Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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Supporting Information for:

Conformational Stability Studies of a Stapled Hexa-β^3-Peptide Library

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1. Peptide synthesis

1.1 General information

Fmoc-protected β-amino acids were purchased from PepTech (Cambridge, MA, USA). 2-(1H-7-Azabenzotriazol-1-yl)--1,1,3,3-tetramethyl uronium hexafluorophosphate (HATU), *O*-benzotriazole-*N*,*N*,*N'*,*N'*-tetramethyl-uronium-hexafluoro-phosphate (HBTU), *N*-hydroxybenzotriazole (HoBt), and Wang resin were purchased from GL Biochem (Shanghai, China). Dimethylformamide (DMF, stored over 4Å MS), *N*-methyl-2-pyrrolidone (NMP), dichloromethane (CH_2Cl_2, distilled from P_2O_5 and stored over 4Å MS), and piperidine were purchased from Merck (Darmstadt, Germany). Trifluoroacetic acid (TFA) and diisopropylethylamine (DIPEA) were purchased from Auspep (Melbourne, Australia). All other reagents were purchased from Sigma-Aldrich.

1.2 β-Peptide preparation

All β-Peptides were synthesized on a 50 μmol scale using standard Fmoc chemistry on Wang resin (0.9 mmol/g loading, GL Biochem, Shanghai, China). The resin was washed (3 x 30 s) with NMP and the Fmoc-protected β-amino acid (3.1 eq. to resin loading) was dissolved in NMP along with HBTU (3 eq. to resin loading), HoBt (3 eq. to resin loading) and DIPEA (4.5 eq. to resin loading). 4-Dimethylaminopyridine (0.1 eq. to resin loading) in NMP was added dropwise and the reaction proceeded overnight.

At this stage peptide synthesis of all peptides proceeded as normal. Thus, following the overnight coupling, the resin was washed with NMP (5 x 30 s) and CH_2Cl_2 (5 x 30 s) and peptide synthesis was continued. One cycle of peptide elongation consisted of the following steps. The loaded resin was first washed with NMP (3 x 30 sec) and the terminal Fmoc protecting group was removed with 20% piperidine/DMF (2 x 15 min). The deprotected resin was then washed with NMP (5 x 30 s) and treated for 90 min with a solution containing 3.1 eq. of the appropriate β-amino acid, 3 eq. HATU, and 4.5 eq. DIPEA. The resin was then washed three times with NMP (3 x 30 s), unreacted amino groups were acetylated upon treatment with 10% v/v acetic anhydride and 1% v/v DIPEA in NMP (2 x 20 min), and the capped resin washed with NMP (3 x 30 s). These steps were repeated until the β-peptide sequence was complete. Once the final Fmoc-protecting group had been removed, the resin was treated with 10% v/v acetic anhydride and 1% v/v DIPEA in NMP (2 x 20 min) to afford an acetyl-capped N-terminus. The resin was subsequently washed with NMP (5 x 30 s) and CH_2Cl_2 (5 x 30 s), dried for 20 min under vacuum, and then treated for 90 min with a cleavage solution containing 2.5% v/v water and 2.5% v/v triisopropylsilane in TFA. The cleaved resin was washed twice with

the cleavage solution (2 x 30 s) and the cleaved β-peptide in TFA was collected. The TFA was evaporated under a stream of N_2 and the peptide was precipitated by the addition of diethyl ether. The precipitate was filtered and reconstituted in H_2O/acetonitrile (1:1) for lyophilization.

1.3. Ring-closing metathesis

Ring closing metathesis (RCM) of all peptides was performed on Fmoc-protected peptides on Wang resin. The resin was swelled in TFE:CH_2Cl_2 (4:1 ratio, 10 mM) and Hoveyda-Grubbs II generation catalyst (35 mol%) was added to the solution and the reaction was allowed to proceed for 48 h (monitored by HPLC and ESI-MS upon cleaving a small sample off resin). The resin was then washed in a solution of DMSO:DMF (1:1) overnight. Final deprotection (and acetylation) and subsequent cleavage of the peptide from the resin was performed using the protocol described above (Section 1.2).

2. Peptide purification and analysis

Mass spectra were acquired with an Agilent 1100 MSD SL ion trap mass spectrometer. Reverse-phase HPLC was performed using an Agilent HP1200 system fitted with a Vydac^{TM} analytical (C18, 300 Å, 5 μm, 4.6 mm x 150 mm) or preparative (C18, 300 Å, 5 μm, 10 mm x 250 mm) columns. Preparative HPLC columns were heated to 60^{o}C in a water bath. The eluents were 0.1% aqueous TFA and 0.1% TFA in acetonitrile.

The success of each synthesis was assessed first by HPLC and ESI-MS analysis of the crude reaction mixture. β-Peptides were then purified to homogeneity by reverse-phase HPLC. The identities and purities of purified β-peptides were assessed by analytical HPLC and mass spectrometry (Table S1). HPLC retention times were observed following analytical HPLC with a solvent gradient of 0-70% 0.1% acetonitrile over 40 min.

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Table **S1**: Analytical data for all peptide alkenes

Peptide Sequence	#	Mass calc.	Mass obs. $[M+H]^-$	Purity %
Ac- β^3(Val-aSer-Leu-Val-aSer-Leu)-OH	3a	823.09	822.6	>95
Ac- β^3(Val-aSer-Leu-Val-aSer-Leu)-OH	4a	795.04	795.5	>95
Ac- β^3(Val-aSer-Leu-Leu-aSer-Leu)-OH	3b	837.11	836.6	74.6
Ac- β^3(Val-aSer-Leu-Leu-aSer-Leu)-OH	4b	809.05	810.6	89.3
Ac- β^3(Val-aSer-Val-Val-aSer-Leu)-OH	3c	809.06	808.6	>95
Ac- β^3(Val-aSer-Val-Val-aSer-Leu)-OH	4c	781.00	780.7	>95
Ac- β^3(Val-aSer-Ala-Leu-aSer-Leu)-OH	3d	795.03	794.6	89.5
Ac- β^3(Val-aSer-Ala-Leu-aSer-Leu)-OH	4d	766.97	767.4	92.5
Ac-β^3(Val-aSer-Ala-Val-aSer-Leu)-OH	3e	781.01	780.6	87.0
Ac-β^3(Val-aSer-Ala-Val-aSer-Leu)-OH	4e	752.95	752.7	91.1
Ac-β^3(Val-aSer-Ala-Ala-aSer-Leu)-OH	3f	752.95	752.8	>95
Ac-β^3(Val-aSer-Ala-Ala-aSer-Leu)-OH	4f	724.89	724.7	89.0
Ac-β^3(Val-aSer-Ala-Ile-aSer-Leu)-OH	3g	795.03	794.9	91.5
Ac-β^3(Val-aSer-Ala-Ile-aSer-Leu)-OH	4g	766.97	766.6	>95
Ac-β^3(Val-aSer-Ile-Ala-aSer-Leu)-OH	3h	795.03	794.7	94.0
Ac-β^3(Val-aSer-Ile-Ala-aSer-Leu)-OH	4h	766.97	766.9	>95
Ac-β^3(Val-aSer-Phe-Ala-aSer-Leu)-OH	3i	829.05	828.7	91.8
Ac-β^3(Val-aSer-Phe-Ala-aSer-Leu)-OH	4i	800.99	800.7	92.4
Ac-β^3(Val-aSer-Phe-Val-aSer-Leu)-OH	3j	857.10	856.6	>95
Ac-β^3(Val-aSer-Phe-Val-aSer-Leu)-OH	4j	829.04	828.7	94.9
Ac-β^3(Glu-aSer-Val-Lys-aSer-Leu)-OH	3k	867.61	866.4	>95
Ac-β^3(Glu-aSer-Val-Lys-aSer-Leu)-OH	4k	839.55	838.4	>95
Ac-β^3(Arg-aSer-Val-Lys-aSer-Leu)-OH	3l	894.67	893.5	>95
Ac-β^3(Arg-aSer-Val-Lys-aSer-Leu)-OH	4l	866.61	865.5	92.9
Ac-β^3(Glu-aSer-Val-Glu-aSer-Leu)-OH	3m	868.55	867.4	93.0
Ac-β^3(Glu-aSer-Val-Glu-aSer-Leu)-OH	4m	840.49	840.5	>95

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Ac- β^3(aSer-Leu-Val-aSer-Leu-Val)-OH	5	823.09	822.6	94.5
Ac- β^3(aSer-Leu-Val-aSer-Leu-Val)-OH	6	795.04	795.0	93.4
Ac- β^3(Leu-Val-aSer-Leu-Val-aSer)-OH	7	823.09	822.6	94.0
Ac- β^3(Leu-Val-aSer-Leu-Val-aSer)-OH	8	795.04	795.0	94.5
Ac-β^3(Val-aGly-Leu-Val-aSer-Leu)-OH	9a	793.06	792.8	89.7
Ac-β^3(Val-aGly-Leu-Val-aSer-Leu)-OH	10a	765.00	764.7	94.0
Ac-β^3(Val-aGly-Leu-Val-aGly-Leu)-OH	9b	763.03	762.7	85.5
Ac-β^3(Val-aGly-Leu-Val-aGly-Leu)-OH	10b	734.97	734.7	89.5
Ac-β^3(Arg-aSer-Val-Lys-aGly-Leu)-OH	9c	865.61	864.6	>95
Ac-β^3(Arg-aSer-Val-Lys-aGly-Leu)-OH	10c	837.55	836.5	>95
Ac-β^3(Arg-aGly-Val-Lys-aGly-Leu)-OH	9d	834.65	833.5	89.0
Ac-β^3(Arg-aGly-Val-Lys-aGly-Leu)-OH	10d	806.59	805.5	88.2

Table **S2**: Analytical data for all peptides possessing a functionalised staple

Peptide	% Yield	Mass Calc.	Mass Obs. $[M+H]^+$
12	95.05	797.5388	797.5392
13	13.54	955.3577	955.3565
15	98.03	829.5286	829.5281
17	17.59	440.2747*	440.5210 *

** seen as M+2 peak*

Yield values for peptides 13 and 17 include SPPS.

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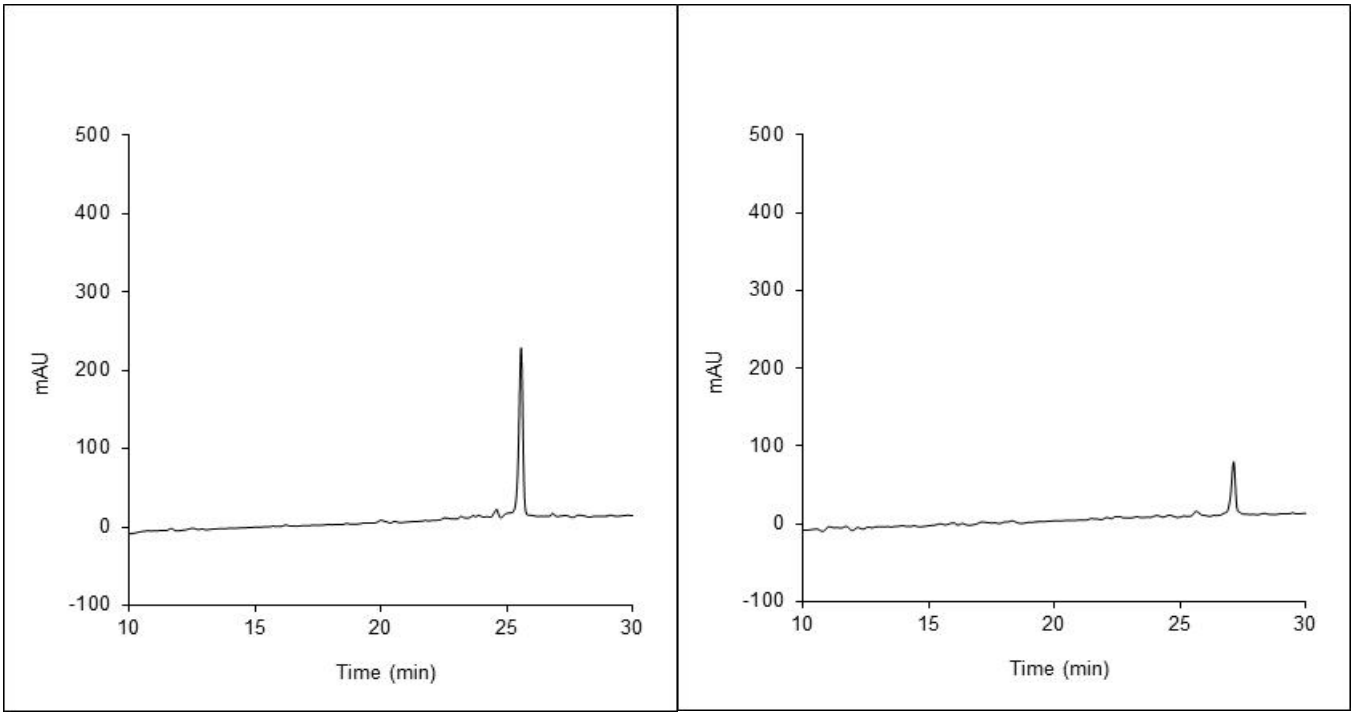
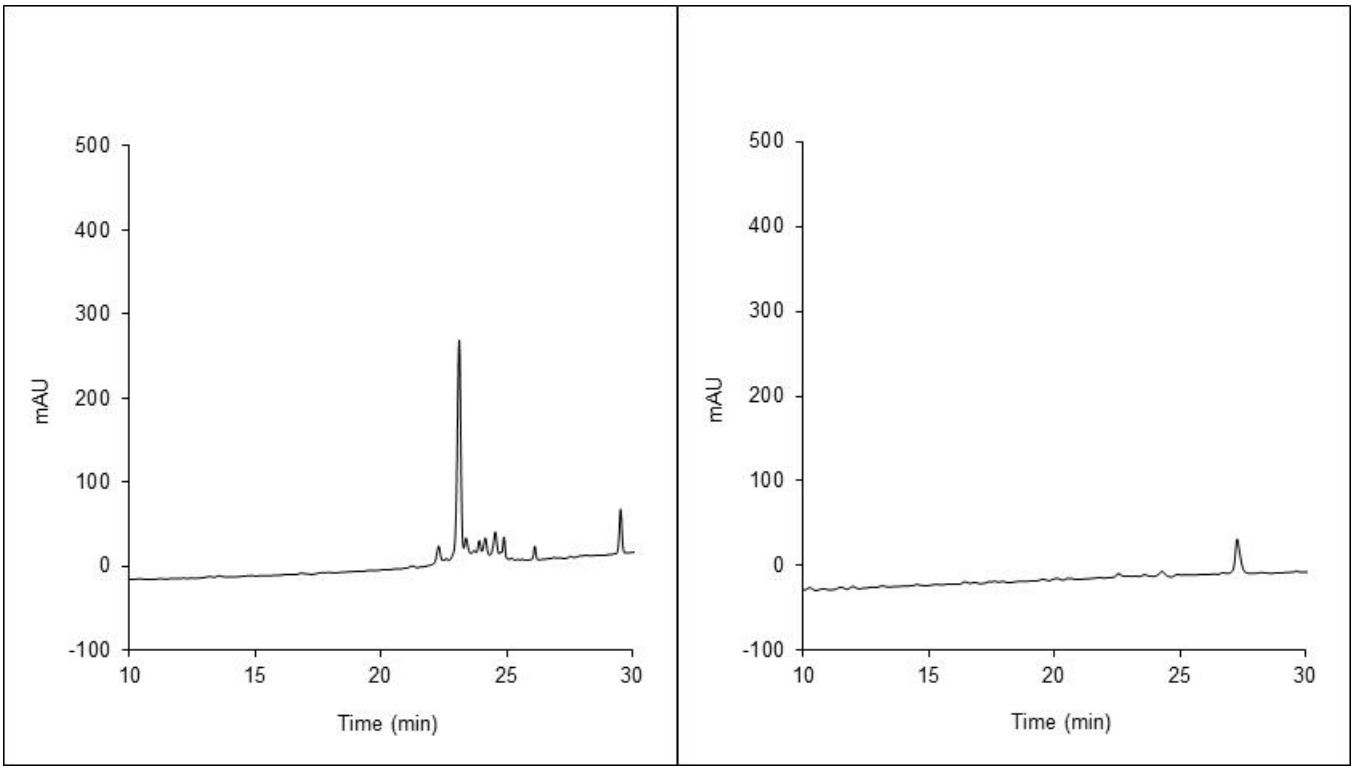


