Supporting Material

Synthesis and Evaluation of Novel Cyclic Peptide Inhibitors of Lysine-Specific Demethylase 1

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Chemical synthesis

N^{α}–Fmoc amino acids were purchased from the Advanced Chemtech (Louisville, KY) and AAPPTec (Louisville, KY). Fmoc-rink linker and aminomethylated polystyrene resin was purchased from the Novabiochem (Gibbstown, NJ). Reagent grade Piperidine was purchased from the Sigma Aldrich. All the other solvents were purchased from the VWR and Fischer and used without further purification. All of the cyclic and linear peptide analogues were synthesized by using standard N^{α}-Fmoc/tBu solid-phase peptide synthesis. Three channel PS3 automated peptide synthesizer from Protein Technologies, Inc. Tucson Arizona was used for peptide synthesis. The aminomethylated polystyrene resin (0.25 mmol, 0.36 mmol/g) was placed in a 40 mL glass reaction vessel in the synthesizer and allowed to swell in 15 mL of DMF solution for 30 min. Then the resin was washed with 15 mL of DMF (3 x 2min). The Fmoc linker was introduced to the swelled resin using mixture of Fmoc linker (1.0 mmol, 4 equiv), HBTU (1 mmol, 4 equiv) and NMM (2 mmol, 8 equiv) in DMF for 60 min. After coupling of Fmoc linker to the resin, the resin was washed with 15 mL of DMF solution (5 x 2 min). The Fmoc protecting group on the resin was removed with 15 mL of 20% piperidine in DMF (2 x 15 min) followed by washing with 15 mL of DMF (5 x 2 min). Then, a preactivated Fmoc-amino acid prepared by mixing a Fmoc-amino acid (4 equiv), HBTU (4 equiv), and NMM (8 equiv) in DMF and was delivered to the reaction vessel. It was allowed to react for 1 hour with the resin. The deprotection and coupling steps were repeated for each amino acid until desired sequence is obtained.

For linear peptides, once the desired sequence is obtained on resin, it was washed with 15 mL of DMF (5 x 2min), DCM (5 x 2min) and methanol (5 x 2min) and dried in the vacuum overnight at $O^{0}C$. Then it was allowed to cleave from the resin for 2.5 hours using cleavage mixture of 18 mL containing TFA, 0.5 mL of dimethylsulfide, 0.5 mL of 1,2-ethanedithiol, and 1 mL of thioanisole. The TFA solution containing cleaved peptide was filtered, and the resin was further washed with trifluoroacetic acid (10 mL). The combined TFA solution was concentrated to a volume of approximately 3 mL with a gentle stream of nitrogen, and the

peptide was precipitated with cold diethyl ether (30 mL). The precipitated peptide was vortexed for 1 min and centrifuged. The ether solution was decanted to remove the scavengers. Washing with cold diethyl ether was repeated for four to five times and the peptide was dried in vacuum.

For cyclic peptides, once the fully protected peptide having desired sequence is obtained on resin (0.25 mmol), orthogonal protective groups of the peptide (alloc protecting groups of Lys and allyl group of Glu) were selectively removed using mixture of $Pd(PPh_3)_4$ (30 mg, 0.1 equiv), and DMBA (390 mg,10 equiv) in 6 mL DMF:DCM (1:3) in the 40 mL reaction vessel under N_2 atmosphere for 30 min for two times. Then the resin was washed with 15 mL of DMF (5x2min) and washed with 15 mL of 0.1 M LiCl in DMF solution (3 x 2 min). It was again washed with 15 ml of DMF (3x2min). The resin was treated with PyBOP/HOBt/DIPEA (6, 6, and 12 equiv) in 6 mL of DCM: DMF: NMP (1:1:1) for 6 h twice for formation of the lactam bridge. Then resin was washed with DMF (3x 2 min) and the cyclic peptide was cleaved from the resin as very similar to the procedure described above for the linear peptide.

Structural characterization of cyclic peptides

The purified peptides were characterized by HRMS and LC-MS (See Table S1 for the characterization data for synthesized peptides). High resolution mass spectrometric data was taken in the positive ion mode using Brucker AUTOFLEX III MALDI-TOF instrument. LC-MS data was obtained in the positive ion mode using Waters LC-MS instrument [having Waters 2545 quaternary gradient module, Waters 2767 sample manager, Waters SFO fluidic organizer, Waters 3100 mass detector containing single quadrapole, and Waters PDA detector 2998] on Waters Xterra C18 column (3.0 x 100 mm, 5 μM). UPLC chromatograms obtained using Waters Aquity UPLC H class [having PDA detector, sample manager FTN and quaternary solvent manager] on Waters BEH C18 column (2.1 x 100 mm, 1.7 μM).

Entry	Purity	Retention Time (min)		Molecular formula	Molar Mass		
		$(t_r)_A$	$(t_r)_B$		Calcd.	Observed (m/z)[a] MALDI -TOF	Observed (m/z)[b] LC-MS
(6) Isu114-106	>95	5.33	0.71	C93H169N35O28S	2256.26	$[M+H]^+$ = 2255.968 $[M+Fe+H]^+$=2311.985	$[M+3H]^{+3}$ =753.92, $[M+4H]^{+4}$ = 566.02, $[M+5H]^{+5}$=452.81, $[M+2H]^{+2}$=1130.24
(7) Isu114-71	>95	0.22	0.82	C93H170N36O27S	2255.28	$[M+H]^+$ = 2255.168 $[M+4F]^+$= 2479.779	$[M+4H]^{+4}$ = 565.19, $[M+3H]^{+3}$=753.20, $[M+2H]^{+2}$=1129.35
(8) Isu114-76	>95	0.26	0.48	C92H163N33O28S	2210.21	$[M+Na+K]^+$ = 2272.951 $[M+4Fe+2K]^+$ = 2502.773	$[M+3H]^{+3}$ = 738.34, [M+3Na]+3=761.56, [M+2H]+2=1107.23
(9) Isu114-86/60	>95	5.04	0.68	C96H174N36O26S	2279.31	[M+3Fe+3Na]+=2572.987	$[M+3Na]^{+3}$ = 784.99 [M+3H]+3=761.26, [M+2H]+2=1141.45
(10) Isu114-59	>95	0.26	0.49	C95H170N34O27S	2251.27	$[M+2Na+K]^+$ = 2336.369 $[M+H]^+$ = 2251.185	$[M+4Fe]^{+4}$ = 619.00 $[M+3Na]^{+3}$ = 775.58 [M+3H]+3=751.87, [M+2H]+2= 1127.49
(11) Isu114-89	>95	0.18	7.69	C96H172N34O28S	2281.28	$[M+5K+2H]^+$= 2478.792	$[M+2H]^+$ = 1141.42, [M+3H]+3=762.66
(12) Isu114-77	>95	5.20	0.48	C93H167N33O27S	2210.24	$[M+4Fe+2K]^+$ = 2503.613	$[M+3H]^{+3}$= 738.19, [M+Na]+3=761.60, [M+2H]+2=1106.47, [M+K]+3=594.38
(13) Isu114-73	>95	5.01	0.47	C92H163N35O28S	2238.21	$[M+H]^+$= 2239.796	$[M+3H]^{+3}$= 747.64, [M+2H]+2=1120.74, [M+4H]+4=560.93

Table S1. Characterization table for the cyclized and linear peptides synthesized in this study. Retention time $(t_r)_A$ was determined using Waters UPLC system running gradient 10 - 90 % acetonitrile in H_2O over 10 min at flow rate of 0.5 mL/min and retention time $(t_r)_B$ was determined using Waters LC-MS system running gradient 10-90 % acetonitrile in H_2O over 20 min at flow rate of 1.0 mL/min. Experimental observed mass for peptides (**6)** - (**13)** obtained by [a]Bruker AutoFlex III MALDI-TOF and [b]Waters LC-MS system having a single quadrapole.

***In vitro* LSD1 enzyme inhibition assay**

The % LSD1 enzyme inhibition for the cyclic and linear peptides was determined *in vitro*[20, 21] *by* using the LSD1 fluorescent assay kit from the BPS Biosciences (cat # 50106) according to the manufacturer's instructions. The LSD1 assay kit comes with the 96 microtiter well plate, purified LSD1 recombinant enzyme, Amplex red reagent, horseradish peroxidase reagent (HRP) and 2 X LSD1 assay buffer for 96 enzyme reactions. The total final LSD1 assay volume was 50 μL. Assay was carried out in 96-well microtiter plate (n =3). The stock solutions of the cyclic and linear peptides were prepared by dissolving them in sterile water and verlindamycin was prepared by dissolving it in DMSO. All the succeeding dilutions for the test inhibitors, and verlindamycin were carried out in the LSD1 assay buffer. LSD1 inhibition of all test compounds were carried out at 10 μM fixed concentrations in final 50 μL of total assay volumes. Enzymatic reaction was initiated by adding methylated peptide substrate (200 μM) to LSD1 assay mixture contained LSD1 (150 ng), test inhibitor (50 μM) and 50 μL of solution containing mixture of 50 μL of 10 mM Amplex red and 100 μL of 10 U/ml HRP in 4850 mL of 1X LSD1 buffer. Positive control contained all the above components except test inhibitor. The substrate control contained all the above components except methylated peptide substrate and test inhibitor. The blank contained all the above components except test inhibitor. Assay components were incubated at room temperature for 25 min before reading the fluorescence at wavelengths 530 nm (excitation) and wavelengths 590 nm (emission) using the SpectraMax M5 instrument from Molecular Devices. Blank fluorescent reading was subtracted from all fluorescent measurement readings. % enzymatic remaining activity was calculated by following equation. [(Test inhibitor fluorescent reading) / (Positive control fluorescence reading) x 100%]. % LSD1 inhibition was calculated by following the equation. [% LSD1 inhibition = 100 – % LSD1 enzymatic activity remaining]. In order to determine the IC_{50} for **(9),** varying

	(6)	(7)	(8)	(9)	(10)	(11)	(12)
Number of values	3	3	3	3	3	3	3
Minimum	91.60	90.65	32.20	93.24	25.59	47.87	28.48
25% Percentile	91.60	90.65	32.20	93.24	25.59	47.87	28.48
Median	97.90	90.88	38.61	94.04	30.89	49.14	32.31
75% Percentile	100.0	90.99	45.54	94.16	86.26	49.78	67.86
Maximum	100.0	90.99	45.54	94.16	86.26	49.78	67.86
Mean	96.50	90.84	38.78	93.81	47.58	48.93	42.88
Std. Deviation	4.372	0.1735	6.672	0.5001	33.60	0.9722	21.72
Std. Error	2.524	0.1002	3.852	0.2888	19.40	0.5613	12.54
Lower 95% CI of mean	85.64	90.41	22.21	92.57	-35.89	46.52	-11.06
Upper 95% CI of mean	107.4	91.27	55.36	95.06	131.1	51.34	96.83
Sum	289.5	272.5	116.4	281.4	142.7	146.8	128.7

Table S2. Statistics involved in calculation of the % LSD1 inhibition of linear peptides **6** and **7** and cyclic peptides **8-13**.

concentrations of **(9)** was preincubated for 5 min at room temperature prior to initiation of the reaction via the methylated peptide substrate addition. IC_{50} was calculated based nonlinear regression analysis of percent LSD1 inhibition data collected from triplicate using GraphPad Prism 5 software (GraphPad, San Diego, California).

Determination of IC_{50} using the MTS cell proliferation assay

The human breast (MCF-7) and lung cancer cell lines (Calu-6) were cultured using 10% (v/v) fetal bovine serum in Eagles's minimum essential medium (EMEM) in the presence of 1% penstrap at 37^0 C in 5% CO_2. The assay was done in triplicate for each test inhibitor involved in IC_{50} calculations. A 100 µL of MCF-7 and Calu-6 cell solution having a concentration of 20,000 cells per ml were dispensed in 96 well plates and were allowed to incubate for 24 hour in 5% CO_2 at 37^0 C. After 24 hours, the cells were treated with varying concentrations of 50 µL of test inhibitor solutions of linear (**7**) and cyclic peptide (**9**) analogues and reference compound (verlindamycin) and were allowed to incubate for 72 hours in 5% CO_2 at 37 ^{0}C. Then, the cells were treated with 20 µL of Promega CellTiter 96® Aq_{ueous} One Solution and were allowed to incubate at 37^0 C in 5% CO_2 for 2h. Absorbance at wavelength 490 nm was measured using SpectraMax M5 instrument from Molecular Devices. Dose response curve was constructed for each inhibitor and IC_{50} values were calculated using GraphPad Prism 5 software (GraphPad, San Diego, California). Data was normalized to the largest value in the dataset.

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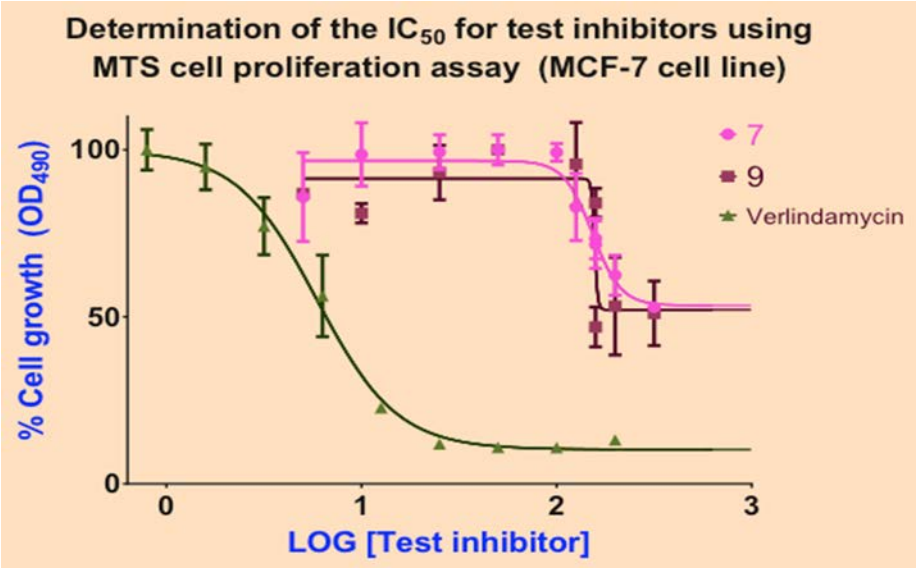


Figure S1. Determination of the IC_{50} value for test inhibitors **3**, **7** and **9** using the MTS cell proliferation assay.in the MCF7 human breast cancer cell line. Inhibition values were gathered at concentrations between 5.0 and 300 µM for (**7**) and (**9**), and 0.4 and 200 µM for verlindamycin. The calculated IC_{50} values are 152.6, 156.6 and 5.9 µM for (**7**), (**9**), and verlindamycin respectively.

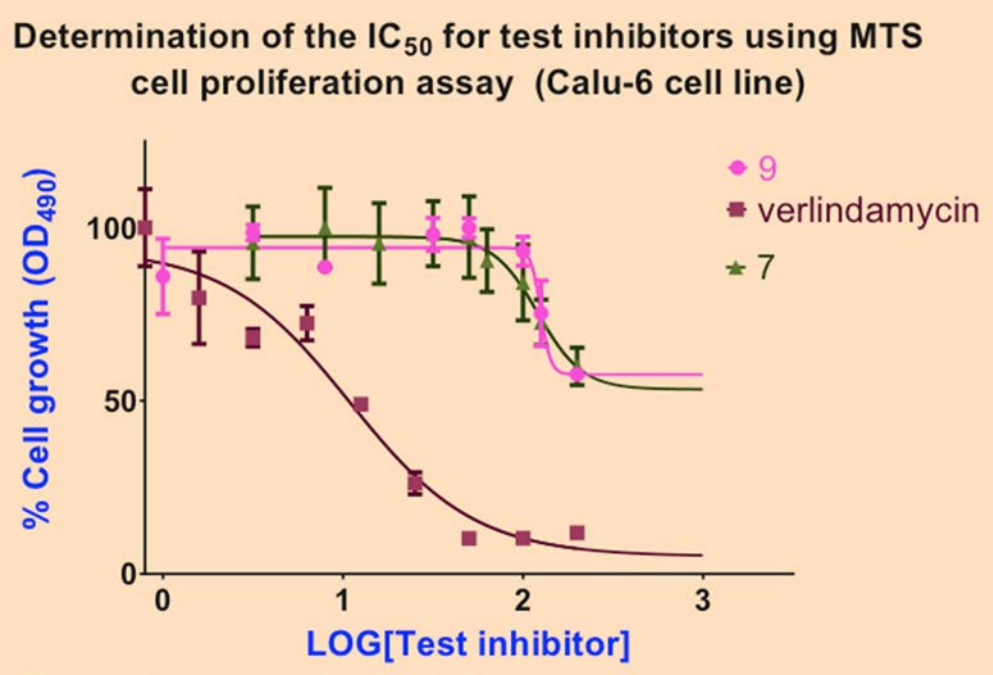


Figure S2. Determination of the IC_{50} value for inhibitors 3 and **9** using MTS cell proliferation assay in the Calu-6 Human lung adenocarcinoma cell line. Inhibition values were gathered at concentrations between 0.76 and 200 µM for verlindamycin, and 1 and 200 µM for (**7**) and (**9**). The calculated IC_{50} values are 125.3, 120.7, and 10.9 µM for (**9**), (**7**), and verlindamycin respectively.

