CN128, a new orally active hydroxypyridinone iron chelator

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Supporting Information

General procedure for biological evaluation

Preparation of ^{59}Fe-ferritin

250-320 g, spontaneously breathing male SD rats were anaesthetised with 2-3% inhaled isofluorane and placed on a heated mat. Buprenorphine (Vetergesic) was administered i.m. (20 μg/kg) to provide post-operative analgesia. The dorsal and ventral neck regions were shaved and a 1.5 cm midline incision was made at both sites. A skin tunnelling needle passed subcutaneously over the right shoulder from the ventral to the dorsal incision site. A vascular access port catheter (3Fr, Silastic catheter, Harvard Instruments cat no# PY 72-4376) was threaded subcutaneously to the ventral neck region, leaving the saline filled port at the dorsal incision site. The catheter was cut to the appropriate length and flushed with 50 IU/ml heparin in clinical grade saline (0.9%). The right jugular vein was carefully isolated with 2 suture threads, and the anterior portion completely occluded. A small incision was made with a 23 gauge needle and the catheter inserted posterially for approximately 1 cm past the suture and secured in place. The ventral neck region was sutured and the region sterilised. The port portion of the catheter was placed subcutaneously at the dorsal incision site and the incision site sutured and sterilised. Animals were recovered in a heated chamber (30 ^{o}C for 1-2 h) before being placed in the holding room.

24 h post vascular access port implantation, no animals displayed any visual clinical signs of distress or infection. Animals were re-anaesthetised with inhaled isofluorane 2-3% as before and the lines flushed with heparinised saline followed by 3 mL of warmed packed red blood cells (0.5 mL/min) using a Huber needle followed by a further heparinised saline flush (1.5 mL/kg). Animals were recovered in a heated chamber and returned to the holding room. Packed red blood cells were prepared from two adult rats (250-300 g) anaesthetised as above. Approximately 10 mL of blood was removed from each rat and added to 80 mL of phosphate buffered saline containing 2 mM citrate at 4 ^{o}C. The diluted blood was centrifuged (2500 Rev/min) for 5 min, washed with phosphate buffered saline and re-suspended in buffered saline.

24 h post red blood cell infusion; animals did not display any visual clinical signs of distress or infection. Animals were re-anaesthetised with inhaled isofluorane 2-3% as before and the lines flushed with heparinised saline. 200 μCi of ^{59}Fe in 0.5ml of sodium citrate (50 mM, pH 7.0) was administered via the access port and flushed through with heparinised saline. Animals were recovered and placed in a designated holding room for a further 24 hours. The animals were sacrificed and the livers removed. The combined livers (60-70 g) were homogenised in 4 vol (w/v) of sodium acetate (0.25 M, pH 4.8) the homogenate was heated to 70 ^{o}C and maintained at 70-75 ^{o}C for 10 min. After cooling to 4 ^{o}C the coagulated proteins were removed by centrifugation at 2500 g for 15 min. the supernatant which contained the ferritin was treated with solid ammonium sulphate, 95 g added to 325 mL (50% saturation) and allowed to stand for 30 min at 4 ^{o}C. The resulting precipitate was separated by centrifugation at 3500 g for 15 min at 4 ^{o}C. The precipitate was re-dissolved in a small volume (15 mL) of sodium acetate (0.25 M, pH 4.8). Undissolved precipitate was removed by passage through PD-10 columns (Sephadex G25; 1 column can treat 3 mL). The combined eluate was centrifuged at 4 ^{o}C for 2 h at 100,000 g. The resulting precipitate was dissolved in sodium phosphate solution (50 mM, pH 7.4) and stored at 4 ^{o}C. The ferritin iron concentration was determined by overnight hydrolysis in 10 M HCl at 37 ^{o}C and subsequent analysed with ferrozine. The specific activity of the ferritin iron was typically determined as 0.06-0.10 μCi/μg.

Iron mobilisation was determined using the following equation:

$$\text{Iron mobilisation (\%)} = (\text{Activity}_{(\text{gut and faeces})}) / (\text{Activity}_{(\text{gut and faeces})} + \text{Activity}_{(\text{liver})})) \times 100$$

$$\text{Efficacy (\%)} = \text{Iron mobilisation (\%)} - \text{Control (\%)}$$

To determine technical success, the recovery of ^{59}Fe-ferritin was determined. The activity of sample of ^{59}Fe-ferritin equal to one dose was determined by gamma counting, and used in the following equation:

$$\text{Recovery (\%)} = ((\text{Activity}_{(\text{gut and faeces})} + \text{Activity}_{(\text{liver})}) / \text{Activity}_{(\text{total})}) \times 100$$

Metabolism study of AAHPOs. 1) Glucuronidation One milligram of liver microsomes (rat, guinea pig or human (Celsis IVT, Neuss, Germany), were incubated with 2 mM uridine 5'-diphosphoglucuronic acid trisodium salt, 5 mM magnesium chloride, and 0.2 mM iron chelator. This solution was made up to 1 mL with tris-HCL (50 mM, pH 7.4), with the addition of 3 µl triton x-100 (all chemicals from Sigma, Dorset, UK). Samples were included with no microsomes as controls. Samples were placed in a shaking water bath at 37 ^{o}C for 1 h. After this incubation, 0.1 mL of 25% trichloroacetic acid was added to the samples to stop the reaction, and 0.1 mL of 2 mM CP95 as an internal control. Samples were then centrifuged at 2000 rpm for 10 min, and the supernatants analysed by HPLC. To determine whether the glucuronidation was reversed by the addition of β-glucuronidase, samples were removed from the water bath, and 5000 units of β-glucuronidase (Sigma, UK) were added. Samples were returned to the water bath at 37 ^{o}C for 16 h before the addition of tricholoracetic acid and the internal standard as before. Samples were syringed filtered before analysis by HPLC. **2) Phase 1 oxidation** One milligram of liver microsomes (rat, guinea pig or human (Celsis IVT, Neuss, Germany), were incubated with 2 mM NADPH, 5 mM magnesium chloride, and 0.2 mM iron chelator. This solution was made up to 1 mL with tris-HCl (pH 7.4) (all chemicals from Sigma, Dorset, UK). Samples were included with no microsomes as controls. Samples were placed in a shaking water bath at 37 ^{o}C for 1 h. After this incubation, 0.1 mL of 2 mM CP95 as an internal control. Samples were then centrifuged at 2000 rpm for 10 minutes, and the supernatants analysed by HPLC. For HPLC, the gradient mobile phase system included: a) 1-heptane sulfonic acid solution (5 mM, pH = 2); and b) acetonitrile. The percentage of acetonitrile was increased from 2-40% over 20 min and maintained for a further 25 min. The flow rate was 1 mL/min and the analytes were monitored at 280 nm. A reversed-phase polymer HPLC column (PLRP-S 100Å, 25 x 0.46 cm ID, 5 µm (Polymer Laboratories Ltd, Church Stretton, Shropshire, UK) was used for the chromatography.

Physicochemical characterisation of 8p

pKa and iron stability constants The automatic titration system used in this study comprised of an autoburette (Metrohm Dosimat 765 liter ml syringe) and Mettler Toledo MP230 pH meter with Metrohm pH electrode (6.0133.100) and a reference electrode (6.0733.100). 0.1 M KCl electrolyte solution was used to maintain the ionic strength. The temperature of the test solutions was maintained in a thermostatic jacketed titration vessel at 25 ^{o}C ± 0.1 ^{o}C by using a Techne TE-8J temperature controller. The solution under investigation was stirred vigorously during the experiment. A Gilson Mini-plus#3 pump with speed capability (20 mL/min) was used to circulate the test solution through a Hellem quartz flow cuvette. For the stability constant determinations, a 50 mm path length cuvette was used, and for pKa determinations, a cuvette path length of 10 mm was used. The flow cuvette was mounted on an HP 8453 UV-visible spectrophotometer. All instruments were interfaced to a computer and controlled by a Visual Basic program. Automatic titration and spectral scans adopted the following strategy: the pH of a solution was increased by 0.1 pH unit by the addition of KOH from the autoburette; when pH readings varied by <0.001 pH unit over a 3 s period, an incubation period was activated. For pK_a determinations, a period of 1 min was adopted; for stability constant determinations, a period of 5 min was adopted. At the end of the equilibrium period, the spectrum of the solution was then recorded. The cycle was repeated automatically until the defined end point pH value was achieved. All the titration data were analysed with the pHab program.[1] The species plots were calculated with the Hyss program.[2] Analytical grade

reagent materials were used in the preparation of all solution.

Electrochemical Measurements Cyclic voltammetry (CV) measurements were performed with a CS-120 device (Corrtest). All measurements were conducted under N_2 in a jacketed, one-compartment cell with a mercury working electrode (geometric area: 0.07 cm^2) (Corrtest), a platinum wire counter electrode (Corrtest) and a Ag/AgCl reference electrode. The sweep rate was 200 mV/s. Due to the limited solubility of the CN128 iron complex, 40% DMSO was utilised. A reversible peak was observed during experiment. An aqueous solution of [Fe] = 1 mM, [**8p**] = 4 mM, in 40% DMSO, MOPS (0.1 M, pH 7.4) was investigated using a mercury electrode. Cathodic and anodic peaks were located at -0.631 and -0.576 V vs Ag/AgCl resulting with $E_{1/2} = (E_{cathodic} - E_{anodic})$ = -603 mV (vs SHE). This value is slightly higher than that of deferiprone (-620 mV) [3] but lower than that of DFO (-440 mV), [4] thus confirming that the iron complex of **8p** will not redox cycle under biological conditions. Using this data together with the stability constant acquired from spectrophotometric titration with iron(III), the affinity constant for iron(II) was calculated from **eq1** which is derived from the Nernst equation.

$$59.16\ (\log\beta Fe^{3+} - \log\beta Fe^{2+}) - E_{complex}(Fe^{3+} / Fe^{2+}) \qquad \textbf{eq1}$$

where $E(Fe^{3+} / Fe^{2+})$ is the redox potential of the iron pair in the absence of the ligand and $E_{complex}(Fe^{3+} / Fe^{2+})$ is the redox potential in the presence of the ligand. The calculated value $\log\beta_2$ = 13.57 for iron (II) (deferiprone is 12.4). This generates a pFe^{2+} value of 6.22 (ligand total concentration 10 μM and ferrous total concentration 1 μM at pH 7.4). An estimated $\log K_1$ value 7.32 for ferrous ion was based on the ratio statistic assumption of $\log K_1 = 0.54 \log\beta_2$.

References:

1. Gans, P.; Sabatini, A. Determination of equilibrium constants from spectrophometric data obtained from solutions of known pH: the program pHab. *Annali, di. Chimica*, **1999**, *89*, 45-49.
2. Alderighi, L.; Gans, P.; Ienco, A.; Peters, D.; Sabatini, A.; Vacca, A. Hyperquad simulation and speciation (hyss): a utility program for the investigation of equilibria involving soluble and partially soluble species. *Coordination Chem. Rev.*, **1999**, *184*, 311-318.
3. Merkofer, M.; Kissner, R.; Hider, R. C.; Brunk, U. T.; Koppeol, W. H. Fenton chemistry and iron chelation under physiologically relevant conditions: electrochemistry and kinetics. *Chem. Res. Toxicol.*, **2006**, *19*, 1263-1269.
4. Kitazawa, M.; Iwasaki, K.; Sakamoto, K. Iron chelators may help prevent photoaging. *J. Cosmetic Dermatology*, **2006**, *5*, 210-217.

HPLC analysis of all final compounds

System: Agilent 1290 Series

Mobile phase: Mobile phase A: H_2O containing 0.1% formic acid, Mobile phase B: CH_3OH containing 0.1 % formic acid

Detector: DAD at 254 nm

Column: PhenomenexLuna 5µ C18, 100 Å, 150 X 4.60 mm 5 micron column

Flow rate: 0.5 mL/min

Using the listed gradients:

Mobile phase B was increased linearly from 5% to 95% over 7 min and 95% over the next 2 min, after which the column was equilibrated to 5% for 1 min.

Table S1. Purity of all biological evaluated compounds

Compound	Purity/%	Retention time/ min
8a(CN106)	98.5	1.993
8b(CN108)	95.5	2.007
8c(CN206)	98.8	2.533
8d(CN208)	96.3	2.573
8e(CN116)	98.3	3.180
8f(CN118)	97.0	3.200
8g(CN216)	92.2	3.780
8h(CN218)	97.7	3.740
8i(CN136)	98.7	3.853
8j(CN236)	97.1	4.360
8k(CN146)	100	4.507
8l(CN148)	100	4.000
8m(CN246)	100	4.013
8n(CN248)	97.1	4.520
8o(CN126)	97.6	4.047
8p(CN128)	100	4.587
8q(CN226)	100	4.453
8r(CN228)	100	4.467
8s(CN150)	100	1.573
8t(CN250)	100	2.093

Supplement Tables

Table S2. Comparison of metal affinities of 8p with those of deferiprone.

For divalent metals ($\log\beta_2$)

	Mg^{2+}	Co^{2+}	Ni^{2+}	Zn^{2+}	Cu^{2+}
8p	13.64	11.56	11.96	13.16	19.29
deferiprone	N.D.	11.73	12.13	13.53	19.61

For trivalent metals ($\log\beta_3$)

	Fe^{3+}	Al^{3+}
8p	36.79	29.58
deferiprone	37.4	30.7

Data of deferiprone was cited from a) Martell *et al. Inorg. Chim. Acta*, **1994**, 223, 21-29. b) A. Cilibrizzi *et al Chem. Rev.* **2018**, 118, 7657-7701. c) S. Gama *et al. J. Inorg. Biochem.***2009**, 103, 288-298.

Table S3. The peak area of M1 and M2 in rat bile after orally administration of 8p (150 μmol/kg)

Peak area	Ⅰ♂	Ⅱ♂	Ⅲ♂	Ⅳ♀	Ⅴ♀	Ⅵ♀
M1	245.6	286.7	377.9	413.1	361.1	441.4
M2	1691.2	1862	2018.6	1248.1	842	1238
Ratio (M2/M1)	6.9	6.5	5.3	3.0	2.3	2.8

Table S4. Inhibition of 8p against CYPs in human liver microsomes

	1A2	2B6	2C8	2C9	2C19	2D6	3A4/5
8p (200 μM)	2.14±1.8	0.00	3.06±3.6	0.00	0.00	17.0±1.6	22.4±1.2
Furafyllie (10 μM)	55.0±2.2	/	/	/	/	/	/
Sertraline (10 μM)	/	8.72±4.2	/	/	/	/	/
Quercetin (10 μM)	/	/	57.0±0.63	/	/	/	/
Sulfaphenazole (4 μM)	/	/	/	44.1±3.5	/	/	/
Ticlopidine (20 μM)	/	/	/	/	50.2±1.2	/	/
quinidine (0.4 μM)	/	/	/	/	/	73.4±0.82	/
ketoconazole (0.2 μM)	/	/	/	/	/	/	77.2±0.59

Table S5. Pharmacokinetic parameters of **8p** (150 μmol/kg, 1 day and continuously administered 7 days) in rats (*n*=8, mean±SD)

Time	AUC(0-∞) mg/L·h	MRT(0-∞) H	$t_{1/2z}$ h	CLz L/h/kg	Vz L/kg	Cmax mg/L
1 day	37.66±12	2.71±0.41	3.32±1.0	1.14±0.37	5.29±1.7	19.88±4.5
7 days	35.03±9	3.78±1.1	5.57±1.9	1.18±0.30	9.2±3.1	18.16±9

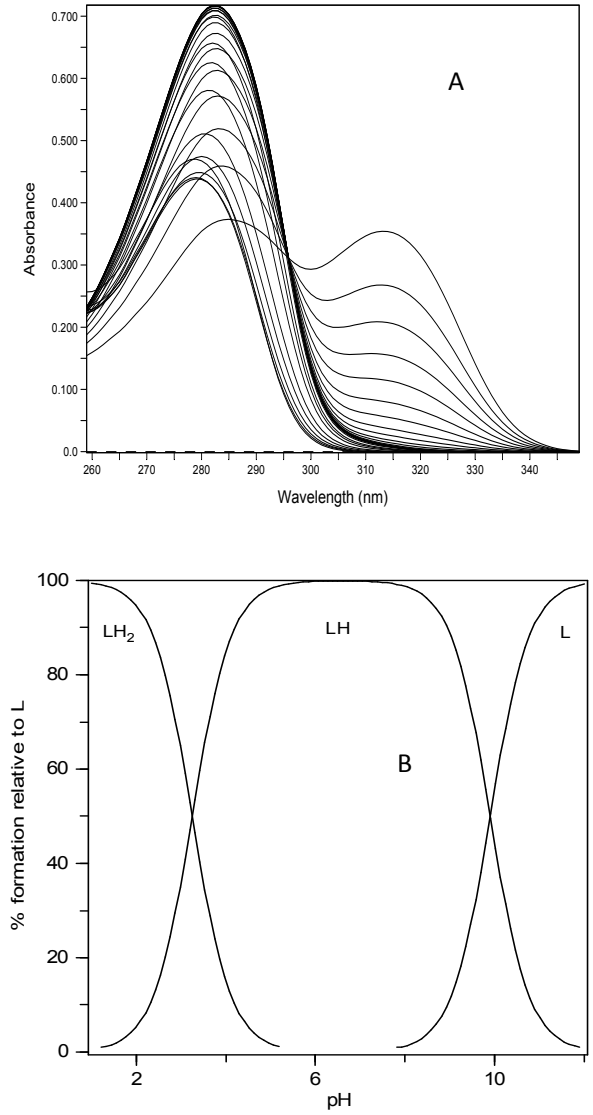
Table S6. The mean concentration of **8p** (150 μmol/kg) in various tissues 10 min after orally administration.

	Mean concentration (μg /g or μg/mL)	Tissue/plasma
plasma	12.19±3.1	
heart	18.18±5.0	1.503
liver	40.40±24	3.275
spleen	15.15±3.7	1.228
lung	11.99±4.6	0.961
kidney	57.76±14	4.760
stomach	107.7±36	8.80
intestine	52.34±36	4.820
skeletal muscle	9.71±3.6	0.7917
brain	1.007±0.17	0.0863
thymus	8.87±2.7	0.7195
adrenal gland	42.84±112	3.701
bone-marrow	1.196±0.36	0.0989

Table S7. The mean concentration of **8p** (150 μmol/kg) in various tissues 6h after orally administration

	Mean concentration (μg /g or μg/mL)
plasma	0.898±0.36
heart	1.106±0.34
liver	3.271±1.2
kidney	3.526±1.2
stomach	3.120±1.1

Figure S1. Determination of pK_a value of 8p

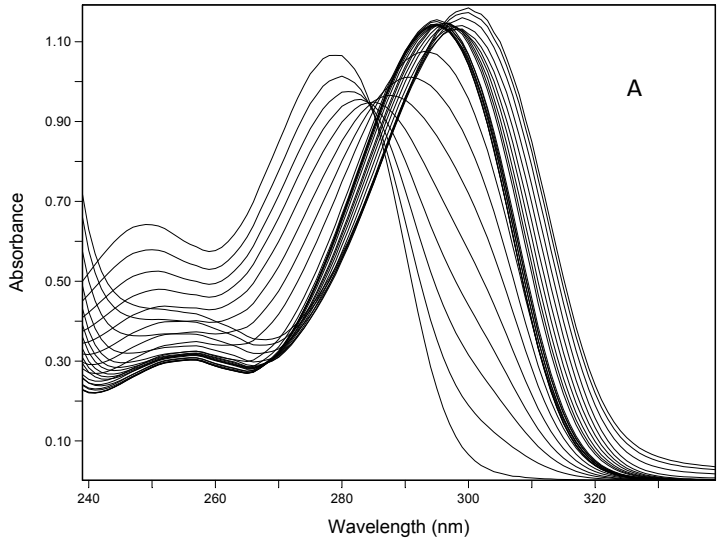


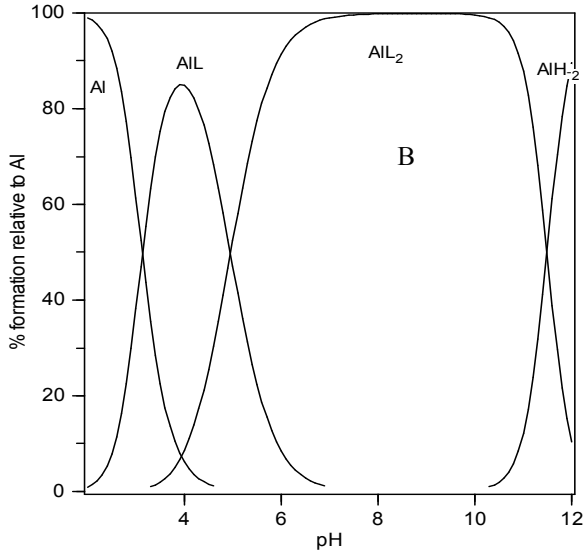
A: Experiment with [L]= 728.3 μM. Start in 15.186 mL KCl (0.1M) at 25 °C, pH=1.858-10.976

B: Speciation plot of CN128 ligand over pH 1-12.

	pK_a values
LH	9.90±0.003
LH_2	3.26±0.003

Figure S2. Determination of logK values for Aluminium (III)





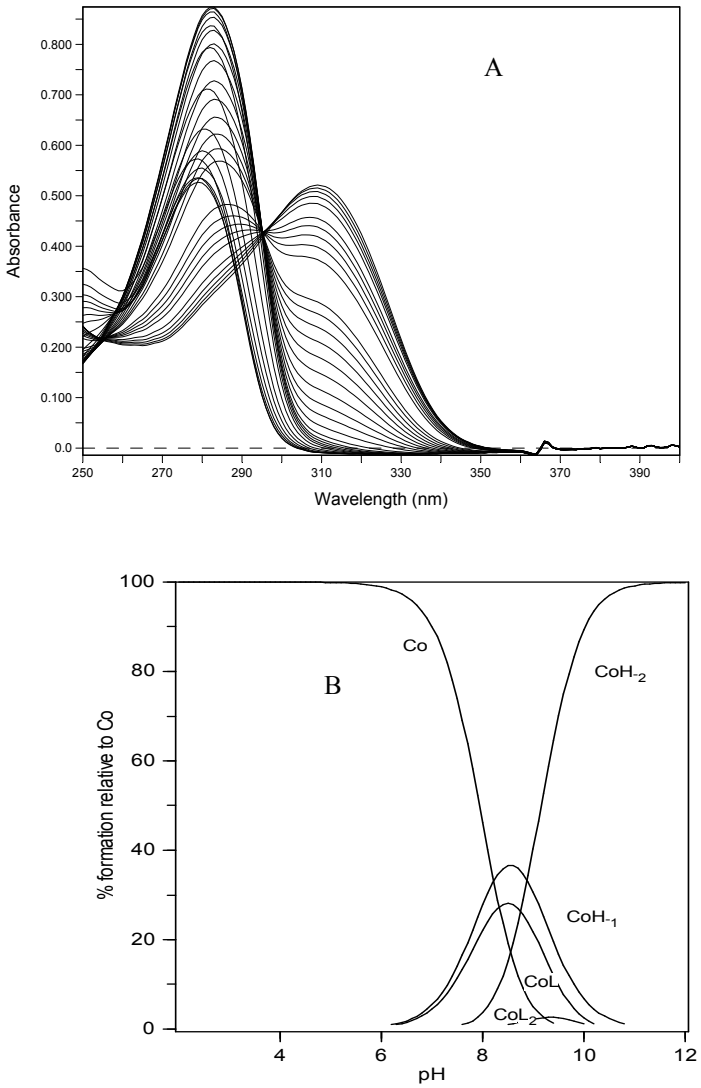
A: Experiment with [L] = 1813.6 μM, [M] = 599.2 μM, ratio of L:M = 3, start in 20.840 mL KCl (0.1M) at 25 °C, pH = 2.047-8.089

B: Speciation plot under the conditions [M]=10^{-6} M, [**8p**] = 10^{-5} M.

