Customizing Morphology, Size, and Response Kinetics of Matrix Metalloproteinase-responsive Nanostructures by Systematic Peptide Design

Jiye Son,[a-c] Daniela Kalafatovic,[a] Mohit Kumar, [a] Barney Yoo,[f] Mike A. Cornejo,[b] María Contel,[b-e] and Rein V. Ulijn*[a,c,f]

[a]Advanced Science Research Center at The Graduate Center of the City University of New York, 85 Saint Nicholas Terrace, New York, NY 10031, USA; [b]Department of Chemistry, Brooklyn College, City University of New York, 2900 Bedford Avenue, Brooklyn, NY 11210, USA; Ph.D. Program in [c]Chemistry, [d]Biochemistry, and [e]Biology, The Graduate Center of the City University of New York, 365 Fifth Avenue, New York, NY 10016, USA; [f]Department of Chemistry, Hunter College, City University of New York, 695 Park Avenue, New York, NY 10065, USA.

Supporting Information

Table of Contents

1. High Resolution Mass Spectrometry (HRMS)

HRMS data were obtained on an Agilent 6550 QToF, with a dual sprayer ESI source, coupled to an Agilent 1290 Infinity LC system. Samples were analyzed by FIA (flow injection analysis) using a mobile phase of 50% acetonitrile in water (0.1% formic acid) with a flow rate of 0.4 mL/min.

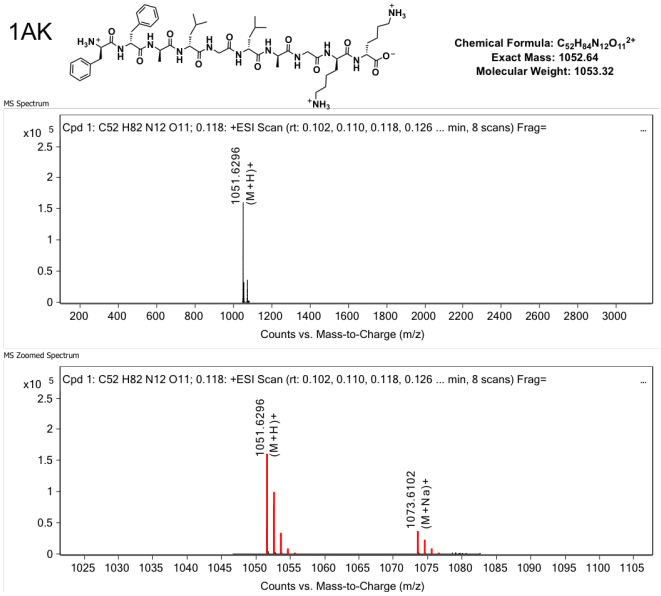


Figure S1 A. MS spectra of **1 AK**.

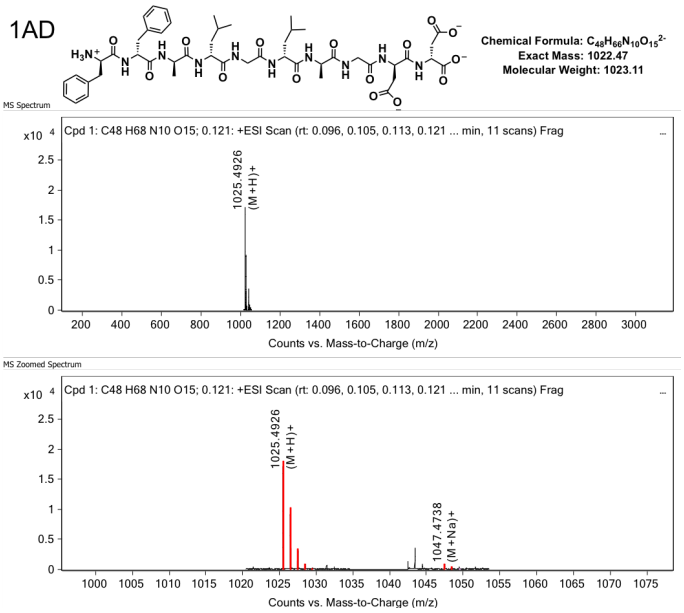


Figure S1 B. MS spectra of **1 AD**.

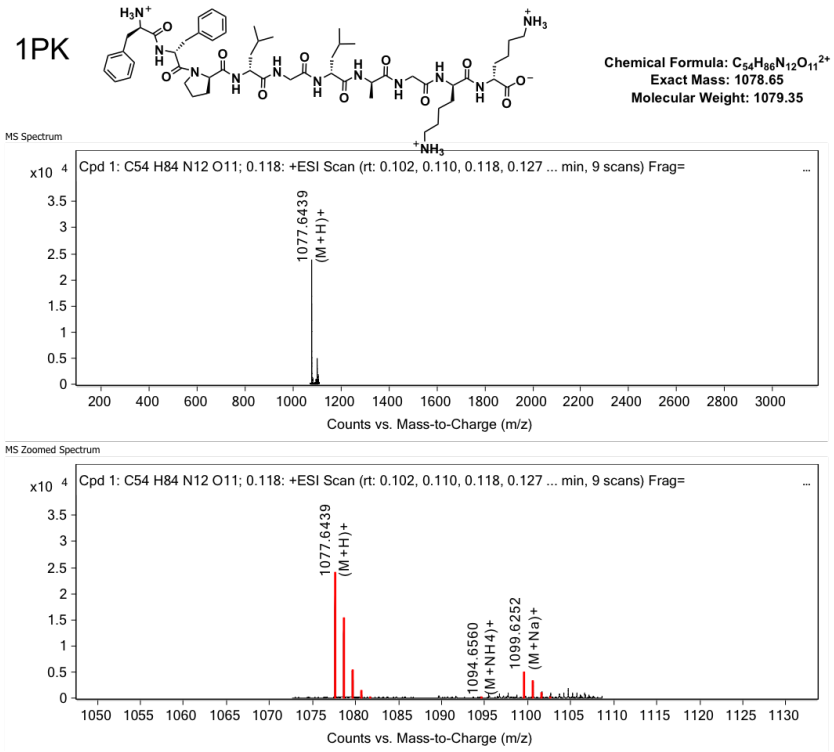


Figure S1 C. MS spectra of **1 PK**.

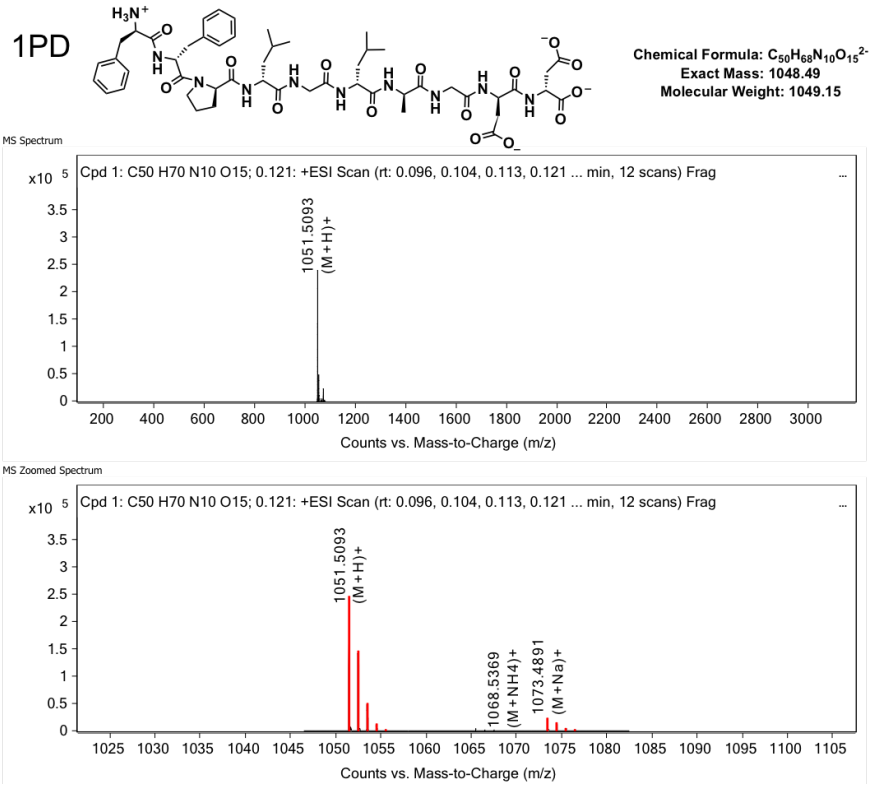


Figure S1 D. MS spectra of **1 PD**.

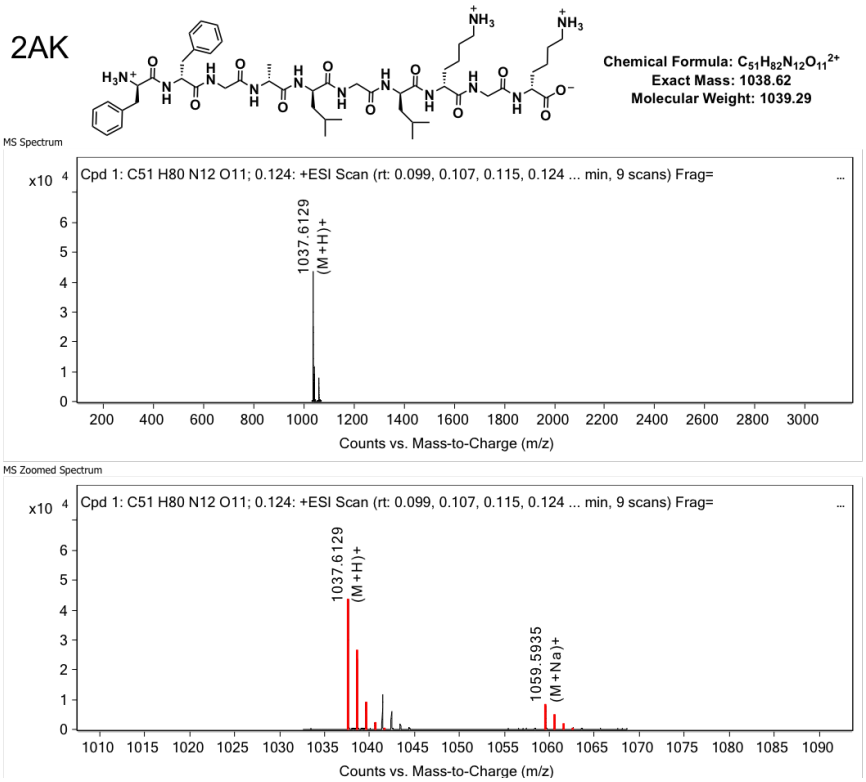


Figure S1 E. MS spectra of **2 AK**.

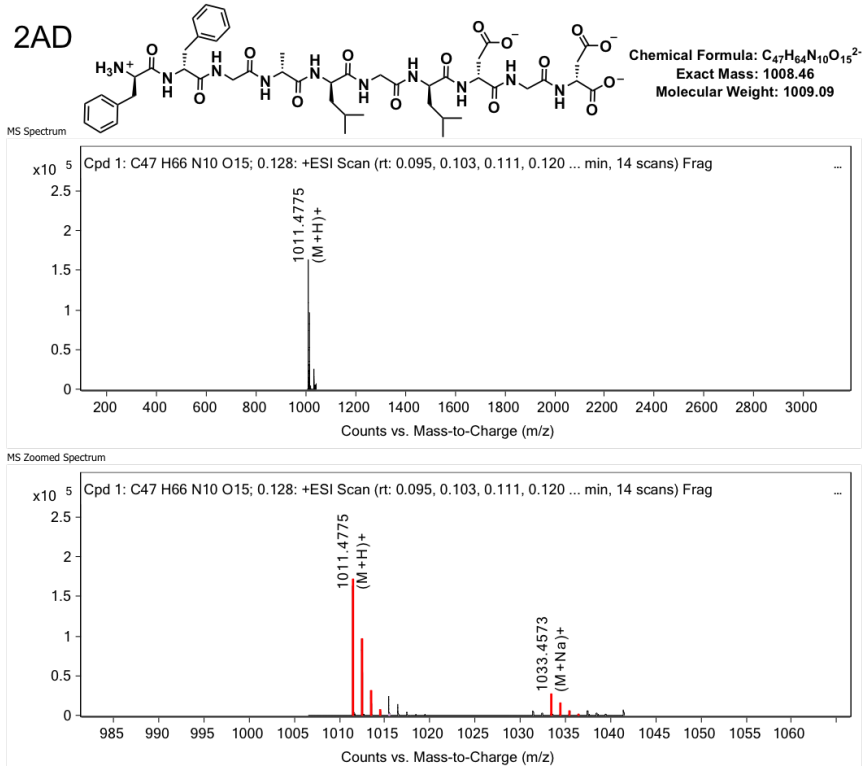


Figure S1 F. MS spectra of **2 AD**.

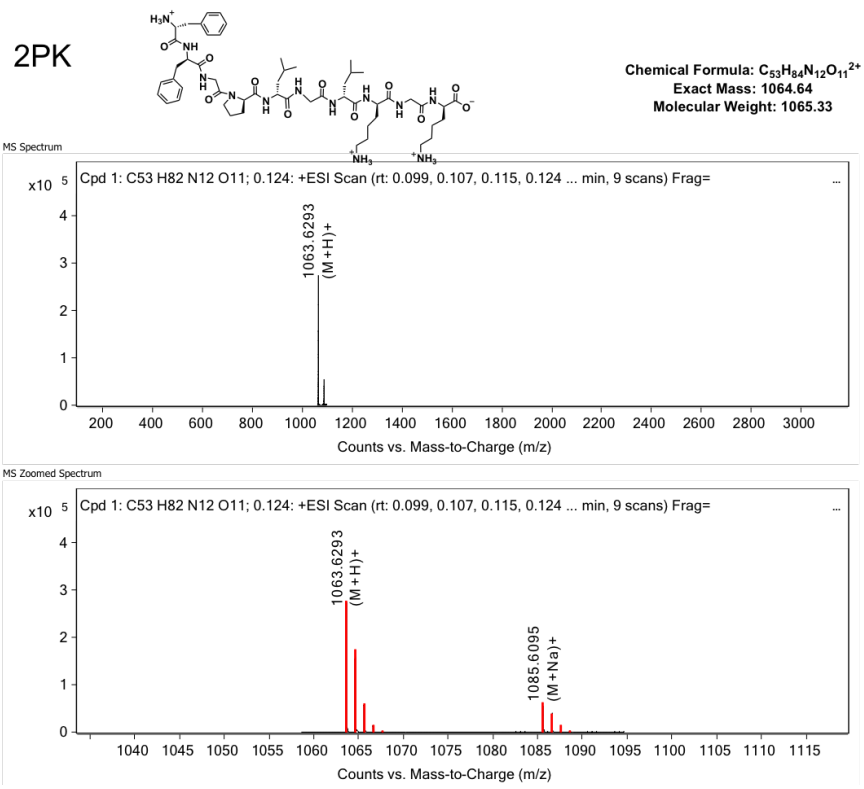


Figure S1 G. MS spectra of **2 PK**.

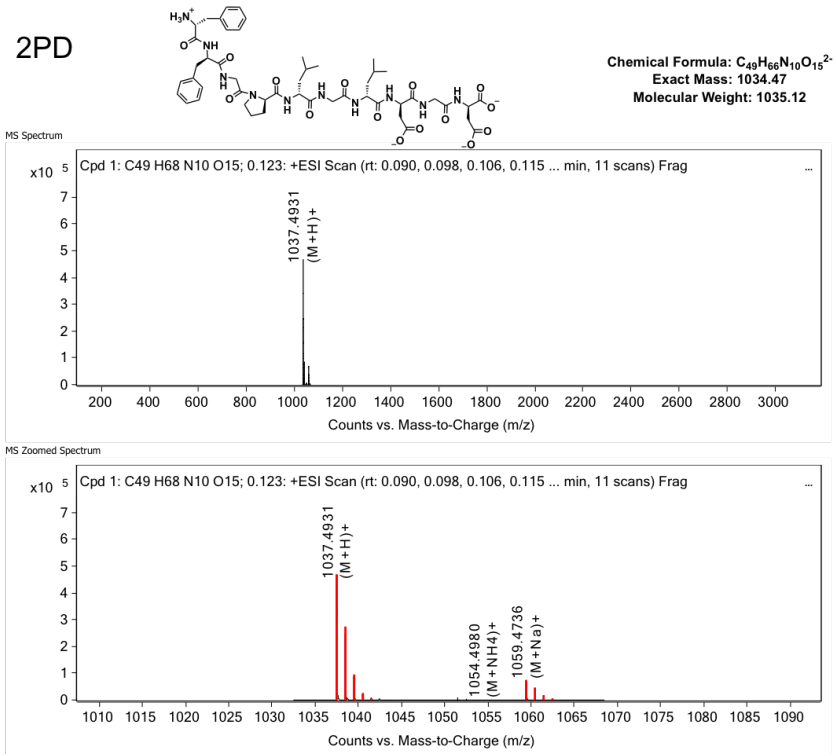


Figure S1 H. MS spectra of **2 PD**.

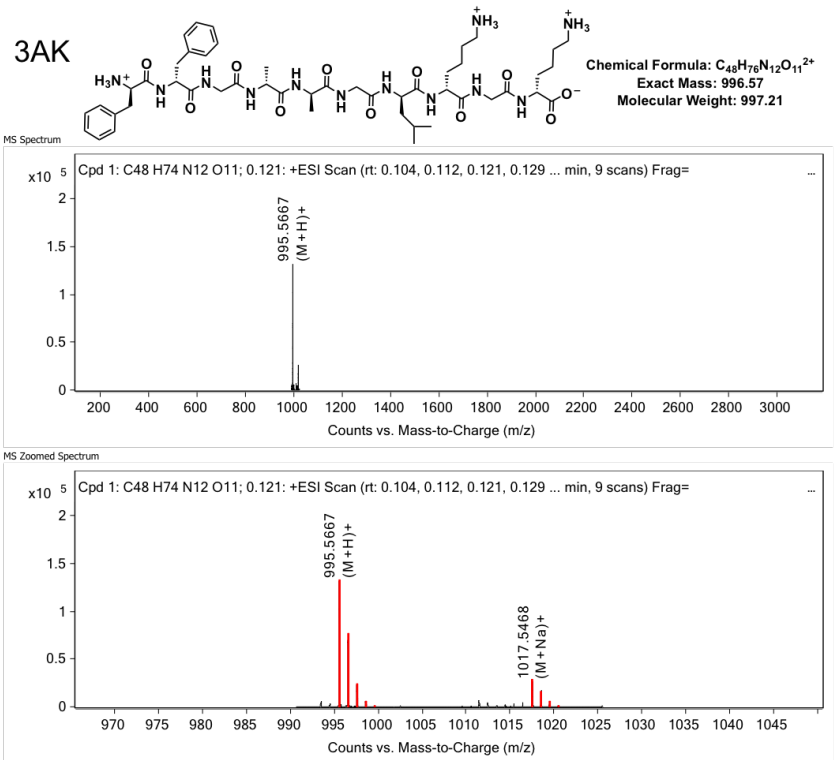


Figure S1 I. MS spectra of **3 AK**.

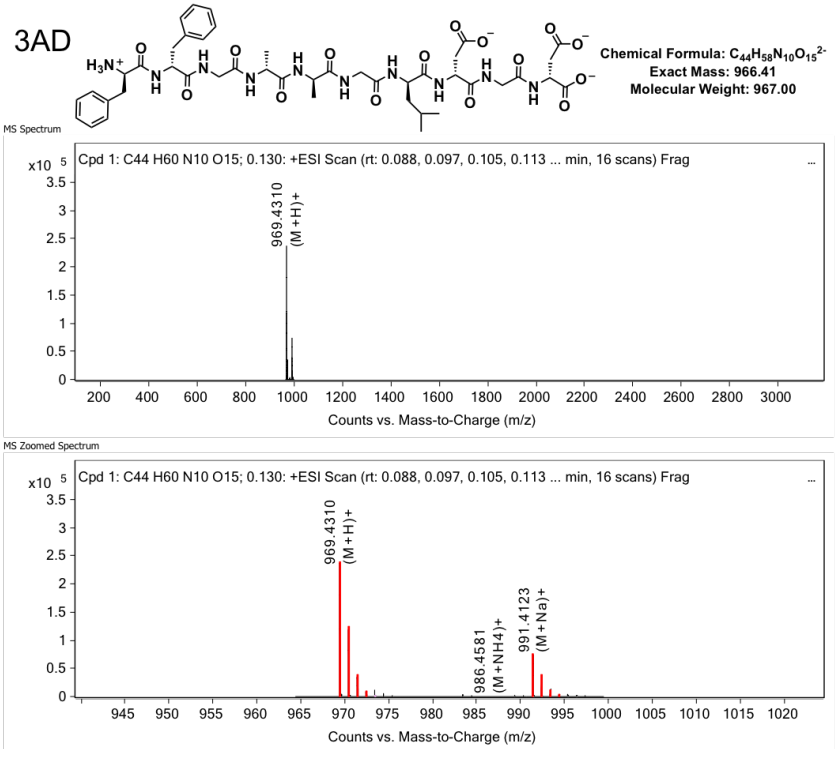


Figure S1 J. MS spectra of **3 AD**.

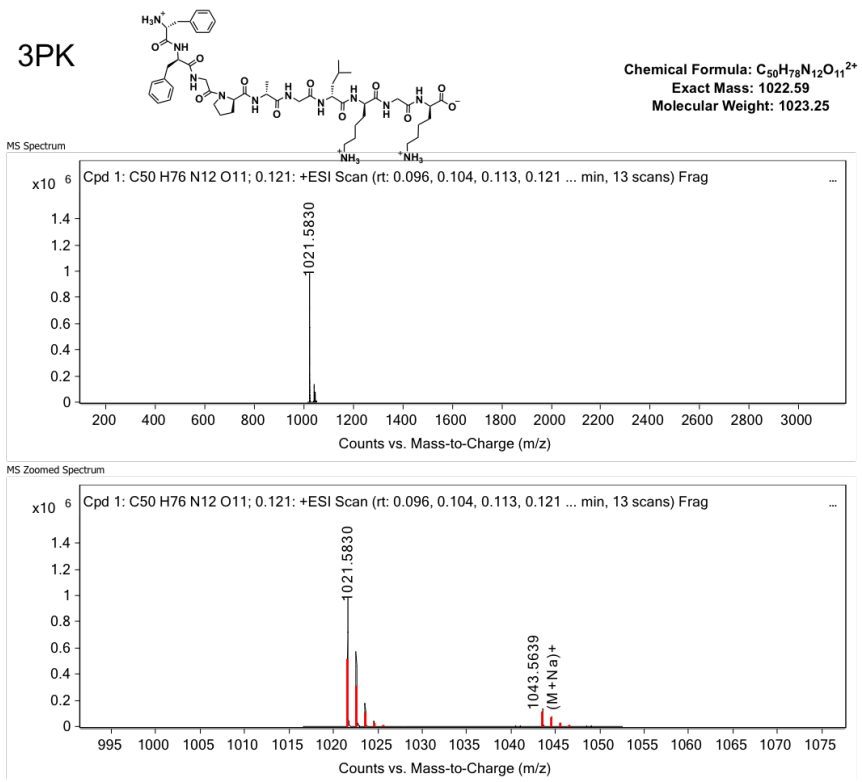


Figure S1 K. MS spectra of **3 PK**.

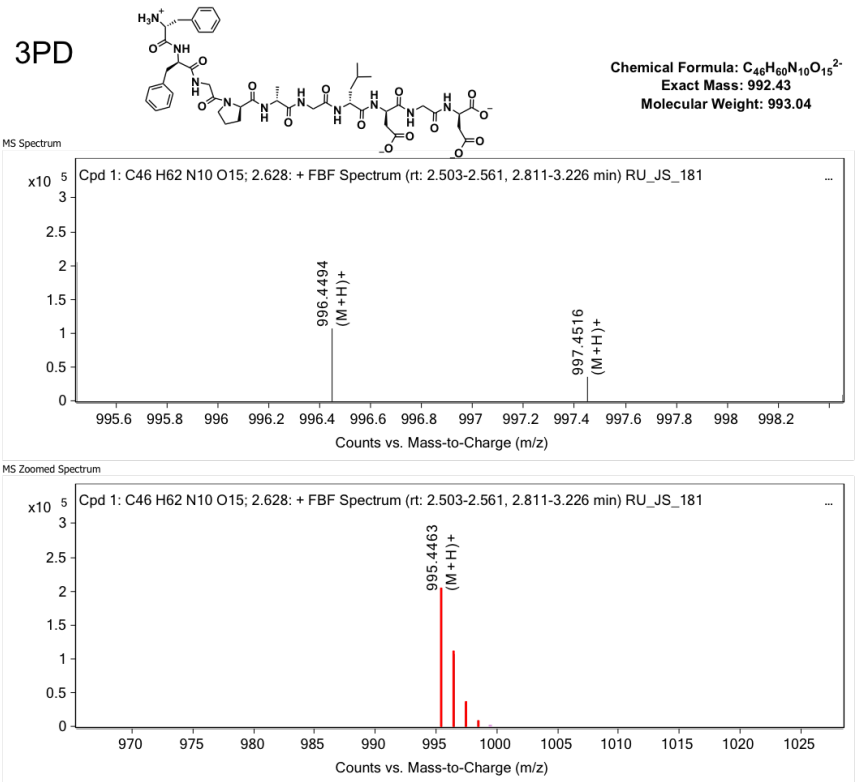


Figure S1 L. MS spectra of **3 PD**.

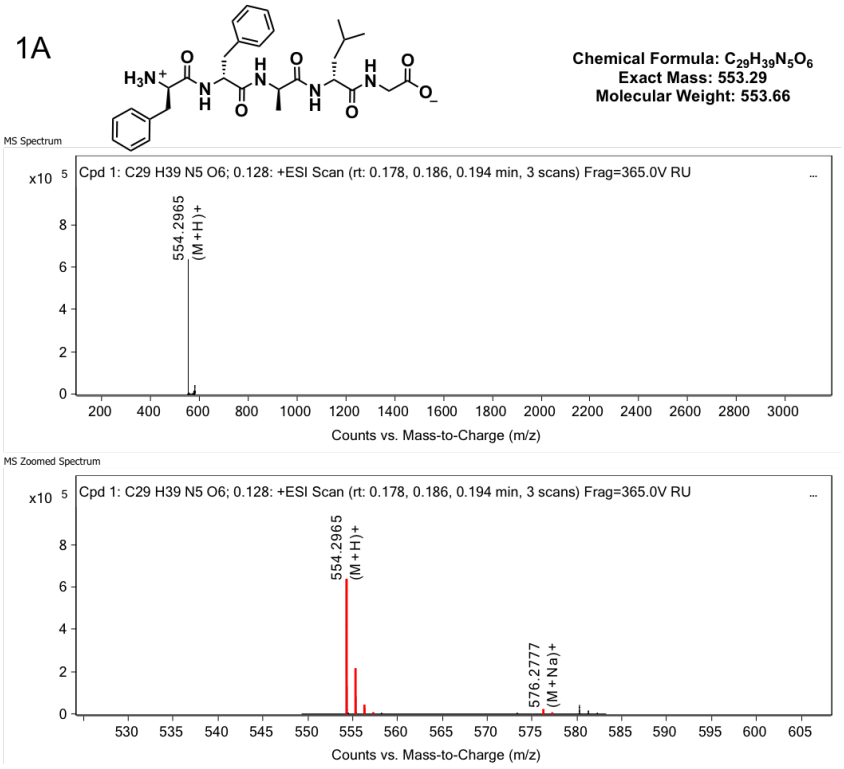


Figure S1 M. MS spectra of **1 A**.

