SUPPORTING INFORMATION

Selectively Fluorinated PAMAM-Arginine Conjugates as Gene Delivery Vectors

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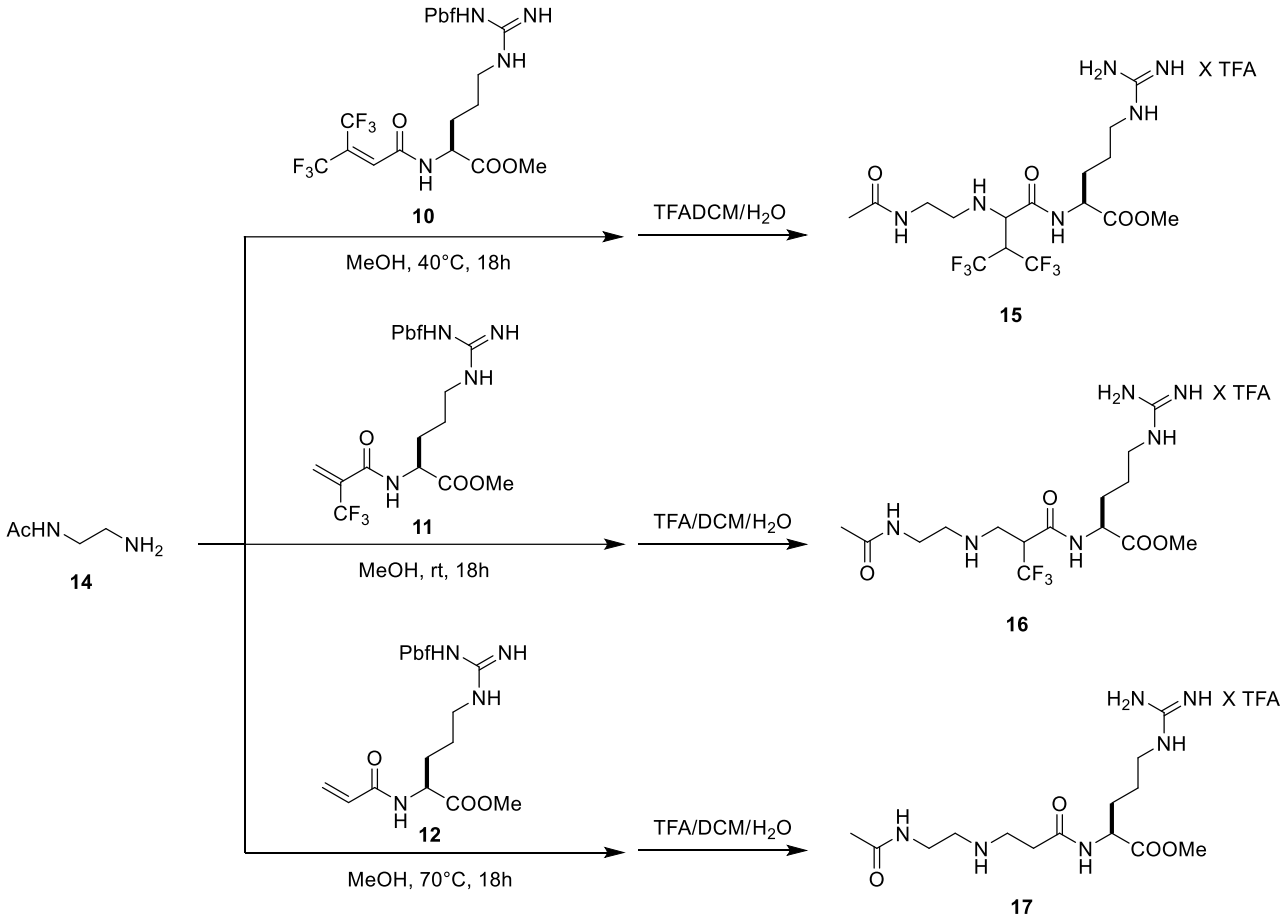
Scheme S1. Synthesis of model compounds **15-17**



General procedure. To a solution of the Michael acceptors **10-12** (1.0 equiv.) in MeOH (0.1 M solution) a solution of N-(2-aminoethyl)acetamide **14** (1.2 equiv.) in MeOH (0.5 M solution) was added dropwise and the solution stirred overnight at a given temperature (40 °C for the reaction with **10**, rt with **11**, and 70°C with **12**). The solvent was evaporated, the crude dissolved in AcOEt and washed with an aqueous 1M solution of HCl and brine. The organic layer was separated, dried over Na_2SO_4, filtered and the solvent removed under pressure. The crude dissolved in a DCM/TFA/H_2O (25:70:5) mixture and the mixture stirred at rt for three hours. The DCM was evaporated and compounds **15-17** were precipitate in diethyl ether.

methyl N^{ω}-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)-N2-(4,4,4-trifluoro-3-(trifluoromethyl)but-2-enoyl)-L-argininate, 10. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 8.0 Hz, 1H), 7.08 (s, 1H), 6.30-6.28 (br s, 3H), 4.56-4.51 (m, 1H), 3.69 (s, 3H), 3.20-3.18 (m, 2H), 2.94 (s, 2H), 3.31 (s, 3H), 3.29 (s, 3H), 2.07 (s, 3H), 1.88-1.86 (m, 1H), 1.81-1.79 (m, 1H), 1.60.1,57 (m, 2H), 1.45 (s, 6H); ^{19}F NMR ($CDCl_3$, 376 MHz) δ – 62.1 (q, *J* = 6.8 Hz, 3F), 67.0 (q, *J* = 6.8 Hz, 3F); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.7, 169.4, 160.0, 157.1, 154.3, 136.4, 134.0, 130.3, 122.9, 117.9 (q, *J* = 276.7), 115.8, 84.7, 58.5, 50.6, 50.5, 41.2, 38.7, 27.0, 26.6, 23.3, 19.1, 17.2, 15.8, 12.2, 10.4, the carbon bonding the two CF_3 groups did not appear due to low intensity; ESI m/z 631.3 $[M+H, (100)]^+$; Anal. calcd. for $C_{25}H_{32}F_6N_4O_6S$: C 47.62, H 5.12, N, 8.88; found: C 47.63, H 5.11, N, 8.88.

methyl N^{ω}-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)-N^2-(2-(trifluoromethyl)acryloyl)-L-argininate, 11. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, *J* = 7.2 Hz, 1H), 6.43 (s, 1H), 6.34 (s, 1H), 6.28 (br s, 2H), 6.16 (s, 1H), 4.56 (br q, *J* = 4.8 Hz, 1H), 3.72 (s, 3H), 3.22-3.20 (m, 2H), 2.97 (s, 2H), 2.55 (s, 3H), 2.48 (s, 3H), 2.10 (s, 3H), 1.96-1.94 (m, 1H), 1.93-1.91 (m, 1H), 1.63-1.61 (m, 2H), 1.46 (s, 6H); ^{19}F NMR ($CDCl_3$, 376 MHz) δ – 64.5 (s, 3F); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.1, 162.0, 158.9, 156.4, 138.2, 133.4 (d, *J* = 31.3 Hz), 132.6, 132.1, 128.6, 124.8, 121.8 (q, *J* = 274.7 Hz), 117.6, 86.5, 60.5, 52.6, 43.2, 40.5, 29.1, 28.6, 25.4, 19.2, 17.9, 14.2, 12.4; ESI m/z 563.3 $[M+H, (100)]^+$; ESI m/z 585.3 $[M+Na, (20)]^+$; Anal. calcd. for $C_{24}H_{33}F_3N_4O_6S$: C 51.24, H 5.91, N 9.96; found: C 51.22, H 5.90, N 9.97.

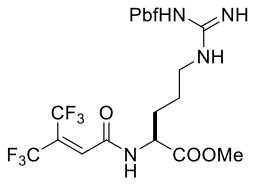
methyl N^2-acryloyl-N^{ω}-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)-L-argininate, 12. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.02 (d, *J* = 7.6 Hz, 1H), 6.48 (br s, 3H), 6.26 (br s, 1H), 6.25 (m, 1H), 5.65 (dd, *J* = 8.8 and 2.8 Hz, 1H), 4.58 (br q, *J* = 4.8 Hz, 1H), 3.73 (s, 3H), 2.25-2.22 (m, 2H), 2.97 (s, 2H), 2.57 (s, 3H), 2.51 (s, 3H), 2.11 (s, 3H), 1.90-1.86 (m, 1H), 1.81-1.78 (m, 1H), 1.65-1.61 (m, 2H), 1.48 (s, 3H), 1.47 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 166.1, 159.1, 156.0, 138.6, 132.5, 130.2, 127.5, 124.8, 117.7, 86.6, 52.5, 43.2, 40.7, 29.6, 28.6, 25.3, 19.3, 17.9, 12.5; ESI m/z 517.3 $[M+Na, (100)]^+$; Anal. calcd. for $C_{23}H_{34}N_4O_6S$: C 55.85, H 6.93, N 11.33; found: C 55.87, H 6.93, N 11.34.

methyl (2-((2-acetamidoethyl)amino)-4,4,4-trifluoro-3-(trifluoromethyl)butanoyl)-L-argininate, 15. ^{1}H NMR (D_2O, 400 MHz), mixture of two diastereoisomers: δ 4.47-4.46 (m, 1H), 4.44 (br s, 1H), 4.34-4.32 (m, 1H), 3.74 (s, 1,5H, one diast.) 3.73 (s, 1,5H, one diast.), 3.48-3.46 (m, 1H), 3.41-3.39 (m, 1H), 3.16-3.14 (m, 2H), 3.13-2.98 (m, 2H), 1.98 (s, 3H), 1.96-1.94 (m, 1H), 1.82-1.79 (m, 1H), 1.61-1.58 (m, 2H). ^{19}F NMR (D_2O, 376 MHz), mixture of two diastereoisomers: δ – 62.6 (quintet, *J* = 9.8

Hz, 3F one diast.), – 62.9 (br s, 6F, one diast.), – 63.3 (quintet, *J* = 9.8 Hz, 3F one dist.); ESI m/z 481.2 [M+H, (100)]$^+$; Anal. calcd. for $C_{16}H_{26}F_6N_6O_4$: C 40.00, H 5.46, N, 17.49; found: C 39.98, H 5.46, N, 17.48.

methyl (2-(((2-acetamidoethyl)amino)methyl)-3,3,3-trifluoropropanoyl)-L-argininate, 16. ^{1}H NMR (D_2O, 400 MHz), mixture of two diastereoisomers: δ 4.59 (dd, *J* = 9.2 and 4.4 Hz, 0.5H, one diast.), 4.17 (dd, *J* = 8.4 and 4.8 Hz, 0.5H, one dist.), 3.95-3.87 (m, 1H), 3.76 (s, 1.5H, one dist.), 3.74 (s, 1.5H, one diast.), 3.70-3.65 (m, 1H), 3.62-3.57 (m, 1H), 3.54-3.16 (m, 2H), 3.27-3.24 (m, 2H), 3.20-3.17 (m, 2H), 1.99 (s, 3H), 1.98-1.94 (m, 1H), 1.83-1.78 (m, 1H), 1.68-1.61 (m, 2H). ^{19}F NMR (D_2O, 376 MHz), mixture of two diastereoisomers: δ – 66.9 (d, *J* = 7.5 Hz, 3F one diast.), – 67.1 (d, *J* = 7.5 Hz, 3F one dist.); ESI m/z 413.1 [M+H, (100)]$^+$; Anal. calcd. for $C_{15}H_{27}F_3N_6O_4$: C 43.69, H 6.60, N 20.38; found: C 43.70, H 6.61, N 20.40.

