Supporting Information

Structure-activity relationships of noncovalent immunoproteasome β5i selective dipeptides

*Wenhu Zhan,[1‡] Pradeep K Singh,[2‡†] Yi Ban,[1] Xiaoping Qing,[3] Marie Dominique Ah Kioon,[3] Hao Fan,[1] Quanju Zhao,[1] Rong Wang,[4] George Sukenick,[4] Jane Salmon,[3] J David Warren,[2] Xiaojing Ma,[1] Franck J. Barrat, [1,3] Carl F. Nathan,[1] Gang Lin[1]**

[1]Department of Microbiology & Immunology, Weill Cornell Medicine, 1300 York Ave, New York, NY 10065; [2]Department of Biochemistry, Milstein Chemistry Core Facility, Weill Cornell Medicine, 1300 York Ave, New York, NY 10065; [3]Autoimmunity and Inflammation Program, HSS Research Institute, Hospital for Special Surgery, New York, NY 10065, USA.[4]NMR Analytical Core Facility, Memorial Sloan Kettering Cancer Center.

Supporting Information

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Table S1. IC50s of the select compounds against β1i, β2i of the immunoproteasome and β1c, β2c of the constitutive proteasome

ID	IC_{50} (μM)			
	β1i - PAL	β1c - LLE	β2i - VLR	β2c - VLR
PKS3053	> 33.3	> 33.3	> 33.3	> 33.3
PKS21210	> 33.3	> 33.3	> 33.3	> 33.3
PKS21272	> 33.3	> 33.3	> 33.3	> 33.3
PKS21209	> 33.3	> 33.3	> 33.3	> 33.3
PKS21265	> 33.3	> 33.3	> 33.3	> 33.3
PKS21266	> 33.3	> 33.3	> 33.3	> 33.3

PAL: Ac-PAL-AMC; LLE: Z-LLE-AMC; VLR: Z-VLR-AMC

Table S2. Protease selectivity of select compounds against Chymotrypsin, Cathepsin L and Trypsin. Percentages of remaining hydrolysis activity of each protease were measured in the presence of the compounds at concentrations 100, 33.3 and 11.1 μM and calculated relative to enzymatic activity in the presence of DMSO. Results are means from two independent experiments and each in triplicate.

	Enzyme activity % at indicated compound concentrations								
	Chymotrypsin			Cathepsin L			Trypsin		
	100 μM	33.3 μM	11.1 μM	100 μM	33.3 μM	11.1 μM	100 μM	33.3 μM	11.1 μM
PKS3053	0.0	16.0	77.6	60.7	109.2	112.8	154.6	106.7	84.8
PKS21210	118.4	109.1	95.3	91.1	101.6	105.6	100.6	122.7	120.0
PKS21272	102.4	99.3	99.1	117.2	114.5	112.0	186.5	120.5	89.5
PKS21209	114.8	106.3	104.2	115.3	109.0	105.8	268.8	179.5	134.0
PKS21265	104.1	99.0	96.4	126.2	112.2	112.0	244.7	178.0	144.1
PKS21266	105.6	101.2	101.7	114.8	113.2	108.5	175.9	199.1	159.2

Supplementary Experimental section

Materials: α-Chymotrypsin (cat. No. C4129), Trypsin from porcine pancreas (cat. No. T-8253) and Cathepsin L from human liver (cat. No. C6854) were purchased from Sigma-Aldrich. Suc-LLVY-AMC, Z-LLE-AMC, Z-LRR-AMC, Ac-ANW-AMC and Suc-LY-AMC (cat. No. S-260) was from Boston Biochem and Z-FR-AMC (cat. No. GWB-E9DD43) was from GenWay Biotech. Proteasome-Glo™ Chymotrypsin-Like Cell-Based Assay kit (cat. No. G8660) and CellTiter-Glo® Luminescent Cell Viability Assay kits (cat. No. G7570) were from Promega (Madison, WI). Complete Media (CM) is RPMI 1640 (LTI 11875-085) with 10% FCS (cat. No. 26140), 1% L-glutamine (cat. No. 25030-081), 50μg/ml gentamicin (cat. No. 15750-060) were from Gibco. Separation Media (SM) is RPMI 1640 (LTI 11875-085) with 2.5mM EDTA (Gibco 15575-038). Lympholyte®-H Lymphocyte Separation Medium (CL5020) was from Cedarlane Laboratories. 96-well round bottom plates (Falcon 3077). Unifilter GF/C plates (cat. No. 5007185) were from Packard. ^{3}H-thymidine (Net-027005MC) was from PerkinElmer.

Protease selectivity assays

Protease selectivity of select compounds was performed as reported with minor changes.[1] Briefly, 1 μL of select compound at concentrations of 100, 33.3 and 11.1 μM in DMSO were spotted into the bottom of a black 96-well plate. Reaction buffer (100 μL, see table below) containing proteases and their substrates were dispensed into each well, and the plate was then spun in a simple benchtop plate centrifuge at 1000 rpm for 1 min, followed by recording the fluorescence of the product AMC (λex 360 nm and λem 460 nm) on a SpectraMax M5 plate reader over 1.5 h. Percentages of remaining activity of proteases were calculated relative to DMSO.

Protease (final con.)	Substrate (final con.)	Buffer
α-Chymotrypsin (2 nM)	Suc-Leu-Tyr-AMC (50 μM)	50 mM Tris, 100 mM NaCl, 5 mM $CaCl_2$, 0.01% Tween 20, pH 8.0
Trypsin from porcine pancreas (4 nM)	Z-FR-AMC (12 μM)	50 mM Tris, 100 mM NaCl, 5 mM $CaCl_2$, 0.01% Tween 20, pH 8.0
Cathepsin L from human liver (2 nM)	Z-FR-AMC (37.5 μM)	100 mM sodium acetate, 5 mM DTT, 1 mM EDTA, pH 5.5

Human PBMC Proliferation Assay

All Informed consents were obtained from blood donors after the nature and possible consequences of the studies were explained.

PBMC Isolation- The procedure was carried out as our previous report.[1] In brief, the EDTA-treated blood samples from donors were separated by Ficoll as follows: The syringes of blood were inverted to mix and divided equally among 50ml tubes (~20ml/tube) containing 20ml separation media (SM), RPMI 1640 with 2.5mM EDTA, and carefully loaded on top of 10ml of Ficoll in each tube. Tubes were spun at 1800 rpm for 20 min (low brake). The interfaces from each two tubes were combined into one 50ml tube, brought up to 45ml with SM and spun at 1700 rpm for 8 min. Supernatant was aspirated; and cells were resuspended in 45ml SM and spun at 1100 rpm (to remove platelets) for 8 min. Cells were then resuspended in Complete Media (CM).

Methods: These experiments were carried out as our previously reported with slight changes.[1] Compounds were tested in a series 3x concentration dilution (final concentrations were from 3 μM to 0.56 nM as indicated in plate map). 10μl of each appropriate dilution in CM were added

to the appropriate wells. The PBMC cells in CM at 1 x 10^6/mL, 100µl/well (100,000 cells/well) were then added to the appropriate wells of the plates and were incubated for 1hr at 37°C, 5% CO_2 prior to adding the stimuli, 50µl Anti-human CD3 at 0.5 µg/ml. All wells were brought up to 200µl with 0.1% DMSO/CM. The plates were incubated at 37°C, 5% CO_2 for 5 days. On day 5, 100µl supernatant was removed from each well of the PBMC assay plates, and 0.5µCi of ^{3}H-Thymidine in 100µl CM was added to each well, and incubated for 6 hours. The assay plates were harvested on a Packard Filtermate Harvester according to the manufacturer's instruction. The plates were dried overnight at room temperature or for 1hr in a 37°C vacuum oven and then 50µl/well PerkinElmer Microscint-20 scintillant was added. The plates were then counted on a Packard TopCount scintillation counter.

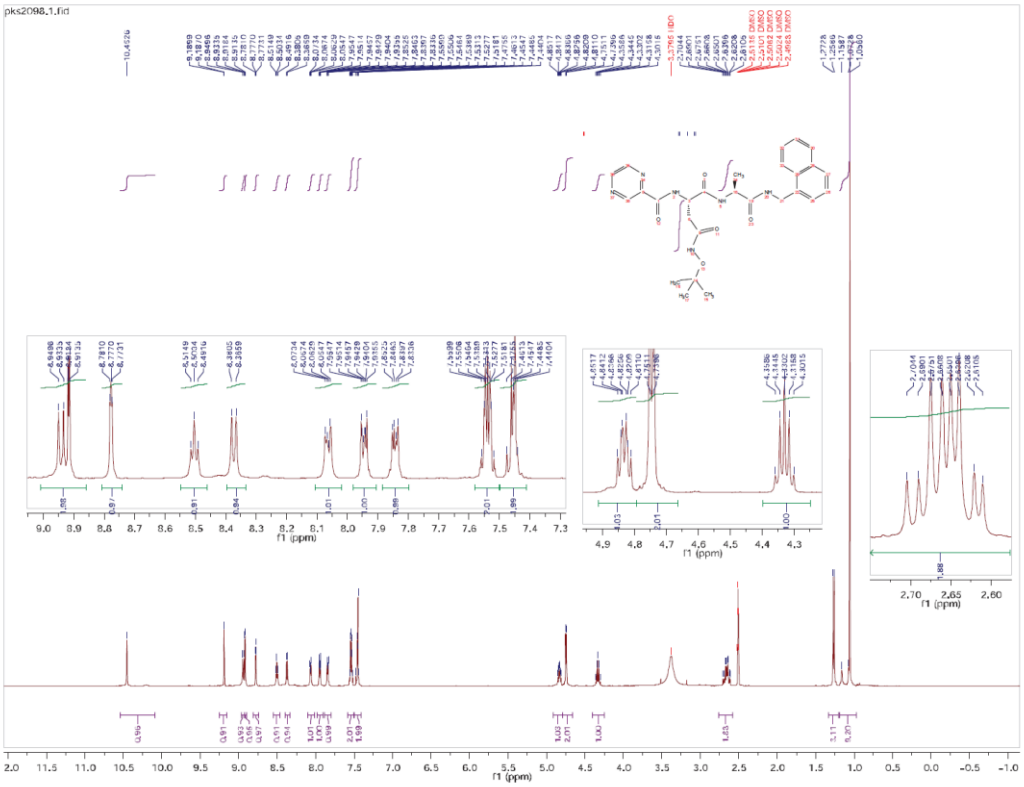
Plate map for assay of anti-human CD3 antibody stimulated human PBMC Proliferation

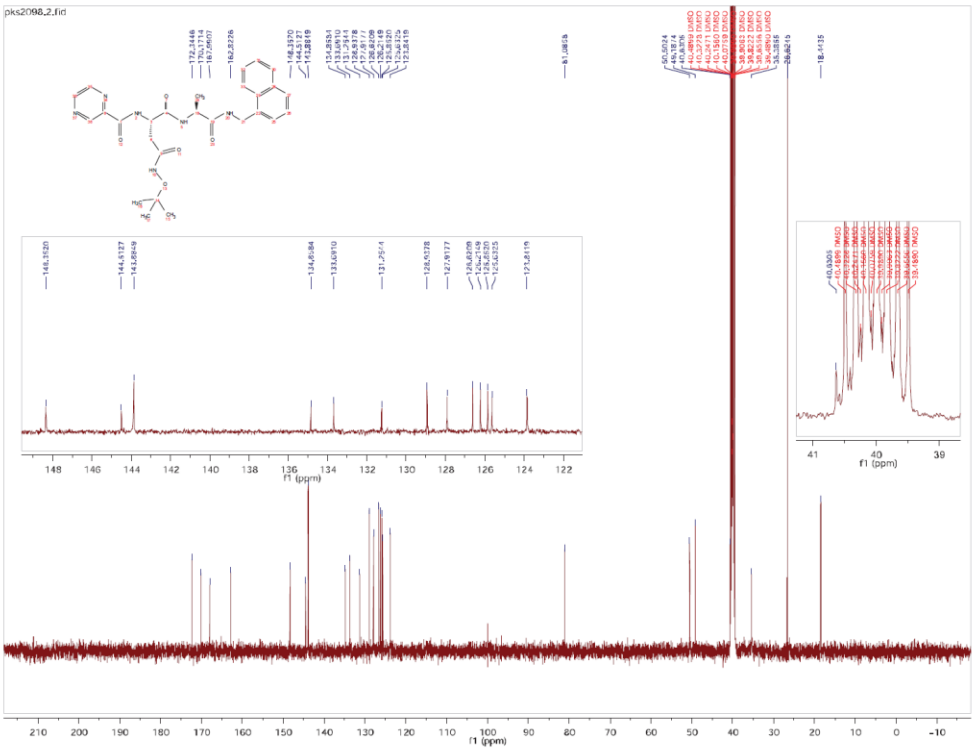
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	Cmpd1	3000 nM	900 nM	270 nM	81 nM	24 nM	7.29 nM	2.19 nM	0.56 nM	PBMC + xCD3	PBMC only	
C		"	"	"	"	"	"	"	"	"	"	
D		"	"	"	"	"	"	"	"	"	"	
E	Cmpd2	3000 nM	900 nM	270 nM	81 nM	24 nM	7.29 nM	2.19 nM	0.56 nM	PBMC + xCD3	PBMC only	
F		"	"	"	"	"	"	"	"	"	"	
G		"	"	"	"	"	"	"	"	"	"	
H												

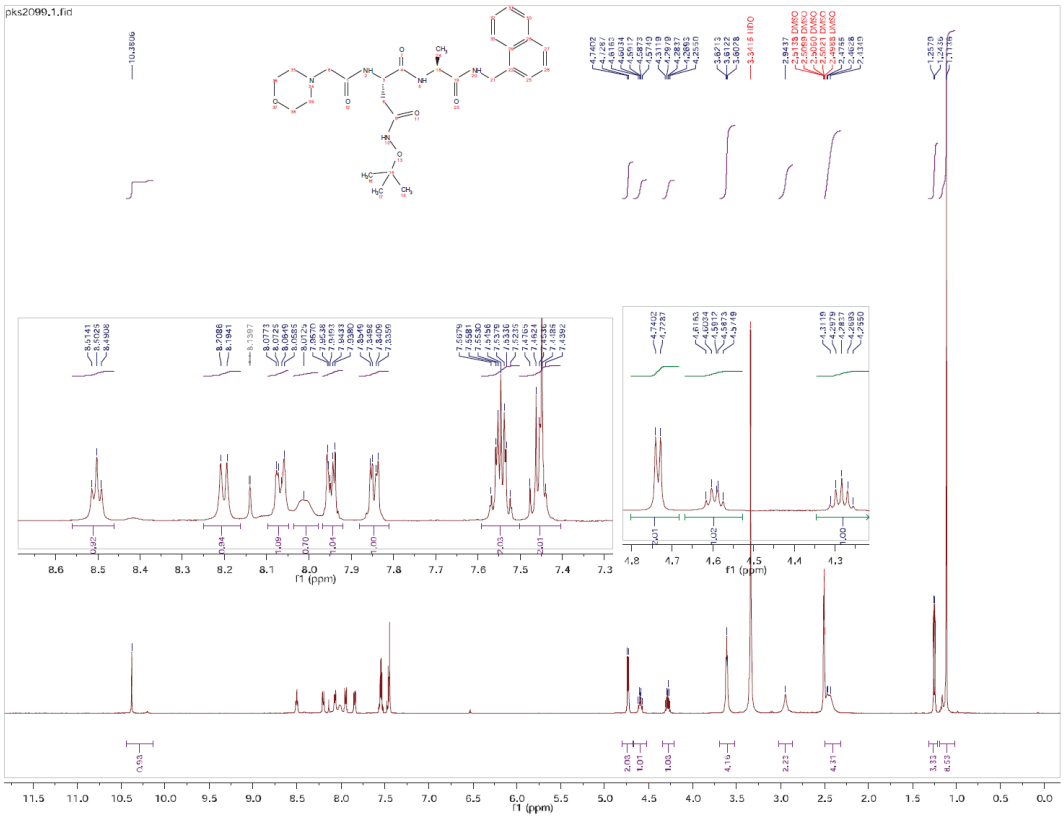
References

1. Singh, P. K.; Fan, H.; Jiang, X.; Shi, L.; Nathan, C. F.; Lin, G., Immunoproteasome beta5i-Selective Dipeptidomimetic Inhibitors. *ChemMedChem* **2016,** *11* (19), 2127-2131.