HPLC trace of **3a** (left) and **4a** (right)



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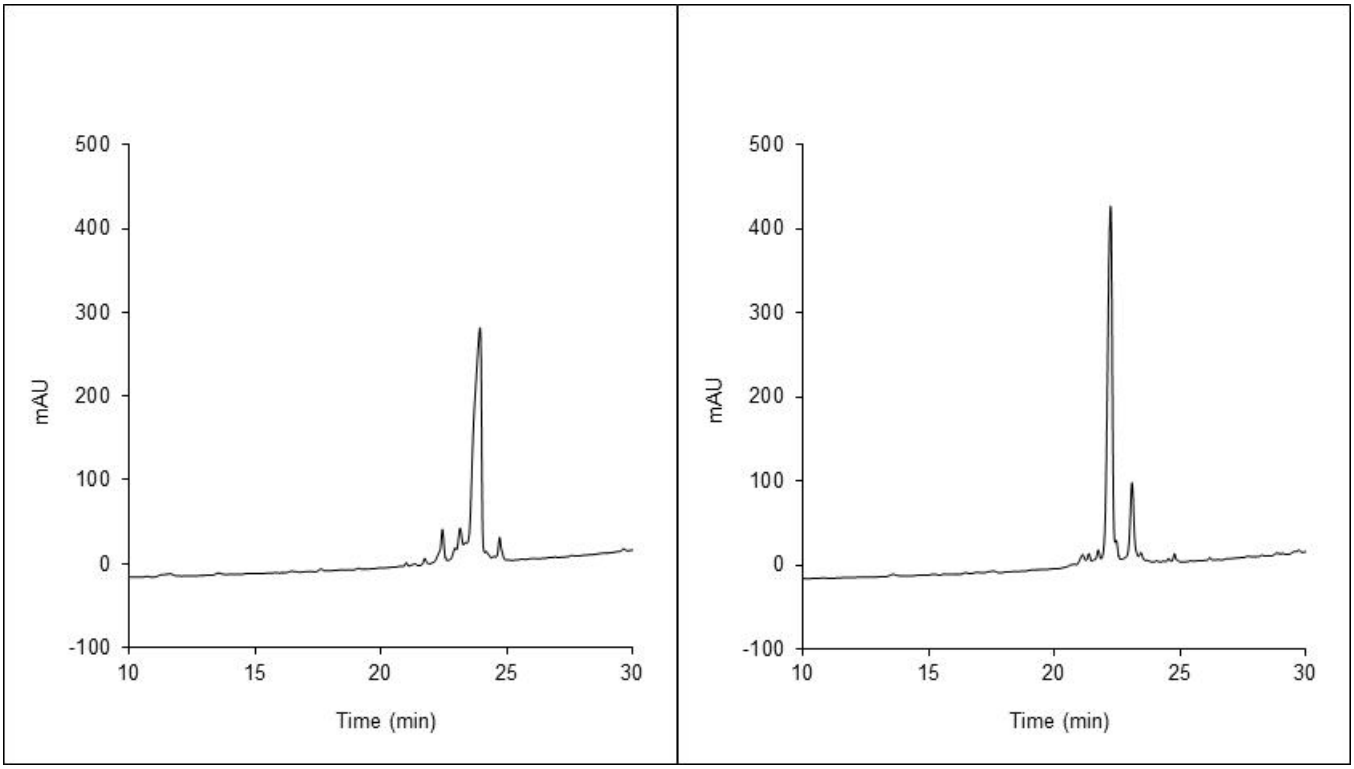
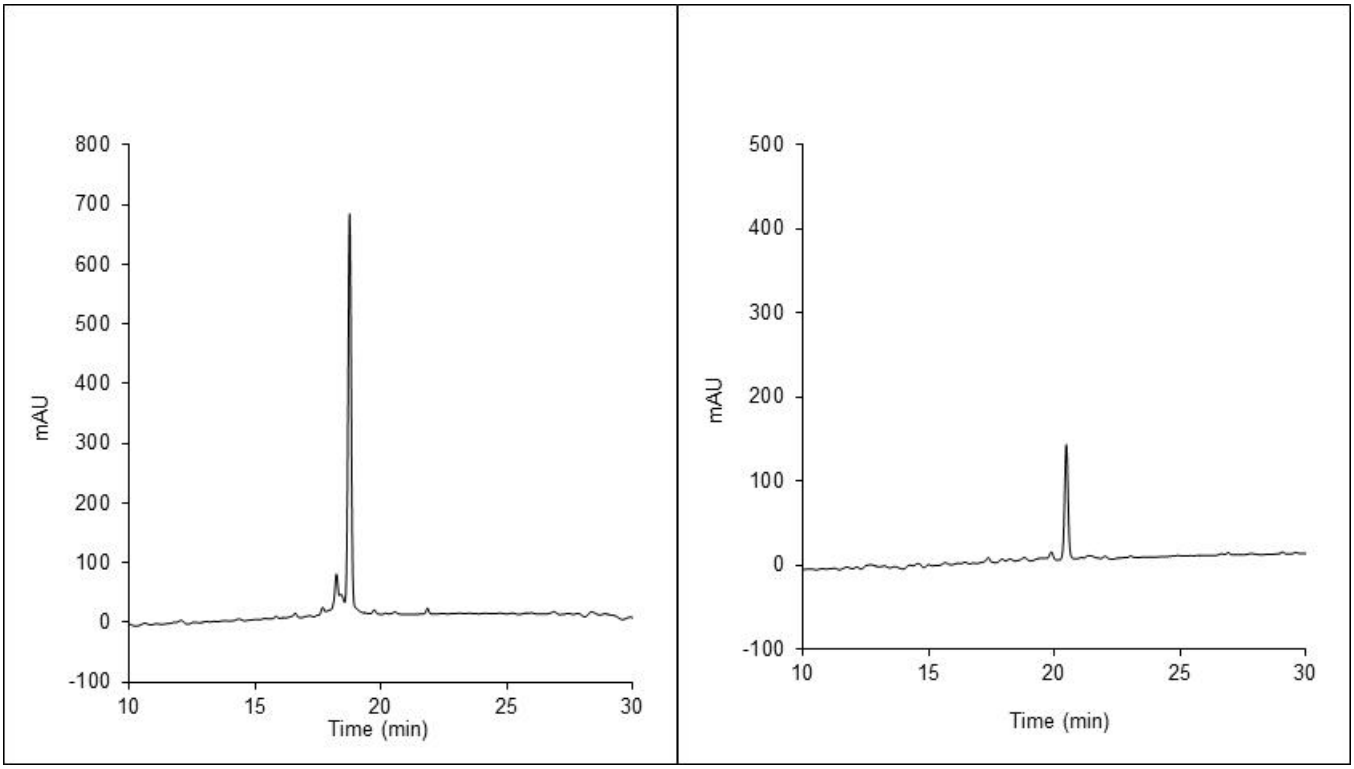


HPLC trace of **3c** (left) and **4c** (right)



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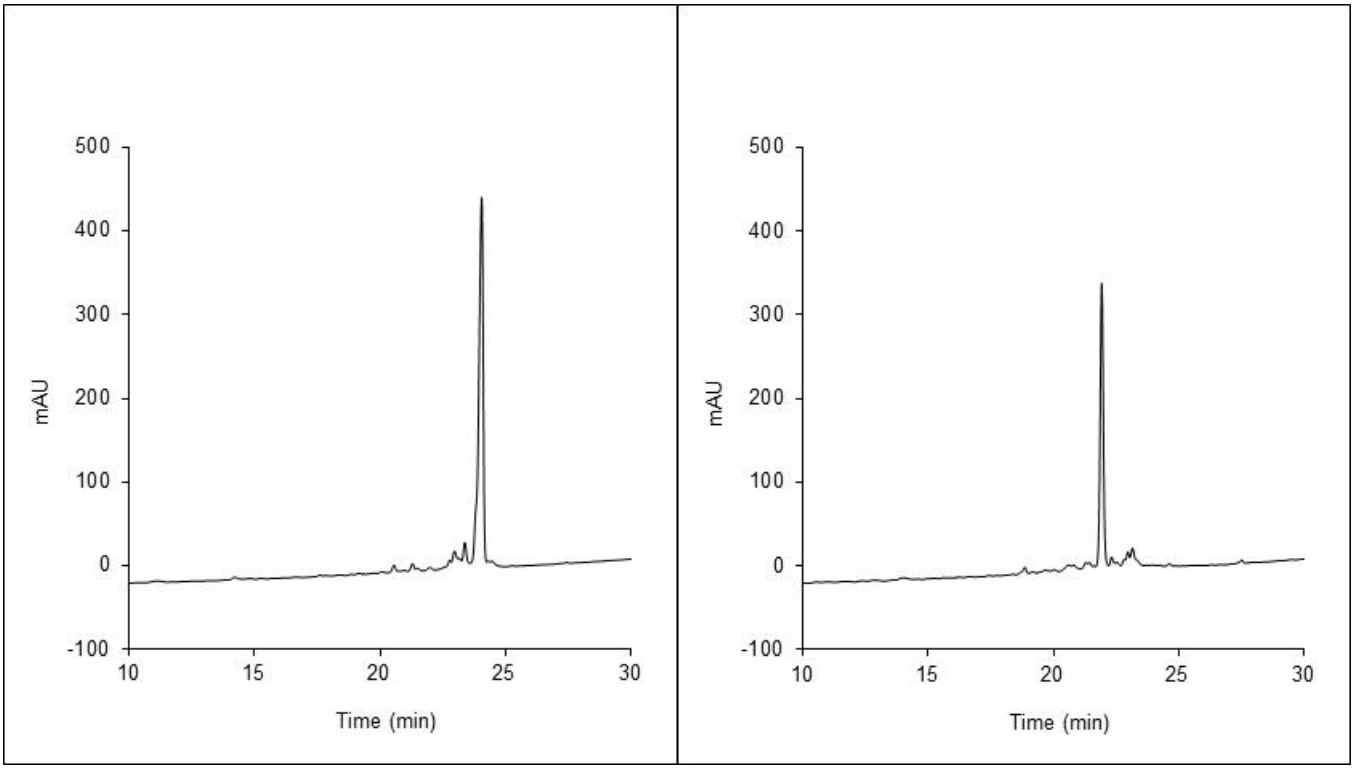

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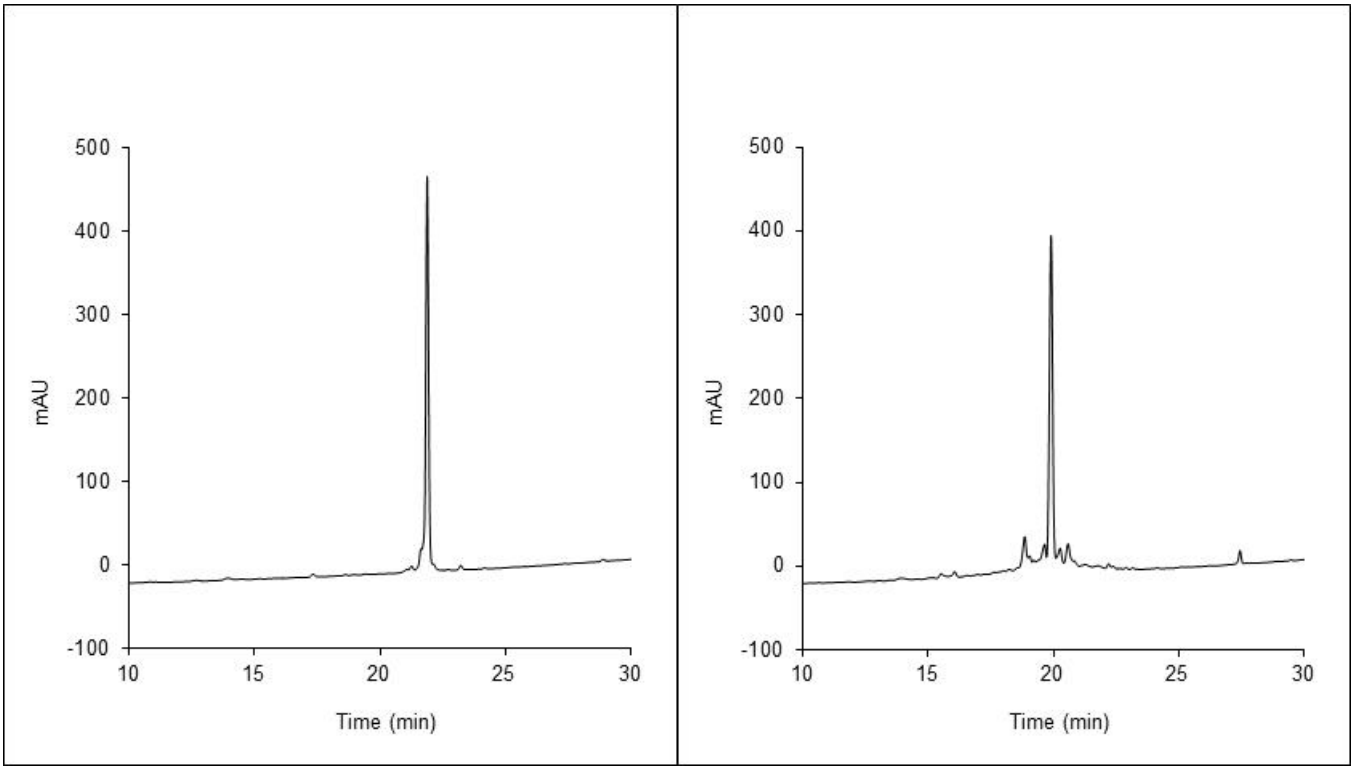

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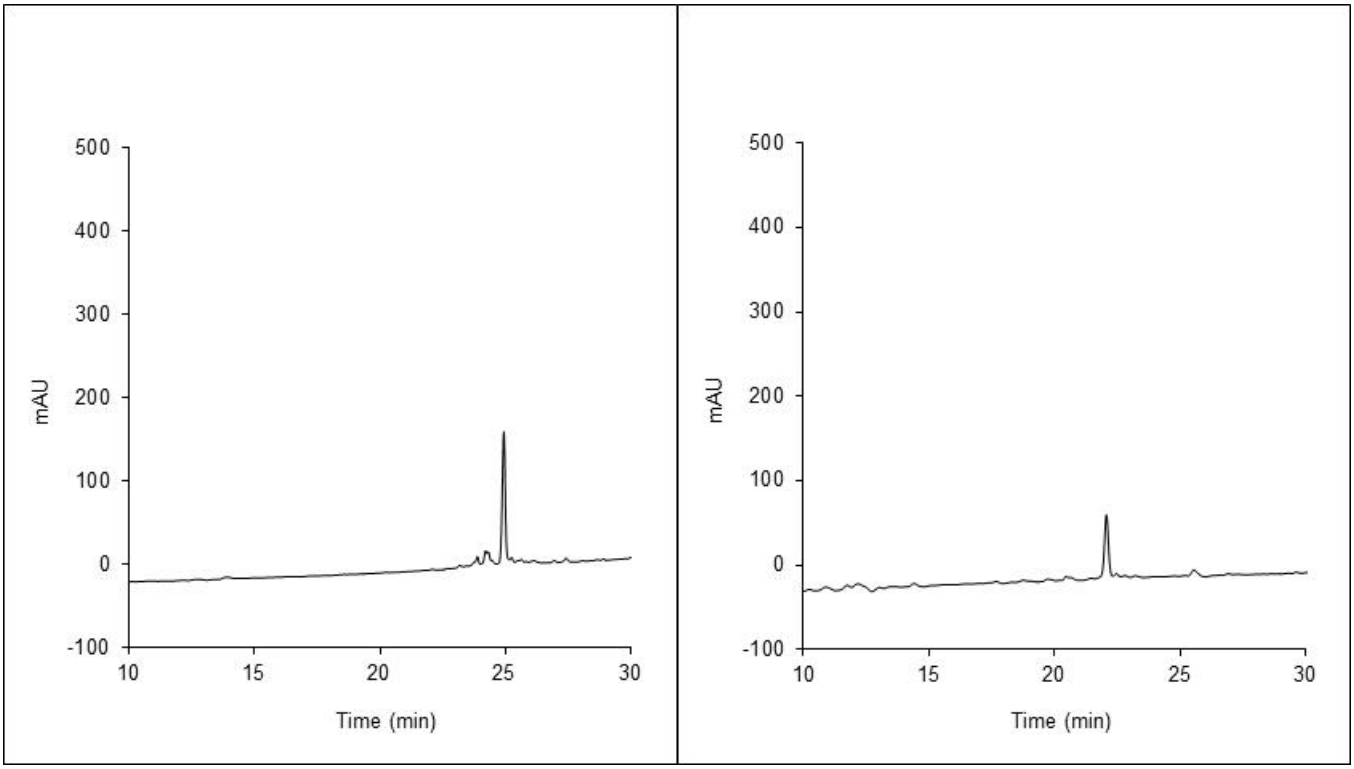