	Affinities (log) for Al^{3+}
$logK_1$	12.155±0.0602
$log\beta_2$	22.181±0.011
$log\beta_3$	29.579±0.041
$pM_{7.4}$	13.0

Determination of logK values for divalent metals: Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Mg^{2+}

Figure S3. Cobalt (II)

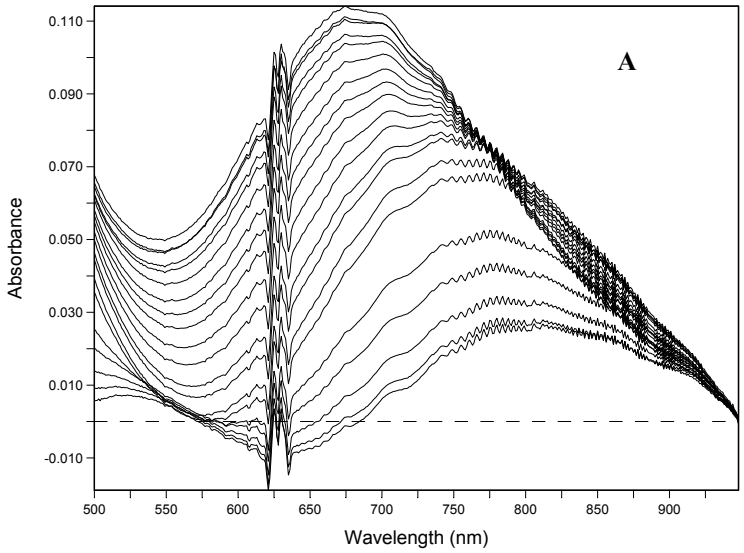


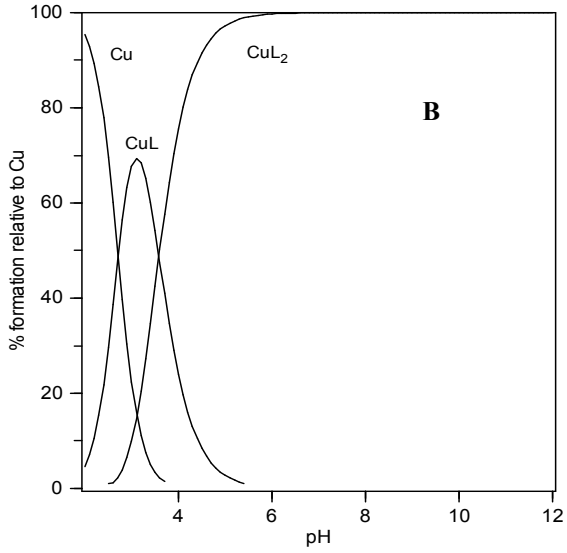
A: Experiment with [L] = 899 μM, [M]= 299.4 μM, ratio of L: M= 3, start in 23.122 mL KCl (0.1M) at 25 ^{o}C, pH = 1.713-11.010

B: Speciation plot under the conditions [M] =10^{-6} M, [**8p**] =10^{-5} M.

	Affinities (log) for Co^{2+}
$logK_1$	6.5957±0.004
$log\beta_2$	11.559±0.014
$pM_{7.4}$	6.11

Figure S4. Copper (II)



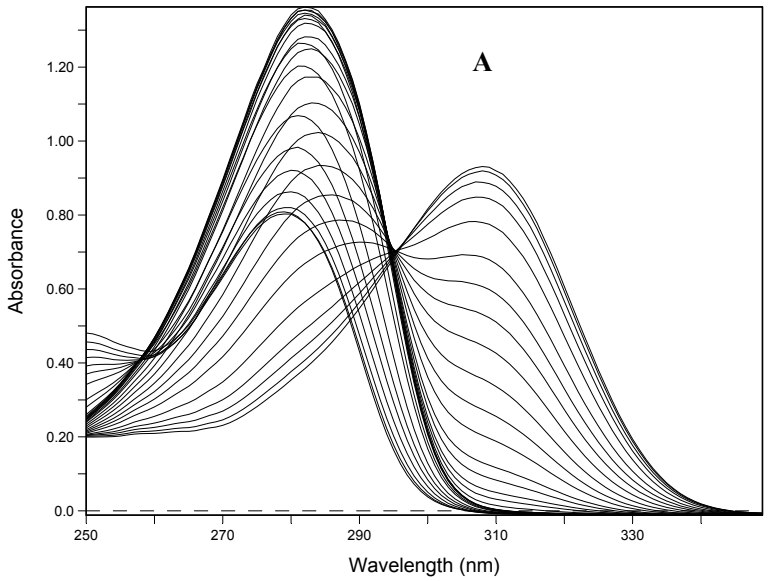


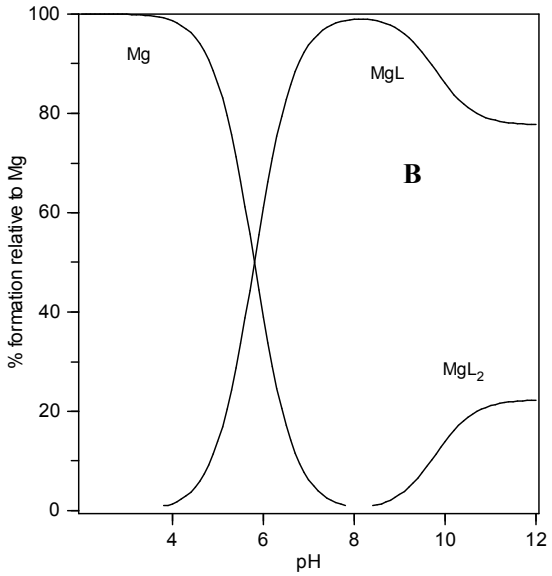
A: Experiment with [L] = 2505.6 μM, [M]= 899.7 μM, ratio of L: M=2.8, start in 21.514 mL KCl (0.1M) at 25 ^{o}C, pH = 1.737-9.795

B: Speciation plot under the conditions [M] =10^{-6} M, [**8p**] =10^{-5} M.

	Affinities (log) for Cu^{2+}
logK_1	10.423±0.045
logβ_2	19.286±0.08
p$M_{7.4}$	8.31

Figure S5. Magnesium (II)



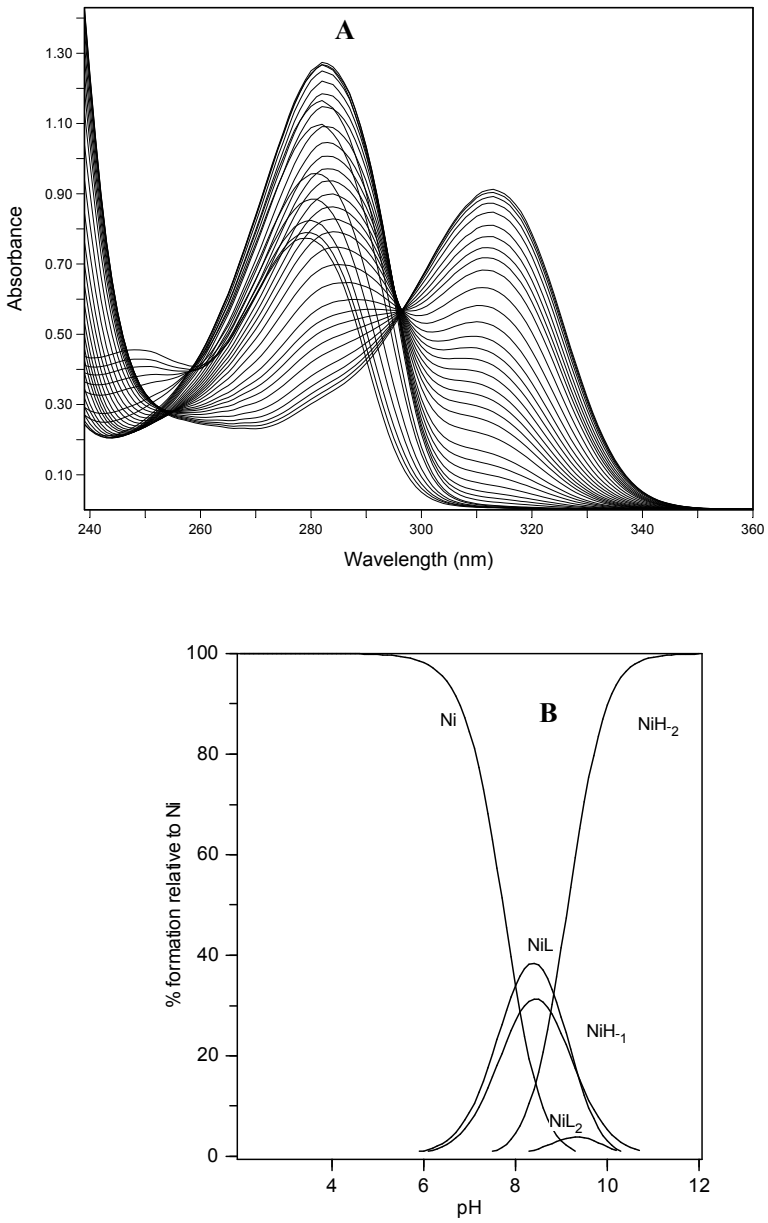


A: Experiment with [L] = 1247.4 µM, [M]= 412.6 µM, ratio of L : M=3, start in 20.301 mL KCl (0.1M)

B: Speciation plot under the conditions [M] = 10^{-6} M, [**8p**] = 10^{-5} M.

	Affinities (log) for Mg^{2+}
$logK_1$	9.122±0.04
$log\beta_2$	13.639±0.041
$pM_{7.4}$	7.59

Figure S6. Nickel (II)

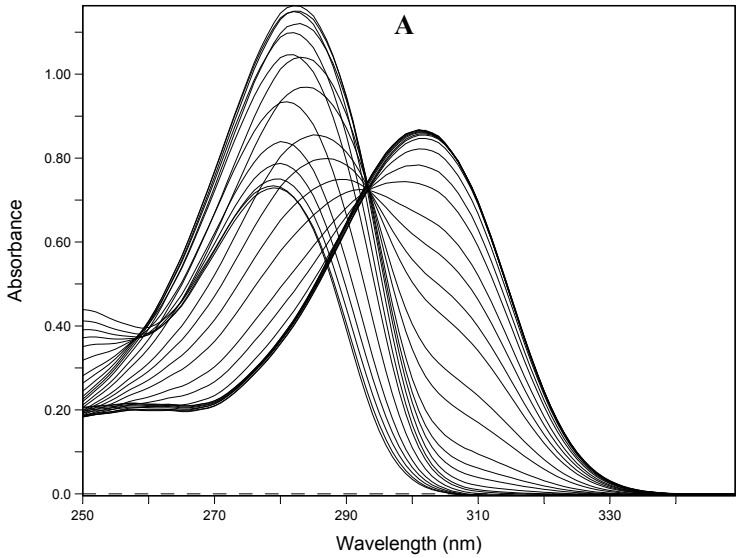


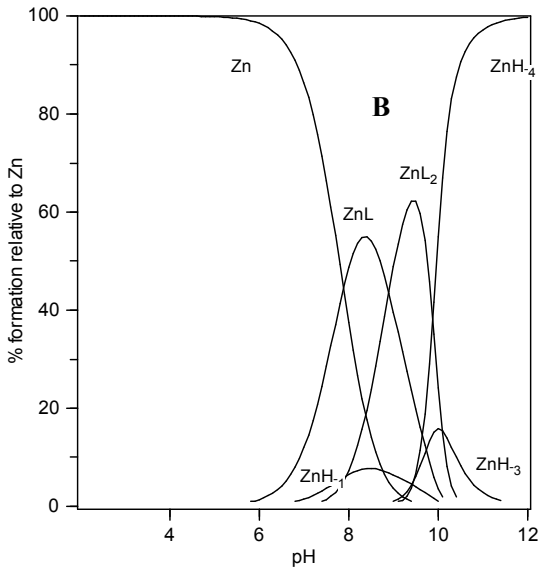
A: Experiment with [L] = 1125.9 μM, [M]= 332.8 μM, ratio of L : M =3.4, start in 20.520 mL KCl (0.1M) at 25 ^{o}C, pH=2.242 -11.013

B: Speciation plot under the conditions [M]=10^{-6} M, [**8p**]=10^{-5}M.

	Affinities (log) for Ni^{2+}
$logK_1$	6.92±0.03
$log\beta_2$	11.962±0.074
$pM_{7.4}$	6.17

Figure S7. Zinc (II)





A: Experiment with [L] = 741.6 μM, [M] = 370.9 μM, ratio of L:M=2, start in 20.613 mL KCl (0.1M) at 25 ^{o}C, pH=1.767-10.996

B: Speciation plot under the conditions [M] = 10^{-6} M, [**8p**] = 10^{-5} M.

	Affinities (log) for Zn^{2+}
$logK_1$	7.0485±0.021
$log\beta_2$	13.156±0.025
$pM_{7.4}$	6.15

Metabolites from rat bile after oral administration of 8p (150 µmol/kg)

Figure S8. HPLC profile of rat bile

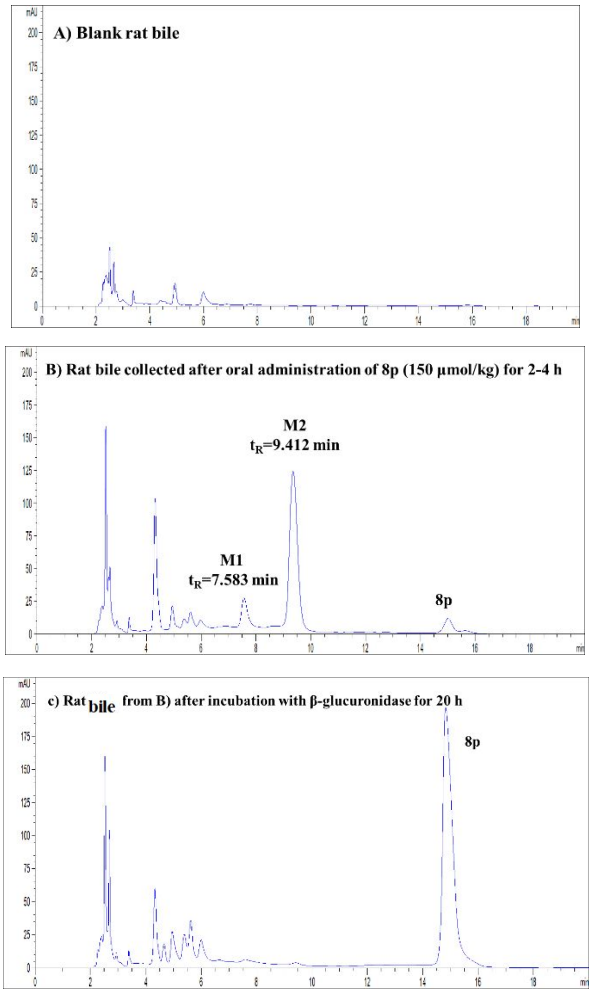
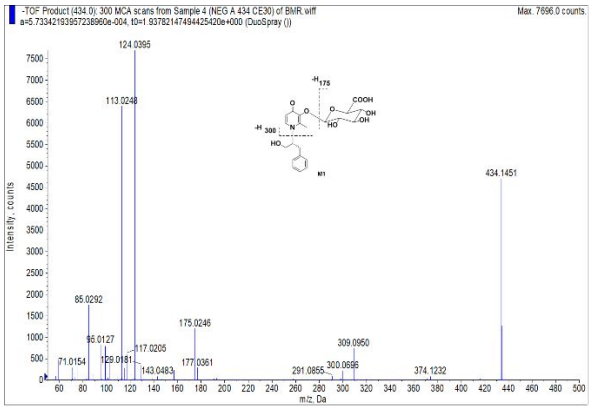


Figure S9. MS-MS Profile (Negative mode)



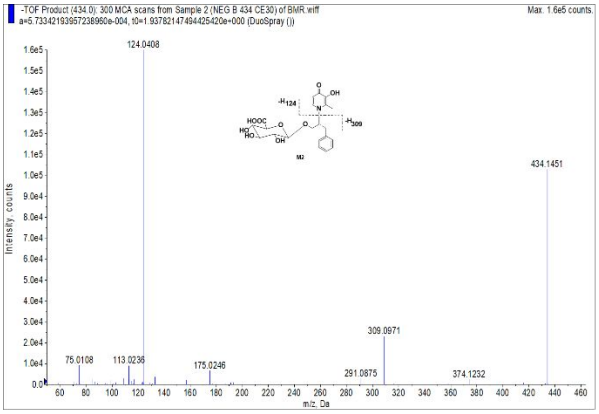
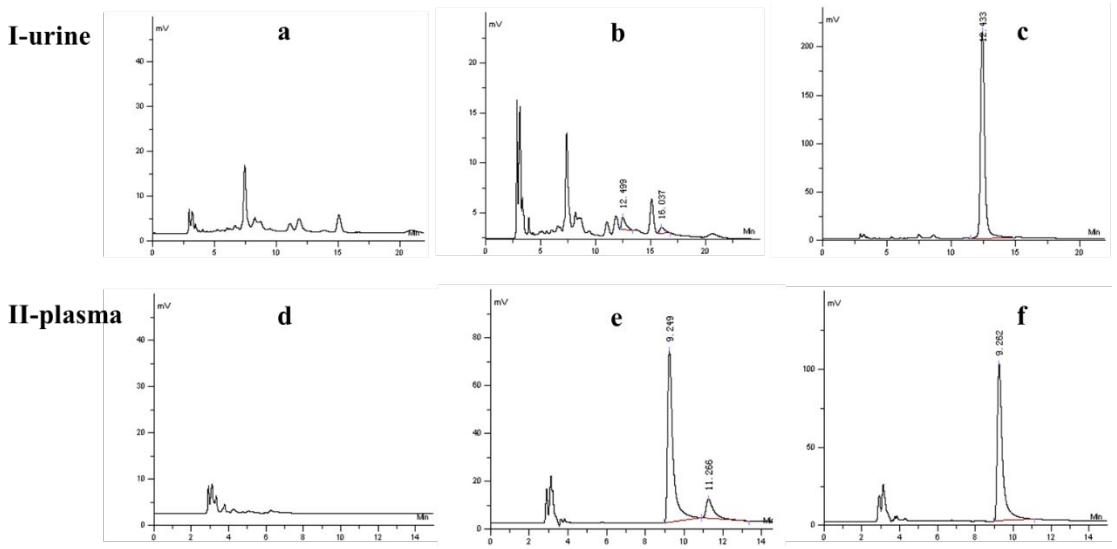
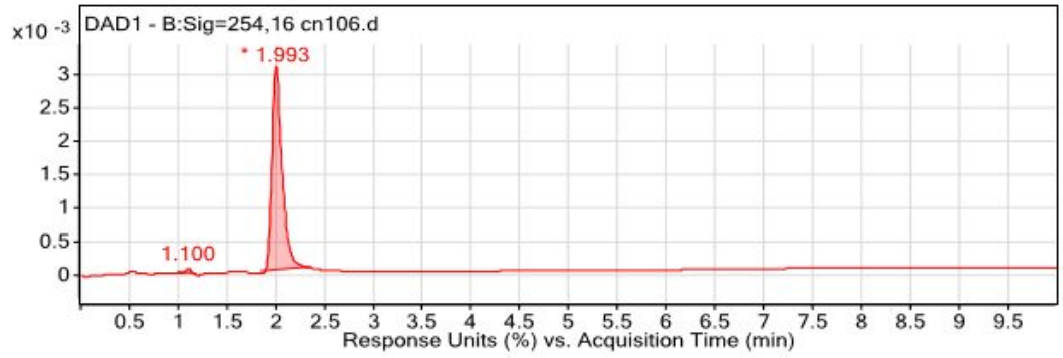


Figure S10. Chiral transformation study of **8p** in rats. I-urine: a) blank urine; b) **8p** (5 μg/mL) and **8o** (**8p enantiomer**) (5 μg/mL) in blank urine; c) urine collected from rats treated with **8p** (90 mg/kg), retention time: 12.50 min (**8p**), 16.04 min (**8o**). II-plasma: d) blank plasma; e) **8p** (100 μg/mL) and **8o** (10 μg/mL) in blank plasma; f) plasma collected from rats treated with **8p** (90 mg/kg), retention time: 9.25 min (**8p**), 11.27 min (**8o**).



HPLC of final products

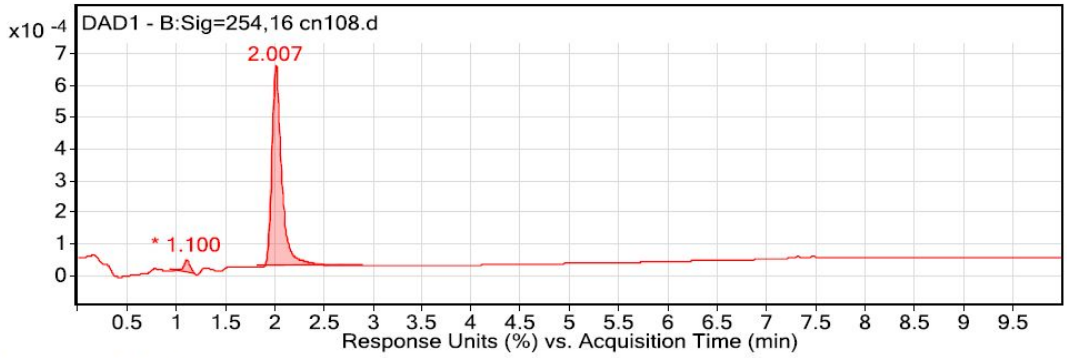
8a



Integration Peak List

Start	RT	End	Height	Area
0.993	1.1	1.187	10.81	44.27
1.84	1.993	2.36	394.41	2898.84

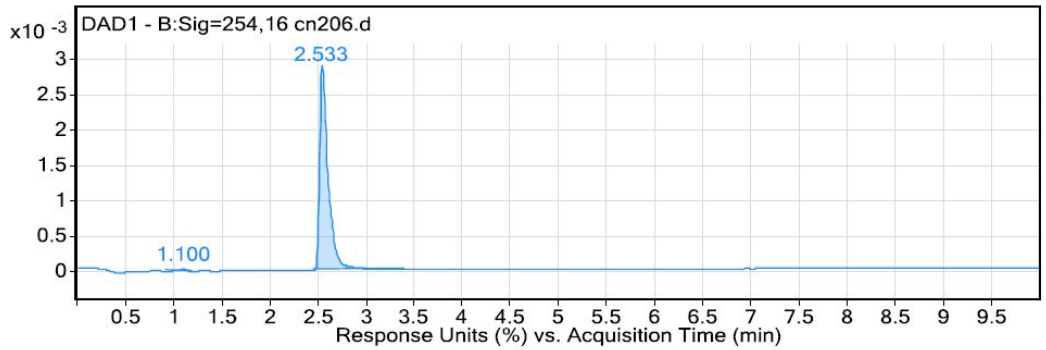
8b



Integration Peak List

Start	RT	End	Height	Area
0.933	1.1	1.193	11.12	55.84
1.82	2.007	2.9	165.16	1178.88

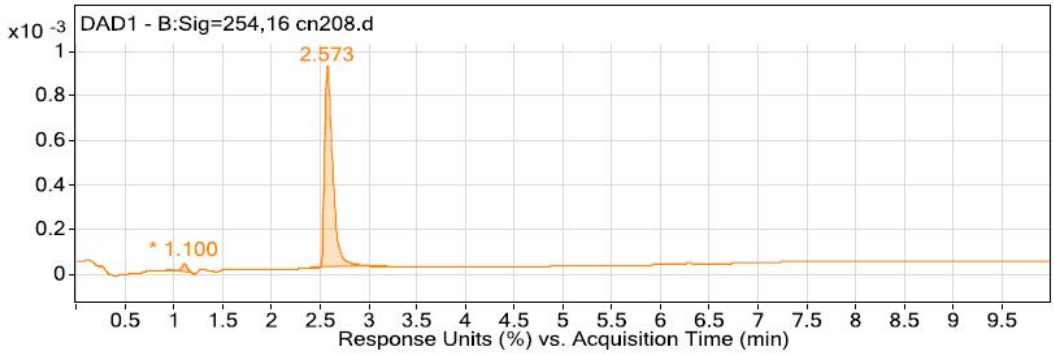
8c



Integration Peak List

Start	RT	End	Height	Area
0.913	1.1	1.193	10.91	57.52
2.447	2.533	3.4	757.22	4761.73

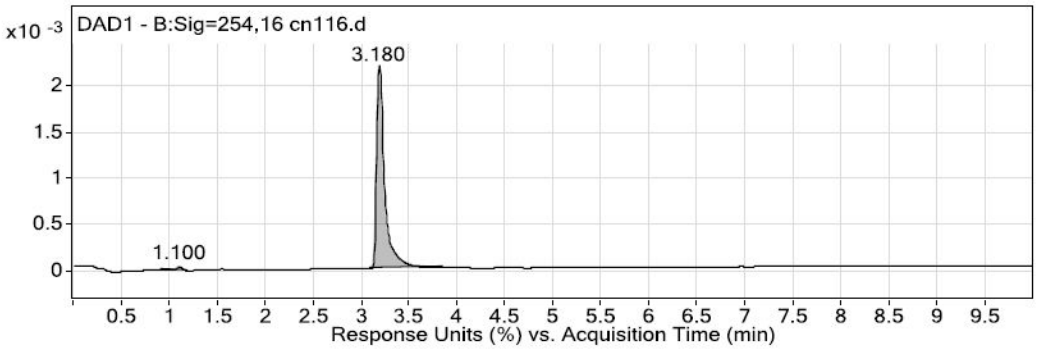
8d



Integration Peak List

Start	RT	End	Height	Area
0.92	1.1	1.193	10.5	54.07
2.4	2.573	3.193	236.56	1403.6

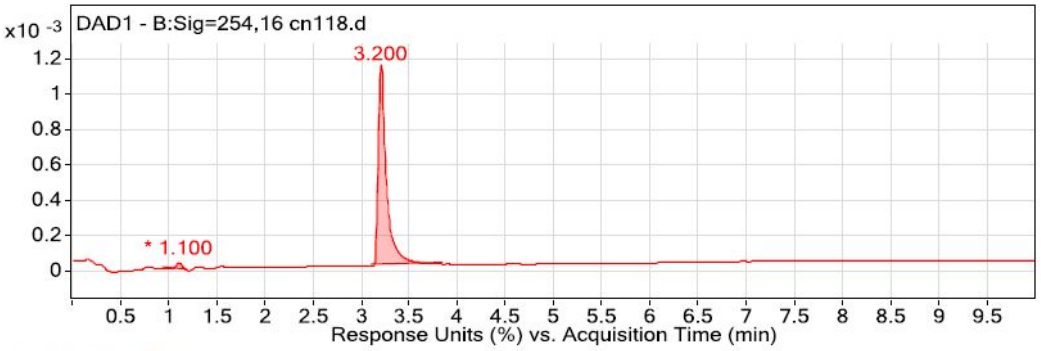
8e



Integration Peak List

Start	RT	End	Height	Area
0.907	1.1	1.193	10.97	57.17
3.087	3.18	3.853	572.82	3343.66

