Supporting Information

Use of Enzymatically Activated Carbon Monoxide Donors for Sensitizing Drug-Resistant Tumor Cells

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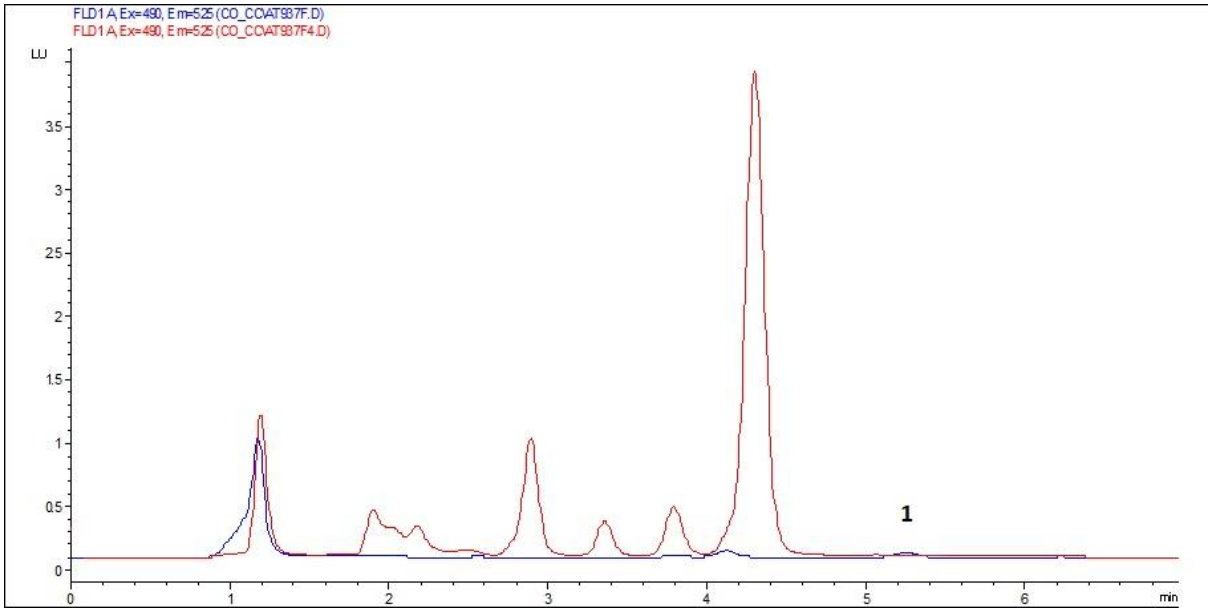

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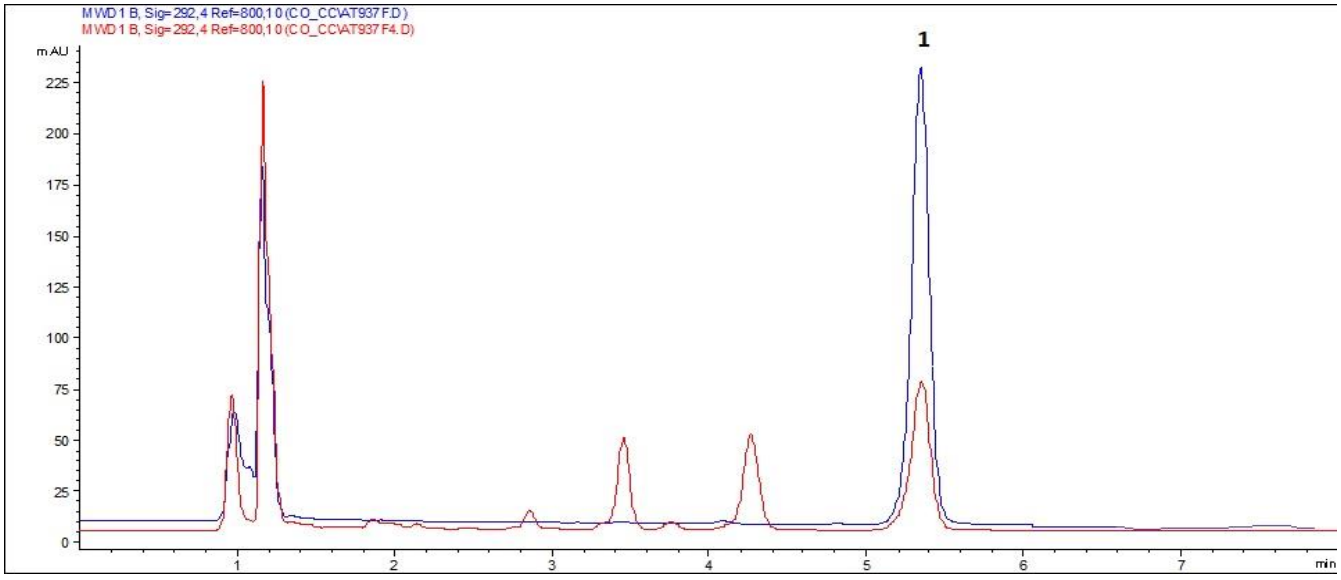


Figure S1. Degradation of compound **1** in human serum: chromatogram of **1** at time 0 (blue track) and after 180 min (red track) of incubation at 37°C in human serum;
(**A**) Fluorescence detection (exc. 490, emiss. 525 nm); (**B**) UV detection at 292 nm (ref 800 nm).

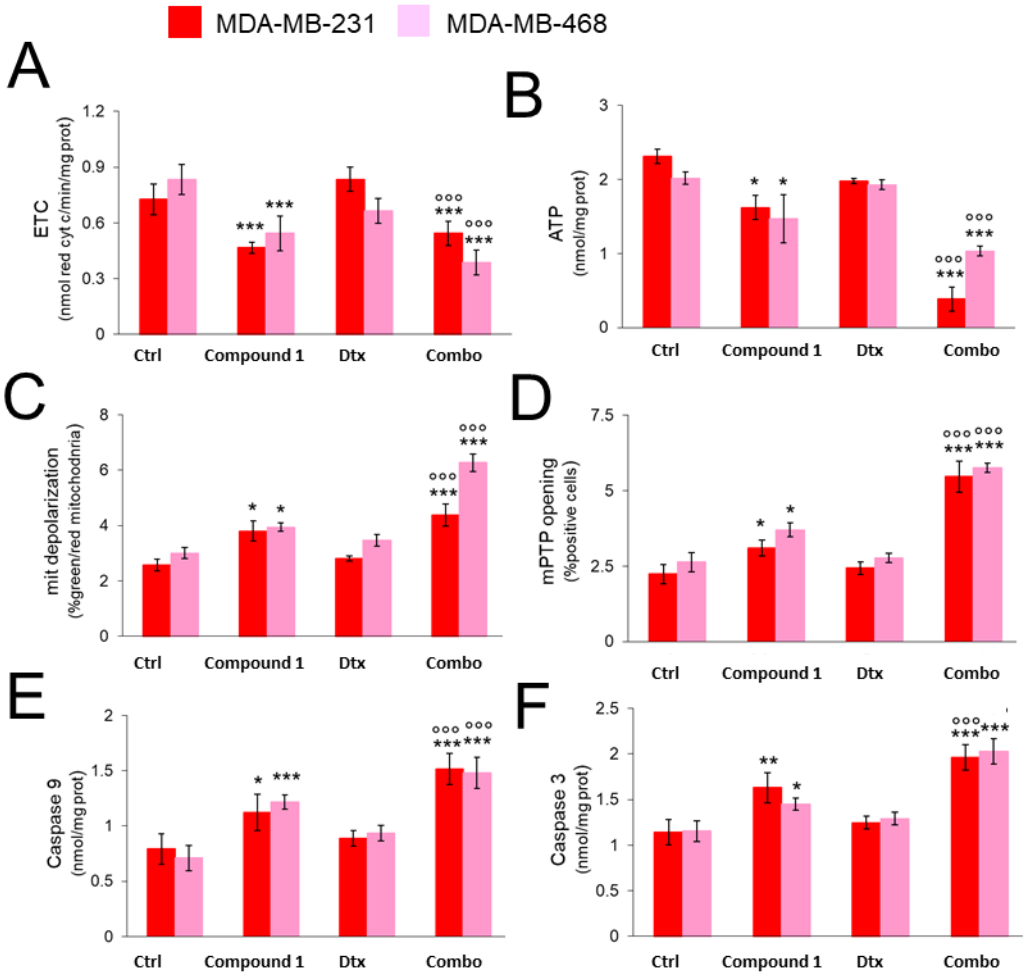


Figure S2. Mitochondrial parameters and caspases activation in triple negative breast cancer cells treated with compound 1 and docetaxel. Human triple negative breast cancer MDA-MB-231 and MDA-MB-468 cells were treated 24 h with 1 µM compound 1+1 µM docetaxel (Dtx), alone or in association (Combo). Ctrl: cells grown in fresh medium. (**A**) Electron transport chain measured spectrophotometrically. (**B**) Mitochondria ATP measured by chemiluminescence-based assay. (**C-D**) Percentage of mitochondria with depolarized membrane, as index of mitochondrial damage, assessed fluorometrically (**C**), and characterized by open mitochondrial permeability transition pore (mPTP), measured by flow cytometry (**D**). (**E-F**) Activity of caspase-9 (**E**) and caspase-3 (**F**), measured fluorometrically. All assays were performed in triplicate (n=3 biological replicates). Data are means ± SD. **p*<0.05, **p<0.01, ****p*<0.001: compound 1+chemotherapeutic drug versus untreated cells; °°°*p*<0.001: compound 1+chemotherapeutic drug versus chemotherapeutic drug alone.

