

Dimerization of a heat shock protein 90 inhibitor enhances inhibitory activity

Hendra Wahyudi, Yao Wang, and Shelli R. McAlpine*

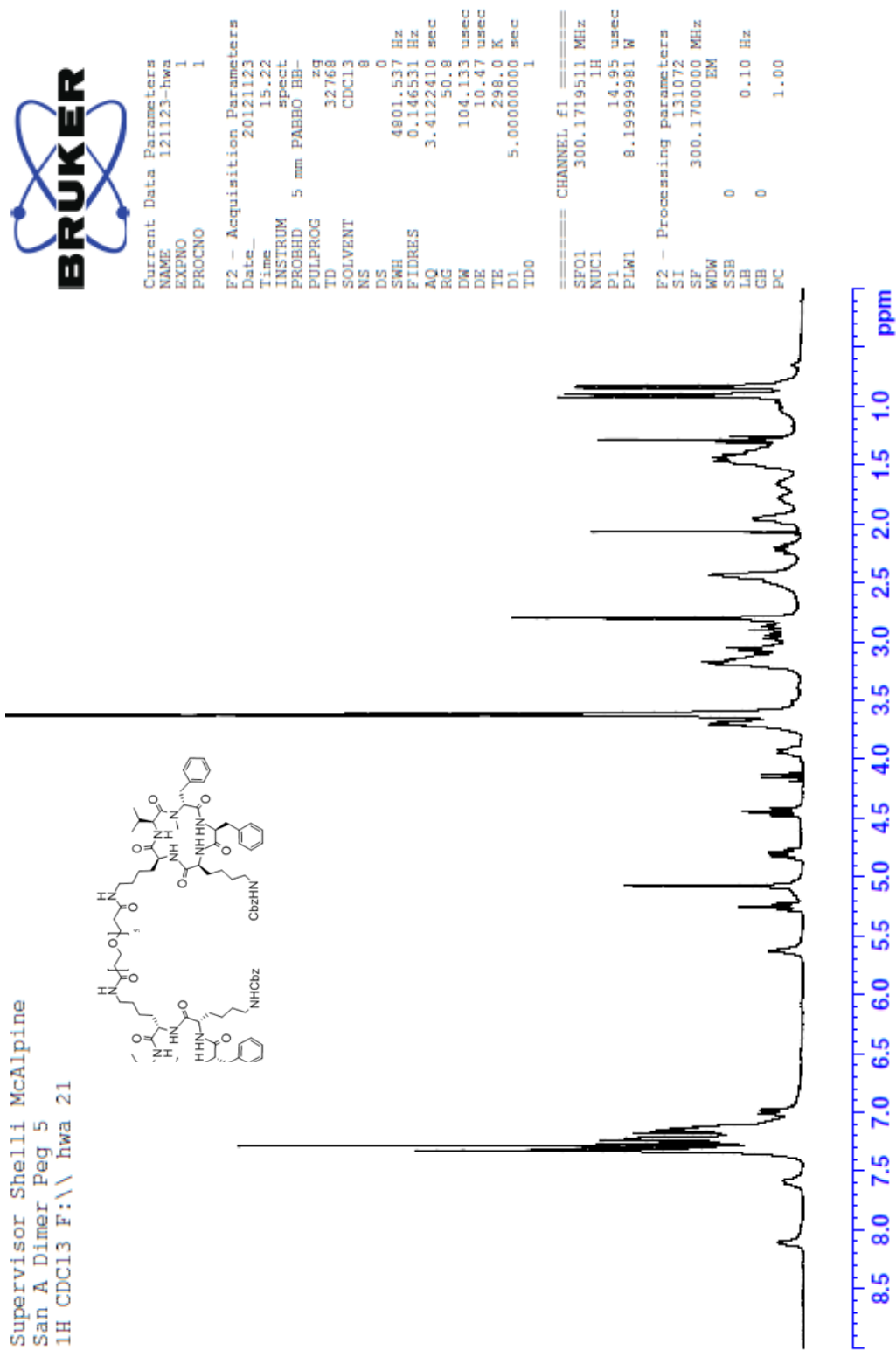
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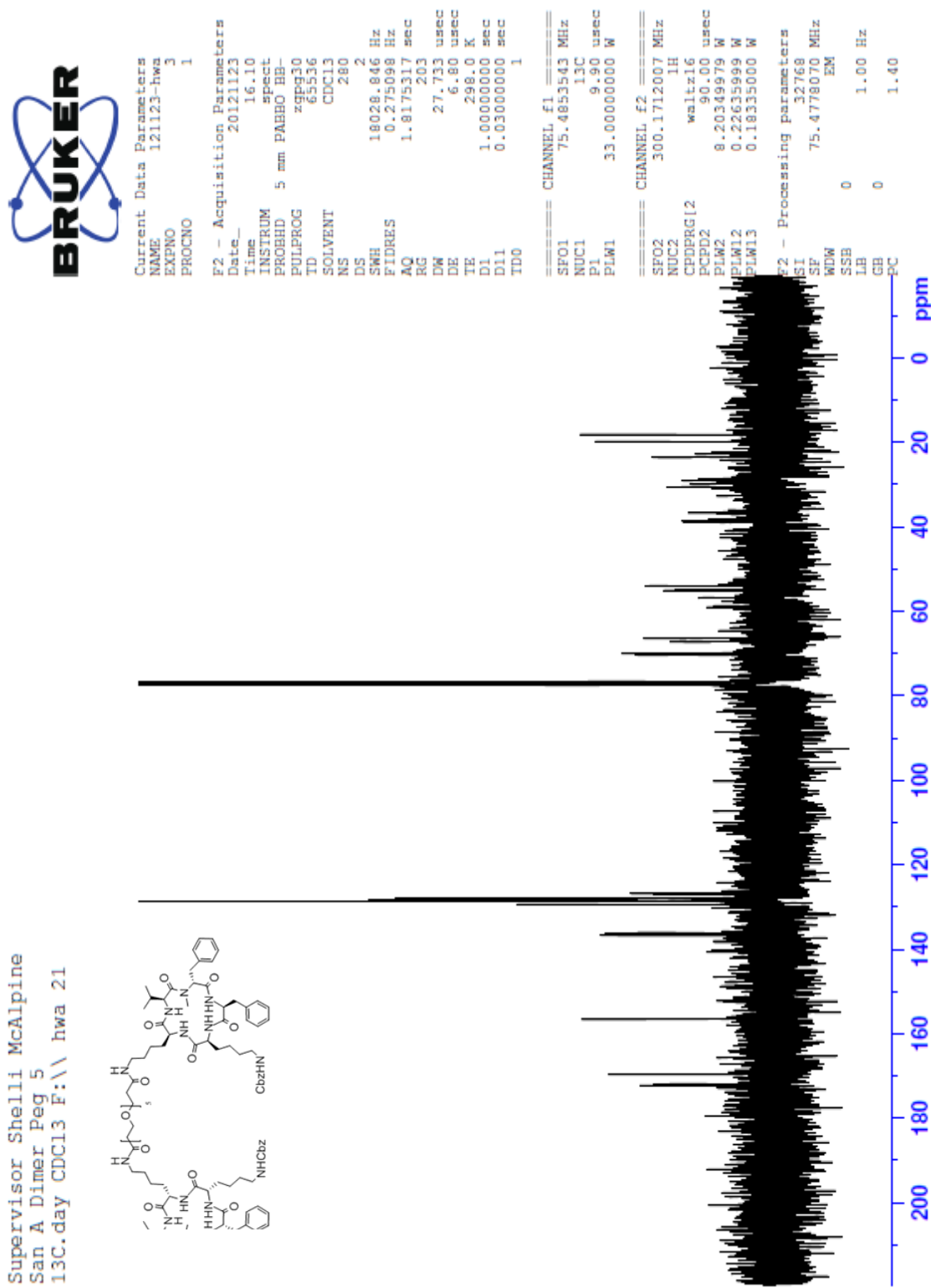
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¹H NMR SM122-PEG-5 DIMER



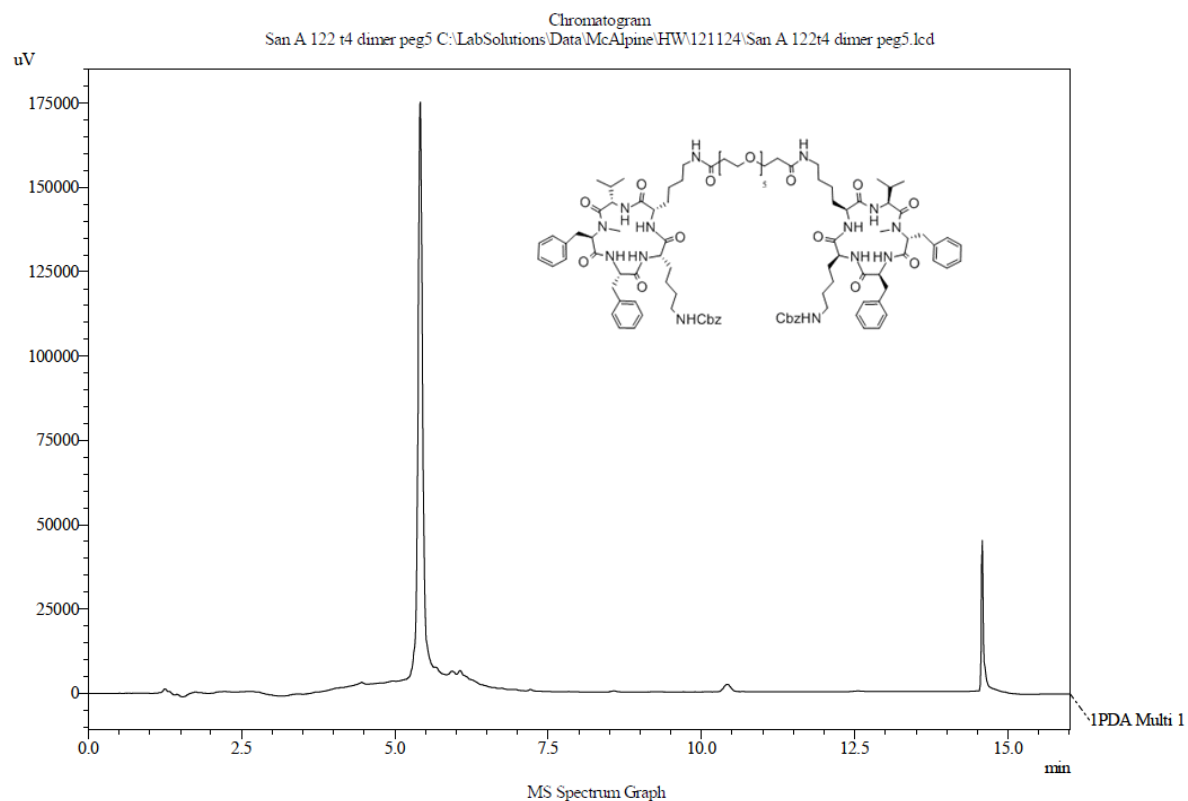
¹³C NMR SM122-PEG-5 DIMER



Supplementary material

LCMS-ESI/UV SM122-PEG-5 DIMER

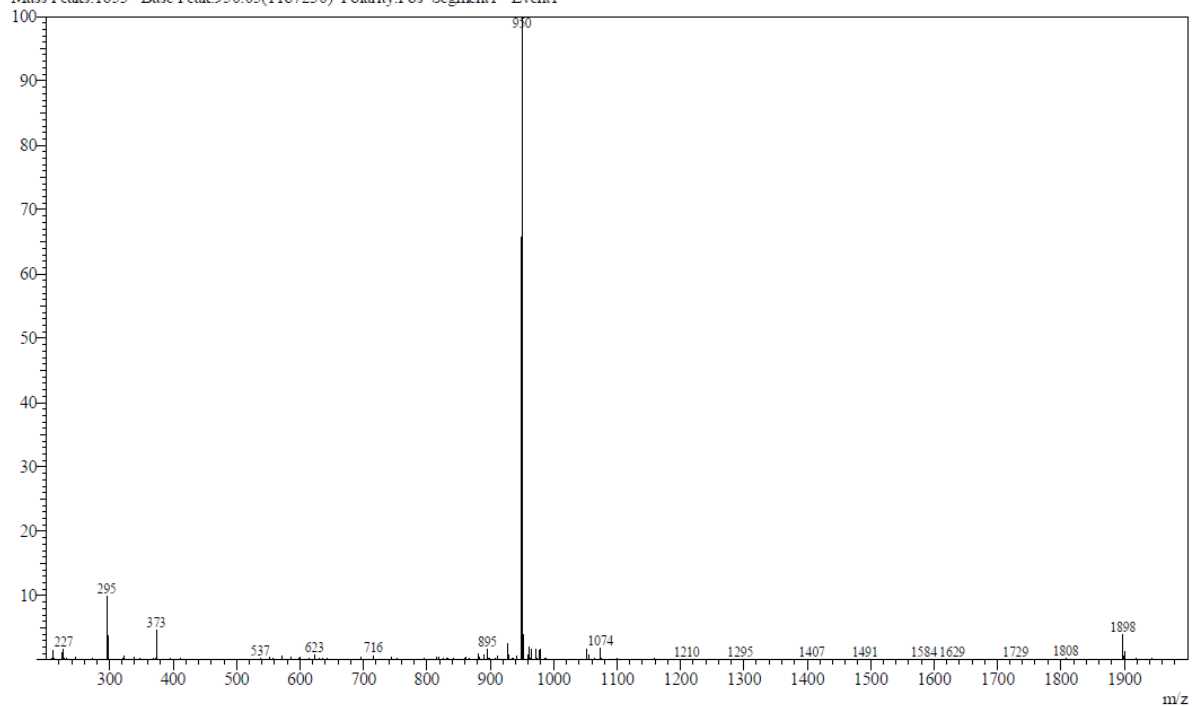
==== Shimadzu LCMSsolution Analysis Report ====



Ret. Time: 5.417 (Scan#: 266)

BG Mode: ?