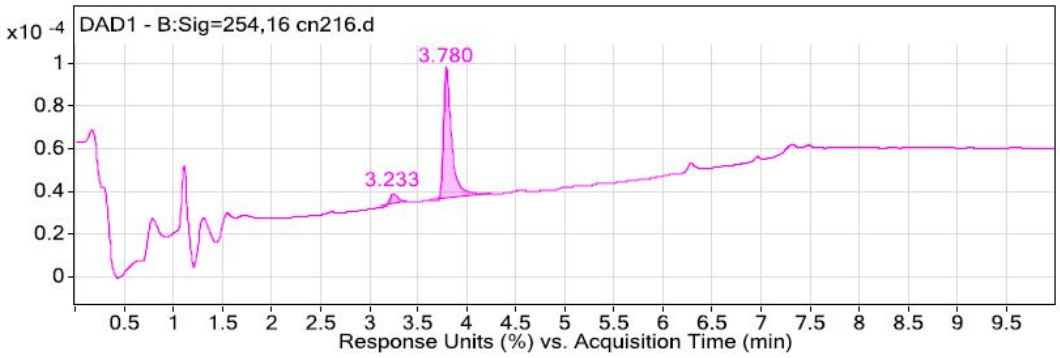
8f



Integration Peak List

Start	RT	End	Height	Area
0.933	1.1	1.2	10.84	54
3.1	3.2	3.847	295.14	1755.89

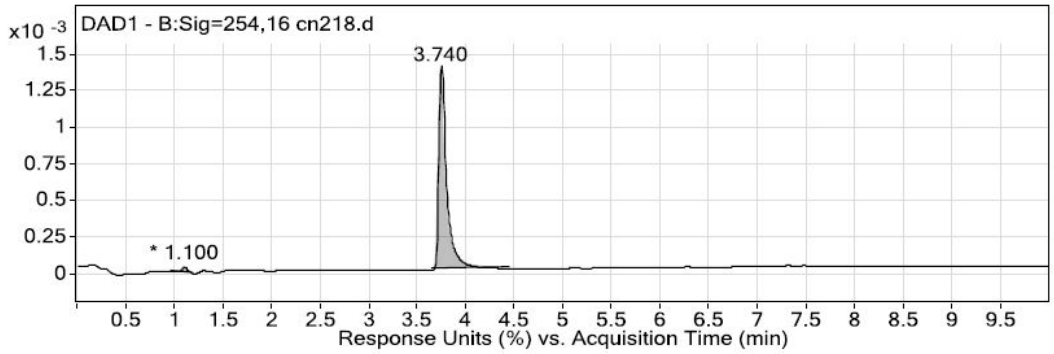
8g



Integration Peak List

Start	RT	End	Height	Area
3.113	3.233	3.387	1.29	8.01
3.613	3.78	4.247	16.17	95.39

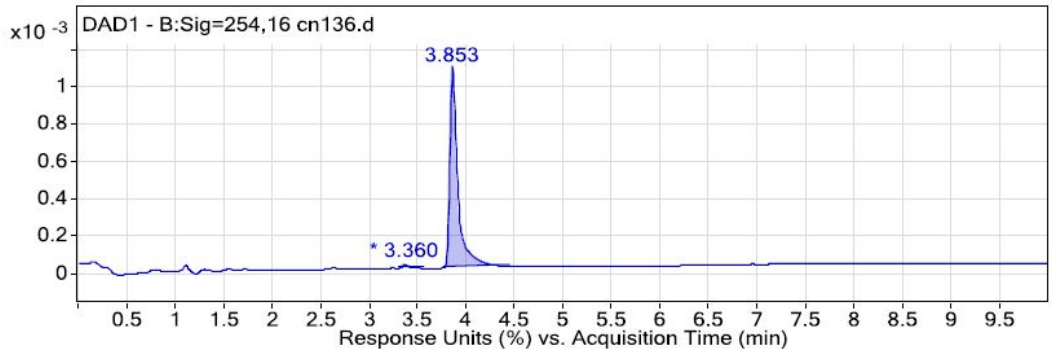
8h



Integration Peak List

Start	RT	End	Height	Area
0.96	1.1	1.193	10.68	49.28
3.647	3.74	4.453	361.67	2061.65

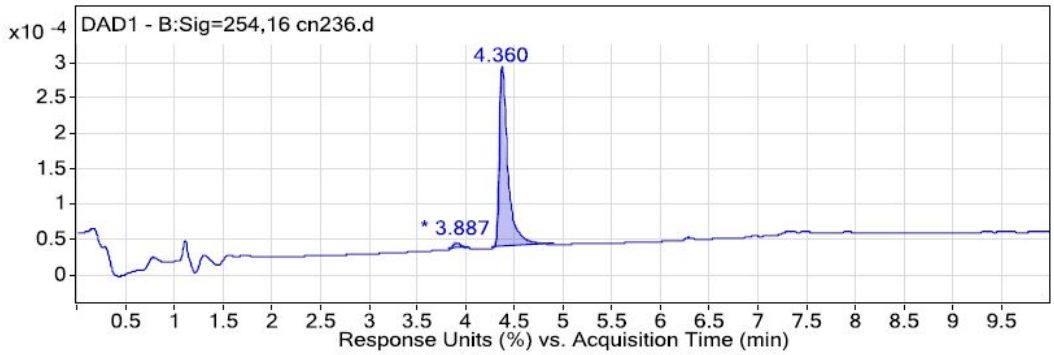
8i



Integration Peak List

Start	RT	End	Height	Area
3.3	3.36	3.567	4.44	21.64
3.76	3.853	4.453	280.32	1625.4

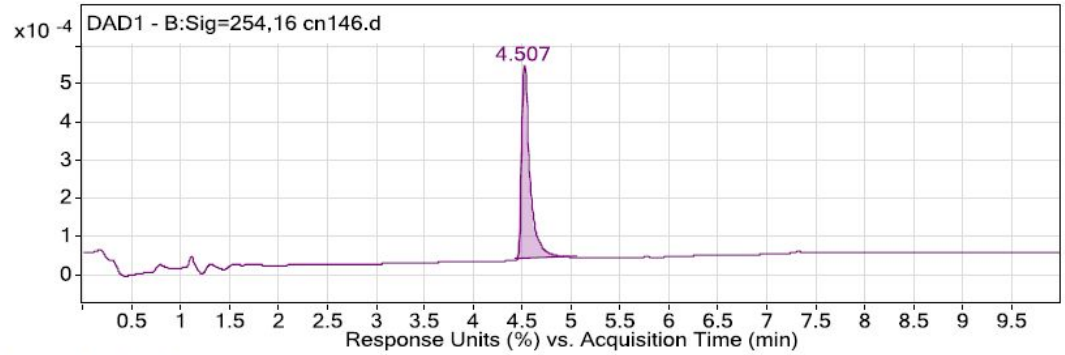
8j



Integration Peak List

Start	RT	End	Height	Area
3.813	3.887	4.027	2.28	12.46
4.26	4.36	4.907	66.6	414.24

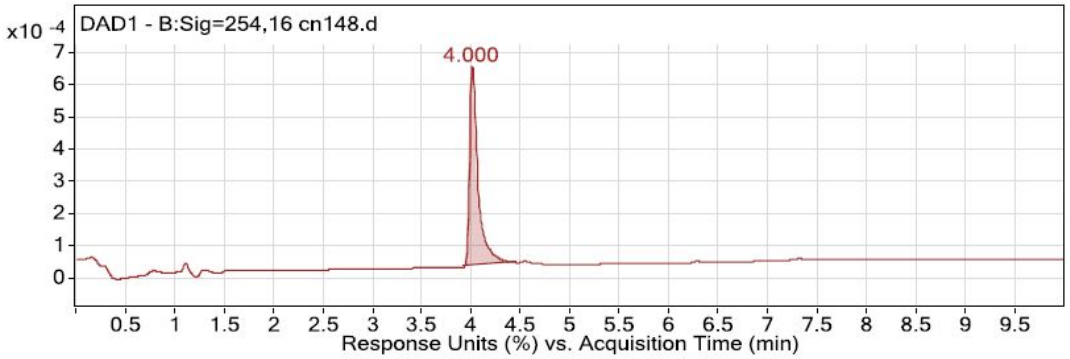
8k



Integration Peak List

Start	RT	End	Height	Area
4.42	4.507	5.06	132.39	795.48

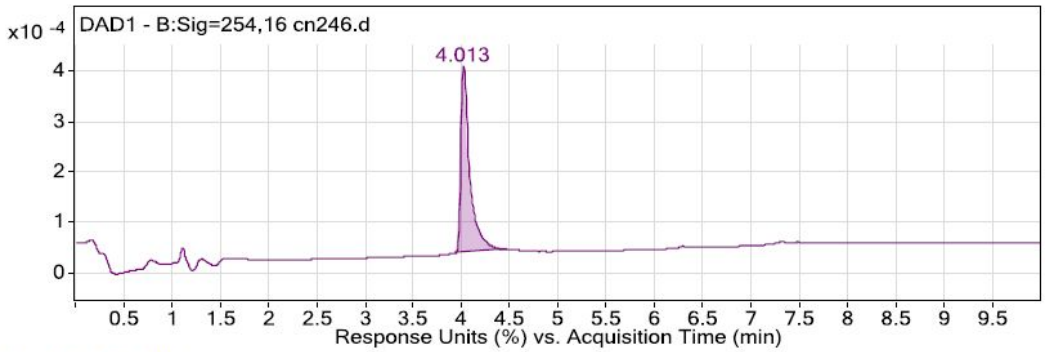
8l



Integration Peak List

Start	RT	End	Height	Area
3.913	4	4.46	161.43	969.39

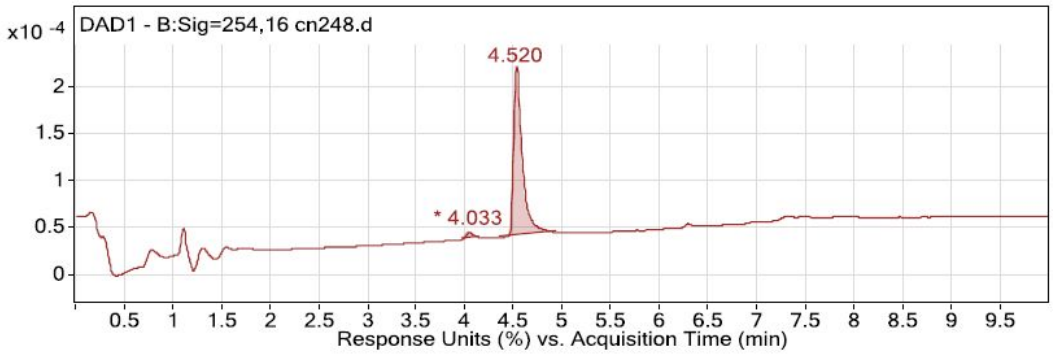
8m



Integration Peak List

Start	RT	End	Height	Area
3.927	4.013	4.473	96.53	625.39

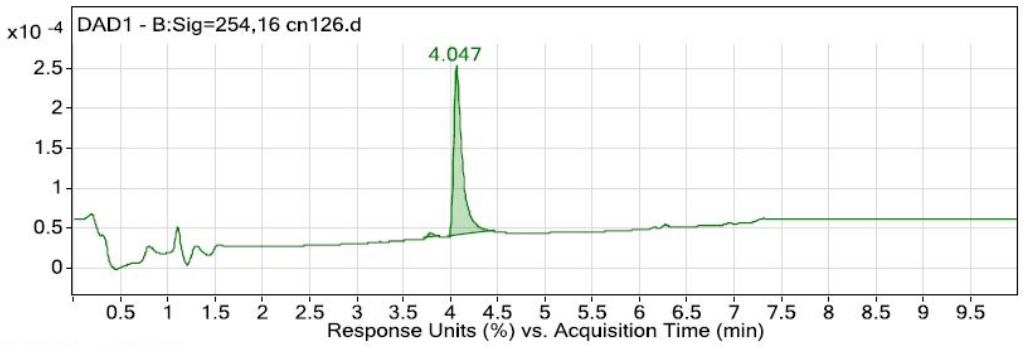
8n



Integration Peak List

Start	RT	End	Height	Area
3.967	4.033	4.147	1.71	8.6
4.353	4.52	4.94	46.88	291.09

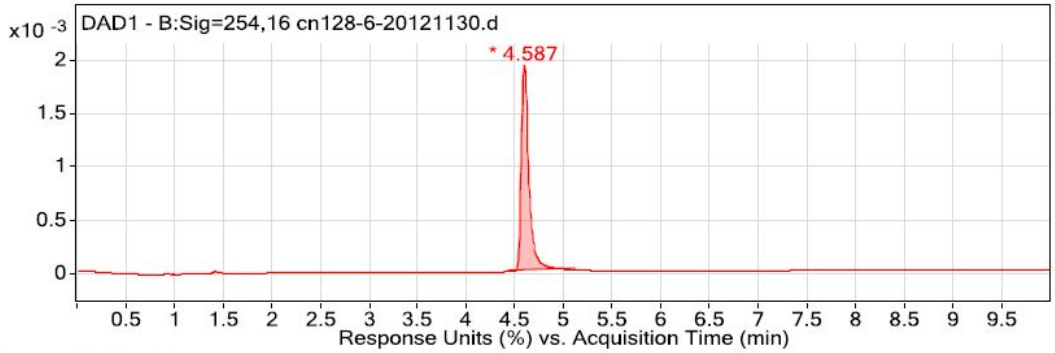
8o



Integration Peak List

Start	RT	End	Height	Area
3.707	3.773	3.887	1.73	8.67
3.96	4.047	4.467	55.77	353.33

