

# The Discovery and Characterization of ML218: A Novel, Centrally Active T-Type Calcium Channel Inhibitor with Robust Effects in STN Neurons and in a Rodent Model of Parkinson's Disease

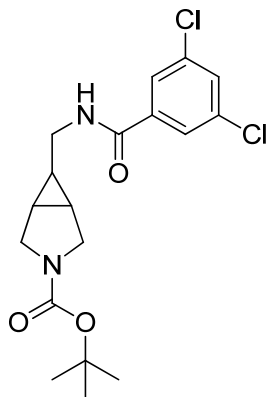
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## Supporting Information

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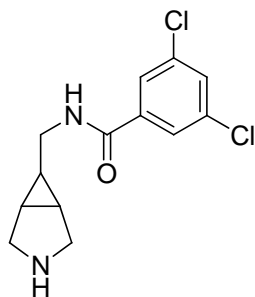
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## ML218 Synthesis Experimental.



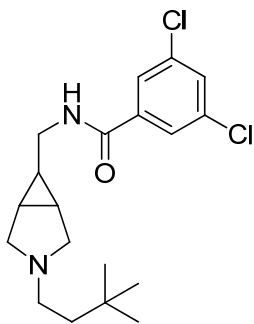
### ***tert*-butyl 6-((3,5-dichlorobenzamido)methyl)-3-azabicyclo[3.1.0]hexane-3-carboxylate (25).**

To a solution of *exo*-3-Boc-6-aminomethyl-3-azabicyclo[3.1.0]hexane (1.0 eq.) and triethylamine (1.0 eq.) in CH<sub>2</sub>Cl<sub>2</sub> at 0 °C was added a solution of 3,5-dichlorobenzoyl chloride (1.0 eq.) in CH<sub>2</sub>Cl<sub>2</sub>. The reaction stirred at 0 °C for approximately 4 hrs. The mixture was then concentrated *in vacuo* and purified by reverse phase chromatography (MeCN/H<sub>2</sub>O/0.1% TFA) to afford the product as a white solid (93% yield). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ (ppm): 7.83 (d, *J* = 1.92 Hz, 2H); 7.66 (t, *J* = 1.88 Hz, 1H); 3.57 (m, 2H); 3.35 (m, 4H); 1.60 (m, 2H); 1.46 (s, 9H); 0.90 (m, 1H). <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD) δ (ppm): 167.96, 157.61, 139.75, 137.33, 133.02, 127.99, 81.86, 43.65, 29.56, 24.46, 24.02, 23.2; HRMS calc'd for C<sub>18</sub>H<sub>22</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub>, 385.1087 [M+H]; found 385.1089.



***N*-(3-azabicyclo[3.1.0]hexan-6-ylmethyl)-3,5-dichlorobenzamide.** To a vial containing *tert*-

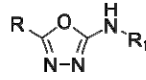
butyl 6-((3,5-dichlorobenzamido)methyl)-3-azabicyclo[3.1.0]hexane-3-carboxylate, was added 4.0 M HCl in dioxane. This mixture was allowed to stir at room temp for 2 hrs. The reaction was then concentrated *in vacuo* and purified by reverse phase chromatography (MeCN/H<sub>2</sub>O/0.1% TFA) to afford the product as a white solid (95% yield). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ (ppm): 7.82 (d, *J* = 1.92 Hz, 2H); 7.68 (m, 1H); 3.45 (s, 4H); 3.34 (m, 2H); 1.90 (m, 2H); 1.21 (m, 1H). <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) δ (ppm): 168.04, 163.95, 139.57, 137.40, 133.17, 127.97, 43.02, 23.35, 22.48; HRMS calc'd for C<sub>13</sub>H<sub>14</sub>Cl<sub>2</sub>N<sub>2</sub>O, 285.0563 [M+H]; found 285.0566.



### **3,5-dichloro-N-((3-(3,3-dimethylbutyl)-3-azabicyclo[3.1.0]hexan-6-yl)methyl)benzamide**

**(ML218).** To a solution of 3,3-dimethylbutyraldehyde (1.0 eq.) in CH<sub>2</sub>Cl<sub>2</sub> was added a solution of *N*-(3-azabicyclo[3.1.0]hexan-6-yl)methyl)-3,5-dichlorobenzamide (1.0 eq.) in CH<sub>2</sub>Cl<sub>2</sub> followed by addition of polymer-supported sodium triacetoxymethylborohydride (1.2 eq.). The reaction was left to stir overnight at room temperature after which the mixture was filtered through a pad of Celite eluting with CH<sub>2</sub>Cl<sub>2</sub> and concentrated *in vacuo* resulting in a crude oil which was then purified by reverse phase chromatography (MeCN/H<sub>2</sub>O/0.1% TFA) to afford the product as an off-white solid (88% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 7.78 (d, *J* = 1.82 Hz, 2H); 7.57 (br, 1H); 7.42 (ap. t, *J* = 1.76 Hz, 1H); 3.64 (d, *J* = 10.56 Hz, 2H); 3.32 (t, *J* = 5.98 Hz, 2H); 2.95-2.83 (m, 4H); 1.95 (m, 1H); 1.74 (s, 2H); 1.62 (m, 2H); 0.88 (s, 9H). <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) δ (ppm): 165.41, 137.46, 135.50, 131.46, 126.36, 55.69, 53.11, 41.94, 39.42, 30.07, 29.44, 21.71; HRMS calc'd for C<sub>19</sub>H<sub>26</sub>Cl<sub>2</sub>N<sub>2</sub>O, 369.1500 [M+H]; found 369.1501.

