

## Supplementary Information:

### Ultrapotent Inhibitor of *Clostridioides difficile* Growth, which Suppresses Recurrence *in vivo*

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<sup>b</sup> Department of Comparative Pathobiology, Purdue University College of Veterinary Medicine, West Lafayette, Indiana 47907, United States

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<sup>d</sup> Purdue Institute of Inflammation, Immunology, and Infectious Disease, West Lafayette, Indiana 47907, United States

I. Bacterial Strains Used in Study

II. <sup>1</sup>H, <sup>13</sup>C, and <sup>19</sup>F Spectra of Analogs

III. HPLC Traces of Compounds **1-17**

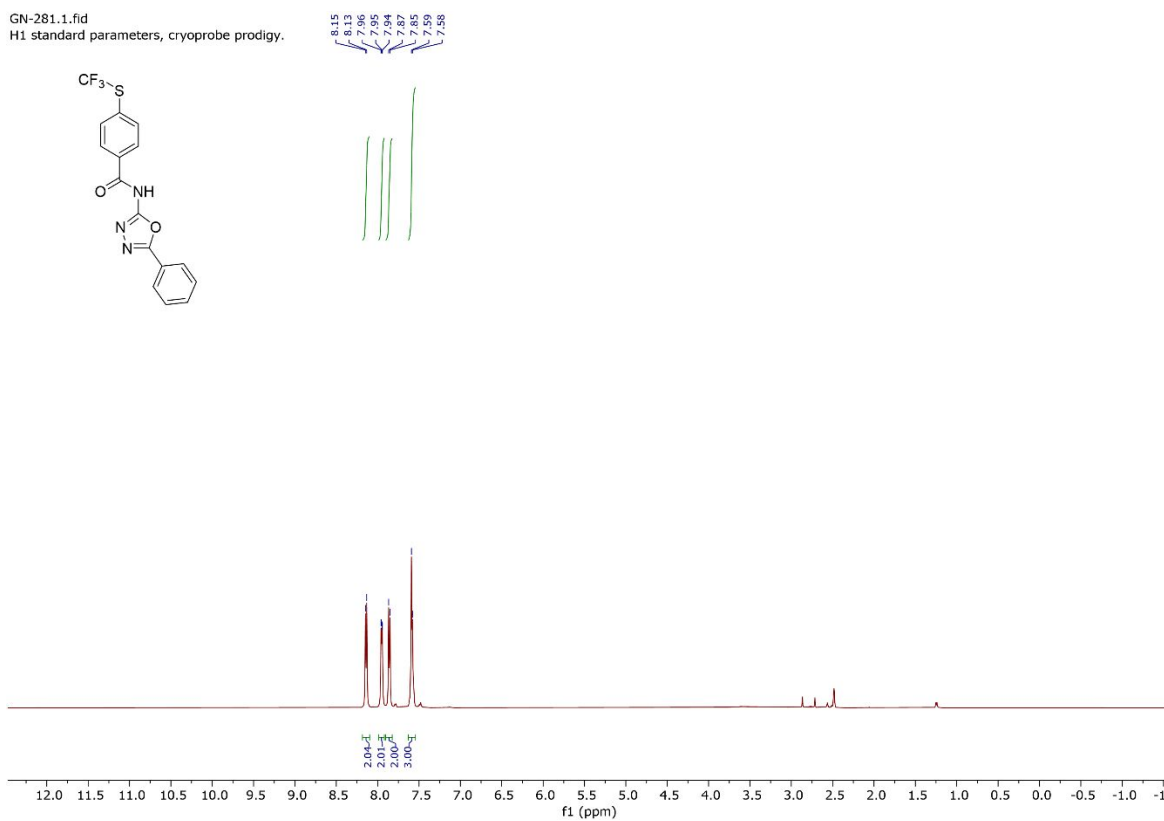
**I. Table 1S. Bacterial strains used in this study.**

<b>Bacterial Strains</b>	<b>Source/Description</b>
<i>C. difficile</i> ATCC BAA-1801 (3232)	Human feces of an adult in Belgium. Non-toxigenic, Ribotype 010.
<i>C. difficile</i> NR-13432 (Isolate 6)	Human patient in USA, 2008/2009.
<i>C. difficile</i> NR-13435 (Isolate 9)	Human patient in USA, 2008/2009.
<i>C. difficile</i> P2 (NR-32883)	Human patient in Western Pennsylvania, USA, 2001. Toxigenic
<i>C. difficile</i> P13 (NR-32891)	Human patient in Western Pennsylvania, USA, 2005. Toxigenic
<i>C. difficile</i> P19 (NR-32895)	Human patient in Western Pennsylvania, USA, 2005. Toxigenic
<i>C. difficile</i> P30 (NR-32904)	Asymptomatic human patient in Western Pennsylvania, USA, 2009. Non-toxigenic
<i>C. difficile</i> ATCC 43255 (VPI 10463)	An abdominal wound. Ribotype 087, <i>tcdA<sup>a</sup></i> and <i>tcdB<sup>b</sup></i> genes
<i>Lactobacillus gasseri</i> HM-400 (EX336960VC03)	From a human mid-vaginal wall in March 2010 in Richmond, Virginia. Reference genome for the Human Microbiome Project (HMP).
<i>Lactobacillus crispatus</i> HM-103 (JV-V01)	From normal human vaginal flora. Reference genome for the Human Microbiome Project (HMP).
<i>Lactobacillus crispatus</i> HM-371 (EX849587VC02)	The mid-vaginal wall of a human patient, Virginia, March 2010 Reference genome for the Human Microbiome Project (HMP).
<i>Bacteroides fragilis</i> HM-711 (CL05T00C42)	Healthy adult human feces, Massachusetts, USA. Reference genome for the Human Microbiome Project (HMP).
<i>Bacteroides fragilis</i> HM-709 (CL07T00C01)	Healthy adult human feces, Massachusetts, USA. Reference genome for the Human Microbiome Project (HMP).
<i>Bacteroides dorei</i> HM-719 (CL02T12C06)	Healthy adult human feces, Massachusetts, USA. Reference genome for the Human Microbiome Project (HMP).

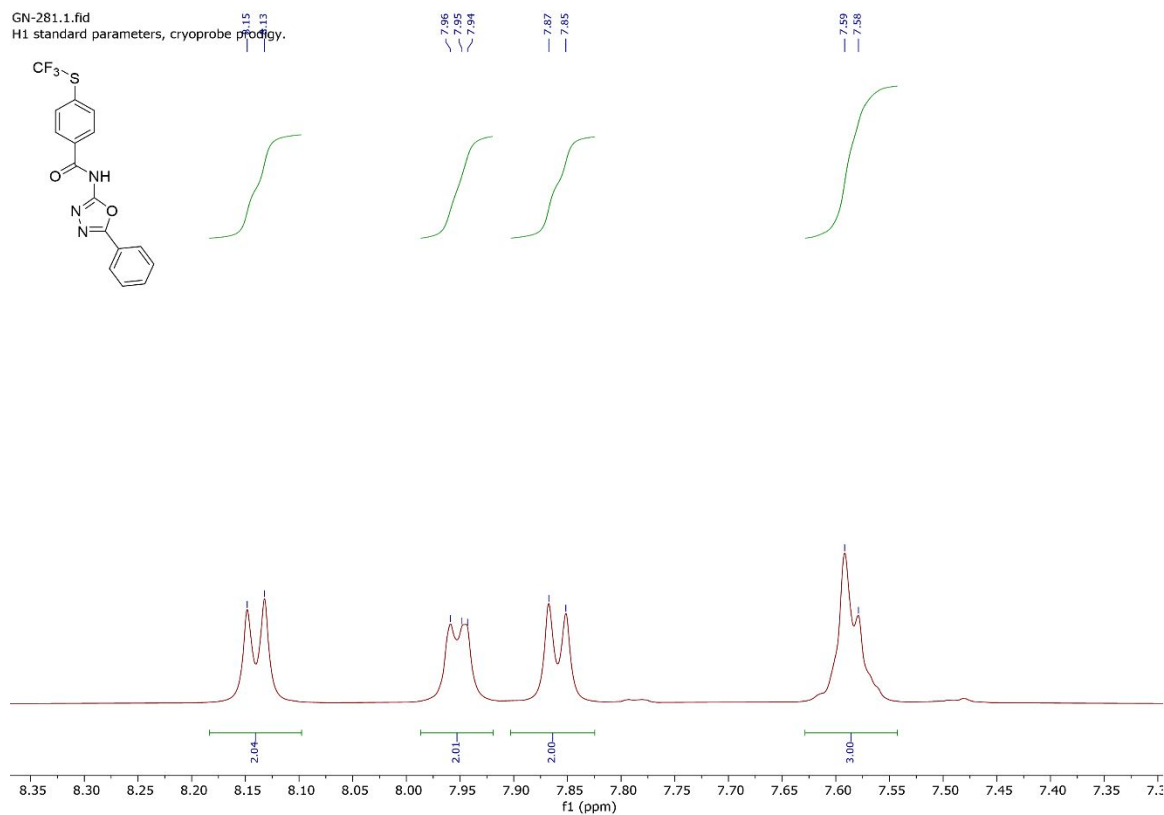
<sup>a</sup>*tcdA*, toxin A gene; <sup>b</sup>*tcdB*, toxin B gene

## II. $^1\text{H}$ , $^{13}\text{C}$ , and $^{19}\text{F}$ NMR Spectra of Analogs:

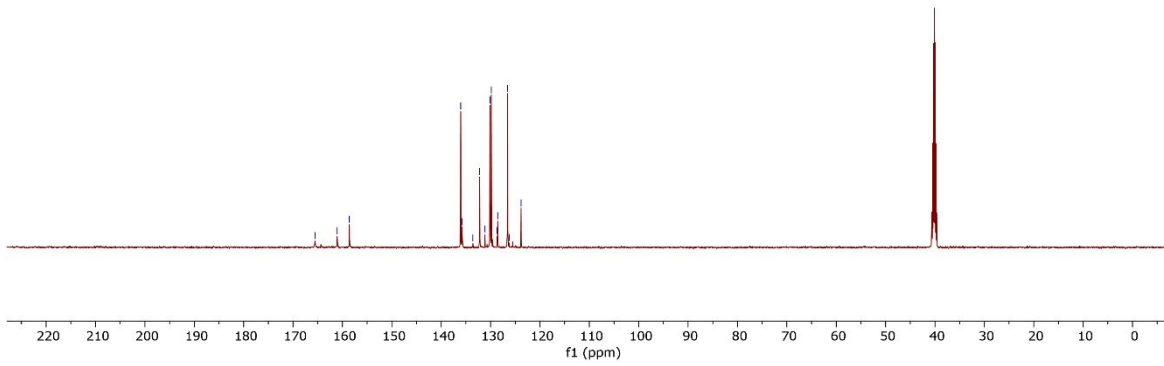
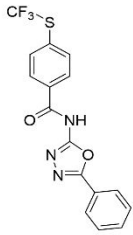
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H1 standard parameters, cryoprobe prodigy.



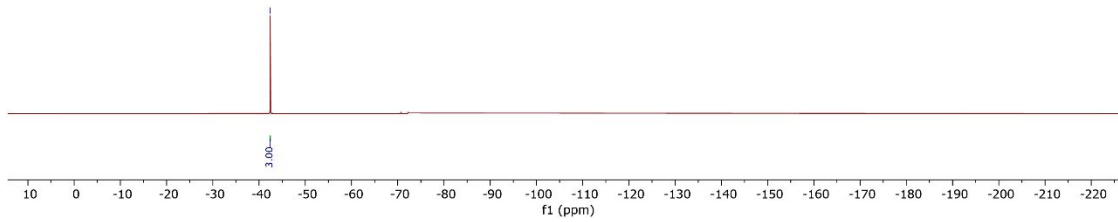
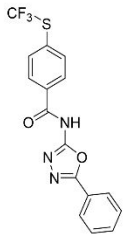
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H1 standard parameters, cryoprobe prodigy.



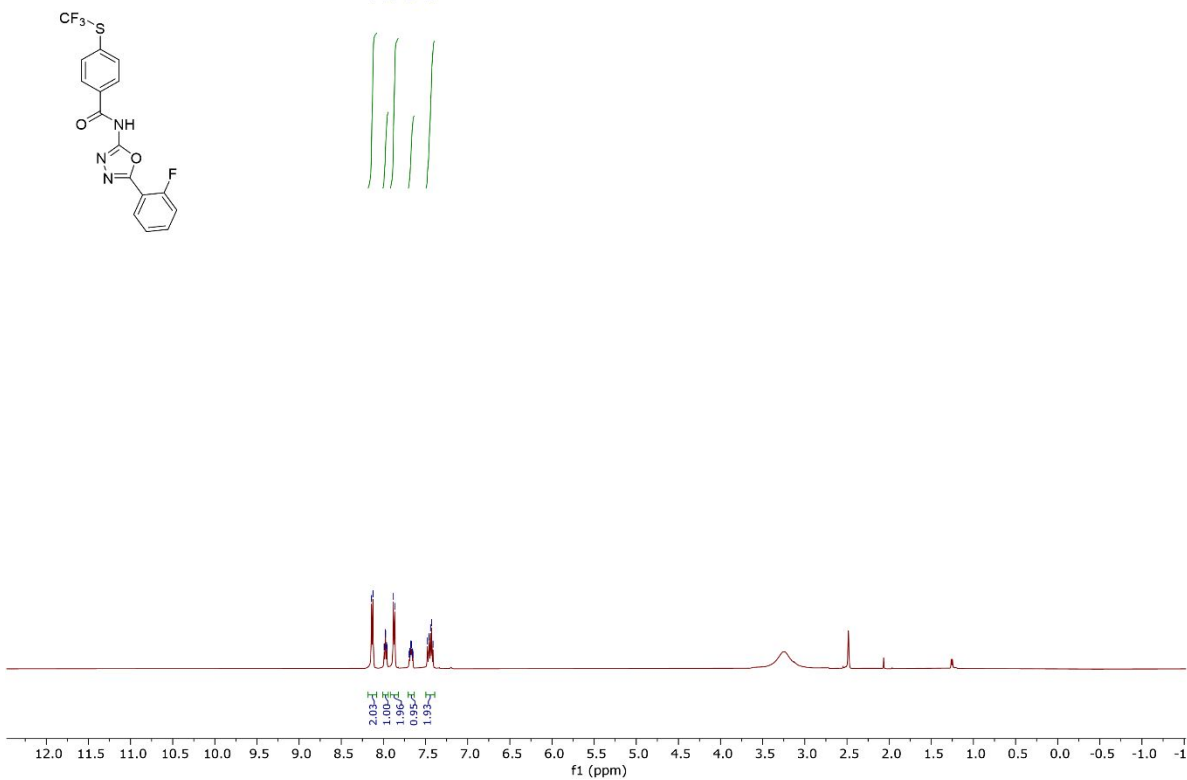
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 C13 standard parameters, cryoprobe prodigy. PULPROG = zgpg30



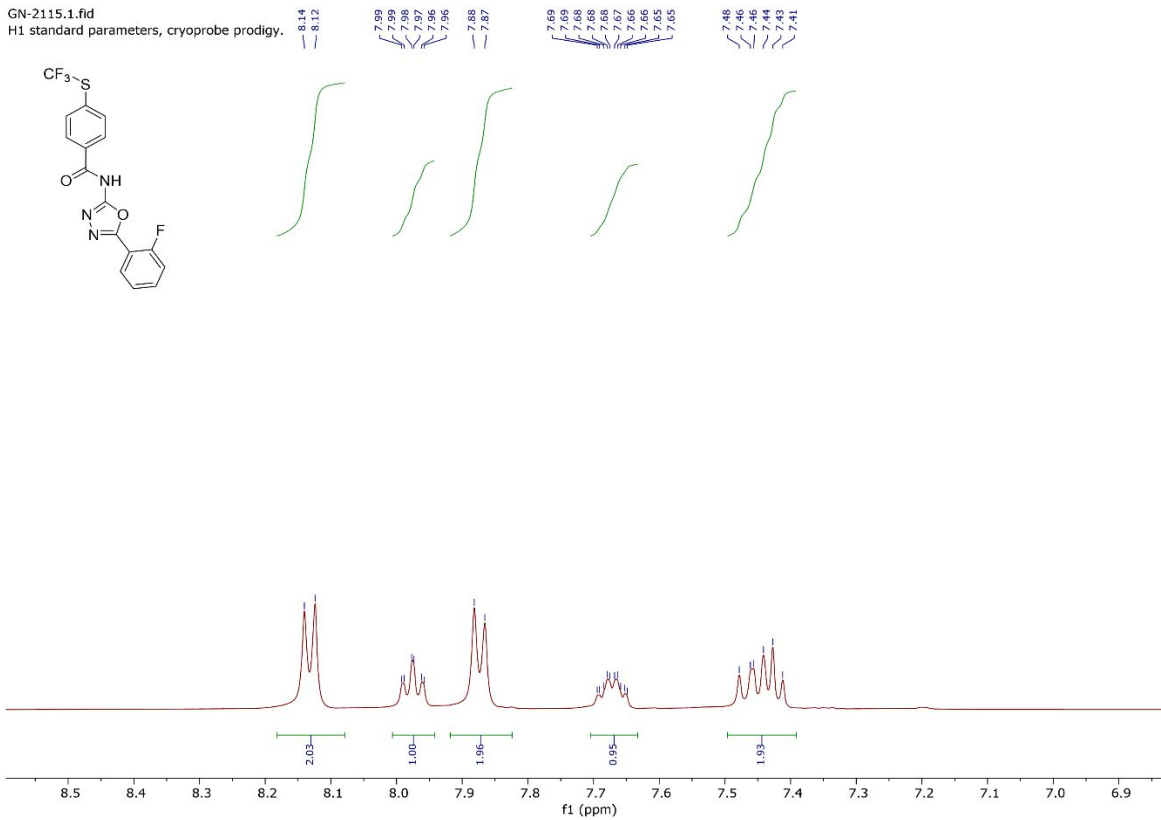
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 F19 standard parameters, cryoprobe prodigy.  
 DE is lengthened compared to default.



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H1 standard parameters, cryoprobe prodigy

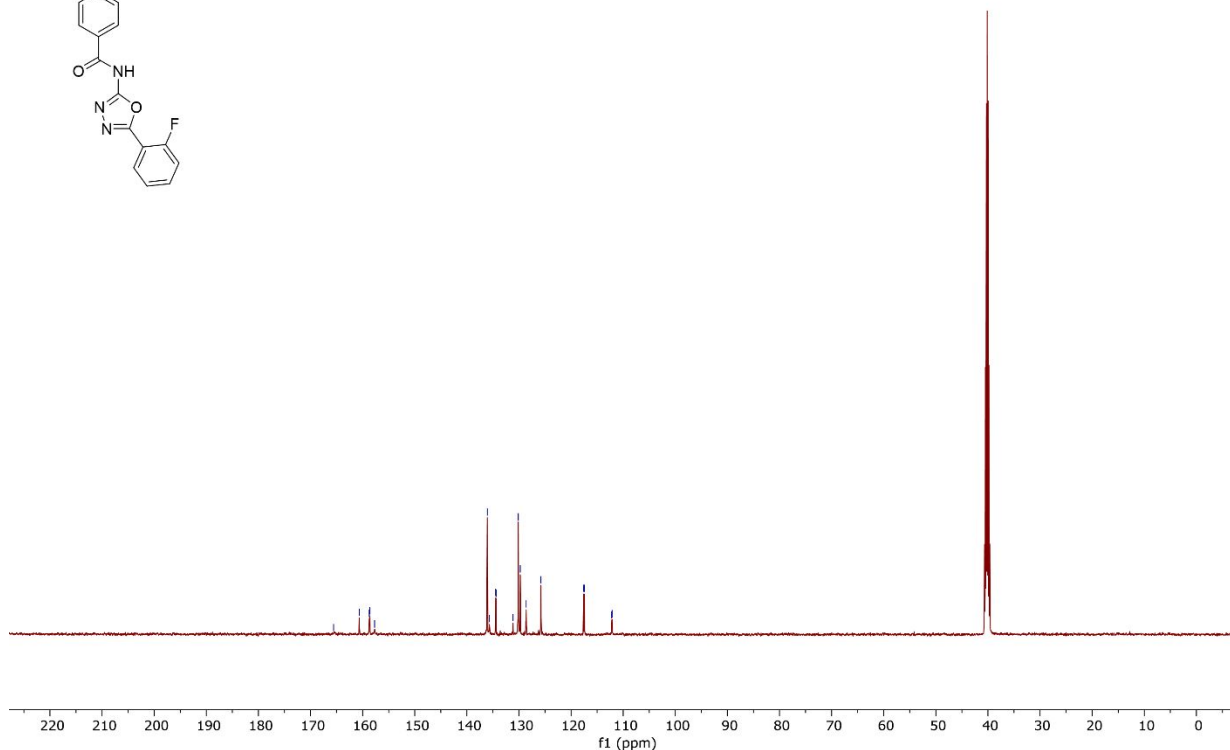
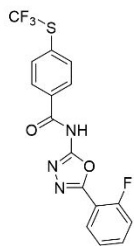


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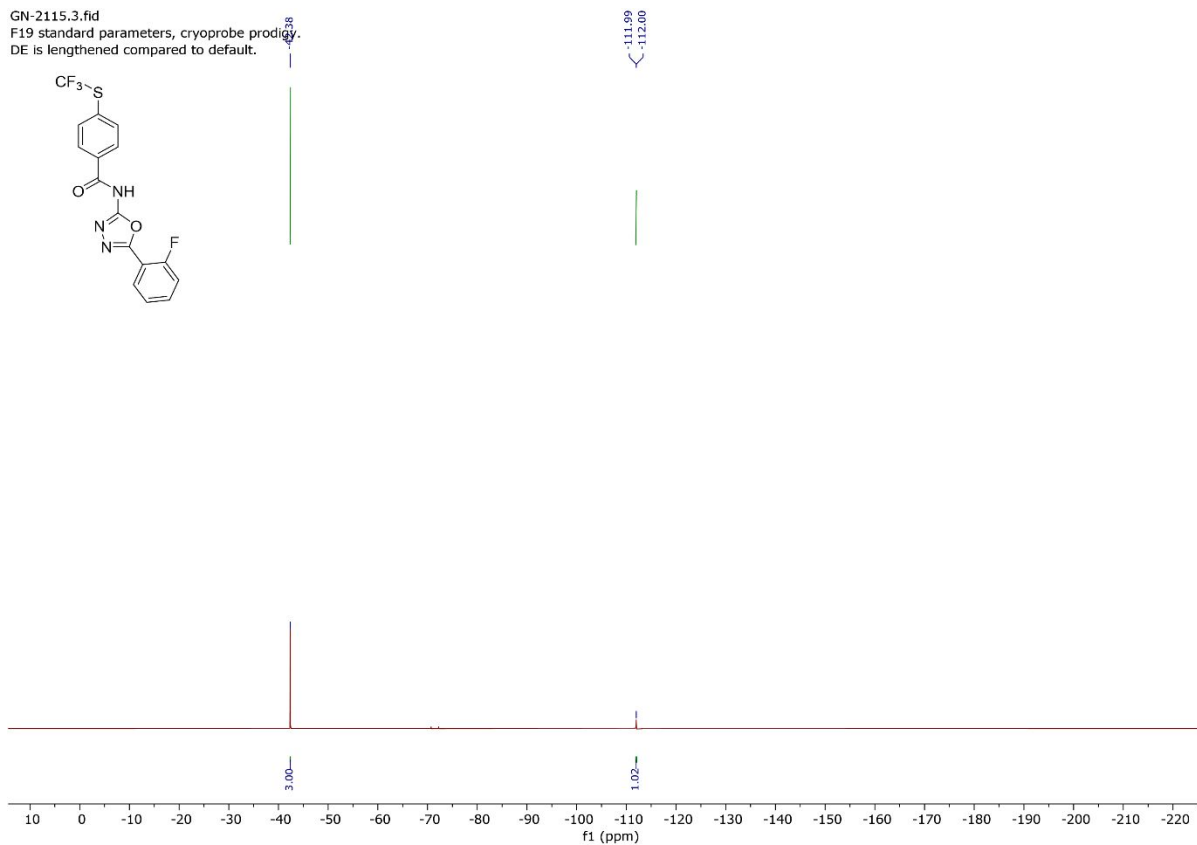
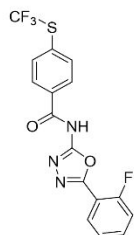
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C13 standard parameters, cryoprobe prodigy.

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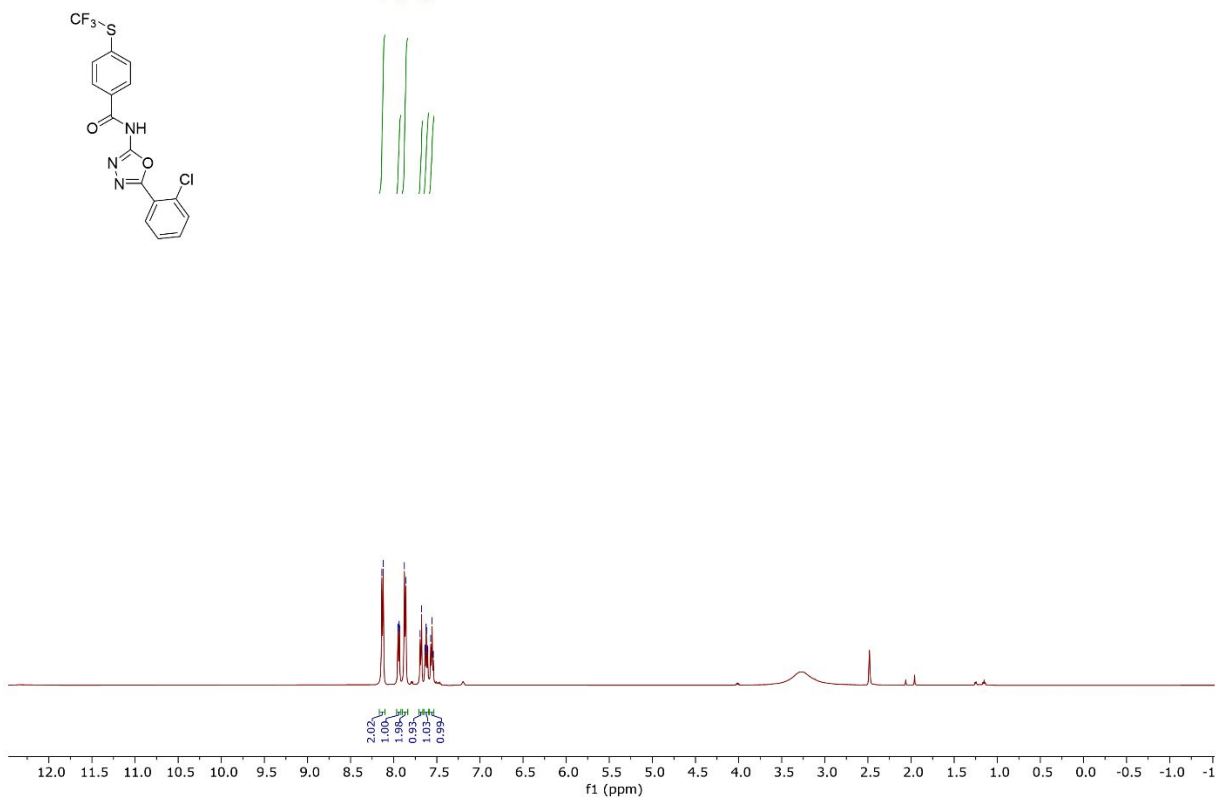


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DE is lengthened compared to default.

