

Supporting Information for

Original article

Discovery of [1,2,3]triazolo[4,5-*d*]pyrimidine derivatives as highly potent, selective, and cellularly active USP28 inhibitors

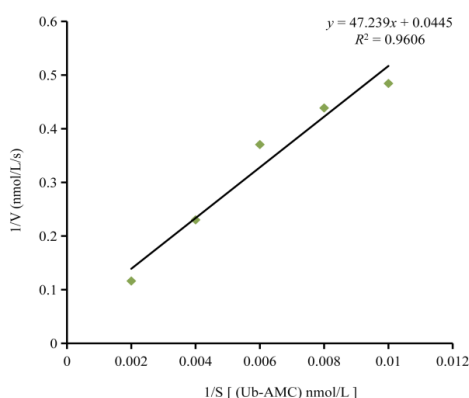
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School of Pharmaceutical Sciences, Zhengzhou University, Zhengzhou 450001, China

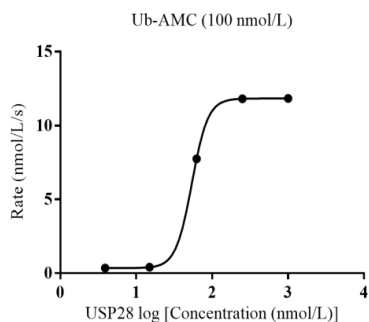
Received 12 August 2019; received in revised form 20 October 2019; accepted 6 December 2019

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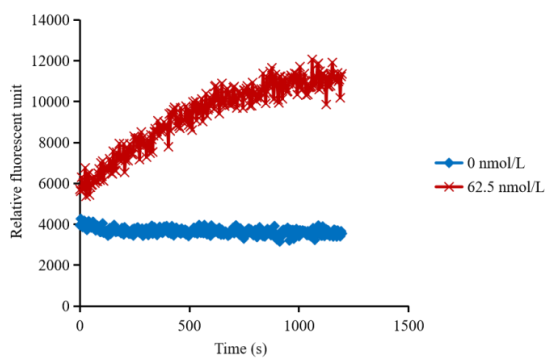
E-mail addresses: yubin@zzu.edu.cn (Bin Yu), shixiaojing@zzu.edu.cn (Xiaojing Shi), liuhm@zzu.edu.cn (Hongmin Liu).



**Figure S1** Determination of the  $K_m$  of USP28 for Ubiquitin-AMC. Varying concentrations of Ubiquitin-AMC were incubated in the presence of 15.625 nmol/L USP28. The diagram shows the mean of  $n = 3$  data for three independent experiments. The initial rate data were fitted to the Michaelis-Menten equation, giving a  $K_m$  value of 1061 nmol/L.

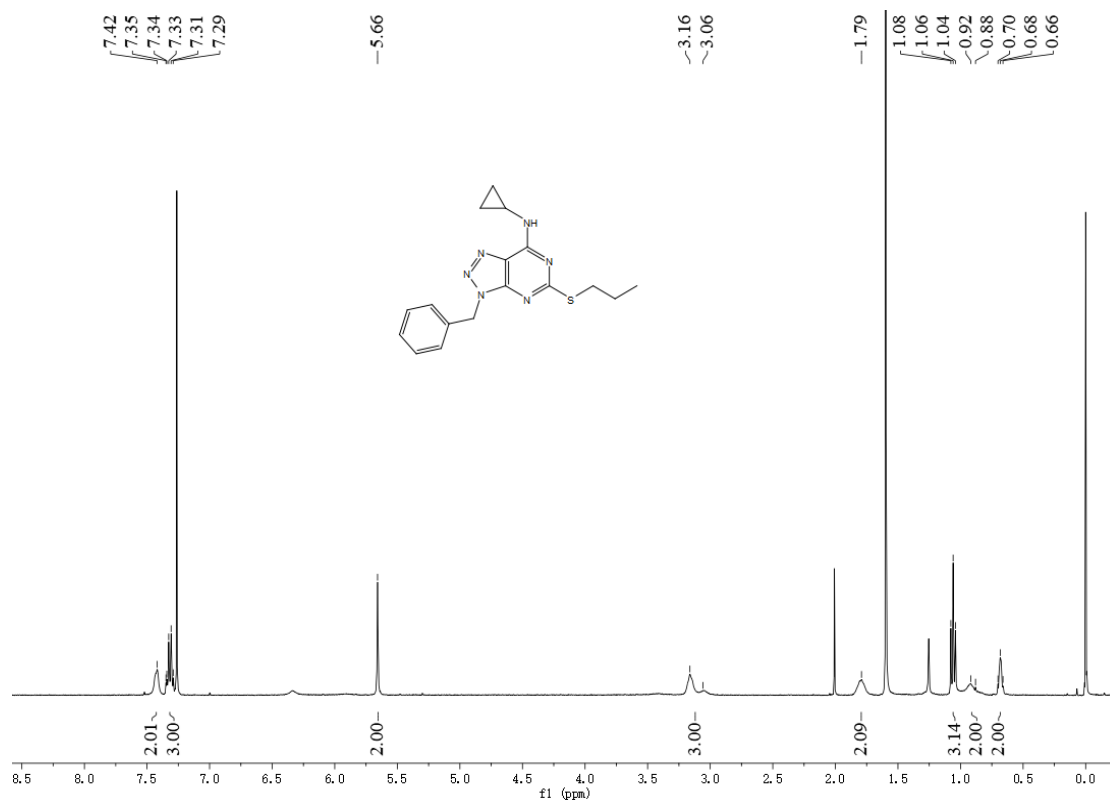


**Figure S2** We employed a screening strategy using purified recombinant USP28 enzyme and a fluorogenic substrate Ub-AMC (100 nmol/L). The  $EC_{50}$  is 54.07 nmol/L. At last, we decided to screen small molecule compounds using purified recombinant USP28 for 62.5 nmol/L.

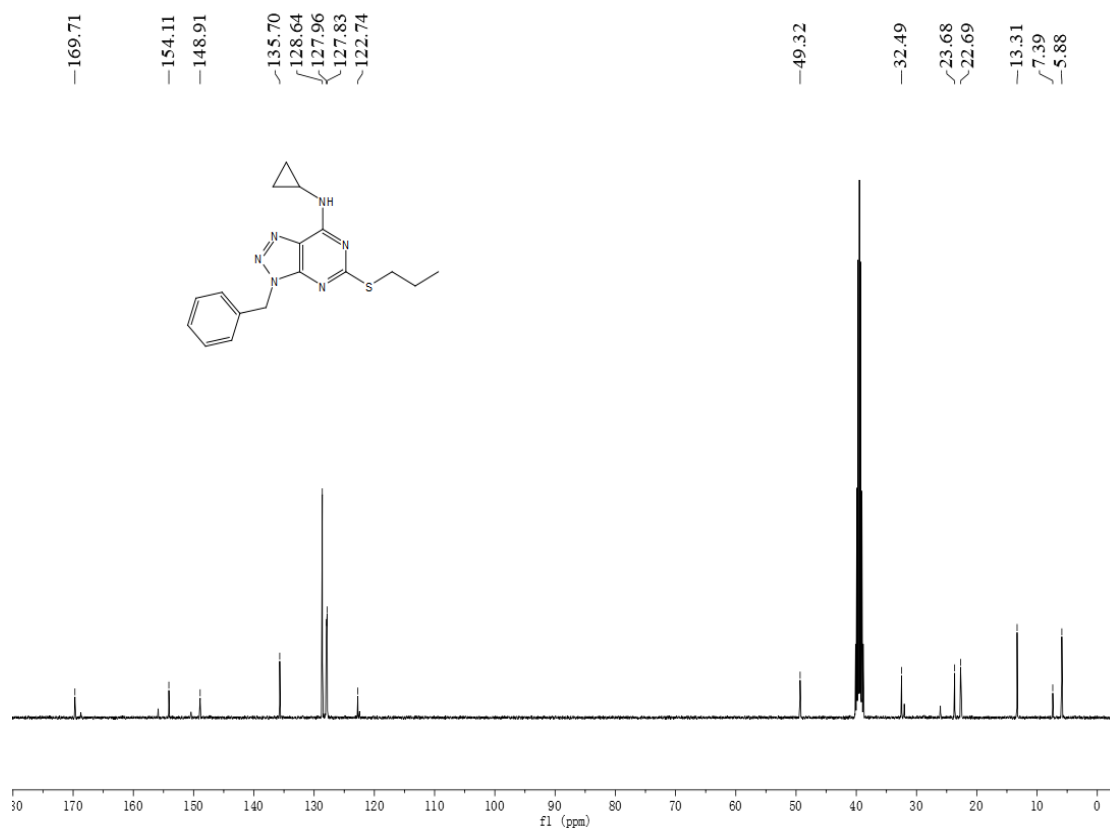


**Figure S3** Representative reaction progress and assay signal following the cleavage of Ubiquitin-AMC by USP28. Reaction time course of 100 nmol/L Ubiquitin-AMC and 62.5 nmol/L USP28. The graph shows representative data for three independent experiments.

### <sup>1</sup>H NMR spectrum of compound 3

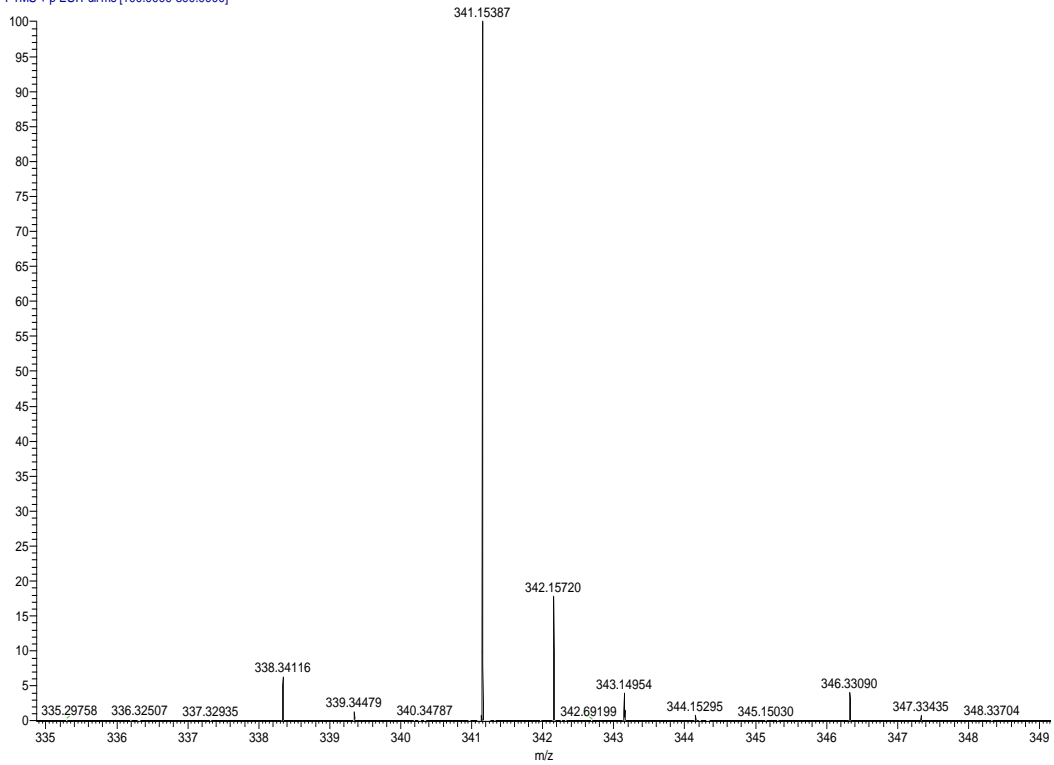


### <sup>13</sup>C NMR spectrum of compound 3

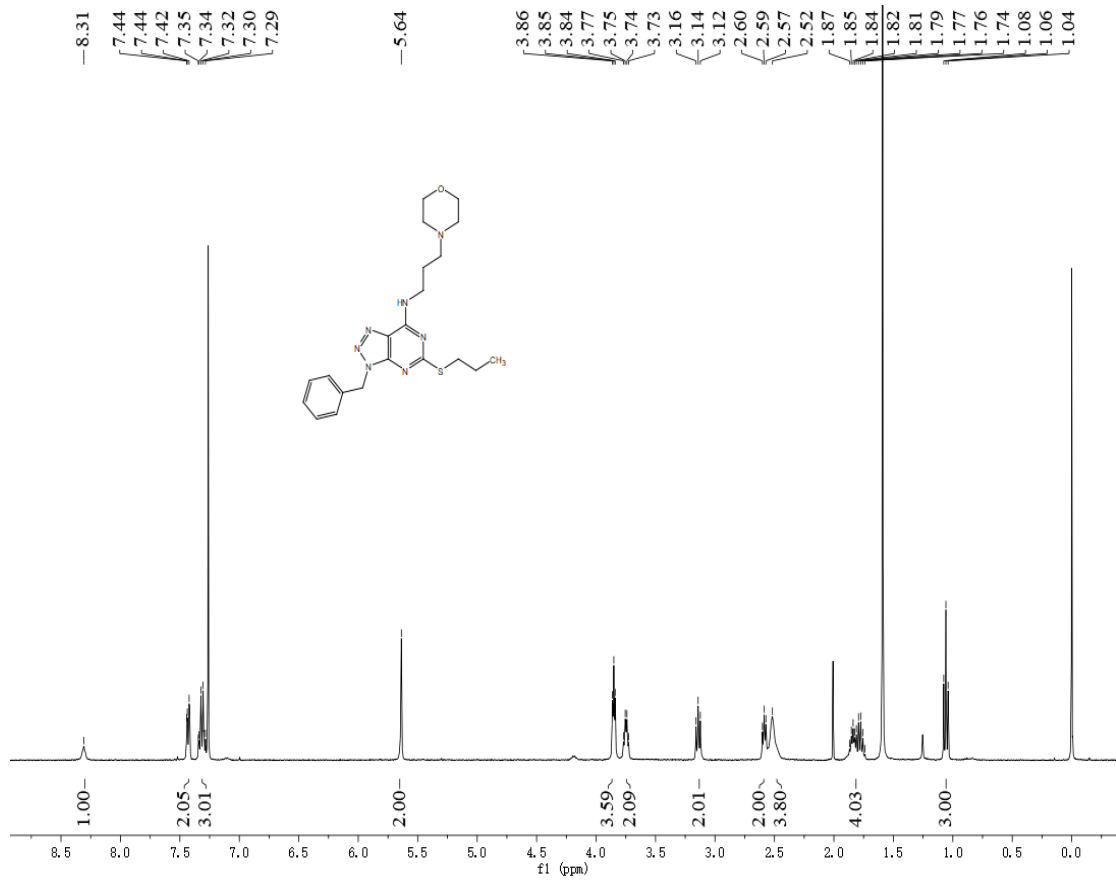


### MS spectrum of compound 3

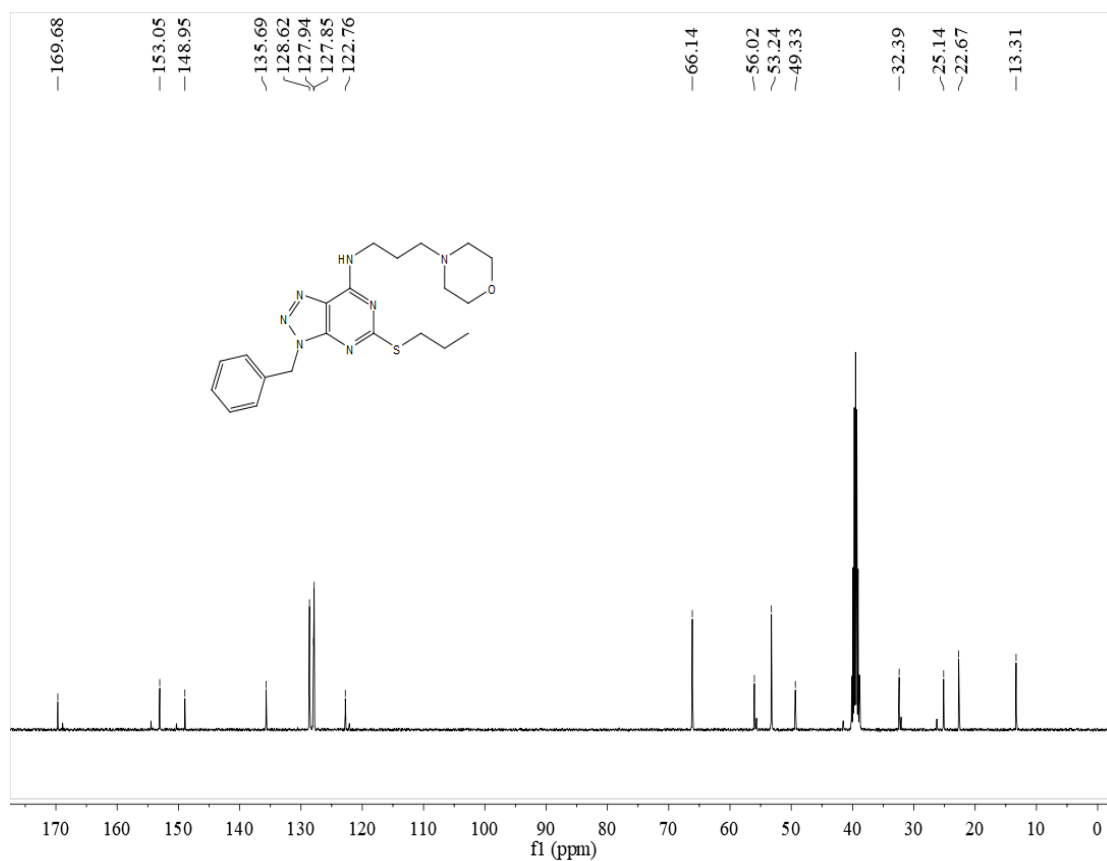
585 #19 RT: 0.18 AV: 1 NL: 4.94E8  
T: FTMS + p ESI Fullms [100.0000-800.0000]



### <sup>1</sup>H NMR spectrum of compound 4

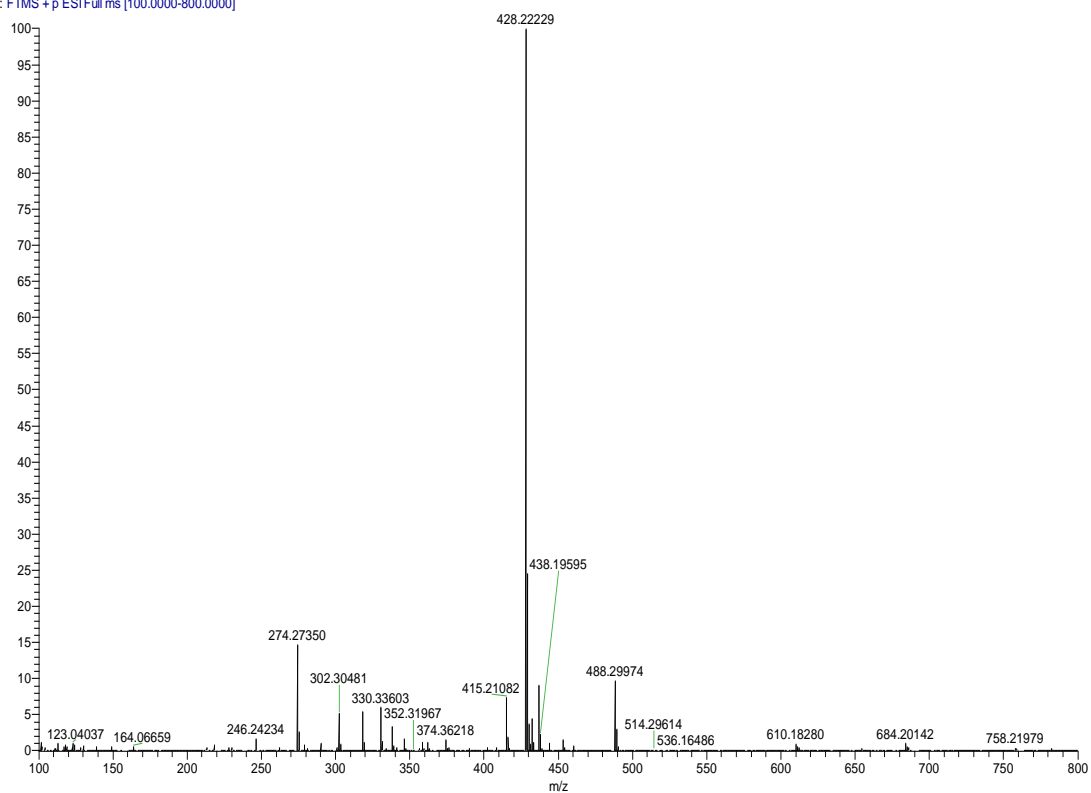


### <sup>13</sup>C NMR spectrum of compound 4

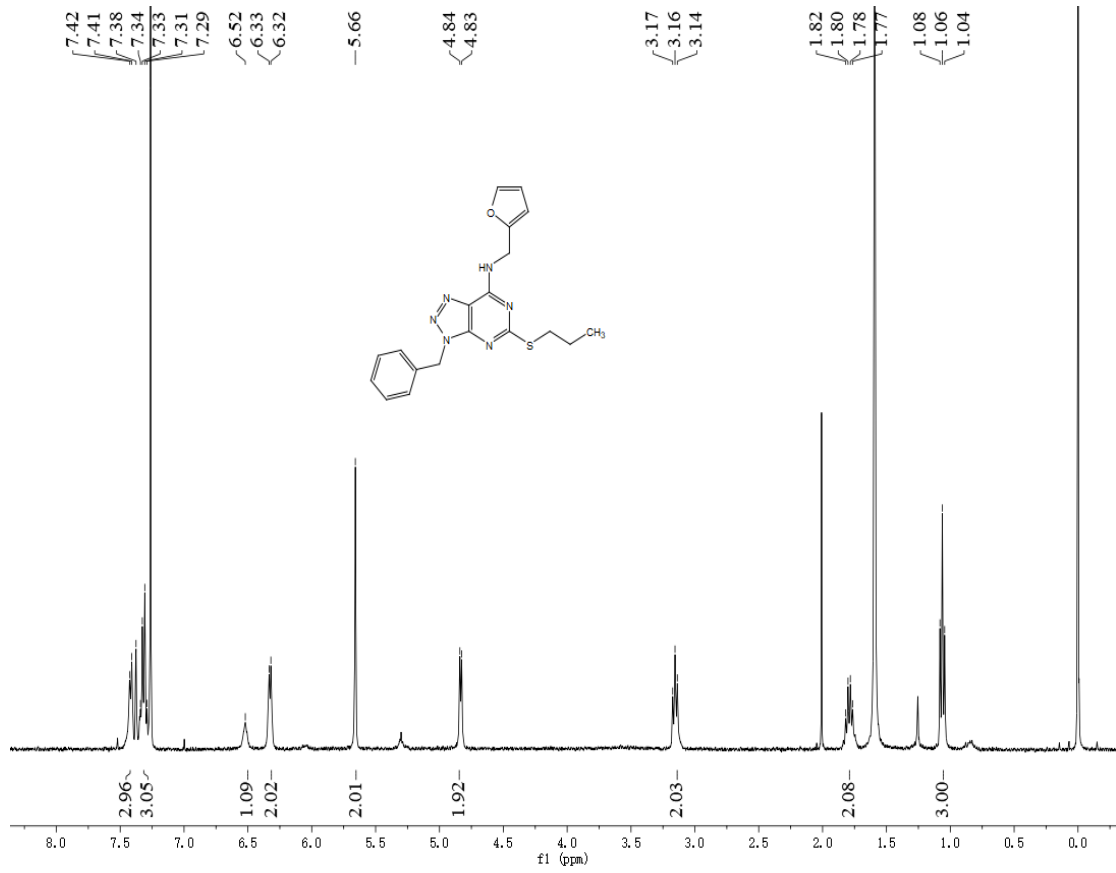


### MS spectrum of compound 4

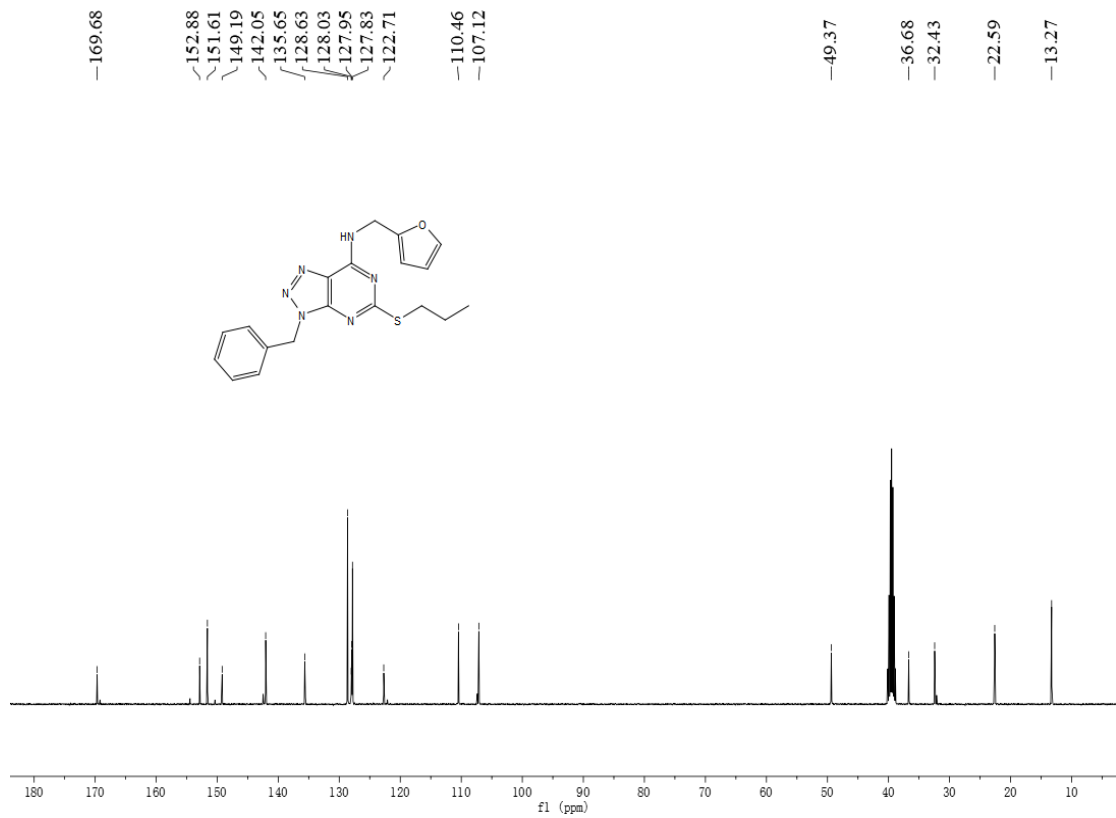
588 #19 RT: 0.18 AV: 1 NL: 1.45E9  
T: FTMS +p ESI Full ms [100.0000-800.0000]



