Electronic Supplementary Material (ESI) for ChemComm.

SUPPORTING INFORMATION

Variation in the Yaa Position of Collagen Peptides Containing AzaGlycine

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ABBREVIATIONS

Boc	tert-Butyloxycarbonyl
CD	Circular dichroism
CDT	1,1'-Carbonylditriazole
CHCA	α-Cyano-4-hydroxycinnamic acid
CMP	Collagen model peptide
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DIEA	*N*,*N*-Diisopropylethylamine
DMF	*N*,*N*-Dimethylformamide
Fmoc	9-Fluorenylmethoxycarbonyl
HATU	1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
HCTU	O-(6-Chlorobenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate
HOBt	1-Hydroxybenzotriazole
HPLC	High-performance liquid chromatography
MALDI-TOF MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
NMM	4-Methylmorpholine
PBS	Phosphate-buffered saline
RT	Room temperature
SPPS	Solid-phase peptide synthesis
tBu	tert-Butyl
TIPS	Triisopropylsilane
Trt	trityl
TFA	Trifluoroacetic acid

INSTRUMENTATION

All peptides were purified using semi-preparative reversed-phase HPLC on a Jasco PU-2080 Plus HPLC system using a Phenomenex Luna Omega 5 µm PS C18 100 Å LC column. Varying gradients of acetonitrile and 0.1% TFA in H2O were used depending on the individual CMP. Analytical HPLC (spectra shown for each peptide) to check purity was carried out on an Agilent 1260 Infinity II system using a Phenomenex Luna Omega 5 µm PS C18 100 Å LC column, with the exception of CMPs **1a** and **18a**, for which a Phenomenex Luna PFP(2) 5 µm PS C18 100 Å LC column was used. Mass spectrometry was performed using a Bruker MALDI-TOF MS Ultraflex III mass spectrometer and CHCA as the matrix. Peptides were lyophilized using a Labconco FreeZone Plus 12 Liter Cascade Console Freeze Dry system. UV-Vis absorption spectrophotometry was performed using a Jasco V-650 Spectrophotometer and 1mm path length quartz cells. Circular dichroism experiments were performed using a Jasco J-1500 Circular Dichroism Spectrometer and 1cm quartz cuvettes.

REAGENTS

All commercially available reagents were used as received. Rink Amide AM Resin was purchased from Novabiochem. HATU and HCTU were purchased from Oakwood Chemical. CHCA, DIEA, DMF, and ether were purchased from Sigma-Aldrich. NMM and 10X PBS were purchased from Fisher. DBU and TFA were purchased from Acros Organics. CDT was purchased from Chem-Impex Int'l. Inc. Fmoc-carbazate was purchased from Ark Pharm, Inc. HOBt was purchased from EMD Millipore.

Fmoc-Amino Acids:	Supplier:
Fmoc-Pro-OH	Chem-Impex Int'l. Inc.
Fmoc-Hyp(tBu)-OH	Ark Pharm, Inc.
Fmoc-Gly-OH	Chem-Impex Int'l Inc.
Fmoc-Asp(tBu)-OH	Chem-Impex Int'l. Inc.
Fmoc-Glu(tBu)-OH	Novabiochem
Fmoc-Lys(Boc)-OH	Novabiochem
Fmoc-Ser(tBu)-OH	Chem-Impex Int'l. Inc.
Fmoc-Thr(tBu)-OH	Novabiochem
Fmoc-Cys(Trt)-OH	Novabiochem
Fmoc-Asn(Trt)-OH	Novabiochem
Fmoc-Gln(Trt)-OH	Novabiochem
Fmoc-Ala-OH	Novabiochem
Fmoc-Val-OH	Chem-Impex Int'l. Inc.
Fmoc-Leu-OH	Novabiochem
Fmoc-Ile-OH	Chem-Impex Int'l. Inc.
Fmoc-(t-Leu)-OH	Ark Pharm, Inc.
Fmoc-Met-OH	Chem-Impex Int'l. Inc.
Fmoc-Phe-OH	Novabiochem
Fmoc-Trp(Boc)-OH	Novabiochem
Fmoc-Tyr(tBu)-OH	Chem-Impex Int'l. Inc.
Fmoc-His(Trt)-OH	Novabiochem

Fmoc-Pro-Hyp(tBu)-Gly-OH was synthesized using methods previously described by our group[1,2].

SYNTHESIS, PURIFICATION, SAMPLE PREPARATION, AND CD MEASUREMENTS

a. Resin Preparation

All peptides in this study were prepared on a 0.02mmol (1 equivalent) scale on Rink Amide AM Resin (loading density: 0.56mmol/g), 30mg of resin was transferred to a 10mL SPPS vessel. The resin was swelled in DMF for 30min. The Fmoc group was removed using 1mL of a 1% HOBt (w/v), 2 % DBU (v/v) in DMF and stirring for 1min. This deprotection step was repeated twice more. The resin was then thoroughly washed with DMF. Finally, a coupling solution containing Fmoc-Pro-Hyp(tBu)-Gly-OH (described below) was added to the resin and stirred for 90min at RT. The solution was then drained, the resin was washed with DMF, the Fmoc group on the growing peptide chain was removed as previously described, and the resin was washed with DMF. For all CMPs except **14c**, **17c**, **20c**, and **21c** this POG coupling was repeated for a total four times to give H_2N-$(POG)_4$ on resin. For CMPs **14c**, **17c**, **20c**, and **21c** only one POG coupling was performed.

b. Standard Amino Acid Couplings

Following Fmoc deprotection and washing, amino acid coupling solutions were added to the free amine on the growing peptide chain. The coupling solutions contained three equivalents of the appropriate Fmoc-amino acid (listed above), three equivalents of HATU (22.8mg, 0.06mmol) or HCTU (24.8mg, 0.06mmol), and six equivalents of DIEA (20µL, 0.12mmol) or NMM (13µL, 0.12mmol) in 0.7mL of DMF. Prior to addition to resin, the coupling solutions were allowed to sit for 5-10min. The coupling solution was stirred with the resin for 90min before being drained. The resin was then washed with DMF, the Fmoc group was removed using 1mL of a 1% HOBt (w/v), 2 % DBU (v/v) in DMF and stirring for 1min (x3), and the resin was washed again with DMF.

c. Fmoc-Pro-Hyp(tBu)-Gly-OH Coupling

The amino acid trimer Fmoc-Pro-Hyp(tBu)-Gly-OH coupling solution was prepared in the same manner as the single amino acids. The coupling solution contained three equivalents of Fmoc-Pro-Hyp(tBu)-Gly-OH (33.8mg, 0.06mmol), three equivalents of HATU (22.8mg, 0.06mmol) or HCTU (24.8mg, 0.06mmol), and six equivalents of DIEA (20µL, 0.12mmol) or NMM (13µL, 0.12mmol) in 0.7mL of DMF. Prior to addition to resin, the coupling solution was allowed to sit for 5-10min. The coupling solution was stirred with the resin for 90min before being drained. The resin was then washed with DMF, the Fmoc group was removed using 1mL of a 1% HOBt (w/v), 2 % DBU (v/v) in DMF and stirring for 1min (x3), and the resin was washed again with DMF. Whenever possible and appropriate, POG coupling solutions were used while synthesizing each CMP.

d. AzaGlycine Coupling

Five equivalents of Fmoc-carbazate (24.5mg, 0.10mmol) and five equivalents of CDT (16.4mg, 0.10mmol) were combined in 1.0mL of DMF and activated at RT for 5-10min before being added to the growing peptide chain on resin. The solution and resin were stirred for 24hr before draining the coupling solution and washing the resin with DMF. The Fmoc group was removed using 1mL of a 1% HOBt (w/v), 2 % DBU (v/v) in DMF and stirring for 1min (x3), and the resin was washed again with DMF.

e. Cleavage from Resin and Precipitation

Following all appropriate couplings, the final Fmoc group was removed using 1mL of a 1% HOBt (w/v), 2% DBU (v/v) in DMF and stirring for 1min (x3), and the resin was washed first with DMF and then with DCM. A 4mL cleavage cocktail containing 95% TFA (3.8mL), 2.5% TIPS (0.1mL), and 2.5% H_2O (0.1mL) was added to the resin. The mixture was stirred for 2hr before being collected into cold ether, causing the peptide to precipitate. The solid was collected by centrifugation, resuspended in cold ether, and collected by centrifugation again (3x). The final pellet was then dissolved in 5mL of H_2O and stored at 4 ˚C.

f. Purification

The peptide solutions were purified using semi-preparative reversed-phase HPLC using acetonitrile and 0.1% TFA in H_2O. Prior to loading onto the column, peptides were heated for 15min at 80 ˚C to ensure the denatured, single stranded form was dominant over the triple helical form. The absorbance at 215nm was monitored to determine collection, collected fractions were analyzed using MALDI-TOF MS in positive ion mode. Appropriate fractions were combined and lyophilized to yield the desired peptide as a white solid.

g. Sample Preparation

After obtaining the pure product, each peptide was then dissolved in a small amount of 1X pH 7.4 PBS (200µL). Using UV-Vis spectrophotometry, the concentration of each sample was determined by measuring the absorbance at 214nm and using an extinction coefficient of 60$mM^{-1}cm^{-1}$ as described by Engel *et al.*[3]. The stock solution was then diluted using PBS to a final concentration of 0.2mM and stored at 4 ˚C for 24hr before carrying out CD measurements.

h. CD Measurements and T_m Determination

For each peptide, approximately 250µL of the 0.2mM solution was placed into a 1mm quartz cuvette. The ellipticity of these solutions was then measured from 260 to 190nm while holding the temperature at 20 ˚C. Measurements were obtained in triplicate and then converted to mean residue ellipticity and averaged to generate the CD scan curves included for each peptide below. Following this, the solutions were then heated at a rate of 12 ˚C/hr starting at 20 ˚C (or 4 ˚C as necessary) and ending at 92 ˚C while monitoring the absorbance at 210, 215, 220, and 225nm. These measurements, obtained in triplicate, were converted to mean residue ellipticity and averaged. The melting temperate for each peptide, T_m, was determined by using the program GraphPad Prism 7 by fitting the data to a two-state model to find the temperature at which 50% of starting ellipticity was lost, as described previously[1].

i. Molecular Dynamics Simulations

Molecular dynamics were performed using GROMACS 5.1[4–9] utilizing the AMBER99SB force field. The model system used the peptide sequence $(POG)_3(P(t\text{-Leu})azG)(POG)_4$. Azaglycine and *tert*-leucine parameters were generated utilizing the restrained electrostatic potential (RESP) approach as previously described[1]. Quantum mechanical optimizations were performed using Gaussian 09[10]. The B3LYP/6- 31g(d) basis set was used for geometry optimizations and the HF/6-31g(d) basis set was used for electrostatic potential calculations. The production run was carried out for 20 nanoseconds (ns) using 2 femtosecond (fs) time steps. Coordinates were saved every 10 picosecond (ps) while

maintaining a temperature of 300 K and a pressure of one bar. The velocity rescaling modified Berendsen thermostat was used to control temperature and Parrinello–Rahman barostat was used to control pressure[11]. The particle mesh Ewald (PME) method was used to calculate the electrostatic interactions. Post processing was performed using UCSF Chimera[12].

SUPPORTING FIGURES

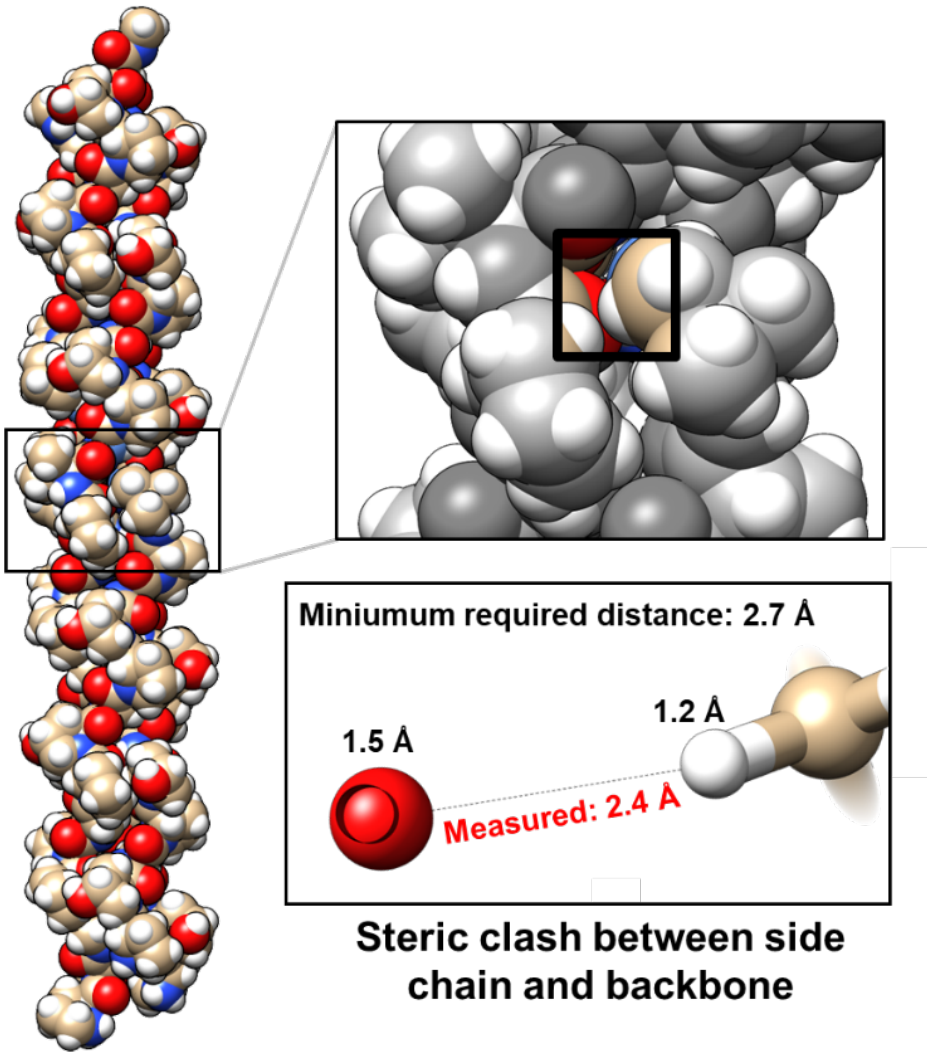


Figure S1. (Left) MD model of a *t*-Leu and azGly-containing CMP. (Right, upper) Enlarged view of the site of the steric clash between the hydrogen of the *t*-Leu side chain and backbone oxygen. (Right, bottom) Comparison of van der Waals radii to the measured distance between the two atoms.

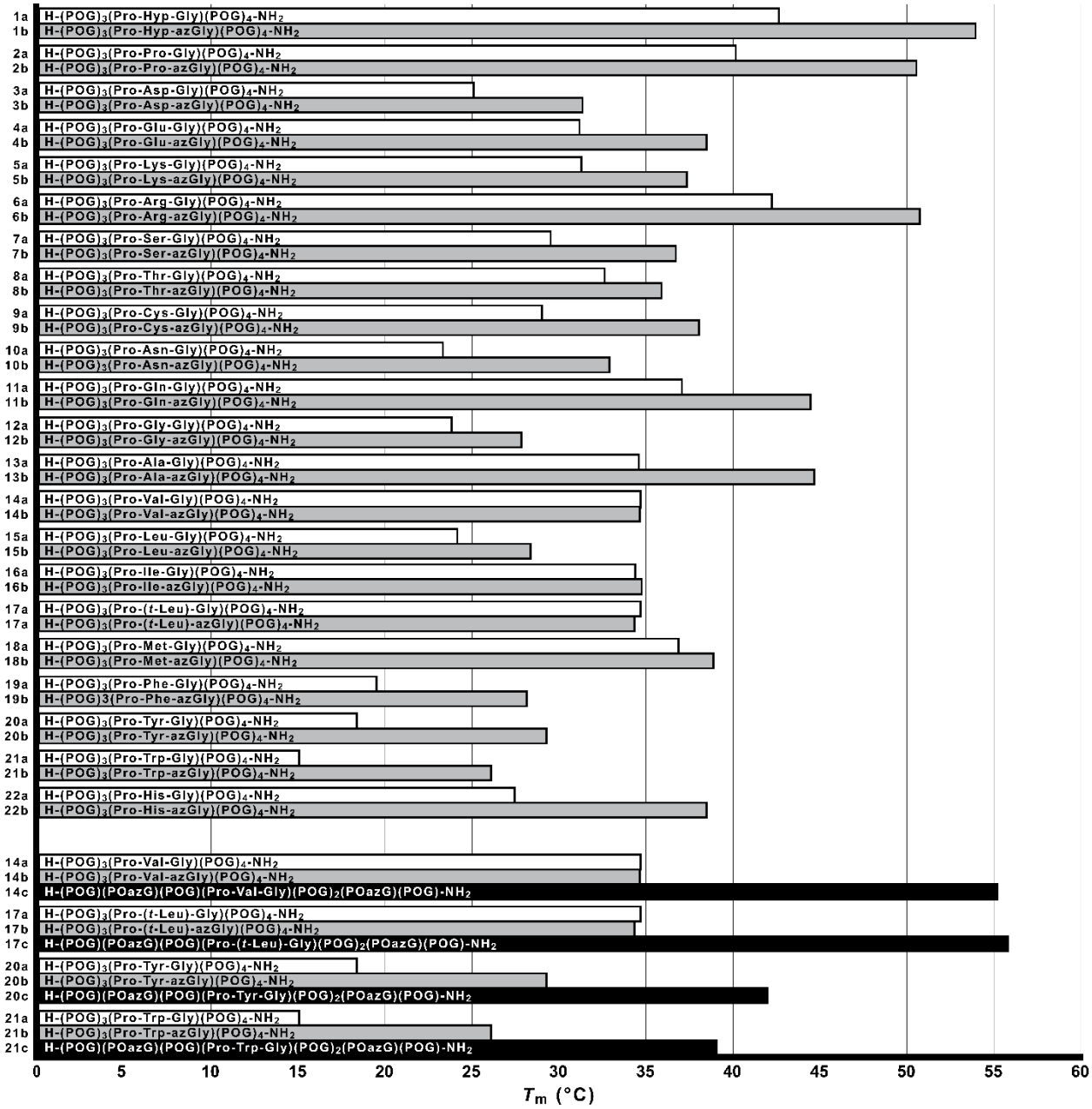
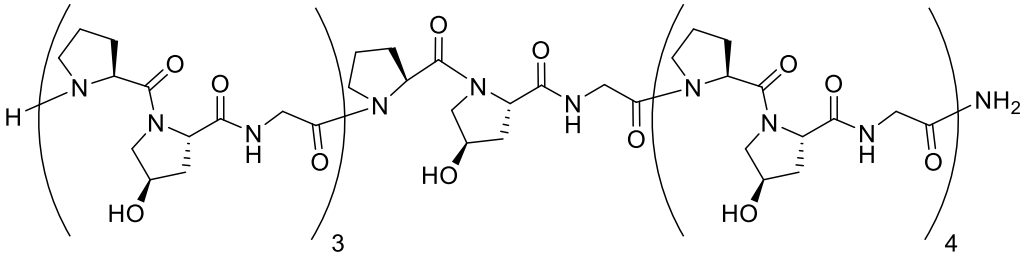


Figure S2. Graphical representation of helical stability of each CMP. White, control sequences. Grey, central azGly-containing sequences. Black, clamped sequences. **14a**, **14b**, **17a**, **17b**, **20a**, **20b**, **21a**, and **21b** are shown twice for clarity in comparison to the clamped variants **14c**, **17c**, **20c**, and **21c**.

MALDI-TOF MASS SPECTROMETRY RESULTS, THERMAL DENATURATION CURVES, AND HPLC TRACES

CMP **1a**: H-(Pro-Hyp-Gly)$_3$(Pro-Hyp-Gly)(Pro-Hyp-Gly)$_4$-NH_2

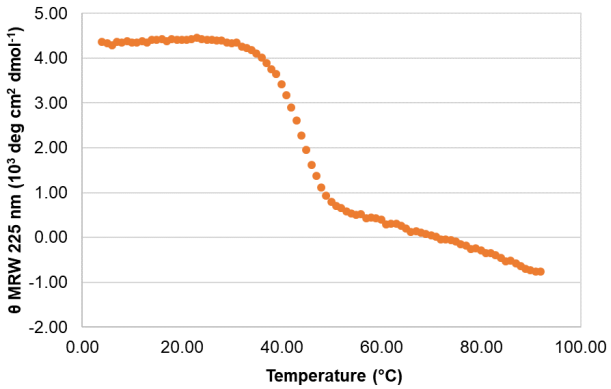


MALDI-TOF MS:

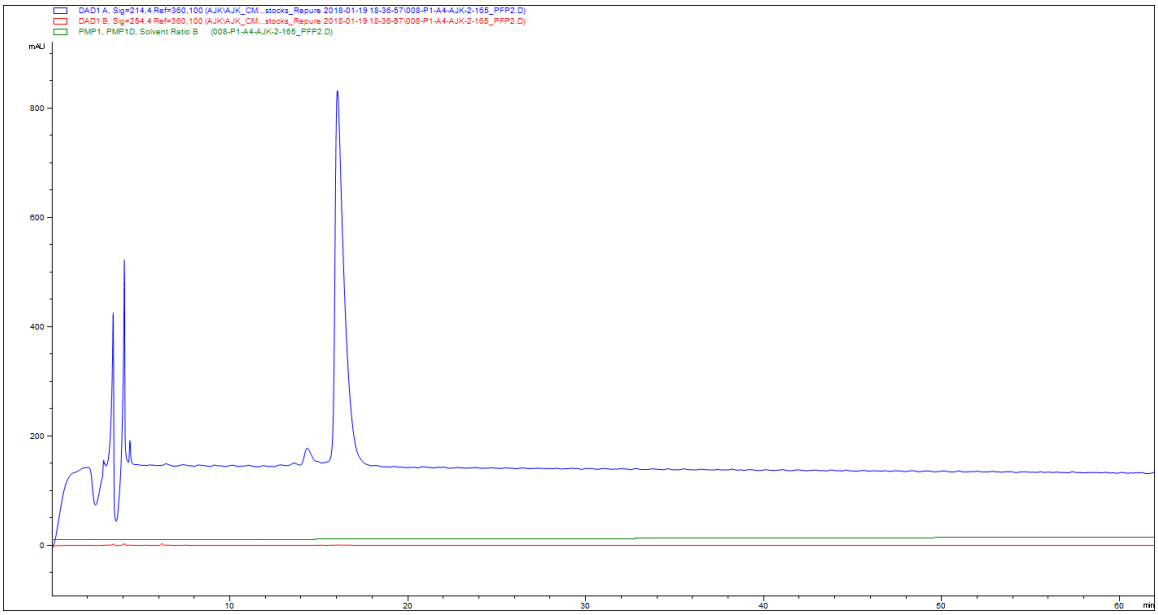
Calculated $[M+Na]^+$: 2178.00

Found: 2176.99

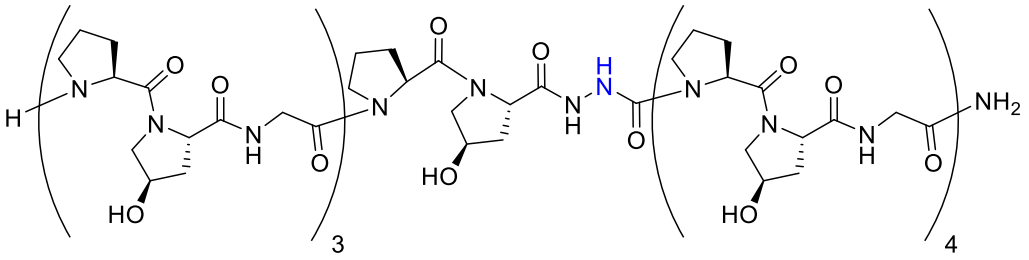
THERMAL DENATURATION CURVE:



HPLC: 10-15% CH_3CN in 0.1% TFA H_2O over 60min at 80 ˚C, PFP(2) column



CMP **1b**: H-(Pro-Hyp-Gly)$_3$(Pro-Hyp-azGly)(Pro-Hyp-Gly)$_4$-NH_2

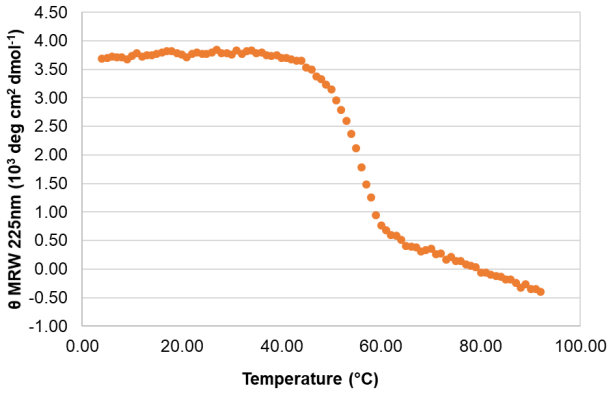


MALDI-TOF MS:

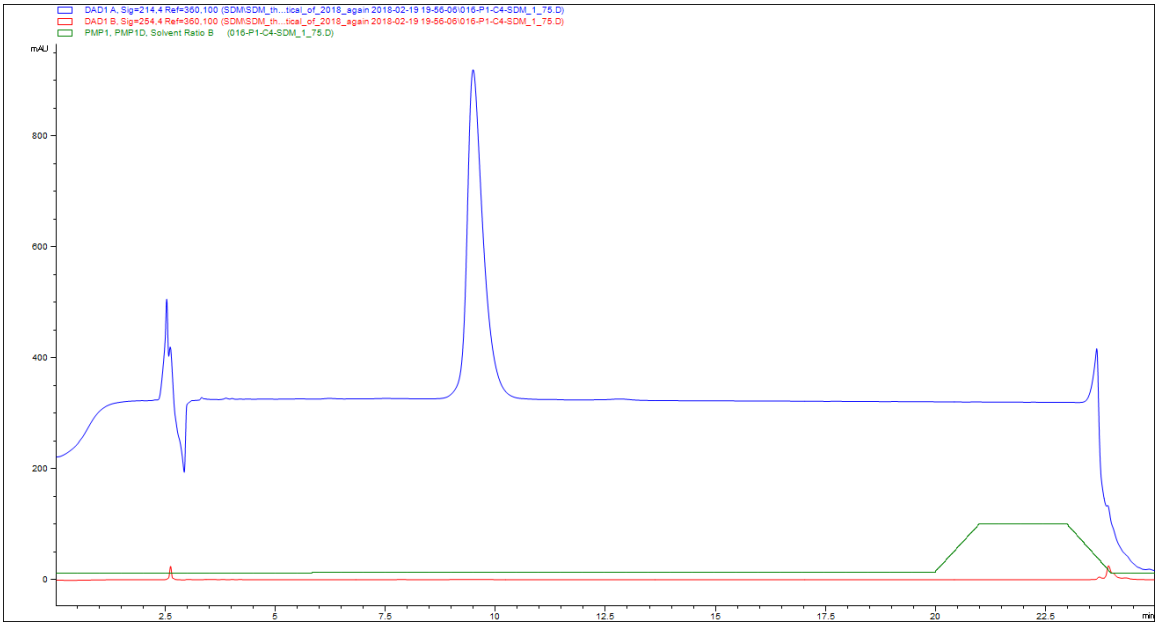
Calculated $[M+Na]^+$: 2178.99

Found: 2179.31

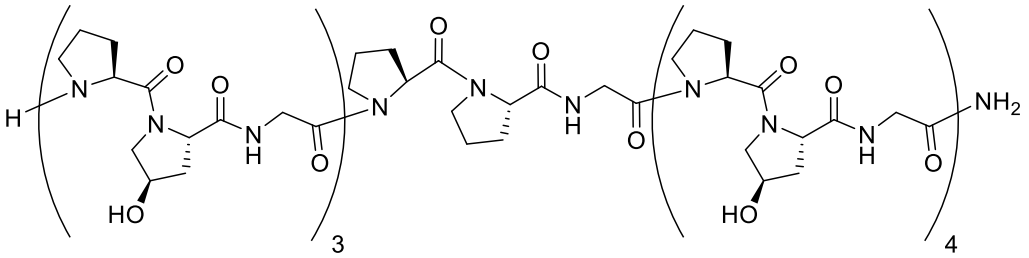
THERMAL DENATURATION CURVE:



HPLC: 12-14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **2a**: H-(Pro-Hyp-Gly)$_3$(Pro-Pro-Gly)(Pro-Hyp-Gly)$_4$-NH_2

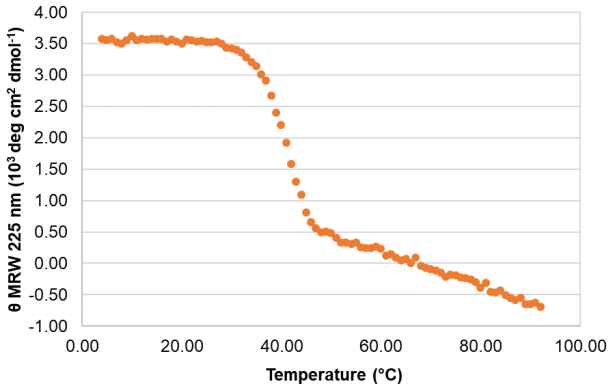


MALDI-TOF MS:

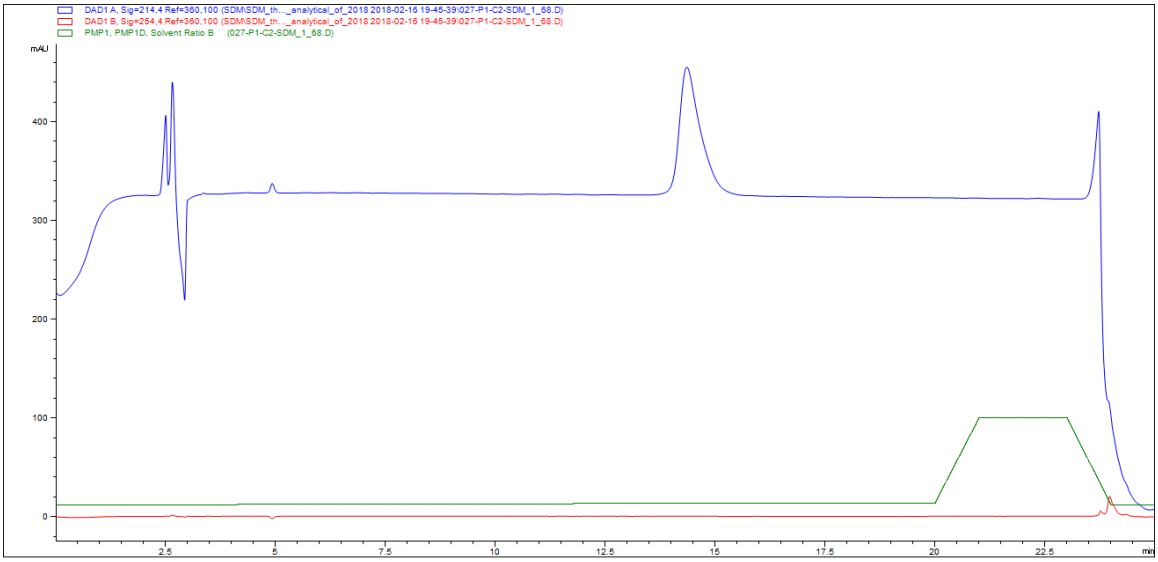
Calculated $[M+Na]^+$: 2162.00

Found: 2162.24

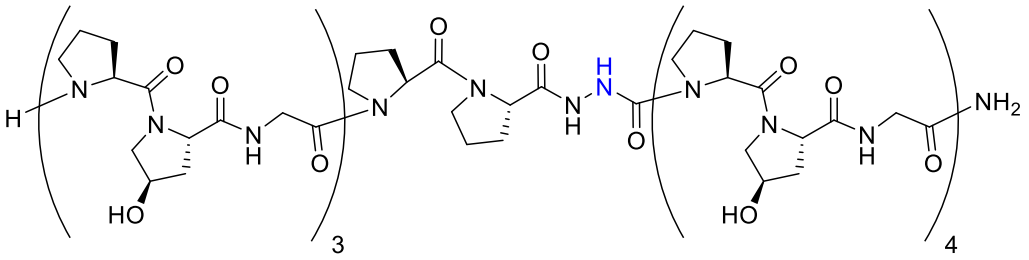
THERMAL DENATURATION CURVE:



HPLC: 12-14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **2b**: H-(Pro-Hyp-Gly)$_3$(Pro-Pro-azGly)(Pro-Hyp-Gly)$_4$-NH_2

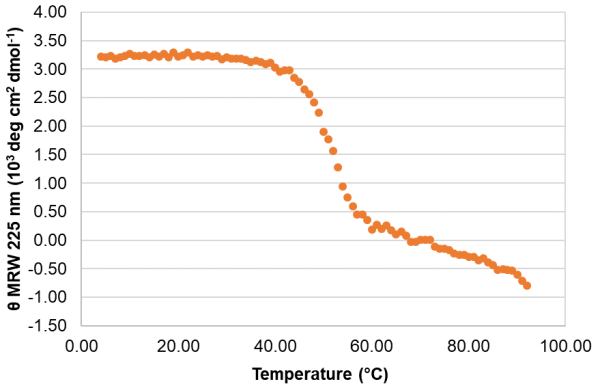


MALDI-TOF MS:

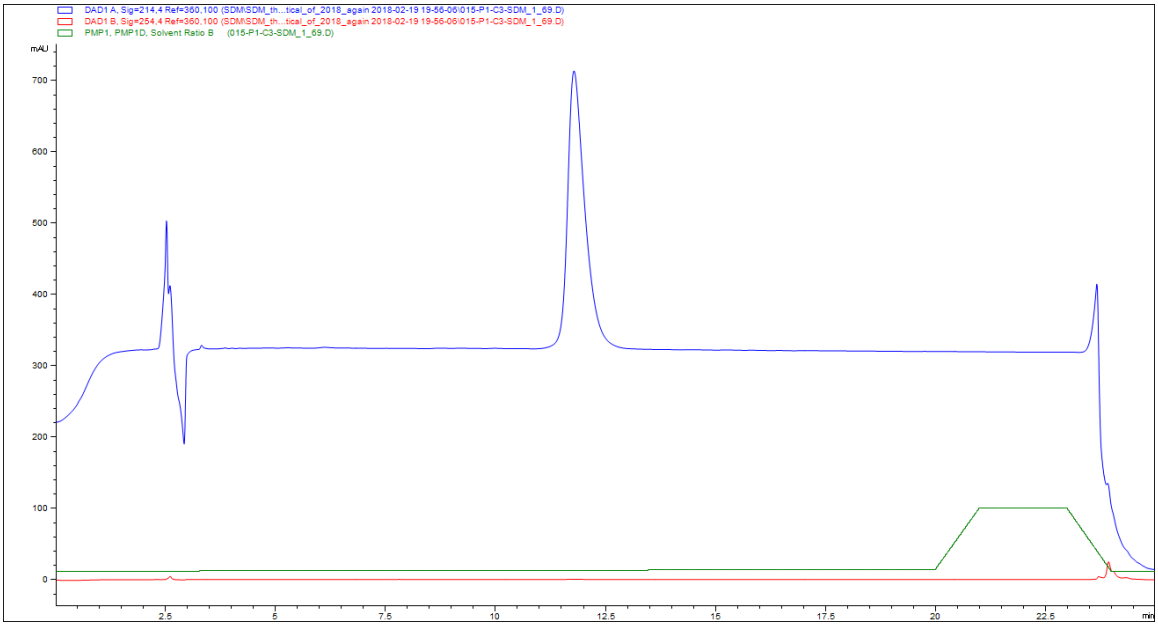
Calculated $[M+Na]^+$: 2163.00

Found: 2163.34

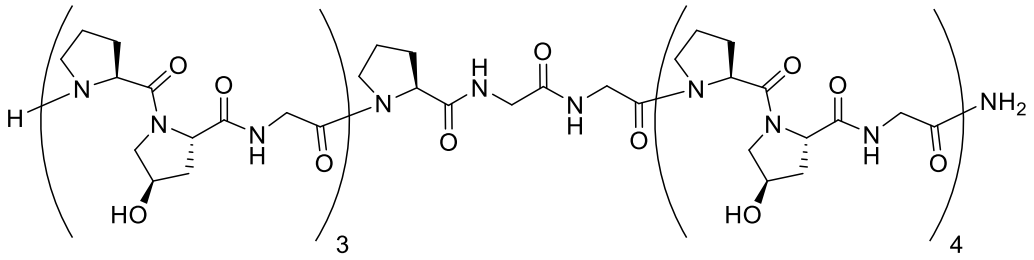
THERMAL DENATURATION CURVE:



HPLC: 12-14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **3a**: H-(Pro-Hyp-Gly)$_3$(Pro-Gly-Gly)(Pro-Hyp-Gly)$_4$-NH_2

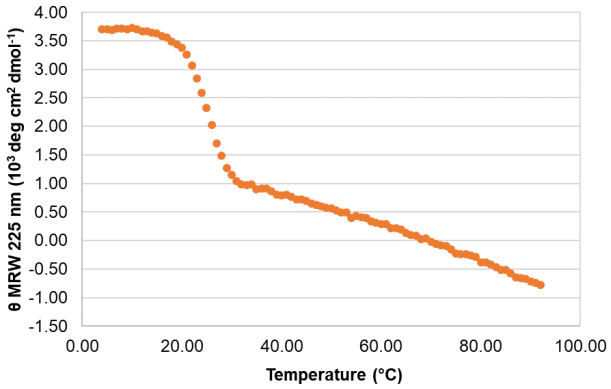


MALDI-TOF MS:

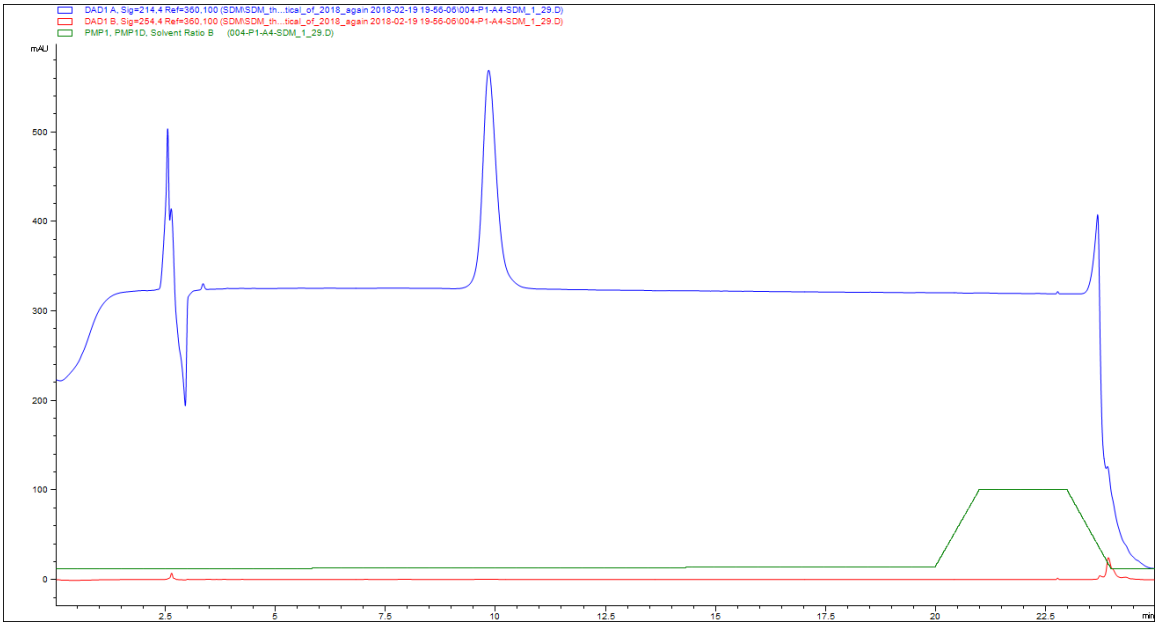
Calculated $[M+Na]^+$: 2121.97

Found: 2122.22

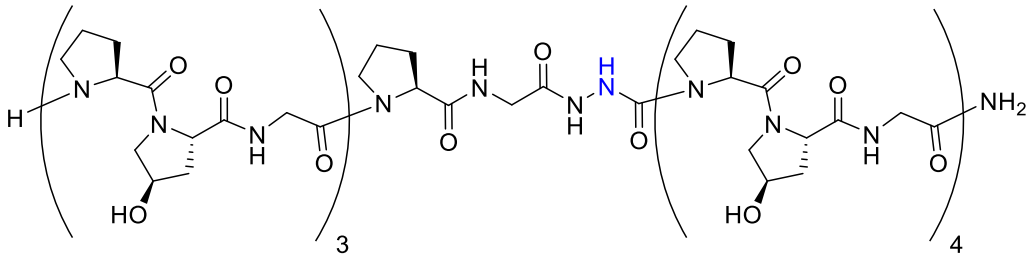
THERMAL DENATURATION CURVE:



HPLC: 12-14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **3b**: H-(Pro-Hyp-Gly)$_3$(Pro-Gly-azGly)(Pro-Hyp-Gly)$_4$-NH_2

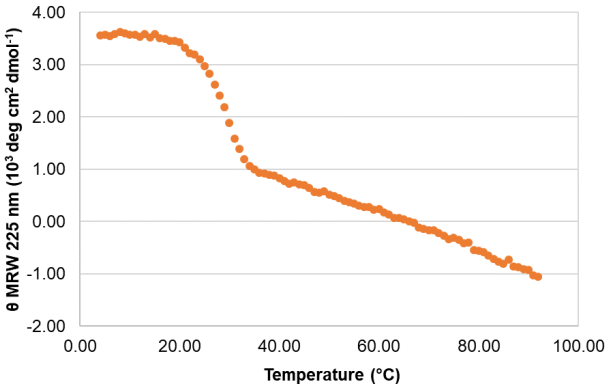


MALDI-TOF MS:

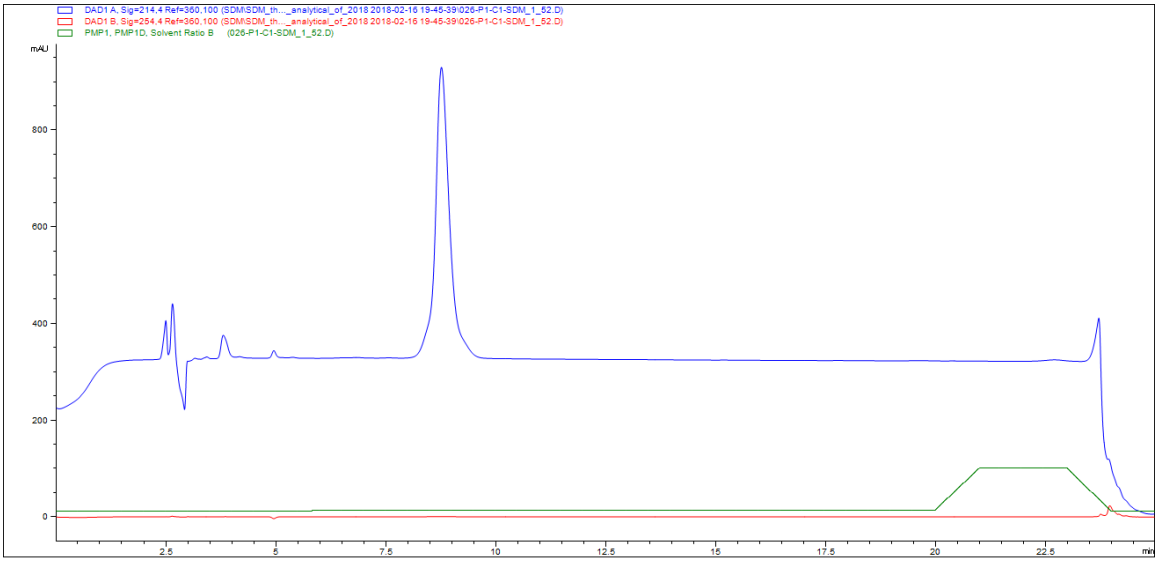
Calculated $[M+Na]^+$: 2122.97

Found: 2123.24

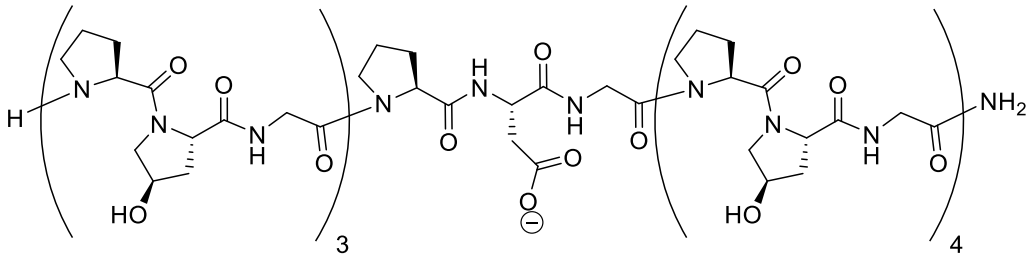
THERMAL DENATURATION CURVE:



HPLC: 12-14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **4a**: H-(Pro-Hyp-Gly)$_3$(Pro-Asp-Gly)(Pro-Hyp-Gly)$_4$-NH_2

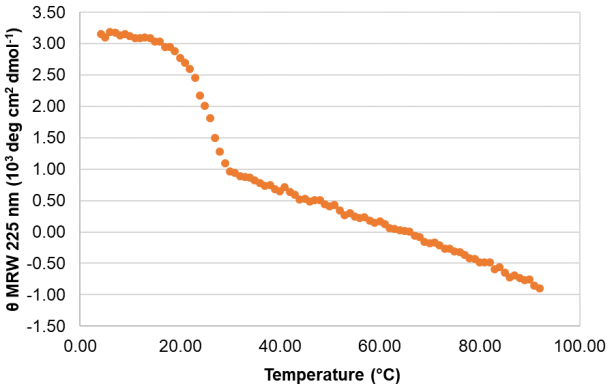


MALDI-TOF MS:

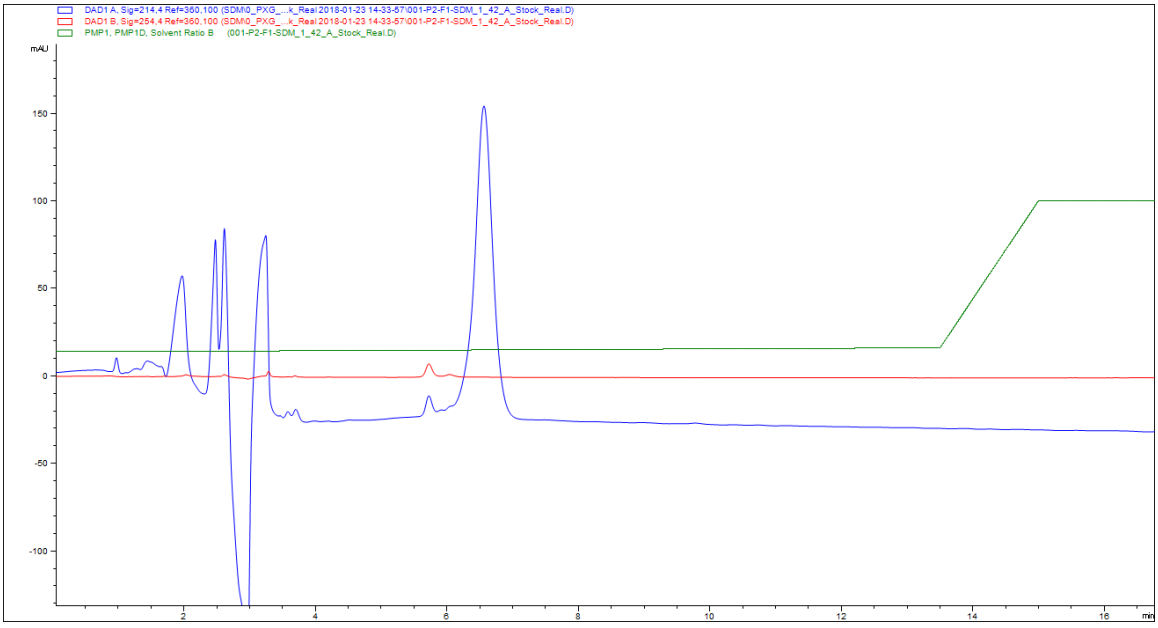
Calculated $[M+Na]^+$: 2179.97

Found: 2180.35

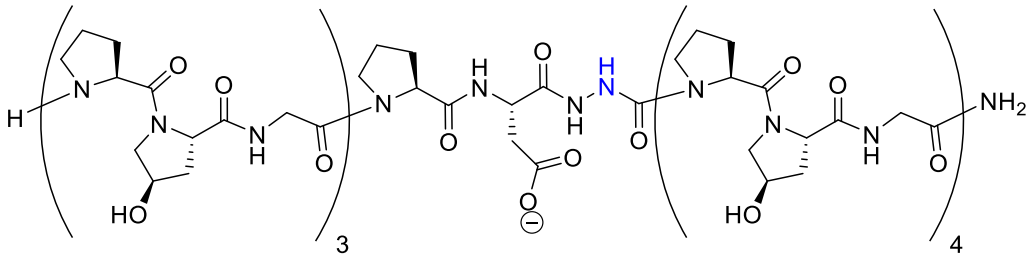
THERMAL DENATURATION CURVE:



HPLC: 14-16.5% CH_3CN in 0.1% TFA H_2O over 13.5min at 80 ˚C



CMP **4b**: H-(Pro-Hyp-Gly)$_3$(Pro-Asp-azGly)(Pro-Hyp-Gly)$_4$-NH_2



MALDI-TOF MS:

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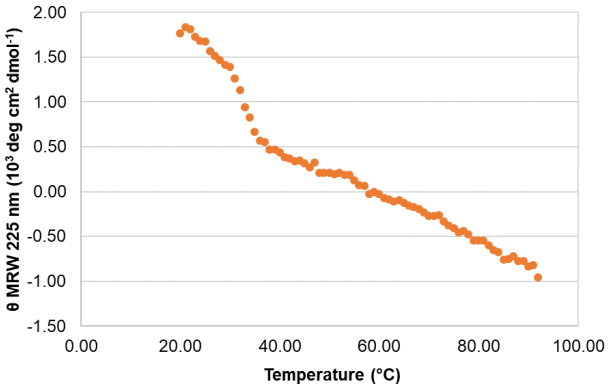
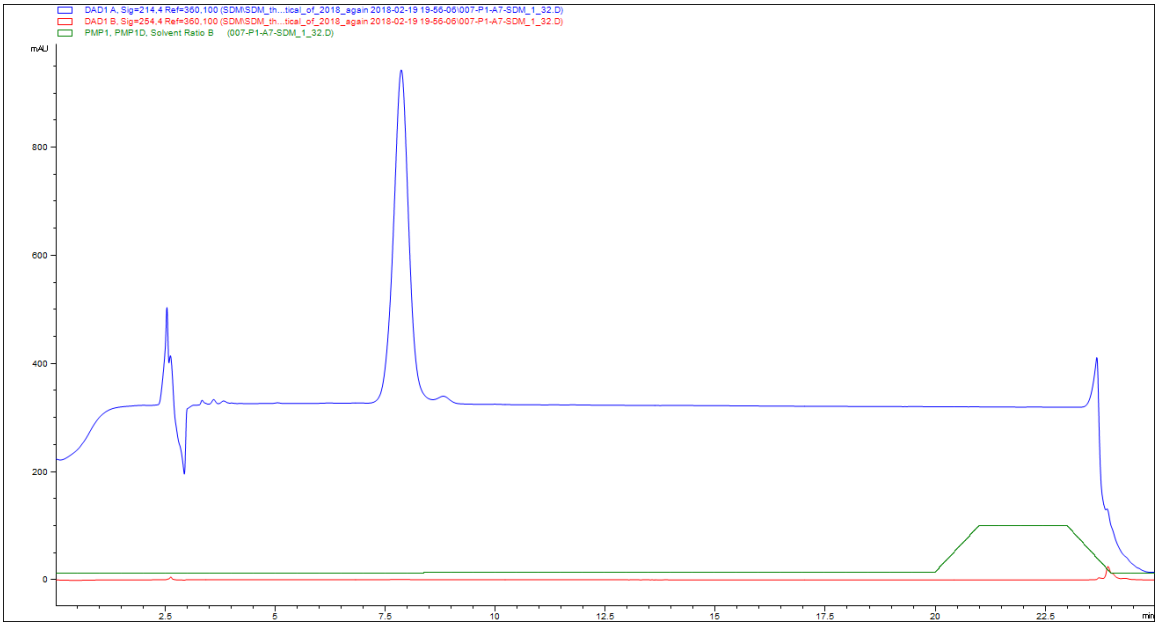
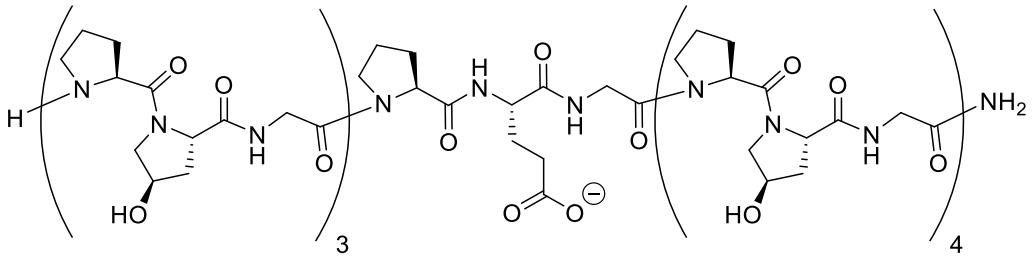
THERMAL DENATURATION CURVE:



HPLC: 12-14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **5a**: H-(Pro-Hyp-Gly)$_3$(Pro-Glu-Gly)(Pro-Hyp-Gly)$_4$-NH_2

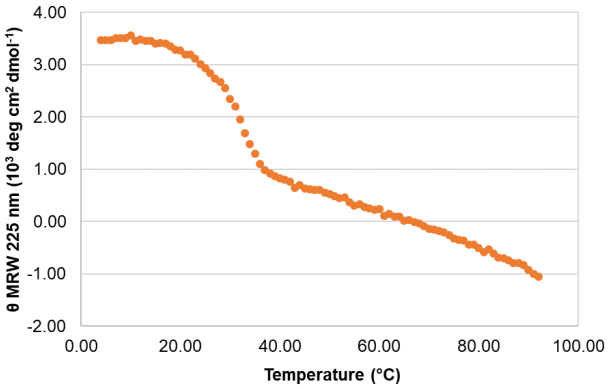


MALDI-TOF MS:

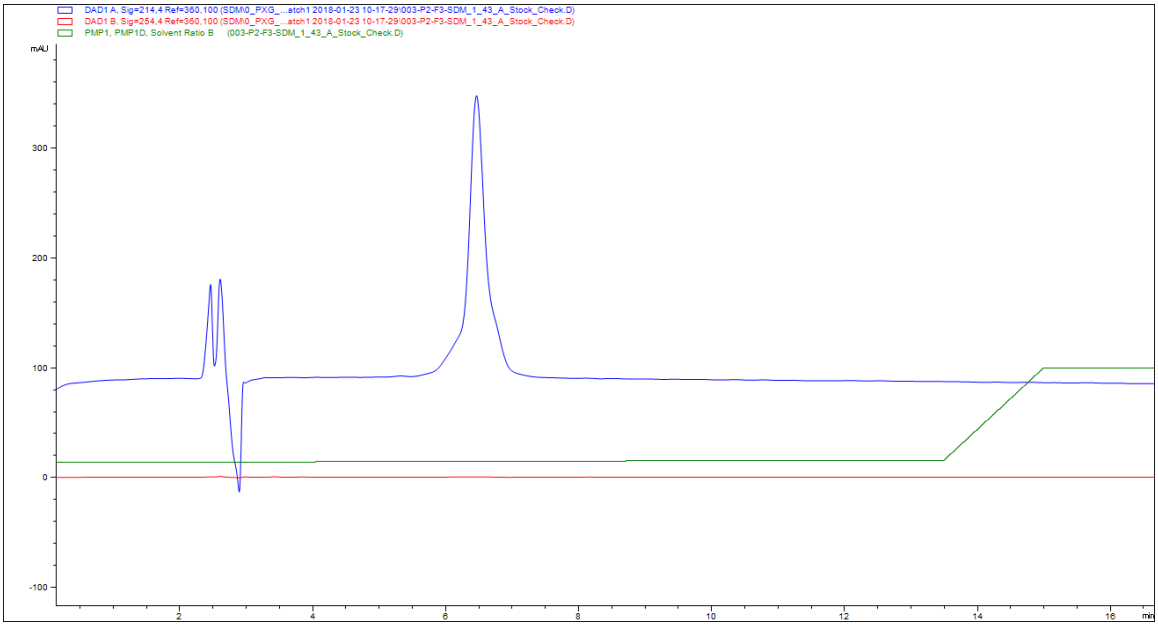
Calculated $[M+Na]^+$: 2193.99

Found: 2193.22

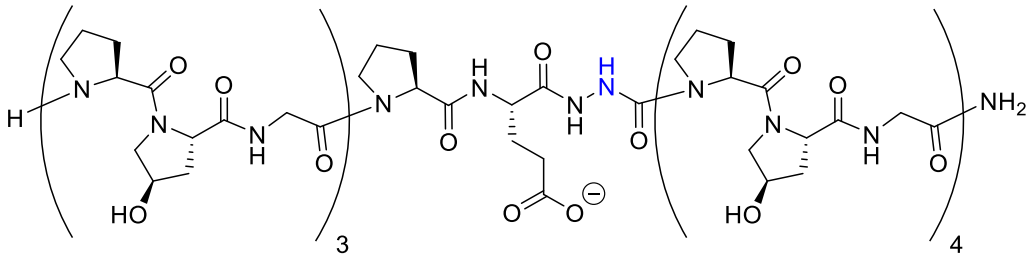
THERMAL DENATURATION CURVE:



HPLC: 14-16.5% CH_3CN in 0.1% TFA H_2O over 13.5min at 80 ˚C



CMP **5b**: H-(Pro-Hyp-Gly)$_3$(Pro-Glu-azGly)(Pro-Hyp-Gly)$_4$-NH_2

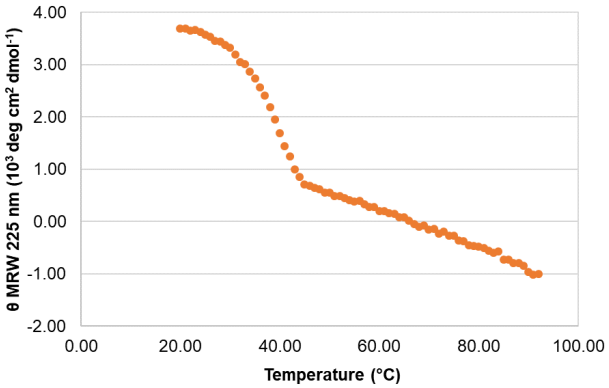


MALDI-TOF MS:

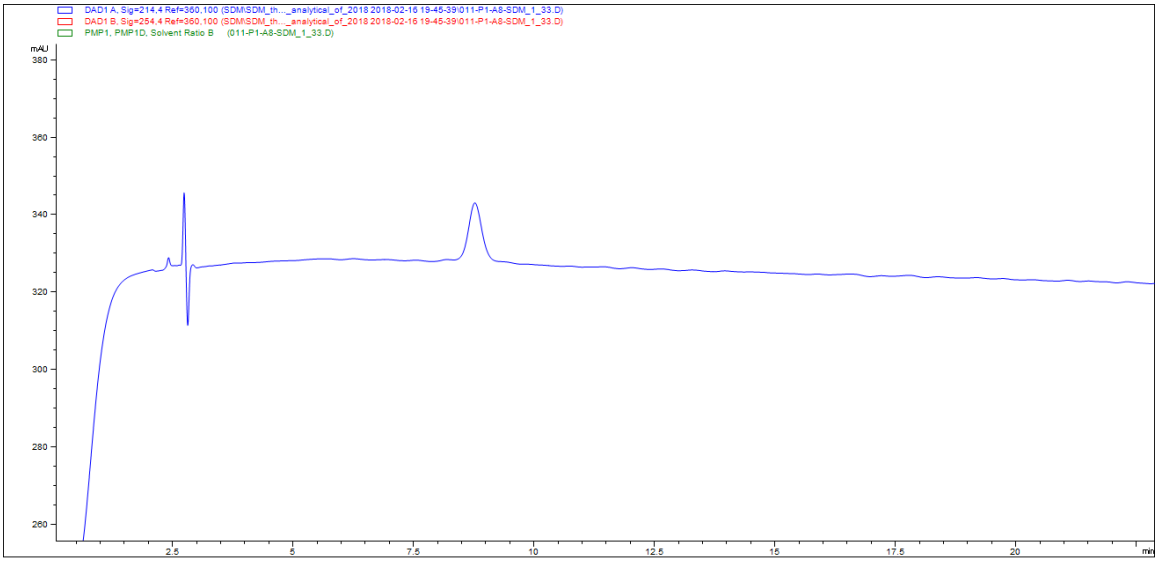
Calculated $[M+Na]^+$: 2194.99

Found: 2194.80

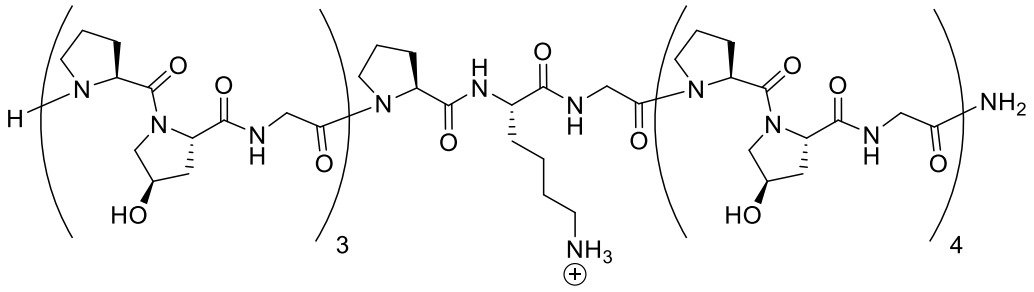
THERMAL DENATURATION CURVE:



HPLC: 12-14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **6a**: H-(Pro-Hyp-Gly)$_3$(Pro-Lys-Gly)(Pro-Hyp-Gly)$_4$-NH_2

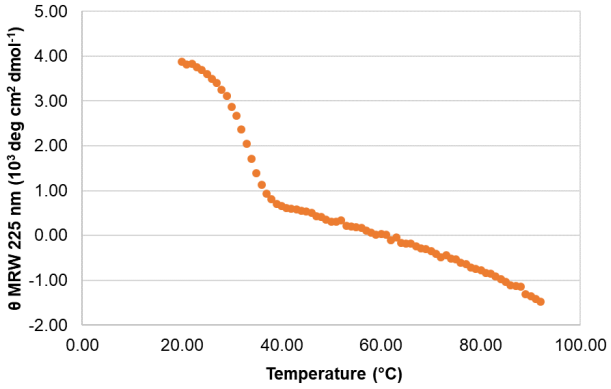


MALDI-TOF MS:

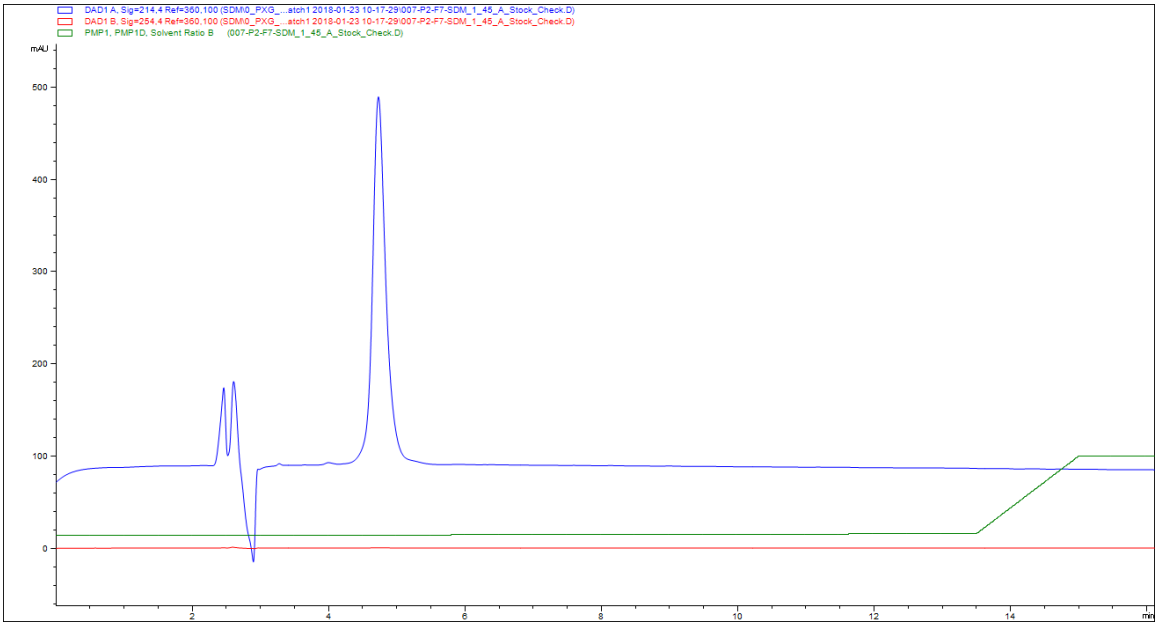
Calculated $[M+Na]^+$: 2193.04

Found: 2192.96

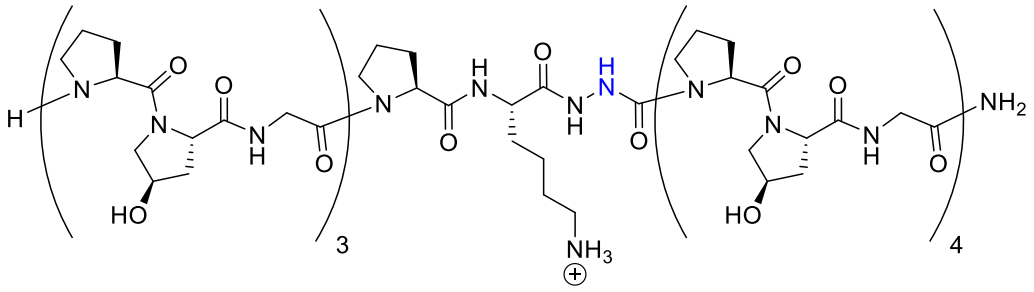
THERMAL DENATURATION CURVE:



HPLC: 14-16.5% CH_3CN in 0.1% TFA H_2O over 13.5min at 80 ˚C



CMP **6b**: H-(Pro-Hyp-Gly)$_3$(Pro-Lys-azGly)(Pro-Hyp-Gly)$_4$-NH_2

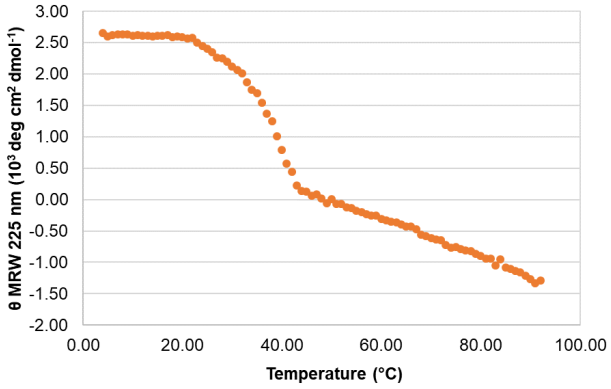


MALDI-TOF MS:

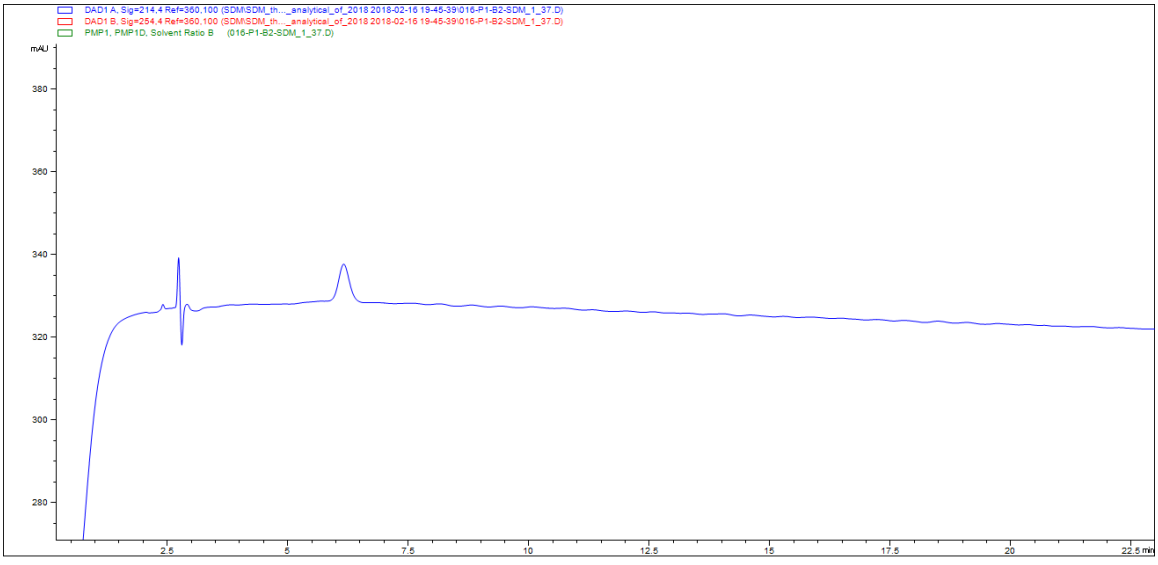
Calculated $[M+Na]^+$: 2194.04

Found: 2194.02

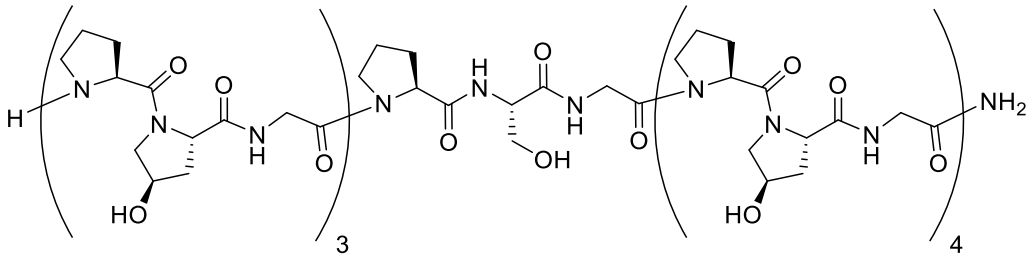
THERMAL DENATURATION CURVE:



HPLC: 12-14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **8a**: H-(Pro-Hyp-Gly)$_3$(Pro-Ser-Gly)(Pro-Hyp-Gly)$_4$-NH_2

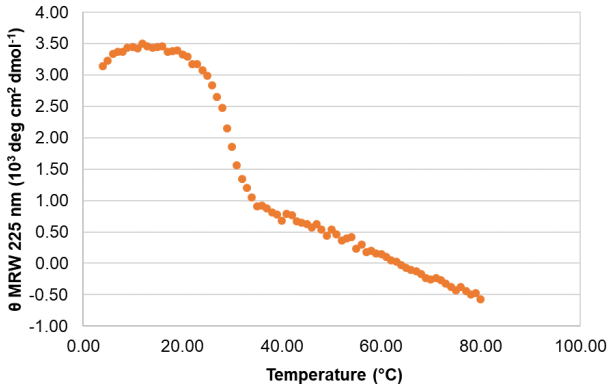


MALDI-TOF MS:

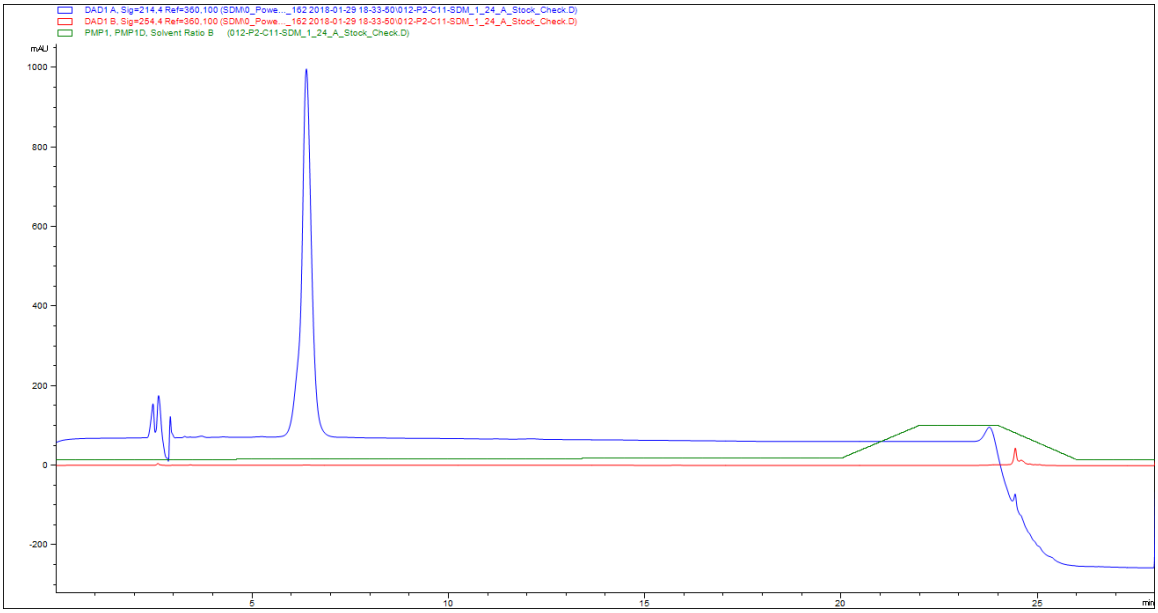
Calculated $[M+Na]^+$: 2151.98

Found: 2152.13

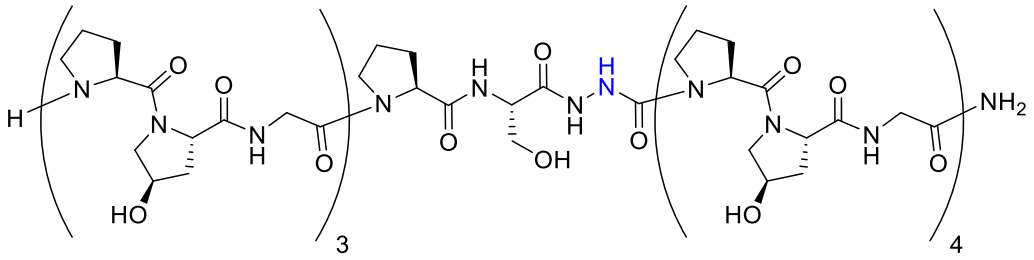
THERMAL DENATURATION CURVE:



HPLC: 14-17% CH_3CN in 0.1% TFA H_2O over 28min at 80 ˚C



CMP **8b**: H-(Pro-Hyp-Gly)$_3$(Pro-Ser-azGly)(Pro-Hyp-Gly)$_4$-NH_2

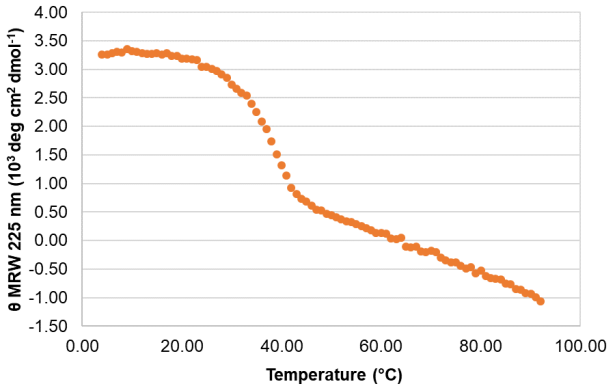


MALDI-TOF MS:

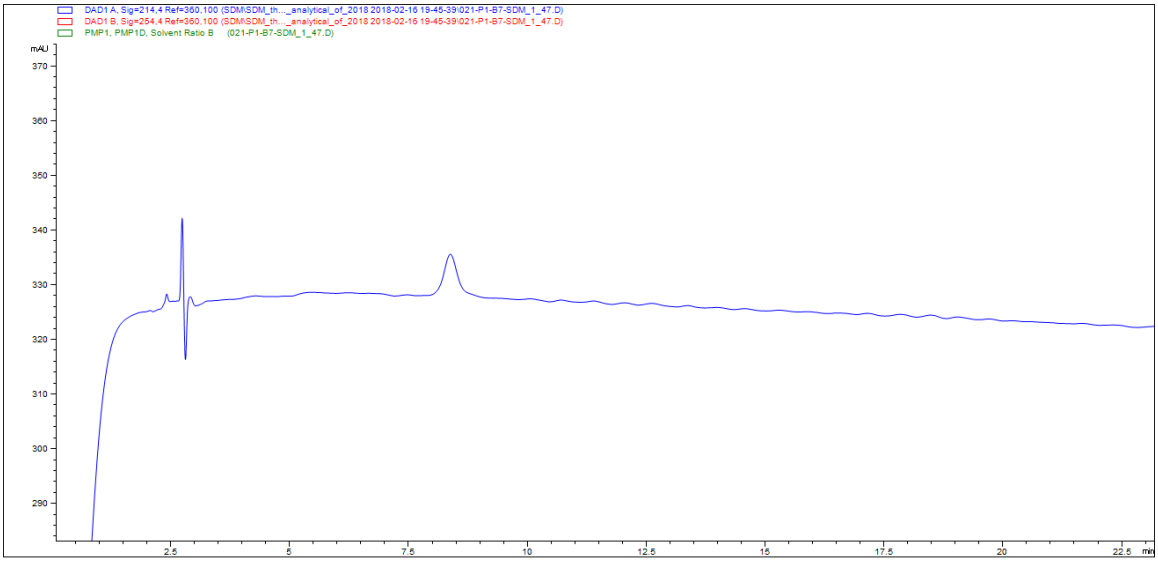
Calculated $[M+Na]^+$: 2152.98

Found: 2153.19

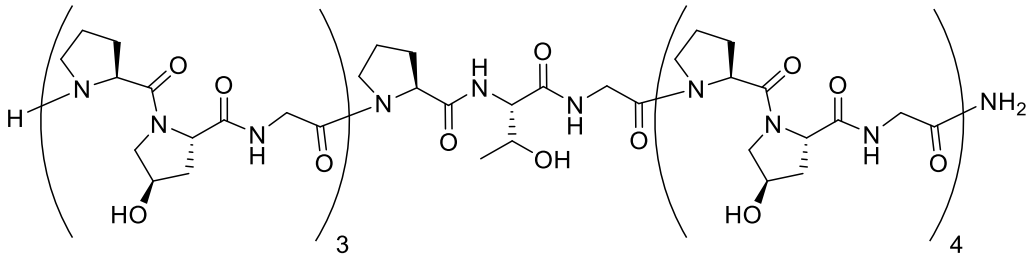
THERMAL DENATURATION CURVE:



HPLC: 12-14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **9a**: H-(Pro-Hyp-Gly)$_3$(Pro-Thr-Gly)(Pro-Hyp-Gly)$_4$-NH_2

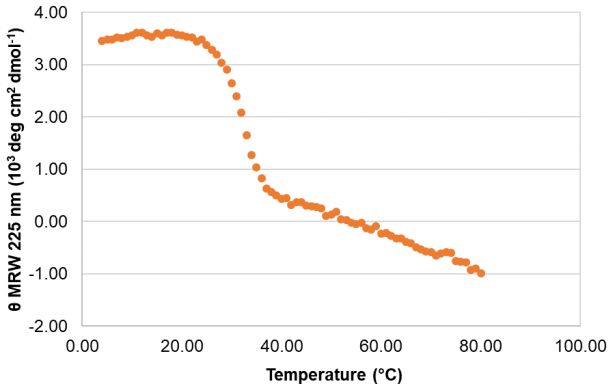


MALDI-TOF MS:

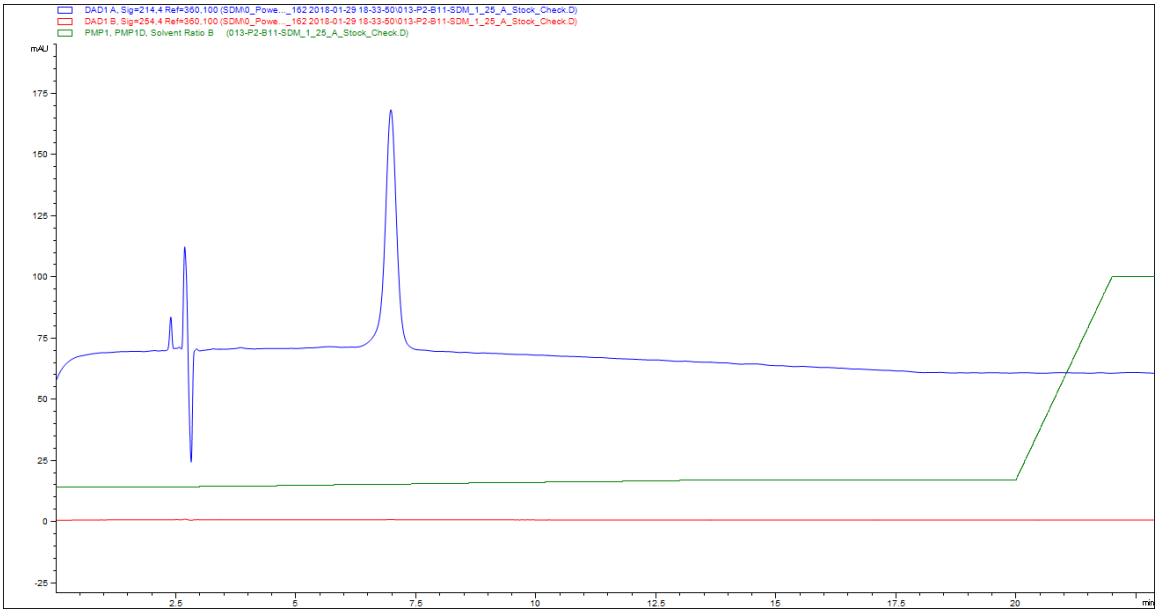
Calculated $[M+Na]^+$: 2166.00

Found: 2166.94

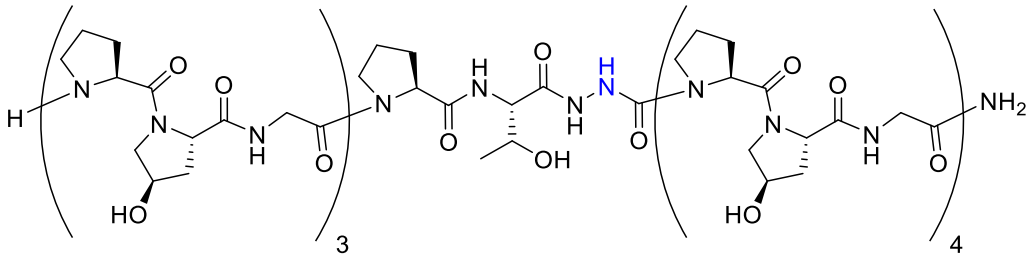
THERMAL DENATURATION CURVE:



HPLC: 14-17% CH_3CN in 0.1% TFA H_2O over 28min at 80 ˚C



CMP **9b**: H-(Pro-Hyp-Gly)$_3$(Pro-Thr-azGly)(Pro-Hyp-Gly)$_4$-NH_2

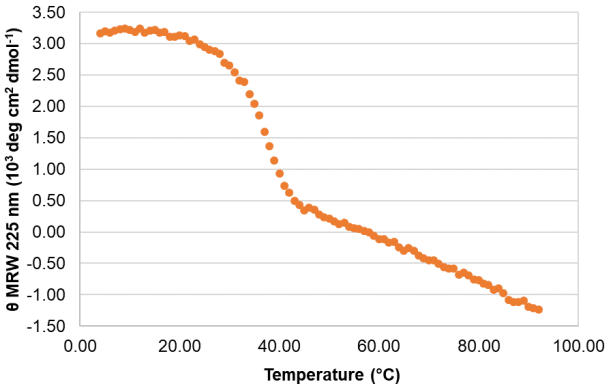


MALDI-TOF MS:

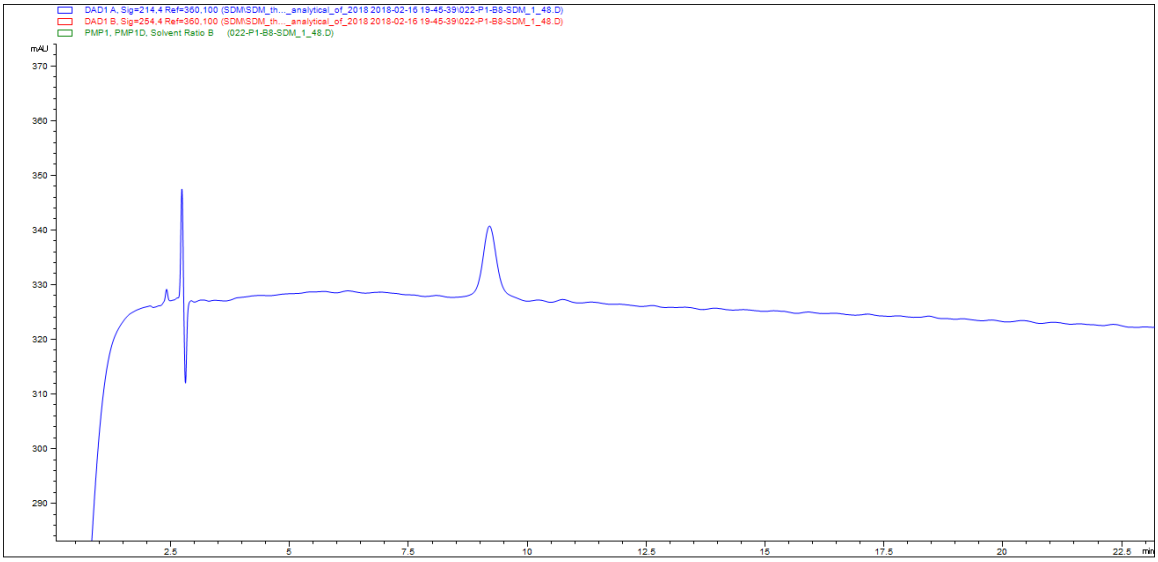
Calculated $[M+Na]^+$: 2166.99

Found: 2167.08

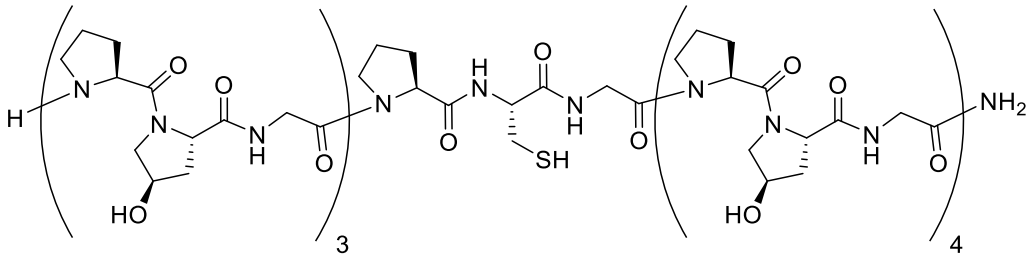
THERMAL DENATURATION CURVE:



HPLC: 12-14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **10a**: H-(Pro-Hyp-Gly)$_3$(Pro-Cys-Gly)(Pro-Hyp-Gly)$_4$-NH_2

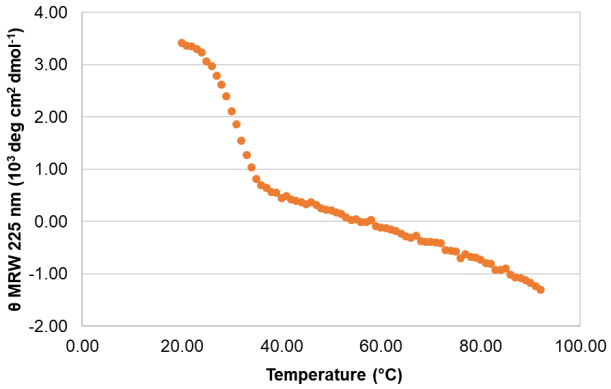


MALDI-TOF MS:

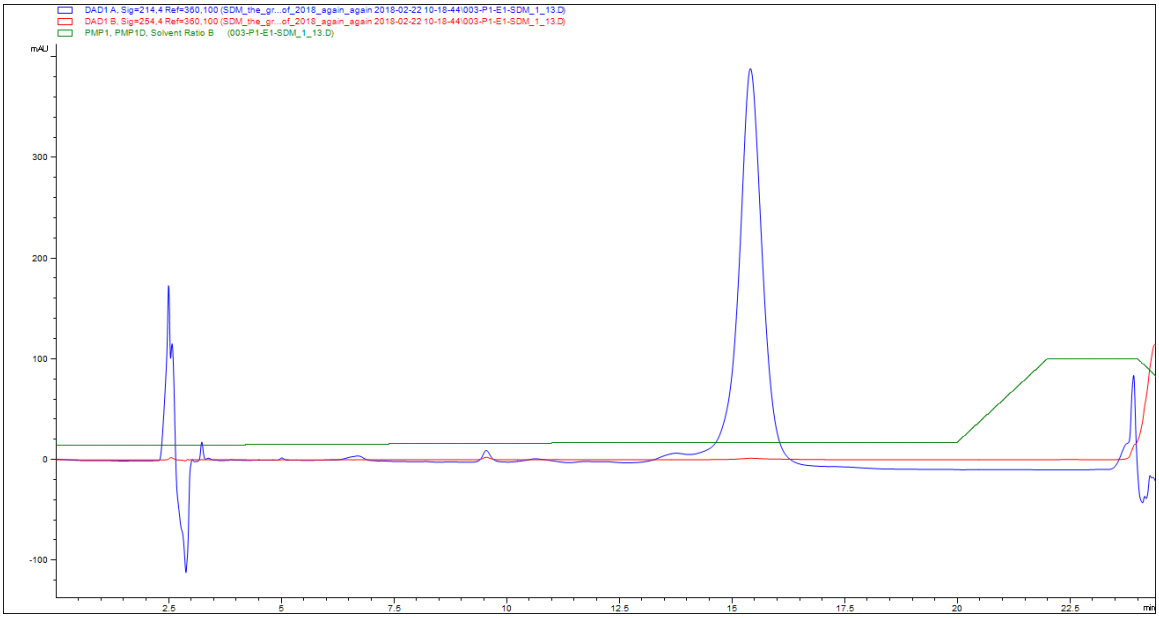
Calculated $[M+Na]^+$: 2167.96

Found: 2167.71

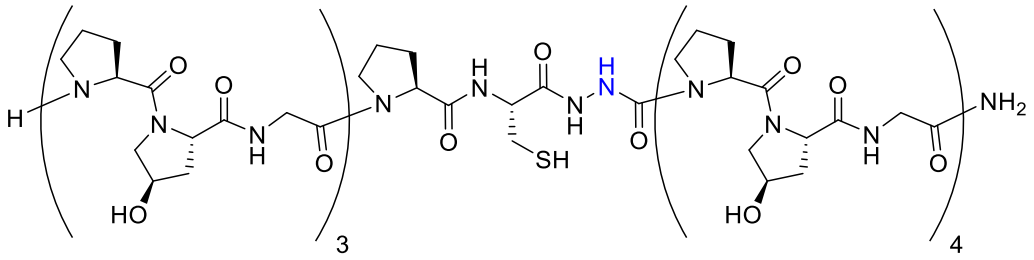
THERMAL DENATURATION CURVE:



HPLC: 14-17% CH_3CN in 0.1% TFA H_2O over 28min at 80 ˚C



CMP **10b**: H-(Pro-Hyp-Gly)$_3$(Pro-Cys-azGly)(Pro-Hyp-Gly)$_4$-NH_2

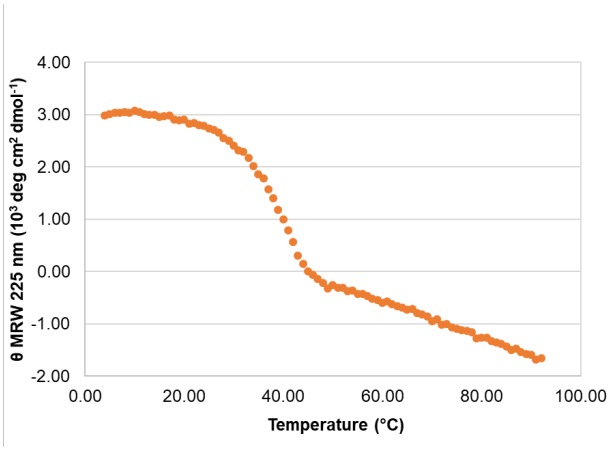


MALDI-TOF MS:

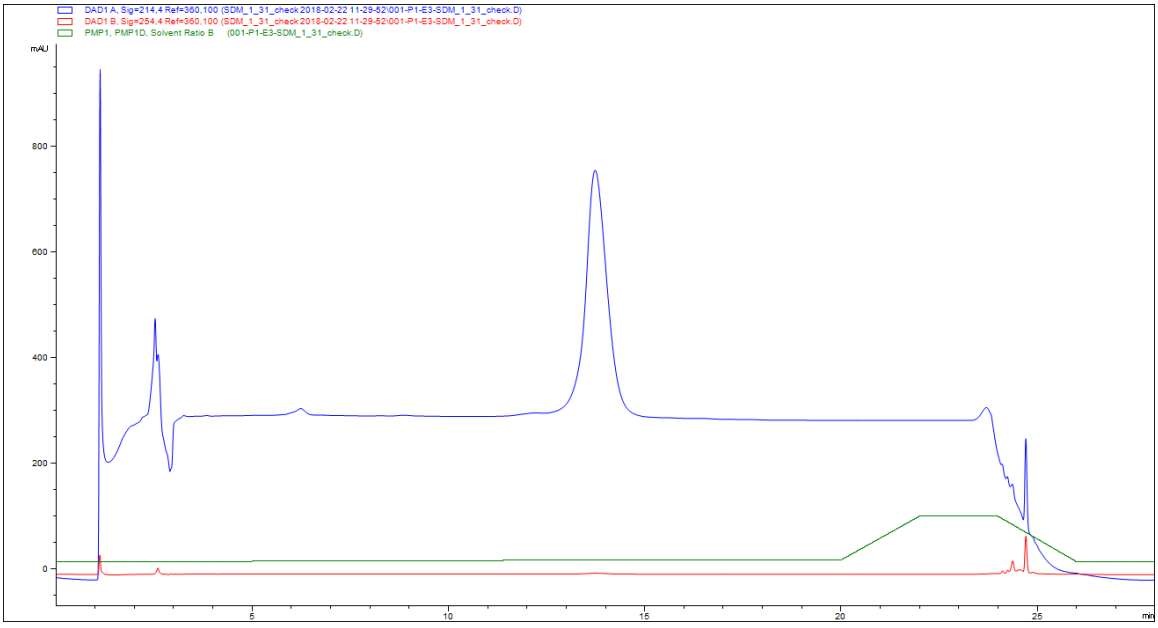
Calculated $[M+Na]^+$: 2168.95

Found: 2166.91

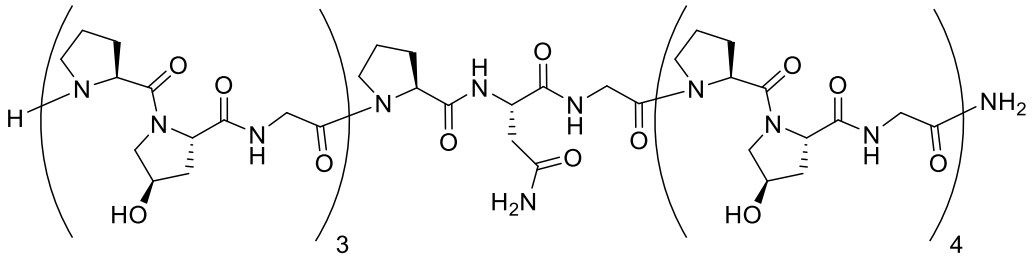
THERMAL DENATURATION CURVE:



HPLC: 14-17% CH_3CN in 0.1% TFA H_2O over 28min at 80 ˚C



CMP **11a**: H-(Pro-Hyp-Gly)$_3$(Pro-Asn-Gly)(Pro-Hyp-Gly)$_4$-NH_2

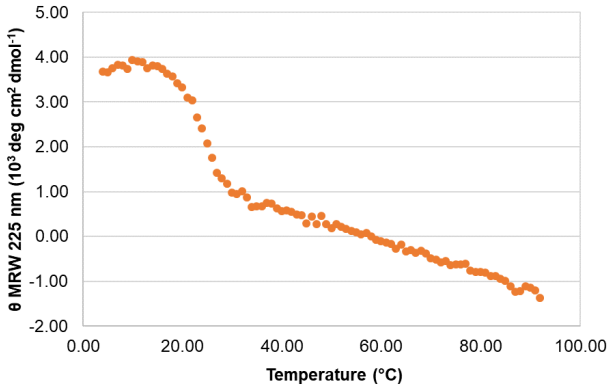


MALDI-TOF MS:

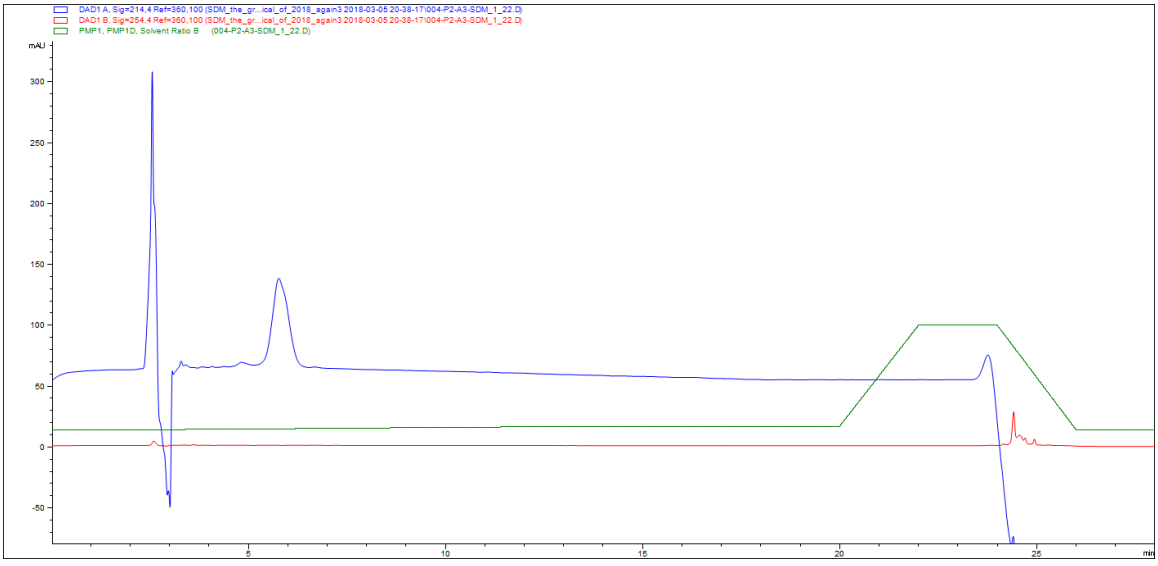
Calculated $[M+Na]^+$: 2178.99

Found: 2179.32

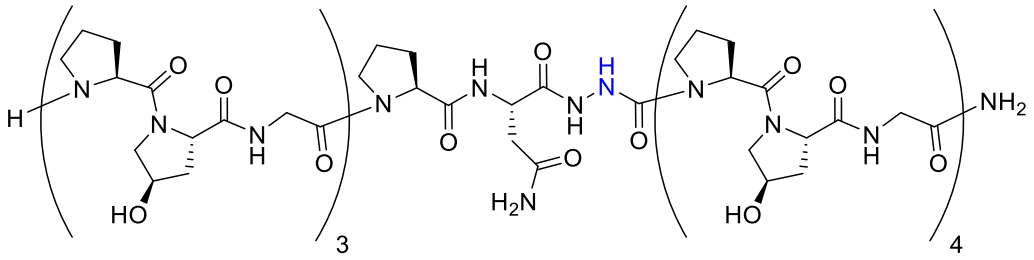
THERMAL DENATURATION CURVE:



HPLC: 14-17% CH_3CN in 0.1% TFA H_2O over 28min at 80 ˚C



CMP **11b**: H-(Pro-Hyp-Gly)$_3$(Pro-Asn-azGly)(Pro-Hyp-Gly)$_4$-NH_2

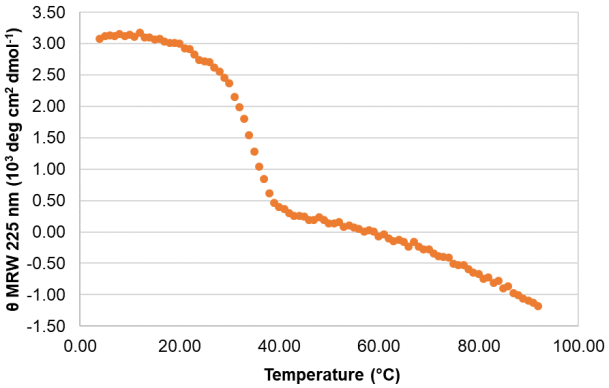


MALDI-TOF MS:

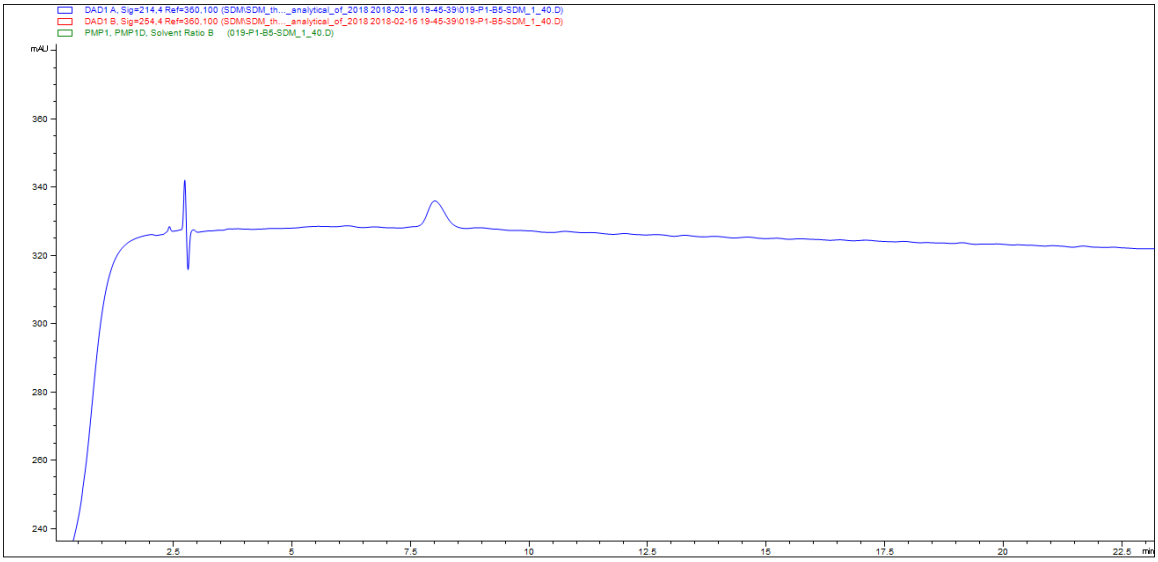
Calculated $[M+Na]^+$: 2179.99

Found: 2181.67

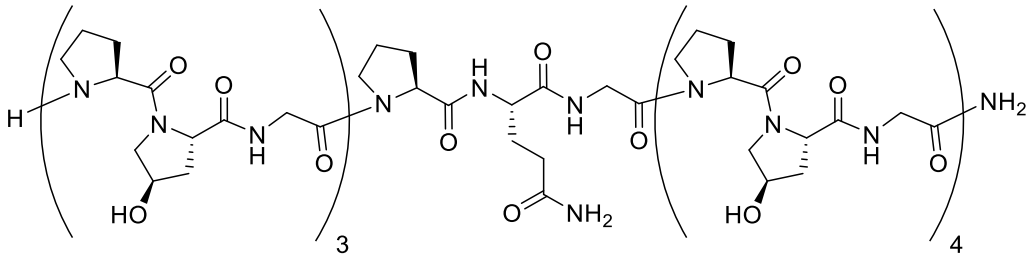
THERMAL DENATURATION CURVE:



HPLC: 12-14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **12a**: H-(Pro-Hyp-Gly)$_3$(Pro-Gln-Gly)(Pro-Hyp-Gly)$_4$-NH_2

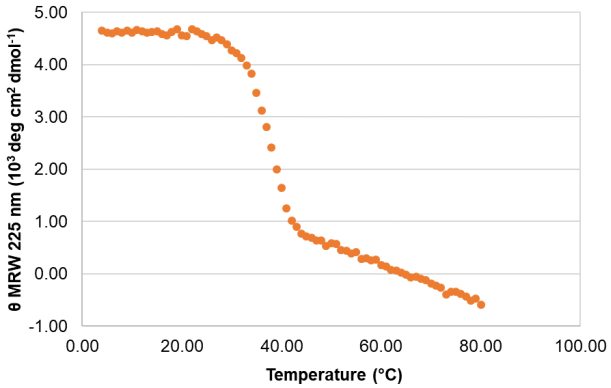


MALDI-TOF MS:

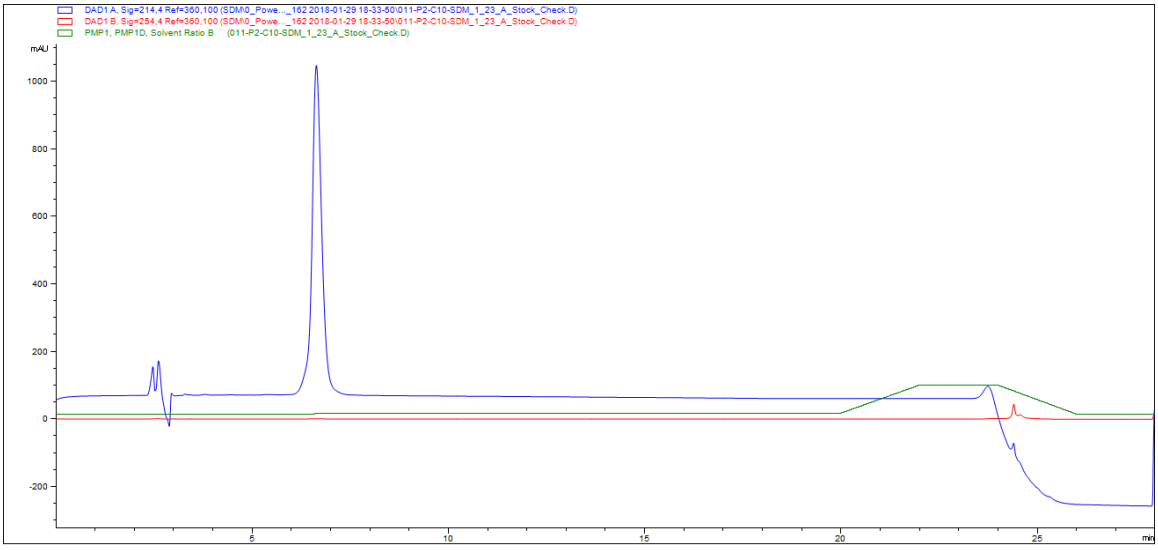
Calculated $[M+Na]^+$: 2193.01

Found: 2193.03

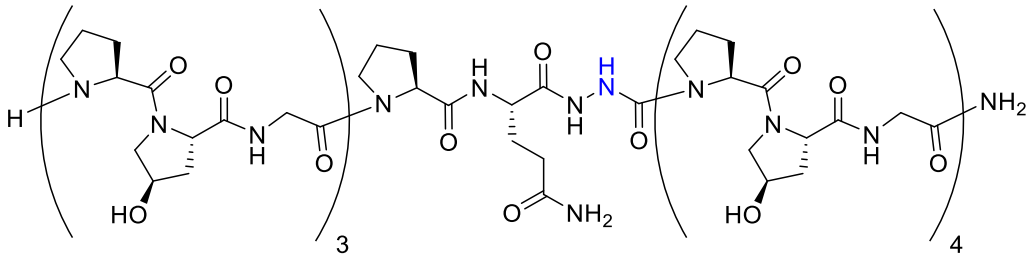
THERMAL DENATURATION CURVE:



HPLC: 14-17% CH_3CN in 0.1% TFA H_2O over 28min at 80 ˚C



CMP **12b**: H-(Pro-Hyp-Gly)$_3$(Pro-Gln-azGly)(Pro-Hyp-Gly)$_4$-NH_2

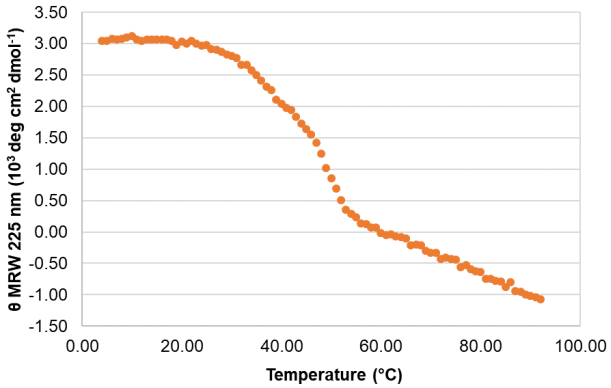


MALDI-TOF MS:

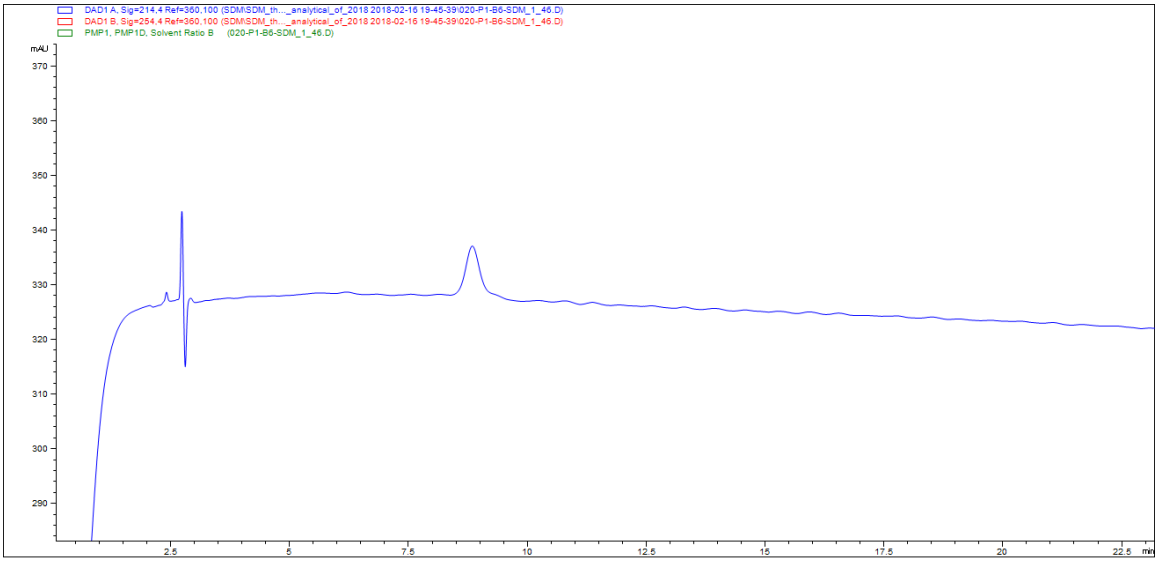
Calculated $[M+Na]^+$: 2194.00

Found: 2194.29

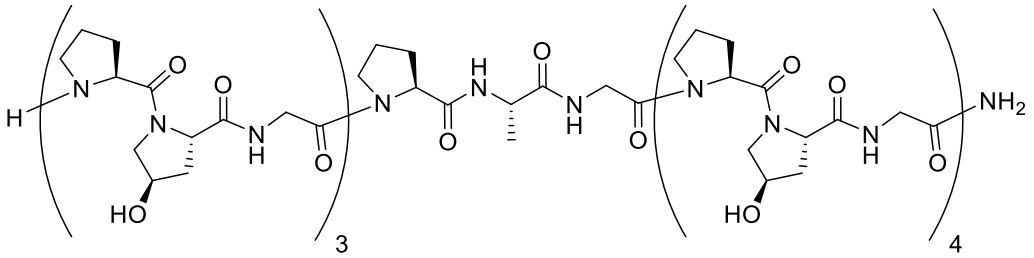
THERMAL DENATURATION CURVE:



HPLC: 12-14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **13a**: H-(Pro-Hyp-Gly)$_3$(Pro-Ala-Gly)(Pro-Hyp-Gly)$_4$-NH_2

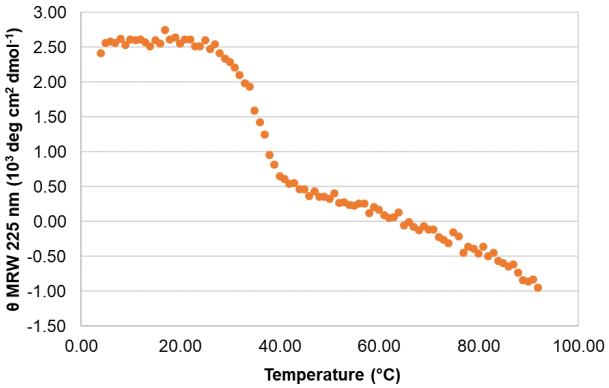


MALDI-TOF MS:

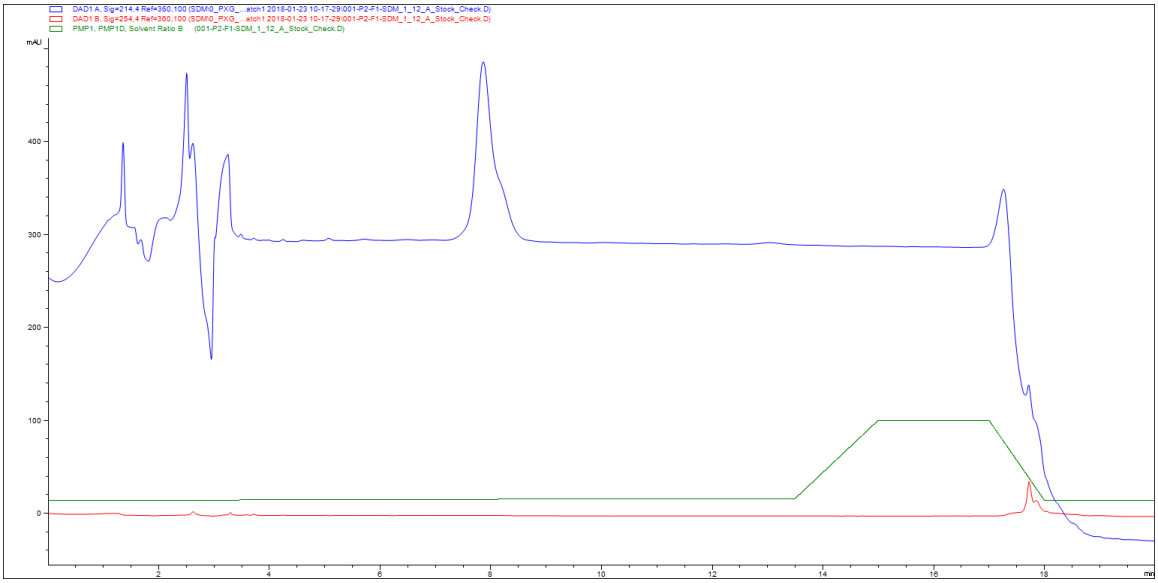
Calculated $[M+Na]^+$: 2135.98

Found: 2136.20

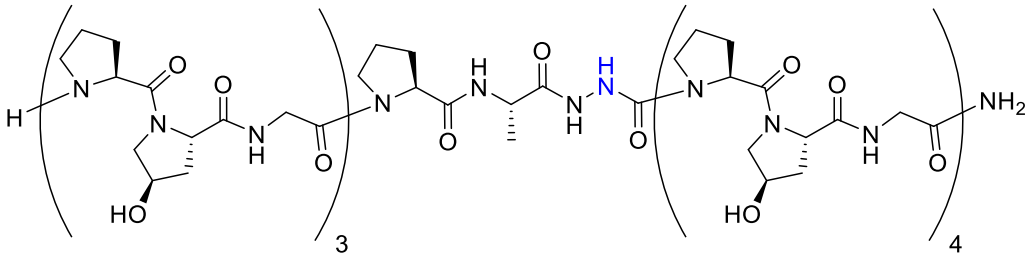
THERMAL DENATURATION CURVE:



HPLC: 14-17% CH_3CN in 0.1% TFA H_2O over 28min at 80 ˚C



CMP **13b**: H-(Pro-Hyp-Gly)$_3$(Pro-Ala-azGly)(Pro-Hyp-Gly)$_4$-NH_2

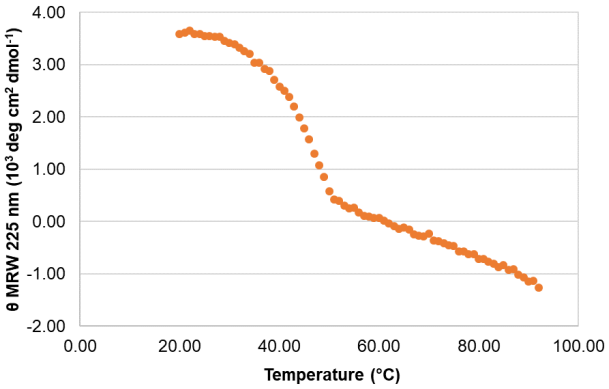


MALDI-TOF MS:

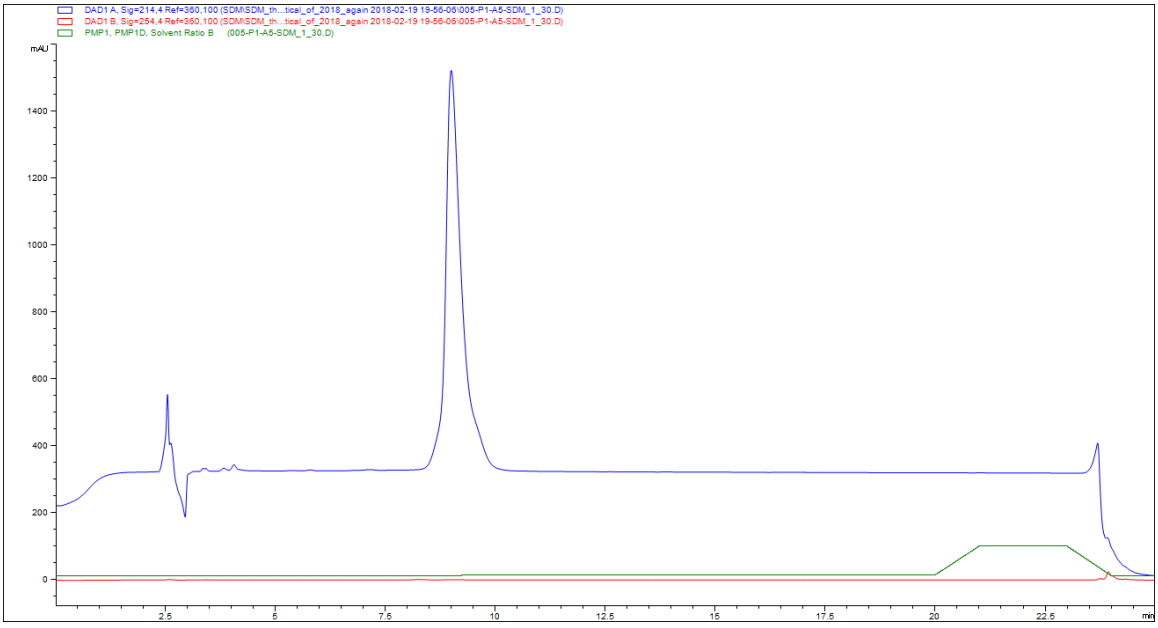
Calculated $[M+Na]^+$: 2136.98

Found: 2136.80

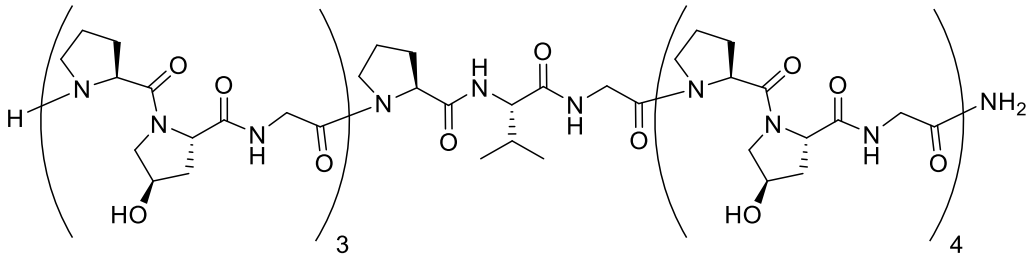
THERMAL DENATURATION CURVE:



HPLC: 12-14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **14a**: H-(Pro-Hyp-Gly)$_3$(Pro-Val-Gly)(Pro-Hyp-Gly)$_4$-NH_2

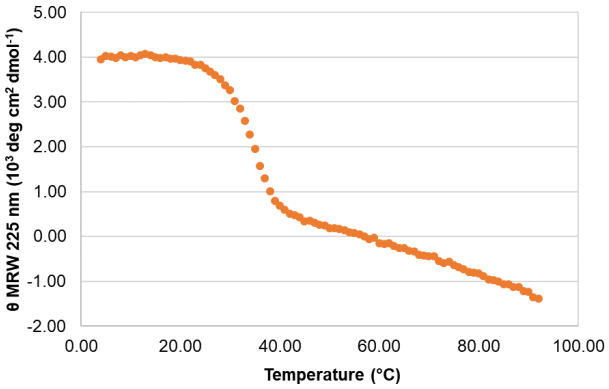


MALDI-TOF MS:

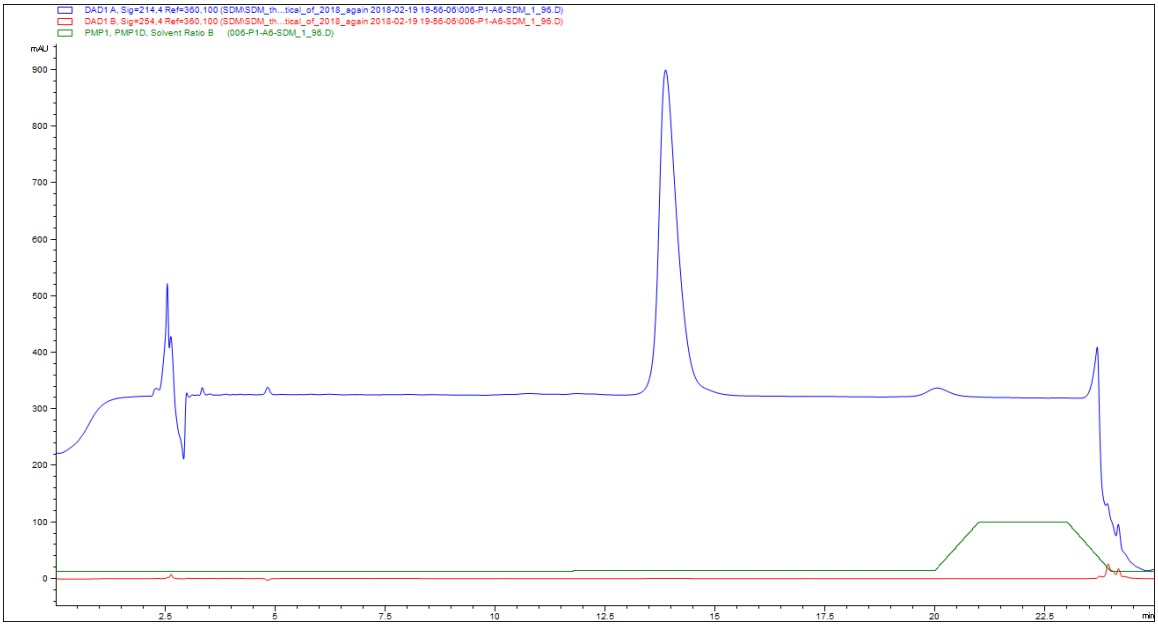
Calculated $[M+Na]^+$: 2164.02

Found: 2164.22

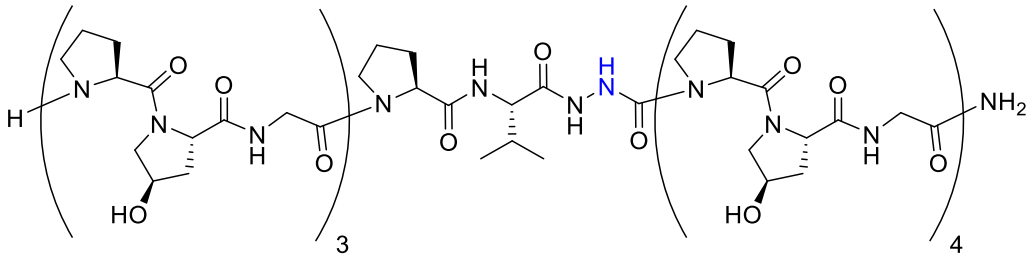
THERMAL DENATURATION CURVE:



HPLC: 12-14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **14b**: H-(Pro-Hyp-Gly)$_3$(Pro-Val-azGly)(Pro-Hyp-Gly)$_4$-NH_2

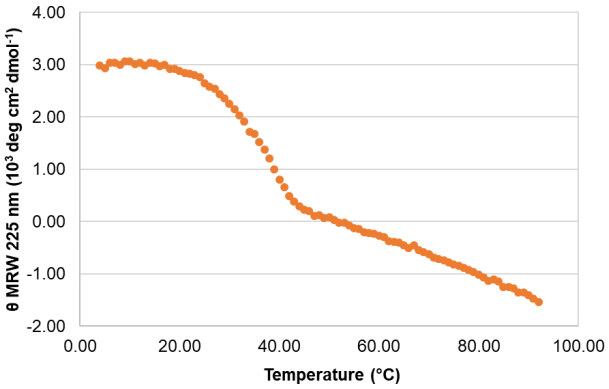


MALDI-TOF MS:

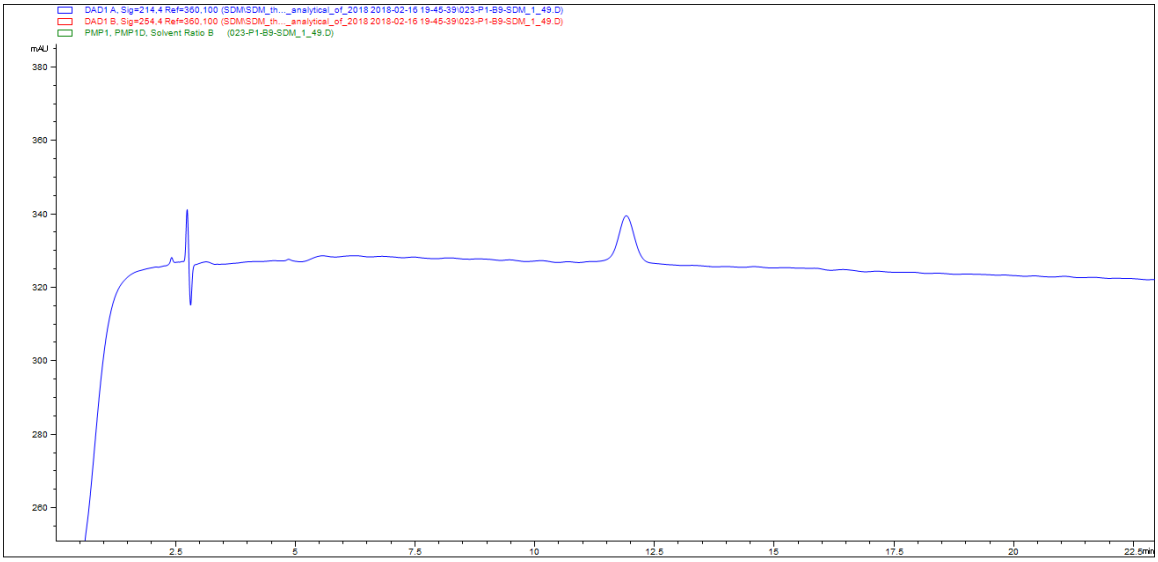
Calculated $[M+Na]^+$: 2165.01

Found: 2164.20

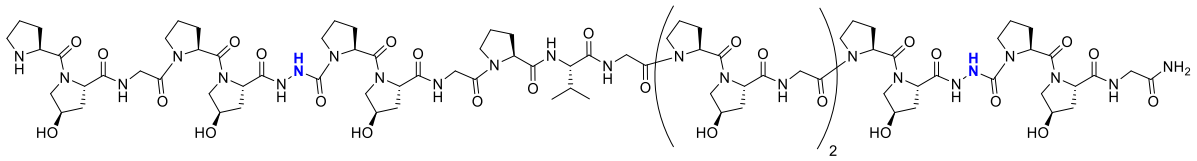
THERMAL DENATURATION CURVE:



HPLC: 12-14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **14c**: H-(Pro-Hyp-Gly)(Pro-Hyp-azGly)(Pro-Hyp-Gly)(Pro-Val-azGly)(Pro-Hyp-Gly)$_2$(Pro-Hyp-Gly)(Pro-Hyp-Gly)-NH_2

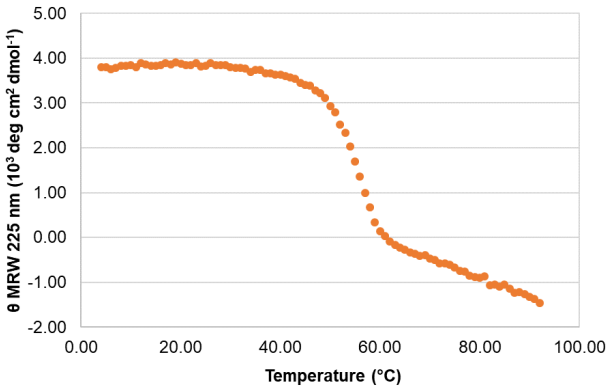


MALDI-TOF MS:

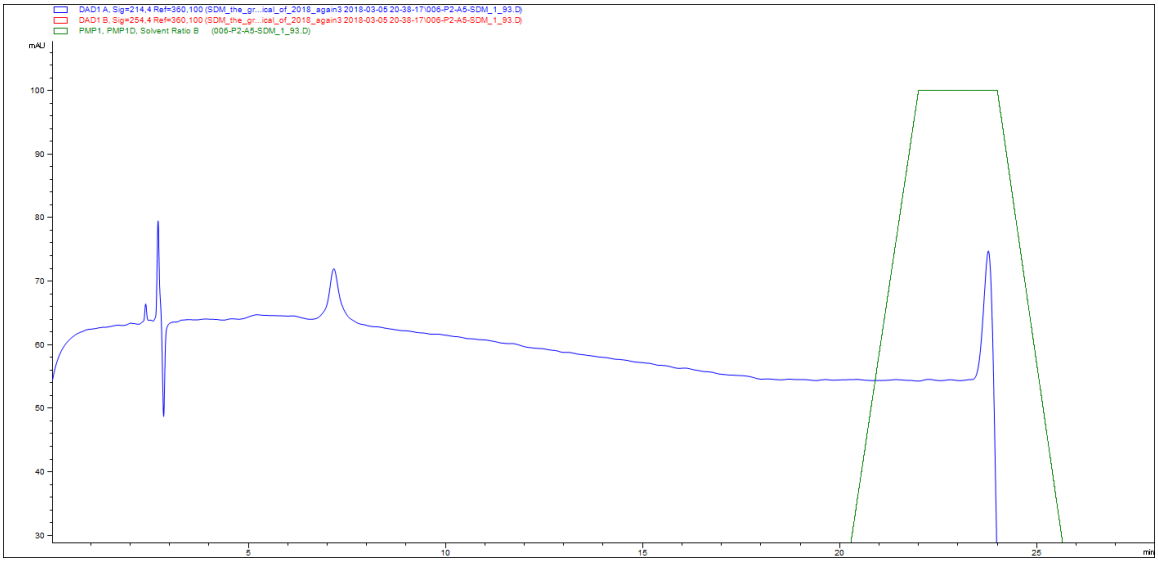
Calculated $[M+Na]^+$: 2166.01

Found: 2166.24

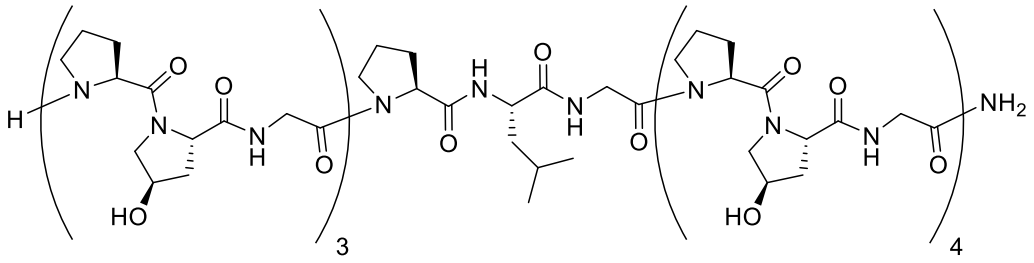
THERMAL DENATURATION CURVE:



HPLC: 14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **15a**: H-(Pro-Hyp-Gly)$_3$(Pro-Leu-Gly)(Pro-Hyp-Gly)$_4$-NH_2

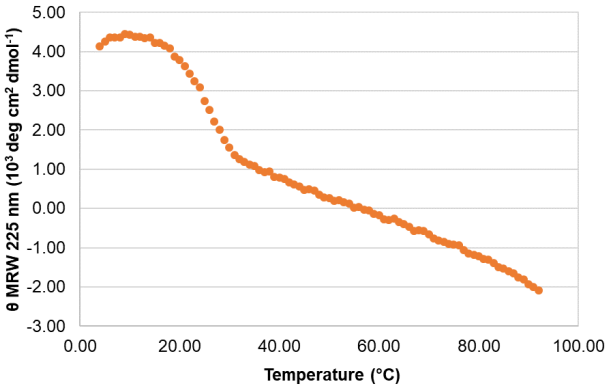


MALDI-TOF MS:

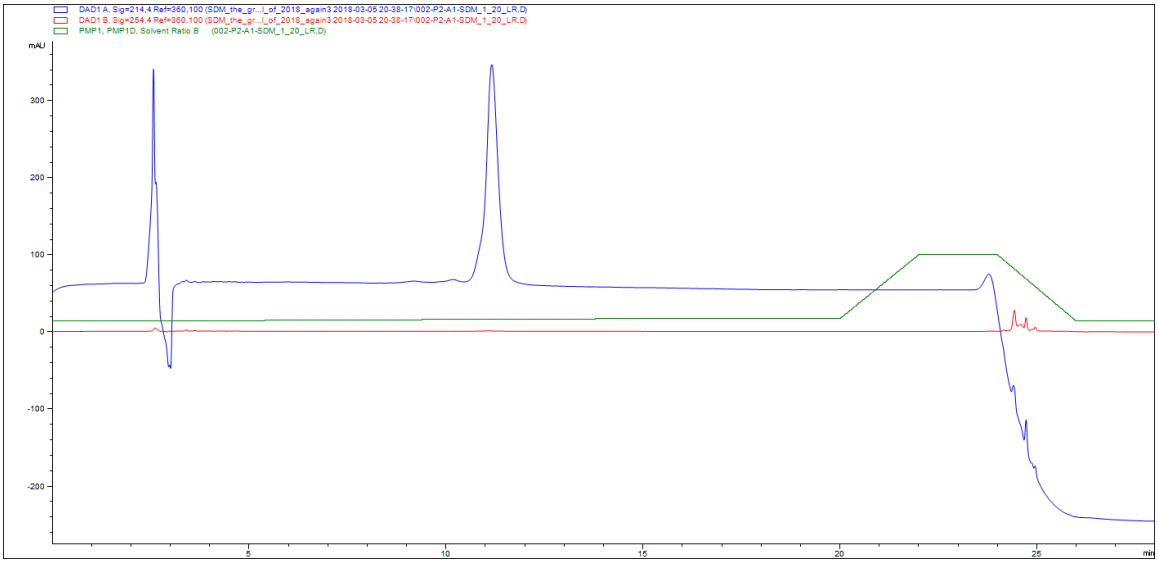
Calculated $[M+Na]^+$: 2178.03

Found: 2177.94

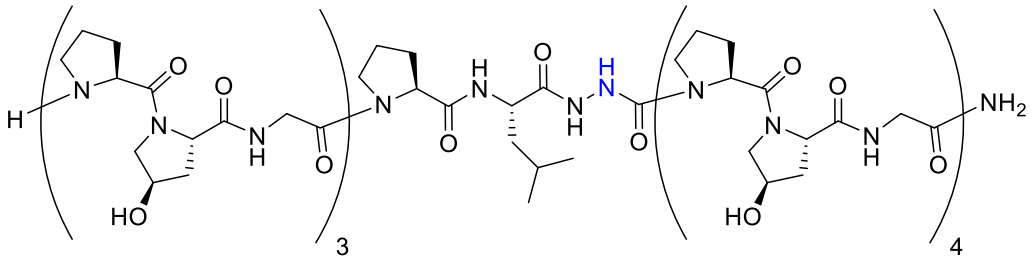
THERMAL DENATURATION CURVE:



HPLC: 14-17% CH_3CN in 0.1% TFA H_2O over 28min at 80 ˚C



CMP **15b**: H-(Pro-Hyp-Gly)$_3$(Pro-Leu-azGly)(Pro-Hyp-Gly)$_4$-NH_2

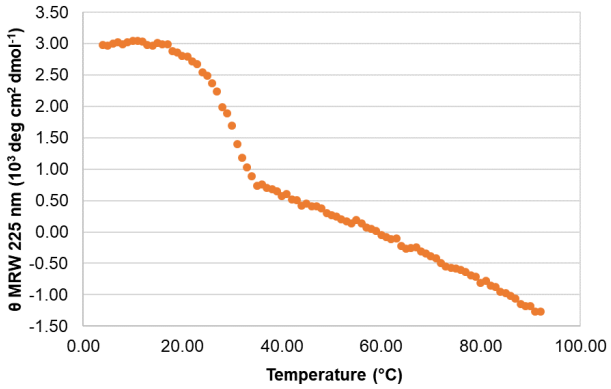


MALDI-TOF MS:

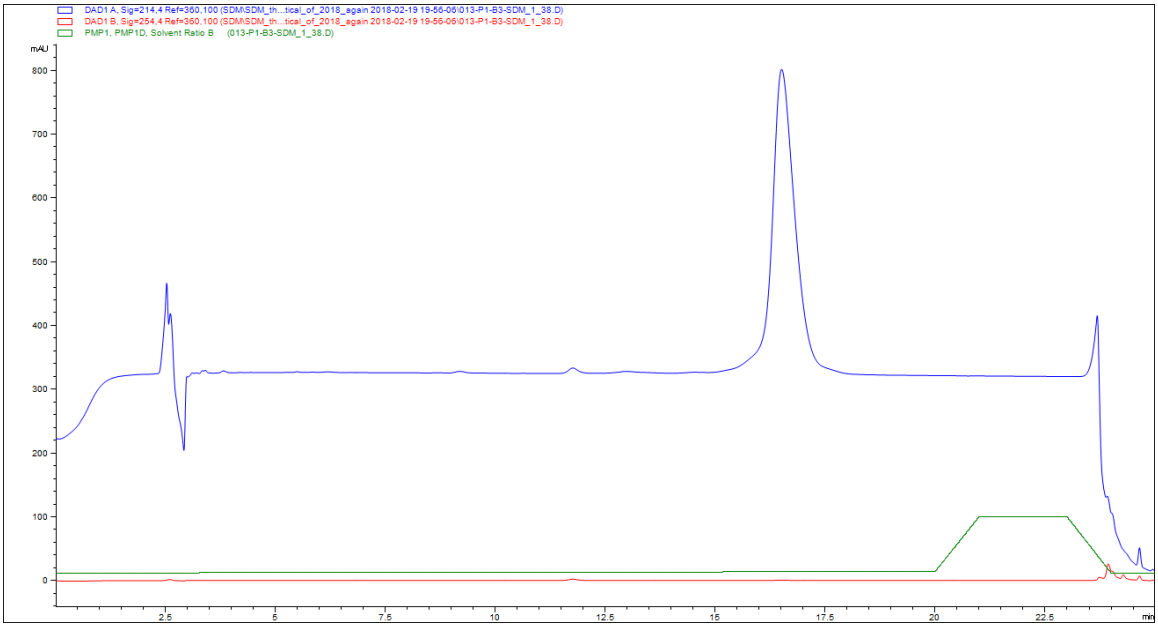
Calculated $[M+Na]^+$: 2179.03

Found: 2179.25

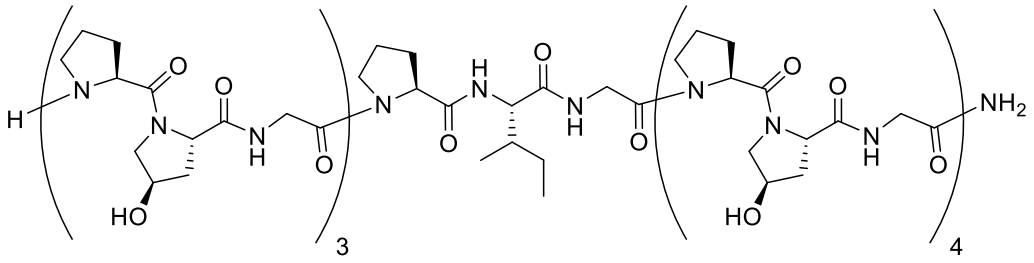
THERMAL DENATURATION CURVE:



HPLC: 12-14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **16a**: H-(Pro-Hyp-Gly)$_3$(Pro-Ile-Gly)(Pro-Hyp-Gly)$_4$-NH_2

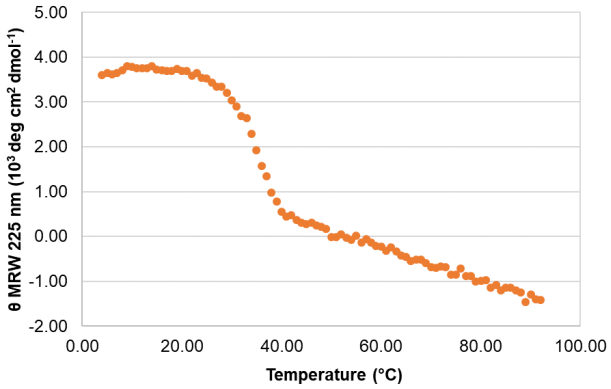


MALDI-TOF MS:

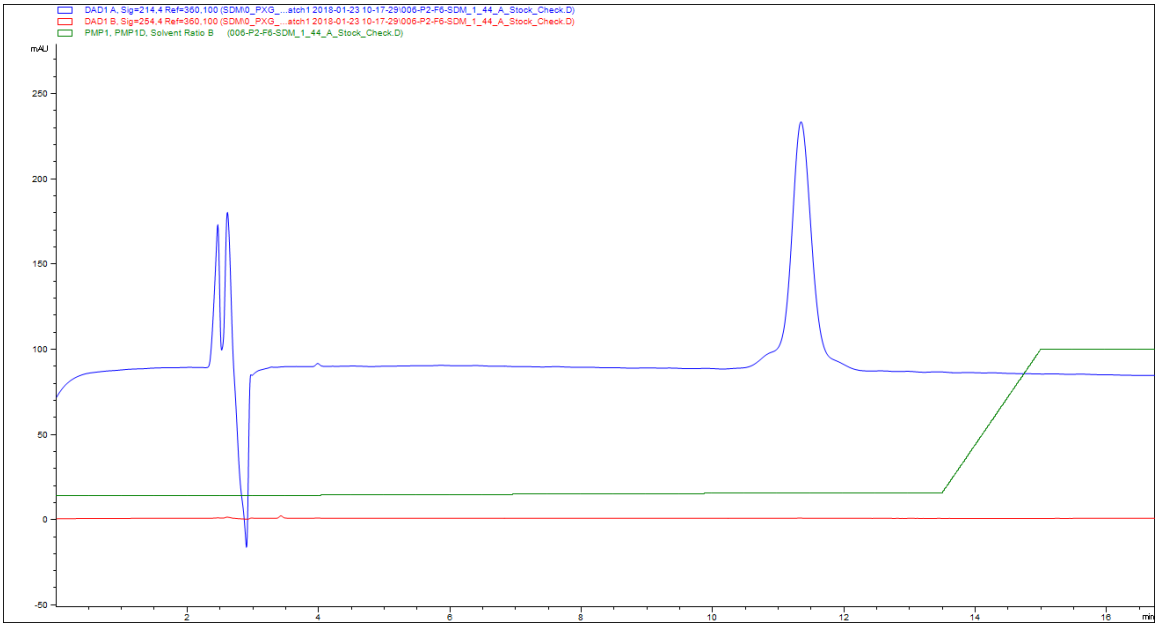
Calculated $[M+Na]^+$: 2178.03

Found: 2177.90

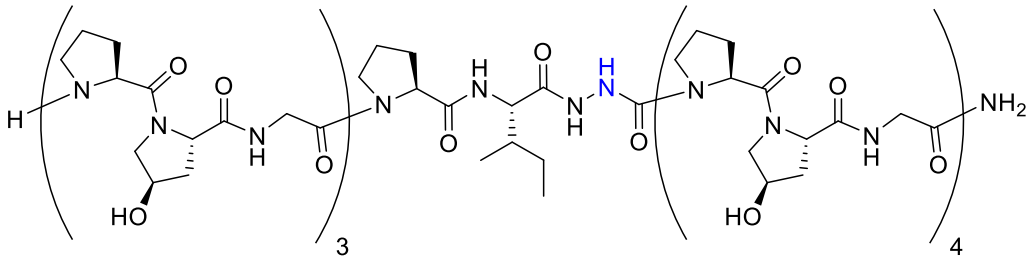
THERMAL DENATURATION CURVE:



HPLC: 14-16.5% CH_3CN in 0.1% TFA H_2O over 13.5min at 80 ˚C



CMP **16b**: H-(Pro-Hyp-Gly)$_3$(Pro-Ile-azGly)(Pro-Hyp-Gly)$_4$-NH_2

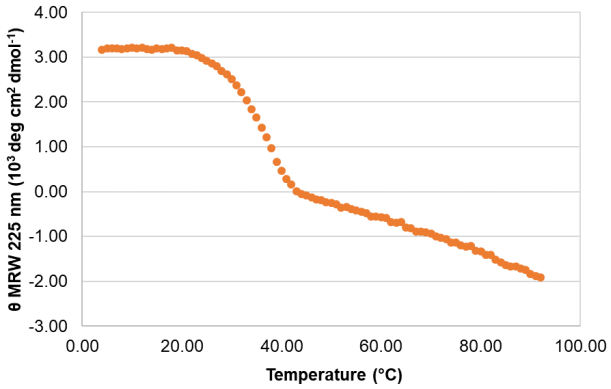


MALDI-TOF MS:

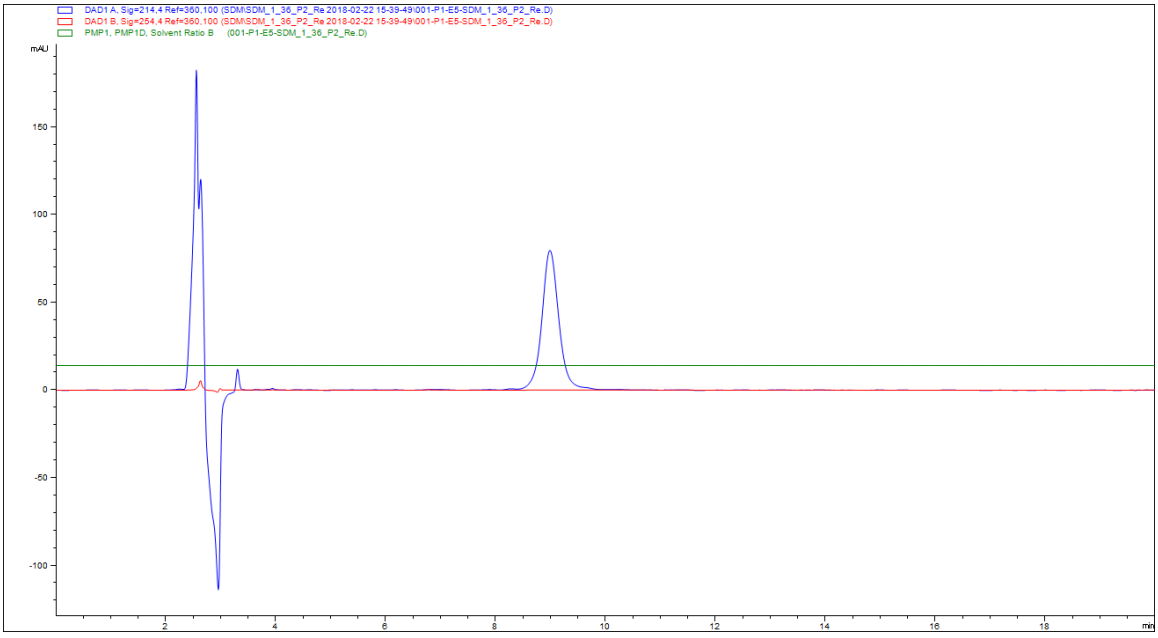
Calculated $[M+Na]^+$: 2179.03

Found: 2179.09

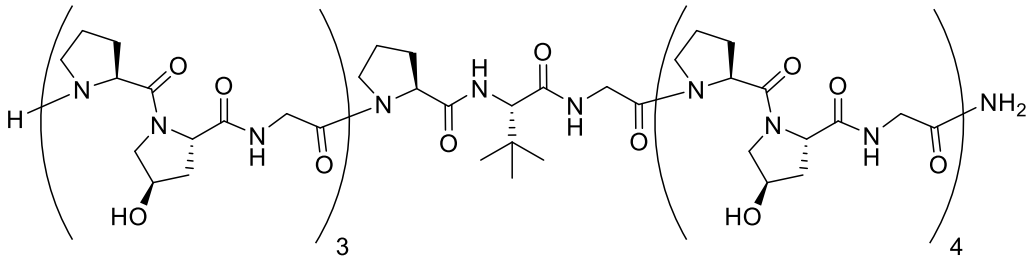
THERMAL DENATURATION CURVE:



HPLC: 14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **17a**: H-(Pro-Hyp-Gly)$_3$(Pro-(t-Leu)-Gly)(Pro-Hyp-Gly)$_4$-NH_2

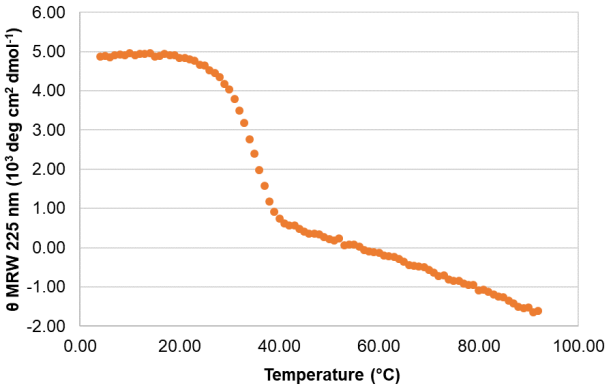


MALDI-TOF MS:

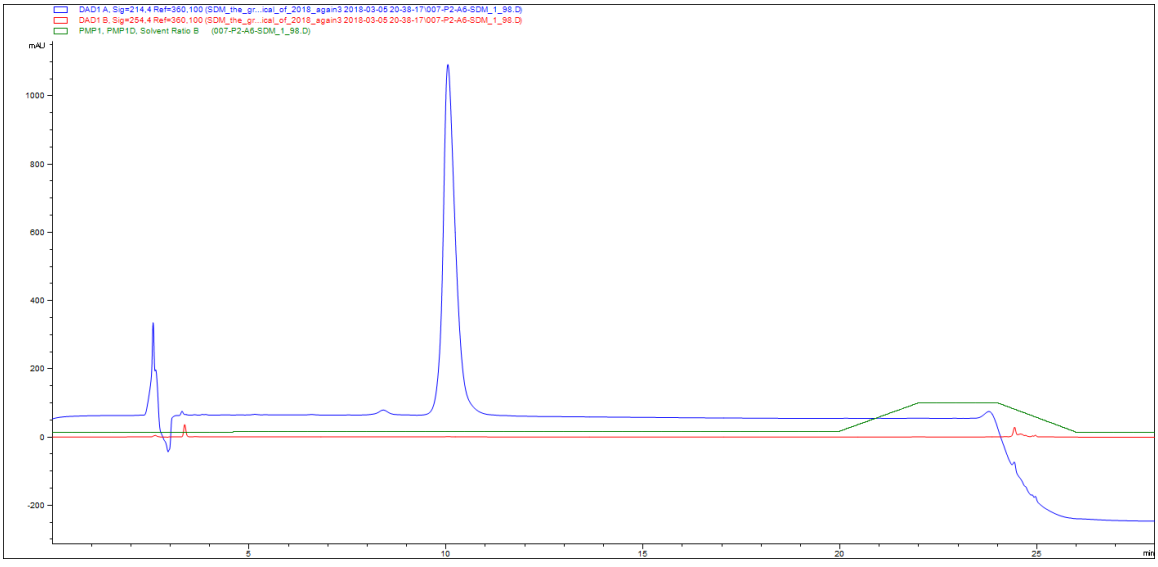
Calculated $[M+Na]^+$: 2178.03

Found: 2178.67

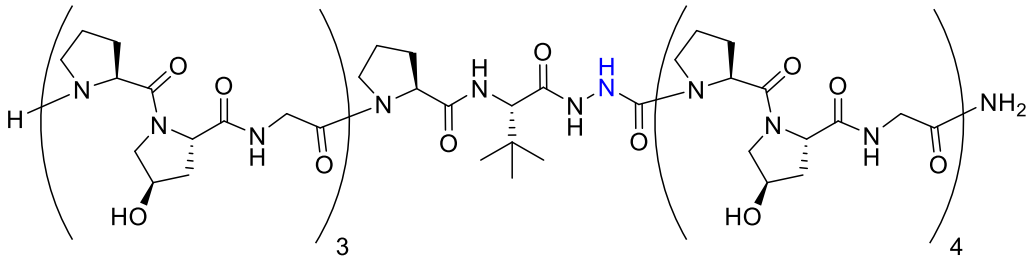
THERMAL DENATURATION CURVE:



HPLC: 14-17% CH_3CN in 0.1% TFA H_2O over 28min at 80 ˚C



CMP **17b**: H-(Pro-Hyp-Gly)$_3$(Pro-(t-Leu)-azGly)(Pro-Hyp-Gly)$_4$-NH_2

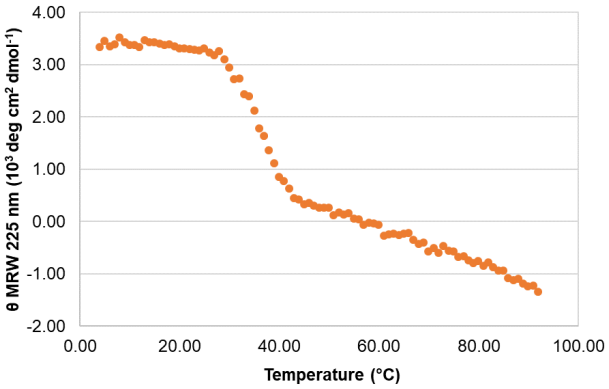


MALDI-TOF MS:

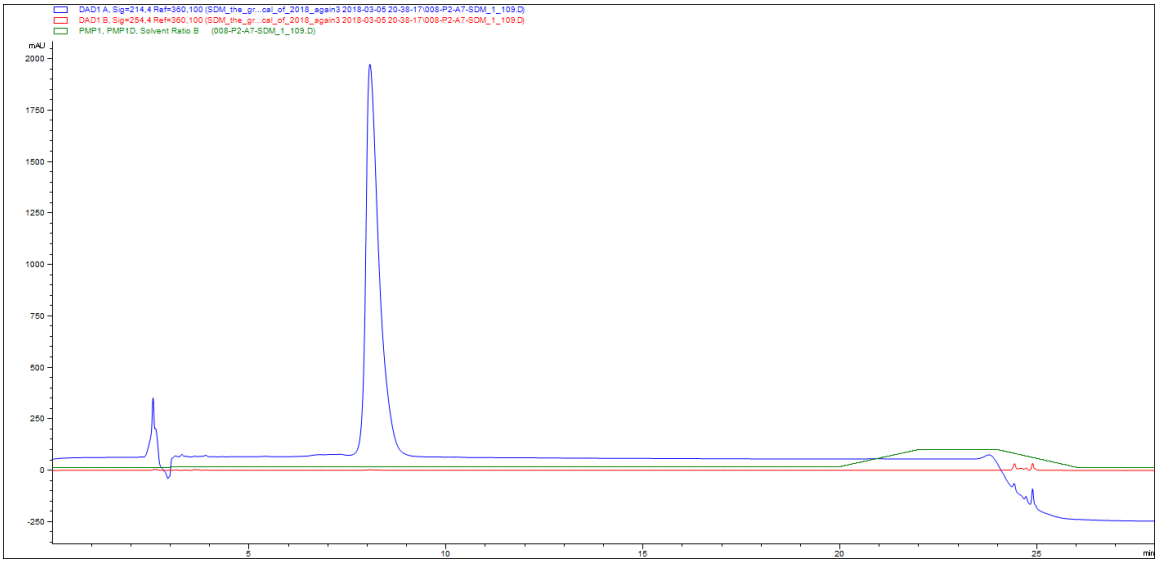
Calculated $[M+Na]^+$: 2179.03

Found: 2719.58

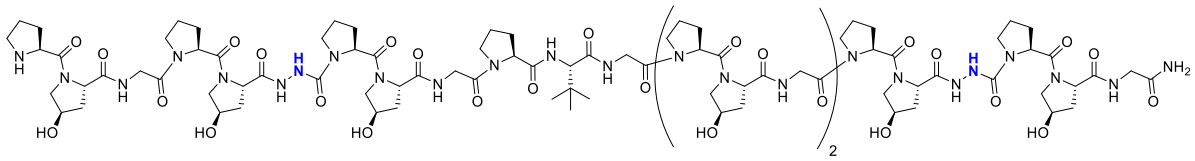
THERMAL DENATURATION CURVE:



HPLC: 14-17% CH_3CN in 0.1% TFA H_2O over 28min at 80 ˚C



CMP **17c**: H-(Pro-Hyp-Gly)(Pro-Hyp-azGly)(Pro-Hyp-Gly)(Pro-(*t*-Leu)-azGly)(Pro-Hyp-Gly)$_2$(Pro-Hyp-Gly)(Pro-Hyp-Gly)-NH_2

