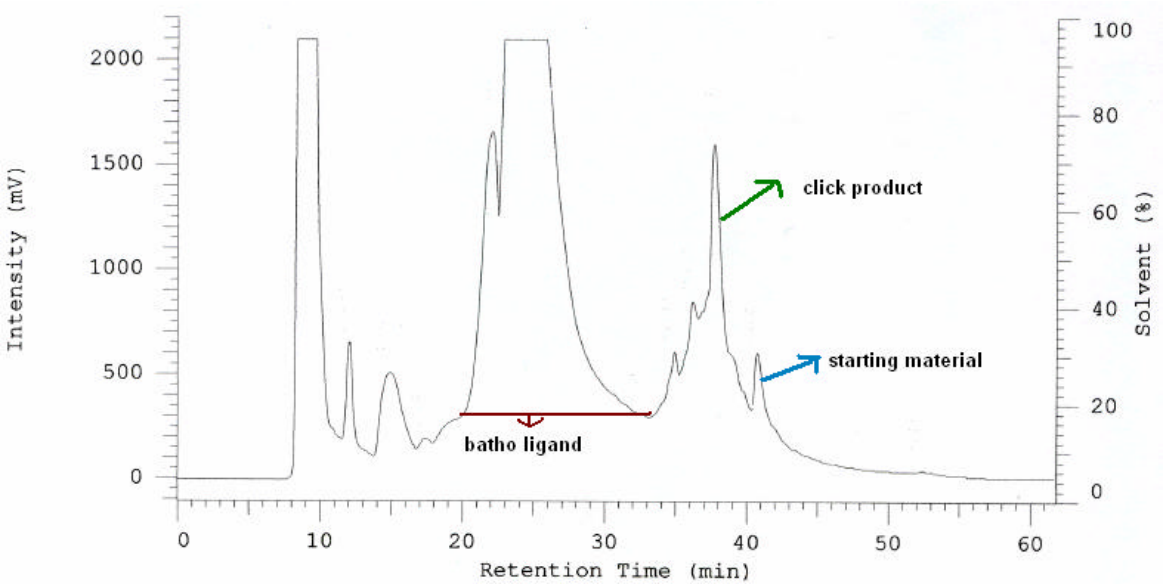


Figure A1. HPLC trace: reaction of peptide 2 and linker 9 after 2.5 hours (Yield = 40 %).

Click chemistry on solid phase (procedure 1).

All solutions were degassed by argon sparge prior to use. The resin-bound peptide, ABA-KIAzGLGAz-KIE-NH_2, (1 equiv) was stirred in 700 uL DMF for 30 minutes. Large excess of 1,5-hexadiyne (5.15 M in pentane, 50 equiv), CuI (356 mM in CH_3CN, 3 equiv), DIEA (356 mM in CH_3CN, 3 equiv) were added to the resin. The heterogeneous mixture was stirred overnight under Ar. The organic solvents were removed and the beads were washed with DMF (3x). The resin was subjected to another round of click chemistry. This time the beads were treated with CuI (356 mM stock solution in CH_3CN, 3 equiv) and DIEA (356 mM stock solution in CH_3CN, 3 equiv). The mixture was stirred for 4-6 hours under Ar. The organic solvents were removed and the beads were washed with DMF (3x), MeOH (3x), Et_2O (3x) and

dried under vacuum. The cyclization product was cleaved from the resin using TFA/TIS/H_2O (95:2.5:2.5). The crude peptide was purified by reverse HPLC using C_{18} column (yield = 40-50%).

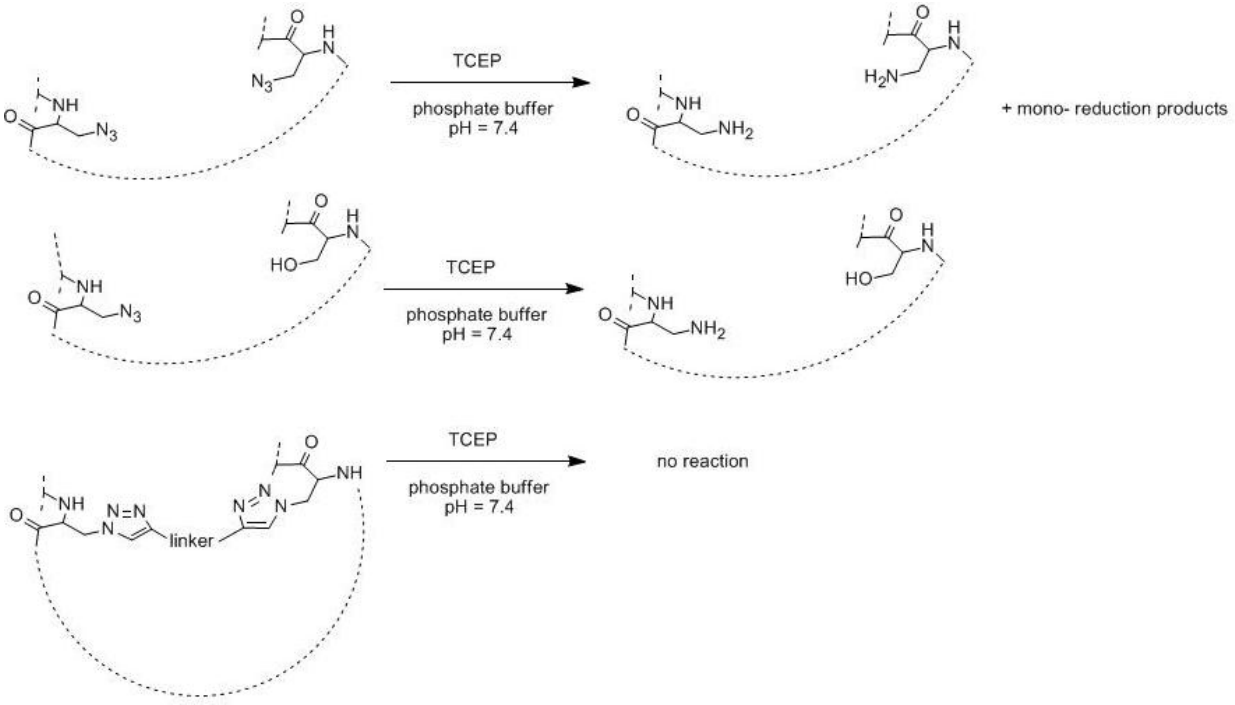
Click chemistry on solid phase (procedure 2).

All solutions were degassed by argon sparge prior to use. Resin-bound **2** (1 equiv) was stirred in 753 µL of DMF for 30 min. Large excess of propargyl alcohol (2.5 M in DMSO, 50 equiv), CuI (356 mM in CH_3CN, 3 equiv), DIEA (356 mM in CH_3CN, 3 equiv) were added to the resin. The heterogeneous mixture was stirred for 3-4 hours under Ar. The organic solvents were removed and the beads were washed with DMF (3x), MeOH (3x), Et_2O (3x) and dried under vacuum. The peptide was cleaved from the resin using TFA/TIS/H_2O (95:2.5:2.5). Reverse HPLC using C_{18} column showed **10a**, monoalkylated peptide and **2**.

Table A1. Adducts prepared from Click Chemistry

Adduct	Peptide + linker	MW (g/mol)
6a	2 + 6	2844
7a	2 + 7	2935
8a	2 + 8	2935
9a	2 + 9	2916
10a	2 + propargyl alcohol (2 equiv)	2877
6b	3 + 6	2819
7b	3 + 7	2910
8b	3 + 8	2910
9b	3 + 9	2891

B) Staudinger reduction



Scheme S6. Staudinger reduction in aqueous solution.

Staudinger Reduction in Aqueous solution. All solutions were degassed by argon sparge prior to use. Stock solution of peptide was diluted with phosphate buffer (pH= 7.4). To this solution, freshly prepared tris (2-carboxyethyl) phosphine hydrochloride (TCEP) was added. The mixture was stirred under N_2 for 3-5 hours. The appearance of new peaks on HPLC trace indicates the presence of free azido group(s).

	Peptide	**TCEP**
Equivalence	1	10
Stock Solution (mM)	5.43*	27.2*
Final Concentration (mM)	0.272	2.72

Total Volume: 200 µL. *-stock solution in pure H_2O

Staudinger Reduction on resin-bound peptide. All solutions were degassed by argon sparge prior to use. The resin-bound peptide (1 equiv) was stirred in 1.5 mL DMF for 30 min. To this mixture, PPh_3 (10 equiv) was added. The reaction mixture was stirred under Ar for 12 h.

