Supporting Information for

Original article

Design and optimization of piperidine-substituted thiophene[3,2-*d*]pyrimidine-based HIV-1 NNRTIs with improved drug resistance and pharmacokinetic profiles

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Contents

1. Water solubility measurement.

Compound **15a** and its hydrochloride form **15a·HCl** (0.1, 1, 10 mg) were dissolved in deionized water (pH = 2.0, 7.0, and 7.4) until the solution was clear. The assays were measured at least in duplicate.

2. Pharmacokinetics assays.

6 male Sprague Dawley rats were randomly divided into 2 groups, which was performed to tail vein injection (2 $mg \cdot kg^{-1}$) and oral administration (10 $mg \cdot kg^{-1}$), respectively. The solution of compound **15a·HCl** was prepared by dissolving in a mixture of PEG400/normal saline (70/30, V/V). Blood samples of the intravenous group were collected from the retrobulbar vein is into heparinized centrifugation tubes at 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h and 12 h after dosing, and blood samples of the oral administration group were collected at 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 10 h, and 12 h after dosing. All the samples were centrifuged at 8000 rpm for 8 min to separate plasma. LC-MS/MS analysis was used to determine the concentration of **15a·HCl** in plasma. Briefly, 50 μL of plasma was added to 50 μL of internal standard and 300 μL of methanol in a 5 mL centrifugation tube, which was centrifuged at 3000g for 10 min. The supernatant layer was collected and a 20 μL aliquot was injected for LC-MS/MS analysis. Standard curves for **15a·HCl** in blood were generated by the

addition of various concentrations of **15a·HCl** together with internal standard to blank plasma. Then all samples were quantified with an Agilent 1200 LC/MSD (Agilent, USA).

3. Acute toxicity experiment.

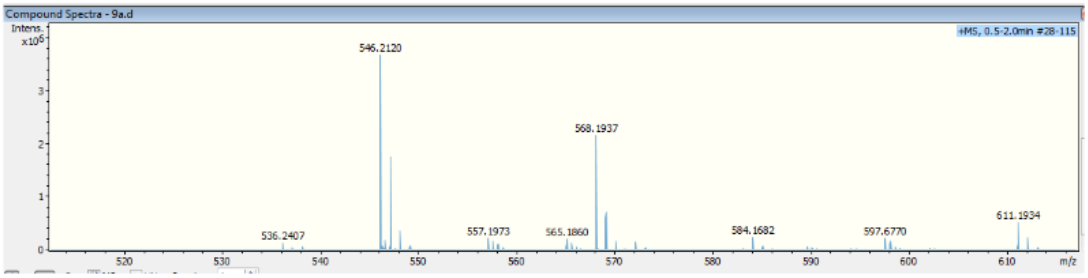
Twenty Kunming mice (18-20 g) were purchased from the animal experimental center of Shandong University and divided into two groups. Compound **15a·HCl** was suspended in PEG-400/normal saline (70/30, V/V) and normal saline at concentrations of 100 $mg \cdot mL^{-1}$. After the mice had been fasted for 12 h, they were administered intragastrical by gavage (2000 mg/kg).

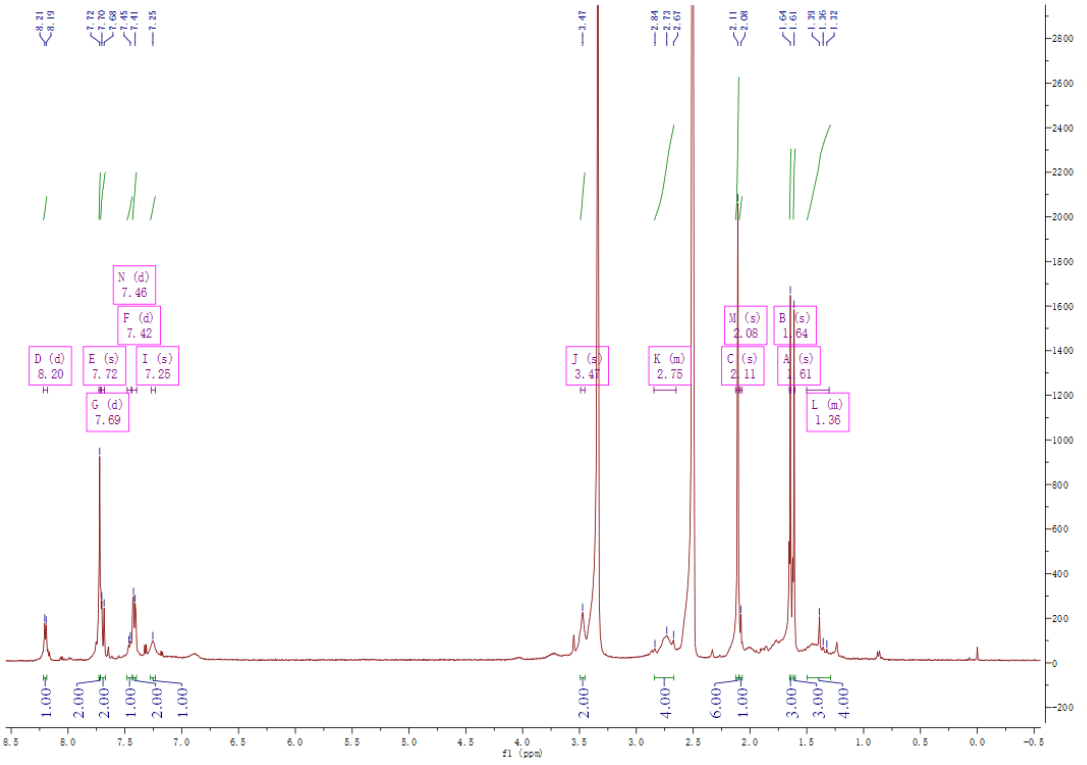
4. Subacute toxicity experiment.

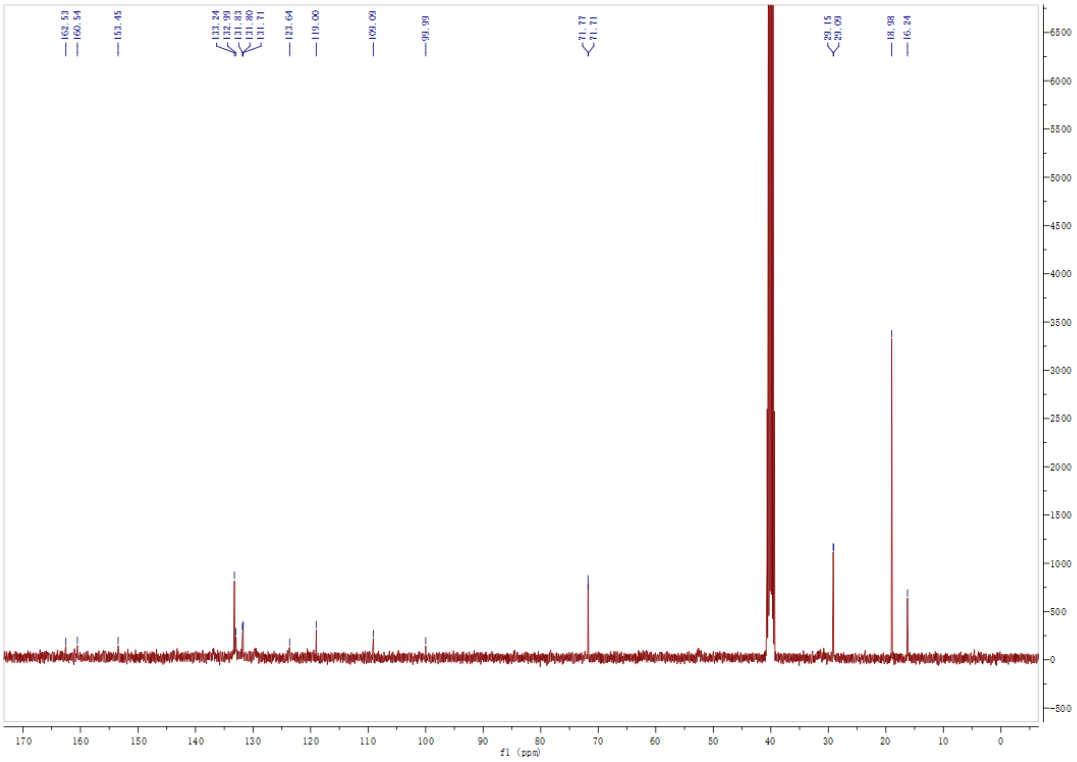
A batch of eight Kunming mice (purchased from the animal experimental center of Shandong University) was randomly divided into four groups (n = 4): control group and test group. All mice were deprived of feed for 12 h and then the mice in the test groups were given 50 $mg \cdot kg^{-1}$ p.o. of **15a·HCl** every two days for 14 days, while the mice in control groups received the same volume of vehicle solution. The mice were weighed before each dosing. All the mice were killed and dissected at Day 14, and the heart, liver, spleen, lung, and kidney were extracted. These organs were examined by HE staining.

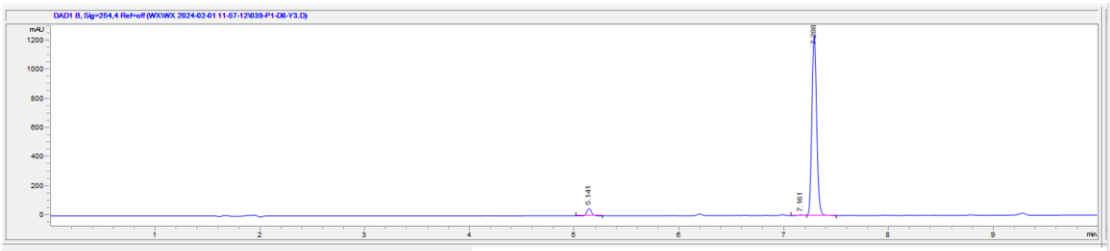
5. Experimental details, ^{1}H and ^{13}C NMR spectra, and HRMS spectra.

4-((2-((1-(4-(dimethylphosphoryl)benzyl)piperidin-4-yl)amino)thieno[3,2-d]pyrimidin-4-yl)oxy)-3,5-dimethylbenzonitrile (9a)


Compound Spectra - 9a.d
Intens.
x10^6
+MS, 0.5-2.0min #28-115
546.2120
568.1937
536.2407
557.1973
565.1860
584.1682
597.6770
611.1934
m/z

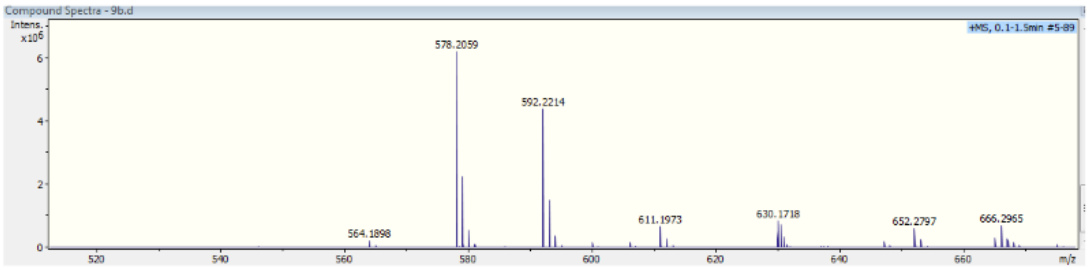

N (d) 7.46
F (d) 7.42
D (d) 8.20
E (s) 7.72
I (s) 7.25
G (d) 7.69
J (s) 3.47
K (m) 2.75
M (s) 2.08
C (s) 2.11
B (s) 1.64
A (s) 1.61
L (m) 1.36
f1 (ppm)

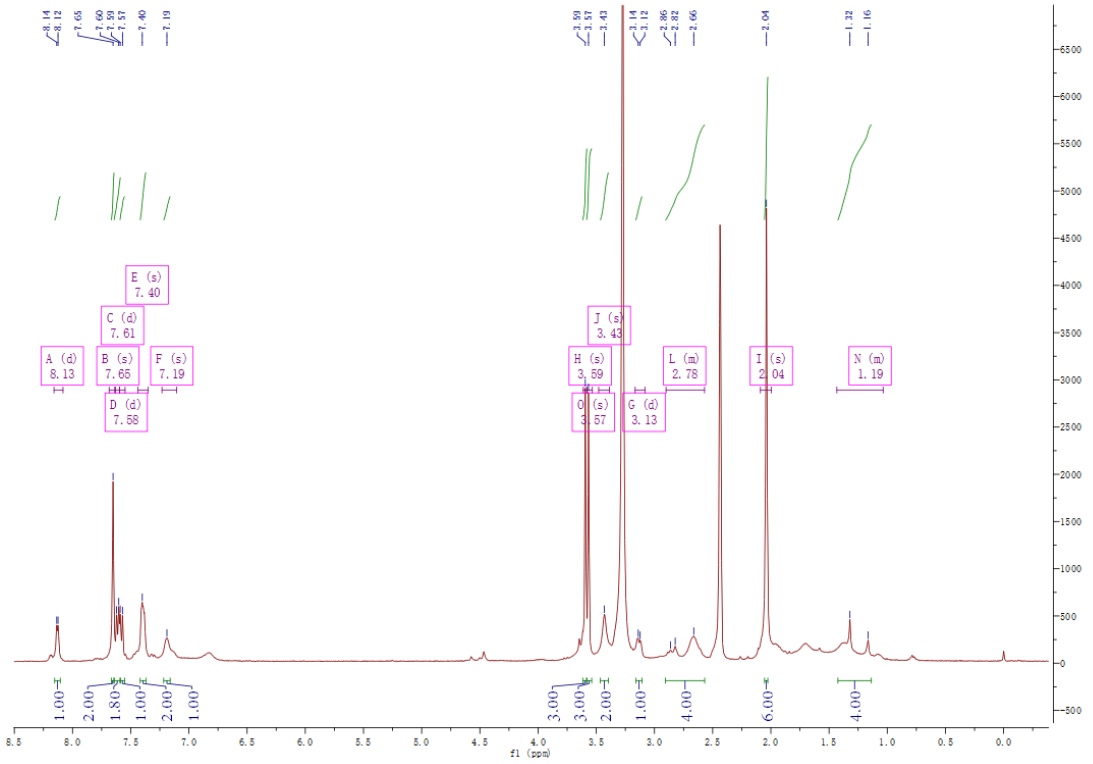


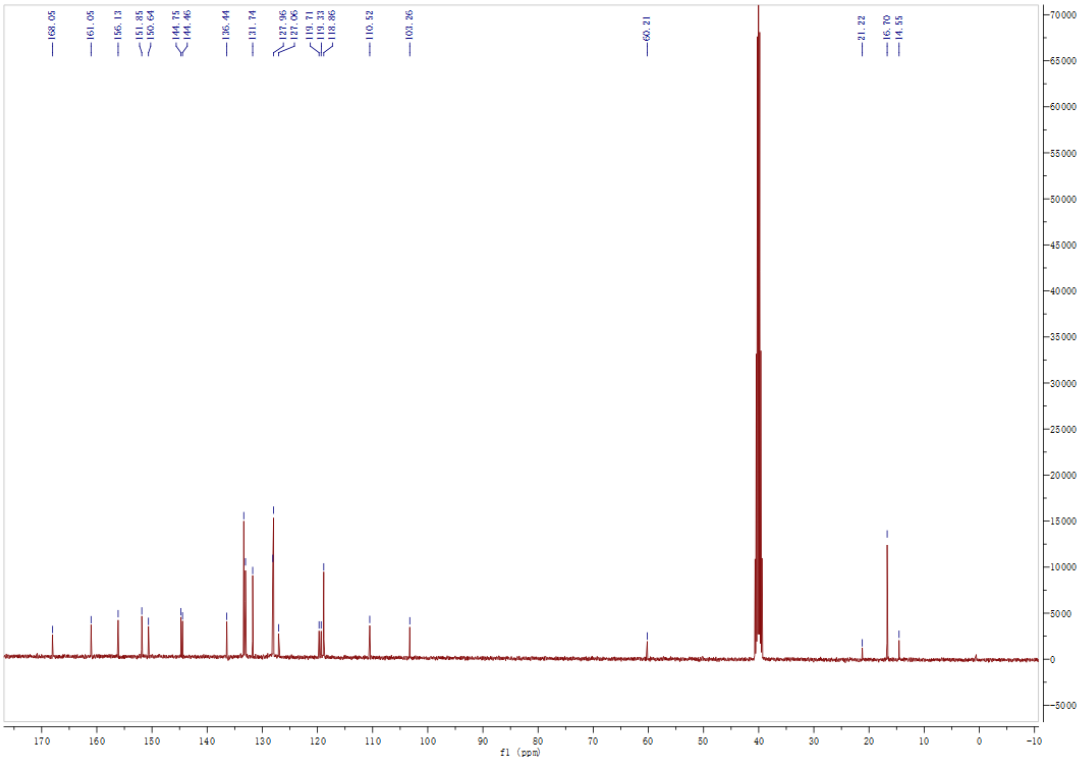


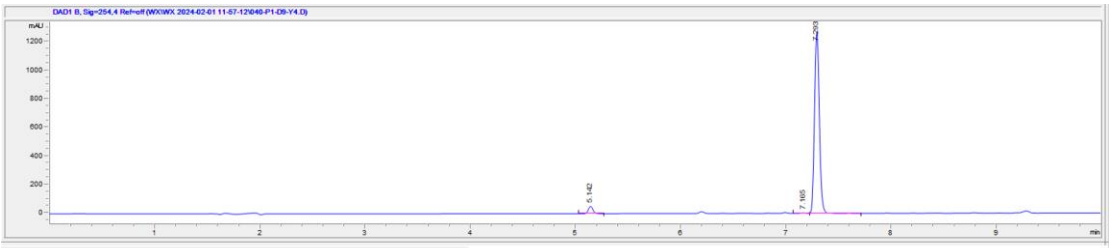
#	时间	峰面积	峰高	峰宽	峰面积 %	对称因子	类型
1	5.141	149.3	48.9	0.0496	3.630	0.877	BB
2	7.161	16.5	4.8	0.0539	0.401	1.007	VV E
3	7.288	3946.8	1243.5	0.051	95.970	0.897	VB R

dimethyl (4-((4-((4-(4-cyano-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl)amino)piperidin-1-yl)methyl)phenyl)phosphonate (9b)



