Supplementary materials for:

Synthesis of SARS-CoV-2 M^{pro} Inhibitors bearing a Cinnamic Ester Warhead with *In Vitro* Activity against Human Coronaviruses

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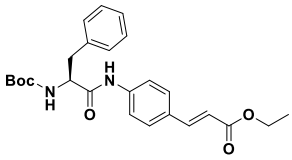
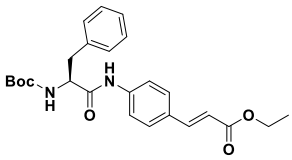
1. ^{1}H- and ^{13}C- Spectra of the Compounds



8a

^{1}H NMR, 400 MHz, $CDCl_3$



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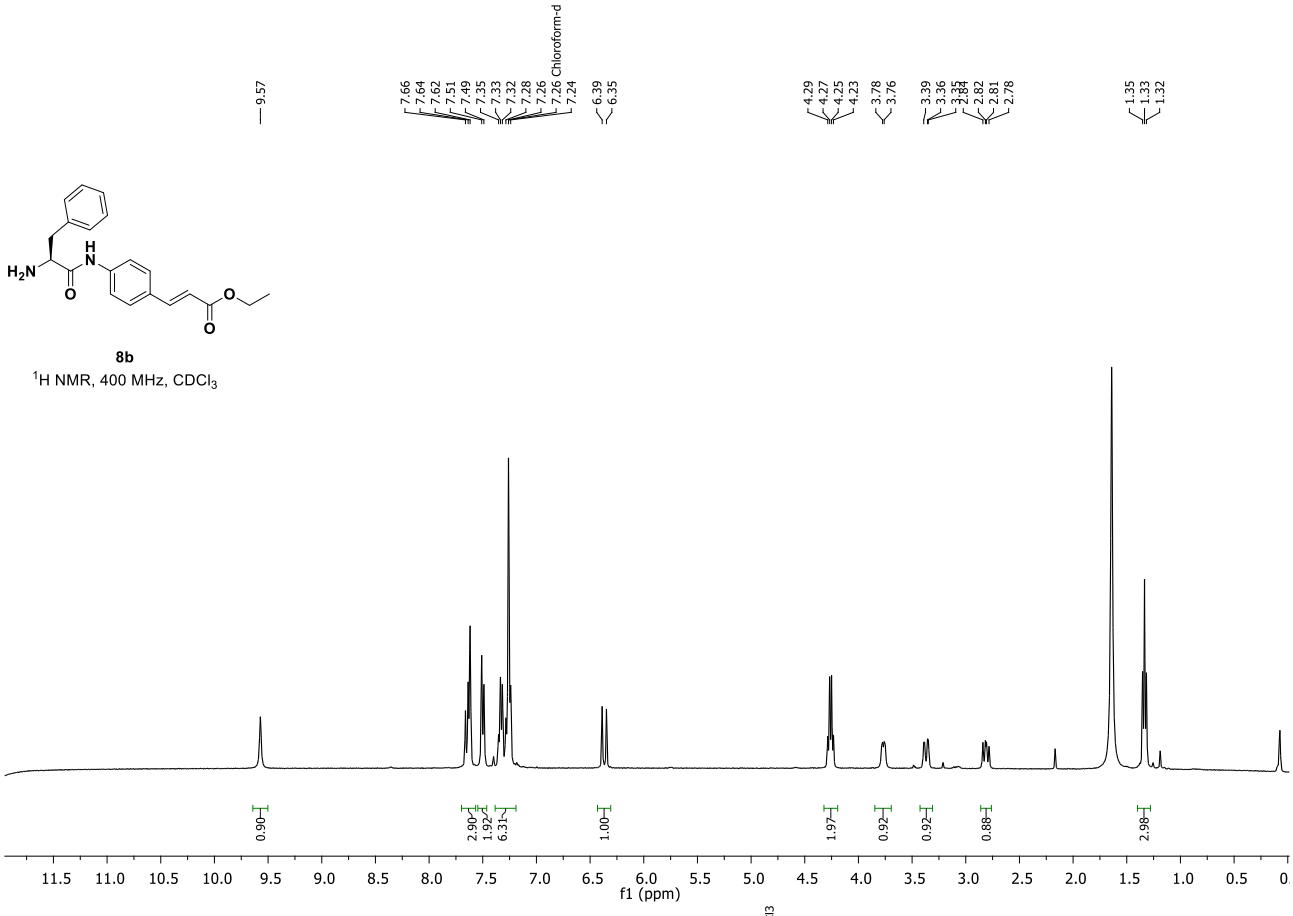

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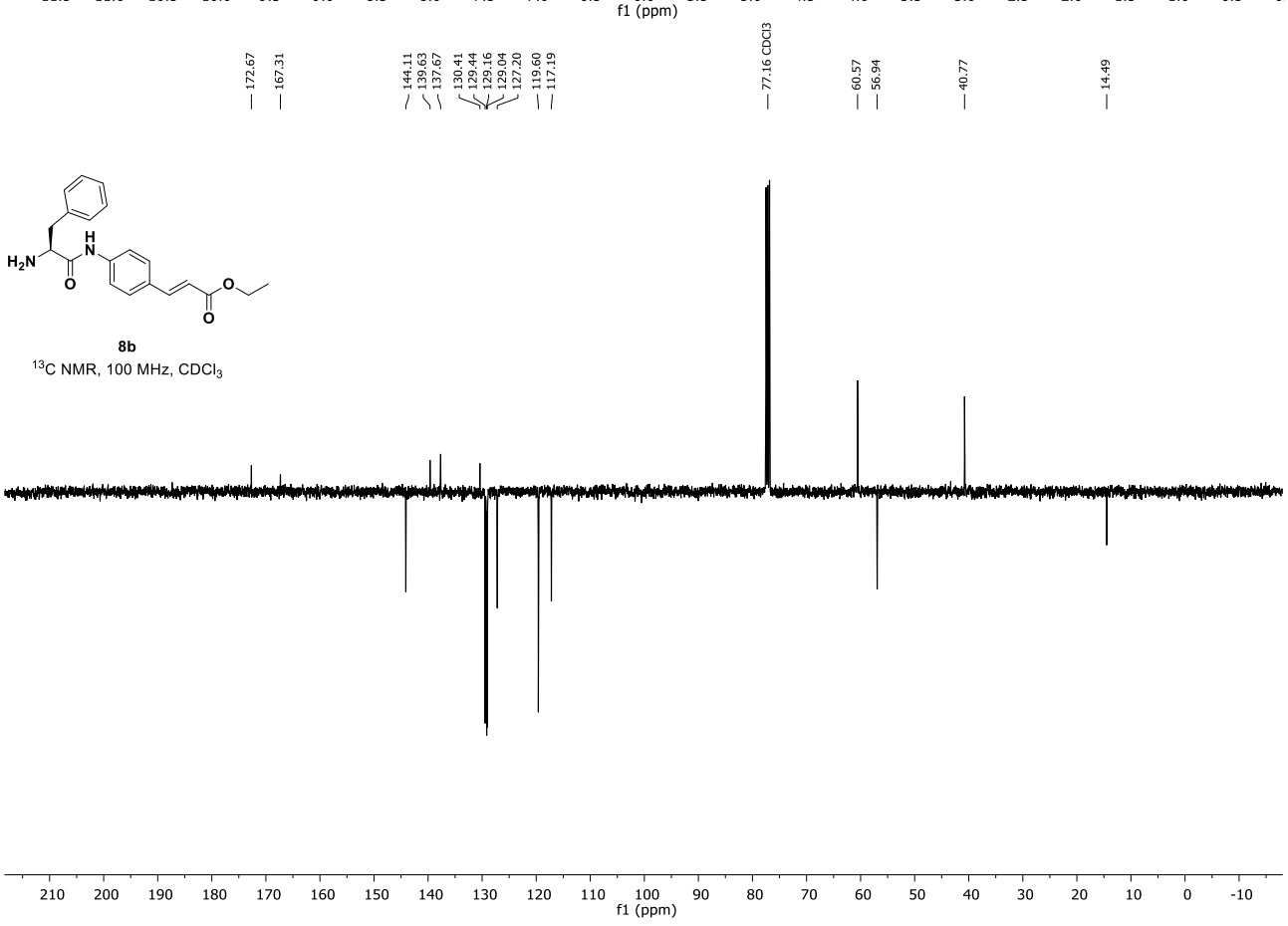

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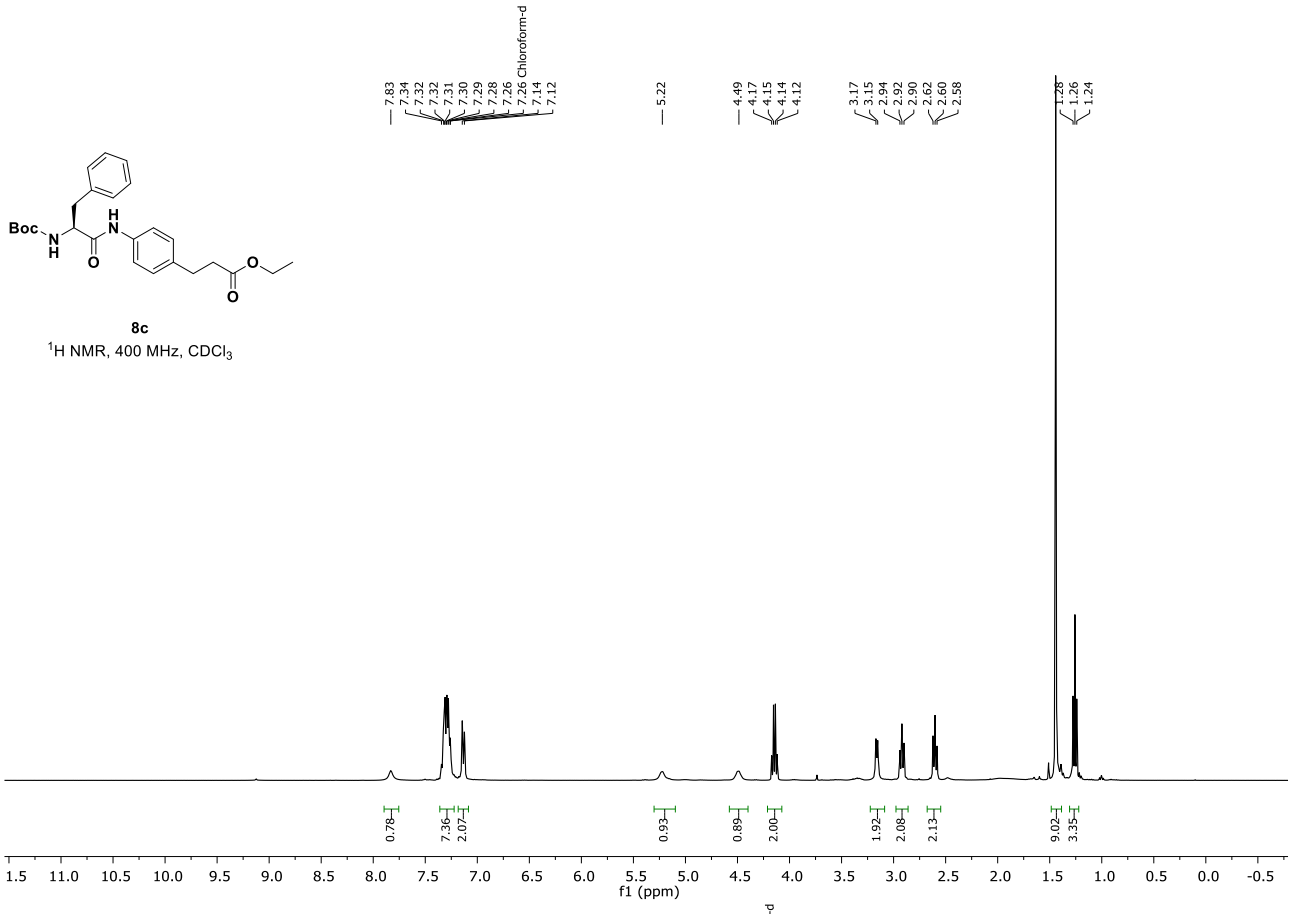

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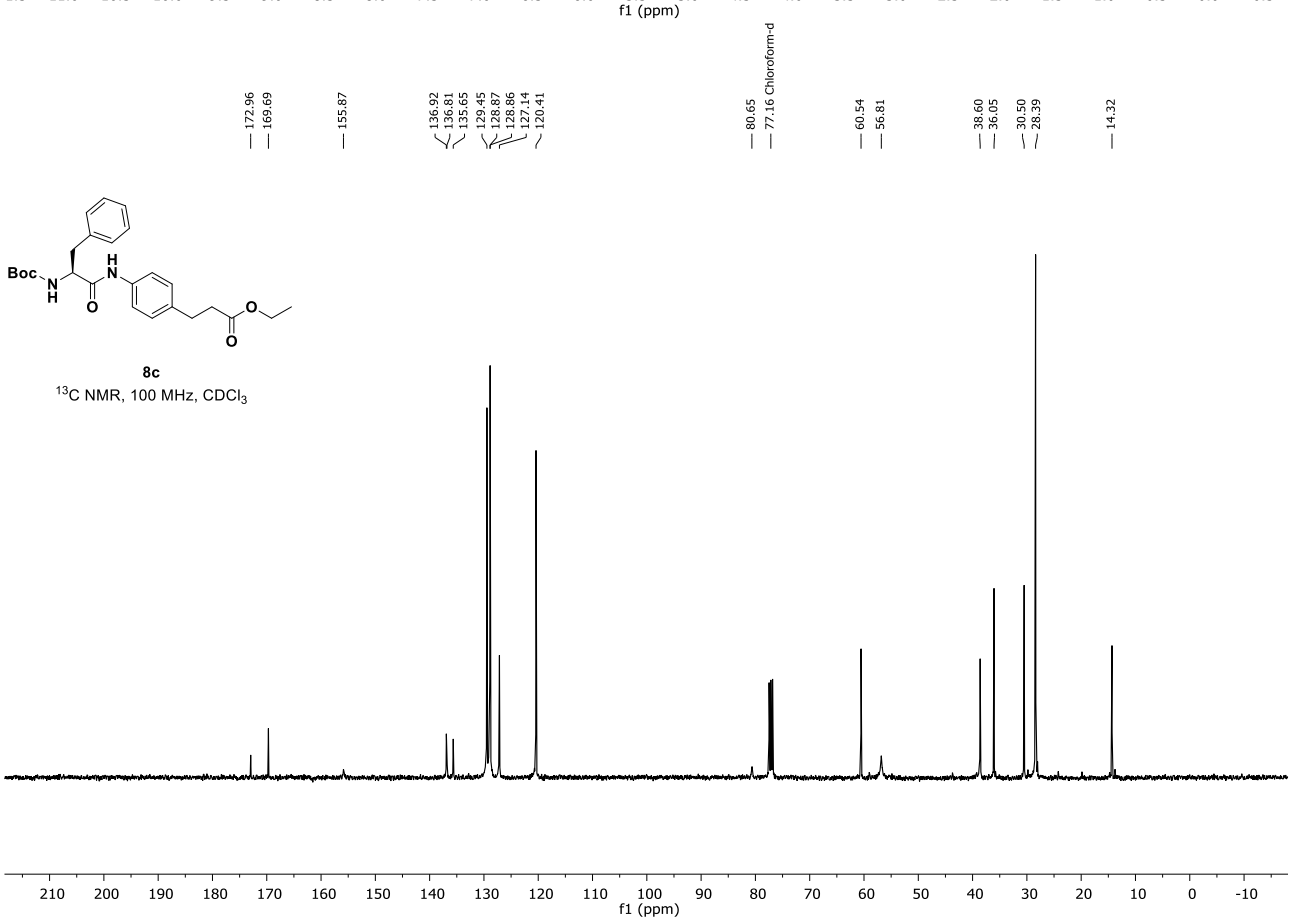