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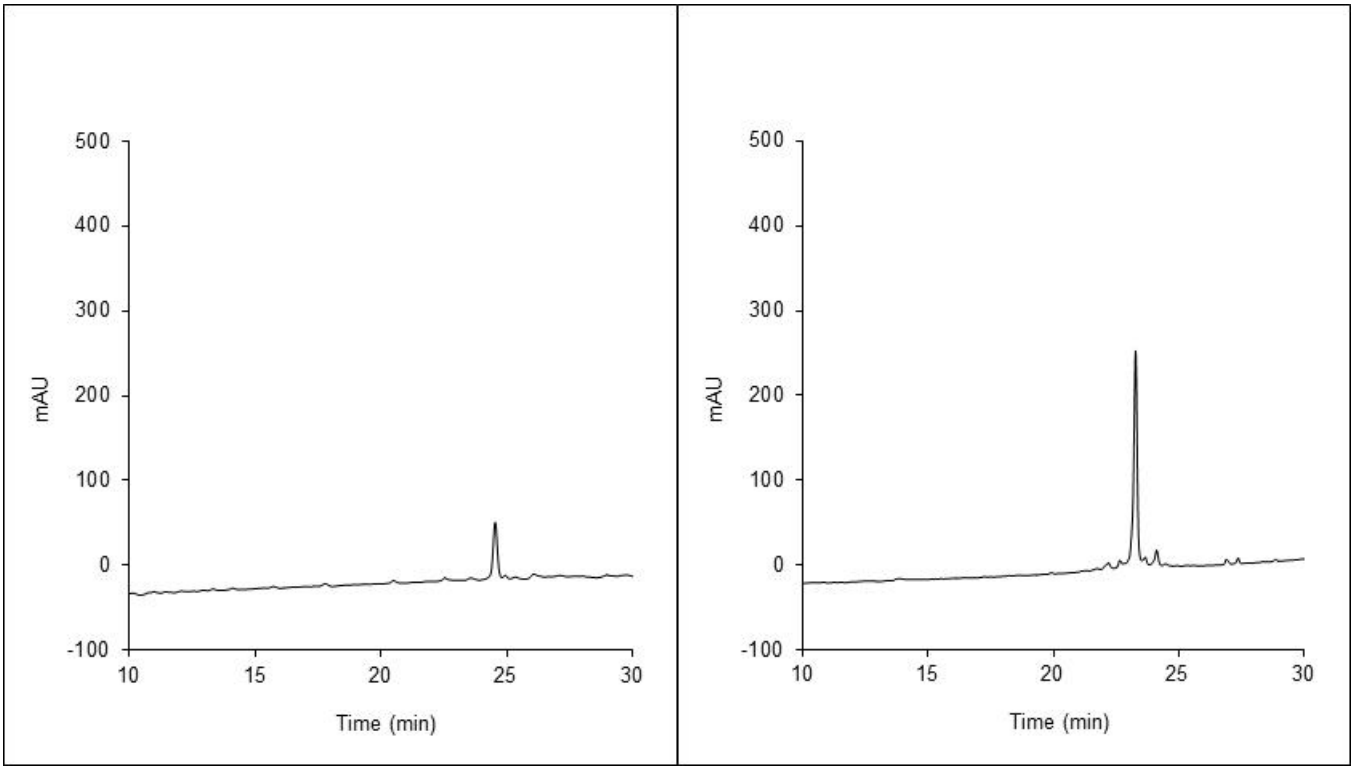

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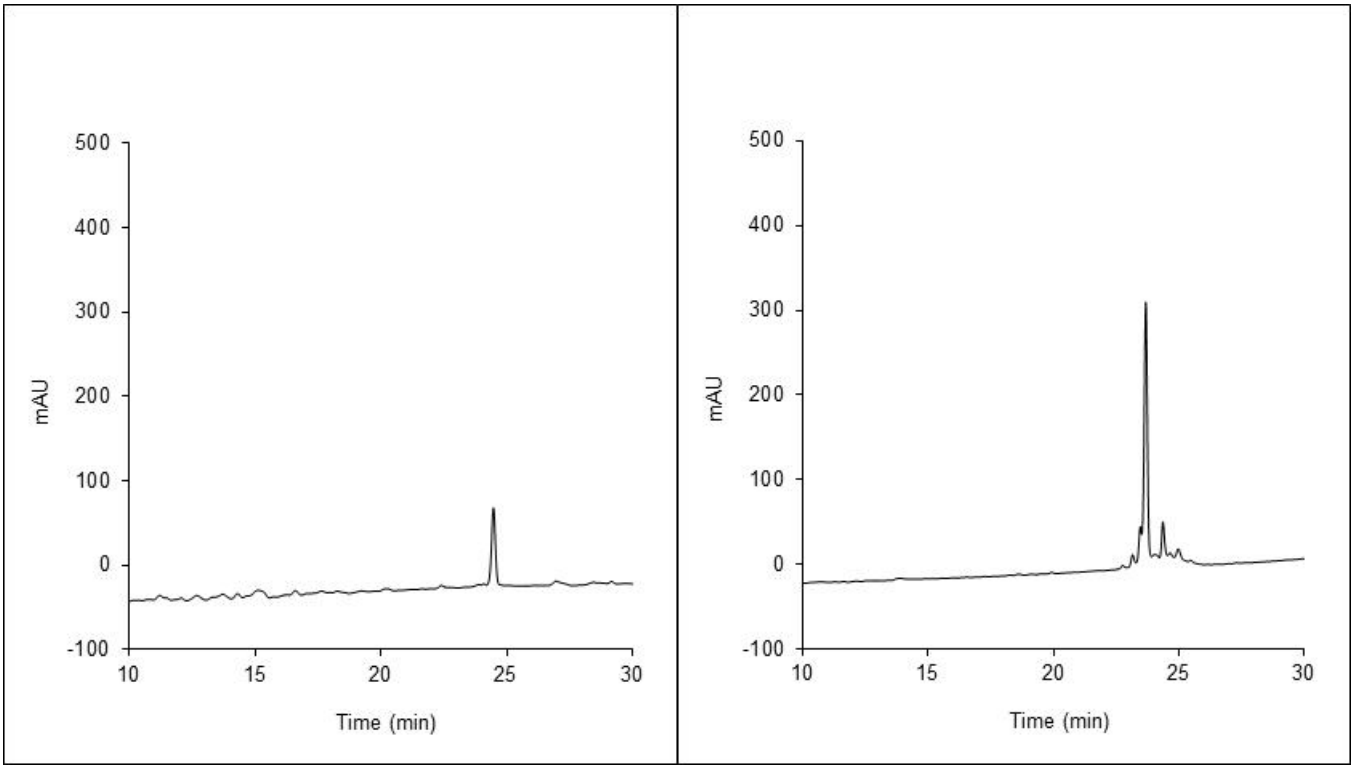

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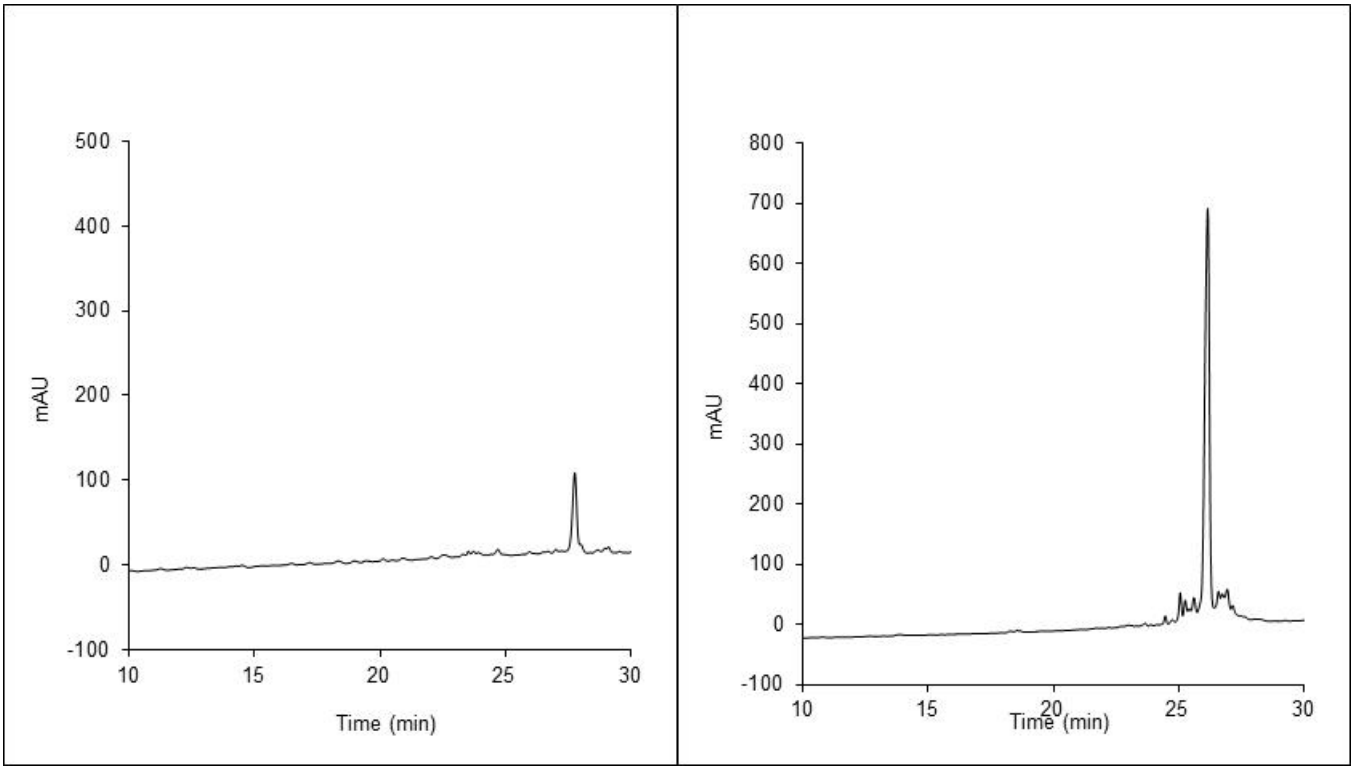

HPLC trace of **3j** (left) and **4j** (right)

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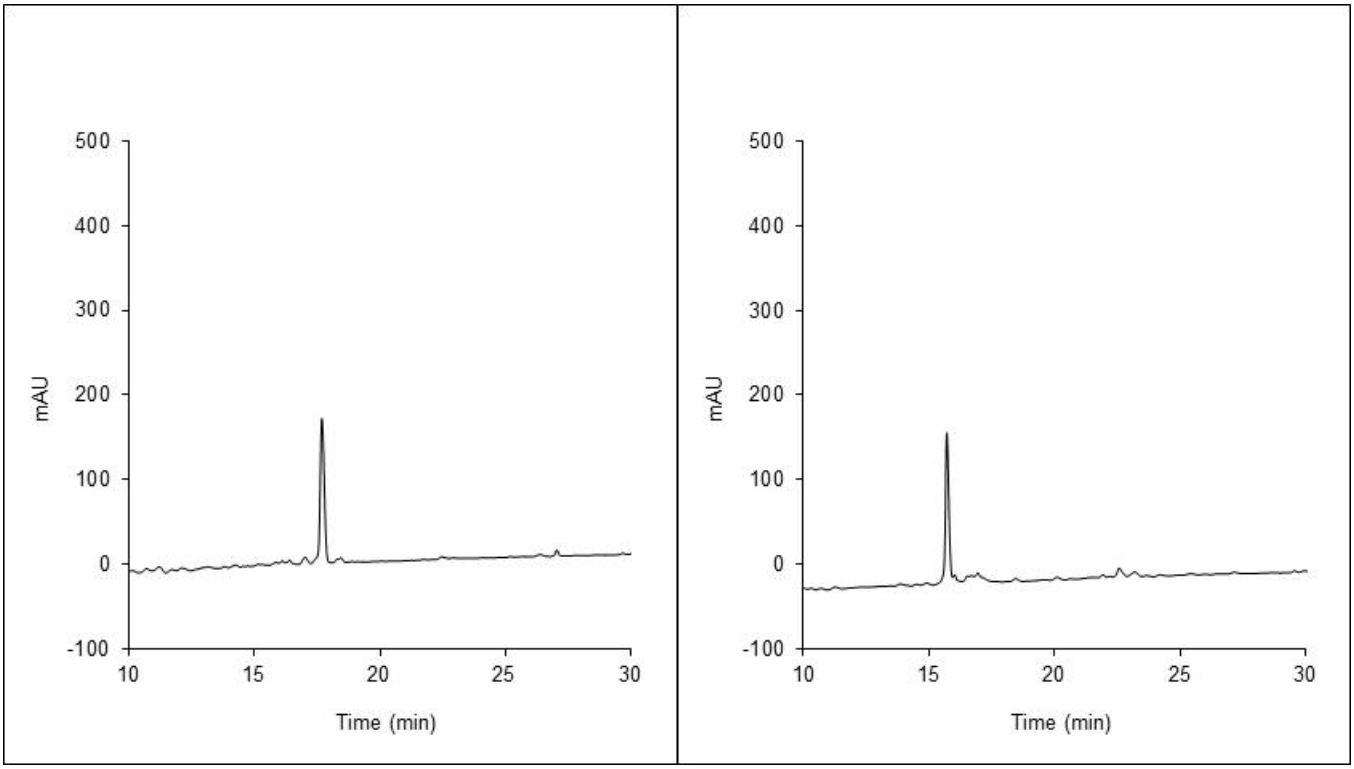