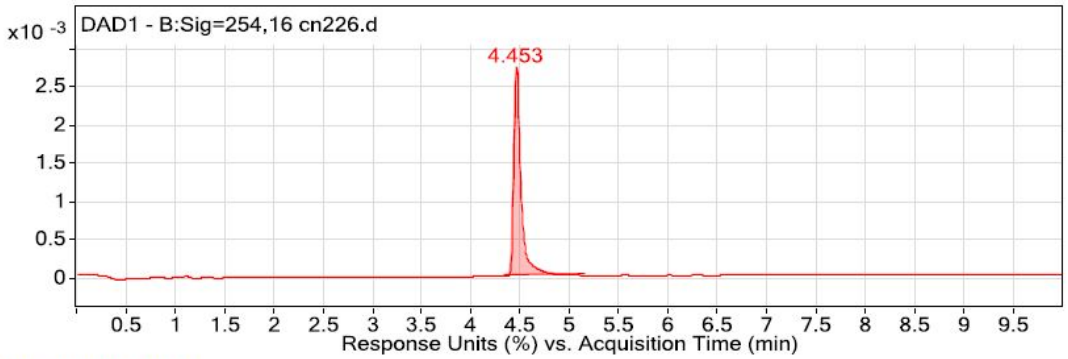
8p



Integration Peak List

Start	RT	End	Height	Area
4.433	4.587	5.127	657.08	3595.45

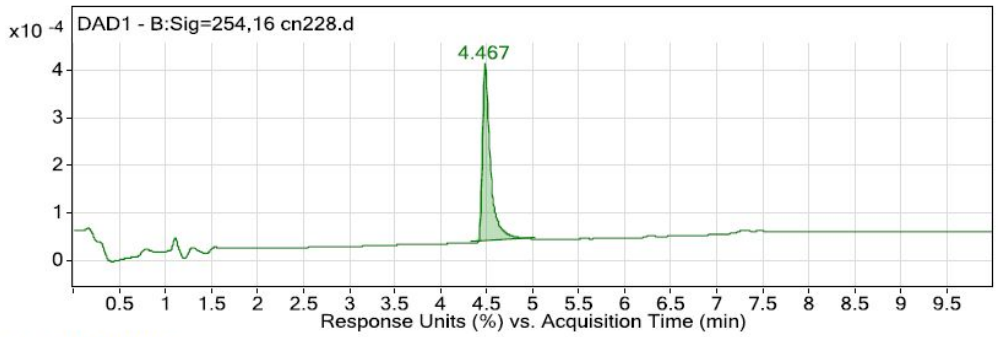
8q



Integration Peak List

Start	RT	End	Height	Area
4.333	4.453	5.16	711.91	3718.44

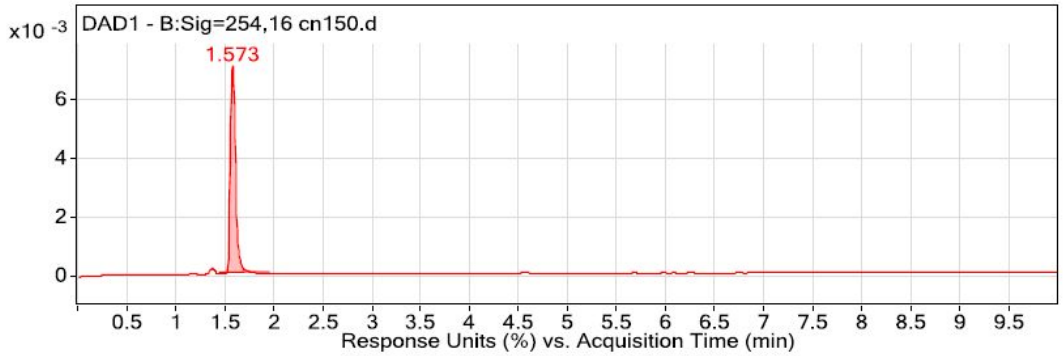
8r



Integration Peak List

Start	RT	End	Height	Area
4.32	4.467	5.027	97.88	601.42

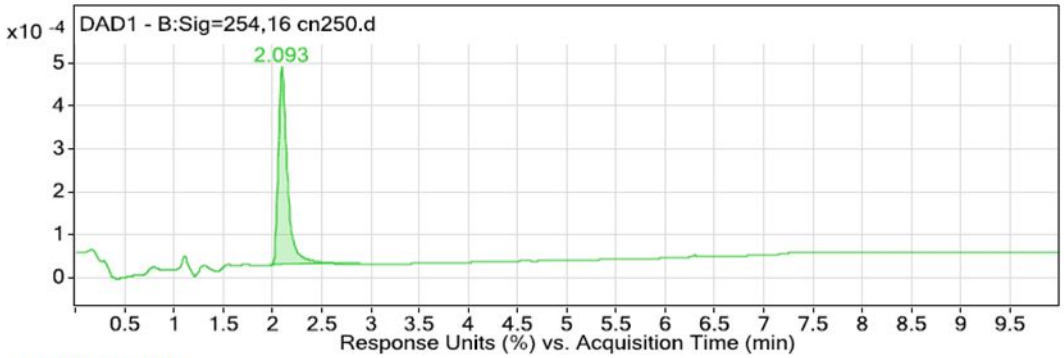
8s



Integration Peak List

Start	RT	End	Height	Area
1.44	1.573	1.96	458.81	1717.07

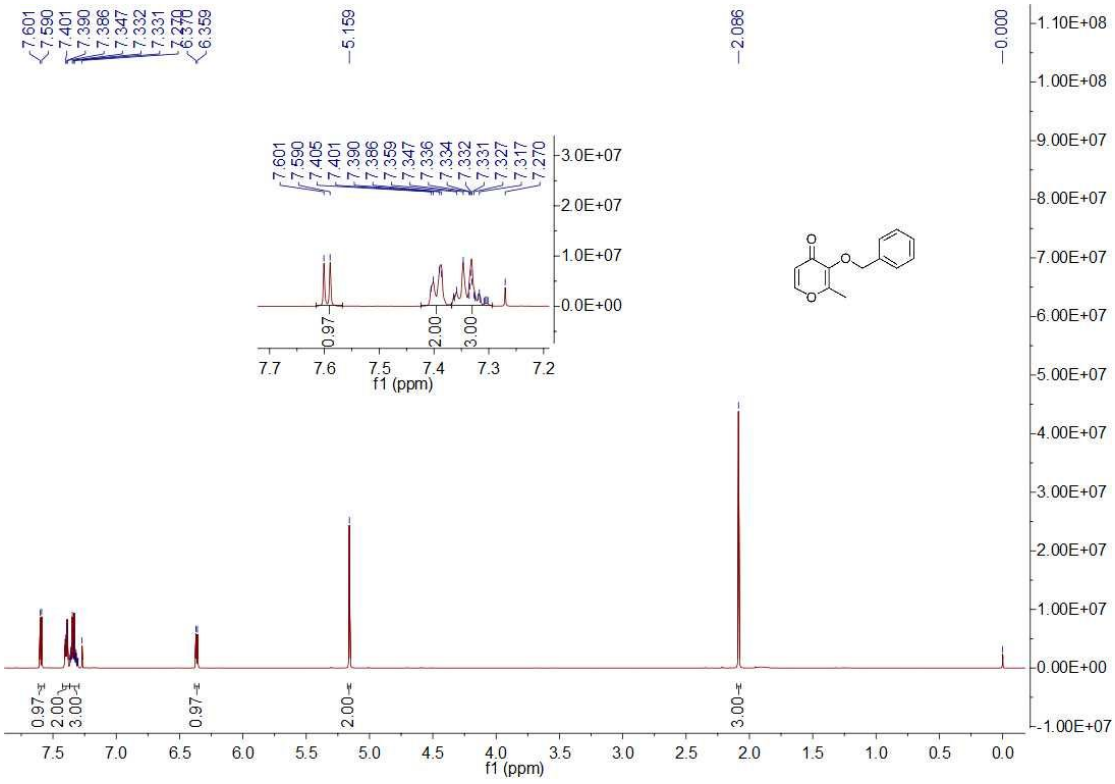
8t

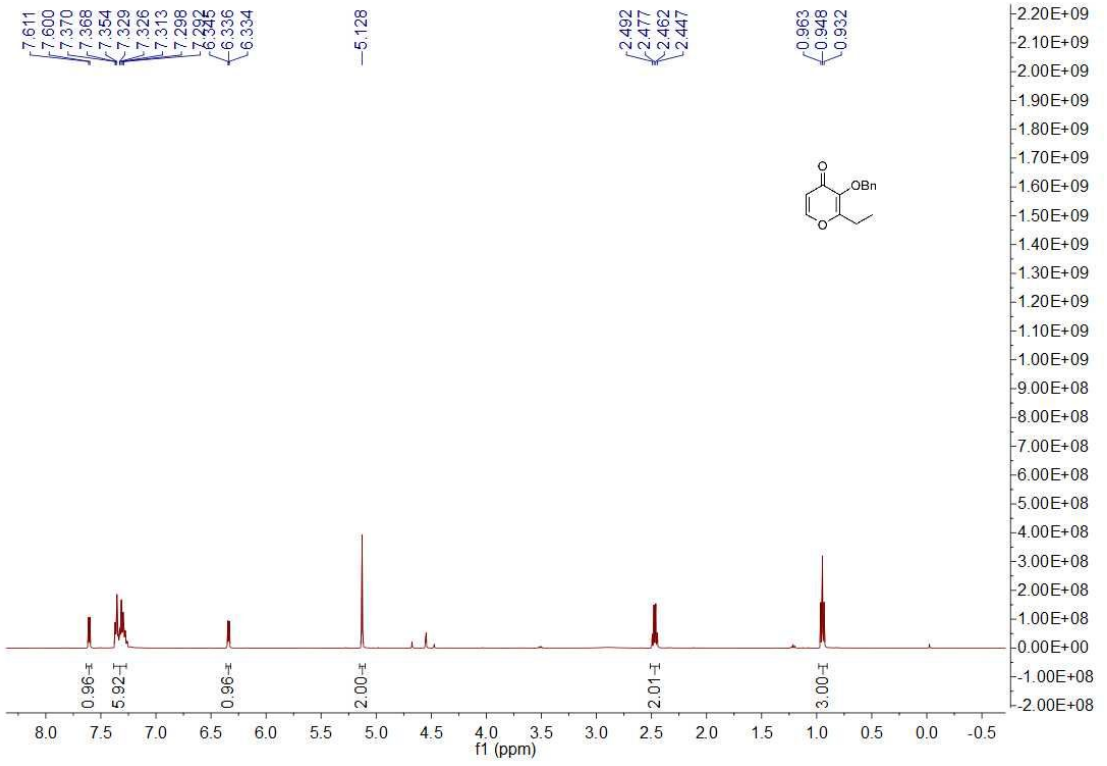


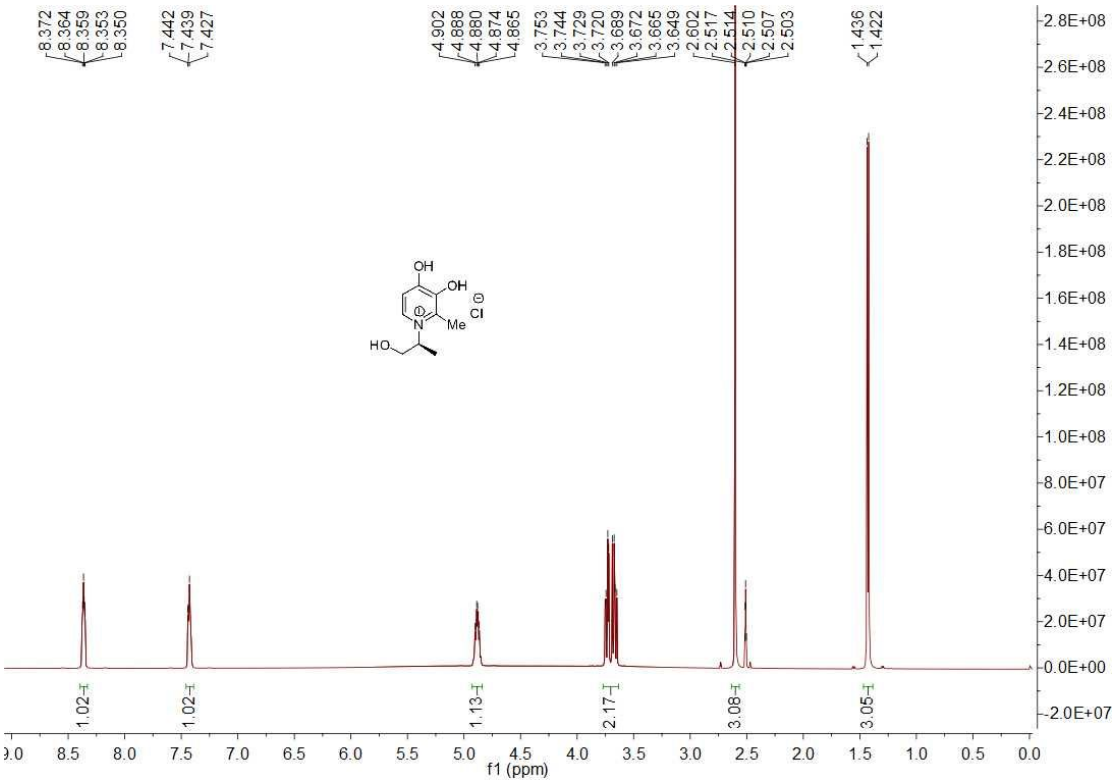
Integration Peak List

Start	RT	End	Height	Area
1.973	2.093	2.893	121.15	796.23

NMR spectrum of intermediates and final products



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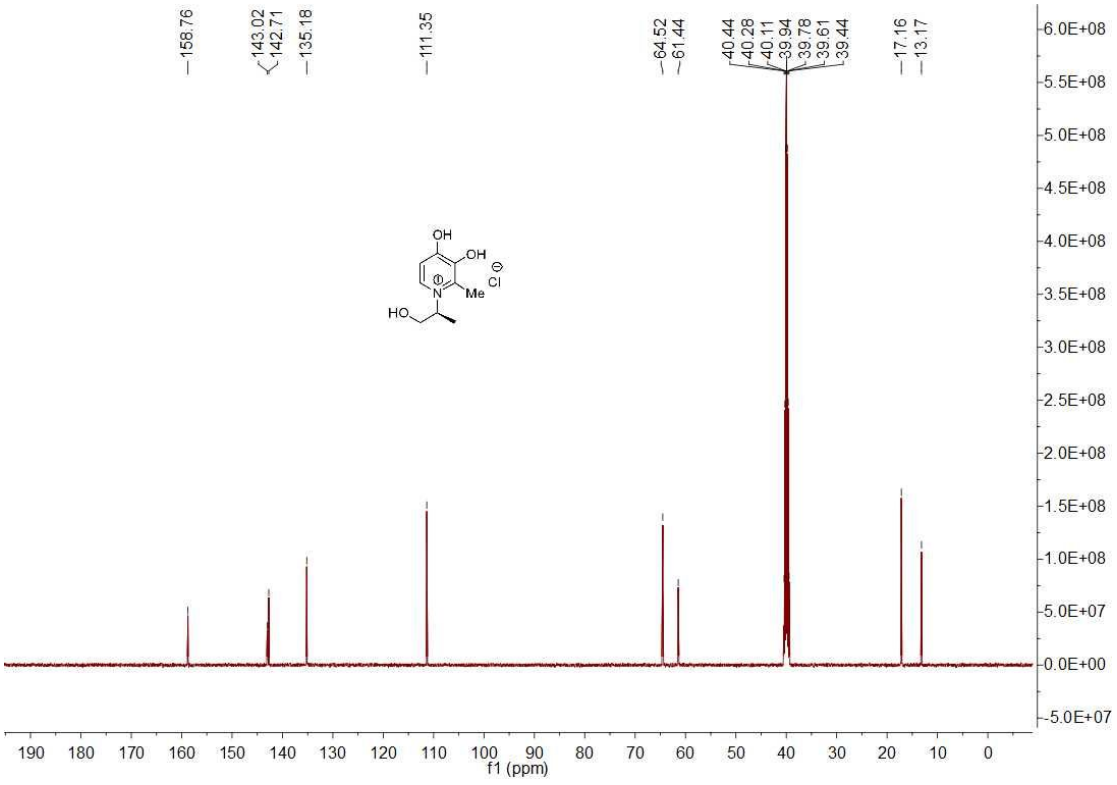
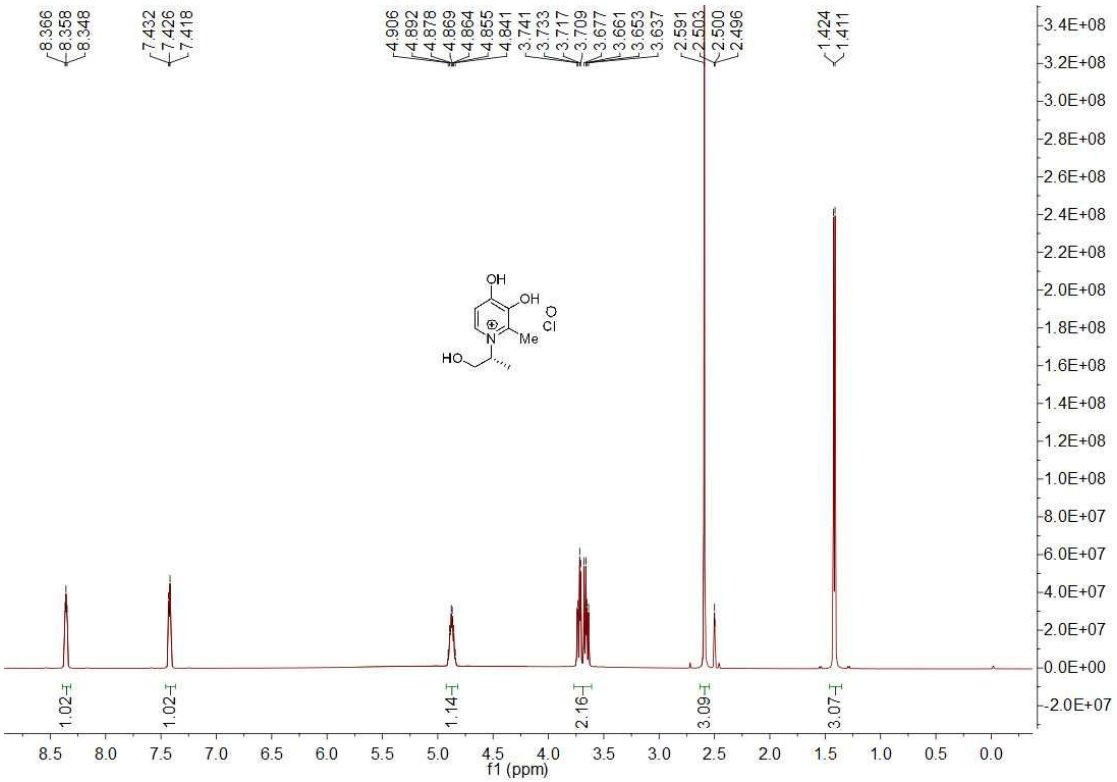
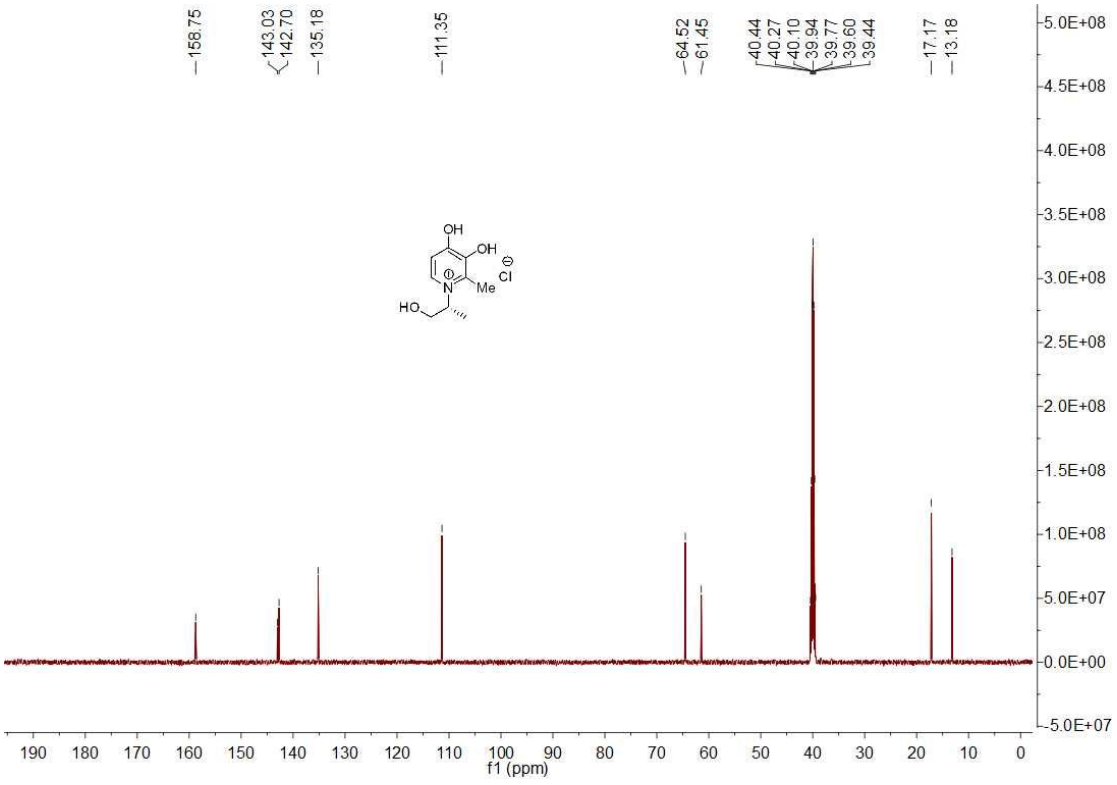
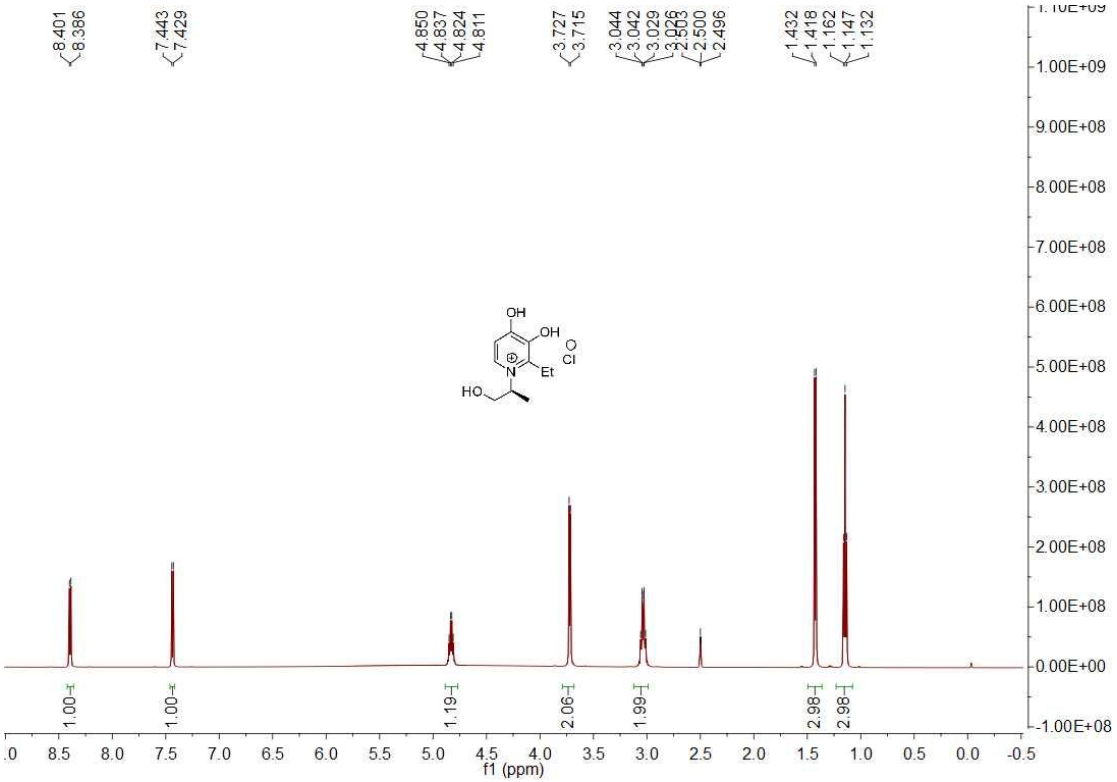
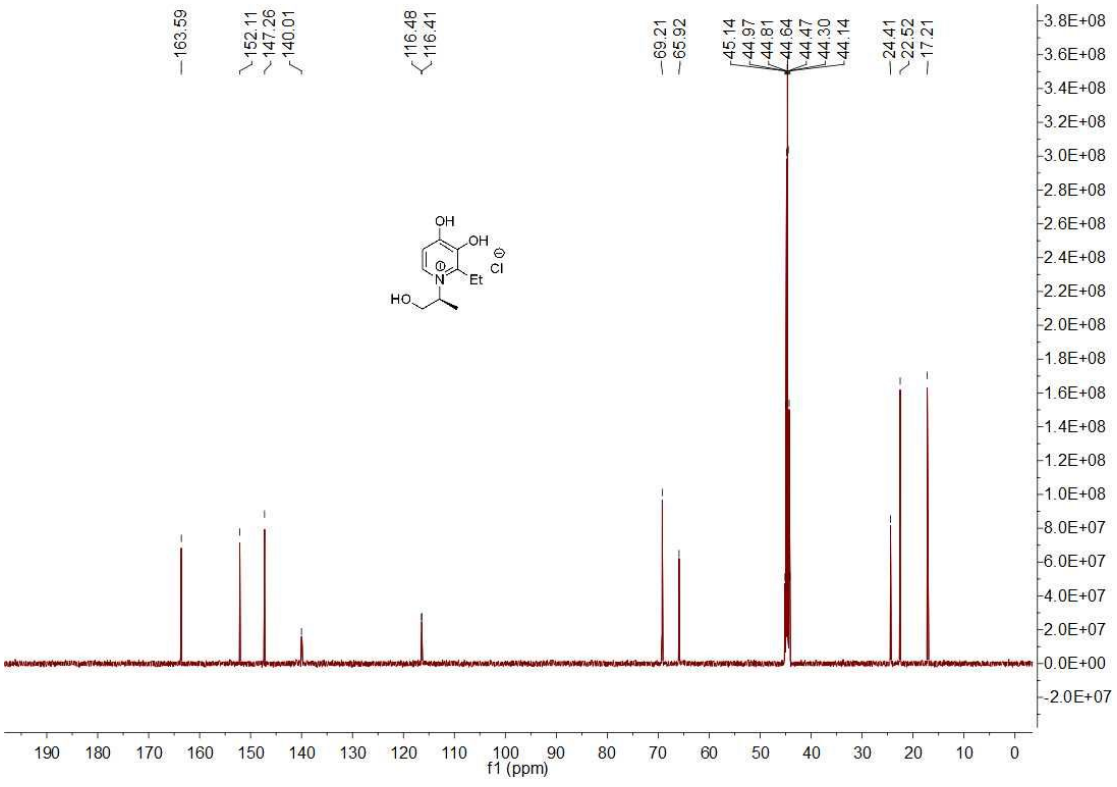
8.359
8.353
8.350
7.442
7.439
7.427
4.902
4.888
4.880
4.874
4.865
3.753
3.744
3.729
3.720
3.689
3.672
3.665
3.649
2.602
2.517
2.514
2.510
2.507
2.503
1.436
1.422
OH
OH
Me
Cl
HO
1.02
1.02
1.13
2.17
3.08
3.05
f1 (ppm)

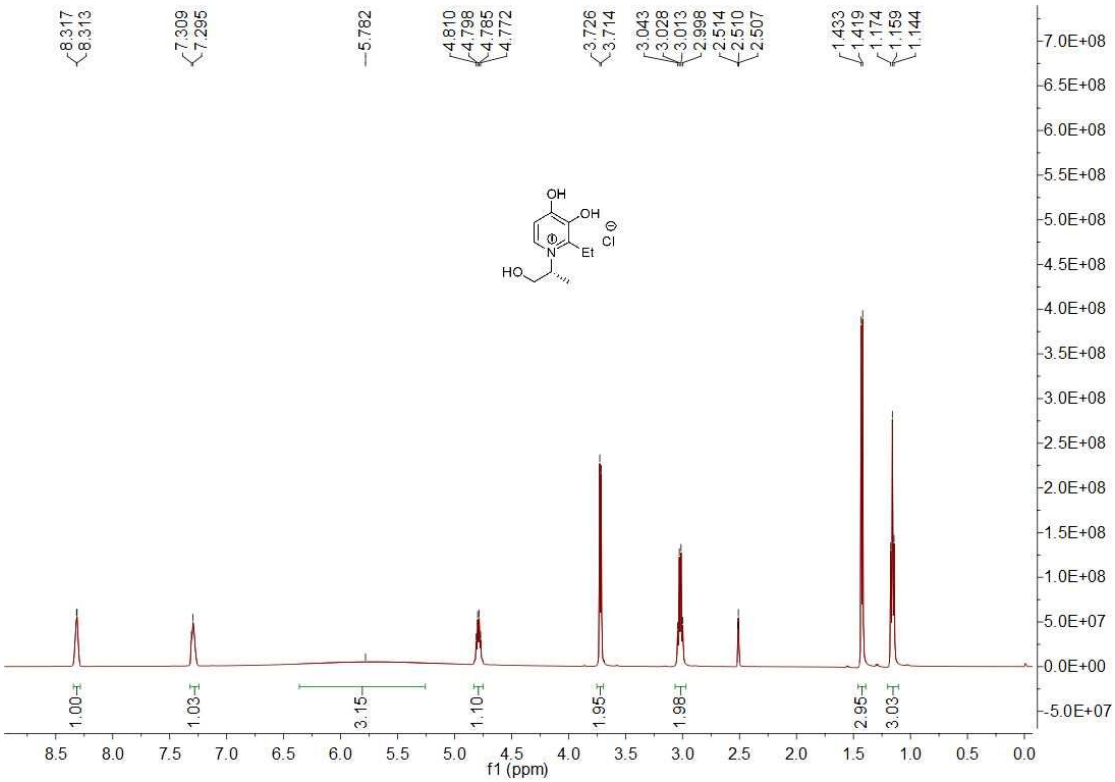

158.76
143.02
142.71
135.18
111.35
64.52
61.44
40.44
40.28
40.11
39.94
39.78
39.61
39.44
17.16
13.17
OH
OH
Me
Cl
HO
f1 (ppm)

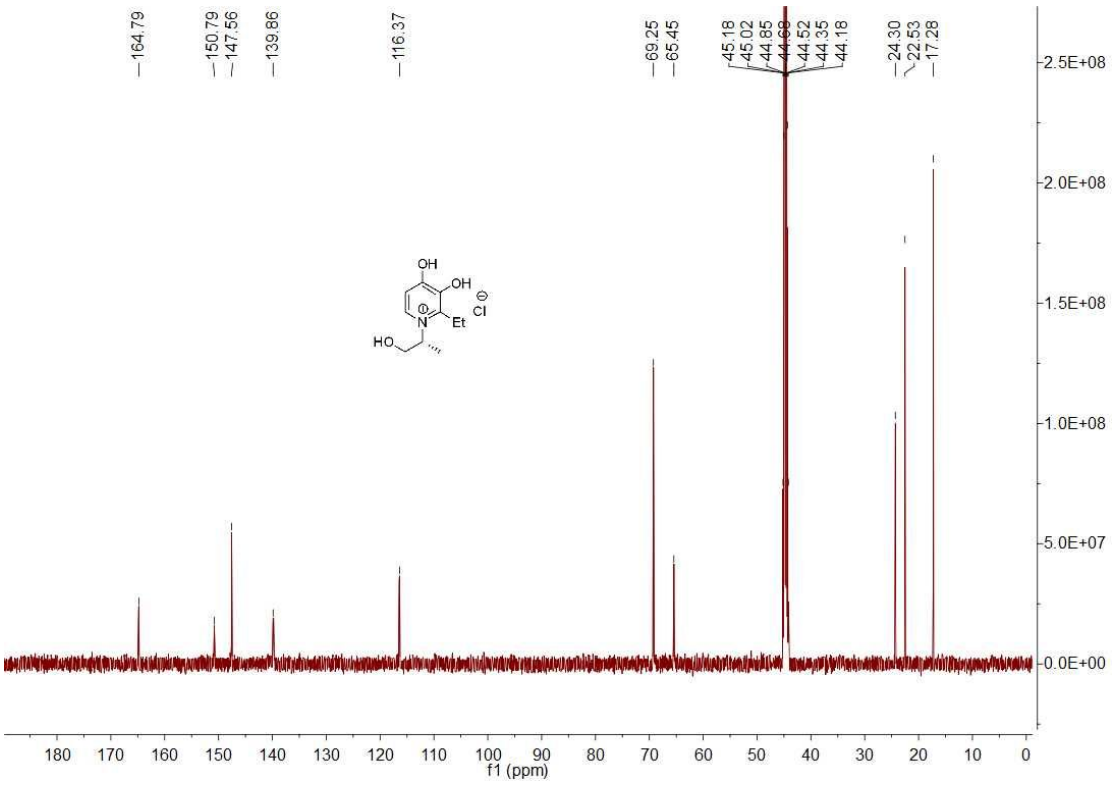

8.366
8.358
8.348
7.432
7.426
7.418
4.906
4.892
4.878
4.869
4.864
4.855
4.841
3.741
3.733
3.717
3.709
3.677
3.661
3.653
3.637
2.591
2.503
2.500
2.496
1.424
1.411
OH
OH
Cl
Me
HO
1.02
1.02
1.14
2.16
3.09
3.07
f1 (ppm)

