Supporting Information

Design of β-amyloid aggregation inhibitors from a predicted structural motif.

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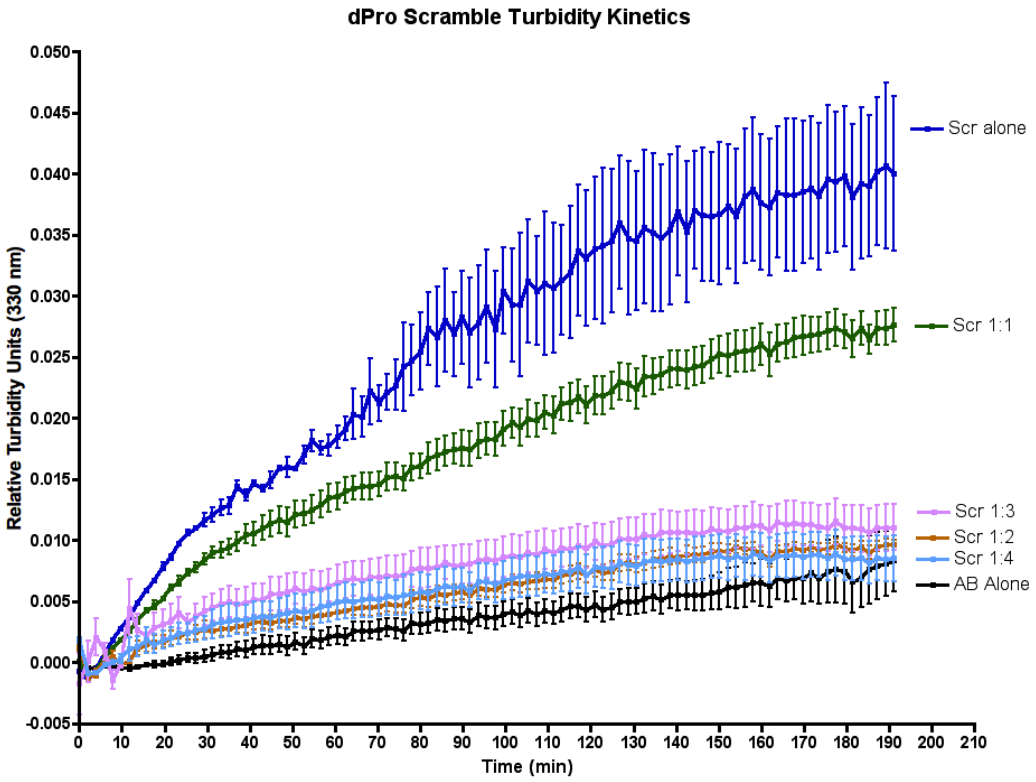
TOC

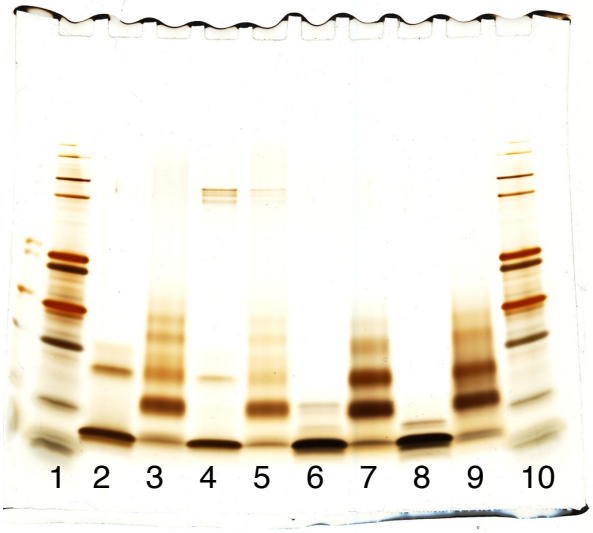
(A) ASIHKGGAVGGAKVEEDDNFVdPFLISYLEIFGAHVVMDVQHR

(B)



Supporting Figure 1 - A) Sequence of the $A\beta_{scramble}$, produced by rearranging the residues found in [D-Pro^{37}]$A\beta_{42}$. B) The results of a kinetic turbidity assay on $A\beta_{scramble}$, WT $A\beta_{42}$, and solutions with various molar ratios of the two peptides. For example, the solution labeled 1:1 contains 25 µM each of $A\beta_{scramble}$ and WT $A\beta_{42}$, while the 1:4 solution contains 25 µM of WT $A\beta_{42}$ and only 6.25 µM of $A\beta_{scramble}$. Turbidity was measured by absorbance of light at 330 nm over 3 hours at 37 °C.

(A)



Lane 1 = MW standard

Lane 2 = Uncrosslinked $A\beta_{42}$ from Bachem

Lane 3 = Crosslinked Bachem $A\beta_{42}$

Lane 4 = Uncrosslinked $A\beta_{42}$ from lab synthesis (homemade)

Lane 5 = Crosslinked homemade $A\beta_{42}$

Lane 6 = Uncrosslinked [Nle^{35},D-Pro^{37}]$A\beta_{42}$

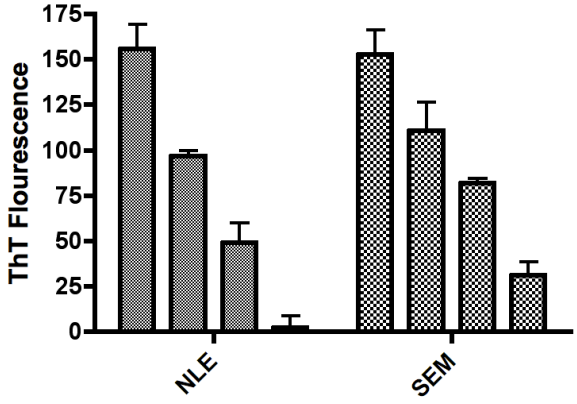
Lane 7 = Crosslinked [Nle^{35},D-Pro^{37}]$A\beta_{42}$