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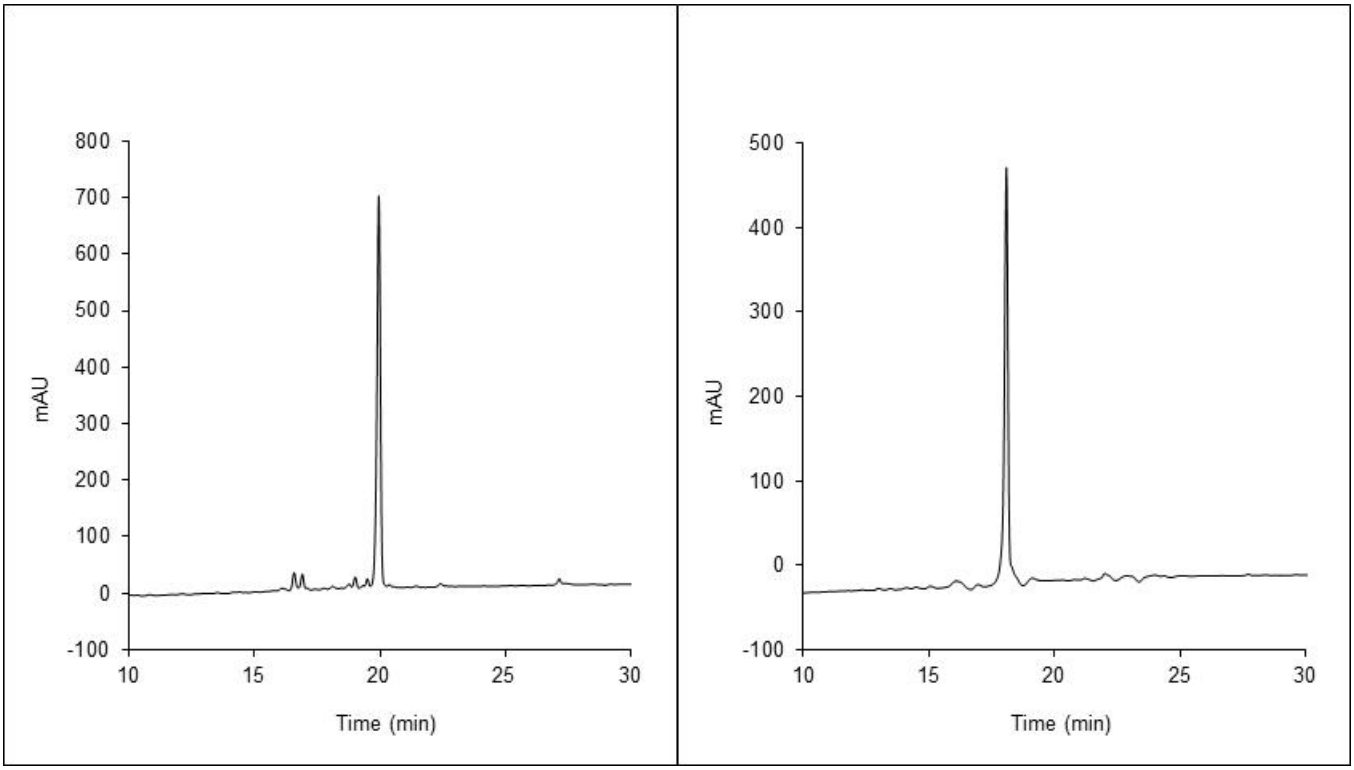

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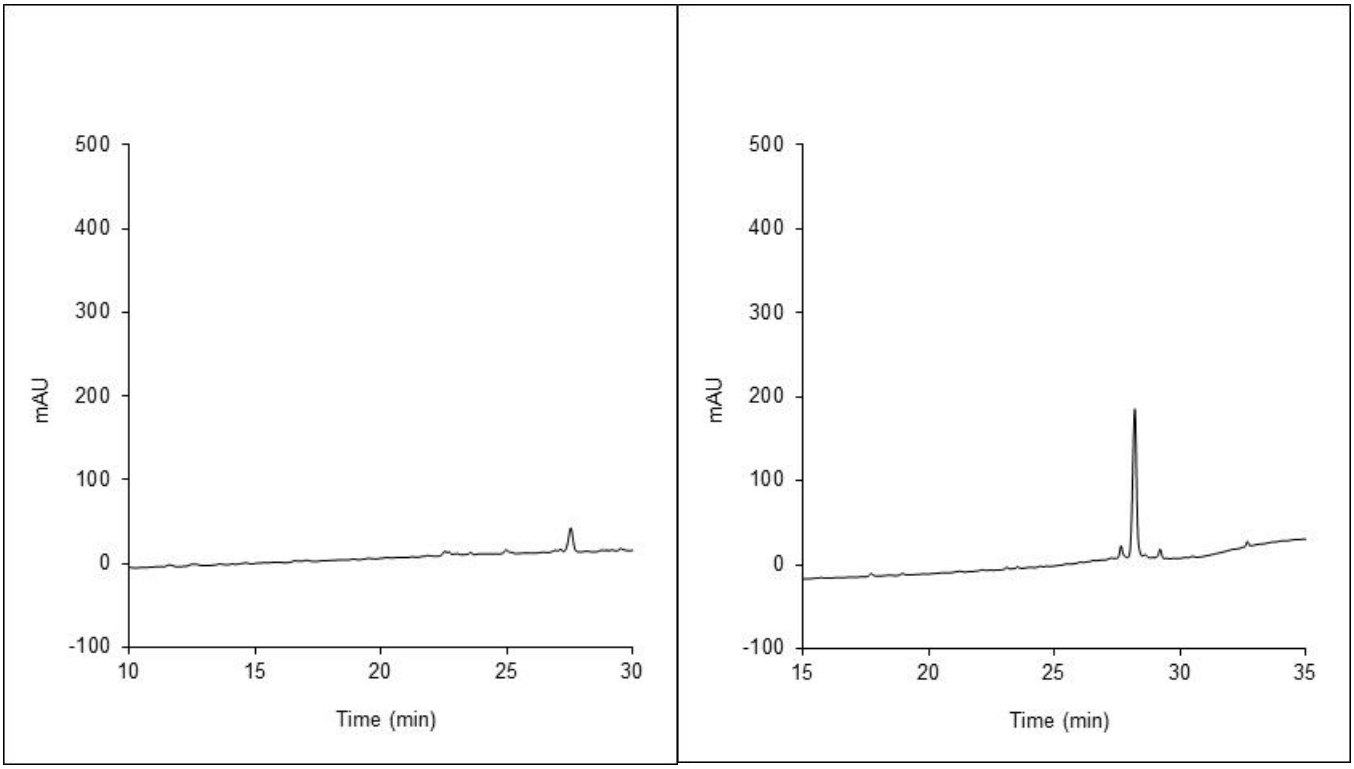

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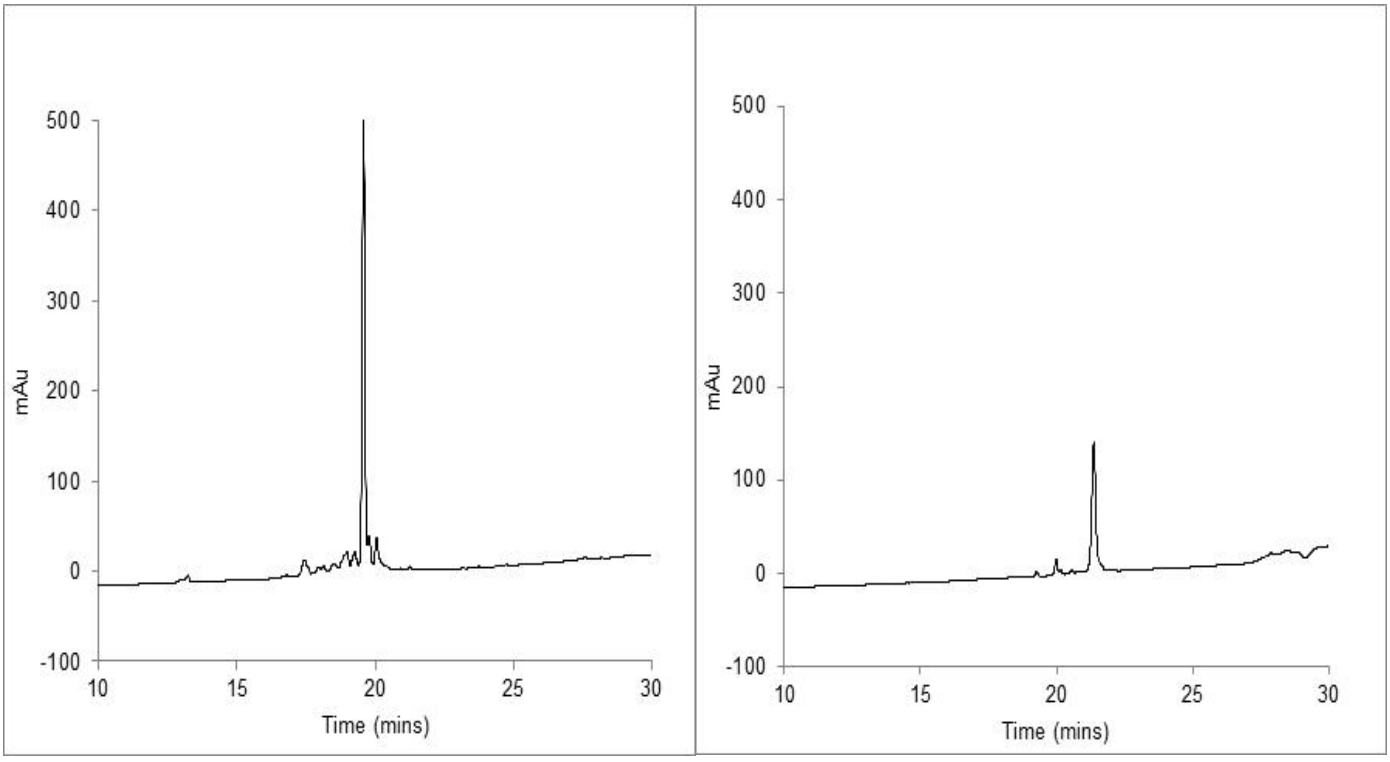

HPLC trace of **5** (left) and **6** (right)

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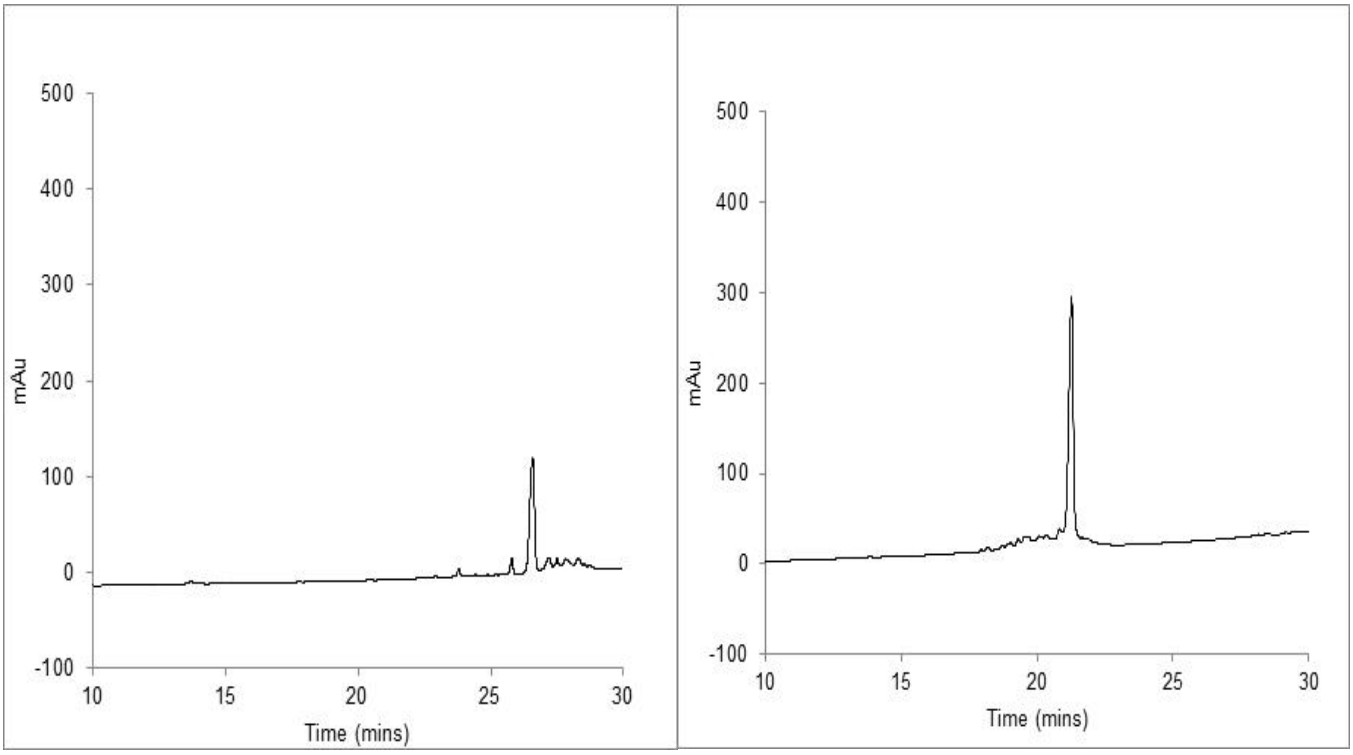

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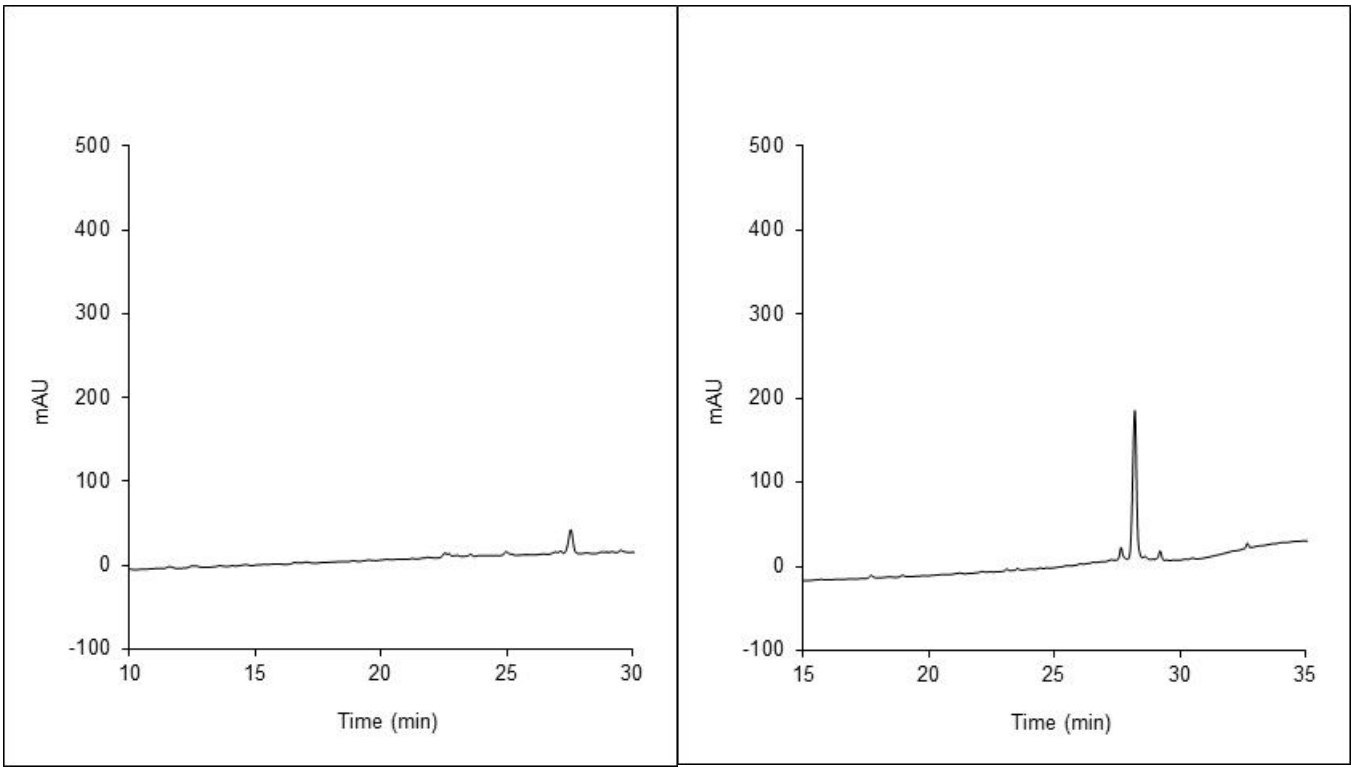

HPLC trace of **9a** (left) and **10a** (right)

