

**Potent, Selective, Allosteric Inhibition of Human Plasmin by Sulfated Non-Saccharide
Glycosaminoglycan Mimetics**

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Table S1. Dissociation Equilibrium Constants (K_D) and Maximal Fluorescence Change (ΔF_{MAX}) for the Interactions of NSGMs and UFH with Human Plasmin.^a

<i>Inhibitor</i>	K_D (μM)	ΔF_{MAX} (%)
2	0.7 ± 0.1^b	-112 ± 3
4	1.0 ± 0.1	-90 ± 2
10	3.6 ± 0.6	-101 ± 7
13	1.9 ± 0.2	-108 ± 3
UFH	6.7 ± 0.8	-97 ± 7

^a Measured using the intrinsic tryptophan fluorescence change in pH 7.4 buffer at 37 °C. See Experimental Part for details.

^b Errors represent standard error calculated using global fit of the data.

Table S2. Salt-Dependence of Plasmin Inhibition by NSGM 2.^a

<i>[NaCl]</i> (mM)	IC_{50} (μM)	<i>HS</i>	ΔY (%)
0	2.8 ± 0.3^b	1.0 ± 0.3	90 ± 7
100	6.3 ± 0.4	0.7 ± 0.1	93 ± 4
200	17.1 ± 2.7	0.8 ± 0.2	71 ± 7

^a The IC_{50} , *HS*, and ΔY values were obtained following non-linear regression analysis of direct inhibition of human plasmin in appropriate TrisHCl buffers of pH 7.4 at 37 °C. Inhibition was monitored spectro-photometrically. See Experimental Section for details. ^b Errors represent ± 1 S.E.

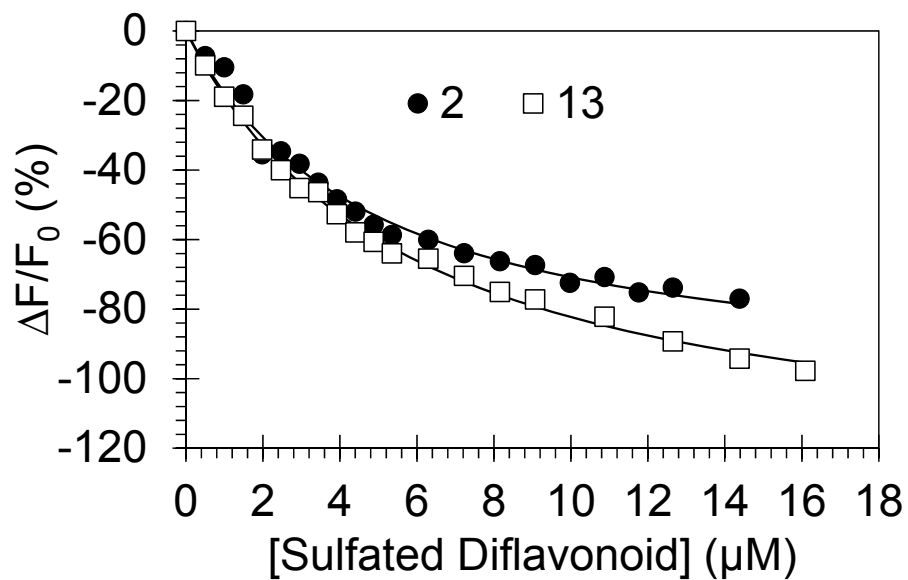


Figure S1: Spectrofluorometric measurement of the affinity of inhibitors **2** (●) and **13** (□) for active-site-blocked, dansyl-EGR-cmk- plasmin at pH 7.4 and 37 °C using the intrinsic tryptophan fluorescence ($\lambda_{EM} = 348$ nm, $\lambda_{EX} = 280$ nm). Solid lines represent the nonlinear regressional fits using quadratic equation 3.

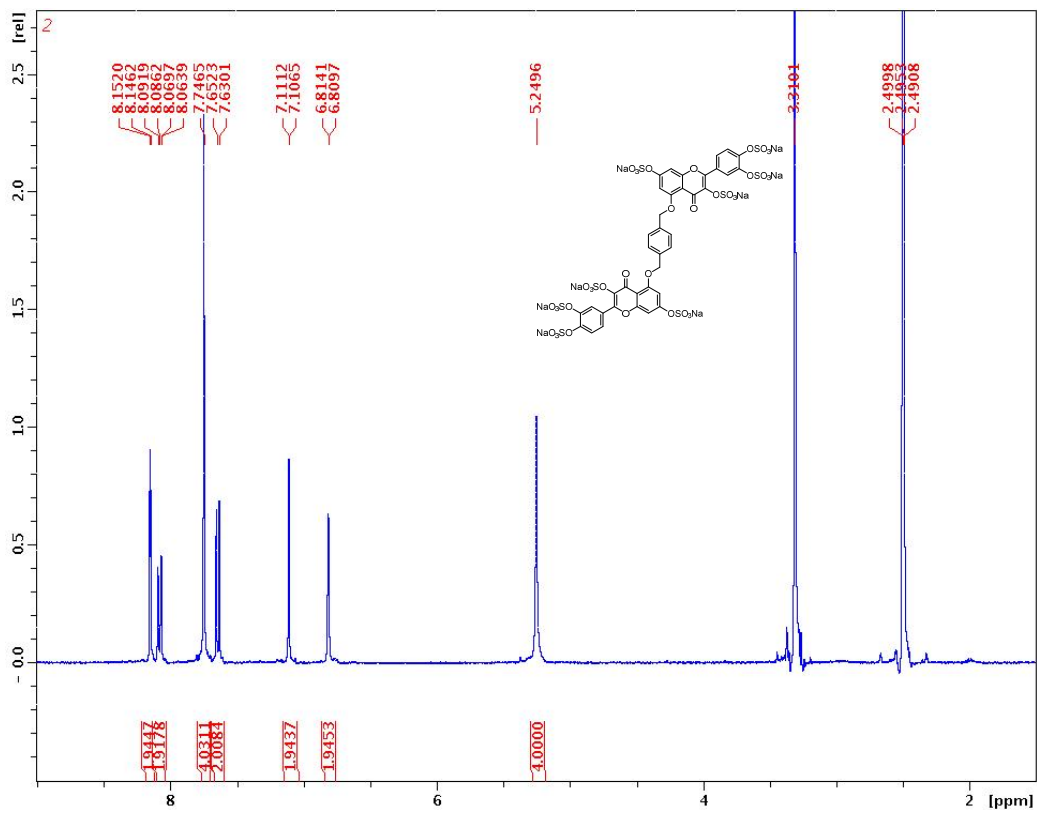


Figure S1a: ¹H NMR (400 MHz, DMSO-d₆) for NSGM 2

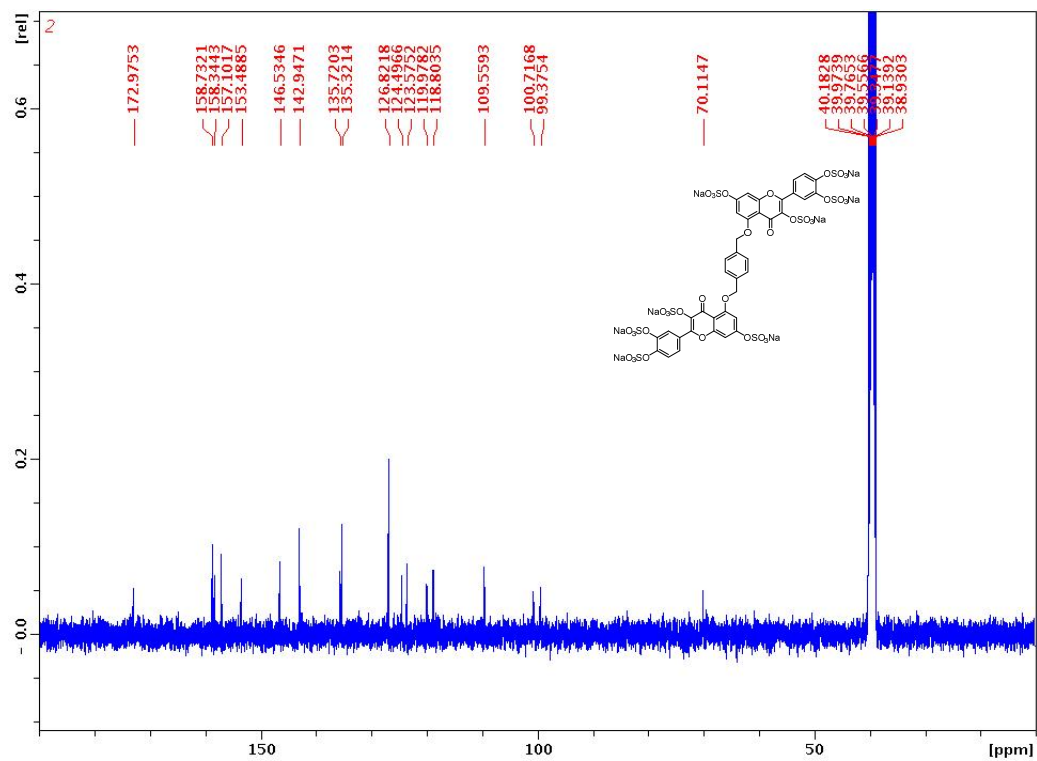


Figure S1b: ¹³C NMR (100 MHz, DMSO-d₆) for NSGM 2

2

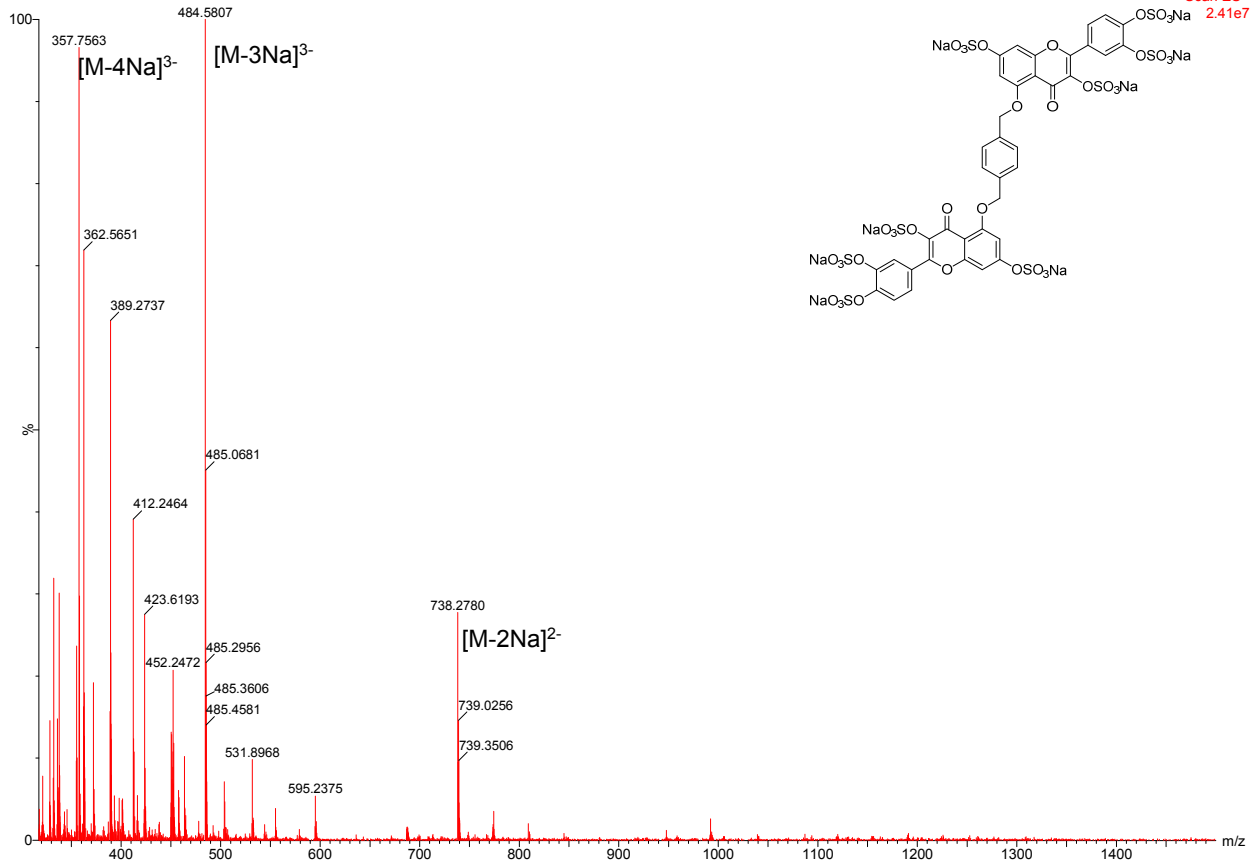


Figure S1c: Mass Spectrum for NSGM 2

DANIEL SAMPLE 0
DA_SFD_2 Sm (Mn, 5x5)