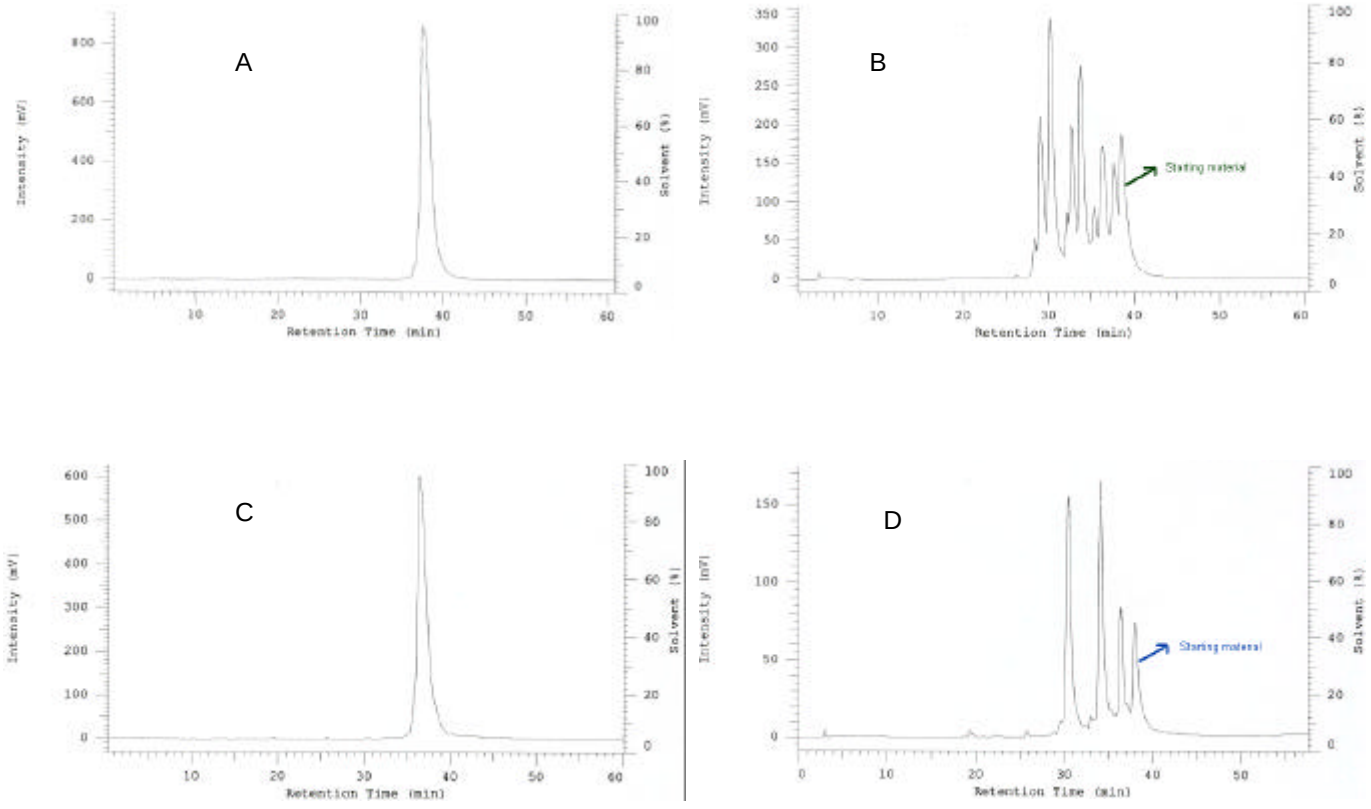


Figure B1. HPLC trace: Staudinger reduction (A) 2 (B) 2 after 5 h (C) 3 (D) 3 after 3 h.

C) Mass Spectrometry

The masses of small molecules and some peptides were determined using Electrospray Ionization on a Bruker Esquire LC/MS plus ion trap spectrometer (Bruker-Daltonics). The electrospray was operated in positive ion mode or negative ion mode depending on the ease

of ionization of the compound. For small molecules, the positive ion mode produced protonated molecular mass ion ($M+H^+$) or molecular mass with positive ions (i.e. Na^+, Li^+). For peptides with MW > 2200 g/mol, the positive ion mode resulted in a doubly charged species $[M+2H]^+$ as a major peak. The negative ion mode, on the other hand, gave deprotonated species $[M-H]^+$.

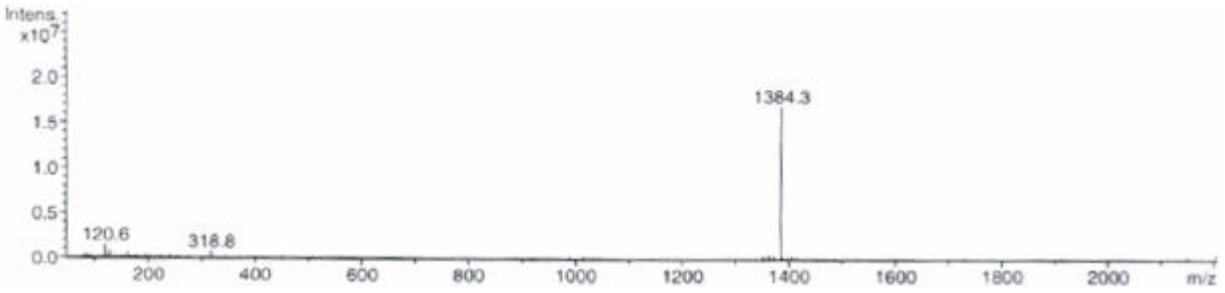


Figure C1. Mass Spectrum: **2,** MW= 1384 ($M+2H^+$).

MALDI was perfomed on Bruker Reflex III MALDI-TOF instrument for other peptides. Concentrated stock sample in pure water (0.5 uL) was mixed quickly with 1uL of a saturated solution of a-cyano-4-hydroxycinnamic acid (H_2O/ACN (50:50) with 0.1% TFA) and spotted in a MALDI plate (Bruker Daltonics).

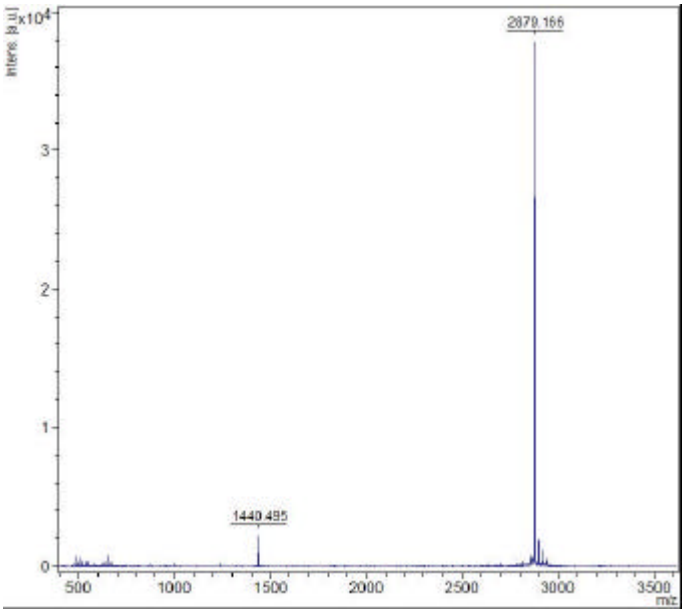


Figure C2. Mass Spectrum: **10a,** MW= 2878 $[M+H]^+$, 1440 $[M+2H]^+$.

D) Quadrupole-time of flight MS/MS

Accurate molecular weight and further detailed sequence information of these peptides were determined on a Micromass Q-Tof II apparatus (Wythenshawe, UK) equipped with an orthogonal electrospray source (Z-spray) and operated in positive ion mode. For external

mass calibration, NaI was used over the *m/z* range of 200 to 2,500. The peptides were dissolved in the mixture of H_2O-CH_3OH-HAc (50: 50:2.5) and directly infused into the electrospray source at a 2 µL/min flow rate. To achieve the optimal electrospray, capillary voltage was set at 3,000 V, source temperature was 150°C, and cone voltage was 60 V. The first quadrupole, Q1, was set to pass ions between 200 and 2,500 *m/z*. The target ion was isolated and fragmented within the second quadrupole by adding a voltage of between 20 and 40 V. The fragment ions were then analyzed in the time-of-flight tube. Data were acquired in continuum mode until well-averaged data were obtained.

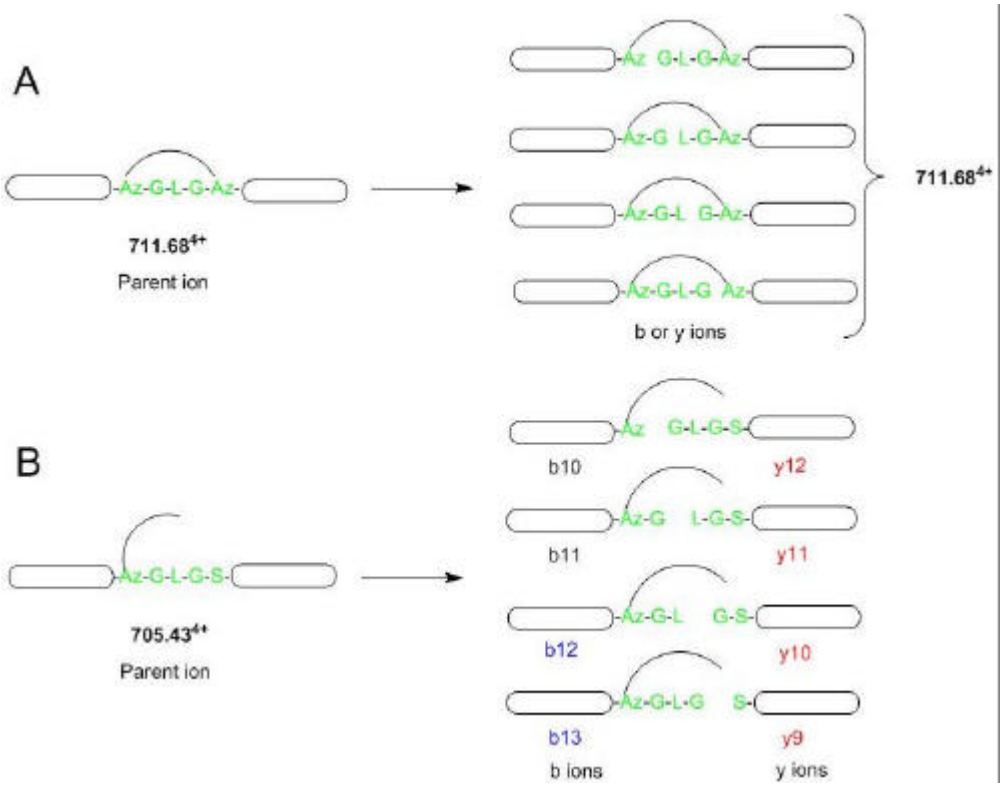


Figure D1. Cartoon representing the strategy employed in Figure 2 of the manuscript.