methyl (3-((2-acetamidoethyl)amino)propanoyl)-L-argininate, 17. ^{1}H NMR (D_2O, 400 MHz): δ 4.41 (dd, *J* = 8.8 and 5.2 Hz, 1H), 3.50 (br t, *J* = 5.6 Hz, 2H), 3.47 (s, 3H), 3.32 (br t, *J* = 7.2 Hz, 2H), 3.10-3.16 (m, 4H), 2.77 (t, *J* = 6.4 Hz, 2H), 1.99 (s, 3H), 1.90-1.88 (m, 1H), 1.77-1.75 (m, 1H), 1.65-1.63 (m, 2H) ; ESI m/z 345.1 [M+H, (100)]$^+$; Anal. calcd. for $C_{14}H_{28}N_6O_4$: C 48.82, H 8.19, N 24.40; found: C 48.82, H 8.20, N 24.41.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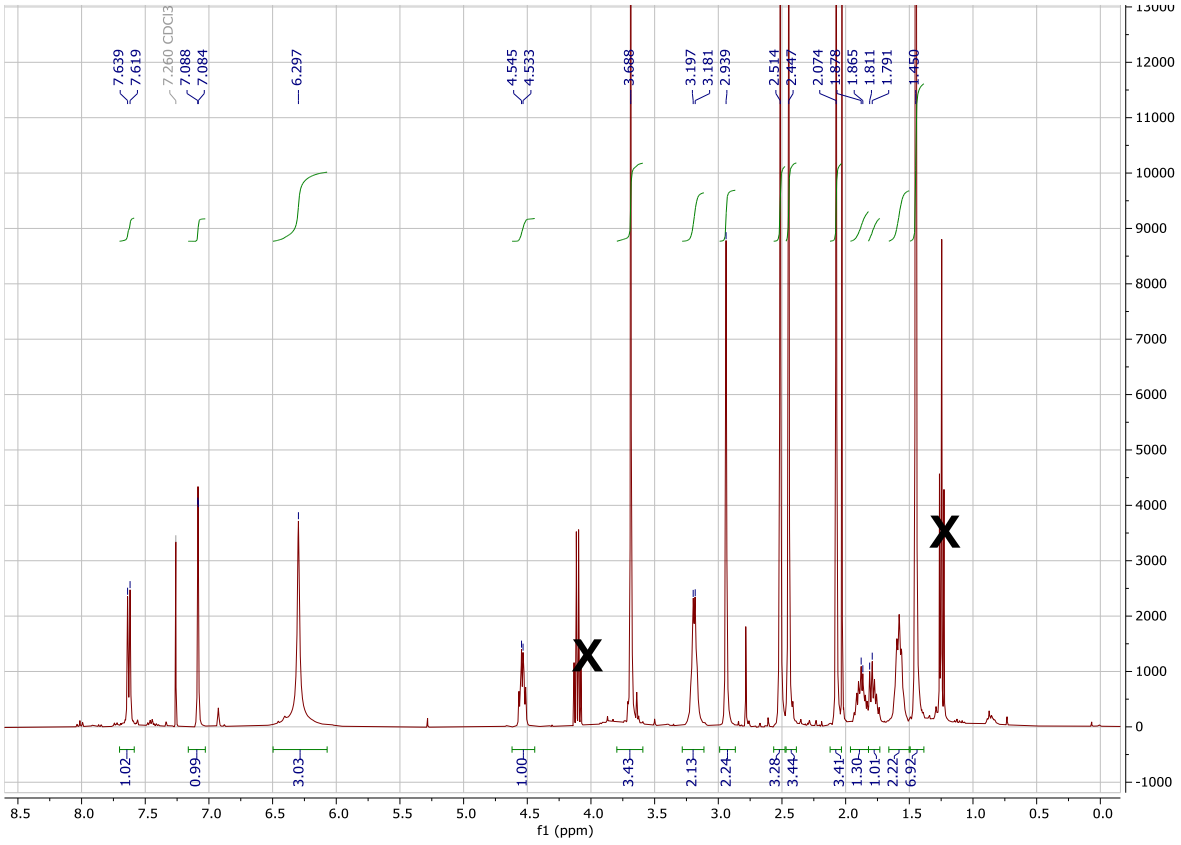
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2.074
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X
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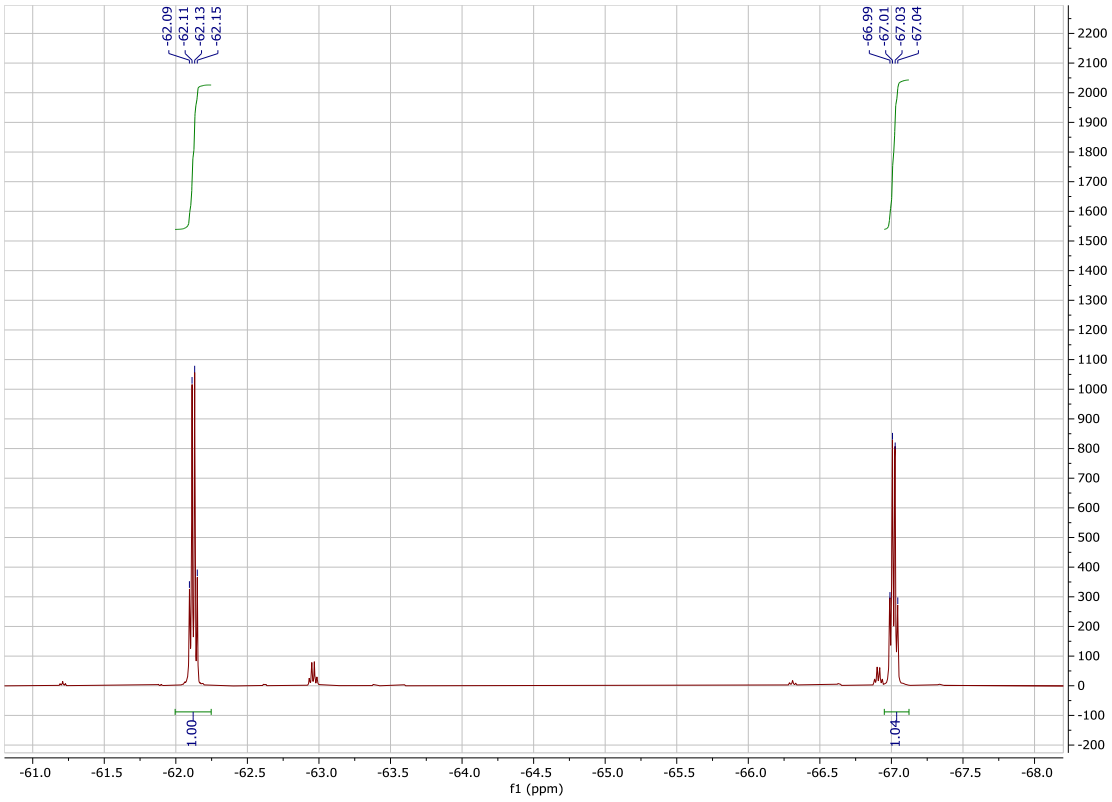
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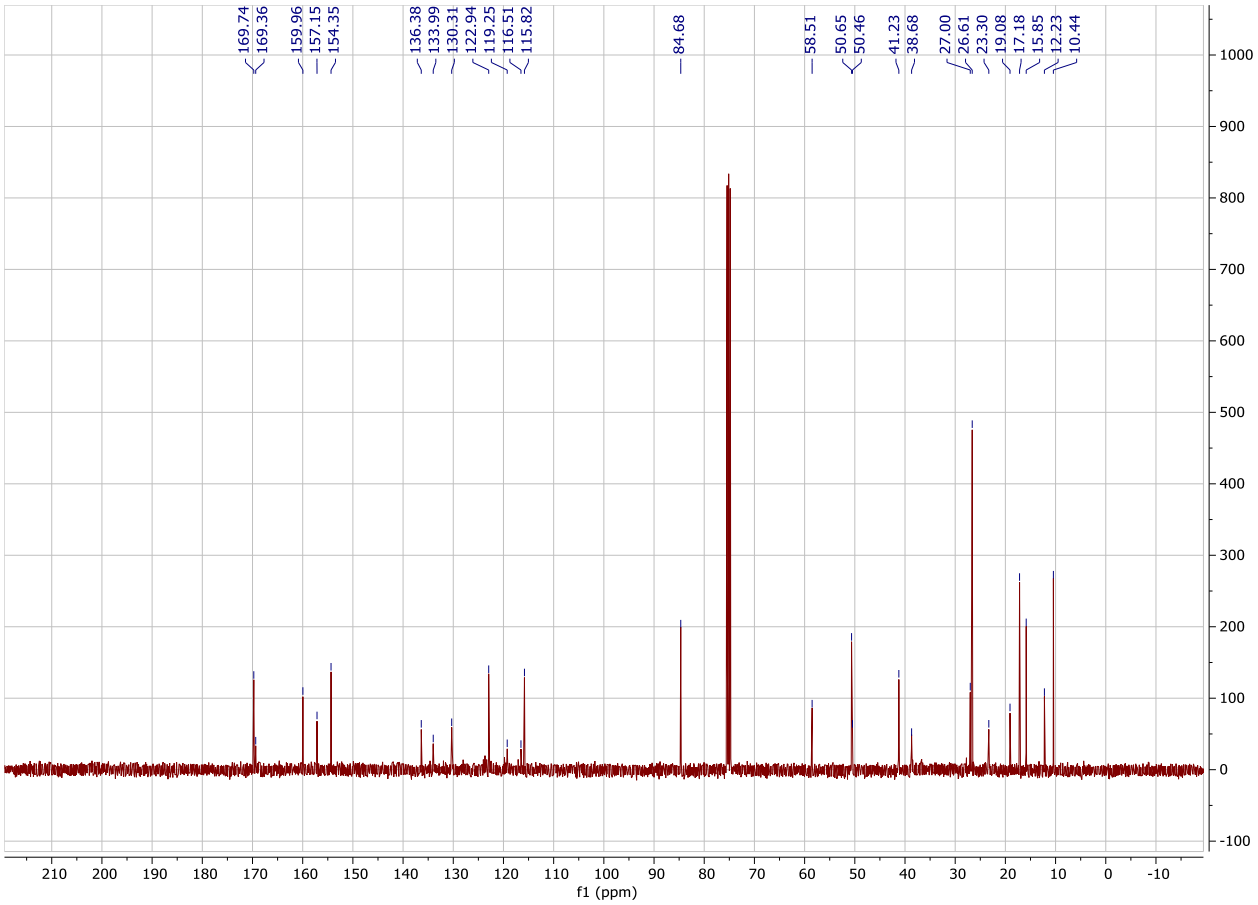

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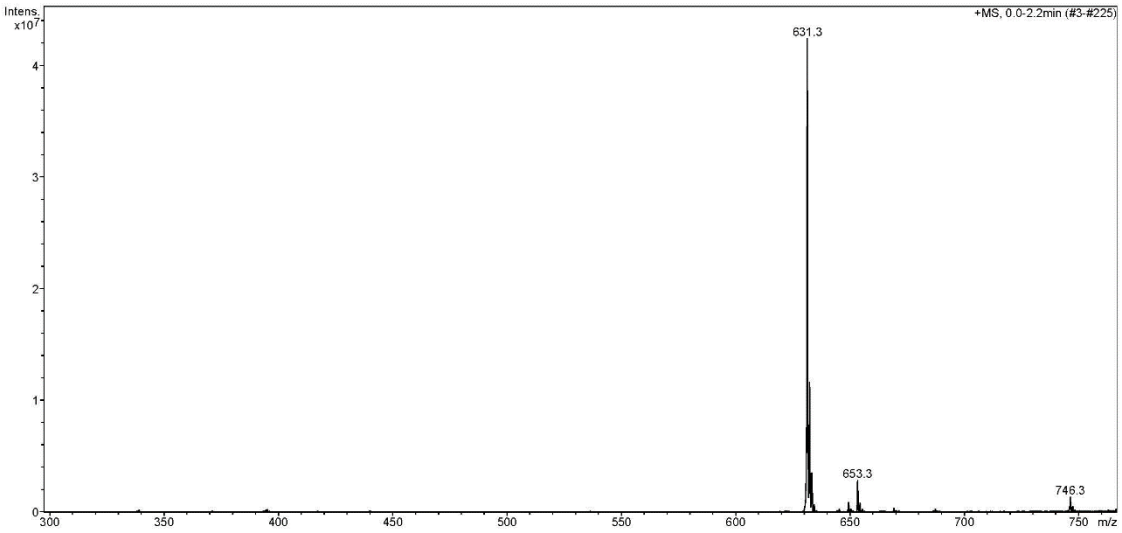

