

Electronic Supporting Information (ESI)

De Novo design of stereochemically-bent sixteen-residue β -hairpin as a hydrolase mimic

Bhupesh Goyal*¹, Kirti Patel, Kinshuk Raj Srivastava and Susheel Durani

Department of Chemistry, Indian Institute of Technology Bombay,
Mumbai–400076, India

*Corresponding author

E-mail address: bhupesh@iitbombay.org

¹**Present Address**

Department of Chemistry, School of Basic and Applied Sciences, Sri Guru Granth Sahib
World University, Fatehgarh Sahib–140406, Punjab, India.

Supporting Information captions

Fig. S1: Mass spectra of sixteen-residue peptide variants.

Fig. S2: $^1\text{H-NMR}$ spectra of peptide variants recorded in 90% $\text{H}_2\text{O}/10\%$ D_2O at 298 K on a 700 MHz spectrometer.

Fig. S3: CD spectrum of peptide variants in water at 298 K in 20–100 μM concentration range. Y-axis represents the mean residue molar ellipticity and X-axis represents wavelength in nm.

Fig. S4: Quenching of tryptophan fluorescence of peptide variants (15 μM) in sodium phosphate buffer (pH 7.0, 20 mM) at 298 K on progressive titration with increasing pNPP concentration (0–300 μM) (**left panel**), and plot of relative fluorescence intensity as a function of pNPP concentration (0–300 μM) (**right panel**).

Fig. S5: UV monitored rate of hydrolysis of pNPA with peptide variants in sodium phosphate buffer (pH 7.0, 20 mM) at 298 K on progressive titration with increasing pNPA concentration (0–75 μM) in the form of Michaelis–Menten plot (**left panel**) and Lineweaver–Burk plot (**right panel**).

Elemental Composition Report

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions

.1196 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Micromass : Q-ToF micro (YA-105)

Department Of Chemistry I.I.T.(B)

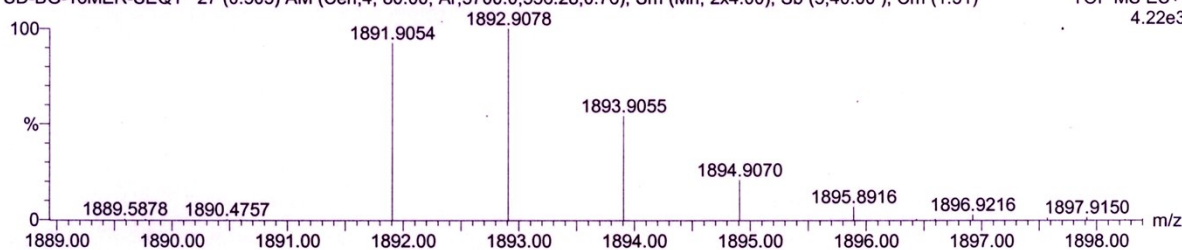
C87H122N22O26

SD-BG-16MER-SEQ1 27 (0.505) AM (Cen,4, 80.00, Ar,5700.0,556.28,0.76); Sm (Mn, 2x4.00); Sb (5,40.00); Cm (1:31)