**Supporting Table 1.** Structures and Activities of the 8x7 Library of Analogs **16**.



CID	R	R <sub>1</sub>	Cav3.2 IC <sub>50</sub> (μM)	CID	R	R <sub>1</sub>	Cav3.2 IC <sub>50</sub> (μM)	CID	R	R <sub>1</sub>	Cav3.2 IC <sub>50</sub> (μM)	CID	R	R <sub>1</sub>	Cav3.2 IC <sub>50</sub> (μM)
85285665			>10	85285667			1.1	85285662			2.6	85285680			2.1
85285666			>10	NA			NA	85285682			3.1	85285681			3.0
85285668			2.1	85285668			3.7	NA			NA	85285682			>10
85285690			2.8	85285670			1.5	85285682			>10	NA			NA
85285684			1.7	NA			NA	85285693			>10	85285679			>10
85285667			2.4	NA			NA	NA			NA	NA			NA
NA			NA	NA			NA	NA			NA	NA			NA
85285669			1.3	85285669			>10	85285694			>10	85285663			>10

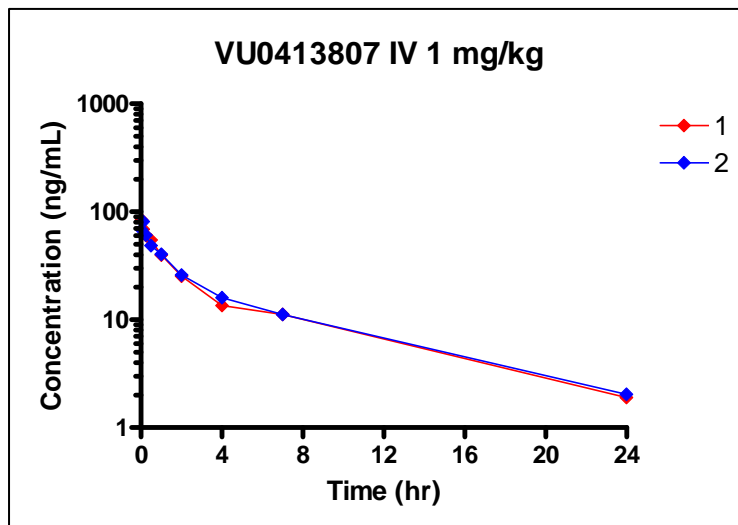
CID	R	R <sub>1</sub>	Cav3.2 IC <sub>50</sub> (μM)	CID	R	R <sub>1</sub>	Cav3.2 IC <sub>50</sub> (μM)	CID	R	R <sub>1</sub>	Cav3.2 IC <sub>50</sub> (μM)
85285673			1.1	85301181			1.5	85301174			3.6
85285674			1.3	85301182			>10	85301175			4.8
85285676			>10	85301194			>10	85301177			2.5
85285678			1.4	85301188			1.8	85301179			>10
85285371			2.3	85301180			>10	85301172			>10
85285370			>10	85301183			1.7	85301176			>10
85285372			2.1	NA			NA	85301172			1.1
85285377			2.6	85301185			>10	85301178			2.7

NA, not assayed

### Additional DMPK Data

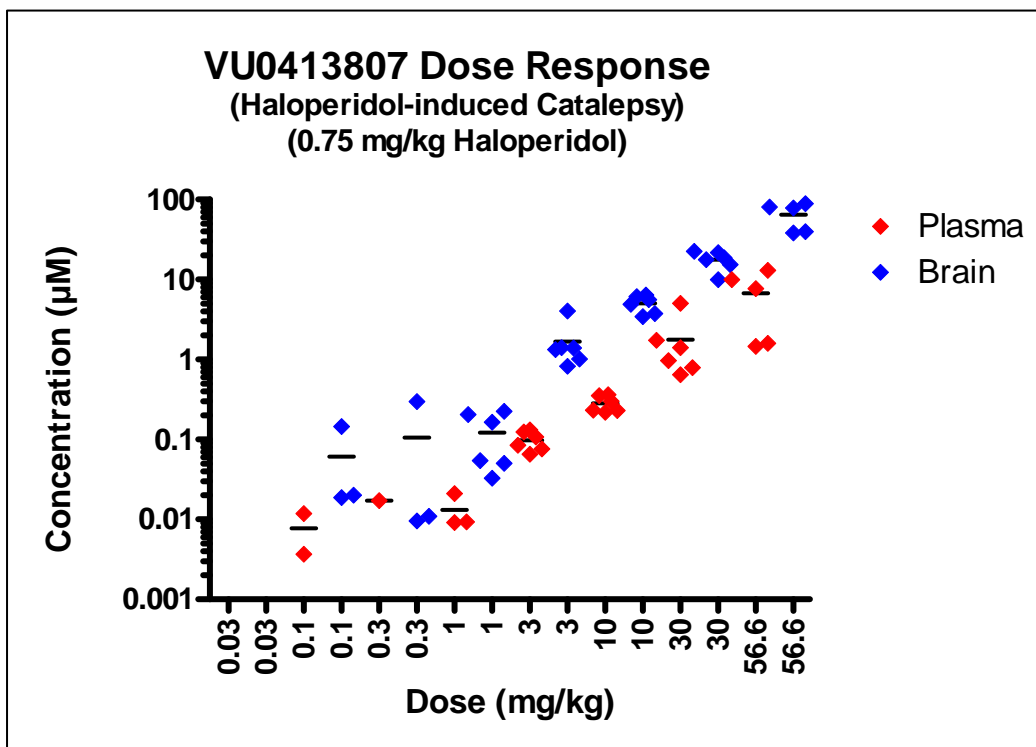
IV - VU0413807 (ML218)			
Dose (mg/kg)	Time (hr)	Concentration (ng/mL)	
		1	2
1	0.0333	84.4	68.0
	0.117	69.1	81.0
	0.25	60.4	60.8
	0.5	54.9	48.5
	1	39.7	40.4
	2	25.3	25.9
	4	13.5	16.0
	7	11.2	11.2
	24	1.90	2.04