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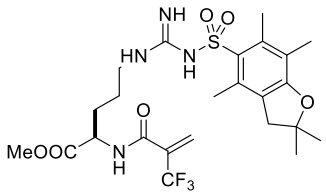
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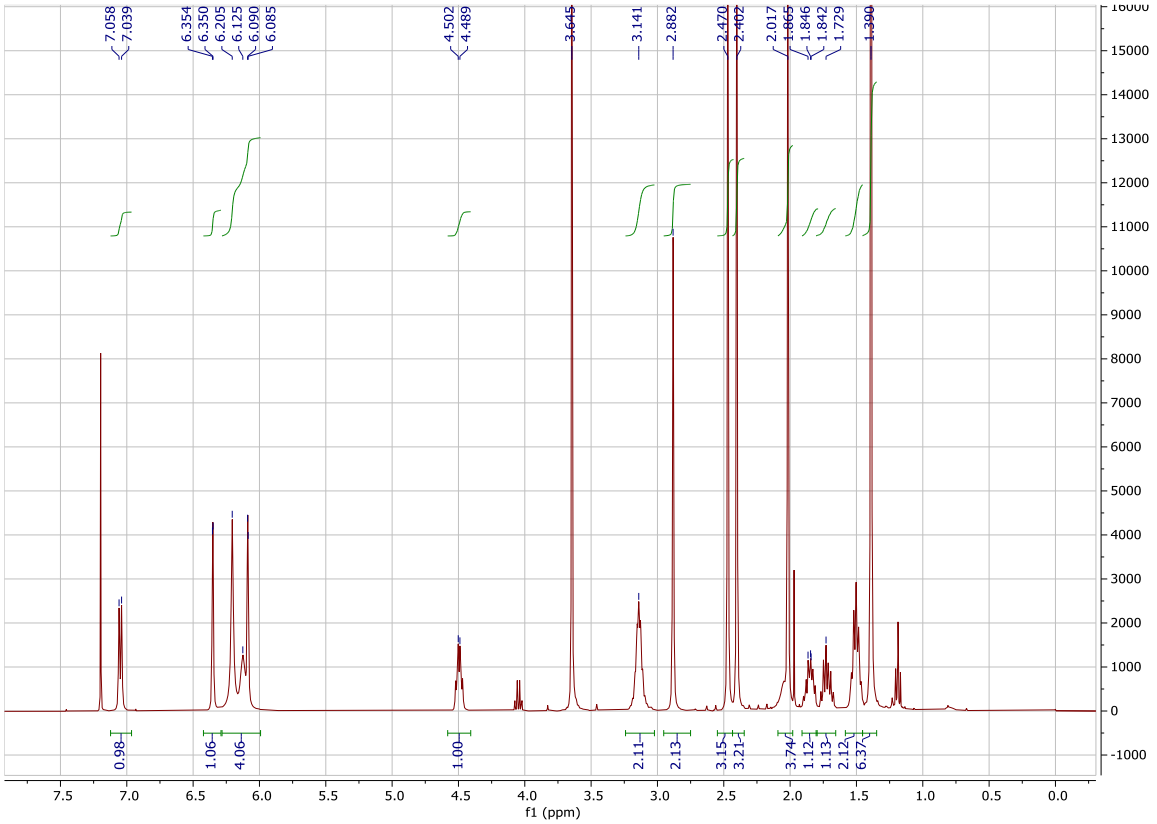
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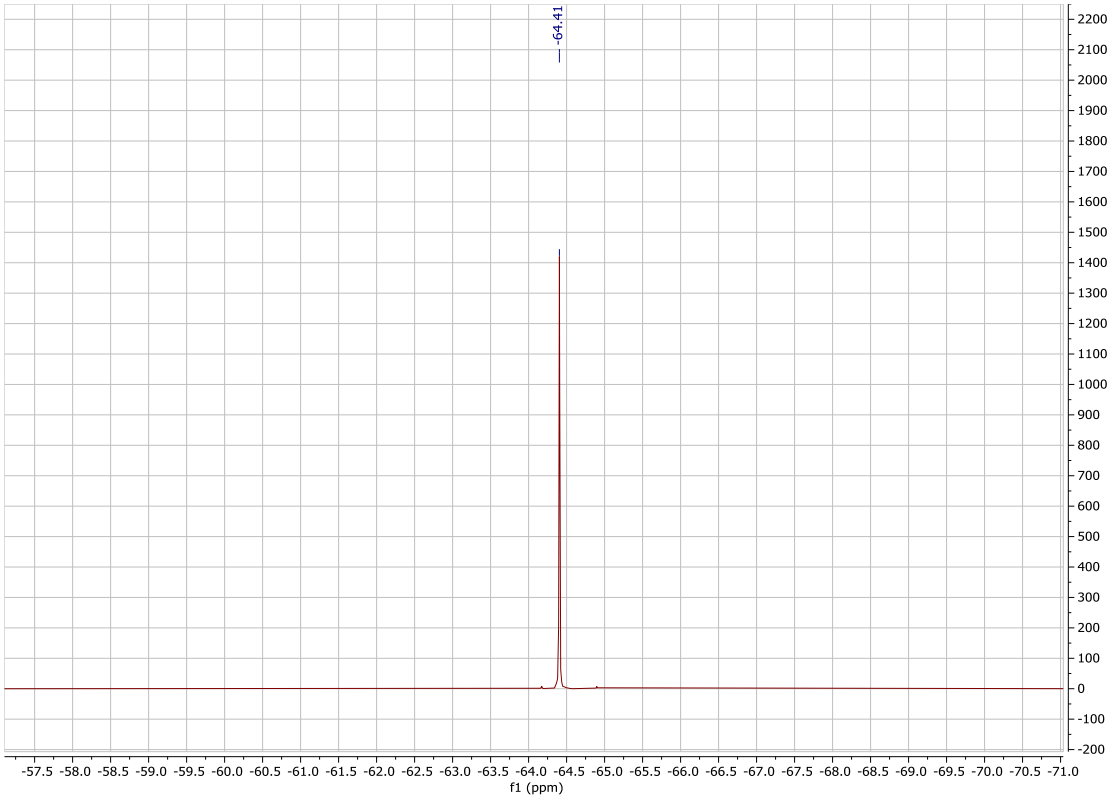
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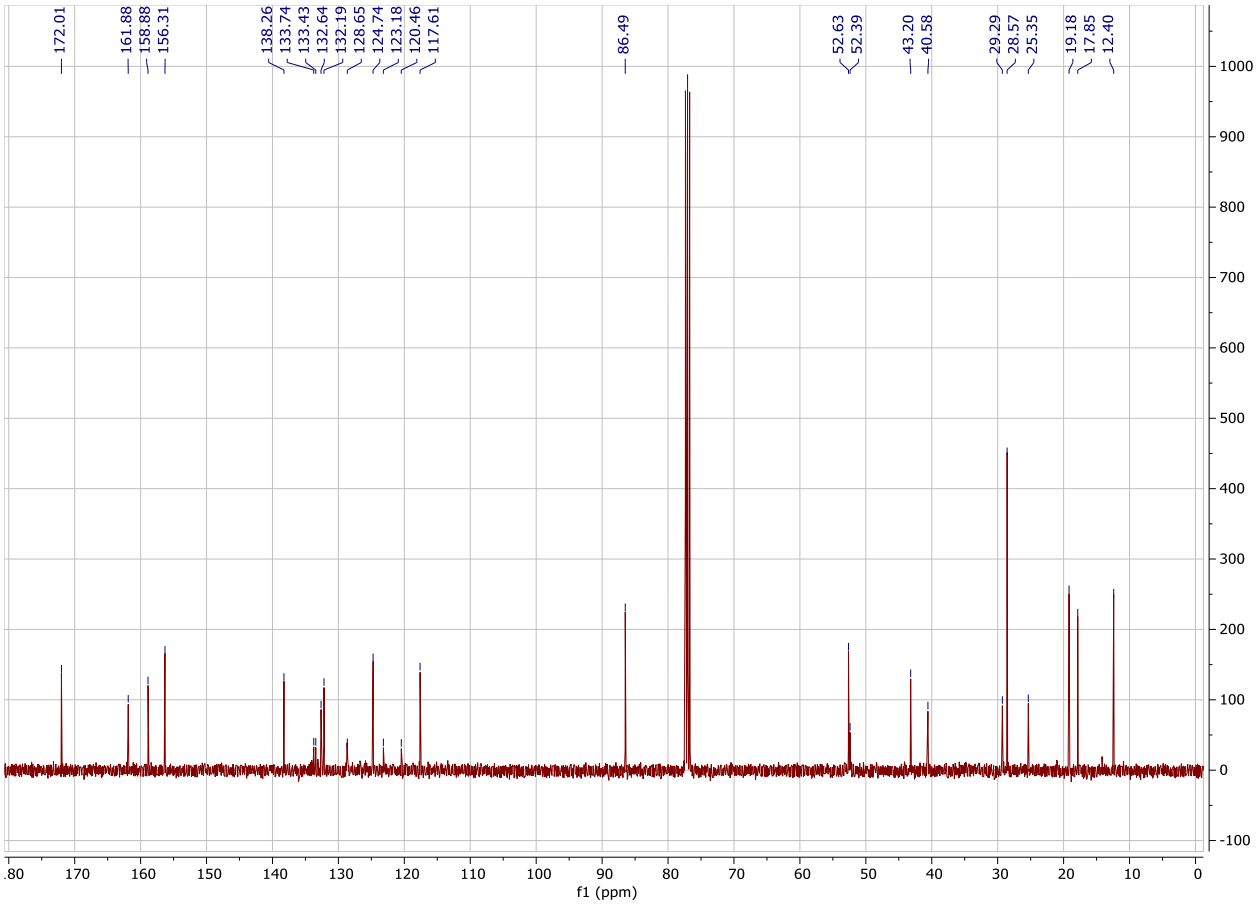

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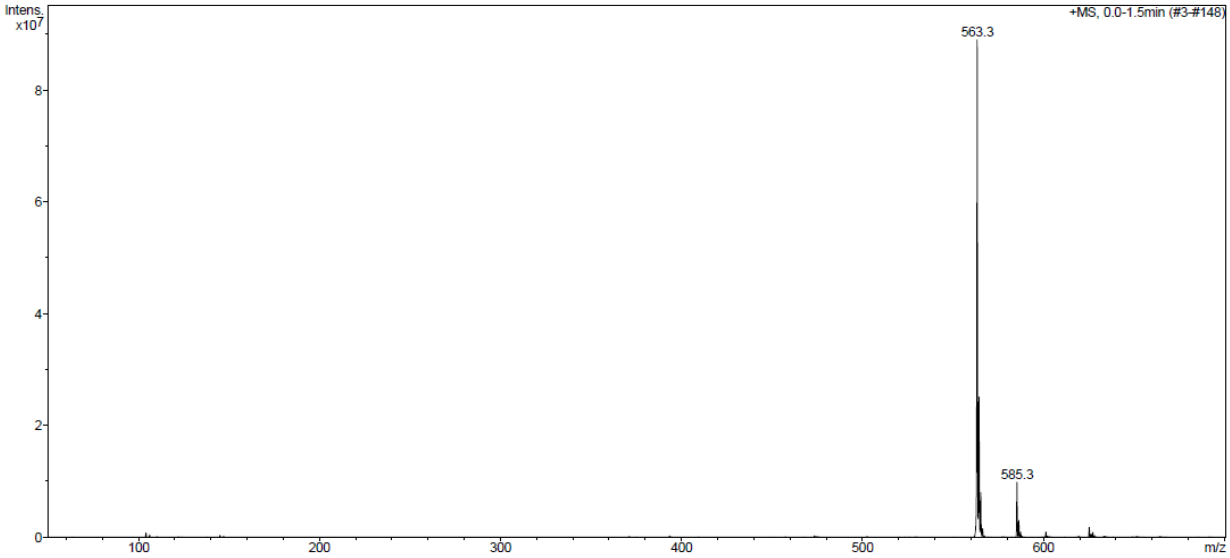

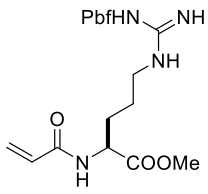
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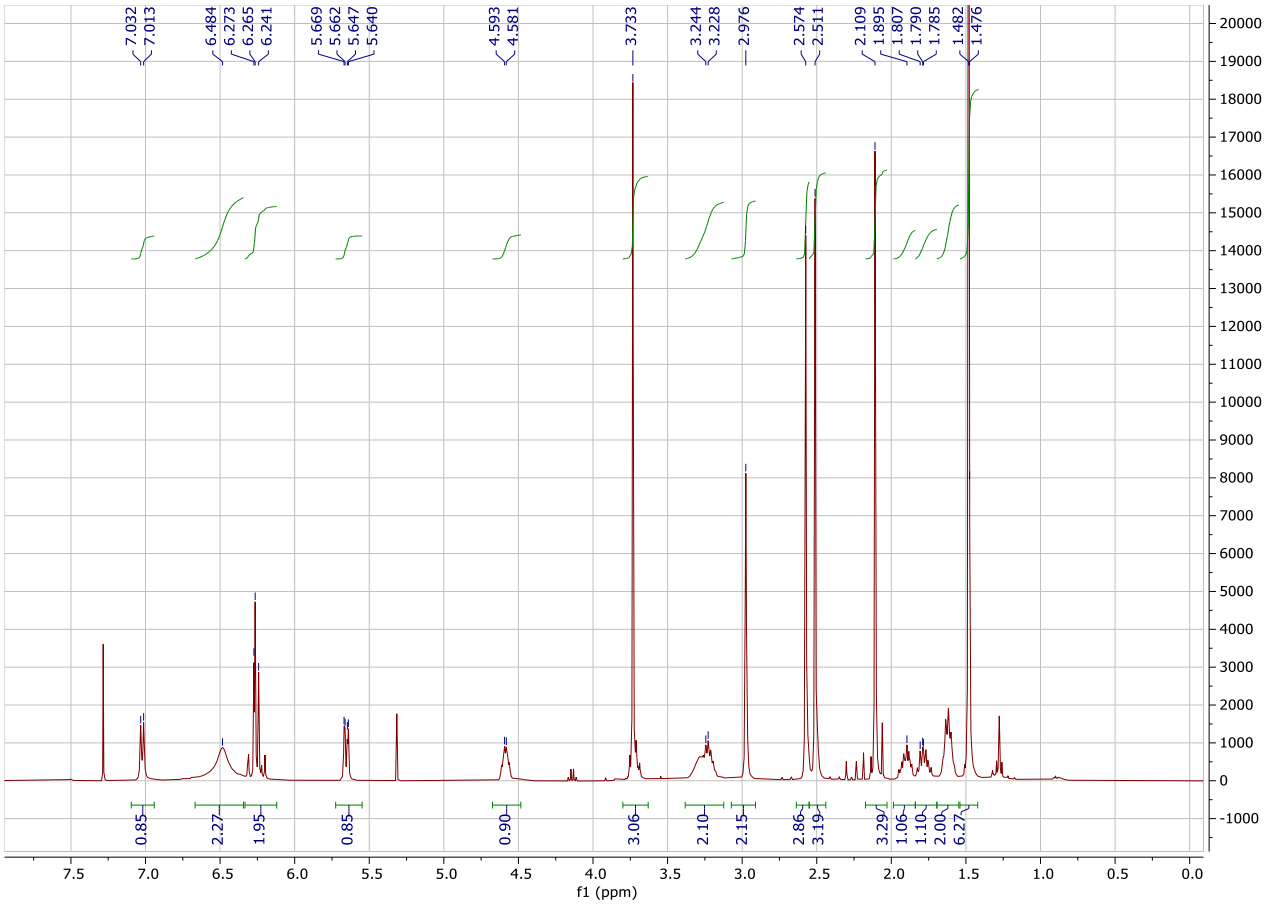
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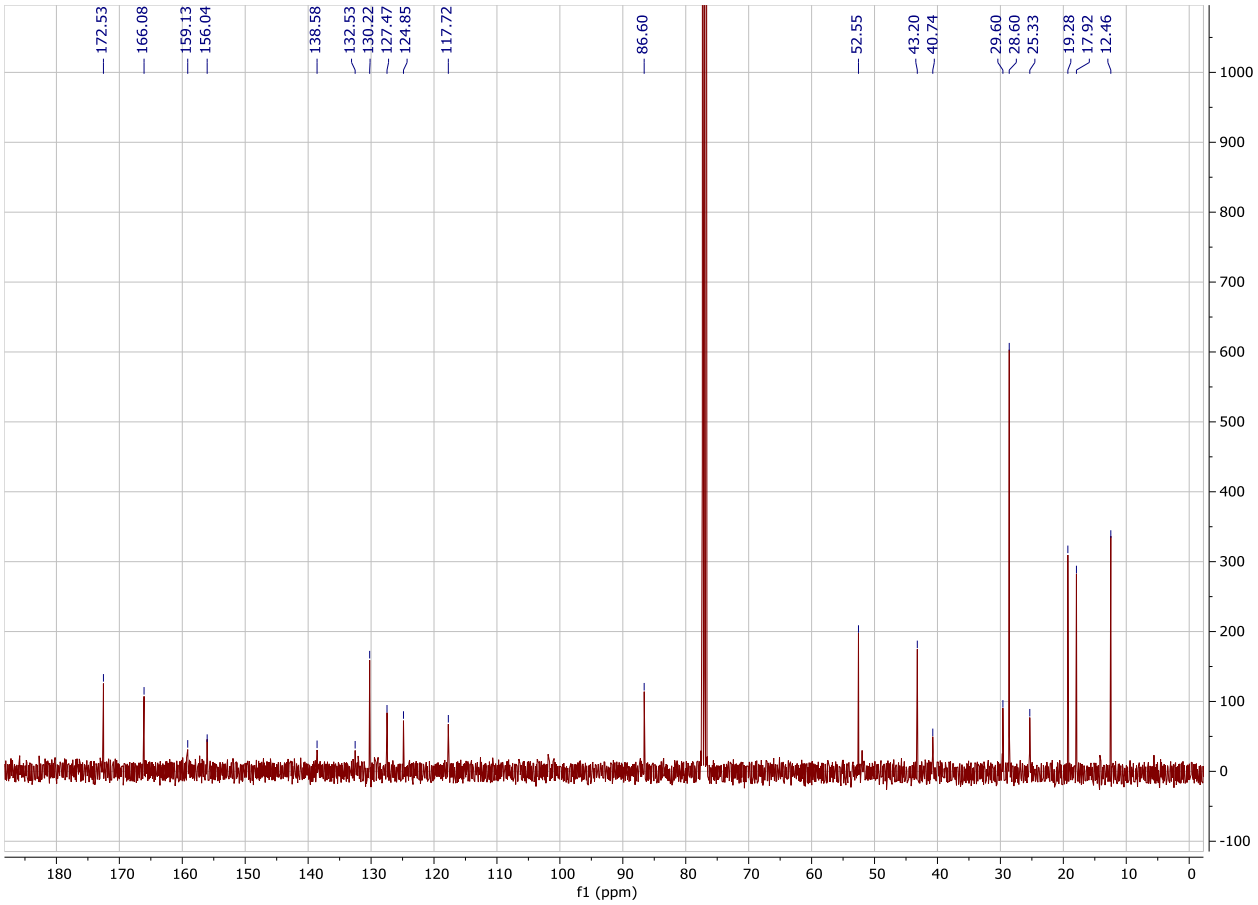