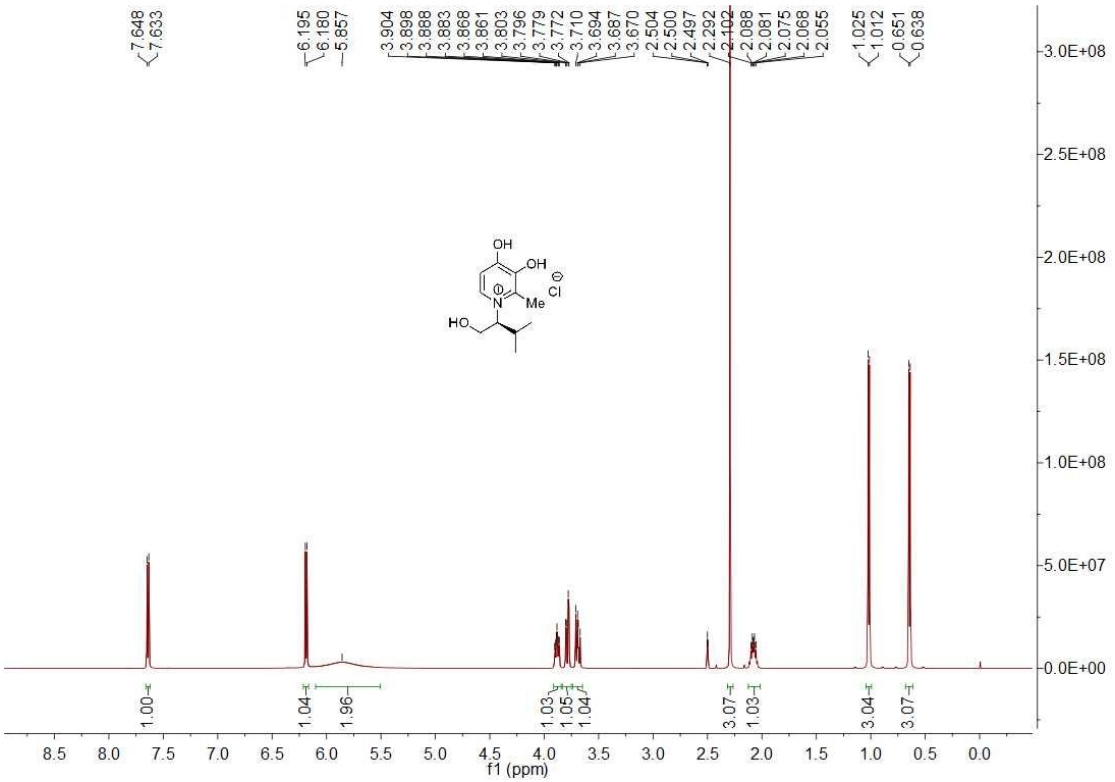

158.75
143.03
142.70
135.18
111.35
64.52
61.45
40.44
40.27
40.10
39.94
39.77
39.60
39.44
17.17
13.18
OH
OH
Cl
Me
HO
f1 (ppm)

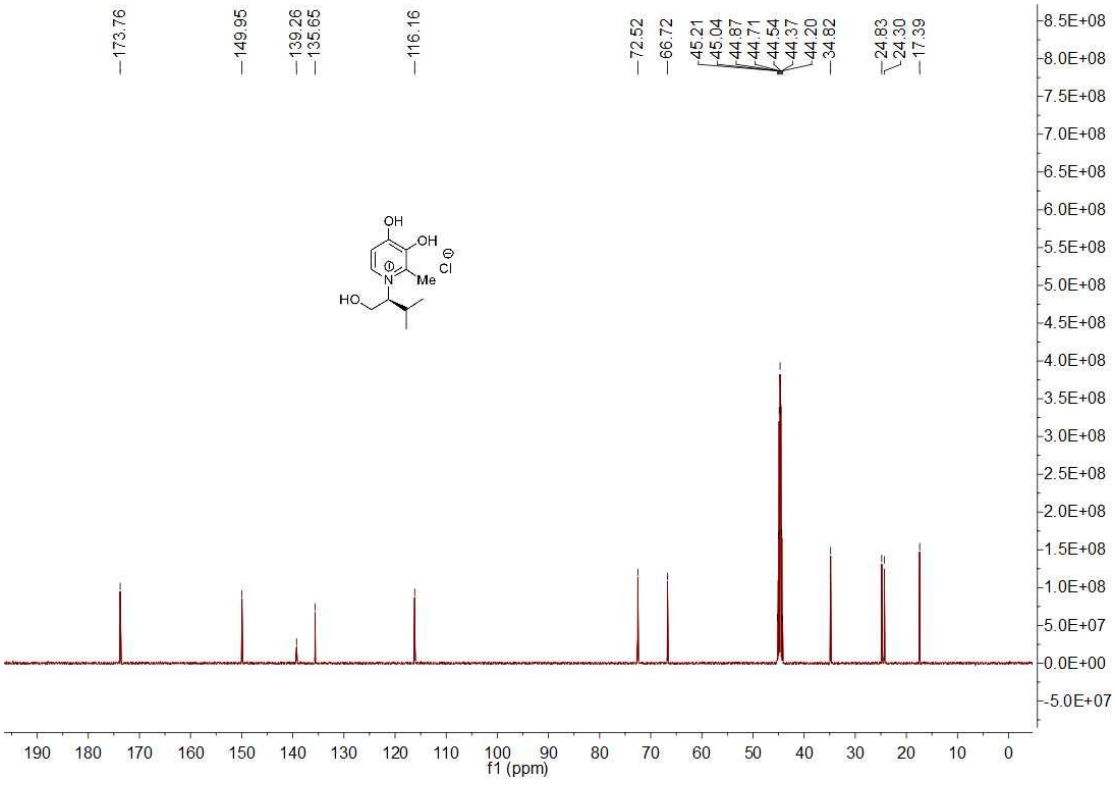

8.401
8.386
7.443
7.429
4.850
4.837
4.824
4.811
3.727
3.715
3.044
3.042
3.029
3.026
2.503
2.500
2.496
1.432
1.418
1.162
1.147
1.132
OH
OH
O
Cl
Et
N
HO
1.00
1.00
1.19
2.06
1.99
2.98
2.98
f1 (ppm)


163.59
152.11
147.26
140.01
116.48
116.41
69.21
65.92
45.14
44.97
44.81
44.64
44.47
44.30
44.14
24.41
22.52
17.21
OH
OH
Cl
Et
N
HO
f1 (ppm)


8.317
8.313
7.309
7.295
5.782
4.810
4.798
4.785
4.772
3.726
3.714
3.043
3.028
3.013
2.998
2.514
2.510
2.507
1.433
1.419
1.174
1.159
1.144
OH
OH
Et
Cl
HO
1.00
1.03
3.15
1.10
1.95
1.98
2.95
3.03
f1 (ppm)


164.79
150.79
147.56
139.86
116.37
69.25
65.45
45.18
45.02
44.85
44.68
44.52
44.35
44.18
24.30
22.53
17.28
OH
OH
Et
Cl
HO
f1 (ppm)


7.648
7.633
6.195
6.180
5.857
3.904
3.898
3.888
3.883
3.868
3.861
3.803
3.796
3.779
3.772
3.710
3.694
3.687
3.670
2.504
2.500
2.497
2.292
2.102
2.088
2.081
2.075
2.068
2.055
1.025
1.012
0.651
0.638
OH
OH
Cl
Me
N
HO
1.00
1.04
1.96
1.03
1.05
1.04
3.07
1.03
3.04
3.07
f1 (ppm)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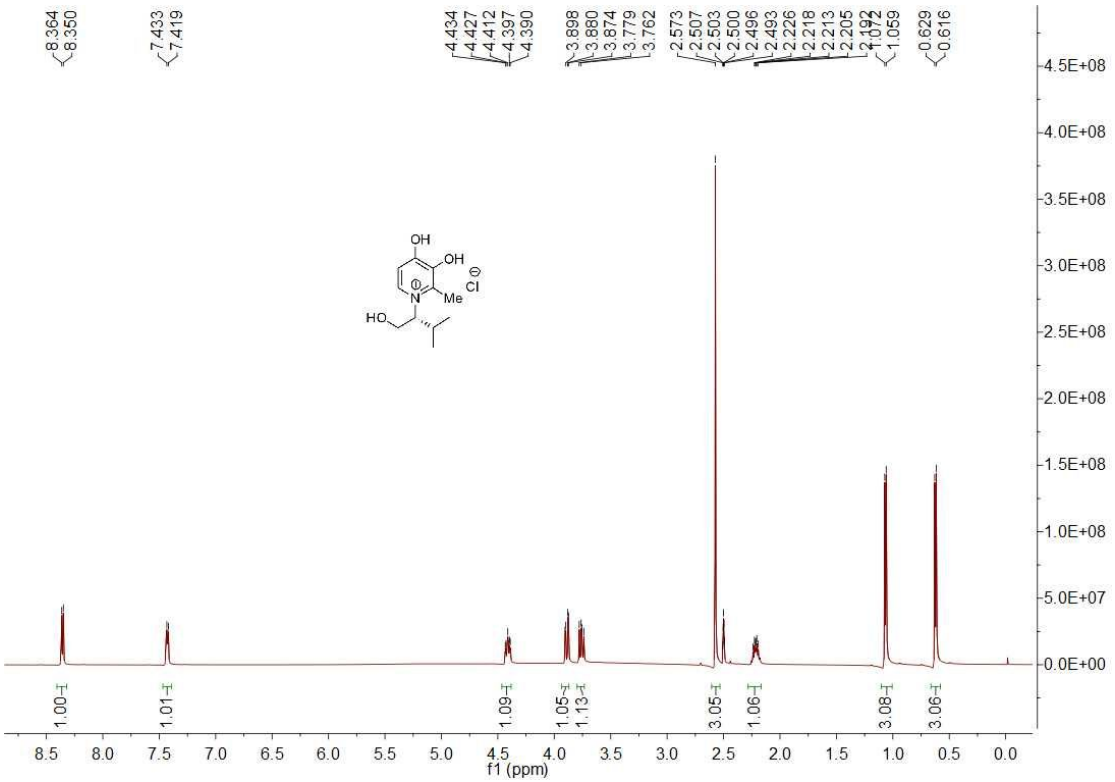
149.95
139.26
135.65
116.16
72.52
66.72
45.21
45.04
44.87
44.71
44.54
44.37
44.20
34.82
24.83
24.30
17.39
OH
OH
Cl
Me
N
HO
f1 (ppm)


8.364
8.350
7.433
7.419
4.434
4.427
4.412
4.397
4.390
3.898
3.880
3.874
3.779
3.762
2.573
2.507
2.503
2.500
2.496
2.493
2.226
2.218
2.213
2.205
2.192
2.072
1.059
0.629
0.616
OH
OH
Me
Cl
HO
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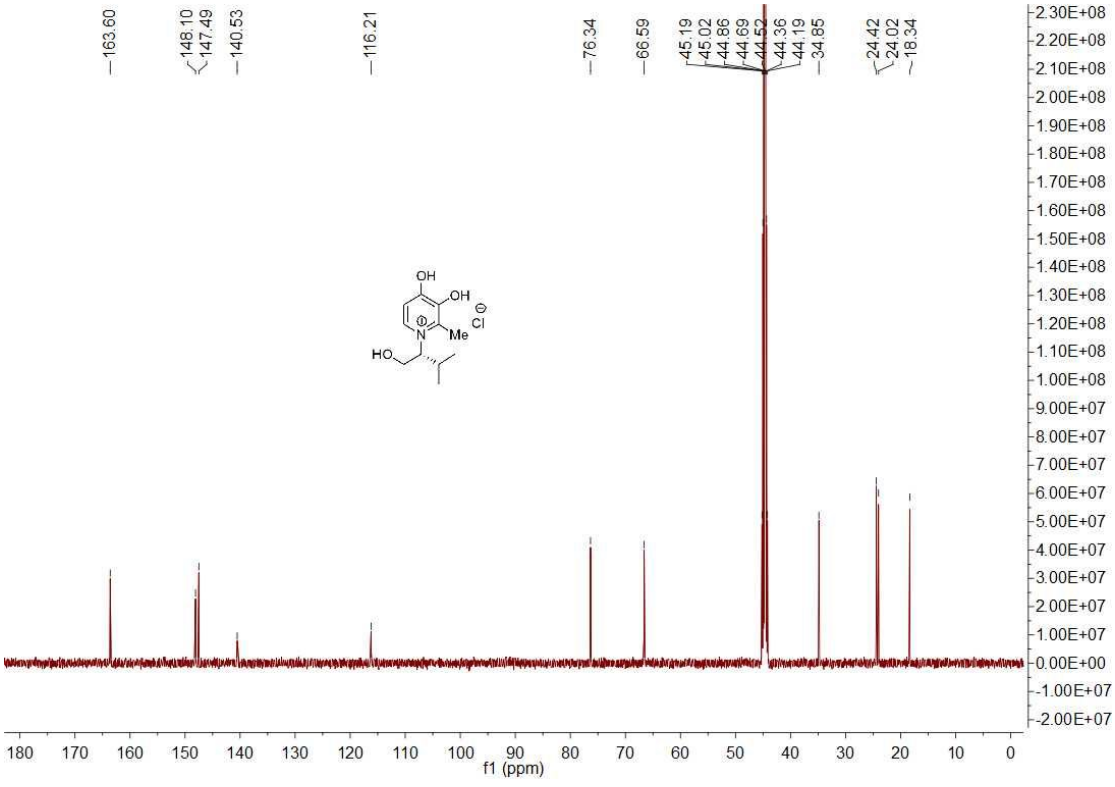
1.01
1.09
1.05
1.13
3.05
1.06
3.08
3.06
f1 (ppm)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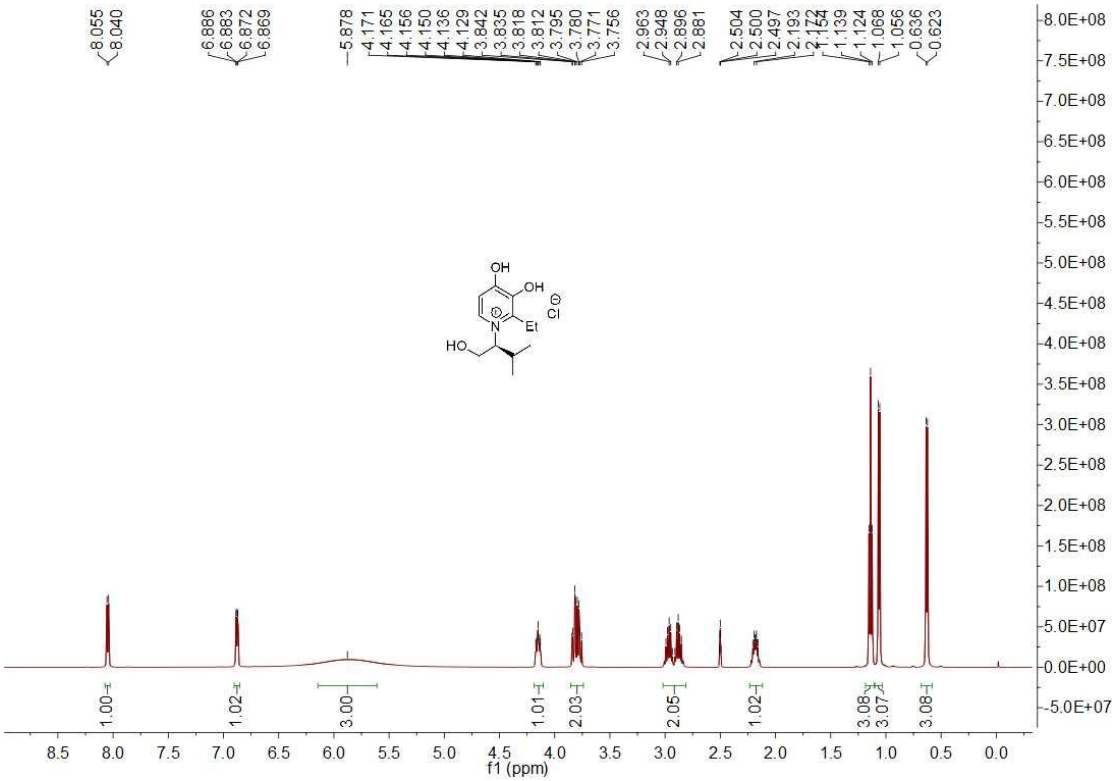
148.10
147.49
140.53
116.21
76.34
66.59
45.19
45.02
44.86
44.69
44.52
44.36
44.19
34.85
24.42
24.02
18.34
OH
OH
Me
Cl
HO
f1 (ppm)


8.055
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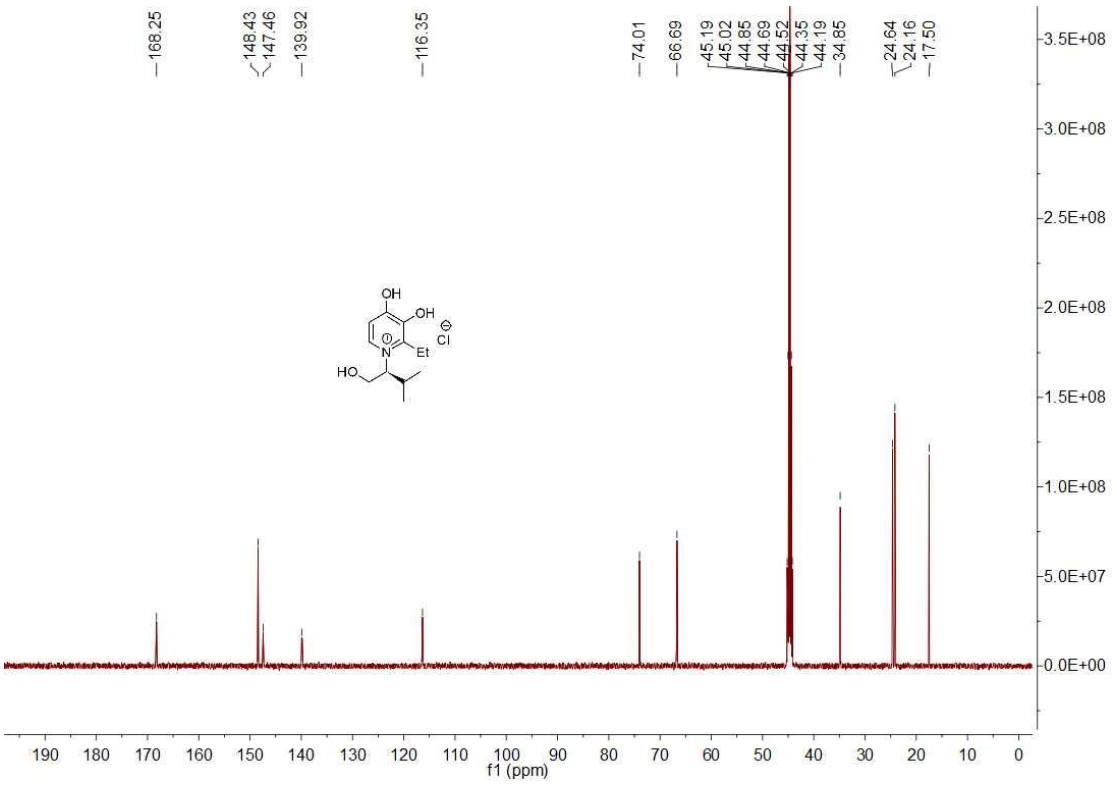
6.883
6.872
6.869
5.878
4.171
4.165
4.156
4.150
4.136
4.129
3.842
3.835
3.818
3.812
3.795
3.780
3.771
3.756
2.963
2.948
2.896
2.881
2.504
2.500
2.497
2.193
2.172
2.154
1.139
1.124
1.068
1.056
0.636
0.623
OH
OH
Et
Cl
HO
1.00
1.02
3.00
1.01
2.03
2.05
1.02
3.08
3.07
3.08
f1 (ppm)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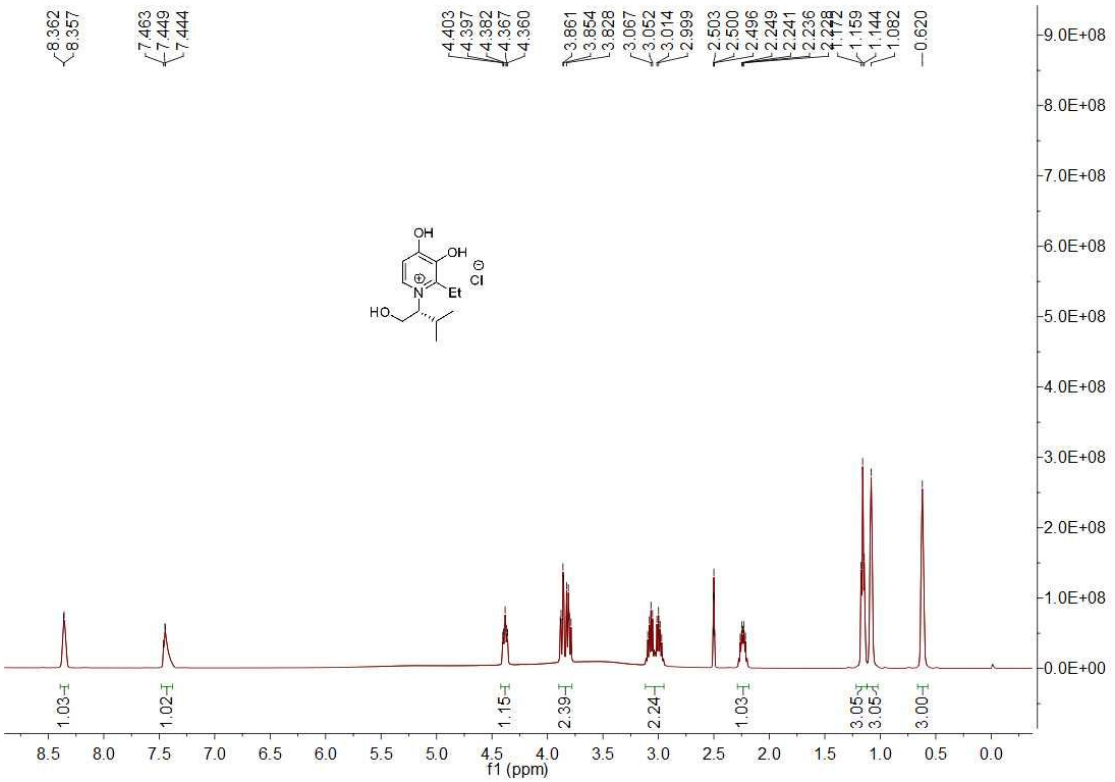
148.43
147.46
139.92
116.35
74.01
66.69
45.19
45.02
44.85
44.69
44.52
44.35
44.19
34.85
24.64
24.16
17.50
OH
OH
Et
Cl
HO
f1 (ppm)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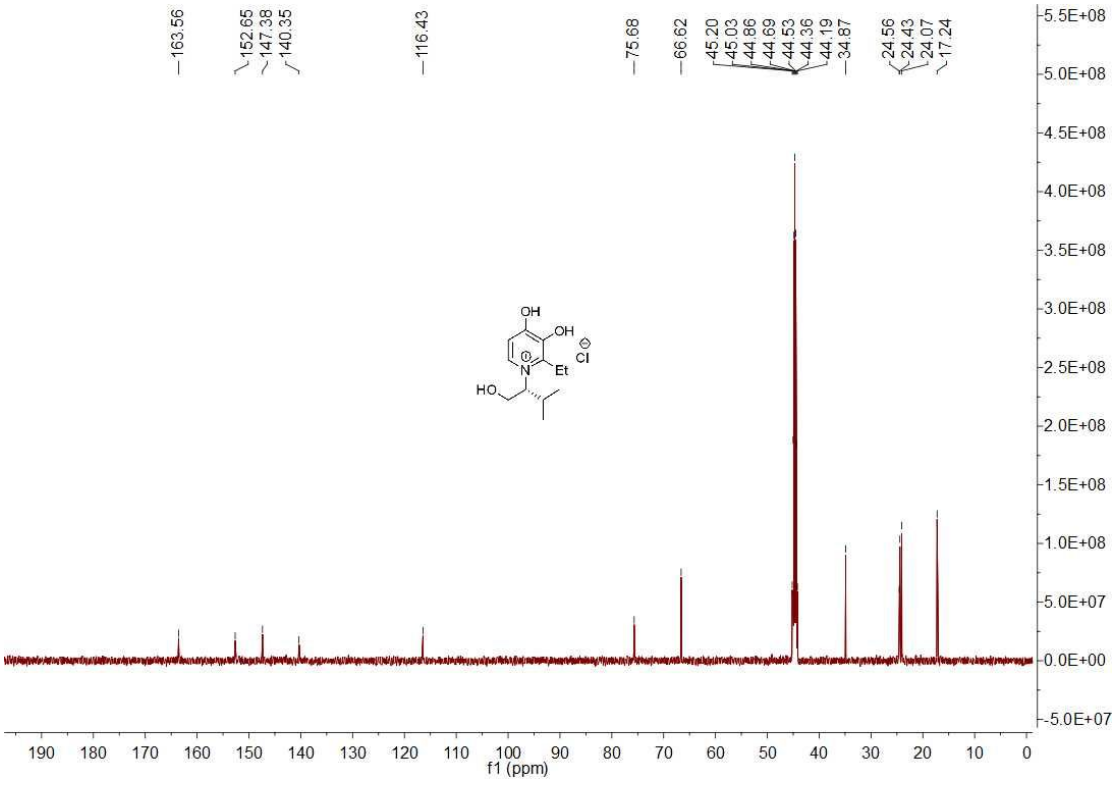
7.463
7.449
7.444
4.403
4.397
4.382
4.367
4.360
3.861
3.854
3.828
3.067
3.052
3.014
2.999
2.503
2.500
2.496
2.249
2.241
2.236
1.159
1.144
1.082
0.620
OH
OH
Cl
Et
HO
1.03
1.02
1.15
2.39
2.24
1.03
3.05
3.05
3.00
f1 (ppm)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152.65
147.38
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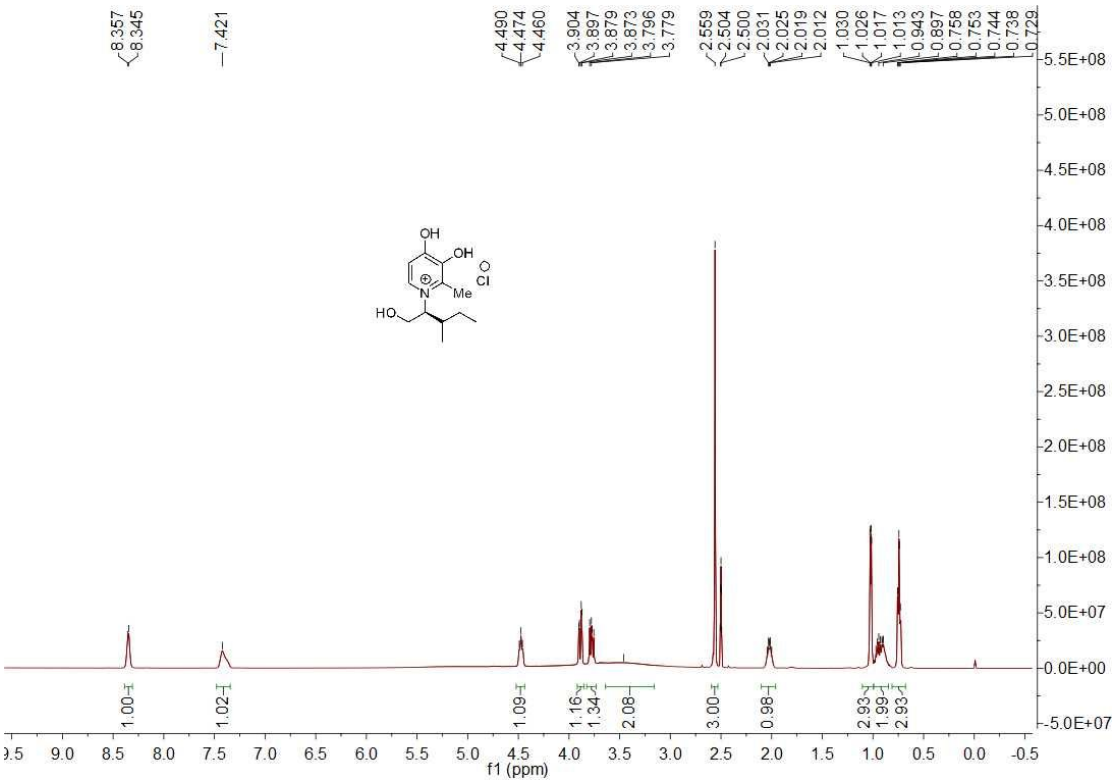
116.43
75.68
66.62
45.20
45.03
44.86
44.69
44.53
44.36
44.19
34.87
24.56
24.43
24.07
17.24
OH
OH
Cl
Et
HO
f1 (ppm)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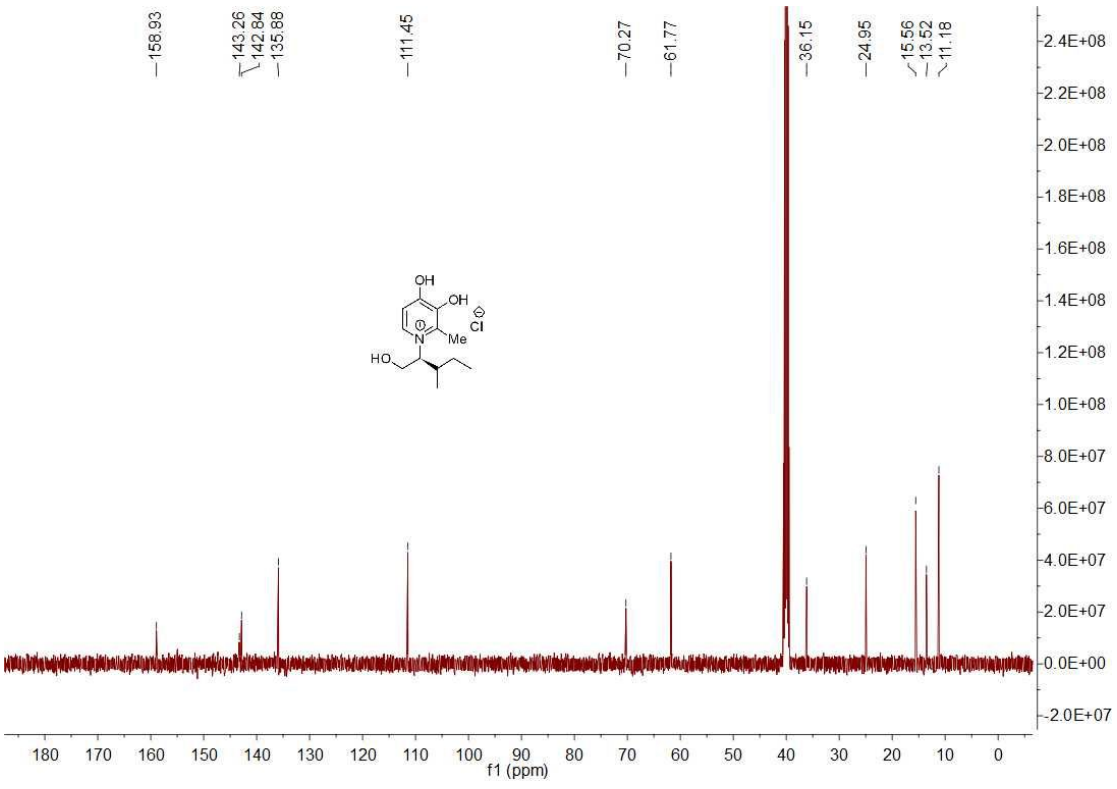
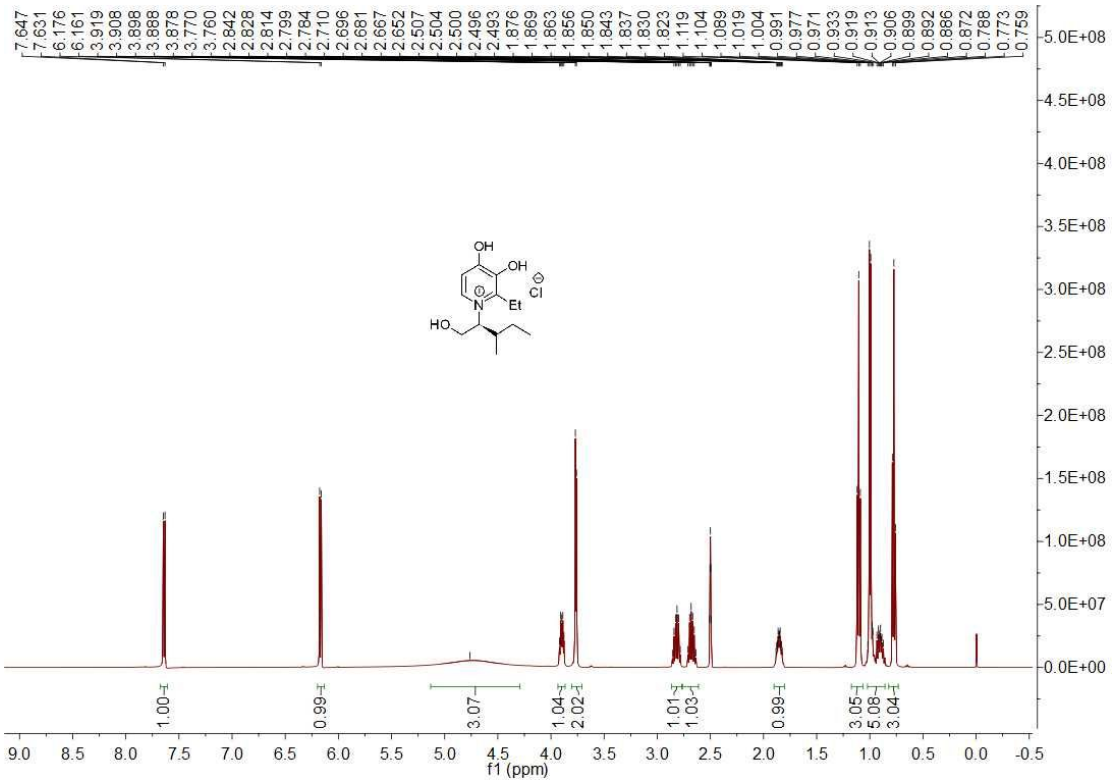
8.345
7.421
4.490
4.474
4.460
3.904
3.897
3.879
3.873
3.796
3.779
2.559
2.504
2.500
2.031
2.025
2.019
2.012
1.030
1.026
1.017
1.013
0.943
0.897
0.758
0.753
0.744
0.738
0.729
OH
OH
Cl
Me
HO
1.00
1.02
1.09
1.16
1.34
2.08
3.00
0.98
2.93
1.99
2.93
f1 (ppm)