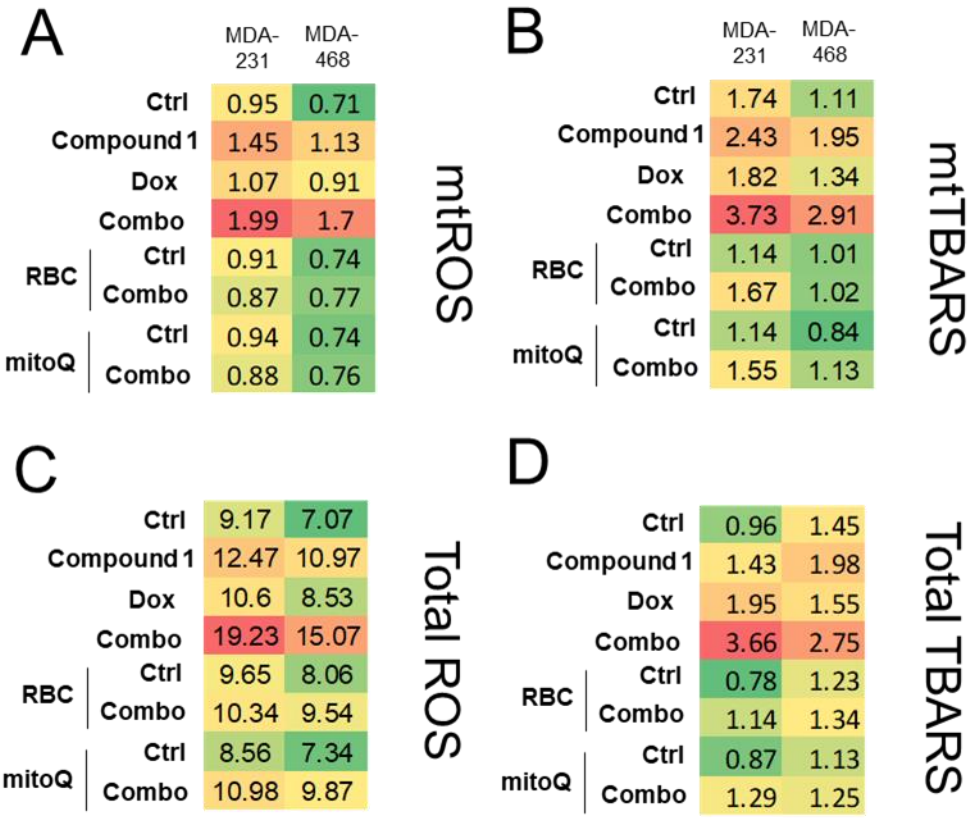
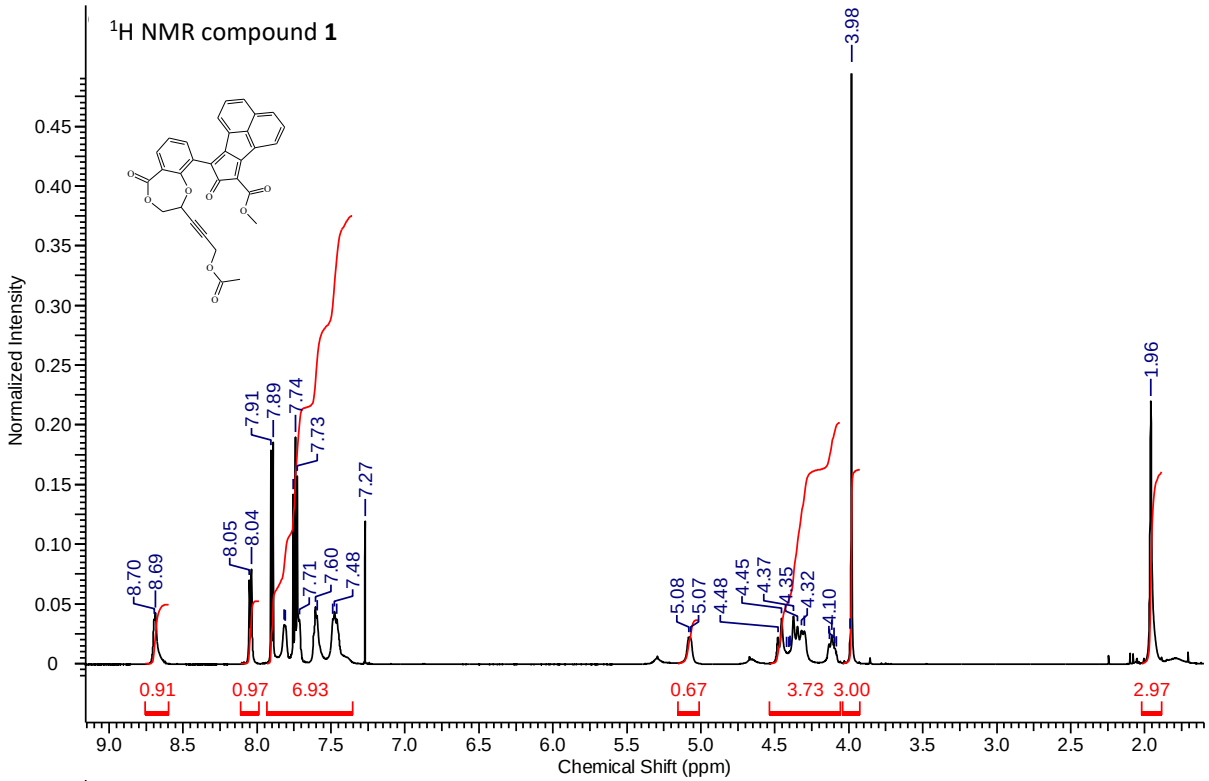


Figure S3. Mitochondrial and total ROS and lipoperoxidation in triple negative cancer cell lines treated with compound 1 and docetaxel. Human triple negative breast cancer MDA-MB-231 and MDA-MB-468 cells were treated 24 h with 1 µM compound 1+1 µM docetaxel (Dtx), alone or in association (Combo). Ctrl: cells grown in fresh medium. When indicated, 10 µg/mL packed red blood cells (RBC), as a CO scavenger, or 0.4 µM mitoquinol (mitoQ), as a mitochodrial ROS scavenger, were co-incubated. (**A-B**) ROS, as an index of oxidative stress, measured fluorometrically and TBARS, as an index of lipoperoxidation, determined spectrophotometrically