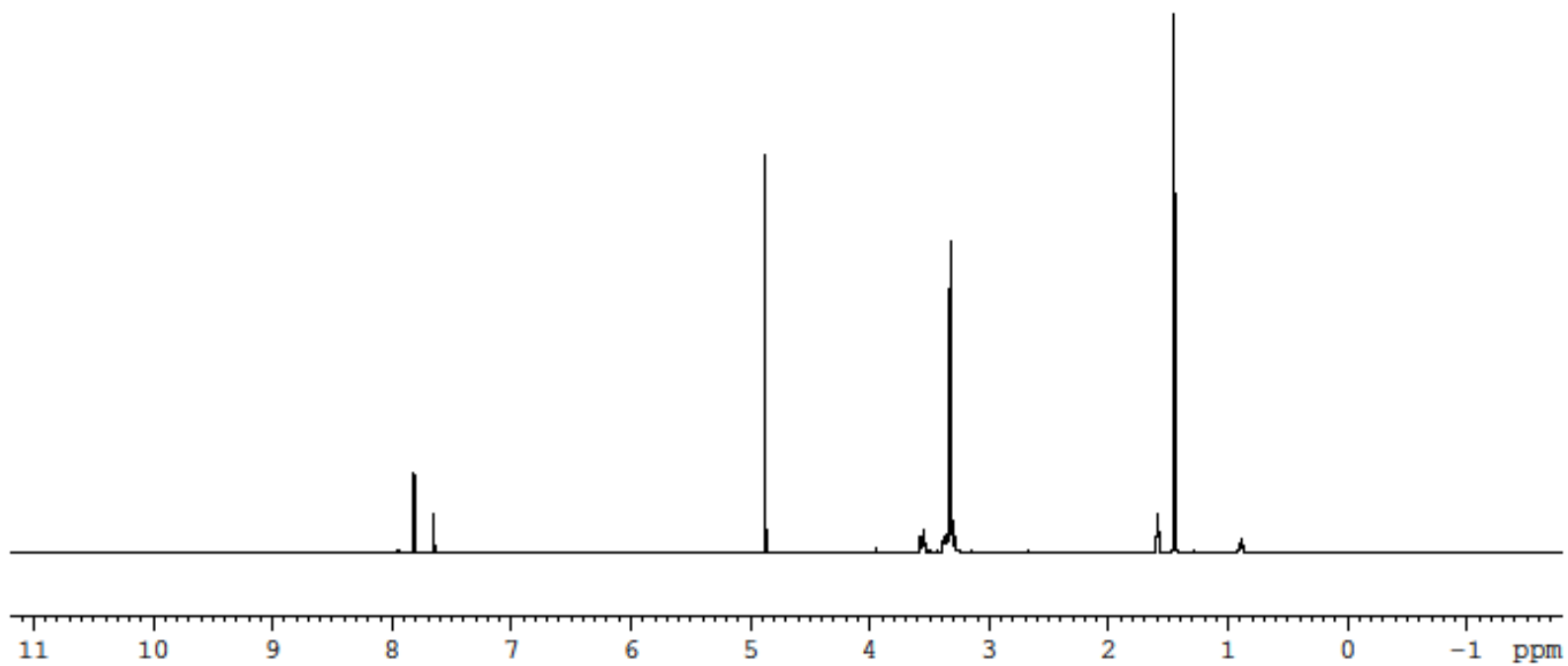
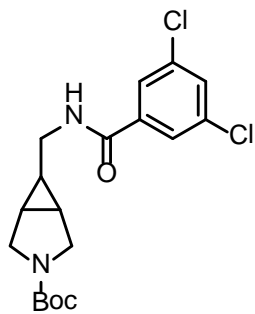
PK Parameter		1	2
t 1/2 (term)	min	415	408
MRT	min	422	428
Cl_obs	mL/min/kg	57	55
Vss	L/kg	24	24
AUC (IV)	hr*ng/mL	295	302