There are three different types of bonds that can be fragmented along the peptide backbone: the CH-CO, CO-NH and NH-CH bonds. When the peptide is fragmented by using collision induced dissociation (i.e. collision of peptide with Ar gas), cleavage occurs predominantly at CO-NH. Fragmentation along the amide bond generates the b and/or the y ions, in which the charge is retained on the N and C terminus, respectively. For closed ad-ducts (i.e. **6a**), cleavage of an amide bond within the 23-membered ring results in a b or y ion having the same mass as the parent ion $[M+4H]^+$ (A). For open adducts, fragmentation on the same cleavage sites results in b10-b13 and y9-y12 ions (B). A representative mass spectra for **6a** (closed adduct) and **6b** (open adduct) were given in succeeding pages to depict the strategy in Figure D1.

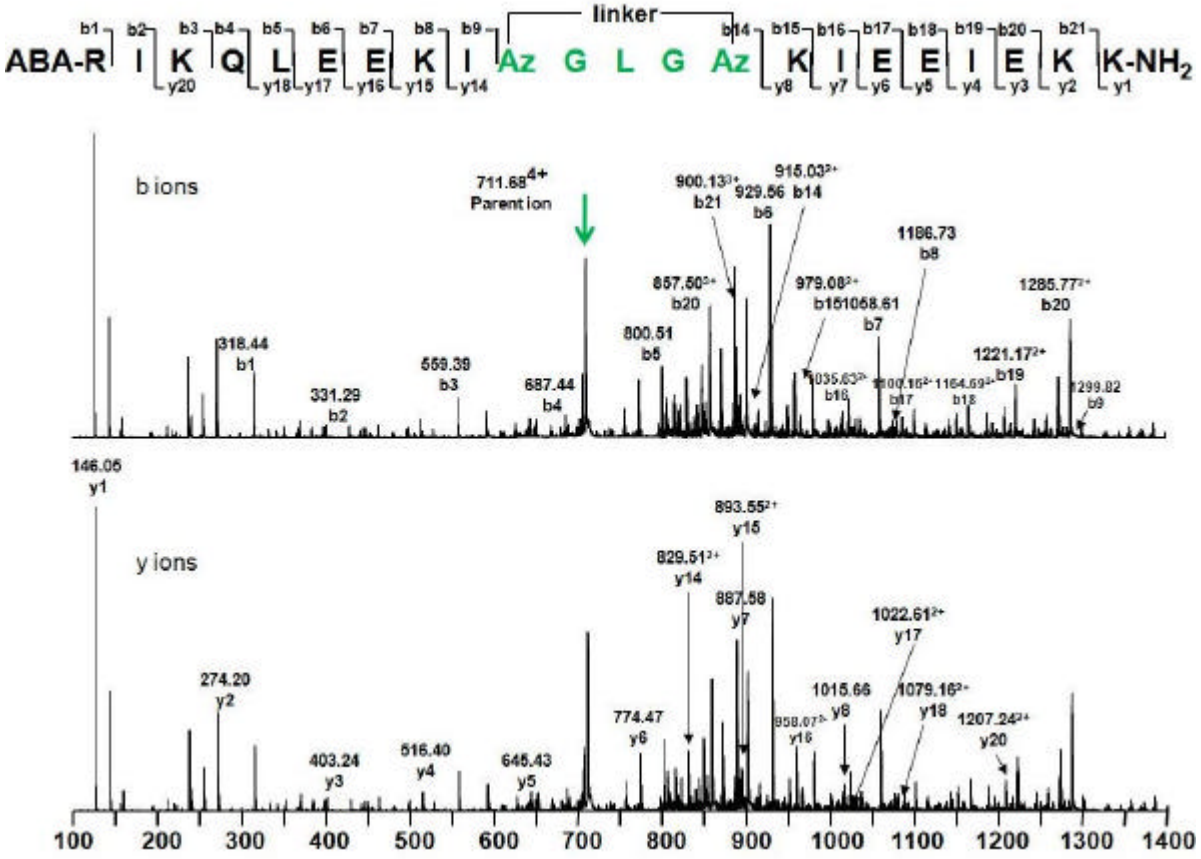


Figure D2. MS/MS spectrum of **6a** (refer to Table D1 for the mass list).

The top and bottom figures in Figure D2 were the same MS/MS spectrum. For clarity, all b ions were labeled in one figure (top) and all y ions were labeled in another figure (bottom). Cleavage of an amide bond within the 23-membered ring resulted in a b or y ion having the same mass as the parent ion. The prominent peak indicated by the green arrow corresponds to the mass of parent ion.

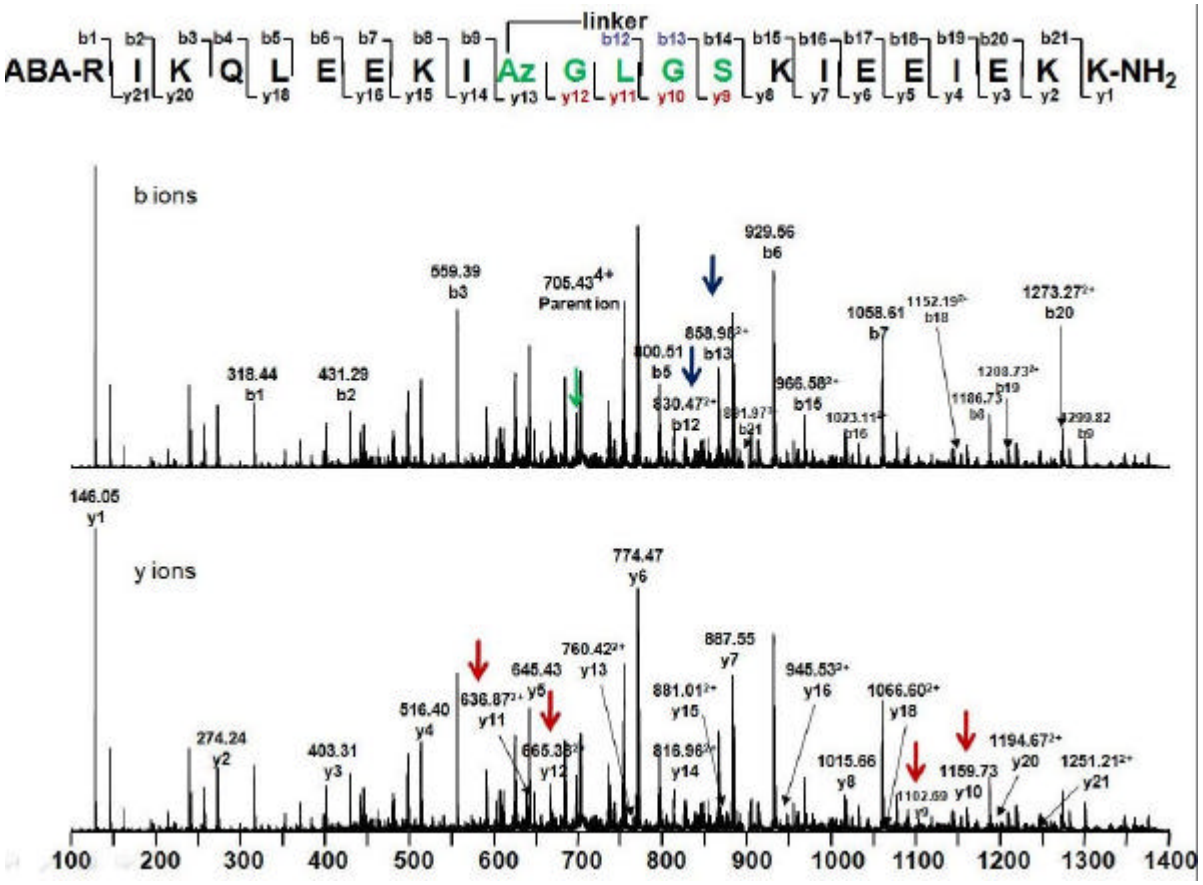
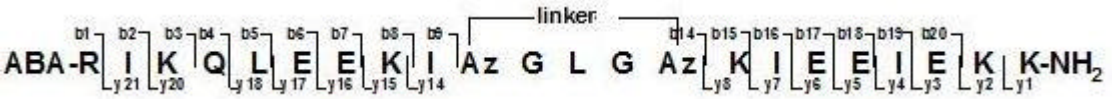


Figure D3. MS/MS spectrum of **6b** (refer to Table D2 for the mass list).