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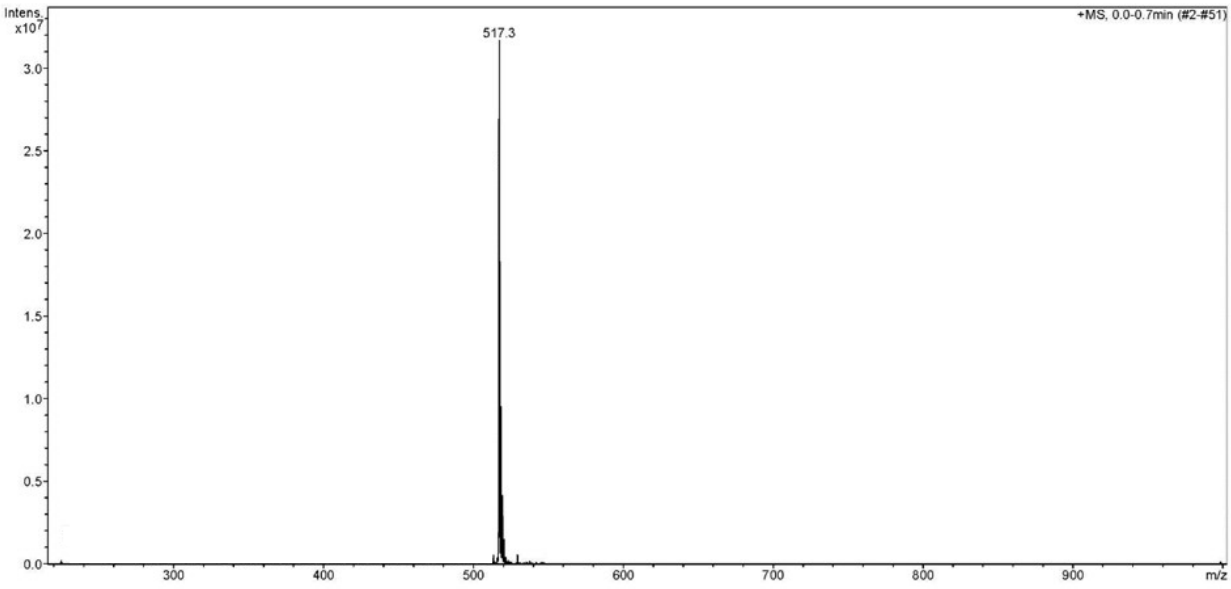

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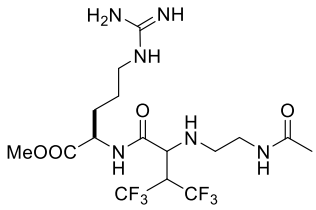

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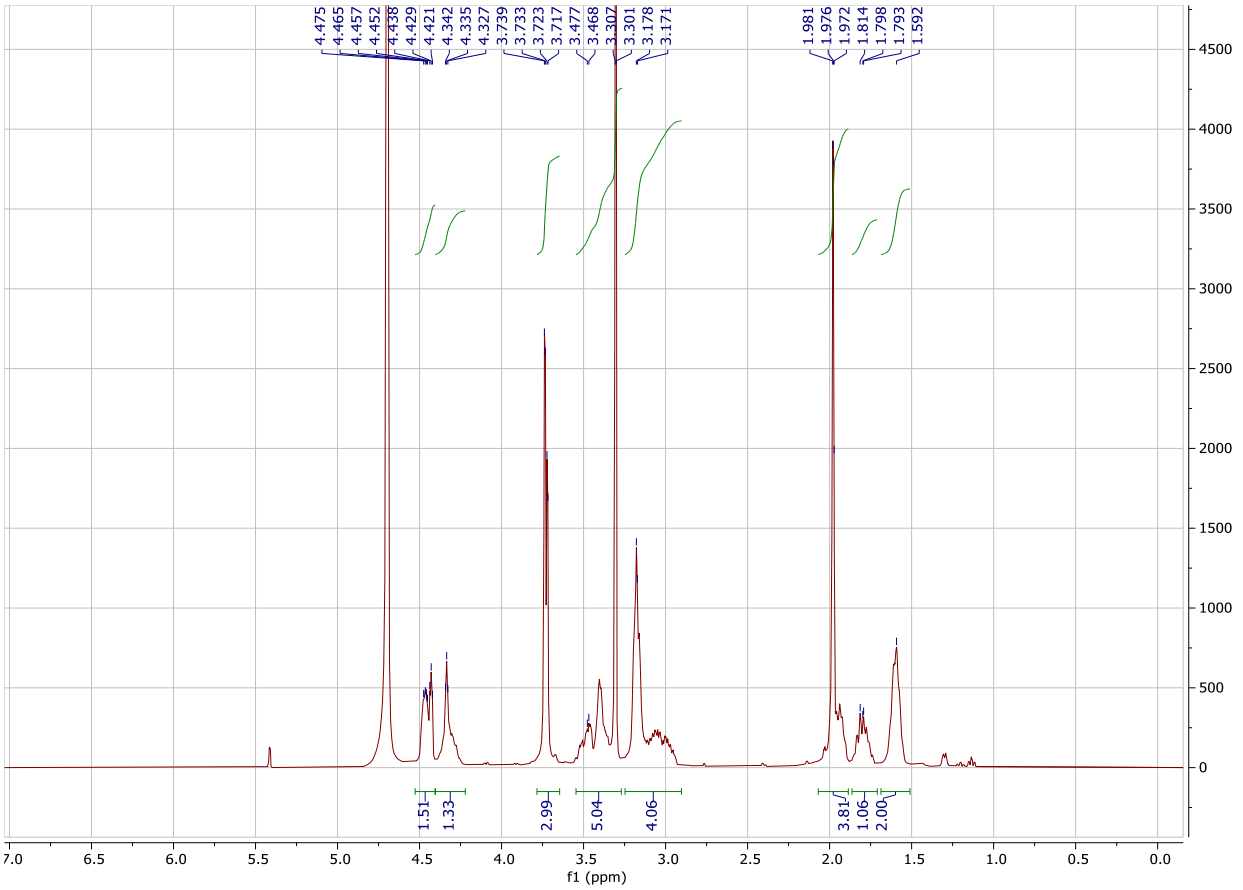
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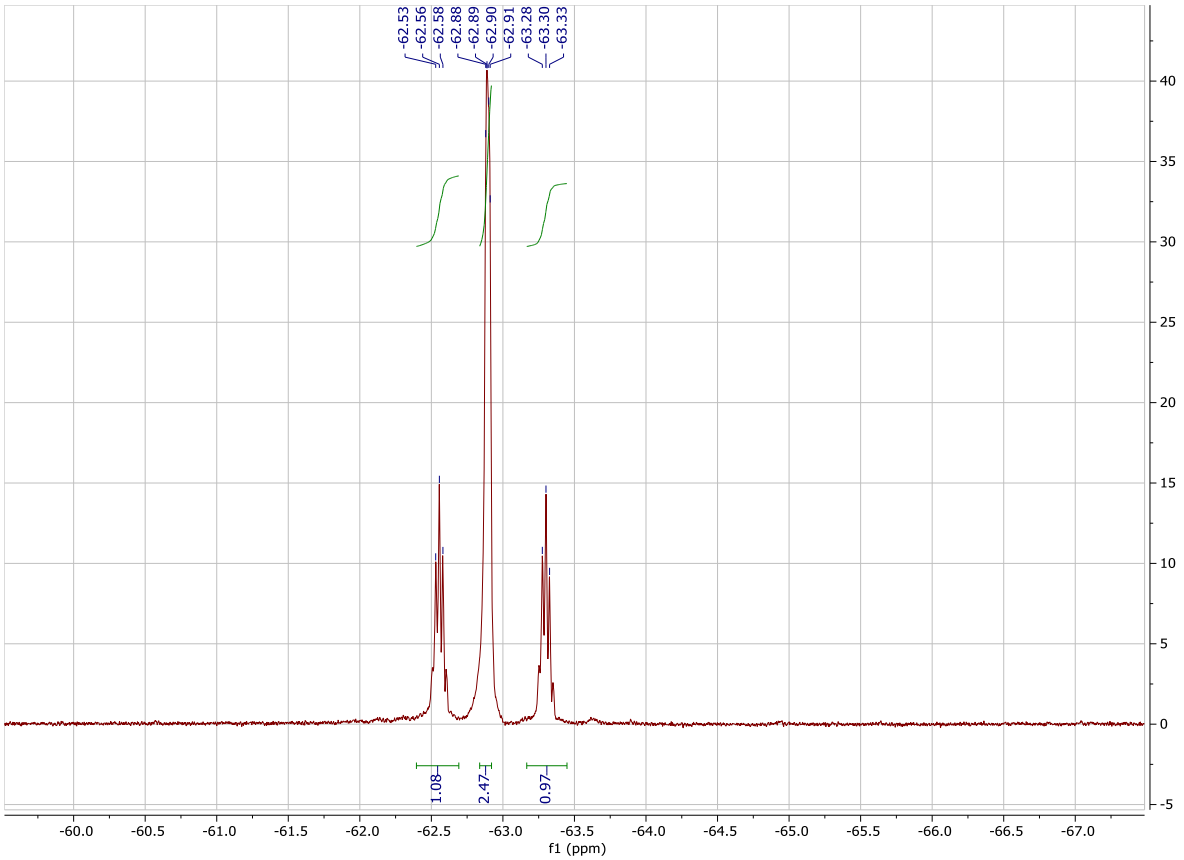

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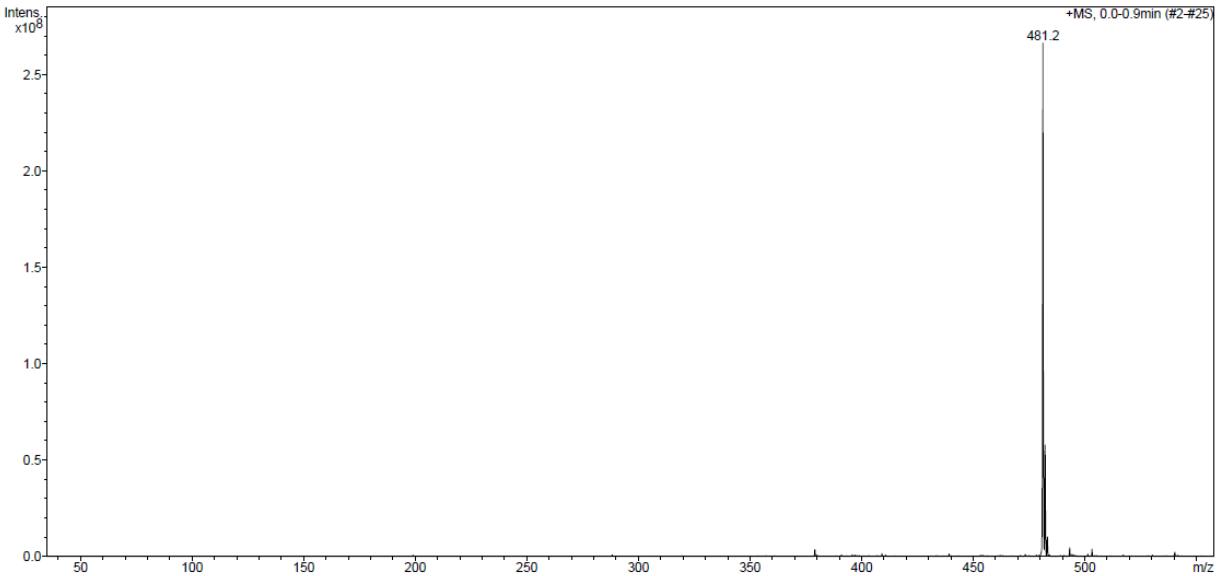