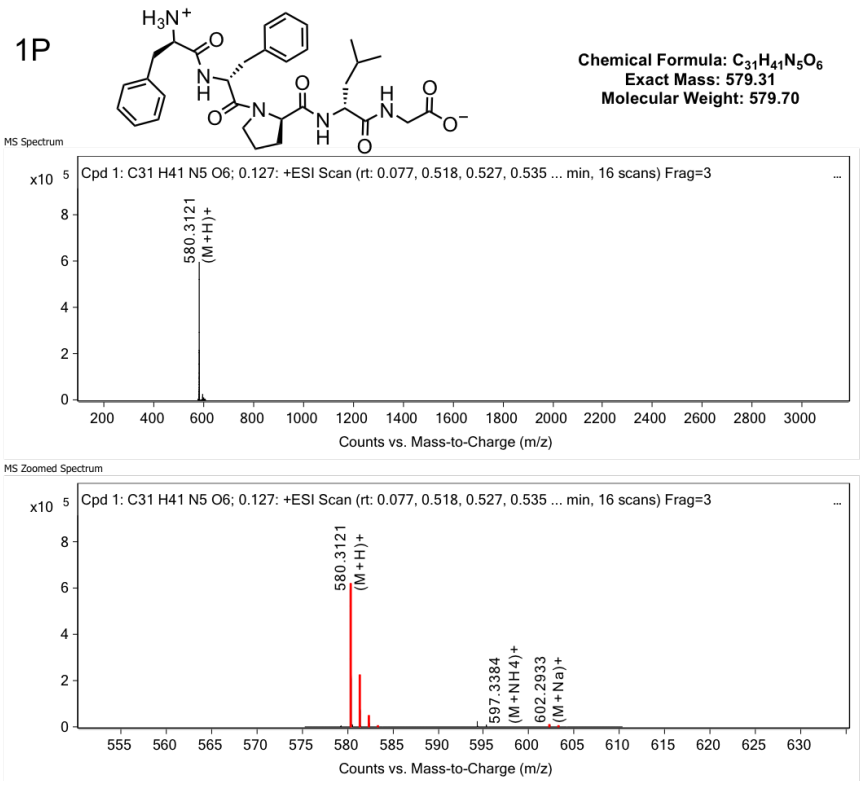


Figure S1 N. MS spectra of **1 P**.

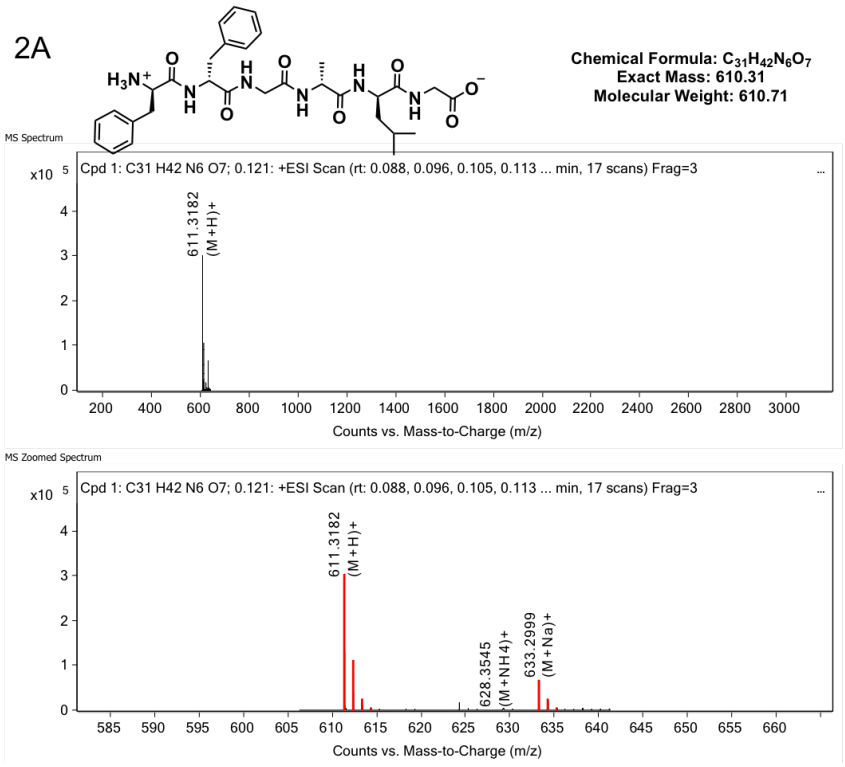


Figure S1 O. MS spectra of **2 A**.

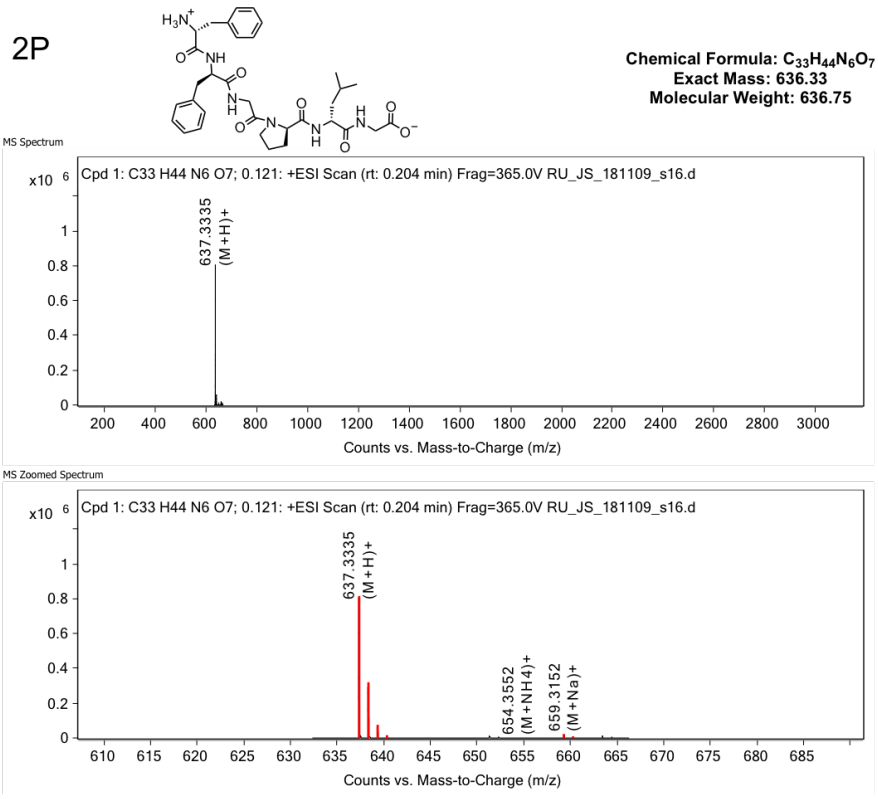


Figure S1 P. MS spectra of **2 P**.

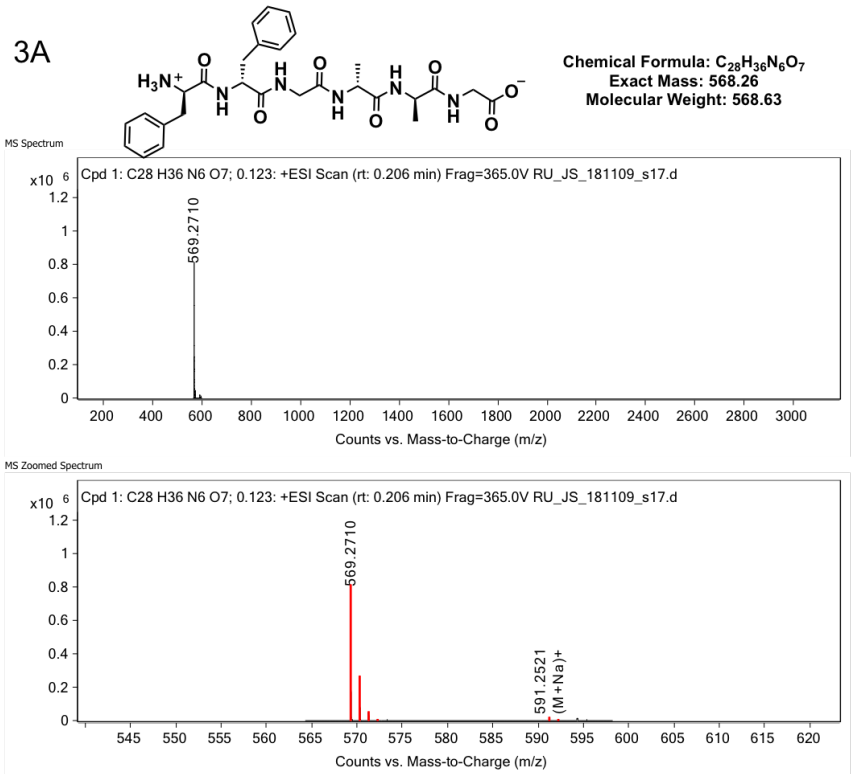


Figure S1 Q. MS spectra of **3 A**.

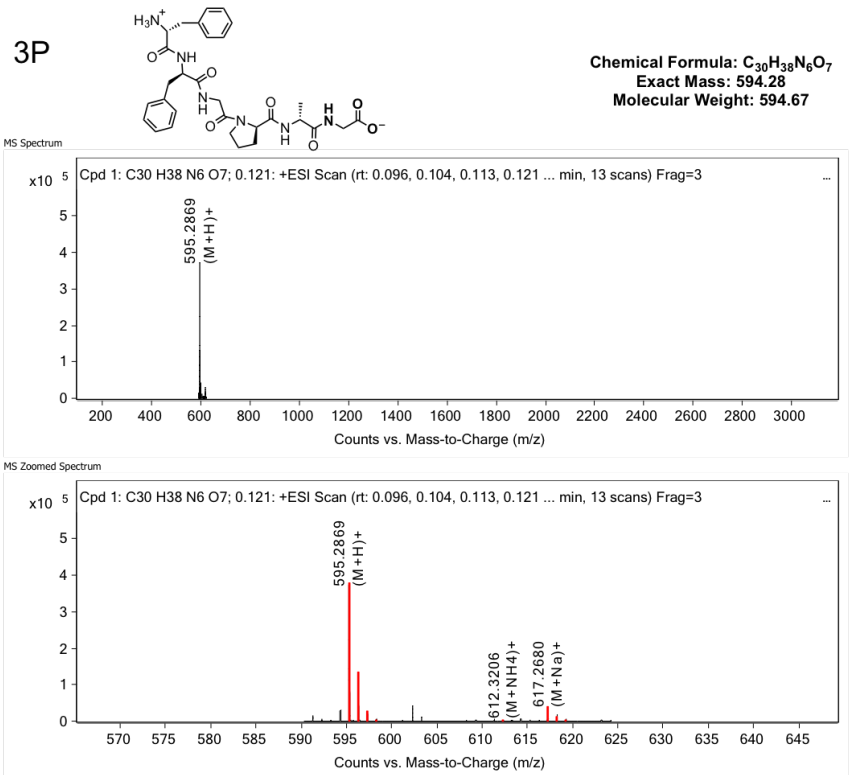
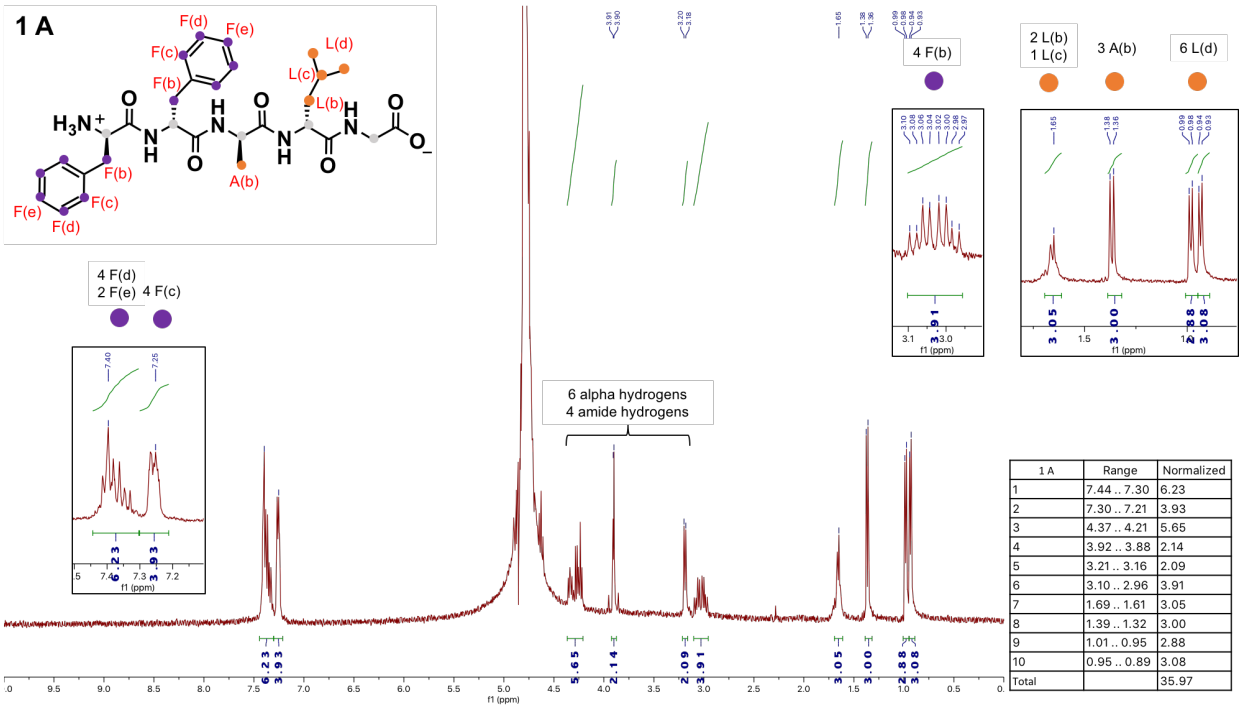


Figure S1 R. MS spectra of **3 P**.

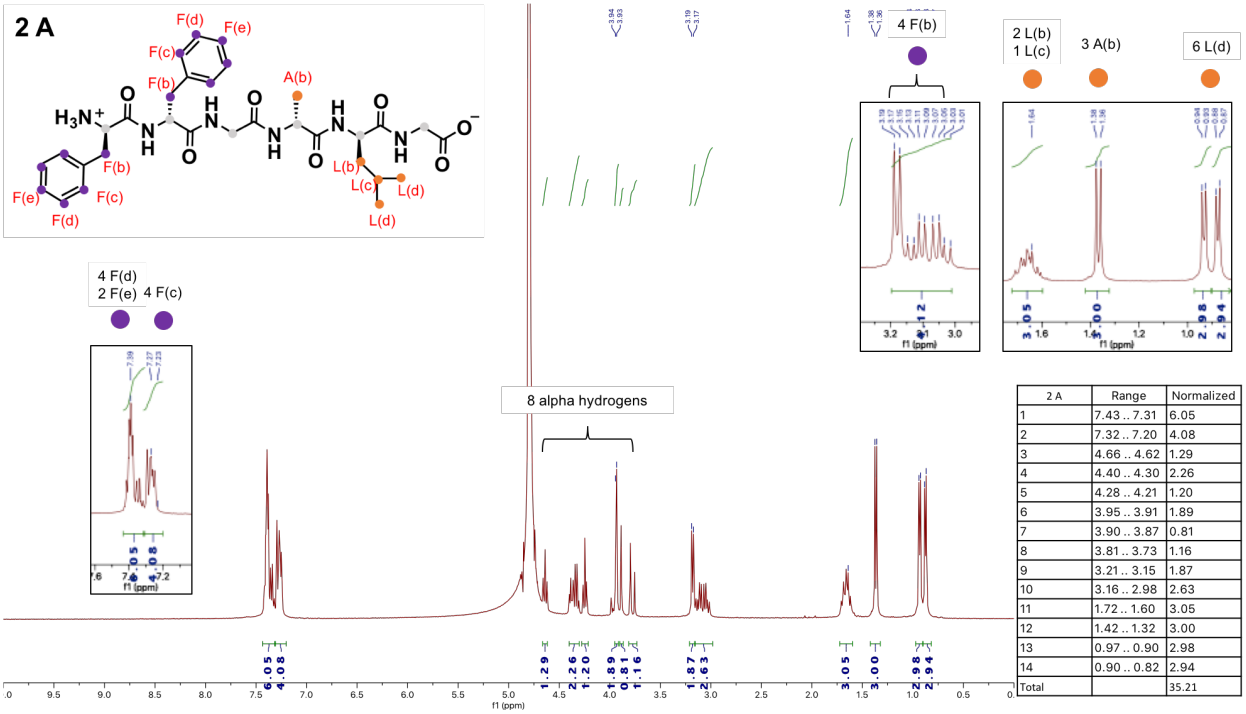
2. ^{1}H Nuclear Magnetic Resonance (NMR) Spectroscopy

^{1}H NMR spectra were recorded in a Bruker AV400 at 400 MHz. Chemical shifts (δ) are given in ppm using D_2O as solvent.



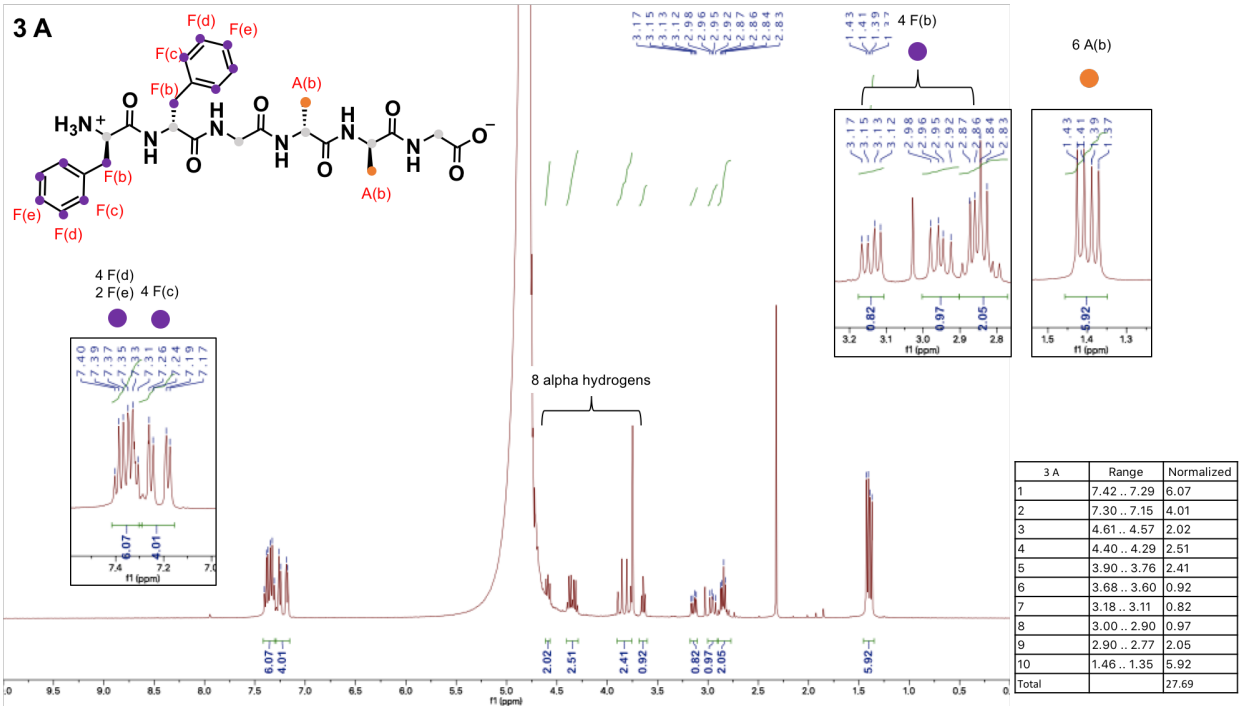
1 A	Range	Normalized
1	7.44 .. 7.30	6.23
2	7.30 .. 7.21	3.93
3	4.37 .. 4.21	5.65
4	3.92 .. 3.88	2.14
5	3.21 .. 3.16	2.09
6	3.10 .. 2.96	3.91
7	1.69 .. 1.61	3.05
8	1.39 .. 1.32	3.00
9	1.01 .. 0.95	2.88
10	0.95 .. 0.89	3.08
Total		35.97

Figure S2 A. ^{1}H NMR spectra of **1 A**.



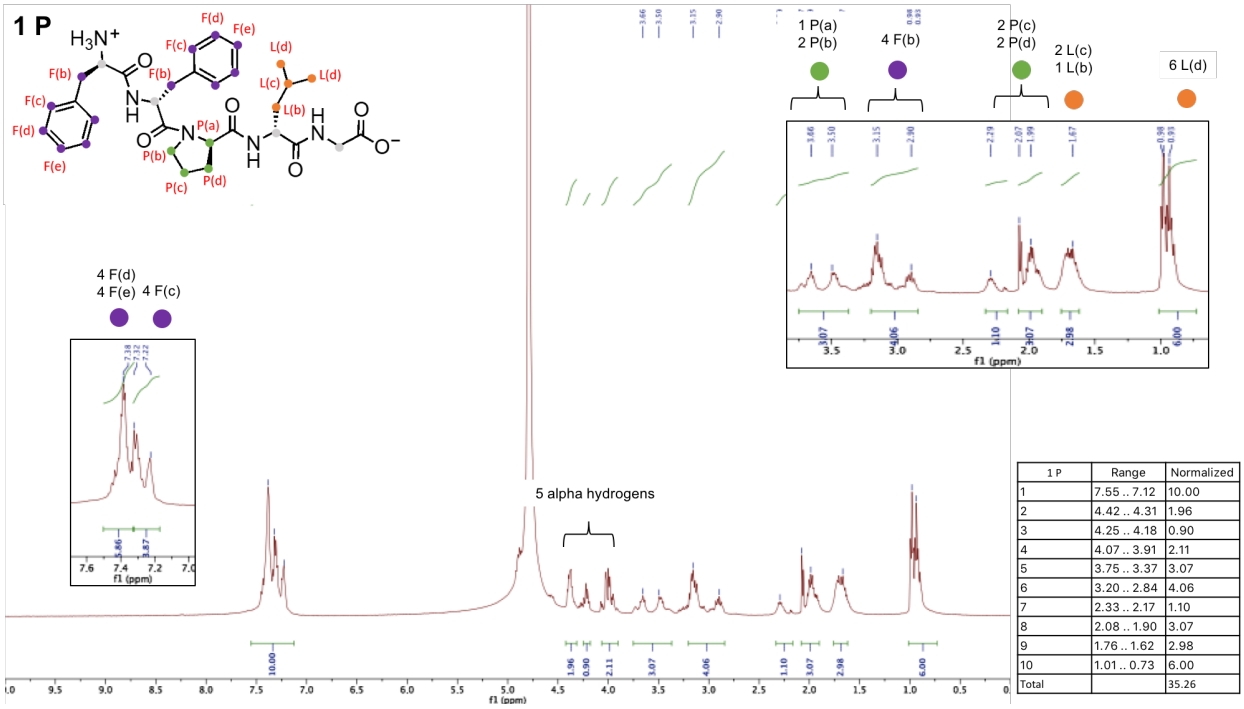
2 A	Range	Normalized
1	7.43 .. 7.31	6.05
2	7.32 .. 7.20	4.08
3	4.66 .. 4.62	1.29
4	4.40 .. 4.30	2.26
5	4.28 .. 4.21	1.20
6	3.95 .. 3.91	1.89
7	3.90 .. 3.87	0.81
8	3.81 .. 3.73	1.16
9	3.21 .. 3.15	1.87
10	3.16 .. 2.98	2.63
11	1.72 .. 1.60	3.05
12	1.42 .. 1.32	3.00
13	0.97 .. 0.90	2.98
14	0.90 .. 0.82	2.94
Total		35.21

Figure S2 B. ^{1}H NMR spectra of **2 A**.



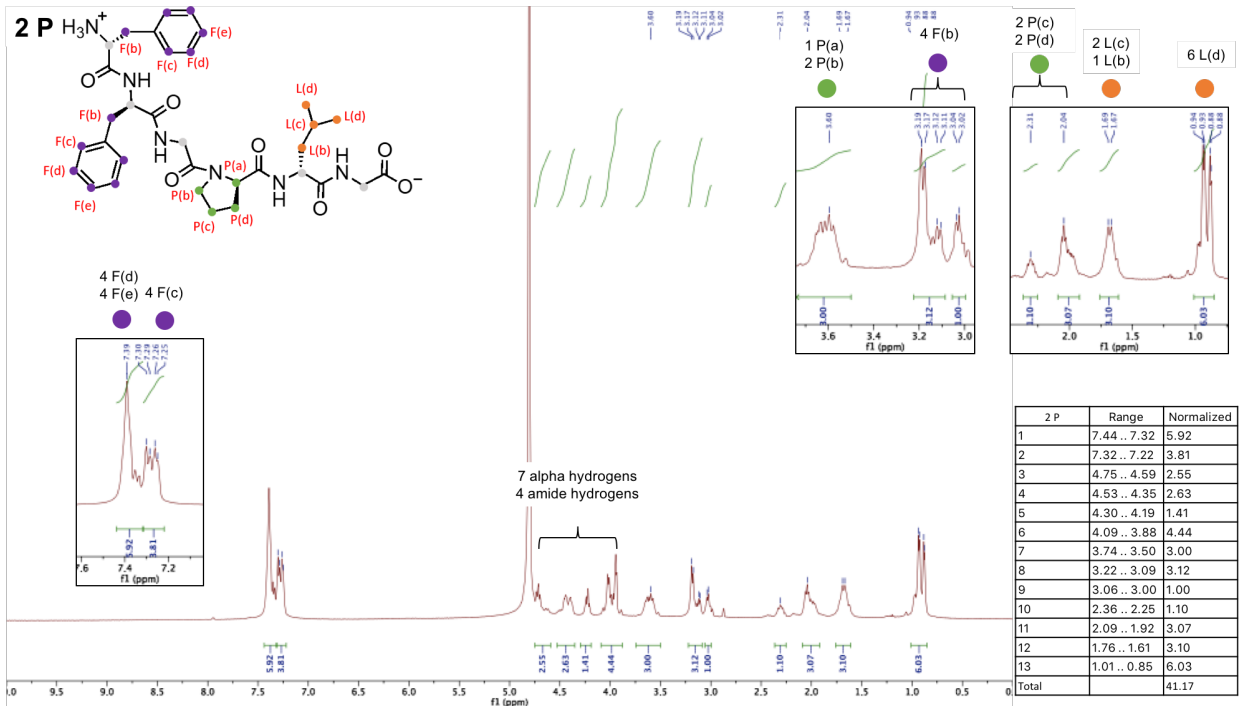
3 A	Range	Normalized
1	7.42 .. 7.29	6.07
2	7.30 .. 7.15	4.01
3	4.61 .. 4.57	2.02
4	4.40 .. 4.29	2.51
5	3.90 .. 3.76	2.41
6	3.68 .. 3.60	0.92
7	3.18 .. 3.11	0.82
8	3.00 .. 2.90	0.97
9	2.90 .. 2.77	2.05
10	1.46 .. 1.35	5.92
Total		27.69

Figure S2 C. ^{1}H NMR spectra of **3 A**.