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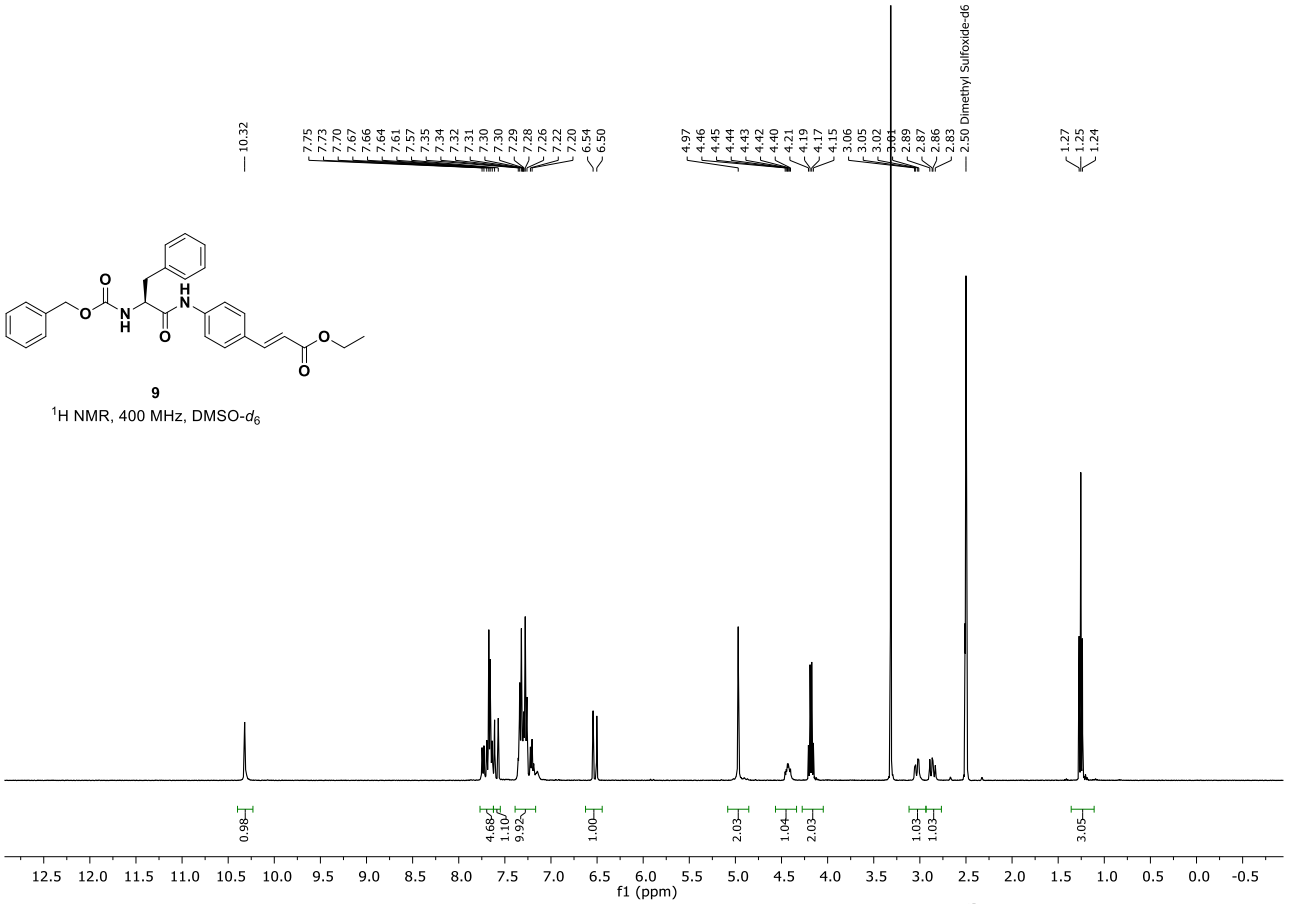

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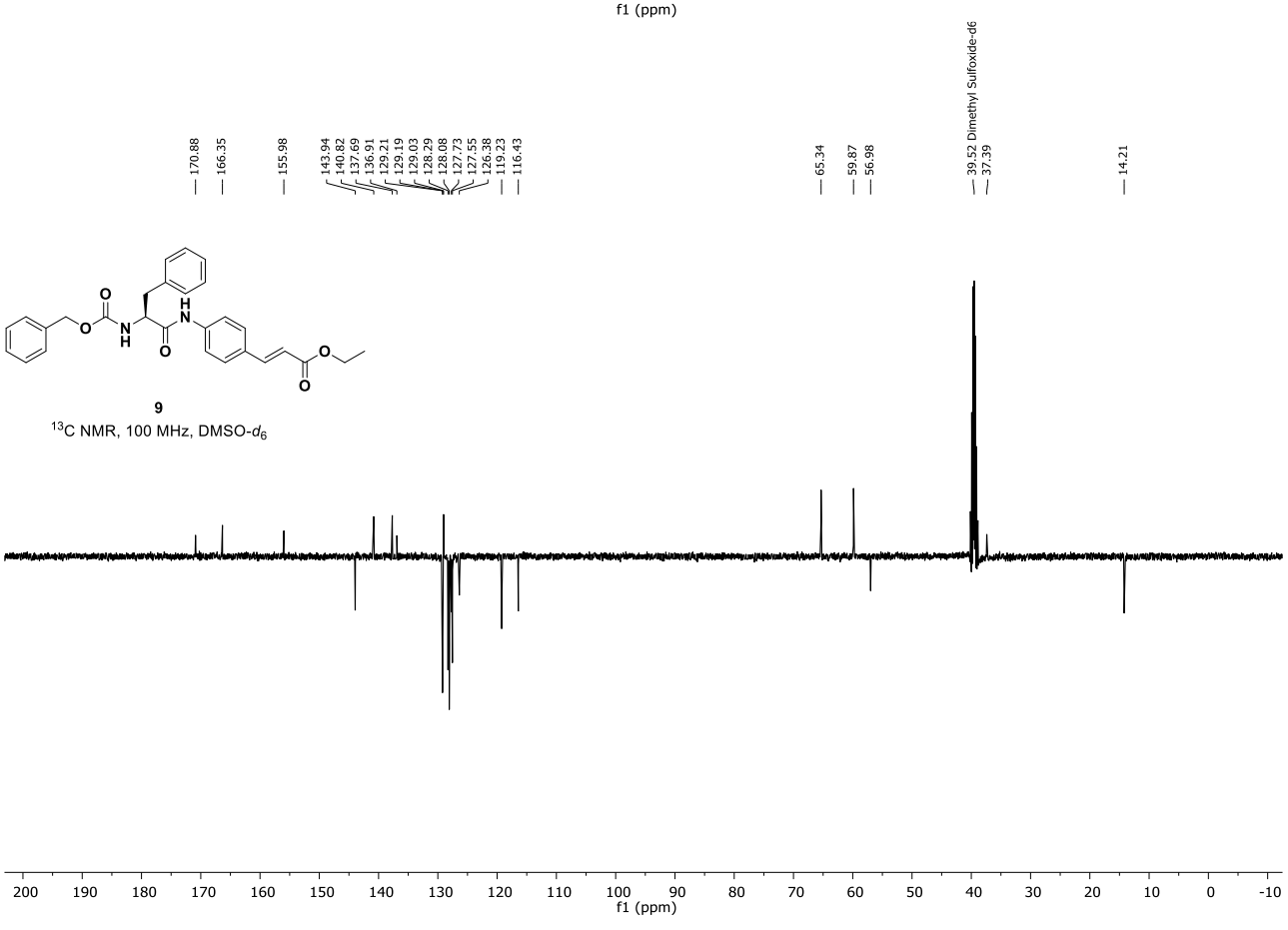

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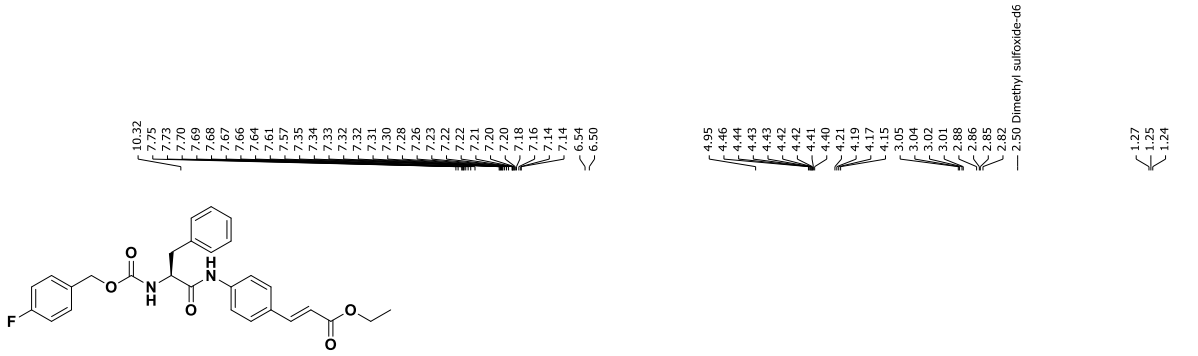

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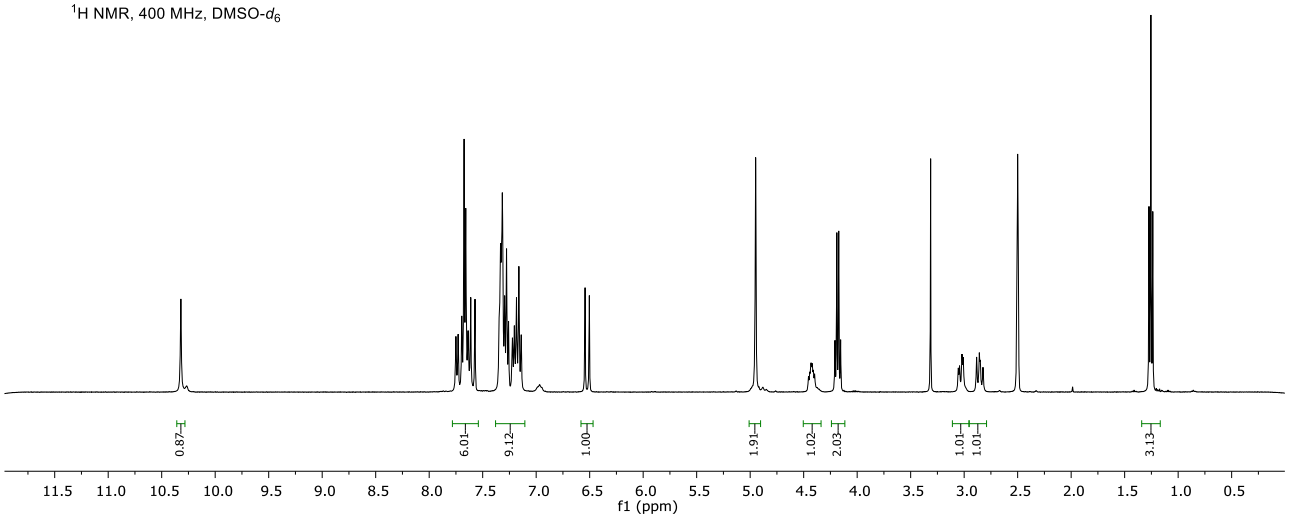

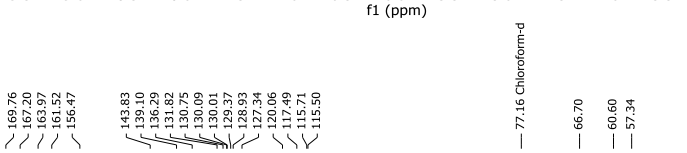

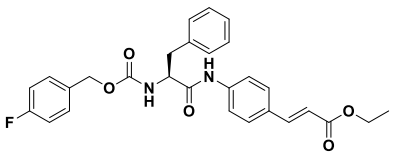

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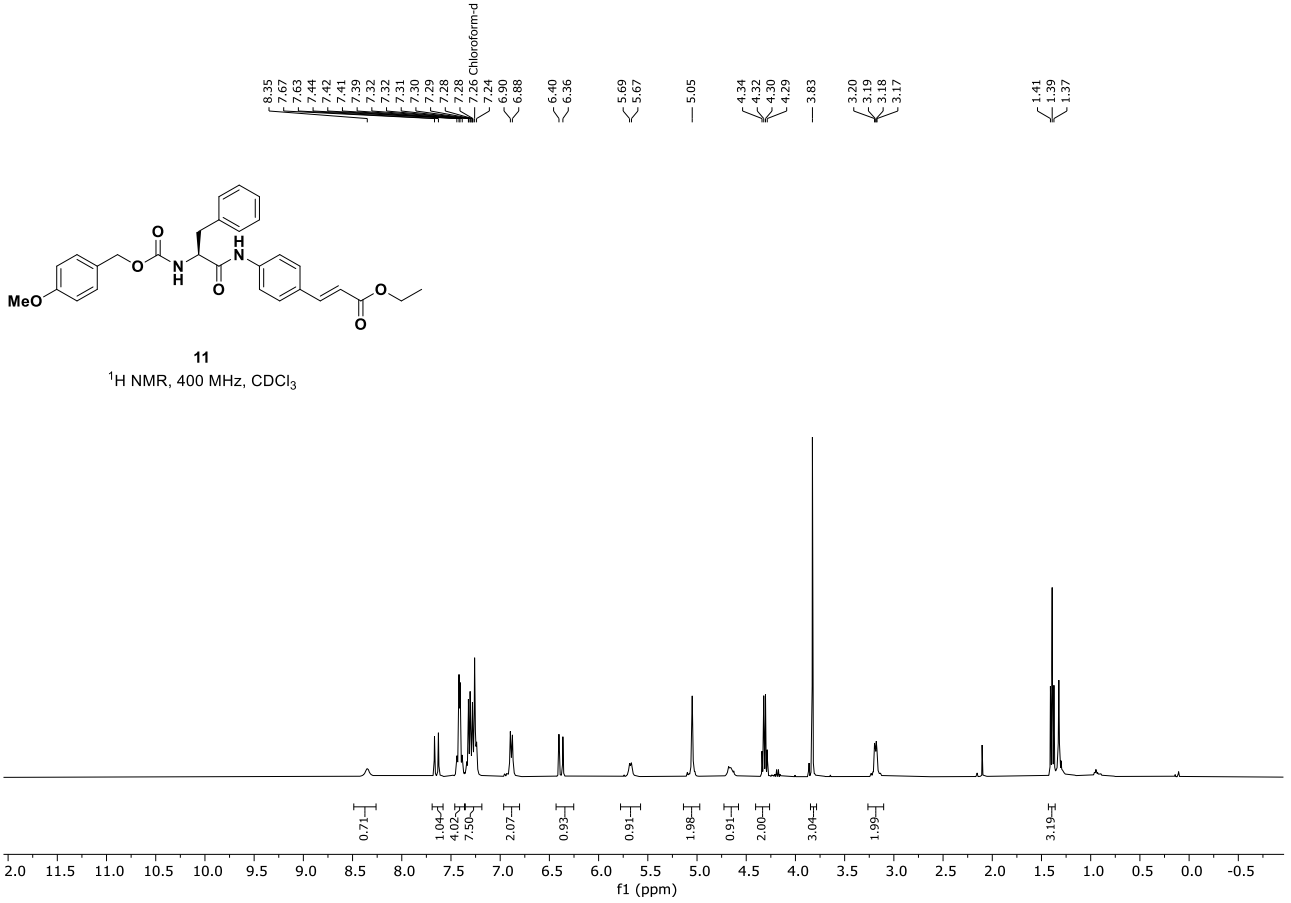

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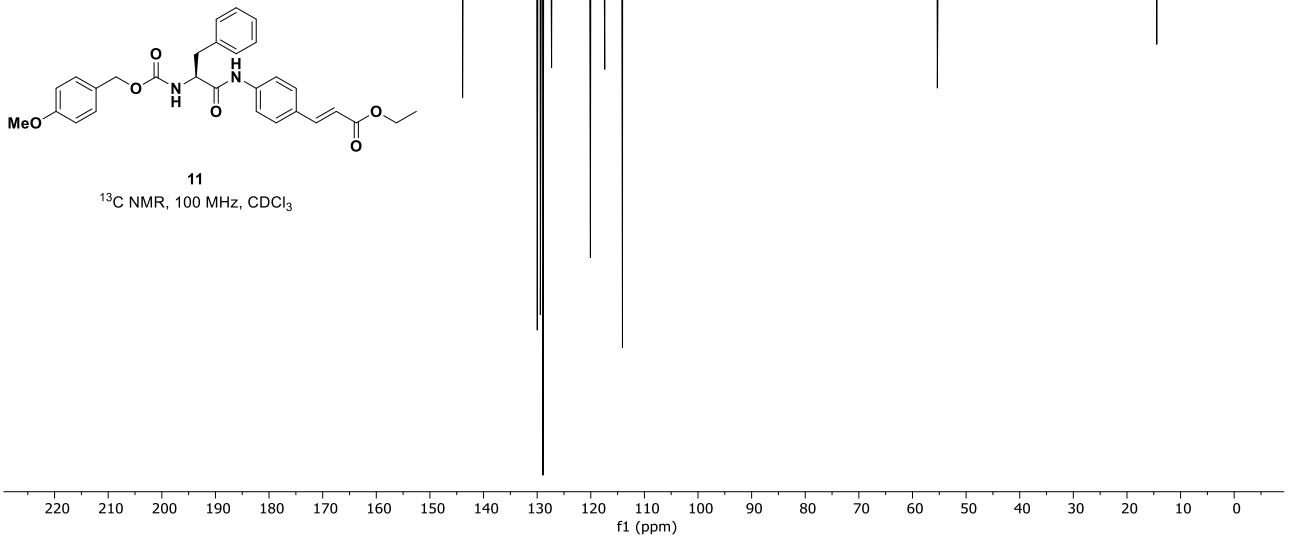

