

Supporting Information

Structure-based Discovery of a Series of NSD2-PWWP1

Inhibitors

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1. Table S1 Statistics for data processing and model refinement of NSD2 with an imidazol-5-yl benzonitrile compound

PDB code	7VLN
Synchrotron	SSRF
Beam line	BL17U1
Wavelength (Å)	0.97919
Space group	P3121
<i>a, b, c</i> (Å)	70.12, 70.12, 230.67
α, β, γ (°)	90.00, 90.00, 120.00
Total reflections	245676
Unique reflections	12759
Resolution (Å)	3.09(3.17-3.09)
R-merge (%)	13.4(185.1)
Mean <i>I</i> / σ (<i>I</i>)	18.9/2.6
Completeness (%)	99.8(99.9)
Redundancy	19.3(19.2)
Resolution (Å)	30.36-3.09
<i>R</i> _{work} / <i>R</i> _{free} (%)	28.75/34.92
Atoms	3804
Mean temperature factor (Å ²)	93.04
Bond lengths (Å)	0.009
Bond angles (°)	1.24
Ramachandran plot (%)	
Preferred	96.69
Allowed	3.31
outliers	0

Values in parentheses are for the highest-resolution shell. The detailed validation file is

also provided as supporting information.

2. Table S2. Pharmacokinetic parameters for compound 38 in ICR (CD-1) Mice.

Parameter	Compound 38
dose (mg/kg, PO)	20
$t_{1/2}$ (h)	3.65
T_{max} (h)	1.50
C_{max} (ng/mL)	94.9
AUC_{last} (h·ng/mL)	180
AUC_{INF_obs} (h·ng/mL)	178

Mice (n = 3). Parameters were calculated from composite mean plasma concentration–time data.

3. Figure S1

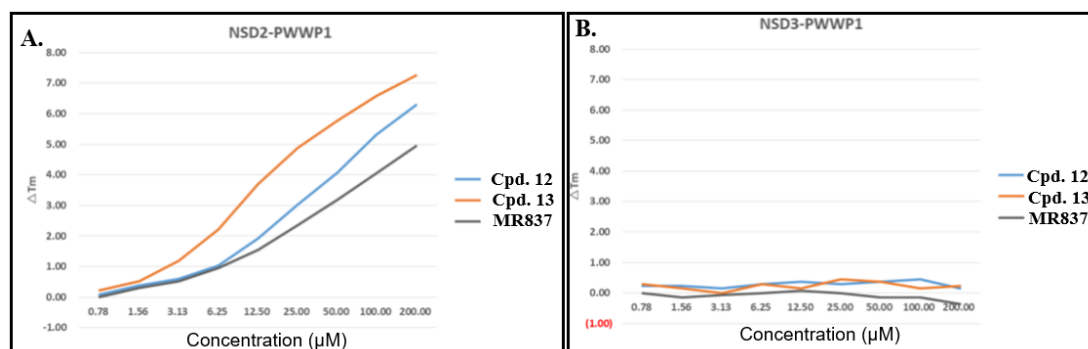


Figure S1. (A) The thermal shift assay displayed that **13** showed more potency binding to NSD2-PWWP1 protein than compound **12**. (B) The compounds showed no effect on the melting temperature of NSD3-PWWP1 protein in a thermal shift assay. Both compounds exhibited remarkable selectivity against NSD2-PWWP1 protein NSD3-PWWP1.

4. Figure S2

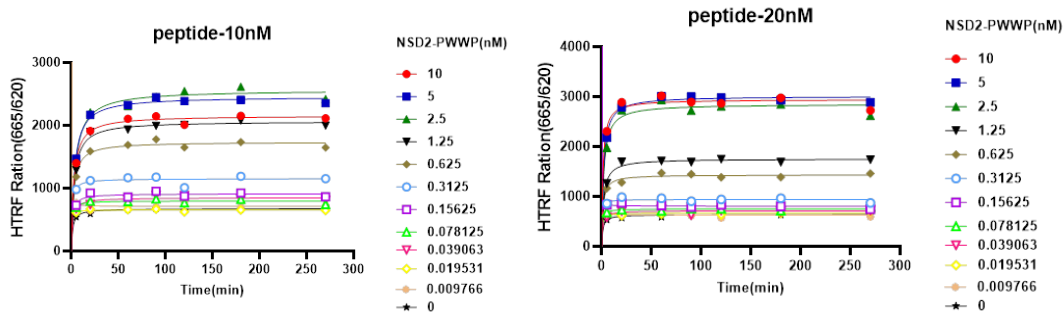
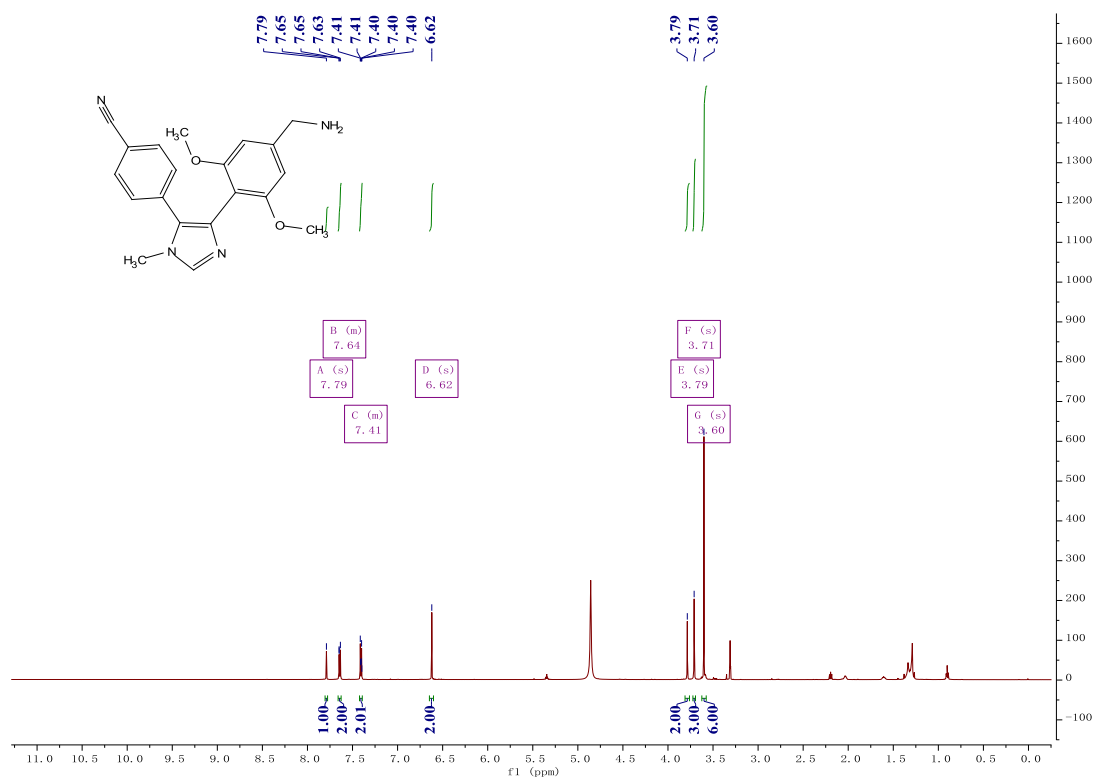
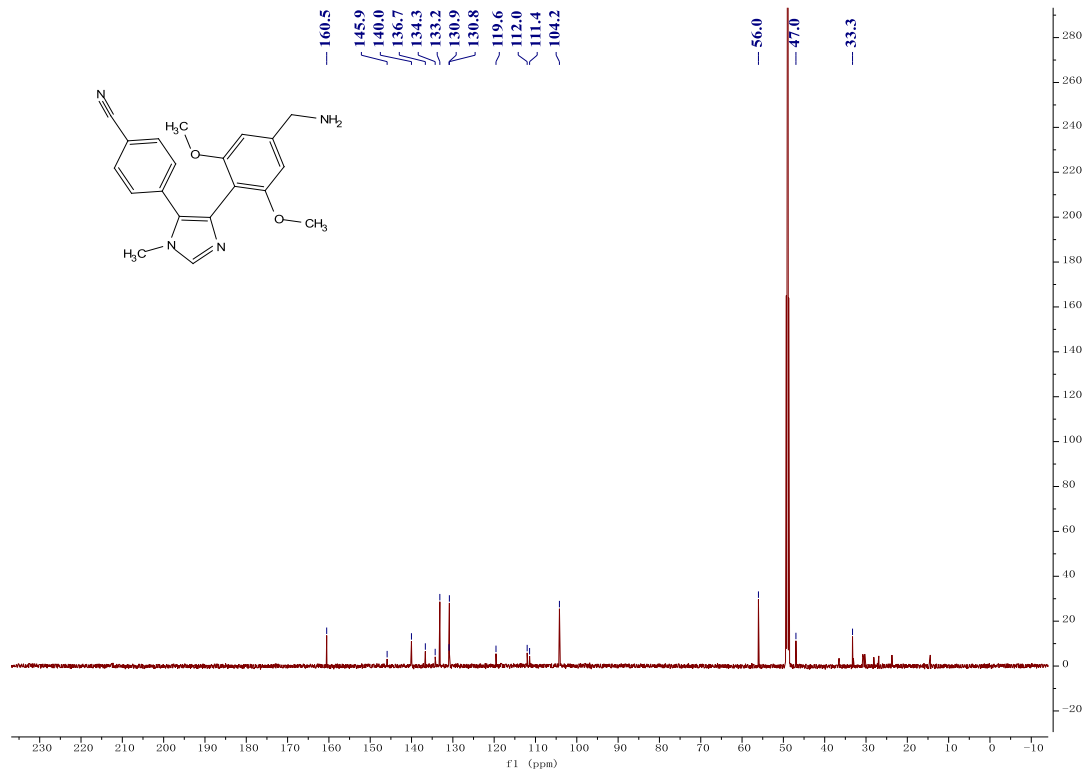


Figure S2. Two peptide concentrations were used to check the sensitive range of the concentration of NSD2-PWWP1 protein in assay development.

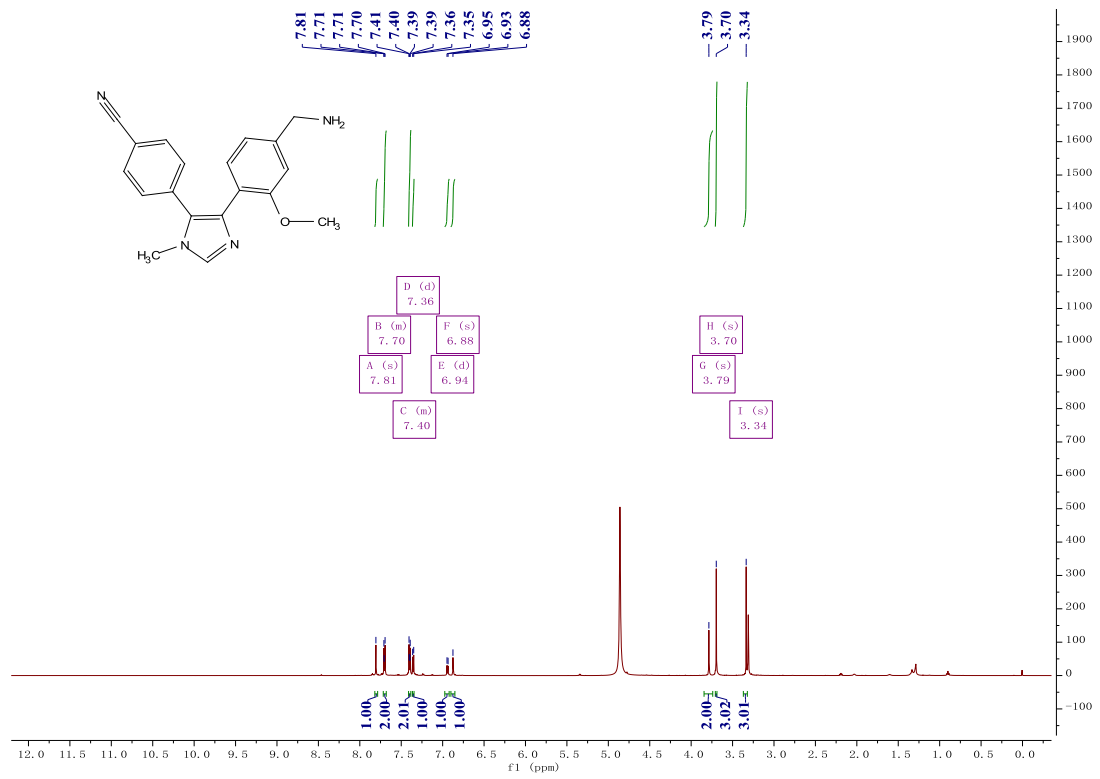
5. Copies of NMR data for selected compounds

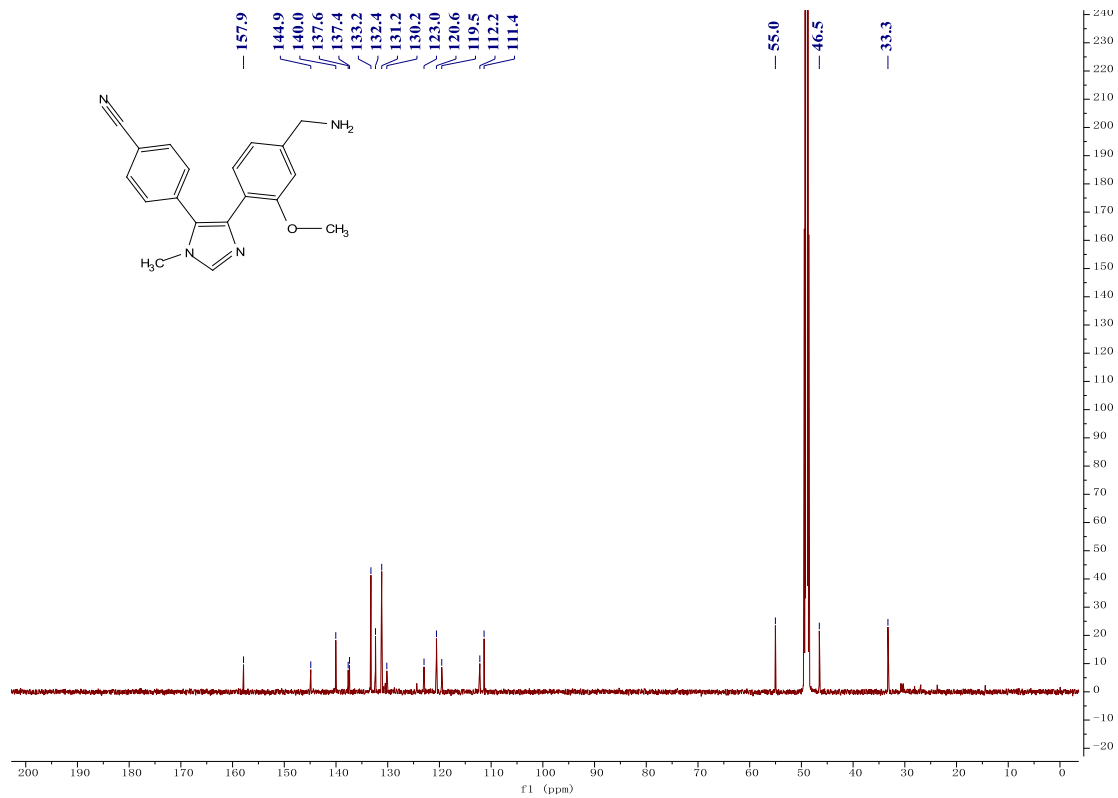
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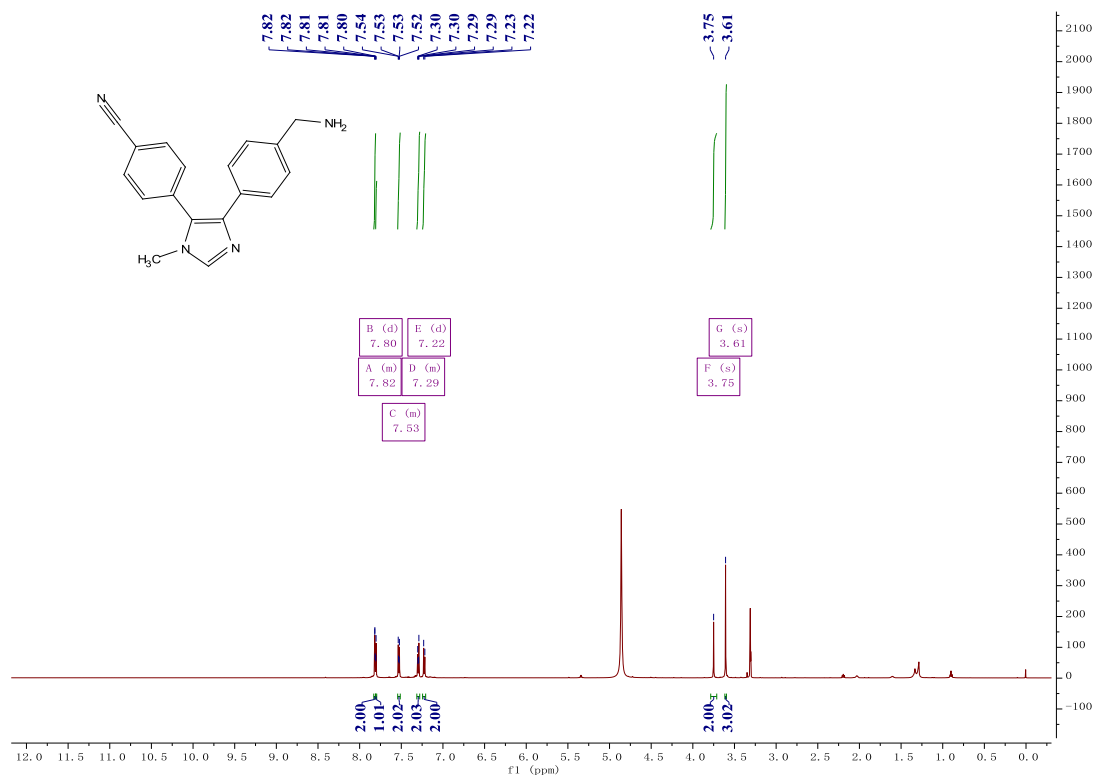


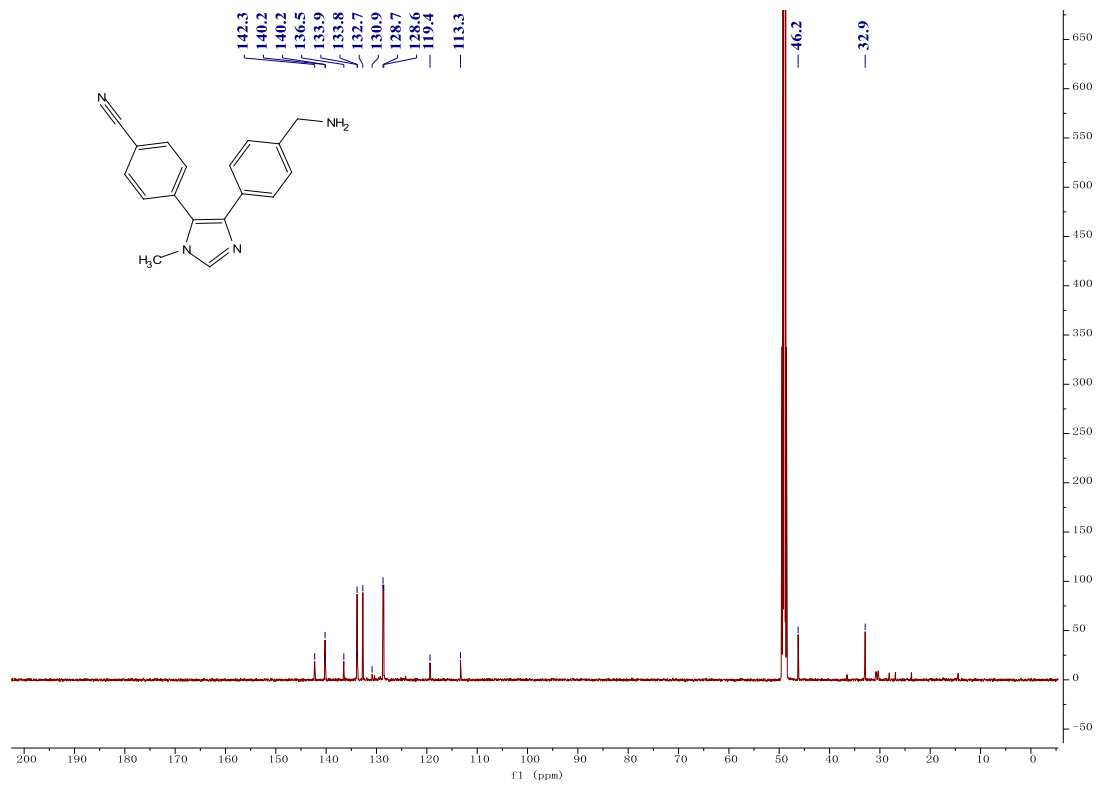
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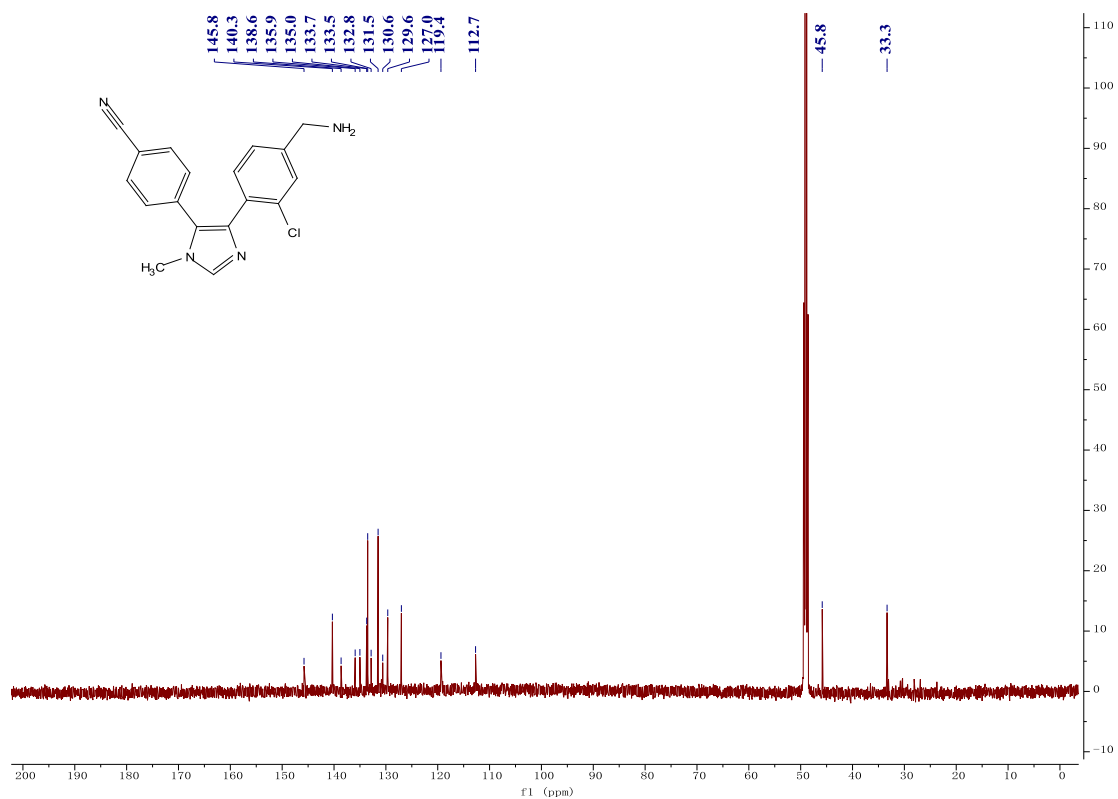
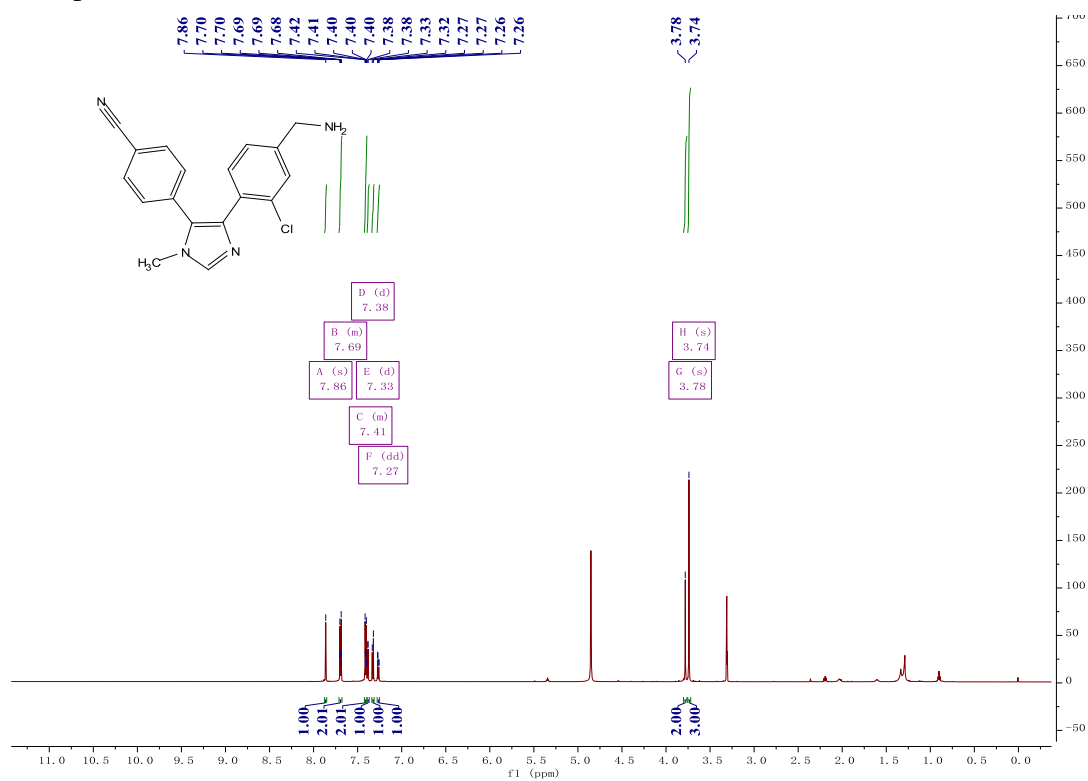