<sup>1</sup>H NMR spectrum of compound 5

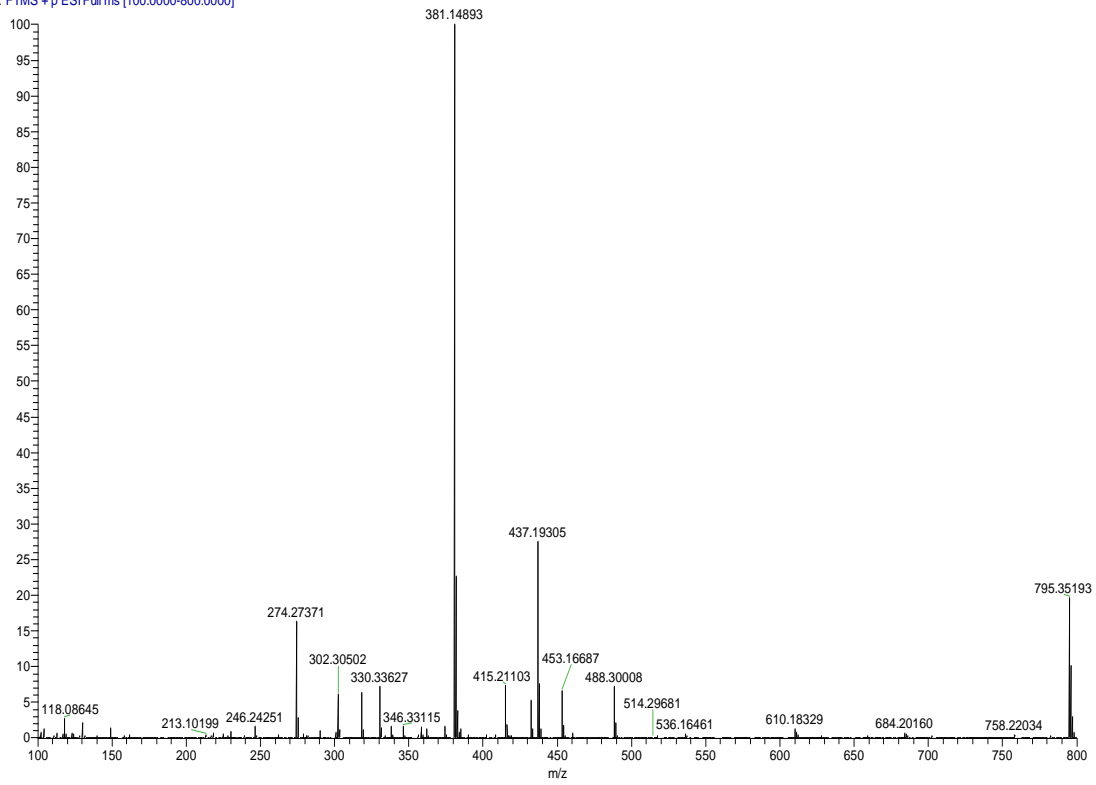


<sup>13</sup>C NMR spectrum of compound 5



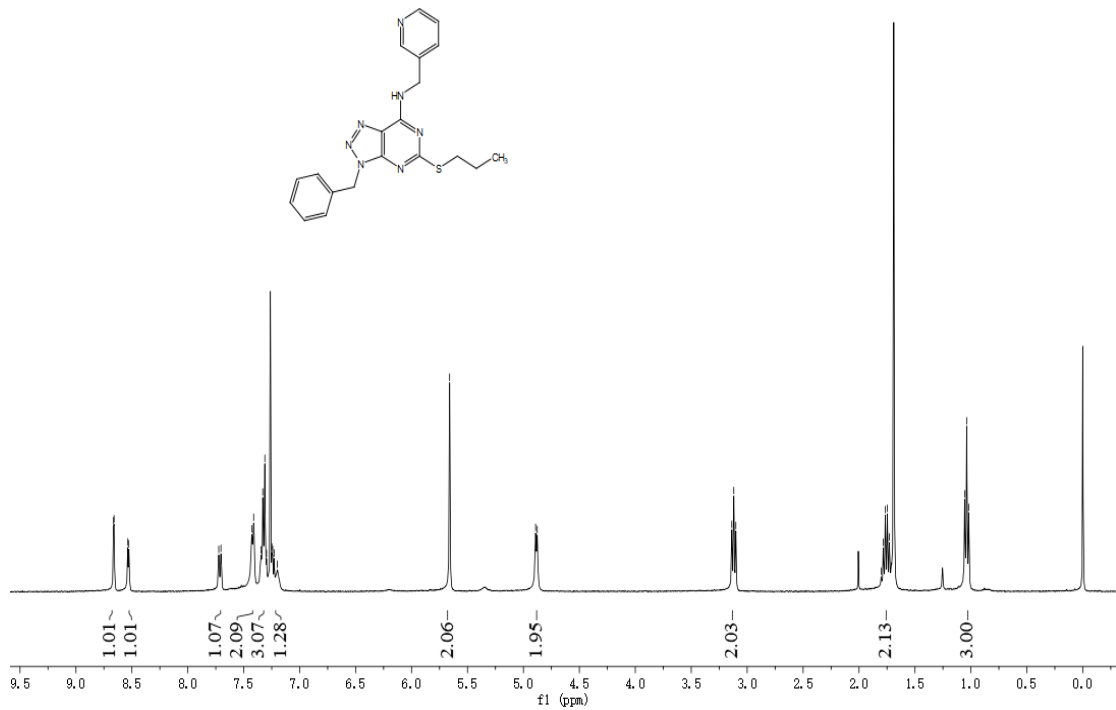
# MS spectrum of compound 5

583 #19 RT: 0.18 AV: 1 NL: 1.25E9  
T: FTMS + p ESI Full ms [100.0000-800.0000]

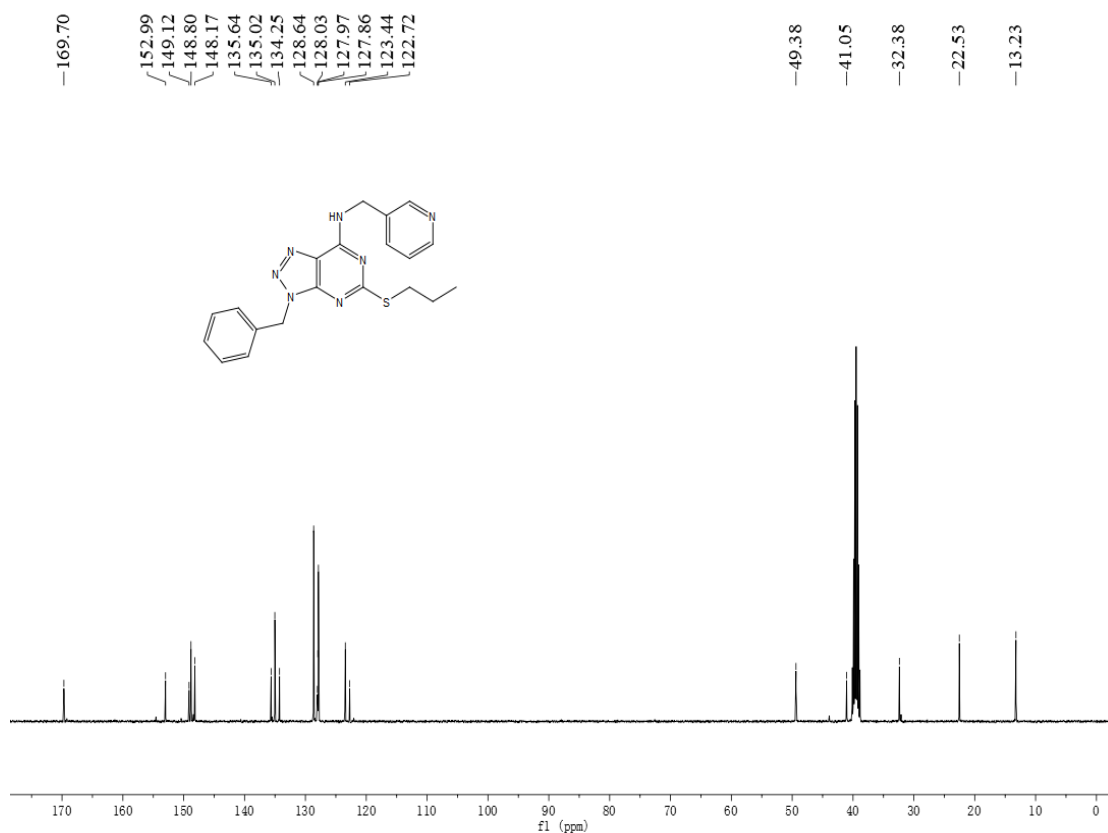


# <sup>1</sup>H NMR spectrum of compound 6

8.66, 8.54, 8.53, 7.72, 7.70, 7.43, 7.41, 7.34, 7.33, 7.31, 7.30, 7.25, 7.24, 7.23, 7.20, -5.66, 4.89, 4.88, 3.14, 3.12, 3.10, 1.80, 1.78, 1.77, 1.75, 1.73, 1.06, 1.04, 1.02

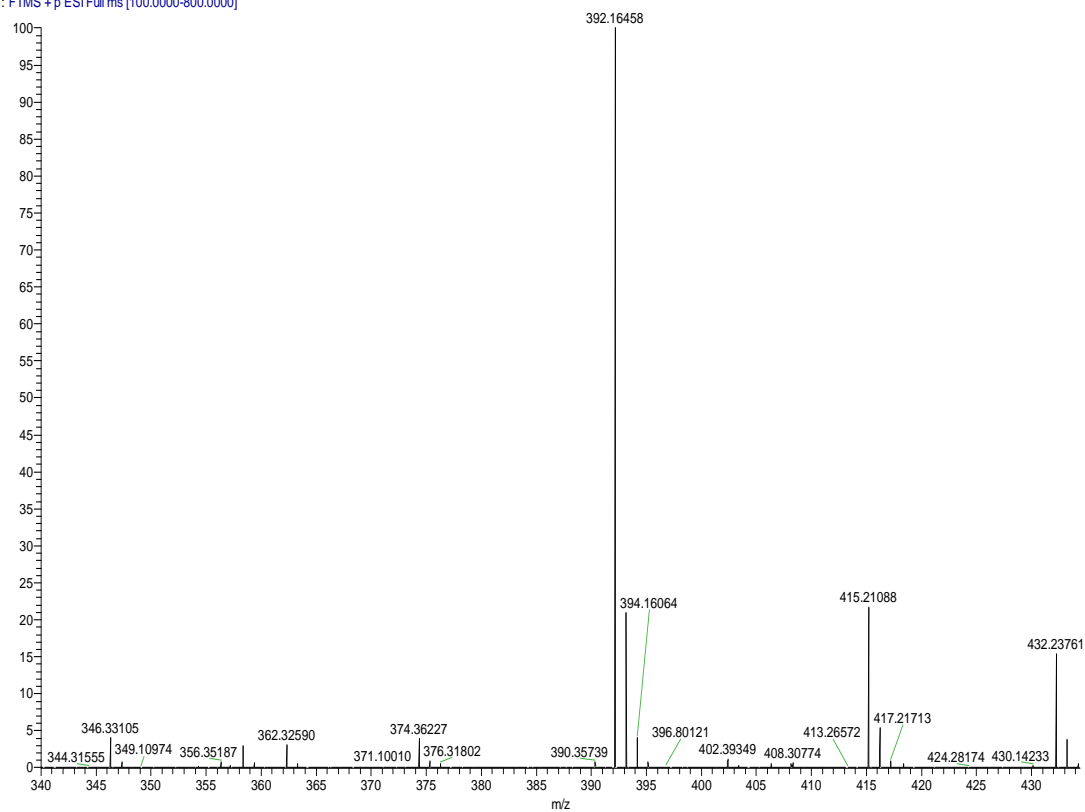


### <sup>13</sup>C NMR spectrum of compound 6



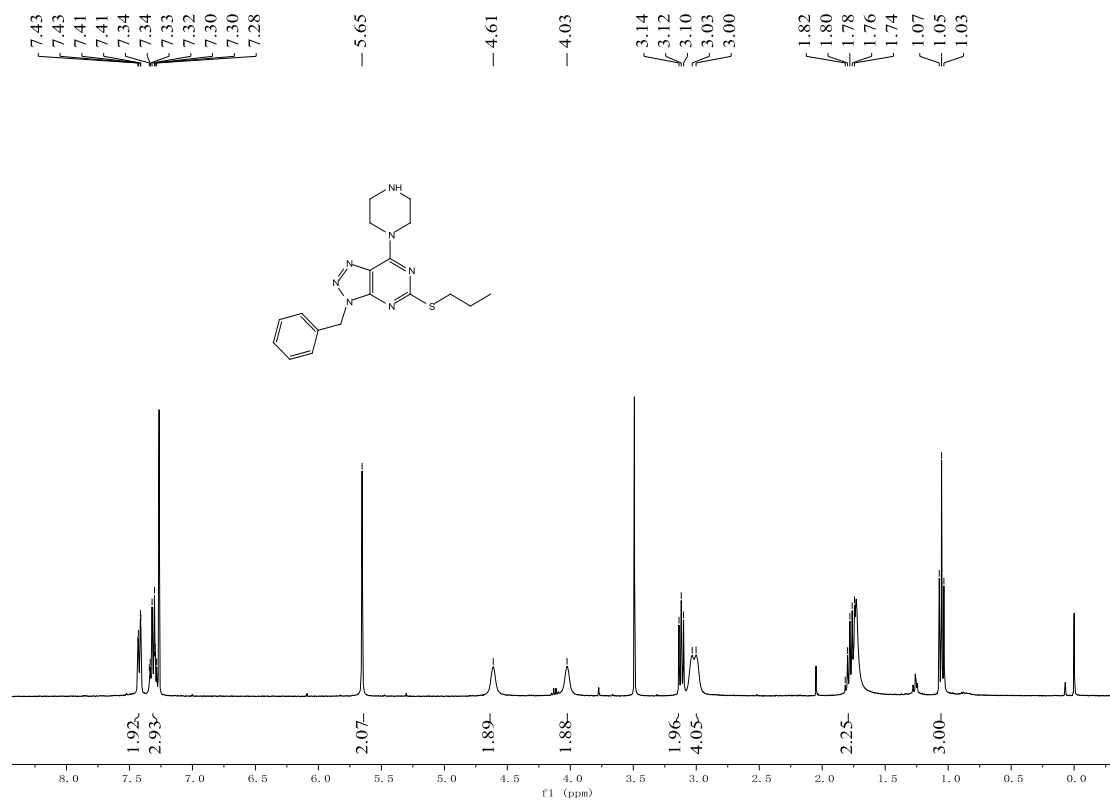
### MS spectrum of compound 6

586 #19 RT: 0.18 AV: 1 NL: 5.05E8  
T: FTMS +p ESI Full ms [100.0000-800.0000]

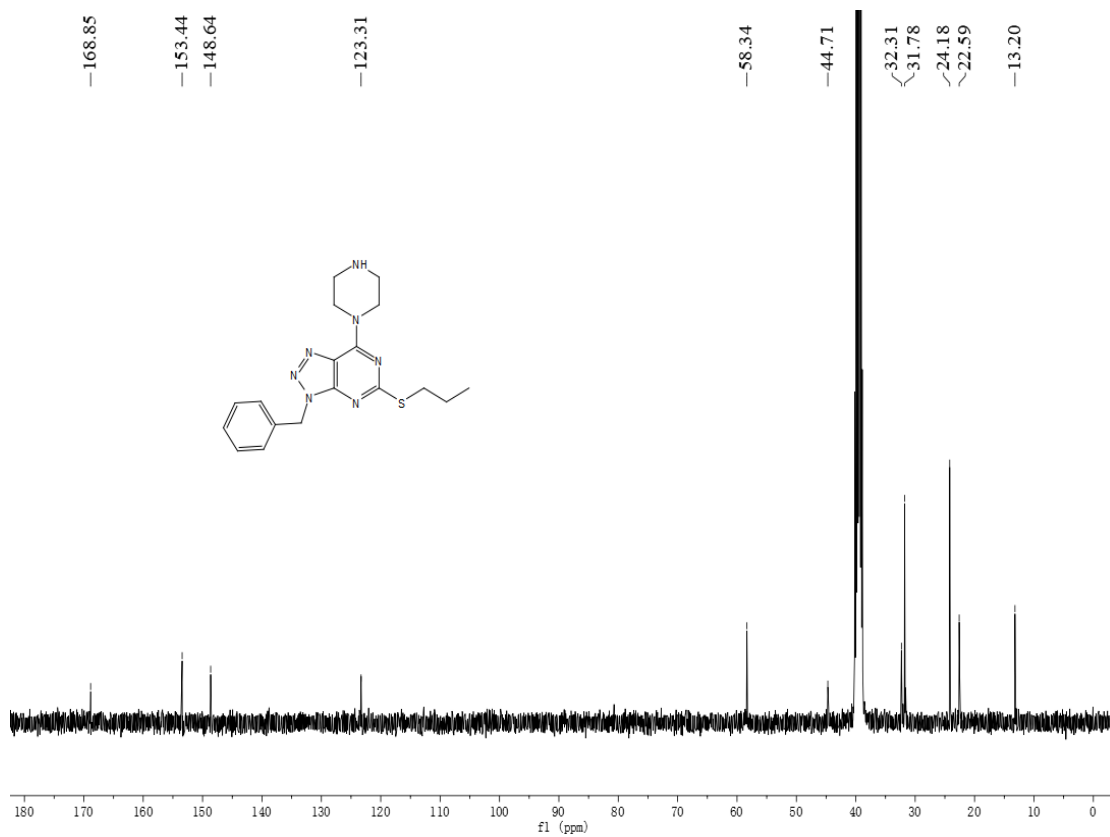




### <sup>1</sup>H NMR spectrum of compound 7

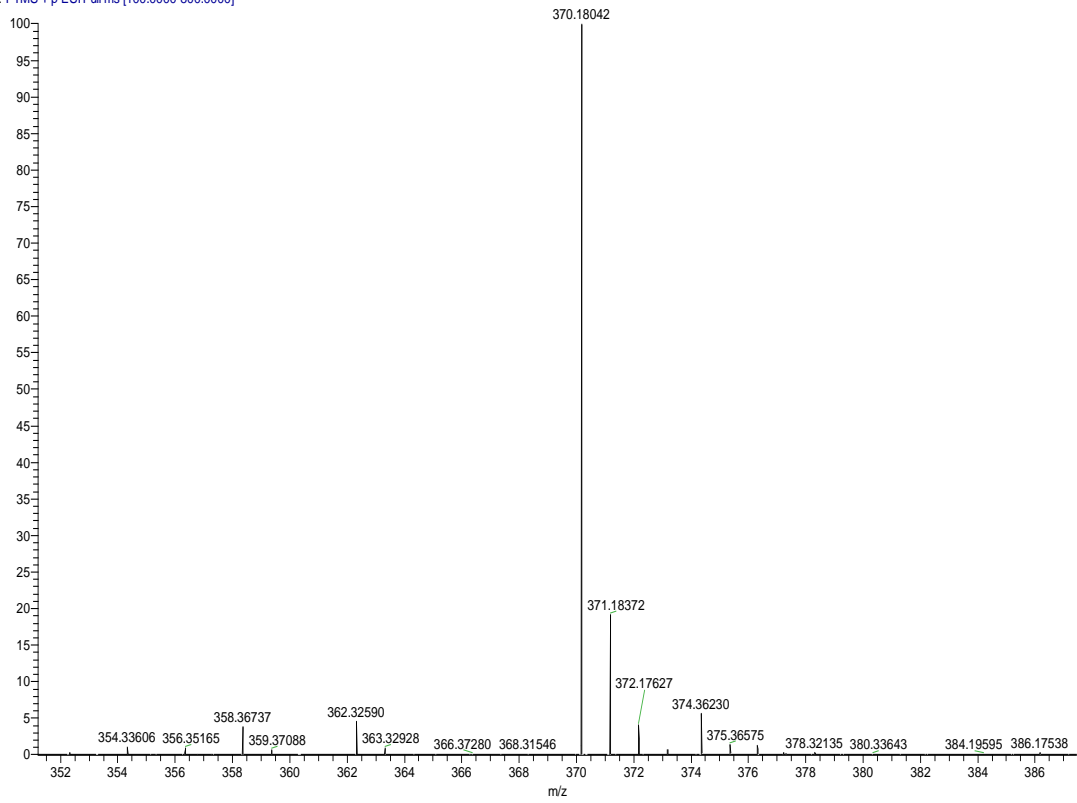


### <sup>13</sup>C NMR spectrum of compound 7



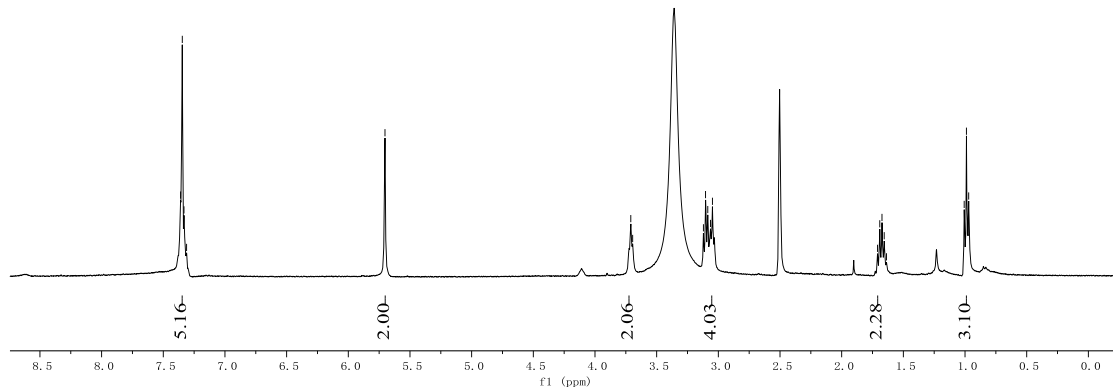
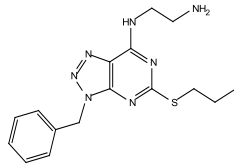
# MS spectrum of compound 7