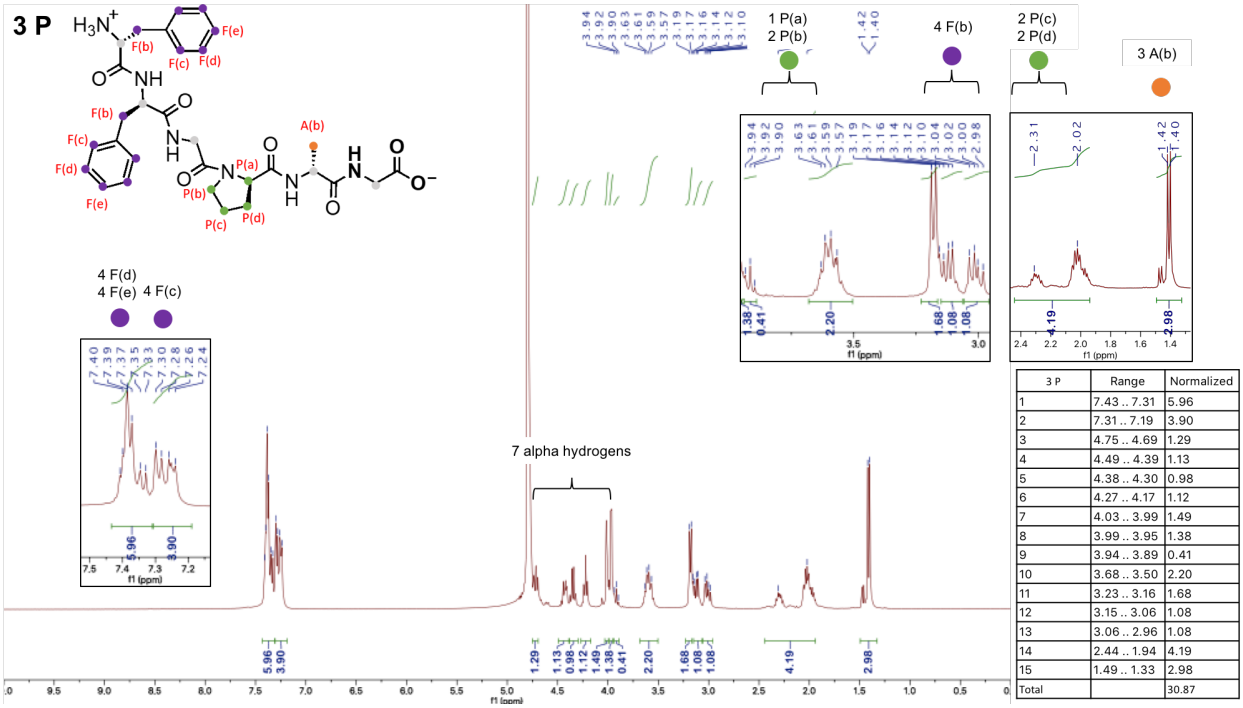
1 P	Range	Normalized
1	7.55 .. 7.12	10.00
2	4.42 .. 4.31	1.96
3	4.25 .. 4.18	0.90
4	4.07 .. 3.91	2.11
5	3.75 .. 3.37	3.07
6	3.20 .. 2.84	4.06
7	2.33 .. 2.17	1.10
8	2.08 .. 1.90	3.07
9	1.76 .. 1.62	2.98
10	1.01 .. 0.73	6.00
Total		35.26

Figure S2 D. ^{1}H NMR spectra of **1 P**.



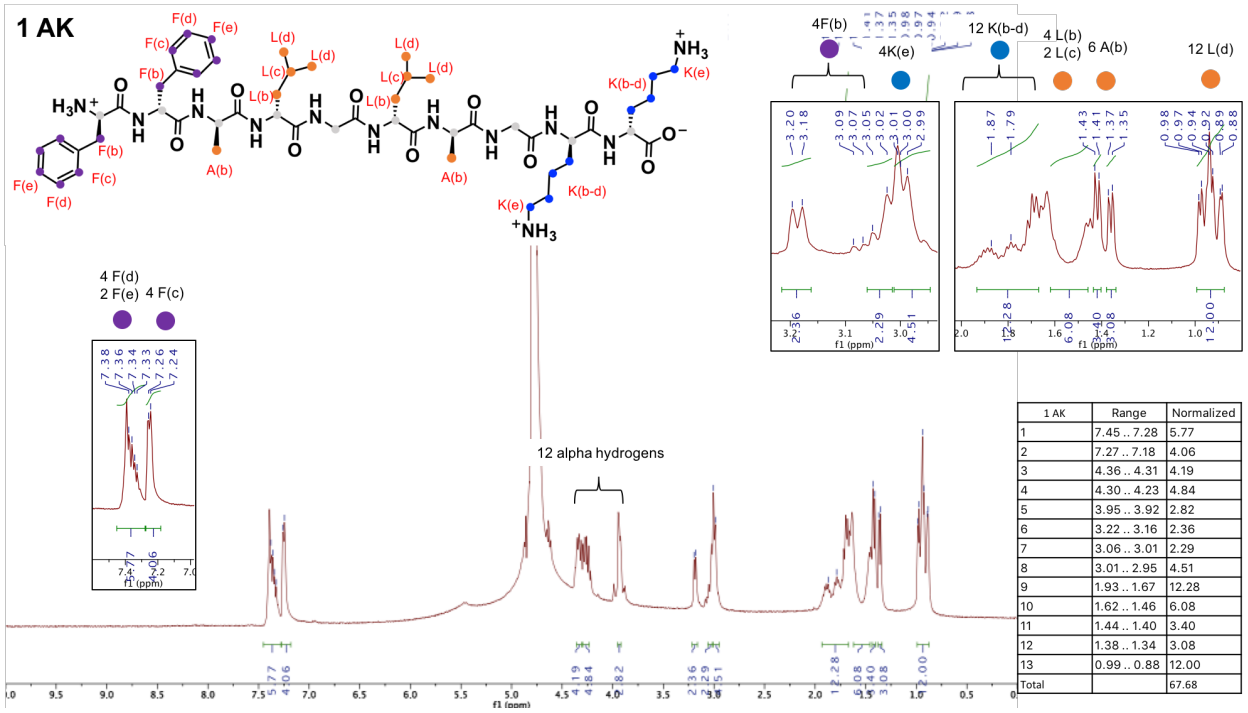
2 P	Range	Normalized
1	7.44 .. 7.32	5.92
2	7.32 .. 7.22	3.81
3	4.75 .. 4.59	2.55
4	4.53 .. 4.35	2.63
5	4.30 .. 4.19	1.41
6	4.09 .. 3.88	4.44
7	3.74 .. 3.50	3.00
8	3.22 .. 3.09	3.12
9	3.06 .. 3.00	1.00
10	2.36 .. 2.25	1.10
11	2.09 .. 1.92	3.07
12	1.76 .. 1.61	3.10
13	1.01 .. 0.85	6.03
Total		41.17

Figure S2 E. ^{1}H NMR spectra of **2 P**.



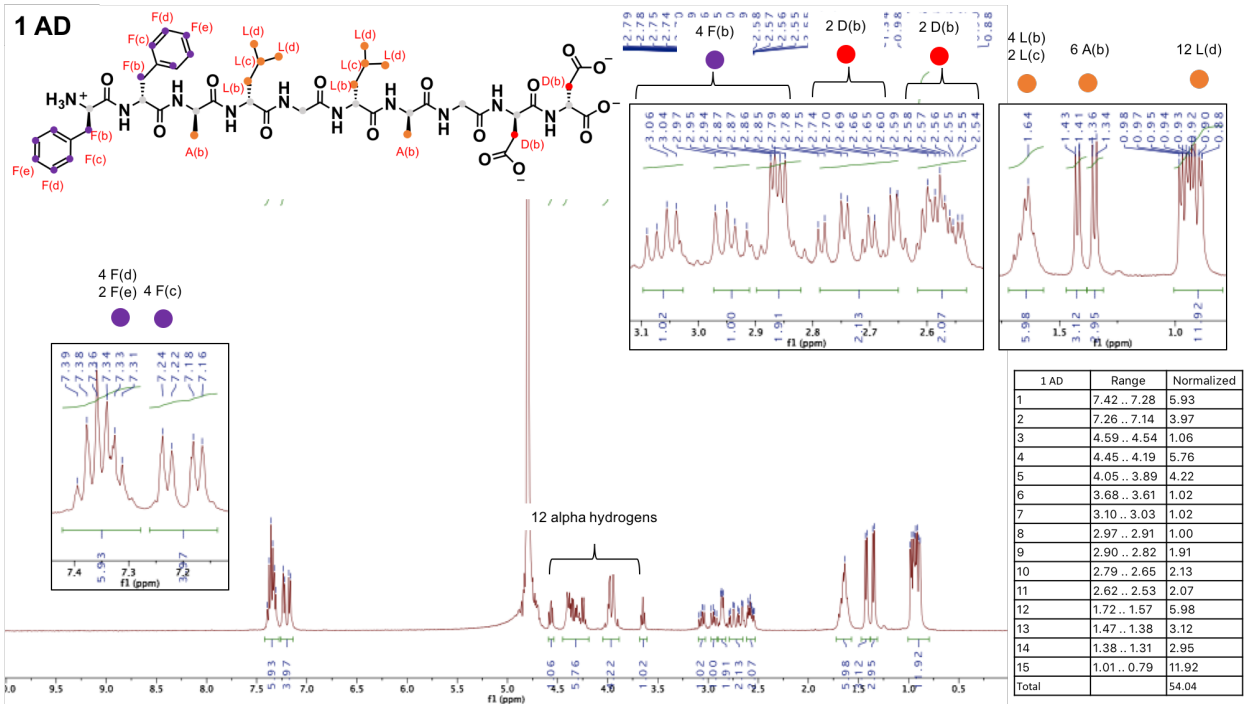
3 P	Range	Normalized
1	7.43 .. 7.31	5.96
2	7.31 .. 7.19	3.90
3	4.75 .. 4.69	1.29
4	4.49 .. 4.39	1.13
5	4.38 .. 4.30	0.98
6	4.27 .. 4.17	1.12
7	4.03 .. 3.99	1.49
8	3.99 .. 3.95	1.38
9	3.94 .. 3.89	0.41
10	3.68 .. 3.50	2.20
11	3.23 .. 3.16	1.68
12	3.15 .. 3.06	1.08
13	3.06 .. 2.96	1.08
14	2.44 .. 1.94	4.19
15	1.49 .. 1.33	2.98
Total		30.87

Figure S2 F. ^{1}H NMR spectra of **3 P**.



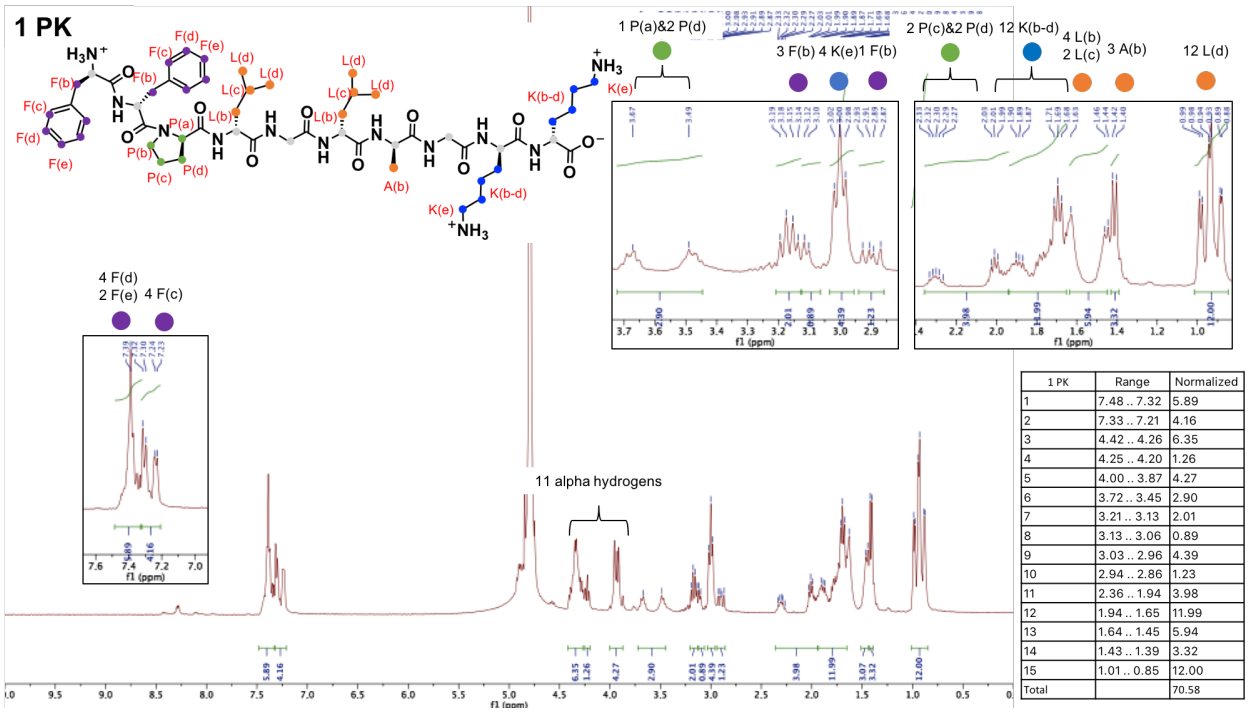
1 AK	Range	Normalized
1	7.45 .. 7.28	5.77
2	7.27 .. 7.18	4.06
3	4.36 .. 4.31	4.19
4	4.30 .. 4.23	4.84
5	3.95 .. 3.92	2.82
6	3.22 .. 3.16	2.36
7	3.06 .. 3.01	2.29
8	3.01 .. 2.95	4.51
9	1.93 .. 1.67	12.28
10	1.62 .. 1.46	6.08
11	1.44 .. 1.40	3.40
12	1.38 .. 1.34	3.08
13	0.99 .. 0.88	12.00
Total		67.68

Figure S2 G. ^{1}H NMR spectra of **1 AK**.



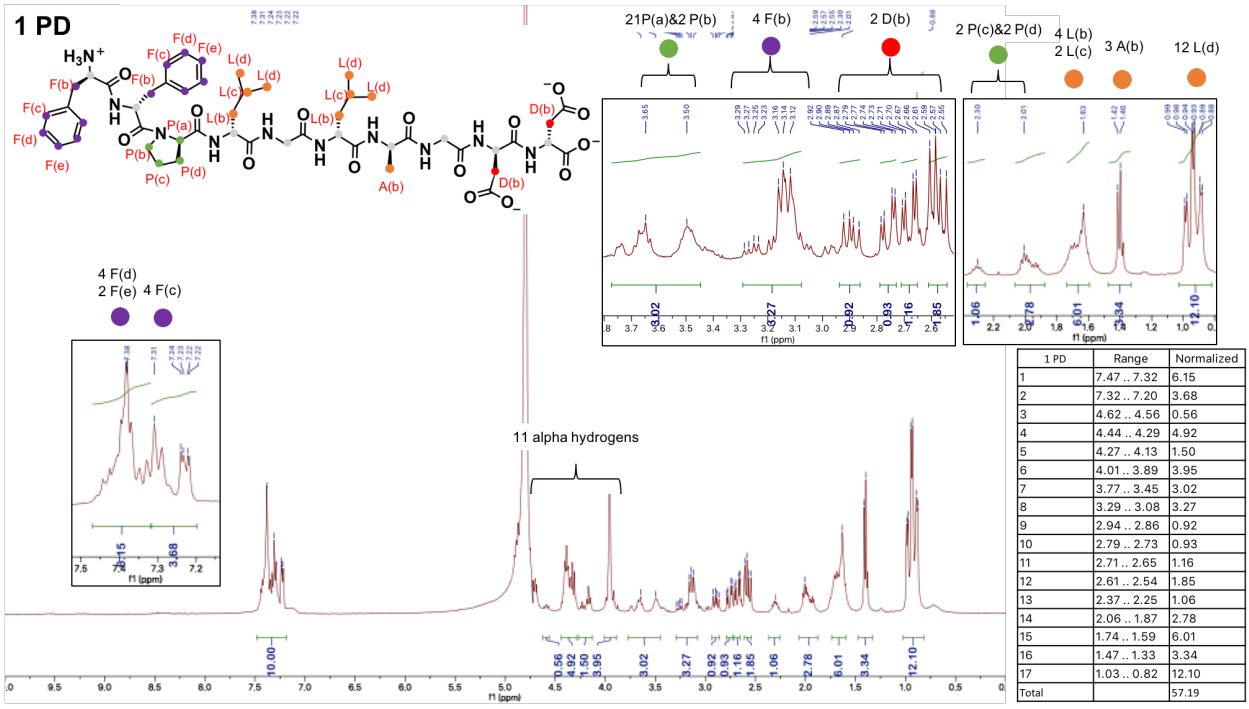
1 AD	Range	Normalized
1	7.42 .. 7.28	5.93
2	7.26 .. 7.14	3.97
3	4.59 .. 4.54	1.06
4	4.45 .. 4.19	5.76
5	4.05 .. 3.89	4.22
6	3.68 .. 3.61	1.02
7	3.10 .. 3.03	1.02
8	2.97 .. 2.91	1.00
9	2.90 .. 2.82	1.91
10	2.79 .. 2.65	2.13
11	2.62 .. 2.53	2.07
12	1.72 .. 1.57	5.98
13	1.47 .. 1.38	3.12
14	1.38 .. 1.31	2.95
15	1.01 .. 0.79	11.92
Total		54.04

Figure S2 H. ^{1}H NMR spectra of **1 AD**.



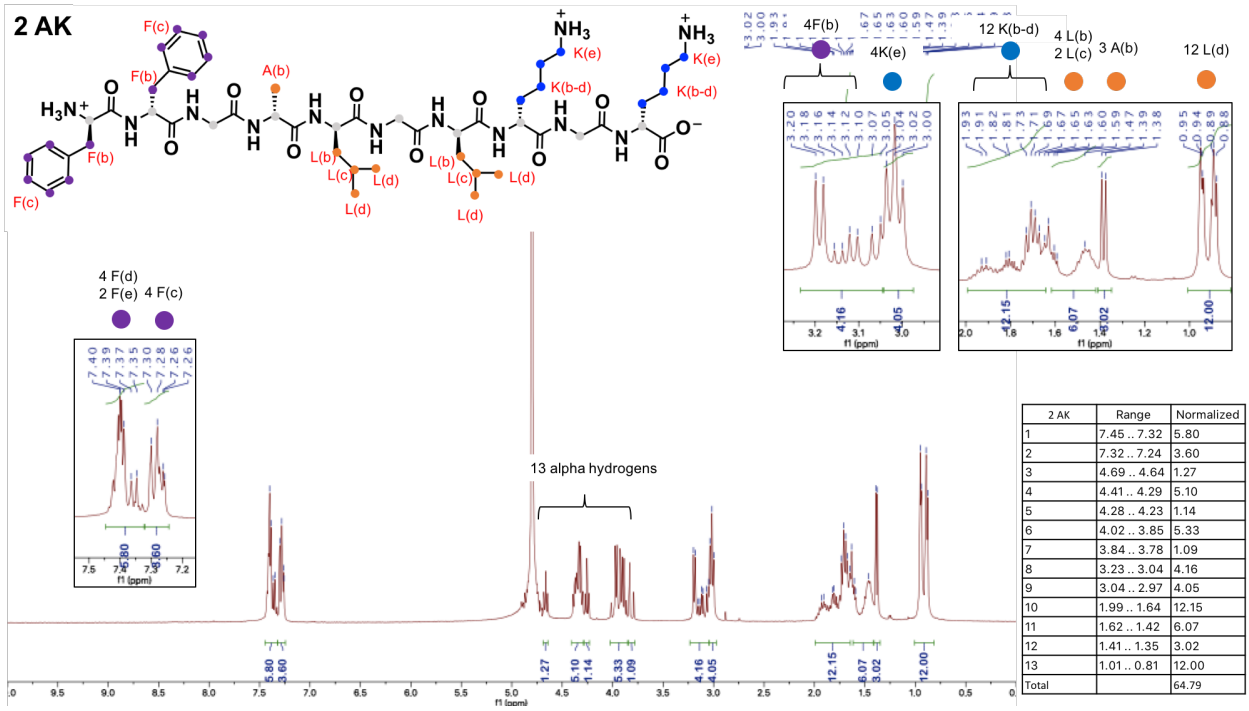
1 PK	Range	Normalized
1	7.48 .. 7.32	5.89
2	7.33 .. 7.21	4.16
3	4.42 .. 4.26	6.35
4	4.25 .. 4.20	1.26
5	4.00 .. 3.87	4.27
6	3.72 .. 3.45	2.90
7	3.21 .. 3.13	2.01
8	3.13 .. 3.06	0.89
9	3.03 .. 2.96	4.39
10	2.94 .. 2.86	1.23
11	2.36 .. 1.94	3.98
12	1.94 .. 1.65	11.99
13	1.64 .. 1.45	5.94
14	1.43 .. 1.39	3.32
15	1.01 .. 0.85	12.00
Total		70.58

Figure S2 I. ^{1}H NMR spectra of **1 PK**.



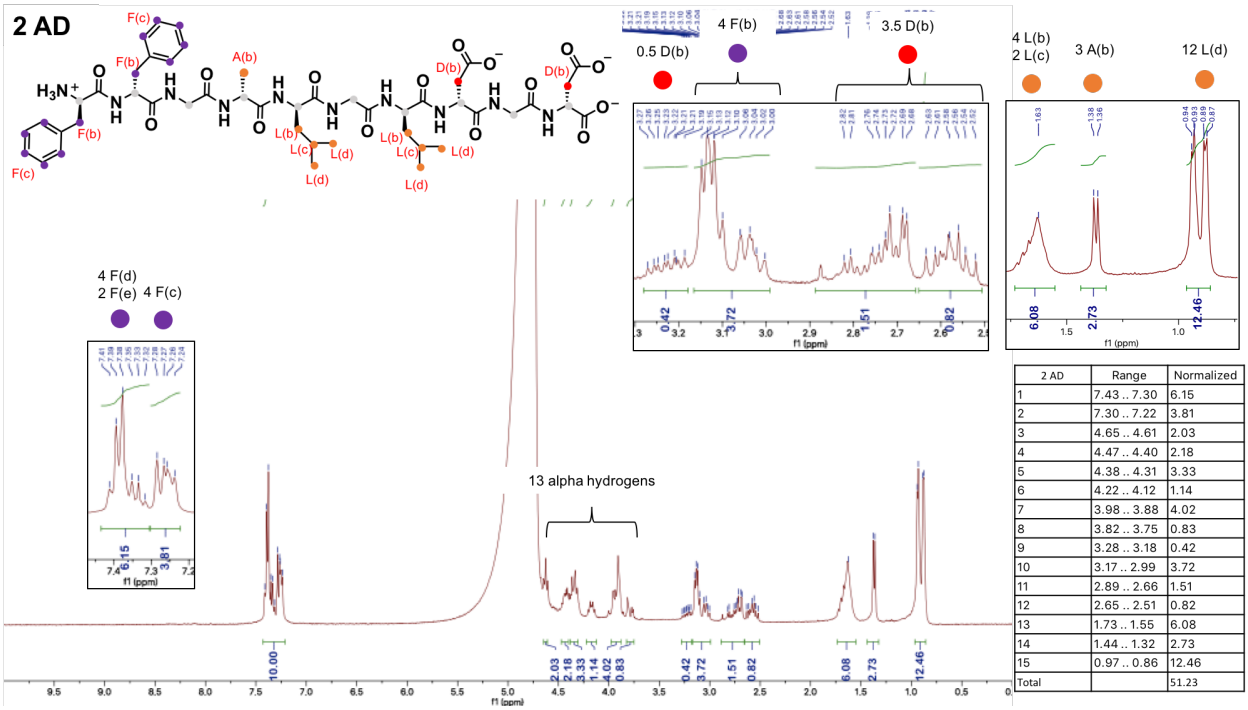
1 PD	Range	Normalized
1	7.47 .. 7.32	6.15
2	7.32 .. 7.20	3.68
3	4.62 .. 4.56	0.56
4	4.44 .. 4.29	4.92
5	4.27 .. 4.13	1.50
6	4.01 .. 3.89	3.95
7	3.77 .. 3.45	3.02
8	3.29 .. 3.08	3.27
9	2.94 .. 2.86	0.92
10	2.79 .. 2.73	0.93
11	2.71 .. 2.65	1.16
12	2.61 .. 2.54	1.85
13	2.37 .. 2.25	1.06
14	2.06 .. 1.87	2.78
15	1.74 .. 1.59	6.01
16	1.47 .. 1.33	3.34
17	1.03 .. 0.82	12.10
Total		57.19

Figure S2 J. ^{1}H NMR spectra of **1 PD**.