The top and bottom figures in Figure D3 were the same MS/MS spectrum. Fragmentation on the same cleavage sites (i.e. the supposed 23- membered ring) resulted in b and y ions with different masses from the parent ion. The appearance of b12-b13 (blue arrows) and y9-y13 ions (red arrows), the low intensity of the parent ion relative to other peaks (i.e. y1), and the over-all appearance of the fragmentation pattern in the mass spectrum were strong indications that **6b** was indeed open.

The mass list for MS/MS fragmentation of **6a**-**9a** and **6a-9b** were given in Tables D1-D12. Measured and theoretical m/z of the peptide were compared to evaluate if the given adduct was closed or open. For open adducts, b10-b13 and y9-y12 ions were typically observed.

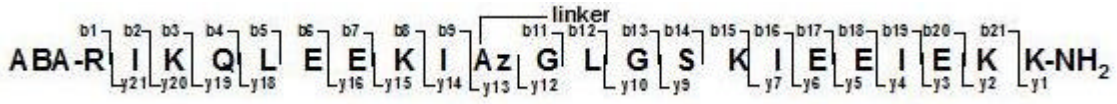
Table D1. MS/MS of **6a**



Measured m/z is 711.68^{4+} so *M*+H = 2843.70 Da. Theoretical molecular weight is 2843.58Da

Measured m/z	Theoretical m/z	Ion name
146.05	146.10	$\mathbf{y_1}$
274.25	274.20	$\mathbf{y_2}$
318.20	318.16	$\mathbf{b_1}$
403.31	403.24	$\mathbf{y_3}$
431.29	431.24	$\mathbf{b_2}$
516.40	516.33	$\mathbf{y_4}$
559.39	559.34	$\mathbf{b_3}$
645.43	645.37	$\mathbf{y_5}$
687.44	687.39	$\mathbf{b_4}$
774.47	774.41	$\mathbf{y_6}$
800.51	800.48	$\mathbf{b_5}$
887.55	887.50	$\mathbf{y_7}$
929.56	929.52	$\mathbf{b_6}$
1015.66	1015.59	$\mathbf{y_8}$
1058.61	1058.56	$\mathbf{b_7}$
1186.73	1186.66	$\mathbf{b_8}$
$1299.82/650.42^{+2}$	$1299.74/650.37^{+2}$	$\mathbf{b_9}$
829.47^{+2}	829.47^{+2}	$\mathbf{y_{14}}$
893.56^{+2}	893.51^{+2}	$\mathbf{y_{15}}$
900.19^{+3}	900.16^{+3}	$\mathbf{b_{21}}$
915.04^{+2}	915.00^{+2}	$\mathbf{b_{14}}$
958.08^{+2}	958.04^{+2}	$\mathbf{y_{16}}$
979.08^{+2}	979.05^{+2}	$\mathbf{b_{15}}$
1022.61^{+2}	1022.56^{+2}	$\mathbf{y_{17}}$
1035.63^{+2}	1035.59^{+2}	$\mathbf{b_{16}}$
1079.16^{+2}	1079.10^{+2}	$\mathbf{y_{18}}$
1100.16^{+2}	1100.11^{+2}	$\mathbf{b_{17}}$
$1164.69^{+2}/776.79^{+3}$	$1164.63^{+2}/776.76^{+3}$	$\mathbf{b_{18}}$
$1207.22^{+2}/805.15^{+3}$	$1207.18^{+2}/805.12^{+3}$	$\mathbf{y_{20}}$
$1221.23^{+2}/814.49^{+3}$	$1221.17^{+2}/814.45^{+3}$	$\mathbf{b_{19}}$
842.83^{+3}	842.81^{+3}	$\mathbf{y_{21}}$
$1285.77^{+2}/857.50^{+3}$	$1285.70^{+2}/857.47^{+3}$	$\mathbf{b_{20}}$

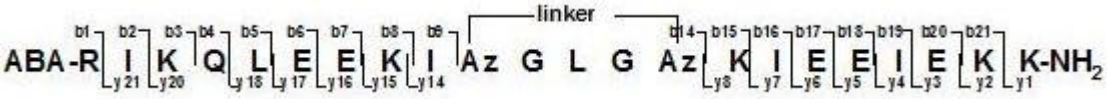
Table D2. MS/MS of **6b**



Measured m/z is 705.43^{4+} so *M*+H = 2818.70 Da. Theoretical molecular weight is 2818.57Da

Measured m/z	Theoretical m/z	Ion name
146.05	146.10	$\mathbf{y_1}$
274.25	274.20	$\mathbf{y_2}$
318.20	318.16	$\mathbf{b_1}$
403.31	403.24	$\mathbf{y_3}$
431.29	431.24	$\mathbf{b_2}$
516.40	516.33	$\mathbf{y_4}$
559.39	559.34	$\mathbf{b_3}$
645.43	645.37	$\mathbf{y_5}$
687.44	687.39	$\mathbf{b_4}$
774.47	774.41	$\mathbf{y_6}$
800.51	800.48	$\mathbf{b_5}$
887.55	887.50	$\mathbf{y_7}$
929.56	929.52	$\mathbf{b_6}$
1058.61	1058.56	$\mathbf{b_7}$
1102.69	1102.62	$\mathbf{y_9}$
1159.73	1159.64	$\mathbf{y_{10}}$
$1186.71/593.88^{+2}$	$1186.66/593.83^{+2}$	$\mathbf{b_8}$
$1299.82/650.42^{+2}$	$1299.74/650.37^{+2}$	$\mathbf{b_9}$
$1329.82/665.42^{+2}$	$1329.75/665.38^{+2}$	$\mathbf{y_{12}}$
$1519.98/760.46^{+2}$	$1519.84/760.42^{+2}$	$\mathbf{y_{13}}$
1546.95	1546.85	$\mathbf{b_{11}}$
817.00^{+2}	816.96^{+2}	$\mathbf{y_{14}}$
830.50^{+2}	830.47^{+2}	$\mathbf{b_{12}}$
859.01^{+2}	858.98^{+2}	$\mathbf{b_{13}}$
881.04^{+2}	881.01^{+2}	$\mathbf{y_{15}}$
902.53^{+2}	902.50^{+2}	$\mathbf{b_{14}}$
945.56^{+2}	945.53^{+2}	$\mathbf{y_{16}}$
966.58^{+2}	966.54^{+2}	$\mathbf{b_{15}}$
1023.11^{+2}	1023.09^{+2}	$\mathbf{b_{16}}$
1066.64^{+2}	1066.60^{+2}	$\mathbf{y_{18}}$
1087.65^{+2}	1087.61^{+2}	$\mathbf{b_{17}}$
1130.68^{+2}	1130.62^{+2}	$\mathbf{y_{19}}$
1152.18^{+2}	1152.13^{+2}	$\mathbf{b_{18}}$
1194.71^{+2}	1194.67^{+2}	$\mathbf{y_{20}}$
1208.73^{+2}	1208.67^{+2}	$\mathbf{b_{19}}$
1251.26^{+2}	1251.21^{+2}	$\mathbf{y_{21}}$
1273.27^{+2}	1273.19^{+2}	$\mathbf{b_{20}}$
891.86^{+3}	891.83^{+3}	$\mathbf{b_{21}}$

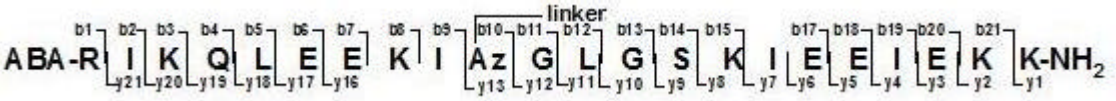
Table D3. MS/MS of **7a**



Measured m/z is 734.67^{4+} so *M*+H = 2935.66 Da. Theoretical molecular weight is 2935.57Da

Measured m/z	Theoretical m/z	Ion name
146.05	146.10	$\mathbf{y_1}$
274.25	274.20	$\mathbf{y_2}$
318.19	318.16	$\mathbf{b_1}$
403.32	403.24	$\mathbf{y_3}$
431.30	431.24	$\mathbf{b_2}$
516.41	516.33	$\mathbf{y_4}$
559.39	559.34	$\mathbf{b_3}$
645.44	645.37	$\mathbf{y_5}$
687.44	687.39	$\mathbf{b_4}$
774.47	774.41	$\mathbf{y_6}$
800.50	800.48	$\mathbf{b_5}$
887.55	887.50	$\mathbf{y_7}$
929.55	929.52	$\mathbf{b_6}$
1015.65	1015.59	$\mathbf{y_8}$
1058.61	1058.56	$\mathbf{b_7}$
1186.70	1186.66	$\mathbf{b_8}$
$1299.82/650.42^{+2}$	$1299.74/650.37^{+2}$	$\mathbf{b_9}$
875.51^{+2}	875.46^{+2}	$\mathbf{y_{14}}$
939.55^{+2}	939.51^{+2}	$\mathbf{y_{15}}$
961.04^{+2}	961.00^{+2}	$\mathbf{b_{14}}$
1004.08^{+2}	1004.03^{+2}	$\mathbf{y_{16}}$
1025.09^{+2}	1025.04^{+2}	$\mathbf{b_{15}}$
1068.61^{+2}	1068.55^{+2}	$\mathbf{y_{17}}$
1081.62^{+2}	1081.58^{+2}	$\mathbf{b_{16}}$
1125.16^{+2}	1125.09^{+2}	$\mathbf{y_{18}}$
$1146.15^{+2}/764.44^{+3}$	$1146.11^{+2}/764.41^{+3}$	$\mathbf{b_{17}}$
$1210.69^{+2}/807.46^{+3}$	$1210.63^{+2}/807.42^{+3}$	$\mathbf{b_{18}}$
$1253.23^{+2}/835.80^{+3}$	$1253.17^{+2}/835.78^{+3}$	$\mathbf{y_{20}}$
$1267.23^{+2}/845.14^{+3}$	$1267.17^{+2}/845.12^{+3}$	$\mathbf{b_{19}}$
1309.75^{+2}	1309.71^{+2}	$\mathbf{y_{21}}$
1331.77^{+2}	1331.69^{+2}	$\mathbf{b_{20}}$
930.85^{+3}	930.83^{+3}	$\mathbf{b_{21}}$