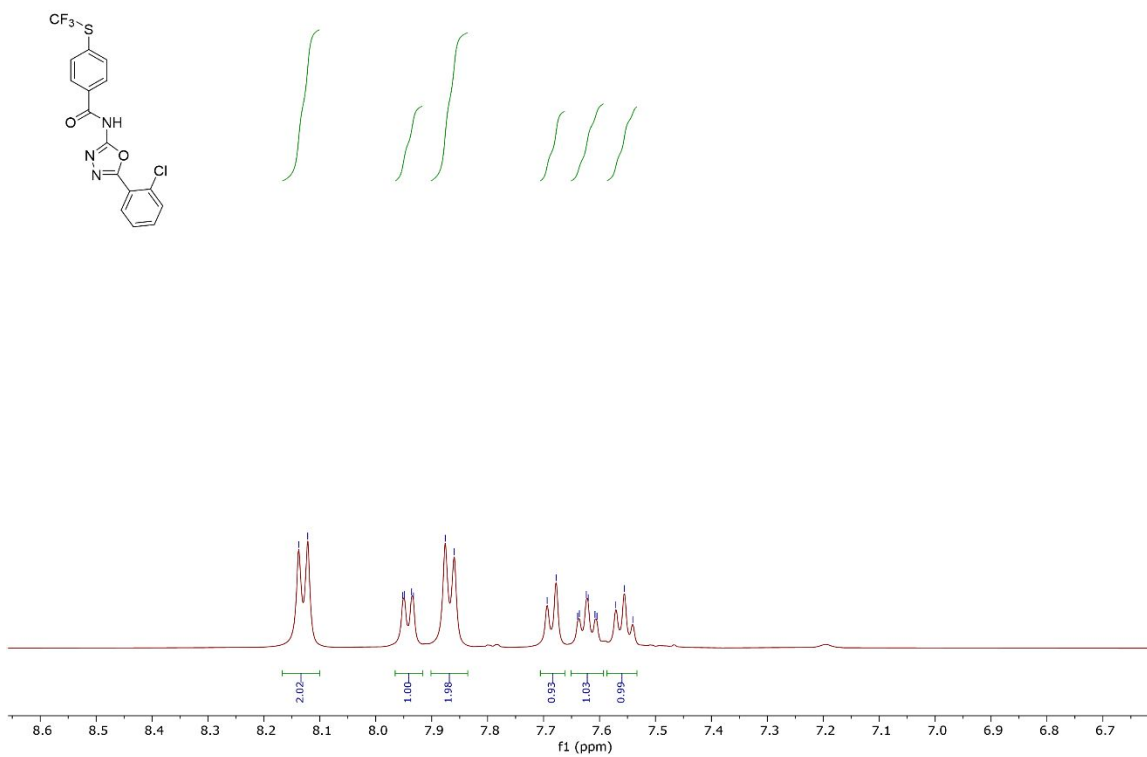
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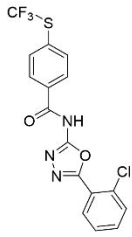


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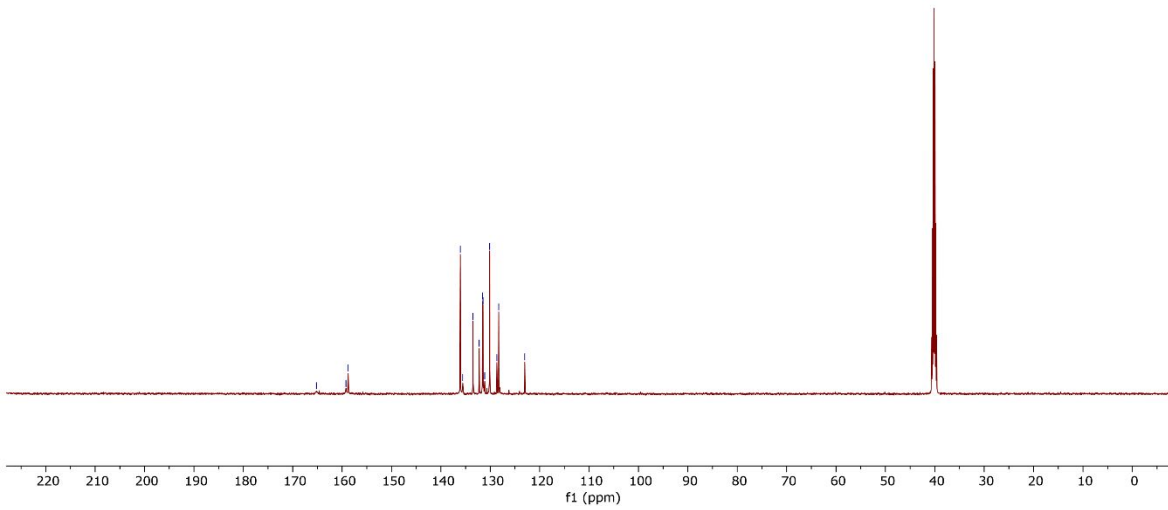


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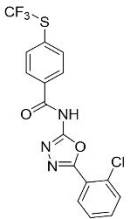


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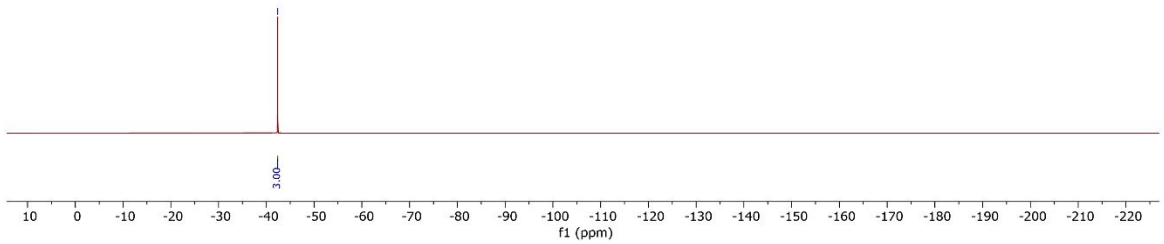


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F19 standard parameters, cryoprobe prodigy.  
DE is lengthened compared to default.

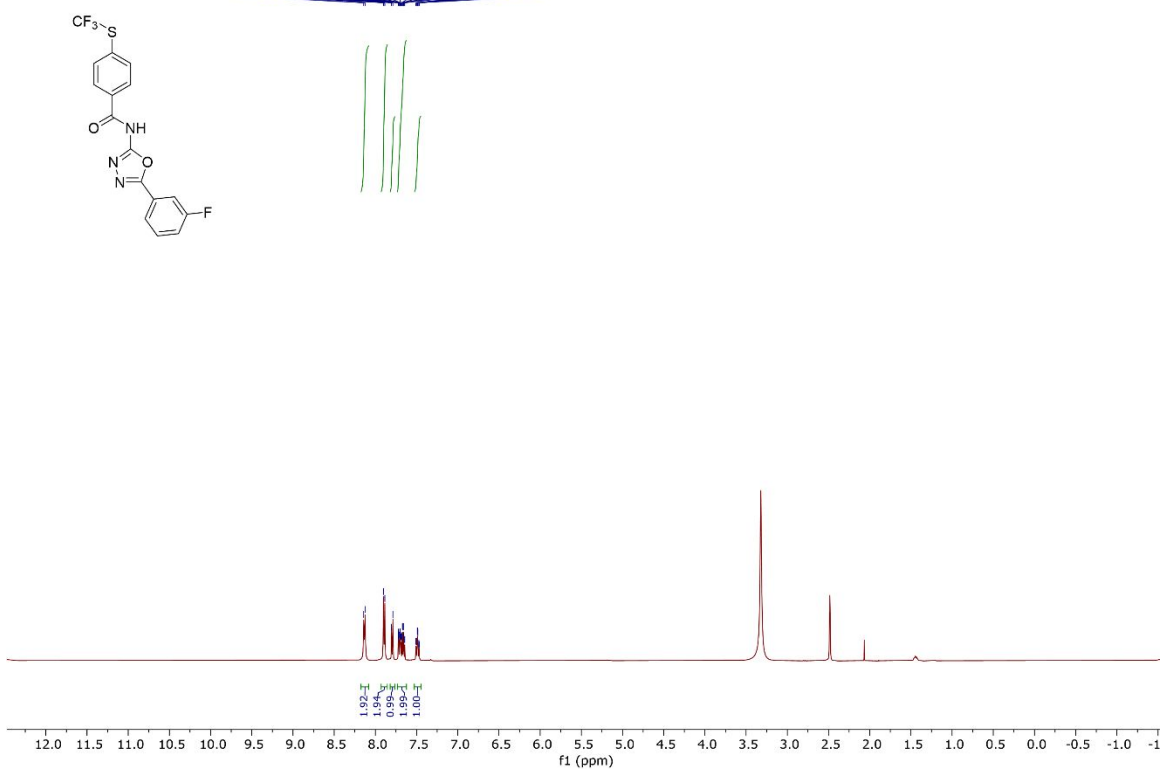


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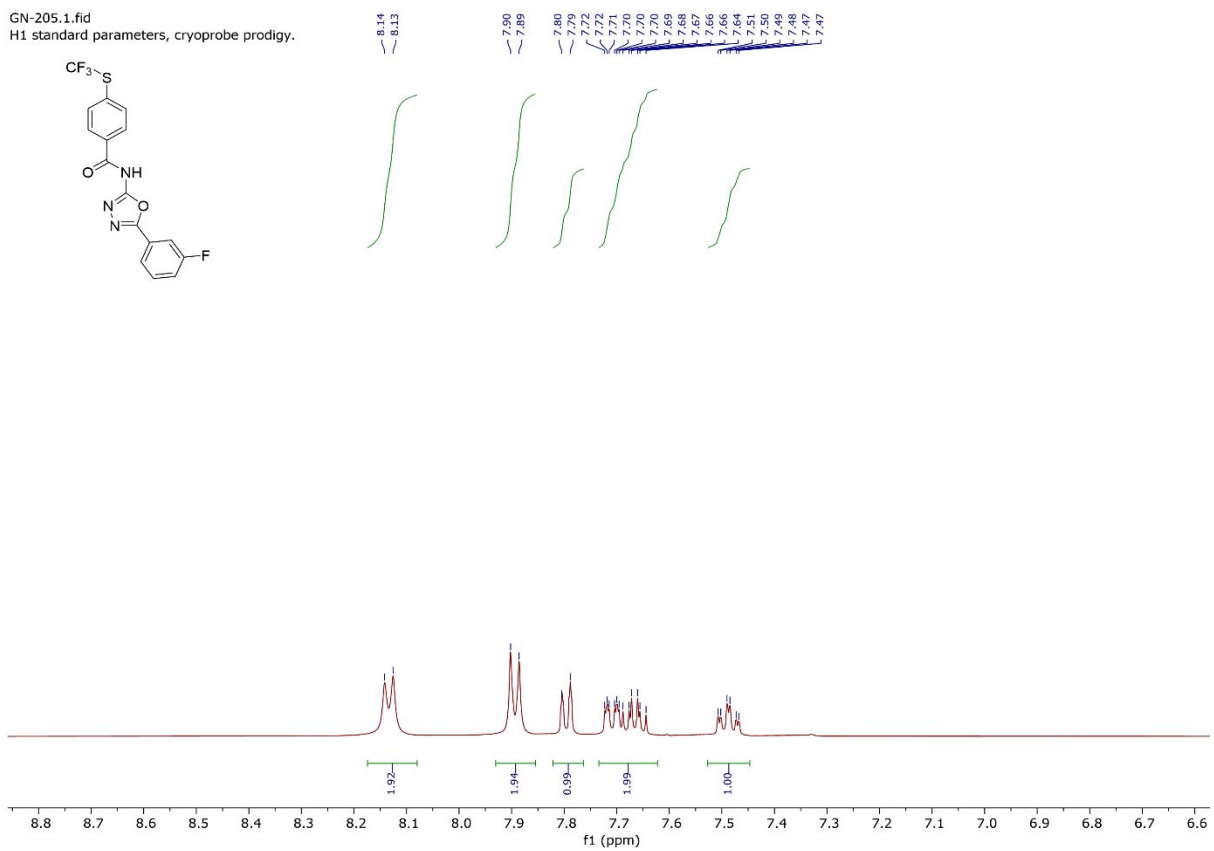




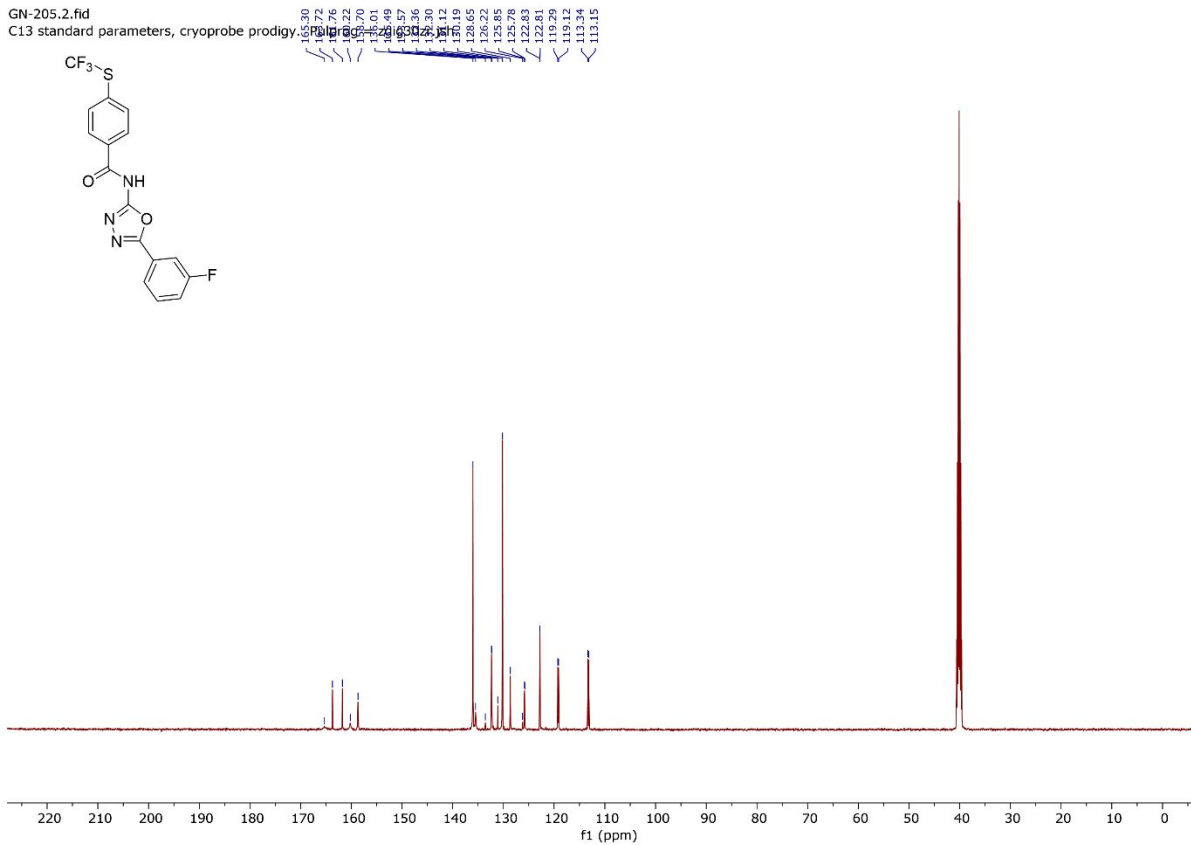
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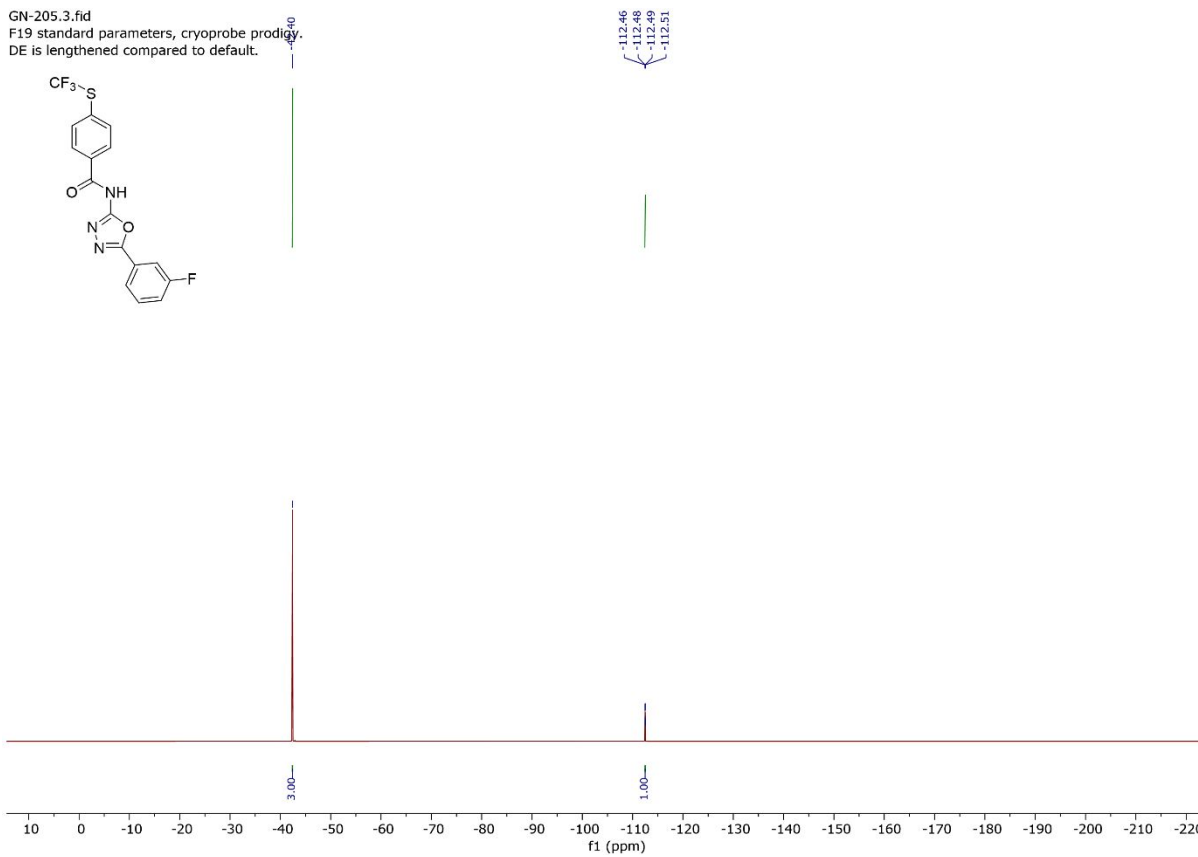
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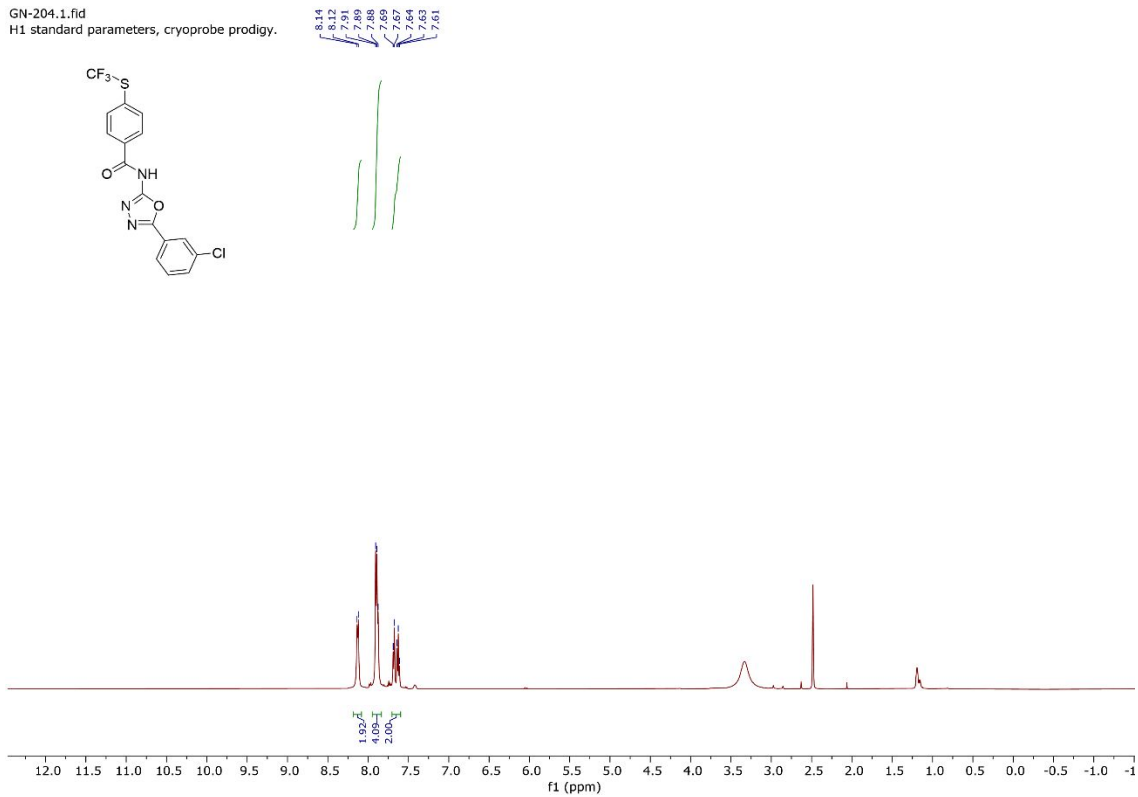
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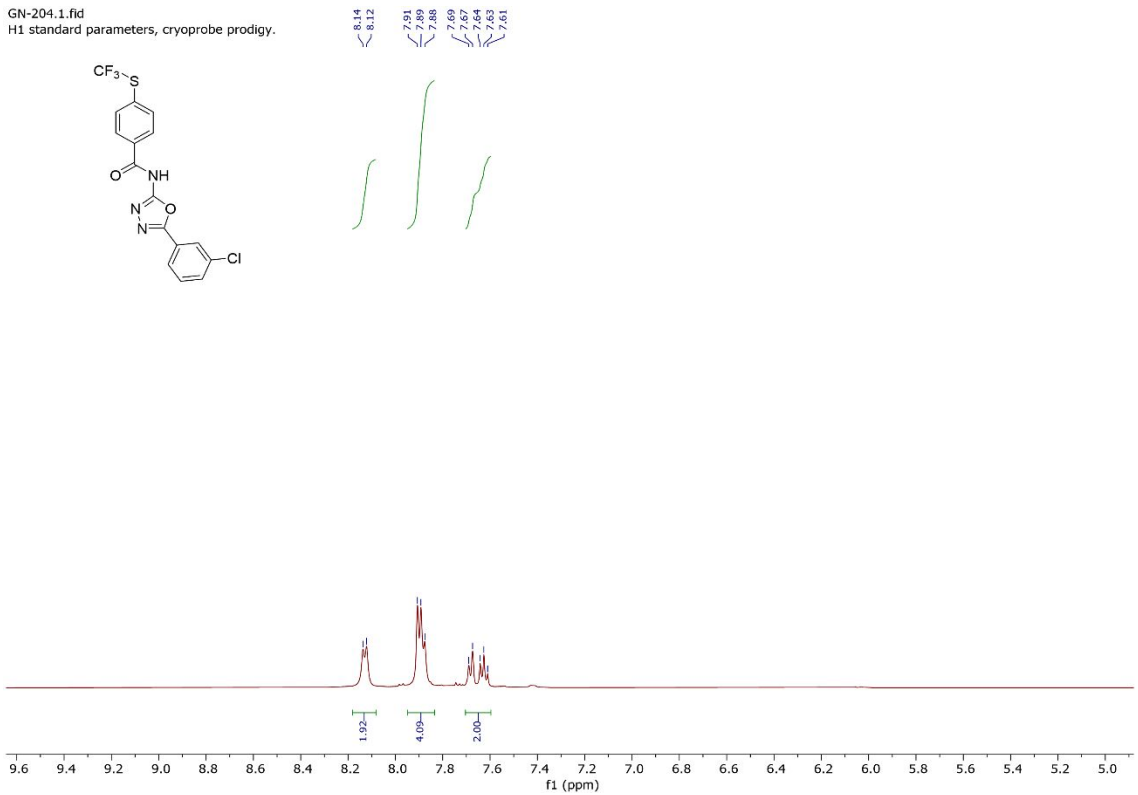
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DE is lengthened compared to default.



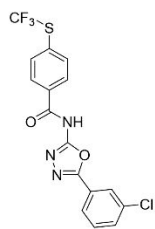
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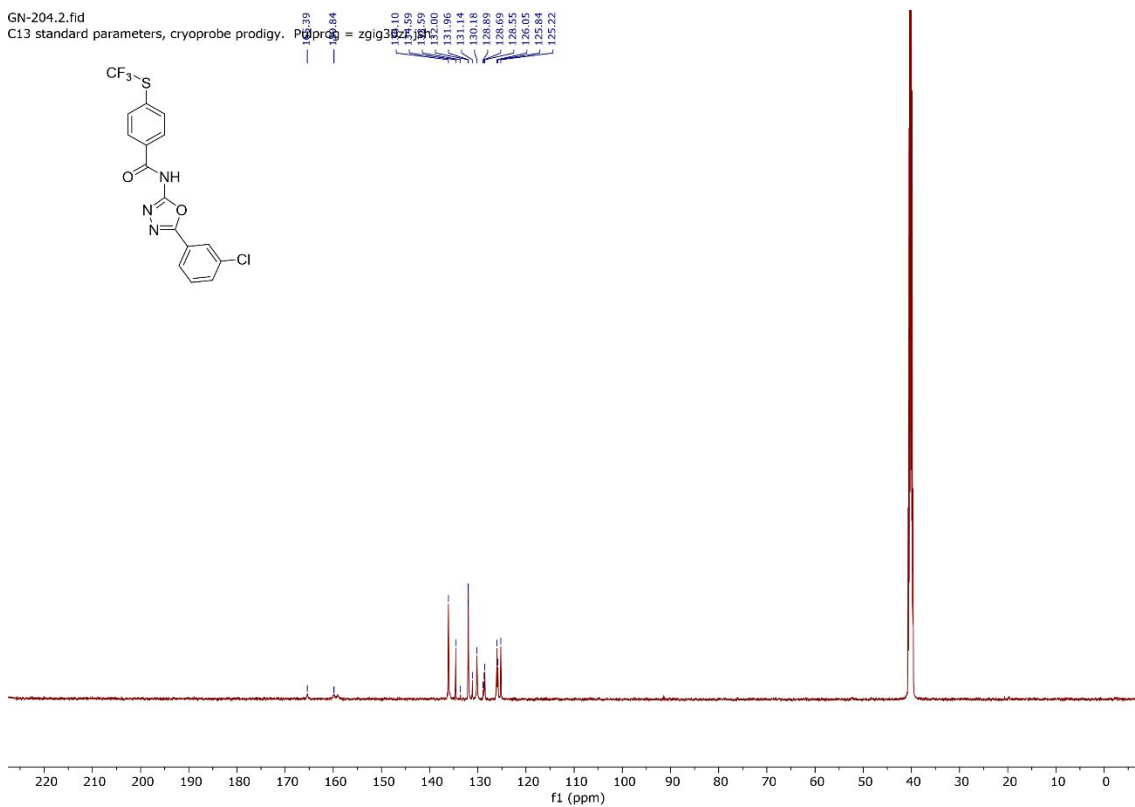
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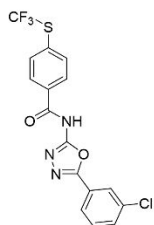
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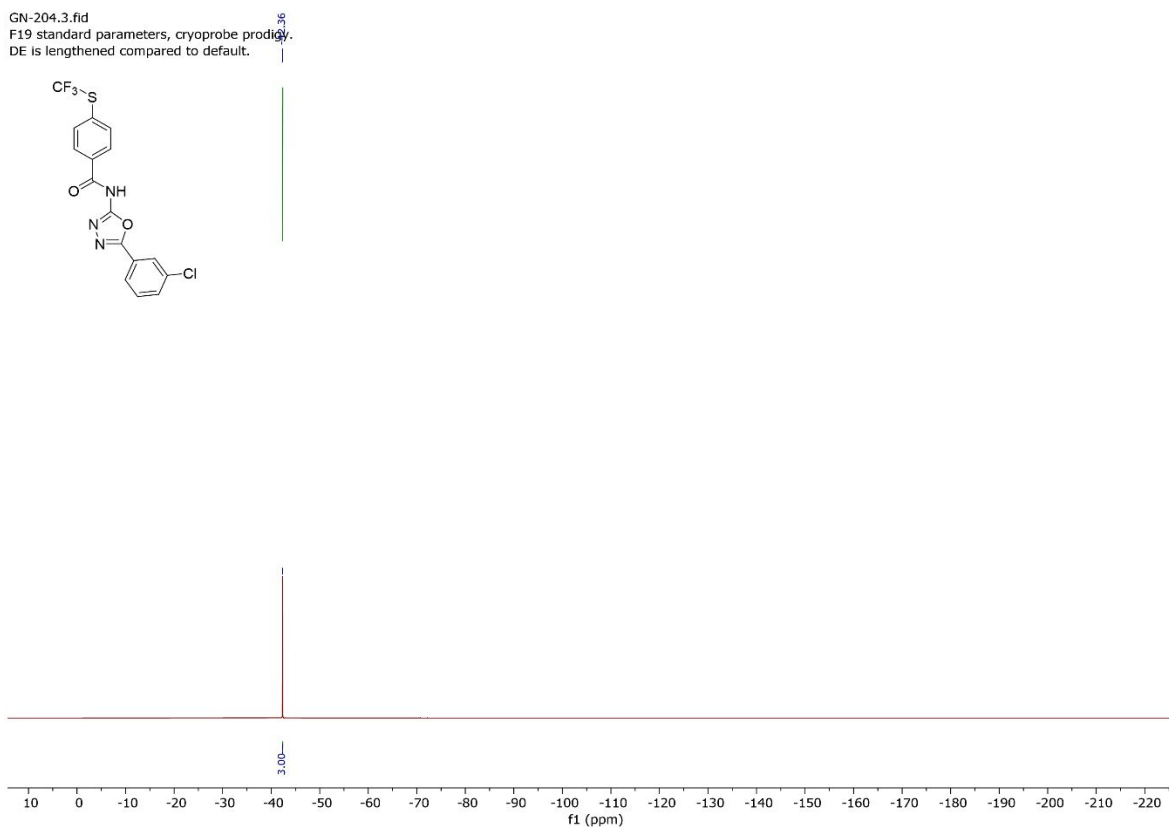
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128.69  
126.05  
125.84  
125.22



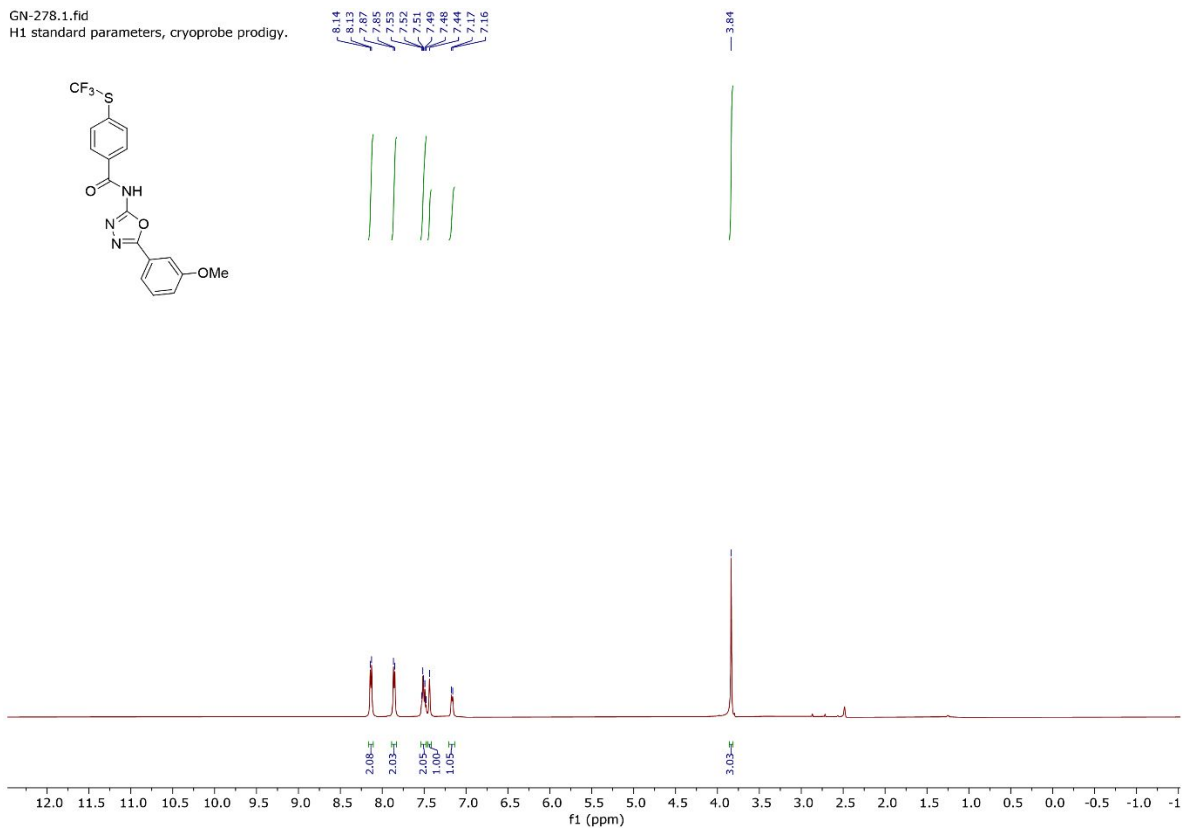
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DE is lengthened compared to default.



