

## **Supporting Information**

### **Design and SAR of withangulatin A analogs that act as covalent TrxR inhibitors through Michael addition reaction in cancer treatment**

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Figure S1. The crystal structure of **3**

Figure S2. The expression of TrxR in 4 tumor cells and 2 normal cells was detected using Western blot.

Figure S3. PharmMapper to screen targets of the potential protein.

Figure S4. The structure of compound **13a'**

Table S1 Crystal data and structure refinement for **3**.

Figure S5. The 2D predict of ligand interactions of the covalent docking adducts of TrxR (PDB: 3EAN mutated Sec498 to Cys498) and **1**, **8e**, **9c** and **13a**.

Table S2. The Covalent Docking Interactions.

Figure S6. The vitro binding ability of **WA** and **13a** to sulphydryl groups.

Figure S7. **WA**, **13a** with NAC and DTT were detected by LC-MS.

Spectra of compound **1-13**

HRMS for compound **1-13**

HPLC for compound **1-13**

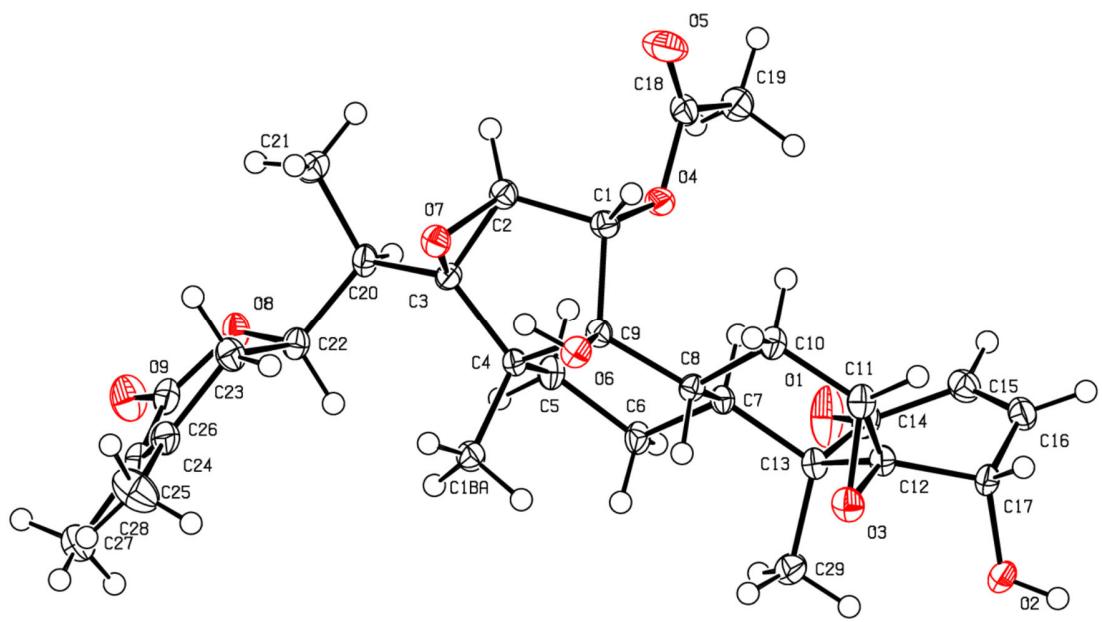


Figure S1. The crystal structure of **3** (CCDC-2008080)

Table S1 Crystal data and structure refinement for **3**.

Identification code	3
Empirical formula	C <sub>30</sub> H <sub>38</sub> O <sub>9</sub>
Formula weight	542.60
Temperature/K	130.0
Crystal system	orthorhombic
Space group	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
a/Å	7.4020(2)
b/Å	13.4298(4)
c/Å	26.9766(9)
α/°	90
β/°	90
γ/°	90
Volume/Å <sup>3</sup>	2681.67(14)
Z	4
ρ <sub>calcd</sub> /cm <sup>3</sup>	1.344
μ/mm <sup>-1</sup>	0.814
F(000)	1160.0
Crystal size/mm <sup>3</sup>	0.12 × 0.08 × 0.03
Radiation	CuKα ( $\lambda = 1.54178$ )
2Θ range for data collection/°	6.552 to 149.01
Index ranges	-9 ≤ h ≤ 5, -16 ≤ k ≤ 16, -33 ≤ l ≤ 31
Reflections collected	20906
Independent reflections	5373 [R <sub>int</sub> = 0.0737, R <sub>sigma</sub> = 0.0636]
Data/restraints/parameters	5373/2/366
Goodness-of-fit on F <sup>2</sup>	0.995
Final R indexes [I>=2σ (I)]	R <sub>1</sub> = 0.0468, wR <sub>2</sub> = 0.0996
Final R indexes [all data]	R <sub>1</sub> = 0.0644, wR <sub>2</sub> = 0.1108
Largest diff. peak/hole / e Å <sup>-3</sup>	0.28/-0.25
Flack parameter	0.05(14)

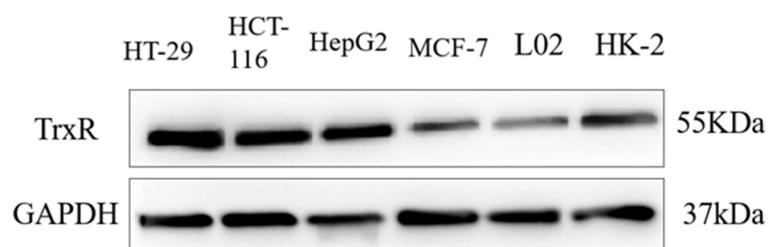


Figure S2. The expression of TrxR in 4 tumor cells and 2 normal cells was detected using Western blot.

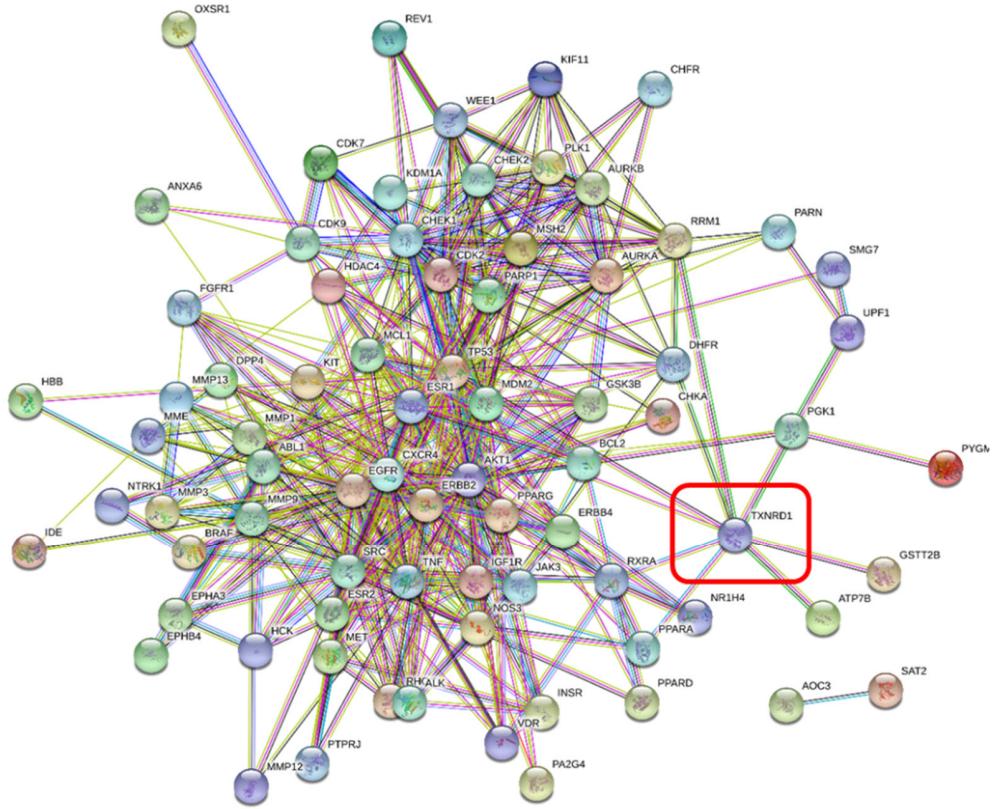
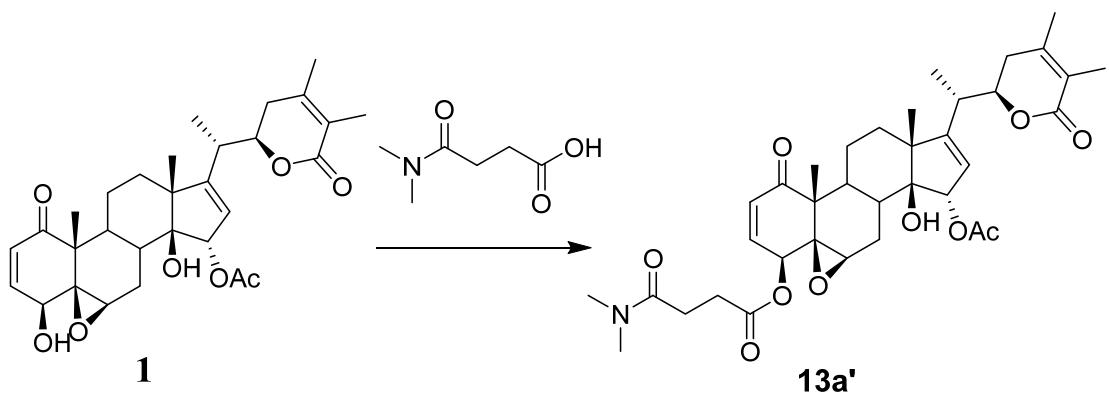
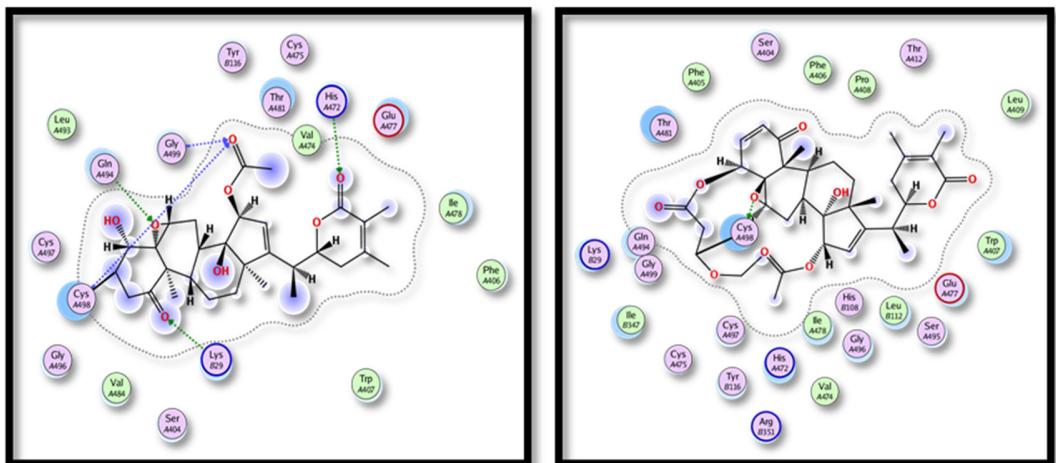


Figure S3. PharmaMapper identified TXNRD1 gene (TrxR1) as a potential target for **13a**



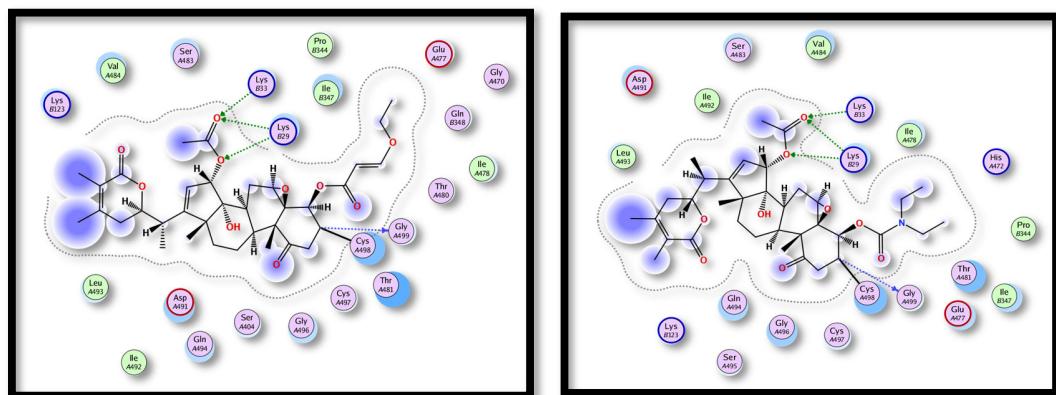
Reagents and conditions: DMAP, EDCI, Et<sub>3</sub>N, anhydrous CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 12h

Figure S4. The preparation of compound **13a'**



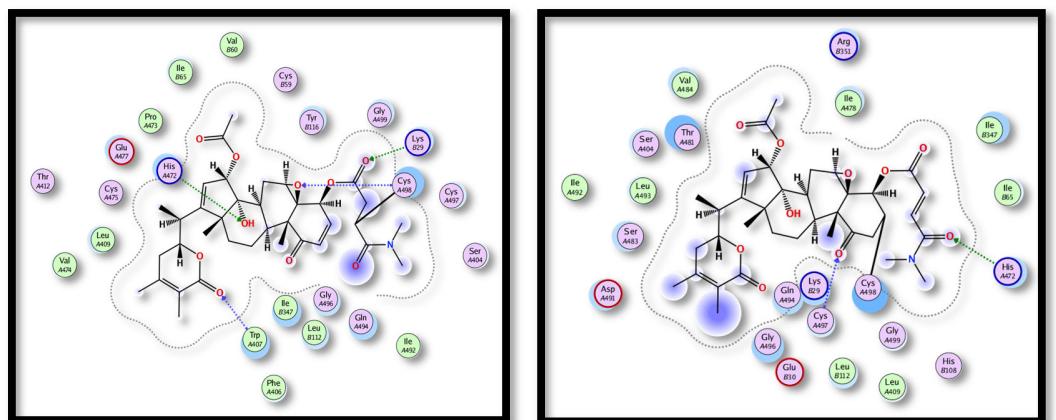
**A (1, WA, position 3)**

**B (8e, position 3')**



**C (8e, position 3)**

**D (9c, position 3)**



**E (13a, position 3')**

**F (13a, position 3)**

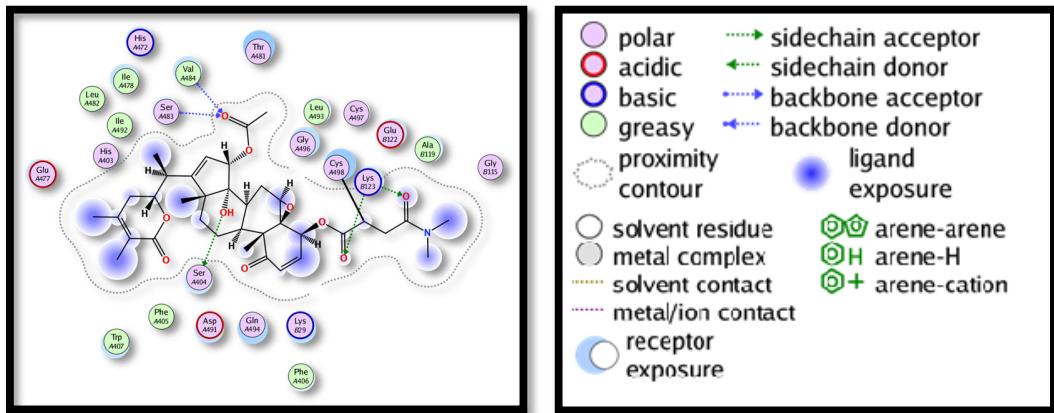


Figure S5. The 2D predict of ligand interactions of the covalent docking adducts of TrxR

Table S2. The Covalent Docking Interactions.

Compd.	Binding <sup>a</sup>	Noncovalent Interactions							Score <sup>d</sup>	IC <sub>50</sub> <sup>e</sup>		
		Covalent		Noncovalent								
		1 <sup>b</sup>	5,6	14	15	26	4-OR <sup>c</sup>					
		C=C-C=O	C=O	epoxy	OH	OAc	C=O	C=O				
<b>1(WA)</b>	3	B29 <sup>f</sup>	A494	NI <sup>g</sup>	A499	A472	NI	-5.73	2.48			
<b>8e</b>	3'	NI	A498	NI	NI	NI	NI	-5.61	0.23			
<b>8e</b>	3	NI	NI	NI	B29/B33	NI	NI	-5.16	0.23			
<b>9c</b>	3	NI	NI	NI	B29/B33	NI	NI	-4.63	0.75			
<b>13a</b>	3'	NI	A498	A472	NI	A407	B29	-5.61	0.08			
<b>13a</b>	3	A497	NI	NI	NI	NI	A472	-5.03	0.08			
<b>13a</b>	2'	NI	NI	A404	A483/A484	NI	B123	-3.82	0.08			

*a:* The covalent docking position. 3 represents the  $\beta$ -position of the  $\alpha,\beta$ -unsaturated carbonyl on ring A of WA; 3' represents the  $\beta$ -position of the  $\alpha,\beta$ -unsaturated carbonyl on the side chain at 4-OH; 2' represents the  $\alpha$ -position of the  $\alpha,\beta$ -unsaturated carbonyl on the side chain at 4-OH. *b:* 1, 4, 5, 6, 14, 15, and 26 are the atom numbers of WA in Figure 1. *c:* 4-OR represents the substitution R of the corresponding compounds at 4-OH of WA; the carbonyl of the R group interacted with the protein through H-bonding. *d:*

Docking score. *e*: Cytotoxic activity (IC<sub>50</sub> values in  $\mu\text{M}$ ) of the compound against HT-29 cells. *f*: The interaction residue ID on chains A or B of TrxR (PDB: 3EAN mutated Sec498 to Cys498). *g*: NI indicates that no interaction was found at that location.



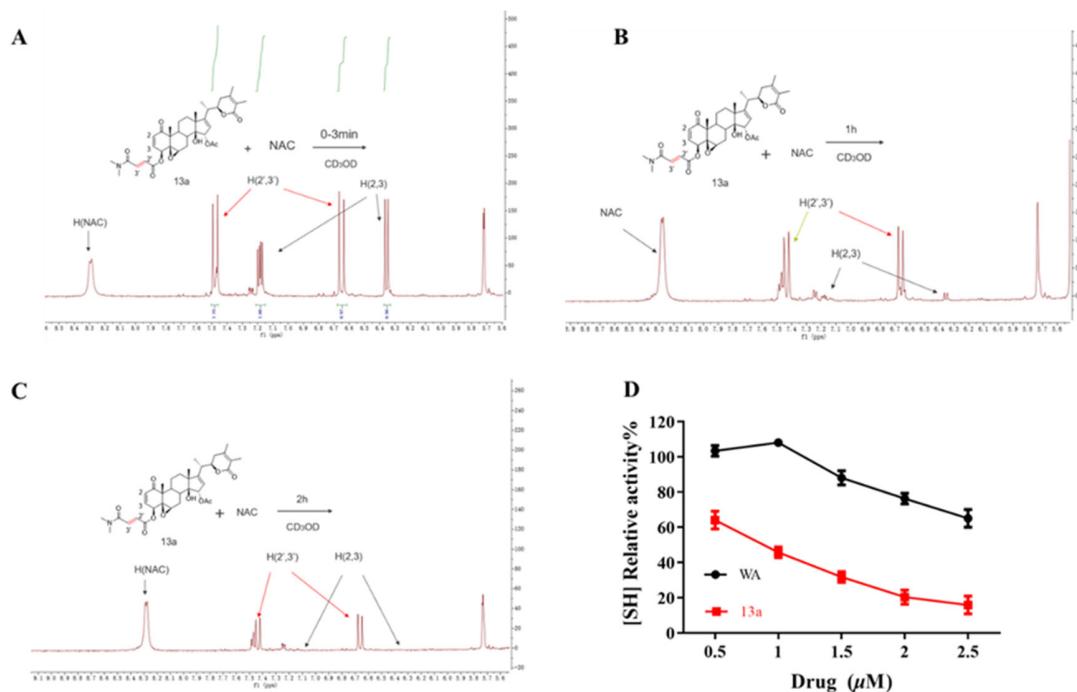


Figure S6. In vitro binding ability of WA and **13a** to sulphhydryl groups. (A) **13a** and NAC were incubated for 0-3min. (B) **13a** and NAC were incubated for 1h. (C) **13a** and NAC were incubated for 2h. (D) The effect of the compounds on the total amount of sulphhydryl groups in HT-29 cells.

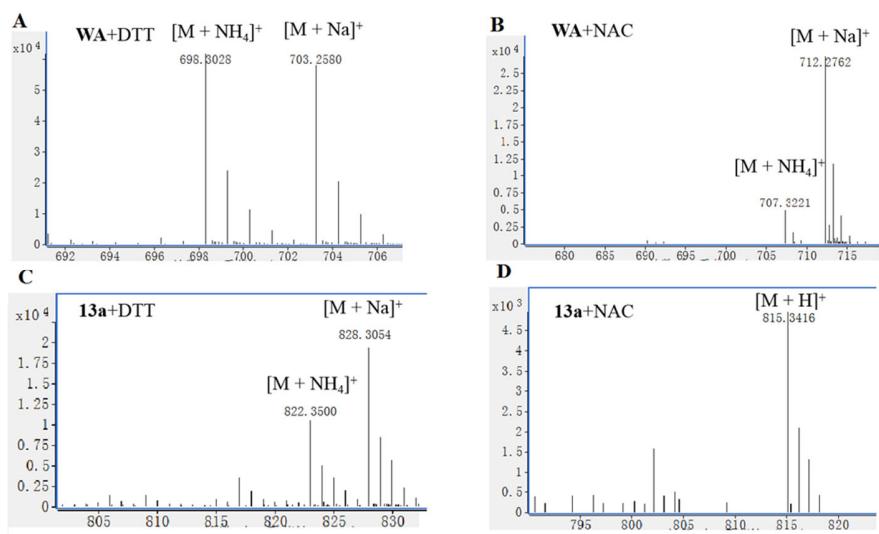
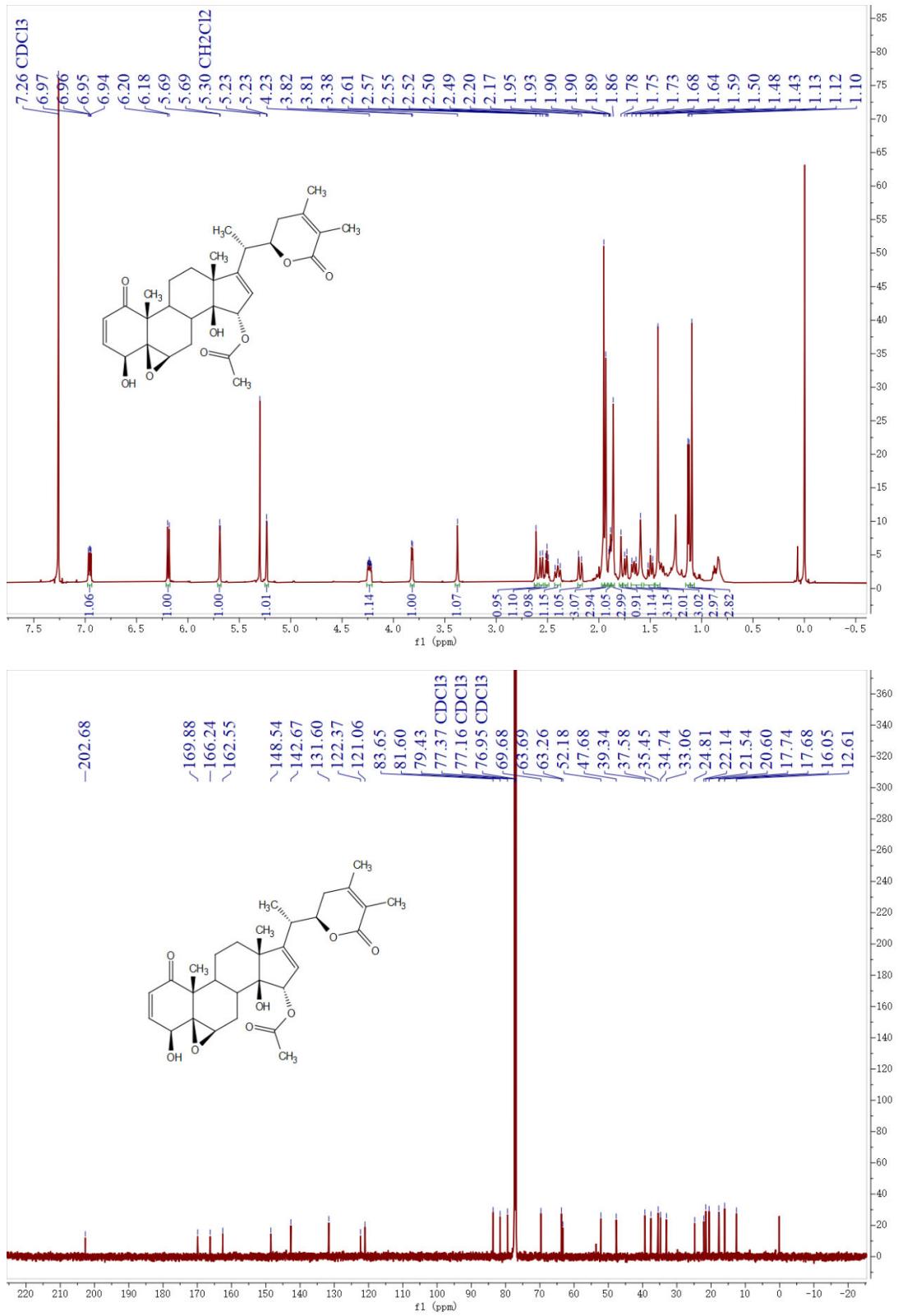
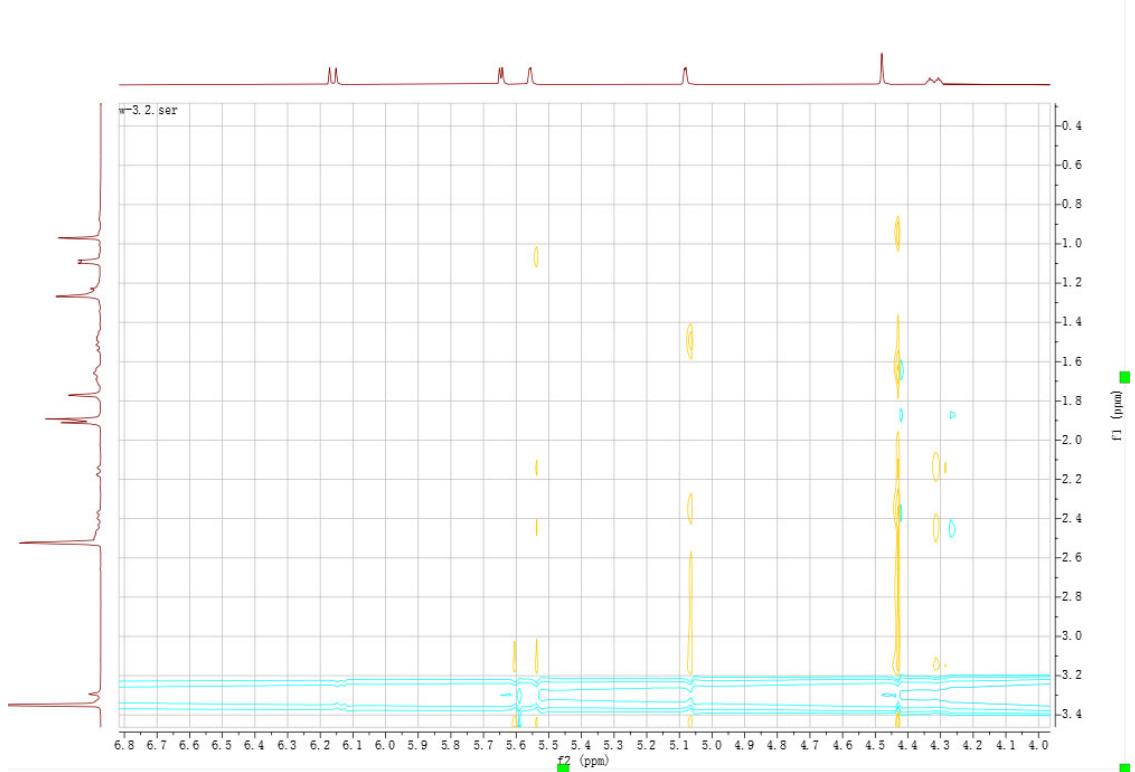
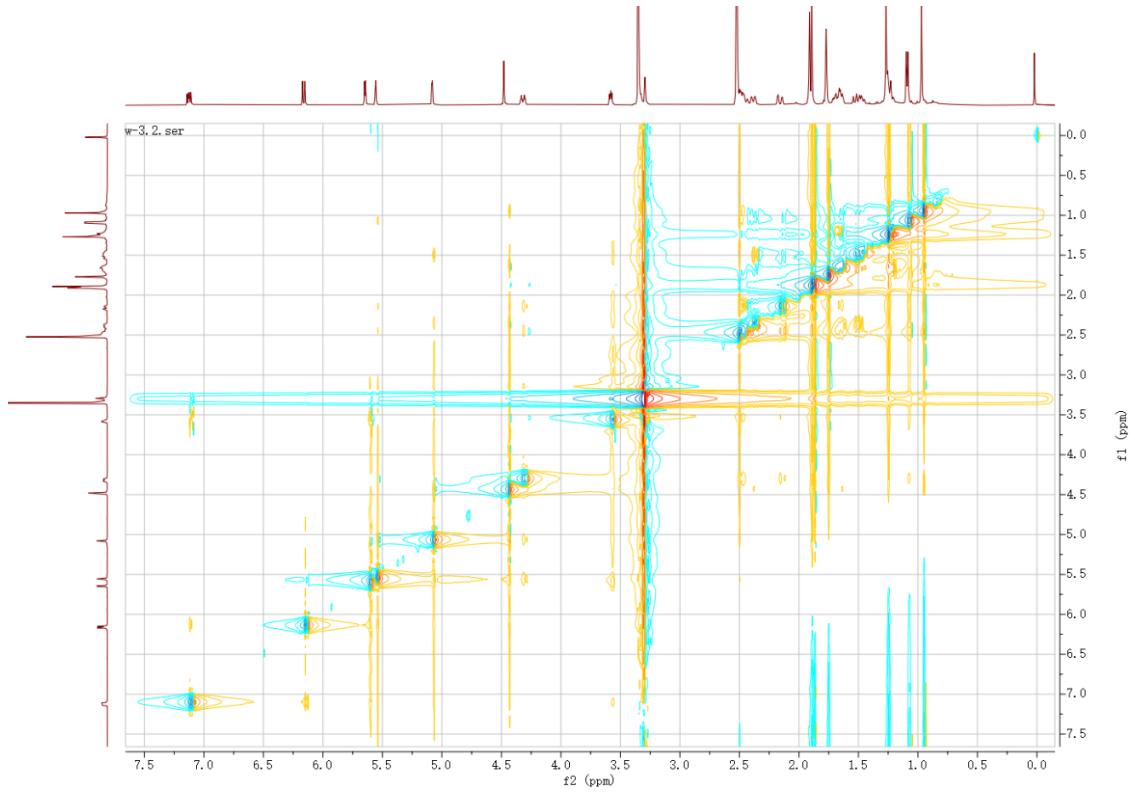