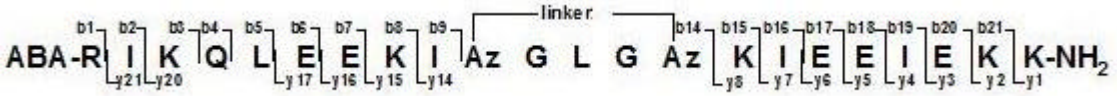
Table D4. MS/MS of **7b**



Measured m/z is 728.43^{4+} so *M*+H = 2910.70 Da. Theoretical molecular weight is 2910.56Da

Measured m/z	Theoretical m/z	Ion name
146.05	146.10	$\mathbf{y_1}$
274.25	274.20	$\mathbf{y_2}$
318.20	318.16	$\mathbf{b_1}$
403.31	403.24	$\mathbf{y_3}$
431.29	431.24	$\mathbf{b_2}$
516.40	516.33	$\mathbf{y_4}$
559.39	559.34	$\mathbf{b_3}$
645.43	645.37	$\mathbf{y_5}$
687.44	687.39	$\mathbf{b_4}$
774.47	774.41	$\mathbf{y_6}$
800.52	800.48	$\mathbf{b_5}$
887.55	887.50	$\mathbf{y_7}$
929.56	929.52	$\mathbf{b_6}$
508.36^{+2}	508.30^{+2}	$\mathbf{y_8}$
1102.69	1102.62	$\mathbf{y_9}$
1159.73	1159.64	$\mathbf{y_{10}}$
1186.73	1186.66	$\mathbf{b_8}$
1272.83	1272.73	$\mathbf{y_{11}}$
1299.82	1299.74	$\mathbf{b_9}$
665.41^{+2}	665.38^{+2}	$\mathbf{y_{12}}$
791.44^{+2}	791.41^{+2}	$\mathbf{b_{10}}$
806.45^{+2}	806.42^{+2}	$\mathbf{y_{13}}$
1638.99	1638.84	$\mathbf{b_{11}}$
876.51^{+2}	876.47^{+2}	$\mathbf{b_{12}}$
905.01^{+2}	904.98^{+2}	$\mathbf{b_{13}}$
948.54^{+2}	948.49^{+2}	$\mathbf{b_{14}}$
991.57^{+2}	991.53^{+2}	$\mathbf{y_{16}}$
1012.58^{+2}	1012.54^{+2}	$\mathbf{b_{15}}$
1056.11^{+2}	1056.05^{+2}	$\mathbf{y_{17}}$
1112.64^{+2}	1112.59^{+2}	$\mathbf{y_{18}}$
1133.65^{+2}	1133.60^{+2}	$\mathbf{b_{17}}$
1176.69^{+2}	1176.62^{+2}	$\mathbf{y_{19}}$
1198.20^{+2}	1198.12^{+2}	$\mathbf{b_{18}}$
$1240.73^{+2}/827.47^{+3}$	$1240.67^{+2}/827.45^{+3}$	$\mathbf{y_{20}}$
1254.76^{+2}	1254.67^{+2}	$\mathbf{b_{19}}$
1297.25^{+2}	1297.21^{+2}	$\mathbf{y_{21}}$
$1319.27^{+2}/879.82^{+3}$	$1319.19^{+2}/879.79^{+3}$	$\mathbf{b_{20}}$
922.51^{+3}	922.49^{+3}	$\mathbf{b_{21}}$

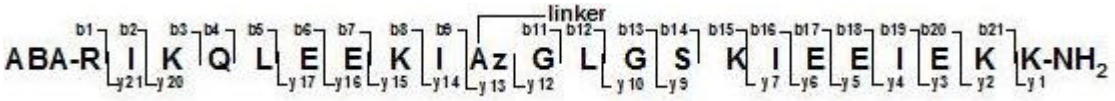
Table D5. MS/MS of **8a**



Measured m/z is 734.75^{4+} so *M*+H = 2935.97 Da. Theoretical molecular weight is 2935.57Da

Measured m/z	Theoretical m/z	Ion name
146.06	146.10	$\mathbf{y_1}$
274.27	274.20	$\mathbf{y_2}$
318.22	318.16	$\mathbf{b_1}$
403.36	403.24	$\mathbf{y_3}$
431.34	431.24	$\mathbf{b_2}$
516.45	516.33	$\mathbf{y_4}$
559.45	559.34	$\mathbf{b_3}$
645.50	645.37	$\mathbf{y_5}$
687.50	687.39	$\mathbf{b_4}$
774.55	774.41	$\mathbf{y_6}$
800.59	800.48	$\mathbf{b_5}$
887.64	887.50	$\mathbf{y_7}$
929.65	929.52	$\mathbf{b_6}$
1015.75	1015.59	$\mathbf{y_8}$
1058.71	1058.56	$\mathbf{b_7}$
1186.83	1186.66	$\mathbf{b_8}$
$1299.94/650.47^{+2}$	$1299.74/650.37^{+2}$	$\mathbf{b_9}$
875.59^{+2}	875.46^{+2}	$\mathbf{y_{14}}$
939.63^{+2}	939.51^{+2}	$\mathbf{y_{15}}$
961.12^{+2}	961.00^{+2}	$\mathbf{b_{14}}$
$1025.18^{+2}/683.80^{+3}$	$1025.04^{+2}/683.70^{+3}$	$\mathbf{b_{15}}$
1004.16^{+2}	1004.03^{+2}	$\mathbf{y_{16}}$
1068.72^{+2}	1068.55^{+2}	$\mathbf{y_{17}}$
1081.72^{+2}	1081.58^{+2}	$\mathbf{b_{16}}$
$1146.27^{+2}/764.51^{+3}$	$1146.11^{+2}/764.41^{+3}$	$\mathbf{b_{17}}$
$1210.80^{+2}/807.54^{+3}$	$1210.63^{+2}/807.42^{+3}$	$\mathbf{b_{18}}$
1253.34^{+2}	1253.17^{+2}	$\mathbf{y_{20}}$
1267.35^{+2}	1267.17^{+2}	$\mathbf{b_{19}}$
1309.87^{+2}	1309.71^{+2}	$\mathbf{y_{21}}$
$1331.90^{+2}/888.24^{+3}$	$1331.69^{+2}/888.13^{+3}$	$\mathbf{b_{20}}$
930.95^{+3}	930.83^{+3}	$\mathbf{b_{21}}$

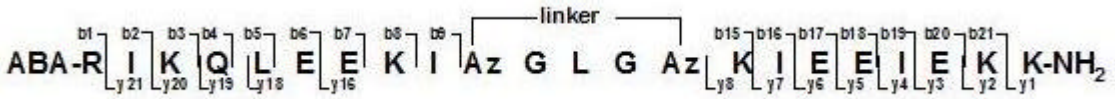
Table D6. MS/MS of **8b**



Measured m/z is 970.73^{3+} so *M*+H = 2910.19 Da. Theoretical *M*+H is 2910.56 Da .

Measured m/z	Theoretical m/z	Ion name
146.06	146.10	$\mathbf{y_1}$
274.21	274.20	$\mathbf{y_2}$
318.15	318.16	$\mathbf{b_1}$
403.37	403.24	$\mathbf{y_3}$
431.23	431.24	$\mathbf{b_2}$
516.33	516.33	$\mathbf{y_4}$
559.31	559.34	$\mathbf{b_3}$
645.34	645.37	$\mathbf{y_5}$
687.34	687.39	$\mathbf{b_4}$
774.36	774.41	$\mathbf{y_6}$
800.40	800.48	$\mathbf{b_5}$
887.42	887.50	$\mathbf{y_7}$
929.42	929.52	$\mathbf{b_6}$
$1058.45/529.76^{+2}$	$1058.56/529.79^{+2}$	$\mathbf{b_7}$
1102.53	1102.62	$\mathbf{y_9}$
1159.56	1159.64	$\mathbf{y_{10}}$
$1186.54/593.79^{+2}$	$1186.66/593.83^{+2}$	$\mathbf{b_8}$
1272.64	$1272.73/636.87^{+2}$	$\mathbf{y_{11}}$
$1299.63/650.32^{+2}$	$1299.74/650.37^{+2}$	$\mathbf{b_9}$
1329.65	1329.75	$\mathbf{y_{12}}$
1611.75	$1611.83/806.42^{+2}$	$\mathbf{y_{13}}$
1638.74	1638.84	$\mathbf{b_{11}}$
1724.82	1724.91	$\mathbf{y_{14}}$
$1751.82/876.36^{+2}$	$1751.92/876.47^{+2}$	$\mathbf{b_{12}}$
$1808.81/904.87^{+2}$	$1808.94/904.98^{+2}$	$\mathbf{b_{13}}$
1852.88	1853.00	$\mathbf{y_{15}}$
$1895.83/948.39^{+2}$	$1895.98/948.49^{+2}$	$\mathbf{b_{14}}$
991.42^{+2}	991.53^{+2}	$\mathbf{y_{16}}$
1012.43^{+2}	1012.54^{+2}	$\mathbf{b_{15}}$
1055.96^{+2}	1056.05^{+2}	$\mathbf{y_{17}}$
1068.96^{+2}	1069.08^{+2}	$\mathbf{b_{16}}$
1133.48^{+2}	1133.60^{+2}	$\mathbf{b_{17}}$
1198.00^{+2}	1198.12^{+2}	$\mathbf{b_{18}}$
1240.54^{+2}	1240.67^{+2}	$\mathbf{y_{20}}$
1254.55^{+2}	1254.67^{+2}	$\mathbf{b_{19}}$
1297.07^{+2}	1297.21^{+2}	$\mathbf{y_{21}}$
$1319.06^{+2}/879.68^{+3}$	$1319.19^{+2}/879.79^{+3}$	$\mathbf{b_{20}}$
$1383.12^{+2}/922.39^{+3}$	$1383.23^{+2}/922.49^{+3}$	$\mathbf{b_{21}}$