Ionisation Mode

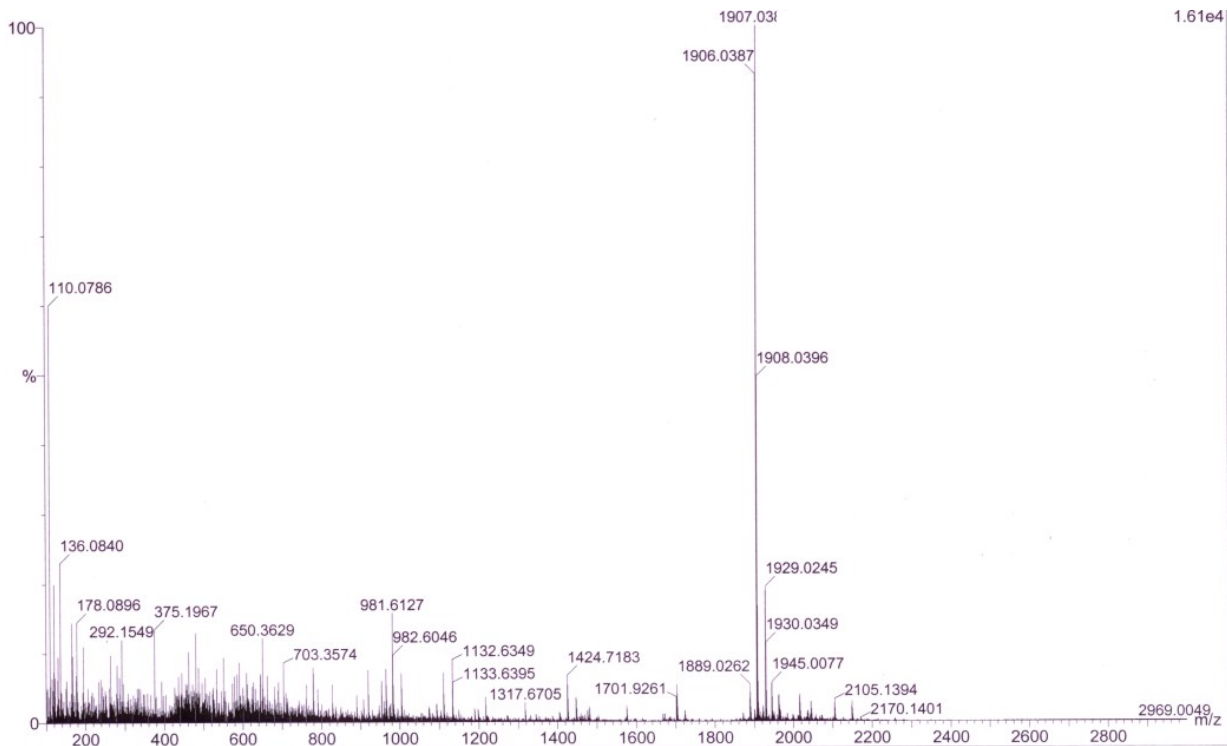
TOF MS ES+

4.22e3



Minimum: -1.5
Maximum: 200.0 10.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
1891.9054	1891.8979	7.5	4.0	37.5	1	C87 H123 N22 O26



Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions

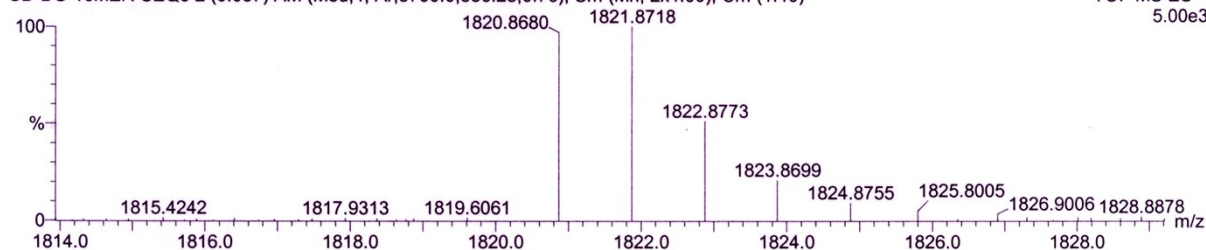
1098 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Micromass : Q-ToF micro (YA-105)

Department Of Chemistry I.I.T.(B)

C84H117N21O25

SD-BG-16MER-SEQ3 2 (0.037) AM (Med,4, Ar,5700.0,556.28,0.76); Sm (Mn, 2x4.00); Cm (1:40)

Ionisation Mode
TOF MS ES+
5.00e3

Minimum: -1.5
Maximum: 200.0 10.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
1820.8680	1820.8608	7.3	4.0	36.5	1	C84 H118 N21 O25