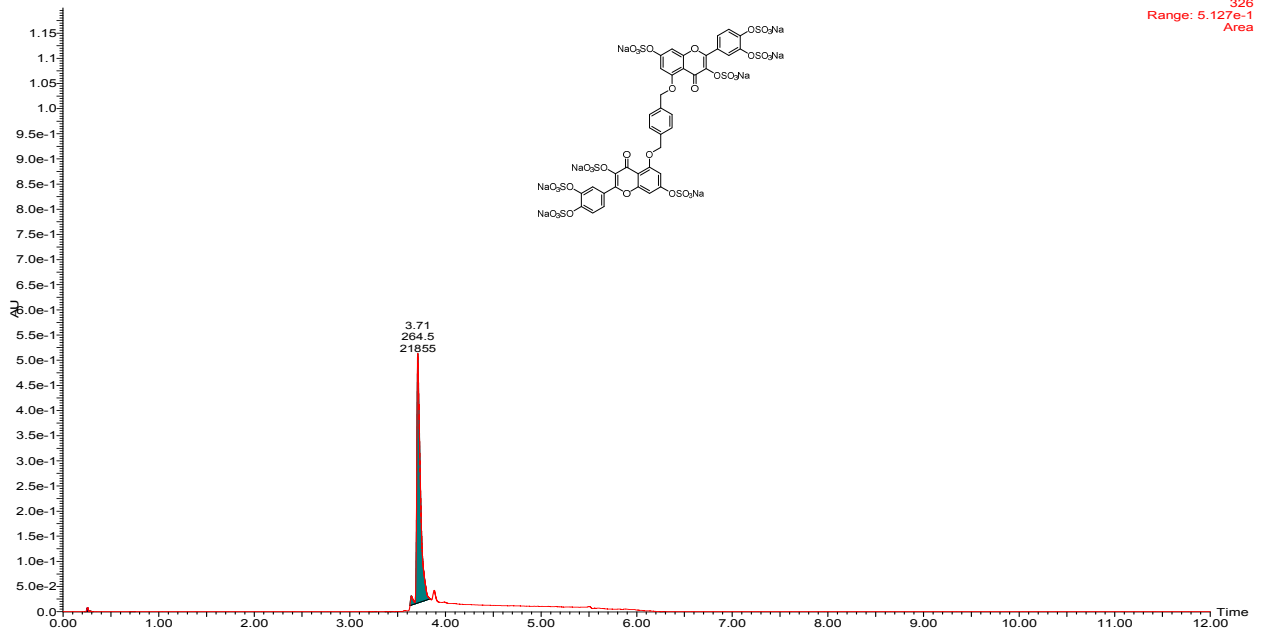


Figure S1d: UPLC profile for NSGM 2

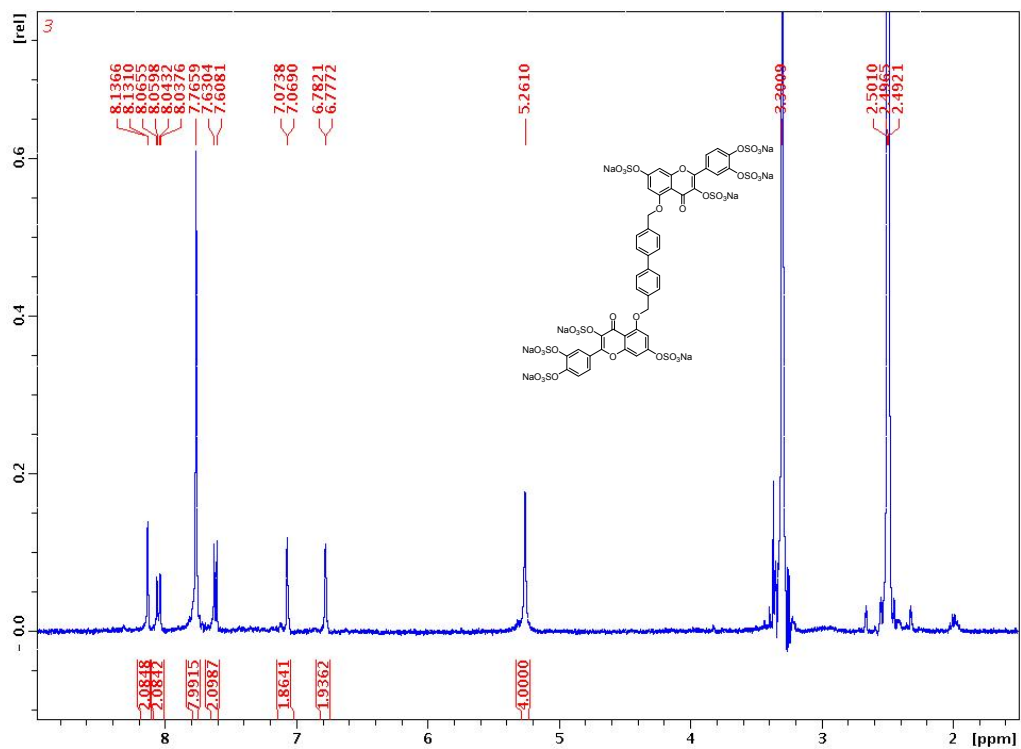


Figure S2a: ^1H NMR (400 MHz, DMSO- d_6) for NSGM 3

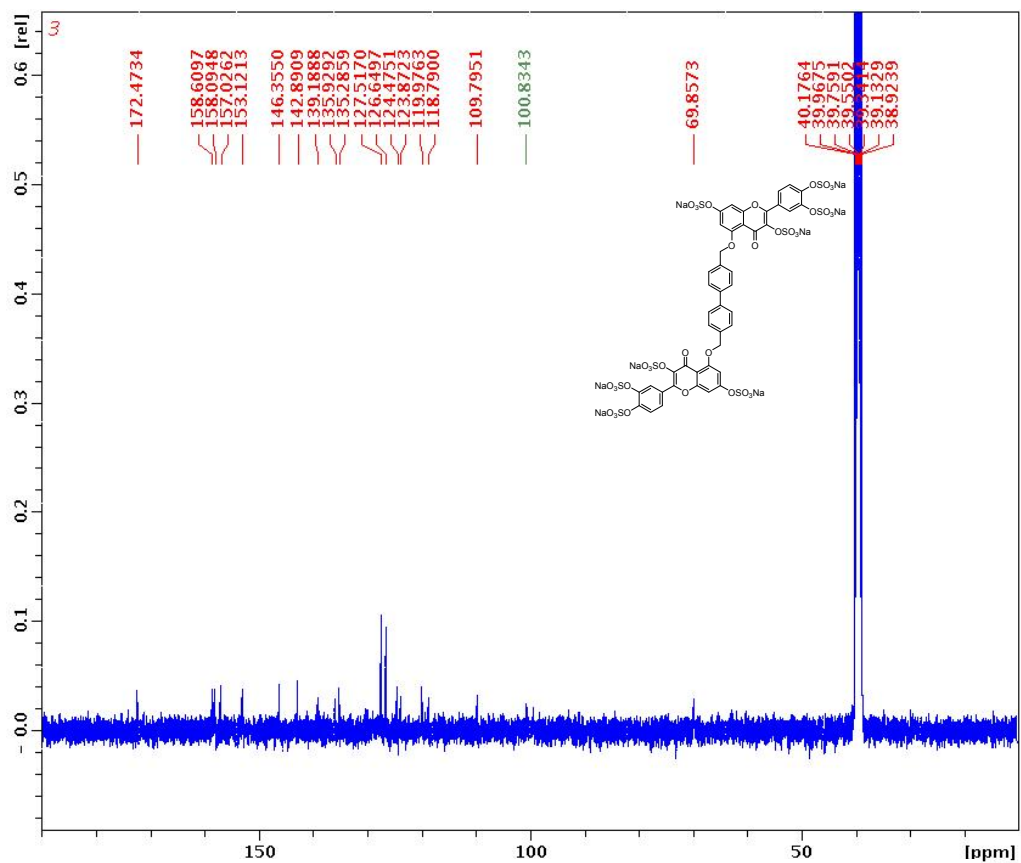


Figure S2b: ^{13}C NMR (100 MHz, DMSO- d_6) for NSGM 3

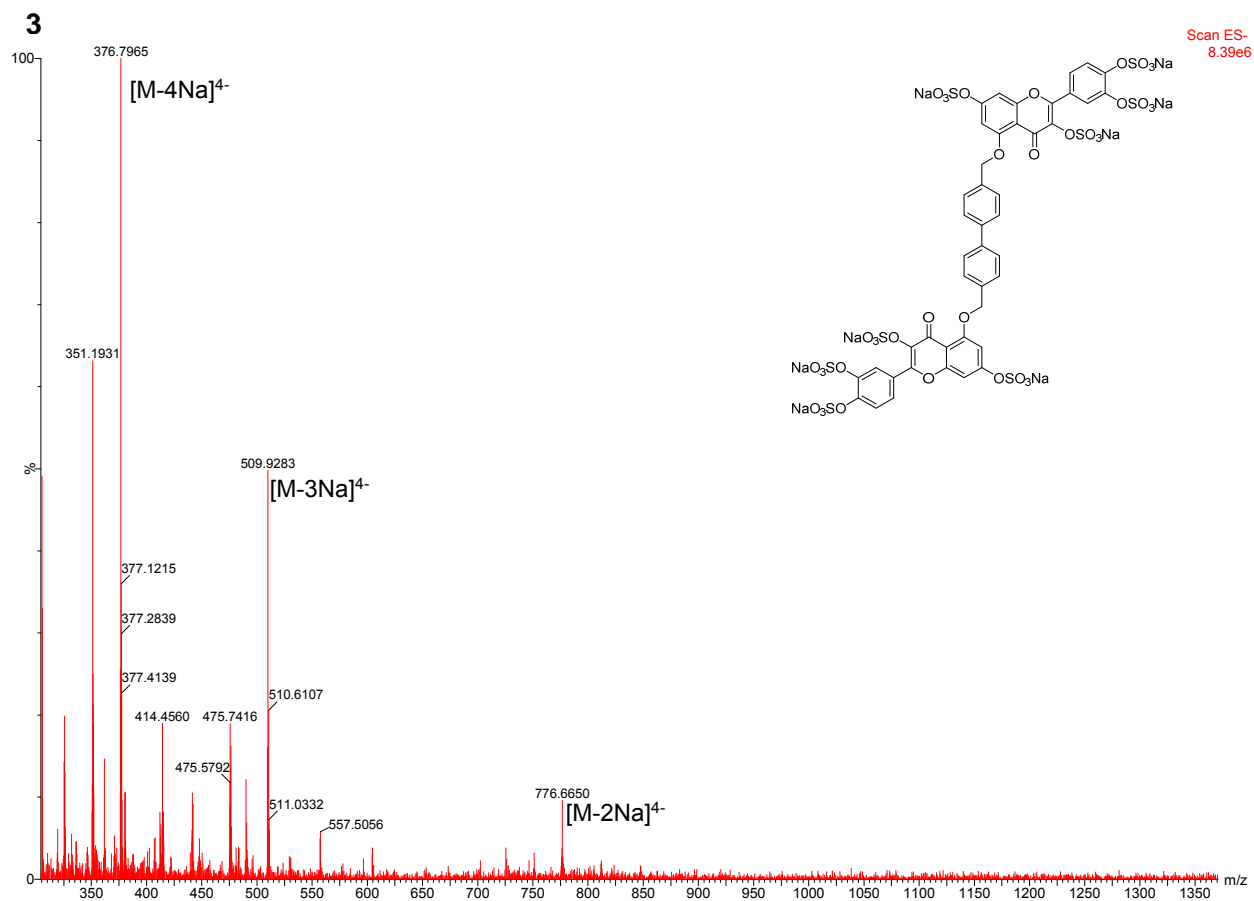


Figure S2c: Mass Spectrum for NSGM 3

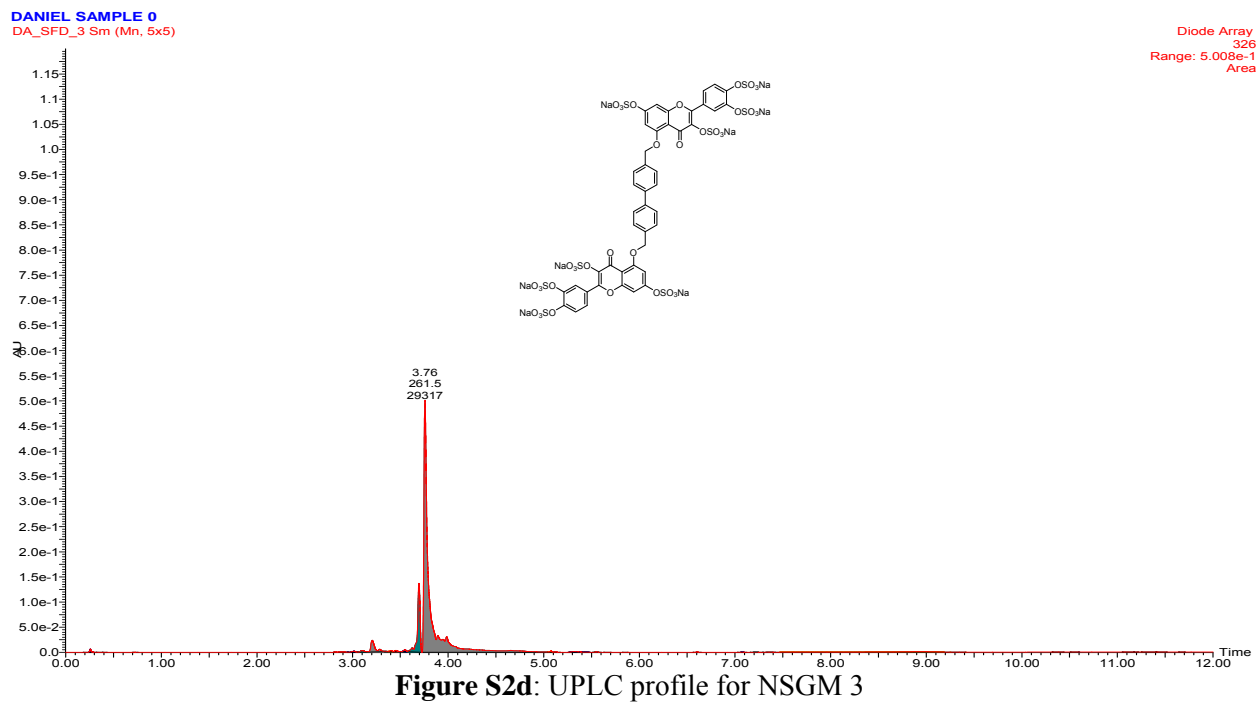


Figure S2d: UPLC profile for NSGM 3

4

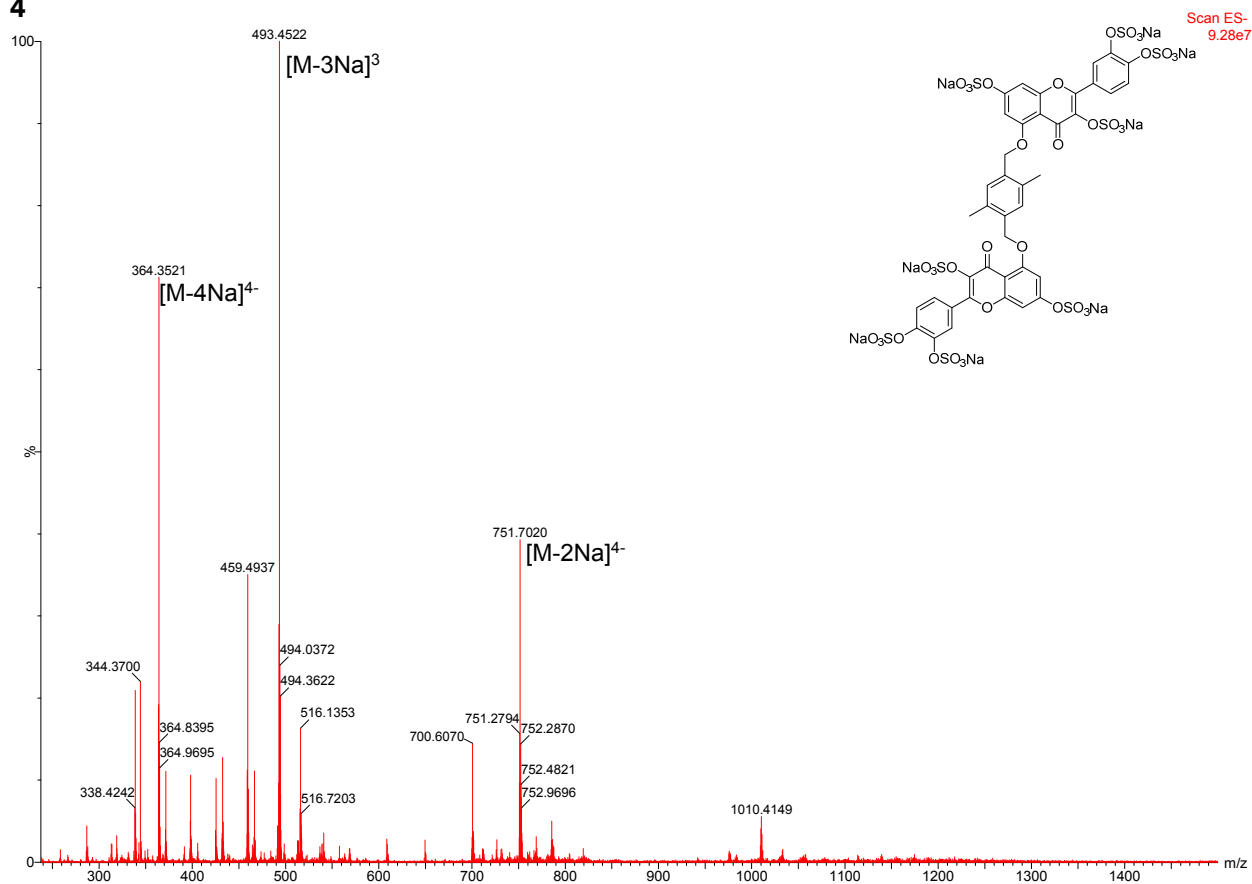


Figure S3c: Mass Spectrum for NSGM 4

DANIEL SAMPLE 0
DA_SFD_4 Sm (Mn, 5x5)

