

Supporting Information

Synthesis and Biological Evaluation of Benzo[*a*]phenazin-5(7*H*)-one Derivatives as a Dual Inhibitor of Topoisomerase I and II

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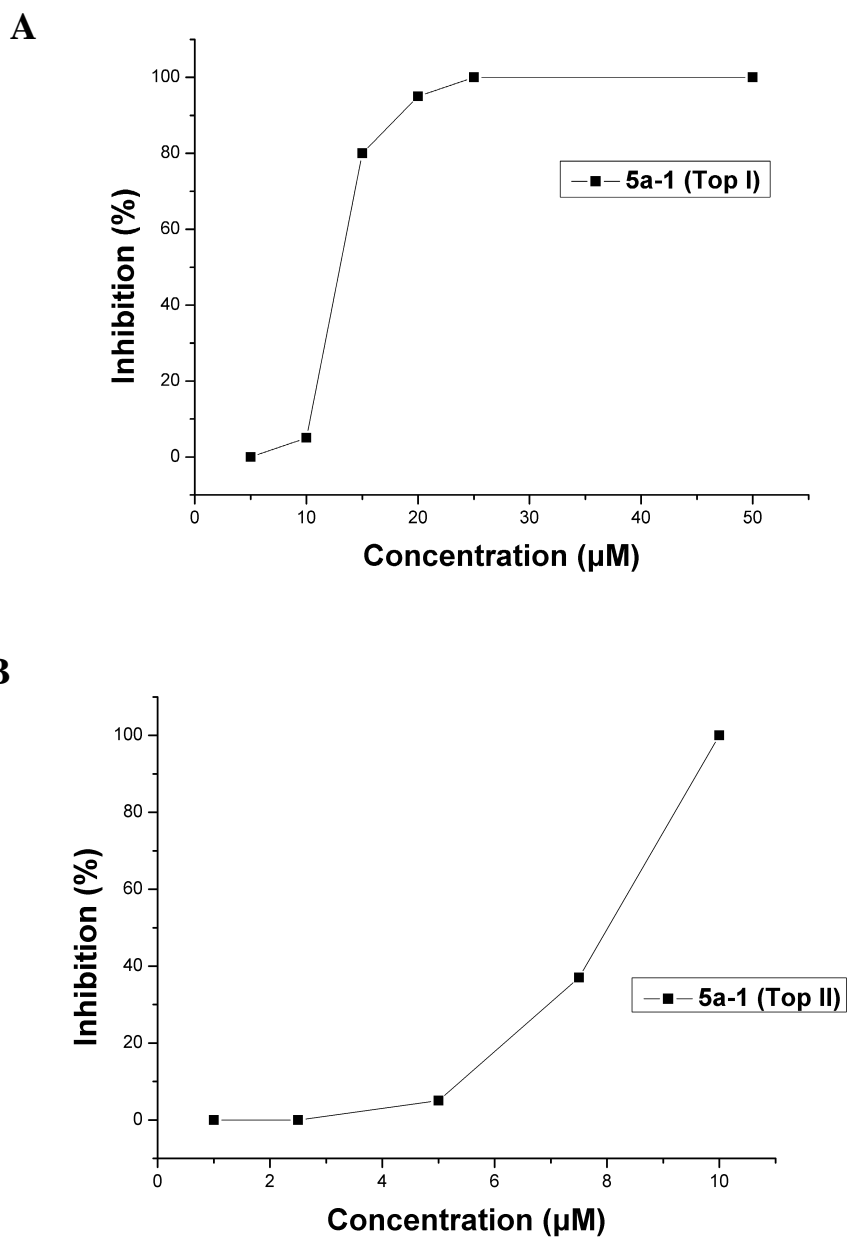


Fig. S1 A and B, Quantitates the effects of **5a-1** on DNA relaxation catalyzed by Topo I and Topo II respectively. The fit data gave IC_{50} values of 12.5 μM and 8.2 μM for Topo I inhibition and Topo II inhibition respectively.

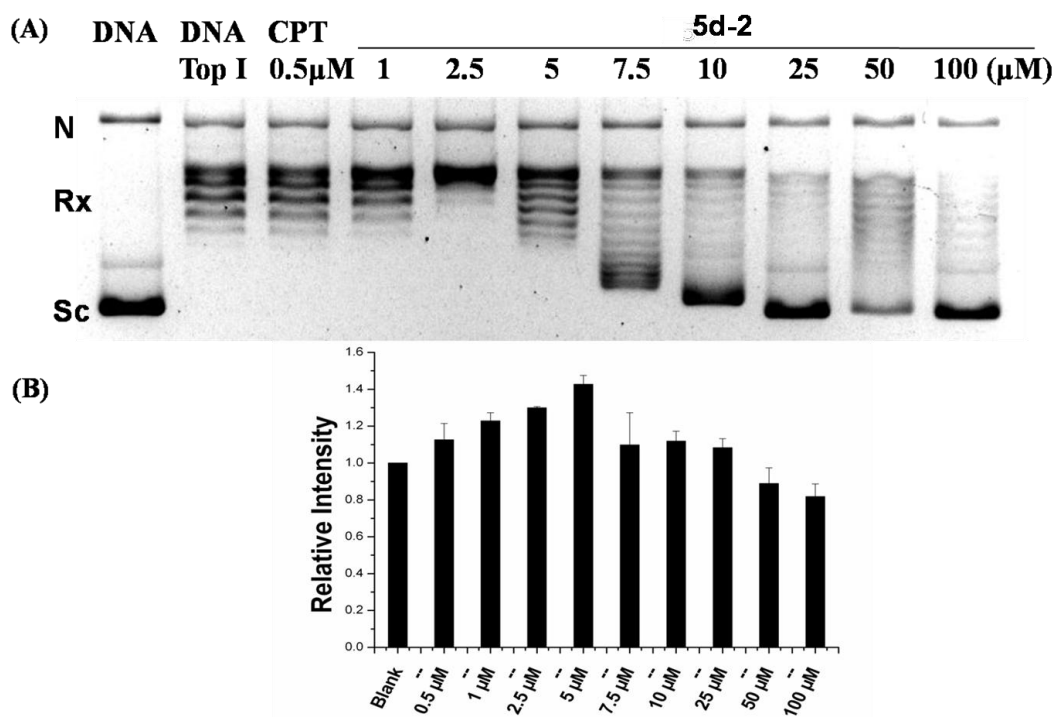


Fig. S2 Effects of **5d-2** on topoisomerase I-DNA cleavage complexes formation at various concentrations.

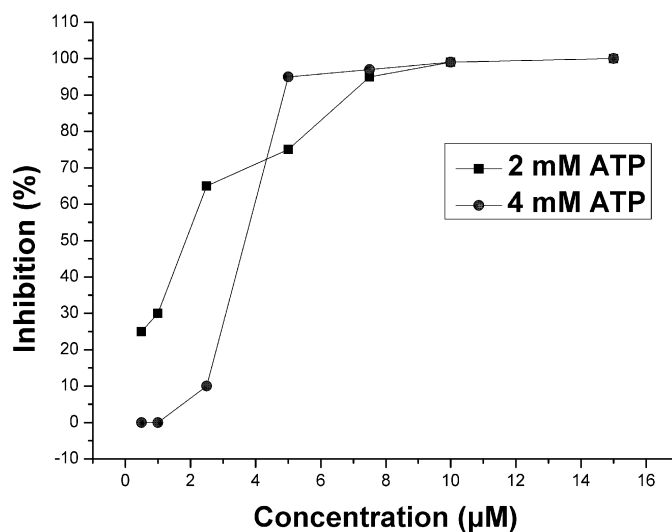


Figure S3. Quantitates the effects of **5d-2** on DNA relaxation catalyzed by Topo II at 2 mM and 4 mM ATP respectively. The fit data gave IC_{50} values of 2.0 μ M and 3.3 μ M for reaction containing 2 mM and 4 mM ATP respectively.

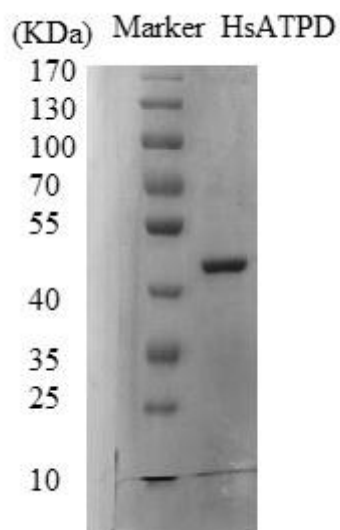


Fig. S4 Identification of HsATPase. 2 μg each of molecular size markers and purified HsATPase were run in a 10% SDS-polyacrylamide gel electrophoresis and stained with Coomassie brilliant blue.

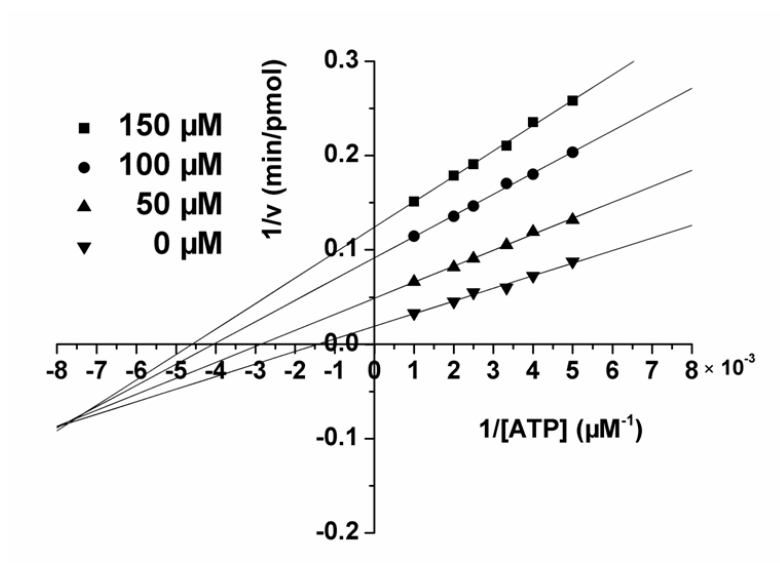


Fig. S5 The double reciprocal Lineweaver-Burk plot for ATP-hydrolysis activity of ATPase in the absence of DNA.

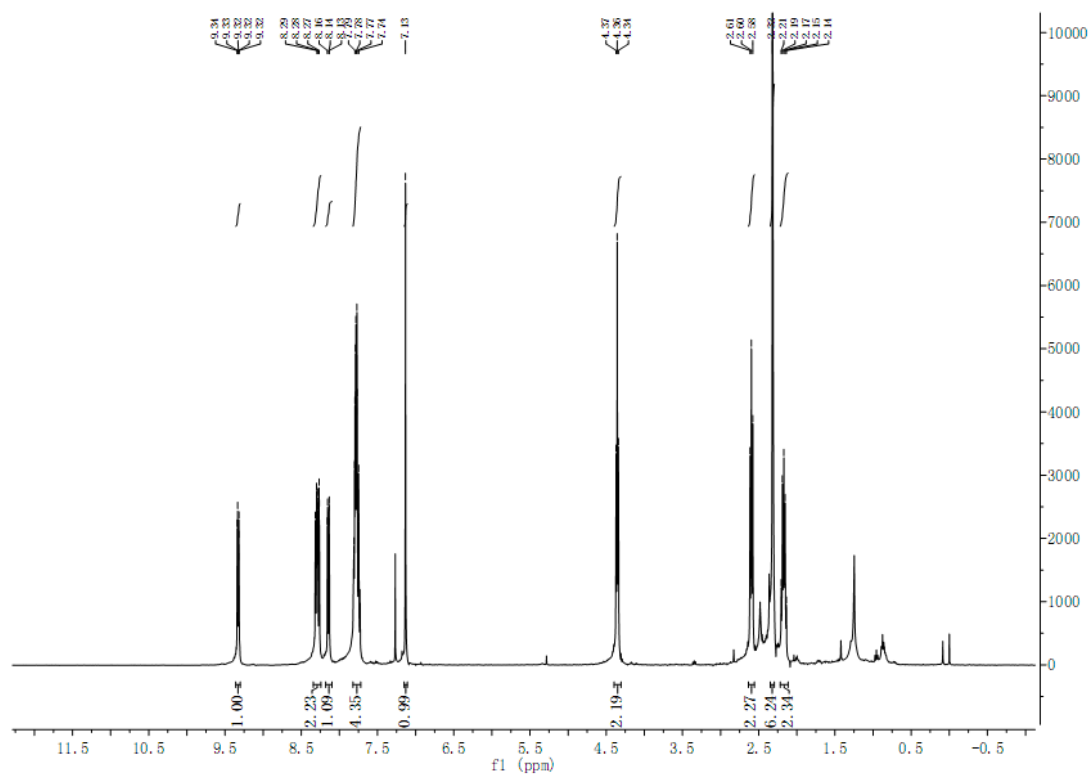


Figure S6-1. ^1H NMR spectra of compound **5a-1** (400 MHz, CDCl_3)

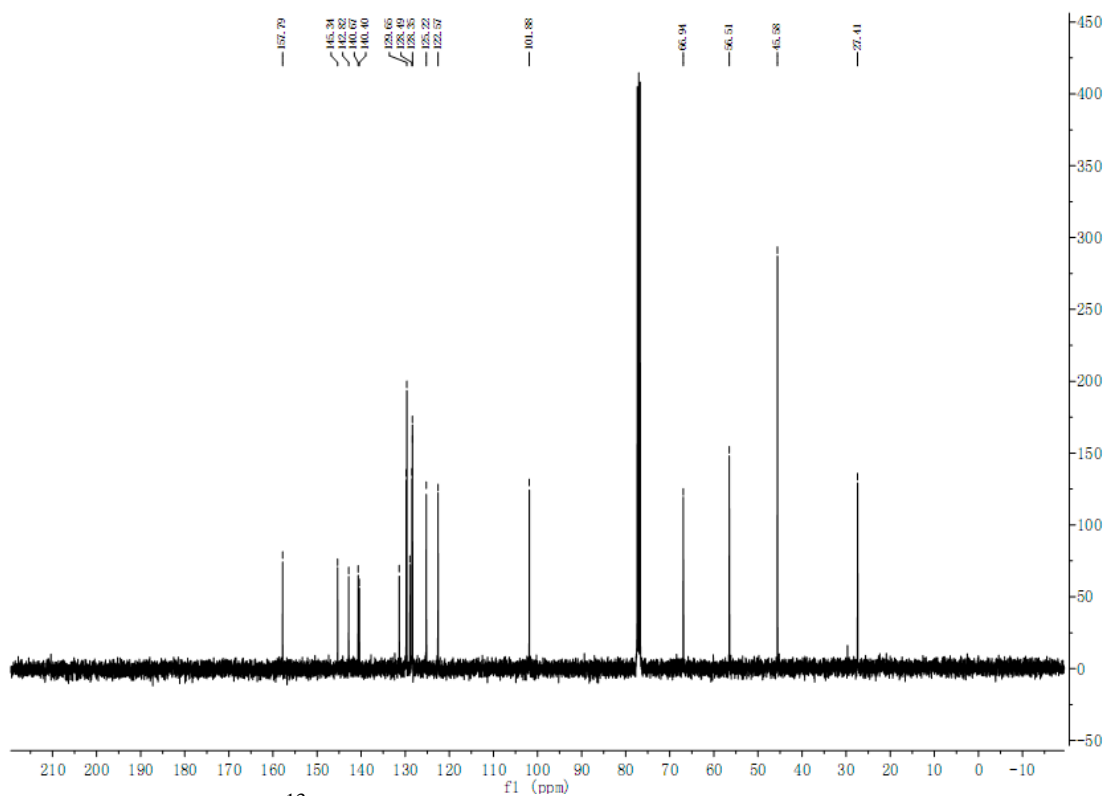


Figure S6-2. ^{13}C NMR spectra of compound **5a-1** (101 MHz, CDCl_3)

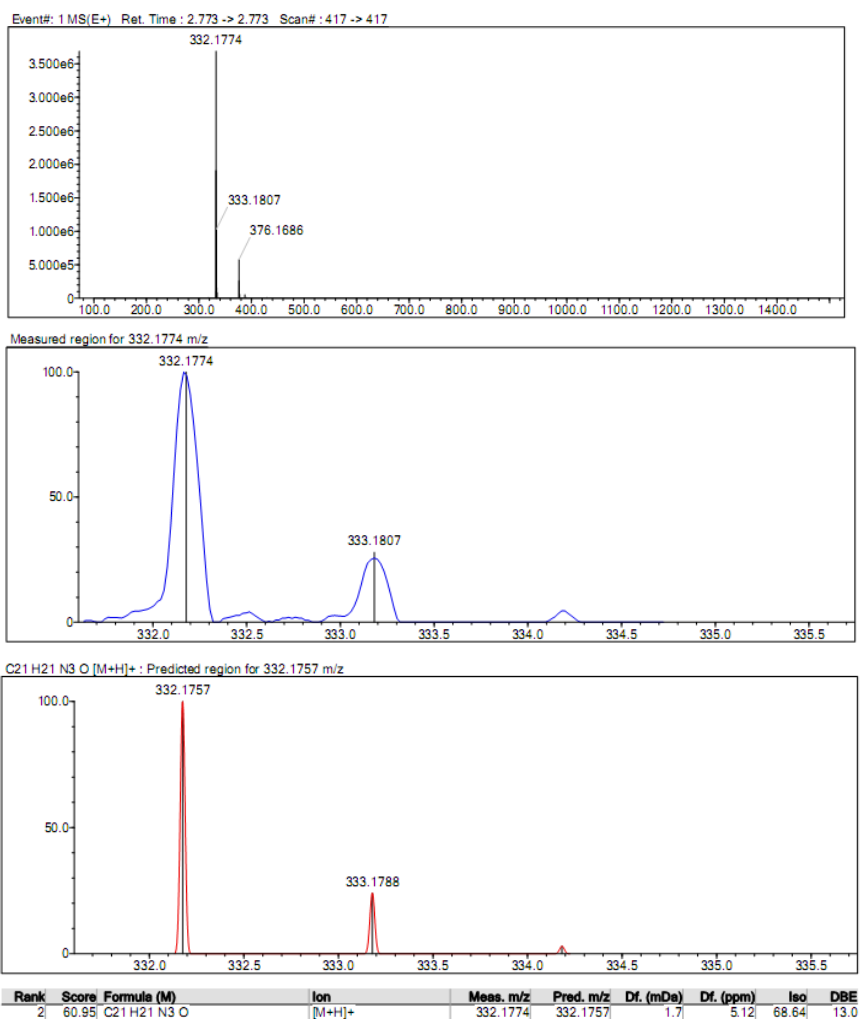
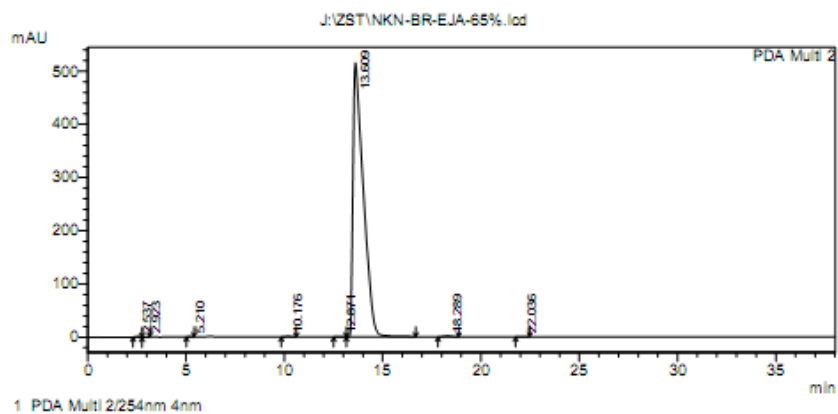


Figure S6-3. HRMS of compound 5a-1

<Chromatogram>



Peak Table

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.537	2661.5	1914	0.141	0.367
2	2.923	5883	434	0.031	0.083
3	5.210	6438	527	0.034	0.101
4	10.176	22960	1359	0.122	0.260
5	12.871	4439	223	0.025	0.043
6	13.609	18778847	515571	99.378	98.753
7	18.289	46062	1835	0.244	0.352
8	22.036	5179	216	0.027	0.041
Total		18896423	522079	100.000	100.000

Figure S6-4. HPLC of compound 5a-1

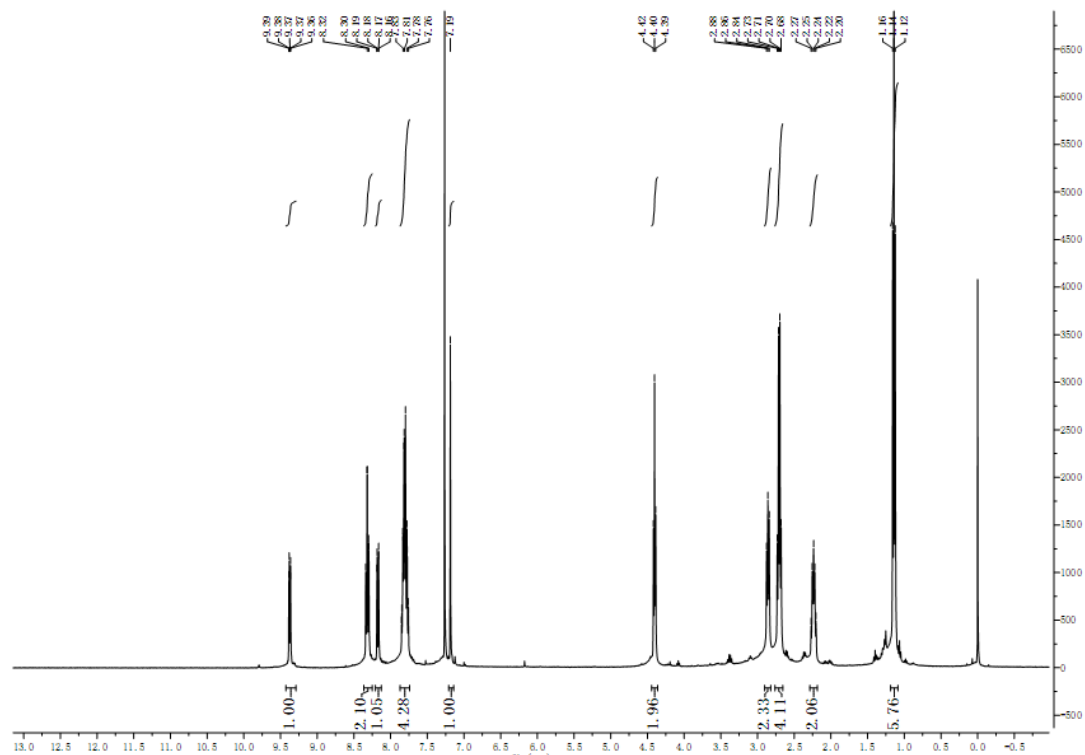


Figure S7-1. ^1H NMR spectra of compound **5a-2** (400 MHz, CDCl_3)

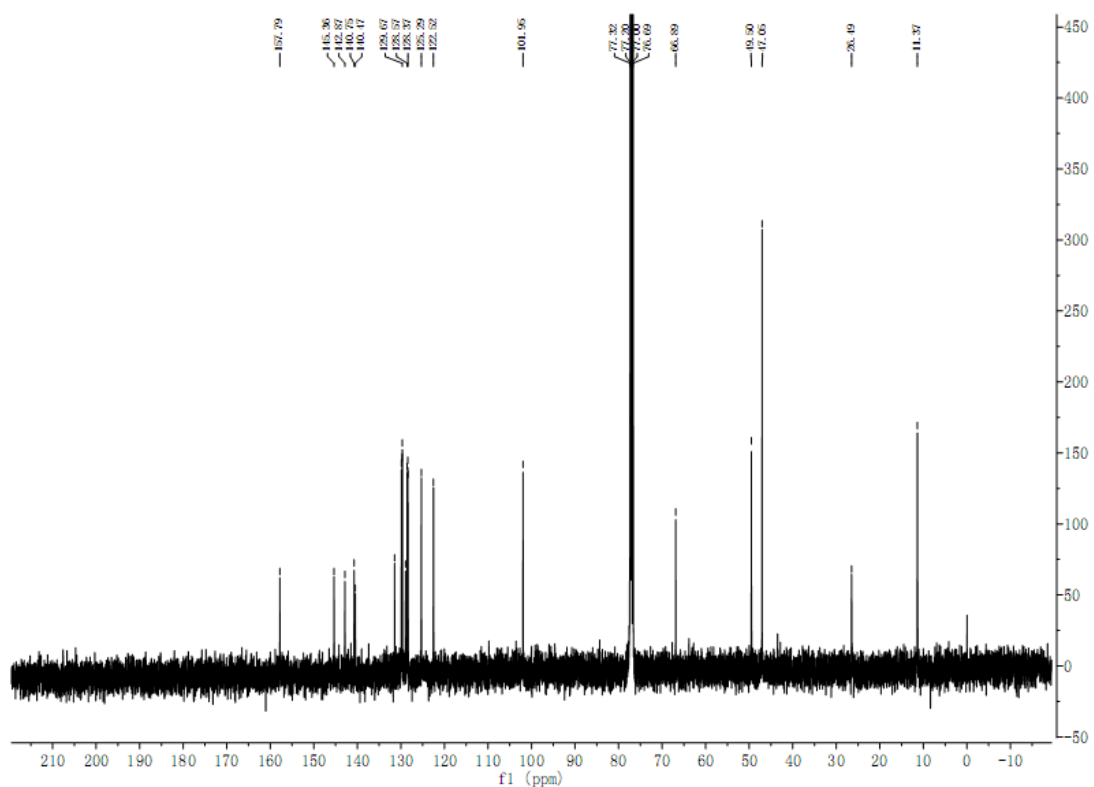


Figure S7-2. ^{13}C NMR spectra of compound **5a-2** (101 MHz, CDCl_3)