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions

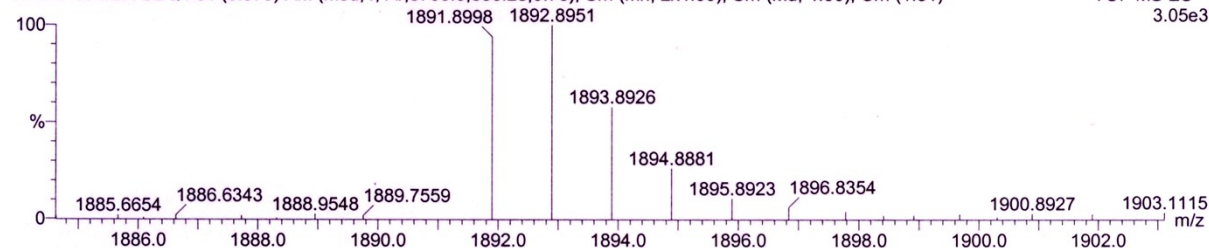
1196 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Micromass : Q-ToF micro (YA-105)

Department Of Chemistry I.I.T.(B)

C87H122N22O26

SD-BG-16MER-SEQ4 31 (0.575) AM (Med,4, Ar,5700.0,556.28,0.76); Sm (Mn, 2x4.00); Sm (Md, 4.00); Cm (1:31)

Ionisation Mode
TOF MS ES+
3.05e3

Minimum: -1.5
Maximum: 200.0 10.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
1891.8998	1891.8979	1.9	1.0	37.5	1	C87 H123 N22 O26

Elemental Composition Report

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions

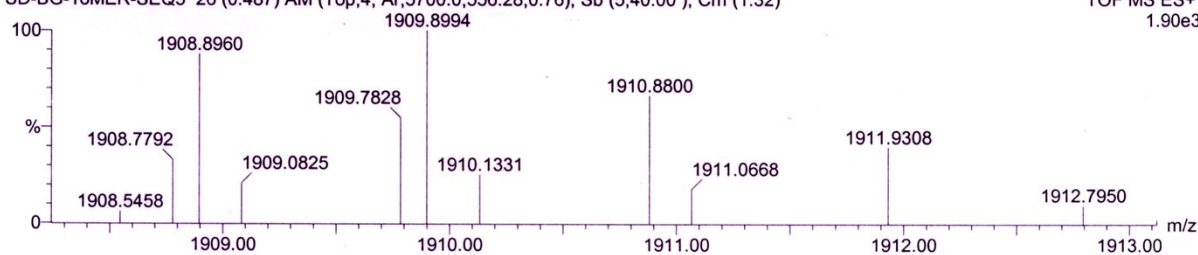
1298 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Micromass : Q-ToF micro (YA-105)

Department Of Chemistry I.I.T.(B)

C86H121N23O27

SD-BG-16MER-SEQ5 26 (0.487) AM (Top,4, Ar,5700.0,556.28,0.76); Sb (5,40.00); Cm (1:32)

Ionisation Mode
TOF MS ES+
1.90e3

Minimum:

Maximum: 200.0 10.0 -1.5 50.0

Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
1908.8960	1908.8881	7.9	4.2	37.5	1	C86 H122 N23 O27

Fig. S1: Mass spectra of sixteen-residue peptide variants.