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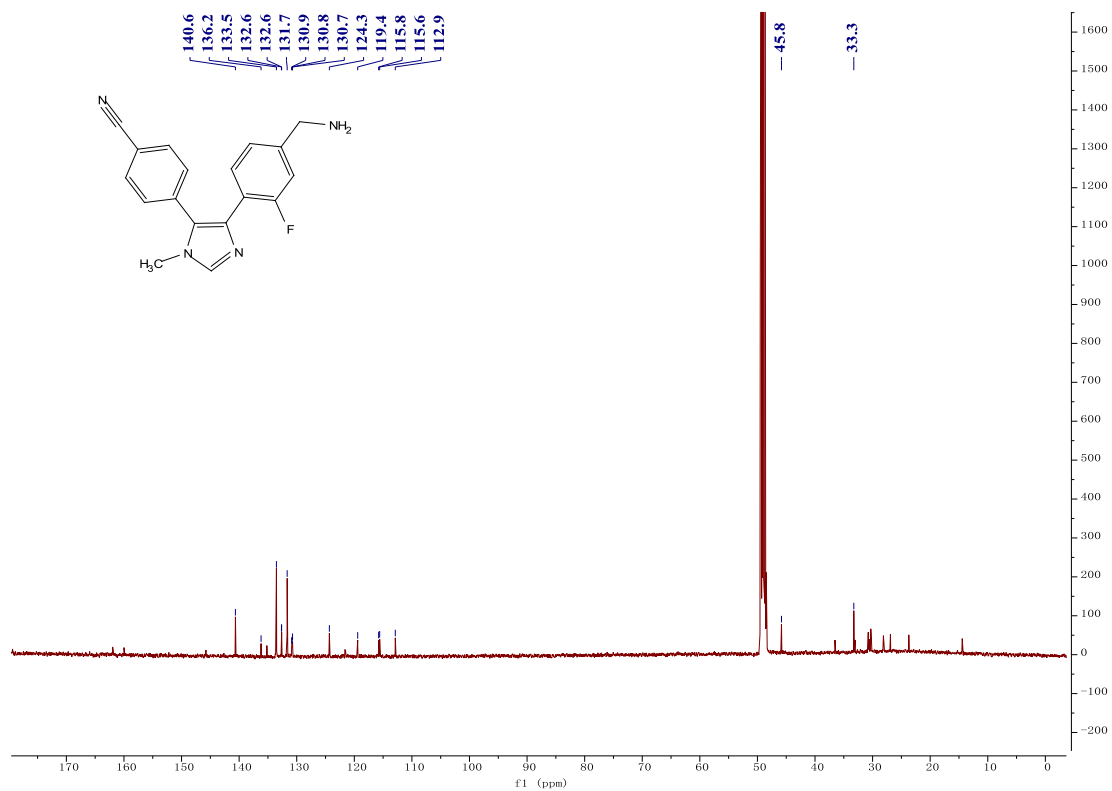
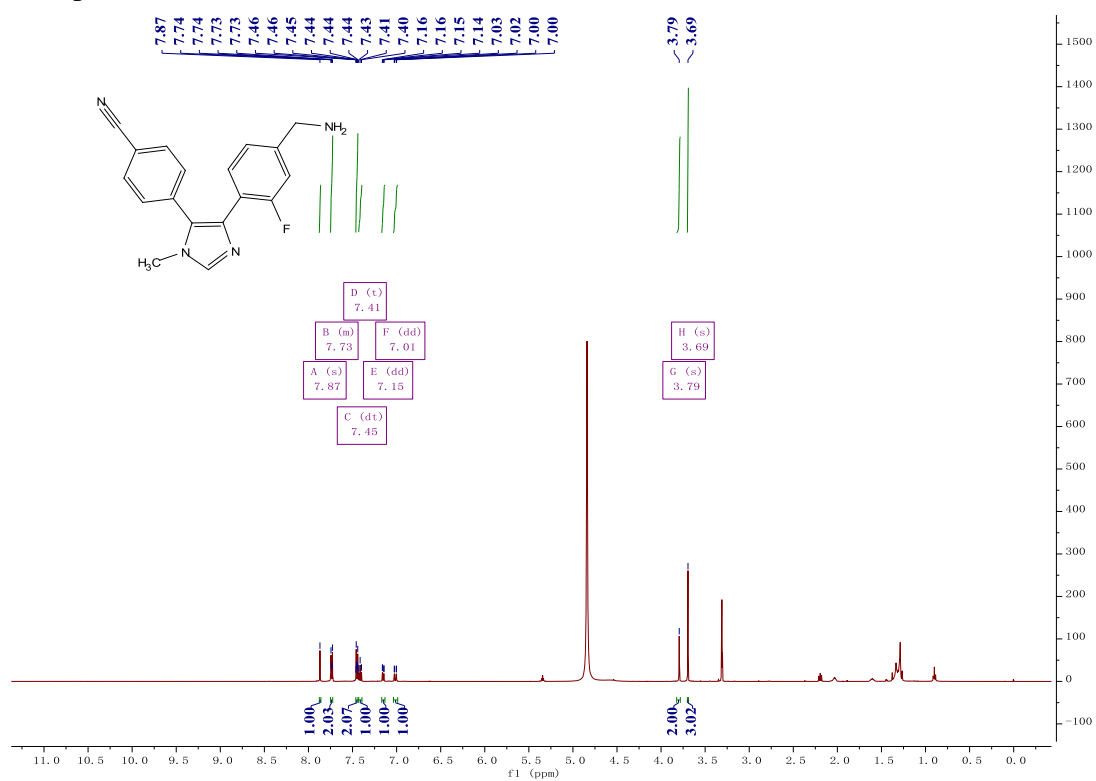