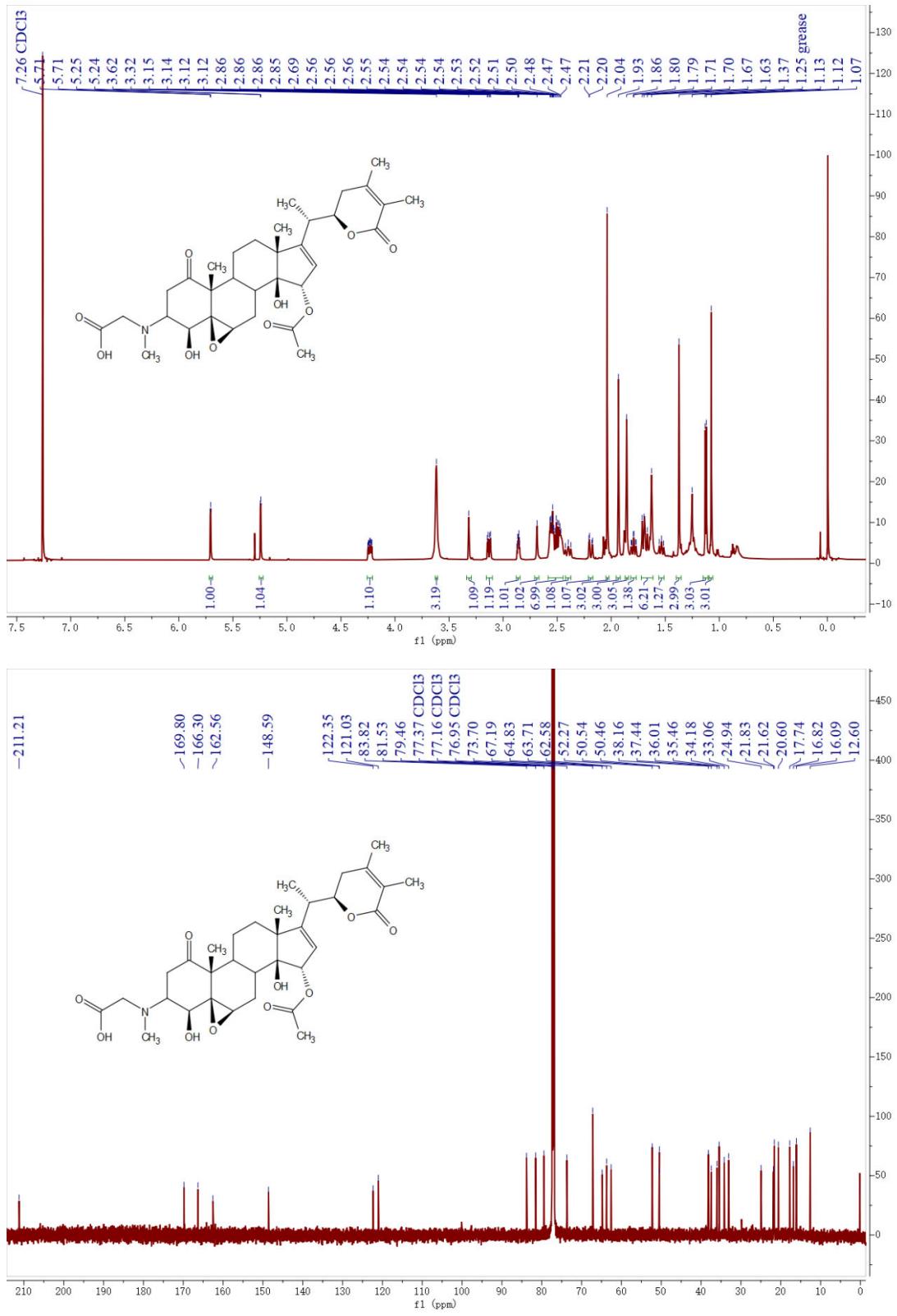
Figure S7. LC-MS analysis of the covalent bond between WA, **13a** and NAC and DTT.

Spectra of compound **1** (WA)