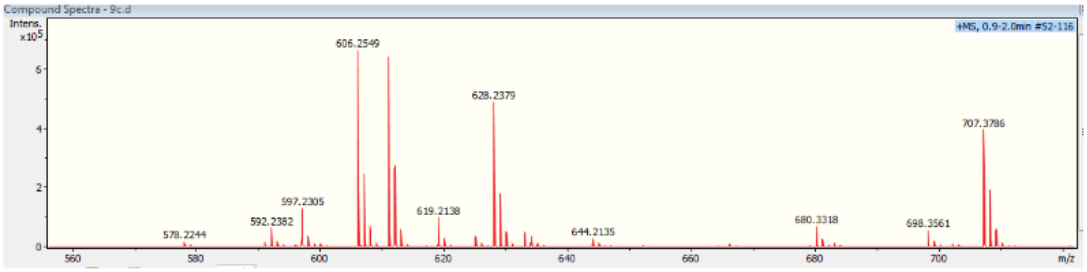


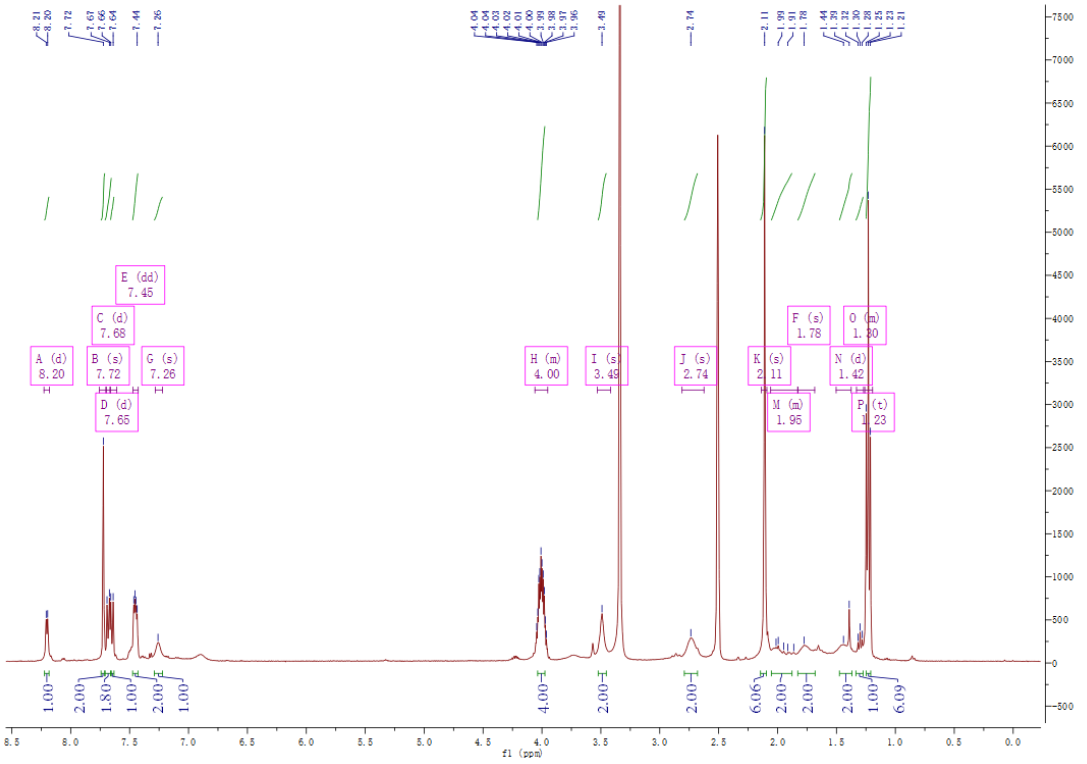


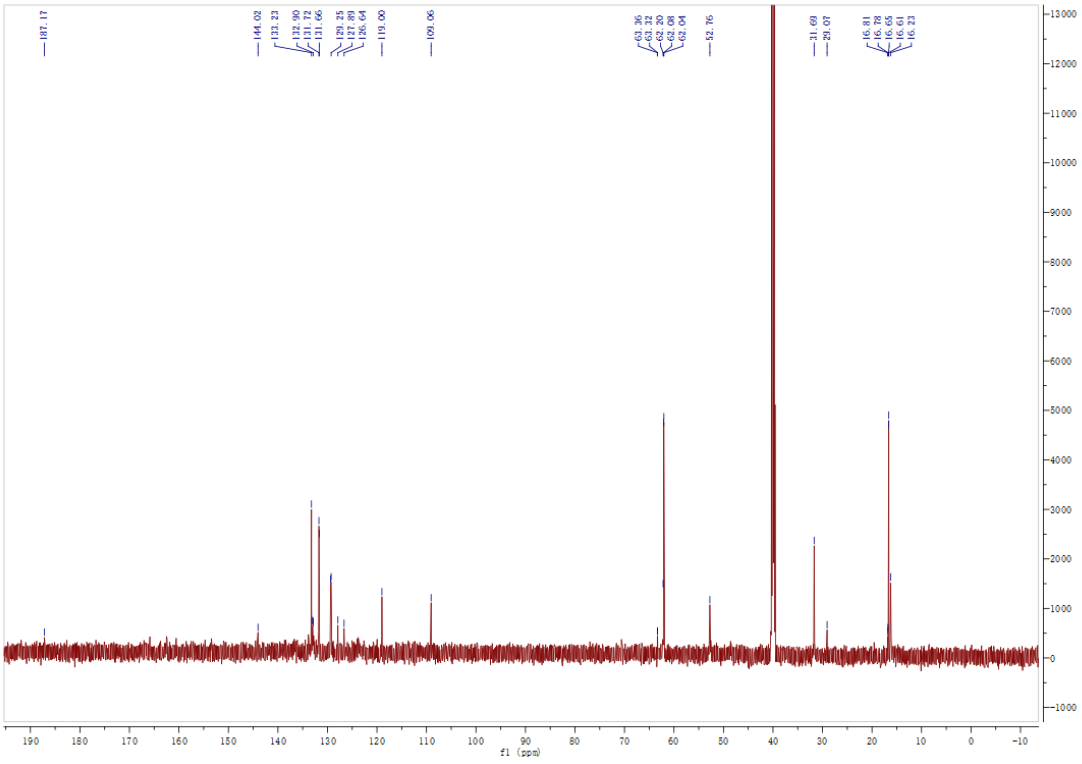


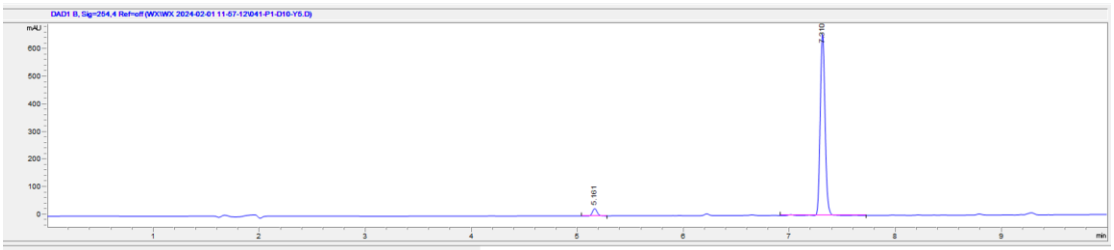
#	时间	峰面积	峰高	峰宽	峰面积 %	对称因子	类型
1	5.142	154.8	50.7	0.0496	3.644	0.879	BB
2	7.165	15.2	4.1	0.0554	0.358	1.138	VV E
3	7.293	4077	1278.2	0.0492	95.998	0.898	VB R

diethyl (4-((4-((4-(4-cyano-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl)amino)piperidin-1-yl)methyl)phenyl)phosphonate (9c)



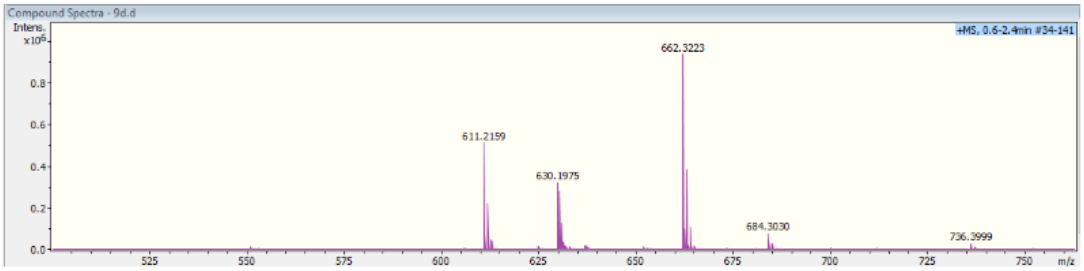


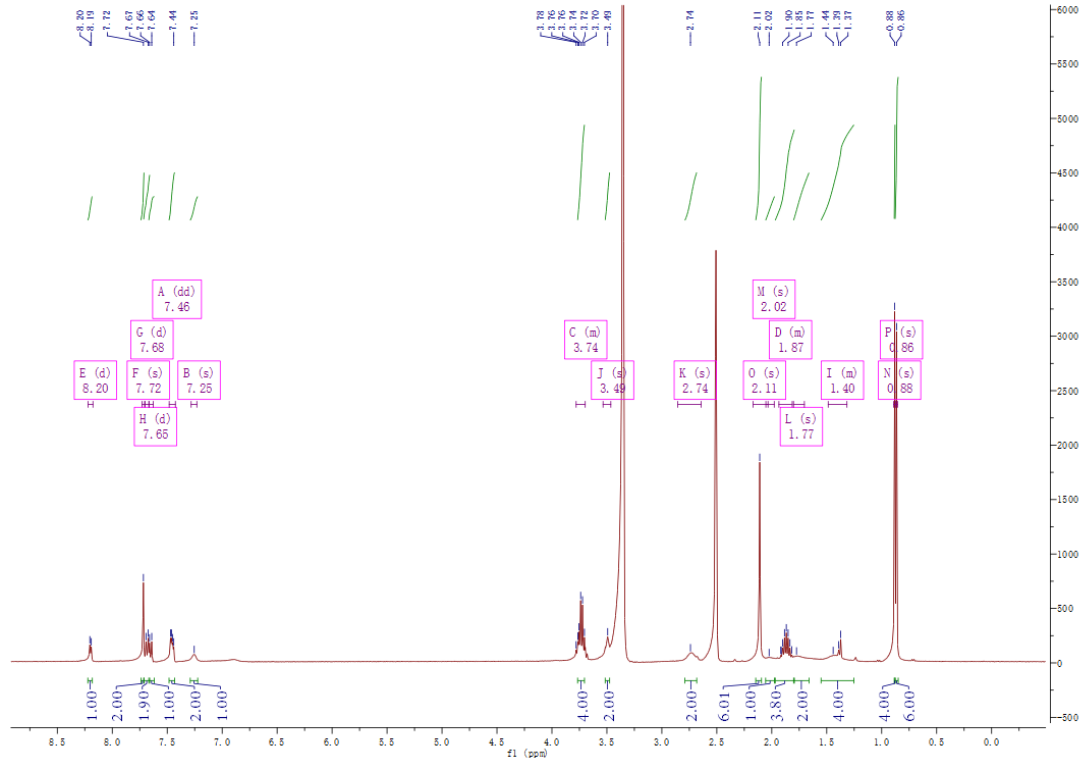


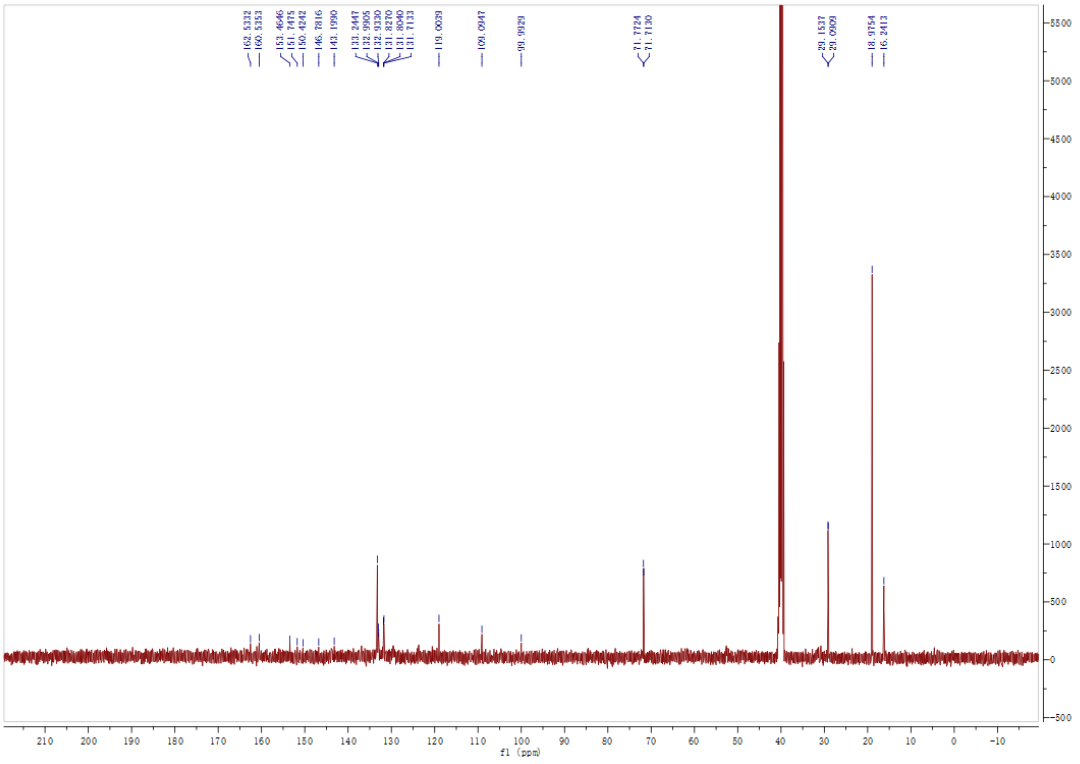


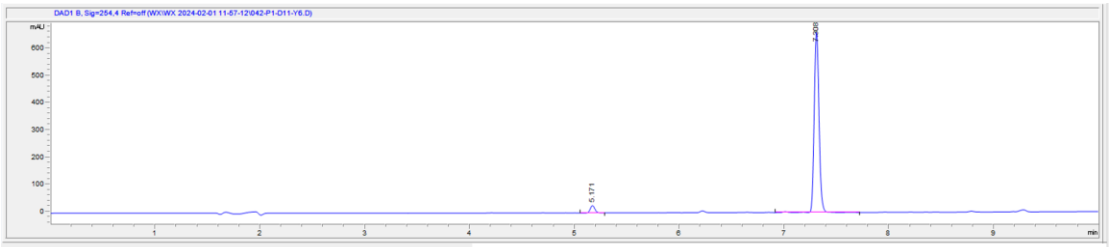
#	时间	峰面积	峰高	峰宽	峰面积 %	对称因子	类型
1	5.161	80.7	26.5	0.0476	3.666	0.893	BB
2	7.31	2119.5	661.7	0.051	96.334	0.912	BB R

dibutyl (4-((4-((4-(4-cyano-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl)amino)piperidin-1-yl)methyl)phenyl)phosphonate (9d)



