SUPPORTING INFORMATION

Discovery of the first Vitamin K analog as a potential treatment of pharmacoresistant seizures

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Table S1. Compound **3l** in the acute, electrically evoked mouse seizure models.

Test	Dose	Time	N/T[a]
Rotarod (Tox)	100 mg/kg	0.25 hr	0/8
	100 mg/kg	0.5 hr	0/8
	100 mg/kg	1 hr	0/8
6 Hz 22 mA	100 mg/kg	0.25 hr	1/8
	100 mg/kg	0.5 hr	1/8
	100 mg/kg	1 hr	0/8
Rotarod (Tox)	200 mg/kg	0.25 hr	0/8
	200 mg/kg	0.5 hr	0/8
	200 mg/kg	1 hr	0/8
6 Hz 22 mA	200 mg/kg	0.25 hr	2/8
	200 mg/kg	0.5 hr	3/8
	200 mg/kg	1 hr	1/8
Rotarod (Tox)	400 mg/kg	0.5 hr	0/8
6 Hz 22 mA	400 mg/kg	0.5 hr	2/8

Note: [a]N = number of animals exhibiting toxicity or number of animals protected and T = number of animals tested

Table S2. Time course experiment and toxicity of **3d** in mouse.

Test	Dose	Time	N/T
Rotarod (Tox)	400 mg/kg	0.25 hr	1/8
6 Hz 32 mA	400 mg/kg	0.25 hr	7/8
Rotarod (Tox)	400 mg/kg	0.5 hr	2/8
6 Hz 32 mA	400 mg/kg	0.5 hr	7/8
Rotarod (Tox)	400 mg/kg	1 hr	1/8
6 Hz 32 mA	400 mg/kg	1 hr	8/8

Note: [a]N = number of animals exhibiting toxicity (Rotarod) or protected animals and T = number of animals tested.

Table S3. Brain/plasma concentration ratio of **2h**.

Compounds No.	Matrix	Time	Mean Tissue	Brain/plasma concentration ratio[a]
2h	Plasma	0.5 h	1014 ng/mL	0.325
	Brain	0.5 h	330 ng/g	

Note: **2h** was administrated by intravenous injection (iv) at the concentration of 5 mg/kg. Sample was obtained after 30 min of administration.

Table S4. Brain/plasma concentration ratio of **3d** in mouse and plasma.

Compounds No.	Matrix	Tested Concentration	% Free	% Bound
3d	Mouse plasma	1 μg/mL	1.55 ± 0.052	98.5 ± 0.052
	Human plasma	1 μg/mL	2.57 ± 0.332	97.4 ± 0.332
Warfarin	Mouse plasma	1 μg/mL	7.88 ± 0.56	92.1 ± 0.56
	Human plasma	1 μg/mL	0.058 ± 0.003	99.4 ± 0.003

Note: The assay was conducted with male mouse (CD-1) and human plasma. A Rapid Equilibrium Dialysis (RED) plate from Thermo Scientific was utilized to determine percentage of plasma protein binding. **3d** and positive control Warfarin were tested in duplicate at a final concentration of 1μg/ml. The plasma with K_2EDTA anticoagulant was used in the assay. The assay consisted of adding 100 μL of spiked plasma in the donor (red) sample chamber and applying 300 μL of PBS (pH = 7.4) buffer into the receiver chamber of the RED plate. The samples were then covered with aluminum sealing foil and incubated at 37°C for 4 hours on an orbital shaker at 200 rpm. After incubation, 25 μL of each buffer and plasma sample was placed into cluster tubes. In order to matrixmatch the samples, 25 μL of blank PBS was added to all of the respective plasma samples and 25 μL of blank plasma was applied to all of the buffer samples. Then 150 μL of ACN with 1.0 μg/ml propranolol (internal standard) was added to all samples. The samples were then centrifuged at 2000 g for 10 min prior to analysis of the supernatant by LC-MS/MS.

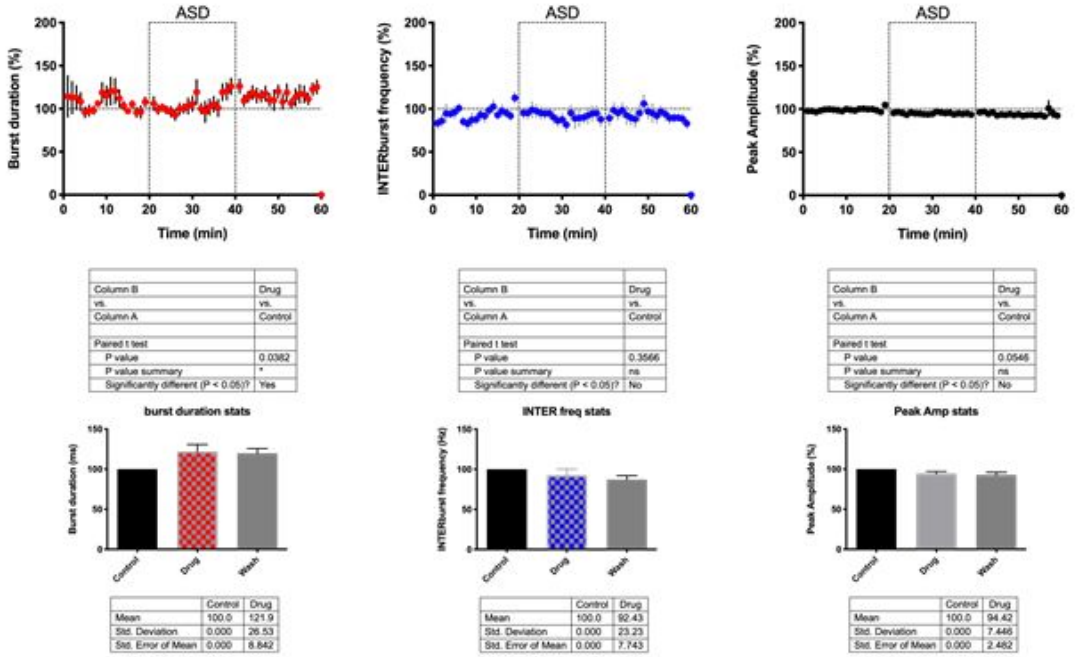


Figure S1. Independent recordings were made in 9 brain slices in this assay, and the average of the data recorded from these slices are presented here. 10 μM compound **3d** failed to significantly alter the burst frequency or amplitude of recurrent epileptiform discharges (REDs) in this assay. The small significant increase of burst duration is not likely to be biologically significant and is more likely due to a small, brief, non-specific change that is not due to compound **3d**. Methods: Horizontal brain slices containing the medial entorhinal cortex (mEC) were prepared form kainite-induced status epilepticus modle of temporal lobe epilepsy rats. REDs were recorded from the superficial layers. Compound **3d** was treated and the effects on the REDs' burst duration, frequency, and peak amplitude were measured. Detailed methods are described in previous study.[1]

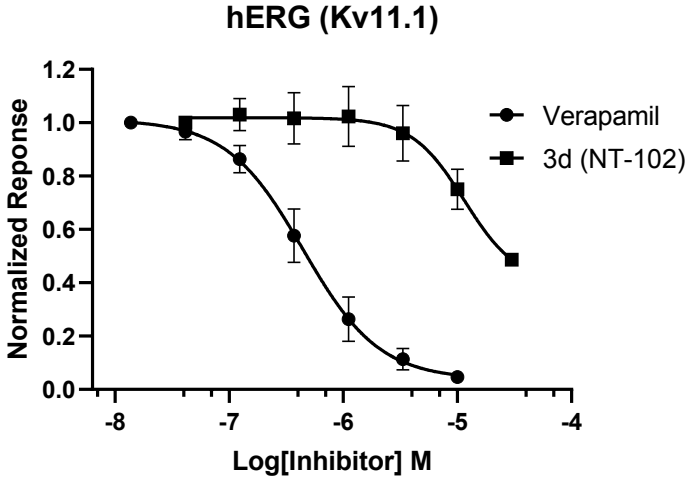


Figure S2. IC_{50} value of **3d** and positive control Verapamil against hERG. hERG inhibitory IC_{50} of **3d** is approximately 30 μM comparing to the positive control Verapamil at 400 nM. Methods: 1.5×10^5 HEK-293 cells stably expressing the hERG channel were plated onto sterile

glass coverslipsin 35 mm^2 dishes. The dishes were stored in at 37 °C in 5% CO_2 until use. Manual patch clamp technique was used to assess the effect on hERG currents in the whole-cell configuration at 35 ± 2 °C. Corresponding vehicle for all concentrations was 0.1% DMSO and was investigated in 3 cells. Compound **3d** was tested in 6-concentration IC_{50} mode, average IC_{50} (n = 3 cells) with 3-fold serial dilution starting at 30 µM. The positive control Verapamil was tested in 6-concentration IC_{50} mode, average IC_{50} (n = 3 cells) with 3-fold dilution starting at 10 µM. Cells were exposed to the test item for approximately 10 min of hERG tail currents were elicited by voltage jumps from -75 mV to 10 mV (500 ms) and then to -40 mV (500 ms) at 0.1 Hz. The composition of the external solution was (in mM): 130 NaCl, 10 HEPES, 5 KCl, 1 $MgCl_2$*$6H_2O$, 1 $CaCl_2$*H_2O, 12.5 dextrose; pH adjusted to 7.4 with 5 M NaOH; ~280 mOsM. The composition of the internal solution was (in mM): 120 K-gluconate, 20 KCl, 10 HEPES, 5 EGTA, 1.5 MgATP; pH adjusted to 7.3 with 1 M KOH; ~280 mOsM. Data capturing and analysis was performed by using Pulse (Heka Electronics, Germany) and Excel. IC_{50} values were calculated based on a sigmoidal does-response model with variable slope.[2]

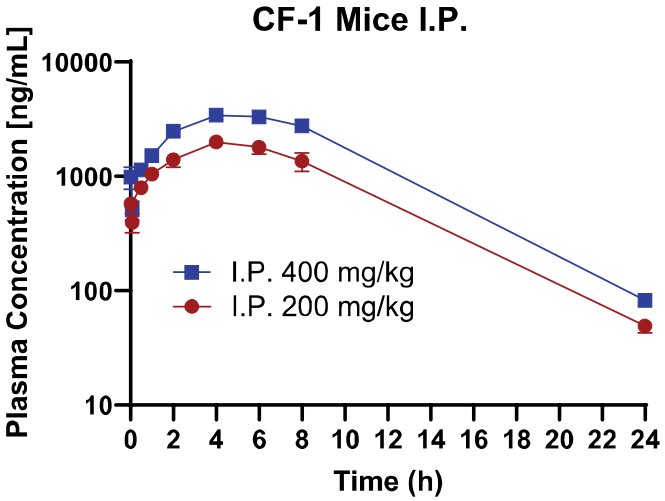


Figure S3. Pharmacokinetic data of **3d** at concentration of 400 mg/kg and 200 mg/kg with ip administration in Male CF-1 Mice. The dose formulation is 95% Miglyol 840: 5% DMSO. C_{max} of 400 mg/kg administration is 3440 ng/mL (14.1 µM), C_{max} of 200 mg/kg administration is 2007 ng/mL (8.2 µM). As brain tissue binding ratio of **3d** is 98.5% (data not shown), the free EC_{50} in mice is approximately 123 nM.

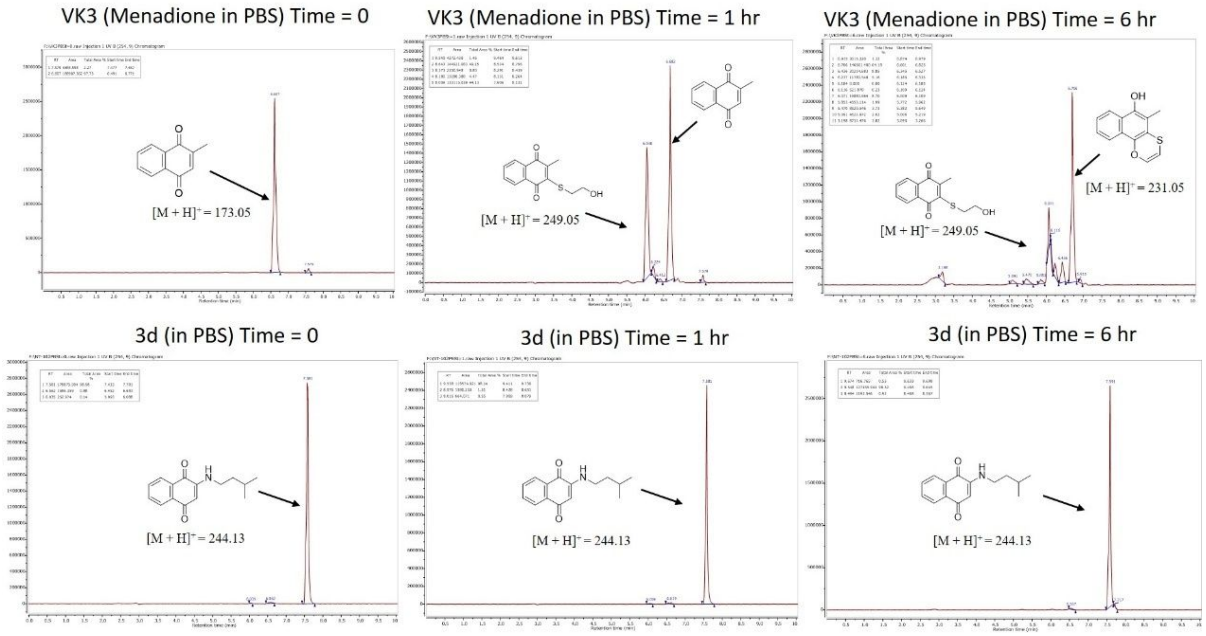
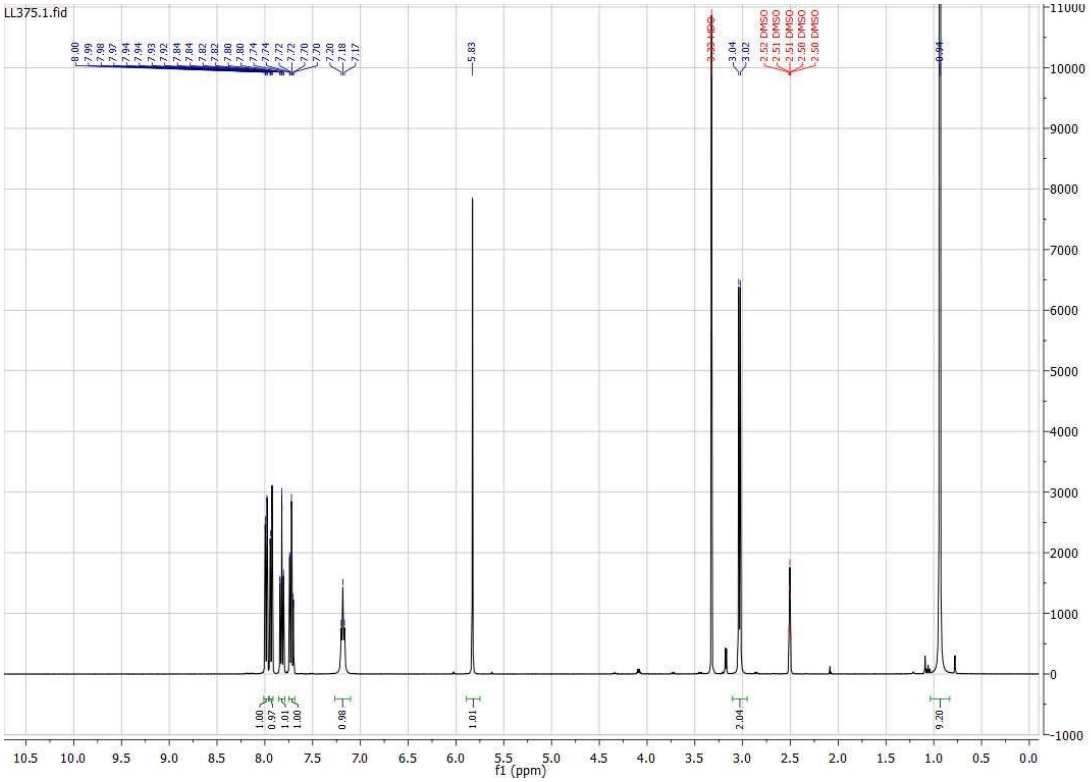


Figure S4. VK3 and **3d** were subjected with 2-mercaptoethanol. VK3 rapidly reacts with 2-mercaptoethanol, while our lead compound **3d** does not react with thiol at all over a 6 h period.



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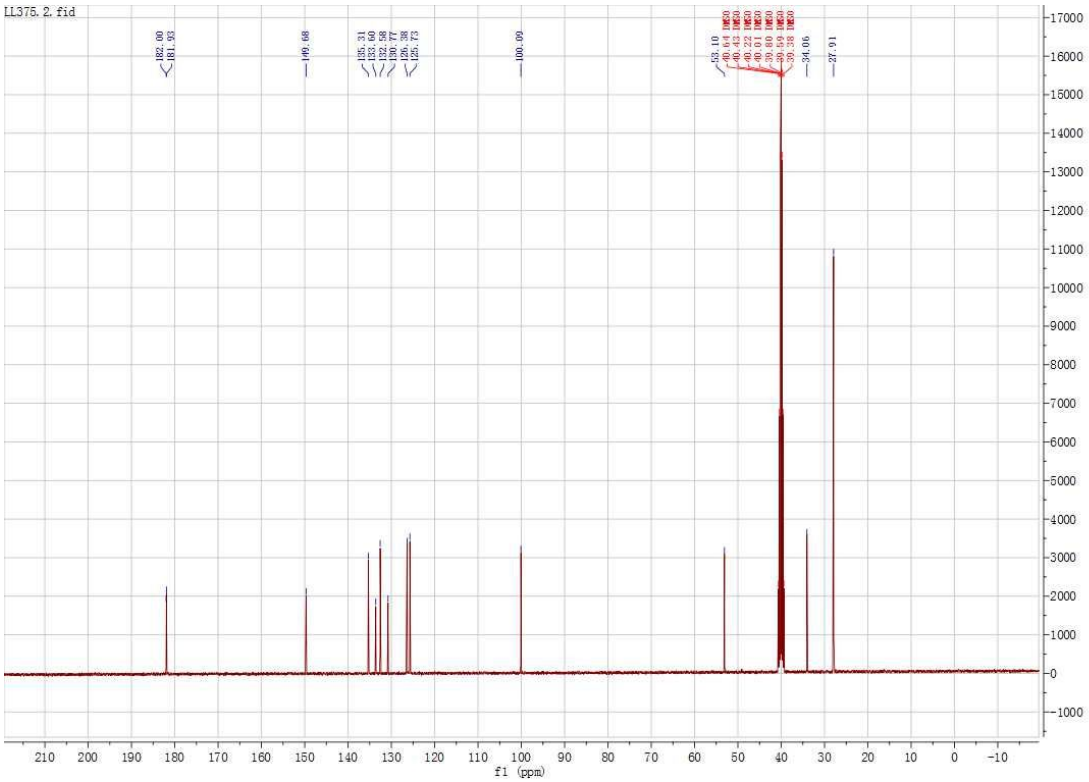
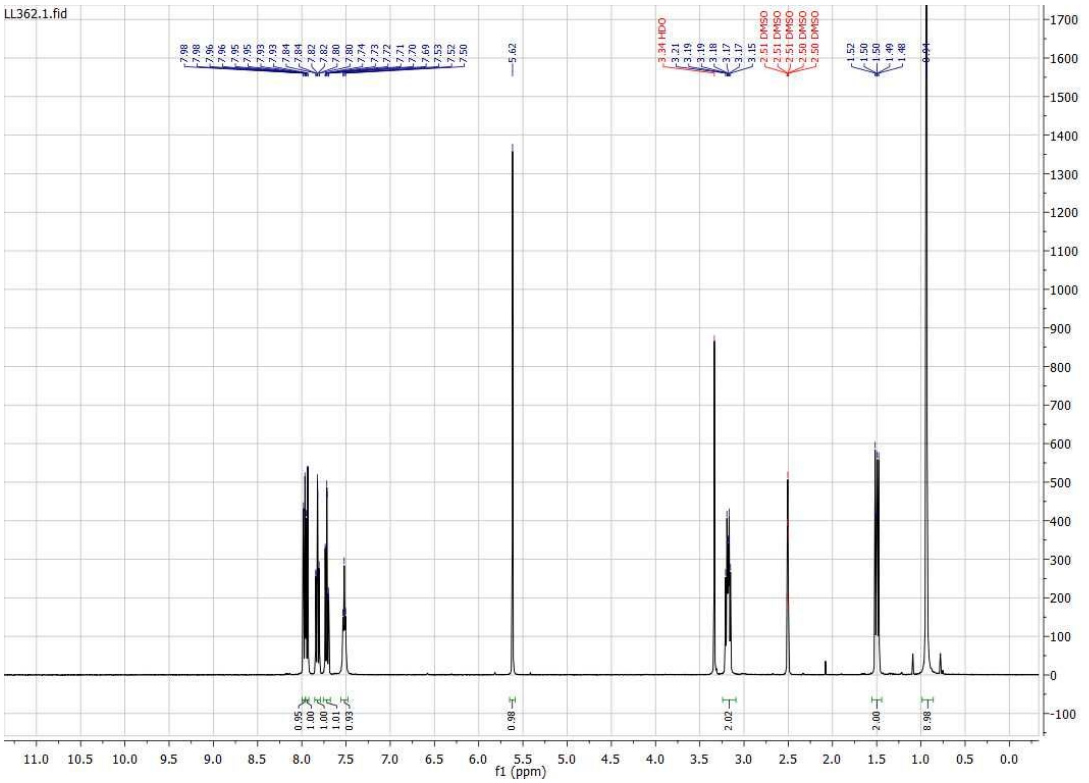


^{13}C NMR of 3a



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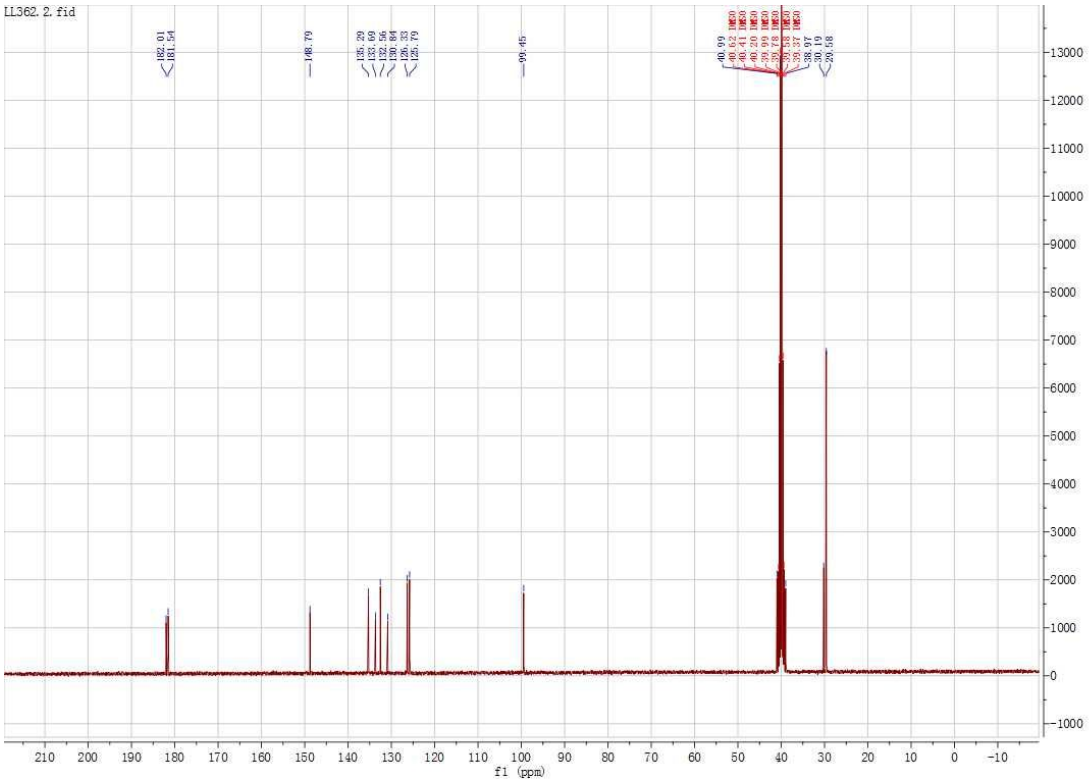

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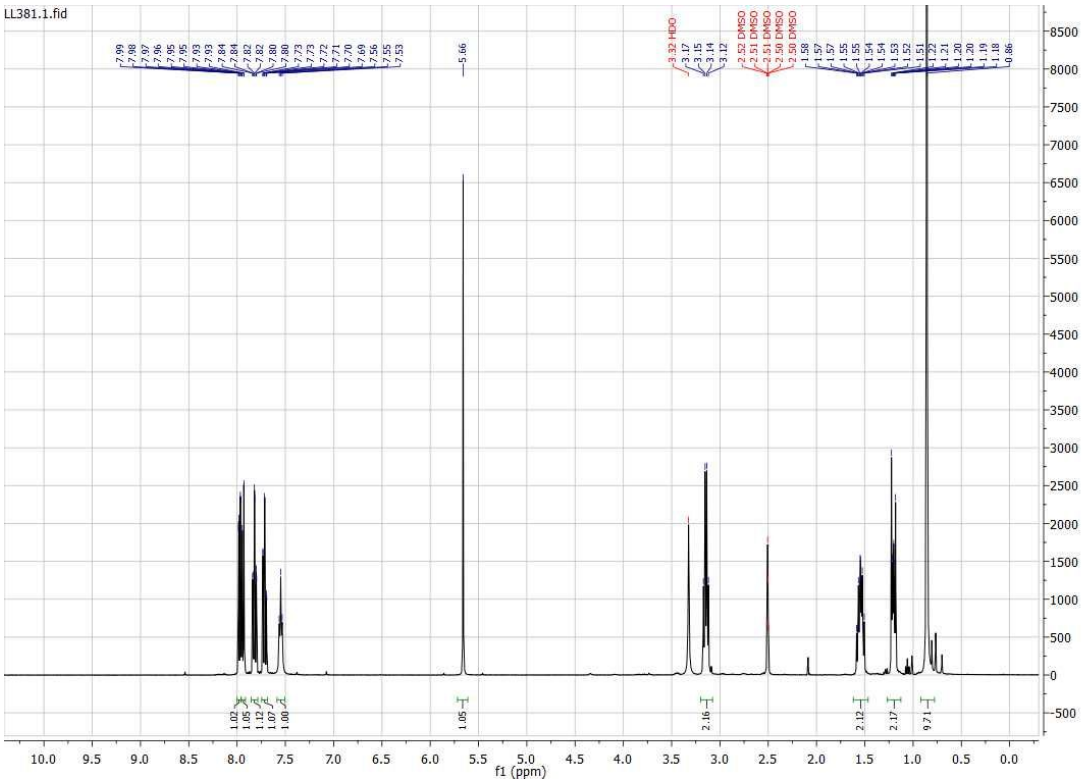