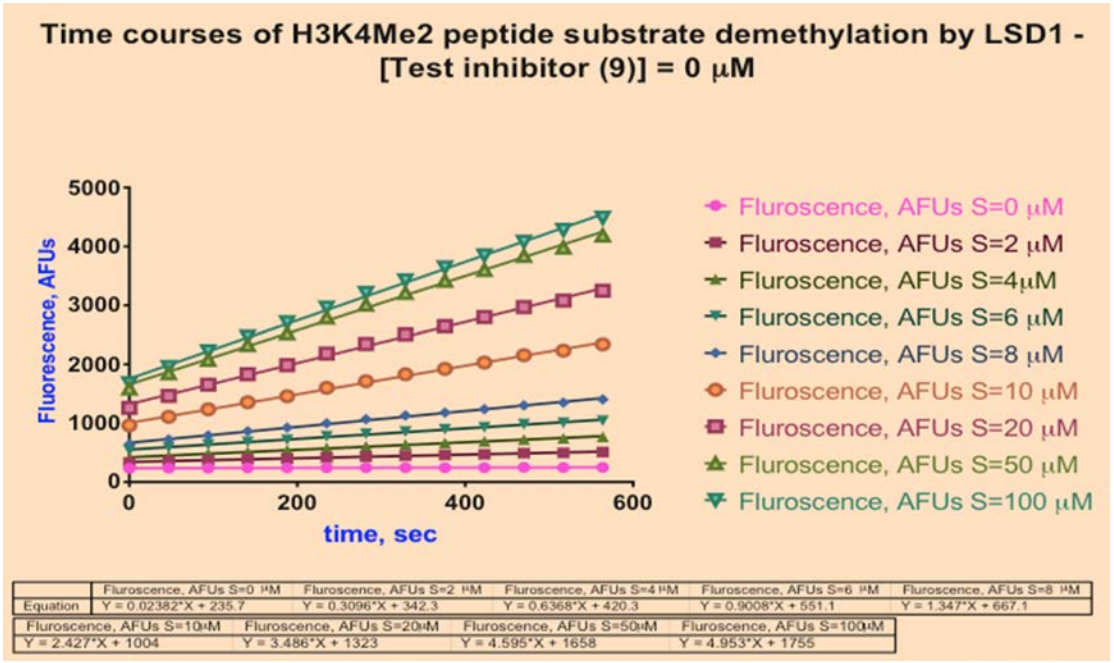
Determination of the *in vitro* stability of the cyclic and linear peptide derivatives using rat plasma

To construct calibration equation for the linear peptide **7** and cyclic peptide **9**, stock solutions were prepared by dissolving 4.9 mg of each peptide in 1000 µL of water. Standard solutions having concentrations of 2.45, 1.22, 0.61, 0.31, 0.15, 0.08, 0.04 mg/mL were made by using (1:1) serial dilution of the stock solutions with water. Each of the standard solutions were injected to the LC-MS running acetonitrile in water gradient (in 1% TFA) and the area under the curve data (AUC) for standard peptide solutions was measured. The calibration equations for linear and cyclic peptides were derived based on the AUC data of standard solutions using linear regression analysis found in GraphPad Prism 5 software (GraphPad, San Diego, California). To determine the half-life ($t_{1/2}$) of linear (**7**) and cyclic peptides (**9**), stock solutions of each peptide were prepared by dissolving 4.9 mg of each peptide in 1000 µL of water in a 2 mL micro tube. Then 1000 µL of rat plasma (non-sterile with heparin, Pel-Freez Biologicals, Rogers, Arkansas, USA) was added to the micro tube and vortexed for 1 min at r.t. For linear peptide, at 0 min, 20 min and 61 min time points, and for cyclic peptides at 20 min, 60 min, 90 min, 180 min and 316 min time points, a 600 µL of solution from the micro tube was transferred to an another micro tube and centrifuged at 10,000 rpm for 15 min. After centrifugation, the supernatant solution was carefully removed, and it was injected to the LC-MS running acetonitrile in water gradient (1% TFA). The area under the curve for each peptide at the different time points was measured. The amount of each peptide remained were calculated using their respective calibration equations derived. Data was normalized . % of the peptide remaining vs. time (sec) was graphically constructed, and the metabolic degradation half-life values ($t_{1/2}$) for each peptide calculated using nonlinear regression analysis found in the GraphPad Prism 5 software (GraphPad, San Diego, California).

Determination of LSD1 enzyme kinetics

Enzyme kinetic analysis was done using Enzo kit (BML-AK544) according to the manufacturer instructions. A 1 mL of substrate (H3K4Me2 peptide) solutions having concentration of 0, 50, 100, 150, 200, 250, 500, 1250, 2500, 5000 µM and 1 mL of test inhibitor solutions having concentration of 0, 0.02, 2, 20 µM were prepared prior to initiate the enzymatic reaction. A 500 µL of stock solution of LSD1 enzyme having concentration of 0.1 µg/ µL in assay medium was prepared. Then, 10 µL of the enzyme solution, 10 µL of assay buffer, and 5 µL of test inhibitor having its concentration ranging from 0 - 200 µM was added per well in 96 well plate and allowed to stay for 30 min. At the end of 30 min, 30 µL of assay buffer containing 2µL of 50X HRP was added to the each well containing enzyme and the inhibitor. Then, 4 µL of substrate solution having its concentration ranging from 0-5000 µM, 1 µL of 100X Cellestial Red, 45 µL of Assay buffer was introduced to each well containing enzyme, the inhibitor, and HRP (See Figure S7). The fluorescence [excitation = 530 nm, emission = 590 nm] was measured using SpectraMax M5 instrument from Molecular Devices over 30 min period per 47 sec intervals at 23^0C. It was found that the fluorescence was linearly increased during the first 564 sec. Fluorescence vs. time (sec) was constructed graphically and the linear regression analysis found in GraphPad Prism 5 software (GraphPad, San Diego, California) was utilized to derive the rate equation for the each test inhibitor and substrate concentration combinations as depicted in the Figures of S8-S12. Initial rate equations representing units of Fluorescence per sec. were converted to no of H_2O_2 molecules generated per sec using calibration equation derived from measuring fluorescence of standard H_2O_2 solutions.

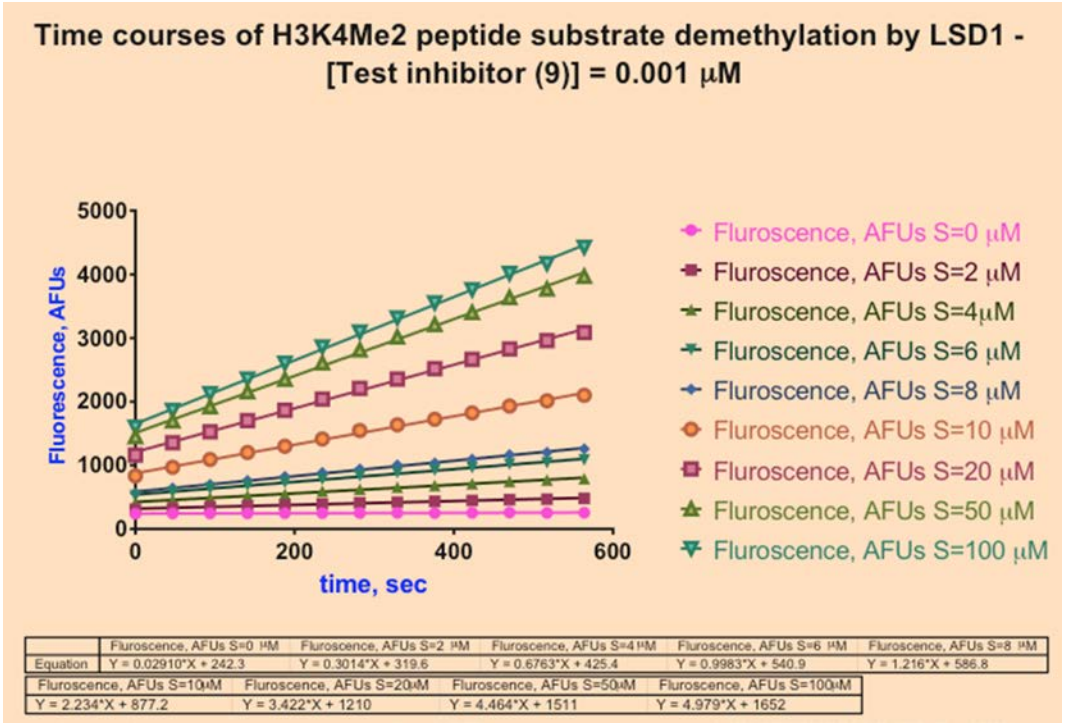
Initial velocity vs. substrate concentrations for different inhibitor concentration combinations were plotted and the Vmax and Km values were calculated using nonlinear regression analysis found in the GraphPad Prism 5 software (GraphPad, San Diego, California). Further, Lineweaver-Burk plot was constructed for different test inhibitor concentrations. Then initial rate vs. substrate concentrations data were fitted for the competitive type inhibition using nonlinear regression analysis found in the GraphPad Prism 5 software (GraphPad, San Diego, California) and V_{max}, K_m and K_i were calculated.



	Fluroscence, AFUs S=0 µM	Fluroscence, AFUs S=2 µM	Fluroscence, AFUs S=4 µM	Fluroscence, AFUs S=6 µM	Fluroscence, AFUs S=8 µM
Equation	Y = 0.02382*X + 235.7	Y = 0.3096*X + 342.3	Y = 0.6368*X + 420.3	Y = 0.9008*X + 551.1	Y = 1.347*X + 667.1

Fluroscence, AFUs S=10µM	Fluroscence, AFUs S=20µM	Fluroscence, AFUs S=50µM	Fluroscence, AFUs S=100µM
Y = 2.427*X + 1004	Y = 3.486*X + 1323	Y = 4.595*X + 1658	Y = 4.953*X + 1755

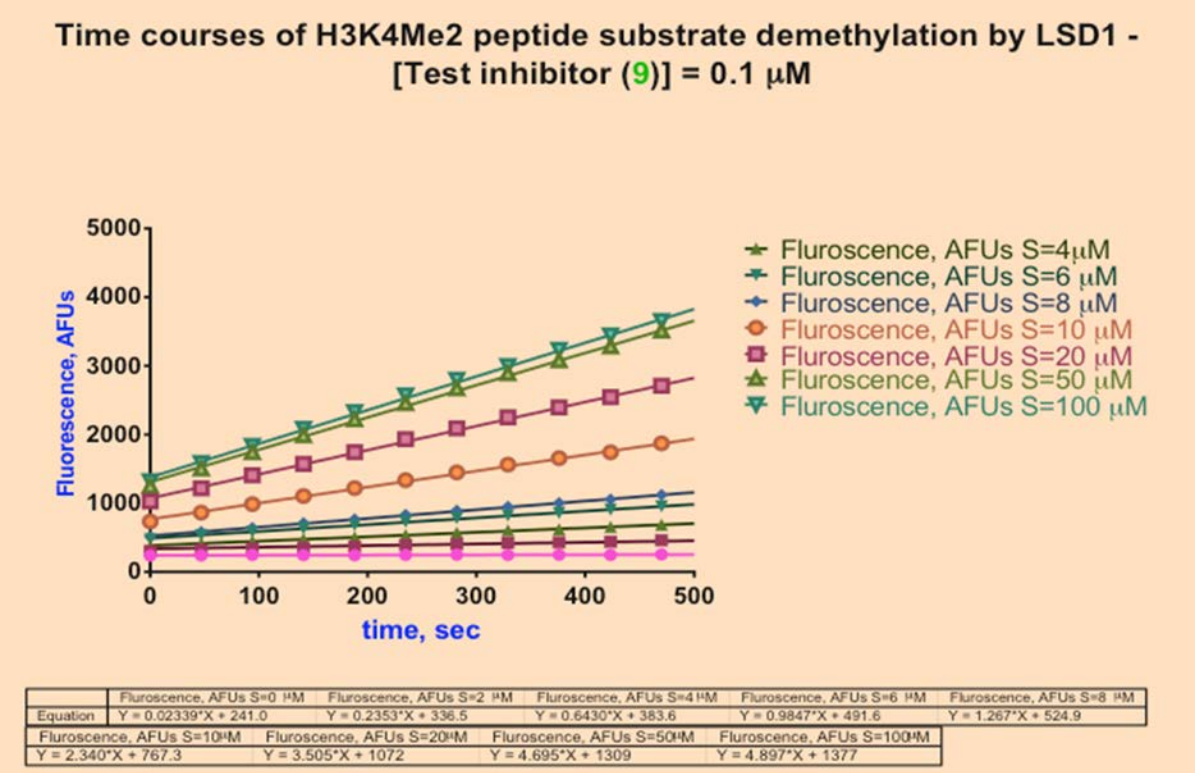
Figure S3. Determination of the rate equation for H3K4Me2 substrate demethylation by LSD1 at varying concentrations of substrate between 0 and 100 µM at fixed concentration {[I]= 0.00 µM} of **9**.



	Fluroscence, AFUs S=0 µM	Fluroscence, AFUs S=2 µM	Fluroscence, AFUs S=4 µM	Fluroscence, AFUs S=6 µM	Fluroscence, AFUs S=8 µM
Equation	Y = 0.02910*X + 242.3	Y = 0.3014*X + 319.6	Y = 0.6763*X + 425.4	Y = 0.9983*X + 540.9	Y = 1.216*X + 586.8

Fluroscence, AFUs S=10µM	Fluroscence, AFUs S=20µM	Fluroscence, AFUs S=50µM	Fluroscence, AFUs S=100µM
Y = 2.234*X + 877.2	Y = 3.422*X + 1210	Y = 4.464*X + 1511	Y = 4.979*X + 1652

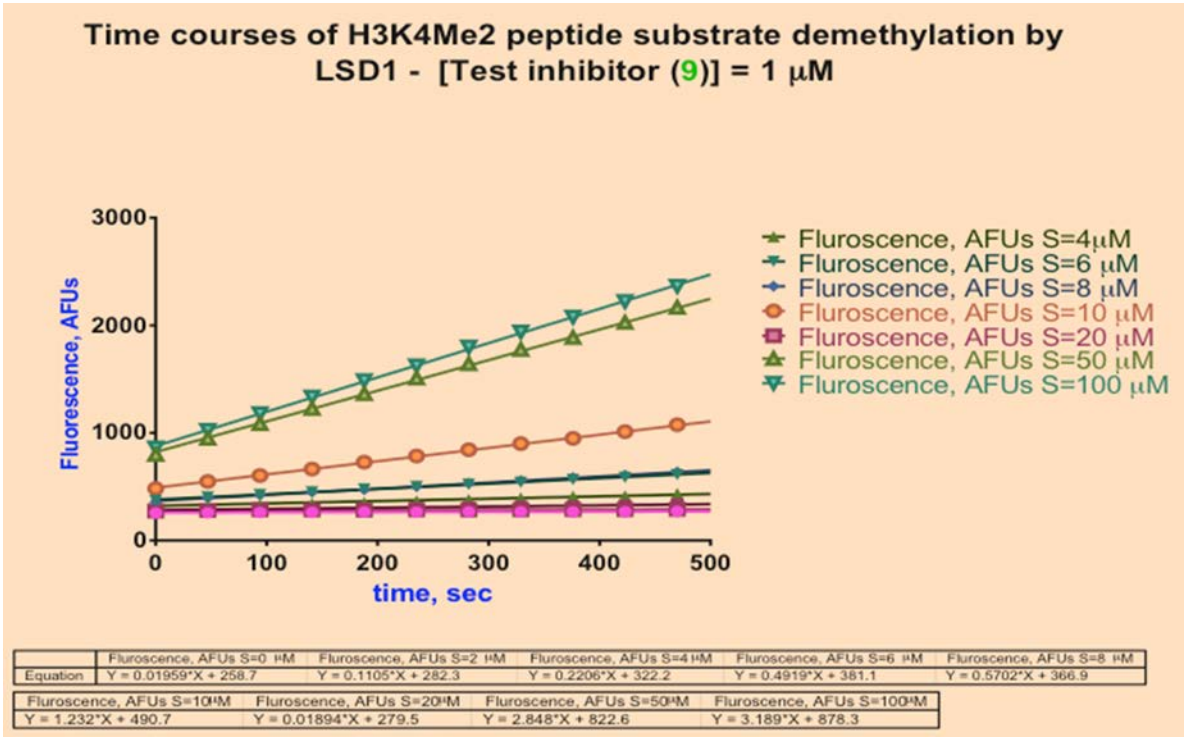
Figure S4. Determination of the rate equation for H3K4Me2 substrate demethylation by LSD1 enzyme for varying concentration of substrate between 0 and 100 µM at fixed concentration {[I]= 0.001 µM} of the test inhibitor, (**9**)



	Fluroscence, AFUs S=0 μM	Fluroscence, AFUs S=2 μM	Fluroscence, AFUs S=4 μM	Fluroscence, AFUs S=6 μM	Fluroscence, AFUs S=8 μM
Equation	Y = 0.02339*X + 241.0	Y = 0.2353*X + 336.5	Y = 0.6430*X + 383.6	Y = 0.9847*X + 491.6	Y = 1.267*X + 524.9

Fluroscence, AFUs S=10 μM	Fluroscence, AFUs S=20 μM	Fluroscence, AFUs S=50 μM	Fluroscence, AFUs S=100 μM
Y = 2.340*X + 767.3	Y = 3.505*X + 1072	Y = 4.695*X + 1309	Y = 4.897*X + 1377

Figure S5. Determination of the rate equation for H3K4Me2 substrate demethylation by LSD1 enzyme for varying concentration of substrate between 0 and 100 μM at fixed concentration {[I]= 0.01 μM} of the test inhibitor, (**9**)



	Fluroscence, AFUs S=0 μM	Fluroscence, AFUs S=2 μM	Fluroscence, AFUs S=4 μM	Fluroscence, AFUs S=6 μM	Fluroscence, AFUs S=8 μM
Equation	Y = 0.01959*X + 258.7	Y = 0.1105*X + 282.3	Y = 0.2206*X + 322.2	Y = 0.4919*X + 381.1	Y = 0.5702*X + 366.9

Fluroscence, AFUs S=10 μM	Fluroscence, AFUs S=20 μM	Fluroscence, AFUs S=50 μM	Fluroscence, AFUs S=100 μM
Y = 1.232*X + 490.7	Y = 0.01894*X + 279.5	Y = 2.848*X + 822.6	Y = 3.189*X + 878.3

Figure S6. Determination of the rate equation for H3K4Me2 substrate demethylation by LSD1 enzyme for varying concentration of substrate between 0 and 100 μM at fixed concentration {[I]= 1 μM} of the test inhibitor, (**9**)

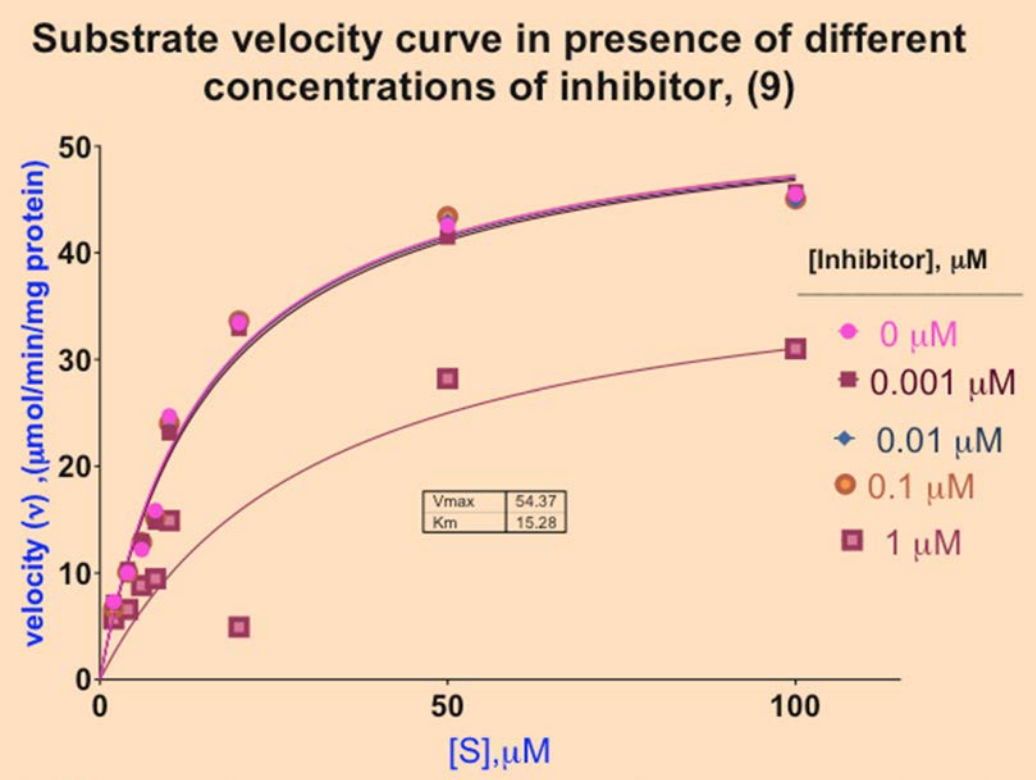
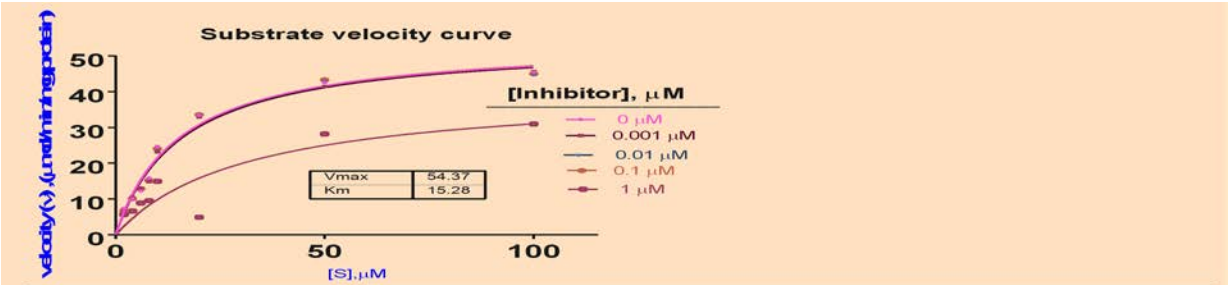


Figure S7. Determination of the V_{max} and K_m values using substrate velocity curve. Initial rate vs. substrate concentrations were plotted against different concentrations of test inhibitor, (**9**). The calculated V_{max} and K_m are 54.37 µmol/min/mg of protein and 15.28 µM respectively.



