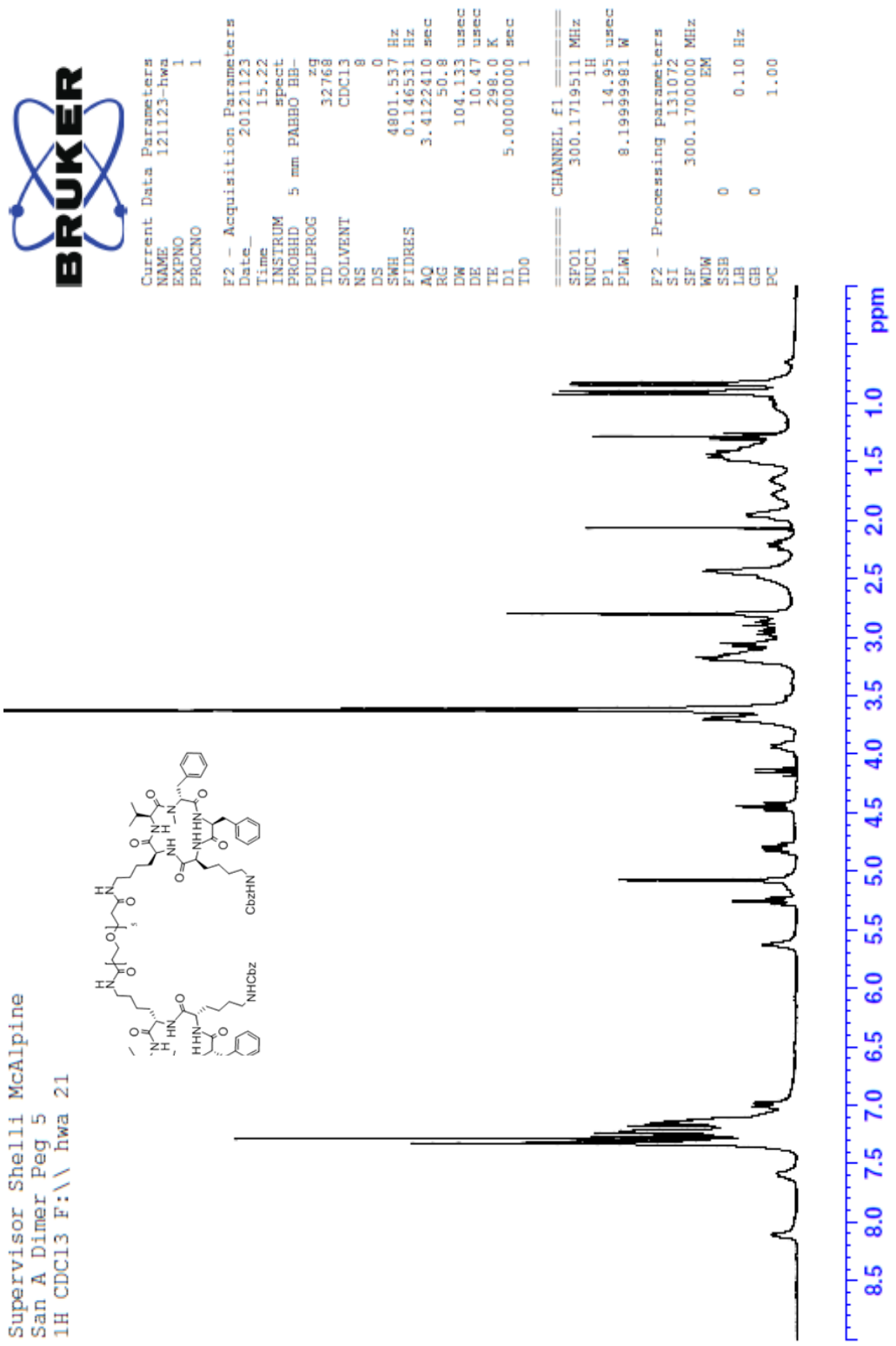
Dimerization of a heat shock protein 90 inhibitor enhances inhibitory activity

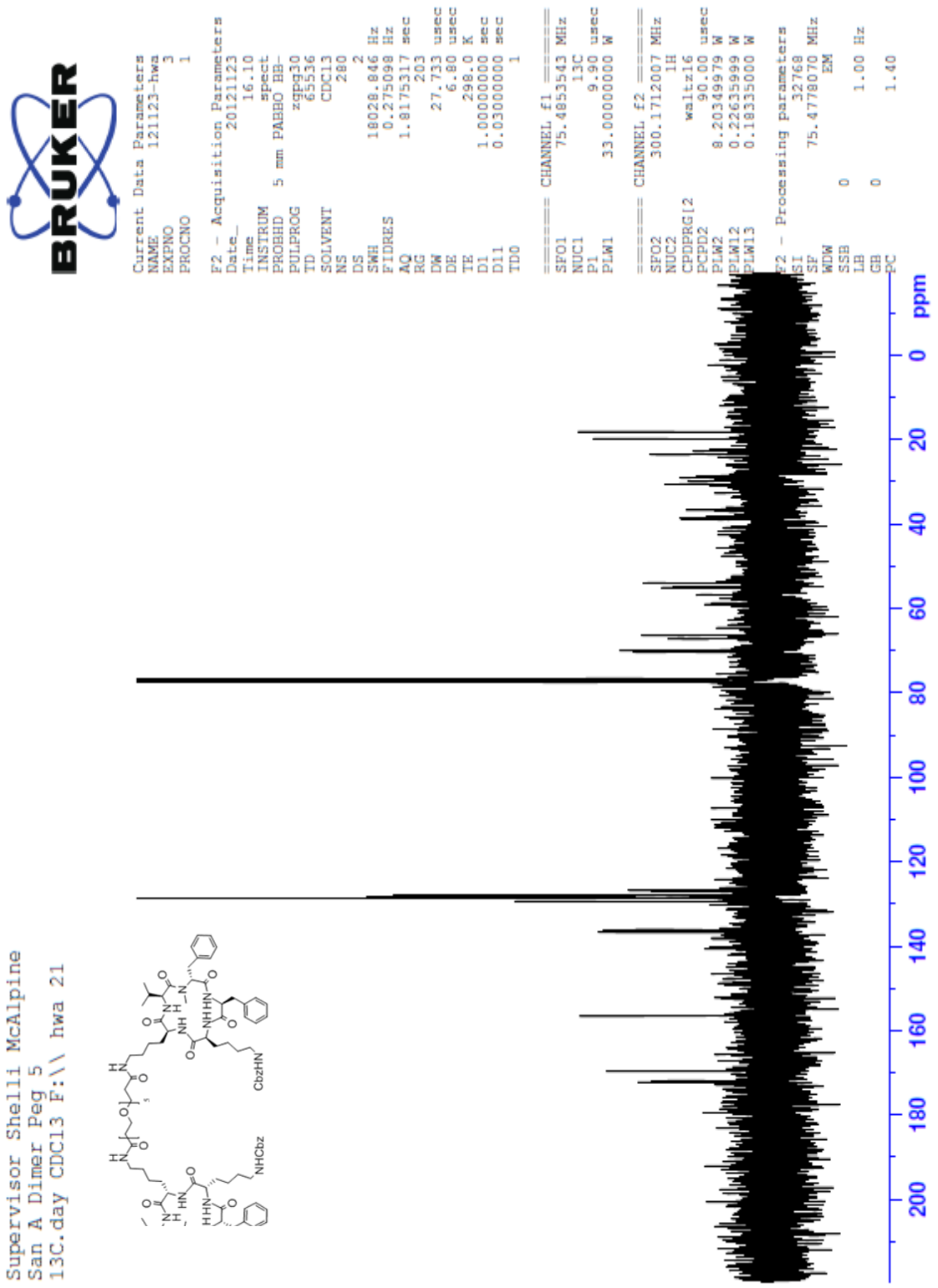
*Hendra Wahyudi, Yao Wang, and Shelli R. McAlpine**
School of Chemistry, University of New South Wales, Sydney, NSW 2052 Australia
s.mcalpine@unsw.edu.au

Contents

^{1}H NMR SM122-PEG-5 DIMER

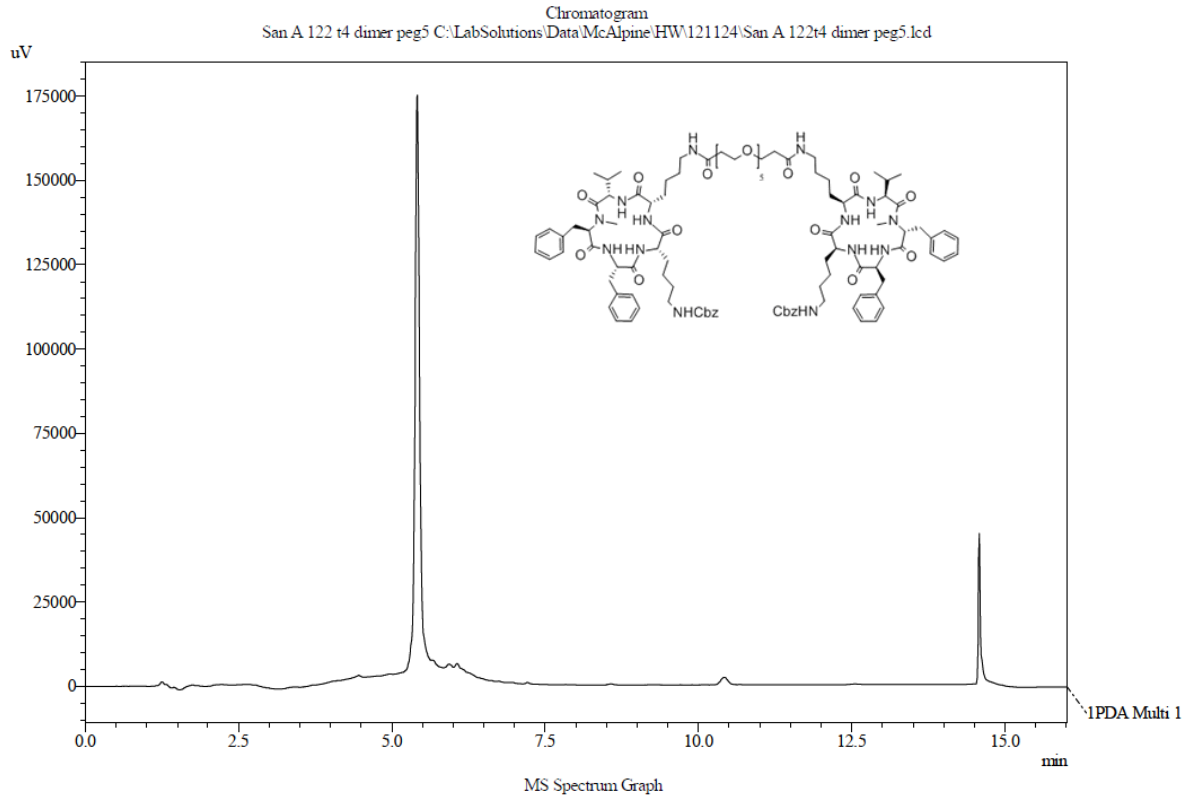


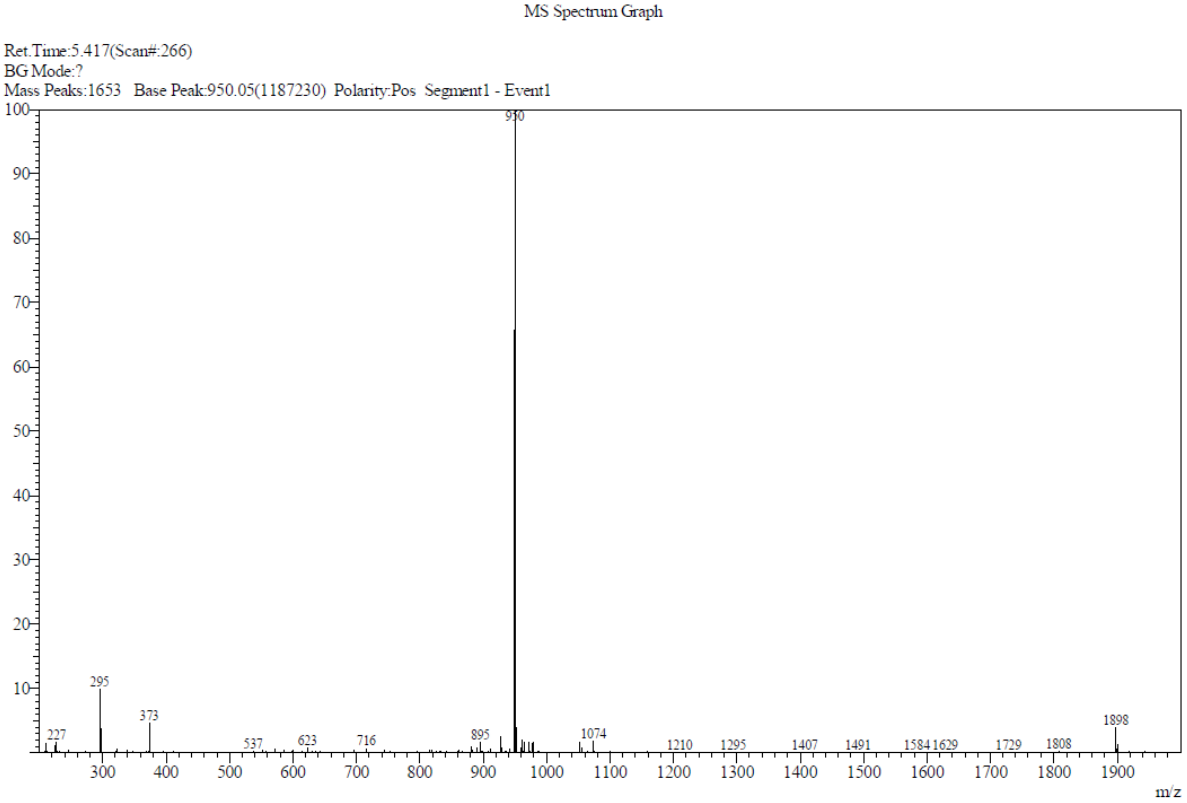
^{13}C NMR SM122-PEG-5 DIMER



LCMS-ESI/UV SM122-PEG-5 DIMER

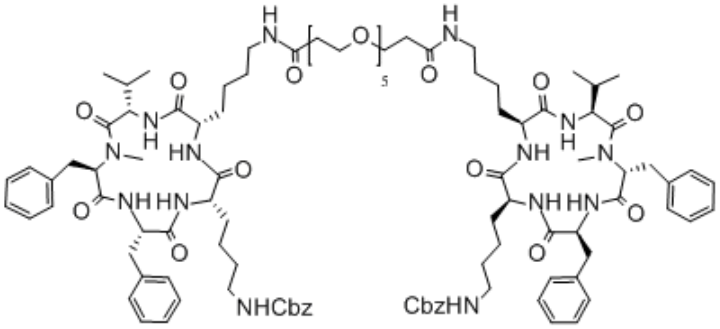
==== Shimadzu LCMSsolution Analysis Report ====