^{1}H and ^{13}C NMR Spectra



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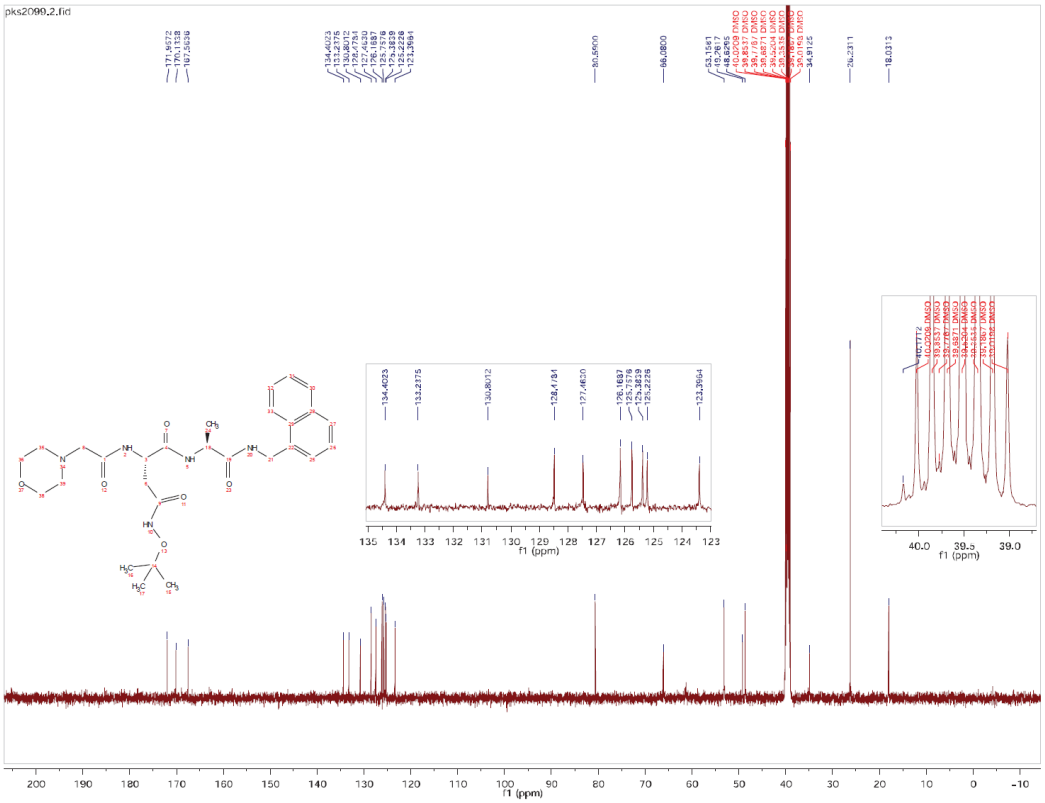
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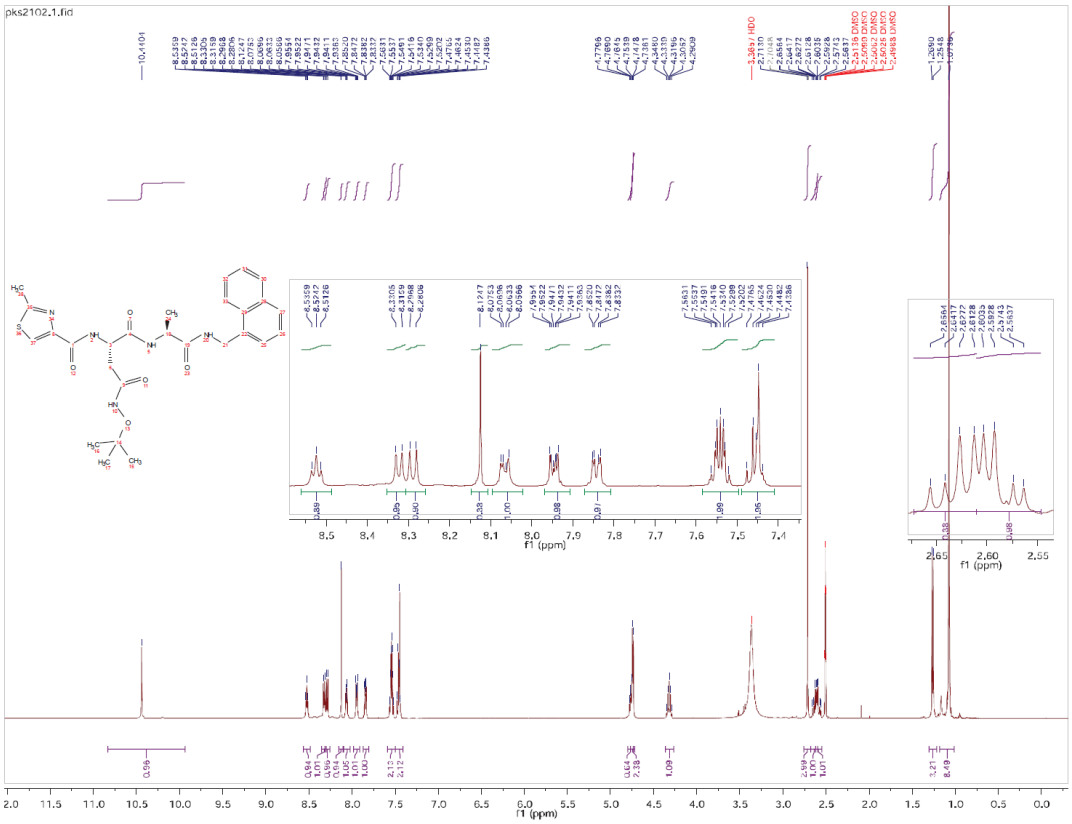
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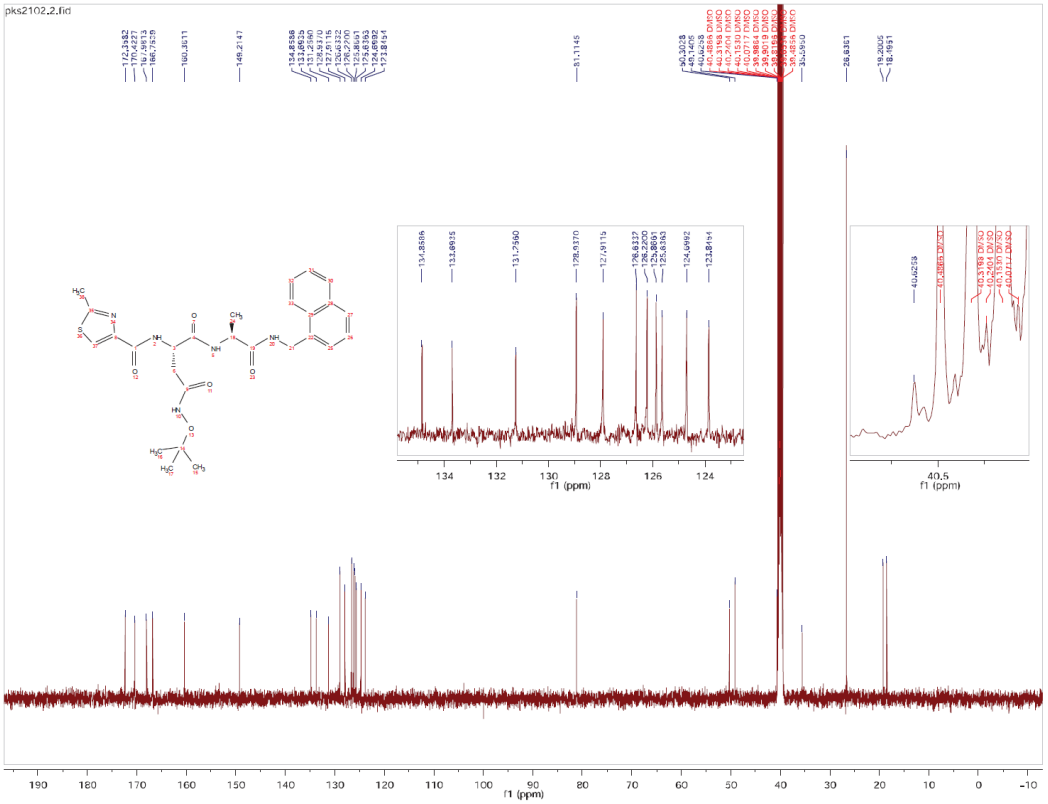
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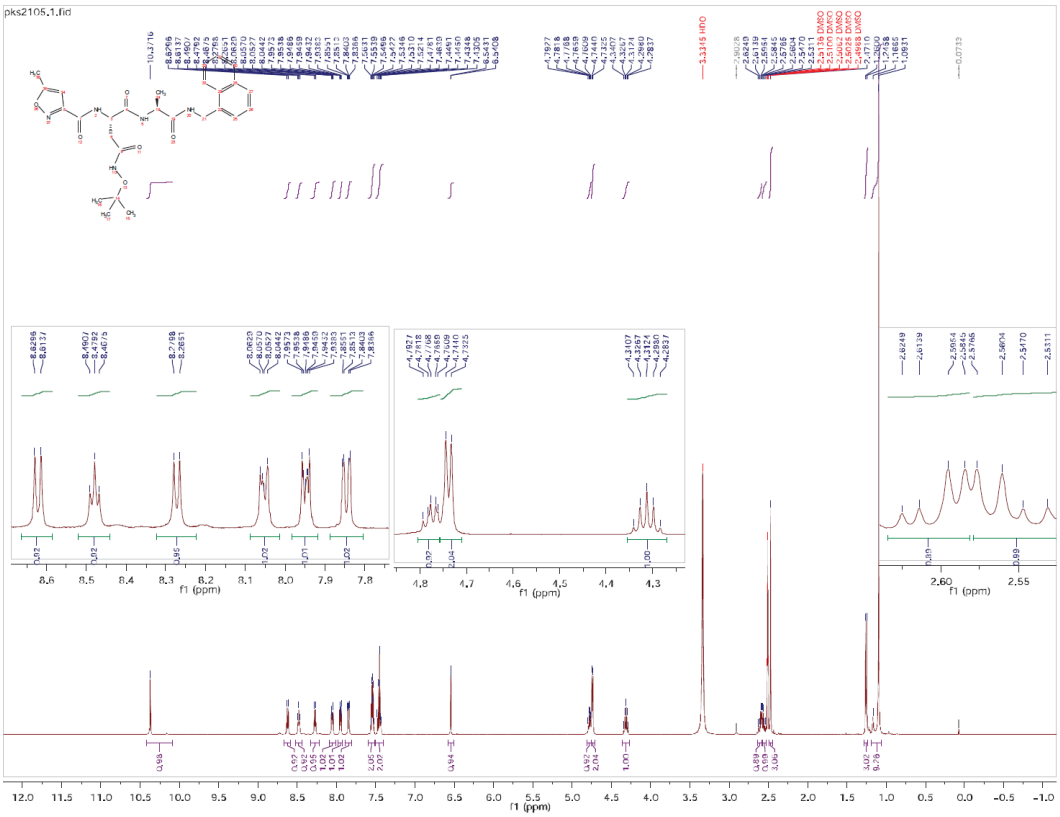
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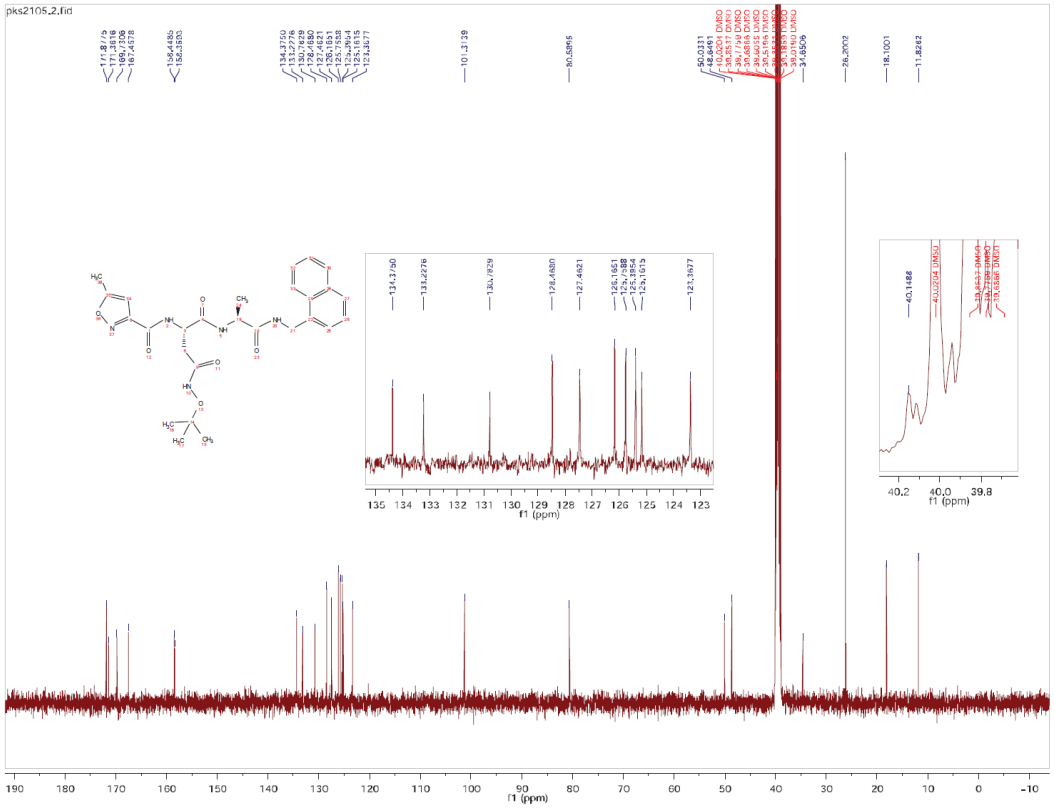
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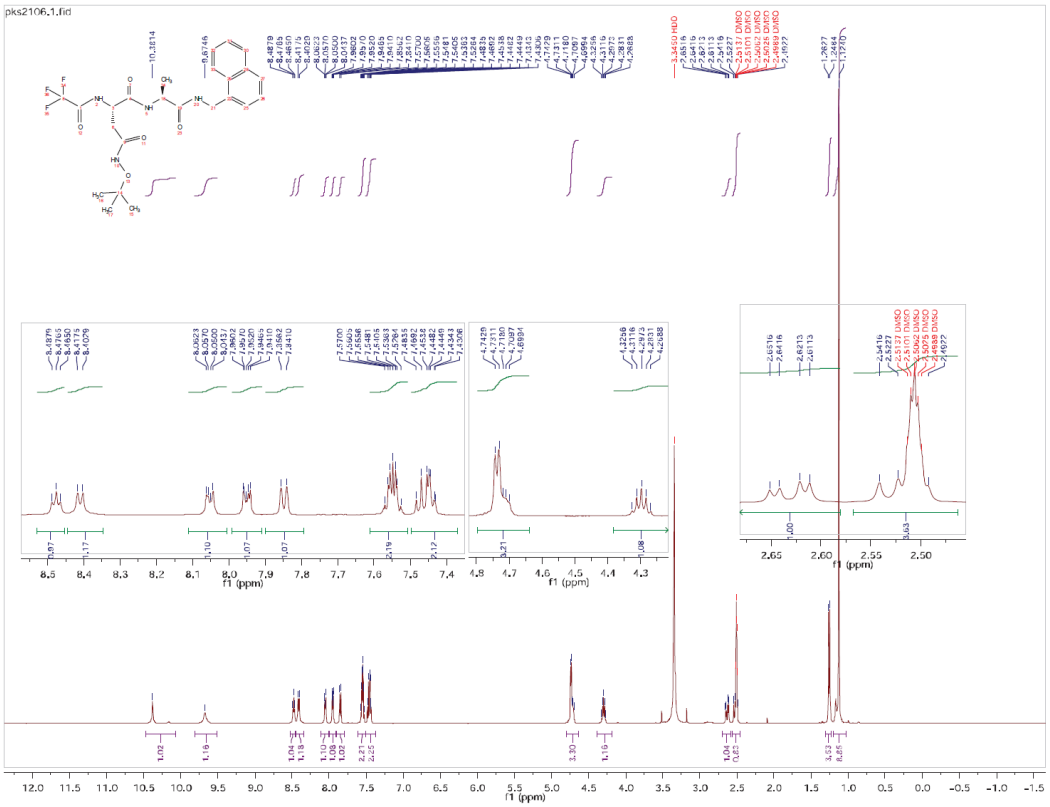
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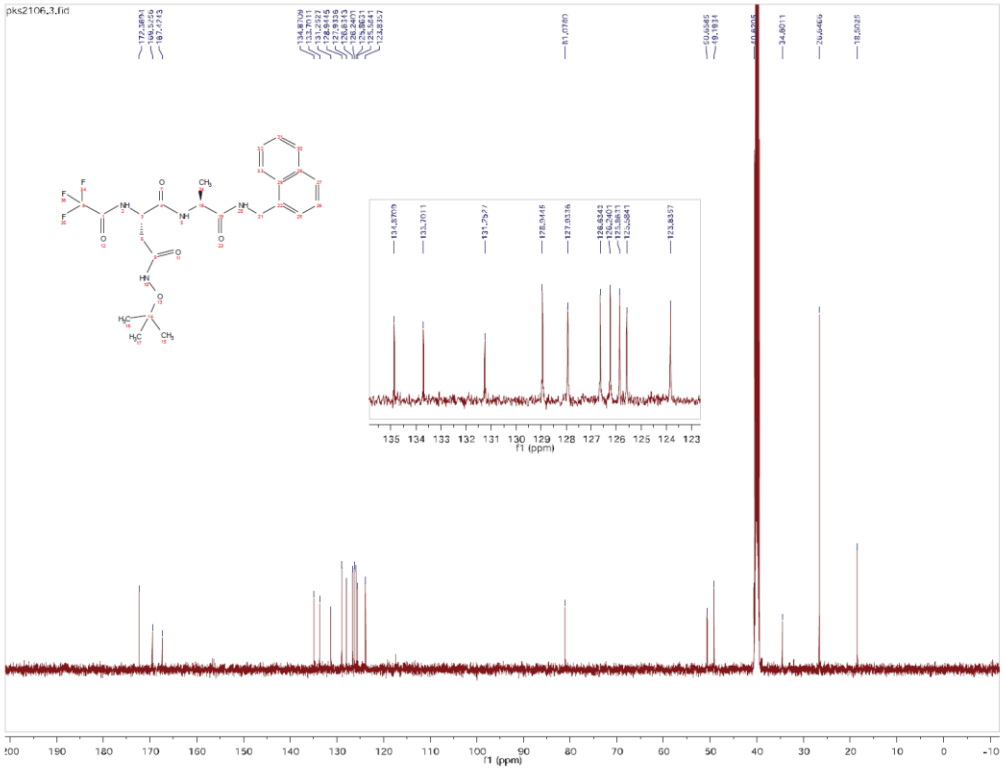
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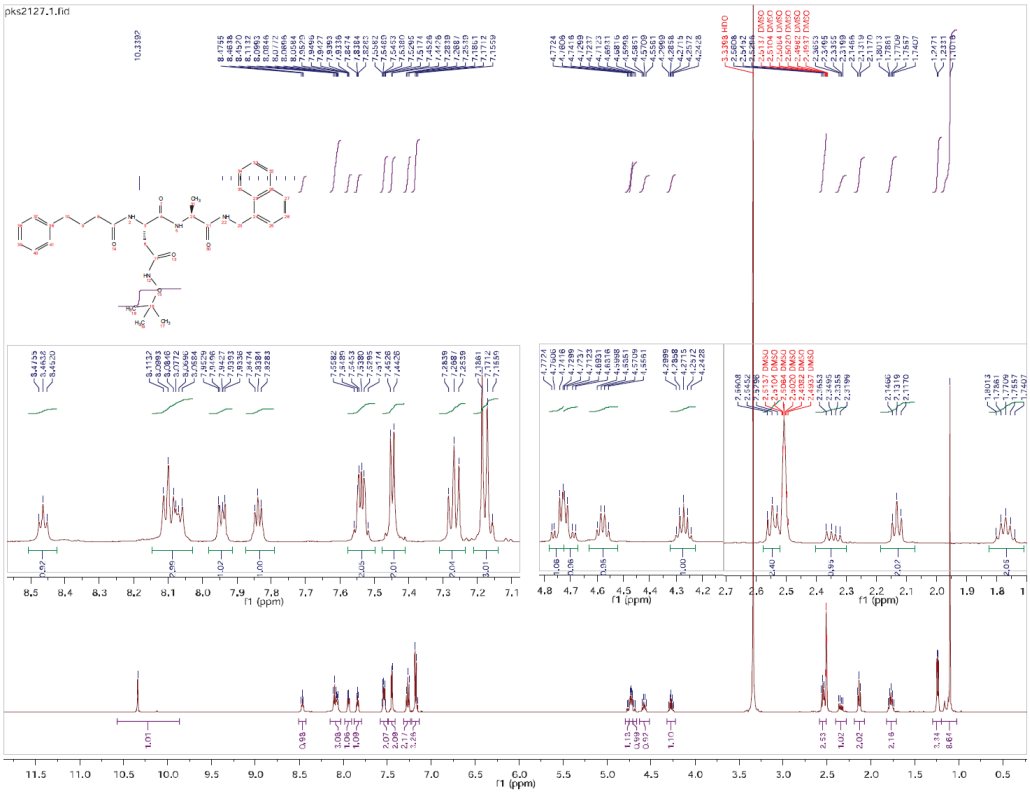
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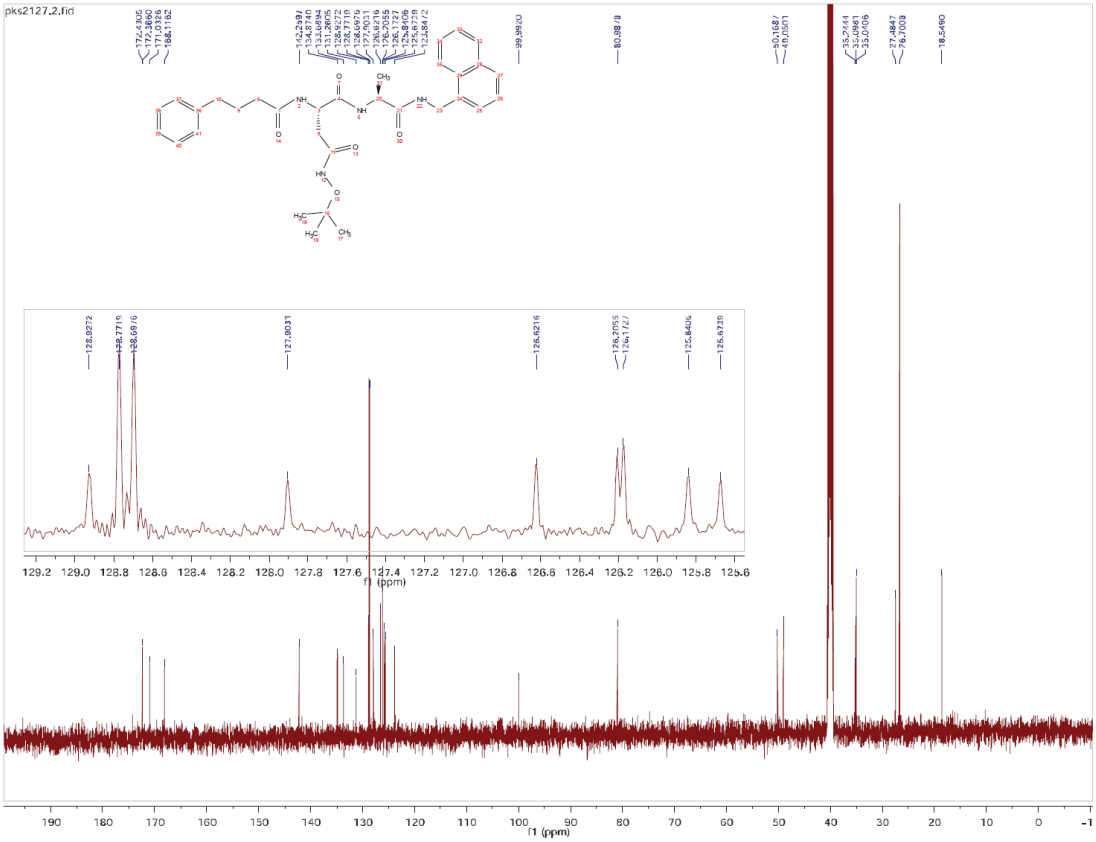
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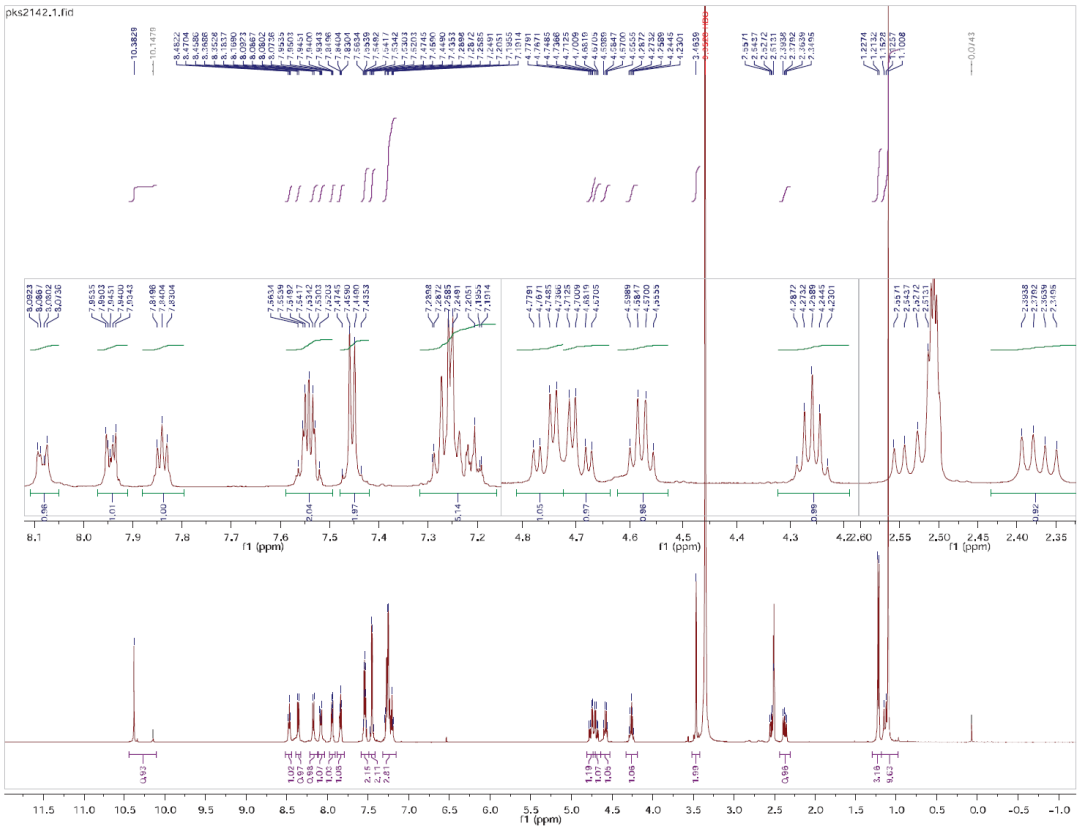
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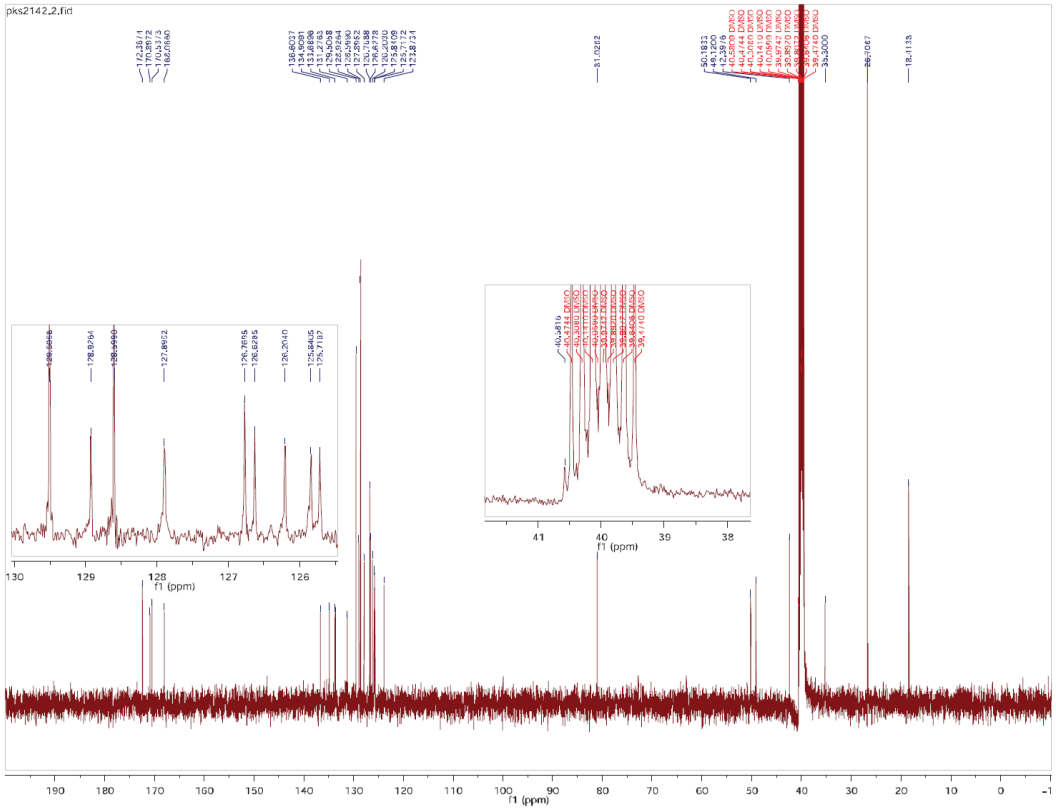
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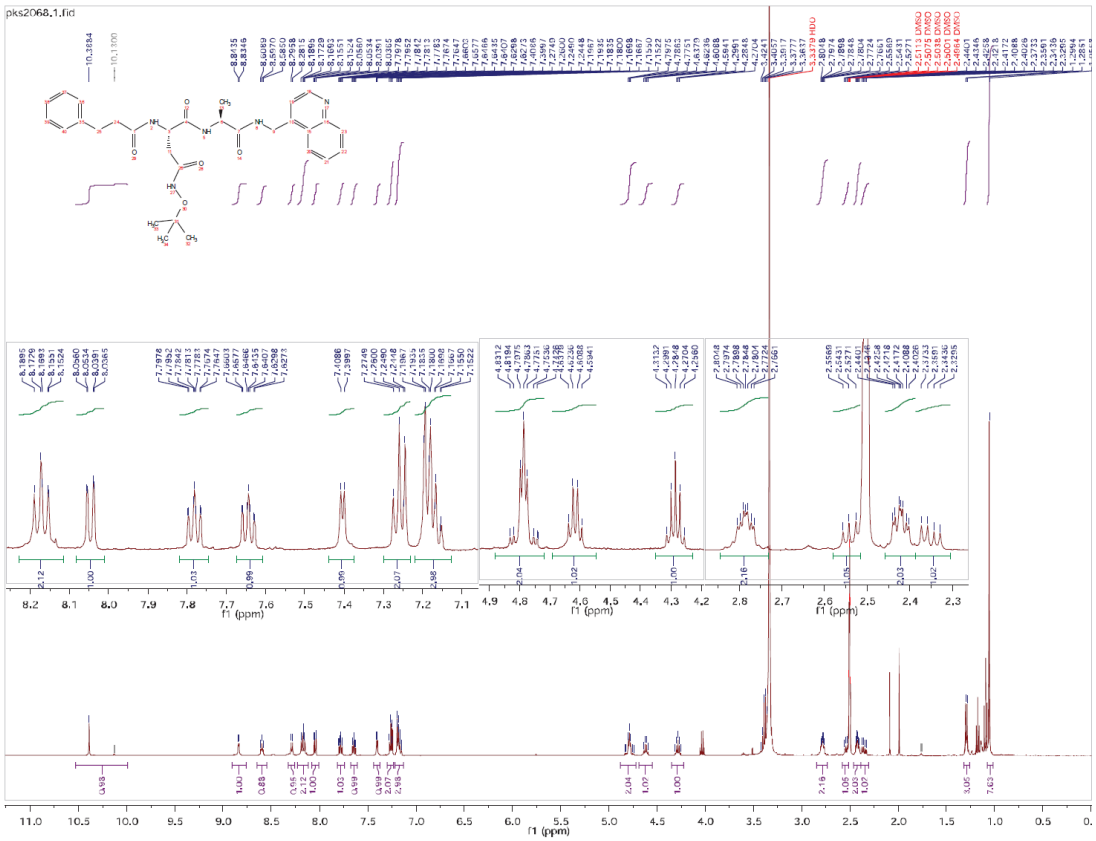