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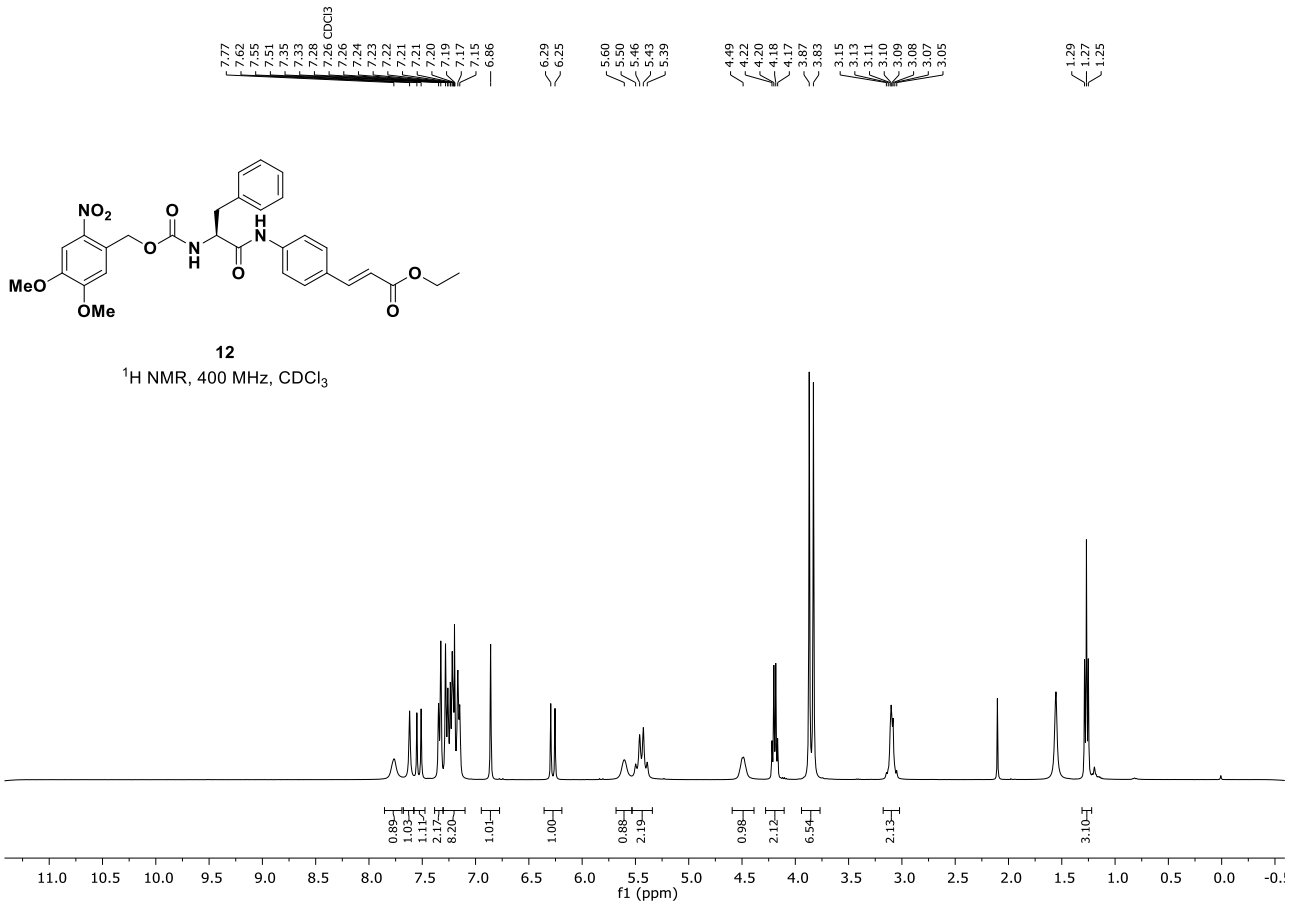

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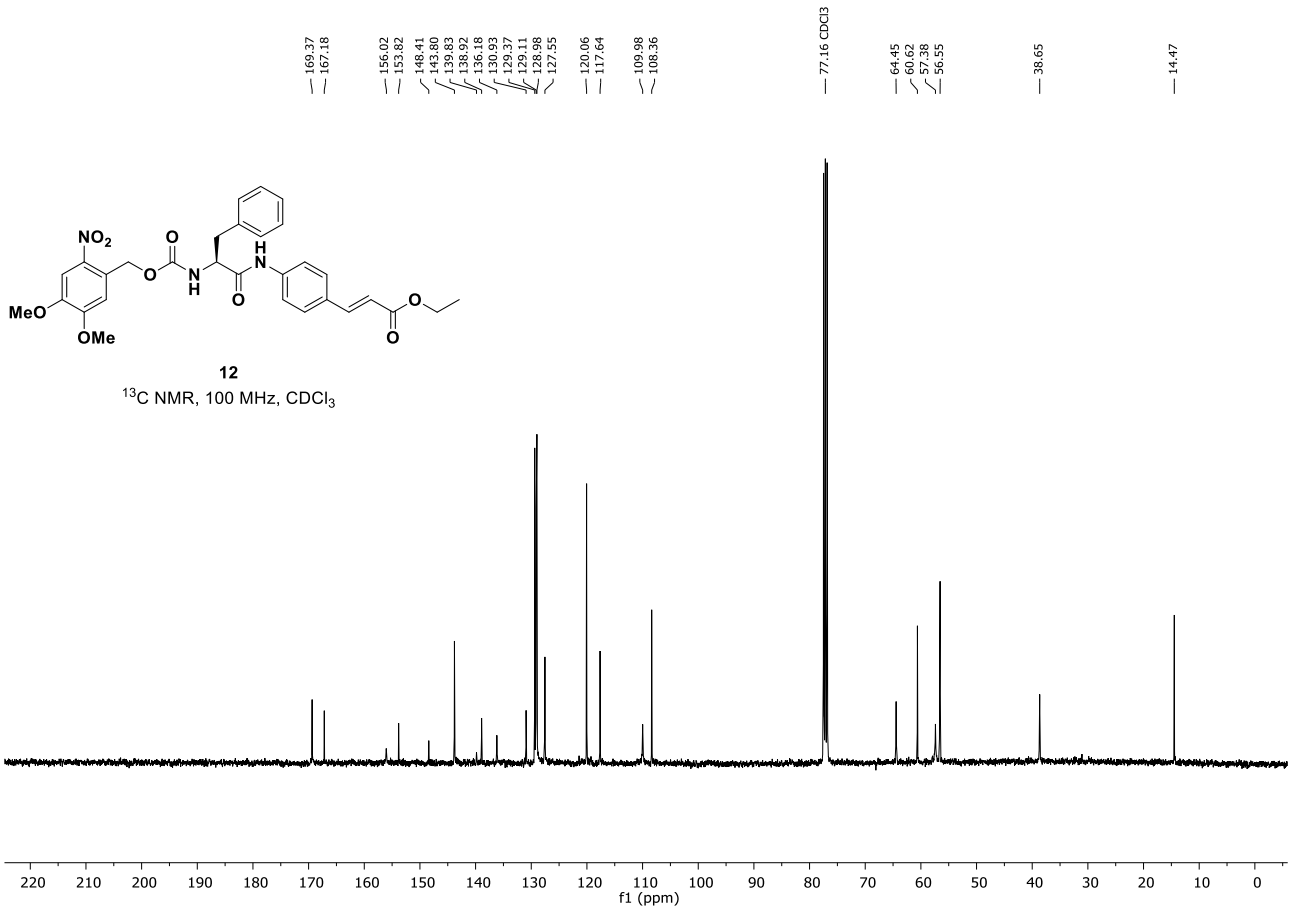

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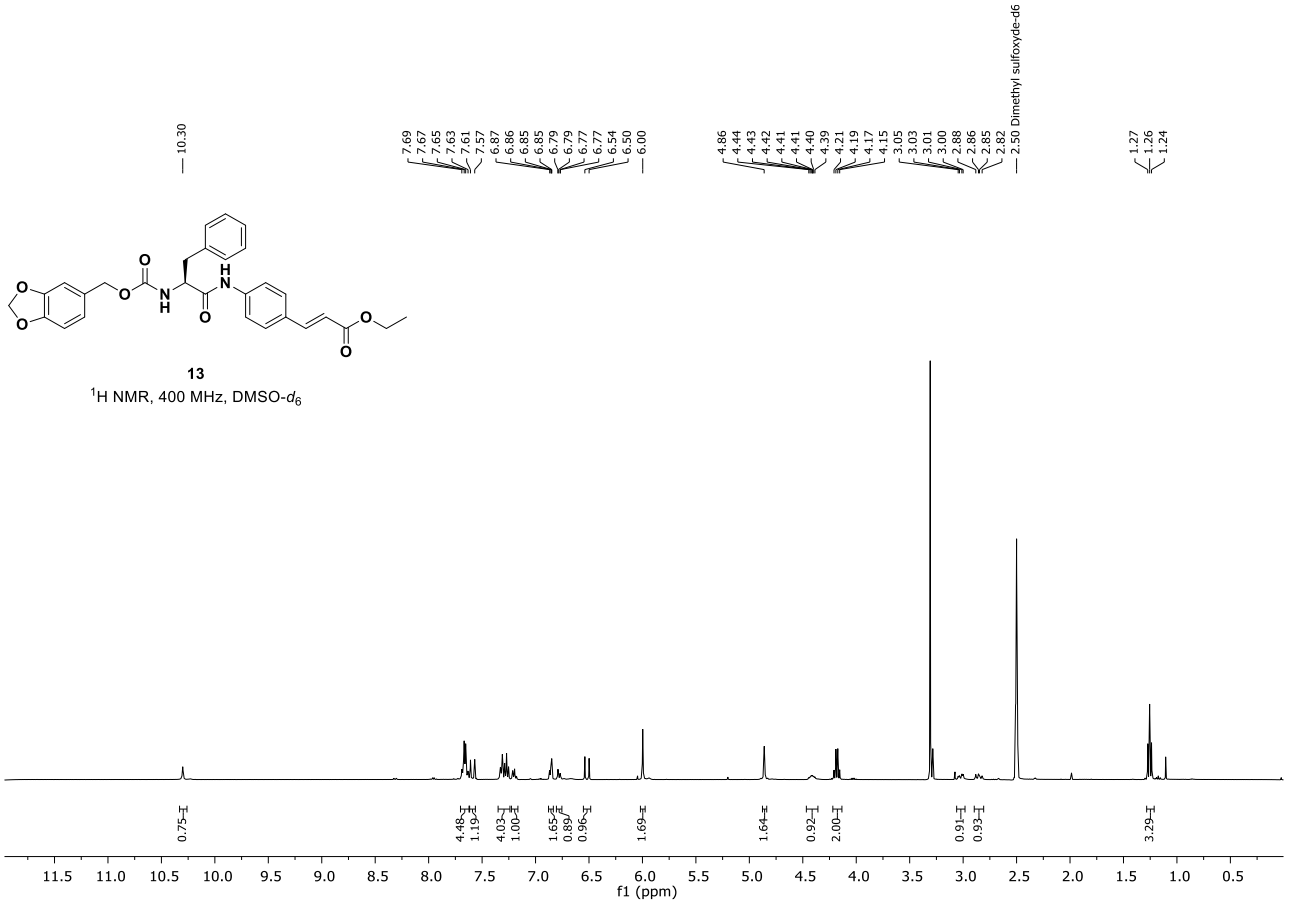

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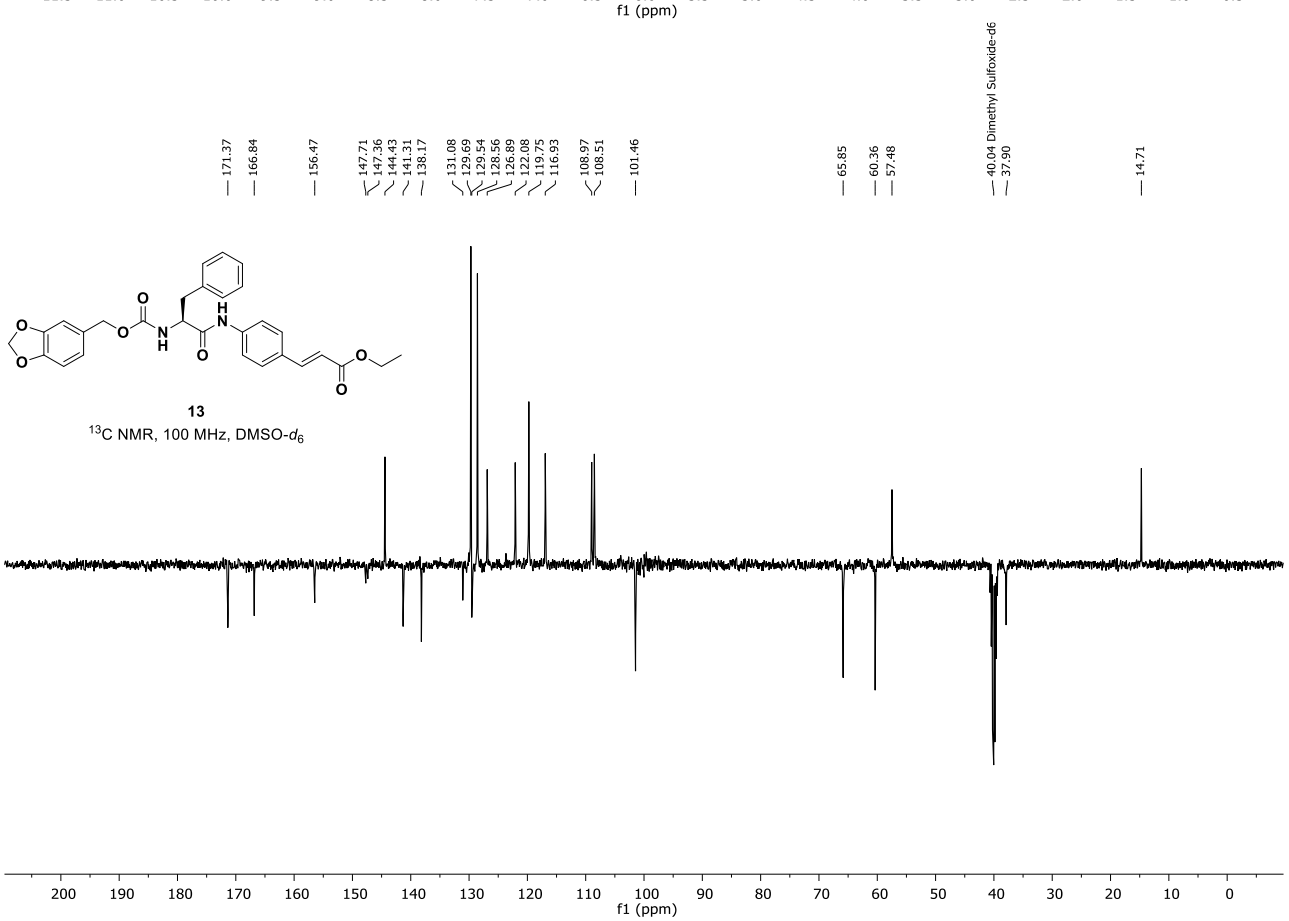

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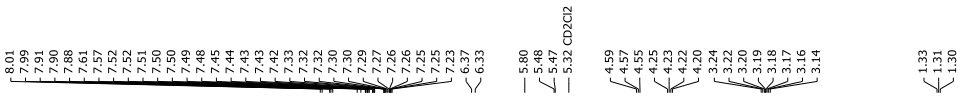

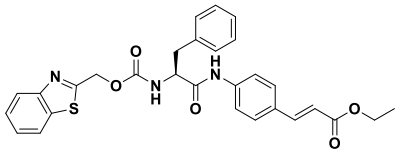

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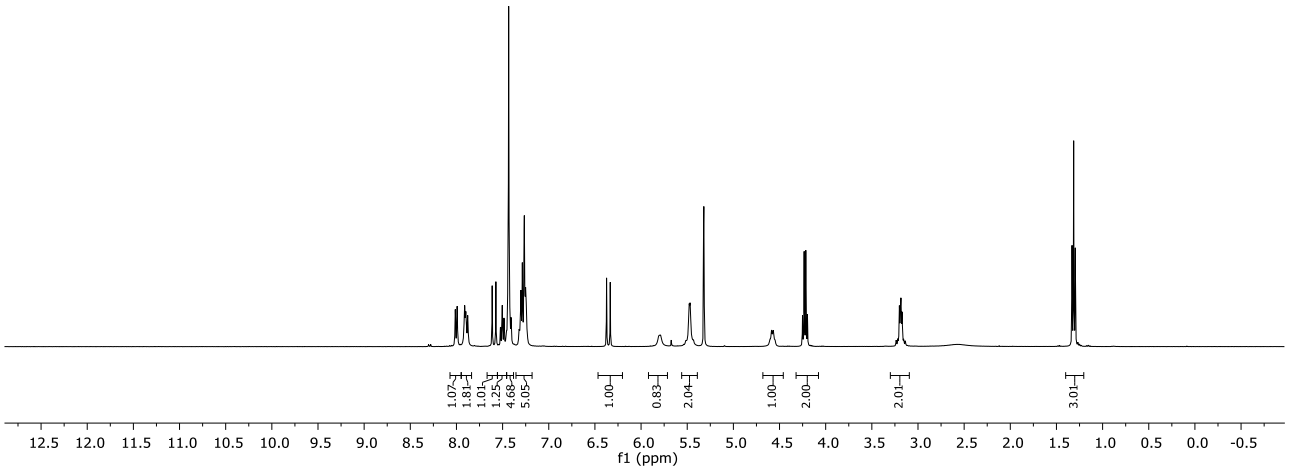
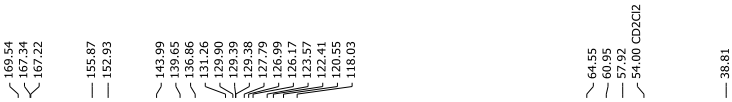
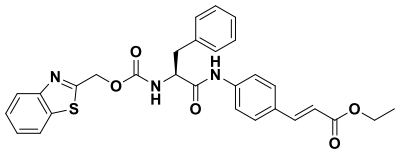
^{1}H NMR, 400 MHz, methylene chloride d_2