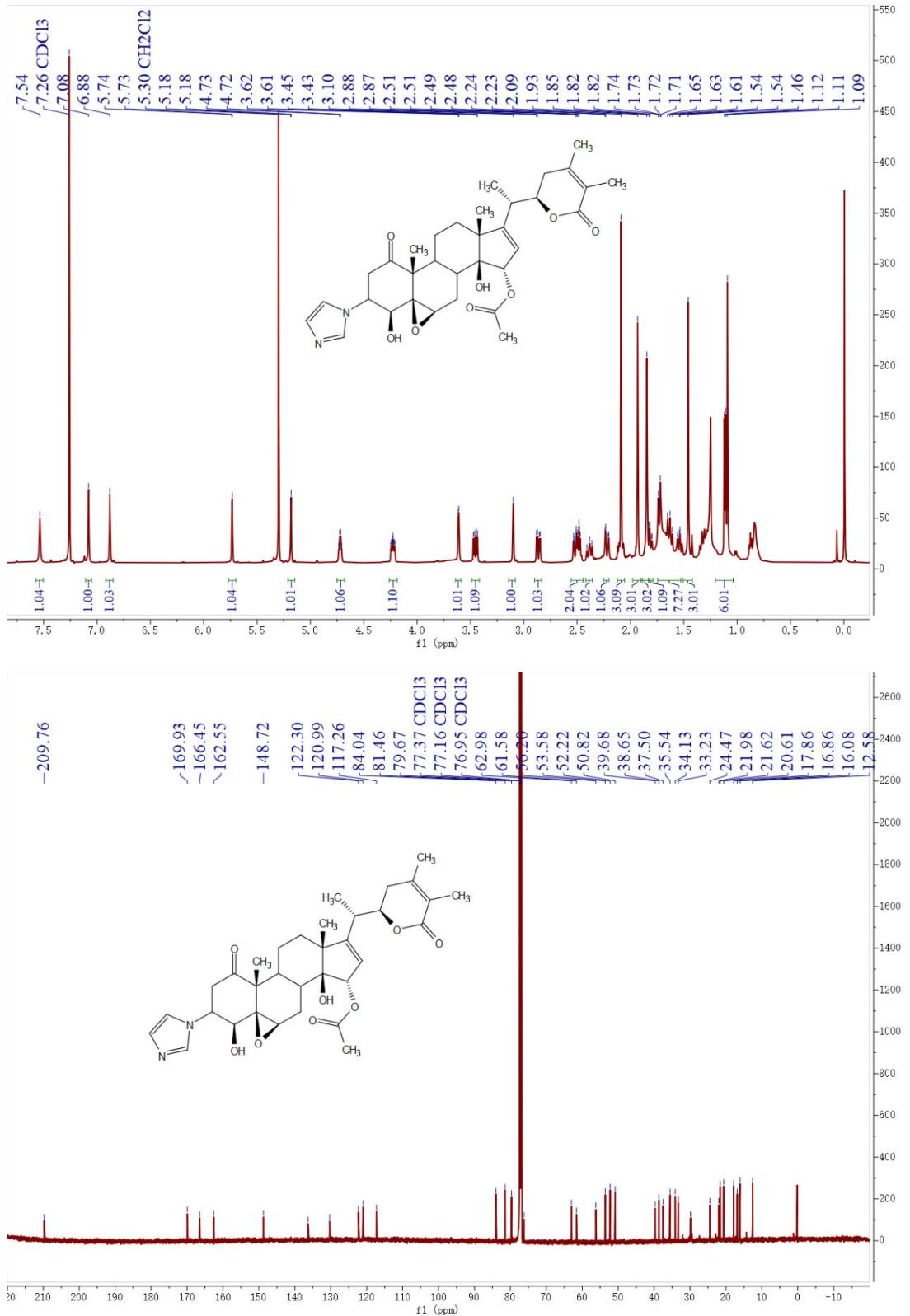




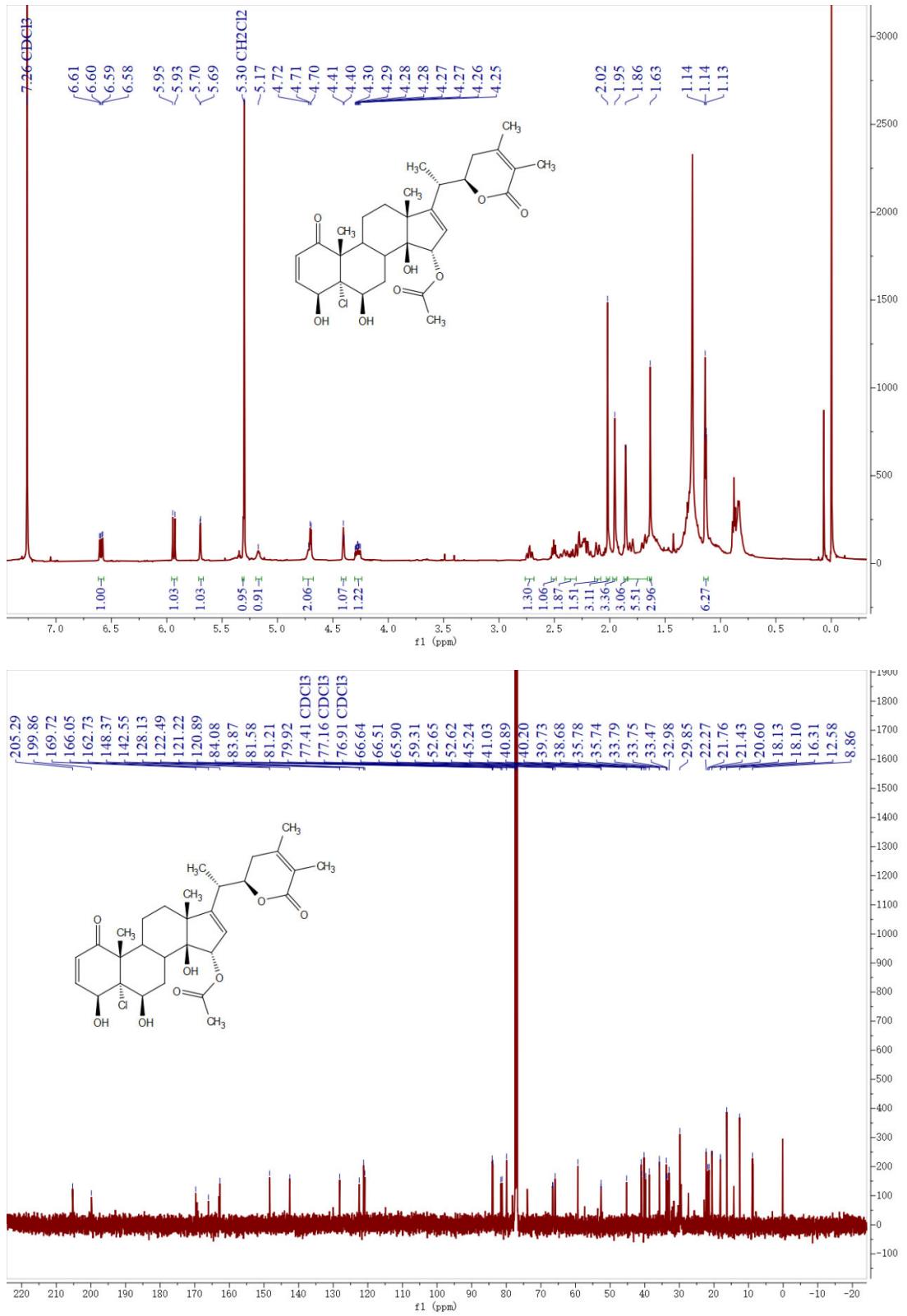
Spectra of compound **2a**



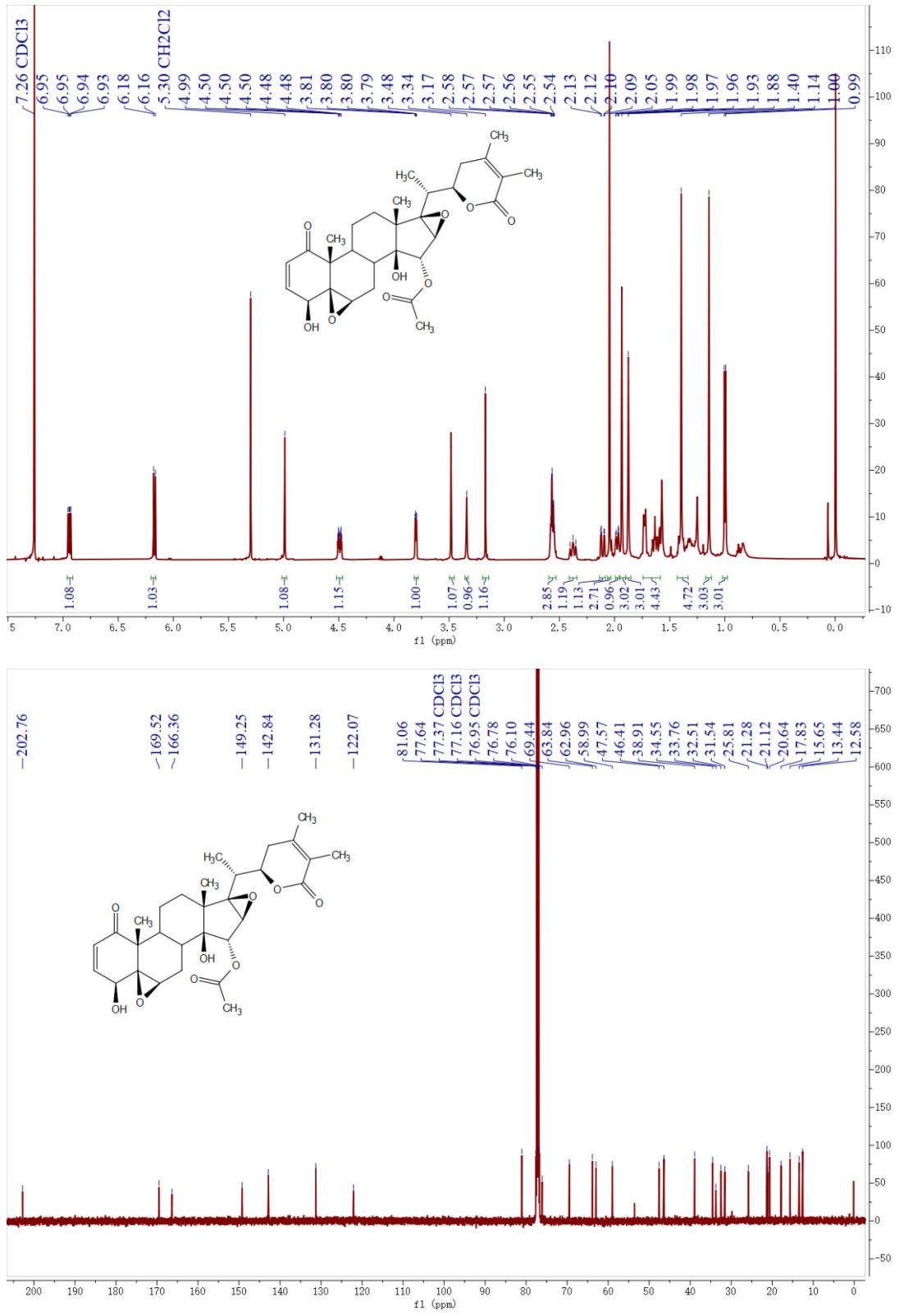
## Spectra of compound **2b**



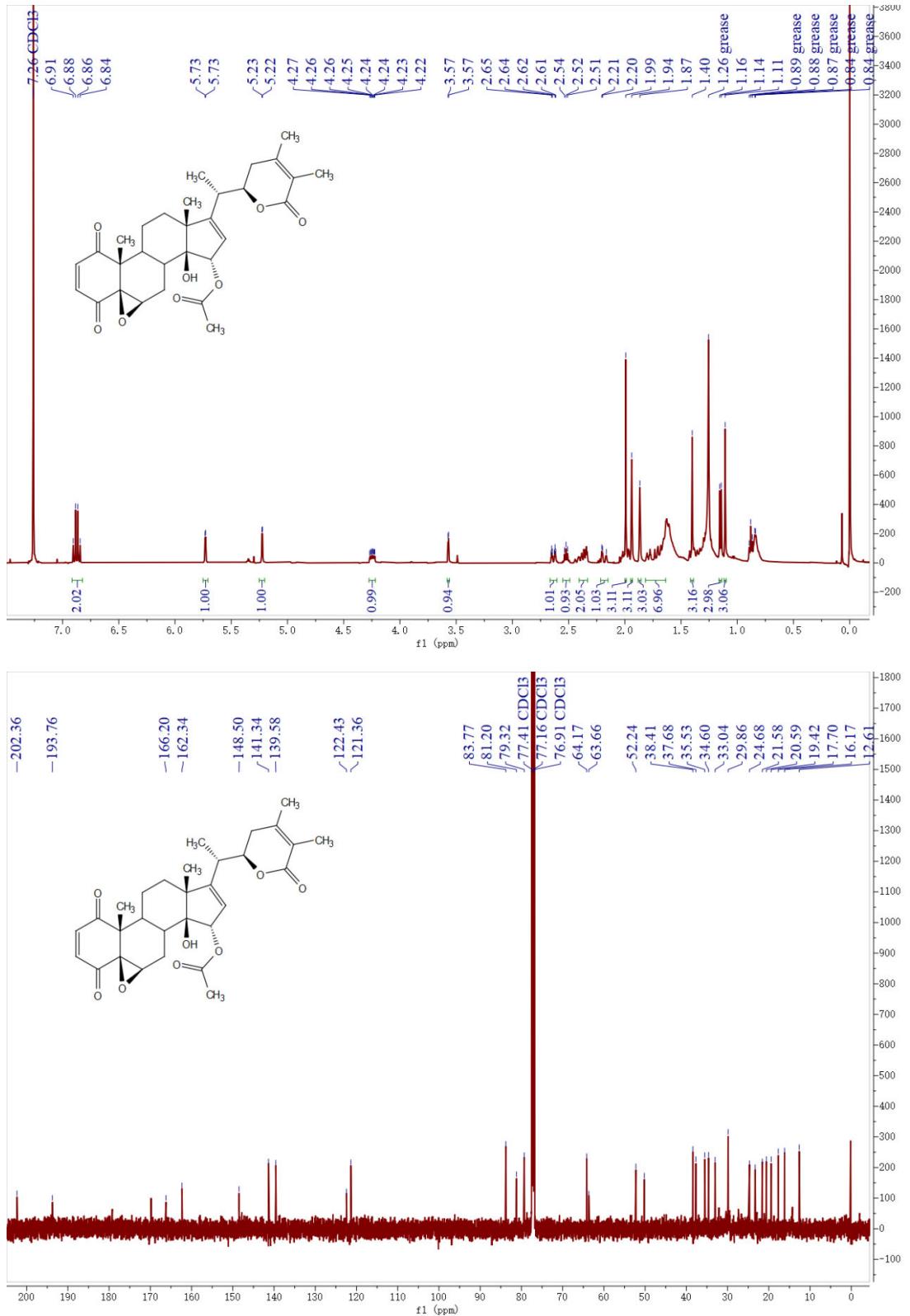
## Spectra of compound 2c



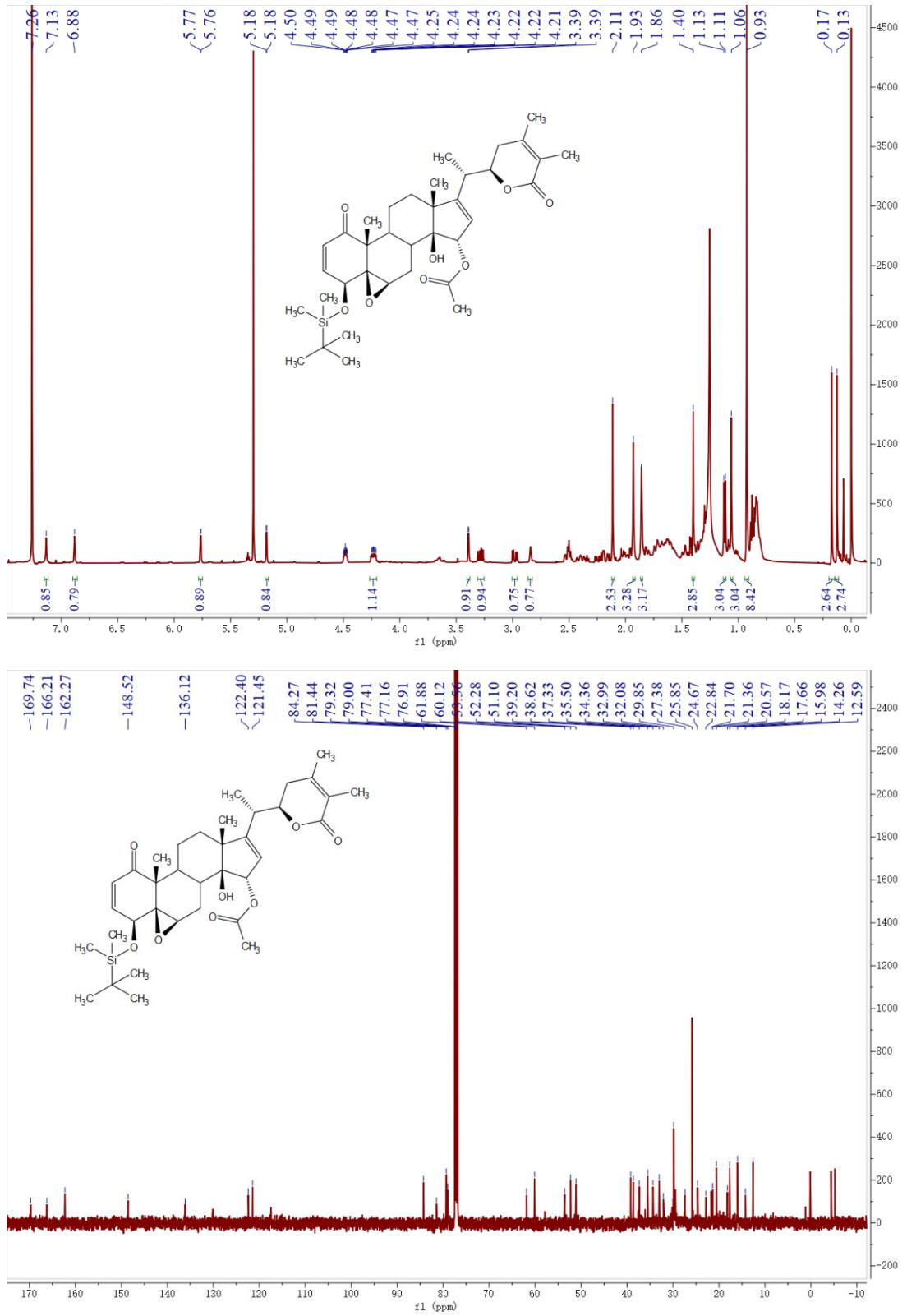
Spectra of compound 3



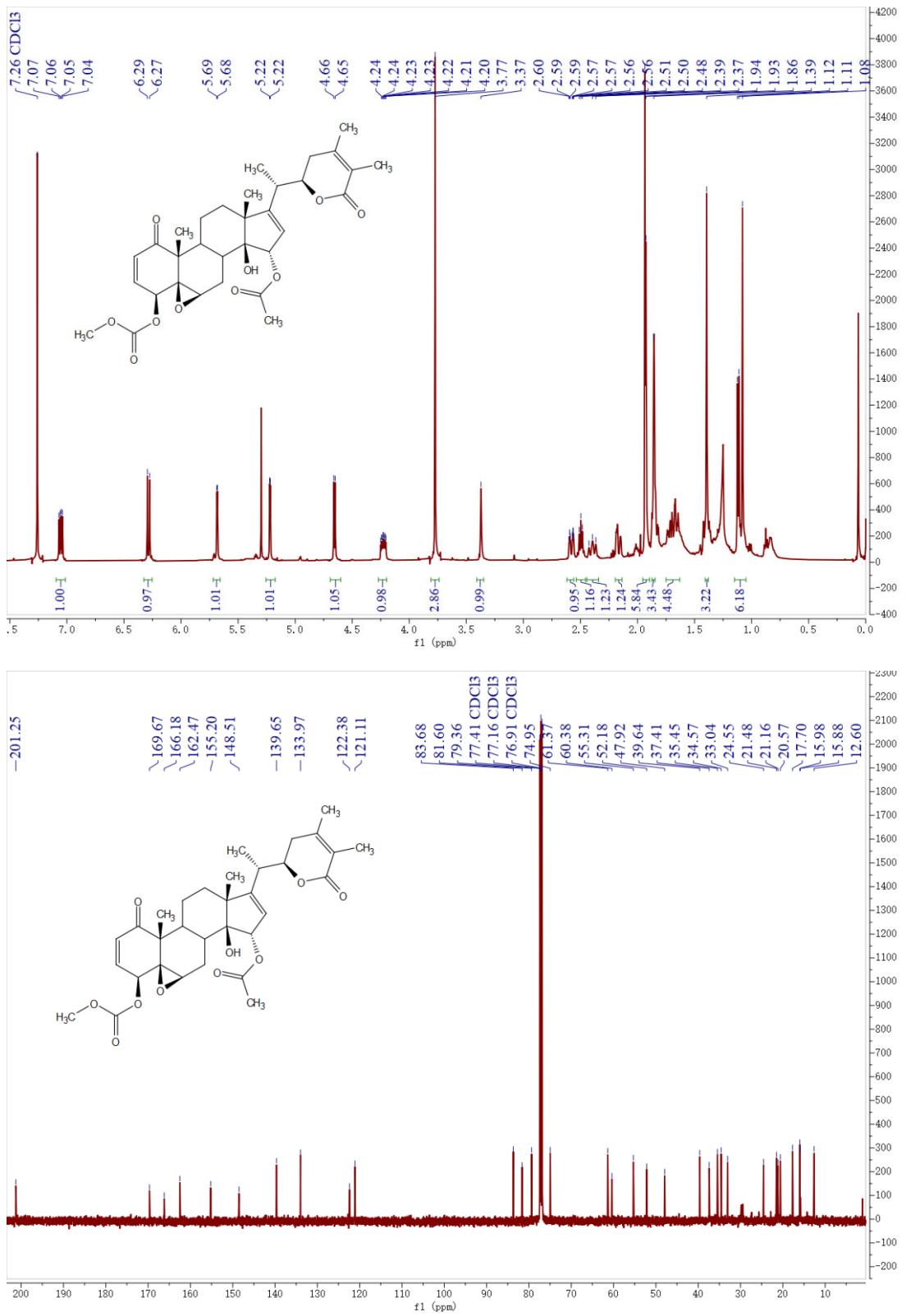
Spectra of compound 4



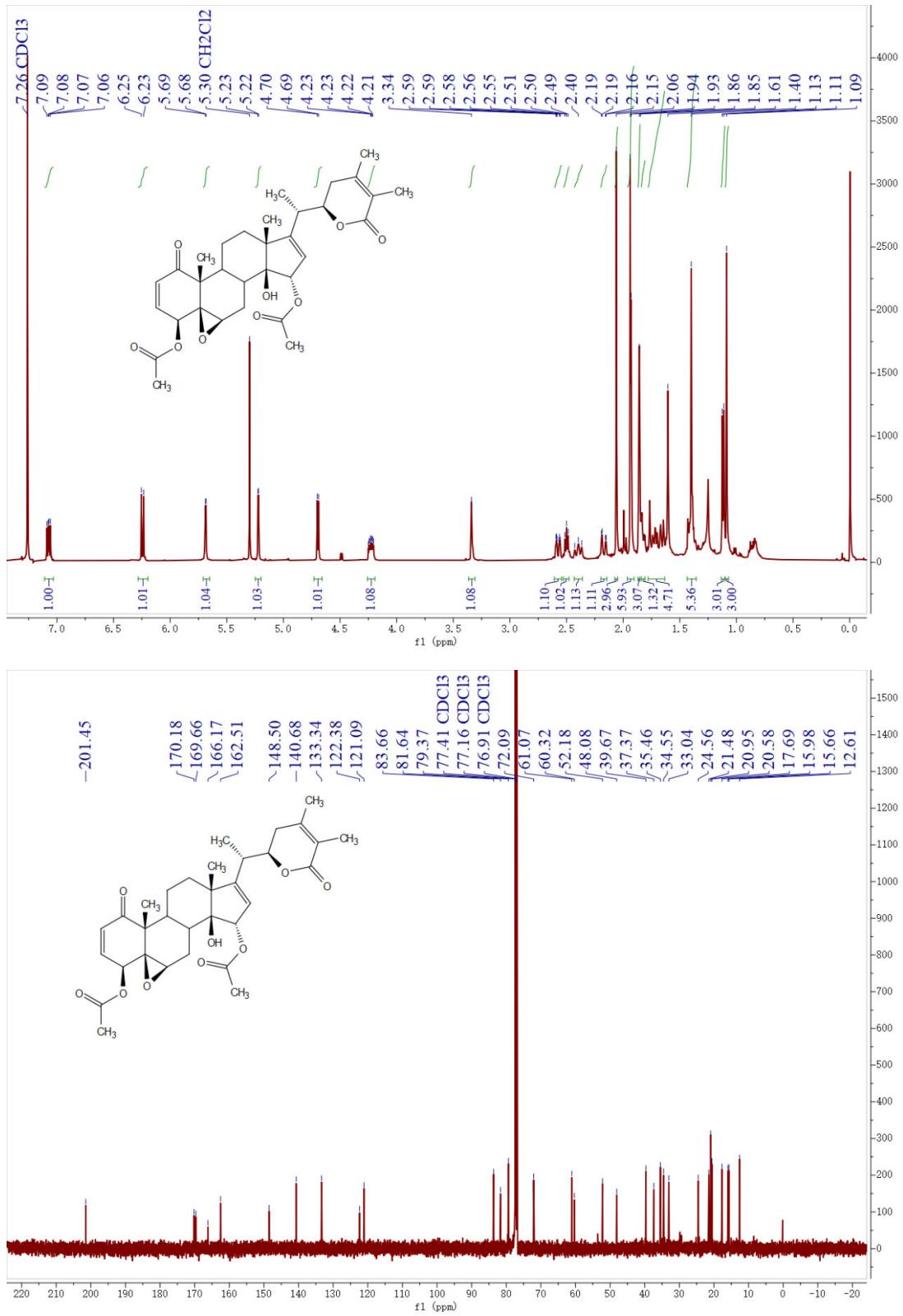
## Spectra of compound 5



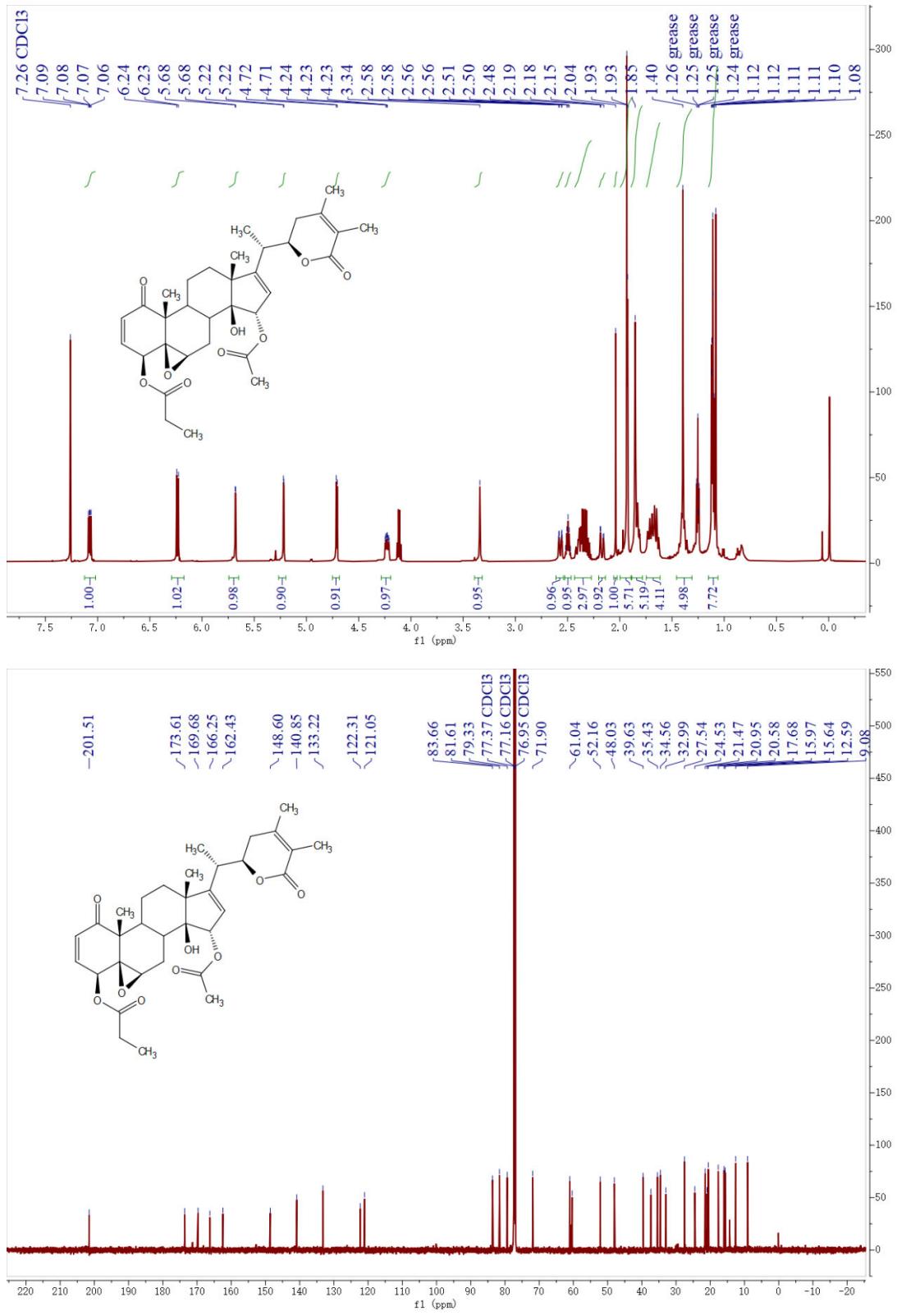
Spectra of compound 6



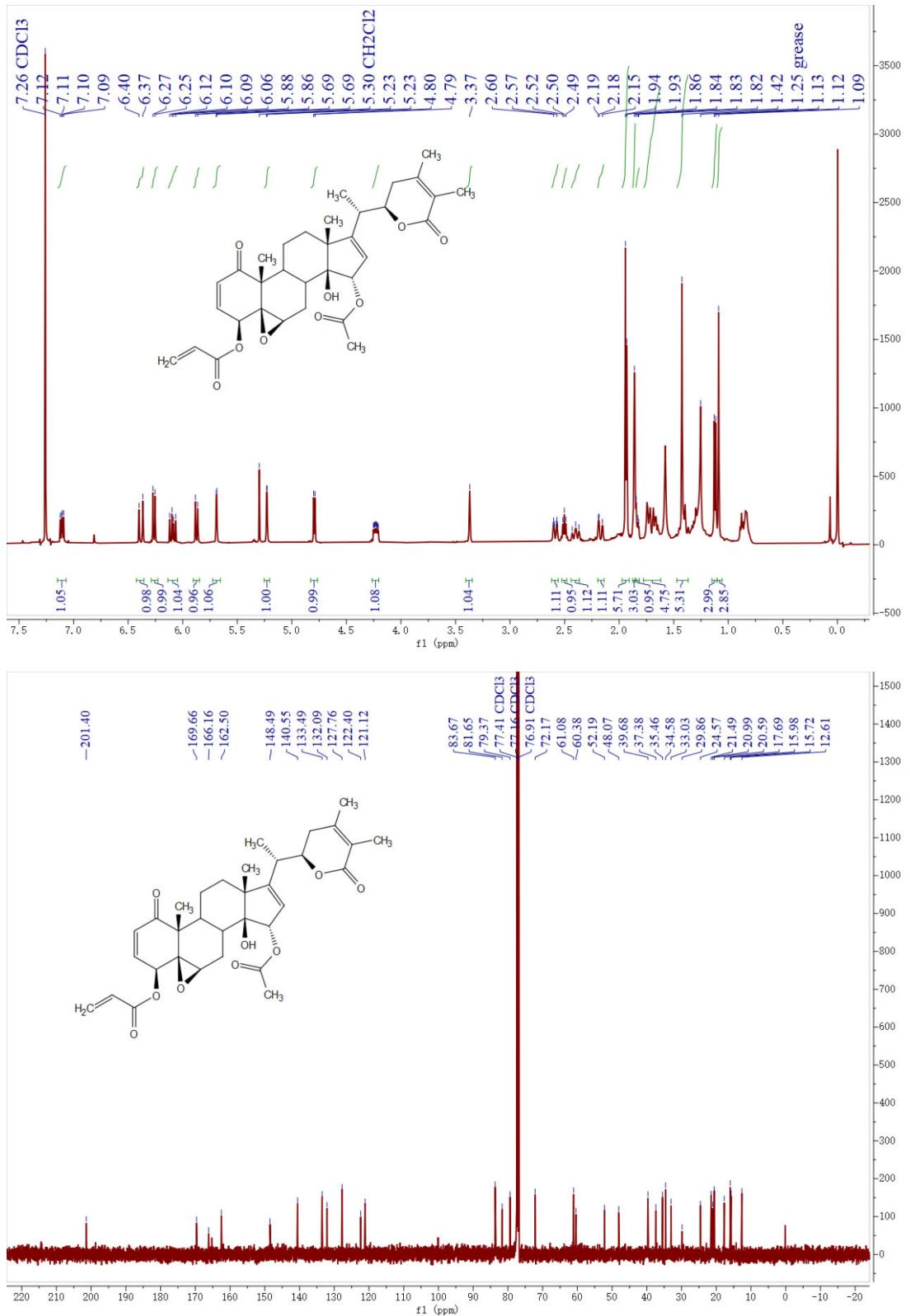