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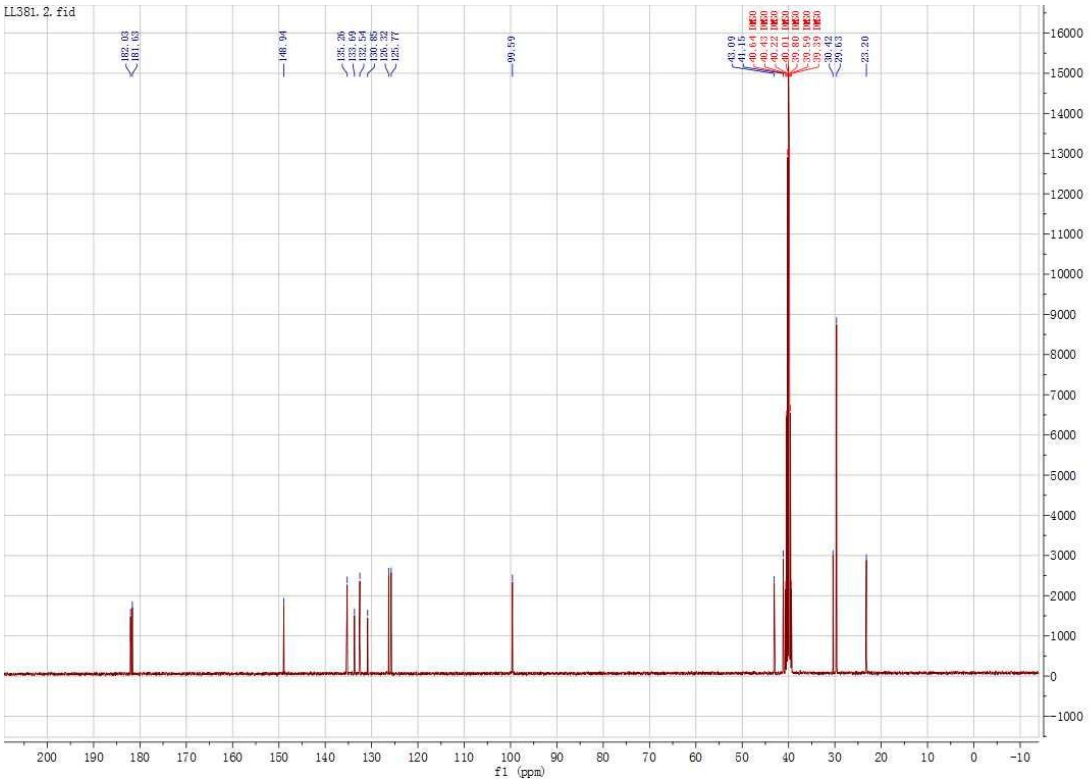

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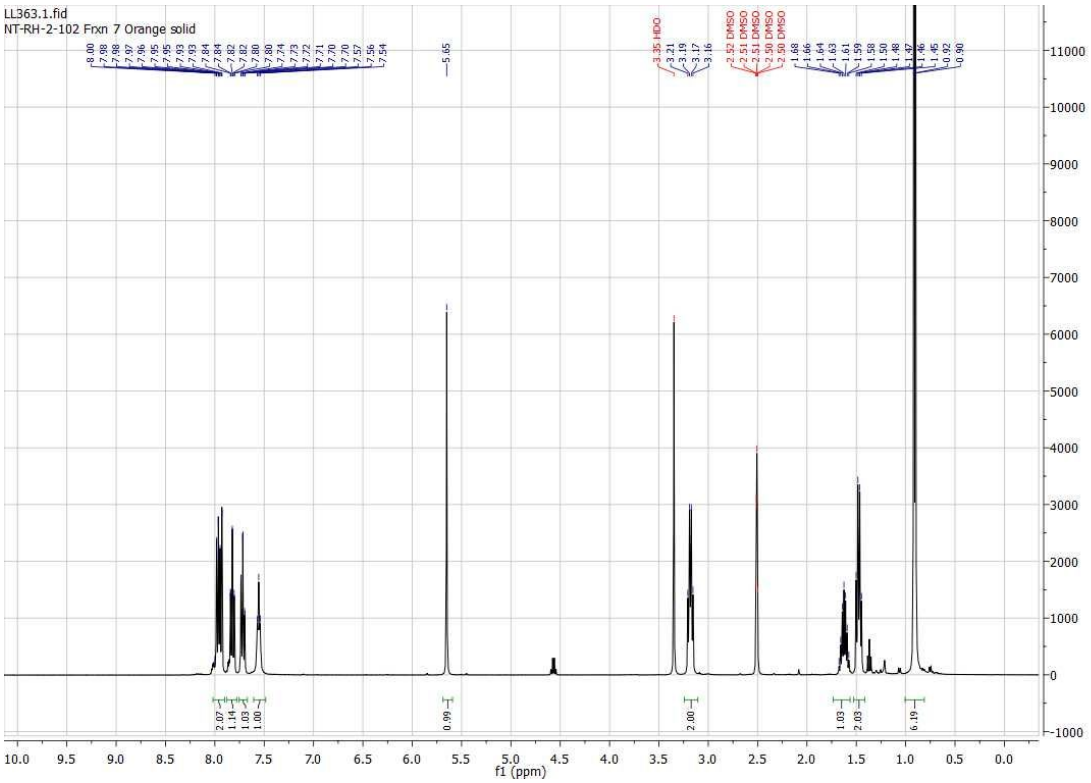

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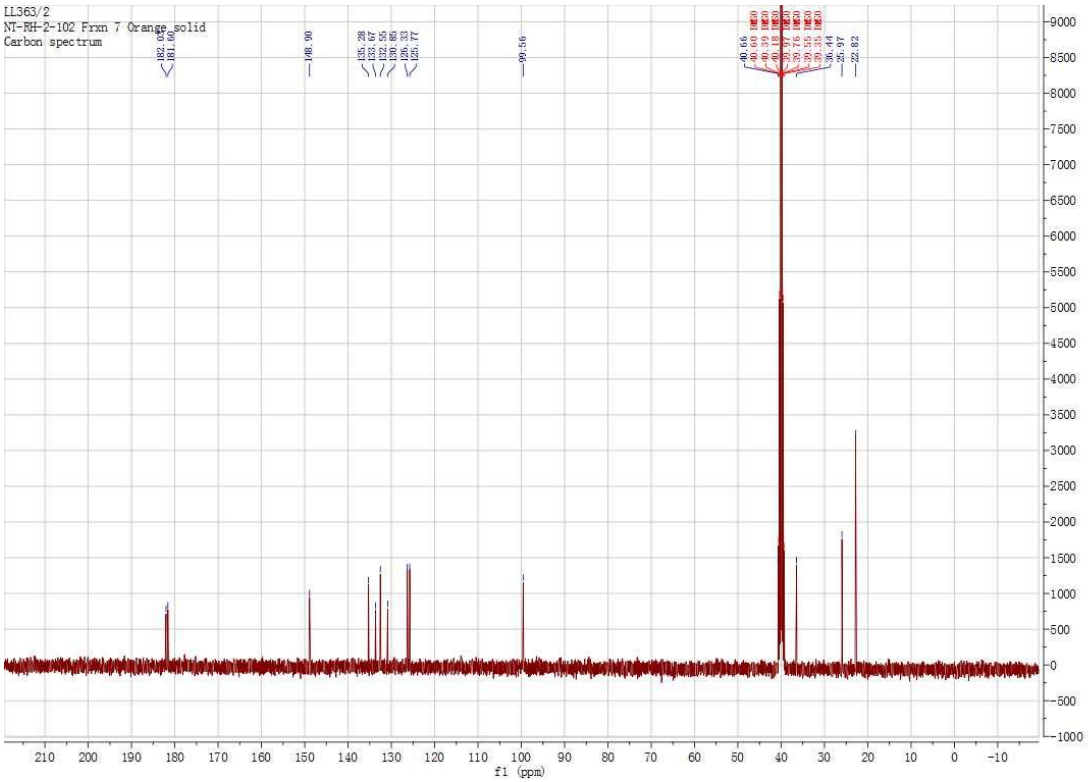

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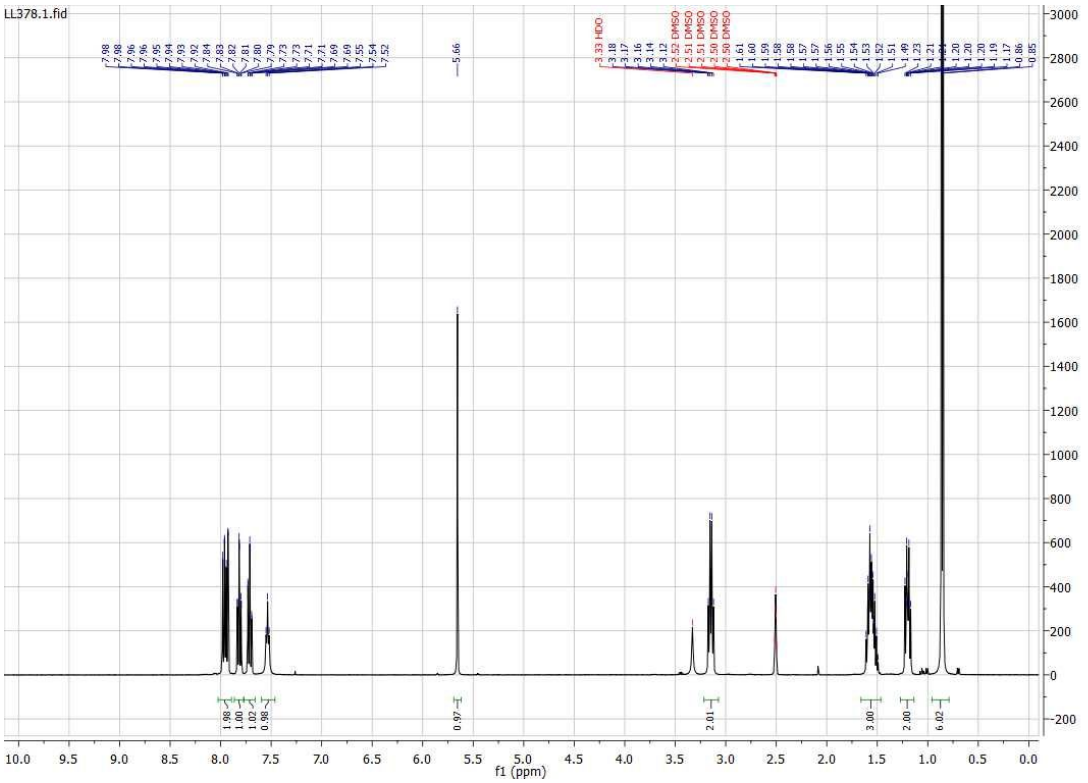

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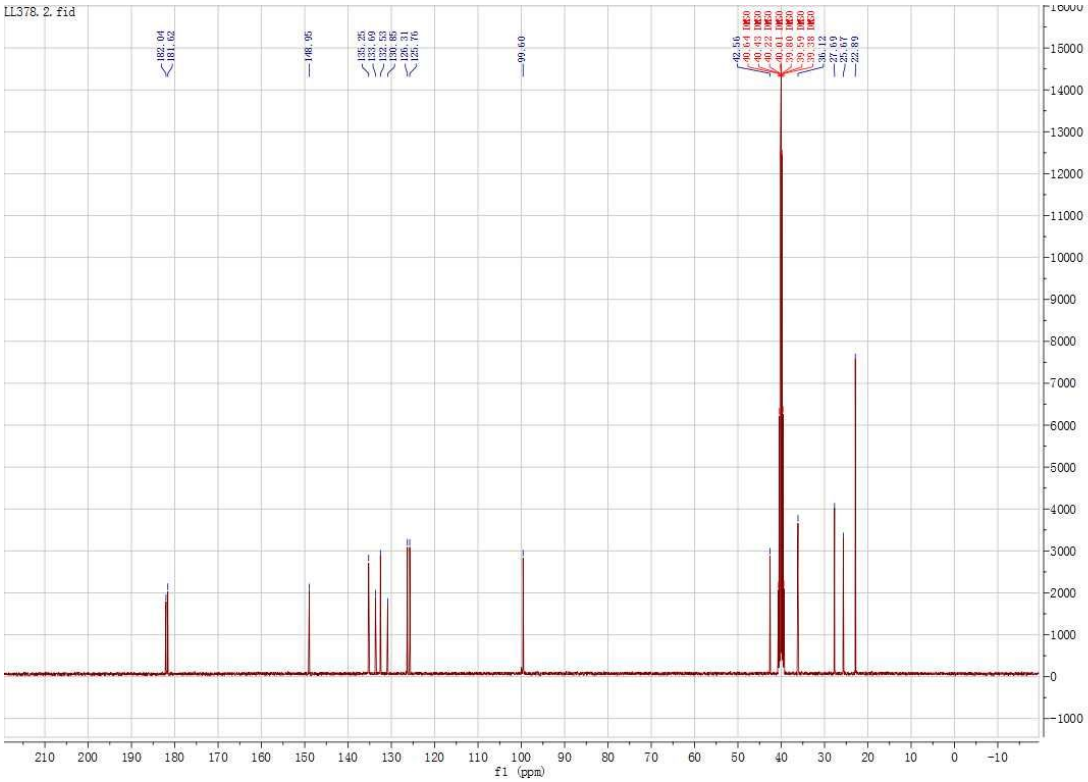

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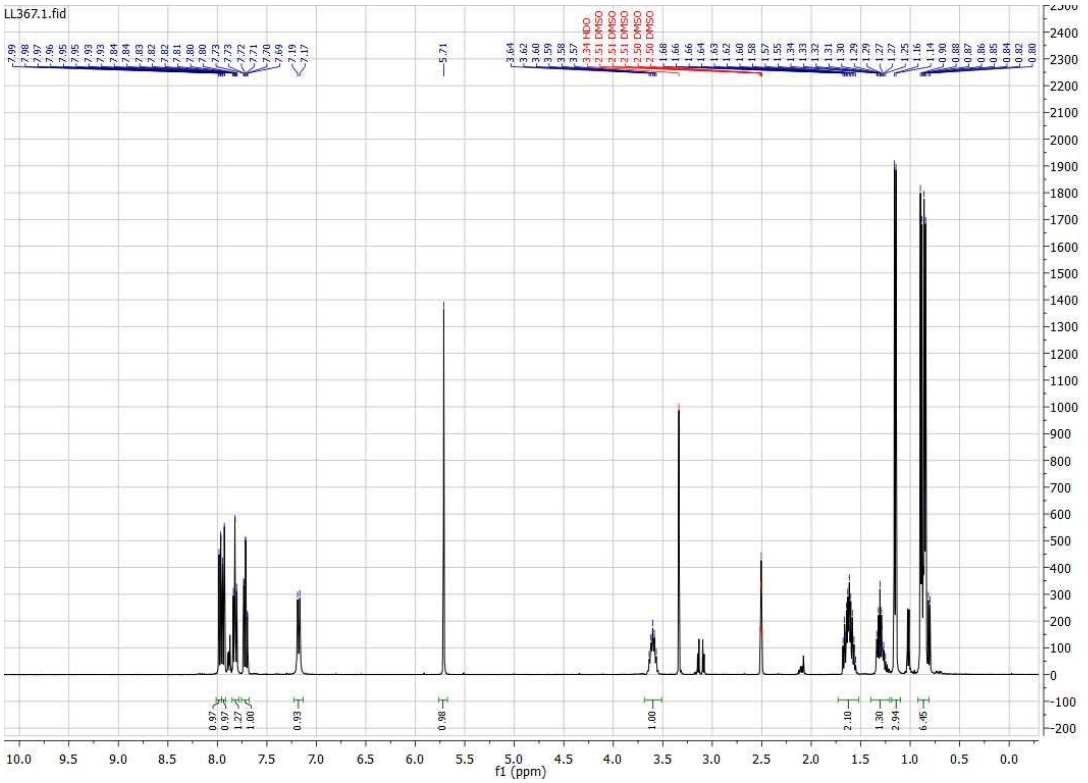

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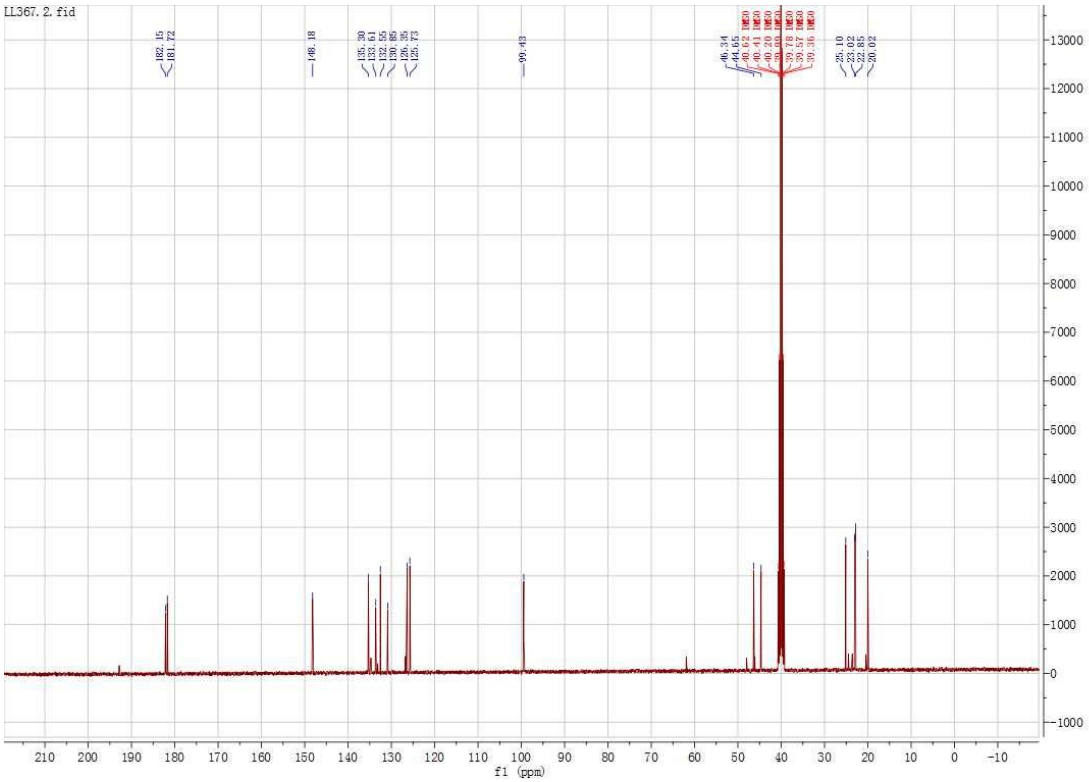

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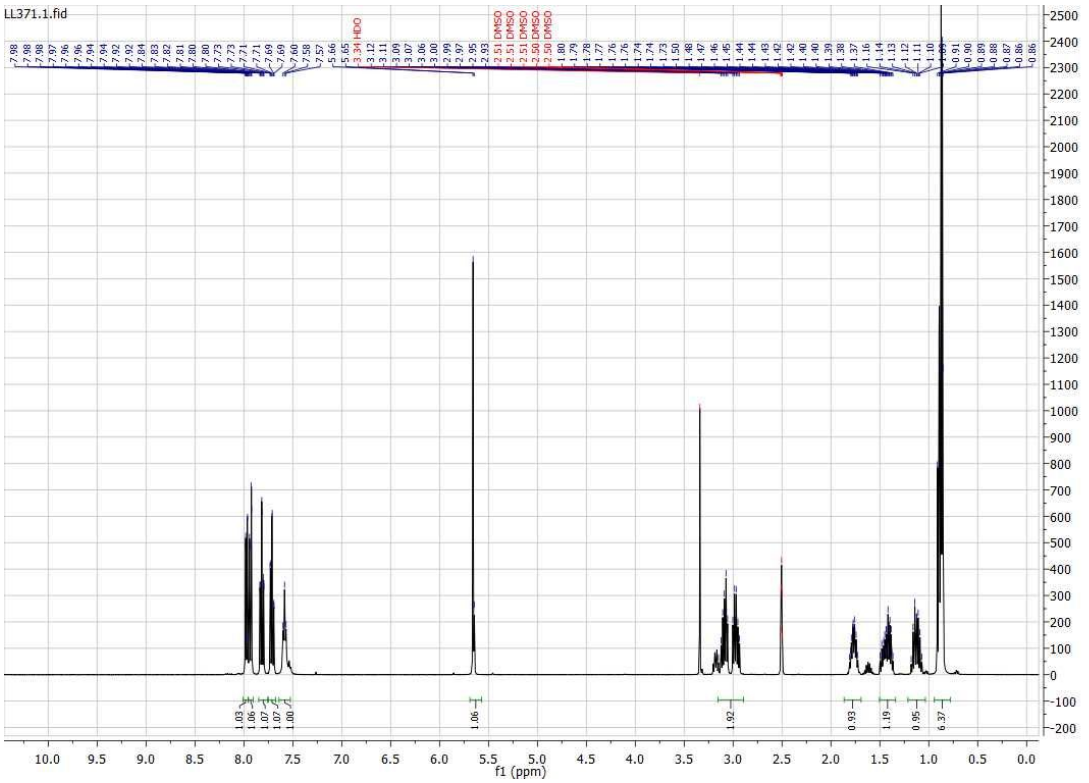

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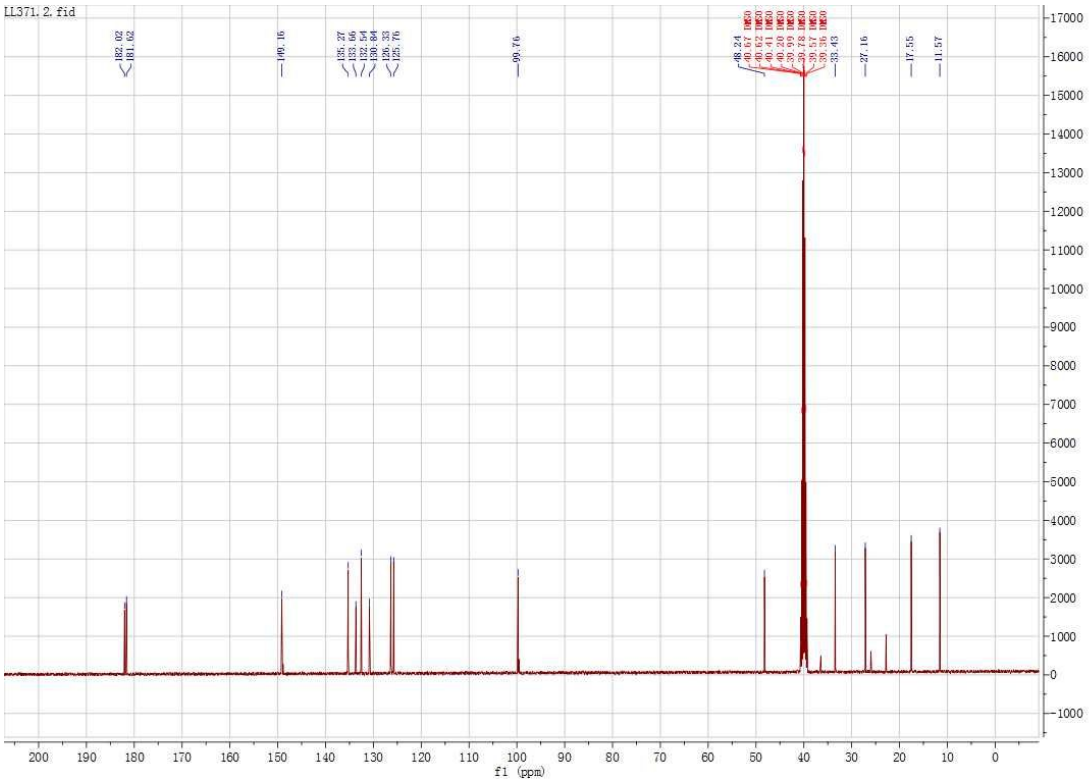

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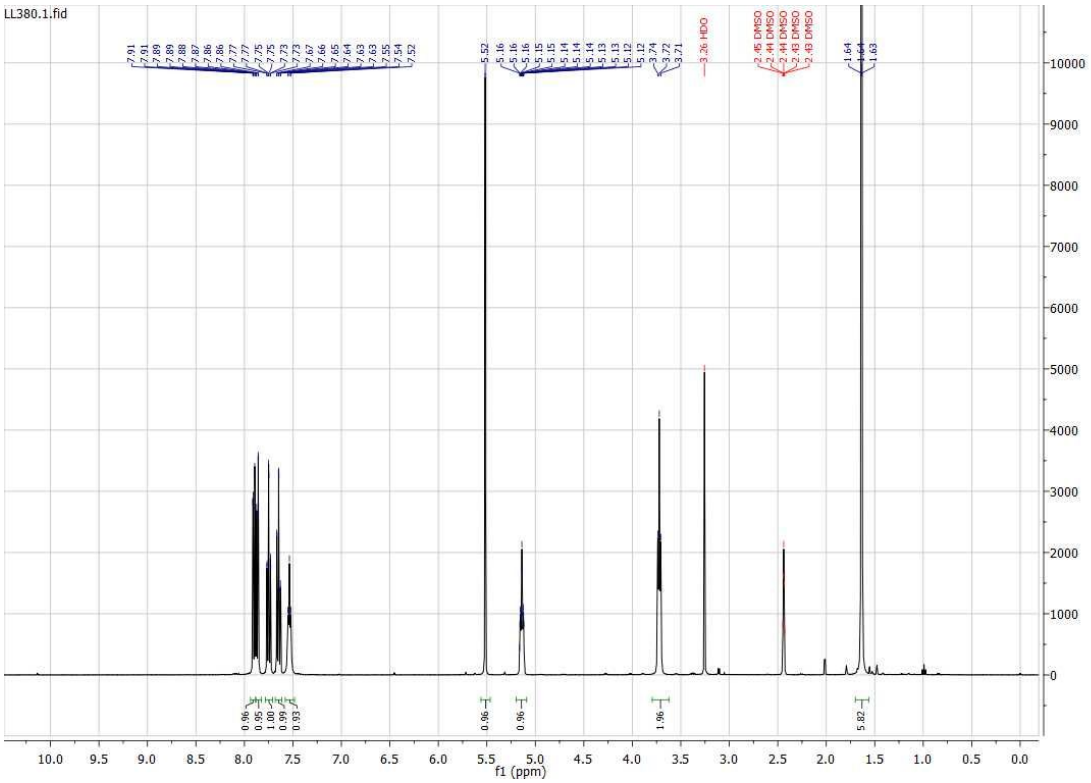