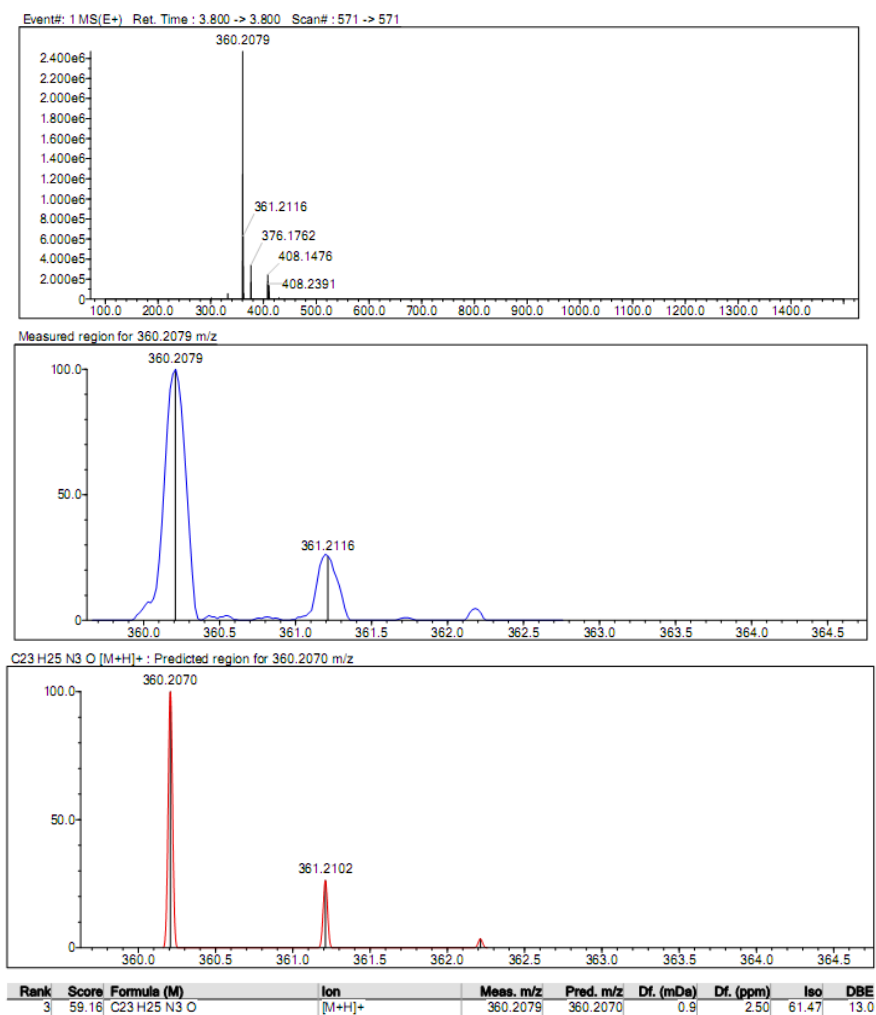
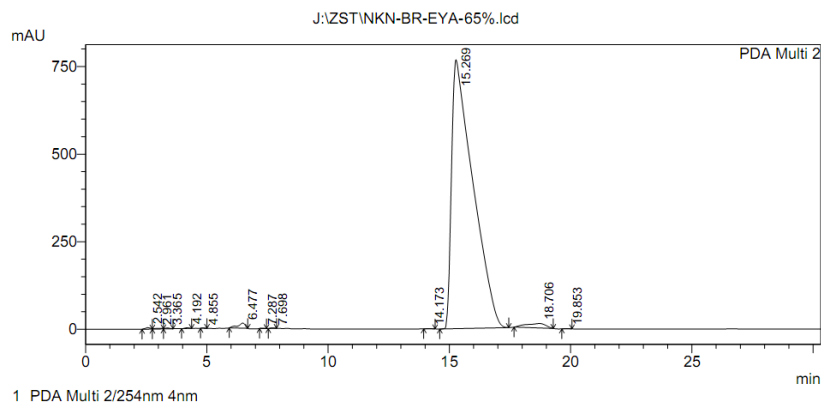


Figure S7-3. HRMS of compound 5a-2

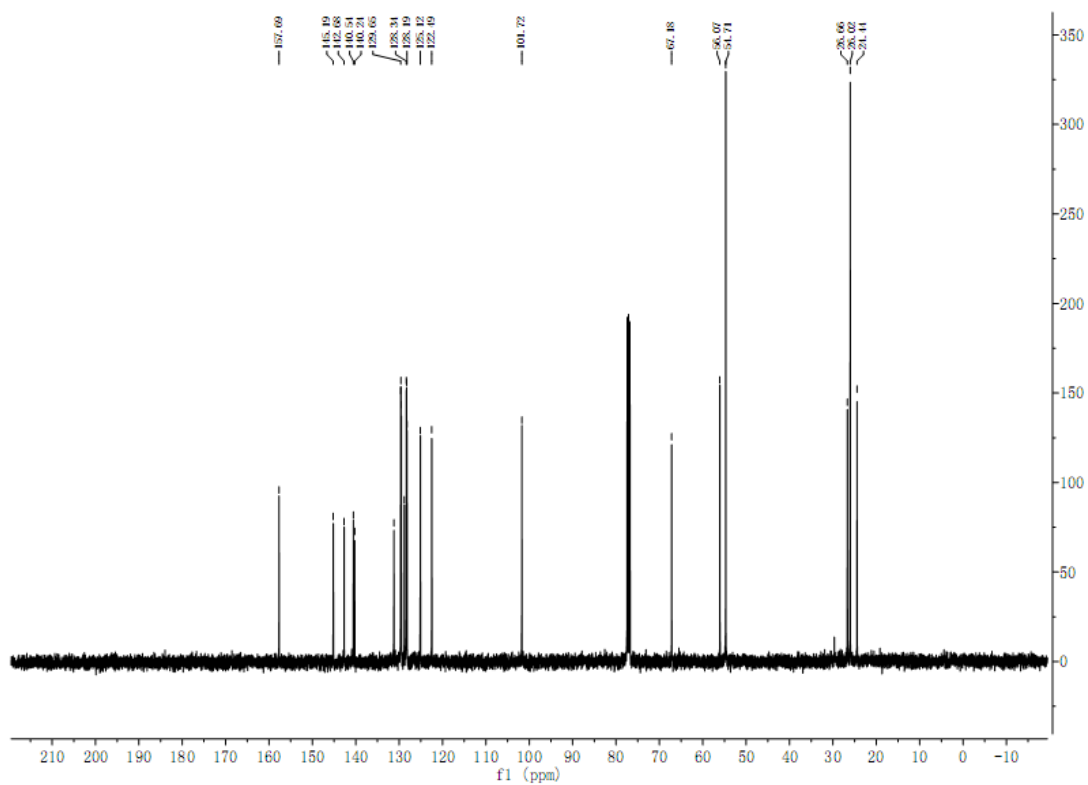
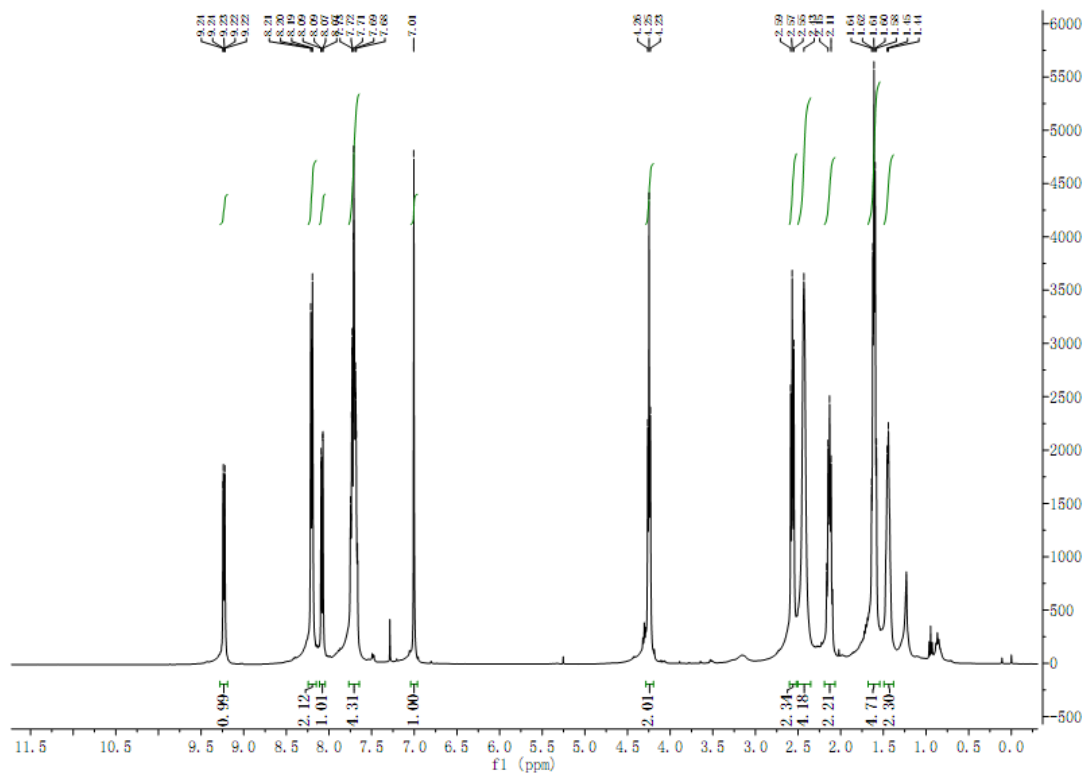
<Chromatogram>



PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.542	66079	4553	0.137	0.561
2	2.961	43156	2033	0.090	0.250
3	3.365	31128	2426	0.065	0.299
4	4.192	27360	2256	0.057	0.278
5	4.855	11554	1205	0.024	0.148
6	6.477	315637	14846	0.656	1.828
7	7.287	13968	1317	0.029	0.162
8	7.698	19979	1535	0.042	0.189
9	14.173	9673	554	0.020	0.068
10	15.269	46864338	767940	97.348	94.570
11	18.706	728127	12700	1.512	1.564
12	19.853	10195	667	0.021	0.082
Total		48141194	812031	100.000	100.000

Figure S7-4. HPLC of compound 5a-2



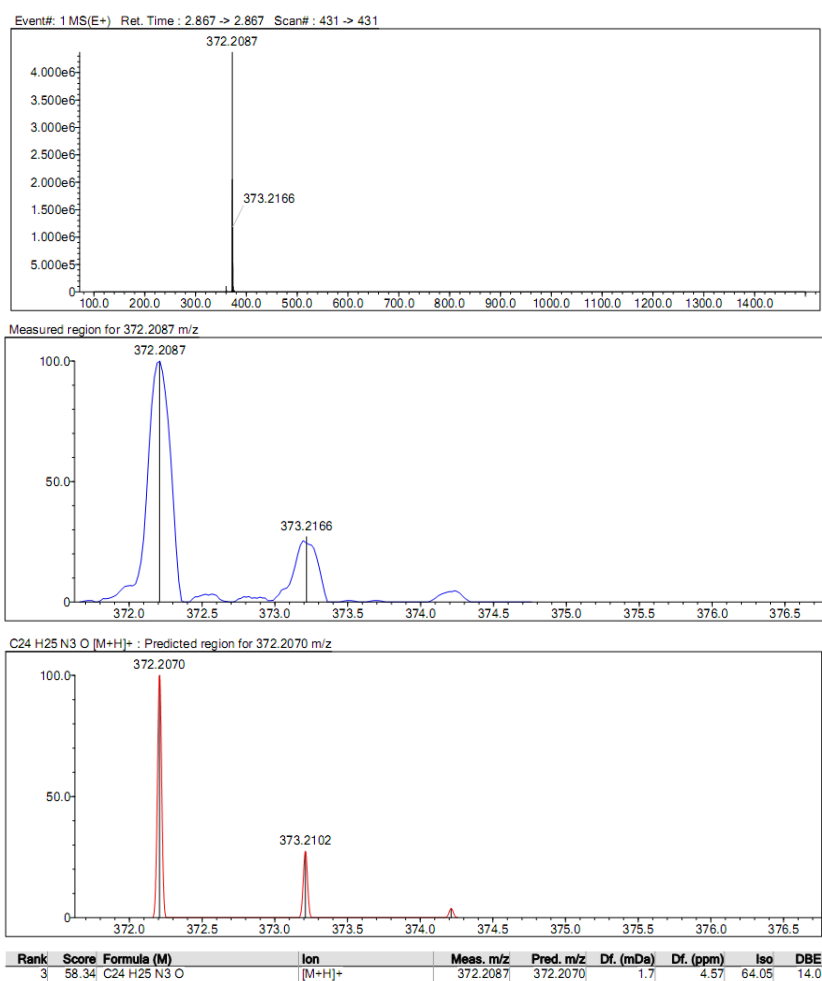
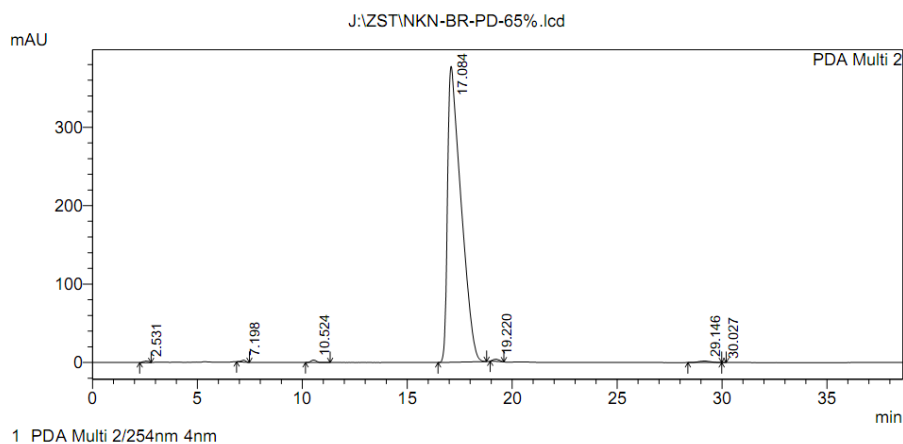


Figure S8-3. HRMS of compound 5a-3

<Chromatogram>



PeakTable

PDA Ch2 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.531	38207	2060	0.223	0.528
2	7.198	36524	2450	0.213	0.628
3	10.524	61044	3252	0.356	0.833
4	17.084	16847384	377553	98.328	96.699
5	19.220	61723	2888	0.360	0.740
6	29.146	86869	2045	0.507	0.524
7	30.027	2071	194	0.012	0.050
Total		17133821	390443	100.000	100.000

Figure S8-4. HPLC of compound 5a-3

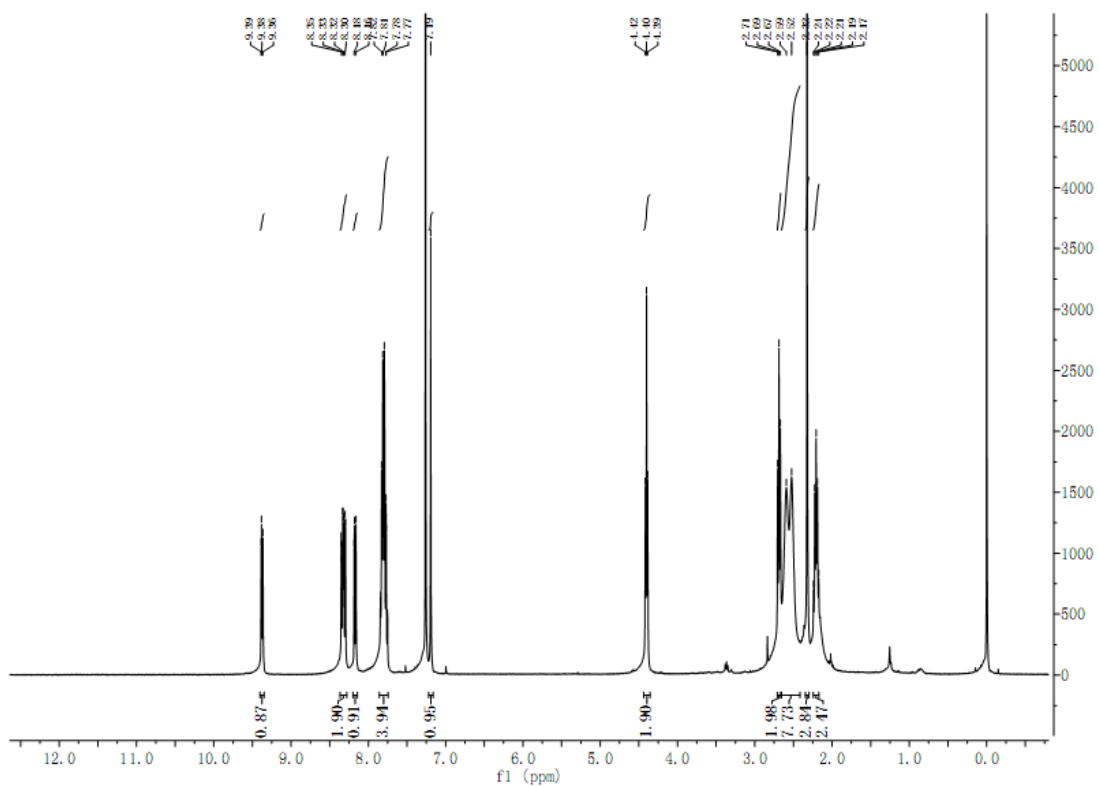


Figure S9-1. ^1H NMR spectra of compound **5a-4** (400 MHz, CDCl_3)

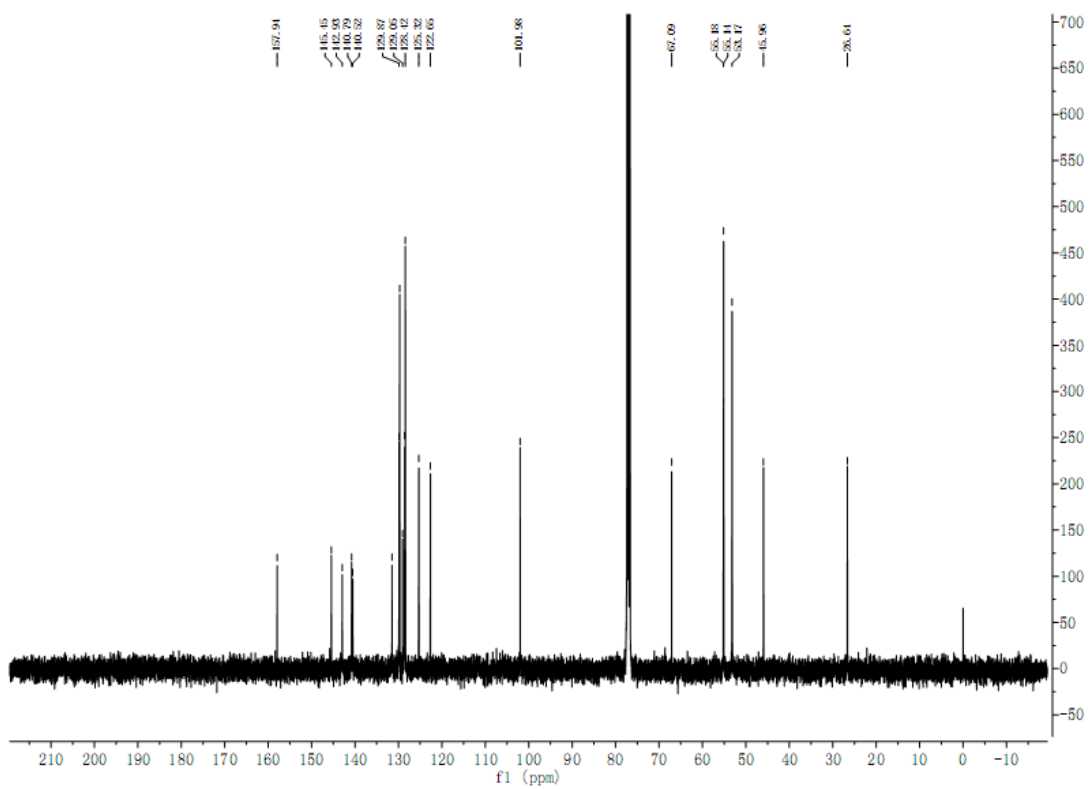


Figure S9-2. ^{13}C NMR spectra of compound **5a-4** (101 MHz, CDCl_3)

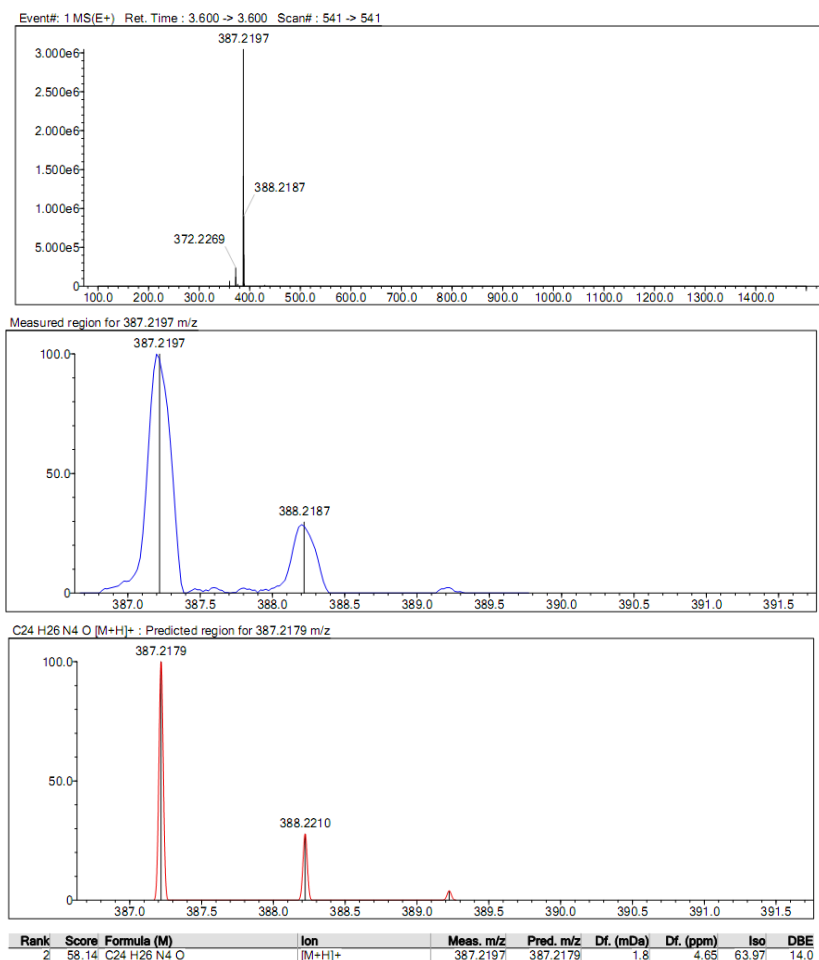
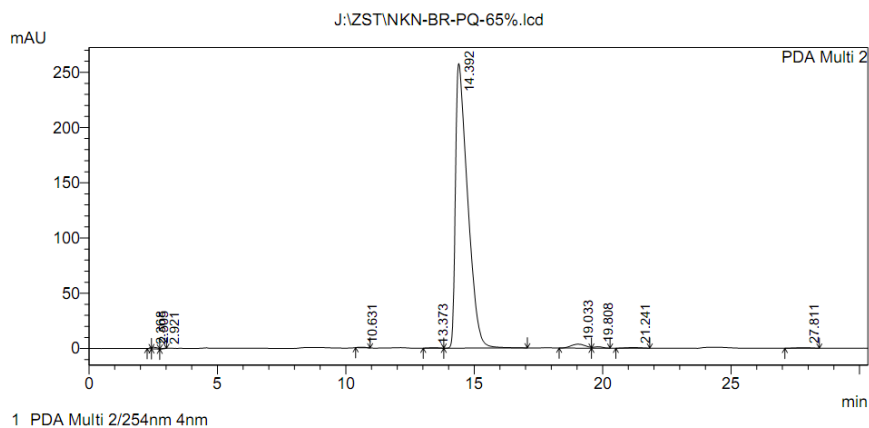


Figure S9-3. HRMS of compound 5a-4

<Chromatogram>



PeakTable

PDA Ch2 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.368	4762	737	0.055	0.276
2	2.505	19201	1625	0.220	0.608
3	2.921	3562	316	0.041	0.118
4	10.631	10149	518	0.116	0.194
5	13.373	10866	417	0.125	0.156
6	14.392	8453686	257657	97.001	96.427
7	19.033	136724	3577	1.569	1.339
8	19.808	30670	1233	0.352	0.462
9	21.241	24207	577	0.278	0.216
10	27.811	21198	549	0.243	0.205
Total		8715026	267205	100.000	100.000

Figure S9-4. HPLC of compound 5a-4

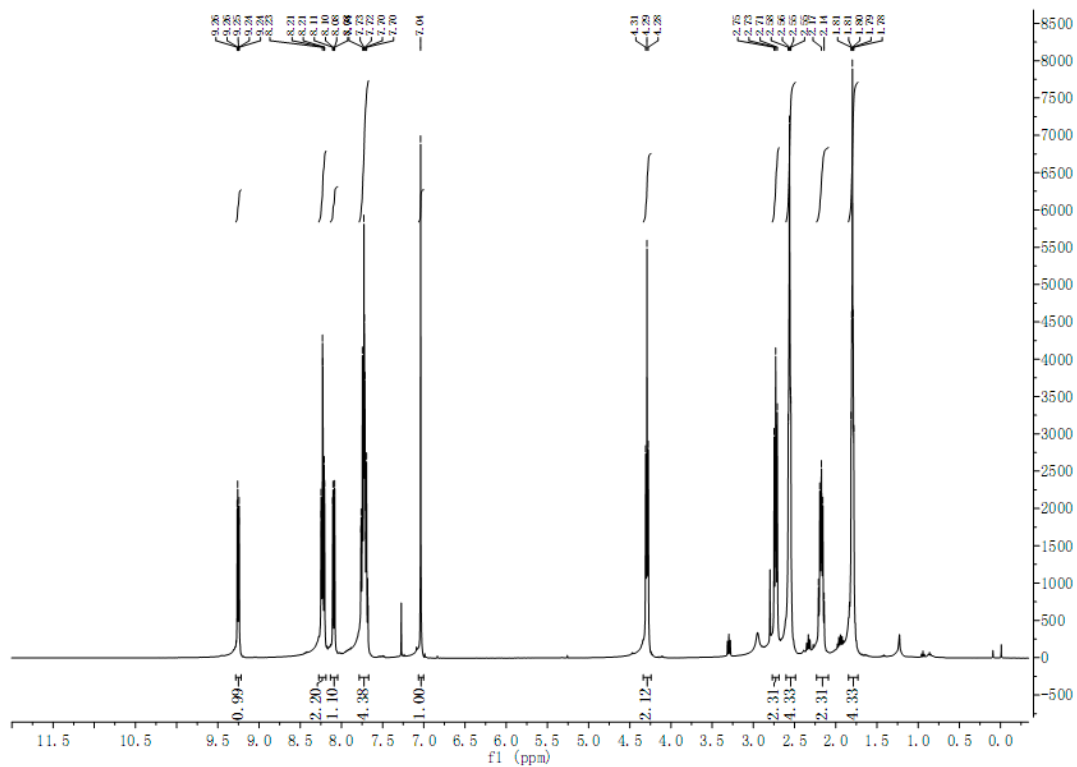


Figure S10-1. ¹H NMR spectra of compound **5a-5** (400 MHz, CDCl₃)