158.93
143.26
142.84
135.88
111.45
70.27
61.77
36.15
24.95
15.56
13.52
11.18
OH
OH
Cl
Me
HO
f1 (ppm)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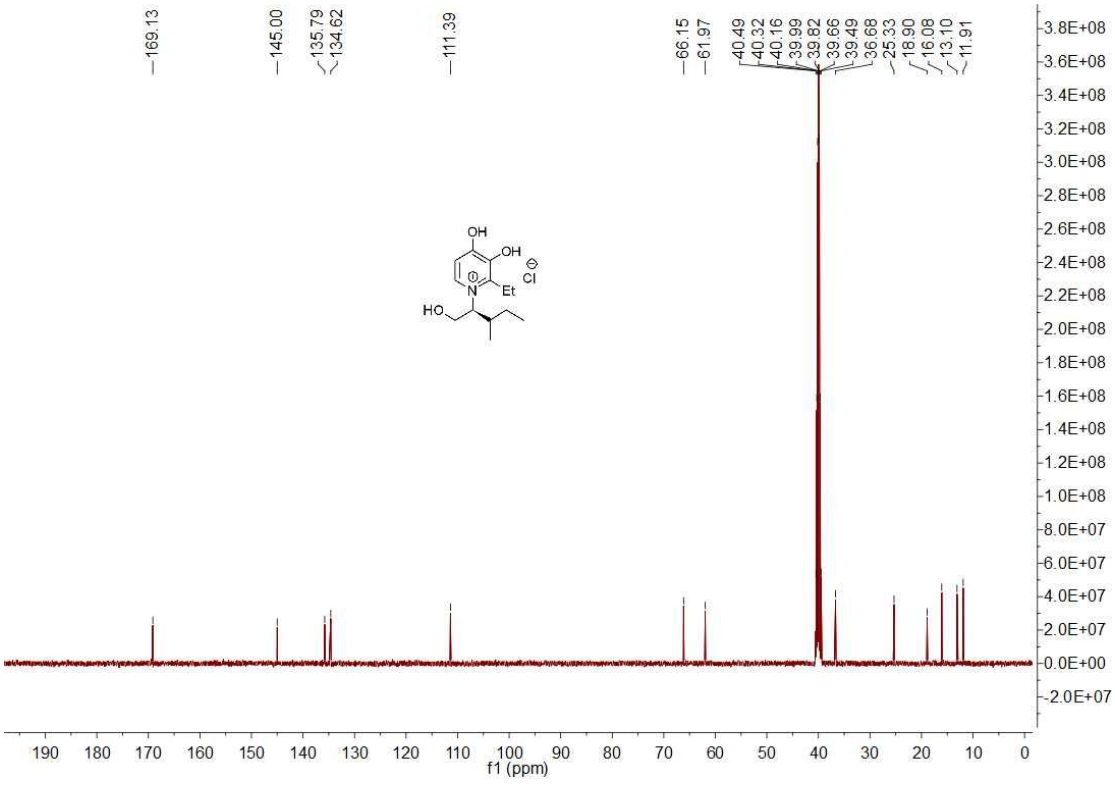
7.631
6.176
6.161
3.919
3.908
3.898
3.888
3.878
3.770
3.760
2.842
2.828
2.814
2.799
2.784
2.710
2.696
2.681
2.667
2.652
2.507
2.504
2.500
2.496
2.493
1.876
1.869
1.863
1.856
1.850
1.843
1.837
1.830
1.823
1.119
1.104
1.089
1.019
1.004
0.991
0.977
0.971
0.933
0.919
0.913
0.906
0.899
0.892
0.886
0.872
0.788
0.773
0.759
OH
OH
Cl
Et
N
HO
1.00
0.99
3.07
1.04
2.02
1.01
1.03
0.99
3.05
5.08
3.04
f1 (ppm)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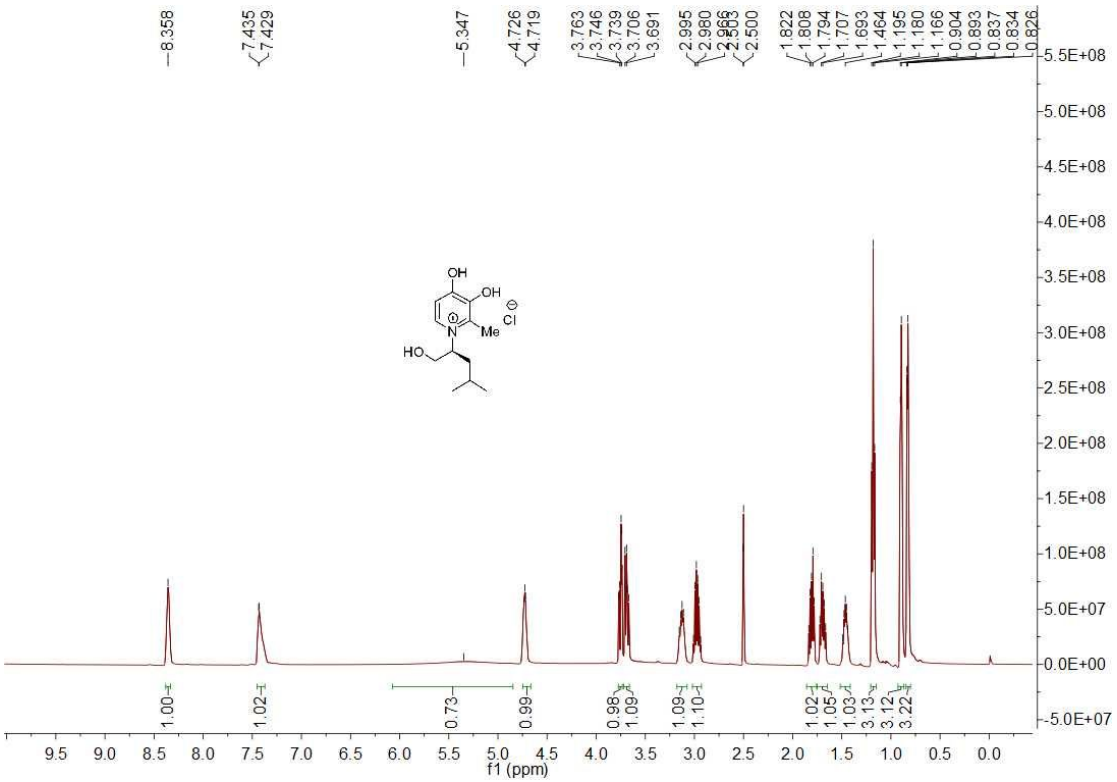
145.00
135.79
134.62
111.39
66.15
61.97
40.49
40.32
40.16
39.99
39.82
39.66
39.49
36.68
25.33
18.90
16.08
13.10
11.91
OH
OH
Cl
Et
N
HO
f1 (ppm)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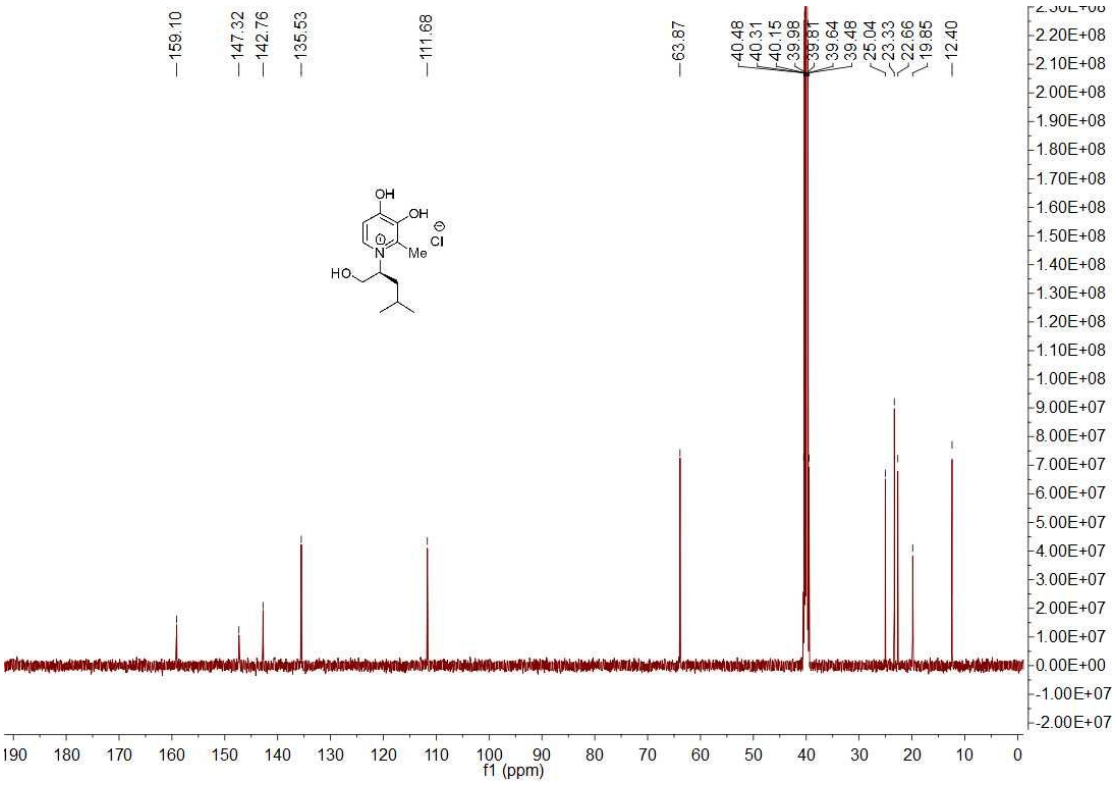
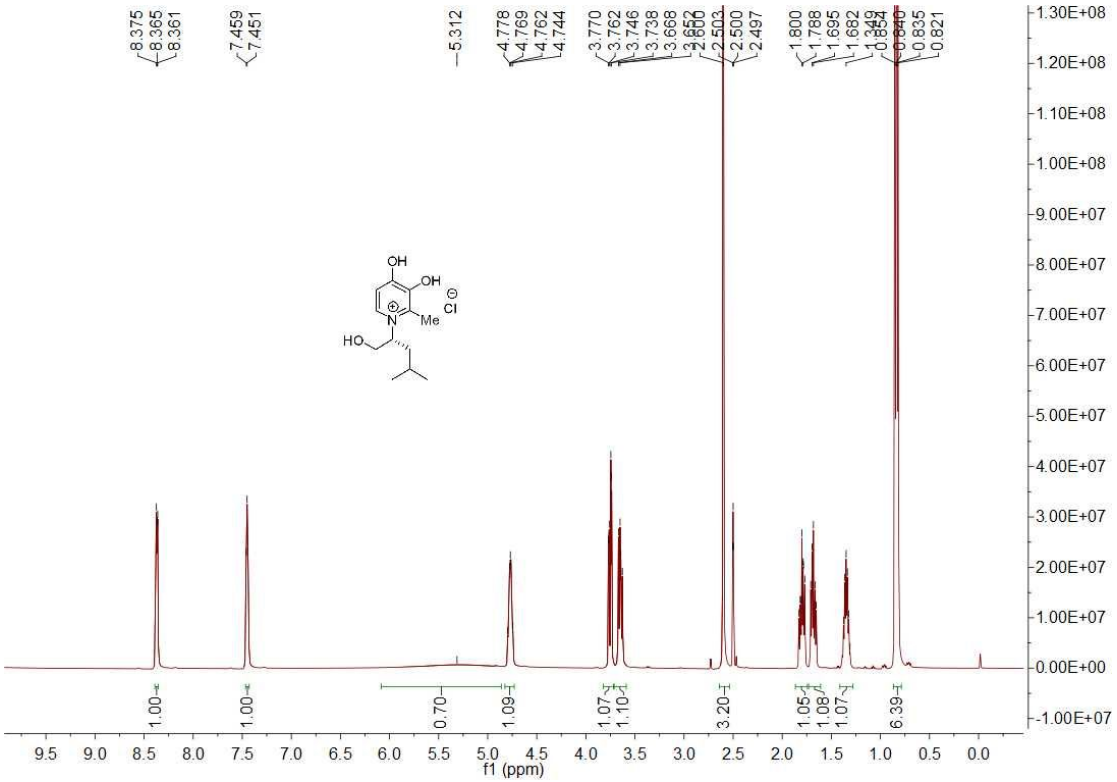
7.435
7.429
5.347
4.726
4.719
3.763
3.746
3.739
3.706
3.691
2.995
2.980
2.966
2.503
2.500
1.822
1.808
1.794
1.707
1.693
1.464
1.195
1.180
1.166
0.904
0.893
0.837
0.834
0.826
OH
OH
Cl
Me
HO
1.00
1.02
0.73
0.99
0.98
1.09
1.09
1.10
1.02
1.05
1.03
3.13
3.12
3.22
f1 (ppm)