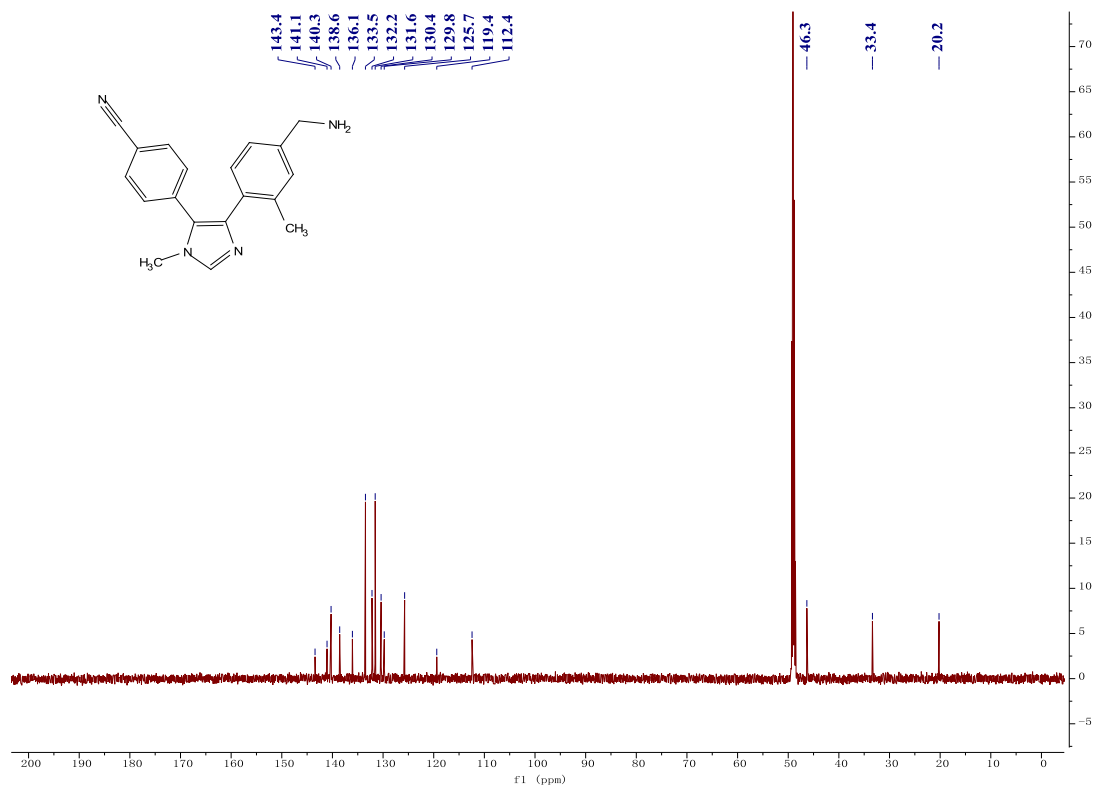
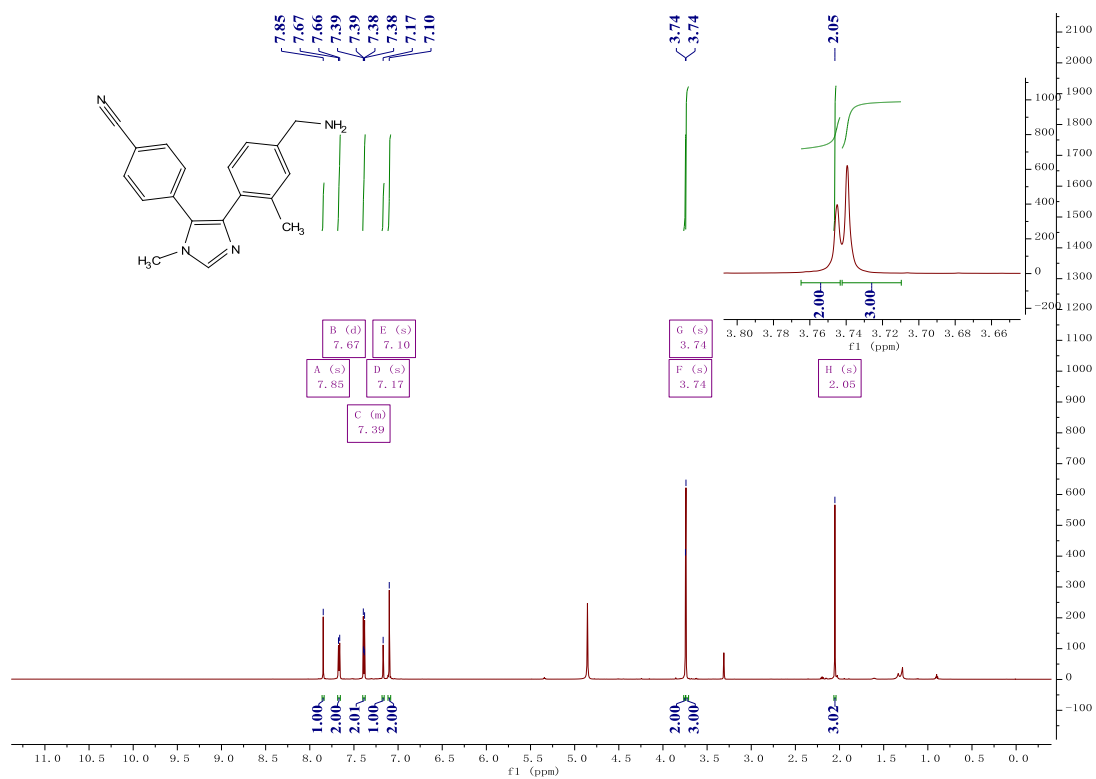
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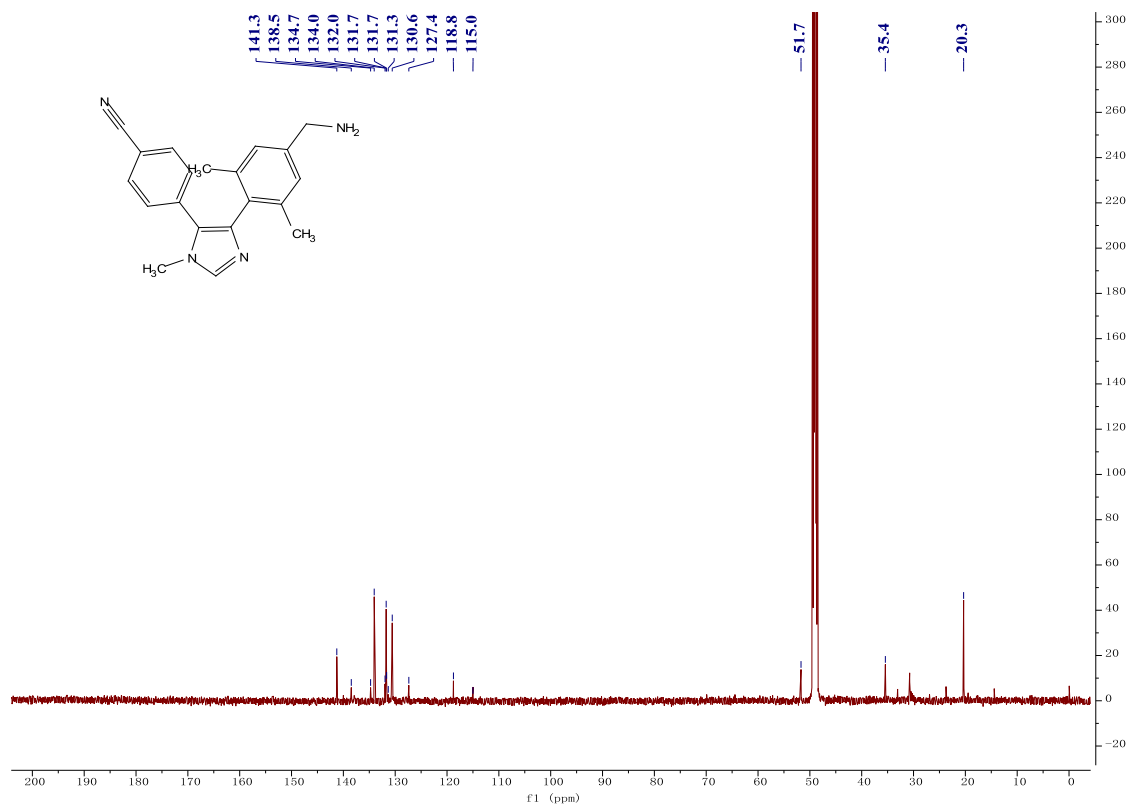
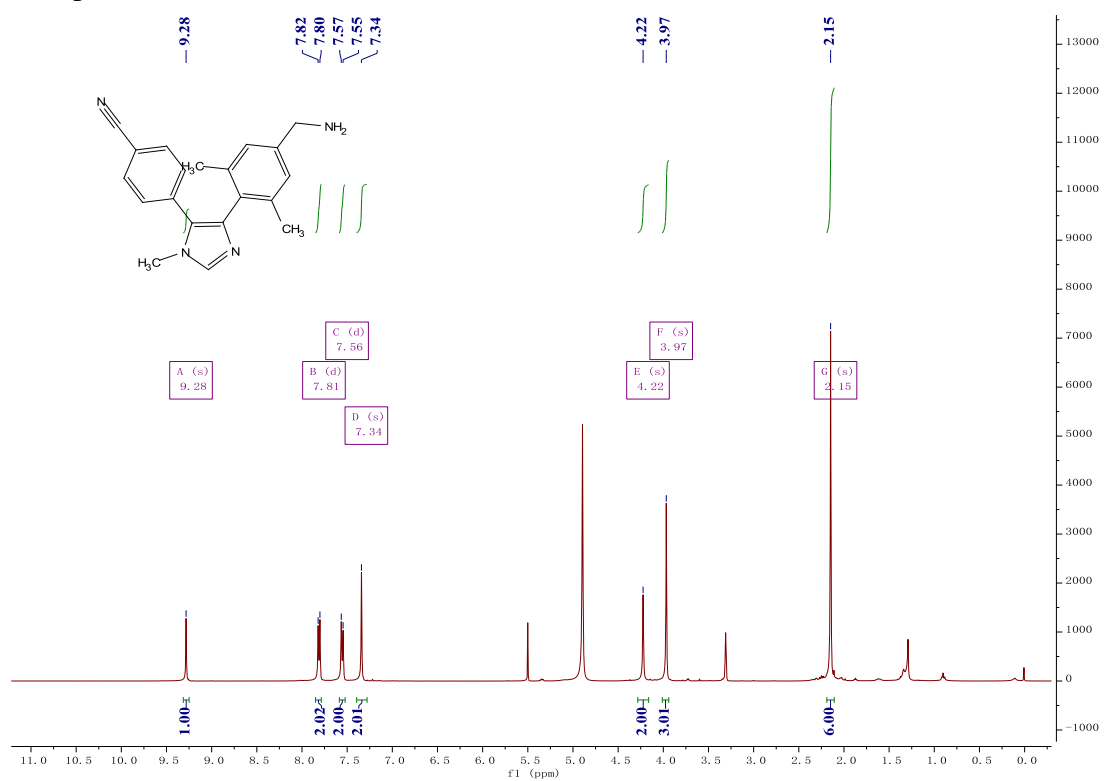
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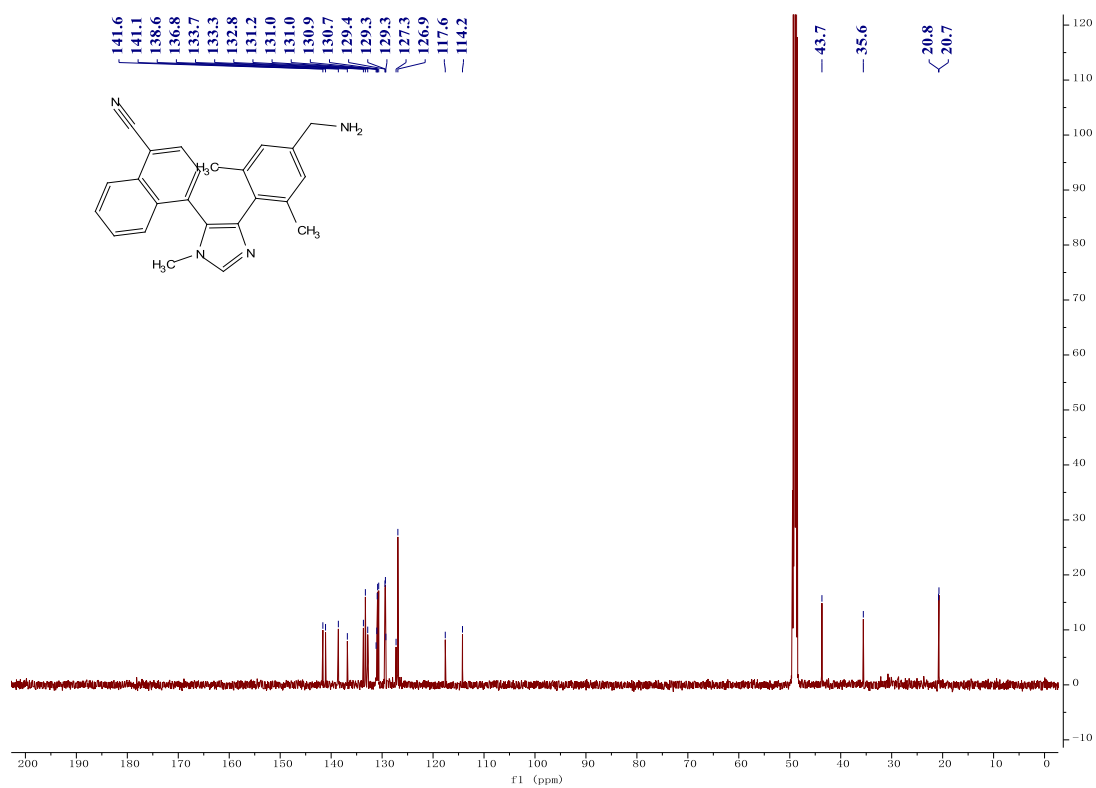
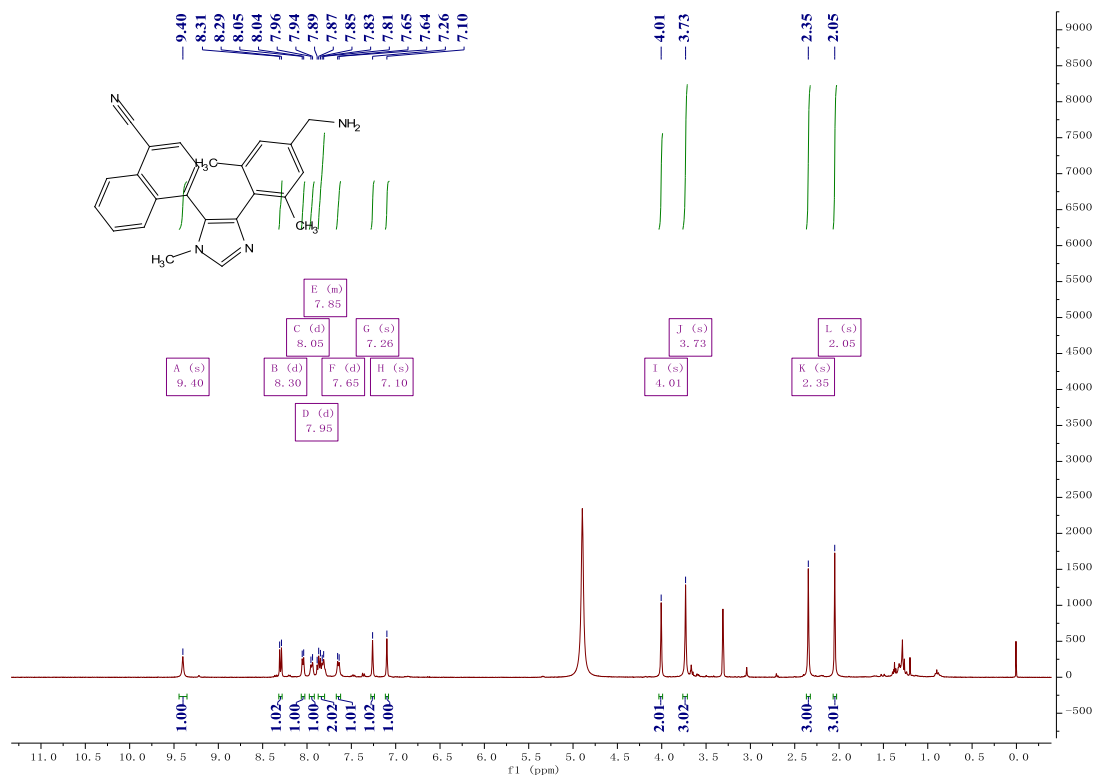
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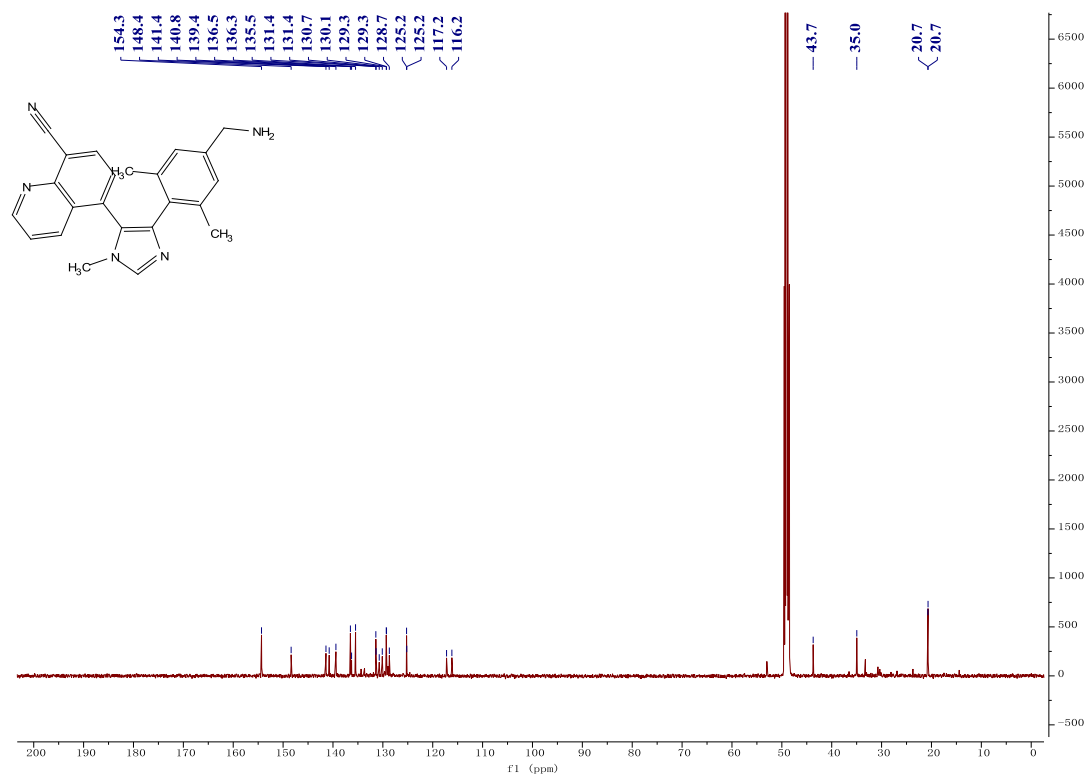
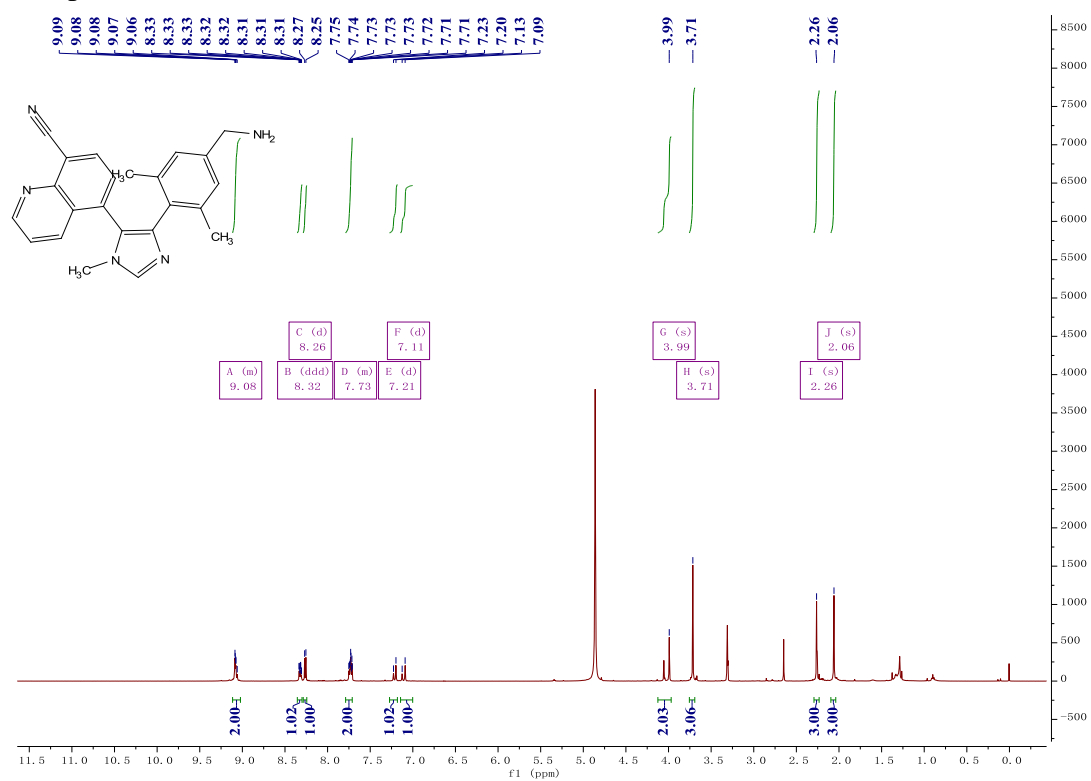
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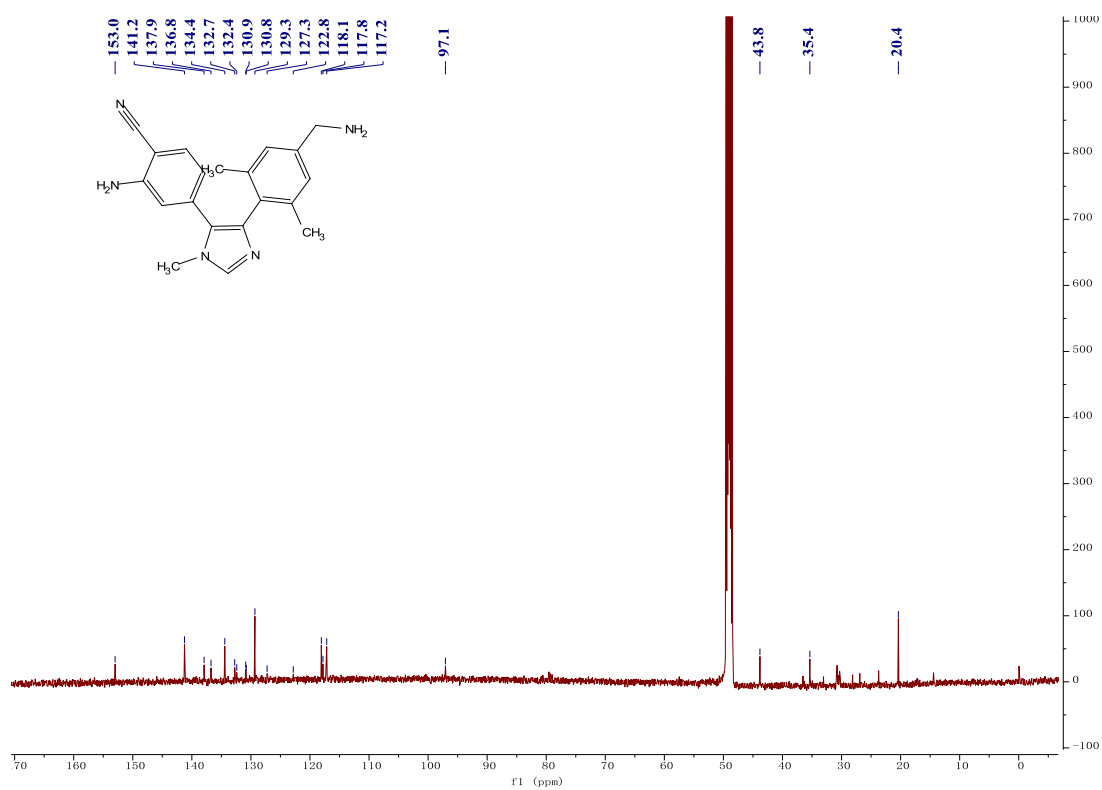
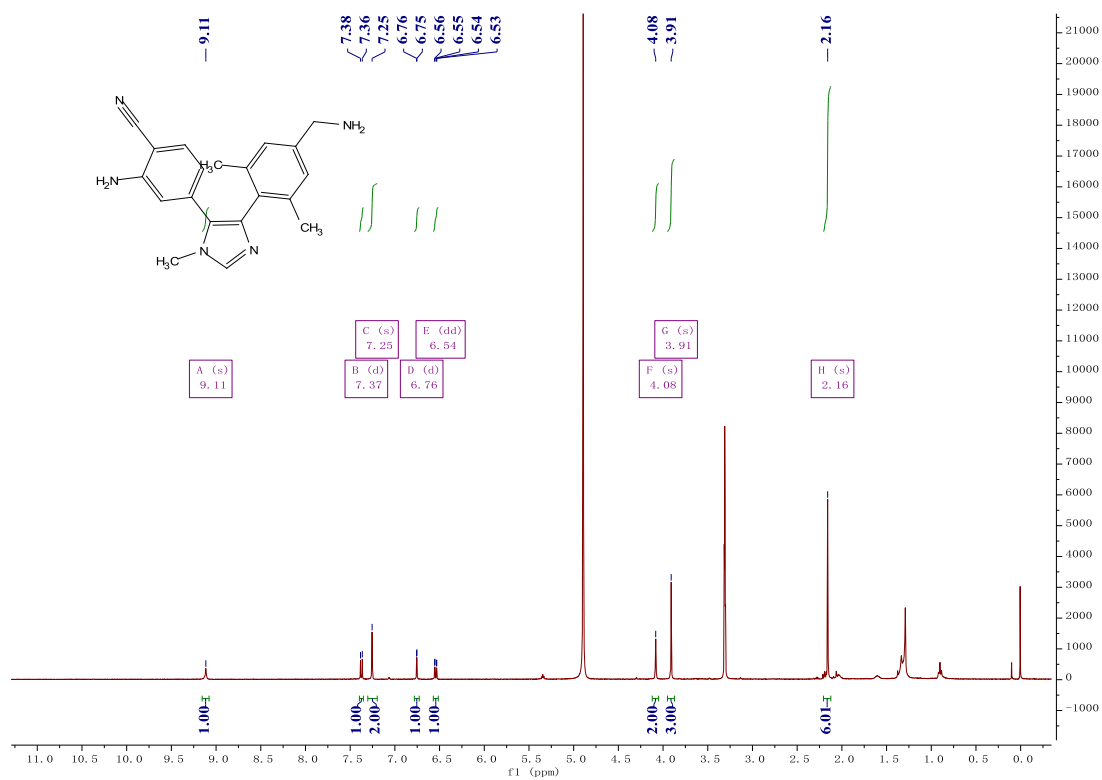
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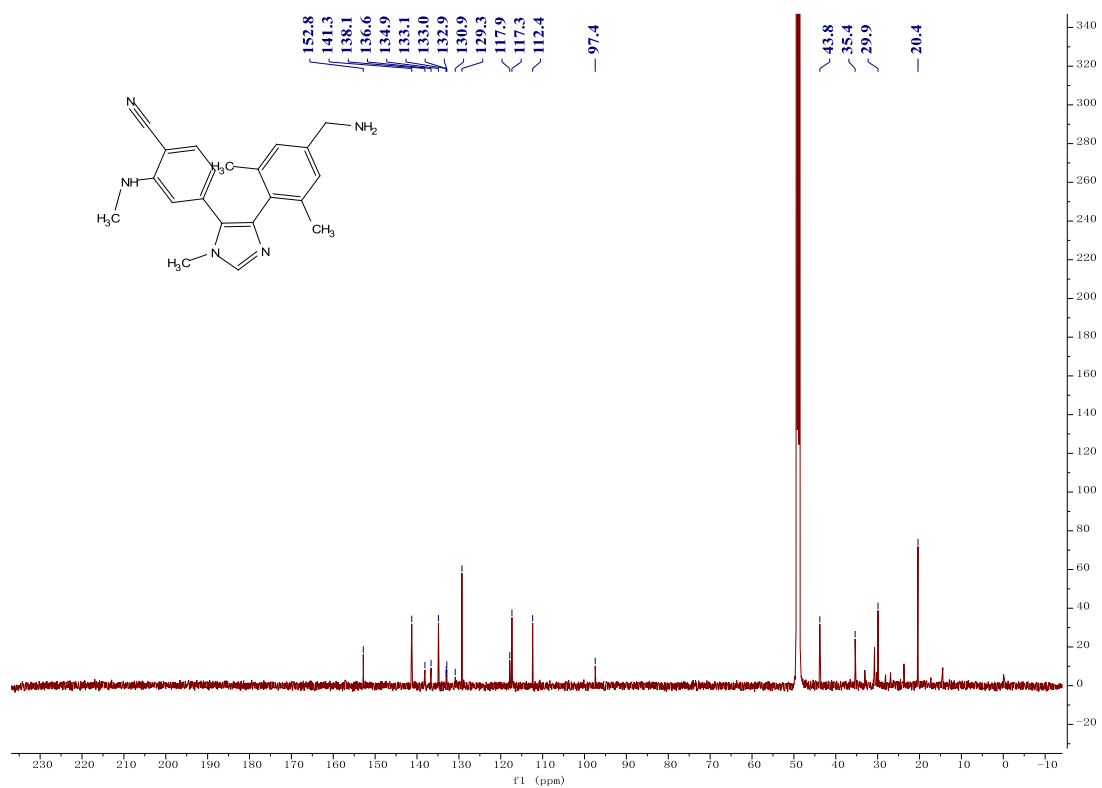
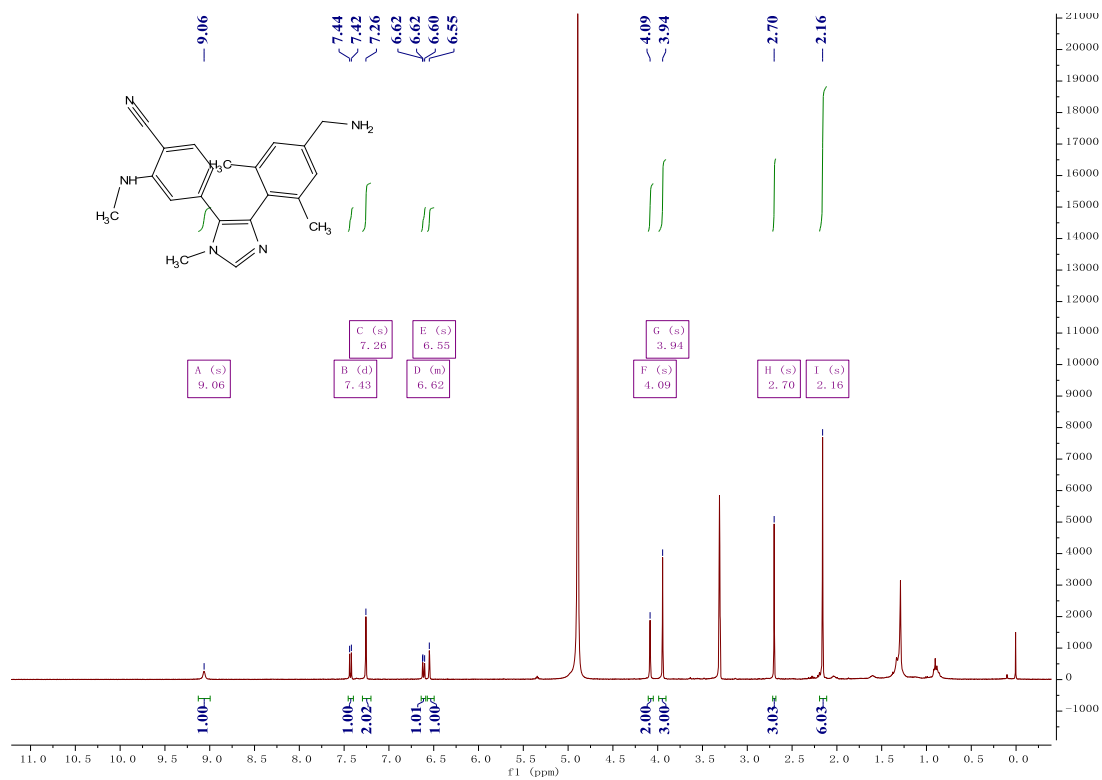
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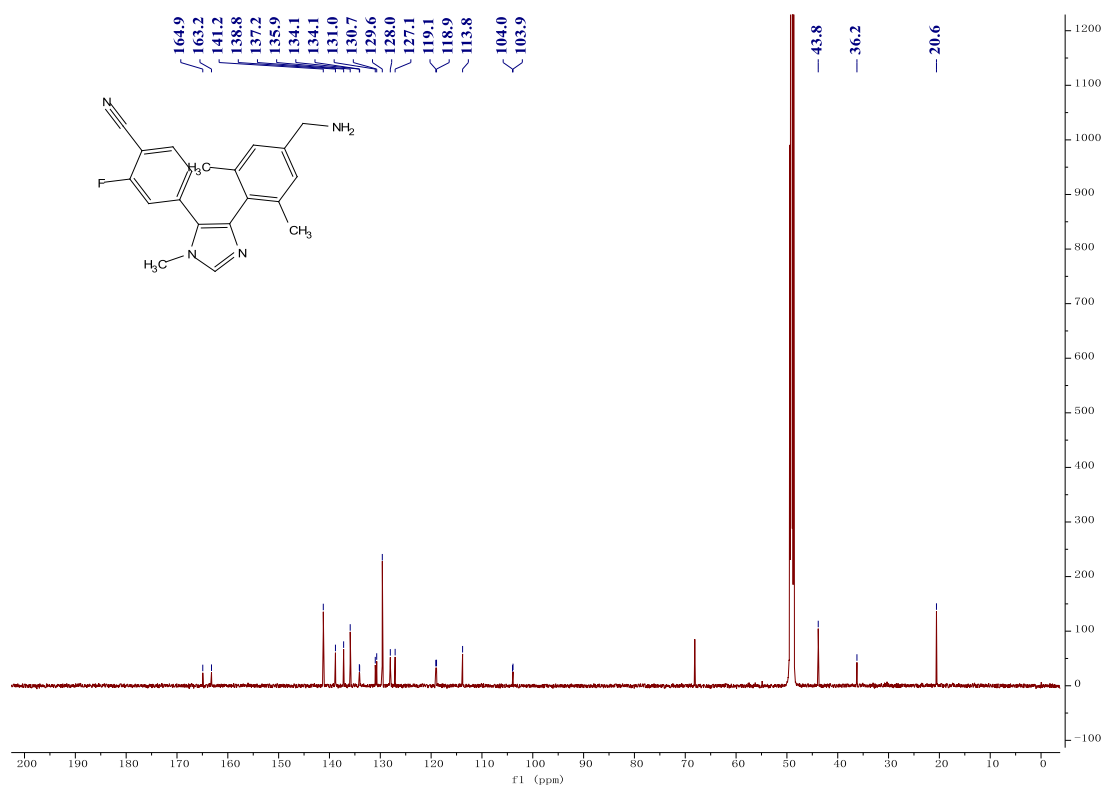
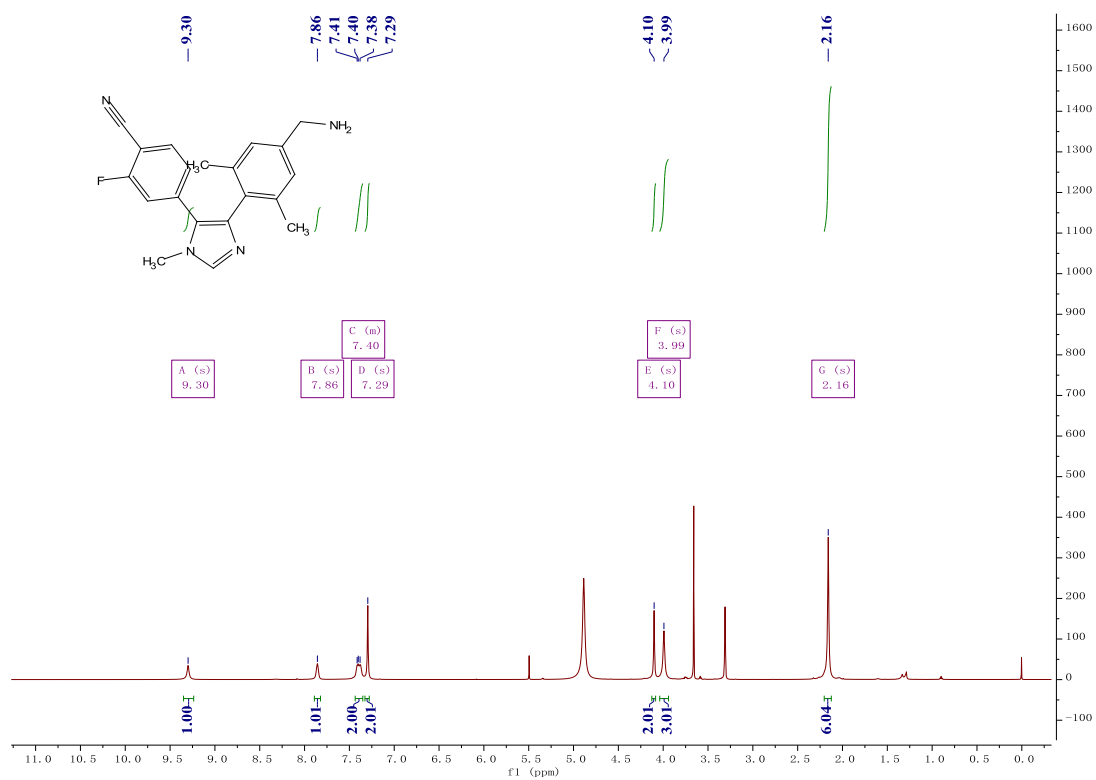