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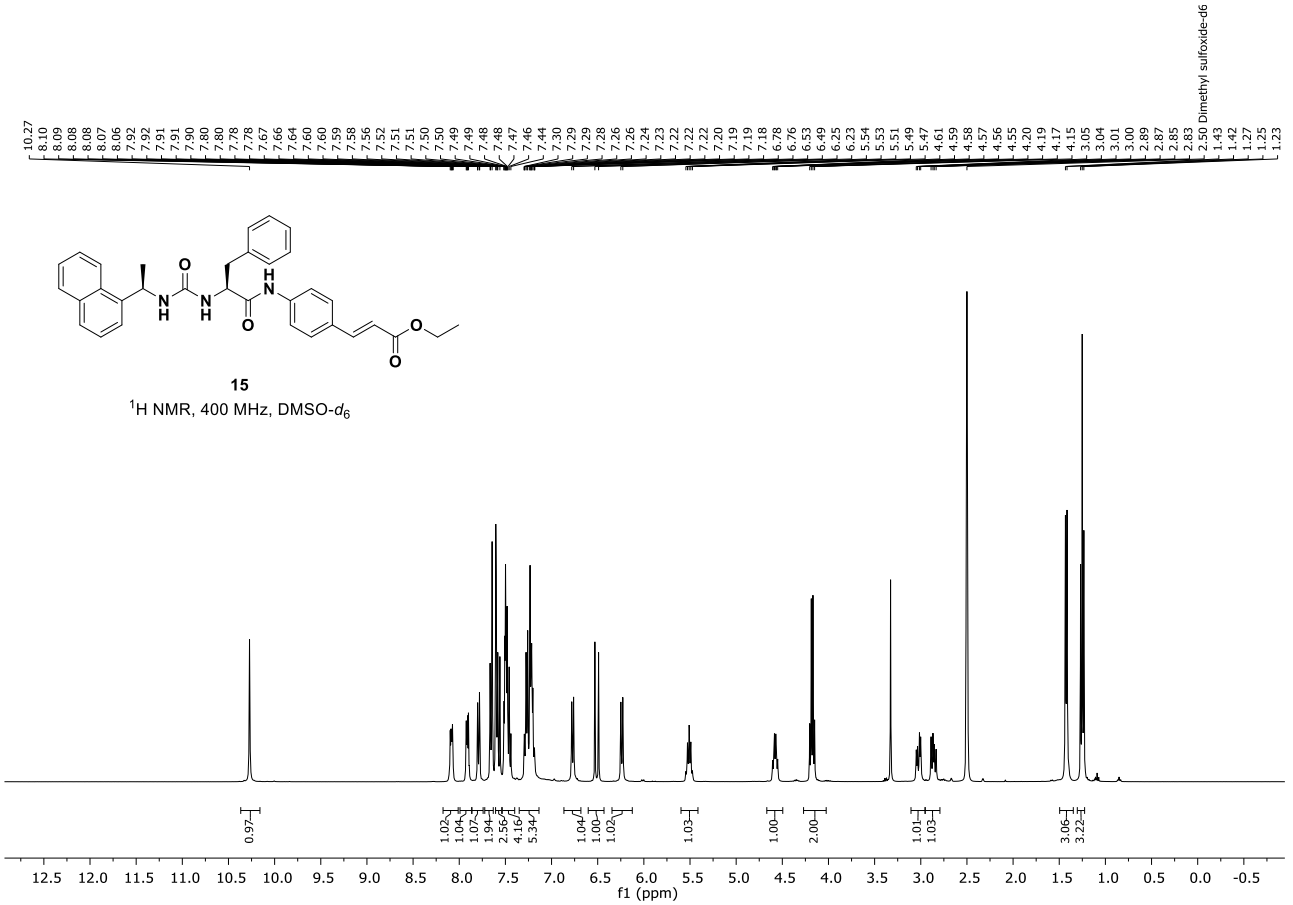
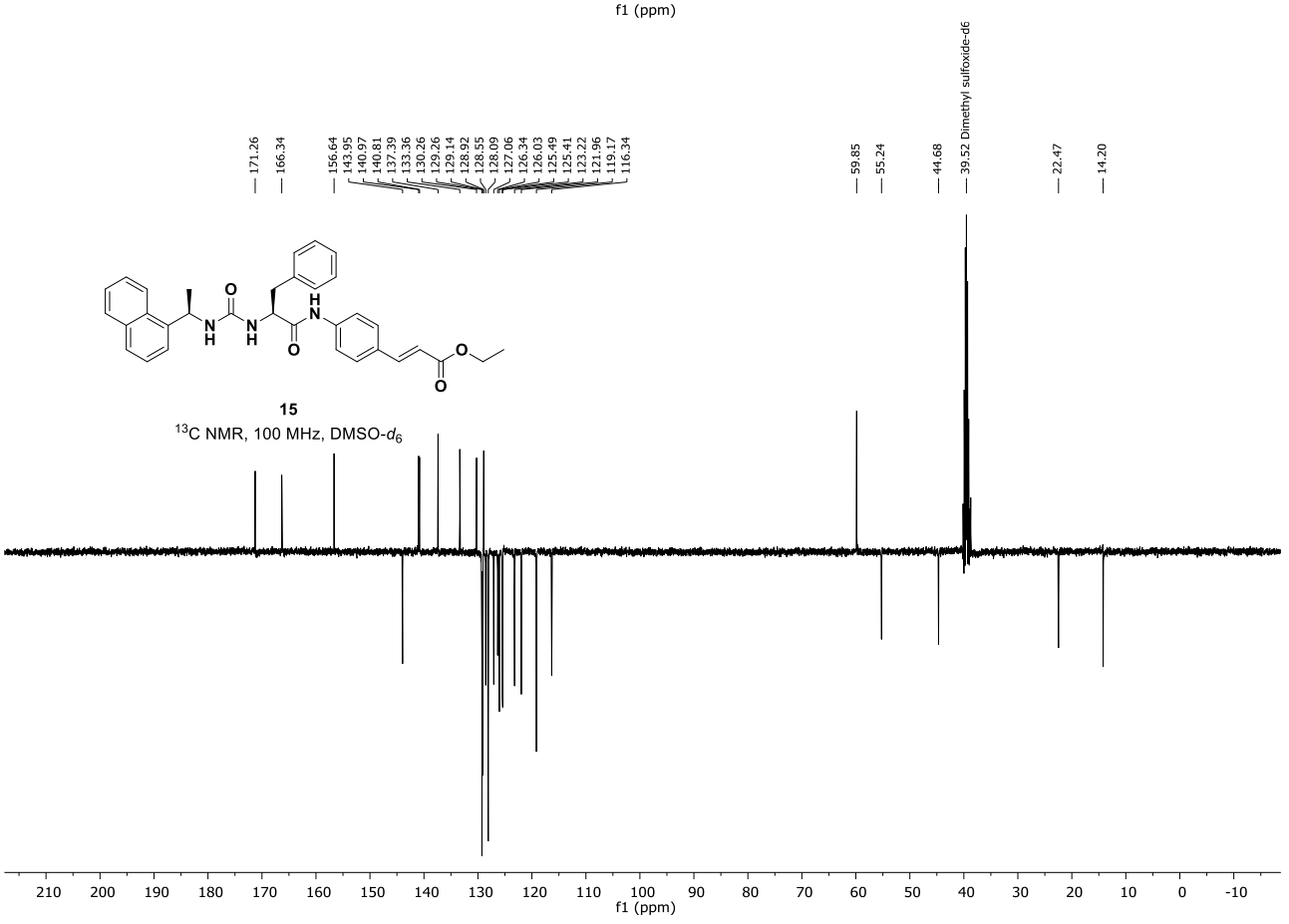
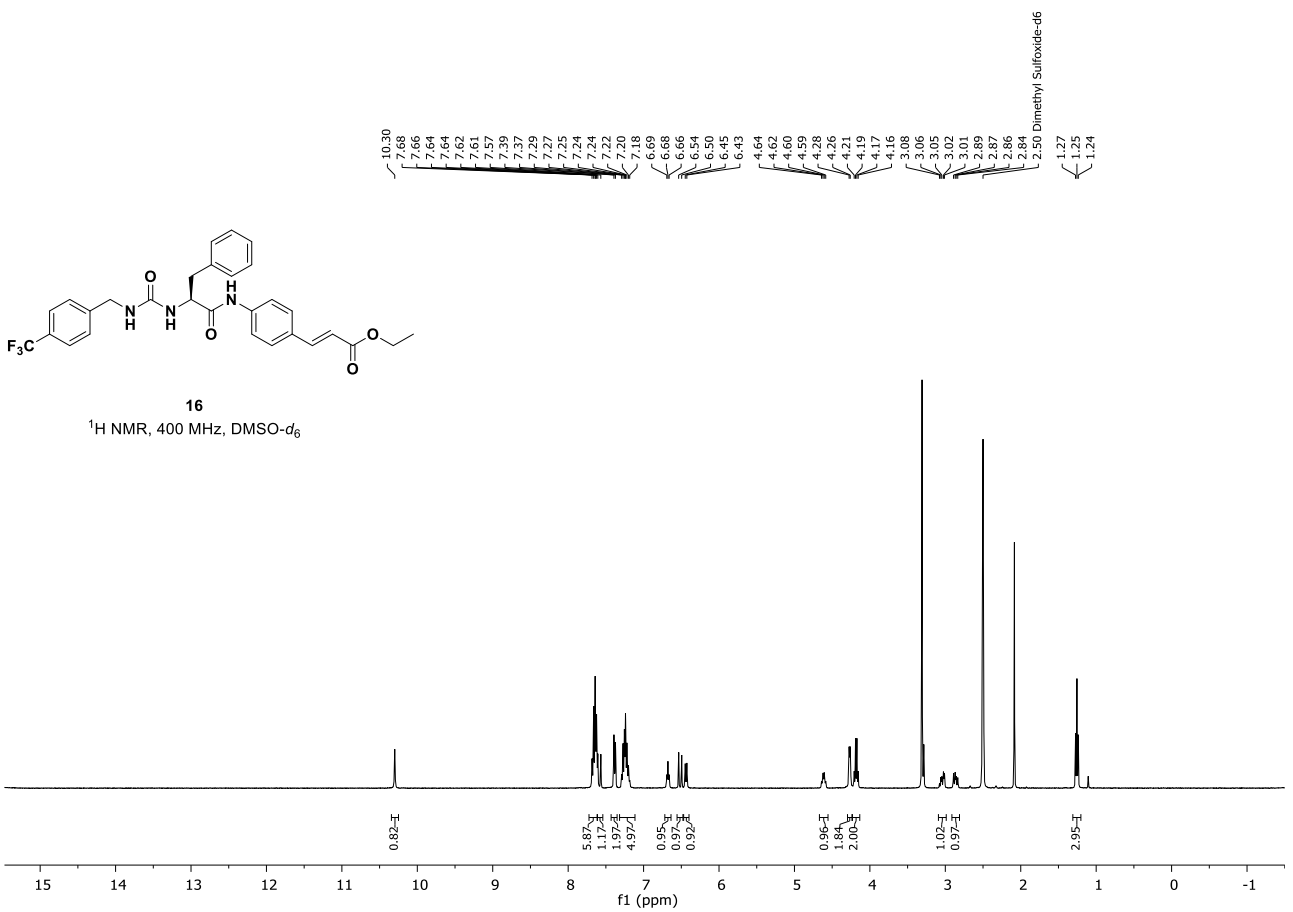
^{13}C NMR, 100 MHz, methylene chloride d_2

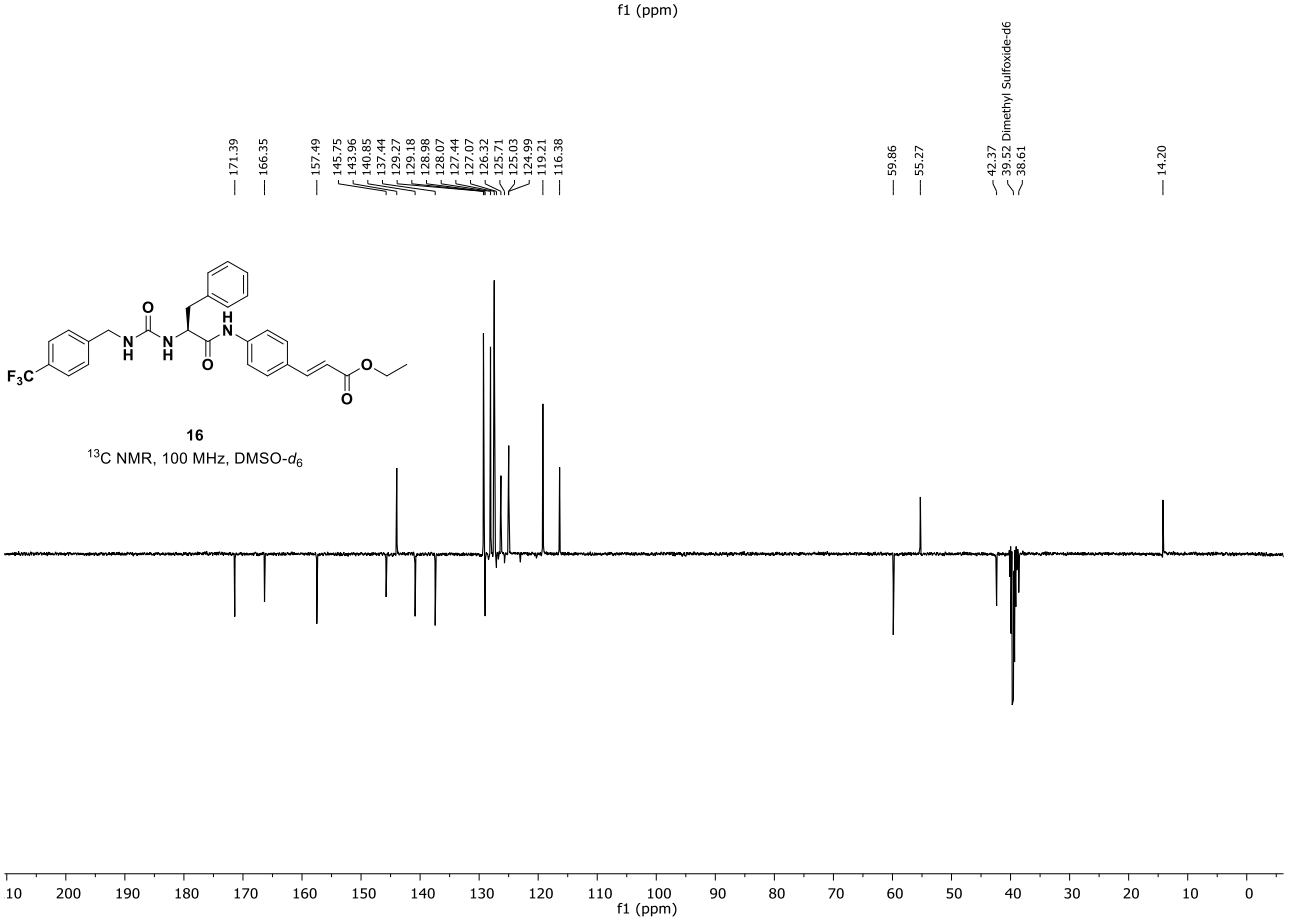

10.27 8.10 8.09 8.08 8.08 8.07 8.06 7.92 7.92 7.91 7.91 7.90 7.80 7.80 7.78 7.78 7.67 7.66 7.64 7.60 7.60 7.59 7.58 7.56 7.52 7.51 7.51 7.50 7.50 7.49 7.49 7.48 7.48 7.47 7.46 7.44 7.30 7.29 7.29 7.28 7.26 7.26 7.24 7.23 7.22 7.22 7.22 7.20 7.19 7.19 7.18 6.78 6.76 6.53 6.49 6.25 6.23 5.54 5.53 5.51 5.49 5.47 4.61 4.59 4.58 4.57 4.56 4.55 4.20 4.19 4.17 4.15 3.05 3.04 3.01 3.00 2.89 2.87 2.85 2.83 2.50 Dimethyl sulfoxide-d6 1.43 1.42 1.27 1.25 1.23
15
1H NMR, 400 MHz, DMSO-d6
0.97 1.02 1.04 1.07 1.94 2.56 4.16 5.34 1.04 1.00 1.02 1.03 1.00 2.00 1.01 1.03 3.06 3.22
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


171.26 166.34 156.64 143.95 140.97 140.81 137.39 133.36 130.26 129.26 129.14 128.92 128.55 128.09 127.06 126.34 126.03 125.49 125.41 123.22 121.96 119.17 116.34 59.85 55.24 44.68 39.52 Dimethyl sulfoxide-d6 22.47 14.20
15
13C NMR, 100 MHz, DMSO-d6
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)