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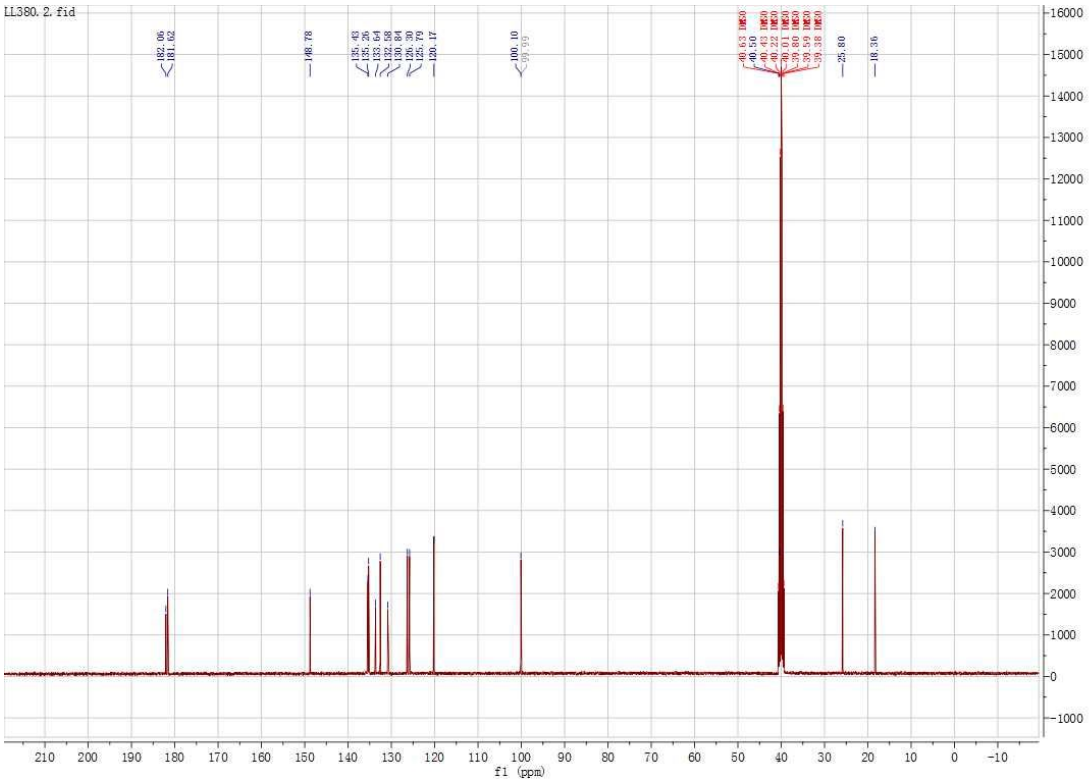

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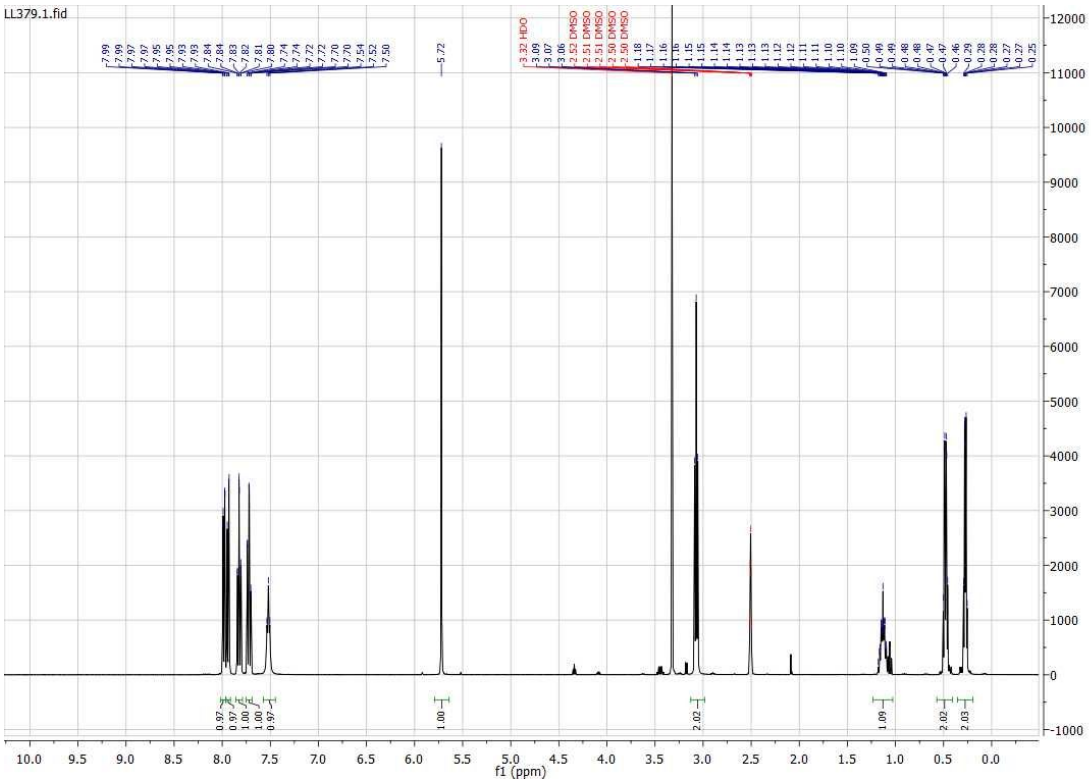

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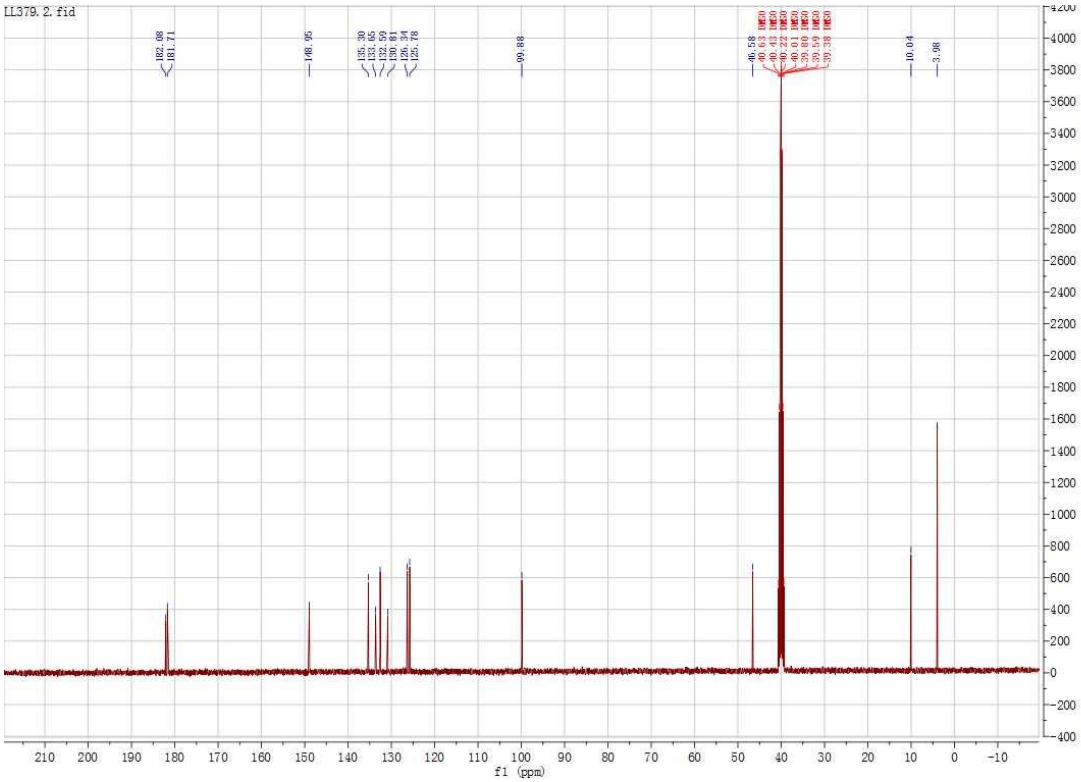

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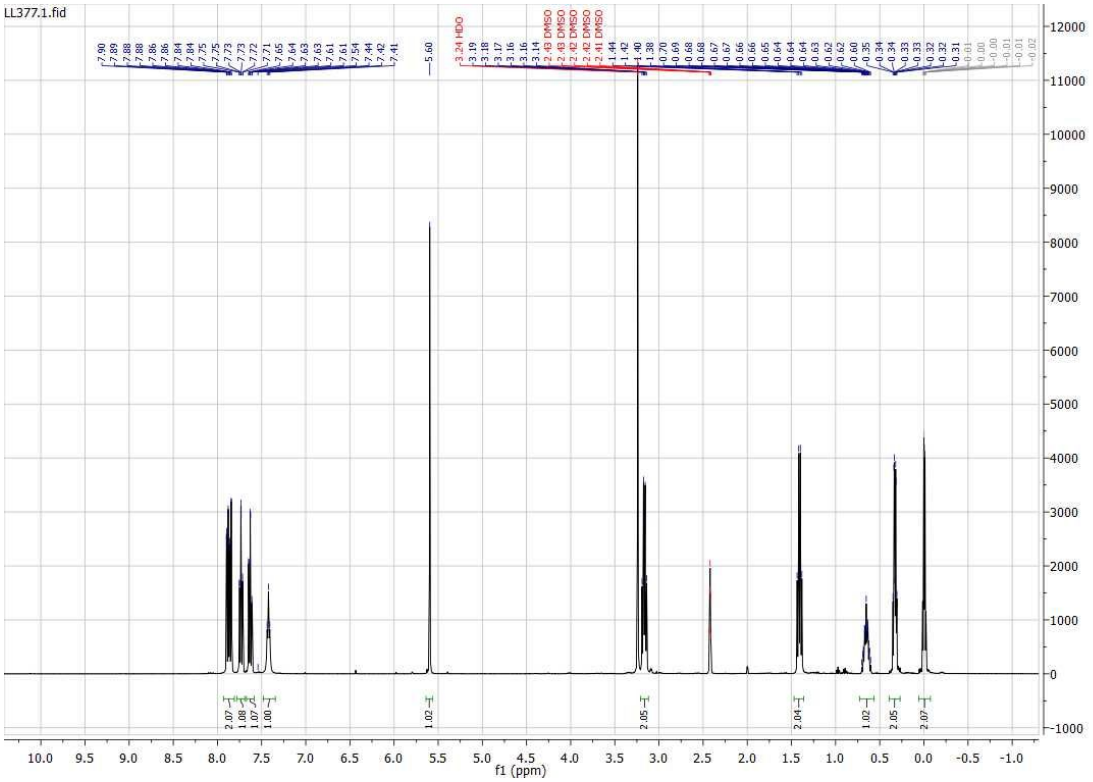

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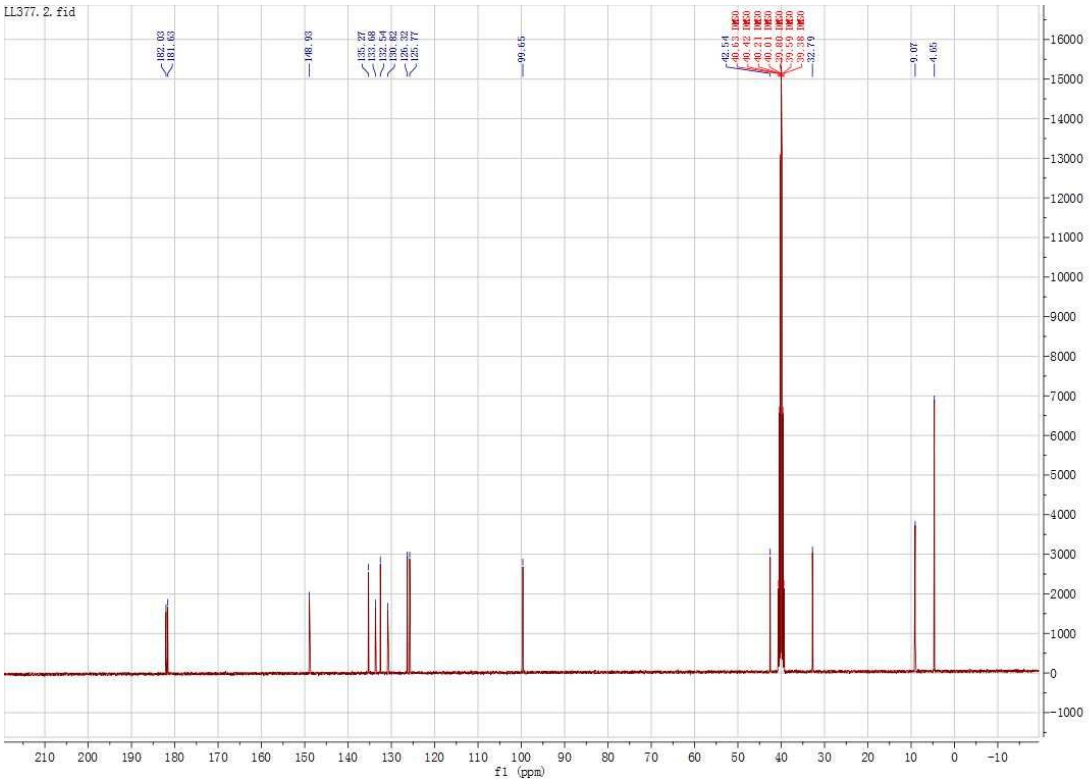

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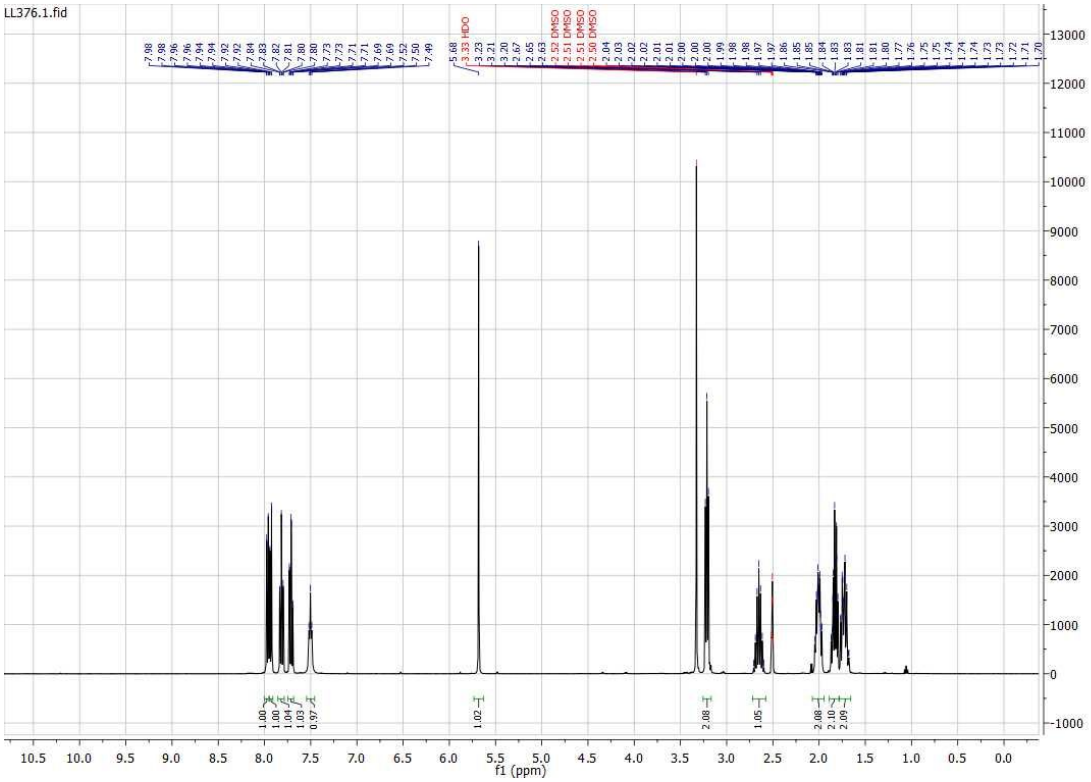

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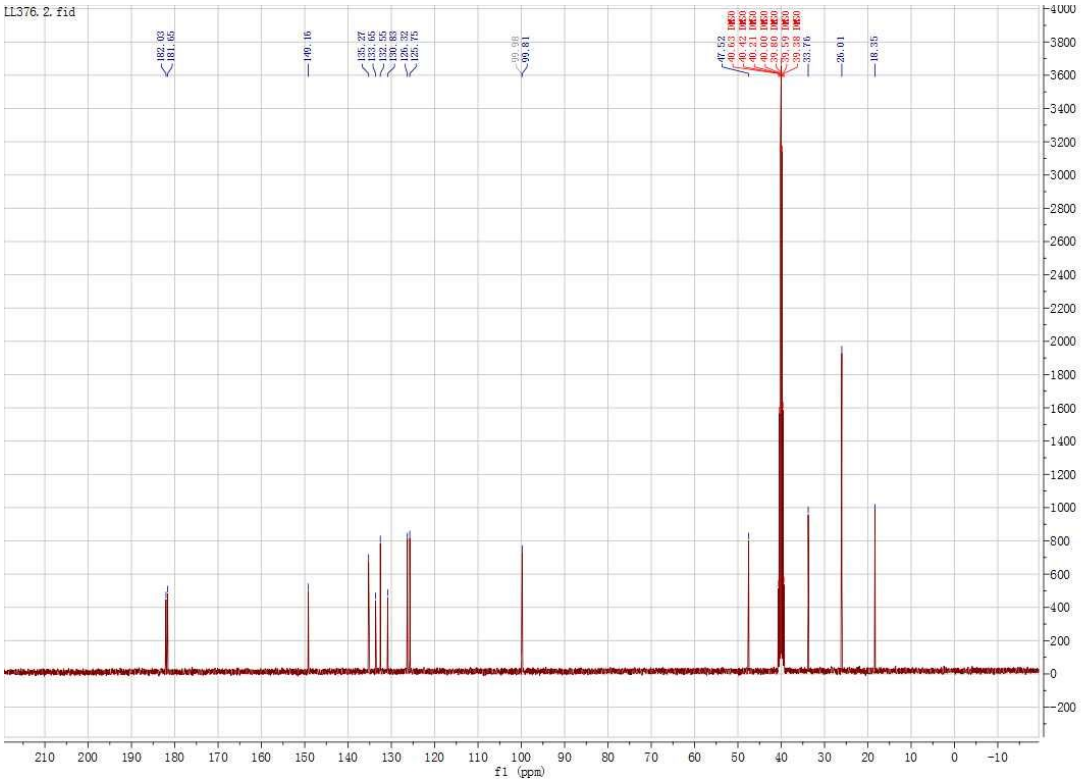

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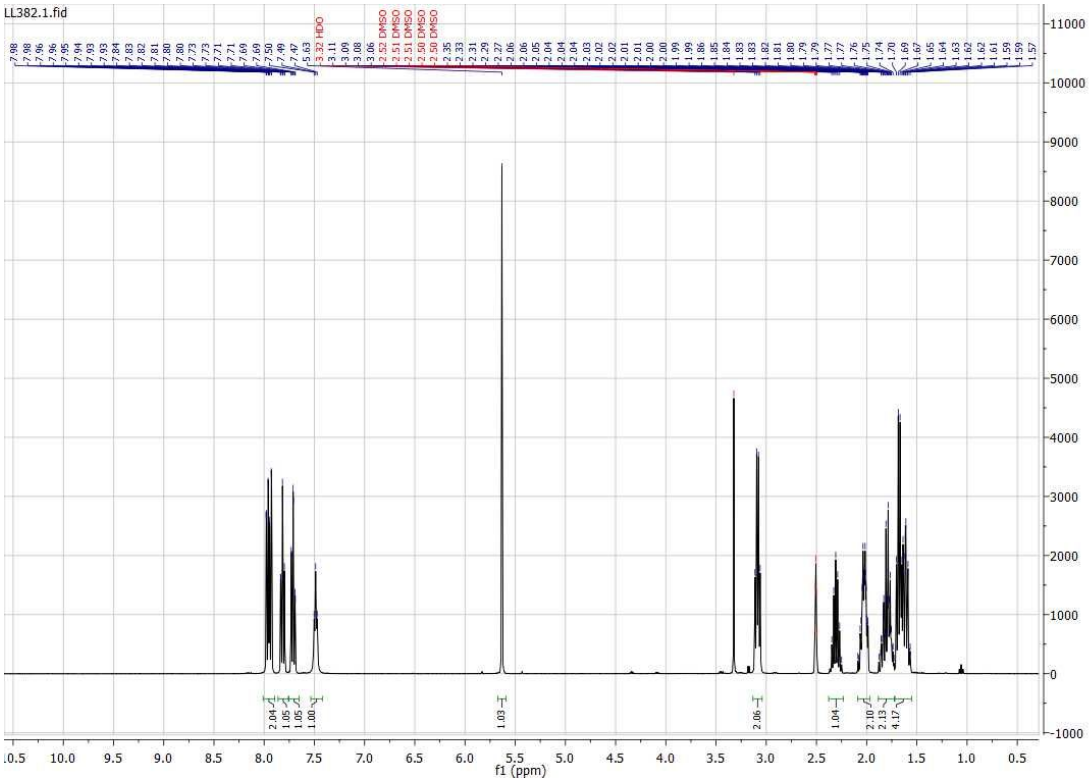

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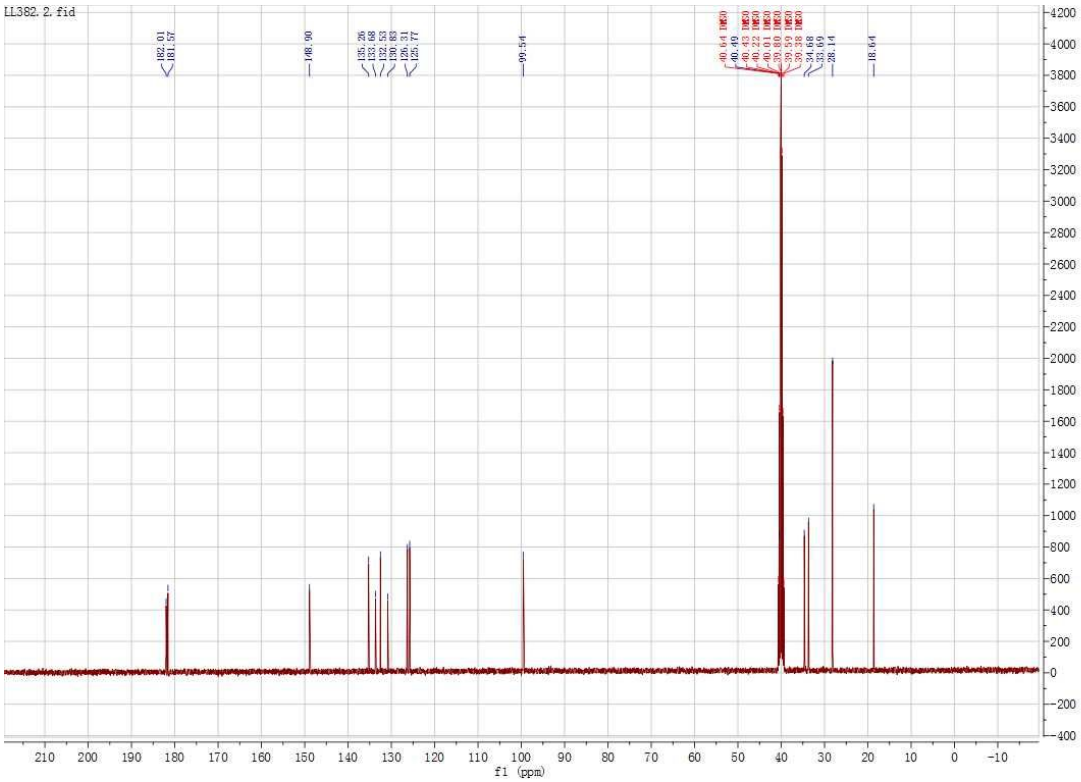

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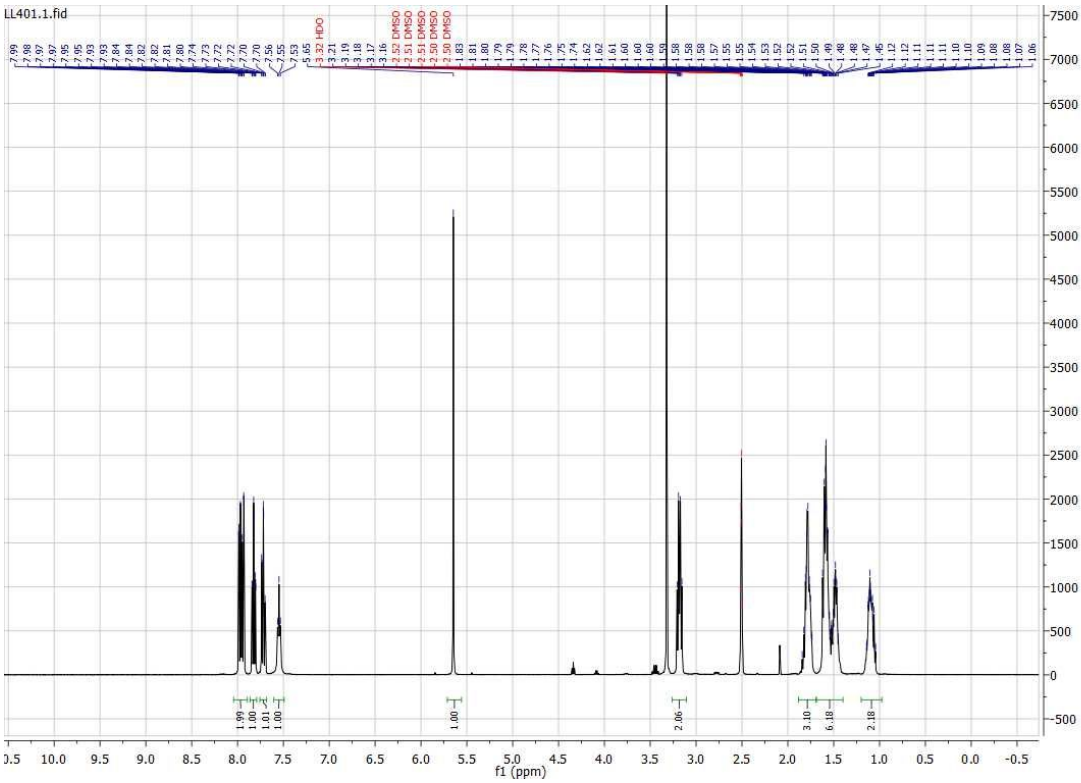

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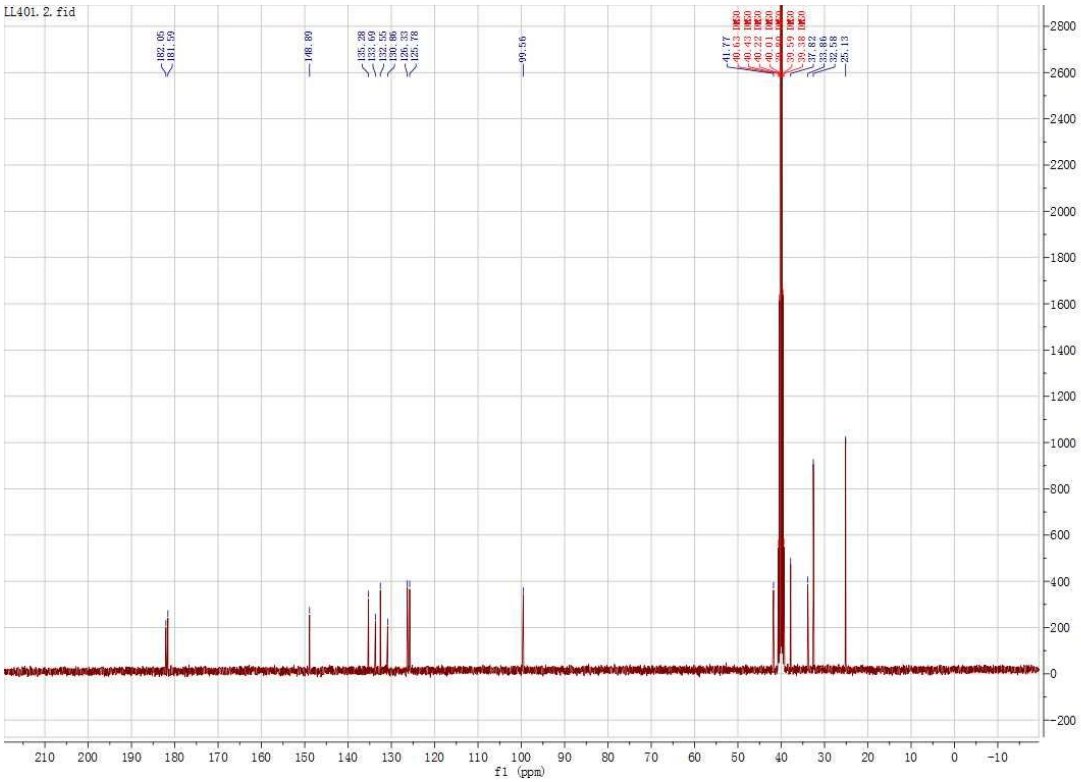