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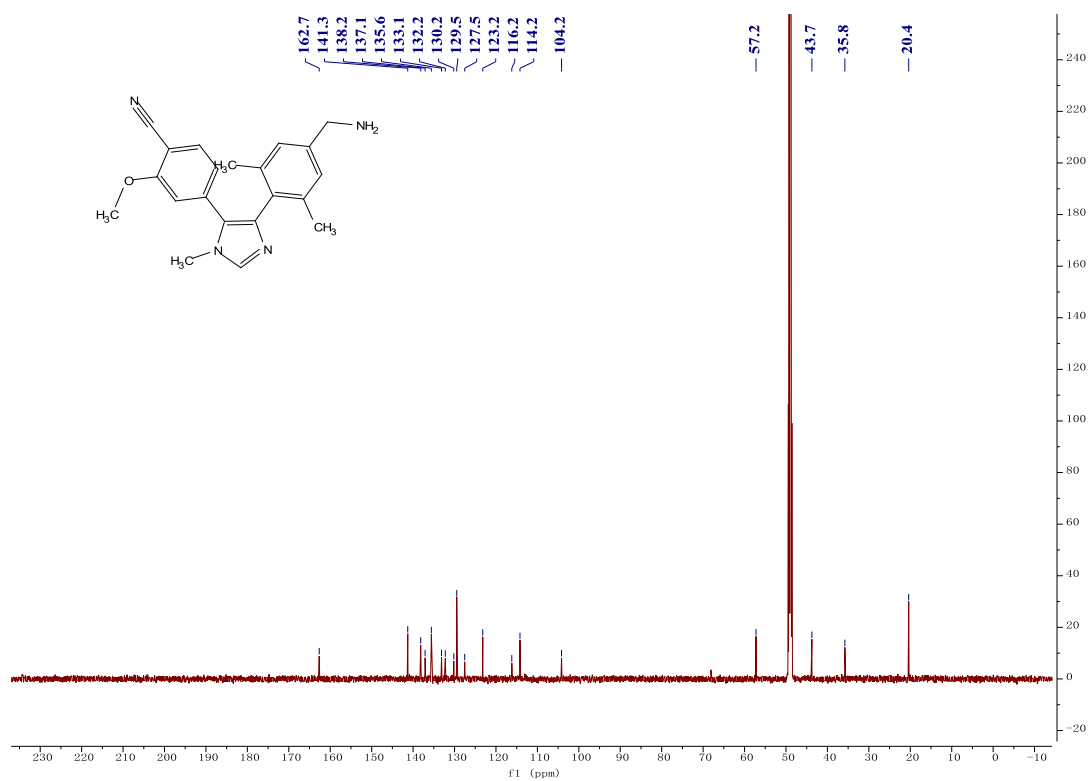
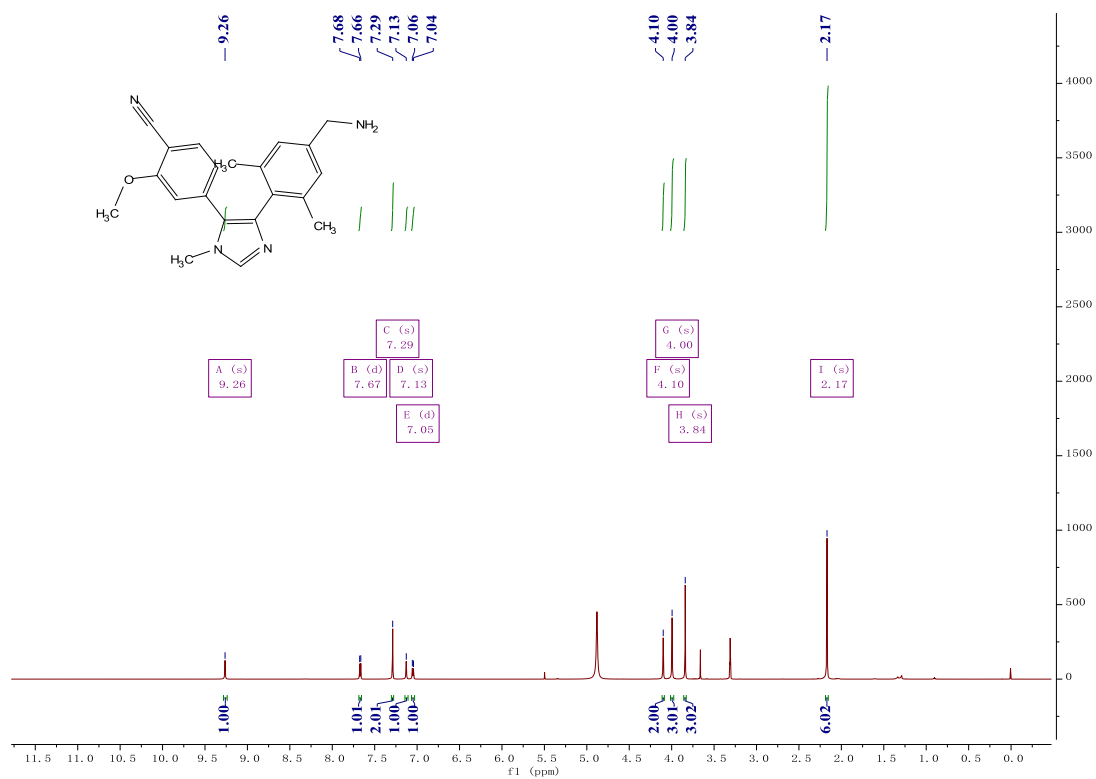
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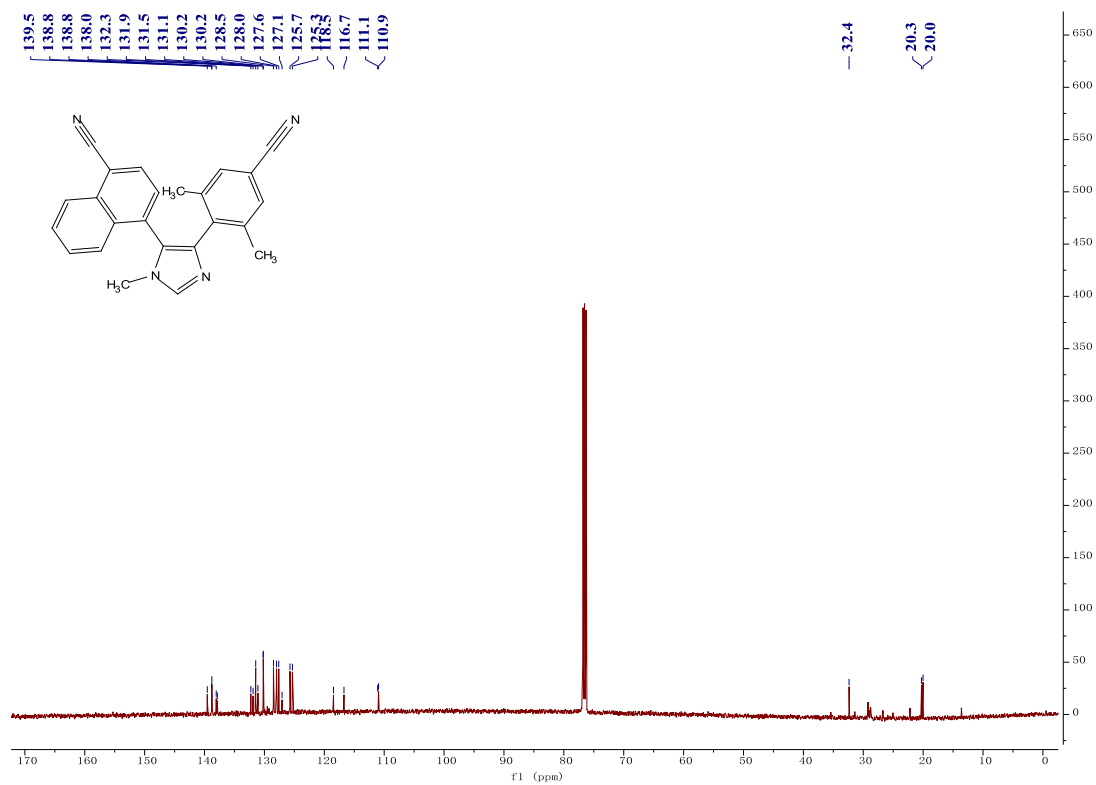
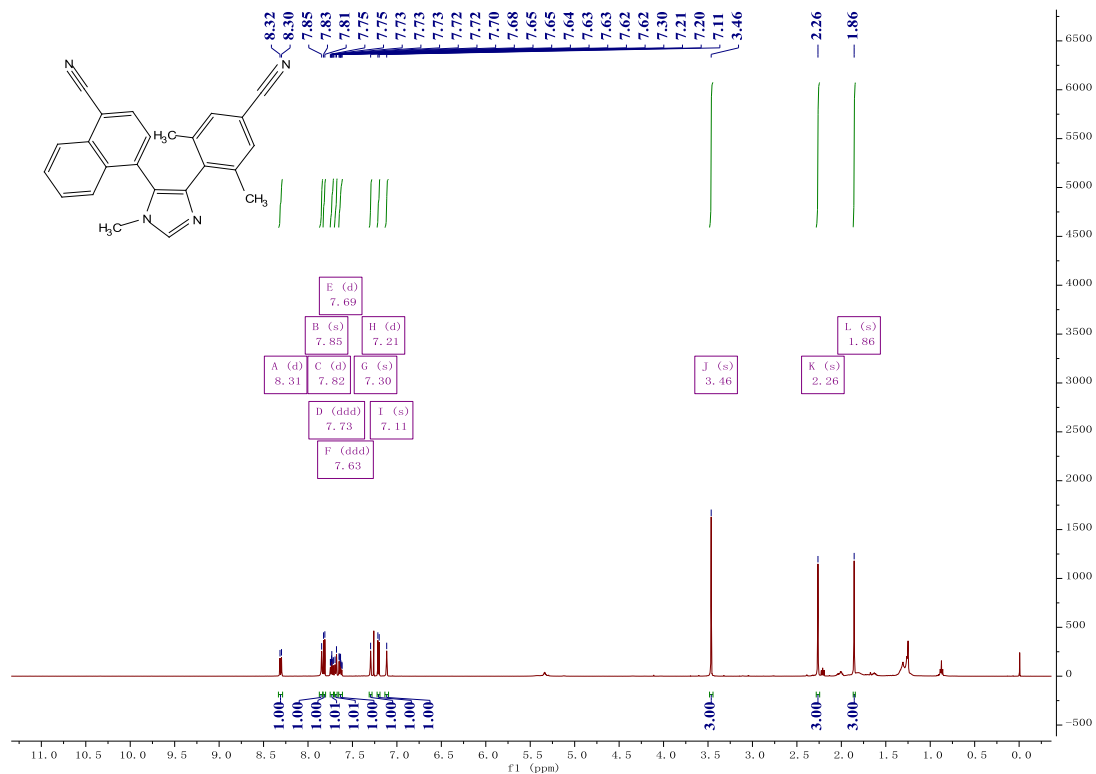
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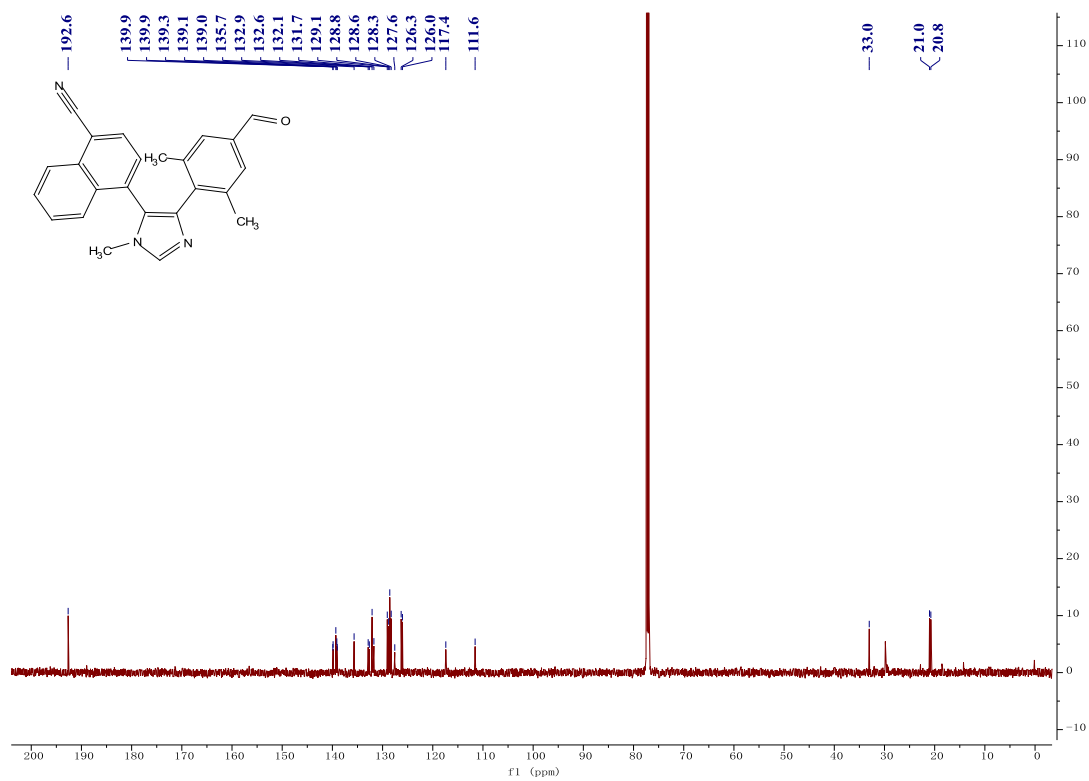
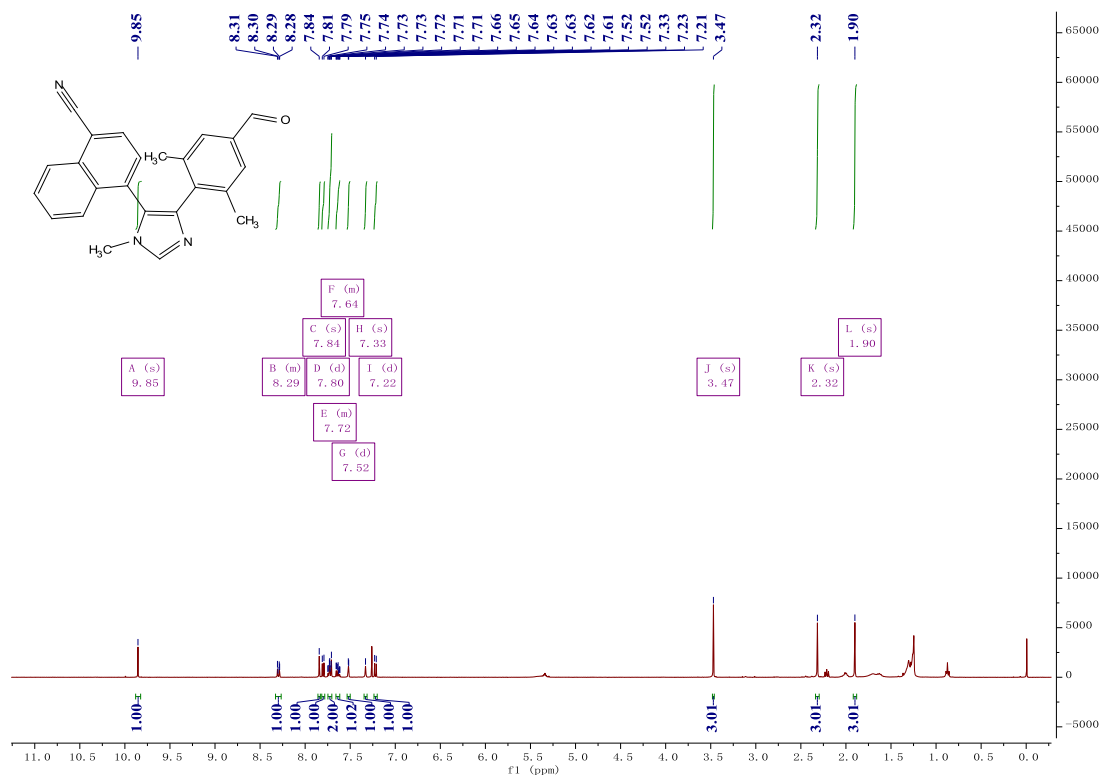
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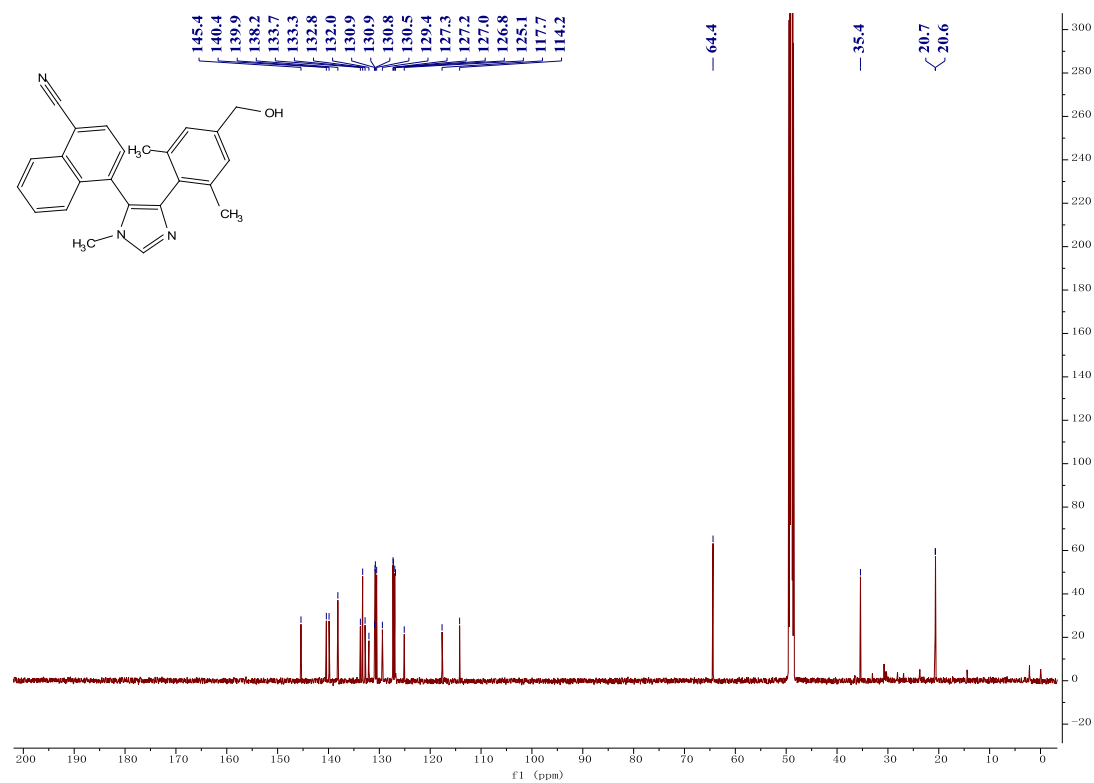
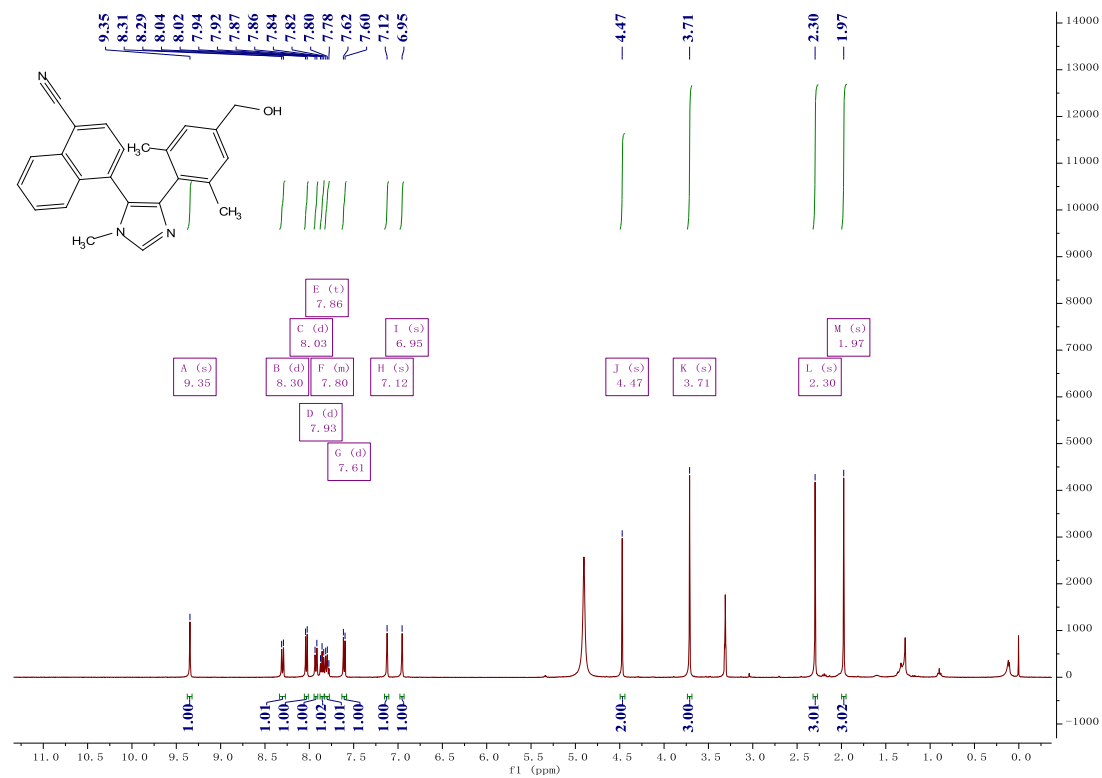
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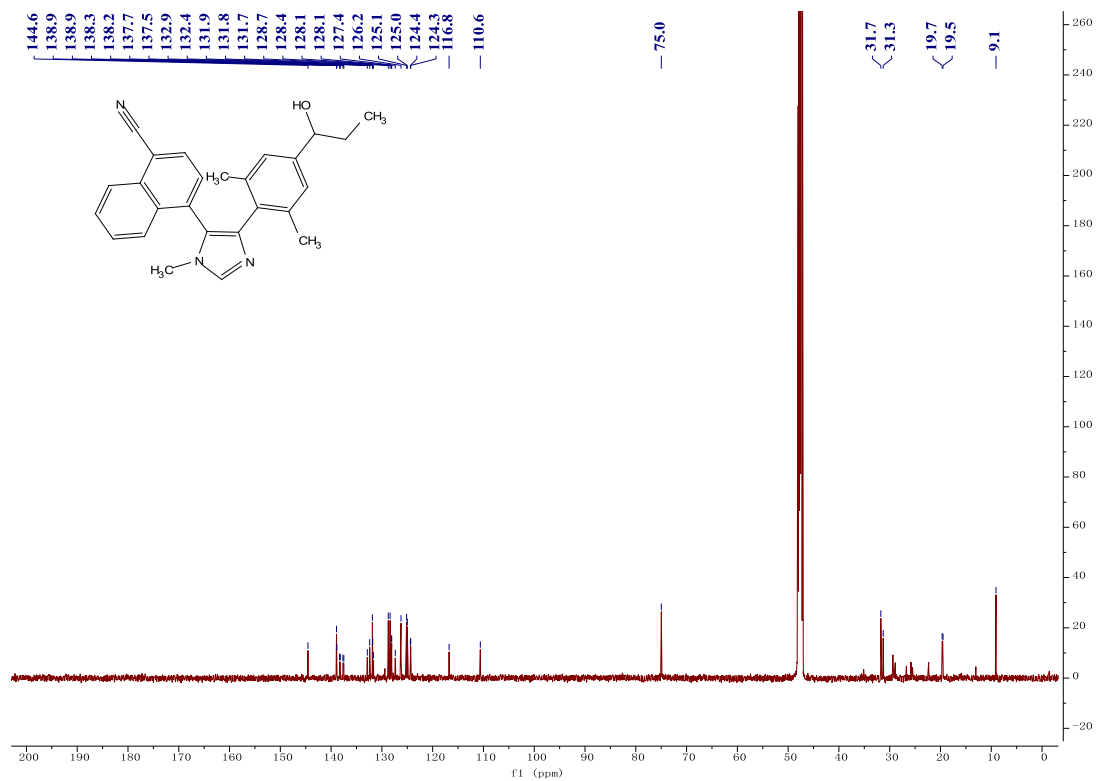
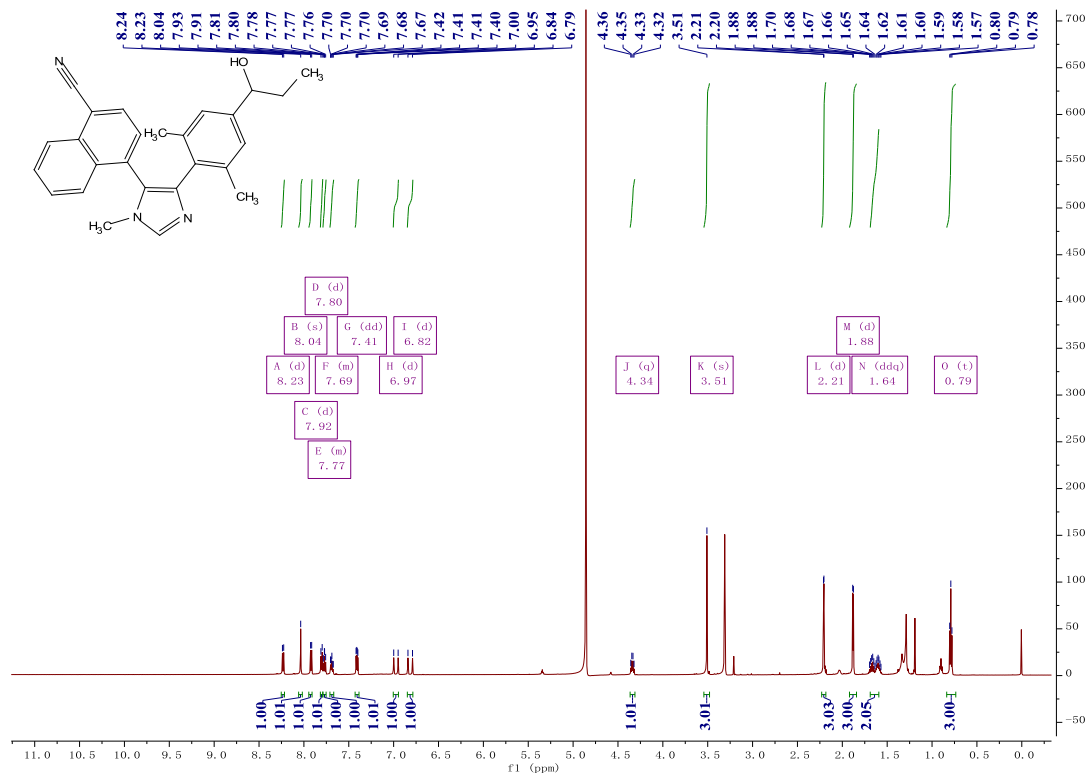
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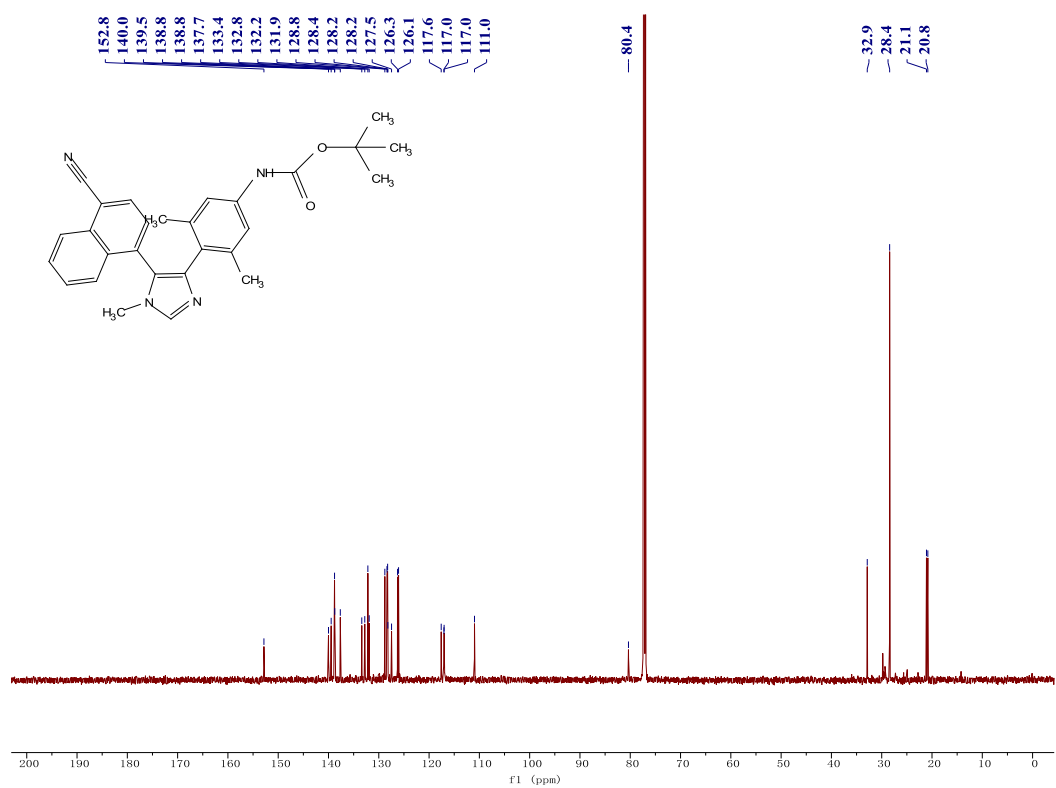
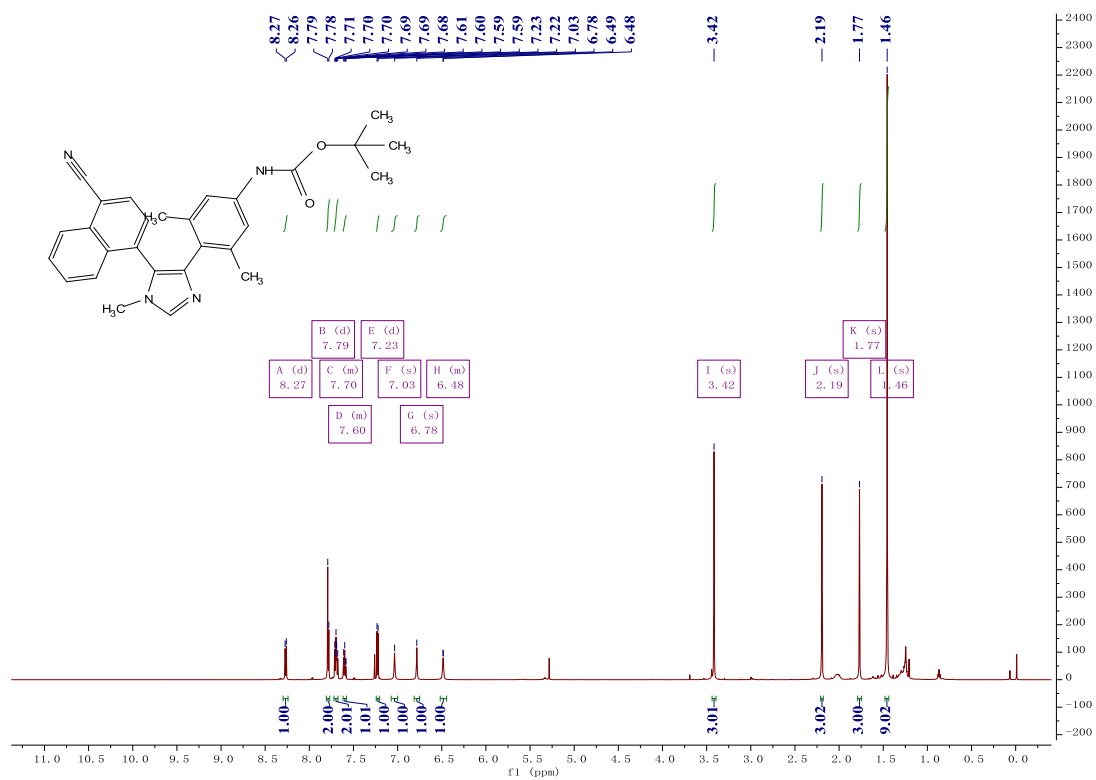
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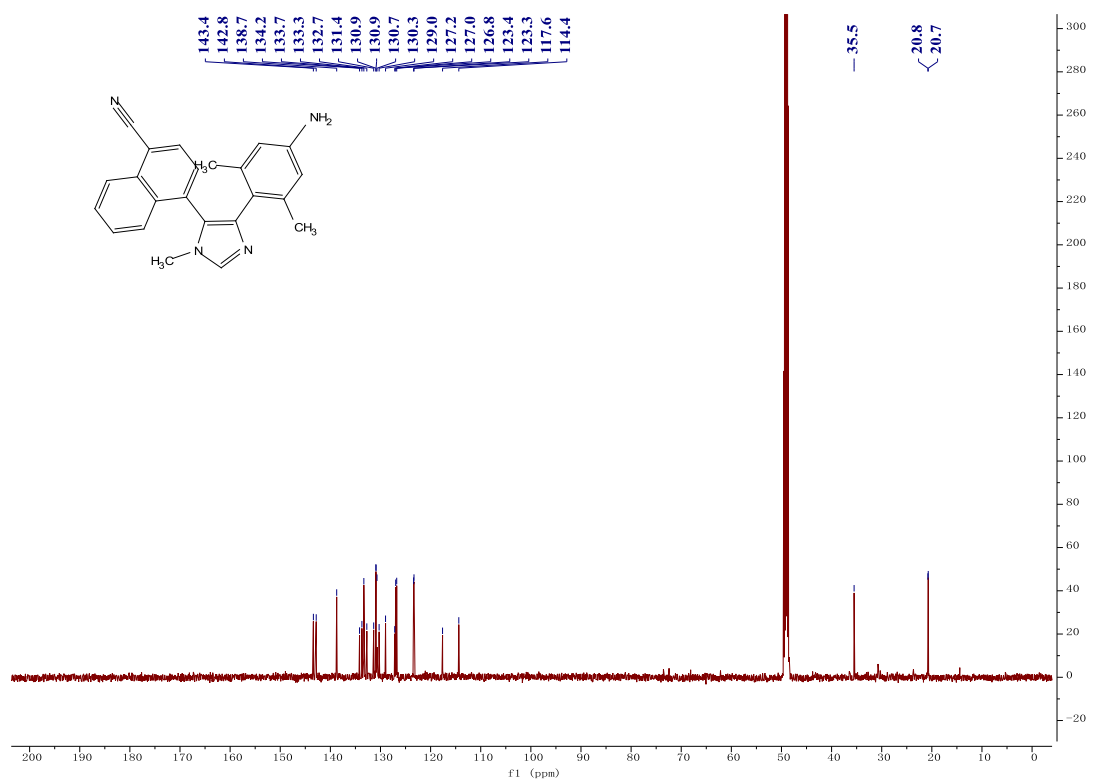