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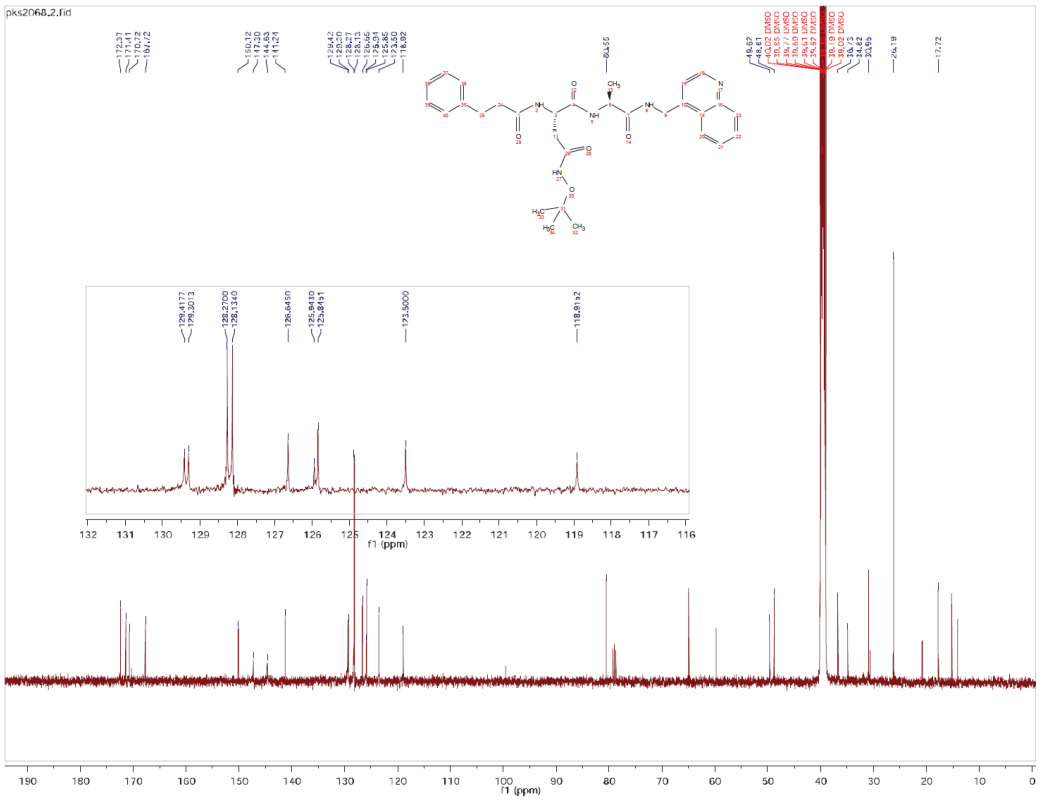
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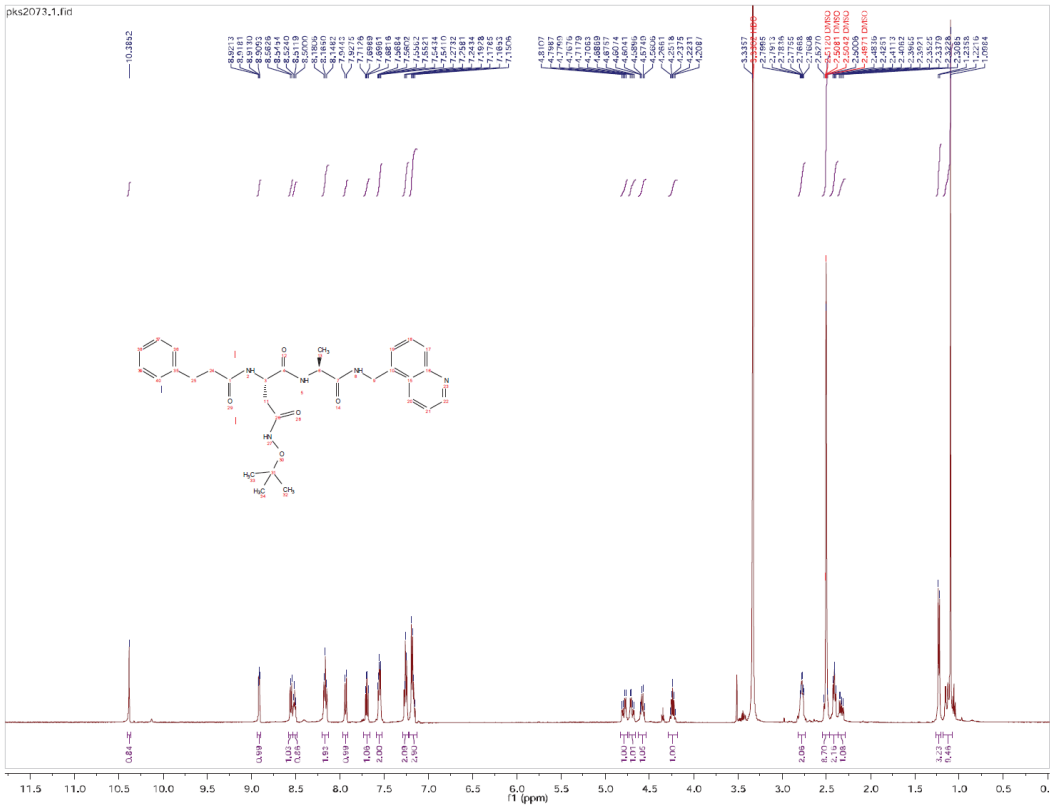
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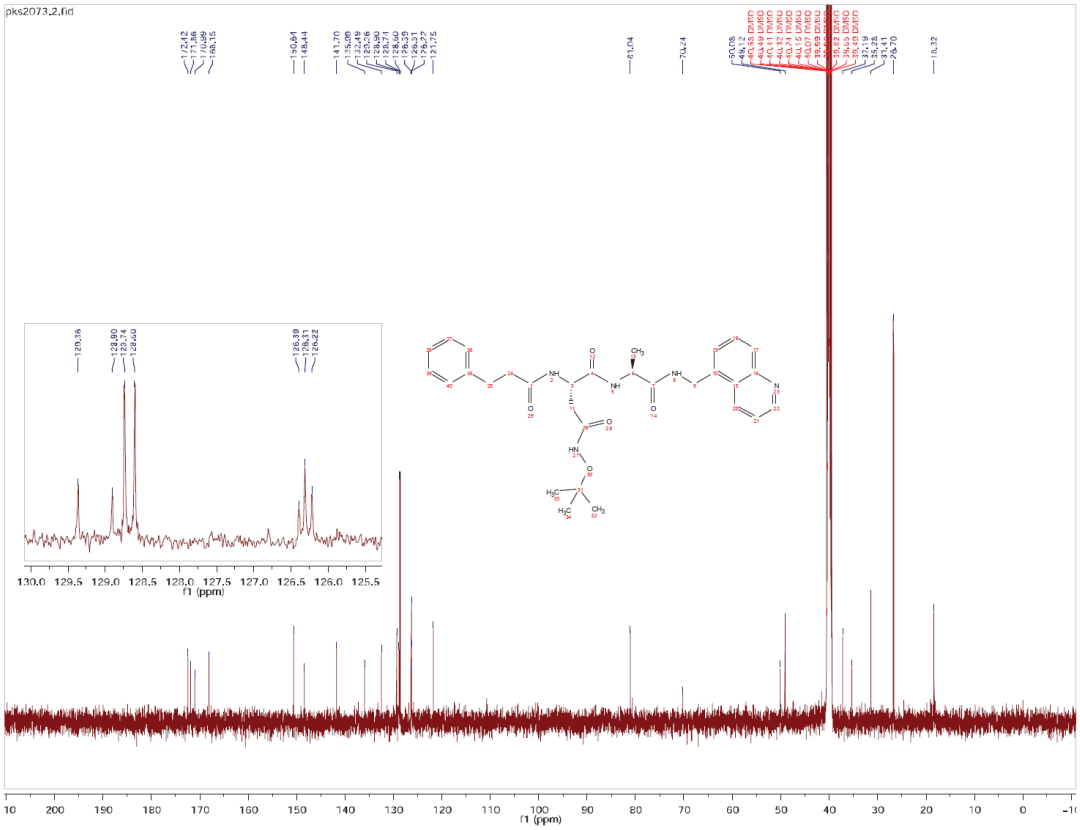
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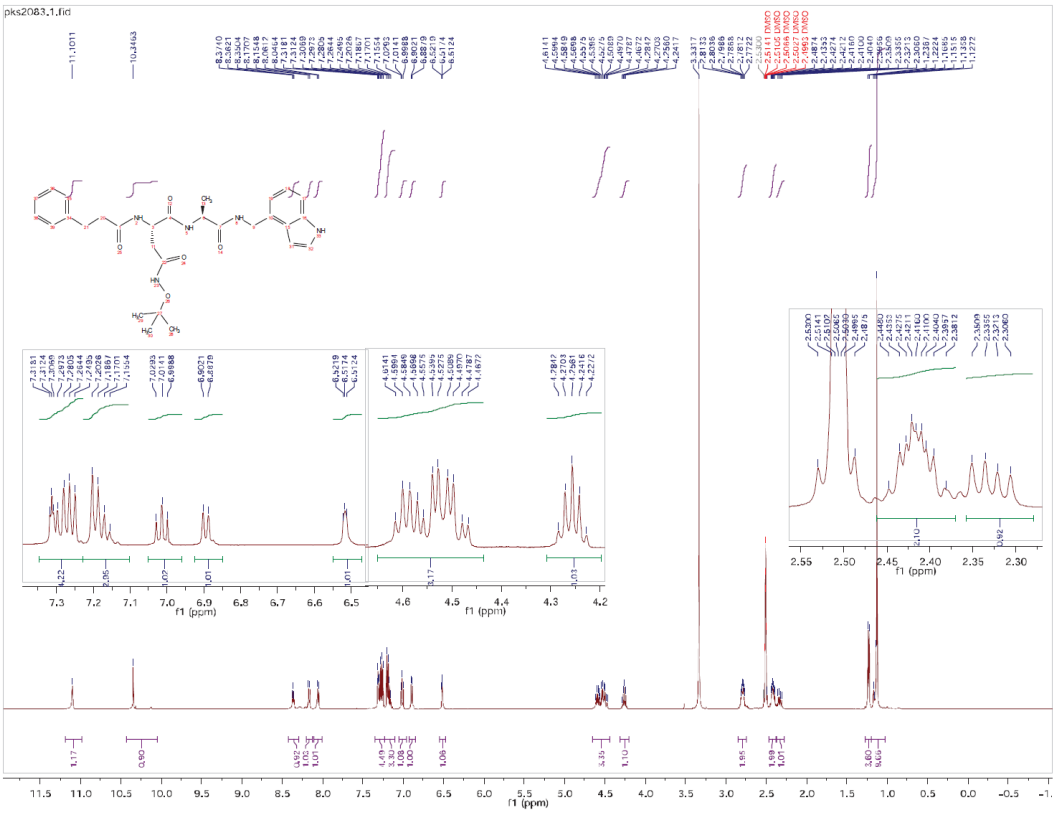
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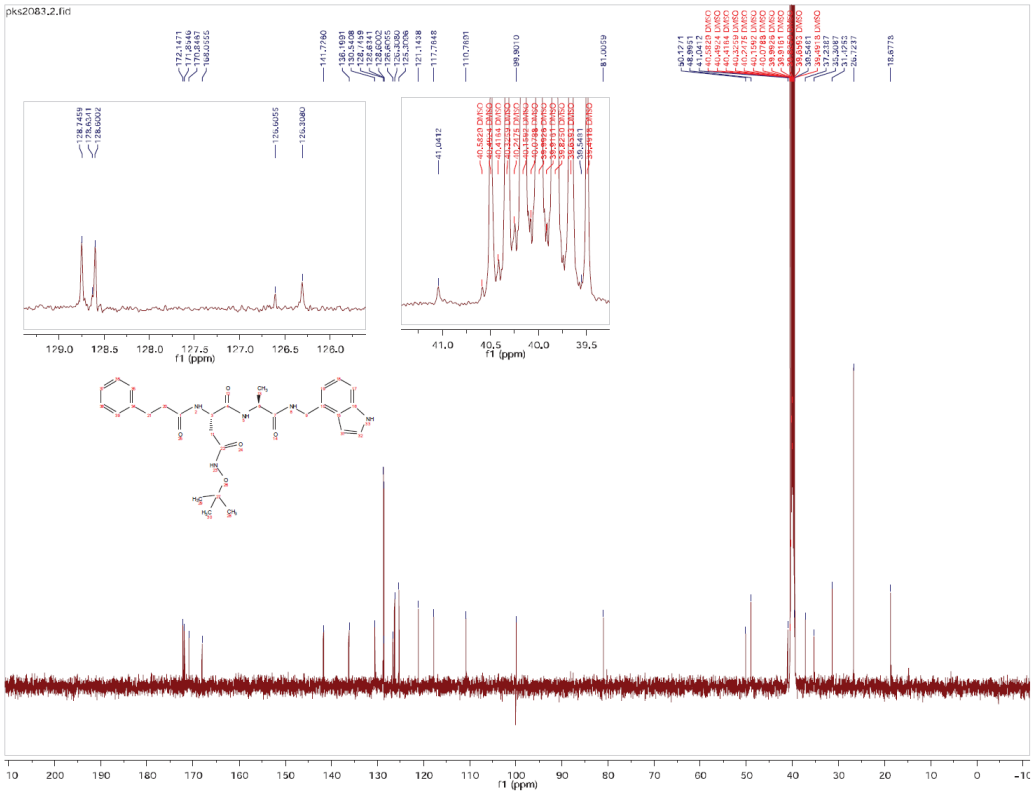
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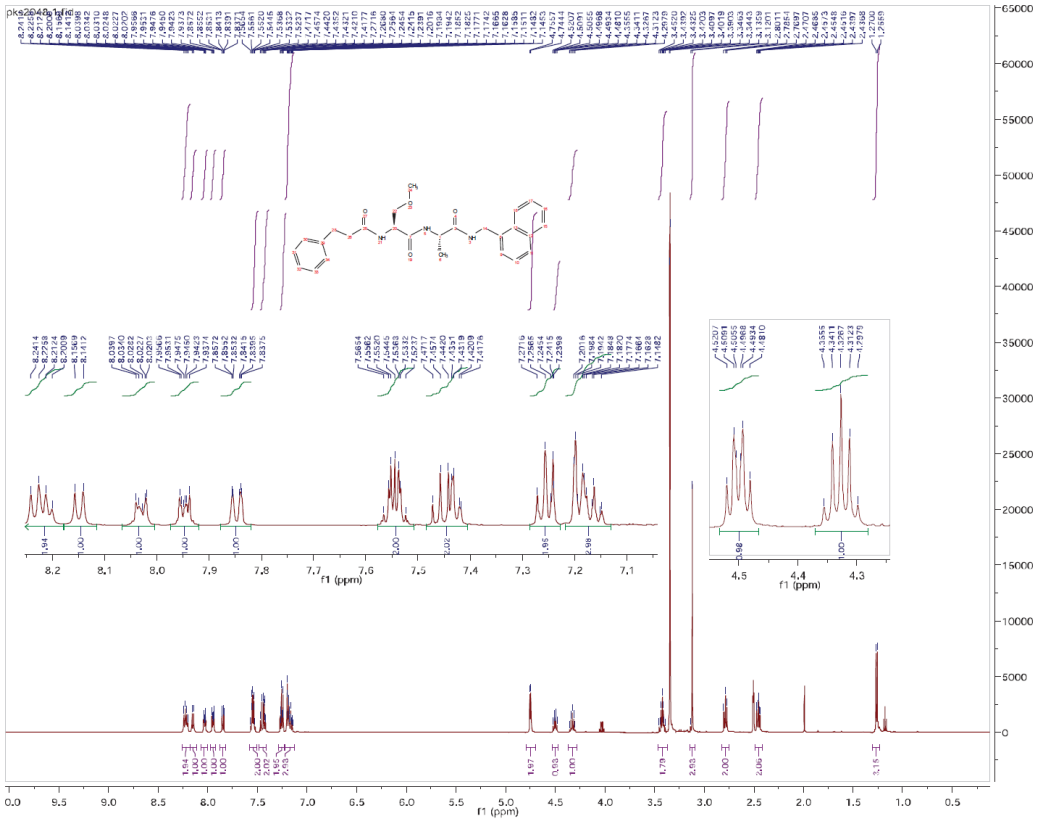
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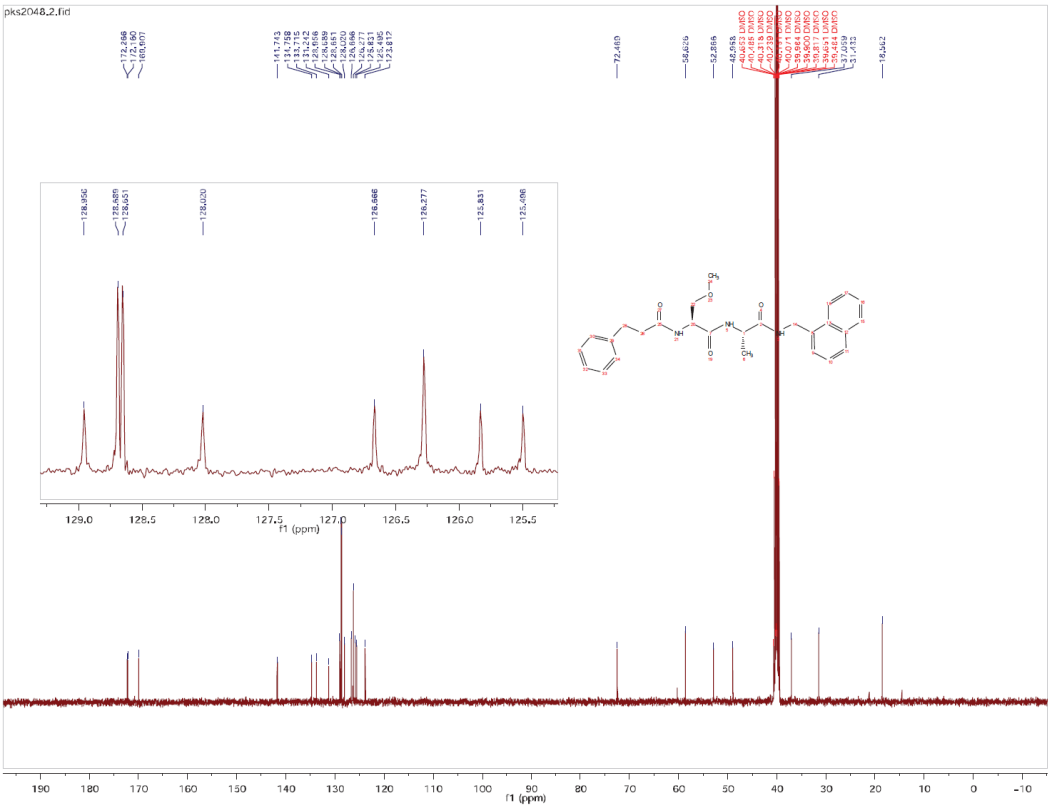
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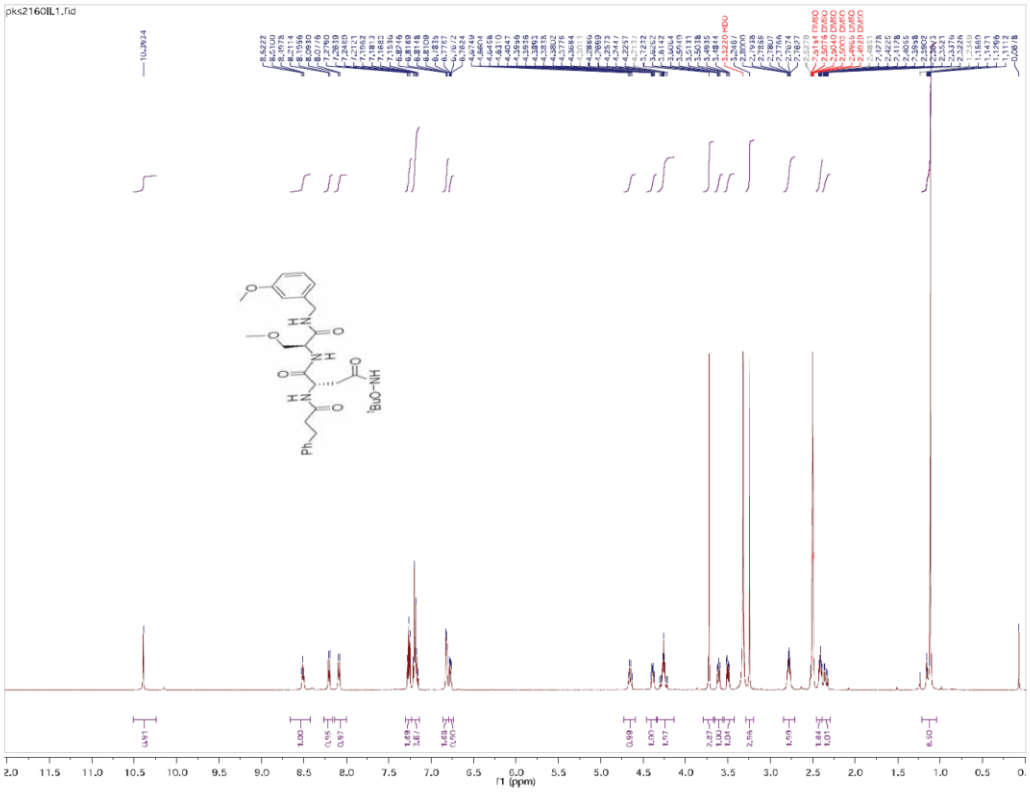
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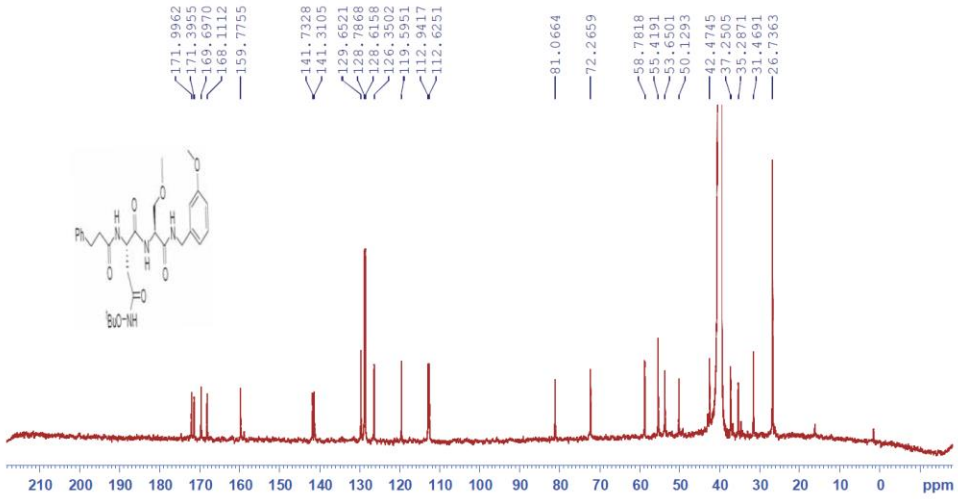
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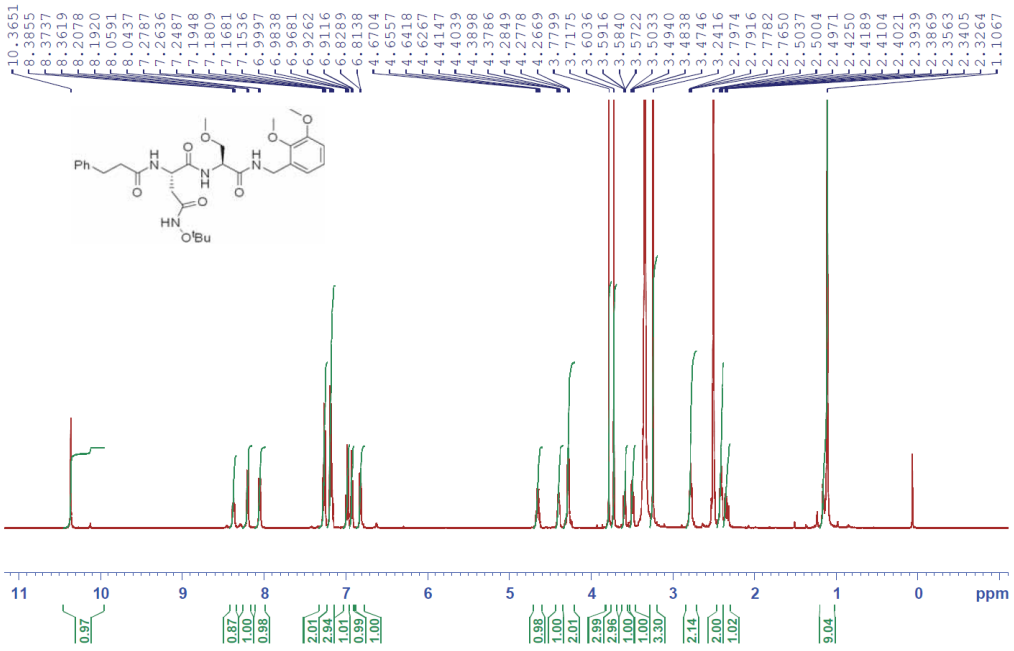
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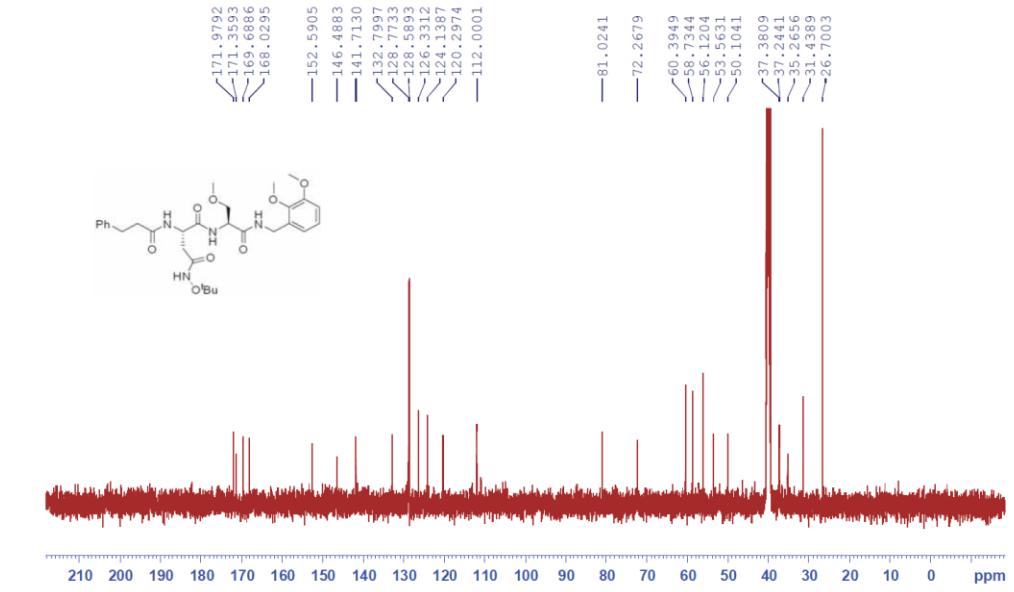
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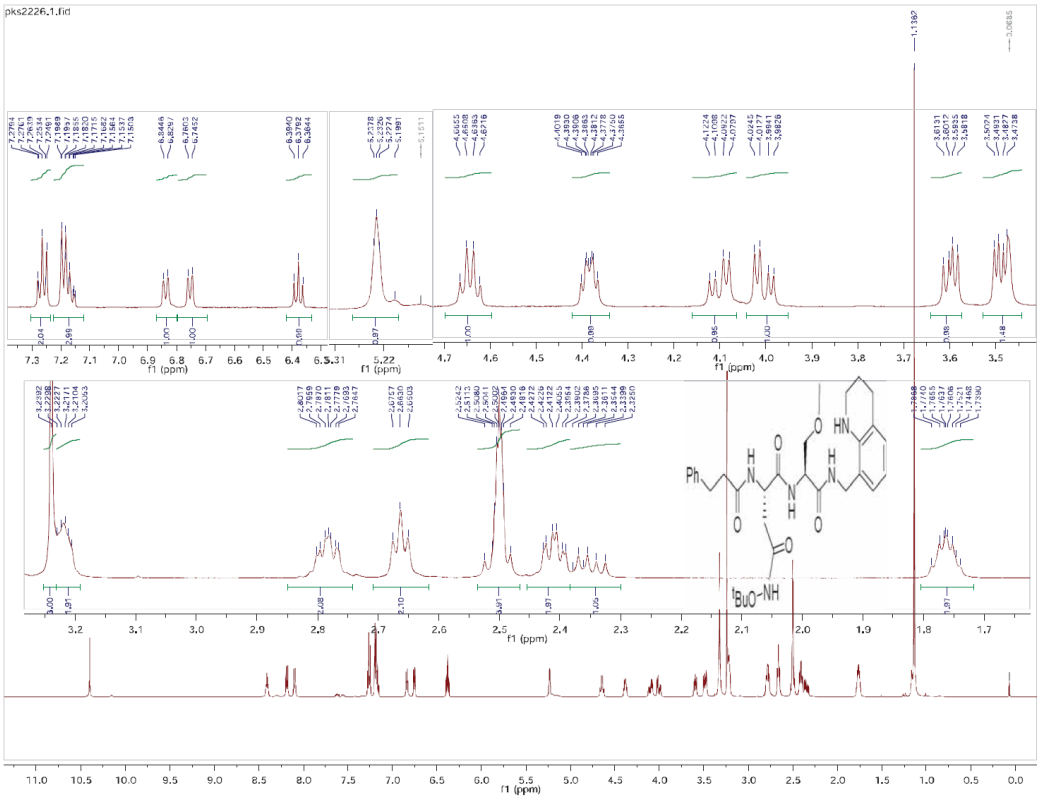