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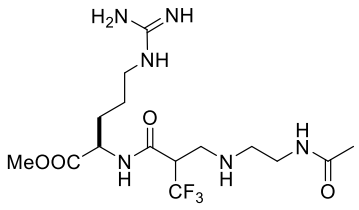

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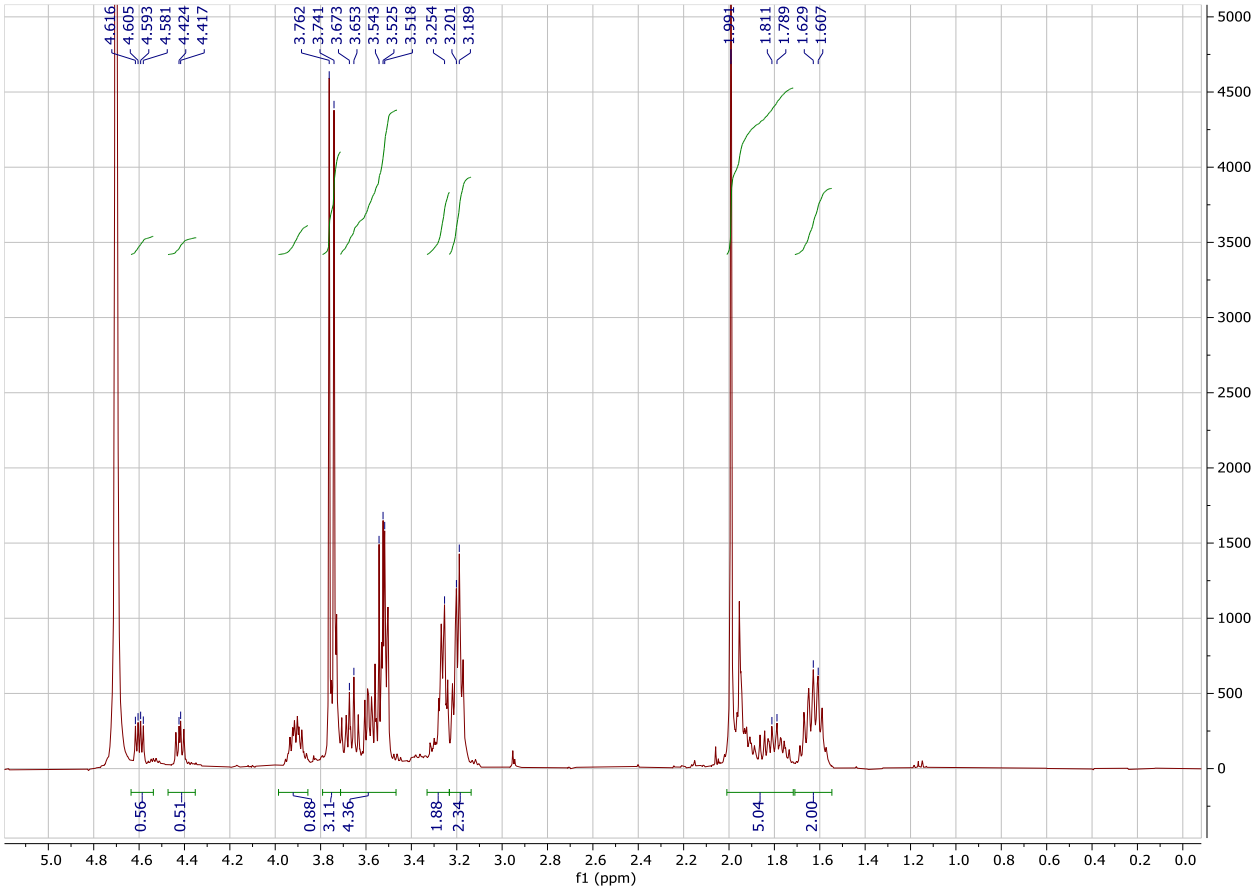
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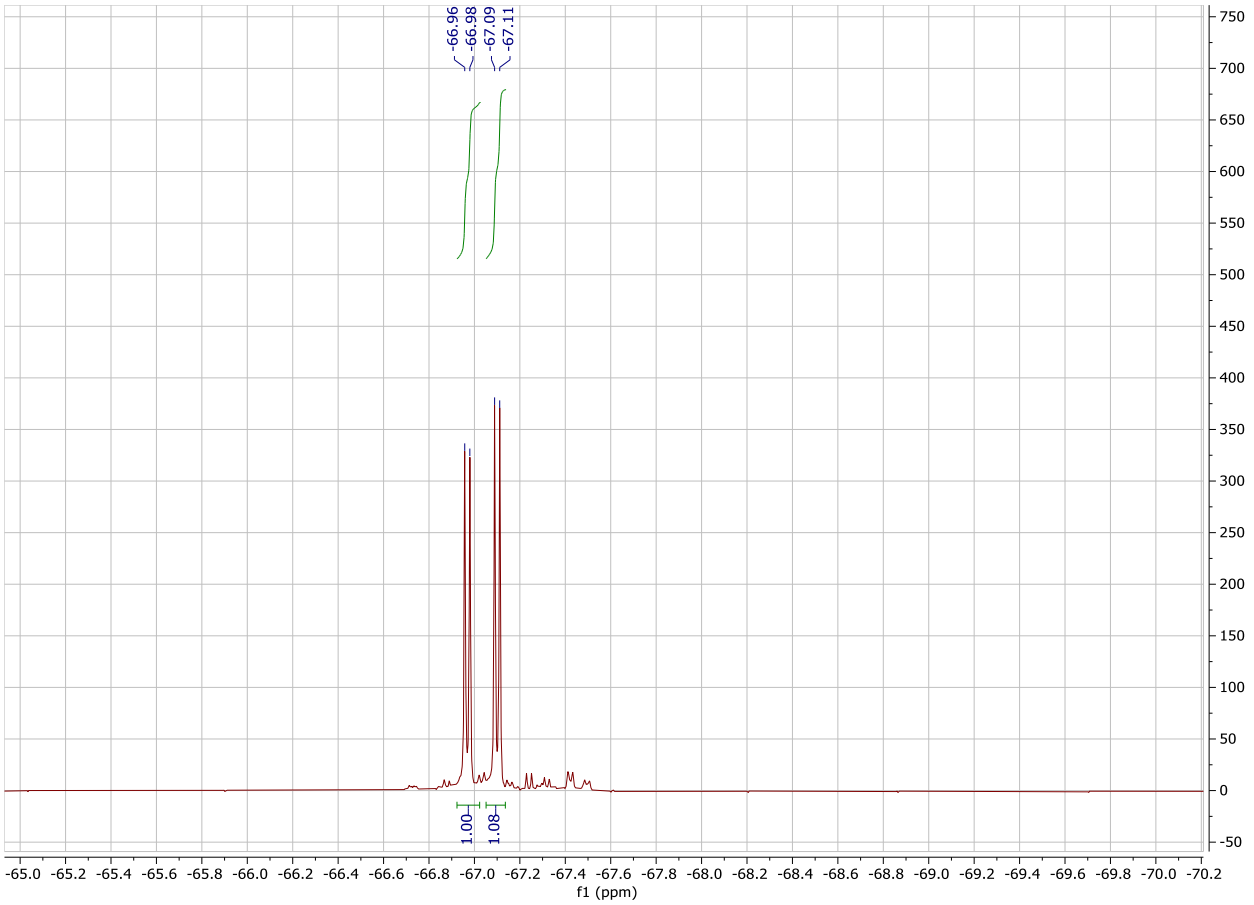

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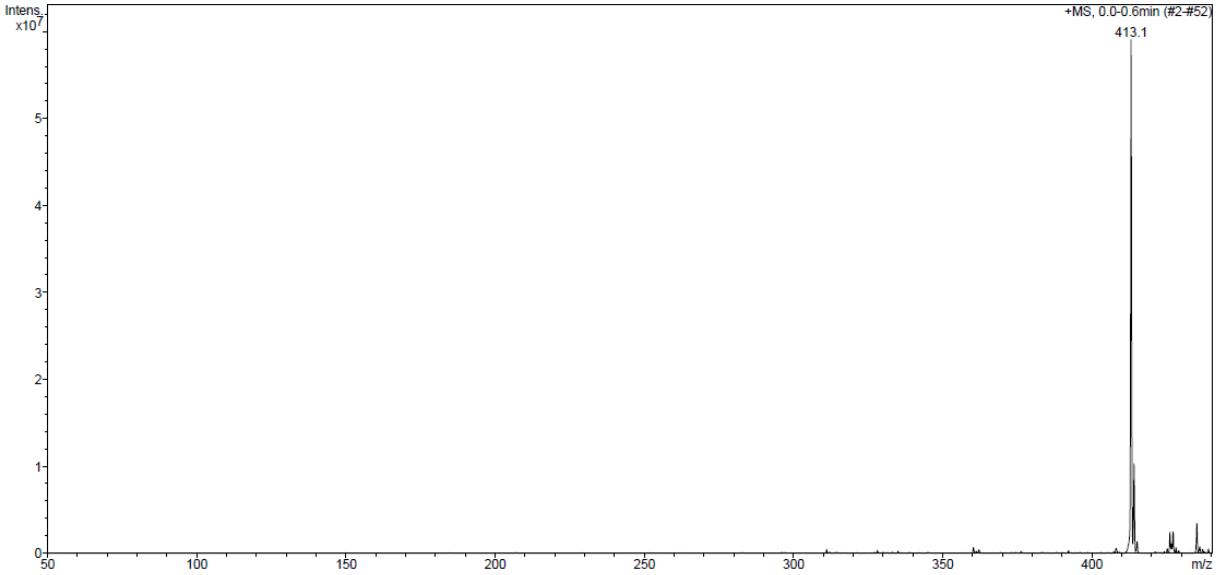