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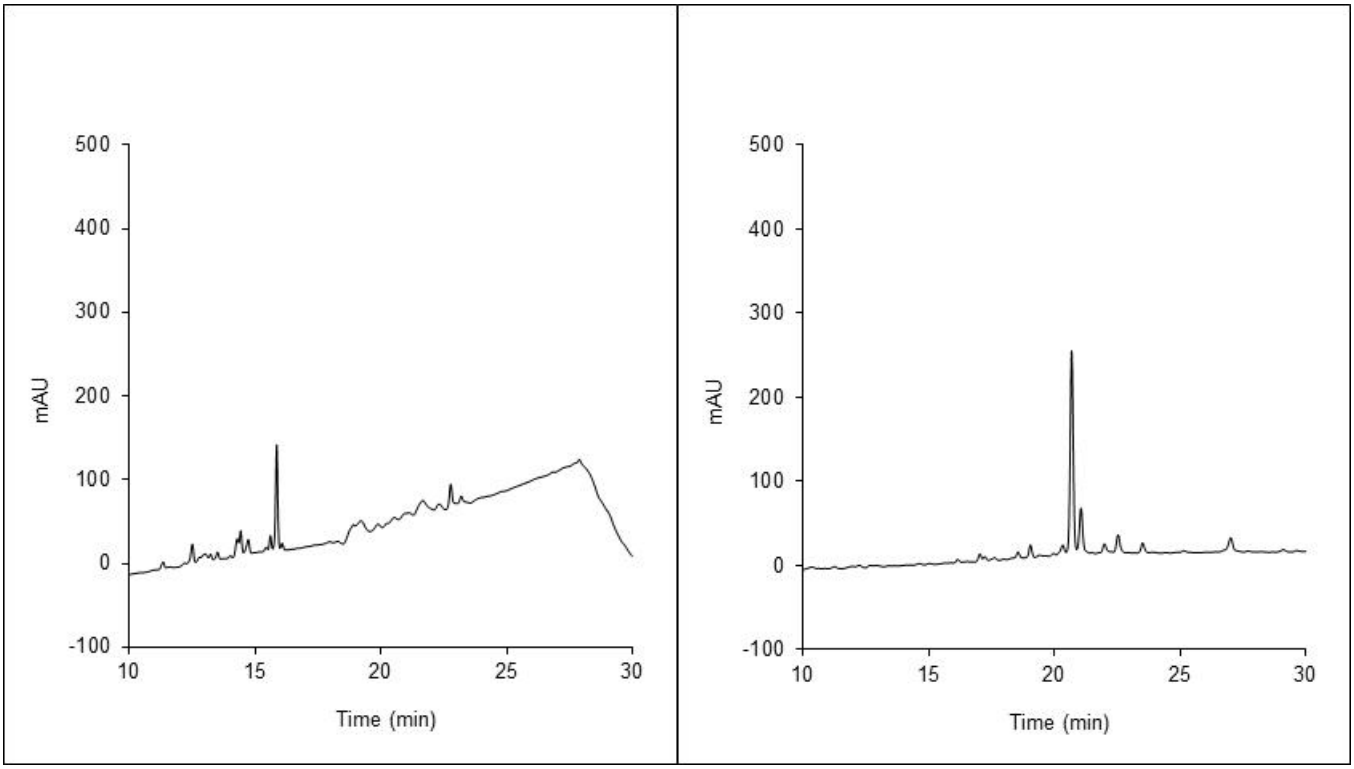
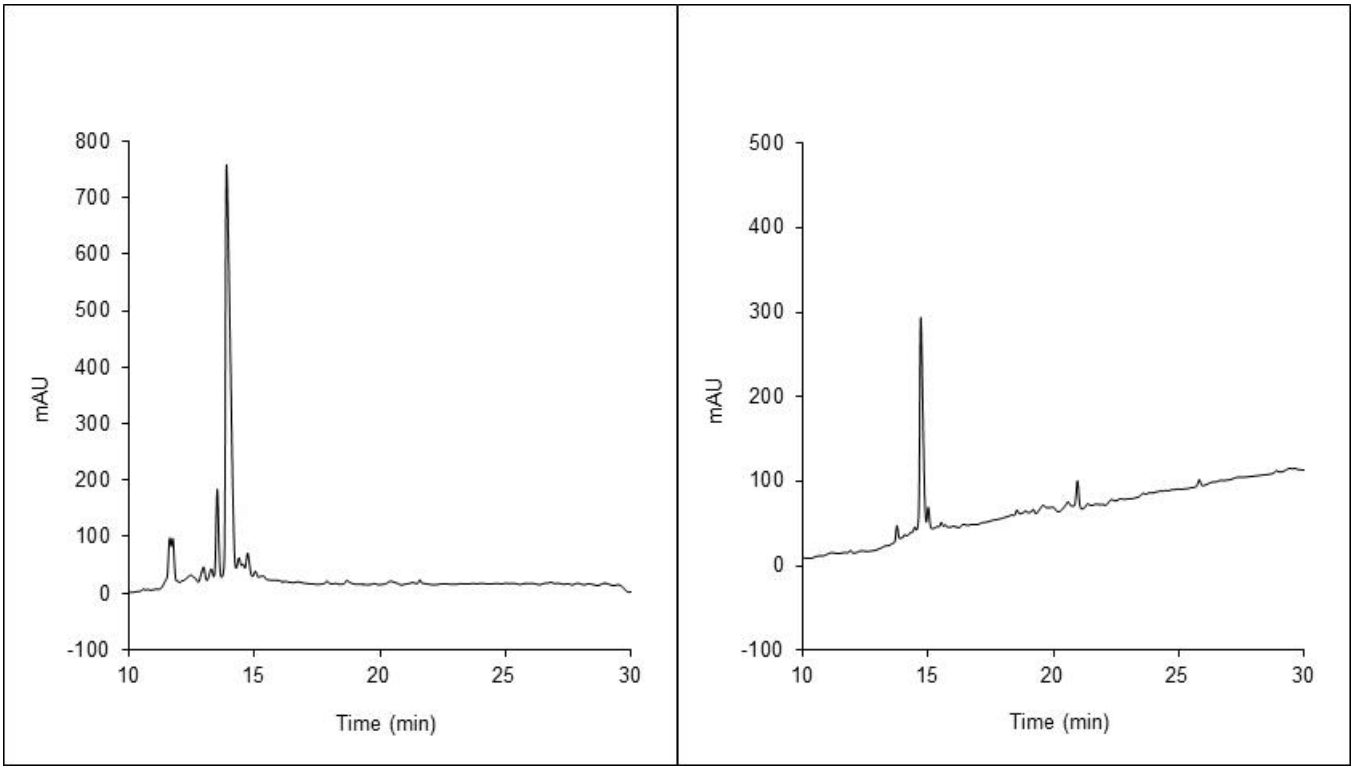


HPLC trace of **9b** (left) and **10b** (right)



HPLC trace of **9c** (left) and **10c** (right)

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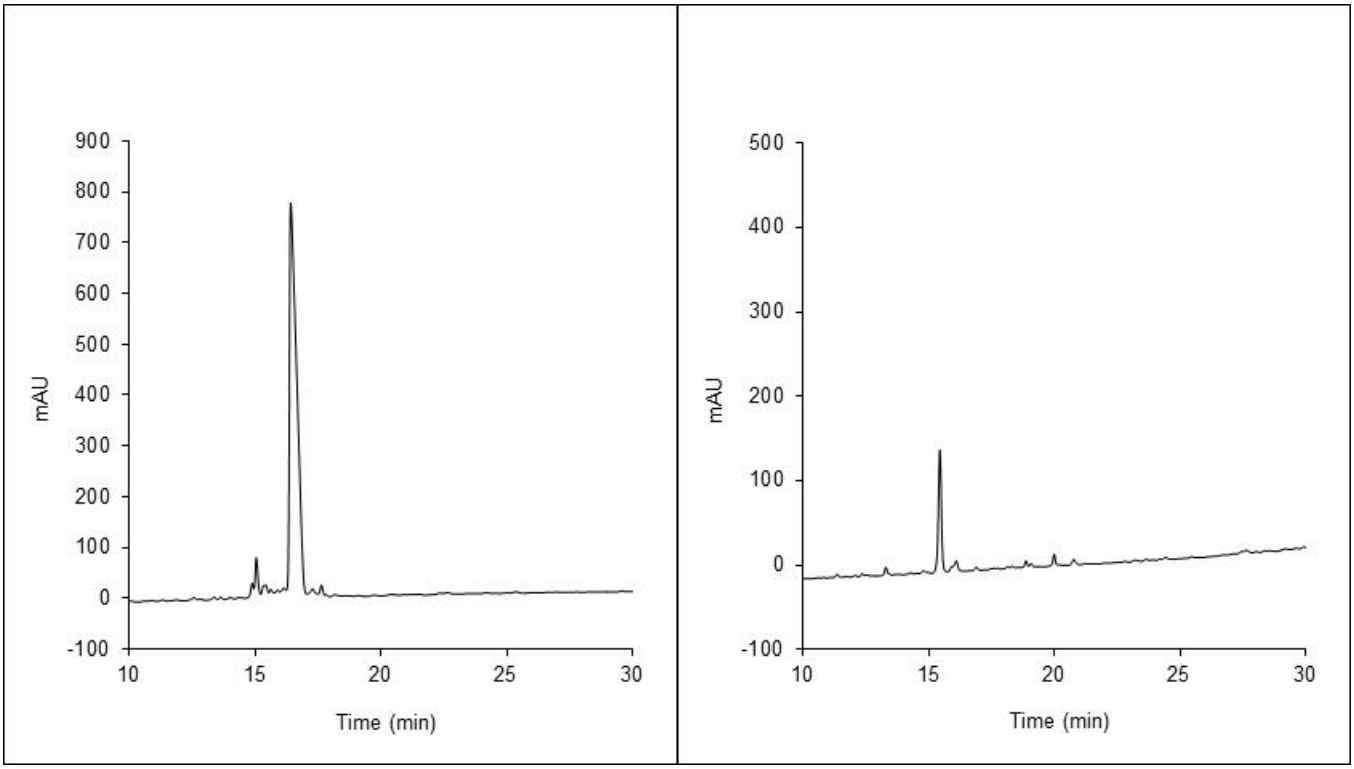

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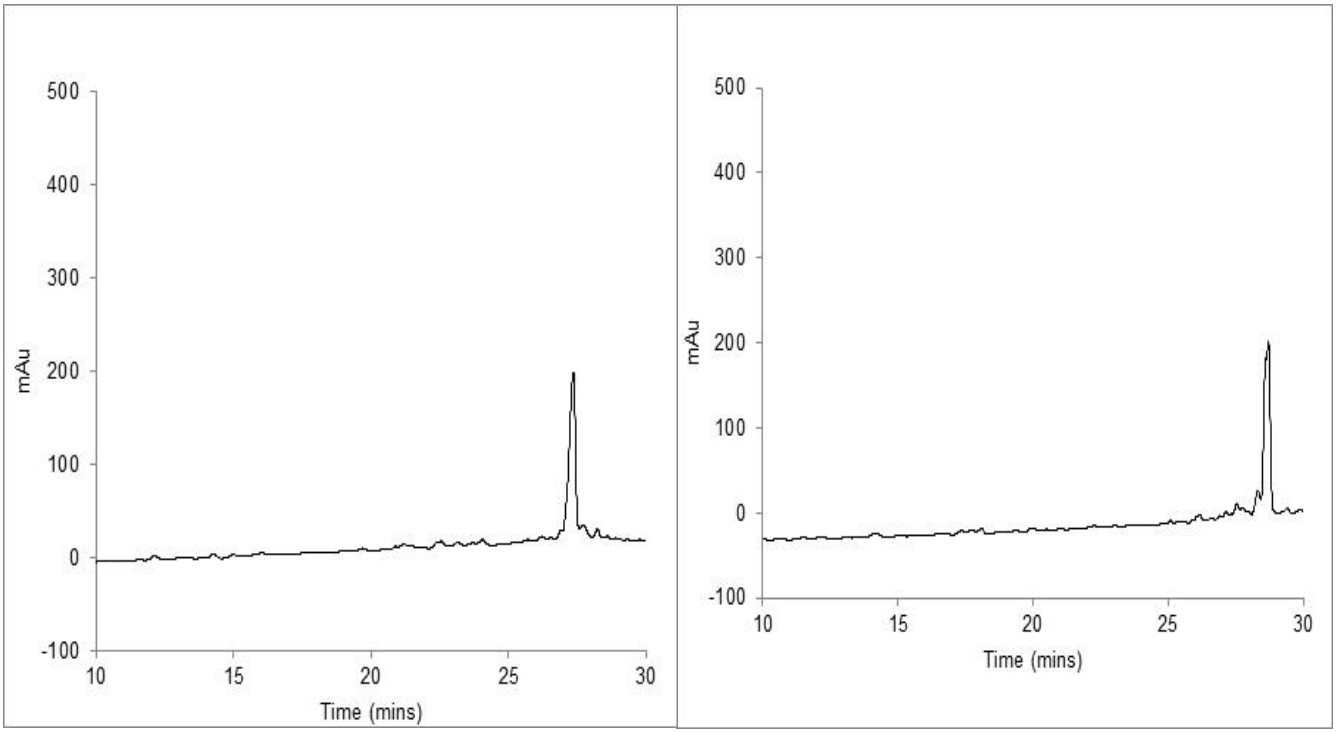

HPLC trace of **12** (left) and **13** (right)

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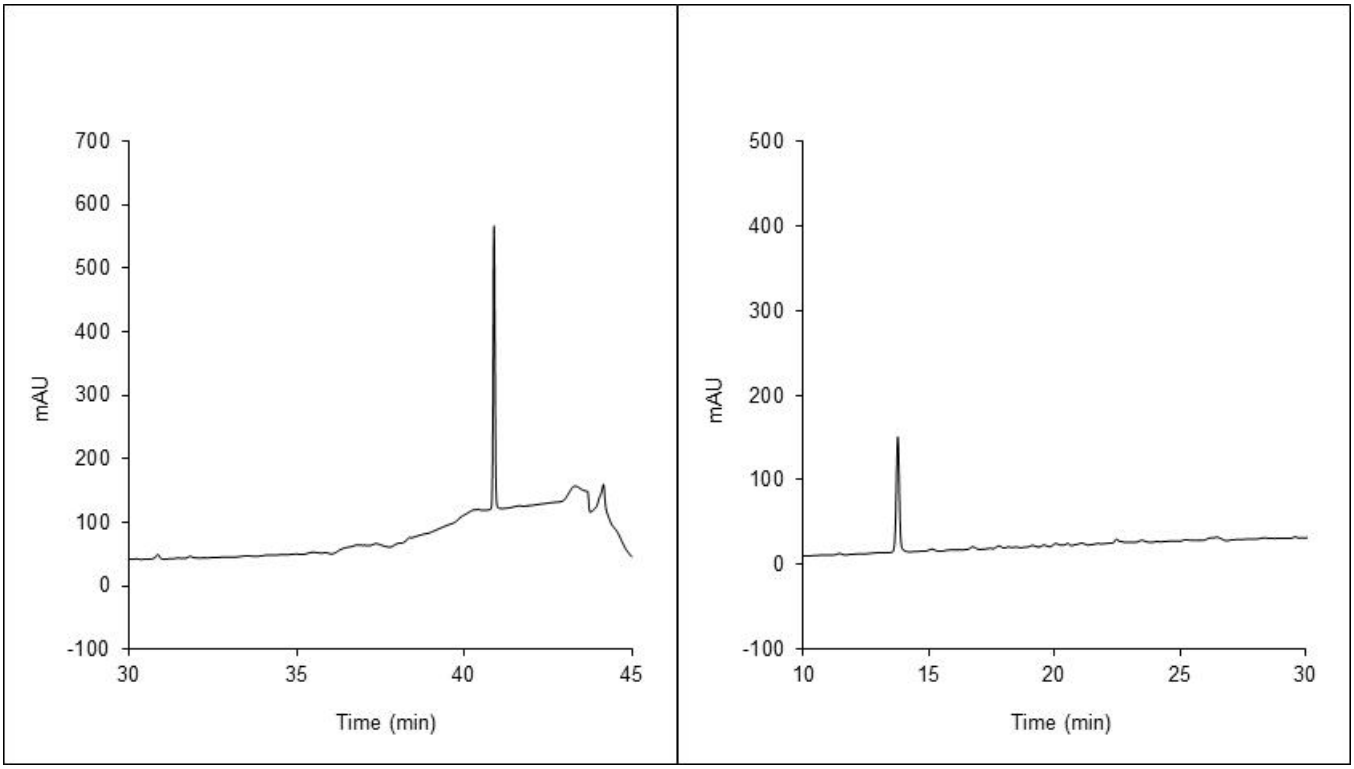

HPLC trace of **15** (left) and **17** (right)

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3. Circular dichroism (CD) analysis

CD measurements for all peptides were performed using a Jasco J-815 Circular Dichroism Spectropolarimeter (Jasco Corp., Japan), calibrated with *d*-10-camphorsulfonic acid. Secondary spectra for all peptides were obtained between wavelengths of 190-260 nm under the same parameters where scan speed was 50 nm/min, bandwidth was 1.0 nm and the resolution was 1 nm with a 1 second response. 0.1 mm quartz cuvettes were used in which three repeat scans were compiled to generate the average spectra. β-peptides were dissolved in methanol, trifluoroethanol or acetonitrile:phosphate buffer (3:1, 5 mM phosphate buffered saline, pH 7.4) and made up to a final concentration of 60 μM. The CD signal resulting from the solvent alone was subtracted from the spectrum of each β-peptide solution.