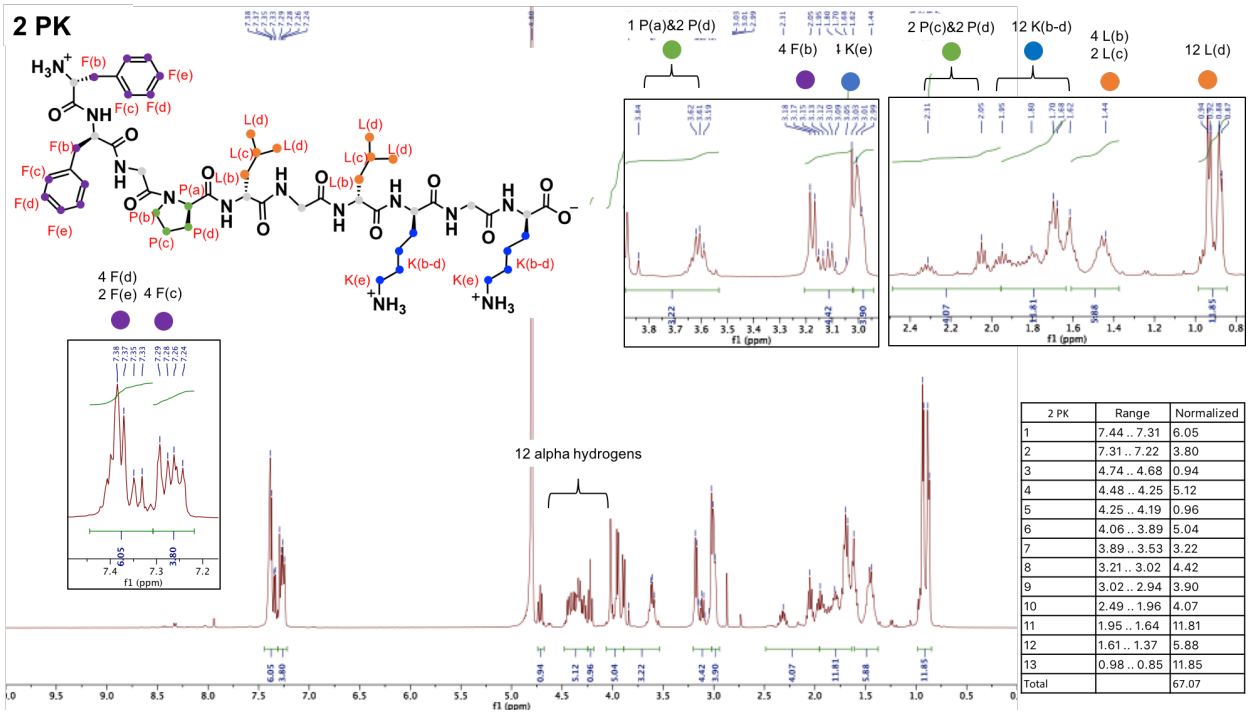
2 AK	Range	Normalized
1	7.45 .. 7.32	5.80
2	7.32 .. 7.24	3.60
3	4.69 .. 4.64	1.27
4	4.41 .. 4.29	5.10
5	4.28 .. 4.23	1.14
6	4.02 .. 3.85	5.33
7	3.84 .. 3.78	1.09
8	3.23 .. 3.04	4.16
9	3.04 .. 2.97	4.05
10	1.99 .. 1.64	12.15
11	1.62 .. 1.42	6.07
12	1.41 .. 1.35	3.02
13	1.01 .. 0.81	12.00
Total		64.79

Figure S2 K. ^{1}H NMR spectra of **2 AK**.



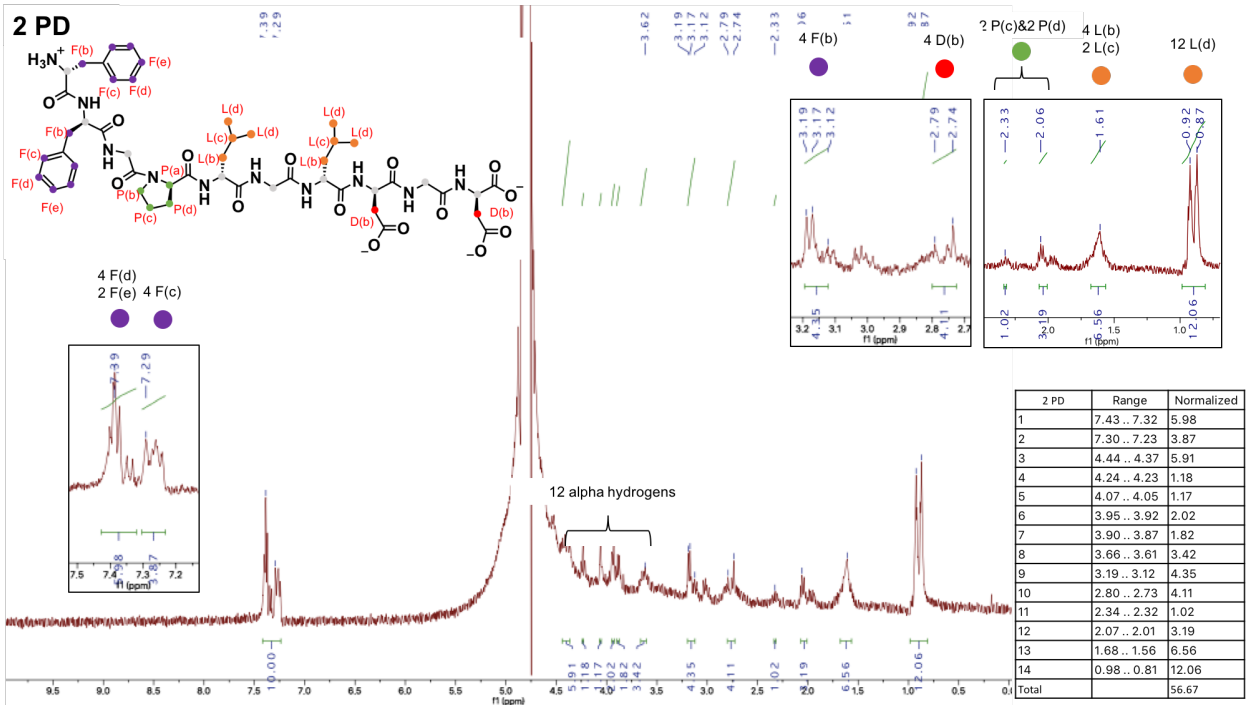
2 AD	Range	Normalized
1	7.43 .. 7.30	6.15
2	7.30 .. 7.22	3.81
3	4.65 .. 4.61	2.03
4	4.47 .. 4.40	2.18
5	4.38 .. 4.31	3.33
6	4.22 .. 4.12	1.14
7	3.98 .. 3.88	4.02
8	3.82 .. 3.75	0.83
9	3.28 .. 3.18	0.42
10	3.17 .. 2.99	3.72
11	2.89 .. 2.66	1.51
12	2.65 .. 2.51	0.82
13	1.73 .. 1.55	6.08
14	1.44 .. 1.32	2.73
15	0.97 .. 0.86	12.46
Total		51.23

Figure S2 L. ^{1}H NMR spectra of **2 AD**.



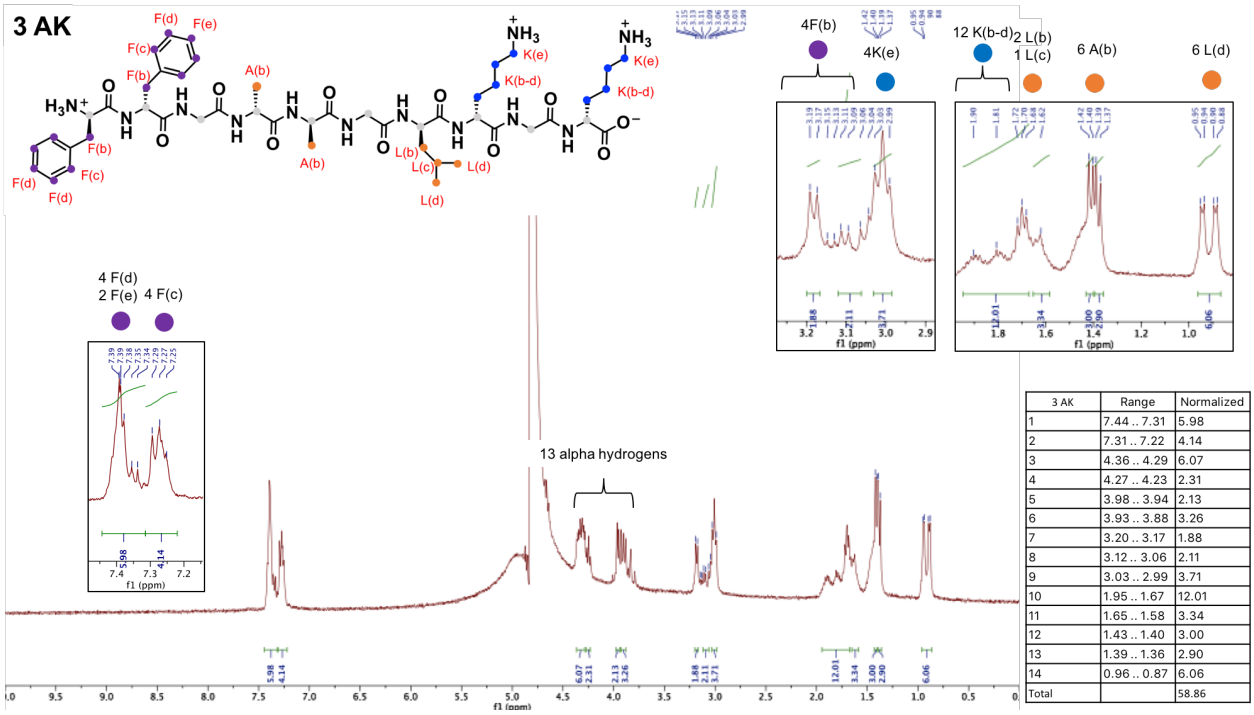
2 PK	Range	Normalized
1	7.44 .. 7.31	6.05
2	7.31 .. 7.22	3.80
3	4.74 .. 4.68	0.94
4	4.48 .. 4.25	5.12
5	4.25 .. 4.19	0.96
6	4.06 .. 3.89	5.04
7	3.89 .. 3.53	3.22
8	3.21 .. 3.02	4.42
9	3.02 .. 2.94	3.90
10	2.49 .. 1.96	4.07
11	1.95 .. 1.64	11.81
12	1.61 .. 1.37	5.88
13	0.98 .. 0.85	11.85
Total		67.07

Figure S2 M. ^{1}H NMR spectra of **2 PK**.



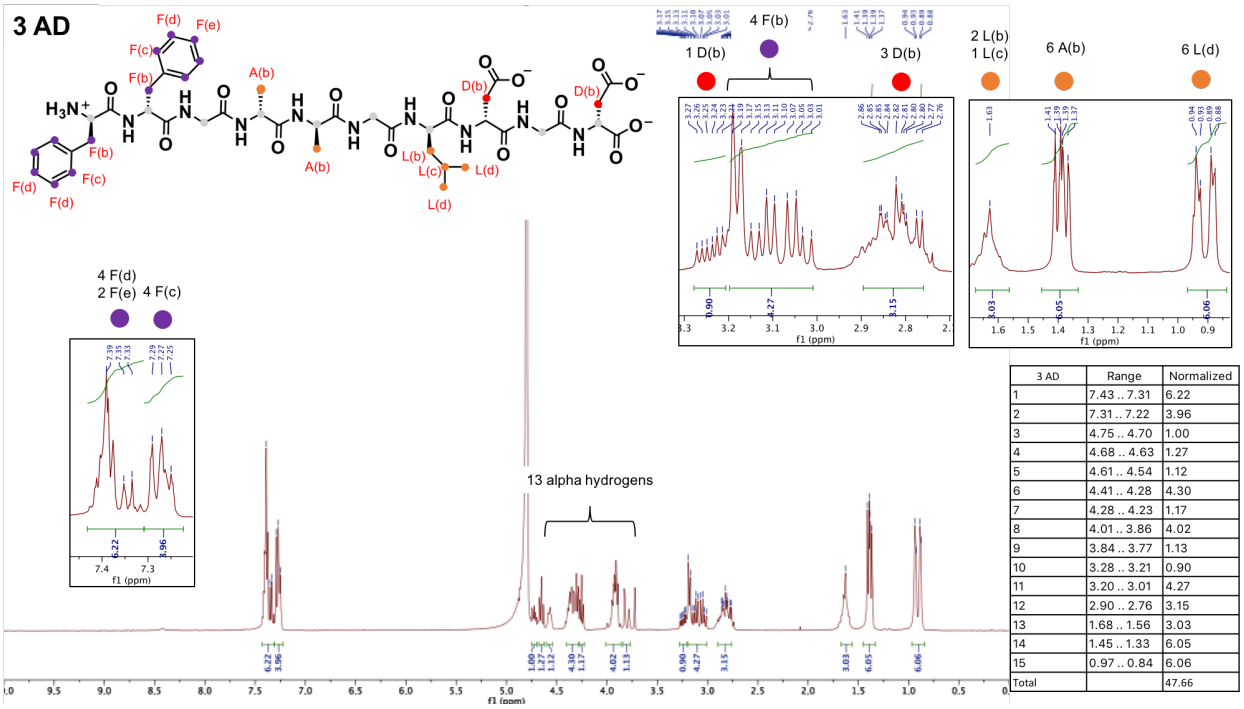
2 PD	Range	Normalized
1	7.43 .. 7.32	5.98
2	7.30 .. 7.23	3.87
3	4.44 .. 4.37	5.91
4	4.24 .. 4.23	1.18
5	4.07 .. 4.05	1.17
6	3.95 .. 3.92	2.02
7	3.90 .. 3.87	1.82
8	3.66 .. 3.61	3.42
9	3.19 .. 3.12	4.35
10	2.80 .. 2.73	4.11
11	2.34 .. 2.32	1.02
12	2.07 .. 2.01	3.19
13	1.68 .. 1.56	6.56
14	0.98 .. 0.81	12.06
Total		56.67

Figure S2 N. ^{1}H NMR spectra of **2 PD**.



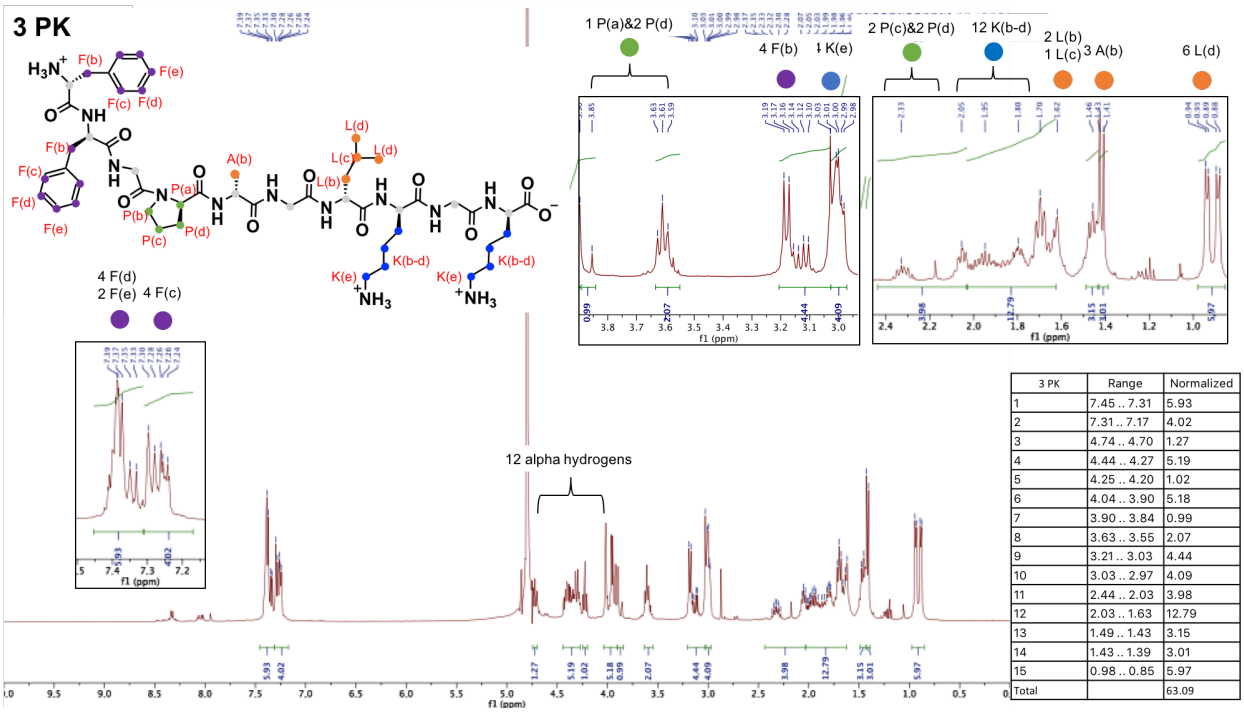
3 AK	Range	Normalized
1	7.44 .. 7.31	5.98
2	7.31 .. 7.22	4.14
3	4.36 .. 4.29	6.07
4	4.27 .. 4.23	2.31
5	3.98 .. 3.94	2.13
6	3.93 .. 3.88	3.26
7	3.20 .. 3.17	1.88
8	3.12 .. 3.06	2.11
9	3.03 .. 2.99	3.71
10	1.95 .. 1.67	12.01
11	1.65 .. 1.58	3.34
12	1.43 .. 1.40	3.00
13	1.39 .. 1.36	2.90
14	0.96 .. 0.87	6.06
Total		58.86

Figure S2 O. ^{1}H NMR spectra of **3 AK**.



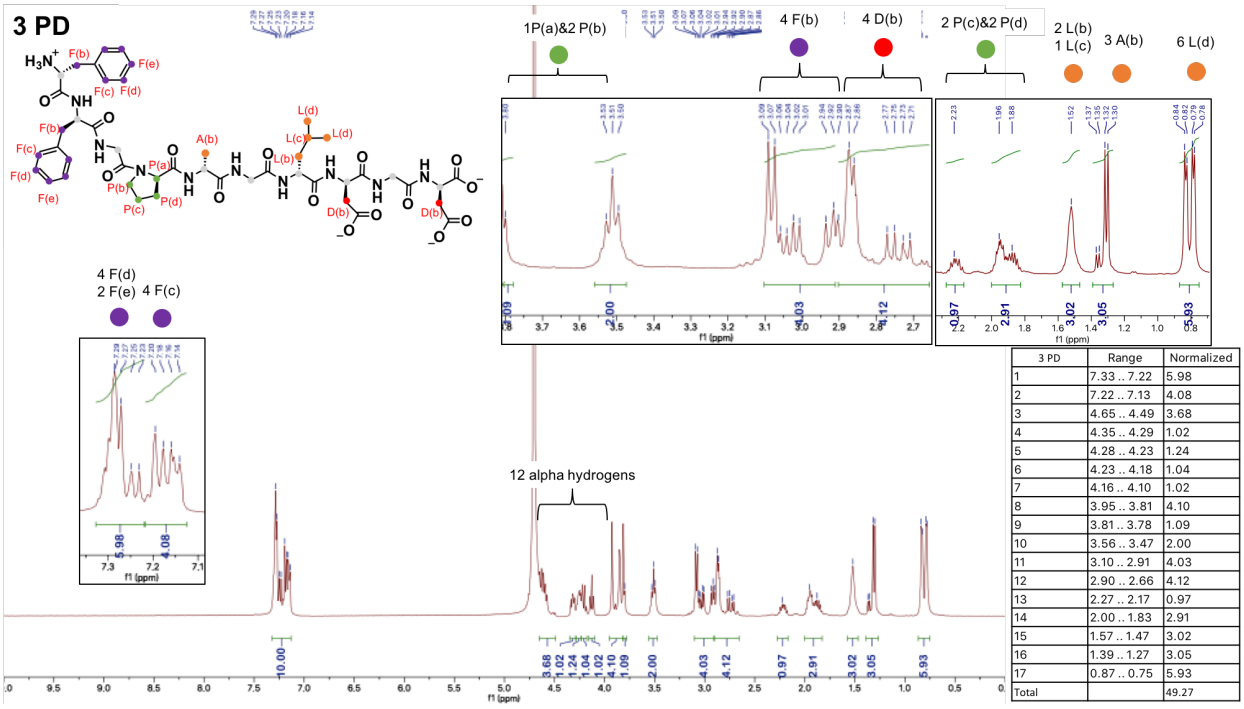
3 AD	Range	Normalized
1	7.43 .. 7.31	6.22
2	7.31 .. 7.22	3.96
3	4.75 .. 4.70	1.00
4	4.68 .. 4.63	1.27
5	4.61 .. 4.54	1.12
6	4.41 .. 4.28	4.30
7	4.28 .. 4.23	1.17
8	4.01 .. 3.86	4.02
9	3.84 .. 3.77	1.13
10	3.28 .. 3.21	0.90
11	3.20 .. 3.01	4.27
12	2.90 .. 2.76	3.15
13	1.68 .. 1.56	3.03
14	1.45 .. 1.33	6.05
15	0.97 .. 0.84	6.06
Total		47.66

Figure S2 P. ^{1}H NMR spectra of **3 AD**.



3 PK	Range	Normalized
1	7.45 .. 7.31	5.93
2	7.31 .. 7.17	4.02
3	4.74 .. 4.70	1.27
4	4.44 .. 4.27	5.19
5	4.25 .. 4.20	1.02
6	4.04 .. 3.90	5.18
7	3.90 .. 3.84	0.99
8	3.63 .. 3.55	2.07
9	3.21 .. 3.03	4.44
10	3.03 .. 2.97	4.09
11	2.44 .. 2.03	3.98
12	2.03 .. 1.63	12.79
13	1.49 .. 1.43	3.15
14	1.43 .. 1.39	3.01
15	0.98 .. 0.85	5.97
Total		63.09

Figure S2 Q. ^{1}H NMR spectra of **3 PK**.



3 PD	Range	Normalized
1	7.33 .. 7.22	5.98
2	7.22 .. 7.13	4.08
3	4.65 .. 4.49	3.68
4	4.35 .. 4.29	1.02
5	4.28 .. 4.23	1.24
6	4.23 .. 4.18	1.04
7	4.16 .. 4.10	1.02
8	3.95 .. 3.81	4.10
9	3.81 .. 3.78	1.09
10	3.56 .. 3.47	2.00
11	3.10 .. 2.91	4.03
12	2.90 .. 2.66	4.12
13	2.27 .. 2.17	0.97
14	2.00 .. 1.83	2.91
15	1.57 .. 1.47	3.02
16	1.39 .. 1.27	3.05
17	0.87 .. 0.75	5.93
Total		49.27

Figure S2 R. ^{1}H NMR spectra of **3 PD**.

3. Determination of peptide hydrolysis rate by MMP-9 using Liquid Chromatography-Mass Spectrometry (LC-MS)

30μL of the MMP-9 reaction solution (or peptide only solution at t=0) was directly added to 50% acetonitrile in water containing 0.1% TFA. Samples were analyzed on an LCMS system comprised of an Agilent 1200 LC system coupled to an Agilent 6340 ion trap mass spectrometer. Samples were injected onto an Agilent Zorbax column (SB-C8, 5μM, 2.1x50mm) using a gradient of 2-50% acetonitrile in water (0.1% formic acid) at a flow rate of 200μL/min over 10minutes followed by a 2min wash step with 95% acetonitrile.

Table S1. The enzymatic products were identified and quantified for two separate trials. Column '#K/D' lists the MW of the 12 peptides and column 'P6-P1↓' lists the MW of the N termini portion of the enzymatic product (and observed major products for the anionic peptides).

	MW (g/mol)		First Trial (% conversion)					Second Trial (% conversion)				
	#K/D	P6-P1↓	0hr	24hr	48hr	72hr	96hr	0hr	24hr	48hr	72hr	96hr
1AK	1050.62	553.65	0	0	0	0	0	0	0	0	0	0
1AD	1024.49	553.65	0	0	0	0	0	0	0	0	0	0
		877.42 (↓P4-P5')	0	14.35	34.14	49.64	57.42	0	11.57	28.94	41.82	50.82
1PK	1075.64	579.69	0	52.61	83.93	100.00	100.00	0	50.07	78.62	87.12	88.67
1PD	1050.5	579.69	0	5.18	6.50	7.27	8.13	0	3.64	5.88	6.30	6.57
2AK	1036.62	610.7	0	28.86	44.68	52.02	57.34	0	14.11	25.47	60.20	60.20
2AD	1010.46	610.7	0	0	0	0	0	0	0	0	0	0
		863.4 (↓P5-P4')	0	0	4.13	4.51	7.04	0	0	7.18	20.93	30.78
2PK	1062.64	636.74	0	61.70	100.00	95.02	89.68	0	69.62	97.49	100.00	85.53
2PD	1036.47	636.74	0	0	0	0	0	0	2.68	5.41	5.17	5.47
		889.42 (↓P5-P4')	0	8.49	40.37	52.96	100.00	0	0	0	0	0
3AK	994.57	568.62	0	2.67	4.50	4.42	4.53	0	10.53	10.62	10.62	14.02
3AD	968.41	568.62	0	0	0	0	0	0	0	0	0	0
3PK	1020.59	594.66	0	48.47	50.28	75.70	82.33	0	70.67	72.04	84.73	100.00
3PD	994.43	594.66	0	0	0	0	0	0	0	0	0	0
		847.37 (↓P5-P4')	0	4.53	41.15	68.38	81.11	0	0	7.98	22.28	39.26

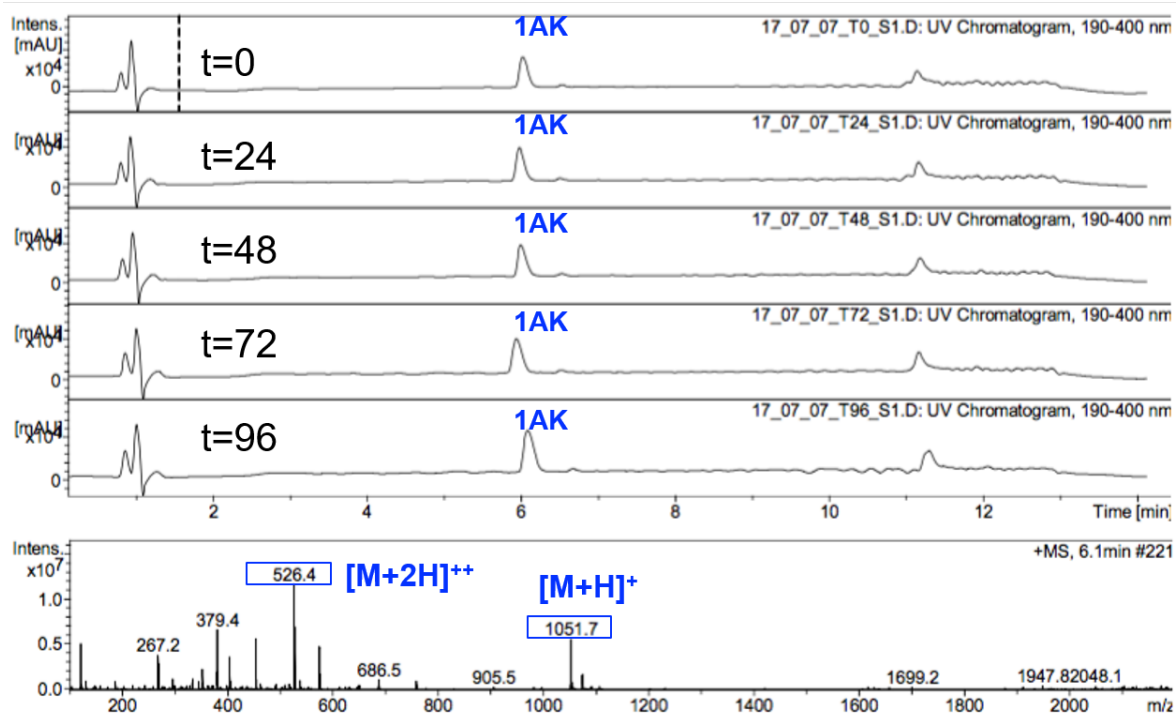


Figure S3 A. First trial LC-MS spectra of **1 AK**.