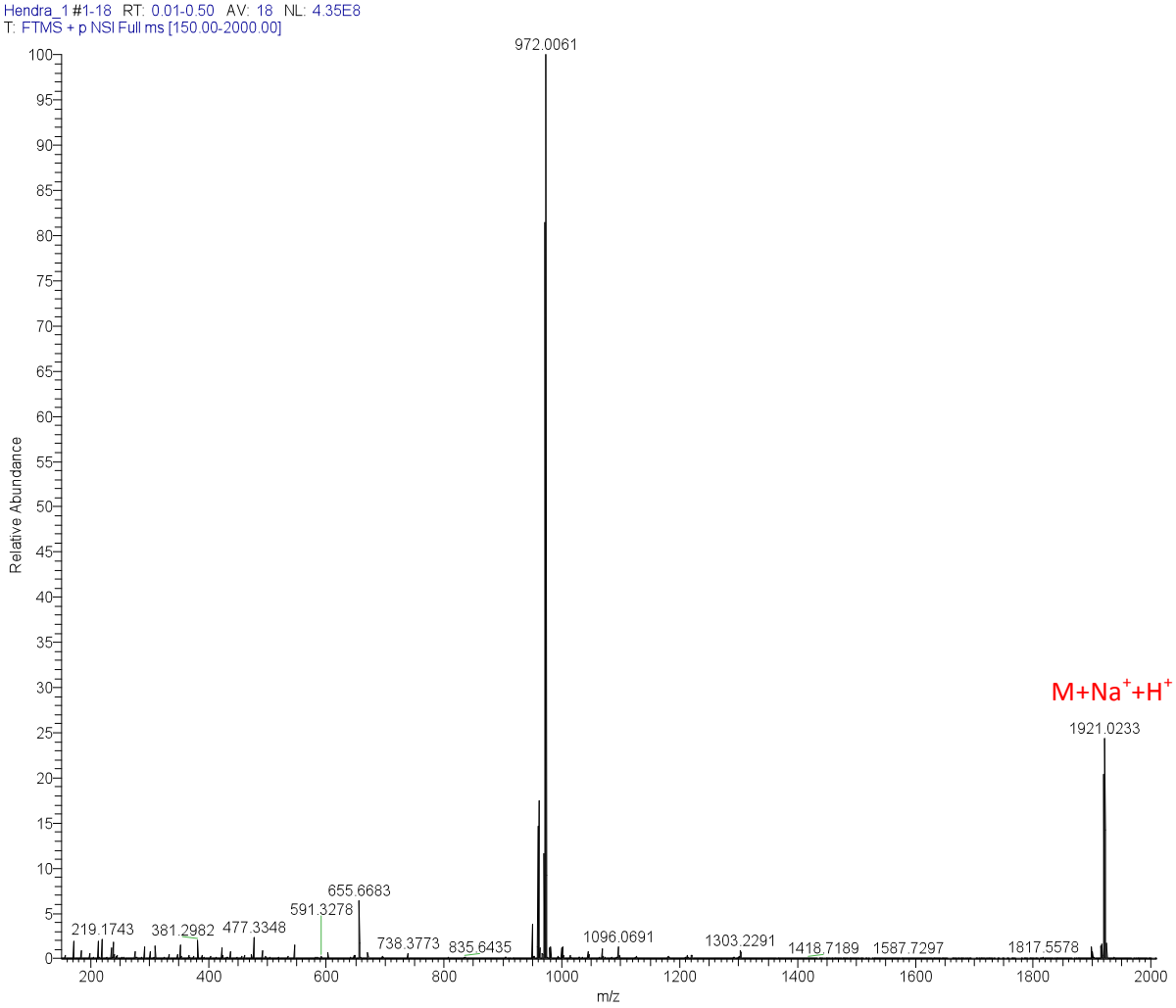




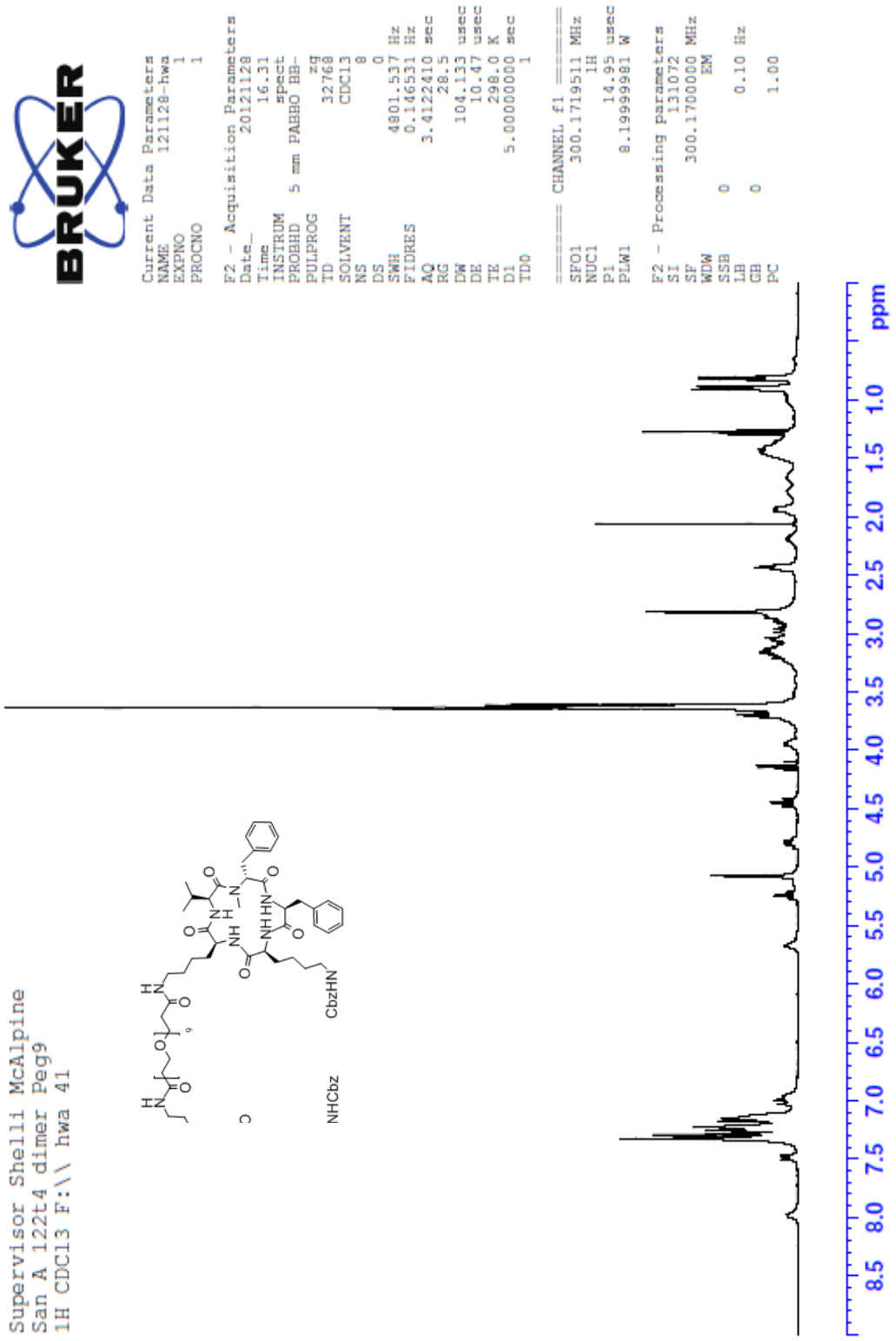
HRMS (ESI-TOF) SM122-PEG-5 DIMER

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

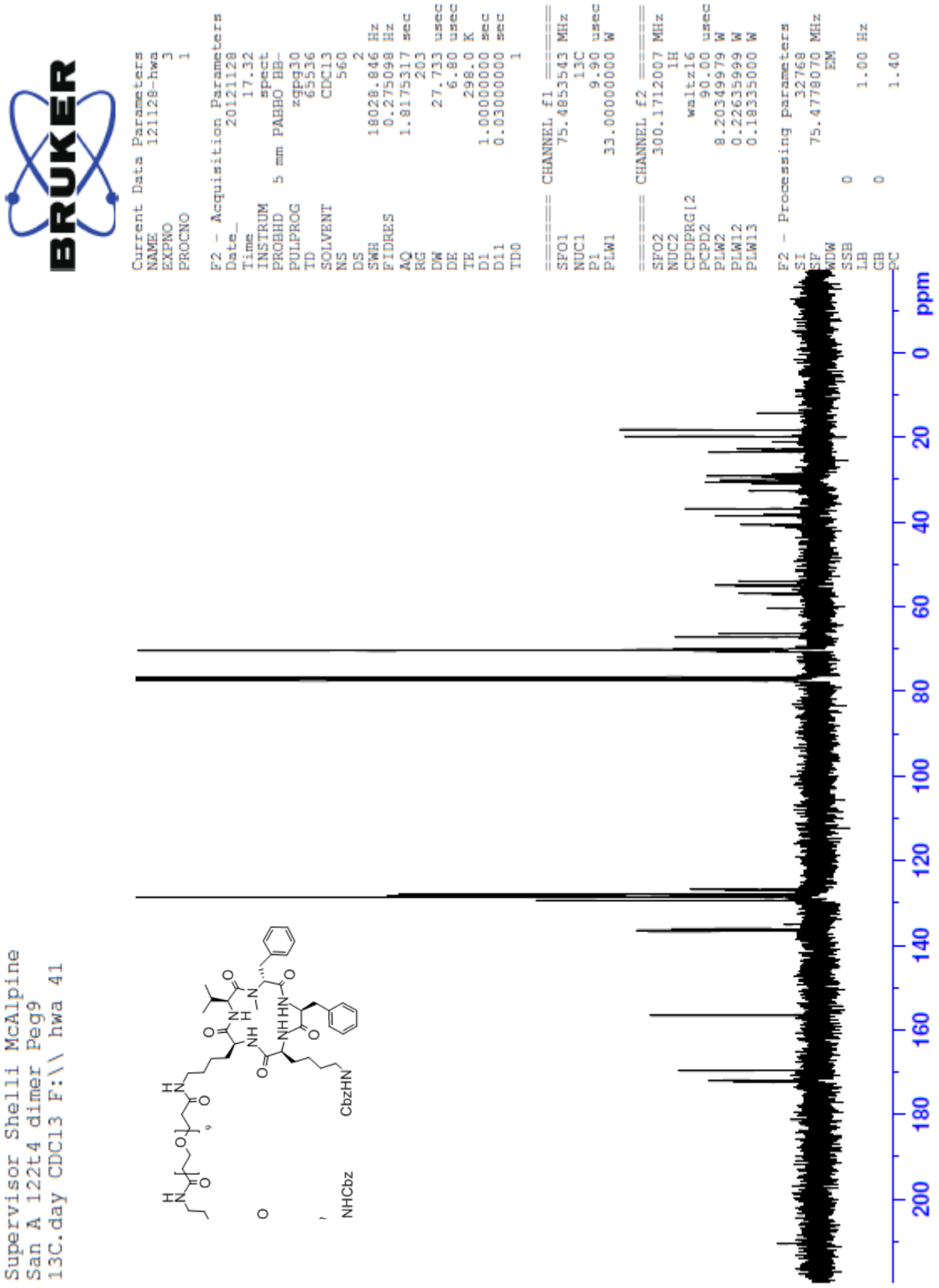




^{1}H NMR SM122-PEG-9 DIMER

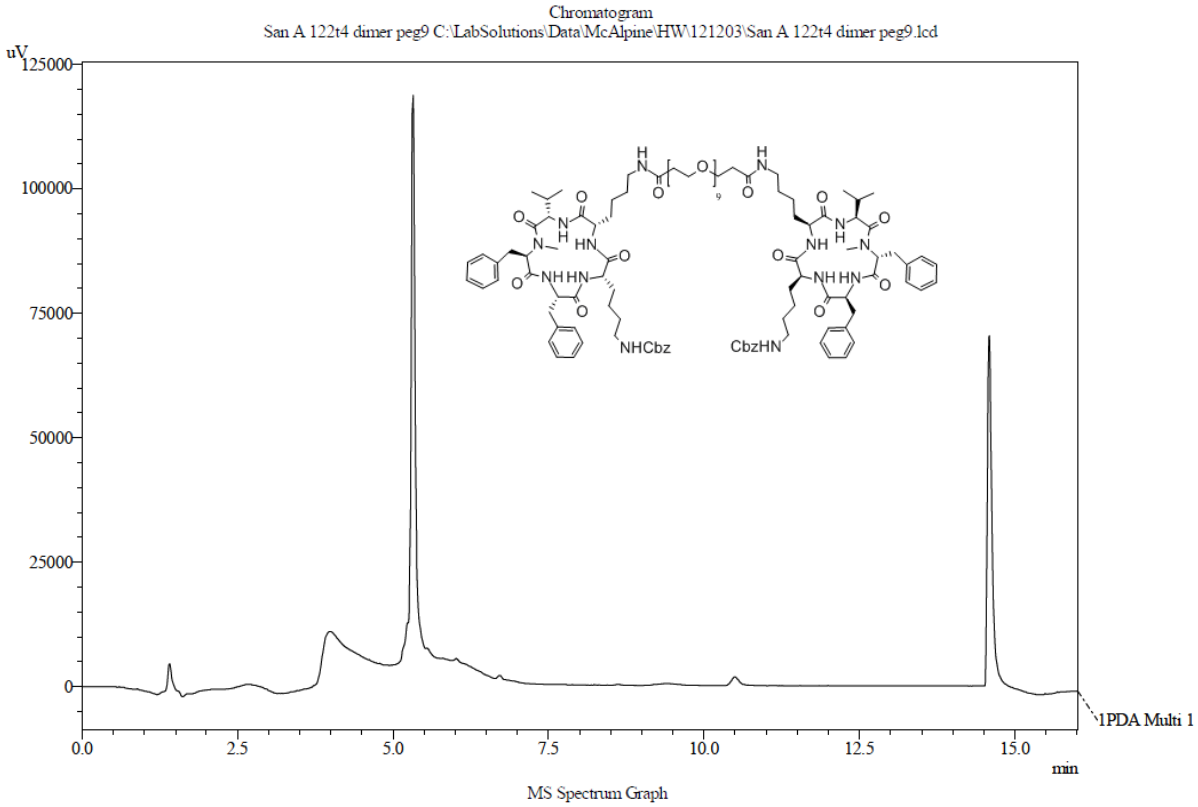


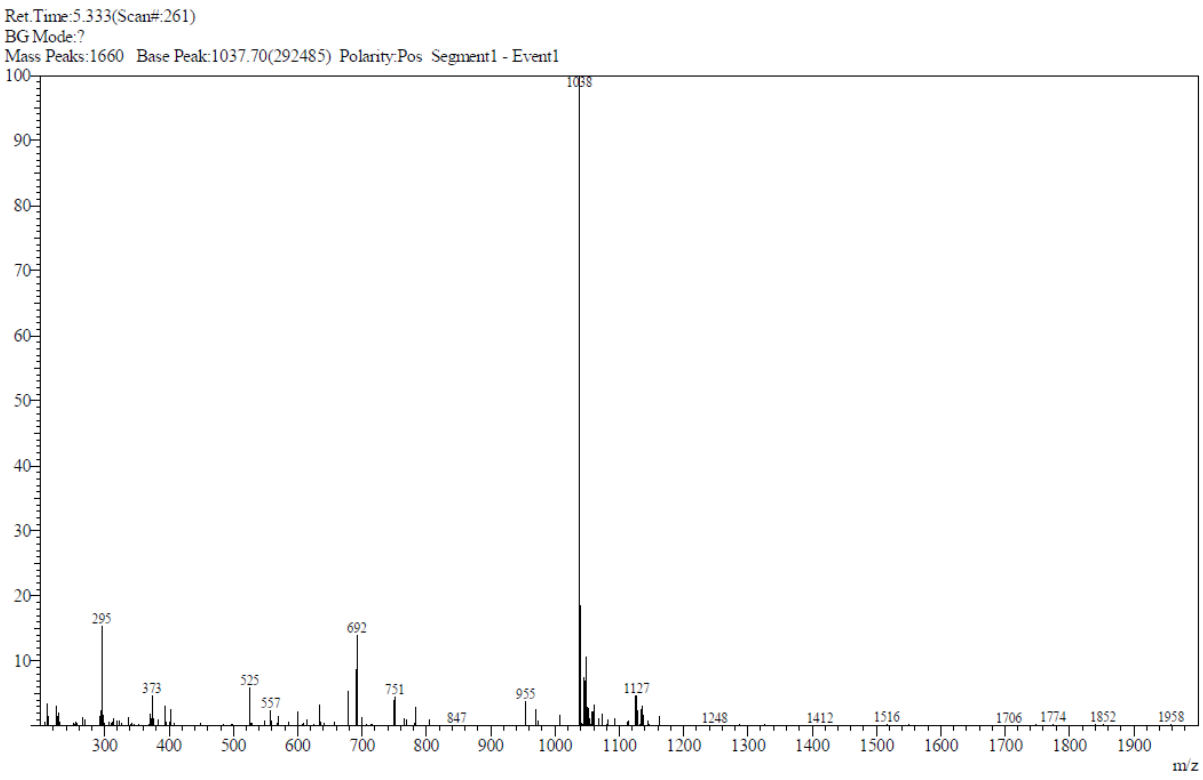
^{13}C NMR SM122-PEG-9 DIMER



LCMS-ESI/UV SM122-PEG-9 DIMER

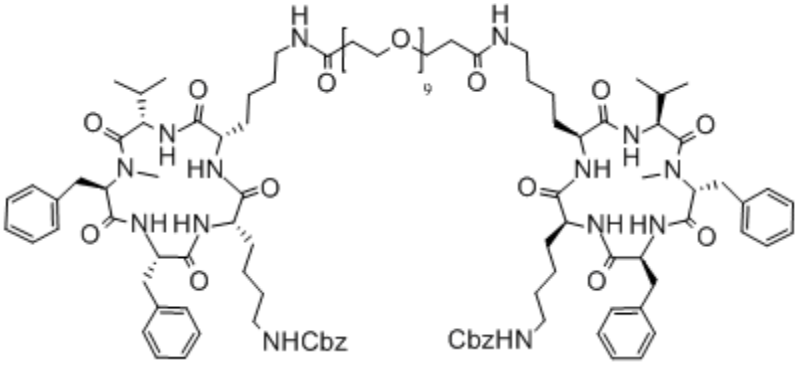
==== Shimadzu LCMSsolution Analysis Report ====