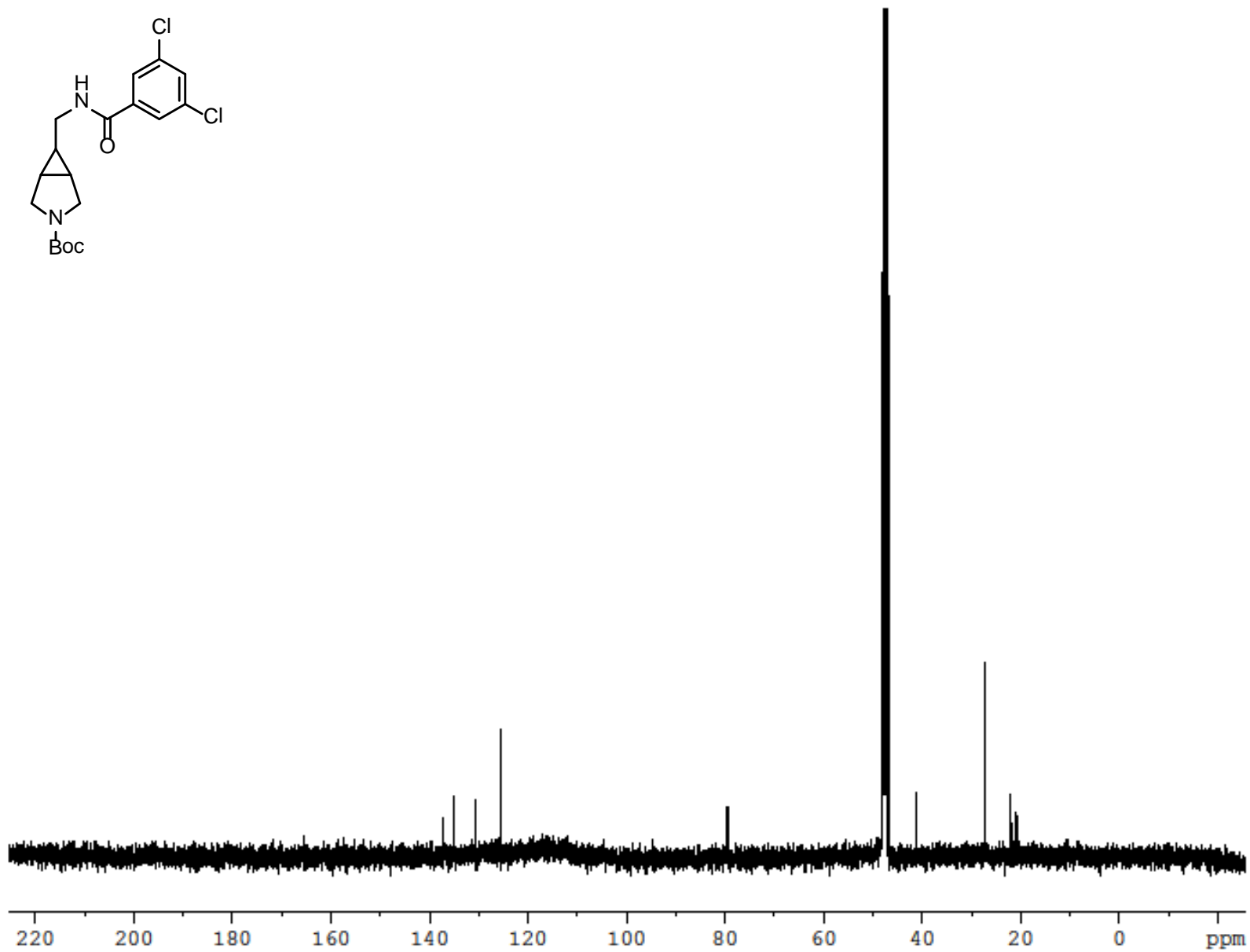
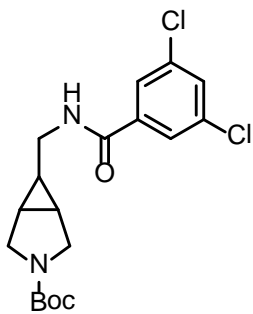


PO PBL – ML218					
Dose (mg/kg)	Time (hr)	Concentration (ng/mL or g)			Brain : Plasma Ratio
		Plasma Systemic	HPV	Brain	
10	1	94.2	418	814	8.64
	1	195	366	1221	6.26

**PK/PD in MI218 AHL Experiment**









Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -0.5, max = 25.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions

244 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 15-500 H: 5-1000 N: 1-200 O: 1-200 Cl: 2-2

MLS-218-Boc

S/N: UH193

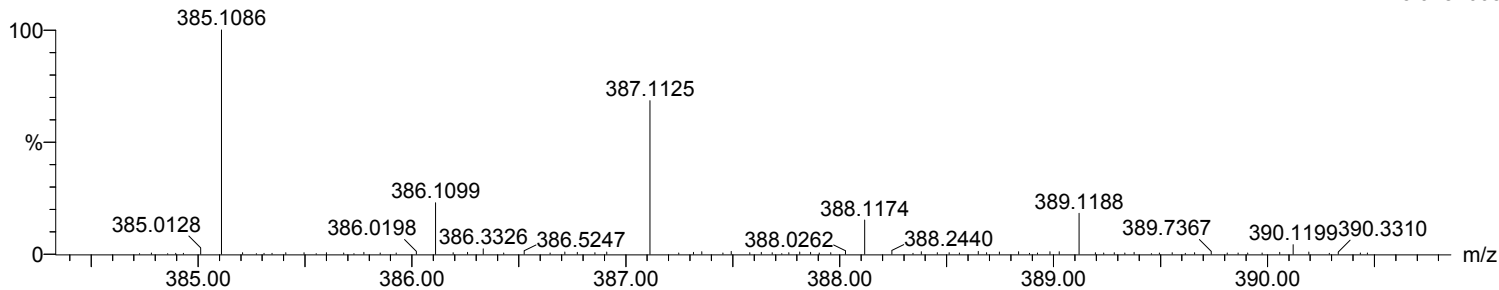
15-Sep-2011

16:03:25

MLS-218-BOC\_091511\_002 96 (1.788) AM (Cen,4, 80.00, Ar,8000.0,556.28,0.70); Sm (SG, 2x1.00); Cm (90:100)