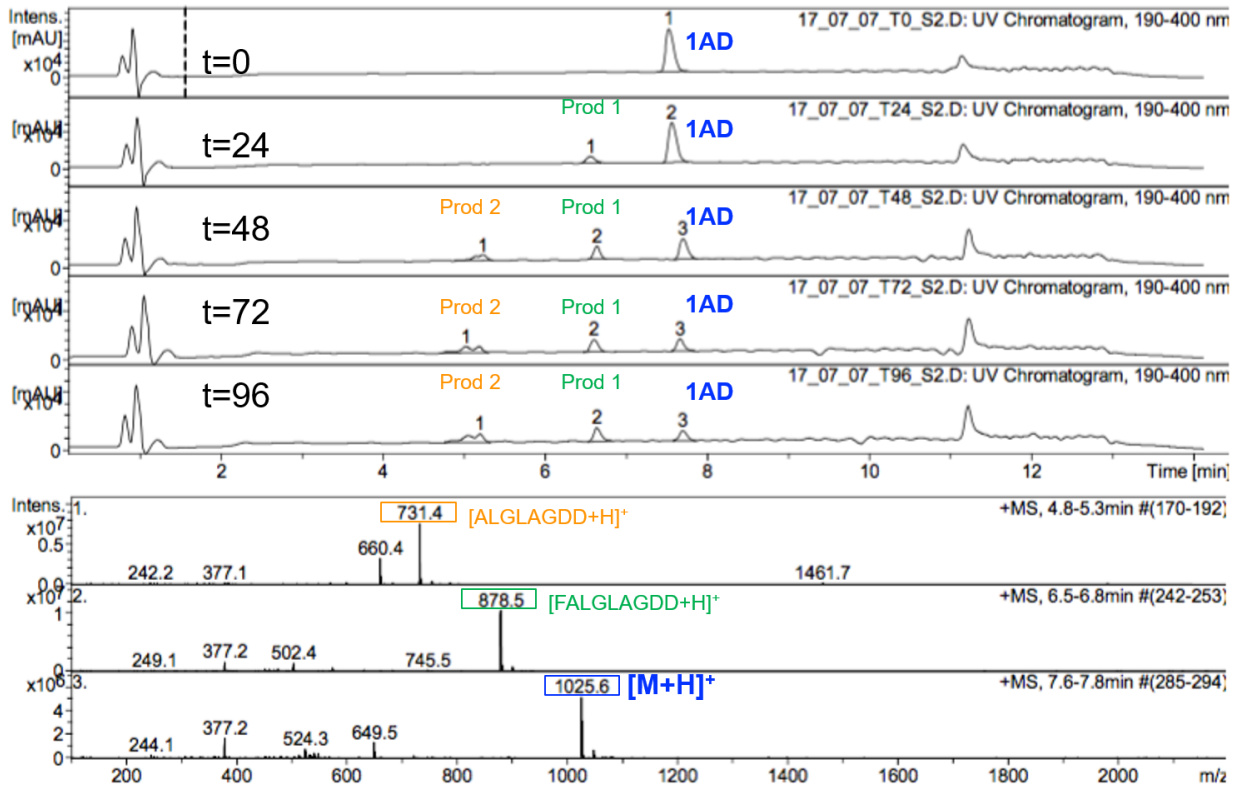


Figure S3 B. First trial LC-MS spectra of **1 AD**.

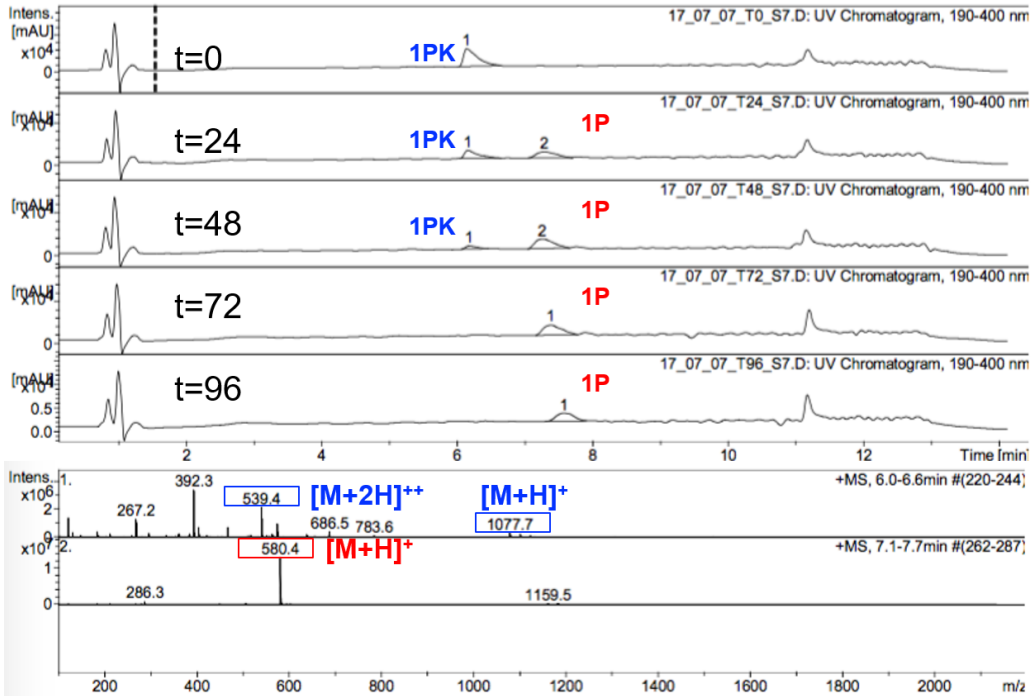


Figure S3 C. First trial LC-MS spectra of **1 PK**.

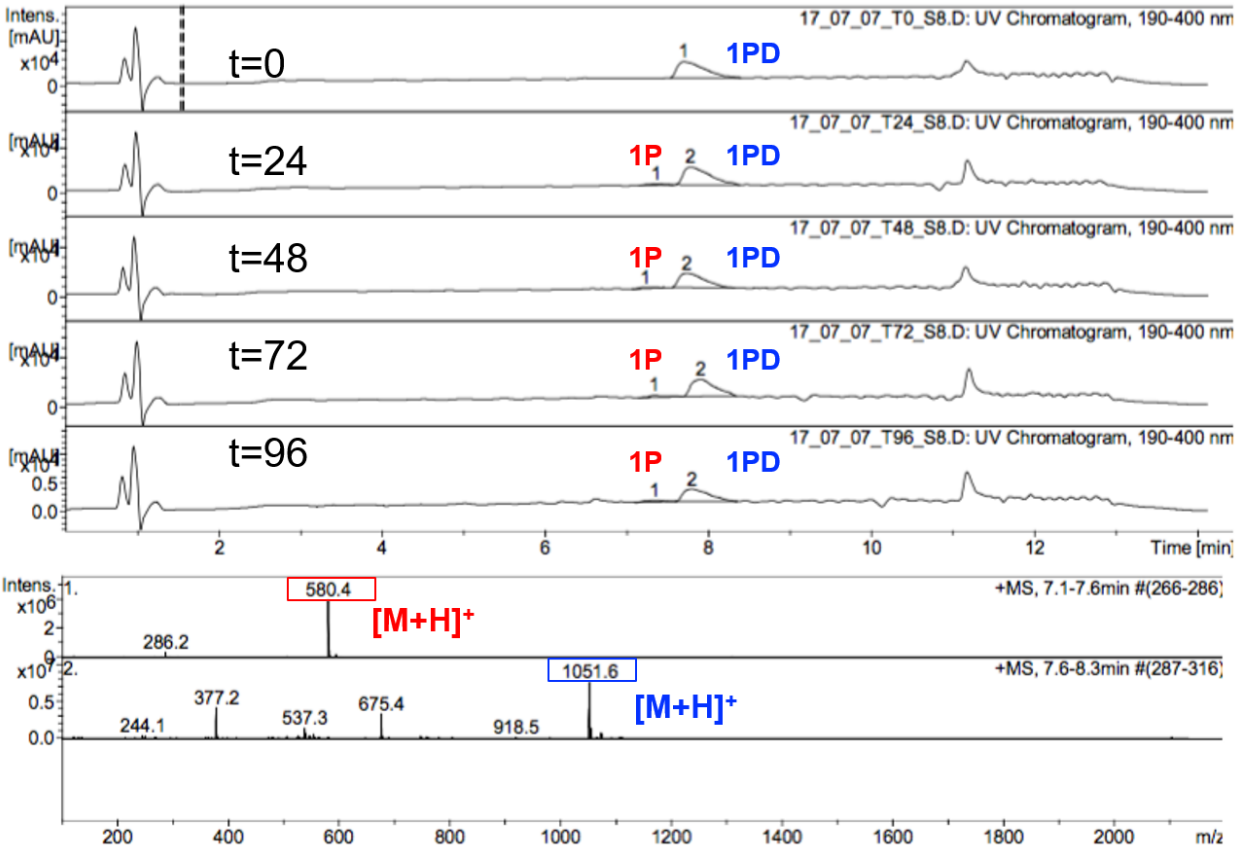


Figure S3 D. First trial LC-MS spectra of **1 PD**.

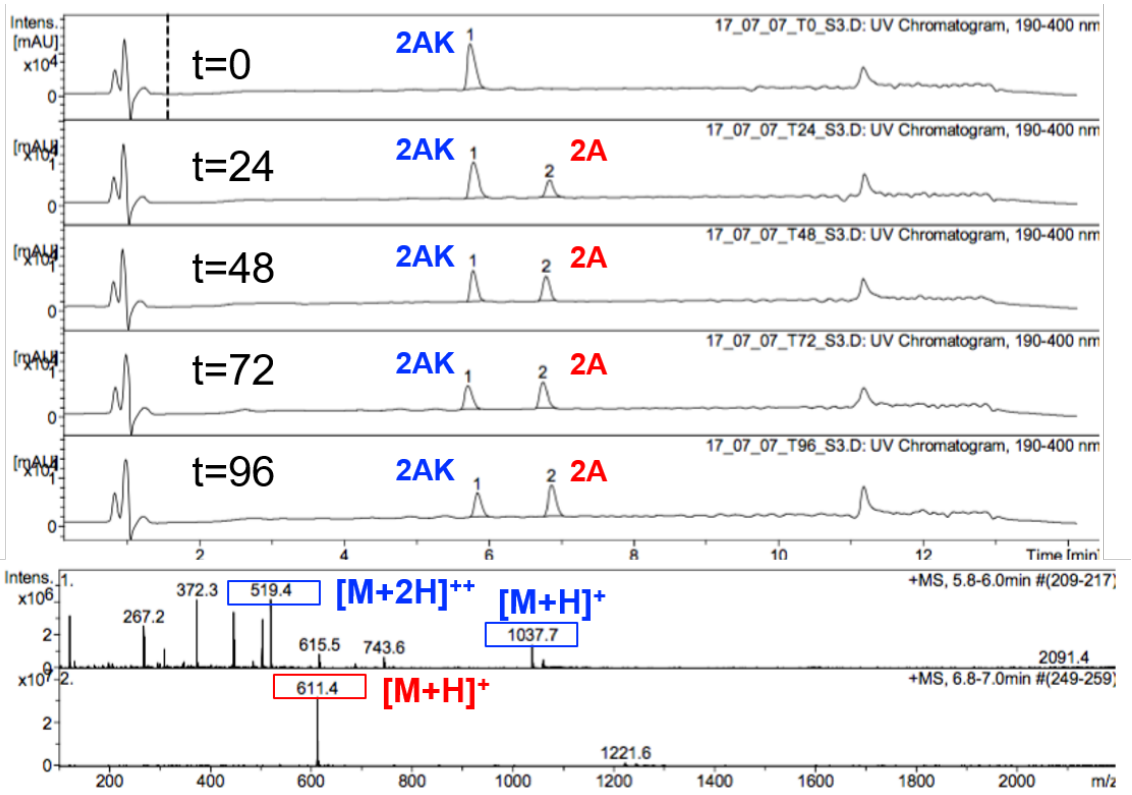


Figure S3 E. First trial LC-MS spectra of **2 AK**.

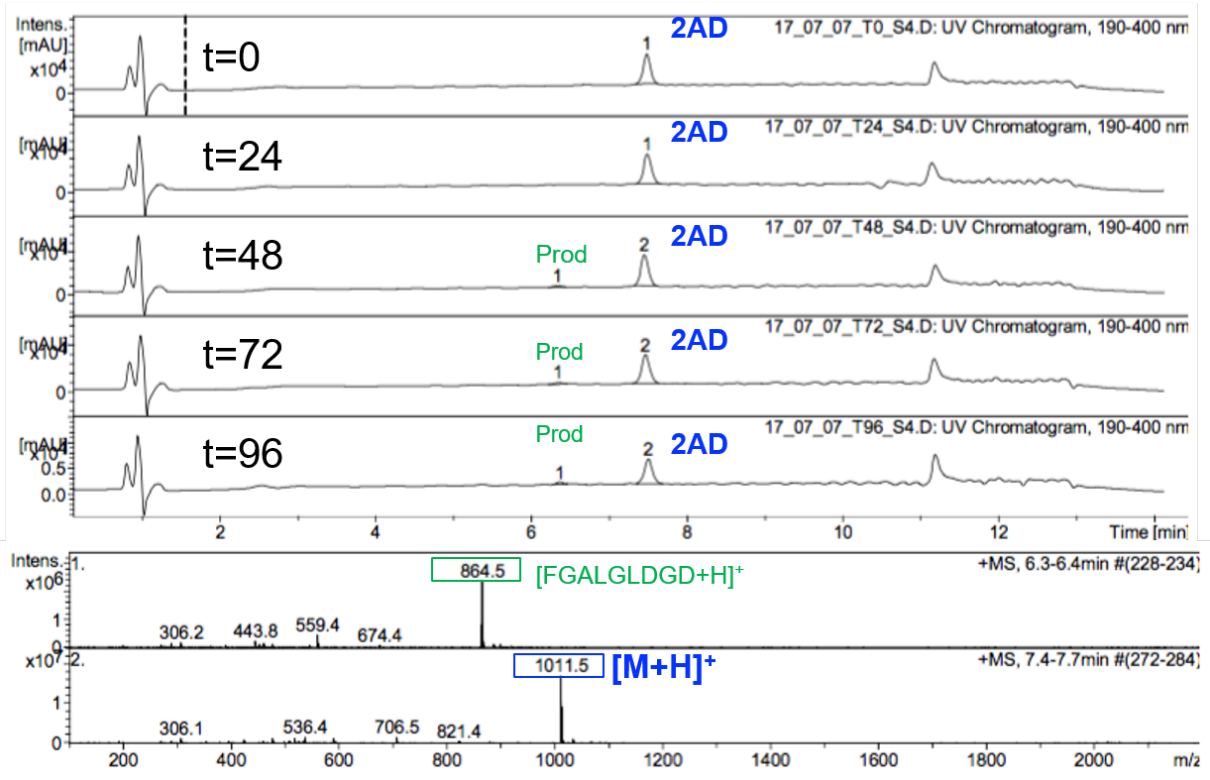


Figure S3 F. First trial LC-MS spectra of **2 AD**.

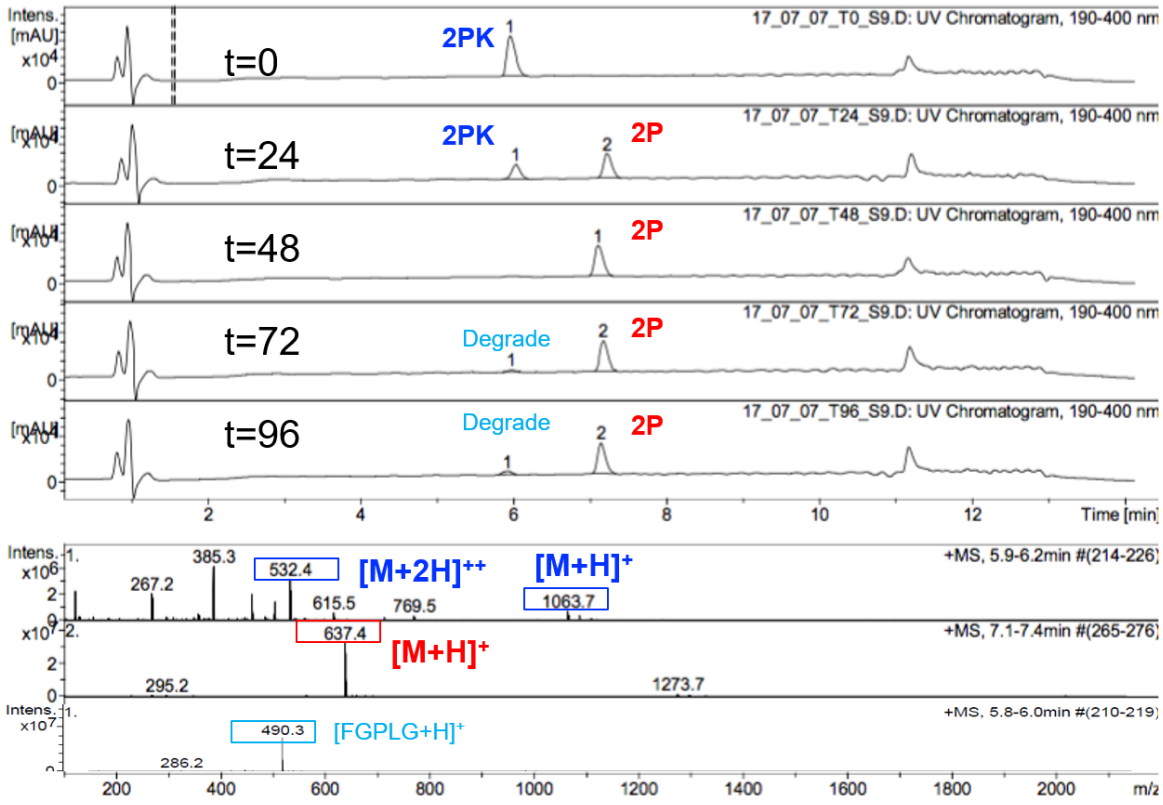


Figure S3 G. First trial LC-MS spectra of **2 PK**.

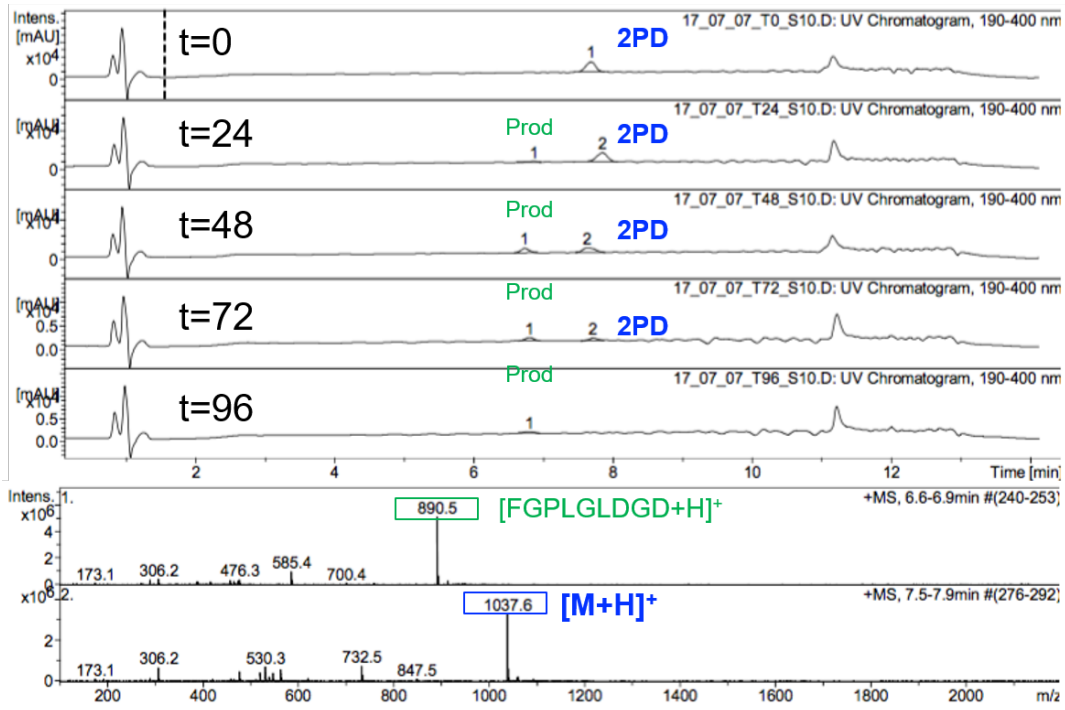


Figure S3 H. First trial LC-MS spectra of **2 PD**.

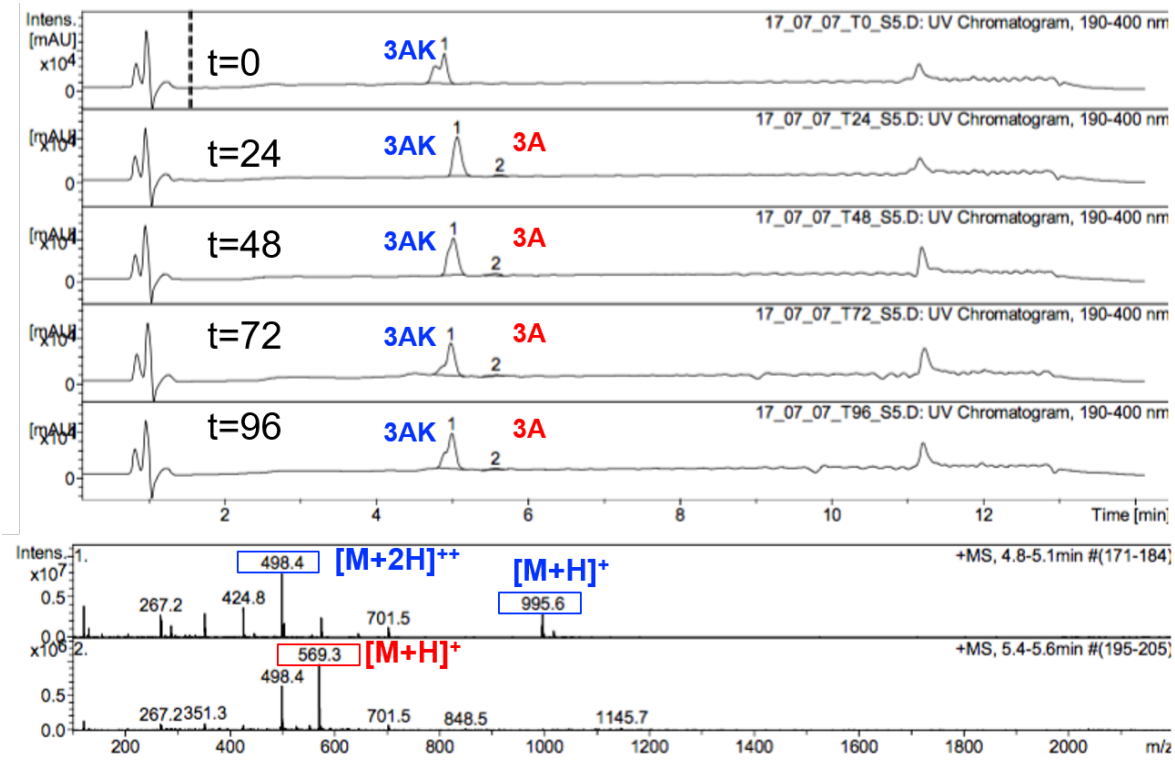


Figure S3 I. First trial LC-MS spectra of **3 AK**.

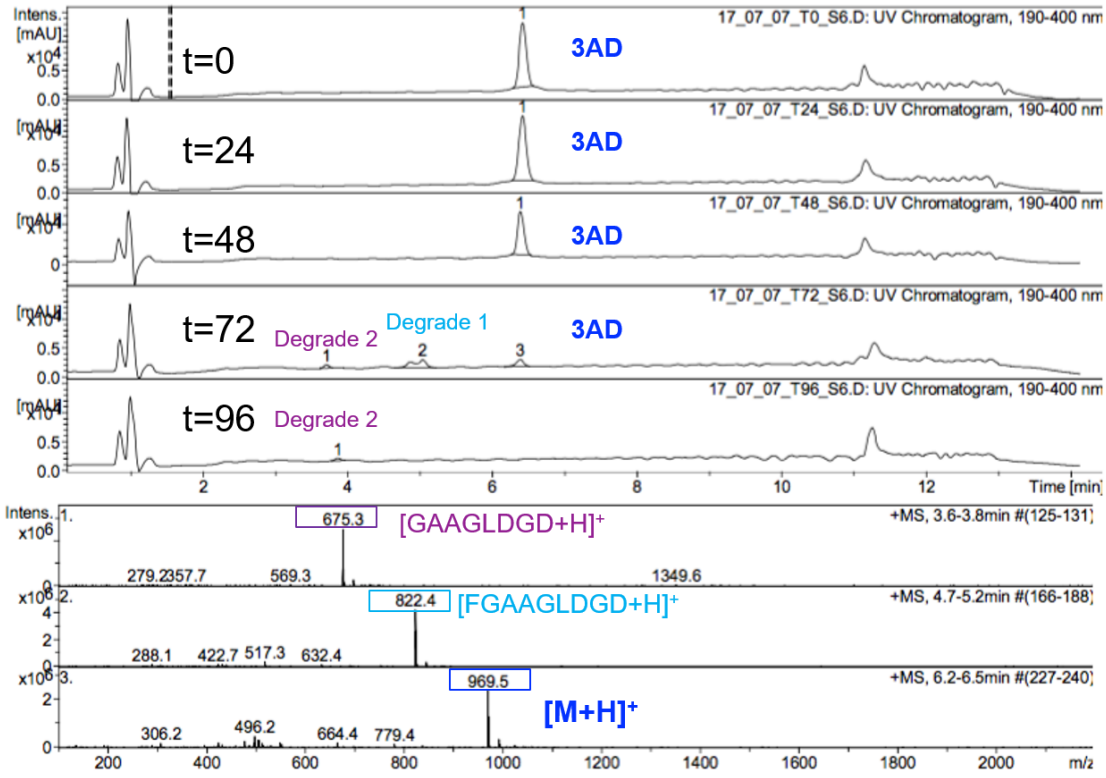


Figure S3 J. First trial LC-MS spectra of **3 AD**.

