The supporting information of the article entitled:

Structure-Based Discovery of SD-36 as a Potent, Selective and Efficacious PROTAC Degrader of STAT3 Protein

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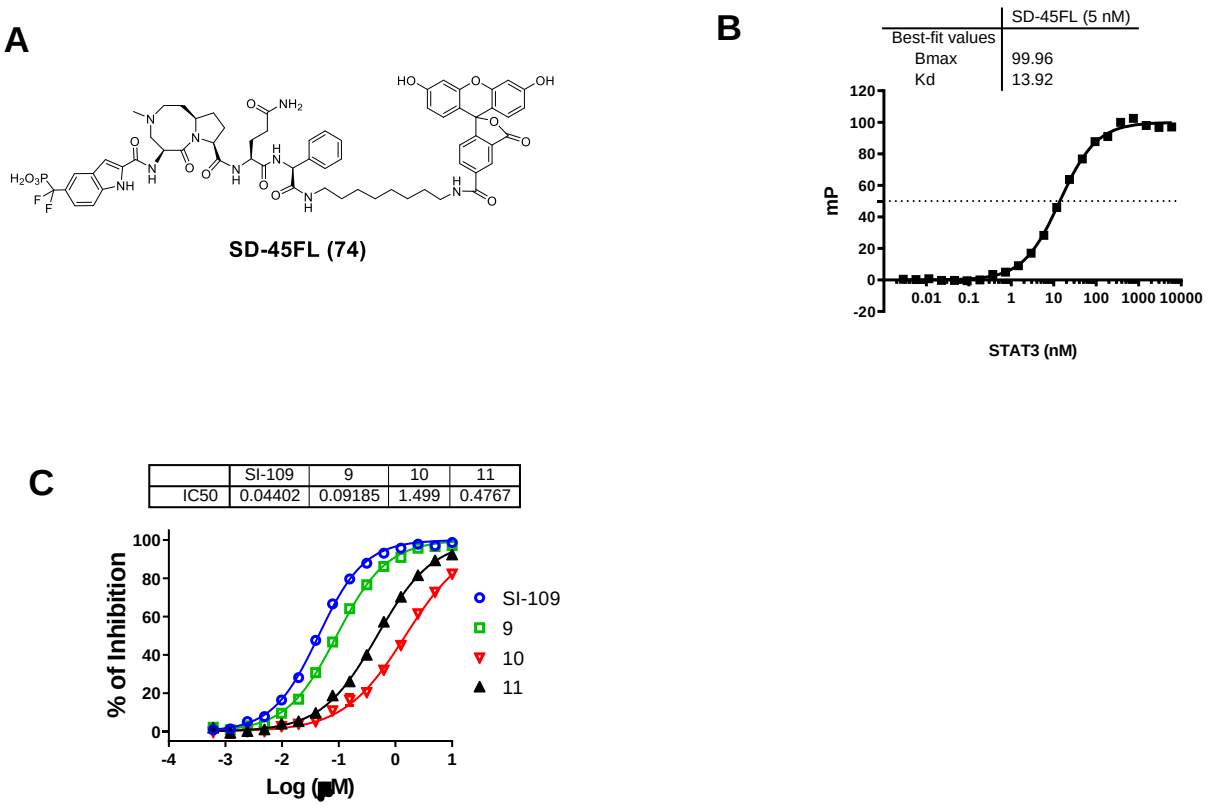


Figure S1. FP assay for the binding of STAT3 SH2 domain inhibitors to purified recombinant STAT3 protein. (A). Fluorescently labeled compound **SD-45FL**. (B). Binding isotherm of **SD-45FL** to STAT3. Fluorescence polarization (FP) saturation binding curve of **SD-45FL** (5 nM) incubated with serial diluted recombinant STAT3 (0.006 to 6 μM). The data are normalized to the highest value for each experiment. The estimated Kd is shown. One representative saturation curve is shown. (C). Competitive binding curves and IC_{50} values of compounds **SI-109**, **9**, **10** and **11** to STAT3 as determined using the FP-based binding assay.

Table S1. Binding affinities of previously reported compound CJ-887 and new STAT3 SH2 inhibitors in a competitive FP-based binding assay.

Cmpds	IC_{50} (nM)	K_i (nM)
1 (CJ-887)	240 ± 12	47 ± 35
2	105 ± 5	25 ± 13
3	2809 ± 155	761 ± 230
4	20 ± 1	7 ± 1
5	44 ± 11	14 ± 3
6	40 ± 0	14 ± 0
7	34 ± 1	12 ± 1
SI-109 (**8**)	42 ± 15	14 ± 4
9	92 ± 12	33 ± 5
10	1628 ± 112	584 ± 40
11	459 ± 82	164 ± 30

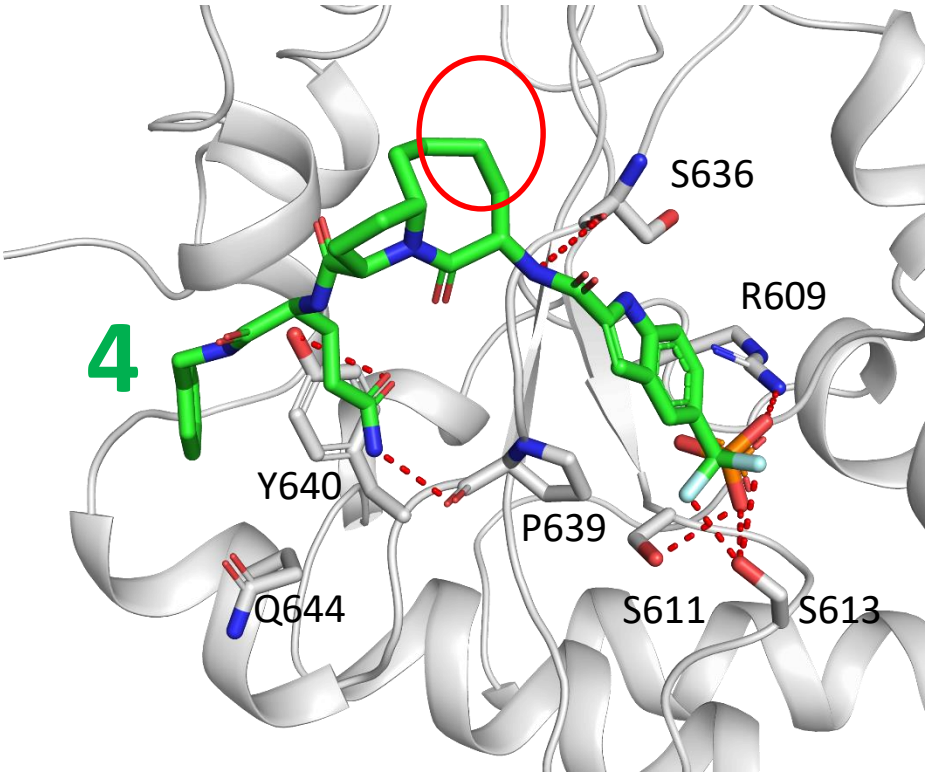


Figure S2. Computational model of compound **4** in complex with STAT3.

Table S2. Crystallography Data Collection and Refinement Statistics

Data Collection	**STAT3-SI109**
PDBID	6NUQ
Space Group	$P4_12_12$
Unit Cell (Å)	84.19 84.19 206.37
Wavelength (Å)	1.07812
Resolution (Å)[1]	3.15 (3.2-3.15)
Rsym[2]	0.109 (0.746)
$\langle I/\sigma I \rangle$[3]	2 (10)
Completeness (%)[4]	94.9 (100)
Redundancy	11.9 (12.0)
Refinement	
Resolution (Å)	3.15
R-Factor [5]	0.233
Rfree [6]	0.260
Protein atoms	4126
Ligands	1
Water Molecules	26
Unique Reflections	12837
R.m.s.d.[7]	
Bonds	0.008
Angles	0.99
MolProbity Score[8]	1.51
Clash Score[8]	2.67
RSCC[9]	0.92
RSR[9]	0.20

[1]Statistics for highest resolution bin of reflections in parentheses.

[2]$R_{sym} = \Sigma_h \Sigma_j \mid I_{hj} - \langle I_h \rangle \mid / \Sigma_h \Sigma_j I_{hj}$, where I_{hj} is the intensity of observation j of reflection h and $\langle I_h \rangle$ is the mean intensity for multiply recorded reflections.

[3]Intensity signal-to-noise ratio.

[4]Completeness of the unique diffraction data.

[5]R-factor = $\Sigma_h \mid IF_oI - IF_cI \mid / \Sigma_h |F_o|$, where F_o and F_c are the observed and calculated structure factor amplitudes for reflection h.

[6]R_{free} is calculated against a 5% random sampling of the reflections that were removed before structure refinement.

[7]Root mean square deviation of bond lengths and bond angles.

[8]Chen et al. (2010) MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallographica D66:12-21.

[9]wwPDB Validation Server.

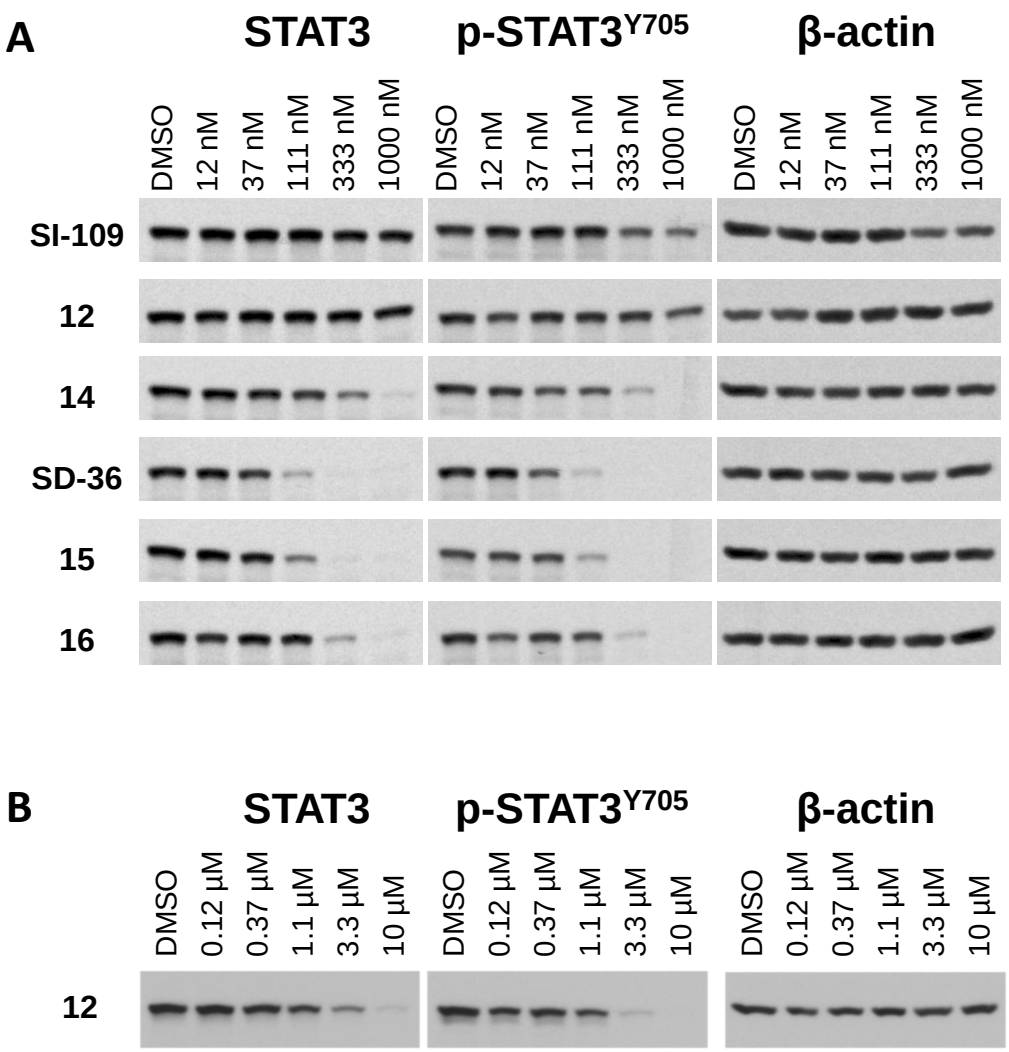


Figure S3. STAT3 degraders with various linker lengths. (A) and (B) Western blotting analysis of total STAT3 and p-STAT3^{Y705} proteins in Molm-16 cells treated with indicated compounds for 4 h.

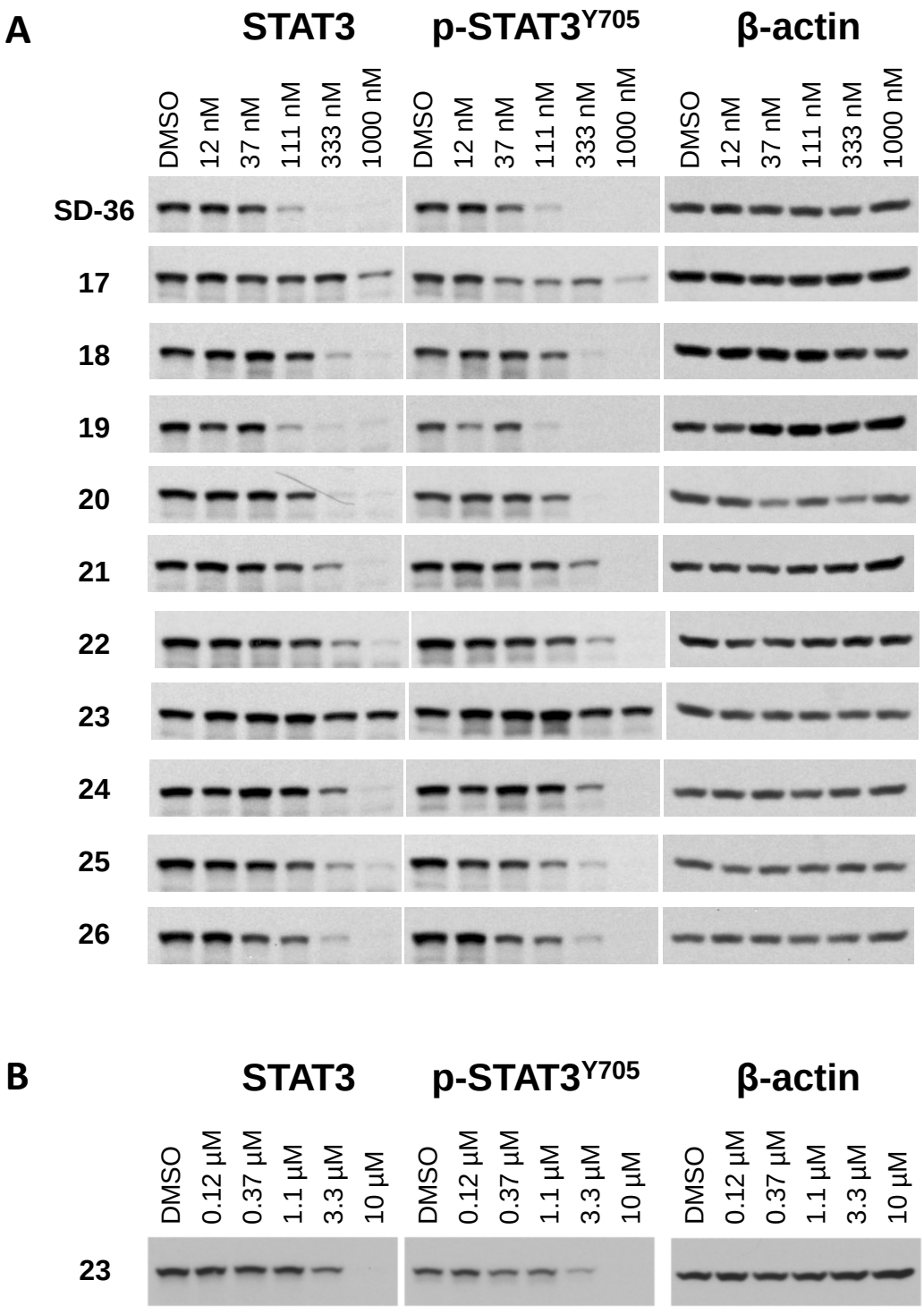


Figure S4. Western blotting analysis of total STAT3 and p-STAT3^{Y705} proteins in Molm-16 cells treated with indicated compounds for 4 h.