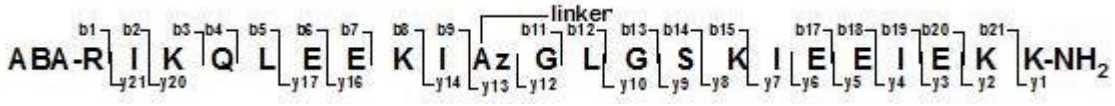
Table D7. MS/MS of **9a**



Measured m/z is 729.93^{4+} so *M*+H = 2916.70 Da. Theoretical molecular weight is 2916.59Da

Measured m/z	Theoretical m/z	Ion name
146.03	146.10	$\mathbf{y_1}$
274.21	274.20	$\mathbf{y_2}$
318.15	318.16	$\mathbf{b_1}$
403.26	403.24	$\mathbf{y_3}$
431.24	431.24	$\mathbf{b_2}$
516.33	516.33	$\mathbf{y_4}$
559.31	559.34	$\mathbf{b_3}$
645.34	645.37	$\mathbf{y_5}$
687.34	687.39	$\mathbf{b_4}$
774.37	774.41	$\mathbf{y_6}$
800.39	800.48	$\mathbf{b_5}$
887.42	887.50	$\mathbf{y_7}$
929.43	929.52	$\mathbf{b_6}$
1015.46	1015.59	$\mathbf{y_8}$
1058.46	1058.56	$\mathbf{b_7}$
$1186.54/593.79^{+2}$	$1186.66/593.83^{+2}$	$\mathbf{b_8}$
$1299.61/650.32^{+2}$	$1299.74/650.37^{+2}$	$\mathbf{b_9}$
994.44^{+2}	994.54^{+2}	$\mathbf{y_{16}}$
1015.46^{+2}	1015.56^{+2}	$\mathbf{b_{15}}$
1071.99^{+2}	1072.10^{+2}	$\mathbf{b_{16}}$
1115.51^{+2}	1115.61^{+2}	$\mathbf{y_{18}}$
1136.51^{+2}	1136.62^{+2}	$\mathbf{b_{17}}$
1179.55^{+2}	1179.64^{+2}	$\mathbf{y_{19}}$
1201.03^{+2}	1201.14^{+2}	$\mathbf{b_{18}}$
1243.57^{+2}	1243.68^{+2}	$\mathbf{y_{20}}$
1257.58^{+2}	1257.68^{+2}	$\mathbf{b_{19}}$
829.37^{+3}	829.46^{+3}	$\mathbf{y_{20}}$
$1322.11^{+2}/881.71^{+3}$	$1322.20^{+2}/881.80^{+3}$	$\mathbf{b_{20}}$
924.41^{+3}	924.50^{+3}	$\mathbf{b_{21}}$

Table D8. MS/MS of **9b**



Measured m/z is 743.60^{4+} so M+H = 2891.74 Da. Theoretical molecular weight is 2891.59Da

Measured m/z	Theoretical m/z	Ion name
146.05	146.10	$\mathbf{y_1}$
274.25	274.20	$\mathbf{y_2}$
318.20	318.16	$\mathbf{b_1}$
403.31	403.24	$\mathbf{y_3}$
431.29	431.24	$\mathbf{b_2}$
516.40	516.33	$\mathbf{y_4}$
559.39	559.34	$\mathbf{b_3}$
645.43	645.37	$\mathbf{y_5}$
687.44	687.39	$\mathbf{b_4}$
774.47	774.41	$\mathbf{y_6}$
800.52	800.48	$\mathbf{b_5}$
887.55	887.50	$\mathbf{y_7}$
929.56	929.52	$\mathbf{b_6}$
1015.64	1015.59	$\mathbf{y_8}$
$1058.61/529.83^{+2}$	$1058.56/529.79^{+2}$	$\mathbf{b_7}$
1102.70	1102.62	$\mathbf{y_9}$
1159.73	1159.64	$\mathbf{y_{10}}$
$1186.73/593.89^{+2}$	$1186.66/593.83^{+2}$	$\mathbf{b_8}$
$1299.84/650.42^{+2}$	$1299.74/650.37^{+2}$	$\mathbf{b_9}$
1329.88	1329.75	$\mathbf{y_{12}}$
796.99^{+2}	796.93^{+2}	$\mathbf{y_{13}}$
810.48^{+2}	810.44^{+2}	$\mathbf{b_{11}}$
853.51^{+2}	853.47^{+2}	$\mathbf{y_{14}}$
867.01^{+2}	866.98^{+2}	$\mathbf{b_{12}}$
895.53^{+2}	895.49^{+2}	$\mathbf{b_{13}}$
939.05^{+2}	939.01^{+2}	$\mathbf{b_{14}}$
982.10^{+2}	982.04^{+2}	$\mathbf{y_{16}}$
1003.10^{+2}	1003.05^{+2}	$\mathbf{b_{15}}$
1046.62^{+2}	1046.56^{+2}	$\mathbf{y_{17}}$
1124.16^{+2}	1124.12^{+2}	$\mathbf{b_{17}}$
1188.72^{+2}	1188.64^{+2}	$\mathbf{b_{18}}$
$1231.23^{+2}/821.15^{+3}$	$1231.18^{+2}/821.12^{+3}$	$\mathbf{y_{20}}$
1245.24^{+2}	1245.18^{+2}	$\mathbf{b_{19}}$
858.84^{+3}	858.82^{+3}	$\mathbf{y_{21}}$
$1309.78^{+2}/873.50^{+3}$	$1309.70^{+2}/873.47^{+3}$	$\mathbf{b_{20}}$
916.20^{+3}	916.17^{+3}	$\mathbf{b_{21}}$

E) Analytical Ultracentrifugation

Apparent molecular masses of peptides were determined by sedimentation equilibrium on a Beckman ProteomeLab™ XL-I ultracentrifuge. Purified peptides (25 μM) were analyzed at three different $NiCl_2$ concentrations (0, 100, and 200 μM) in 10 mM Tris.Cl containing 50 mM NaCl at pH 7.1. The peptides were equilibrated at three rotor speeds (25 000, 32 000, and 45 000 rpm) for 24 and 30 h at 20 °C. Absorbance scans at 270 and 280 nm were fit to Equation (1) describing the equilibrium sedimentation of a homogeneous single ideal species:

$$\mathrm{Abs}(r) = A' \exp[H\chi M(x^2 - x_0^2)] + B \quad (1)$$

here *Abs* = Absorbance at radius *r*, A′= absorbance at reference radius x_0, H = $(1-\bar{\nu}\rho)\cdot\omega^2/2RT$, with $\bar{\nu}$ = partial specific volume of the peptide, ρ = solvent density, ω = angular velocity in radians/second, *M* = apparent molecular weight, *E* = blank absorbance. Data were fit by using Igor Pro v5.03 and partial specific volumes and solution densities were calculated by using the program SEDNTERP.

Table E1. Average fitted molecular weight of **1** using analytical equilibrium sedimentation.[a]

Rotor speed (rpm)	$NiCl_2$ concentration (μM)	average fitted mw (Da)[b]
25,000	0	2756 ± 68
25,000	100	5610 ± 114
25,000	200	6199 ± 123
32,000	0	2493 ± 23
32,000	100	5175 ± 71
32,000	200	5469 ± 15

Table E2. Average fitted molecular weight of **6a** using analytical equilibrium sedimentation.[a]

Rotor speed (rpm)	$NiCl_2$ concentration (μM)	average fitted mw (Da)[b]
25,000	0	5162 ± 100
25,000	100	4849 ± 84
25,000	200	5005 ± 36
32,000	0	4985 ± 93
32,000	100	4624 ± 81
32,000	200	4726 ± 37

Table E3. Average fitted molecular weight of **9a** using analytical equilibrium sedimentation.[a]

Rotor speed (rpm)	$NiCl_2$ concentration (μM)	average fitted mw (Da)[b]
25,000	0	5197 ± 47
25,000	100	5397 ± 32
25,000	200	5407 ± 81
32,000	0	4867 ± 62
32,000	100	5003 ± 97
32,000	200	5075 ± 39

[a]Measurements were made as described in *Methods* using 25 μM of all peptides at 20 °C at 25,000, 32,000, and 45,000 rpm. [b]Data from multiple speeds were fit to equation describing homogeneous single ideal species. Uncertainties represent one SD (*n* = 3, 5, or 6).