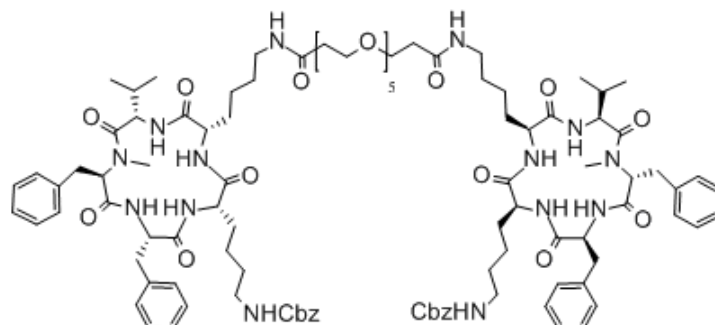
Mass Peaks: 1653 Base Peak: 950.05 (1187230) Polarity: Pos Segment1 - Event1



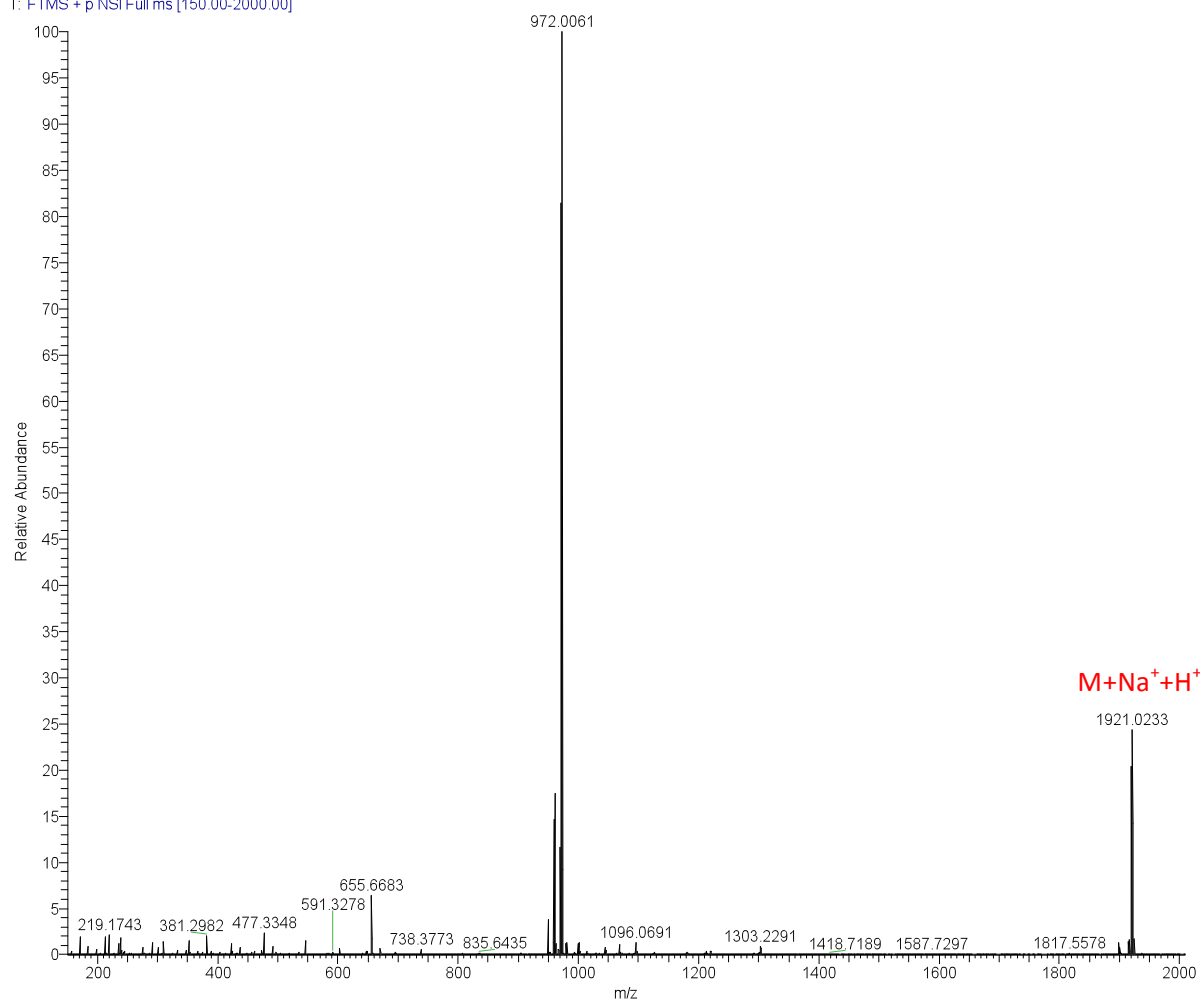
Supplementary material

HRMS (ESI-TOF) SM122-PEG-5 DIMER

Exact mass=1921.0293, found 1921.0233 ($M + Na^+ + H^+$)



Hendra_1 #1-18 RT: 0.01-0.50 AV: 18 NL: 4.35E8
T: FTMS + p NSI Full ms [150.00-2000.00]



Supervisor Shelli McAlpine
San A 122t4 dimer Peg9
¹H CDCl₃ F:\\ hwa 41

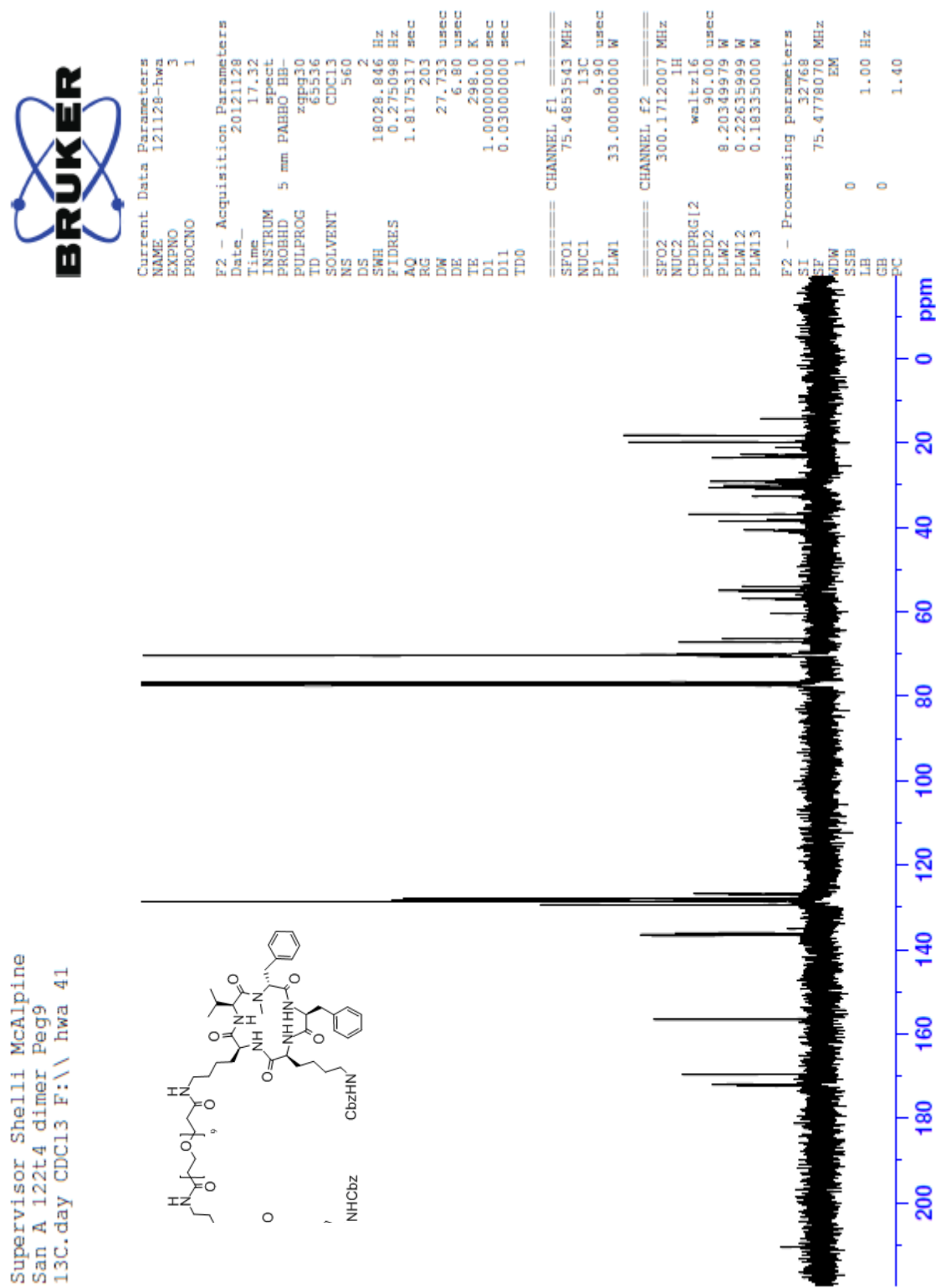
Chemical Structure:

COC(=O)N(CCCNC(=O)C(C)C)CCCNC(=O)[C@H](Cc1ccccc1)C(=O)N[C@@H](Cc2ccccc2)C(=O)NCCCCNC(=O)c3ccccc3

NMR Data:

Current Data Parameters	F2 - Acquisition Parameters	Channel f1	F2 - Processing parameters
NAME 121128-hwa	Date_ 20121128	SFO1 300.1719511 MHz	SI 131072
EXPNO 1	Time 16.31	NUC1 1H	SP 300.1700000 MHz
PROCNO 1	INSTRUM spect	P1 14.95 usec	KDWM EM
	PROBHD 5 mm PABBO BB-	PLW1 8.19999981 W	SSB 0
	PULPROG zg		LB 0
TD 32768	SOLVENT CDCl ₃		GB 0
NS 8	DS 0		PC 1.00
SWH 4801.537 Hz	FIDRES 0.146531 Hz		
AQ 3.4122410 sec	RG 28.5		
DW 104.135 usec	DE 10.47 usec		
TE 298.0 K	D1 5.00000000 sec		
TD0 1			

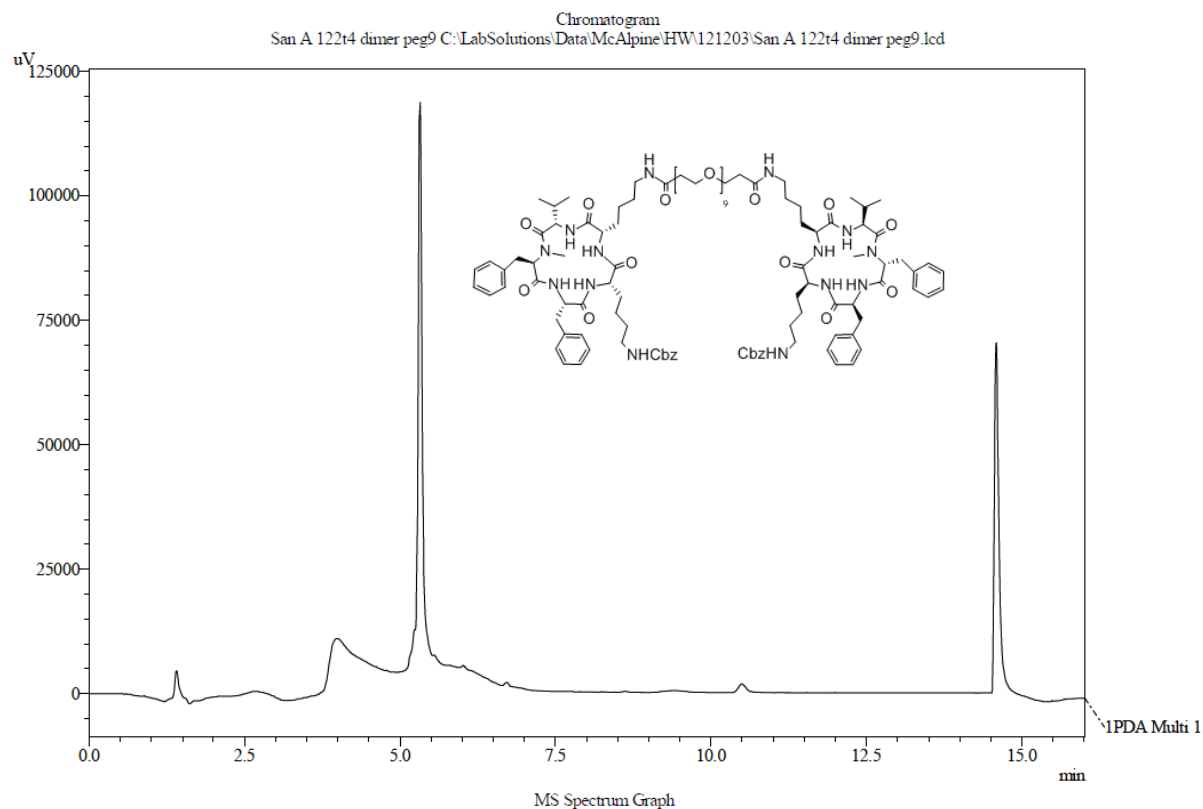
¹³C NMR SM122-PEG-9 DIMER



Supplementary material

LCMS-ESI/UV SM122-PEG-9 DIMER

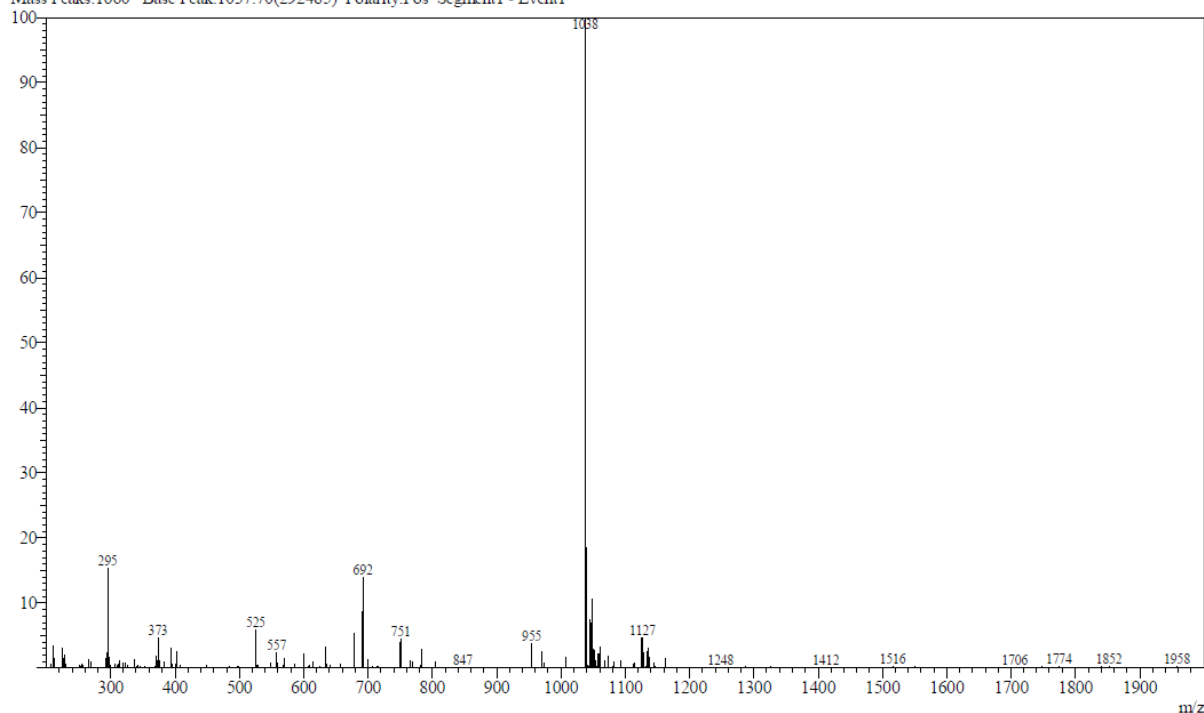
==== Shimadzu LCMSsolution Analysis Report ====



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BG Mode: ?