The quartz cell temperature of 25 ºC was maintained using a thermostatic water bath and stabilised using a Peltier temperature controller. The results were evaluated using the Jasco Spectra Manager and any remaining noise in the spectra was removed using the Jasco Fast Fourier Transform algorithm. Data were converted to ellipticity (deg cm^2 $dmol^{-1}$) according to the equation:

$$[\Theta] = \Psi / (1000nlc),$$

where Ψ is the CD signal in degrees, n is the number of amides, l is the path length in centimetres, and c is the concentration in decimoles per mL.

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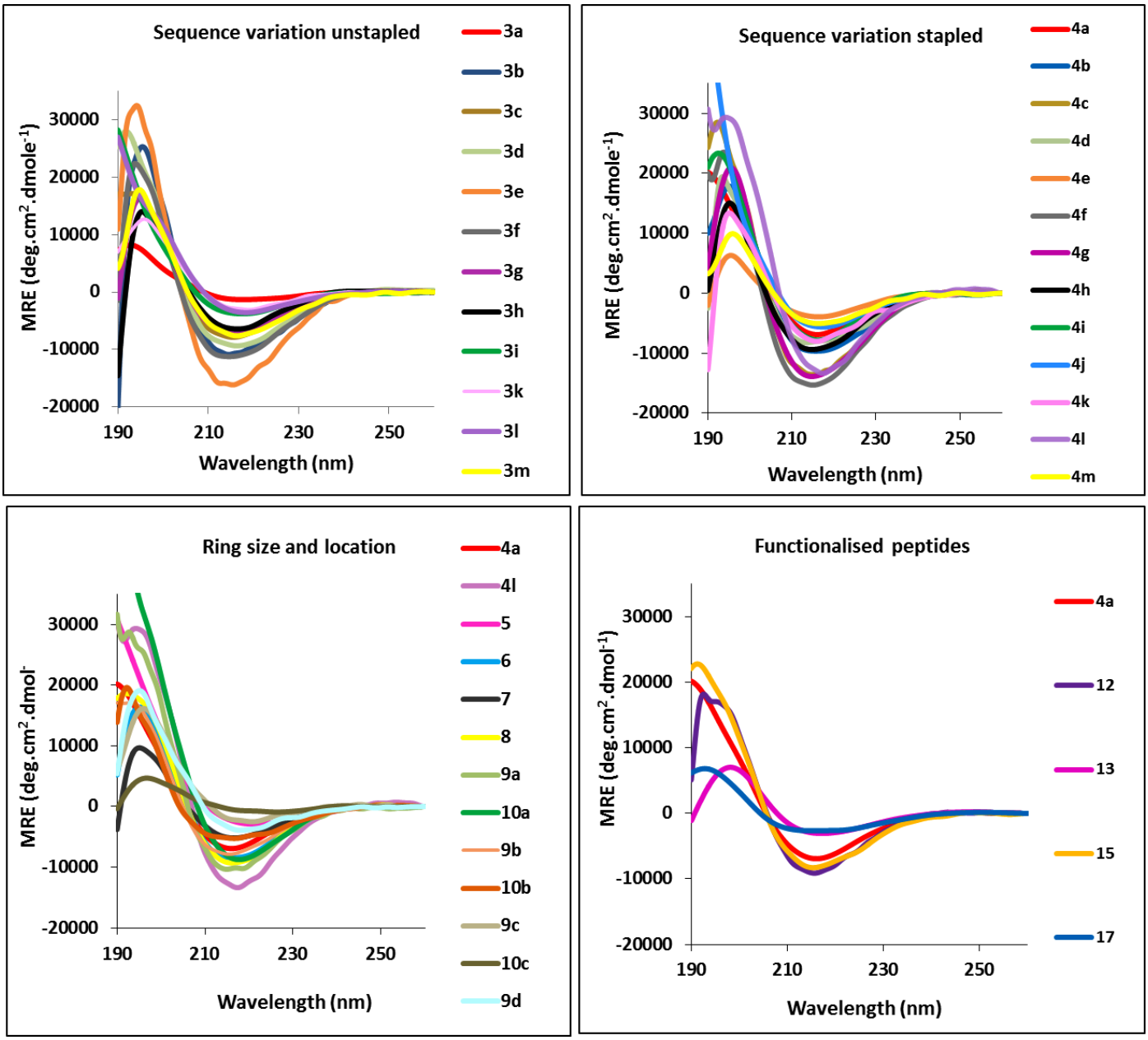

Figure **S1:** Analysis of all peptides by CD in MeOH. Peptides **3a** and **3j** were insoluble in MeOH and could not be assessed.

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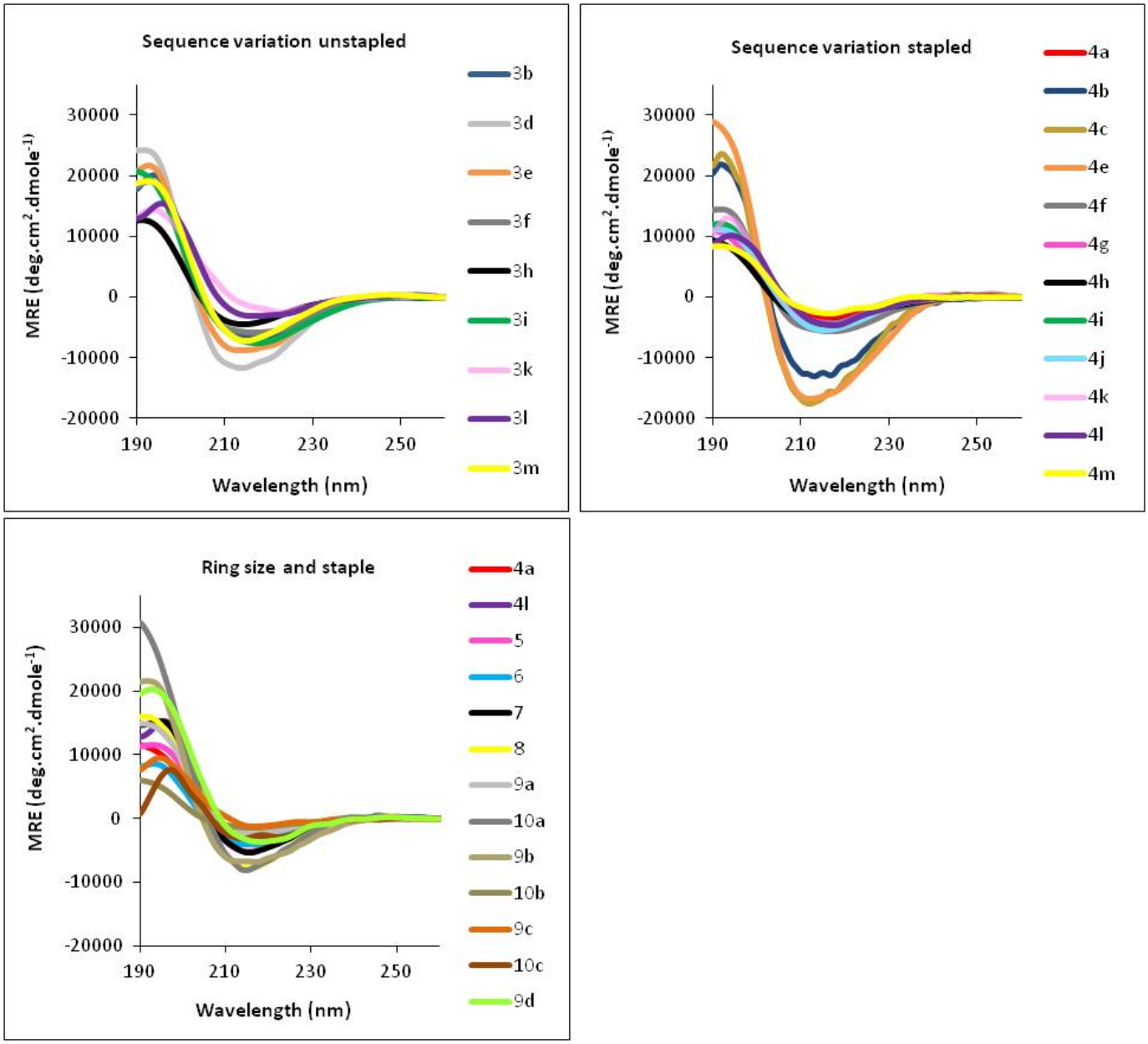

Figure **S2**: CD spectra of all peptides in TFE.

Note: Peptides **3c**, **4d**, **3g**, **3j**, **12**, **13**, **15**, **and 17** were insoluble in TFE.

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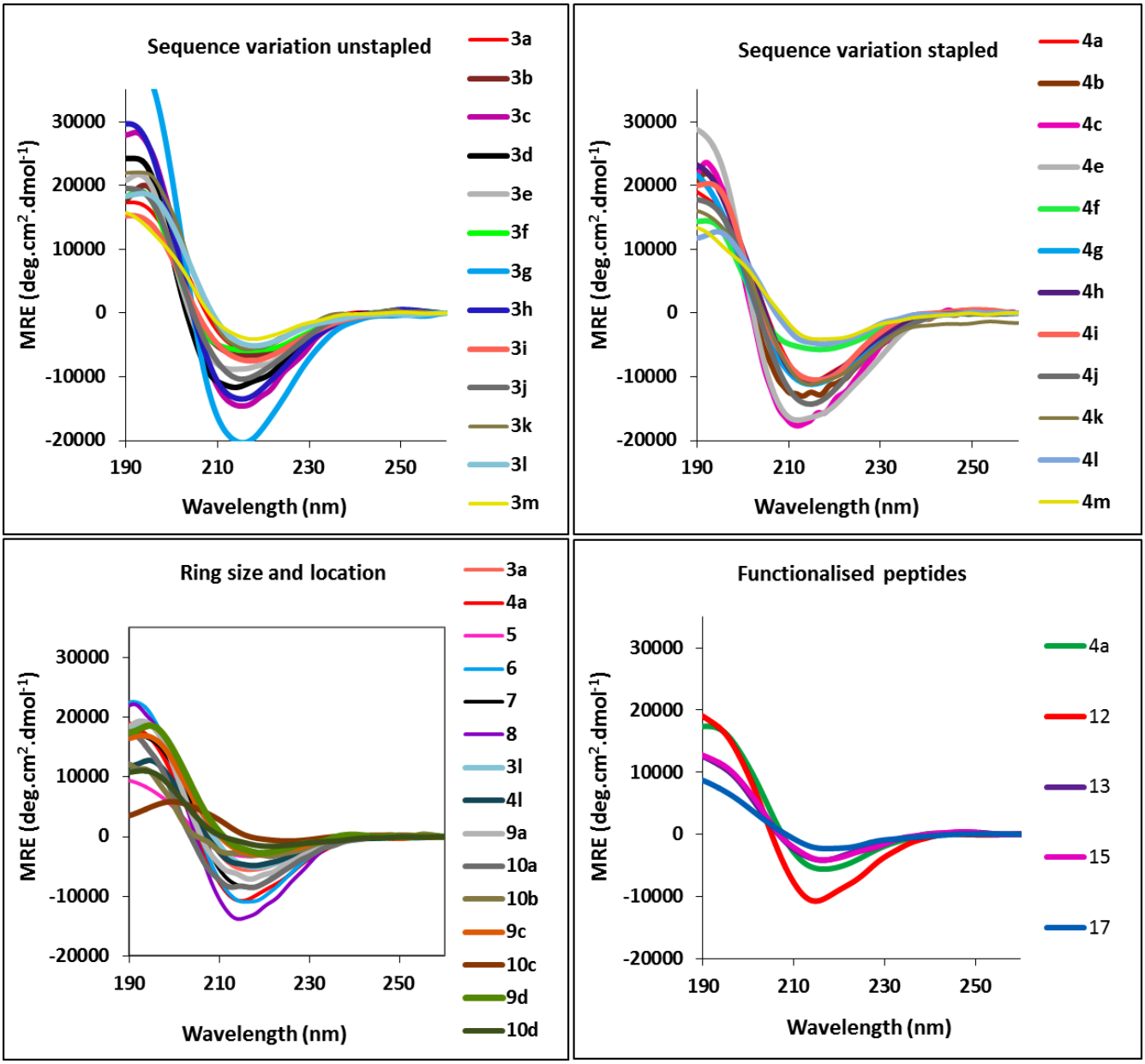

Figure S3: CD spectra of all peptides in ACN/Phosphate buffer.

Note: Peptides **4d** and **9b** were insoluble in ACN:Phosphate buffer.