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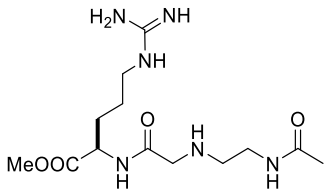
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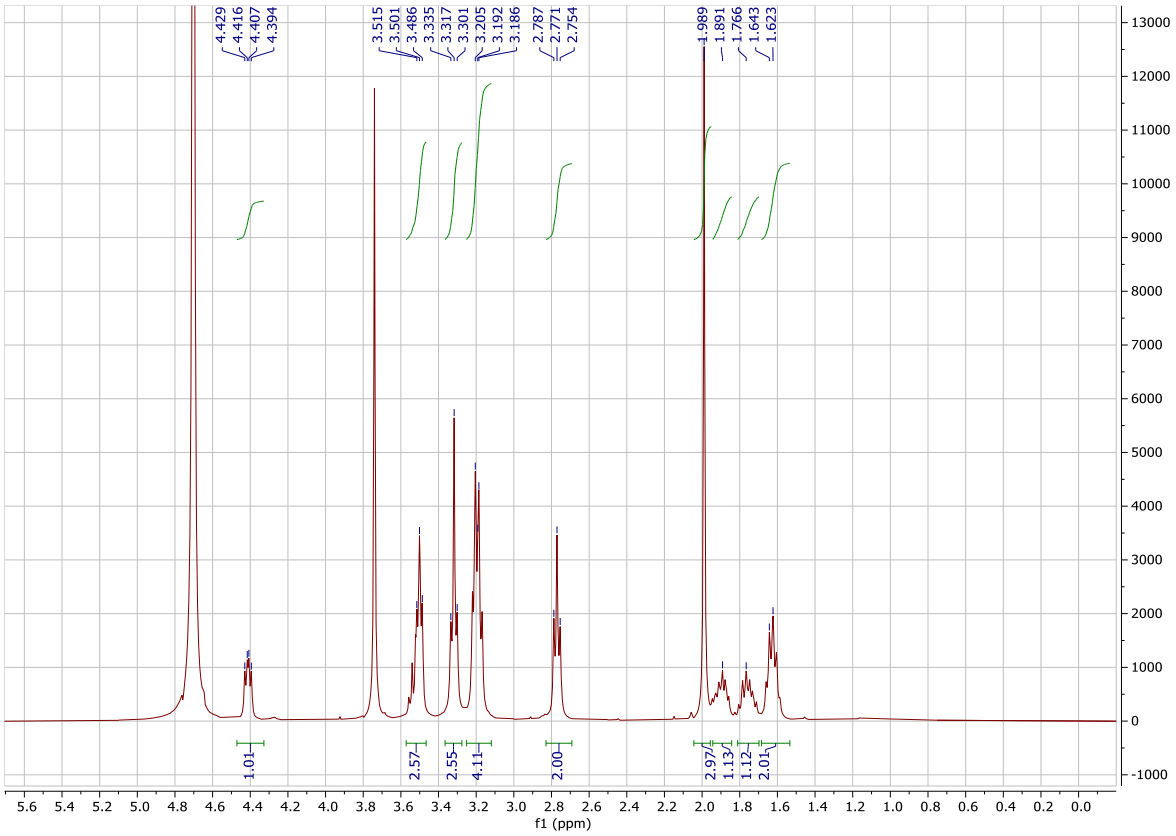
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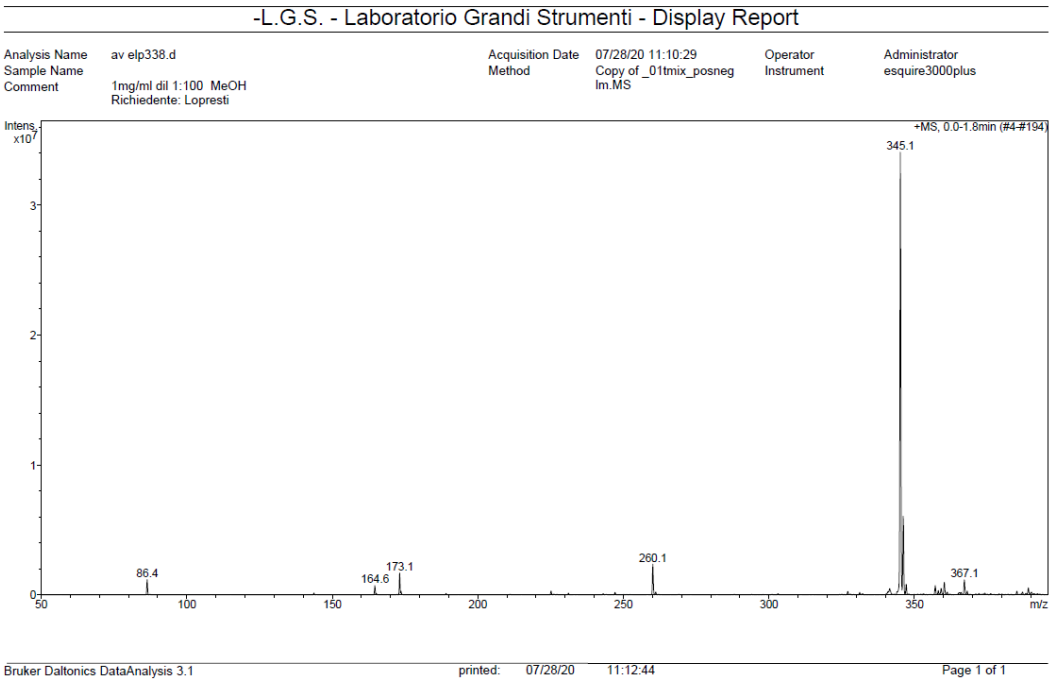
2.01
f1 (ppm)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Sample Name
Comment 1mg/ml dil 1:100 MeOH
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Acquisition Date 07/28/20 11:10:29
Method Copy of _01tmix_posneg lm.MS
Operator
Instrument
Administrator
esquire3000plus
Intens.
x10^7
+MS, 0.0-1.8min (#4-#194)
345.1
260.1
173.1
164.6
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m/z
Bruker Daltonics DataAnalysis 3.1
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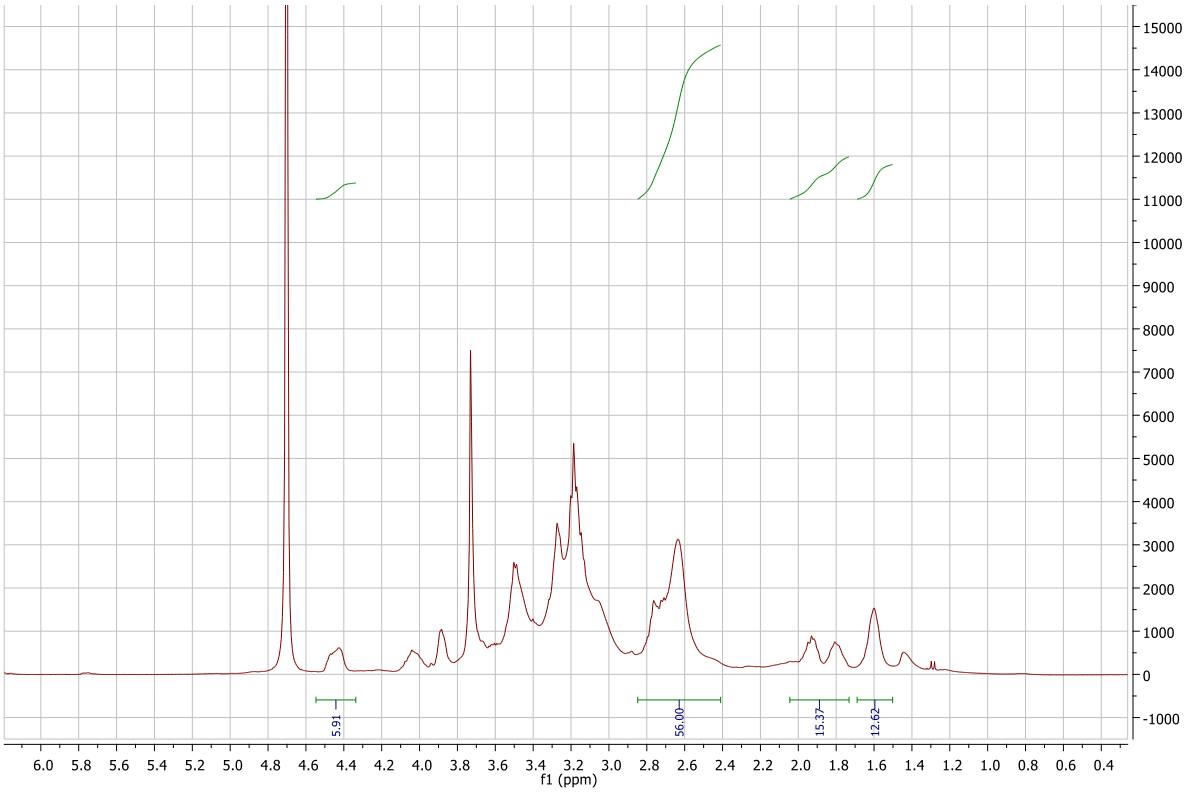
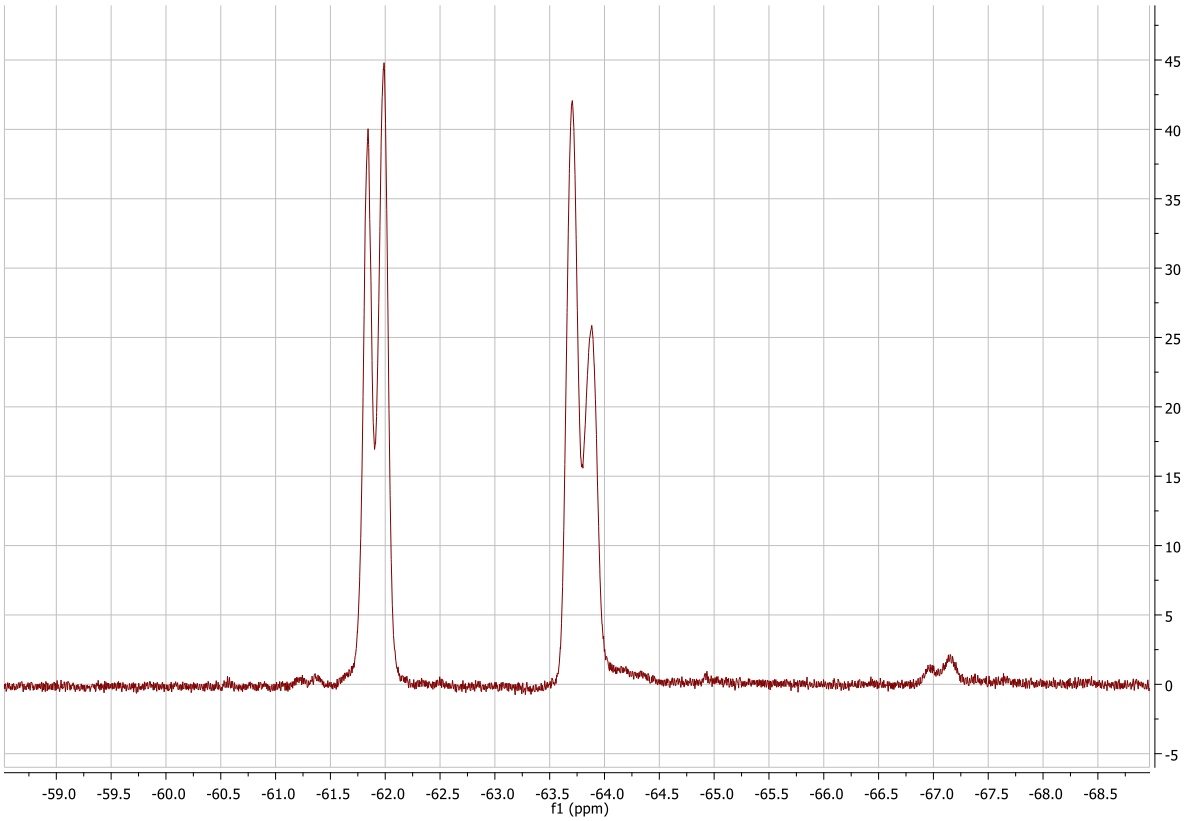
Page 1 of 1

PAMAM G2−hfVal-Arg 1





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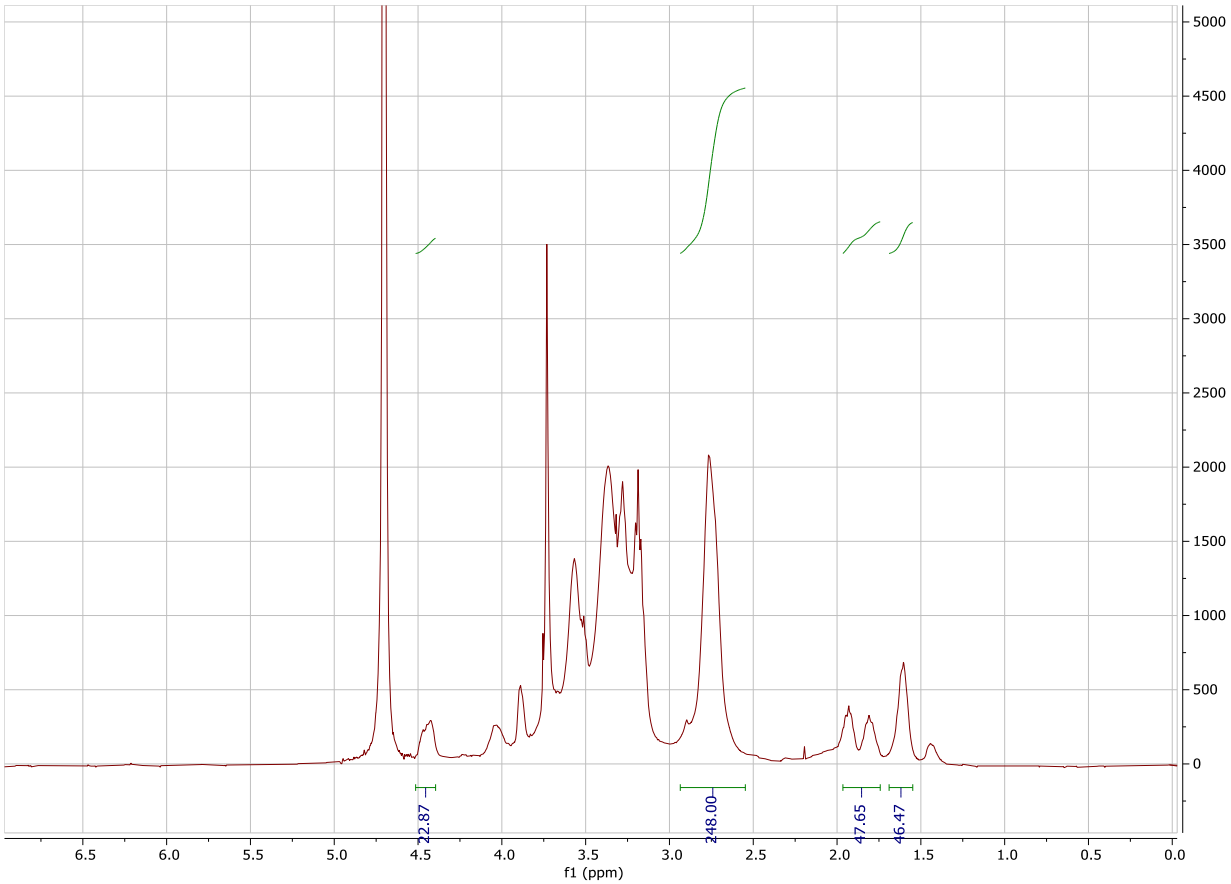