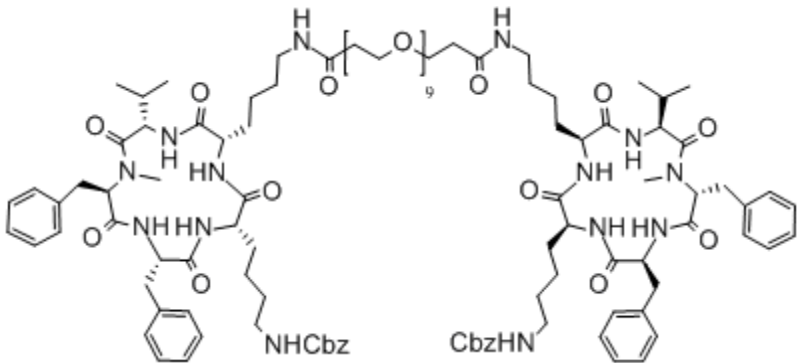
Mass Peaks: 1660 Base Peak: 1037.70 (292485) Polarity: Pos Segment 1 - Event 1



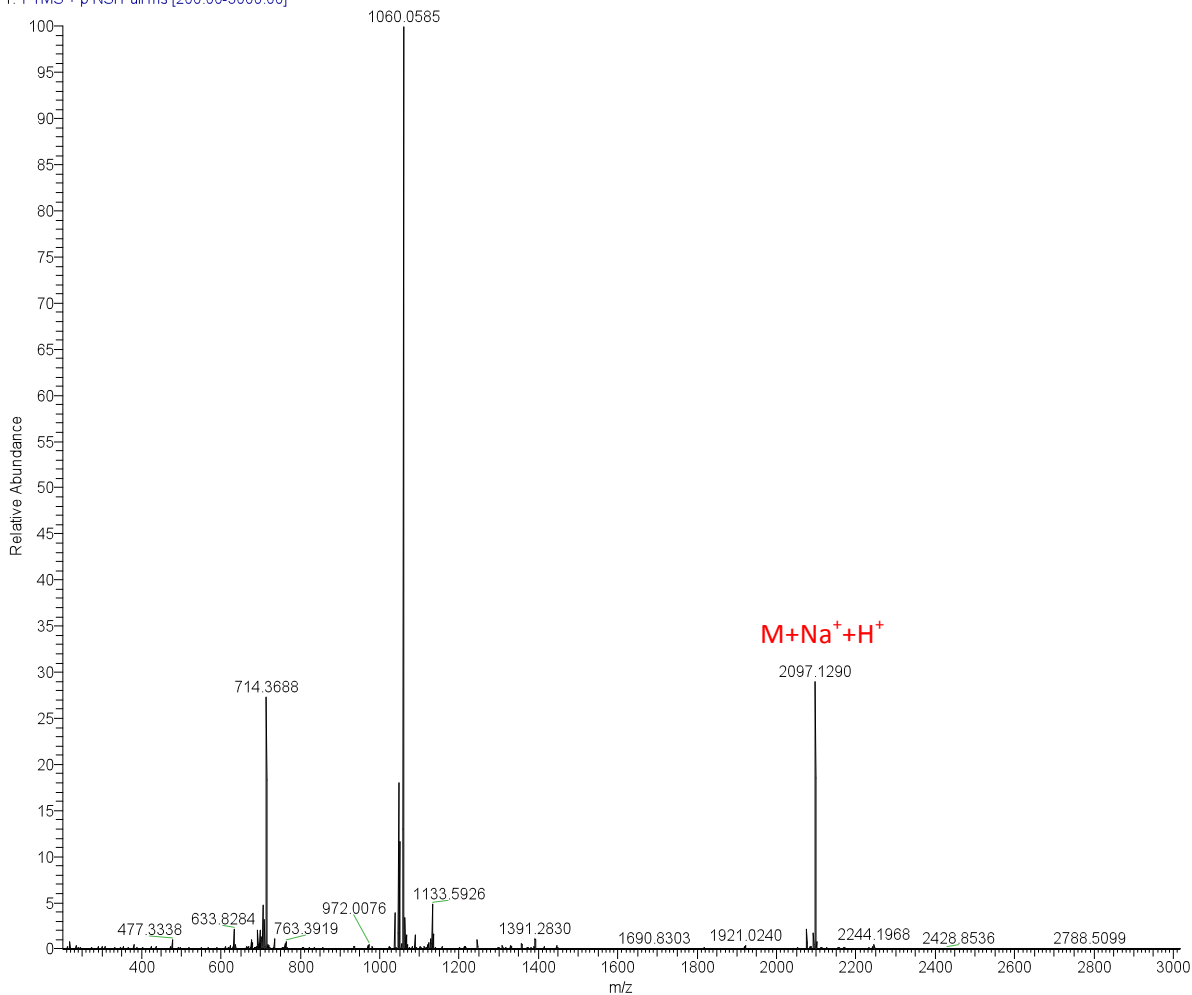
Supplementary material

HRMS (ESI-TOF) SM122-PEG-9 DIMER

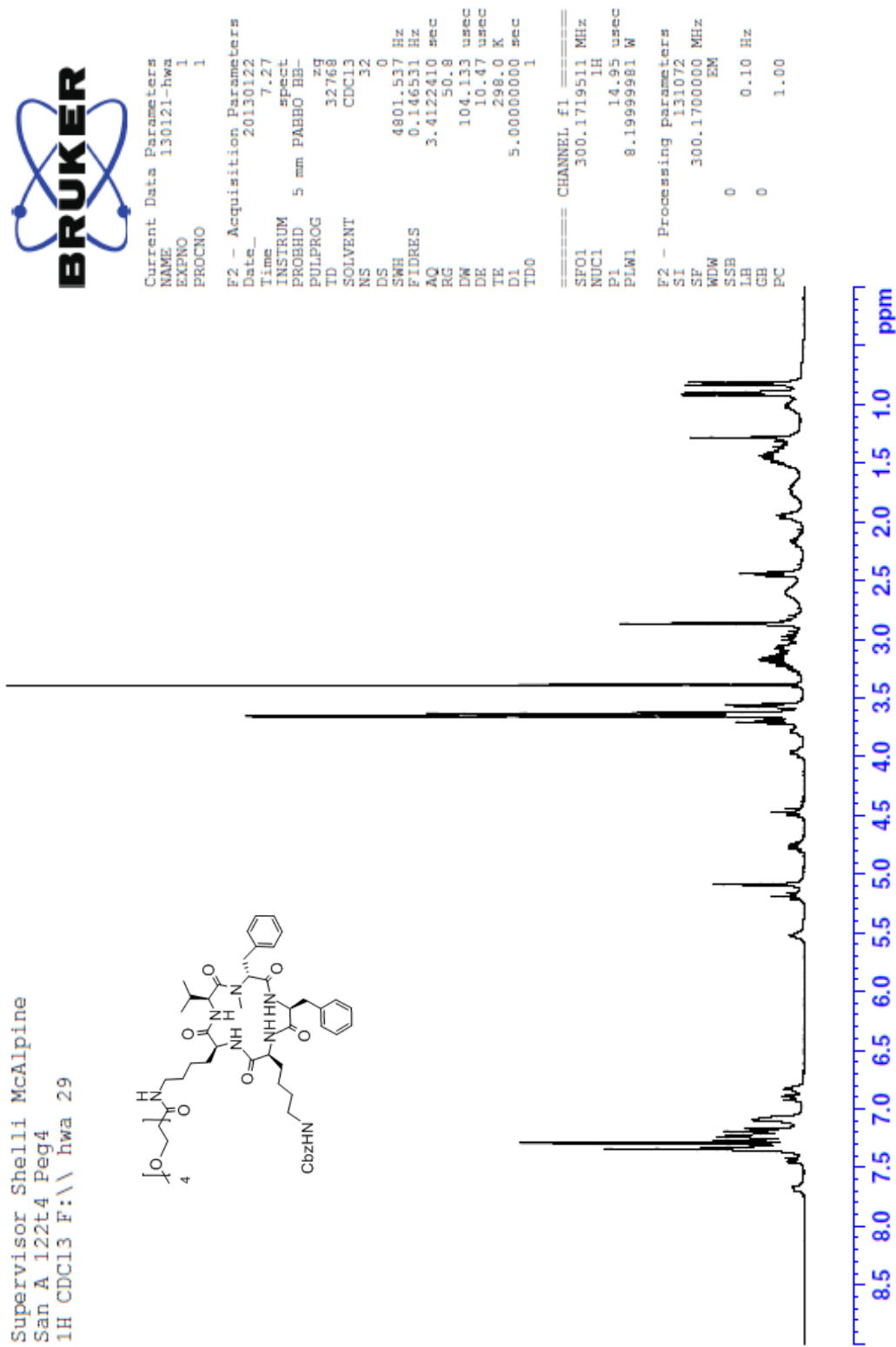
Exact mass=2097.1342, found 2097.1290 ($M + Na^+ + H^+$)



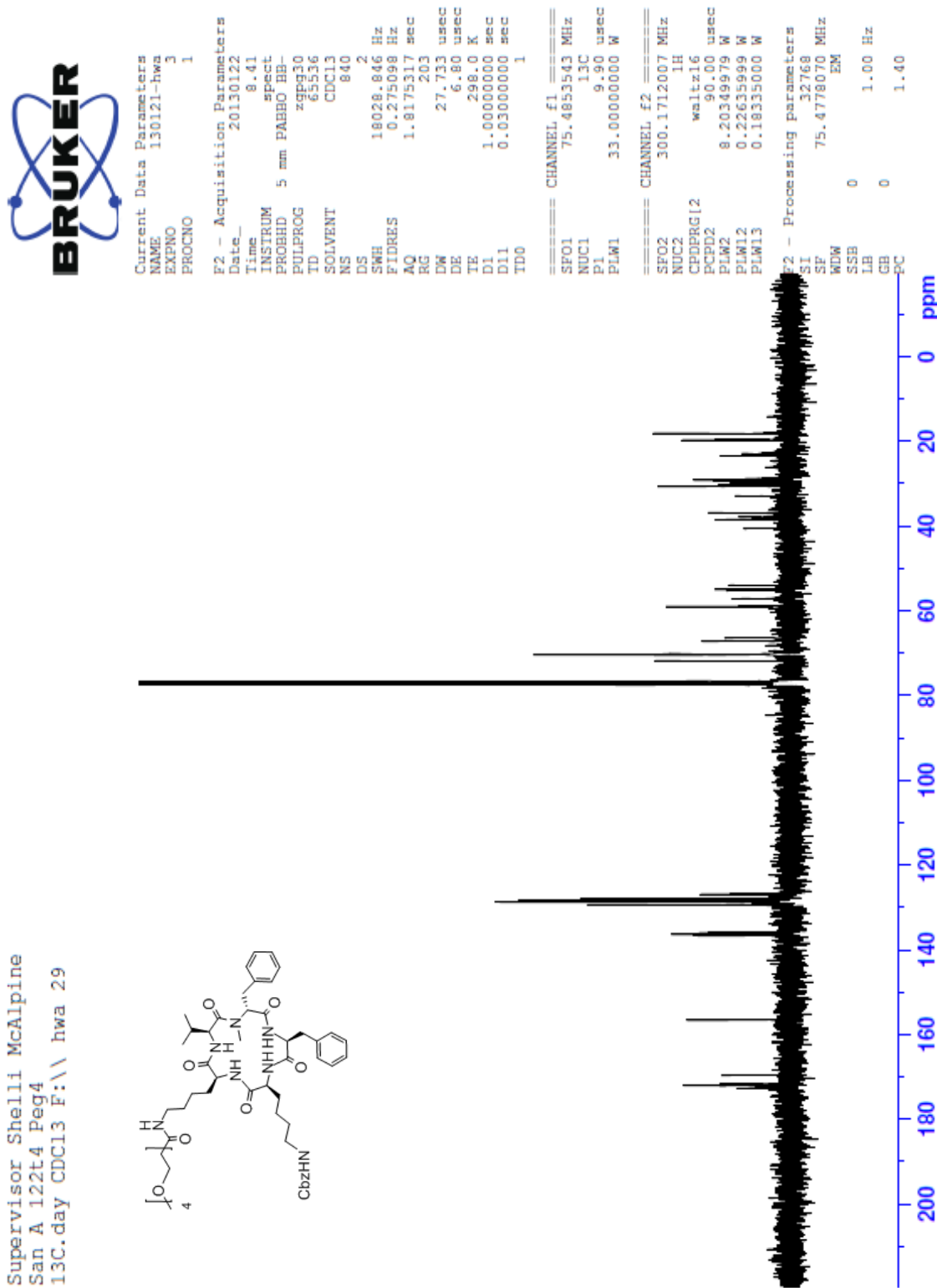
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T: FTMS + p NSI Full ms [200.00-3000.00]