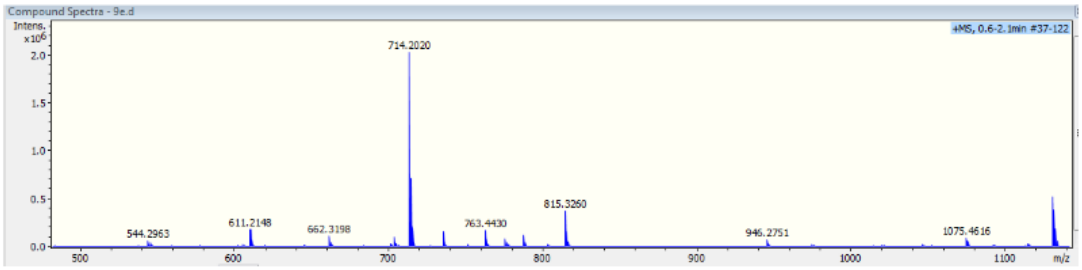


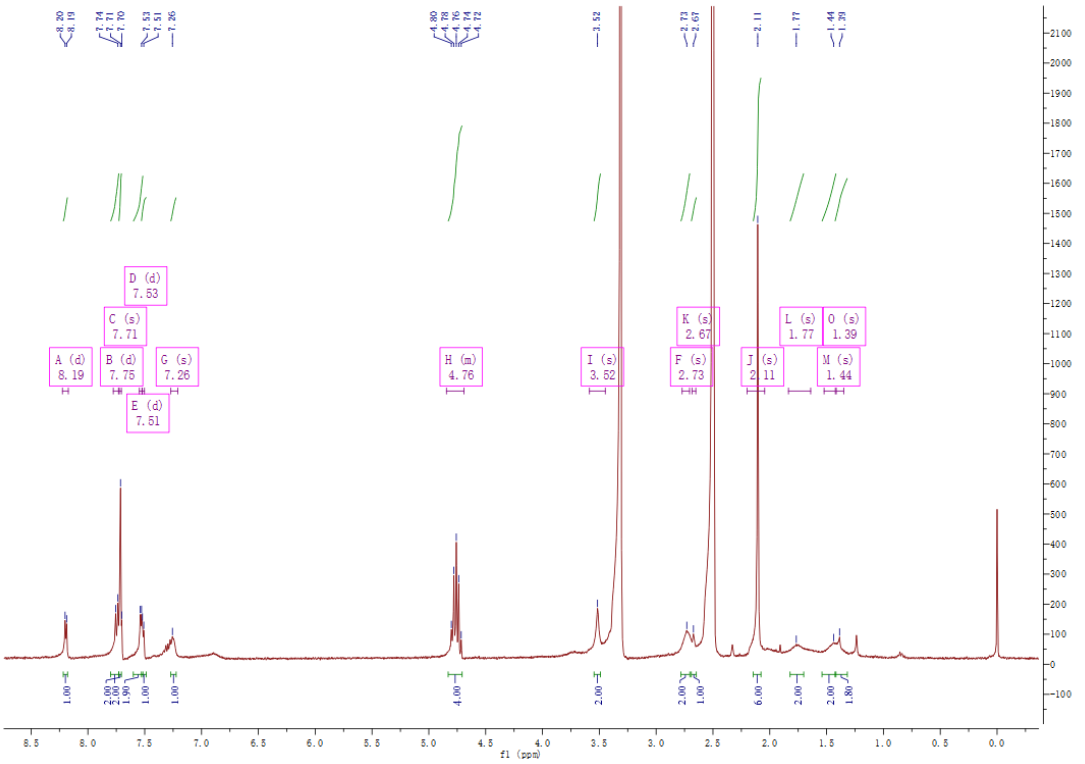


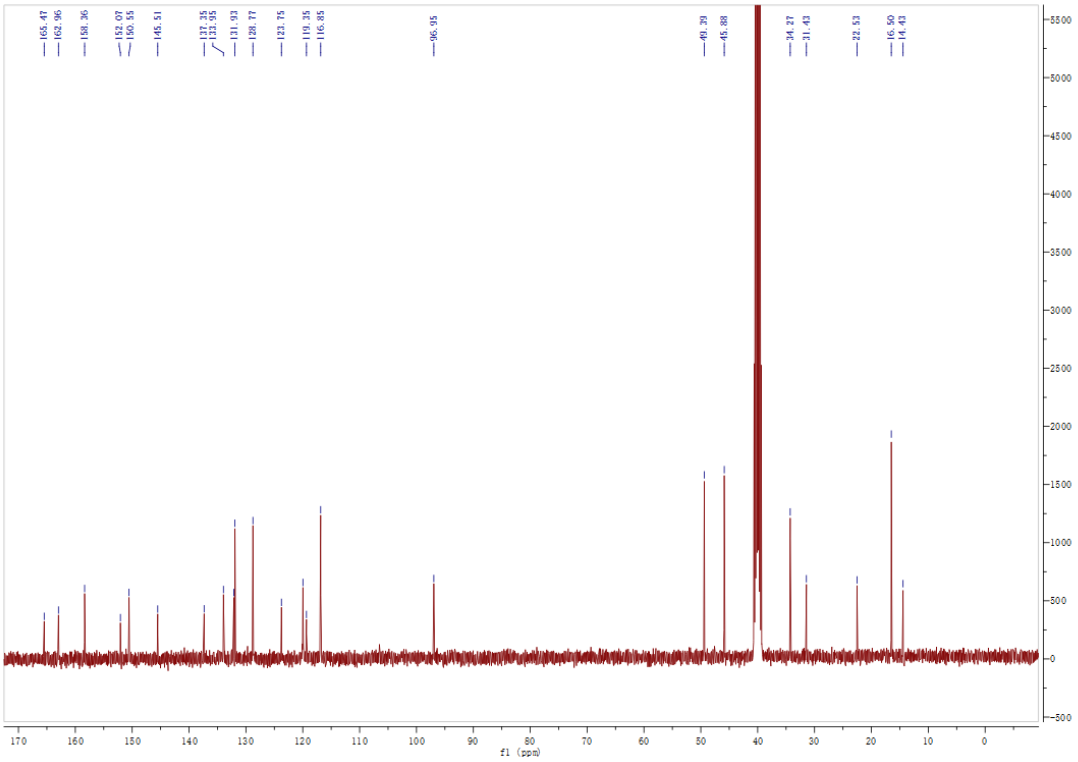


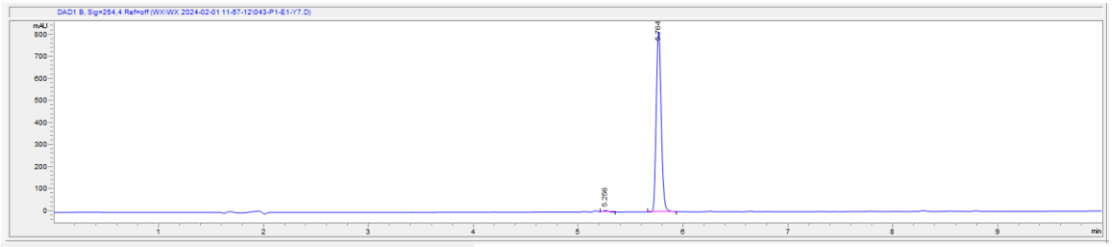
#	时间	峰面积	峰高	峰宽	峰面积 %	对称因子	类型
1	5.171	81.9	26.9	0.0476	3.702	0.89	BB
2	7.308	2131.9	666.3	0.049	96.298	0.902	BB R

bis(2,2,2-trifluoroethyl) (4-((4-((4-(4-cyano-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl)amino)piperidin-1-yl)methyl)phenyl)phosphonate (9e)



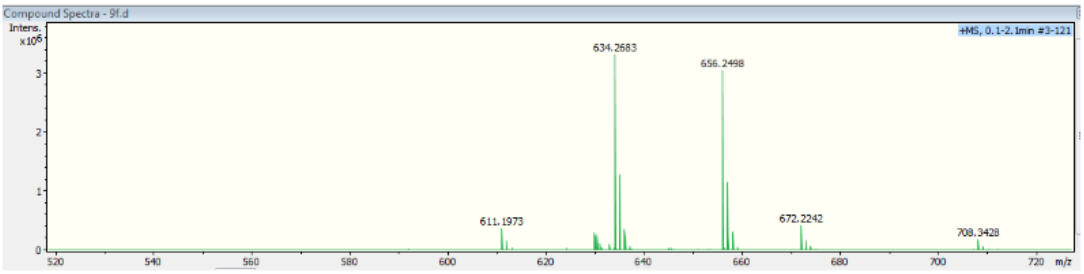


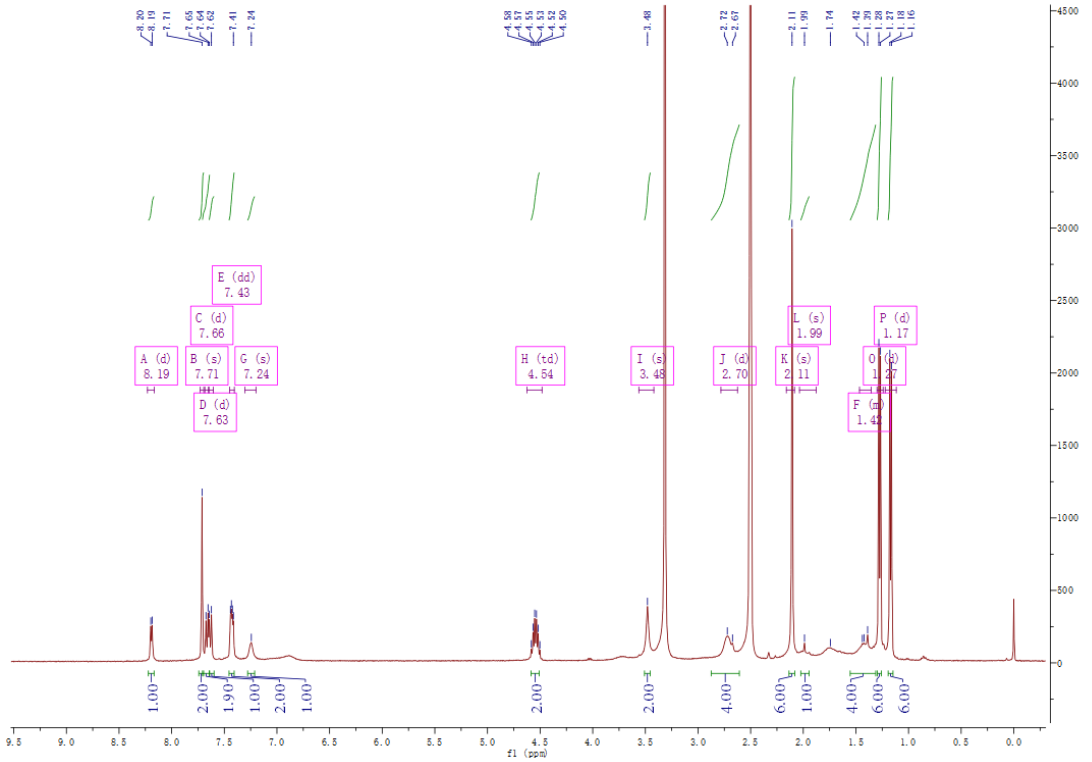


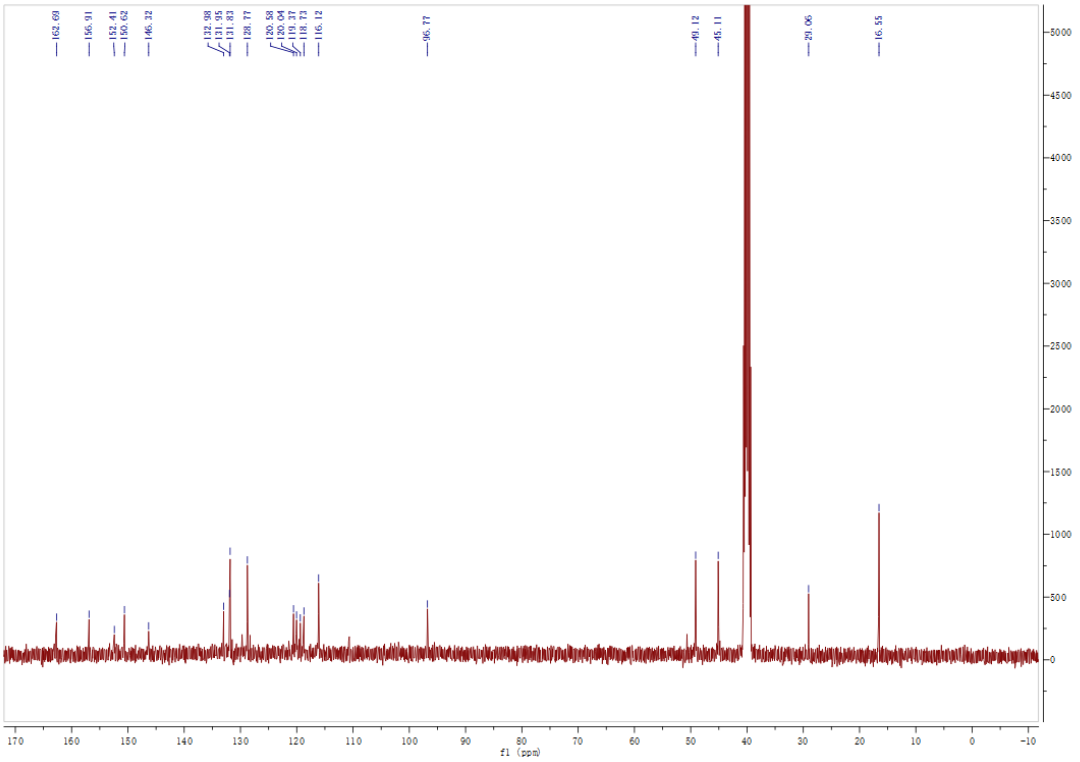


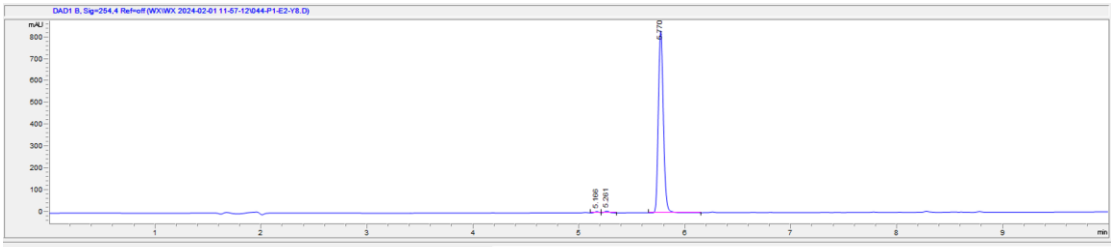
#	时间	峰面积	峰高	峰宽	峰面积 %	对称因子	类型
1	5.256	25.4	7.8	0.0499	0.931	0.949	VB
2	5.764	2705.7	826.9	0.0502	99.069	0.838	BB

diisopropyl (4-((4-((4-(4-cyano-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl)amino)piperidin-1-yl)methyl)phenyl)phosphonate (9f)



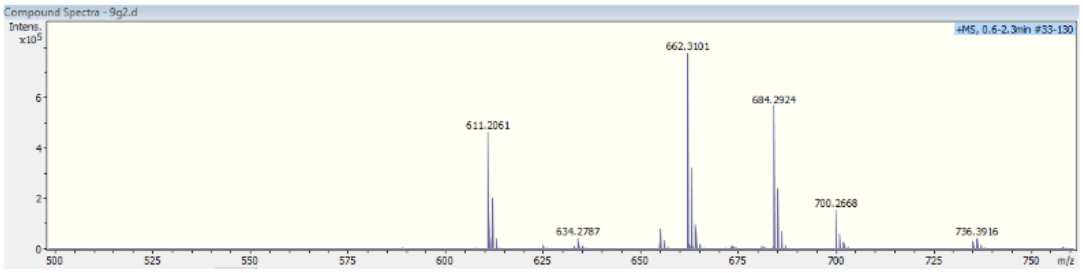


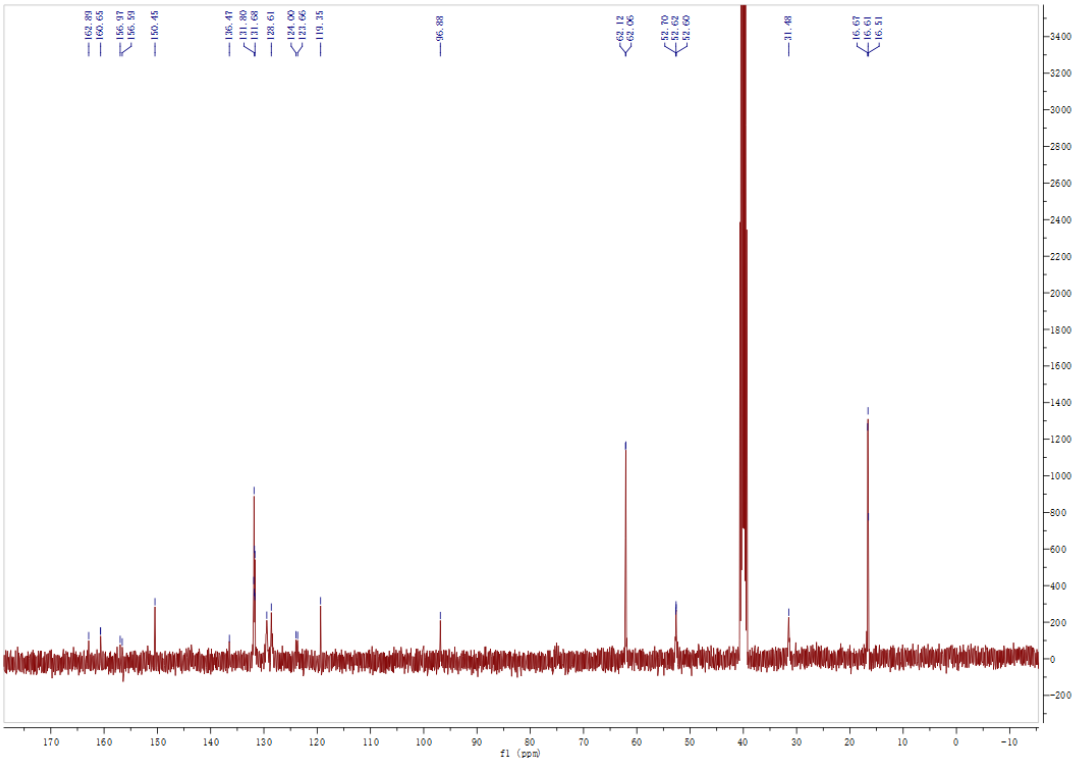


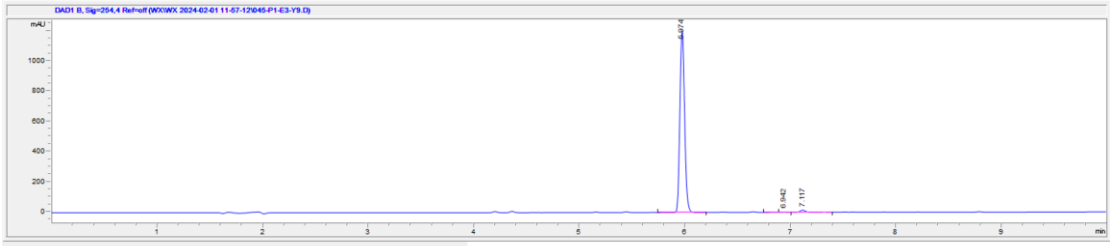


#	时间	峰面积	峰高	峰宽	峰面积 %	对称因子	类型
1	5.166	17.9	5.9	0.0472	0.641	0.931	VV
2	5.261	25.7	7.9	0.05	0.921	0.966	VB
3	5.77	2743.3	836.5	0.0523	98.438	0.832	BB

di-tert-butyl (4-((4-((4-(4-cyano-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl)amino)piperidin-1-yl)methyl)phenyl)phosphonate (9g)