Lane 8 = Uncrosslinked [Sem^{35},D-Pro^{37}]$A\beta_{42}$

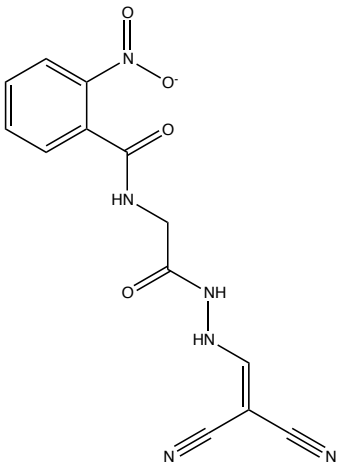
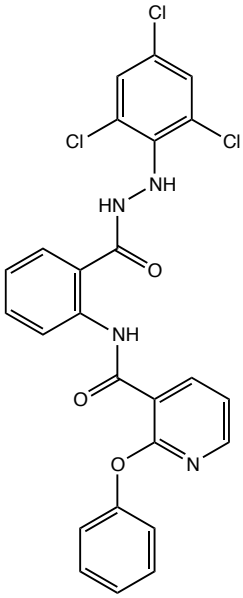
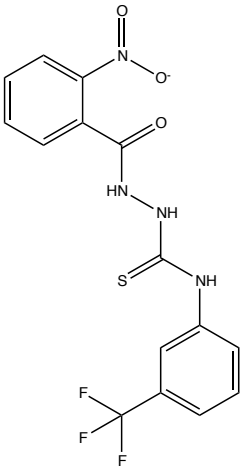
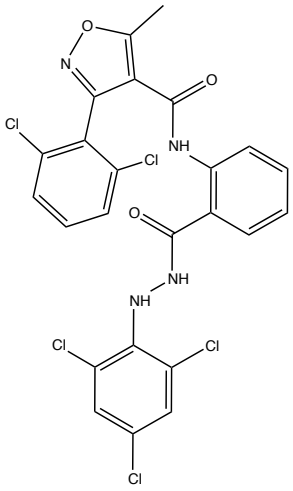
Lane 9 = Crosslinked [Sem^{35},D-Pro^{37}]$A\beta_{42}$

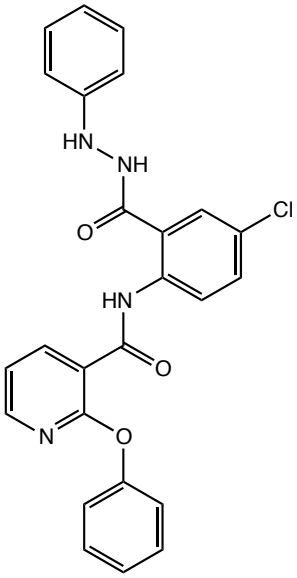
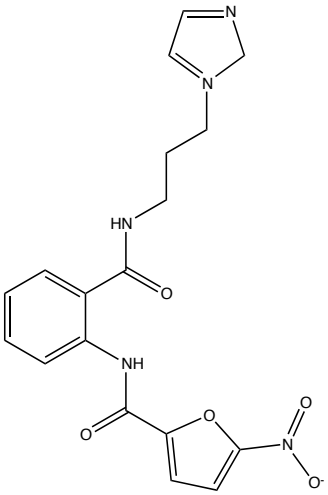
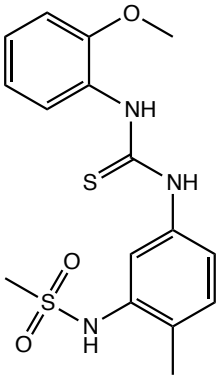
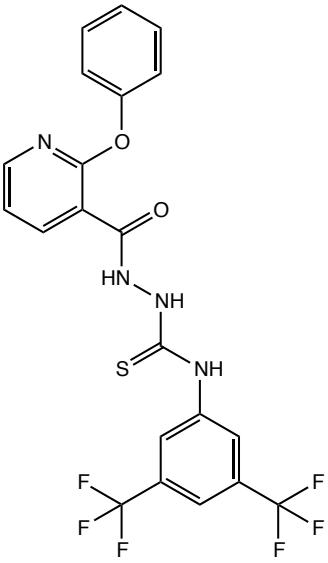
Lane 10 = MW standard

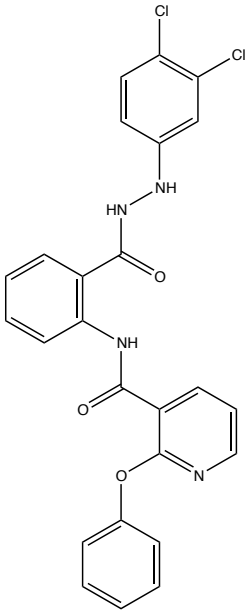
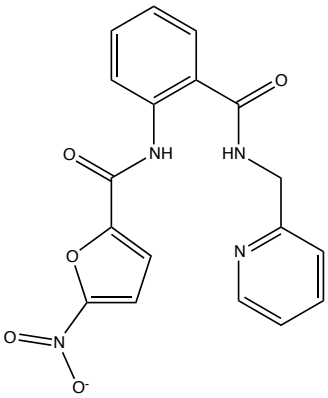
(B)



Supporting Figure 2 - Comparison of the properties of [Nle^{35},D-Pro^{37}]Aβ_{42} and [Sem^{35},D-Pro^{37}] Aβ_{42}. A) Result from a PICUP/SDS-Page experiment. The cross-linked MW distributions of [Nle^{35},D-Pro^{37}]Aβ_{42} (lane7) and [Sem^{35},D-Pro^{37}]Aβ_{42} (lane 9) are similar, and significantly different than either WT Aβ_{42}. B) Results from a ThT experiment measuring fluorescence of the substituted peptides in solution with WT Aβ_{42}. For each set of four bars, the left-most bar is the fluorescence of Aβ_{42} alone, the next bar is a 1:4 molar ratio of substituted to WT peptide, the third bar is a 1:1 ratio, and the fourth bar is the fluorescence of the substituted peptide alone. The dose dependent decrease in fluorescence upon addition of substituted peptide to WT Aβ_{42} is similar for both compounds, with [Nle^{35},D-Pro^{37}]Aβ_{42} demonstrating slightly increased inhibition in comparison to [Sem^{35},D-Pro^{37}]Aβ_{42}.