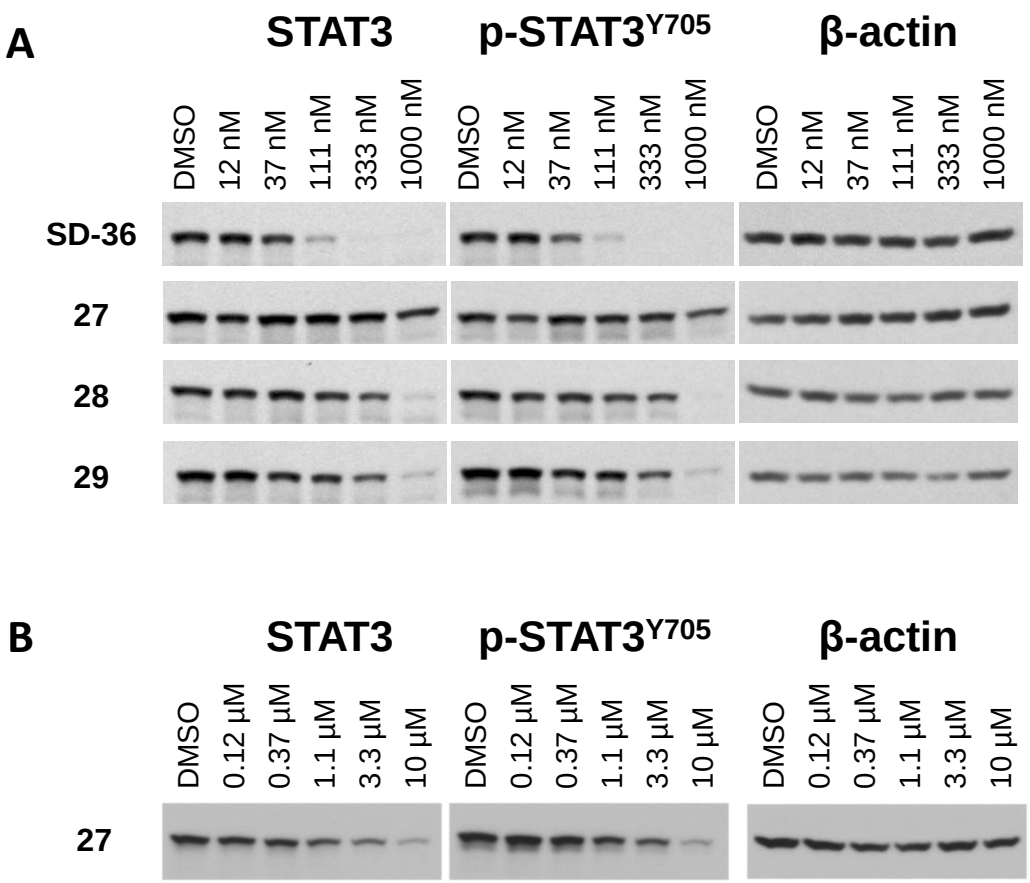


Figure S5. Western blotting analysis of total STAT3 and p-STAT3^{Y705} proteins in Molm-16 cells treated with indicated compounds for 4 h.

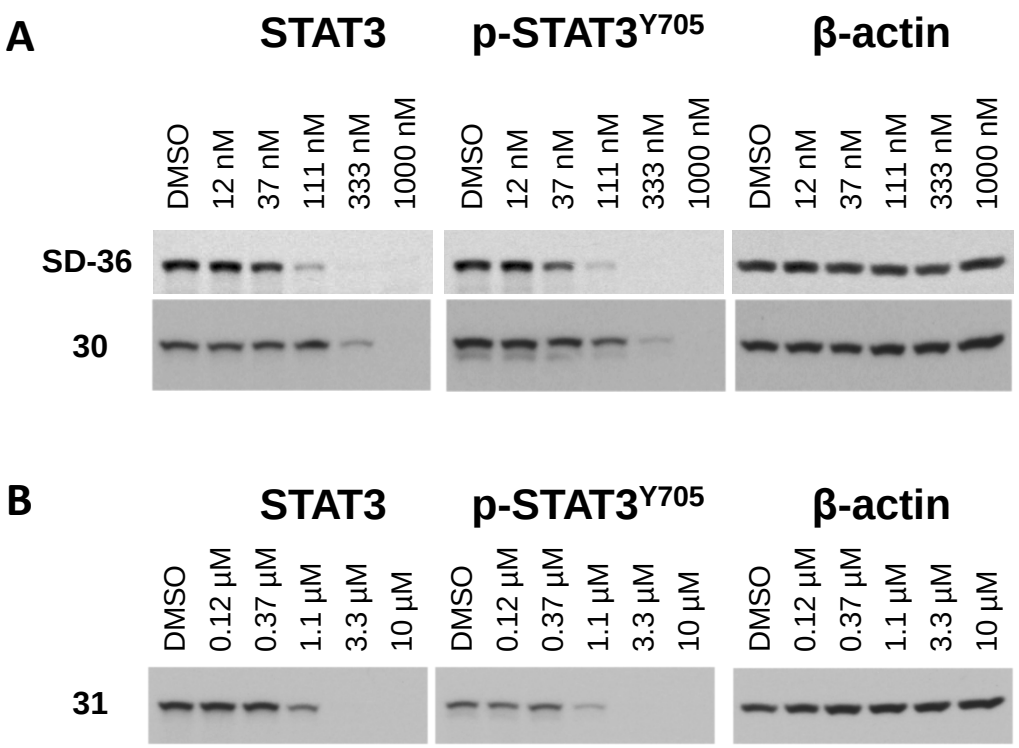


Figure S6. Western blotting analysis of total STAT3 and p-STAT3^{Y705} proteins in Molm-16 cells treated with indicated compounds for 4 h.

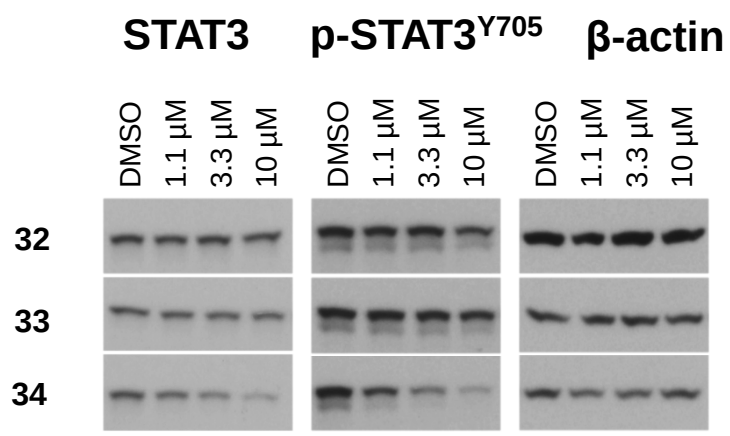


Figure S7. Western blotting analysis of total STAT3 and p-STAT3^{Y705} proteins in Molm-16 cells treated with indicated compounds for 4 h.

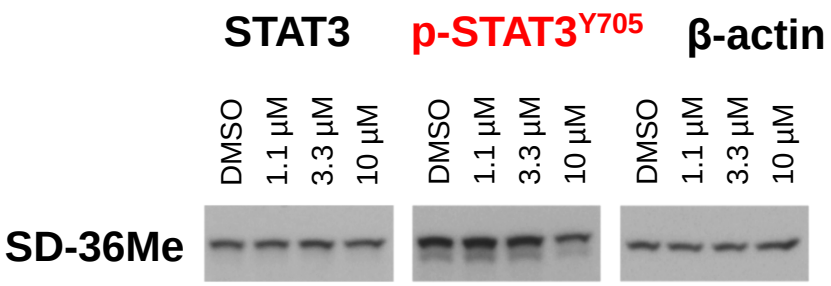


Figure S8. Western blotting analysis of total STAT3 and p-STAT3^{Y705} proteins in Molm-16 cells treated with SD-36Me for 4 h.

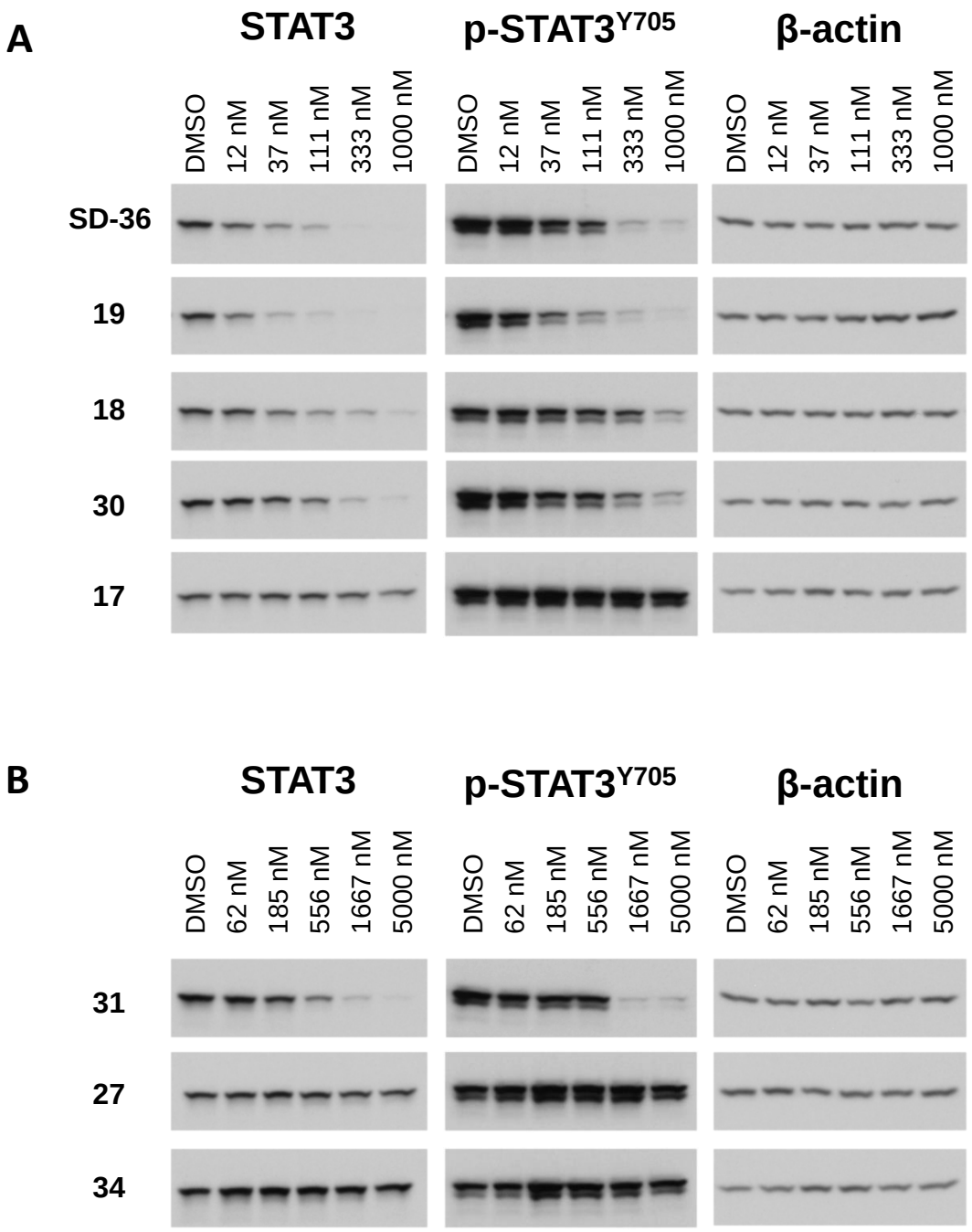
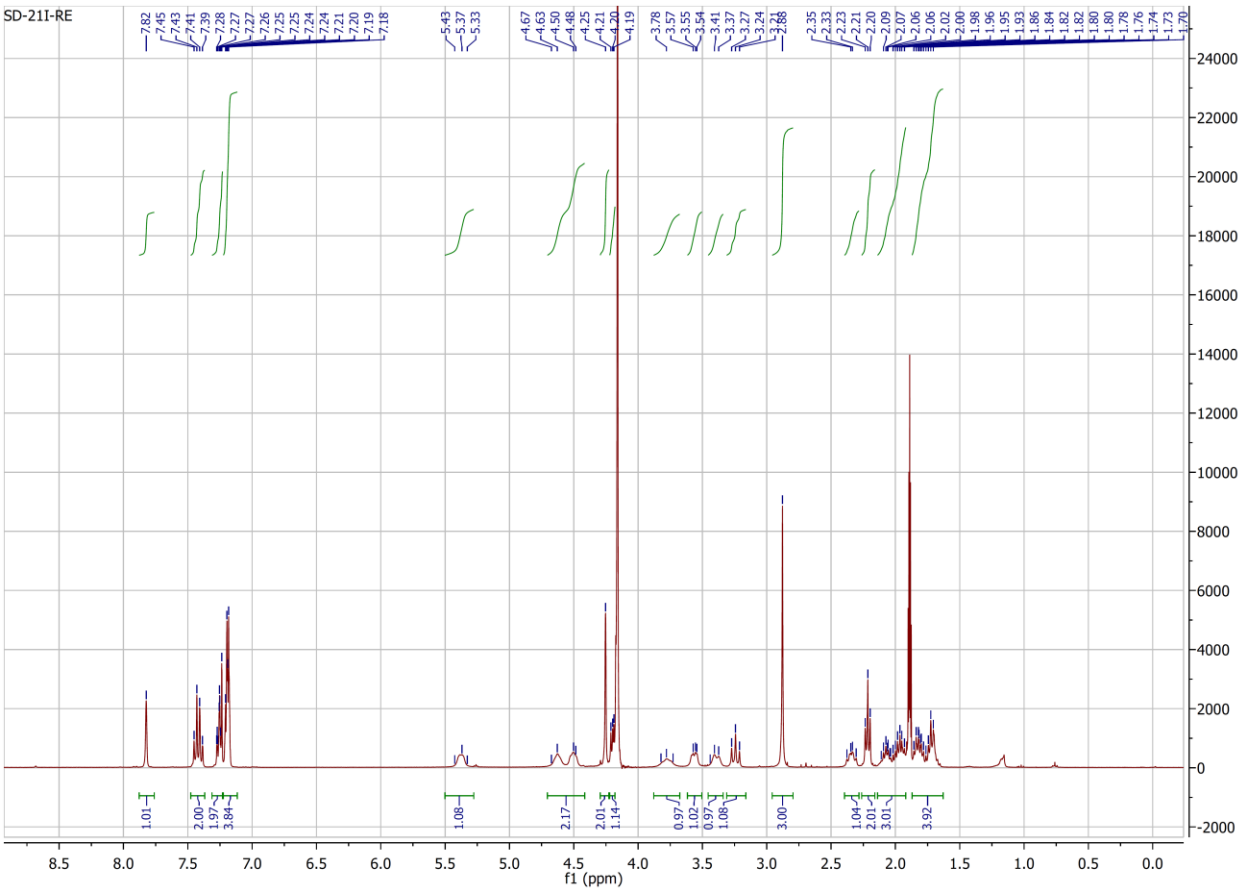


Figure S9. Western blotting analysis of total STAT3 and p-STAT3^{Y705} proteins in SU-DHL-1cell line treated with treated with representative STAT3 degraders for 16 h.

^{1}H NMR for compound **5**.