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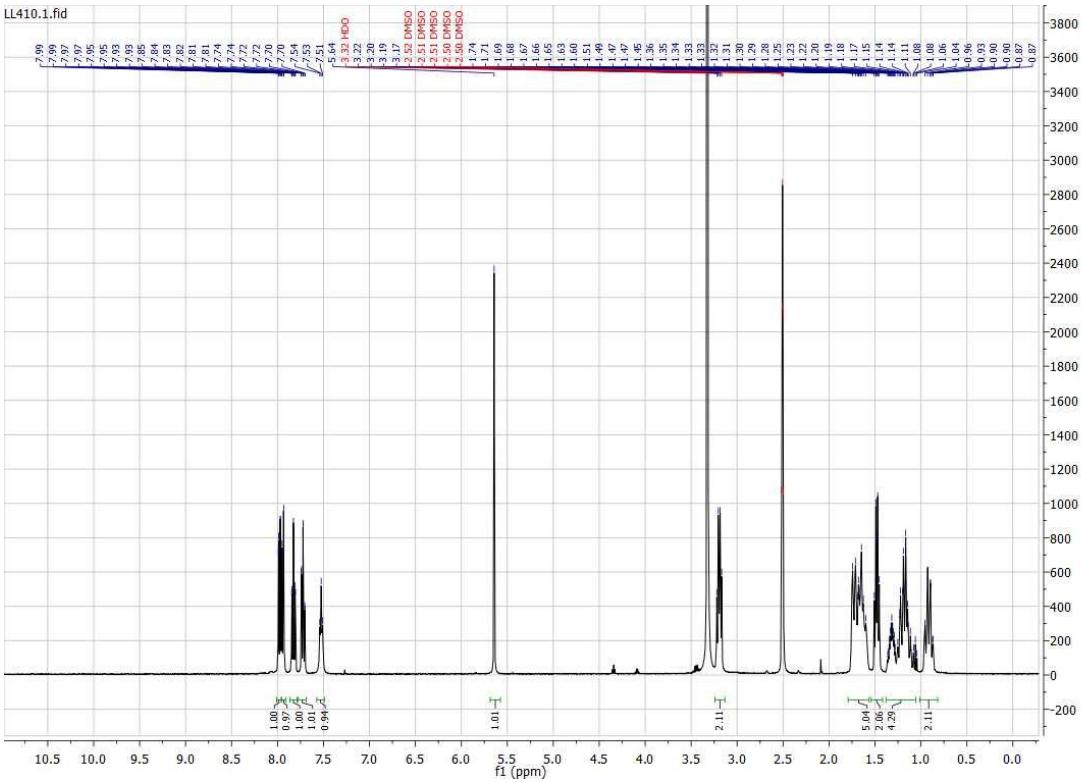

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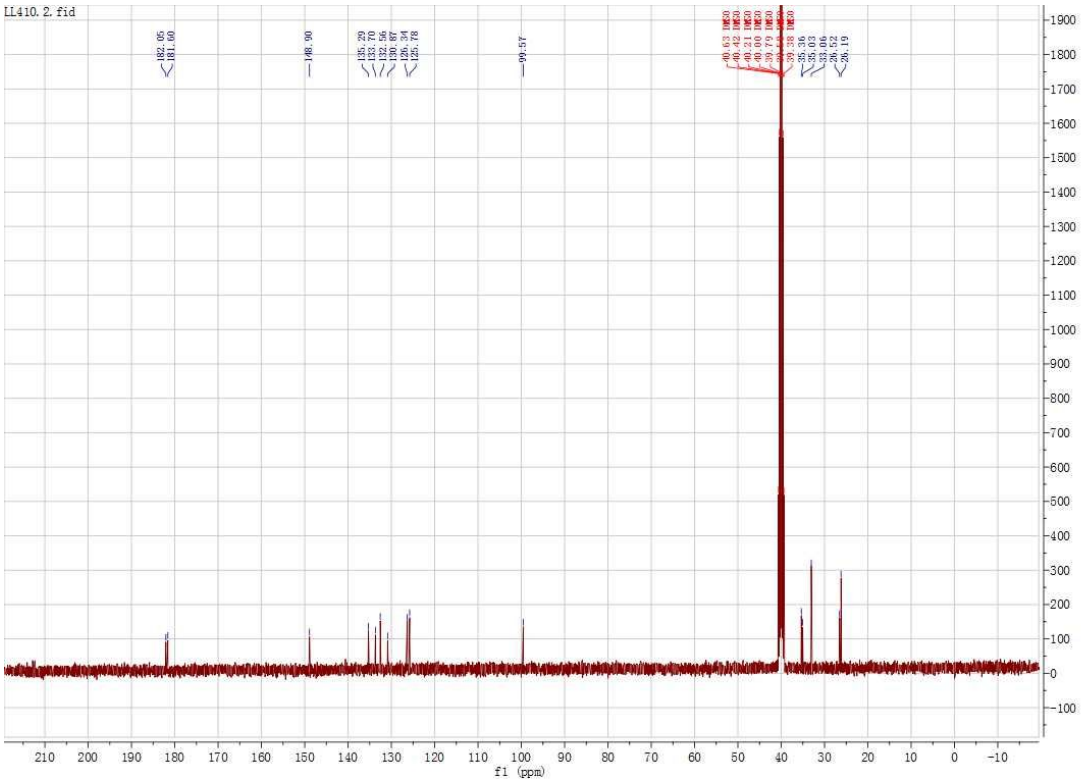

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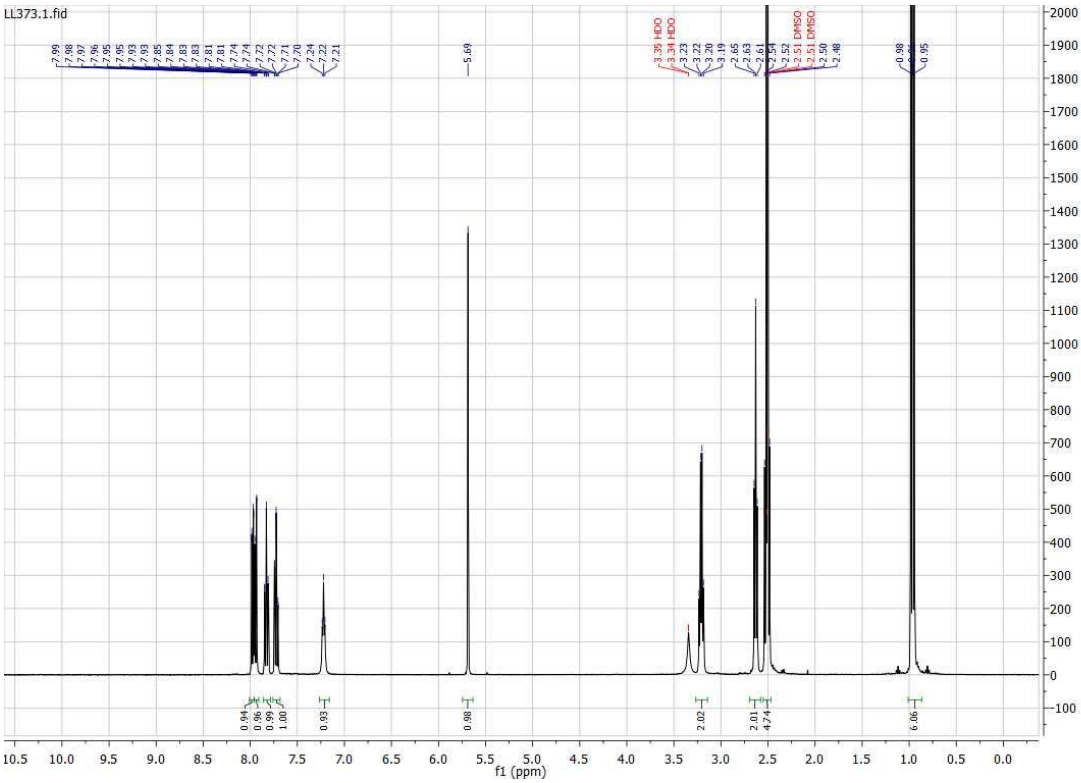

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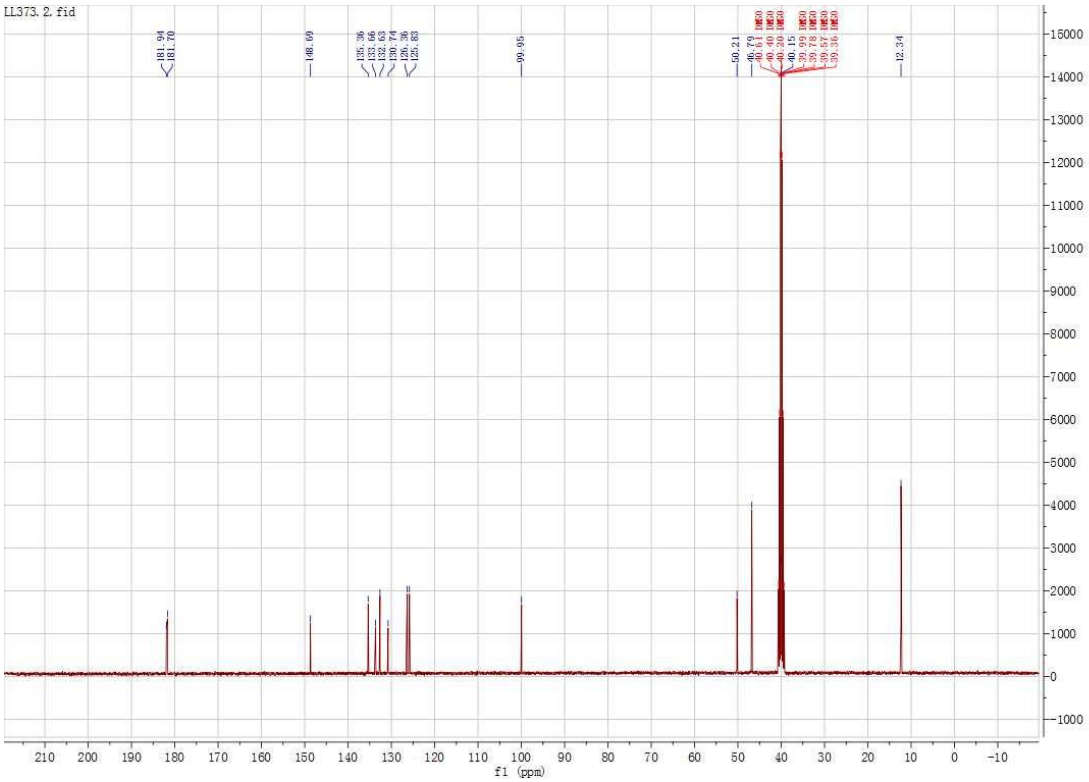

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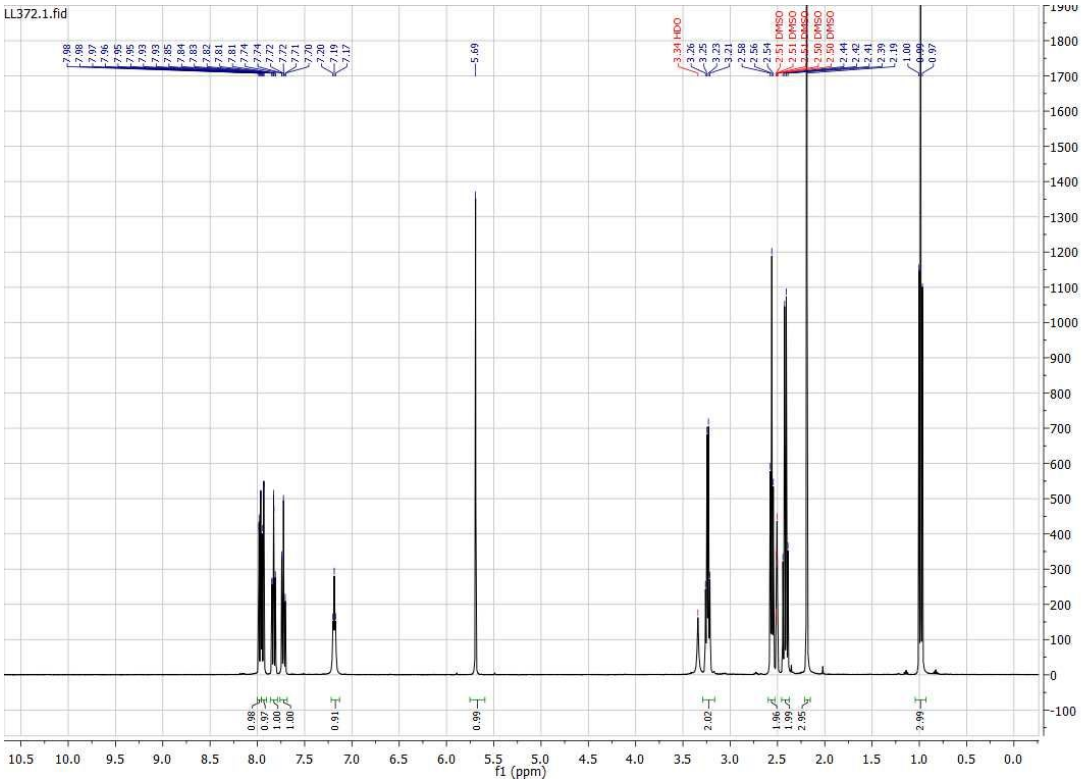

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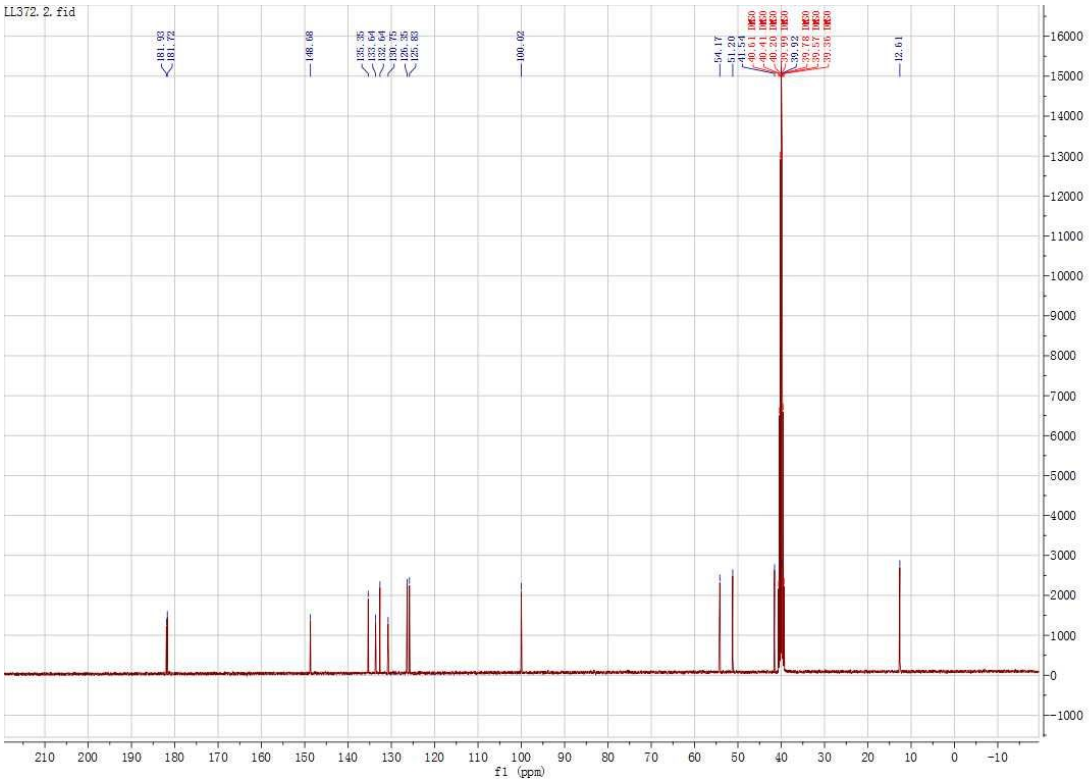

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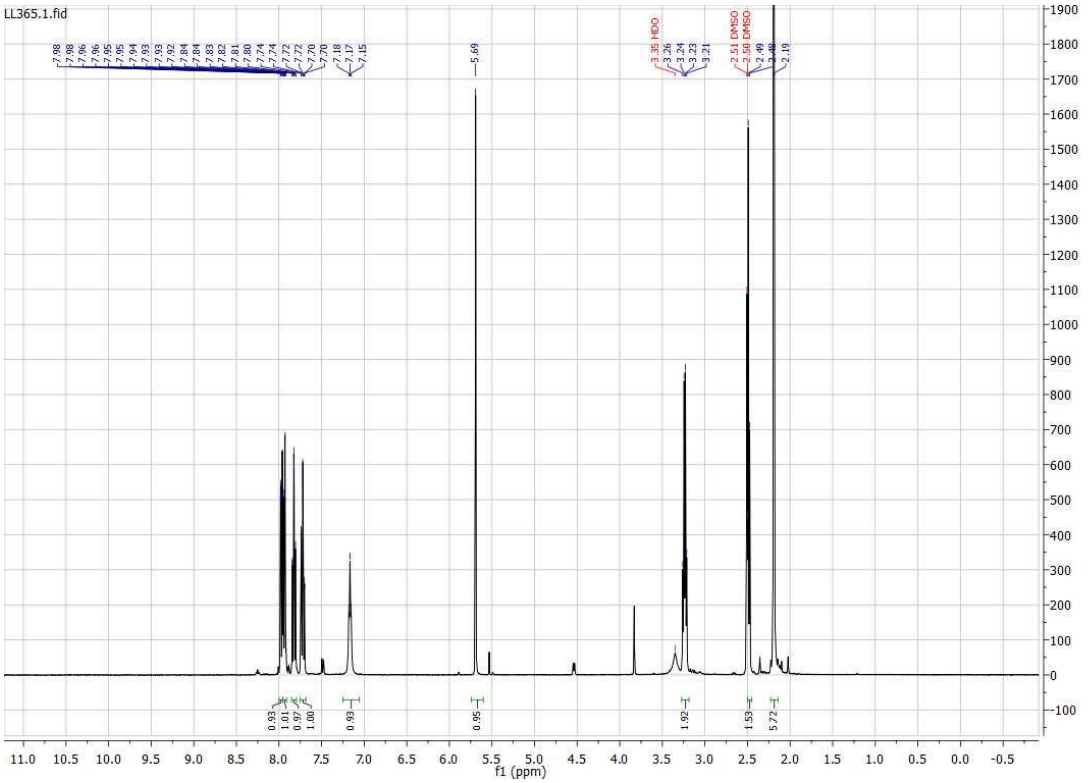

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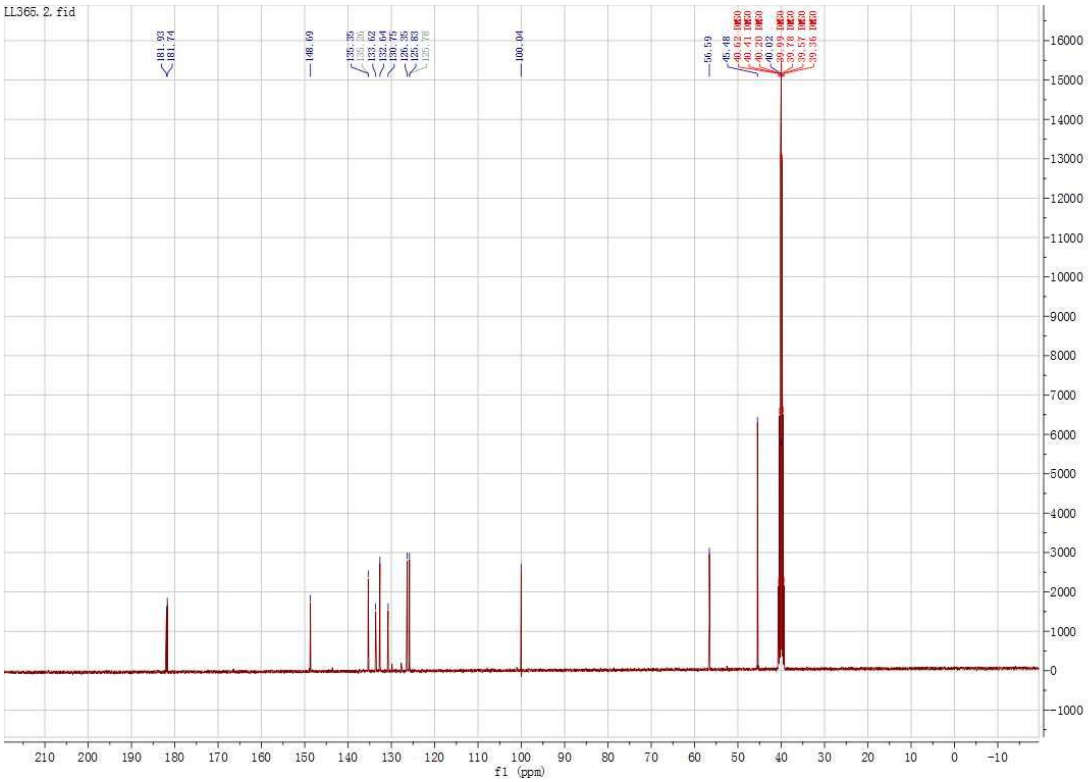

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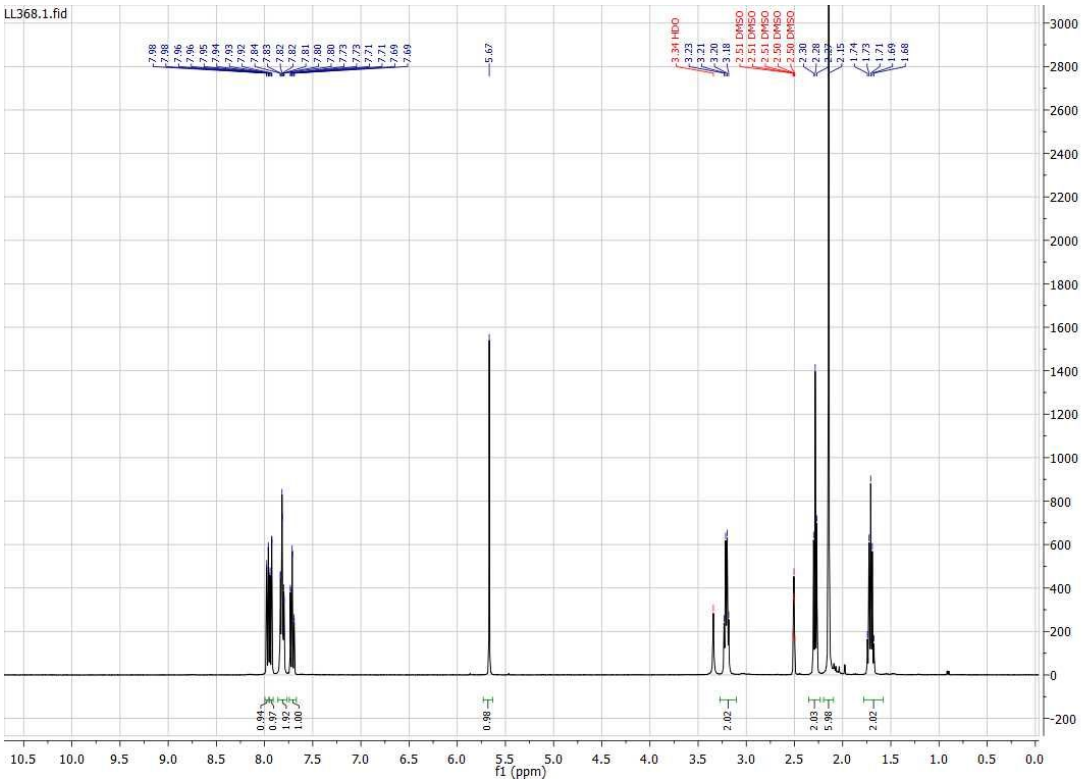

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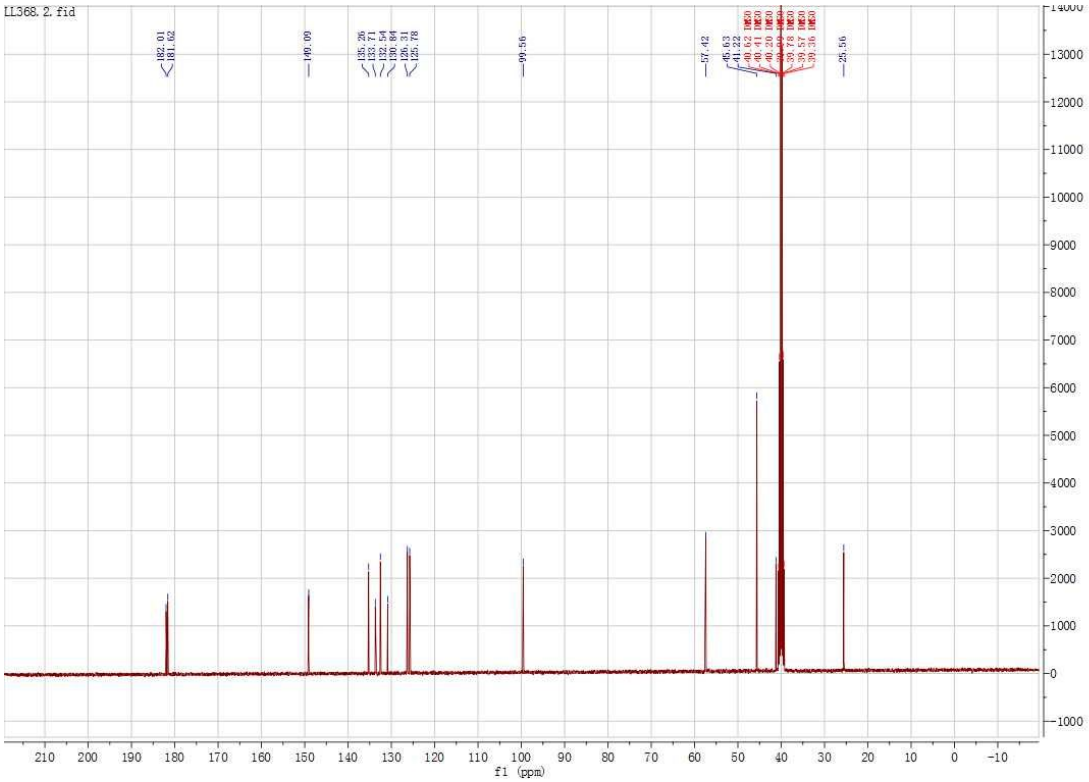