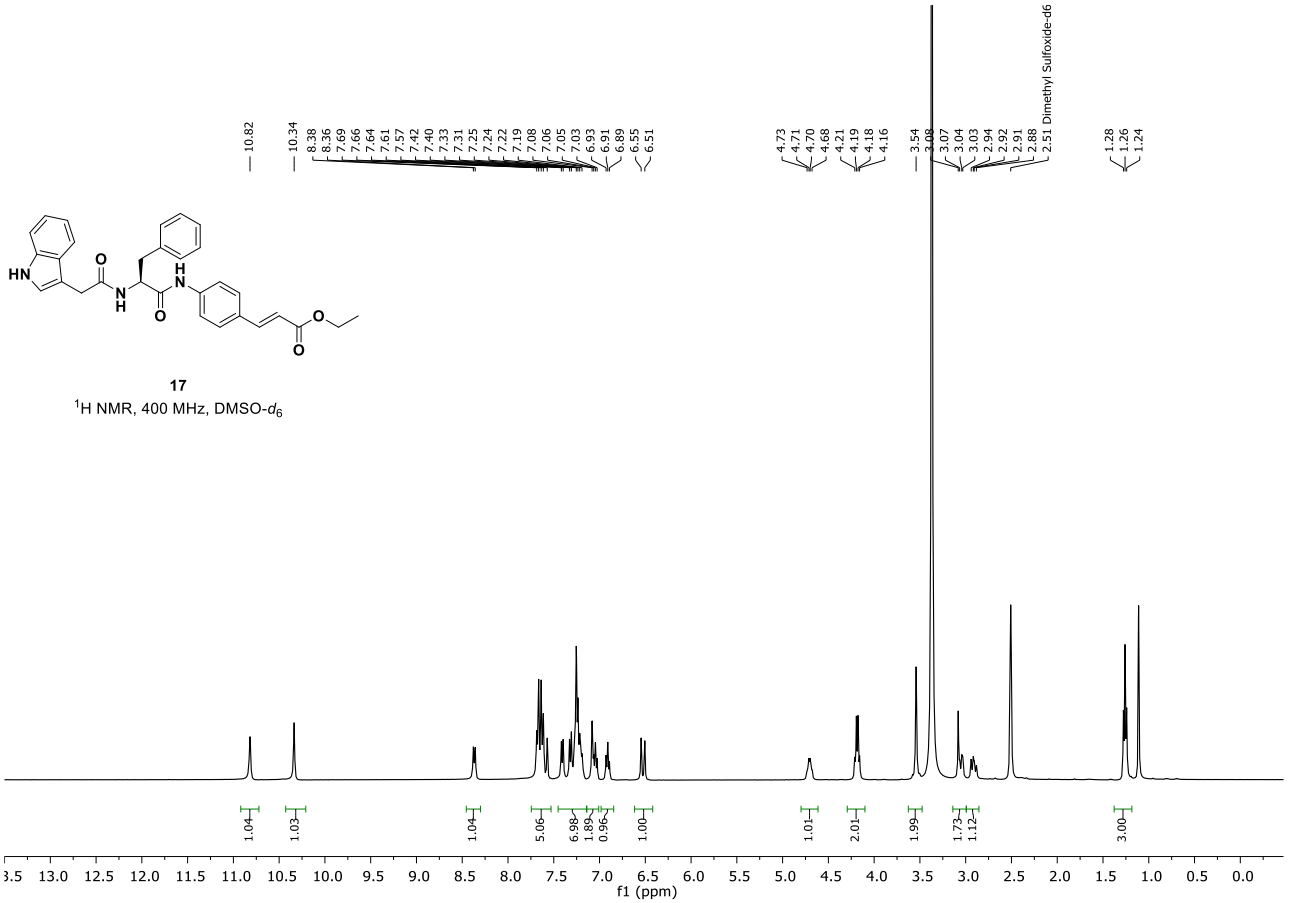
16

^{1}H NMR, 400 MHz, DMSO-d_6



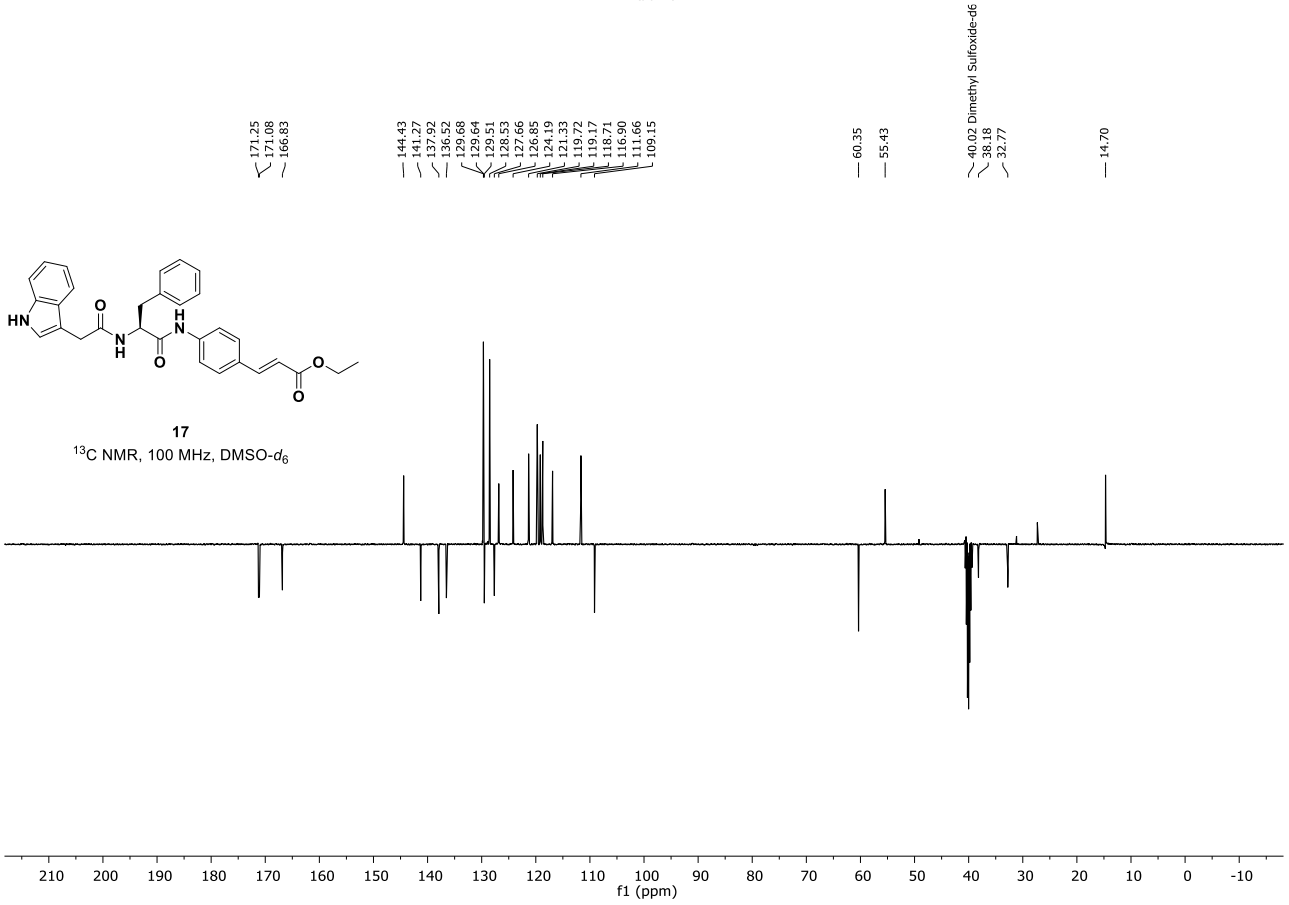
16

^{13}C NMR, 100 MHz, DMSO-d_6



17

^{1}H NMR, 400 MHz, DMSO-d_6



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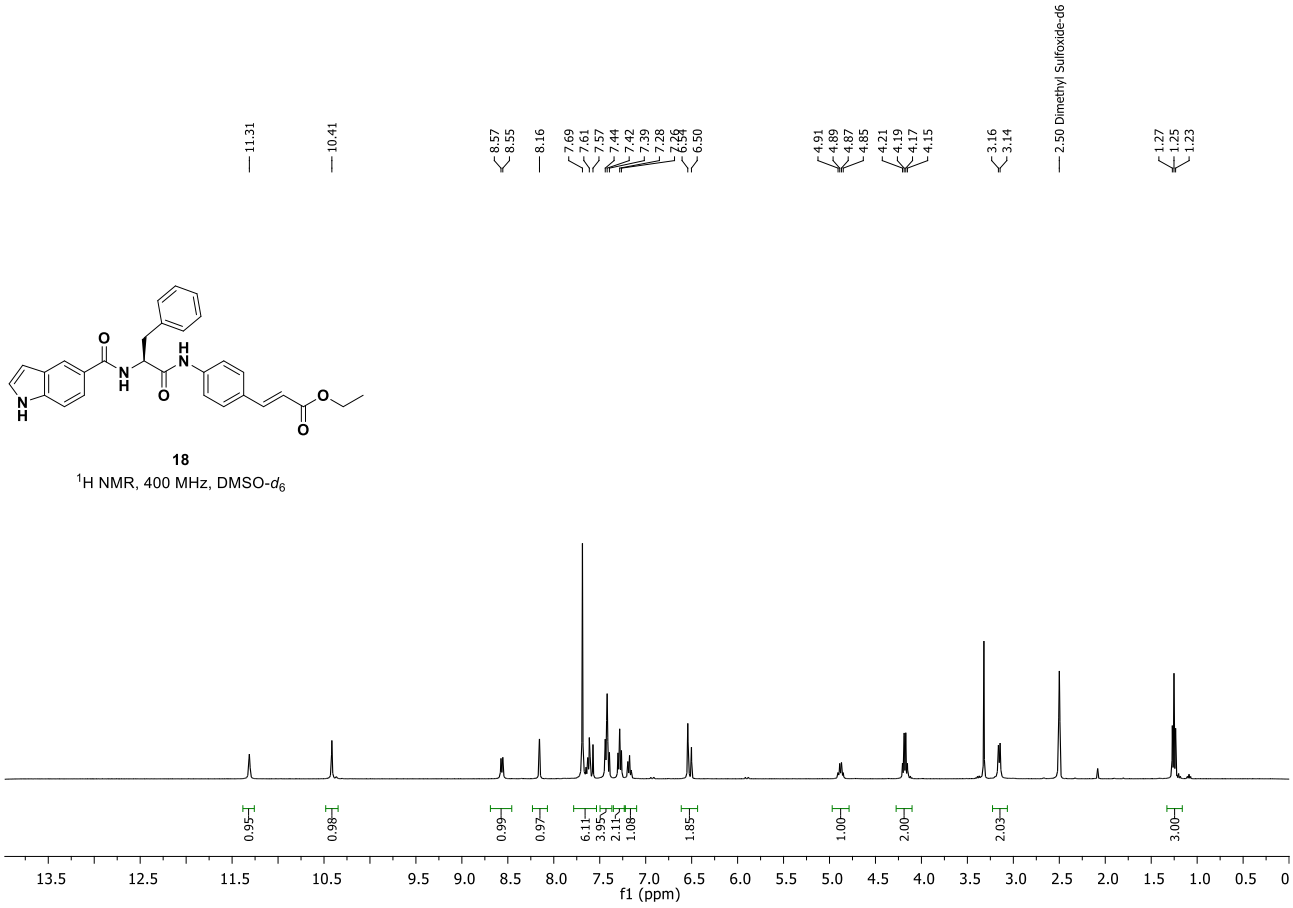
^{13}C NMR, 100 MHz, DMSO-d_6



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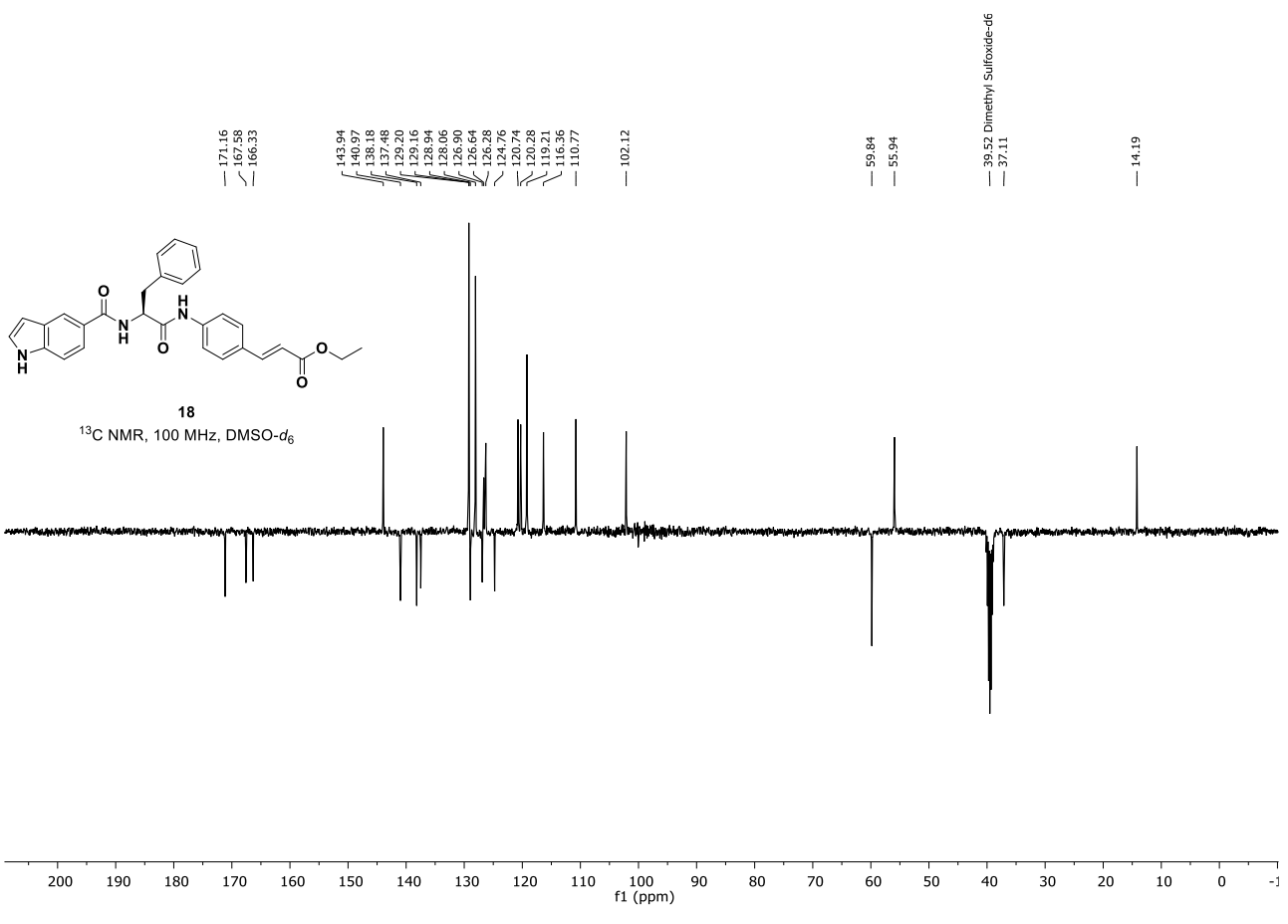
^{1}H NMR, 400 MHz, DMSO-d_6