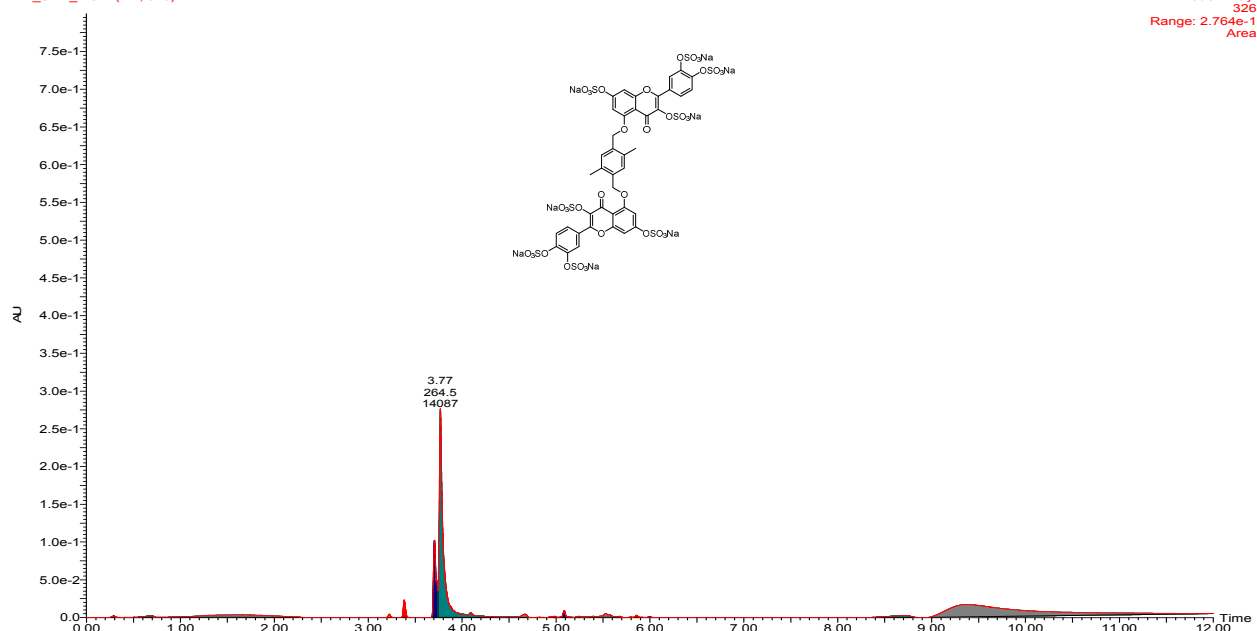


Figure S3d: UPLC profile for NSGM 4

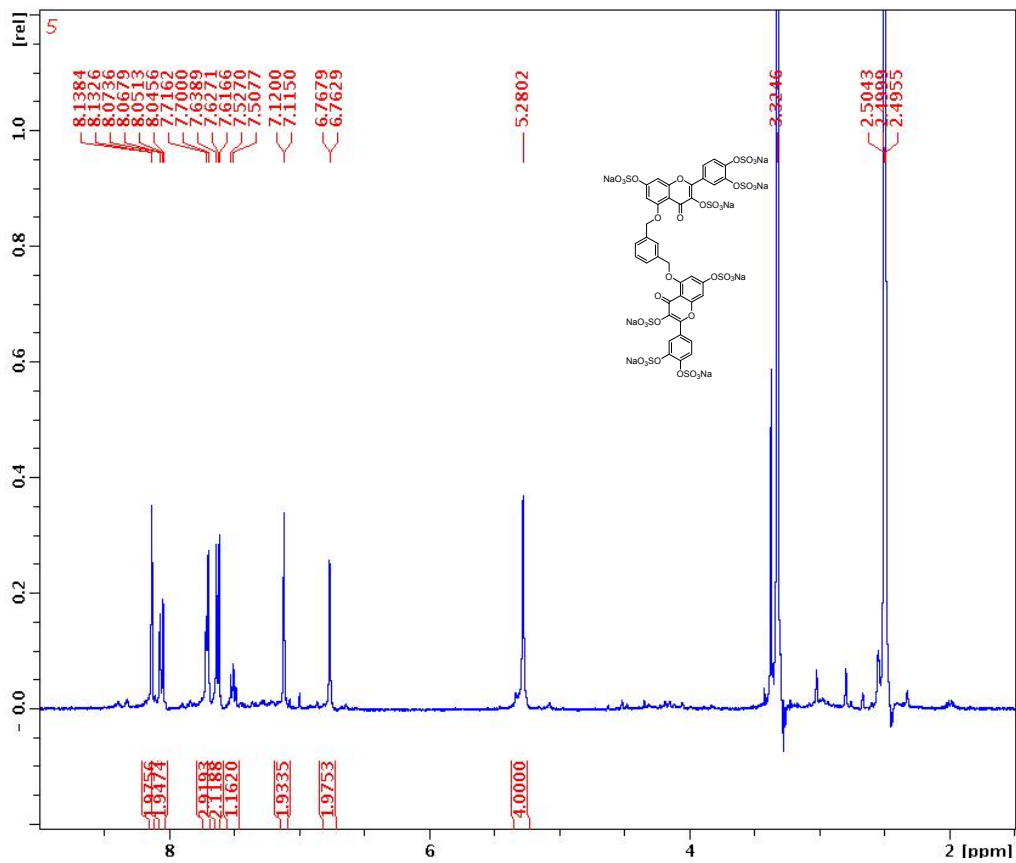


Figure S4a: ¹H NMR (400 MHz, DMSO-d₆) for NSGM 5

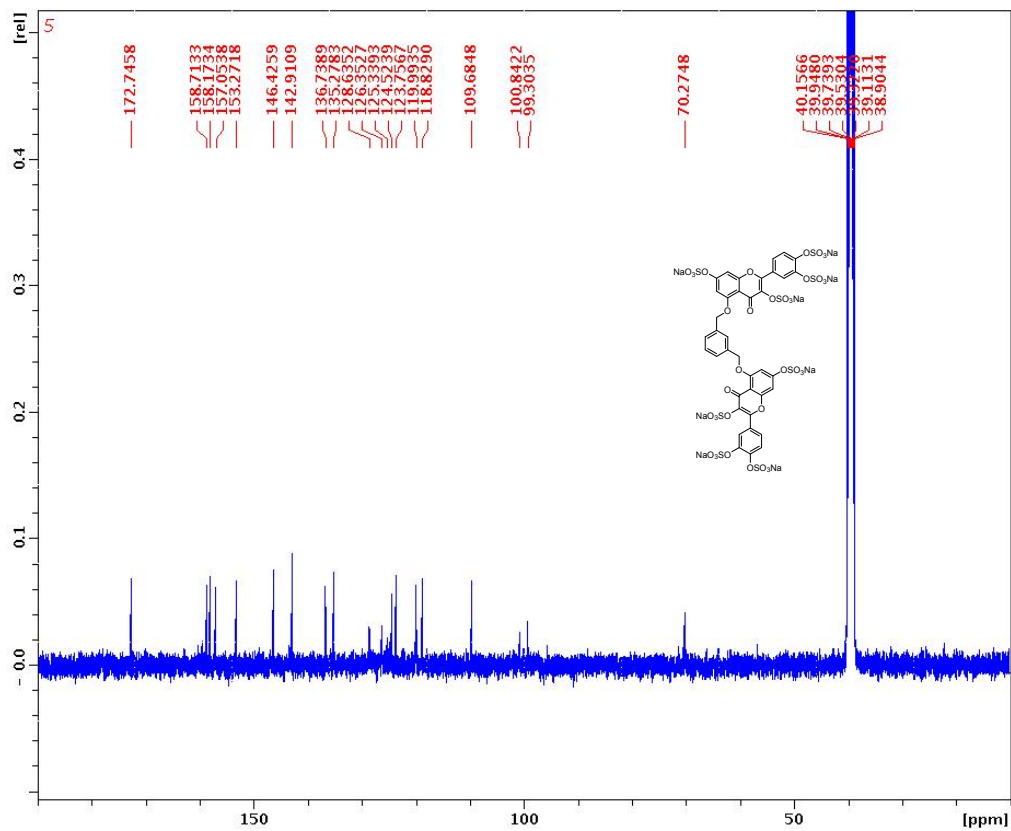


Figure S4b: ¹³C NMR (100 MHz, DMSO-d₆) for NSGM 5

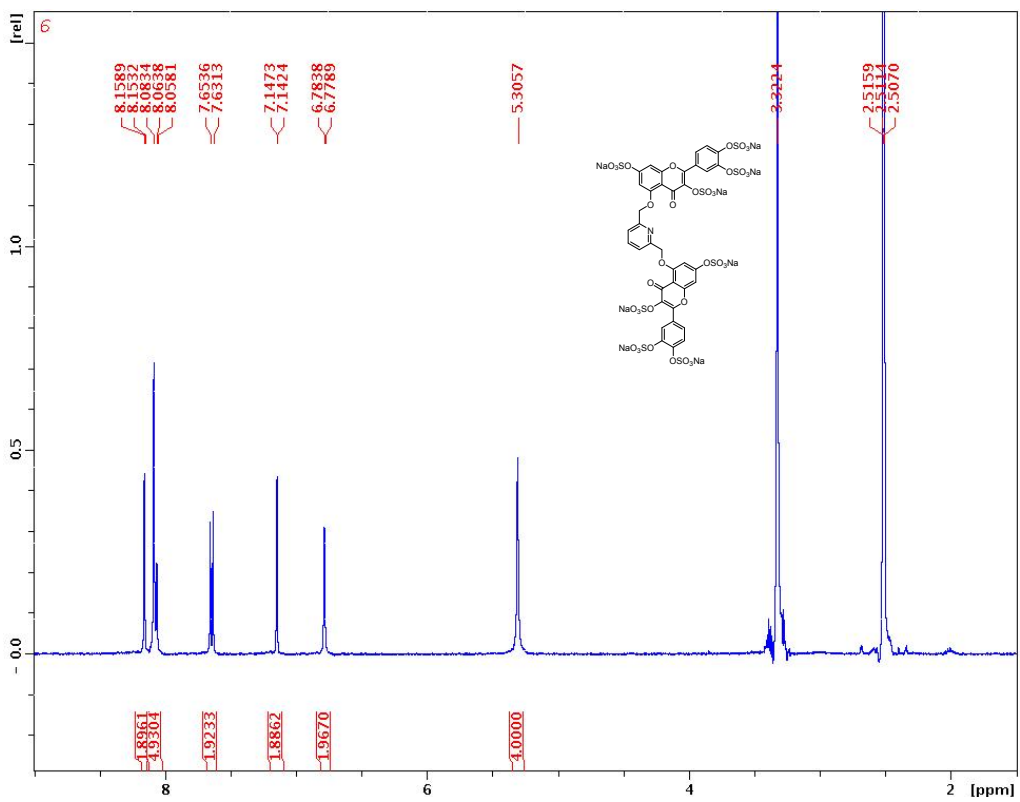


Figure S5a: ^1H NMR (400 MHz, DMSO-d₆) for NSGM 6

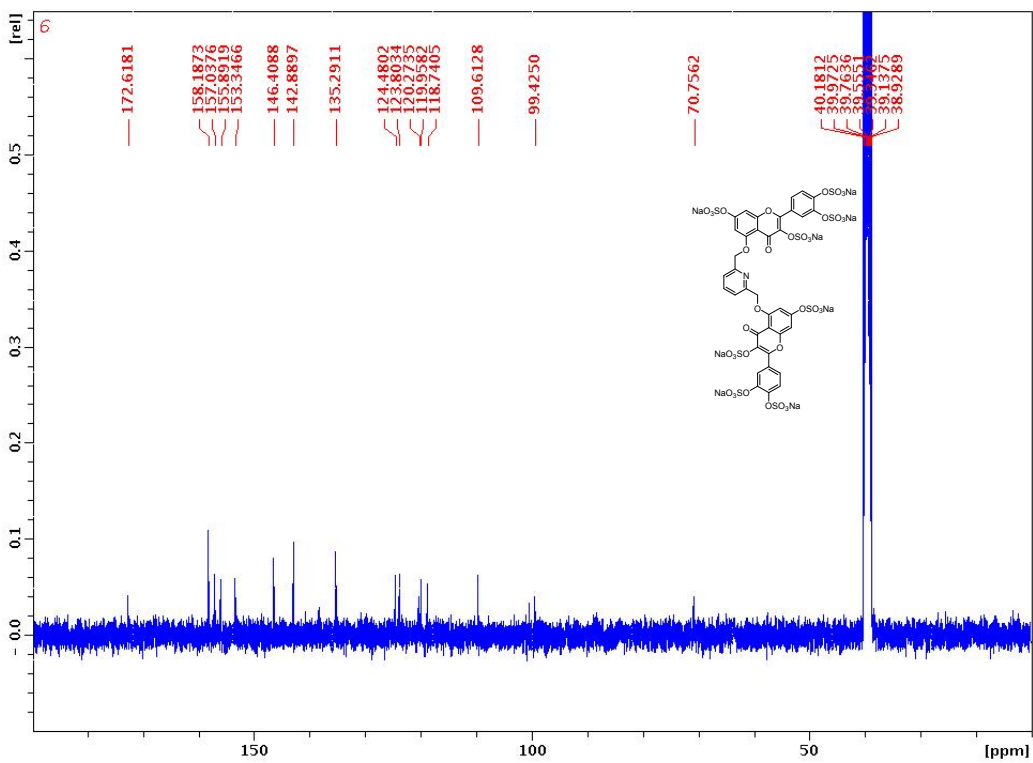


Figure S5b: ^{13}C NMR (100 MHz, DMSO-d₆) for NSGM 6

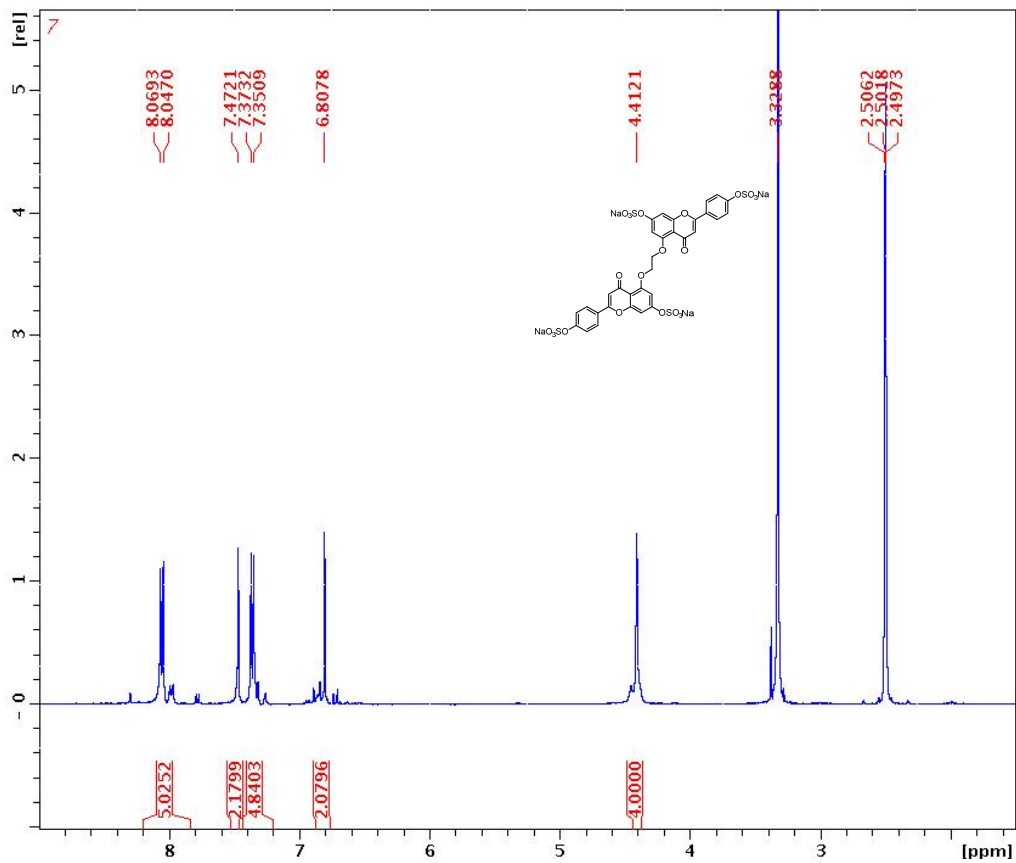


Figure S6a: ^1H NMR (400 MHz, DMSO- d_6) for NSGM 7

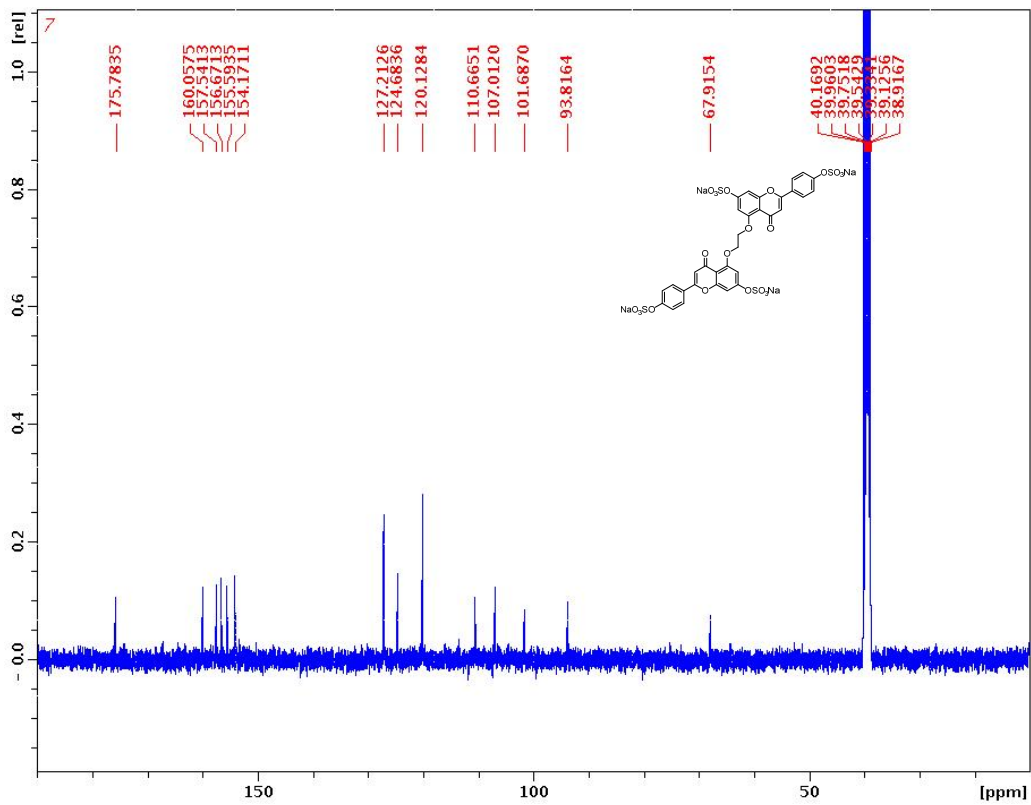