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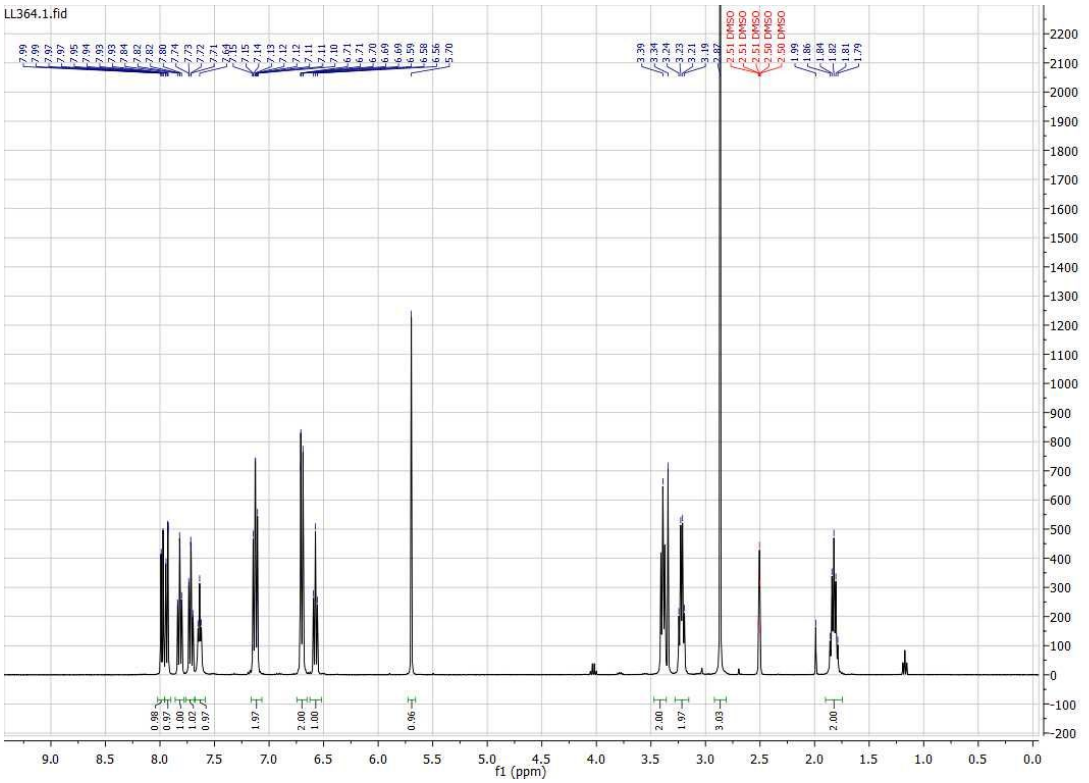

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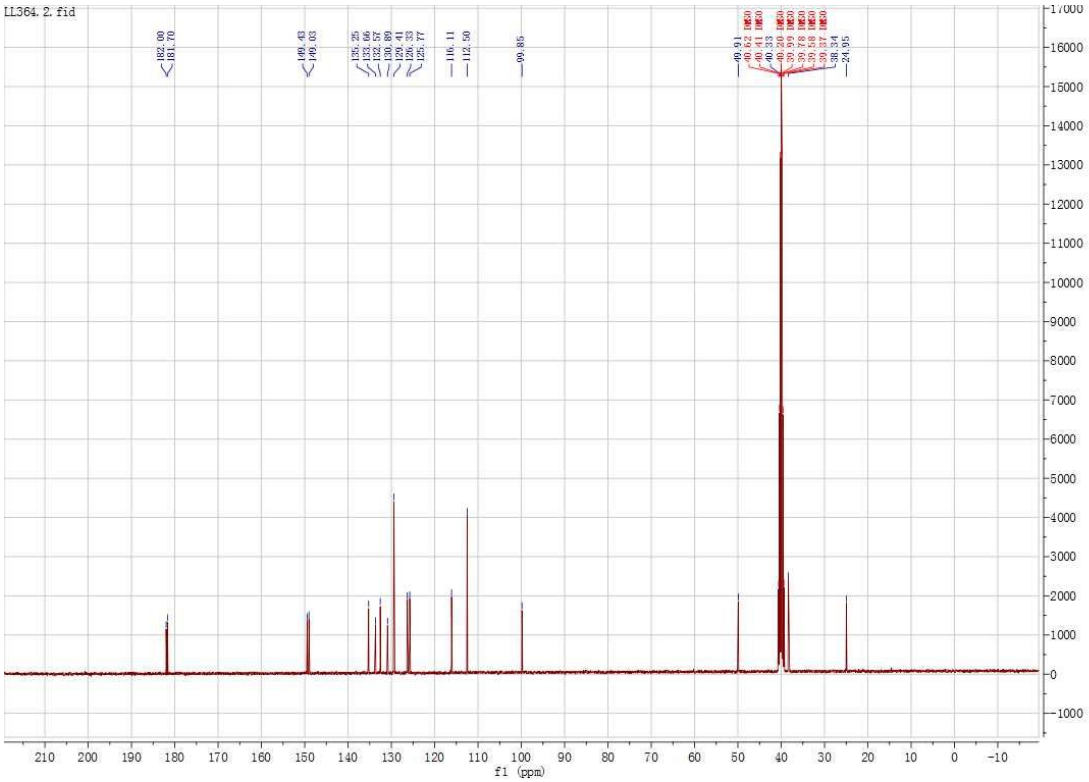

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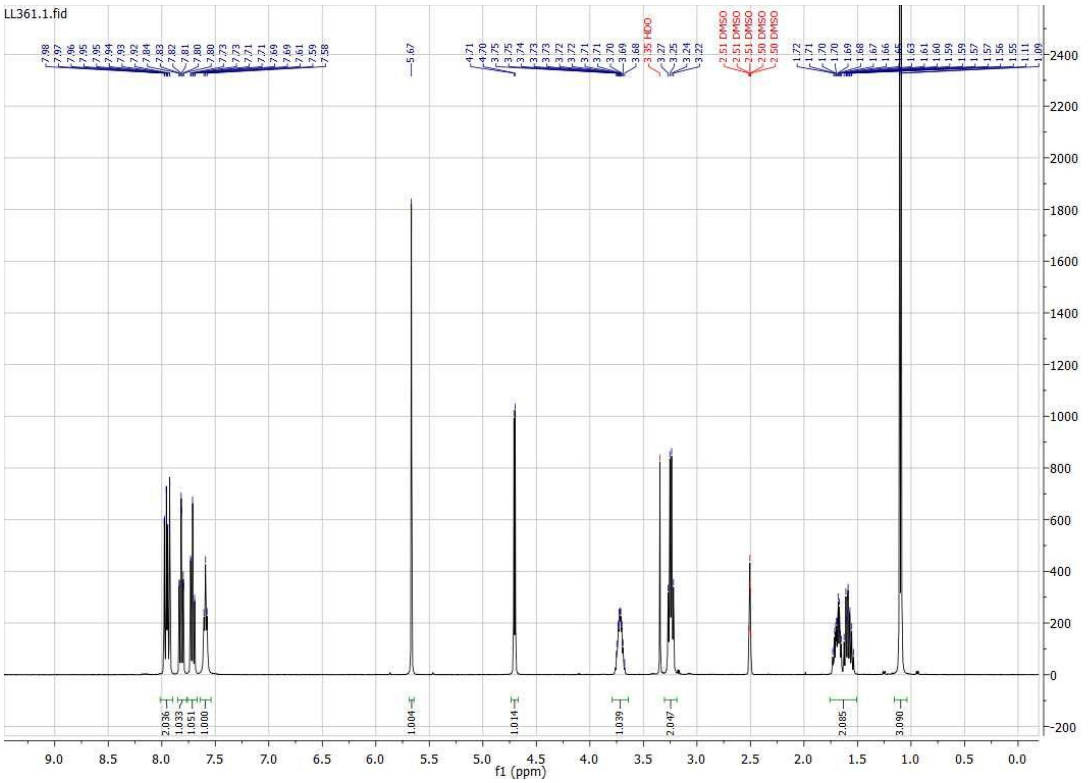
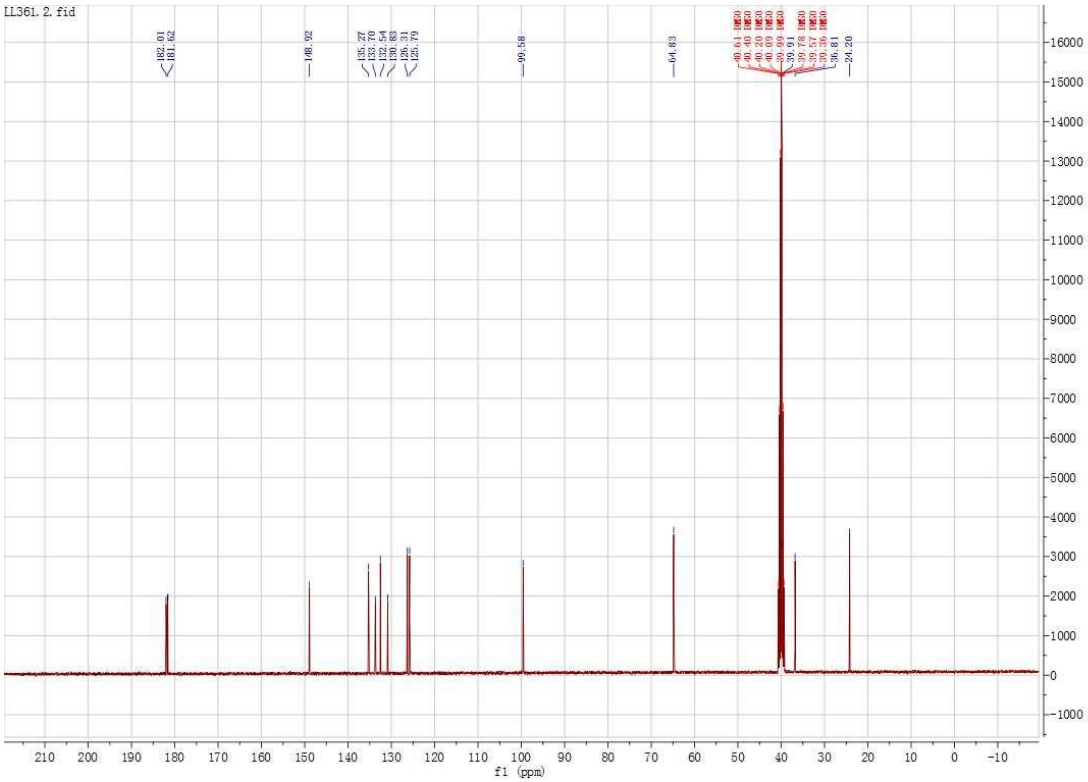
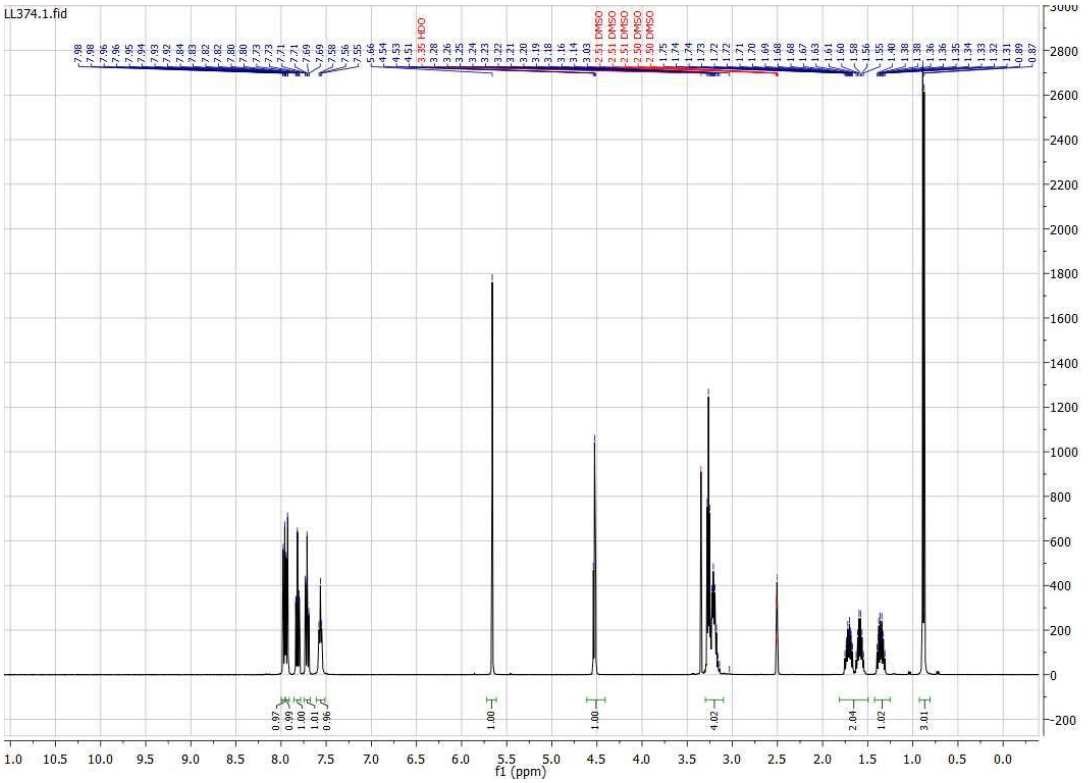