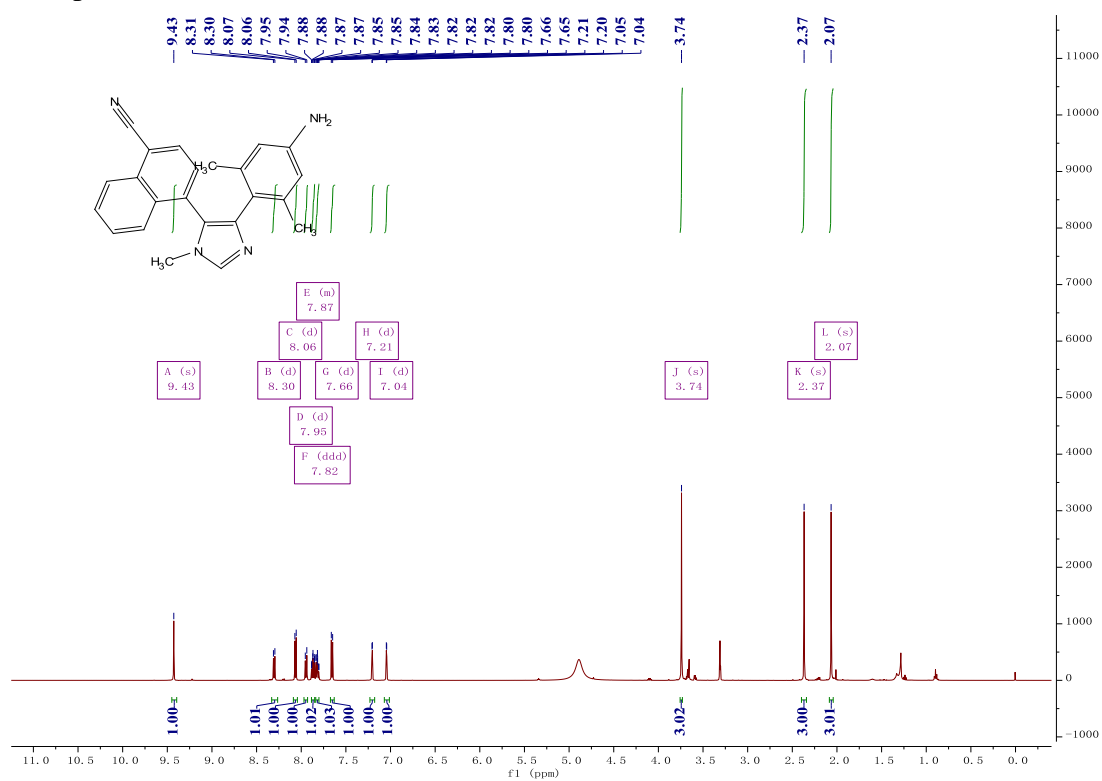
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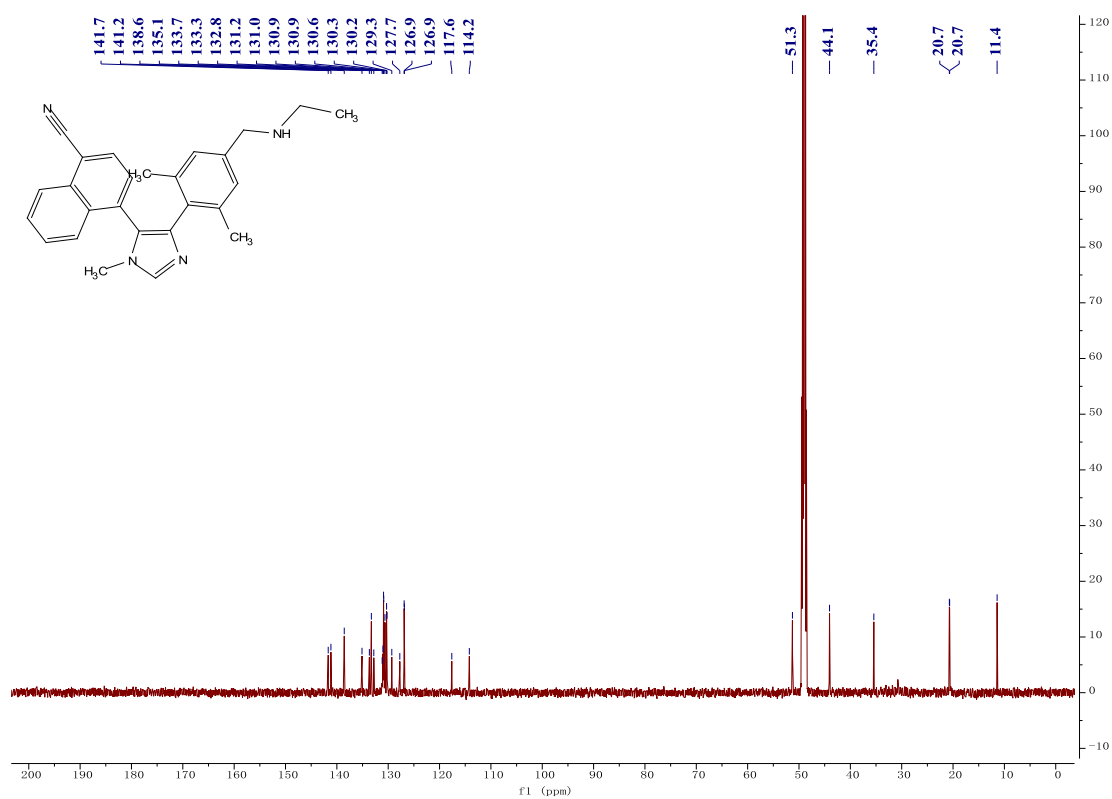
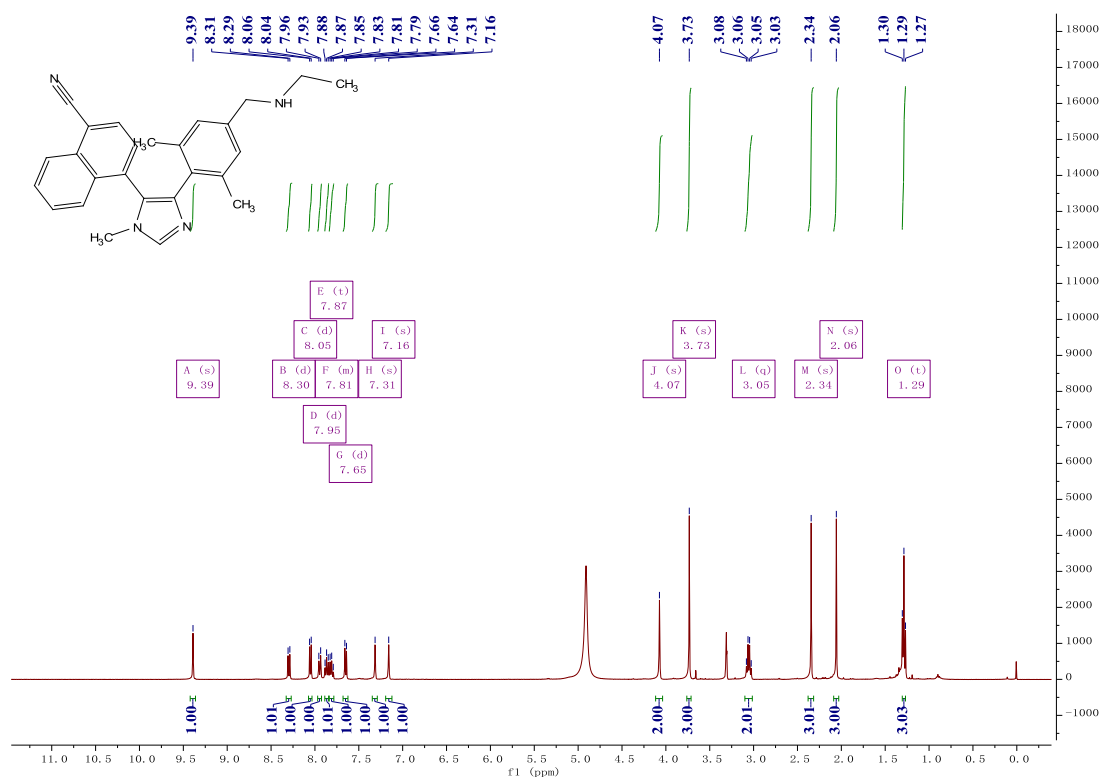
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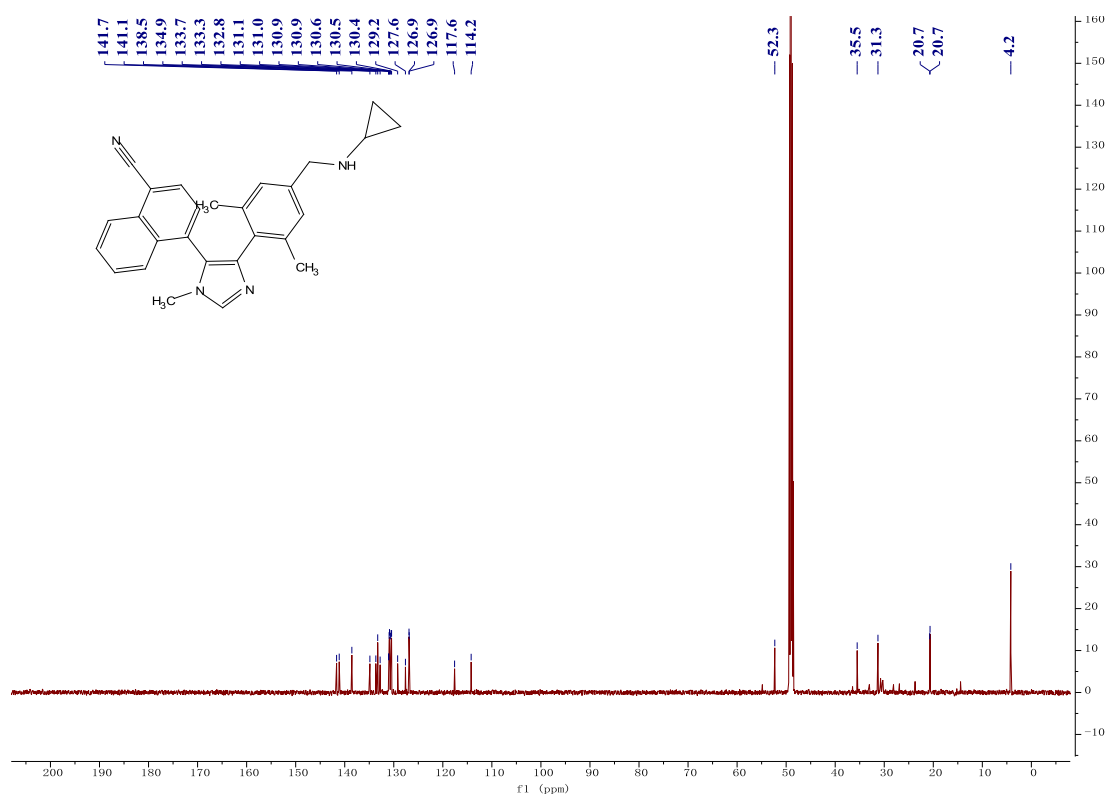
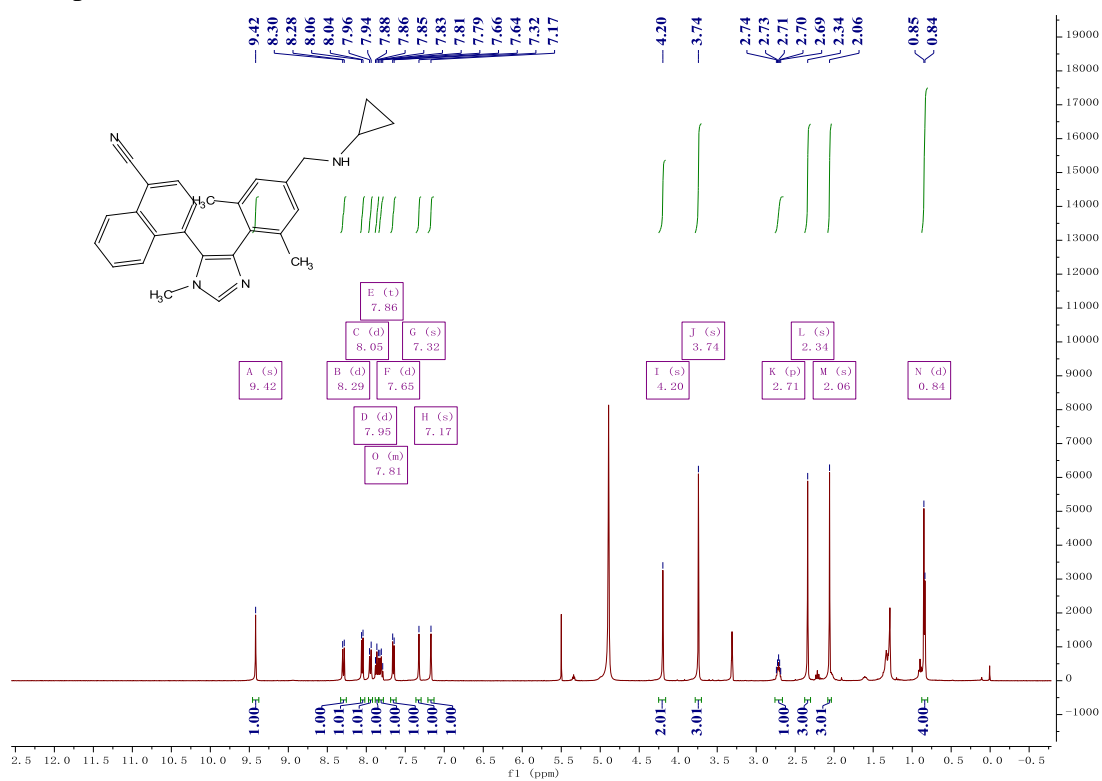
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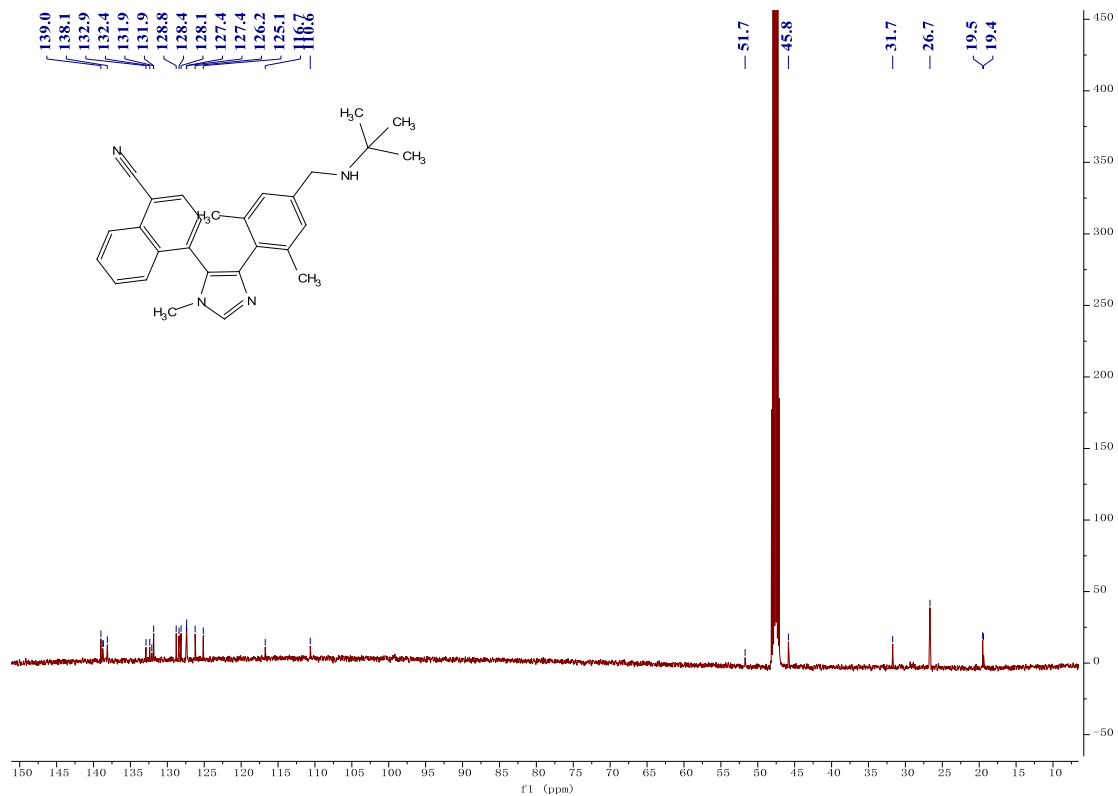
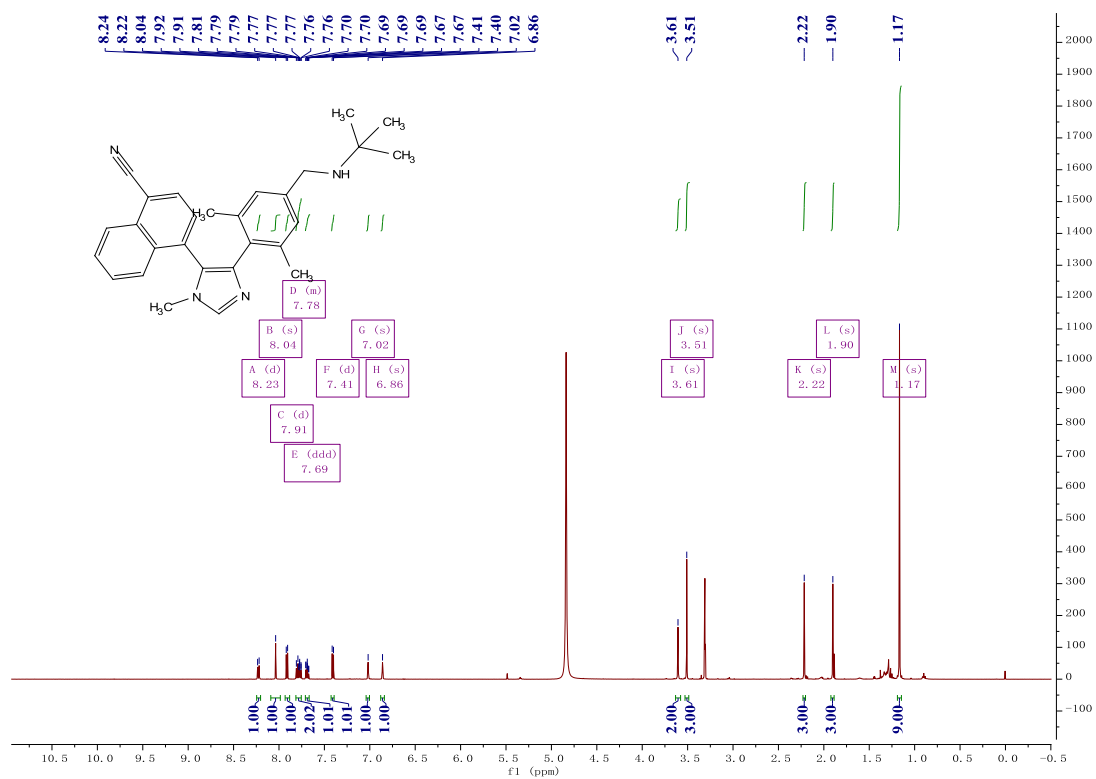
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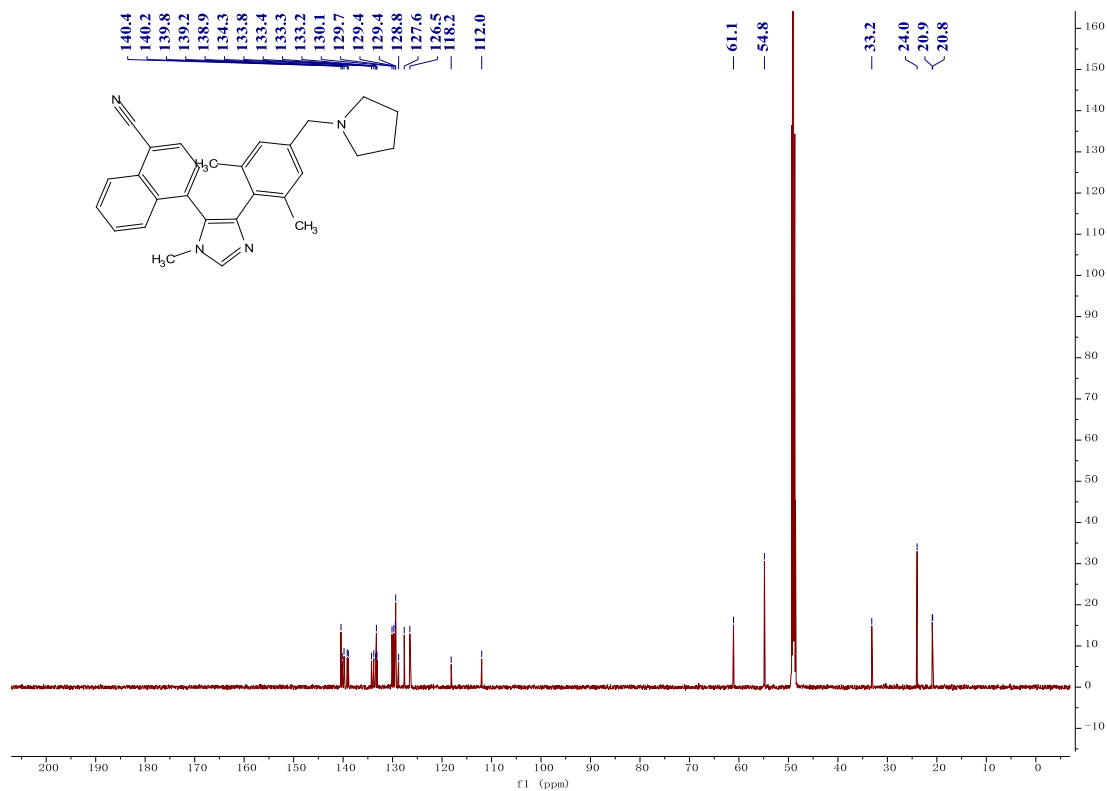
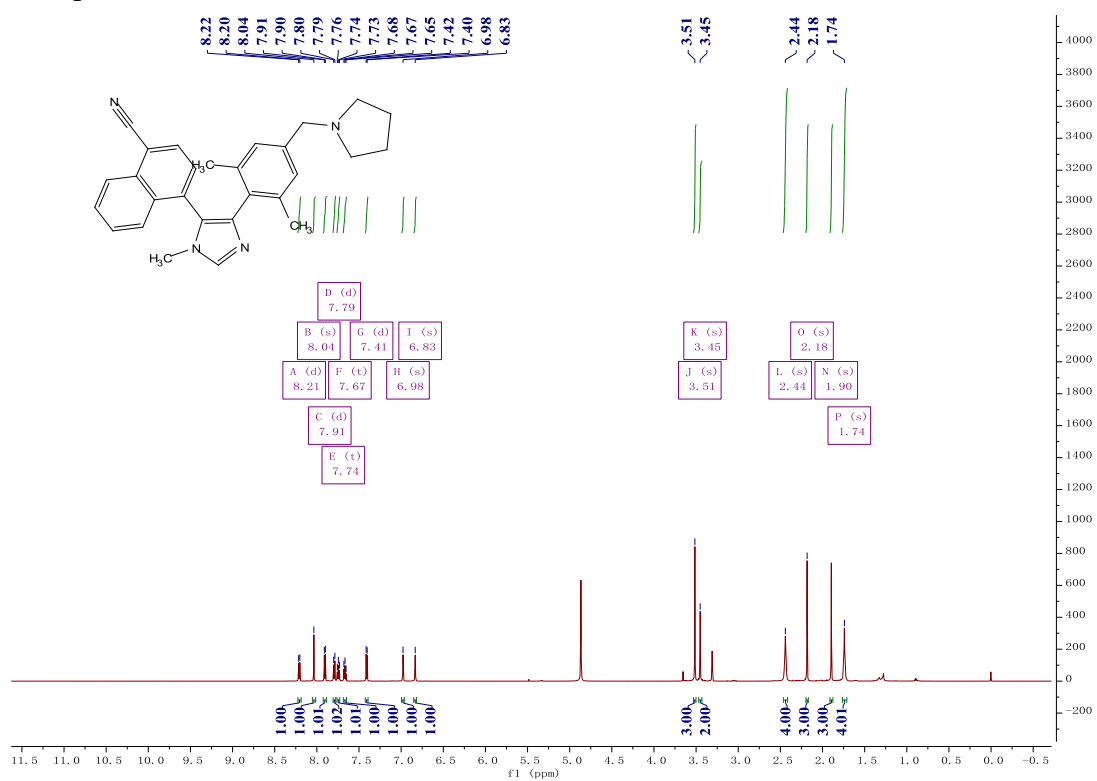
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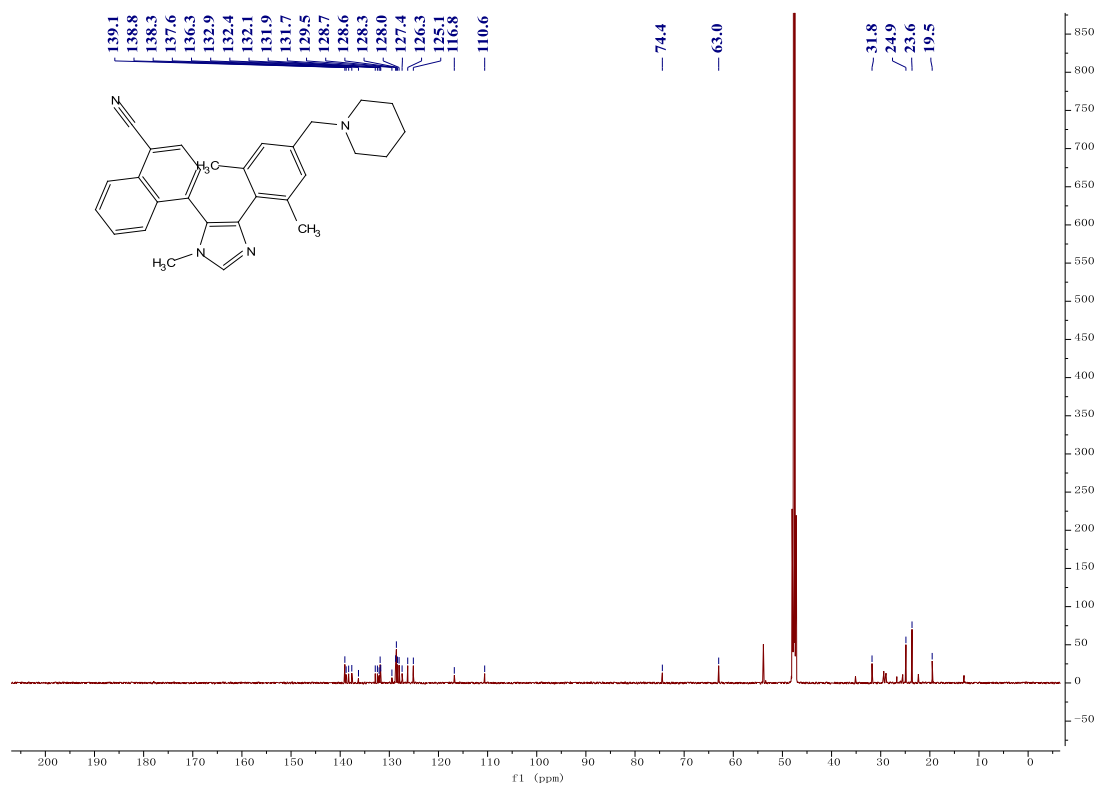
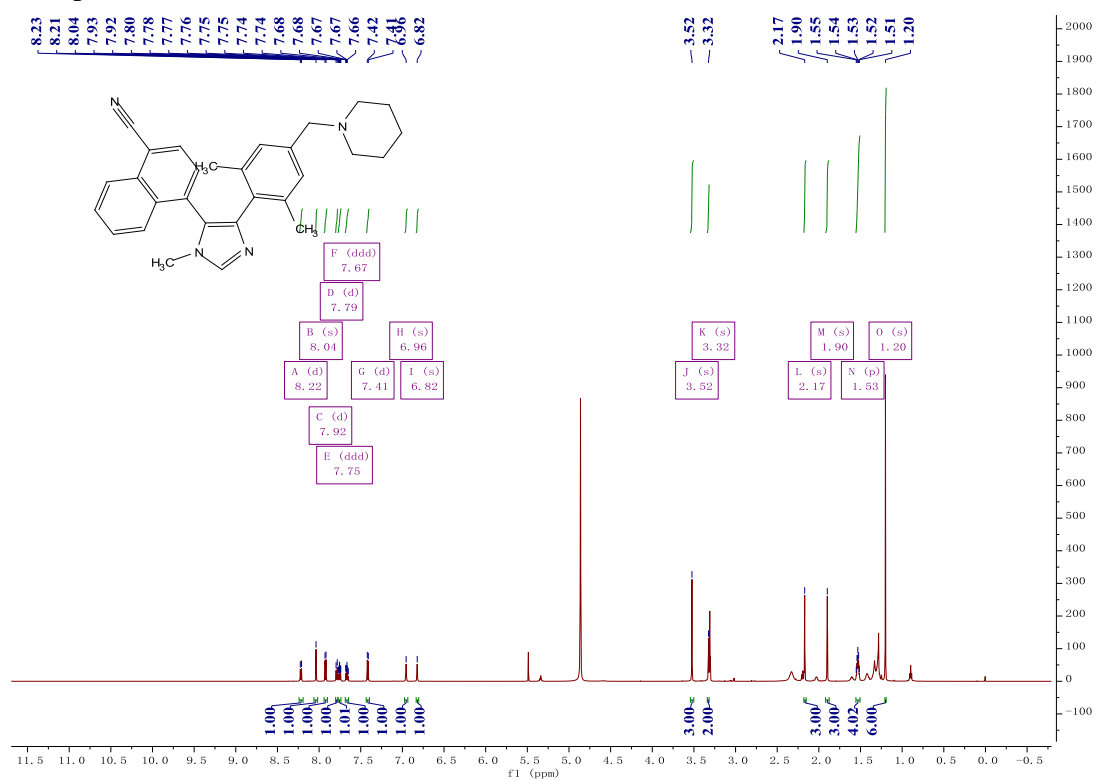
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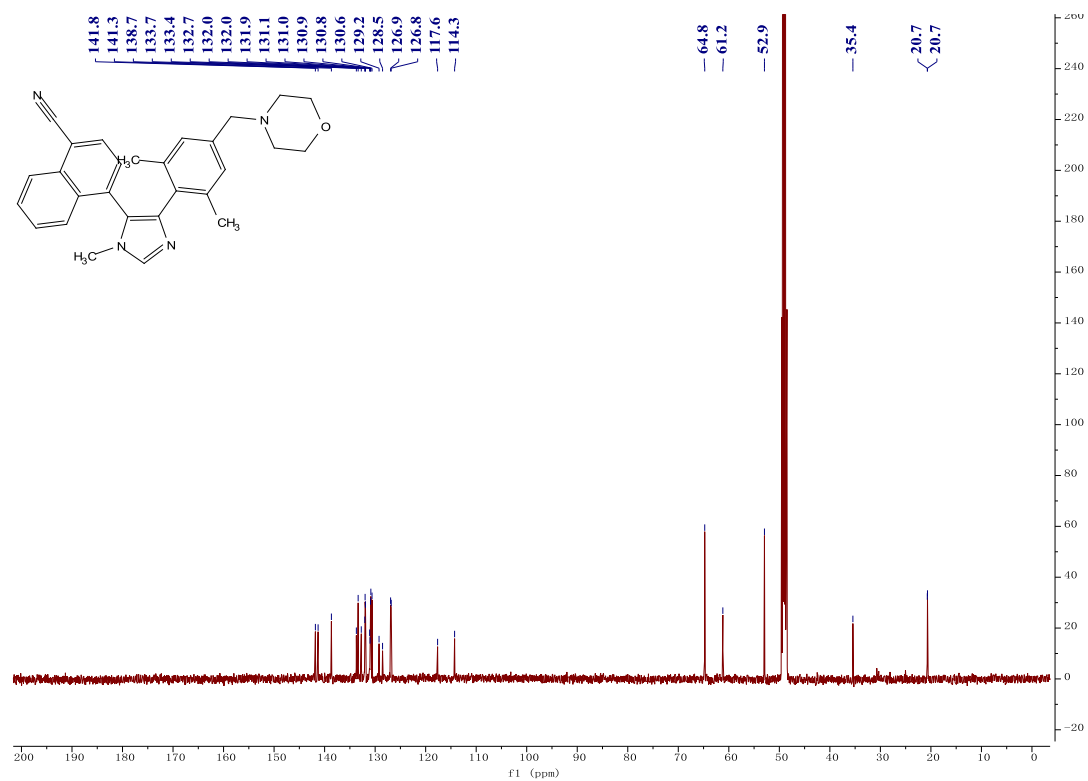
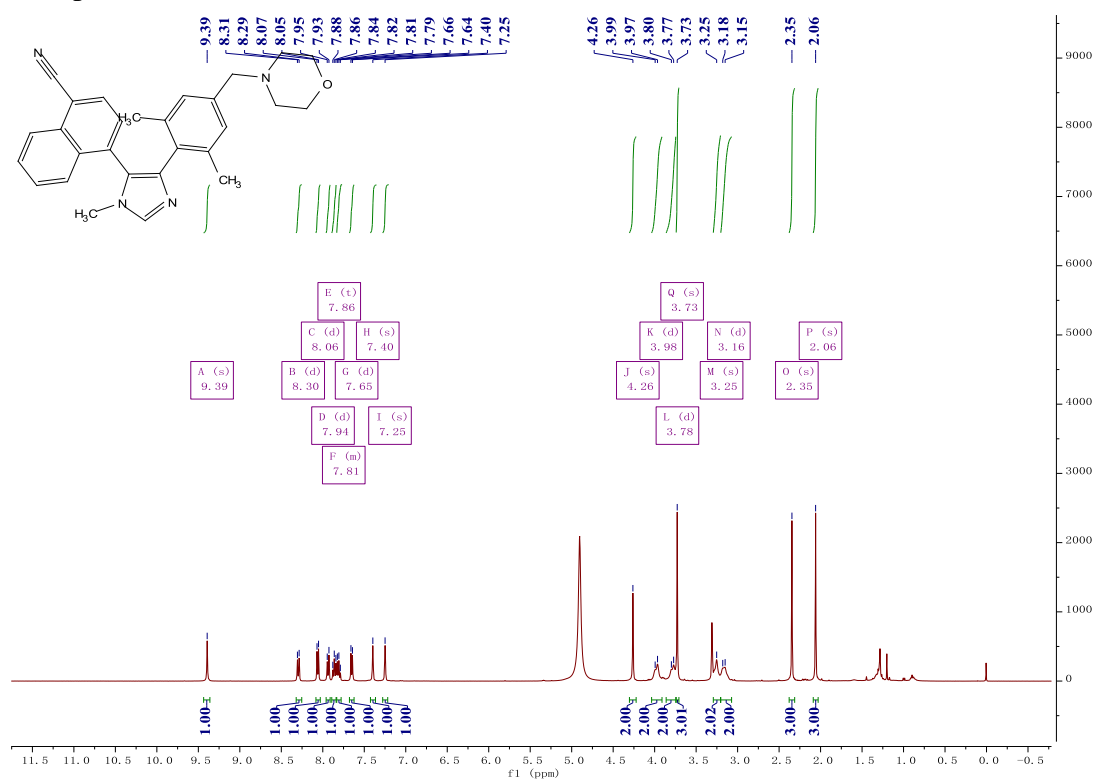
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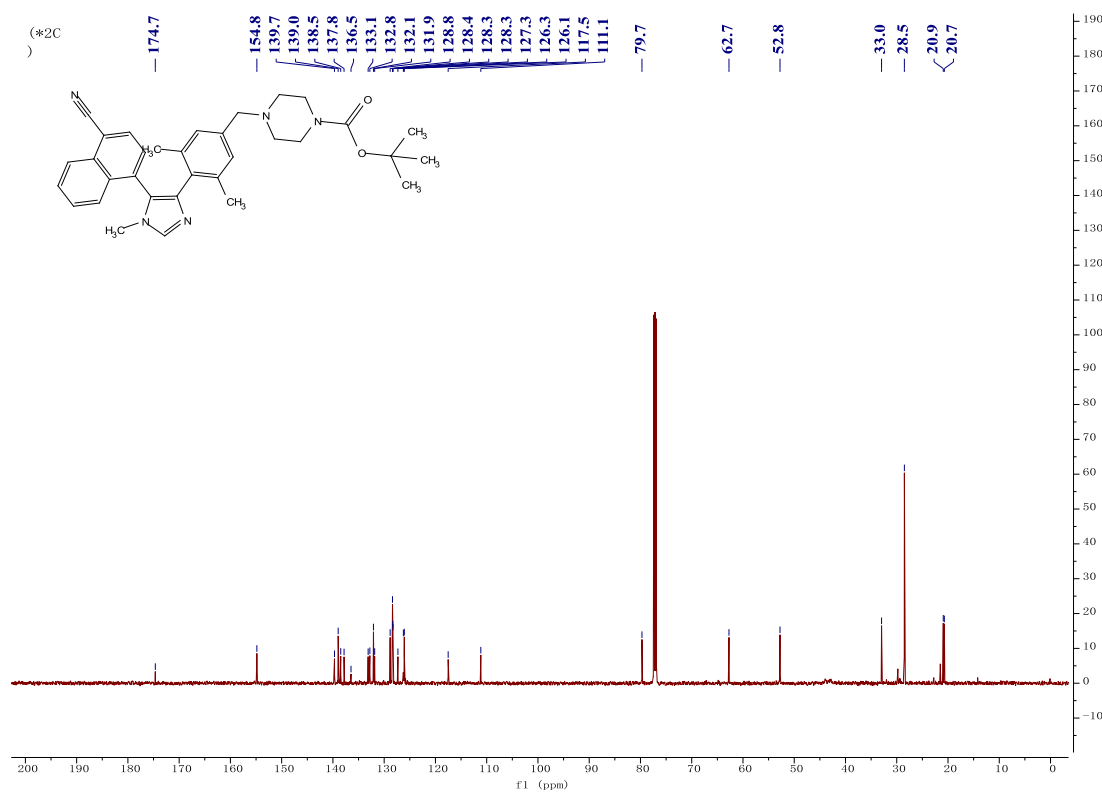