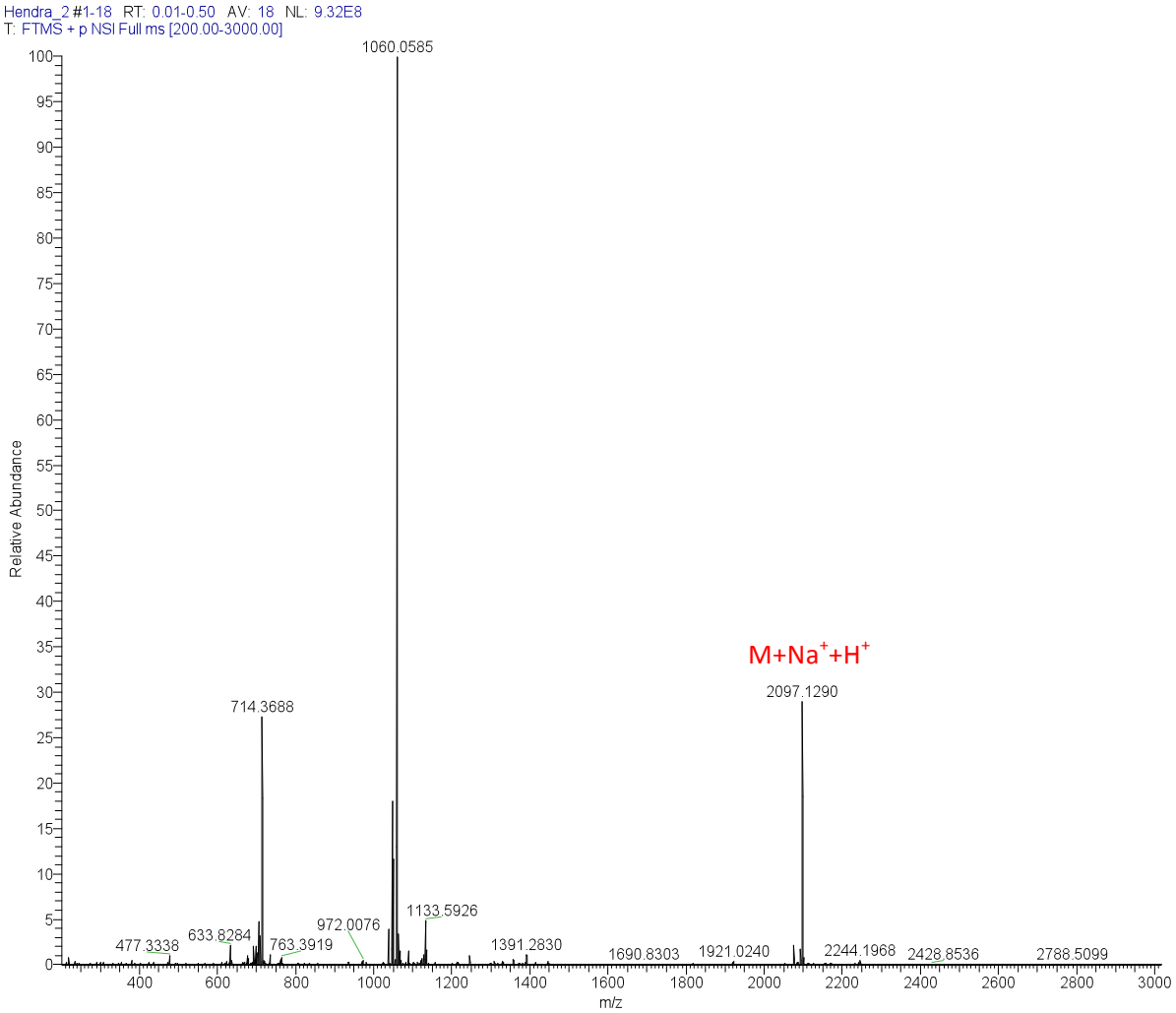




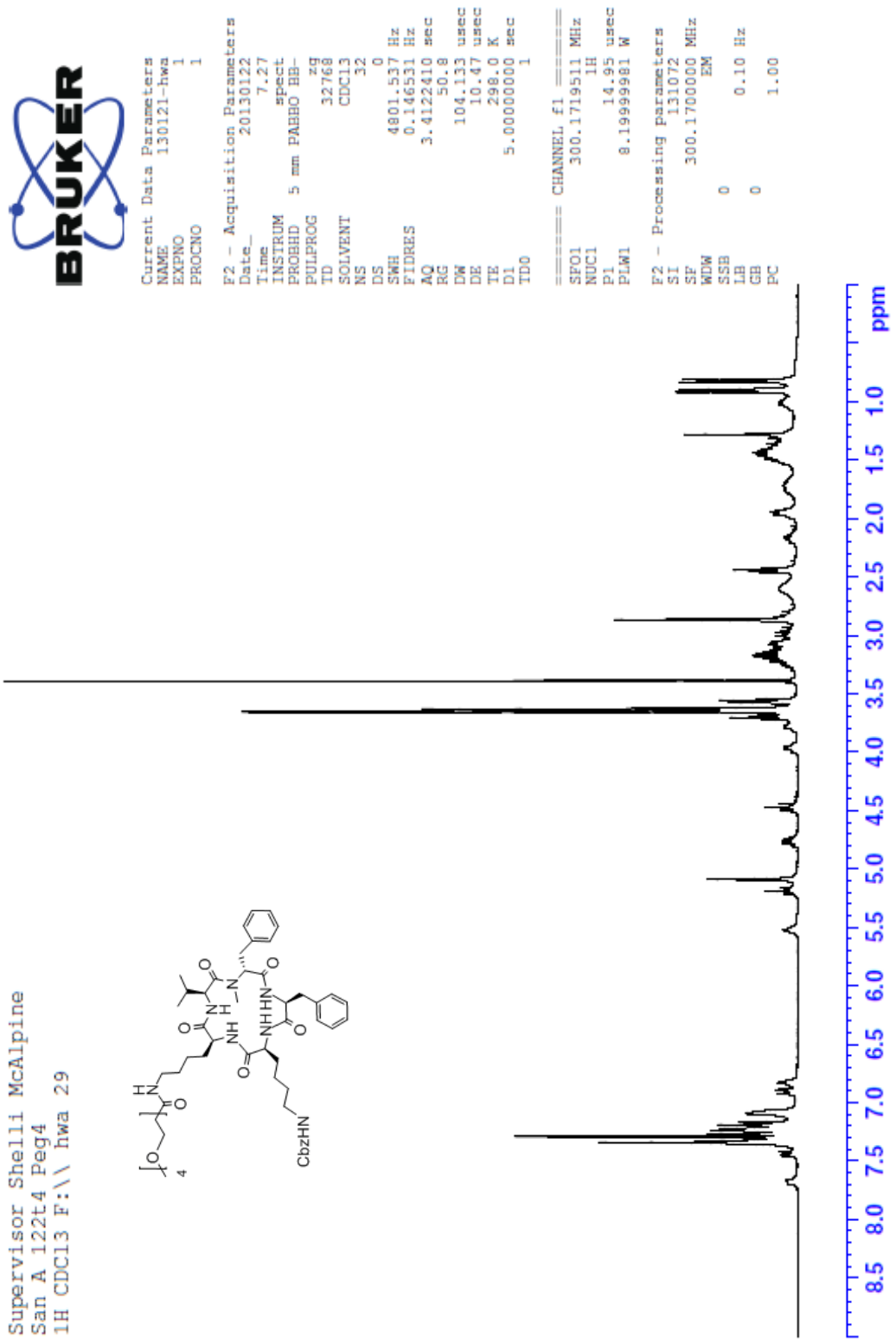
HRMS (ESI-TOF) SM122-PEG-9 DIMER

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

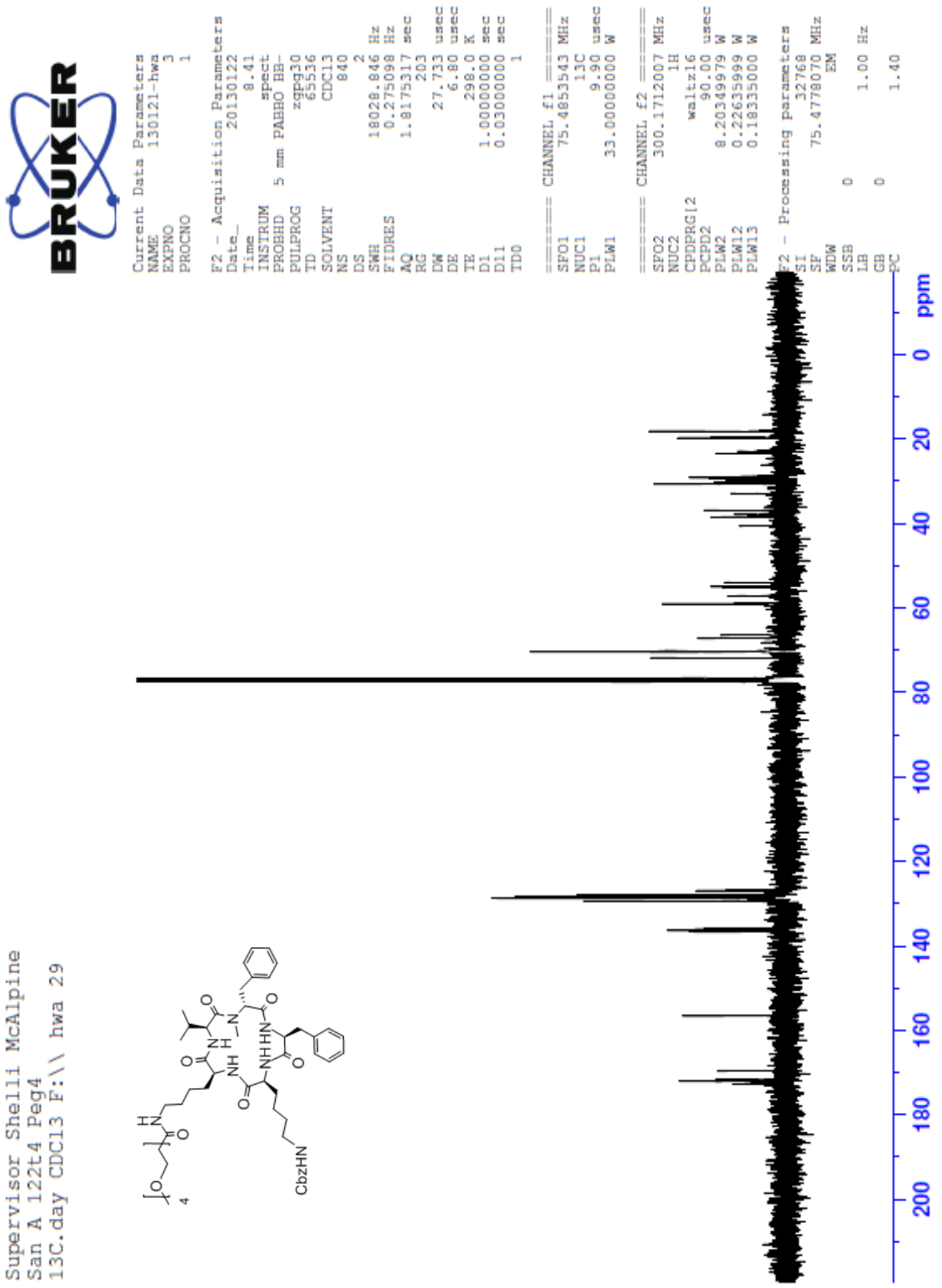




^{1}H NMR SM122-PEG-4

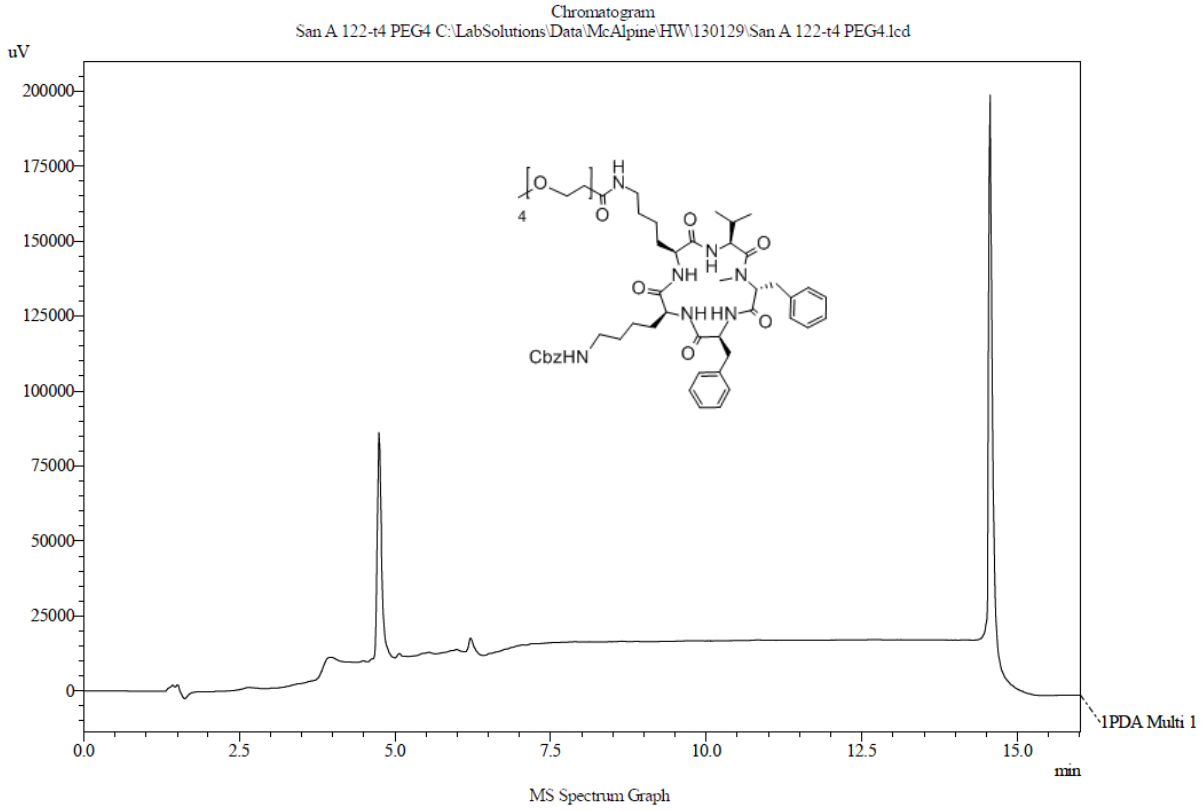


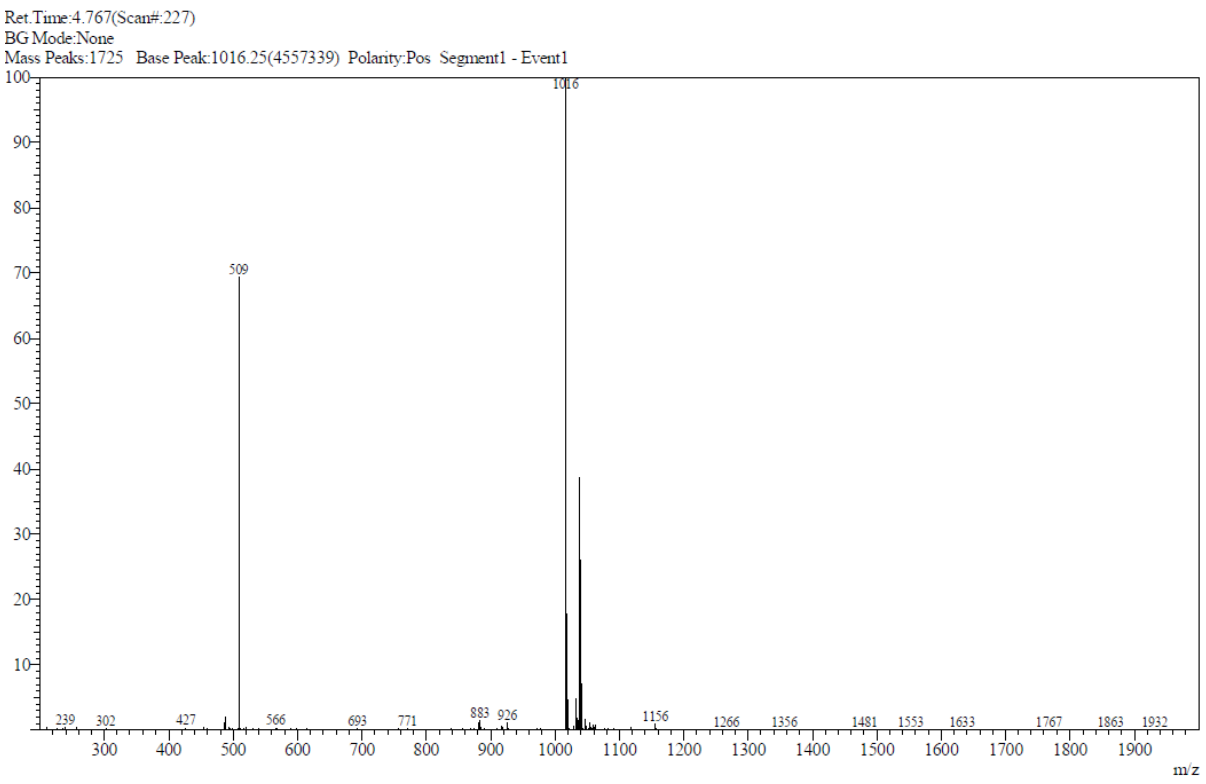
^{13}C NMR SM122-PEG-4



LCMS-ESI/UV SM122-PEG-4

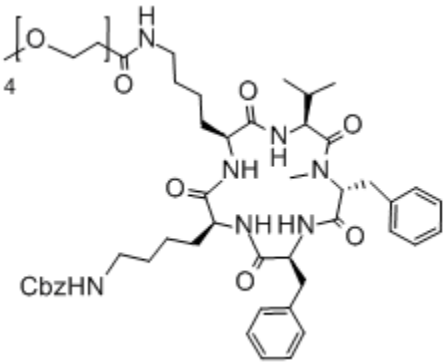
==== Shimadzu LCMSsolution Analysis Report ====