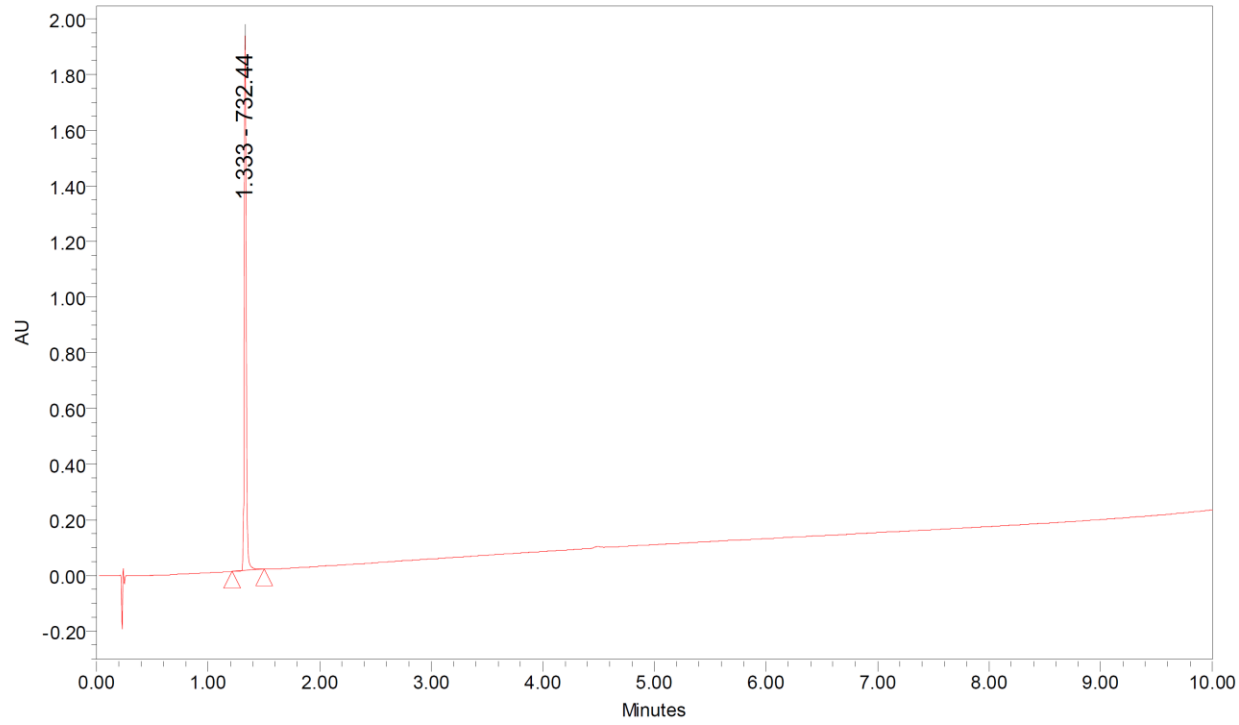


UPLC-MS analysis for compound **5**.

SAMPLE INFORMATION

Sample Name:	ZH-SD-21I	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	3/25/2019 7:30:26 PM EDT
Vial:	1:C,6	Acq. Method Set:	10to100% Bin 10 min_Delay5min
Injection #:	1	Date Processed:	3/28/2019 6:30:31 PM EDT
Injection Volume:	3.00 ul	Processing Method:	Bruce
Run Time:	10.0 Minutes	Channel Name:	230.0nm@1
Sample Set Name:	3	Proc. Chnl. Descr.:	PDA Spectrum PDA 230.0 nm (PDA

Auto-Scaled Chromatogram



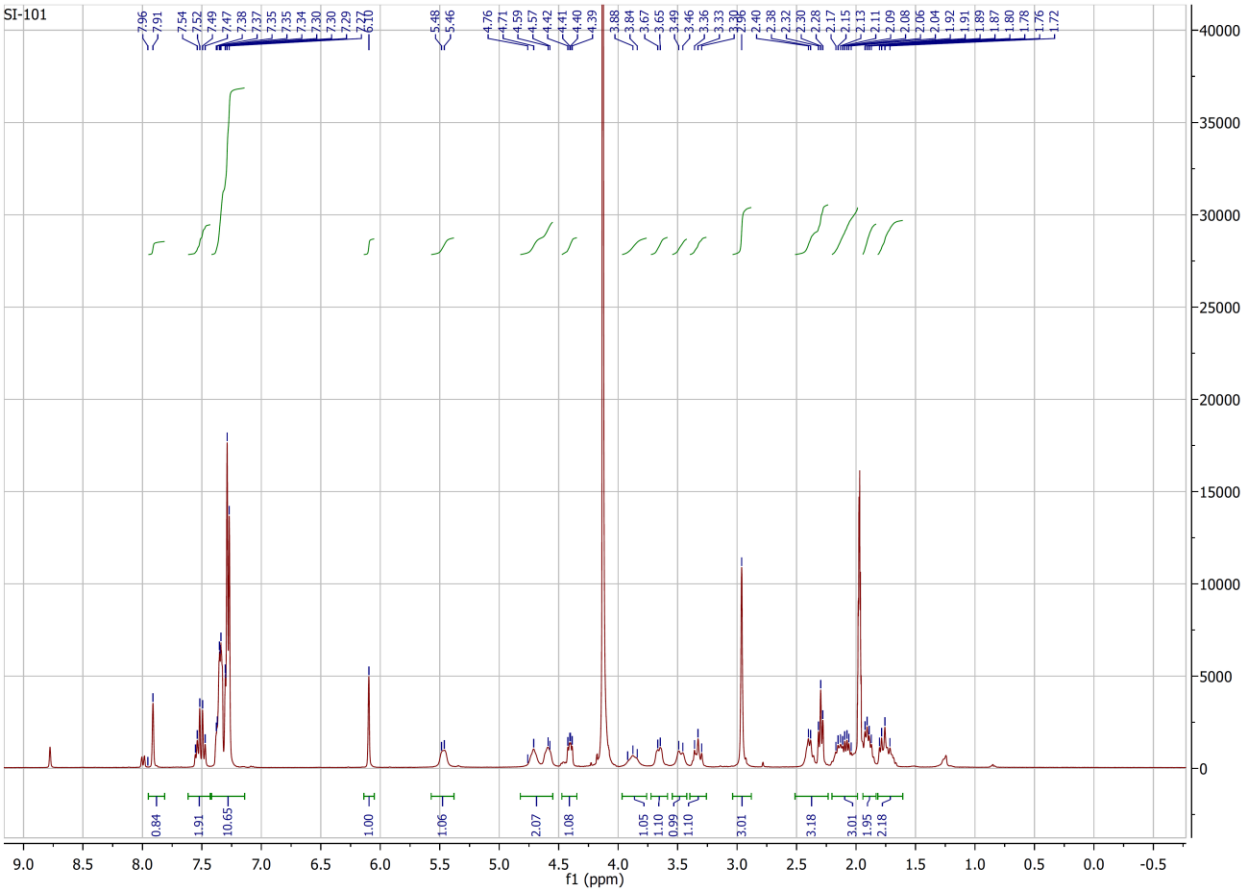
Peak Results

	RT	Area	Height	% Area
1	1.333	2436390	1921951	100.00

Peak Results

	Base Peak (m/z)
1	732.44

^{1}H NMR for compound **7**.

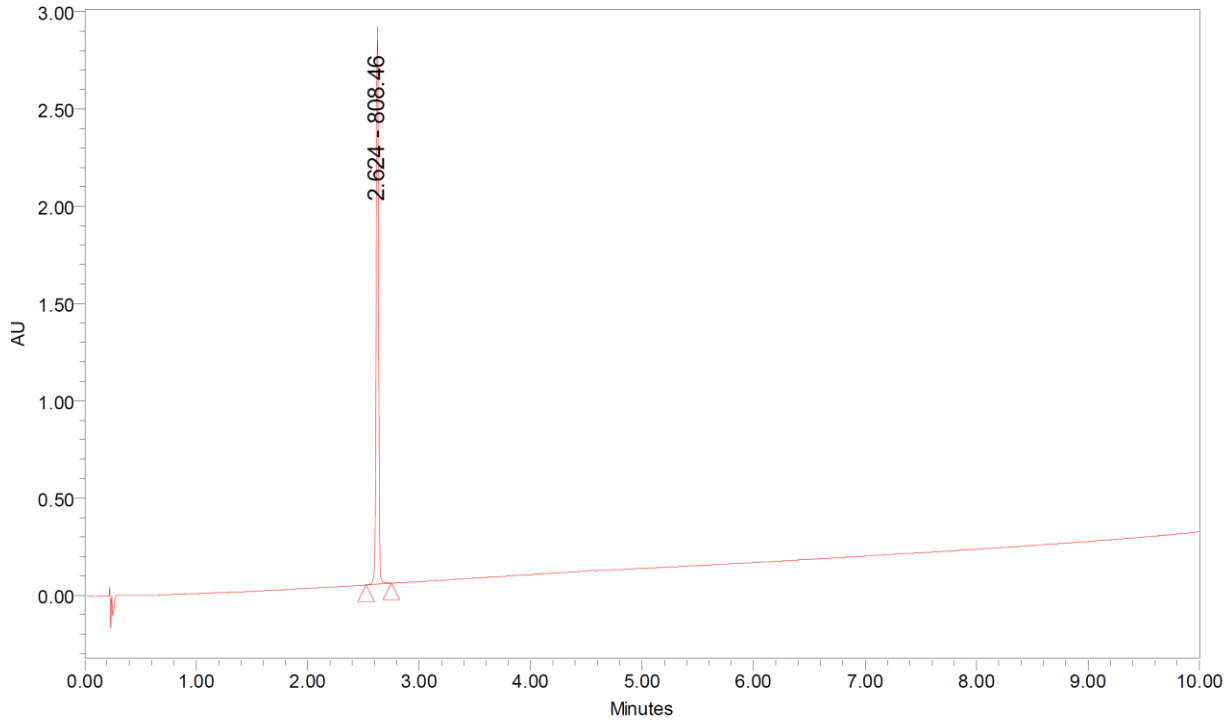


UPLC-MS analysis for compound **7**.

SAMPLE INFORMATION

Sample Name:	zh-si101	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	12/21/2018 2:30:11 PM EST
Vial:	1:A,7	Acq. Method Set:	10to100% Bin 10 min_Delay5min
Injection #:	1	Date Processed:	12/21/2018 2:47:46 PM EST
Injection Volume:	8.00 ul	Processing Method:	Bruce
Run Time:	10.0 Minutes	Channel Name:	230.0nm
Sample Set Name:	aa	Proc. Chnl. Descr.:	PDA Spectrum PDA 230.0 nm (PDA

Auto-Scaled Chromatogram



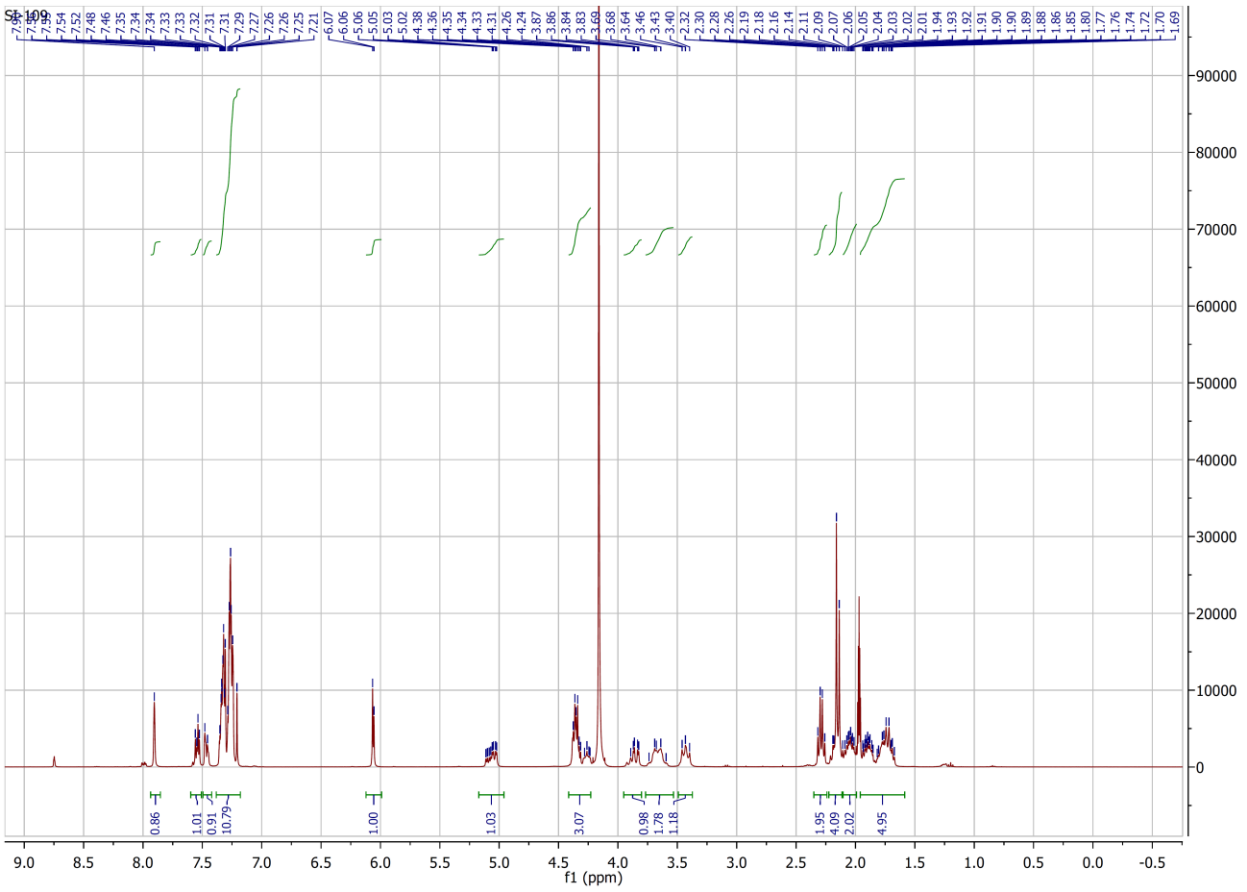
Peak Results

	RT	Area	Height	% Area
1	2.624	4182693	2803694	100.00

Peak Results

	Base Peak (m/z)
1	808.46

^{1}H NMR for SI-109 (**8**).



UPLC-MS analysis for SI-109 (**8**).