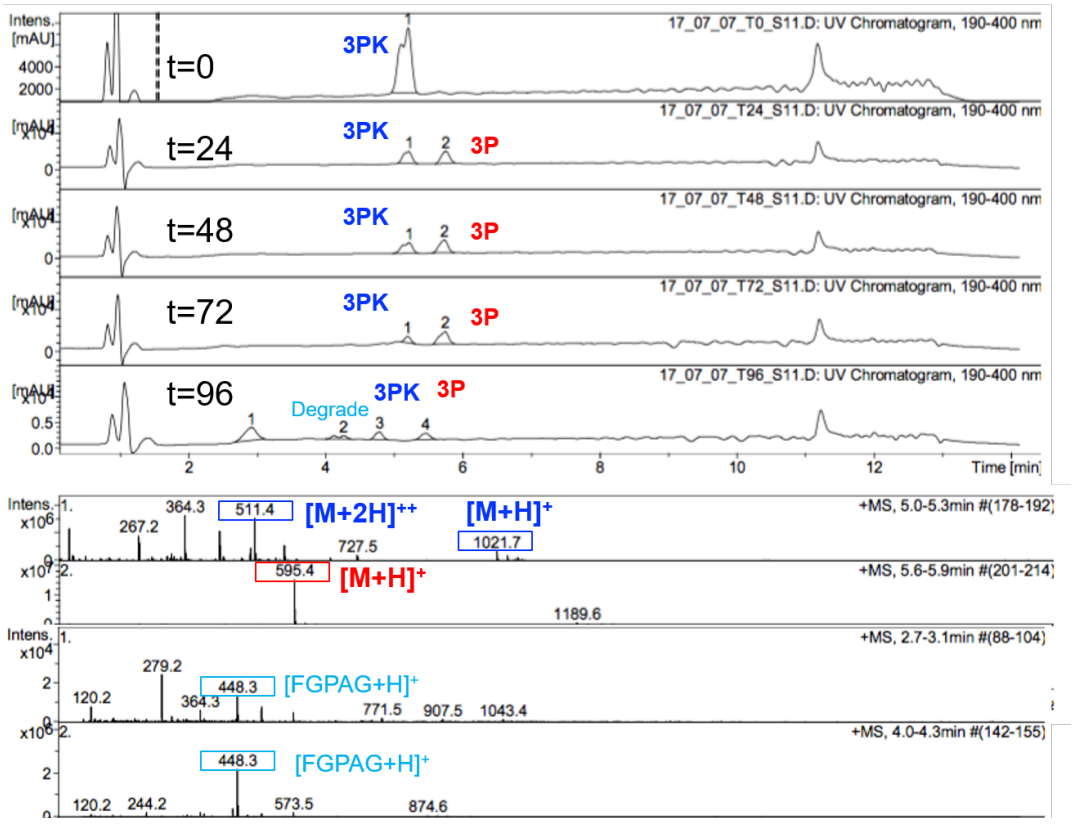


Figure S3 K. First trial LC-MS spectra of **3 PK**.

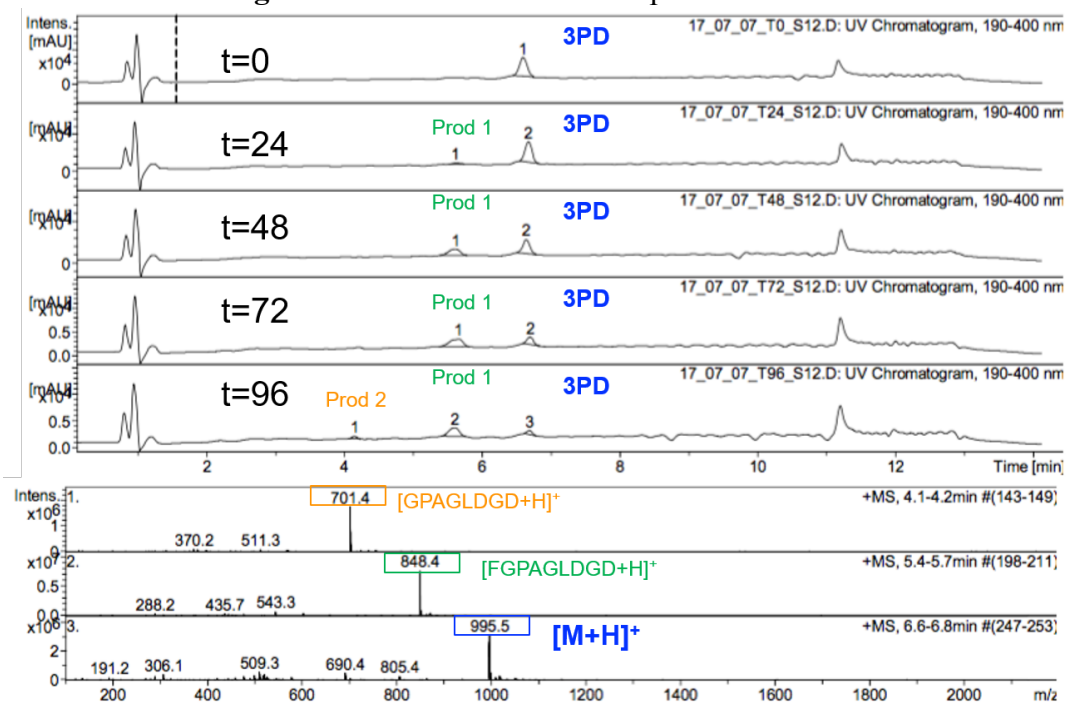


Figure S3 L. First trial LC-MS spectra of **3 PD**.

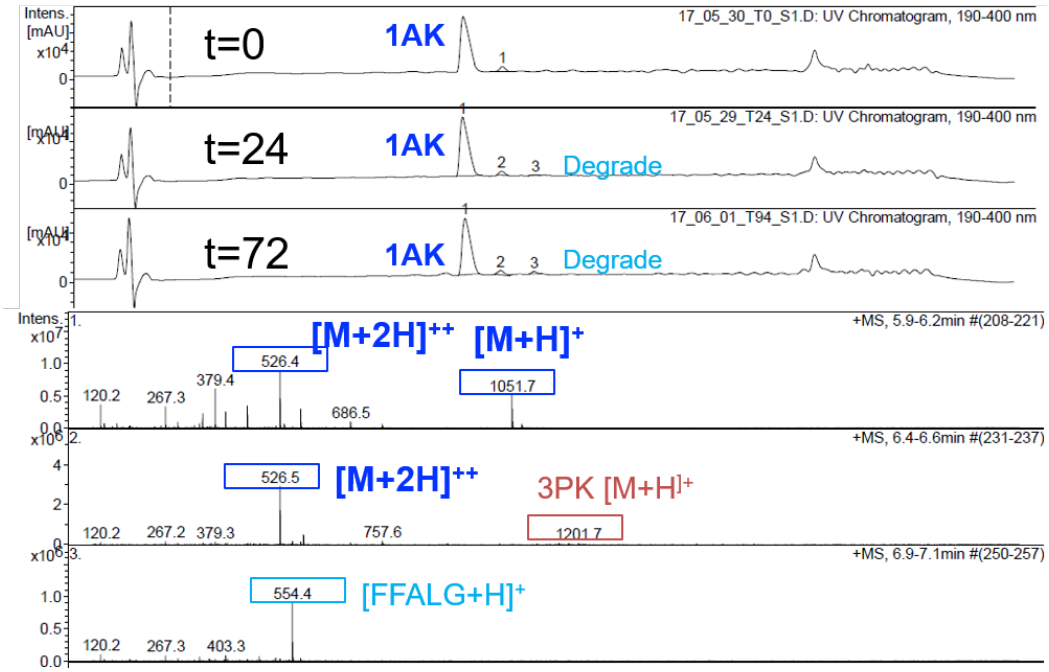


Figure S4 A. Second trial LC-MS spectra of **1 AK**.

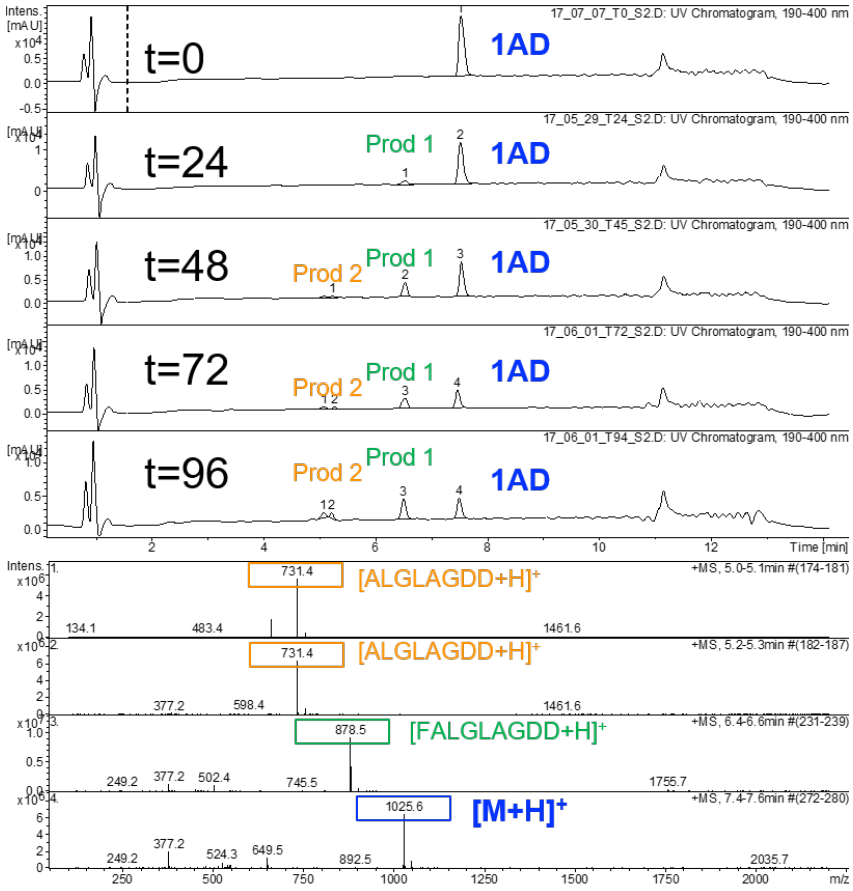


Figure S4 B. Second trial LC-MS spectra of **1 AD**.

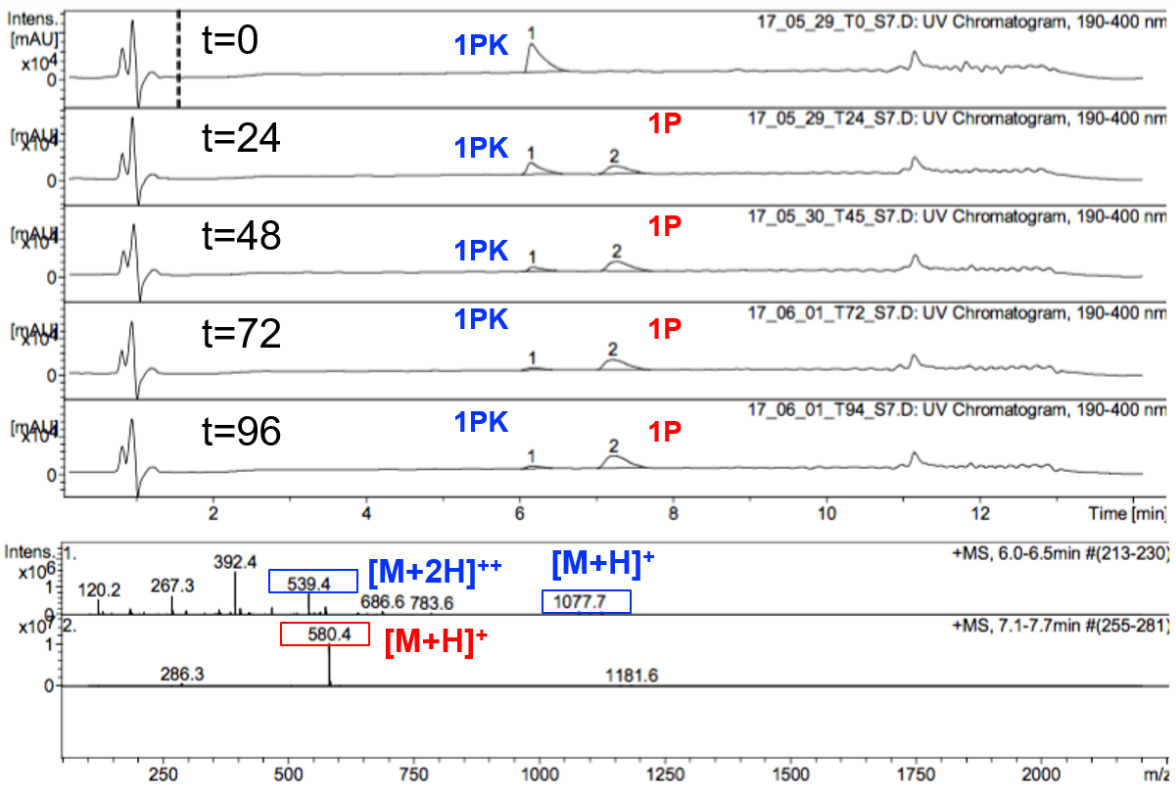


Figure S4 C. Second trial LC-MS spectra of **1 PK**.

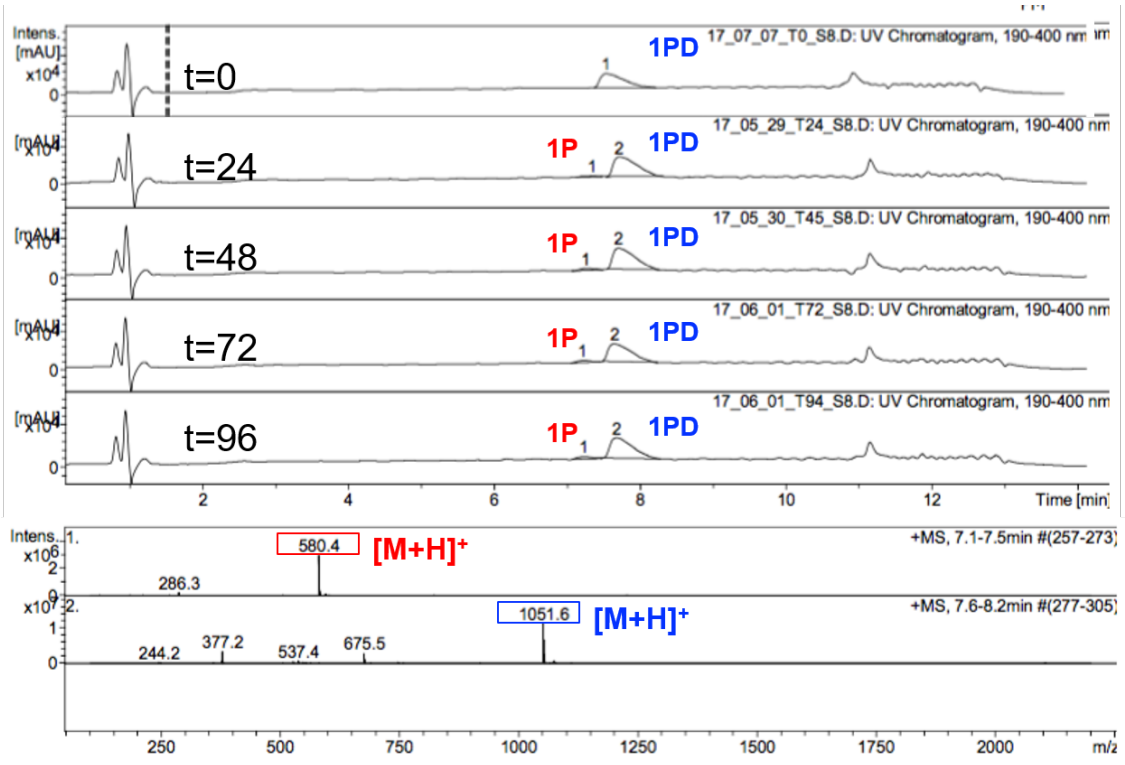


Figure S4 D. Second trial LC-MS spectra of **1 PD**.

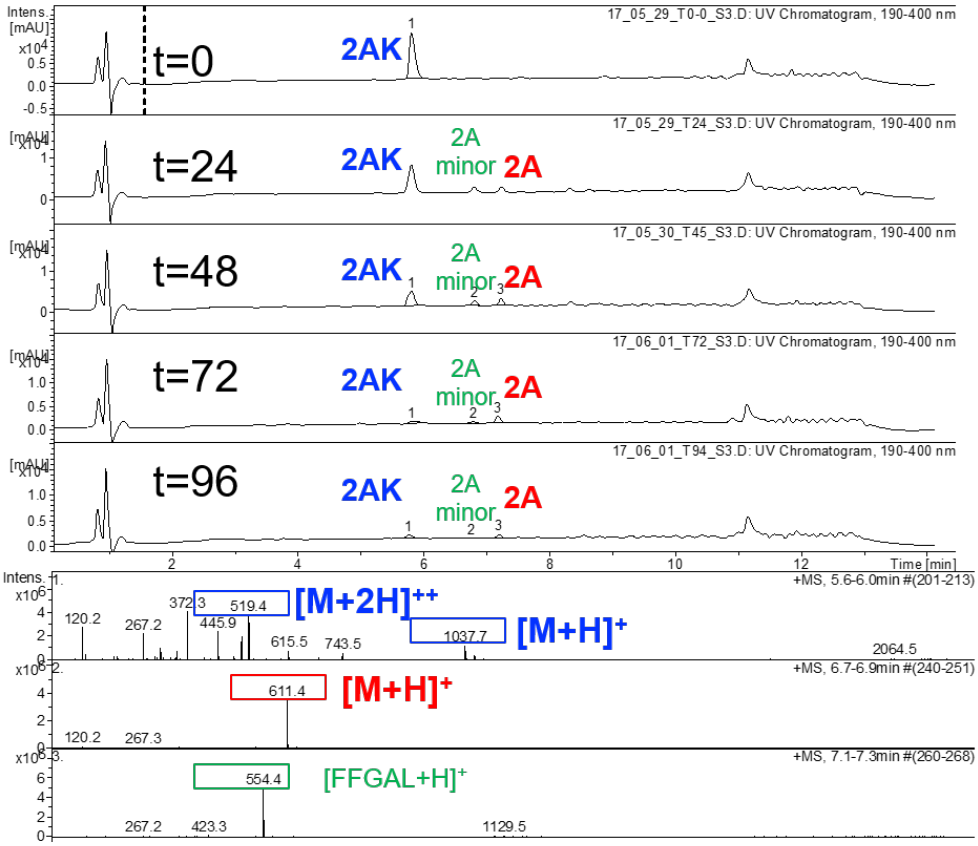


Figure S4 E. Second trial LC-MS spectra of **2 AK**.

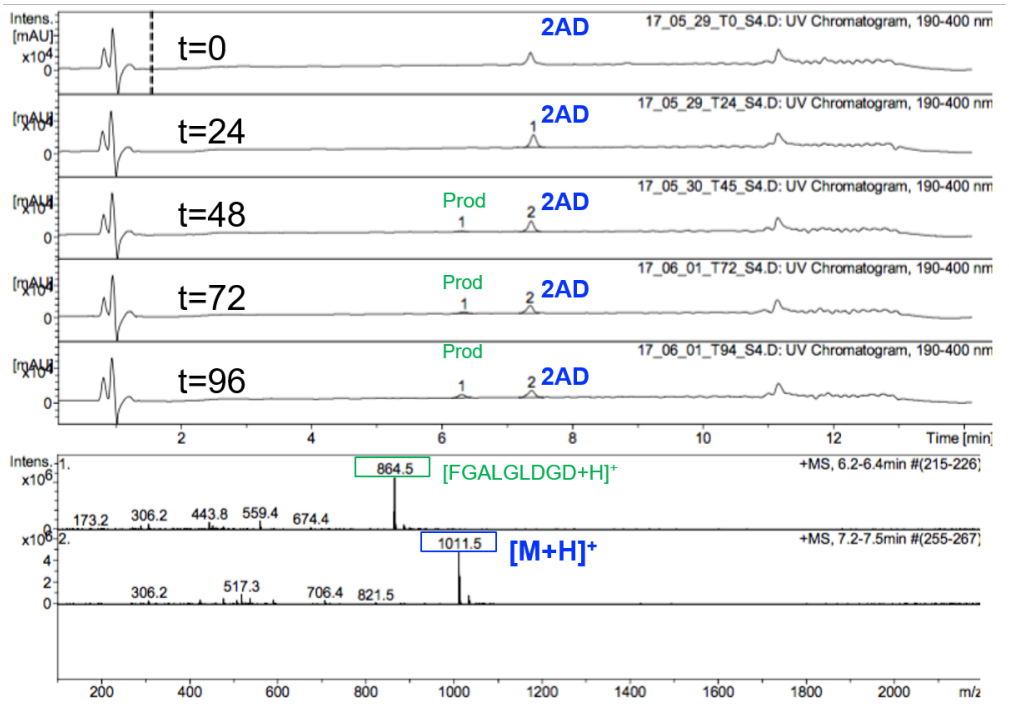


Figure S4 F. Second trial LC-MS spectra of **2 AD**.

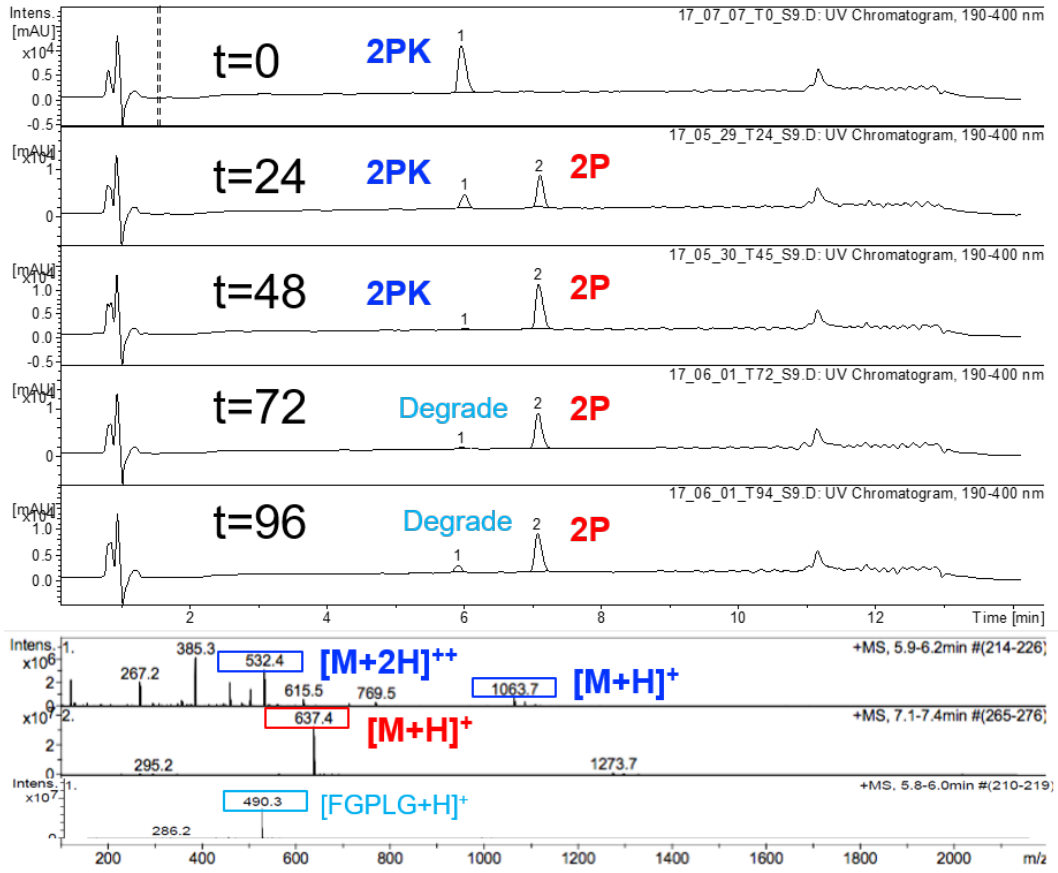


Figure S4 G. Second trial LC-MS spectra of **2 PK**.

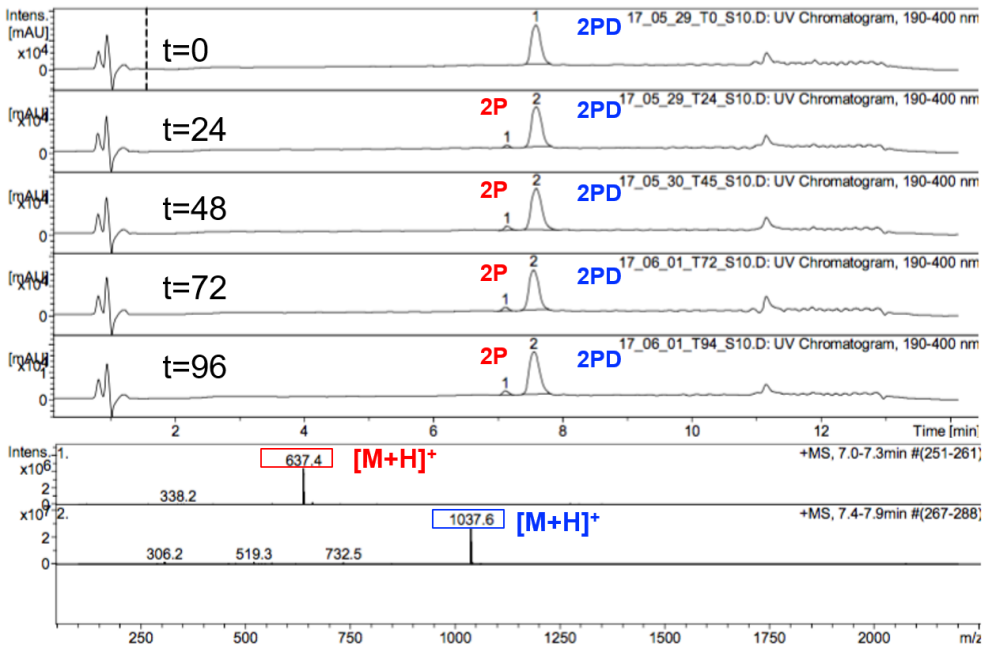


Figure S4 H. Second trial LC-MS spectra of **2 PD**.