in extracted mitochondria. (**C-D**) ROS and TBARS measured in whole cell lysate. All assays were performed in triplicate (n=3 biological replicates). Data are represented as heatmap (numbers correspond to means). For all panels: $^{**}p<0.01$: compound 1 versus untreated cells, $^{***}p<0.001$: compound 1+chemotherapeutic drug versus untreated cells; $^{°°°}p<0.001$: compound 1+chemotherapeutic drug versus chemotherapeutic drug alone; $^{§§§}p<0.001$: compound 1+chemotherapeutic drug versus compound 1 alone; $^{###}p<0.001$: RBC+Combo/mitoQ+Combo versus Combo alone.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8.04
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7.71
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7.48
7.27
5.08
5.07
4.48
4.45
4.37
4.35
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4.10
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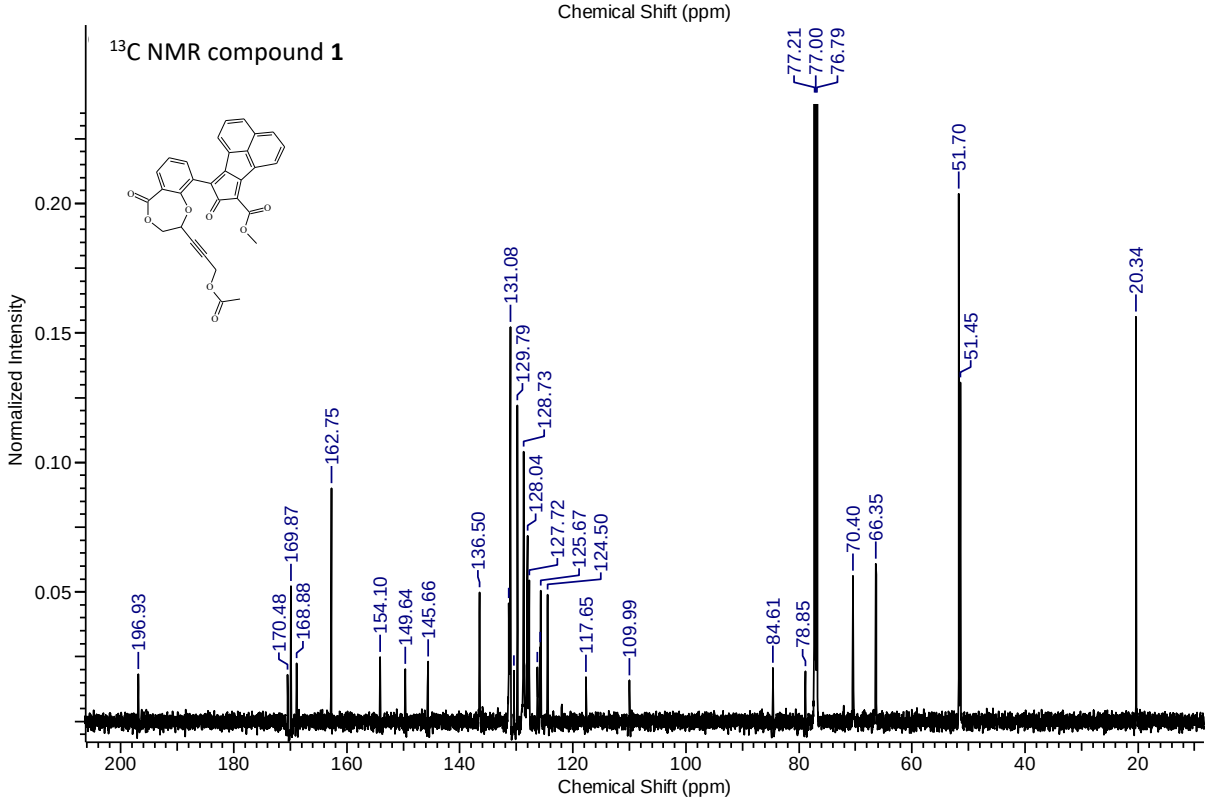
0.97
6.93
0.67
3.73
3.00
2.97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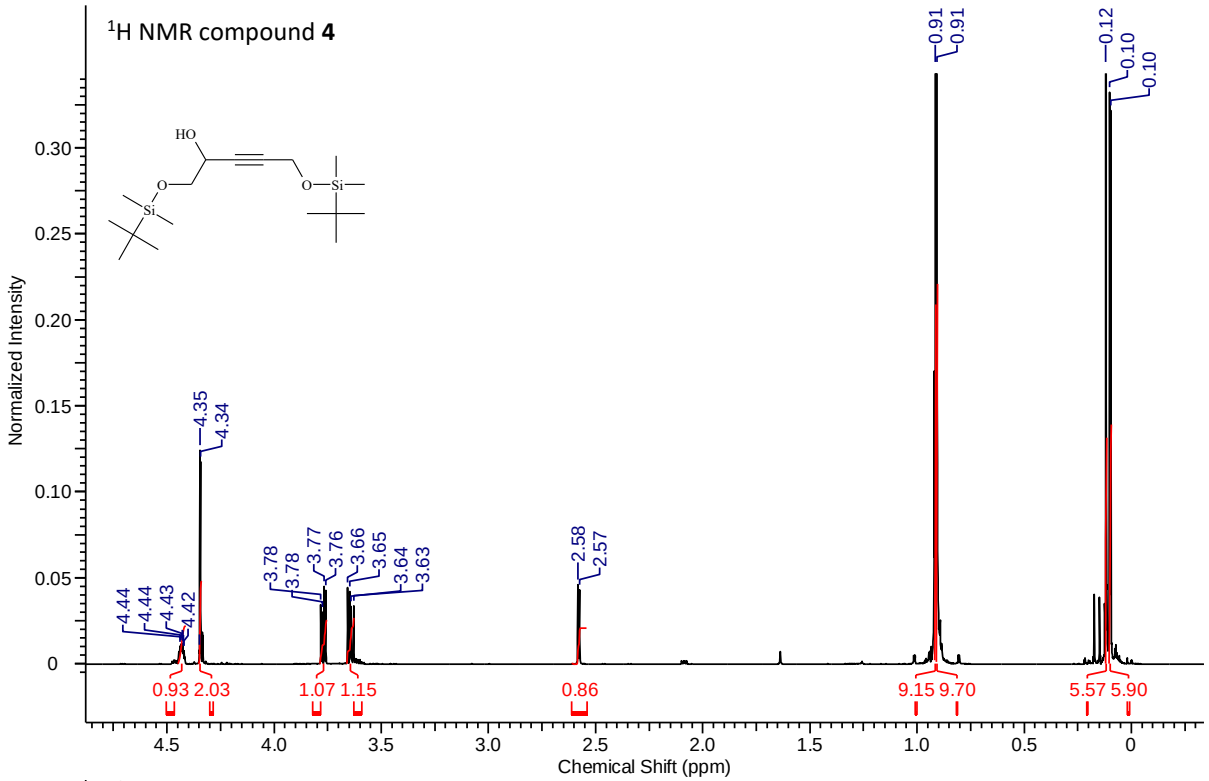
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3.77
3.76
3.66
3.65
3.64
3.63
2.58
2.57
0.91
0.91
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0.10
0.93
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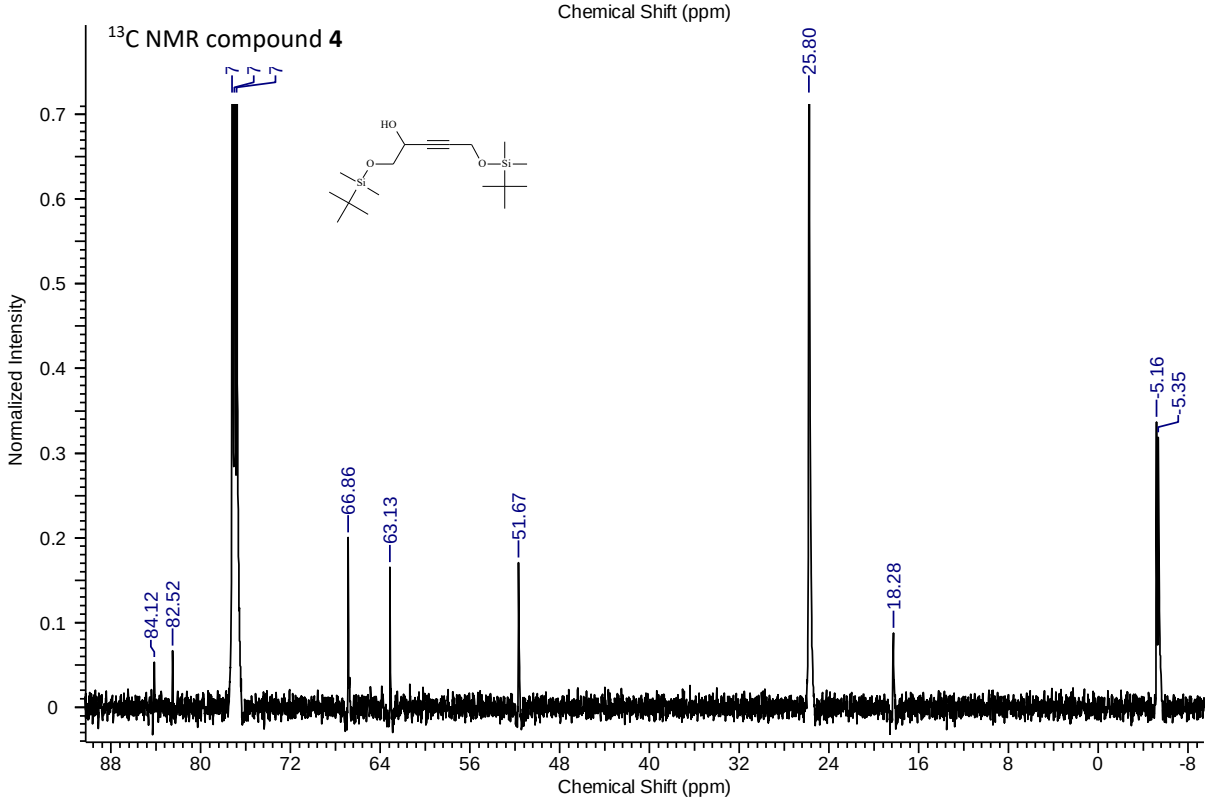
1.07
1.15
0.86
9.15
9.70
5.57
5.90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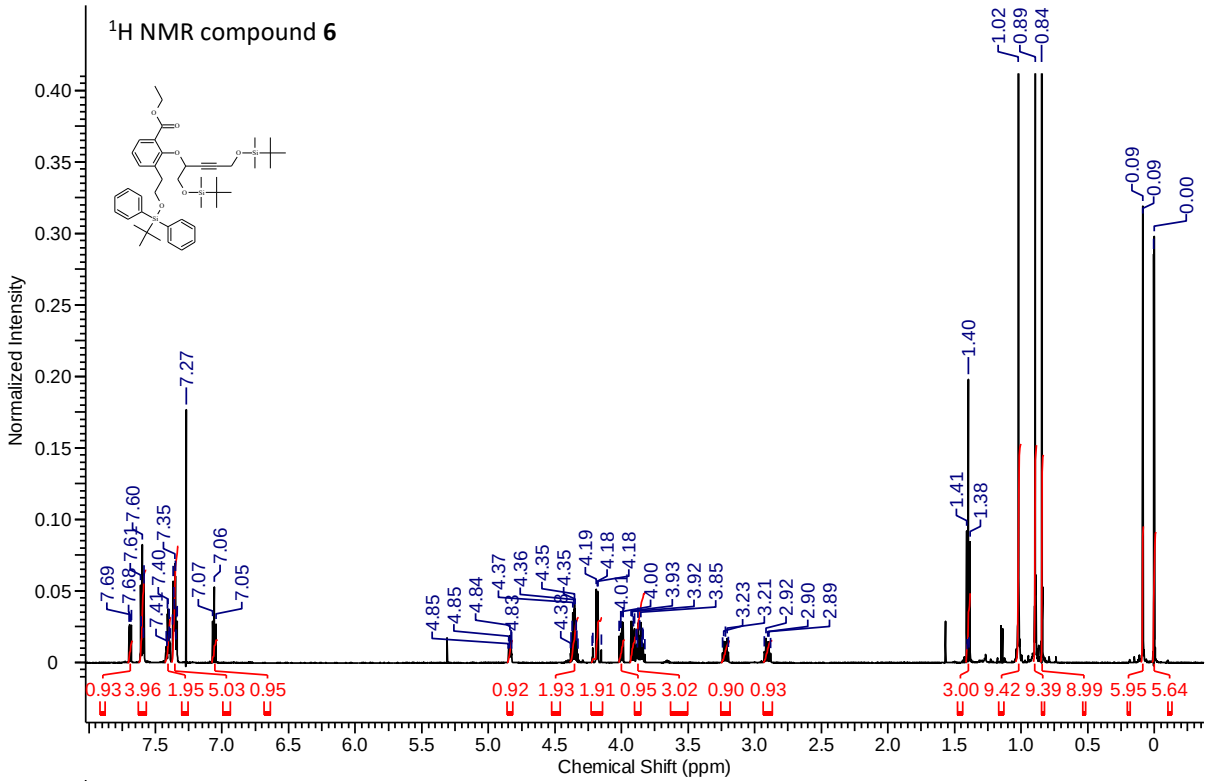