SAMPLE INFORMATION

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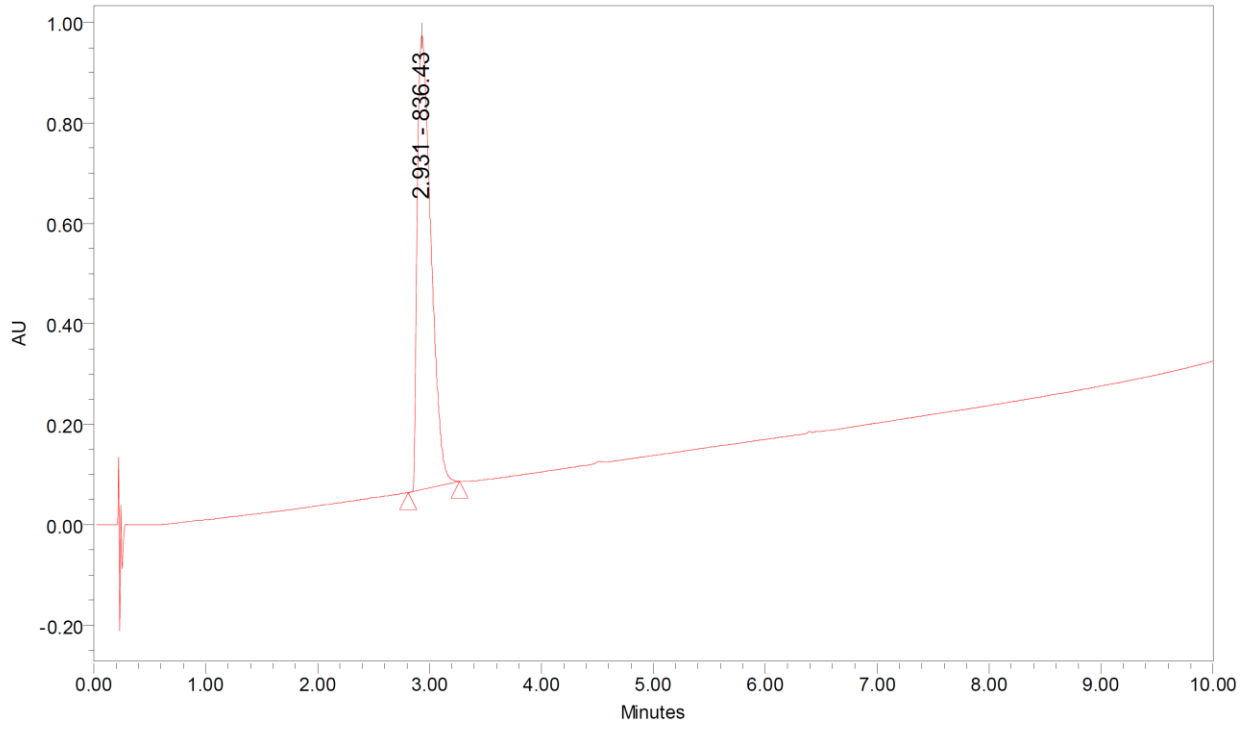
Sample Type:	Unknown	Date Acquired:	12/21/2018 1:19:31 PM EST
Vial:	1:A,5	Acq. Method Set:	10to100% Bin 10 min_Delay5min
Injection #:	1	Date Processed:	12/21/2018 2:15:32 PM EST
Injection Volume:	8.00 ul	Processing Method:	Bruce
Run Time:	10.0 Minutes	Channel Name:	230.0nm
Sample Set Name:	aa	Proc. Chnl. Descr.:	PDA Spectrum PDA 230.0 nm (PDA

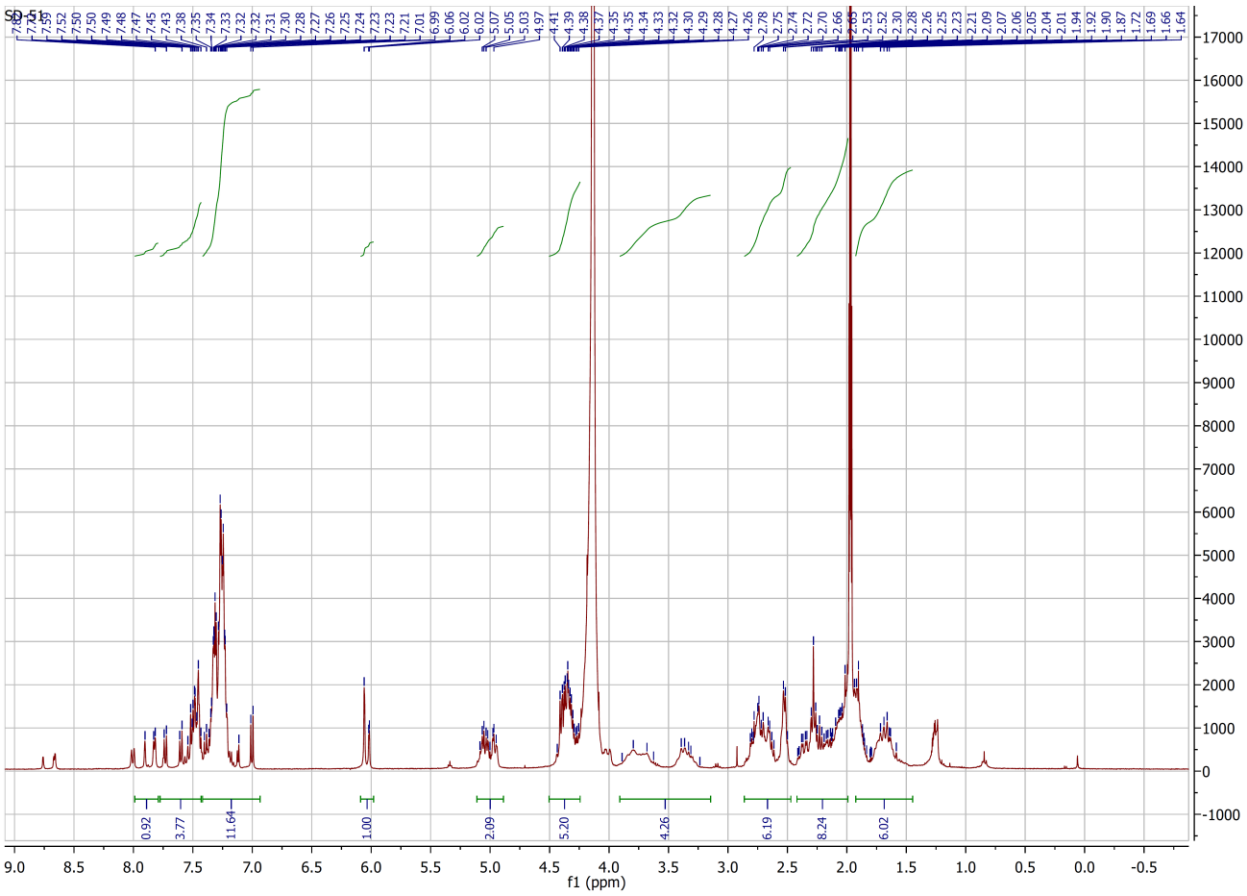
Auto-Scaled Chromatogram



Peak Results

	RT	Area	Height	% Area
1	2.931	7675966	904157	100.00

^{1}H NMR for compound **12**.

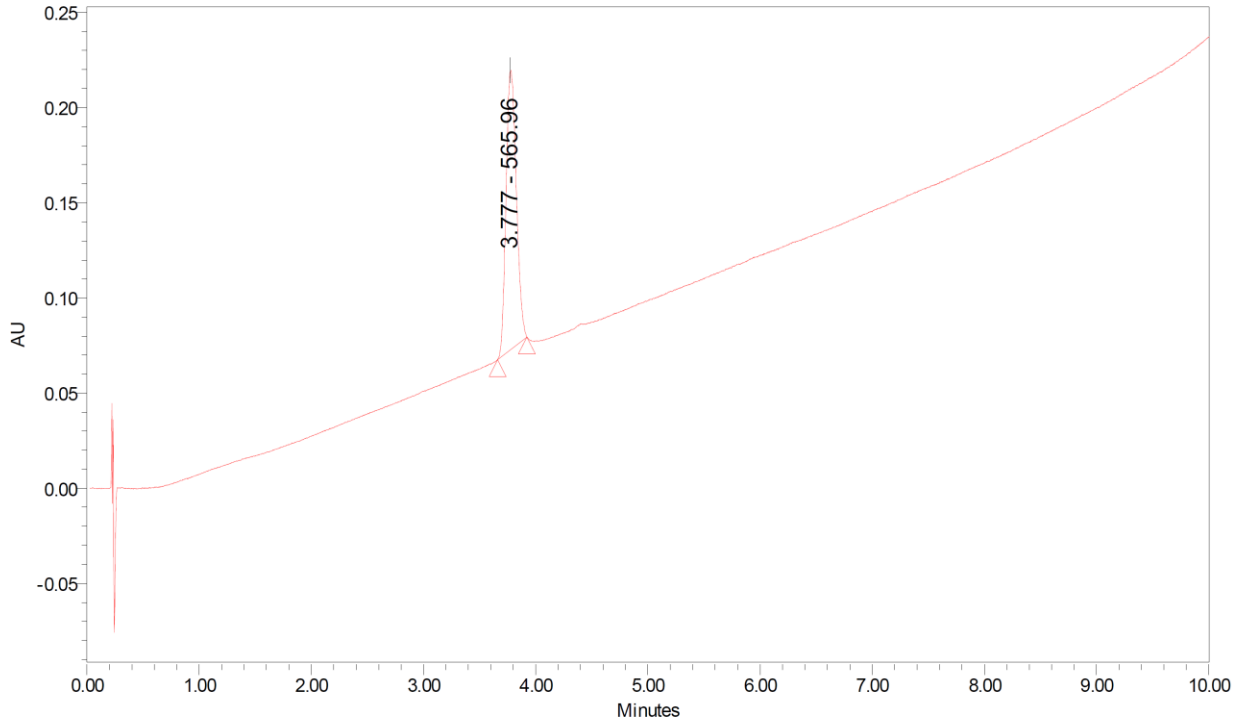


UPLC-MS analysis for compound **12.**

SAMPLE INFORMATION

Sample Name:	ZH-SD-51	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	9/6/2018 11:34:14 AM EDT
Vial:	1:E,5	Acq. Method Set:	10to100% Bin 10
Injection #:	1	Date Processed:	9/6/2018 5:14:49 PM EDT
Injection Volume:	3.00 ul	Processing Method:	Bruce
Run Time:	10.0 Minutes	Channel Name:	230.0nm
Sample Set Name:	Shilin	Proc. Chnl. Descr.:	PDA Spectrum (210-500)nm

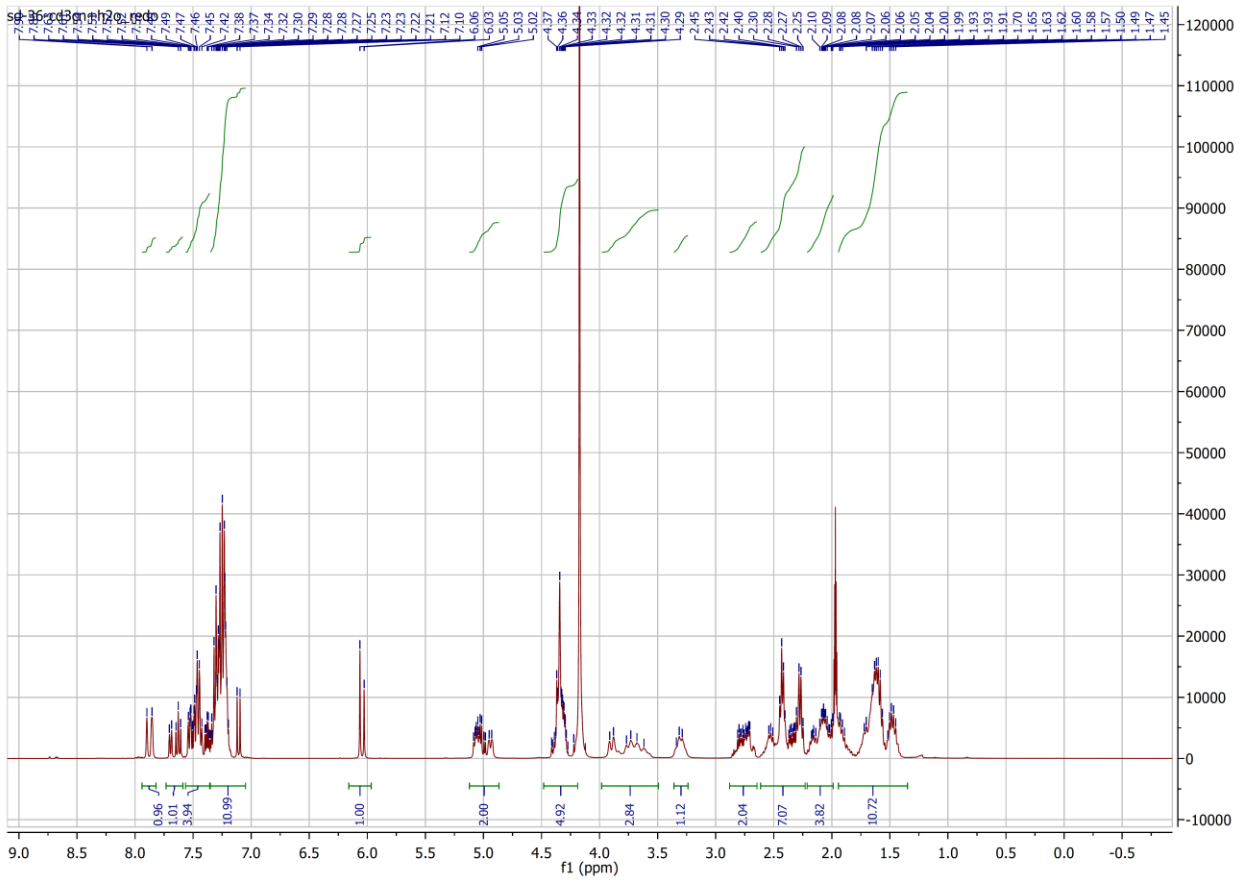
Auto-Scaled Chromatogram



Peak Results

	RT	Area	Height	% Area	Base Peak (m/z)
1	3.777	935129	146756	100.00	565.96

^{1}H NMR for SD-36 (**14**).

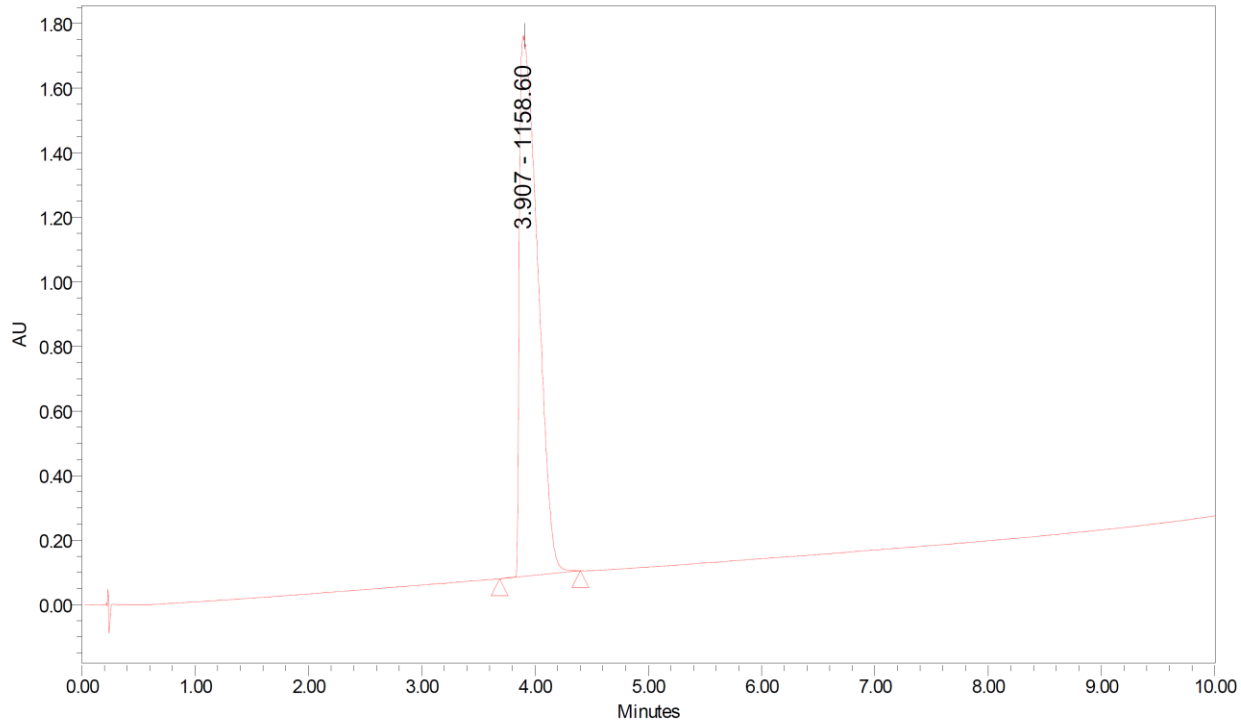


UPLC-MS analysis for SD-36 (**14**).