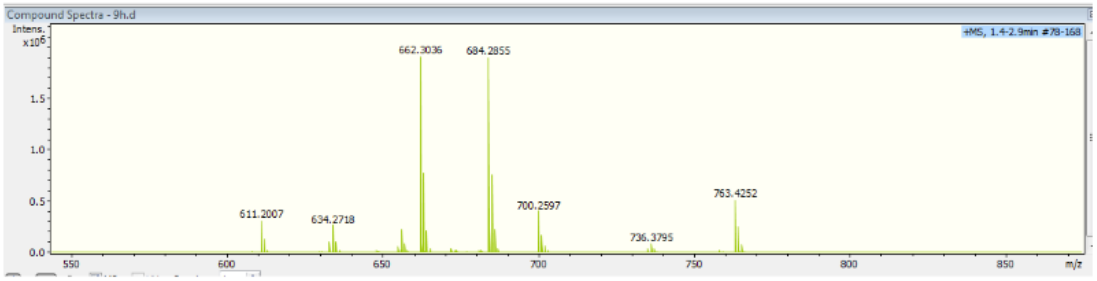






#	时间	峰面积	峰高	峰宽	峰面积 %	对称因子	类型
1	5.974	3754.6	1210.8	0.0501	98.178	0.881	BB R
2	6.942	5.5	1.5	0.0562	0.144	0.946	VV E
3	7.117	64.2	14.6	0.0631	1.678	1.344	BB R

diisobutyl (4-((4-((4-(4-cyano-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl)amino)piperidin-1-yl)methyl)phenyl)phosphonate (9h)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Intens.
x10^6
+MS, 1.4-2.9min #79-168
662.3036
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611.2007
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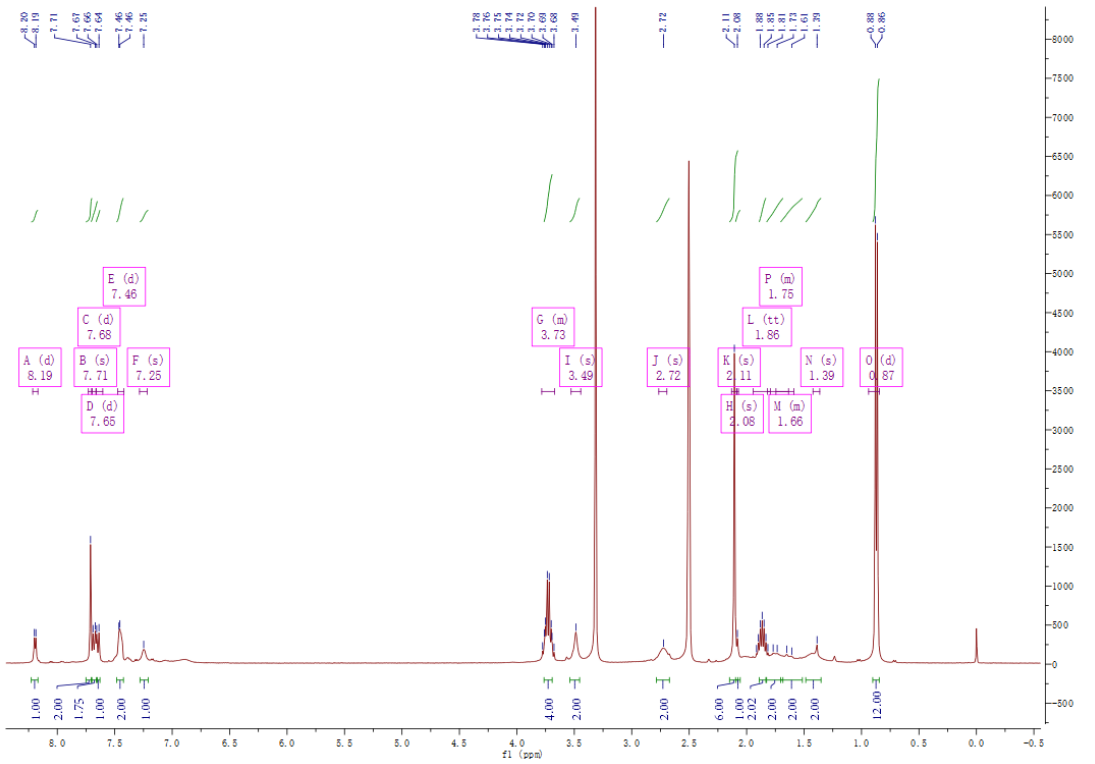
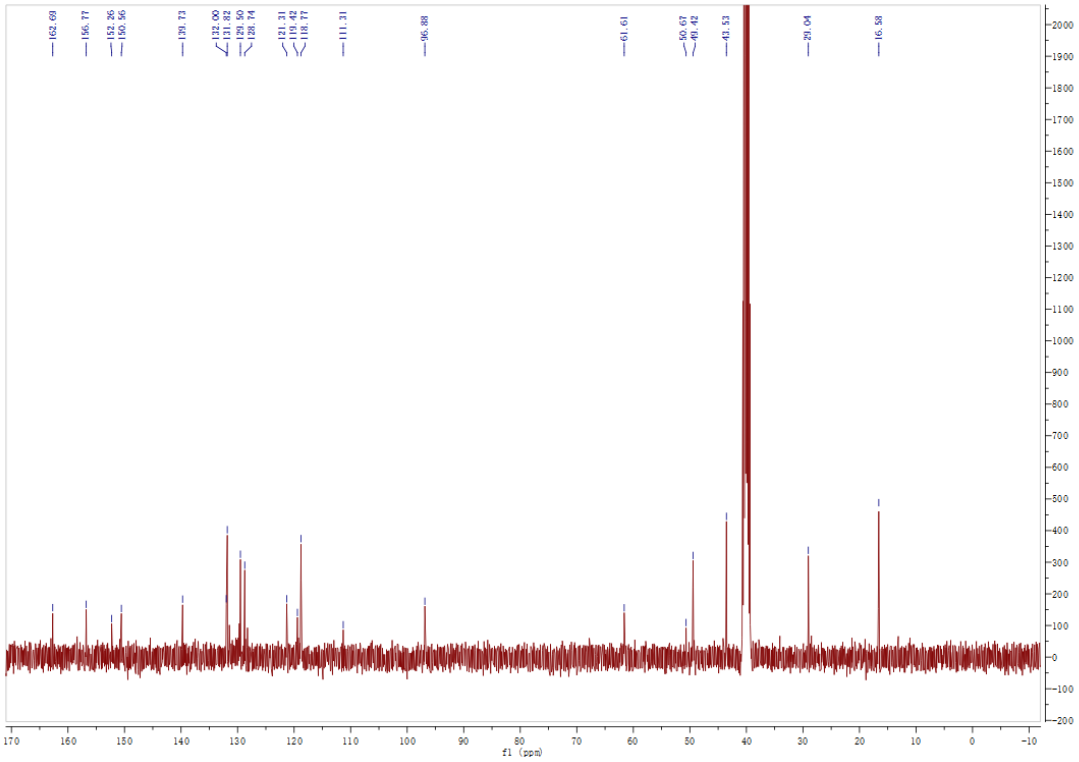
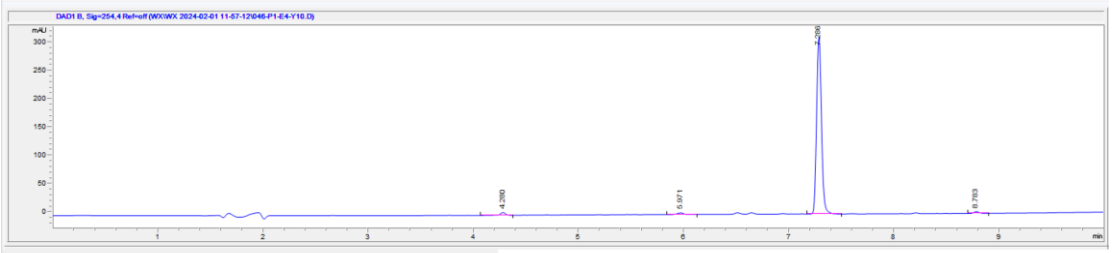
736.3795
763.4252
m/z

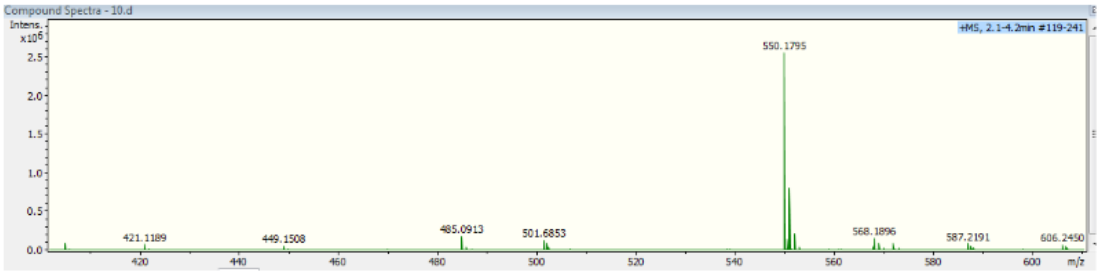

A (d) 8.19
B (s) 7.71
C (d) 7.68
D (d) 7.66
E (d) 7.46
F (s) 7.25
G (m) 3.73
I (s) 3.49
J (s) 2.72
K (s) 2.11
H (s) 2.08
M (m) 1.66
L (tt) 1.86
P (m) 1.75
N (s) 1.39
O (d) 0.87
f1 (ppm)

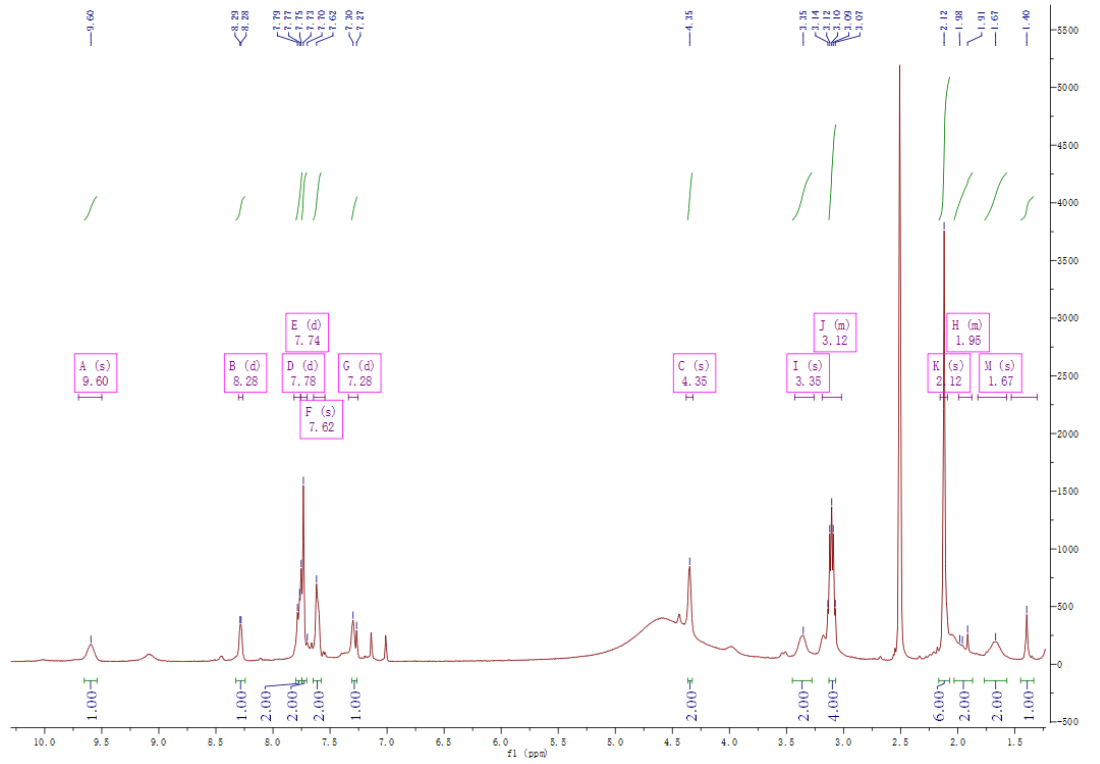


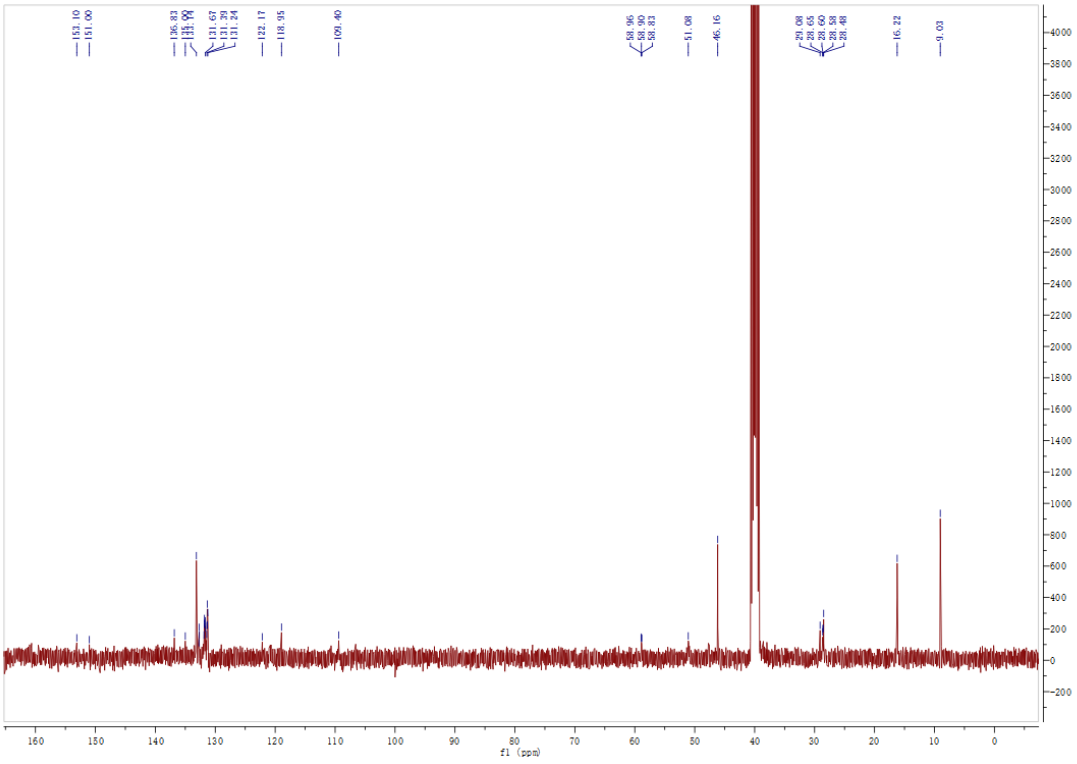


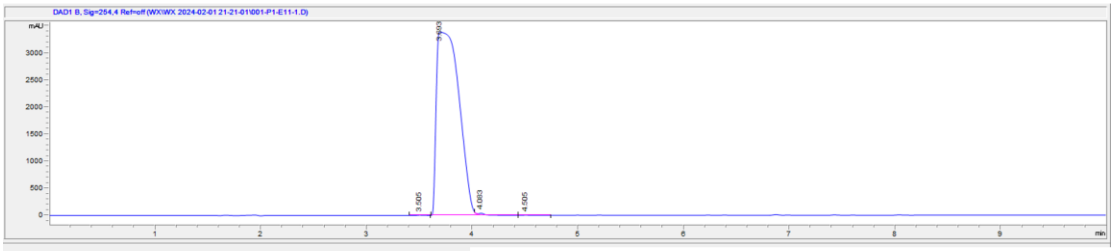
#	时间	峰面积	峰高	峰宽	峰面积 %	对称因子	类型
1	4.28	17.2	5	0.0523	1.627	1.146	BB
2	5.971	12.2	2.9	0.063	1.151	1.216	BB
3	7.286	1018.9	315.7	0.0497	96.167	0.868	BB
4	8.783	11.2	3.2	0.0546	1.054	0.825	BB

(4-((4-((4-(4-cyano-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl)amino)piperidin-1-yl)methyl)phenyl)phosphonic acid (10)