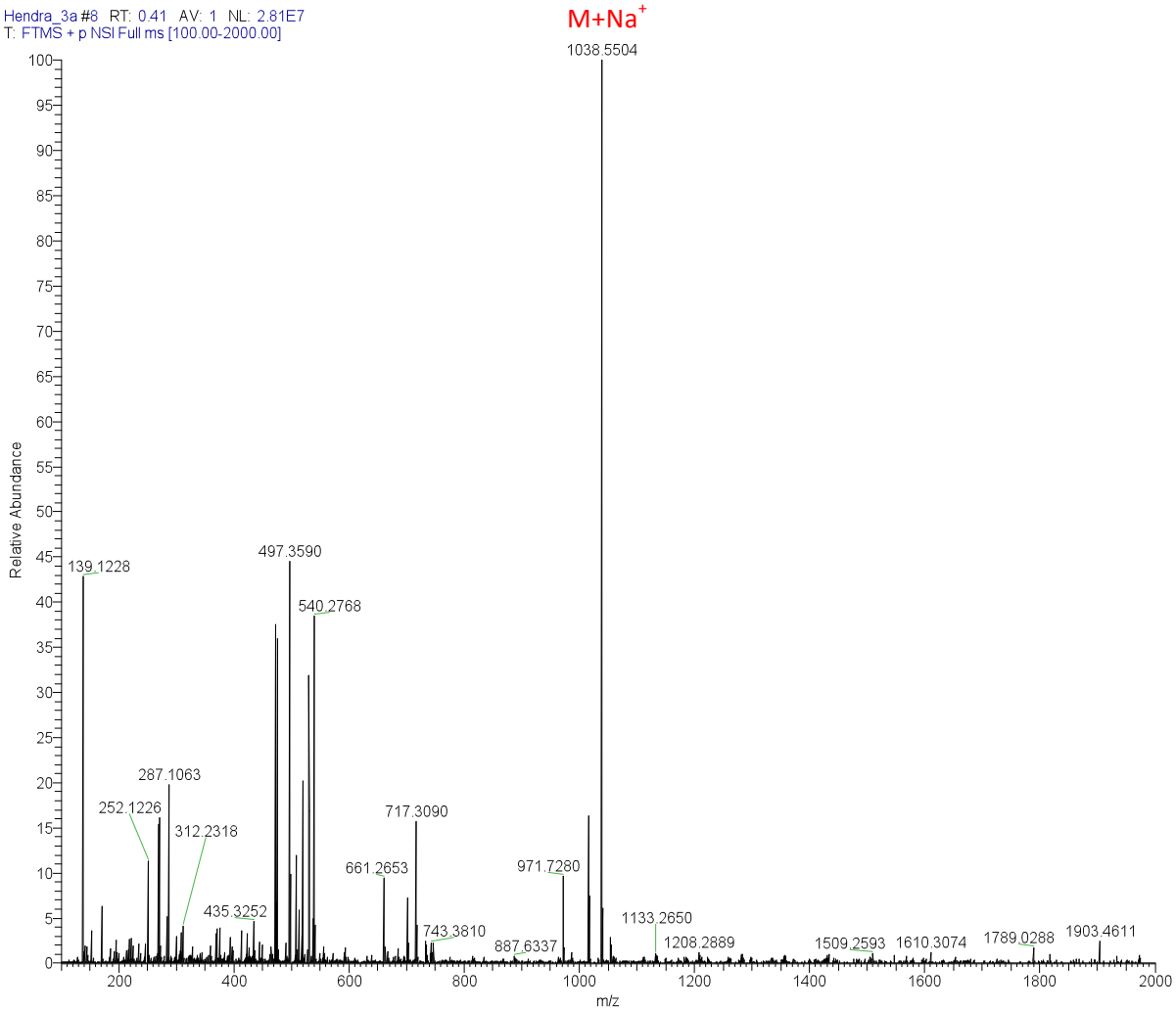




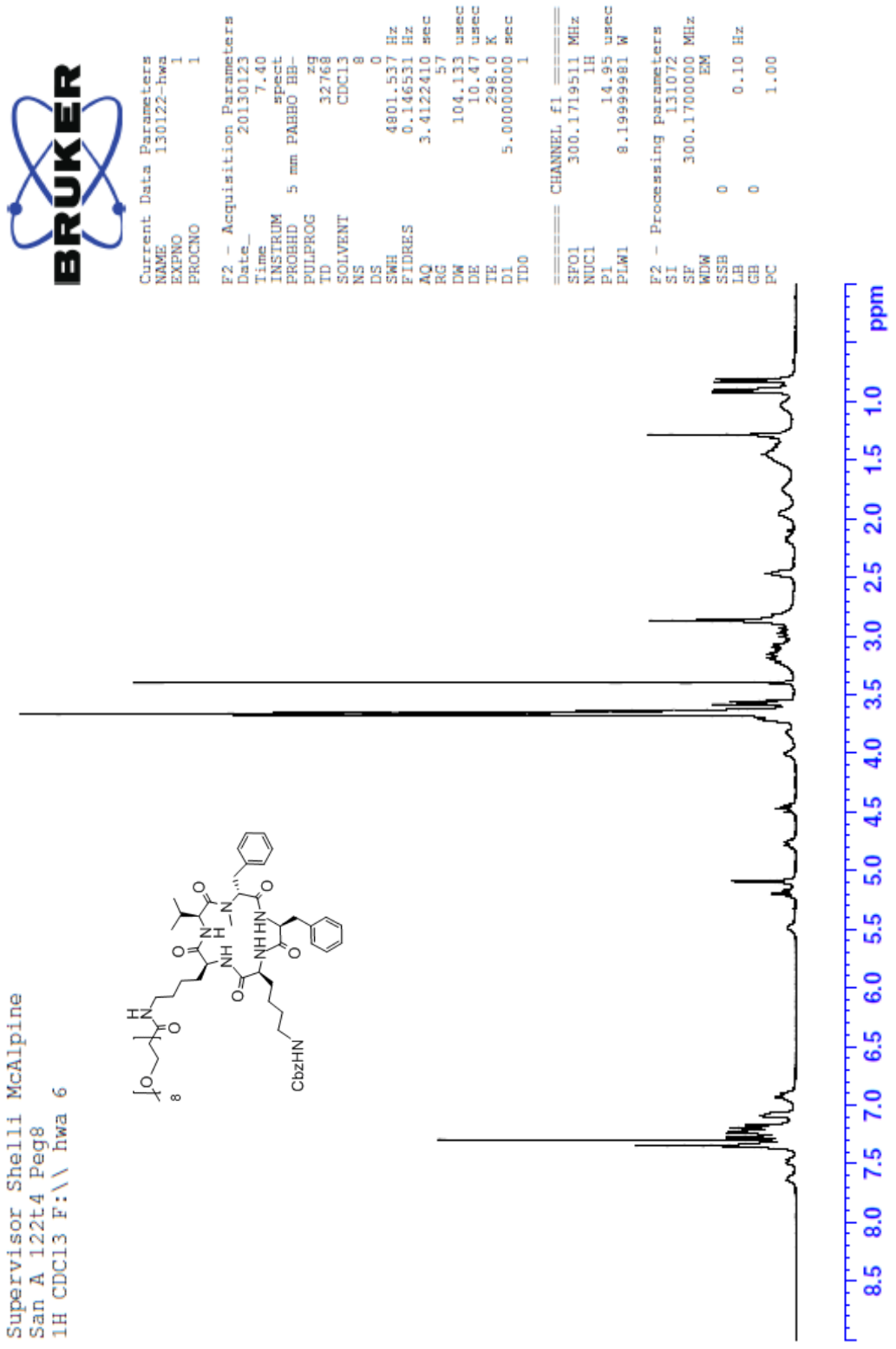
HRMS (ESI-TOF) SM122-PEG-4

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

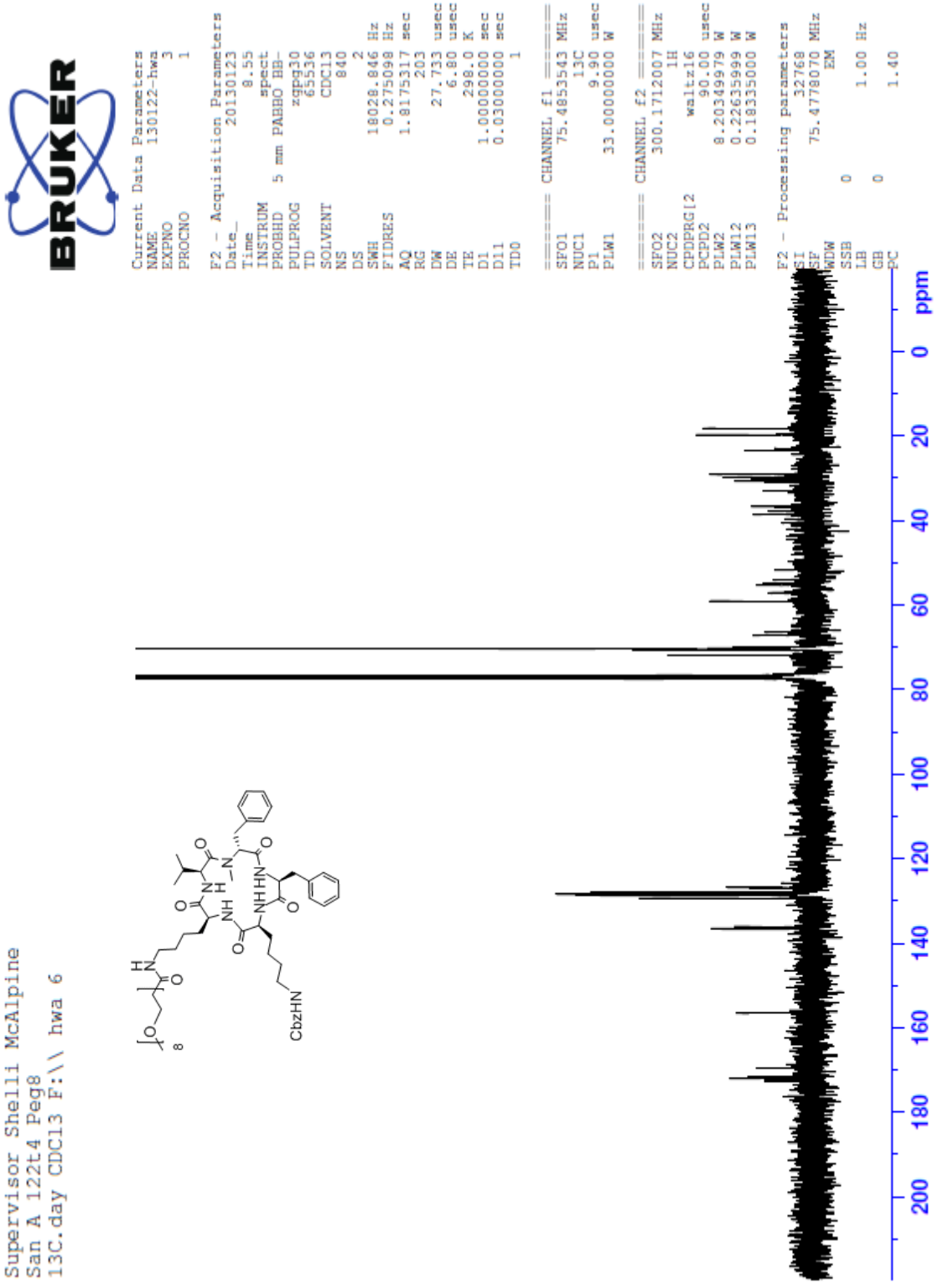




^{1}H NMR SM122-PEG-8

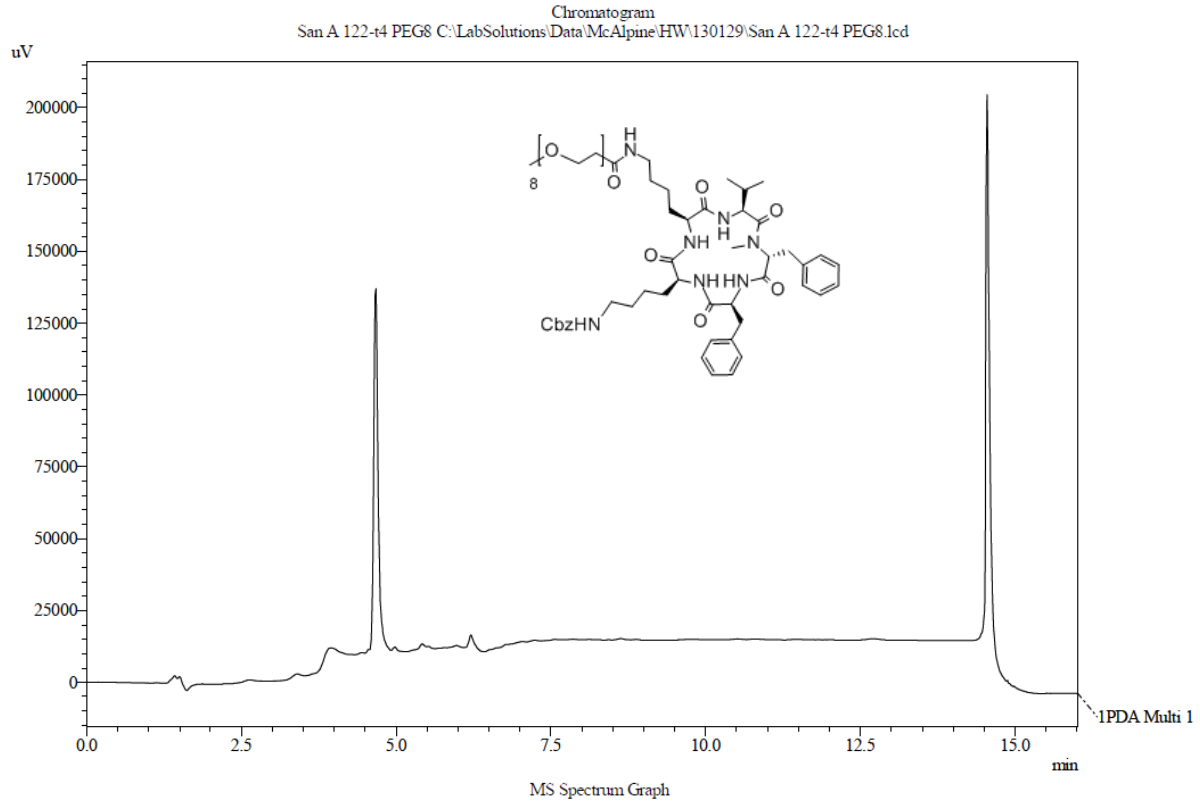


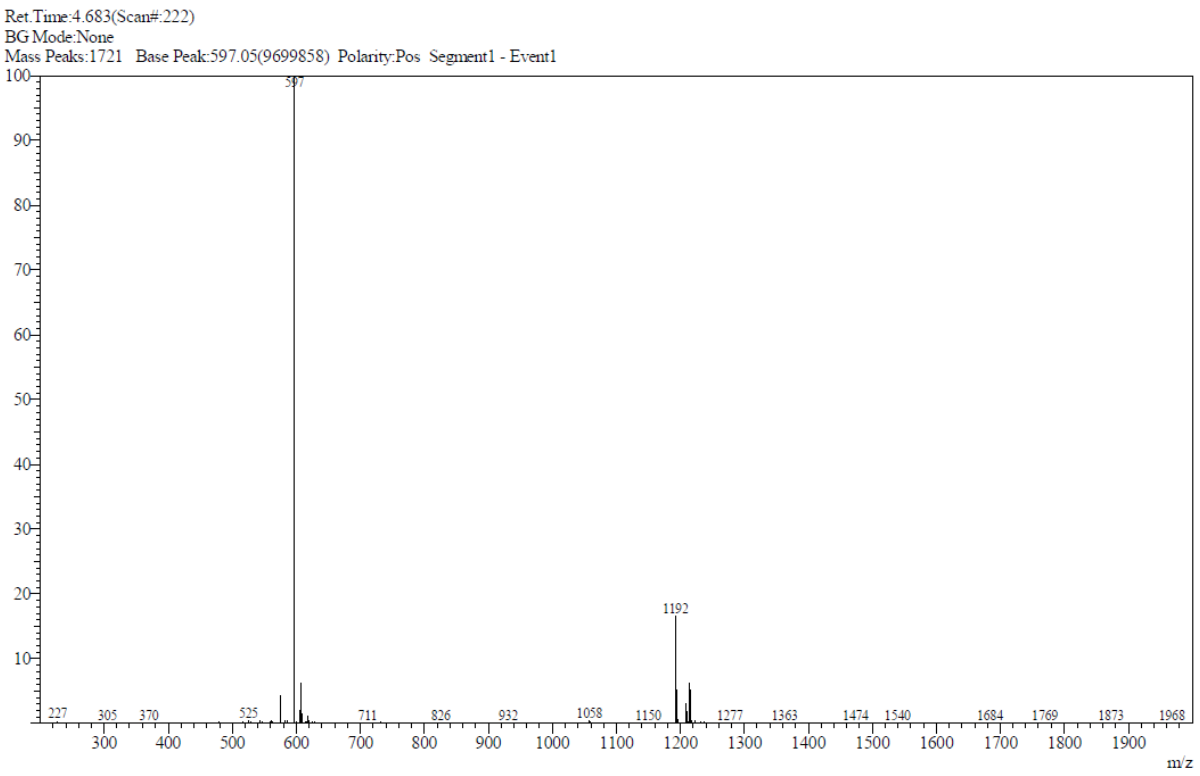
^{13}C NMR SM122-PEG-8



LCMS-ESI/UV SM122-PEG-8

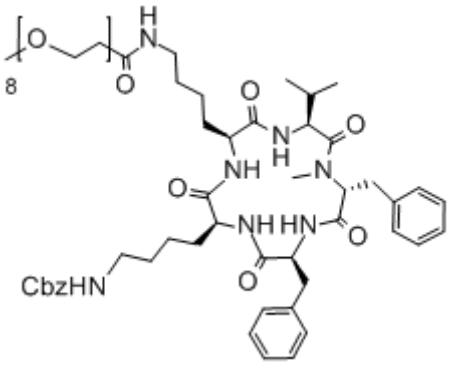
==== Shimadzu LCMSsolution Analysis Report ====