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4.35
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4.18
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4.01
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3.92
3.85
3.23
3.21
2.92
2.90
2.89
1.41
1.40
1.38
1.02
0.89
0.84
0.09
0.09
0.00
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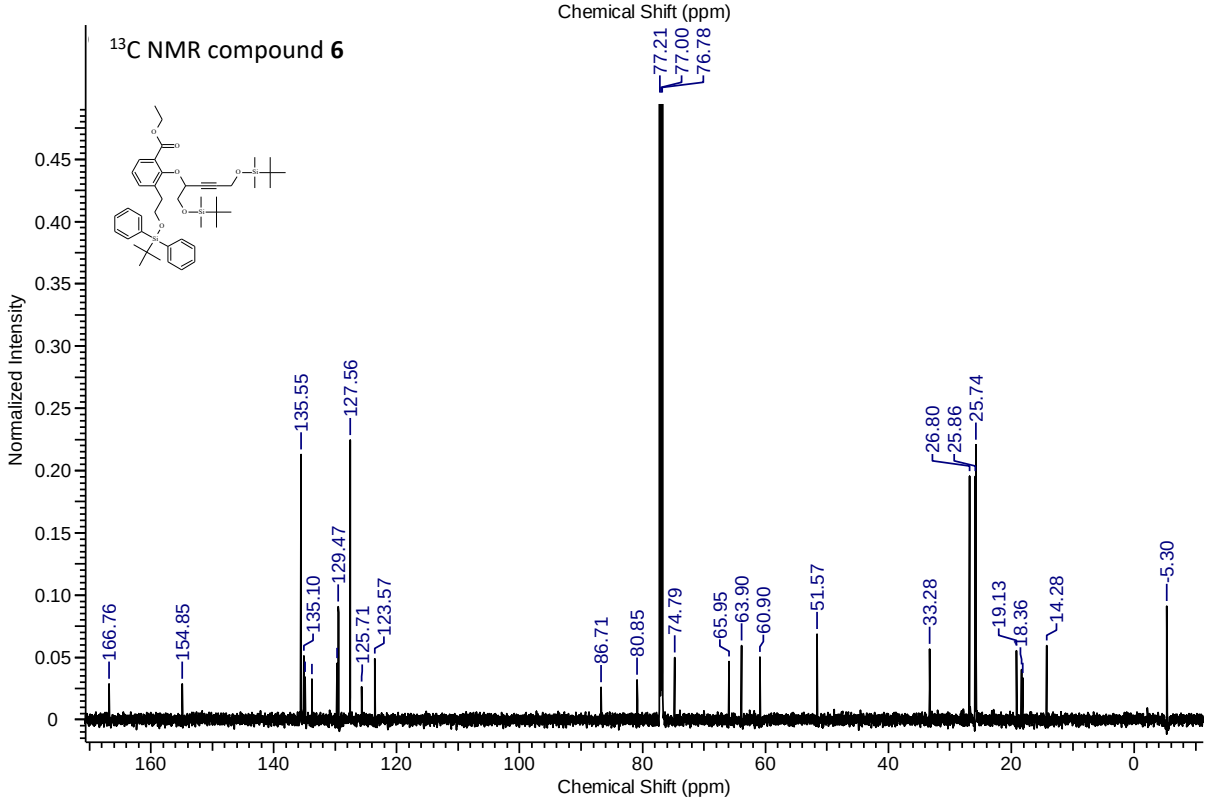
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Chemical Shift (ppm)
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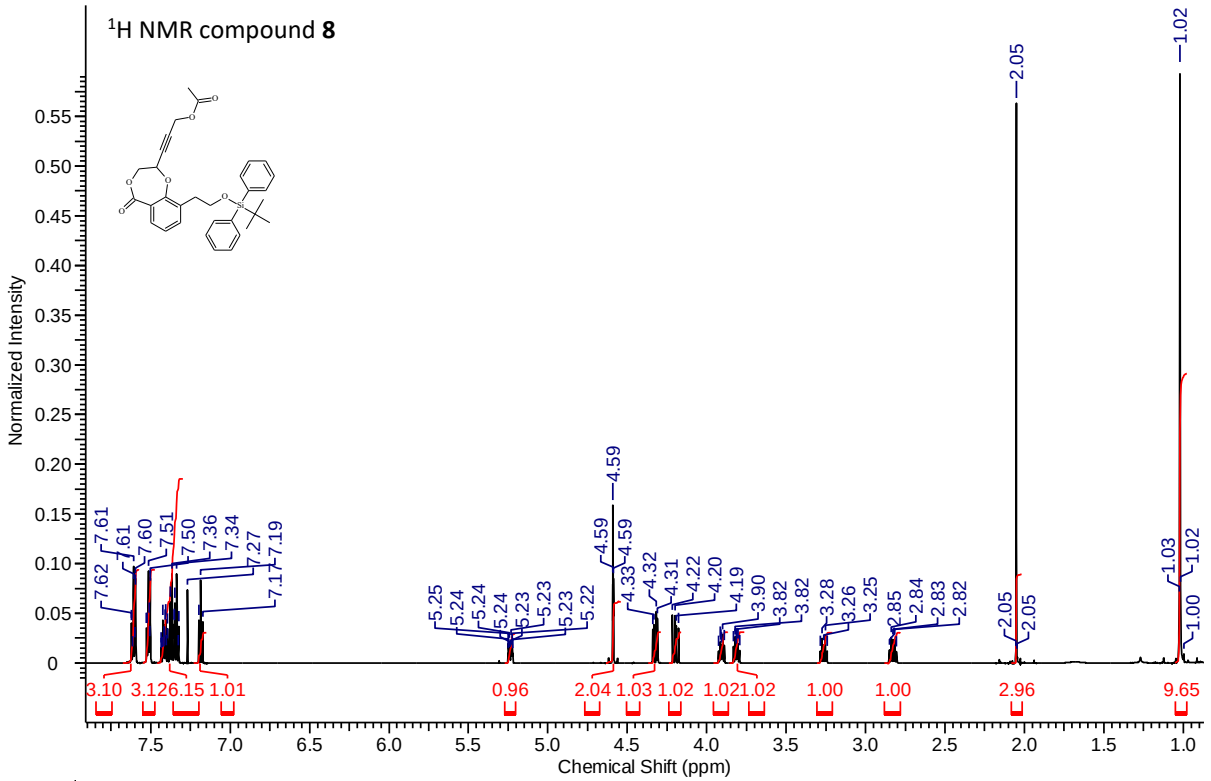
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Chemical Shift (ppm)
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5.25 5.24 5.24 5.24 5.23 5.23 5.23 5.22