Spectra of compound 7a



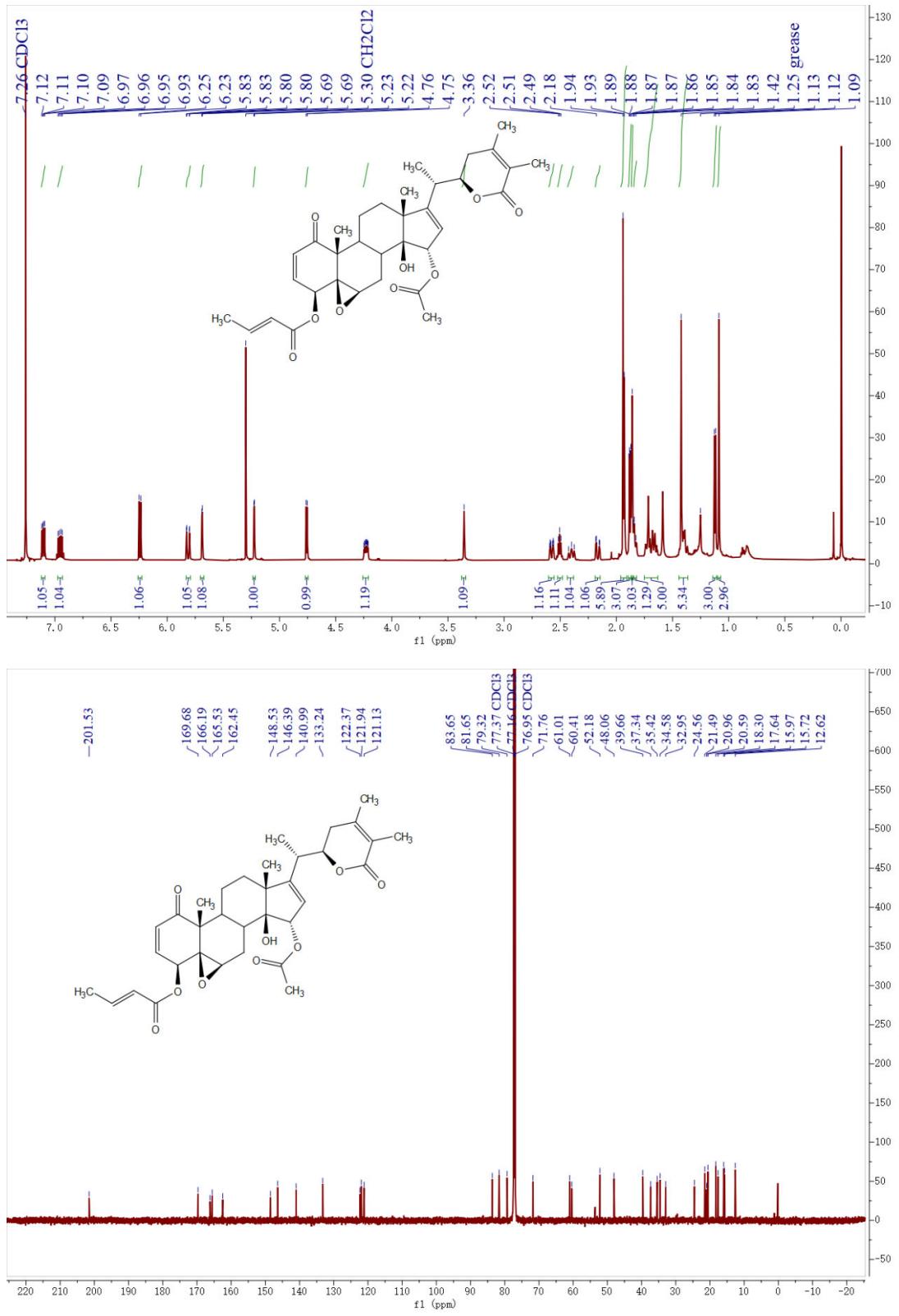
Spectra of compound 7b



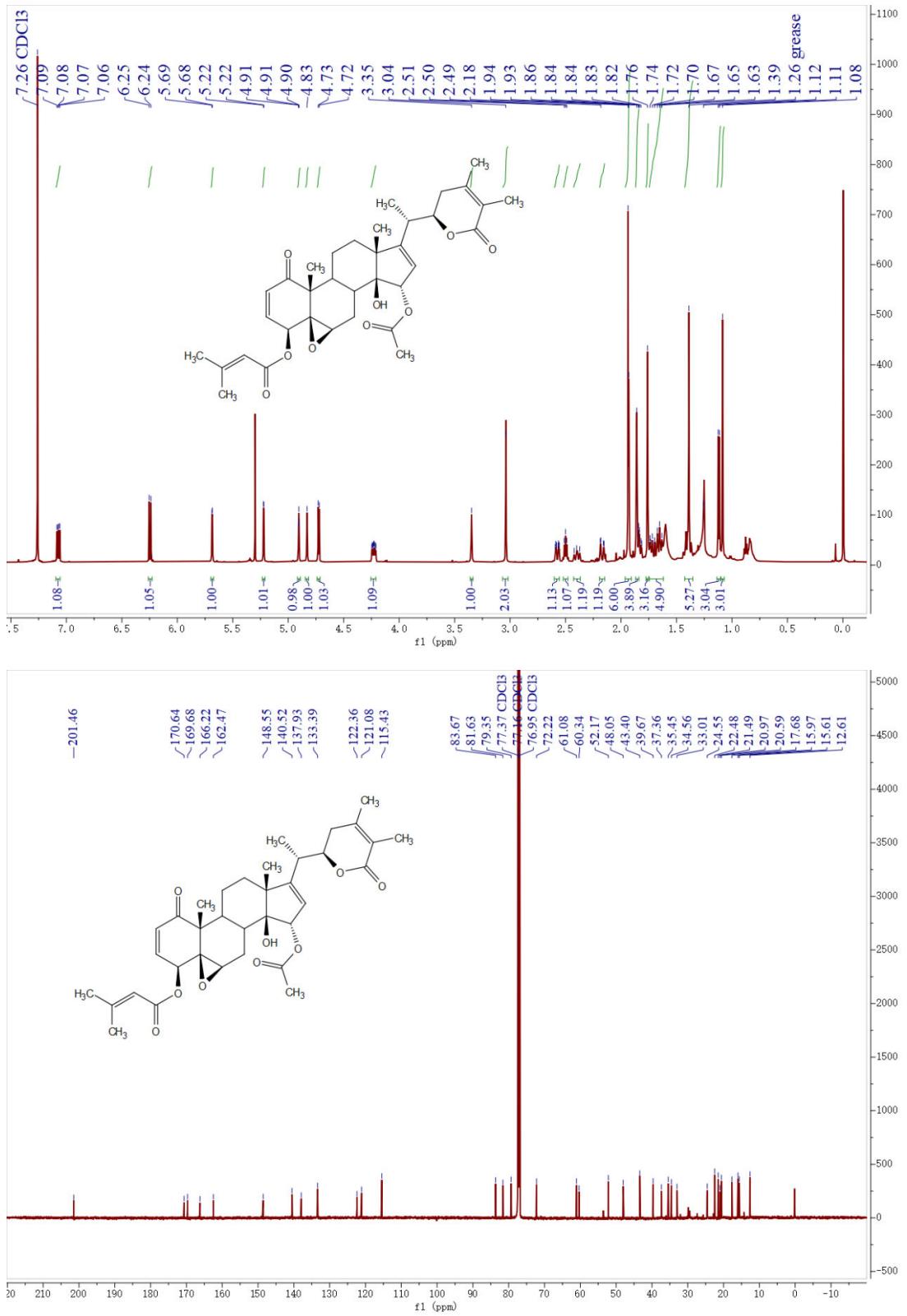
Spectra of compound **8a**



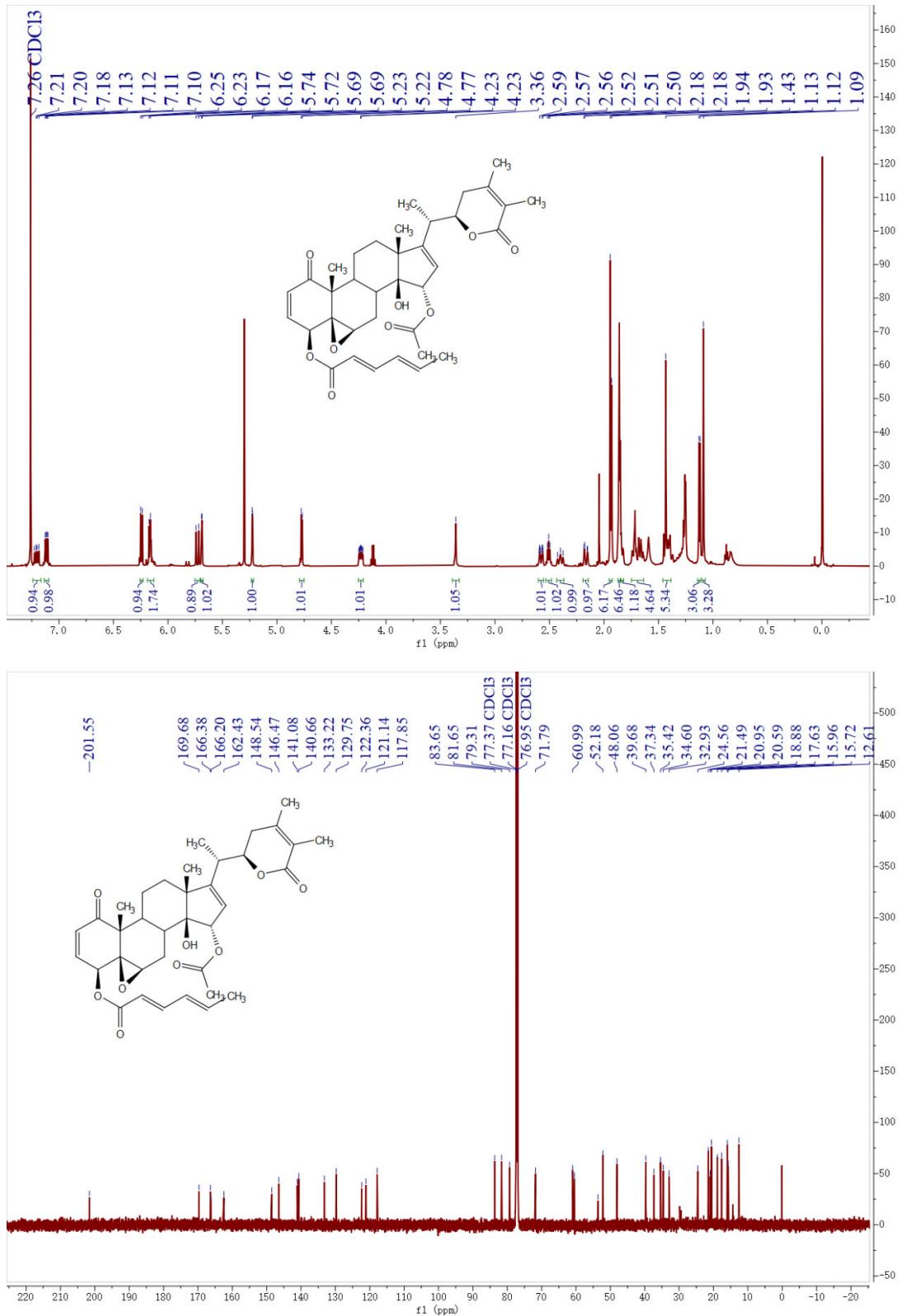
## Spectra of compound **8b**



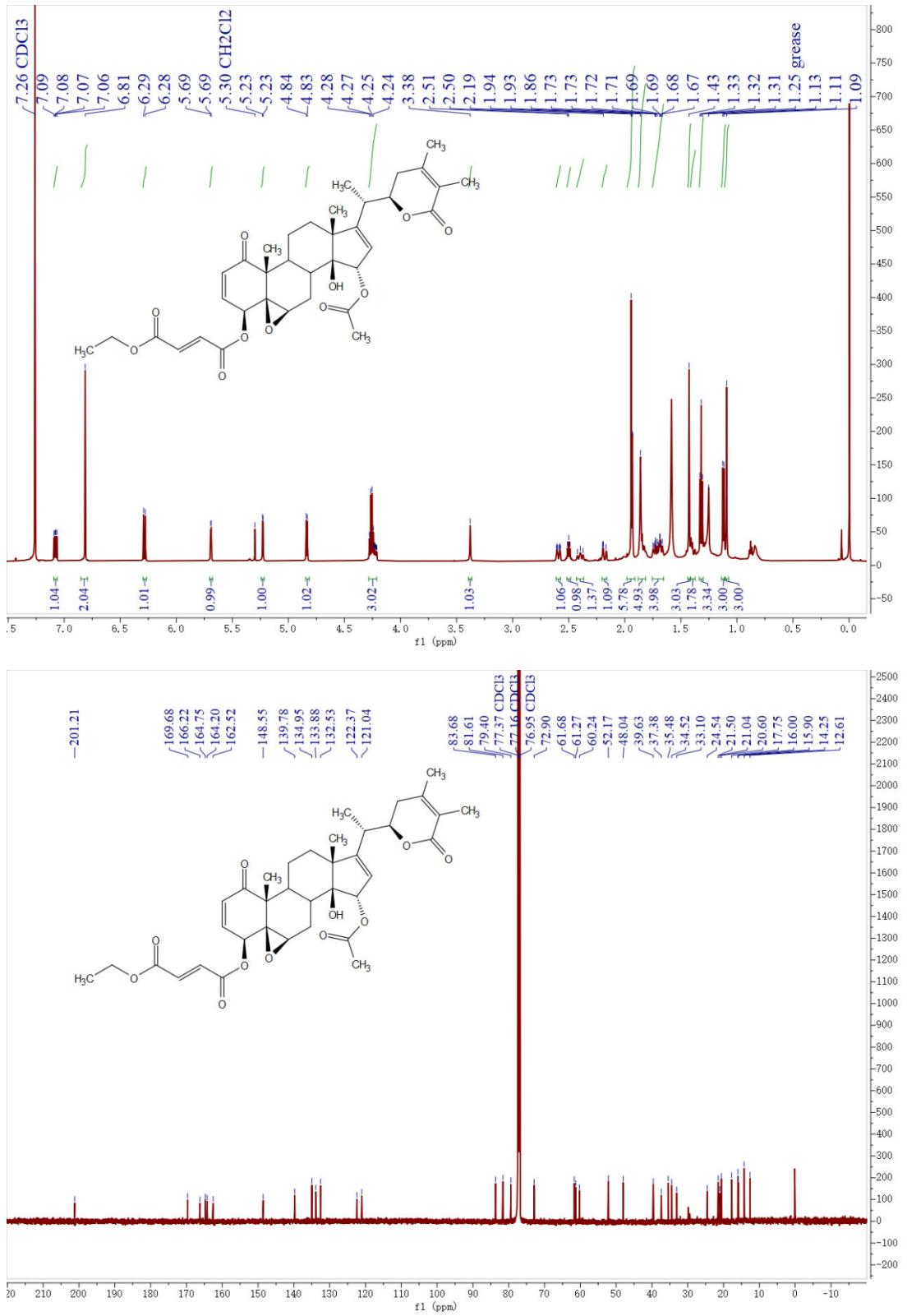
## Spectra of compound **8c**



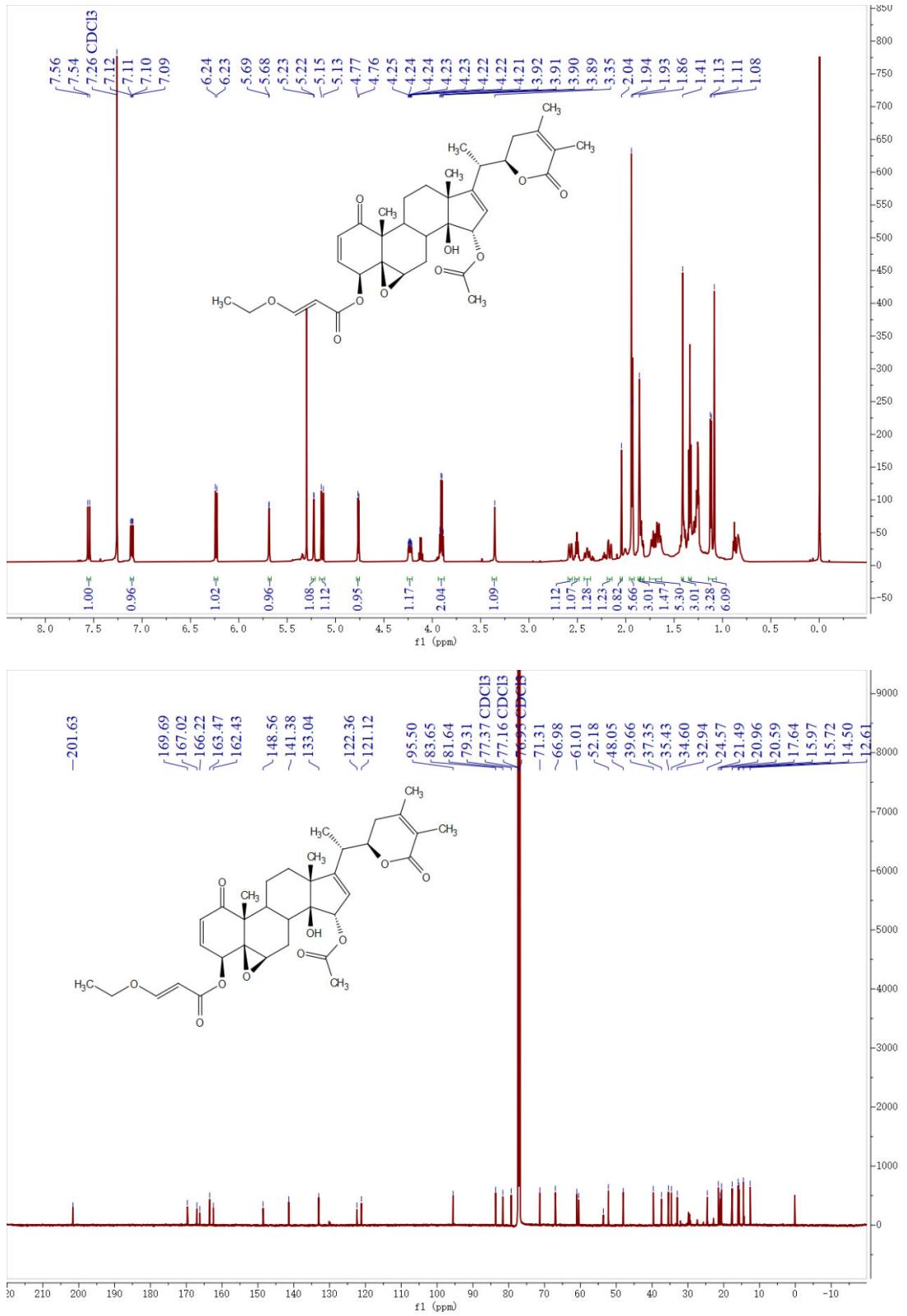
## Spectra of compound **8d**



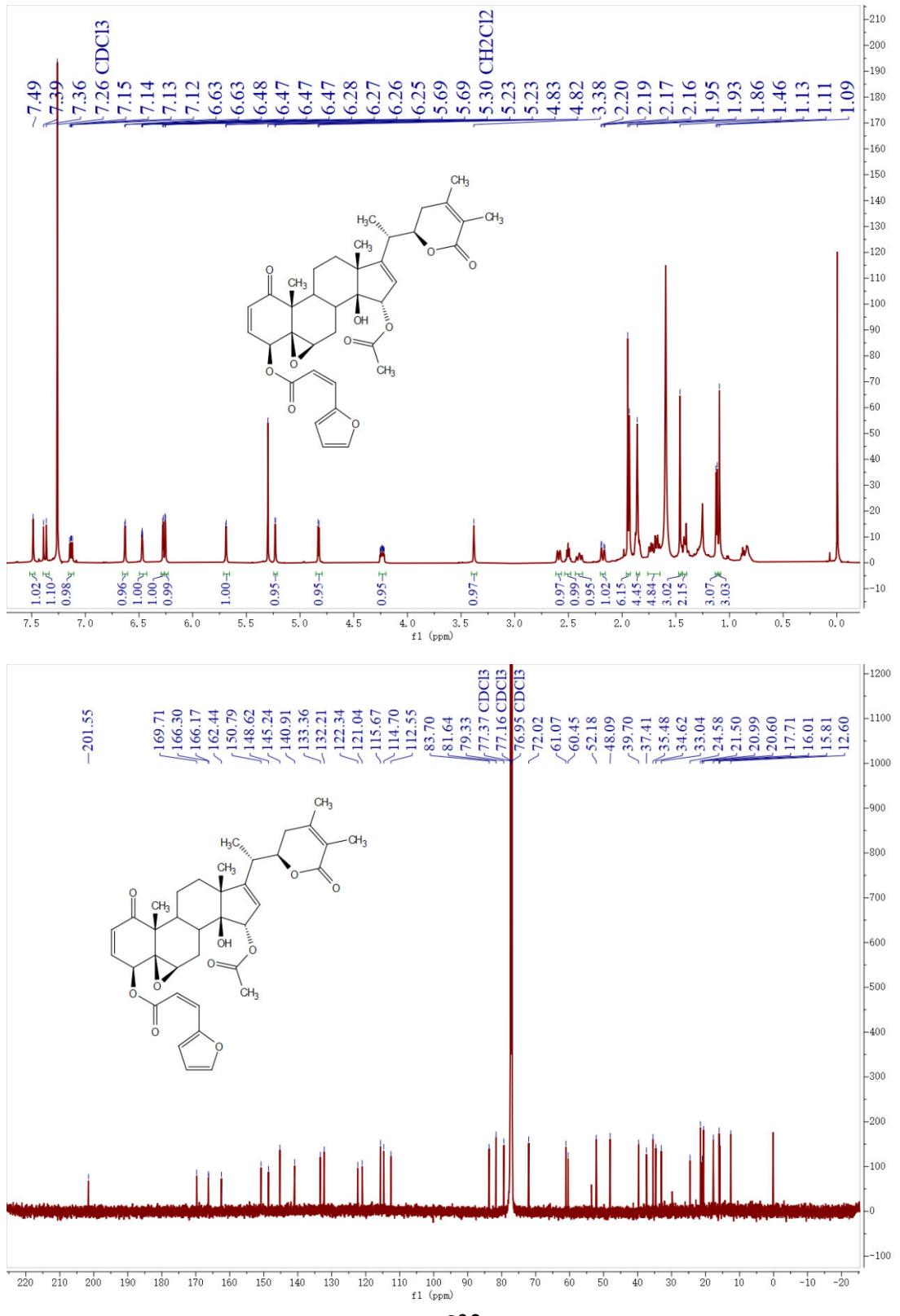
Spectra of compound **8e**



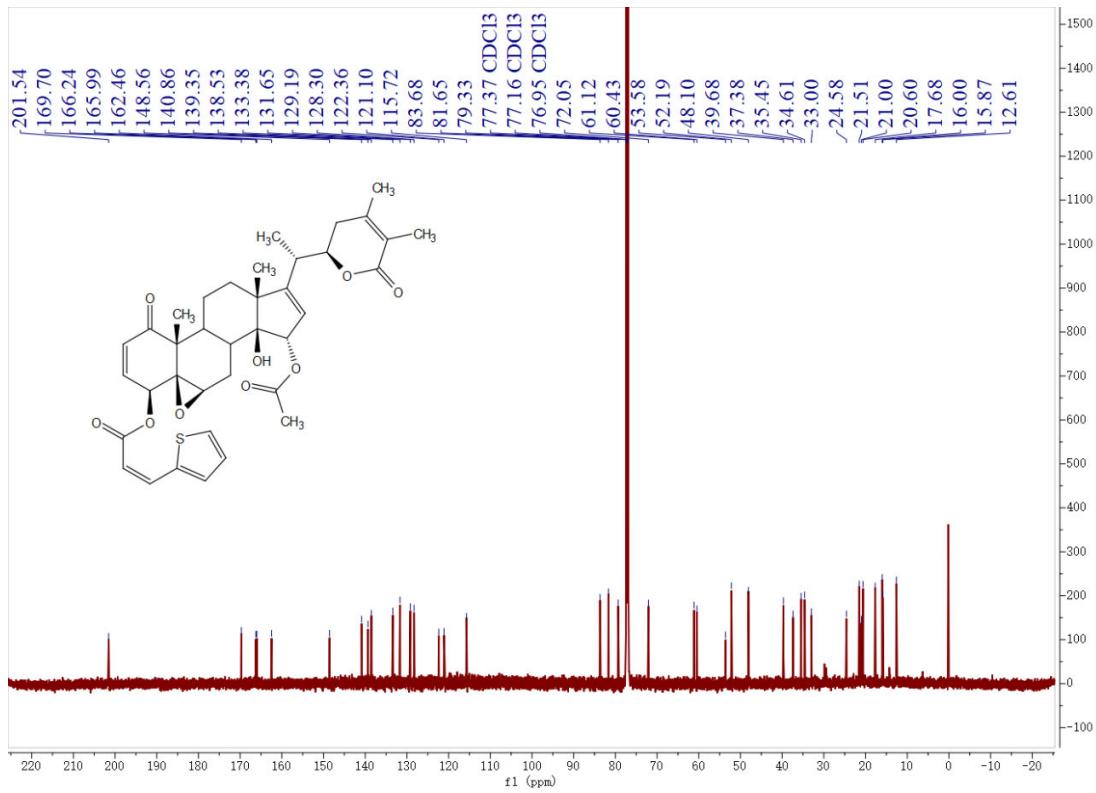
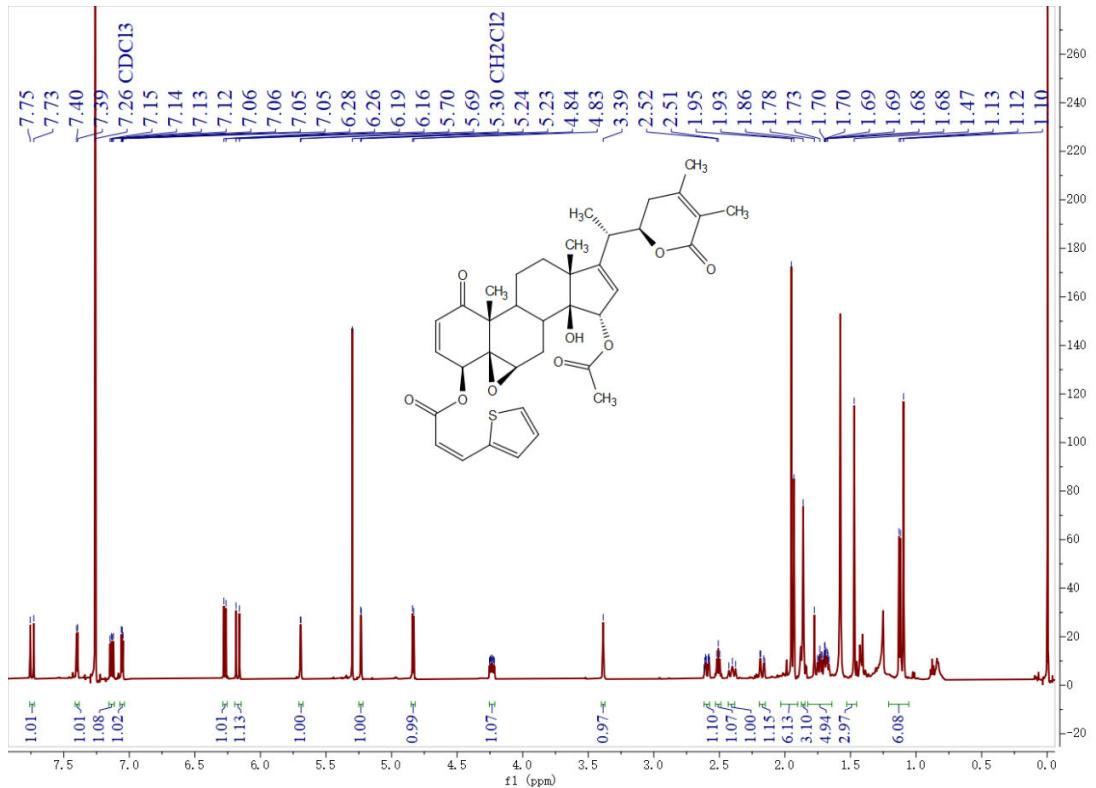
## Spectra of compound **8f**