0224-0 #19 RT: 0.18 AV: 1 NL: 3.42E8  
T: FTMS + p ESI Full ms [100.0000-800.0000]

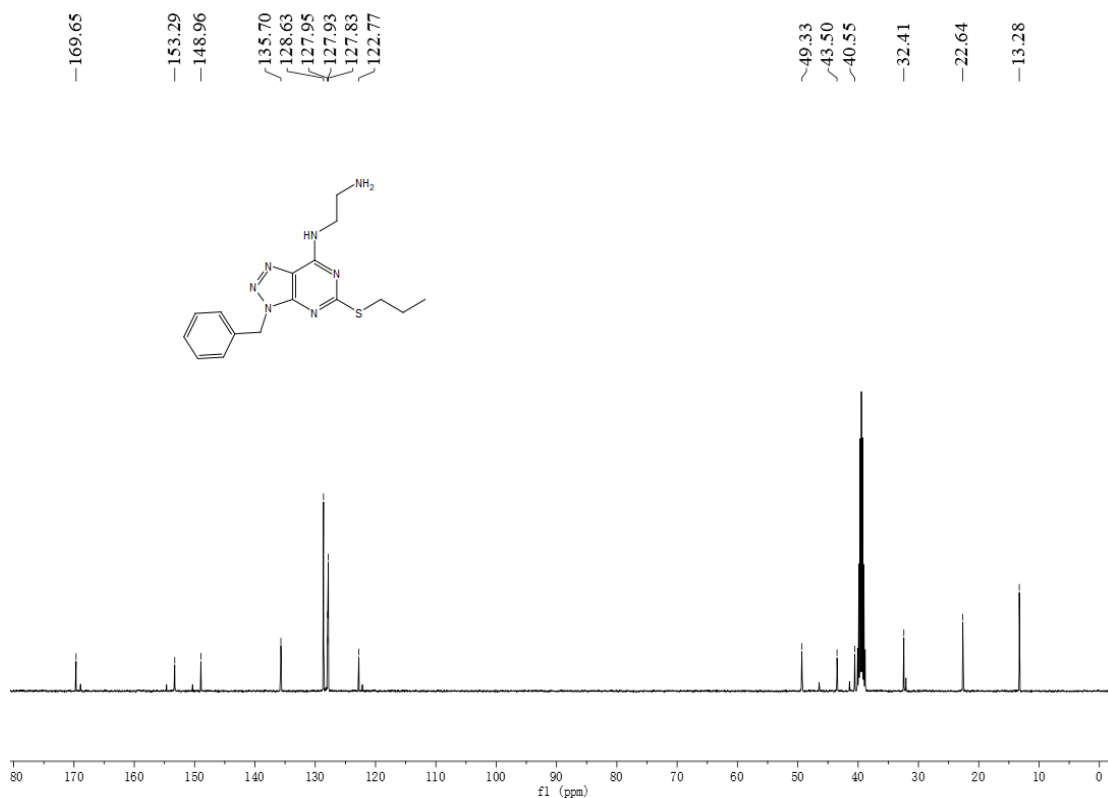


# <sup>1</sup>H NMR spectrum of compound 8

7.36, 7.35, 7.33, 7.31 (multiplet)  
5.70 (singlet)  
3.71, 3.69, 3.12, 3.10, 3.09, 3.06, 3.05 (multiplet)  
1.71, 1.69, 1.67, 1.66, 1.64 (multiplet)  
1.01, 0.99, 0.97 (multiplet)

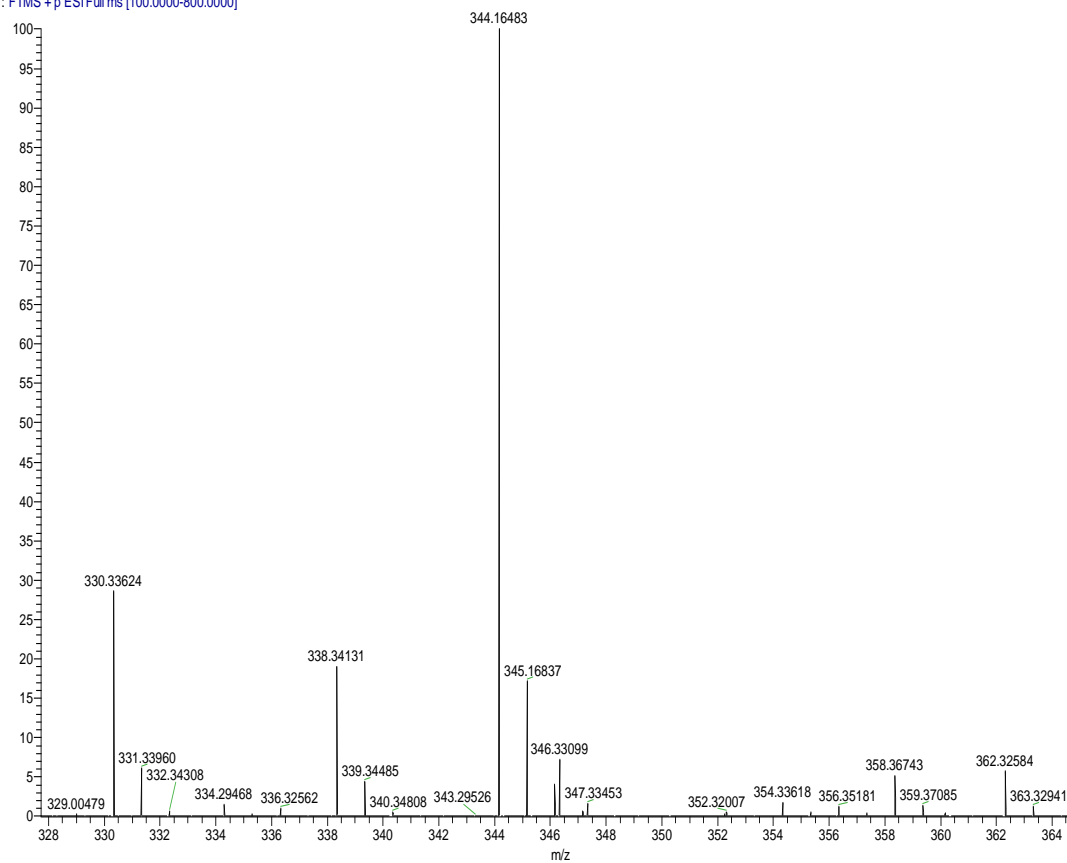


### <sup>13</sup>C NMR spectrum of compound **8**



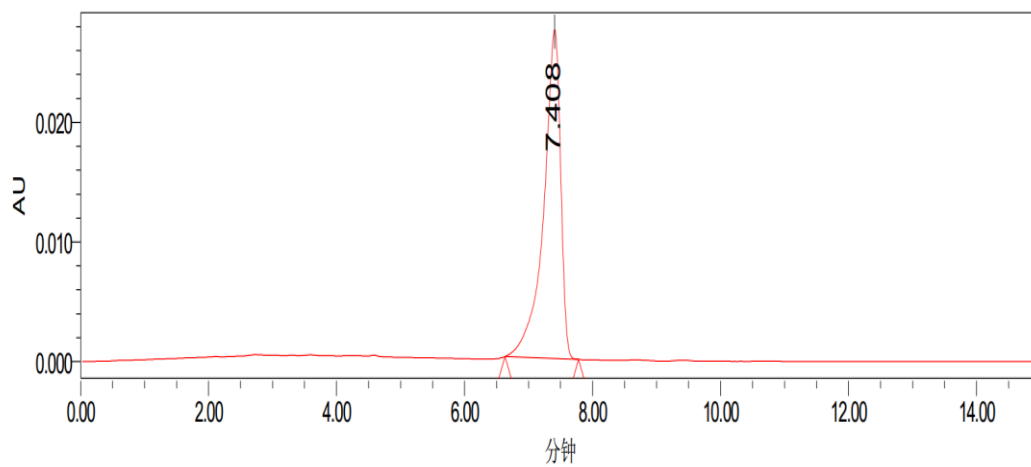
### MS spectrum of compound **8**

0227-1 #19 RT: 0.18 AV: 1 NL: 2.57E8  
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# HPLC spectrum of compound 8

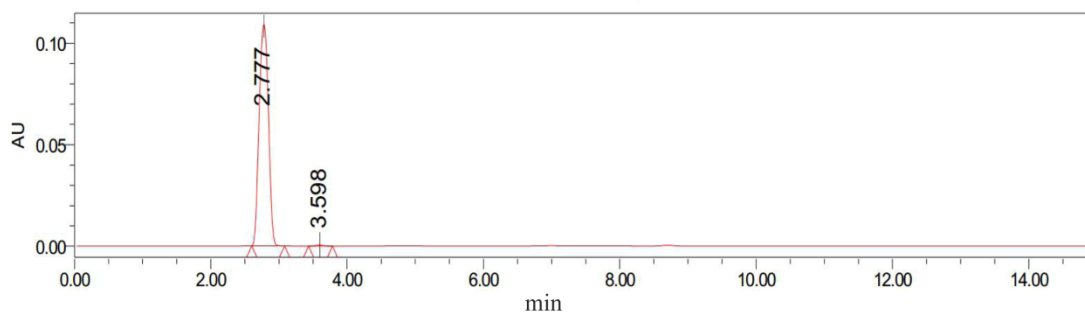
## HPLC chromatogram



## HPLC chromatogram

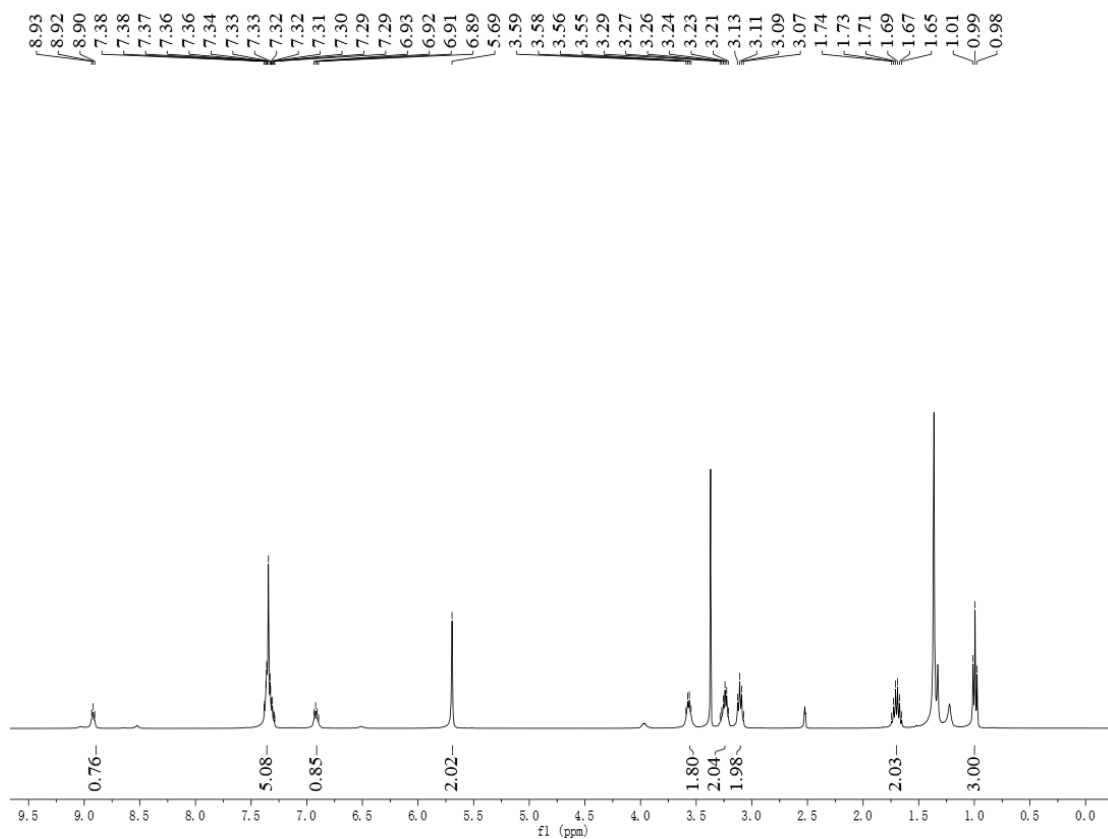
Retention Time(min) (分钟)	Area (微伏*秒)	% Area	
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## HPLC chromatogram

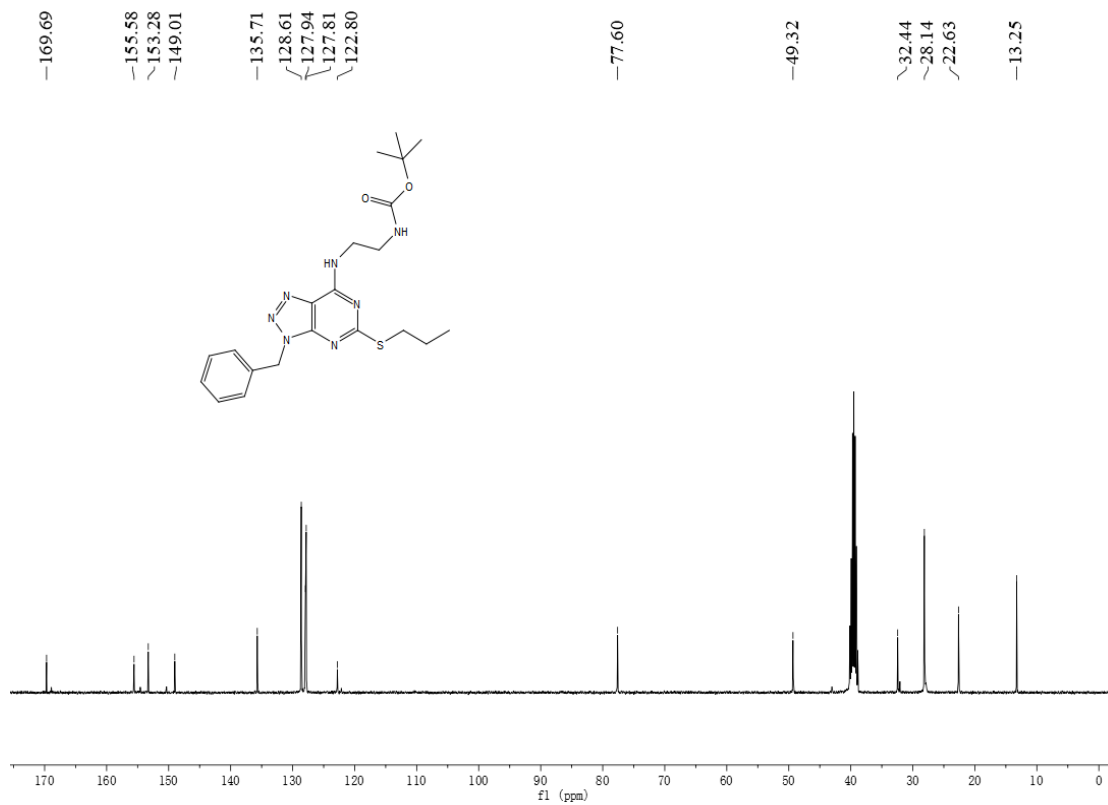


Retention Time (min)	Area( $\mu\text{v}\cdot\text{s}$ )	%Area
7.41	505333	100.00

### <sup>1</sup>H NMR spectrum of compound 9

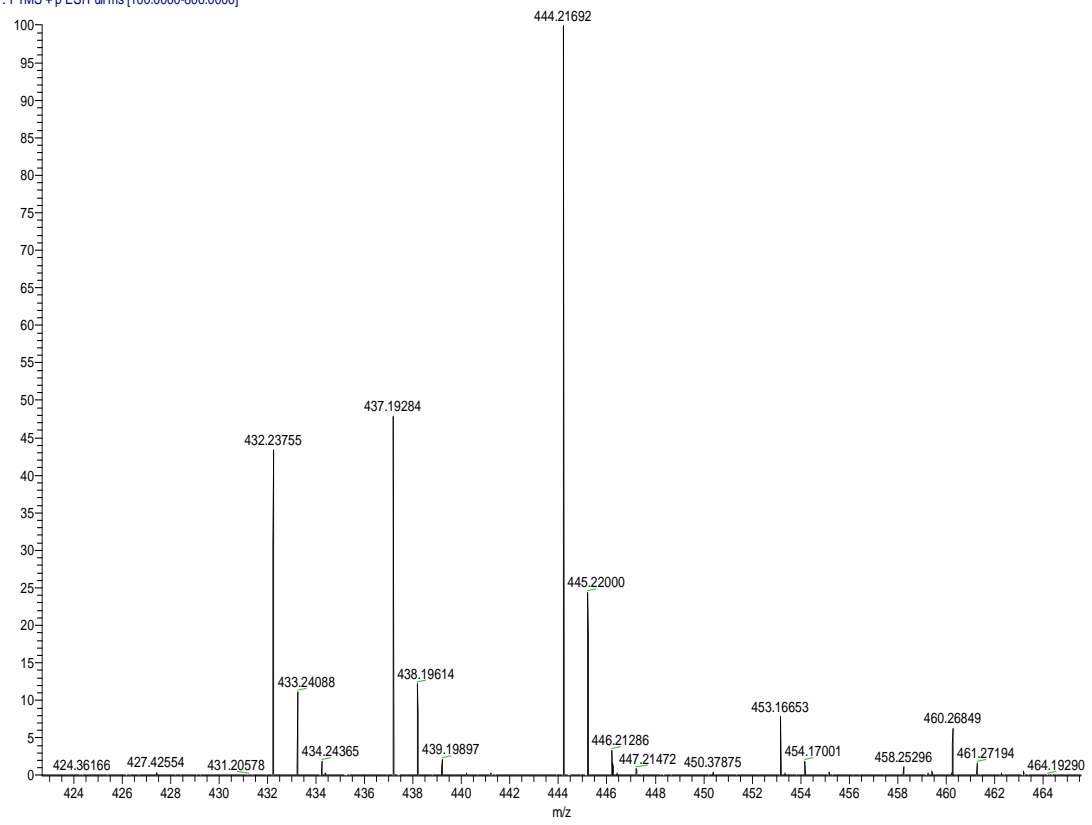


### <sup>13</sup>C NMR spectrum of compound 9

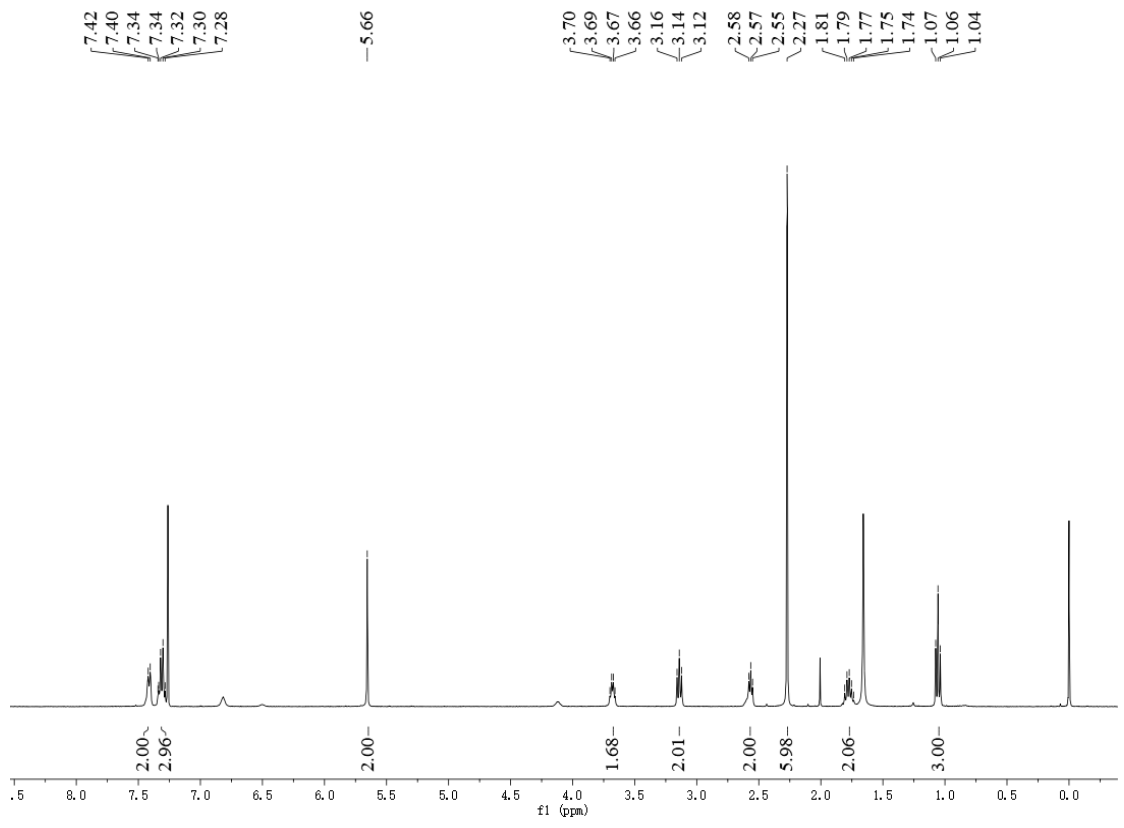


# MS spectrum of compound 9

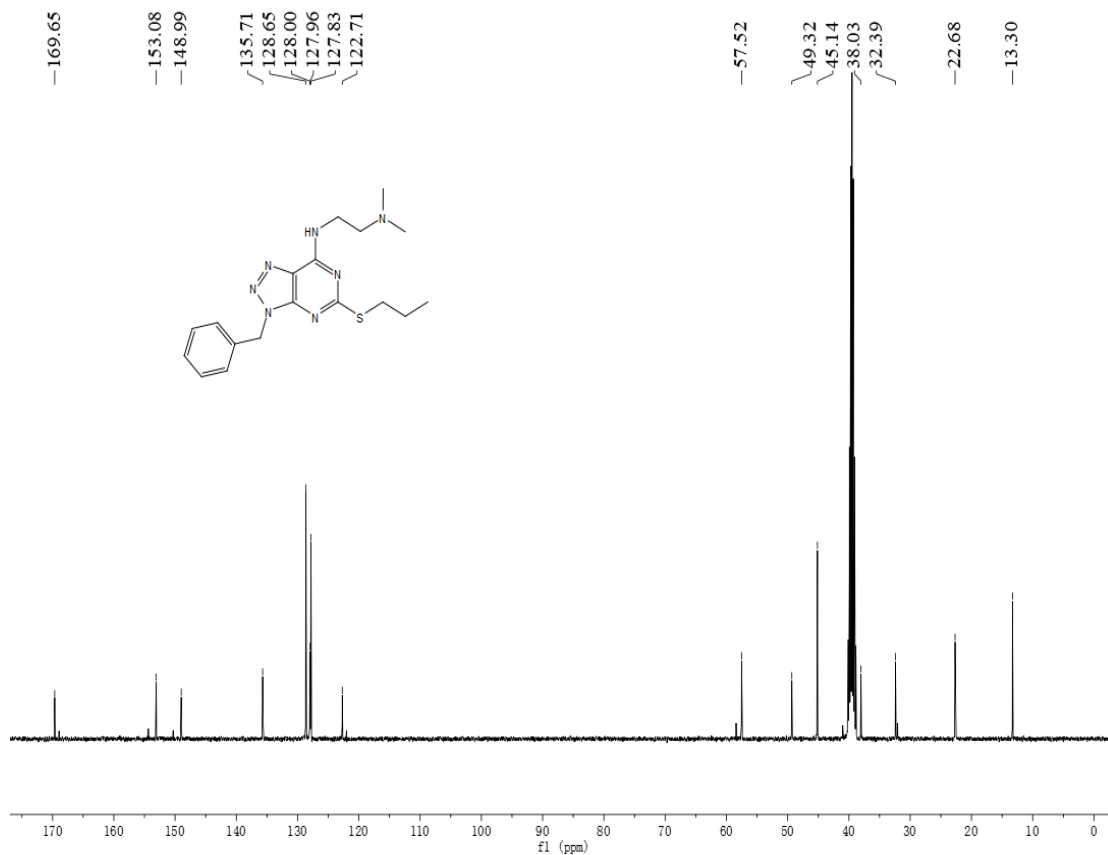
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T: FTMS +p ESI Full ms [100.0000-800.0000]