Figure S6b: ^{13}C NMR (100 MHz, DMSO- d_6) for NSGM 7

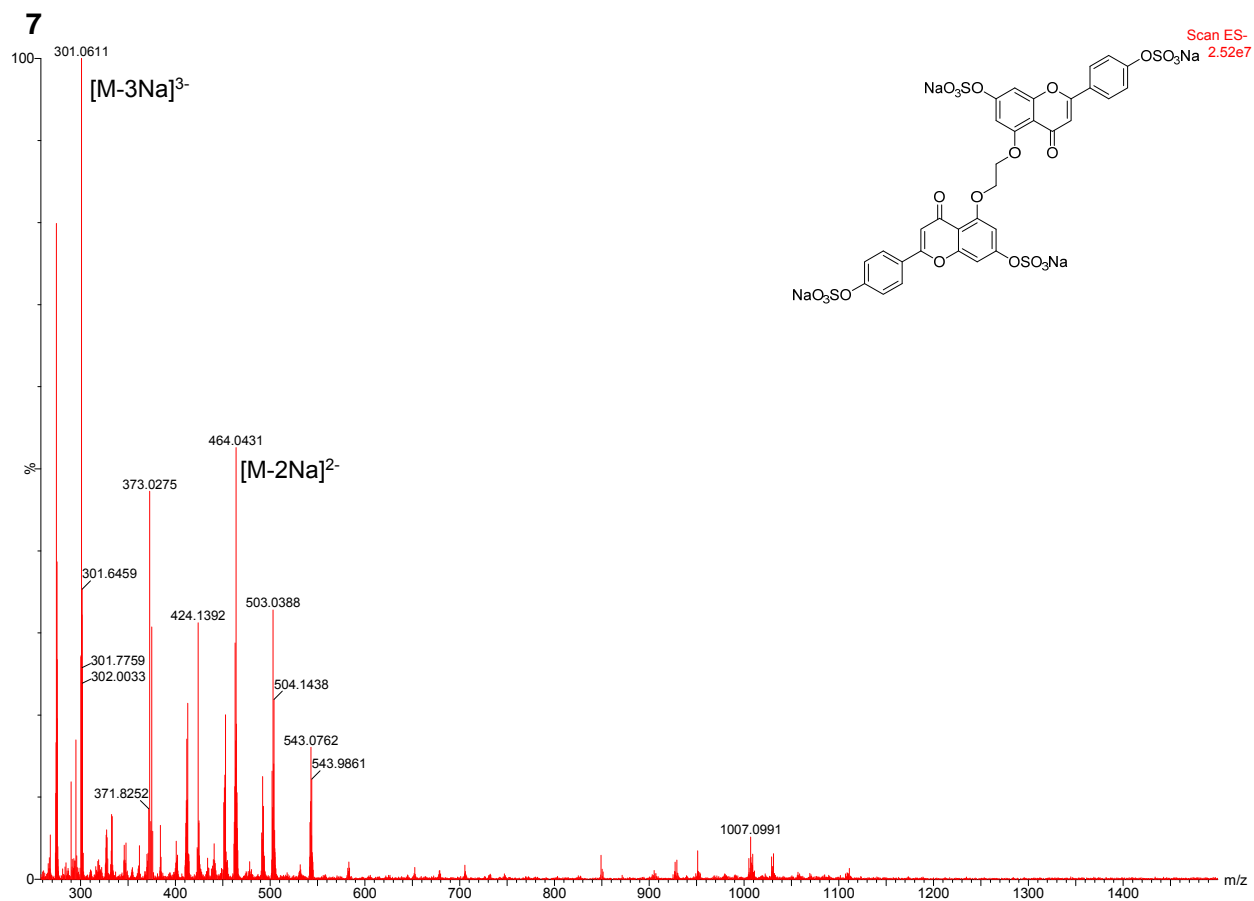


Figure S6c: Mass Spectrum for NSGM 7

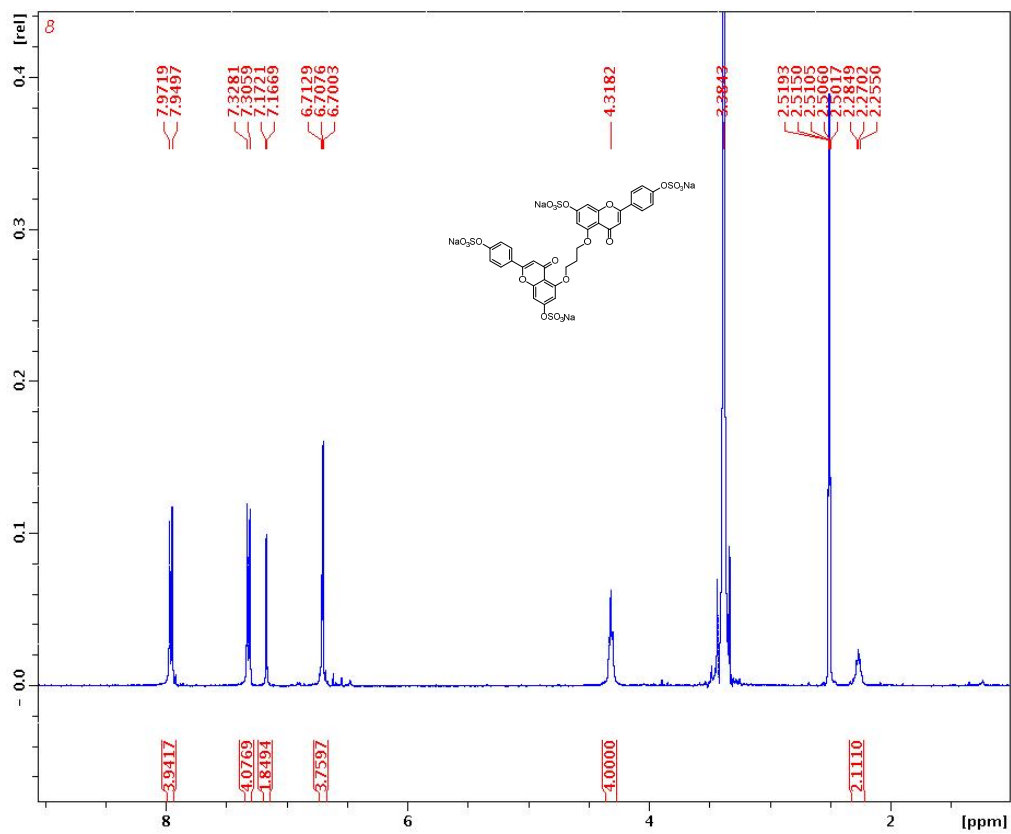


Figure S7a: ^1H NMR (400 MHz, DMSO- d_6) for NSGM 8

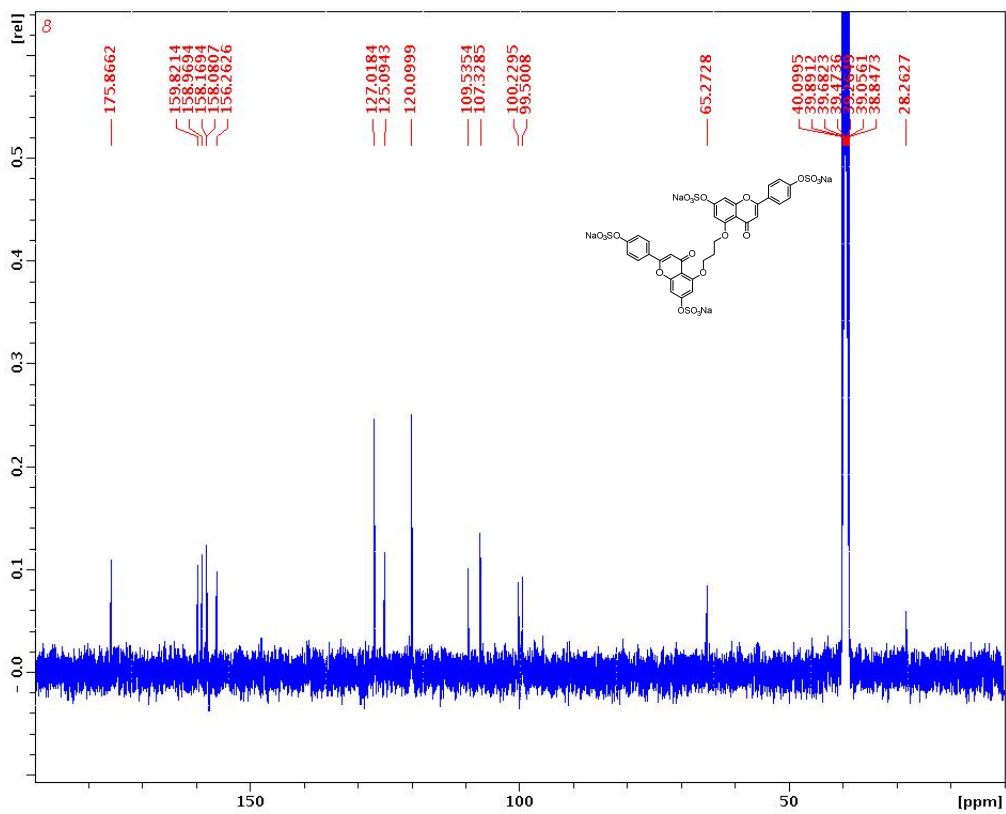


Figure S7b: ^{13}C NMR (100 MHz, DMSO- d_6) for NSGM 8

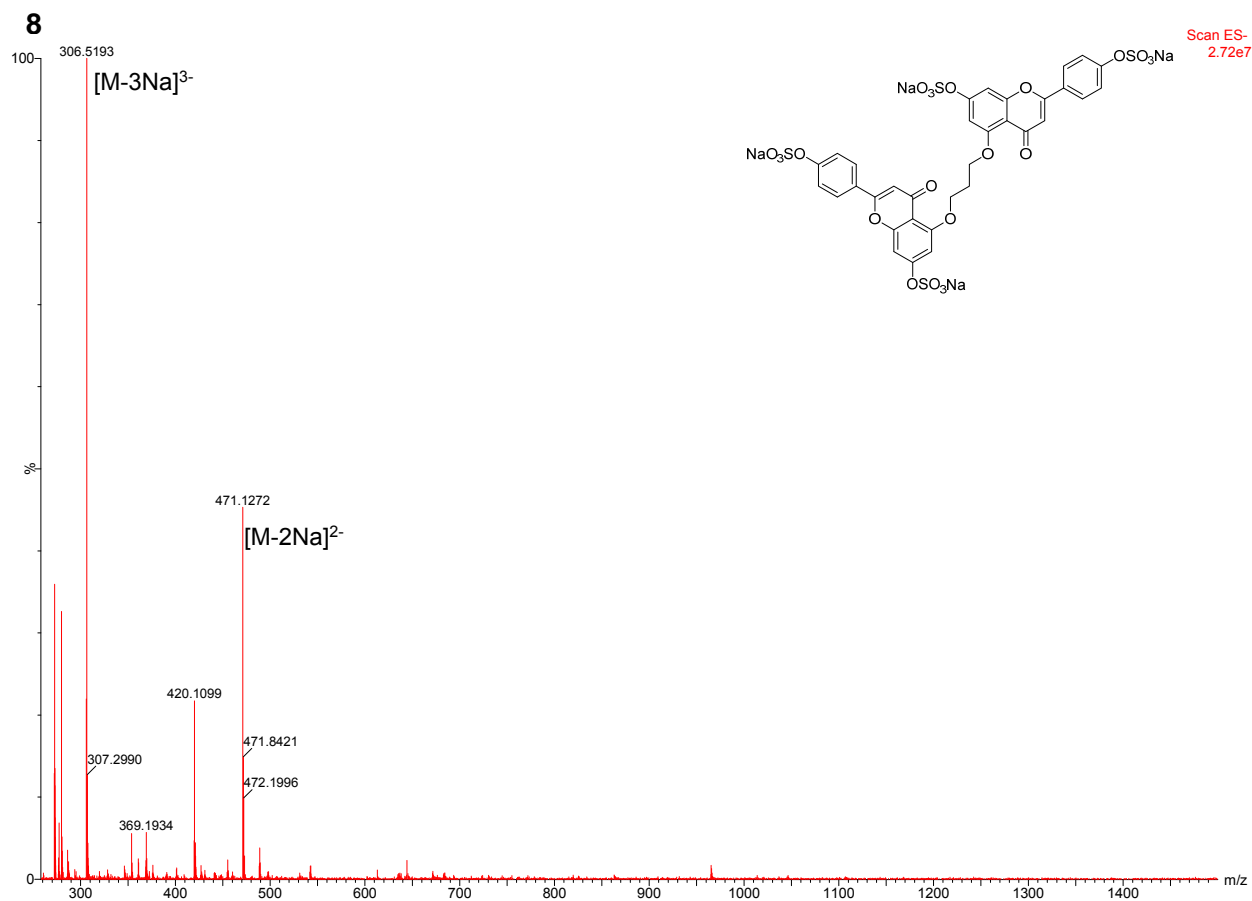


Figure S7c: Mass Spectrum for NSGM 8

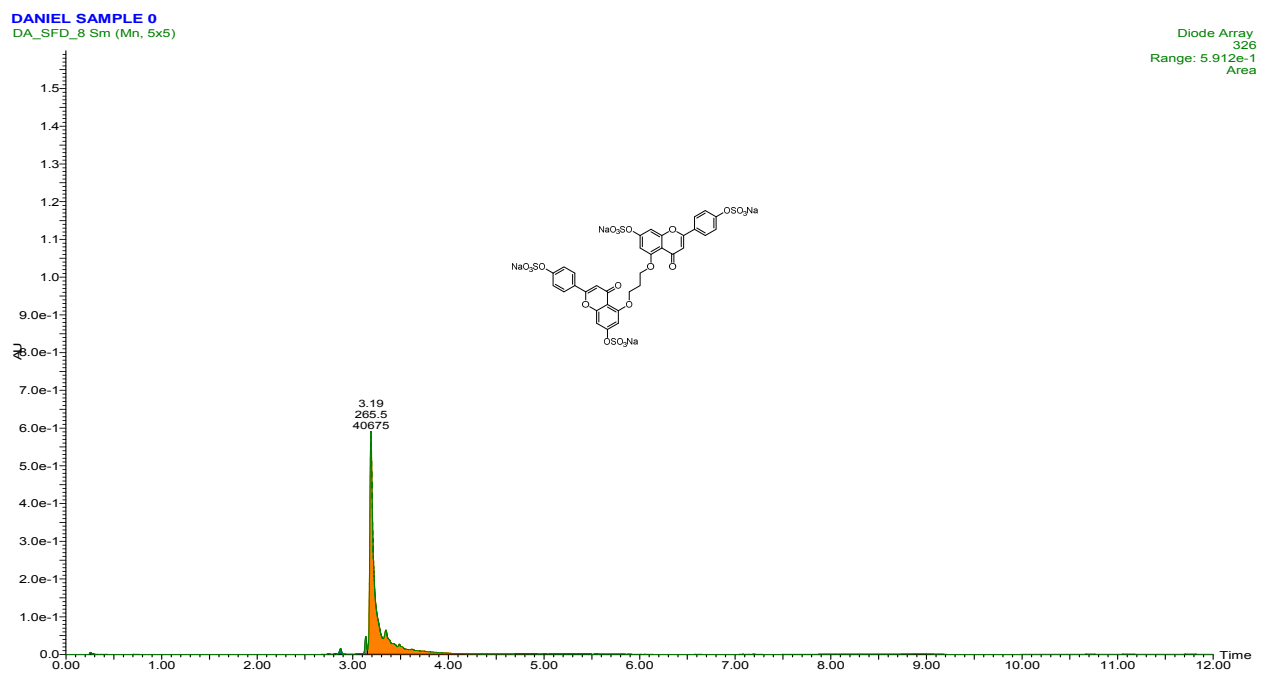


Figure S7d: UPLC profile for NSGM 8

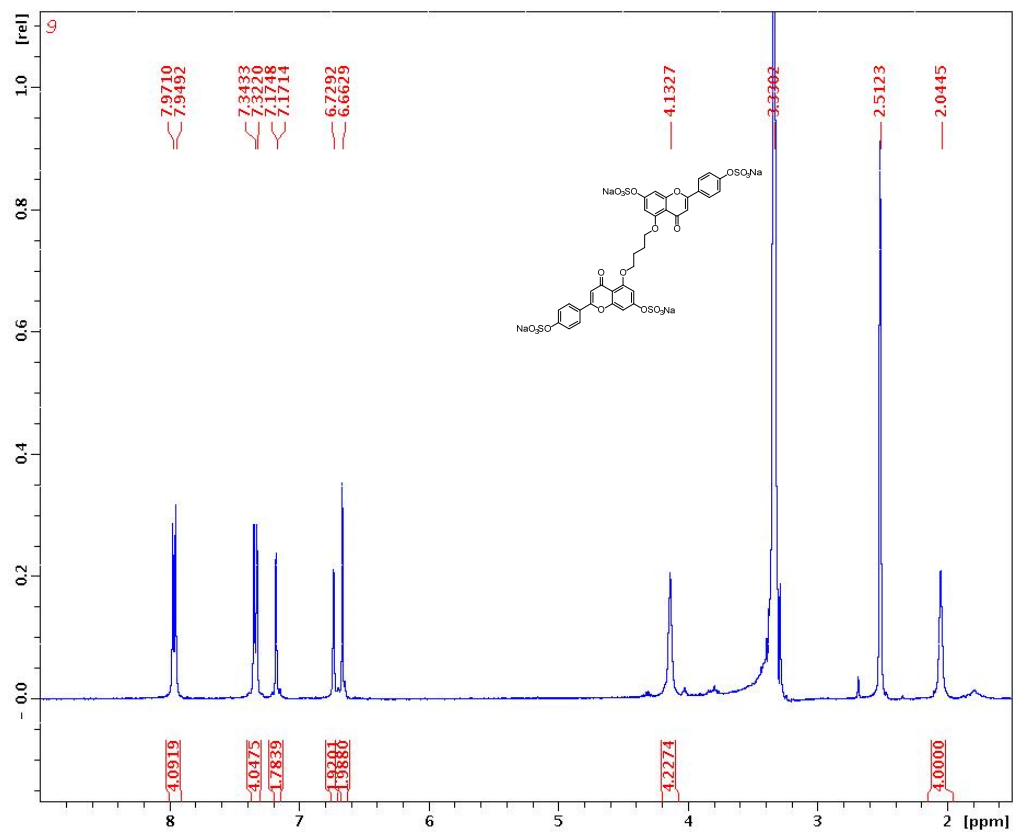


Figure S8a: ^1H NMR (400 MHz, DMSO- d_6) for NSGM 9