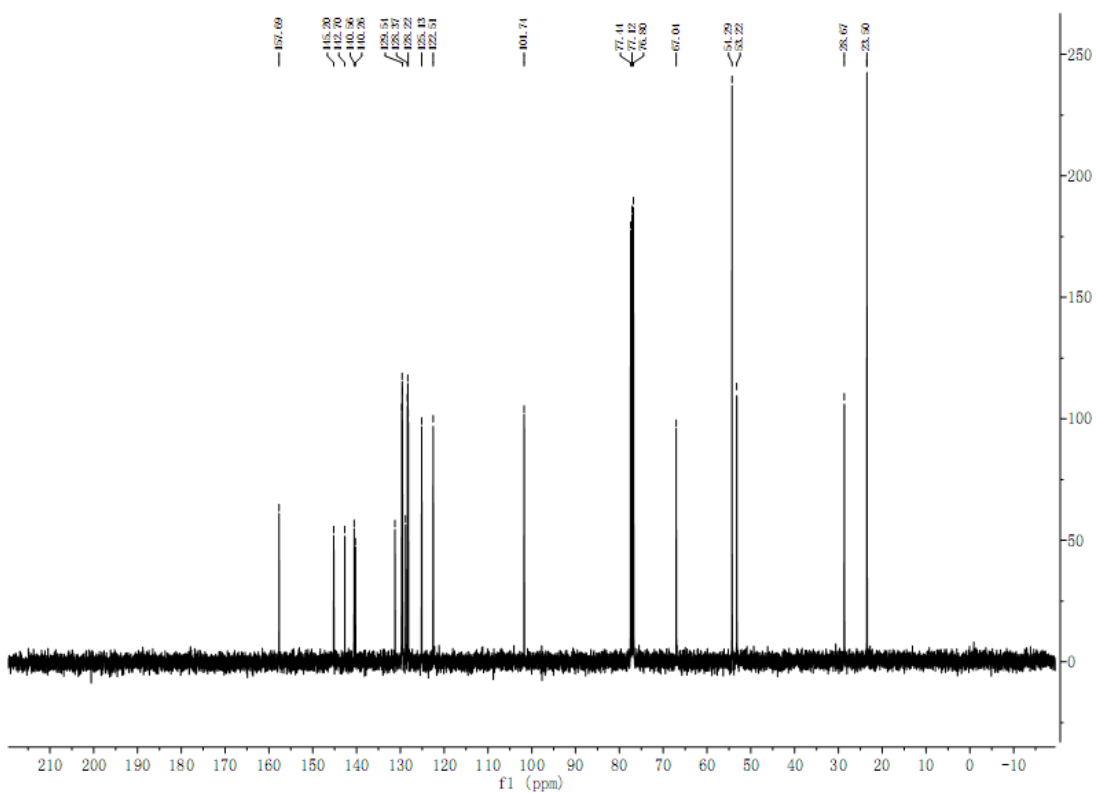


Figure S10-2. ¹³C NMR spectra of compound **5a-5** (101 MHz, CDCl₃)

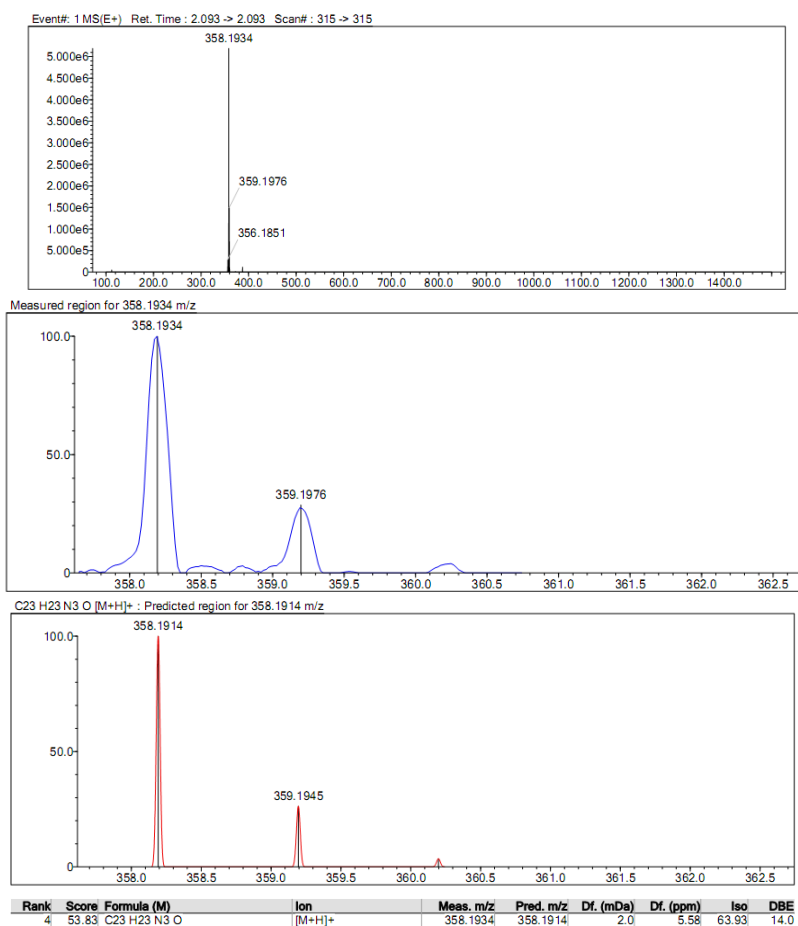
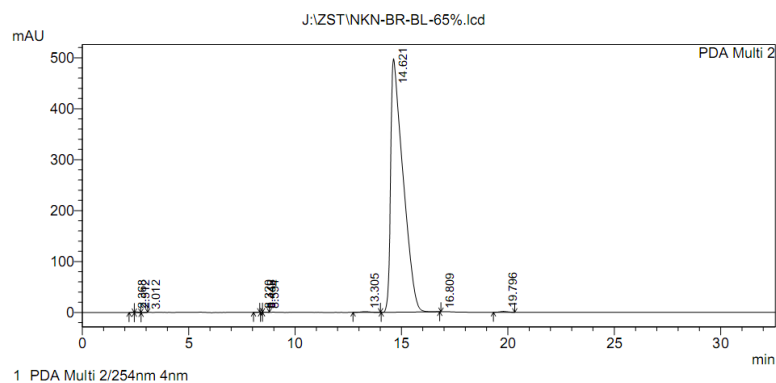


Figure S10-3. HRMS of compound 5a-5

<Chromatogram>



PeakTable

PDA Ch2 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.368	7715	951	0.038	0.188
2	2.512	21548	1735	0.107	0.344
3	3.012	4264	191	0.021	0.038
4	8.320	3024	279	0.015	0.055
5	8.448	1629	320	0.008	0.063
6	8.594	4529	349	0.022	0.069
7	13.305	45998	1462	0.228	0.290
8	14.621	20028655	497705	99.342	98.602
9	16.809	42	28	0.000	0.006
10	19.796	43829	1740	0.217	0.345
Total		20161234	504759	100.000	100.000

Figure S10-4. HPLC of compound 5a-5

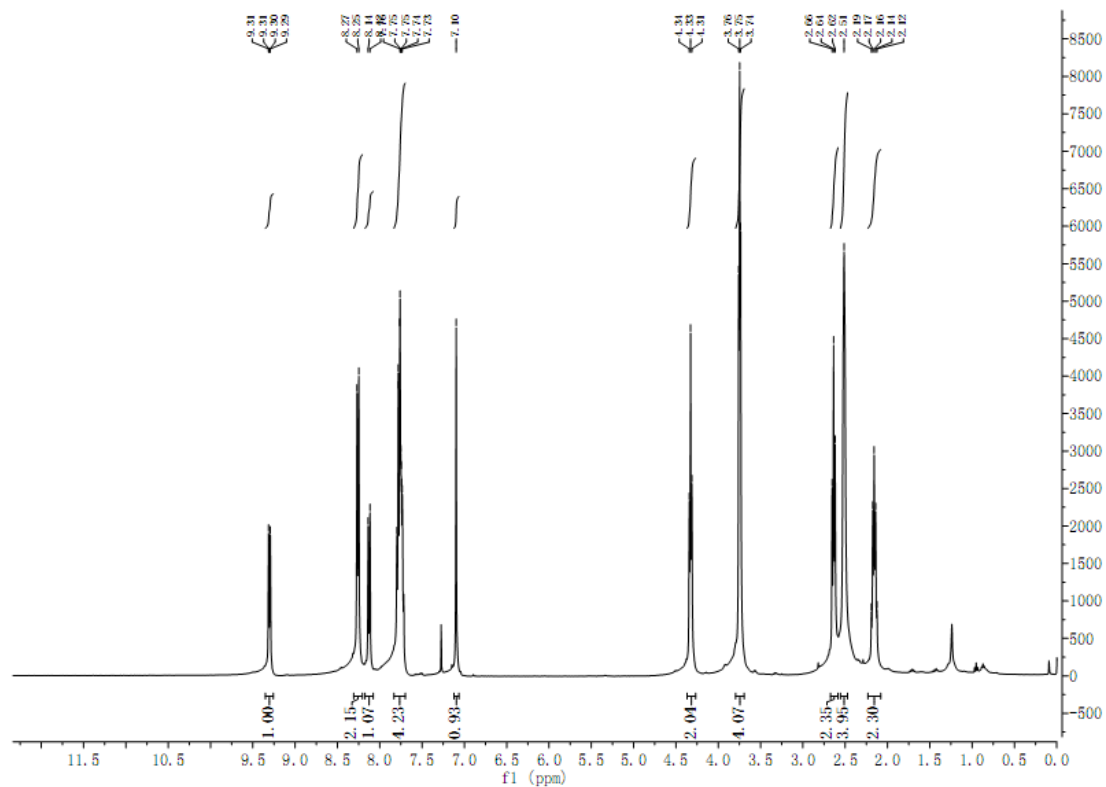


Figure S11-1. ^1H NMR spectra of compound **5a-6** (400 MHz, CDCl_3)

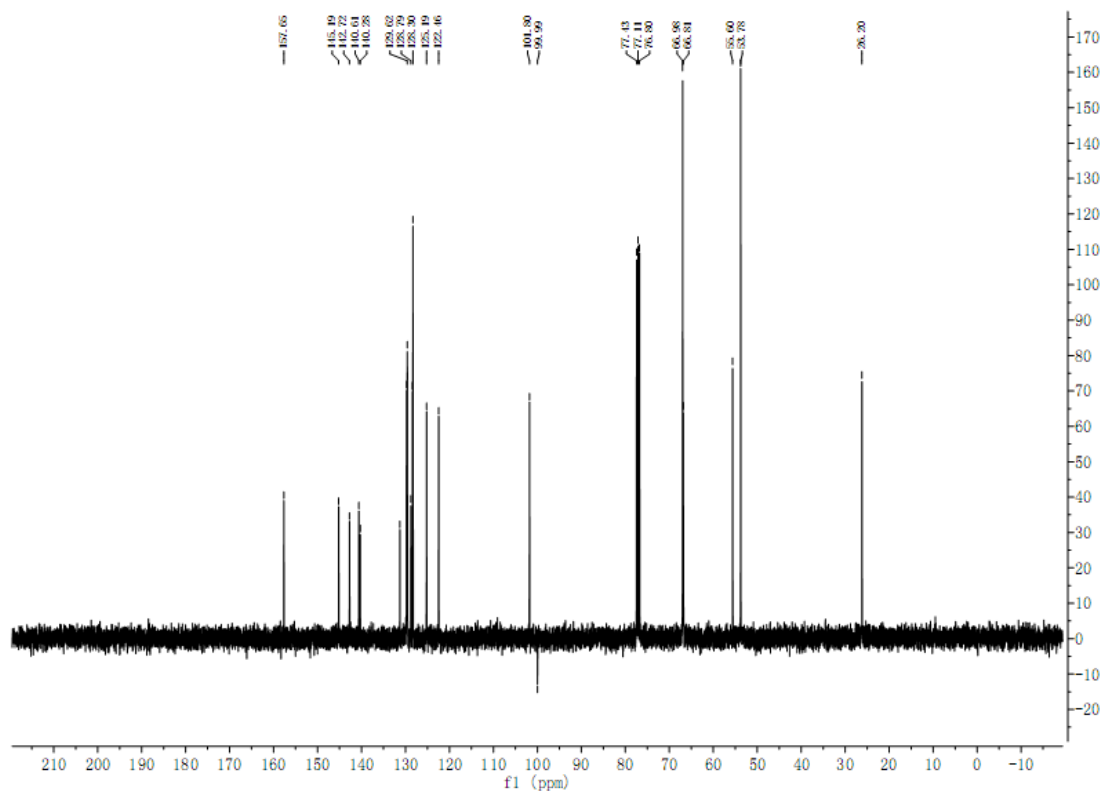


Figure S11-2. ^{13}C NMR spectra of compound **5a-6** (101 MHz, CDCl_3)

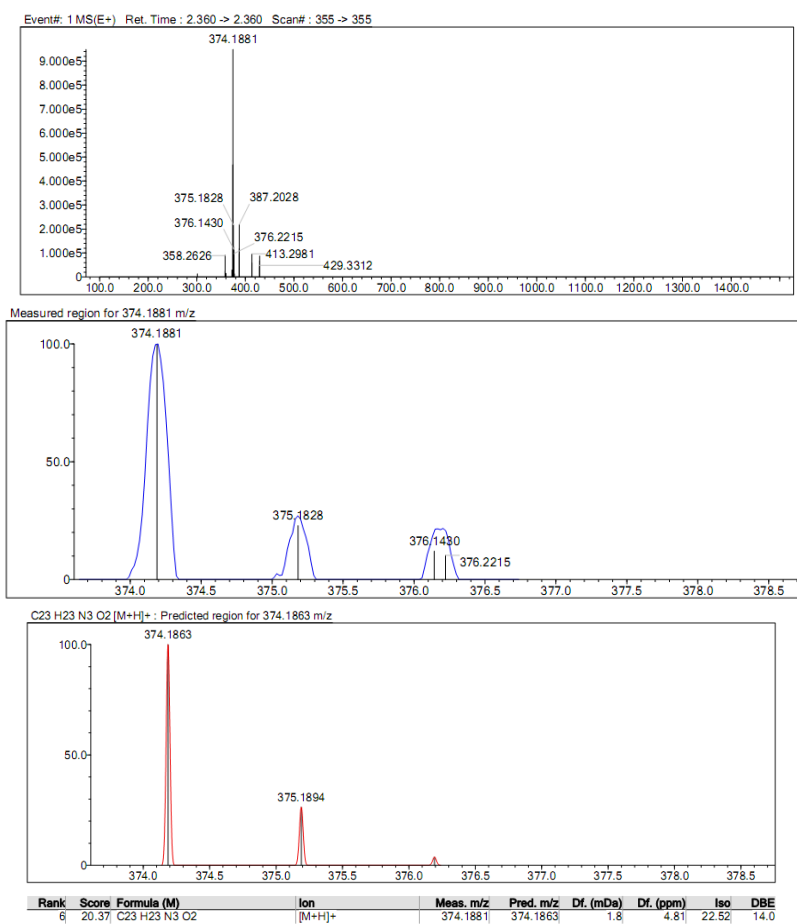
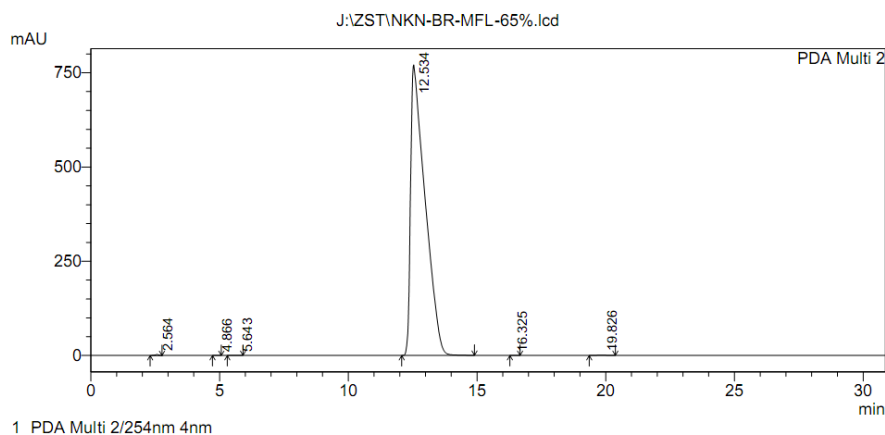


Figure S11-3. HRMS of compound 5a-6

<Chromatogram>

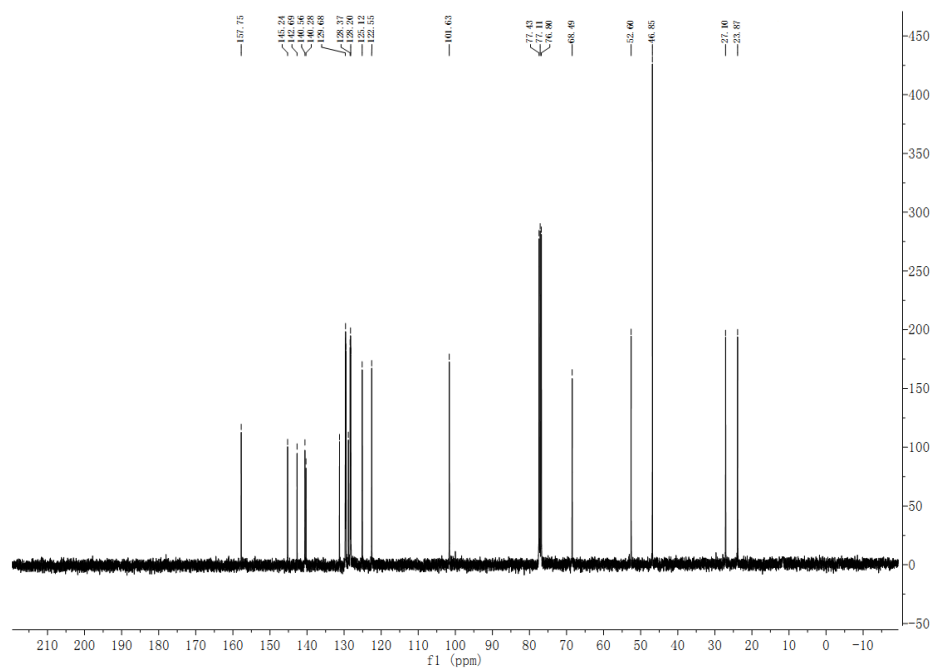
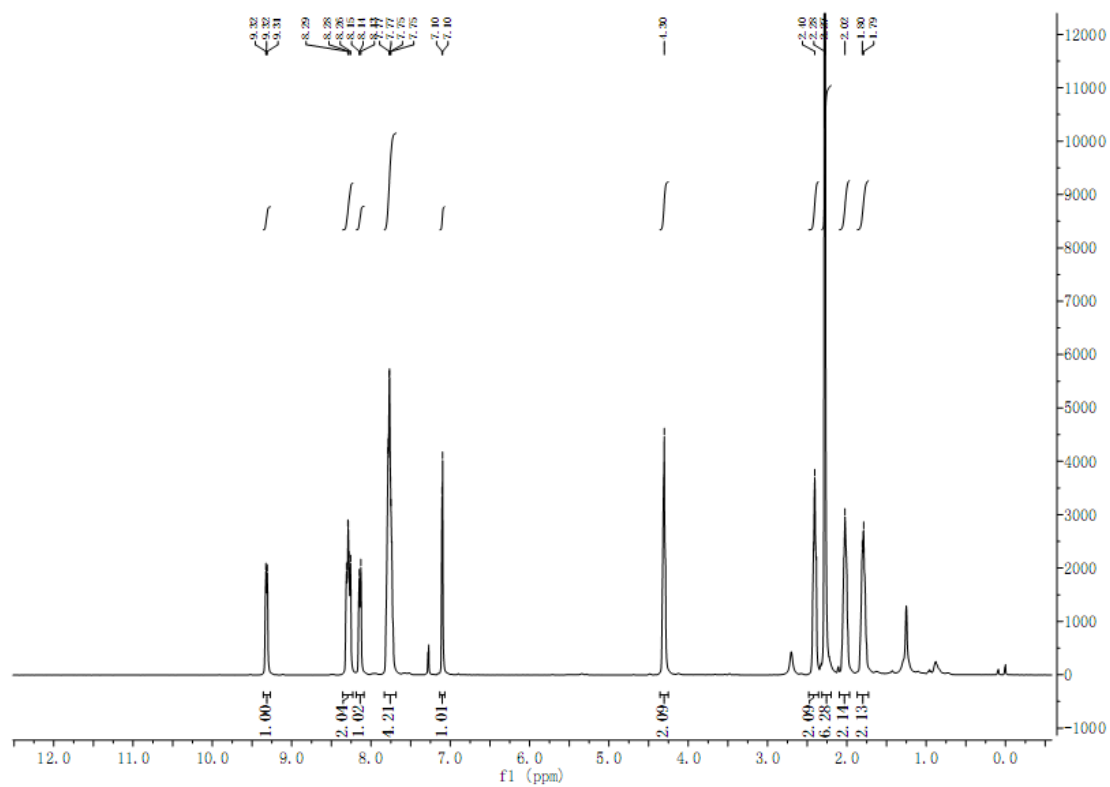


PeakTable

PDA Ch2 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.564	39742	2952	0.132	0.381
2	4.866	2469	244	0.008	0.031
3	5.643	9606	623	0.032	0.080
4	12.534	30004204	770771	99.727	99.337
5	16.325	2828	170	0.009	0.022
6	19.826	27527	1158	0.091	0.149
Total		30086374	775919	100.000	100.000

Figure S11-4. HPLC of compound 5a-6



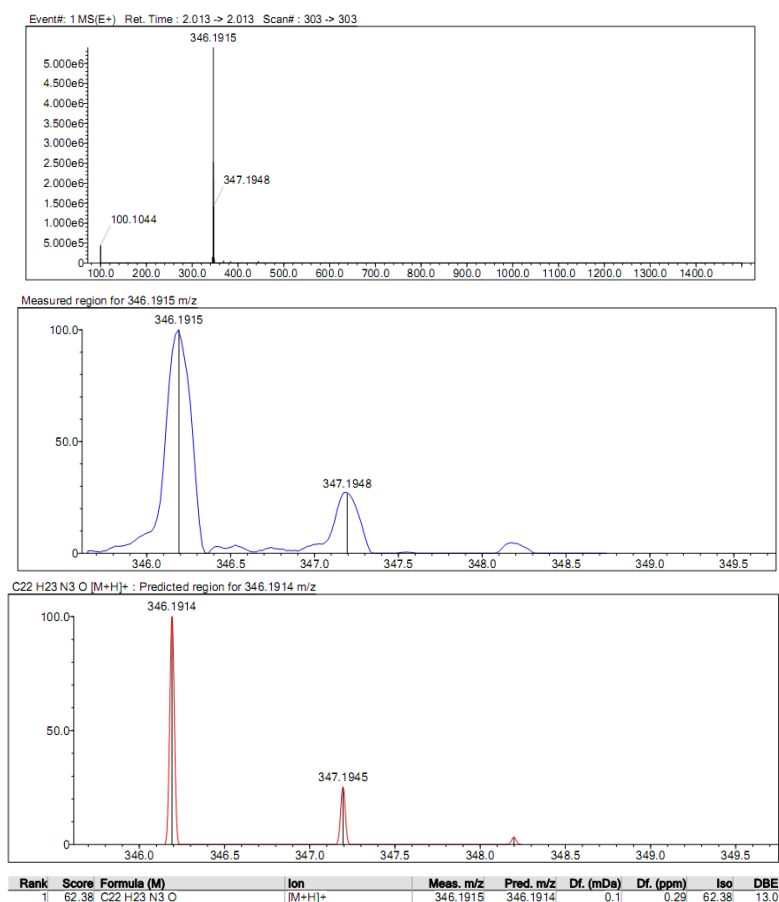
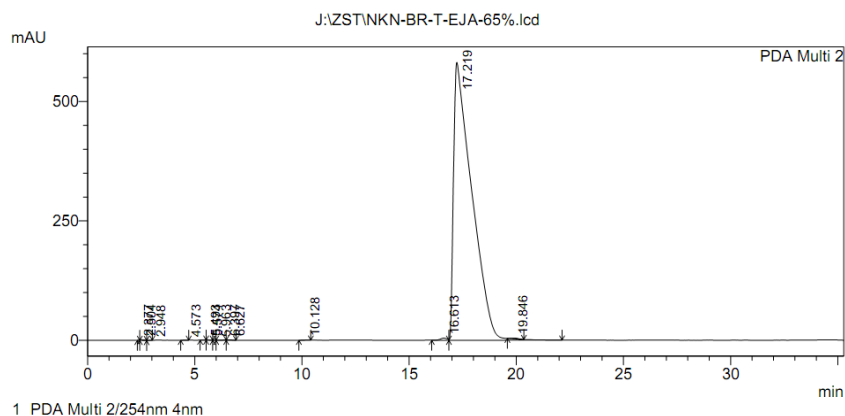


Figure S12-3. HRMS of compound **6a-1**

<Chromatogram>



1 PDA Multi 2/254nm 4nm

PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.377	4020	715	0.012	0.121
2	2.504	20040	1591	0.058	0.268
3	2.948	3304	231	0.010	0.039
4	4.573	2938	282	0.009	0.048
5	5.493	2246	250	0.007	0.042
6	5.574	3600	271	0.010	0.046
7	5.963	955	119	0.003	0.020
8	6.397	9647	542	0.028	0.091
9	6.627	12473	839	0.036	0.141
10	10.128	8175	488	0.024	0.082
11	16.613	96779	4457	0.281	0.751
12	17.219	34275400	581804	99.405	98.069
13	19.846	41082	1667	0.119	0.281
Total		34480660	593258	100.000	100.000

Figure S12-4. HPLC of compound **6a-1**

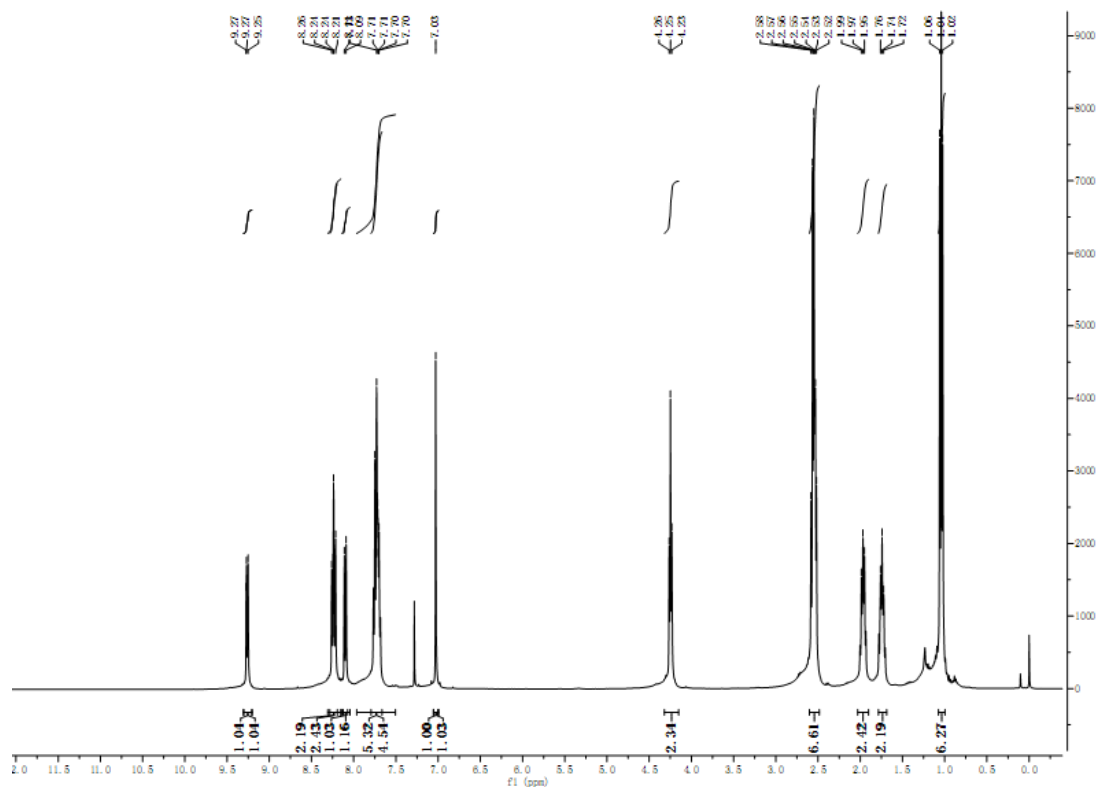


Figure S13-1. ^1H NMR spectra of compound **6a-2** (400 MHz, CDCl_3)