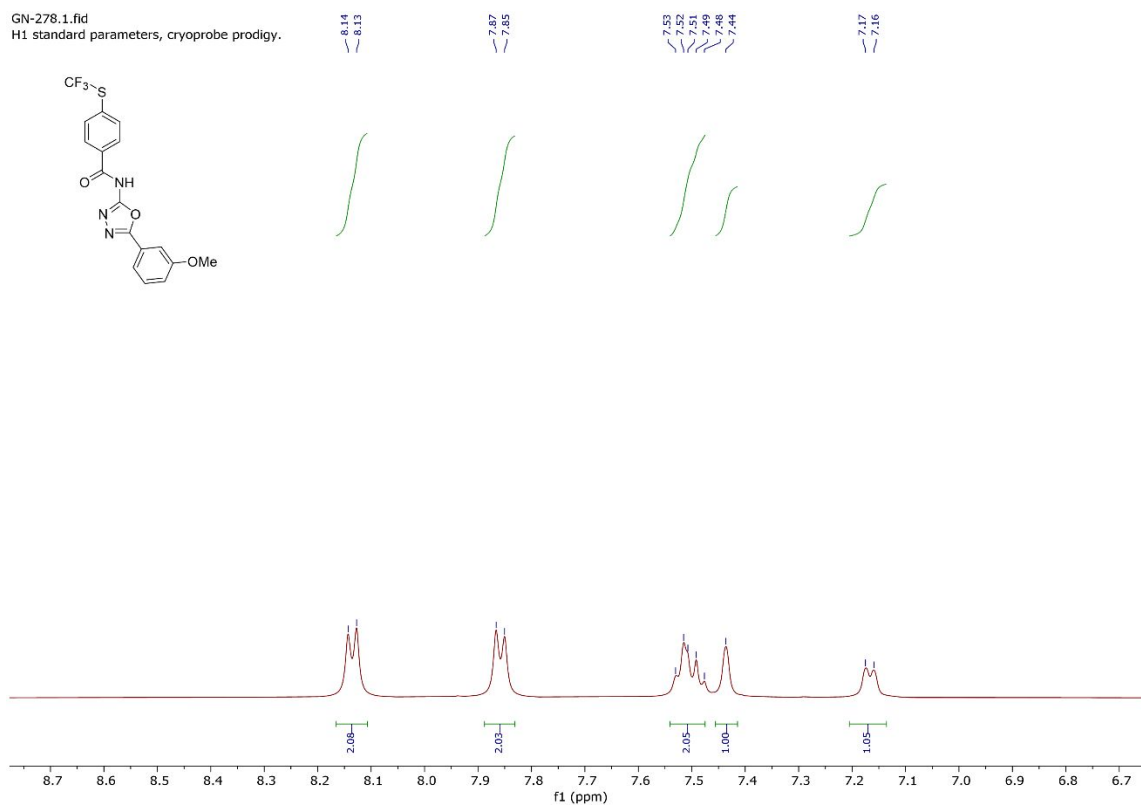
39.236



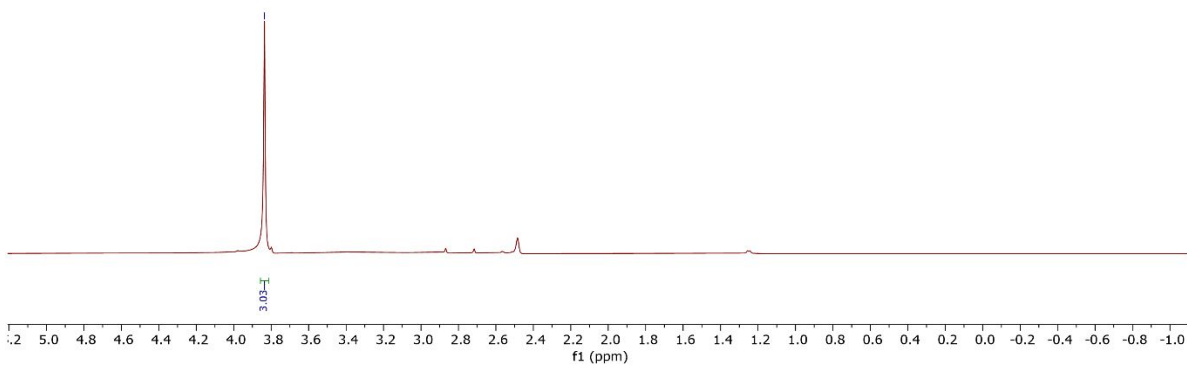
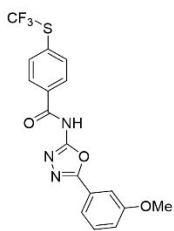
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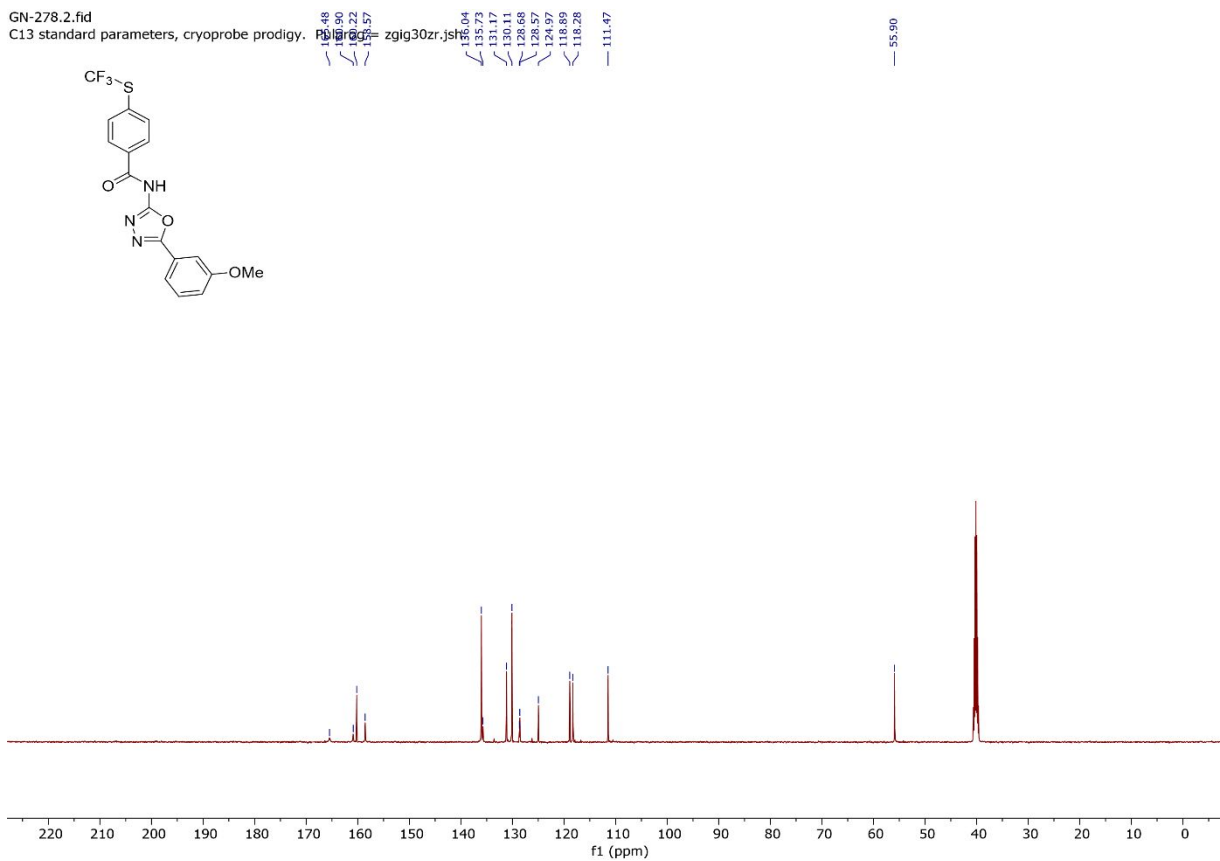
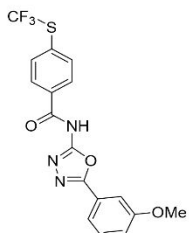
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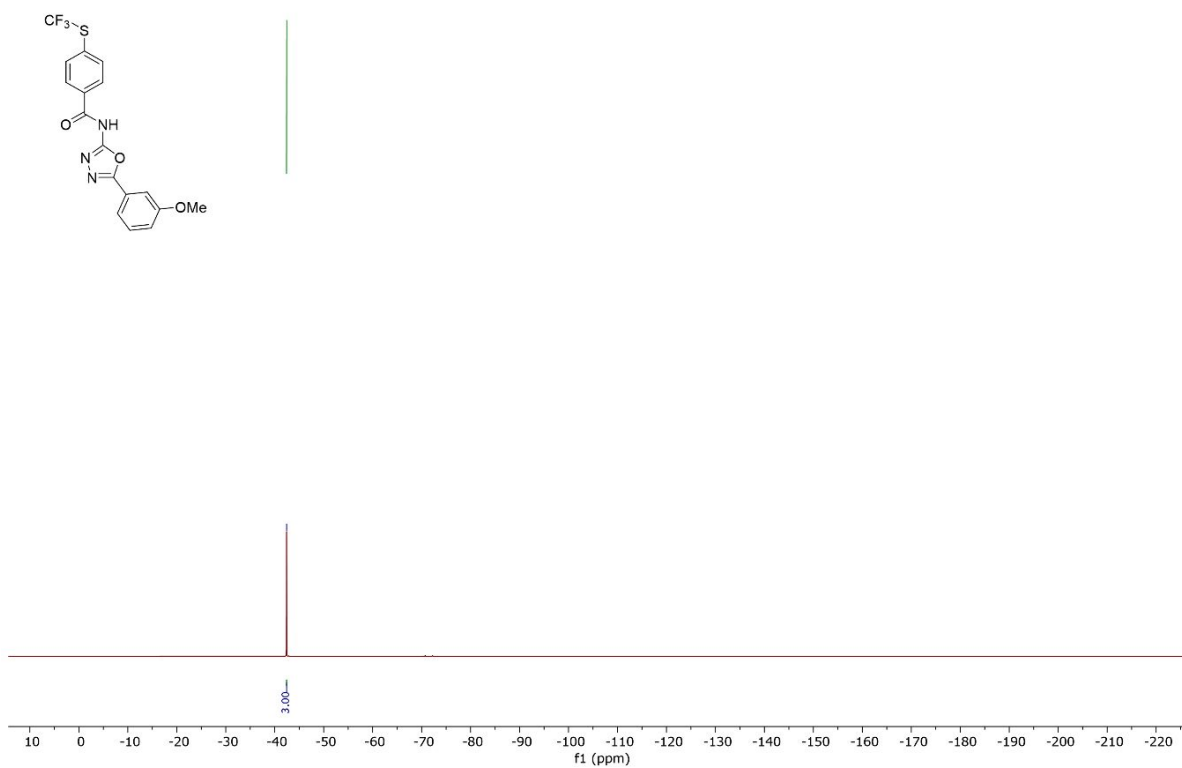
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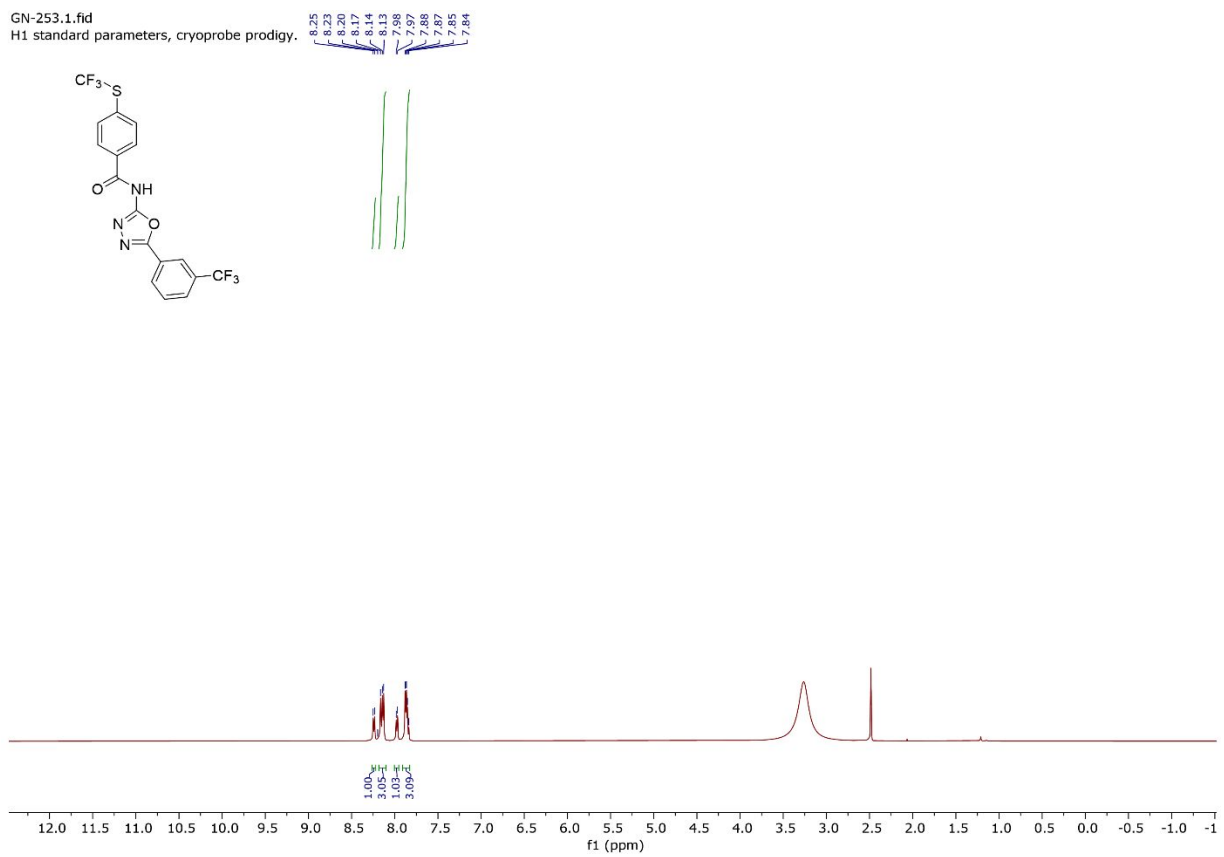
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C13 standard parameters, cryoprobe prodigy.



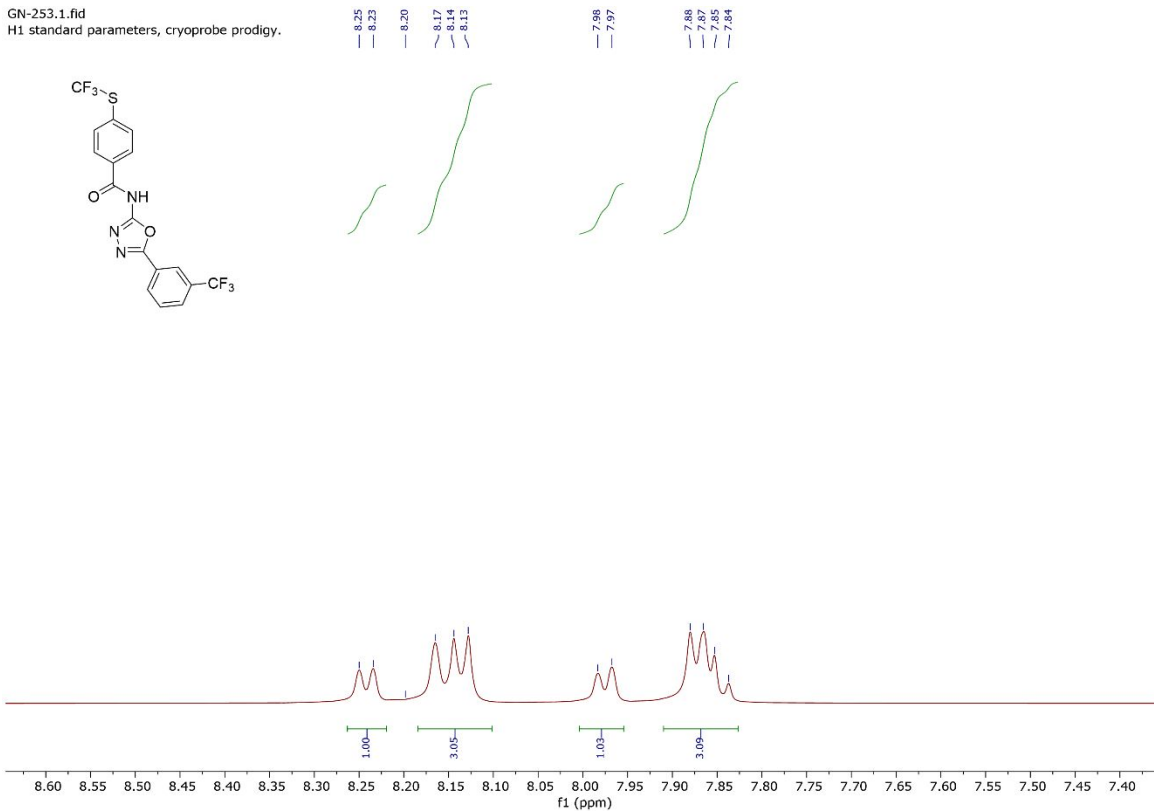
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F19 standard parameters, cryoprobe prodigy.  
DE is lengthened compared to default.



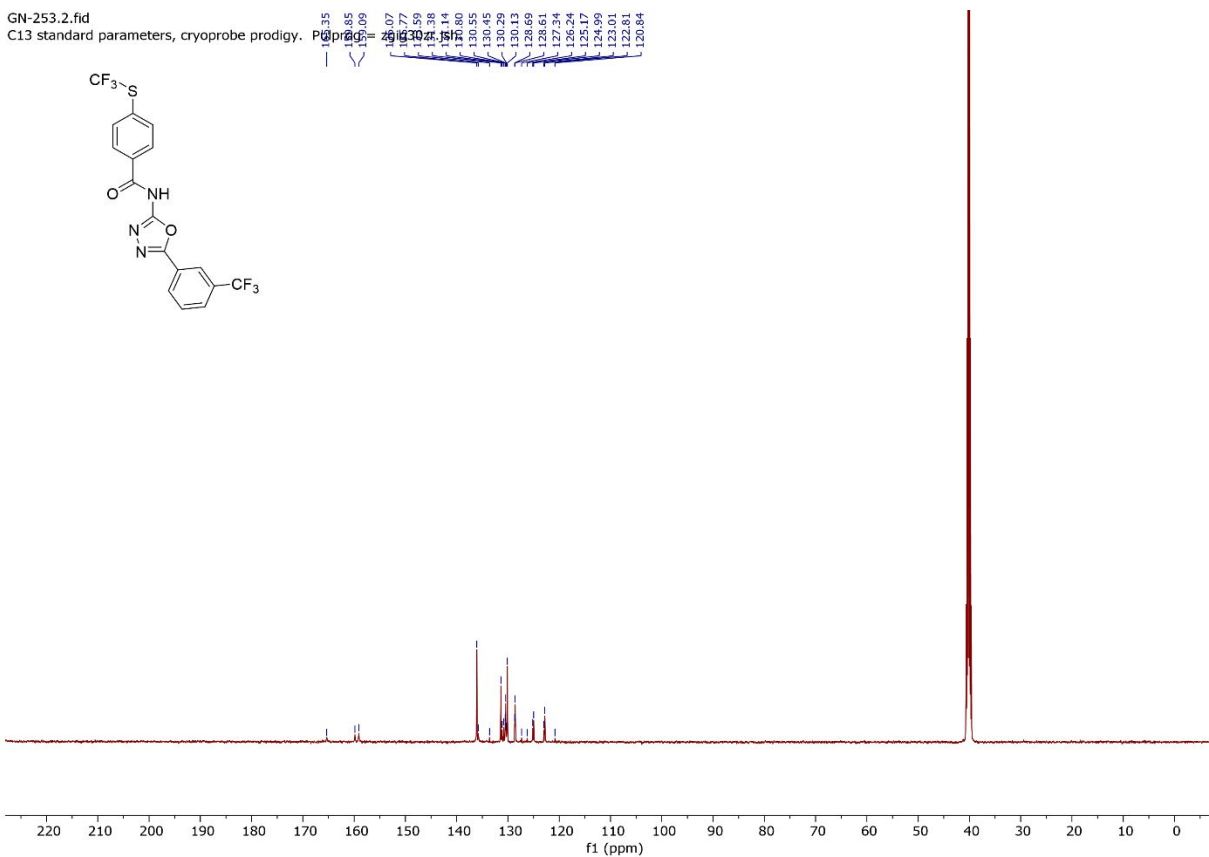
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H1 standard parameters, cryoprobe prodigy.



GN-253.1.fid  
H1 standard parameters, cryoprobe prodigy.

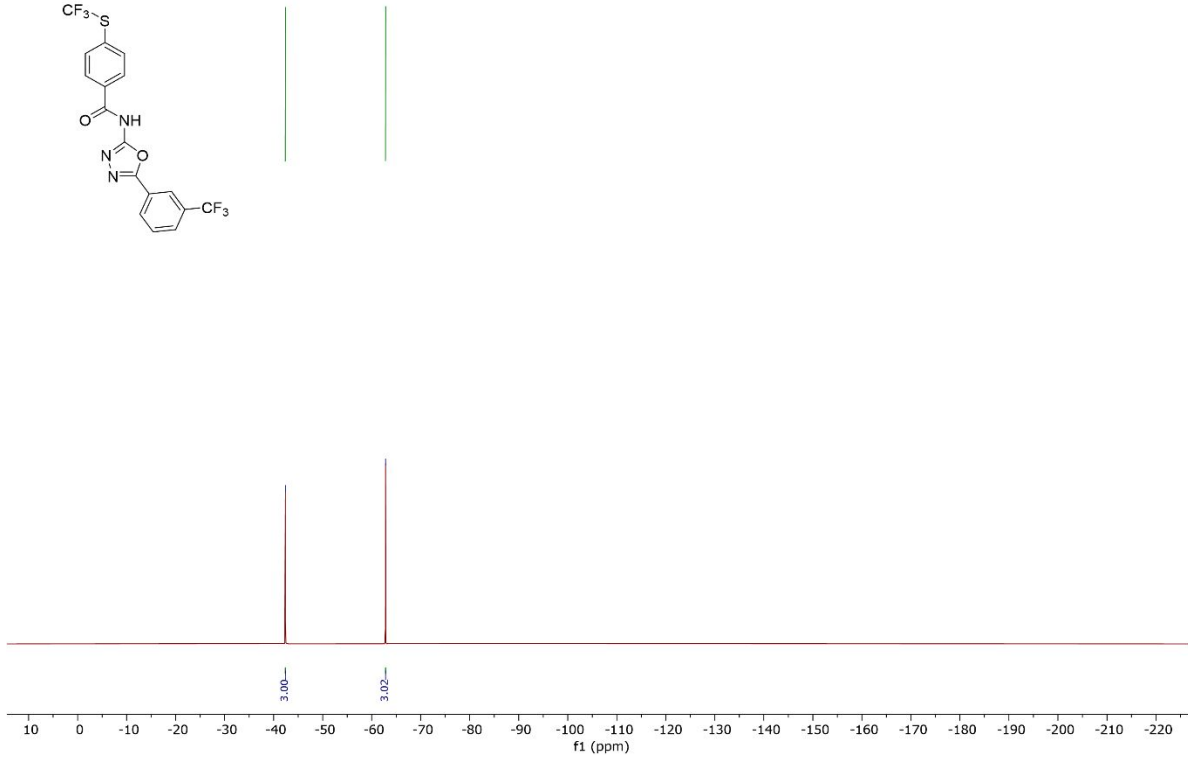


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C13 standard parameters, cryoprobe prodigy.

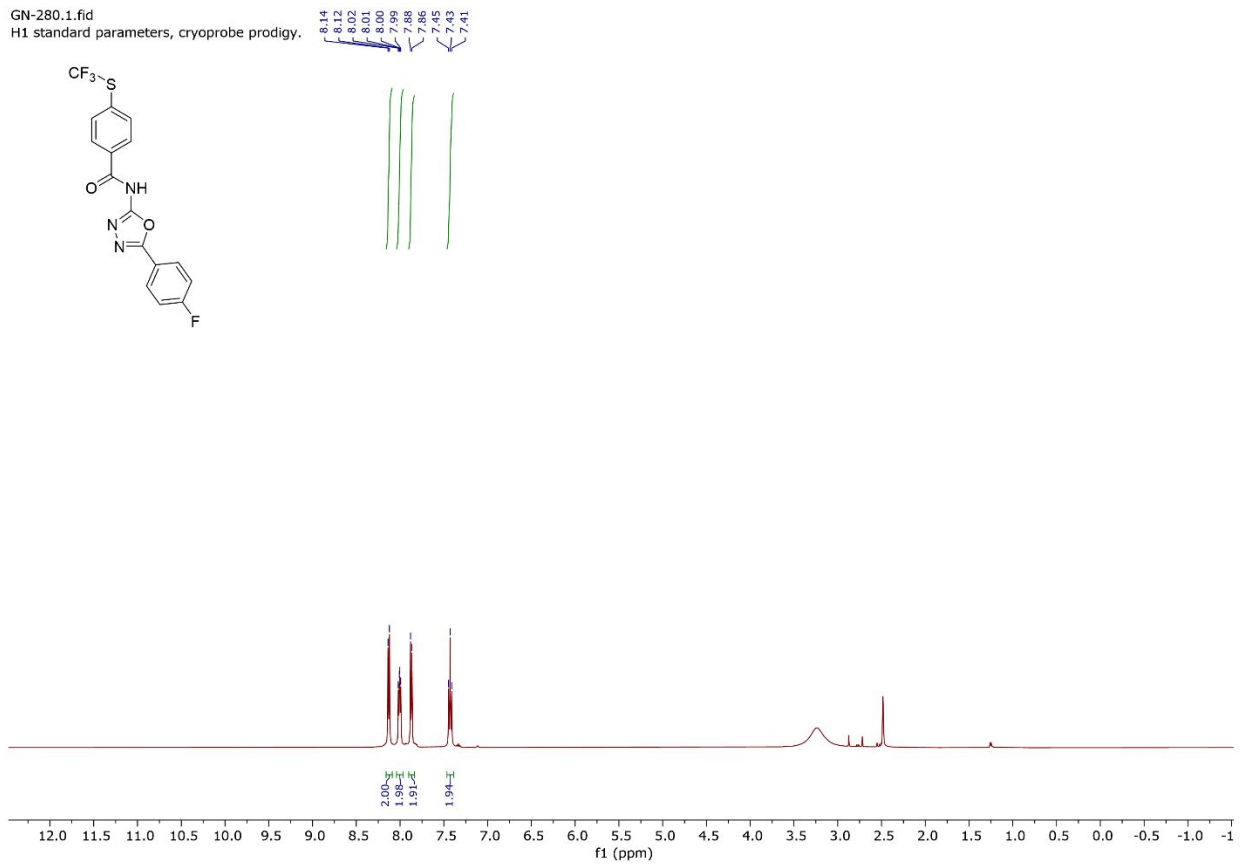




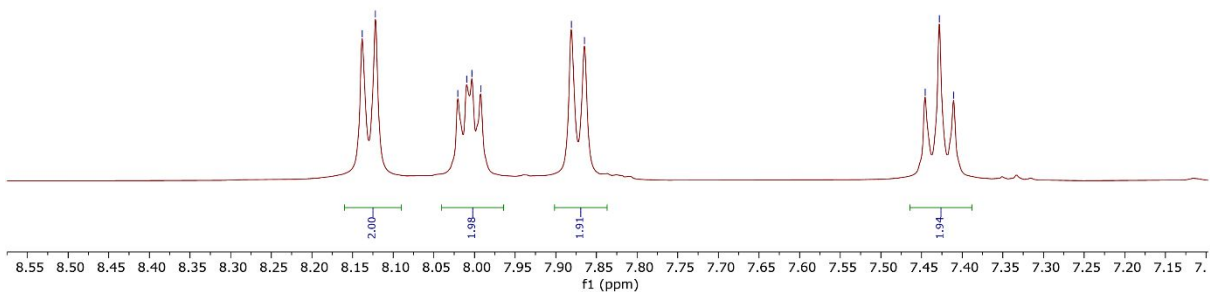
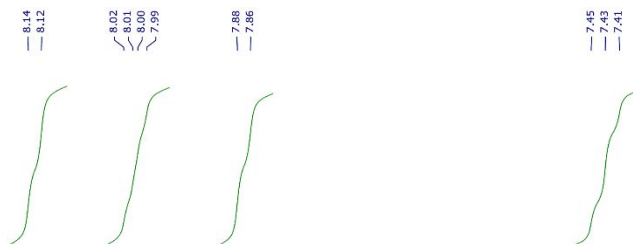
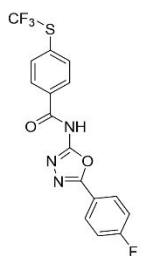
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F19 standard parameters, cryoprobe prodigy.  
DE is lengthened compared to default.