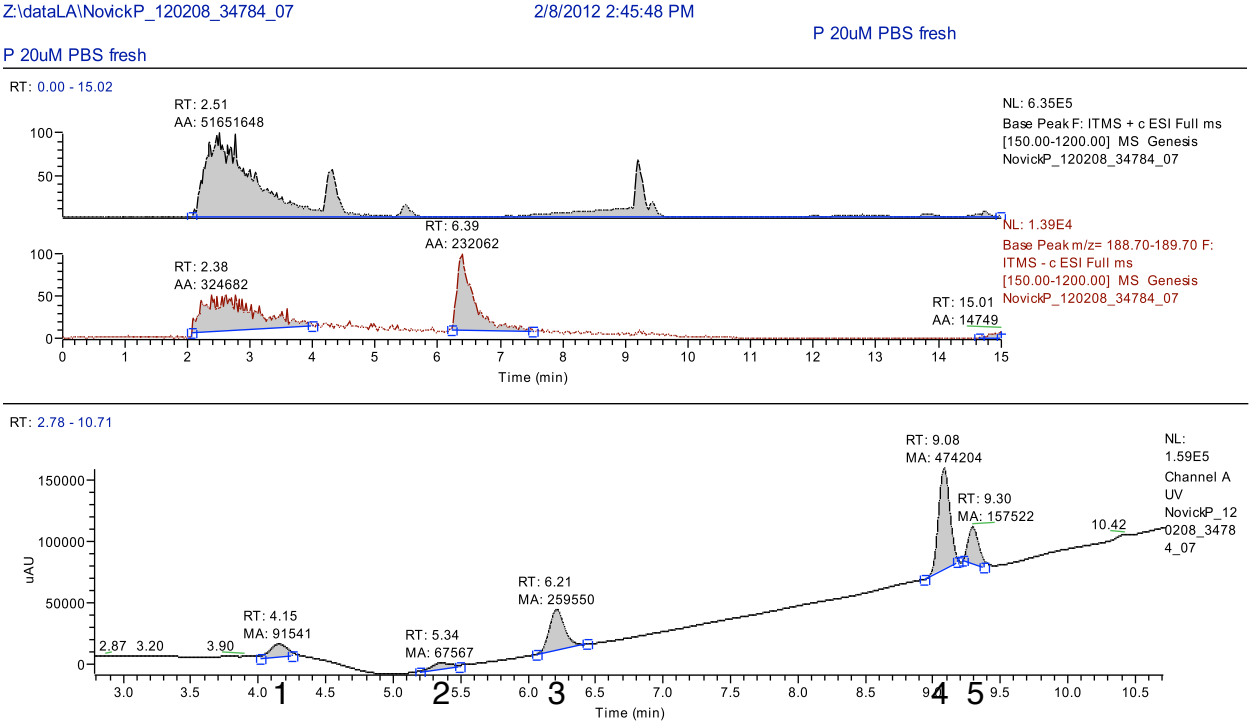
Structure	Rank	Activity		Rank	Activity
 BTB 2612	2	109	 BTB 5441	20	156
 BLT 23	3	83	 BTB 5443	30	167

Structure	Rank	Activity		Rank	Activity
 BTB 4932	5	142	 BTB 5615	31	113.8
 BTB 1394	6	149	 BTB 3068	32	85

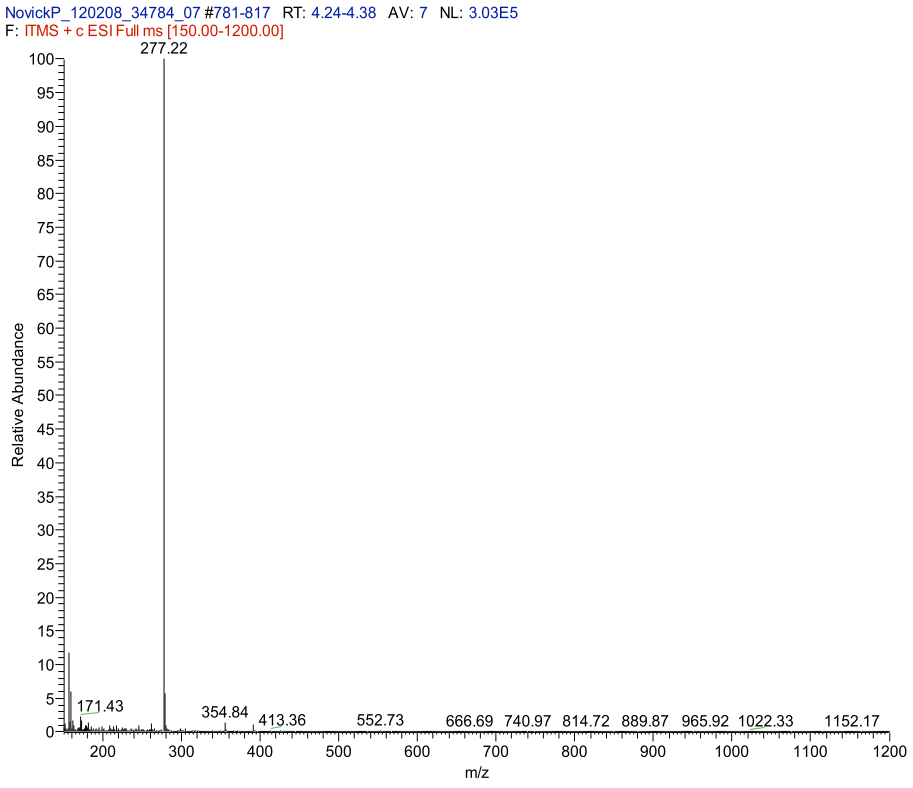
Structure	Rank	Activity		Rank	Activity
 BTB 4940	8	128	 BTB 5618	43	55

Supporting Table 1 - The structures, rank in the virtual screening results, and activity in preliminary single dose ThT experiments (10 μM compound) of the 10 highest ranking compounds from the virtual screen that were tested. Only BTB 5618 showed promising activity out of this group of compounds, however this activity was not consistently reproducible in validation assays.