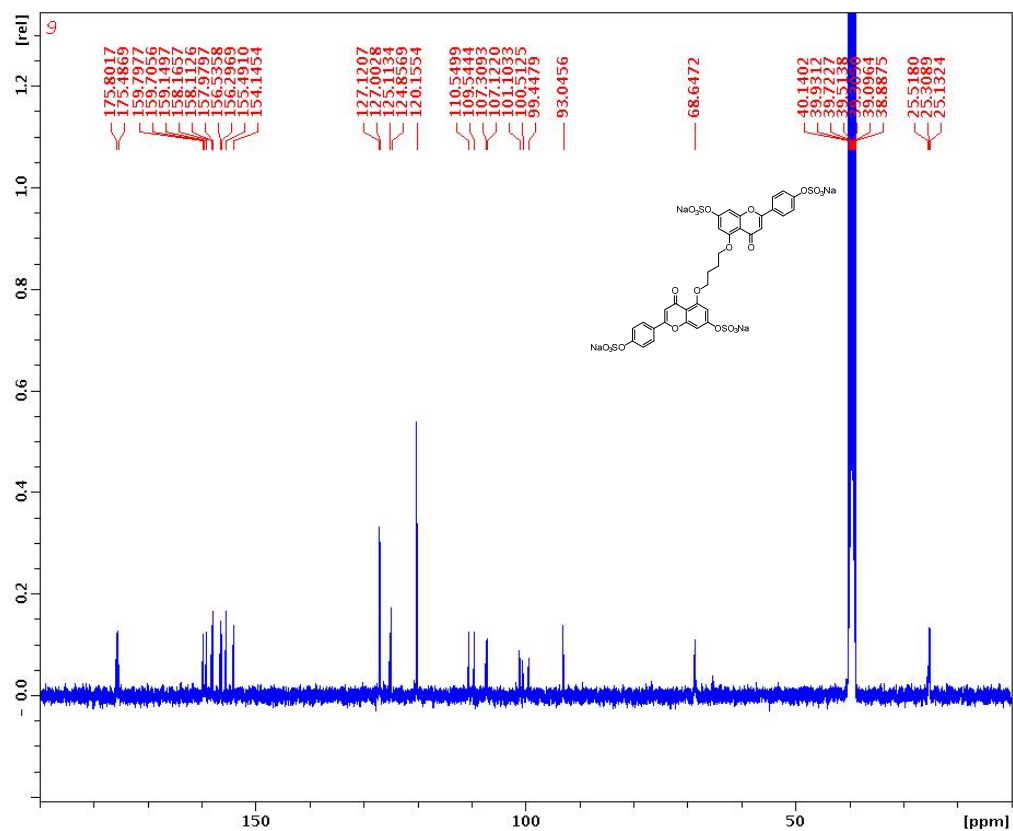


Figure S8b: ^{13}C NMR (100 MHz, DMSO- d_6) for NSGM 9

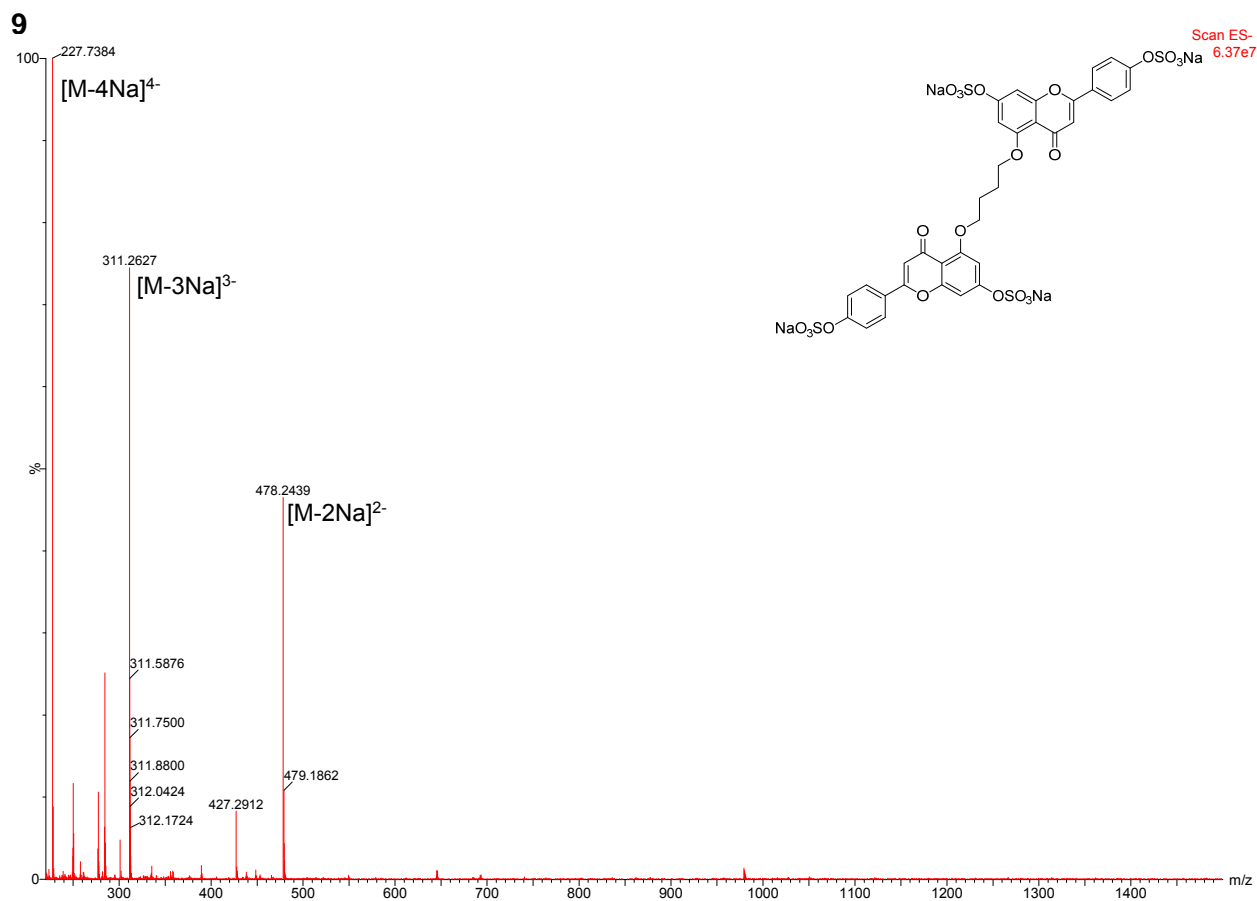


Figure S8c: Mass Spectrum for NSGM 9

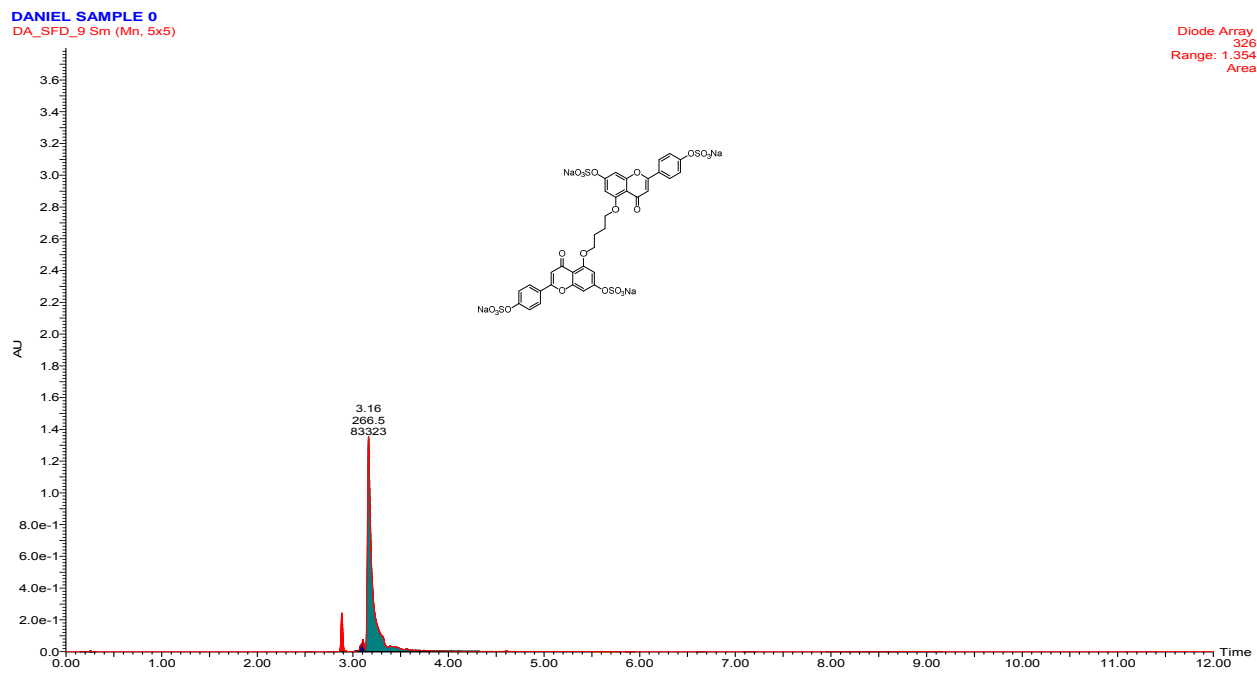


Figure S8c: UPLC profile for NSGM 9

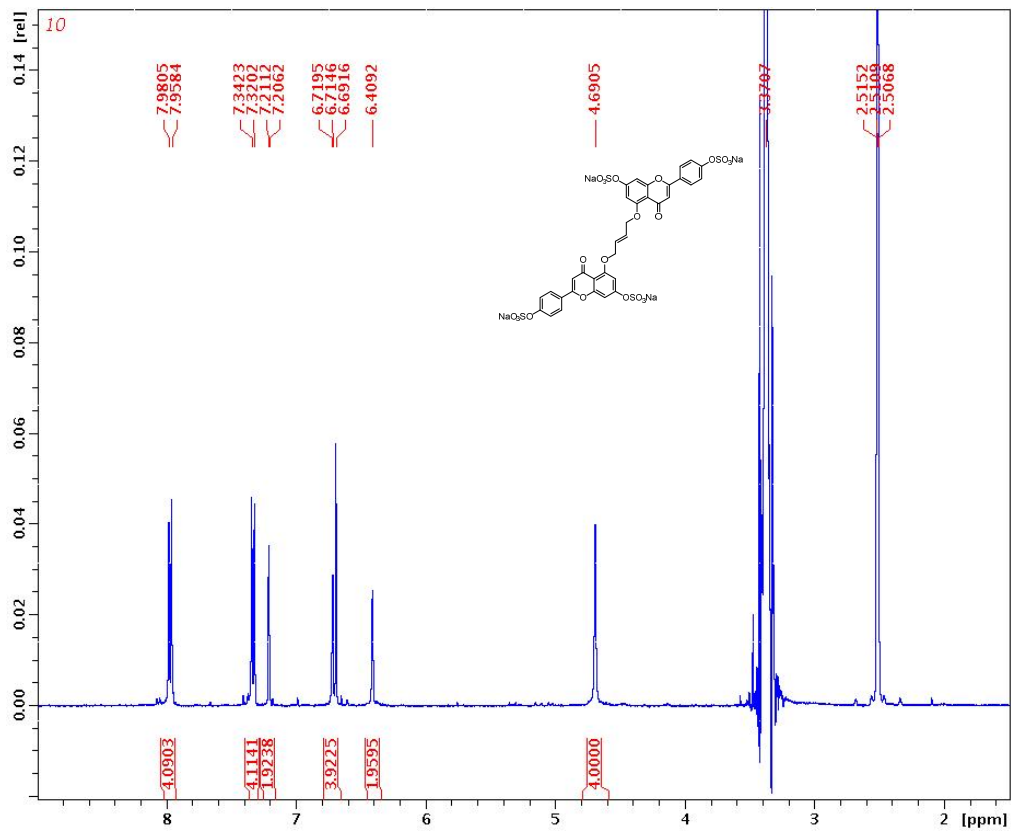


Figure S9a: ^1H NMR (400 MHz, DMSO- d_6) for NSGM 10

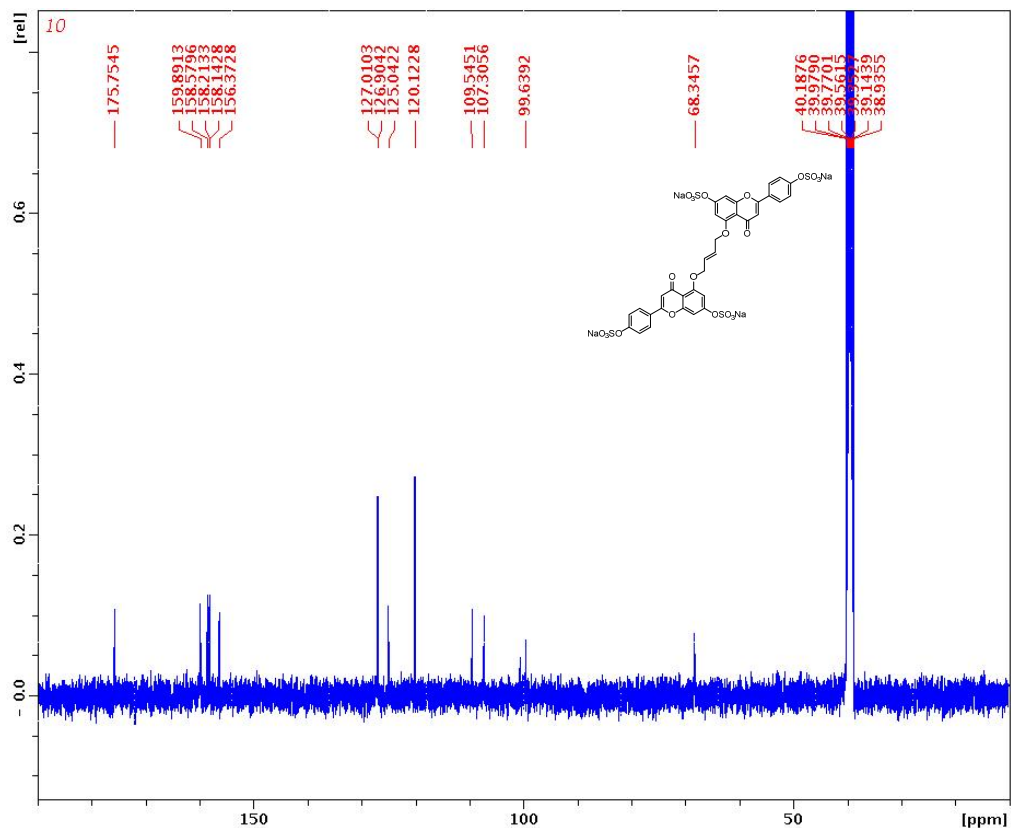


Figure S9b: ^{13}C NMR (100 MHz, DMSO- d_6) for NSGM 10

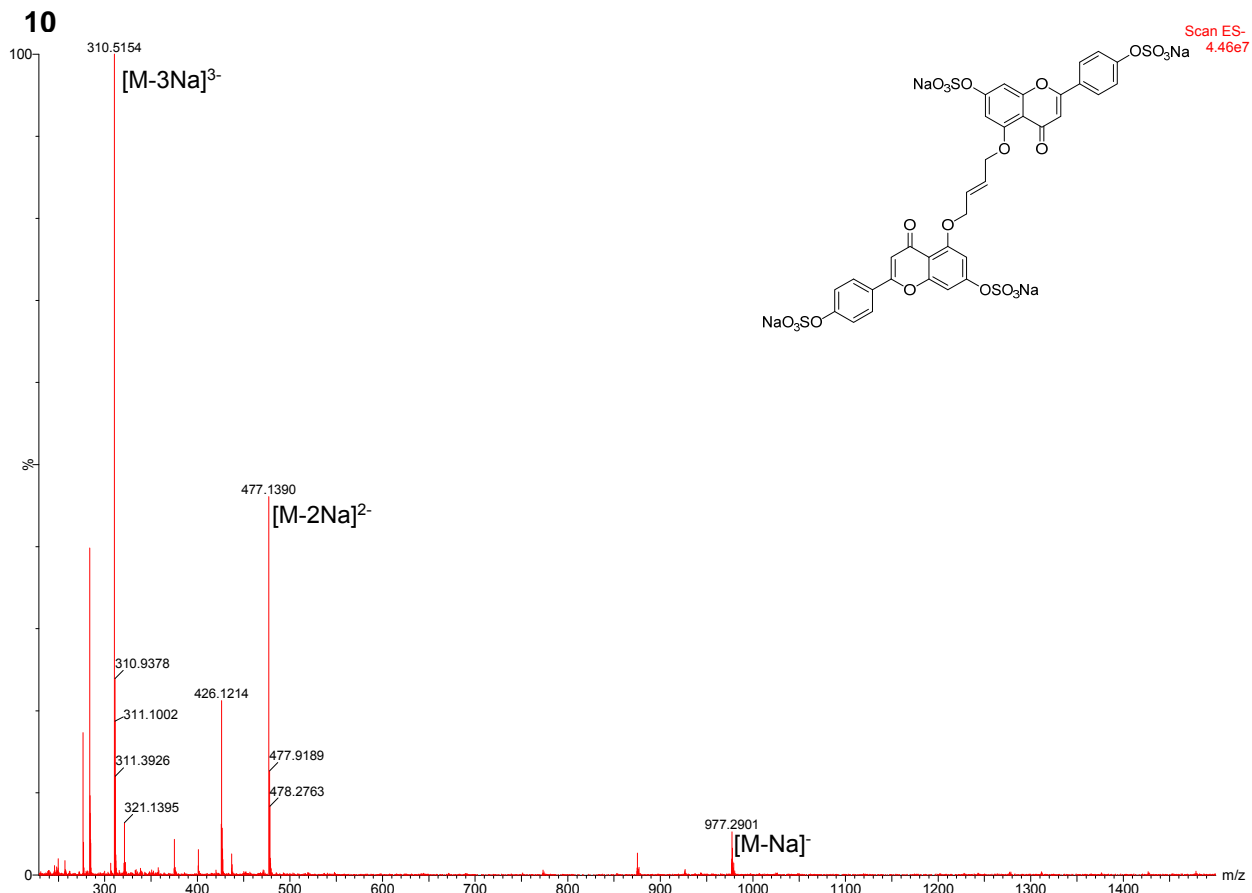


Figure S9c: Mass Spectrum for NSGM 10

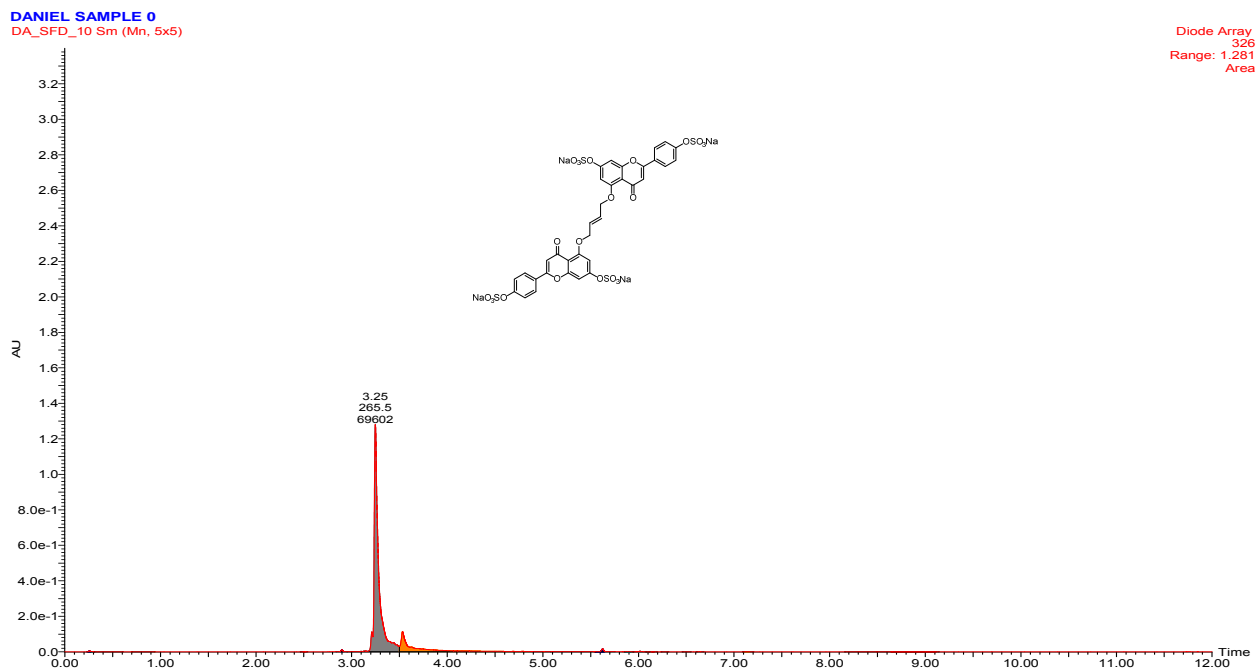