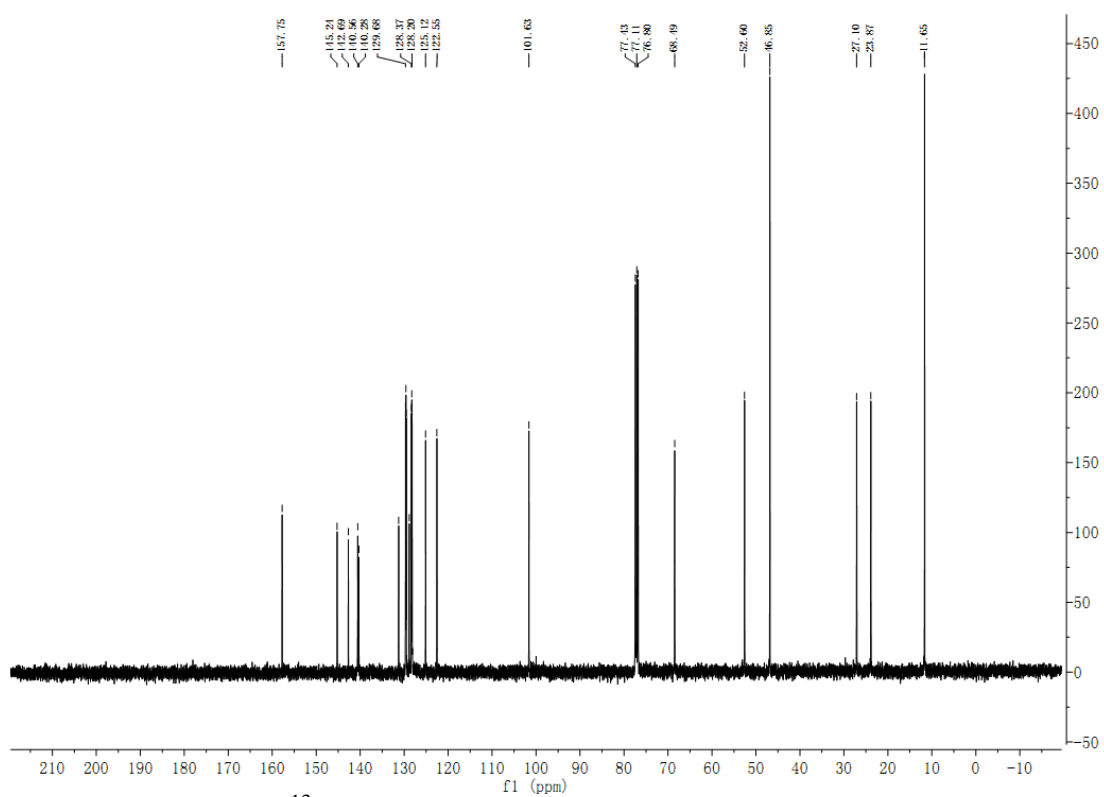


Figure S13-2. ^{13}C NMR spectra of compound **6a-2** (101 MHz, CDCl_3)

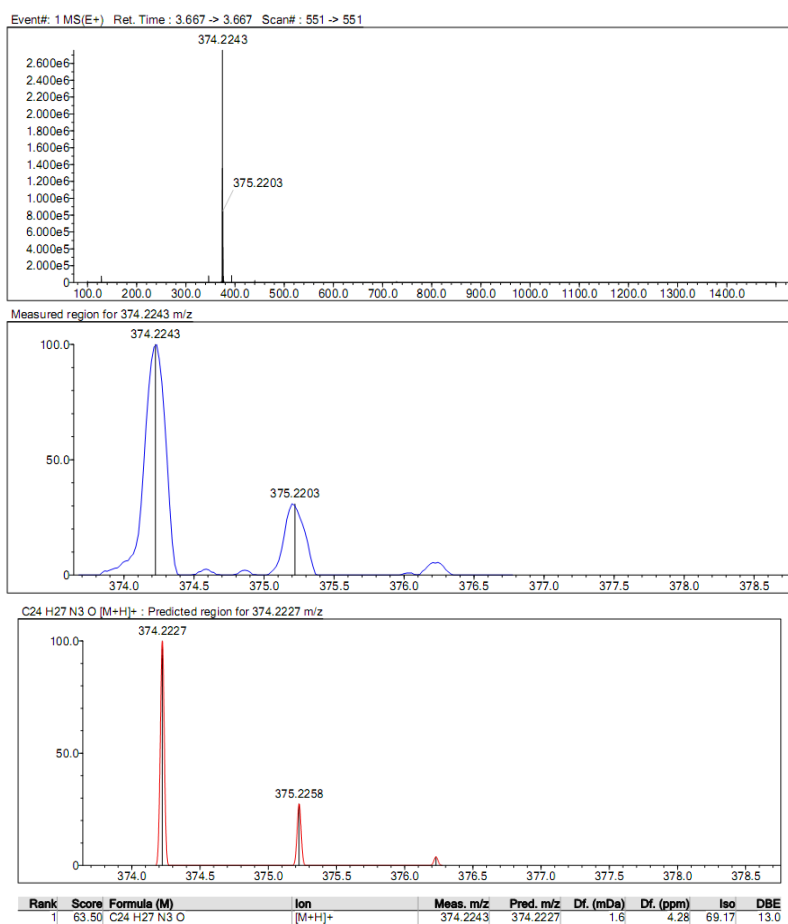
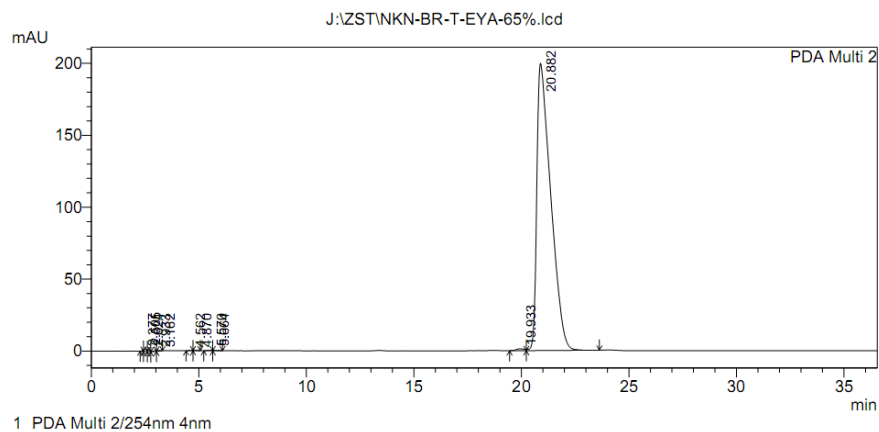


Figure S13-3. HRMS of compound **6a-2**

<Chromatogram>



PeakTable

PDA Ch2 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.377	3890	818	0.043	0.396
2	2.505	13316	1578	0.148	0.763
3	2.624	8011	1276	0.089	0.617
4	2.933	6683	567	0.074	0.274
5	3.162	4490	397	0.050	0.192
6	4.562	3222	282	0.036	0.136
7	4.870	2757	239	0.031	0.116
8	5.570	5091	303	0.056	0.147
9	5.664	3317	273	0.037	0.132
10	19.933	35310	1310	0.391	0.634
11	20.882	8934401	199724	99.046	96.593
Total		9020488	206769	100.000	100.000

Figure S13-4. HPLC of compound **6a-2**

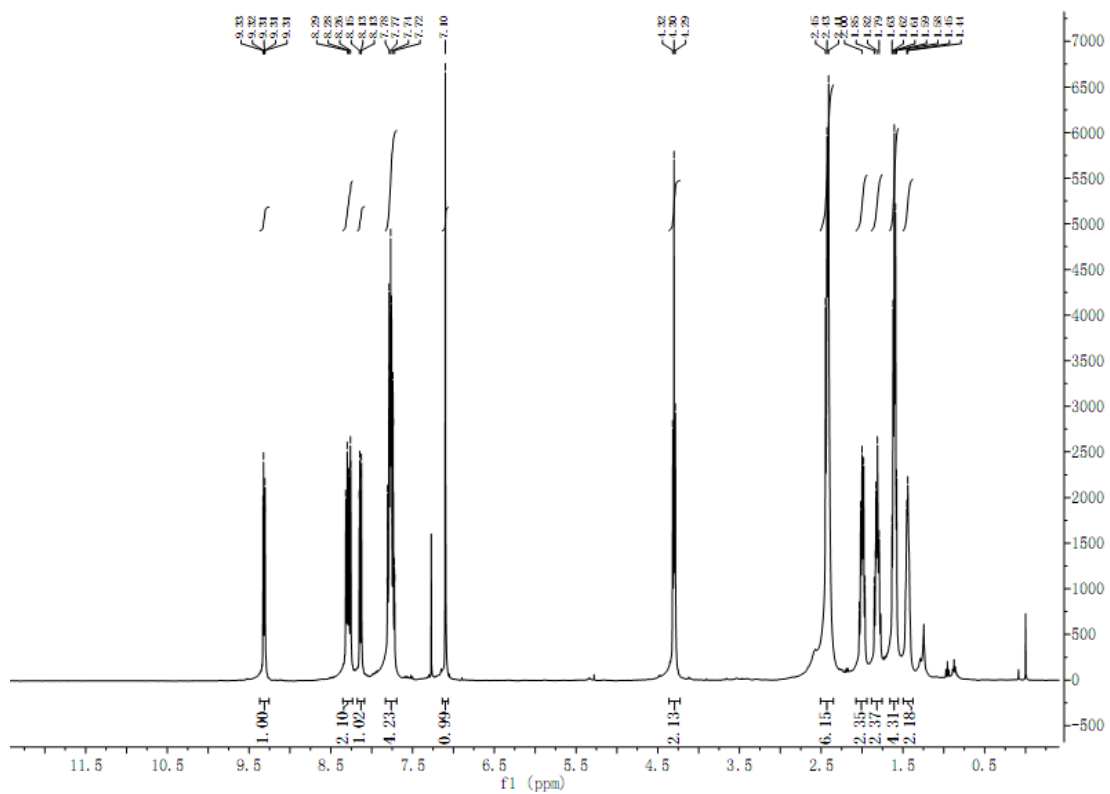


Figure S14-1. ¹H NMR spectra of compound **6a-3** (400 MHz, CDCl₃)

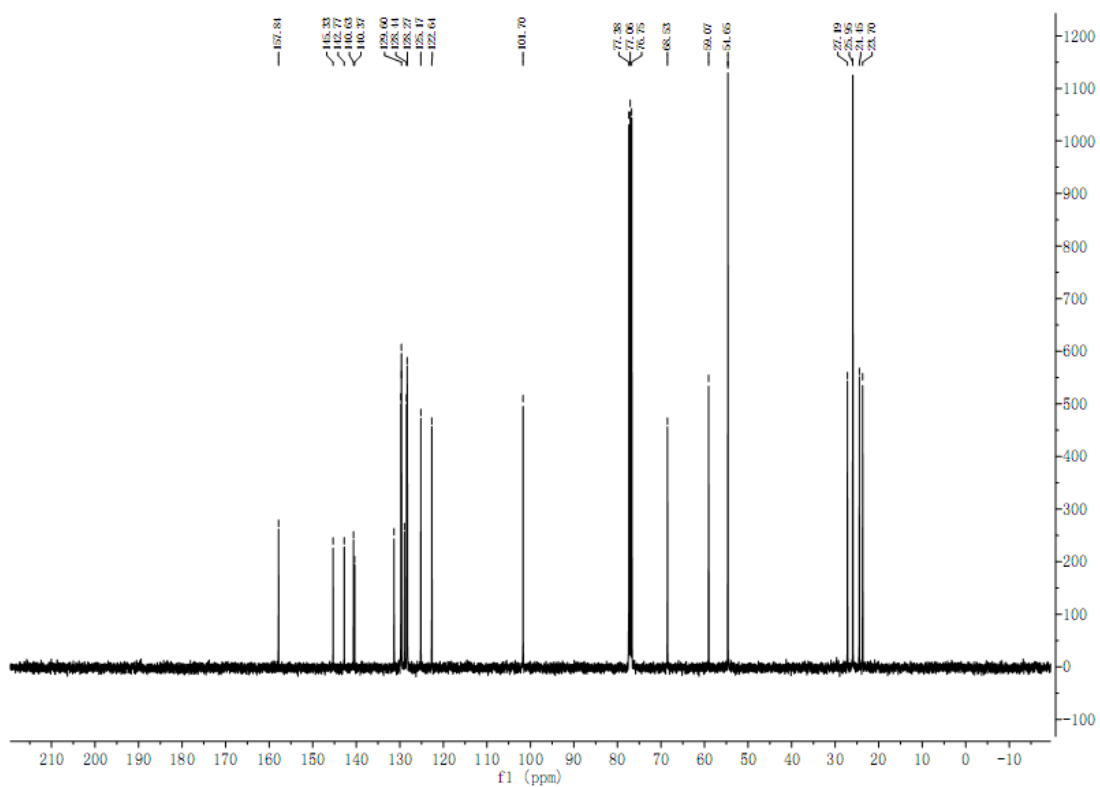


Figure S14-2. ¹³C NMR spectra of compound **6a-3** (101 MHz, CDCl₃)

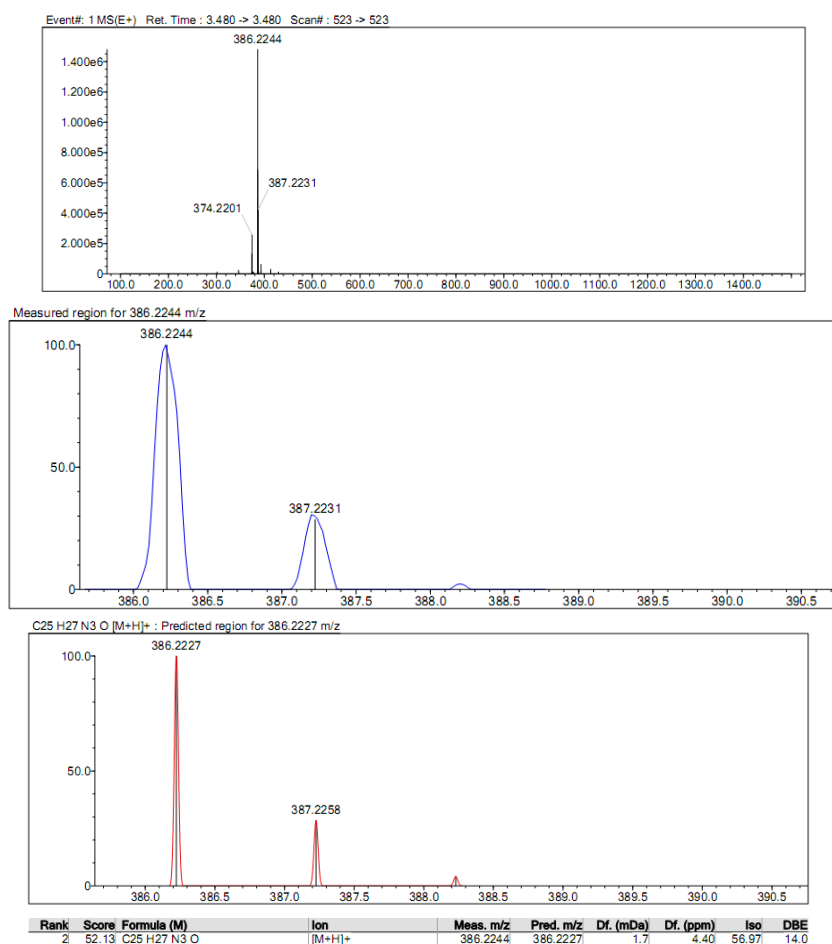
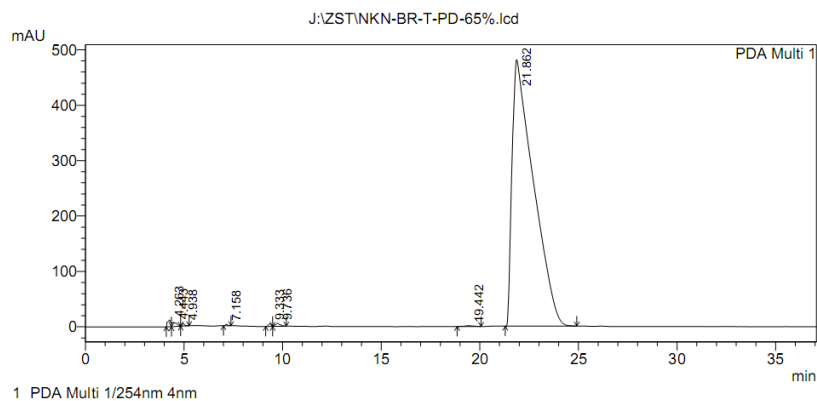


Figure S14-3. HRMS of compound **6a-3**

<Chromatogram>

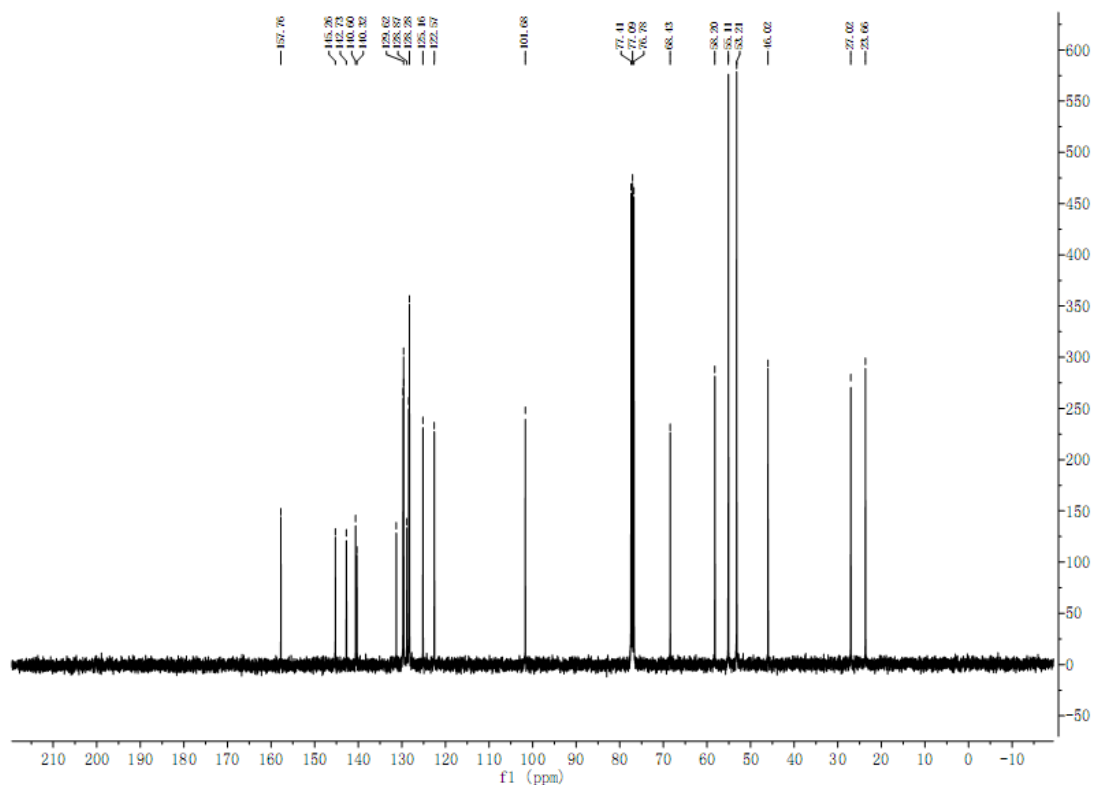
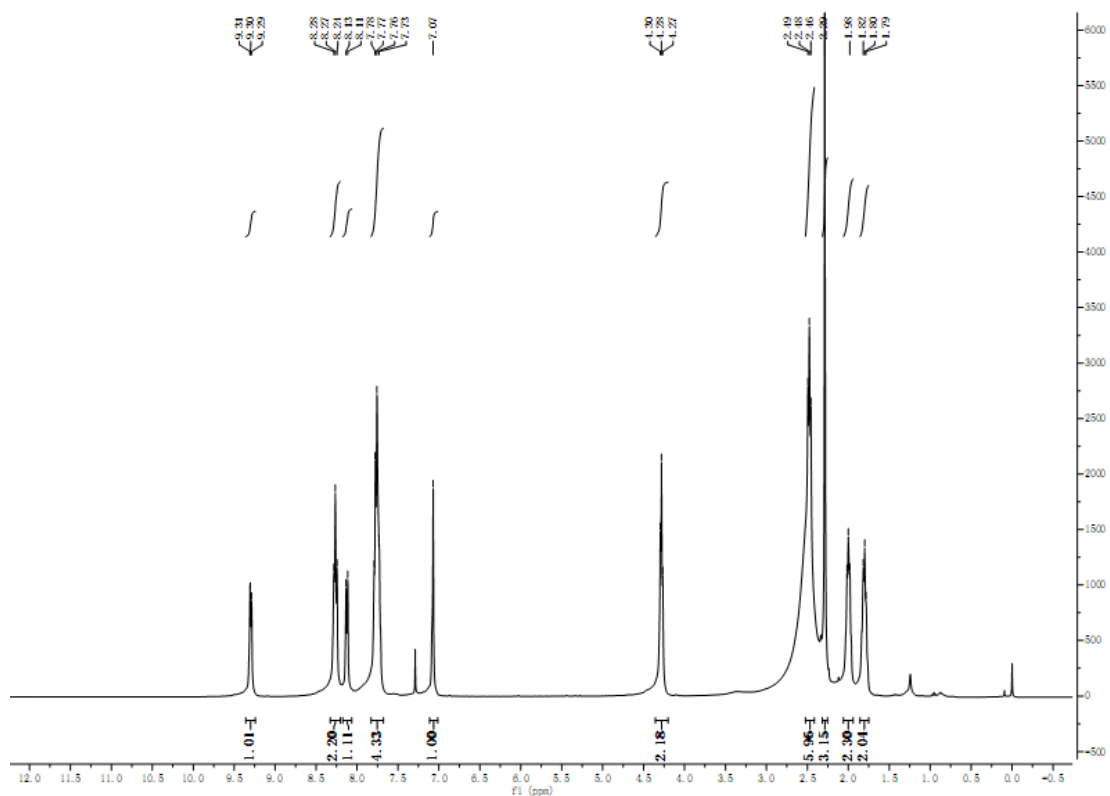


PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.263	140125	12310	0.382	2.371
2	4.443	127567	6857	0.348	1.321
3	4.938	38628	4810	0.105	0.926
4	7.158	22082	2677	0.060	0.516
5	9.333	45262	4543	0.123	0.875
6	9.736	91208	5661	0.248	1.090
7	19.442	39150	1284	0.107	0.247
8	21.862	36204597	481065	98.627	92.654
Total		36708620	519207	100.000	100.000

Figure S14-4. HPLC of compound **6a-3**



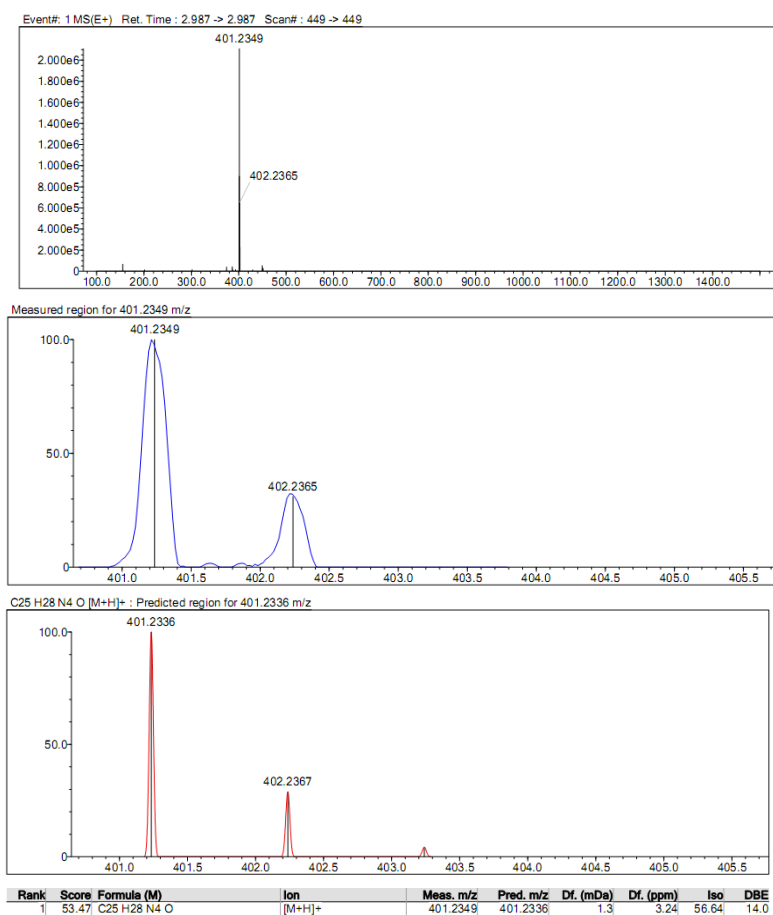
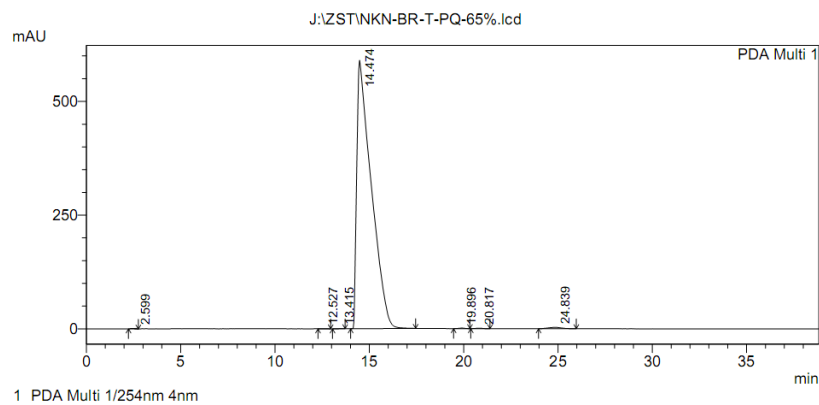