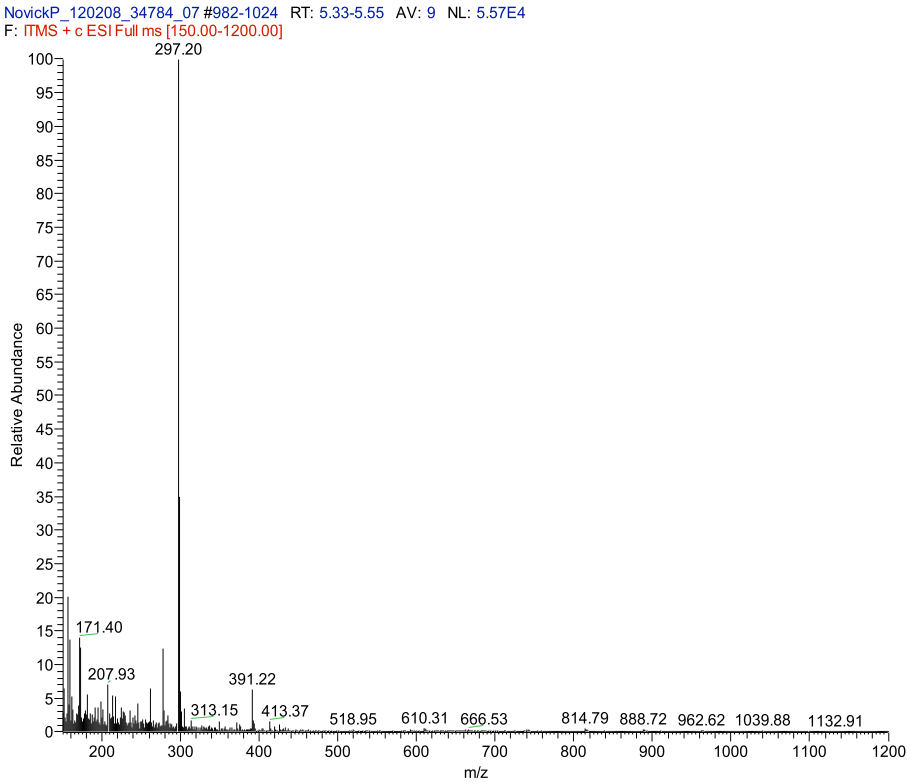
(A)


Z:\dataLA\NovickP_120208_34784_07
2/8/2012 2:45:48 PM
P 20uM PBS fresh
P 20uM PBS fresh
RT: 0.00 - 15.02
RT: 2.51
AA: 51651648
NL: 6.35E5
Base Peak F: ITMS + c ESI Full ms
[150.00-1200.00] MS Genesis
NovickP_120208_34784_07
RT: 6.39
AA: 232062
RT: 2.38
AA: 324682
RT: 15.01
AA: 14749
NL: 1.39E4
Base Peak m/z= 188.70-189.70 F:
ITMS - c ESI Full ms
[150.00-1200.00] MS Genesis
NovickP_120208_34784_07
Time (min)
RT: 2.78 - 10.71
RT: 9.08
MA: 474204
RT: 9.30
MA: 157522
10.42
NL:
1.59E5
Channel A
UV
NovickP_12
0208_3478
4_07
RT: 6.21
MA: 259550
RT: 4.15
MA: 91541
RT: 5.34
MA: 67567
2.87
3.20
3.90
uAU
1
2
3
4
5
Time (min)

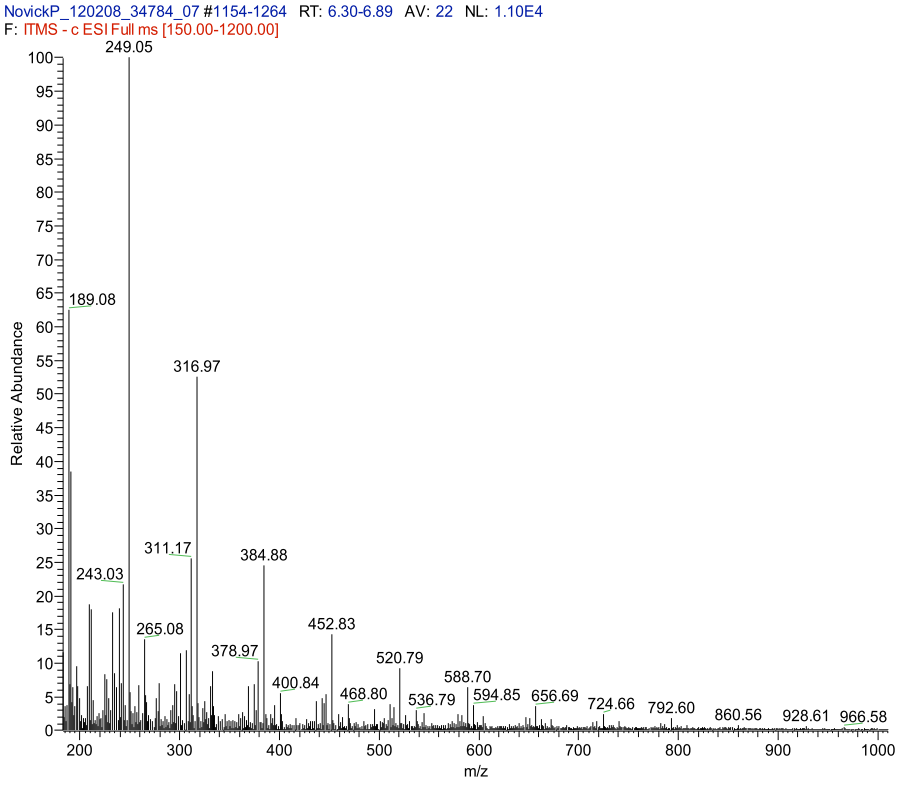
(B)