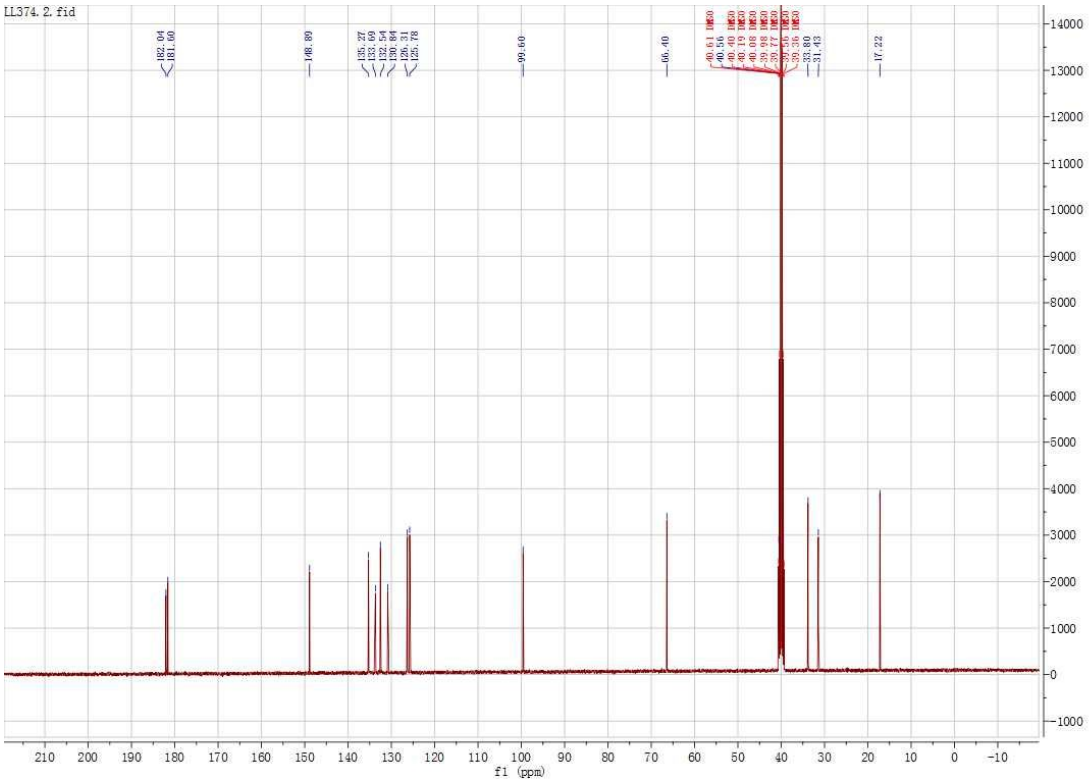
^{1}H NMR of 3t



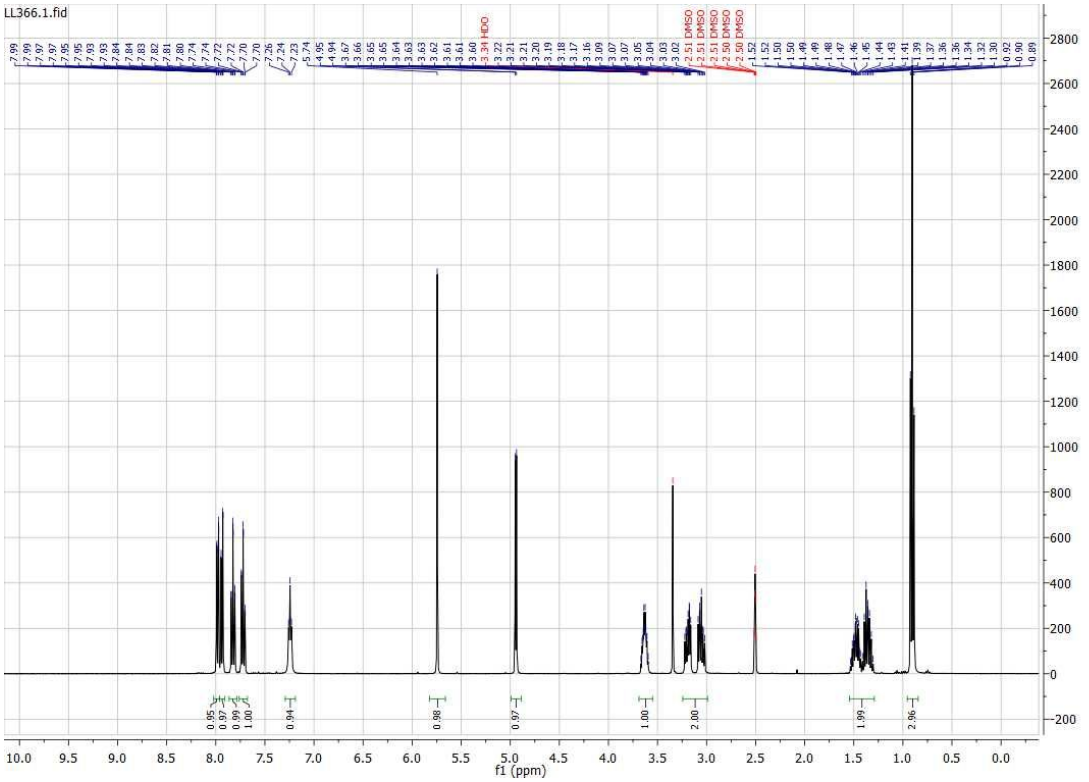
^{13}C NMR of 3t



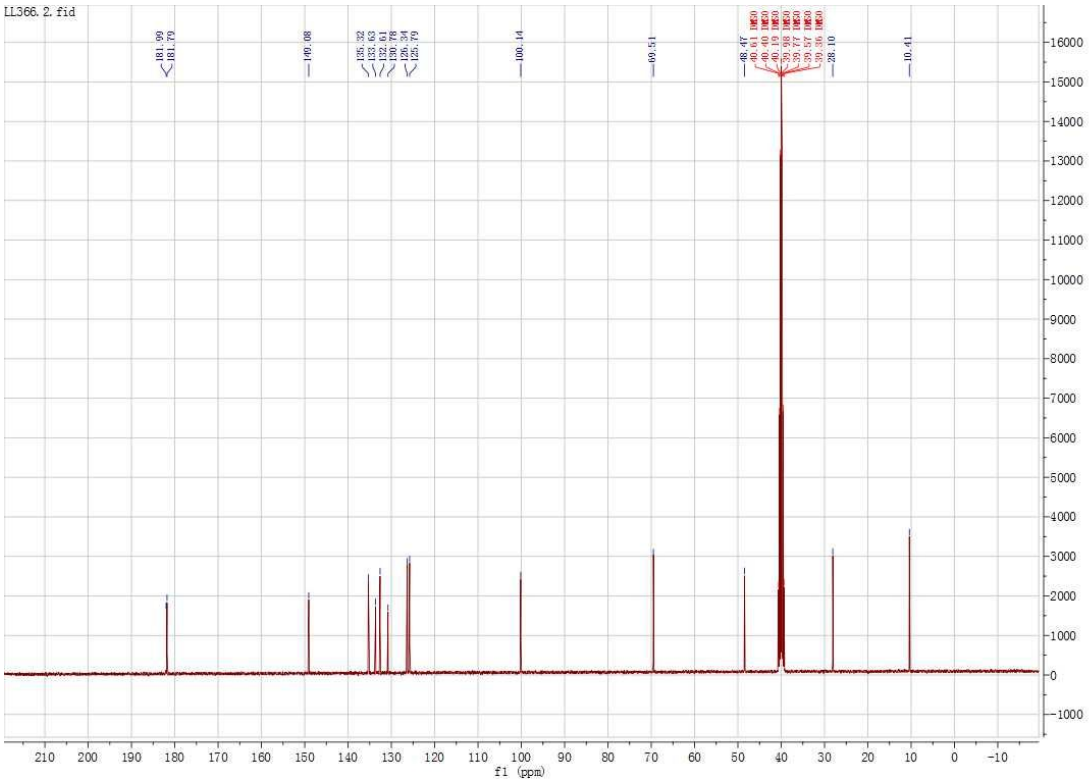
^{1}H NMR of 3u



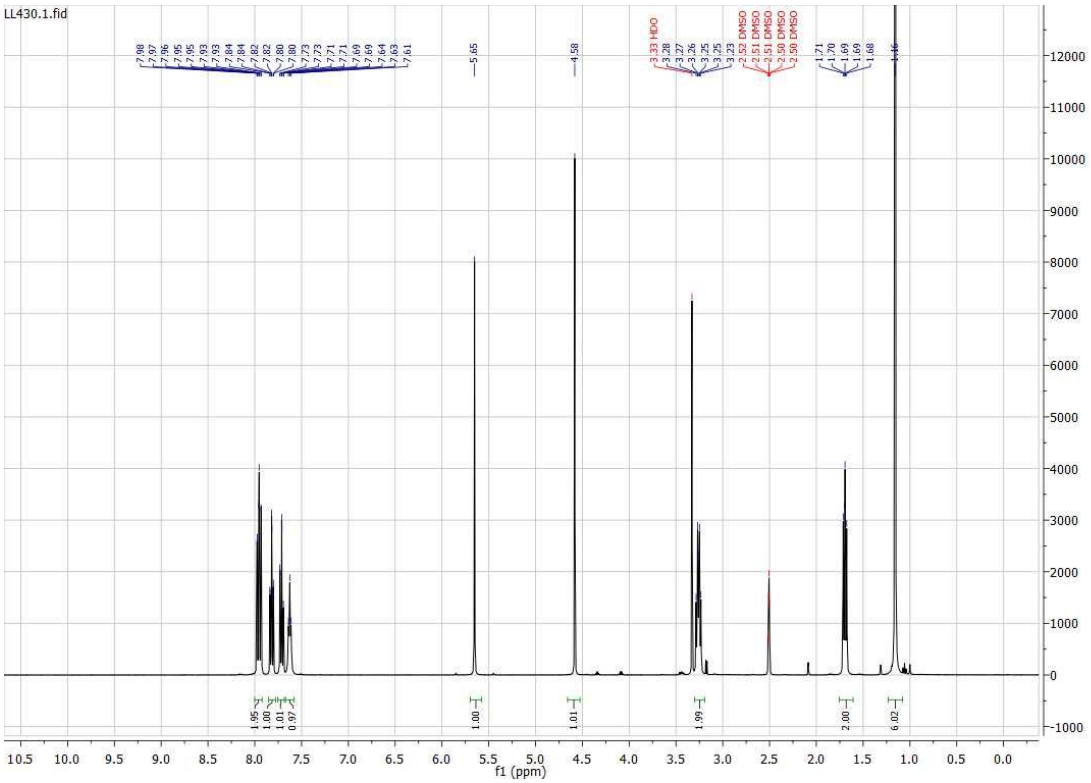
^{13}C NMR of 3u



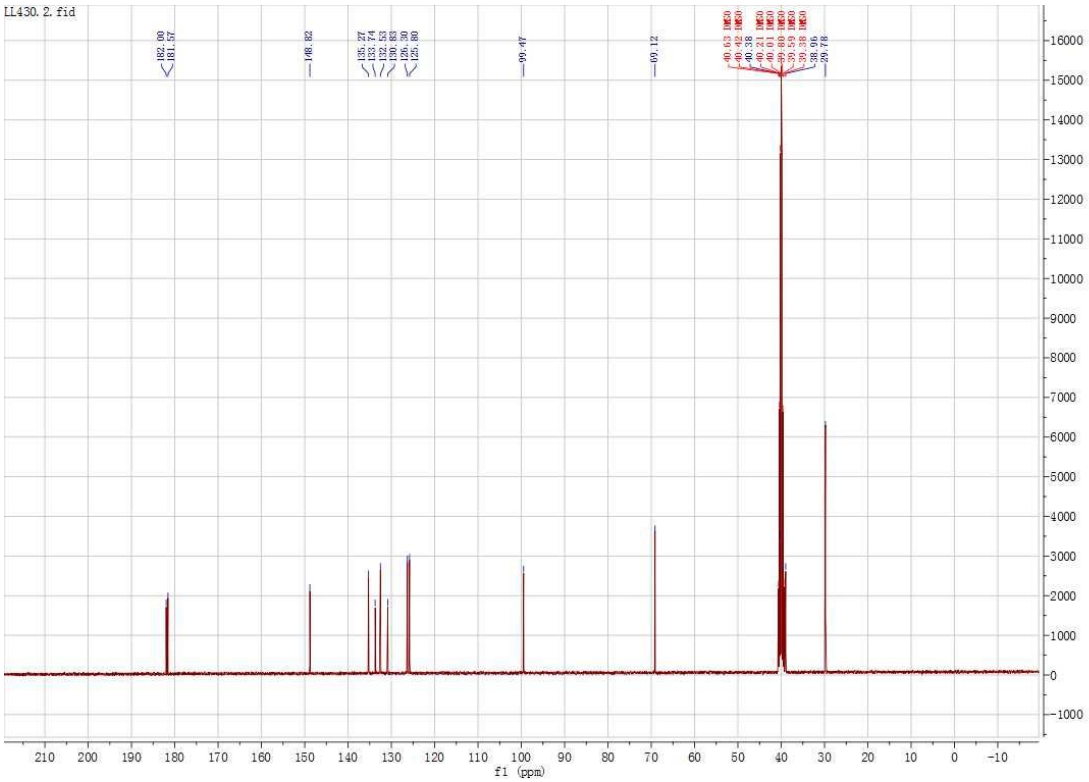
^{1}H NMR of 3v



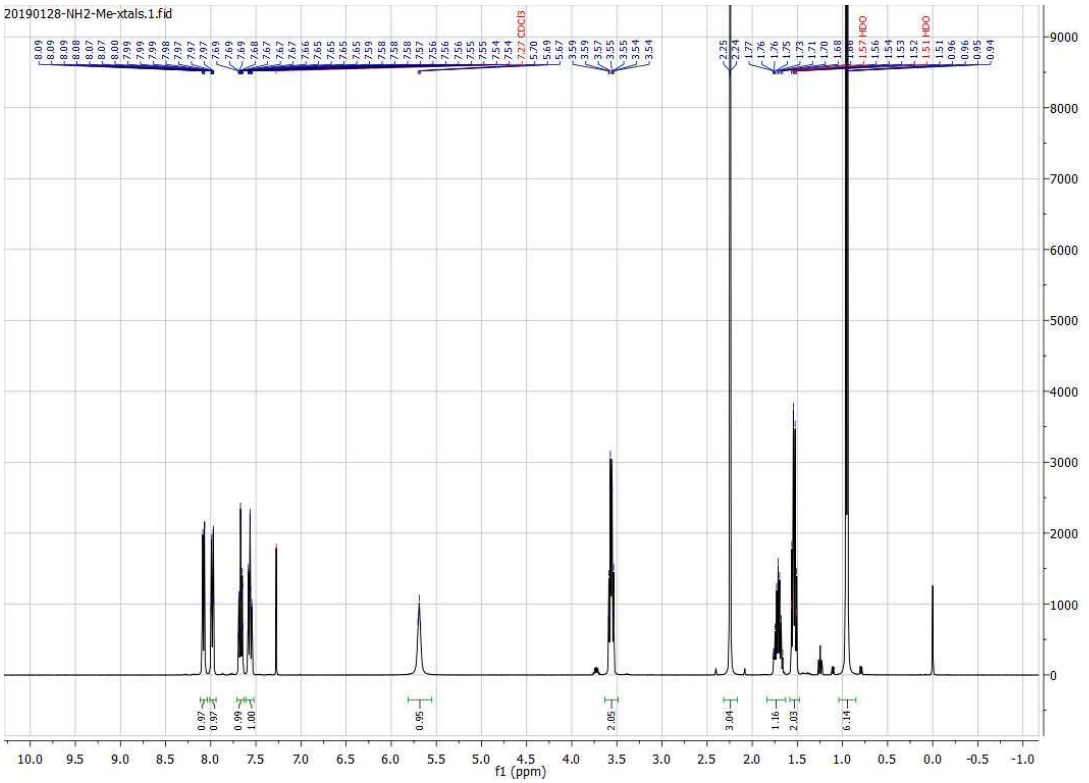
^{13}C NMR of 3v



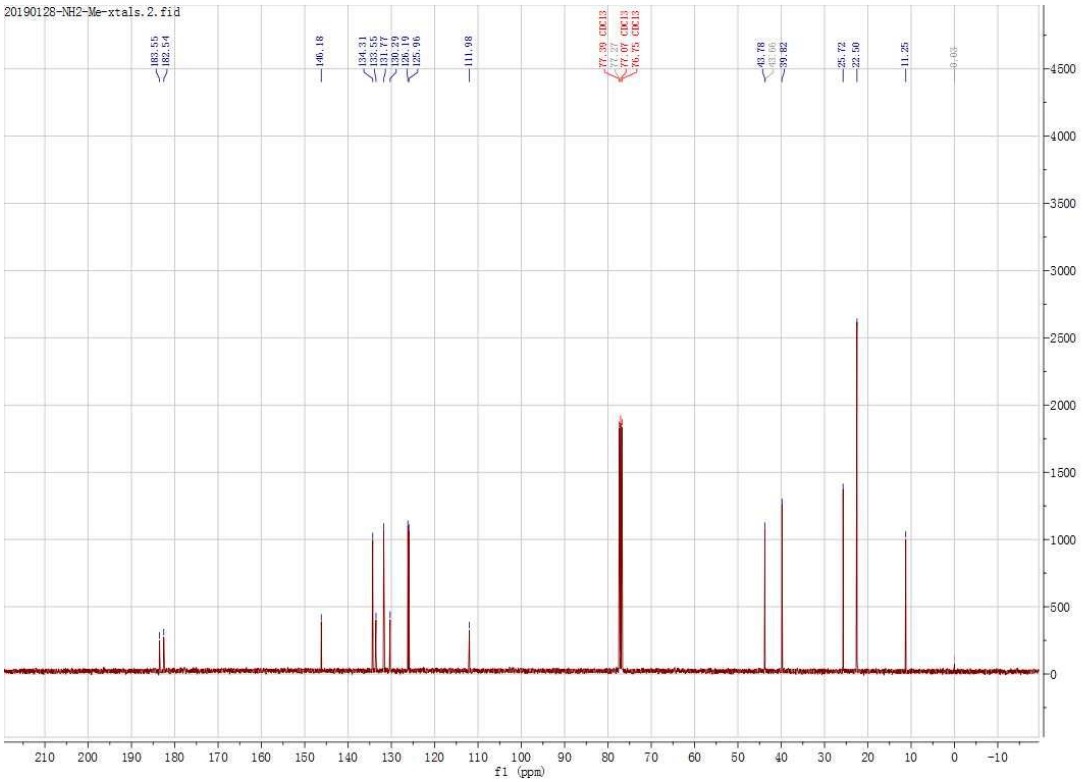
^{1}H NMR of 3w



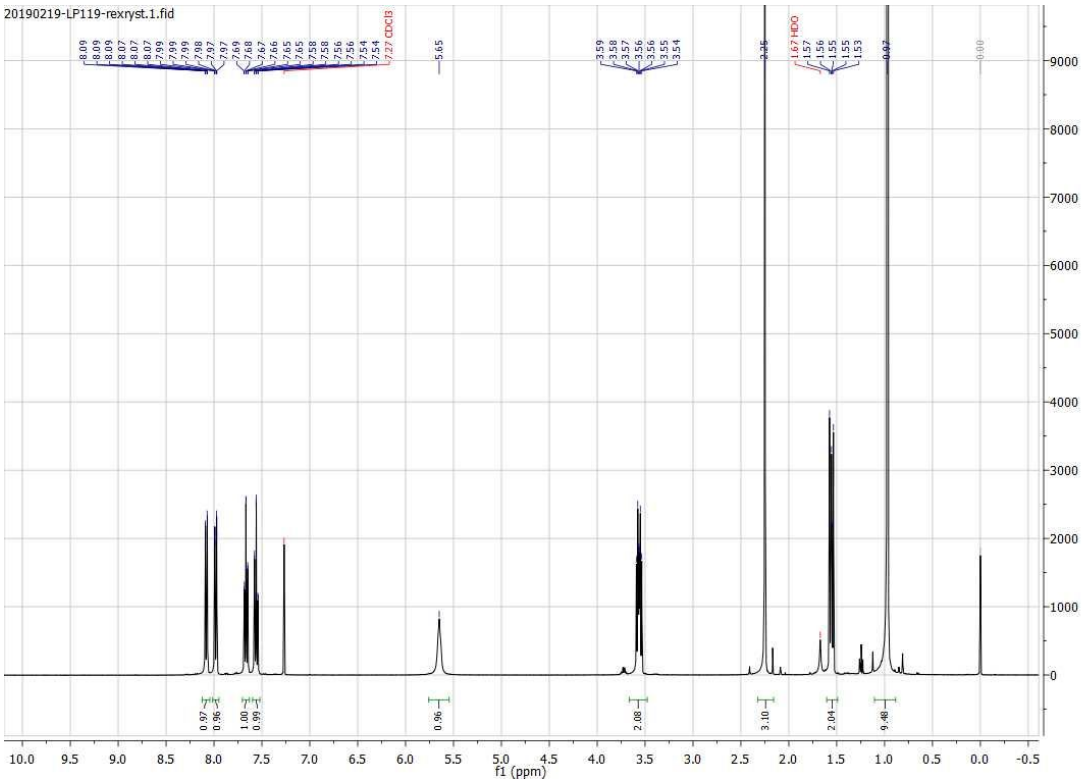
^{13}C NMR of 3w



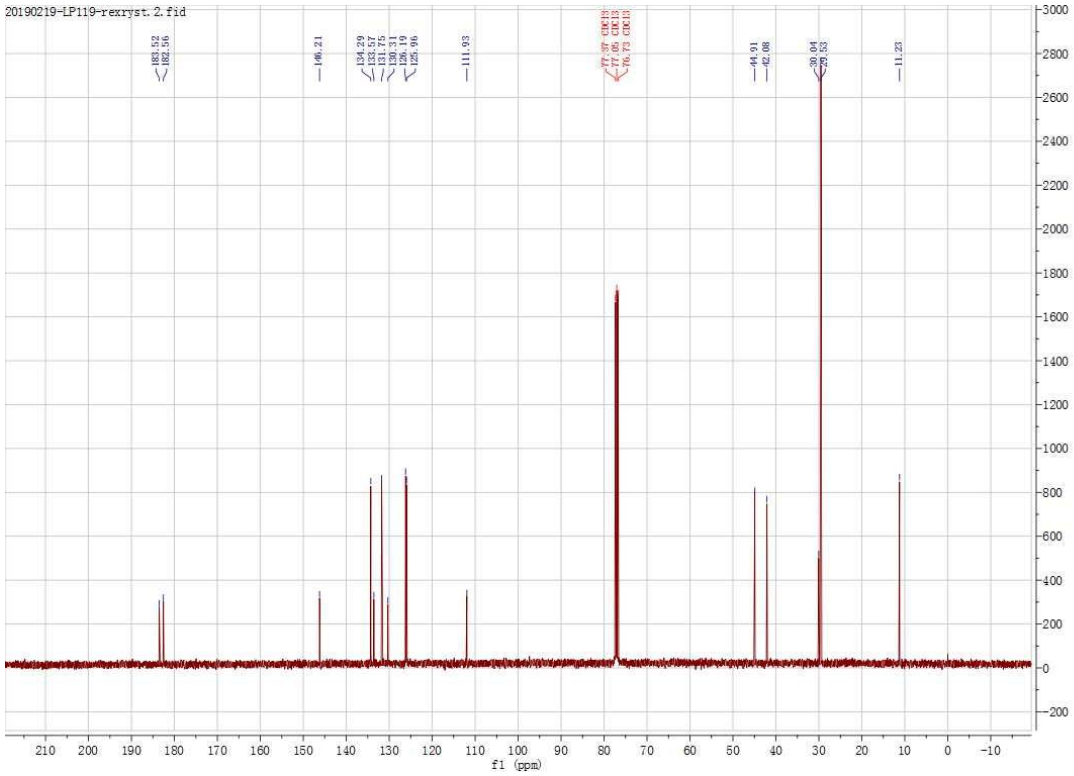
^{1}H NMR of 5a



^{13}C NMR of 5a



^{1}H NMR of 5b

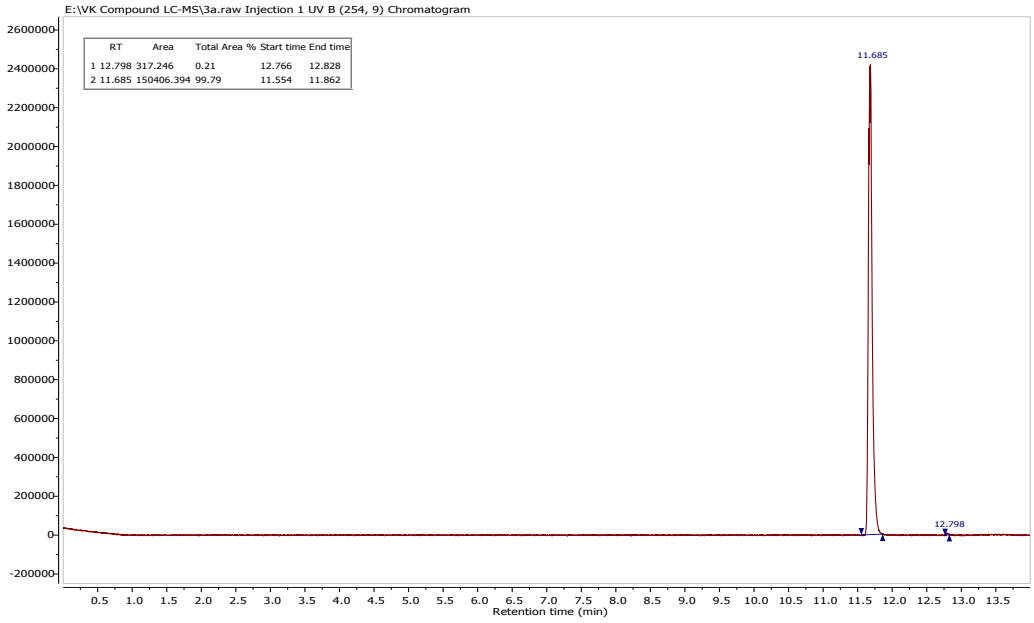


^{13}C NMR of 5b

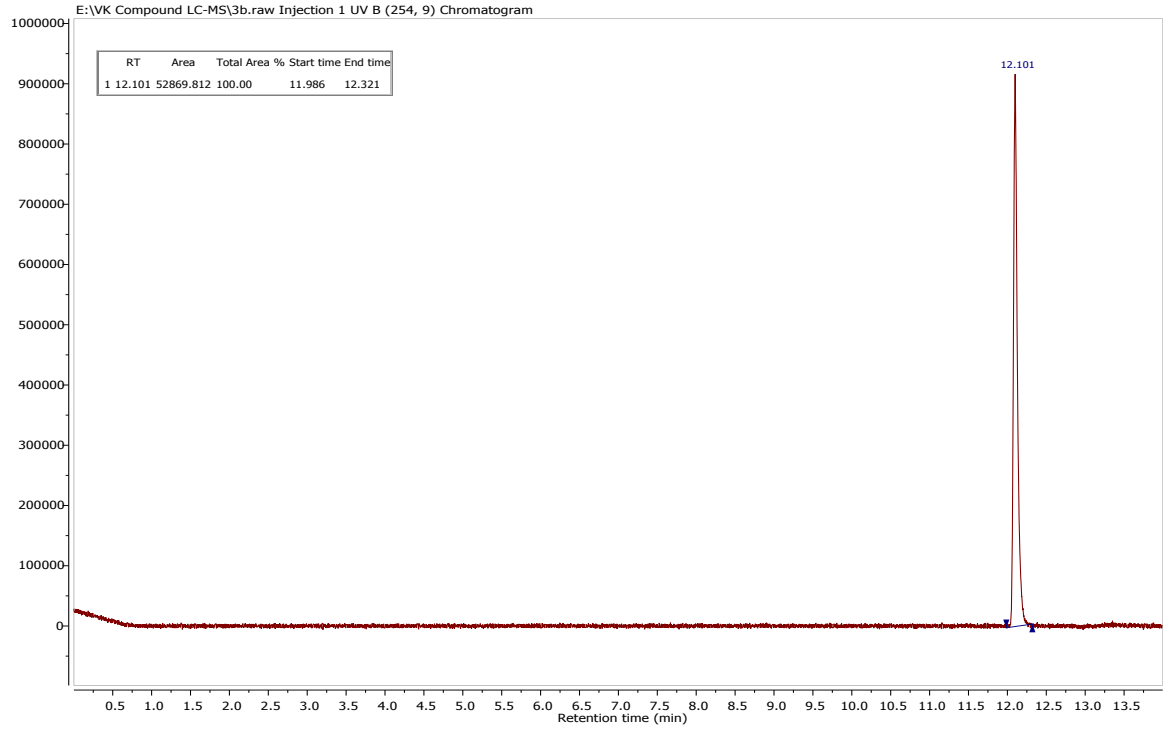
HPLC traces and purity of the target compounds

All target compounds are at least 95% pure as confirmed via UV detection of ESI LCMS, performed on an Agilent 1100 HPLC instrument using an ODS HYPERSIL column (5µm, 4.6 mm × 250 mm) with a gradient of water/methanol plus 0.1% formic acid (3a-3w: 0-13 mins from 0% to 100% methanol, 13-14 mins: from 100% to 0% methanol. 5a and 5b: 0-3minutes from 0% to 90% methanol, 3-17 mins from 90% to 100% methanol, and 17-18 mins from 100% to 0% methanol).

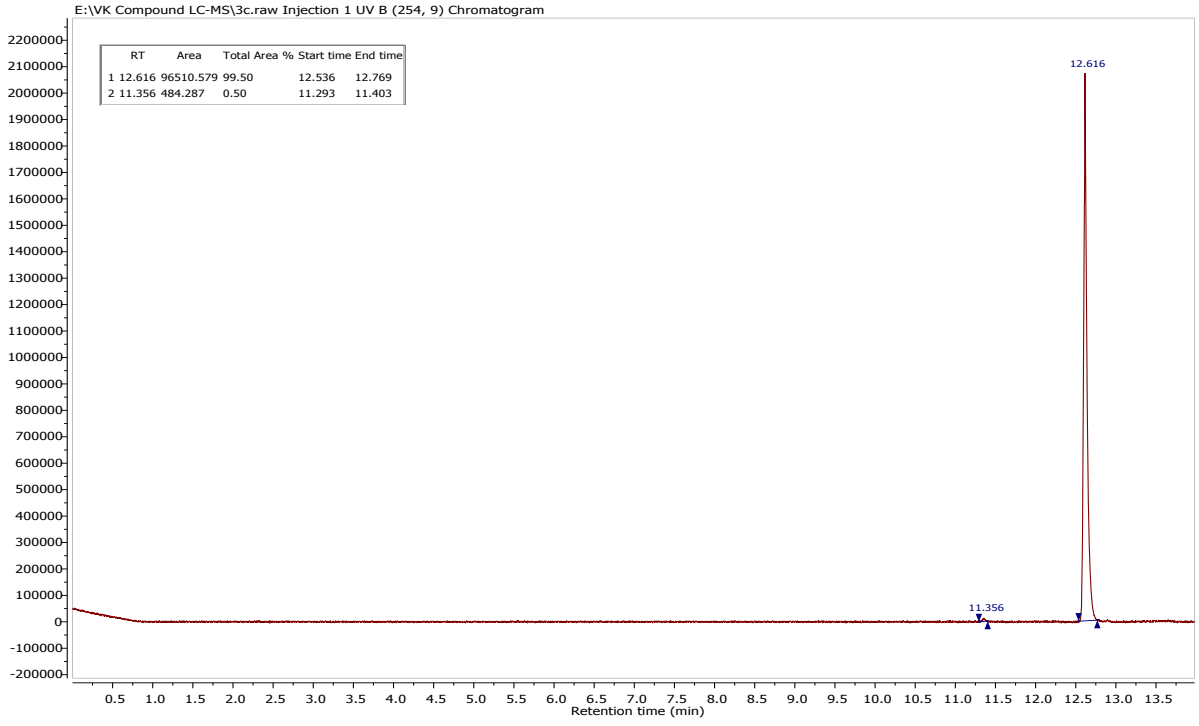
3a



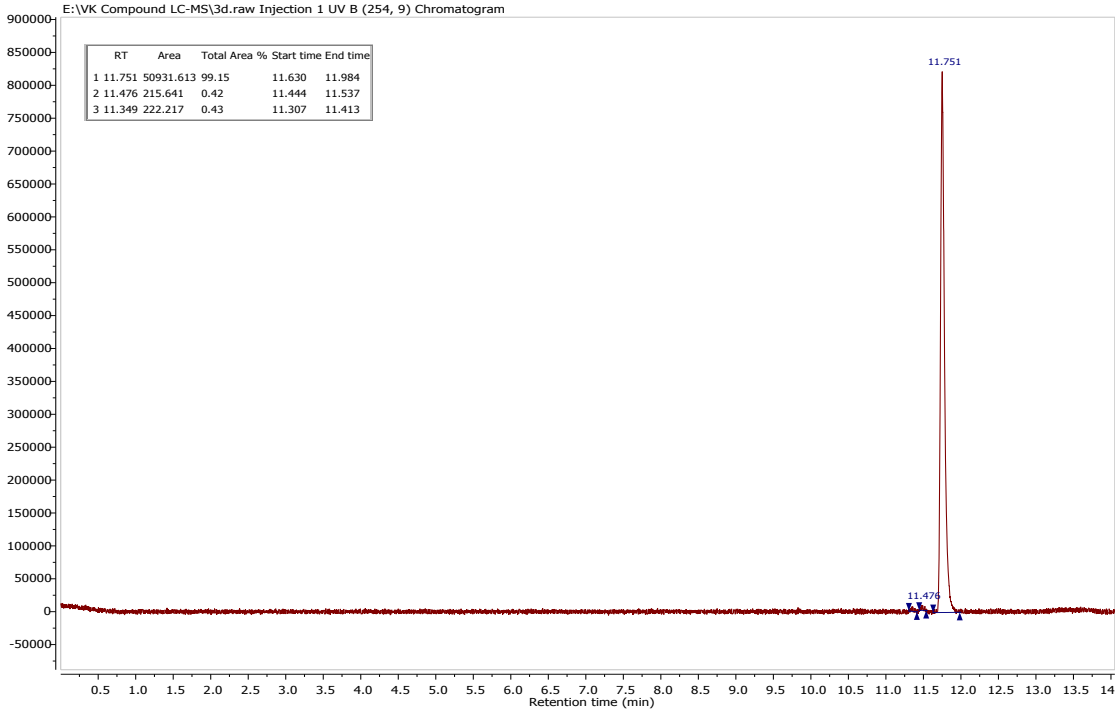
3b


E:\VK Compound LC-MS\3b.raw Injection 1 UV B (254, 9) Chromatogram
RT Area Total Area % Start time End time
1 12.101 52869.812 100.00 11.986 12.321
12.101
Retention time (min)

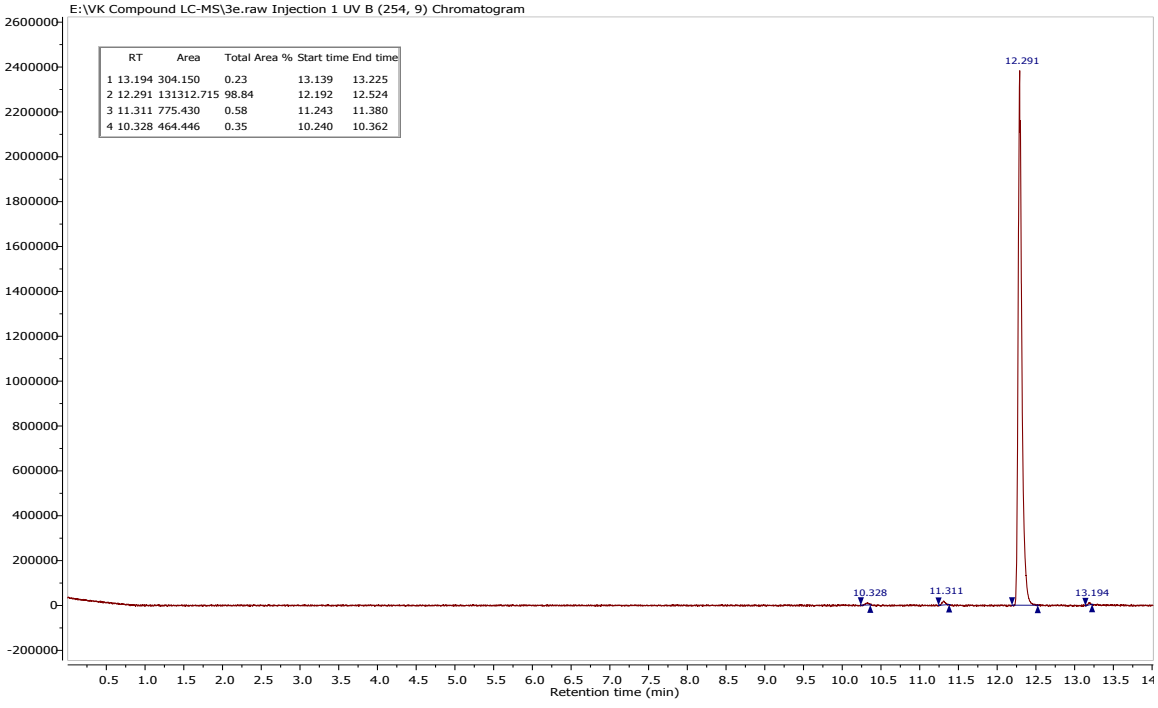
3c


E:\VK Compound LC-MS\3c.raw Injection 1 UV B (254, 9) Chromatogram
RT Area Total Area % Start time End time
1 12.616 96510.579 99.50 12.536 12.769
2 11.356 484.287 0.50 11.293 11.403
12.616
11.356
Retention time (min)

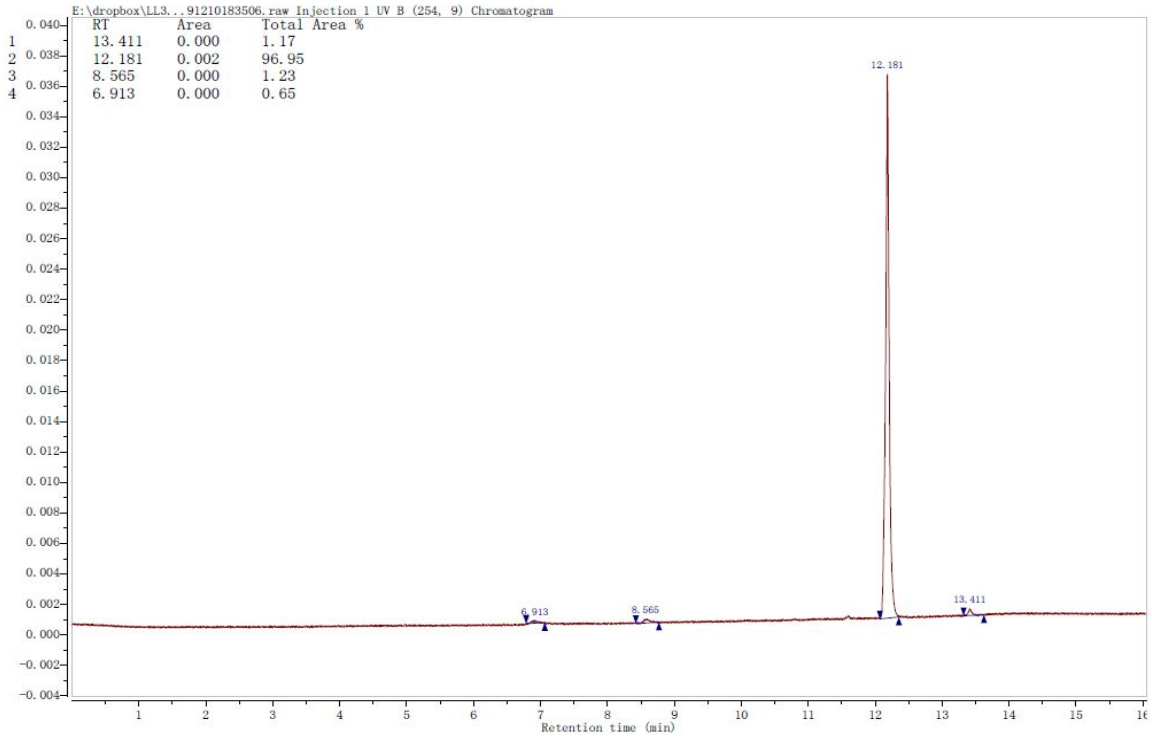
3d


E:\VK Compound LC-MS\3d.raw Injection 1 UV B (254, 9) Chromatogram
RT Area Total Area % Start time End time
1 11.751 50931.613 99.15 11.630 11.984
2 11.476 215.641 0.42 11.444 11.537
3 11.349 222.217 0.43 11.307 11.413
11.751
11.476
Retention time (min)