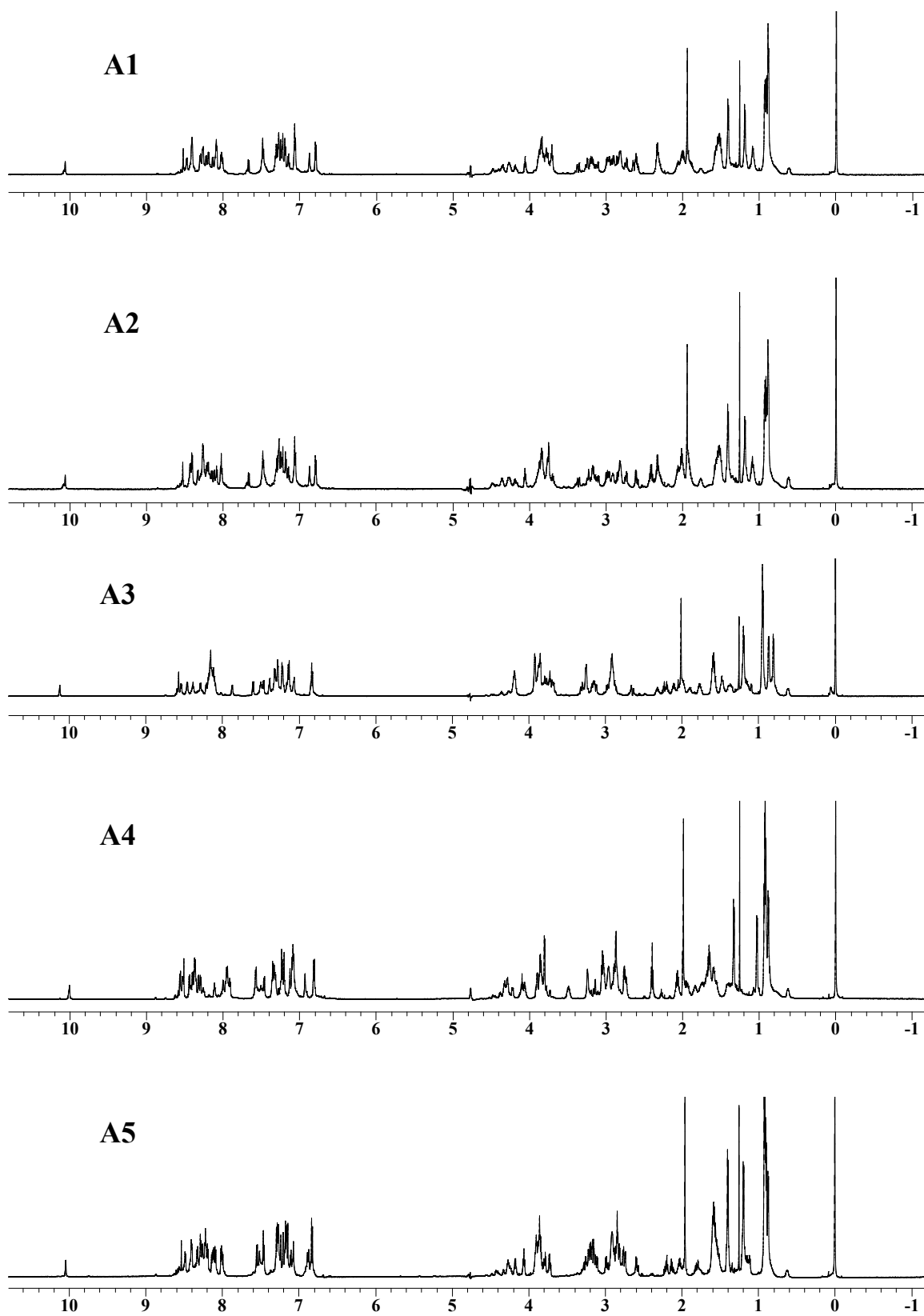
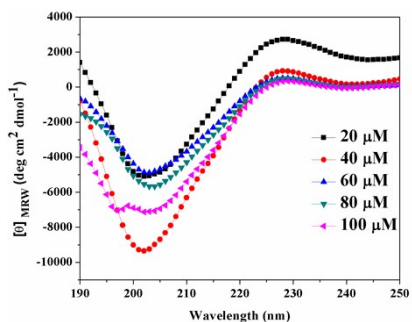
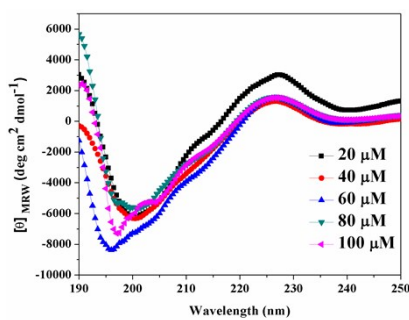


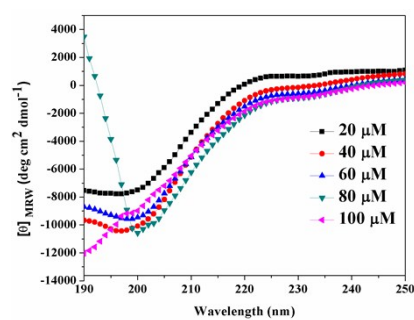
Fig. S2: ¹H-NMR spectra of peptide variants recorded in 90% H₂O/10% D₂O at 298 K on a 700 MHz spectrometer.