4.59 4.59 4.59 4.33 4.32 4.31 4.22 4.20 4.19
3.90 3.82 3.82 3.28 3.26 3.25
2.85 2.84 2.83 2.82
2.05 2.05
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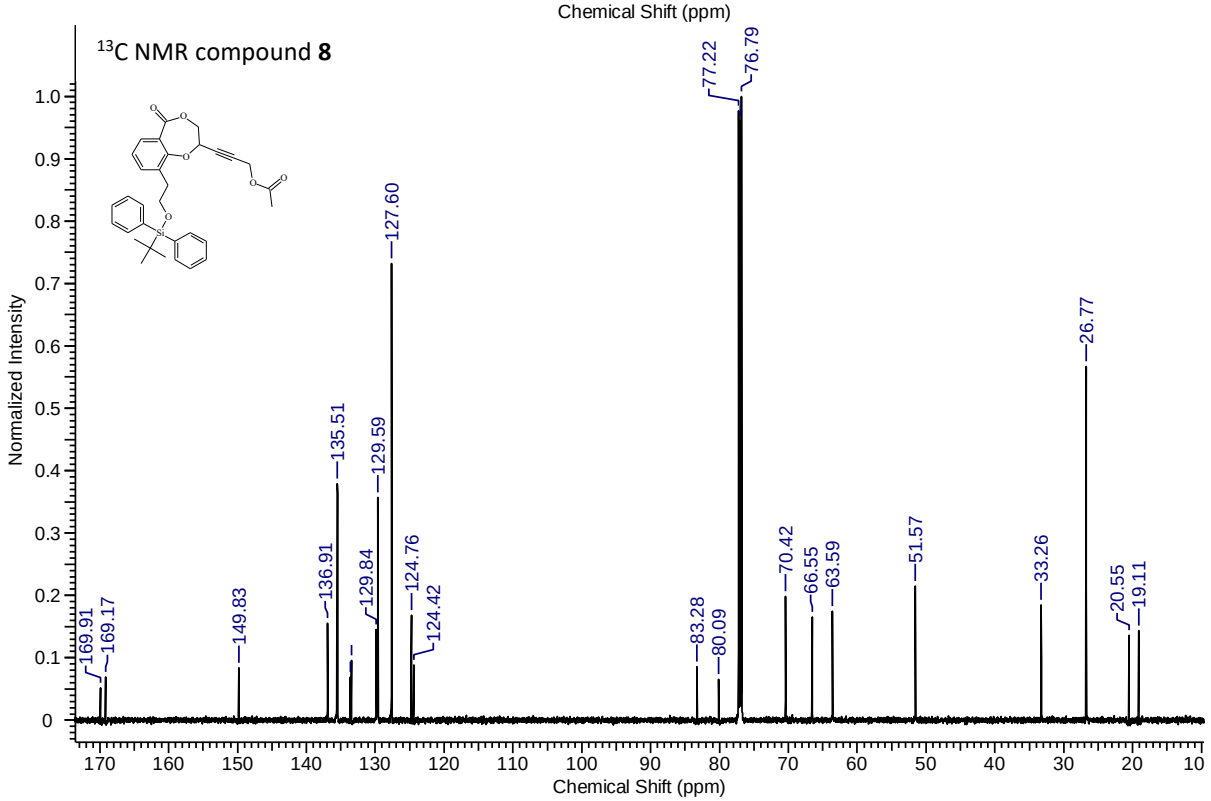
^{13}C NMR compound **8**
Normalized Intensity
Chemical Shift (ppm)
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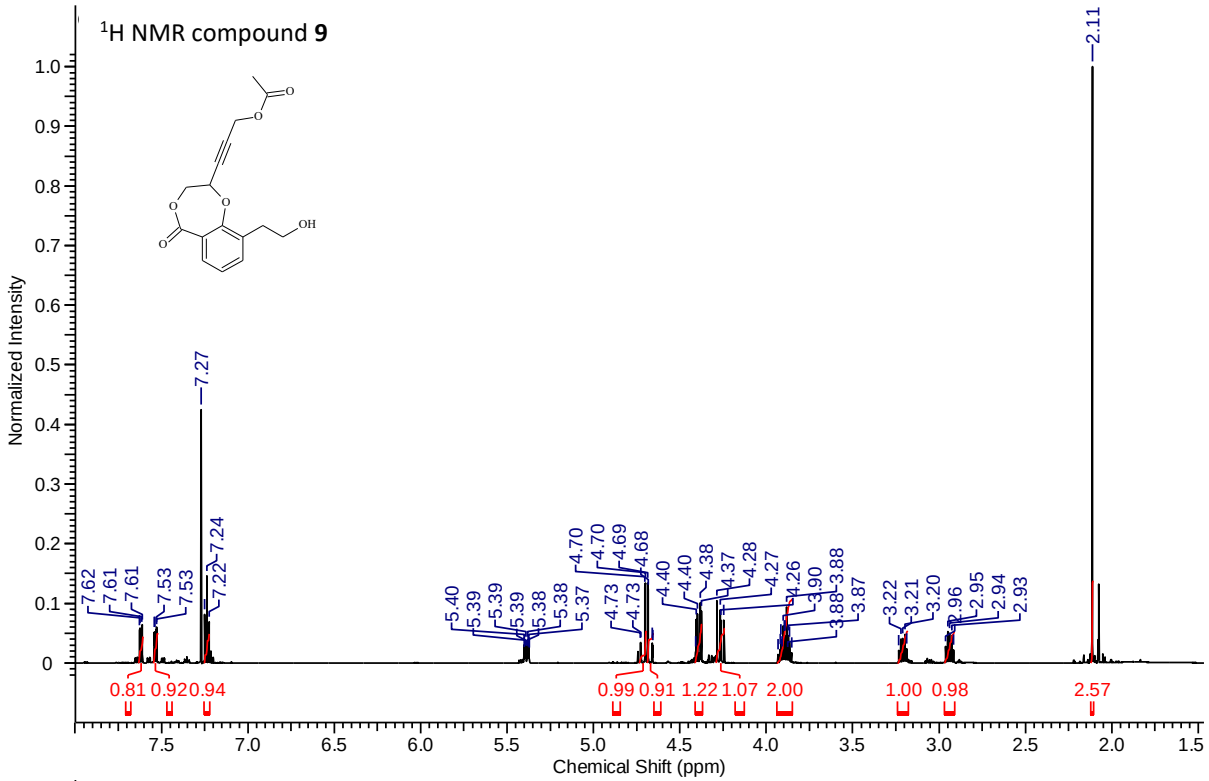
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Chemical Shift (ppm)
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5.37
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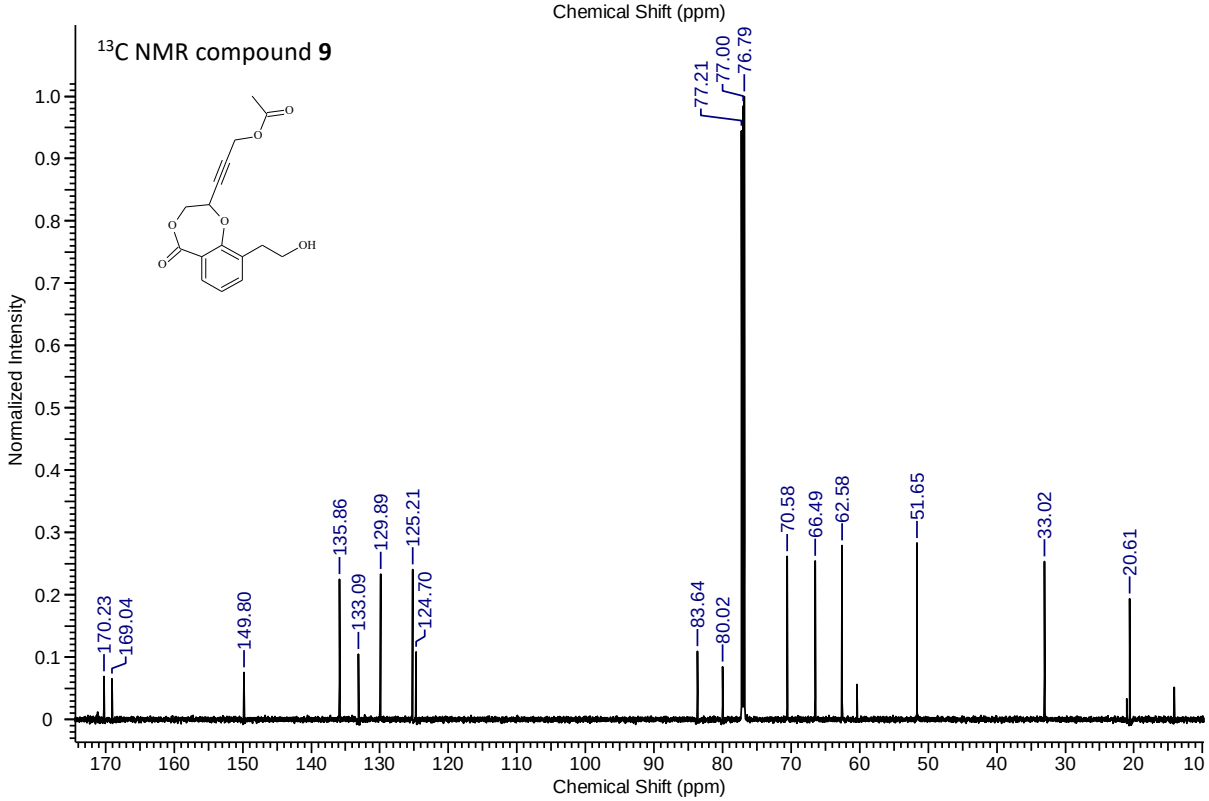
^{13}C NMR compound **9**
Normalized Intensity
Chemical Shift (ppm)
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77.00