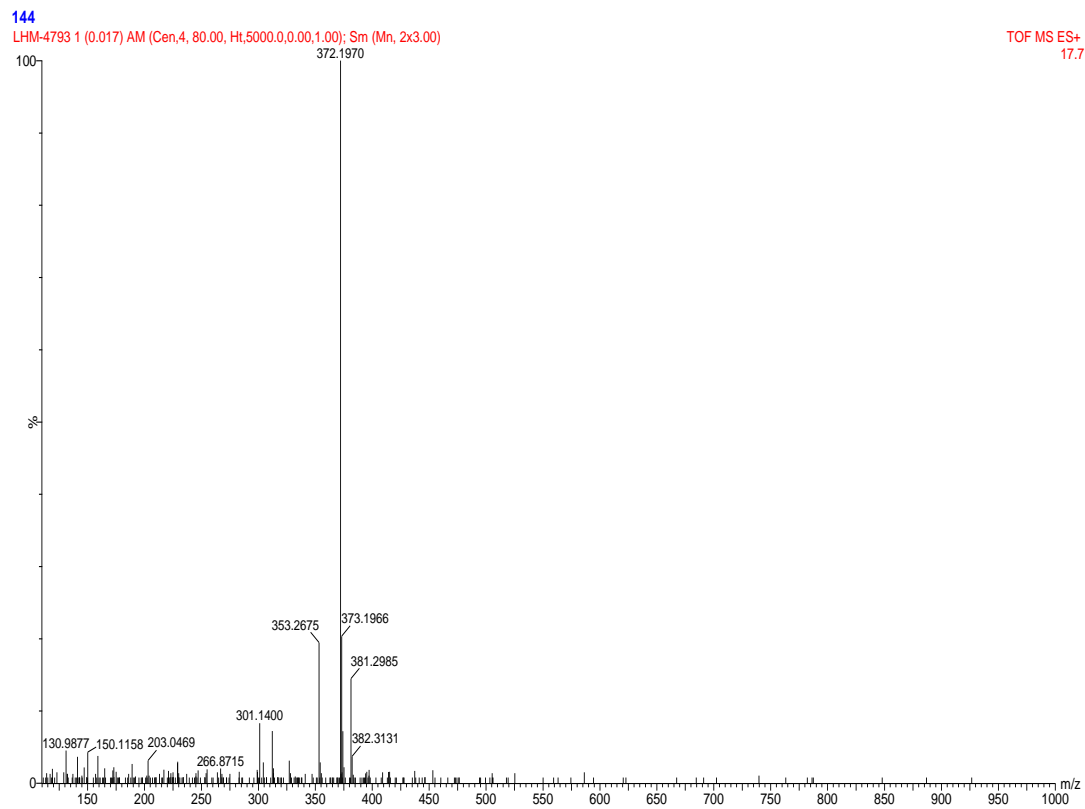
# <sup>1</sup>H NMR spectrum of compound 10



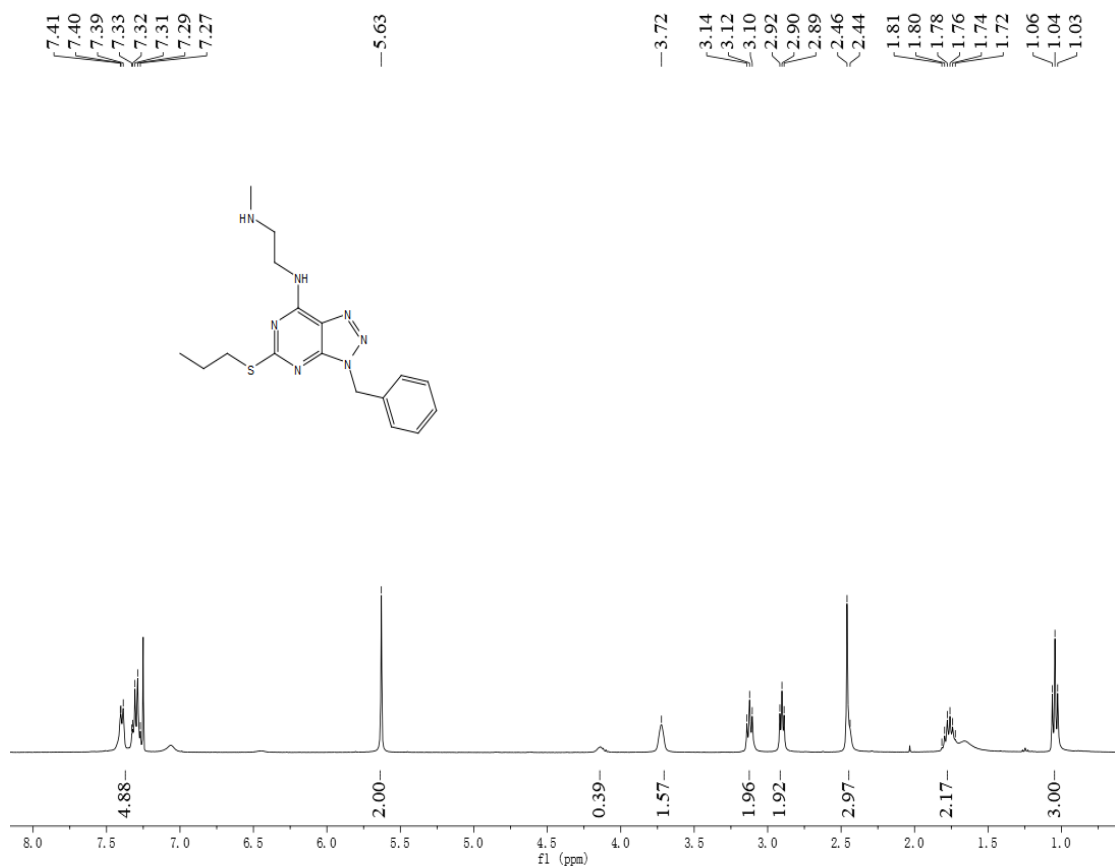
### <sup>13</sup>C NMR spectrum of compound 10



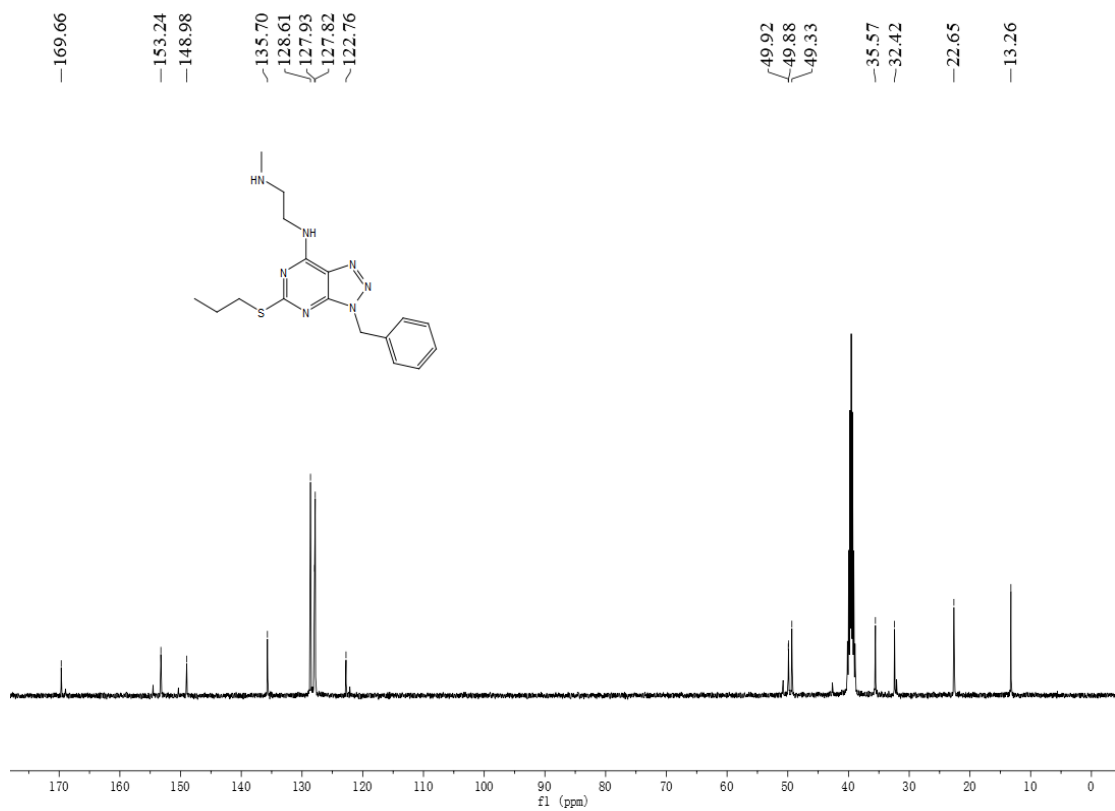
### MS spectrum of compound 10



### <sup>1</sup>H NMR spectrum of compound **11**



### <sup>13</sup>C NMR spectrum of compound **11**



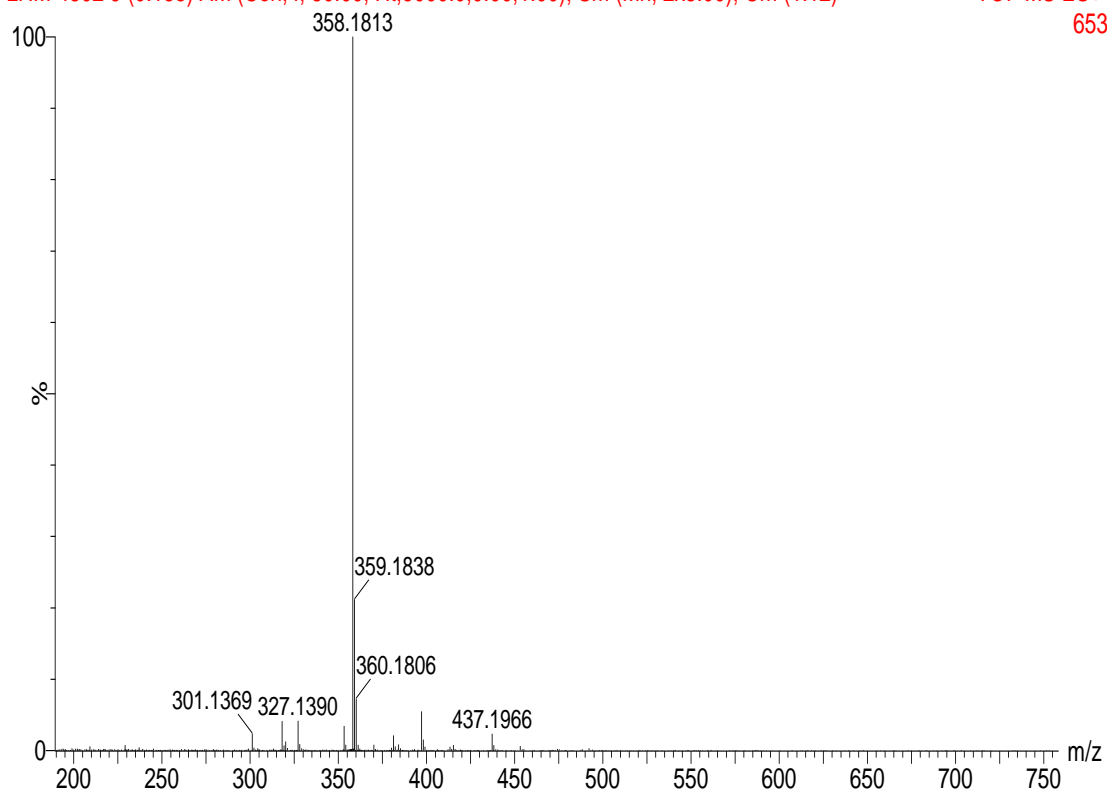


# MS spectrum of compound 11

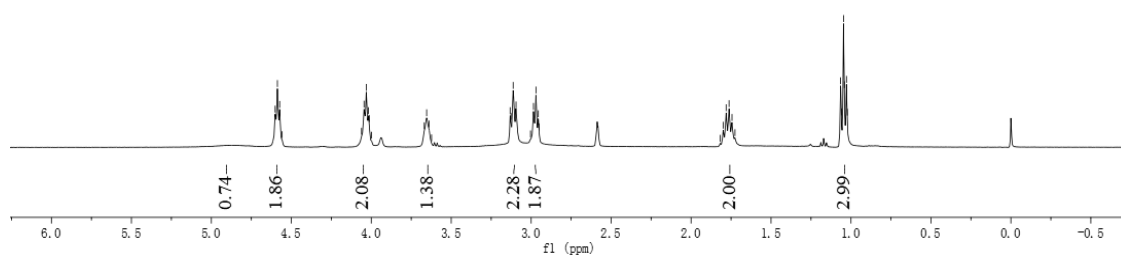
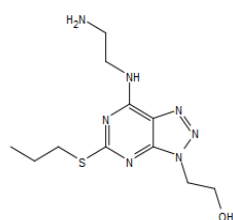
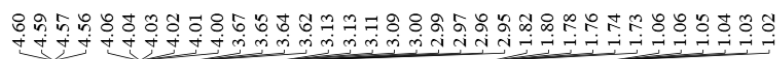
179

LHM-4802 9 (0.155) AM (Cen,4, 80.00, Ht,5000.0,0.00,1.00); Sm (Mn, 2x3.00); Cm (1:12)

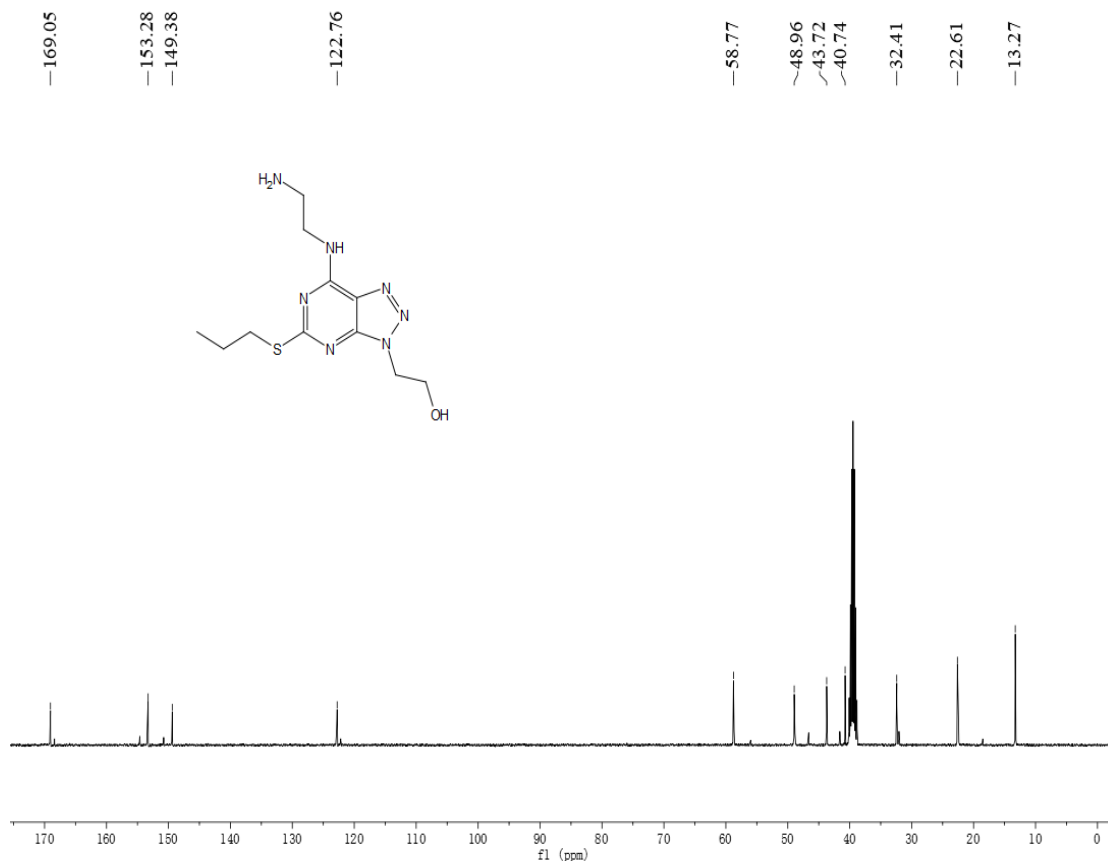
TOF MS ES+  
653



# <sup>1</sup>H NMR spectrum of compound 12



### <sup>13</sup>C NMR spectrum of compound 12

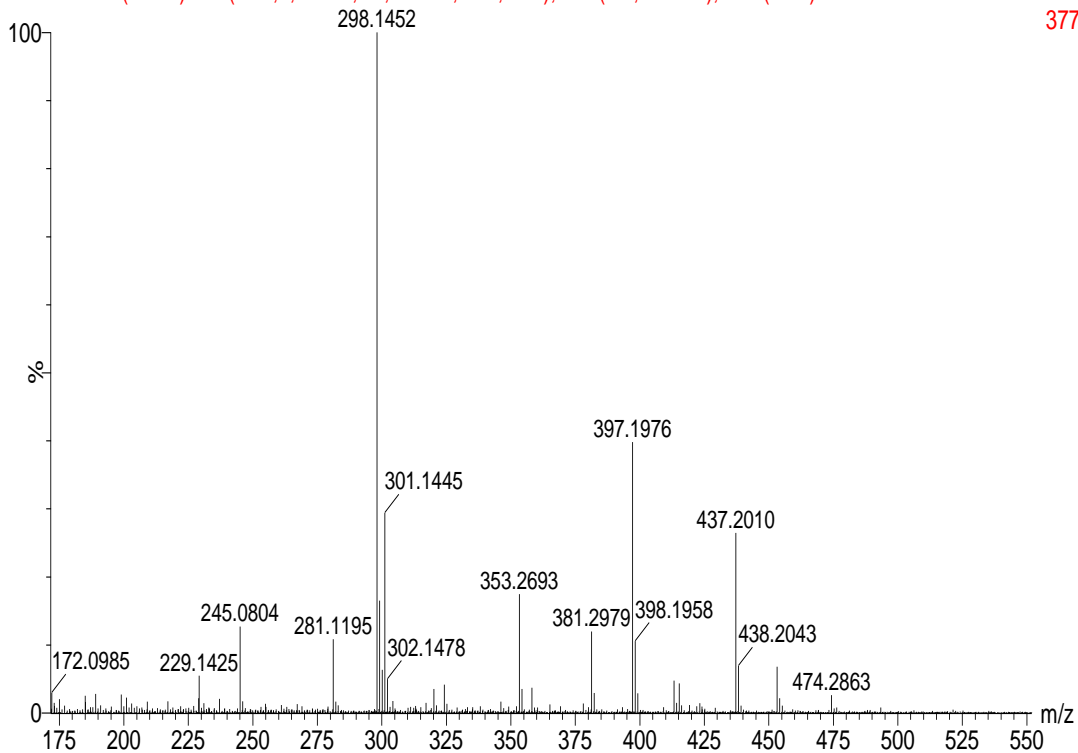


### MS spectrum of compound 12

135

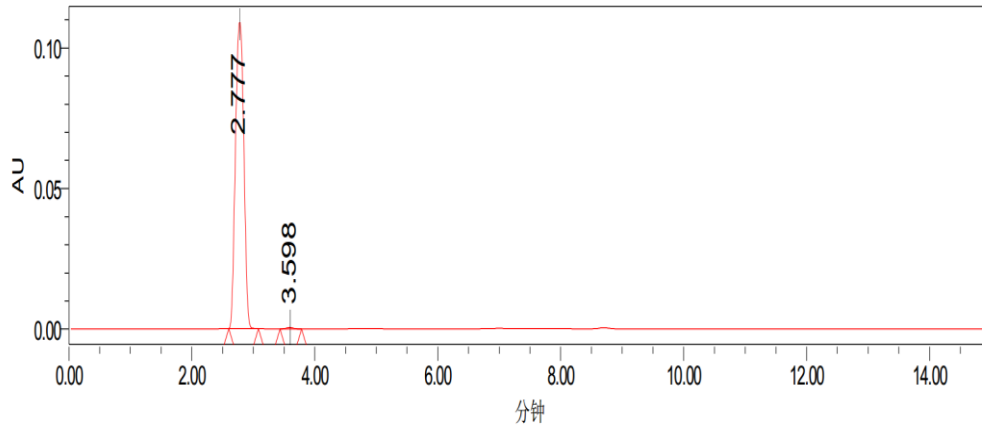
LHM-4786 4 (0.069) AM (Cen,4, 80.00, Ht,5000.0,0.00,1.00); Sm (Mn, 2x3.00); Cm (1:11)

TOF MS ES+  
377



# HPLC spectrum of compound 12

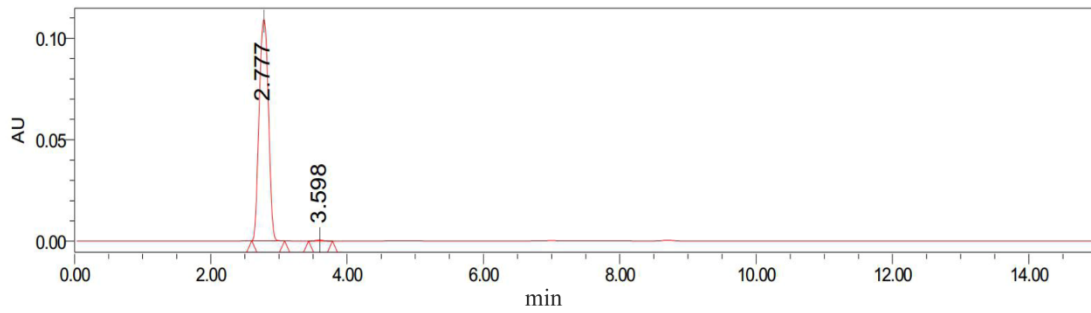
## HPLC chromatogram



## HPLC chromatogram

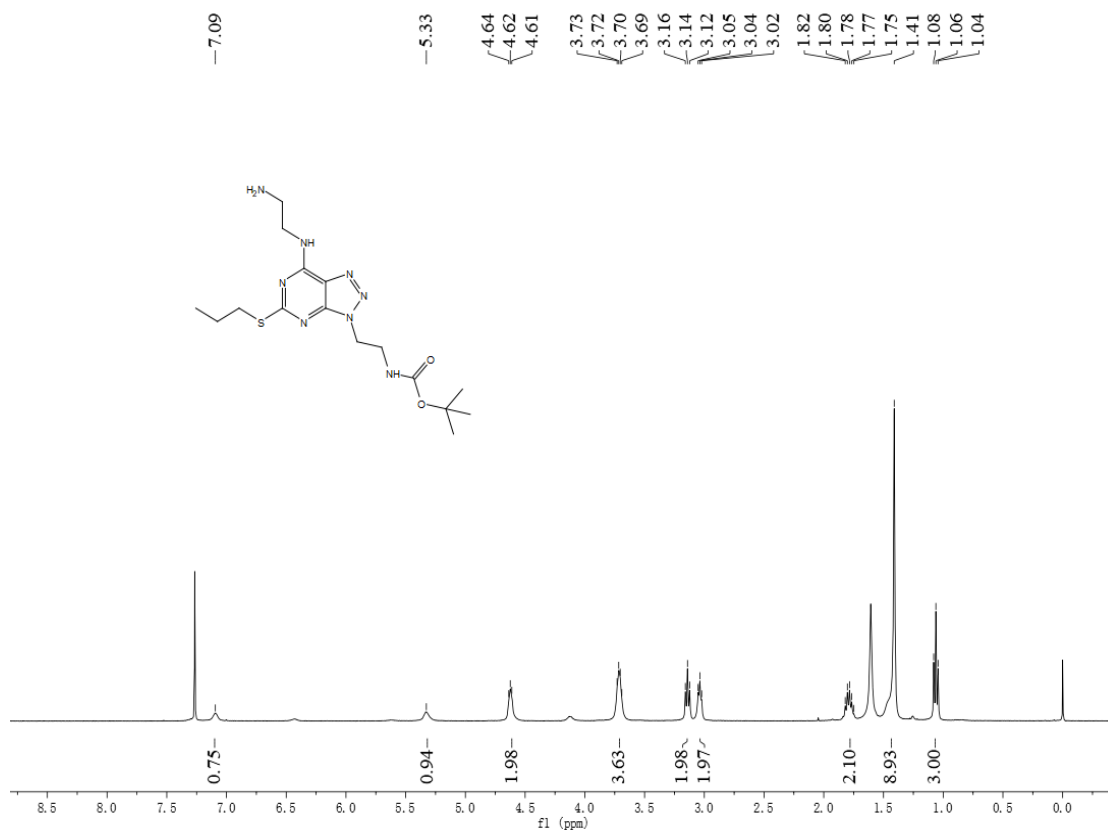
Retention Time(min) (分钟)	Area (微伏*秒)	% Area	
1	2.78	1032299	99.65
2	3.60	3625	0.35