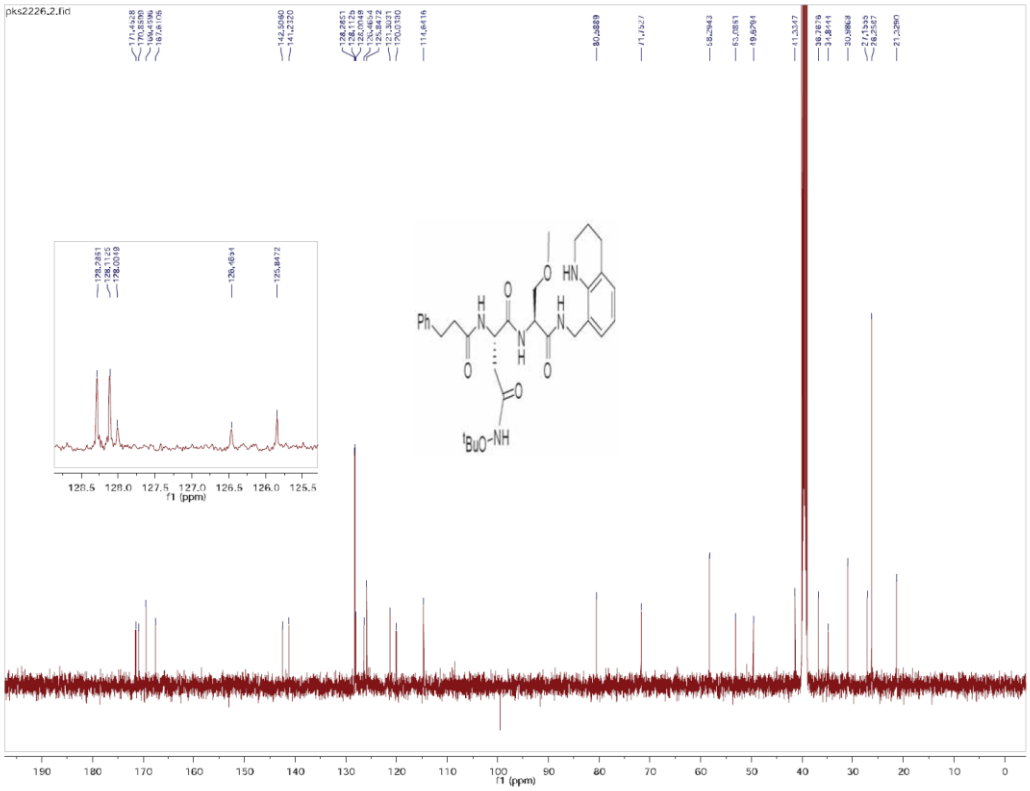

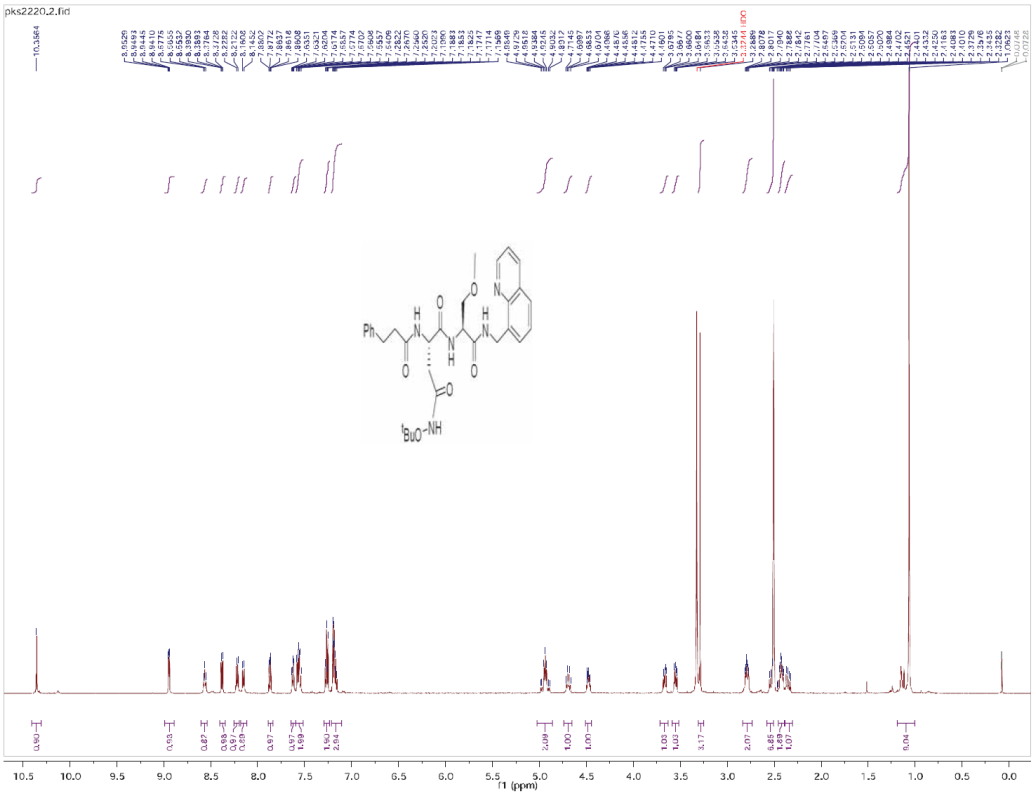
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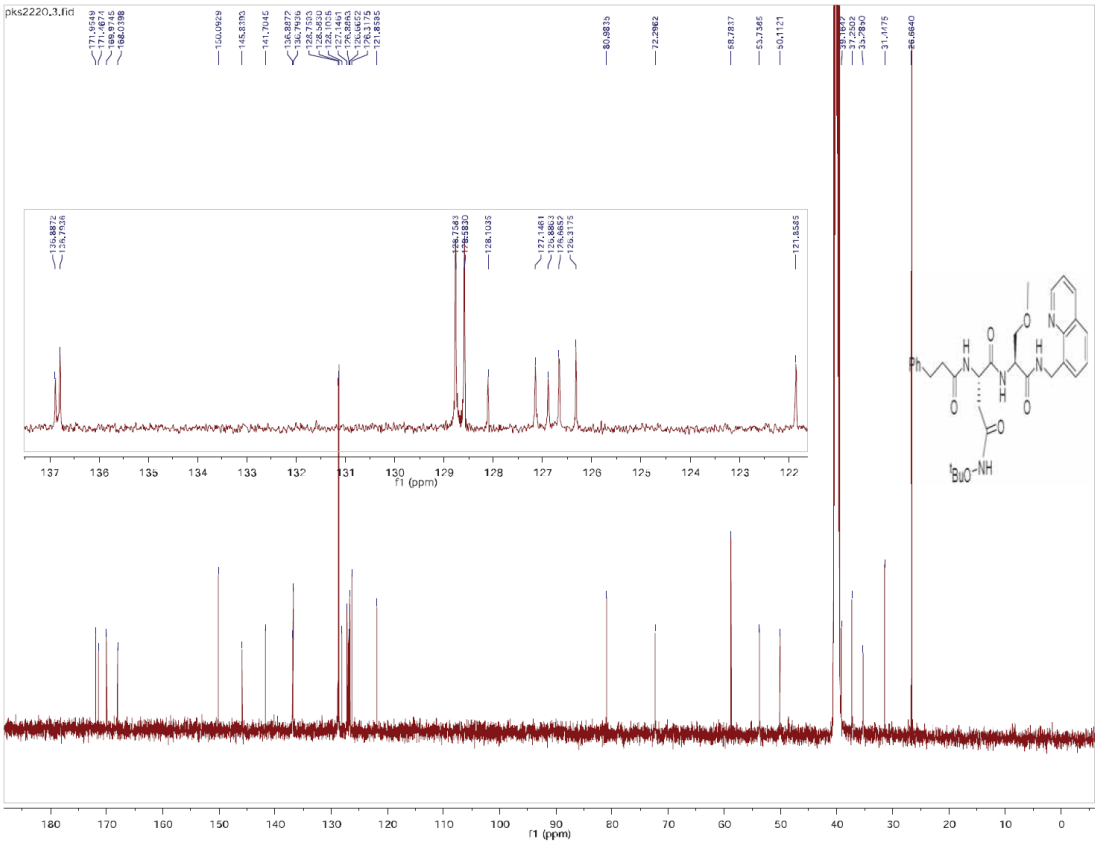
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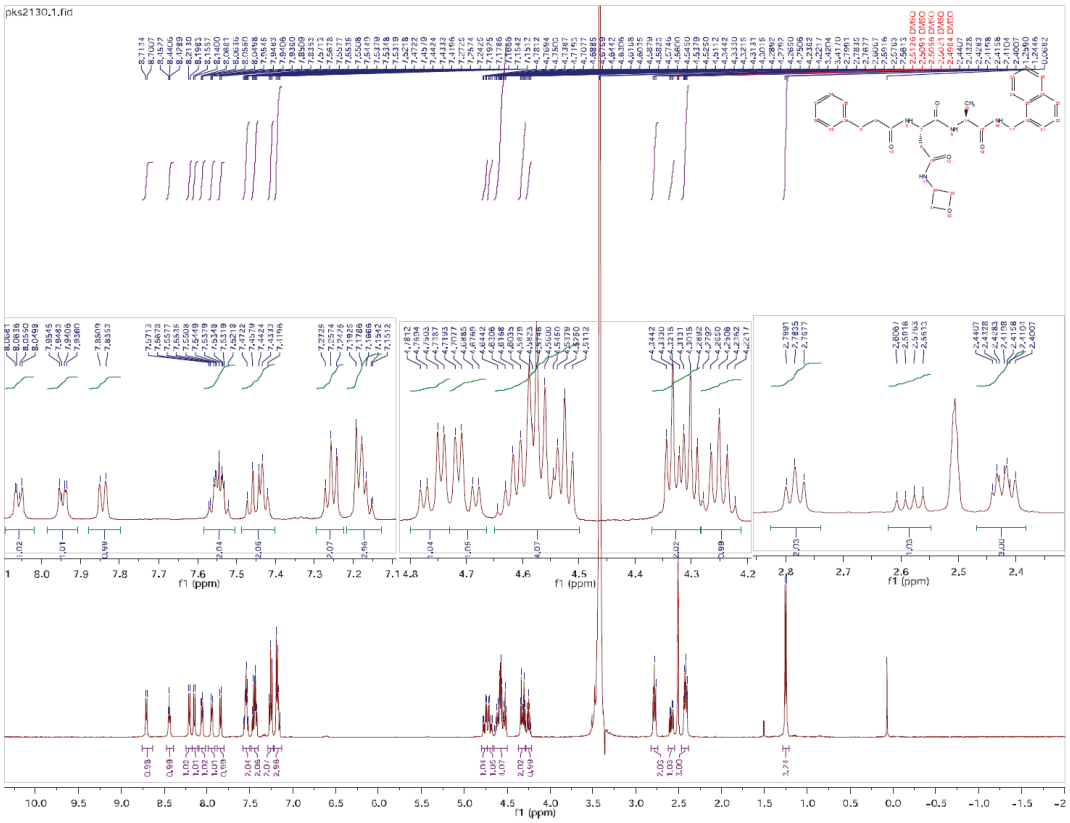
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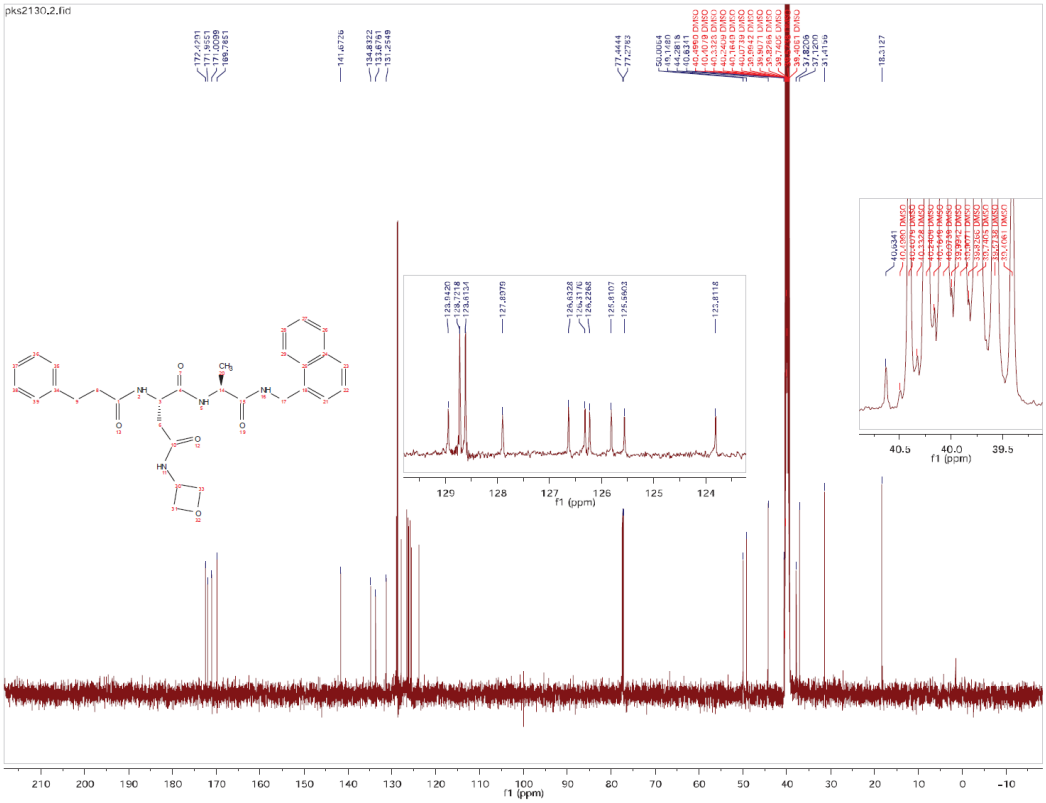
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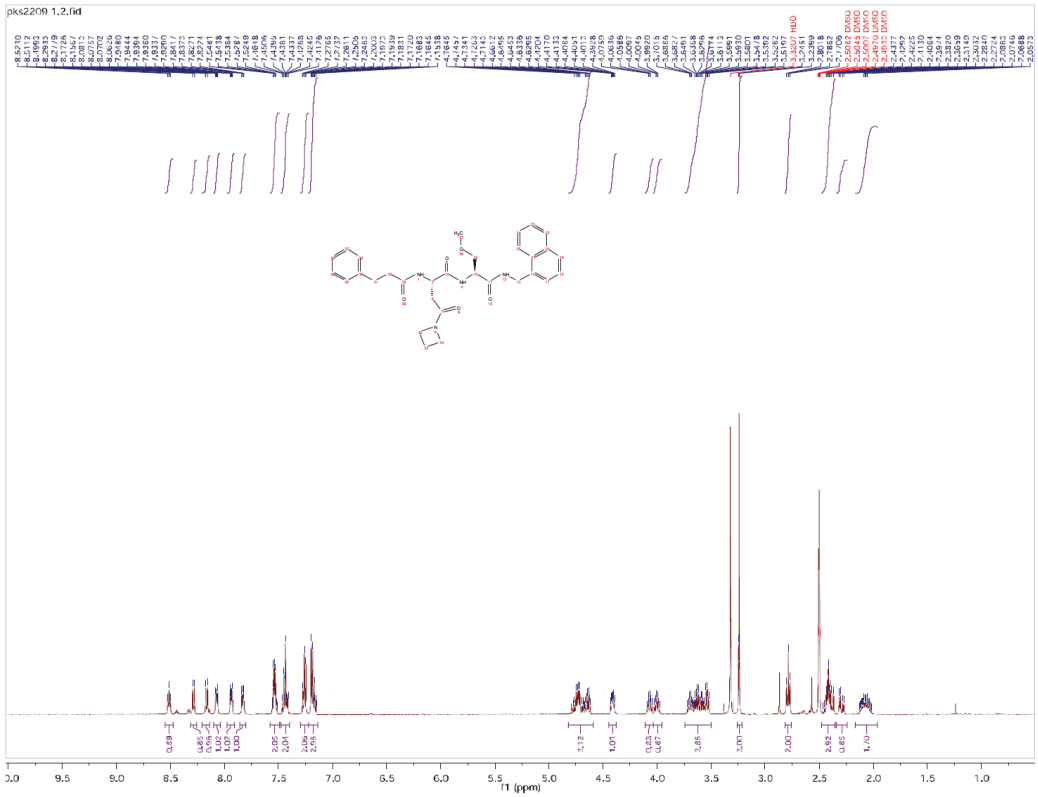
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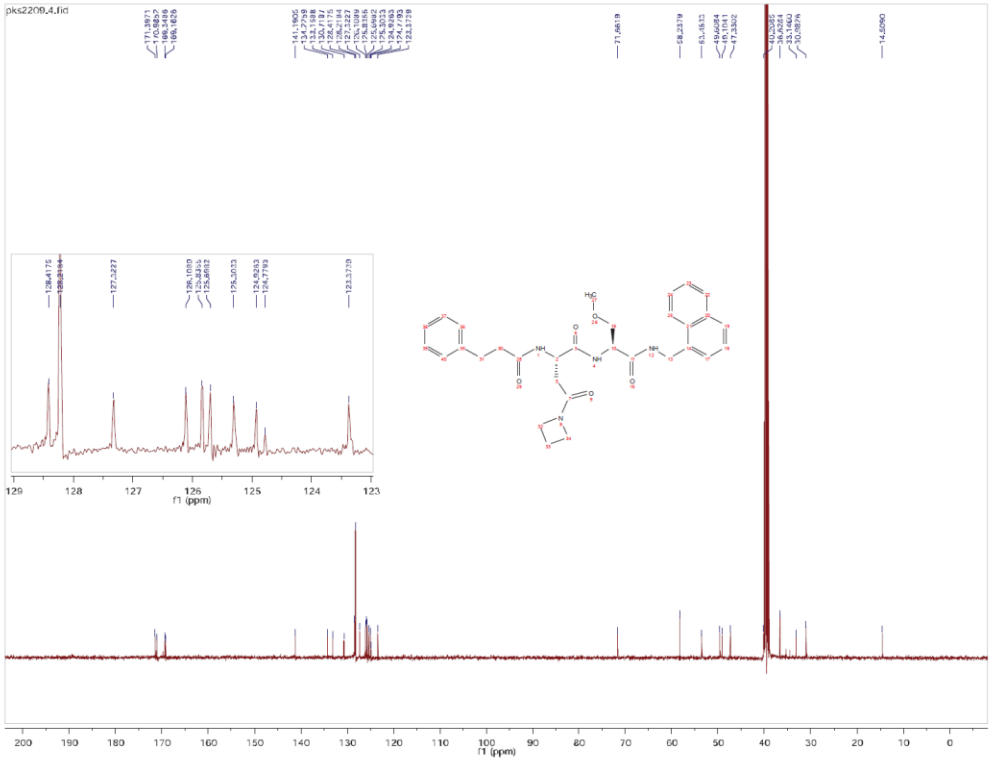
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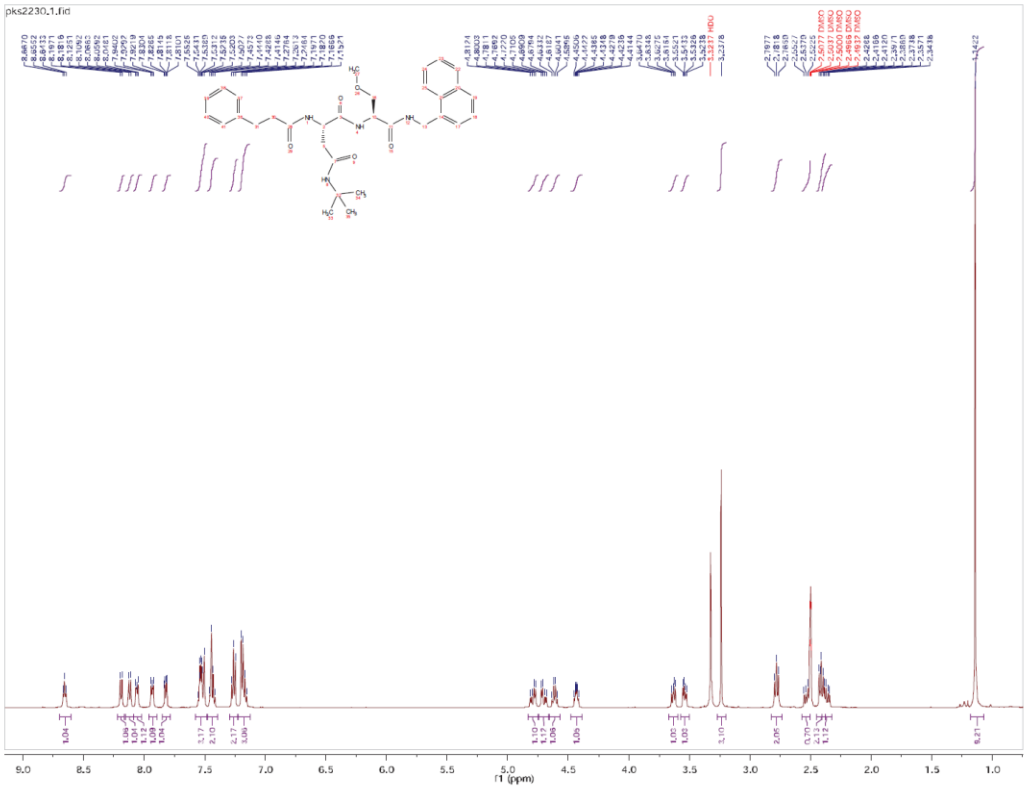
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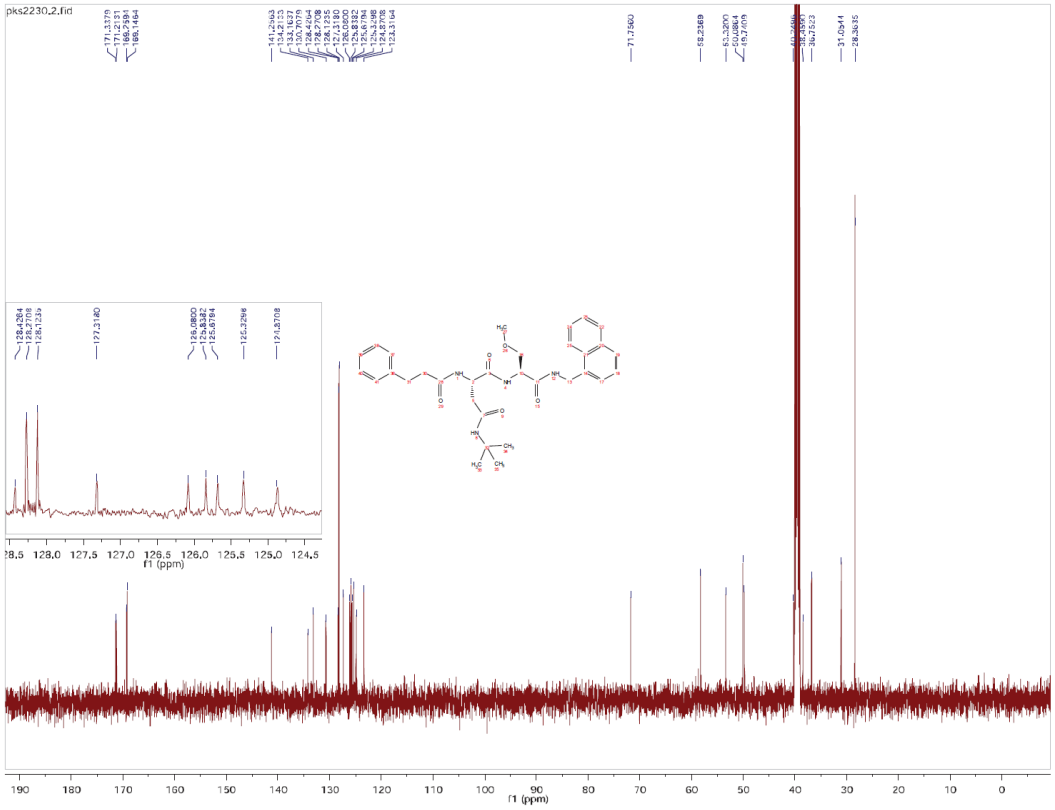
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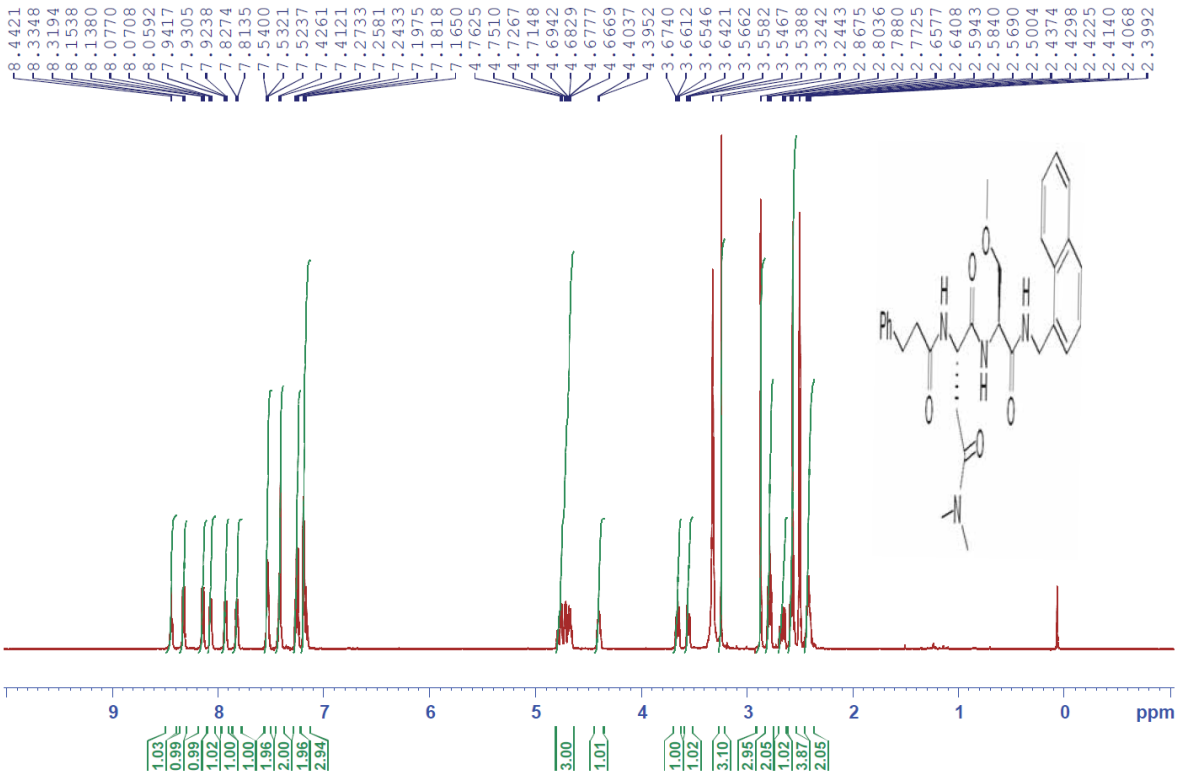
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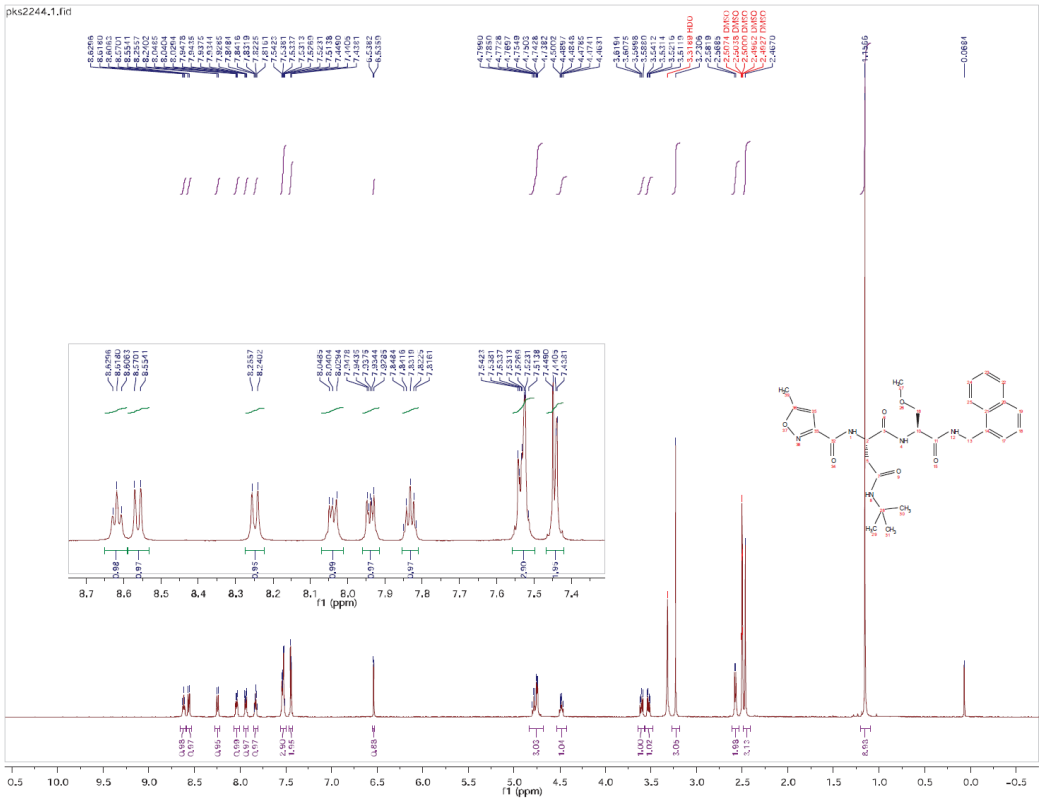
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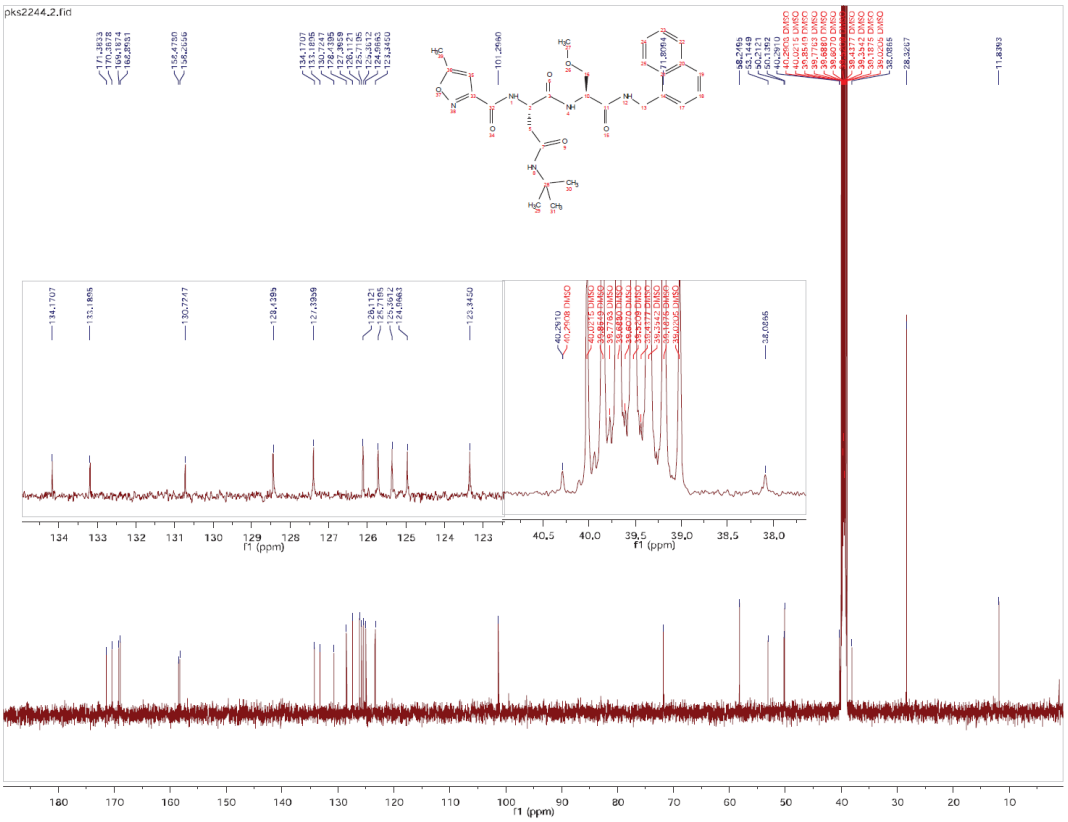
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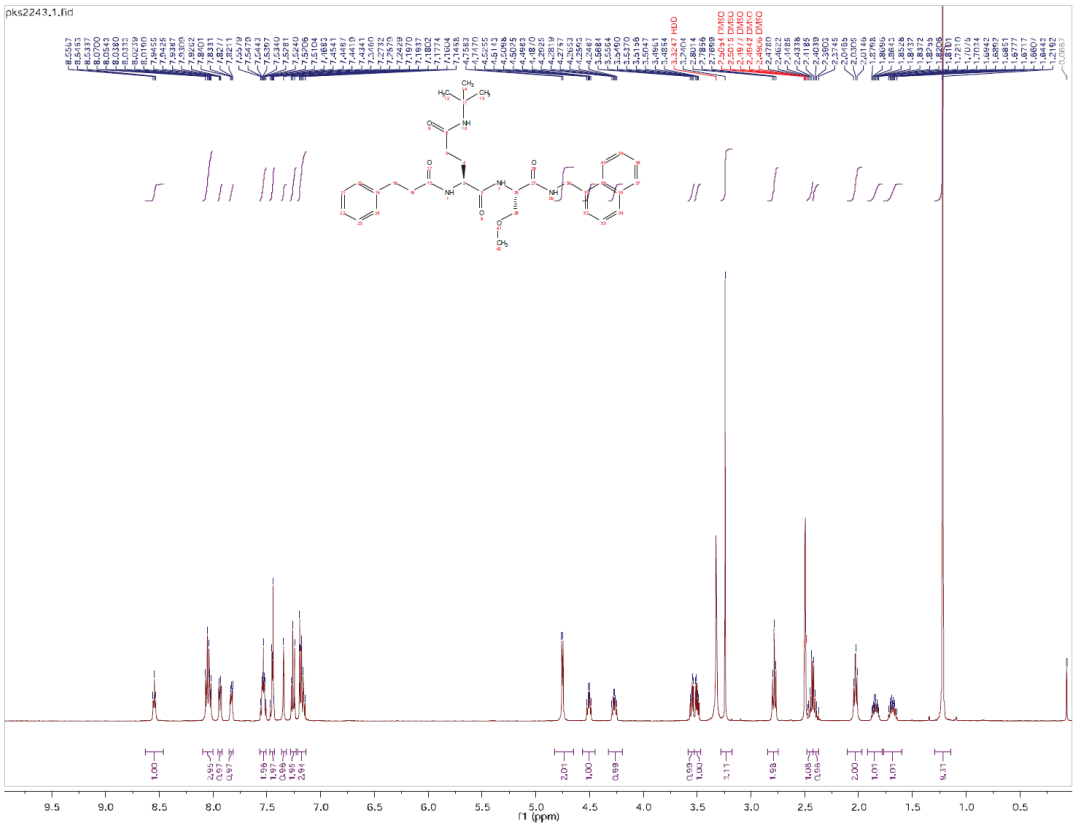
f1 (ppm)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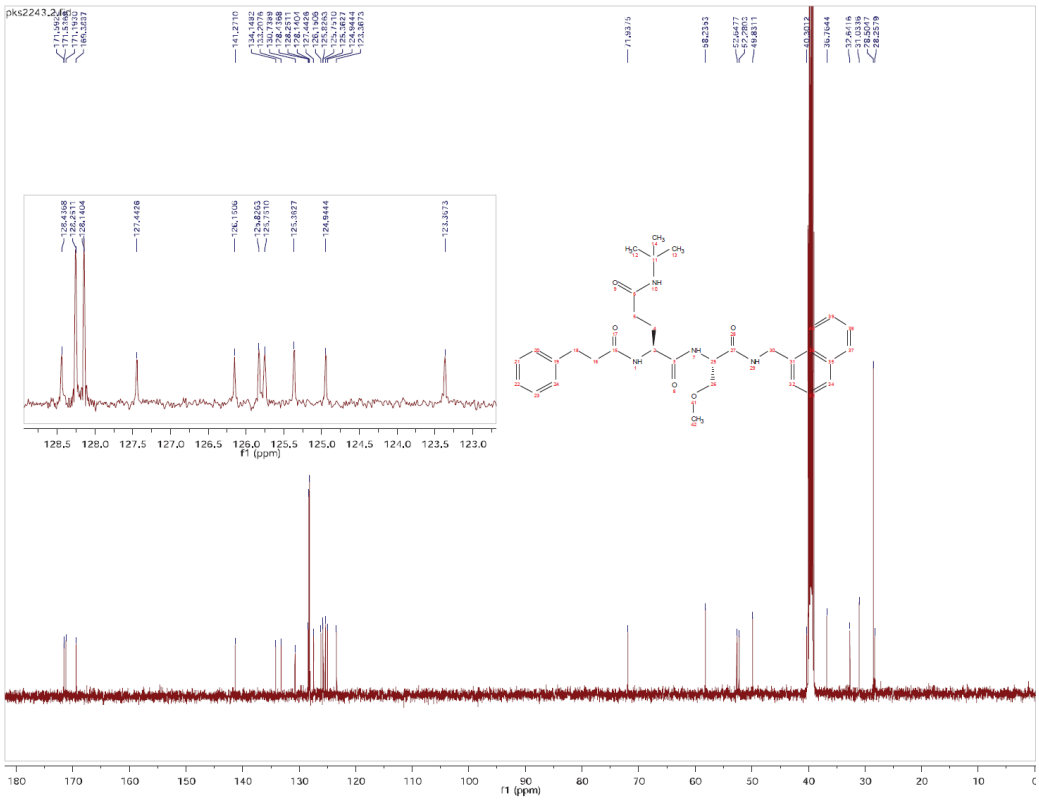
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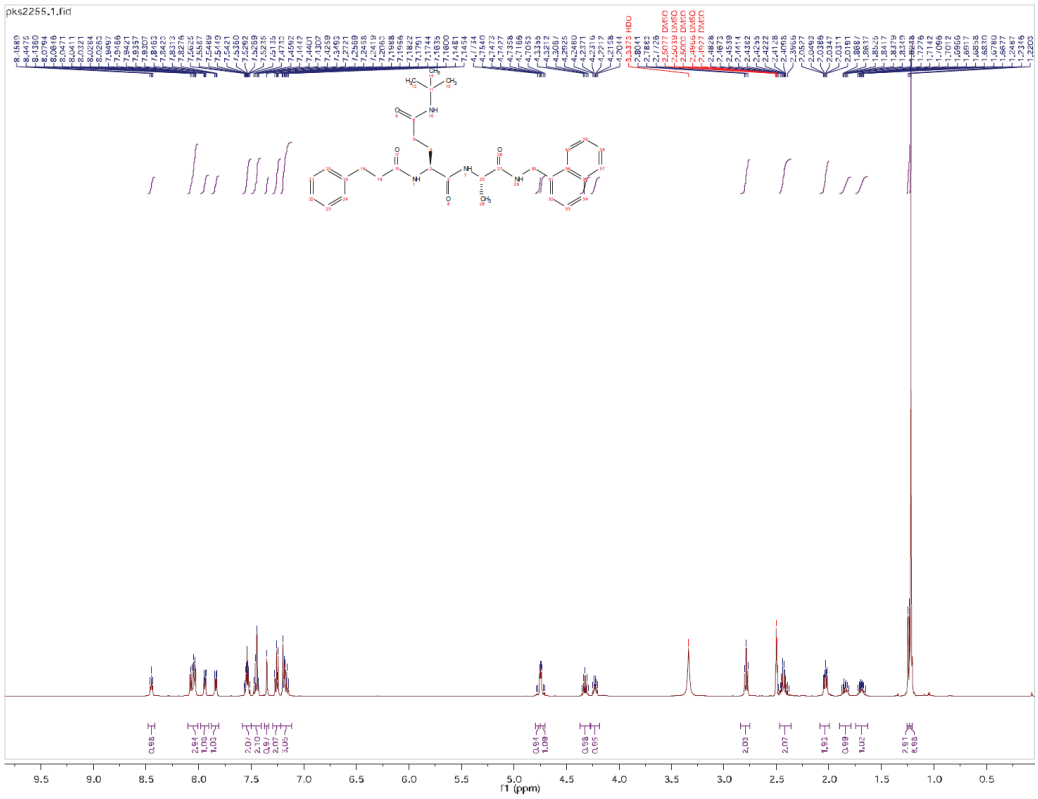
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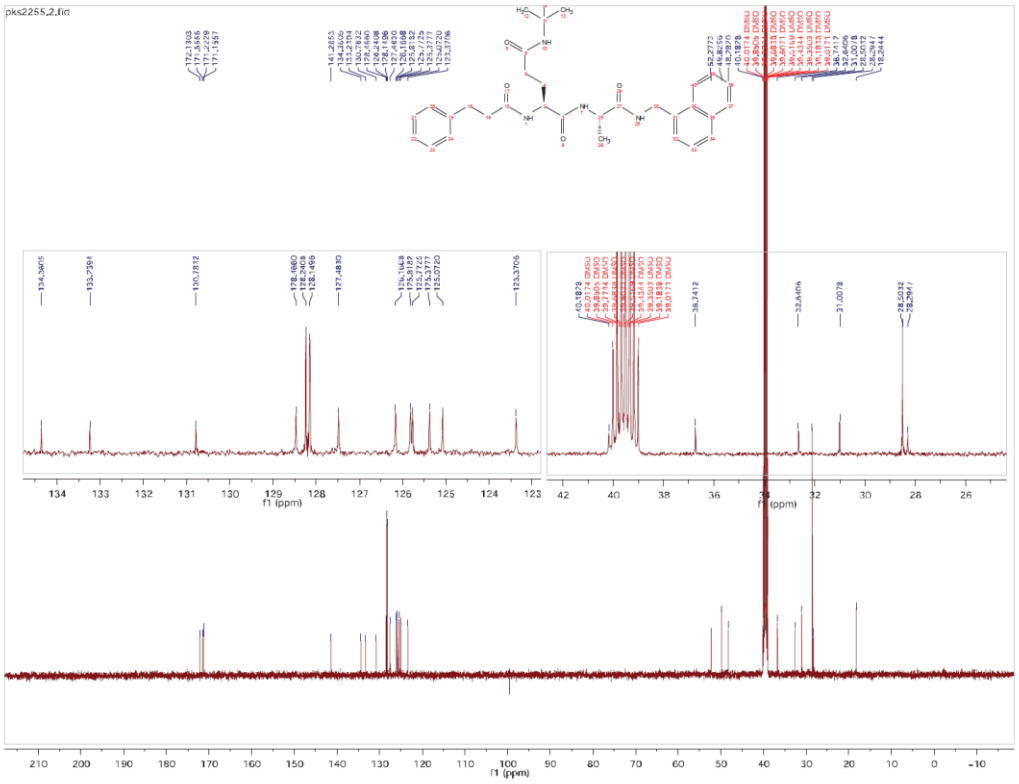
f1 (ppm)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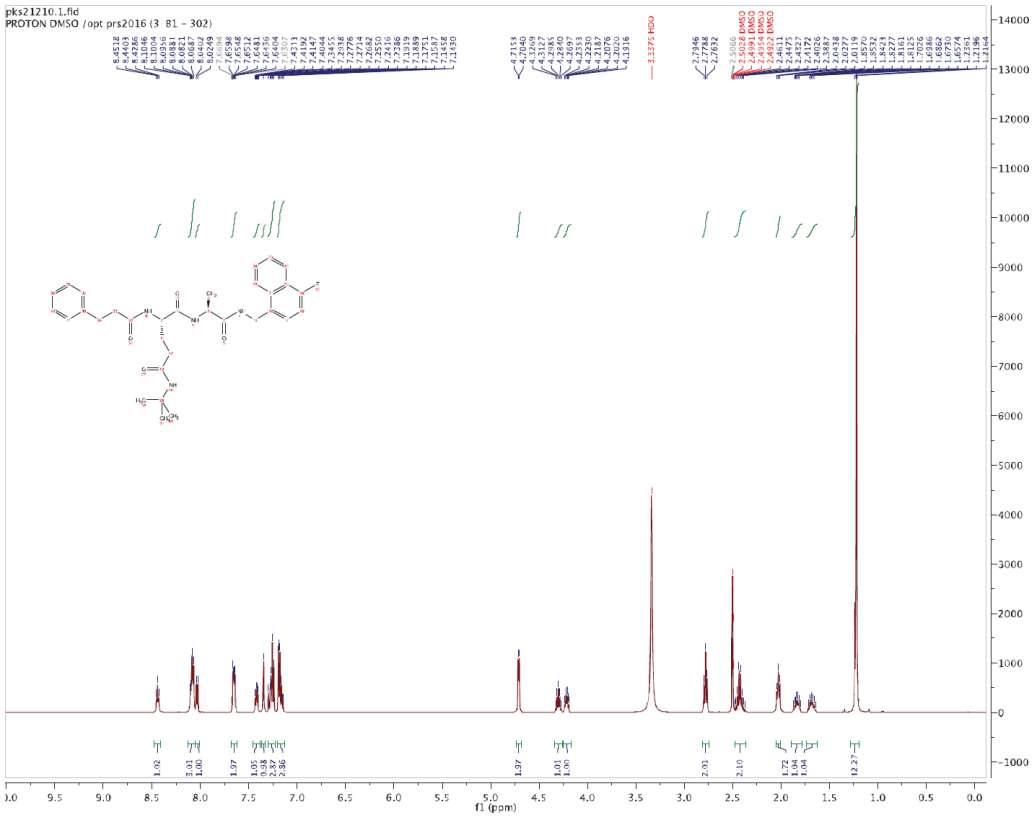