SAMPLE INFORMATION

Sample Name:	ZH-SD-36	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	7/16/2018 6:39:40 PM EDT
Vial:	1:F,7	Acq. Method Set:	10to100% Bin 10
Injection #:	1	Date Processed:	7/16/2018 7:41:00 PM EDT
Injection Volume:	3.00 ul	Processing Method:	Bruce
Run Time:	10.0 Minutes	Channel Name:	230.0nm@1
Sample Set Name:	Shilin	Proc. Chnl. Descr.:	PDA Spectrum (210-500)nm

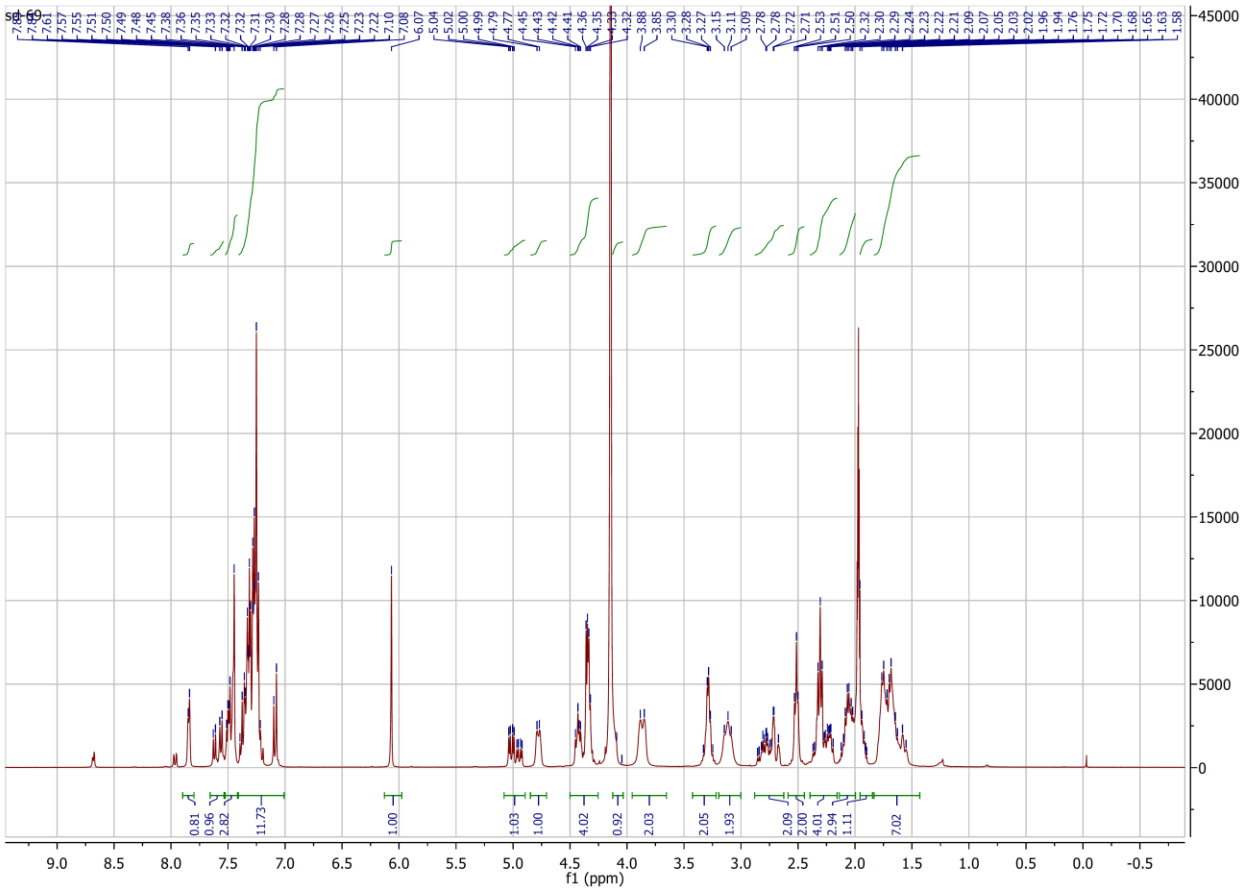
Auto-Scaled Chromatogram



Peak Results

	RT	Area	Height	% Area	Base Peak (m/z)
1	3.907	18527168	1674564	100.00	1158.60

^{1}H NMR for compound **19**.



UPLC-MS analysis for compound **19**.

SAMPLE INFORMATION

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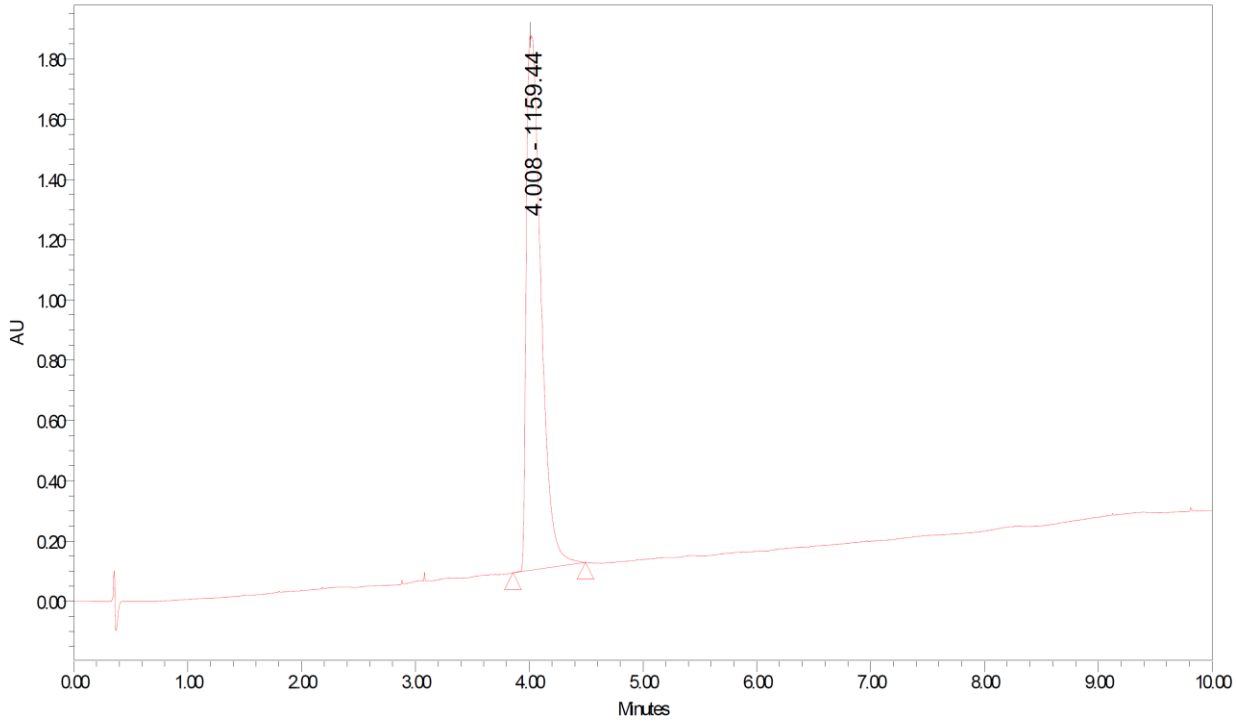
Injection #:	1	Date Processed:	8/16/2018 5:48:00 PM EDT
Injection Volume:	3.00 ul	Processing Method:	Bruce
Run Time:	10.0 Minutes	Channel Name:	230.0nm
Sample Set Name:	SHILIN	Proc. Chnl. Descr.:	PDA Spectrum PDA 230.0 nm (PDA Spectrum

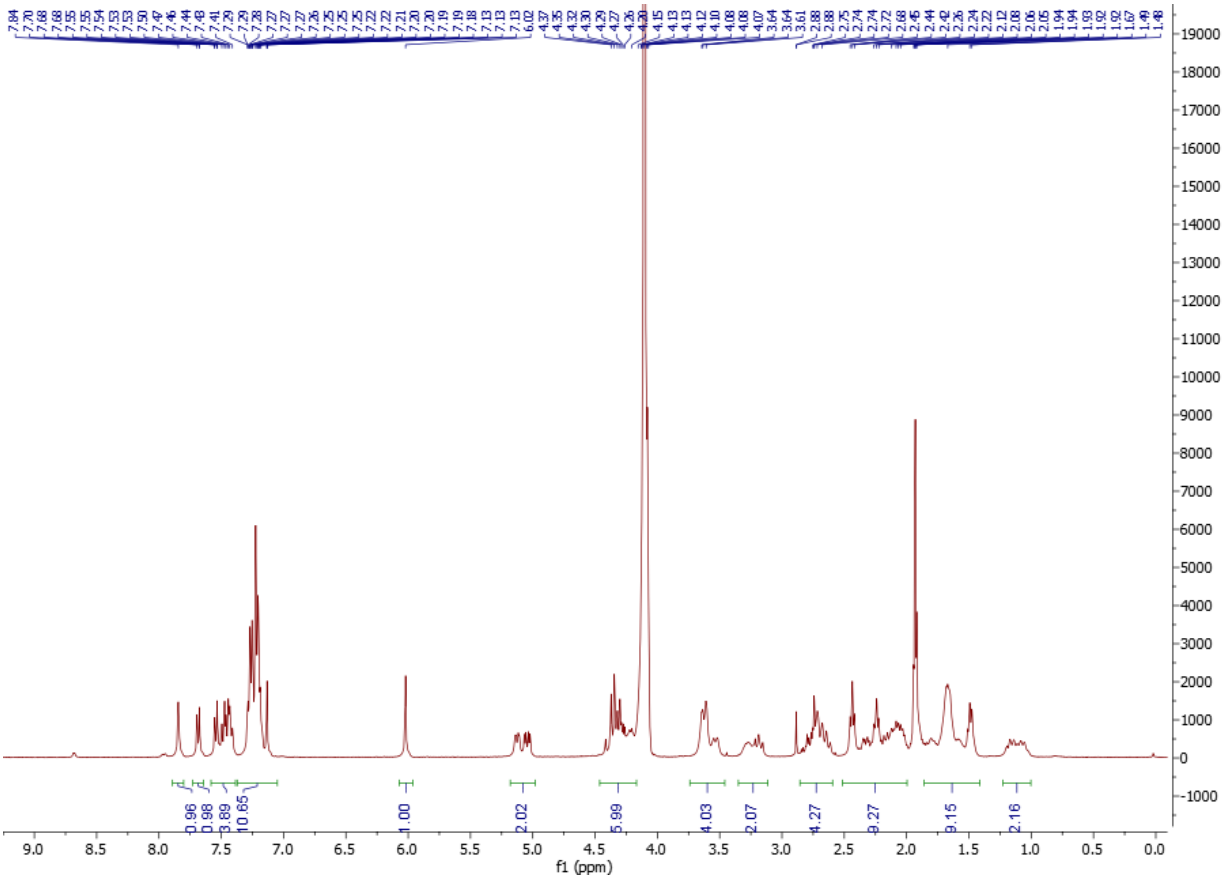
Auto-Scaled Chromatogram



Peak Results

	RT	Area	Height	%Area	Base Peak (m/z)
1	4.008	15901123	1776487	100.00	1159.44

^{1}H NMR for compound **22**.



UPLC-MS analysis for compound **22.**

SAMPLE INFORMATION

Sample Name:	XRQ-SD-356-2	Acquired By:	System
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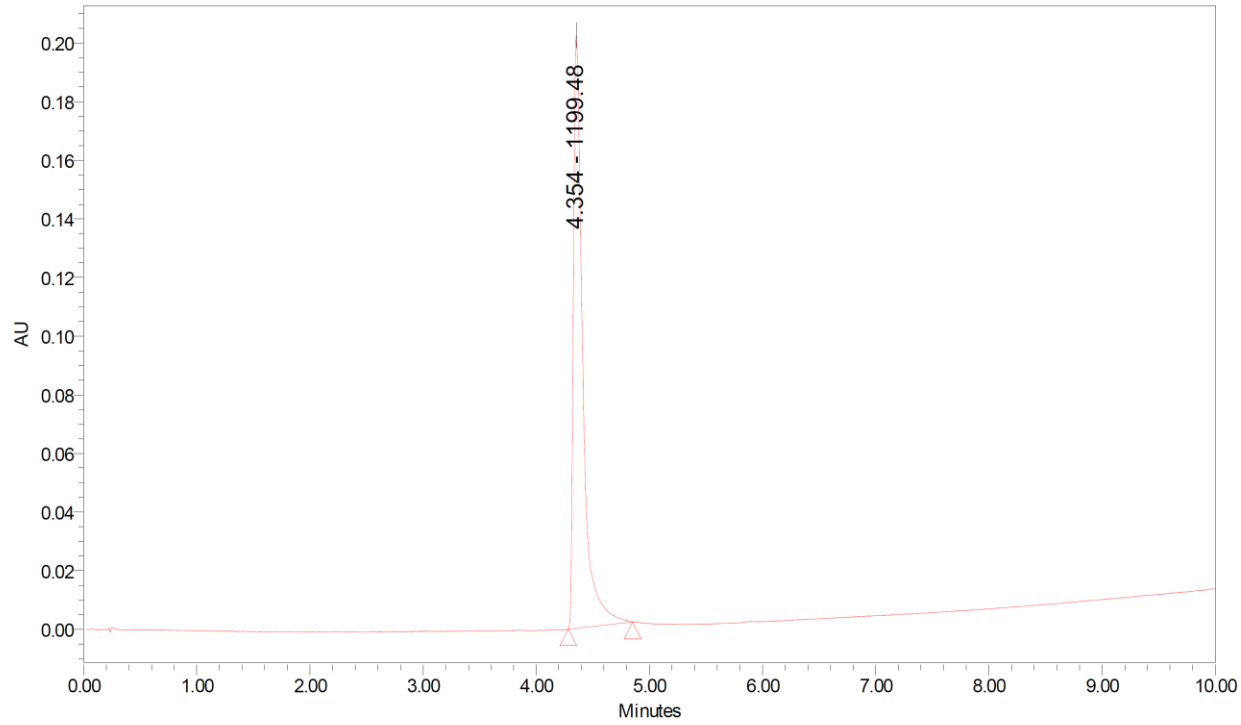
Vial:	1:A,1	Acq. Method Set:	10to100% Bin 10
Injection #:	1	Date Processed:	10/16/2018 9:19:10 PM EDT
Injection Volume:	3.00 ul	Processing Method:	Bruce
Run Time:	10.0 Minutes	Channel Name:	254.0nm@1
Sample Set Name:	Shilin	Proc. Chnl. Descr.:	PDA Spectrum (210-500)nm

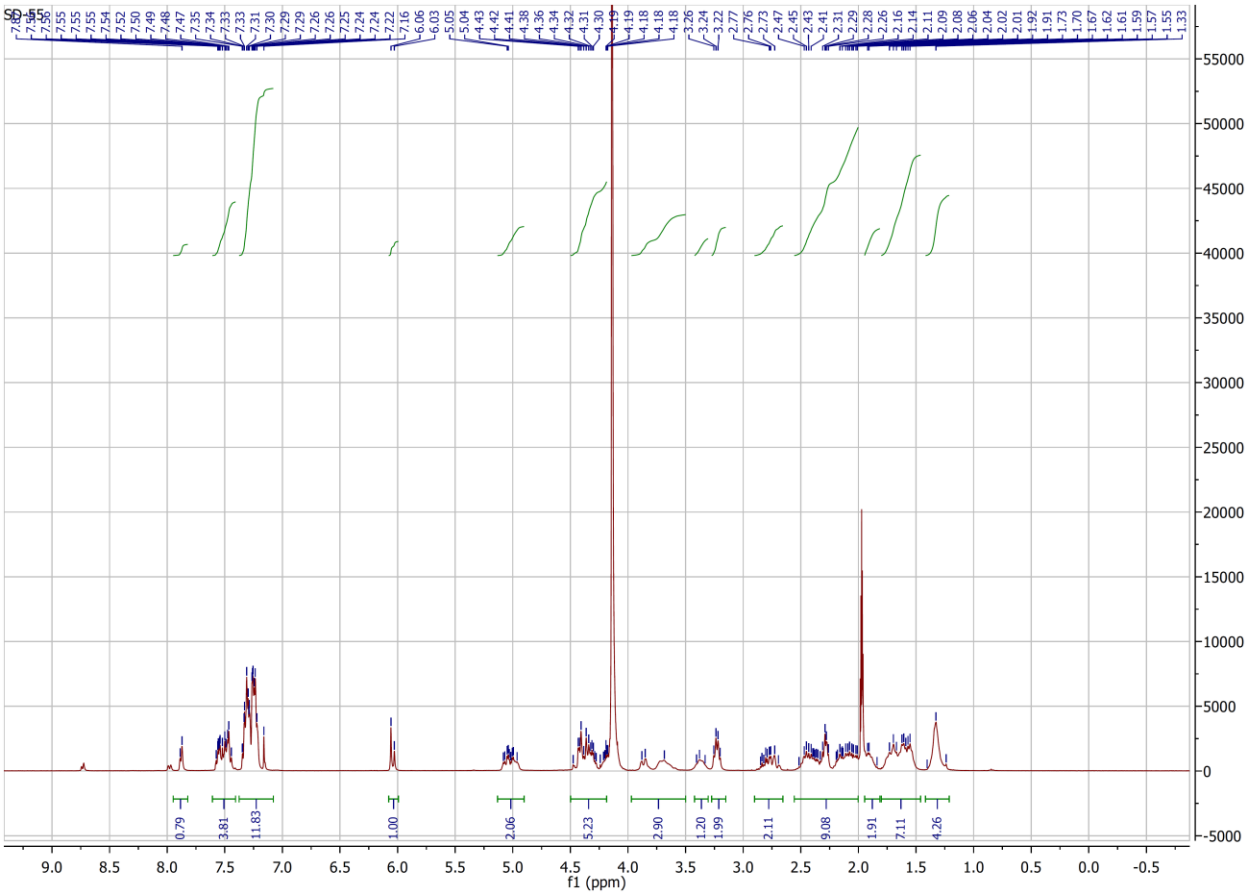
Auto-Scaled Chromatogram



Peak Results

	RT	Area	Height	% Area	Base Peak (m/z)
1	4.354	1169135	202098	100.00	1199.48

^{1}H NMR for compound **26**.