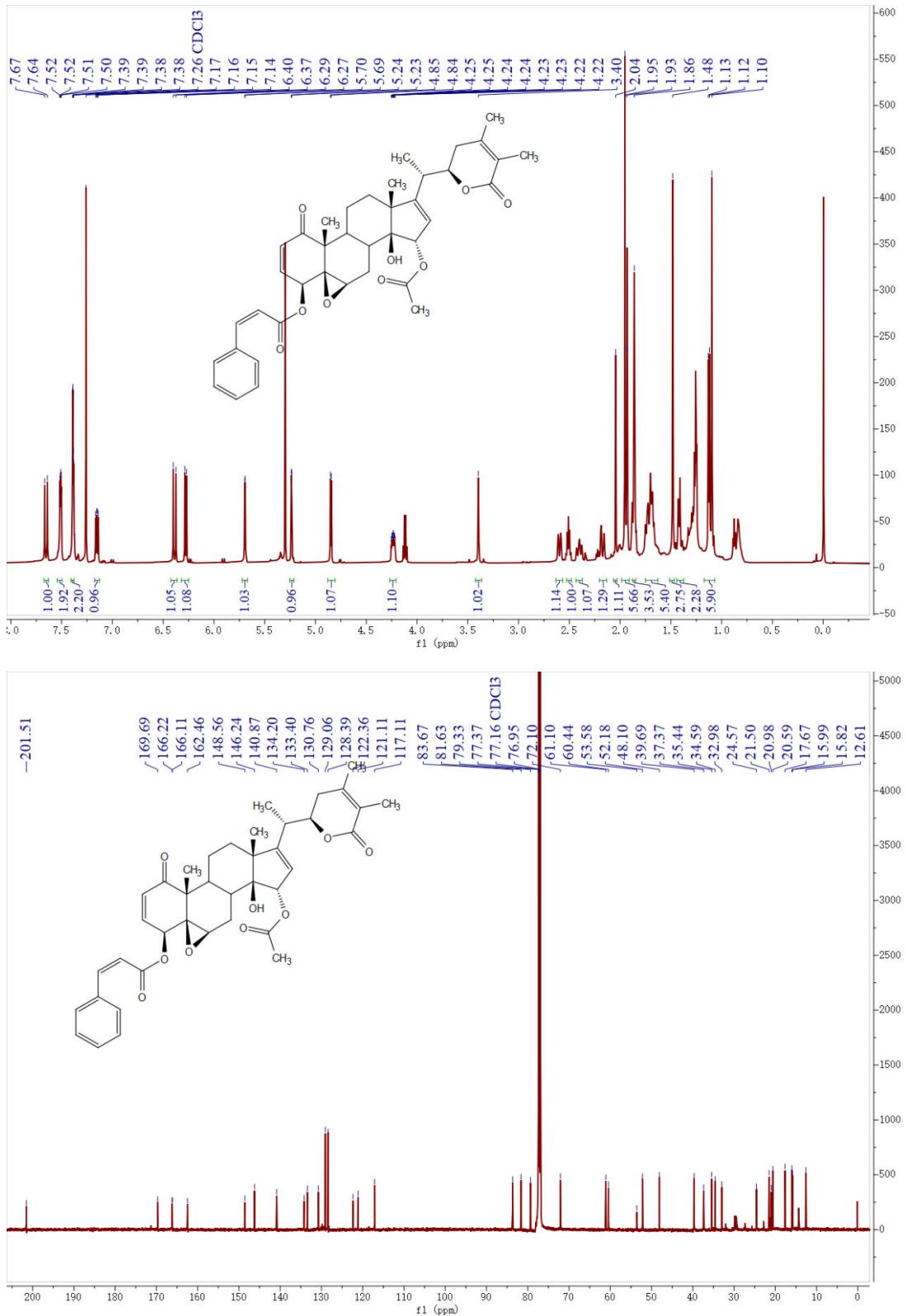
## Spectra of compound **8g**



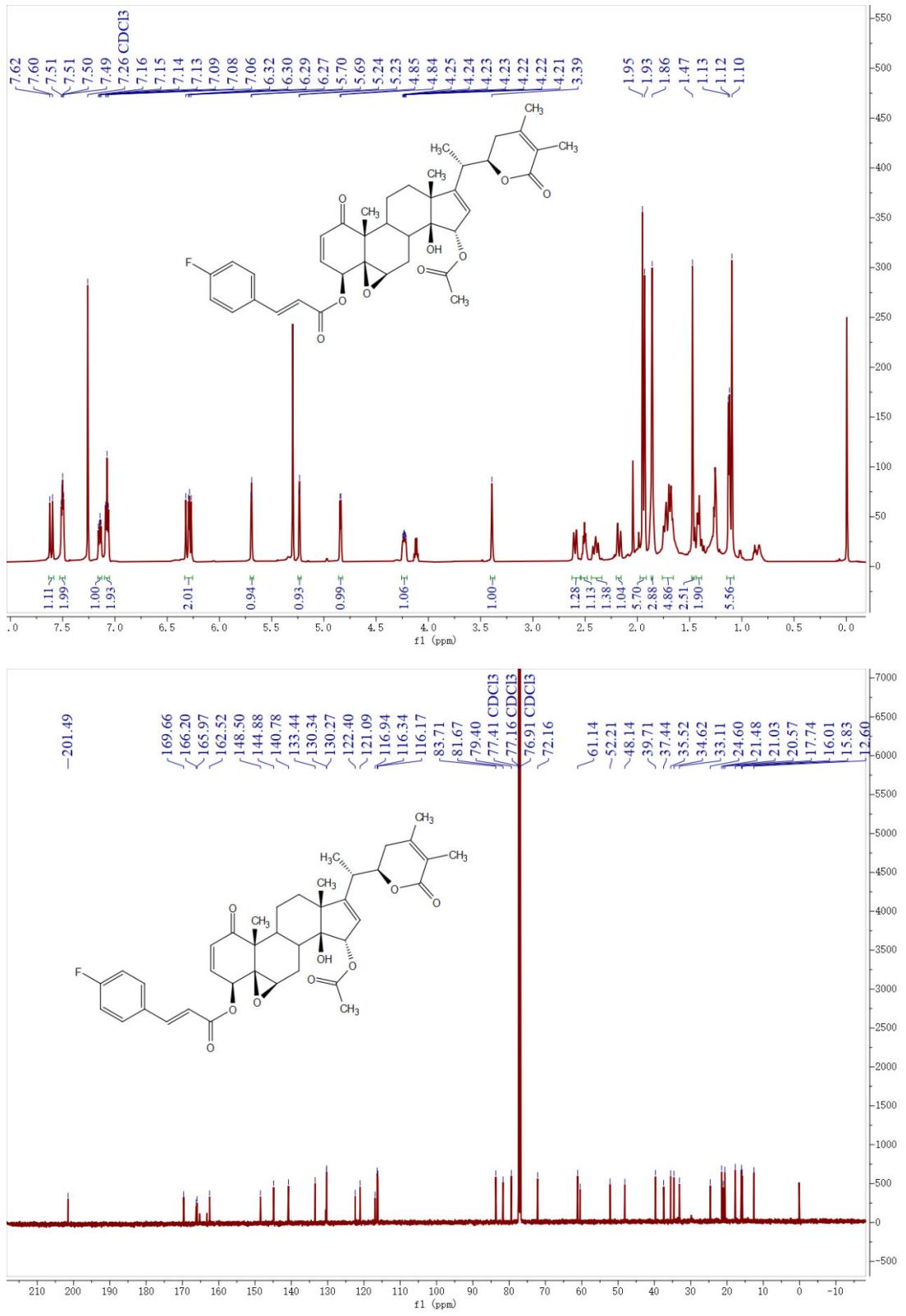
## Spectra of compound **8h**



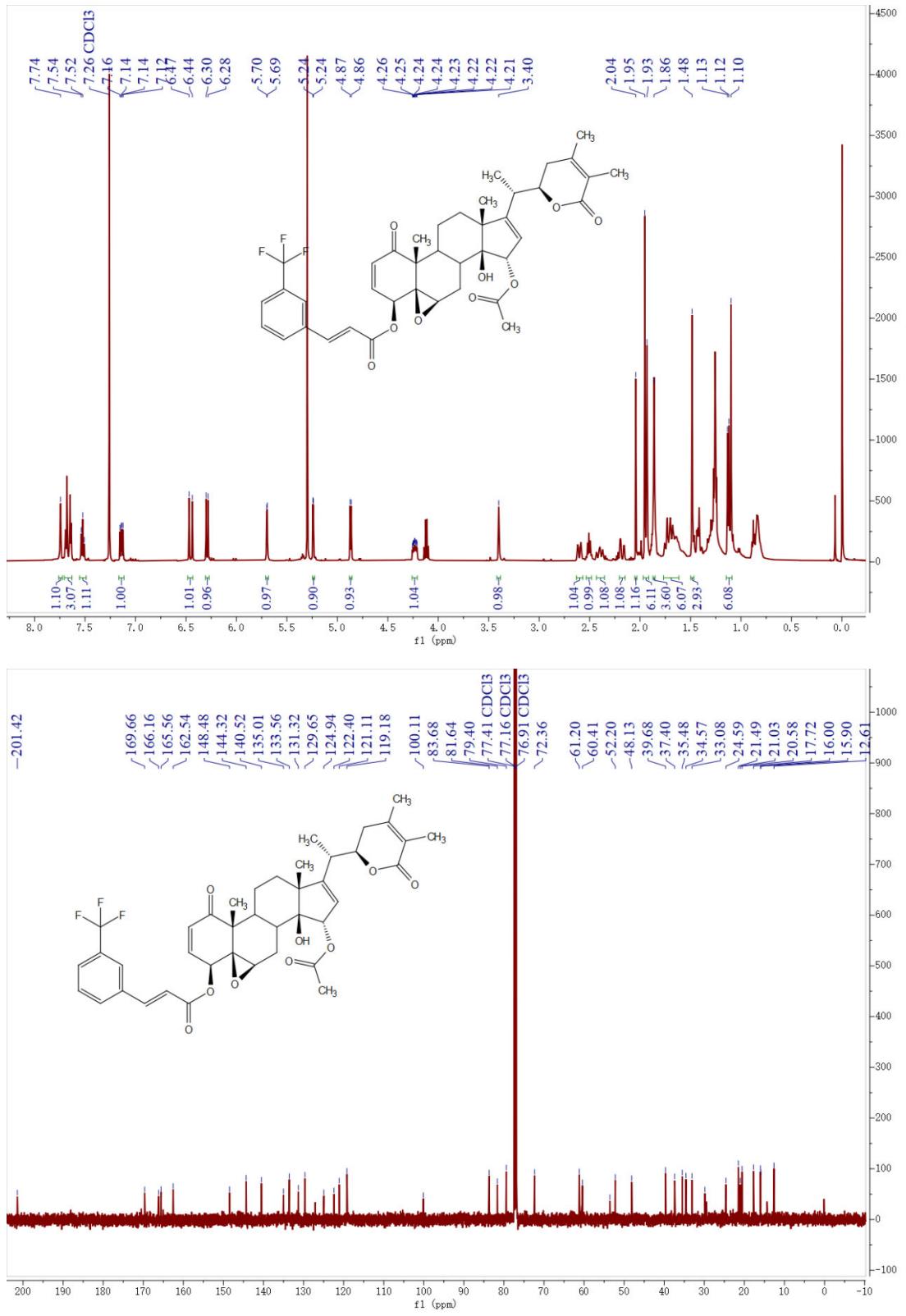
Spectra of compound **8i**



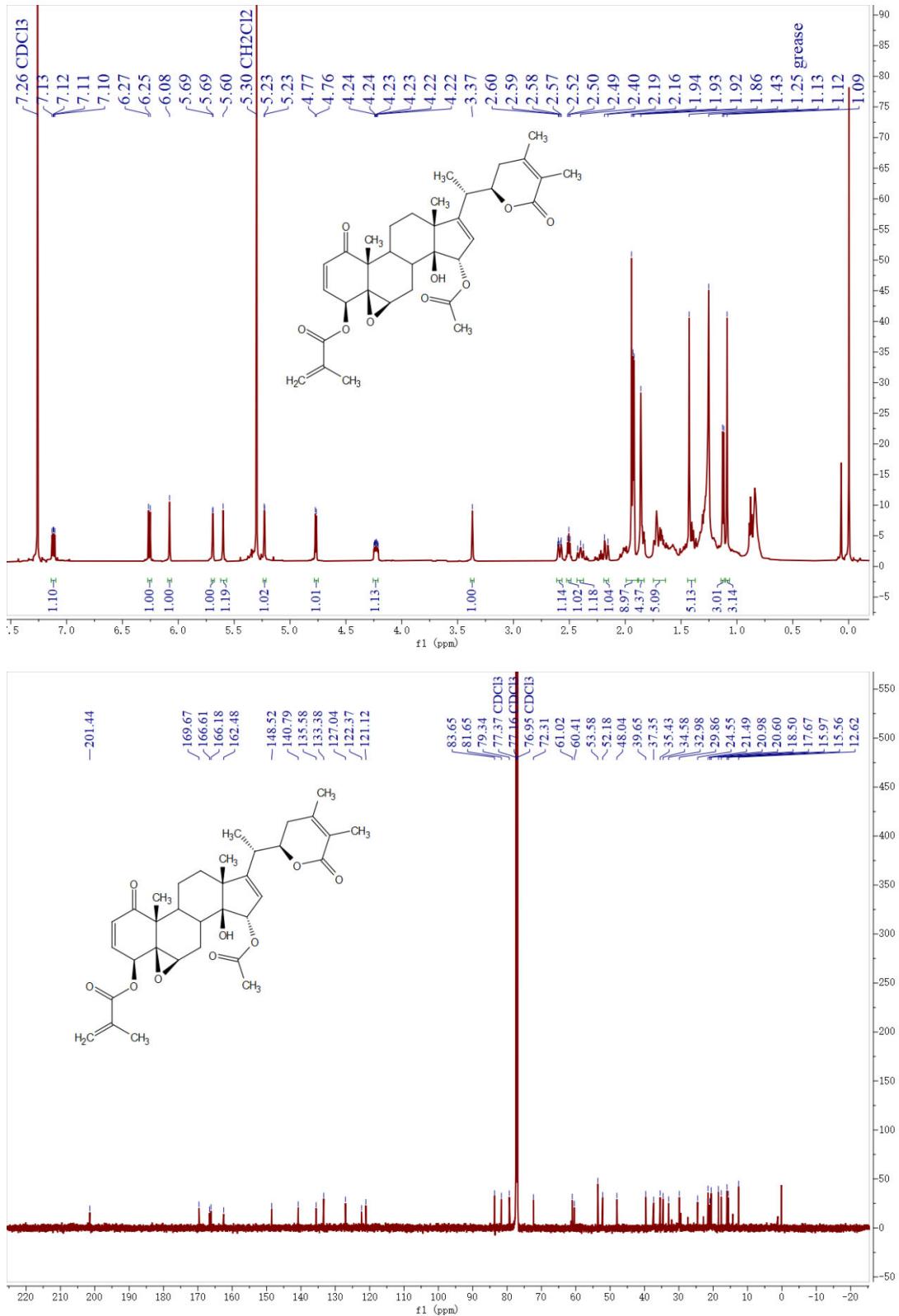
## Spectra of compound 8j



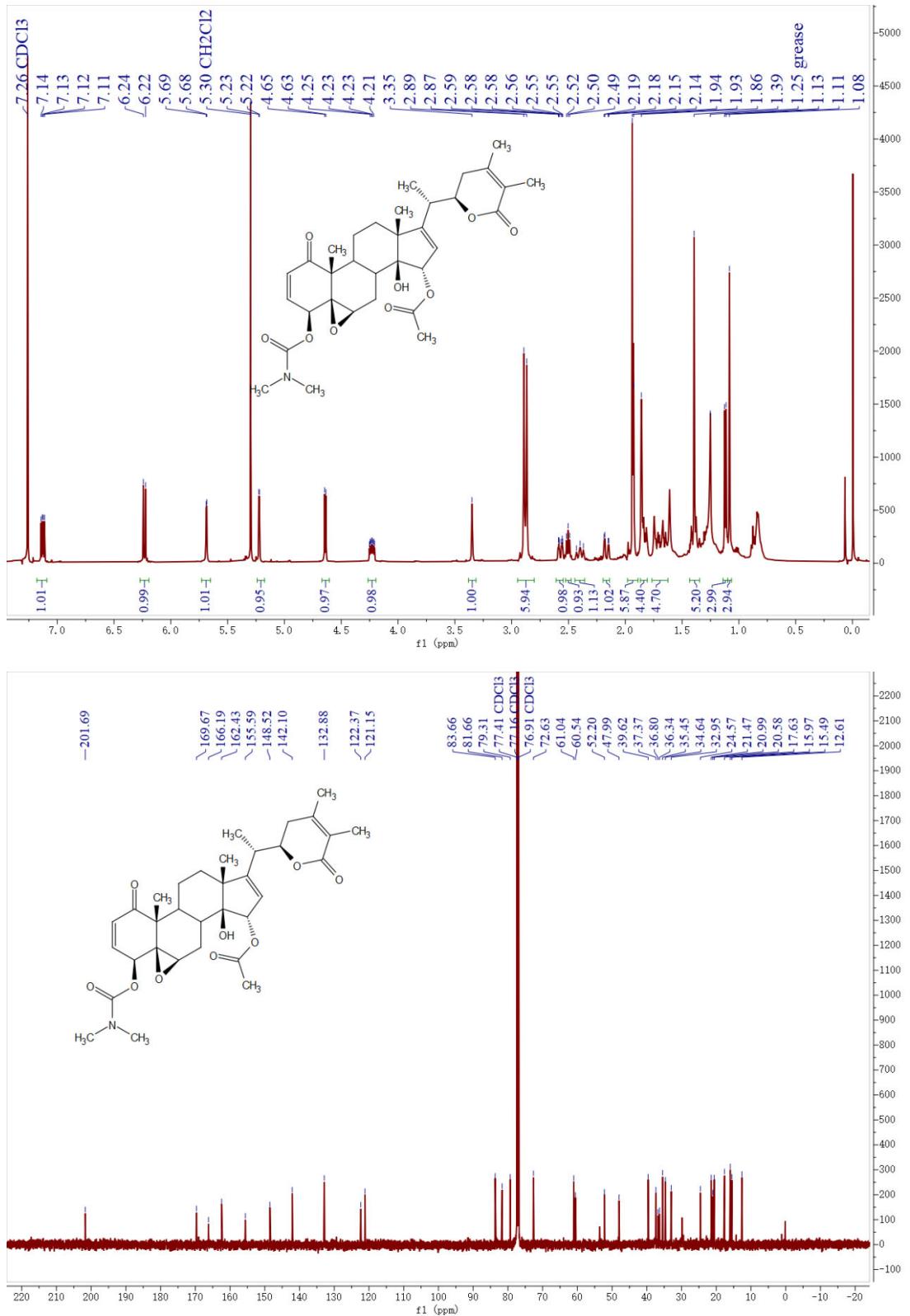
Spectra of compound **8k**



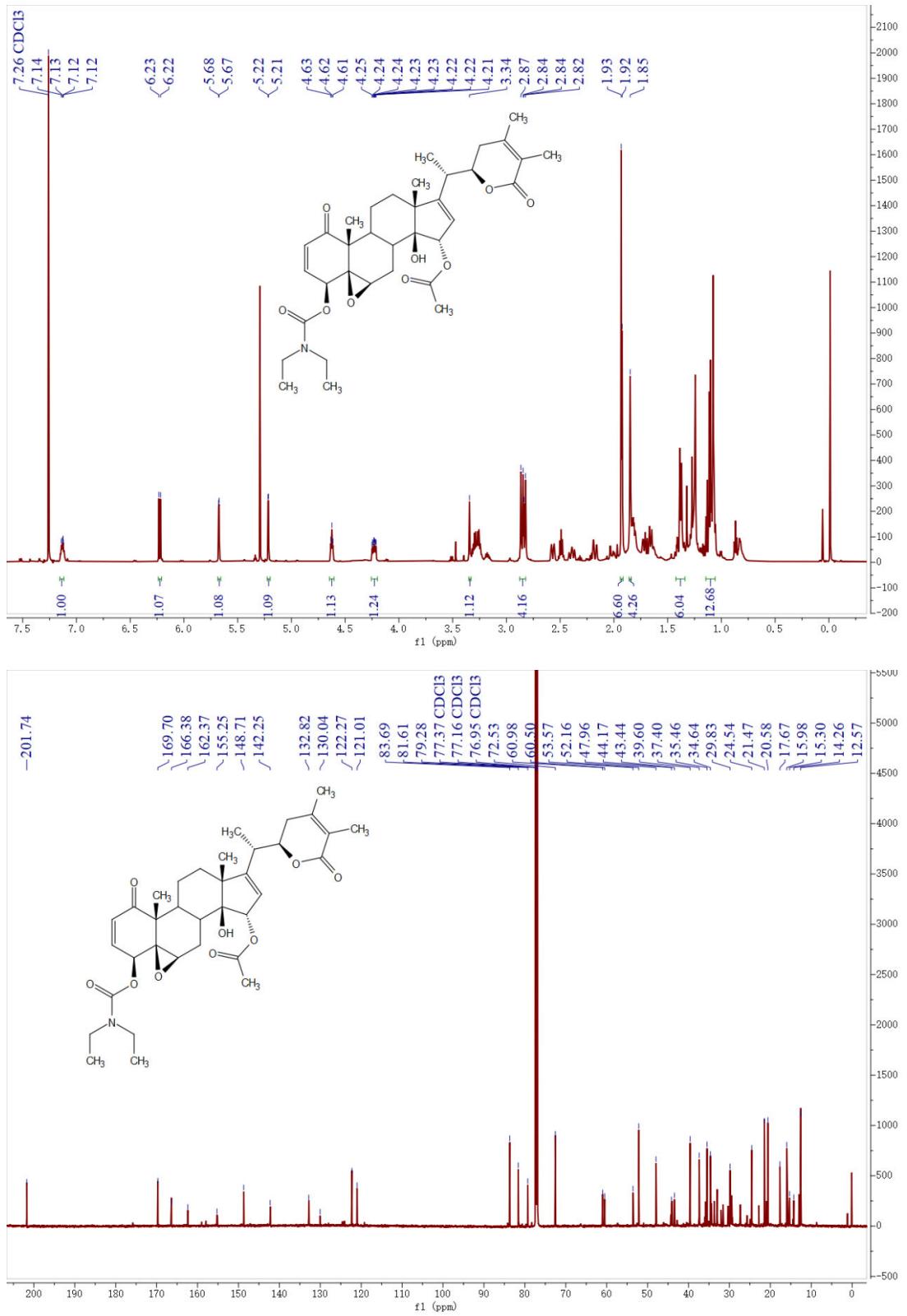
Spectra of compound **8I**



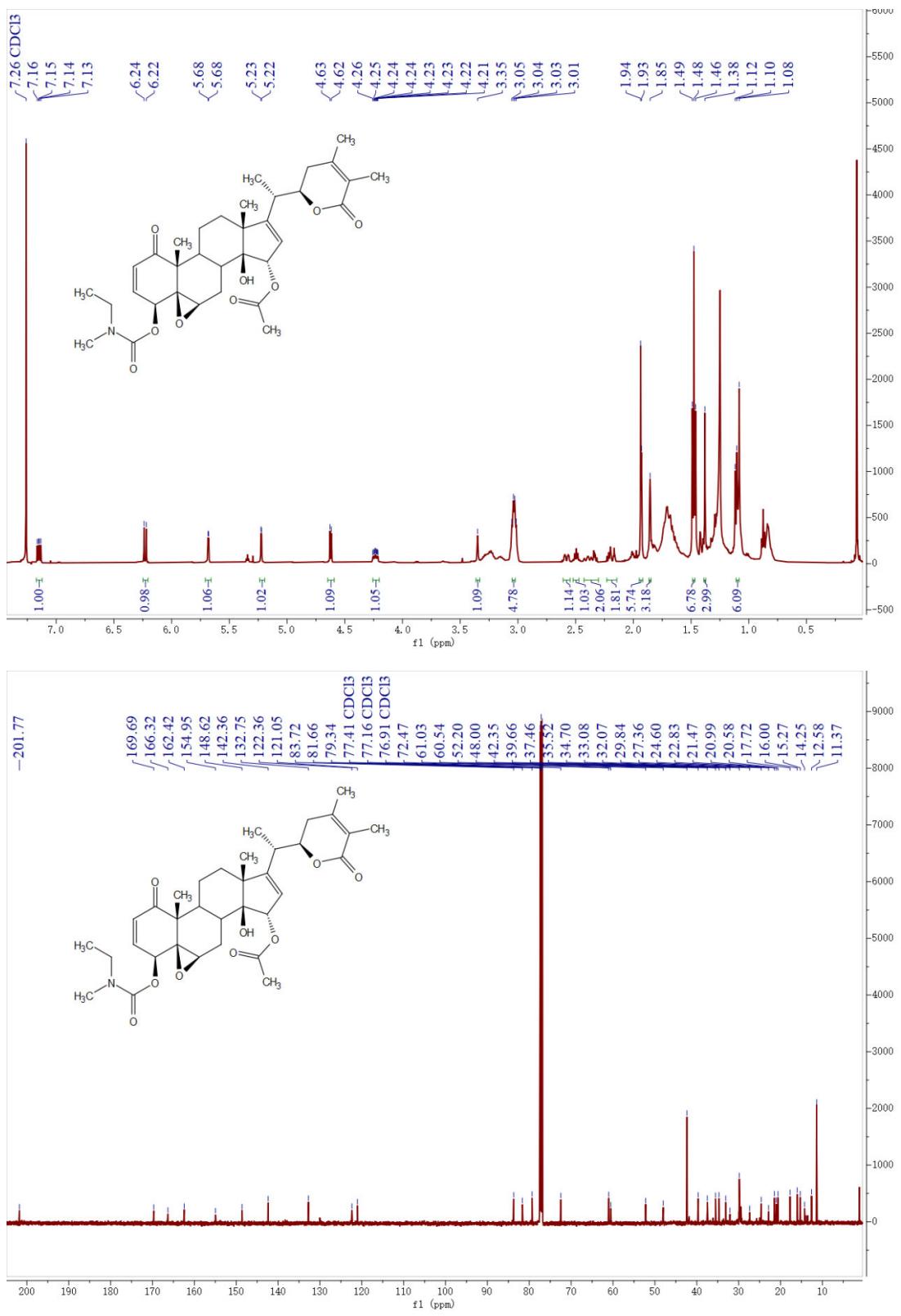
Spectra of compound **9a**



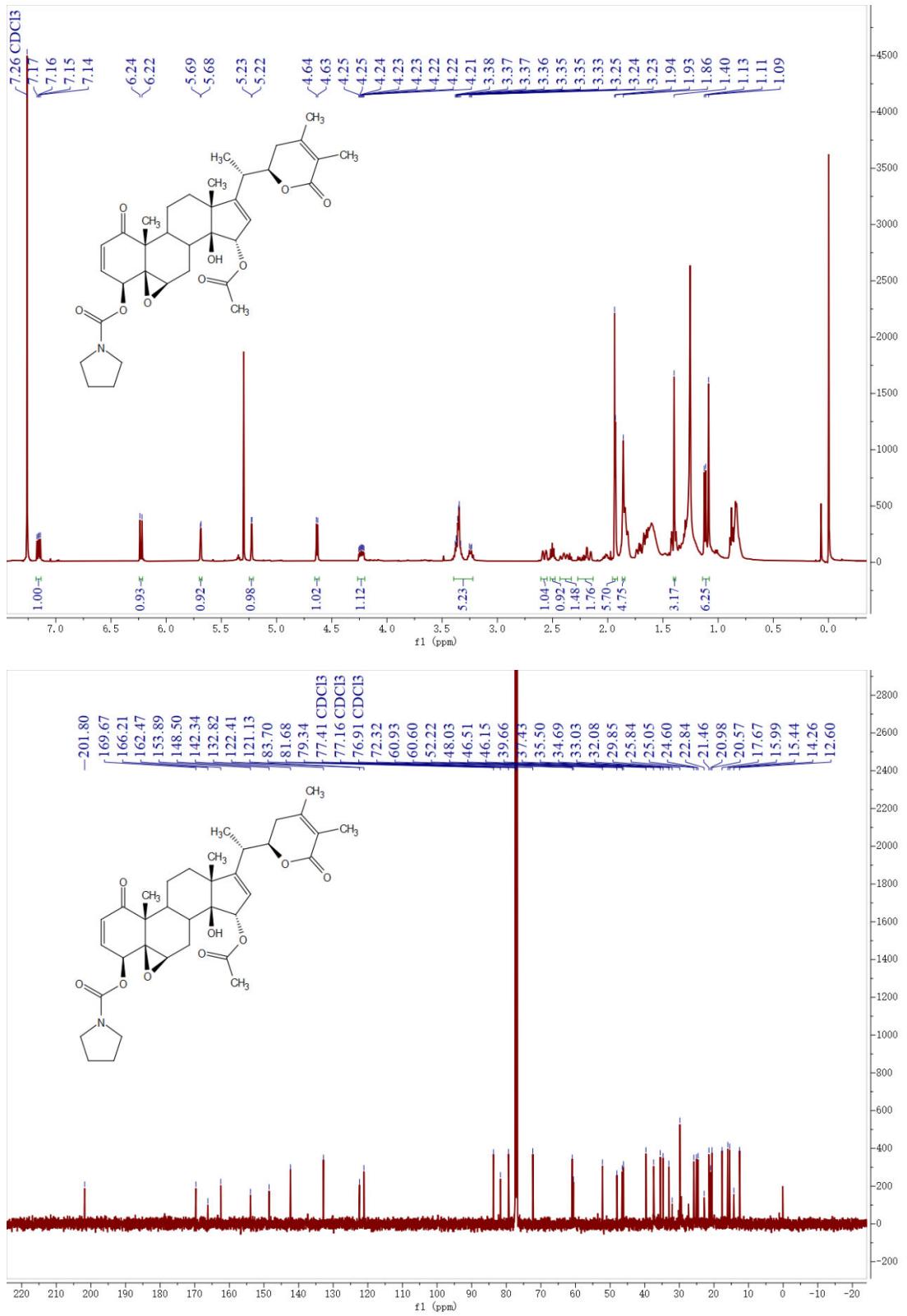
## Spectra of compound **9b**



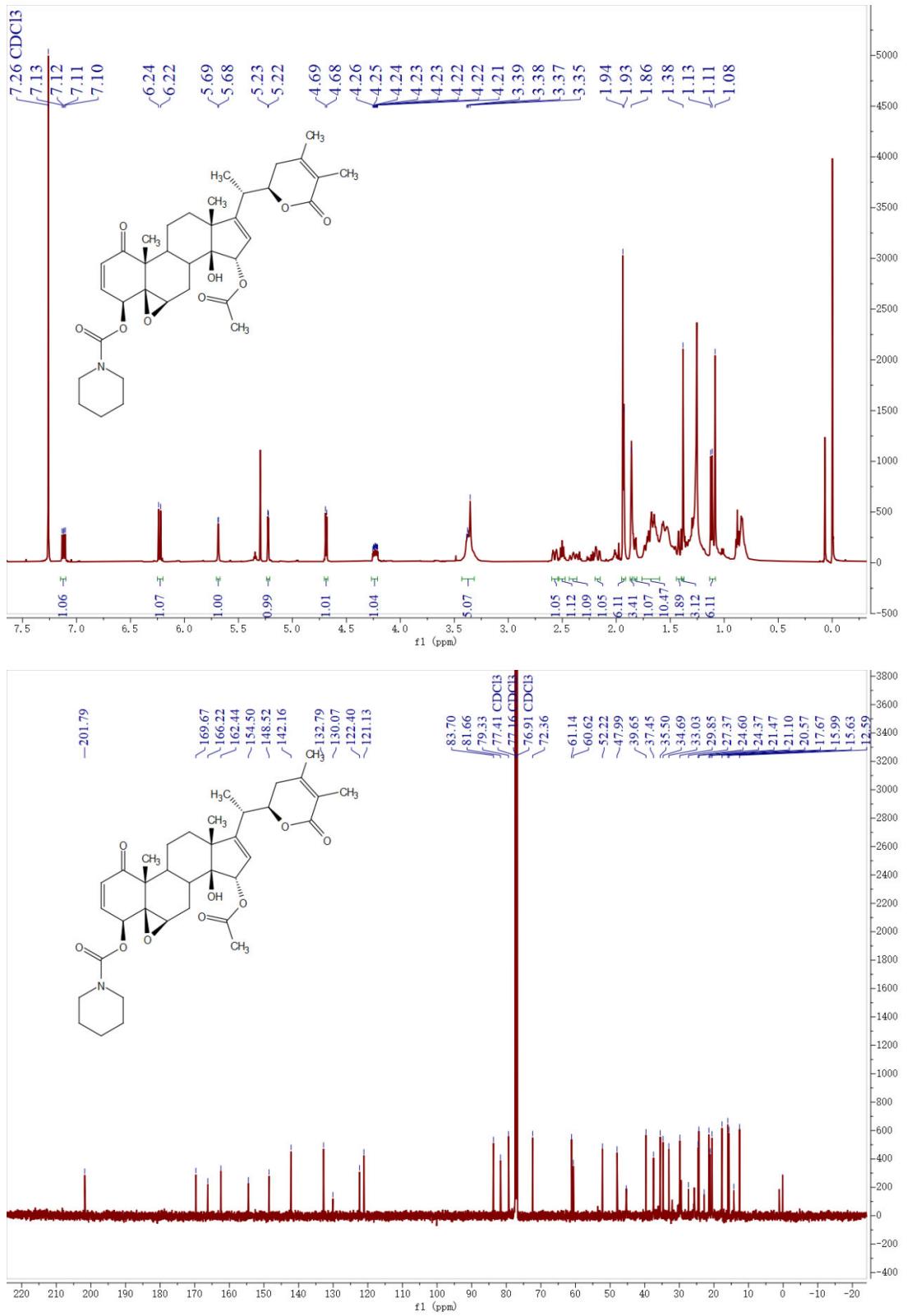
Spectra of compound **9c**



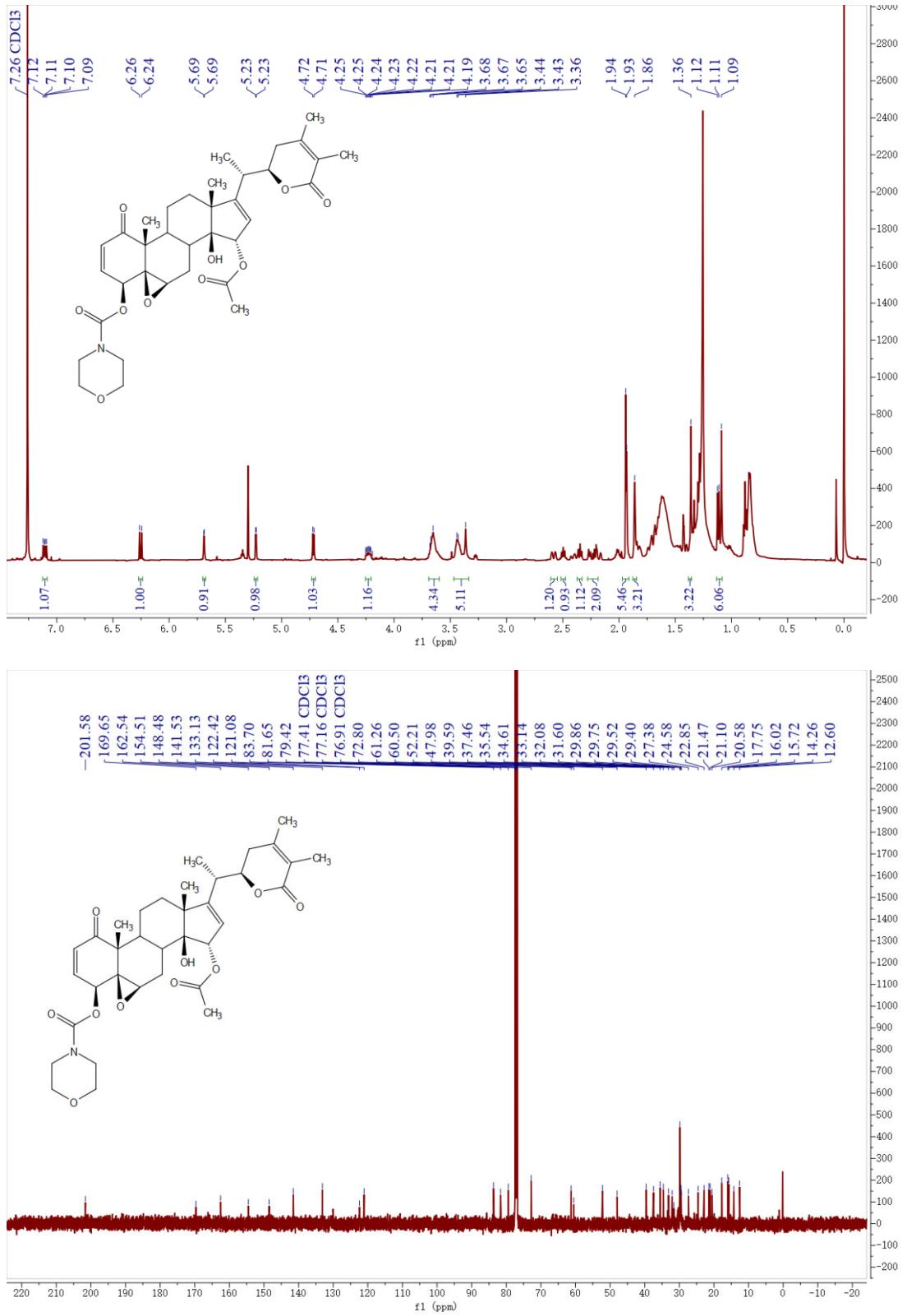
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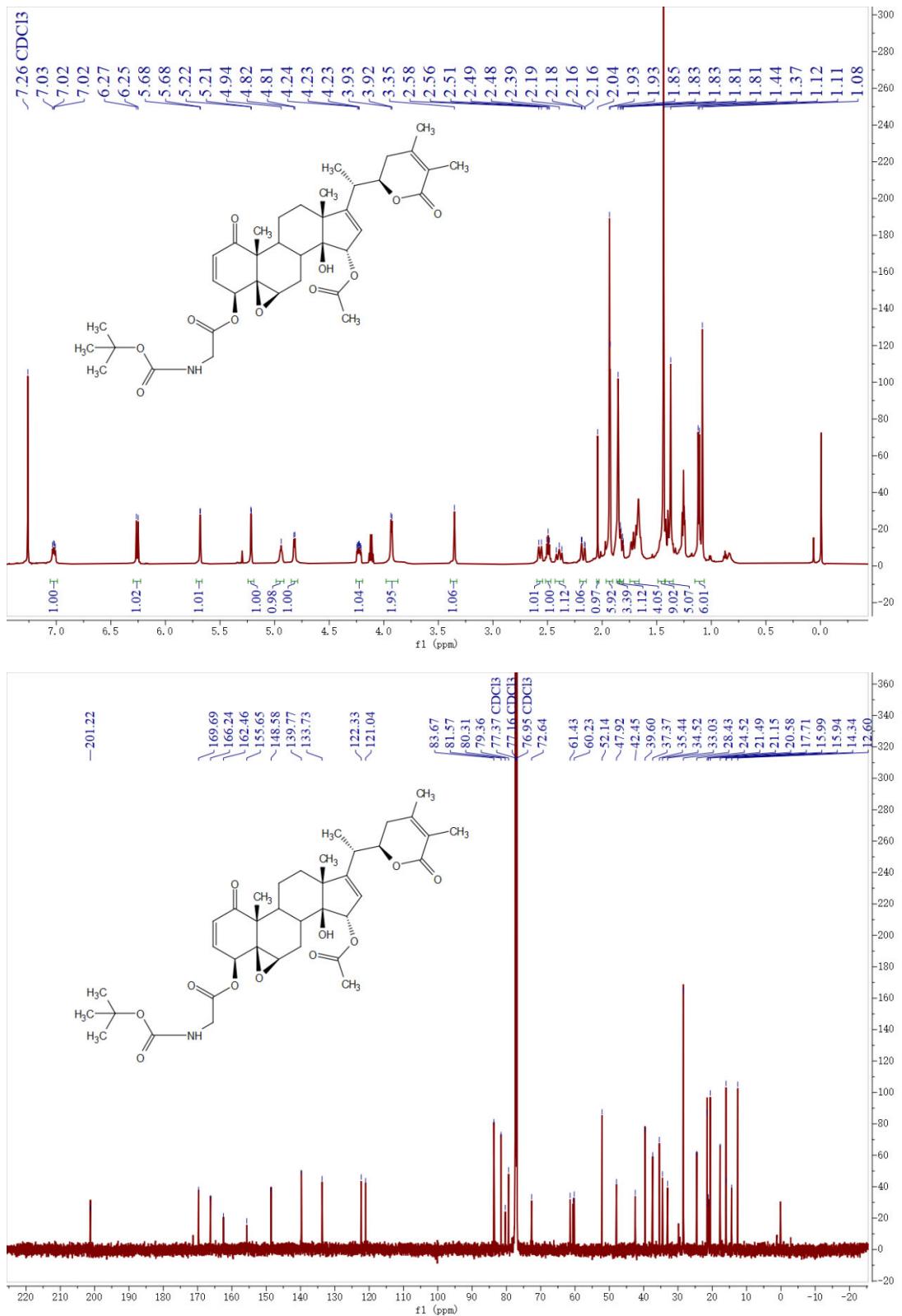
## Spectra of compound **9e**