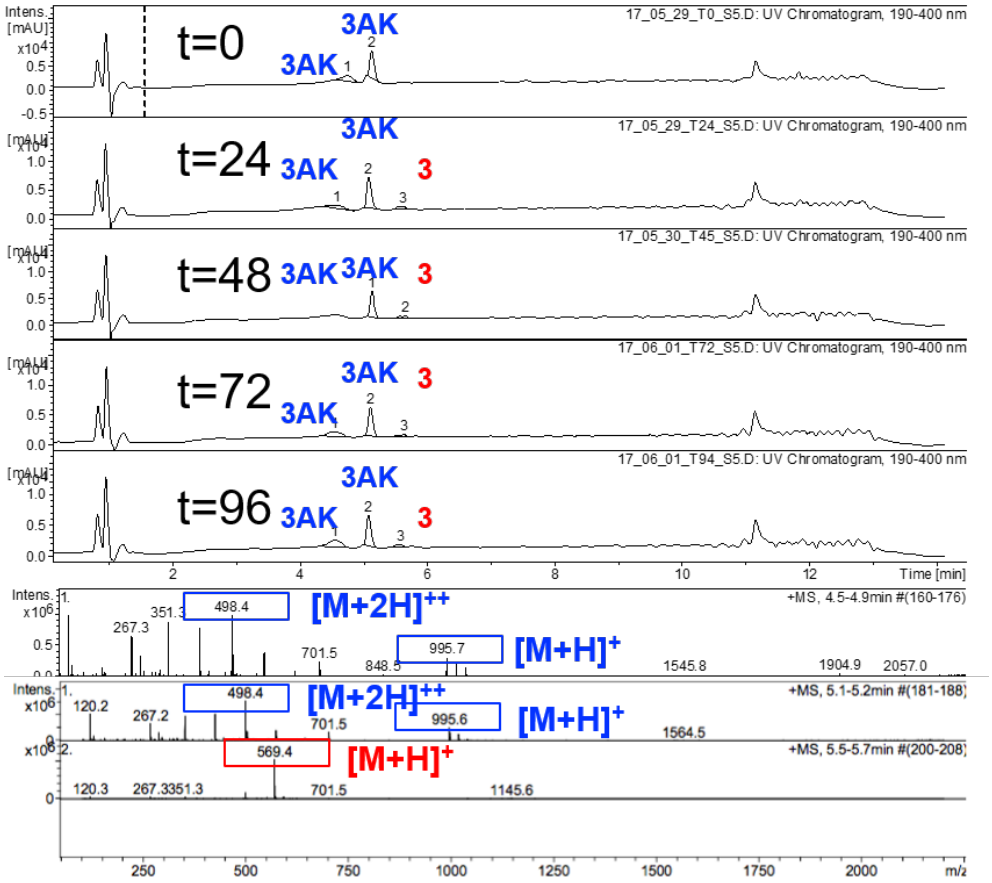


Figure S4 I. Second trial LC-MS spectra of **3 AK**.

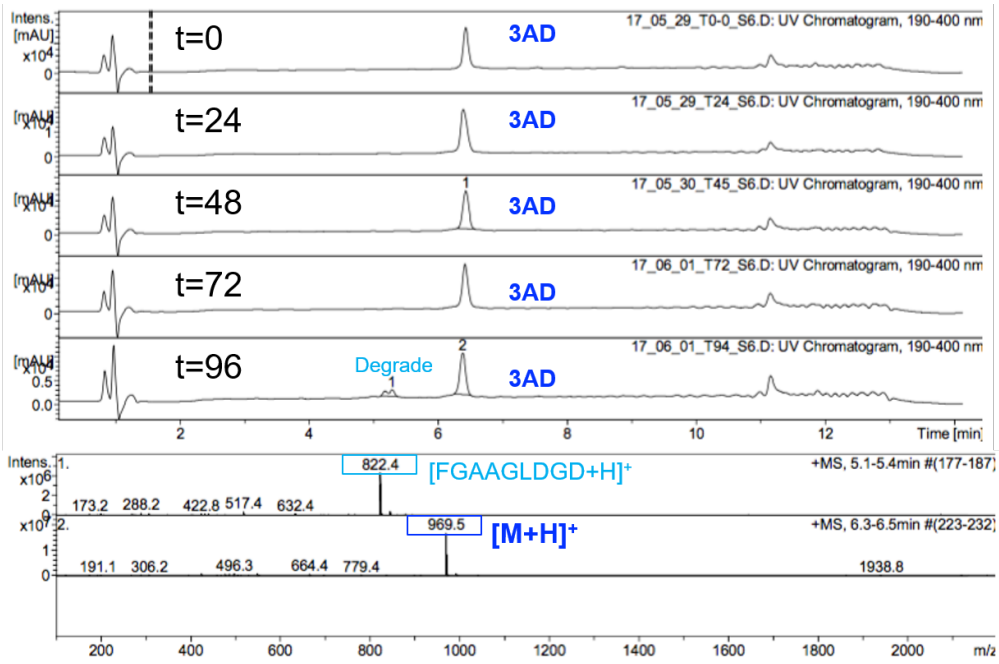


Figure S4 J. Second trial LC-MS spectra of **3 AD**.

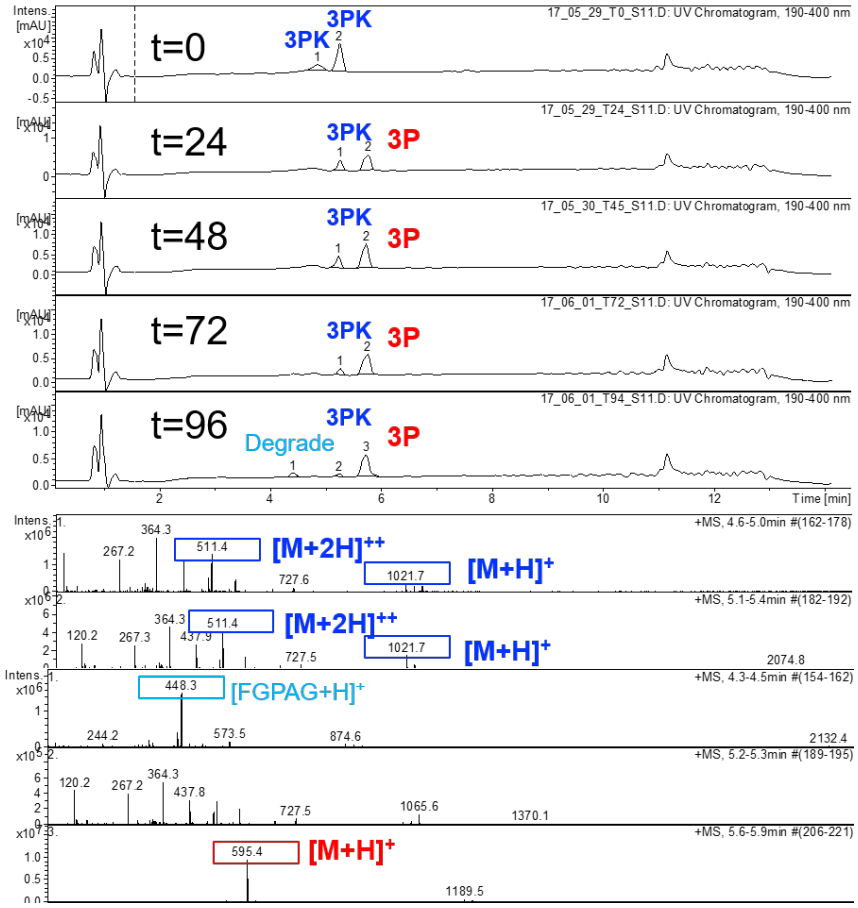


Figure S4 K. Second trial LC-MS spectra of **3 PK**.

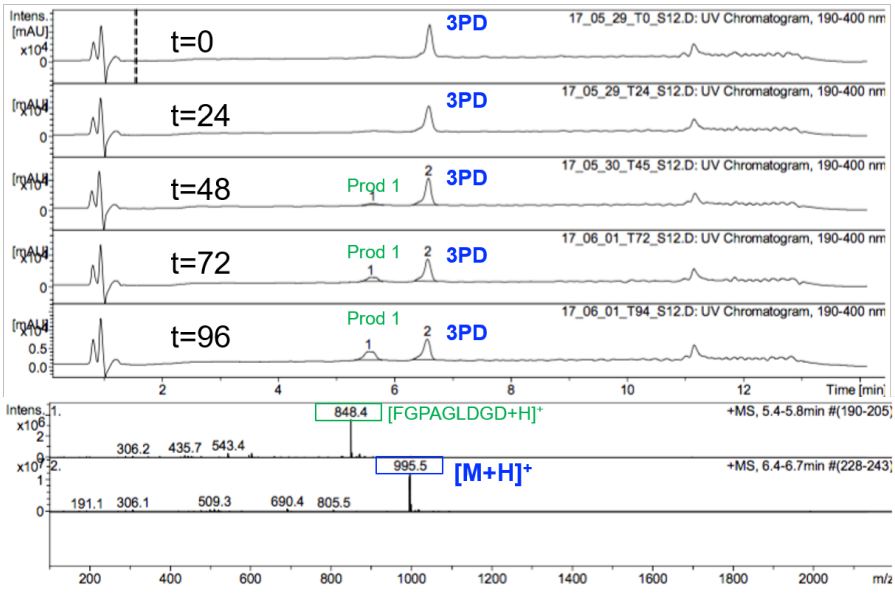


Figure S4 L. Second trial LC-MS spectra of **3 PD**.

4. Atomic Force Microscopy (AFM)

AFM images were taken on Bruker Dimension FastScan using FASTSCAN-B tip on fast scan mode. 1mM of peptide solution was prepared in phosphate buffer (pH 7.4), sonicated for 10minutes and drop casted on freshly cleaved mica and allowed to dry for 48hr before imaging.

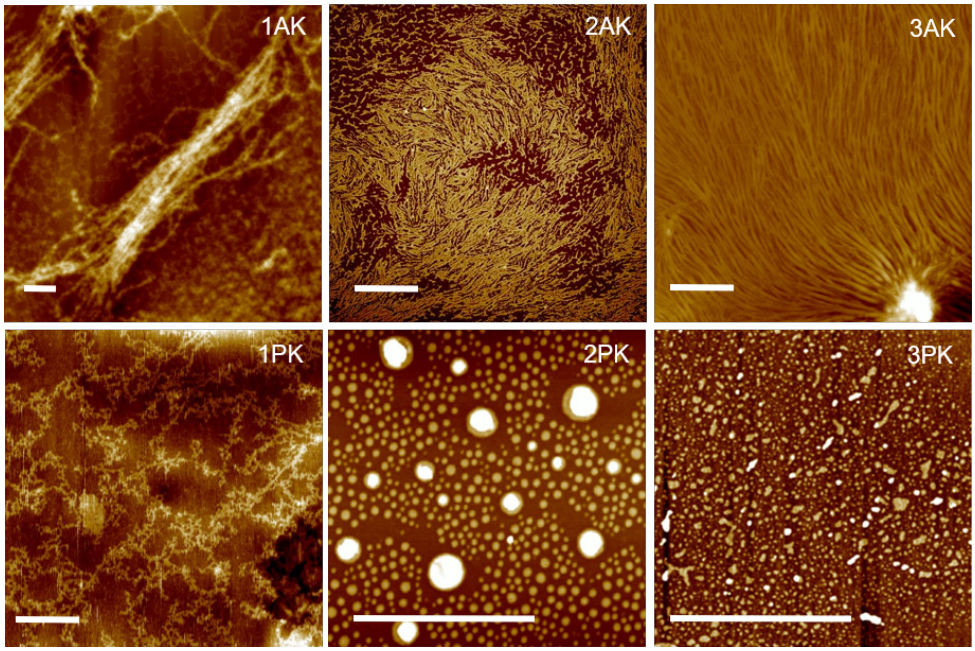


Figure S5 A. AFM images of **1-3 AK** and **1-3 PK**. Scale bar 1µm.

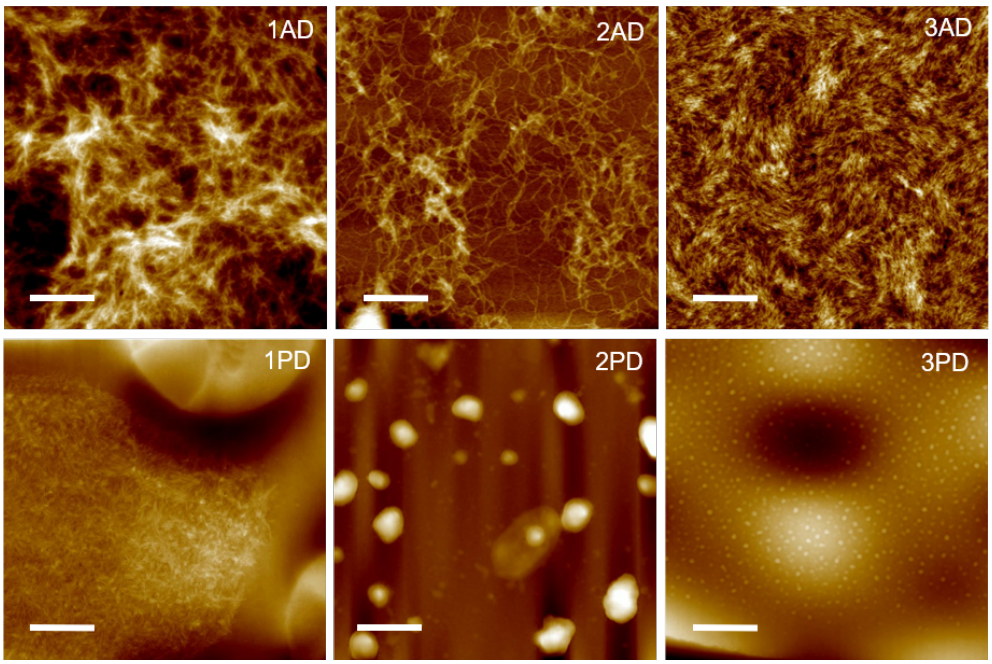


Figure S5 B. AFM images of **1-3AD** and **1-3 PD**. Scale bar 1µm.

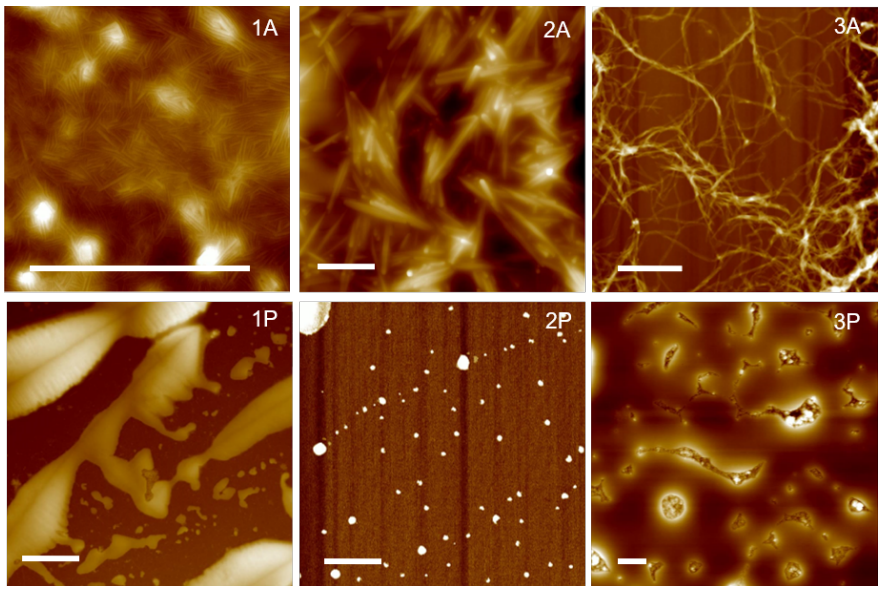


Figure S5 C. AFM images of **1-3AD** and **1-3 PD**. Scale bar 1µm.

5. Transmission Electron Microscopy (TEM)

TEM images were taken on FEI Titan Halo 80-300 microscope. 1mM of peptide solution was prepared in 10mM phosphate buffer (pH 7.4) sonicated for 10minutes and 5µL of the solution was drop casted on a carbon film grid (400 mesh, copper) and dried completely. To the dry grid, 5µL of MilliQ water was drop casted and quickly blotted to wash away the phosphate salts and dried completely. Finally, 5µL of methylamine vanadate based negative stain (NanoVan® by Nanoprobes) was drop casted, blotted away, and dried completely.

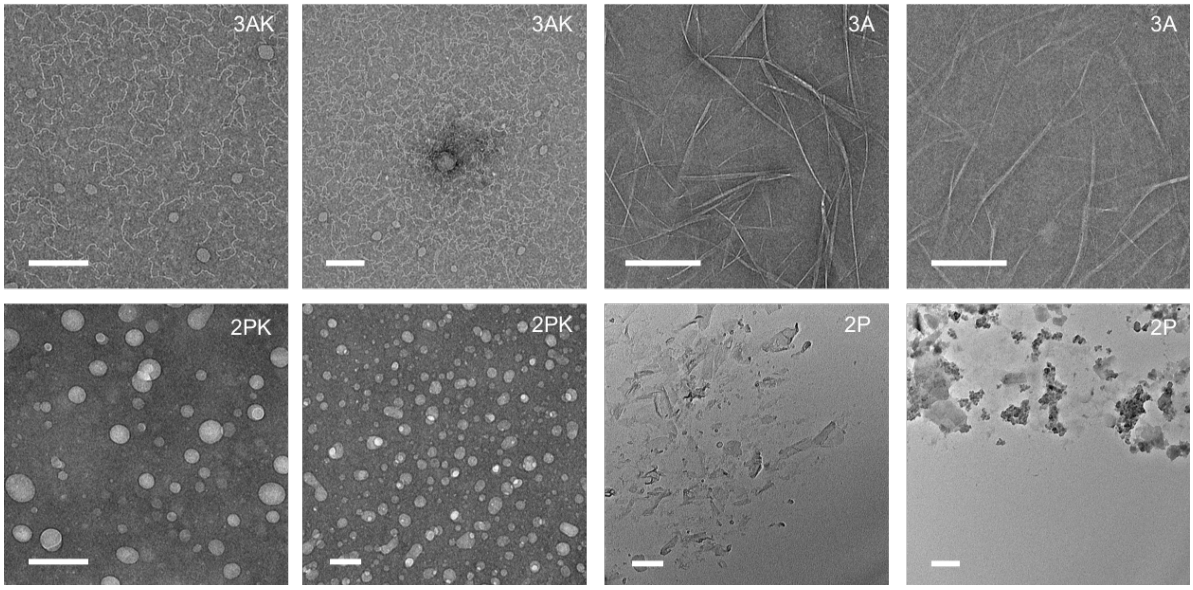


Figure S6. TEM images of **3 AK**, **3 A**, **2 PK**, and **2 P**. Scale bar 200nm.

6. Fourier Transform Infrared Spectroscopy (FTIR)

Absorbance spectra were taken from 4000cm^{-1} to 800cm^{-1} with 64 scans at 4cm^{-1} resolution on the Bruker Vertex 70 spectrometer. 20mM peptide solutions was prepared in deuterated phosphate buffer (pH=8), pH was adjusted to 7.4 using 0.5M NaOH or 0.5M HCl, and sonicated for 10minutes. 5µL of sample solutions were drop casted between two CaF_2 cells with PTFE spacers (12µm thickness x 13mm diameter). For analysis, deuterated phosphate buffer absorbance spectra was subtracted from the sample absorbance and normalized from 1560 to 1655cm^{-1}.

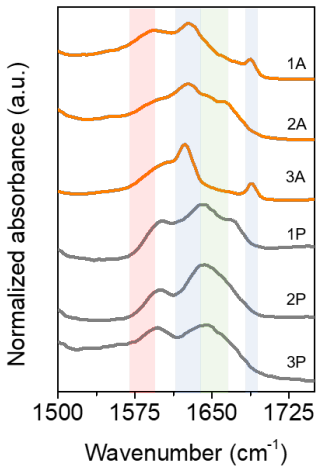


Figure S7. FTIR spectra of the peptides **1-3 A** and **1-3 P** in amide I region. Red shade highlights the carboxylate groups (1580-1590cm^{-1}), blue shade highlights parallel β-sheet (near 1620cm^{-1}) and anti-parallel β-sheet (additional peak at1688cm^{-1}) hydrogen bonding of the peptide backbones, and the green shade highlights disordered hydrogen bonds (1640-1650cm^{-1}).

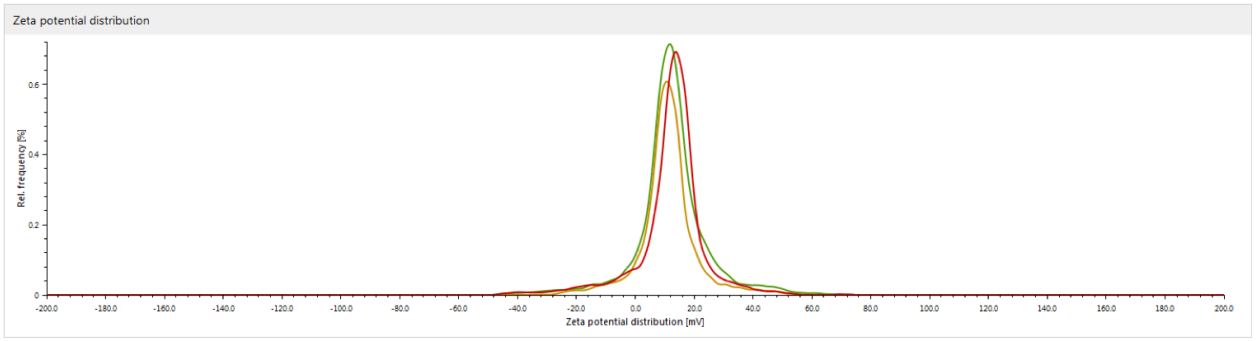
Table S2. FTIR absorbance of peptides in the amide I region.

	FTIR absorbance in the Amide I region (cm^{-1})				
Peptide	**Carboxylate**	**β-sheet**	**Disorder**	**(antiparallel) β-sheet**	**Asp (COOH)**
1AK	1590	1620		1688	-
1AD	1541	1624	-	1697	1716
1A	1593	1627	-	1687	-
1PK	1593	-	1642	-	-
1PD	1584	-	1645 1672*	-	1717
1P	1601	-	1641 1669	-	-
2AK	1591	-	1646	-	-
2AD	1584	-	1649	-	1711
2A	1596	1624	1643 1658	-	-
2PK	1593	-	1645	-	-
2PD	1585	-	1652 1672*	-	1715
2P	1600	-	1643	-	-
3AK	1592	-	1647	-	-
3AD	1584	-	1650	-	1710
3A	1607	1624	-	1688	-
3PK	1592	-	1647	-	-
3PD	1584	-	1649	-	1711
3P	1600	-	1653	-	

*1672cm^{-1} peak is due to the residual TFA

7. Zeta Potential

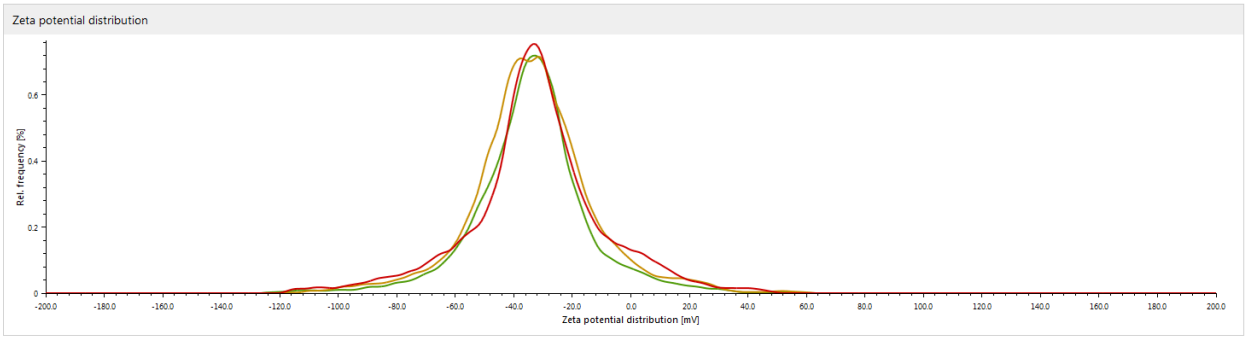
Zeta potential measurements were made on Anton Paar Litesizer 500 Particle Analyzer. 5mM of peptide samples were prepared in 2%PBS and the pH was adjusted to 7.4 using dilute NaOH and HCl. 50uL of samples were pipetted into Univette low volume cuvette and three series of measurements were made at 25°C using Smoluchowski approximation.



Name	Temperature	Mean zeta potential	Distribution peak	Conductivity	Electrophoretic Mobility	Processed runs	Adjusted voltage	Color	Go to
5mMAKin2%PBSpH7.4 1	25.0 °C	11.5 mV	12.0 mV	2.614 mS/cm	0.8930 µm*cm/Vs	100	10.3 V		%
5mMAKin2%PBSpH7.4 2	25.0 °C	10.7 mV	10.9 mV	2.635 mS/cm	0.8348 µm*cm/Vs	120	10.8 V		%
5mMAKin2%PBSpH7.4 3	25.0 °C	12.3 mV	13.8 mV	2.575 mS/cm	0.9585 µm*cm/Vs	100	11.1 V		%

Mean	11.5 mV	12.2 mV	2.608 mS/cm	0.8954 µm*cm/Vs
Standard deviation	0.8 mV	1.5 mV	0.030 mS/cm	0.0619 µm*cm/Vs
Rel. standard deviation	6.91 %	12.10 %	1.16 %	6.91 %

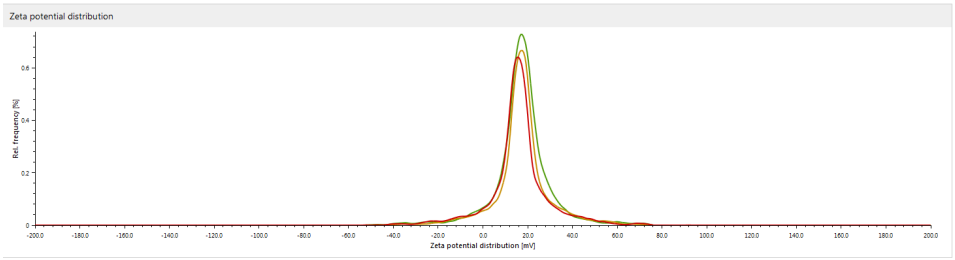
Figure S8 A. Triplicate measurements of zeta potential of **1 AK**.