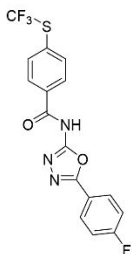
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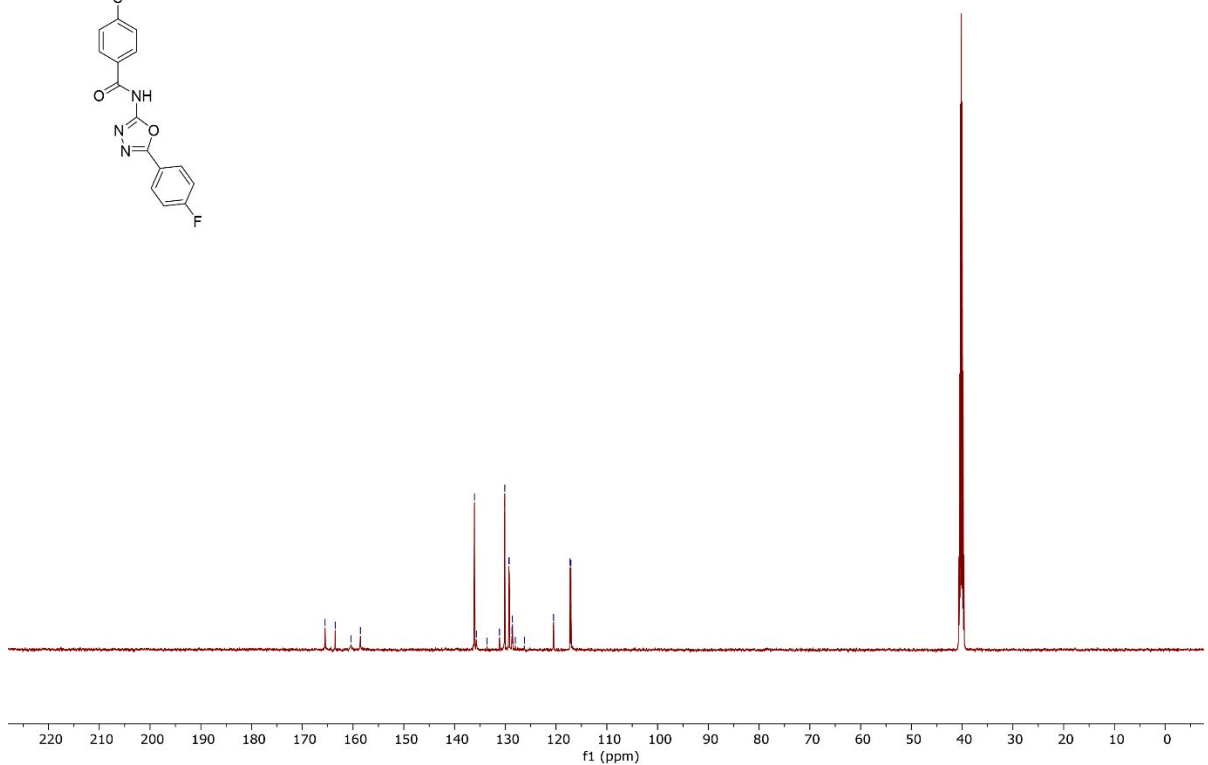
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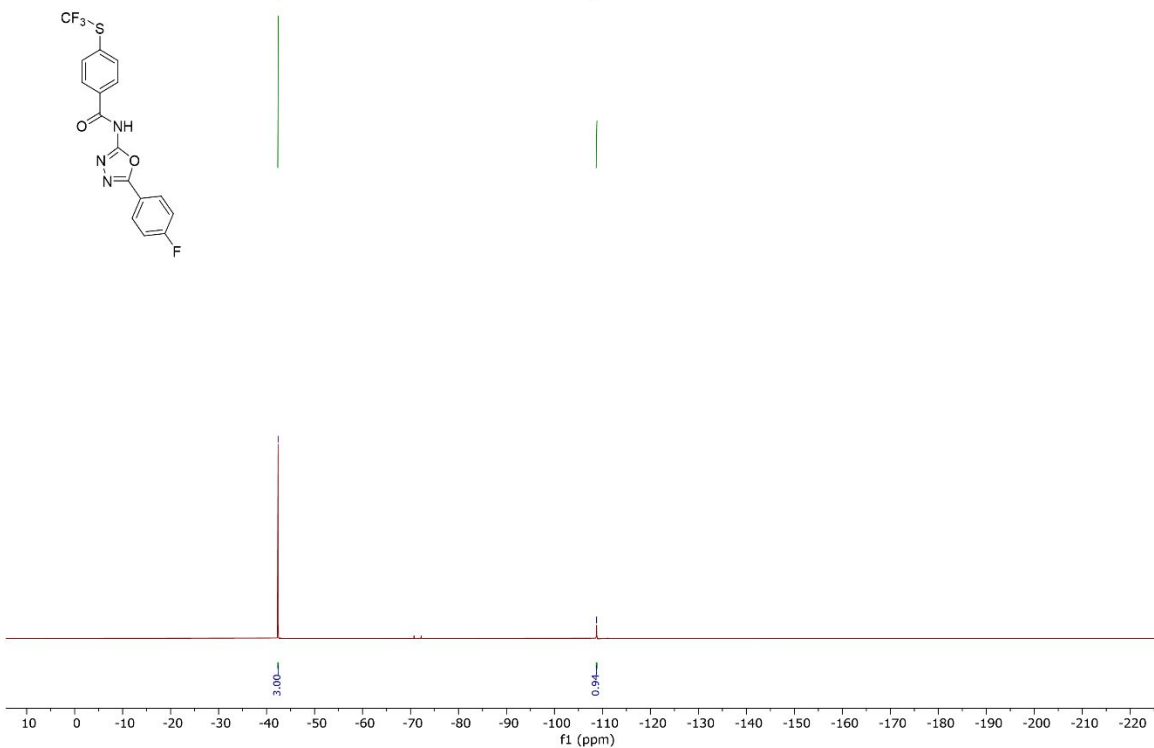
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C13 standard parameters, cryoprobe prodigy.



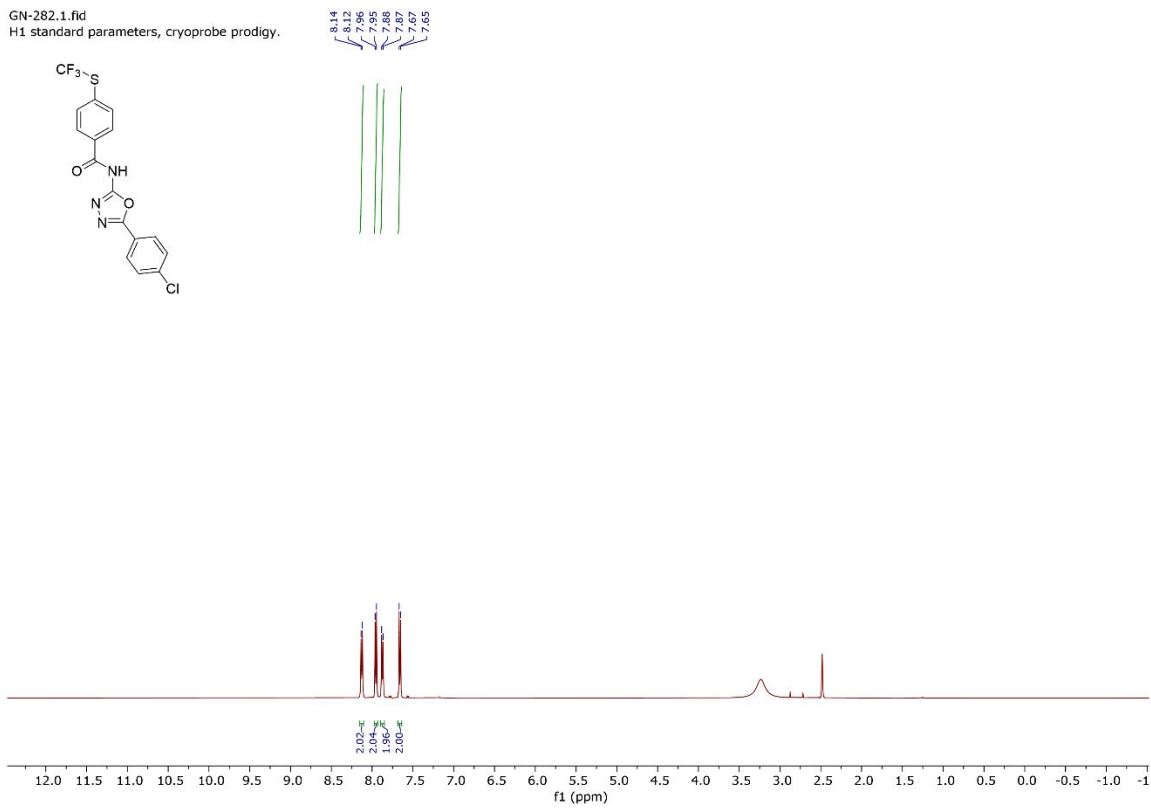
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GN-280.3.fid  
F19 standard parameters, cryoprobe prodigy.  
DE is lengthened compared to default.

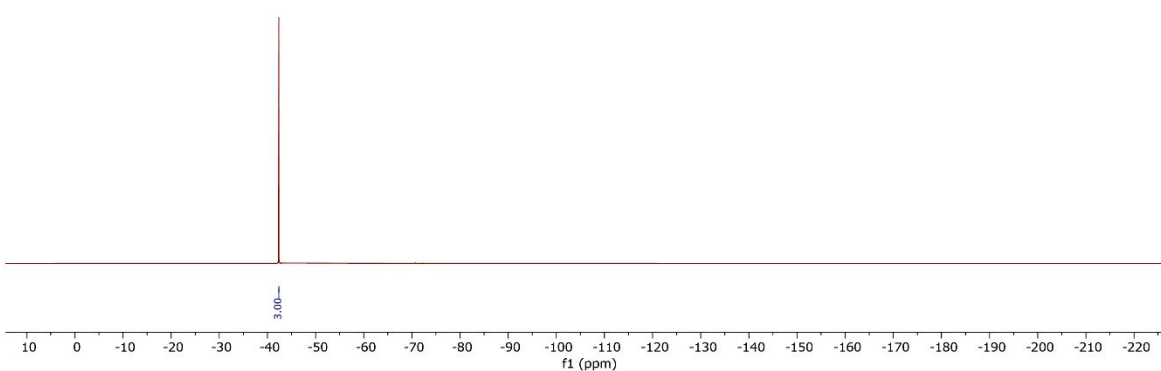
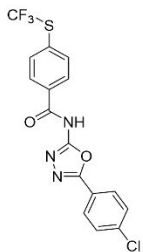


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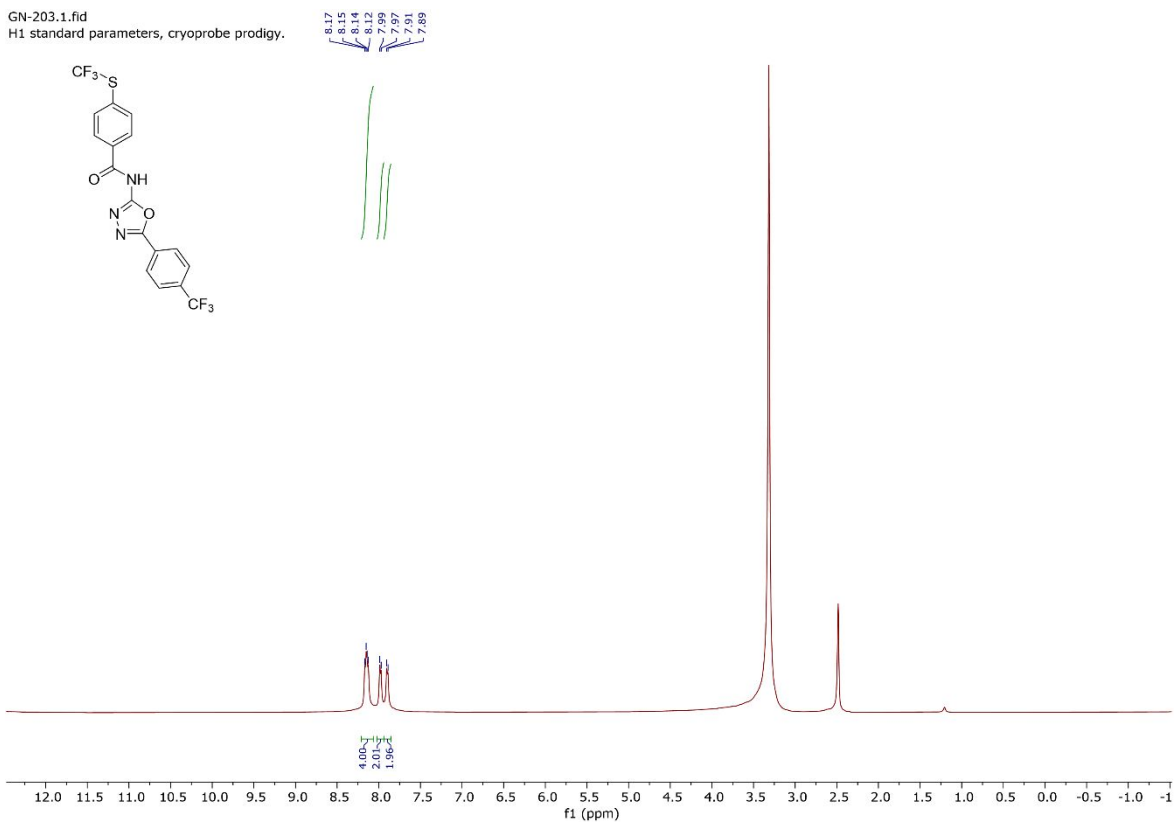
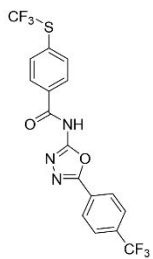




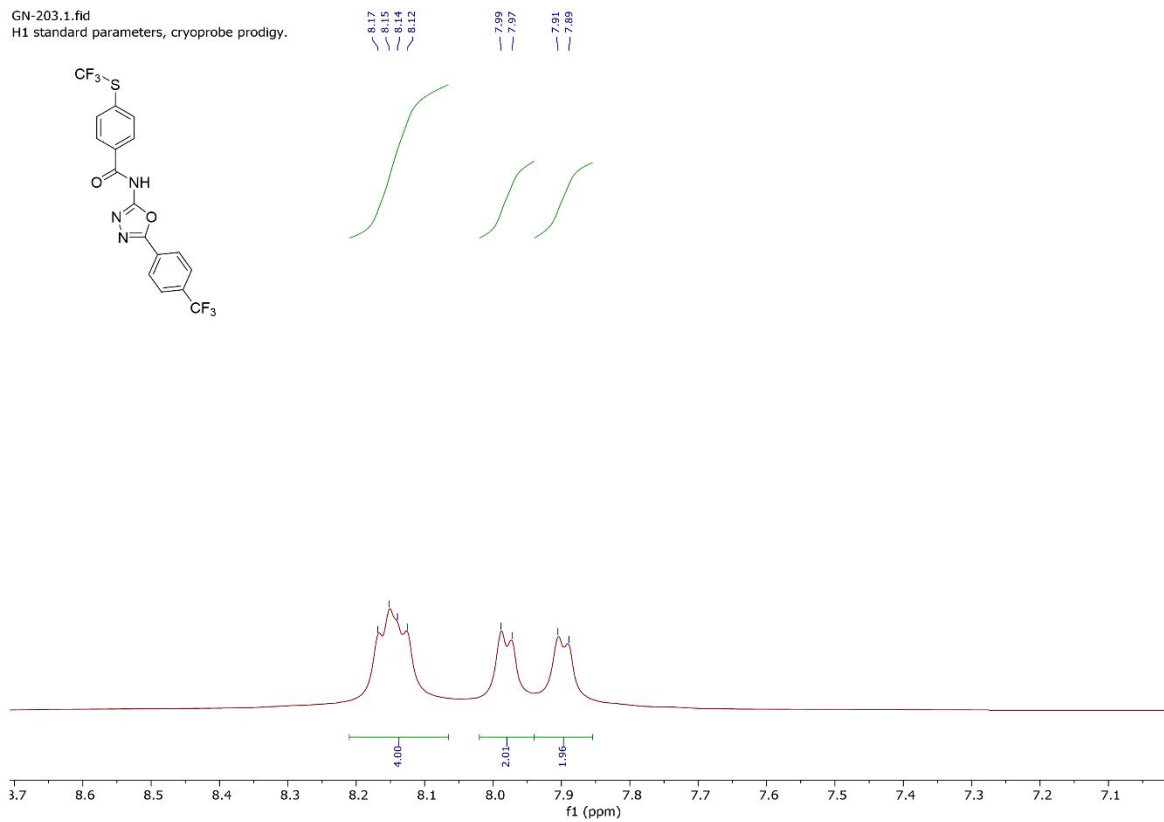
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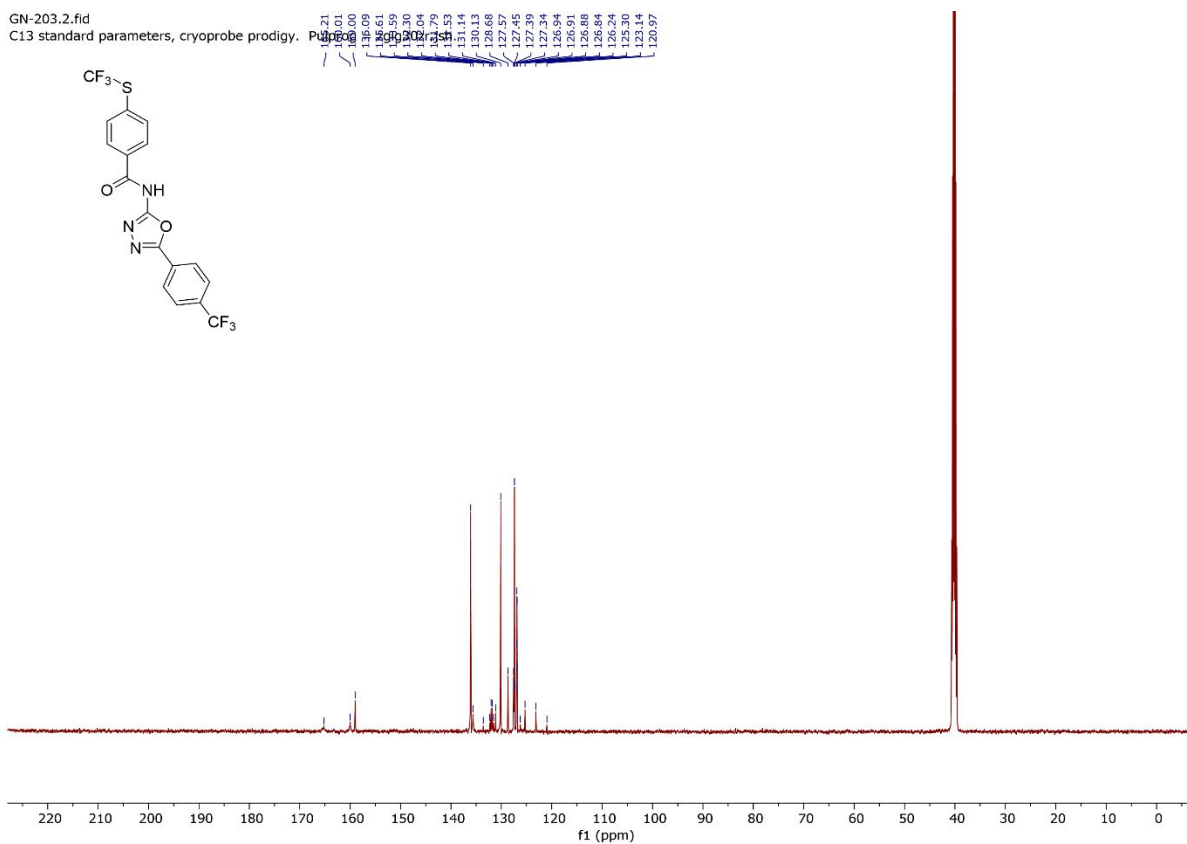
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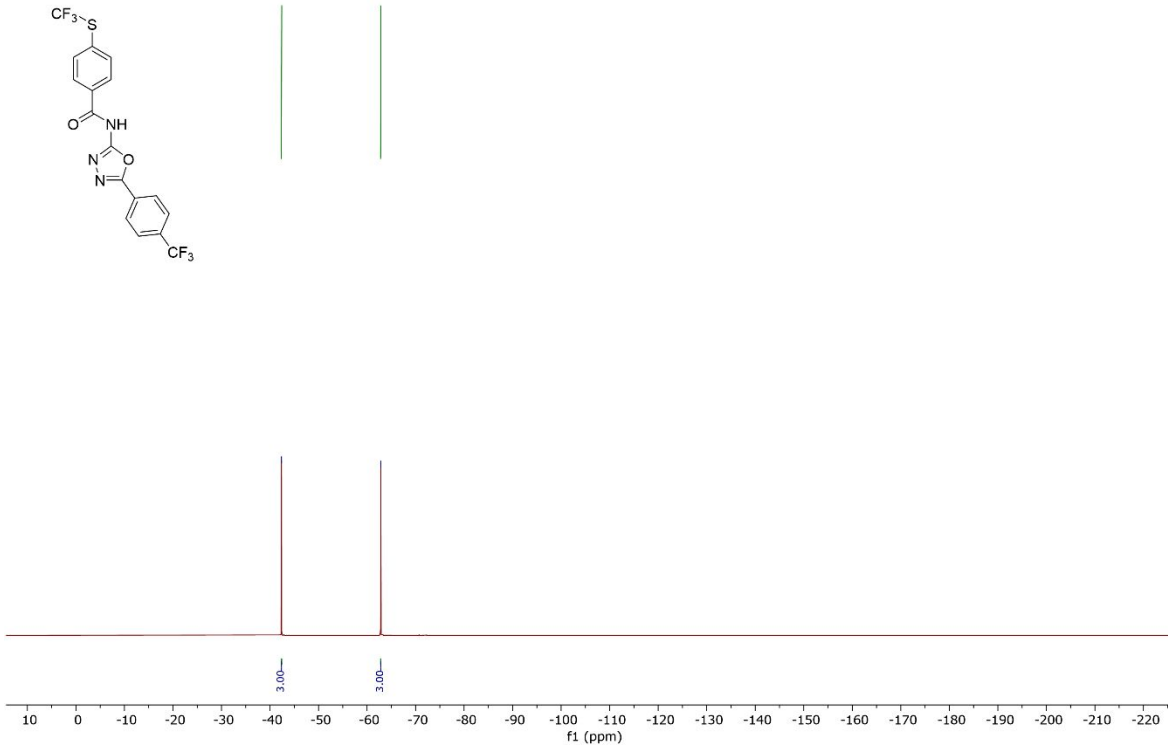
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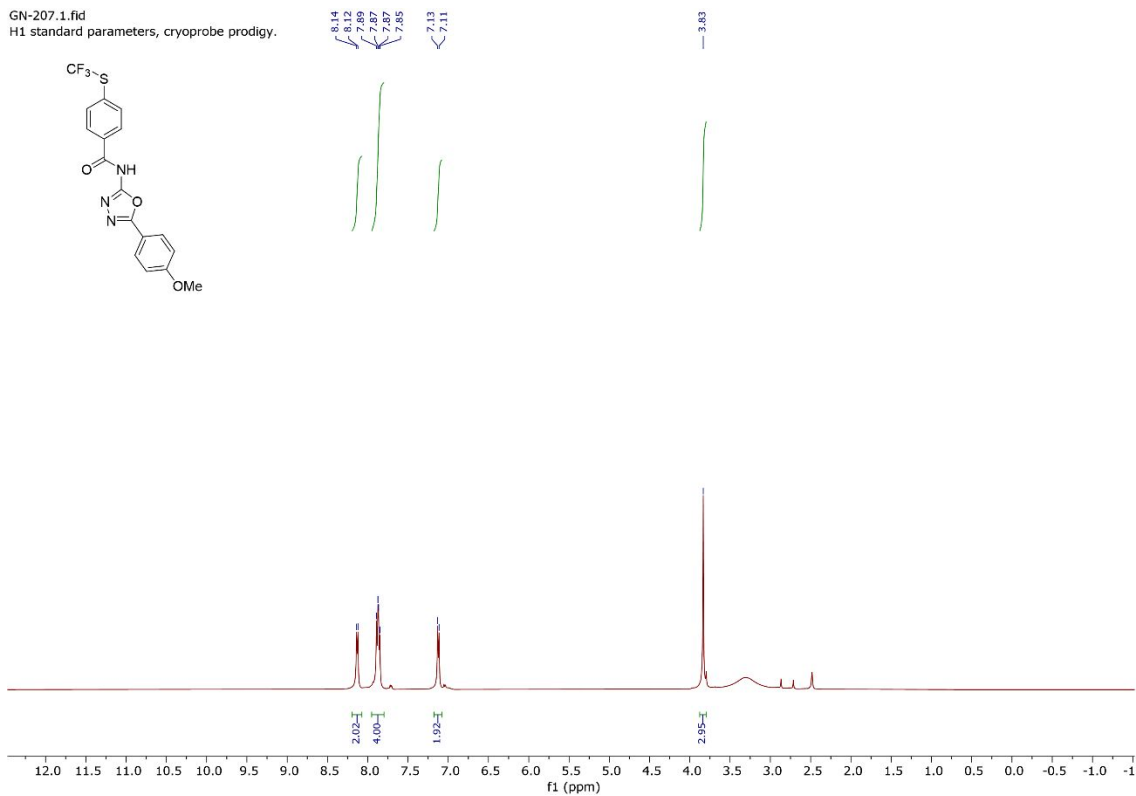
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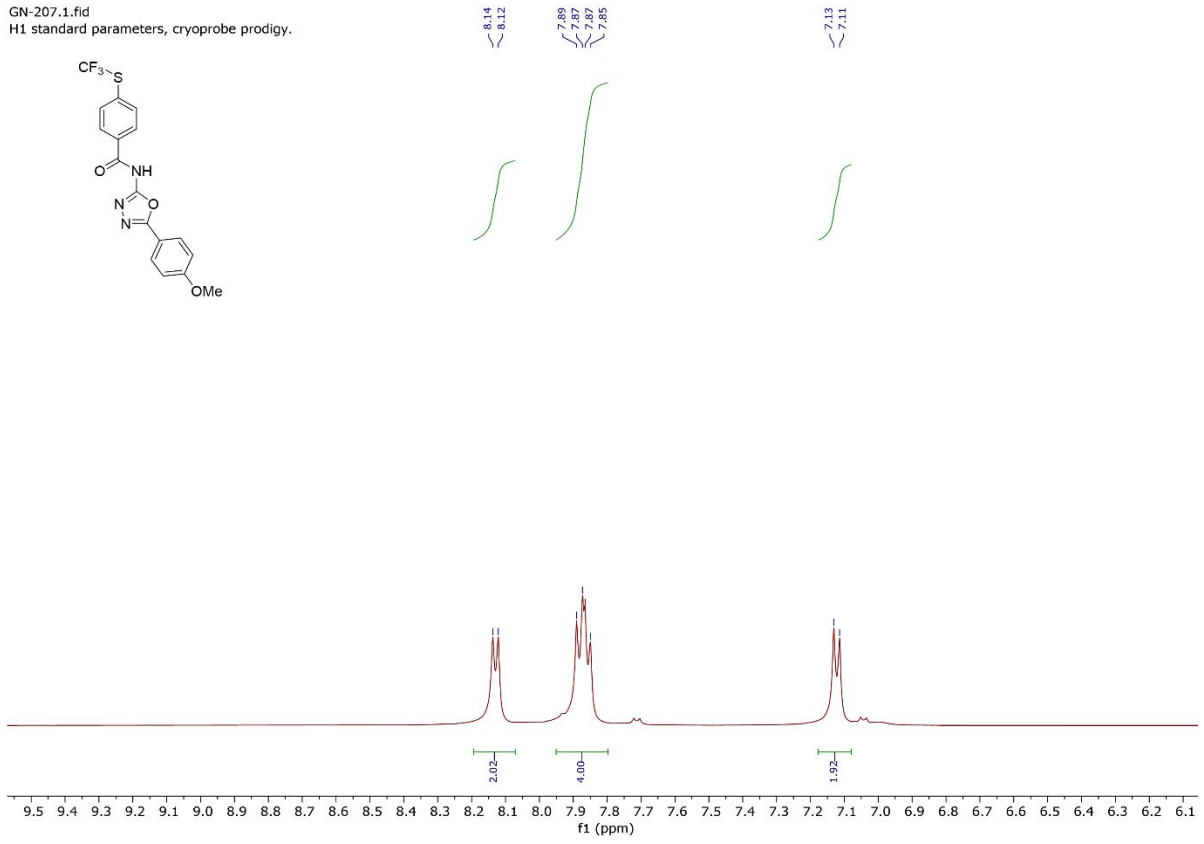
GN-203.3.fid  
F19 standard parameters, cryoprobe prodigy.  
DE is lengthened compared to default.