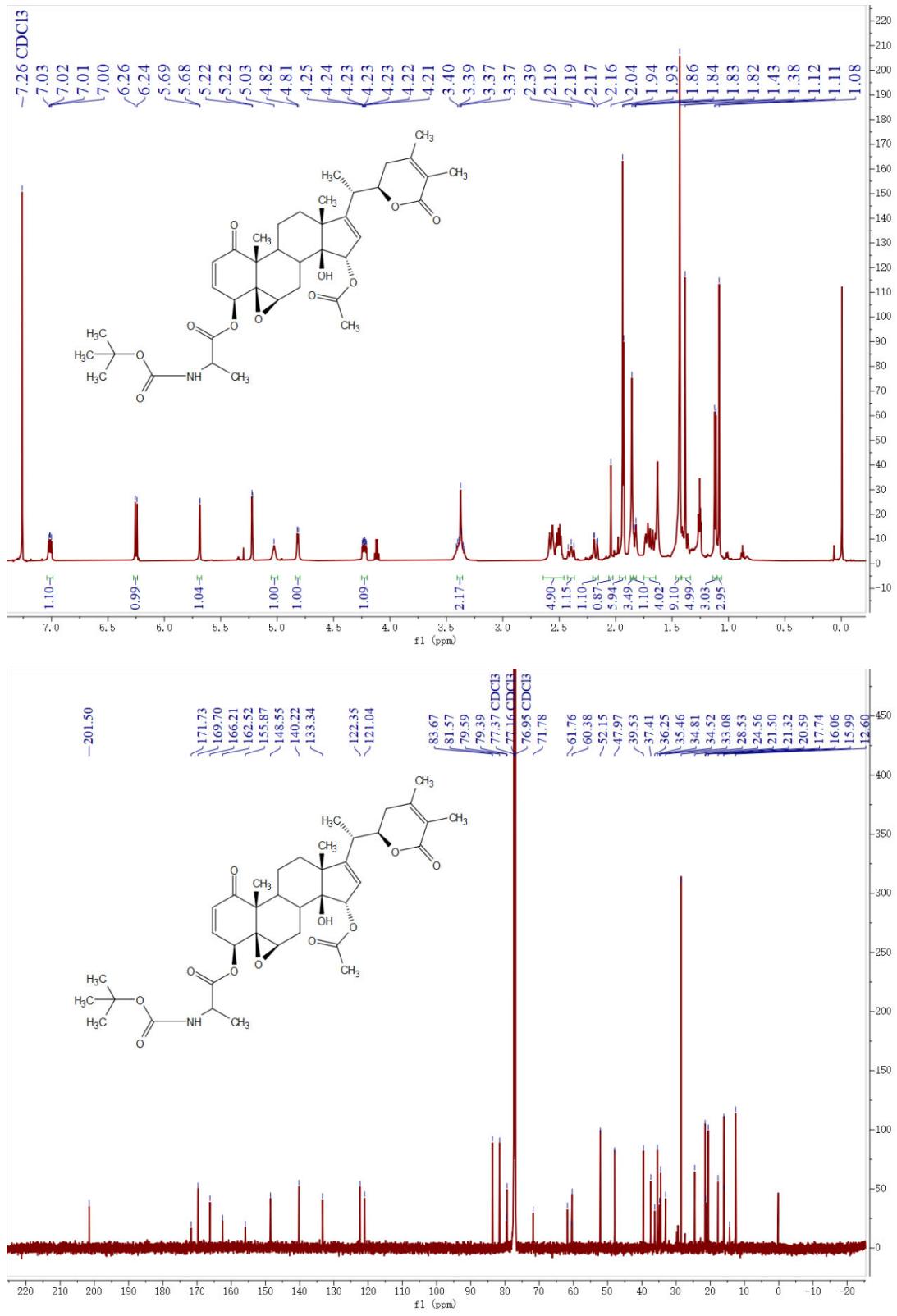
Spectra of compound **9f**



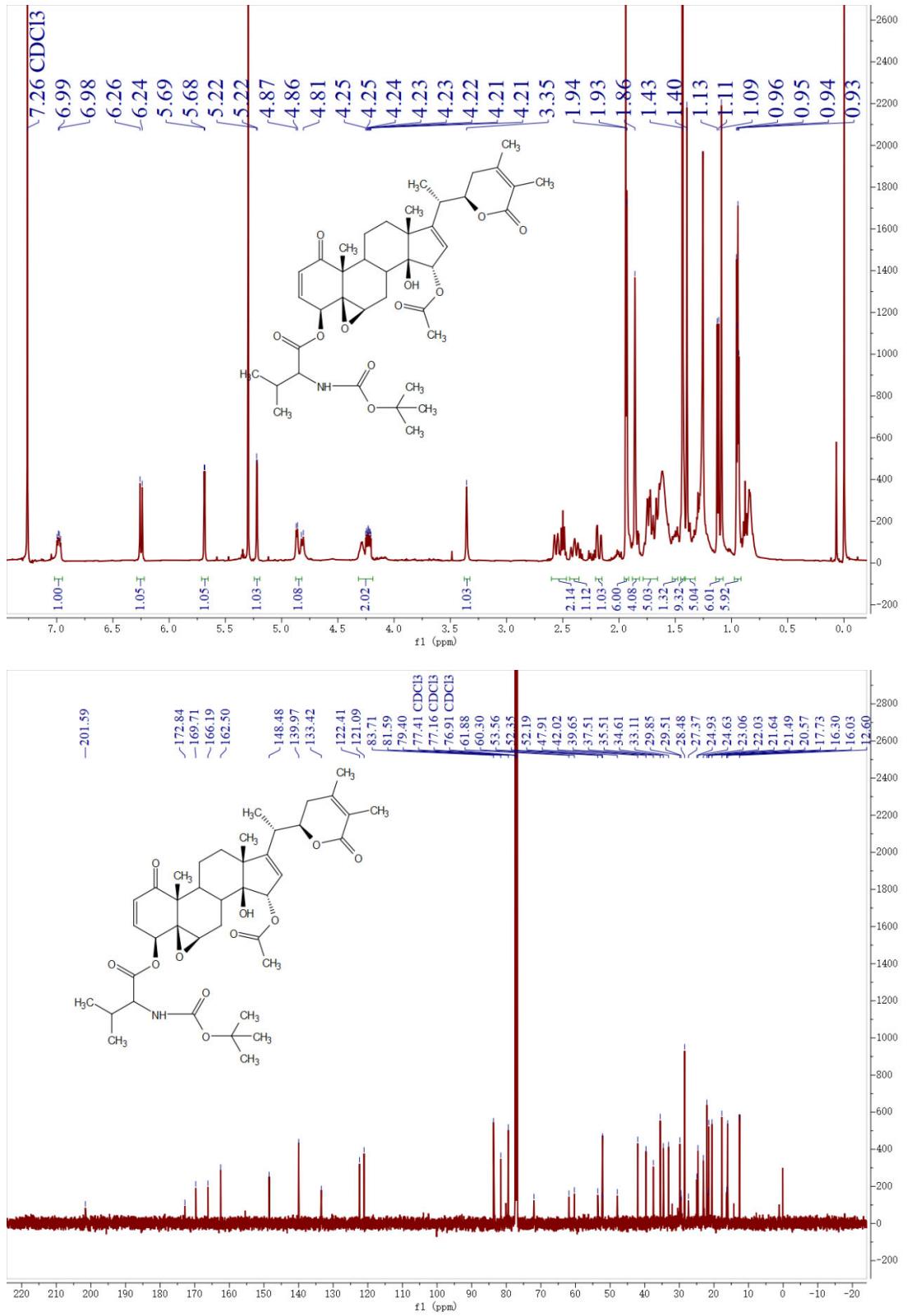
Spectra of compound **10a**



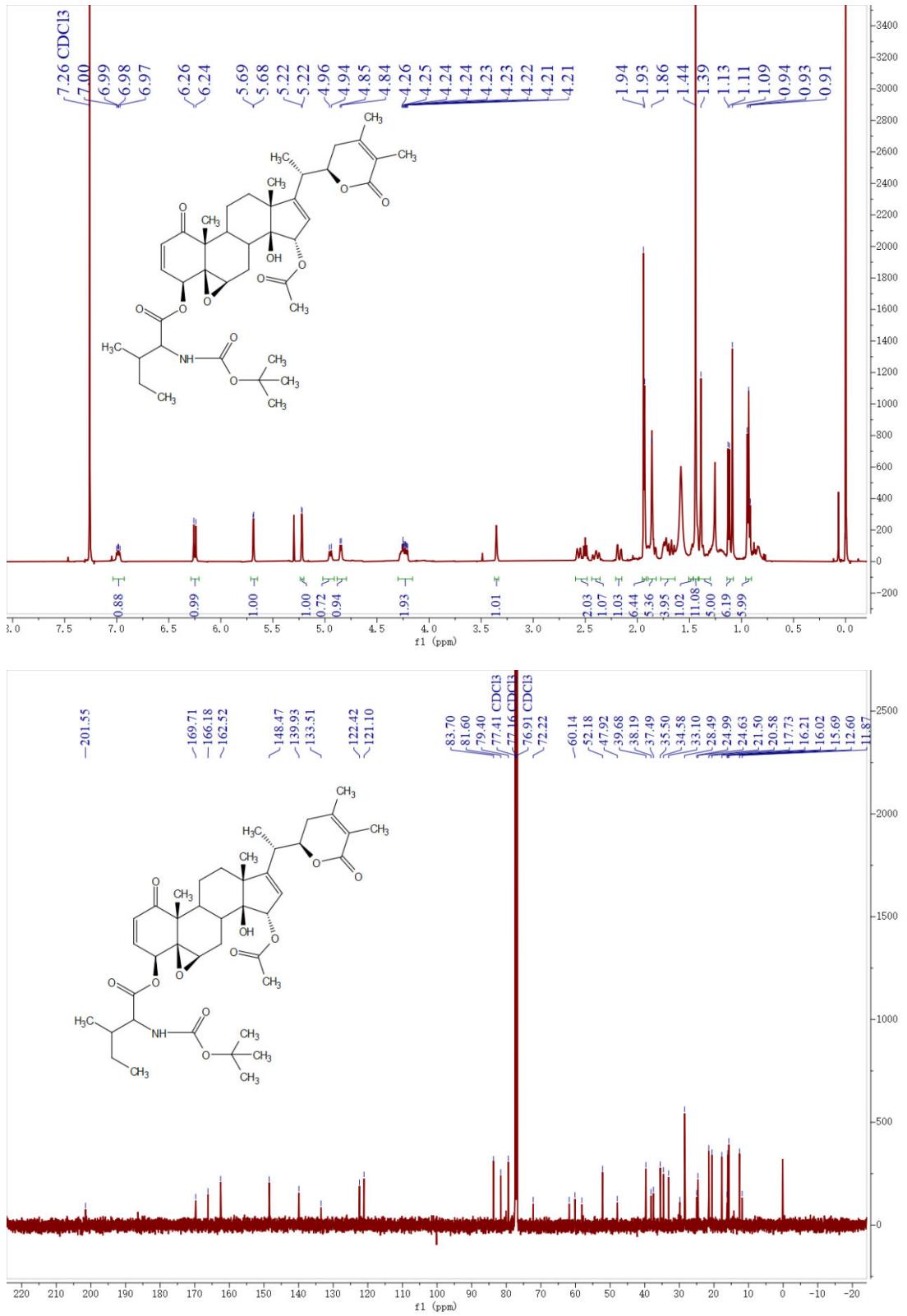
Spectra of compound **10b**



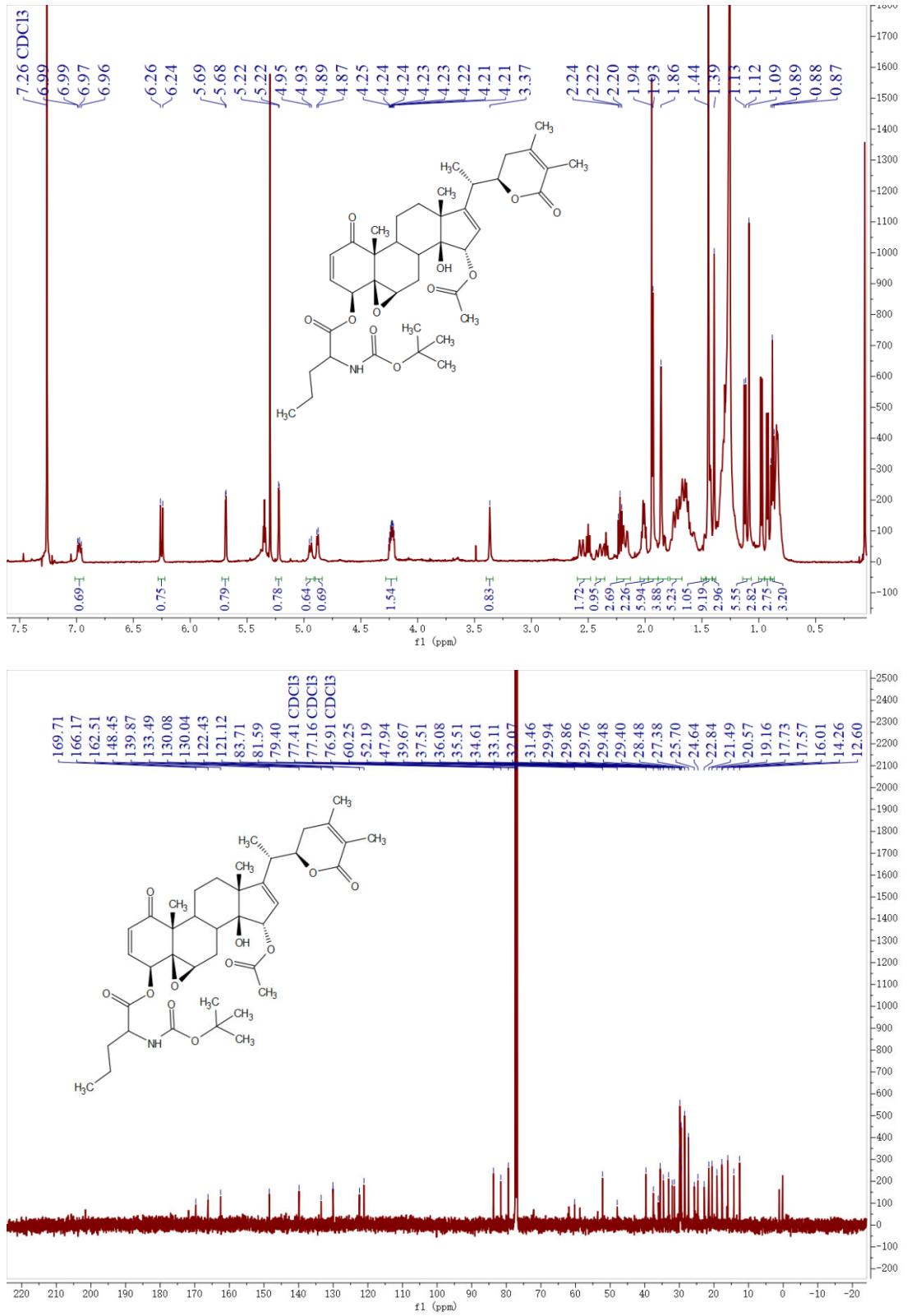
## Spectra of compound **10c**



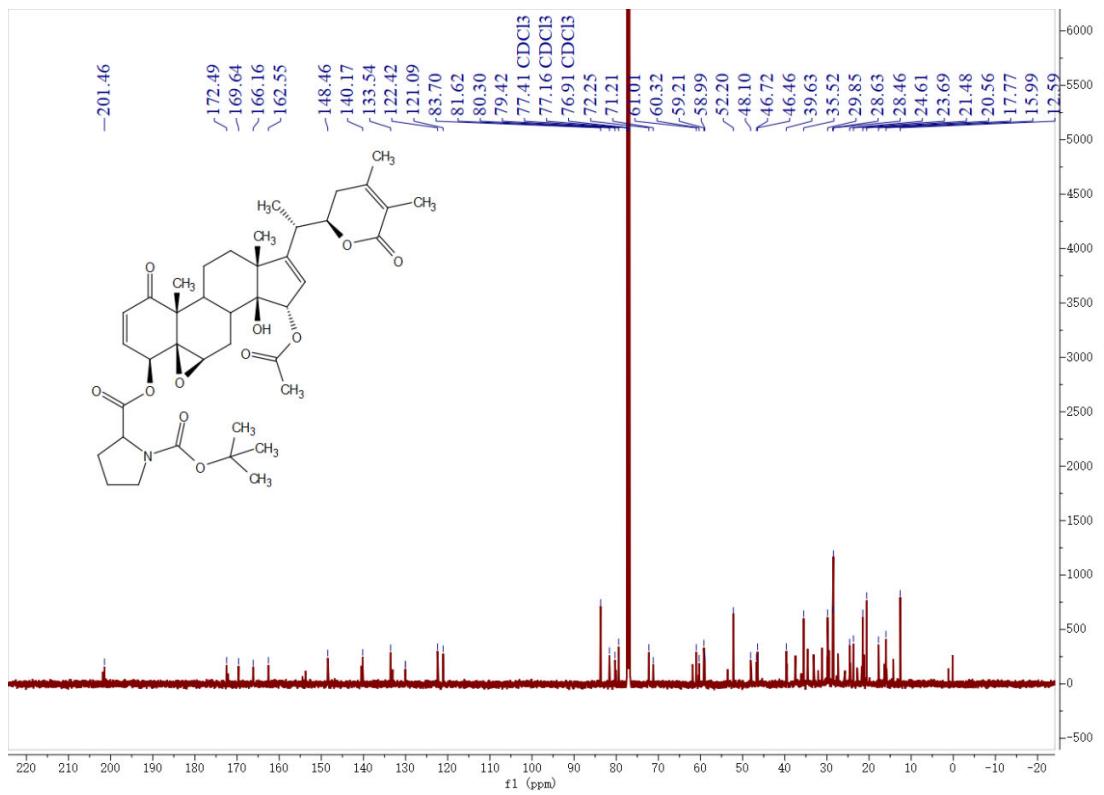
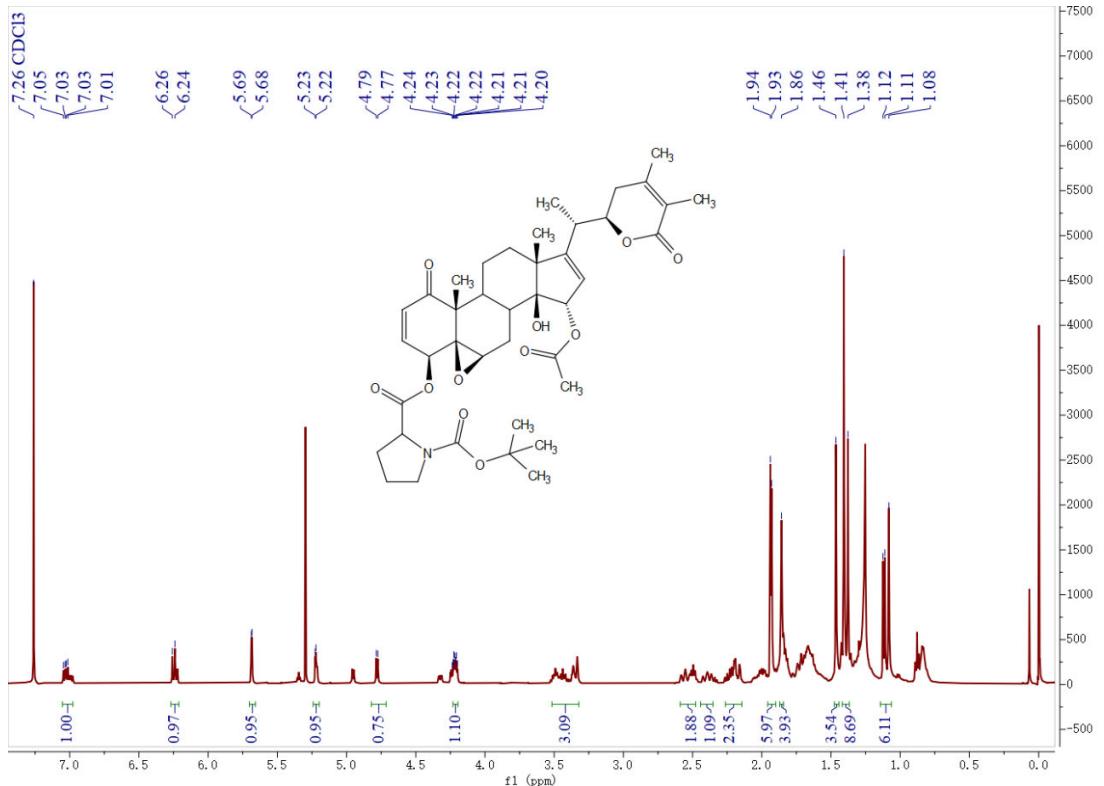
## Spectra of compound **10d**



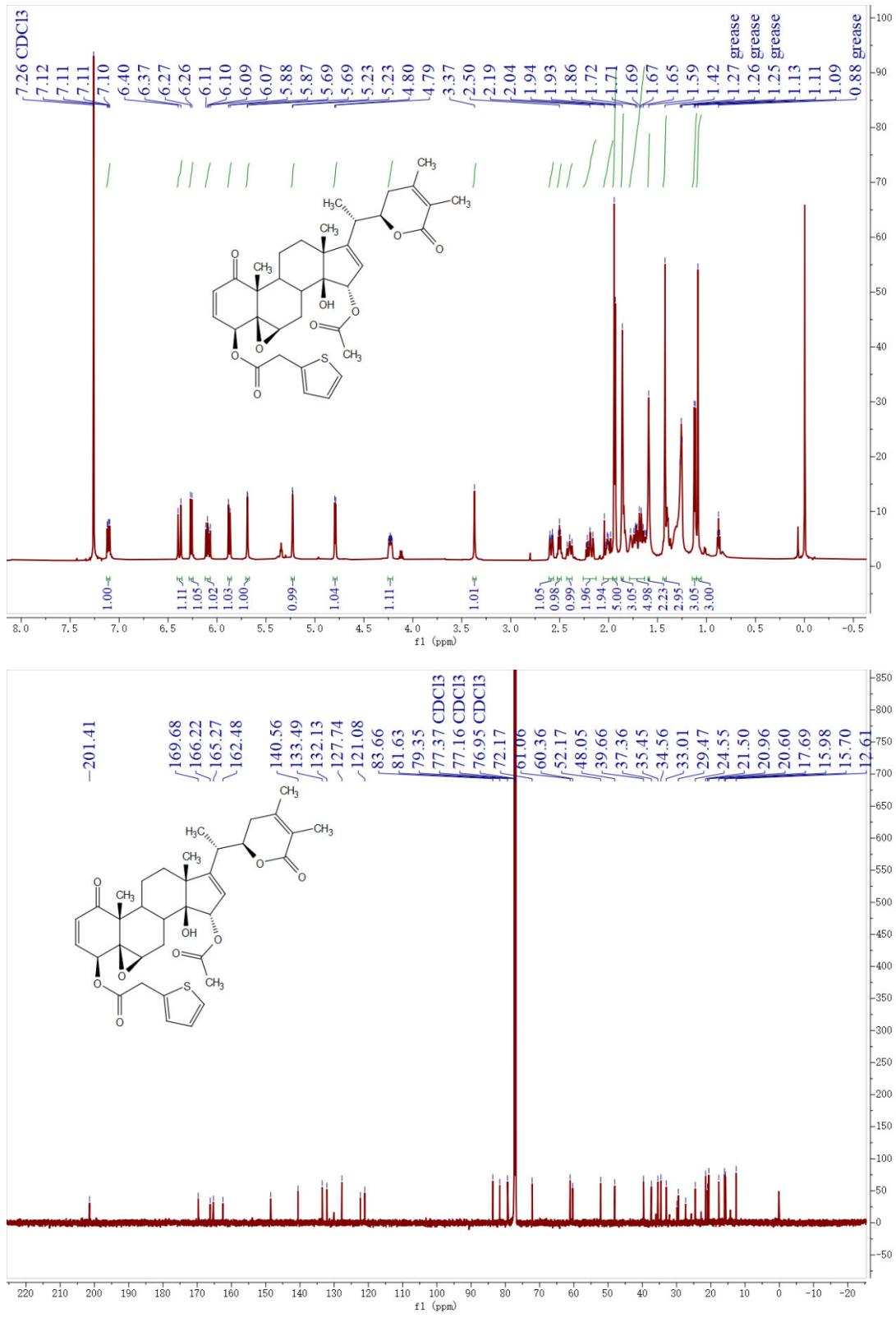
## Spectra of compound **10e**



## Spectra of compound **10f**

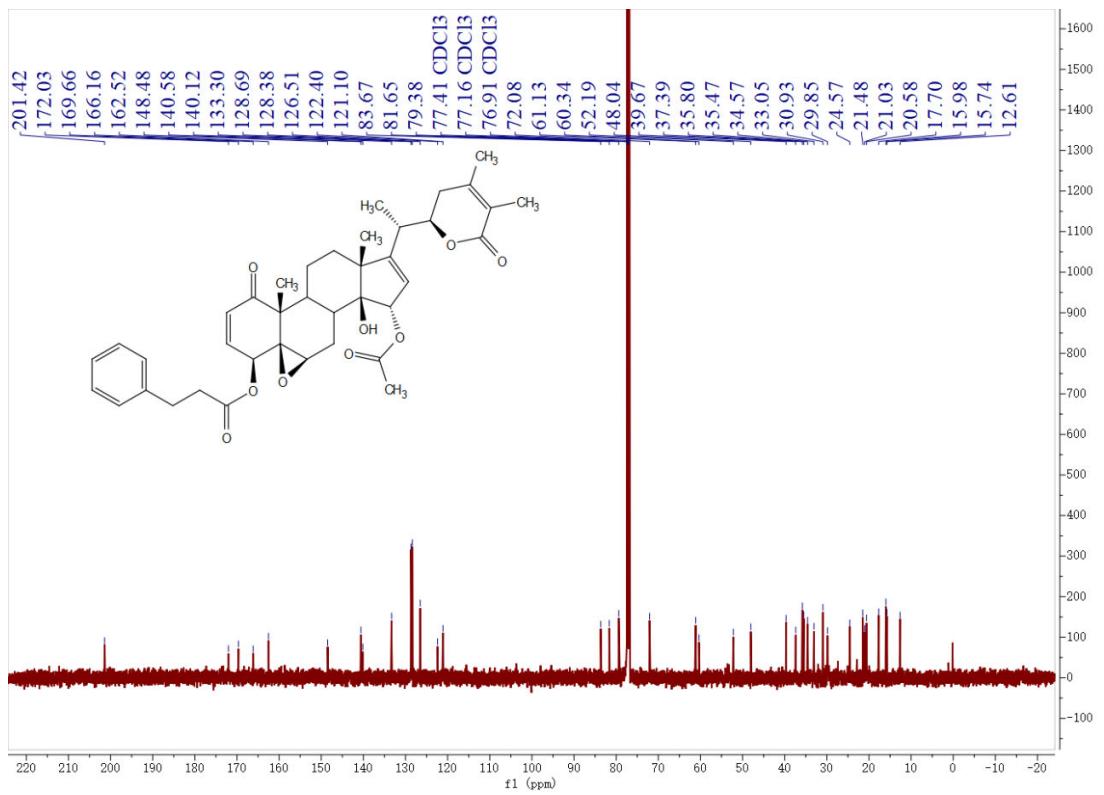
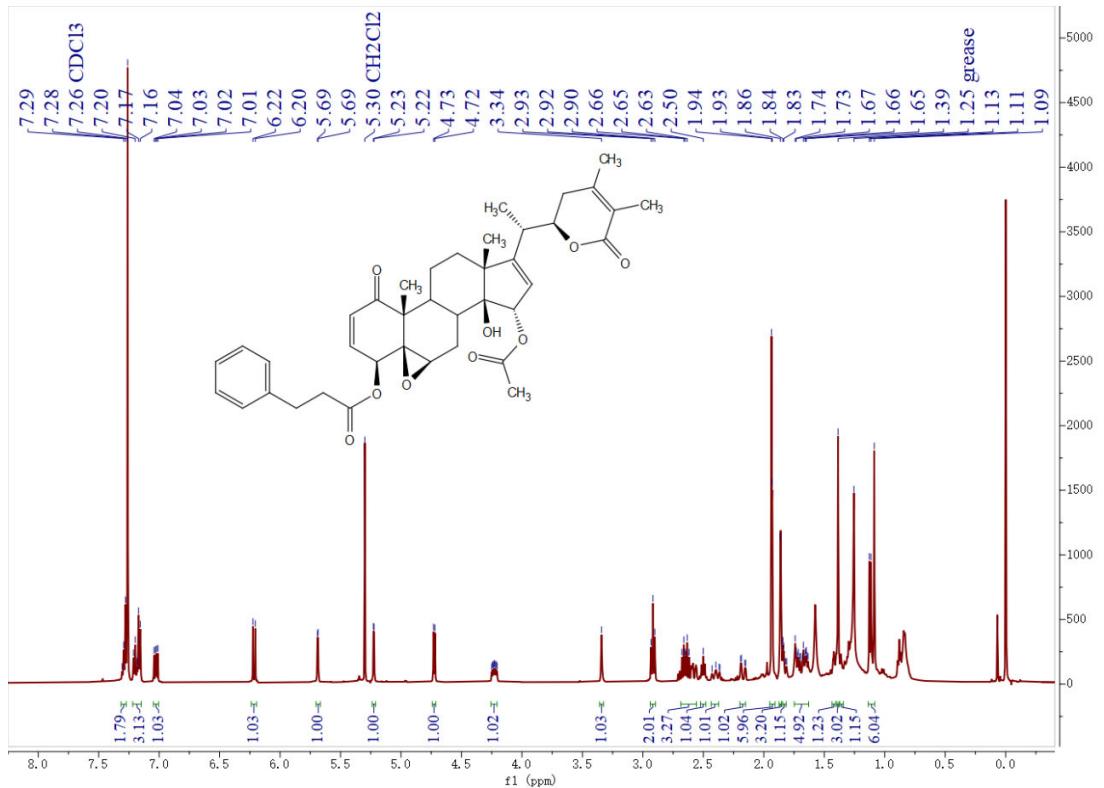


Spectra of compound 11

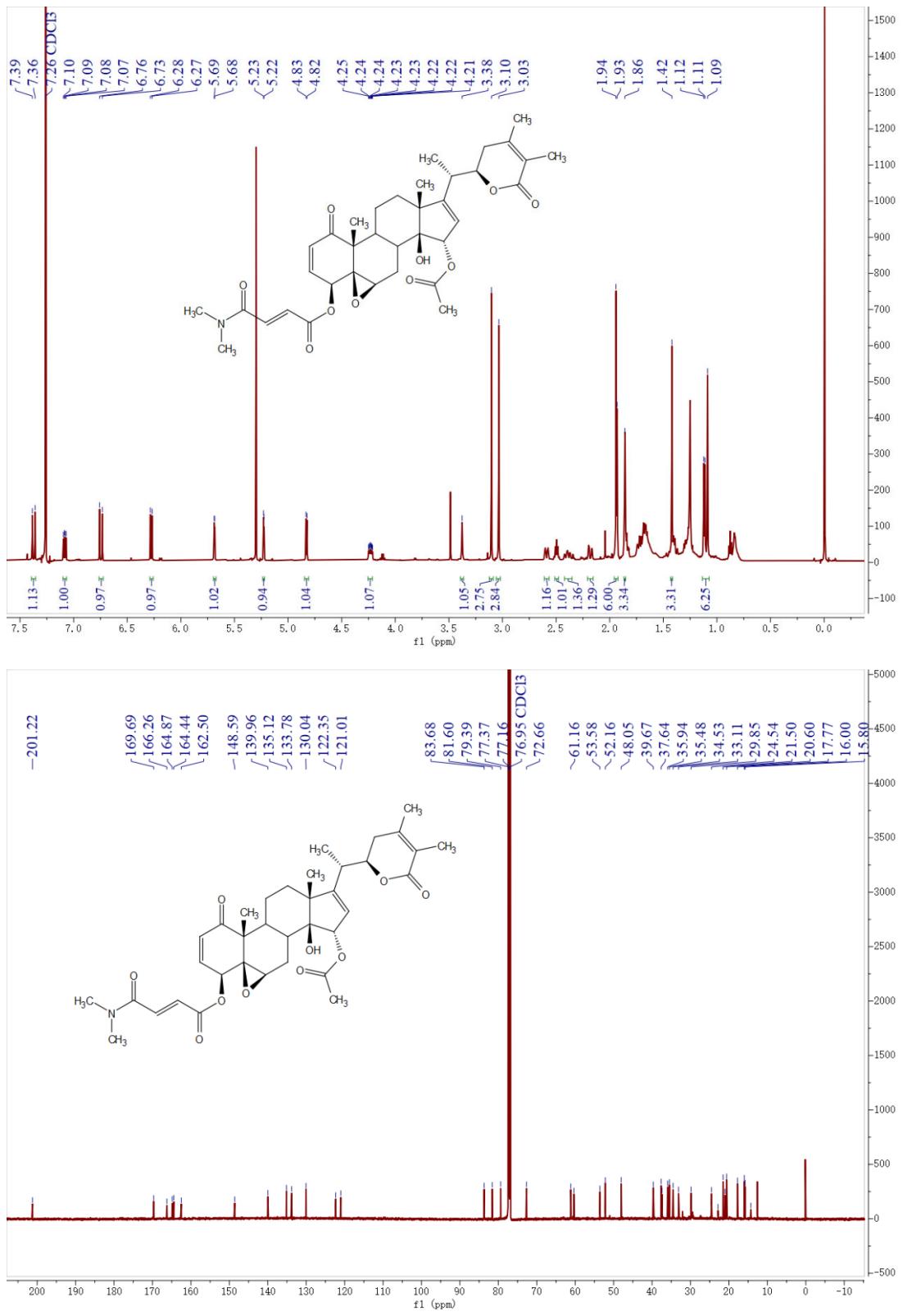


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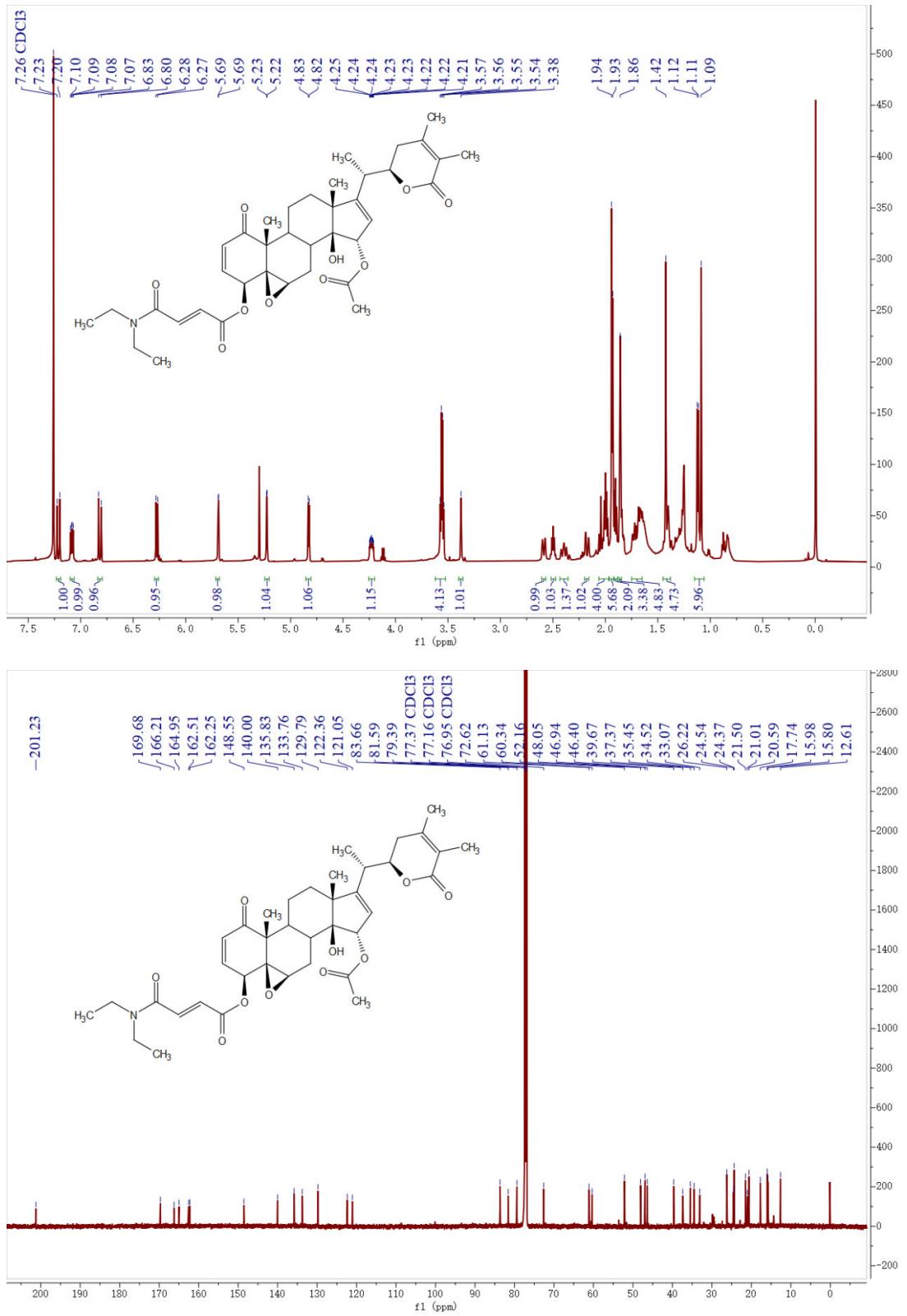
## Spectra of compound 12