	0 uM	0.001 uM	0.01 uM	0.1 uM	1 uM
Michaelis-Menten					
Best-fit values					
Vmax	54.37	54.24	54.27	54.62	40.86
Km	15.28	15.88	15.45	15.53	31.74
Std. Error					
Vmax	3.342	2.906	3.326	3.365	11.24
Km	2.500	2.249	2.516	2.539	20.01
95% Confidence Intervals					
Vmax	46.19 to 62.55	47.13 to 61.35	46.13 to 62.40	46.39 to 62.85	13.35 to 68.37
Km	9.161 to 21.40	10.38 to 21.39	9.296 to 21.61	9.314 to 21.74	0.0 to 80.70
Goodness of Fit					
Degrees of Freedom	6	6	6	6	6
R square	0.9737	0.9803	0.9741	0.9742	0.7635
Absolute Sum of Squares	41.47	30.11	40.60	41.35	176.0
Sy.x	2.629	2.240	2.601	2.625	5.417
Constraints					
Km	Km > 0.0	Km > 0.0	Km > 0.0	Km > 0.0	Km > 0.0
Number of points Analyzed	8	8	8	8	8

Table S3. Statistical data involved in calculation of the V_{max} and K_m values using substrate velocity curves.

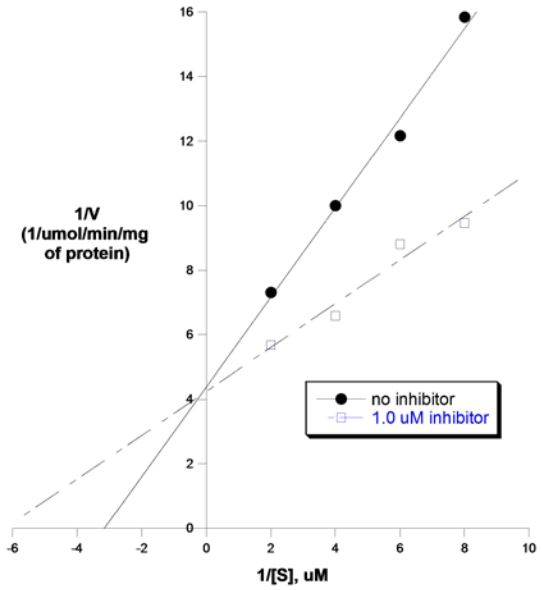


Figure S8. Lineweaver –Burke analysis for inhibition of LSD1 by **9**. 1/initial rate vs. 1/[S] was plotted at 0 and 1.0 mM of compound **9.**

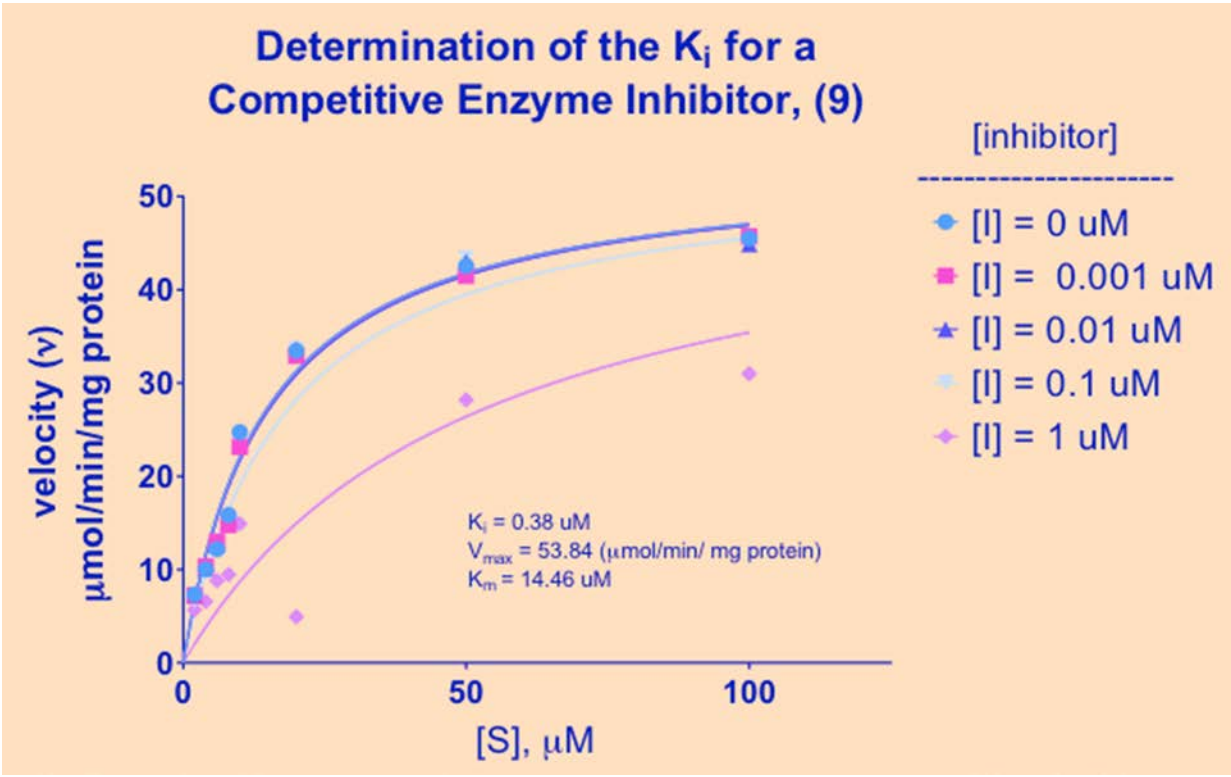


Figure S9. Determination of the V_{max}, K_m, K_i values by fitting substrate velocity data for competitive type inhibition. Initial rate vs. substrate concentrations were plotted against different concentrations of test inhibitor, (**9**). The calculated V_{max}, K_m and K_i are 53.84 µmol/min/mg of protein, 14.46 µM and 0.38 µM

	[I] = 0 uM	[I] = 0.001 uM	[I] = 0.01 uM	[I] = 0.1 uM	[I] = 1 uM	Global (shared)
Competitive inhibition						
Best-fit values						
Km	14.46	14.46	14.46	14.46	14.46	14.46
I	= 0.0	= 0.0010	= 0.0100	= 0.1000	= 1.000	
Ki	0.3851	0.3851	0.3851	0.3851	0.3851	0.3851
Vmax	53.84	53.84	53.84	53.84	53.84	53.84
Std. Error						
Km	1.514	1.514	1.514	1.514	1.514	1.514
Ki	0.08063	0.08063	0.08063	0.08063	0.08063	0.08063
Vmax	2.037	2.037	2.037	2.037	2.037	2.037
95% Confidence Intervals						
Km	11.39 to 17.53	11.39 to 17.53	11.39 to 17.53	11.39 to 17.53	11.39 to 17.53	11.39 to 17.53
Ki	0.2217 to 0.5485	0.2217 to 0.5485	0.2217 to 0.5485	0.2217 to 0.5485	0.2217 to 0.5485	0.2217 to 0.5485
Vmax	49.72 to 57.97	49.72 to 57.97	49.72 to 57.97	49.72 to 57.97	49.72 to 57.97	49.72 to 57.97
Goodness of Fit						
Degrees of Freedom						37
R square	0.9729	0.9770	0.9737	0.9545	0.7328	0.9491
Absolute Sum of Squares	42.66	35.14	41.21	73.00	198.9	390.9
Sy.x						3.250
Constraints						
Km	Km > 0.0 and shared	Km > 0.0 and shared	Km > 0.0 and shared	Km > 0.0 and shared	Km > 0.0 and shared	
I	I = 0.0	I = 0.0010	I = 0.0100	I = 0.1000	I = 1.000	
Ki	Ki > 0.0 and shared	Ki > 0.0 and shared	Ki > 0.0 and shared	Ki > 0.0 and shared	Ki > 0.0 and shared	
Vmax	Vmax > 0.0 and shared	Vmax > 0.0 and shared	Vmax > 0.0 and shared	Vmax > 0.0 and shared	Vmax > 0.0 and shared	
Number of points						
Analyzed	8	8	8	8	8	

Table S4. Statistical data involved in calculation of the V_{max}, K_m and K_i values using substrate velocity curves for competitive inhibition by **9**.

Determination of the conformation of peptide ligands

Conformational analysis was done by AMD quad core computer system installed with MacroModel ver9, and Maestro ver9 graphical interface. Extended structures of cyclic peptides were constructed using the Maestro graphical under interface. The standard bond lengths and angles, stereo configuration of side chains, formal charges of the functional groups of peptides at physiological pH = 7.2 were taken into account. The structures were initially energetically minimized using the OPLS-AA force field and the Polak-Ribier conjugate gradient (PRCG). Process of the energy minimization was continually repeated until their optimizations were converged to a gradient root mean square deviation (RMSD) less than 0.005 kJ/Å mol or a limit of 50000 iterations was reached. Continuum dielectric water solvent model (GB/SA) with extended cutoff distances (8 Å for van der Waals, 20 Å for electrostatics, and 4 Å for H-bonds) was used to simulate the aqueous conditions. MCMM procedure found in Macromodelv9 was used for the conformational analysis. A total of 1000 search steps were set up and conformations with energy differences of 21 kJ/mol or less from the global minimum were allowed to save. Standard Maestro and Discovery Studio graphical interfaces were used to measure interatomic distances, and dihedral angles, and to generate the Ramachandran plots of the cyclic and linear peptides.

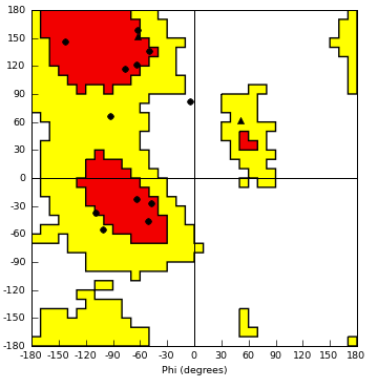


Figure 3A

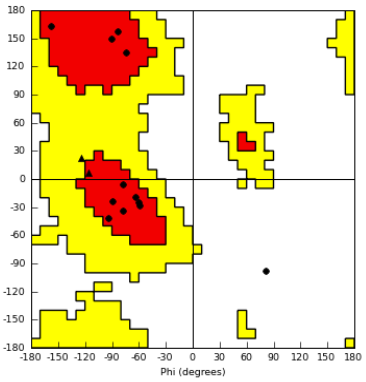


Figure 3B

Figure S10. Ramachandran plots generated from Maestro A) The Ramachandran plot of Xray crystallographic conformation of $[Met]^4$ H3 (1-21)-OH [PDB code: 2V1D]. B) The Ramachandran plot of global minimum energy conformation of **(9)**. Only the amino acids (1-16) of both peptides were shown in the Ramachandran plots.

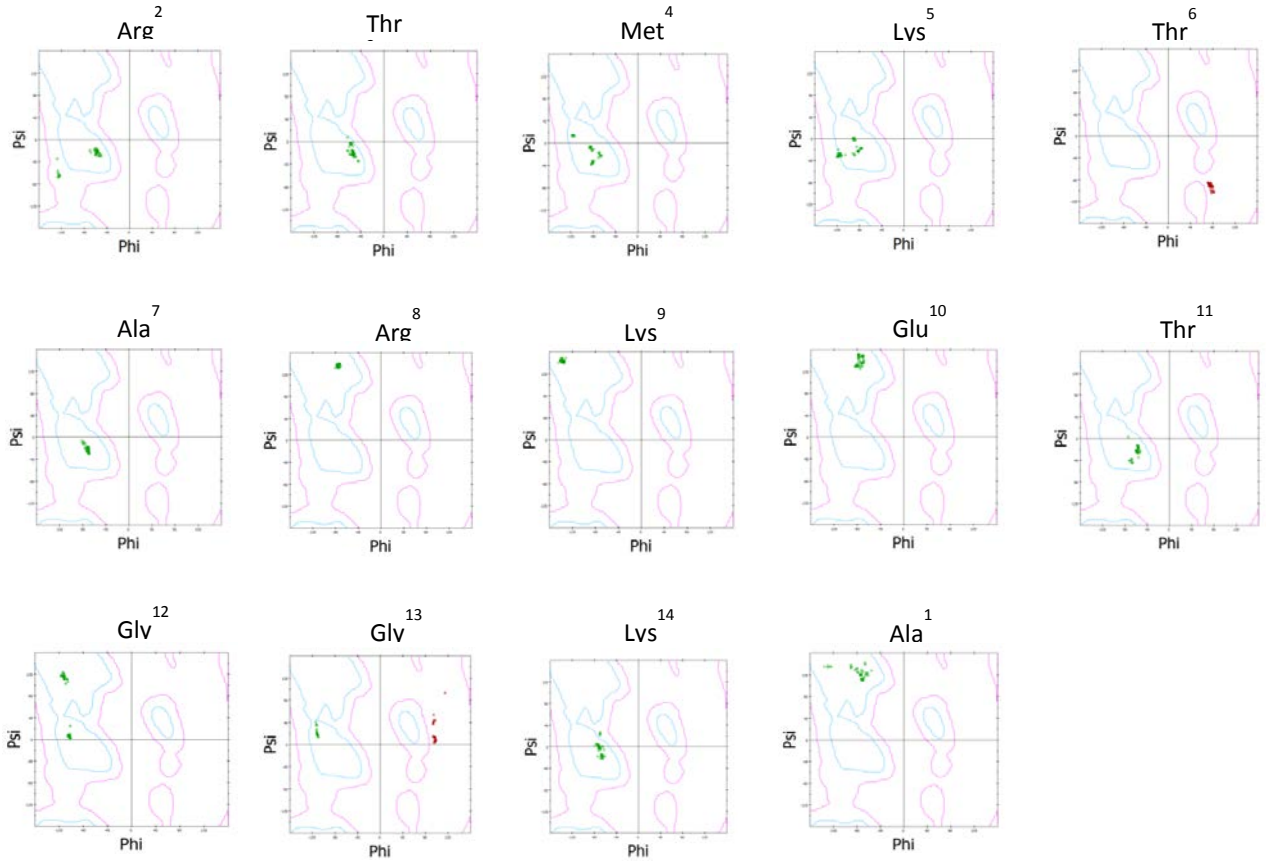


Figure S11. *Φ* and *ψ* dihedral angle distributions (i.e., Ramachandran plots) of the resultant 50 energetically lowest structures obtained from the MCMM algorithm calculations for each amino acids in the peptide (**9**)- amino acids (1-16).

ID vs. Res.	Ala		Arg^2		Thr		Met		Gln		Thr		Ala		Arg		Lys		Ser		Thr		Gly		Gly		Lys^{14}		Ala	
[Met]^4 H3 (1-16)-OH	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ
	-	-	-51	-46	-48	-28	-64	-22	-76	117	-101	-55	-62	159	-5	82	-63	121	-50	136	-109	-37	-63	152	51	62	-143	146	-93	66
	Ala		Arg^2		Thr		Met		Gln		Thr		Ala		Arg		Lys		Ser		Thr		Gly		Gly		Lys^{14}		Ala	
	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ
(9)	-	-	-60	-28	-60	-25	-94	-42	-90	-23	81	-97	-78	-33	-84	157	-158	163	-90	150	-64	-19	-116	6	-124	22	-78	-5	-74	135

Table S5. Comparison of the dihedral angles (ϕ ψ) of global minimum conformation of the cyclic peptide **9** and [Met]^4 H3 (1-16)-OH found in the PDB ID : 2V1D.