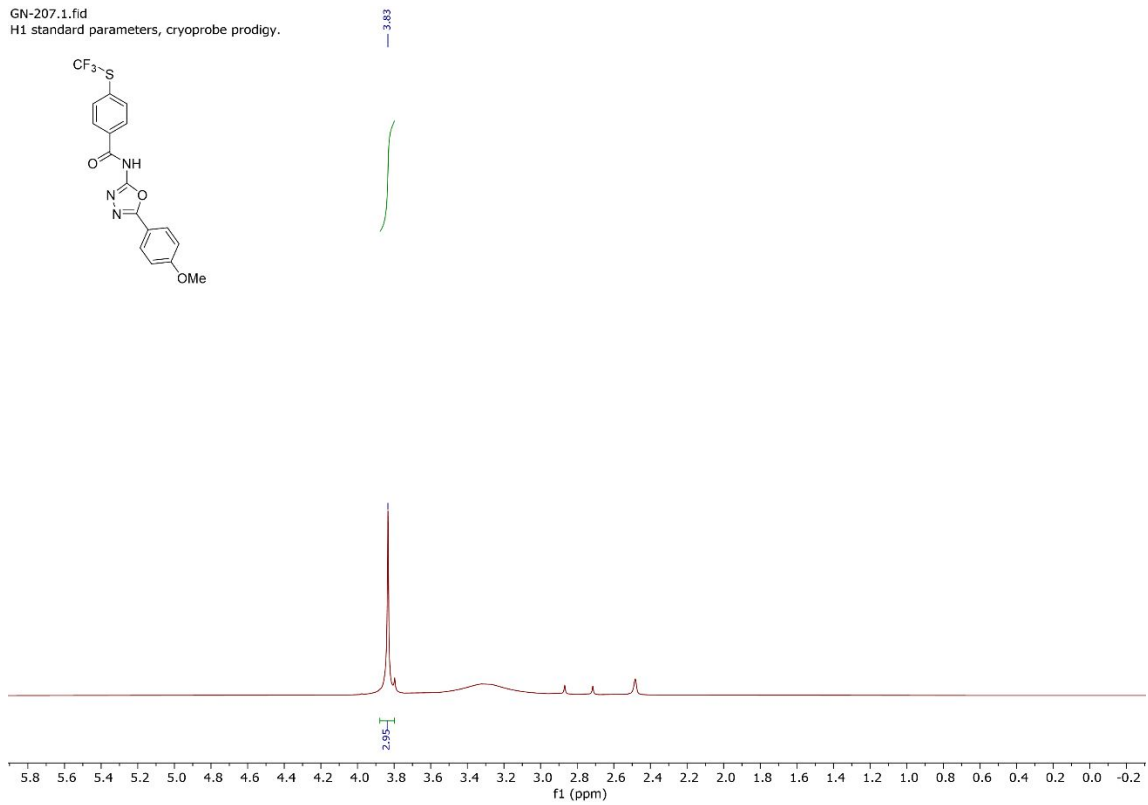
GN-207.1.fid  
H1 standard parameters, cryoprobe prodigy.



GN-207.1.fid  
H1 standard parameters, cryoprobe prodigy.



GN-207.1.fid  
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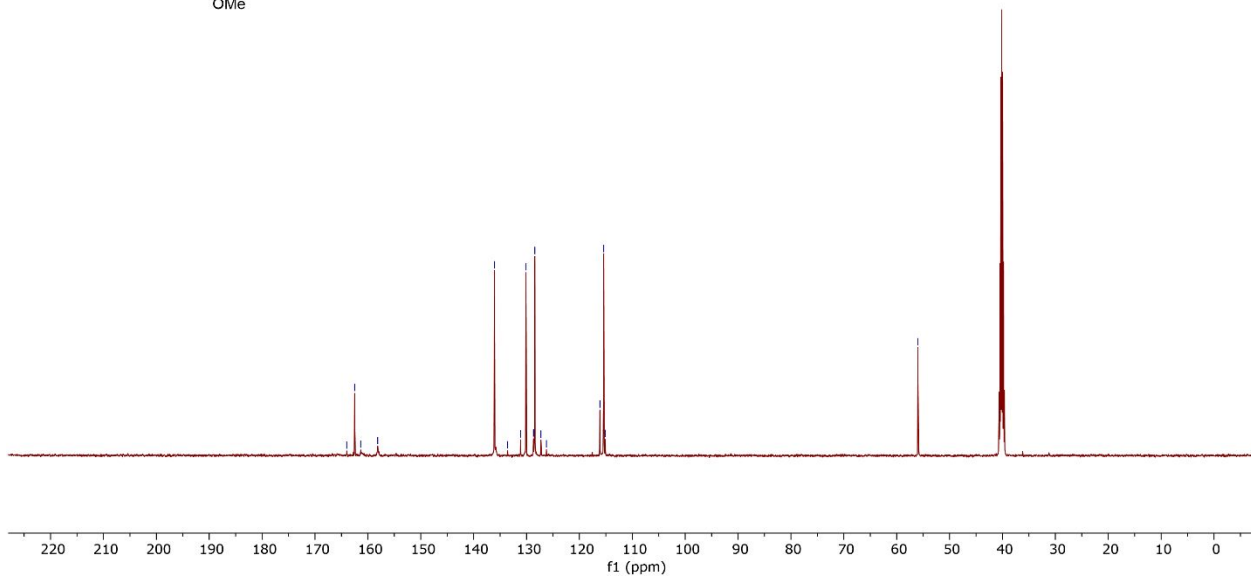
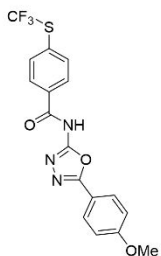


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C13 standard parameters, cryoprobe prodigy.

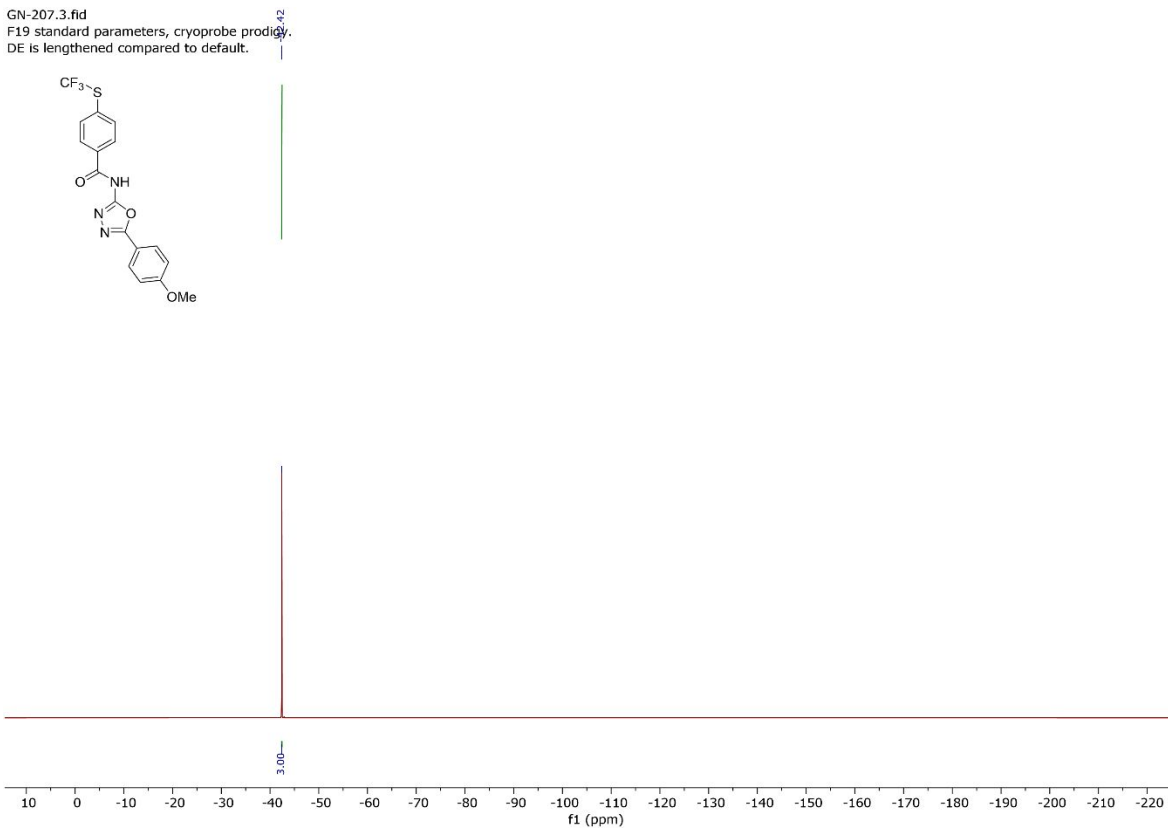
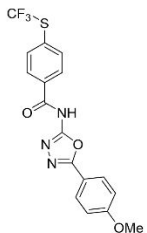
156.99  
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55.98

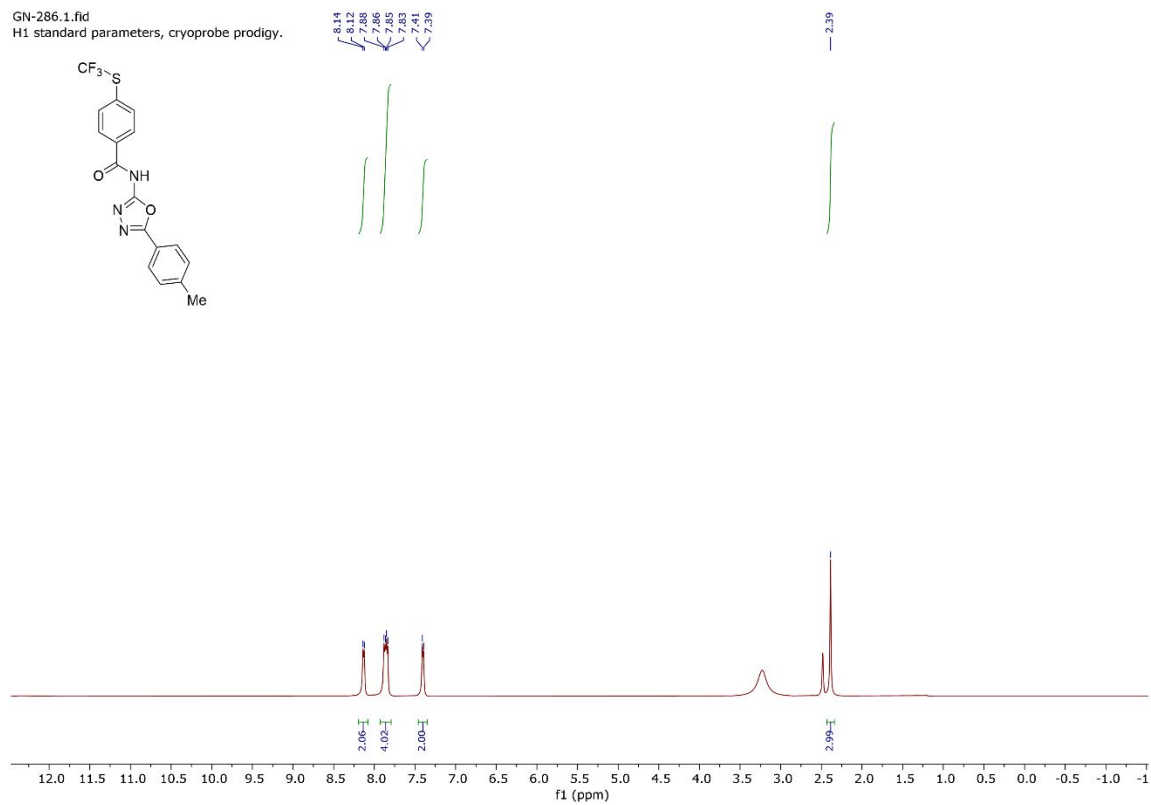


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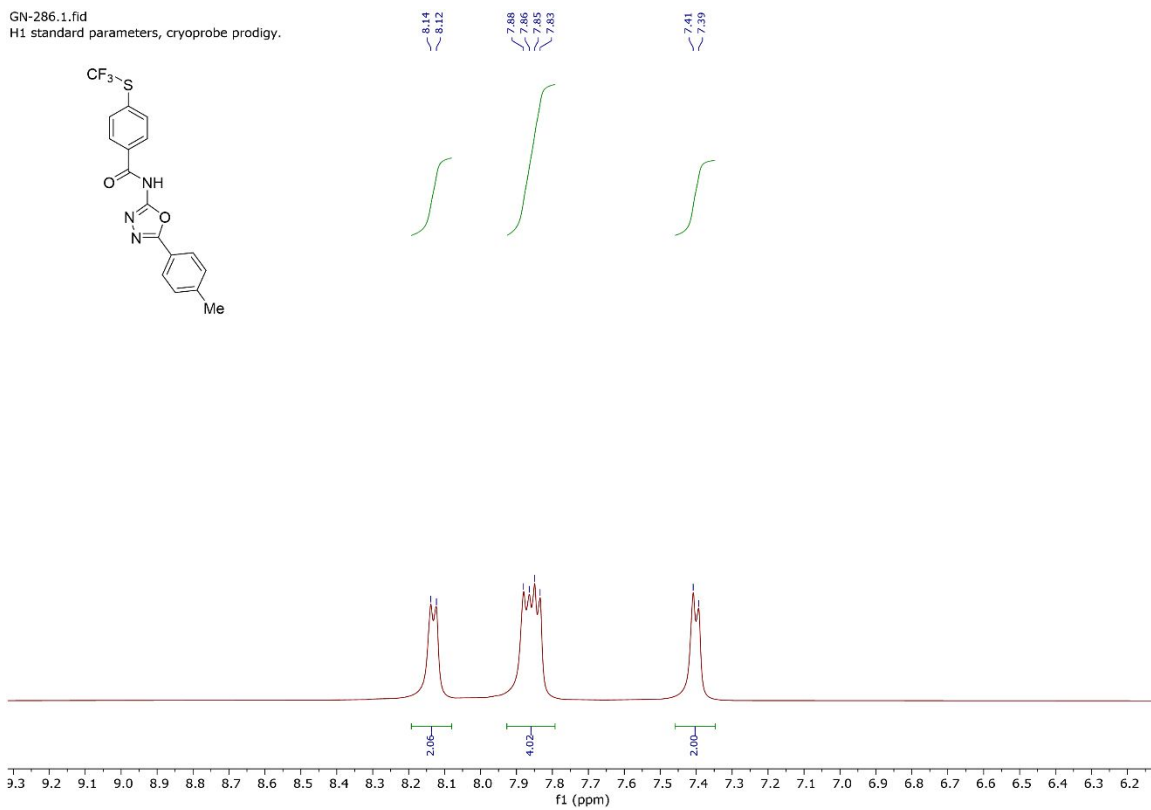
F19 standard parameters, cryoprobe prodigy.  
DE is lengthened compared to default.



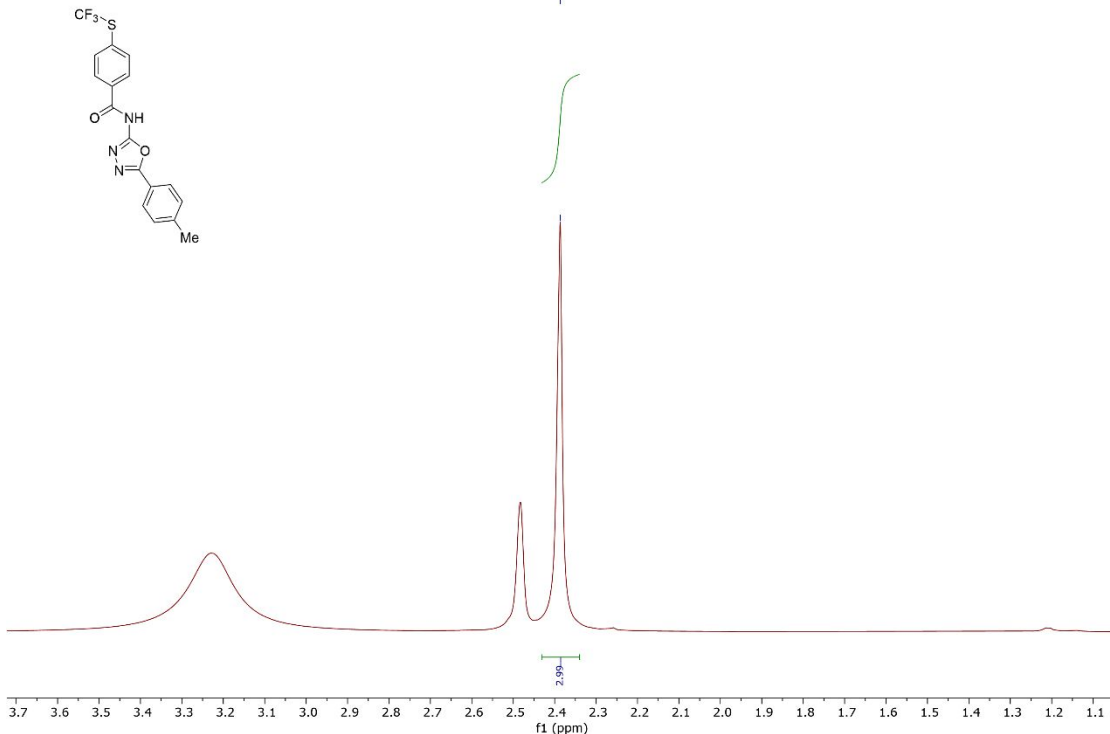
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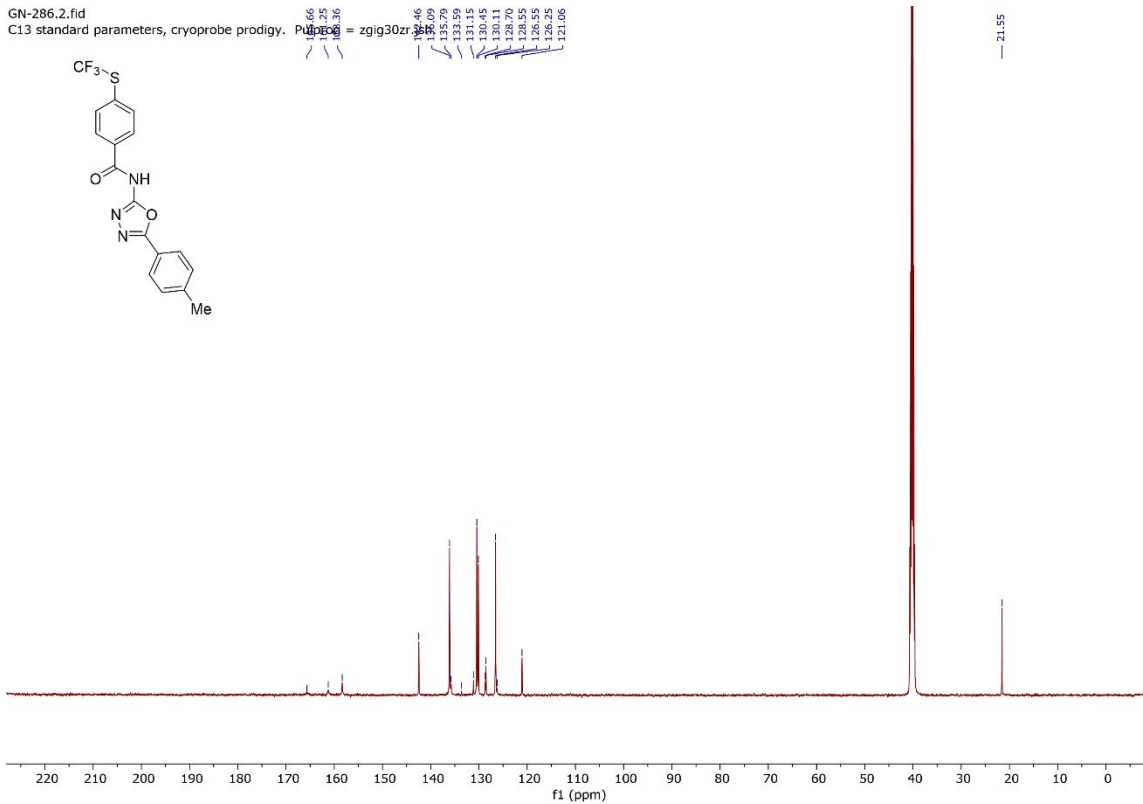
GN-286.1.fid  
H1 standard parameters, cryoprobe prodigy.



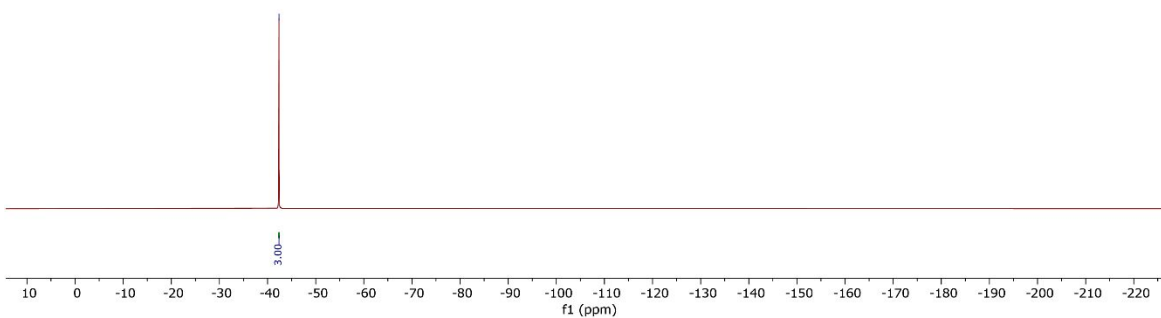
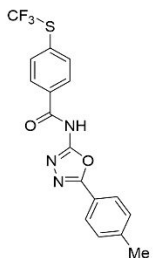
GN-286.1.fid  
H1 standard parameters, cryoprobe prodigy.



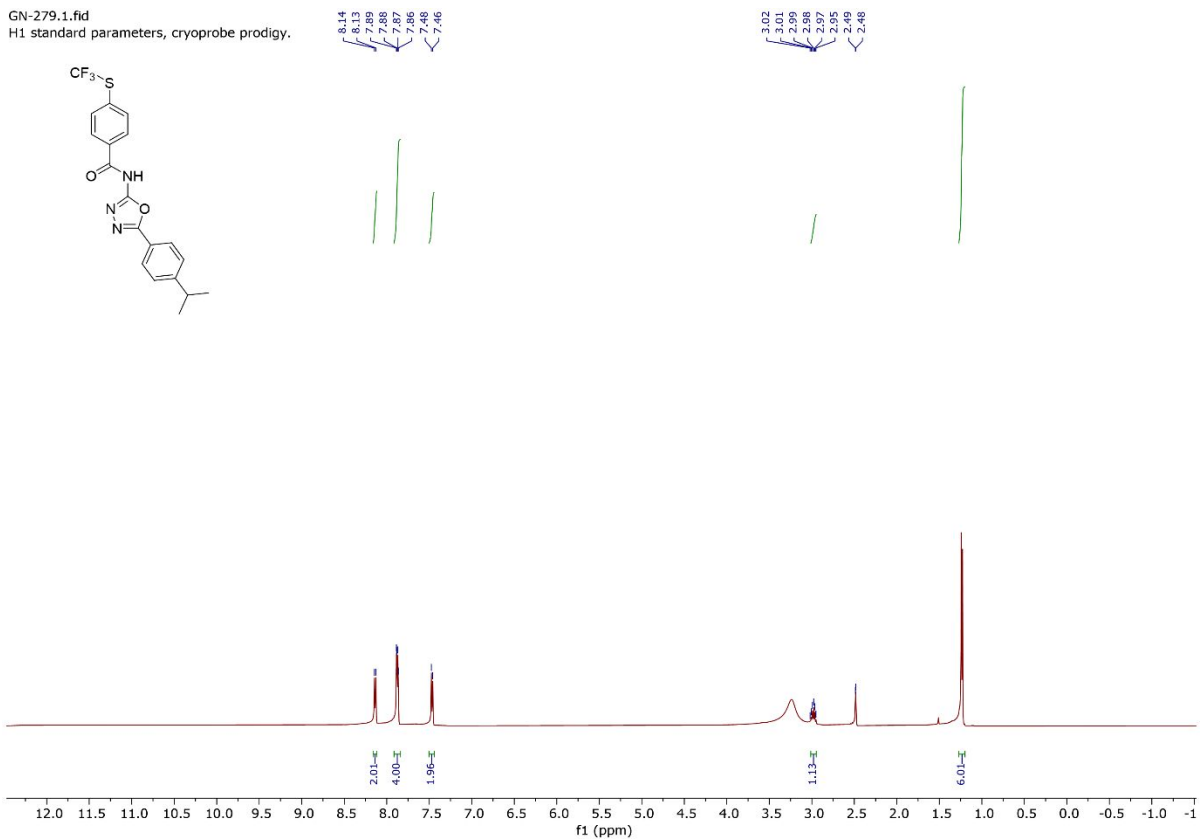
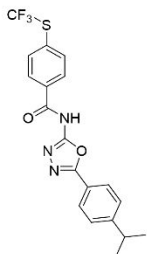
GN-286.2.fid  
C13 standard parameters, cryoprobe prodigy.



GN-286.3.fid  
F19 standard parameters, cryoprobe prodigy.  
DE is lengthened compared to default.

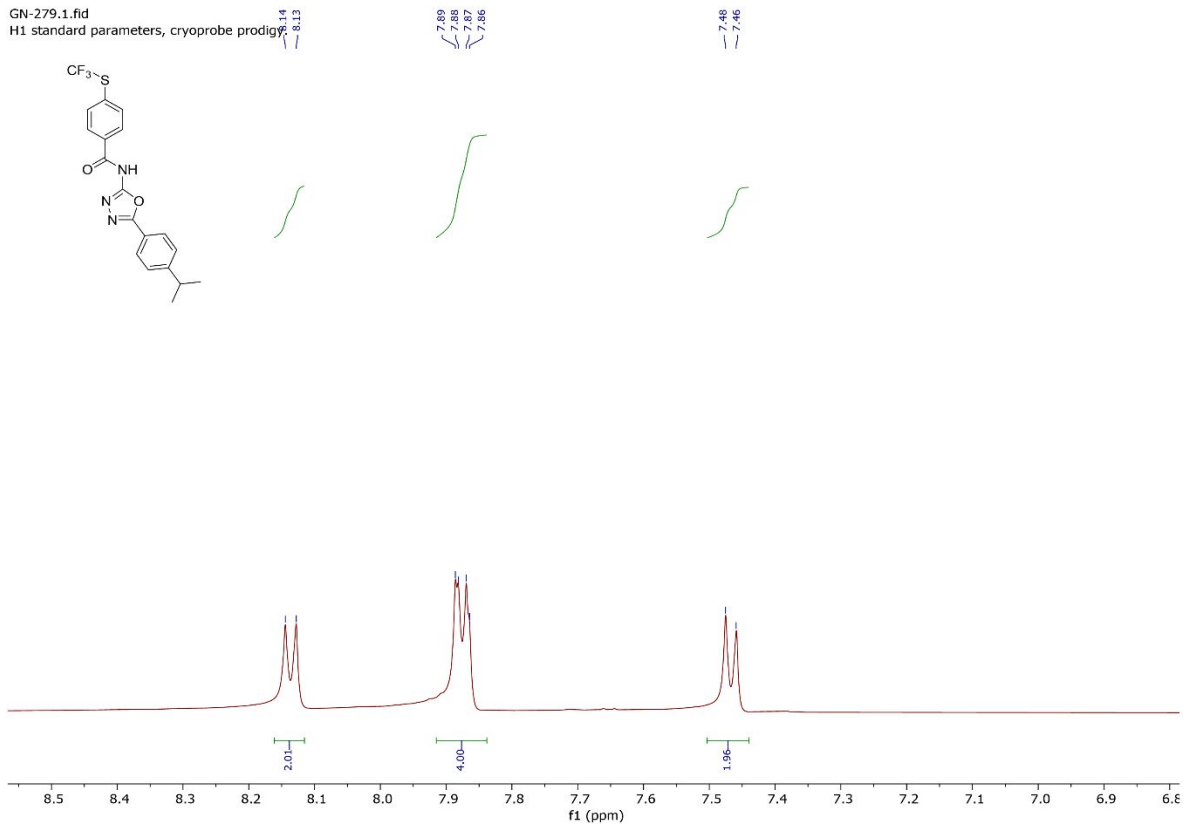


GN-279.1.fid  
H1 standard parameters, cryoprobe prodigy.



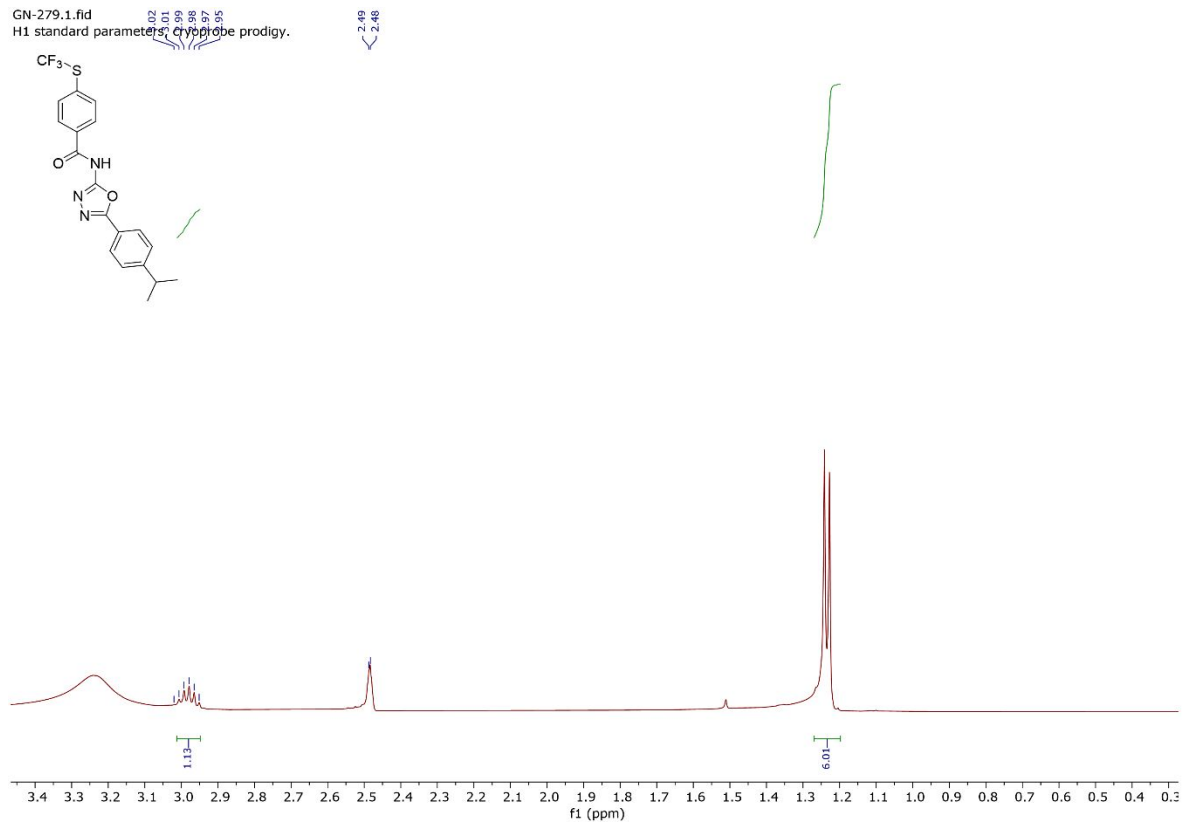
GN-279.1.fid

H1 standard parameters, cryoprobe prodigy.