NovickP_120208_34784_07 #781-817 RT: 4.24-4.38 AV: 7 NL: 3.03E5
F: ITMS + c ESI Full ms [150.00-1200.00]
277.22
171.43
354.84
413.36
552.73
666.69
740.97
814.72
889.87
965.92
1022.33
1152.17
Relative Abundance
m/z

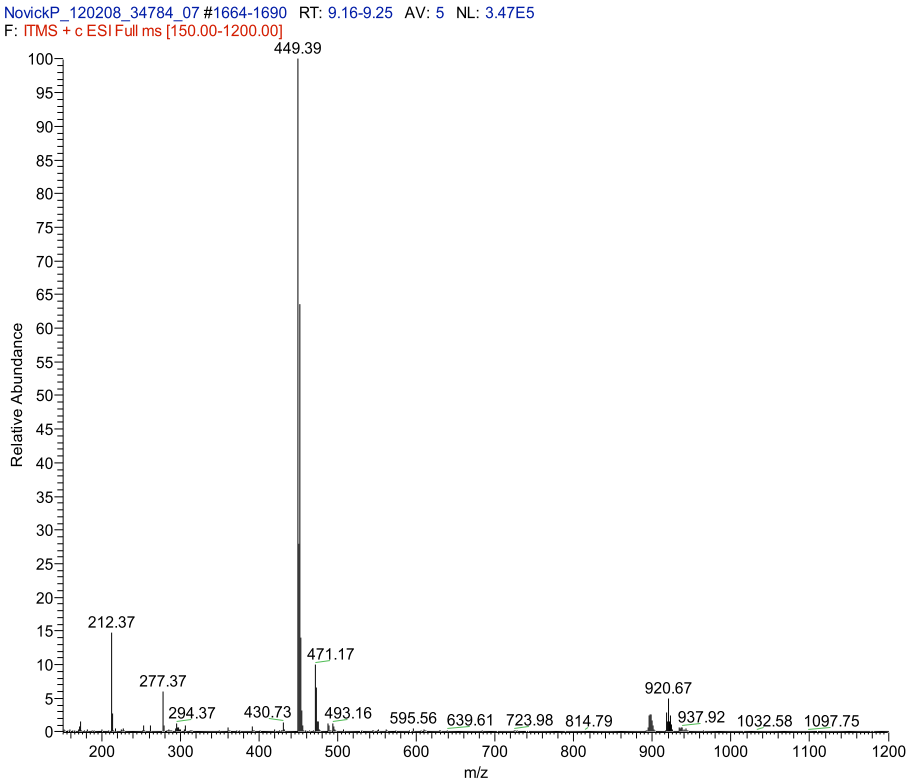
(C)


NovickP_120208_34784_07 #982-1024 RT: 5.33-5.55 AV: 9 NL: 5.57E4
F: ITMS + c ESI Full ms [150.00-1200.00]
297.20
171.40
207.93
391.22
313.15
413.37
518.95
610.31
666.53
814.79
888.72
962.62
1039.88
1132.91
Relative Abundance
m/z

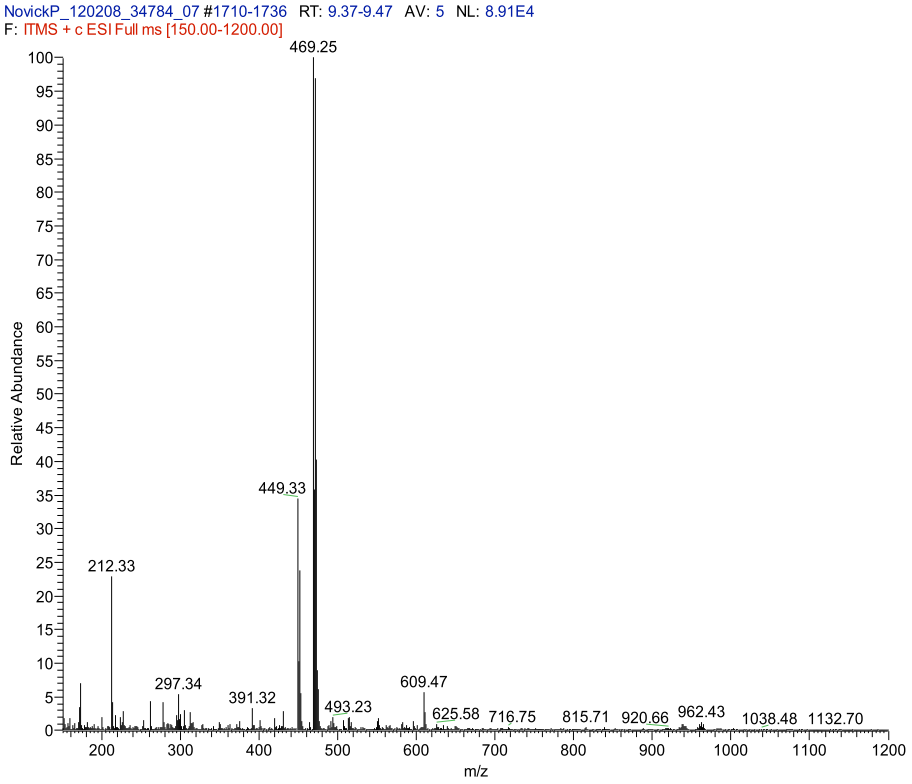
(D)


NovickP_120208_34784_07 #1154-1264 RT: 6.30-6.89 AV: 22 NL: 1.10E4
F: ITMS - c ESI Full ms [150.00-1200.00]
249.05
189.08
316.97
311.17
384.88
243.03
265.08
452.83
378.97
520.79
588.70
400.84
468.80
536.79
594.85
656.69
724.66
792.60
860.56
928.61
966.58
Relative Abundance
m/z