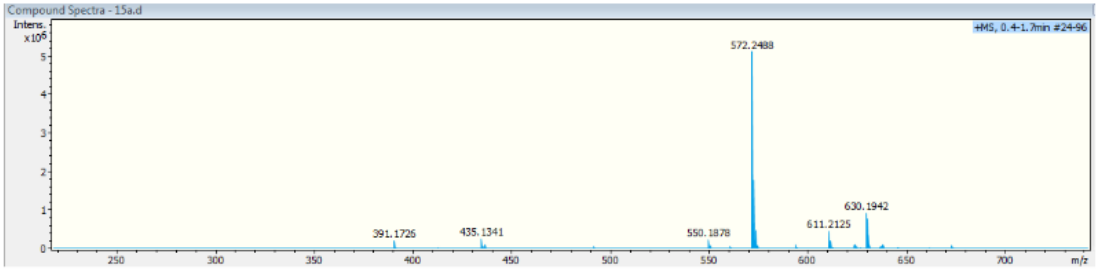


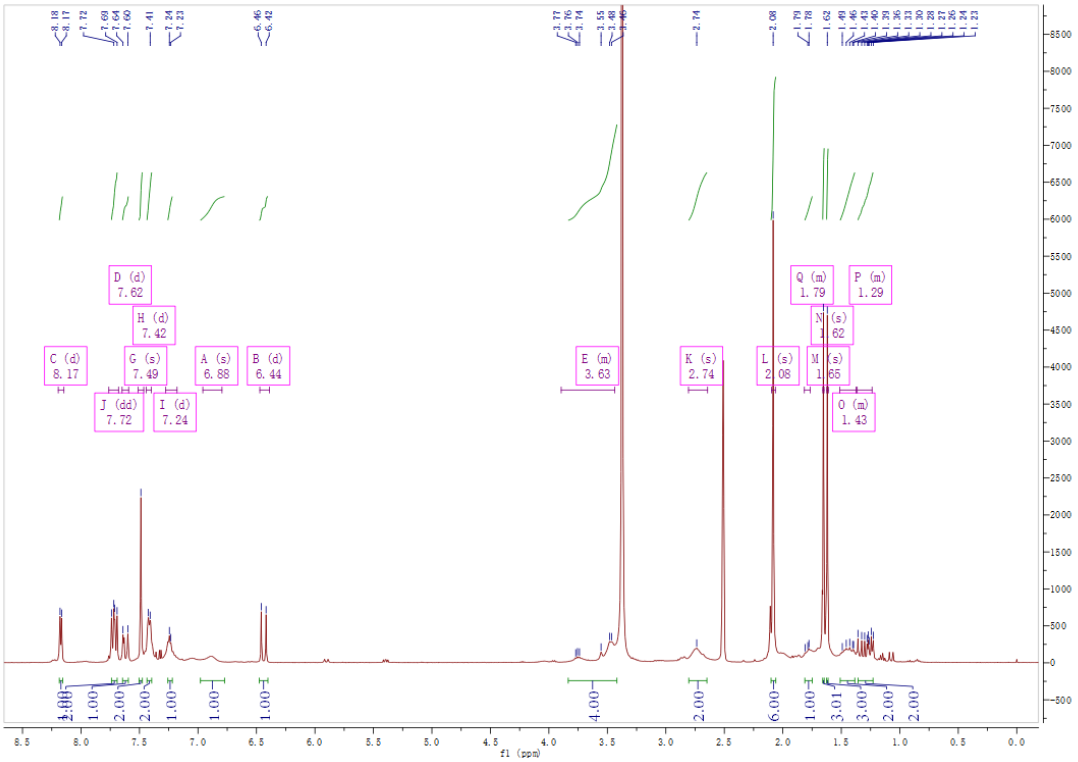


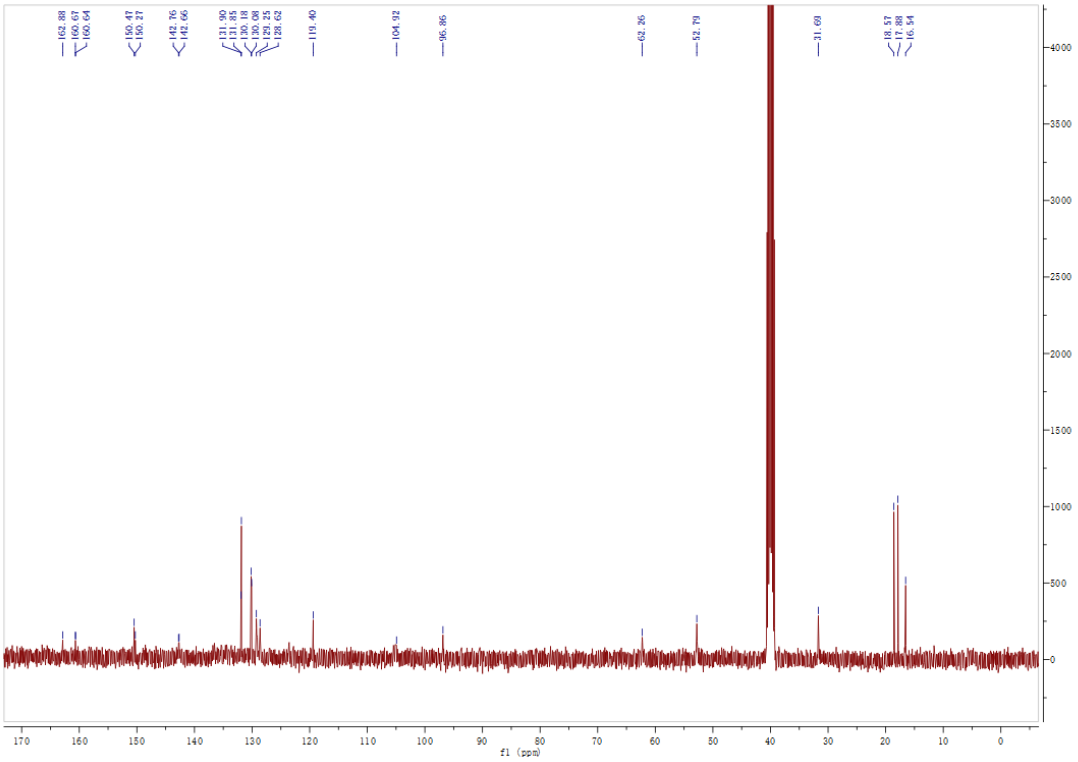


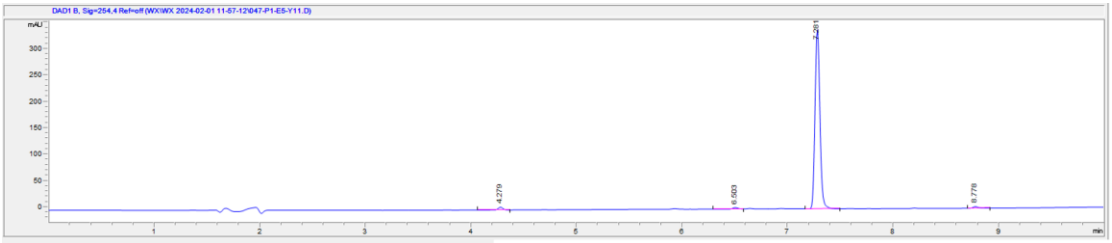
#	时间	峰面积	峰高	峰宽	峰面积 %	对称因子	类型
1	3.505	18.9	5	0.0564	0.037	0.558	BB
2	3.693	50412.9	3395.9	0.2432	99.564	0.198	BB R
3	4.083	181.9	35.9	0.0711	0.359	0.399	VV E
4	4.505	20	3.8	0.0731	0.039	0.718	VB E

(E)-3-(4-((2-((1-(4-(dimethylphosphoryl)benzyl)piperidin-4-yl)amino)thieno[3,2-d]pyrimidin-4-yl)oxy)-3,5-dimethylphenyl)acrylonitrile (15a)



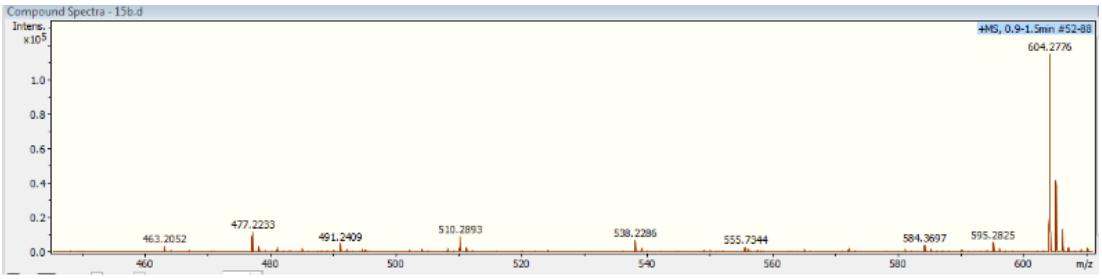


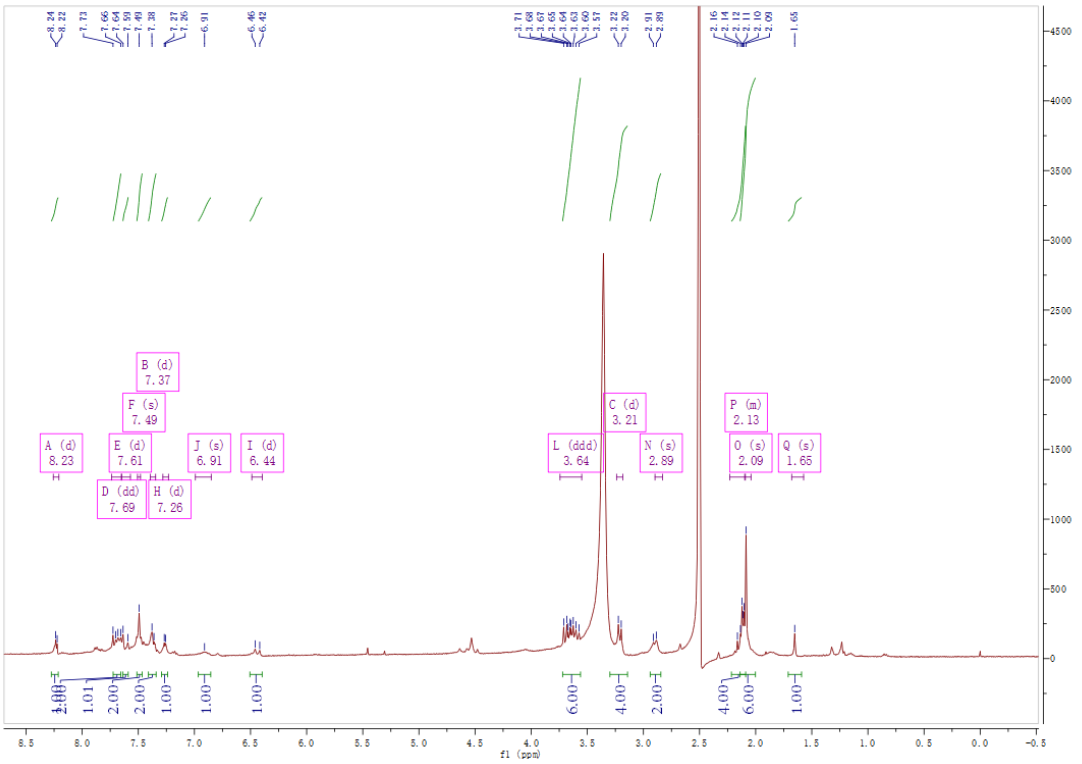


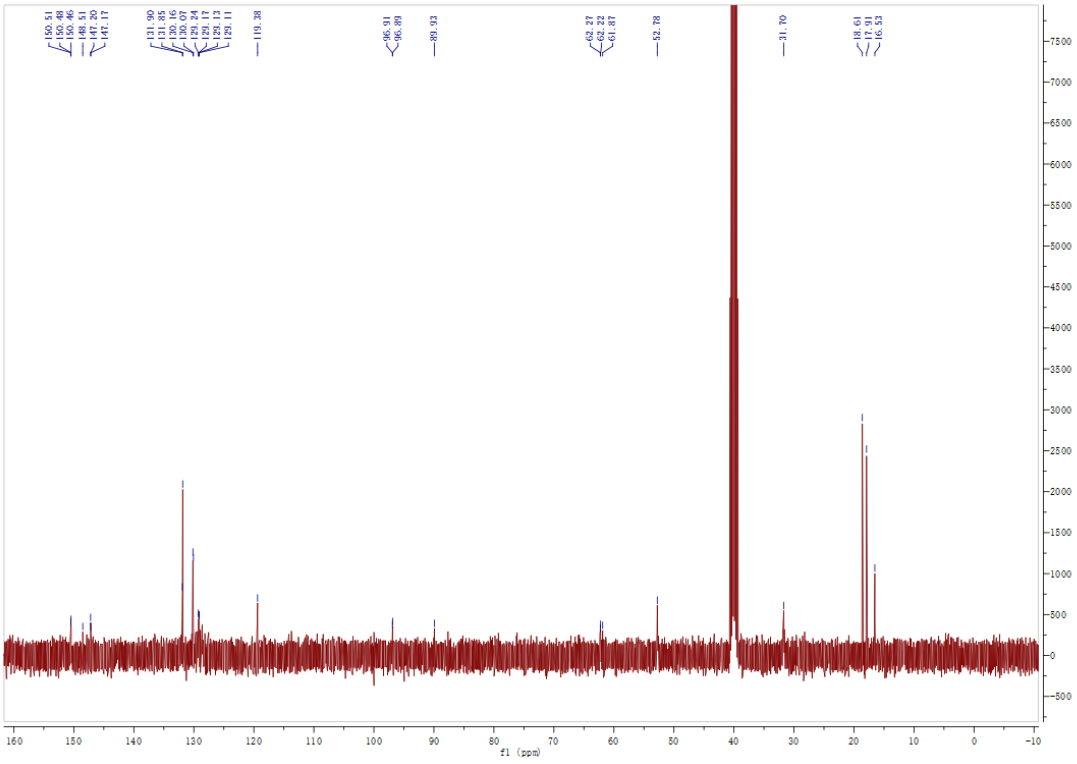


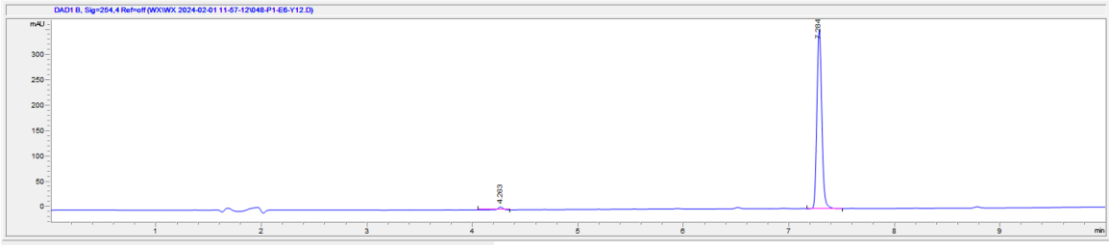
#	时间	峰面积	峰高	峰宽	峰面积 %	对称因子	类型
1	4.279	18.1	5.3	0.052	1.604	1.119	BB
2	6.503	10.5	2.8	0.0575	0.930	0.86	BV
3	7.281	1092.3	339	0.0516	96.533	0.855	BB
4	8.778	10.6	3	0.0536	0.933	0.787	BB

dimethyl (E)-(4-((4-((4-(4-(2-cyanovinyl)-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl)amino)piperidin-1-yl)methyl)phenyl)phosphonate (15b)



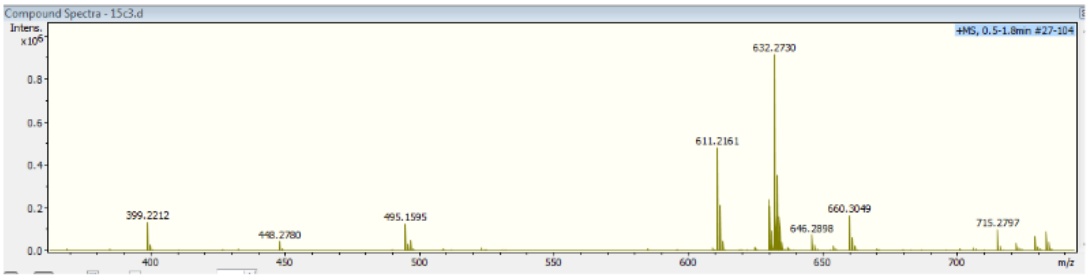


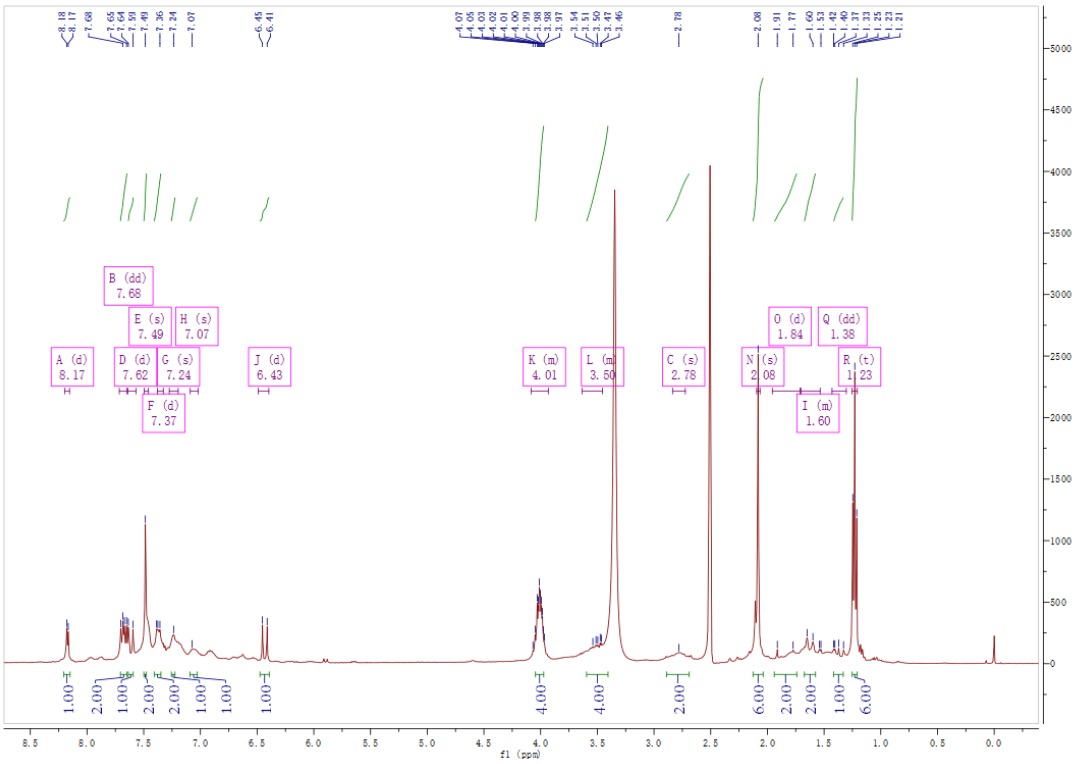


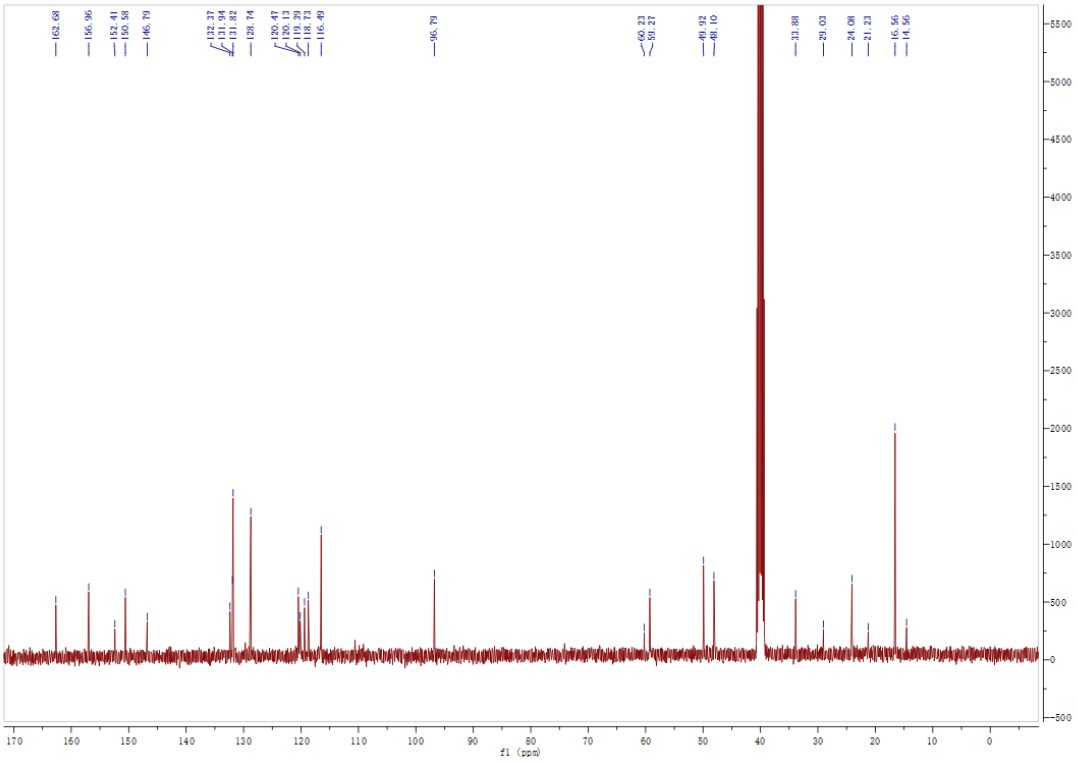


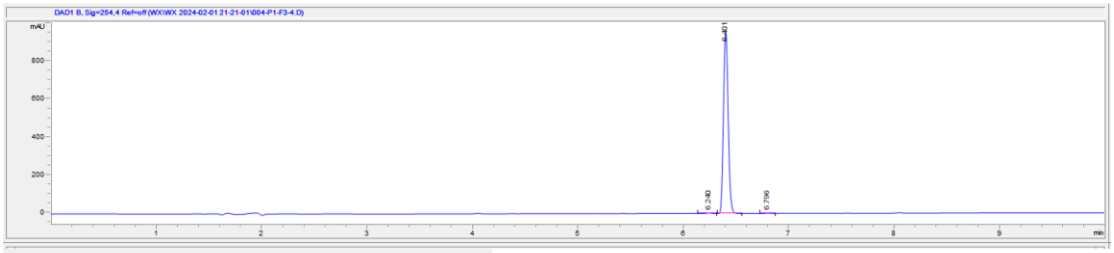
#	时间	峰面积	峰高	峰宽	峰面积 %	对称因子	类型
1	4.263	18.3	5.5	0.0511	1.579	1.068	BB R
2	7.284	1138	354.1	0.0515	98.421	0.856	BB

diethyl (E)-(4-((4-((4-(4-(2-cyanovinyl)-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl)amino)piperidin-1-yl)methyl)phenyl)phosphonate (15c)