Structural Characterization of Title Compounds by LC/MS, MALDI and UPLC

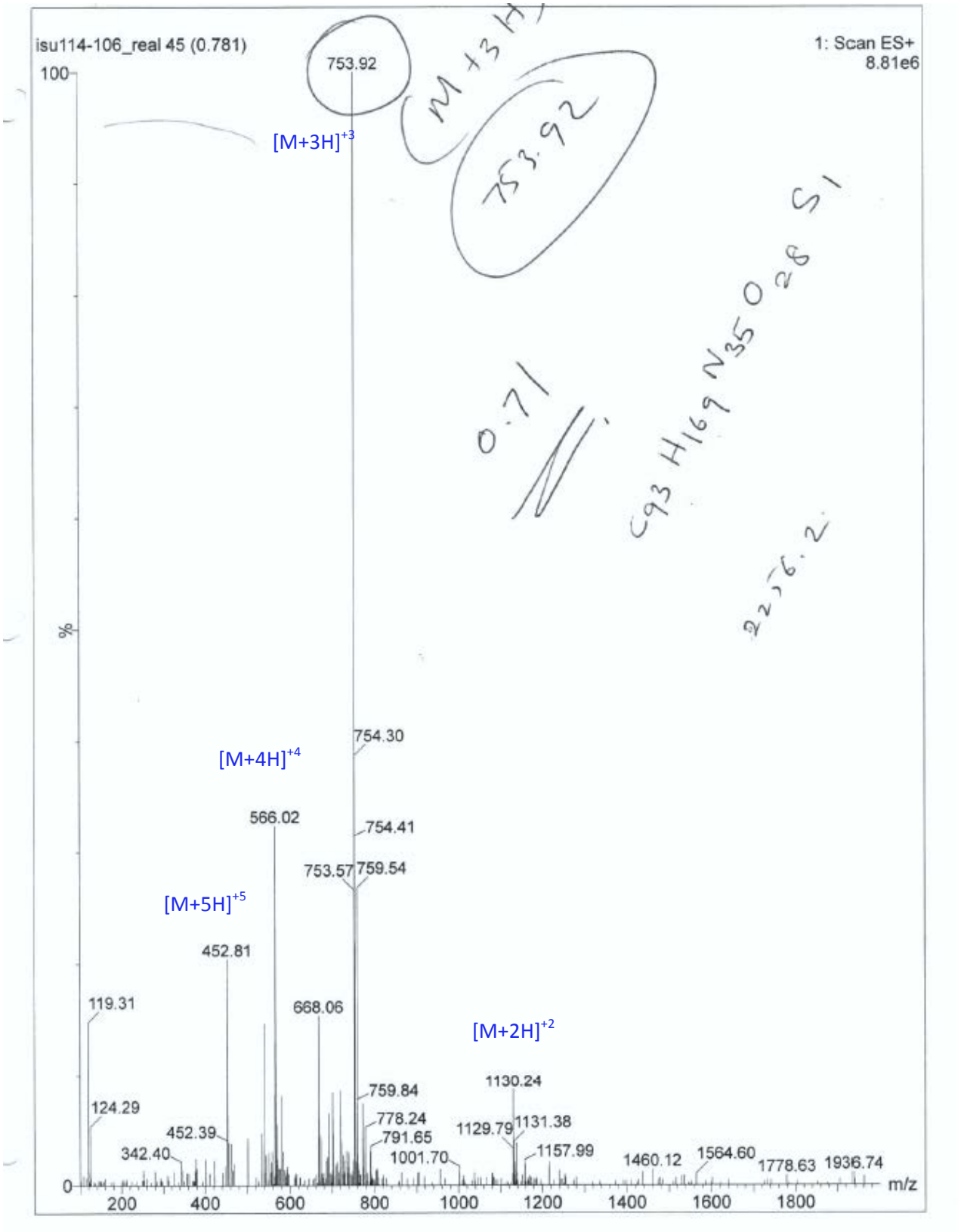


Figure S12. LC-MS mass spectrum of isu114-106 (**6**)

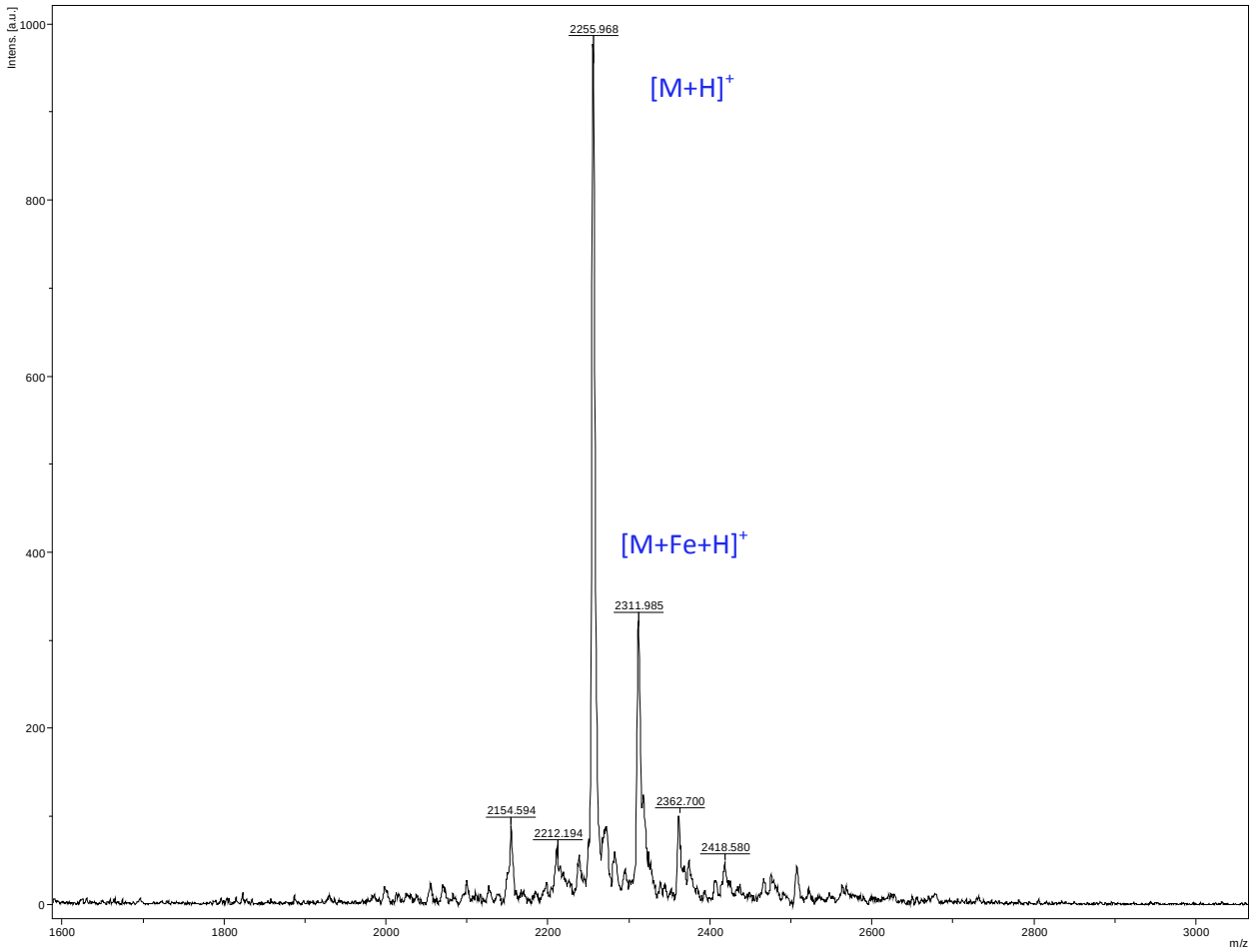


Figure S13. MALDI mass spectrum of isu114-106(**6**)

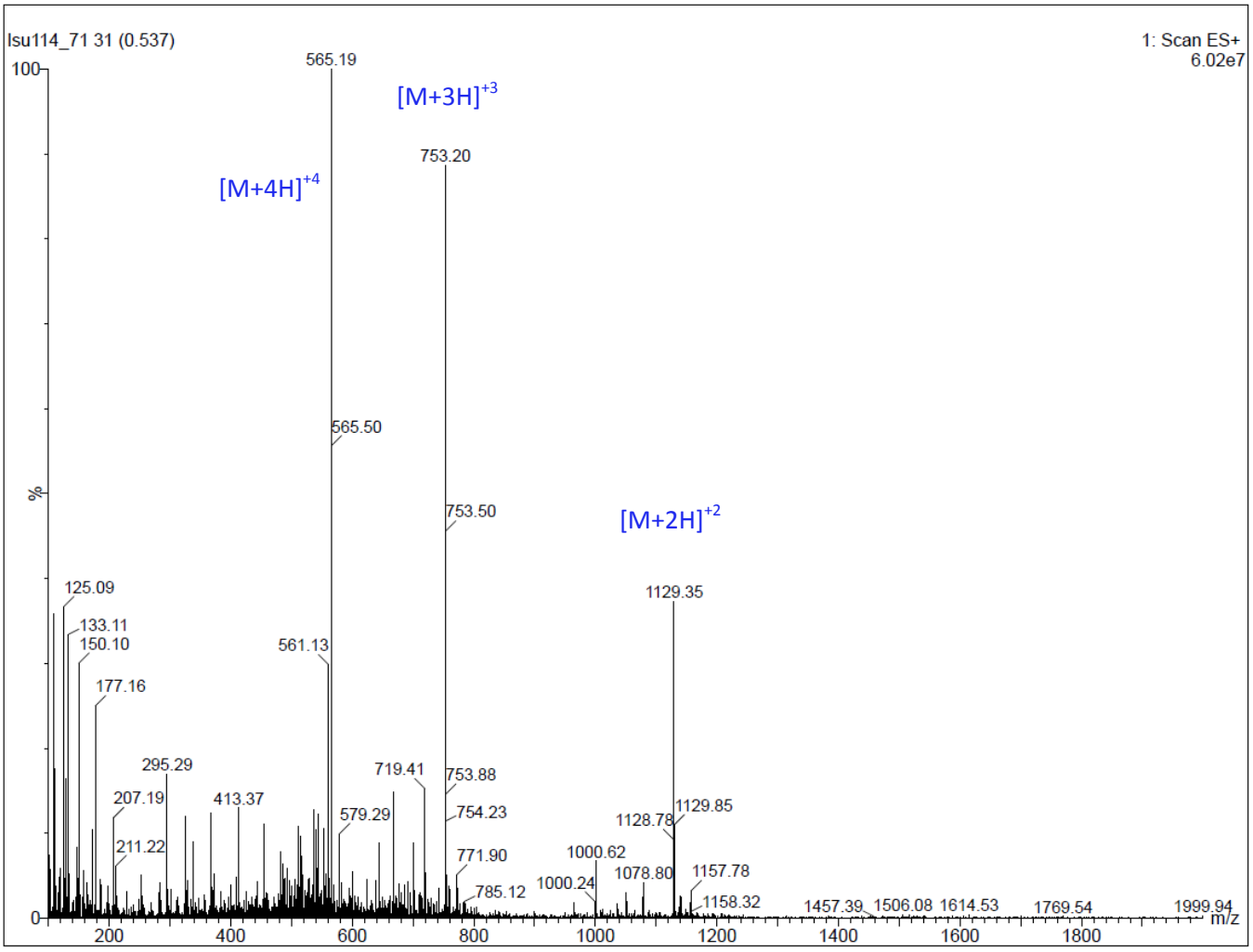


Figure S14. LC-MS mass spectrum of isu114-71(**7**)

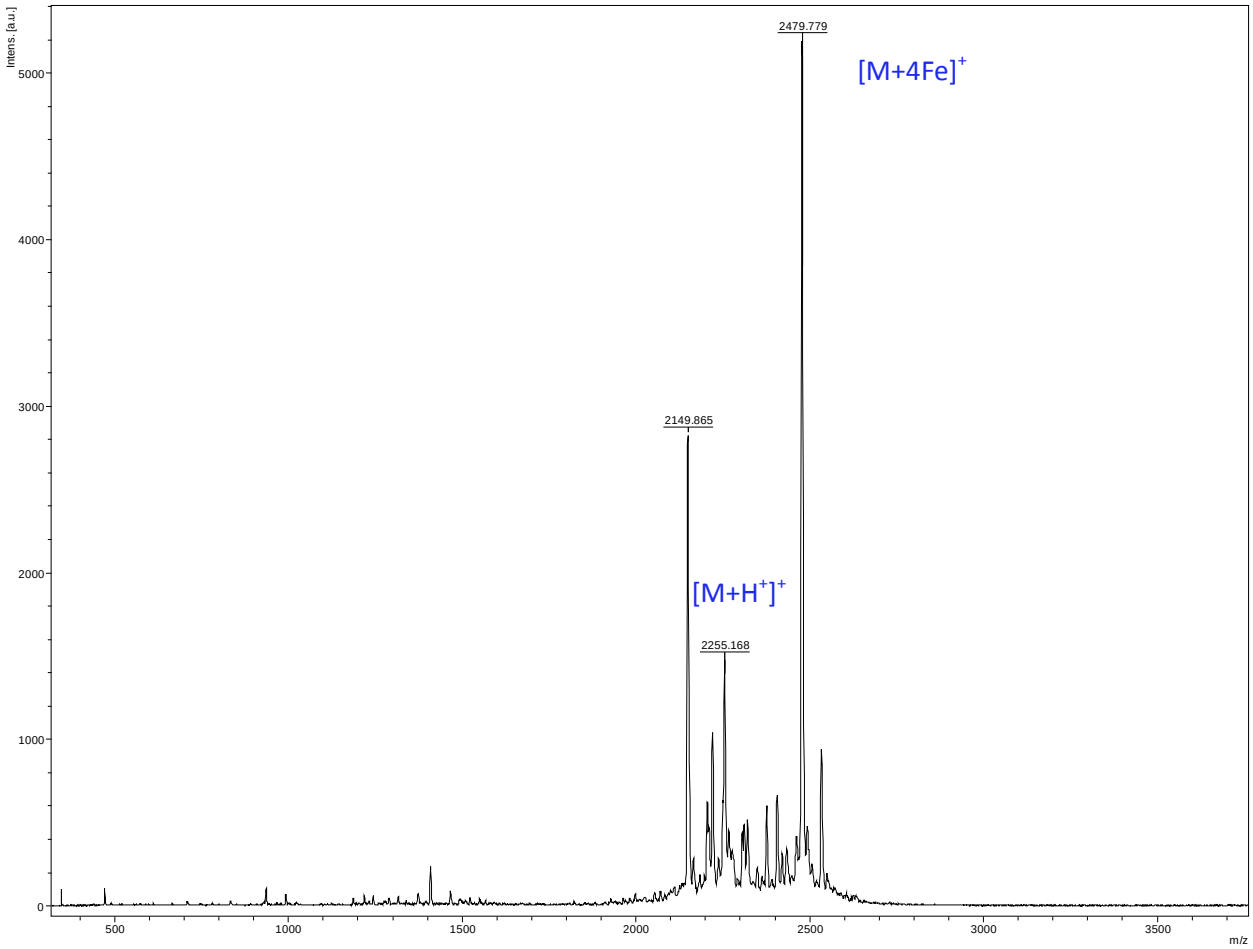


Figure S15. MALDI mass spectrum of isu114-71(**7**)

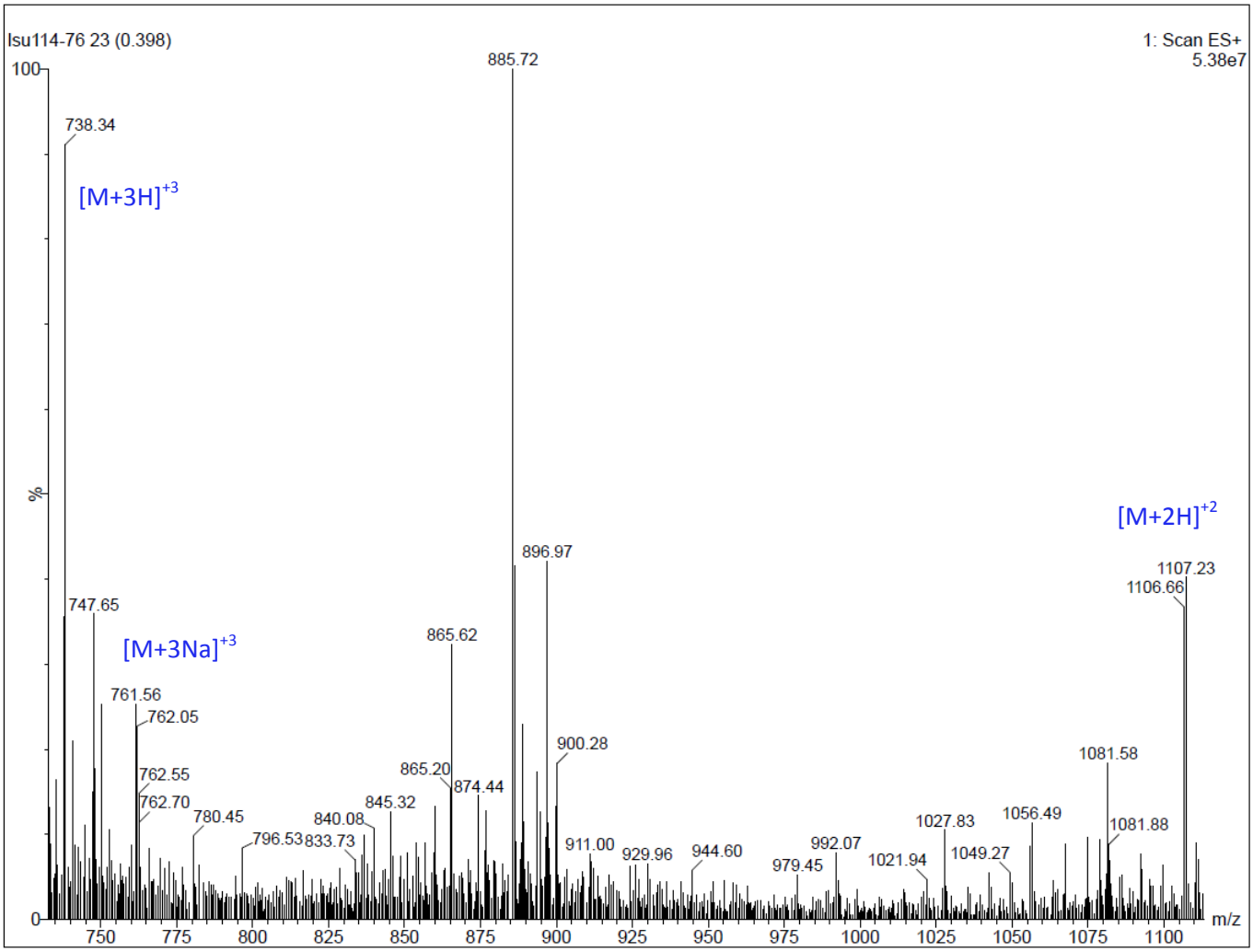


Figure S16. LC-MS mass spectrum of isu114-76(**8**)