76.79
170.23
169.04
149.80
135.86
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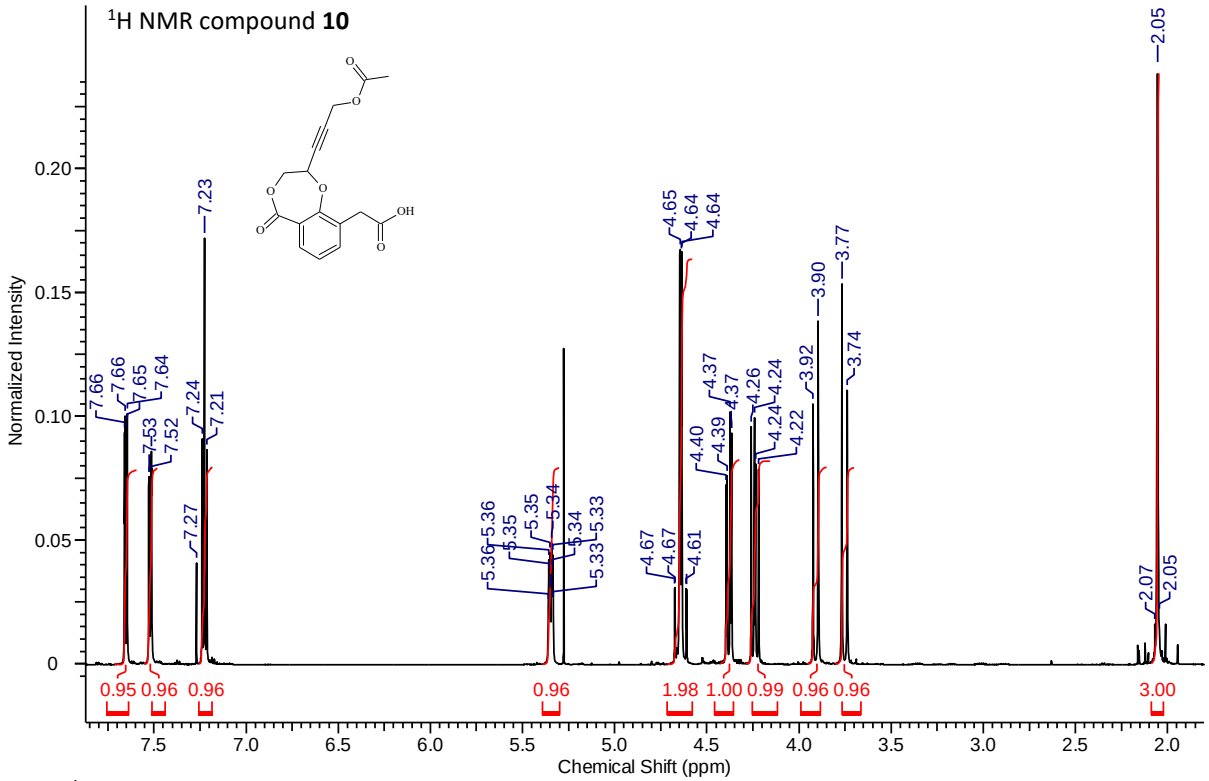
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Chemical Shift (ppm)
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5.36
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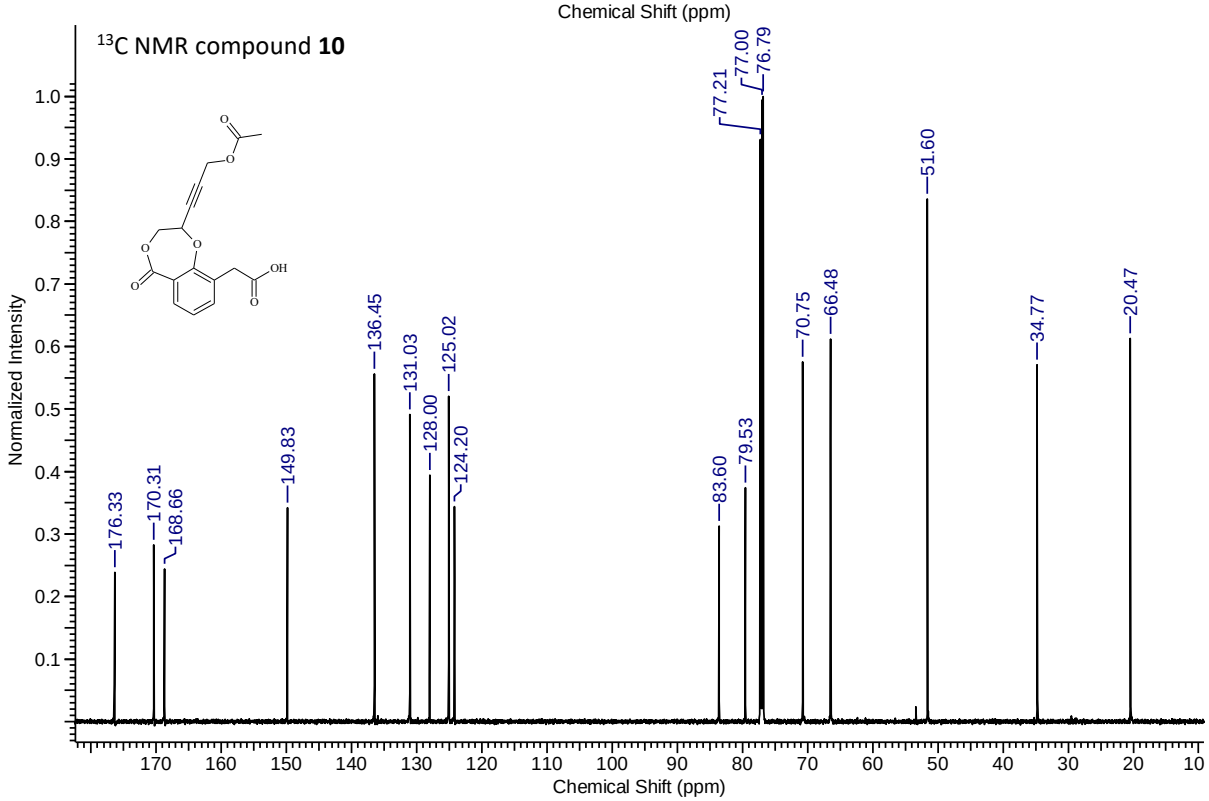
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Normalized Intensity
Chemical Shift (ppm)
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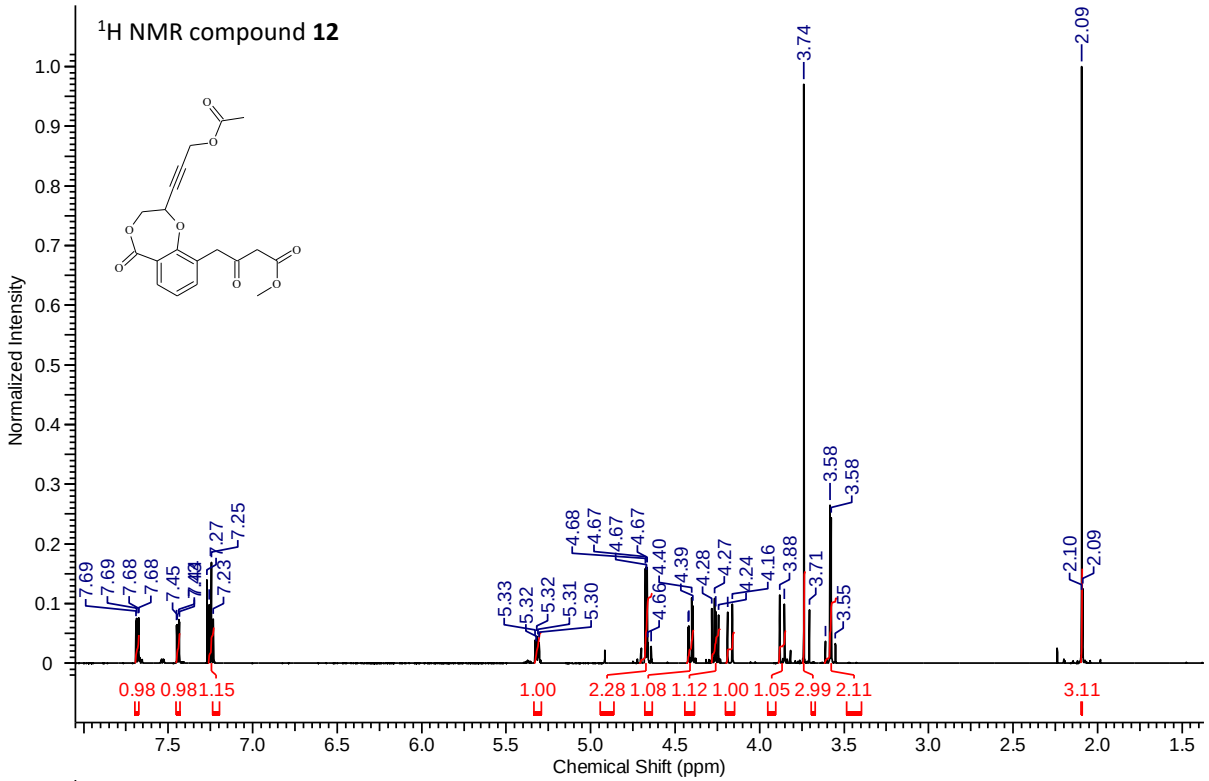
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Chemical Shift (ppm)
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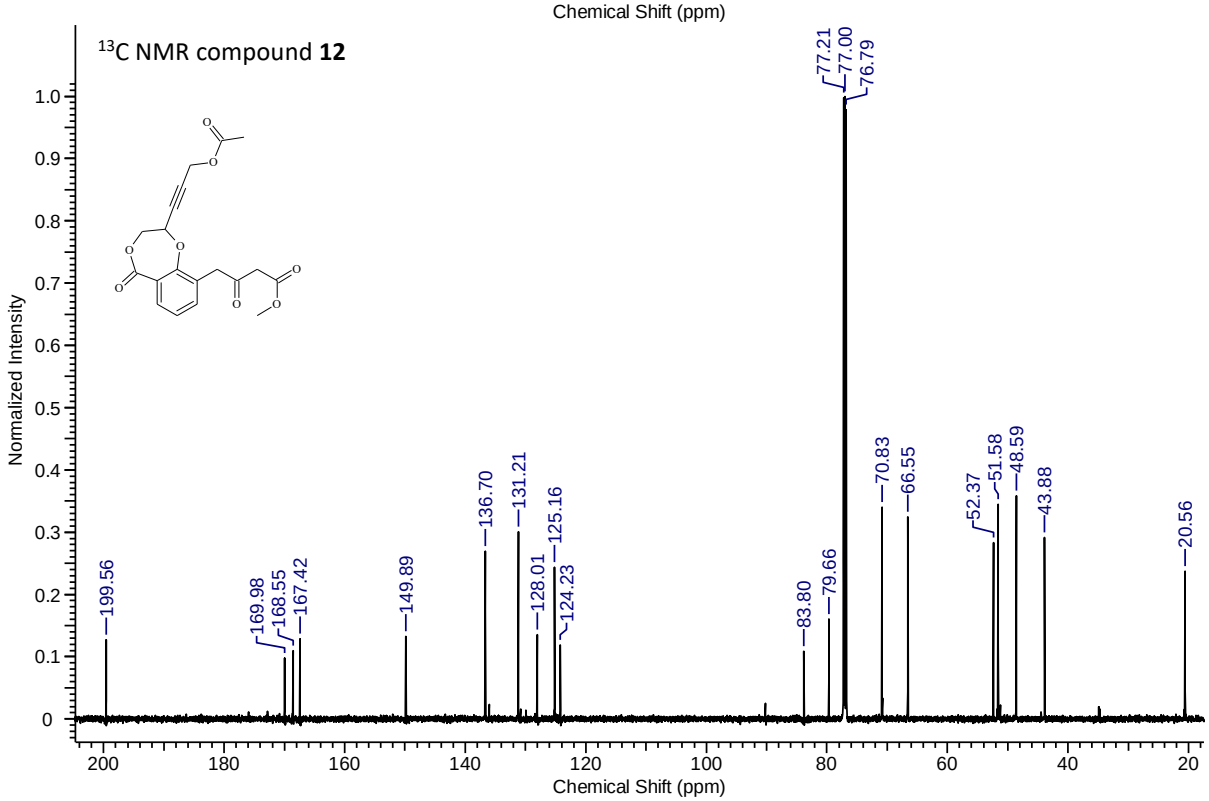