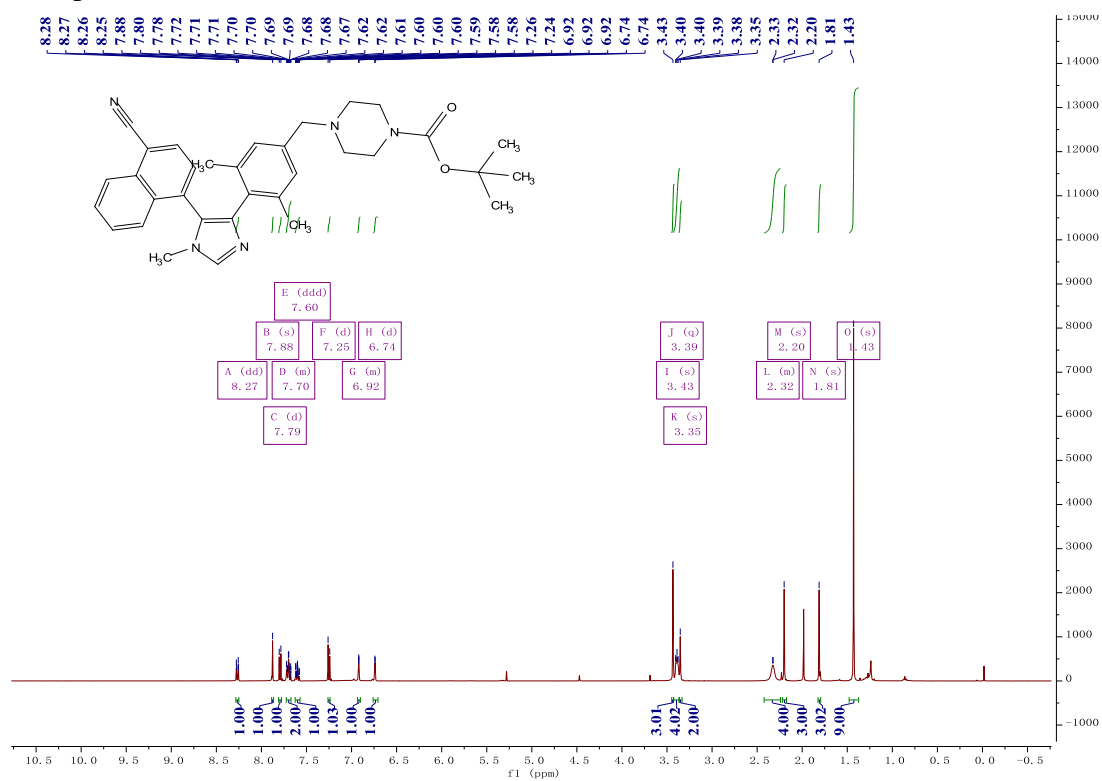
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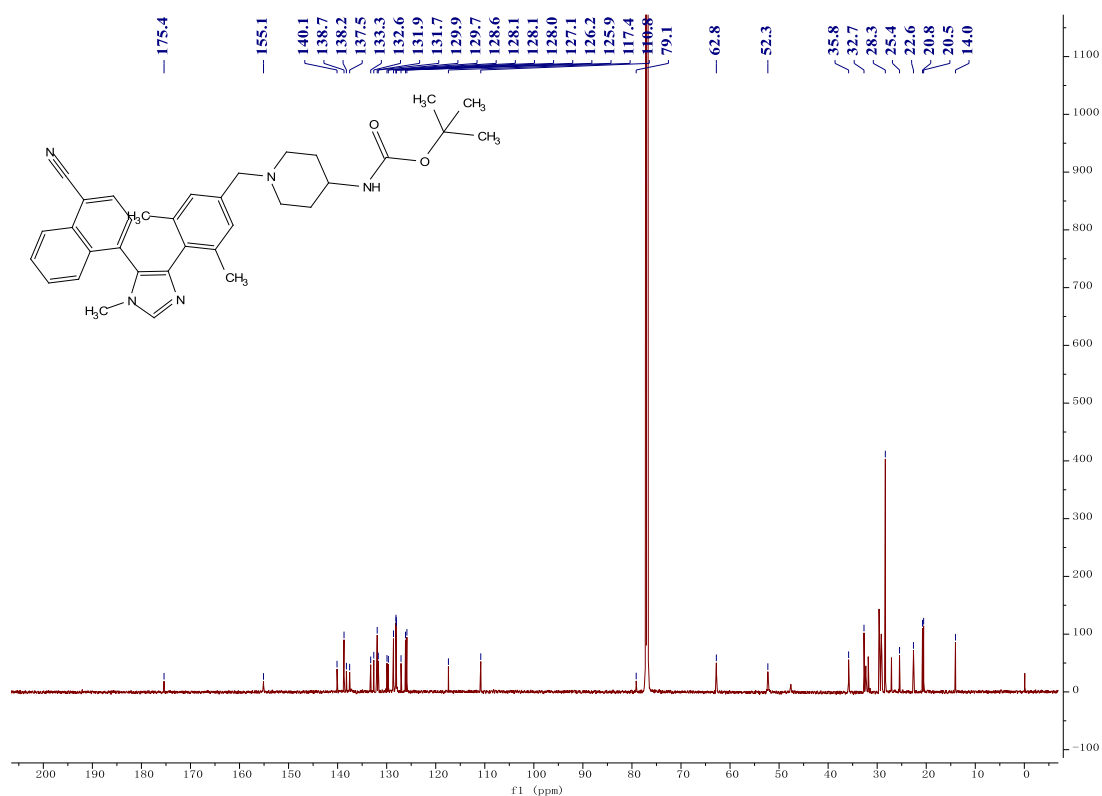
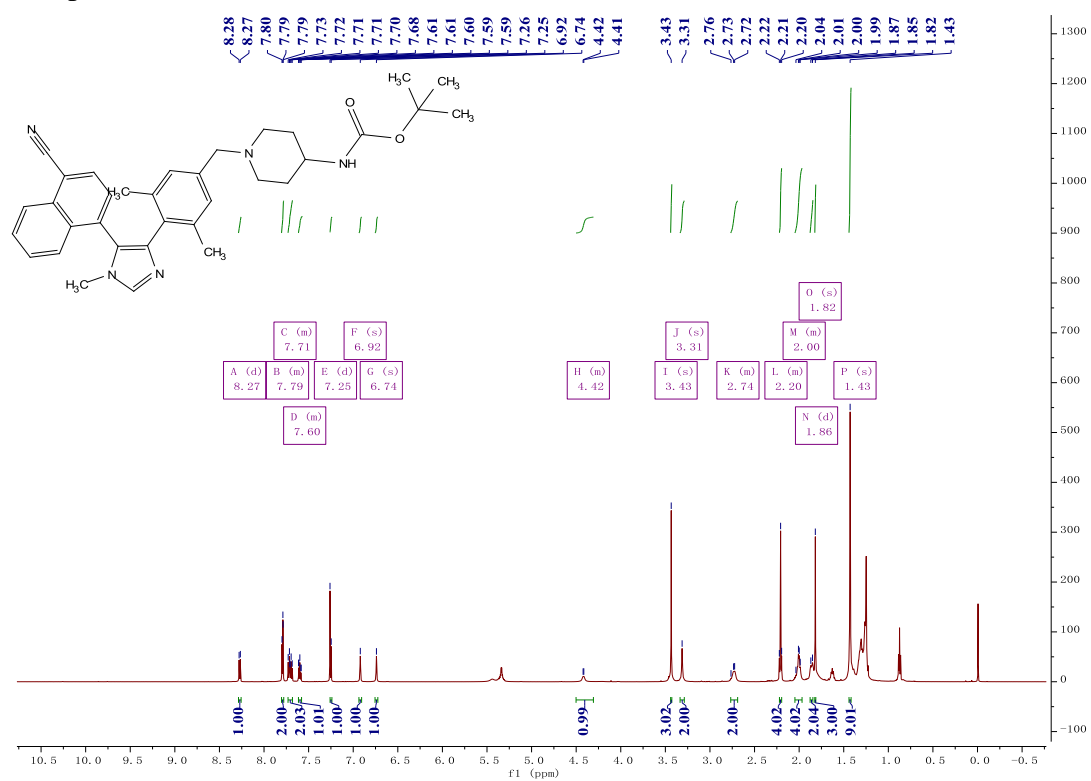
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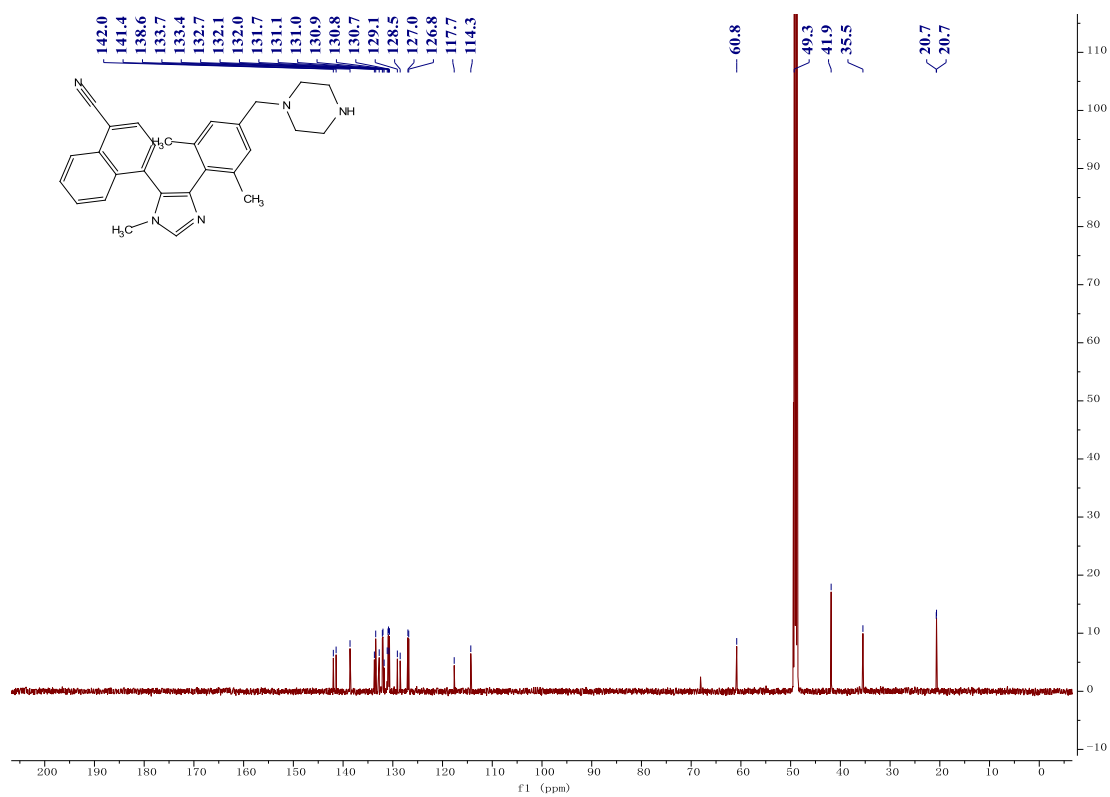
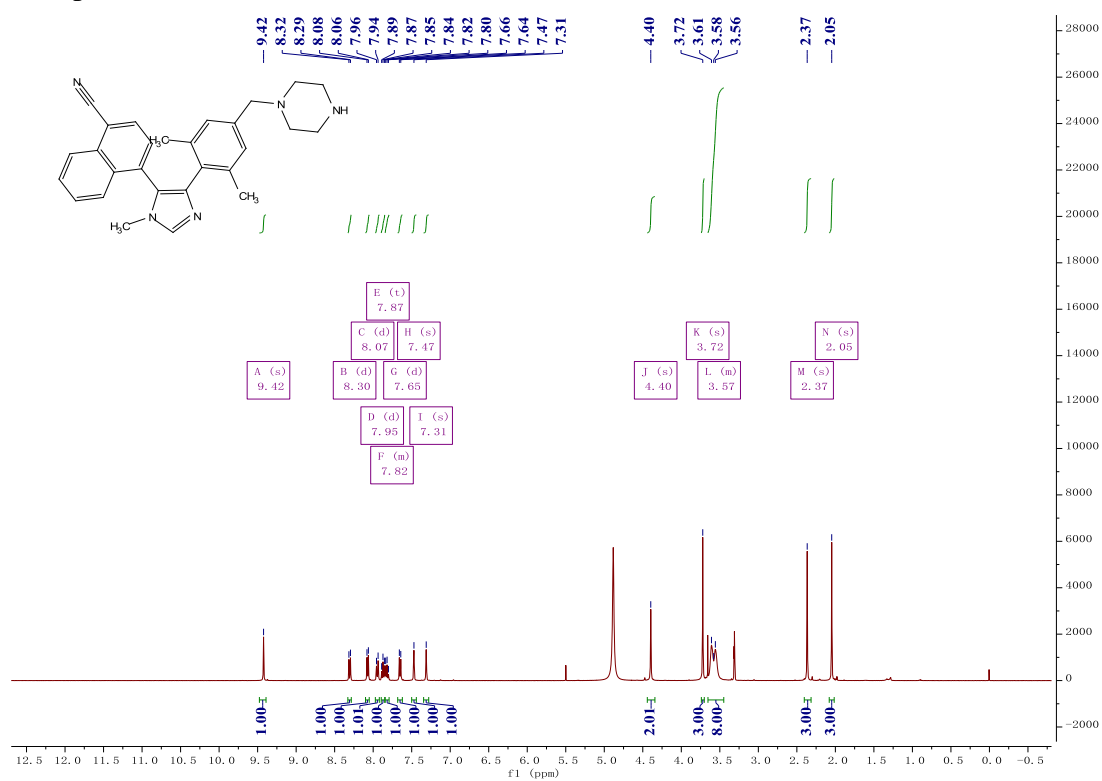
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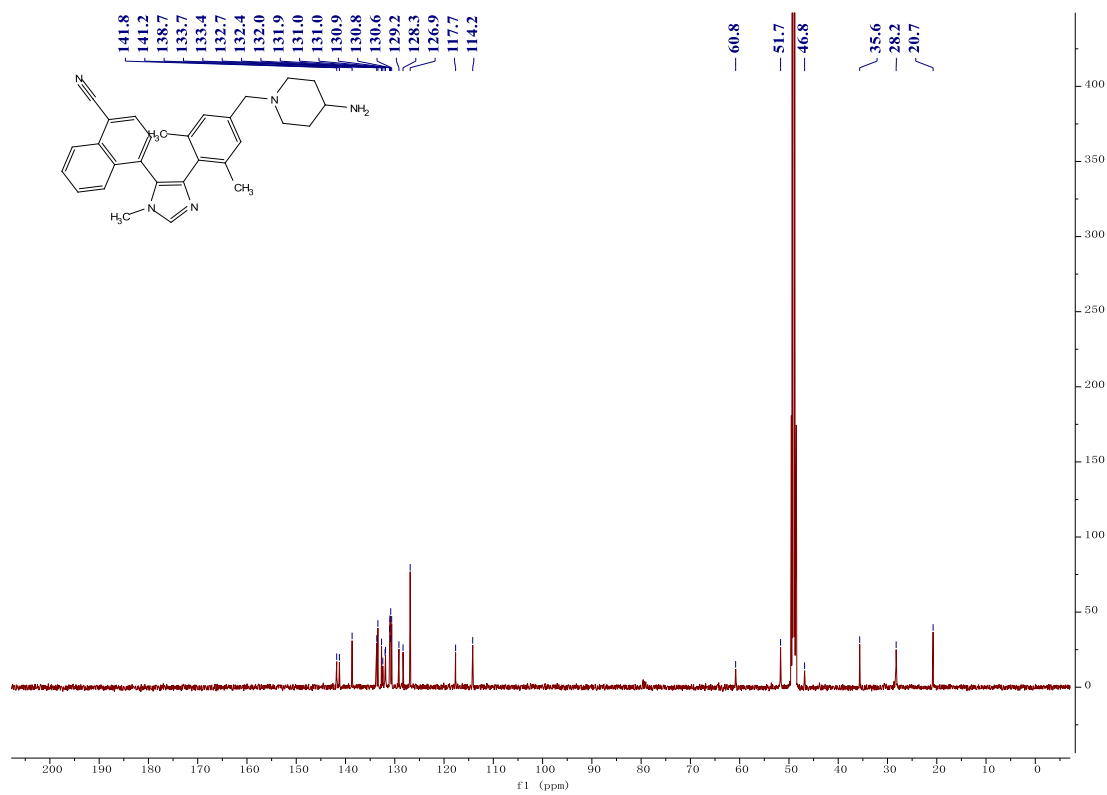
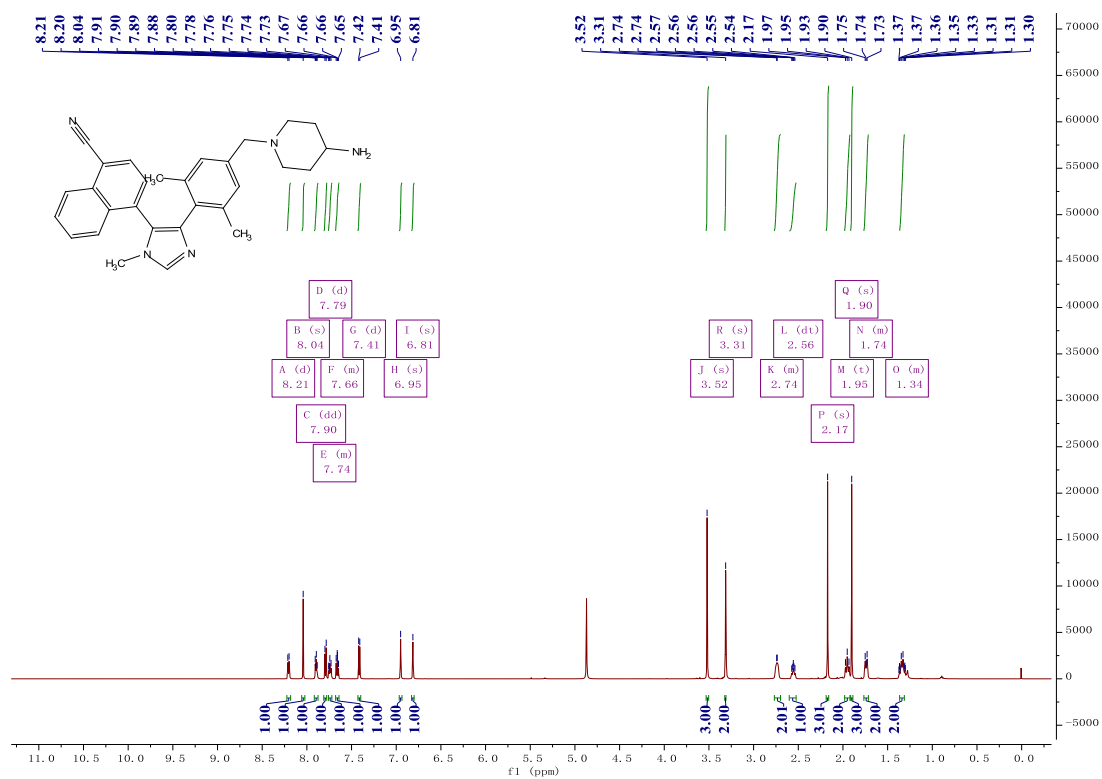
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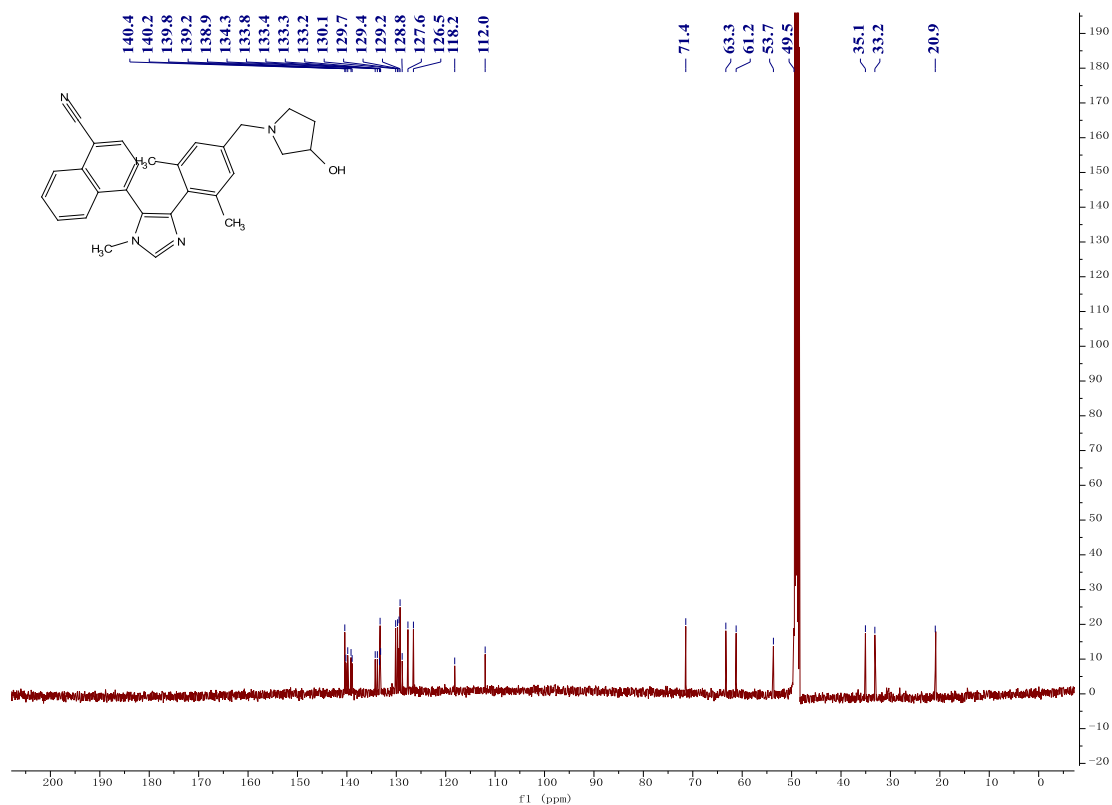
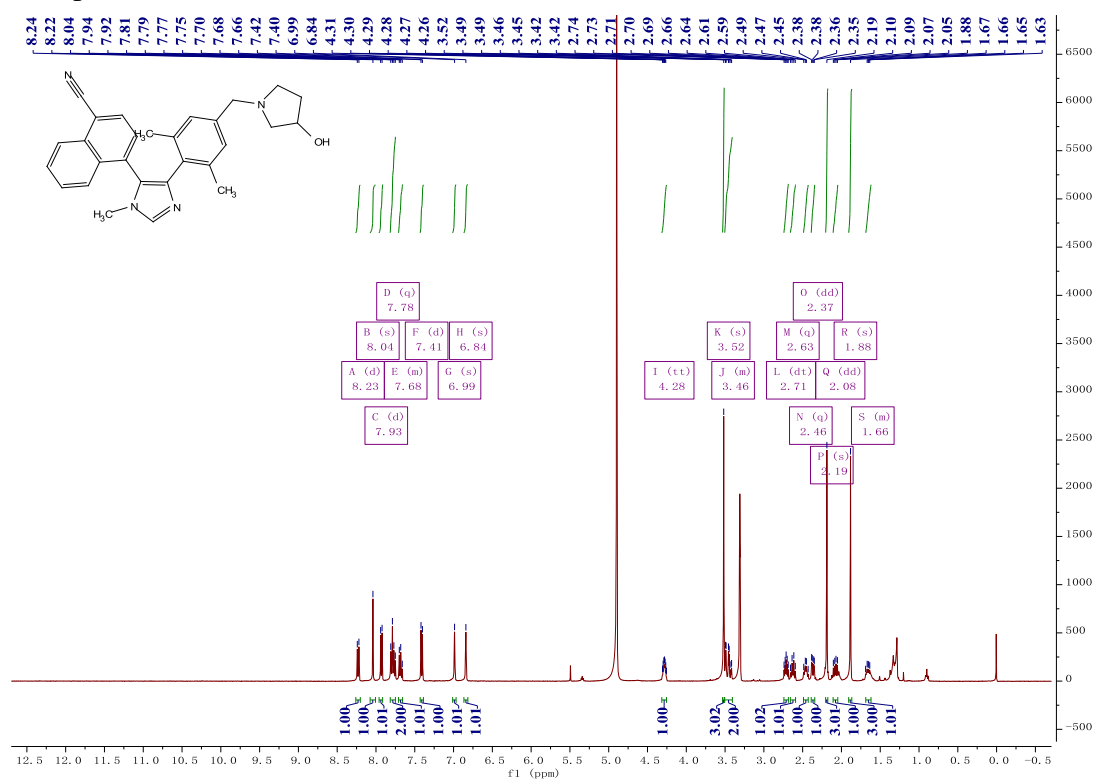
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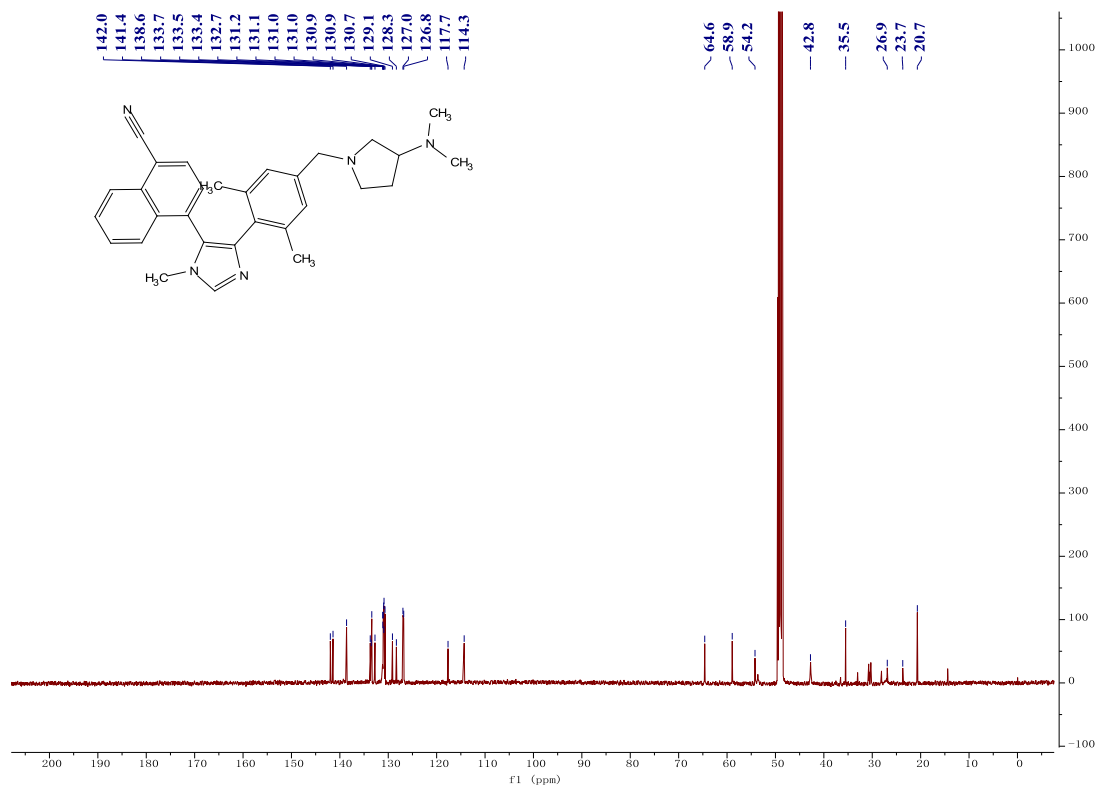
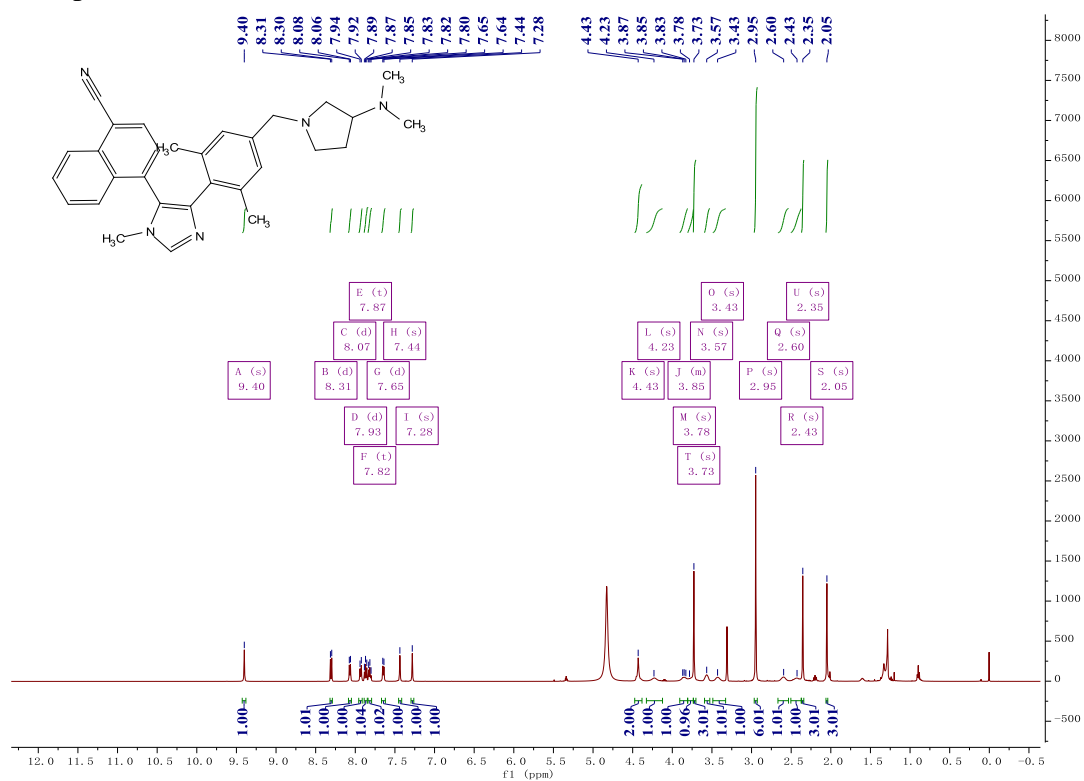
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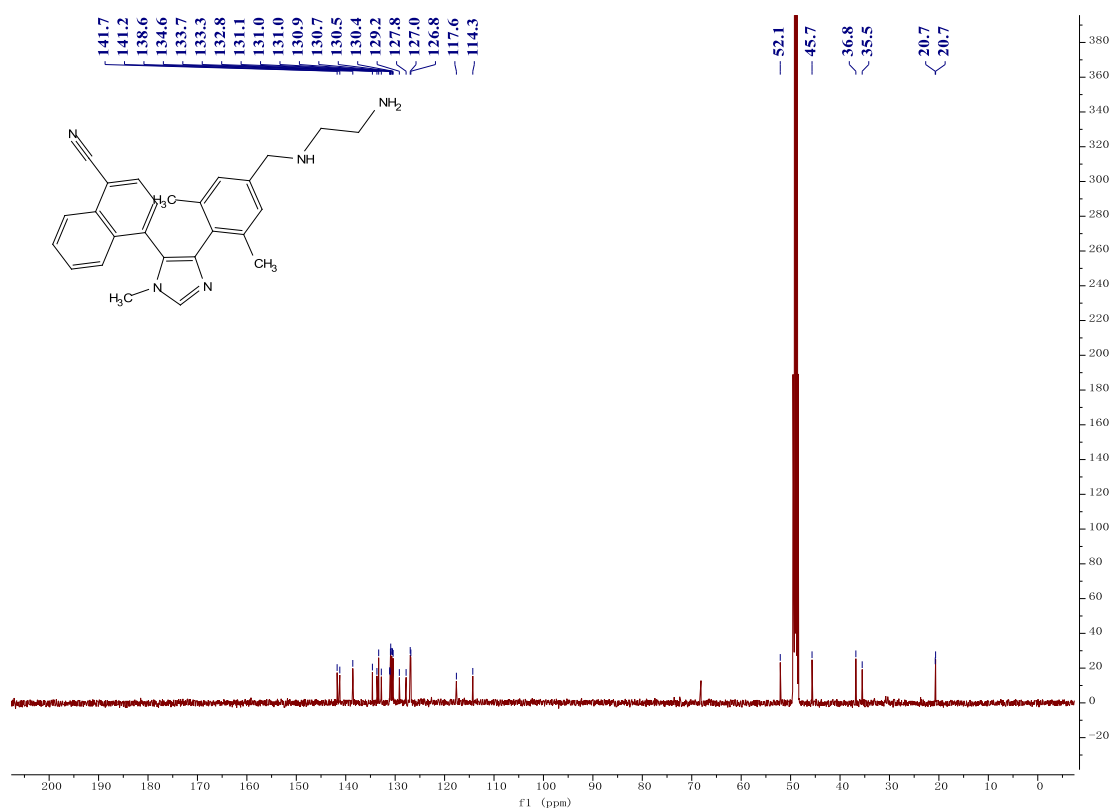
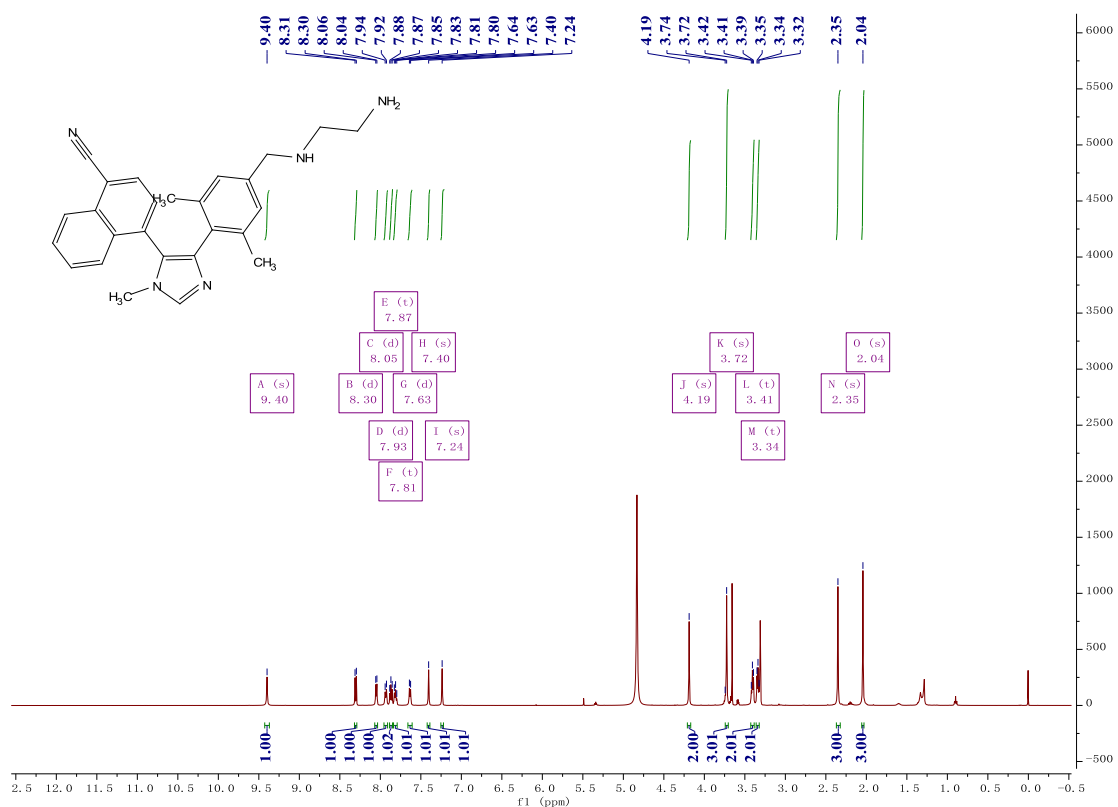
Compound 39