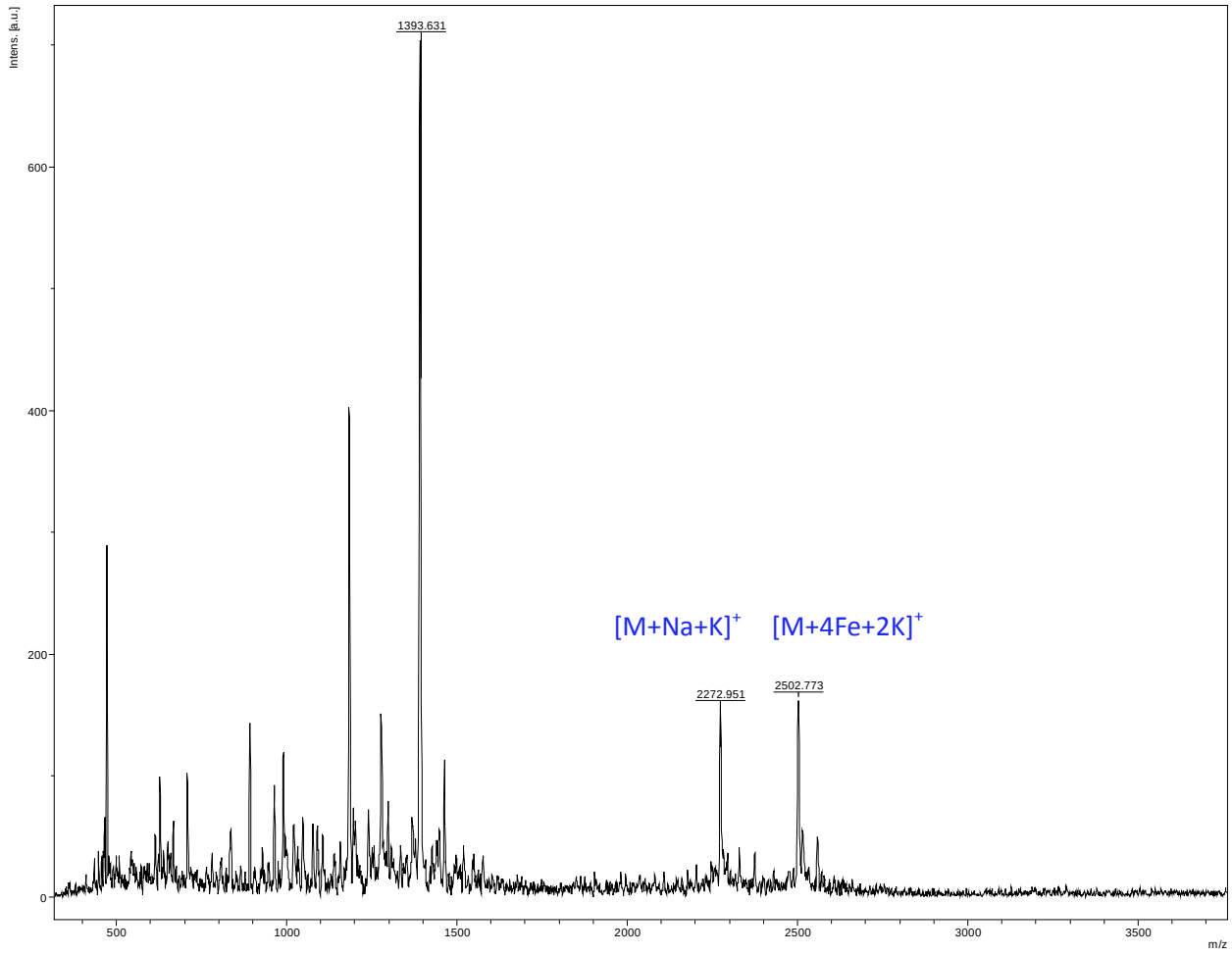


Figure S17. MALDI mass spectrum of isu114-76(**8**)

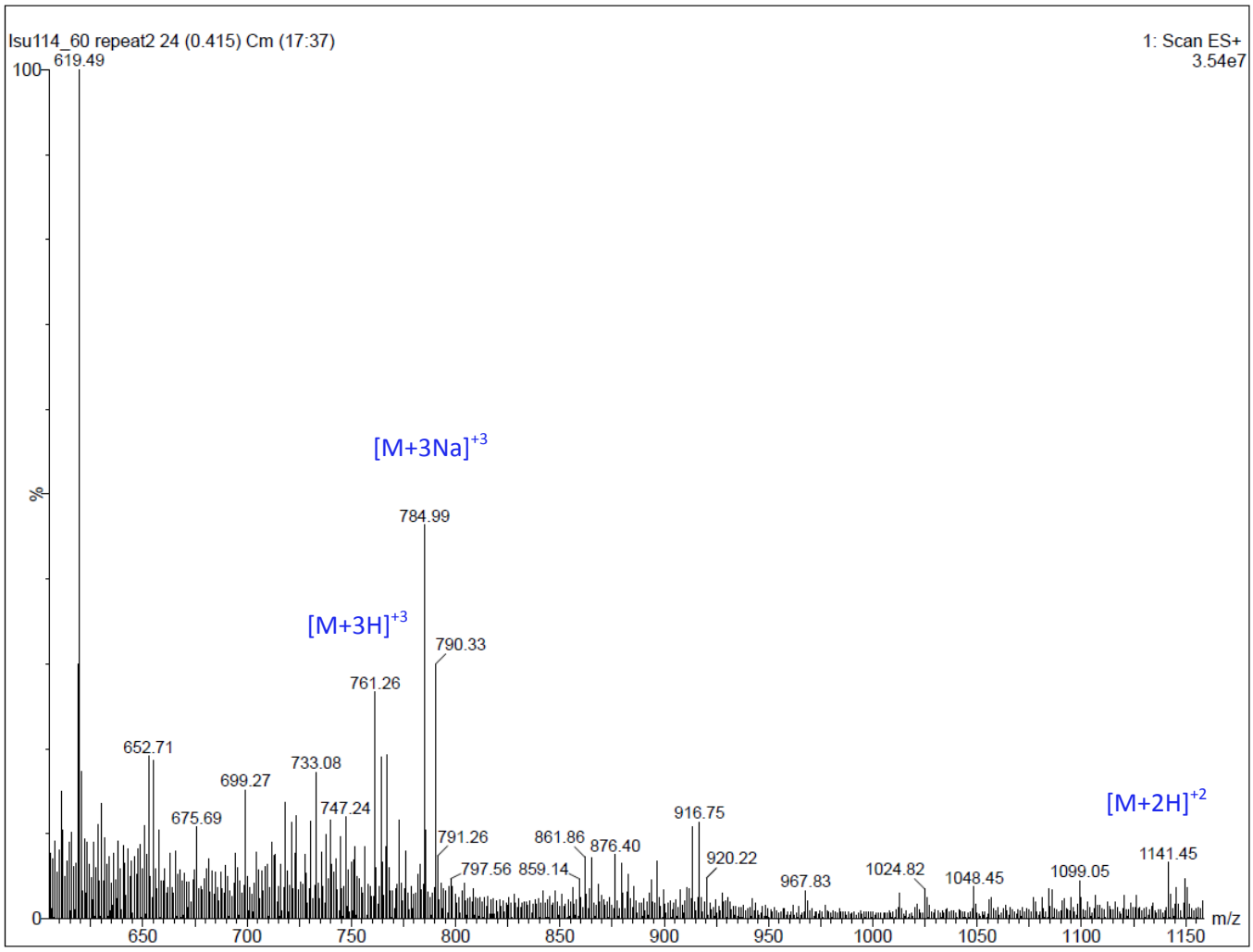


Figure S18. LC-MS mass spectrum of isu114-60(**9**)

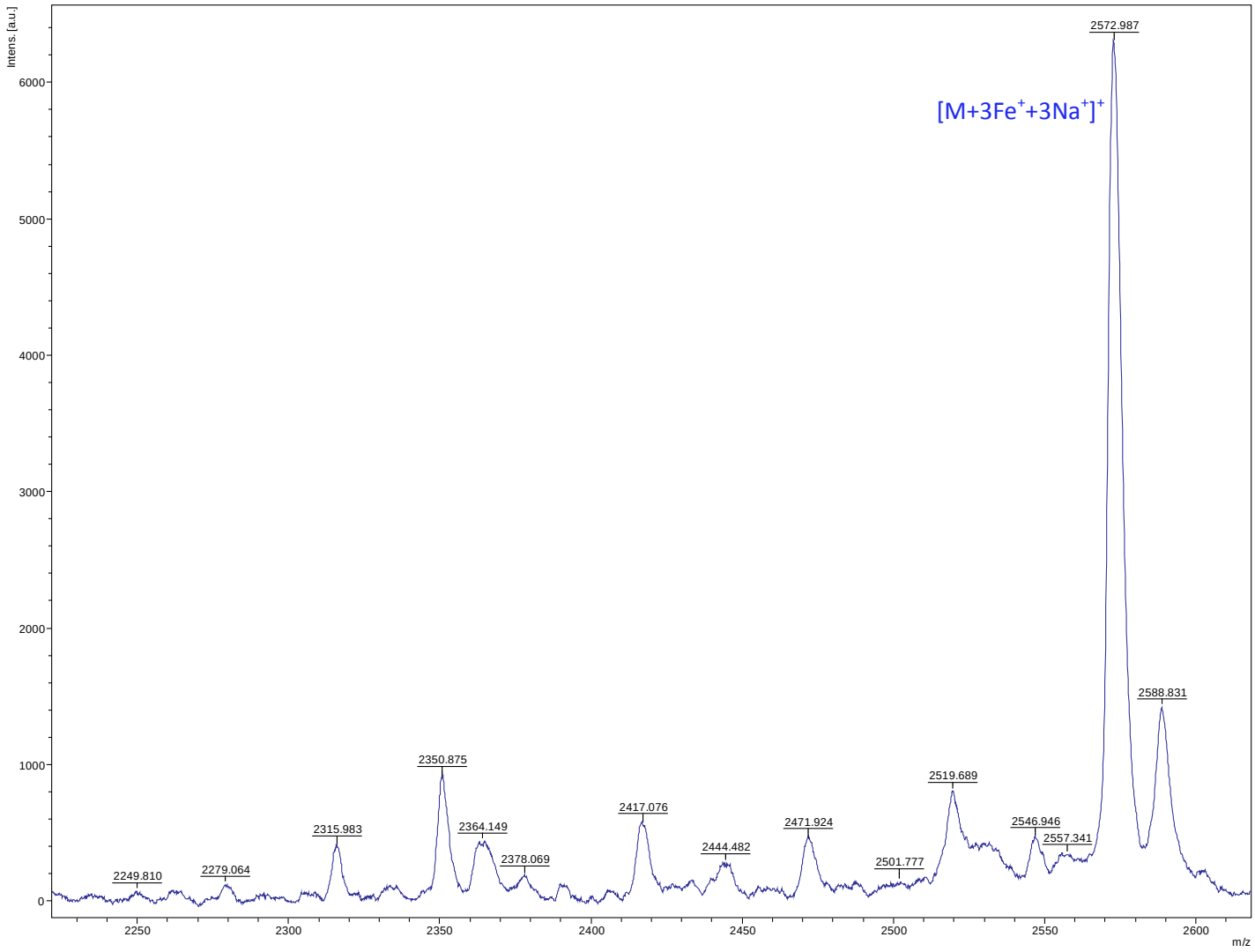


Figure S19. MALDI mass spectrum of isu114-60/86 (**9**)