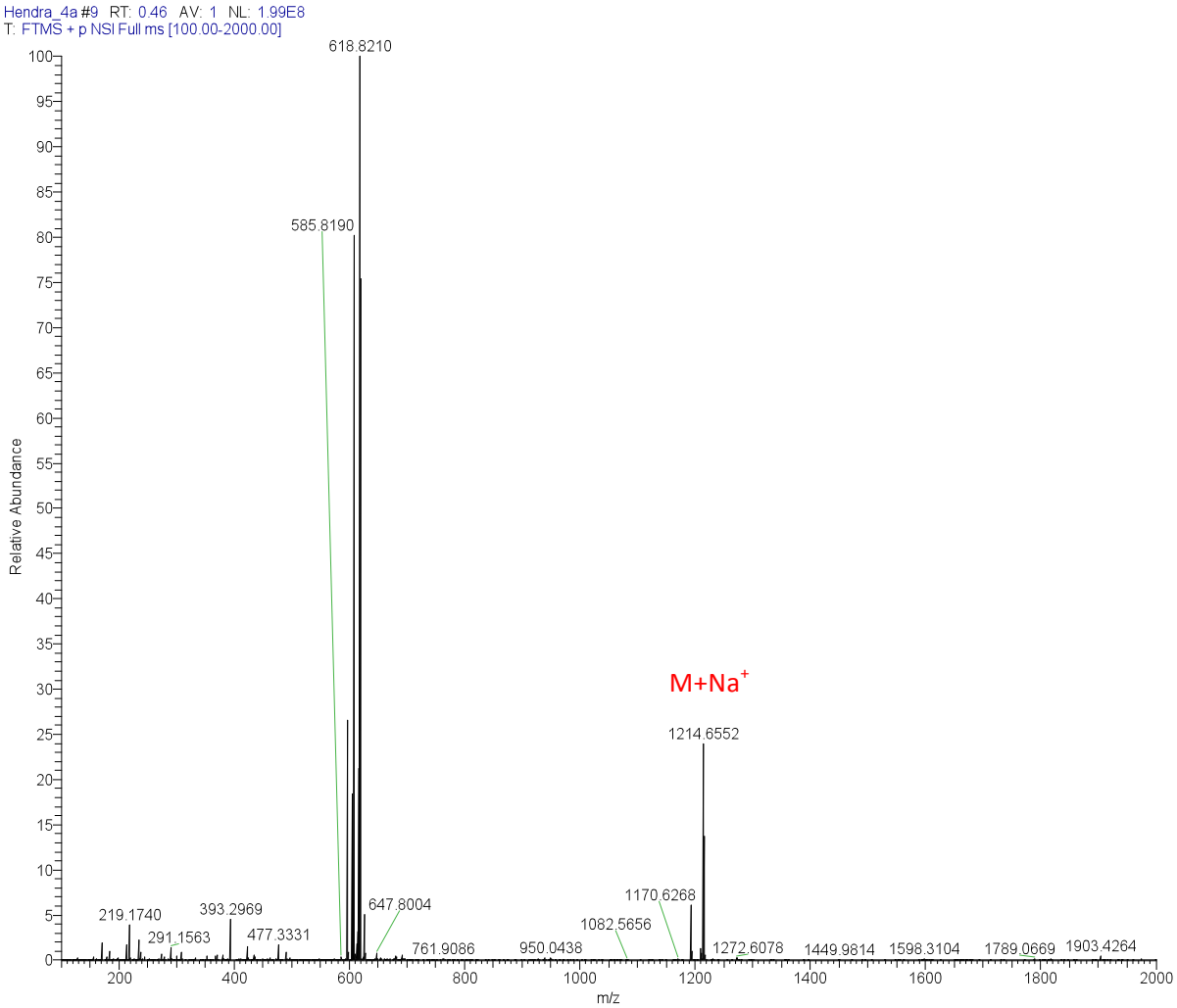




HRMS (ESI-TOF) SM122-PEG-8

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

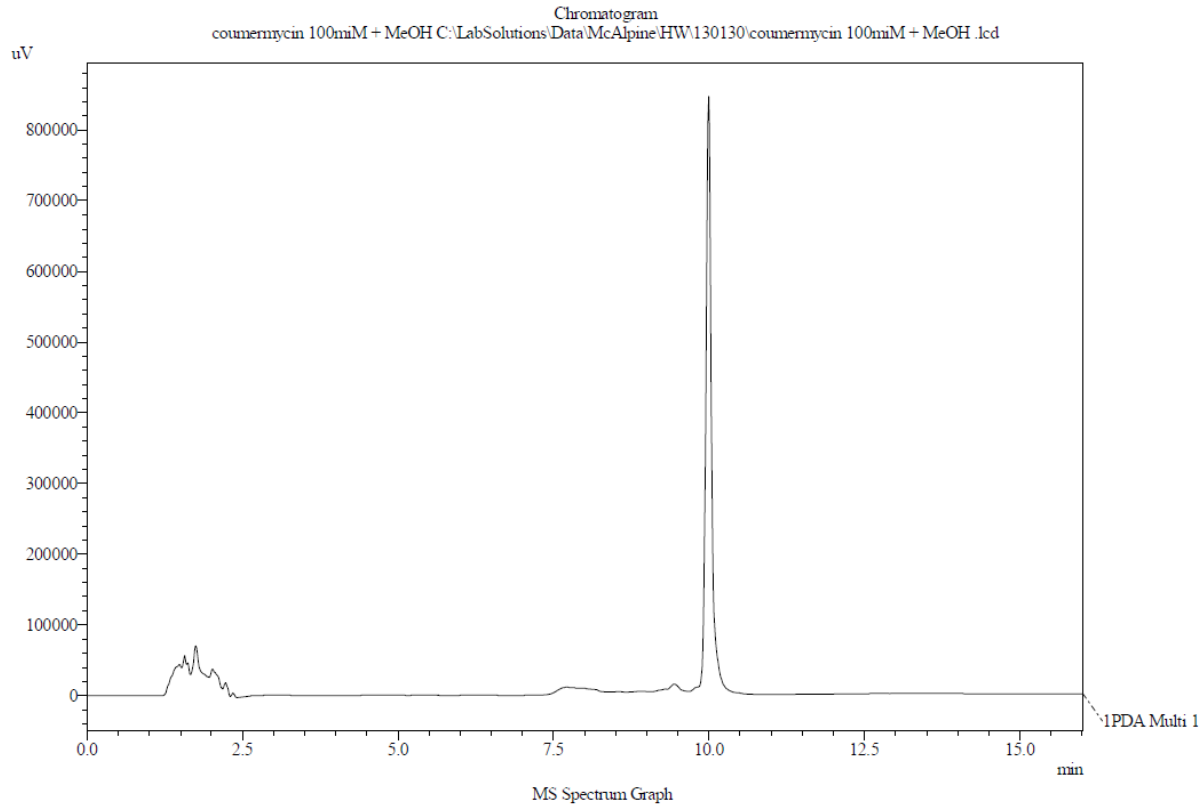


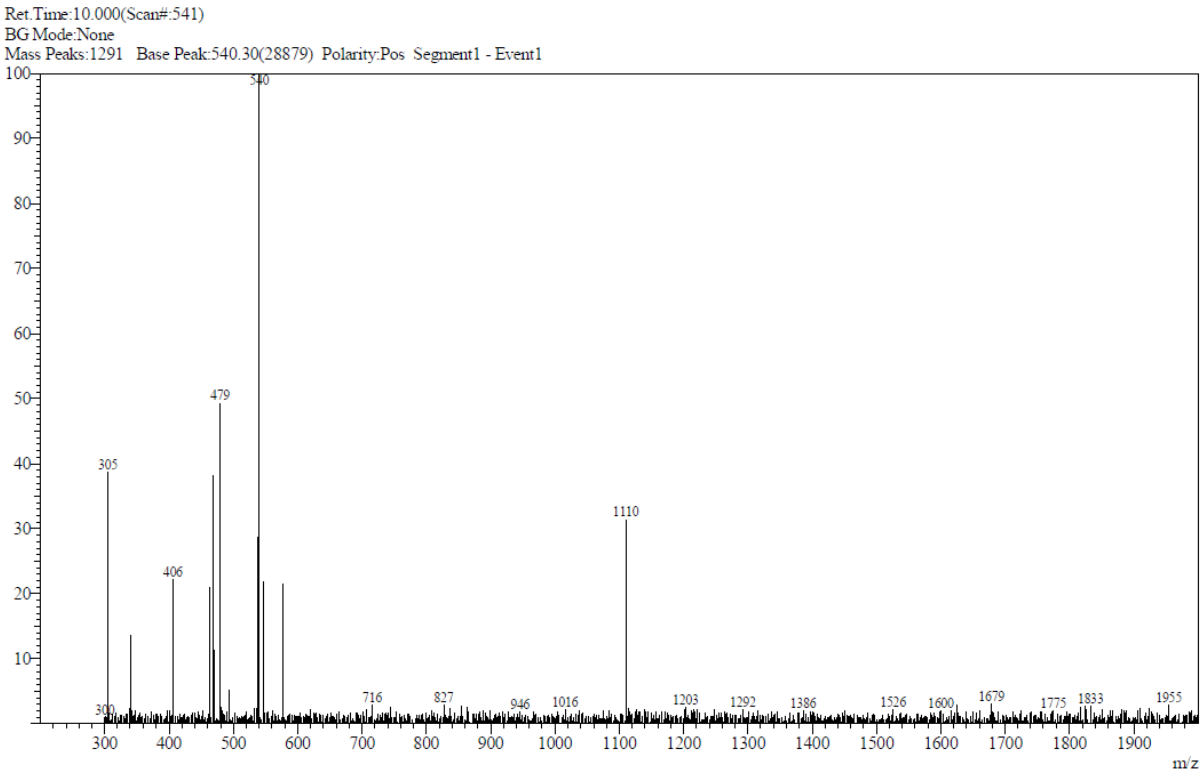


Solubility Data

Internal Standard Coumermycin A1 (10 µM; MW: 1110.08)