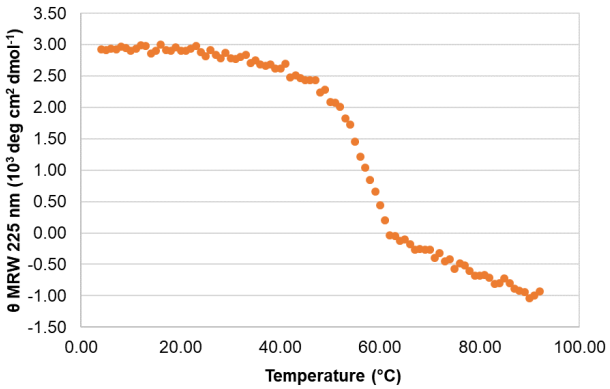


MALDI-TOF MS:

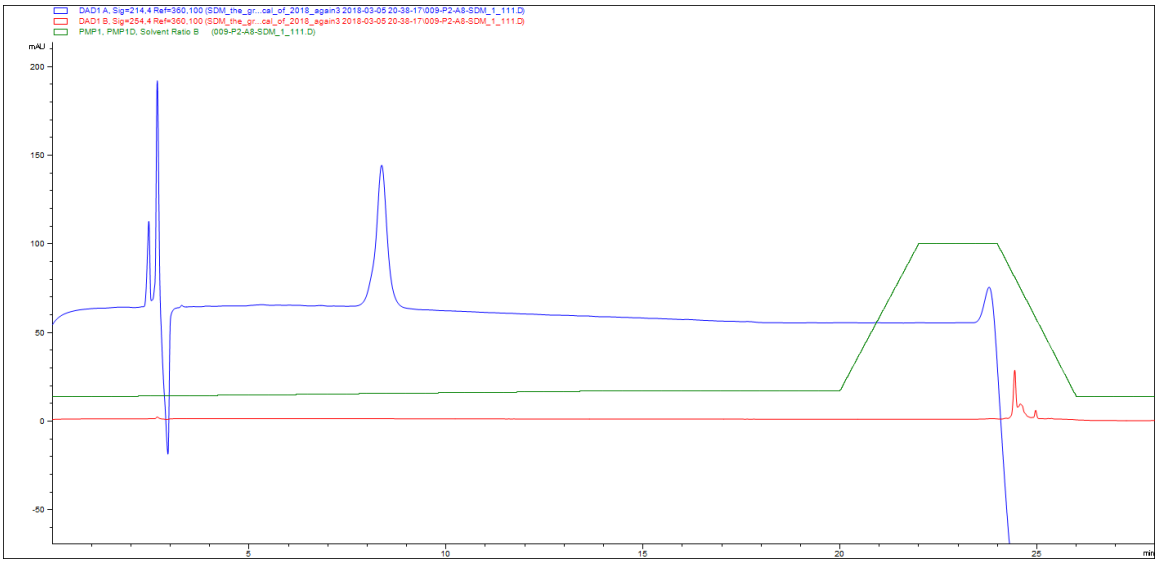
Calculated $[M+Na]^+$: 2180.02

Found: 2180.10

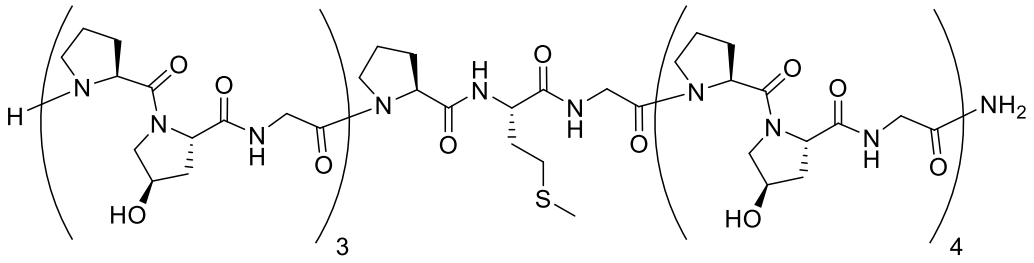
THERMAL DENATURATION CURVE:



HPLC: 14-17% CH_3CN in 0.1% TFA H_2O over 28min at 80 ˚C



CMP **18a**: H-(Pro-Hyp-Gly)$_3$(Pro-Met-Gly)(Pro-Hyp-Gly)$_4$-NH_2

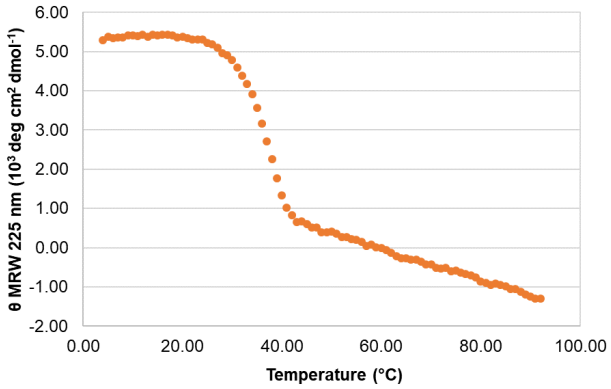


MALDI-TOF MS:

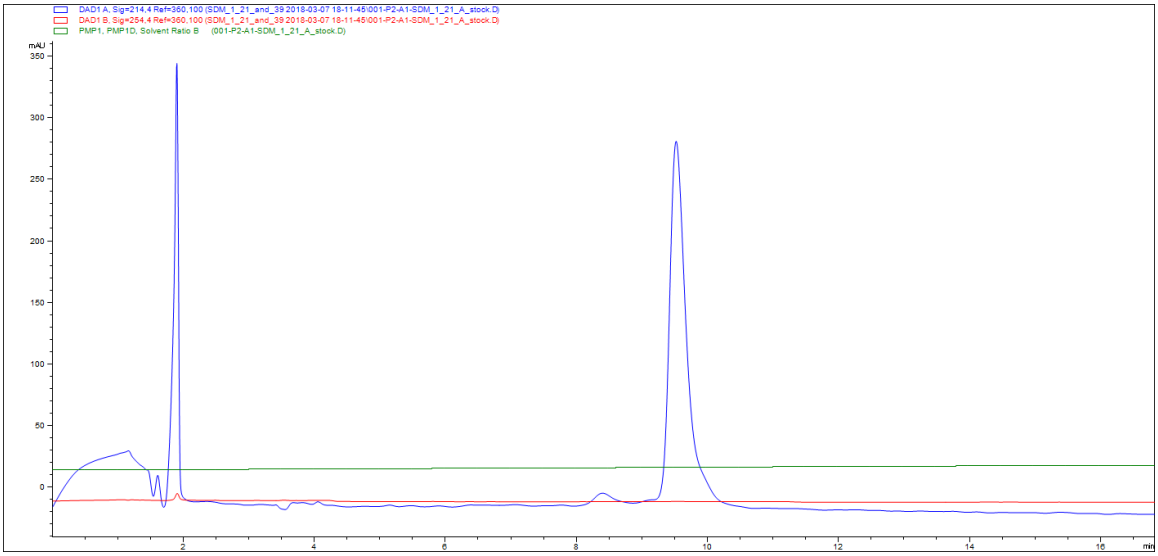
Calculated $[M+Na]^+$: 2195.99

Found: 2196.94

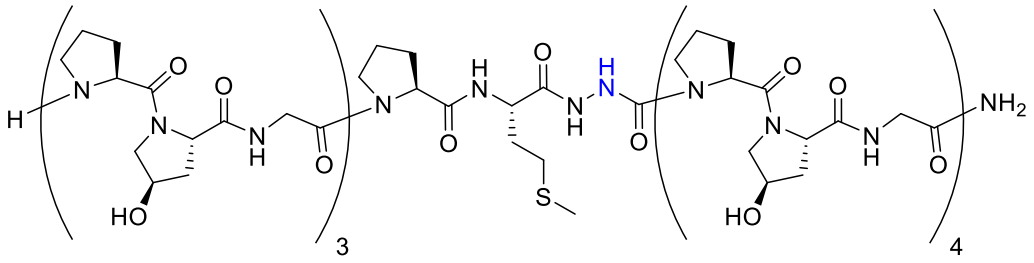
THERMAL DENATURATION CURVE:



HPLC: 12-17% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C, PFP(2) Column



CMP **18b**: H-(Pro-Hyp-Gly)$_3$(Pro-Met-azGly)(Pro-Hyp-Gly)$_4$-NH_2

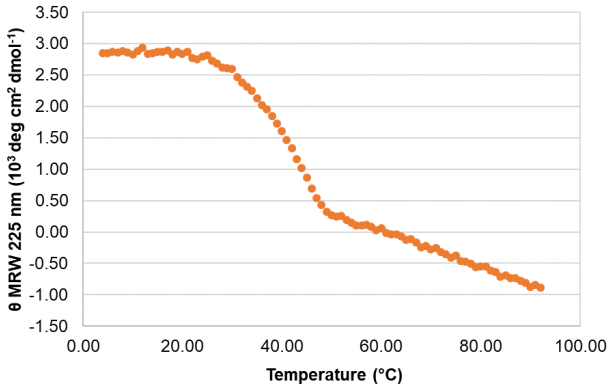


MALDI-TOF MS:

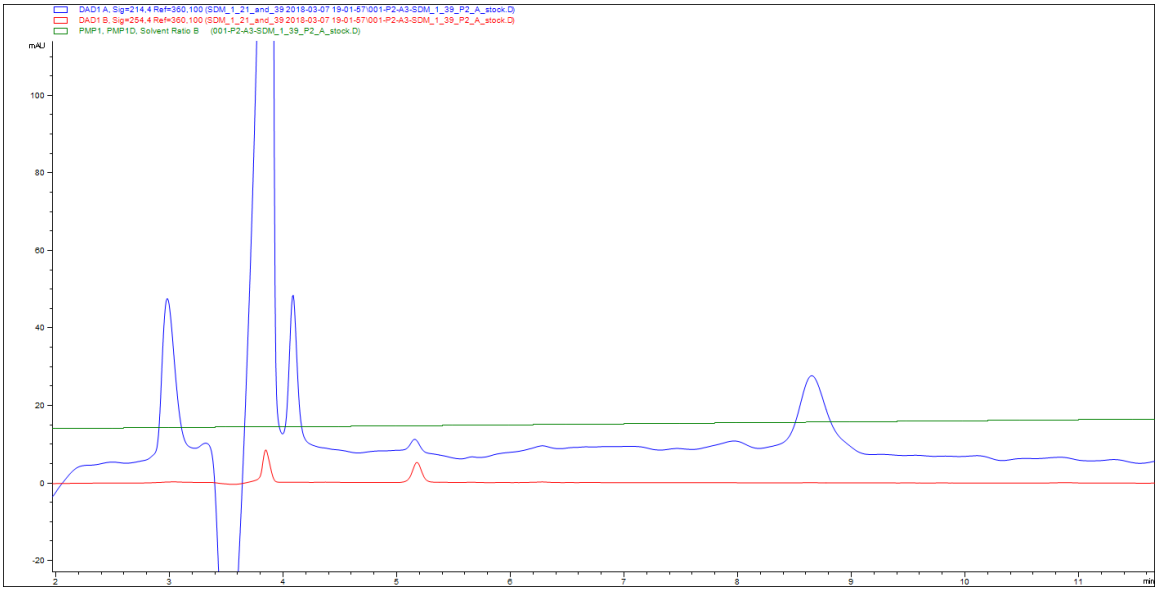
Calculated $[M+Na]^+$: 2196.98

Found: 2197.06

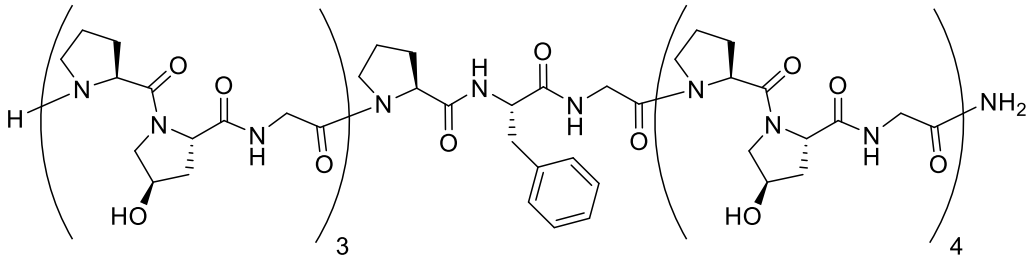
THERMAL DENATURATION CURVE:



HPLC: 14-17% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **19a**: H-(Pro-Hyp-Gly)$_3$(Pro-Phe-Gly)(Pro-Hyp-Gly)$_4$-NH_2

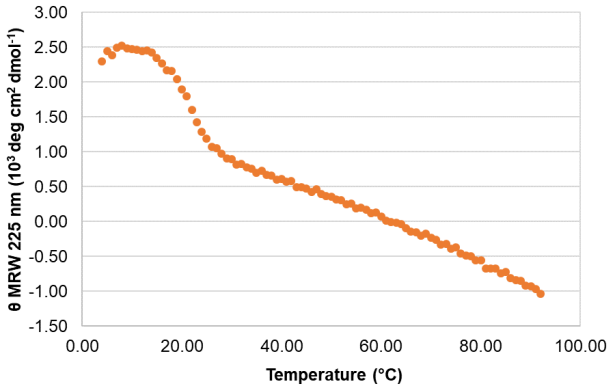


MALDI-TOF MS:

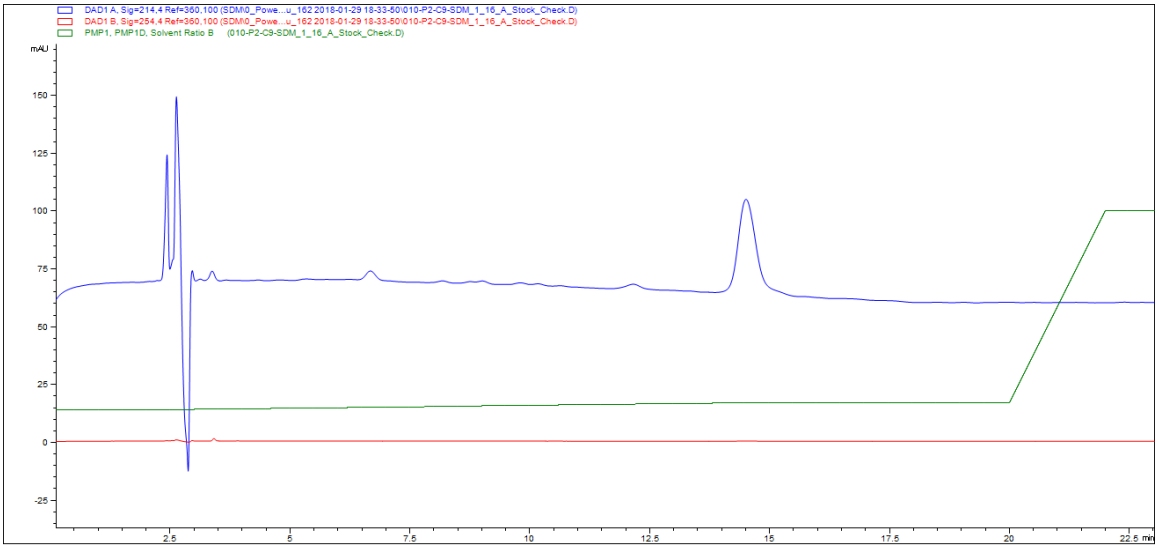
Calculated $[M+Na]^+$: 2212.02

Found: 2212.30

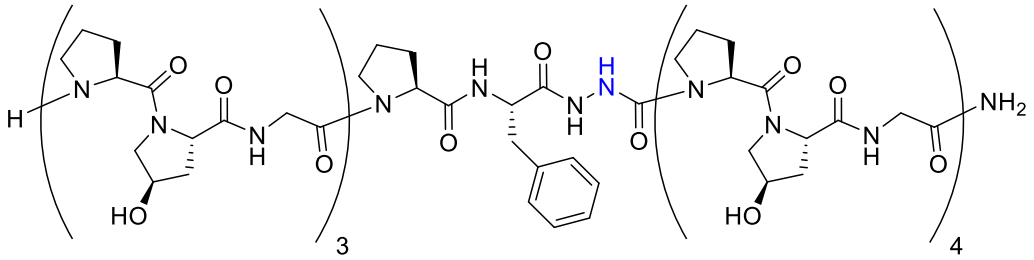
THERMAL DENATURATION CURVE:



HPLC: 14-17% CH_3CN in 0.1% TFA H_2O over 28min at 80 ˚C



CMP **19b**: H-(Pro-Hyp-Gly)$_3$(Pro-Phe-azGly)(Pro-Hyp-Gly)$_4$-NH_2

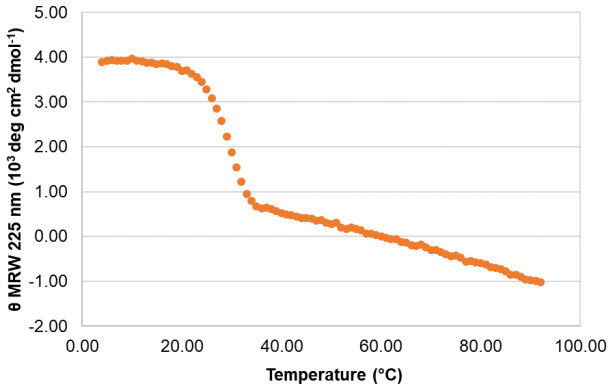


MALDI-TOF MS:

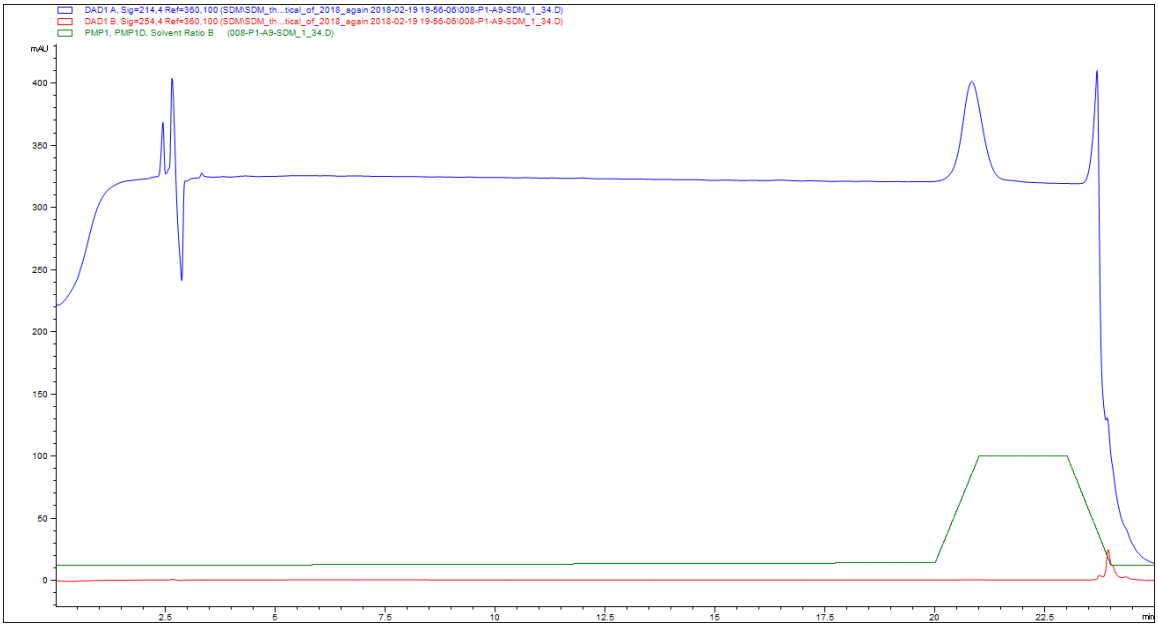
Calculated $[M+Na]^+$: 2213.01

Found: 2213.50

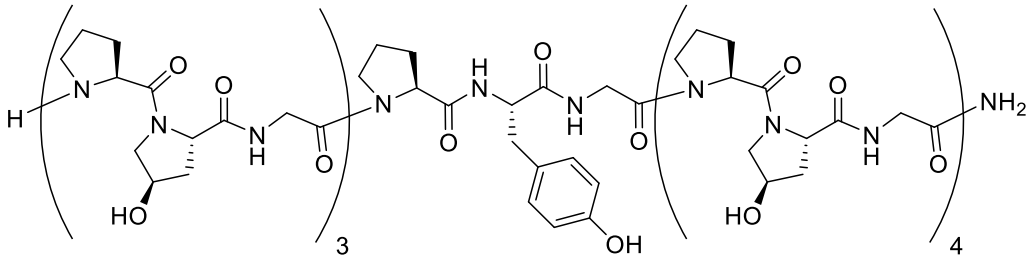
THERMAL DENATURATION CURVE:



HPLC: 12-14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **20a**: H-(Pro-Hyp-Gly)$_3$(Pro-Tyr-Gly)(Pro-Hyp-Gly)$_4$-NH_2

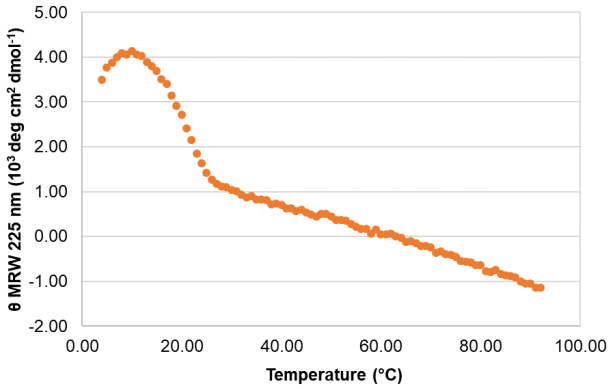


MALDI-TOF MS:

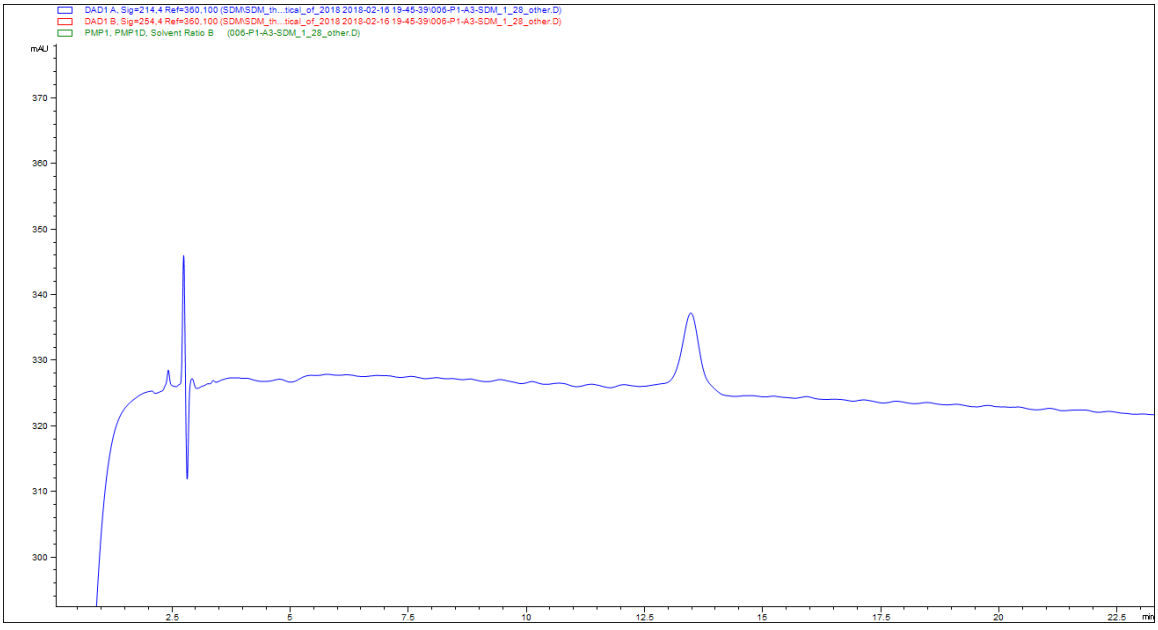
Calculated $[M+Na]^+$: 2229.01

Found: 2228.53

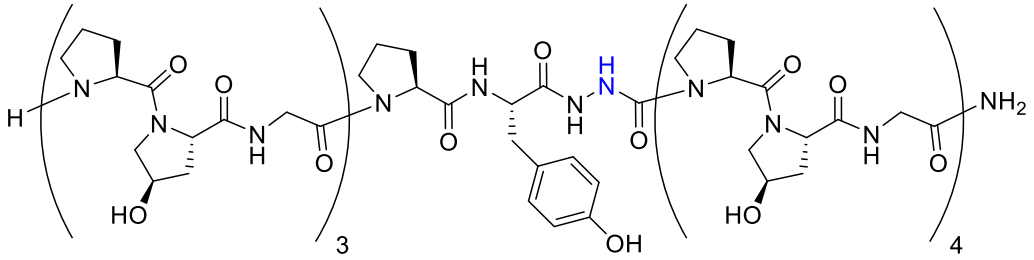
THERMAL DENATURATION CURVE:



HPLC: 12-14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **20b**: H-(Pro-Hyp-Gly)$_3$(Pro-Tyr-azGly)(Pro-Hyp-Gly)$_4$-NH_2

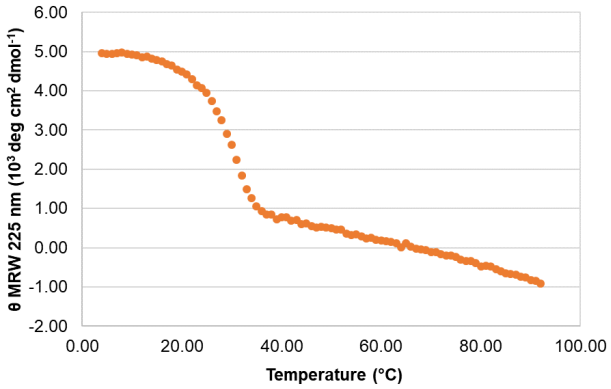


MALDI-TOF MS:

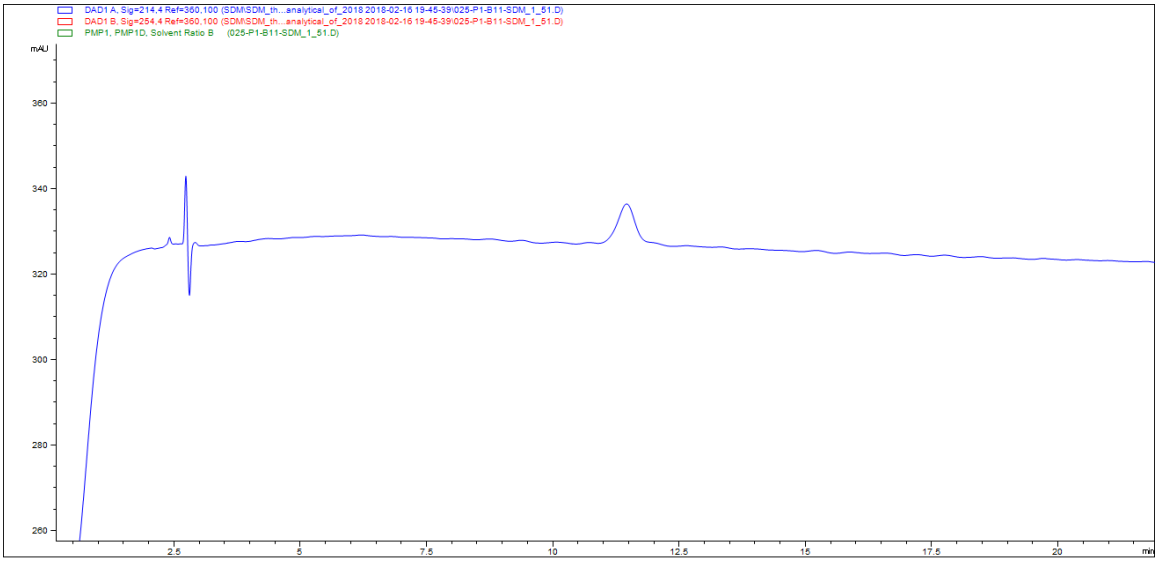
Calculated $[M+Na]^+$: 2230.01

Found: 2229.58

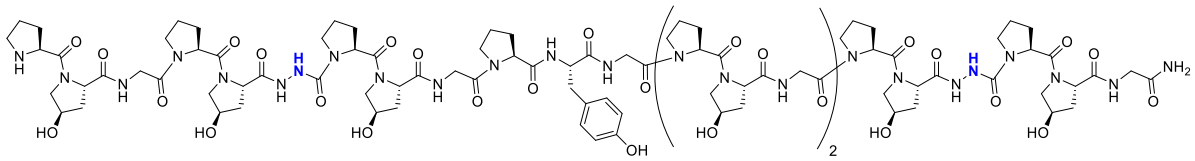
THERMAL DENATURATION CURVE:



HPLC: 12-14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **20c**: H-(Pro-Hyp-Gly)(Pro-Hyp-azGly)(Pro-Hyp-Gly)(Pro-Tyr-azGly)(Pro-Hyp-Gly)$_2$(Pro-Hyp-Gly)(Pro-Hyp-Gly)-NH_2

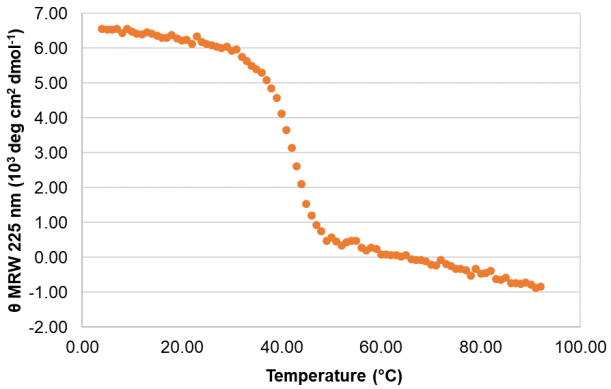


MALDI-TOF MS:

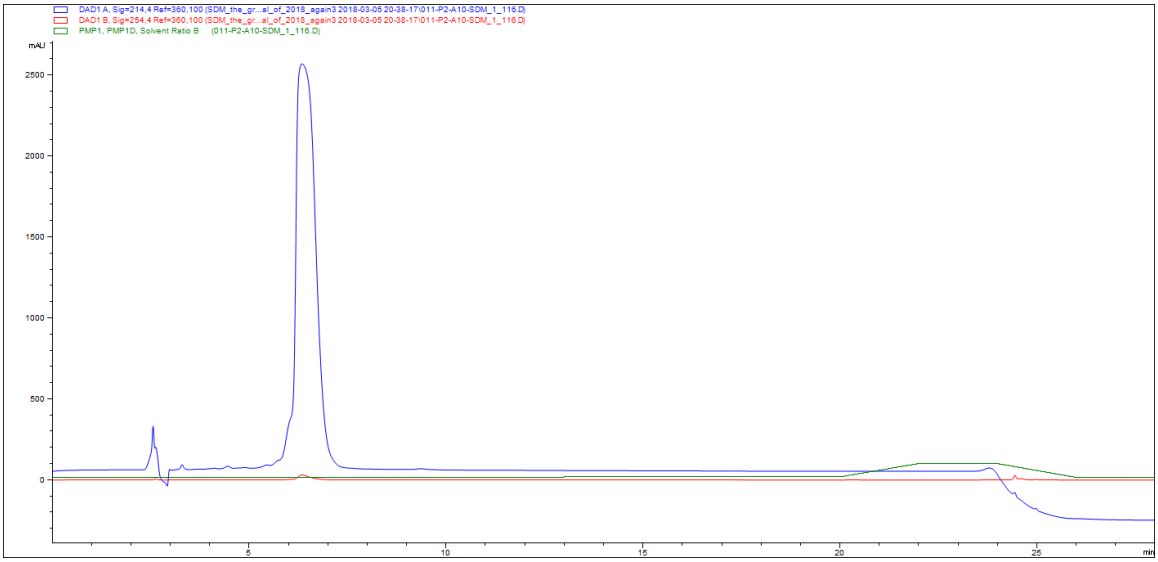
Calculated $[M+Na]^+$: 2230.00

Found: 2229.77

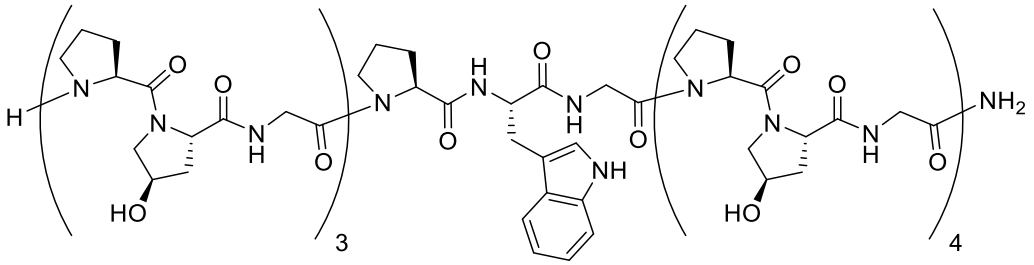
THERMAL DENATURATION CURVE:



HPLC: 14-17% CH_3CN in 0.1% TFA H_2O over 28min at 80 ˚C



CMP **21a**: H-(Pro-Hyp-Gly)$_3$(Pro-Trp-Gly)(Pro-Hyp-Gly)$_4$-NH_2

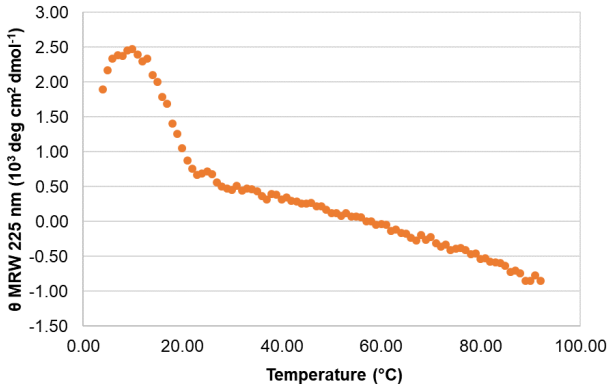


MALDI-TOF MS:

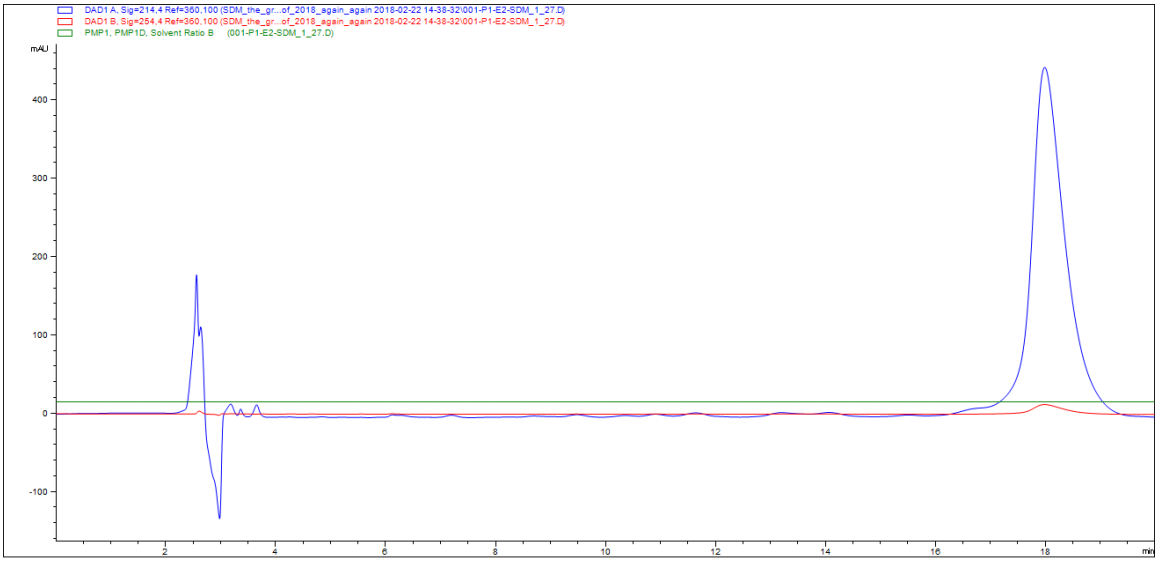
Calculated $[M+Na]^+$: 2251.03

Found: 2251.25

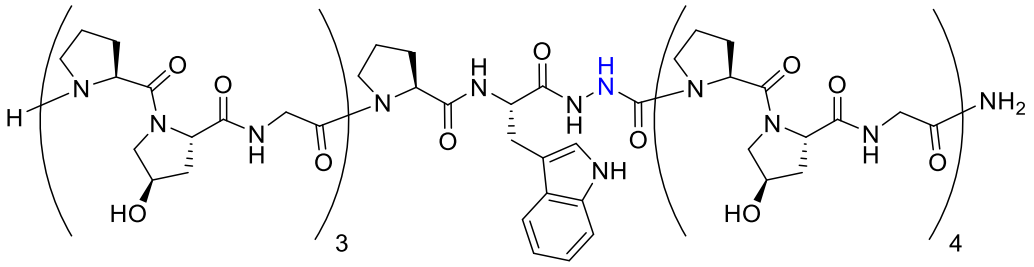
THERMAL DENATURATION CURVE:



HPLC: 14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **21b**: H-(Pro-Hyp-Gly)$_3$(Pro-Trp-azGly)(Pro-Hyp-Gly)$_4$-NH_2

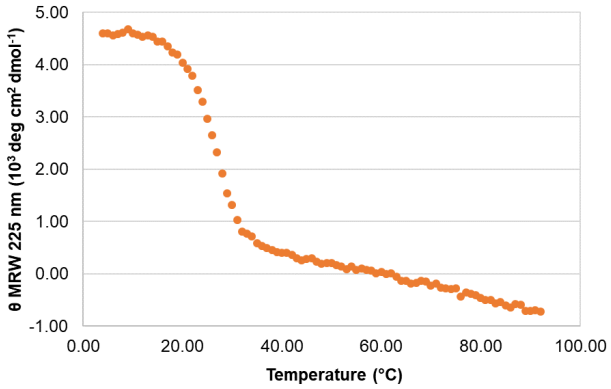


MALDI-TOF MS:

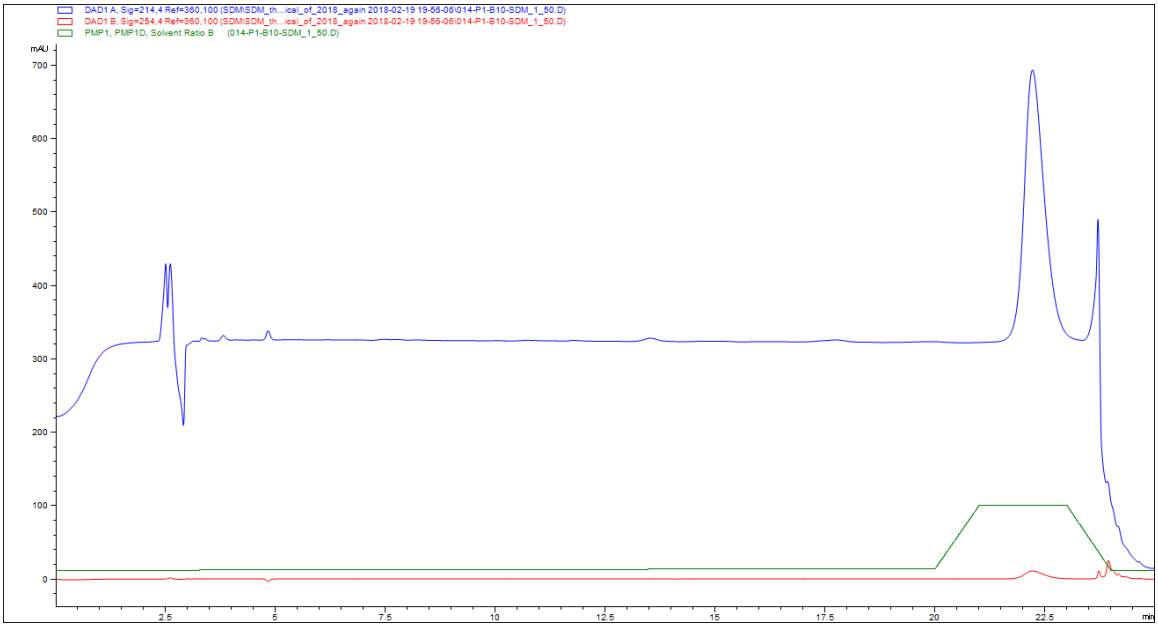
Calculated $[M+Na]^+$: 2252.02

Found: 2252.33

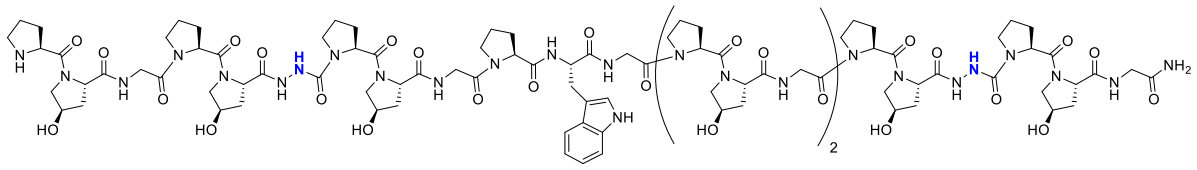
THERMAL DENATURATION CURVE:



HPLC: 12-14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **21c**: H-(Pro-Hyp-Gly)(Pro-Hyp-azGly)(Pro-Hyp-Gly)(Pro-Trp-azGly)(Pro-Hyp-Gly)$_2$(Pro-Hyp-Gly)(Pro-Hyp-Gly)-NH_2

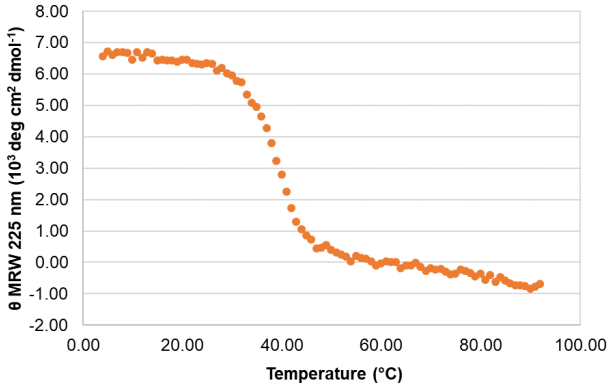


MALDI-TOF MS:

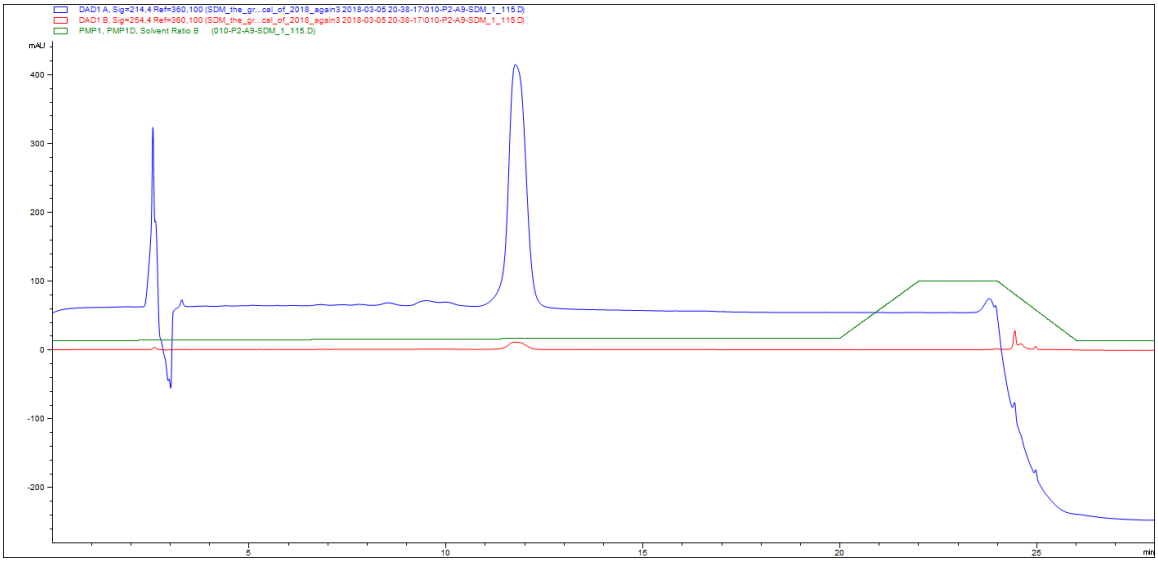
Calculated $[M+Na]^+$: 2253.02

Found: 2253.53

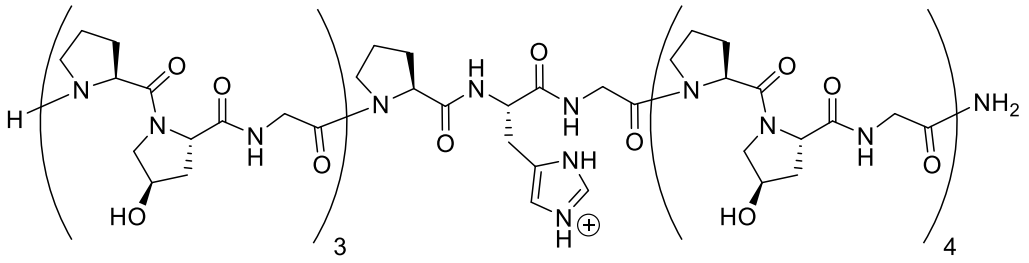
THERMAL DENATURATION CURVE:



HPLC: 14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



CMP **22a**: H-(Pro-Hyp-Gly)$_3$(Pro-His-Gly)(Pro-Hyp-Gly)$_4$-NH_2

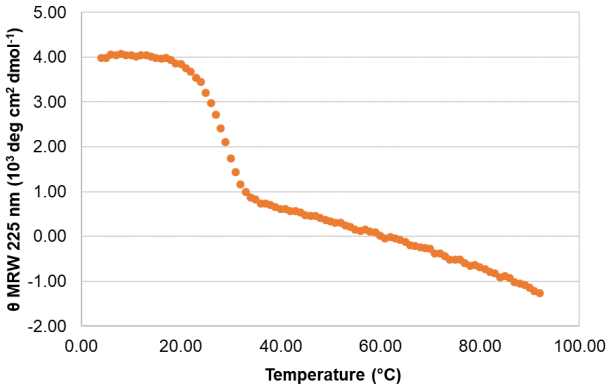


MALDI-TOF MS:

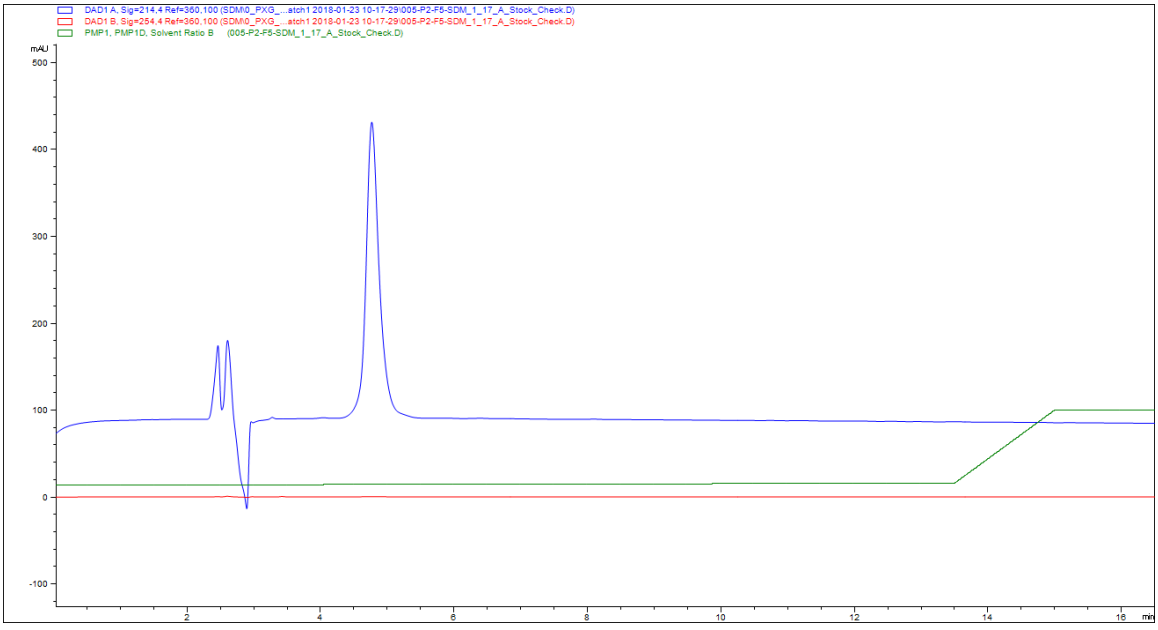
Calculated $[M+Na]^+$: 2202.06

Found: 2203.76

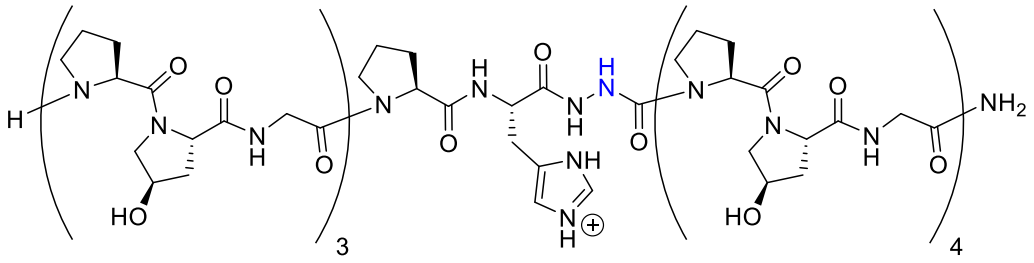
THERMAL DENATURATION CURVE:



HPLC: 14-16.5% CH_3CN in 0.1% TFA H_2O over 13.5min at 80 ˚C



CMP **22b**: H-(Pro-Hyp-Gly)$_3$(Pro-His-azGly)(Pro-Hyp-Gly)$_4$-NH_2

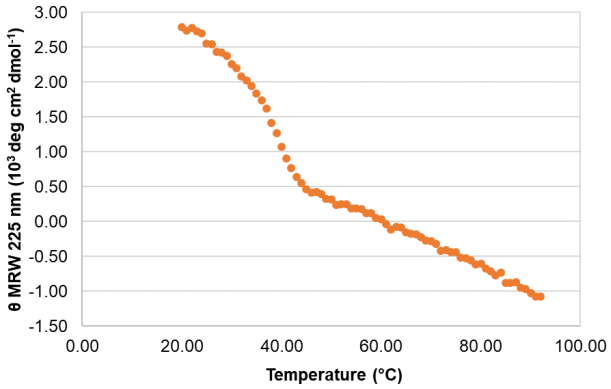


MALDI-TOF MS:

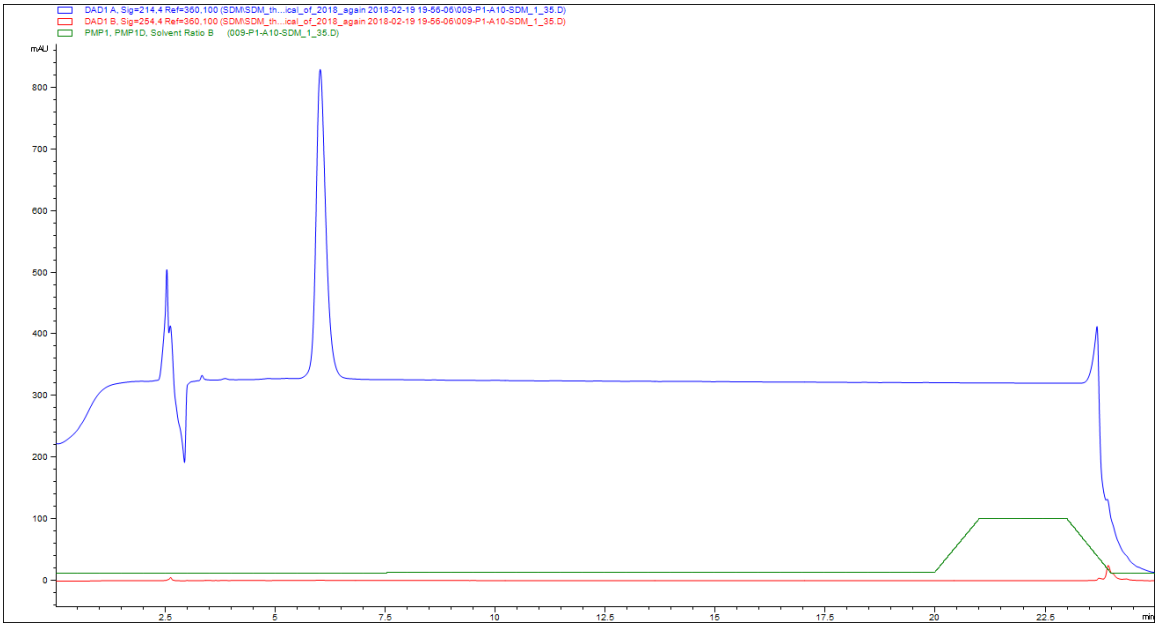
Calculated $[M+Na]^+$: 2203.00

Found: 2203.51

THERMAL DENATURATION CURVE:



HPLC: 12-14% CH_3CN in 0.1% TFA H_2O over 20min at 80 ˚C



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