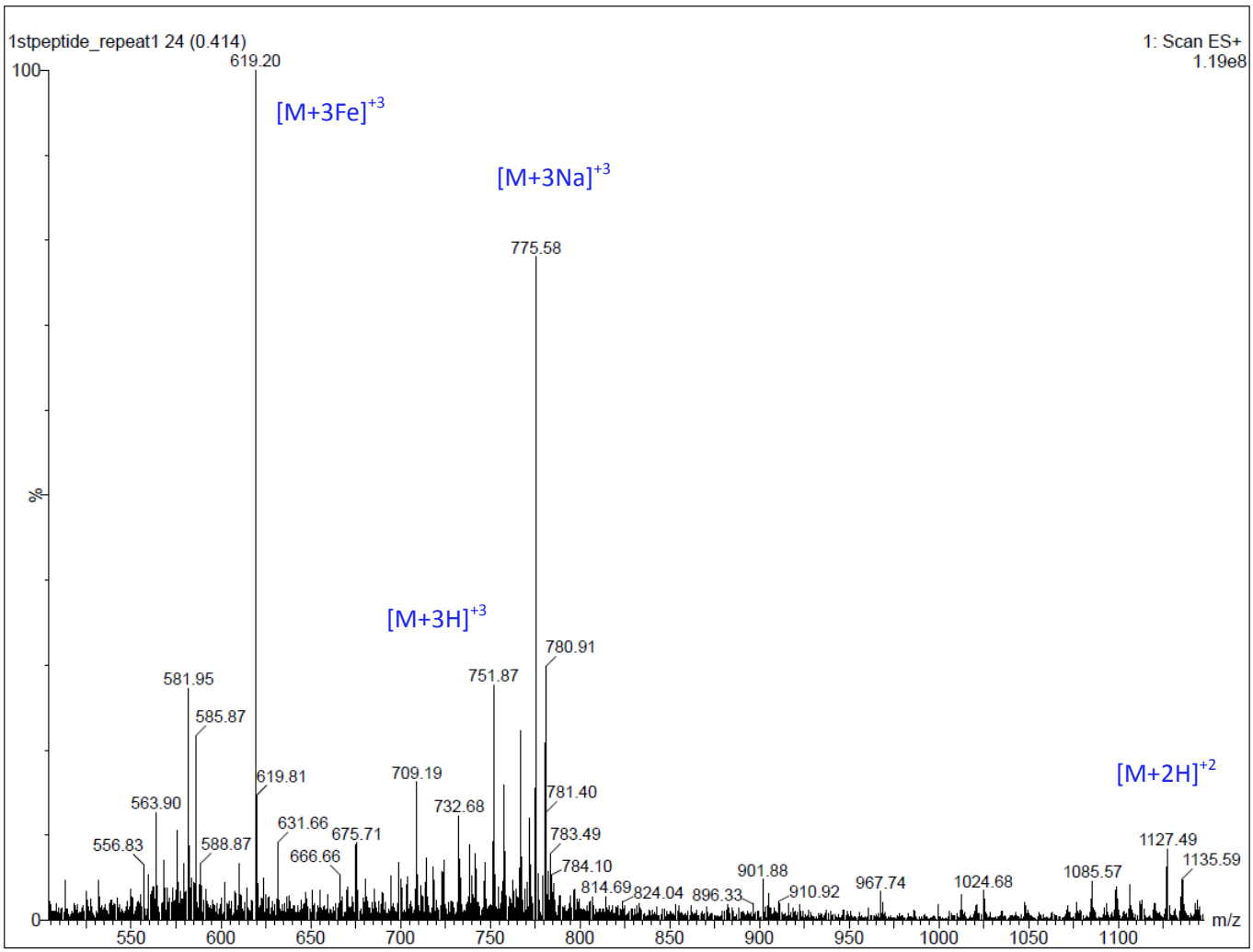


Figure S20. LC-MS mass spectrum of isu1140-59 (**10)**

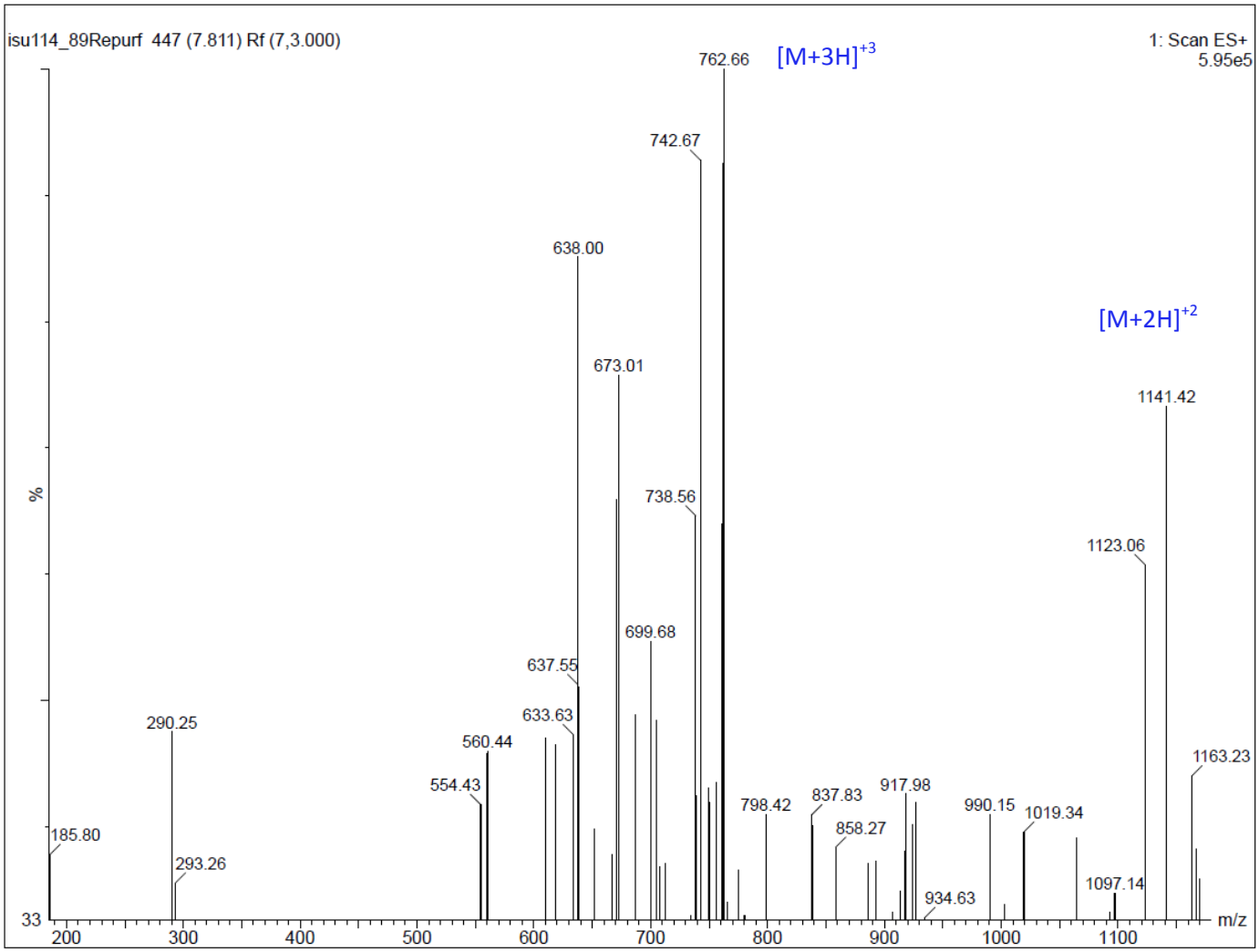


Figure S21. LC-MS mass spectrum of isu114-89 (**11)**

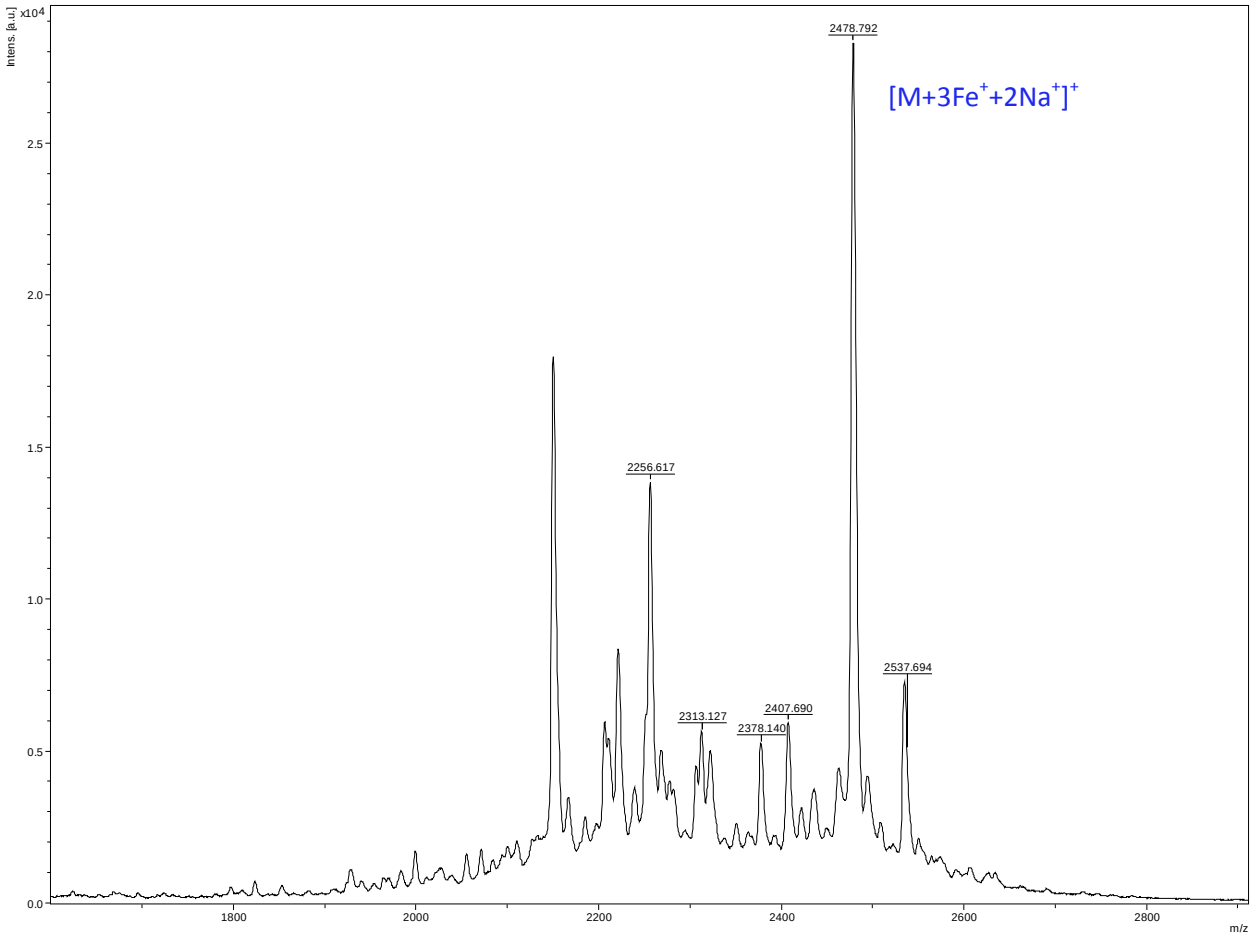


Figure S22. MALDI mass spectrum of isu114-89 (**11**)

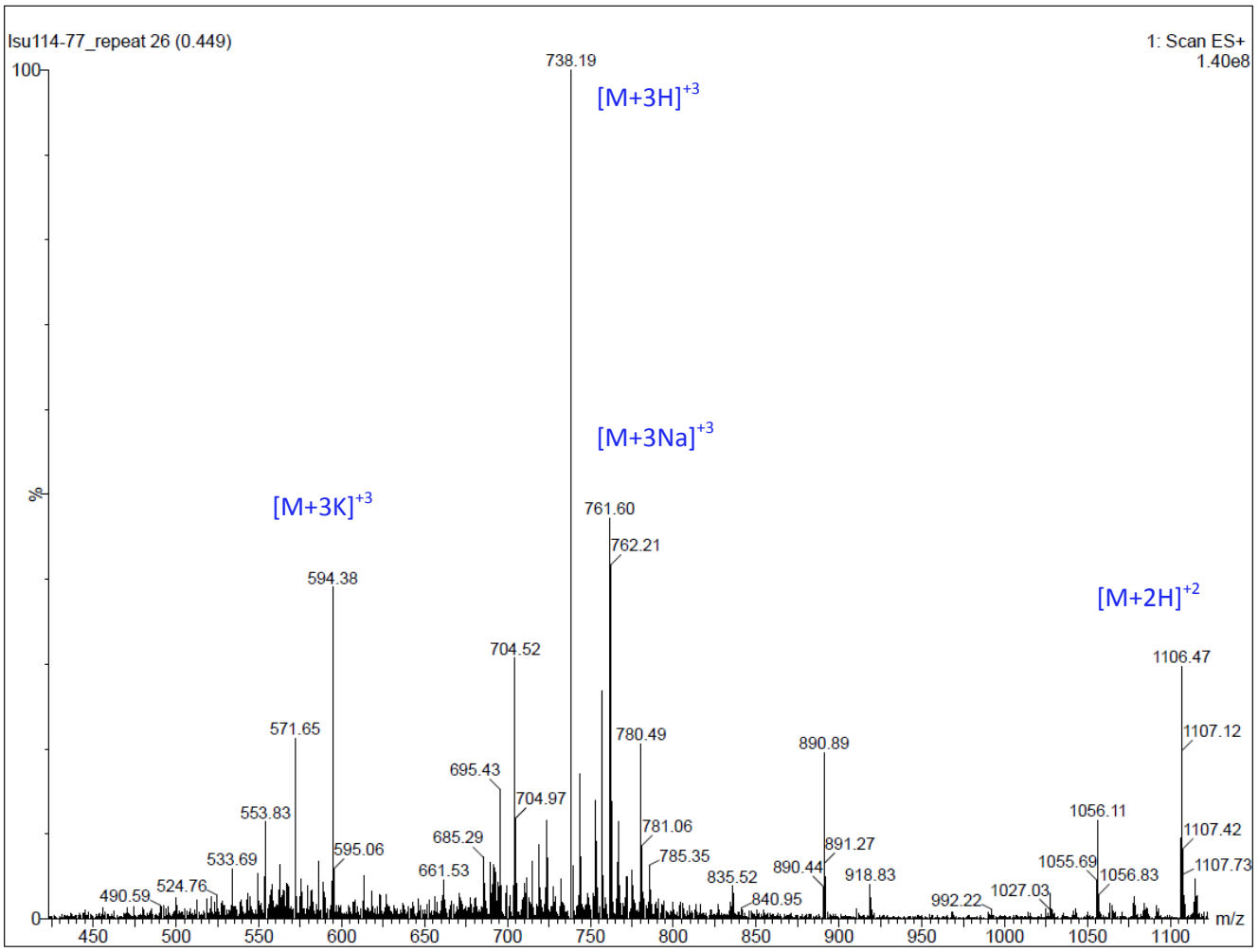


Figure S23. LC-MS mass spectrum of isu114-77 (**12**)

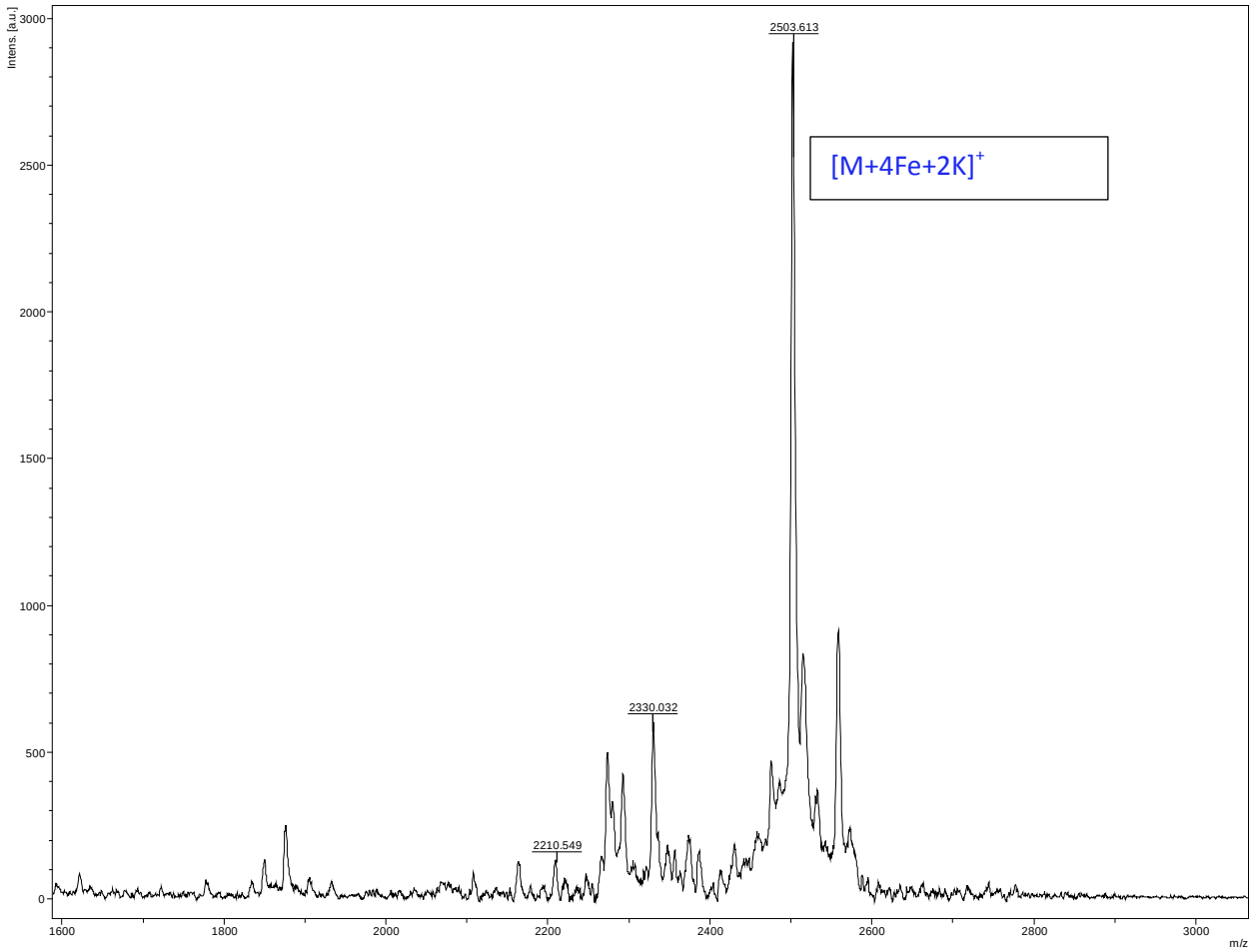


Figure S24. MALDI mass spectrum of isu114-77 (**12**)

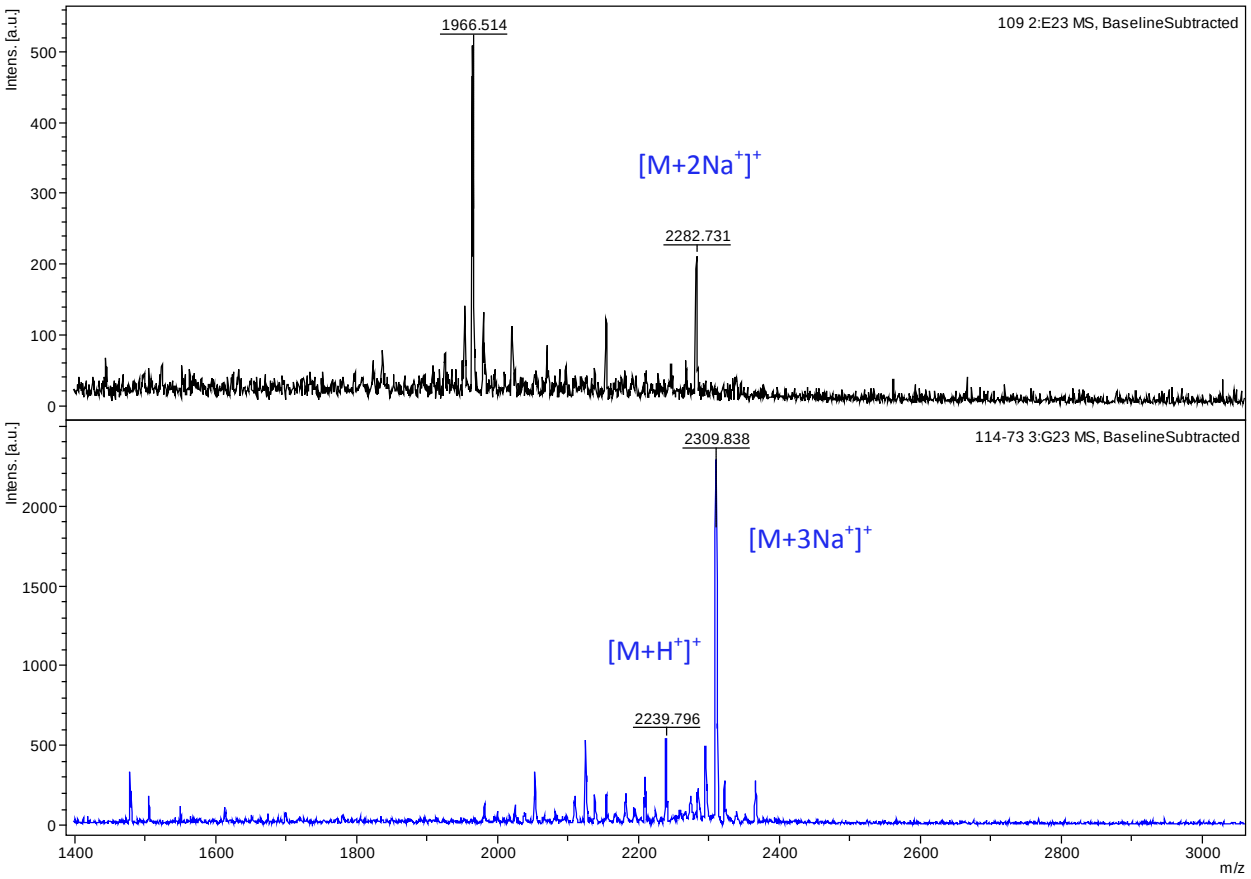


Figure S25. MALDI mass spectrum of isu114-73 (**13**)

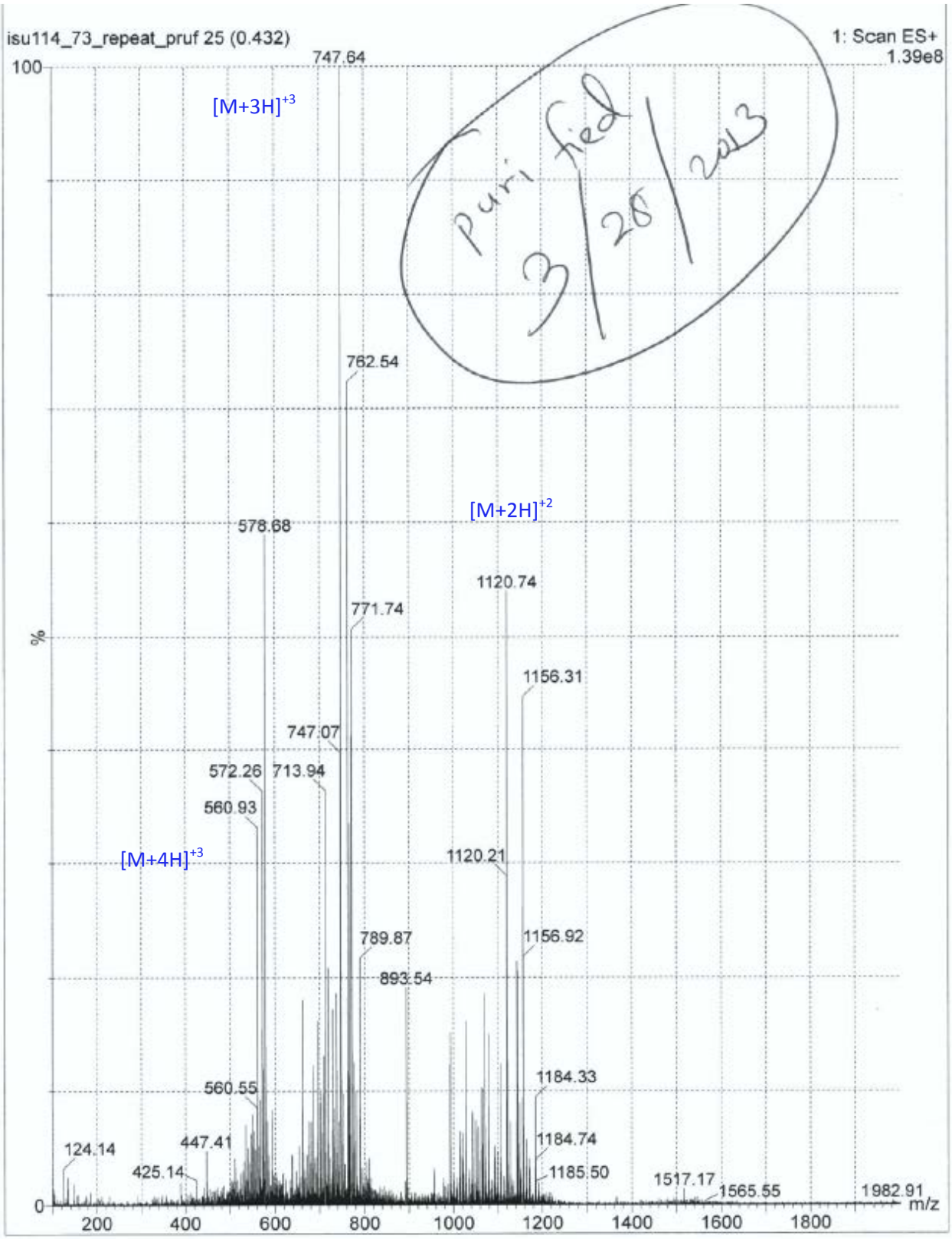


Figure S26. LC-MS mass spectrum of isu114-73 (**13**)