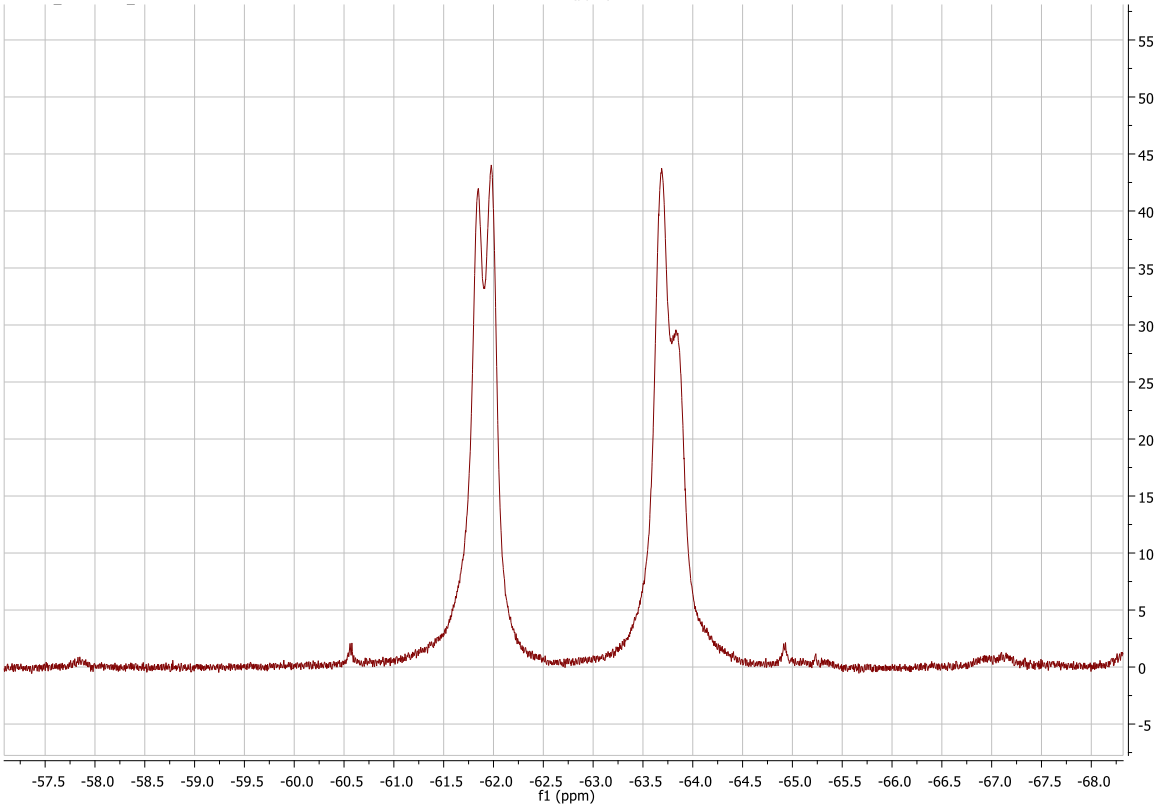

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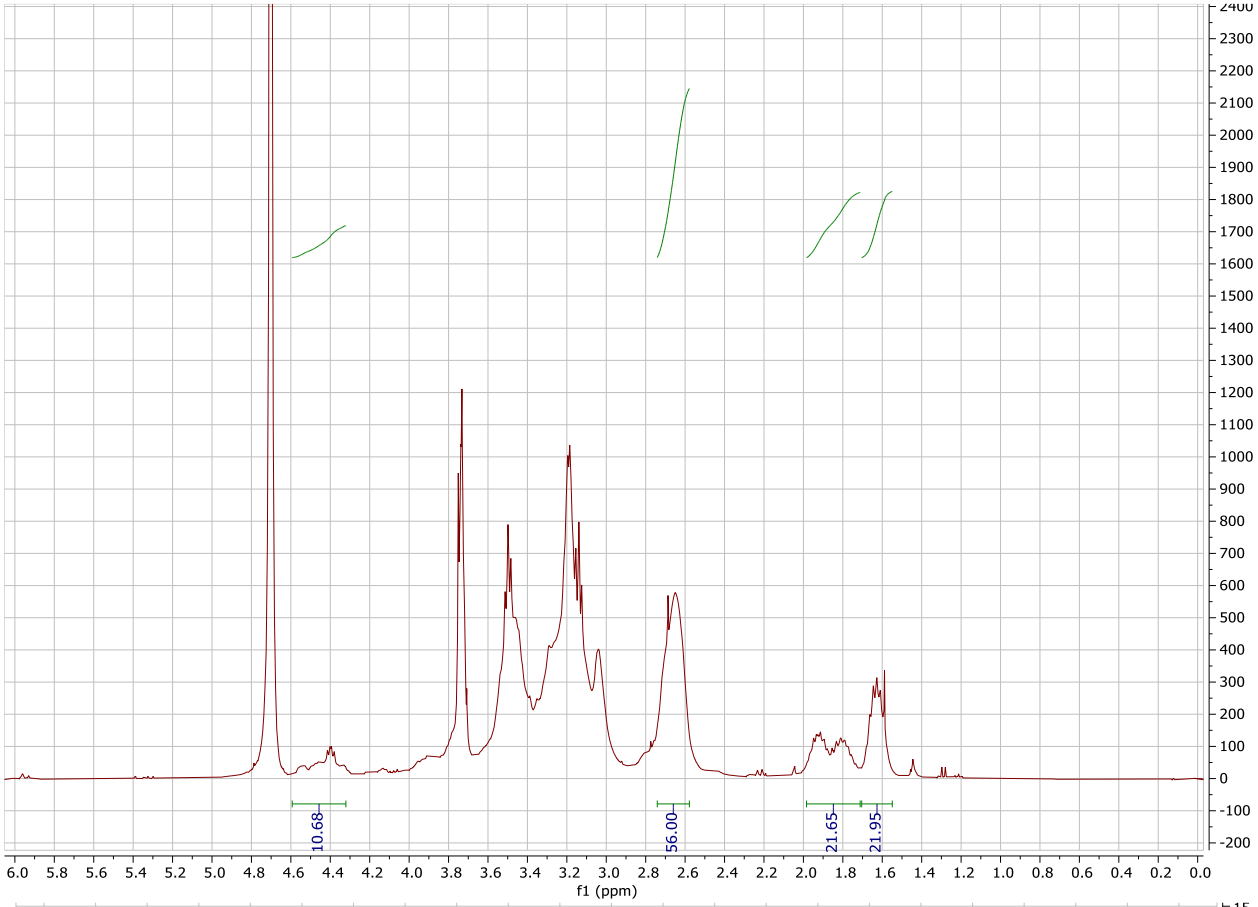

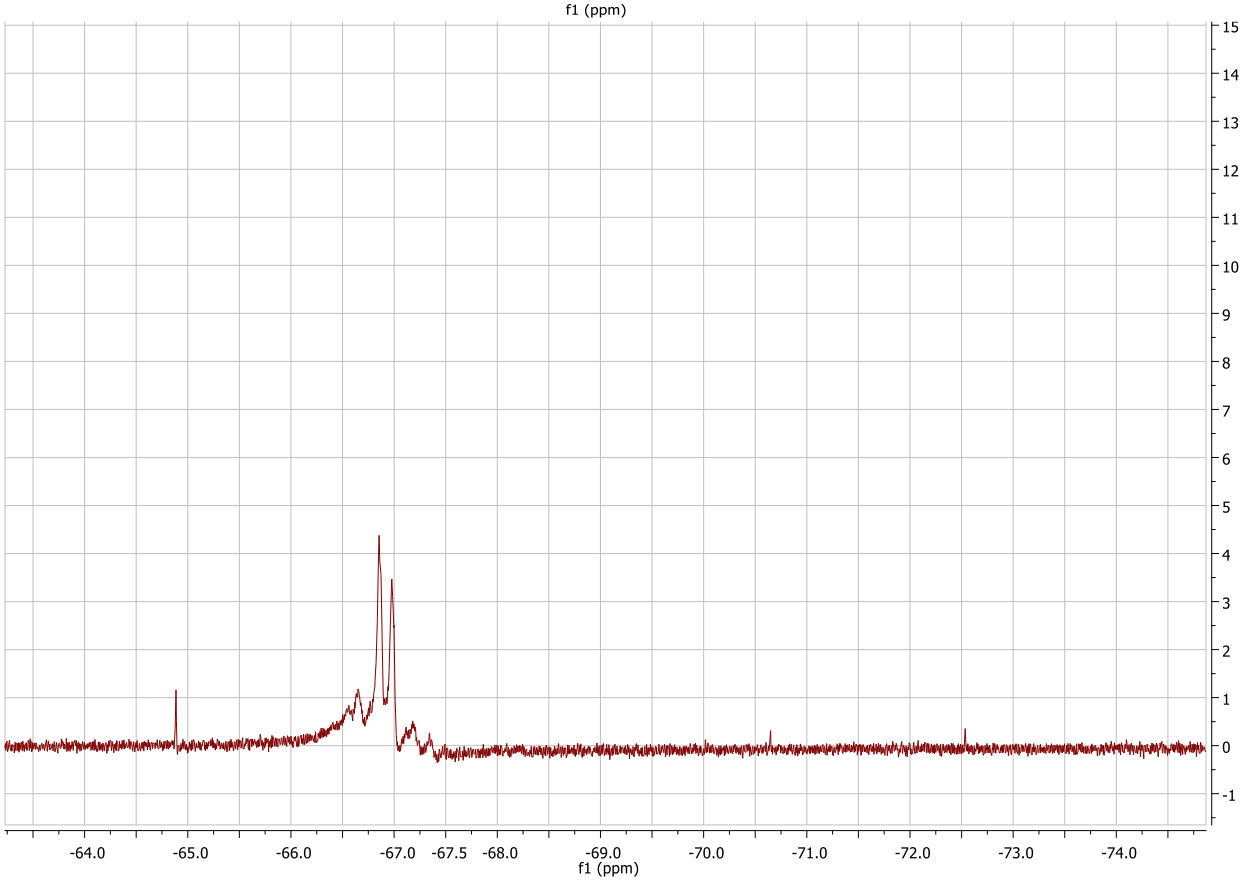

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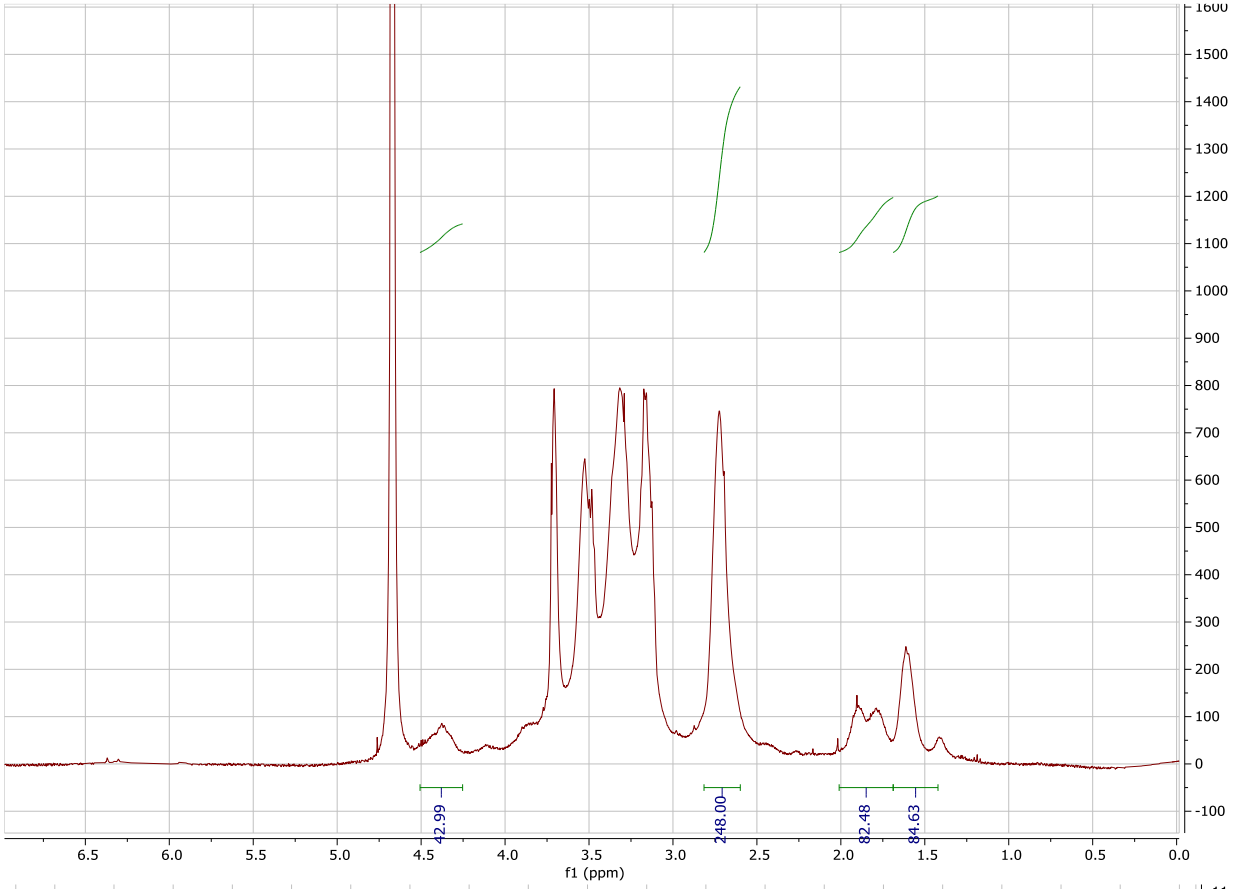