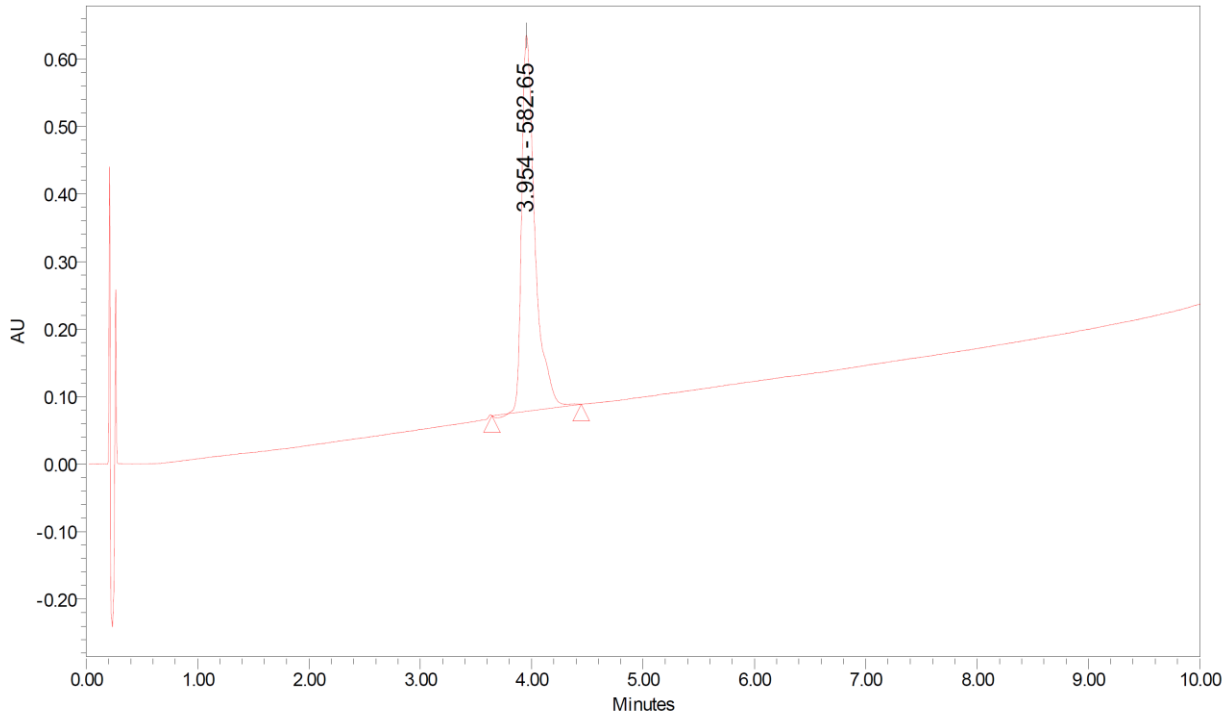


UPLC-MS analysis for compound **26**.

SAMPLE INFORMATION

Sample Name:	XRQ-SD-55	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	9/6/2018 12:05:32 PM EDT
Vial:	1:E,7	Acq. Method Set:	10to100% Bin 10
Injection #:	1	Date Processed:	9/6/2018 5:16:25 PM EDT
Injection Volume:	3.00 ul	Processing Method:	Bruce
Run Time:	10.0 Minutes	Channel Name:	230.0nm
Sample Set Name:	Shilin	Proc. Chnl. Descr.:	PDA Spectrum (210-500)nm

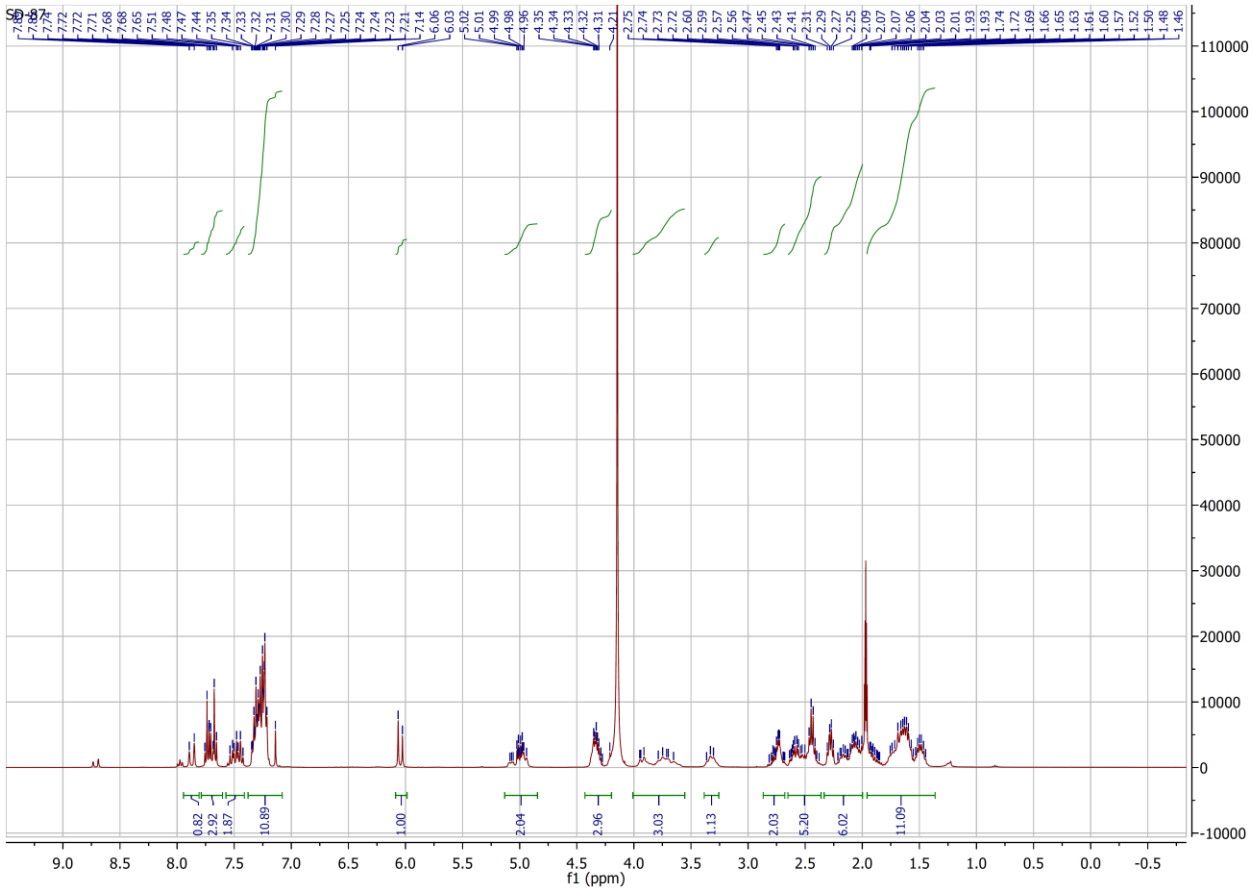
Auto-Scaled Chromatogram



Peak Results

	RT	Area	Height	% Area	Base Peak (m/z)
1	3.954	4619107	556371	100.00	582.65

^{1}H NMR for compound **27**.



UPLC-MS analysis for compound **27.**

SAMPLE INFORMATION

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Sample Type:	Unknown	Date Acquired:	8/9/2018 3:01:42 PM EDT
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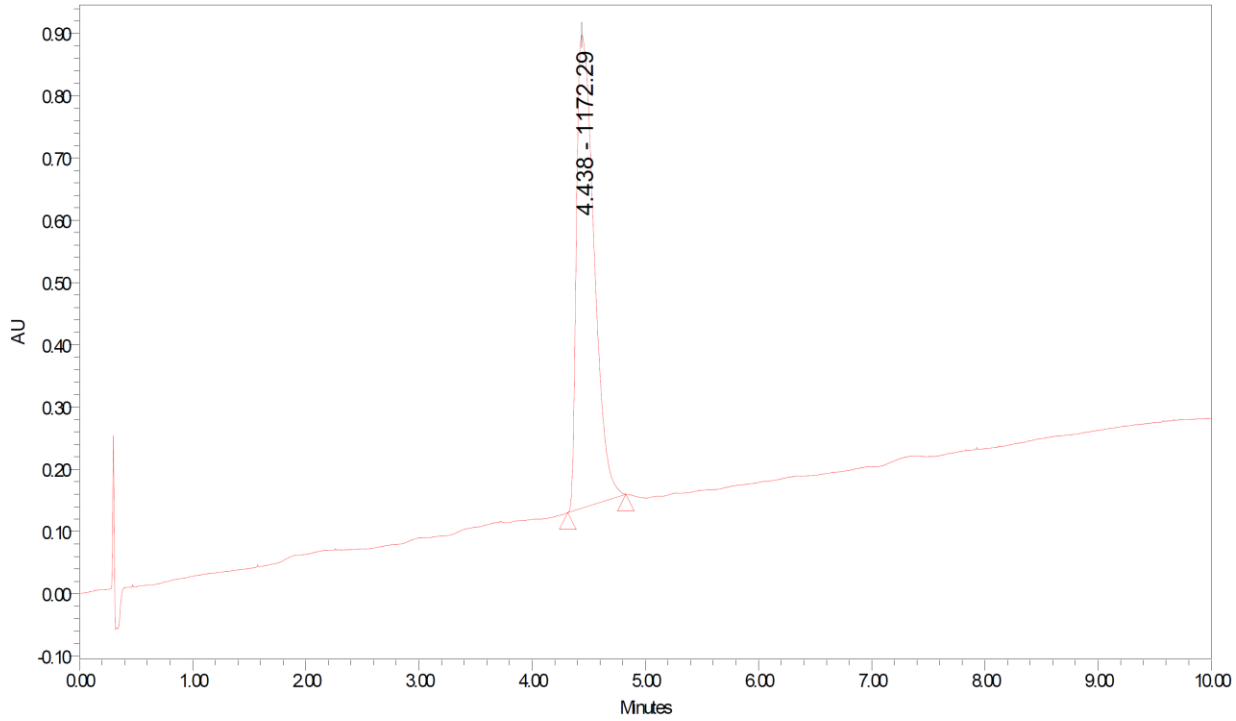
Injection #:	1	Date Processed:	8/9/2018 4:26:41 PM EDT
Injection Volume:	3.00 ul	Processing Method:	Bruce
Run Time:	10.0 Minutes	Channel Name:	230.0nm
Sample Set Name:	SHILIN	Proc. Chnl. Descr.:	PDA Spectrum PDA 230.0 nm (PDA Spectrum

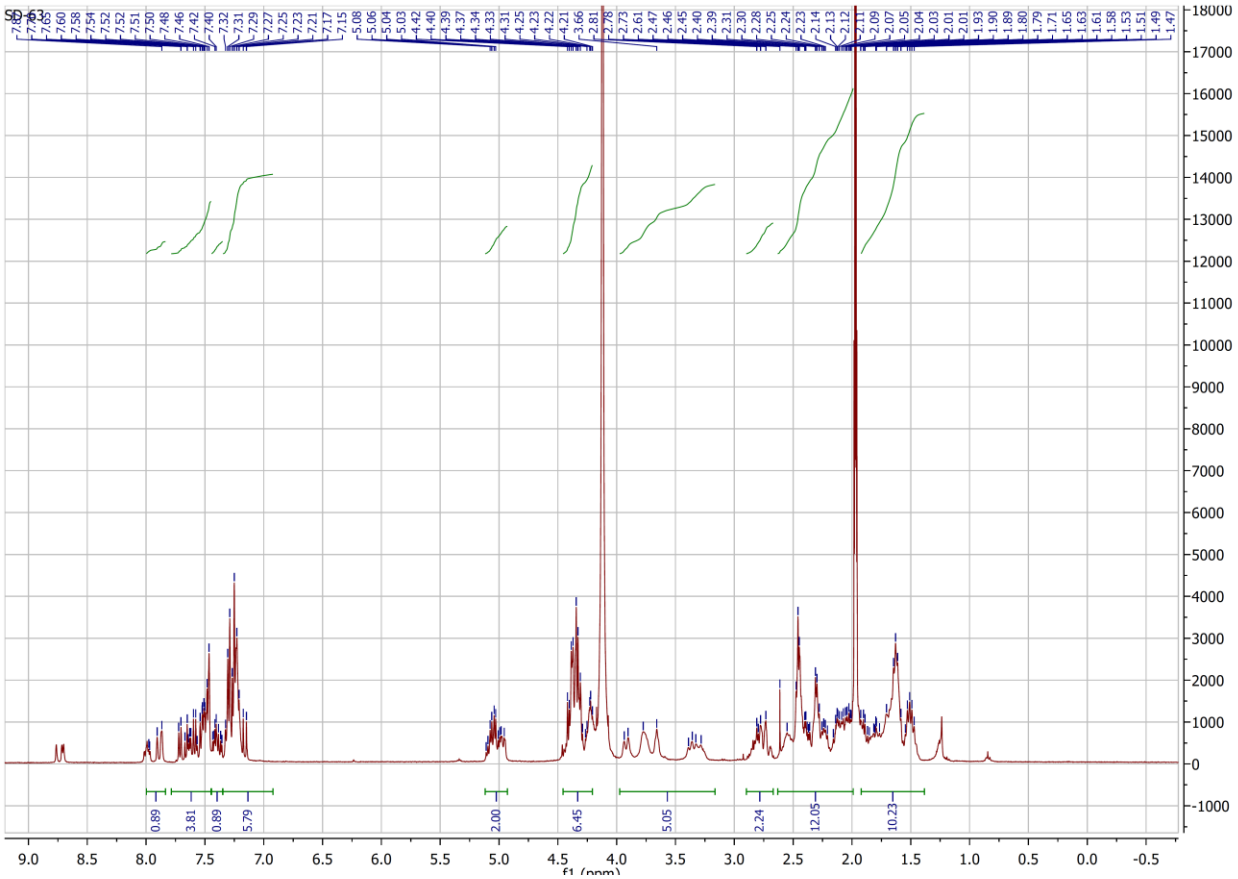
Auto-Scaled Chromatogram



Peak Results

	RT	Area	Height	%Area	Base Peak (m/z)
1	4.438	8206444	760771	100.00	1172.29

^{1}H NMR for compound **30**.



UPLC-MS analysis for compound **30**.