Compound 40

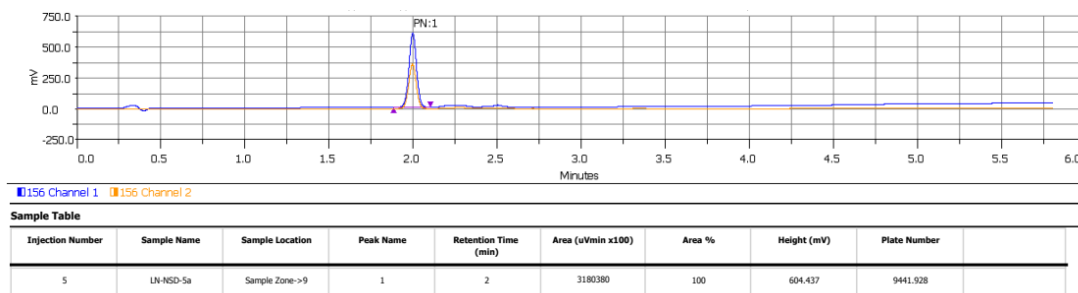


Compound 41

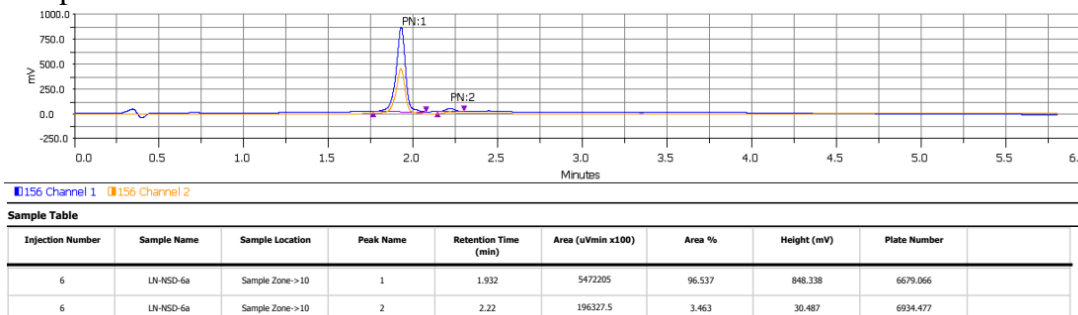


6. HPLC traces for selected compounds

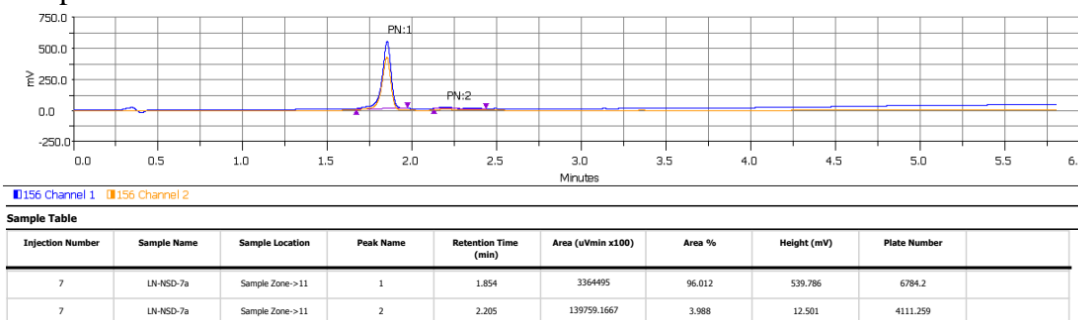
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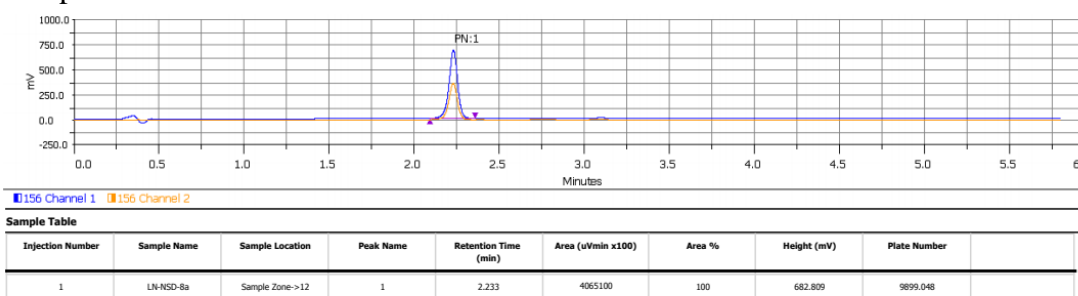
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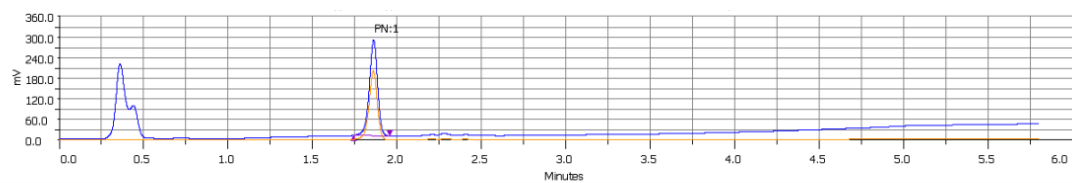
Compound 7