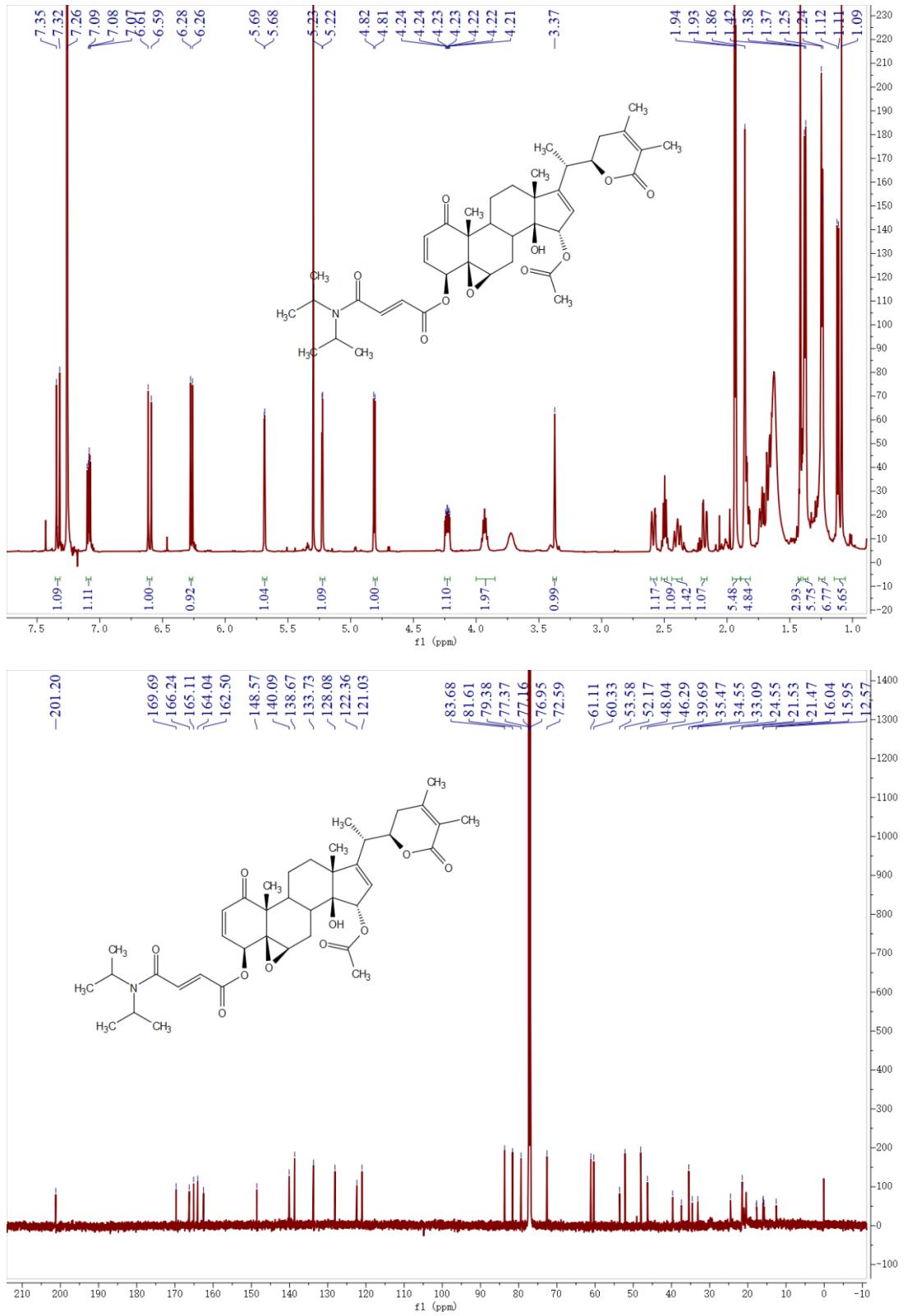
Spectra of compound **13a**



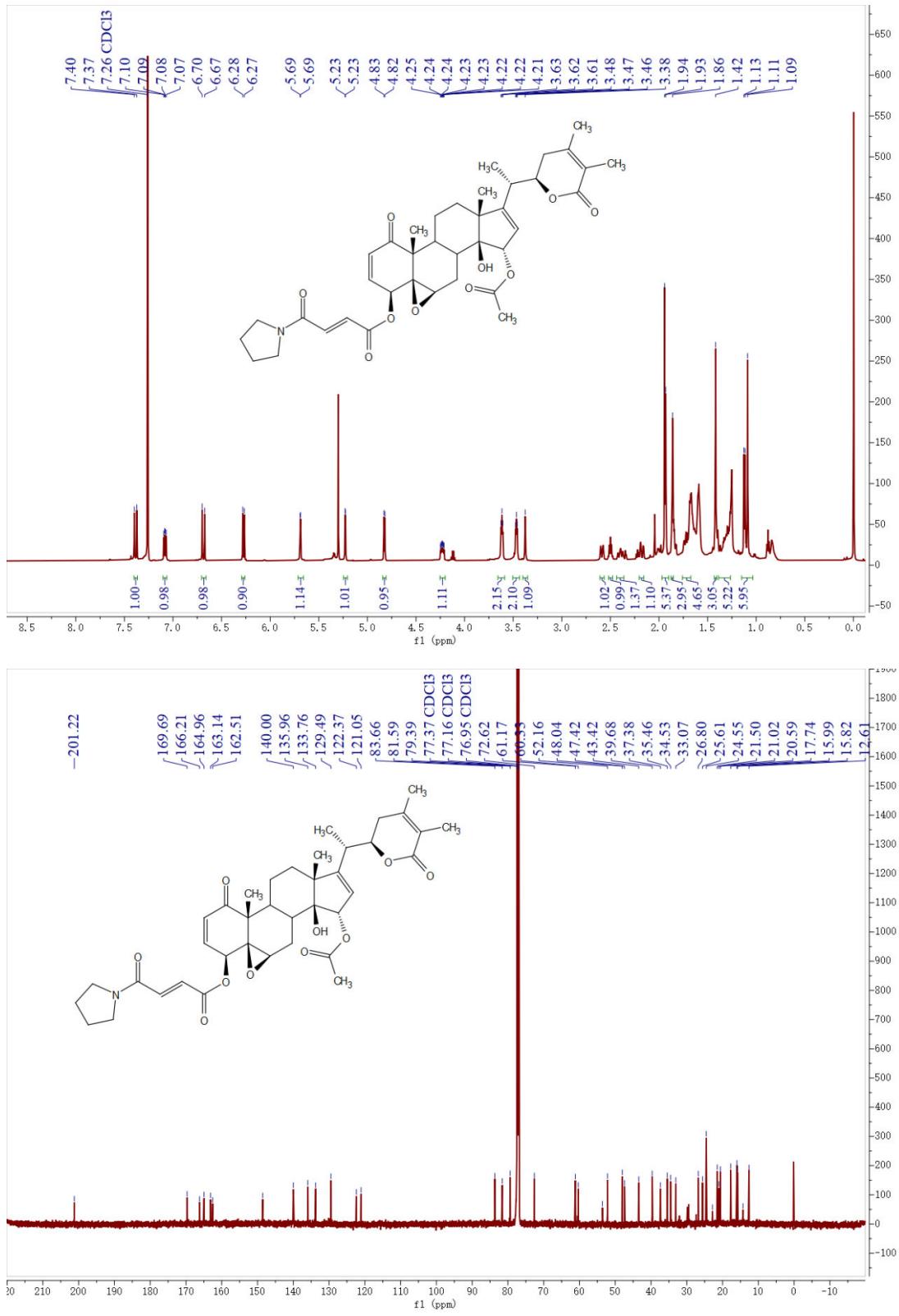
## Spectra of compound **13b**



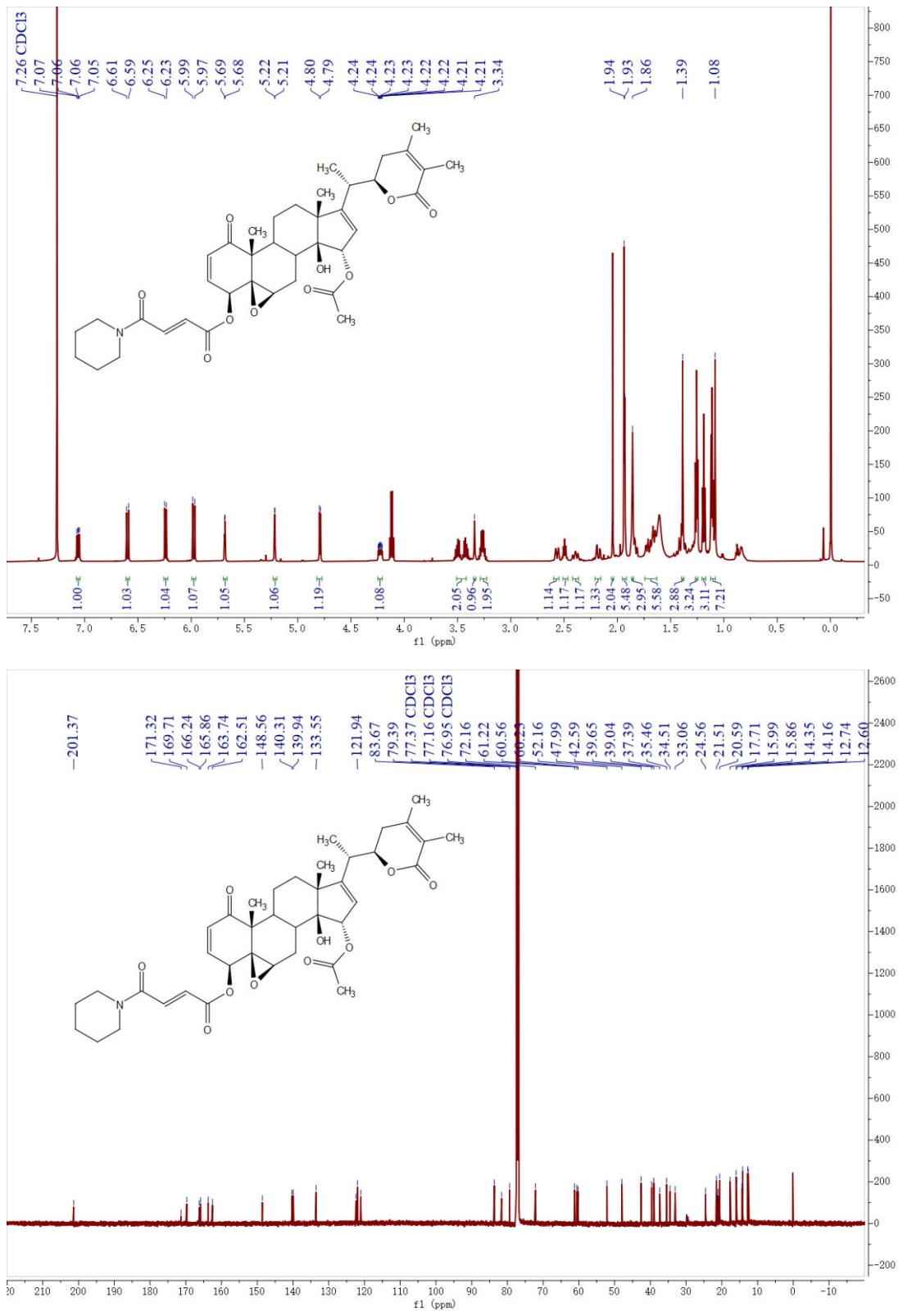
Spectra of compound **13c**



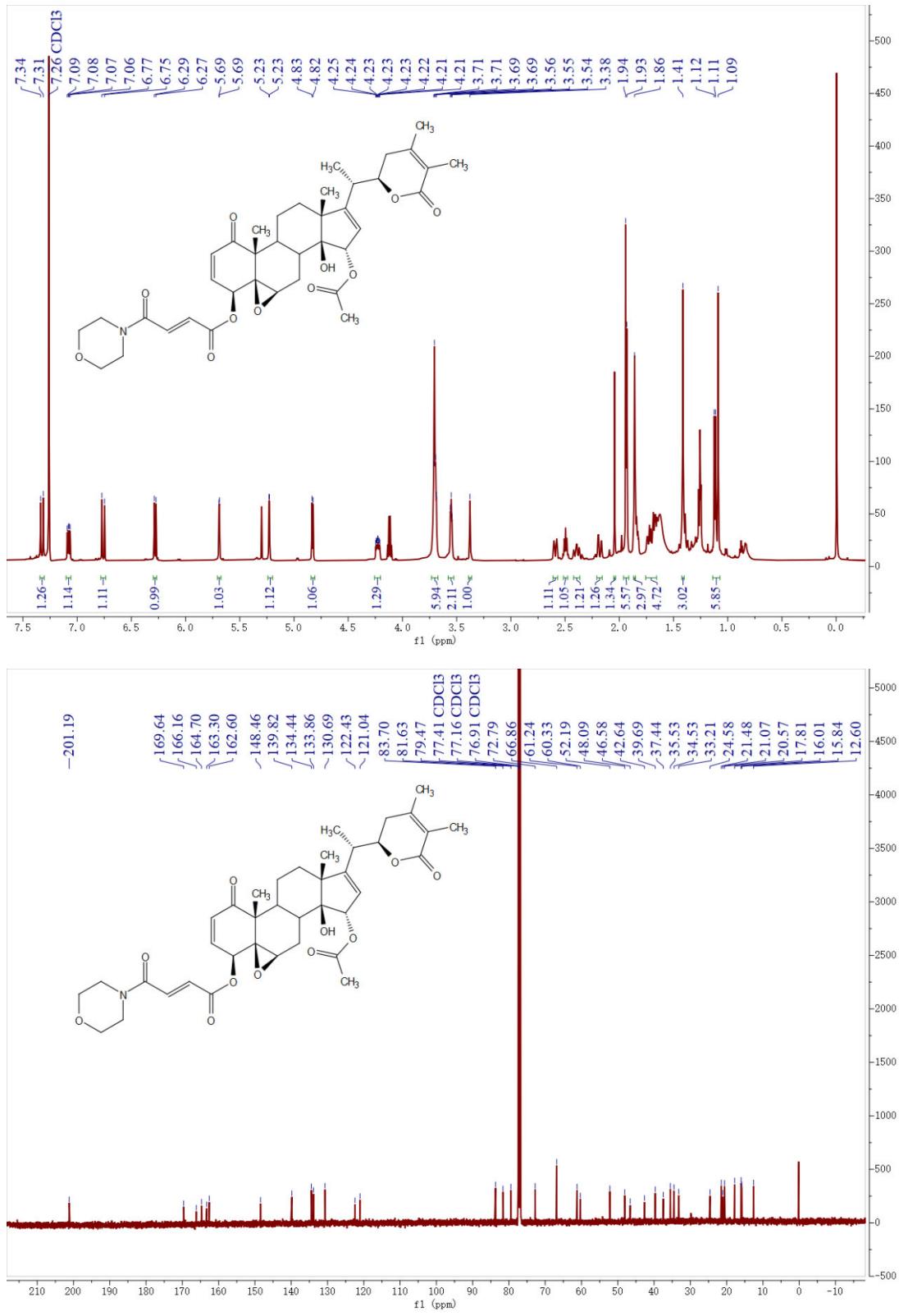
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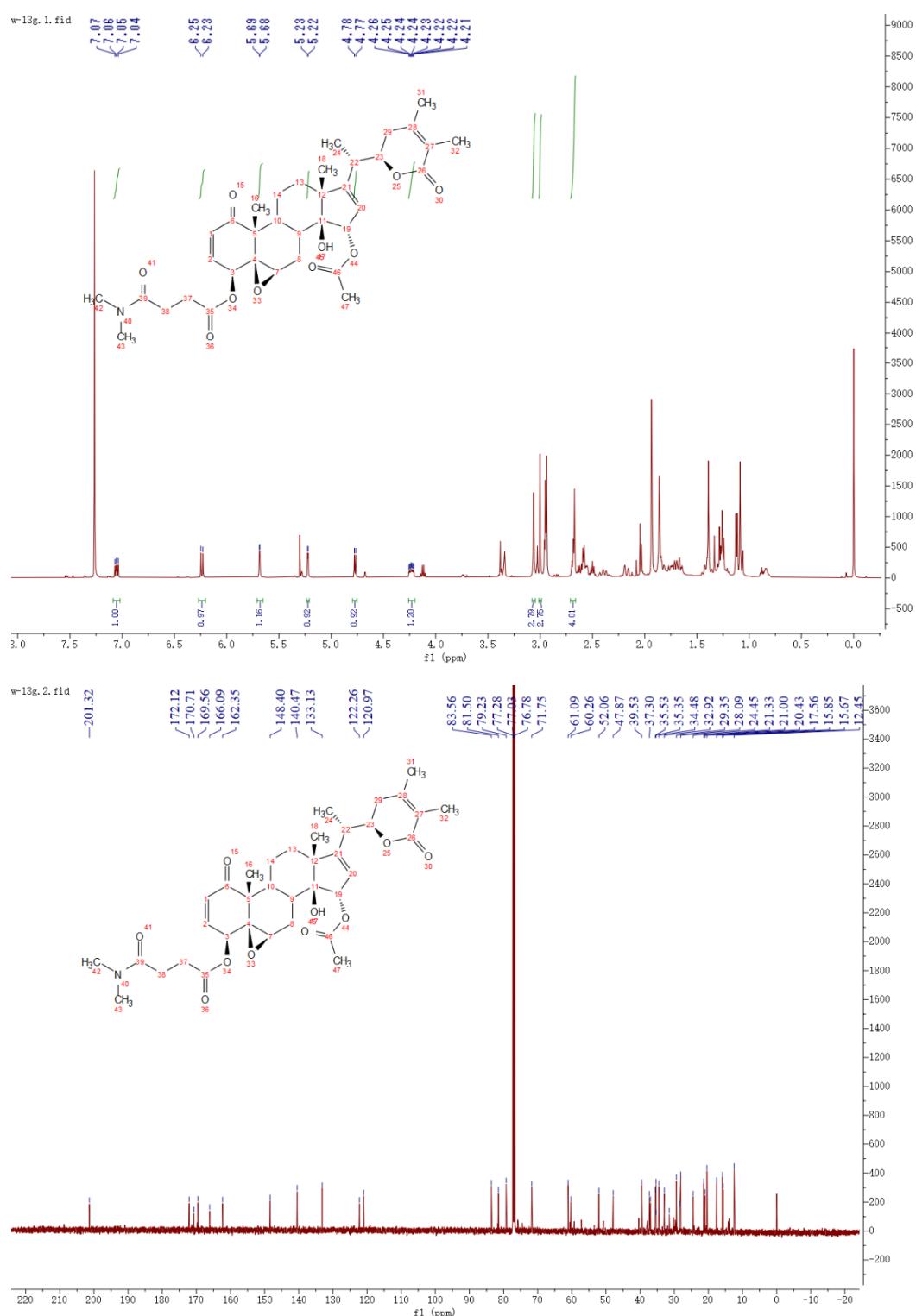
Spectra of compound **13e**



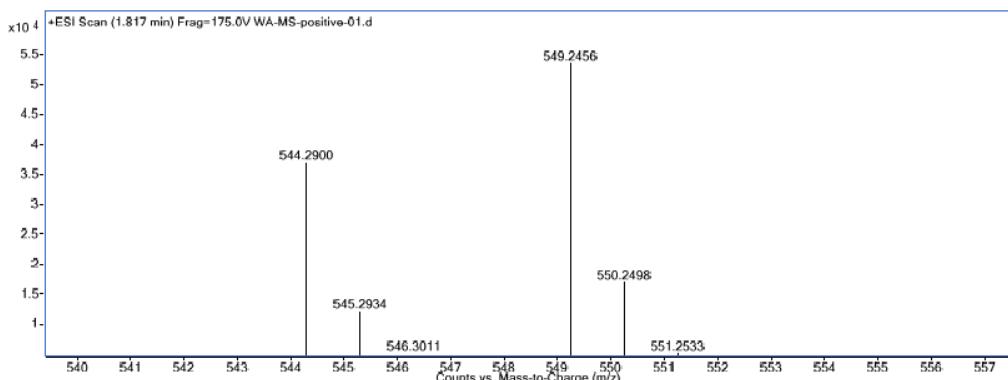
## Spectra of compound 13f



## Spectra of compound 13a'



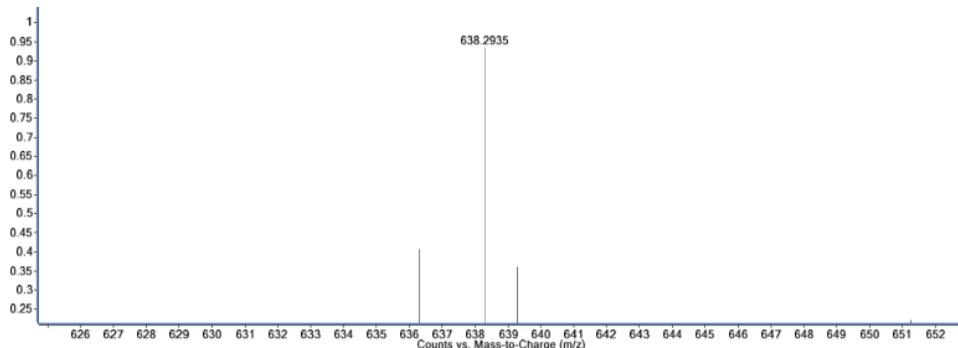
## HRMS for 1(WA)



### Elemental Composition Calculator

Target m/z:	549.2456	Result type:	Positive ions	Species:	[M+Na] <sup>+</sup>
Elements:	C (0-80); H (0-120); O (0-30); Na (0-5) ;				
Ion Formula	Calculated m/z			PPM Error	
C <sub>30</sub> H <sub>38</sub> NaO <sub>8</sub>	549.2459			0.6	

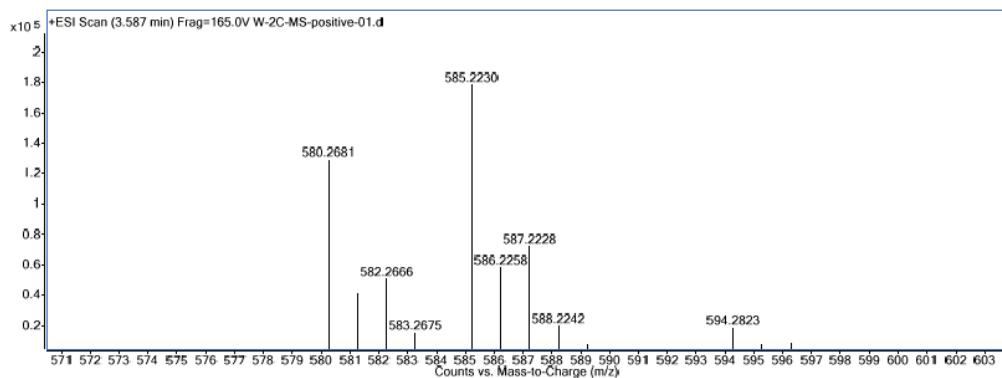
## HRMS for 2a



### Elemental Composition Calculator

Target m/z:	638.2935	Result type:	Positive ions	Species:	[M+Na] <sup>+</sup>
Elements:	C (0-80); H (0-120); O (0-30); N(0-5); Na (0-5)				
Ion Formula	Calculated m/z			PPM Error	
C <sub>33</sub> H <sub>45</sub> NNaO <sub>10</sub>	638.2936			0.15	

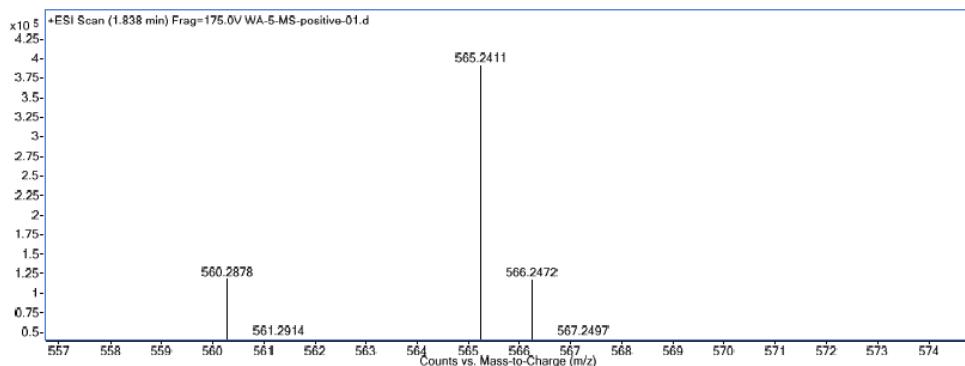
## HRMS for 2c



### Elemental Composition Calculator

Target m/z:	585.2230	Result type:	Positive ions	Species:	[M+Na] <sup>+</sup>
Elements:	C (0-80); H (0-120); O (0-30); Na (0-5); Cl(0-5)				
Ion Formula	Calculated m/z			PPM Error	
C <sub>30</sub> H <sub>39</sub> ClNaO <sub>8</sub>	585.2226			-0.77	

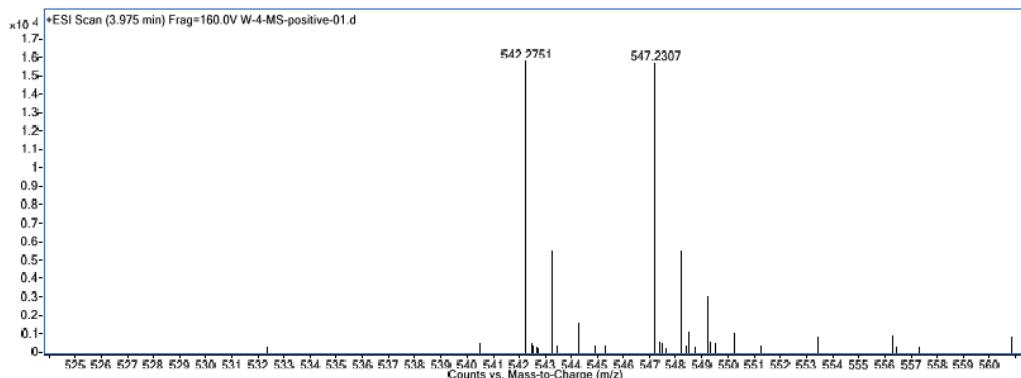
## HRMS for 3



### Elemental Composition Calculator

Target m/z:	565.2411	Result type:	Positive ions	Species:	[M+Na] <sup>+</sup>
Elements:	C (0-80); H (0-120); O (0-30); Na (0-5);				
Ion Formula	Calculated m/z			PPM Error	
C <sub>30</sub> H <sub>38</sub> NaO <sub>9</sub>	565.2408			-0.55	

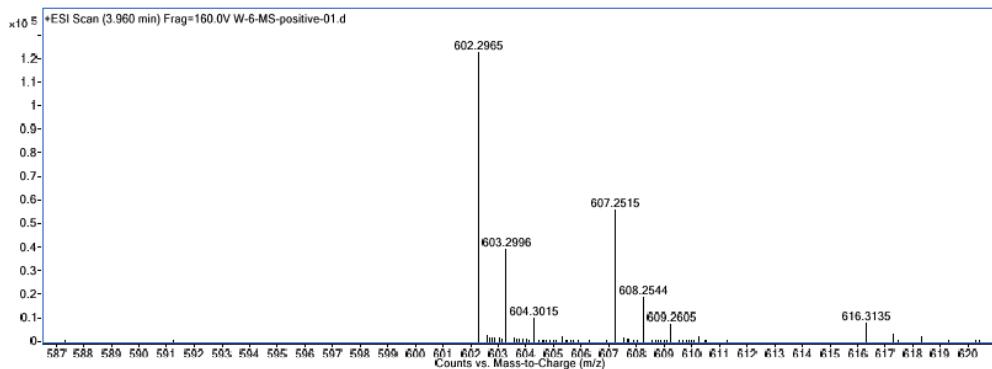
## HRMS for 4



### Elemental Composition Calculator

Target m/z:	542.2751	Result type:	Positive ions	Species:	[M+NH4] <sup>+</sup>
Elements:	C (0-80); H (0-120); O (0-30); N(0-10)				
Ion Formula	Calculated m/z			PPM Error	
C30H40NO8	542.2748			-0.40	

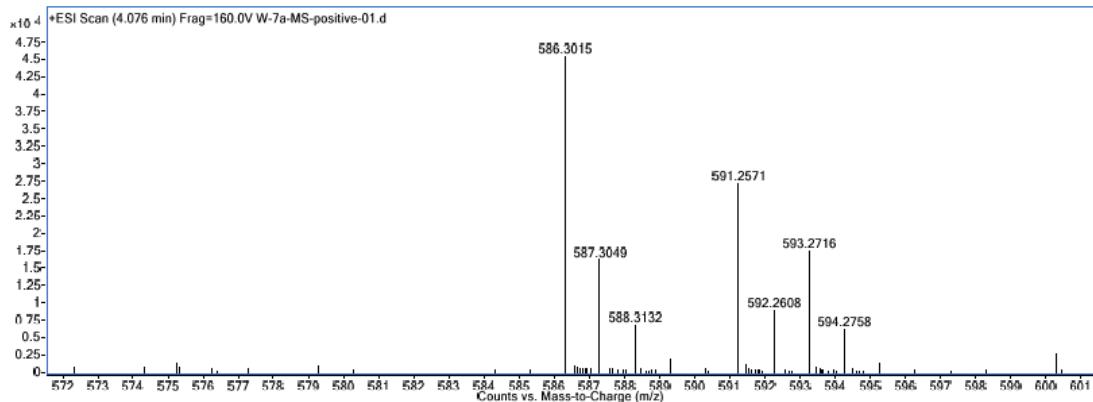
## HRMS for 6



### Elemental Composition Calculator

Target m/z:	602.2965	Result type:	Positive ions	Species:	[M+NH4] <sup>+</sup>
Elements:	C (0-80); H (0-120); O (0-30); N(0-10)				
Ion Formula	Calculated m/z			PPM Error	
C32H44NO10	602.2960			-0.89	

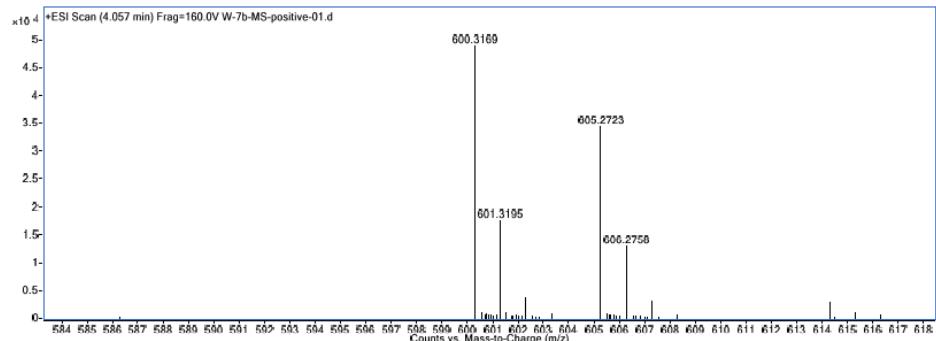
## HRMS for 7a



### Elemental Composition Calculator

Target m/z:	586.3015	Result type:	Positive ions	Species:	[M+NH4] <sup>+</sup>
<b>Elements:</b>		C (0-80); H (0-120); O (0-30); N(0-10)			
<b>Ion Formula</b>		<b>Calcalated m/z</b>			<b>PPM Error</b>
C32H44NO9		586.3011			-0.76