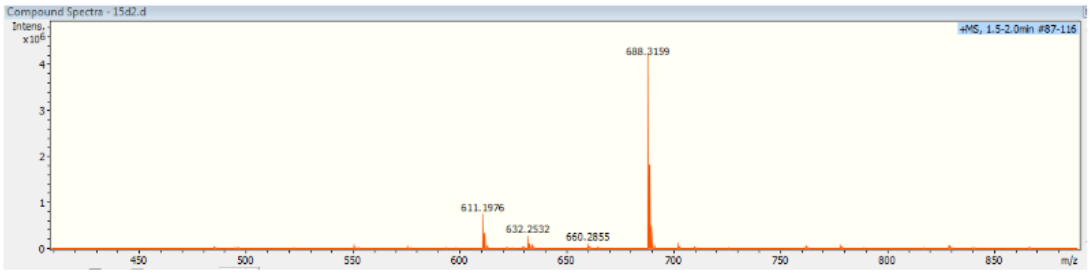


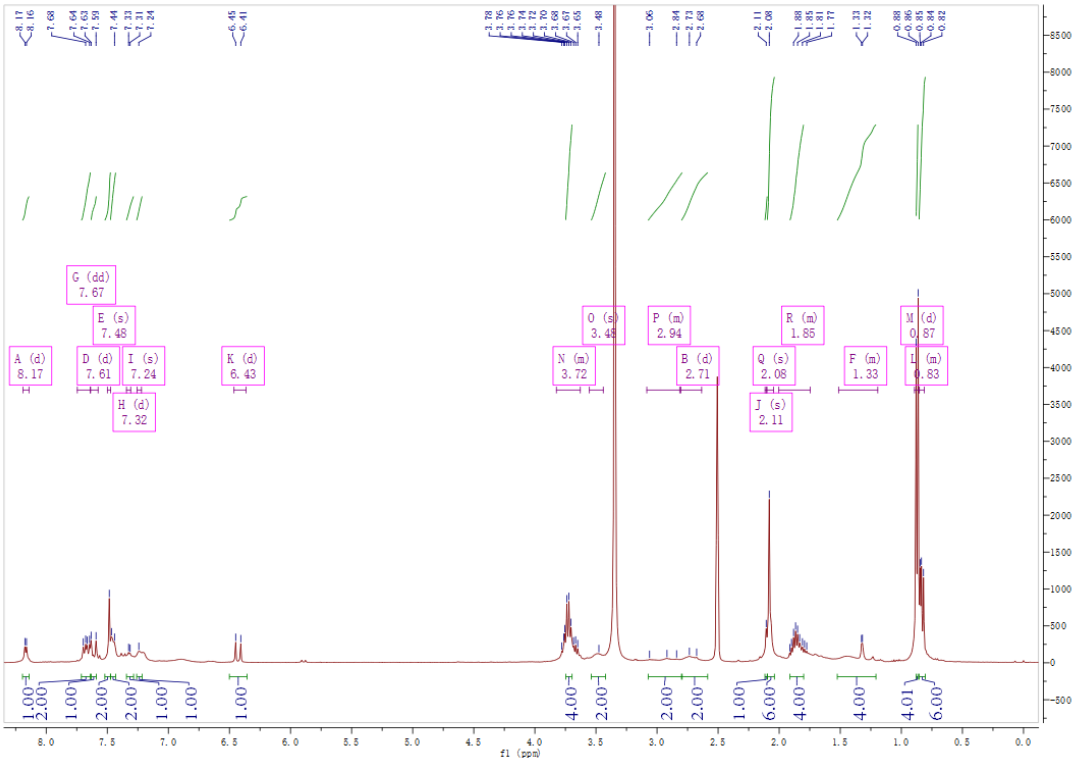


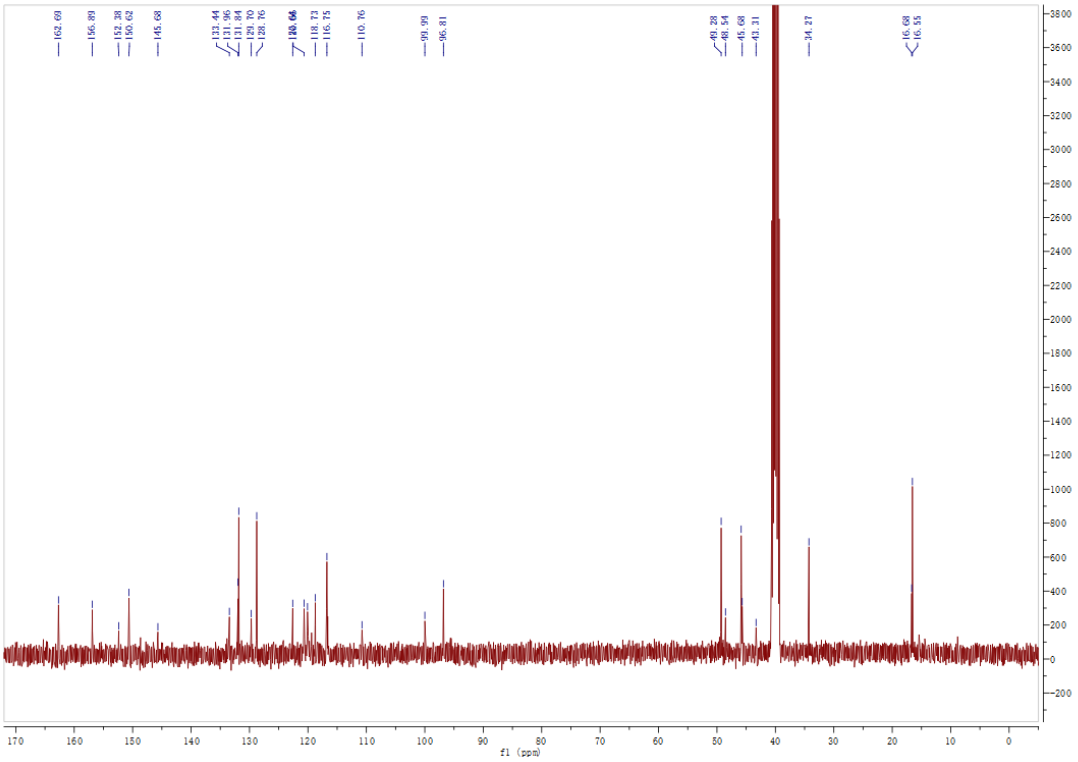


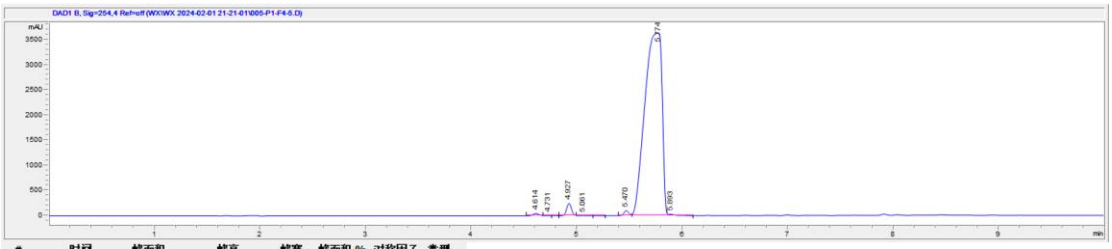
#	时间	峰面积	峰高	峰宽	峰面积 %	对称因子	类型
1	6.24	10.4	3	0.0531	0.350	1.077	BB
2	6.401	2958.3	959.3	0.048	99.470	0.894	BB
3	6.796	5.3	1.6	0.0518	0.179	0.859	BB

dibutyl (E)-(4-((4-((4-(4-(2-cyanovinyl)-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl)amino)piperidin-1-yl)methyl)phenyl)phosphonate (15d)



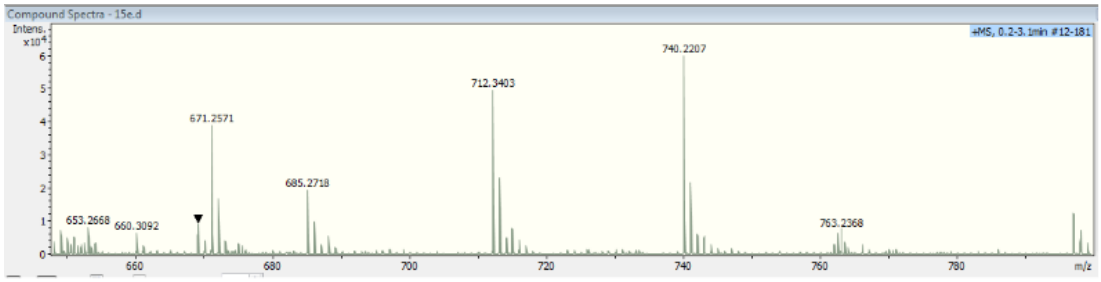


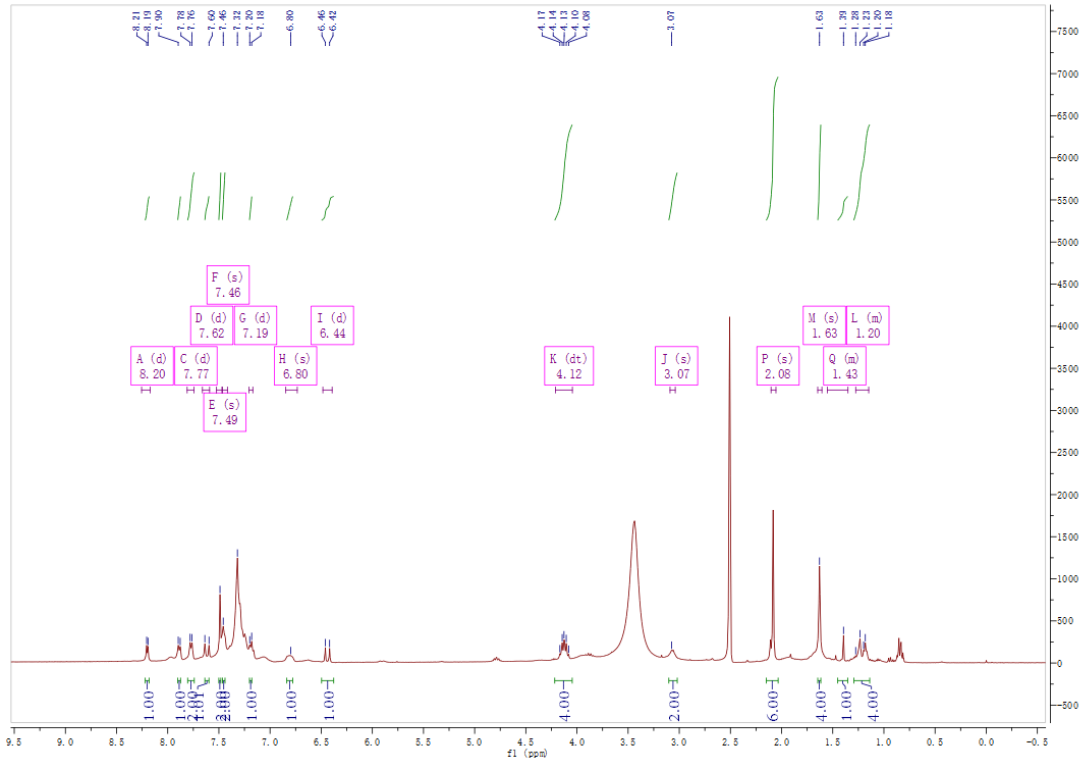


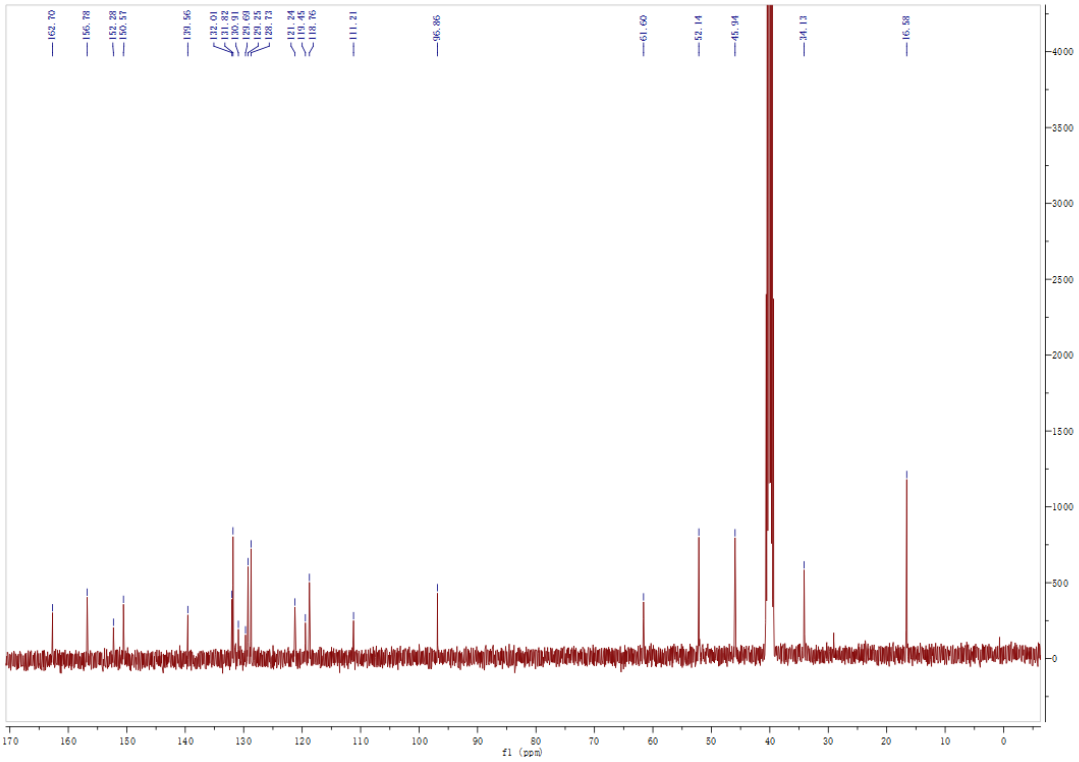


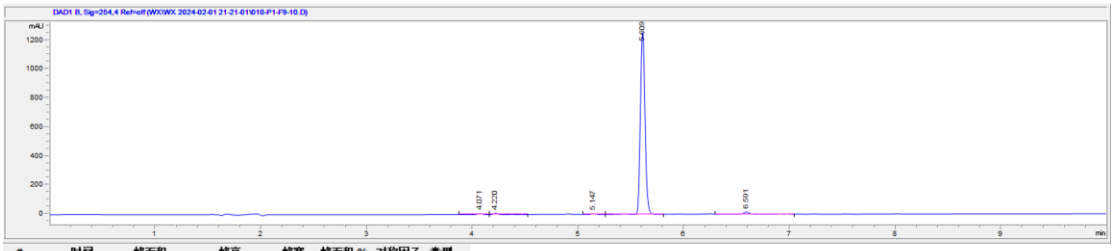
#	时间	峰面积	峰高	峰宽	峰面积 %	对称因子	类型
1	4.614	169.9	44.4	0.0606	0.398	0.882	BB R
2	4.731	5.3	1.9	0.0427	0.012	0.868	VV E
3	4.927	792.1	244.9	0.0497	1.856	0.91	BB R
4	5.061	18.3	4	0.0656	0.043	0.528	VV E
5	5.47	305.8	99.7	0.0498	0.716	0.983	BV E
6	5.774	41301.3	3646.3	0.1861	96.745	3.359	BB R
7	5.893	98.3	24.5	0.0568	0.230	0.434	VB E

bis(2,2,2-trifluoroethyl) (E)-(4-((4-((4-(4-(2-cyanovinyl)-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl)amino)piperidin-1-yl)methyl)phenyl)phosphonate (15e)