Figure S15-3. HRMS of compound **6a-4**

<Chromatogram>



PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.599	30341	1586	0.096	0.265
2	12.527	8313	398	0.026	0.067
3	13.415	6853	336	0.022	0.056
4	14.474	31483616	590061	99.210	98.751
5	19.896	25630	1126	0.081	0.188
6	20.817	28874	1043	0.091	0.175
7	24.839	150832	2975	0.475	0.498
Total		31734459	597525	100.000	100.000

Figure S15-4. HPLC of compound **6a-4**

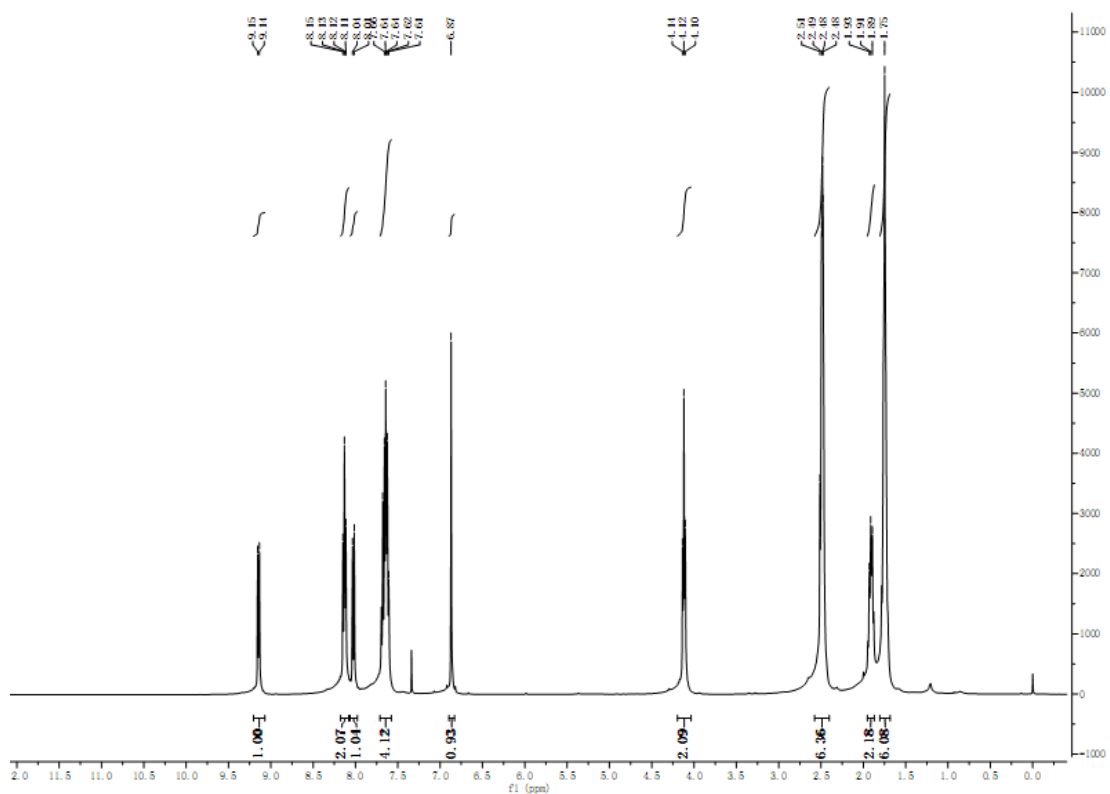


Figure S16-1. ^1H NMR spectra of compound **6a-5** (400 MHz, CDCl_3)

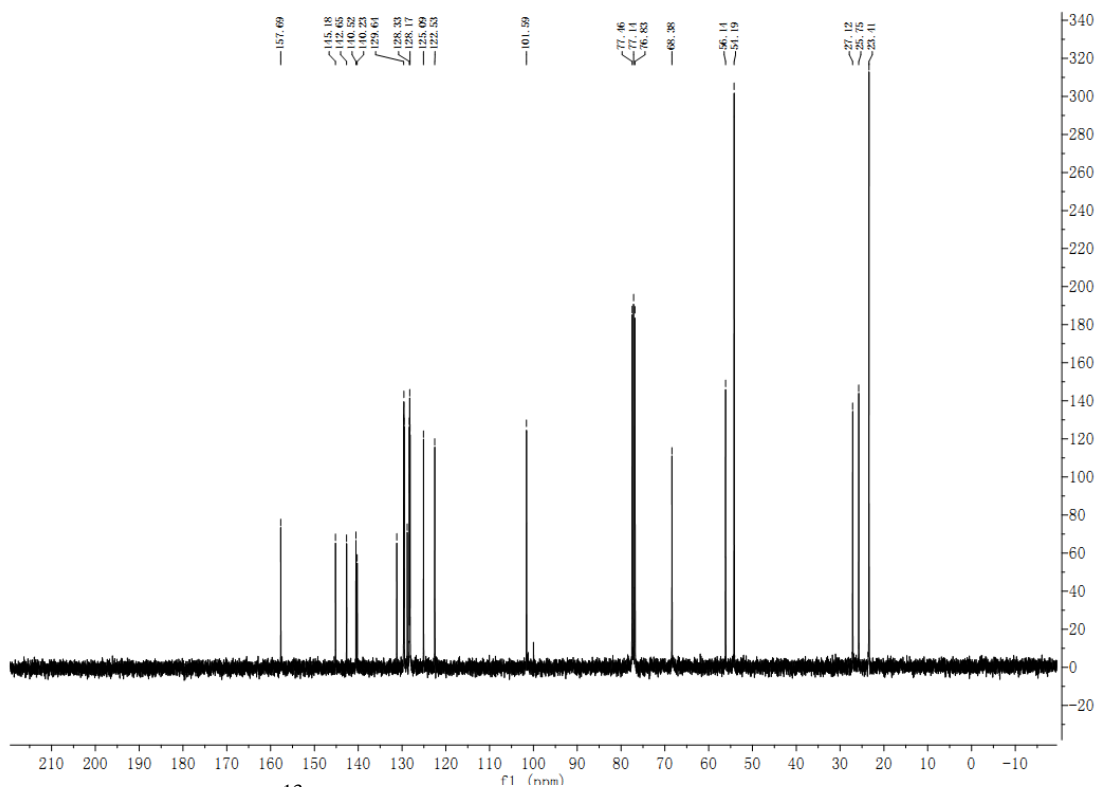


Figure S16-2. ^{13}C NMR spectra of compound **6a-5** (101 MHz, CDCl_3)

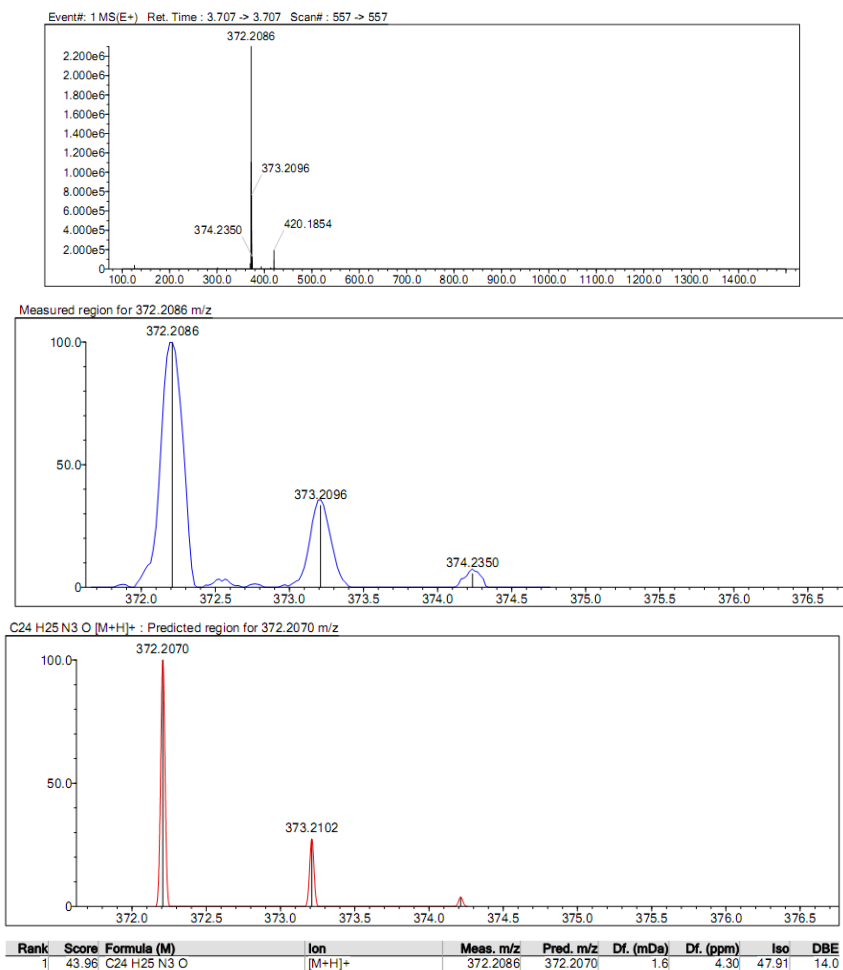
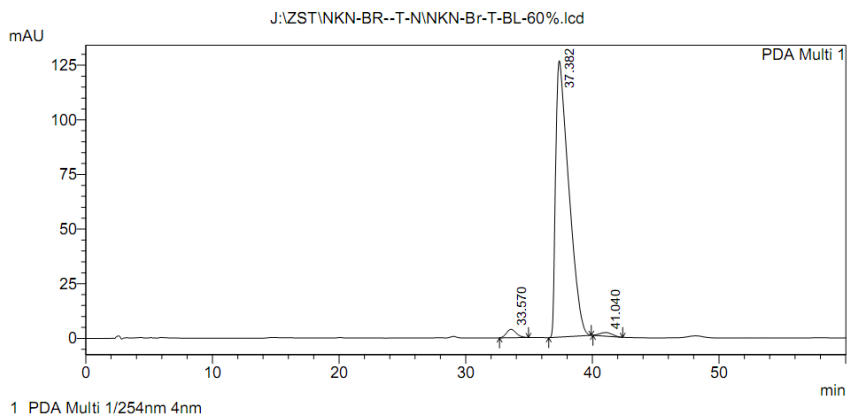


Figure S16-3. HRMS of compound 6a-5

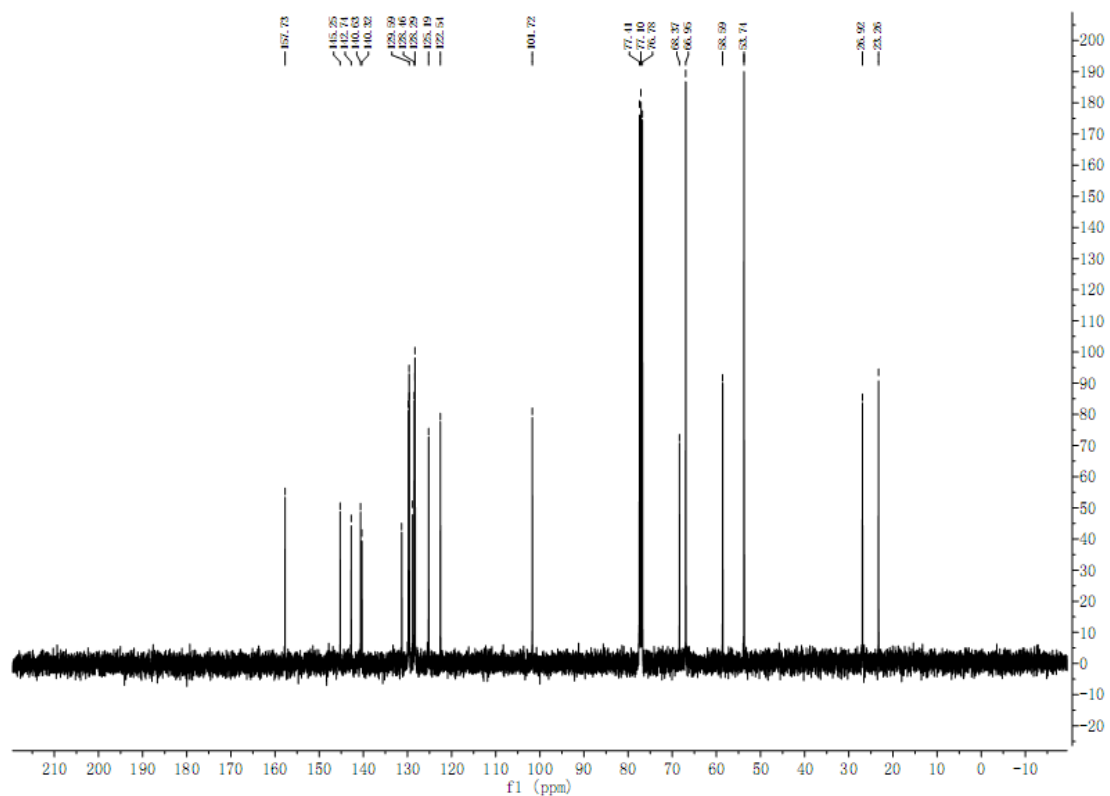
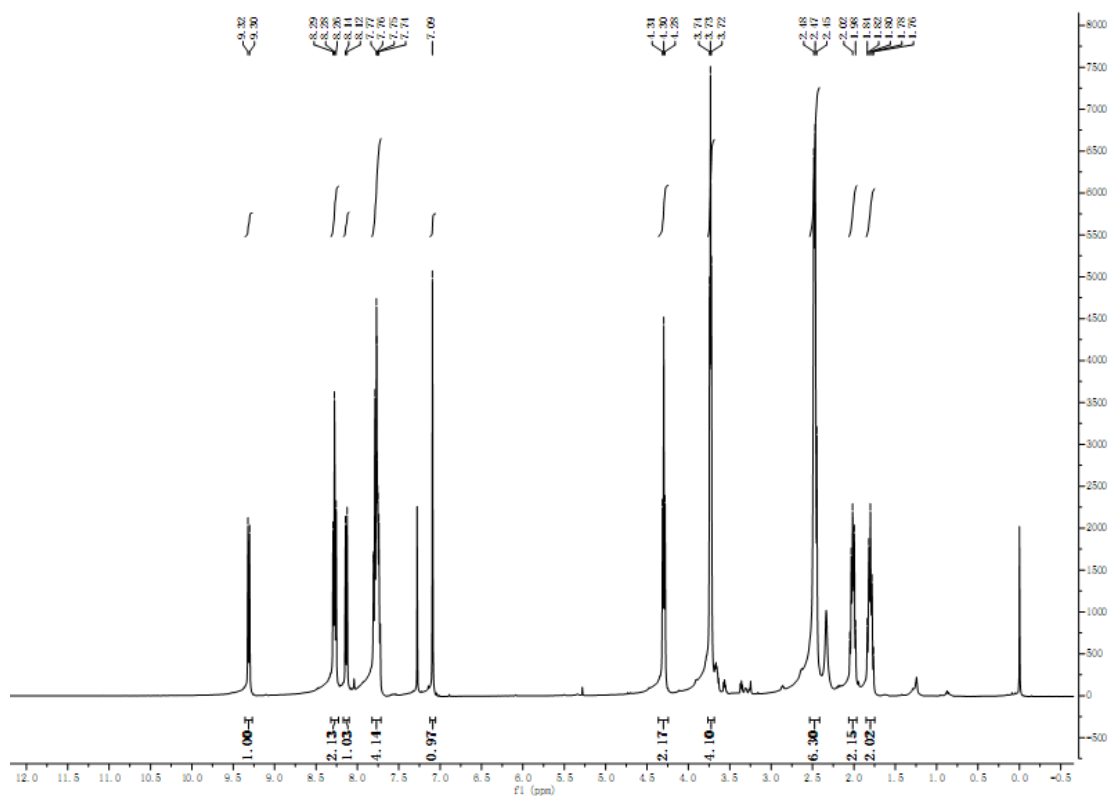
<Chromatogram>



PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	33.570	210262	3866	2.223	2.927
2	37.382	9134583	126532	96.559	95.803
3	41.040	115225	1678	1.218	1.270
Total		9460071	132076	100.000	100.000

Figure S16-4. HPLC of compound 6a-5



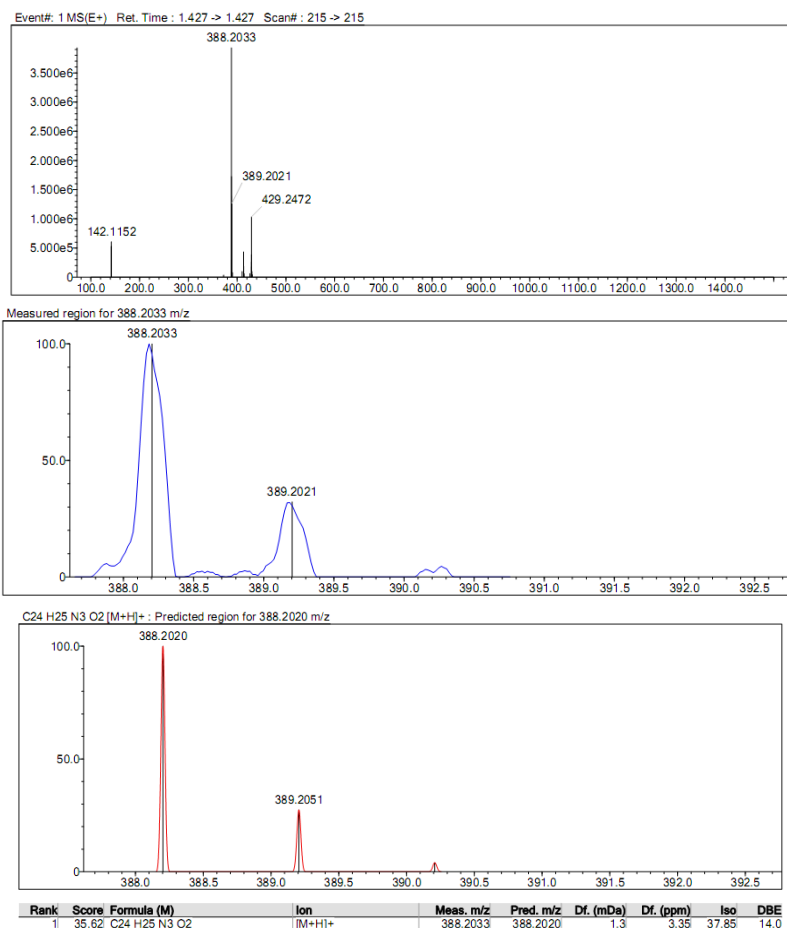
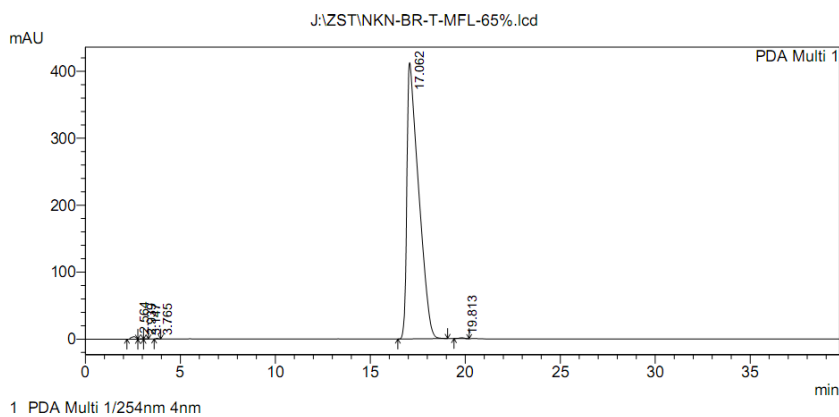


Figure S17-3. HRMS of compound **6a-6**

<Chromatogram>



PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.564	63952	3908	0.360	0.931
2	2.939	7552	540	0.042	0.129
3	3.147	4326	398	0.024	0.095
4	3.765	8555	1016	0.048	0.242
5	17.062	17677247	412763	99.403	98.363
6	19.813	21768	1008	0.122	0.240
Total		17783400	419634	100.000	100.000

Figure S17-4. HPLC of compound **6a-6**

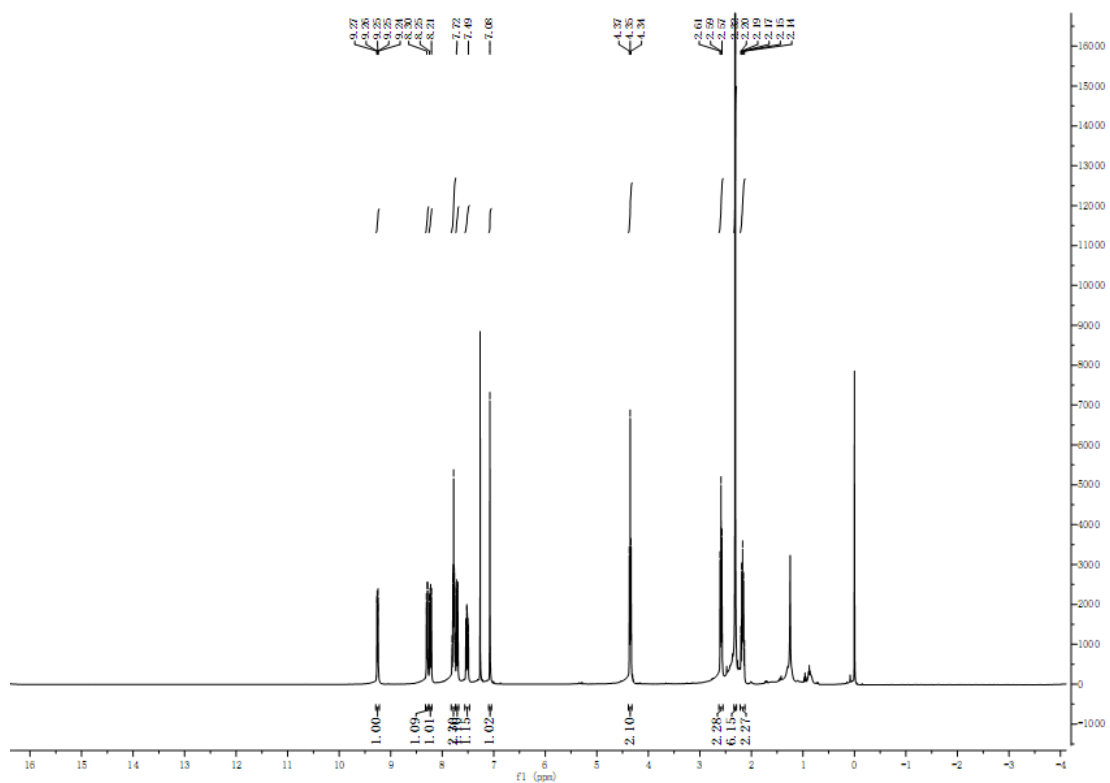


Figure S18-1. ^1H NMR spectra of compound **5b-1** (400 MHz, CDCl_3)

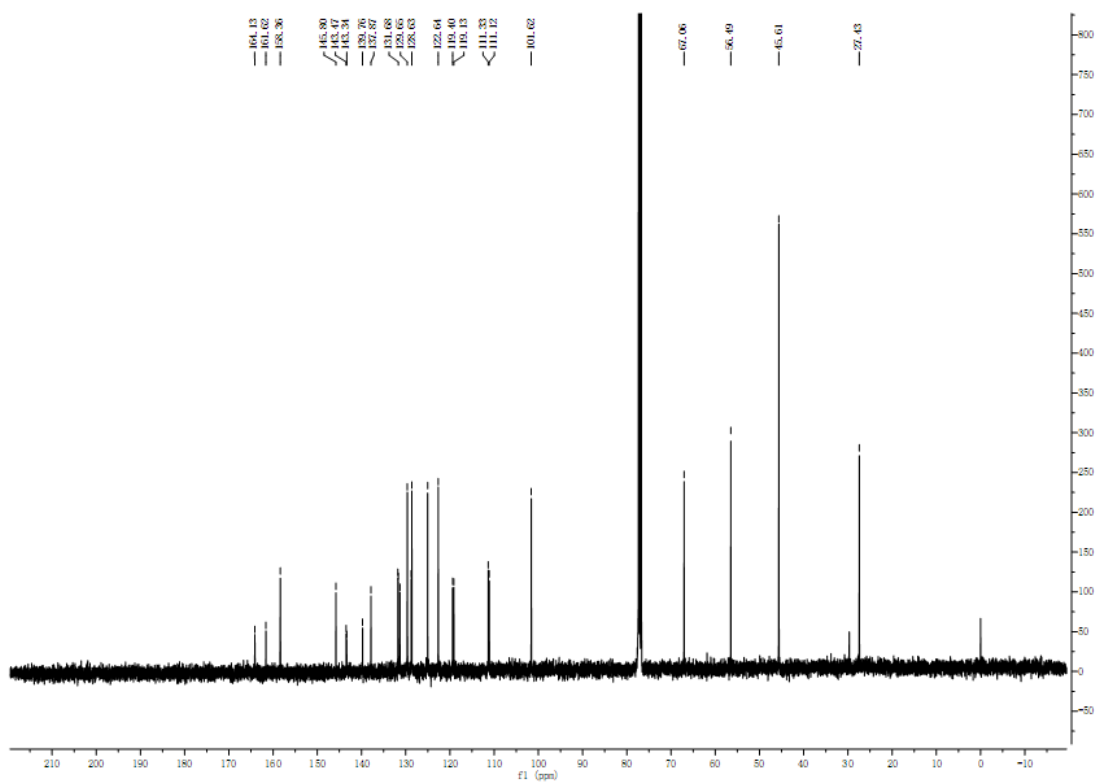


Figure S18-2. ^{13}C NMR spectra of compound **5b-1** (101 MHz, CDCl_3)