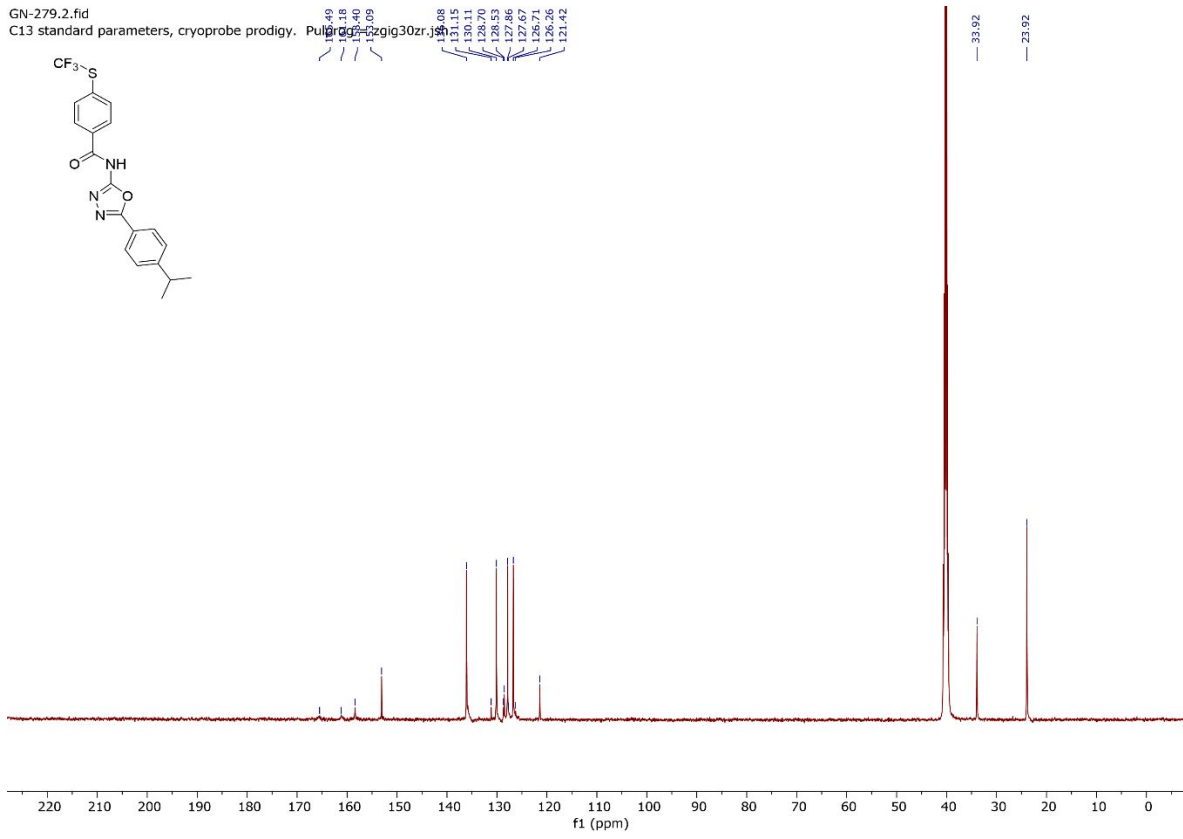


GN-279.1.fid

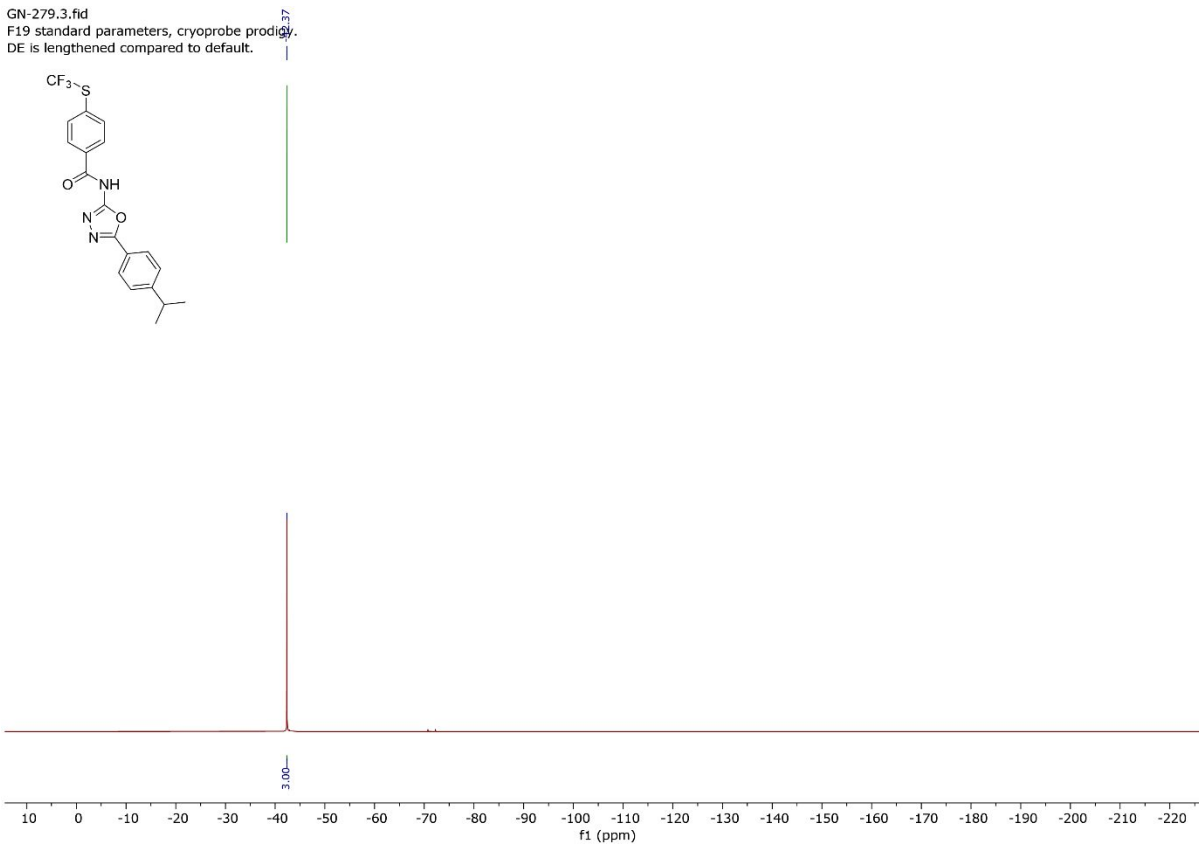
H1 standard parameters, cryoprobe prodigy.



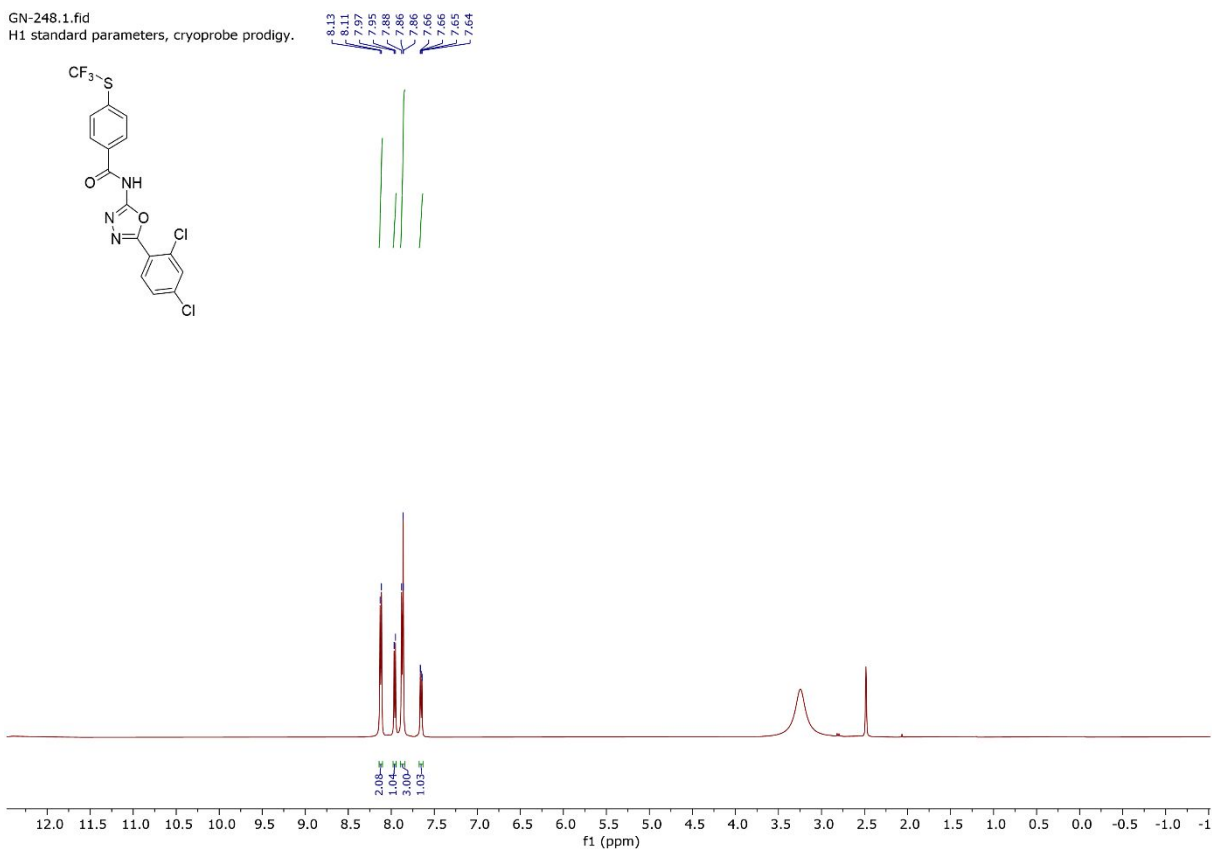
GN-279.2.fid  
C13 standard parameters, cryoprobe prodigy.



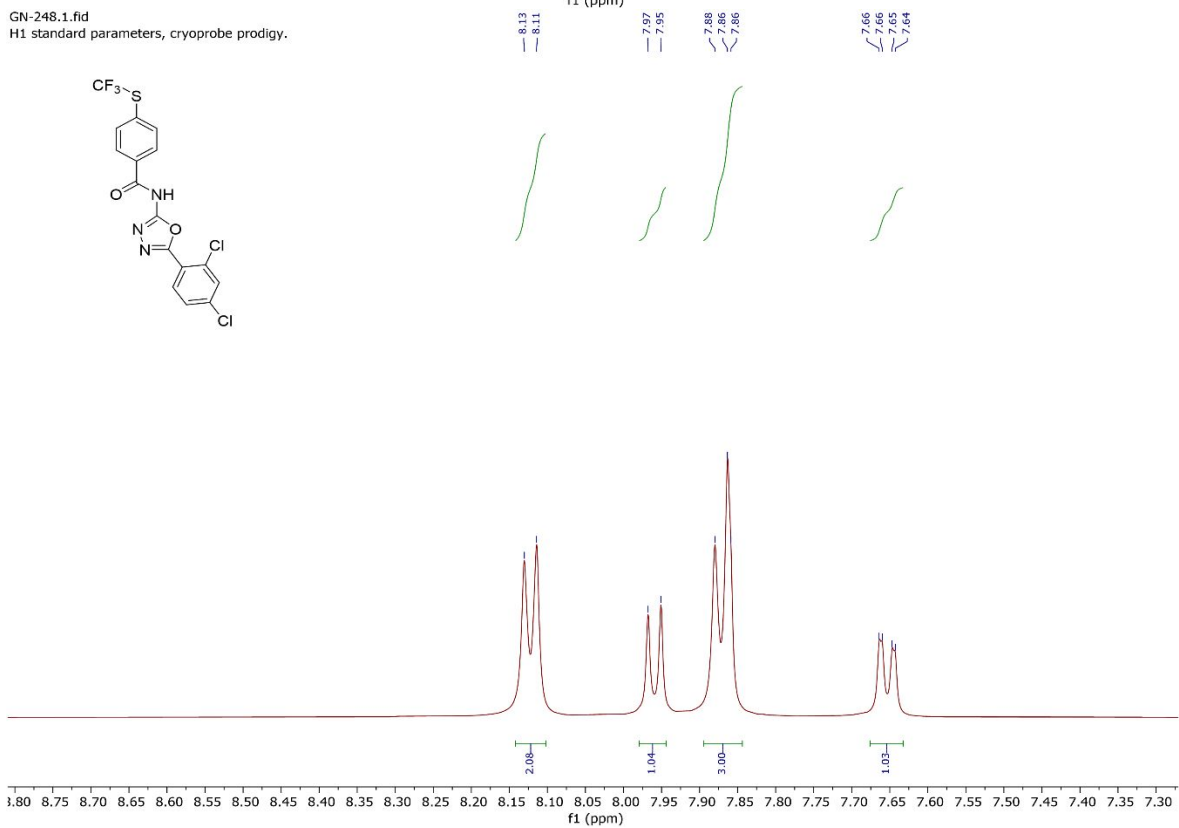
GN-279.3.fid  
F19 standard parameters, cryoprobe prodigy.  
DE is lengthened compared to default.



GN-248.1.fid  
H1 standard parameters, cryoprobe prodigy.

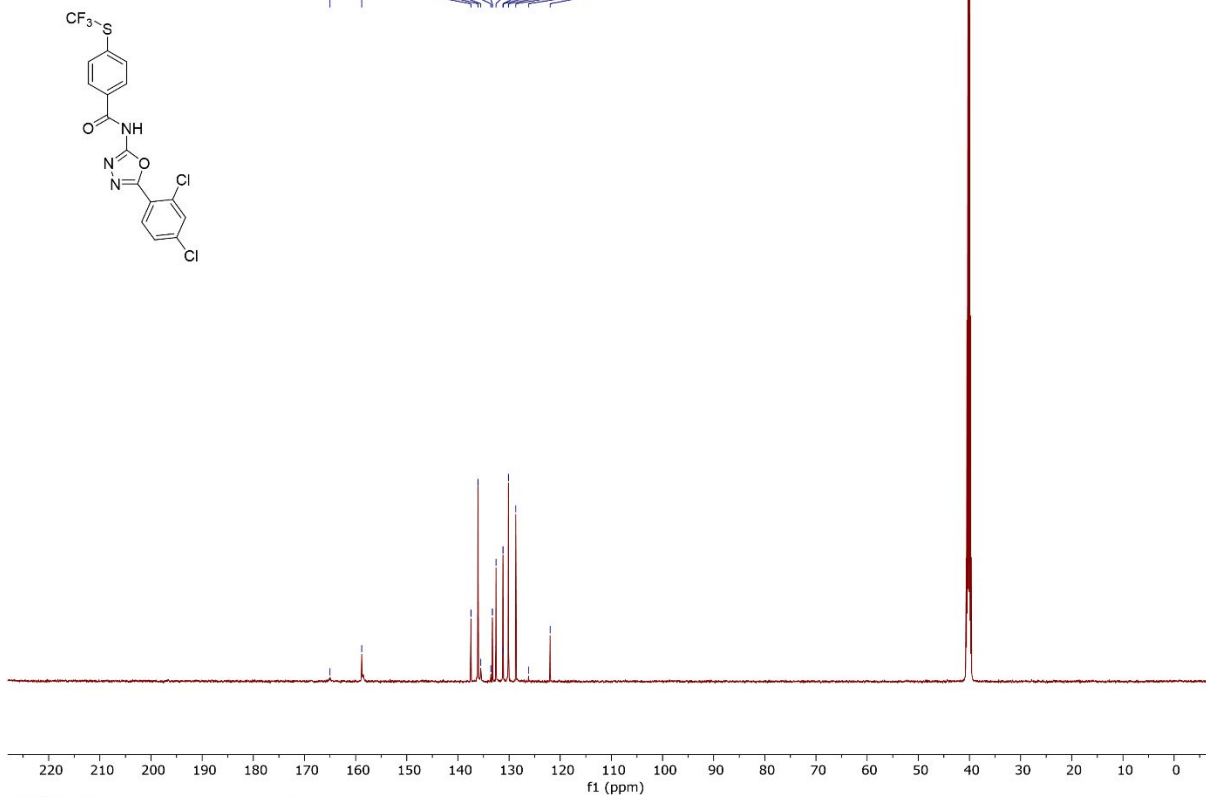


GN-248.1.fid  
H1 standard parameters, cryoprobe prodigy.

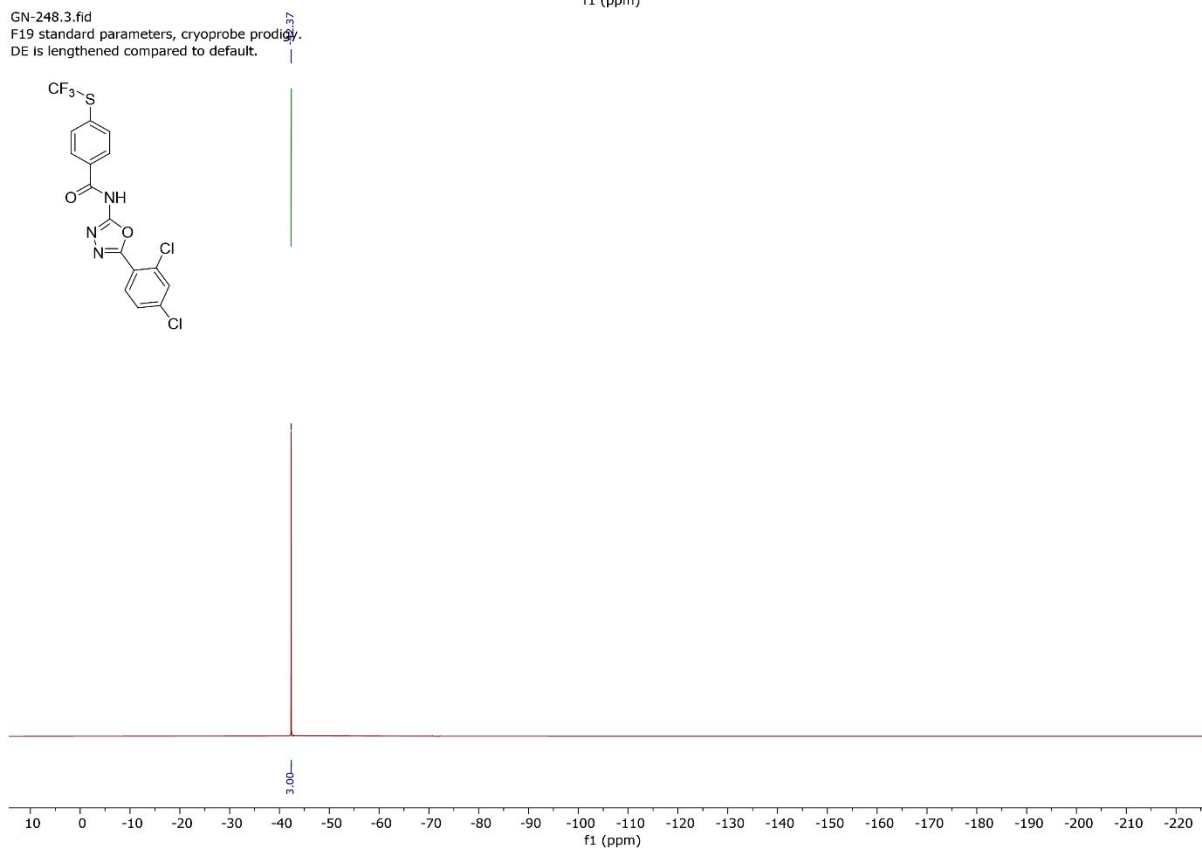


GN-248.2.fid  
C13 standard parameters, cryoprobe prodigy.

prog = zgig3024  
118.00  
118.81  
132.47  
133.07  
133.53  
133.58  
133.25  
132.56  
131.48  
131.13  
130.13  
128.68  
126.23  
121.99

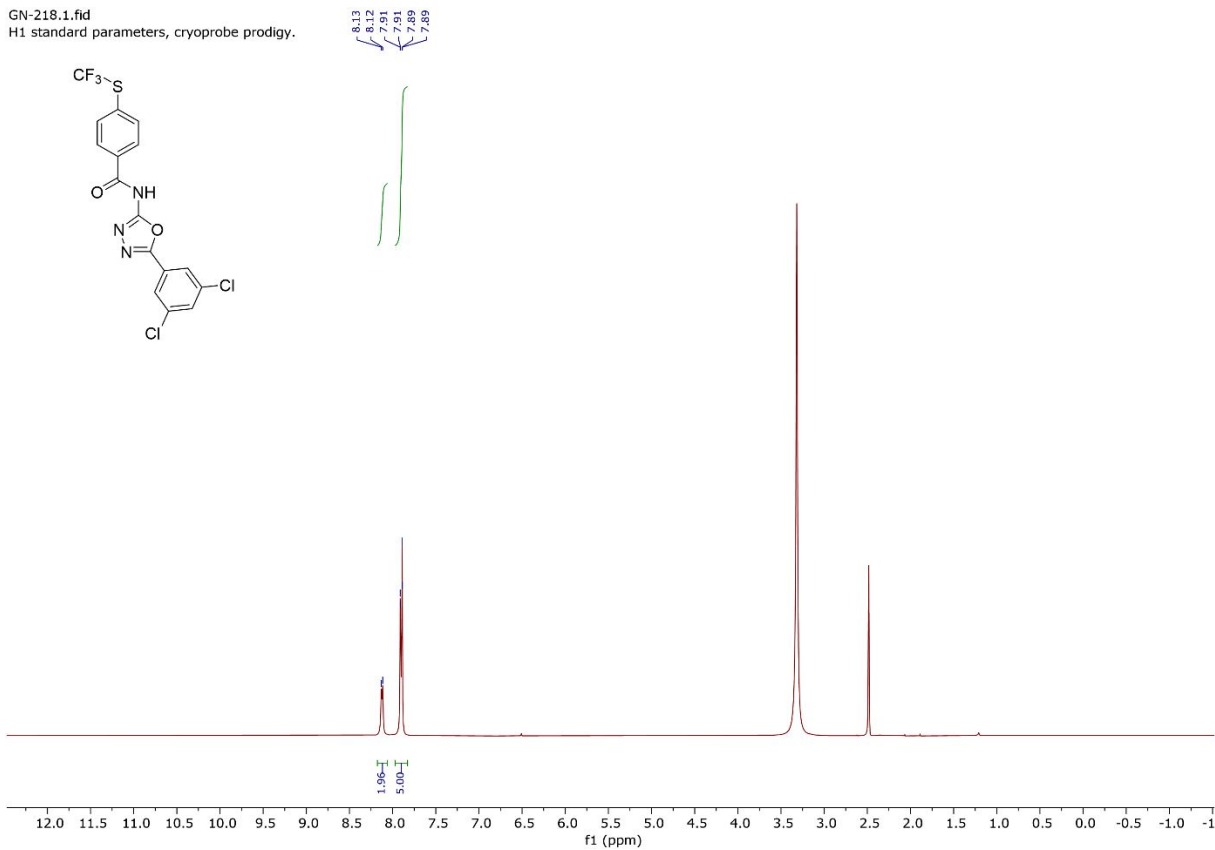


GN-248.3.fid  
F19 standard parameters, cryoprobe prodigy.  
DE is lengthened compared to default.

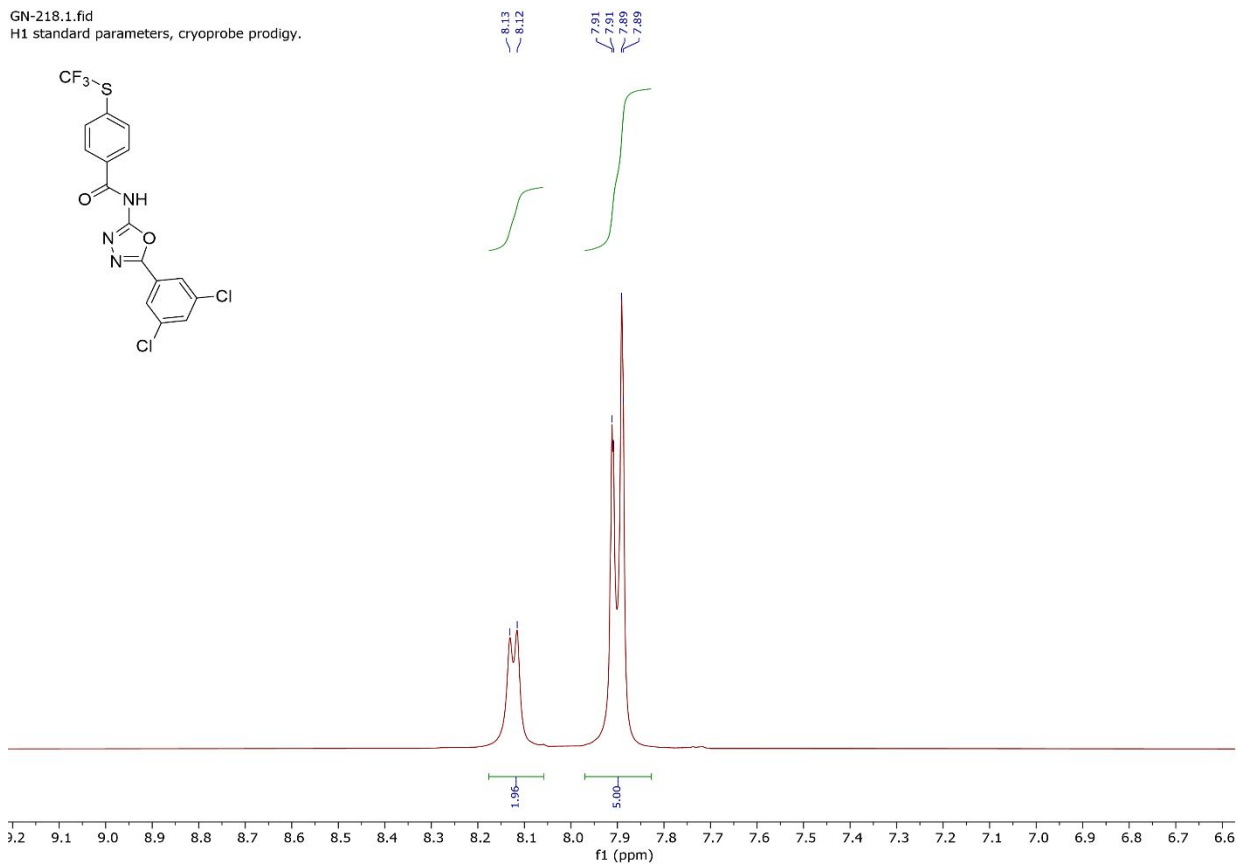




GN-218.1.fid  
H1 standard parameters, cryoprobe prodigy.



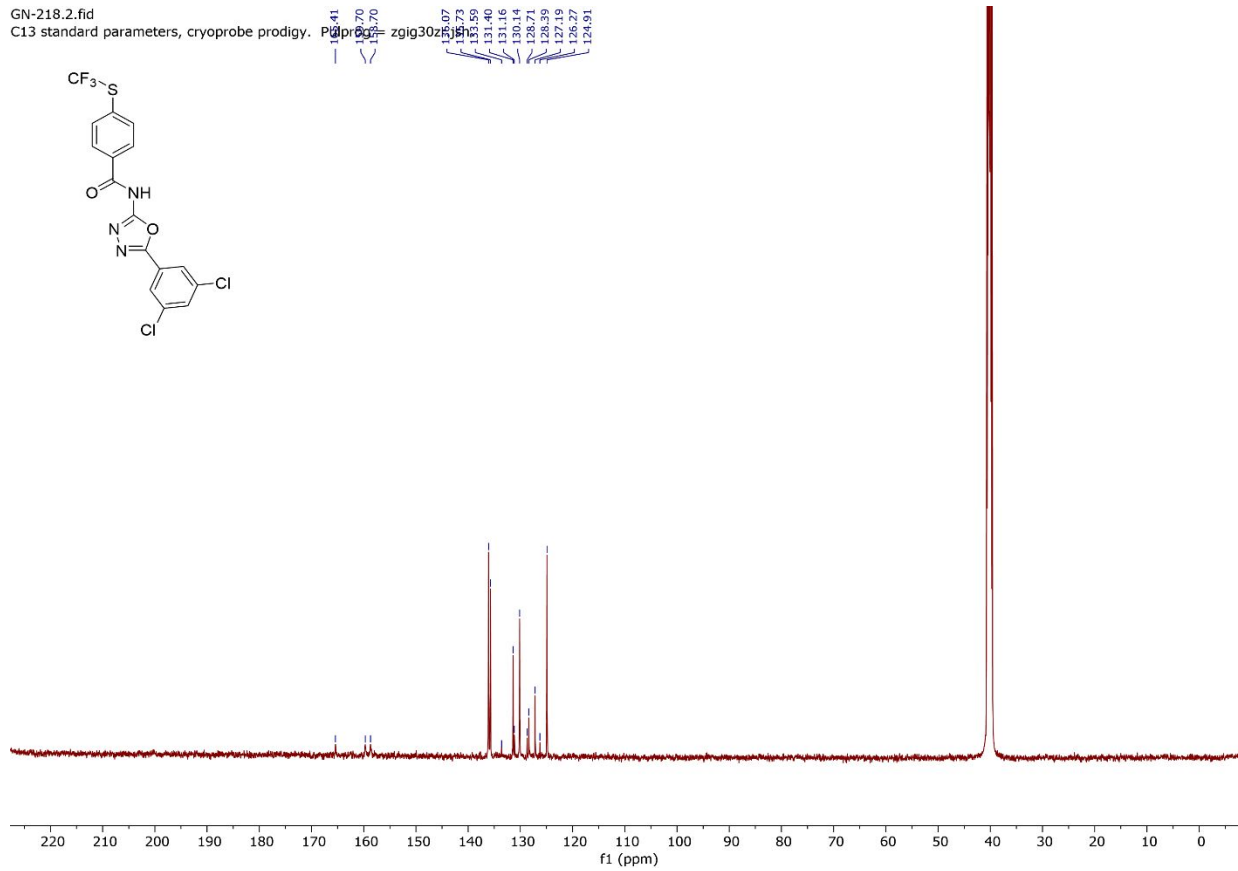
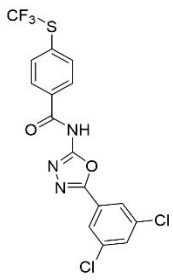
GN-218.1.fid  
H1 standard parameters, cryoprobe prodigy.