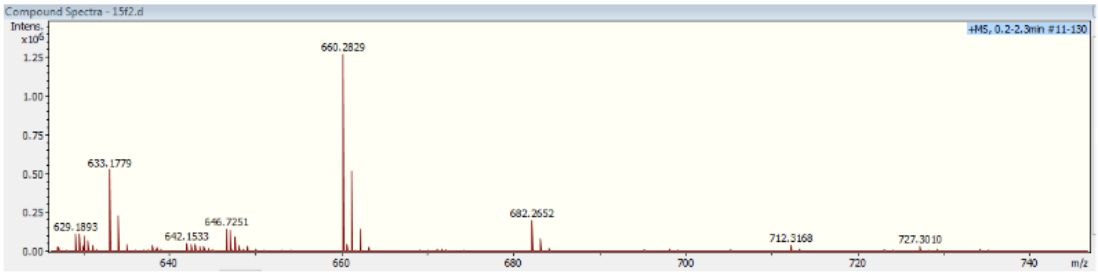


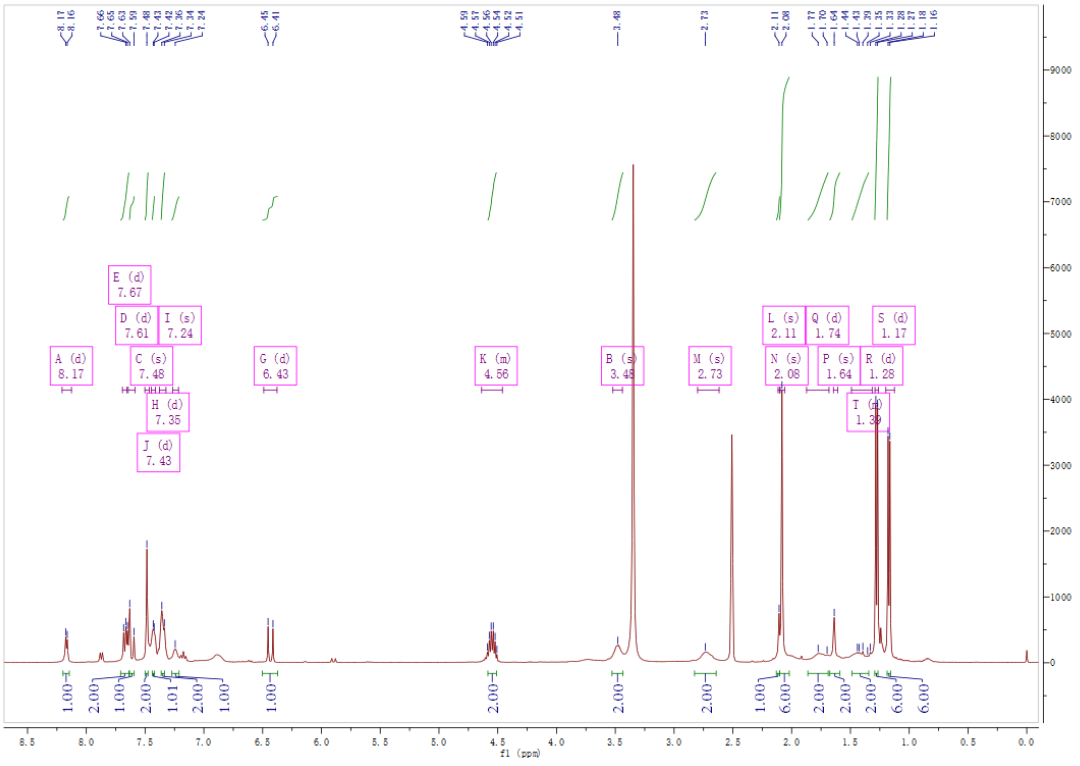


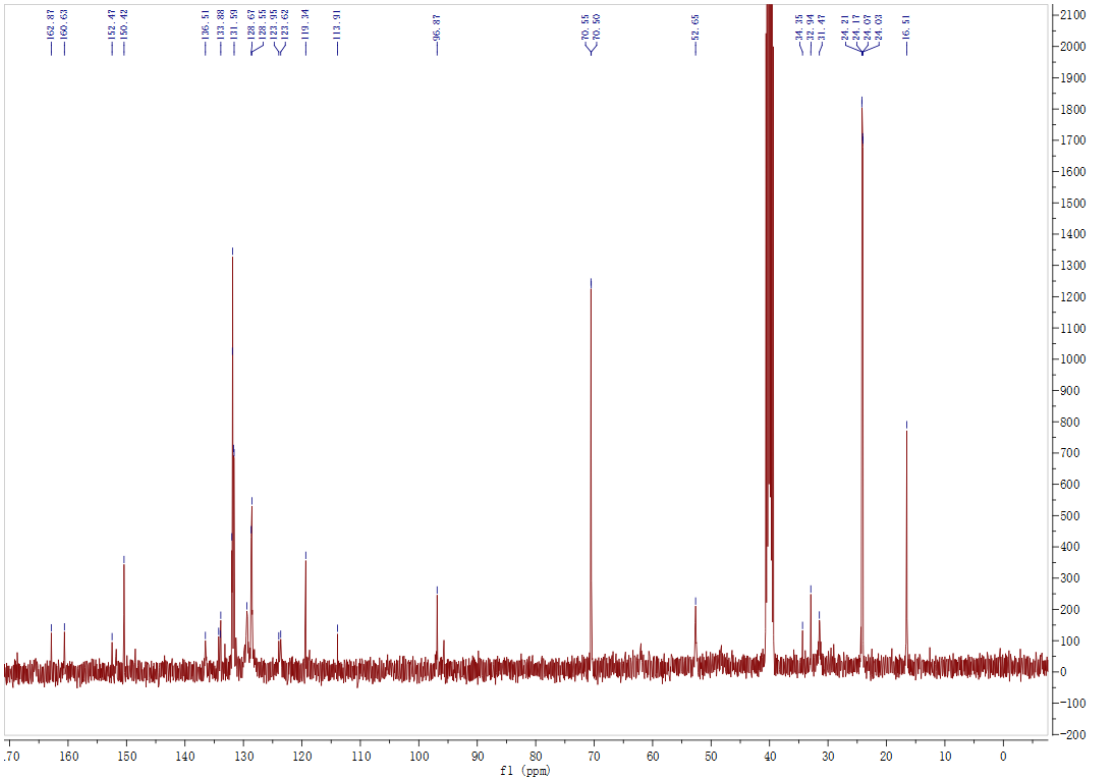


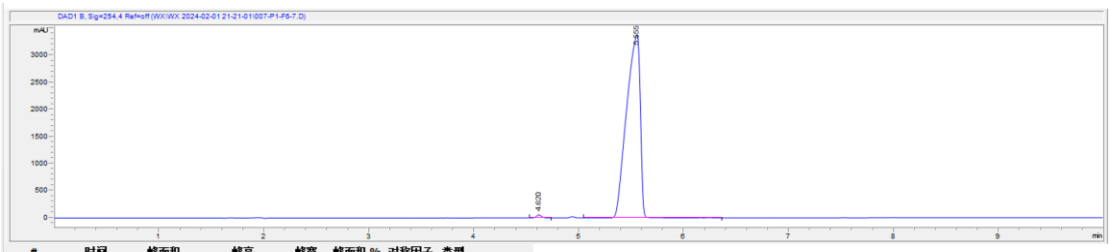
#	时间	峰面积	峰高	峰宽	峰面积 %	对称因子	类型
1	4.071	24.3	6.3	0.0632	0.606	0.958	BV
2	4.22	29.8	9.1	0.0504	0.741	0.704	VB R
3	5.147	17.2	5.5	0.0486	0.429	0.824	BB
4	5.609	3867.4	1264.4	0.0475	96.201	0.909	BB R
5	6.591	81.4	15.6	0.0689	2.024	0.728	BB R

diisopropyl (E)-(4-((4-((4-(4-(2-cyanovinyl)-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl)amino)piperidin-1-yl)methyl)phenyl)phosphonate (15f)



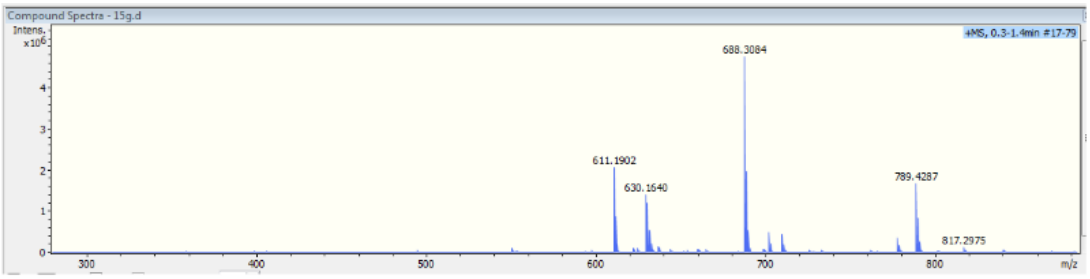


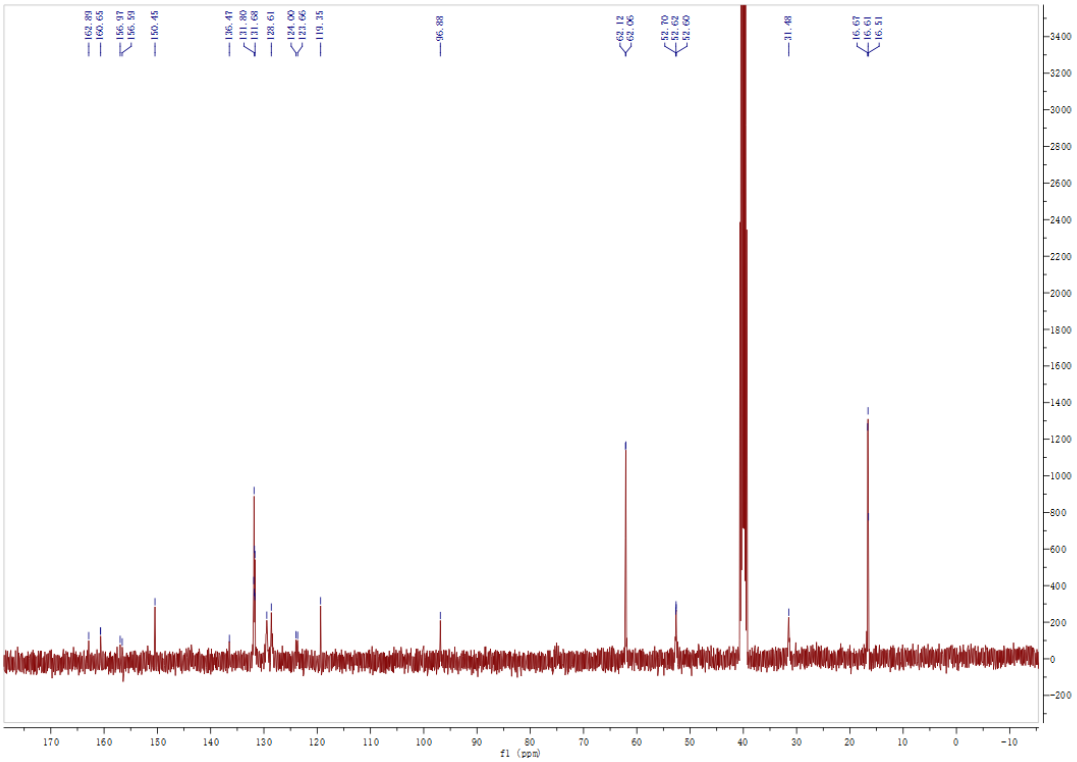


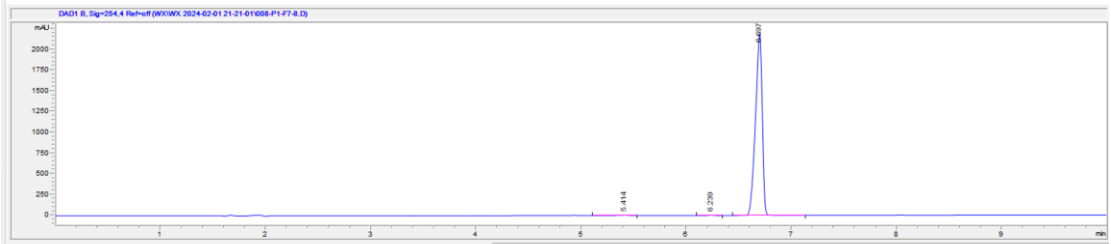


#	时间	峰面积	峰高	峰宽	峰面积 %	对称因子	类型
1	4.62	193.3	55.9	0.0544	0.631	0.905	BV
2	5.555	30463.2	3374.8	0.1573	99.369	2.987	BB R

di-tert-butyl (E)-(4-((4-((4-(4-(2-cyanovinyl)-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl)amino)piperidin-1-yl)methyl)phenyl)phosphonate (15g)

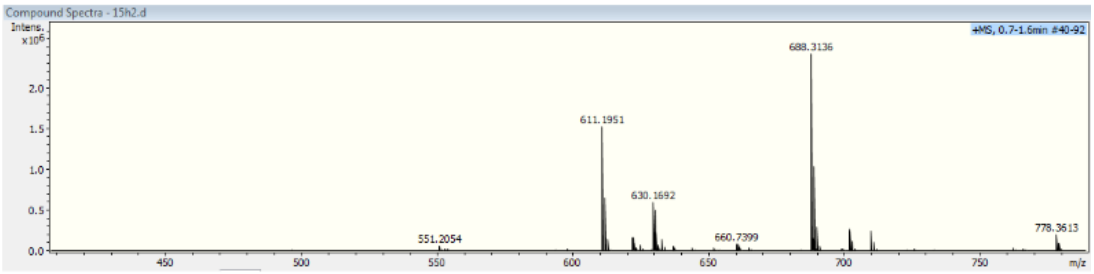


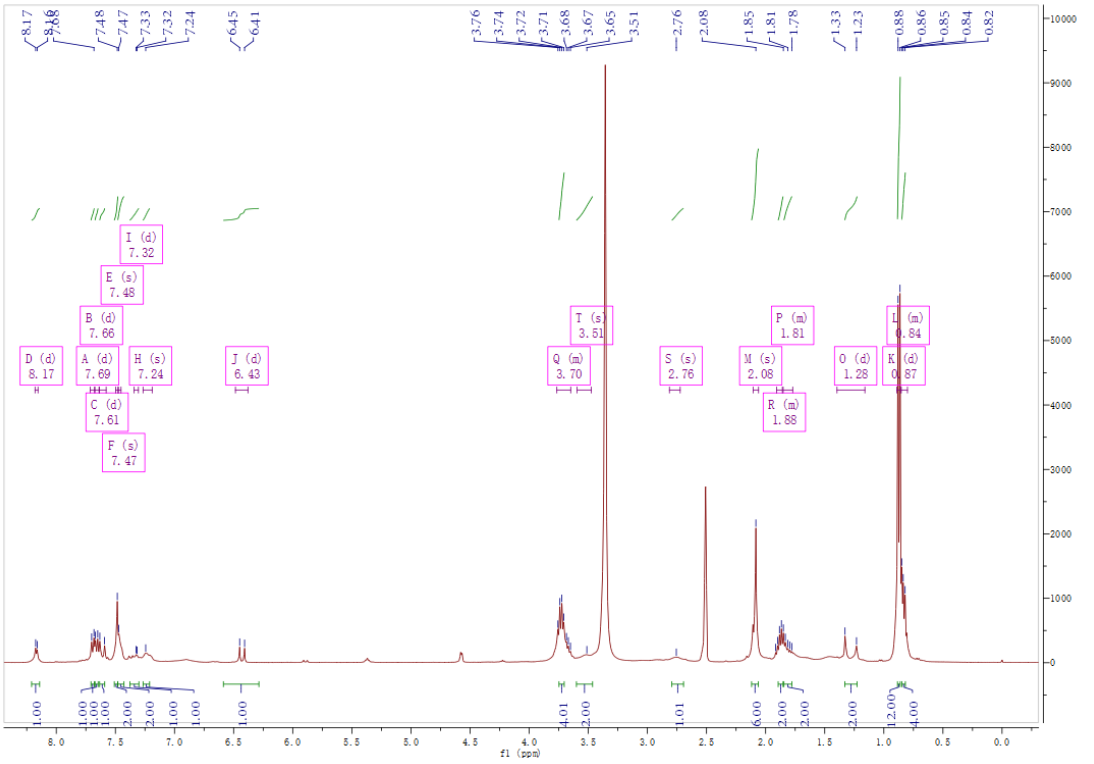


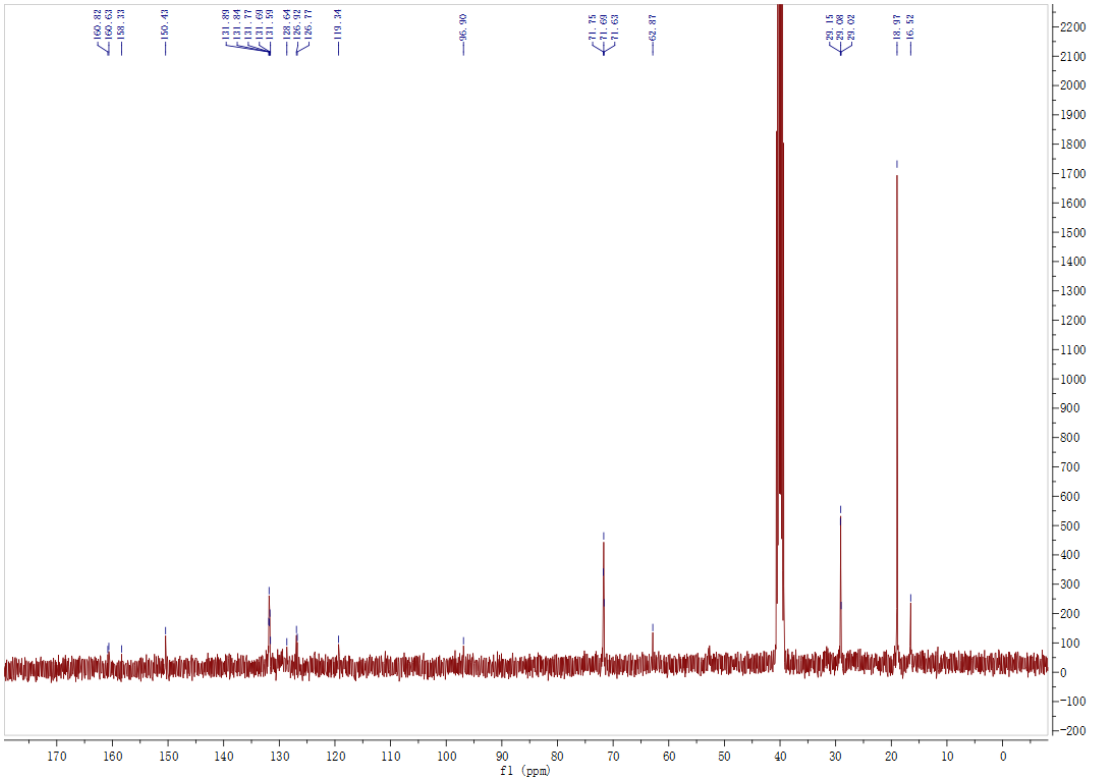


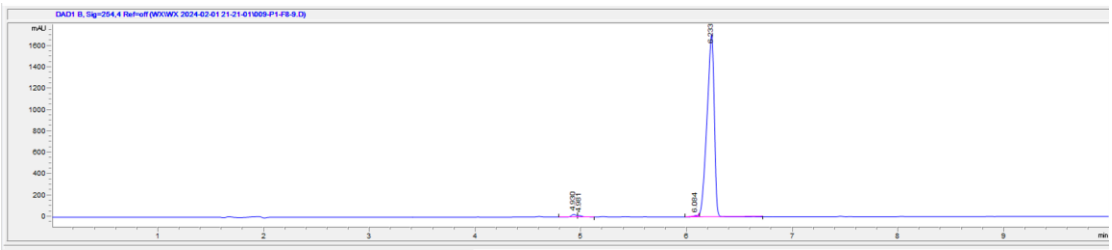
#	时间	峰面积	峰高	峰宽	峰面积 %	对称因子	类型
1	5.414	28.2	3.9	0.1009	0.286	1.657	BV R
2	6.239	13	3.3	0.0598	0.131	1.302	BB R
3	6.697	9823.9	2201	0.0681	99.582	1.499	BB R

diisobutyl (E)-(4-((4-((4-(4-(2-cyanovinyl)-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl)amino)piperidin-1-yl)methyl)phenyl)phosphonate (15h)