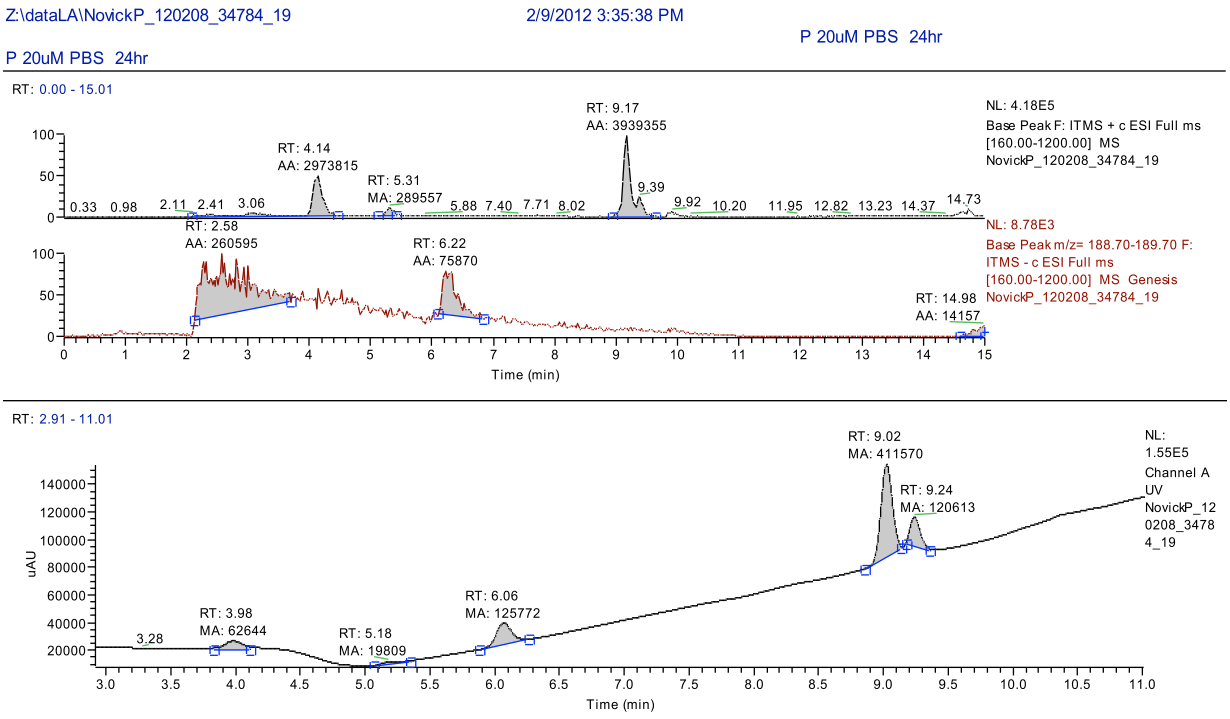
(E)


NovickP_120208_34784_07 #1664-1690 RT: 9.16-9.25 AV: 5 NL: 3.47E5
F: ITMS + c ESI Full ms [150.00-1200.00]
Relative Abundance
m/z
449.39
212.37
277.37
294.37
430.73
471.17
493.16
595.56
639.61
723.98
814.79
920.67
937.92
1032.58
1097.75

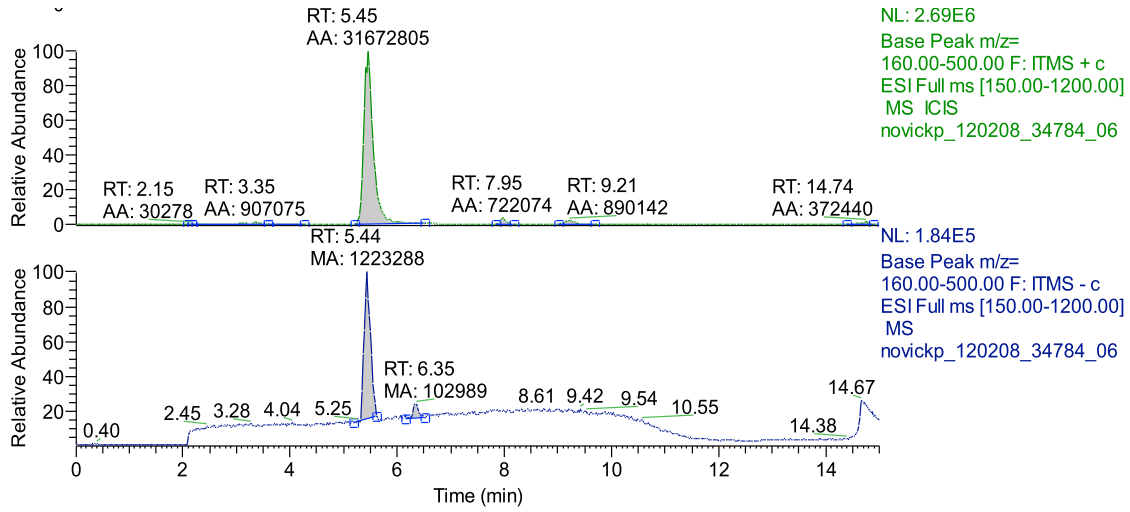
(F)


NovickP_120208_34784_07 #1710-1736 RT: 9.37-9.47 AV: 5 NL: 8.91E4
F: ITMS + c ESI Full ms [150.00-1200.00]
Relative Abundance
m/z
469.25
449.33
212.33
297.34
391.32
493.23
609.47
625.58
716.75
815.71
920.66
962.43
1038.48
1132.70

(G)