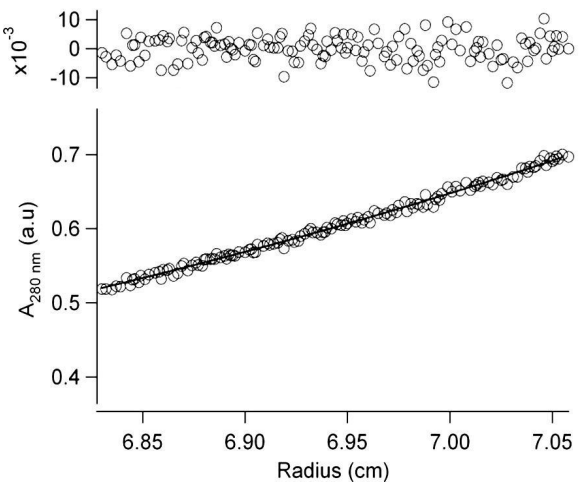


Figure E1. Sedimentation velocity of 1, no $NiCl_2$, 2814 Da

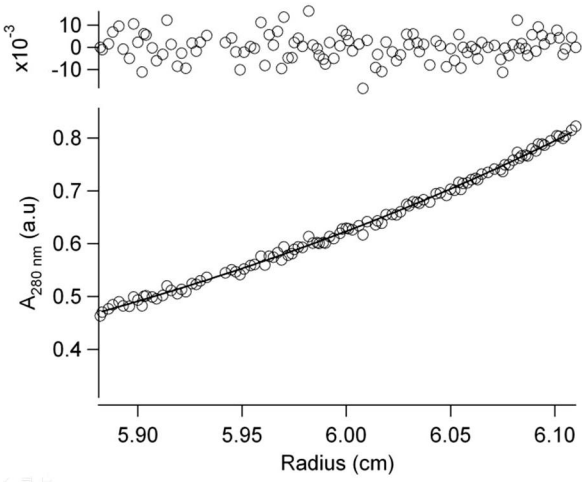


Figure E2. Sedimentation velocity of 1, no $NiCl_2$, 5982 Da

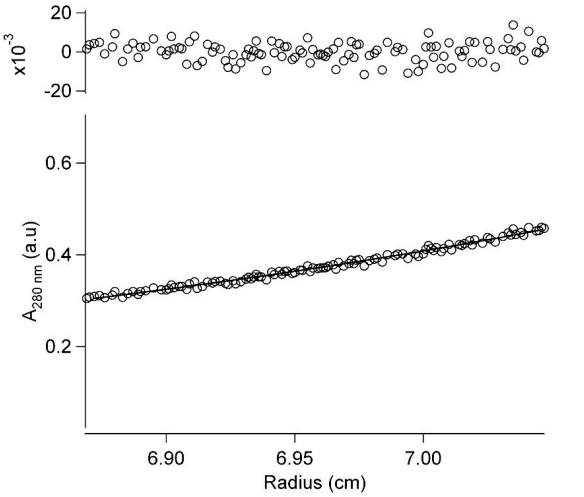


Figure E3. Sedimentation velocity of **6a**, no $NiCl_2$, 5156Da

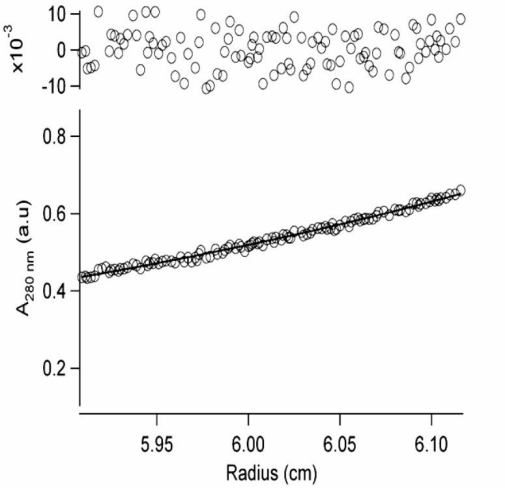


Figure E4. Sedimentation velocity of **6a**, with $NiCl_2$, 4990Da

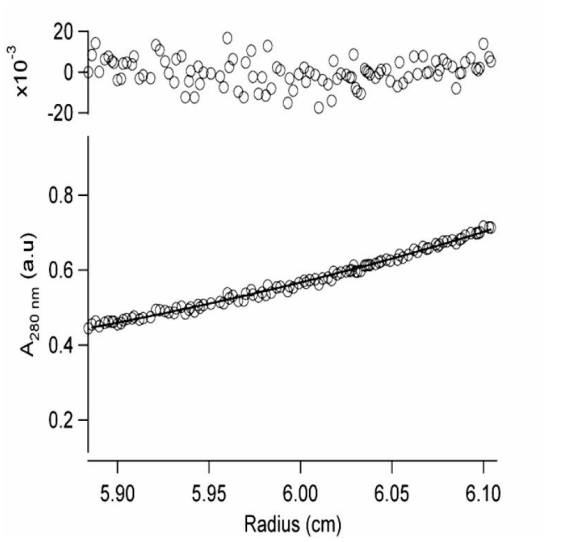


Figure E5. Sedimentation velocity of **9a**, no $NiCl_2$, 5178 Da

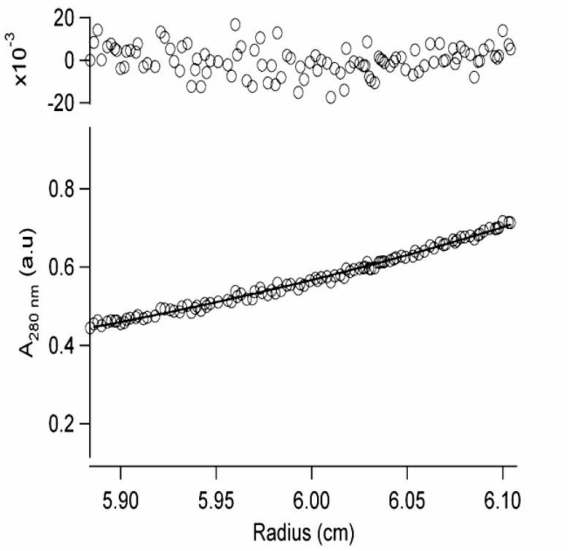


Figure E6. Sedimentation velocity of **9a**, with $NiCl_2$, 5463 Da

F) Circular Dichroism

CD spectra were obtained from AVIV Model 202 Circular dichroism spectropolarimeter by using a 1 mm quartz cell (Helma USA). For CD studies, the following solutions were prepared:

Experiments	Final concentration of solutions
Without Ni^{2+}	50 μM peptide: 10 mM Tris.Cl : 50 mM NaCl pH 7.1
With Ni^{2+}	50 μM peptide: 500 μM $NiCl_2.6H_20$ 10 mM Tris.Cl : 50 mM NaCl

Three consecutive CD scans were taken at 25 °C in 1 nm intervals, 3.0 s averaging time and at least 2 min equilibration time. The average scans were baseline corrected. Temperature melts were run on peptides which fold with Ni^{2+}. CD melting curves were obtained by monitoring the signal at 222 nm from 5 °C- 95 °C with equilibration for 2 min at each temperature. The melting temperature, T_m, was determined from the 1^{st} derivative of the CD melting curve. For the Ni^{2+} titration of peptides, CD scans were acquired at 25 °C from 260 to 190 nm with an averaging time of 3.0 s and equilibration time of at least 2 min. Metal (Ni^{2+}) was added in 5, 10, 15 and 20 μM increments. Signal was corrected for dilution.

Peptides and Peptide Adducts	Sequences
1	ABA-RIKQLEEK-I**HGLGH**K-IEELEKK-NH_2
2	ABA-RIKQLEEK-I**AzGLGAz**K-IEELEKK-NH_2
6a	2 + linker 6
8a	2 + linker 8
9a	2 + linker 9
10a	10 + propargyl alcohol (2eq)

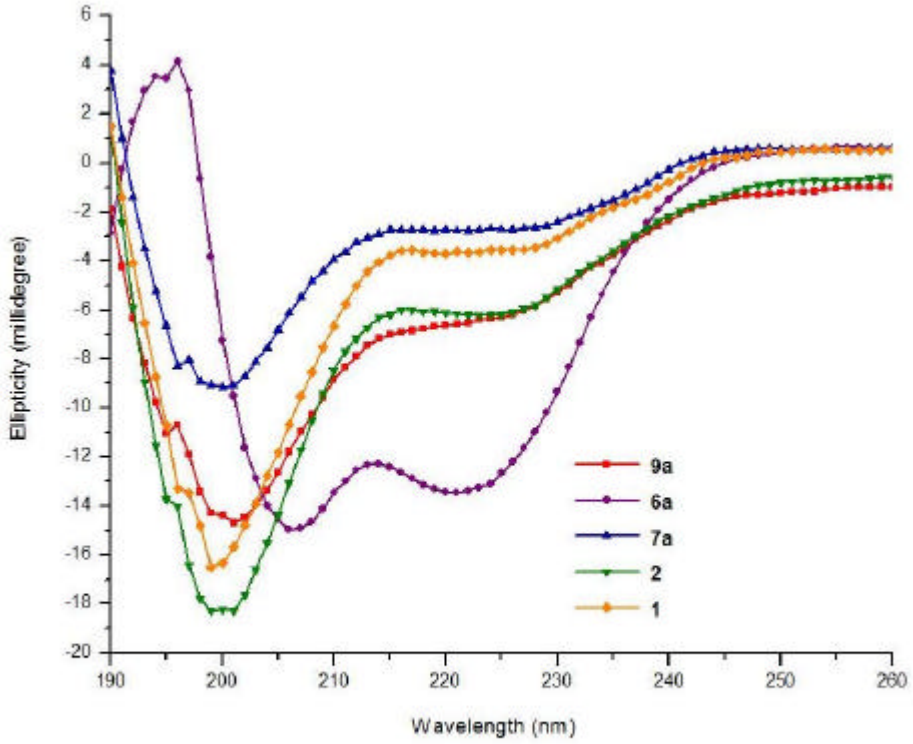


Figure F1. CD Spectra of **1**, **2** and click adducts (**2** + linkers).