Name	Temperature	Mean zeta potential	Distribution peak	Conductivity	Electrophoretic Mobility	Processed runs	Adjusted voltage	Color	Go to
5mMADin2%PBSpH7.4 1	25.0 °C	-33.6 mV	-32.6 mV	7.995 mS/cm	-2.6200 µm*cm/Vs	100	7.0 V		%
5mMADin2%PBSpH7.4 2	25.0 °C	-35.5 mV	-31.1 mV	8.196 mS/cm	-2.7670 µm*cm/Vs	100	6.7 V		%
5mMADin2%PBSpH7.4 3	25.0 °C	-35.7 mV	-33.4 mV	8.225 mS/cm	-2.7811 µm*cm/Vs	120	6.6 V		%

Mean	-34.9 mV	-32.4 mV	8.139 mS/cm	-2.7227 µm*cm/Vs
Standard deviation	1.1 mV	1.2 mV	0.126 mS/cm	0.0892 µm*cm/Vs
Rel. standard deviation	3.28 %	3.60 %	1.55 %	3.28 %

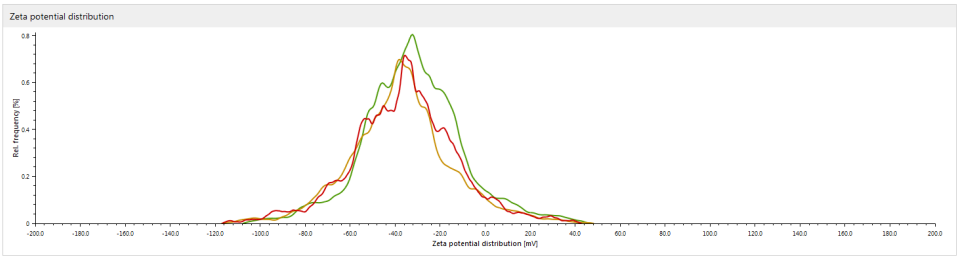
Figure S8 B. Triplicate measurements of zeta potential of **1 AD**.



Name	Temperature	Mean zeta potential	Distribution peak	Conductivity	Electrophoretic Mobility	Processed runs	Adjusted voltage	Color	Go to
5mMPK2%PBSpH7.4 1	25.0 °C	15.7 mV	17.0 mV	2.289 mS/cm	1.2215 µm*cm/Vs	100	11.0 V		% % %
5mMPK2%PBSpH7.4 2	25.0 °C	15.1 mV	17.2 mV	2.322 mS/cm	1.1783 µm*cm/Vs	100	11.1 V		
5mMPK2%PBSpH7.4 3	25.0 °C	15.5 mV	15.7 mV	2.338 mS/cm	1.2095 µm*cm/Vs	100	11.1 V		

Mean	15.4 mV	16.6 mV	2.316 mS/cm	1.2031 µm*cm/Vs
Standard deviation	0.3 mV	0.8 mV	0.025 mS/cm	0.0223 µm*cm/Vs
Rel. standard deviation	1.85 %	4.92 %	1.07 %	1.85 %

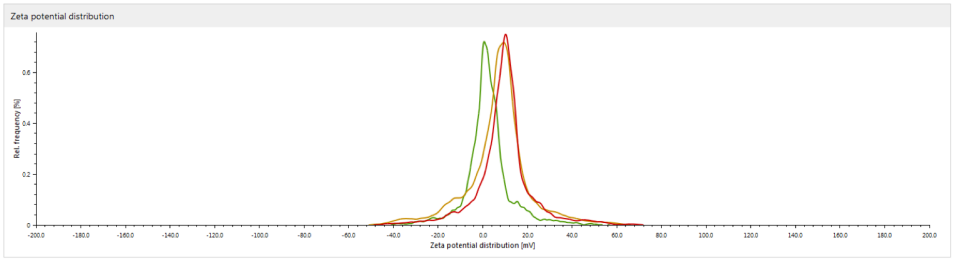
Figure S8 C. Triplicate measurements of zeta potential of **1 PK**.



Name	Temperature	Mean zeta potential	Distribution peak	Conductivity	Electrophoretic Mobility	Processed runs	Adjusted voltage	Color	Go to
5mMPDin2%PBSpH7.4 1	25.0 °C	-37.1 mV	-32.3 mV	3.217 mS/cm	-2.8883 µm*cm/Vs	100	9.7 V		% % %
5mMPDin2%PBSpH7.4 2	25.0 °C	-37.0 mV	-38.3 mV	3.242 mS/cm	-2.8819 µm*cm/Vs	120	9.6 V		
5mMPDin2%PBSpH7.4 3	25.0 °C	-39.9 mV	-35.6 mV	3.211 mS/cm	-3.1095 µm*cm/Vs	200	9.9 V		

Mean	-38.0 mV	-35.4 mV	3.223 mS/cm	-2.9599 µm*cm/Vs
Standard deviation	1.7 mV	3.0 mV	0.017 mS/cm	0.1296 µm*cm/Vs
Rel. standard deviation	4.38 %	8.53 %	0.52 %	4.38 %

Figure S8 D. Triplicate measurements of zeta potential of **1 PD**.



Name	Temperature	Mean zeta potential	Distribution peak	Conductivity	Electrophoretic Mobility	Processed runs	Adjusted voltage	Color	Go to
5mMGALK2%PBSpH7.4 1	25.0 °C	5.1 mV	0.5 mV	2.027 mS/cm	0.3971 µm*cm/Vs	860	11.5 V		% % %
5mMGALK2%PBSpH7.4 2	25.0 °C	9.0 mV	9.5 mV	2.072 mS/cm	0.6991 µm*cm/Vs	100	12.3 V		
5mMGALK2%PBSpH7.4 3	25.0 °C	10.6 mV	10.0 mV	2.089 mS/cm	0.8281 µm*cm/Vs	140	12.1 V		

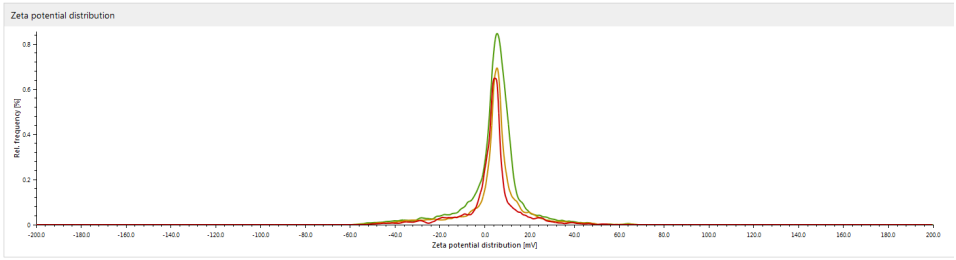
Mean	8.2 mV	6.7 mV	2.063 mS/cm	0.6414 µm*cm/Vs
Standard deviation	2.8 mV	5.4 mV	0.032 mS/cm	0.2212 µm*cm/Vs
Rel. standard deviation	34.49 %	80.19 %	1.56 %	34.49 %

Figure S8 E. Triplicate measurements of zeta potential of **2 AK**.

Name	Temperature	Mean zeta potential	Distribution peak	Conductivity	Electrophoretic Mobility	Processed runs	Adjusted voltage	Color	Go to
5mMGALD2%PBSpH7.4 1	25.0 °C	-28.6 mV	-31.0 mV	2.938 mS/cm	-2.2294 µm*cm/Vs	100	10.1 V		
5mMGALD2%PBSpH7.4 2	25.0 °C	-31.2 mV	-28.3 mV	2.986 mS/cm	-2.4297 µm*cm/Vs	100	10.1 V		
5mMGALD2%PBSpH7.4 3	25.0 °C	-31.0 mV	-28.0 mV	3.008 mS/cm	-2.4158 µm*cm/Vs	100	10.0 V		

Mean	-30.3 mV	-29.1 mV	2.977 mS/cm	-2.3583 µm*cm/Vs
Standard deviation	1.4 mV	1.6 mV	0.036 mS/cm	0.1118 µm*cm/Vs
Rel. standard deviation	4.74 %	5.61 %	1.20 %	4.74 %

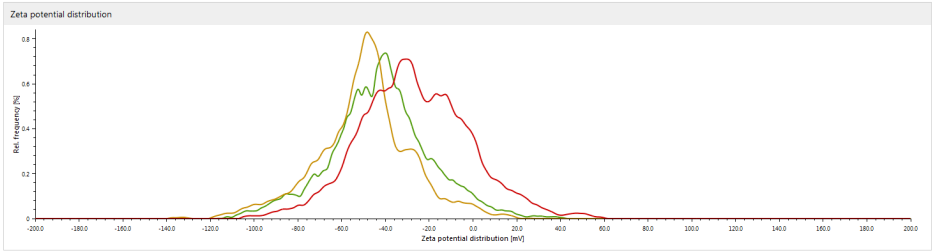
Figure S8 F. Triplicate measurements of zeta potential of **2 AD**.



Name	Temperature	Mean zeta potential	Distribution peak	Conductivity	Electrophoretic Mobility	Processed runs	Adjusted voltage	Color	Go to
5mMGPLK2%PBSpH7.4 (2) 1	25.0 °C	5.0 mV	5.6 mV	1.994 mS/cm	0.3870 µm*cm/Vs	200	12.2 V		
5mMGPLK2%PBSpH7.4 (2) 2	25.0 °C	4.2 mV	5.5 mV	2.032 mS/cm	0.3309 µm*cm/Vs	280	12.2 V		
5mMGPLK2%PBSpH7.4 (2) 3	25.0 °C	3.1 mV	4.4 mV	2.015 mS/cm	0.2392 µm*cm/Vs	760	12.5 V		

Mean	4.1 mV	5.2 mV	2.014 mS/cm	0.3191 µm*cm/Vs
Standard deviation	1.0 mV	0.7 mV	0.019 mS/cm	0.0746 µm*cm/Vs
Rel. standard deviation	23.39 %	12.83 %	0.95 %	23.39 %

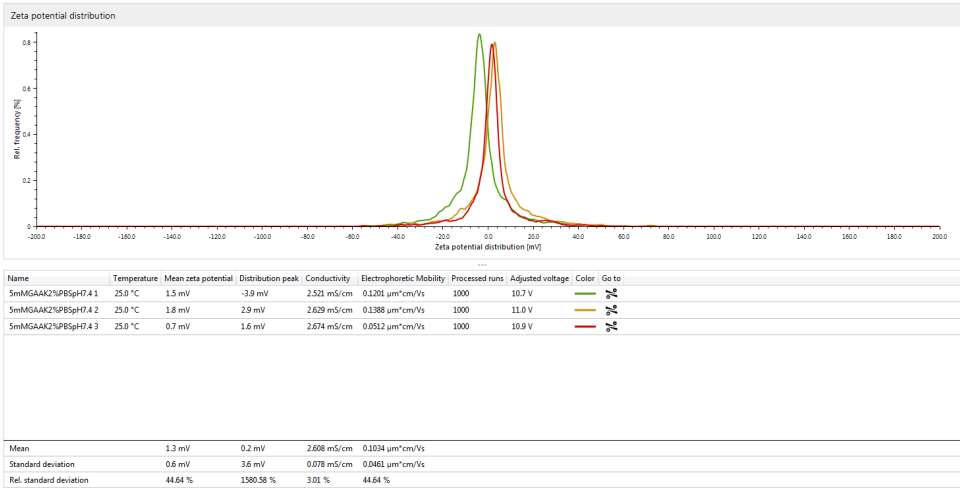
Figure S8 G. Triplicate measurements of zeta potential of **2 PK**.



Name	Temperature	Mean zeta potential	Distribution peak	Conductivity	Electrophoretic Mobility	Processed runs	Adjusted voltage	Color	Go to
5mMGPLDin2%PBSpH7.4 1	25.0 °C	-38.6 mV	-40.2 mV	3.245 mS/cm	-3.0064 µm*cm/Vs	160	9.6 V		
5mMGPLDin2%PBSpH7.4 2	25.0 °C	-42.6 mV	-48.2 mV	3.245 mS/cm	-3.3226 µm*cm/Vs	120	9.8 V		
5mMGPLDin2%PBSpH7.4 3	25.0 °C	-42.5 mV	-30.2 mV	3.260 mS/cm	-3.3143 µm*cm/Vs	100	9.7 V		

Mean	-41.2 mV	-39.6 mV	3.250 mS/cm	-3.2144 µm*cm/Vs
Standard deviation	2.3 mV	9.0 mV	0.008 mS/cm	0.1802 µm*cm/Vs
Rel. standard deviation	5.61 %	22.77 %	0.26 %	5.61 %

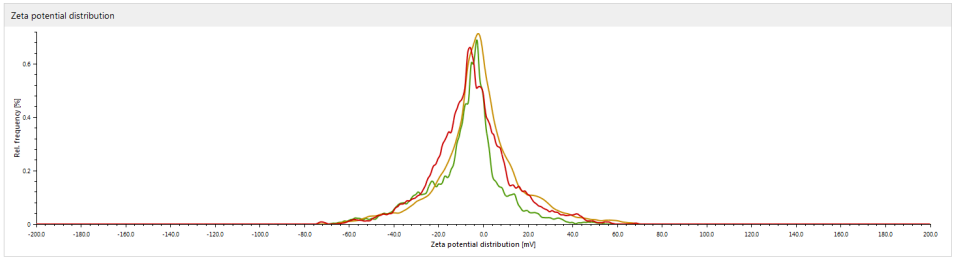
Figure S8 H. Triplicate measurements of zeta potential of **2 PD**.



Name	Temperature	Mean zeta potential	Distribution peak	Conductivity	Electrophoretic Mobility	Processed runs	Adjusted voltage	Color	Go to
5mMGAAK2%PBSpH7.4 1	25.0 °C	1.5 mV	-3.9 mV	2.521 mS/cm	0.1201 µm*cm/Vs	1000	10.7 V		%
5mMGAAK2%PBSpH7.4 2	25.0 °C	1.8 mV	2.9 mV	2.629 mS/cm	0.1388 µm*cm/Vs	1000	11.0 V		%
5mMGAAK2%PBSpH7.4 3	25.0 °C	0.7 mV	1.6 mV	2.674 mS/cm	0.0512 µm*cm/Vs	1000	10.9 V		%

Mean	1.3 mV	0.2 mV	2.608 mS/cm	0.1034 µm*cm/Vs
Standard deviation	0.6 mV	3.6 mV	0.078 mS/cm	0.0461 µm*cm/Vs
Rel. standard deviation	44.64 %	1580.58 %	3.01 %	44.64 %

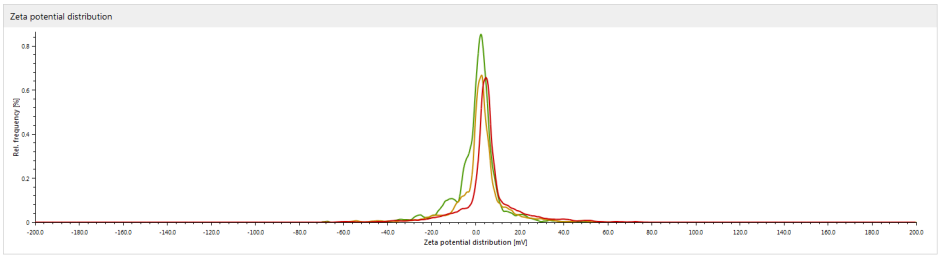
Figure S8 I. Triplicate measurements of zeta potential of **3 AK**.



Name	Temperature	Mean zeta potential	Distribution peak	Conductivity	Electrophoretic Mobility	Processed runs	Adjusted voltage	Color	Go to
5mMGAAD2%PBSpH7.4 1	25.0 °C	-7.0 mV	-3.1 mV	2.492 mS/cm	-0.5435 µm*cm/Vs	480	11.0 V		%
5mMGAAD2%PBSpH7.4 2	25.0 °C	-6.0 mV	-2.1 mV	2.528 mS/cm	-0.4646 µm*cm/Vs	120	10.8 V		%
5mMGAAD2%PBSpH7.4 3	25.0 °C	-9.5 mV	-6.0 mV	2.530 mS/cm	-0.7395 µm*cm/Vs	240	10.7 V		%

Mean	-7.5 mV	-3.7 mV	2.517 mS/cm	-0.5826 µm*cm/Vs
Standard deviation	1.8 mV	2.0 mV	0.021 mS/cm	0.1416 µm*cm/Vs
Rel. standard deviation	24.30 %	54.94 %	0.85 %	24.30 %

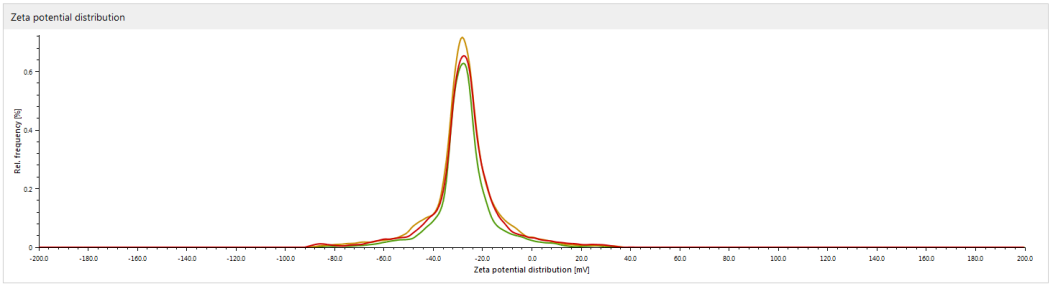
Figure S8 J. Triplicate measurements of zeta potential of **3 AD**.



Name	Temperature	Mean zeta potential	Distribution peak	Conductivity	Electrophoretic Mobility	Processed runs	Adjusted voltage	Color	Go to
5mMGPAK2%PBSpH7.4 1	25.0 °C	-0.5 mV	2.2 mV	2.745 mS/cm	-0.0366 µm*cm/Vs	1000	10.5 V		%
5mMGPAK2%PBSpH7.4 2	25.0 °C	1.1 mV	2.8 mV	2.879 mS/cm	0.0882 µm*cm/Vs	1000	10.7 V		%
5mMGPAK2%PBSpH7.4 3	25.0 °C	2.5 mV	4.8 mV	2.928 mS/cm	0.1967 µm*cm/Vs	1000	10.4 V		%

Mean	1.1 mV	3.3 mV	2.851 mS/cm	0.0828 µm*cm/Vs
Standard deviation	1.5 mV	1.3 mV	0.095 mS/cm	0.1168 µm*cm/Vs
Rel. standard deviation	141.05 %	41.23 %	3.32 %	141.05 %

Figure S8 K. Triplicate measurements of zeta potential of **3 PK**.



Name	Temperature	Mean zeta potential	Distribution peak	Conductivity	Electrophoretic Mobility	Processed runs	Adjusted voltage	Color	Go to
5mMGPAD2%PBSpH7.4 12/17/17 1	25.0 °C	-28.2 mV	-27.7 mV	2.860 mS/cm	-2.2006 µm*cm/Vs	100	10.1 V		%
5mMGPAD2%PBSpH7.4 12/17/17 2	25.0 °C	-28.9 mV	-28.2 mV	2.889 mS/cm	-2.2491 µm*cm/Vs	100	10.1 V		%
5mMGPAD2%PBSpH7.4 12/17/17 3	25.0 °C	-27.5 mV	-27.6 mV	2.903 mS/cm	-2.1438 µm*cm/Vs	100	10.1 V		%

Mean	-28.2 mV	-27.8 mV	2.884 mS/cm	-2.1978 µm*cm/Vs
Standard deviation	0.7 mV	0.3 mV	0.022 mS/cm	0.0527 µm*cm/Vs
Rel. standard deviation	2.40 %	1.16 %	0.77 %	2.40 %

Figure S8 L. Triplicate measurements of zeta potential of **3 PD**.

8. Critical Aggregation Concentration (CAC)

1mM of peptides were prepared in PBS and the pH adjusted to 7.4 using 0.5M NaOH or 0.5M HCl, and serially diluted in PBS with thorough vortexing. Peptide solutions were incubated in 50°C for 15 minutes, then 2µL of stock pyrene solution (100µM in methanol) was added to 100µL of each peptide solution, gently mixed, and incubated for 5 minutes at 50°C, then finally cooled down to room temperature to co-assemble the peptides with pyrene molecules. Pyrene emission spectra was measured from 350-450nm (λ_{ex}=310nm) in a micro fluorescence cuvette (3mm path length) on Jasco FP-8500 Spectrofluorometer (measurement parameter: 20nm excitation and 1nm emission bandwidth, 0.2sec response, medium sensitivity, 0.2cm^{-1} data interval, at 200nm/min). The CAC was determined by plotting the ratio between intensities of the 3rd to 1st peak of the pyrene emission spectra. Increasing peptide concentrations were measured in 0.1mM increments until the slope of the plot had changed, and simultaneously the 3rd peak shifted from 382.4nm to 384.4nm and the 1st peak shifted from 371.8nm to 373.4nm.

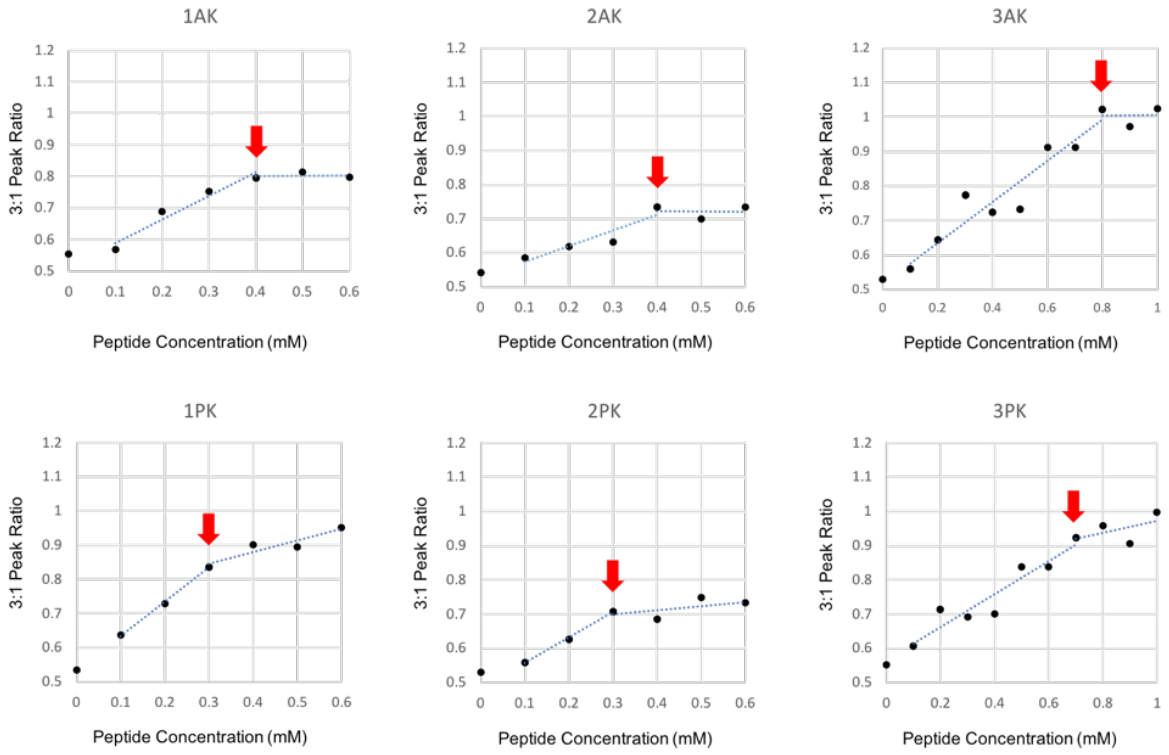


Figure S9 A. 3:1 peak ratio of pyrene co-assembled with **1-3 AK/PK**.

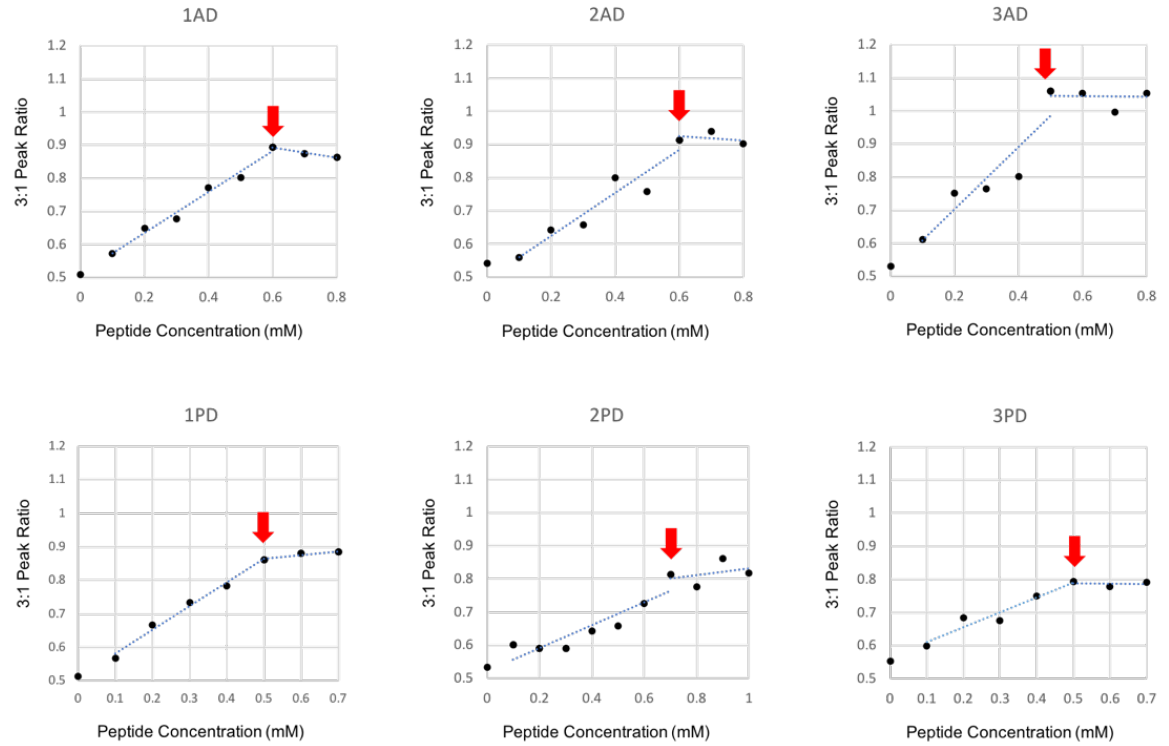


Figure S9 B. 3:1 peak ratio of pyrene co-assembled with **1-3 AD/PD**.