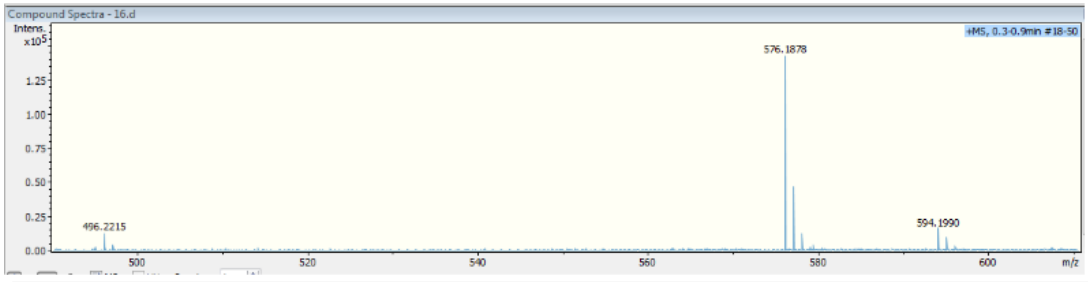


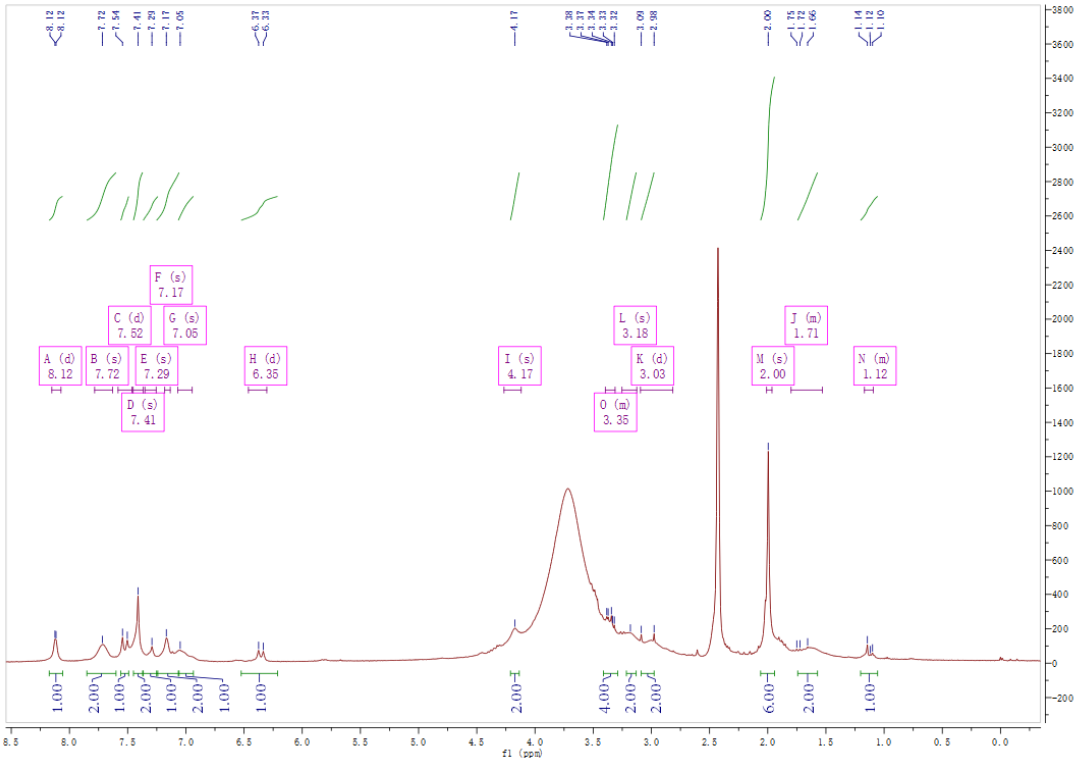


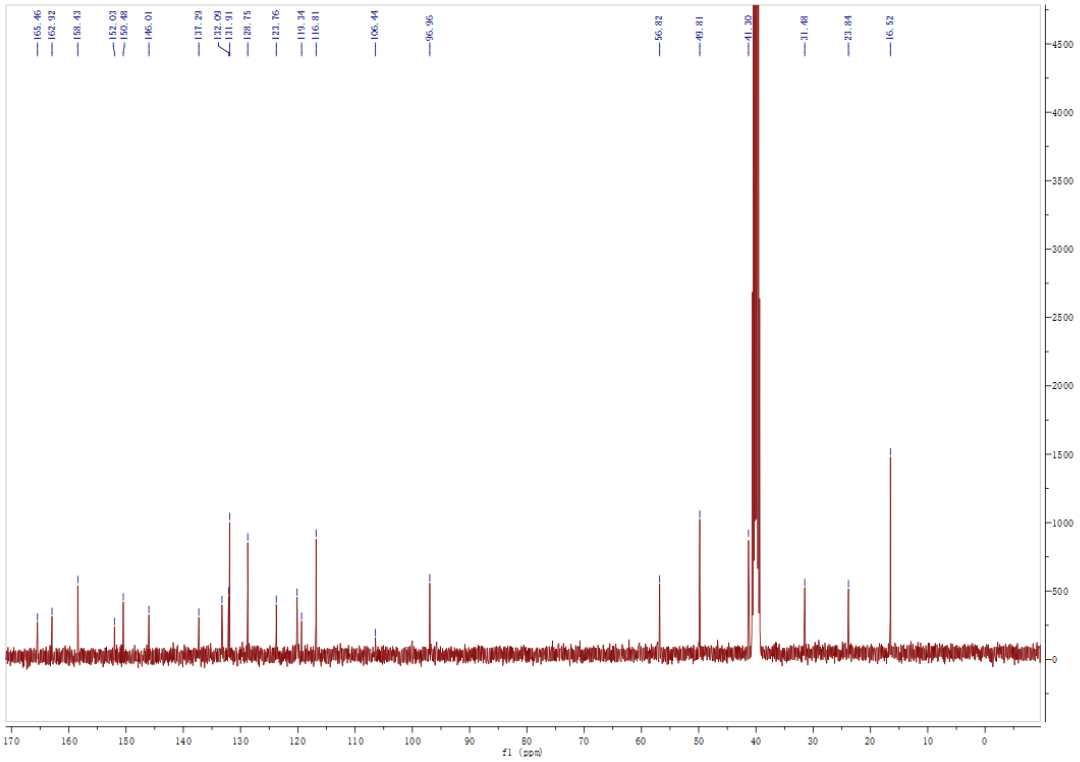


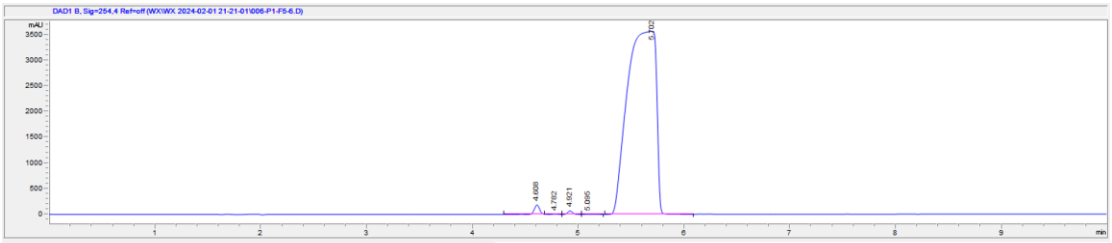
#	时间	峰面积	峰高	峰宽	峰面积 %	对称因子	类型
1	4.93	83	24.9	0.0489	0.989	0.88	BV
2	4.981	39.3	13.4	0.049	0.469	0.266	VV
3	6.084	32	11.9	0.0395	0.382	1.641	BV E
4	6.233	8230.8	1718.4	0.0718	98.160	1.646	BB R

(E)-(4-((4-((4-(4-(2-cyanovinyl)-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl)amino)piperidin-1-yl)methyl)phenyl)phosphonic acid (16)



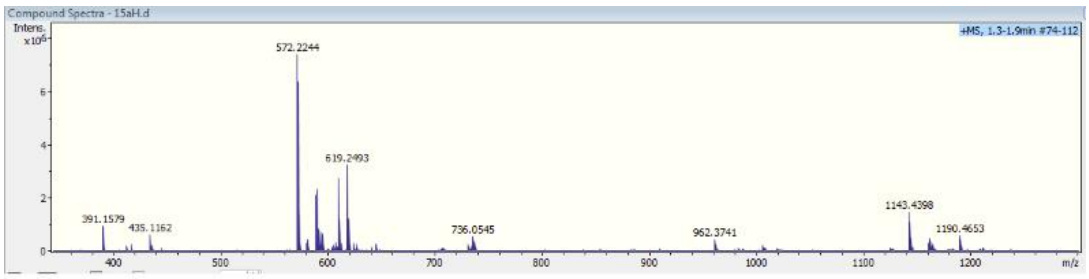


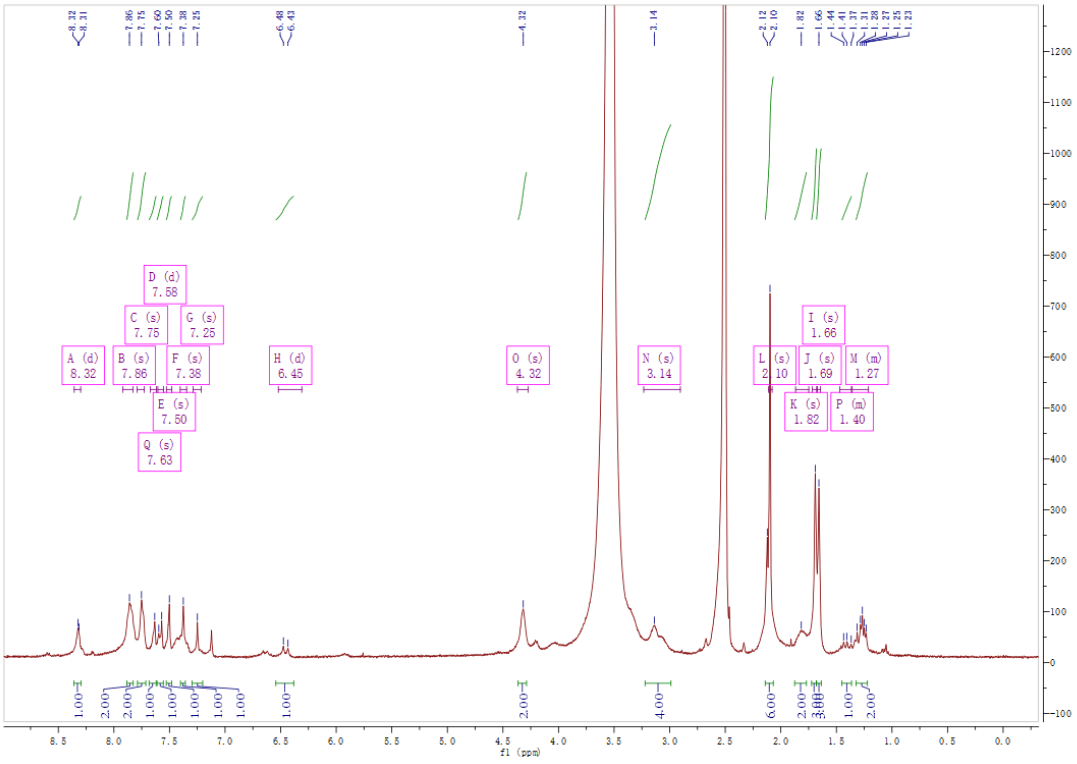


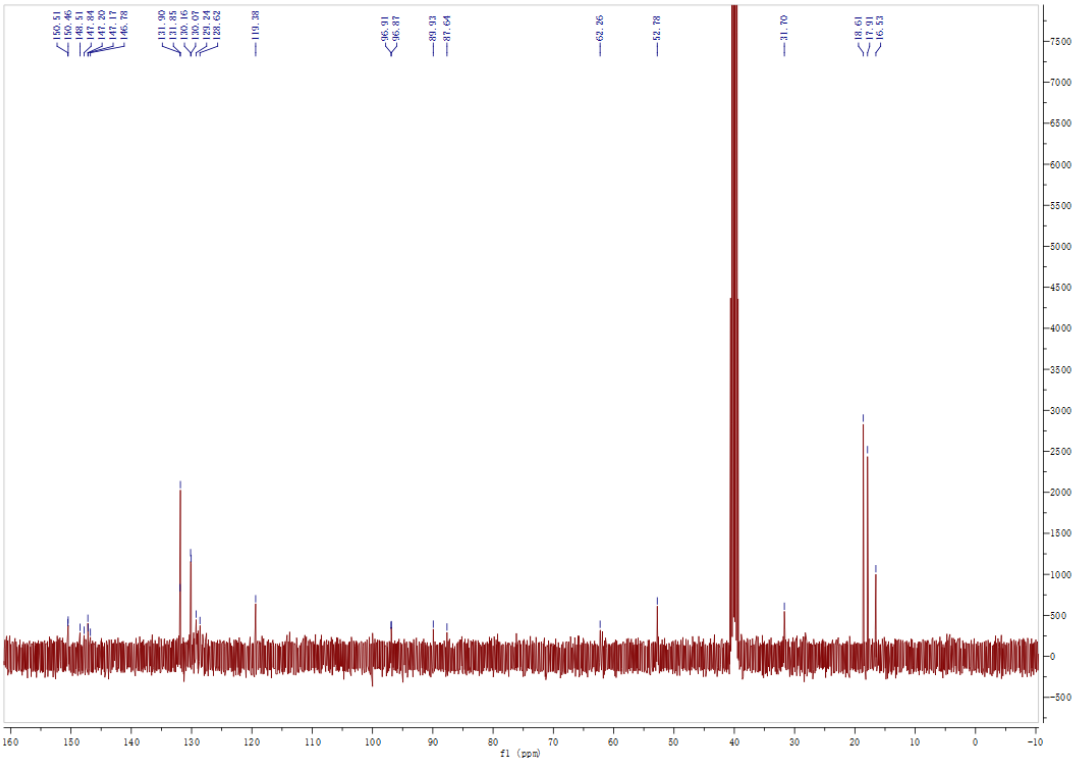


#	时间	峰面积	峰高	峰宽	峰面积 %	对称因子	类型
1	4.608	652.8	185.6	0.0546	0.952	1.003	BV R
2	4.782	20.7	6.7	0.048	0.030	0.963	VV E
3	4.921	216.6	67.6	0.0514	0.316	0.889	VB
4	5.095	6.8	1.6	0.0626	0.010	0.484	BB
5	5.702	67672	3585.8	0.2522	98.692	5.583	BB

15a·HCl




A (d) 8.32
B (s) 7.86
C (s) 7.75
D (d) 7.58
E (s) 7.50
F (s) 7.38
G (s) 7.25
H (d) 6.45
O (s) 4.32
N (s) 3.14
L (s) 2.10
J (s) 1.69
I (s) 1.66
M (m) 1.27
K (s) 1.82
P (m) 1.40
Q (s) 7.63
f1 (ppm)


150.51
150.46
146.51
145.94
142.20
141.17
136.78
131.90
131.85
130.16
130.07
129.24
128.62
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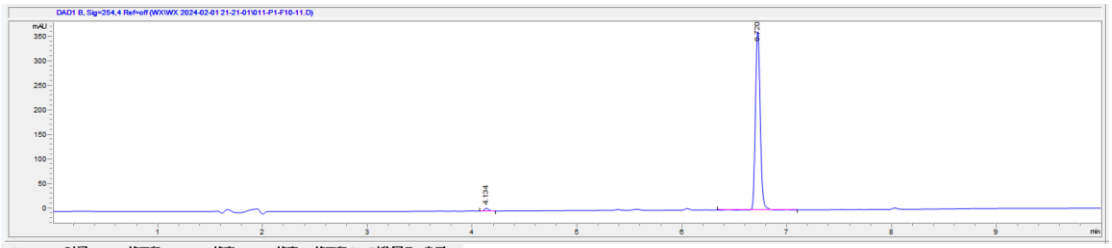
17.91
16.53
f1 (ppm)



#	时间	峰面积	峰高	峰宽	峰面积 %	对称因子	类型
1	4.134	16.7	5.5	0.0473	1.417	0.907	VB
2	6.72	1161.8	365.9	0.049	98.583	0.873	BB R