Compound 8



Compound 9

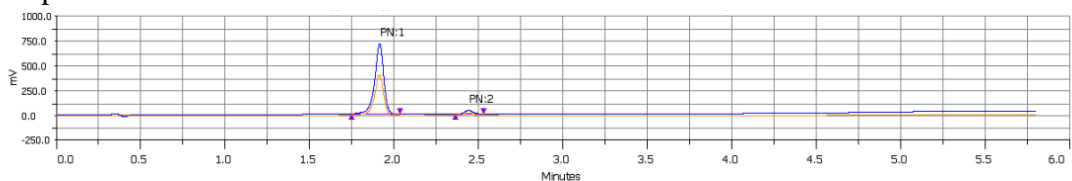


156 Channel 1 156 Channel 2

Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
6	LN-NSD-9a	Sample Zone->13	1	1.864	1581984.1667	100	278.783	7327.921

Compound 11

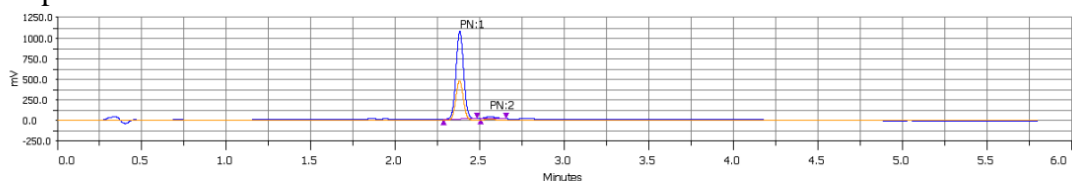


156 Channel 1 156 Channel 2

Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
3	ln-nsd-11	Sample Zone->62	1	1.917	4423045	95.59	709.732	6908.254
3	ln-nsd-11	Sample Zone->62	2	2.445	204067.5	4.41	35.77	12299.024

Compound 12

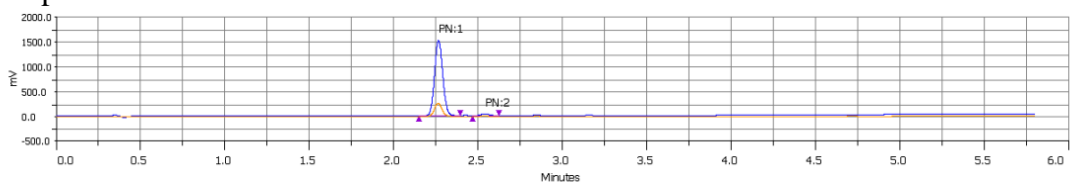


156 Channel 1 156 Channel 2

Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
10	LN-NSD-12a	Sample Zone->14	1	2.383	5596622.9167	96.77	1065.195	13200.097
10	LN-NSD-12a	Sample Zone->14	2	2.561	186785.4167	3.23	22.342	5159.418

Compound 13

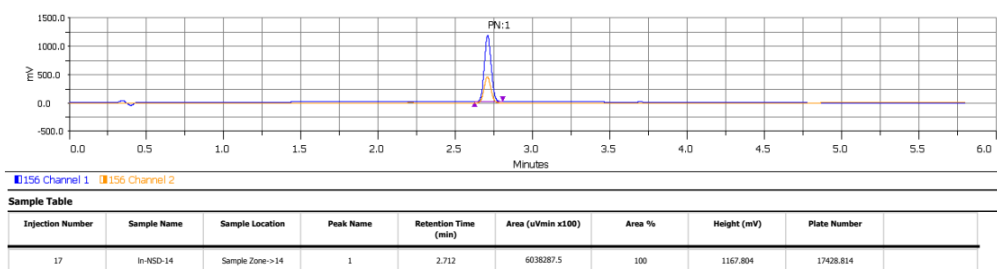


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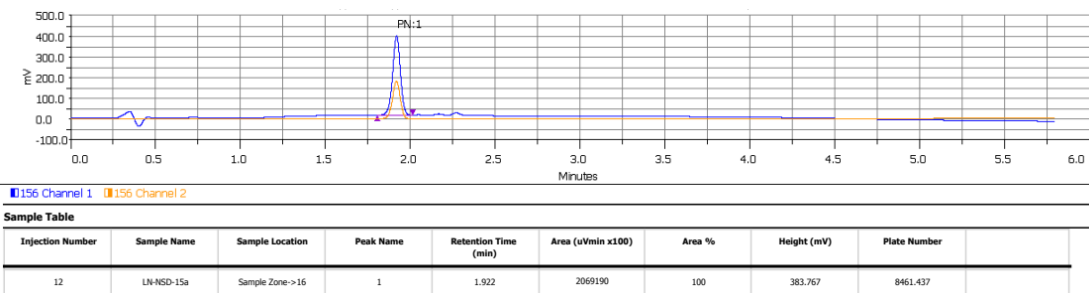
Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
11	LN-NSD-13a	Sample Zone->15	1	2.268	8604348.3333	97.791	1520.183	10105.934
11	LN-NSD-13a	Sample Zone->15	2	2.545	194384.1667	2.209	35.623	13384.065

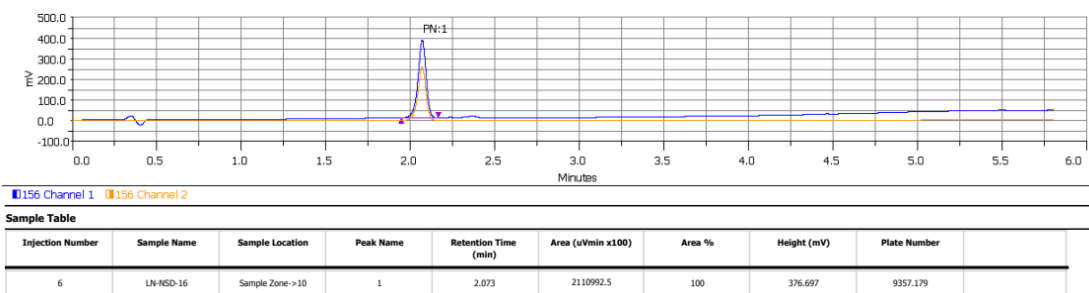
Compound 14



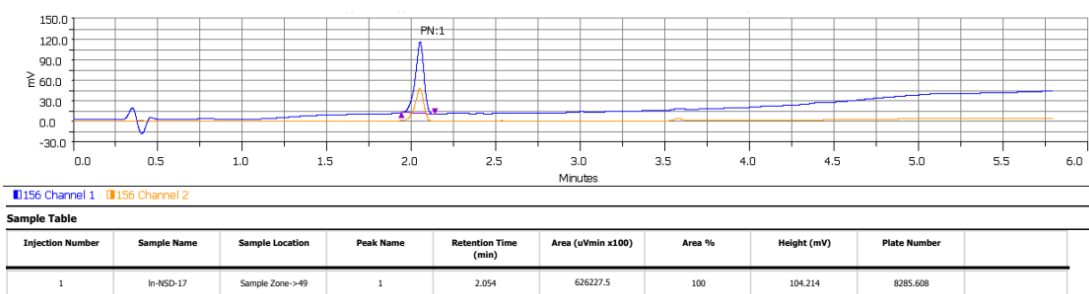
Compound 15



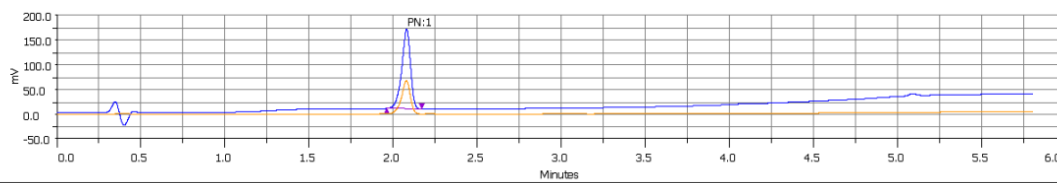
Compound 16



Compound 17



Compound 19

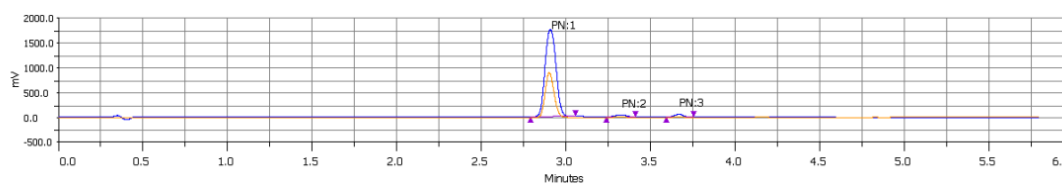


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Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
1	In-nsd-19	Sample Zone->S8	1	2.083	980473.75	100	161.641	8119.048

Compound 21

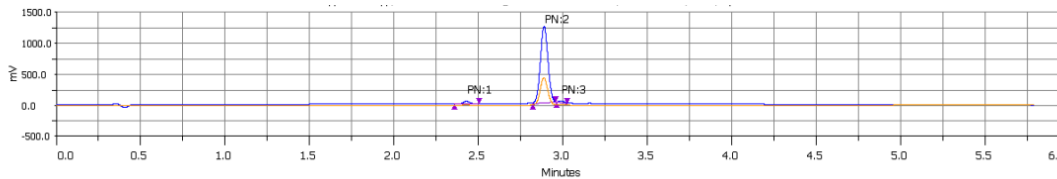


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Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
3	In-NSD-21	Sample Zone->14	1	2.912	13102361.6667	96.036	1752.162	8993.972
3	In-NSD-21	Sample Zone->14	2	3.331	264658.3333	1.94	39.569	14724.819
3	In-NSD-21	Sample Zone->14	3	3.671	276124.1667	2.024	53.546	32035.369

Compound 22

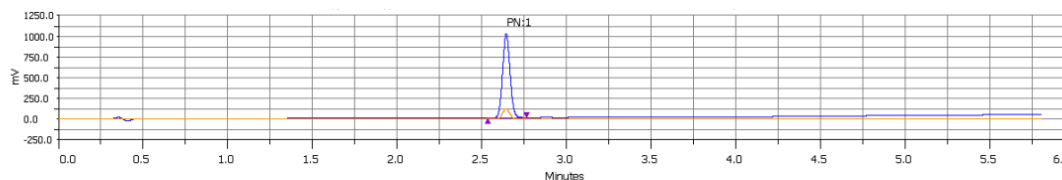


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Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
7	LN-NSD-22	Sample Zone->23	1	2.432	207142.4967	3.063	42.297	15498.121
7	LN-NSD-22	Sample Zone->23	2	2.892	6451667.9622	95.401	1230.675	19051.463
7	LN-NSD-22	Sample Zone->23	3	2.998	103840.4578	1.535	25.506	22080.947

Compound 24

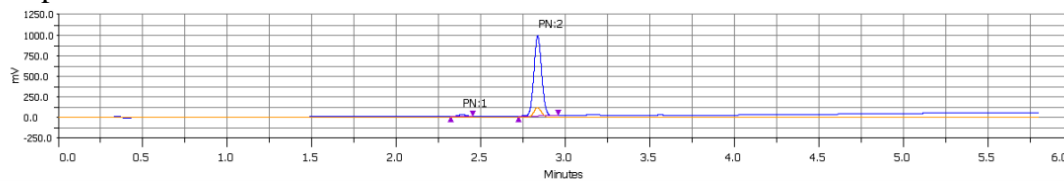


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Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
4	LN-NSD-24	Sample Zone->13	1	2.647	5183107.5	100	1018.07	17309.594

Compound 25

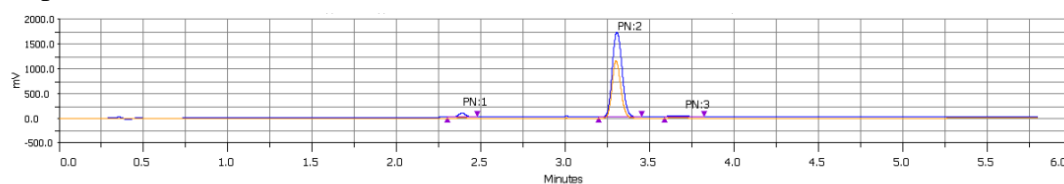


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Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
16	In-nsd-25	Sample Zone->29	1	2.391	101535.4167	1.915	20.41	14579.237
16	In-nsd-25	Sample Zone->29	2	2.839	5201180.8333	98.085	972.846	18111.349

Compound 26

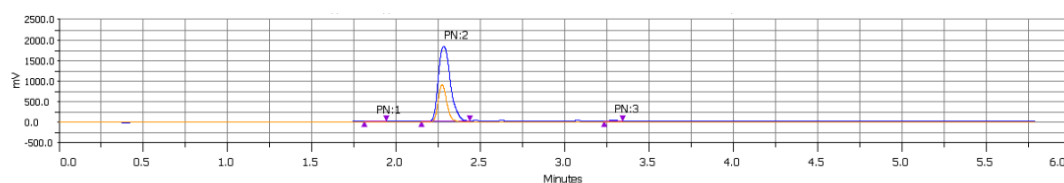


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Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
17	In-nsd-26	Sample Zone->30	1	2.391	403449.5833	3.342	80.473	14533.032
17	In-nsd-26	Sample Zone->30	2	3.31	11560095	95.746	1709.768	14555.818
17	In-nsd-26	Sample Zone->30	3	3.713	110144.1667	0.912	10.447	5567.556

Compound 27

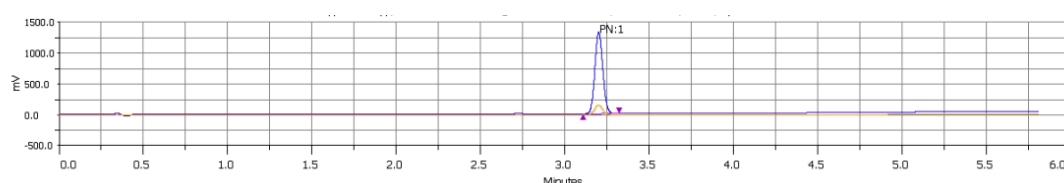


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Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
5	In-nsd-27	Sample Zone->31	1	1.88	100065.4167	0.659	18.347	7243.322
5	In-nsd-27	Sample Zone->31	2	2.285	15007310.8333	98.88	1817.042	4962.992
5	In-nsd-27	Sample Zone->31	3	3.293	69910	0.461	14.164	27304.132

Compound 28

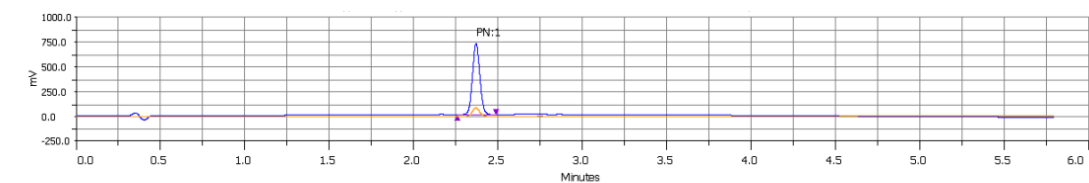


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Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
18	In-NSD-28	Sample Zone->15	1	3.205	7265237.0833	100	1324.747	21682.51

Compound 29

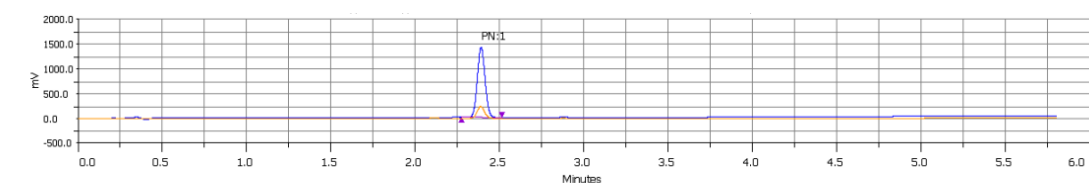


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Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
14	LN-NSD-29a	Sample Zone->18	1	2.373	3657035	100	717.26	13895.037

Compound 30

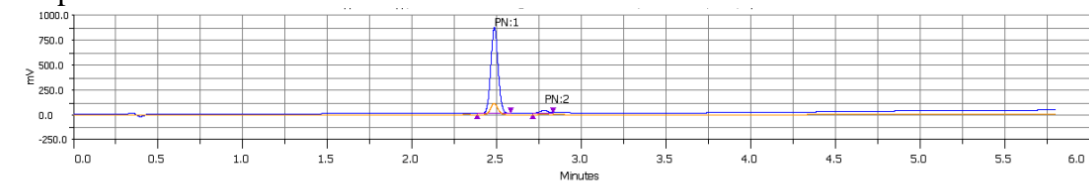


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Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
4	LN-NSD-30	Sample Zone->8	1	2.394	7626912.5	100	1415.922	12534.771

Compound 31

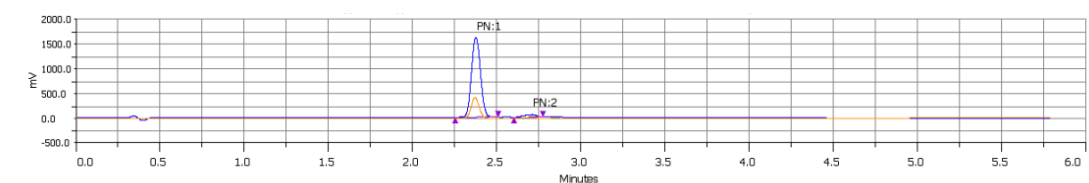


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Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
4	In-NSD-31	Sample Zone->15	1	2.488	4334778.3333	97.202	861.779	15554.329
4	In-NSD-31	Sample Zone->15	2	2.782	124780	2.798	23.983	16833.942

Compound 32

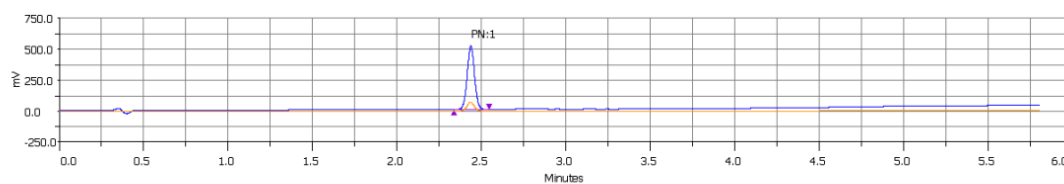


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Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
5	In-NSD-32	Sample Zone->16	1	2.379	10091143.75	96.071	1616.622	8908.778
5	In-NSD-32	Sample Zone->16	2	2.715	412648.3333	3.929	53.441	5847.237

Compound 33

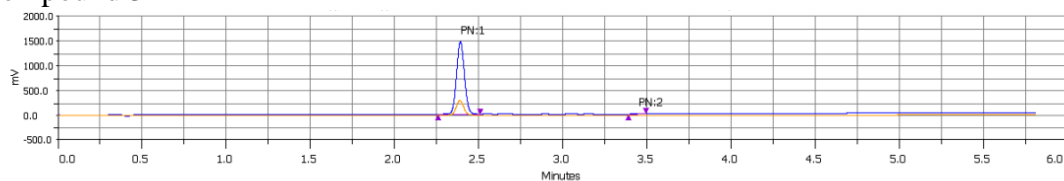


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Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
6	LN-NSD-33	Sample Zone->17	1	2.44	2602007.5	100	514.385	15117.738

Compound 34

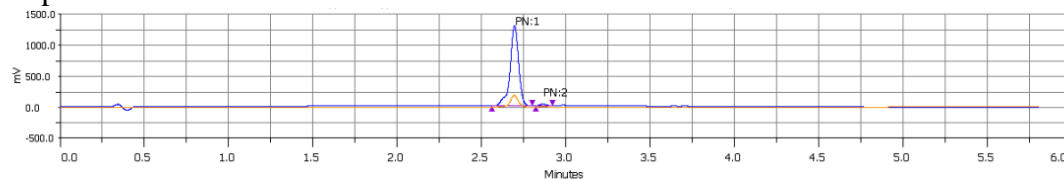


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Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
8	LN-NSD-34	Sample Zone->24	1	2.394	8068676.6667	99.065	1466.201	12080.683
8	LN-NSD-34	Sample Zone->24	2	3.45	76150	0.935	15.643	30130.848

Compound 35

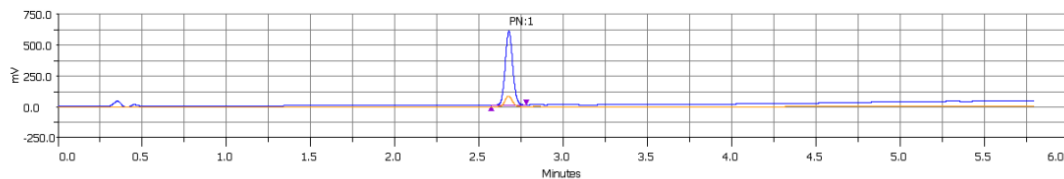


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Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
19	LN-NSD-35	Sample Zone->16	1	2.699	7614661.6667	98.478	1294.429	15561.618
19	LN-NSD-35	Sample Zone->16	2	2.87	117682.9167	1.522	25.687	22887.163

Compound 36

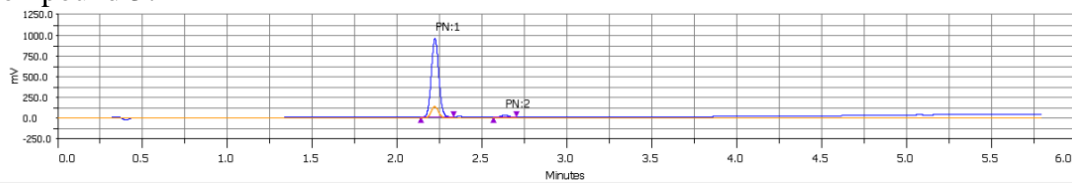


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Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
2	LN-NSD-36b	Sample Zone->25	1	2.68	3087397.5	100	600.639	17455.015

Compound 37

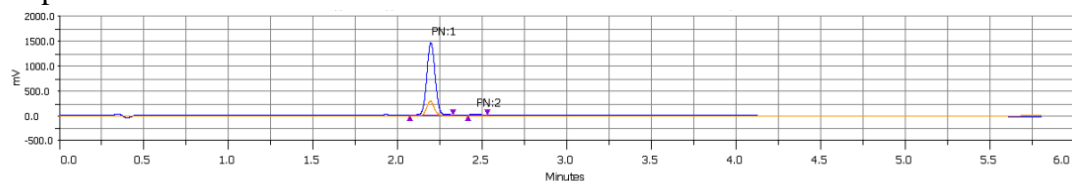


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Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
15	LN-NSD-37a	Sample Zone->19	1	2.225	4792623.3333	97.663	946.922	12279.422
15	LN-NSD-37a	Sample Zone->19	2	2.637	114704.1667	2.337	22.69	17318.092

Compound 38

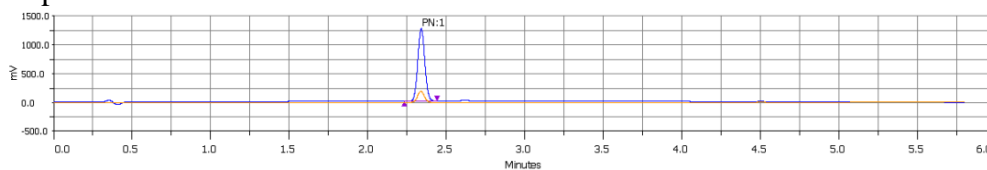


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Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
4	In-nsd-38a	Sample Zone->63	1	2.199	8258107.0833	99.249	1454.355	9482.775
4	In-nsd-38a	Sample Zone->63	2	2.467	62513.75	0.751	12.672	15279.767

Compound 39

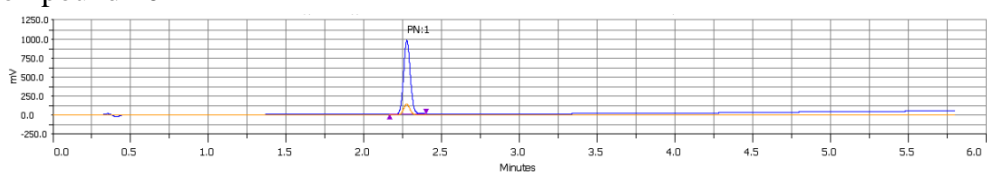


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Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
5	LN-NSD-39	Sample Zone->14	1	2.343	6554845	100	1262.631	12878.904

Compound 40

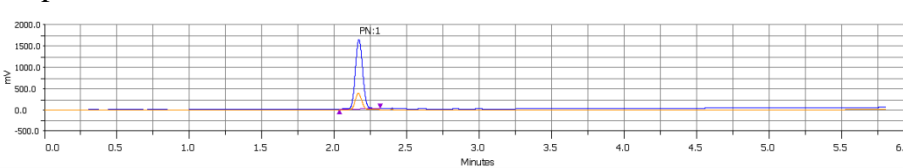


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Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
6	LN-NSD-40	Sample Zone->15	1	2.279	4848737.0833	100	965.78	13251.752

Compound 41



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Sample Table

Injection Number	Sample Name	Sample Location	Peak Name	Retention Time (min)	Area (uVmin x100)	Area %	Height (mV)	Plate Number
20	In-nsd-41	Sample Zone->33	1	2.172	9571755	100	1633.122	8570.731