159.10
147.32
142.76
135.53
111.68
63.87
40.48
40.31
40.15
39.98
39.81
39.64
39.48
25.04
23.33
22.66
19.85
12.40
OH
OH
Cl
Me
HO
f1 (ppm)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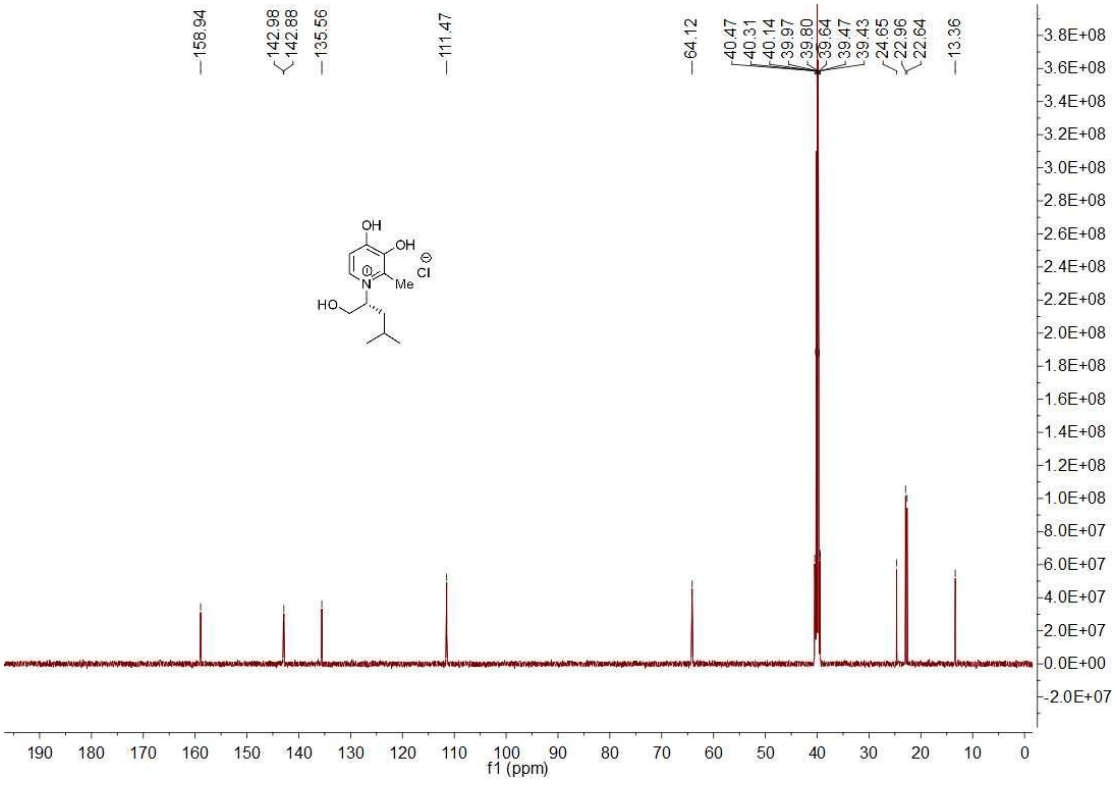
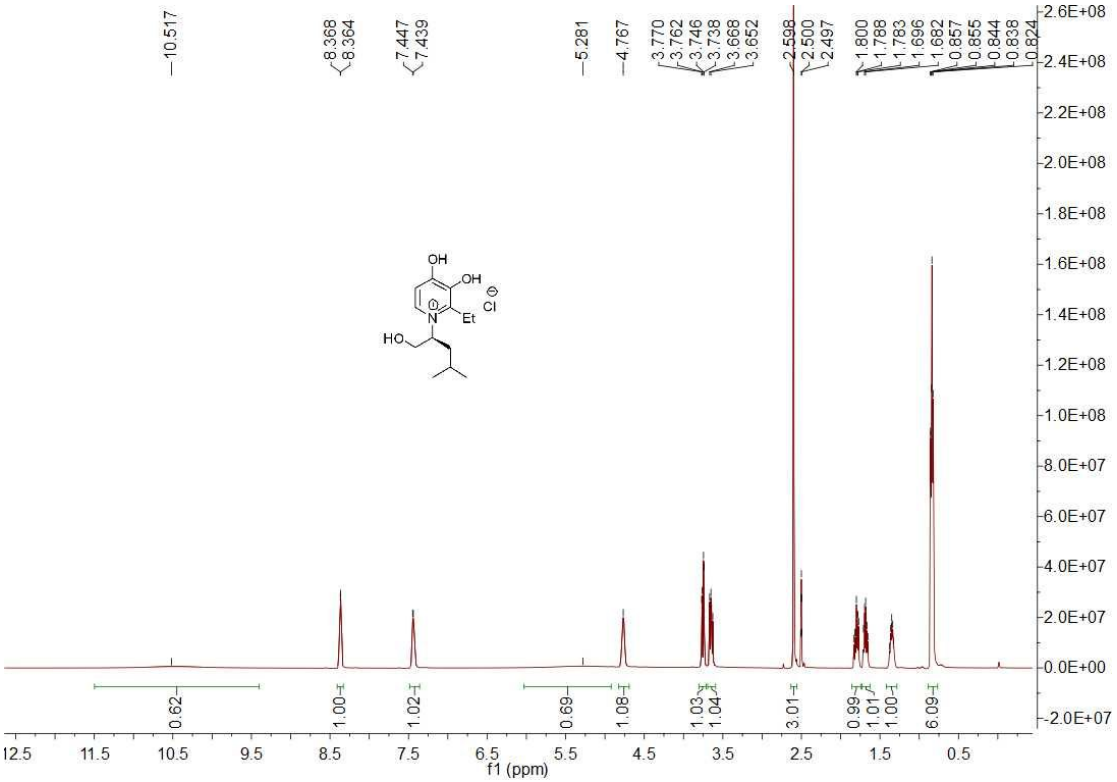
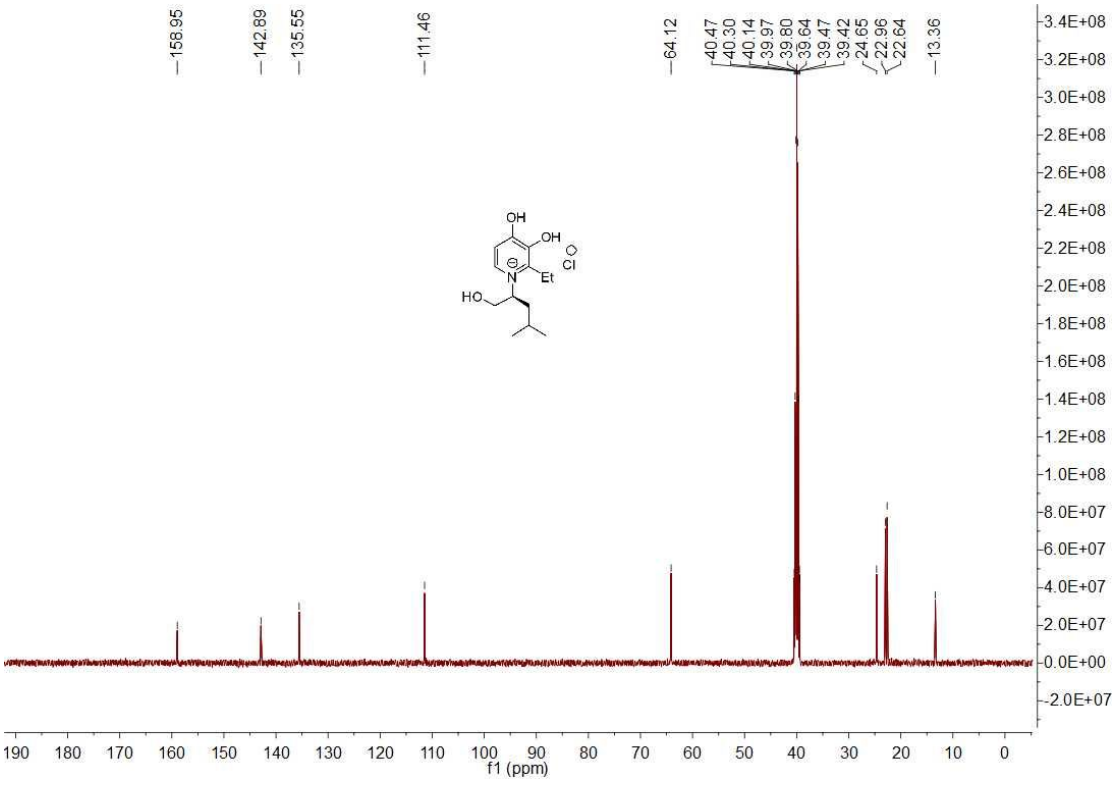
8.365
8.361
7.459
7.451
5.312
4.778
4.769
4.762
4.744
3.770
3.762
3.746
3.738
3.668
2.503
2.500
2.497
1.800
1.788
1.695
1.682
0.840
0.835
0.821
OH
OH
Cl
Me
HO
1.00
1.00
0.70
1.09
1.07
1.10
3.20
1.05
1.08
1.07
6.39
f1 (ppm)


158.94
142.98
142.88
135.56
111.47
64.12
40.47
40.31
40.14
39.97
39.80
39.64
39.47
39.43
24.65
22.96
22.64
13.36
OH
OH
Cl
Me
HO
f1 (ppm)


10.517
8.368
8.364
7.447
7.439
5.281
4.767
3.770
3.762
3.746
3.738
3.668
3.652
2.598
2.500
2.497
1.800
1.788
1.783
1.696
1.682
0.857
0.855
0.844
0.838
0.824
OH
OH
Cl
N
Et
HO
0.62
1.00
1.02
0.69
1.08
1.03
1.04
3.01
0.99
1.01
1.00
6.09
f1 (ppm)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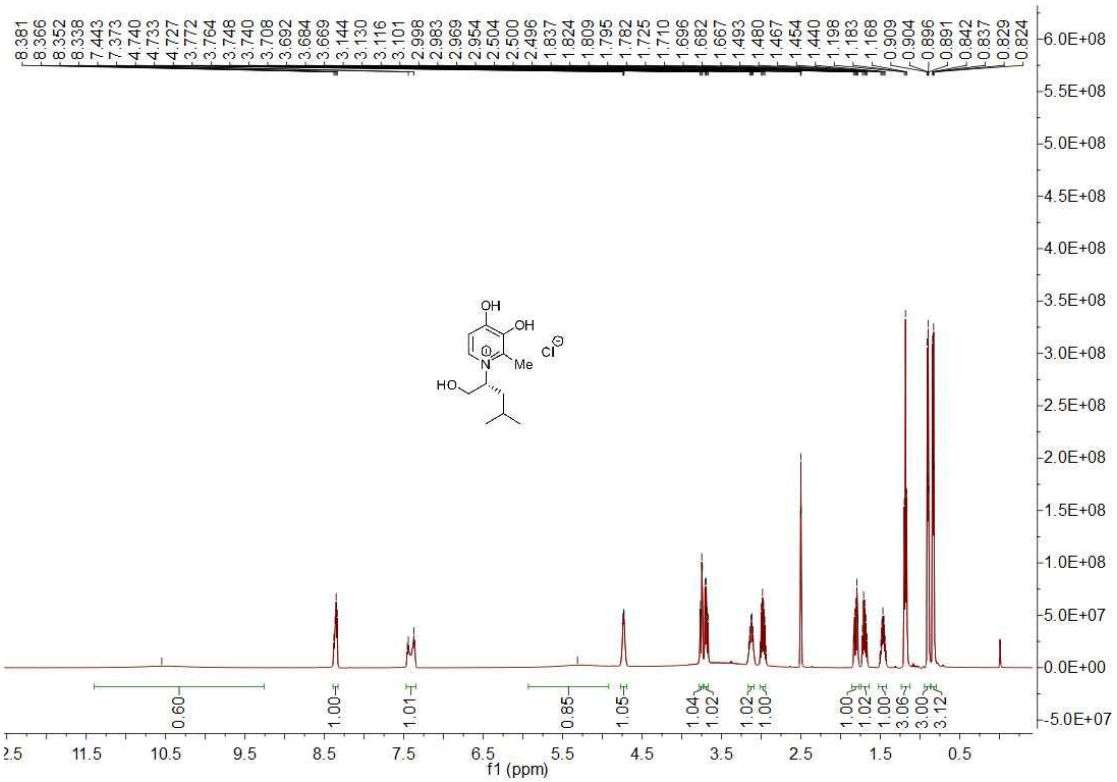
142.89
135.55
111.46
64.12
40.47
40.30
40.14
39.97
39.80
39.64
39.47
39.42
24.65
22.96
22.64
13.36
OH
OH
Cl
N
Et
HO
f1 (ppm)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8.352
8.338
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7.373
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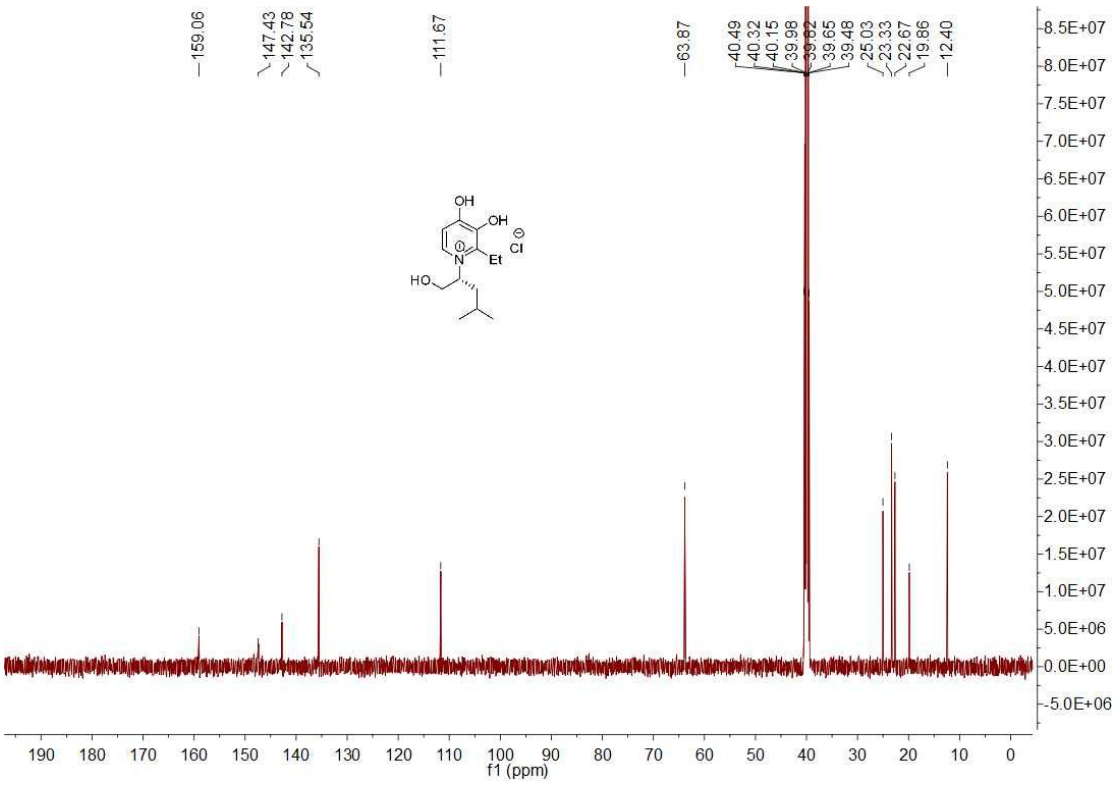
4.733
4.727
3.772
3.764
3.748
3.740
3.708
3.692
3.684
3.669
3.144
3.130
3.116
3.101
2.998
2.983
2.969
2.954
2.504
2.500
2.496
1.837
1.824
1.809
1.795
1.782
1.725
1.710
1.696
1.682
1.667
1.493
1.480
1.467
1.454
1.440
1.198
1.183
1.168
0.909
0.904
0.896
0.891
0.842
0.837
0.829
0.824
OH
OH
Cl
N
Me
HO
0.60
1.00
1.01
0.85
1.05
1.04
1.02
1.02
1.00
1.00
1.02
1.00
3.06
3.00
3.12
f1 (ppm)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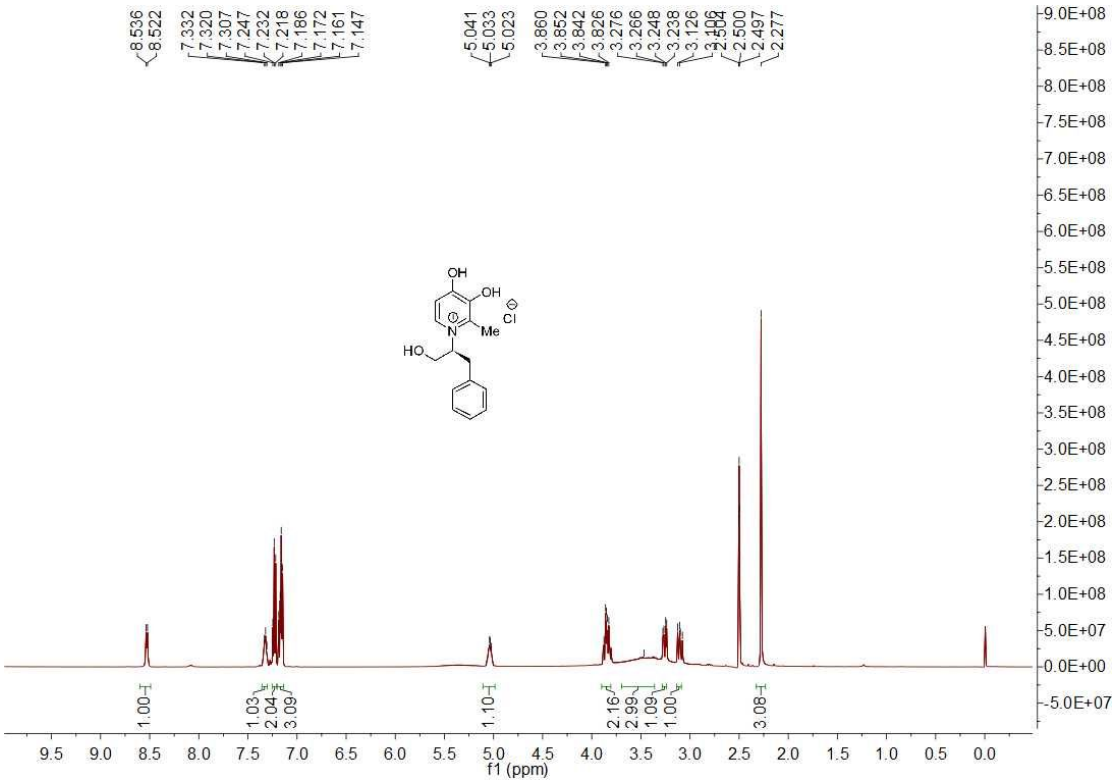
142.78
135.54
111.67
63.87
40.49
40.32
40.15
39.98
39.82
39.65
39.48
25.03
23.33
22.67
19.86
12.40
OH
OH
Cl
N
Et
HO
f1 (ppm)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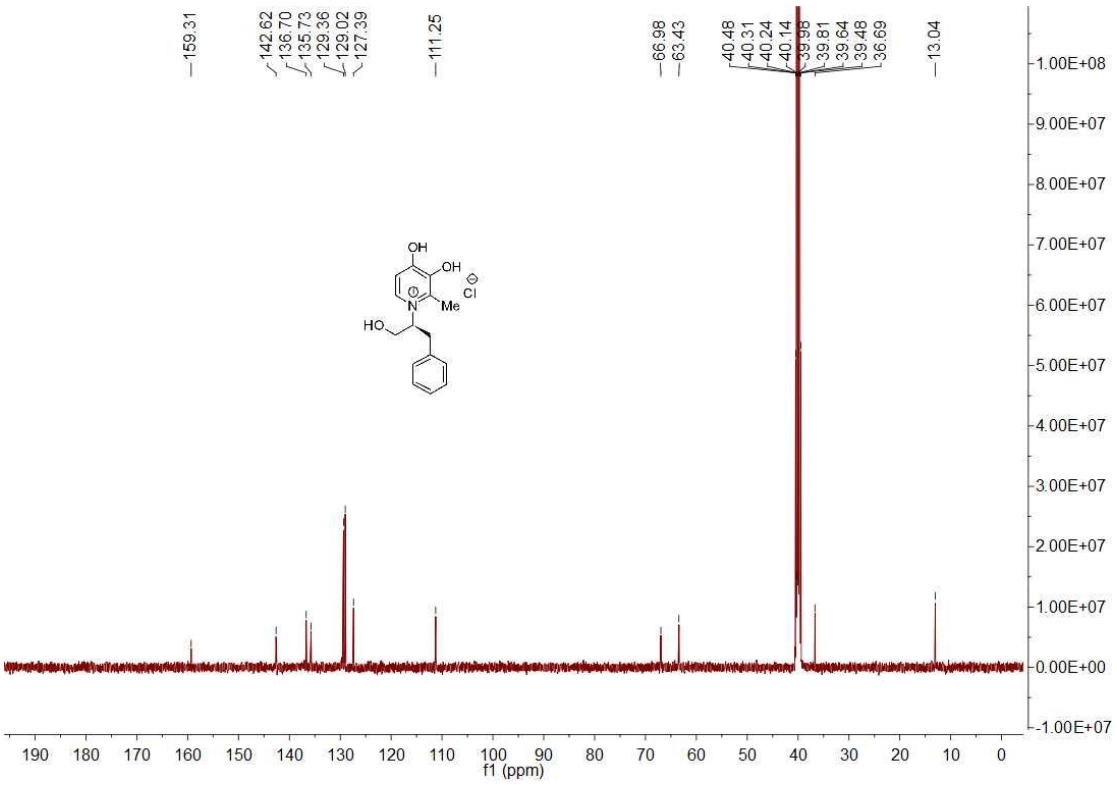
8.522
7.332
7.320
7.307
7.247
7.232
7.218
7.186
7.172
7.161
7.147
5.041
5.033
5.023
3.860
3.852
3.842
3.826
3.276
3.266
3.248
3.238
3.126
2.500
2.497
2.277
OH
OH
Cl
Me
HO
1.00
1.03
2.04
3.09
1.10
2.16
2.99
1.09
1.00
3.08
f1 (ppm)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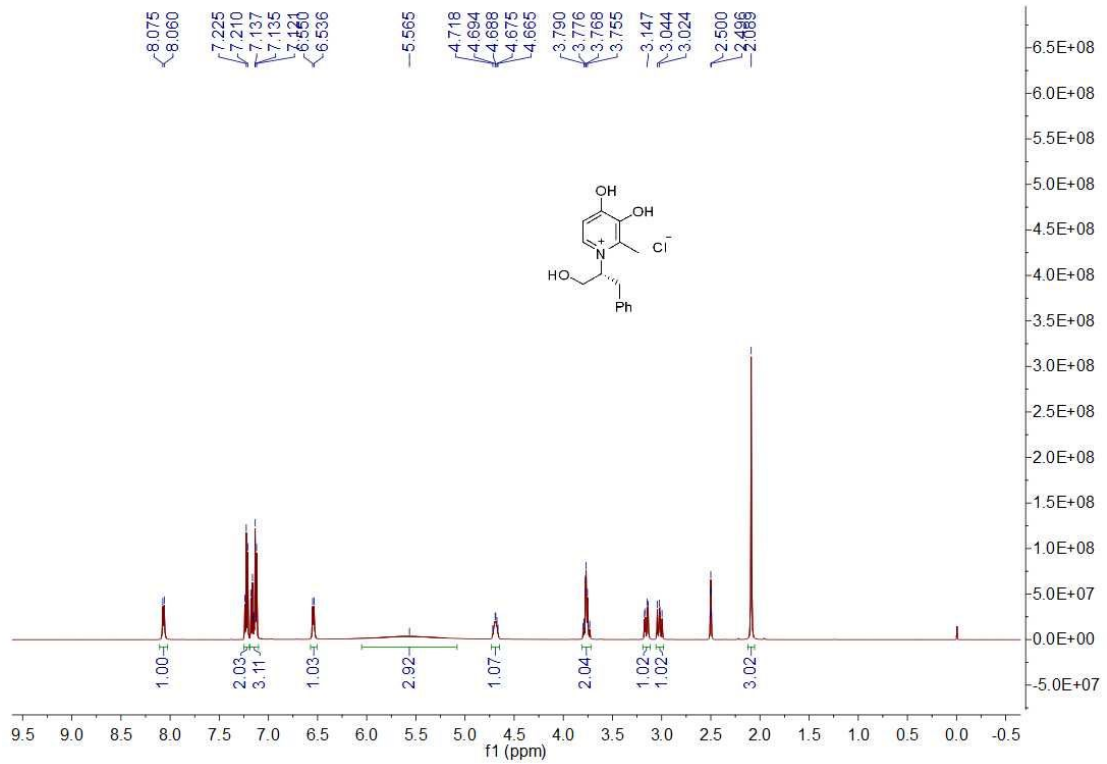
136.70
135.73
129.36
129.02
127.39
111.25
66.98
63.43
40.48
40.31
40.24
40.14
39.98
39.81
39.64
39.48
36.69
13.04
OH
OH
Cl
Me
HO
f1 (ppm)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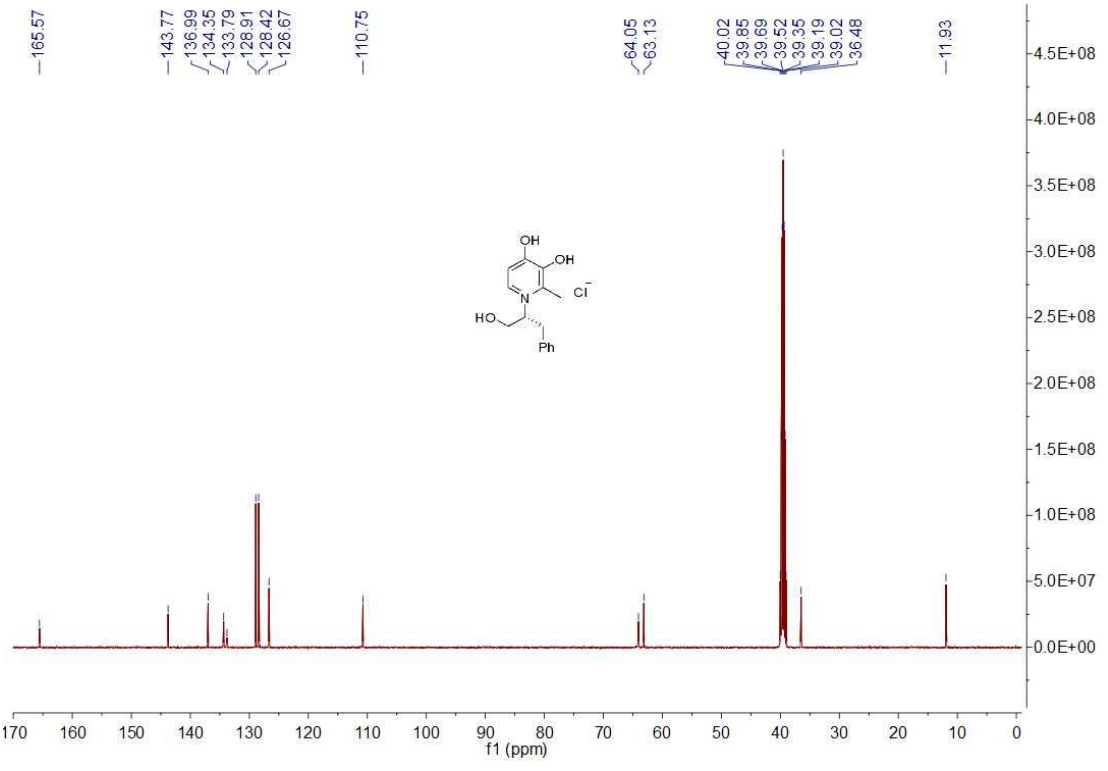
8.060
7.225
7.210
7.137
7.135
7.121
6.551
6.536
5.565
4.718
4.694
4.688
4.675
4.665
3.790
3.776
3.768
3.755
3.147
3.044
3.024
2.500
2.496
2.089
OH
OH
Cl⁻
N
HO
Ph
1.00
2.03
3.11
1.03
2.92
1.07
2.04
1.02
1.02
3.02
f1 (ppm)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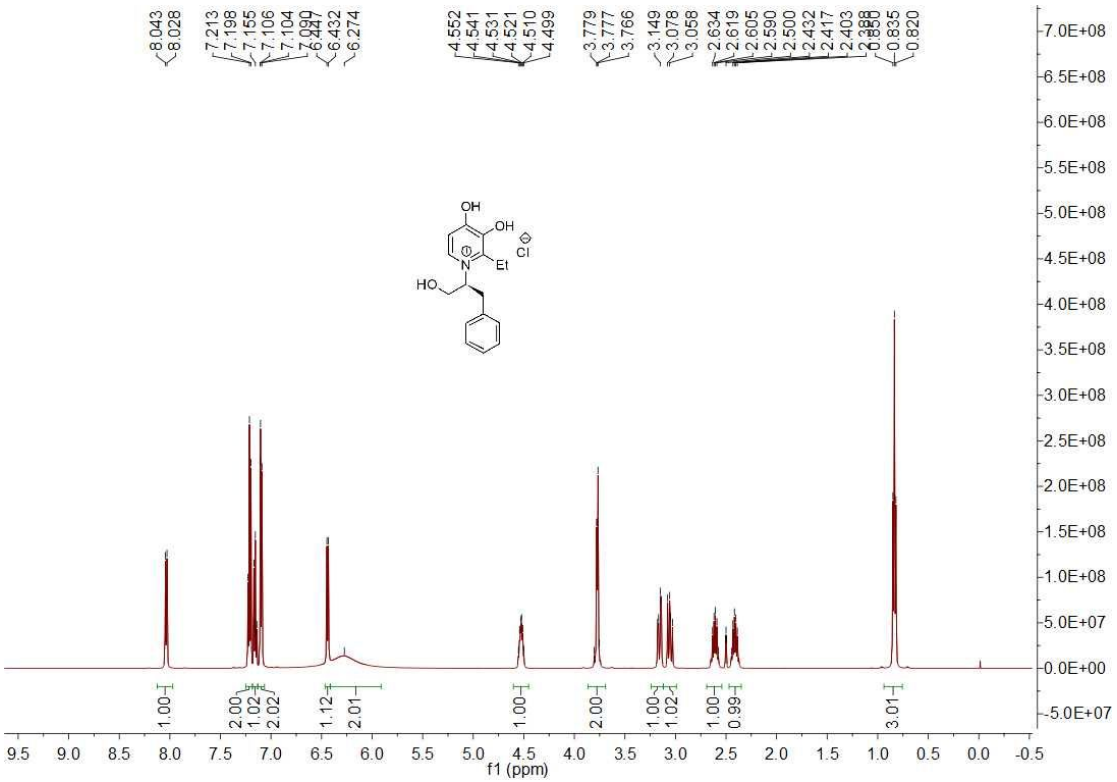
143.77
136.99
134.35
133.79
128.91
128.42
126.67
110.75
64.05
63.13
40.02
39.85
39.69
39.52
39.35
39.19
39.02
36.48
11.93
OH
OH
Cl⁻
N
HO
Ph
f1 (ppm)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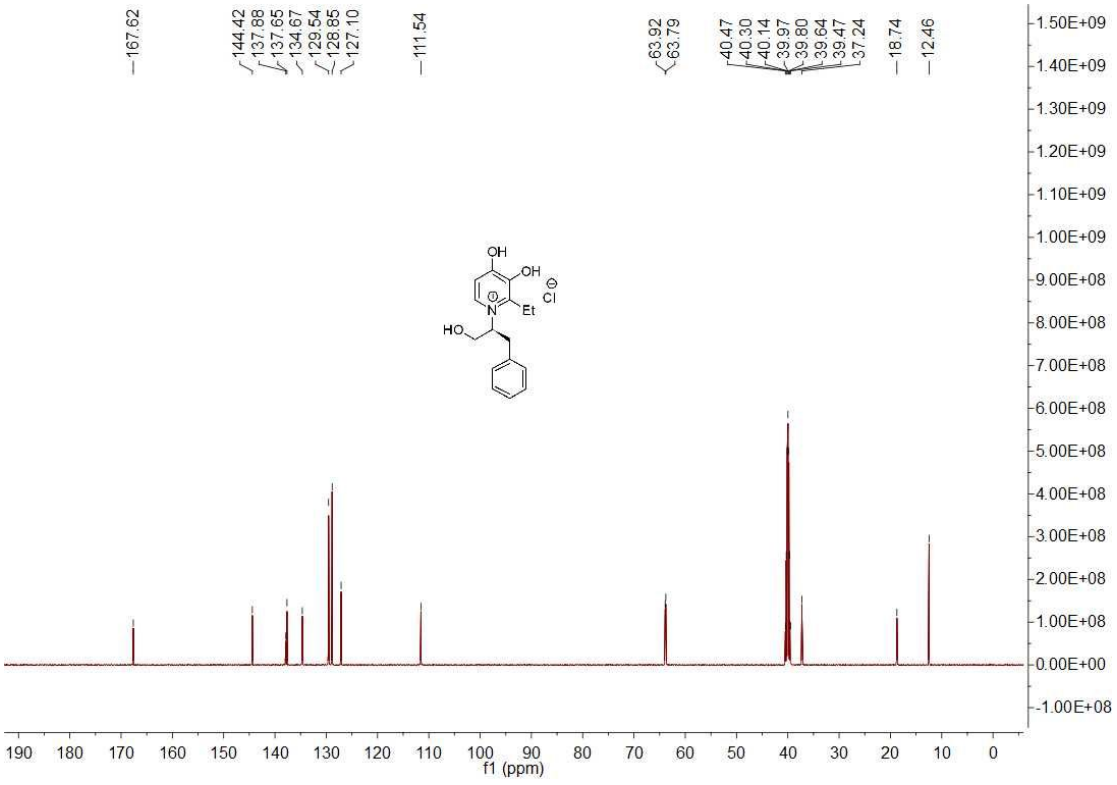
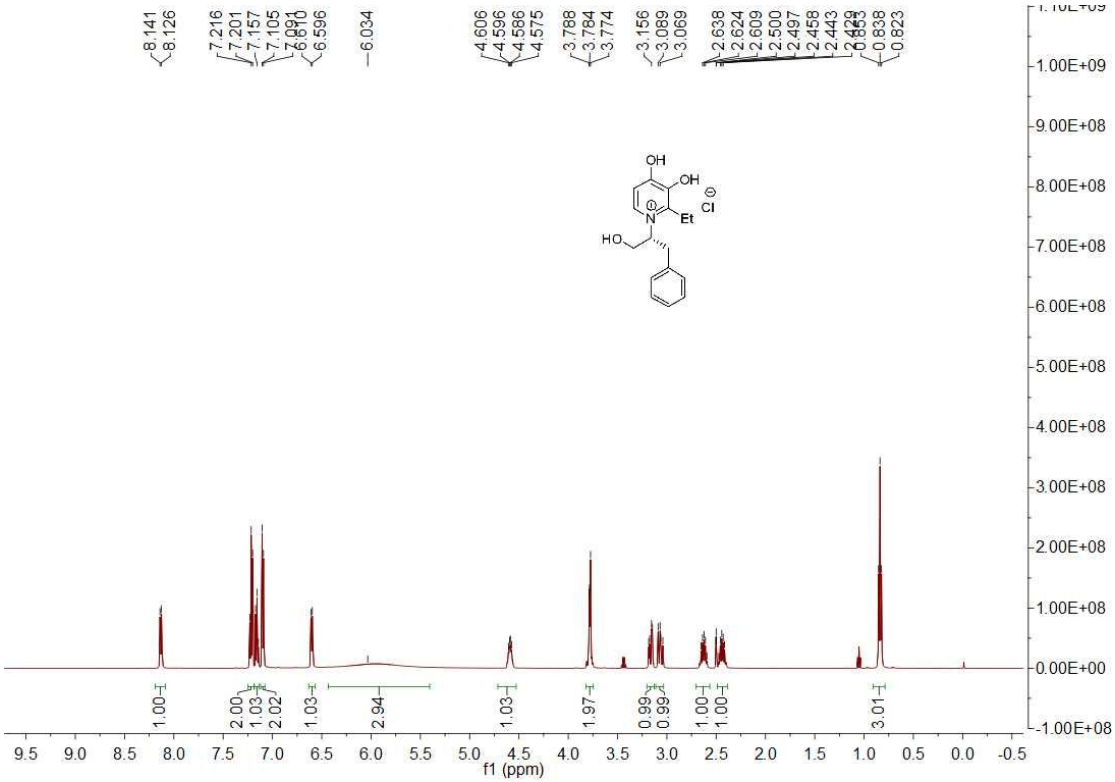
8.028
7.213
7.198
7.155
7.106
7.104
6.432
6.274
4.552
4.541
4.531
4.521
4.510
4.499
3.779
3.777
3.766
3.149
3.078
3.058
2.634
2.619
2.605
2.590
2.500
2.432
2.417
2.403
0.835
0.820
OH
OH
Et
Cl
HO
1.00
2.00
1.02
2.02
1.12
2.01
1.00
2.00
1.00
1.02
1.00
0.99
3.01
f1 (ppm)