SAMPLE INFORMATION

Sample Name:	zh-sd-63	Acquired By:	System
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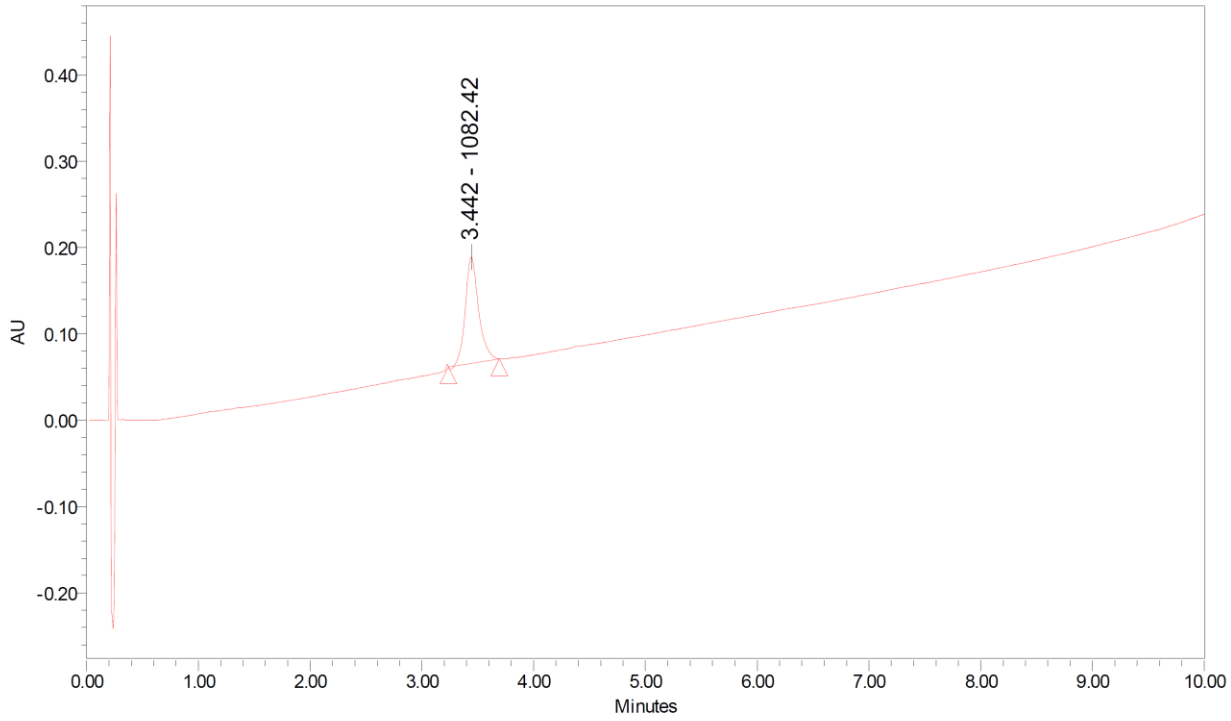
Injection #:	1	Date Processed:	9/10/2018 5:50:38 PM EDT
Injection Volume:	3.00 ul	Processing Method:	Bruce
Run Time:	10.0 Minutes	Channel Name:	230.0nm
Sample Set Name:	aa	Proc. Chnl. Descr.:	PDA Spectrum (210-500)nm

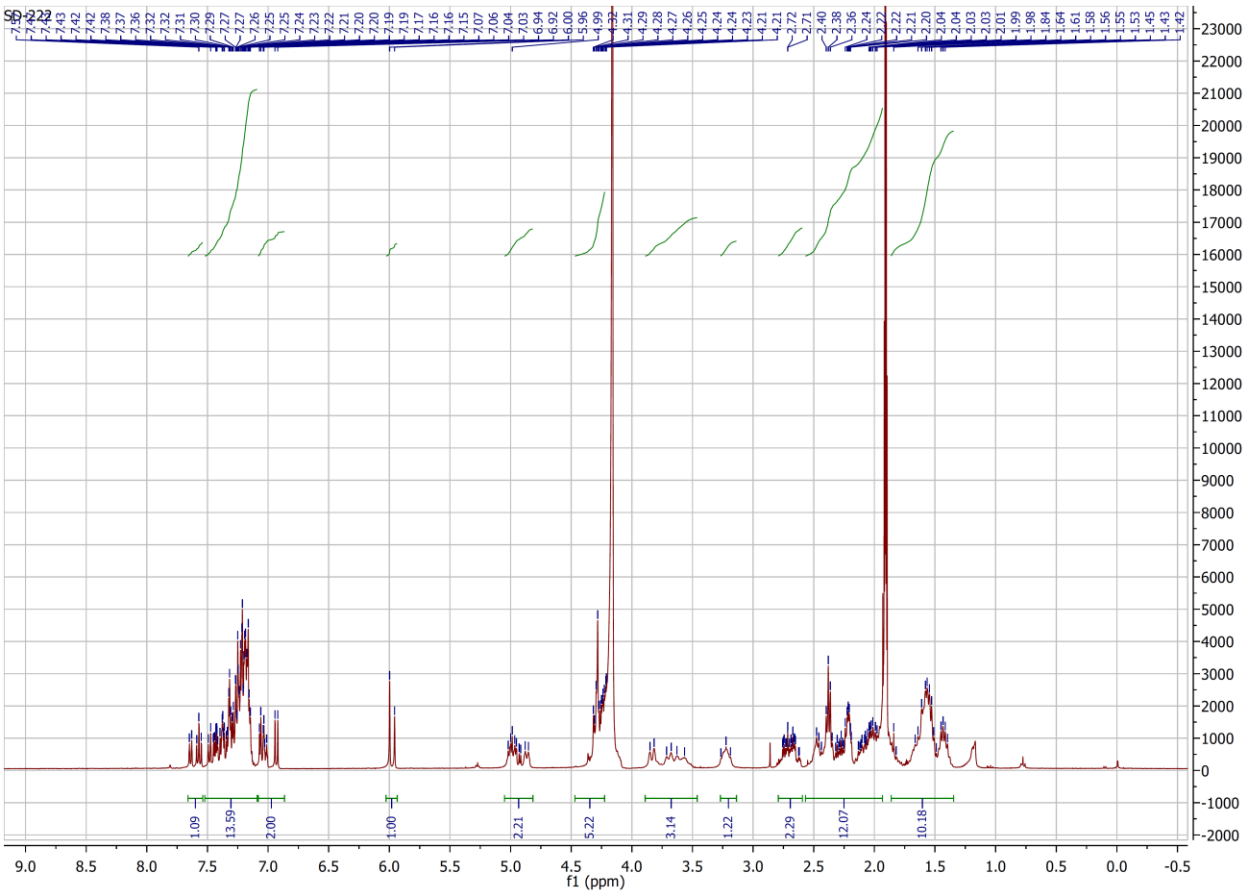
Auto-Scaled Chromatogram



Peak Results

	RT	Area	Height	% Area	Base Peak (m/z)
1	3.442	1023610	123259	100.00	1082.42

^{1}H NMR for compound **32**.



UPLC-MS analysis for compound **32.**

SAMPLE INFORMATION

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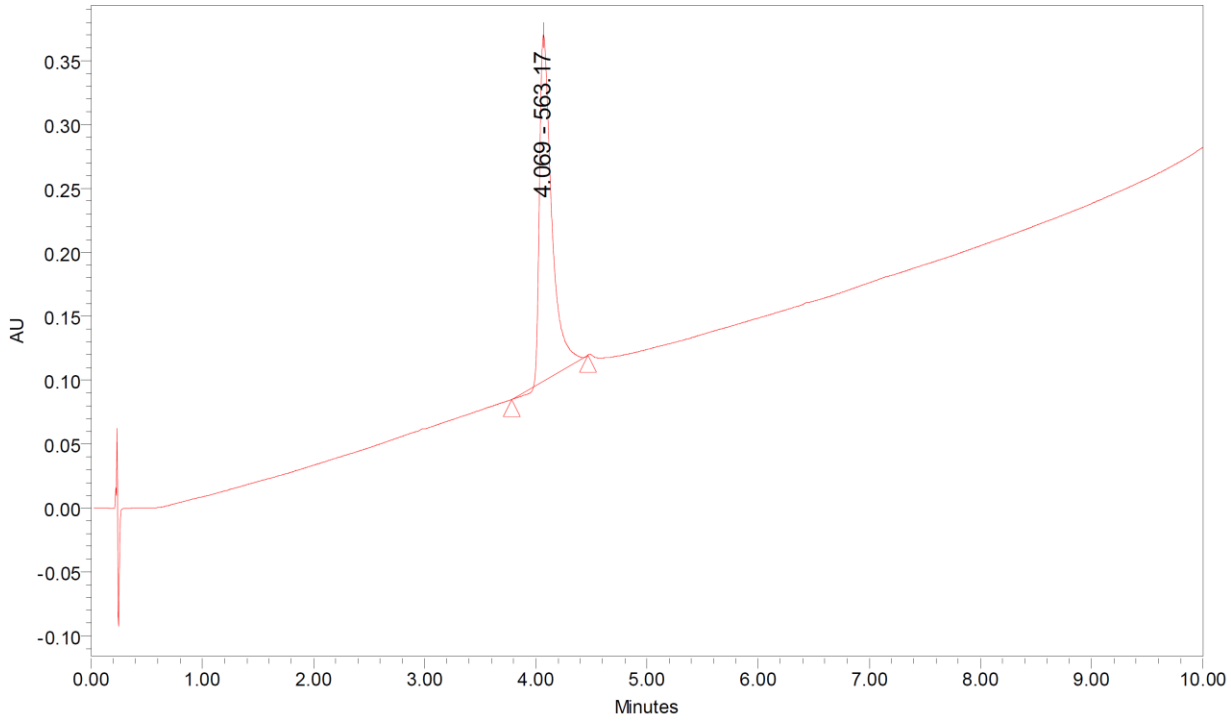
Injection #:	1	Date Processed:	3/25/2019 1:23:08 PM EDT
Injection Volume:	3.00 ul	Processing Method:	Bruce
Run Time:	10.0 Minutes	Channel Name:	230.0nm
Sample Set Name:	3	Proc. Chnl. Descr.:	PDA Spectrum PDA 230.0 nm (PDA

Auto-Scaled Chromatogram



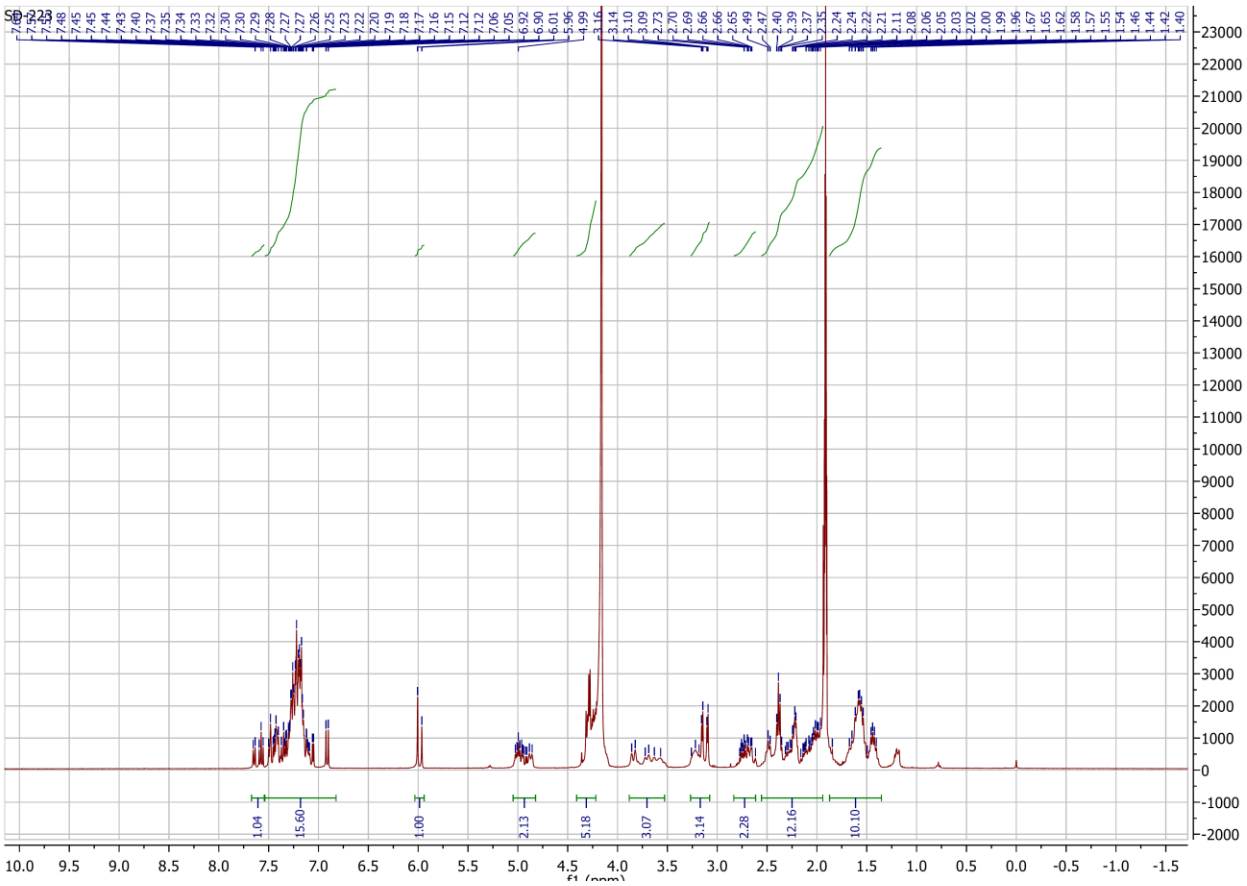
Peak Results

	RT	Area	Height	% Area
1	4.069	2069216	270698	100.00

Peak Results

	Base Peak (m/z)
1	563.17

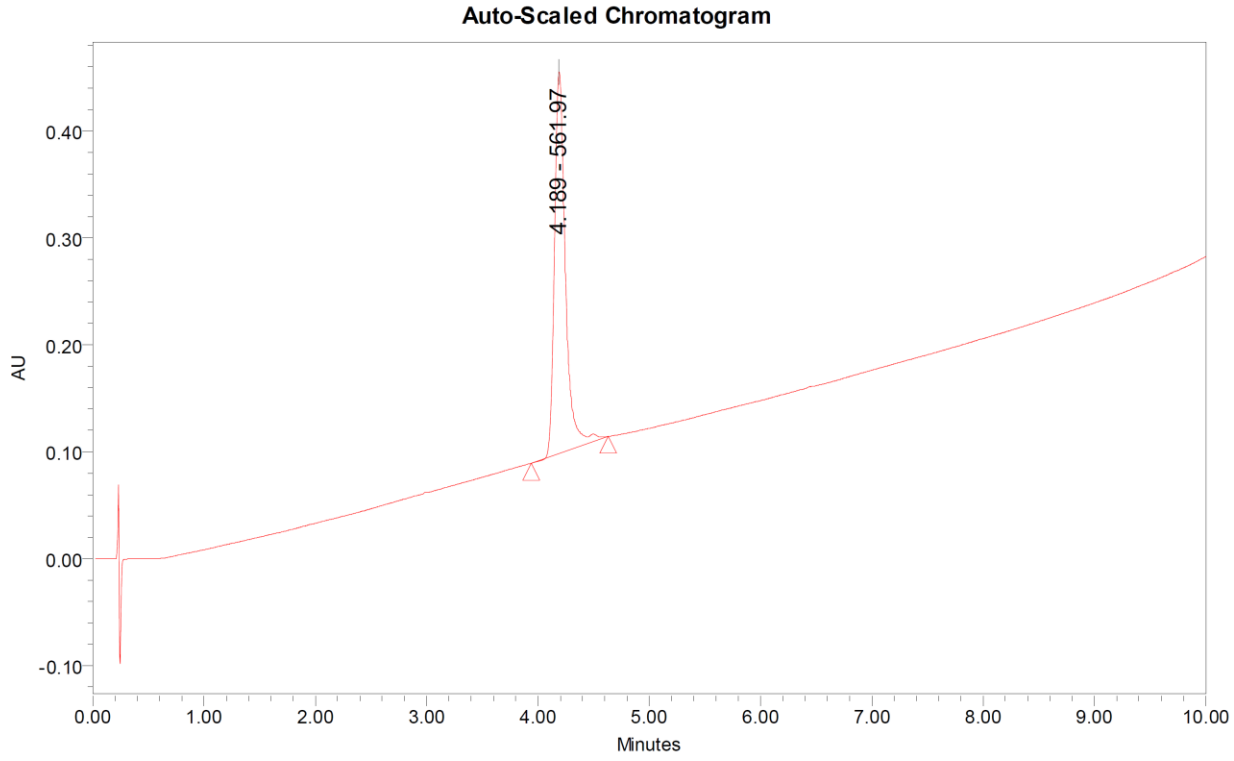
^{1}H NMR for compound **33**.