¹H NMR SM122-PEG-4



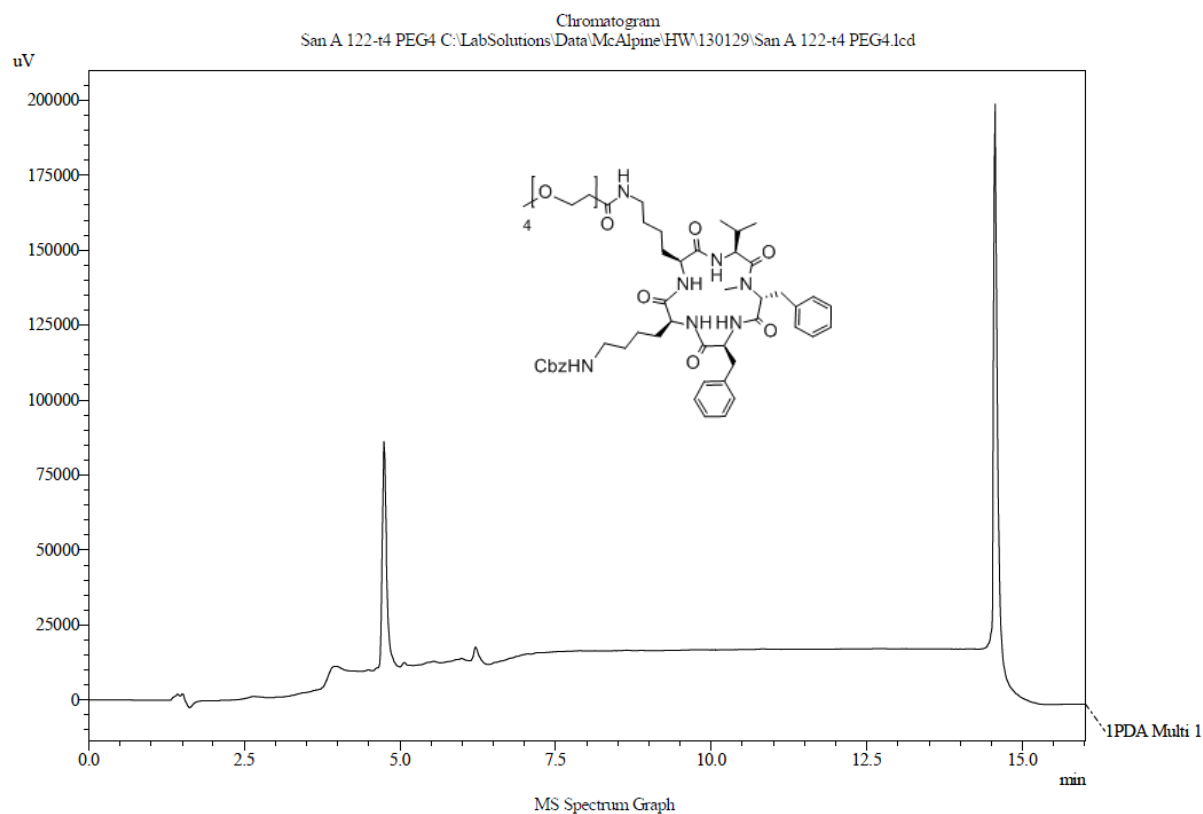
¹³C NMR SM122-PEG-4



Supplementary material

LCMS-ESI/UV SM122-PEG-4

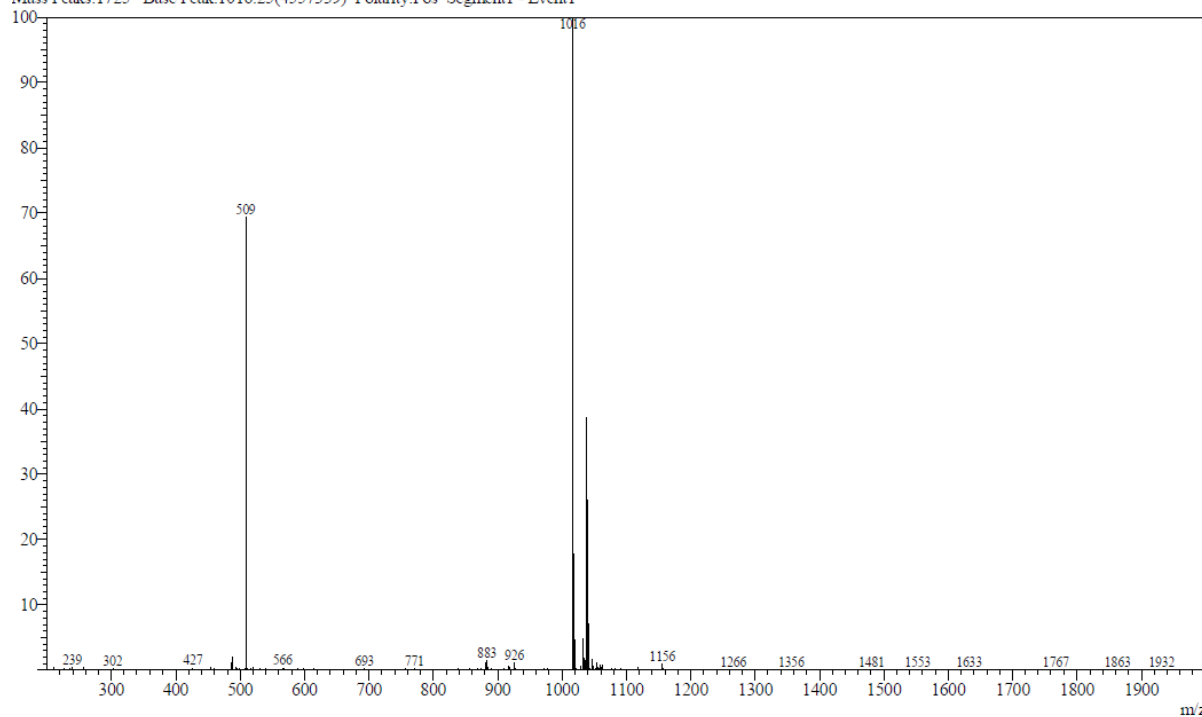
==== Shimadzu LCMSsolution Analysis Report ====



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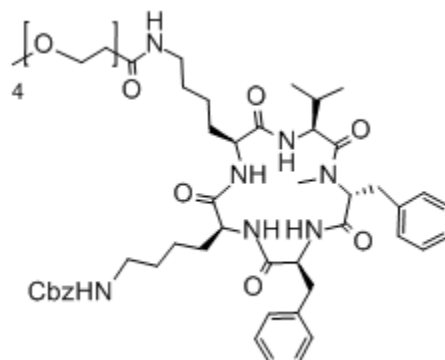
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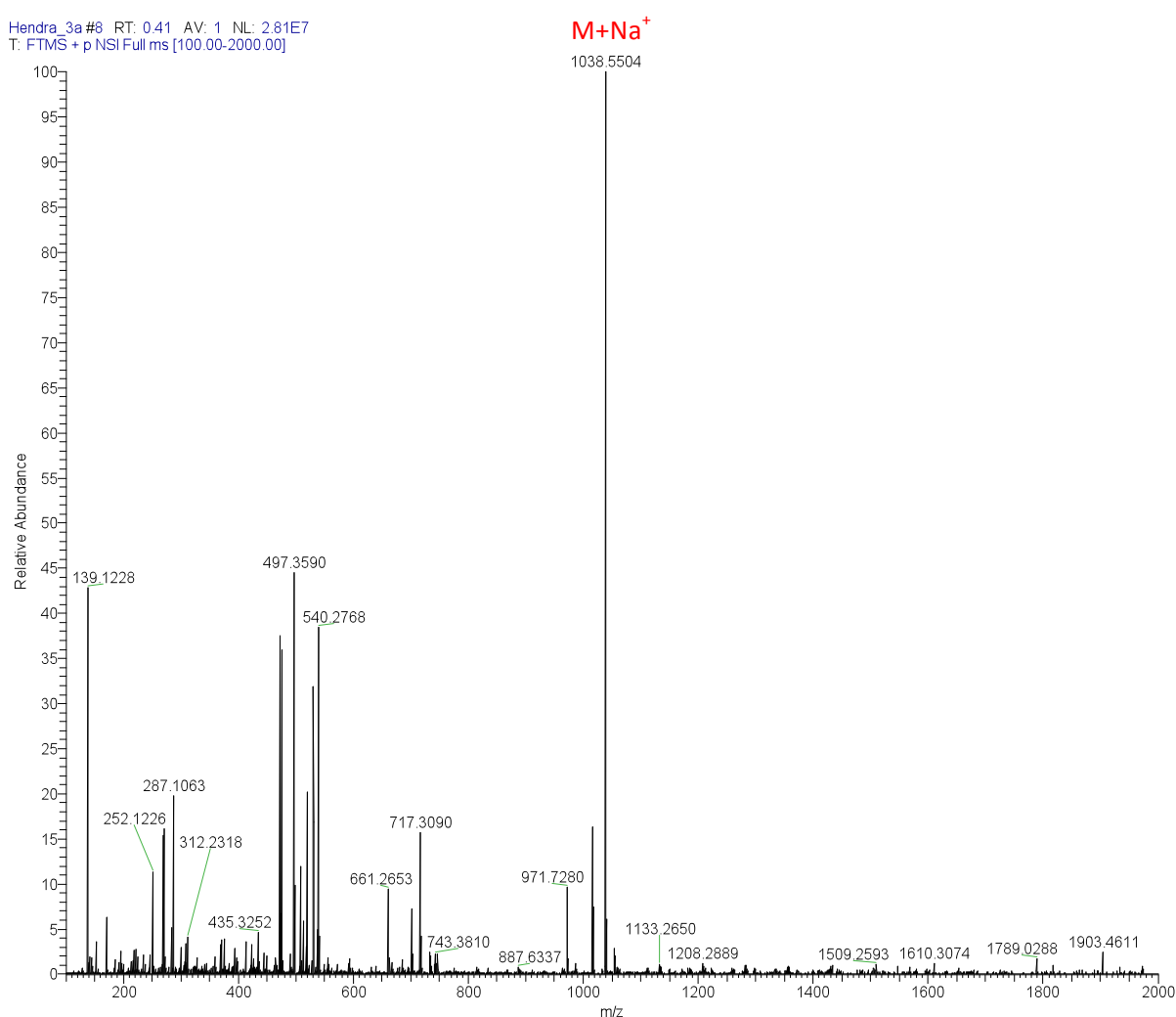
Supplementary material

HRMS (ESI-TOF) SM122-PEG-4

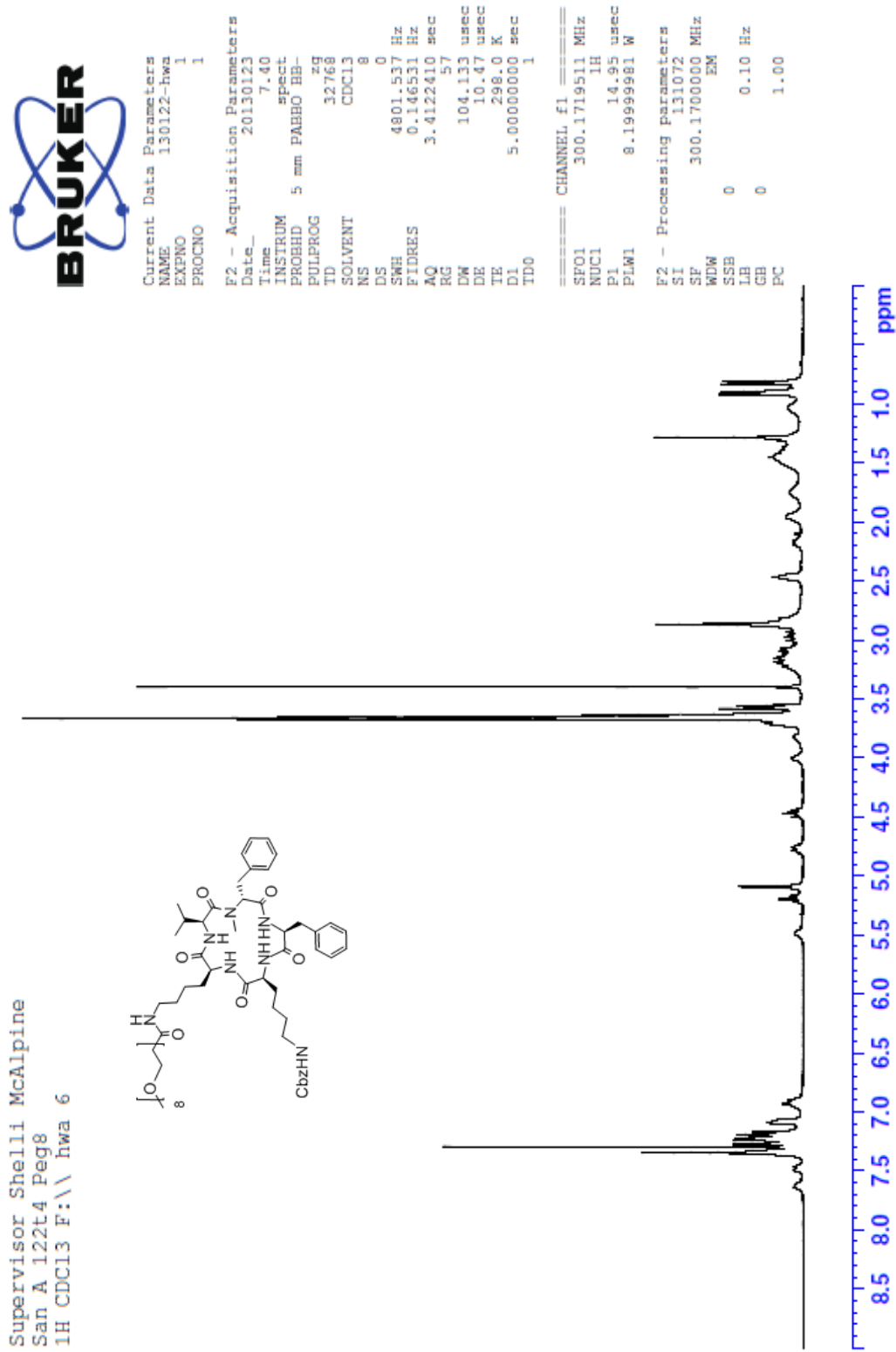
Exact mass=1038.5528, found 1038.5504 ($M + Na^+$)



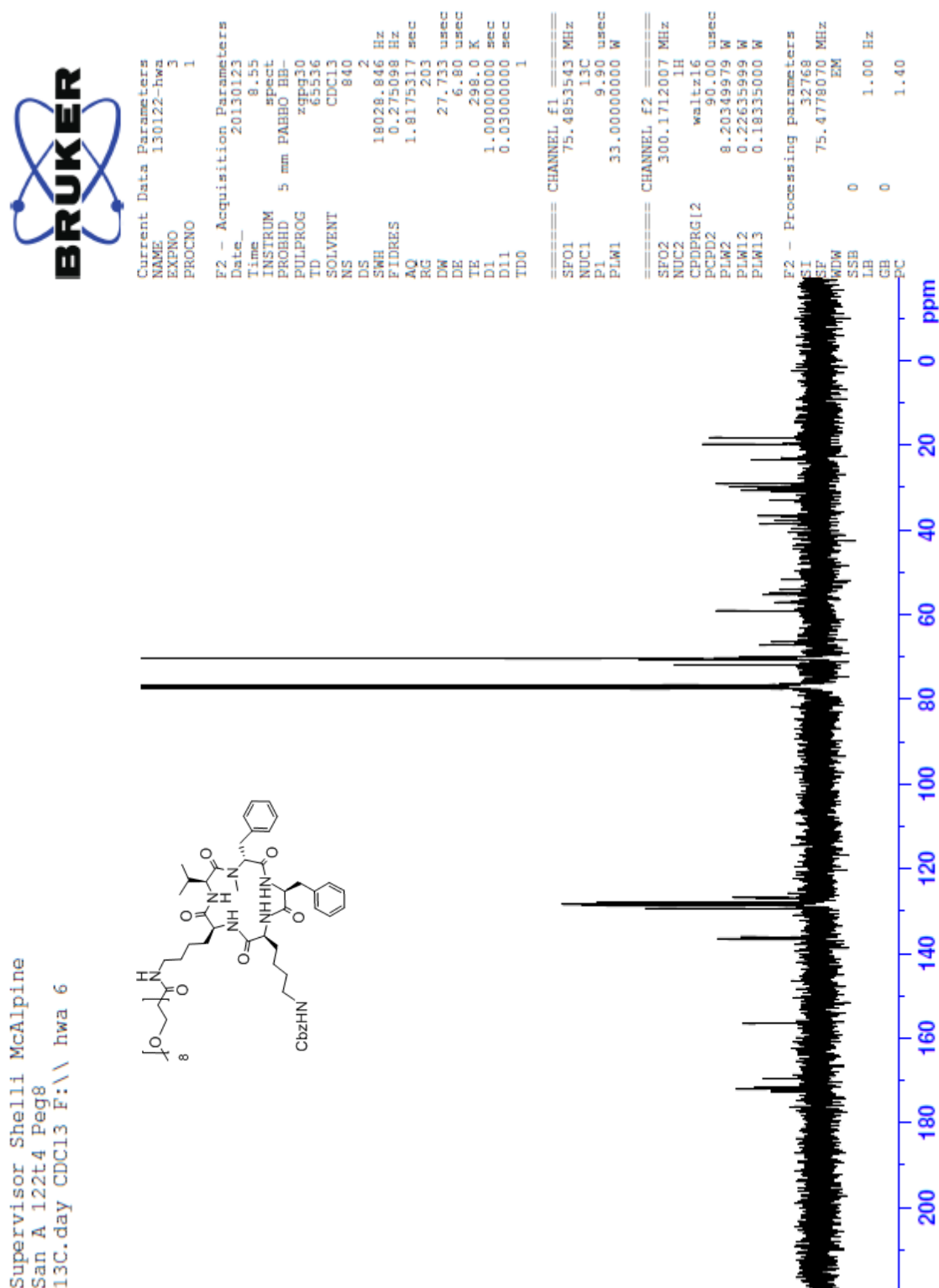
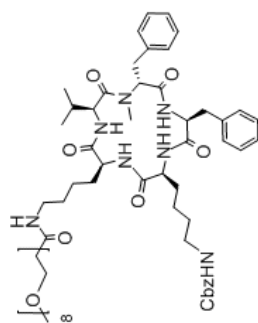
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¹H NMR SM122-PEG-8