## HPLC chromatogram

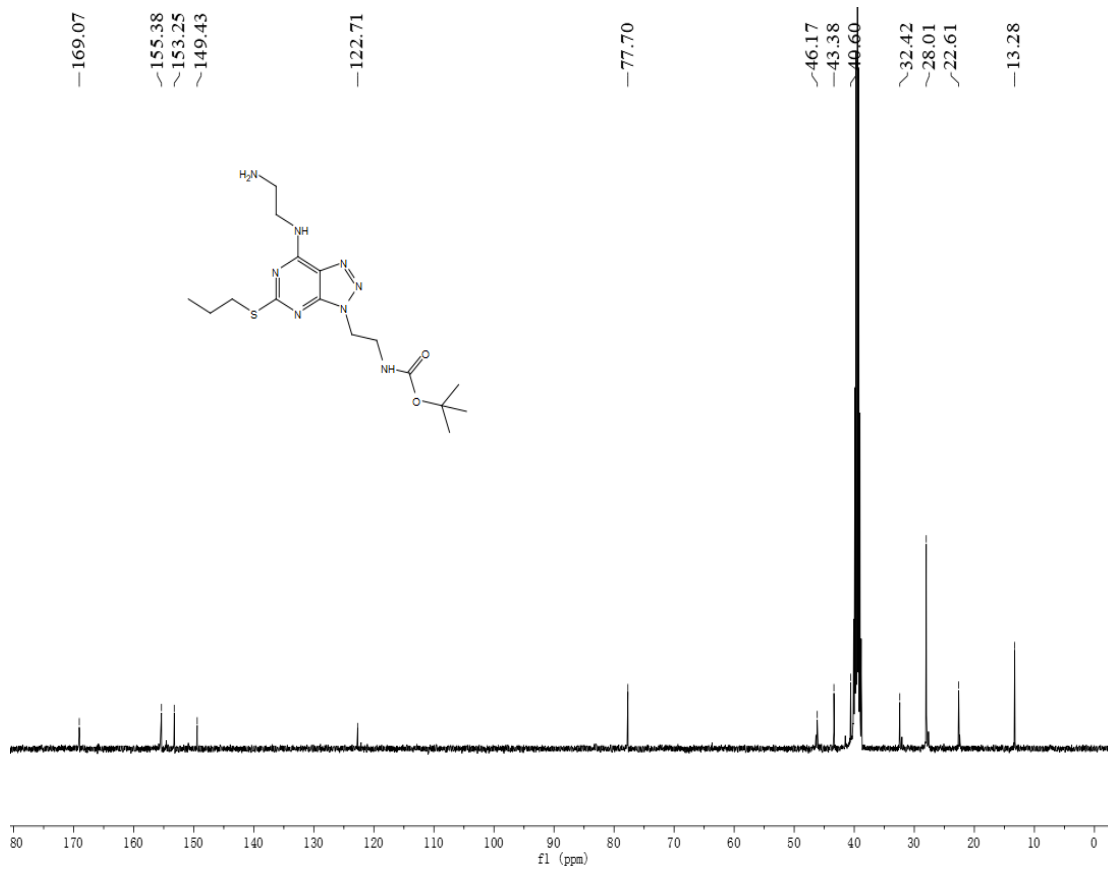


Retention Time (min)	Area( $\mu\text{v}\cdot\text{s}$ )	%Area
7.41	505333	100.00

### <sup>1</sup>H NMR spectrum of compound 13



### <sup>13</sup>C NMR spectrum of compound 13

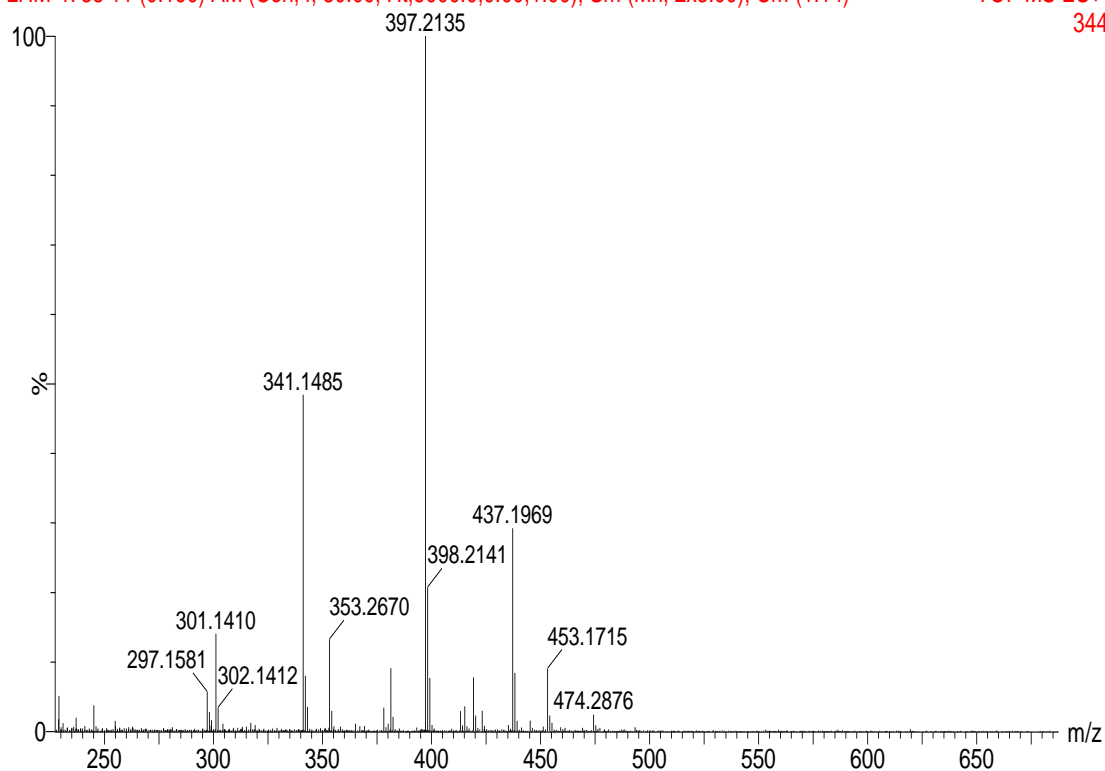


# MS spectrum of compound 13

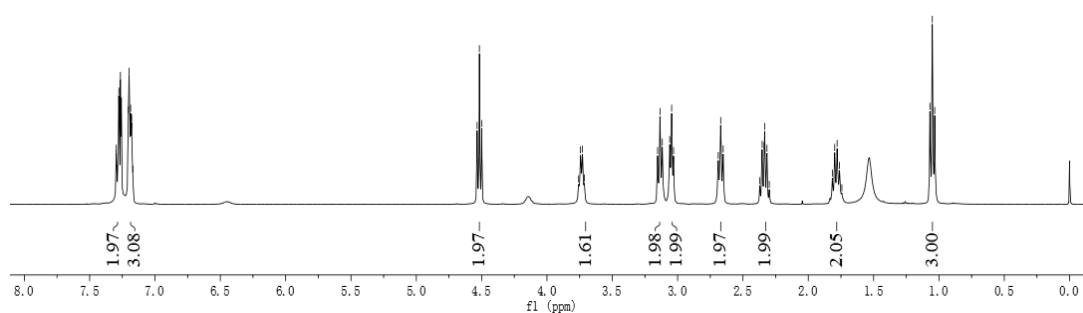
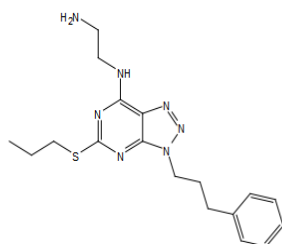
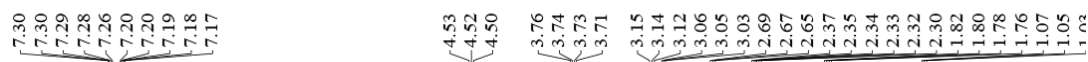
137

LHM-4788 11 (0.190) AM (Cen,4, 80.00, Ht,5000.0,0.00,1.00); Sm (Mn, 2x3.00); Cm (1:14)

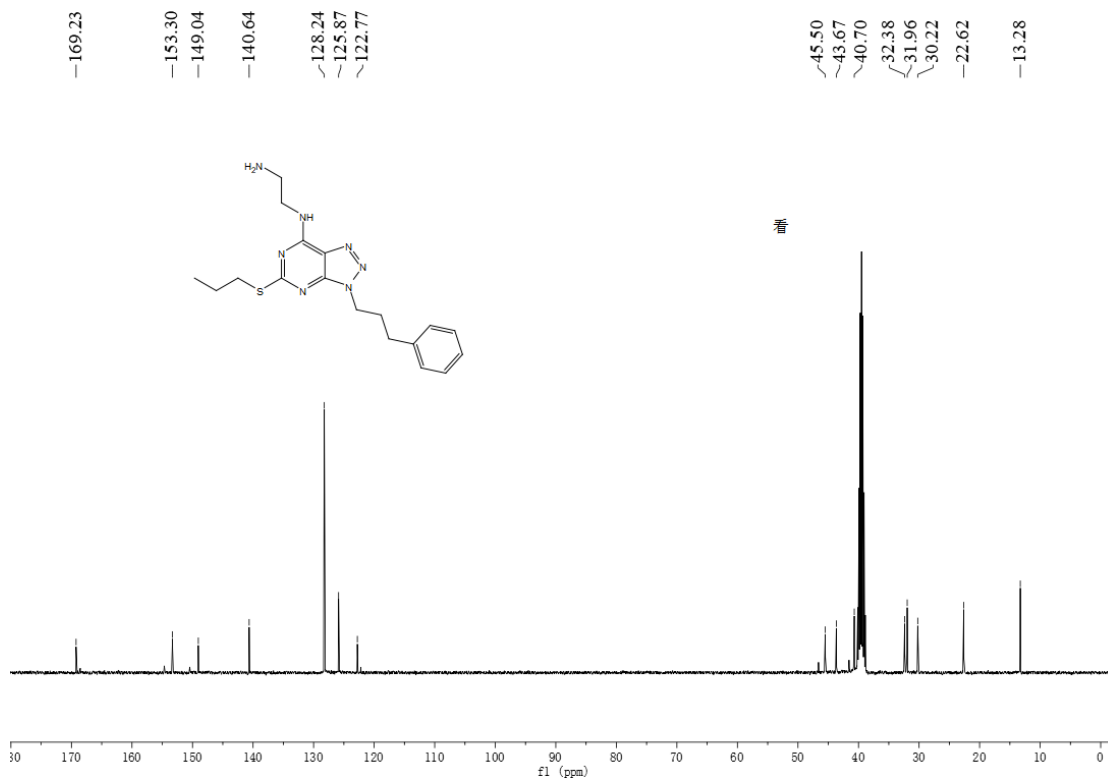
TOF MS ES+  
344



# <sup>1</sup>H NMR spectrum of compound 14



<sup>13</sup>C NMR spectrum of compound **14**

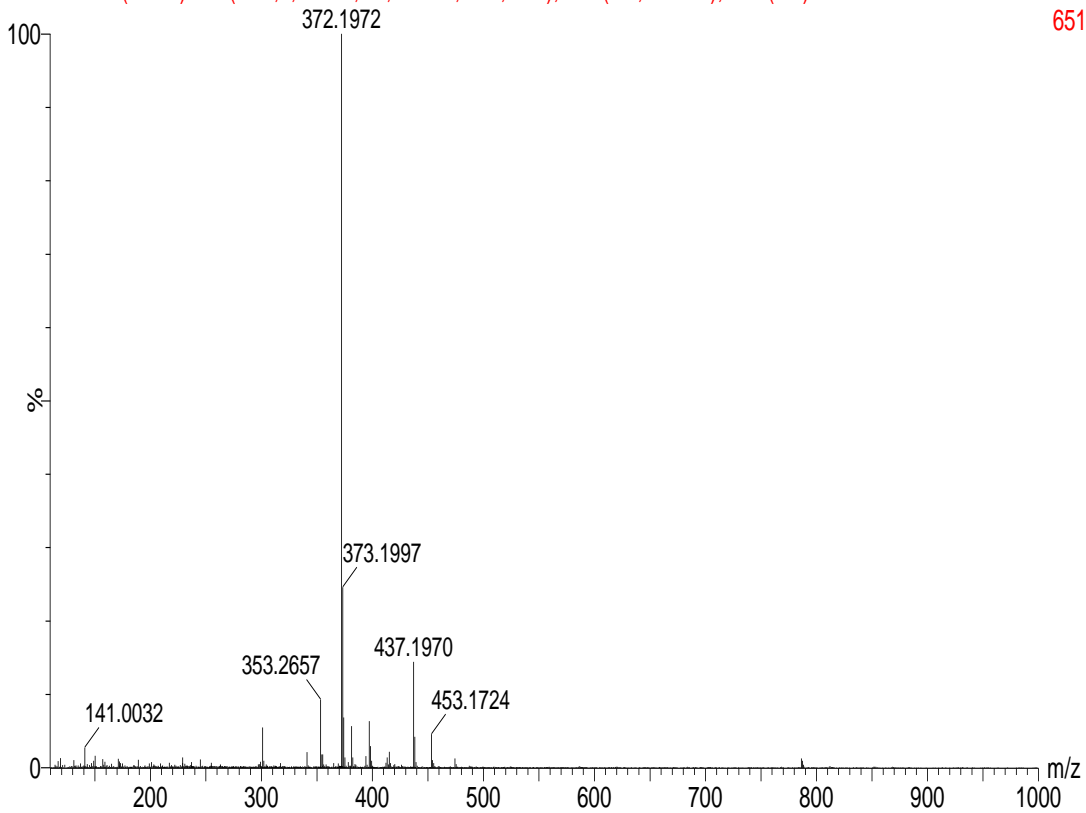


MS spectrum of compound **14**

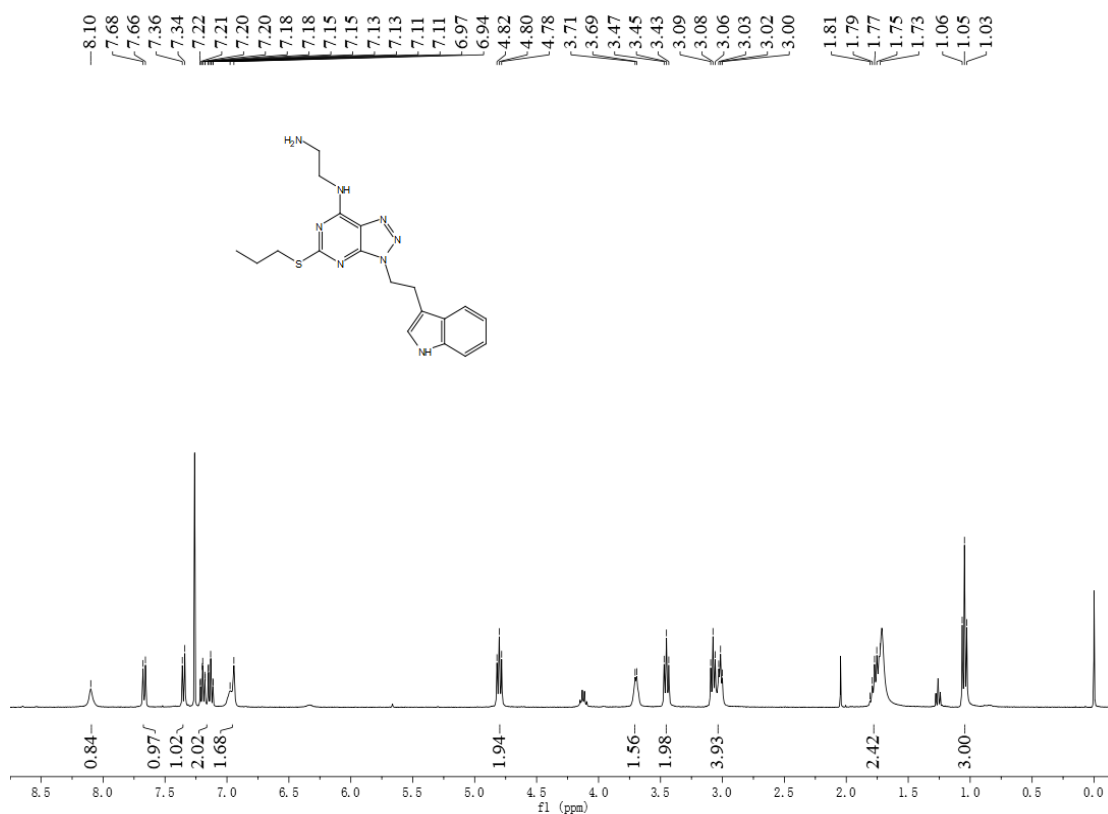
**138**

LHM-4789 1 (0.017) AM (Cen,4, 80.00, Ht,5000.0,0.00,1.00); Sm (Mn, 2x3.00); Cm (1:9)

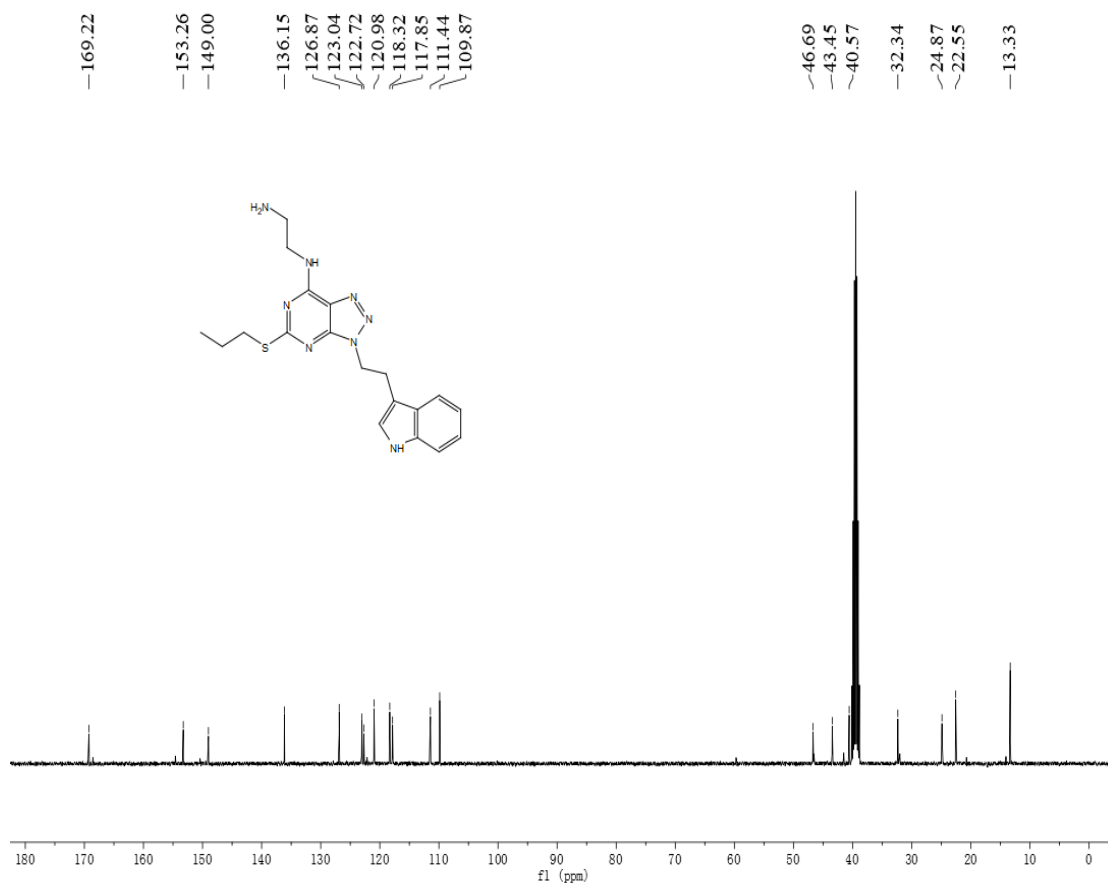
TOF MS ES+  
651



### <sup>1</sup>H NMR spectrum of compound 15



### <sup>13</sup>C NMR spectrum of compound 15

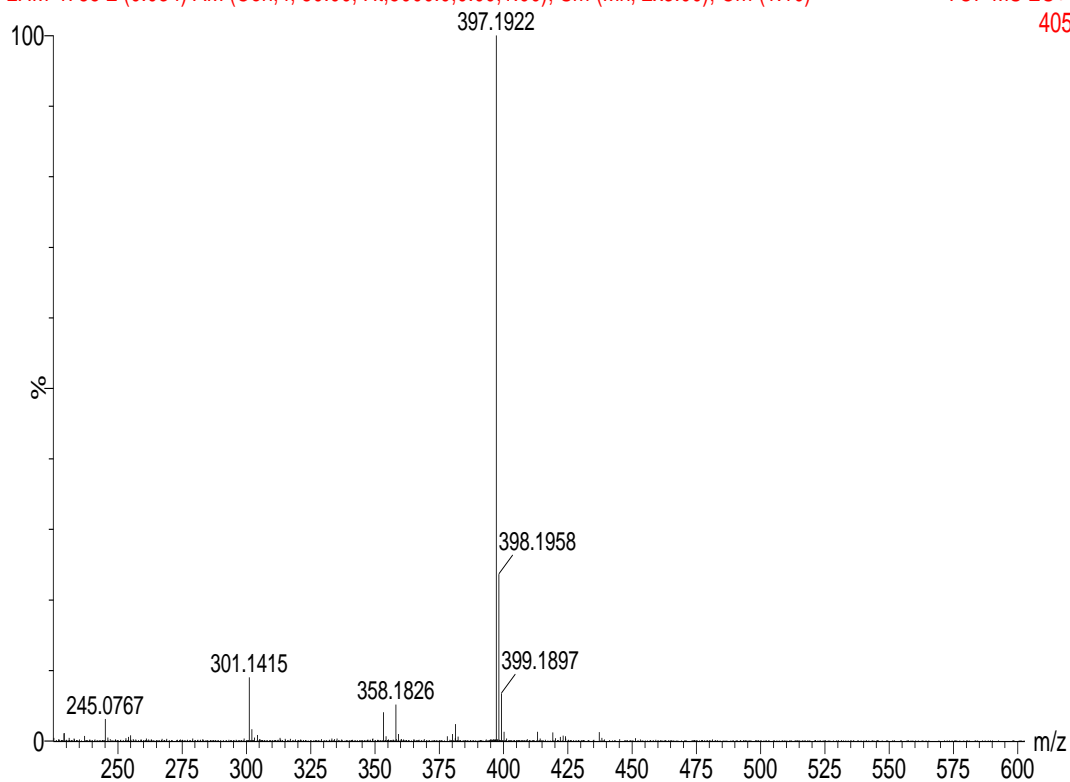


# MS spectrum of compound 15

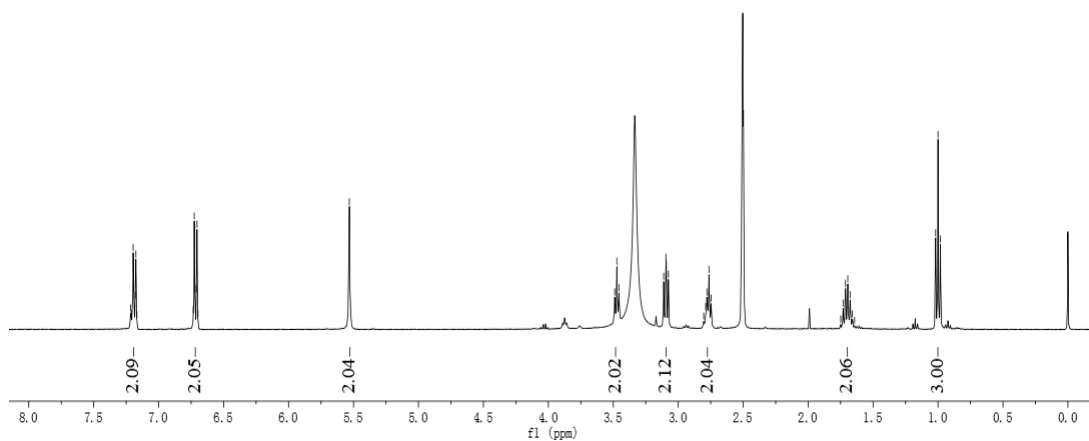
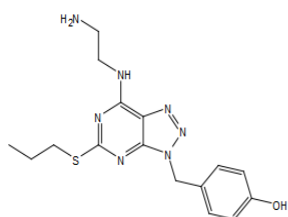
134