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Chemical Shift (ppm)
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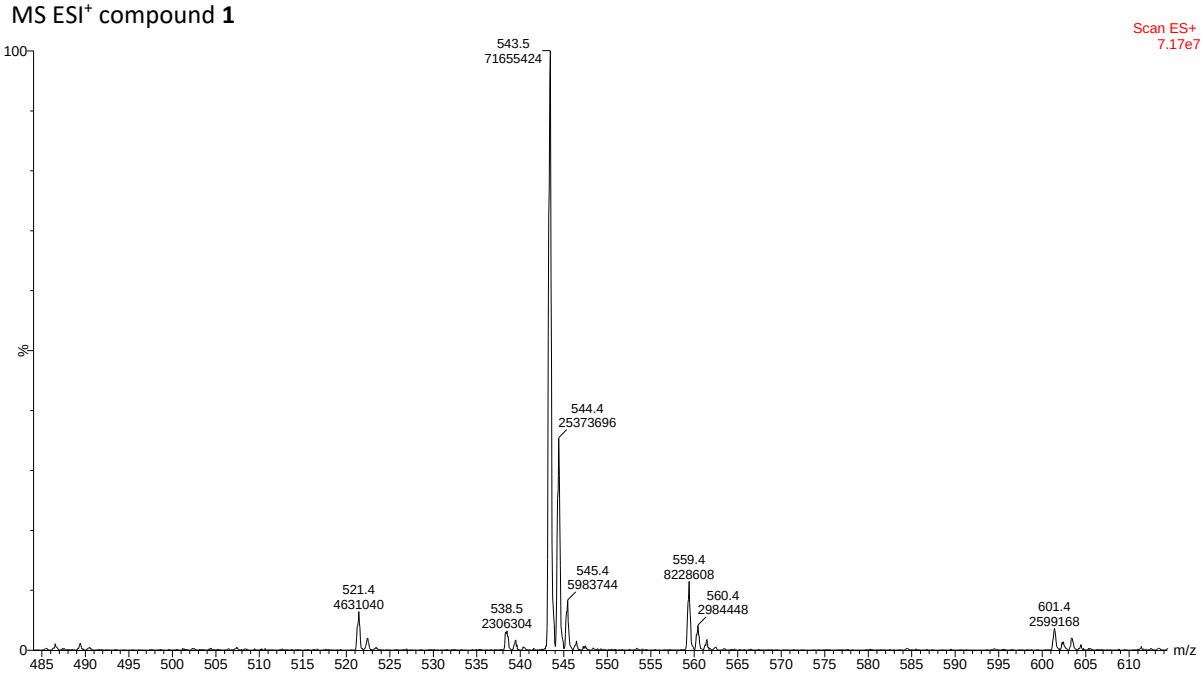

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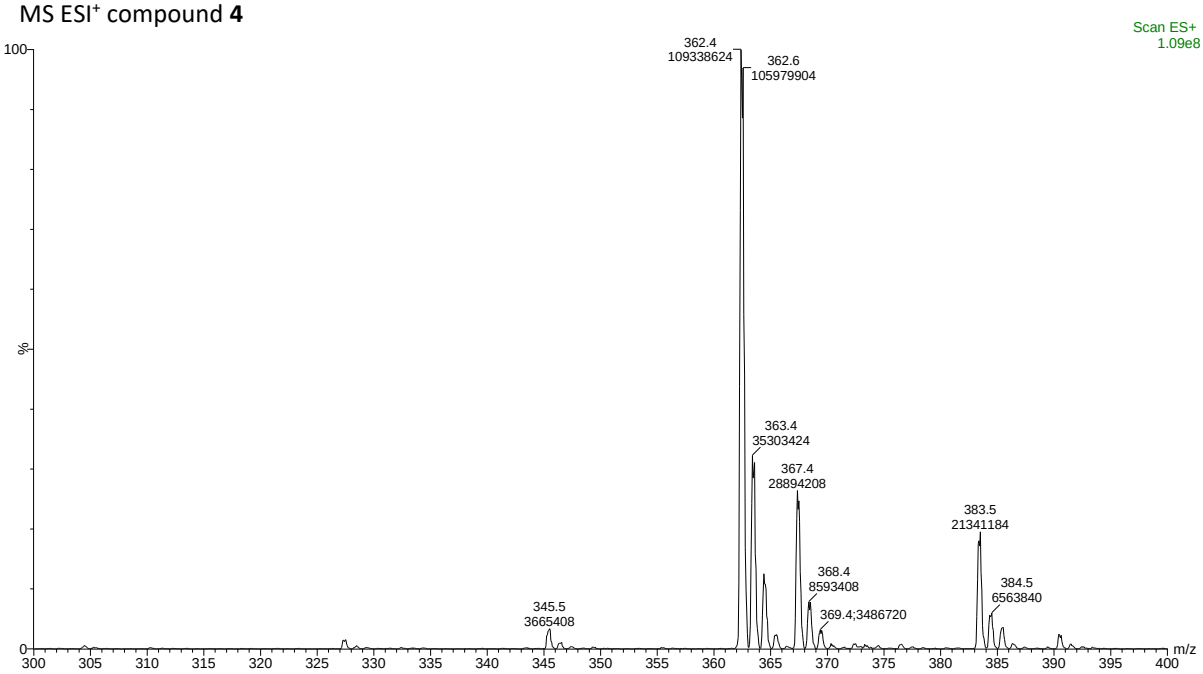

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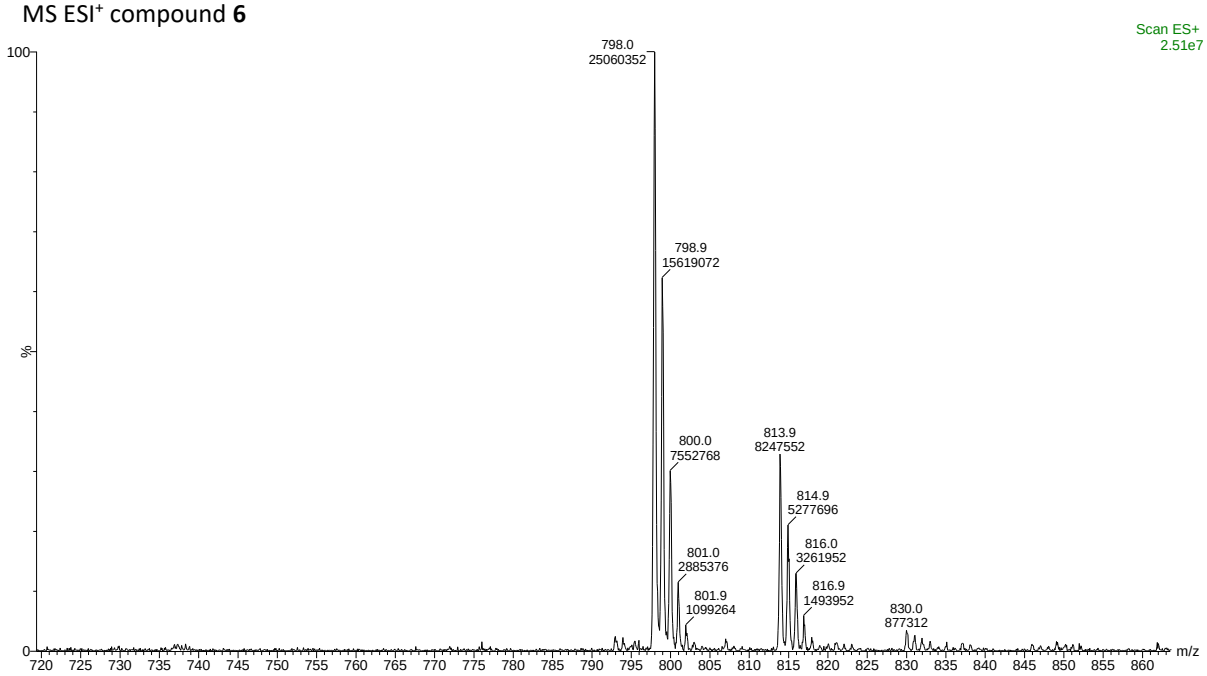

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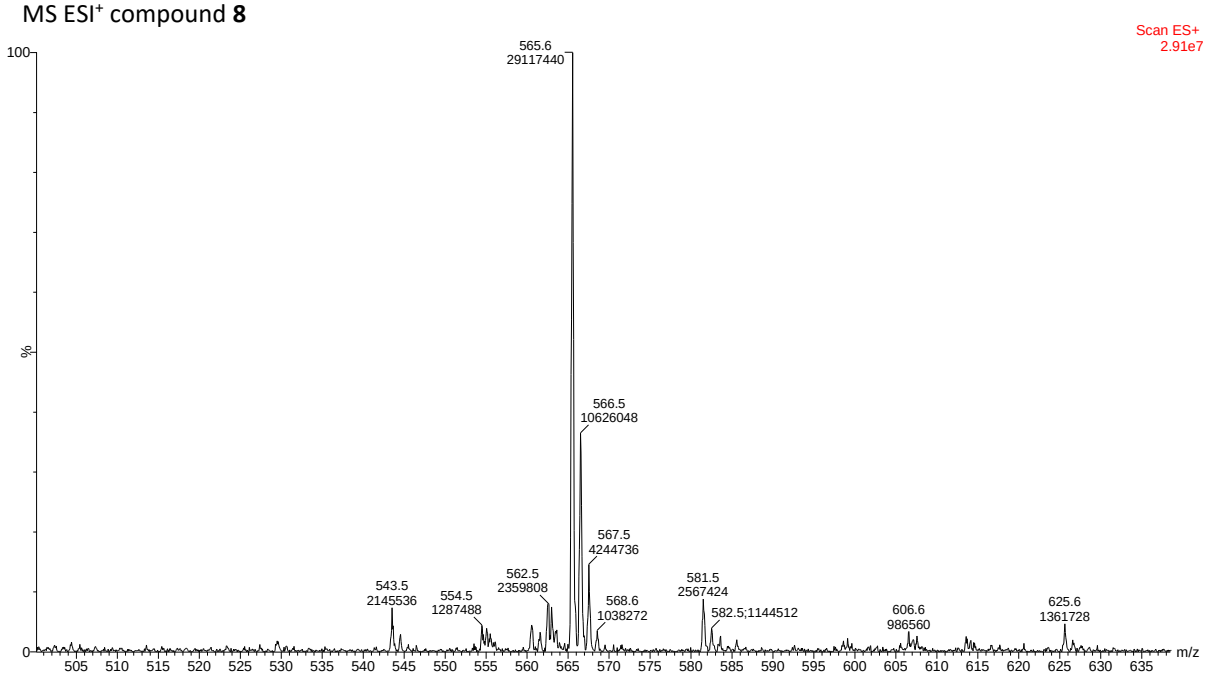

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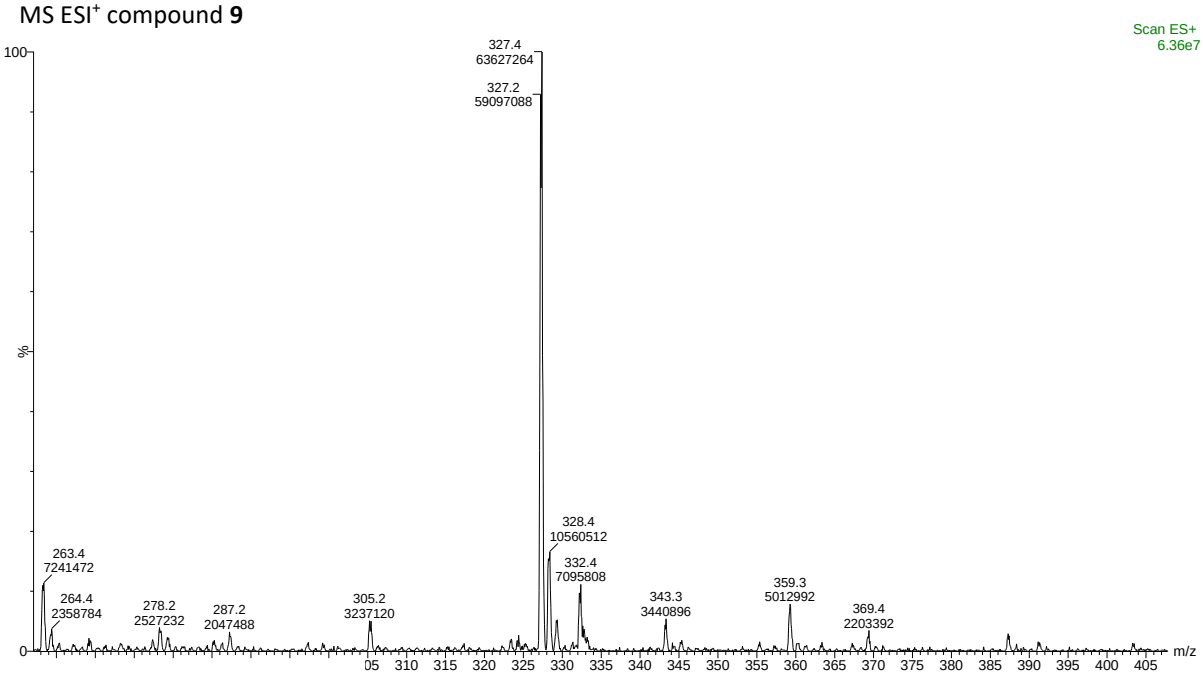

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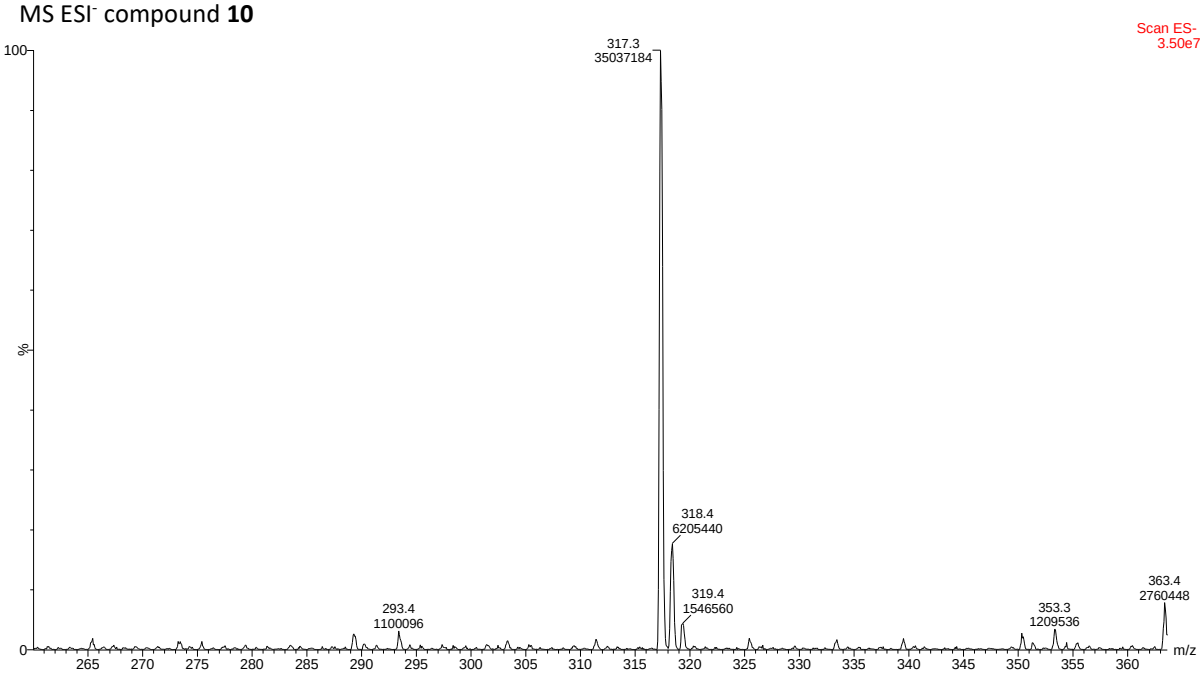

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