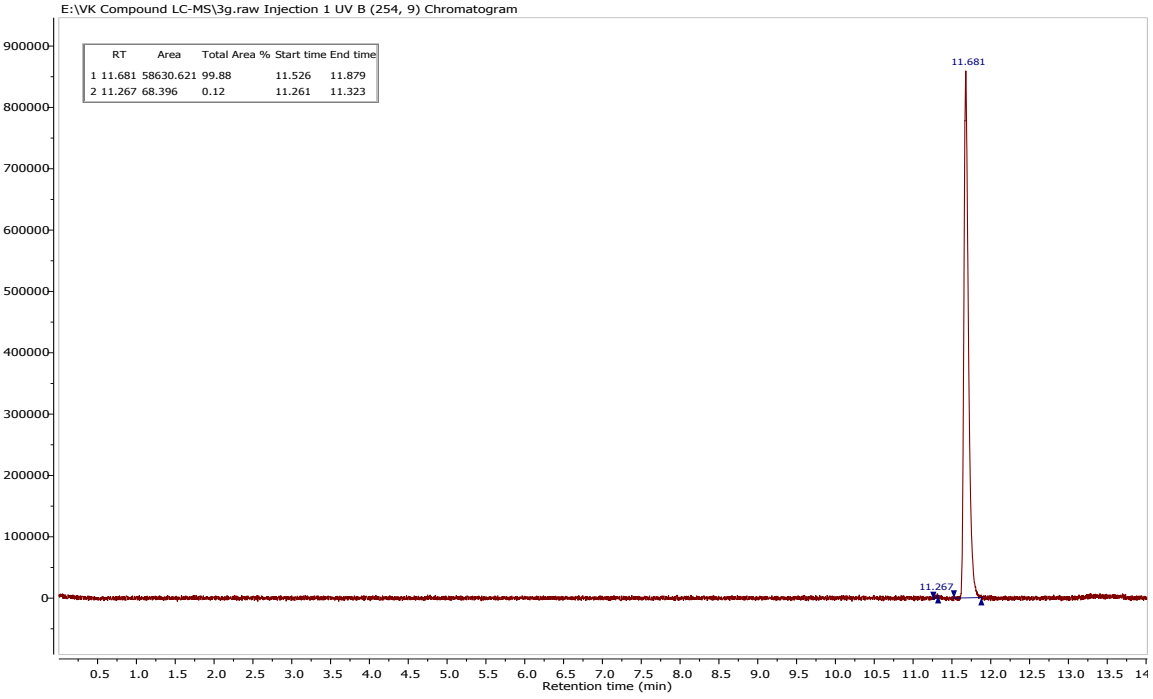
3e


E:\VK Compound LC-MS\3e.raw Injection 1 UV B (254, 9) Chromatogram
RT Area Total Area % Start time End time
1 13.194 304.150 0.23 13.139 13.225
2 12.291 131312.715 98.84 12.192 12.524
3 11.311 775.430 0.58 11.243 11.380
4 10.328 464.446 0.35 10.240 10.362
12.291
10.328
11.311
13.194
Retention time (min)

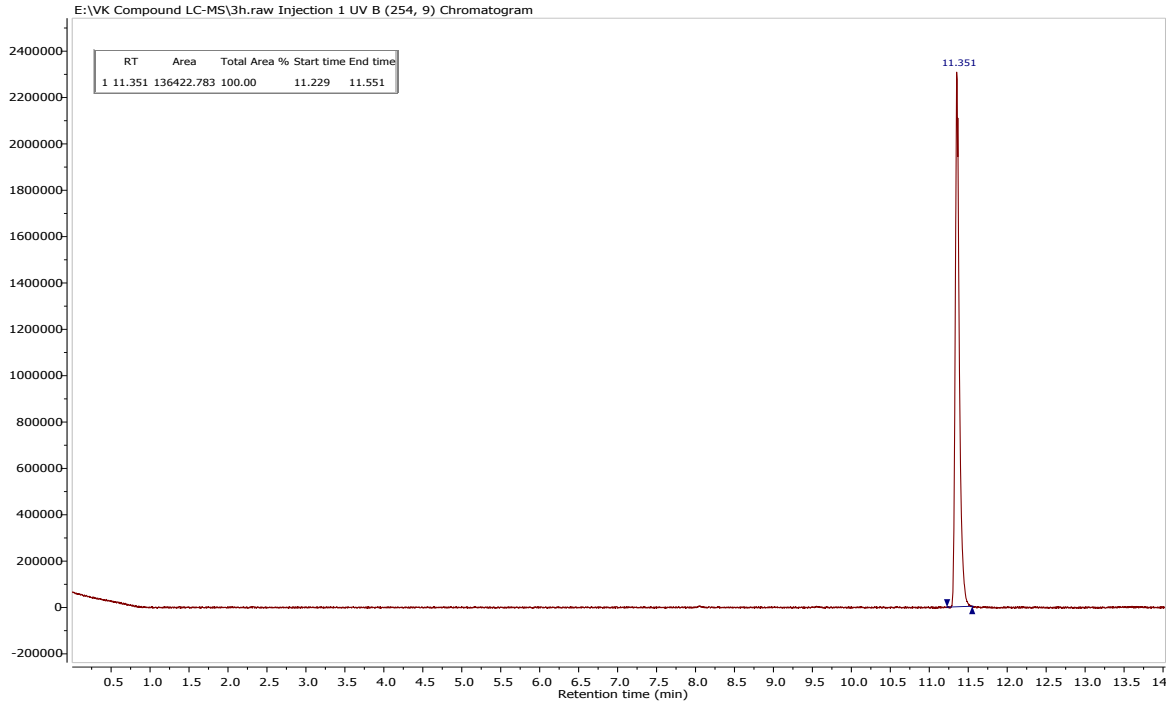
3f


E:\dropbox\LL3...91210183506.raw Injection 1 UV B (254, 9) Chromatogram
RT Area Total Area %
1 13.411 0.000 1.17
2 12.181 0.002 96.95
3 8.565 0.000 1.23
4 6.913 0.000 0.65
12.181
6.913
8.565
13.411
Retention time (min)

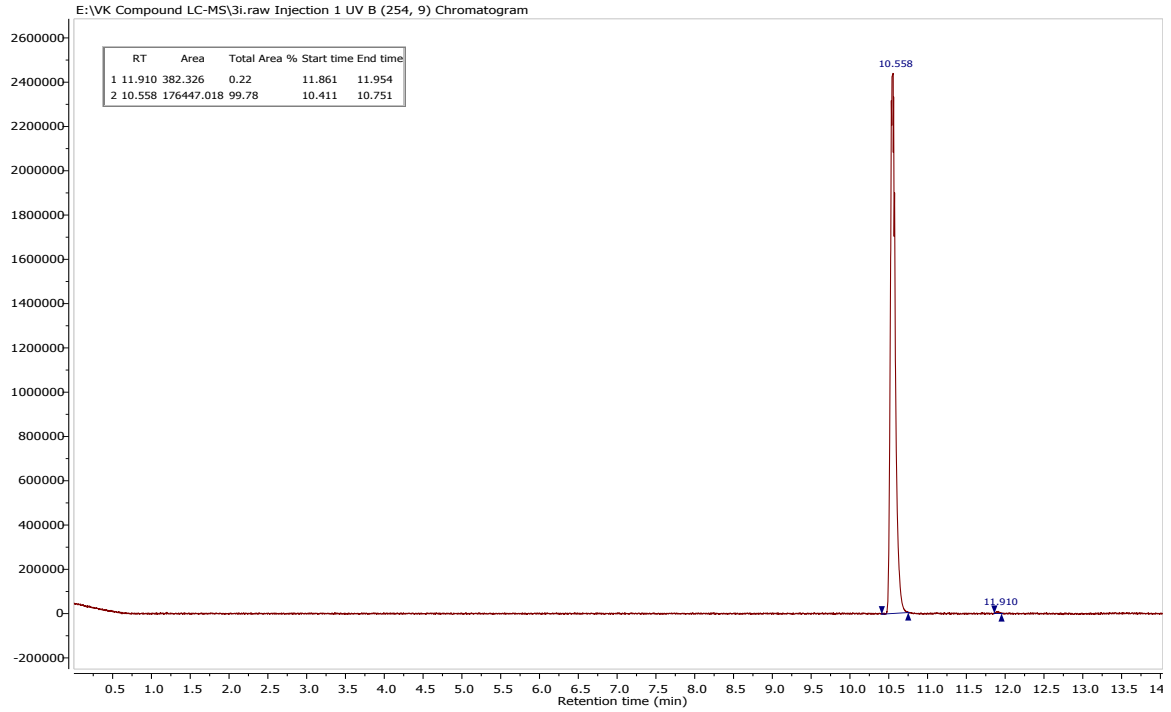
3g


E:\VK Compound LC-MS\3g.raw Injection 1 UV B (254, 9) Chromatogram
RT Area Total Area % Start time End time
1 11.681 58630.621 99.88 11.526 11.879
2 11.267 68.396 0.12 11.261 11.323
11.681
11.267
Retention time (min)

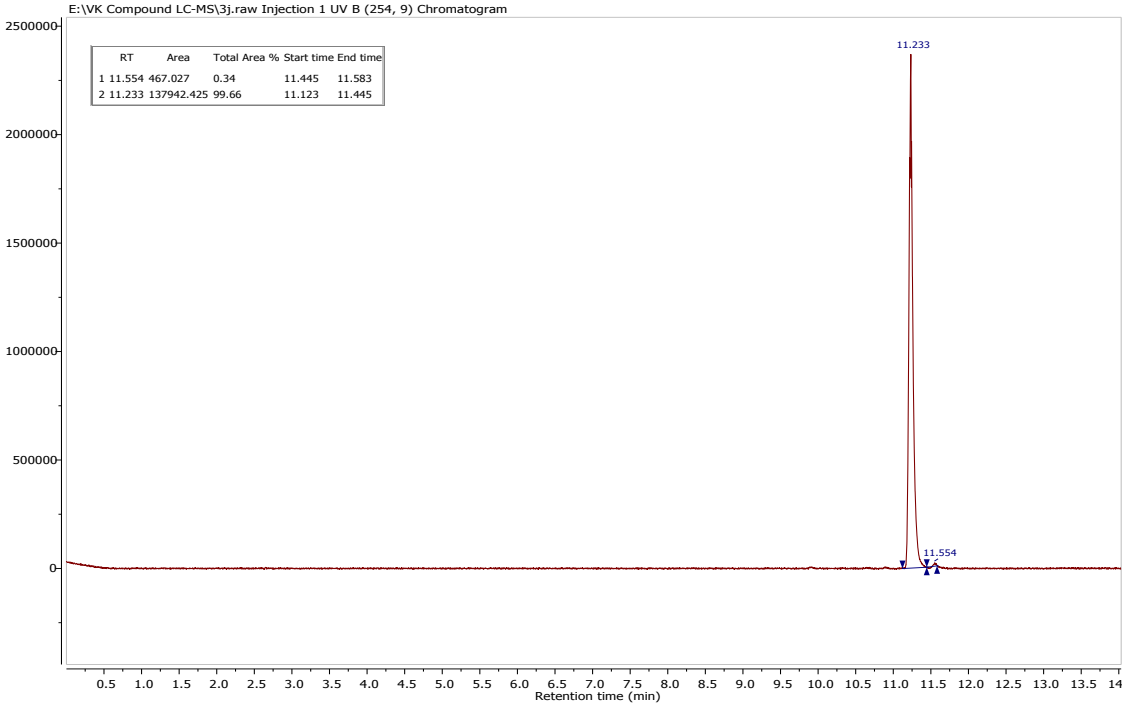
3h


E:\VK Compound LC-MS\3h.raw Injection 1 UV B (254, 9) Chromatogram
RT Area Total Area % Start time End time
1 11.351 136422.783 100.00 11.229 11.551
11.351
Retention time (min)

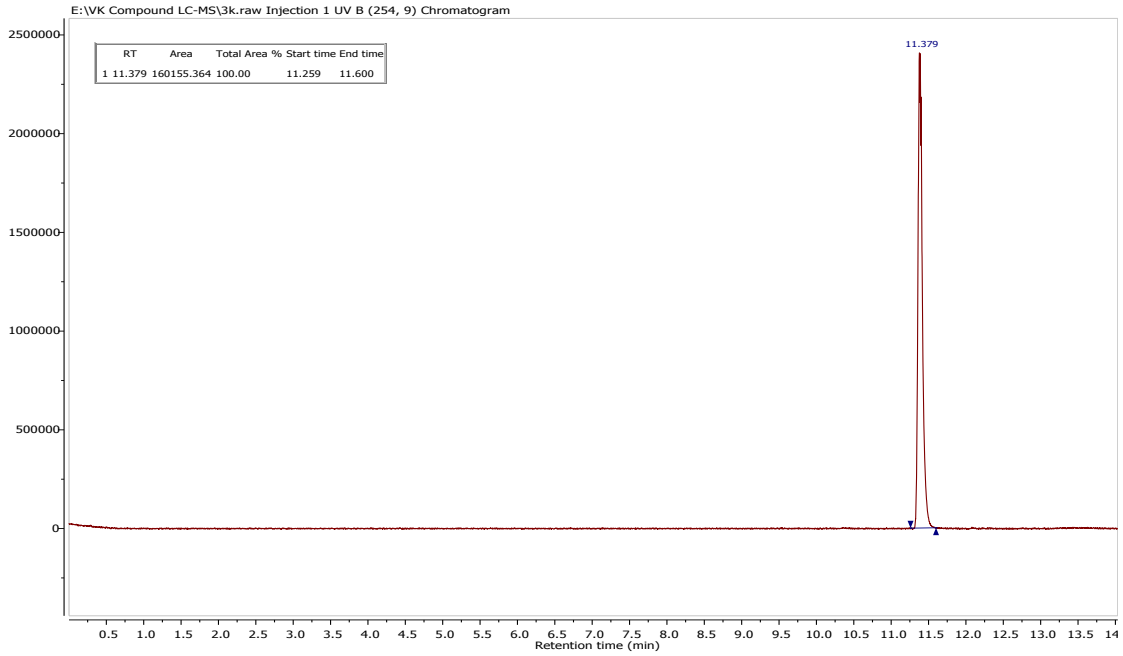
3i


E:\VK Compound LC-MS\3i.raw Injection 1 UV B (254, 9) Chromatogram
RT Area Total Area % Start time End time
1 11.910 382.326 0.22 11.861 11.954
2 10.558 176447.018 99.78 10.411 10.751
10.558
11.910
Retention time (min)

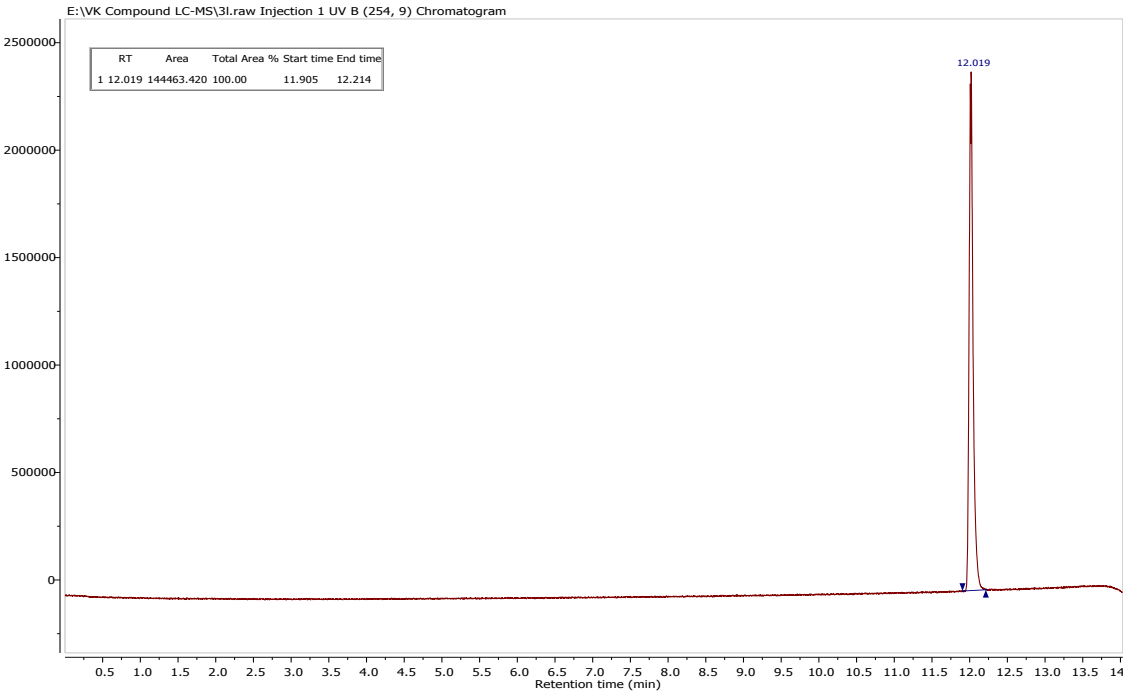
3j


E:\VK Compound LC-MS\3j.raw Injection 1 UV B (254, 9) Chromatogram
RT Area Total Area % Start time End time
1 11.554 467.027 0.34 11.445 11.583
2 11.233 137942.425 99.66 11.123 11.445
11.233
11.554
Retention time (min)

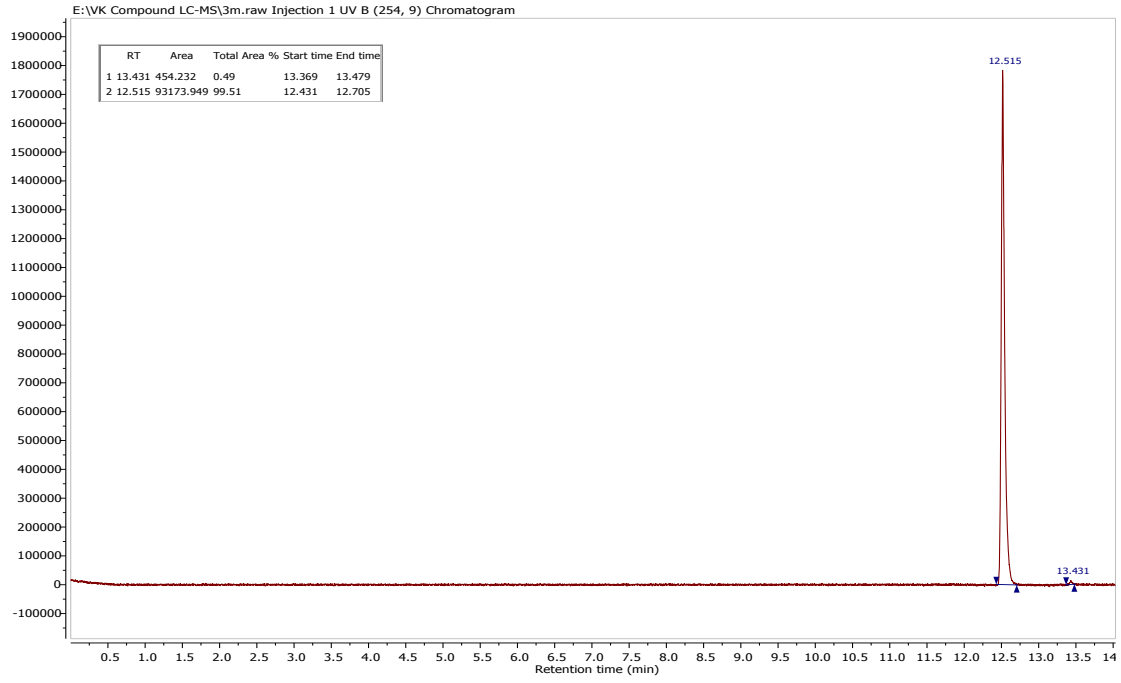
3k


E:\VK Compound LC-MS\3k.raw Injection 1 UV B (254, 9) Chromatogram
RT Area Total Area % Start time End time
1 11.379 160155.364 100.00 11.259 11.600
11.379
Retention time (min)

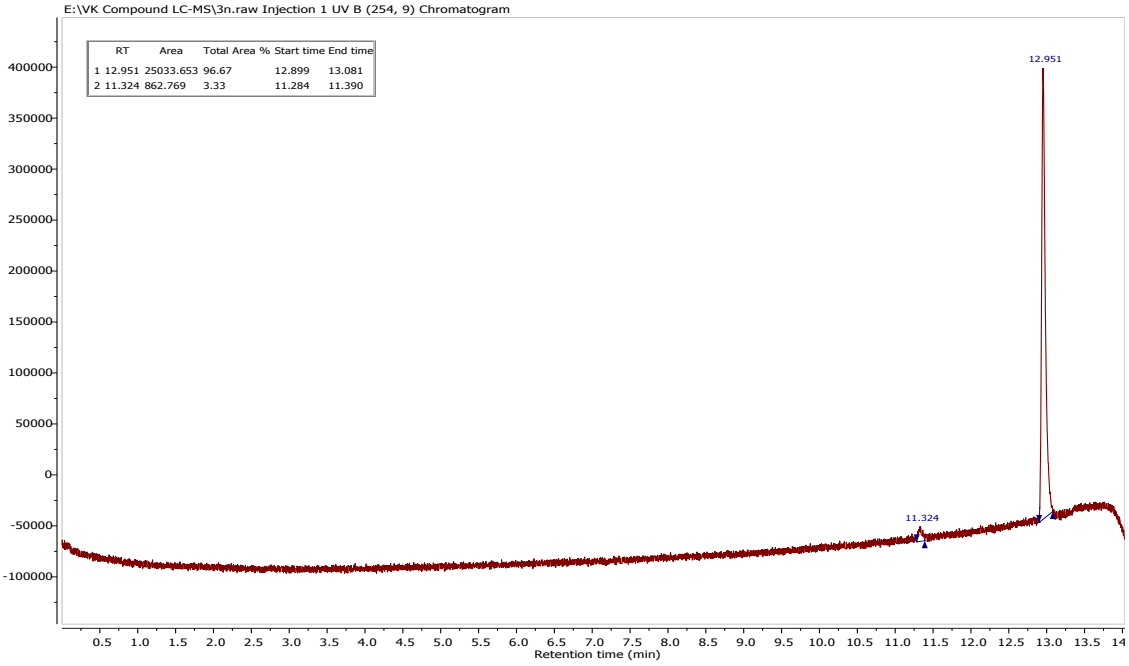
3l


E:\VK Compound LC-MS\3l.raw Injection 1 UV B (254, 9) Chromatogram
RT Area Total Area % Start time End time
1 12.019 144463.420 100.00 11.905 12.214
12.019
Retention time (min)

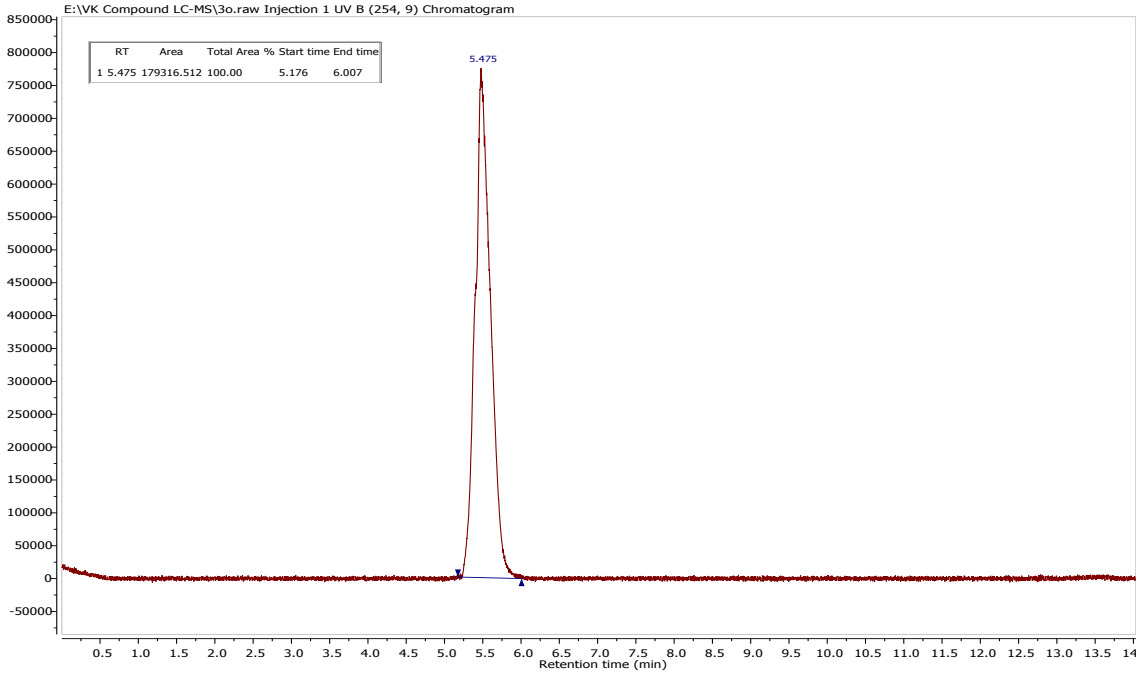
3m


E:\VK Compound LC-MS\3m.raw Injection 1 UV B (254, 9) Chromatogram
RT Area Total Area % Start time End time
1 13.431 454.232 0.49 13.369 13.479
2 12.515 93173.949 99.51 12.431 12.705
12.515
13.431
Retention time (min)

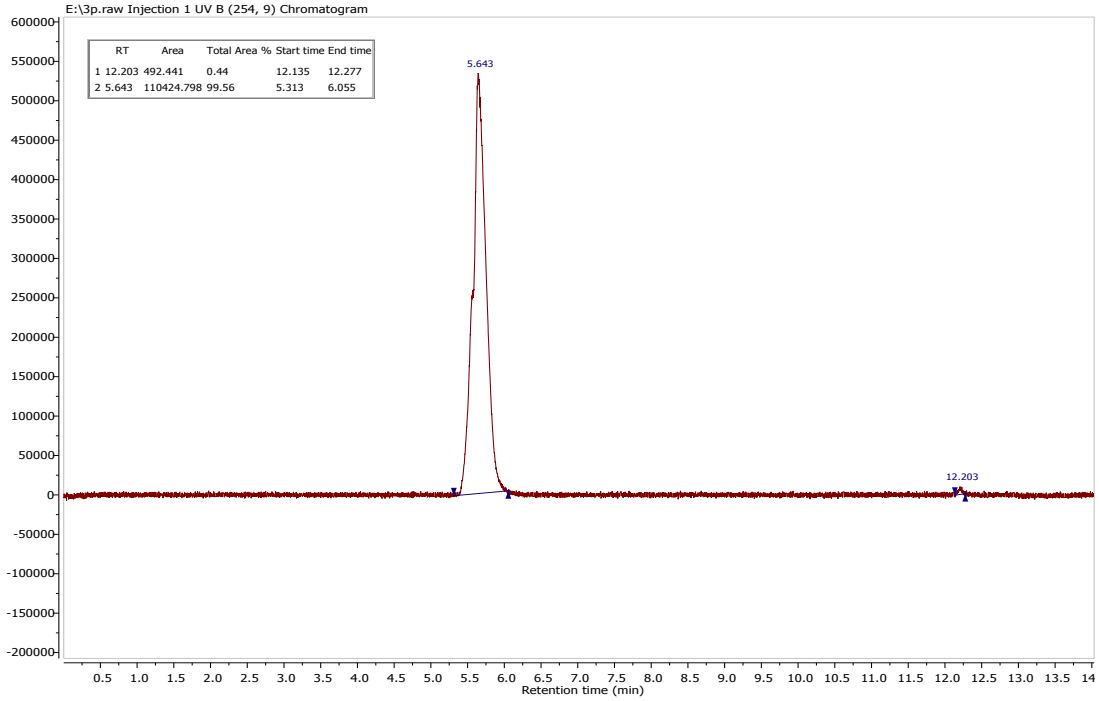
3n


E:\VK Compound LC-MS\3n.raw Injection 1 UV B (254, 9) Chromatogram
RT Area Total Area % Start time End time
1 12.951 25033.653 96.67 12.899 13.081
2 11.324 862.769 3.33 11.284 11.390
12.951
11.324
Retention time (min)