LHM-4785 2 (0.034) AM (Cen,4, 80.00, Ht,5000.0,0.00,1.00); Sm (Mn, 2x3.00); Cm (1:10)

TOF MS ES+  
405

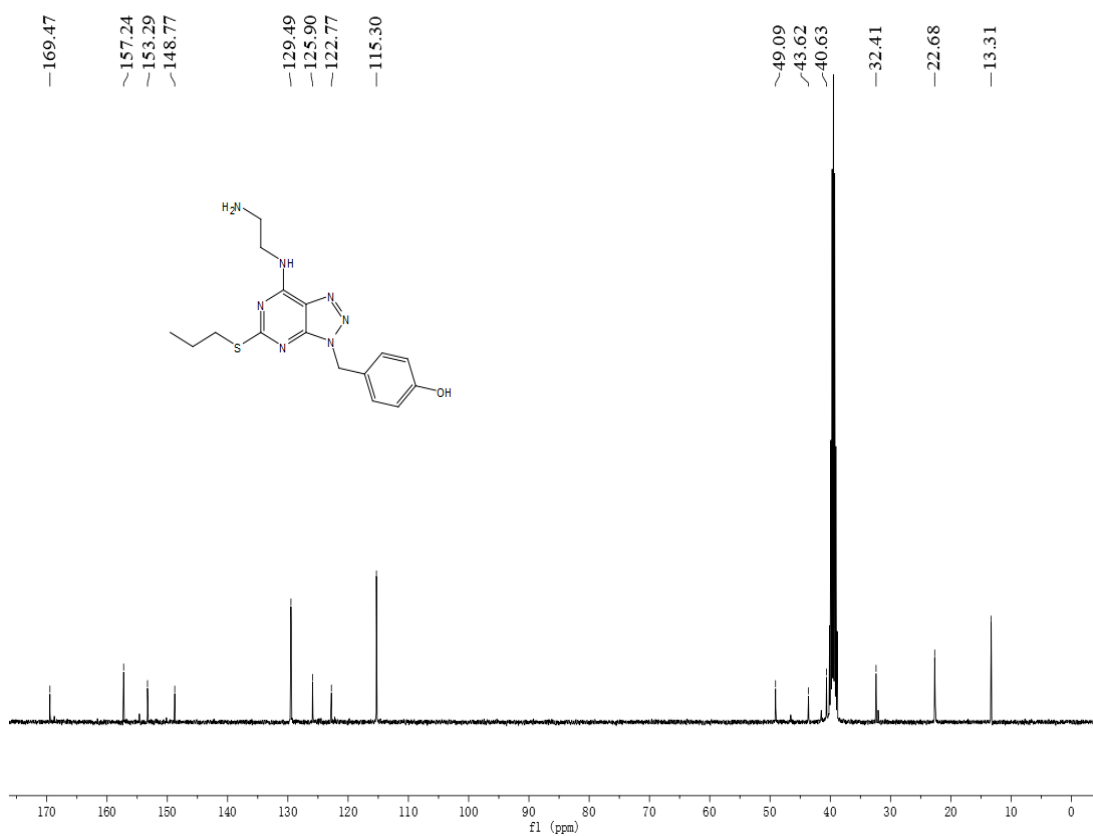


# <sup>1</sup>H NMR spectrum of compound 16





### <sup>13</sup>C NMR spectrum of compound 16

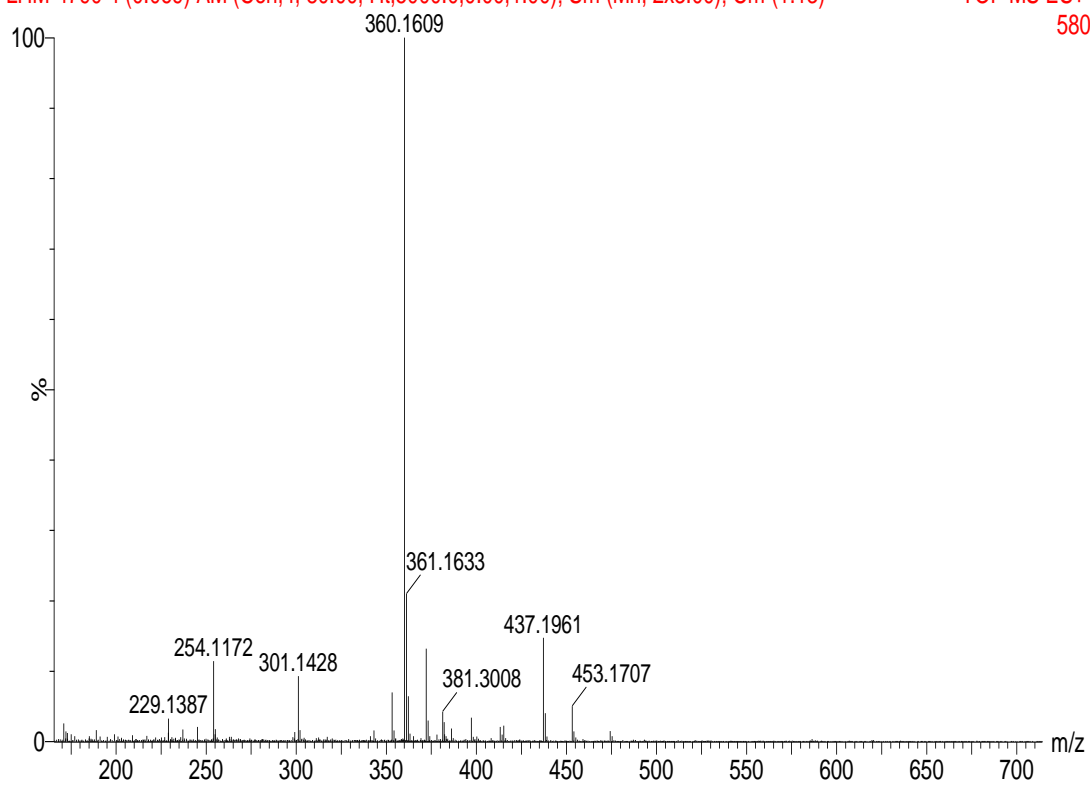


### MS spectrum of compound 16

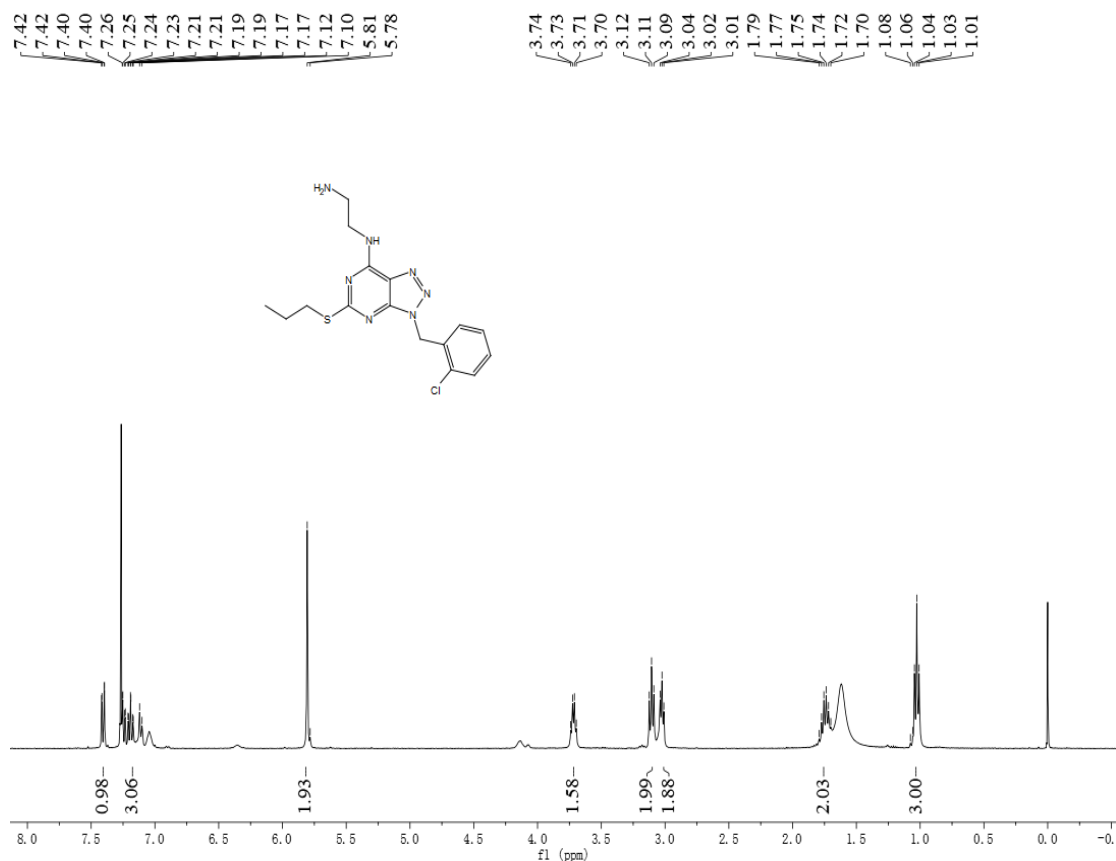
140

LHM-4790 4 (0.069) AM (Cen,4, 80.00, Ht,5000.0,0.00,1.00); Sm (Mn, 2x3.00); Cm (1:13)

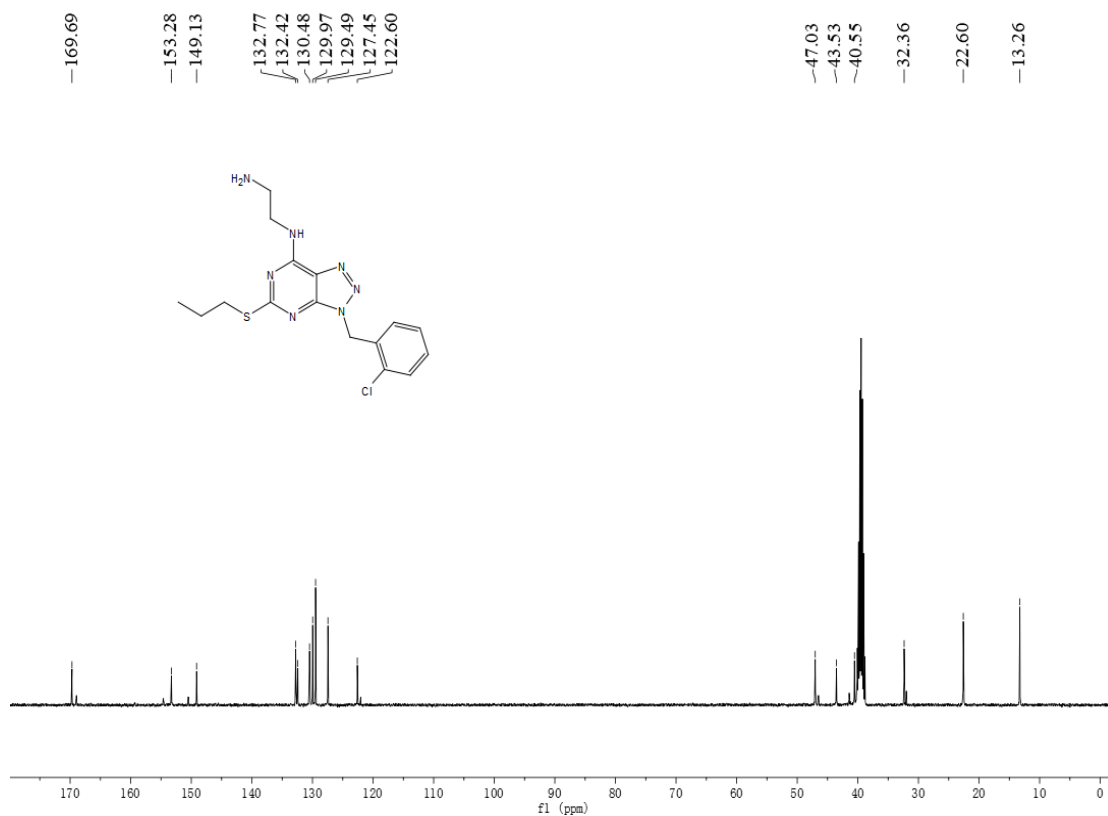
TOF MS ES+  
580



### <sup>1</sup>H NMR spectrum of compound 17



### <sup>13</sup>C NMR spectrum of compound 17

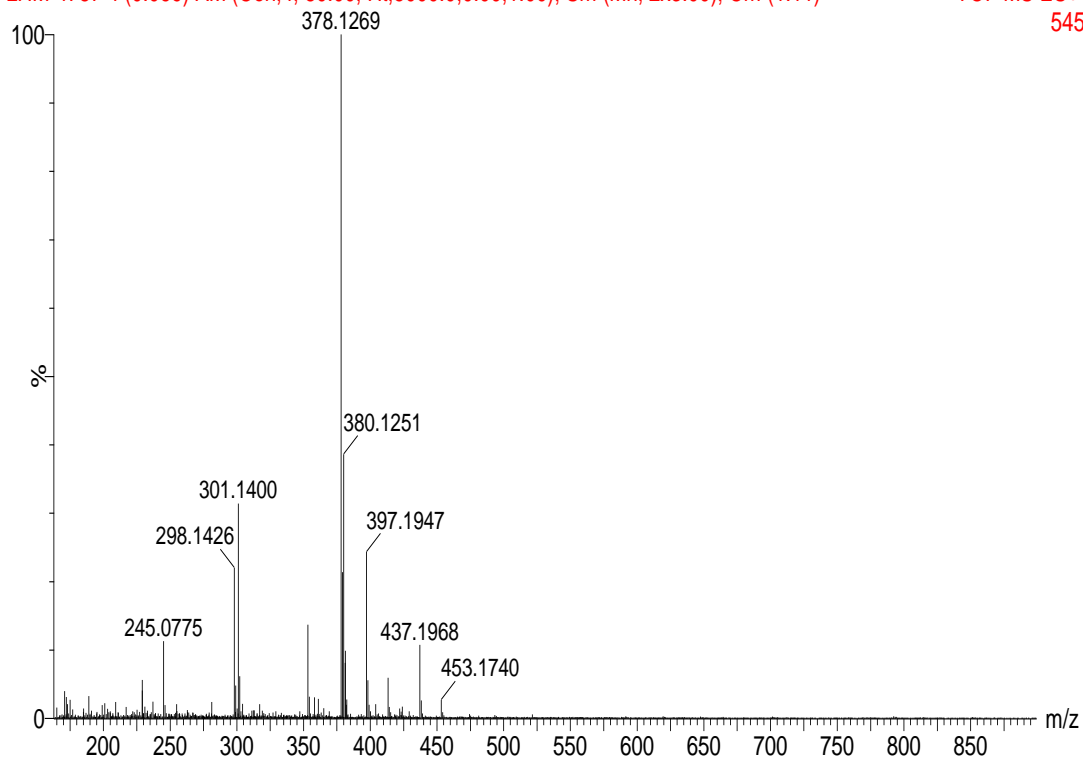


# MS spectrum of compound 17

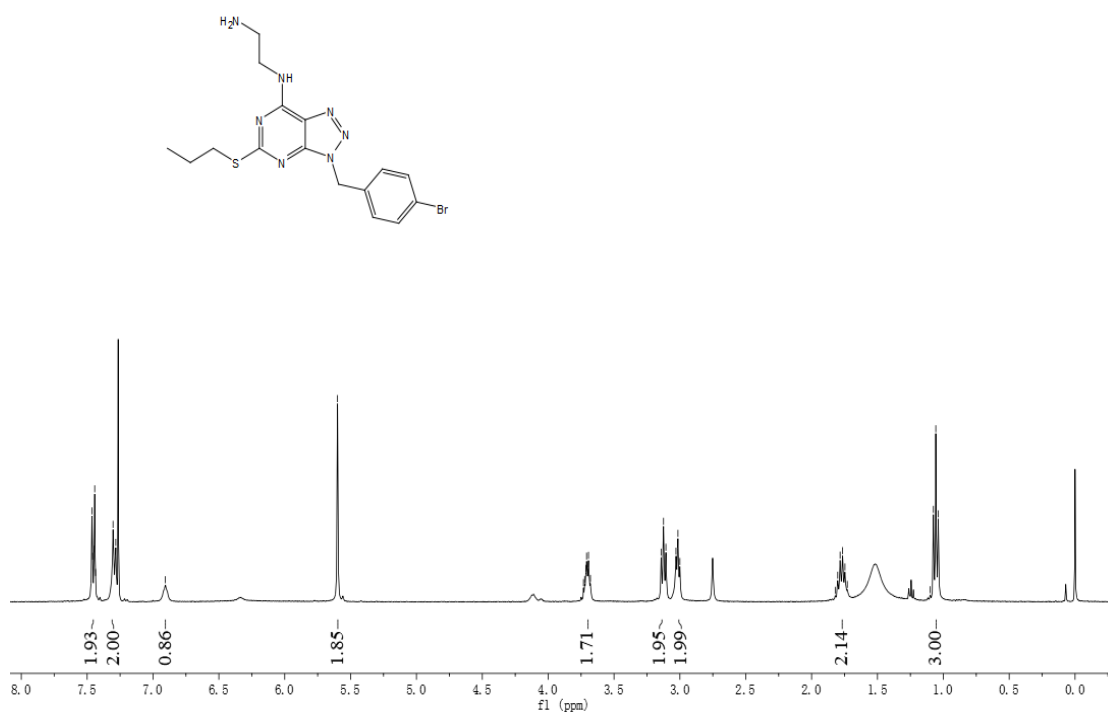
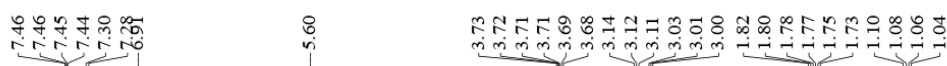
136

LHM-4787 4 (0.069) AM (Cen,4, 80.00, Ht,5000.0,0.00,1.00); Sm (Mn, 2x3.00); Cm (1:11)

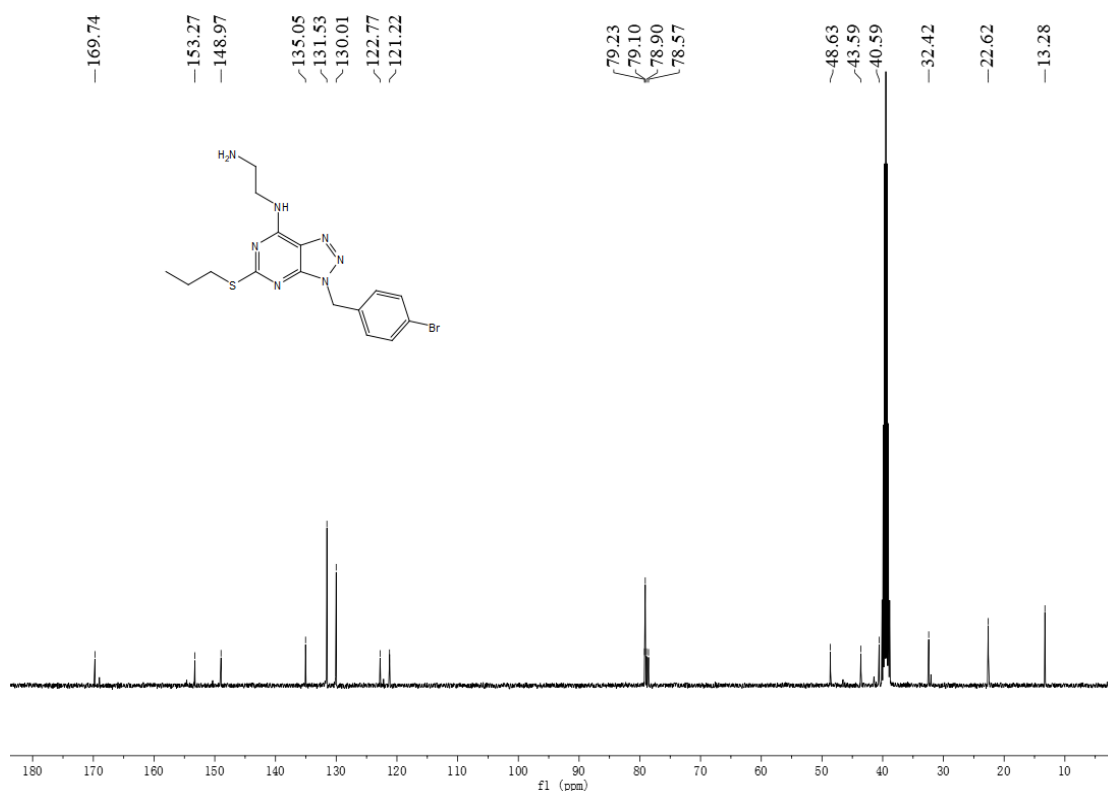
TOF MS ES+  
545



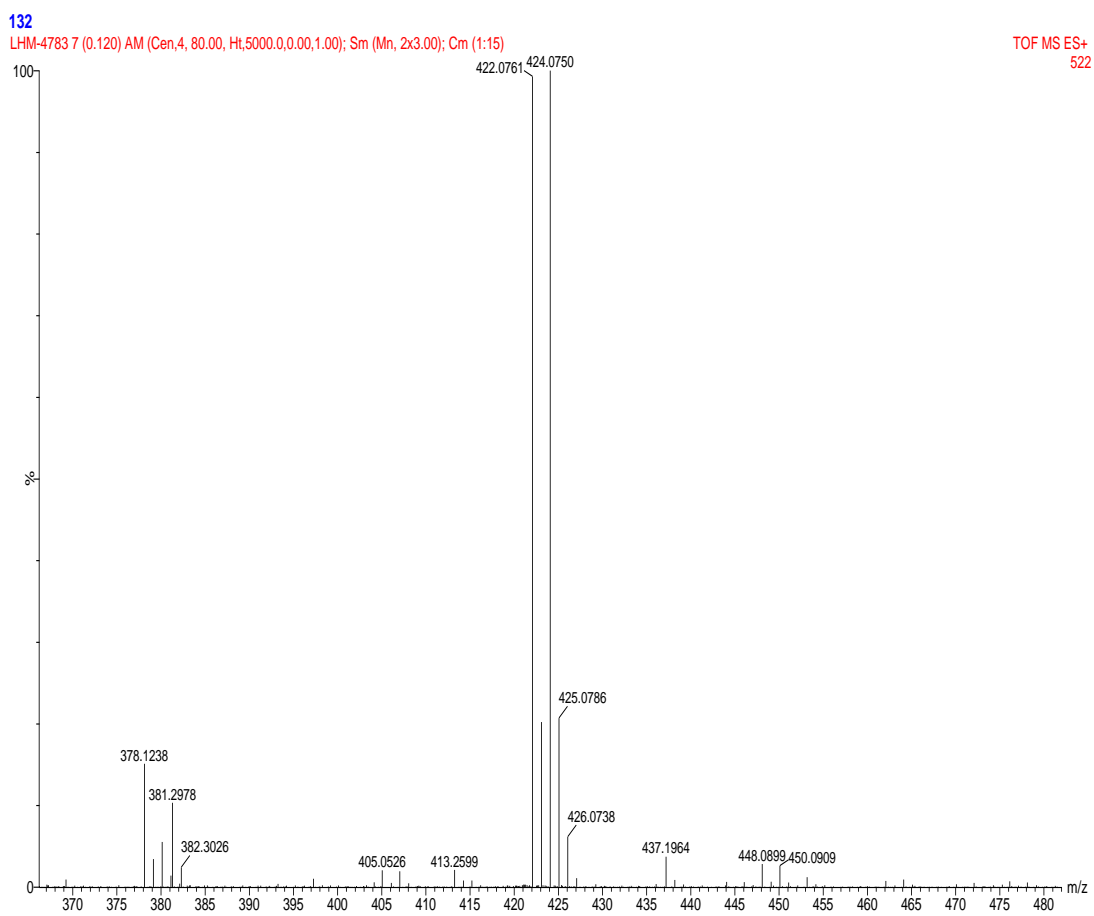
# <sup>1</sup>H NMR spectrum of compound 18