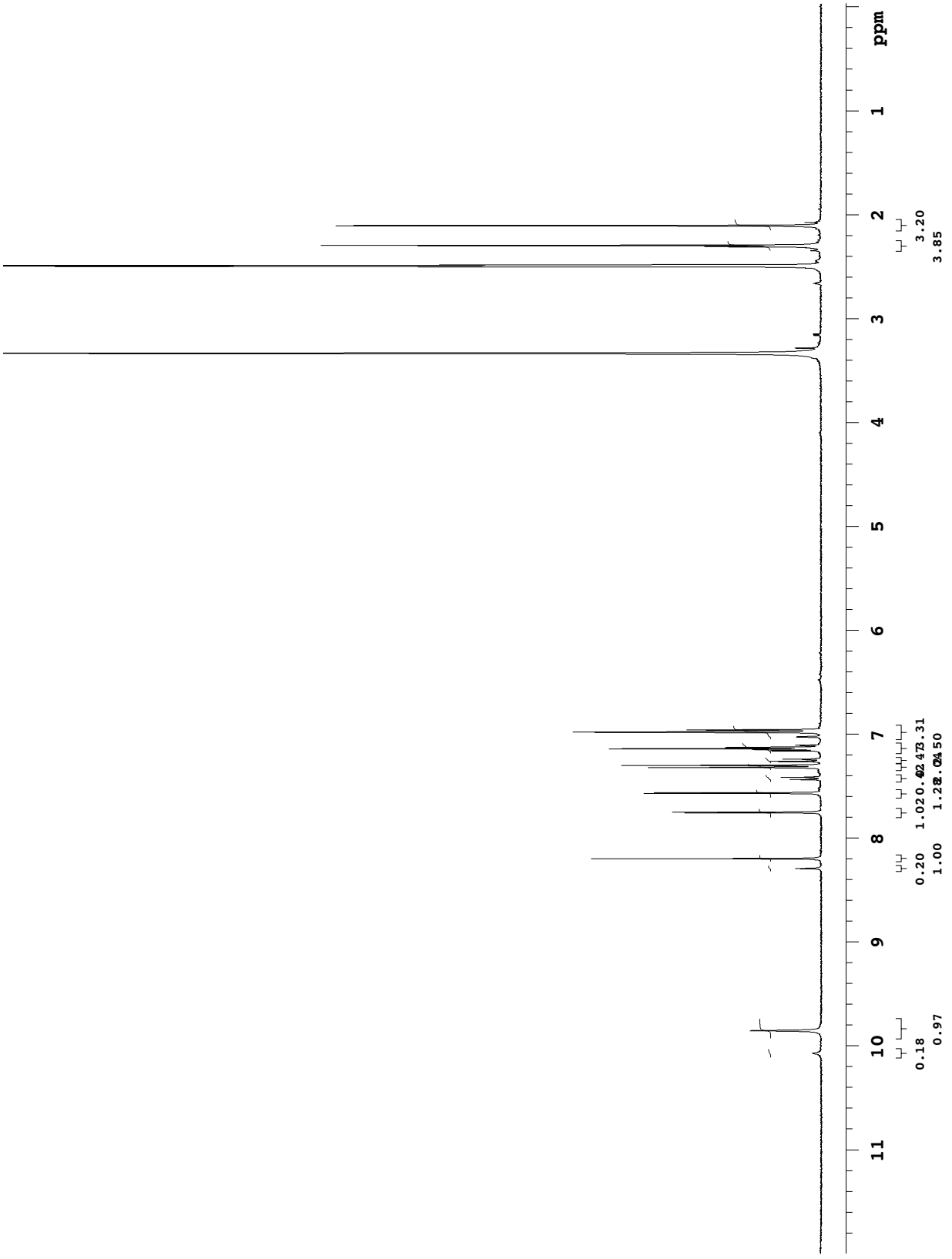
(H)



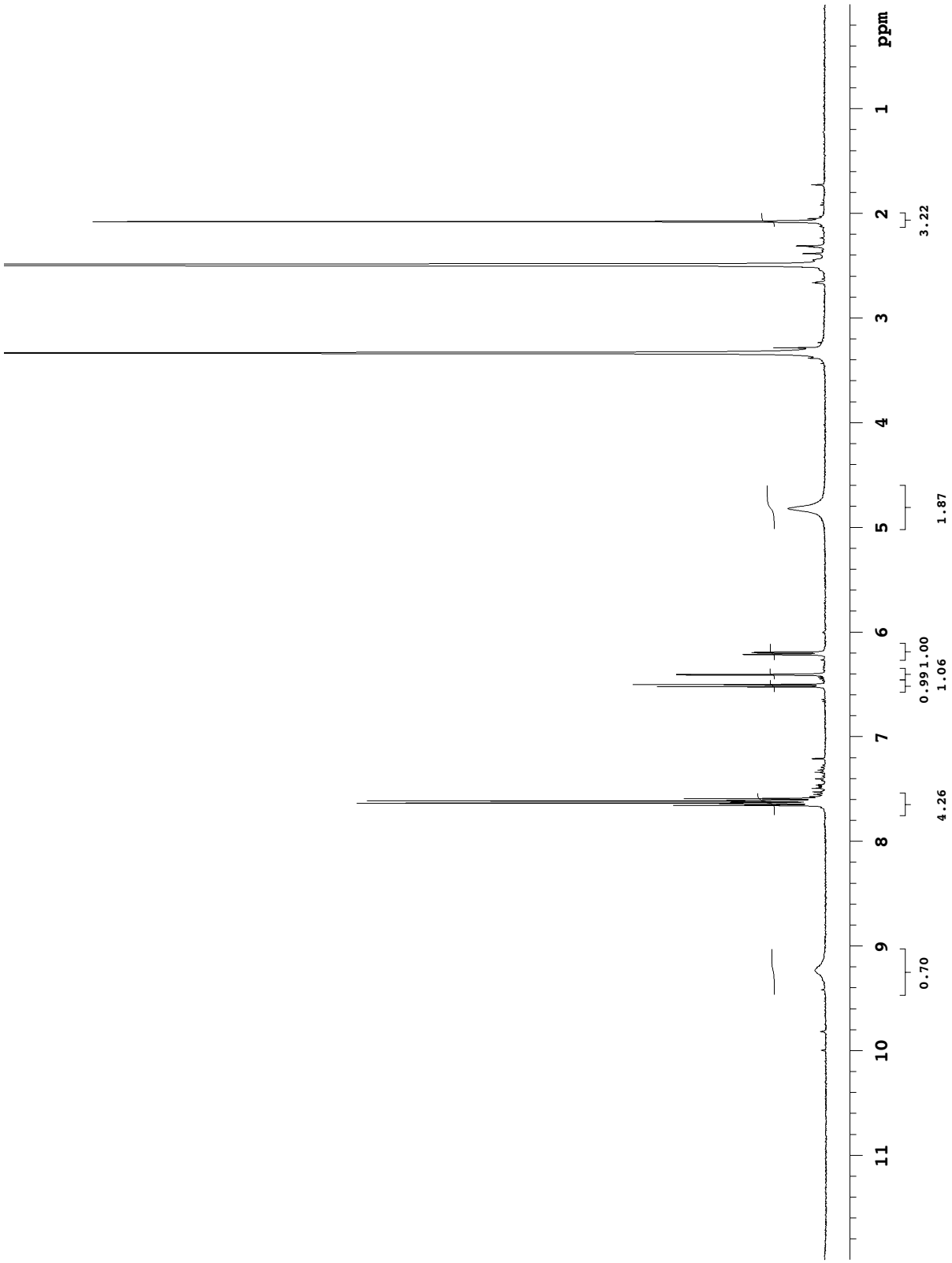
Supporting Figure 3 - LC-MS analysis of **1** and fragments. A) Stock solutions of **1** were diluted into PBS pH=7.4 and immediately analyzed by LC-MS. The signal from mass abundance in ESI positive ionization mode, ESI negative ionization mode, and the UV absorption are shown (top to bottom, respectively). The peaks in the UV absorption are numbered 1-5, and the mass analysis of the peaks is shown in B-F. Peak 1 (**2-20**) corresponds to the mass chromatogram