Figure S9d: UPLC profile for NSGM 10

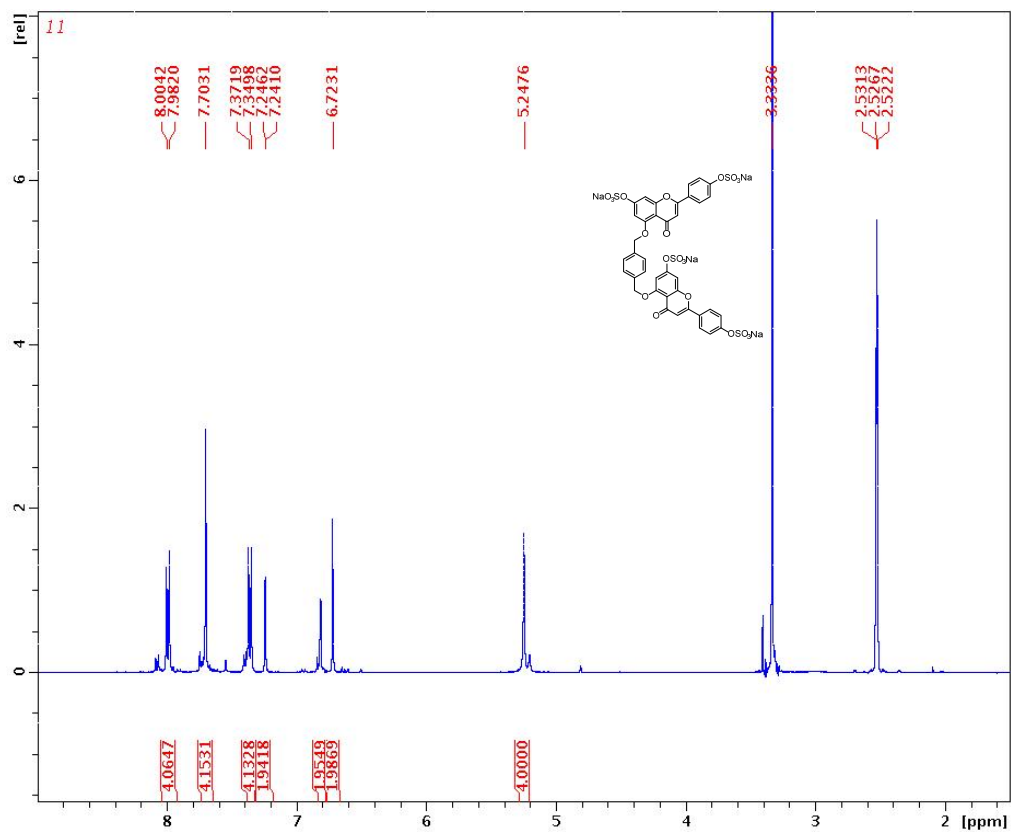


Figure S10a: ^1H NMR (400 MHz, DMSO- d_6) for NSGM 11

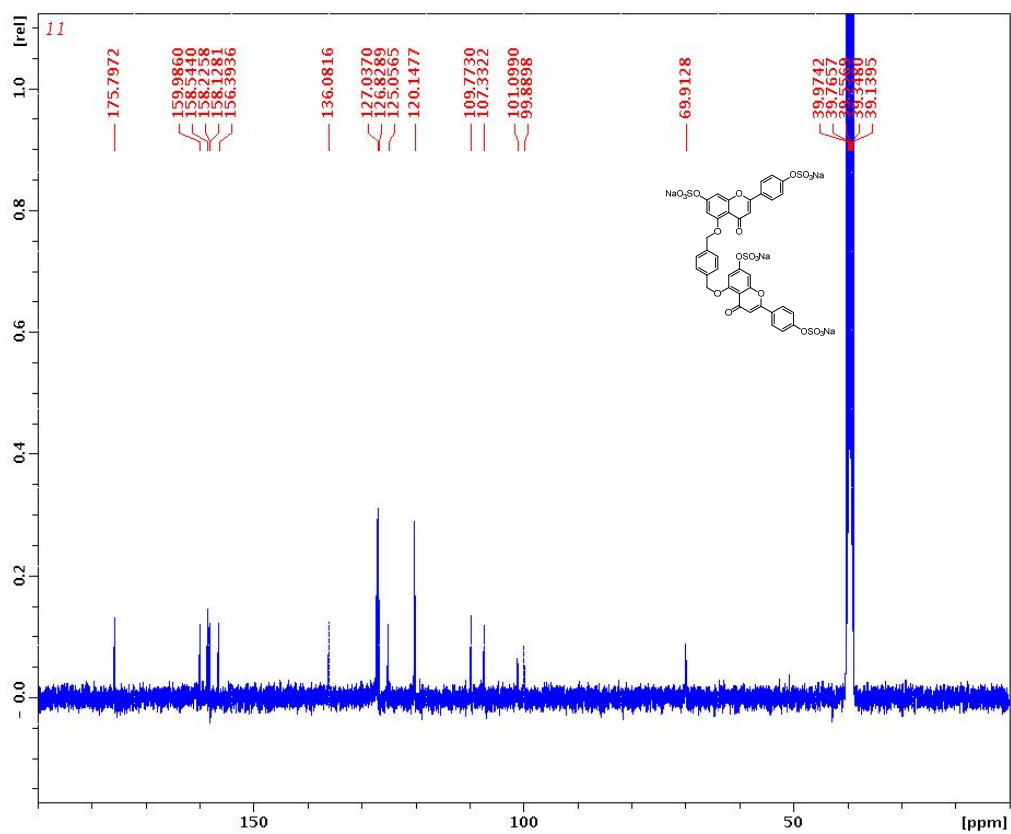


Figure S10b: ^{13}C NMR (100 MHz, DMSO- d_6) for NSGM 11

11

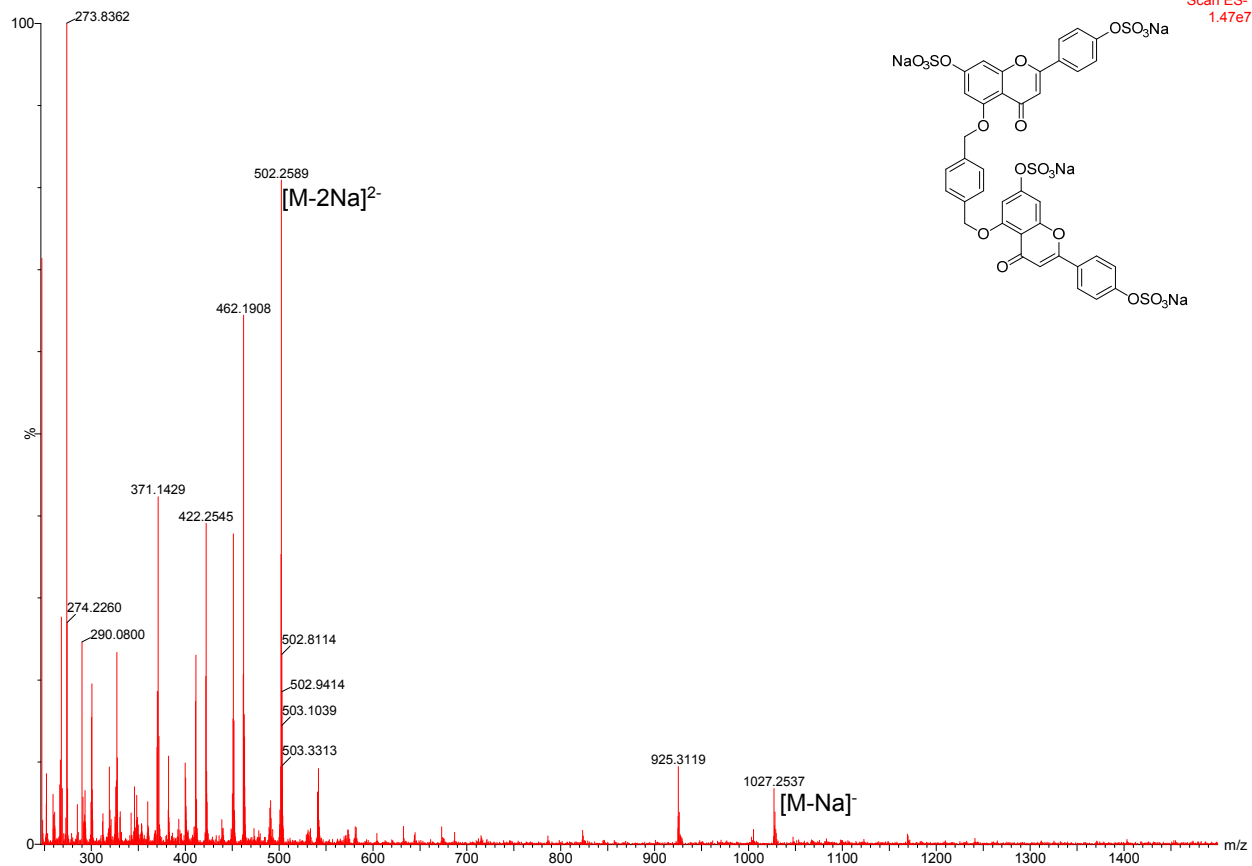


Figure S10c: Mass Spectrum for NSGM 11

DANIEL SAMPLE 0
DA_SFD_11 Sm (Mn, 5x5)

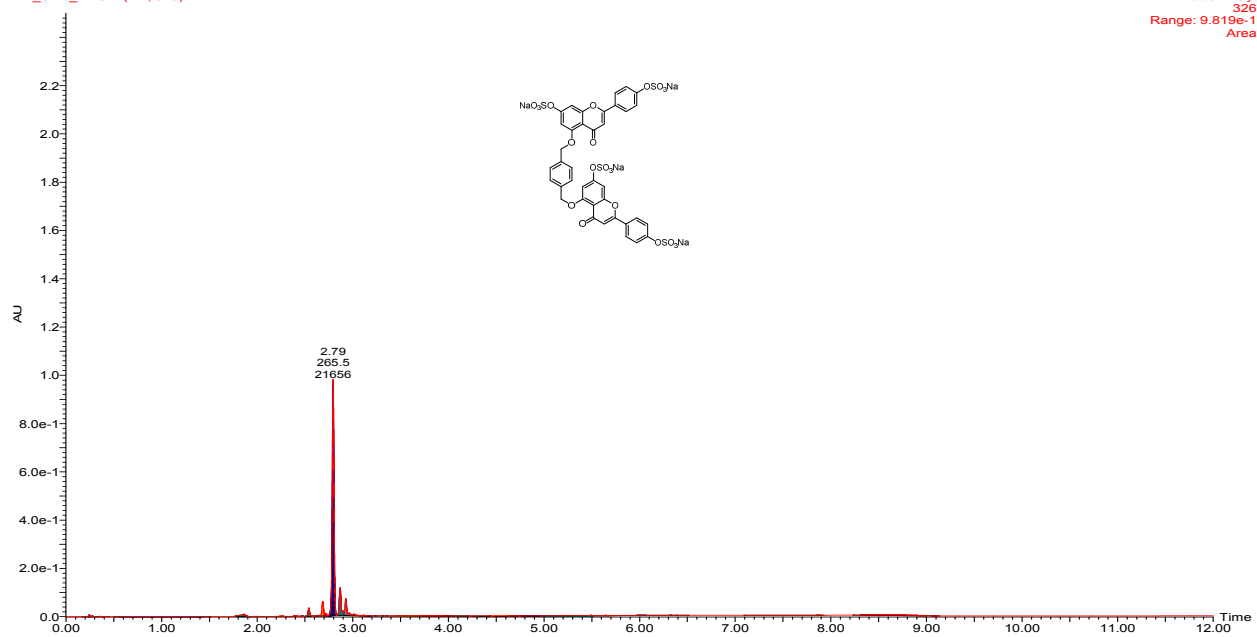


Figure S10d: UPLC profile for NSGM 11

12

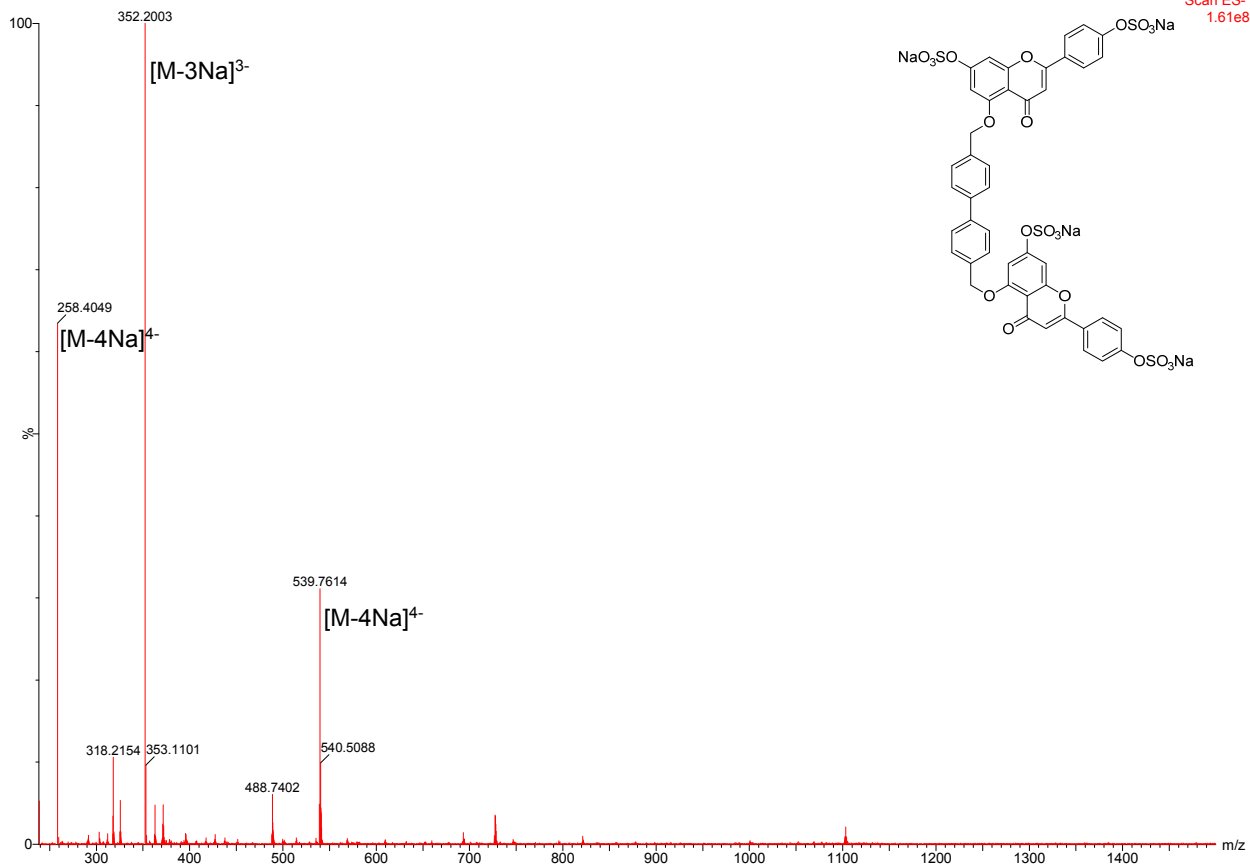


Figure S11c: UPLC profile for NSGM 12

DANIEL SAMPLE 0
DA_SFD_12 Sm (Mn, 5x5)