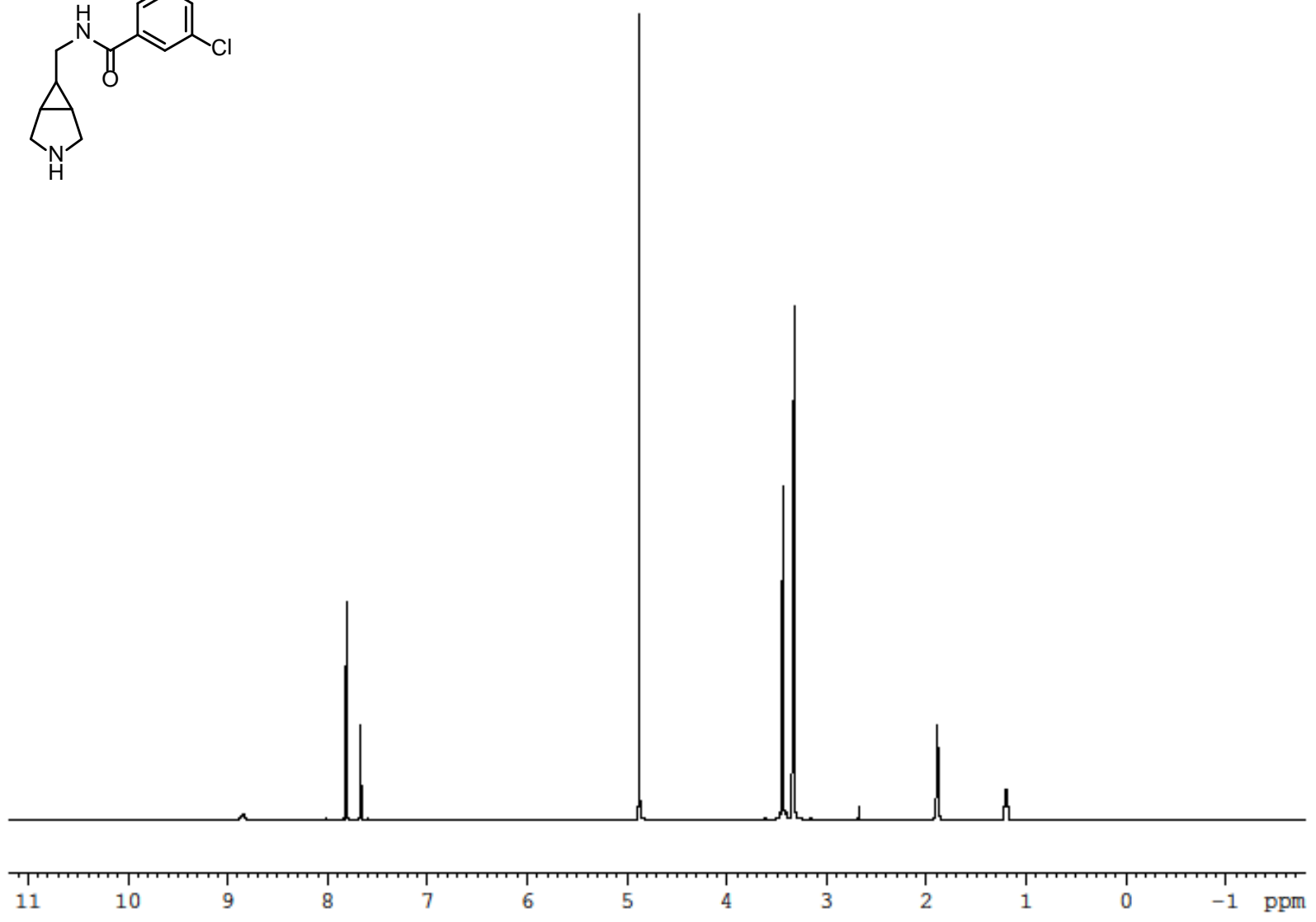
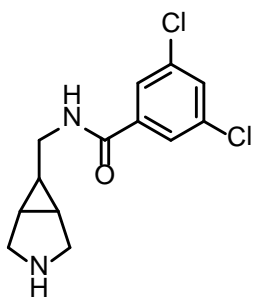
TOF MS ES+