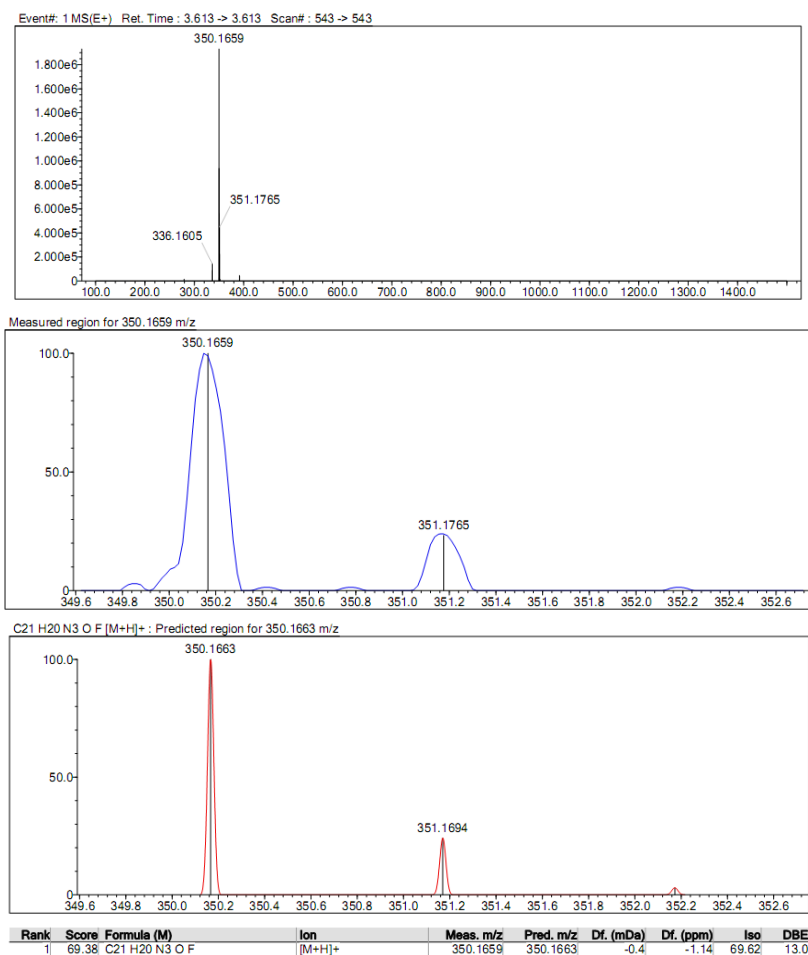
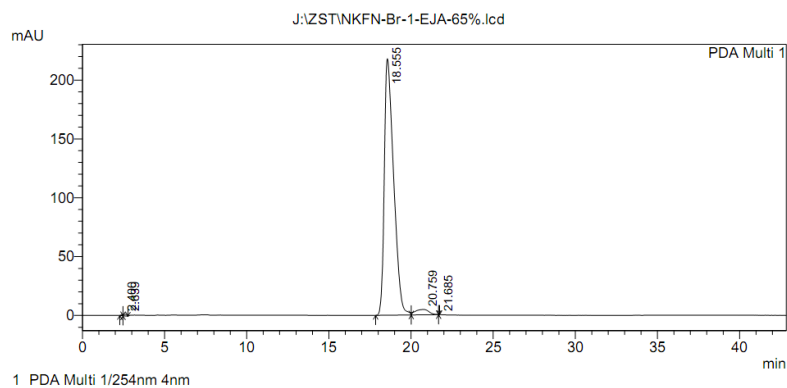


Figure S18-3. HRMS of compound **5b-1**

<Chromatogram>



PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.400	7612	937	0.086	0.416
2	2.639	19328	1492	0.218	0.662
3	18.555	8579698	218065	96.695	96.843
4	20.759	266126	4608	2.999	2.046
5	21.685	168	73	0.002	0.032
Total		8872931	225174	100.000	100.000

Figure S18-4. HPLC of compound **5b-1**

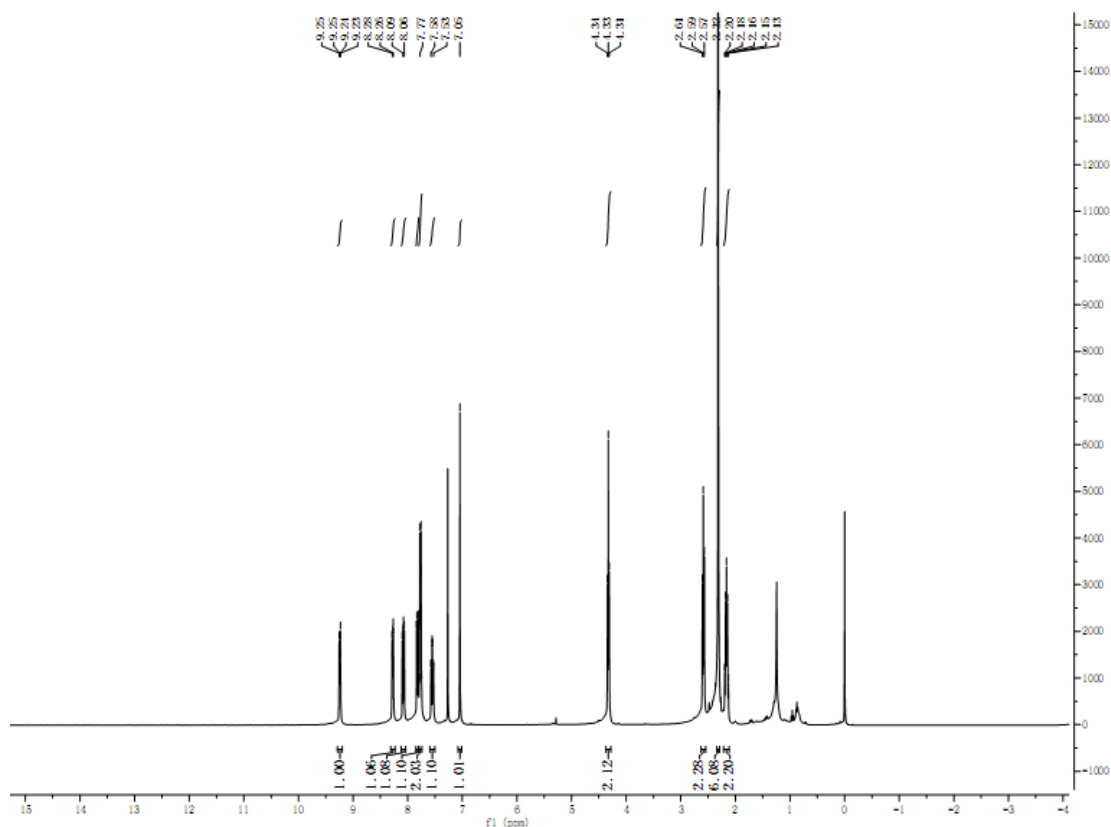


Figure S19-1. ¹H NMR spectra of compound **5b-2** (400 MHz, CDCl₃)

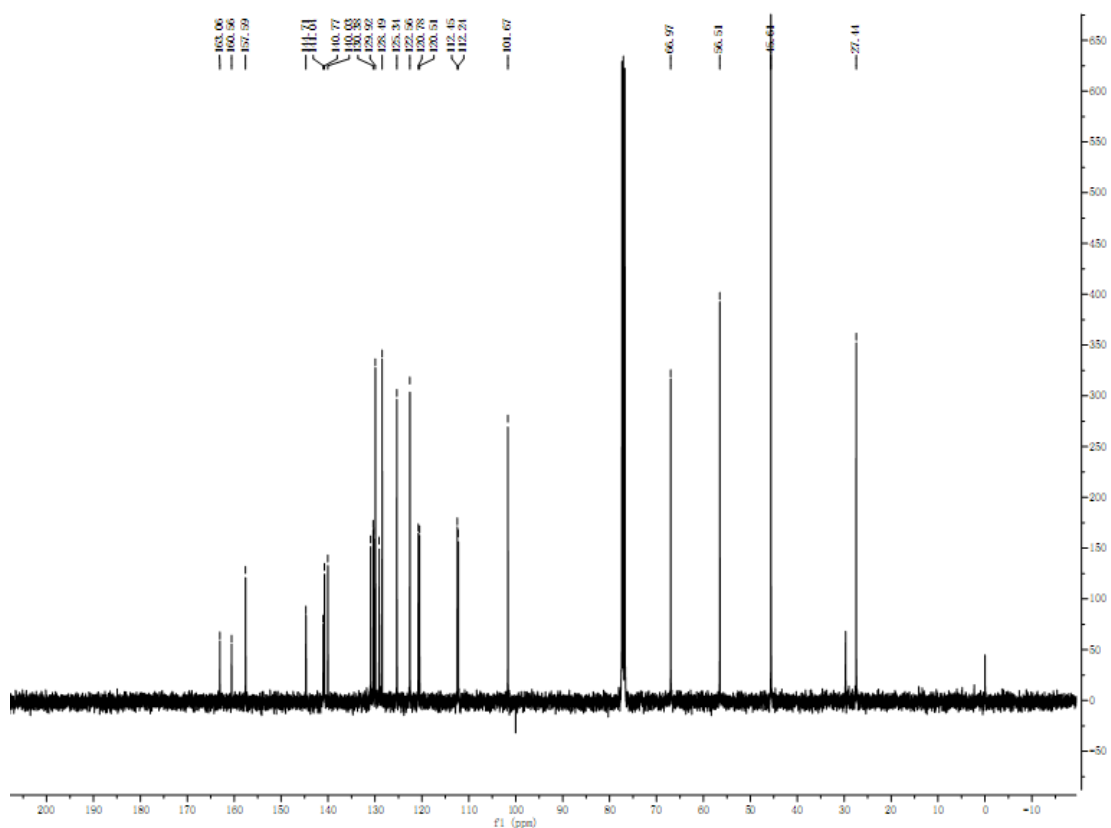


Figure S19-2. ¹³C NMR spectra of compound **5b-2** (101 MHz, CDCl₃)

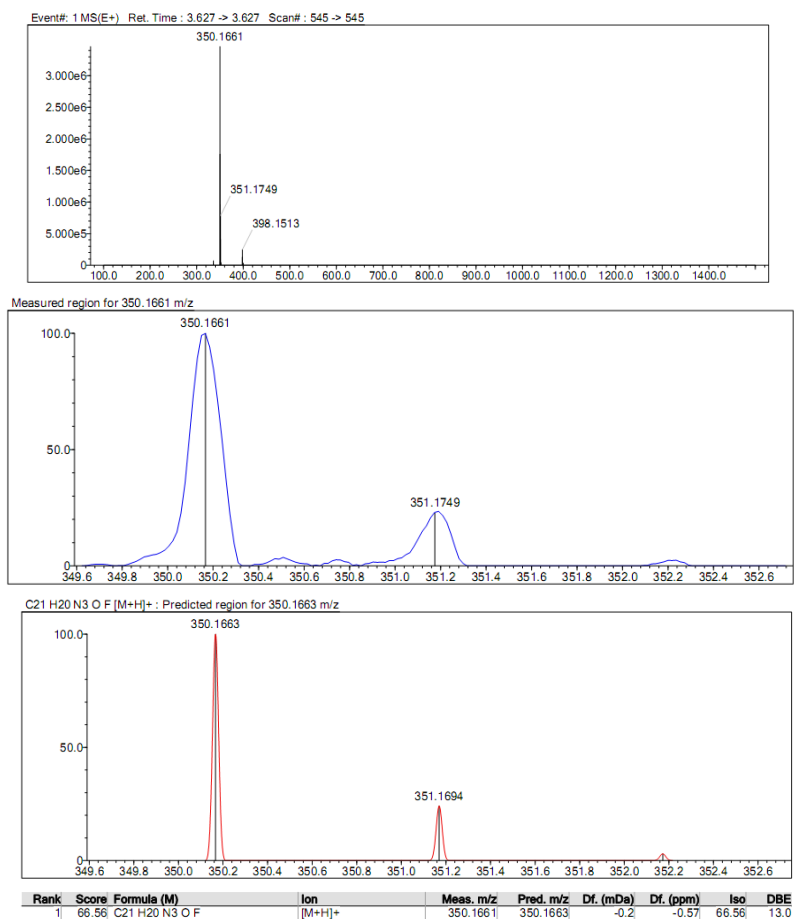
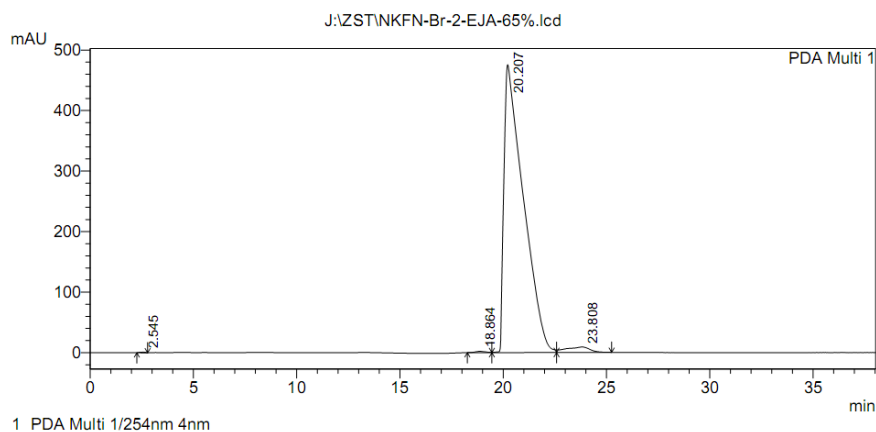


Figure S19-3. HRMS of compound **5b-2**

<Chromatogram>



PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.545	26179	1282	0.082	0.263
2	18.864	70660	2031	0.222	0.416
3	20.207	31024472	475688	97.454	97.499
4	23.808	713644	8890	2.242	1.822
Total		31834955	487891	100.000	100.000

Figure S19-4. HPLC of compound **5b-2**

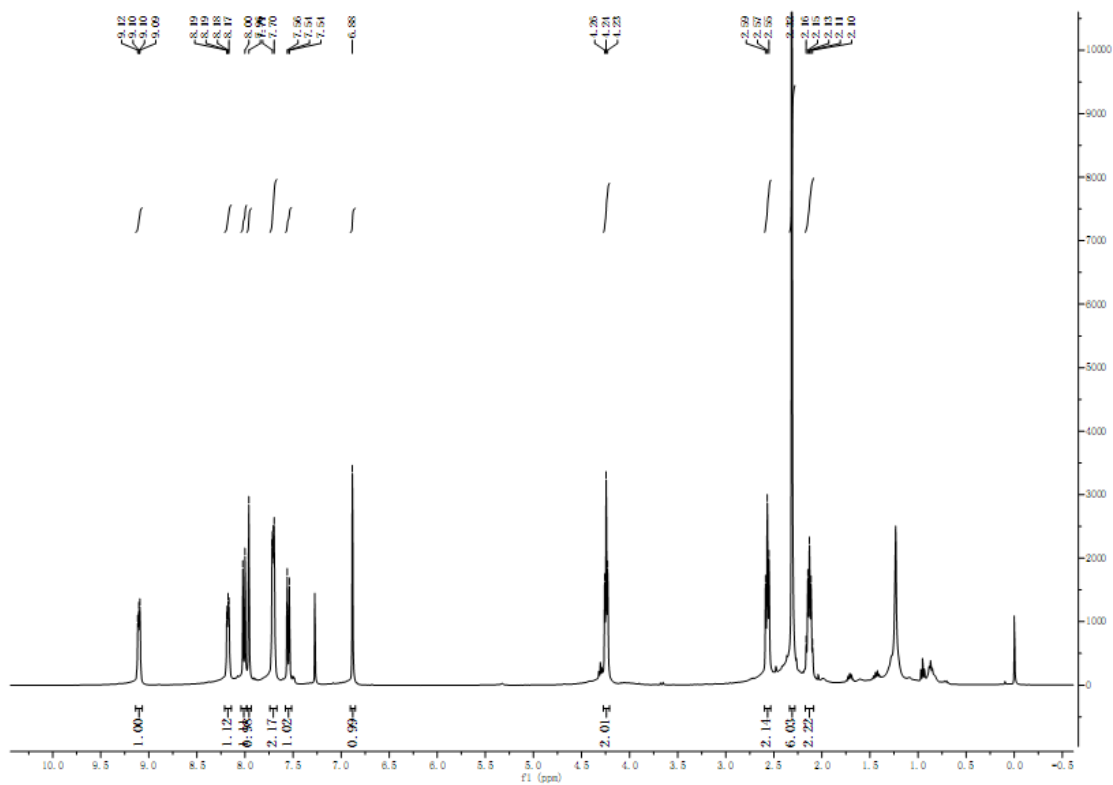


Figure S20-1. ^1H NMR spectra of compound **5c-1** (400 MHz, CDCl_3)

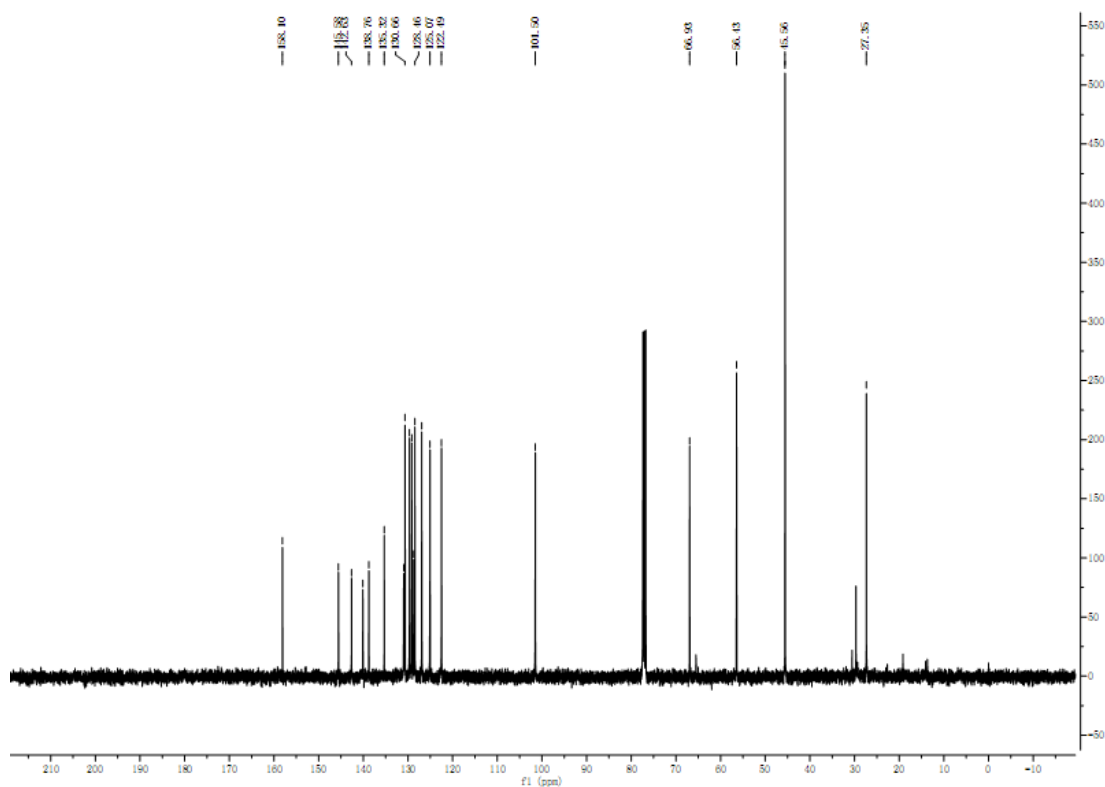


Figure S20-2. ^{13}C NMR spectra of compound **5c-1** (101 MHz, CDCl_3)

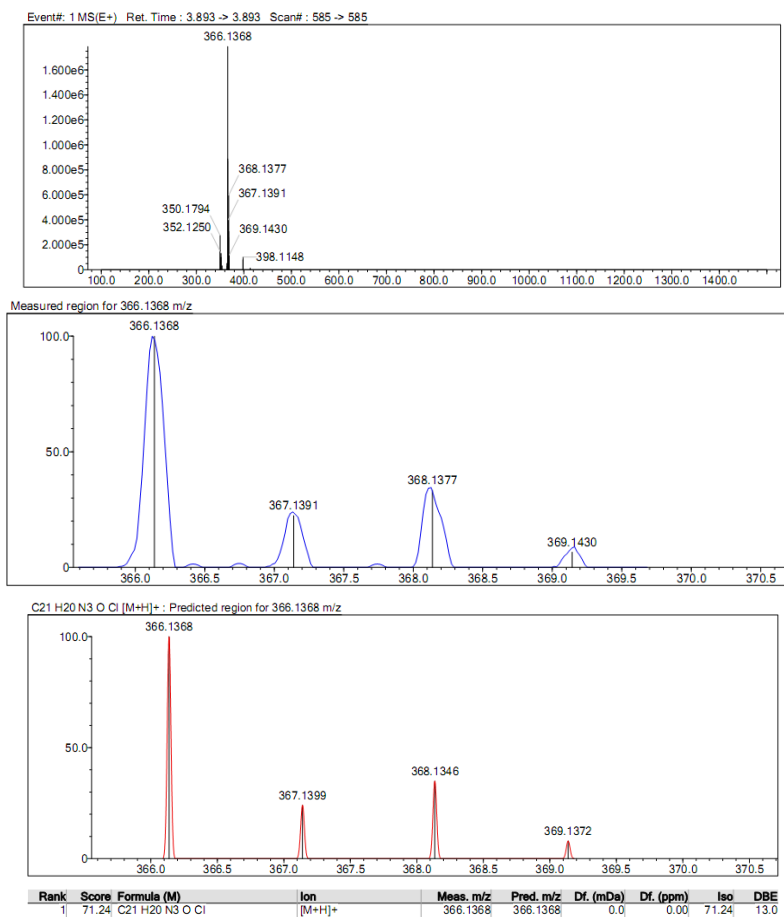
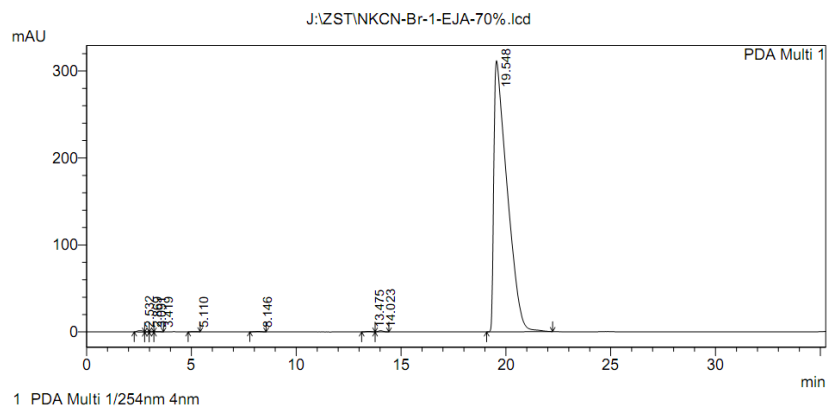


Figure S20-3. HRMS of compound **5c-1**

<Chromatogram>



PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.532	28647	1529	0.207	0.481
2	2.869	5746	550	0.042	0.173
3	3.091	9089	888	0.066	0.279
4	3.419	8662	464	0.063	0.146
5	5.110	7534	382	0.054	0.120
6	8.146	11580	452	0.084	0.142
7	13.475	14805	709	0.107	0.223
8	14.023	23948	1342	0.173	0.422
9	19.548	13723497	311861	99.205	98.014
Total		13833507	318179	100.000	100.000

Figure S20-4. HPLC of compound **5c-1**

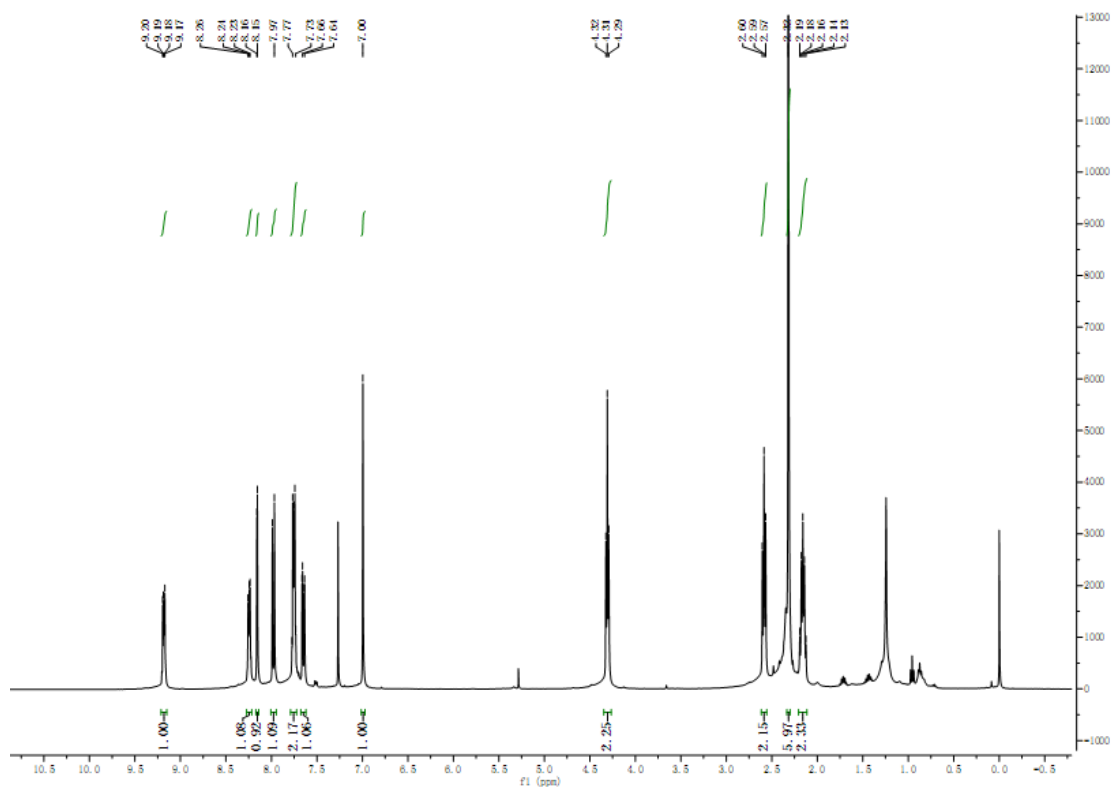


Figure S21-1. ^1H NMR spectra of compound **5c-2** (400 MHz, CDCl_3)

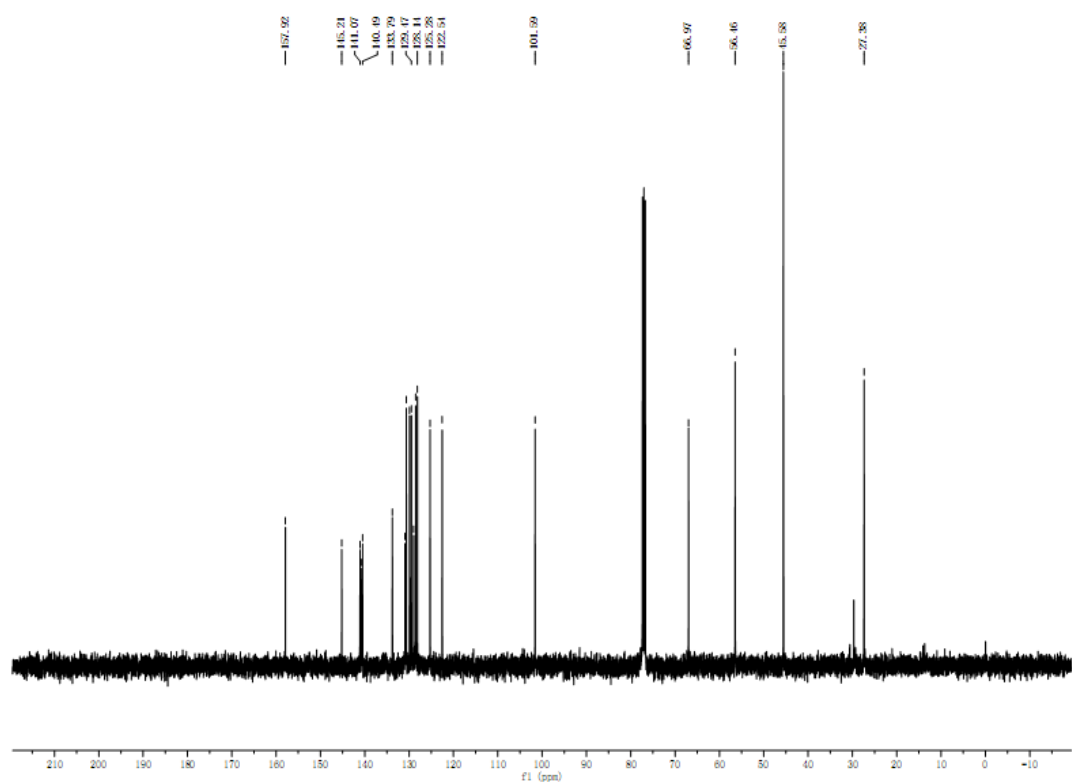


Figure S21-2. ^{13}C NMR spectra of compound **5c-2** (101 MHz, CDCl_3)