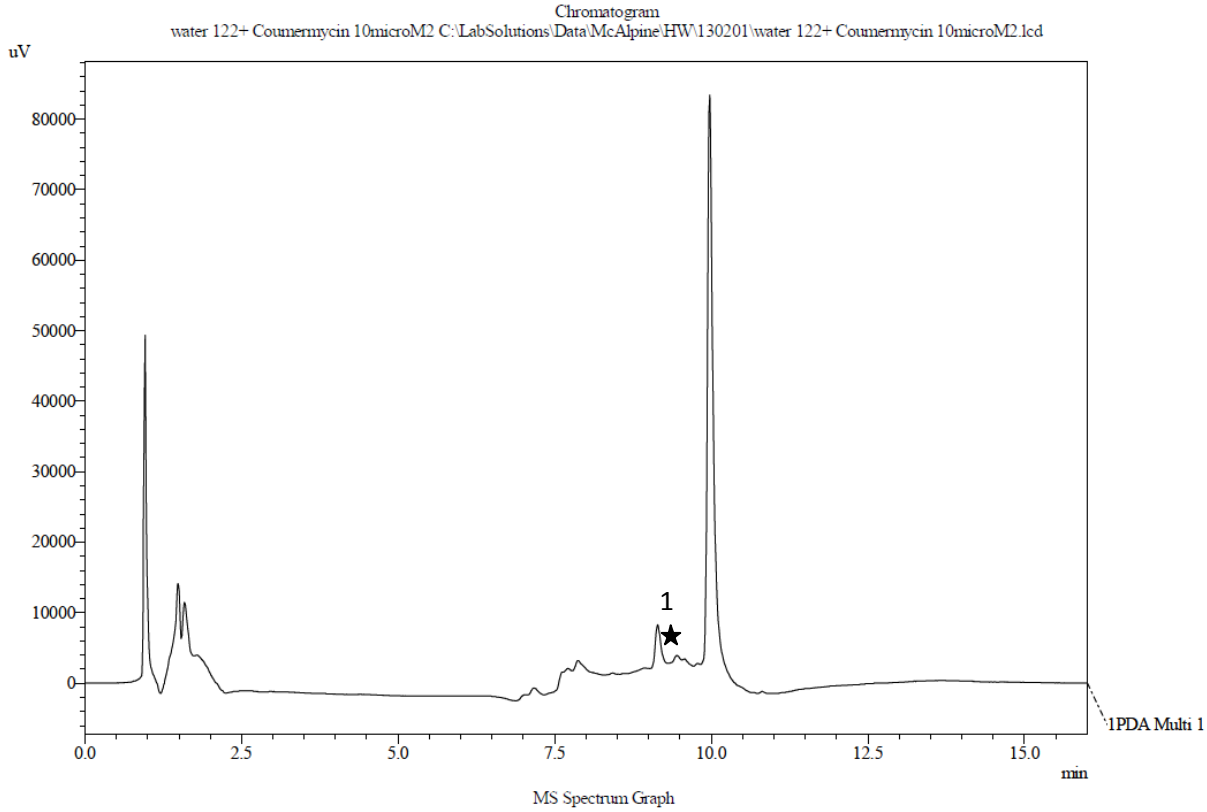
==== Shimadzu LCMSsolution Analysis Report ====

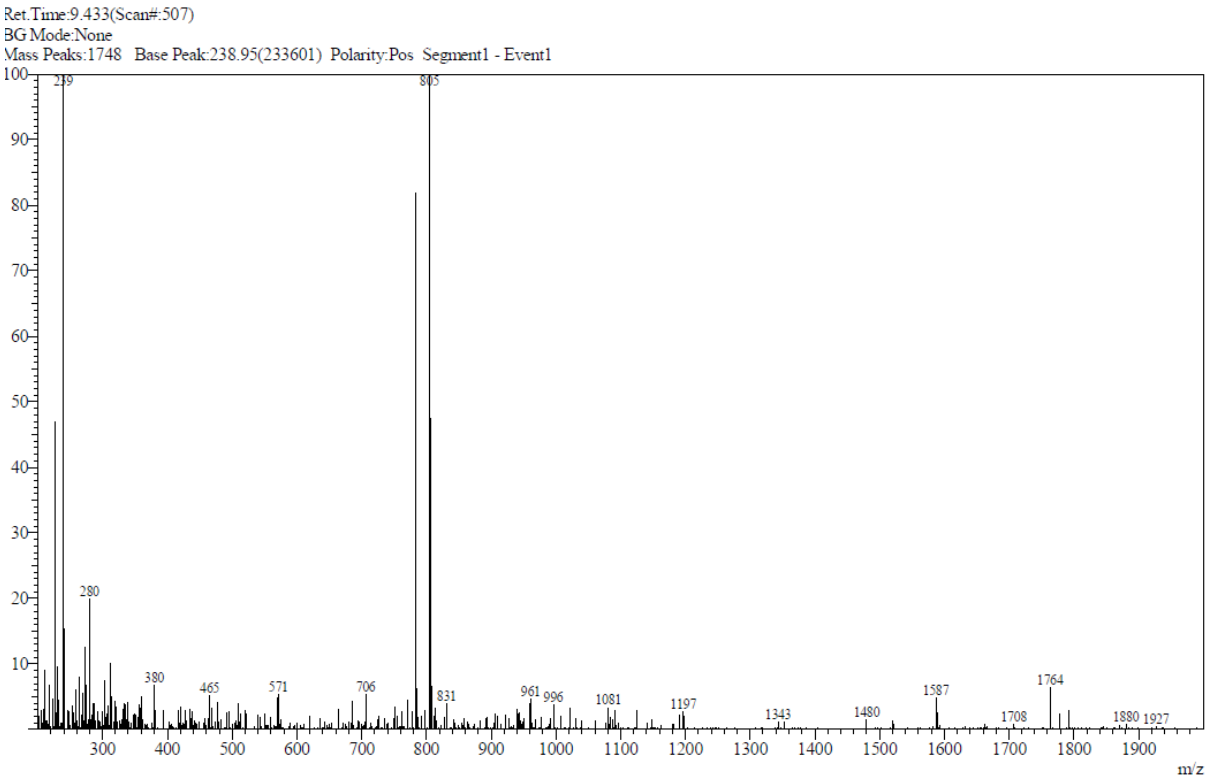




Co-injection of Coumermycin A1 (10 μM) and SM122 (MW: 782.44) solubility sample

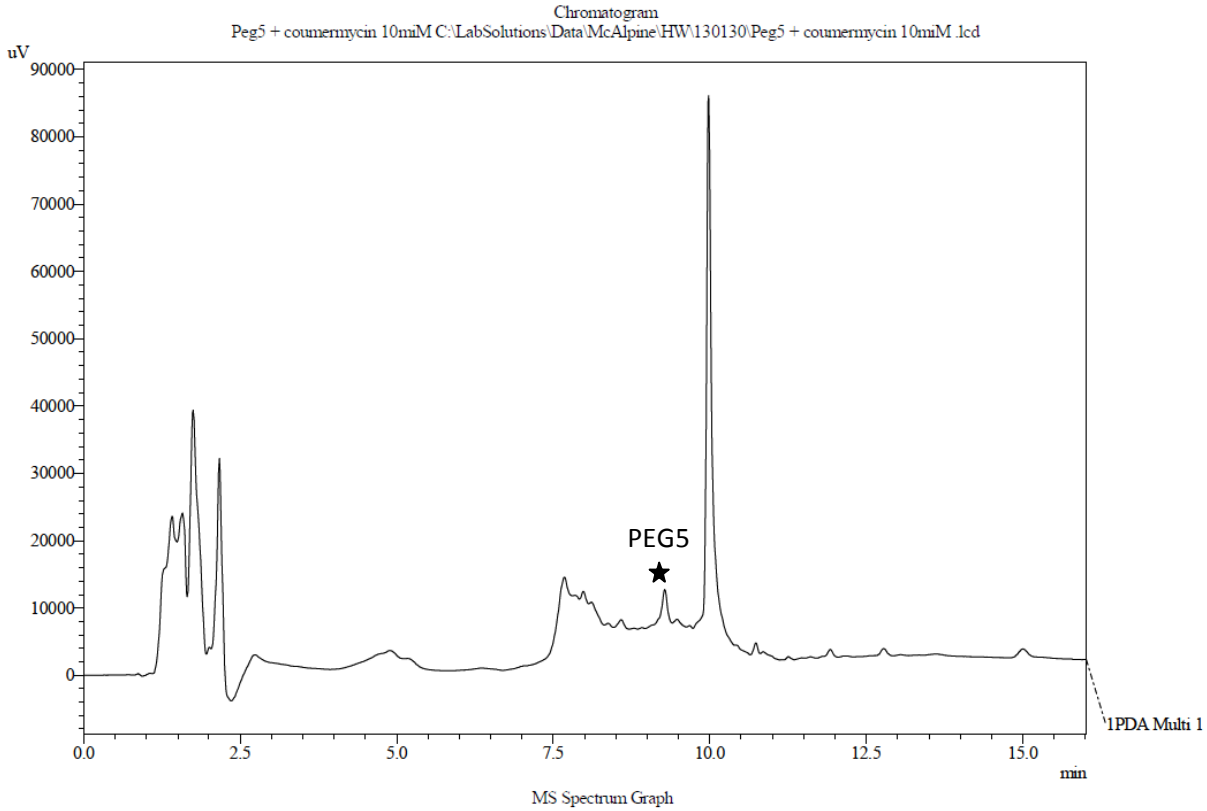
==== Shimadzu LCMSsolution Analysis Report ====