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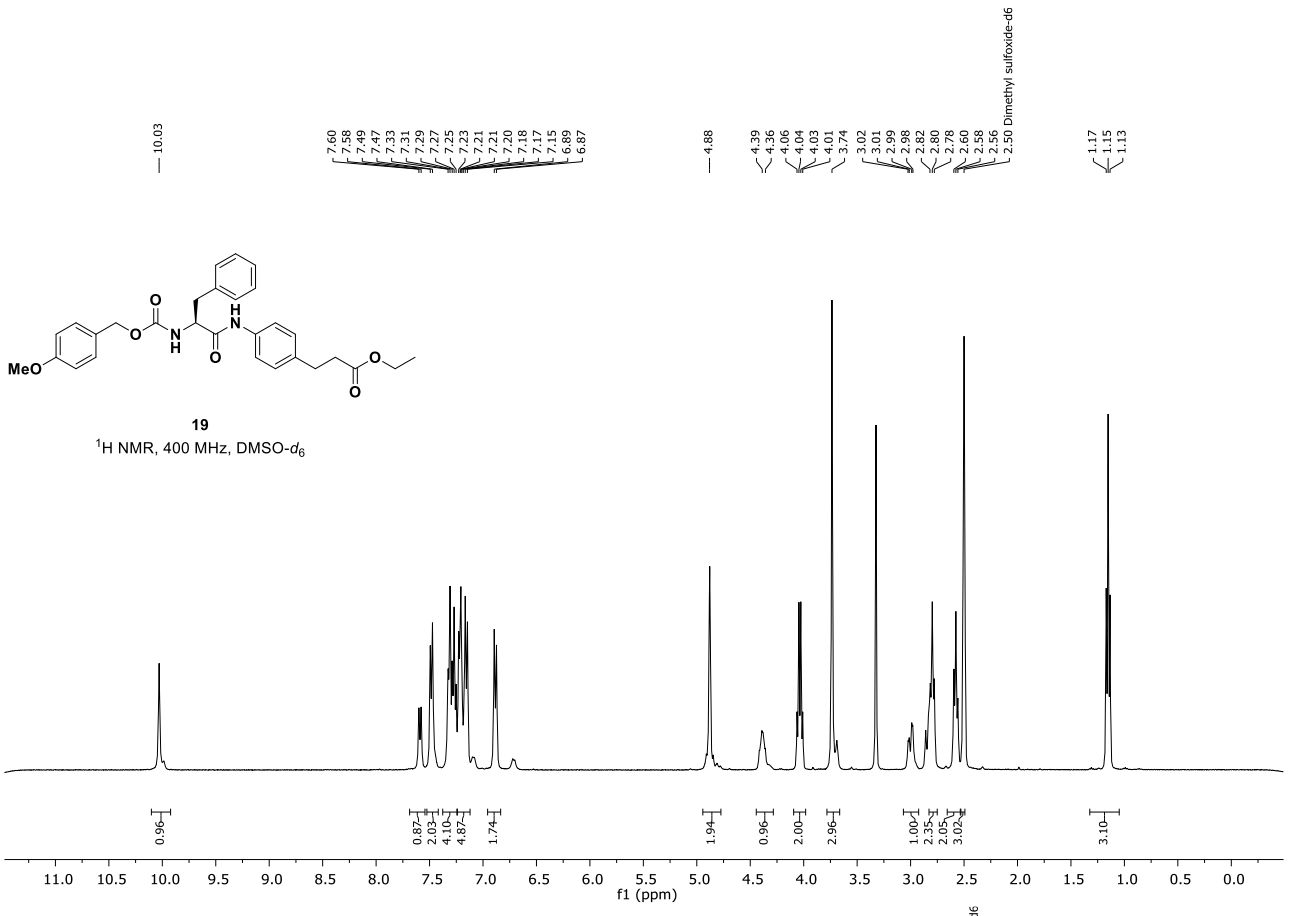

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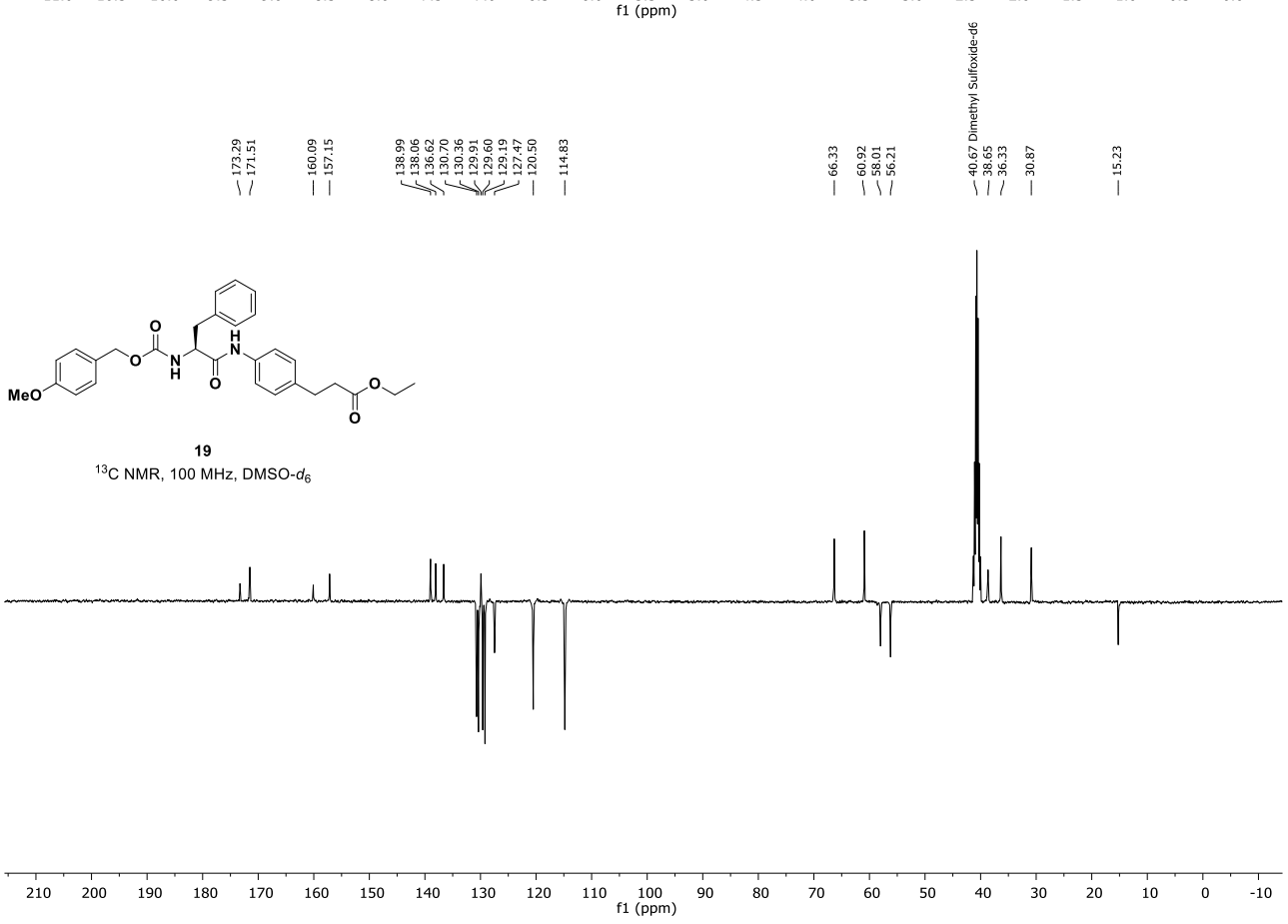

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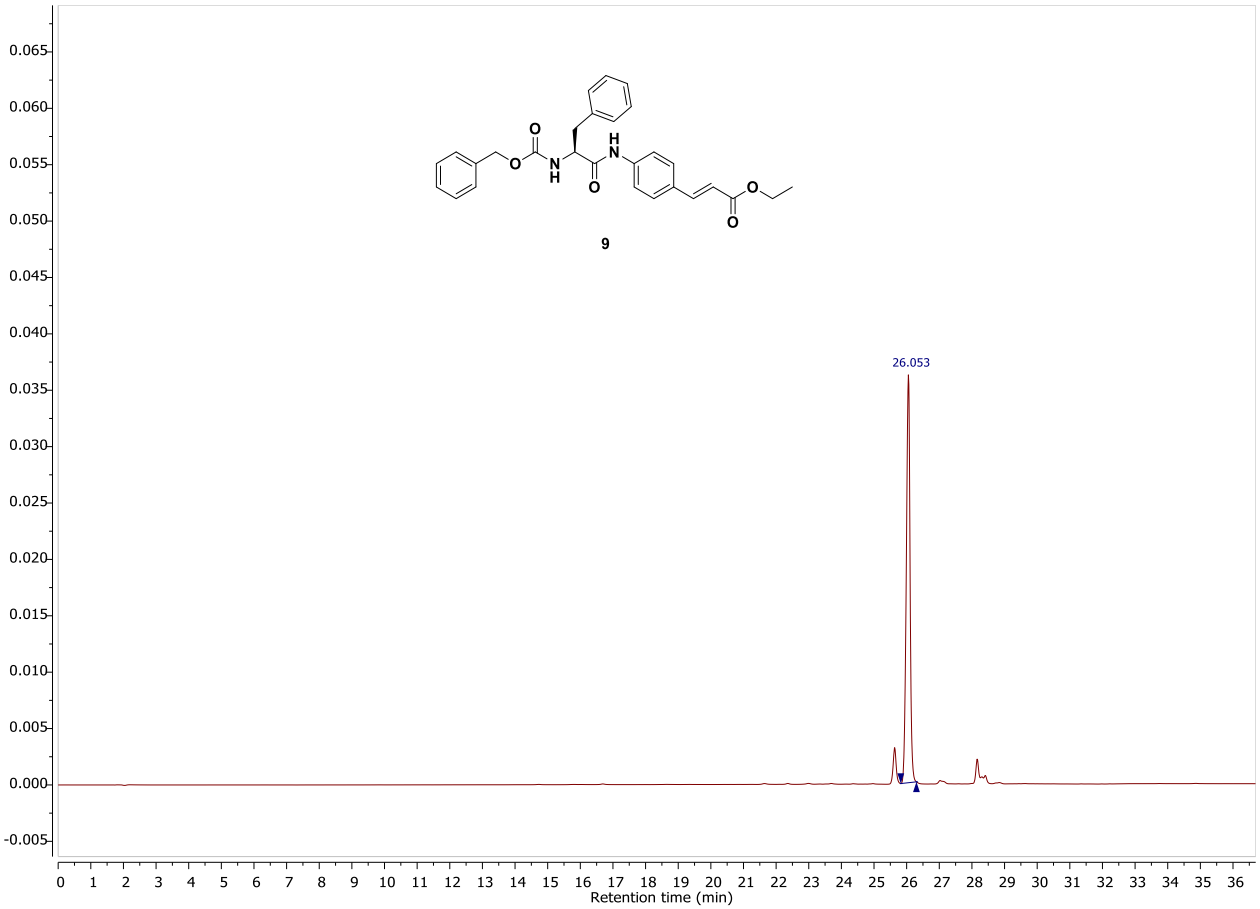
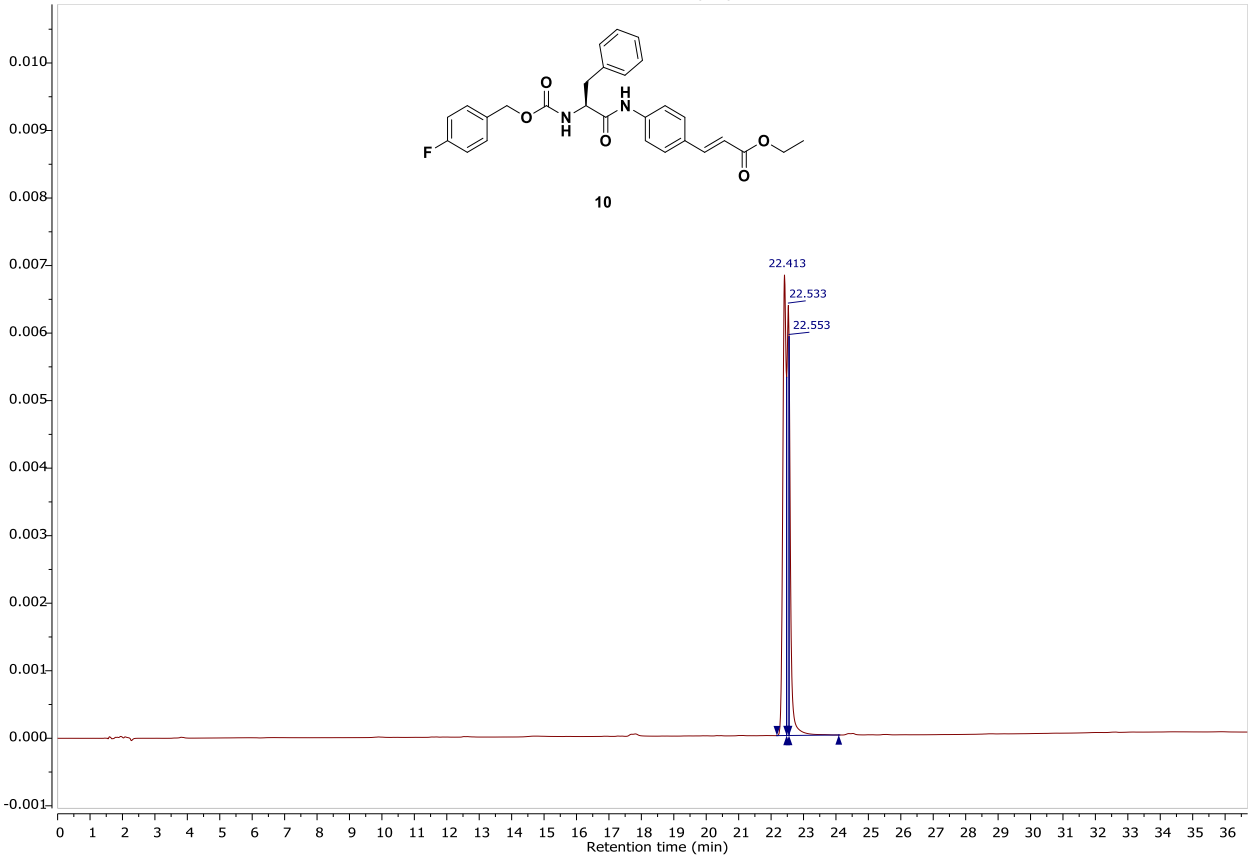
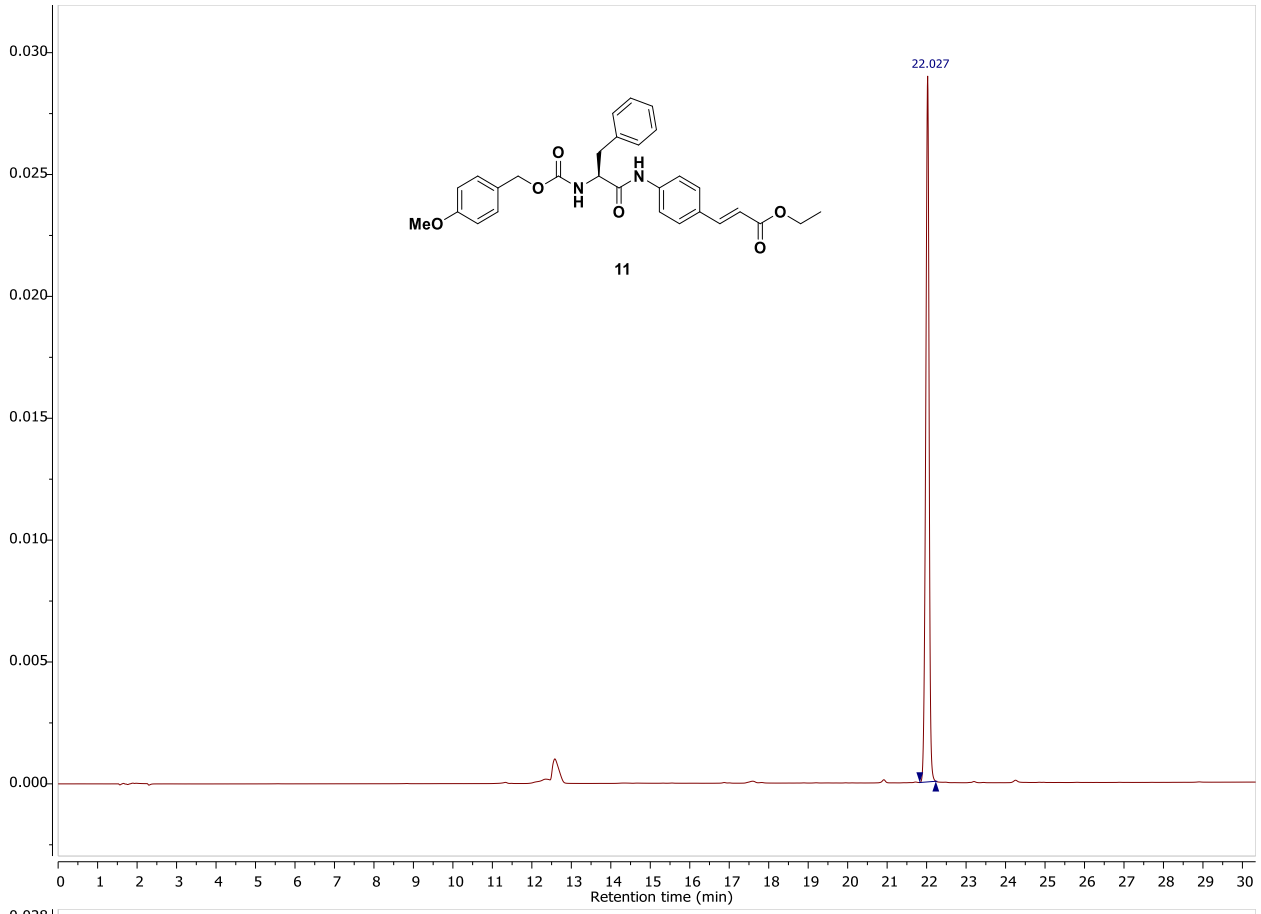
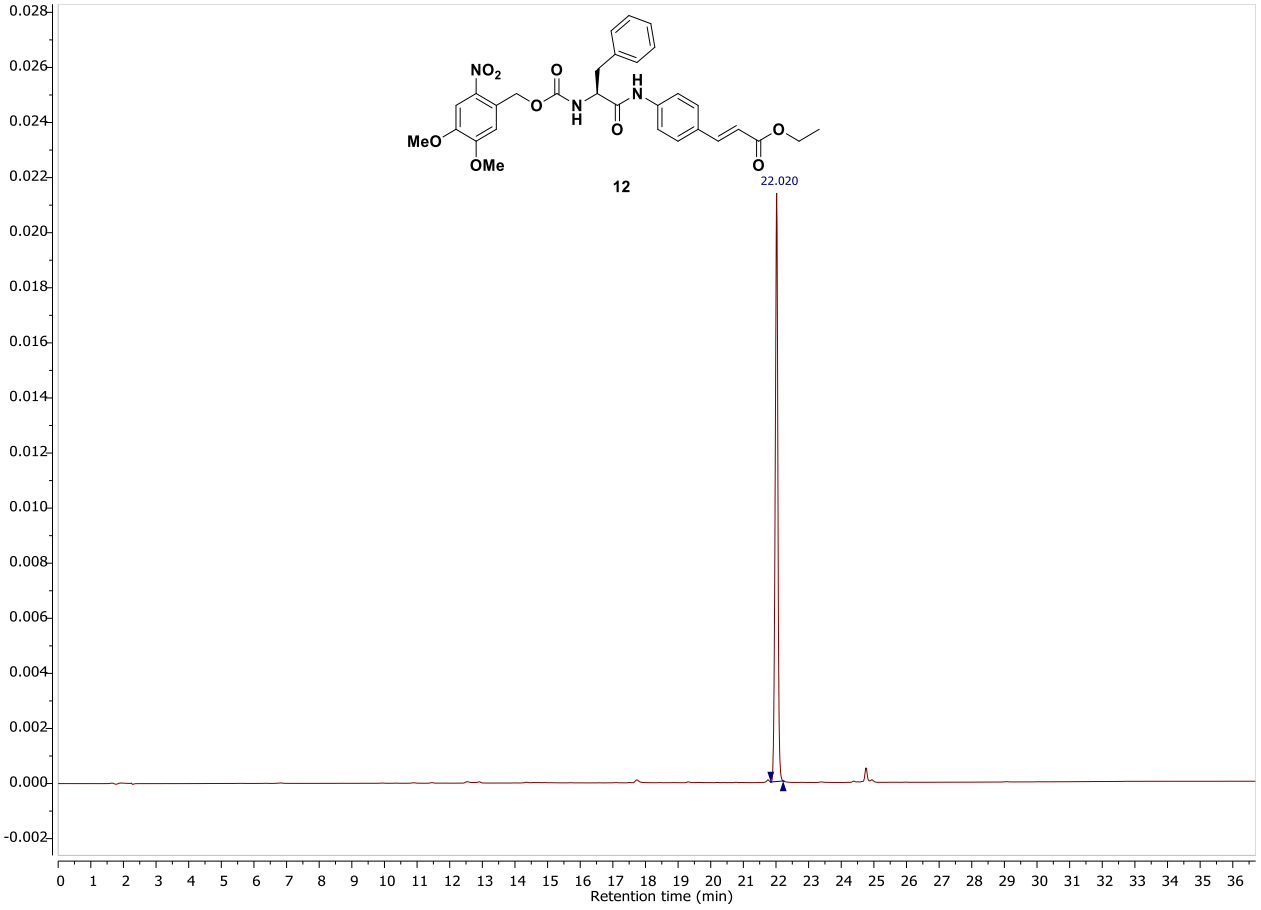
2. HPLC traces of the final compounds