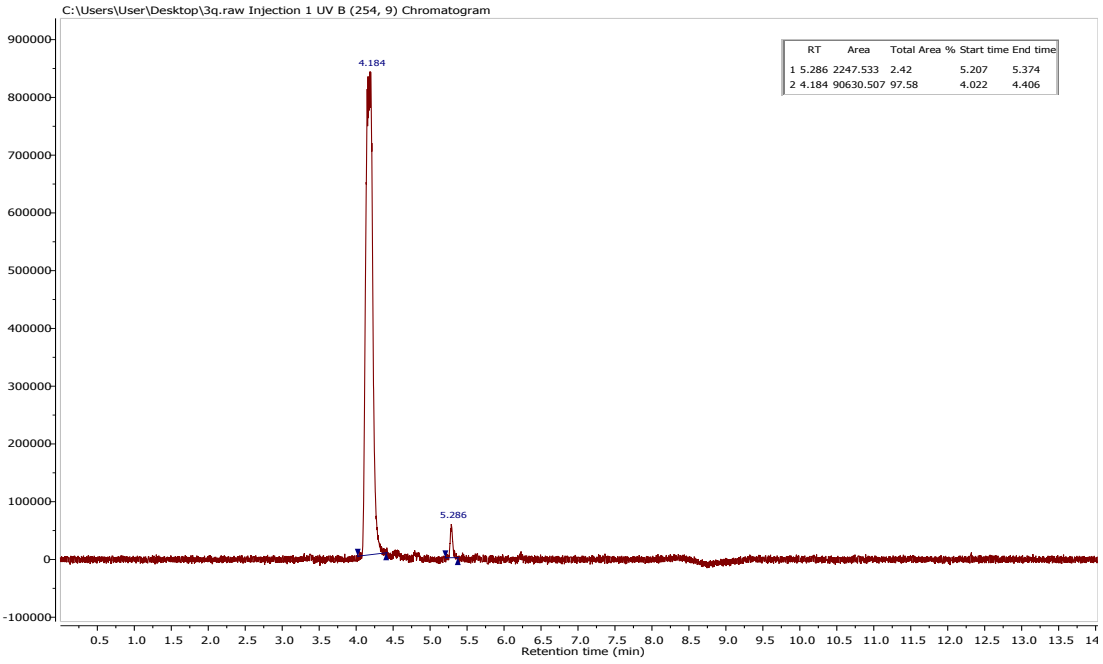
3o


E:\VK Compound LC-MS\3o.raw Injection 1 UV B (254, 9) Chromatogram
RT Area Total Area % Start time End time
1 5.475 179316.512 100.00 5.176 6.007
5.475
Retention time (min)

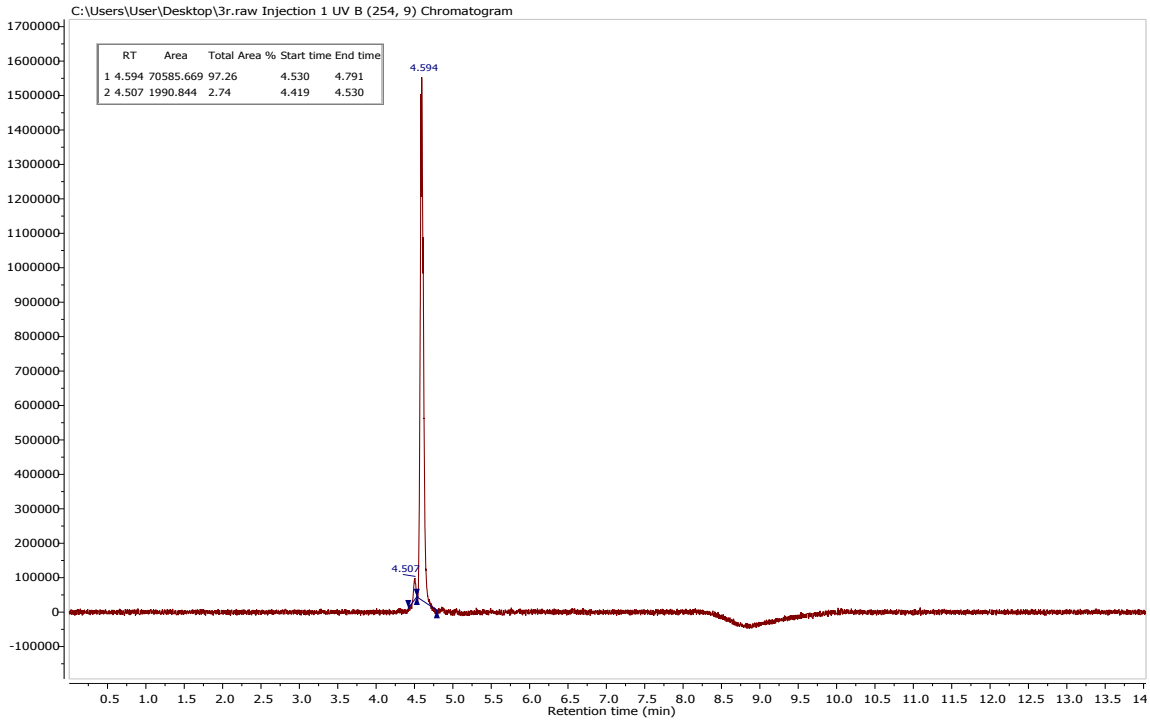
3p


E:\3p.raw Injection 1 UV B (254, 9) Chromatogram
RT Area Total Area % Start time End time
1 12.203 492.441 0.44 12.135 12.277
2 5.643 110424.798 99.56 5.313 6.055
5.643
12.203
Retention time (min)

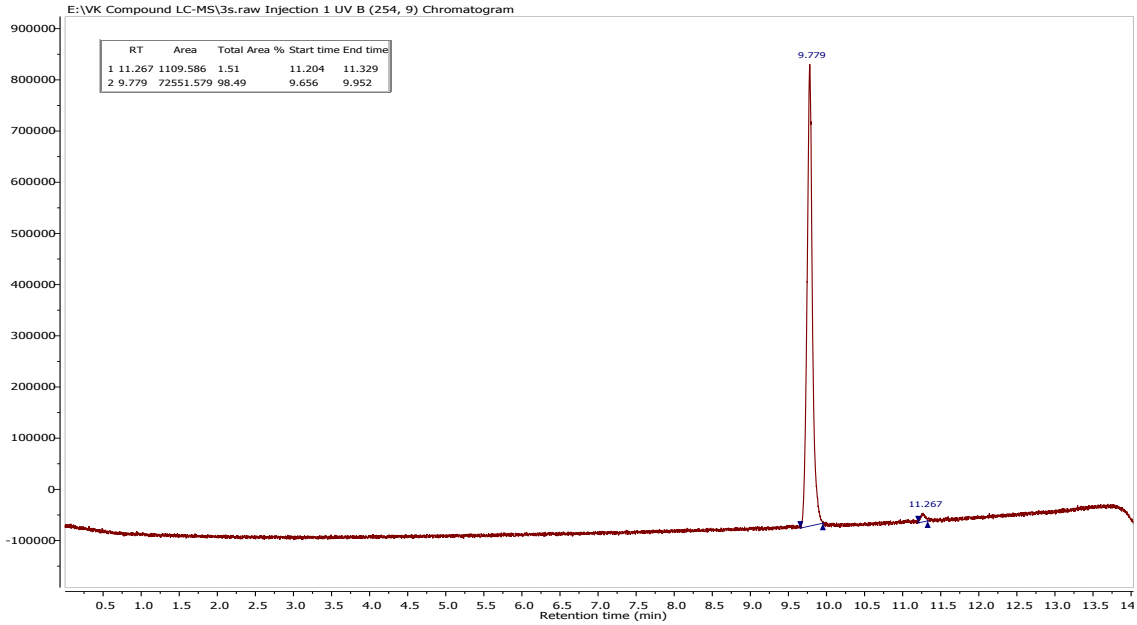
3q


C:\Users\User\Desktop\3q.raw Injection 1 UV B (254, 9) Chromatogram
RT Area Total Area % Start time End time
1 5.286 2247.533 2.42 5.207 5.374
2 4.184 90630.507 97.58 4.022 4.406
4.184
5.286
Retention time (min)

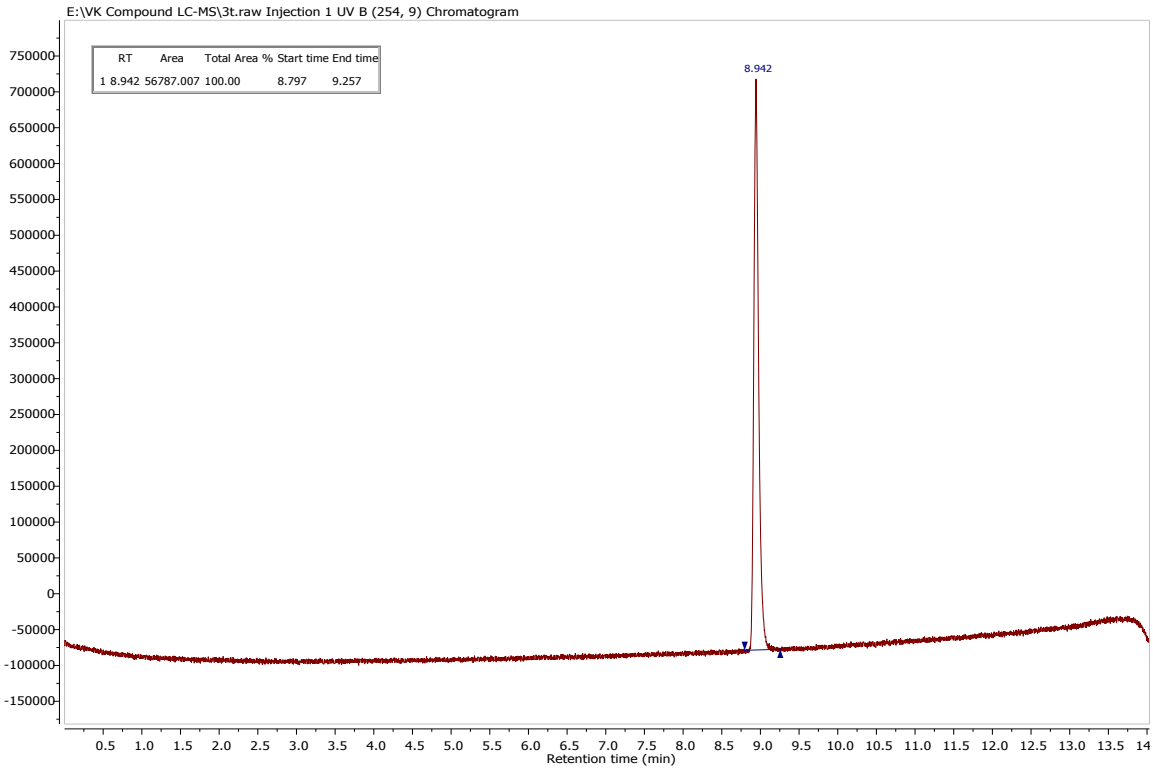
3r


C:\Users\User\Desktop\3r.raw Injection 1 UV B (254, 9) Chromatogram
RT Area Total Area % Start time End time
1 4.594 70585.669 97.26 4.530 4.791
2 4.507 1990.844 2.74 4.419 4.530
4.594
4.507
Retention time (min)

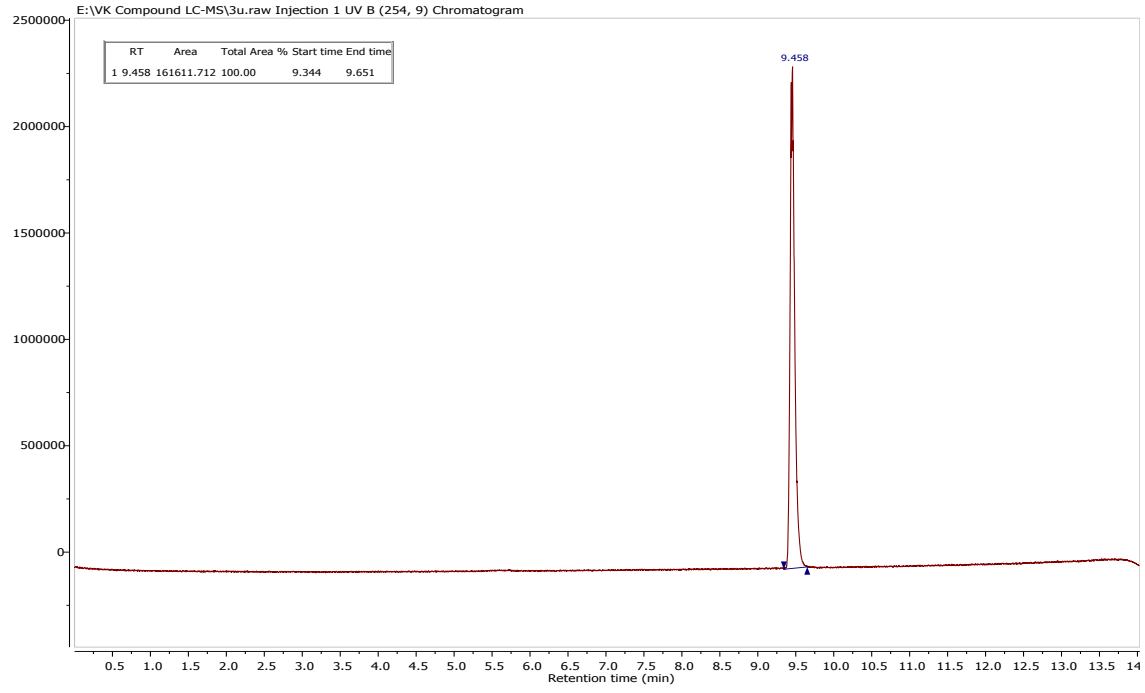
3s


E:\VK Compound LC-MS\3s.raw Injection 1 UV B (254, 9) Chromatogram
RT Area Total Area % Start time End time
1 11.267 1109.586 1.51 11.204 11.329
2 9.779 72551.579 98.49 9.656 9.952
9.779
11.267
Retention time (min)

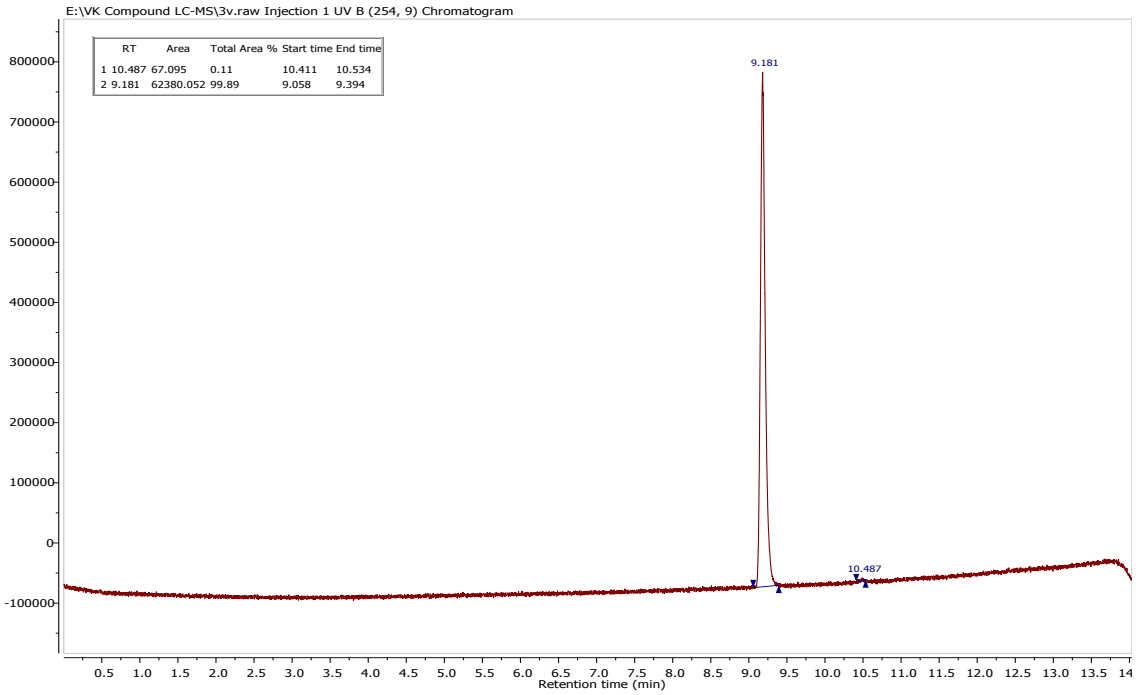
3t


E:\VK Compound LC-MS\3t.raw Injection 1 UV B (254, 9) Chromatogram
RT Area Total Area % Start time End time
1 8.942 56787.007 100.00 8.797 9.257
8.942
Retention time (min)

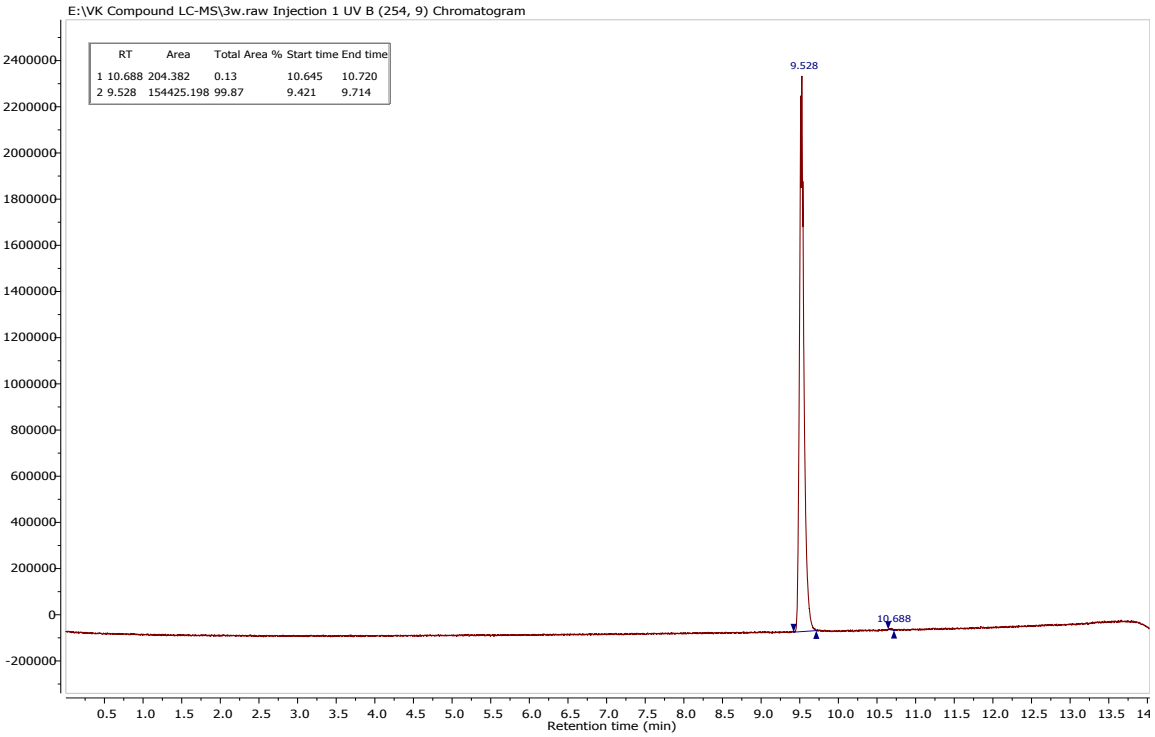
3u


E:\VK Compound LC-MS\3u.raw Injection 1 UV B (254, 9) Chromatogram
RT Area Total Area % Start time End time
1 9.458 161611.712 100.00 9.344 9.651
9.458
Retention time (min)

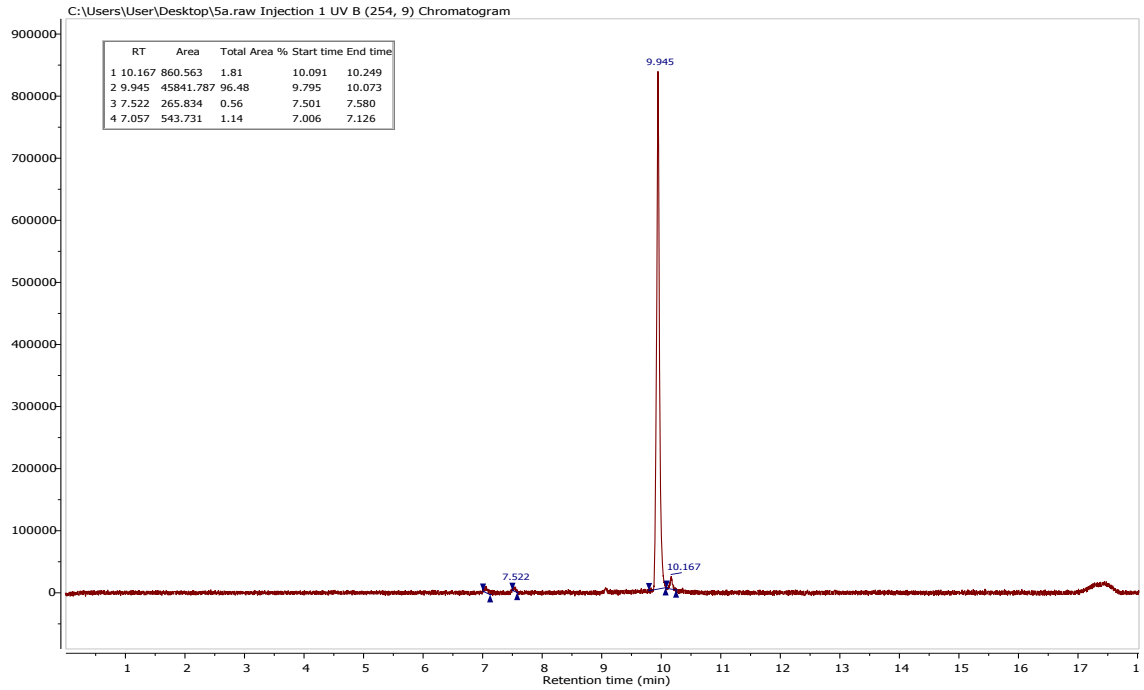
3v


E:\VK Compound LC-MS\3v.raw Injection 1 UV B (254, 9) Chromatogram
RT Area Total Area % Start time End time
1 10.487 67.095 0.11 10.411 10.534
2 9.181 62380.052 99.89 9.058 9.394
9.181
10.487
Retention time (min)

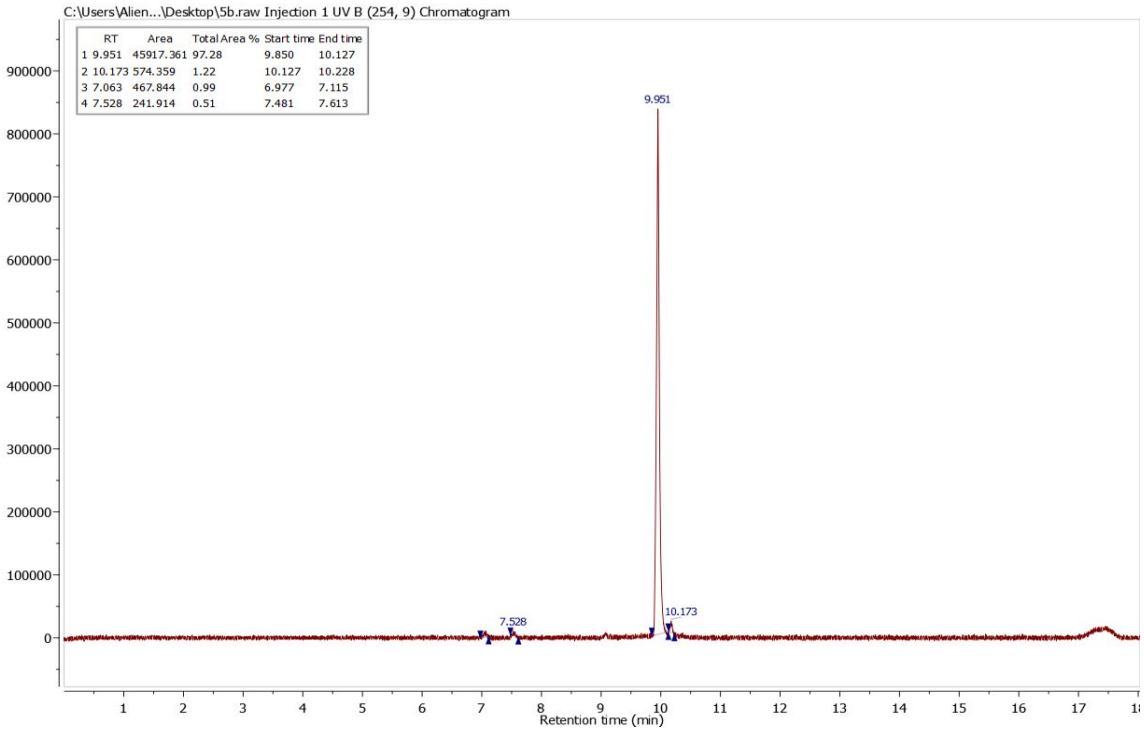
3w


E:\VK Compound LC-MS\3w.raw Injection 1 UV B (254, 9) Chromatogram
RT Area Total Area % Start time End time
1 10.688 204.382 0.13 10.645 10.720
2 9.528 154425.198 99.87 9.421 9.714
9.528
10.688
Retention time (min)

5a



5b



Reference

(1) West, P. J.; Saunders, G. W.; Billingsley, P.; Smith, M. D.; White, H. S.; Metcalf, C. S.; Wilcox, K. S. Recurrent epileptiform discharges in the medial entorhinal cortex of kainate-treated rats are differentially sensitive to antiseizure drugs. *Epilepsia.* **2018**, *59*, 2035-2048.

(2) Shultz, M. D.; Cao, X.; Chen, C. H.; Cho, Y. S.; Davis, N. R.; Eckman, J.; Fan, J.; Fekete,

A.; Firestone, B.; Flynn, J.; Green, J.; Growney, J. D.; Holmqvist, M.; Hsu, M.; Jansson, D.; Jiang, L.; Kwon, P.; Liu, G.; Lombardo, F.; Lu, Q.; Majumdar, D.; Meta, C.; Perez, L.; Pu, M.; Ramsey, T.; Remiszewski, S.; Skolnik, S.; Traebert, M.; Urban, L.; Uttamsingh, V.; Wang, P.; Whitebread, S.; Whitehead, L.; Yan-Neale, Y.; Yao, Y. M.; Zhou, L.; Atadja, P., Optimization of the in vitro cardiac safety of hydroxamate-based histone deacetylase inhibitors. *J. Med. Chem.* **2011**, *54*, 4752-4772.