shown in (B), peak 2 (**2**) corresponds to (C), peak 3 (**3**) corresponds to (D), peak 4 (**1-20**) corresponds to (E), and peak 5 (**1**) corresponds to (F). (G) Analysis of a sample of 1 incubated in PBS for a period of 24hours, with otherwise identical preparation and analysis to (A). The relative peak areas of the 5 components does not vary significantly. (H) Mass abundance in positive (top) and negative (bottom) ionization mode of a freshly made solution containing **2** and **3**. Interestingly, the peak at 4.15 seen in (A) and (G), representing **2-20**, is not found.

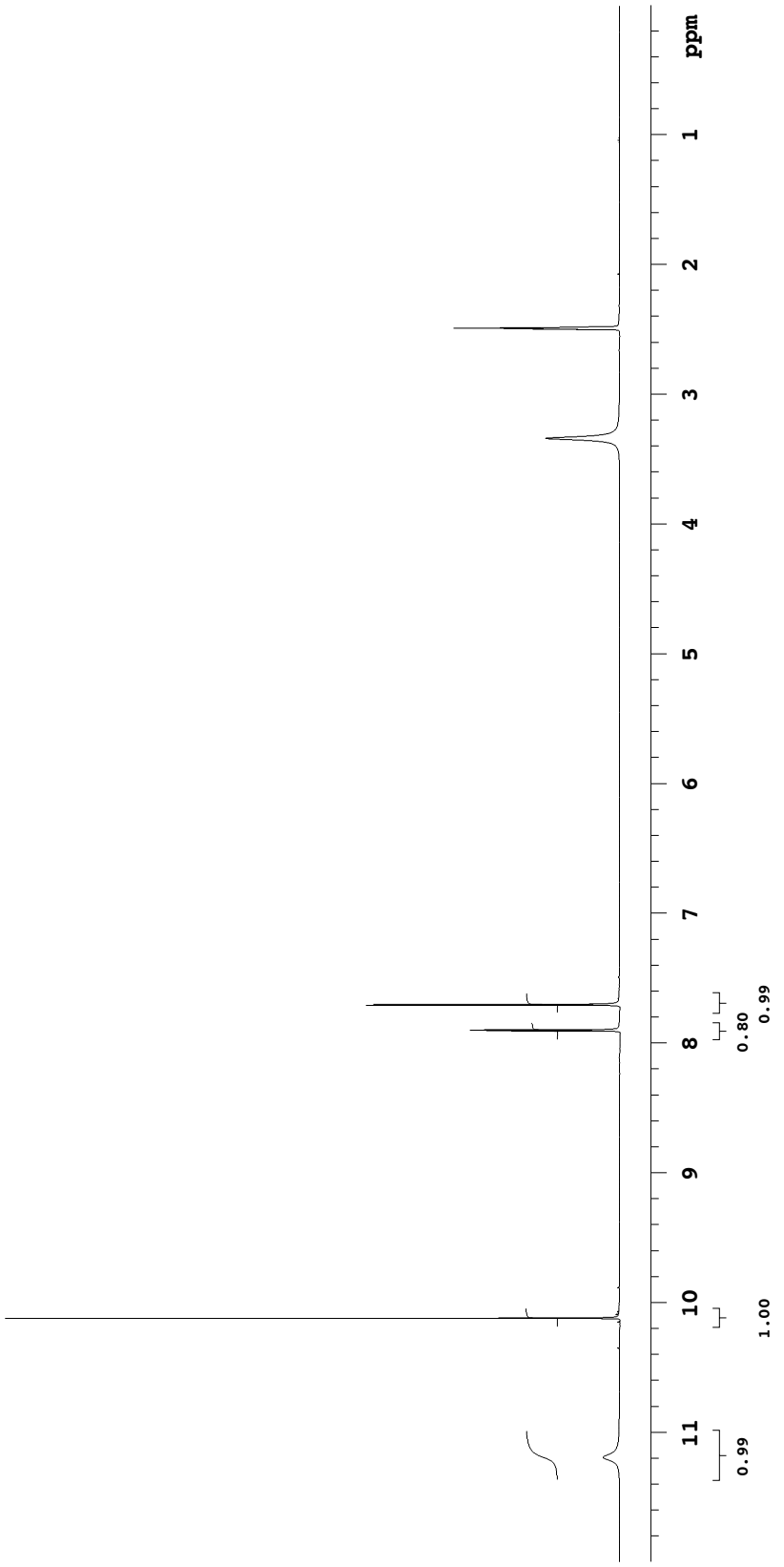
(A)



(B)



(C)



Supporting Figure 4 - 1HNMR of **1** (A), **2** (B), **3** (C) collected in deuterated DMSO at 400 MHz.