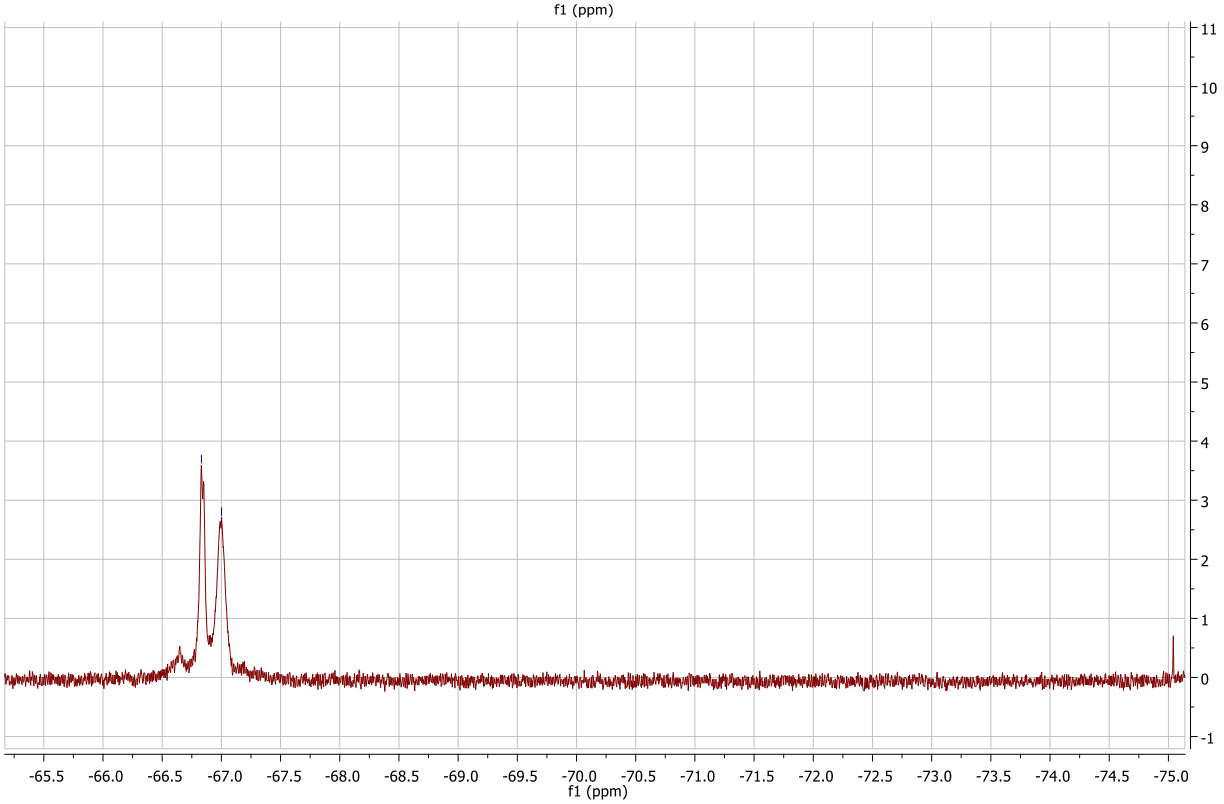

PAMAM G2 -βAla-Arg 13

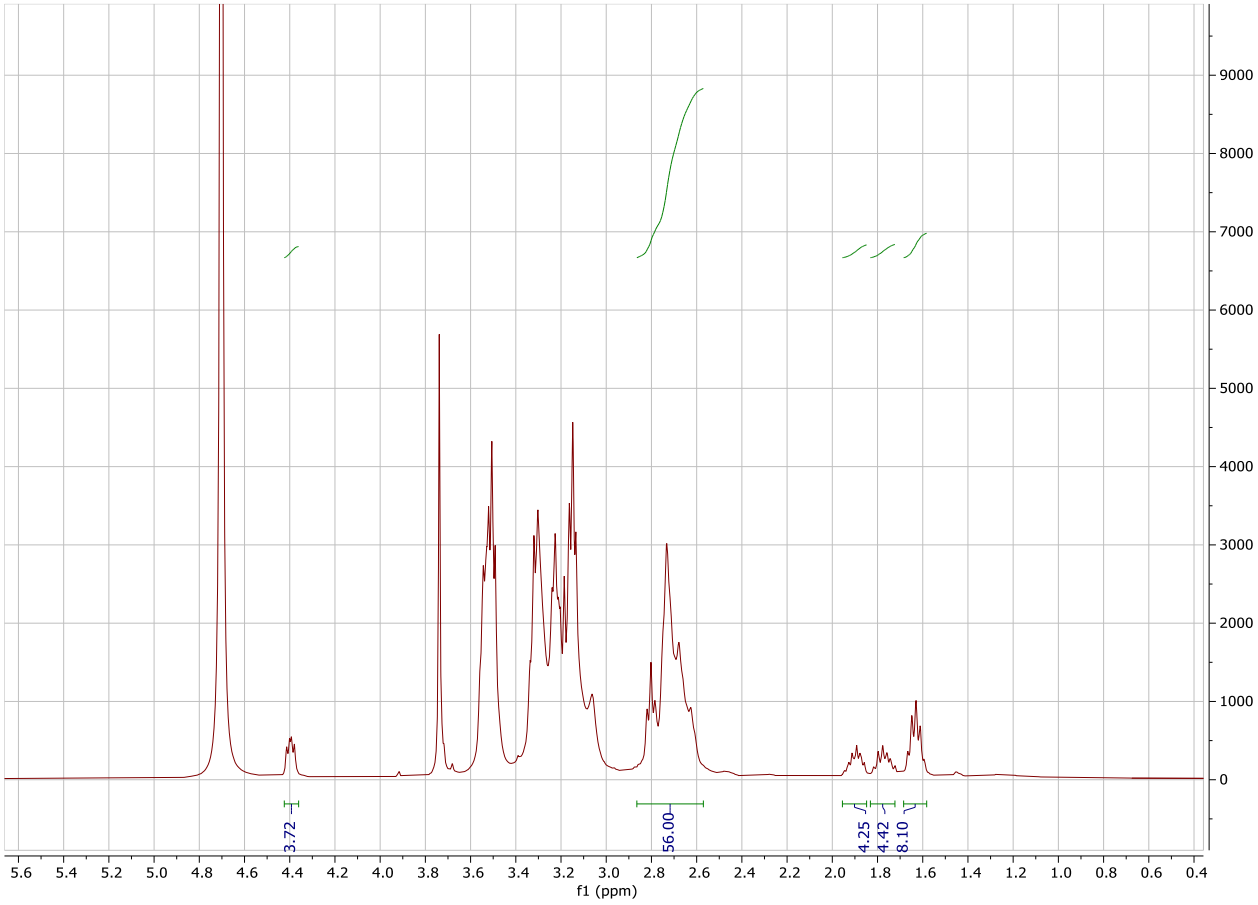

Figure S1. Agarose gel analysis of model DNA (Figure S1a) and pGFP (Figure S1b), used as preliminary complexation behavior analyses for PAMAM G4-αtfm-βAla-Arg **4** and PAMAM G2 dendrimers at different N/P ratios, namely 5, 15 and 30 each. The first lane corresponds to the DNA ladder, while in the last lane free herring sperm DNA or pGFP plasmid were loaded as negative controls.

PAMAM G4-αtfm-βAla-Arg **4** dendrimer at N/P ratio equal to 5 do not complex both model DNA and pGFP, but at higher N/P ratio a good complexation is observed. This is confirmed by gel retardation assay, in which free DNA runs through the gel with a stained nucleic acid intensity decreasing by increasing polymer concentration. Moreover, at higher N/P ratios formed complexes remain stuck in the loading wells. On the other hand, the low generation PAMAM G2 can form complexes with both model DNA and plasmid at all tested N/P ratios, as demonstrated by no detectable signals of nucleic acids bands through the gel but stuck in the loading wells.

Free pGFP plasmid shows a main band at 2.7 kb and two more secondary bands at higher weights (6 kb and >10 kb, respectively). These bands do not correspond to real nucleic acid dimension as it has not been linearized nor fragmented; these bands correspond to the circular plasmid isoforms assumed during gel migration.

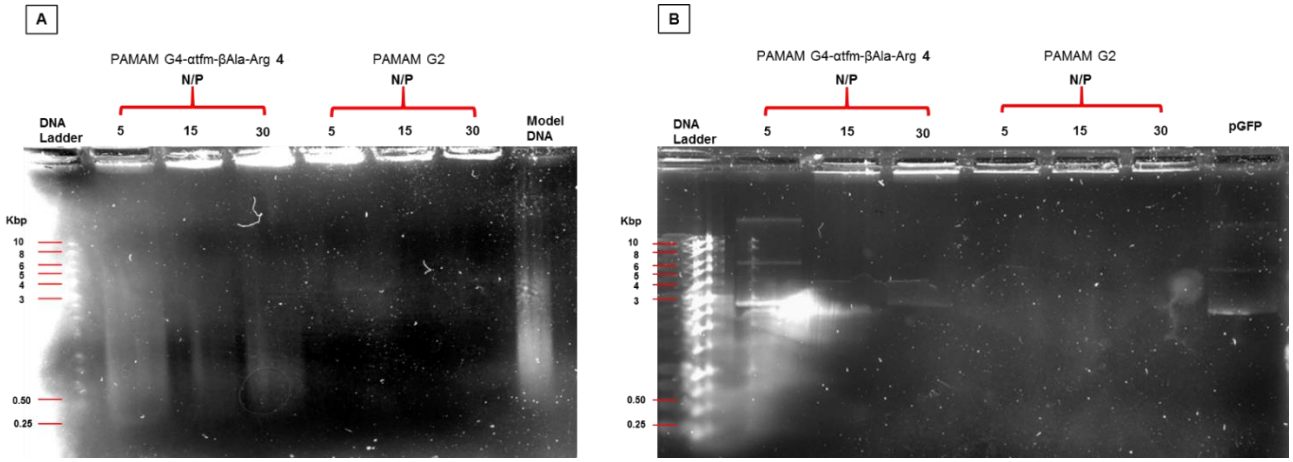

Figure S2. Particle size distribution by intensity (black line), volume (red line) and number (blue line) for the four dendriplexes synthesized: a) PAMAM G2-hfVal-Arg **1**, b) PAMAM G4-hfVal-Arg **2**, c) PAMAM G2-αtfm-βAla-Arg **3**, d) PAMAM G4-αtfm-βAla-Arg **4**

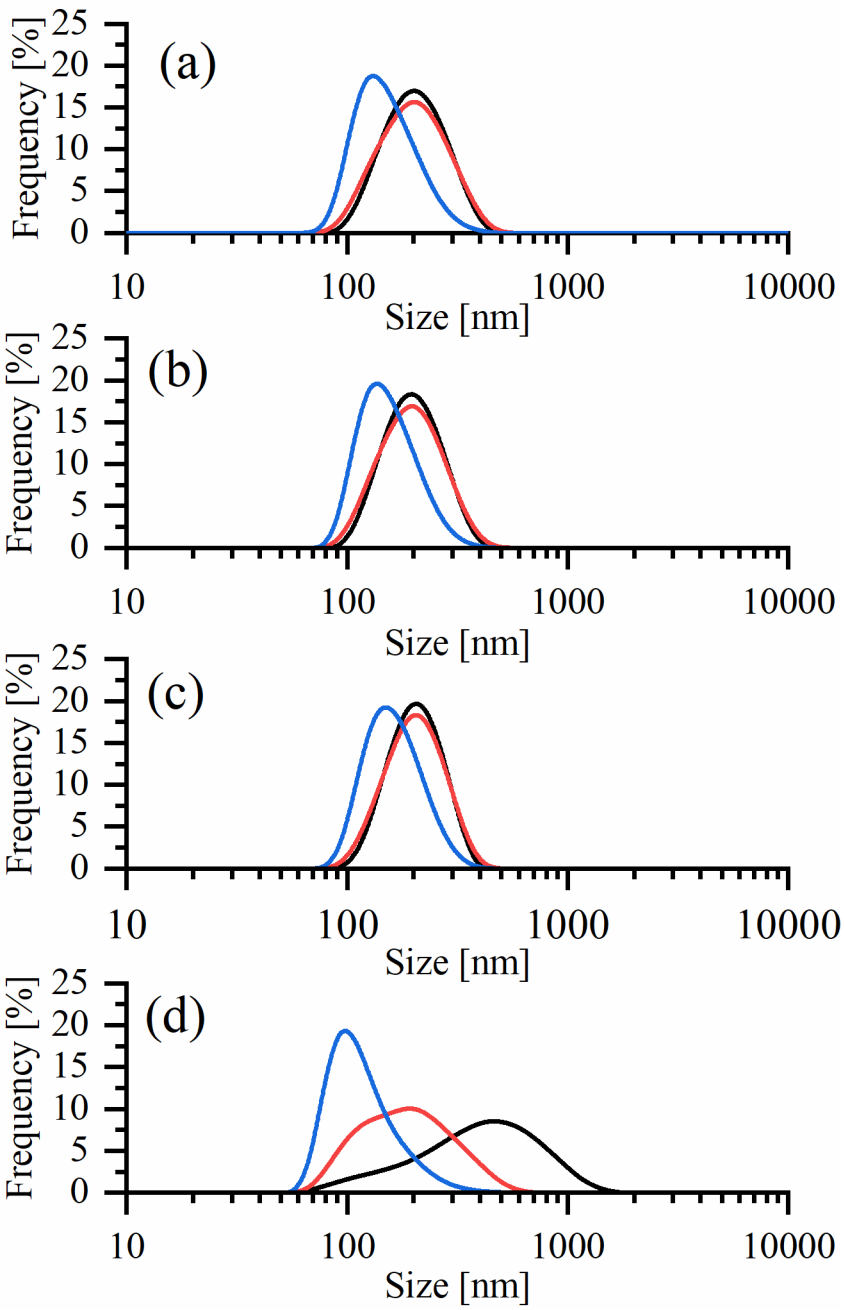

Figure S3. In the graphs below, the Relative Transfection Efficiencies of each tested system at N/P ratio equal to 15 are reported. Single TEs have been normalized by the TE of the positive control bPEI 25kDa. Relative TEs of undecorated PAMAM G2 and G4 dendrimers are reported, underlying a very low transfection efficiency of these systems, although some capability of forming complexes with DNA, as demonstrated by gel retardation assays. On the other hand, the TEs of our proposed systems, resulting in a very depicting the remarkable transfection efficiency of PAMAM G2-hfVal-Arg 1 and PAMAM G4-hfVal-Arg 2 dendrimers, with six-fold and four-fold higher TEs respectively as compared to both the positive control bPEI 25kDa (fixed to 1), and the undecorated PAMAM G2 and PAMAM G4 dendrimers.