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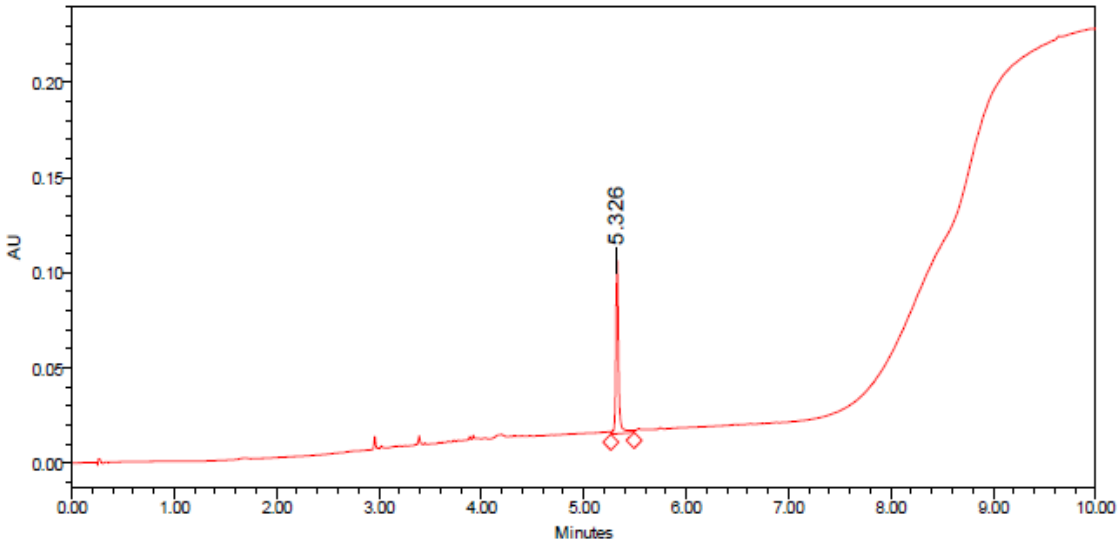
Untitled

SAMPLE INFORMATION

Sample Name:	isu114-106	Acquired By:	System
Sample Type:	Unknown	Sample Set Name	isu114_106
Vial:	1:A,1	Acq. Method Set:	isuru_10_90_10min
Injection #:	1	Processing Method	Default
Injection Volume:	10.00 ul	Channel Name:	254.0nm
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	PDA 254.0 nm
Date Acquired:	7/30/2013 1:21:19 PM EDT		
Date Processed:	10/14/2013 8:22:14 PM EDT		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1		5.326	168919	91047		

PDA Result Table

	Name	RT	Purity1 Angle	Purity1 Threshold	Match1 Spect. Name	Match1 Angle	Match1 Threshold
1		5.326					

Reported by User: System
Report Method: Untitled
Report Method ID: 128
Page: 1 of 1

Project Name: Isuru2
Date Printed:
10/14/2013
8:22:22 PM US/Eastern

Figure S27. UPLC chromatogram of the isu114-106 (**6**)

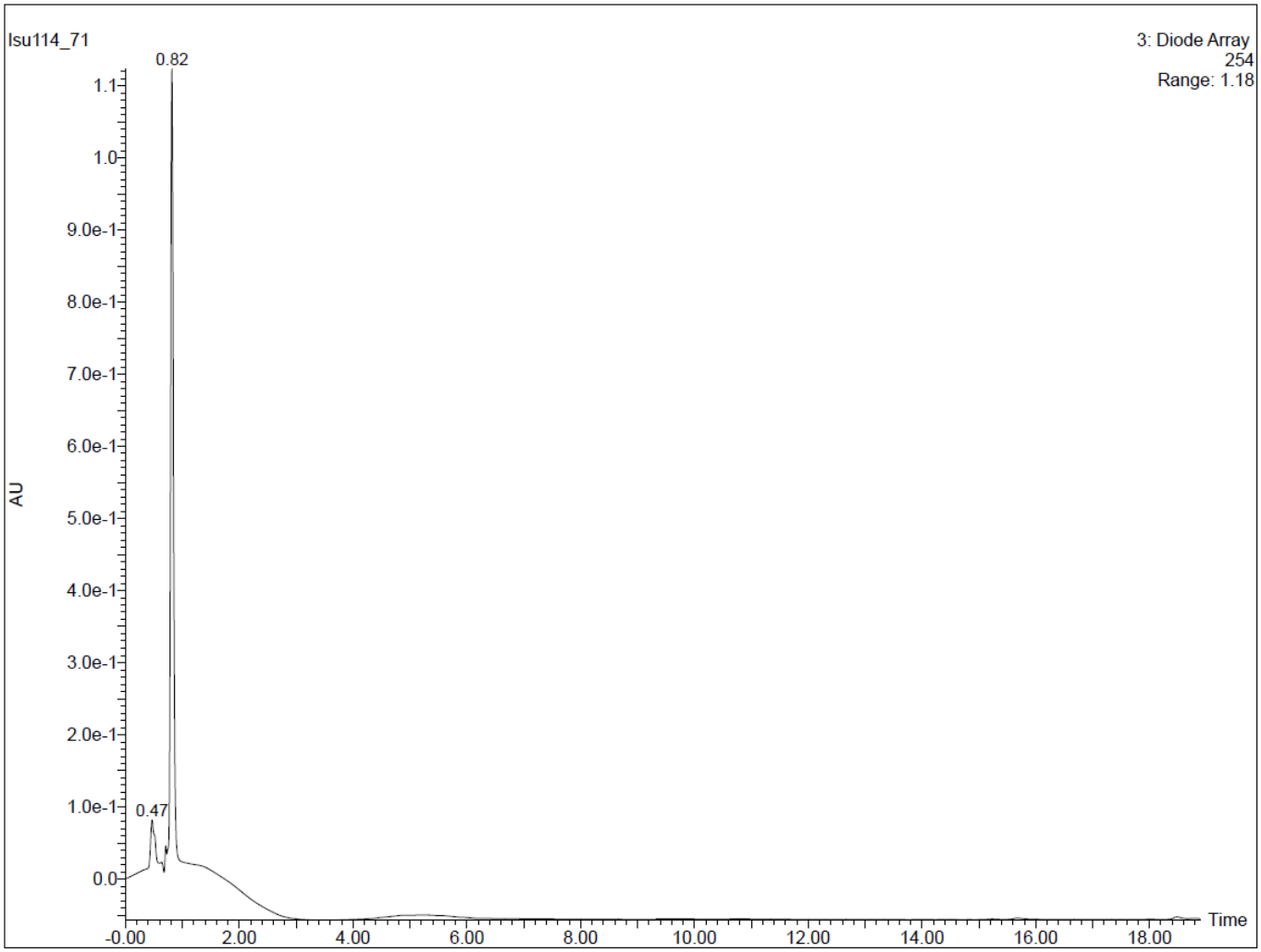


Figure S28. LC-MS chromatogram of the Isu114-71 (**7**)

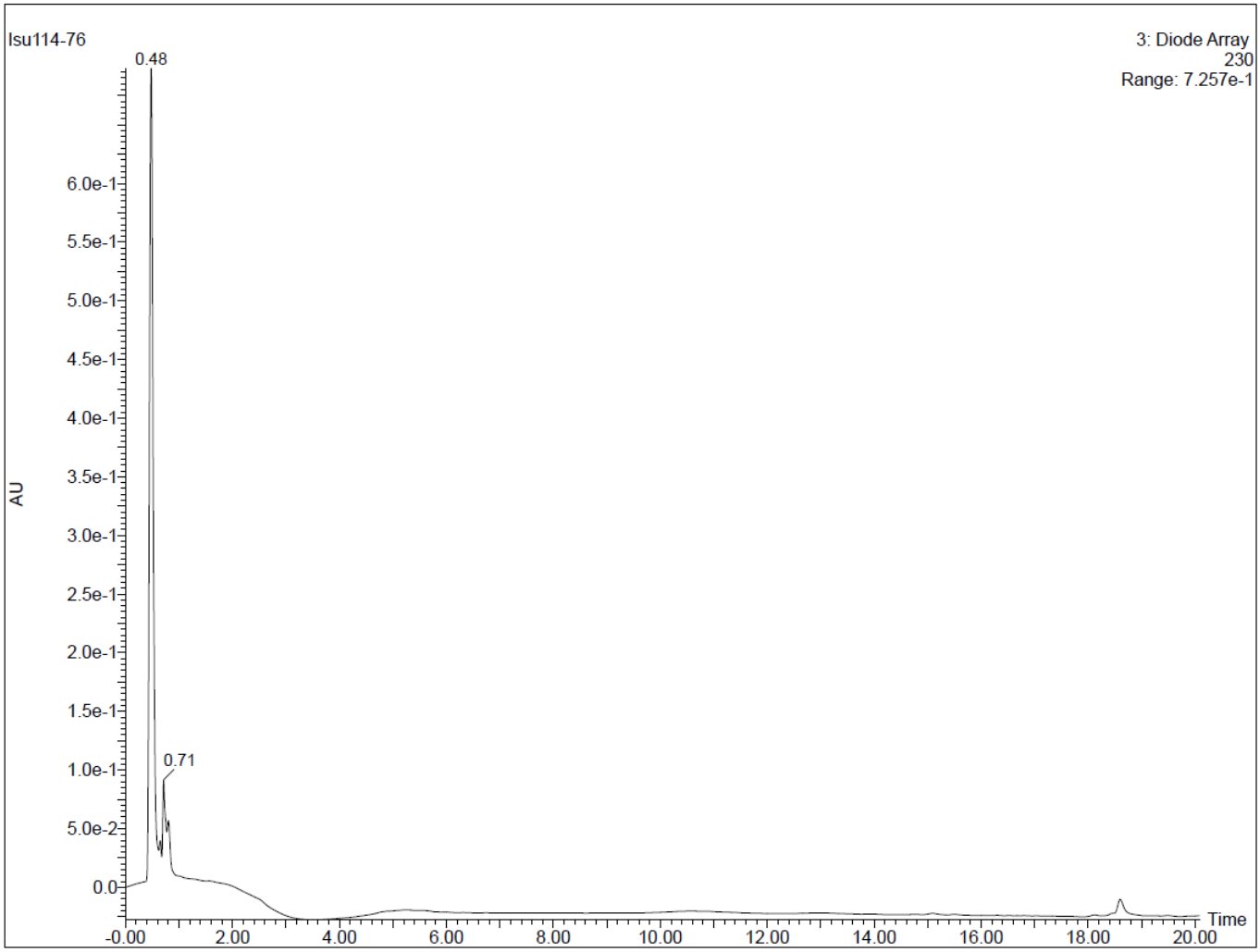


Figure S29. LC-MS chromatogram of the Isu114-76 (**8**)

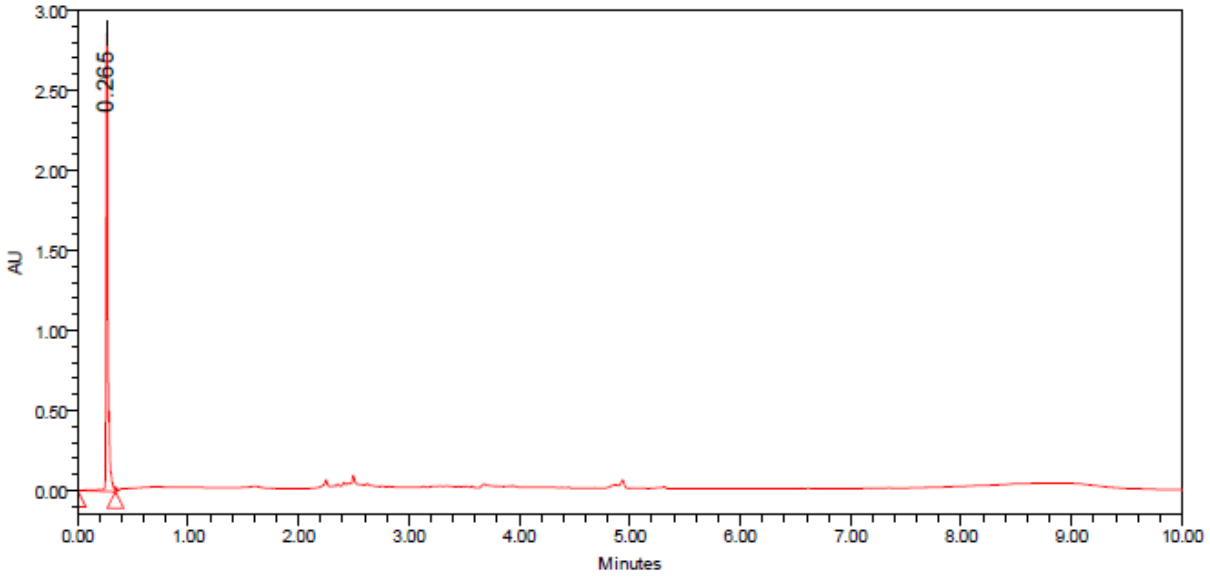
Empower 3 SOFTWARE

Untitled

SAMPLE INFORMATION

Sample Name:	isu114_76_repeat	Acquired By:	System
Sample Type:	Unknown	Sample Set Name	isu114_76
Vial:	1:A,1	Acq. Method Set:	isuru_10_90_10min
Injection #:	1	Processing Method	Default
Injection Volume:	10.00 ul	Channel Name:	230.0nm@1
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	PDA 230.0 nm
Date Acquired:	2/15/2013 10:46:56 AM EST		
Date Processed:	10/14/2013 7:56:50 PM EDT		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1		0.265	3519129	2863412		

PDA Result Table

	Name	RT	Purity1 Angle	Purity1 Threshold	Match1 Spect. Name	Match1 Angle	Match1 Threshold
1		0.265					

Reported by User: System
Report Method: Untitled
Report Method ID: 128
Page: 1 of 1

Project Name: Isuru2
Date Printed:
10/14/2013
7:57:02 PM US/Eastern

Figure S30. UPLC chromatogram of the isu114-76 (**8**)

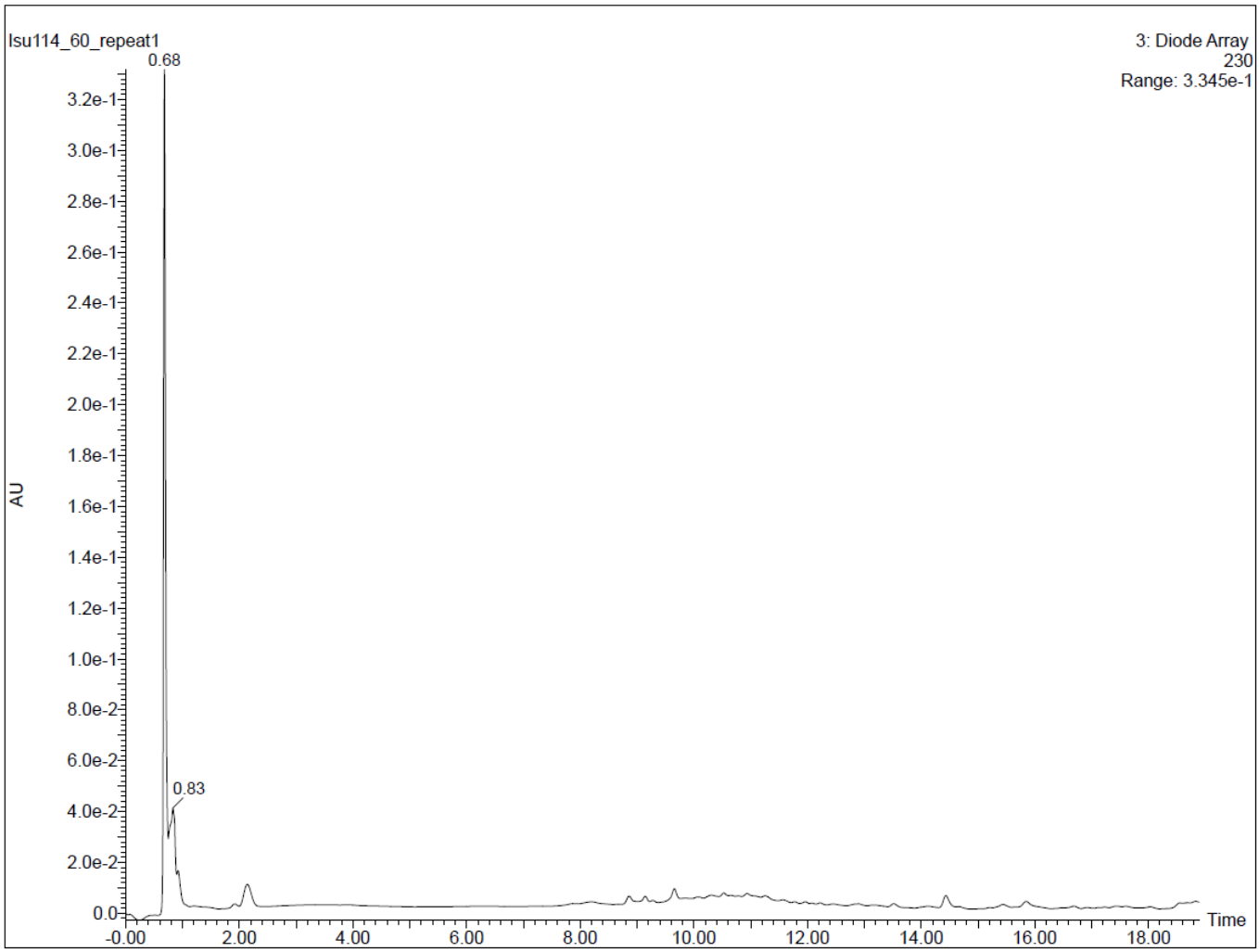


Figure S31. LC-MS chromatogram of the isu114-60 (**9**)