f1 (ppm)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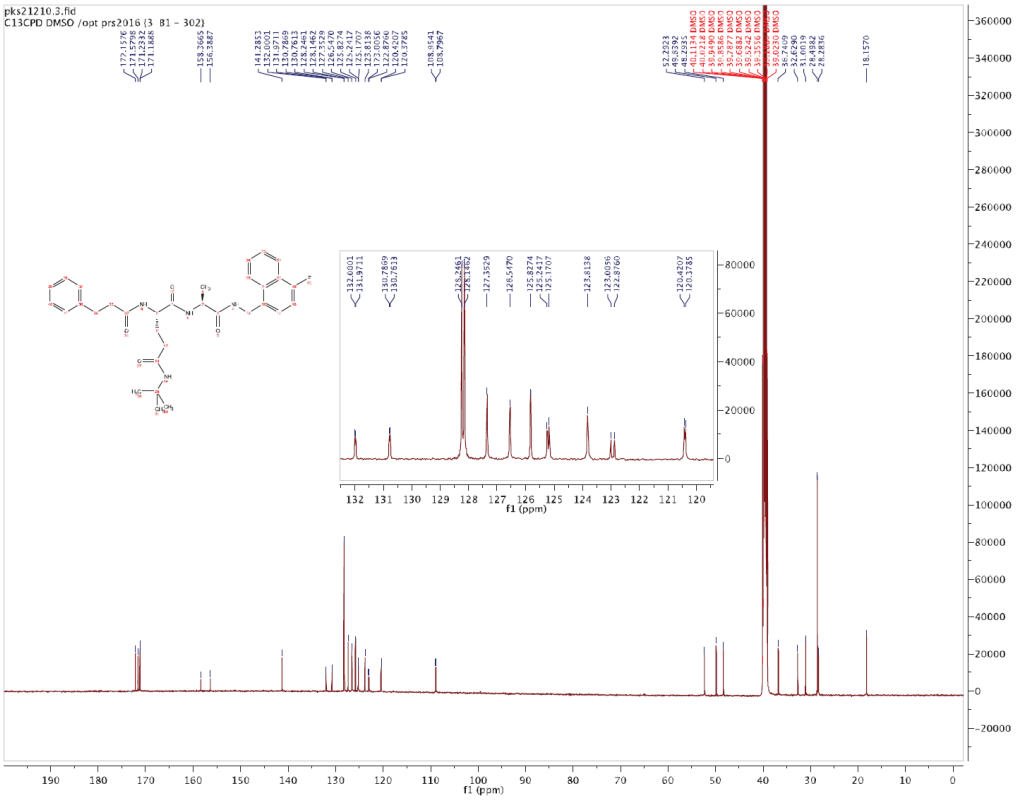
f1 (ppm)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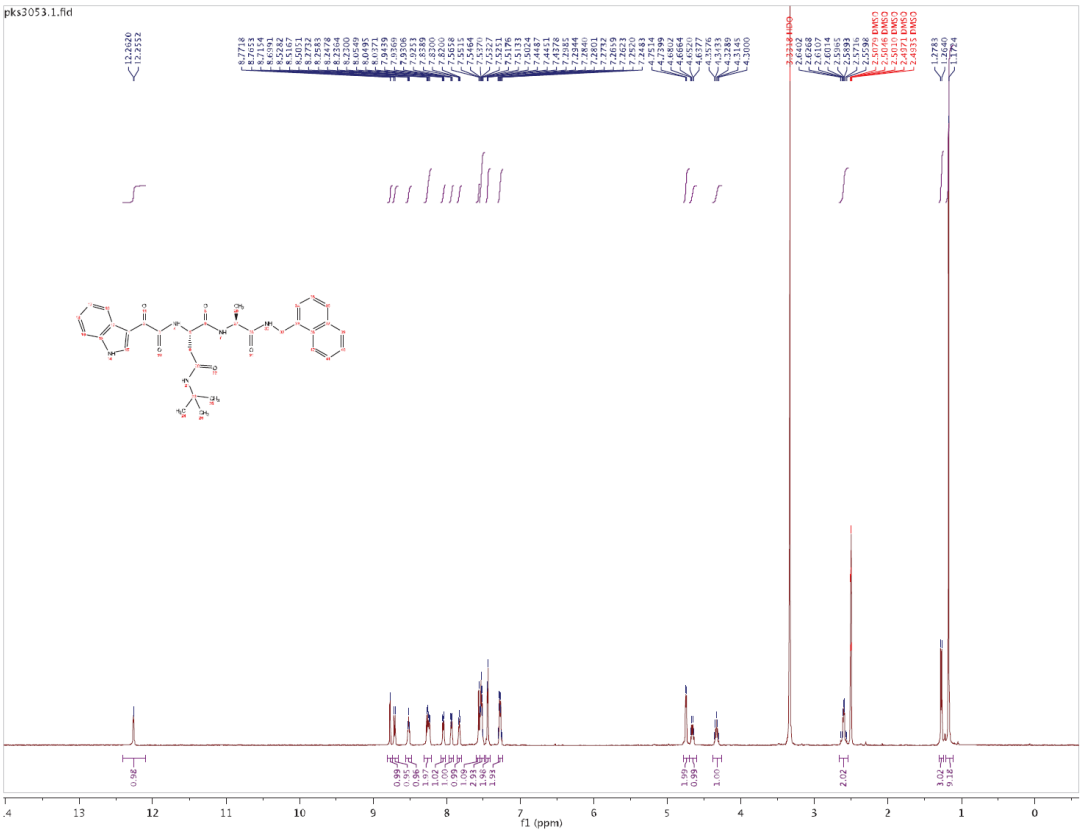
f1 (ppm)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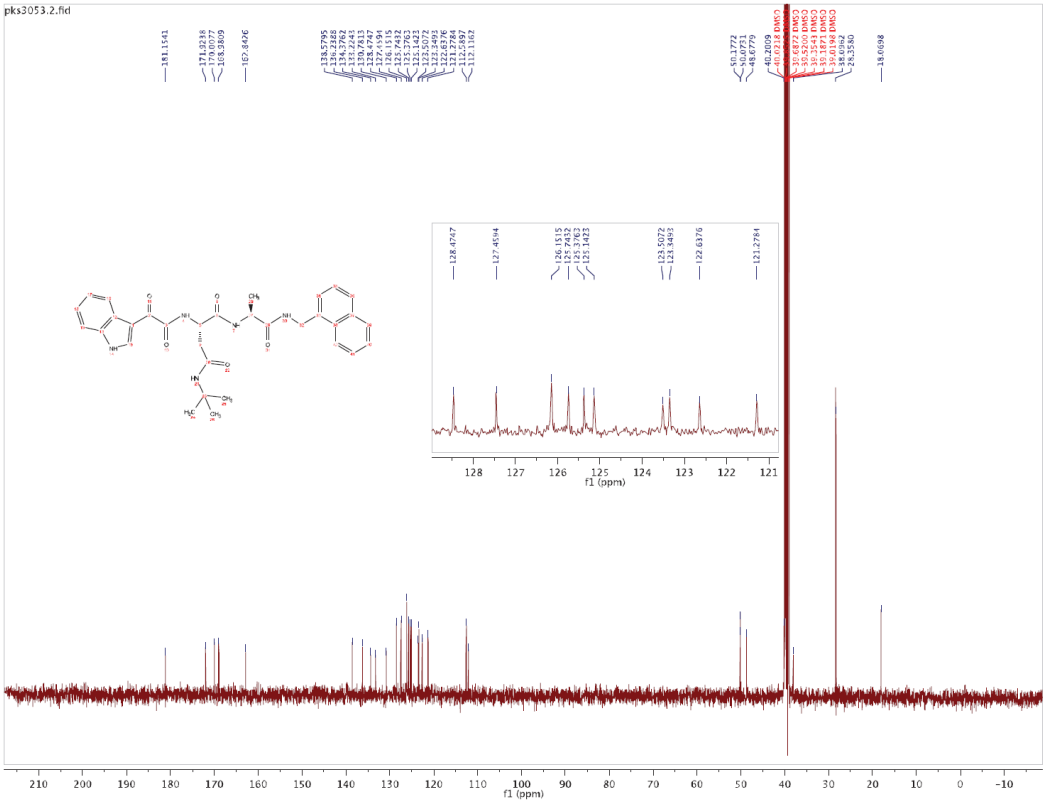
f1 (ppm)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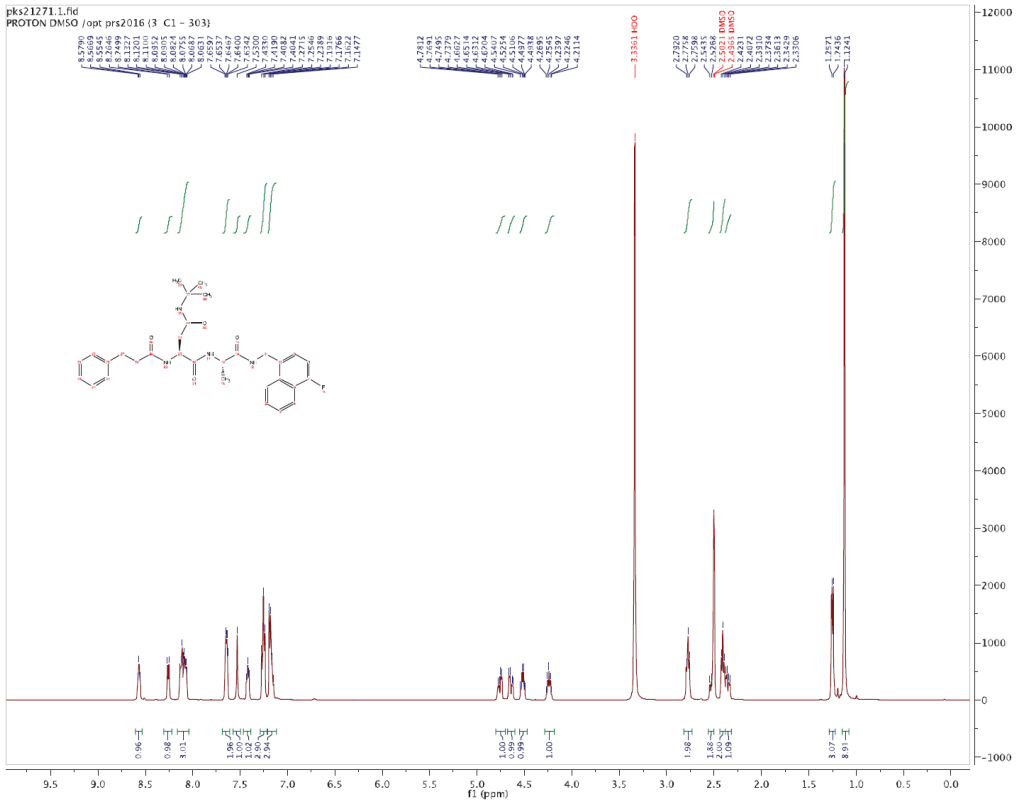
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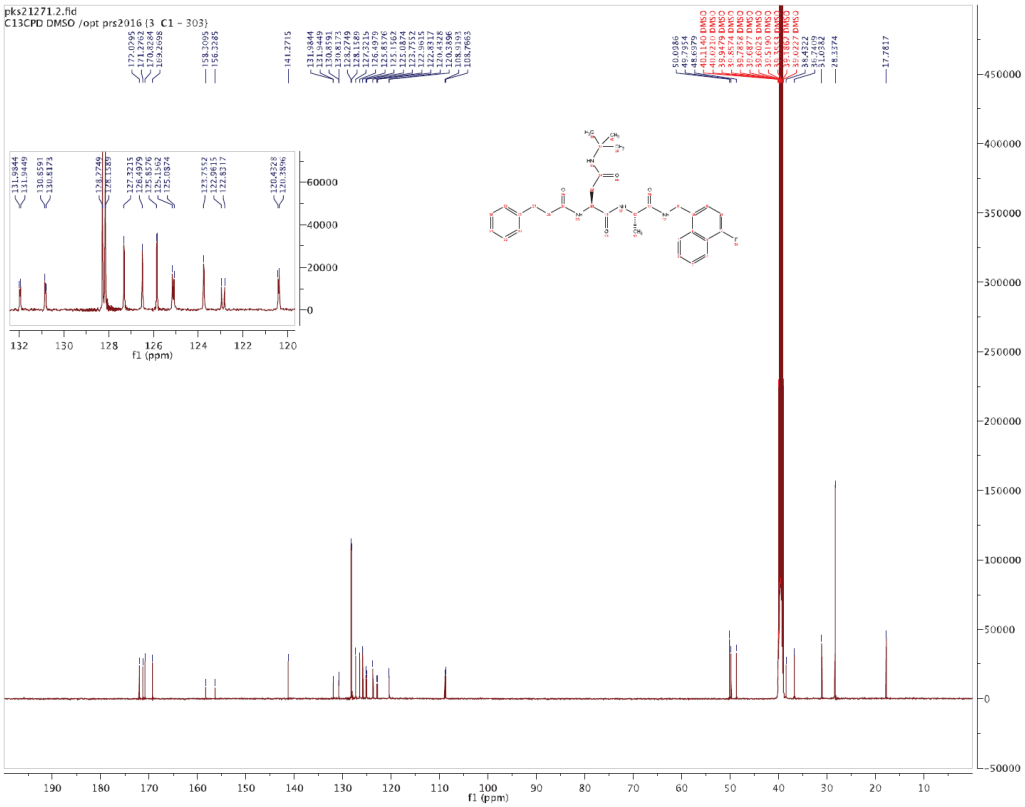
f1 (ppm)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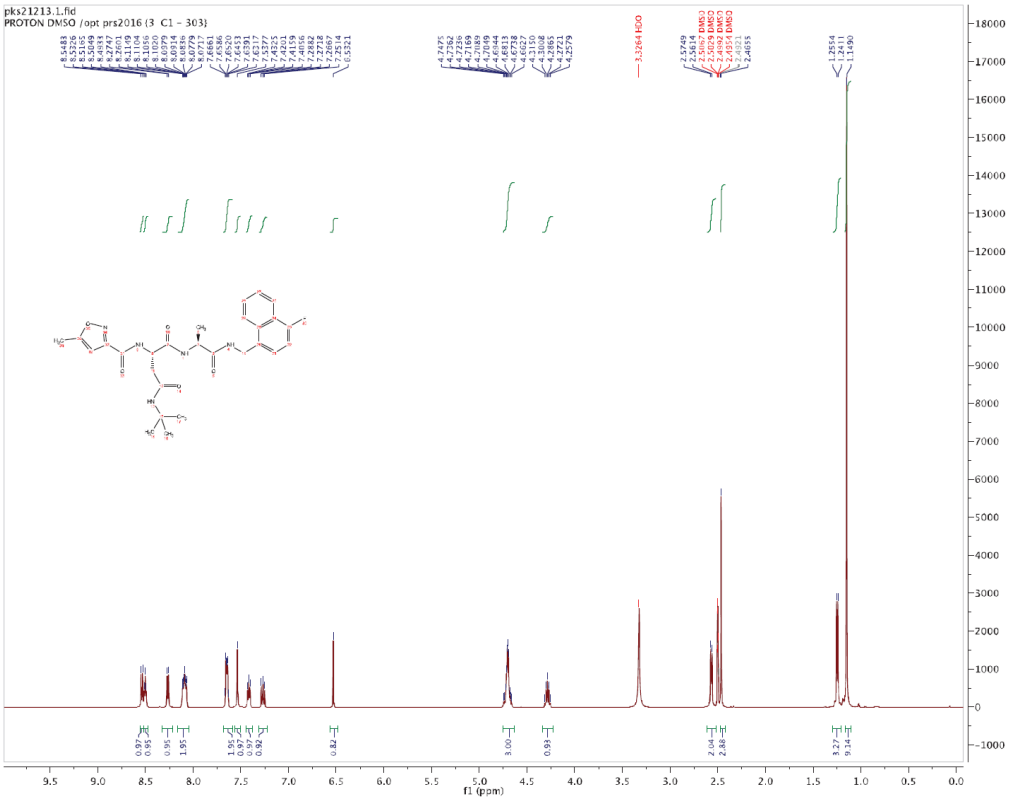
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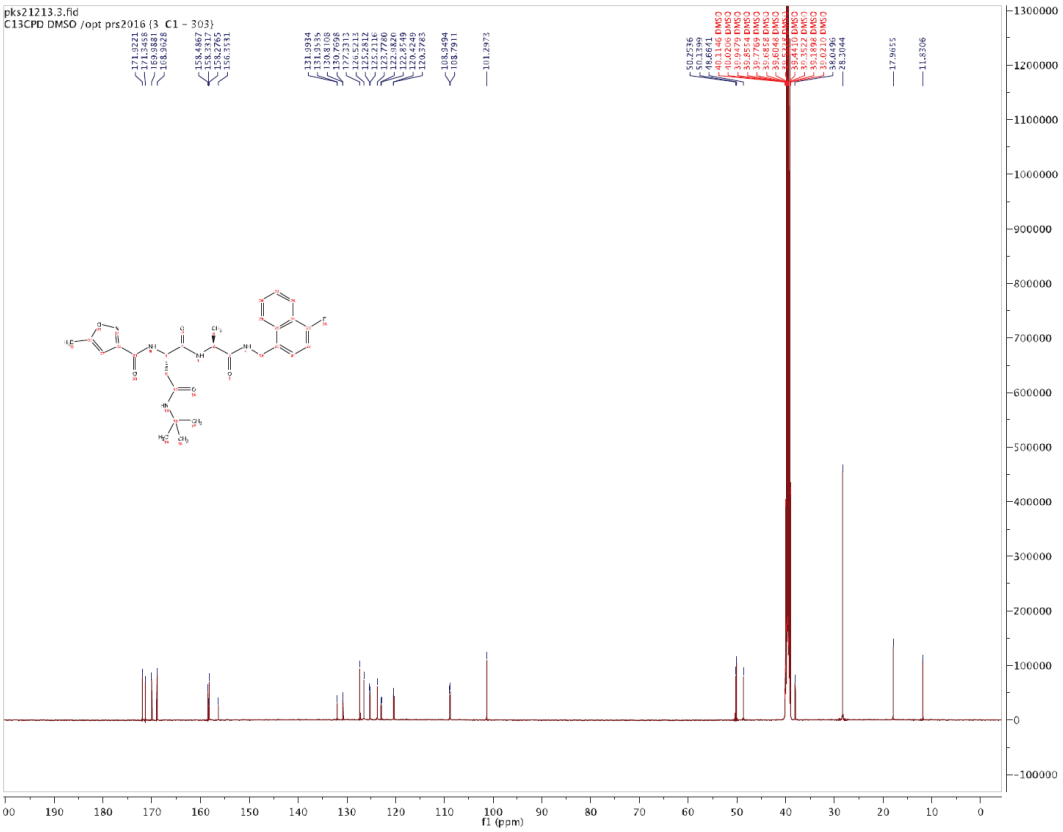
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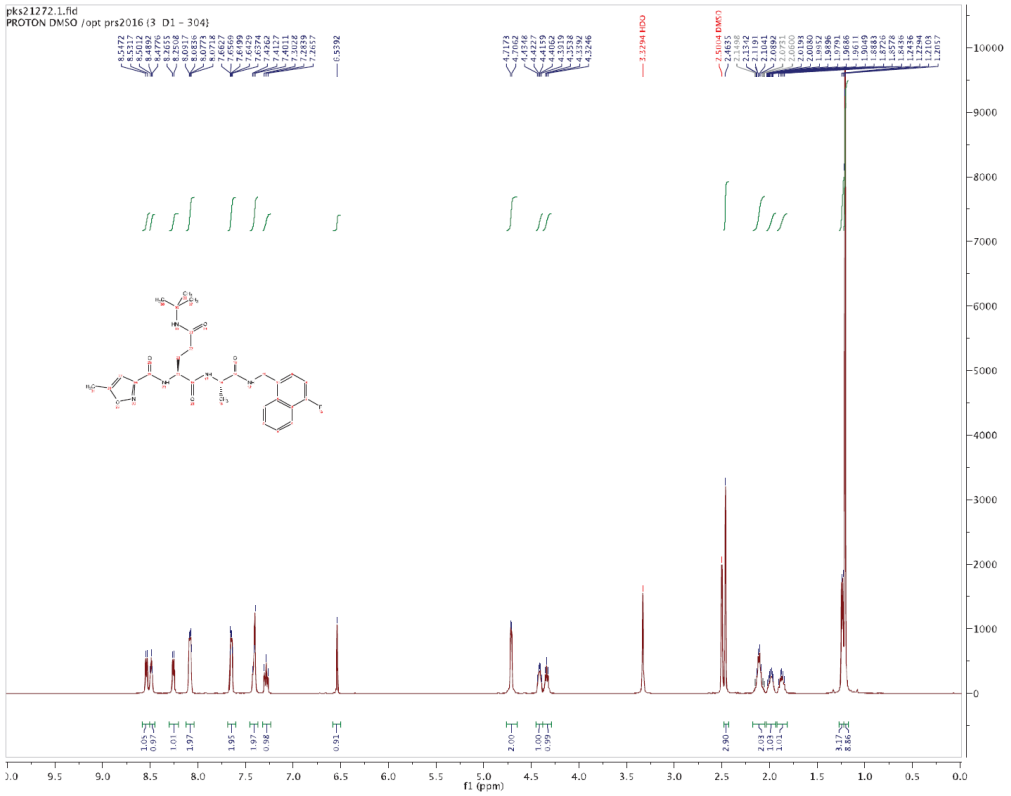
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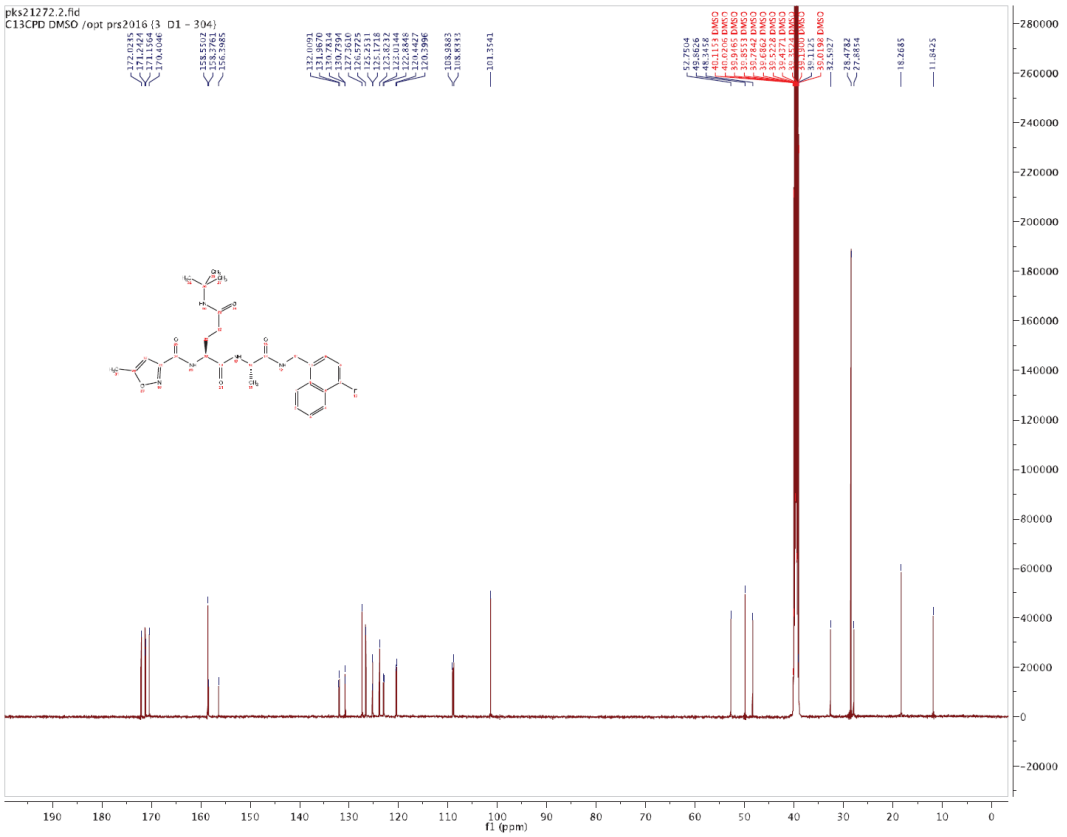
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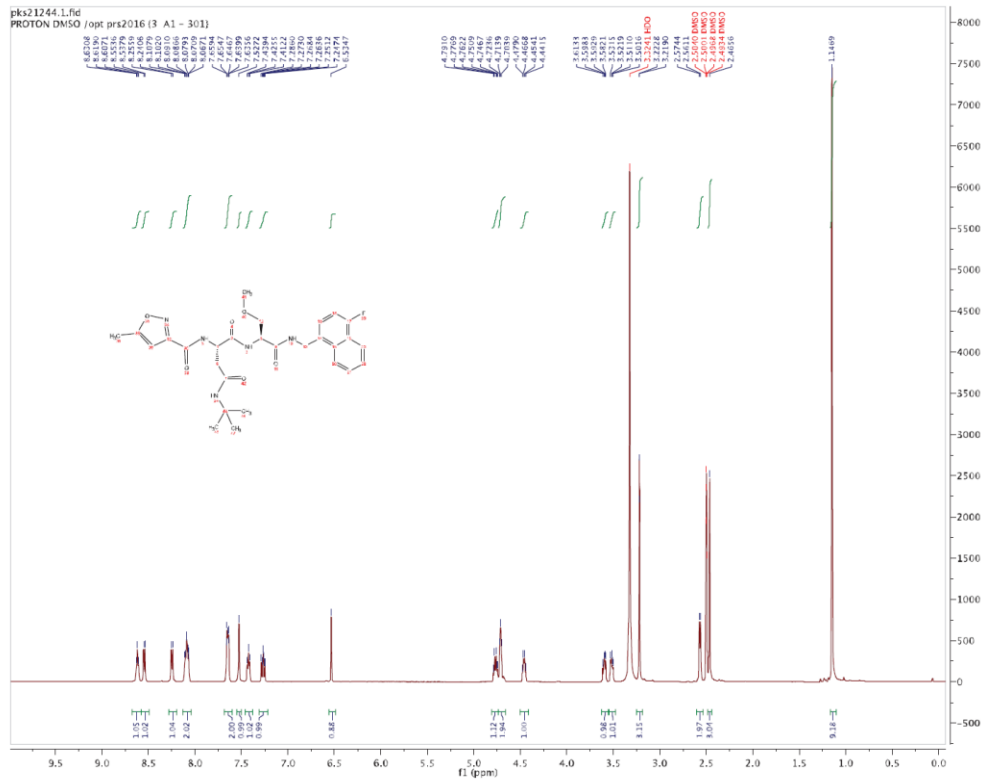
f1 (ppm)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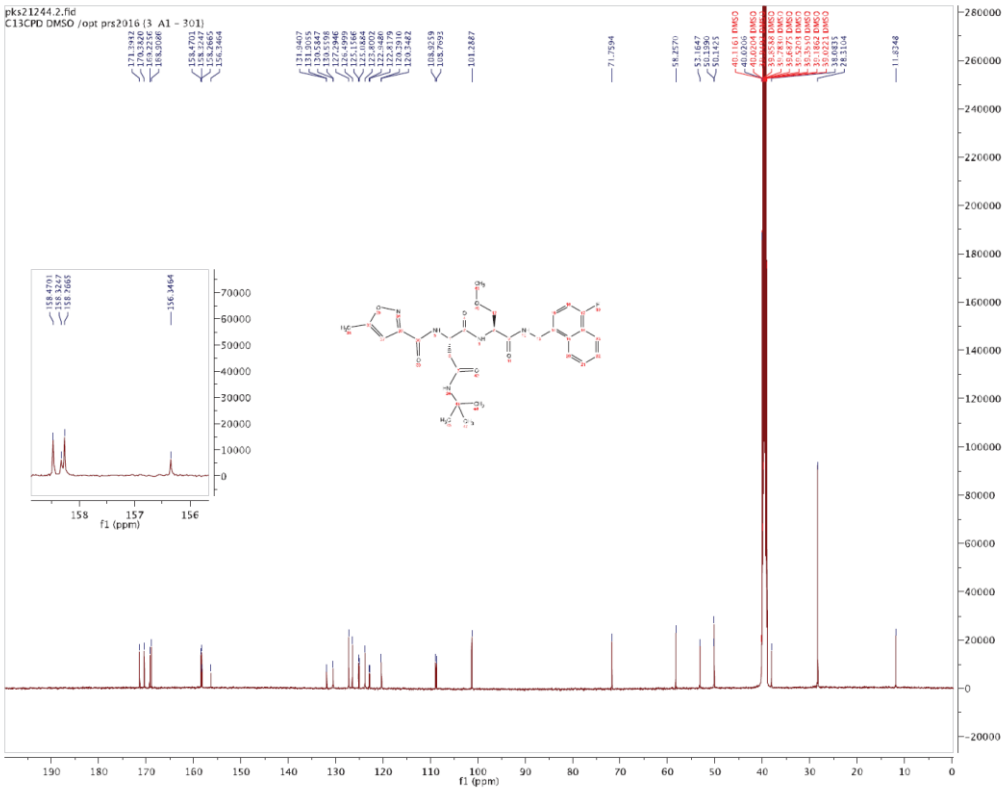
PROTON DMSO /opt prs2016 (3 A1 - 301)
f1 (ppm)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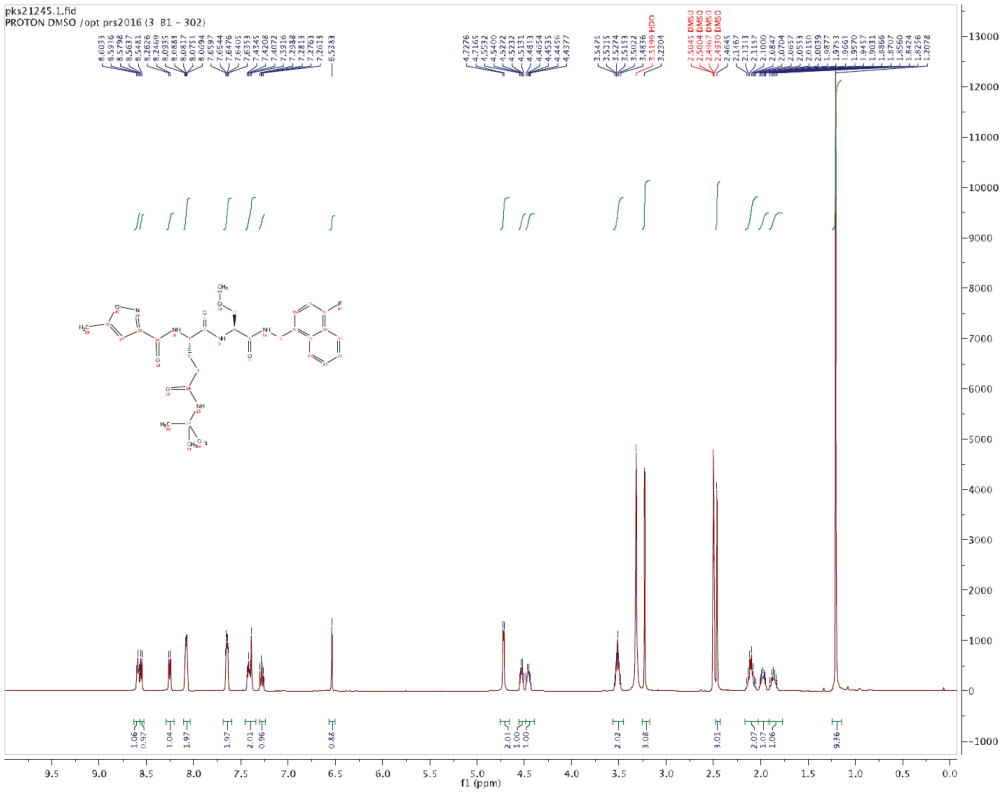
f1 (ppm)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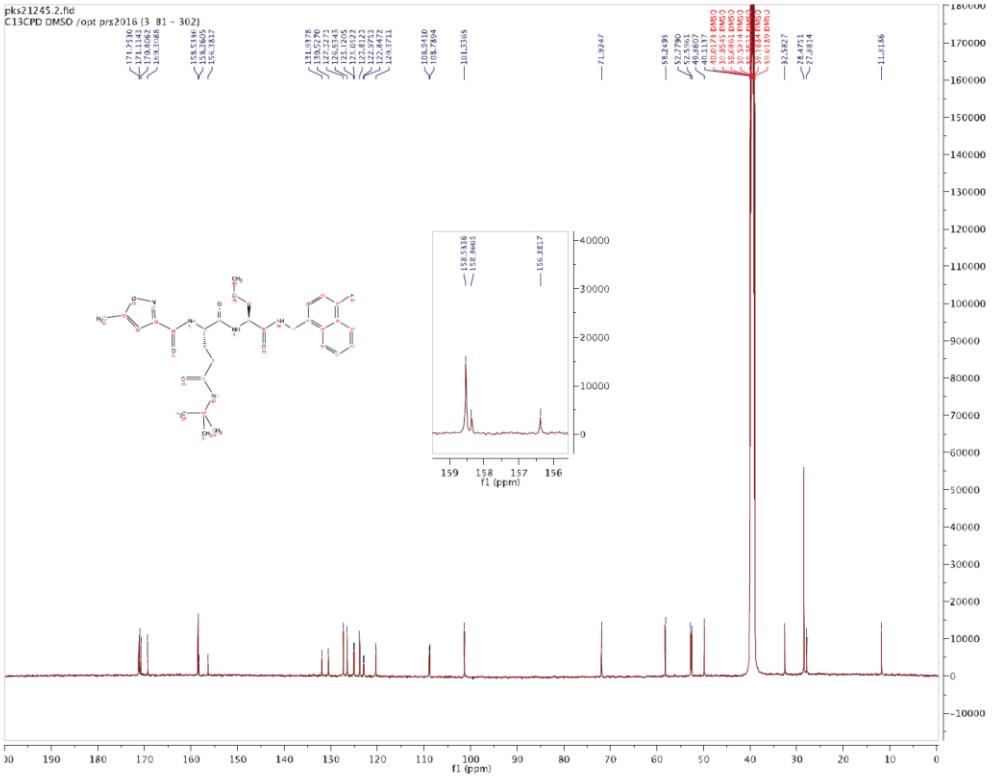
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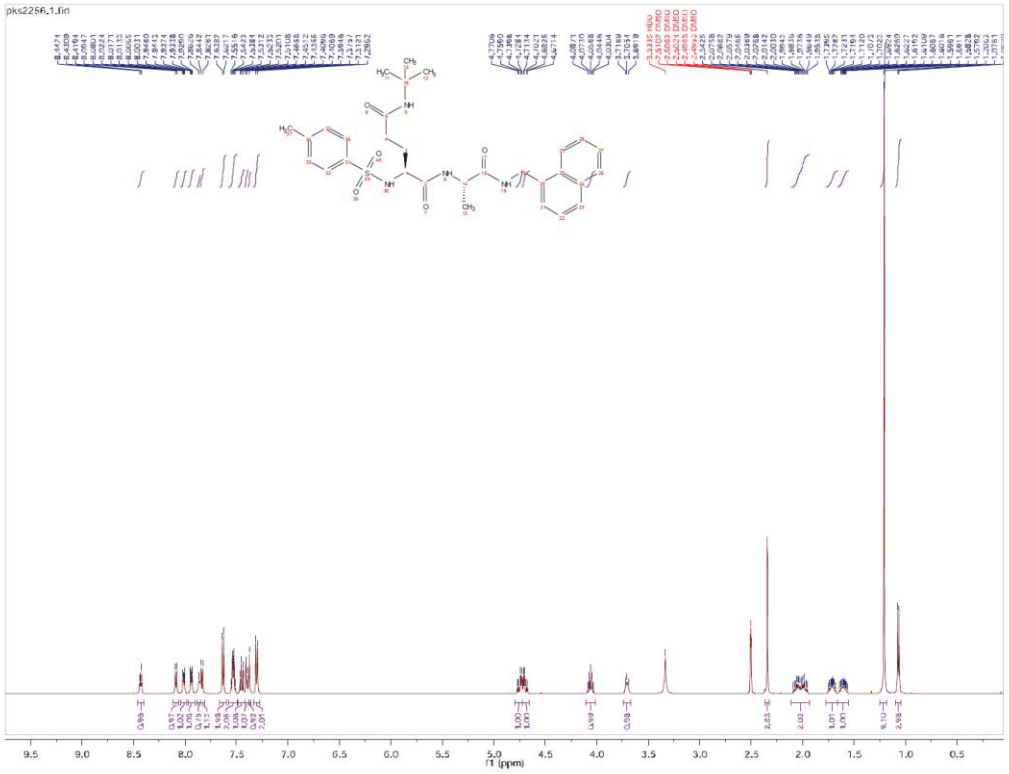
f1 (ppm)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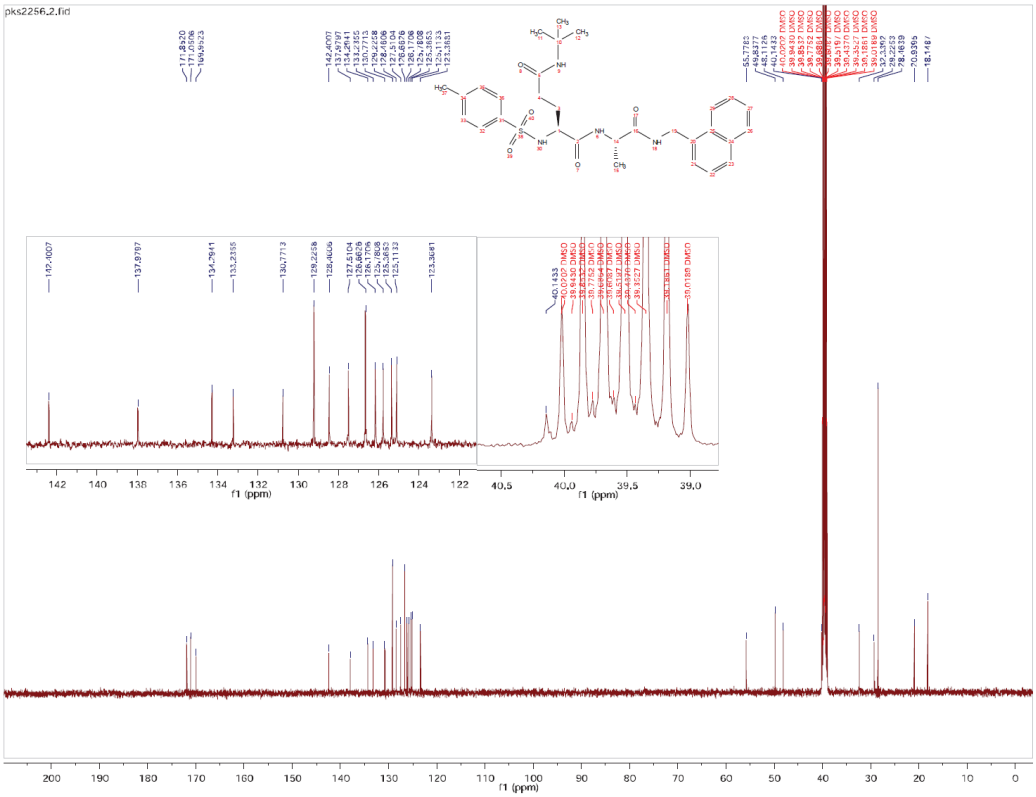