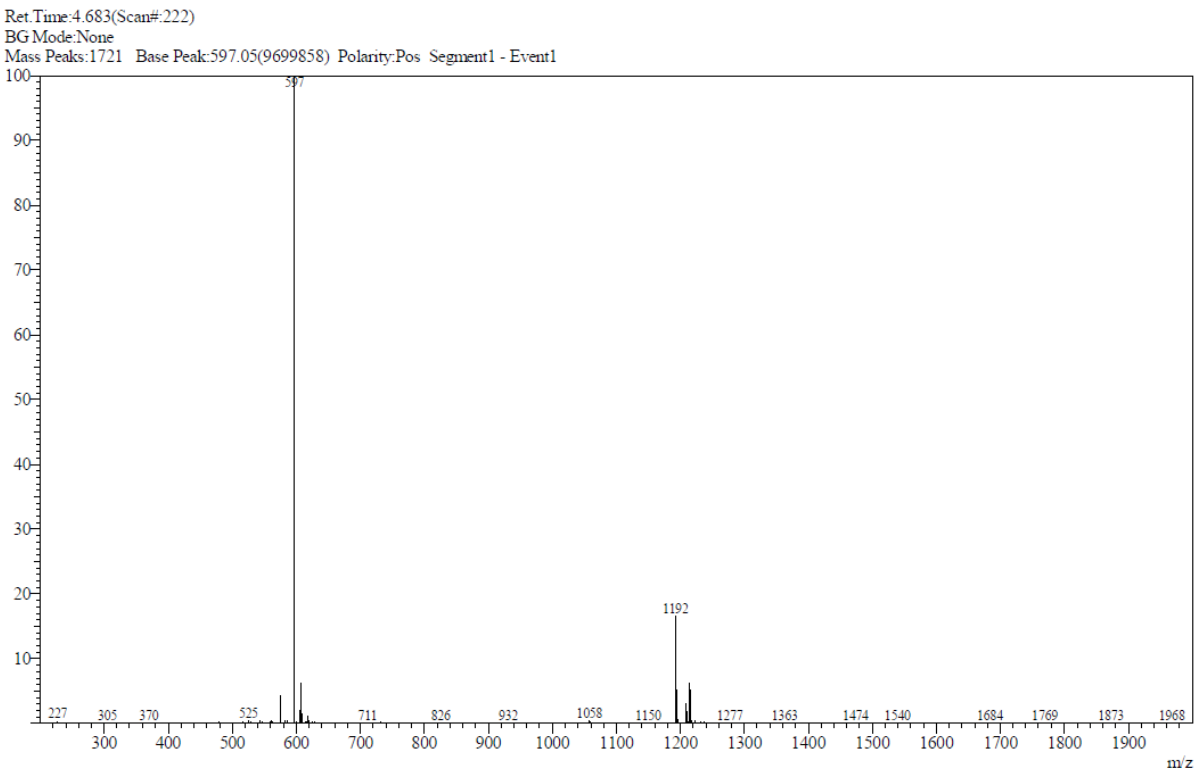
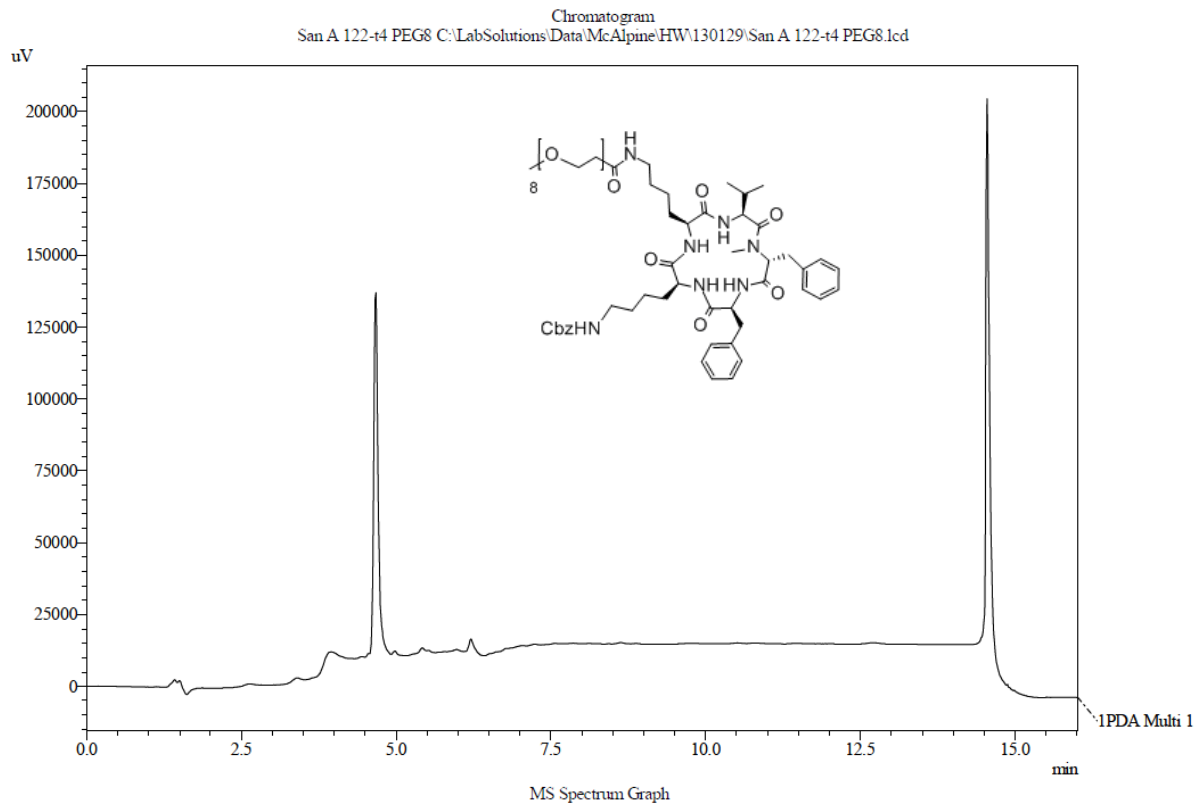


Supervisor Shelli McAlpine
San A 122t4 Peg8
13C.day CDC13 F:\ hwa 6



Supplementary material

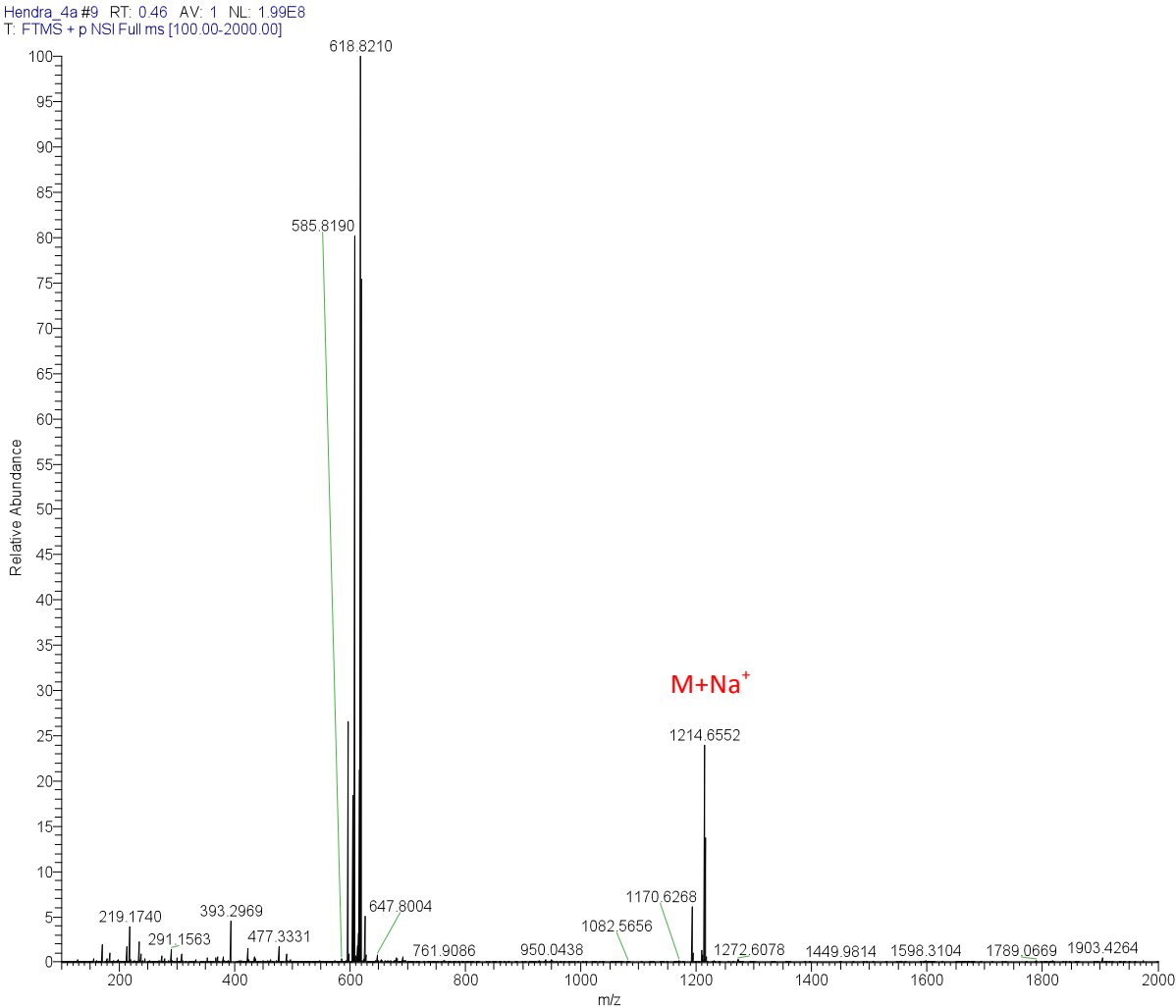
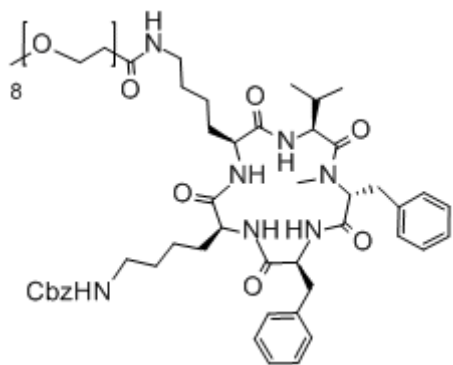
LCMS-ESI/UV SM122-PEG-8
==== Shimadzu LCMSsolution Analysis Report ====



Supplementary material

HRMS (ESI-TOF) SM122-PEG-8

Exact mass=1214.6577, found 1214.6552 (M + Na⁺)



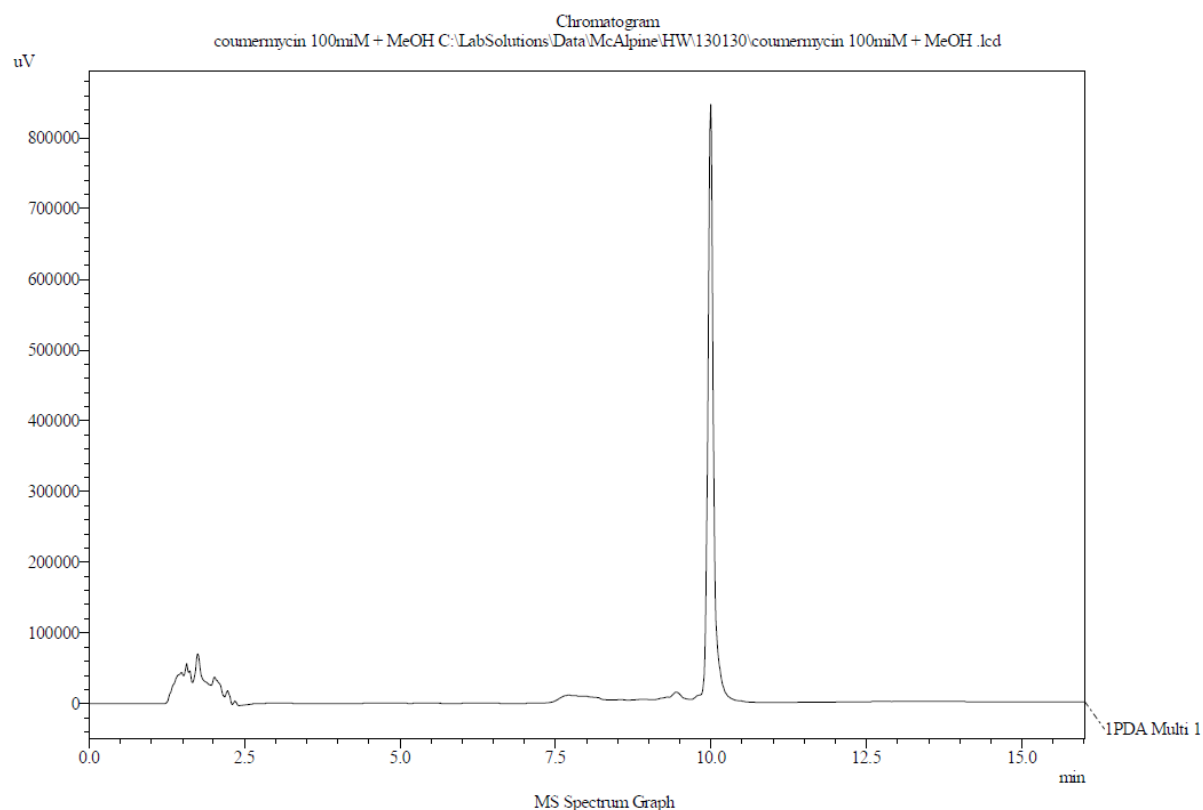
Supplementary material

Solubility Data

Internal Standard Coumermycin A1 (10 μ M; MW: 1110.08)

Supplementary material

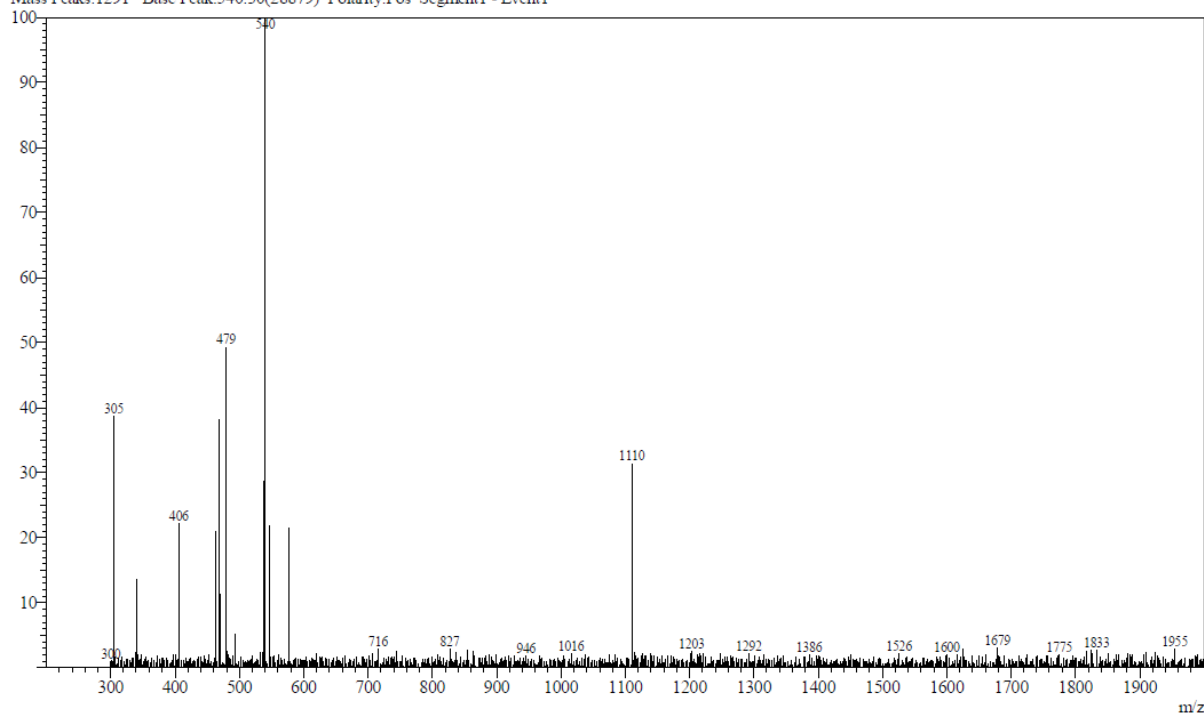
==== Shimadzu LCMSsolution Analysis Report ====