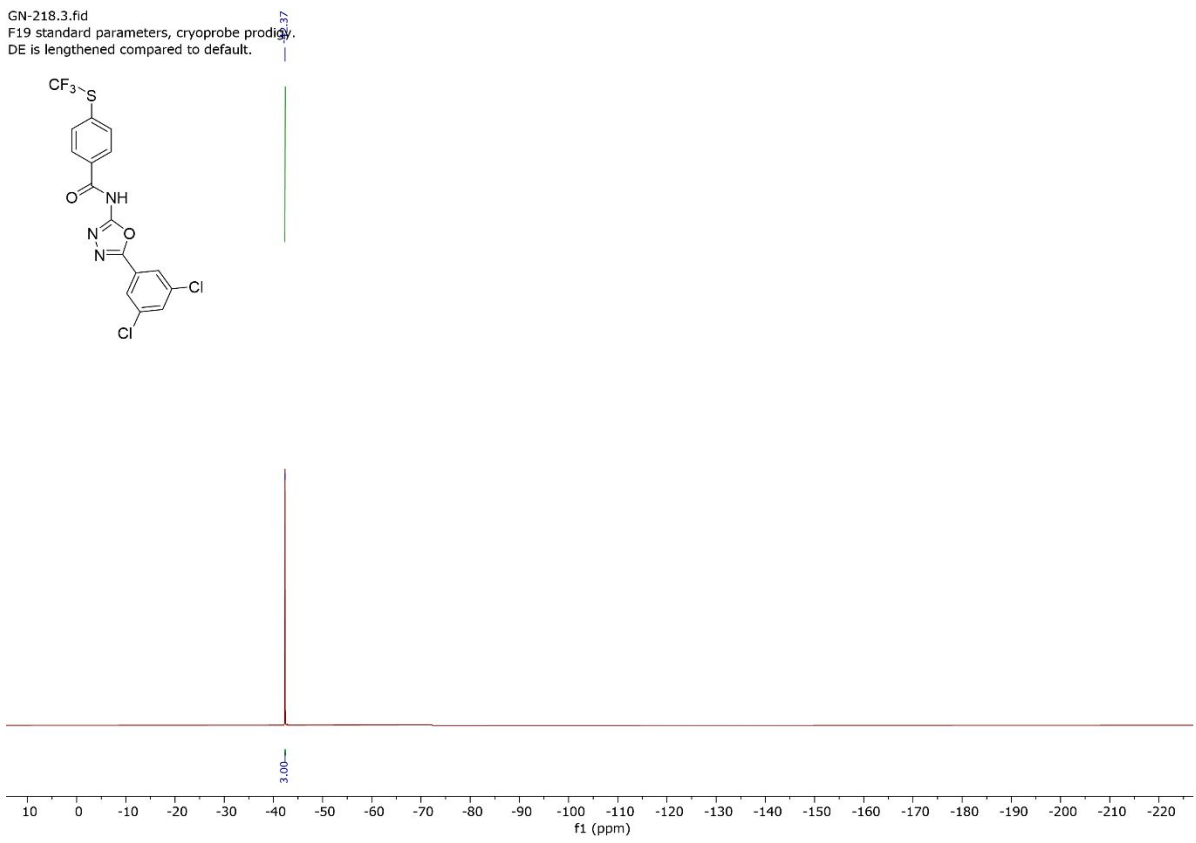
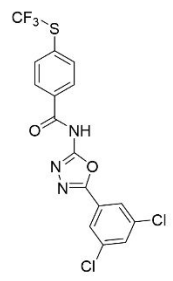
[S33]

GN-218.2.fid  
C13 standard parameters, cryoprobe prodigy.

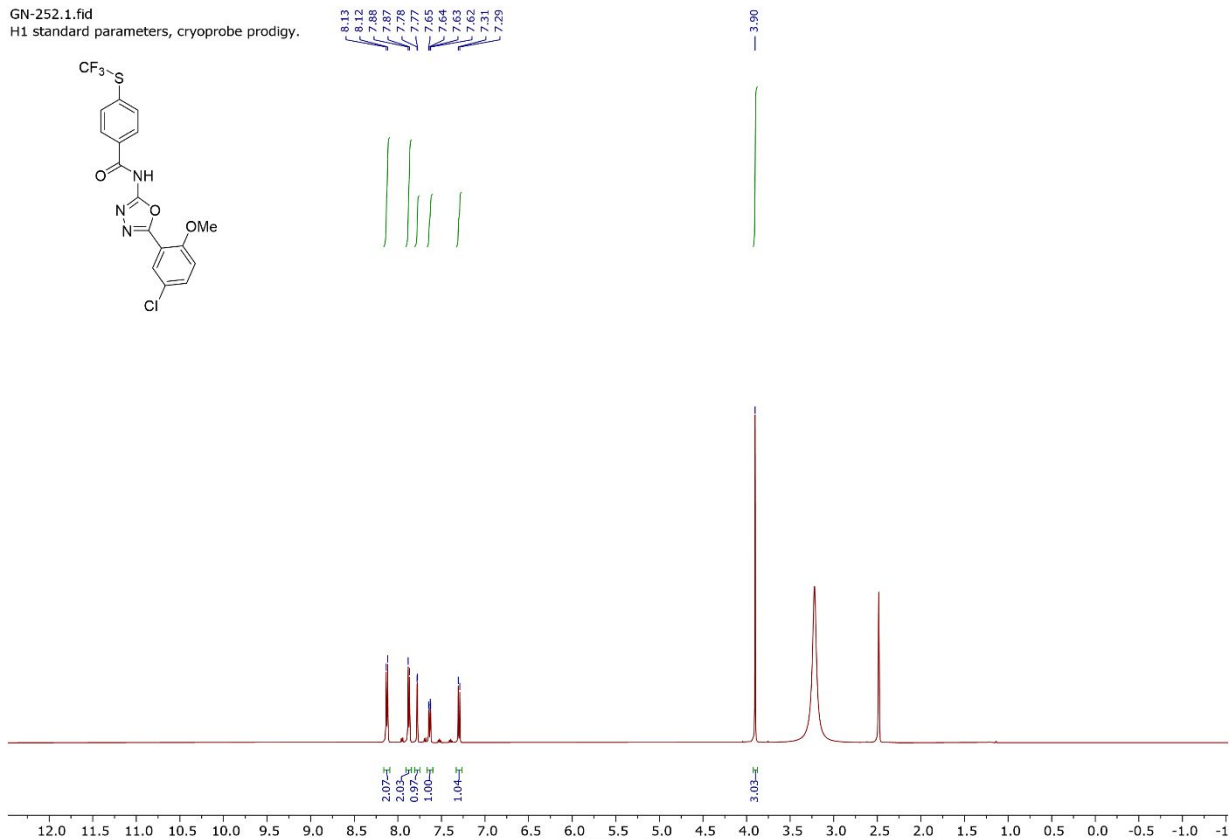
124.41  
124.70  
124.70  
124.07  
124.51  
124.59  
124.40  
124.16  
124.14  
124.51  
124.58  
124.19  
124.27  
124.91



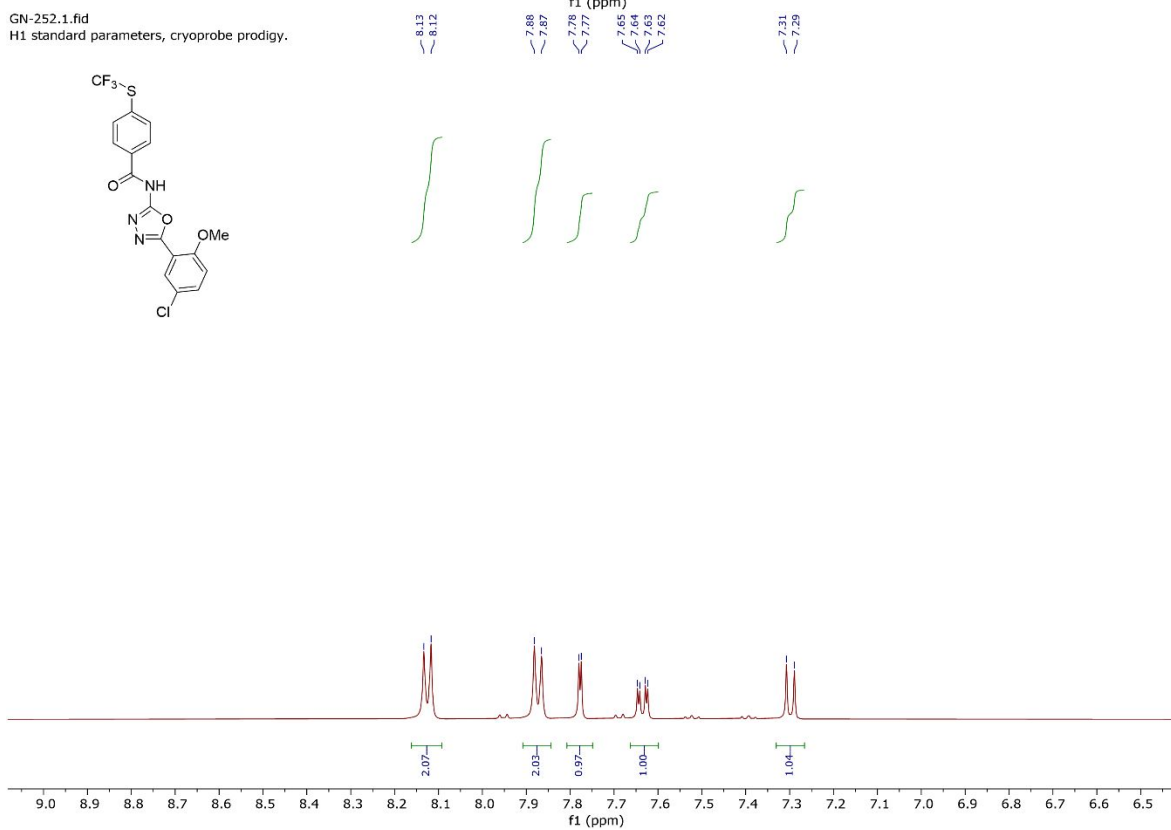
GN-218.3.fid  
F19 standard parameters, cryoprobe prodigy.  
DE is lengthened compared to default.



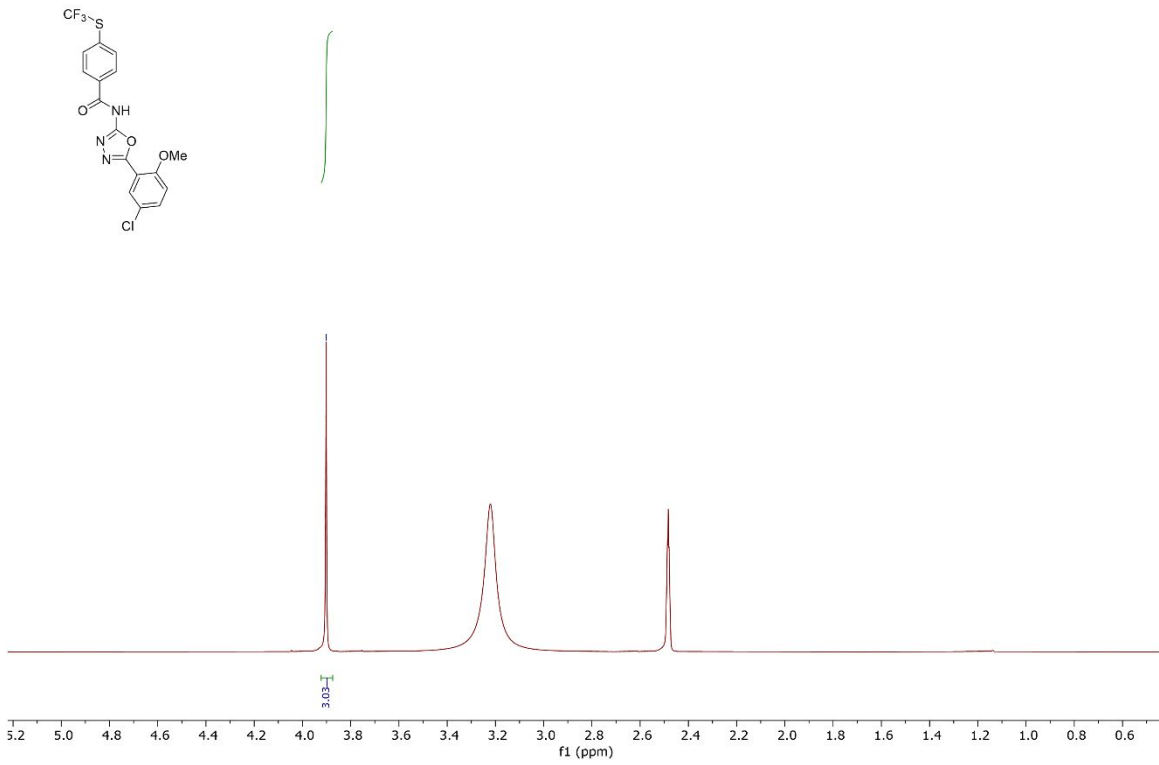
GN-252.1.fid  
H1 standard parameters, cryoprobe prodigy.



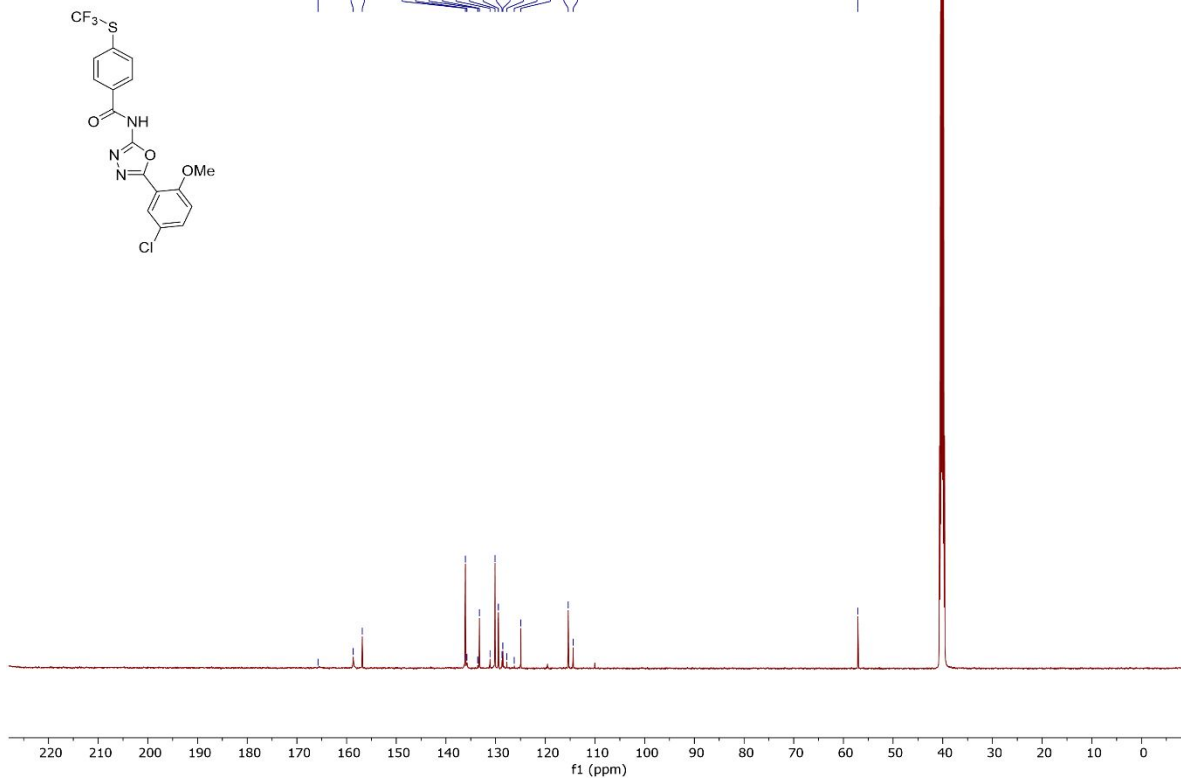
GN-252.1.fid  
H1 standard parameters, cryoprobe prodigy.



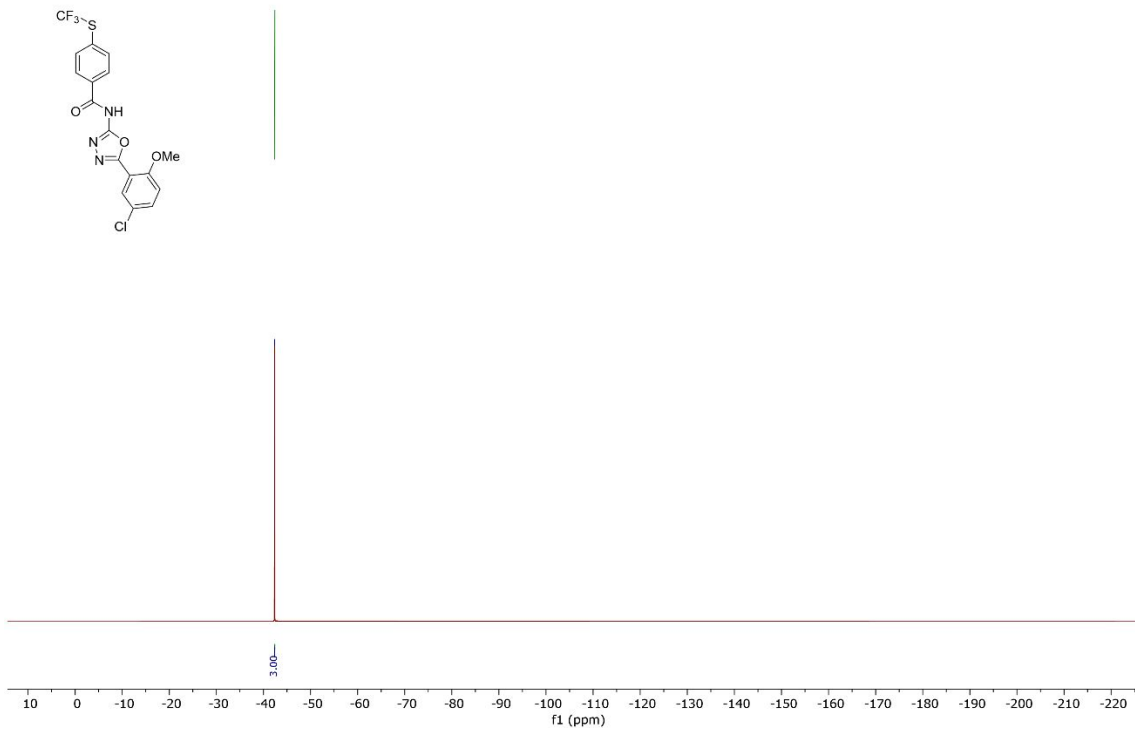
GN-252.1.fid  
H1 standard parameters, cryoprobe prodigy.



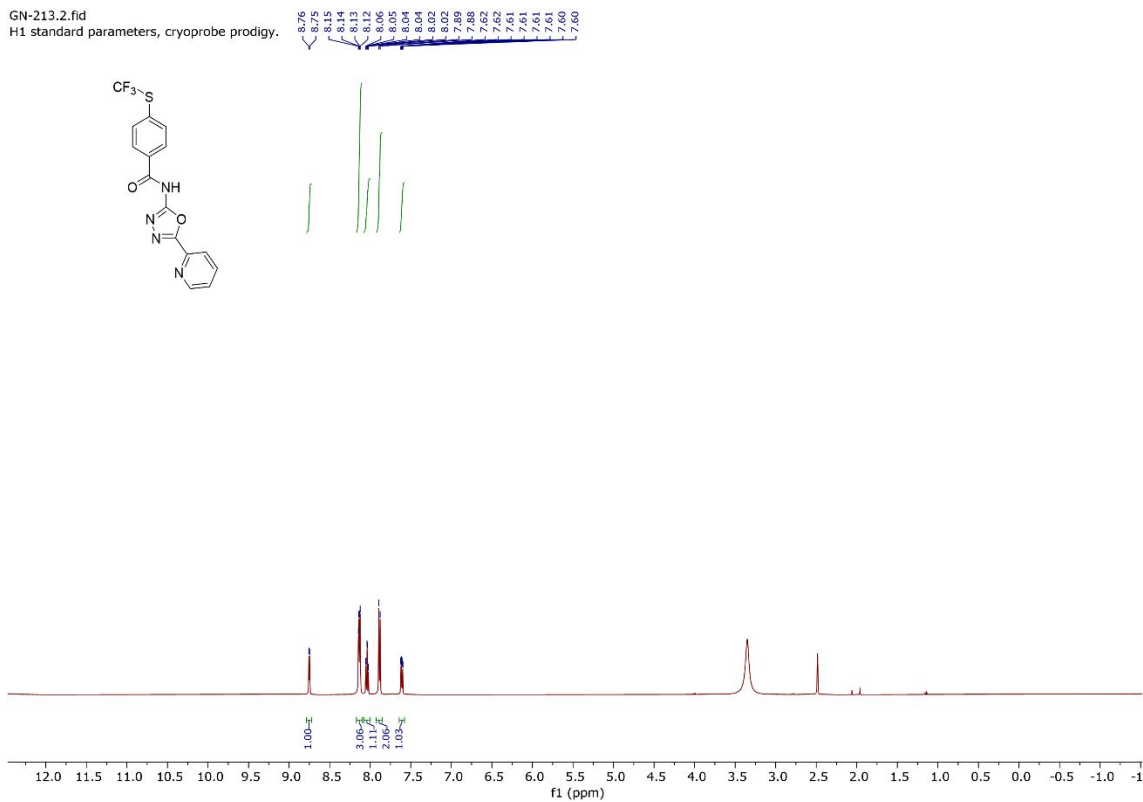
GN-252.2.fid  
C13 standard parameters, cryoprobe prodigy.



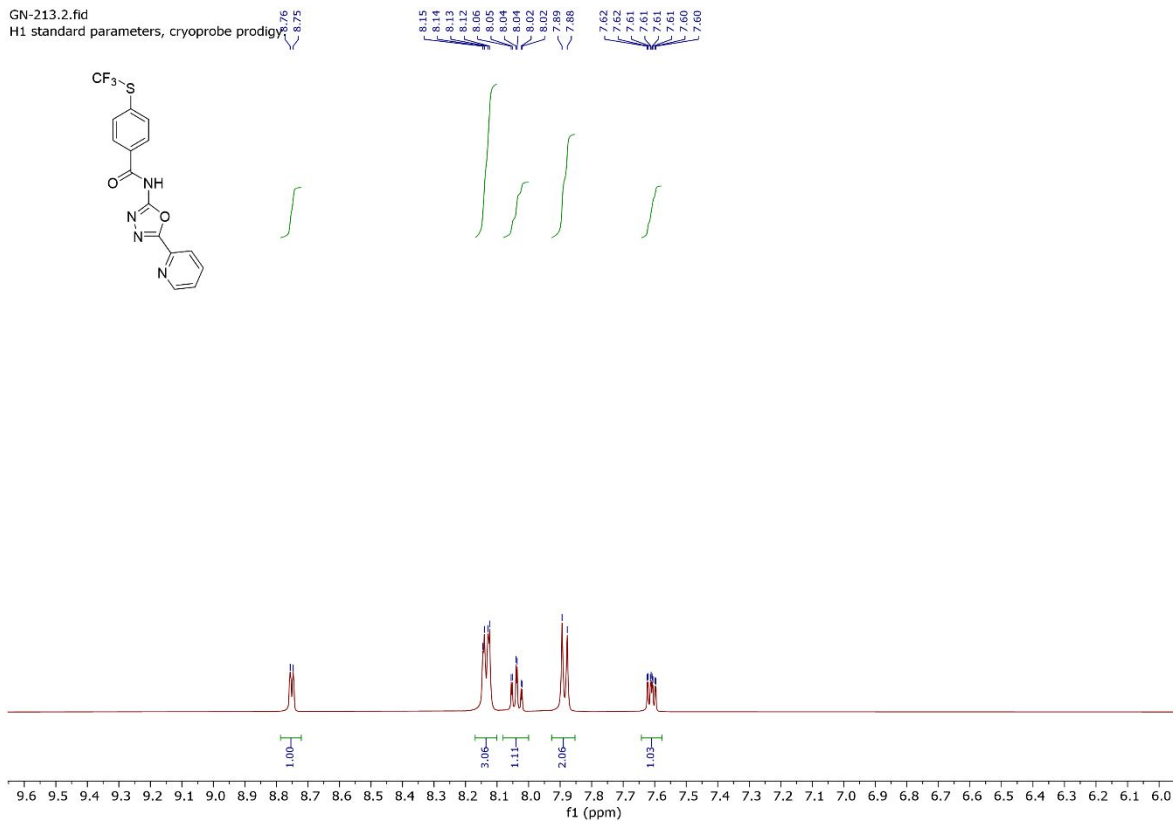
GN-252.3.fid  
F19 standard parameters, cryoprobe prodigy.  
DE is lengthened compared to default.



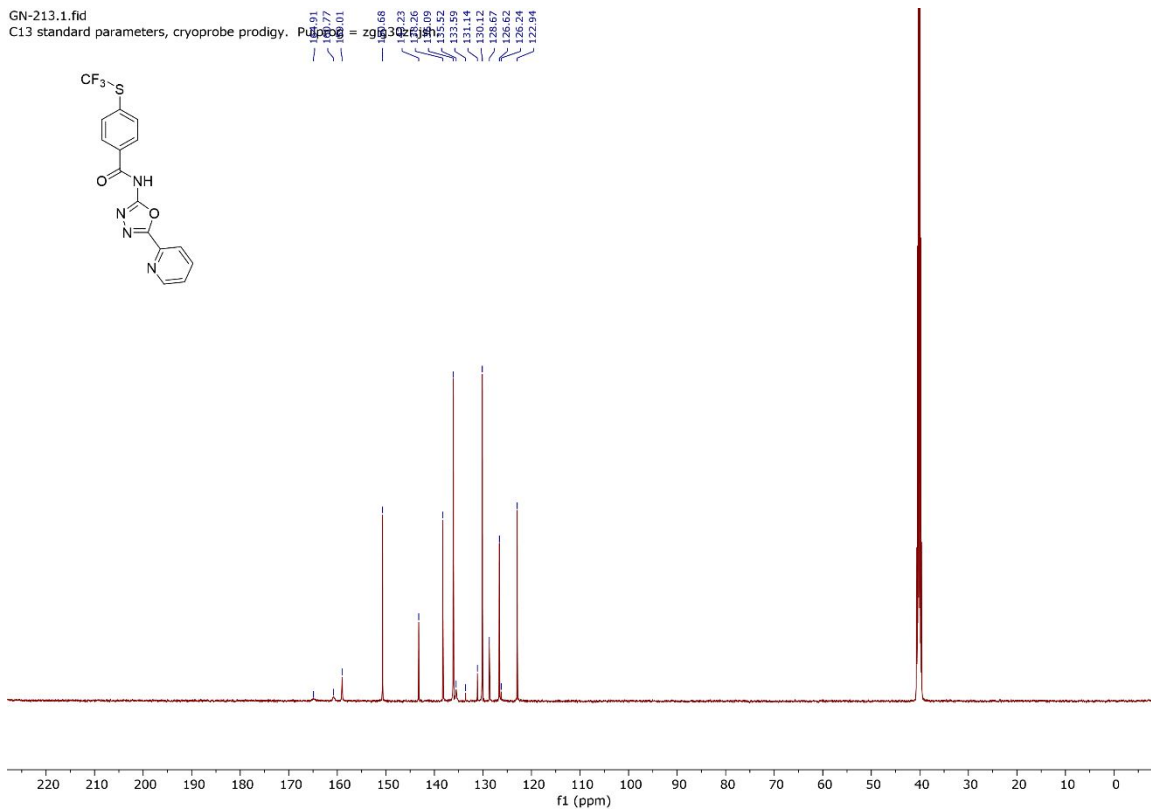
GN-213.2.fid  
H1 standard parameters, cryoprobe prodigy.



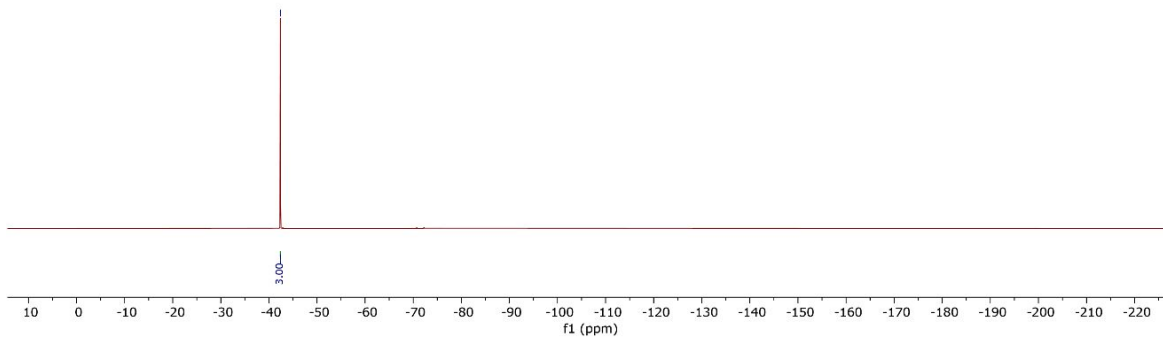
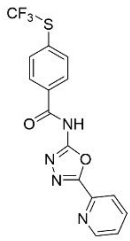
GN-213.2.fid  
H1 standard parameters, cryoprobe prodigy



GN-213.1.fid  
C13 standard parameters, cryoprobe prodigy



GN-213.3.fid  
F19 standard parameters, cryoprobe prodigy.  
DE is lengthened compared to default.