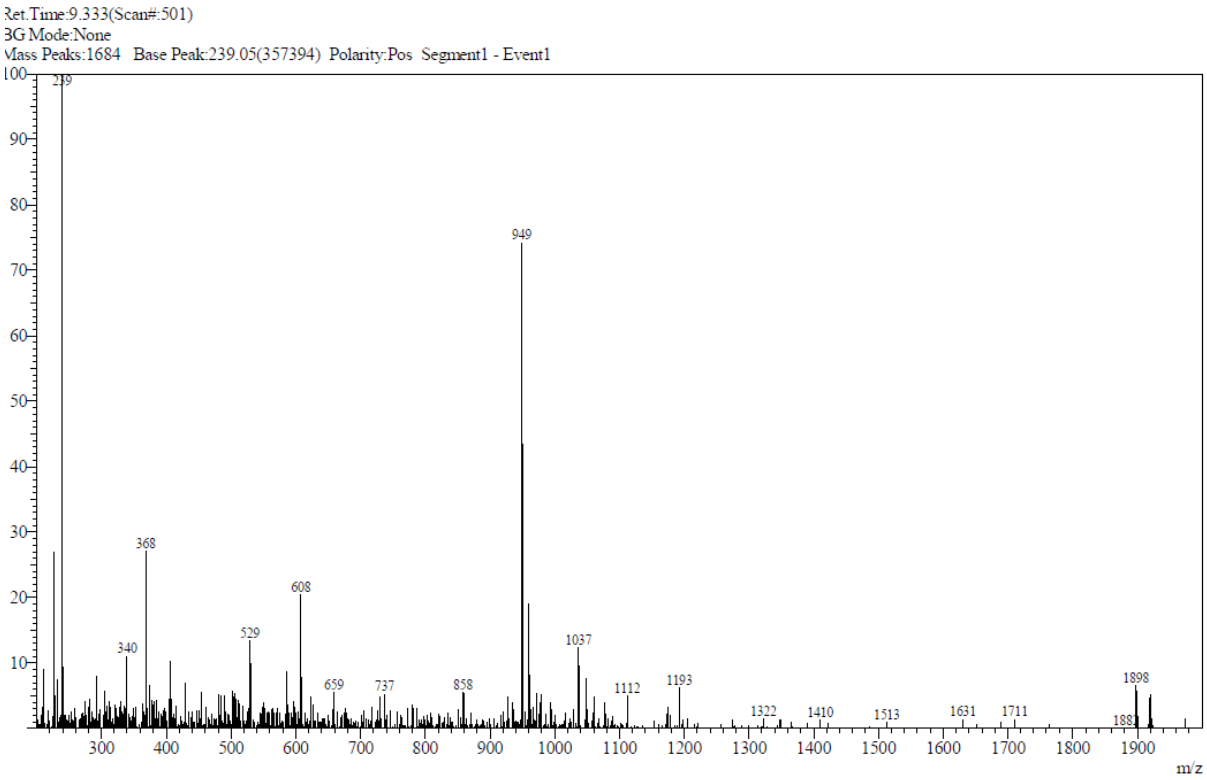




Co-injection of Coumermycin A1 (10 µM) and SM122-PEG-5 DIMER solubility sample

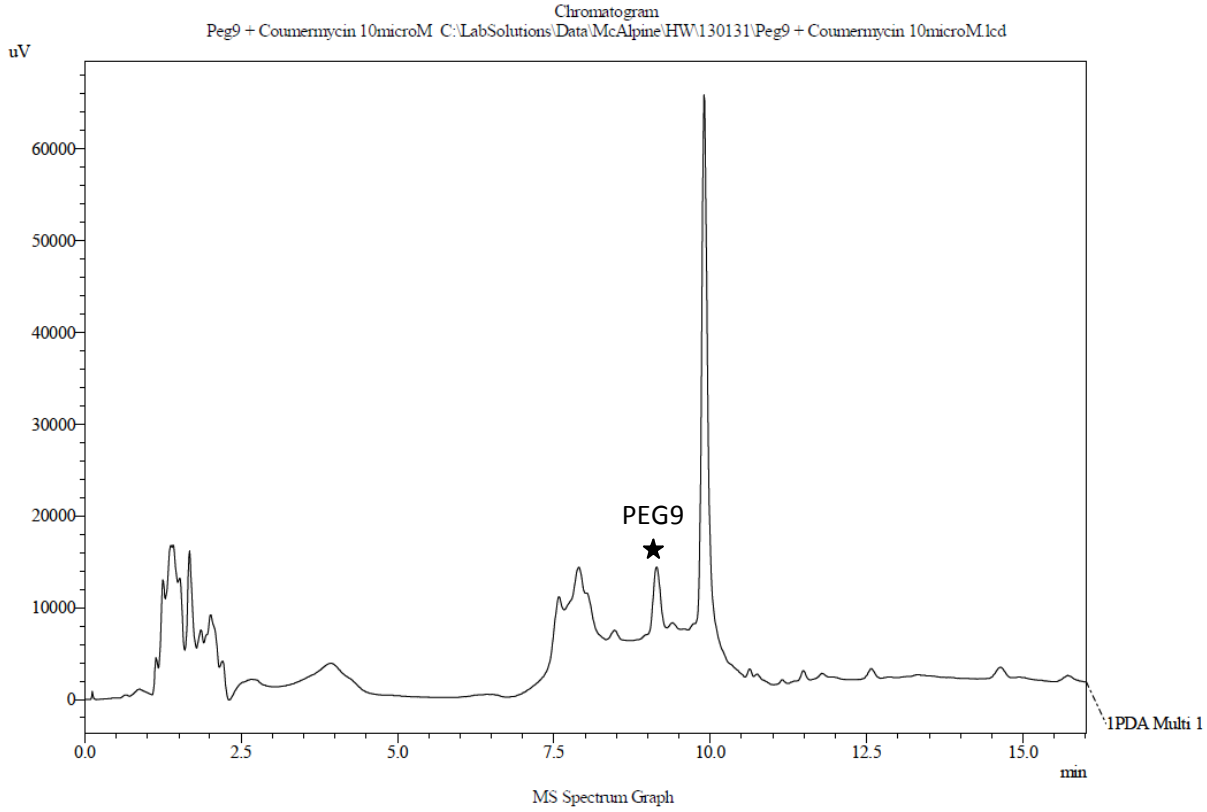
==== Shimadzu LCMSsolution Analysis Report ====

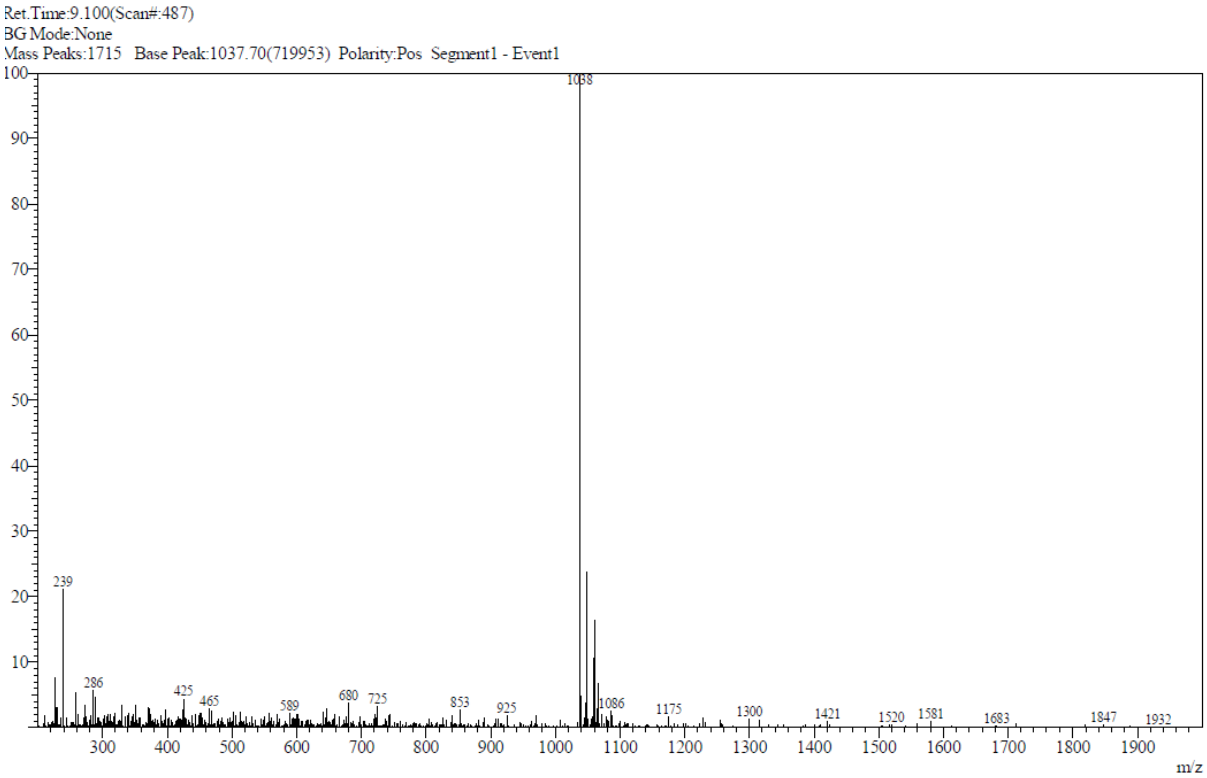




Co-injection of Coumermycin A1 (10 μM) and SM122-PEG-9 DIMER solubility sample

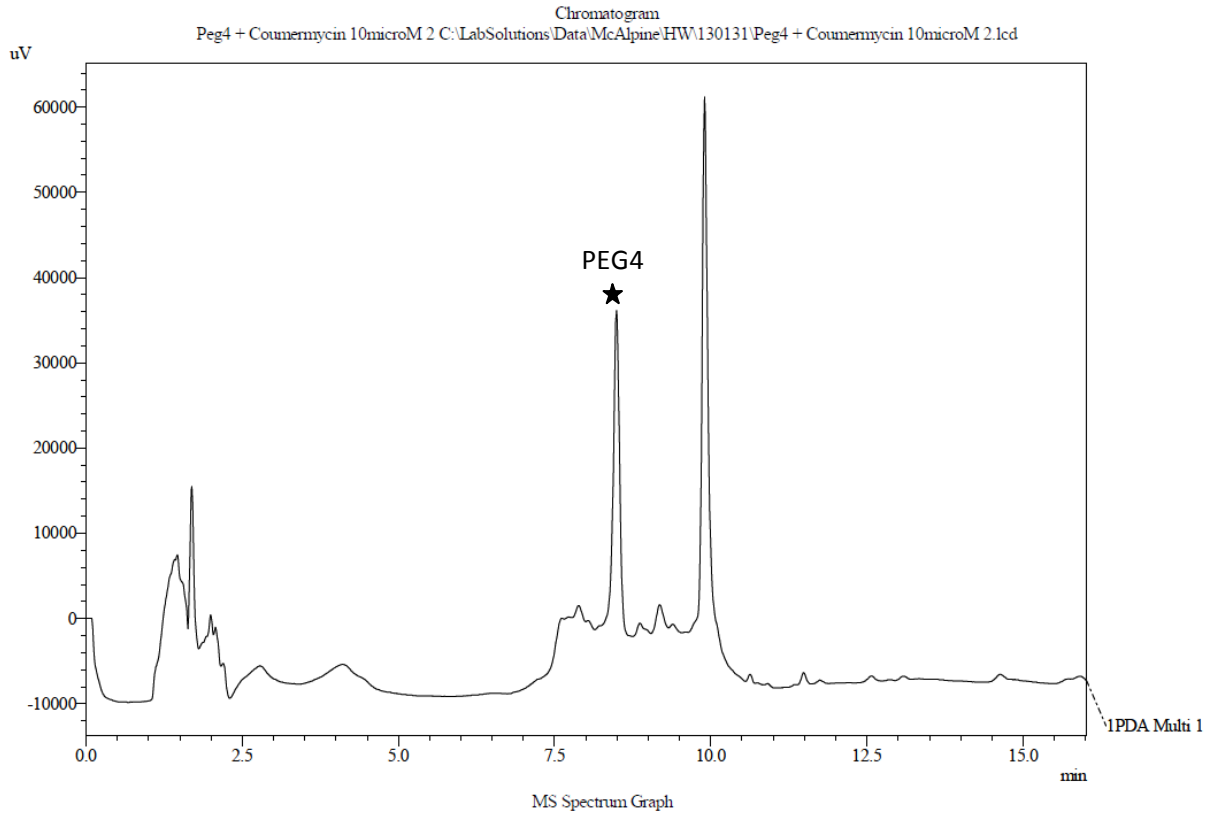
==== Shimadzu LCMSsolution Analysis Report ====

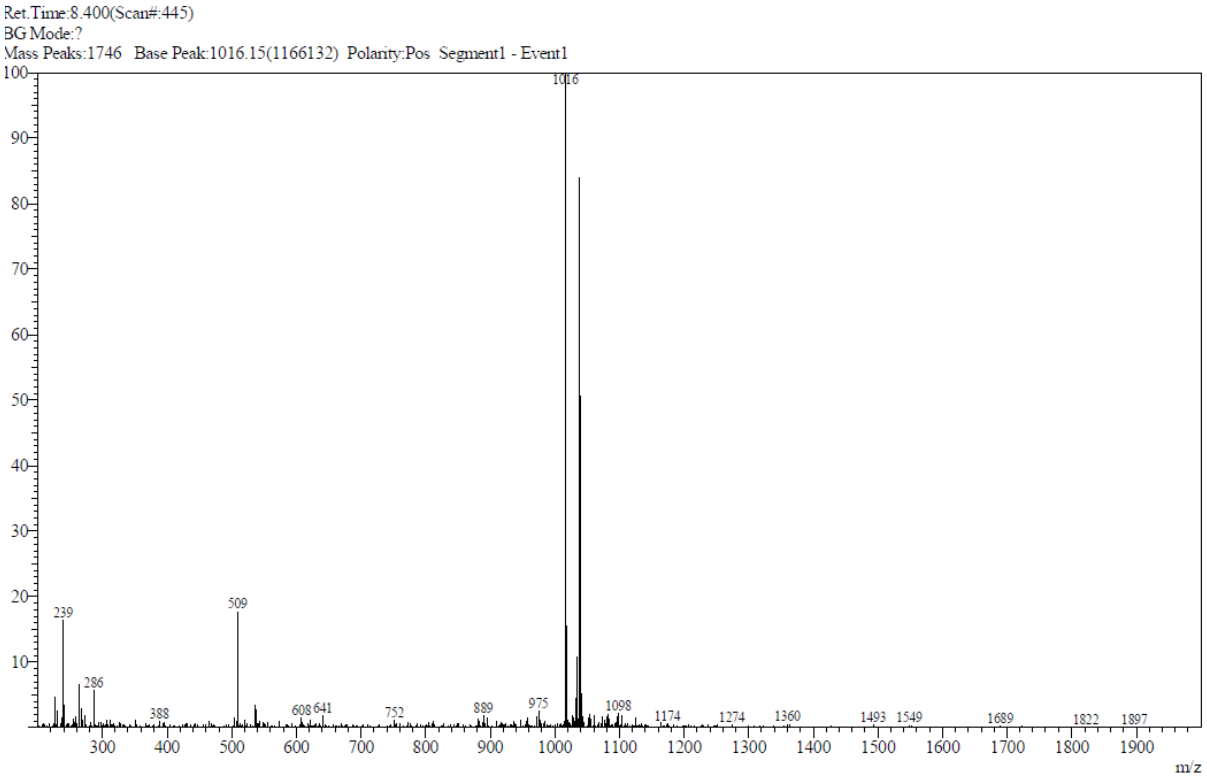




Co-injection of Coumermycin A1 (10 μM) and SM122-PEG4 solubility sample

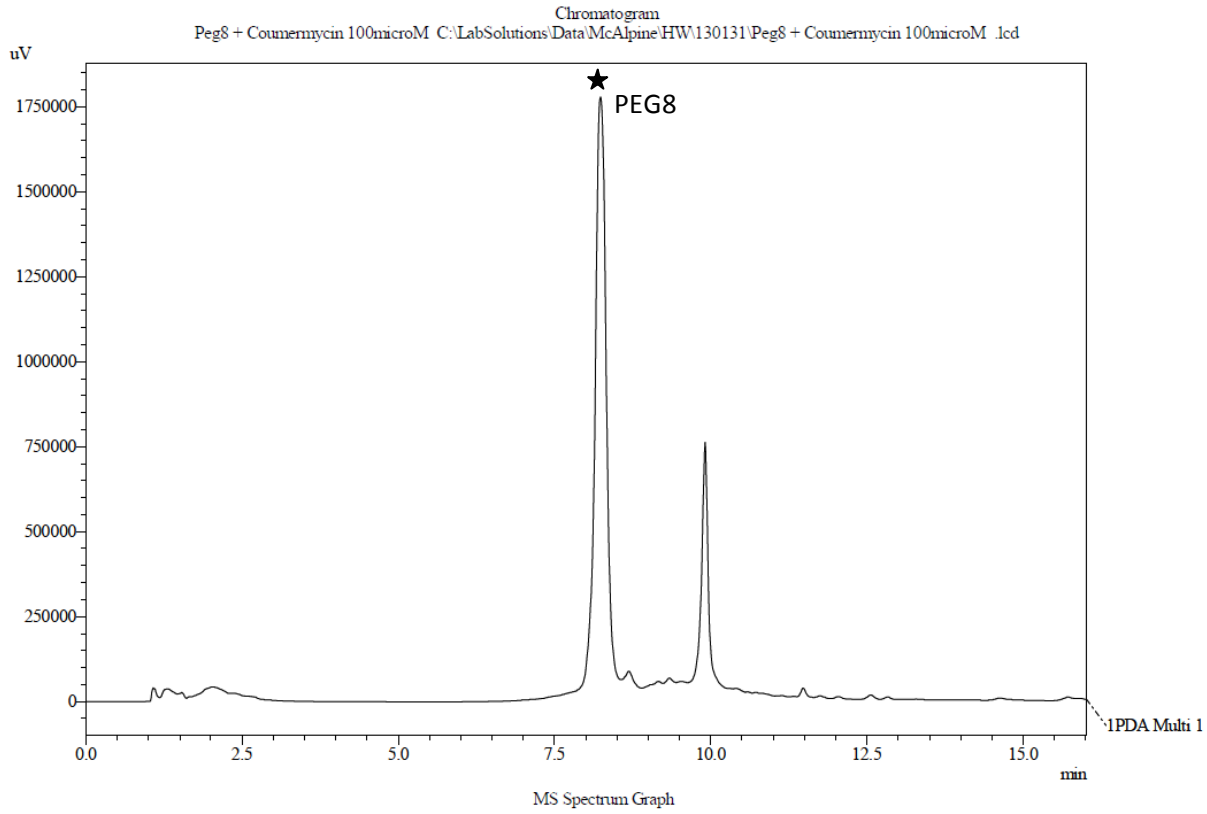
==== Shimadzu LCMSsolution Analysis Report ====