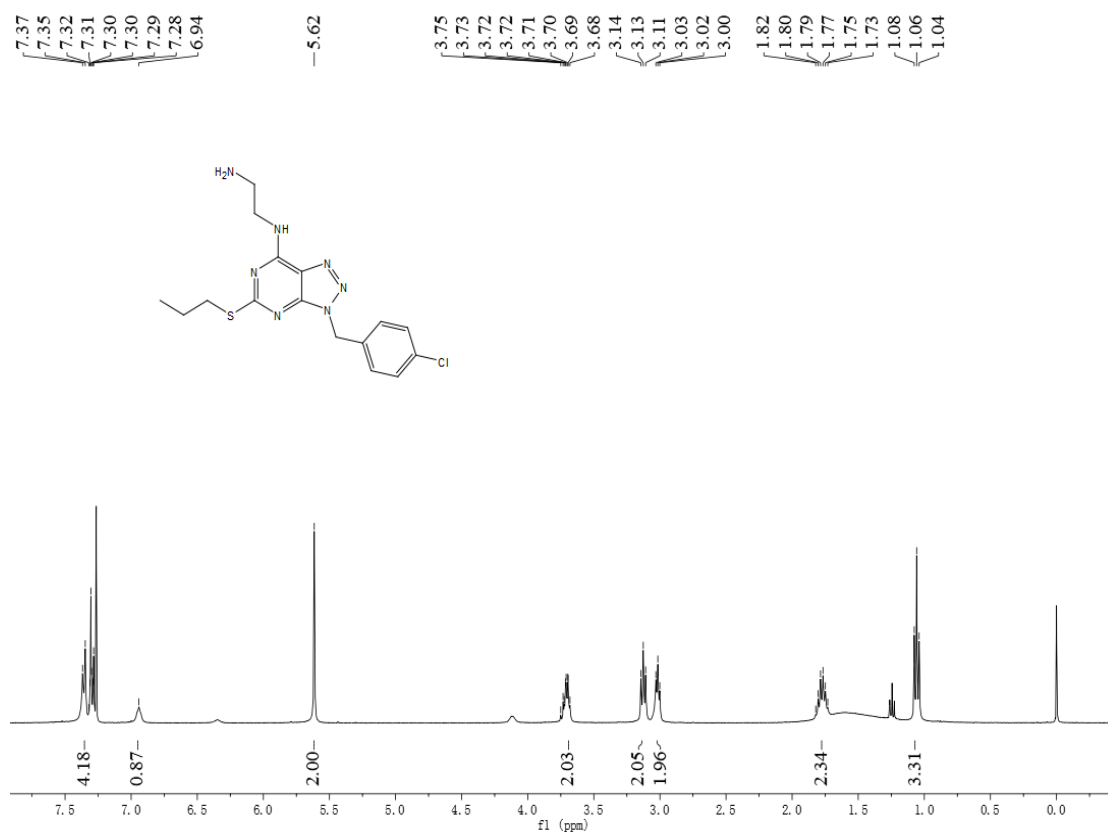
### <sup>13</sup>C NMR spectrum of compound 18



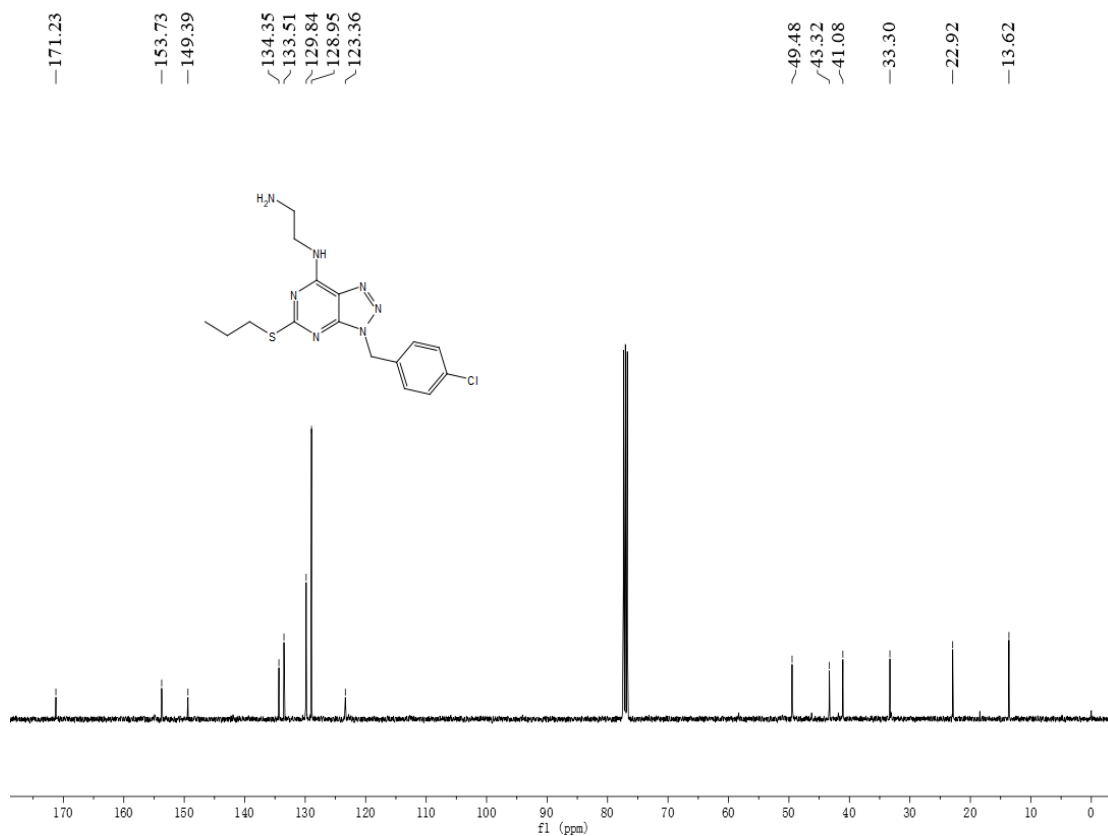
### MS spectrum of compound 18



### <sup>1</sup>H NMR spectrum of compound **19**



### <sup>13</sup>C NMR spectrum of compound **19**

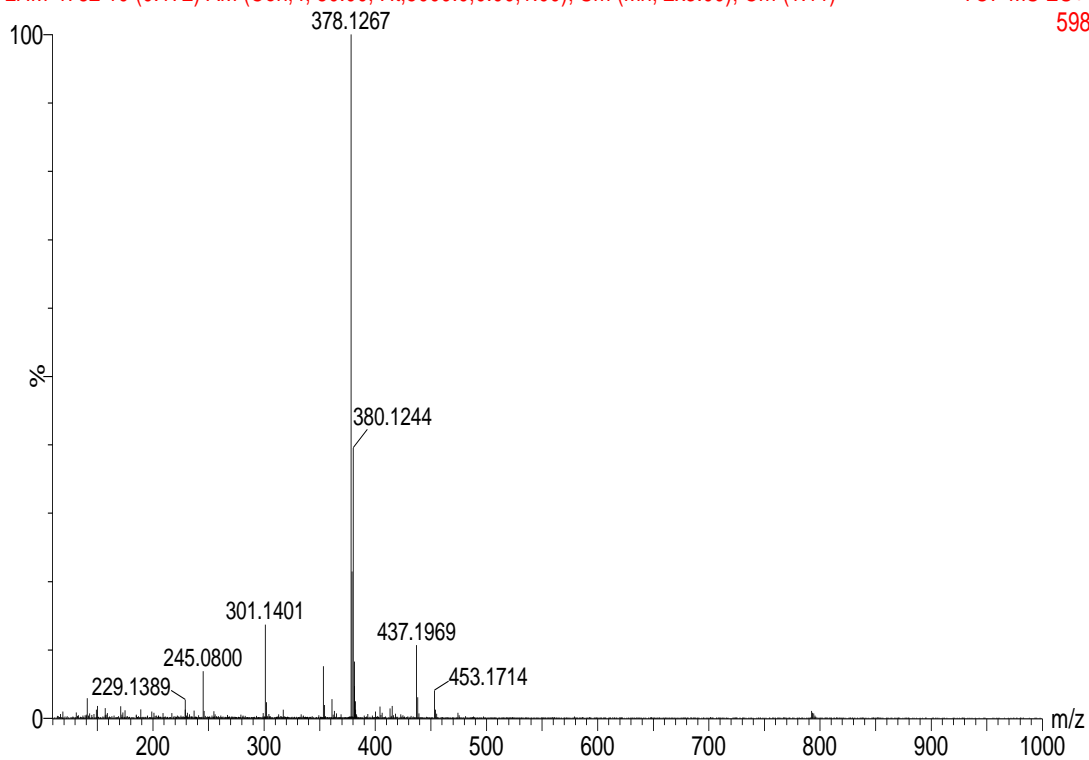


### MS spectrum of compound 19

131

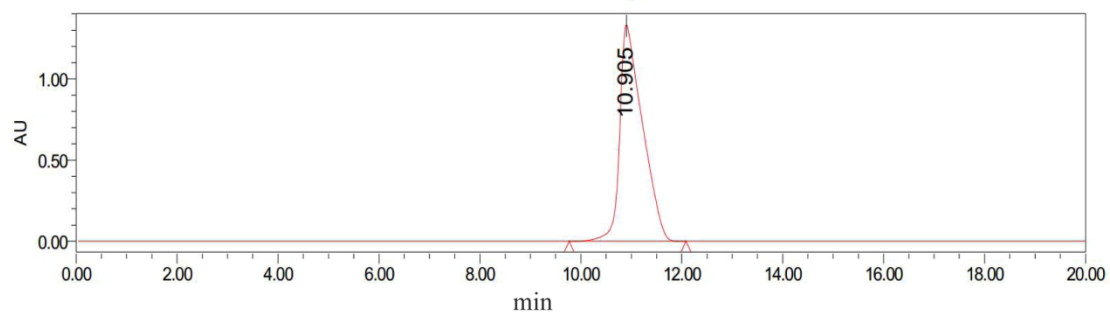
LHM-4782 10 (0.172) AM (Cen,4, 80.00, Ht,5000.0,0.00,1.00); Sm (Mn, 2x3.00); Cm (1:11)

TOF MS ES+  
598



### HPLC spectrum of compound 19

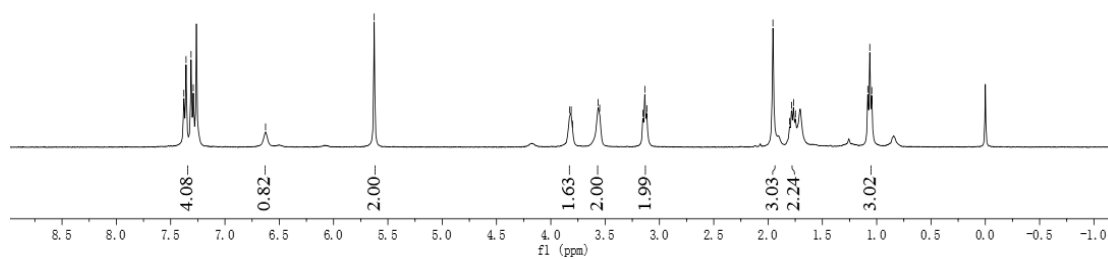
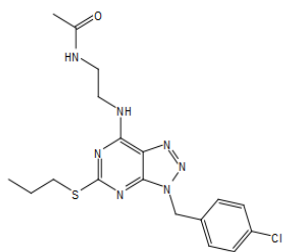
#### HPLC chromatogram



Retention Time (min)	Area( $\mu\text{v}\cdot\text{s}$ )	%Area
10.90	41753297	100.00

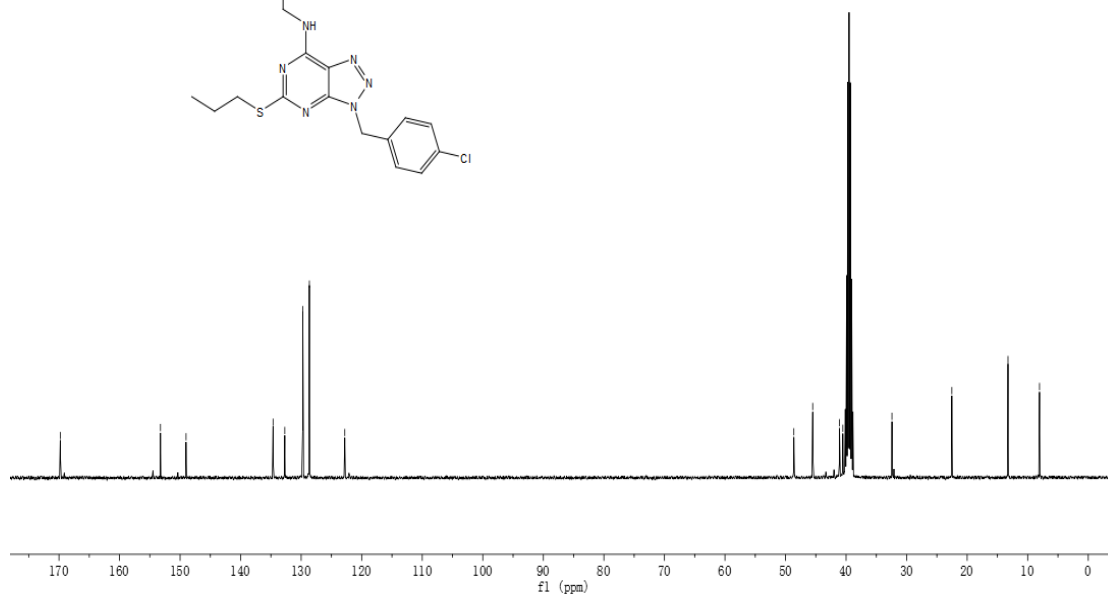
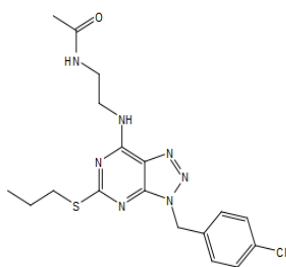
### <sup>1</sup>H NMR spectrum of compound **20**

7.38  
7.36  
7.31  
7.29  
— 6.63  
— 5.63  
3.83  
3.81  
3.80  
3.56  
3.55  
3.15  
3.13  
3.12  
1.96  
1.80  
1.78  
1.77  
1.75  
1.08  
1.06  
1.05



### <sup>13</sup>C NMR spectrum of compound **20**

— 169.79  
— 153.24  
— 149.01  
134.64  
132.70  
129.74  
128.65  
122.80  
48.63  
45.51  
41.08  
40.53  
32.42  
22.53  
13.25  
8.05

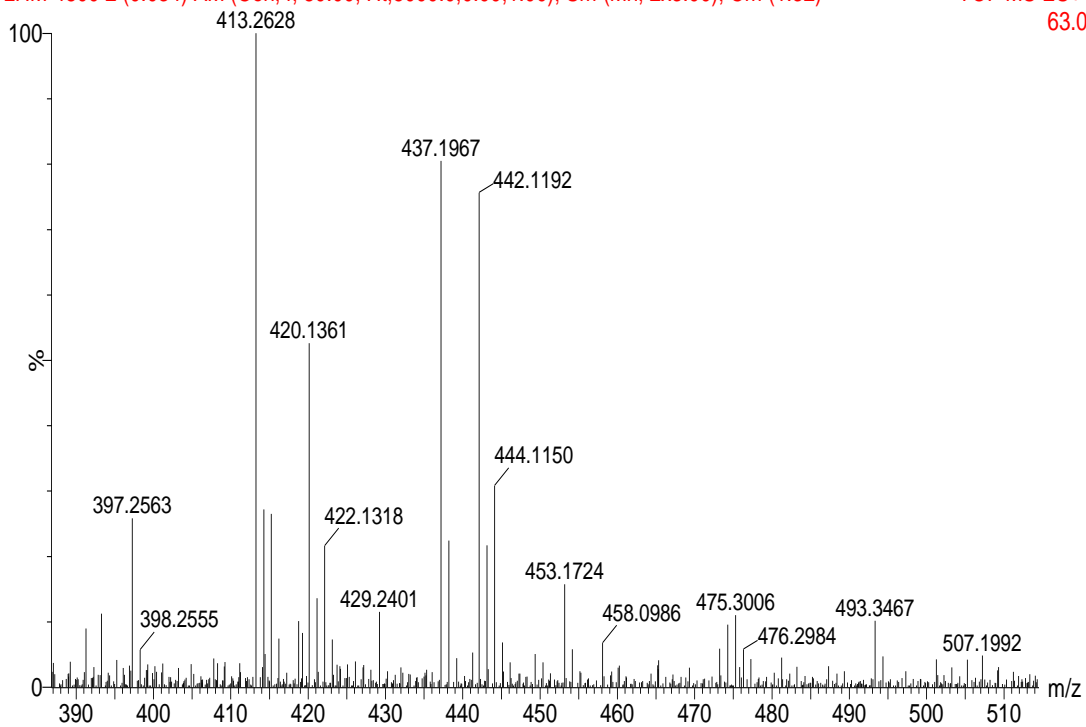


# MS spectrum of compound 20

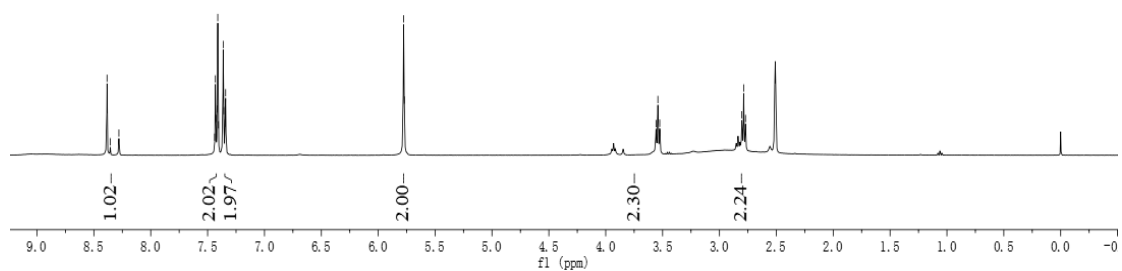
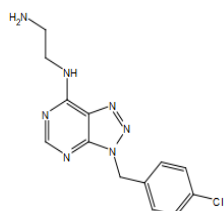
177

LHM-4800 2 (0.034) AM (Cen,4, 80.00, Ht,5000.0,0.00,1.00); Sm (Mn, 2x3.00); Cm (1:32)

TOF MS ES+  
63.0

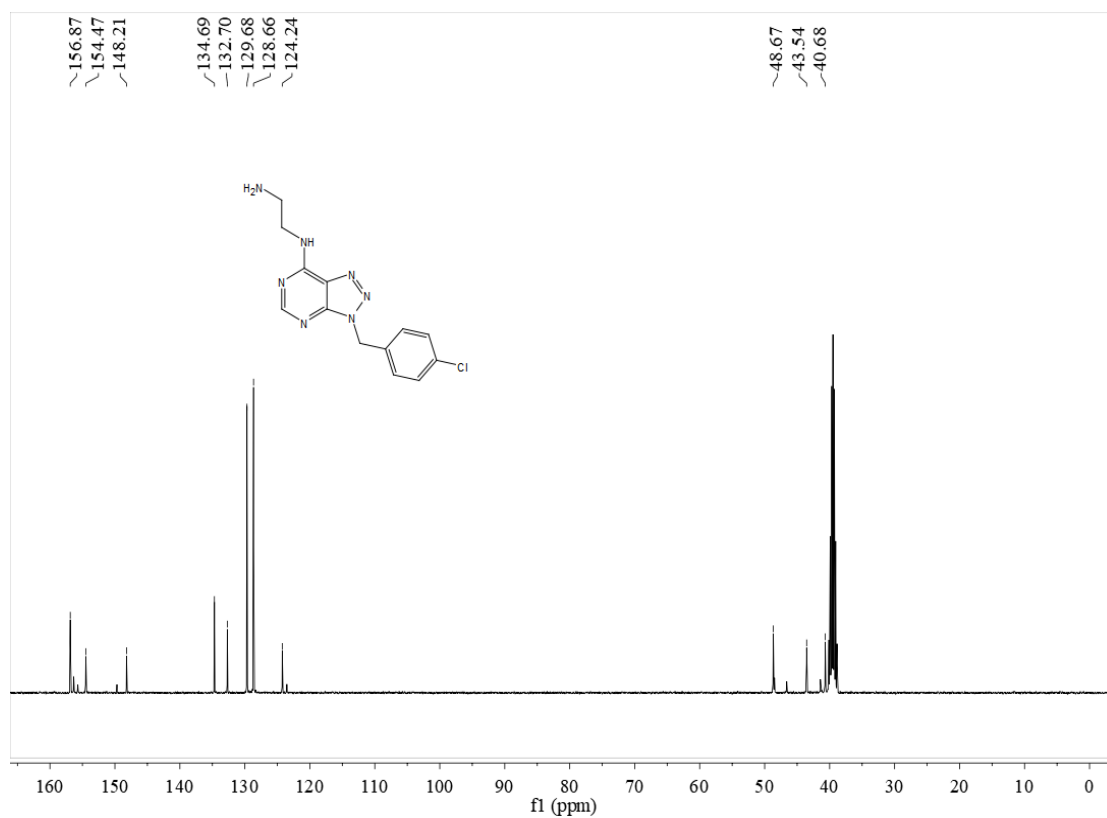


# <sup>1</sup>H NMR spectrum of compound 21





### <sup>13</sup>C NMR spectrum of compound 21

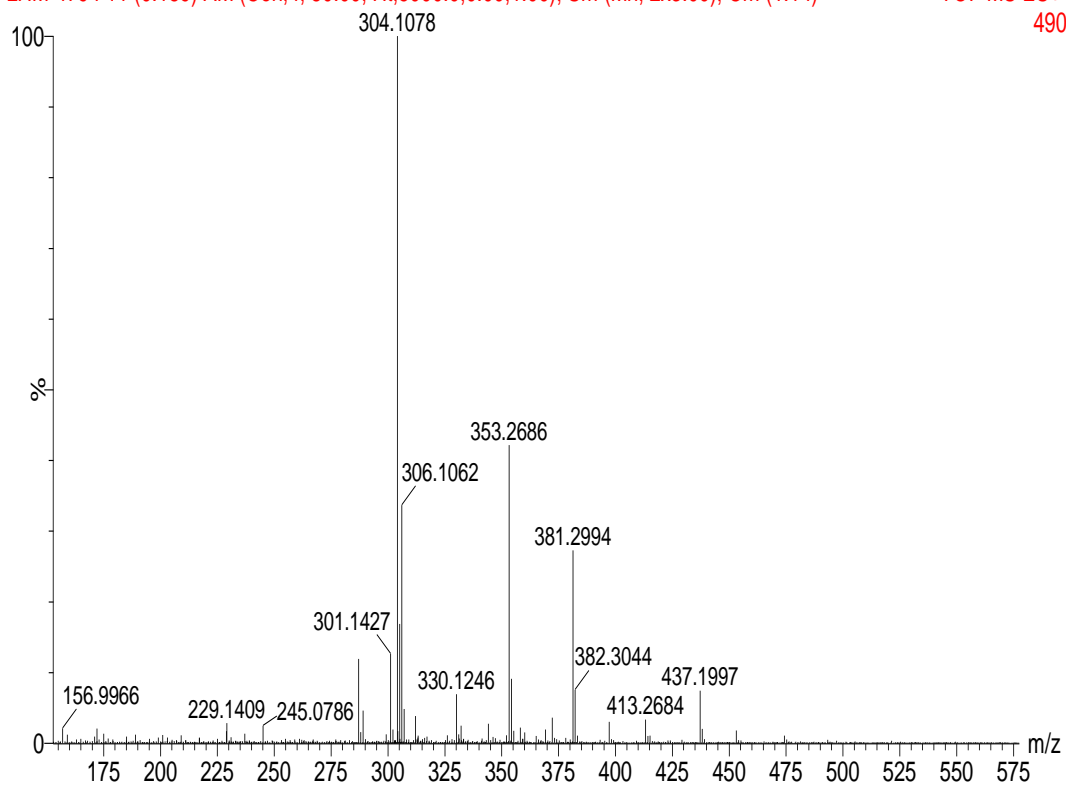


### MS spectrum of compound 21

171

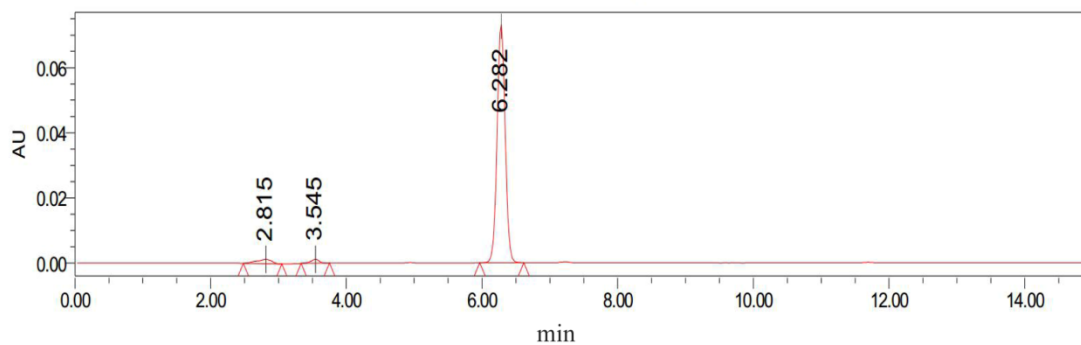
LHM-4794 11 (0.189) AM (Cen,4, 80.00, Ht,5000.0,0.00,1.00); Sm (Mn, 2x3.00); Cm (1:14)