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BG Mode:None

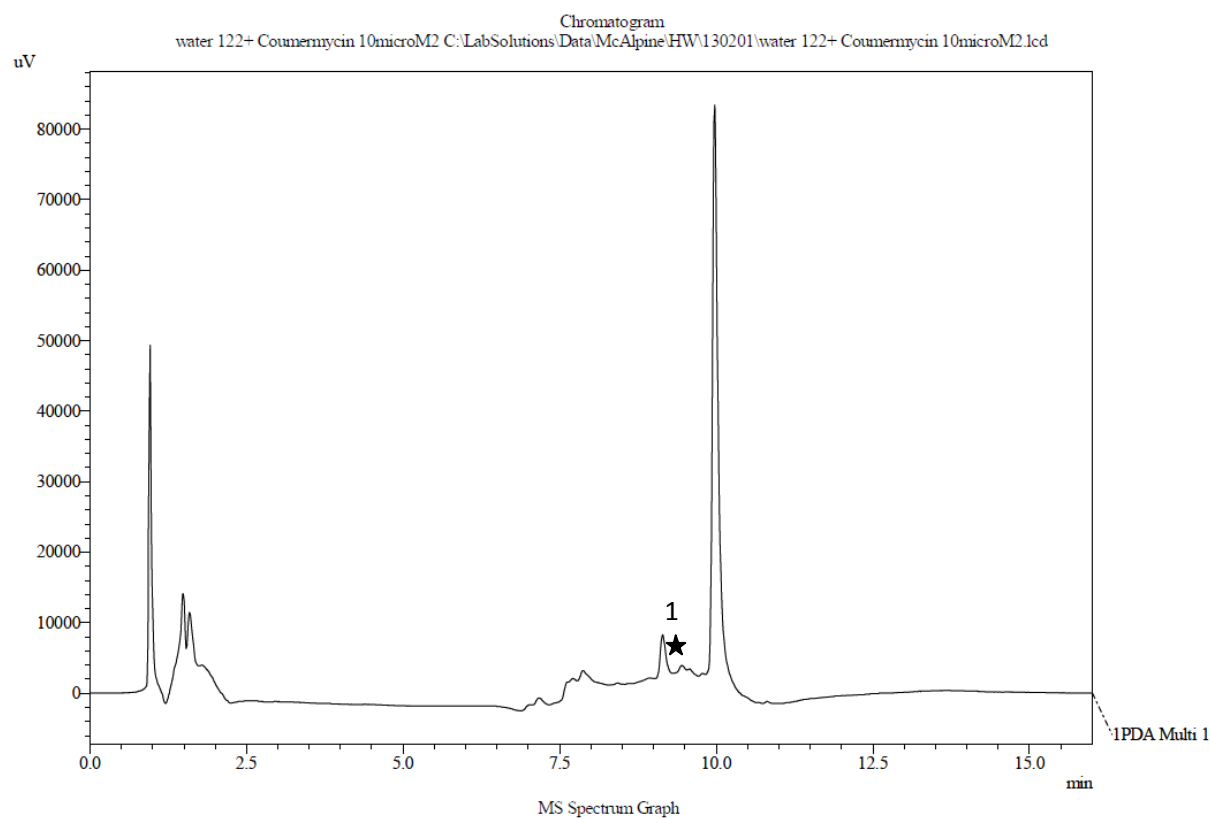
Mass Peaks:1291 Base Peak:540.30(28879) Polarity:Pos Segment1 - Event1



Co-injection of Coumermycin A1 (10 μ M) and SM122 (MW: 782.44) solubility sample

Supplementary material

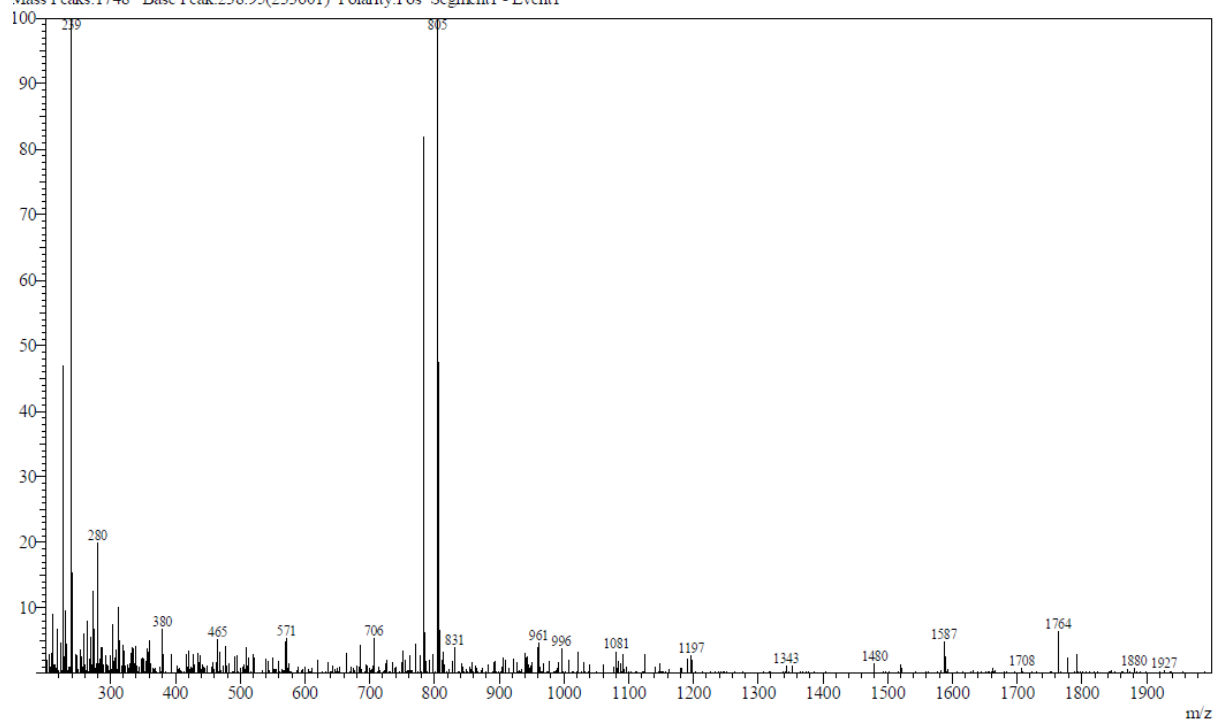
==== Shimadzu LCMSsolution Analysis Report ====



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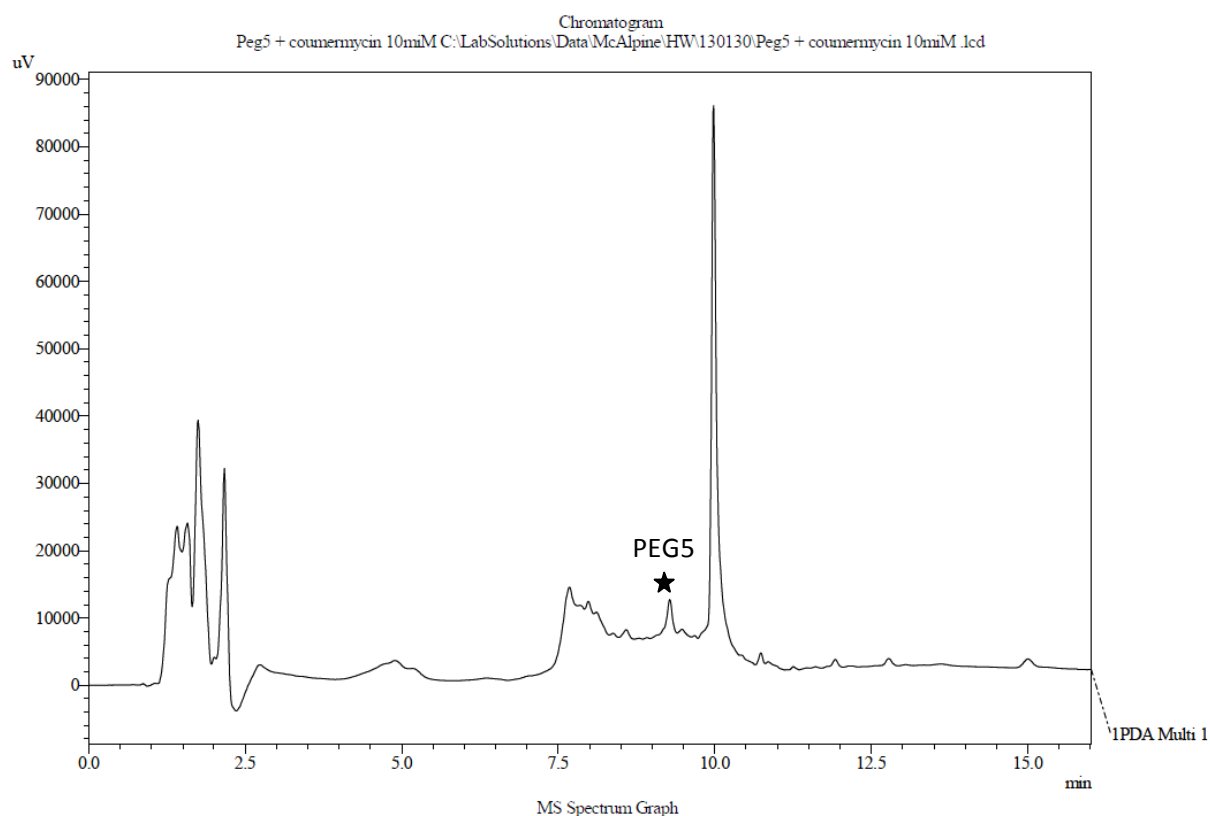
Mass Peaks:1748 Base Peak:238.95(233601) Polarity:Pos Segment1 - Event1



Co-injection of Coumermycin A1 (10 μ M) and SM122-PEG-5 DIMER solubility sample

Supplementary material

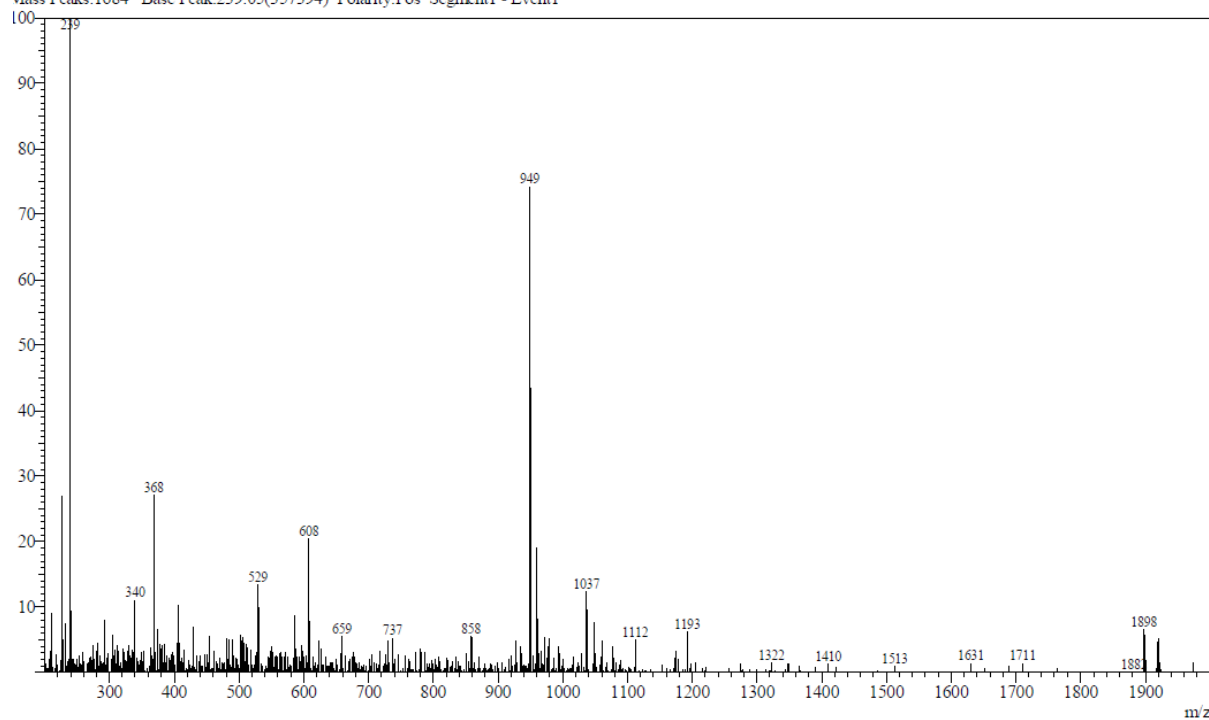
==== Shimadzu LCMSsolution Analysis Report ====