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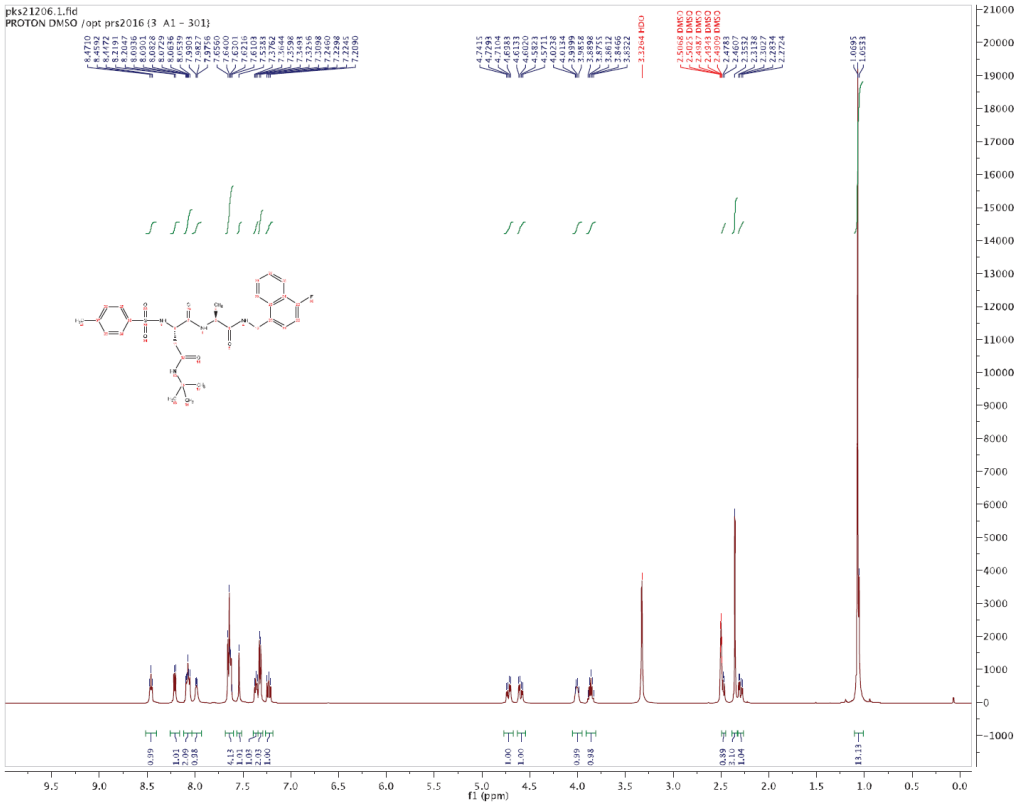
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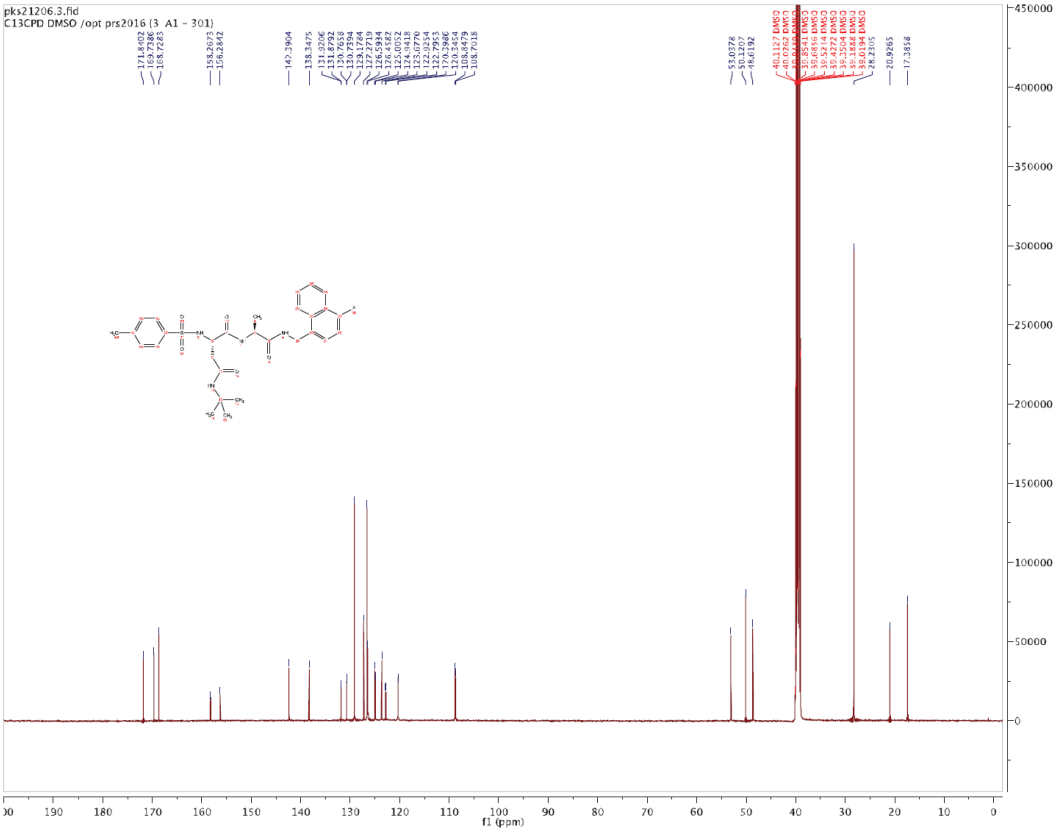
f1 (ppm)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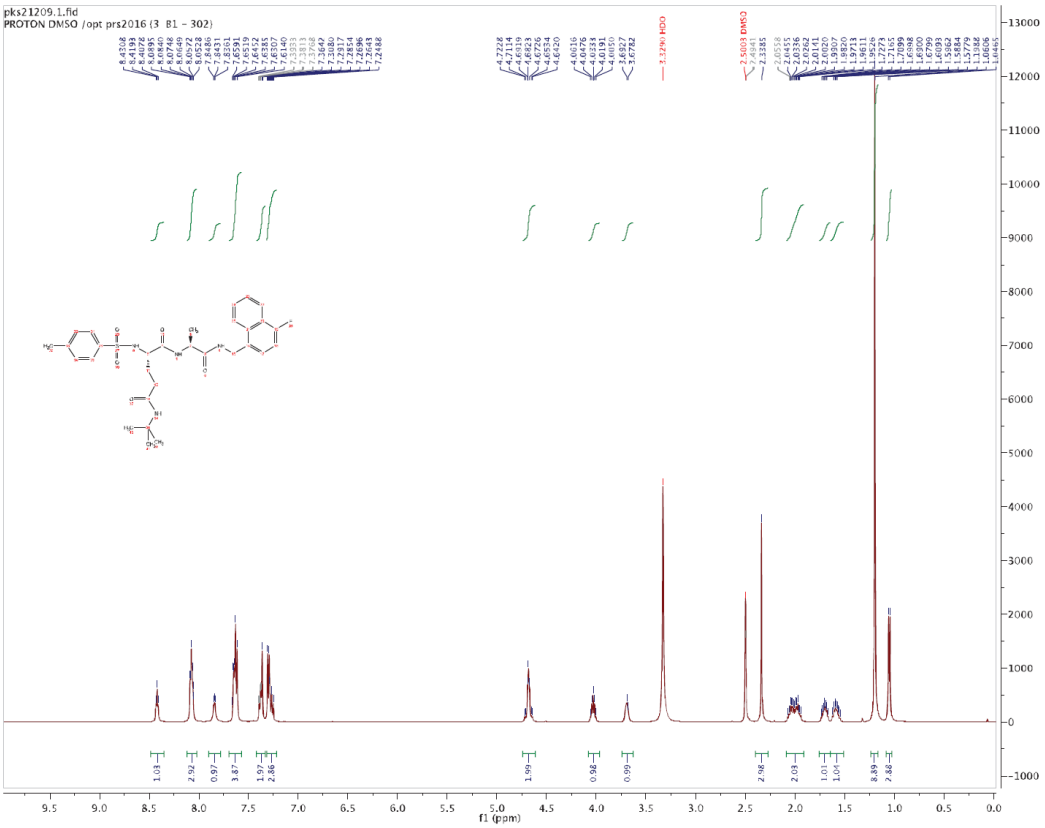
C13CPD DMSO /opt prs2016 (3 A1 - 301)
f1 (ppm)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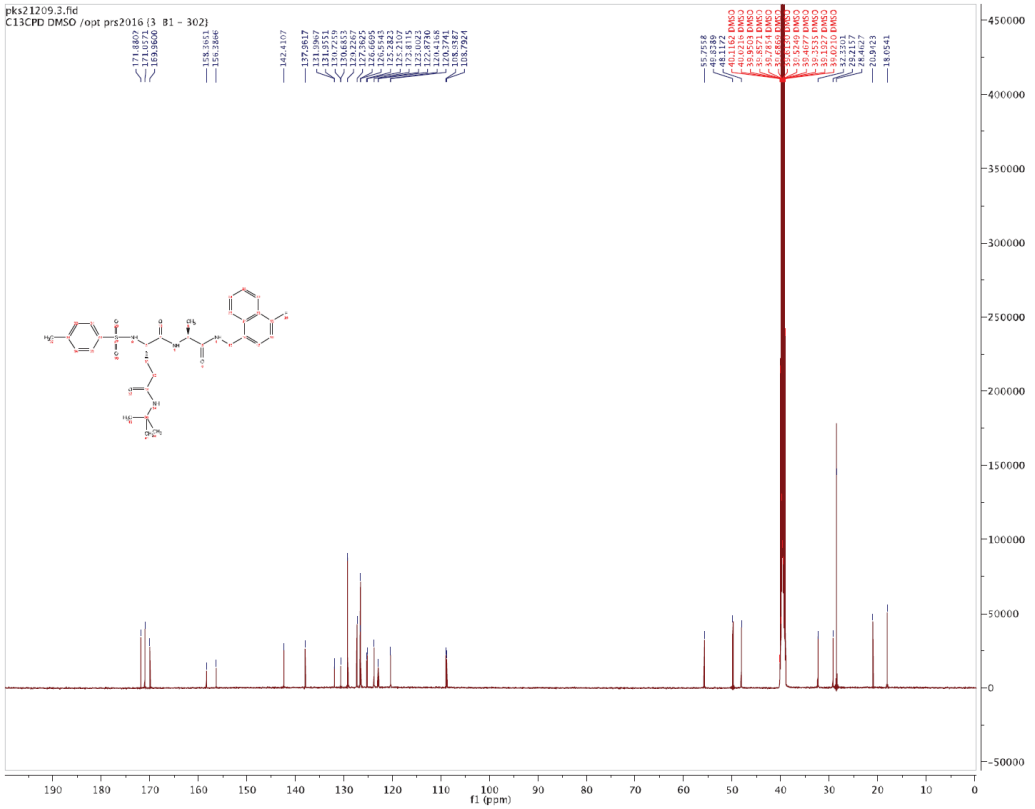
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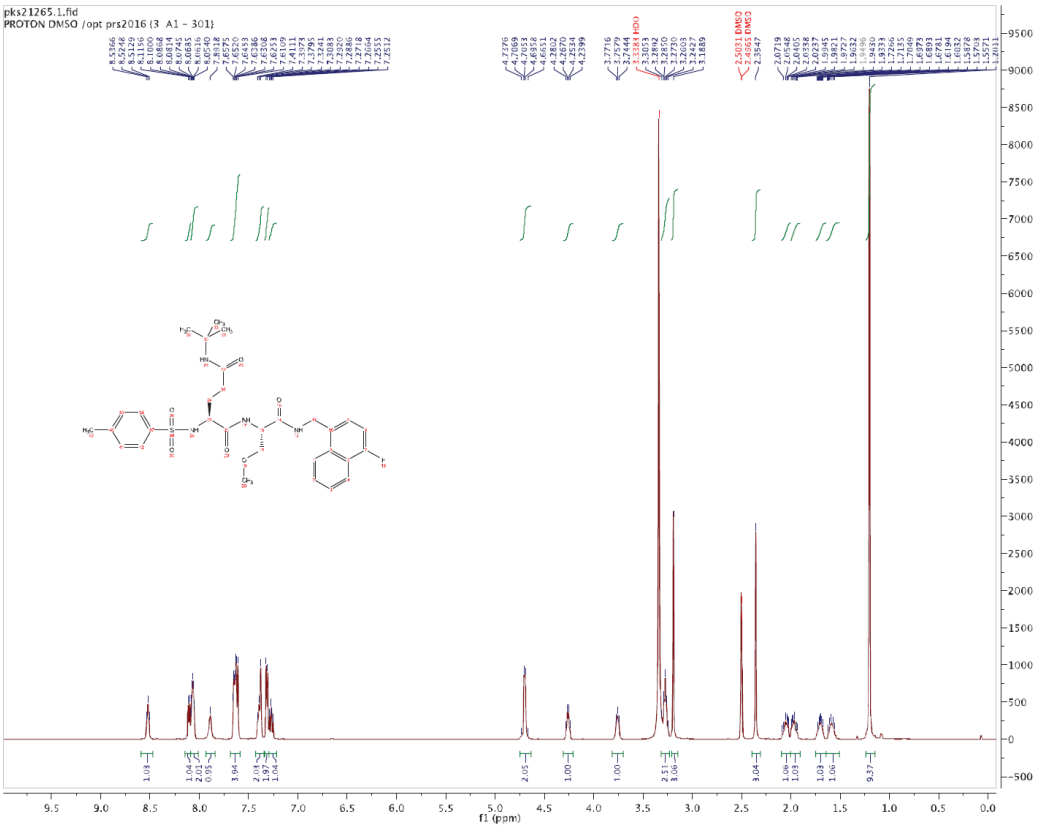
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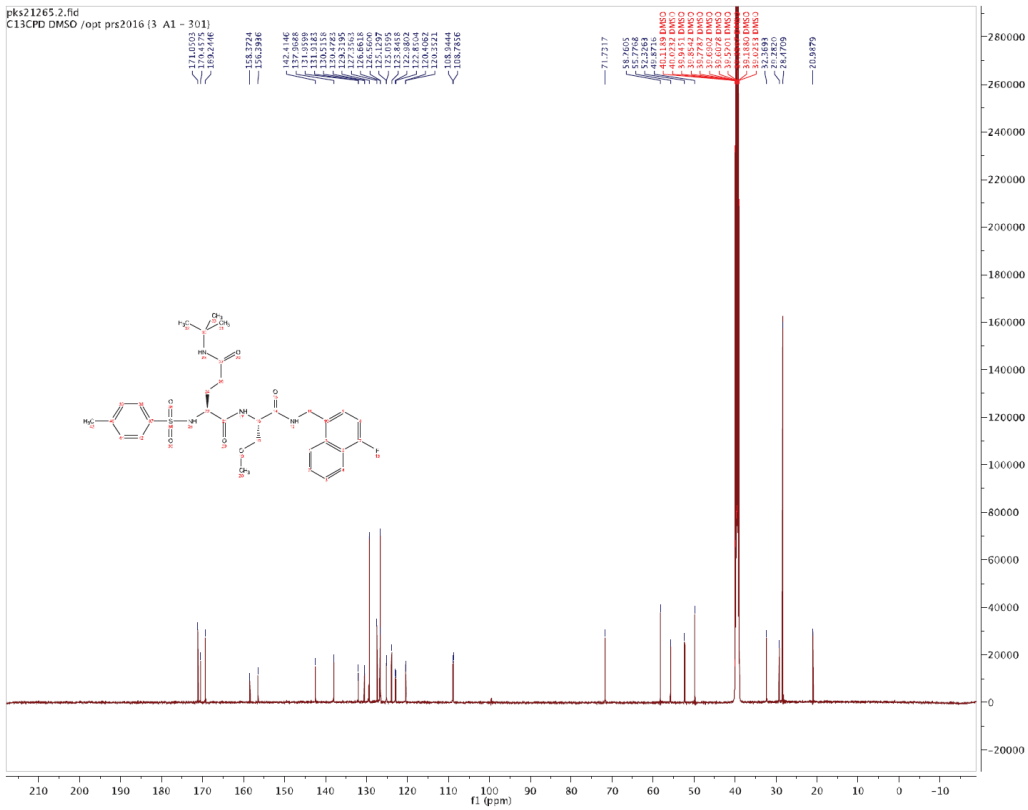
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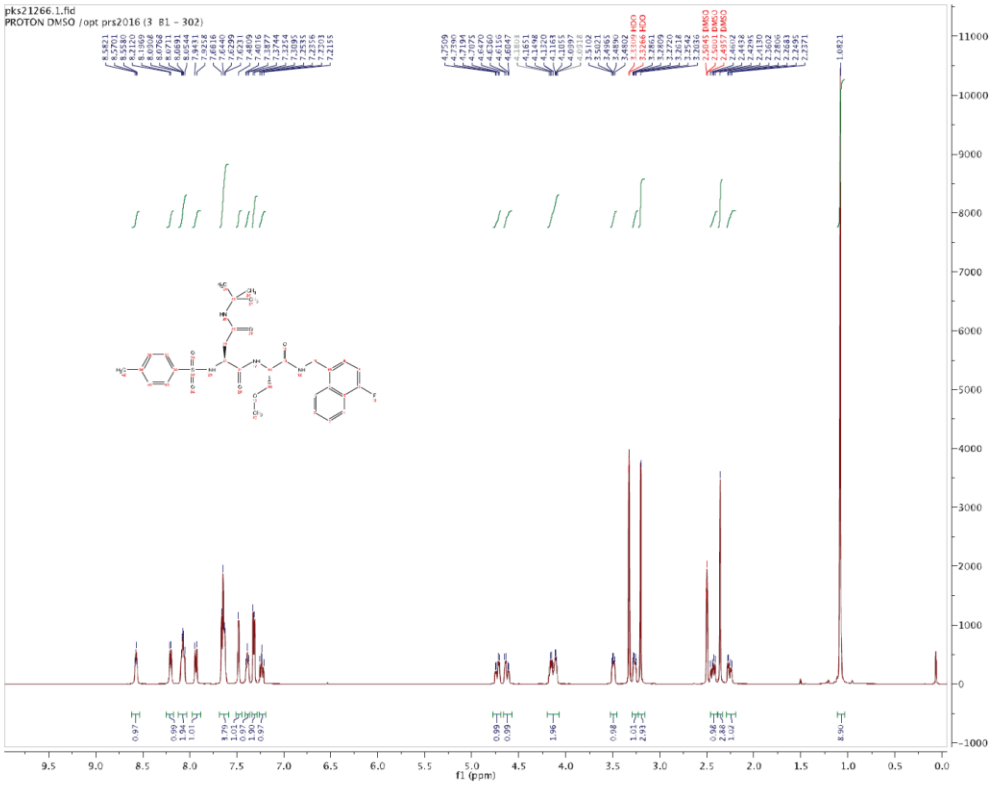
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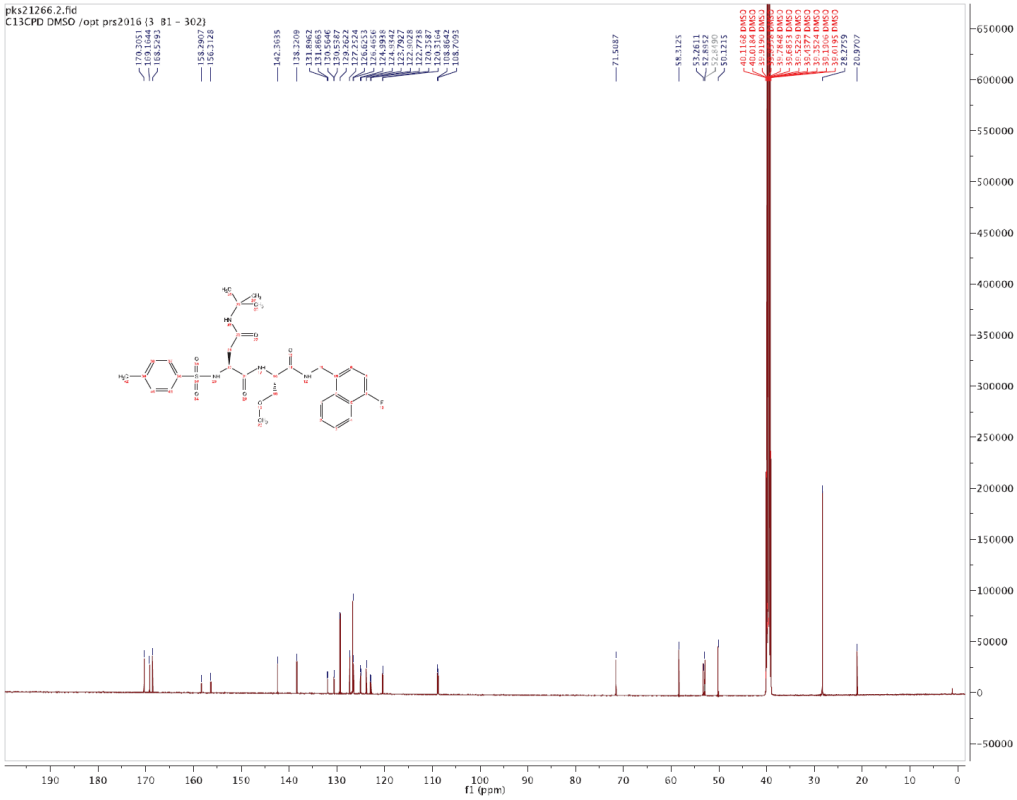
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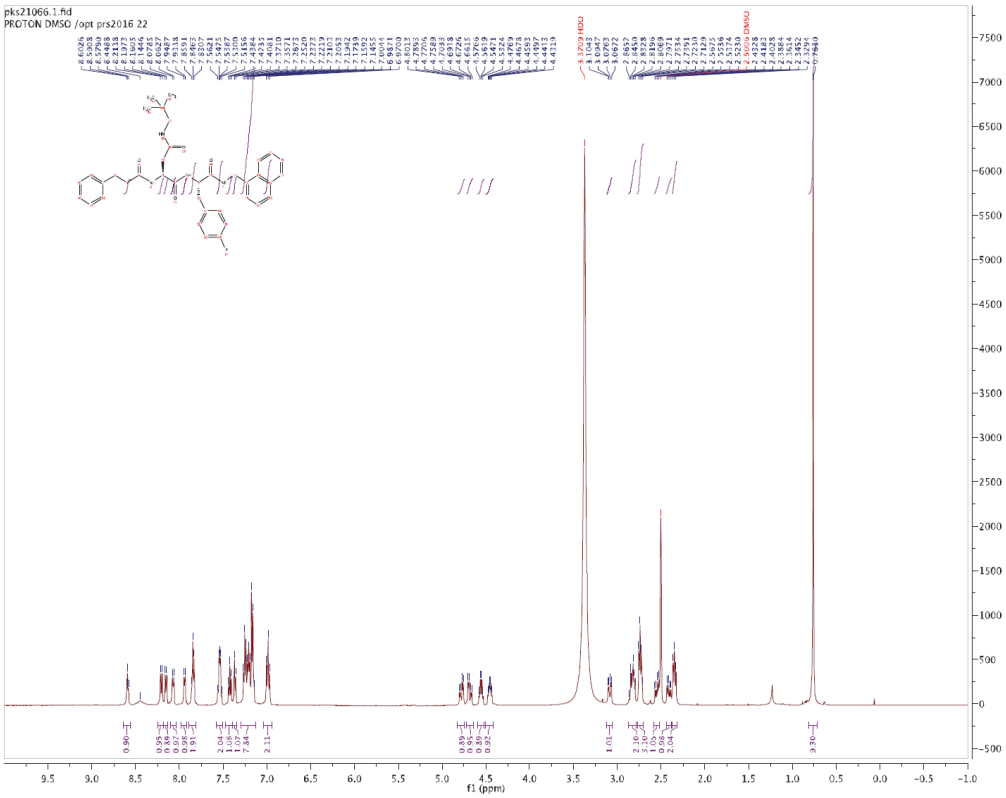
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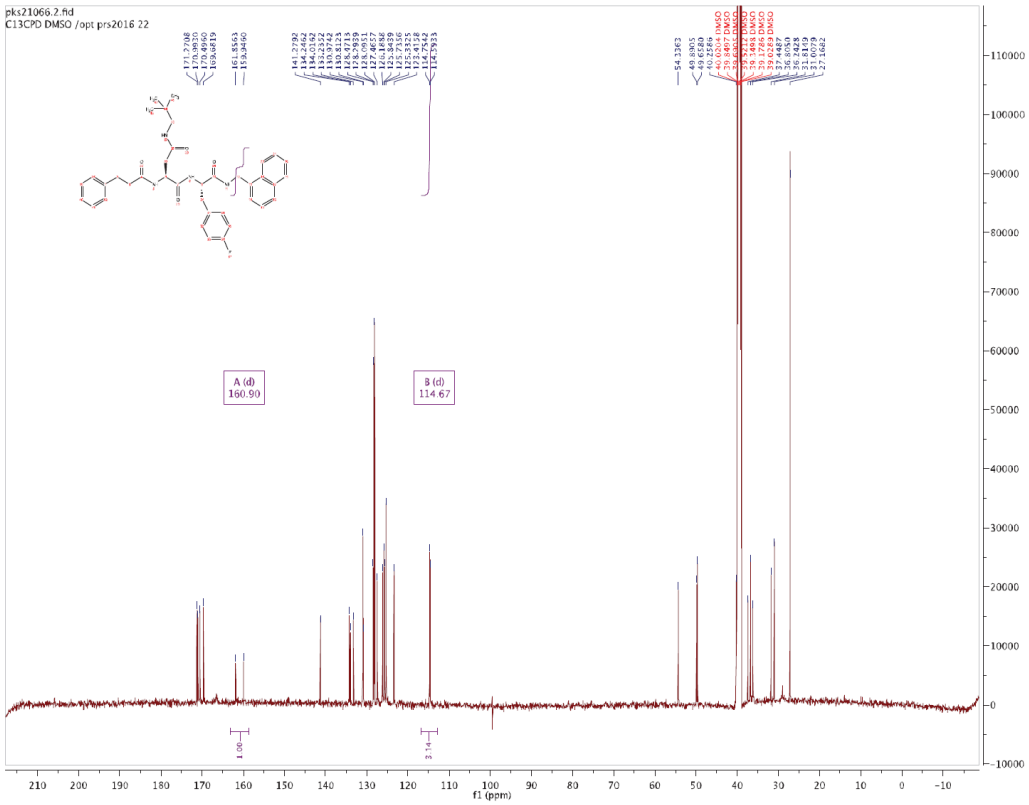
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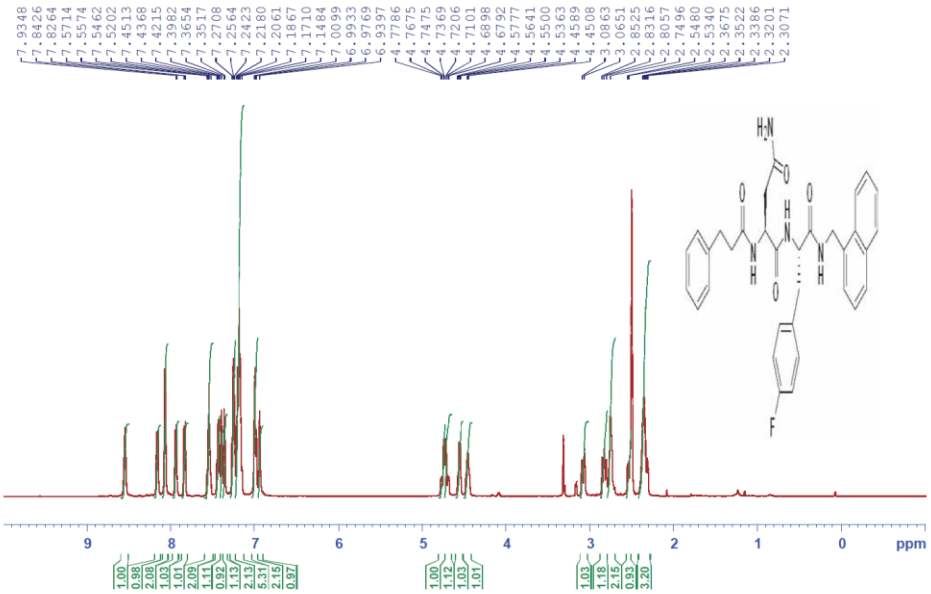
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O
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H
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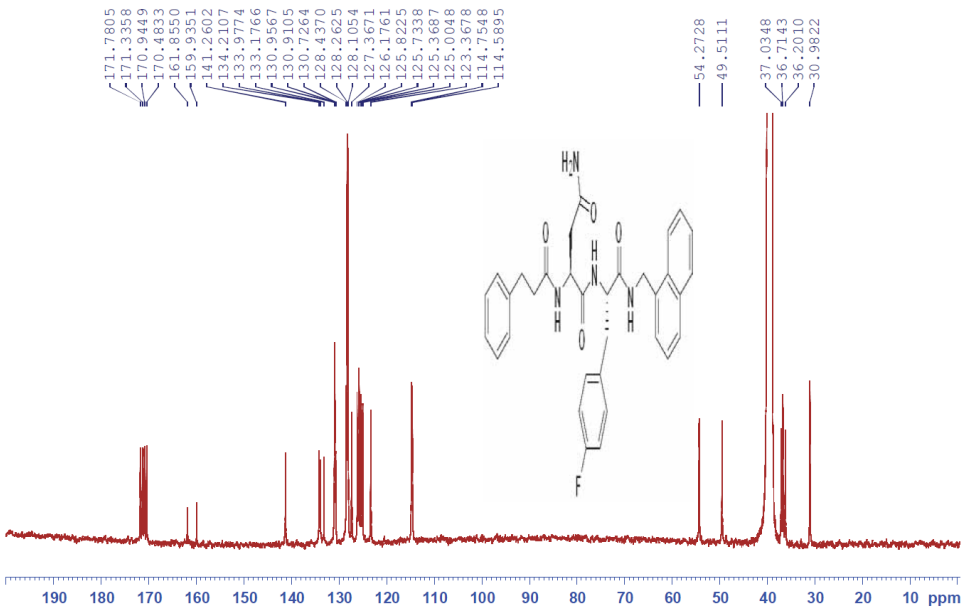
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110
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90
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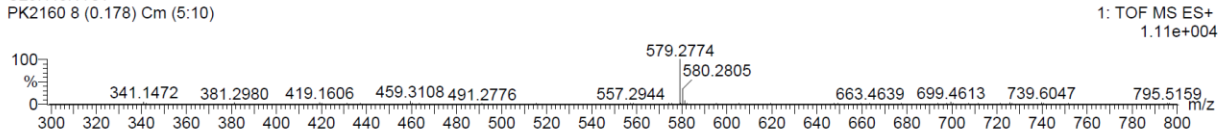
70
60
50
40
30
20
10
ppm