TOF MS ES+  
490



# HPLC spectrum of compound 21

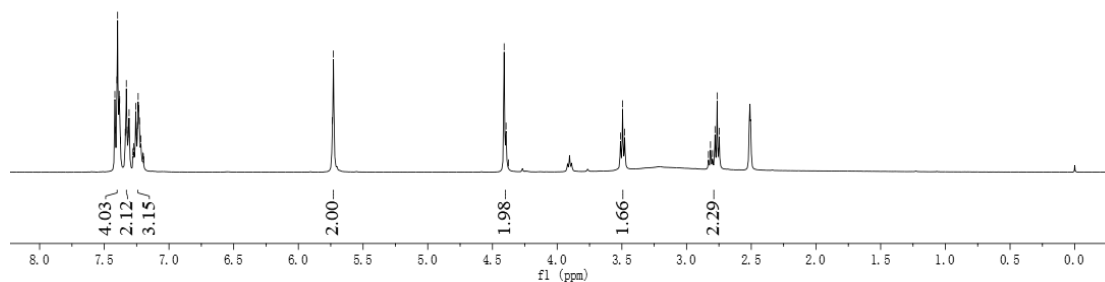
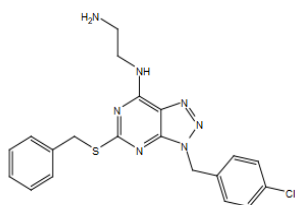
## HPLC chromatogram



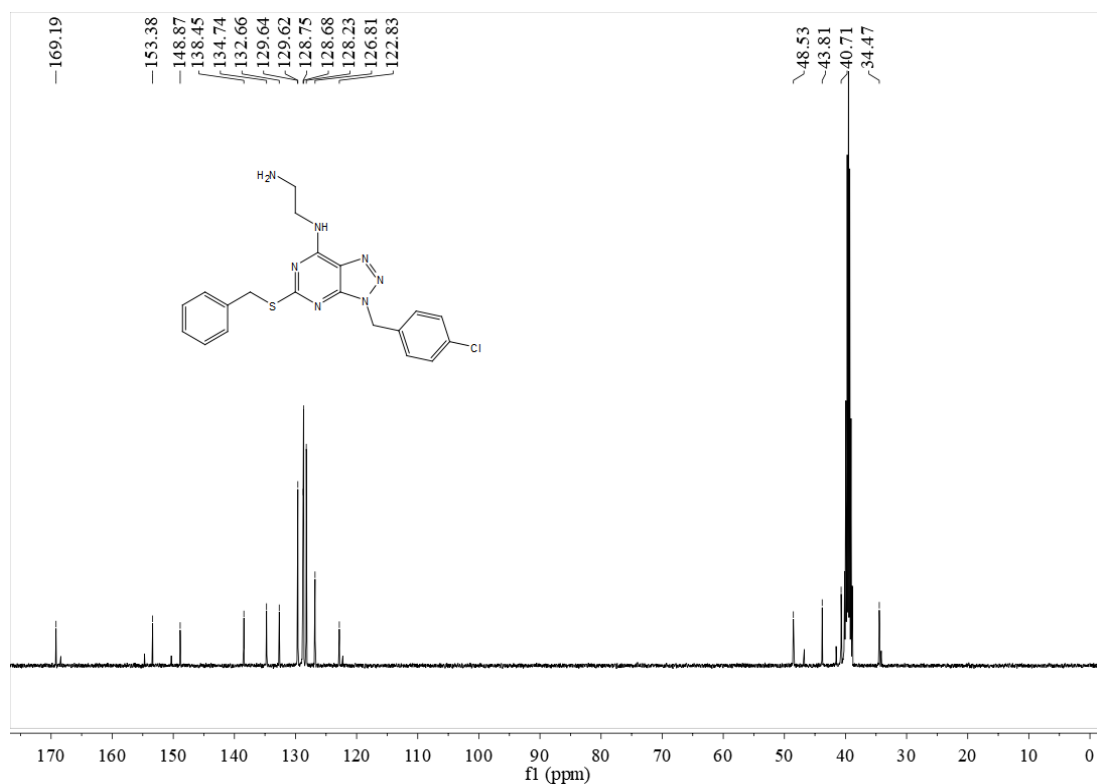
	Retention Time (min)	Area( $\mu\text{v}\cdot\text{s}$ )	%Area
1	2.82	21135	3.34
2	3.55	9728	1.54
3	6.28	602821	95.13

## $^1\text{H}$ NMR spectrum of compound 22

7.42  
7.41  
7.40  
7.40  
7.38  
7.38  
7.37  
7.34  
7.33  
7.32  
7.32  
7.31  
7.28  
7.27  
7.27  
7.26  
7.25  
7.24  
7.24  
7.23  
7.23  
7.22  
7.20  
7.20  
7.19  
5.74  
5.73  
4.41  
4.39  
4.38  
4.38  
3.51  
3.49  
3.48  
2.83  
2.82  
2.80  
2.78  
2.76  
2.75



<sup>13</sup>C NMR spectrum of compound 22

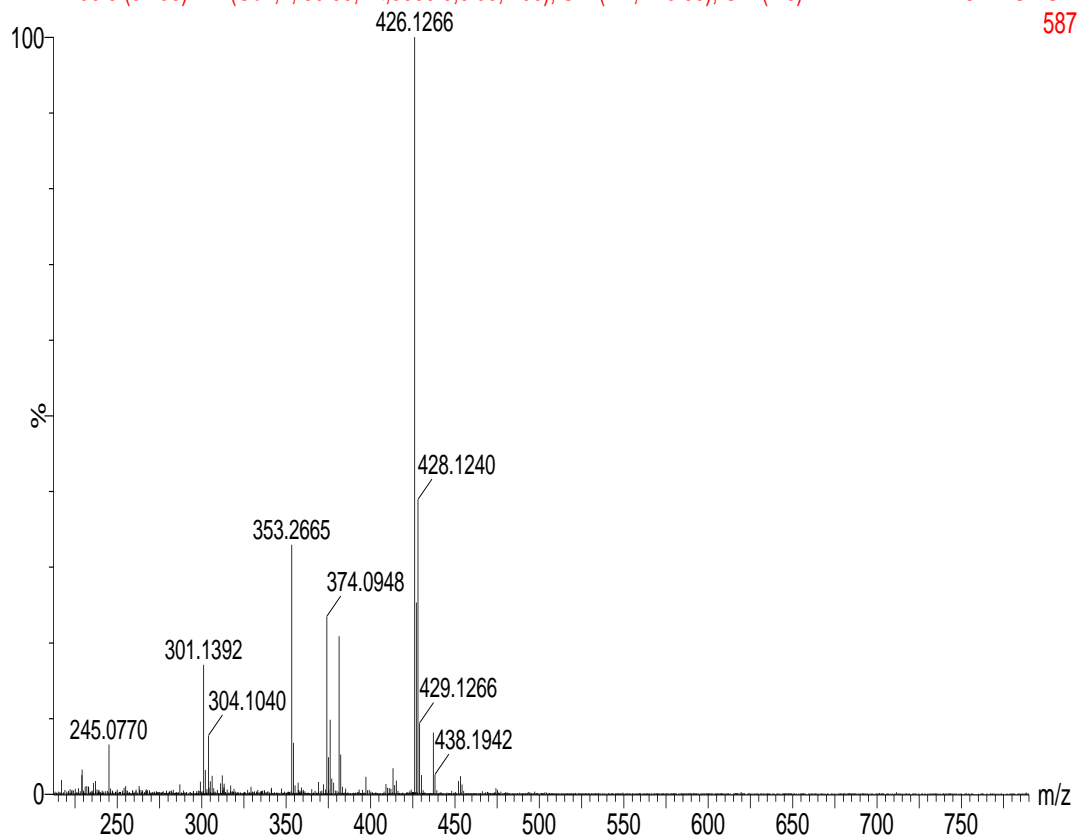


MS spectrum of compound 22

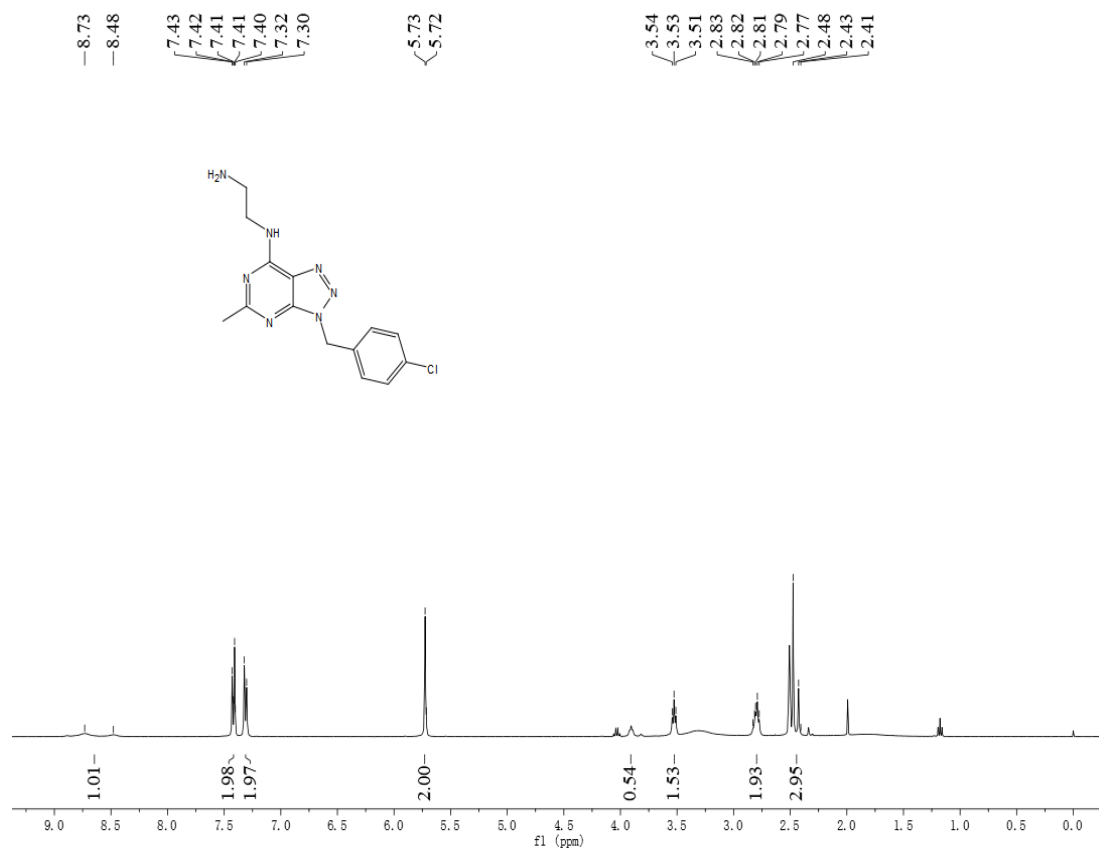
173

LHM-4796 9 (0.156) AM (Cen,4, 80.00, Ht,5000.0,0.00,1.00); Sm (Mn, 2x3.00); Cm (1:9)

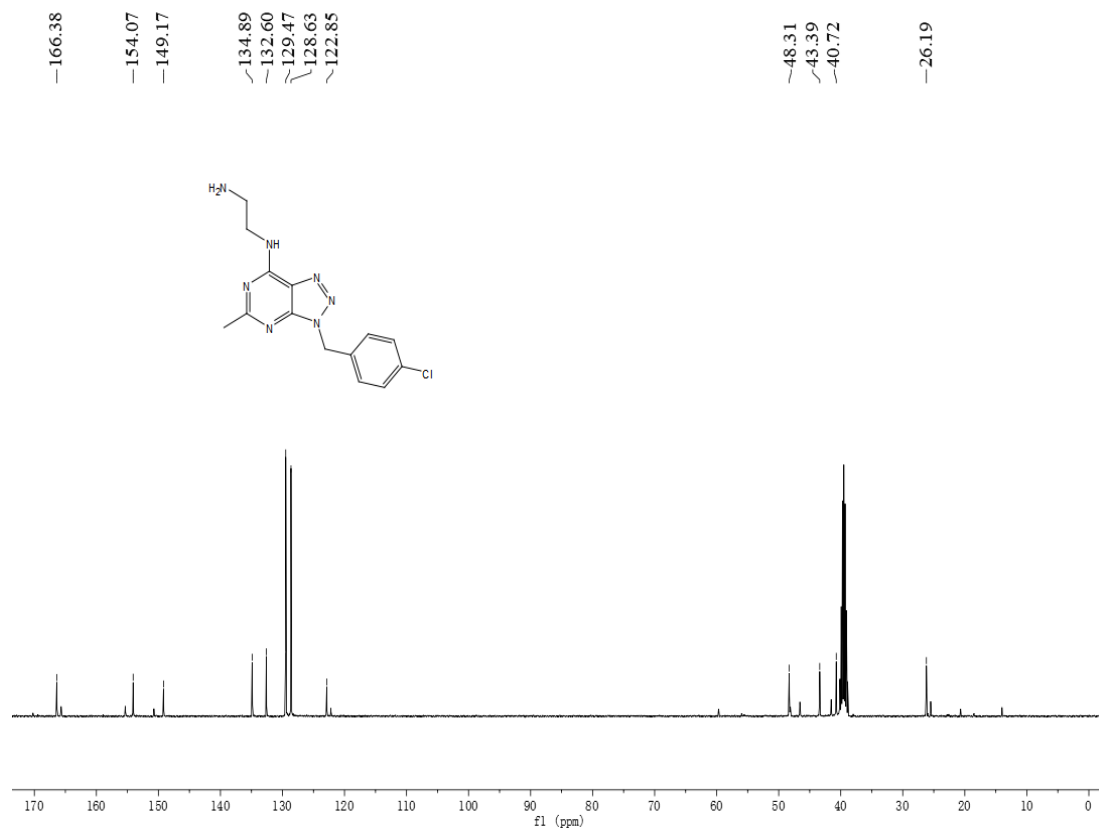
TOF MS ES+  
587



### <sup>1</sup>H NMR spectrum of compound 23



### <sup>13</sup>C NMR spectrum of compound 23

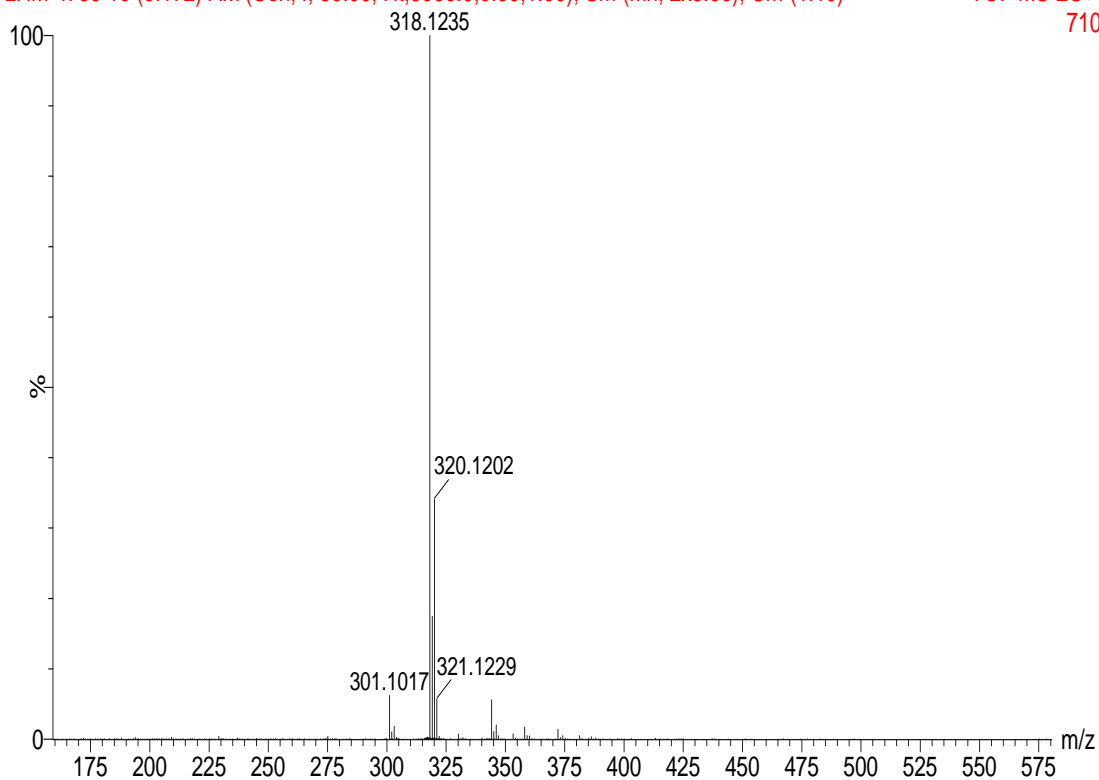


# MS spectrum of compound 23

176

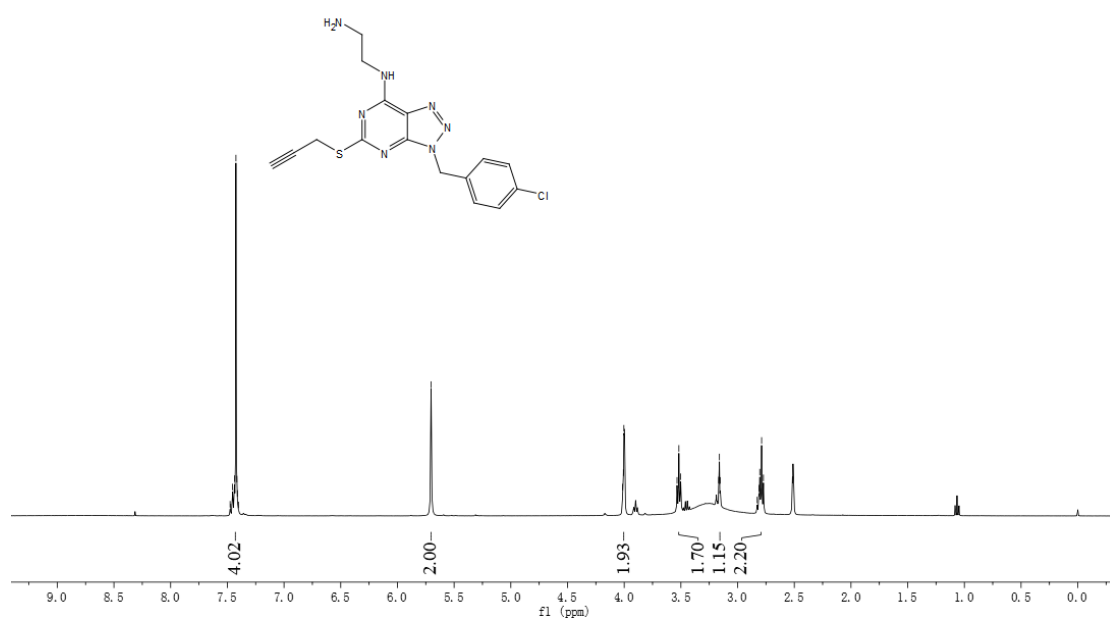
LHM-4799 10 (0.172) AM (Cen,4, 80.00, Ht,5000.0,0.00,1.00); Sm (Mn, 2x3.00); Cm (1:10)

TOF MS ES+  
710

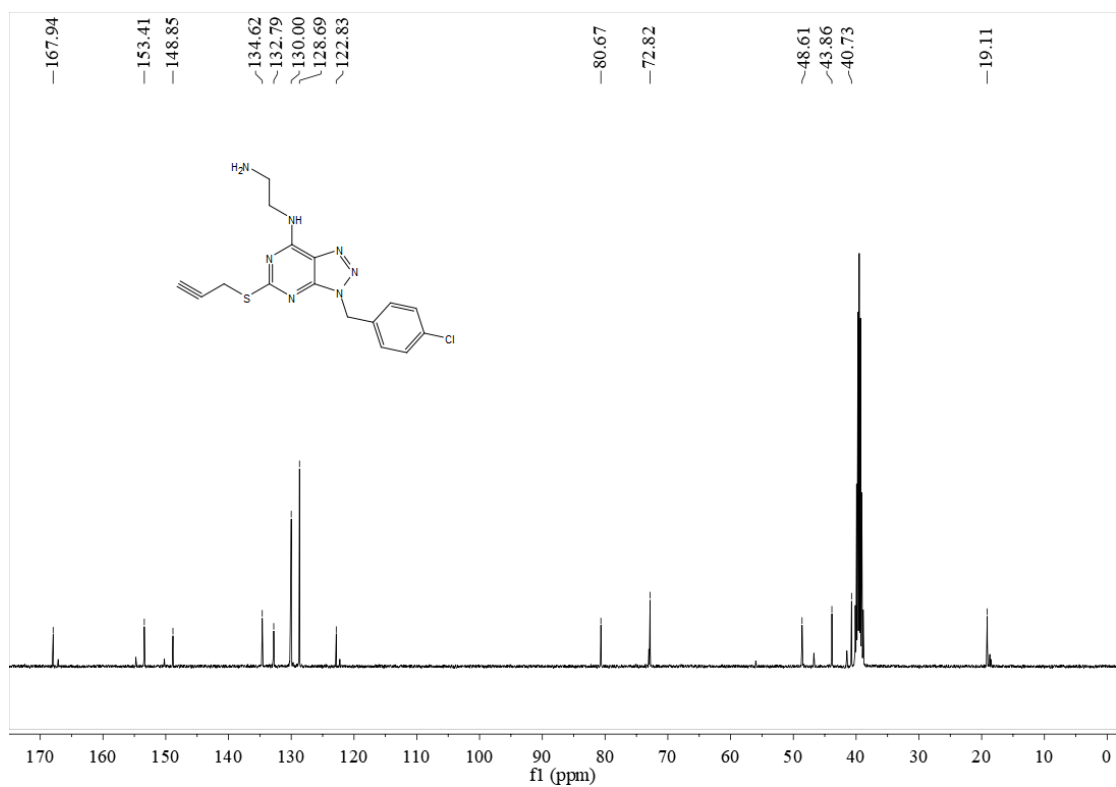


# <sup>1</sup>H NMR spectrum of compound 24

7.48, 7.47, 7.45, 7.44, 7.42, 7.42, 7.41, 7.40, 5.70, 4.01, 4.01, 4.00, 4.00, 3.53, 3.52, 3.50, 3.17, 3.16, 3.15, 2.83, 2.81, 2.80, 2.80, 2.79, 2.77



### $^{13}\text{C}$ NMR spectrum of compound **24**



### MS spectrum of compound **24**

**172**

LHM-4795 3 (0.052) AM (Gen,4, 80.00, Ht,5000.0,0.00,1.00); Sm (Mn, 2x3.00); Cm (1:13)

TOF MS ES+  
470