167.62
144.42
137.88
137.65
134.67
129.54
128.85
127.10
111.54
63.92
63.79
40.47
40.30
40.14
39.97
39.80
39.64
39.47
37.24
18.74
12.46
OH
OH
Et
Cl
HO
f1 (ppm)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8.126
7.216
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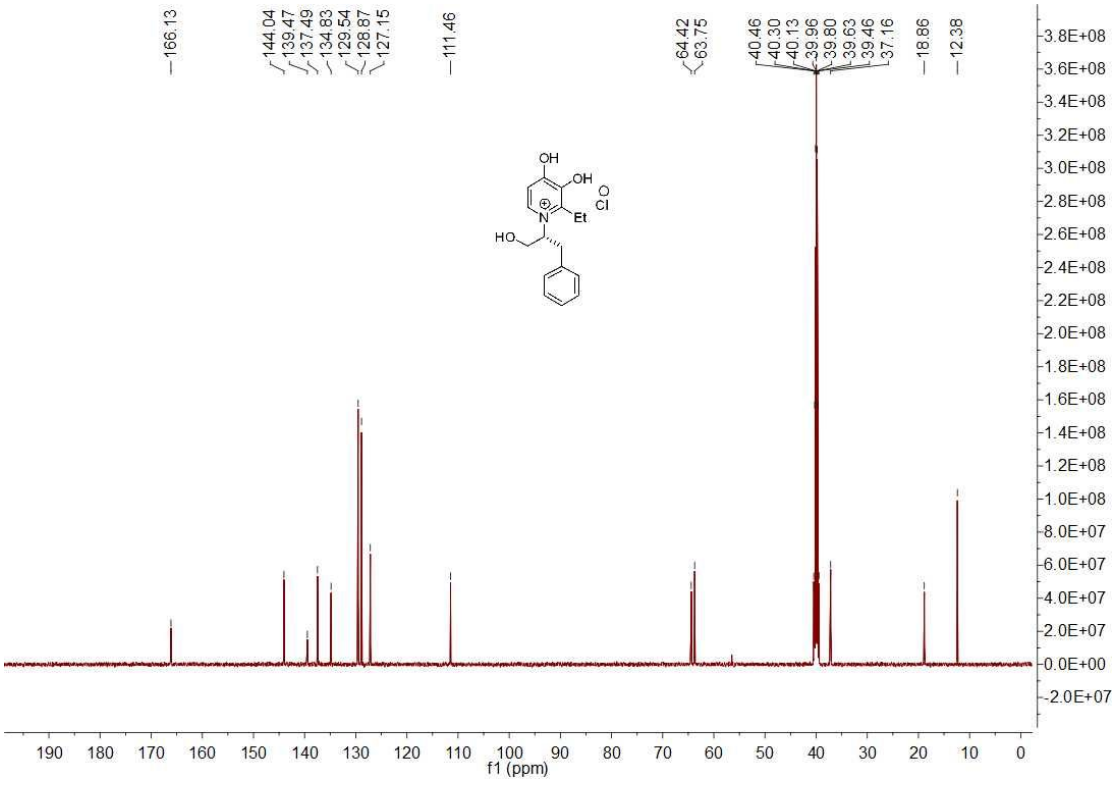
7.157
7.105
7.091
6.810
6.596
6.034
4.606
4.596
4.586
4.575
3.788
3.784
3.774
3.156
3.089
3.069
2.638
2.624
2.609
2.500
2.497
2.458
2.443
0.838
0.823
OH
OH
Cl
Et
HO
1.00
2.00
1.03
2.02
1.03
2.94
1.03
1.97
0.99
0.99
1.00
1.00
3.01
f1 (ppm)


166.13
144.04
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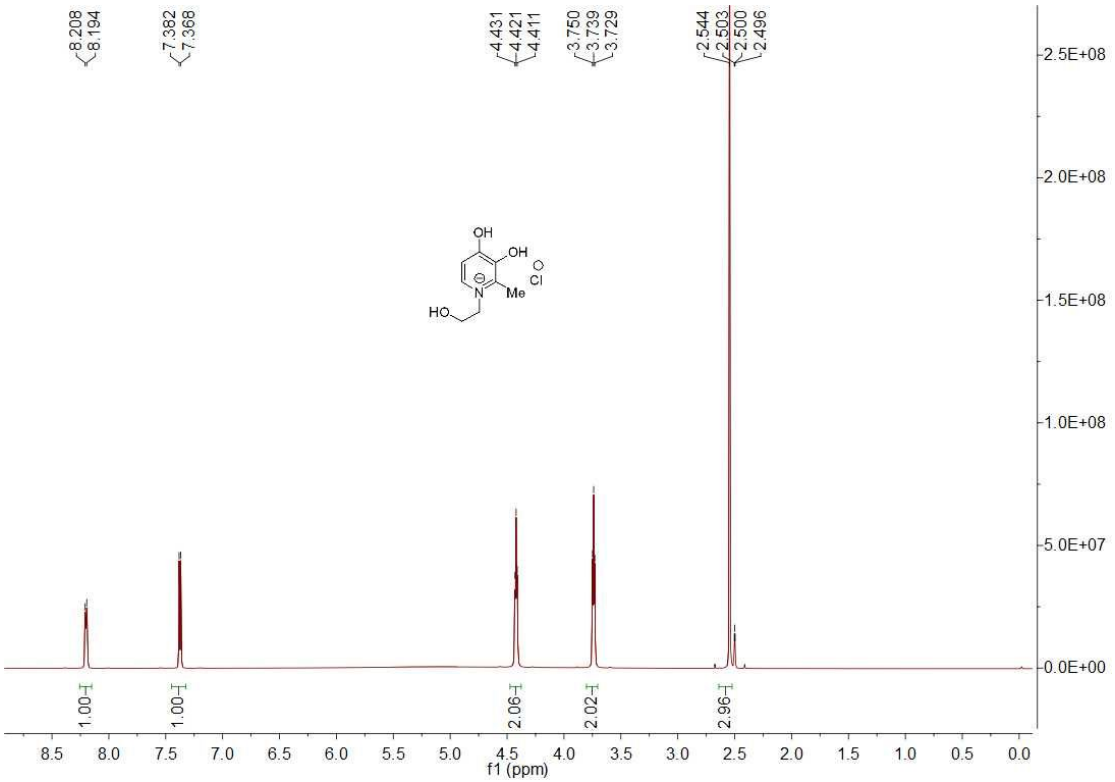
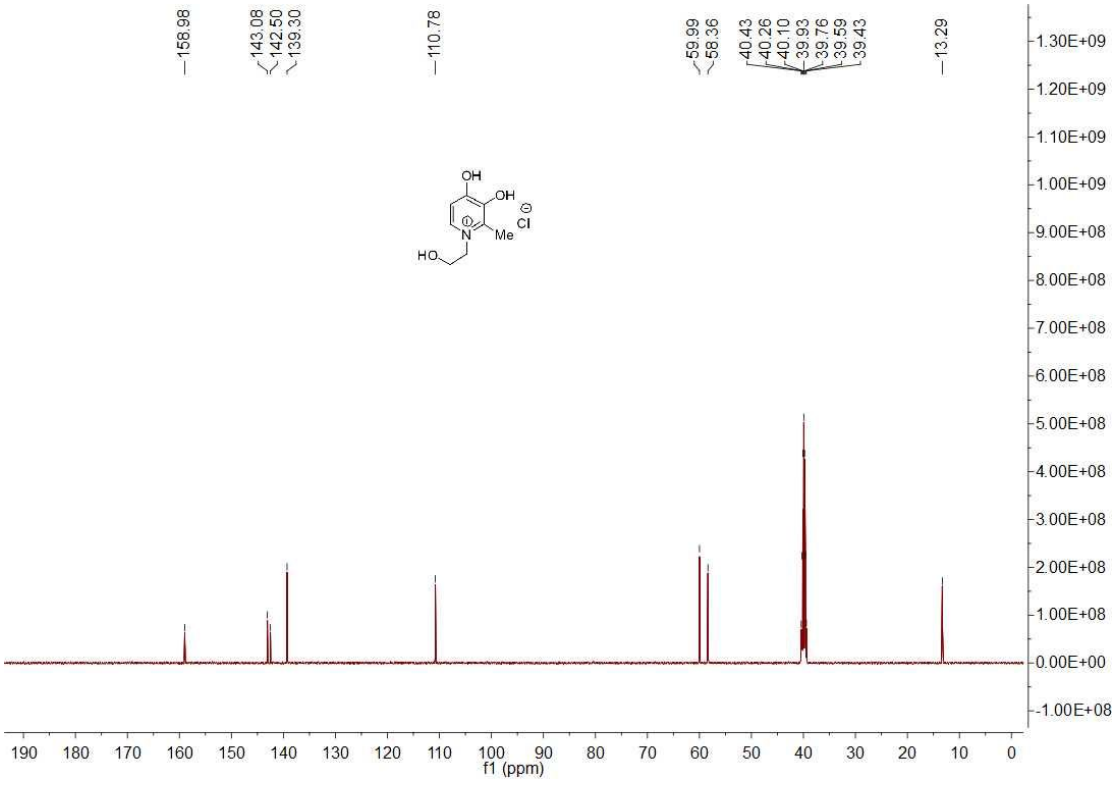
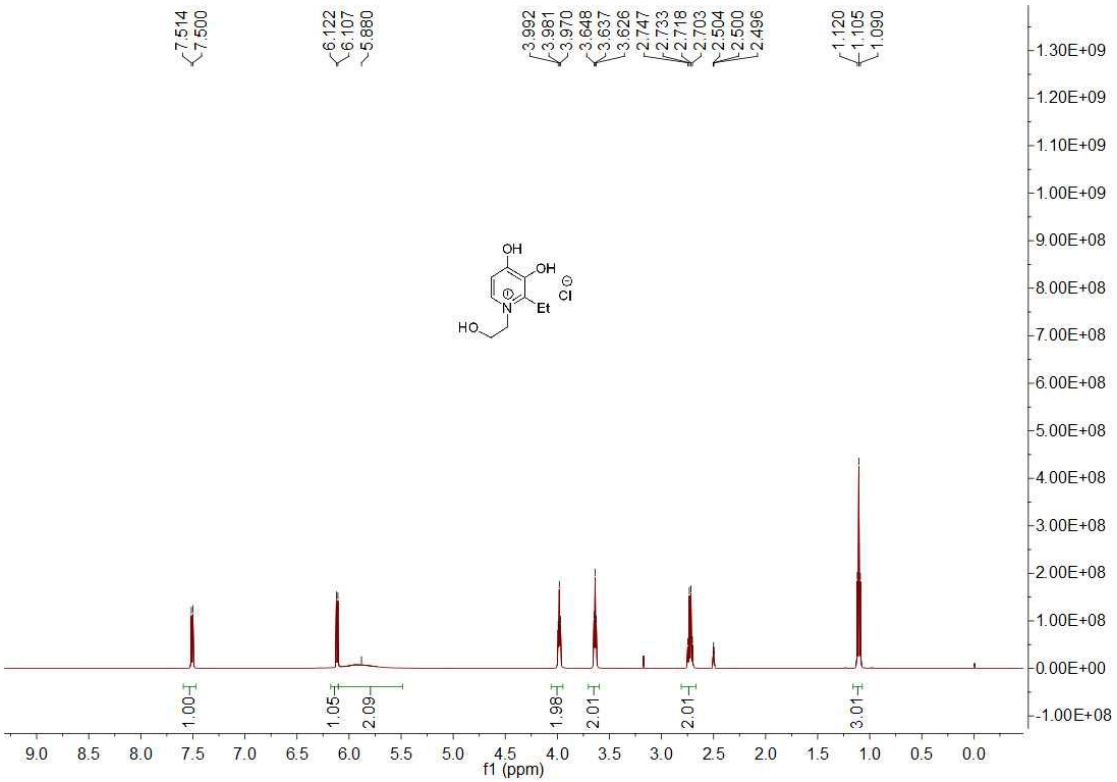
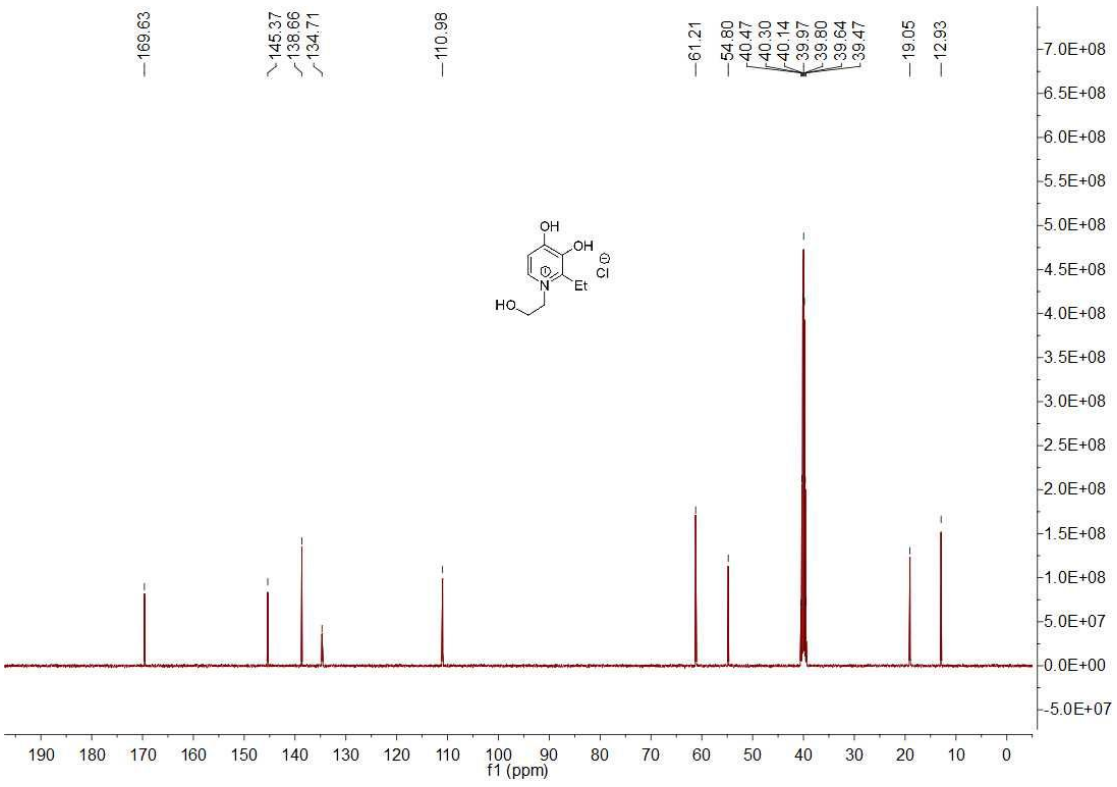
137.49
134.83
129.54
128.87
127.15
111.46
64.42
63.75
40.46
40.30
40.13
39.96
39.80
39.63
39.46
37.16
18.86
12.38
OH
OH
Cl
Et
HO
f1 (ppm)


8.208
8.194
7.382
7.368
4.431
4.421
4.411
3.750
3.739
3.729
2.544
2.503
2.500
2.496
OH
OH
Cl
Me
HO
1.00
1.00
2.06
2.02
2.96
f1 (ppm)


158.98
143.08
142.50
139.30
110.78
59.99
58.36
40.43
40.26
40.10
39.93
39.76
39.59
39.43
13.29
OH
OH
Cl
Me
HO
f1 (ppm)


7.514
7.500
6.122
6.107
5.880
3.992
3.981
3.970
3.648
3.637
3.626
2.747
2.733
2.718
2.703
2.504
2.500
2.496
1.120
1.105
1.090
OH
OH
Cl
N
Et
HO
1.00
1.05
2.09
1.98
2.01
2.01
3.01
f1 (ppm)


169.63
145.37
138.66
134.71
110.98
61.21
54.80
40.47
40.30
40.14
39.97
39.80
39.64
39.47
19.05
12.93
OH
OH
Cl
N
Et
HO
f1 (ppm)