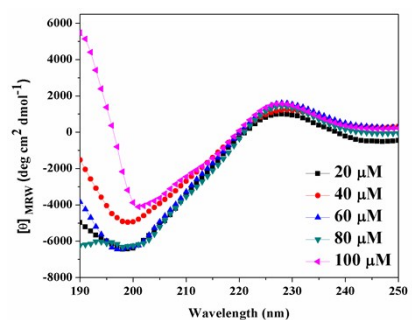
A1



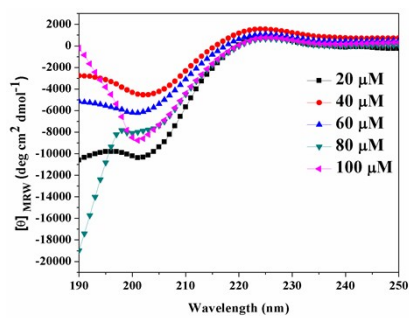
A2



A3



A4



A5

Fig. S3: CD spectrum of peptide variants in water at 298 K in 20–100 μM concentration range. Y-axis represents the mean residue molar ellipticity and X-axis represents wavelength in nm.

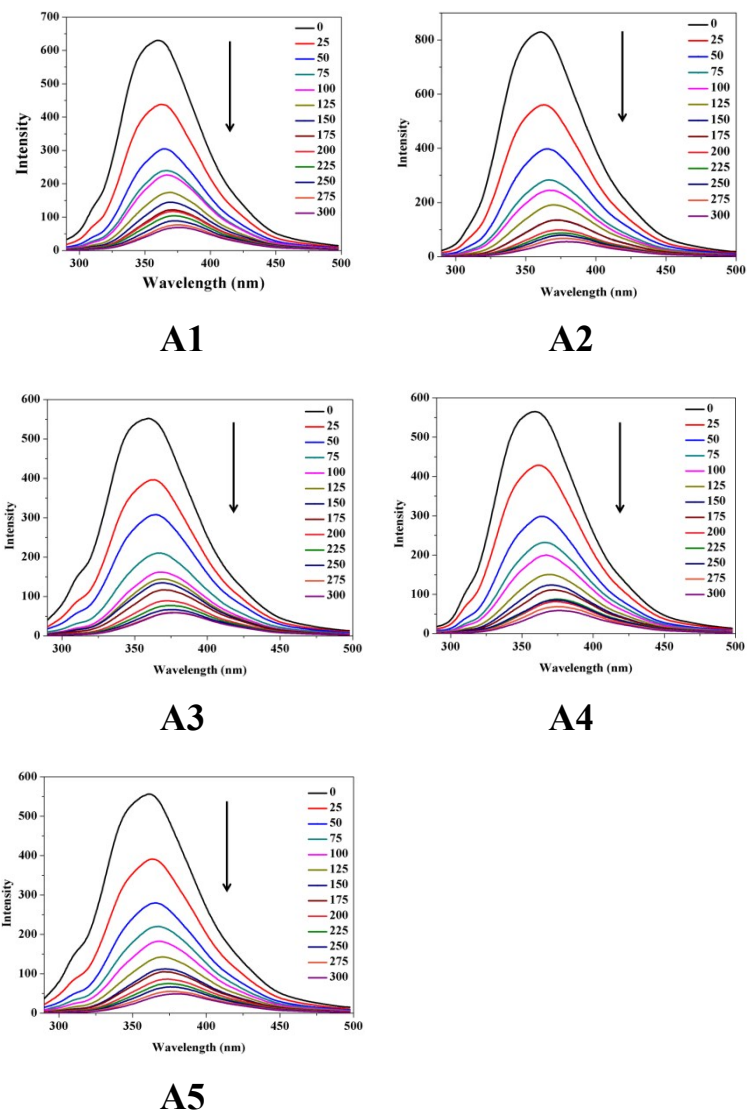


Fig. S4: Quenching of tryptophan fluorescence of peptide variants (15 μM) in sodium phosphate buffer (pH 7.0, 20 mM) at 298 K on progressive titration with increasing pNPP concentration (0–300 μM).