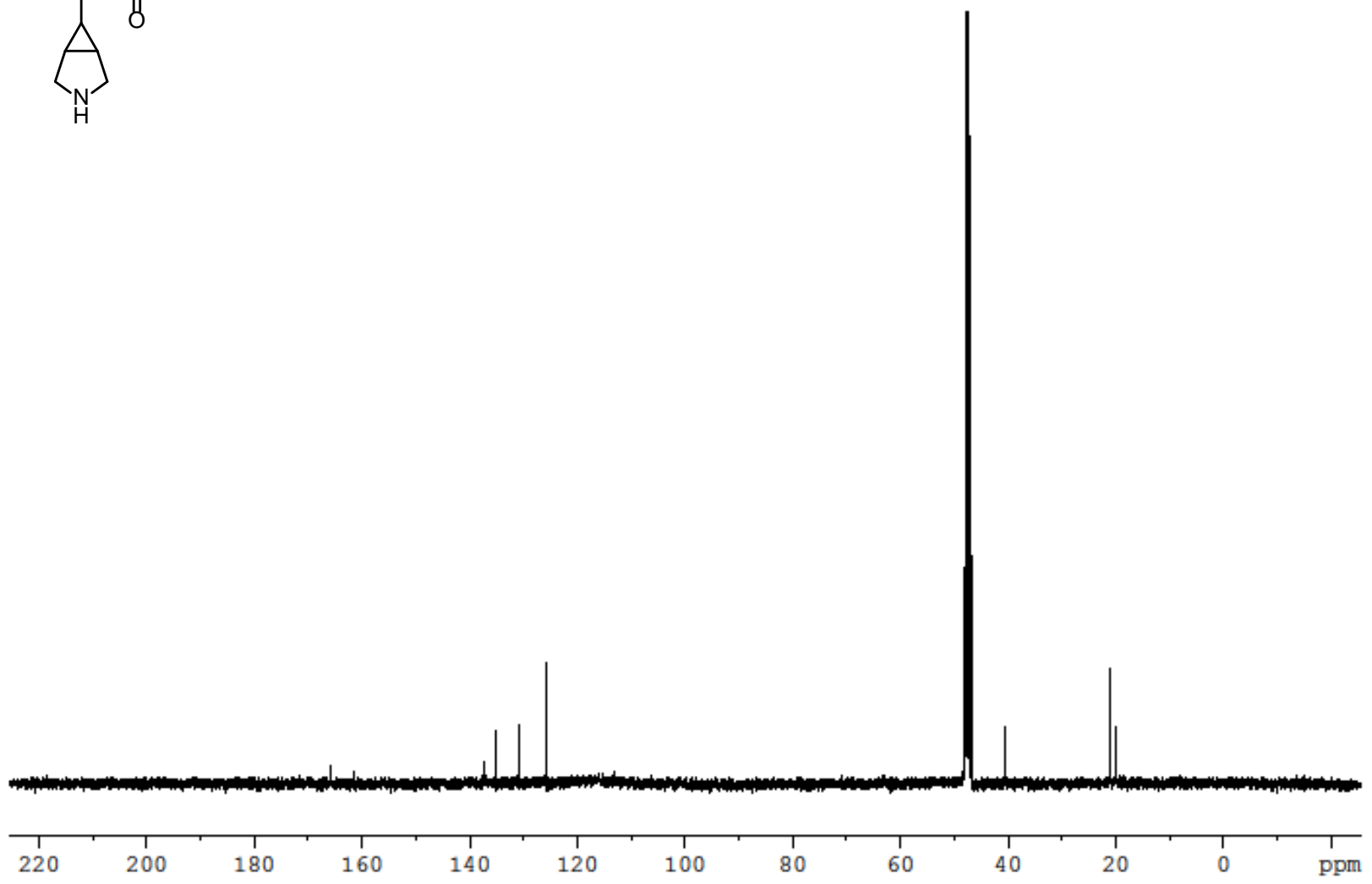
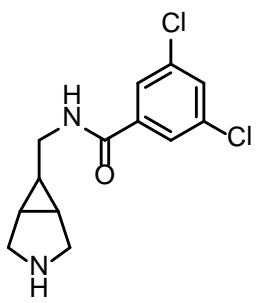
3.94e+003



Minimum: -0.5  
Maximum: 10.0 5.0 25.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
385.1086	385.1086	0.0	0.0	7.5	2.2	C18 H23 N2 O3 C12





Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -0.5, max = 25.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions

90 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 12-500 H: 5-1000 N: 1-200 O: 1-200 Cl: 2-2