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3G Mode:None

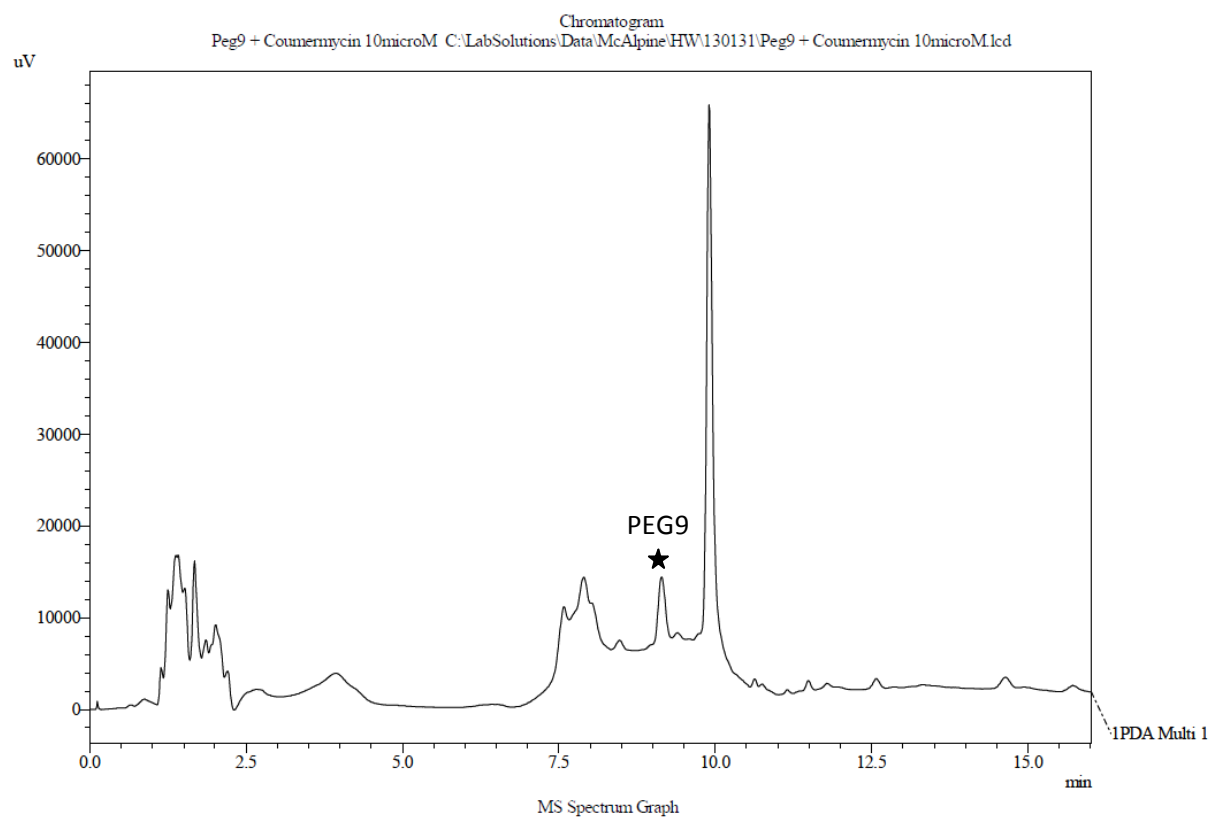
Mass Peaks:1684 Base Peak:239.05(357394) Polarity:Pos Segment1 - Event1



Co-injection of Coumermycin A1 (10 μ M) and SM122-PEG-9 DIMER solubility sample

Supplementary material

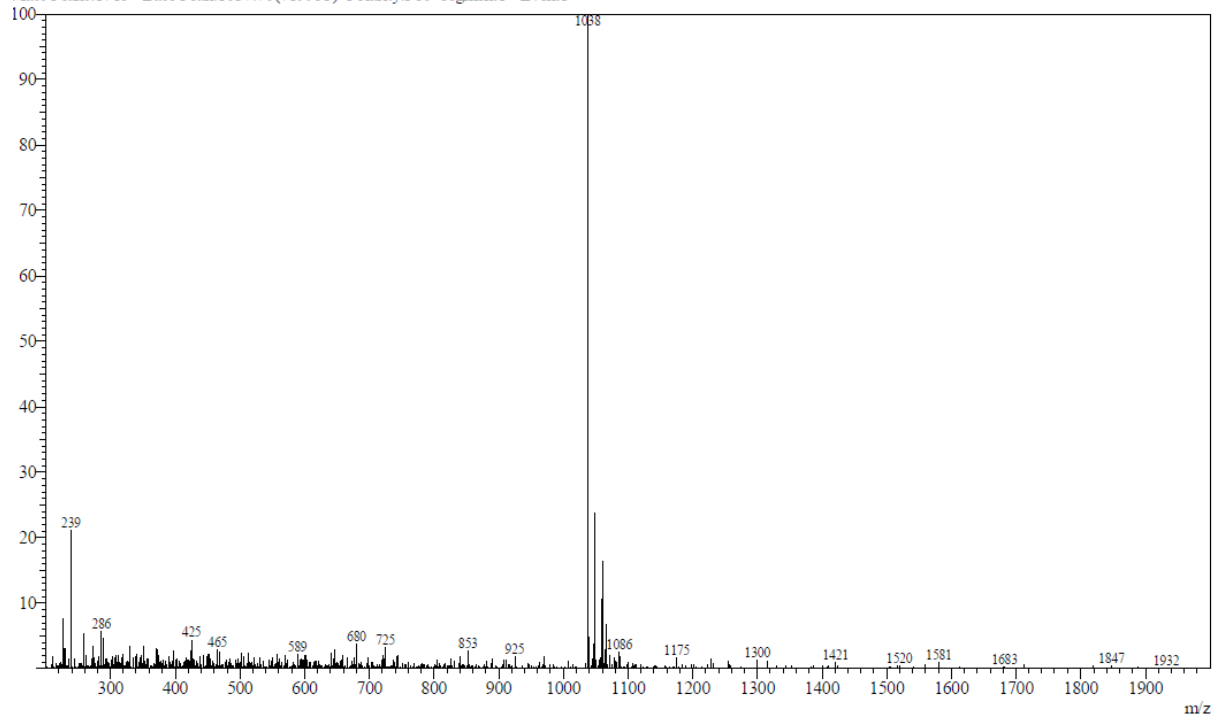
==== Shimadzu LCMSsolution Analysis Report ====



Ret.Time:9.100(Scan#:487)

B/G Mode:None

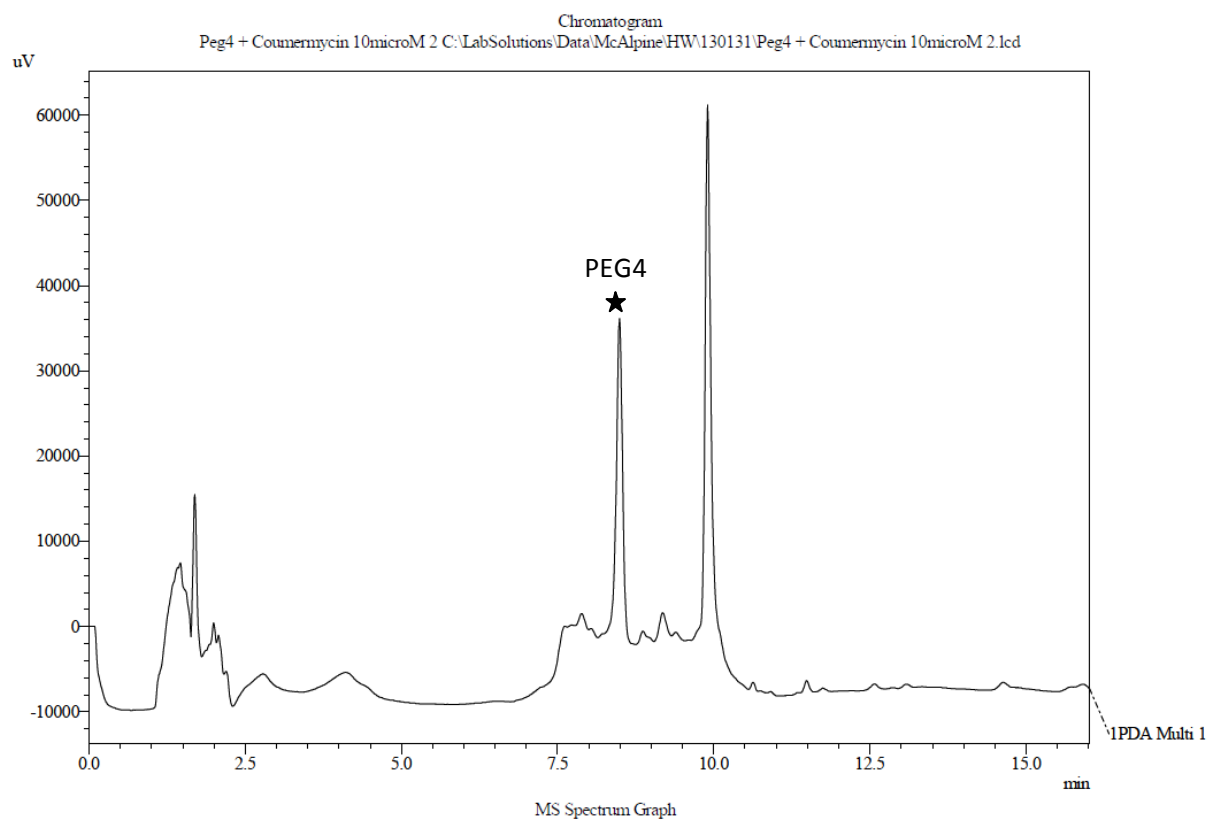
Mass Peaks:1715 Base Peak:1037.70(719953) Polarity:Pos Segment1 - Event1



Co-injection of Coumermycin A1 (10 μ M) and SM122-PEG4 solubility sample

Supplementary material

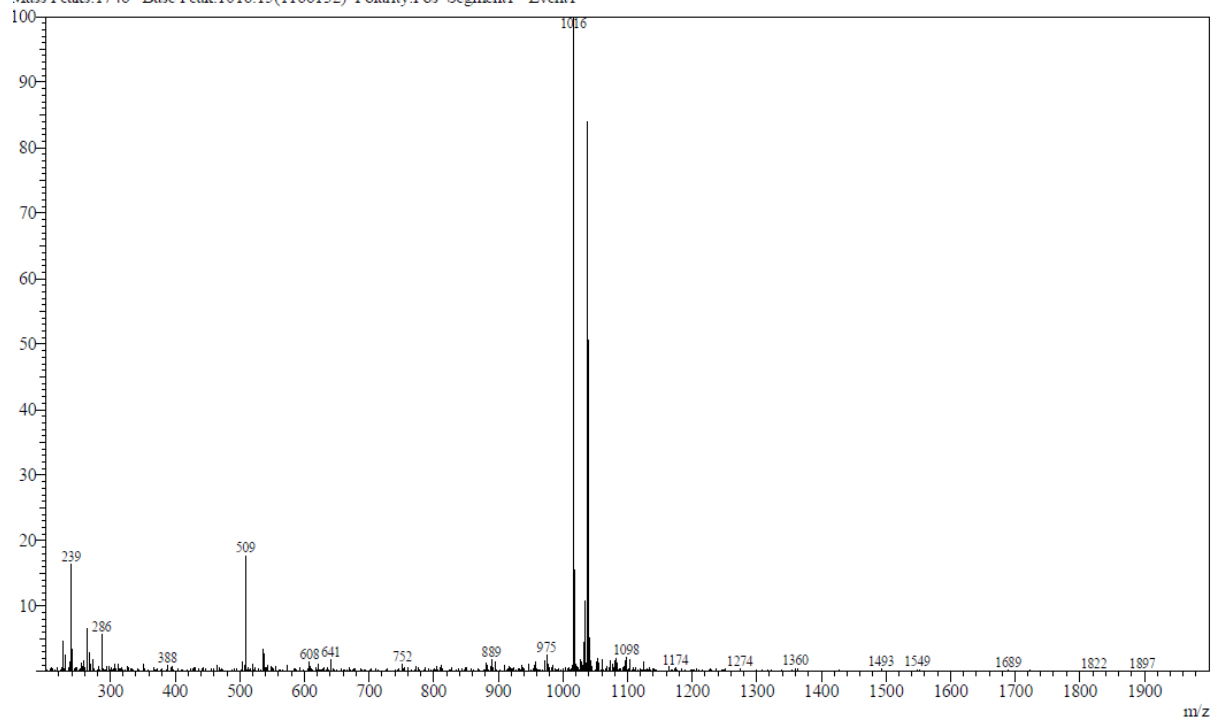
==== Shimadzu LCMSsolution Analysis Report ====



Ret.Time:8.400(Scan#:445)

BG Mode:?

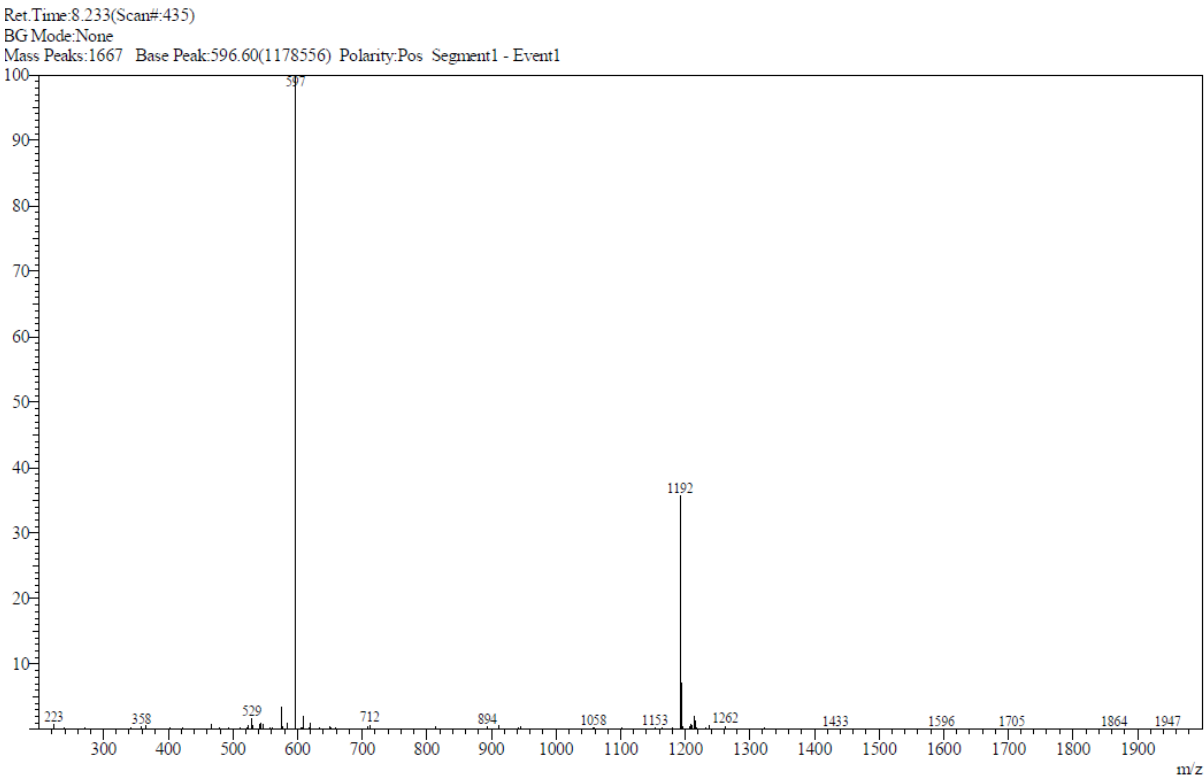
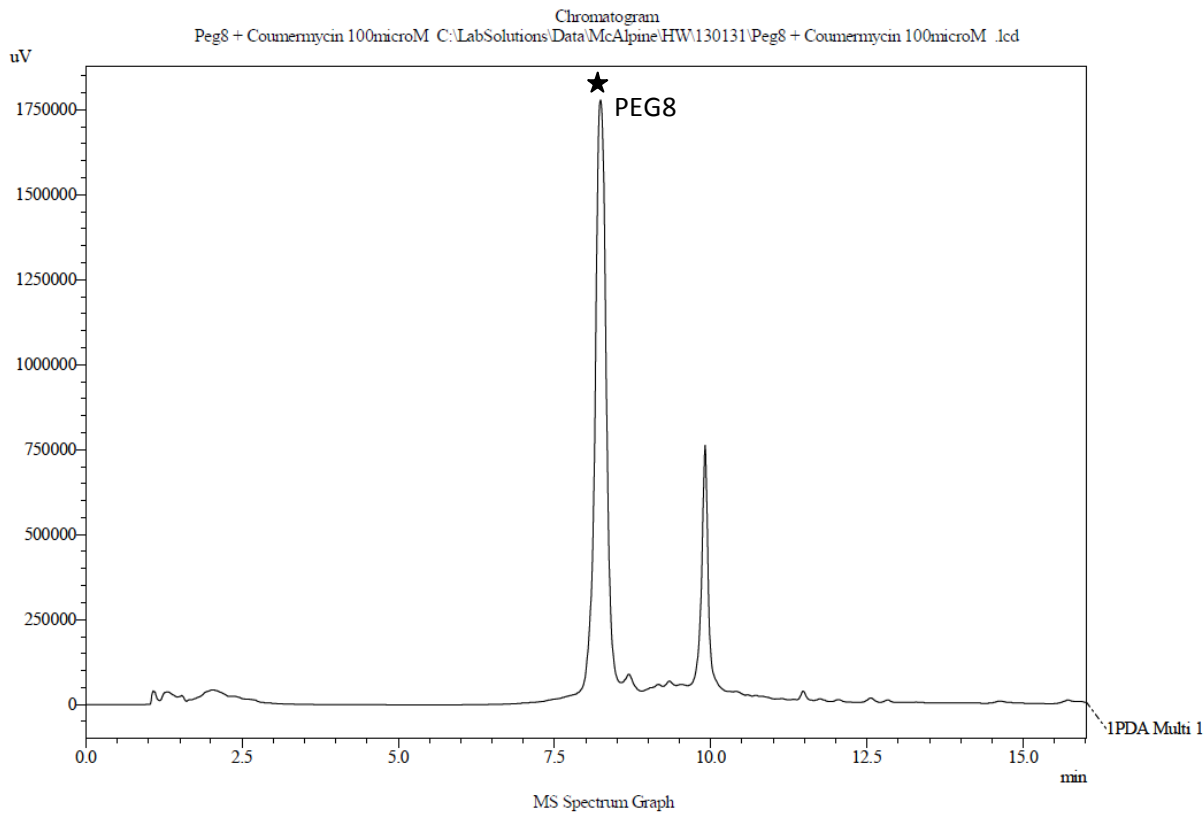
Mass Peaks:1746 Base Peak:1016.15(1166132) Polarity:Pos Segment1 - Event1