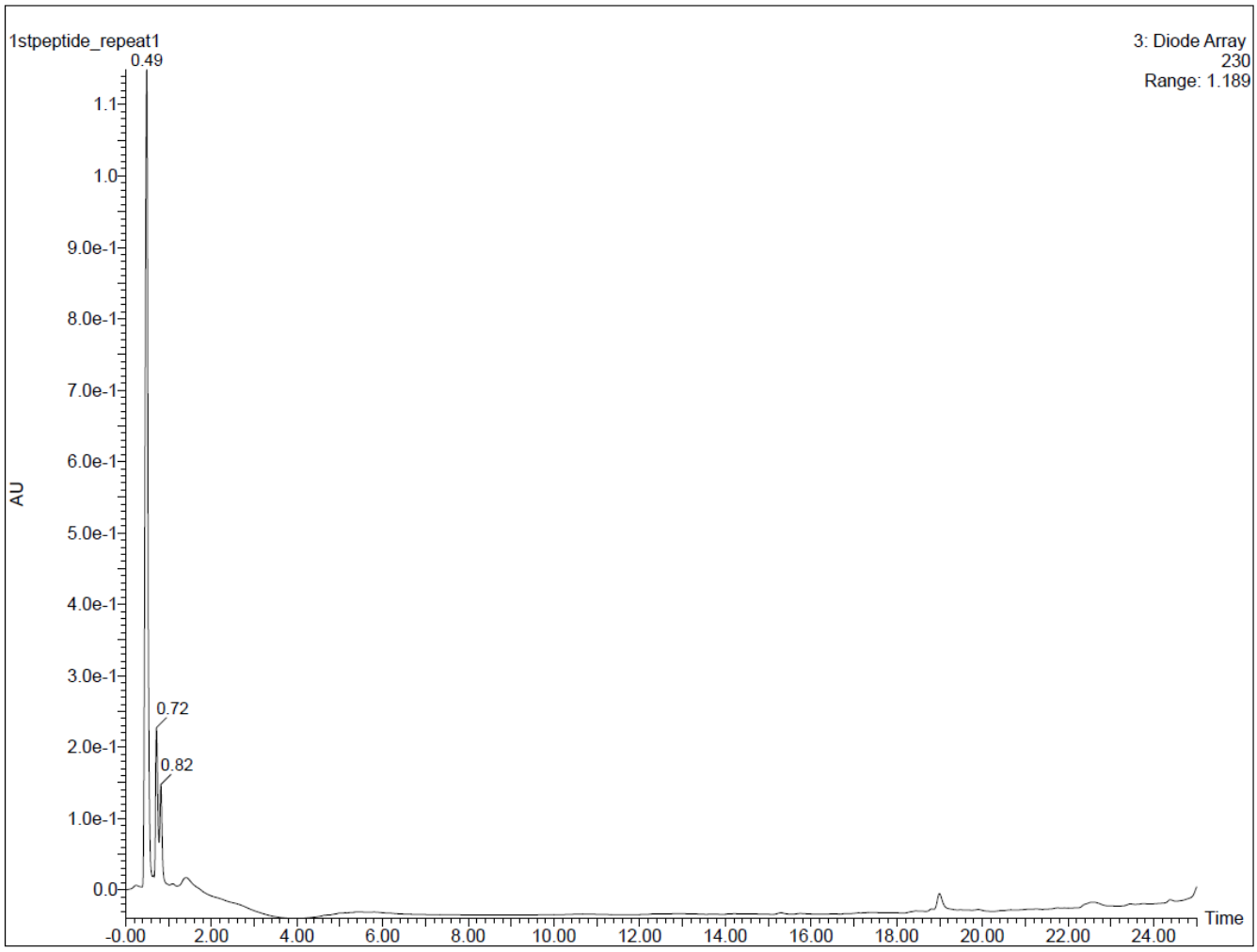


Figure S32. LC-MS chromatogram of the isu114-59 (**10**)

Empower™3 SOFTWARE

Untitled

SAMPLE INFORMATION

Sample Name:	isu114-59_repeat	Acquired By:	System
Sample Type:	Unknown	Sample Set Name	isu114_59
Vial:	1:A,1	Acq. Method Set:	isuru_10_90_10min
Injection #:	1	Processing Method	Default
Injection Volume:	10.00 ul	Channel Name:	230.0nm@1
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	PDA 230.0 nm
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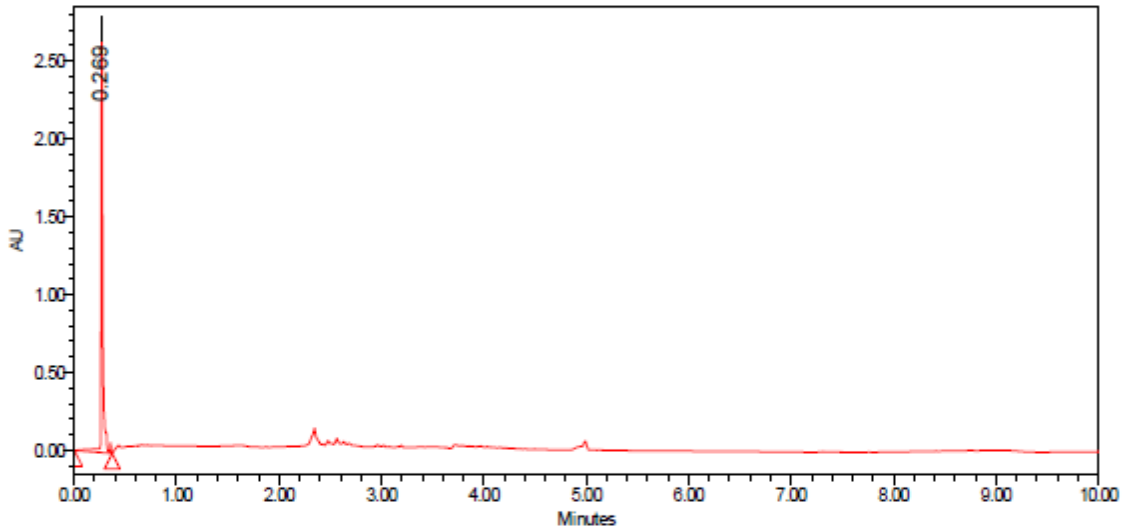
Date Processed:	10/14/2013 7:58:24 PM EDT		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
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PDA Result Table

	Name	RT	Purity1 Angle	Purity1 Threshold	Match1 Spect. Name	Match1 Angle	Match1 Threshold
1		0.269					

Reported by User: System
Report Method: Untitled
Report Method ID: 128
Page: 1 of 1

Project Name: Isuru2
Date Printed:
10/14/2013
7:58:34 PM US/Eastern

Figure S33. UPLC chromatogram of the isu114-59 (**10**)

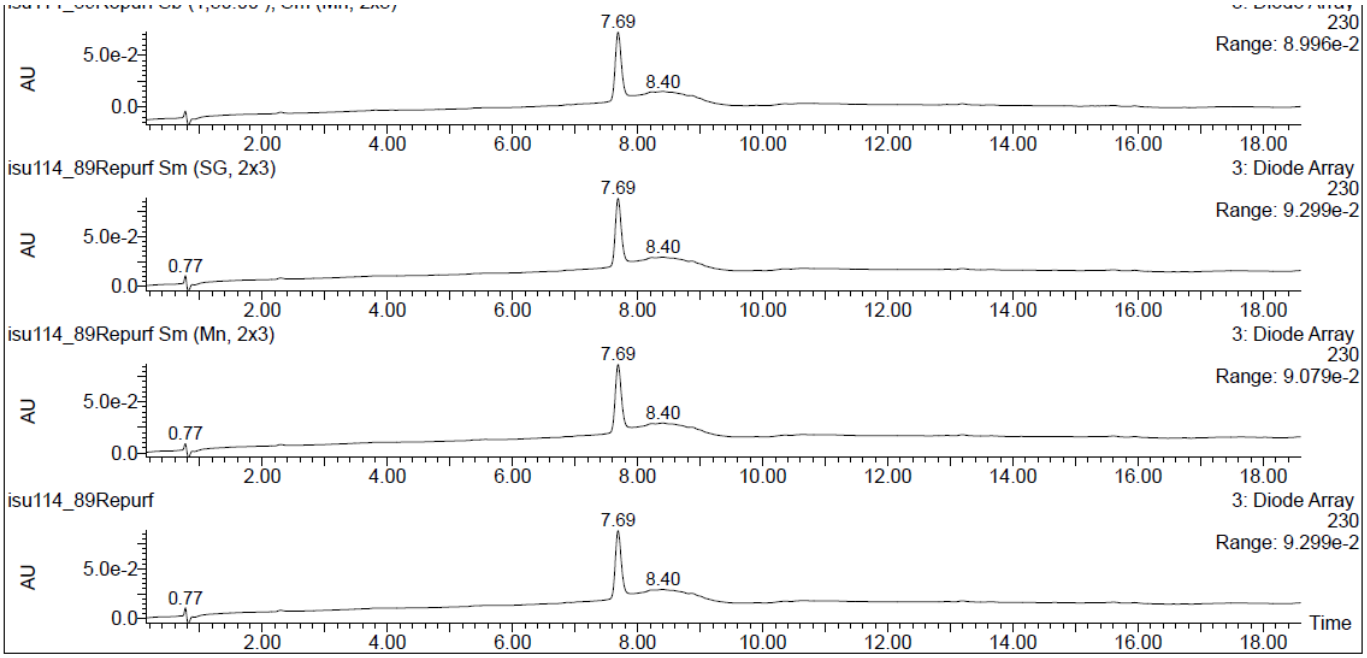

Figure S34. LC-MS chromatogram of the isu114-89 (**11**)

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Untitled

SAMPLE INFORMATION

Sample Name:	isu114_89_v1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name	Isu_all
Vial:	1:A,2	Acq. Method Set:	isuru_10_90_10min
Injection #:	1	Processing Method	Default
Injection Volume:	10.00 ul	Channel Name:	310.0nm
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	PDA 310.0 nm
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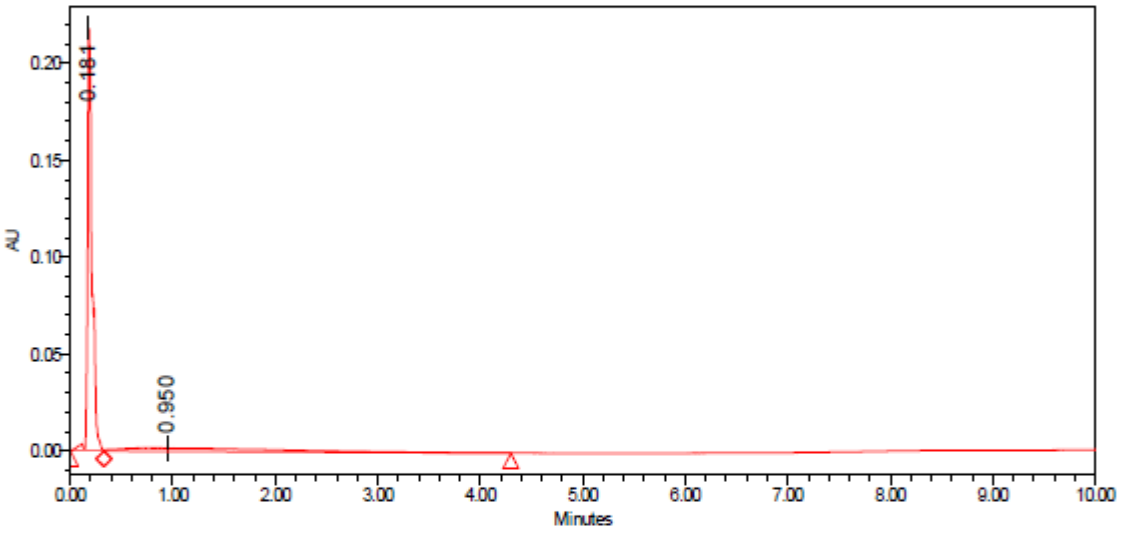
Date Processed:	10/14/2013 8:14:07 PM EDT		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1		0.181	668149	218472		
2		0.950	191240	1688		

PDA Result Table

	Name	RT	Purity1 Angle	Purity1 Threshold	Match1 Spect. Name	Match1 Angle	Match1 Threshold
1		0.181					
2		0.950					

Reported by User: System
Report Method: Untitled
Report Method ID: 128
Page: 1 of 1

Project Name: Isuru2
Date Printed:
10/14/2013
8:14:24 PM US/Eastern

Figure S35. UPLC chromatogram of the isu114-89 (**11**)

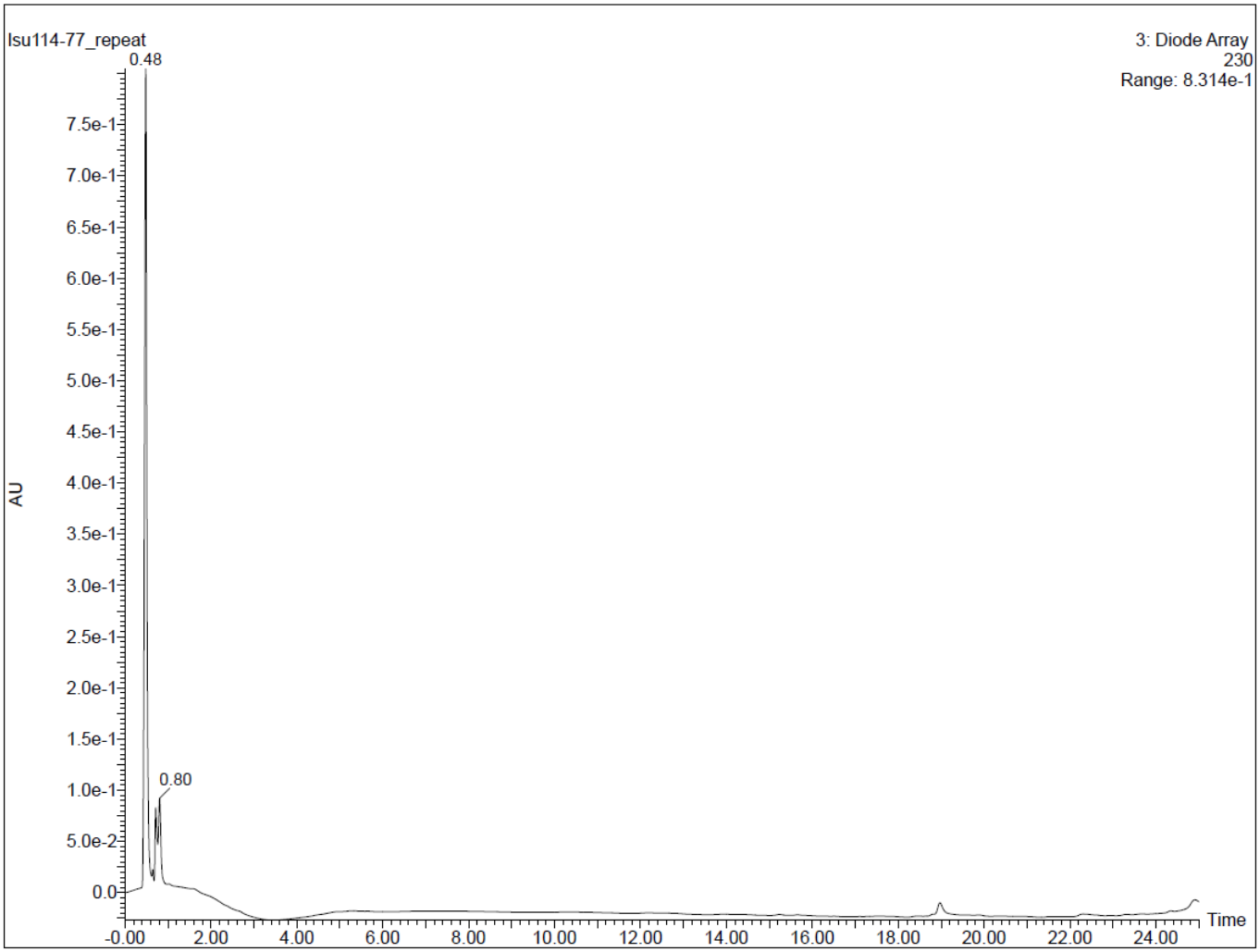


Figure S36. LC-MS chromatogram of the Isu114-77 (**12**)

Empower 3 SOFTWARE

Untitled

SAMPLE INFORMATION

Sample Name:	isu114_73_v1	Acquired By:	System
Sample Type:	Unknown	Sample Set Name	isu114_73
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Injection #:	1	Processing Method	Default
Injection Volume:	10.00 ul	Channel Name:	254.0nm
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	PDA 254.0 nm
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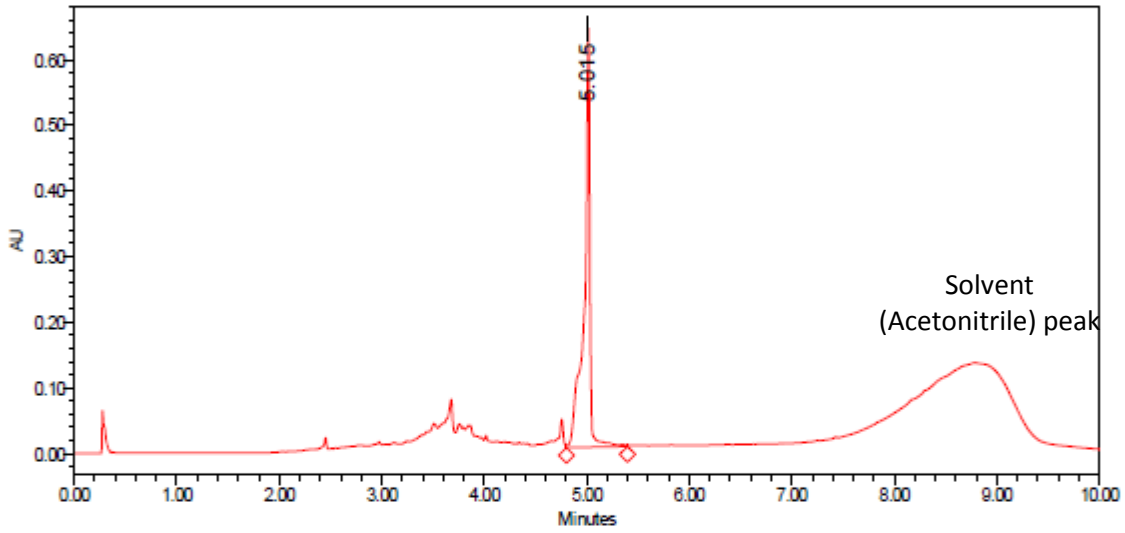
Date Processed:	10/14/2013 8:08:13 PM EDT		

Auto-Scaled Chromatogram



Peak Results

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PDA Result Table

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1		5.015					

Reported by User: System
Report Method: Untitled
Report Method ID: 128
Page: 1 of 1

Project Name: Isuru2
Date Printed:
10/14/2013
8:08:28 PM US/Eastern

Figure S37. UPLC chromatogram of the isu114-73 (**13**)