MLS-218-Deboc

S/N: UH193

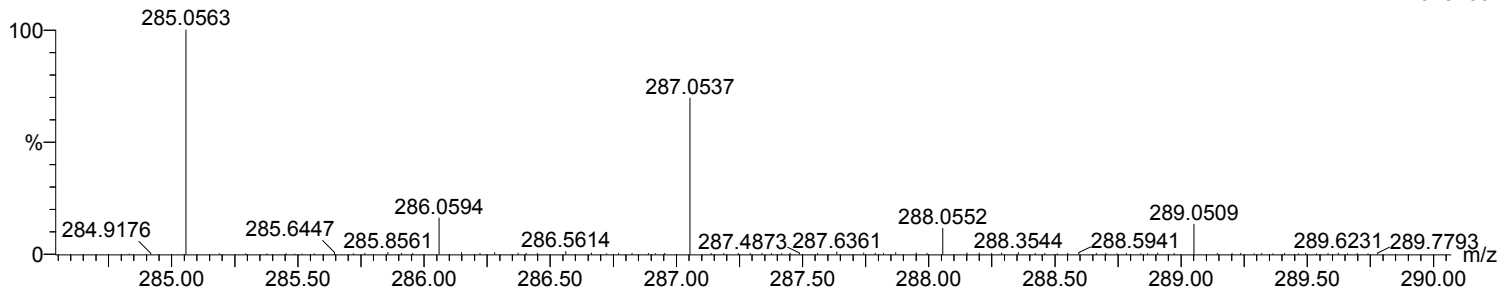
15-Sep-2011

16:14:44

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TOF MS ES+

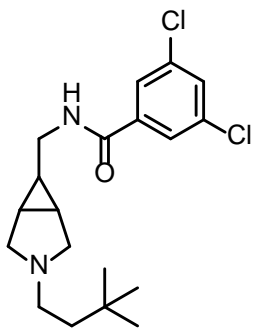
1.07e+004



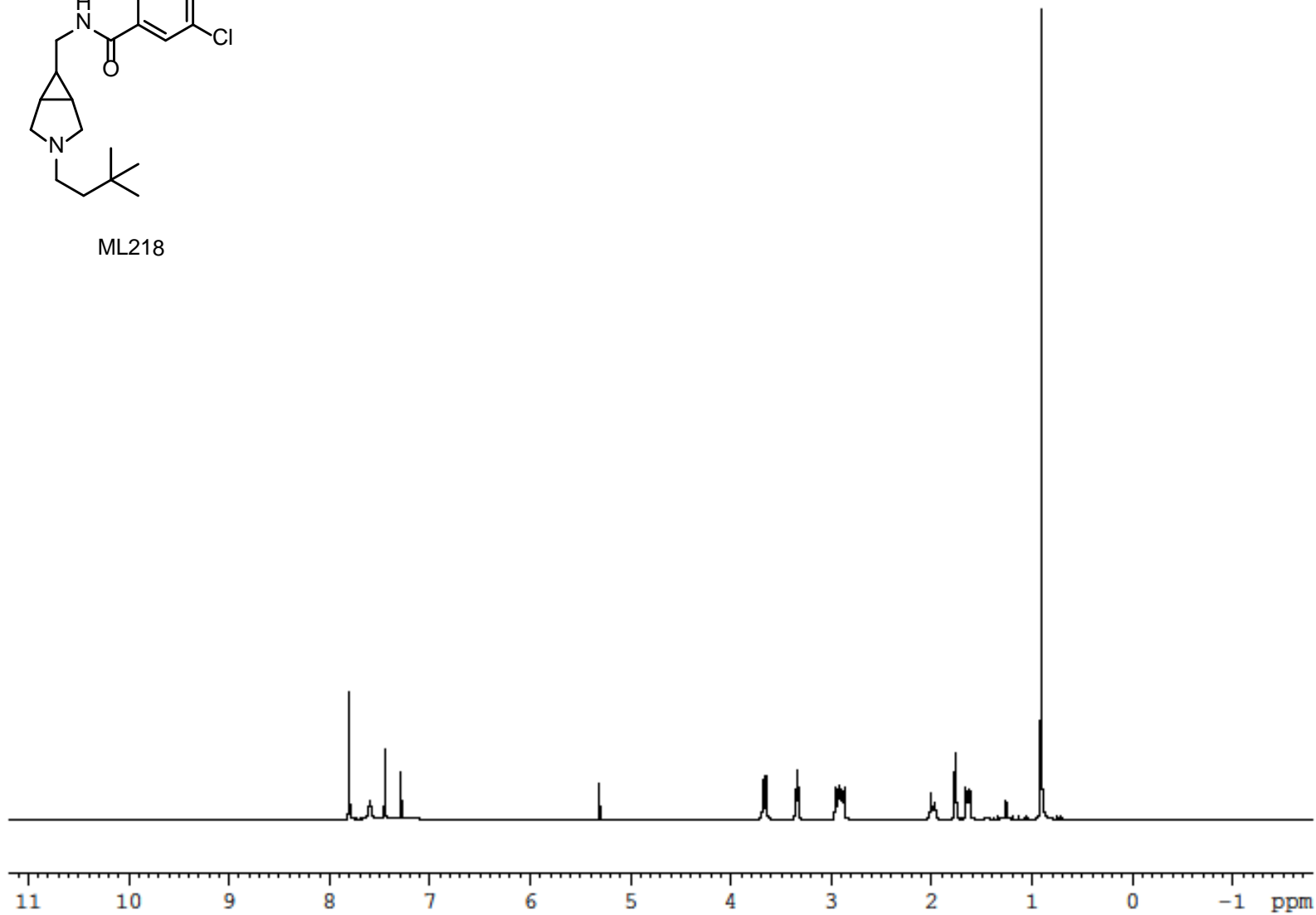
Minimum: -0.5

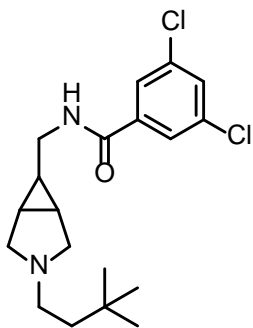
Maximum: 10.0 5.0 25.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
285.0563	285.0561	0.2	0.7	6.5	0.8	C13 H15 N2 O C12