HRMS

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

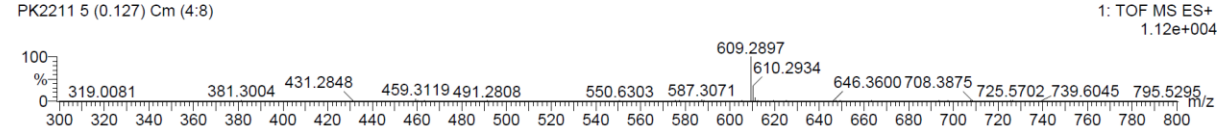
Monoisotopic Mass, Even Electron Ions
89 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-32 H: 0-41 N: 0-5 O: 0-7 Na: 0-1
C29H40N4O7
PK2160 8 (0.178) Cm (5:10)



Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
579.2774	579.2795	-2.1	-3.6	11.5	5.9	C29 H40 N4 O7 Na

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

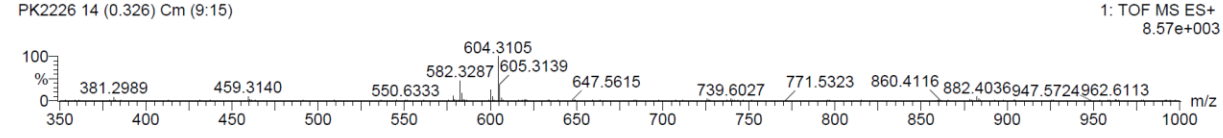
Monoisotopic Mass, Even Electron Ions
98 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-30 H: 0-42 N: 0-5 O: 0-8 Na: 0-1
C30H42N4O8
PK2211 5 (0.127) Cm (4:8)



Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
609.2897	609.2900	-0.3	-0.5	11.5	5.5	C30 H42 N4 O8 Na

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
97 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-31 H: 0-43 N: 0-5 O: 0-8 Na: 0-1
C31H43N5O6
PK2226 14 (0.326) Cm (9:15)



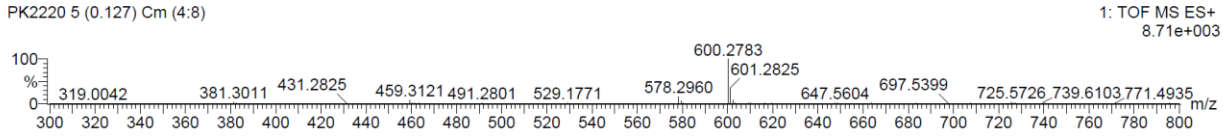
Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
604.3105	604.3111	-0.6	-1.0	12.5	13.0	C31 H43 N5 O6 Na

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
95 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-31 H: 0-42 N: 0-5 O: 0-8 Na: 0-1

C31H39N5O6
PK2220 5 (0.127) Cm (4:8)



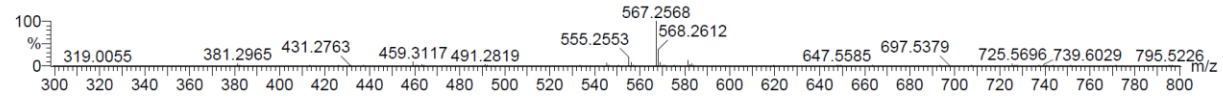
Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
600.2783	600.2798	-1.5	-2.5	14.5	2.1	C31 H39 N5 O6 Na

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
57 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-33 H: 0-41 N: 0-5 O: 0-5 Na: 0-1

C31H36N4O5
PK2209 6 (0.144) Cm (5:9)



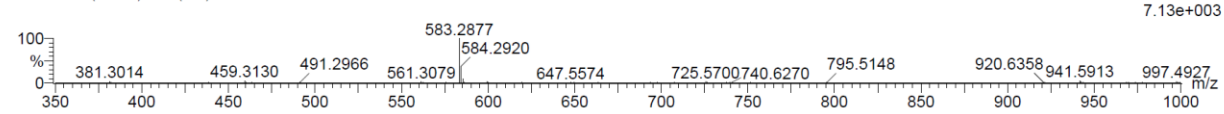
Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
567.2568	567.2583	-1.5	-2.6	15.5	2.4	C31 H36 N4 O5 Na

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
80 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-32 H: 0-40 N: 0-4 O: 0-8 Na: 0-1

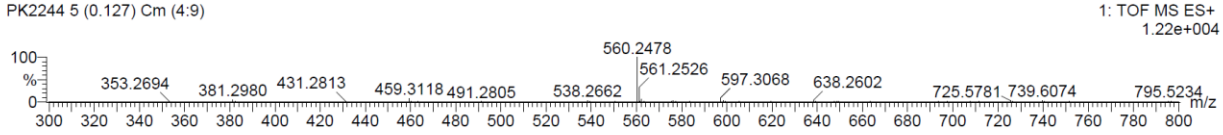
C32H40N4O5
PK2230 6 (0.144) Cm (5:8)



Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
583.2877	583.2896	-1.9	-3.3	14.5	3.6	C32 H40 N4 O5 Na

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

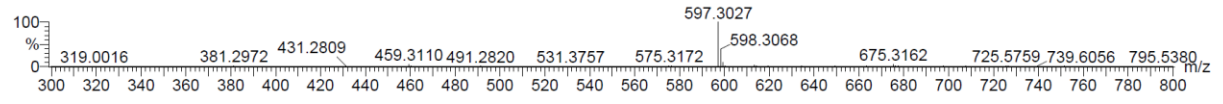
Monoisotopic Mass, Even Electron Ions
89 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-33 H: 0-42 N: 0-5 O: 0-6 Na: 0-1
C28H35N5O6
PK2244 5 (0.127) Cm (4:9)



Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
560.2478	560.2485	-0.7	-1.2	13.5	2.3	C28 H35 N5 O6 Na

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

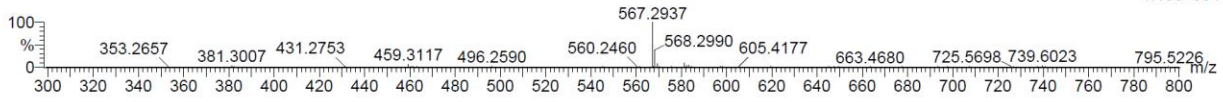
Monoisotopic Mass, Even Electron Ions
52 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-33 H: 0-42 N: 0-4 O: 0-5 Na: 0-1
C33H42N4O5
PK2243 14 (0.326) Cm (11:16)



Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
597.3027	597.3053	-2.6	-4.4	14.5	6.0	C33 H42 N4 O5 Na

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
79 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-33 H: 0-42 N: 0-5 O: 0-6 Na: 0-1
C32H40N4O4
PK2255 6 (0.144) Cm (4:7)



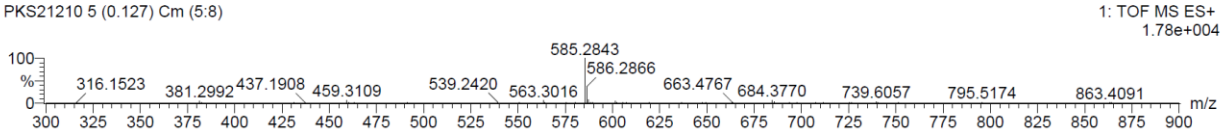
Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
567.2937	567.2947	-1.0	-1.8	14.5	0.4	C32 H40 N4 O4 Na

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
89 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-32 H: 0-39 N: 0-4 O: 0-4 F: 0-1 Na: 0-1

C32H39FN4O4
PKS21210 5 (0.127) Cm (5:8)



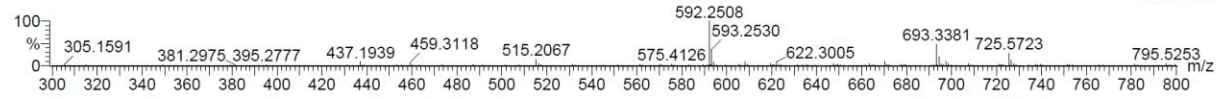
Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
585.2843	585.2853	-1.0	-1.7	14.5	5.3	C32 H39 N4 O4 F Na

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
63 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-32 H: 0-41 N: 0-5 O: 0-5 Na: 0-1

C32H35N5O5
PK3053 26 (0.598) Cm (25:27)