22.027
MeO
11
Retention time (min)


NO2
MeO
OMe
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22.020
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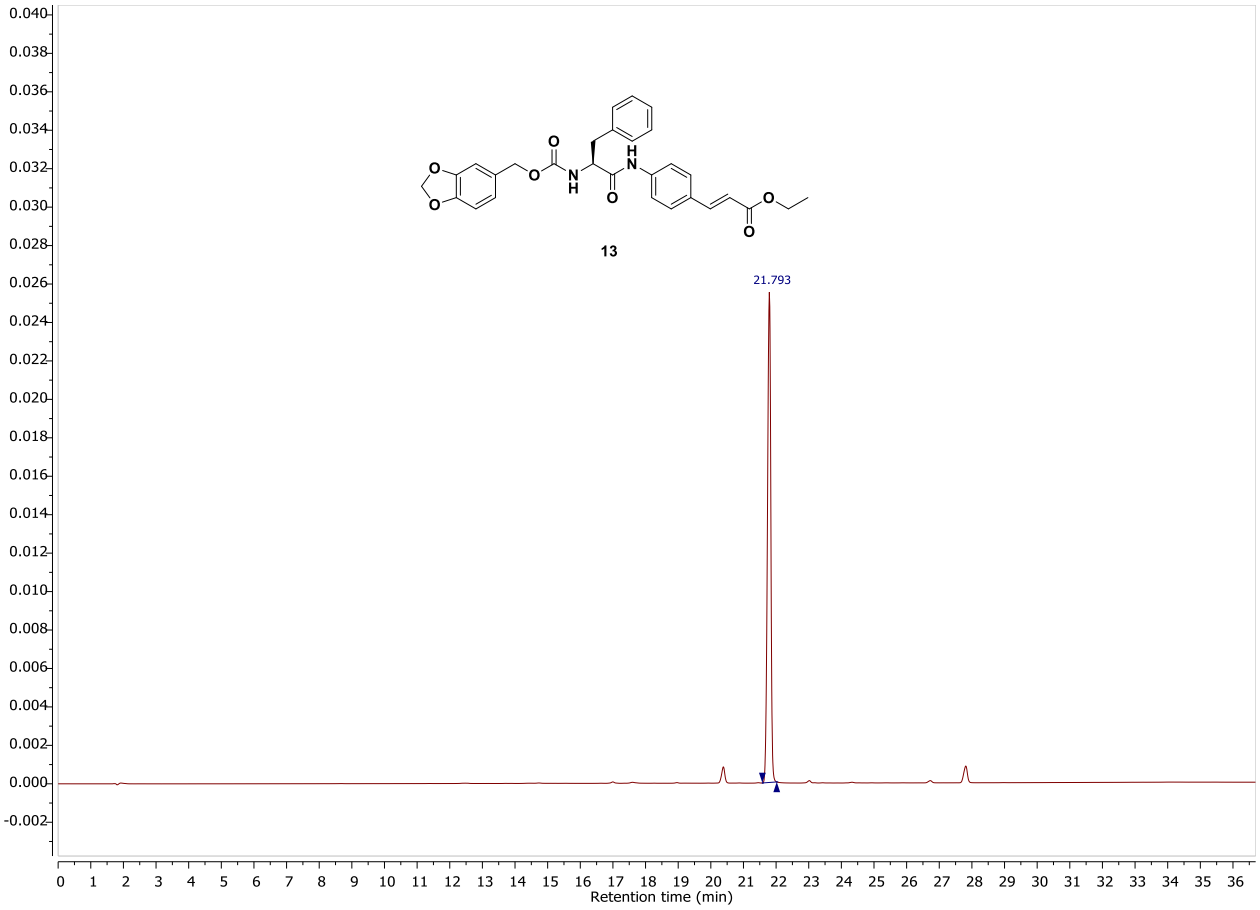
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21.793
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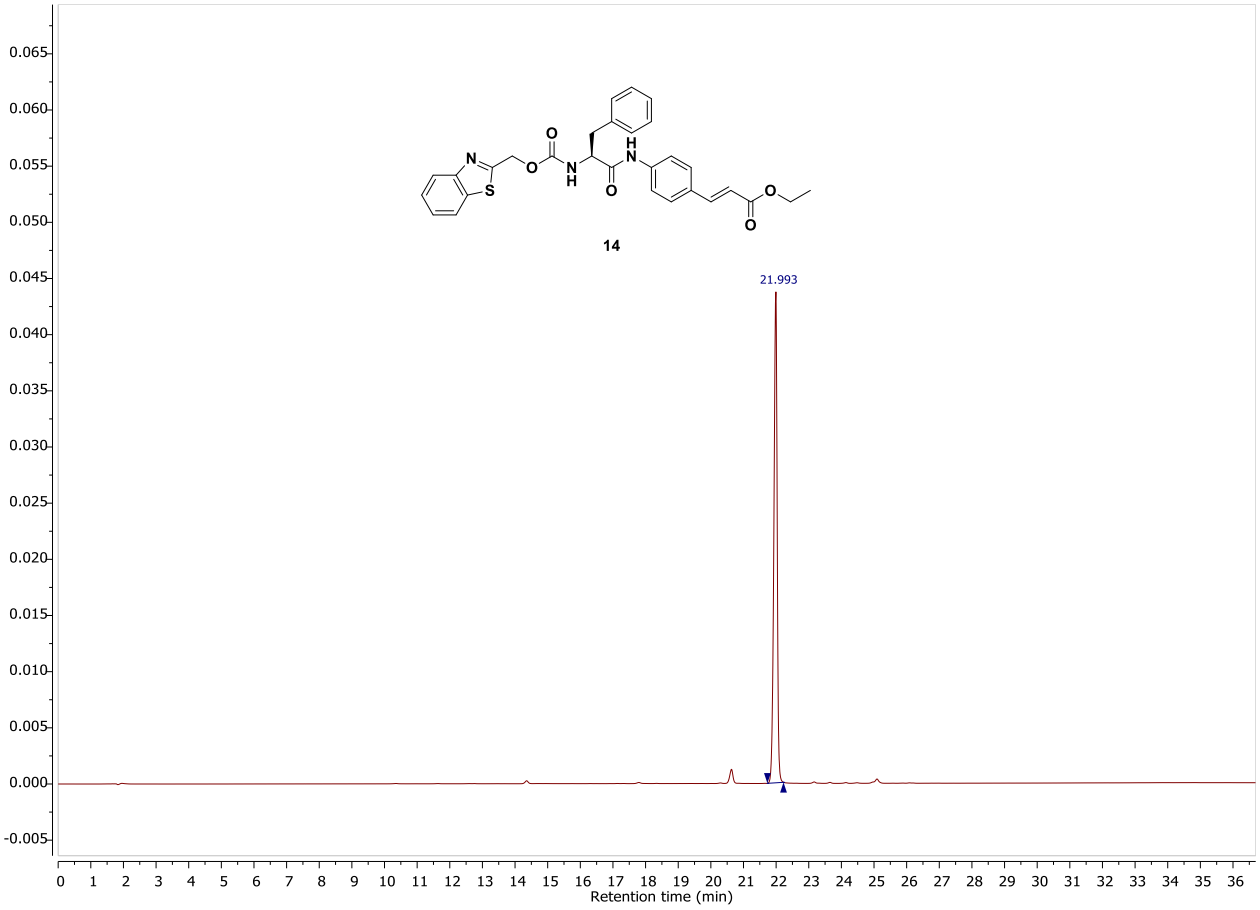
14
21.993
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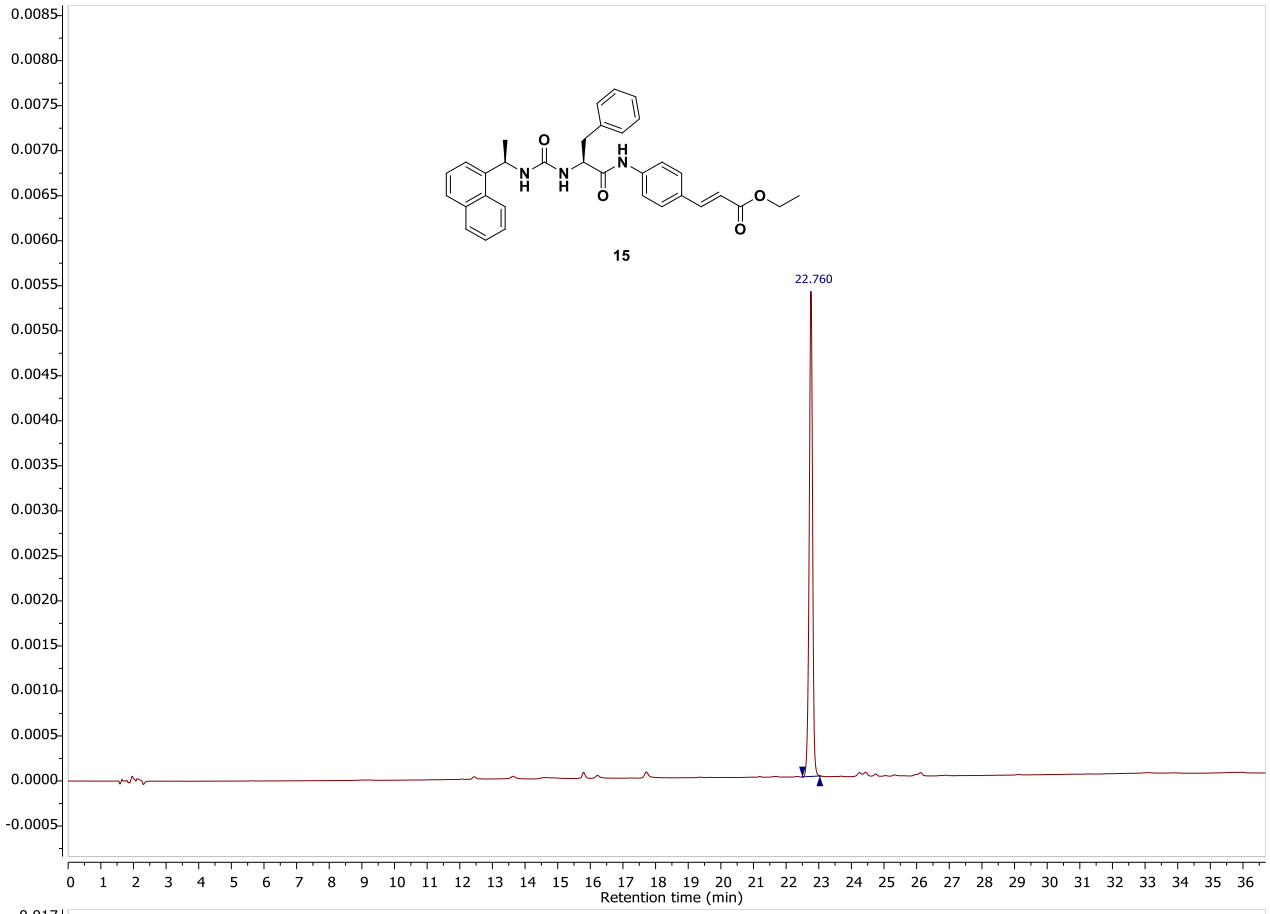
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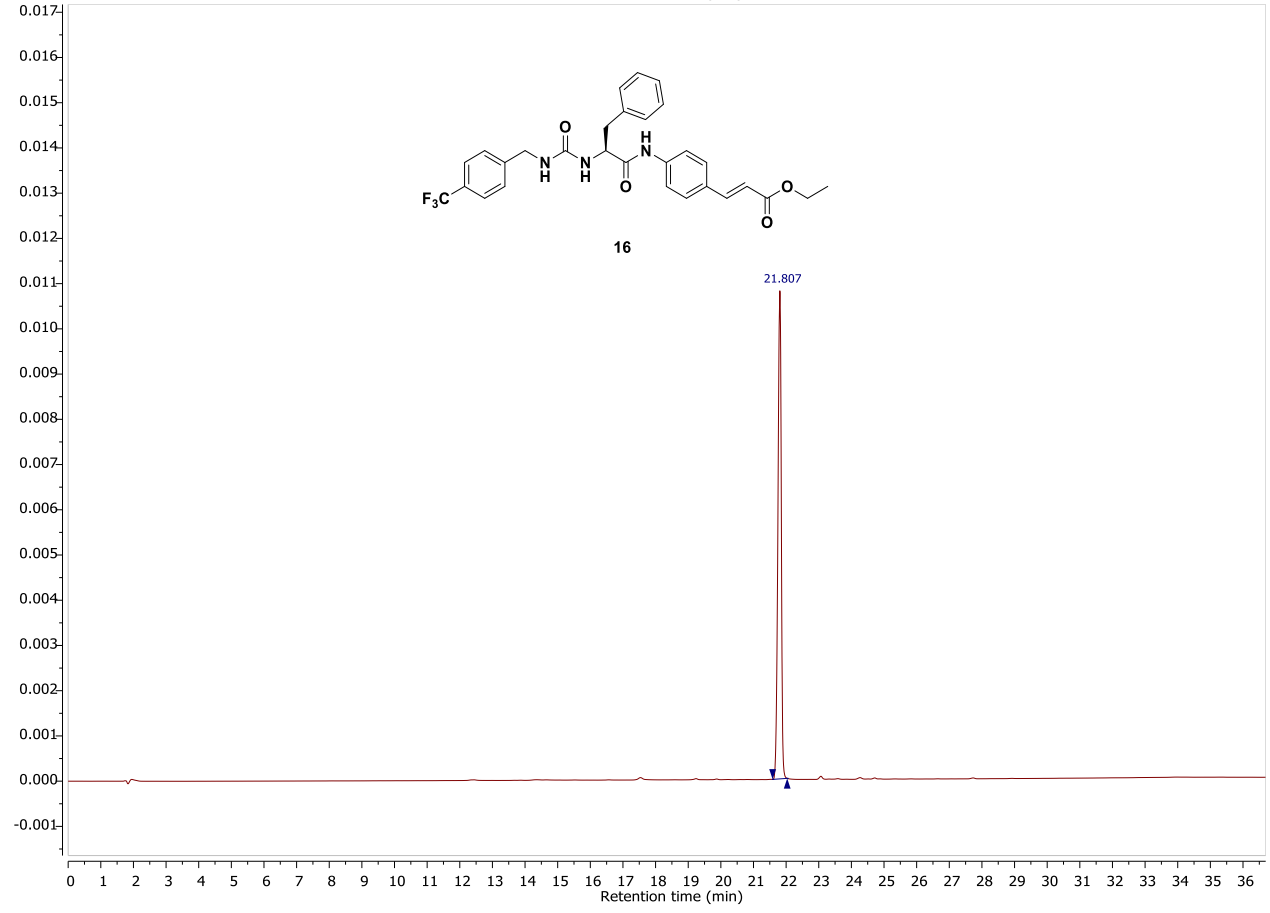
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21.807
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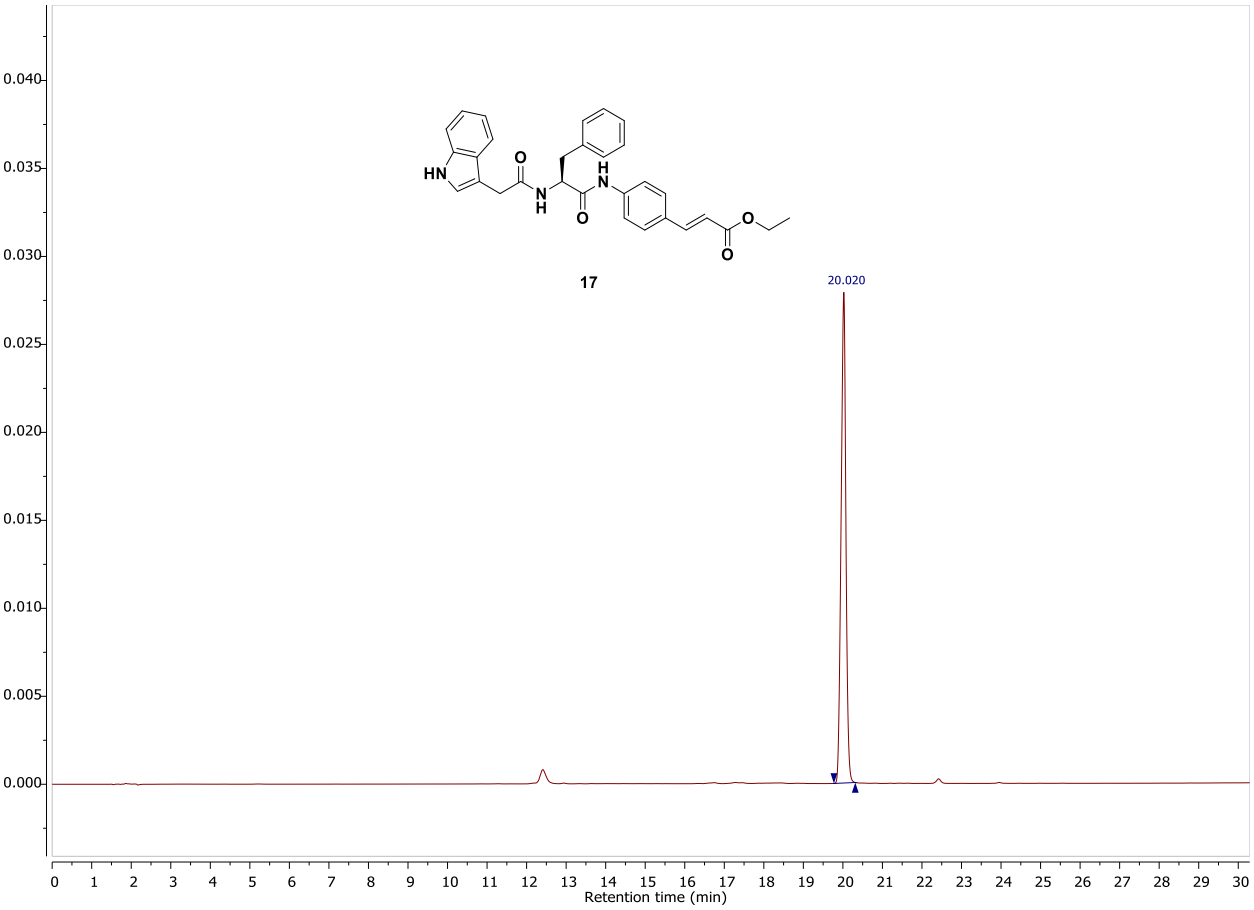
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20.020
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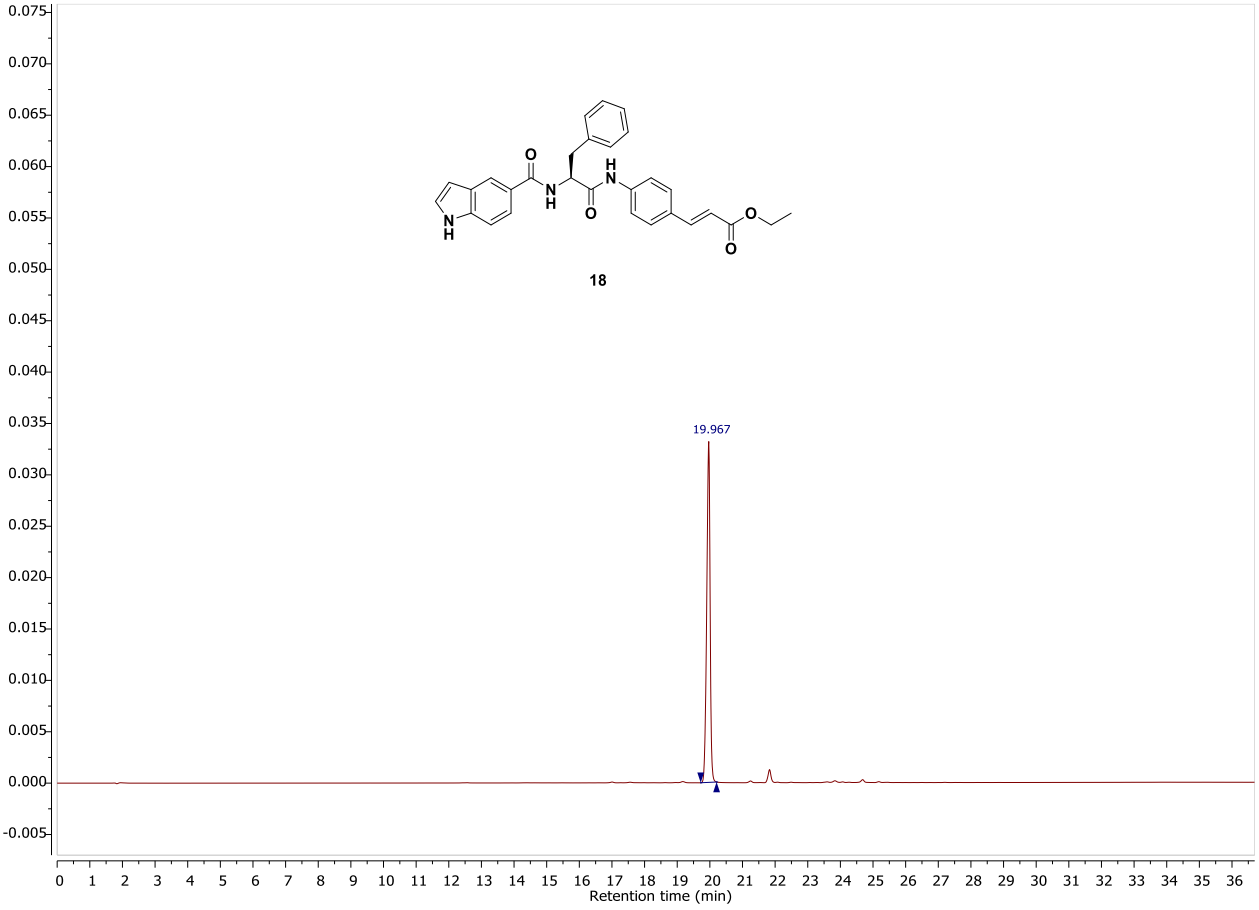
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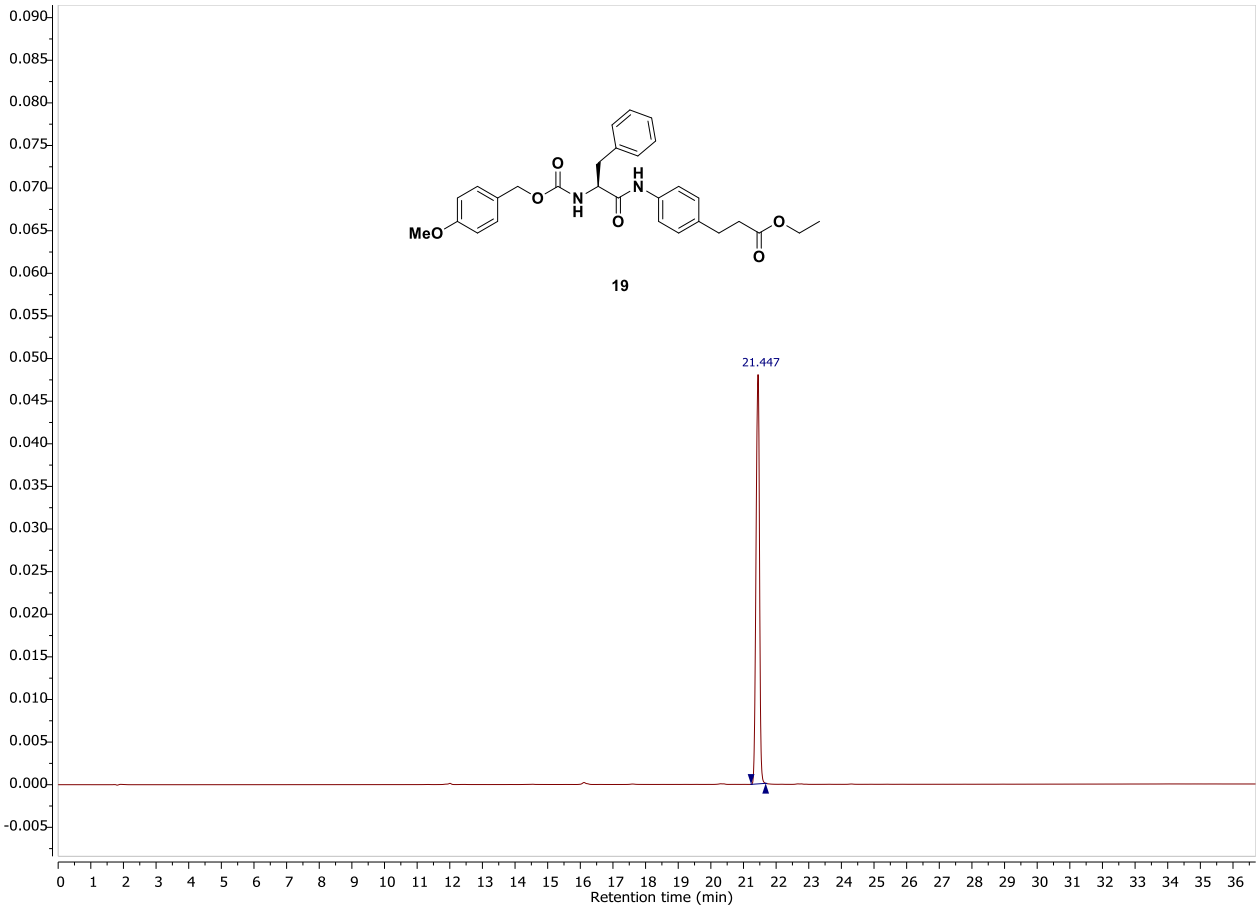
MeO
O
O
N
H
H
N
O
O
O
19
21.447
0.090
0.085
0.080
0.075
0.070
0.065
0.060
0.055
0.050
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
-0.005
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36
Retention time (min)