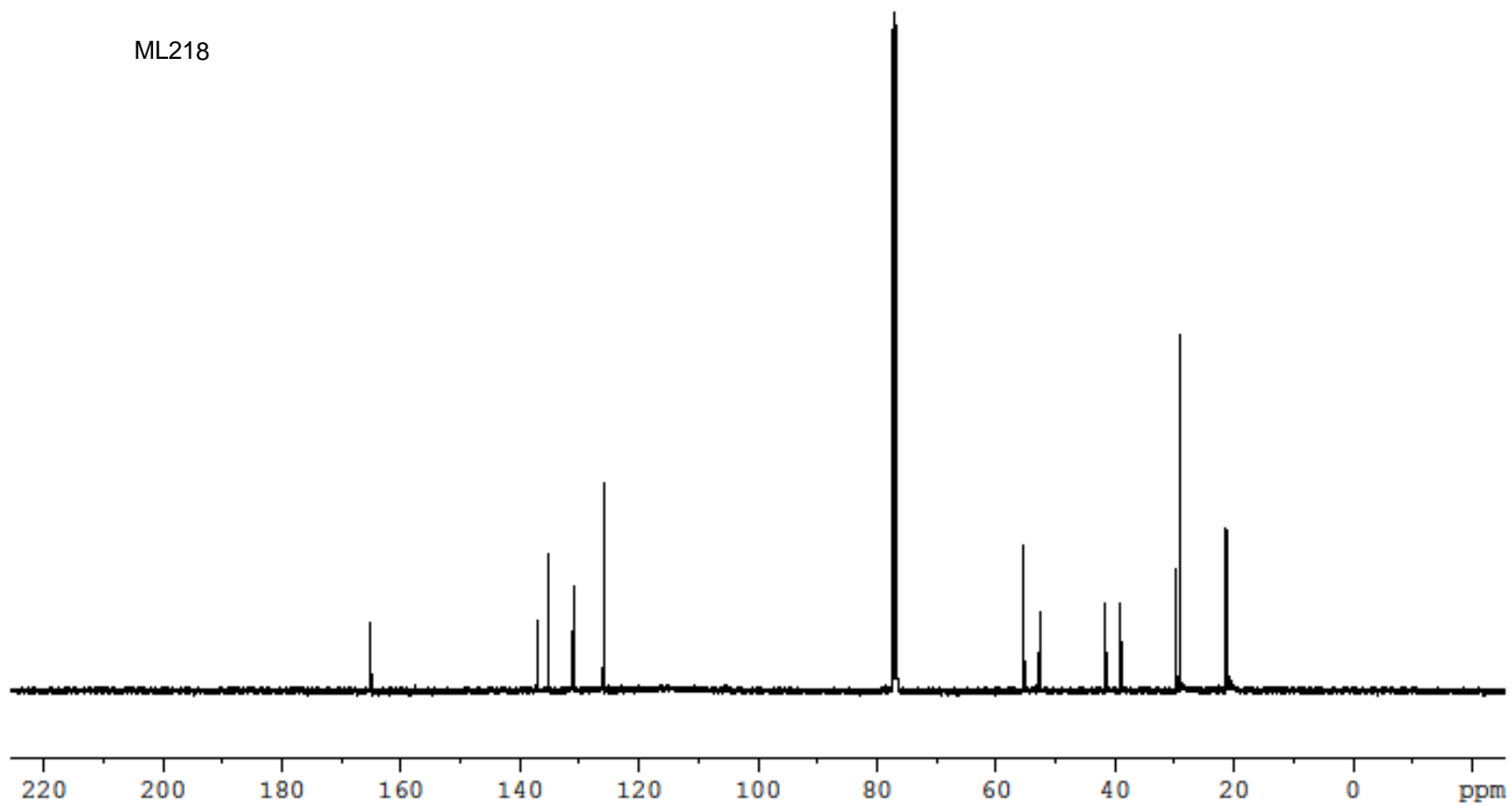


ML218





ML218



Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -0.5, max = 25.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions

207 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 15-500 H: 5-1000 N: 1-200 O: 1-200 Cl: 2-2

ML218

S/N: UH193

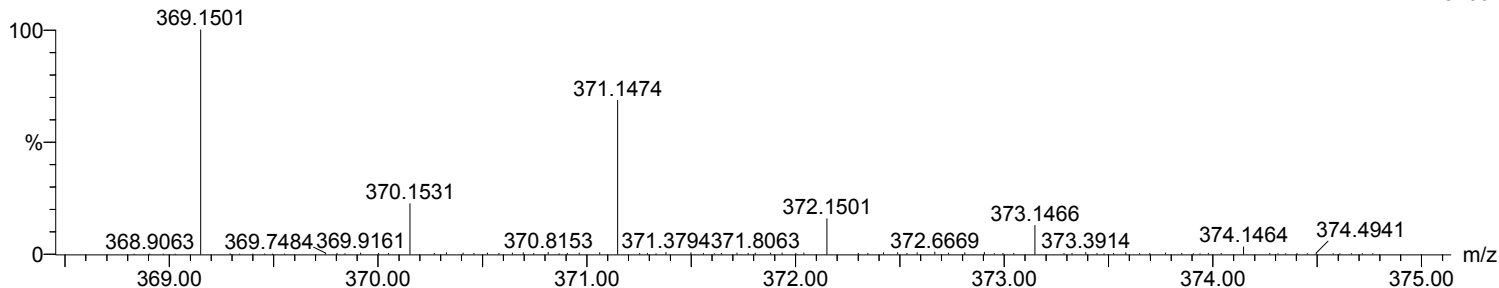
15-Sep-2011

14:38:20

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TOF MS ES+

1.27e+004



Minimum: -0.5

Maximum: 10.0 5.0 25.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
369.1501	369.1500	0.1	0.3	6.5	0.1	C19 H27 N2 O C12