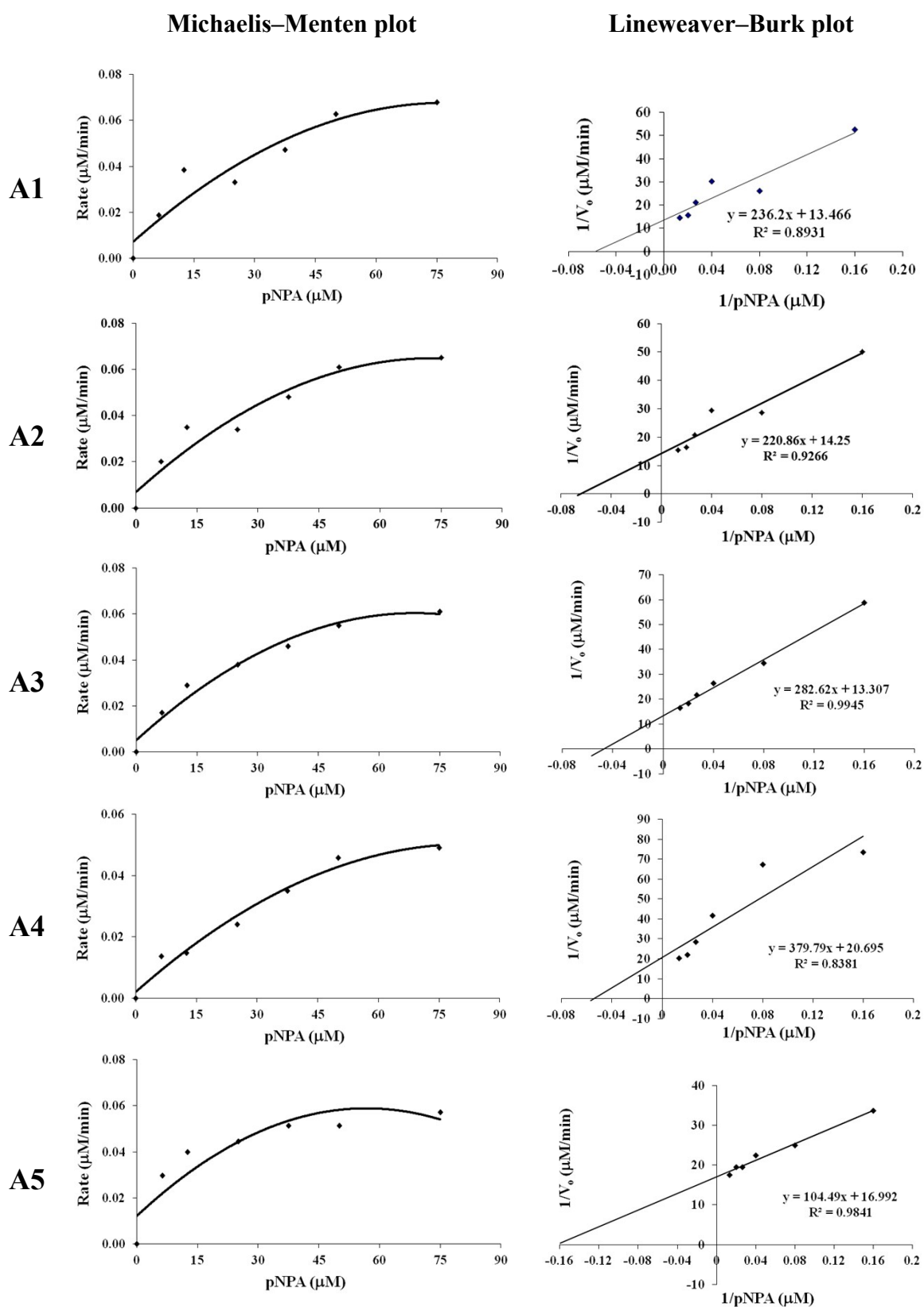


Fig. S5: UV monitored rate of hydrolysis of pNPA with peptide variants in sodium phosphate buffer (pH 7.0, 20 mM) at 298 K on progressive titration with increasing pNPA concentration (0–75 μM) in the form of Michaelis–Menten plot (**left panel**) and Lineweaver–Burk plot (**right panel**).