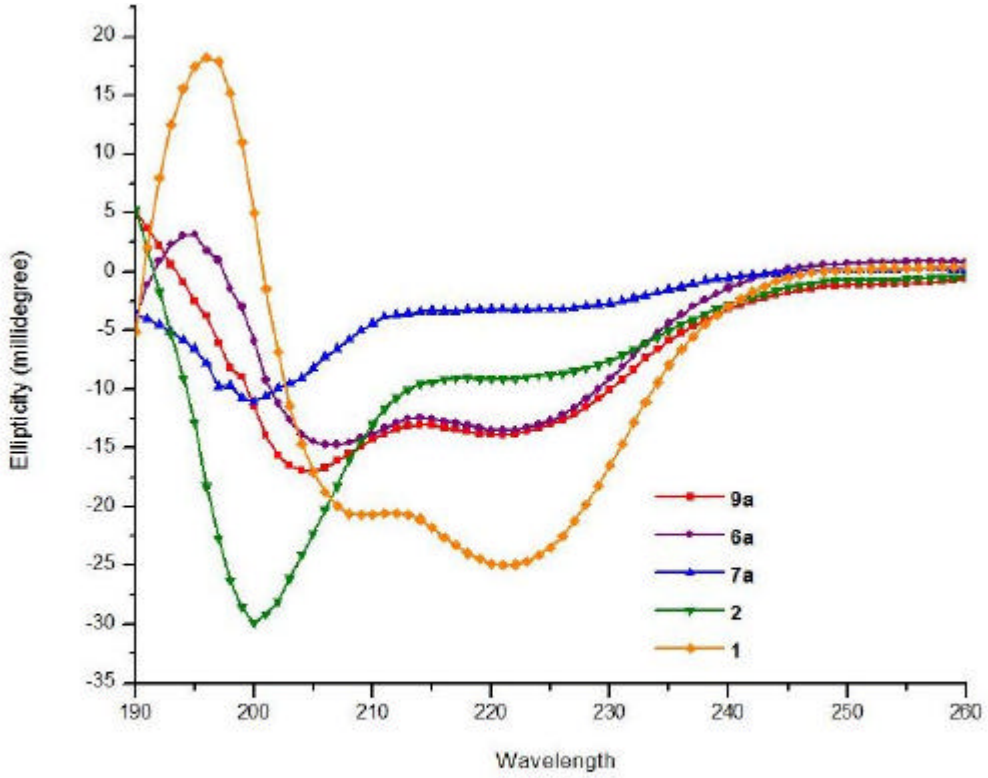


Figure F2. CD Spectra of **1**, **2** and click adducts (**2** + linkers) in the presence $NiCl_2$.

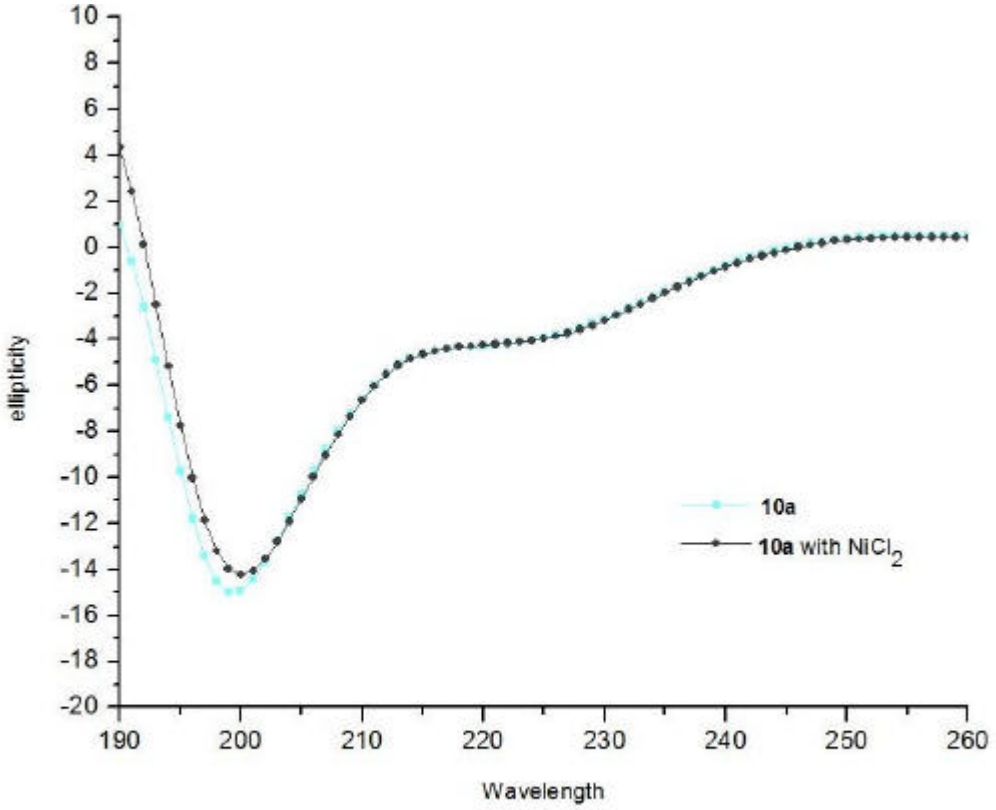


Figure F3. CD Spectra of **10a**, click adduct of **2** + propargyl alcohol (2eq).

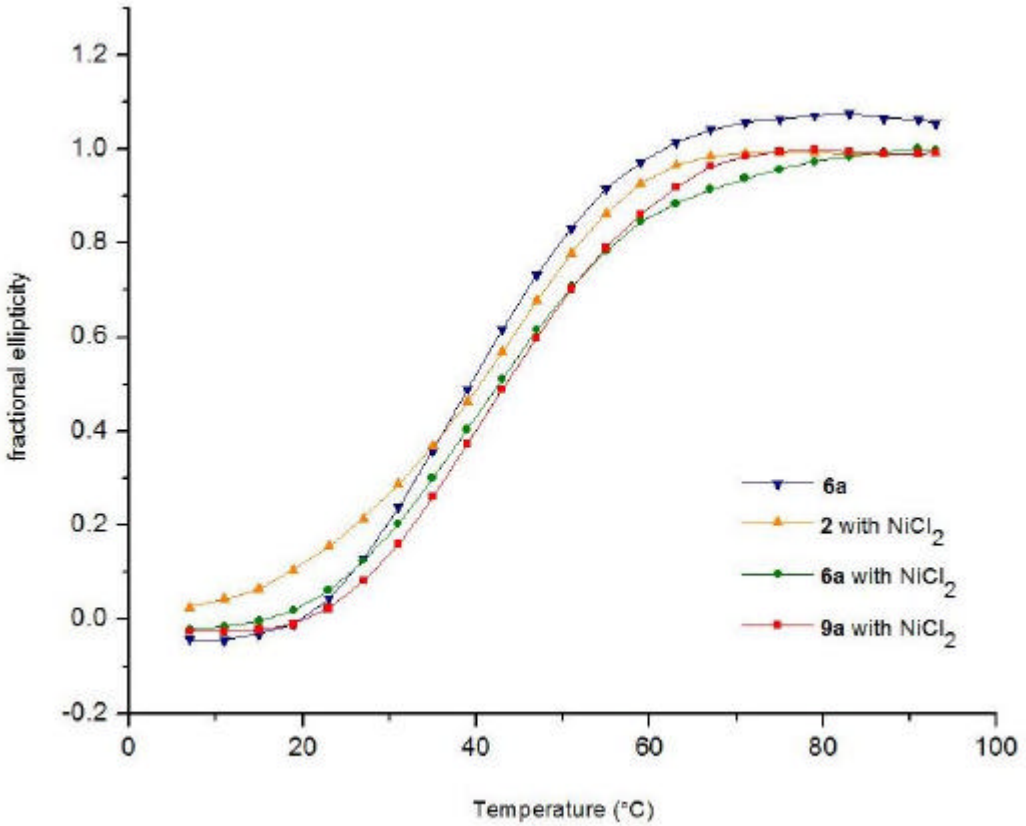


Figure F4. Normalized melts of selected peptides.