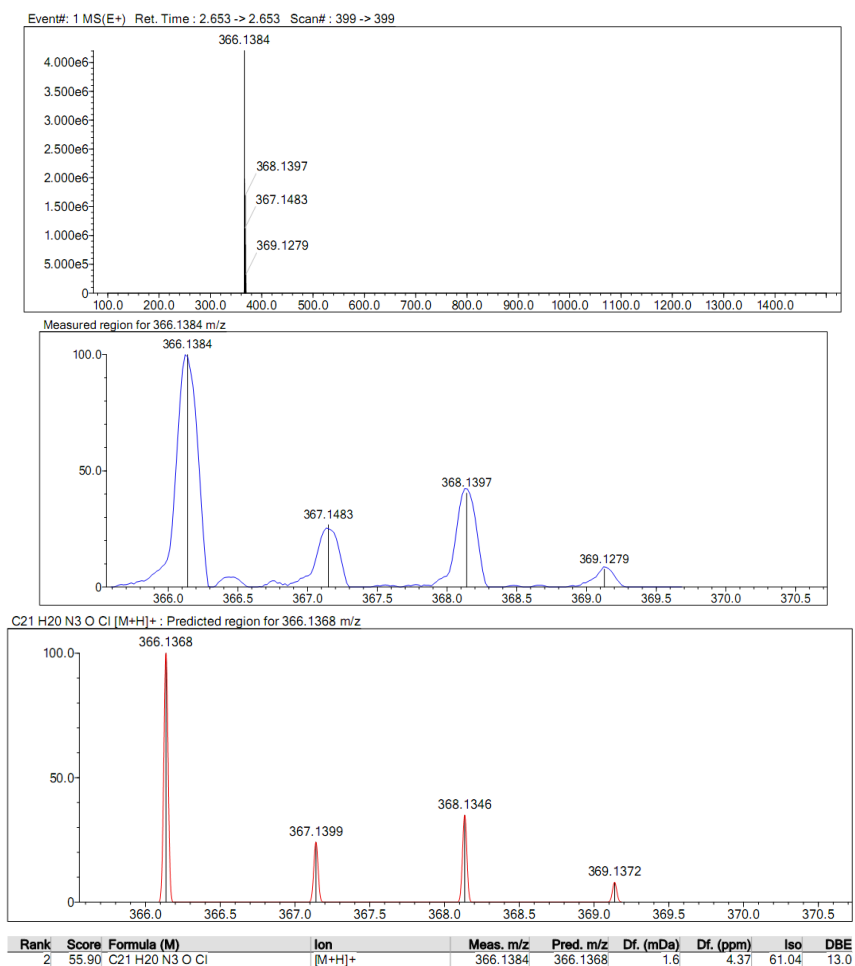
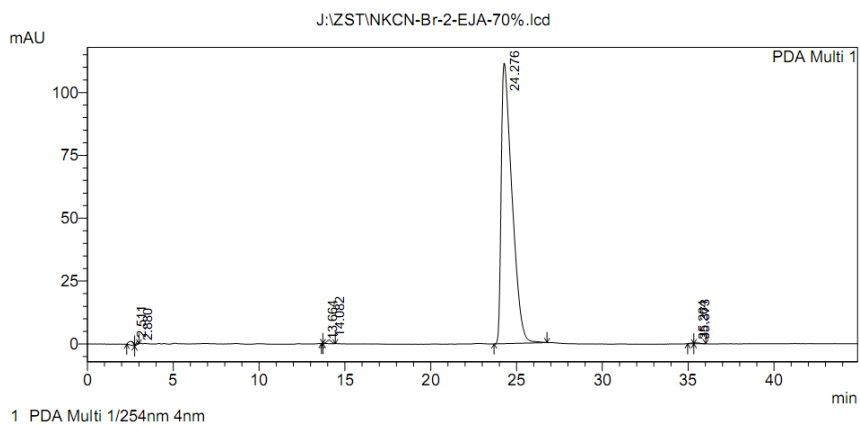


Figure S21-3. HRMS of compound **5c-2**

<Chromatogram>



PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.511	28566	1456	0.578	1.259
2	2.880	4198	423	0.085	0.366
3	13.664	200	34	0.004	0.029
4	14.082	27694	1435	0.560	1.242
5	24.276	4872490	111550	98.523	96.510
6	35.264	3957	313	0.080	0.271
7	35.373	8417	373	0.170	0.322
Total		4945521	115584	100.000	100.000

Figure S21-4. HPLC of compound **5c-2**

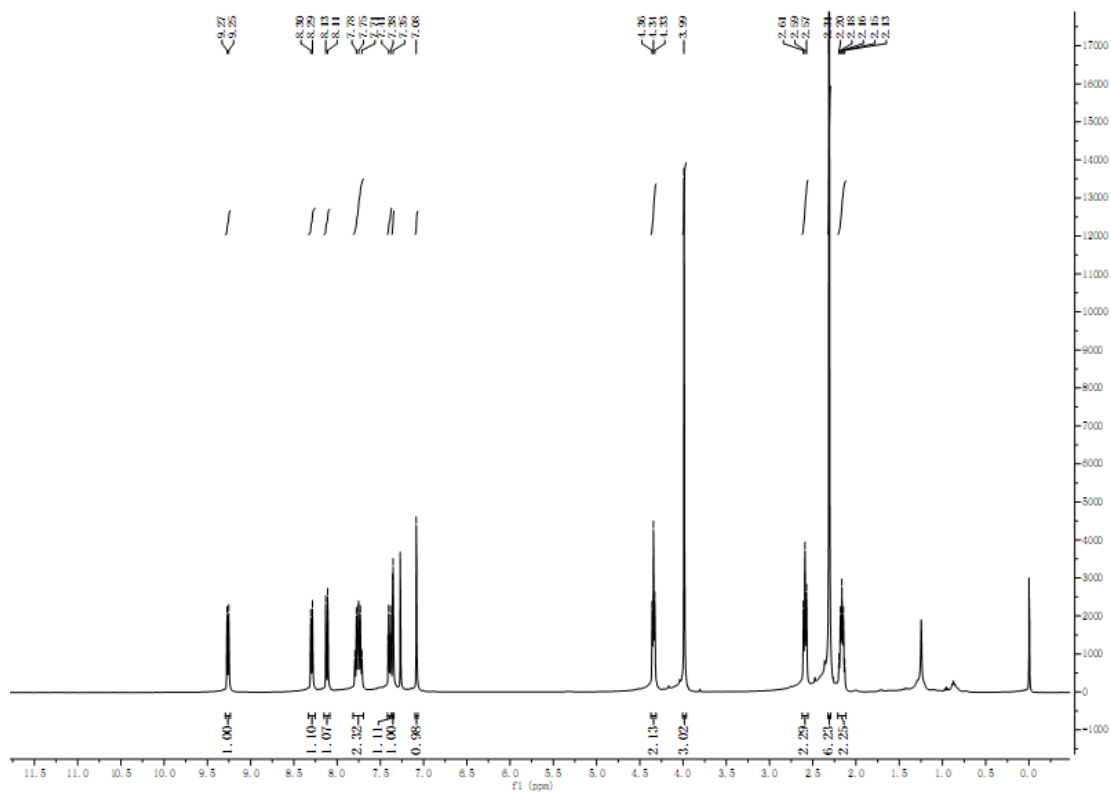


Figure S22-1. ^1H NMR spectra of compound **5d-1** (400 MHz, CDCl_3)

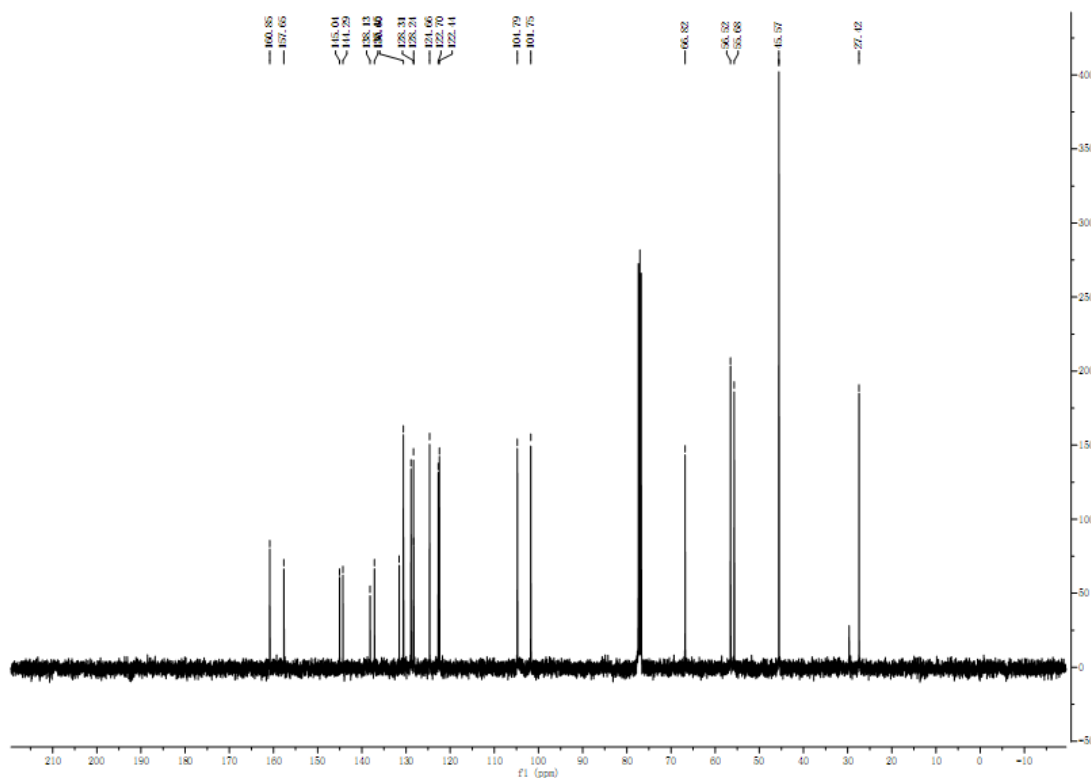


Figure S22-2. ^{13}C NMR spectra of compound **5d-1** (101 MHz, CDCl_3)

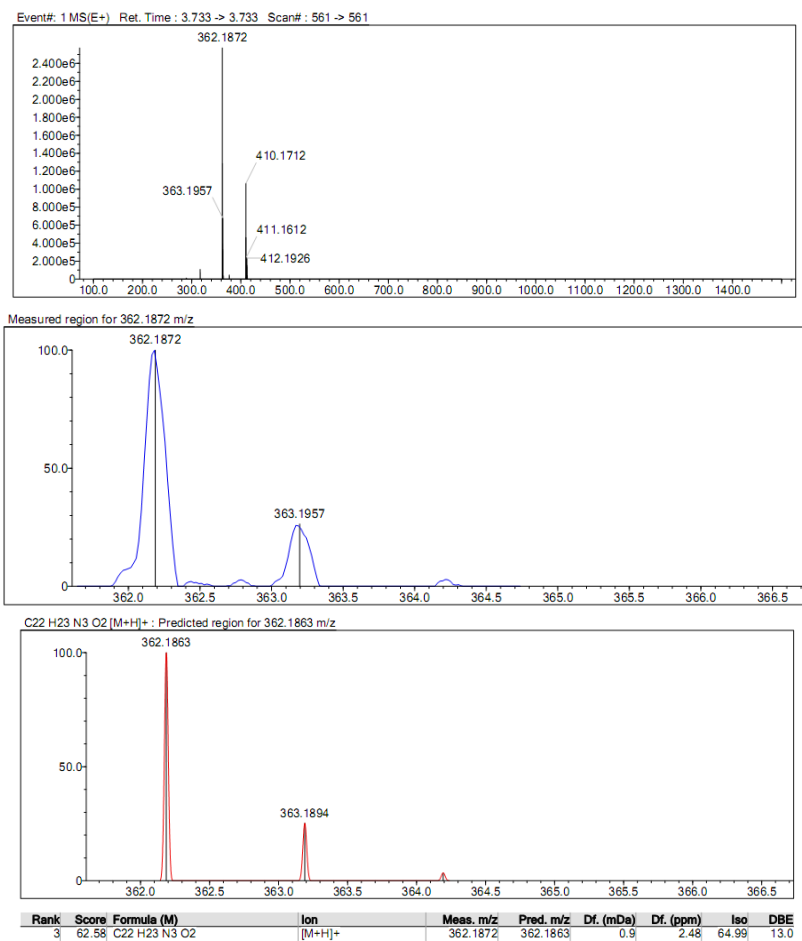
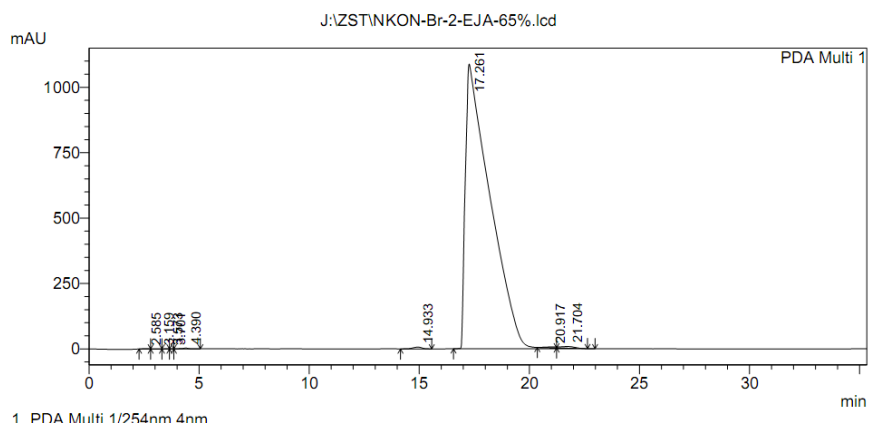


Figure S22-3. HRMS of compound **5d-1**

<Chromatogram>



PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.585	25146	1452	0.030	0.131
2	3.159	16708	767	0.020	0.069
3	3.573	13418	742	0.016	0.067
4	3.701	9677	818	0.011	0.074
5	4.390	100776	3838	0.118	0.345
6	14.933	210523	7074	0.247	0.636
7	17.261	84410195	1088383	99.100	97.876
8	20.917	100282	2637	0.118	0.237
9	21.704	289715	6288	0.340	0.565
Total		85176440	1111999	100.000	100.000

Figure S22-4. HPLC of compound **5d-1**

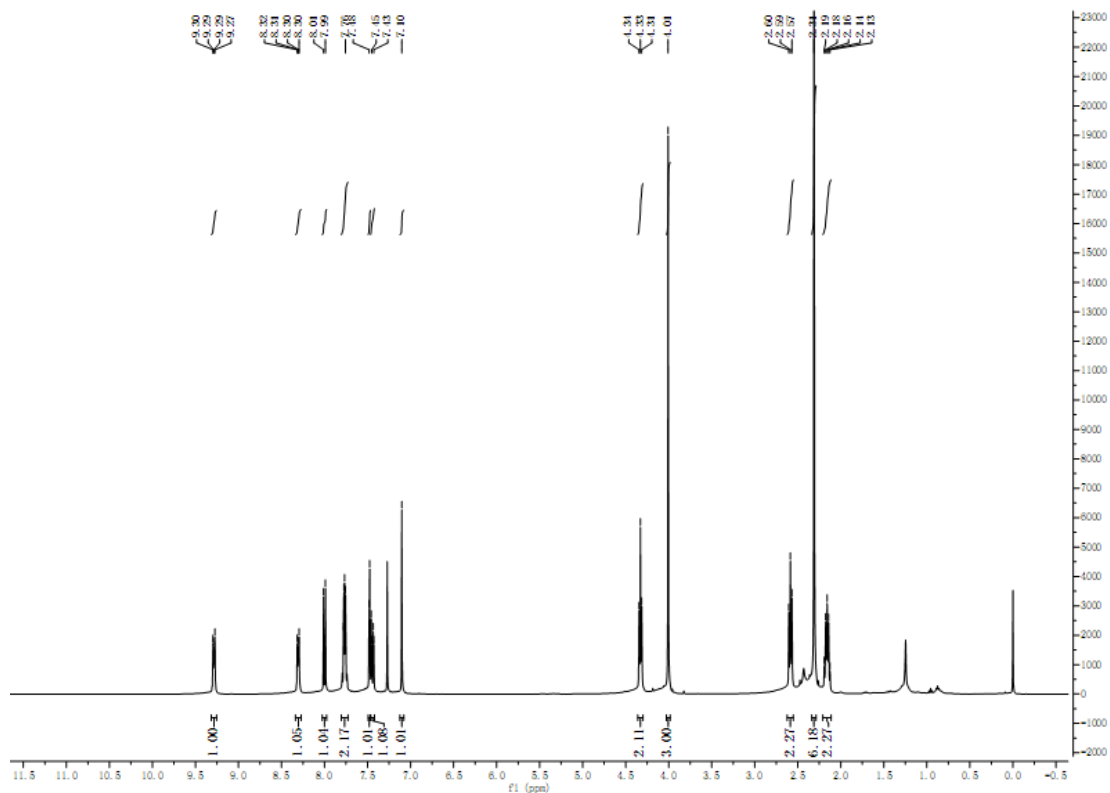


Figure S23-1. ^1H NMR spectra of compound **5d-2** (400 MHz, CDCl_3)

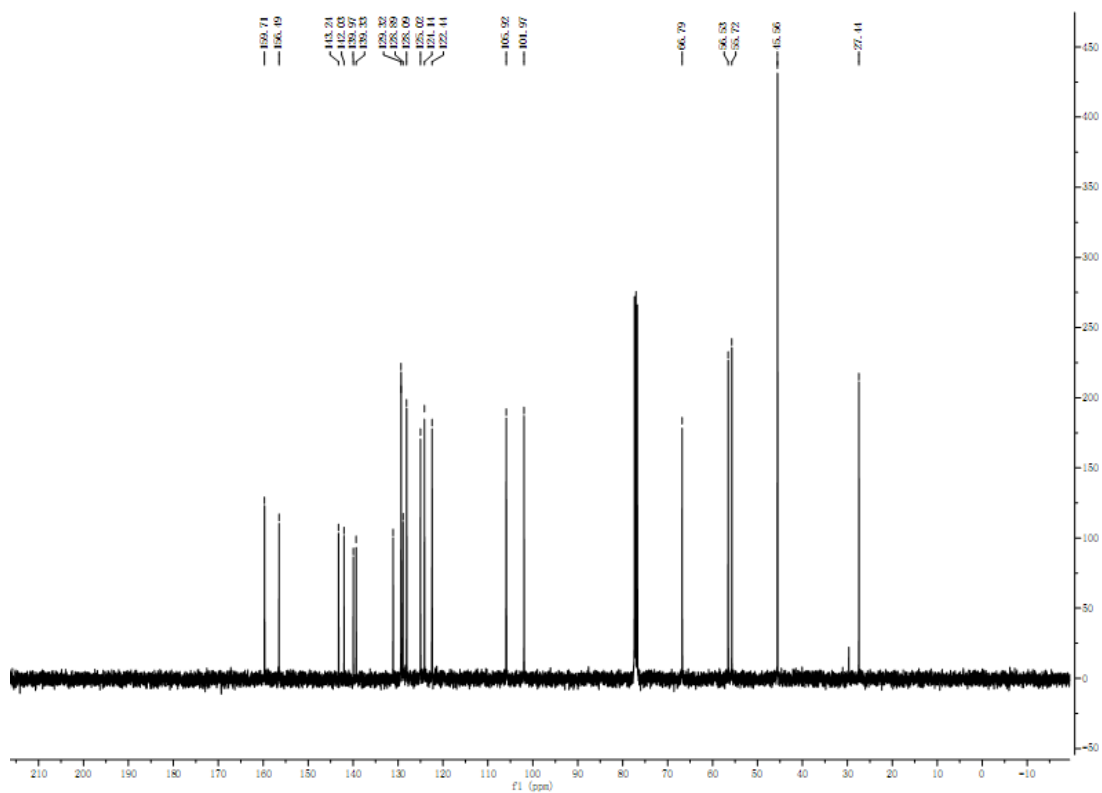


Figure S23-2. ^{13}C NMR spectra of compound **5d-2** (101 MHz, CDCl_3)

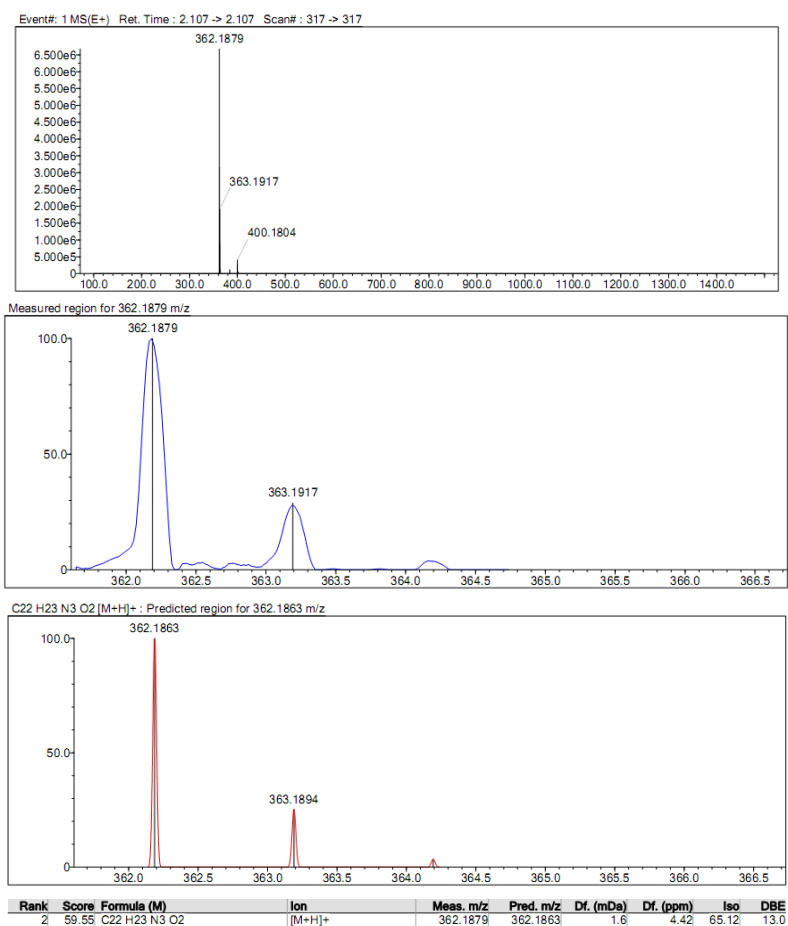
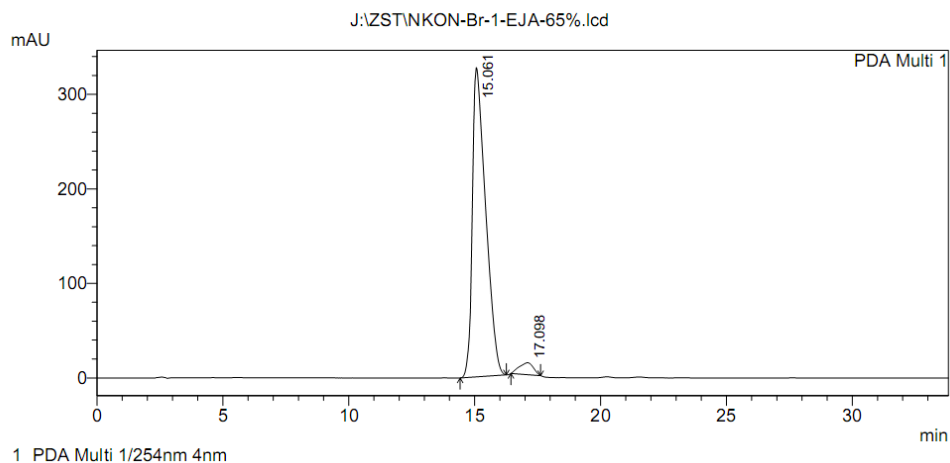


Figure S23-3. HRMS of compound **5d-2**

<Chromatogram>



PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	15.061	11979096	327071	96.016	96.260
2	17.098	497104	12708	3.984	3.740
Total		12476200	339780	100.000	100.000

Figure S23-4. HPLC of compound **5d-2**

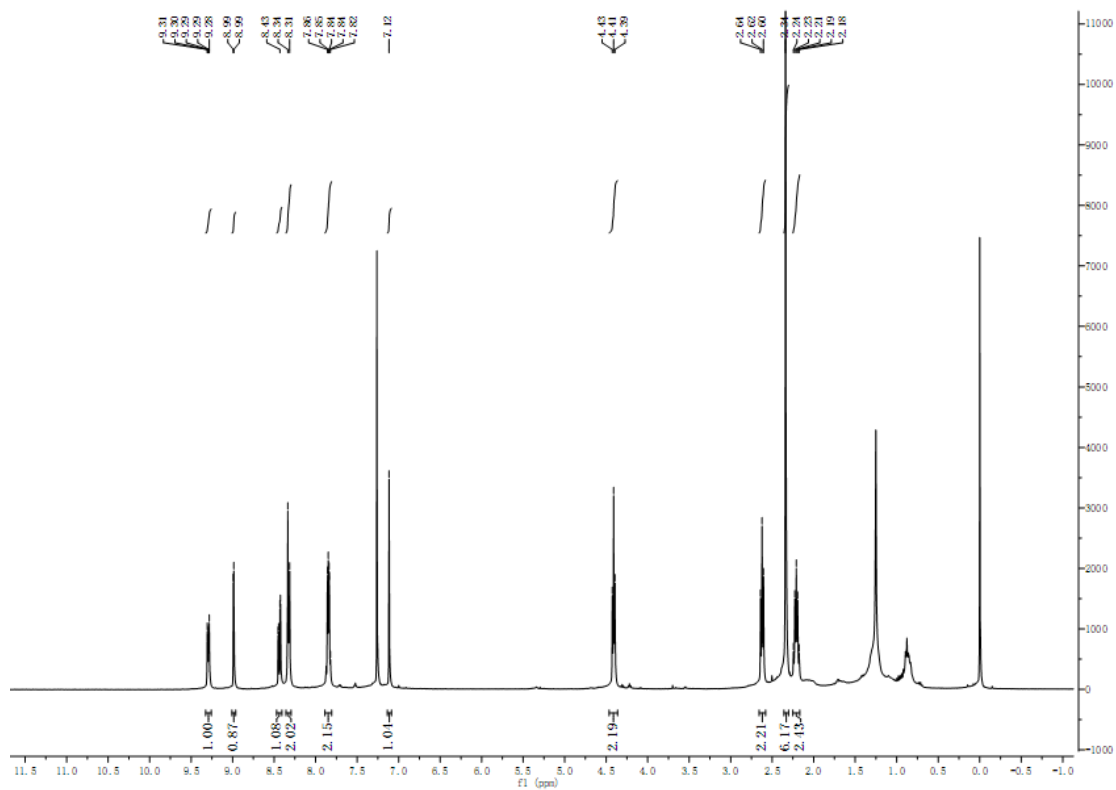


Figure S24-1. ^1H NMR spectra of compound **5e-1** (400 MHz, CDCl_3)