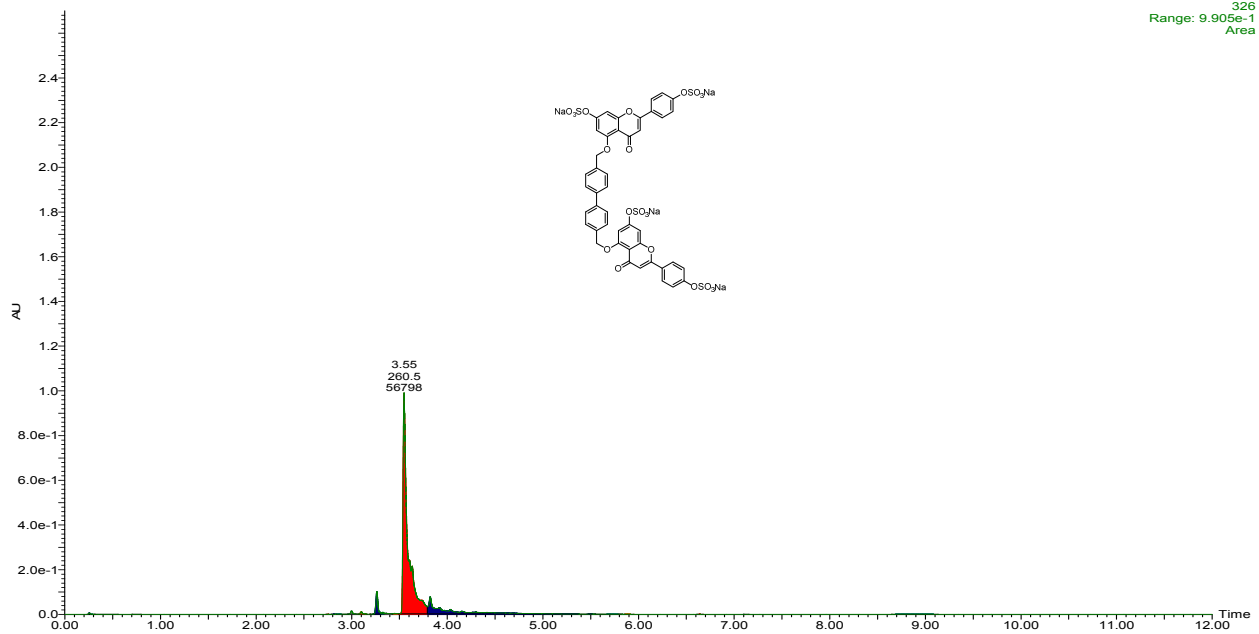


Figure S11d: Mass Spectrum for NSGM 12

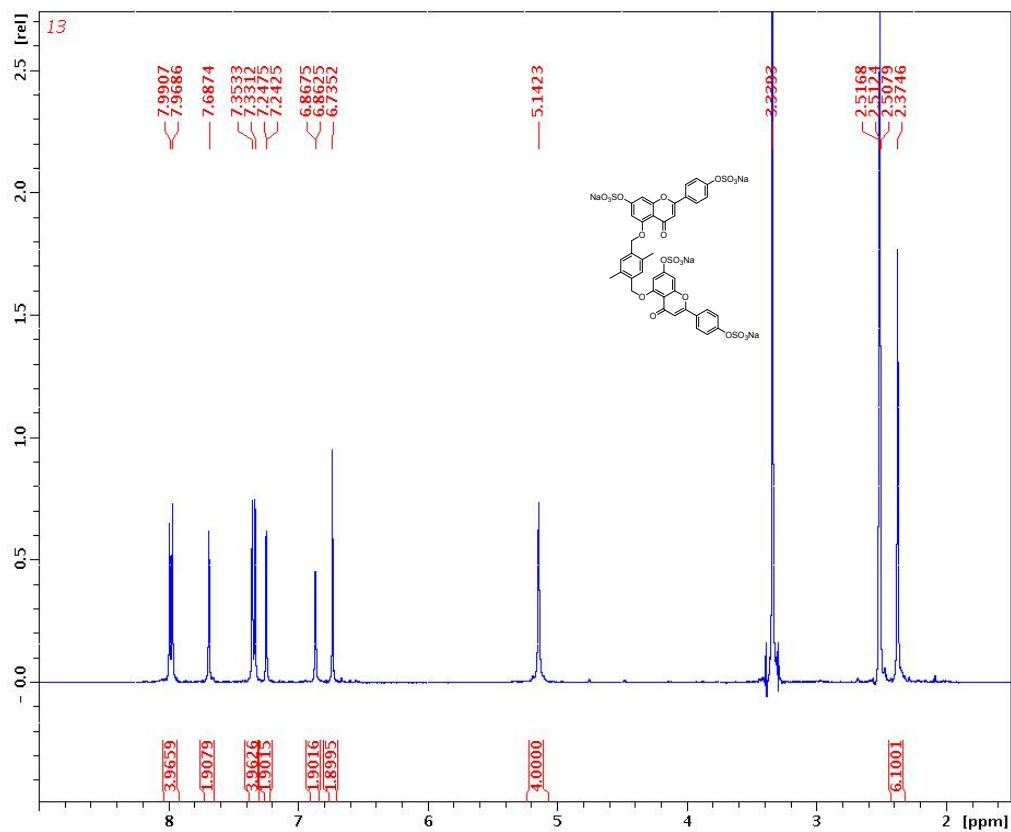


Figure S12a: ^1H NMR (400 MHz, DMSO- d_6) for NSGM 13

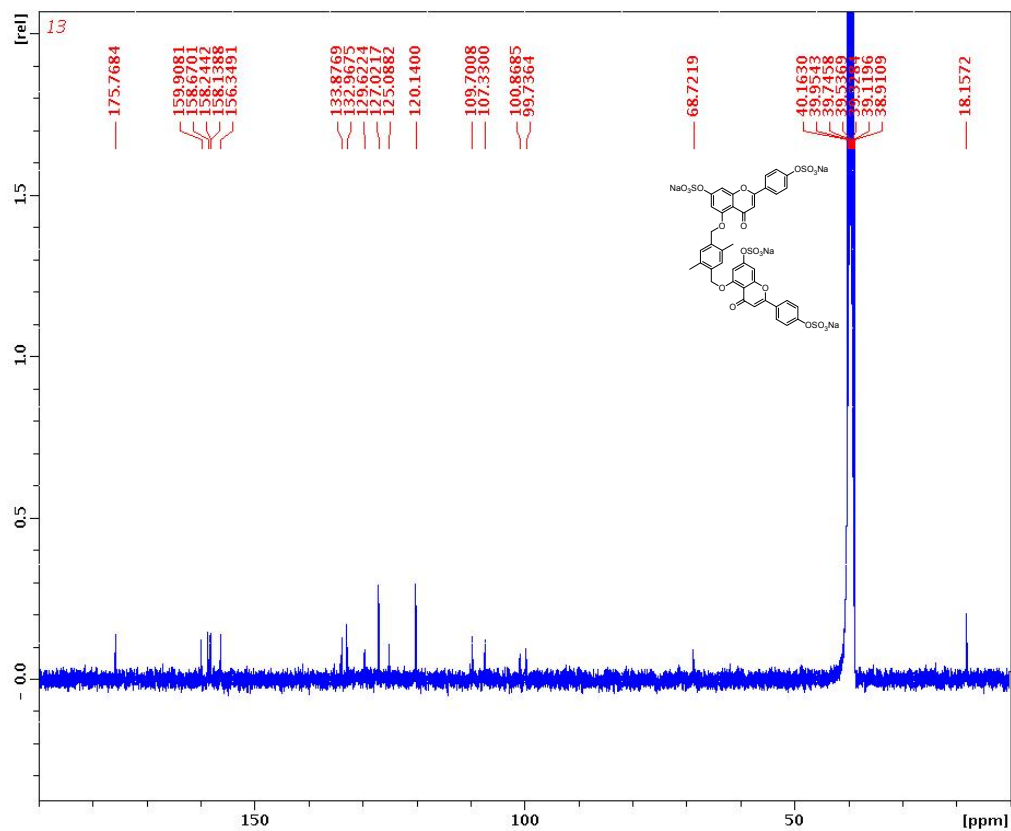


Figure S12b: ^{13}C NMR (100 MHz, DMSO- d_6) for NSGM 13

13

Scan ES-
8.06e7

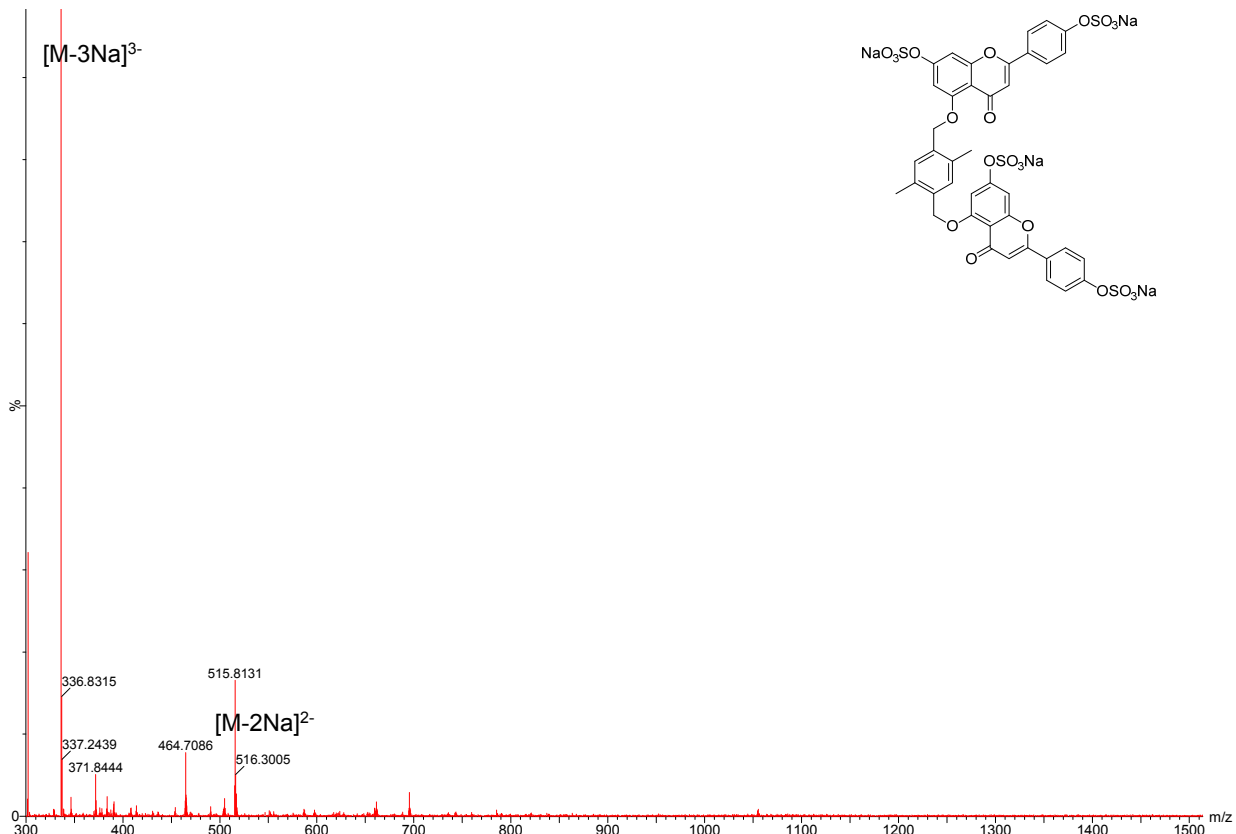


Figure S12c: Mass Spectrum for NSGM 13

DANIEL SAMPLE 0
DA_SFD_13 Sm (Mn, 5x5)