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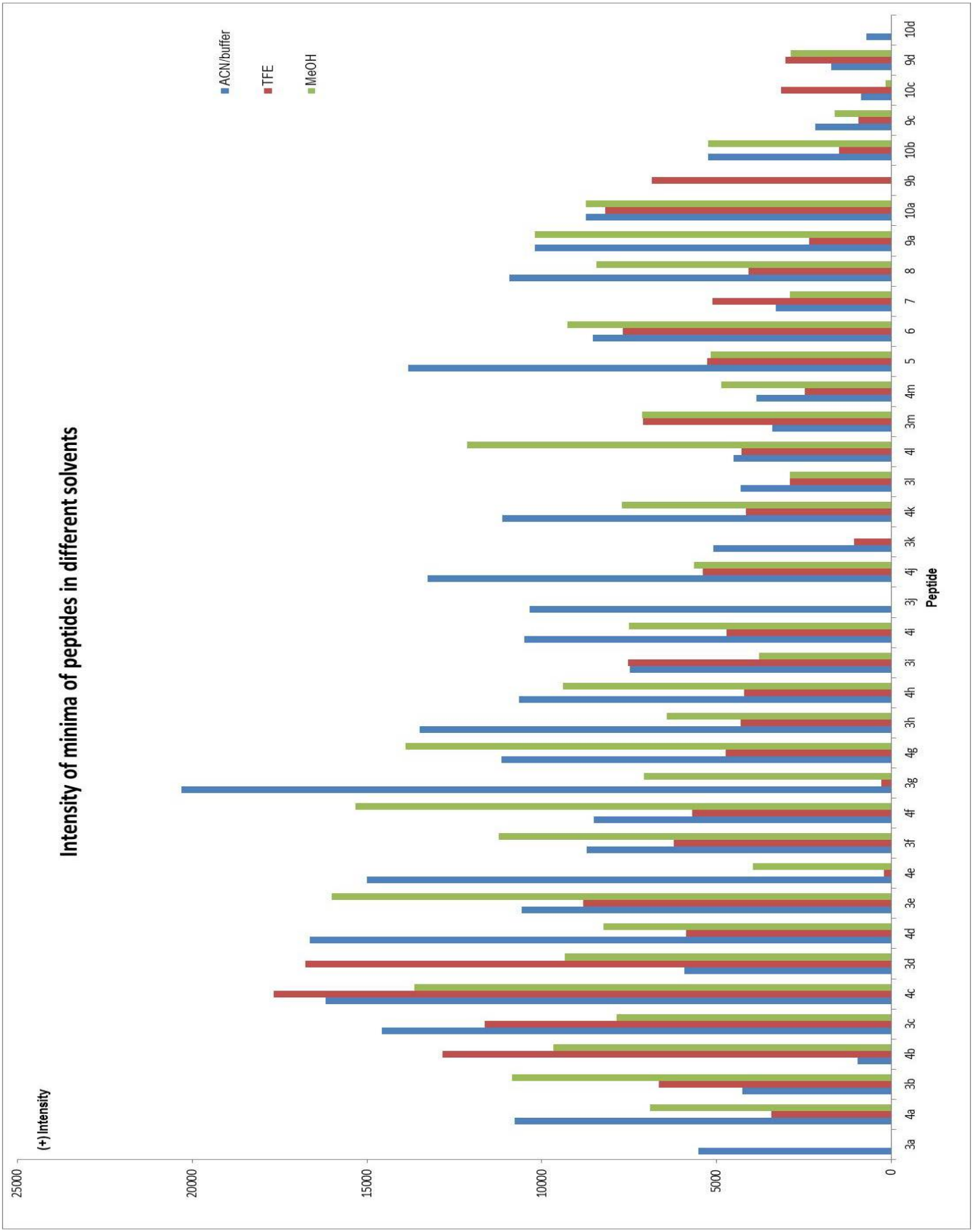

Figure S4: CD minima of all peptides in all solvents studied.

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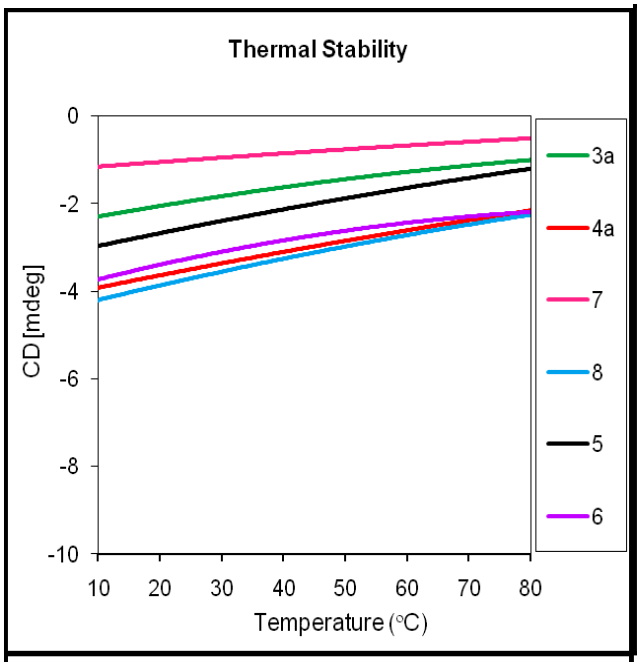

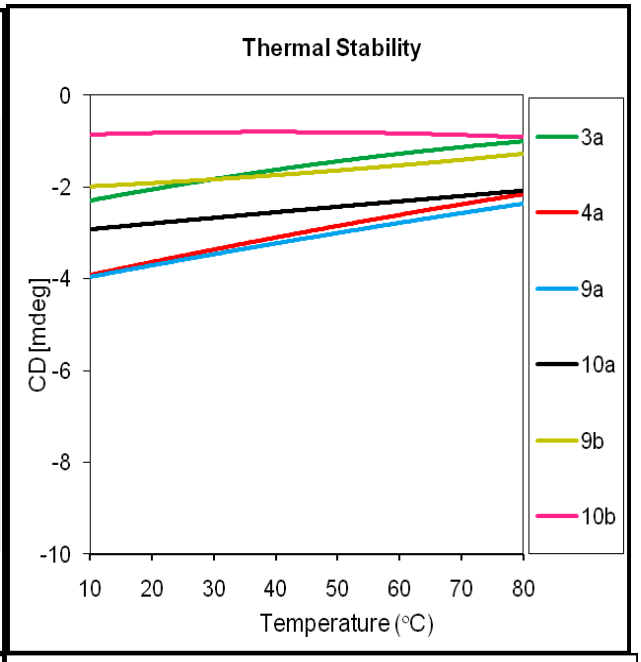

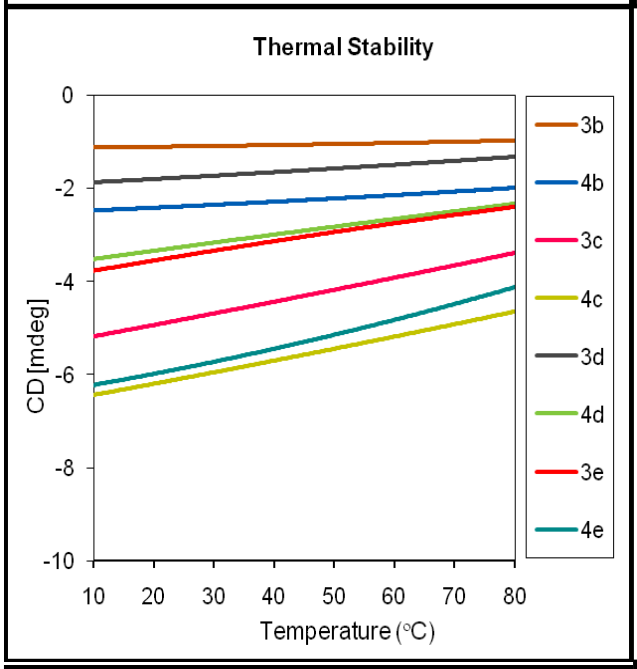

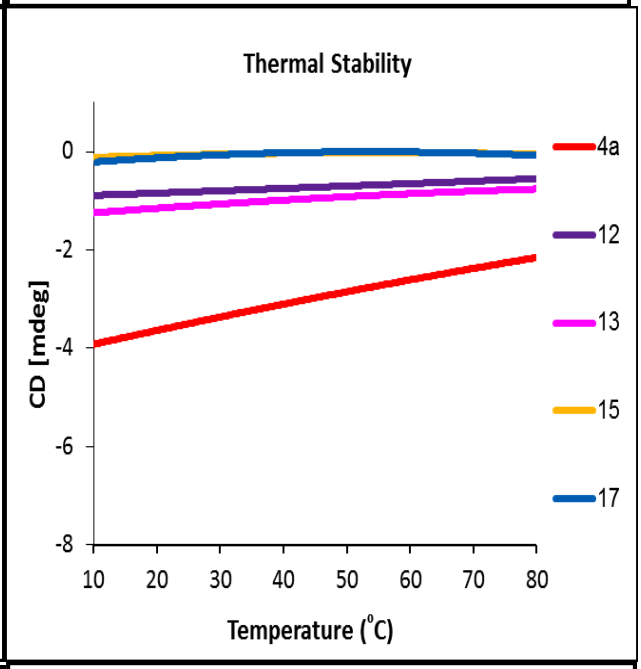

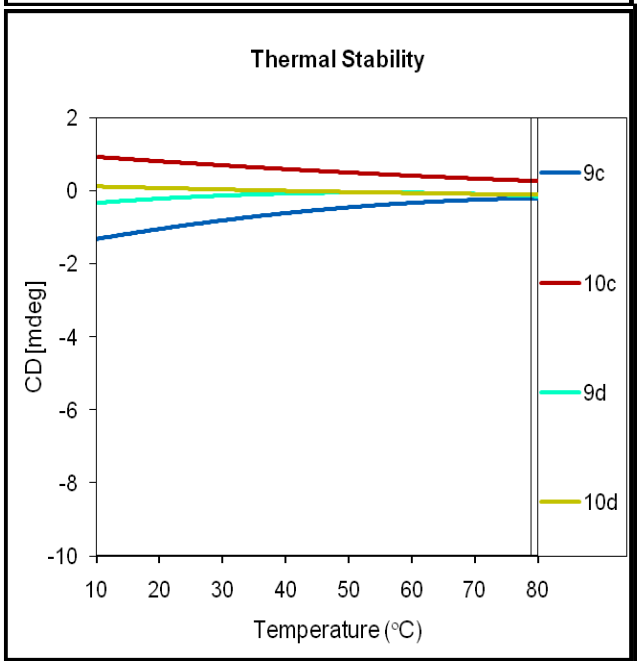

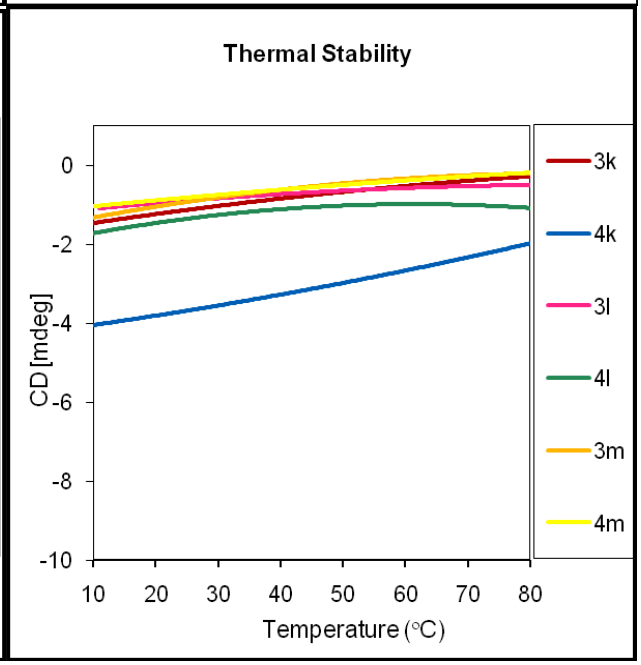

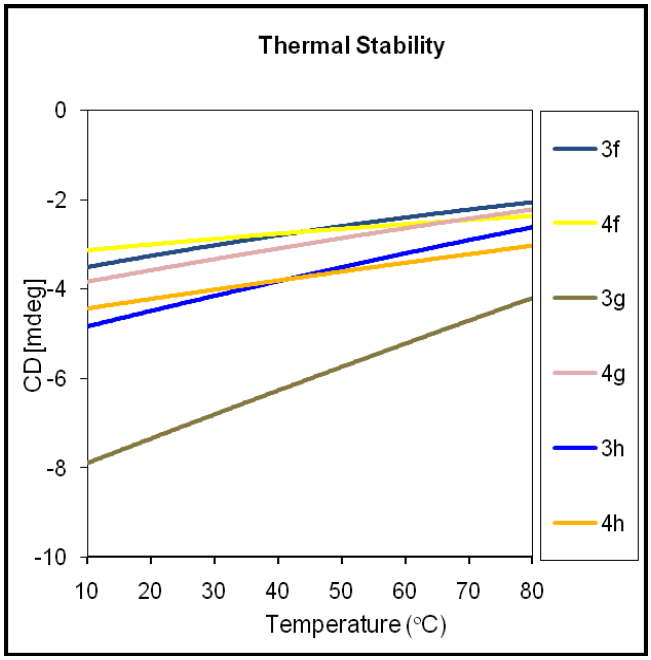

Figure **S5**: Thermal stability of all peptides by CD analysis in ACN:Phosphate buffer (3:1).

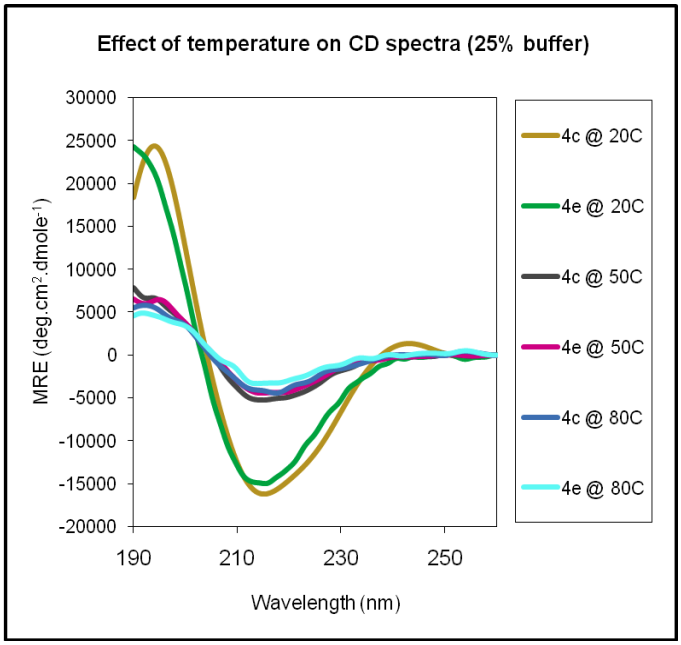

Figure **S6:** CD spectra of peptides **4c** and **4e** indicating that the peptide structure still retains the maxima and minima that are typical of a 14-helix.

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4. NMR analysis

4.1 General information

Proton NMR spectra of all β-peptides were run at 400 MHz or 500 MHz on a Bruker Av400 or a DRX500, respectively. Spectra were recorded in d_3-methanol (CD_3OH) or H_2O:D_2O 90:10 at 298.0 K. For peptides **3f** and **4f**, key resonances are displayed in Tables S2 and S4 and these have been reported as doublet (d), doublet of doublets (dd) or multiplet (m).

TOCSY and NOESY experiments were performed on peptides **1f** and **2f** at 298.0 K on a Bruker DRX500 with mixing times of 120 and 400 ms, respectively.