Co-injection of Coumermycin A1 (100 μ M) and SM122-PEG8 solubility sample

Supplementary material

==== Shimadzu LCMSsolution Analysis Report ====



Supplementary material

Supplemental Table 1: Peak area values

Test	CA-1	SM122-PEG-4	SM122-PEG-5	SM122-PEG-8	SM122-PEG-9	SM122
	DIMER			DIMER		
1	847455	514647	---	---	---	---
2	718067	484622	---	---	---	---
3	694257	433552	---	---	---	---
1	698723	---	285549	---	---	---
2	717285	---	240109	---	---	---
3	651601	---	236551	---	---	---
1	6995989	---	---	11897539	---	---
2	6799004	---	---	11119149	---	---
3	5775530	---	---	9614665	---	---
1	869070	---	---	---	350779	---
2	524656	---	---	---	263268	---
3	526123	---	---	---	299311	---
1	581530	---	---	---	---	41858
2	542472	---	---	---	---	66946
3	805189	---	---	---	---	45169

Supplemental Table 2: Solubility values

Compound	($\mu\text{g/mL}$)	(μM)
SM122	0.66 ± 0.28	0.84 ± 0.35
SM122-PEG-4	6.45 ± 0.36	6.36 ± 0.35
SM122-PEG-5 DIMER	7.00 ± 0.71	3.69 ± 0.37
SM122-PEG-8	198.64 ± 3.89	166.69 ± 3.27
SM122-PEG-9 DIMER	10.18 ± 1.72	4.91 ± 0.83

Calculation method:

Solubility (μM) = (peak area of compound/ peak area of CA-1) $\times$ concentration of CA-1*

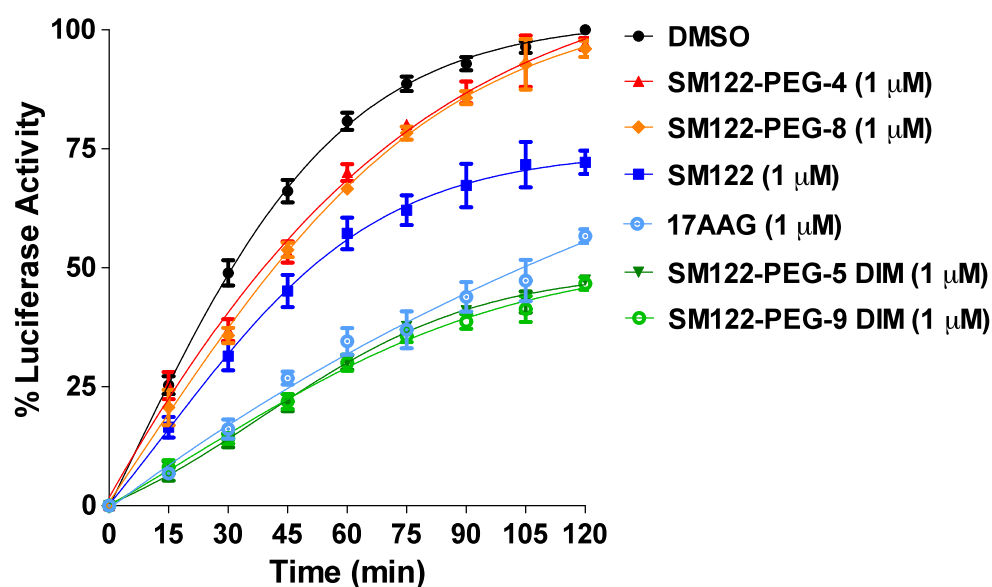
Solubility ($\mu\text{g/mL}$) = Solubility (μM) $\times$ MW of compound (g/mol) $\times 10^{-3}$ (L/mL) $\times 10^6$ ($\mu\text{g/g}$)

* For compound SM122, SM122-PEG-5 DIMER, SM122-PEG-9 DIMER, and SM122-PEG-4, concentration of CA-1 is 10 μM ; for SM122-PEG-8, concentration of CA-1 is 100 μM .

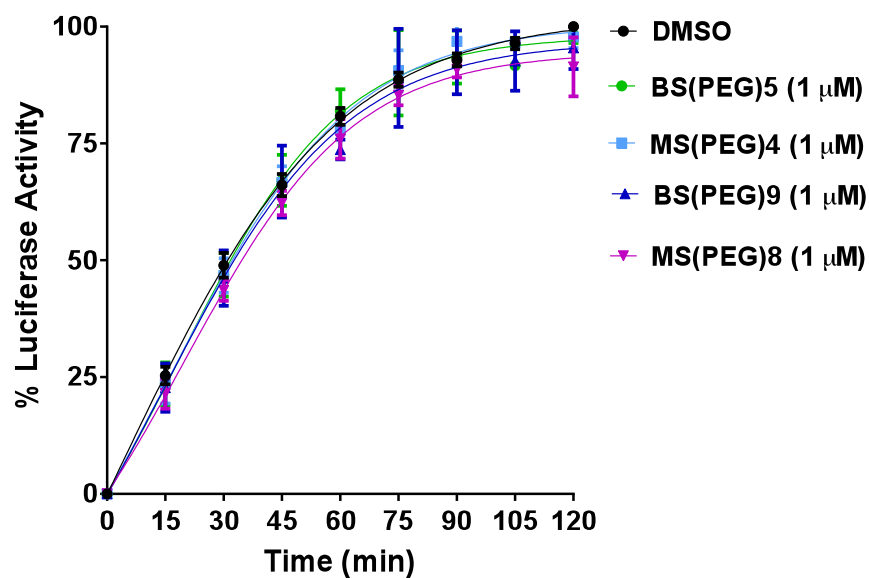
Solubility Method: ~5 mg of SM122-PEG conjugates or DIMERs was stirred in 0.3 mL of Milli-Q water for over 24 hours. The filtrate was filtered through a 45 micron filter to remove all insoluble particulates. The concentration of each sample was measured by LCMS utilizing Coumermycin A1 (CA-1) with known concentrations as the internal standard. The solubility value in water was then calculated (Table 1).

Biological data

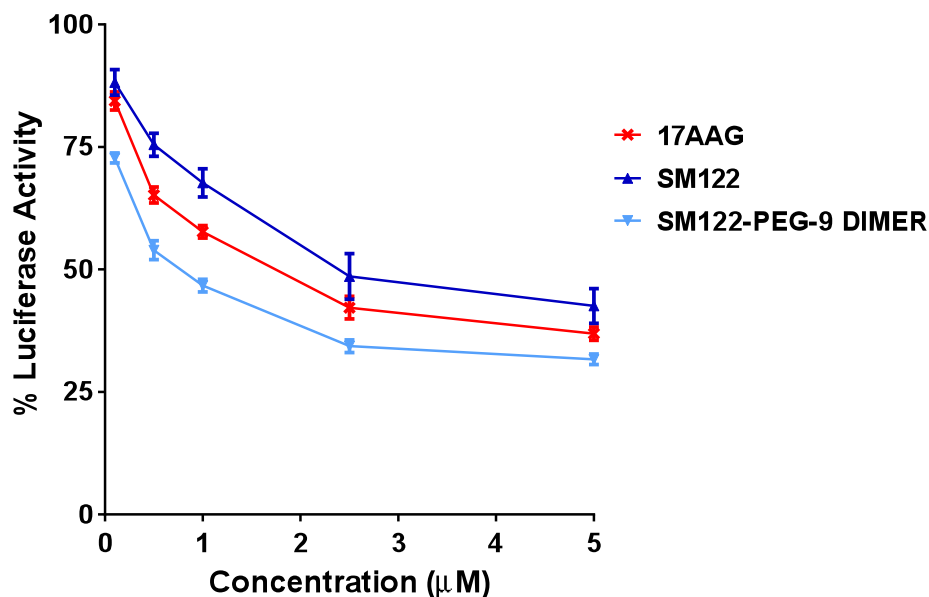
a) Luciferase Refolding Assay in RRL System



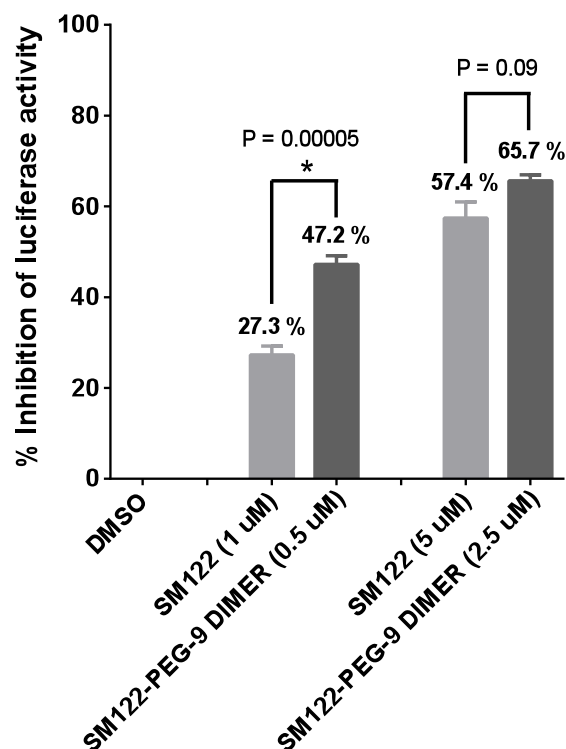
b) Luciferase Refolding Assay in RRL System



**c) Luciferase Refolding Assay
in RRL System (120 min)**



**d) Luciferase Refolding Assay
in RRL System (120 min)**

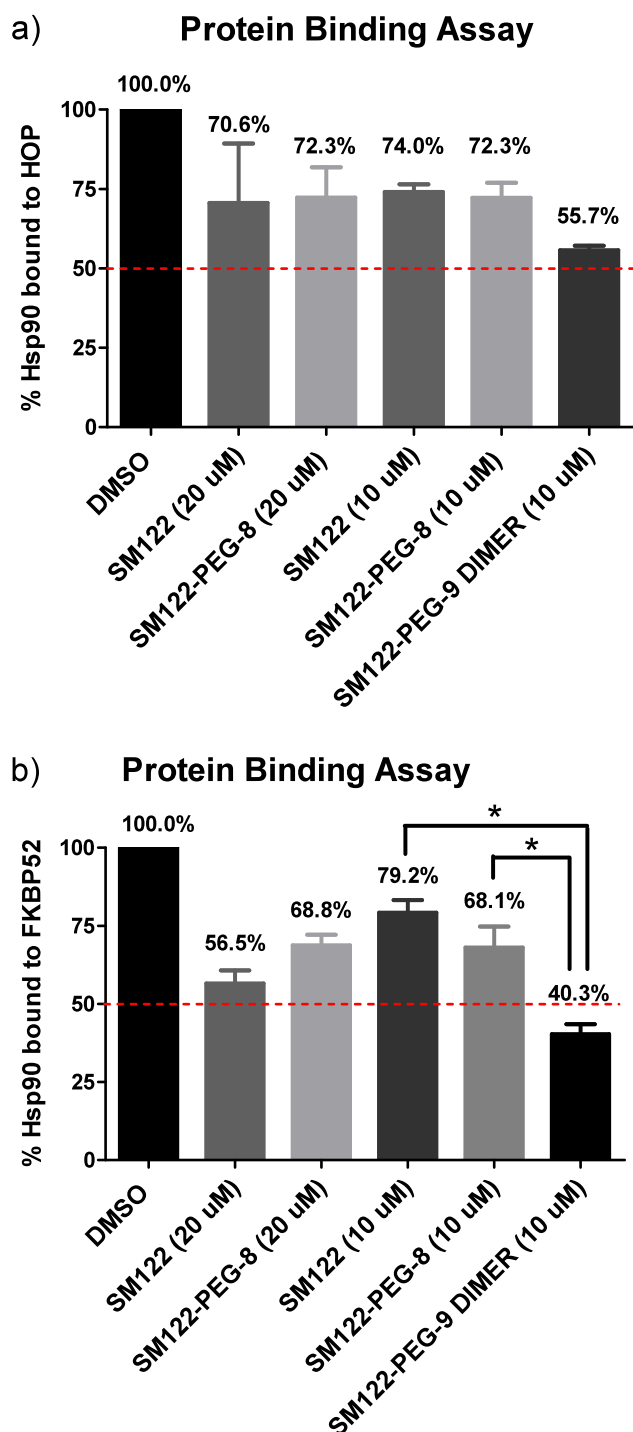


Supplemental Figure 1a-c: Luciferase activity or % inhibition of luciferase activity in rabbit reticulocyte lysate (RRL) refolding system treated with indicated concentrations of 17AAG, SM122, PEG conjugates or DIMERS. RRL was pre-incubated with compounds for 5 hours. Error bars indicate the standard deviation.

Supplementary material

Supplemental Table 3: Inhibition of Luciferase activity.

Compound	Concentration (μM)	% Inhibition of luciferase activity
17AAG	1	43.34 ± 3.15
SM122	1	27.27 ± 4.82
SM122-PEG-4	1	2.94 ± 1.72
SM122-PEG-5 DIMER	1	52.56 ± 1.22
SM122-PEG-8	1	3.63 ± 1.32
SM122-PEG-9 DIMER	1	53.30 ± 2.61



Supplemental Figure 2a-b: The inhibitory effects of 17AAG, SM122, SM122-PEG-8 and SM122-PEG-9 DIMER on the binding between Hsp90 and its co-chaperone HOP and FKBP52.