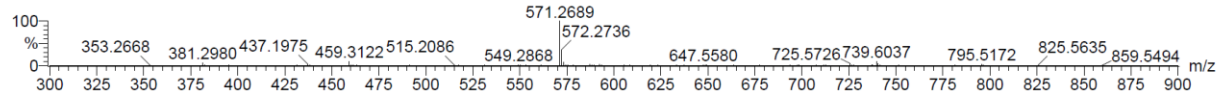
Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
592.2508	592.2536	-2.8	-4.7	17.5	18.9	C32 H35 N5 O5 Na

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
143 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-31 H: 0-39 N: 0-5 O: 0-6 F: 0-1 Na: 0-1

C31H37FN4O4
PKS21271 8 (0.179) Cm (5:8)

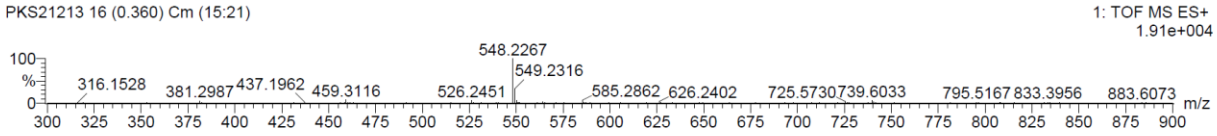


Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
571.2689	571.2697	-0.8	-1.4	14.5	1.1	C31 H37 N4 O4 F Na

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
134 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-27 H: 0-32 N: 0-5 O: 0-5 F: 0-1 Na: 0-1

C27H32FN5O5
PKS21213 16 (0.360) Cm (15:21)

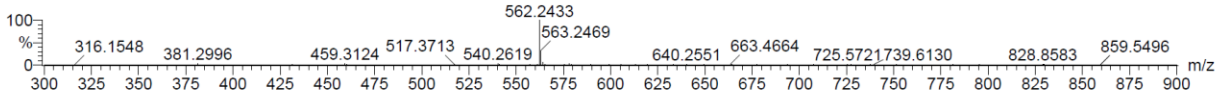


Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
548.2267	548.2285	-1.8	-3.3	13.5	4.5	C27 H32 N5 O5 F Na

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
151 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-28 H: 0-39 N: 0-5 O: 0-6 F: 0-1 Na: 0-1

C28H34FN5O5
PKS21272 16 (0.360) Cm (13:16)

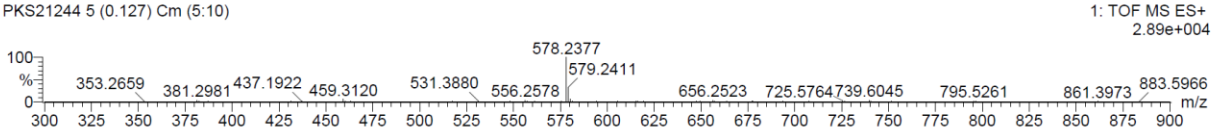


Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
562.2433	562.2442	-0.9	-1.6	13.5	1.1	C28 H34 N5 O5 F Na

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
158 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-28 H: 0-34 N: 0-5 O: 0-6 F: 0-1 Na: 0-1

C28H34FN5O6
PKS21244 5 (0.127) Cm (5:10)

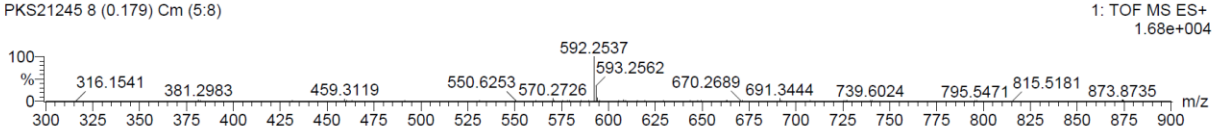


Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
578.2377	578.2391	-1.4	-2.4	13.5	8.4	C28 H34 N5 O6 F Na

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
158 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-29 H: 0-36 N: 0-5 O: 0-6 F: 0-1 Na: 0-1

C29H36FN5O6
PKS21245 8 (0.179) Cm (5:8)

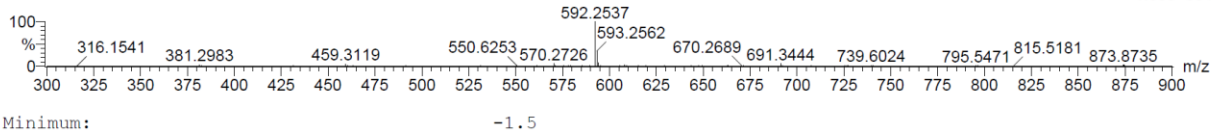


Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
592.2537	592.2547	-1.0	-1.7	13.5	3.0	C29 H36 N5 O6 F Na

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
158 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-29 H: 0-36 N: 0-5 O: 0-6 F: 0-1 Na: 0-1

C29H36FN5O6
PKS21245 8 (0.179) Cm (5:8)

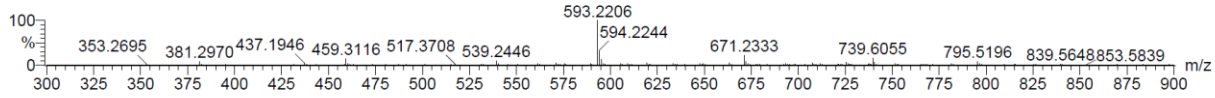


Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
592.2537	592.2547	-1.0	-1.7	13.5	3.0	C29 H36 N5 O6 F Na

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
229 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-29 H: 0-35 N: 0-4 O: 0-5 F: 0-1 Na: 0-1 S: 0-1

C29H35FN4O5S
PKS21206 13 (0.309) Cm (13:18)

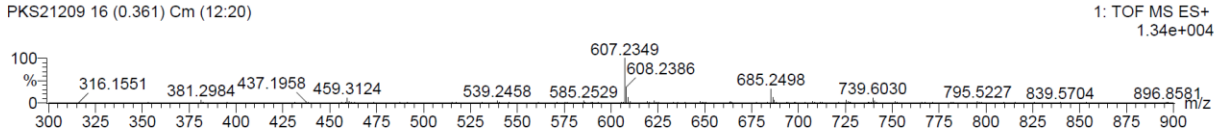


Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
593.2206	593.2210	-0.4	-0.7	13.5	5.1	C29 H35 N4 O5 F Na S

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
229 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-30 H: 0-37 N: 0-4 O: 0-5 F: 0-1 Na: 0-1 S: 0-1

C30H37FN4O5S
PKS21209 16 (0.361) Cm (12:20)

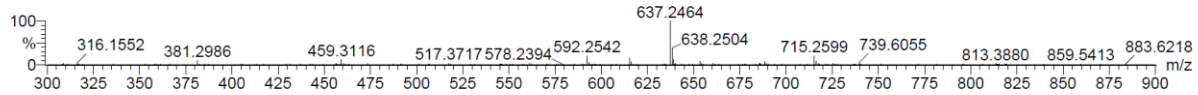


Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
607.2349	607.2366	-1.7	-2.8	13.5	2.0	C30 H37 N4 O5 F Na S

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
314 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-31 H: 0-39 N: 0-5 O: 0-6 F: 0-1 Na: 0-1 S: 0-1

C31H39FN4O6S
PKS21265 10 (0.235) Cm (9:15)

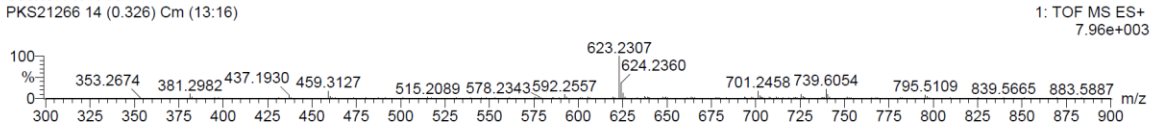


Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
637.2464	637.2472	-0.8	-1.3	13.5	0.4	C31 H39 N4 O6 F Na S

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
302 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-31 H: 0-39 N: 0-5 O: 0-6 F: 0-1 Na: 0-1 S: 0-1

C30H37FN4O6S
PKS21266 14 (0.326) Cm (13:16)

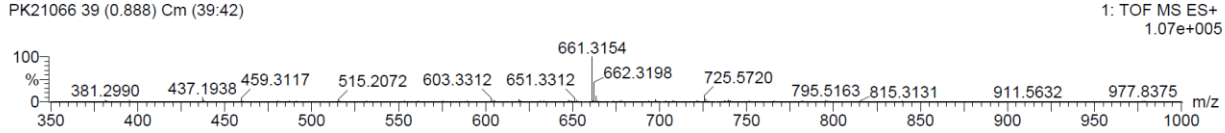


Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
623.2307	623.2316	-0.9	-1.4	13.5	1.4	C30 H37 N4 O6 F Na S

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
96 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-38 H: 0-45 N: 0-5 O: 0-4 F: 0-1 Na: 0-1

C38H43FN4O4
PK21066 39 (0.888) Cm (39:42)

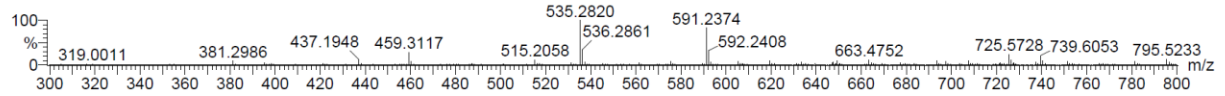


Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
661.3154	661.3166	-1.2	-1.8	18.5	83.7	C38 H43 N4 O4 F Na

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 110.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

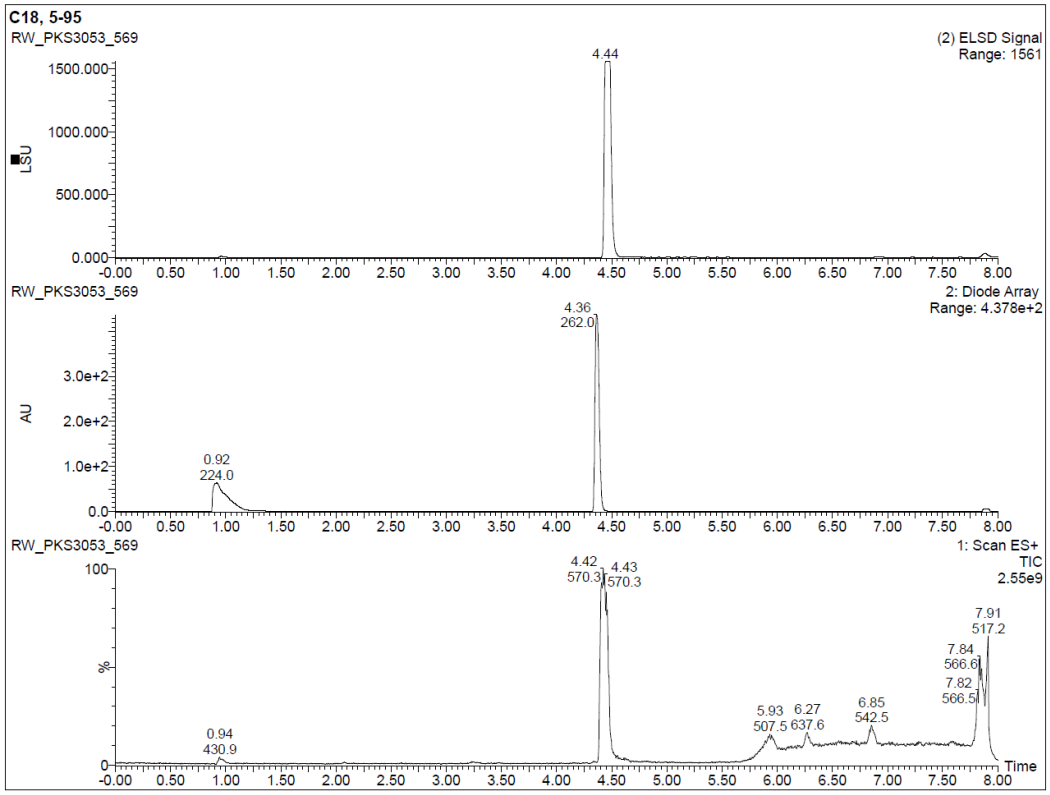
Monoisotopic Mass, Even Electron Ions
95 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 0-33 H: 0-33 N: 0-5 O: 0-4 F: 0-1 Na: 0-1

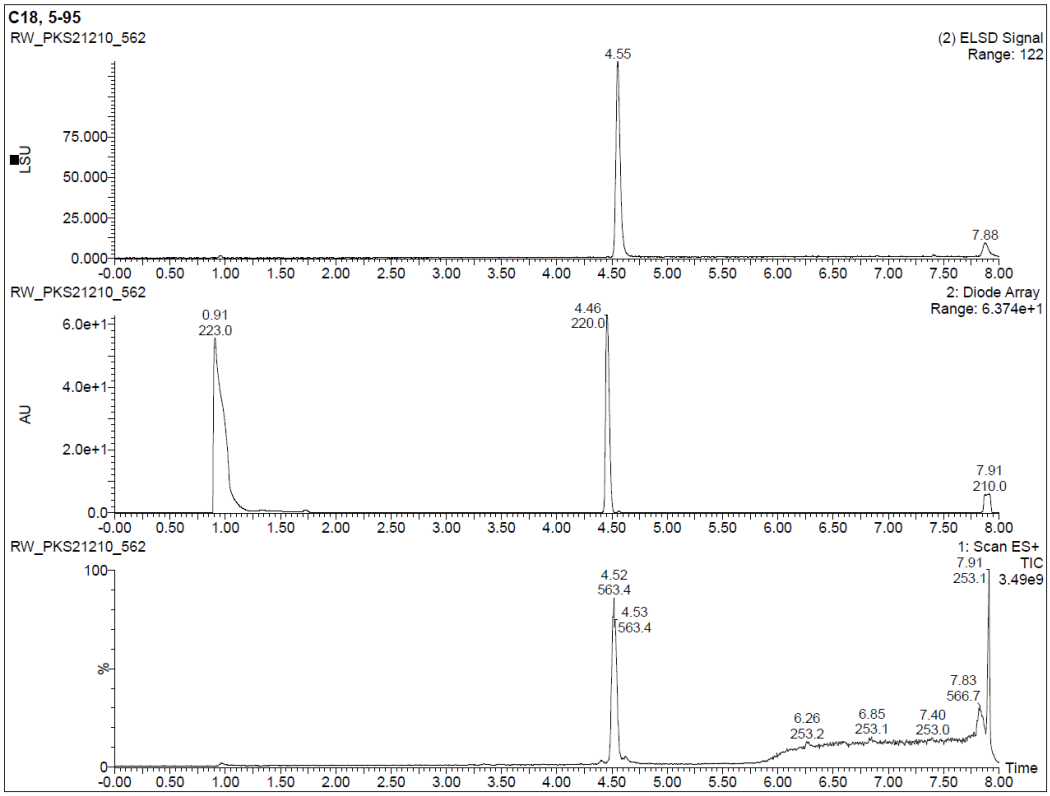
C33H33FN4O4
PK21083 28 (0.632) Cm (27:29)

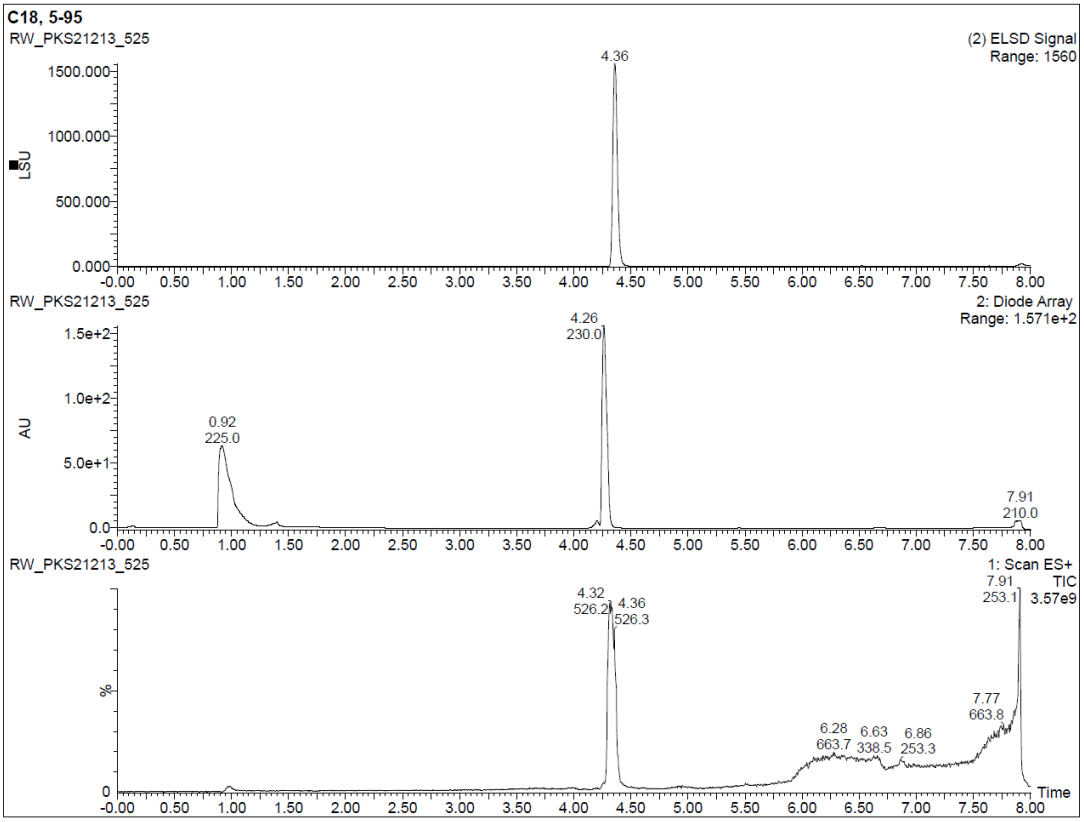


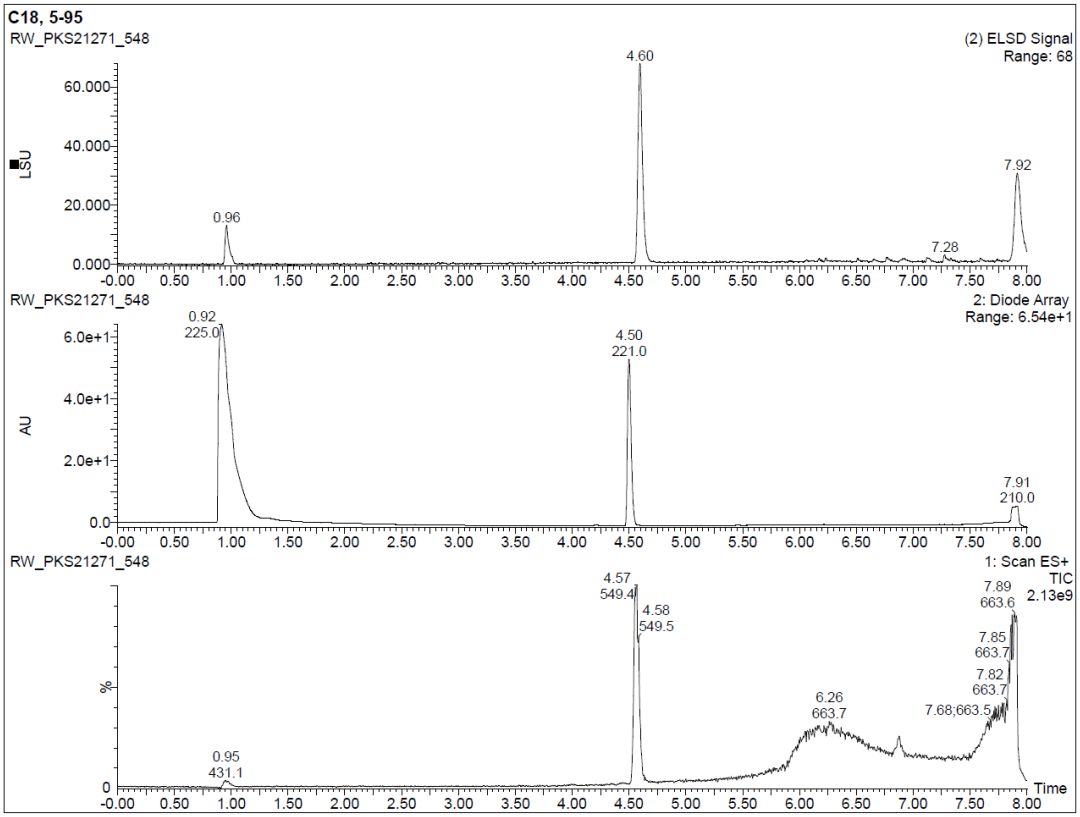
Minimum:				-1.5		
Maximum:		5.0	5.0	110.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
591.2374	591.2384	-1.0	-1.7	18.5	1.2	C33 H33 N4 O4 F Na

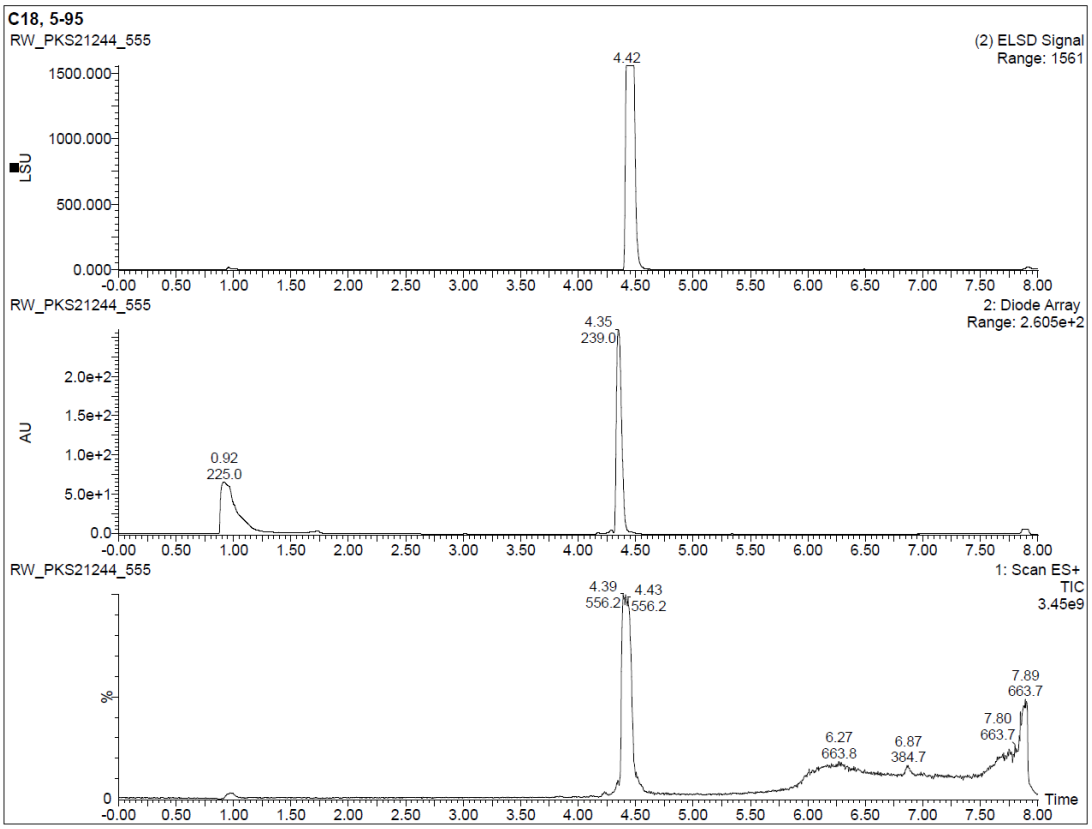
HPLC

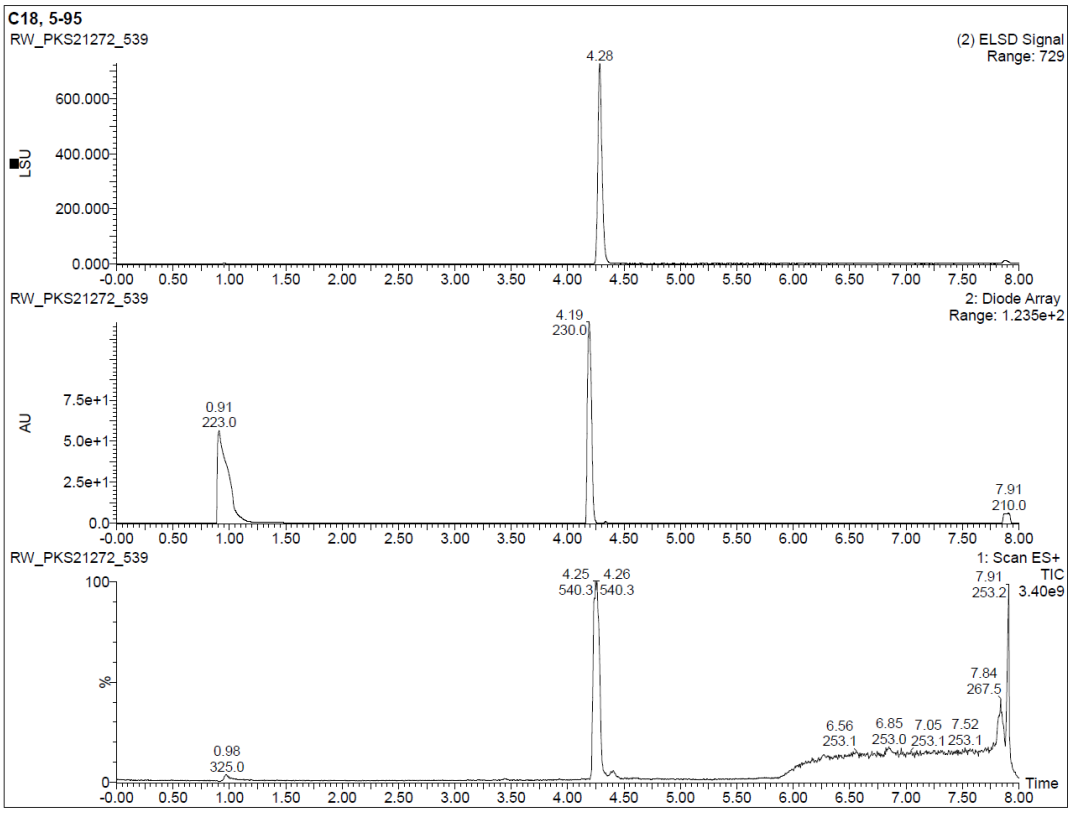

C18, 5-95
RW_PKS3053_569
(2) ELSD Signal
Range: 1561
4.44
LSU
RW_PKS3053_569
2: Diode Array
Range: 4.378e+2
4.36
262.0
0.92
224.0
AU
RW_PKS3053_569
1: Scan ES+
TIC
2.55e9
4.42
570.3
4.43
570.3
7.91
517.2
7.84
566.6
7.82
566.5
5.93
507.5
6.27
637.6
6.85
542.5
0.94
430.9
%
Time


C18, 5-95
RW_PKS21210_562
(2) ELSD Signal
Range: 122
4.55
7.88
LSU
RW_PKS21210_562
2: Diode Array
Range: 6.374e+1
0.91
223.0
4.46
220.0
7.91
210.0
AU
RW_PKS21210_562
1: Scan ES+
TIC
3.49e9
7.91
253.1
4.52
563.4
4.53
563.4
7.83
566.7
6.26
253.2
6.85
253.1
7.40
253.0
%
Time


C18, 5-95
RW_PKS21213_525
(2) ELSD Signal
Range: 1560
4.36
LSU
RW_PKS21213_525
2: Diode Array
Range: 1.571e+2
4.26
230.0
0.92
225.0
7.91
210.0
AU
RW_PKS21213_525
1: Scan ES+
TIC
3.57e9
4.32
526.2
4.36
526.3
7.91
253.1
7.77
663.8
6.28
663.7
6.63
338.5
6.86
253.3
%
Time


C18, 5-95
RW_PKS21271_548
(2) ELSD Signal
Range: 68
4.60
7.92
0.96
7.28
LSU
RW_PKS21271_548
2: Diode Array
Range: 6.54e+1
0.92
225.0
4.50
221.0
7.91
210.0
AU
RW_PKS21271_548
1: Scan ES+
TIC
2.13e9
4.57
549.4
4.58
549.5
7.89
663.6
7.85
663.7
7.82
663.7
7.68;663.5
6.26
663.7
0.95
431.1
%
Time


C18, 5-95
RW_PKS21244_555
(2) ELSD Signal
Range: 1561
4.42
LSU
RW_PKS21244_555
2: Diode Array
Range: 2.605e+2
4.35
239.0
0.92
225.0
AU
RW_PKS21244_555
1: Scan ES+
TIC
3.45e9
4.39
556.2
4.43
556.2
7.89
663.7
7.80
663.7
6.27
663.8
6.87
384.7
%
Time


C18, 5-95
RW_PKS21272_539
(2) ELSD Signal
Range: 729
4.28
LSU
RW_PKS21272_539
2: Diode Array
Range: 1.235e+2
4.19
230.0
0.91
223.0
7.91
210.0
AU
RW_PKS21272_539
1: Scan ES+
TIC
3.40e9
4.25
540.3
4.26
540.3
7.91
253.2
7.84
267.5
6.56
253.1
6.85
253.0
7.05
253.1
7.52
253.1
0.98
325.0
%
Time