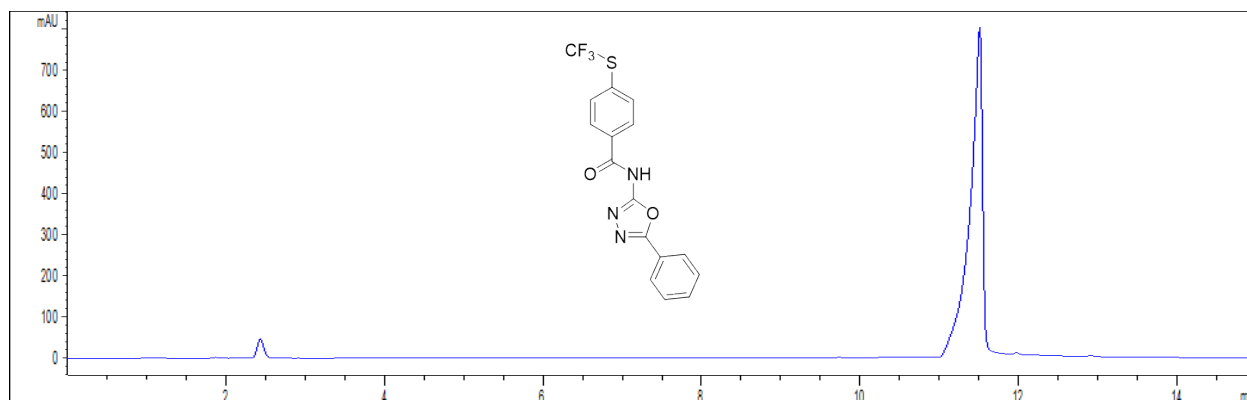
### III. HPLC Traces of Compounds 1 -17:

**Table 2S: HPLC Retention Times and Percent Purity for Compounds 1-17**

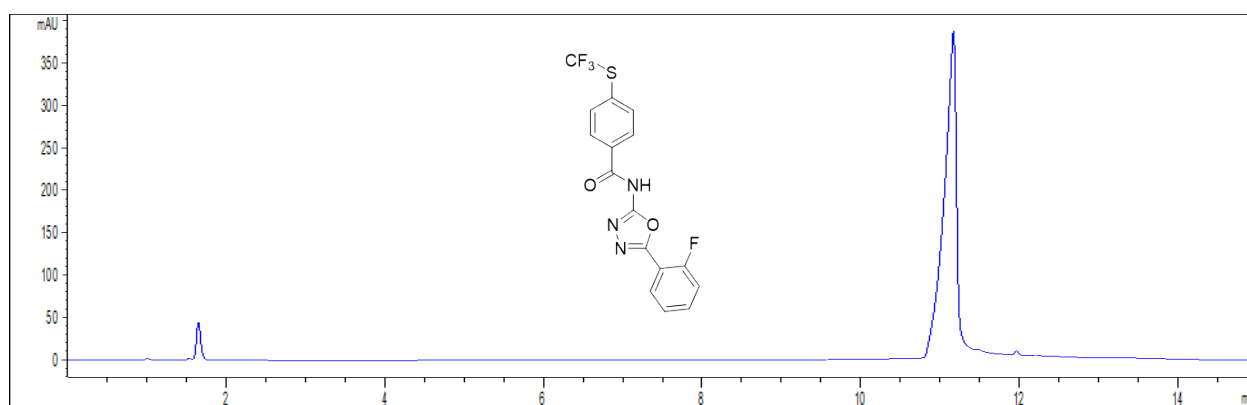
<b>Compound</b>	<b>Retention Time (min)</b>	<b>Purity</b>
<b>1</b>	11.5	96%
<b>2</b>	11.2	96%
<b>3</b>	11.4	96%
<b>4</b>	12.9	98%
<b>5</b>	13.5	96%
<b>6</b>	13.0	98%
<b>7</b>	13.0	98%
<b>8</b>	12.3	96%
<b>9</b>	13.4	97%
<b>10</b>	13.3	99%
<b>11</b>	12.9	99%
<b>12</b>	12.9	98%
<b>13</b>	14.3	97%
<b>14</b>	13.5	99%
<b>HSGN-218</b>	14.2	99%
<b>16</b>	13.1	97%
<b>17</b>	10.3	98%



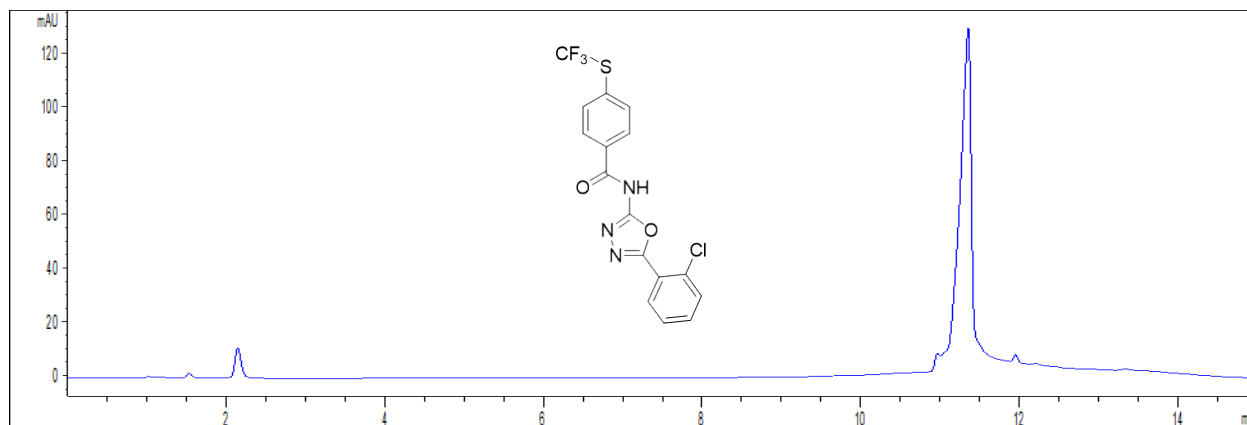
**HPLC Trace at 260 nm for *N*-(5-phenyl-1,3,4-oxadiazol-2-yl)-4-((trifluoromethyl)thio)benzamide (1):**



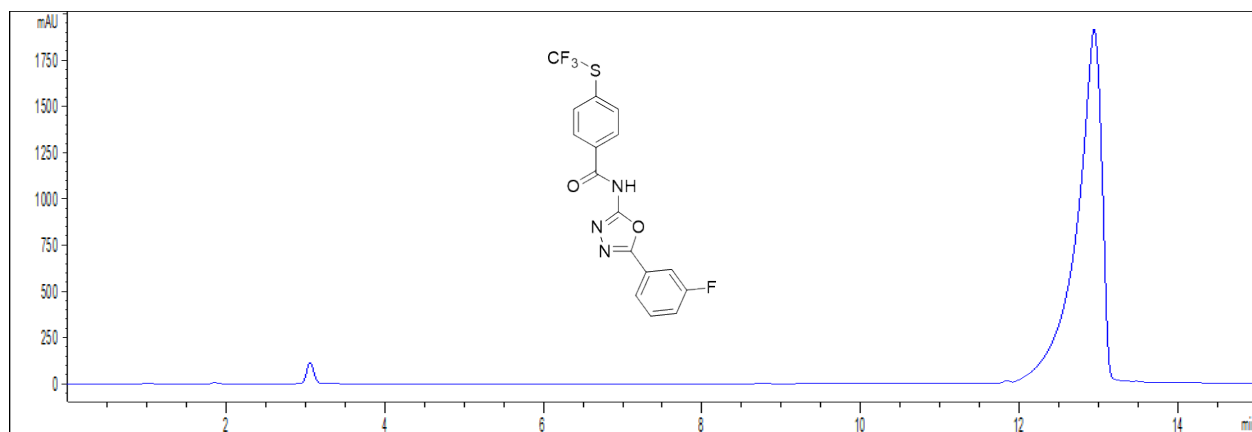
**HPLC Trace at 260 nm for *N*-(5-(2-fluorophenyl)-1,3,4-oxadiazol-2-yl)-4-((trifluoromethyl)thio)benzamide (2):**



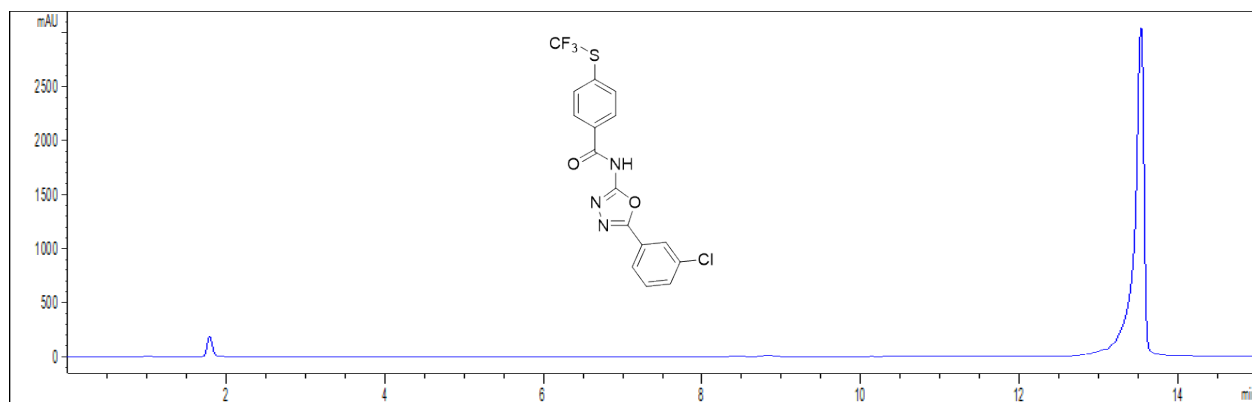
**HPLC Trace at 260 nm for *N*-(5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl)-4-((trifluoromethyl)thio)benzamide (3):**



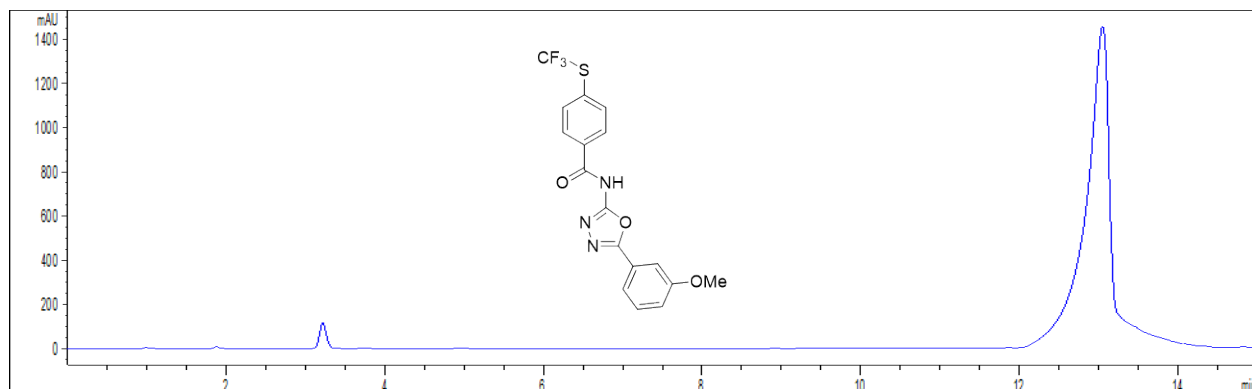
**HPLC Trace at 260 nm for *N*-(5-(3-fluorophenyl)-1,3,4-oxadiazol-2-yl)-4-((trifluoromethyl)thio)benzamide (4):**



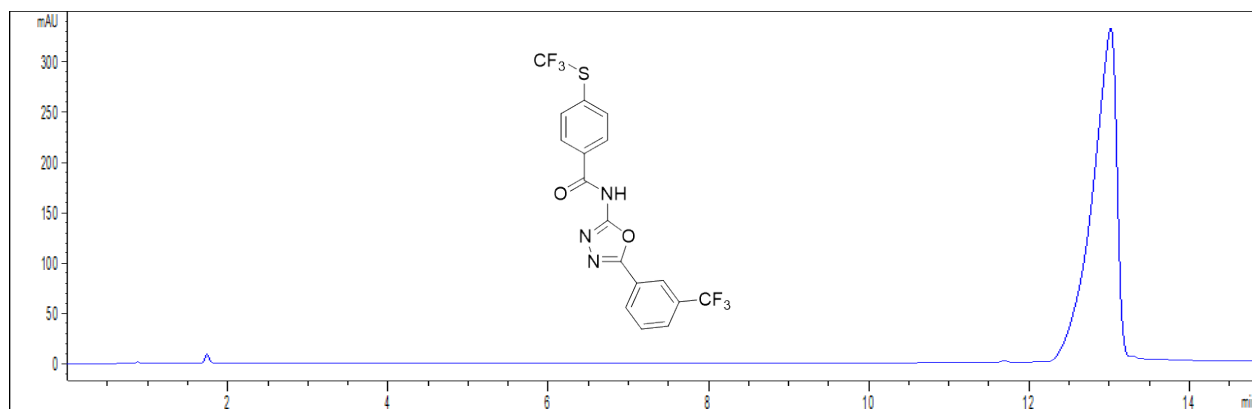
**HPLC Trace at 260 nm for *N*-(5-(3-chlorophenyl)-1,3,4-oxadiazol-2-yl)-4-((trifluoromethyl)thio)benzamide (5):**



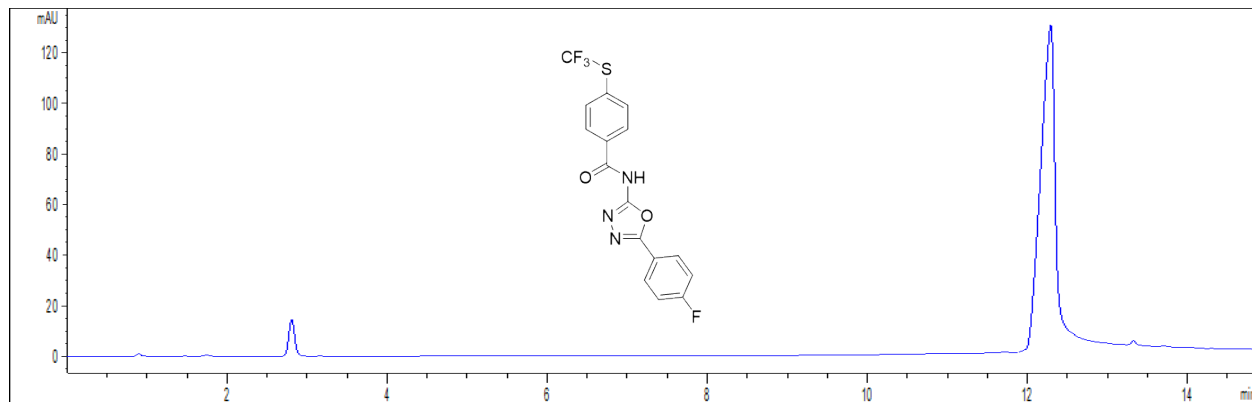
**HPLC Trace at 260 nm for *N*-(5-(3-methoxyphenyl)-1,3,4-oxadiazol-2-yl)-4-((trifluoromethyl)thio)benzamide (6):**



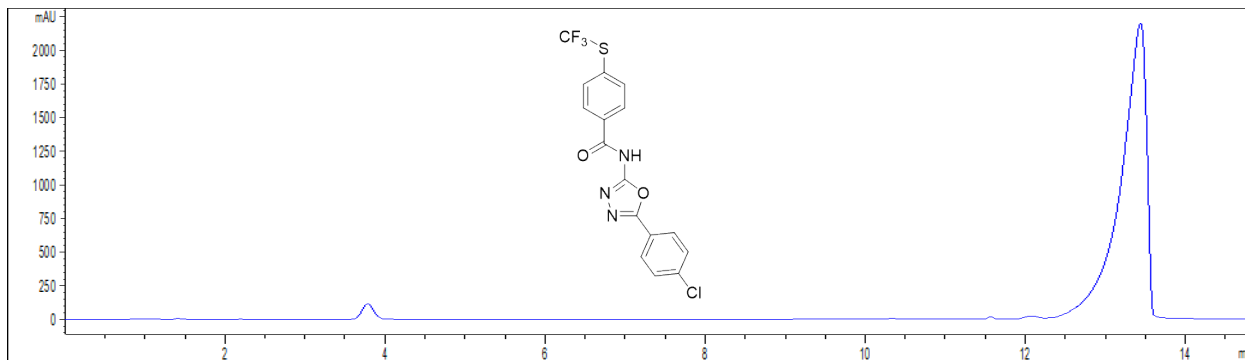
**HPLC Trace at 260 nm for *N*-(5-(3-trifluoromethylphenyl)-1,3,4-oxadiazol-2-yl)-4-((trifluoromethyl)thio)benzamide (7):**



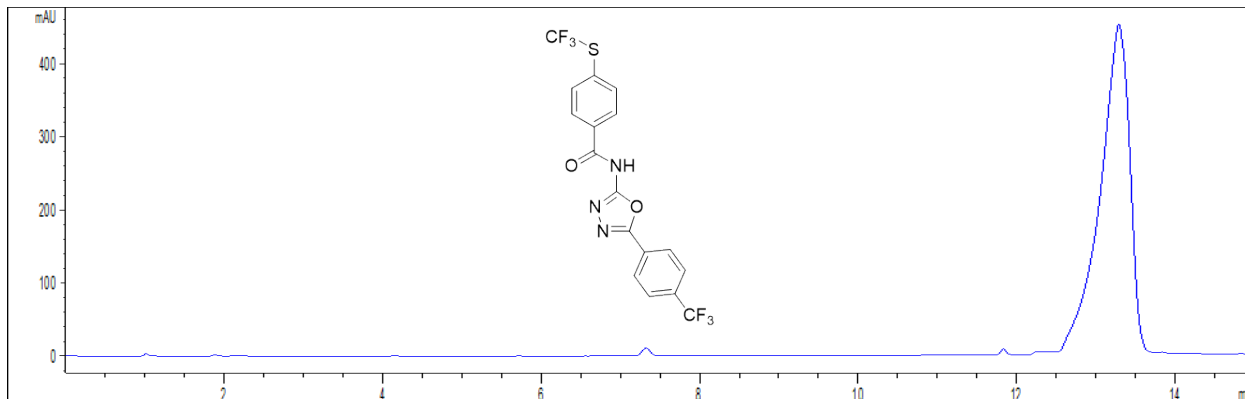
**HPLC Trace at 260 nm for *N*-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)-4-((trifluoromethyl)thio)benzamide (8):**



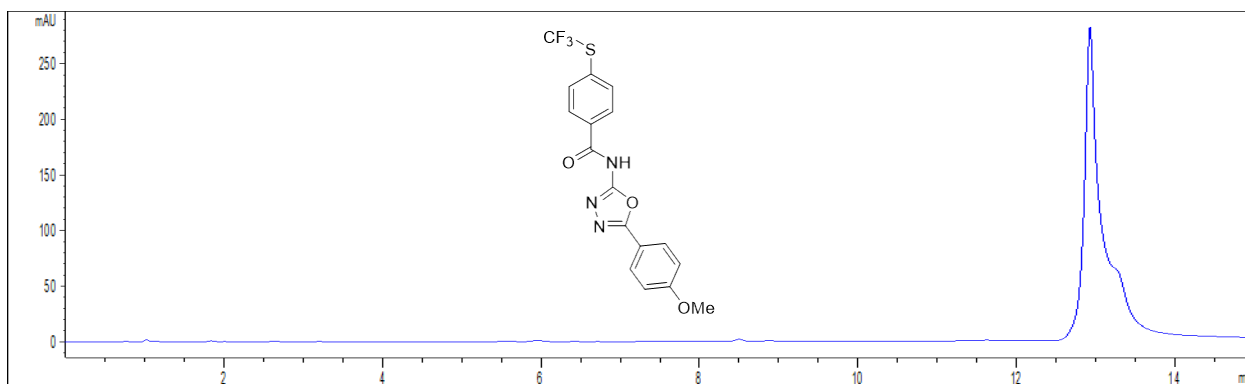
**HPLC Trace at 260 nm for *N*-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-4-((trifluoromethyl)thio)benzamide (9):**



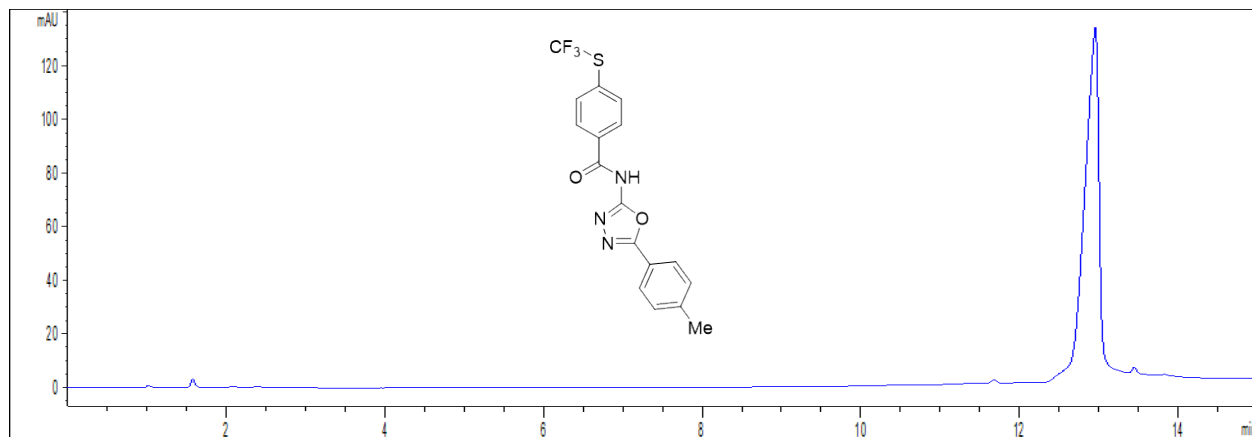
**HPLC Trace at 260 nm for *N*-(5-(4-trifluoromethylphenyl)-1,3,4-oxadiazol-2-yl)-4-((trifluoromethyl)thio)benzamide (10):**



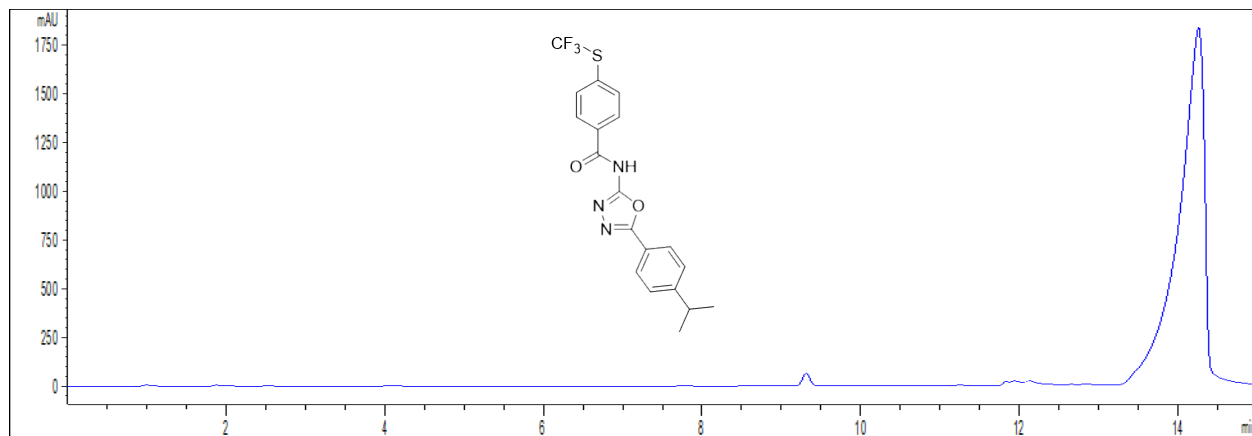
**HPLC Trace at 260 nm for *N*-(5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)-4-((trifluoromethyl)thio)benzamide (11):**



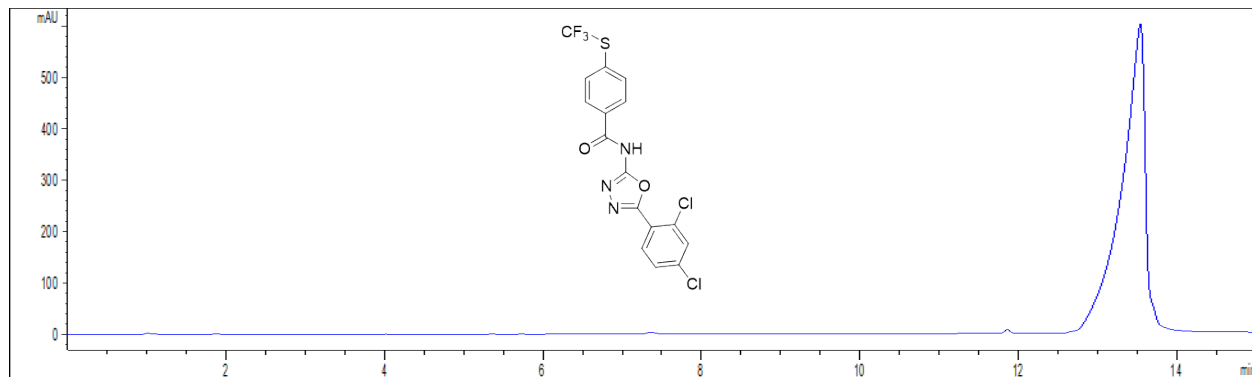
**HPLC Trace at 260 nm for *N*-(5-(4-methylphenyl)-1,3,4-oxadiazol-2-yl)-4-((trifluoromethyl)thio)benzamide (12):**



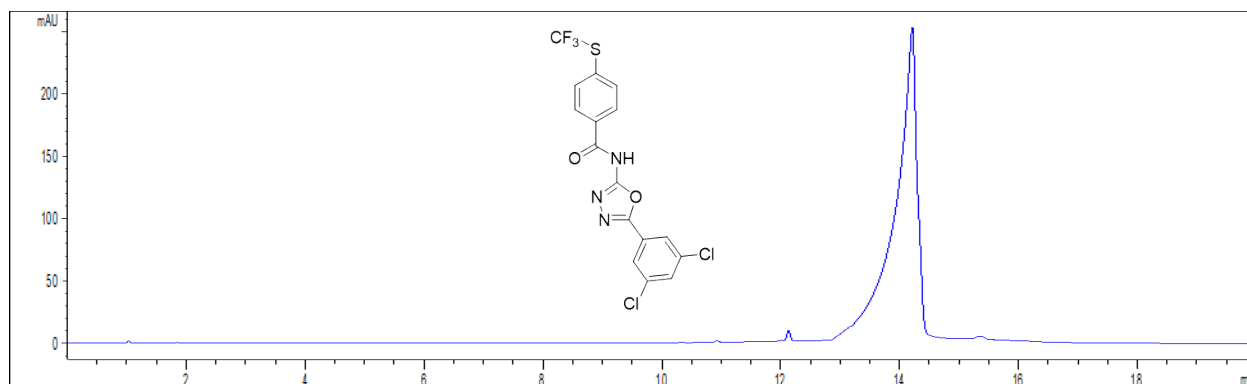
**HPLC Trace at 260 nm for *N*-(5-(4-isopropylphenyl)-1,3,4-oxadiazol-2-yl)-4-((trifluoromethyl)thio)benzamide (13):**



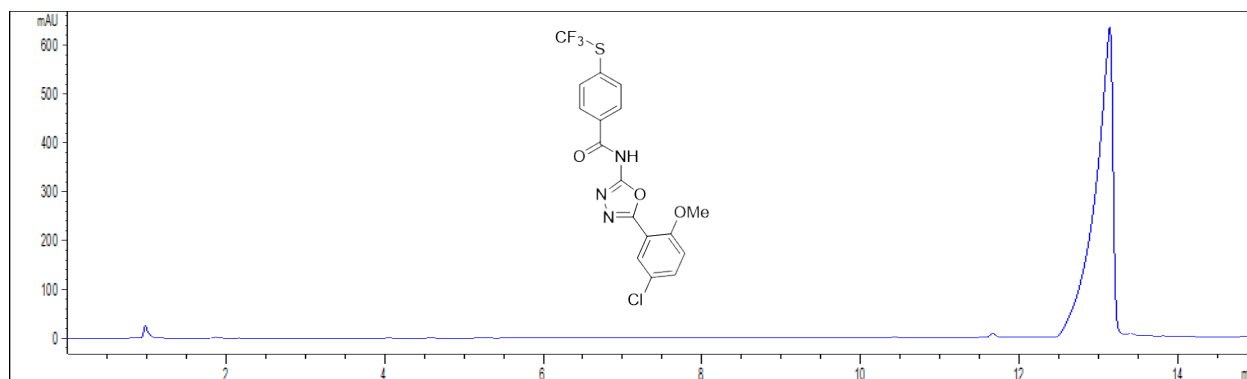
**HPLC Trace at 260 nm for *N*-(5-(2,4-dichlorophenyl)-1,3,4-oxadiazol-2-yl)-4-((trifluoromethyl)thio)benzamide (14):**



**HPLC Trace at 260 nm for *N*-(5-(3,5-dichlorophenyl)-1,3,4-oxadiazol-2-yl)-4-((trifluoromethyl)thio)benzamide (HSGN-218):**



**HPLC Trace at 260 nm for *N*-(5-(5-chloro-2-methoxyphenyl)-1,3,4-oxadiazol-2-yl)-4-((trifluoromethyl)thio)benzamide (16):**



**HPLC Trace at 260 nm for *N*-(5-(pyridin-2-yl)-1,3,4-oxadiazol-2-yl)-4-((trifluoromethyl)thio)benzamide (17):**