Diode Array
326
Range: 1.397
Area

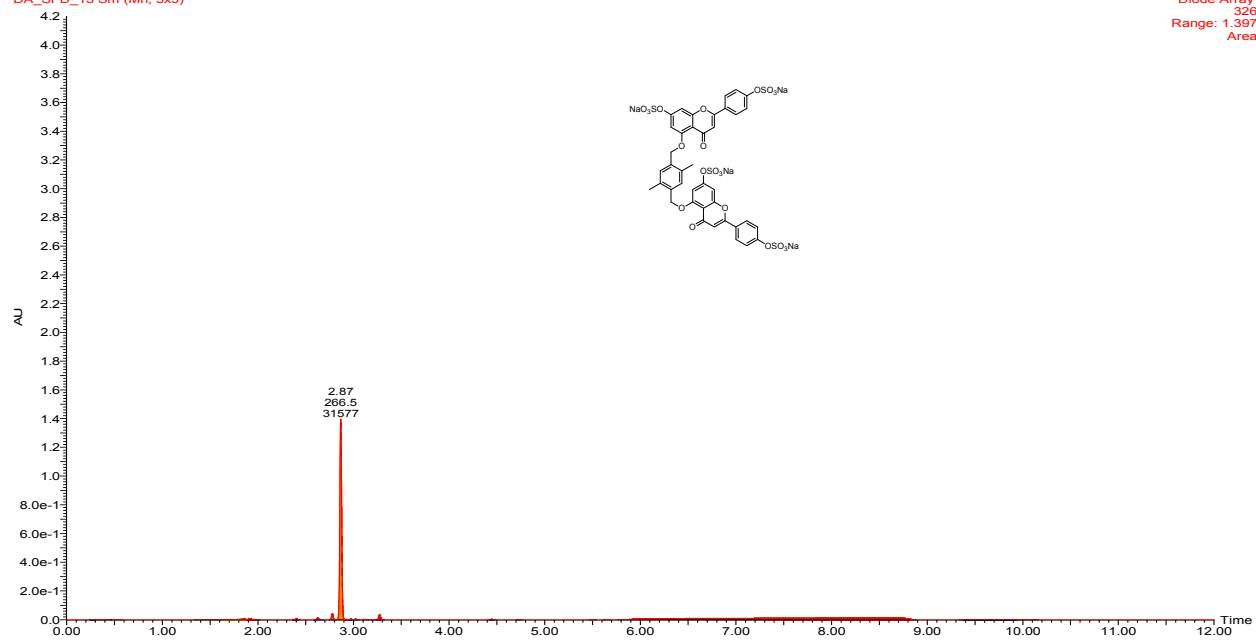


Figure S12d: UPLC profile for NSGM 13

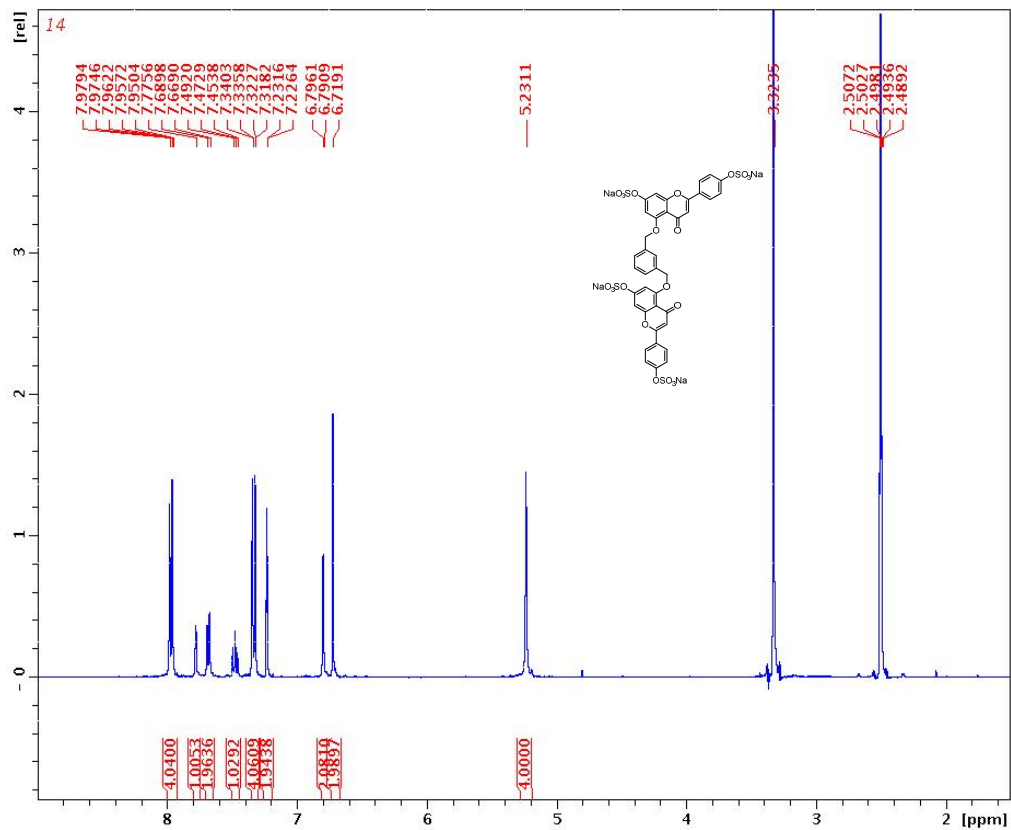


Figure S13a: ^1H NMR (400 MHz, DMSO-d₆) for NSGM 14

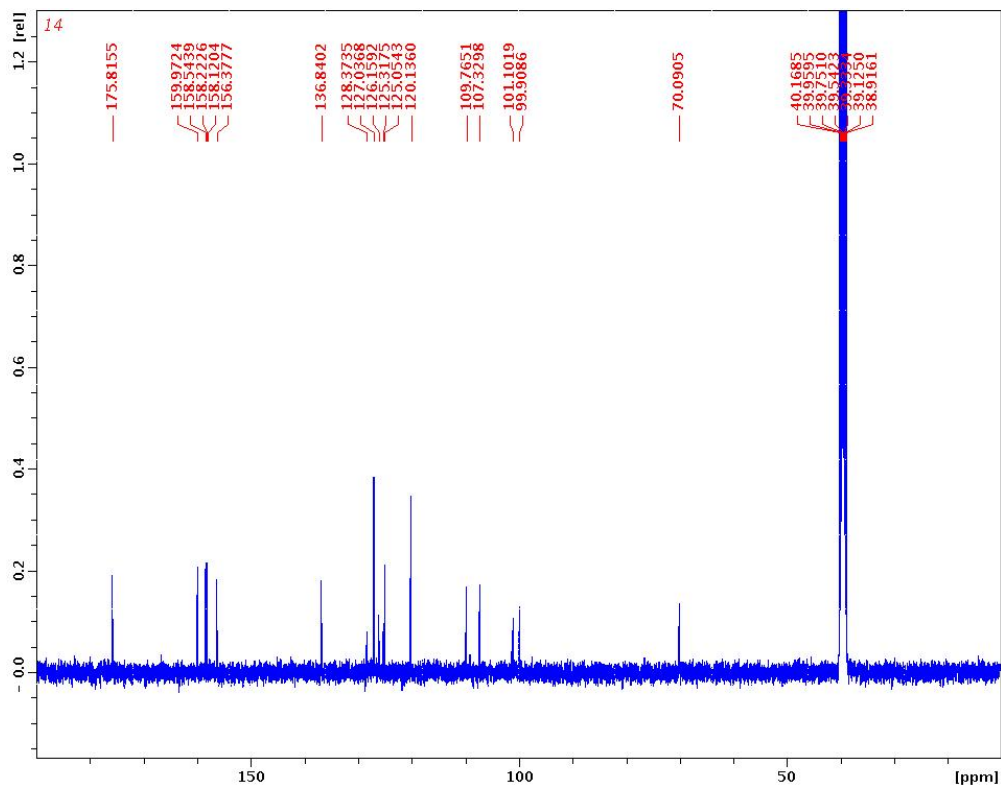


Figure S13b: ^{13}C NMR (100 MHz, DMSO-d₆) for NSGM 14

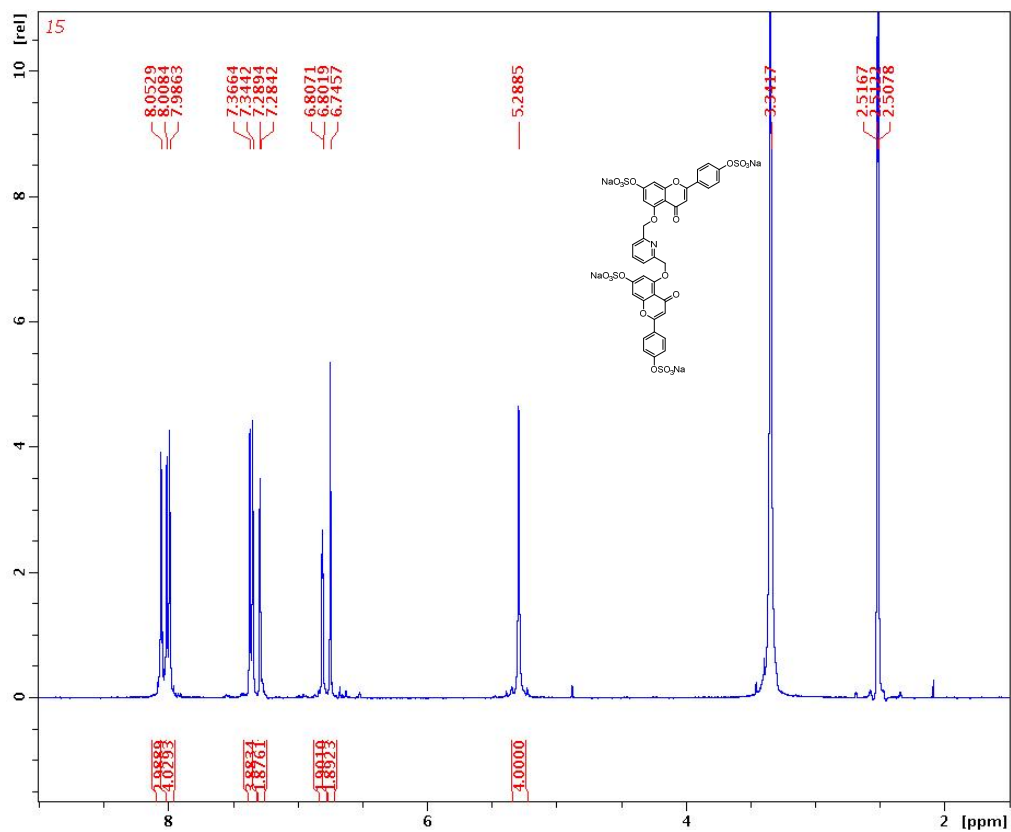


Figure S14a: ^1H NMR (400 MHz, DMSO- d_6) for NSGM 15

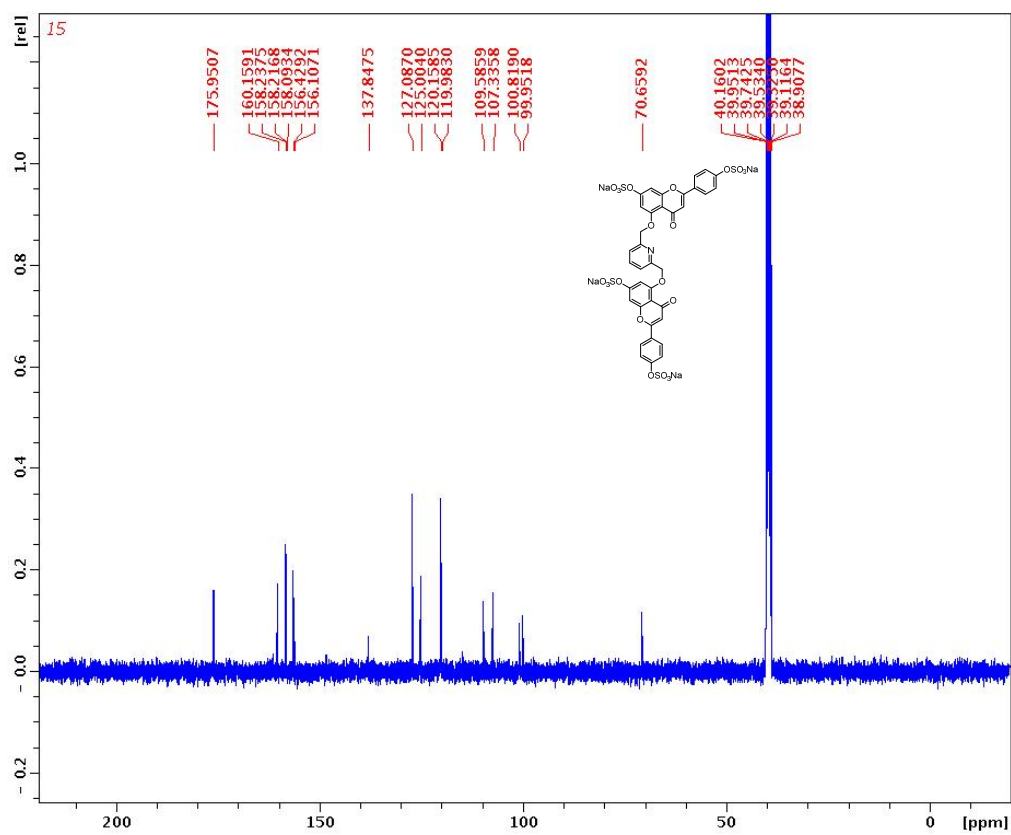
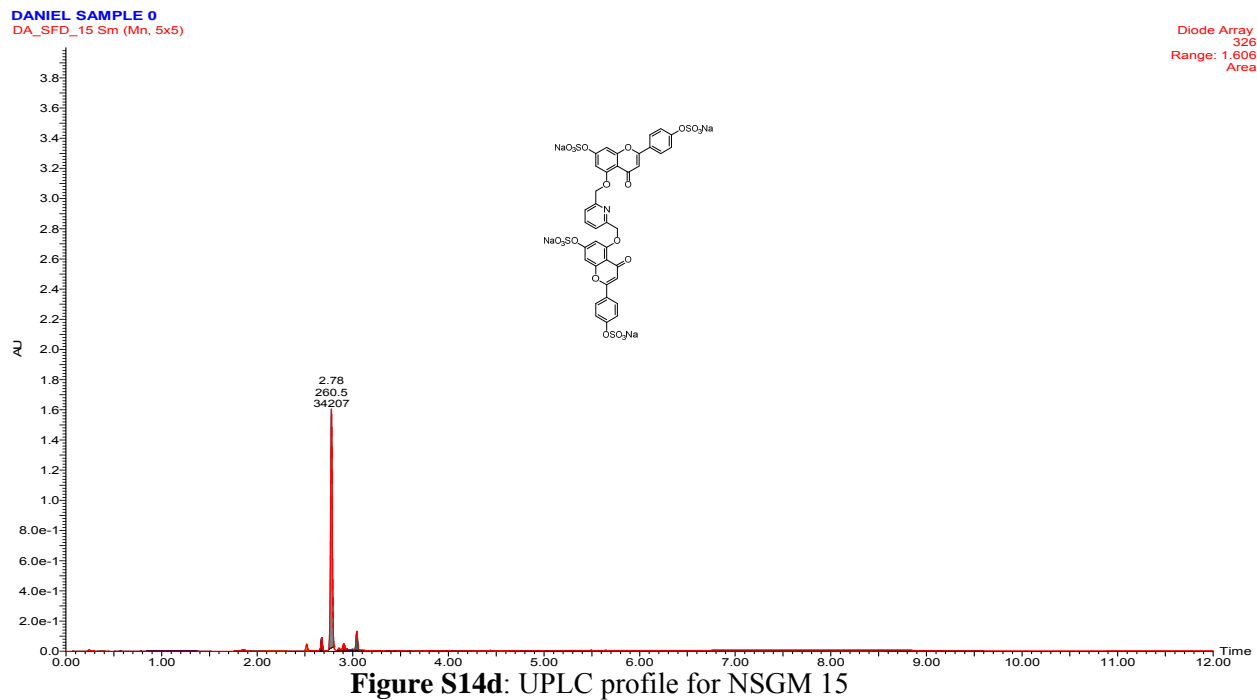
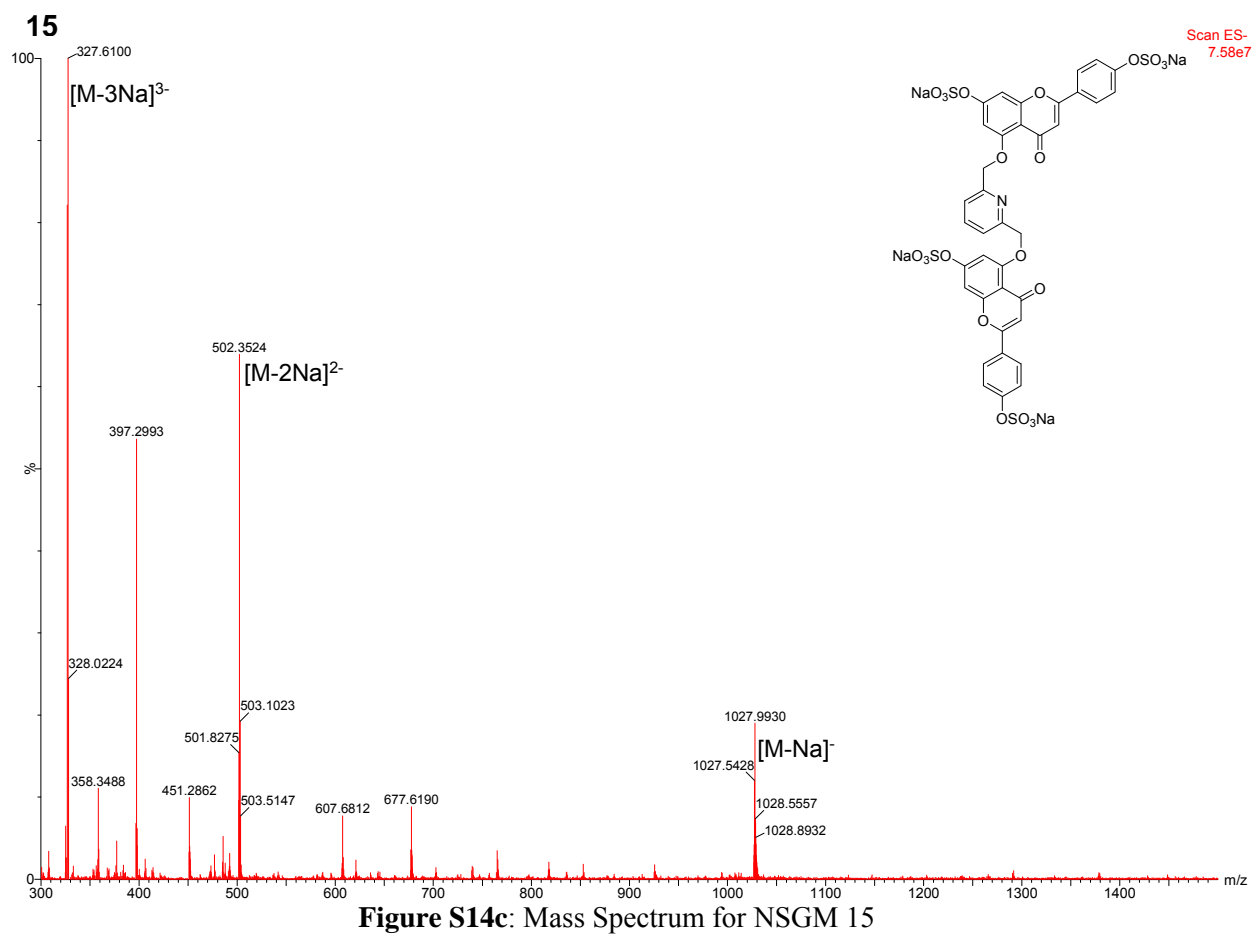


Figure S14b: ^{13}C NMR (100 MHz, DMSO- d_6) for NSGM 15



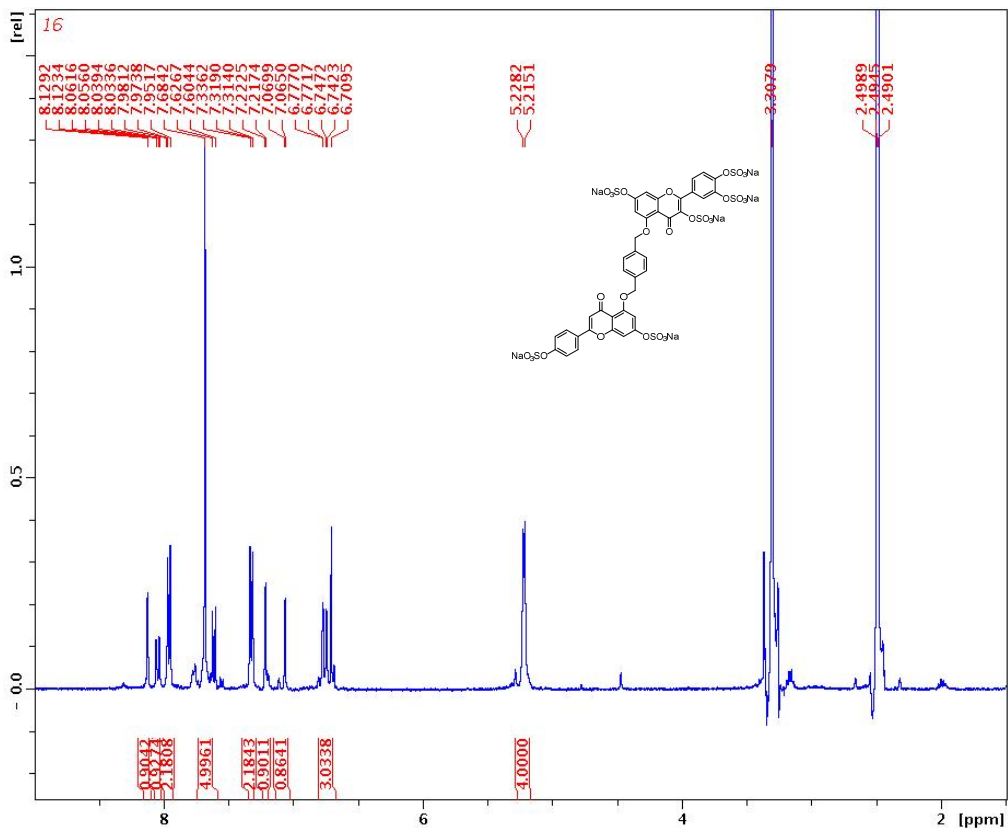


Figure S15a: ¹H NMR (400 MHz, DMSO-d₆) for NSGM 16

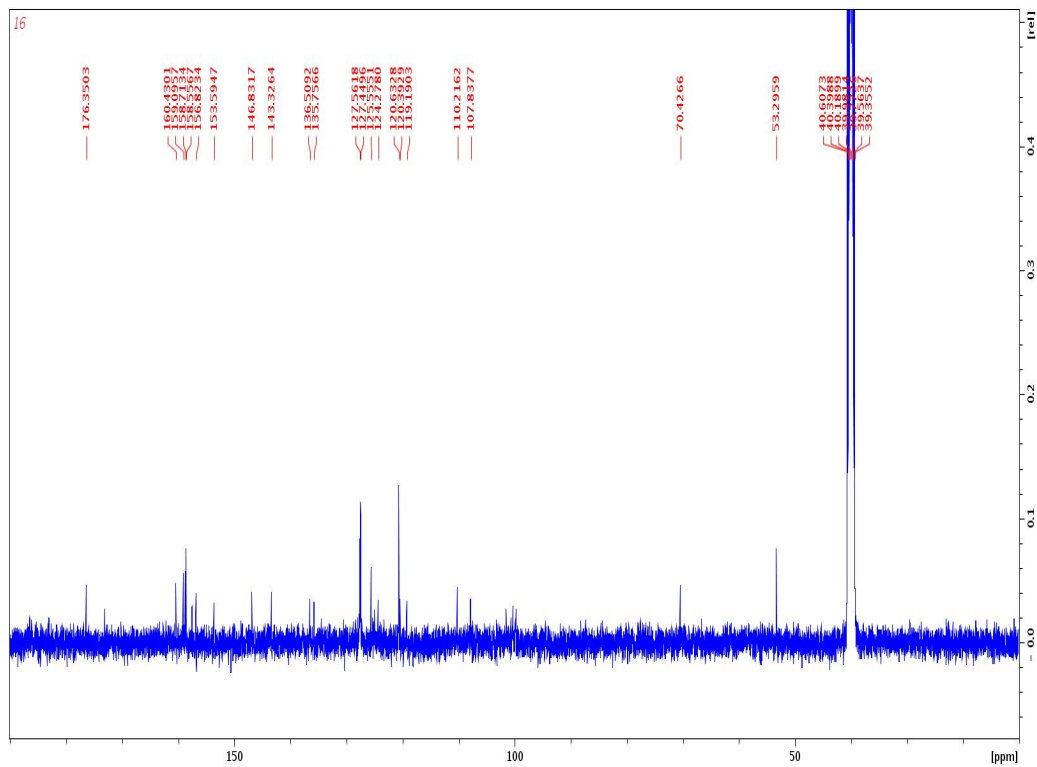


Figure S15a: ¹³C NMR (100 MHz, DMSO-d₆) for NSGM 16

16

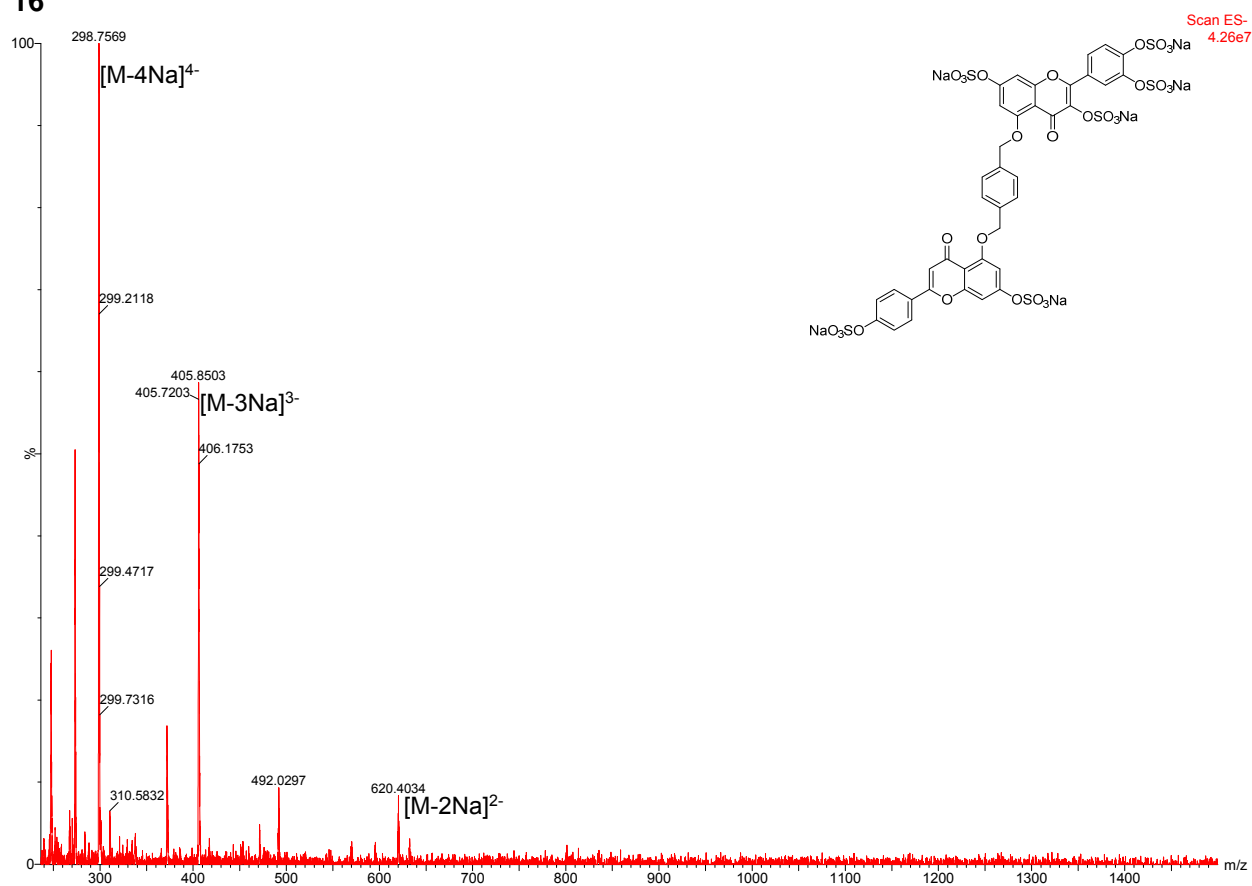


Figure S15c: Mass Spectrum for NSGM 16

PAINS Evaluation

1. The compounds reported in this manuscript are not documented as pan assay interference compounds (PAINS).
2. In multiple chromogenic substrate assay employed to measure inhibition potencies using several log fold range in concentrations, no interference was detected with substrate or other excipients.
3. The compounds themselves are off-white or yellowish in color and therefore do not generate color interference.
4. None of the compounds possess a reactive functional group. The compounds do not react covalently with plasmin or with any of the homologous enzymes studied herein. This is also supported by the fact that reversal of inhibition can be effected with protamine sulfate. This reversal is complete (100%).
5. Finally, no aggregation of compounds in water was observable in the range of concentrations studied (nM to ~500 μ M). All compounds studied are highly water soluble and not known to aggregate in water. Further, the assay buffer used for studying inhibition contained 0.02% Tween80, which is a surfactant that limits induction of aggregation.