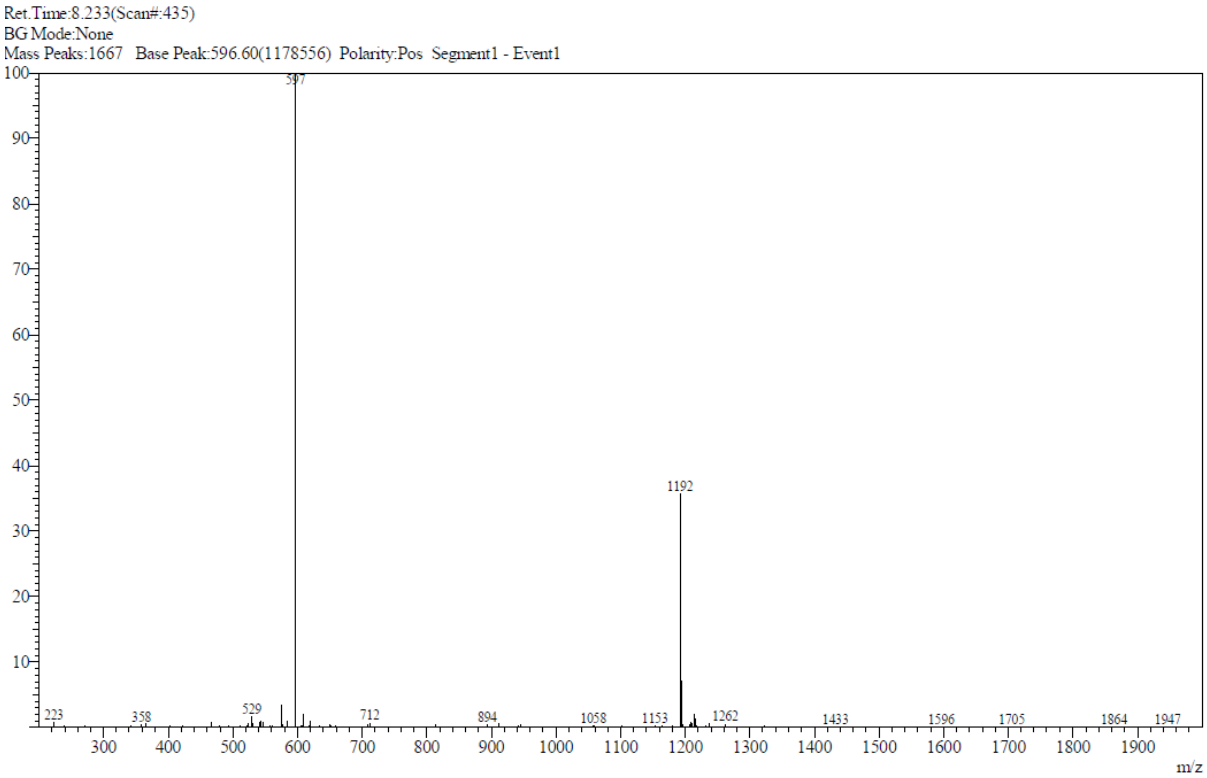




Co-injection of Coumermycin A1 (100 µM) and SM122-PEG8 solubility sample

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





Supplemental Table 1: Peak area values

Test	CA-1	SM122-PEG-4	SM122-PEG-5 DIMER	SM122-PEG-8	SM122-PEG-9 DIMER	SM122
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	(μg/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) × concentration of CA-1*

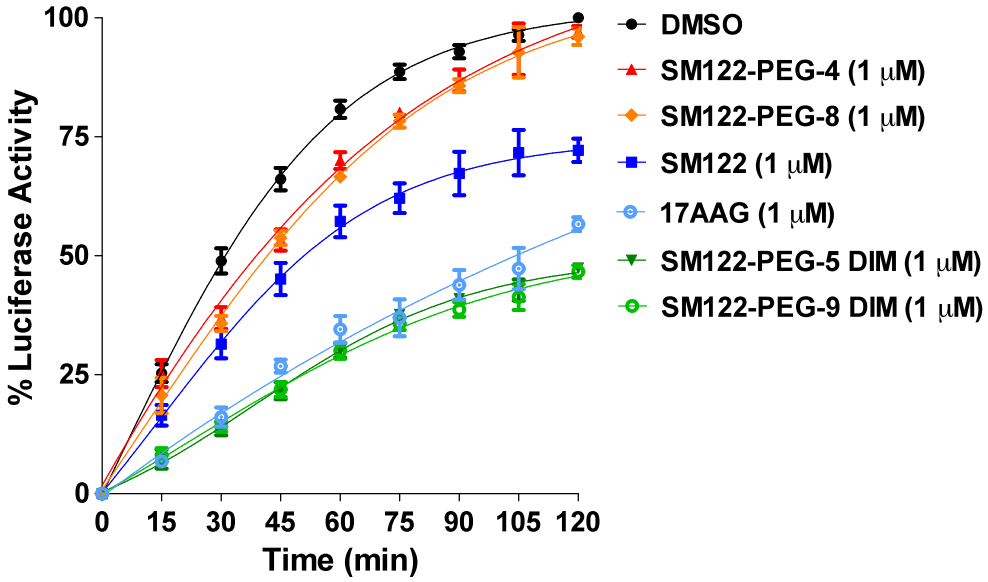
Solubility (μg/mL) = Solubility (μM) × MW of compound (g/mol) × 10^{-3} (L/mL) × 10^{6} (μg/g)

* 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.

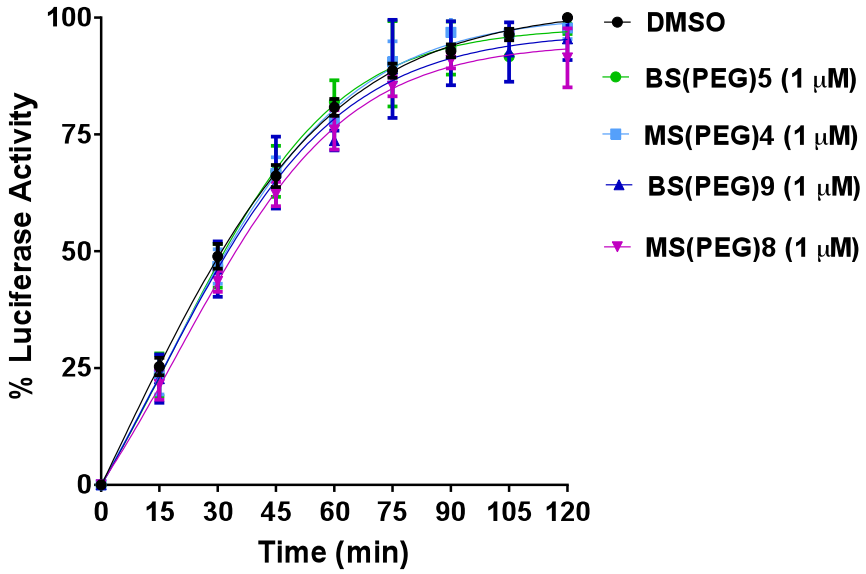
Solubility Method: ~5 mg of SM122-PEG conjugates or DIMERs was stirred in 0.3 mL of Milli-Q water for over 24 hours. The filtrate was filtered through a 45 micron filter to remove all insoluble particulates. The concentration of each sample was measured by LCMS utilizing Coumermycin A1 (CA-1) with known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

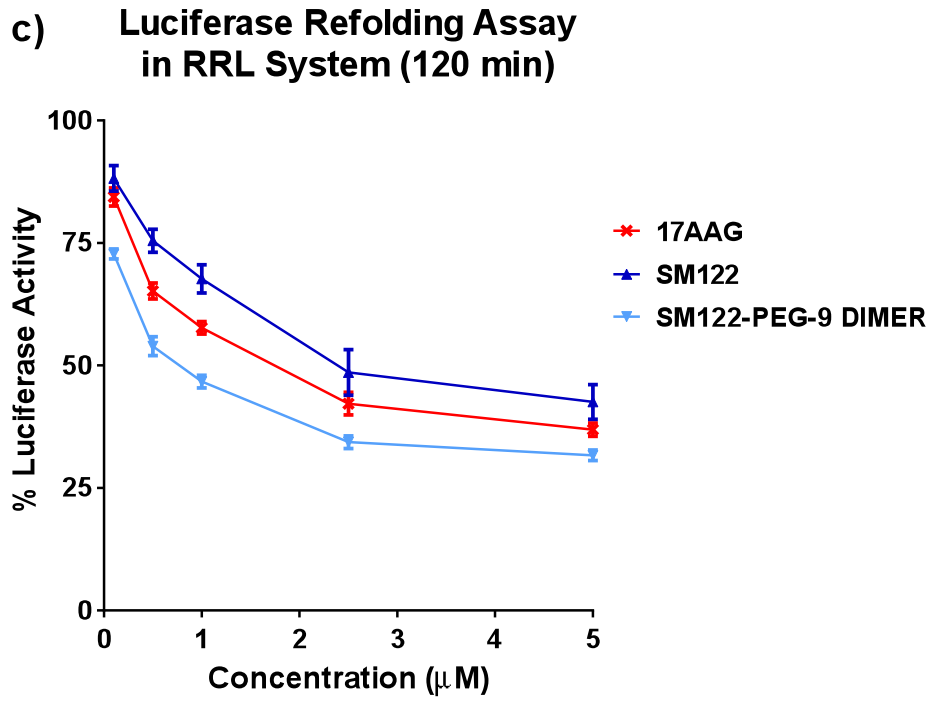
Biological data

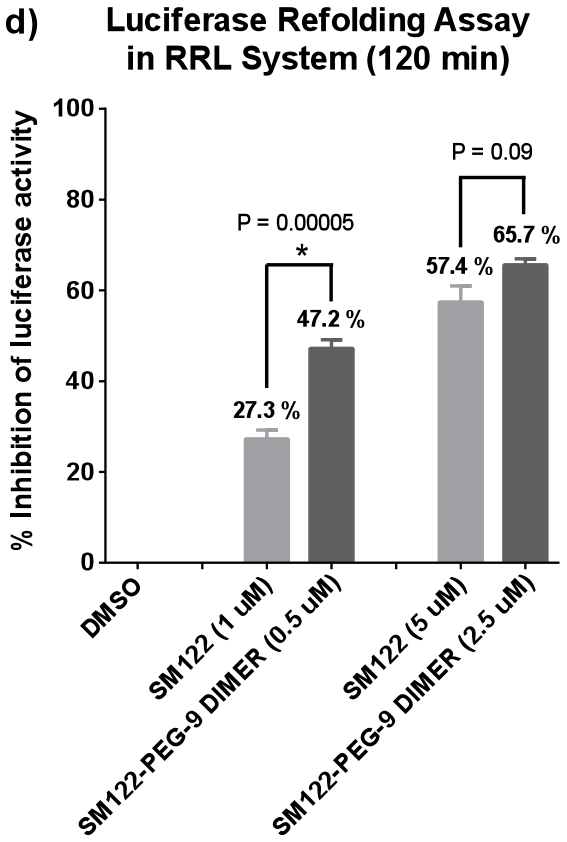
a) **Luciferase Refolding Assay in RRL System**



b) **Luciferase Refolding Assay in RRL System**



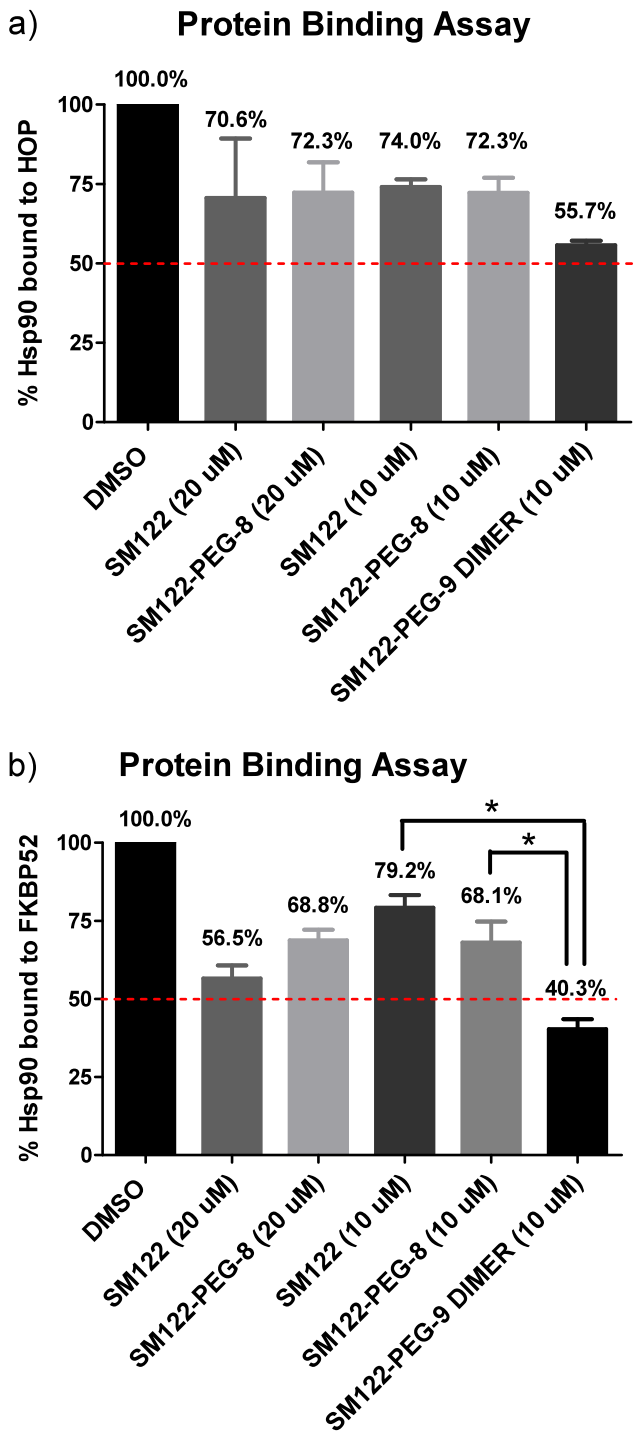




Supplemental Figure 1a-c: Luciferase activity or % inhibition of luciferase activity in rabbit reticulocyte lysate (RRL) refolding system treated with indicated concentrations of 17AAG, SM122, PEG conjugates or DIMERs. RRL was pre-incubated with compounds for 5 hours. Error bars indicate the standard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

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.