3. Figure S1

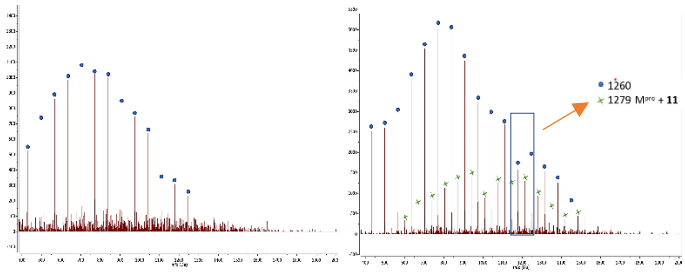

Figure S1. (A) Representative ESI-MS spectrum of a solution containing 0.4 μM SARS-CoV-2 M^{pro} in water/MeOH (1:1) with 0.1% HCOOH. The spectrum was acquired in positive ion mode. The blue dots correspond to the unmodified protein. (B) Representative ESI-MS spectrum of a mixture containing 0.4 mM SARS-CoV-2 M^{pro} after incubation with **11** in water/MeOH (1:1) with 0.1% HCOOH. The spectrum was acquired in positive ion mode. The blue dots correspond to the unmodified protein, and the green stars correspond to the modified protein.

4. Figure S2

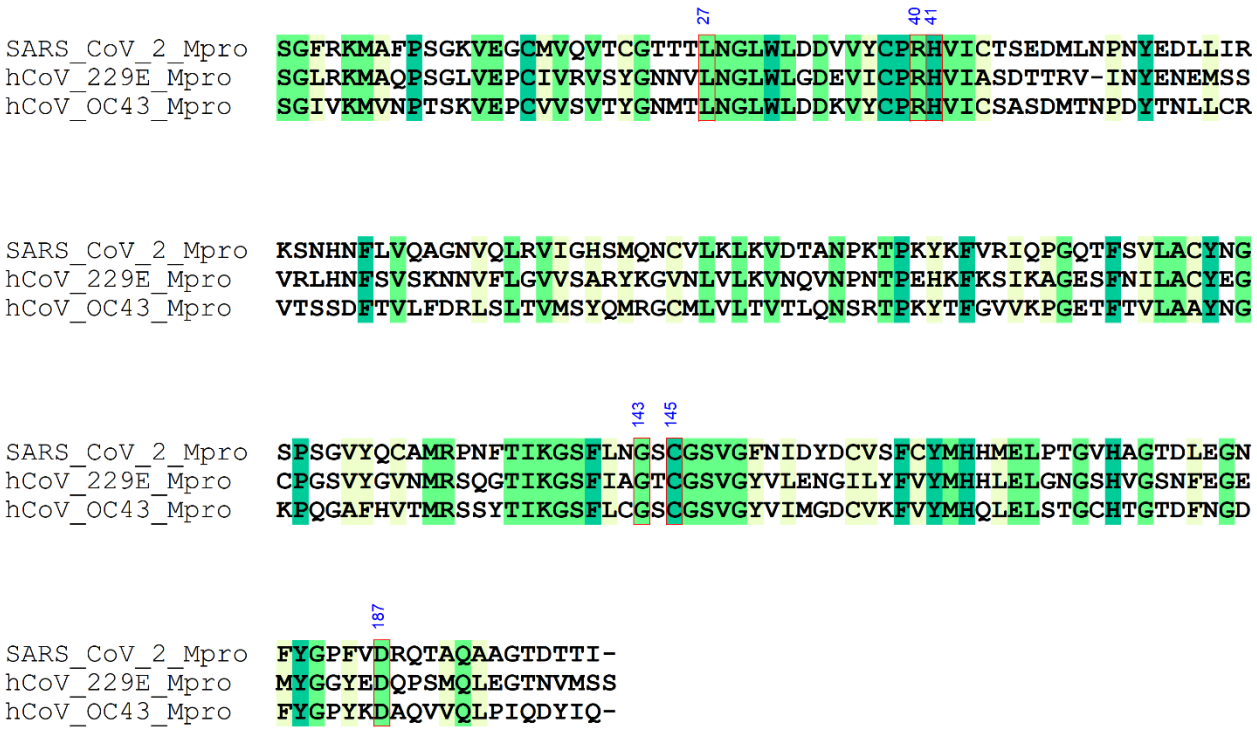

Figure S2. Sequence alignment of SARS-CoV-2 M^{pro}, hCoV-229E Mpro, and hCoV-OC43 M^{pro}. Conserved residues are labeled in dark green, the same residues are indicated in lighter green, and residues with similar properties are yellow. The key residues are highlighted by a red square and the number of sequence positions in navy blue.

Table S1. Antiviral activity of cinnamic esters against hCoV-229E and hCoV-OC43. Notes: EC_{50} was measured for hCoV-229E by evaluating the residual MRC-5 cell viability as a surrogate of viral CPE, or by focus forming reduction assay (FFRA) against hCoV-OC43 in HCT-8 cells.

Compound	hCoV-229E EC_{50} (μM)	hCoV-OC43 EC_{50} (μM)
11	> 50	> 50
12	5.27 ± 0.25	> 50
13	> 50	> 50
15	> 50	> 50
17	> 50	9.14 ± 0.70
18	> 50	10.1 ± 0.17
19	> 50	> 50