UPLC-MS analysis for compound **33.**

SAMPLE INFORMATION

Sample Name:	ZH-SD-223	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	3/15/2019 3:42:59 PM EDT
Vial:	1:E,8	Acq. Method Set:	10to100% Bin 10 min_Delay5min
Injection #:	1	Date Processed:	3/25/2019 1:23:36 PM EDT
Injection Volume:	3.00 ul	Processing Method:	Bruce
Run Time:	10.0 Minutes	Channel Name:	230.0nm
Sample Set Name:	3	Proc. Chnl. Descr.:	PDA Spectrum PDA 230.0 nm (PDA



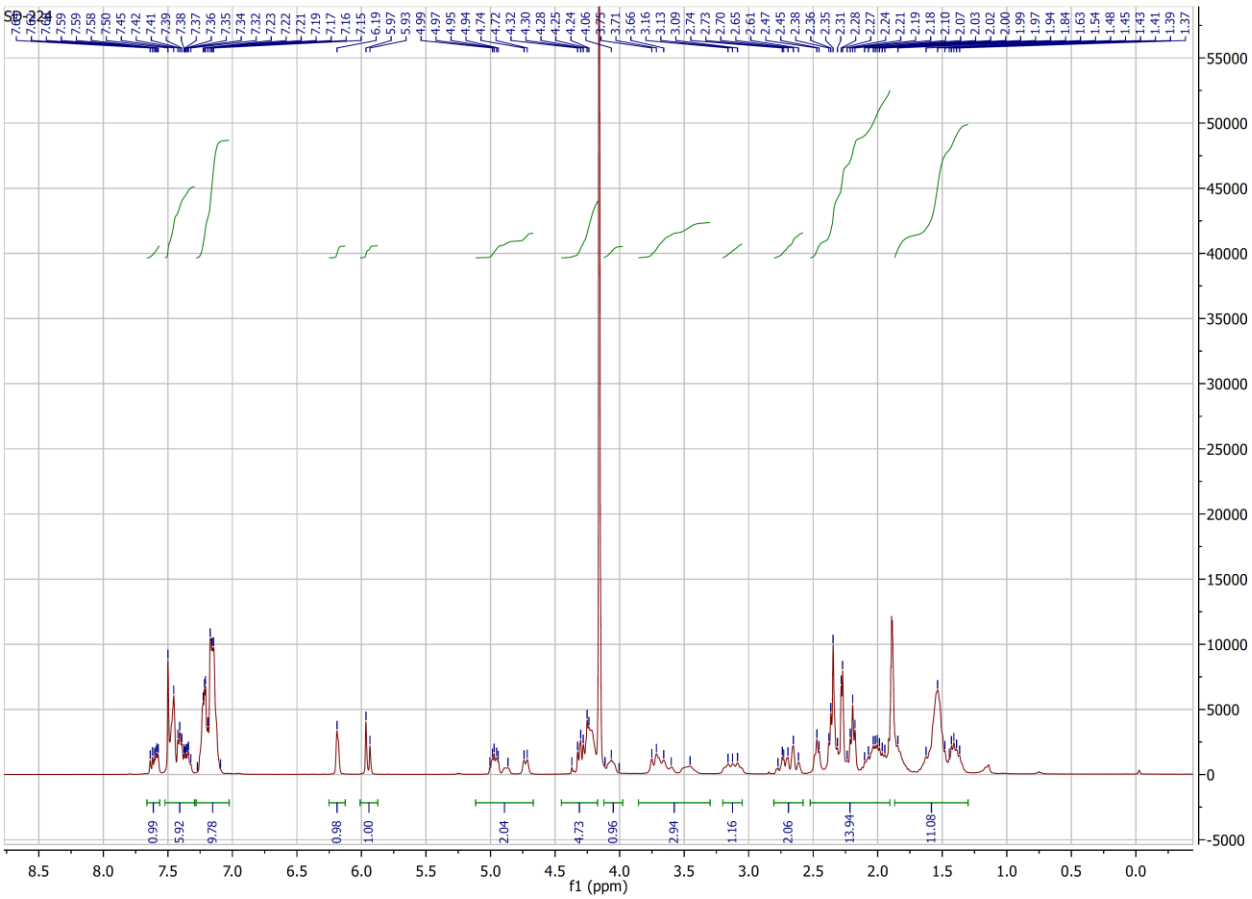
Peak Results

	RT	Area	Height	% Area
1	4.189	2439539	356947	100.00

Peak Results

	Base Peak (m/z)
1	561.97

^{1}H NMR for compound **34**.

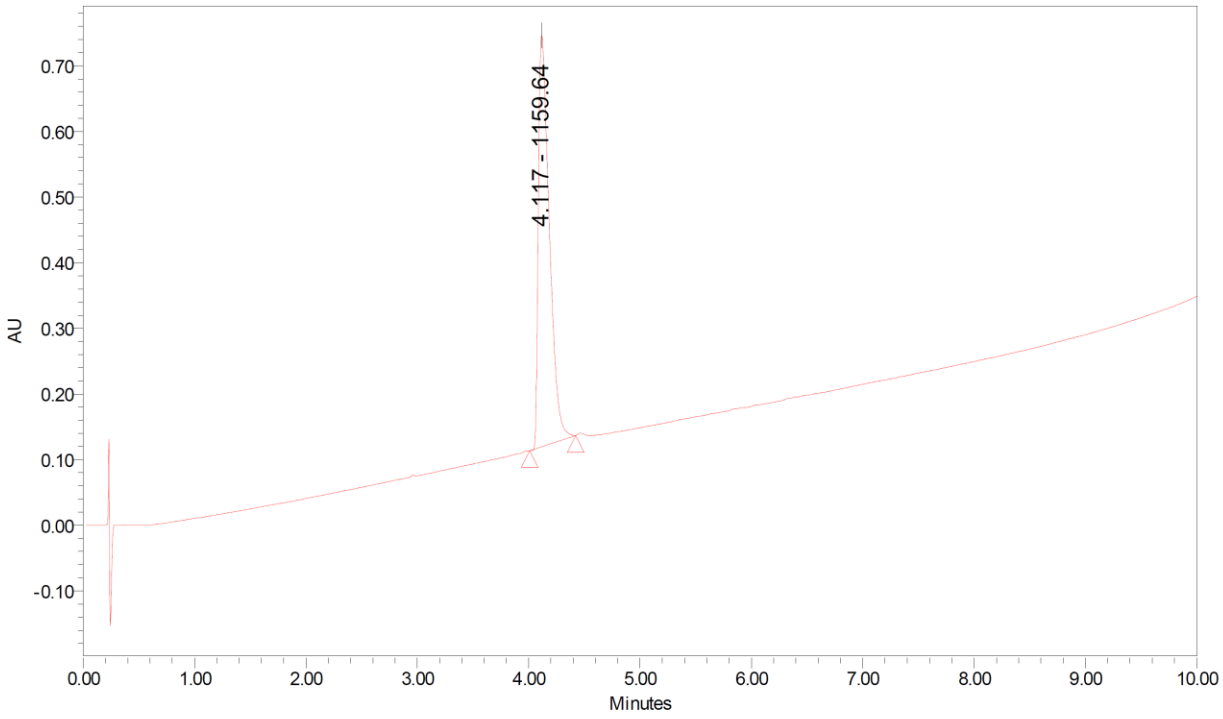


UPLC-MS analysis for compound **34**.

SAMPLE INFORMATION

Sample Name:	ZH-SD-224	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	3/29/2019 10:15:55 AM EDT
Vial:	1:E,6	Acq. Method Set:	10to100% Bin 10
Injection #:	1	Date Processed:	3/29/2019 10:35:01 AM EDT
Injection Volume:	3.00 ul	Processing Method:	Bruce
Run Time:	10.0 Minutes	Channel Name:	230.0nm
Sample Set Name:	111	Proc. Chnl. Descr.:	PDA Spectrum (210-500)nm

Auto-Scaled Chromatogram



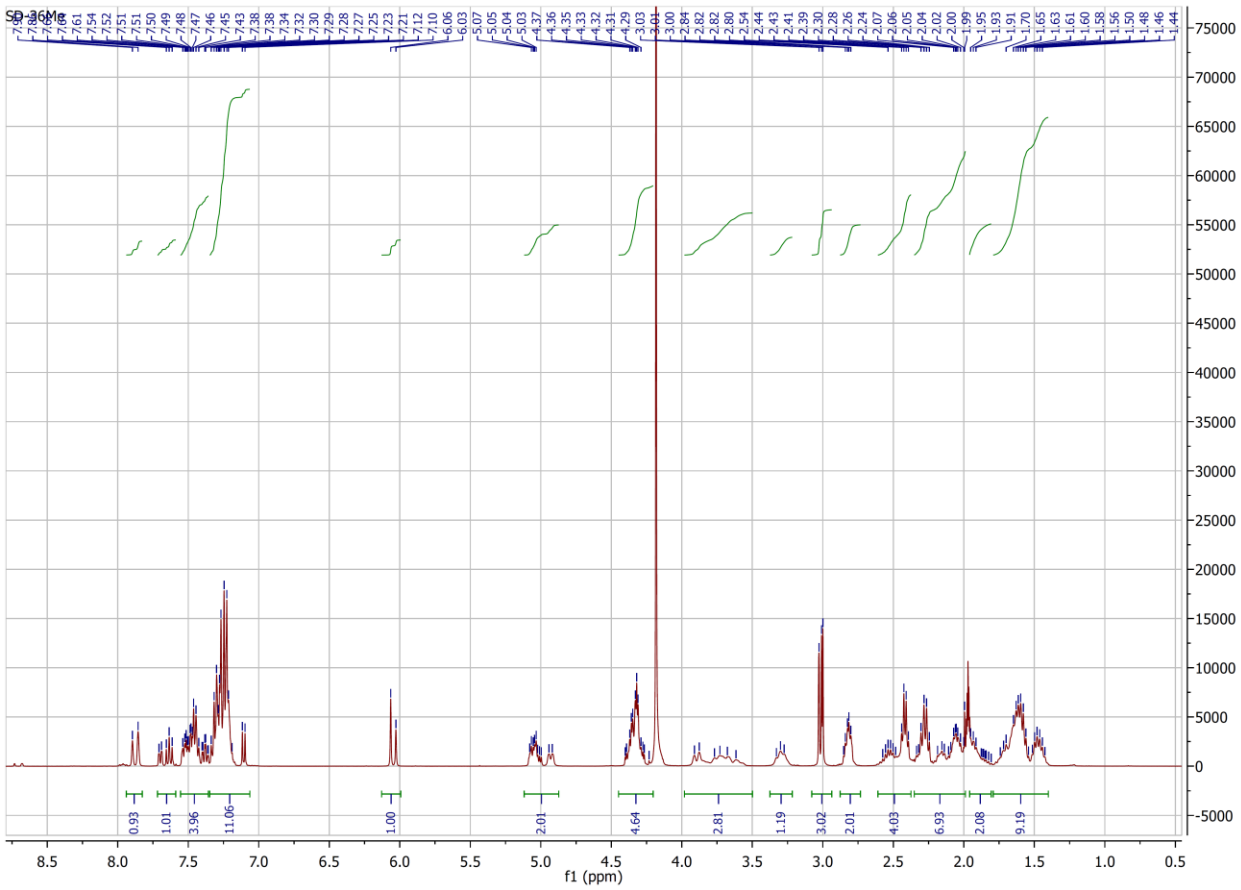
Peak Results

	RT	Area	Height	% Area
1	4.117	4286274	626716	100.00

Peak Results

	Base Peak (m/z)
1	1159.64

^{1}H NMR for SD-36Me (**35**).

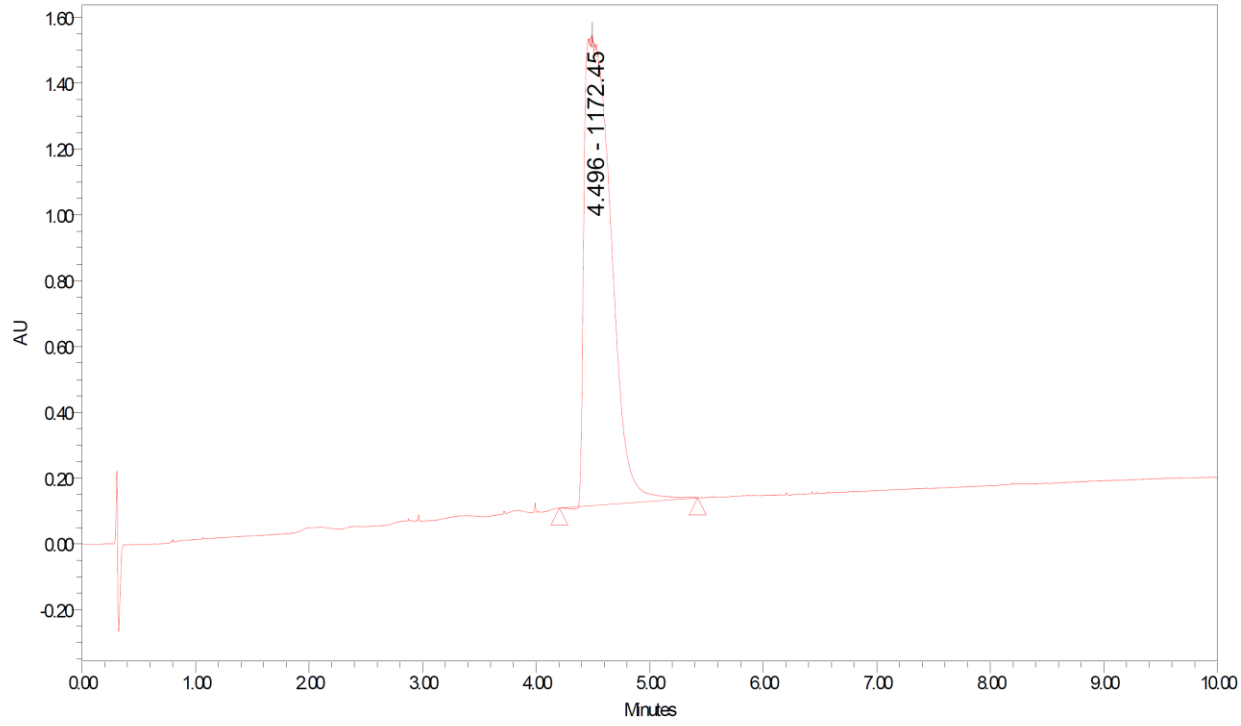


UPLC-MS analysis for SD-36Me (**35**).

SAMPLE INFORMATION

Sample Name:	zh-scl-36m3-hplc	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	8/1/2018 4:00:34 PM EDT
Vial:	1:B,8	Acq. Method Set:	10to100%Bin 10 min_Delay5min
Injection #:	1	Date Processed:	8/2/2018 2:04:05 PM EDT
Injection Volume:	3.00 ul	Processing Method:	Bruce
Run Time:	10.0 Minutes	Channel Name:	220.0nm
Sample Set Name:	3	Proc. Chnl. Descr.:	PDA Spectrum PDA 220.0 nm (PDA Spectrum

Auto-Scaled Chromatogram



Peak Results

	RT	Area	Height	%Area	Base Peak (m/z)
1	4.496	23457871	1430384	100.00	1172.45