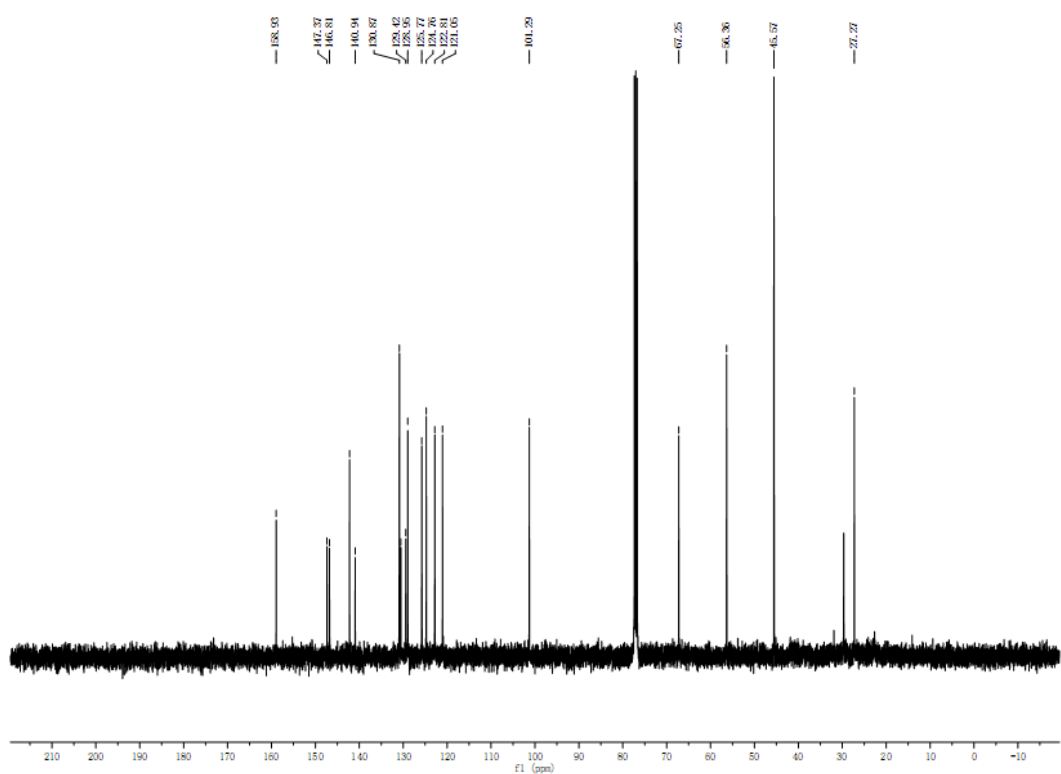


Figure S24-2. ^{13}C NMR spectra of compound **5e-1** (101 MHz, CDCl_3)

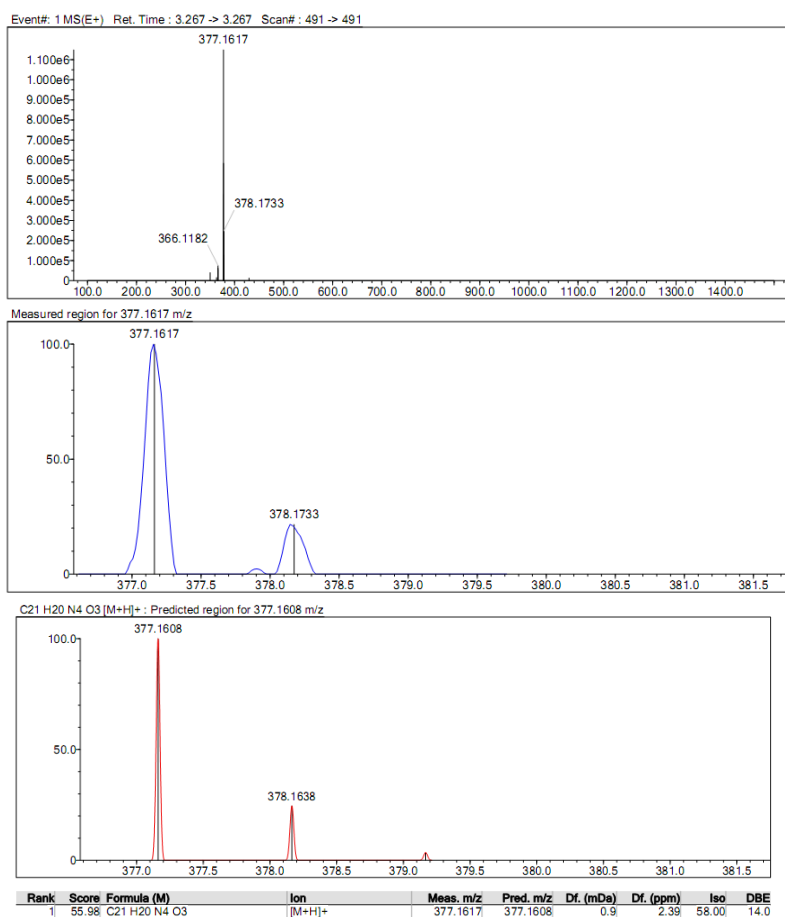
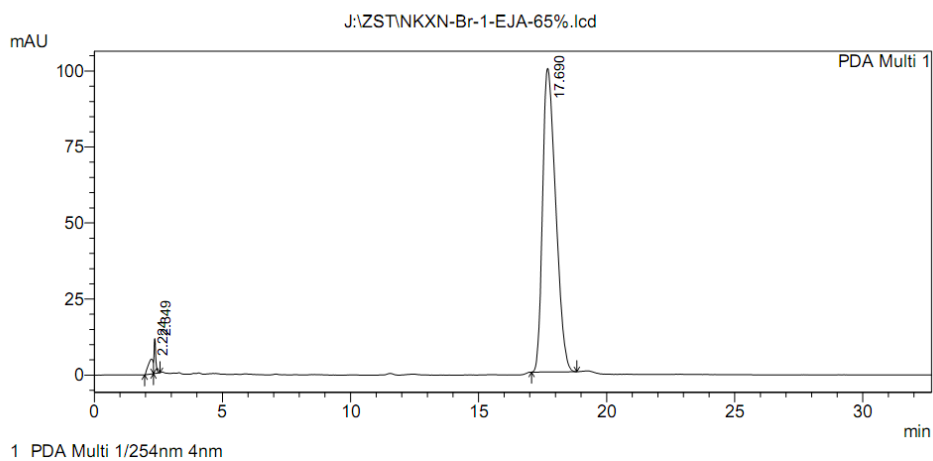


Figure S24-3. HRMS of compound **5e-1**

<Chromatogram>



PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.224	65446	4881	1.780	4.198
2	2.349	53642	11509	1.459	9.897
3	17.690	3557666	99897	96.761	85.906
Total		3676754	116287	100.000	100.000

Figure S24-4. HPLC of compound **5e-1**

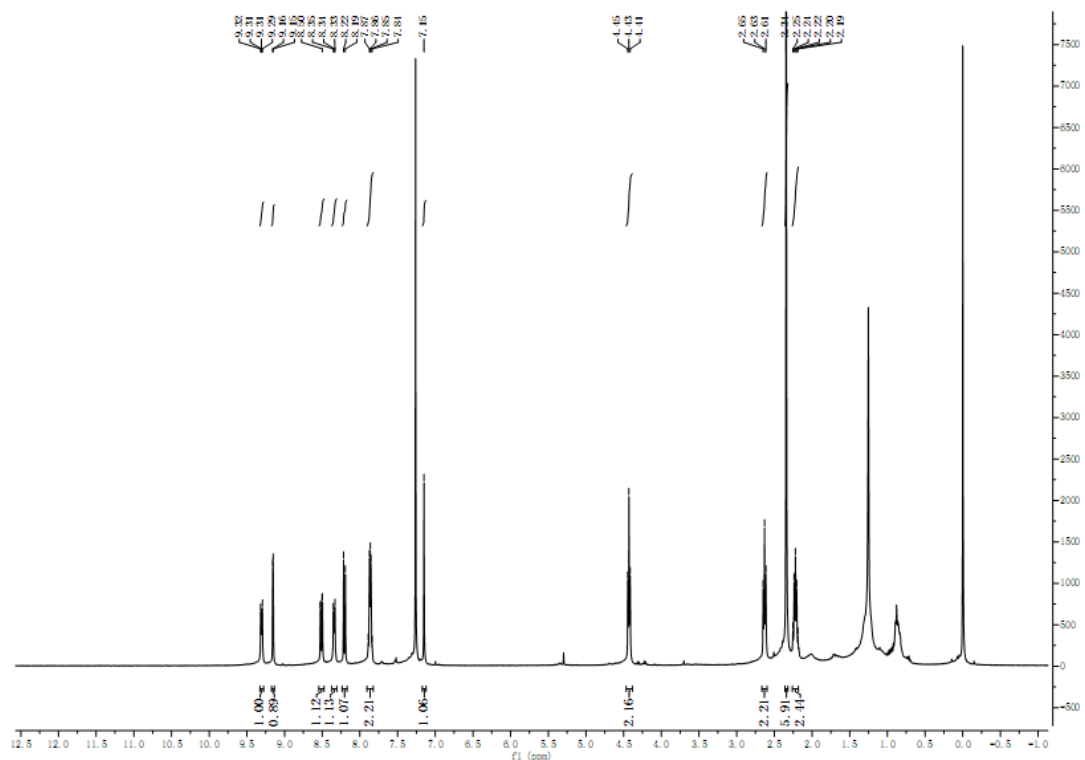


Figure S25-1. ¹H NMR spectra of compound **5e-2** (400 MHz, CDCl₃)

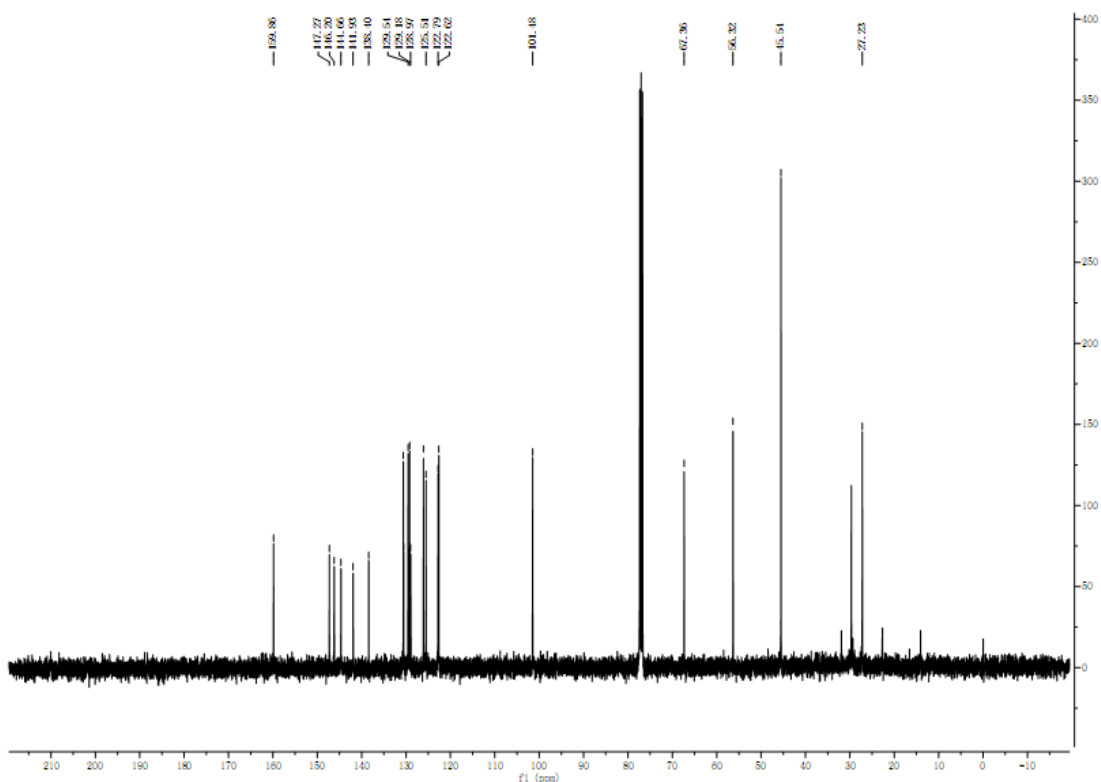


Figure S25-2. ¹³C NMR spectra of compound **5e-2** (101 MHz, CDCl₃)

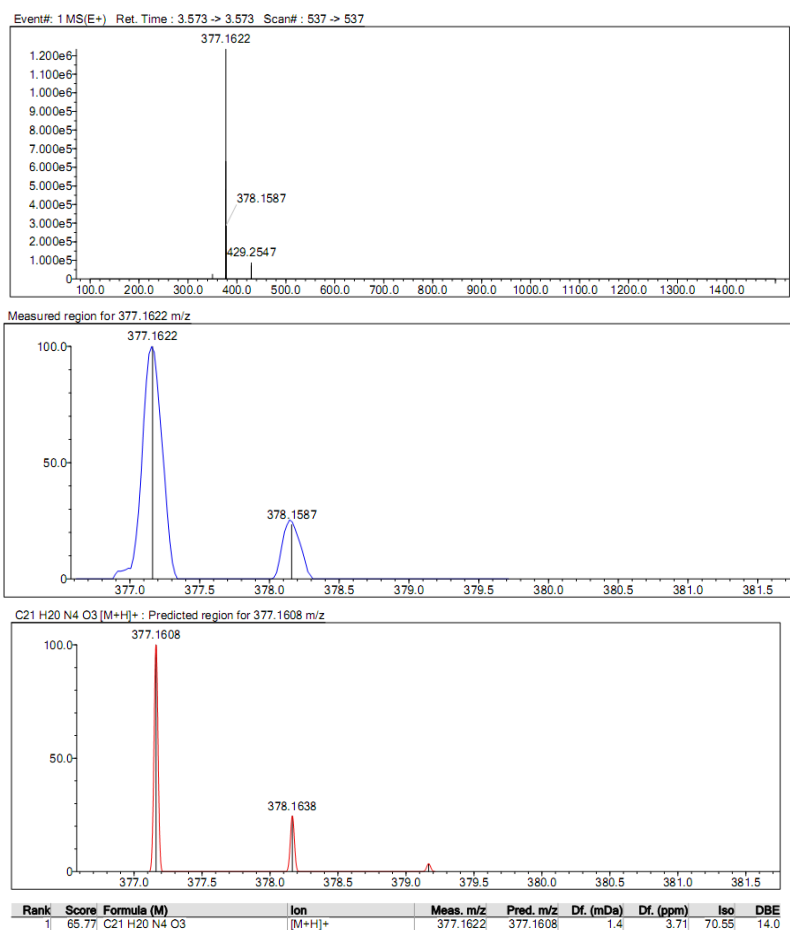
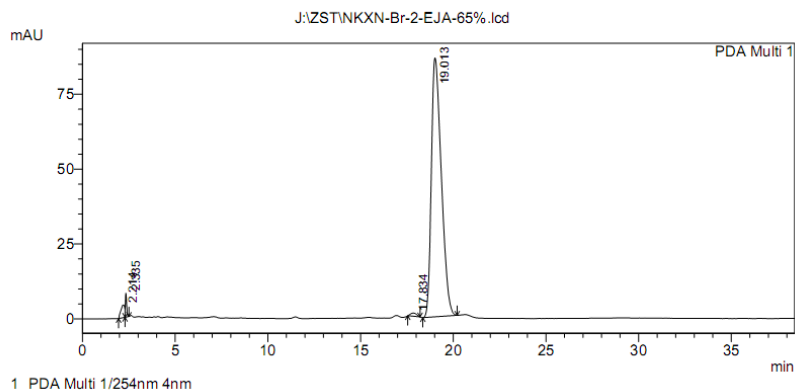


Figure S25-3. HRMS of compound 5e-2

<Chromatogram>

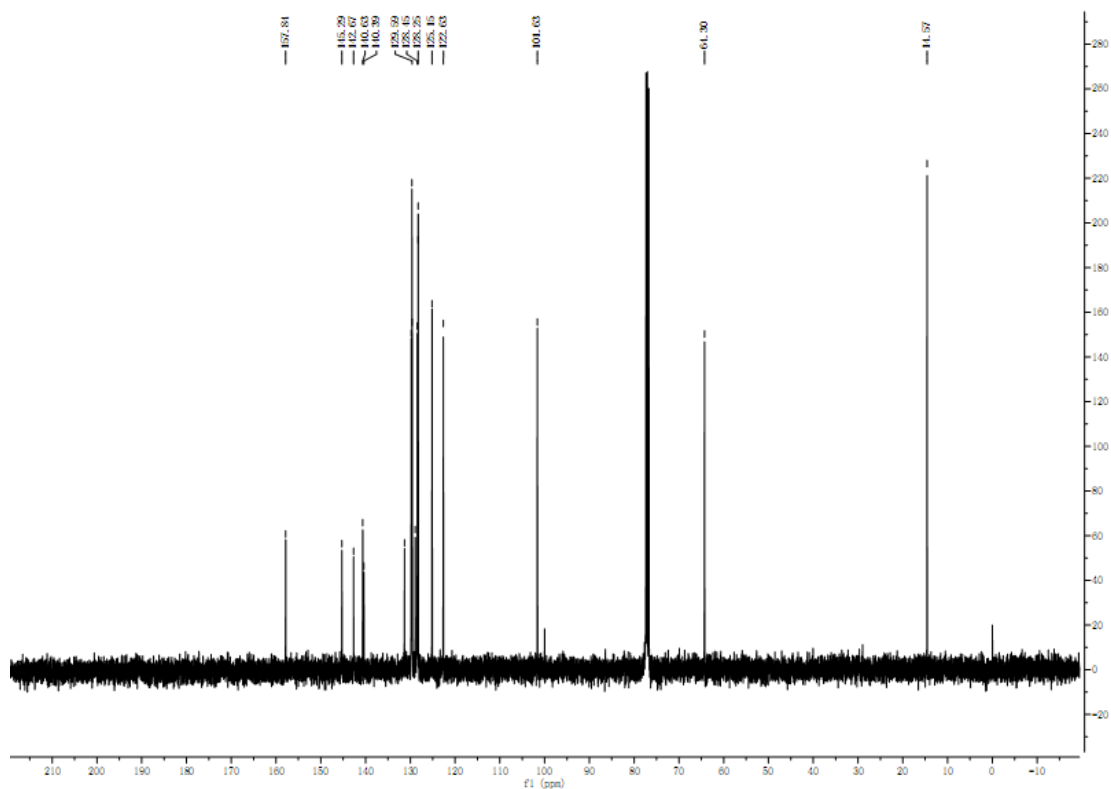
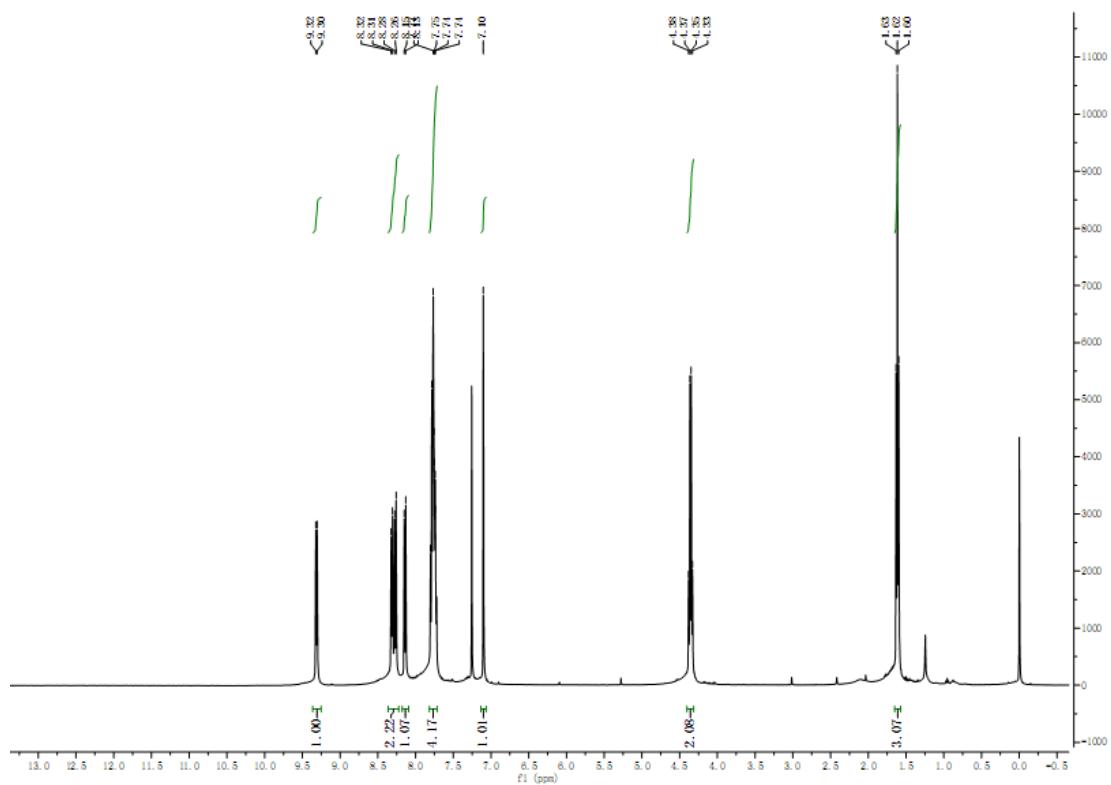


PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.214	60726	4243	1.816	4.253
2	2.335	36717	7990	1.098	8.009
3	17.834	24373	1053	0.729	1.055
4	19.013	3221474	86470	96.356	86.682
Total		3343291	99755	100.000	100.000

Figure S25-4. HPLC of compound 5e-2



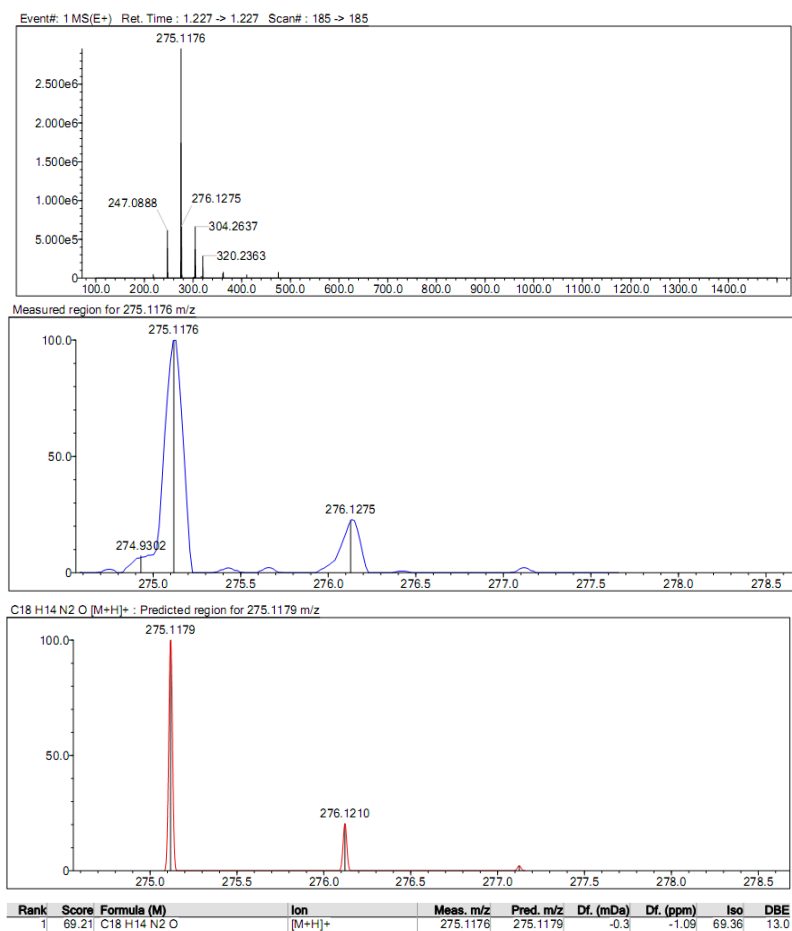
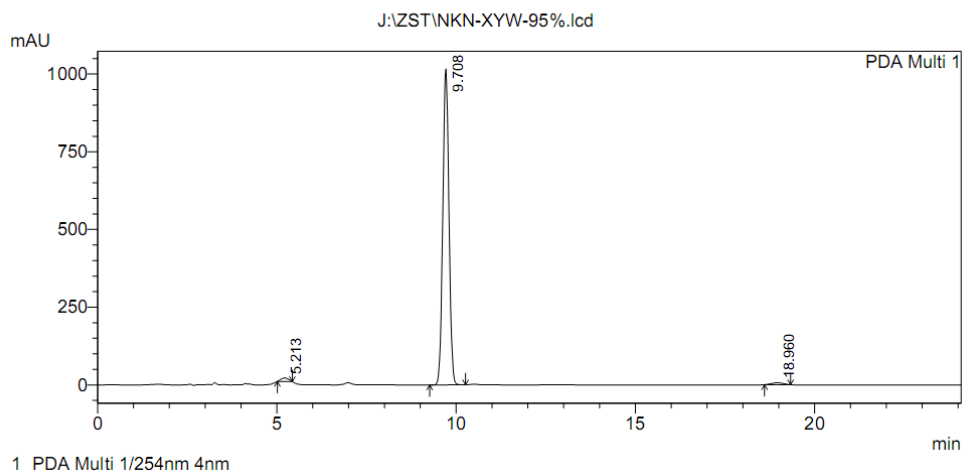


Figure S26-3. HRMS of compound 7

<Chromatogram>



PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.213	172298	11346	1.386	1.097
2	9.708	12112179	1016614	97.456	98.292
3	18.960	143845	6321	1.157	0.611
Total		12428322	1034281	100.000	100.000

Figure S26-4. HPLC of compound 7