4.2 ^{1}H NMR and NOESY spectra for peptide 3f

Table S3: Resonance experiments from TOCSY for peptide **3f**

i	NH (ppm)	J (Hz)	Hβ (ppm)	Hα1 (ppm)	Hα2 (ppm)
1. Val	7.86 (d)	10.0	4.19 (m)	2.34 (m)	2.48 (m)
2. Ser	7.95 (d)	10.0	4.39 (m)	2.34 (m)	2.48 (m)
3. Ala	8.00 (d)	10.0	4.31 (m)	2.28 (m)	2.54 (m)
4. Ala	7.69 (d)	10.0	4.39 (m)	2.28 (m)	2.54 (m)
5. Ser	7.75 (d)	10.0	4.49 (m)	2.34 (m)	2.54 (m)
6. Leu	7.72 (d)	12.5	4.39 (m)	2.34 (m)	2.48 (m)

Table S4: NOE Interactions for **3f**

Interacting Pair	NOE	Connectivity	Strength
Val -1 (NH); Ala-3 (Hβ)	Yes	*i*/*i*+2	medium
Val -1 (NH); Ala-4 (Hβ)	ambiguous	*i*/*i*+3	medium
Ser-2 (NH); Ala-4 (Hβ)	ambiguous	*i*/*i*+2	strong
Ser-2 (NH); Ser-5 (Hβ)	Yes	*i*/*i*+3	strong
Ser-2 (NH); Ser-5 (Hα)	Yes	*i*/*i*+3	strong
Ser-2 (Hα); Ser-5 (Hα)	Yes	*i*/*i*+3	medium
Ala-3 (NH)- Ser-5 (Hβ)	Yes	*i*/*i*+2	strong
Ala-3 (NH)- Leu-6 (Hα)	Yes	*i*/*i*+3	strong
Ala-4 (NH)- Leu-6 (Hβ)	Yes	*i*/*i*+2	strong

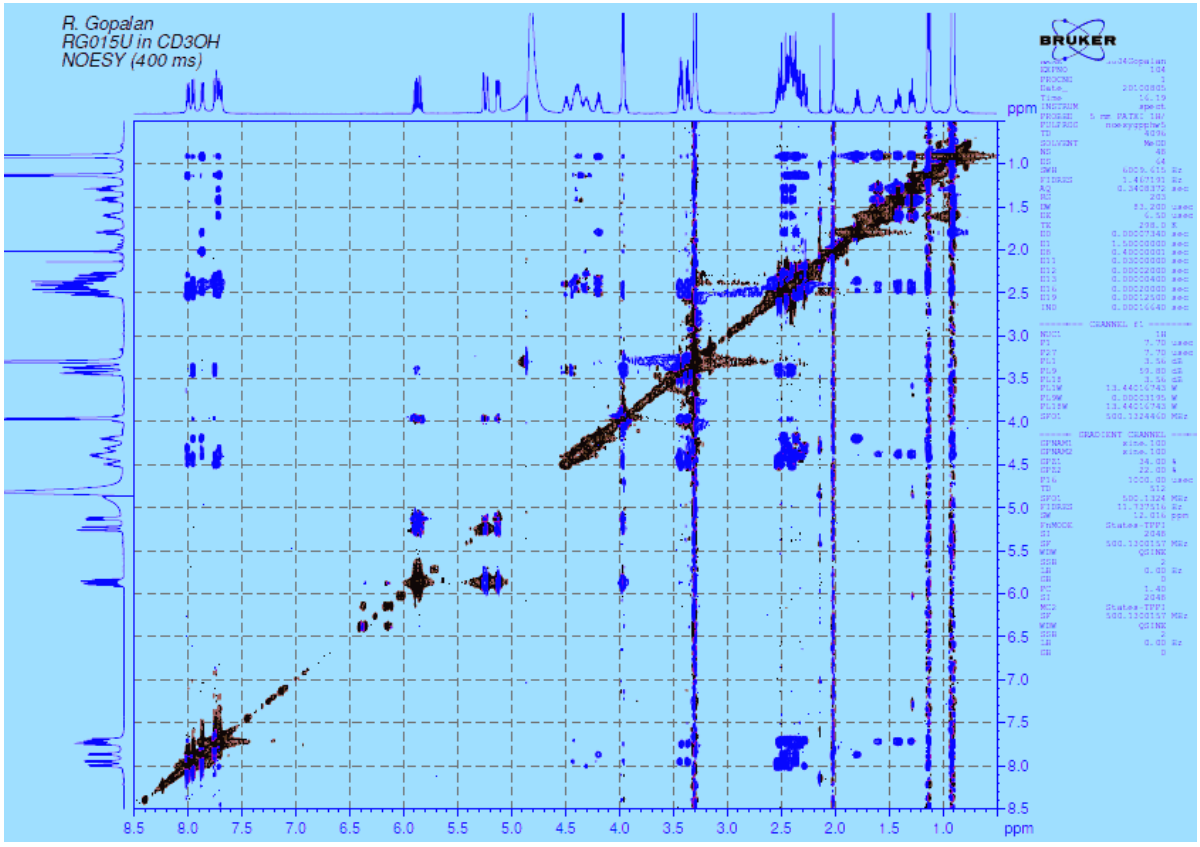

Figure **S7.** NOESY spectrum (500 MHz, CD_3OH) of **3f**

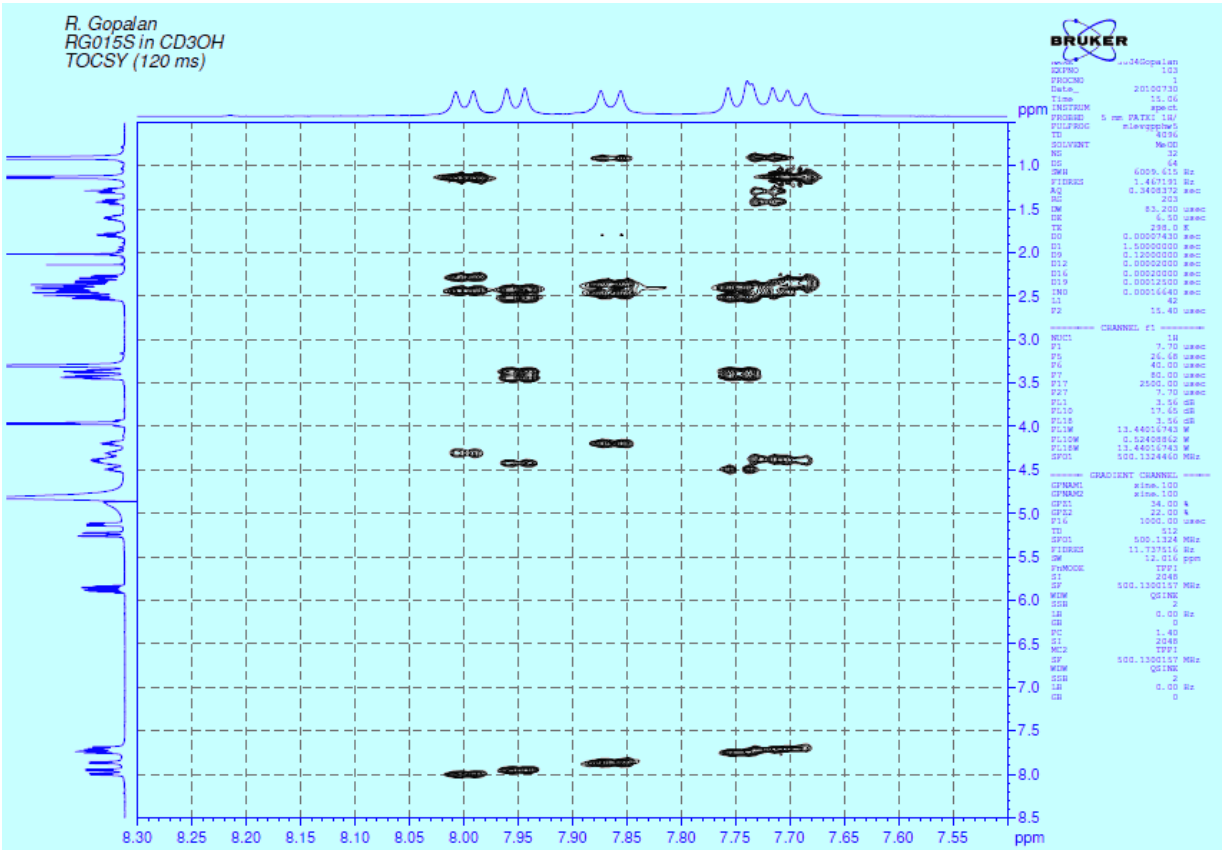

Figure **S8.** NOESY spectrum (500 MHz, CD_3OH) of **3f**

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Table S5: Resonance experiments from TOCSY for peptide **4f**

i	NH (ppm)	J (Hz)	Hβ (ppm)	Hα1 (ppm)	Hα2 (ppm)
1. Val	7.98 (d)	10.0	4.25 (m)	2.30 (m)	2.50 (m)
2. Ser	7.87 (d)	10.0	4.45 (m)	2.30 (m)	2.52 (m)
3. Ala	7.96 (d)	10.0	4.35 (m)	2.23 (m)	2.50 (m)
4. Ala	7.60(d)	10.0	4.45 (m)	2.25 (m)	2.40 (m)
5. Ser	7.55 (d)	10.0	4.51 (m)	2.20 (m)	2.51 (m)
6. Leu	7.67 (d)	10.0	4.40 (m)	2.40 (m)	2.50 (m)

Table S6: NOE Interactions for **4f**

Interacting Pair	NOE	Connectivity	Strength
Val-1 (NH); Ala-4 (Hα)	ambiguous	*i*/*i*+3	strong
Ser-2 (NH); Ala-4 (Hβ)	Yes	*i*/*i*+2	strong
Ser-2 (NH); Ser-5 (Hβ)	Yes	i/i+3	strong
Ala-3 (NH); Ser-5 (Hβ)	Yes	*i*/*i*+2	medium
Ser-5 (NH); Ser-2 (Hβ)	Yes	*i*/*i*+3	weak
Ser-5 (NH); Ala-3 (Hα)	ambiguous	*i*/*i*+2	strong
Leu-6 (NH); Ser-2 (Hα)	yes	*i*/*i*+3	medium
Leu-6 (NH); Ala-3 (Hα)	yes	*i*/*i*+3	strong

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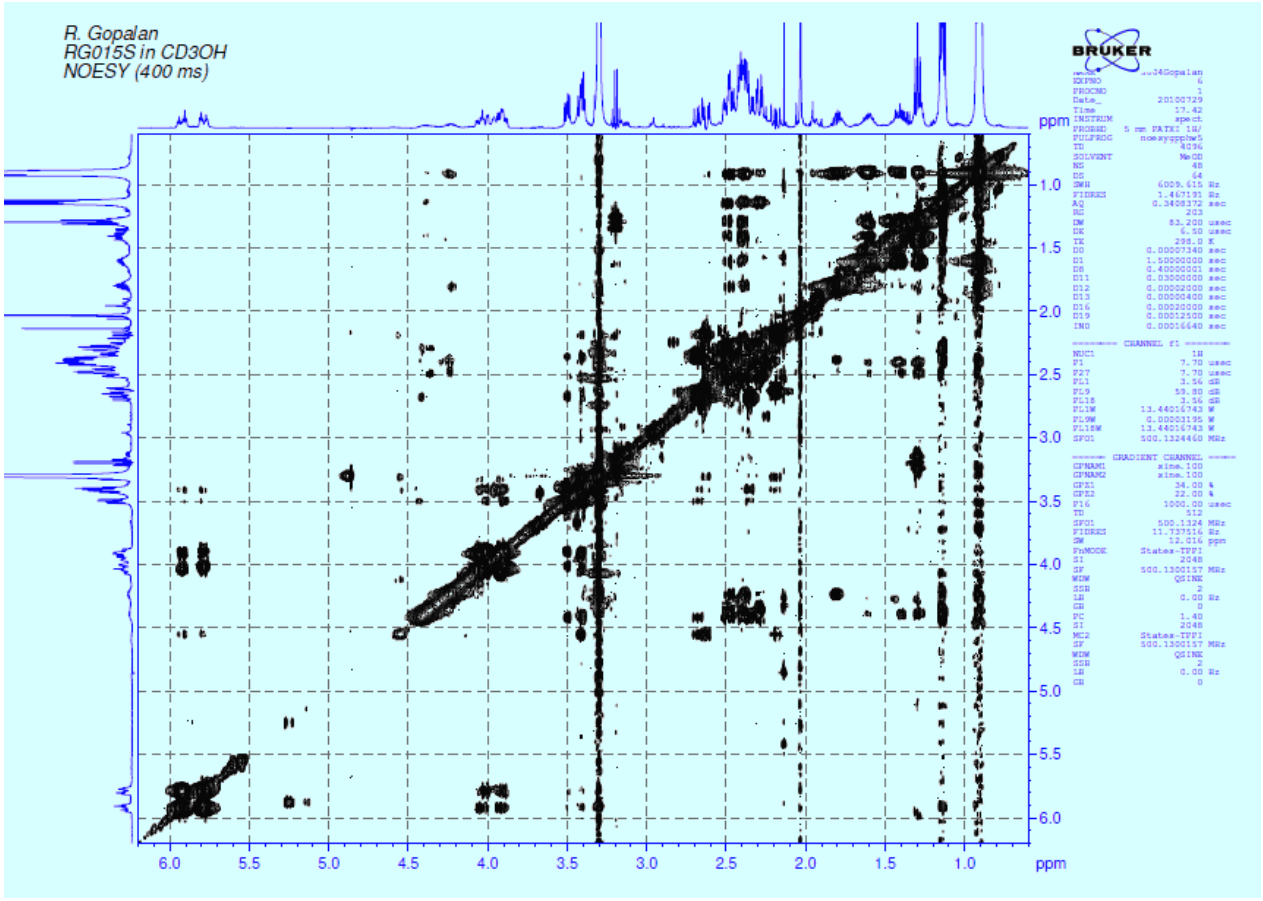

Figure **S9.** NOESY spectrum (500 MHz, CD_3OH) of **4f**

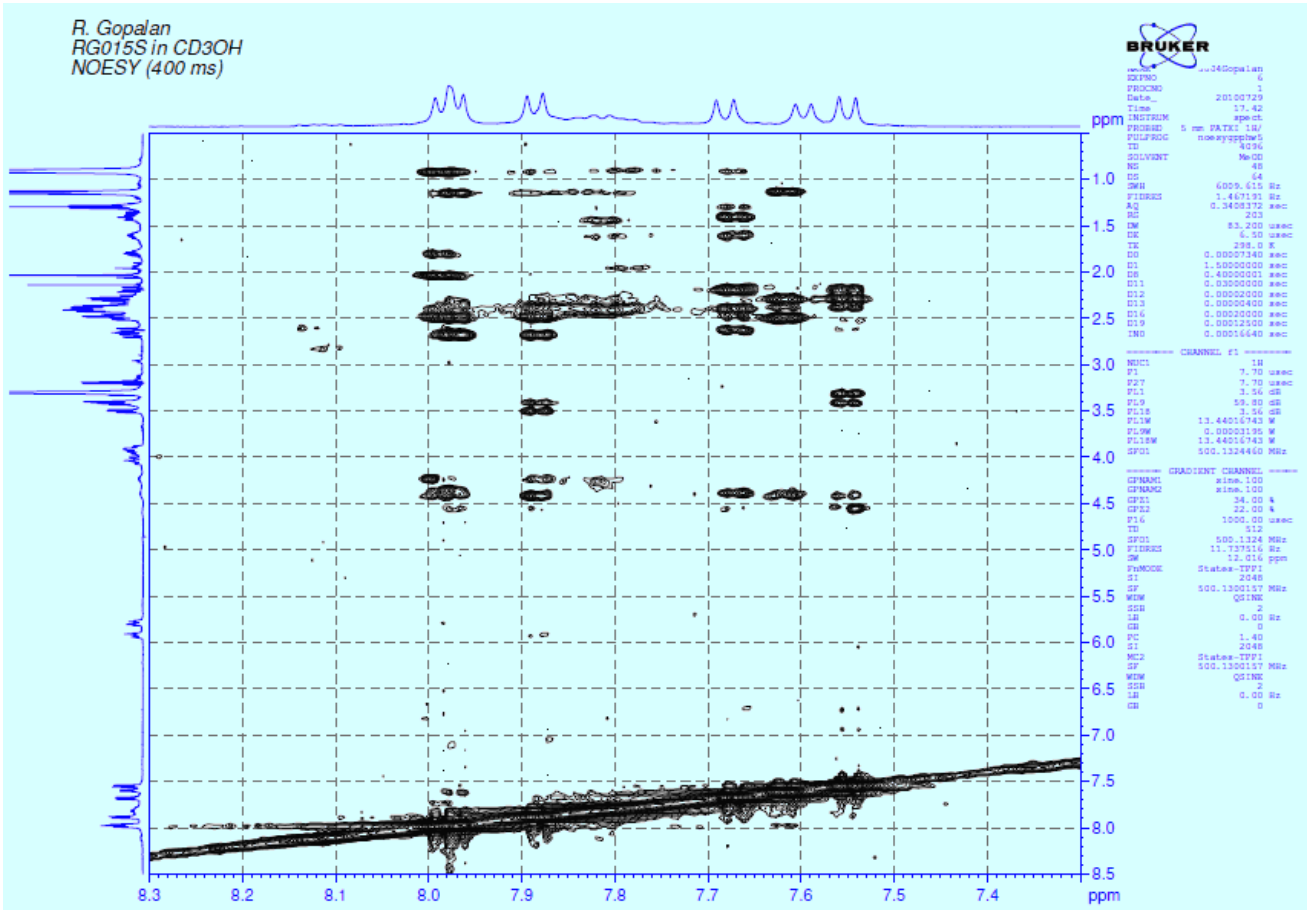

Figure **S10:** NOESY spectrum (500 MHz, CD_3OH) of **4f**