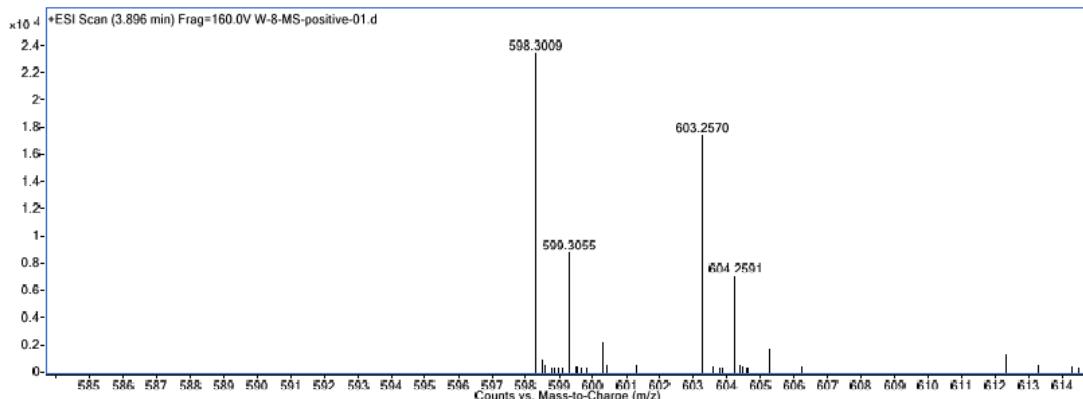
## HRMS for 7b



### Elemental Composition Calculator

Target m/z:	600.3169	Result type:	Positive ions	Species:	[M+NH4] <sup>+</sup>
<b>Elements:</b>		C (0-80); H (0-120); O (0-30); N(0-10)			
<b>Ion Formula</b>		<b>Calcalated m/z</b>			<b>PPM Error</b>
C33H46NO9		600.3167			-0.27

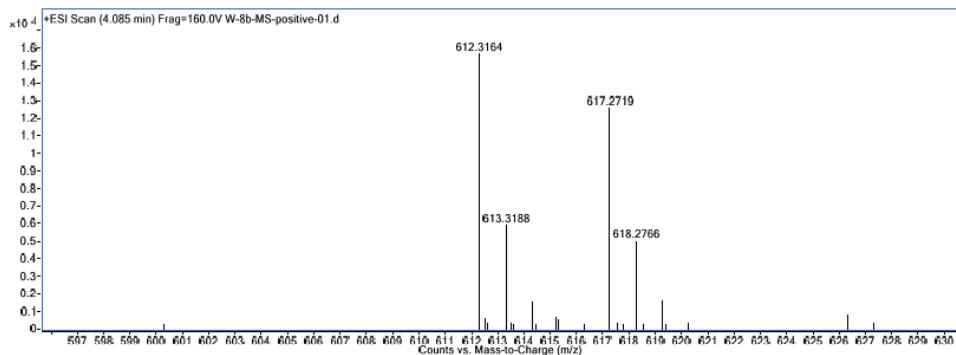
## HRMS for 8a



### Elemental Composition Calculator

Target m/z:	598.3009	Result type:	Positive ions	Species:	[M+NH4] <sup>+</sup>
<b>Elements:</b>		C (0-80); H (0-120); O (0-30); N(0-10)			
<b>Ion Formula</b>		<b>Calculated m/z</b>		<b>PPM Error</b>	
C33H44NO9		598.3011		0.20	

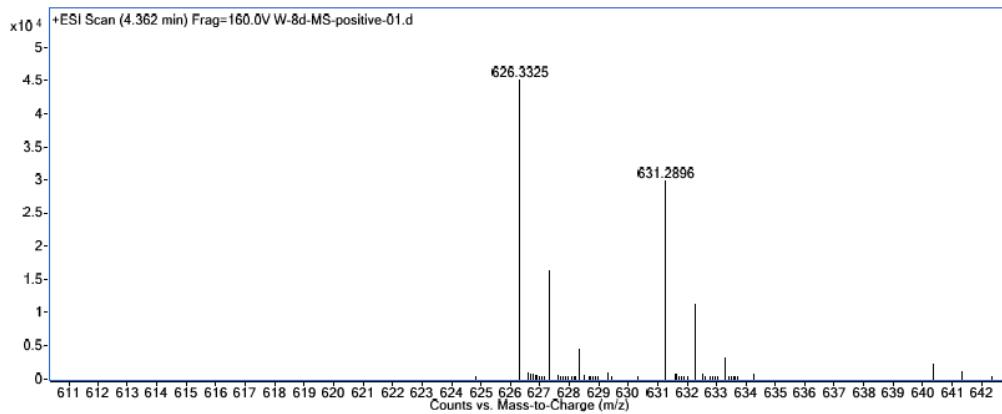
## HRMS for 8b



### Elemental Composition Calculator

Target m/z:	612.3164	Result type:	Positive ions	Species:	[M+NH4] <sup>+</sup>
<b>Elements:</b>		C (0-80); H (0-120); O (0-30); N(0-10)			
<b>Ion Formula</b>		<b>Calculated m/z</b>		<b>PPM Error</b>	
C34H46NO9		612.3167		0.42	

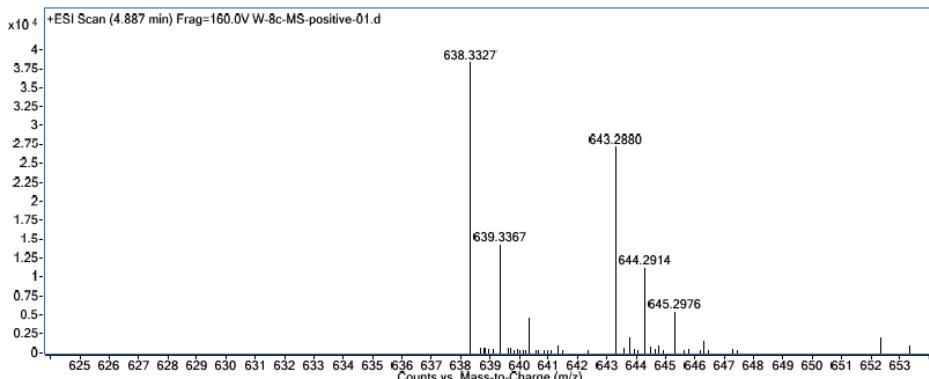
## HRMS for 8c



### Elemental Composition Calculator

Target m/z:	626.3325	Result type:	Positive ions	Species:	[M+NH4] <sup>+</sup>
Elements:	C (0-80); H (0-120); O (0-30); N(0-10)				
Ion Formula	Calculated m/z			PPM Error	
C35H48NO9	626.3324			-0.17	

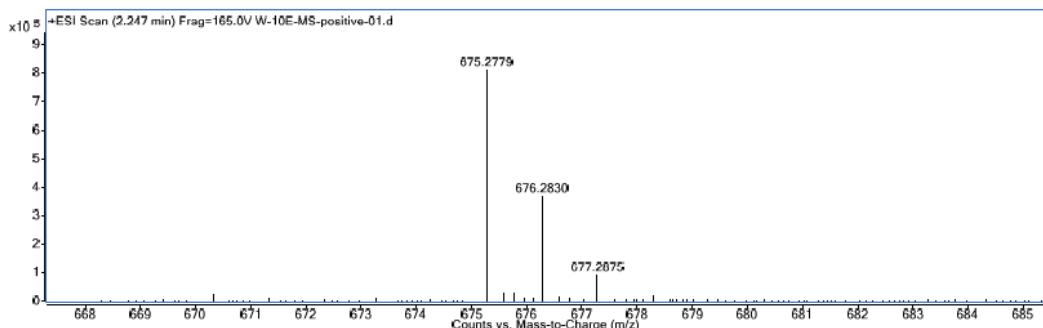
## HRMS for 8d



### Elemental Composition Calculator

Target m/z:	638.3327	Result type:	Positive ions	Species:	[M+NH4] <sup>+</sup>
Elements:	C (0-80); H (0-120); O (0-30); N(0-10)				
Ion Formula	Calculated m/z			PPM Error	
C36H48NO9	638.3324			-0.58	

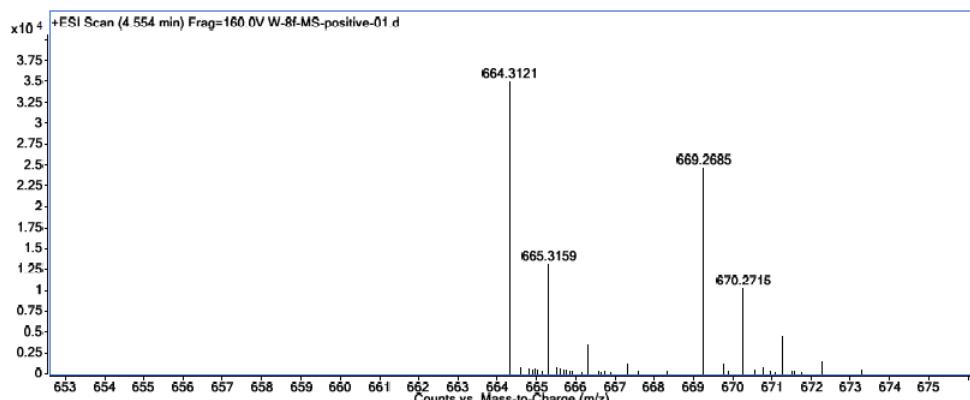
## HRMS for 8e



### Elemental Composition Calculator

Target m/z:	675.2779	Result type:	Positive ions	Species:	[M+Na] <sup>+</sup>
Elements:	C (0-80); H (0-120); O (0-30)				
Ion Formula	Calculated m/z			PPM Error	
C <sub>36</sub> H <sub>44</sub> NaO <sub>11</sub>	675.2776			-0.44	

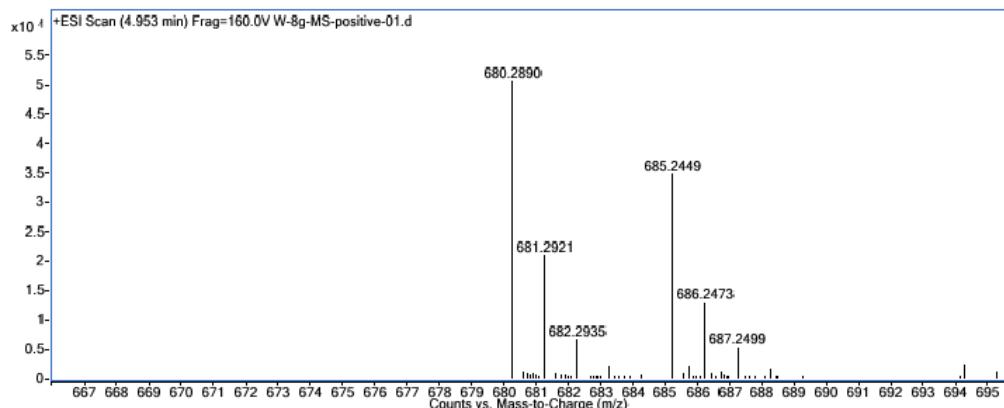
## HRMS for 8g



### Elemental Composition Calculator

Target m/z:	664.3121	Result type:	Positive ions	Species:	[M+NH <sub>4</sub> ] <sup>+</sup>
Elements:	C (0-80); H (0-120); O (0-30); N(0-10)				
Ion Formula	Calcalated m/z			PPM Error	
C <sub>37</sub> H <sub>46</sub> NO <sub>10</sub>	664.3116			-0.74	

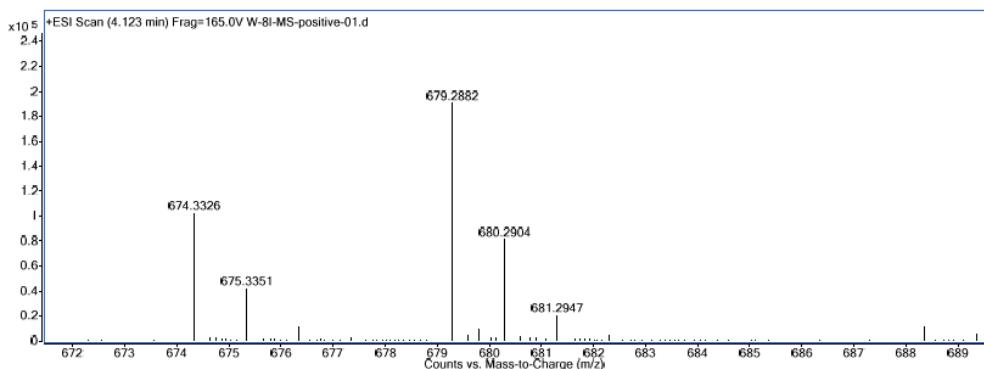
## HRMS for **8h**



### Elemental Composition Calculator

Target m/z:	680.2890	Result type:	Positive ions	Species:	[M+NH4] <sup>+</sup>
Elements:	C (0-80); H (0-120); O (0-30); N(0-10) ; S(0-5)				
Ion Formula	Calculated m/z			PPM Error	
C37H46NO9S	680.2888			-0.32	

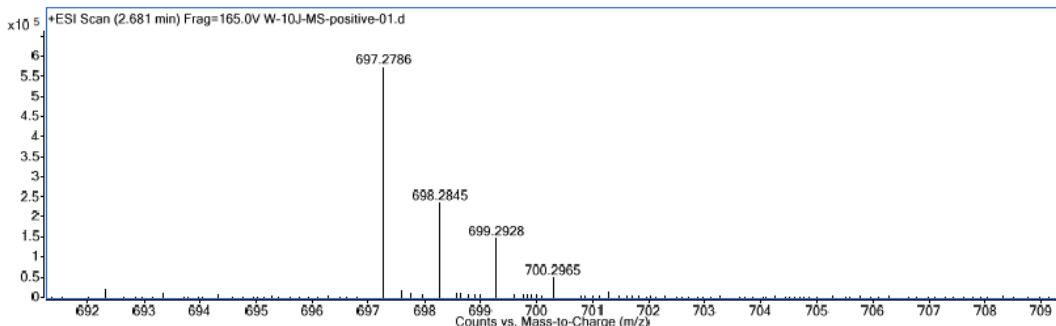
## HRMS for **8i**



### Elemental Composition Calculator

Target m/z:	679.2882	Result type:	Positive ions	Species:	[M+Na] <sup>+</sup>
Elements:	C (0-80); H (0-120); O (0-30); Na (0-5)				
Ion Formula	Calculated m/z			PPM Error	
C39H44NaO9	679.2878			-0.6	

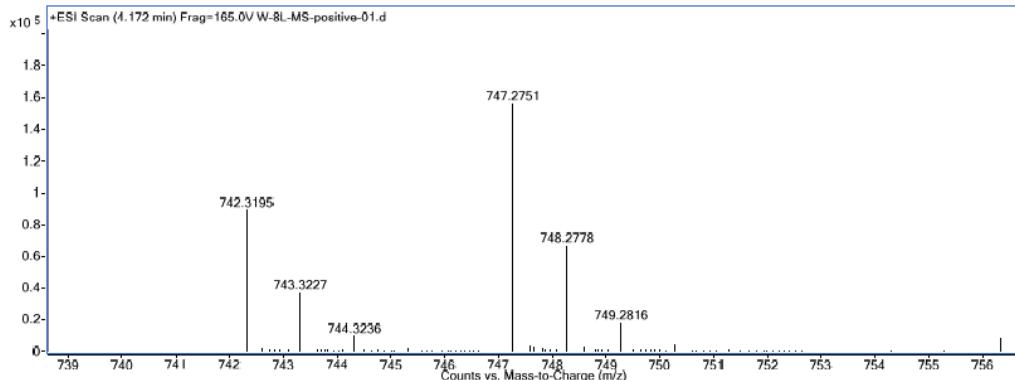
## HRMS for 8j



### Elemental Composition Calculator

Target m/z:	697.2786	Result type:	Positive ions	Species:	[M+Na] <sup>+</sup>
Elements:	C (0-80); H (0-120); O (0-30); F(0-5)				
Ion Formula			Calculated m/z	PPM Error	
C39H43FNaO9			697.2783	-0.36	

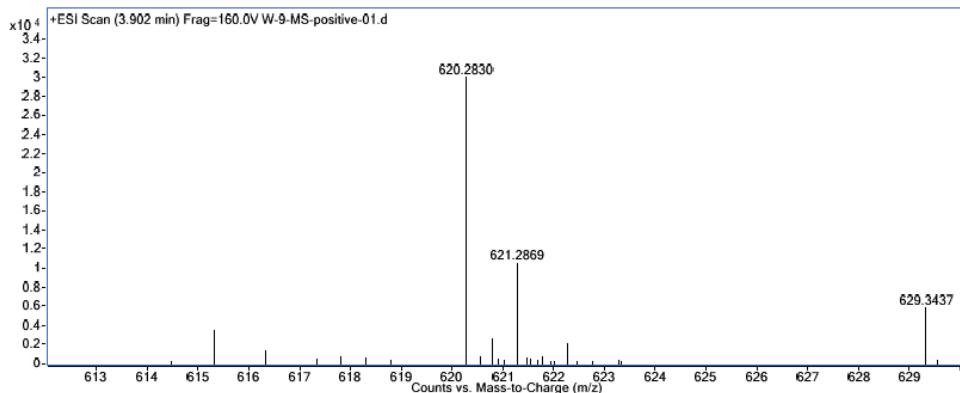
## HRMS for 8k



### Elemental Composition Calculator

Target m/z:	747.2751	Result type:	Positive ions	Species:	[M+Na] <sup>+</sup>
Elements:	C (0-80); H (0-120); O (0-30); Na (0-5) ;F(0-5)				
Ion Formula			Calculated m/z	PPM Error	
C40H43NaF3O9			747.2751	0.12	

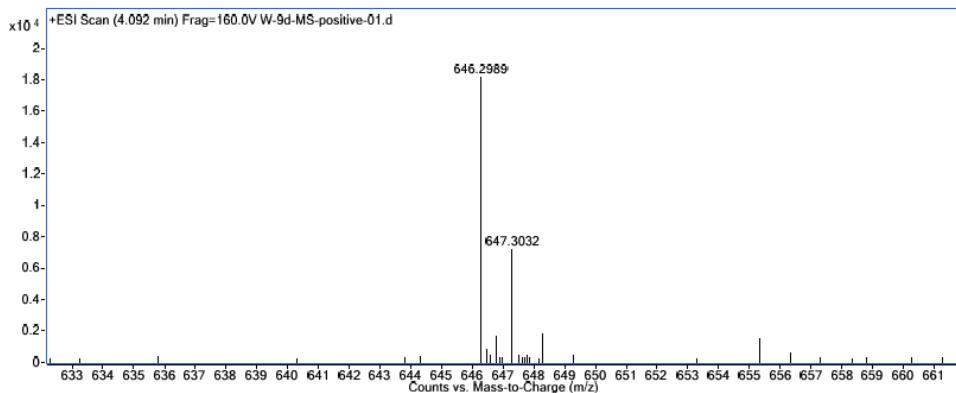
## HRMS for 9a



### Elemental Composition Calculator

Target m/z:	620.2830	Result type:	Positive ions	Species:	[M+Na] <sup>+</sup>
Elements:	C (0-80); H (0-120); O (0-30); Na (0-5); N (0-5)				
Ion Formula	Calculated m/z			PPM Error	
C <sub>33</sub> H <sub>43</sub> NNaO <sub>9</sub>	620.2830			-0.04	

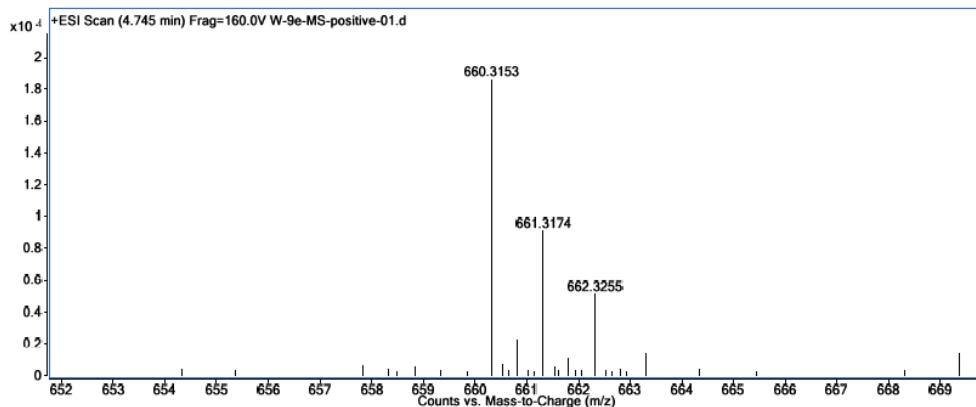
## HRMS for 9d



### Elemental Composition Calculator

Target m/z:	646.2989	Result type:	Positive ions	Species:	[M+Na] <sup>+</sup>
Elements:	C (0-80); H (0-120); O (0-30); Na (0-5); N (0-5)				
Ion Formula	Calculated m/z			PPM Error	
C <sub>35</sub> H <sub>45</sub> NNaO <sub>9</sub>	646.2987			-0.39	

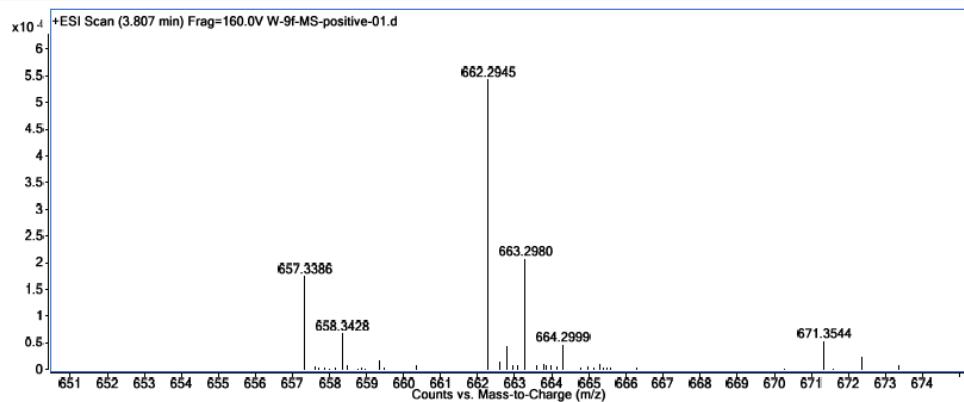
## HRMS for 9e



### Elemental Composition Calculator

Target m/z:	660.3153	Result type:	Positive ions	Species:	[M+Na] <sup>+</sup>
<b>Elements:</b>		C (0-80); H (0-120); O (0-30); Na (0-5); N (0-5)			
<b>Ion Formula</b>		<b>Calculated m/z</b>		<b>PPM Error</b>	
C <sub>36</sub> H <sub>47</sub> NNaO <sub>9</sub>		660.3143		-1.58	

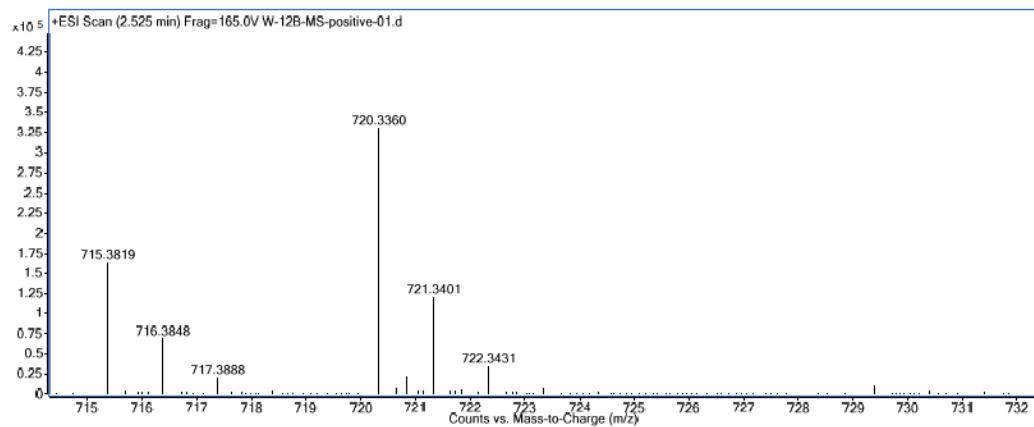
## HRMS for 9f



### Elemental Composition Calculator

Target m/z:	662.2945	Result type:	Positive ions	Species:	[M+Na] <sup>+</sup>
<b>Elements:</b>		C (0-80); H (0-120); O (0-30); Na (0-5); N (0-5)			
<b>Ion Formula</b>		<b>Calculated m/z</b>		<b>PPM Error</b>	
C <sub>35</sub> H <sub>45</sub> NNaO <sub>10</sub>		662.2936		-1.38	

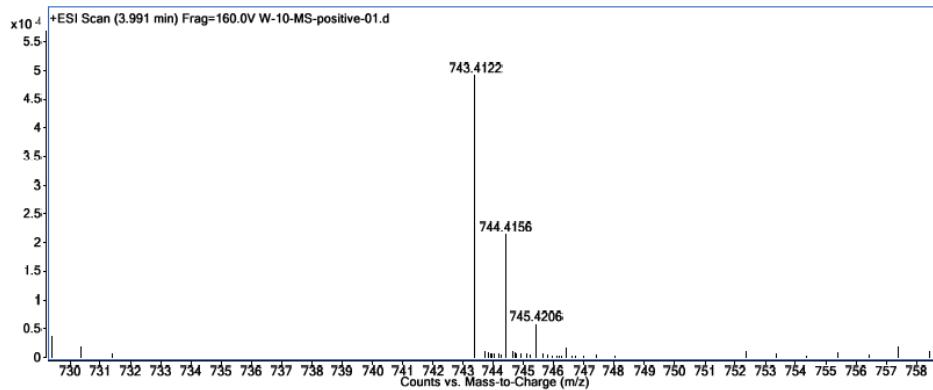
## HRMS for 10b



### Elemental Composition Calculator

Target m/z:	720.3360	Result type:	Positive ions	Species:	[M+Na] <sup>+</sup>
<b>Elements:</b>		C (0-80); H (0-120); O (0-30); N(0-5); Na (0-5)			
<b>Ion Formula</b>		<b>Calculated m/z</b>			<b>PPM Error</b>
C38H51NNaO11		720.3354			-0.85

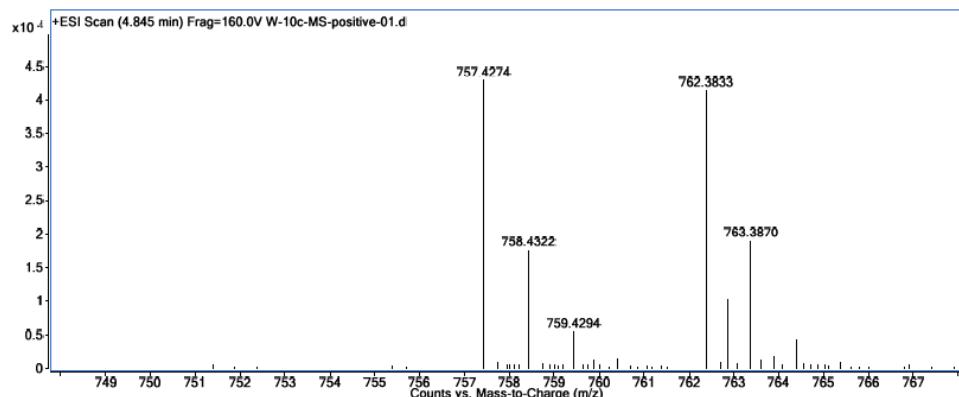
## HRMS for 10c



#### Elemental Composition Calculator

Target m/z:	743.4122	Result type:	Positive ions	Species:	[M+NH4] <sup>+</sup>	
<b>Elements:</b>		C (0-80); H (0-120); O (0-30); N(0-10)				
<b>Ion Formula</b>		Calculated m/z			PPM Error	
C40H59N2O11		743.4113			-1.11	

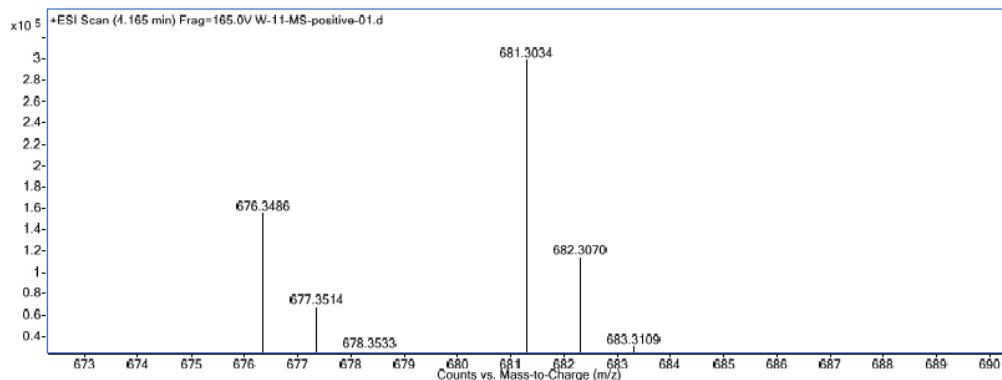
#### HRMS for 10d



#### Elemental Composition Calculator

Target m/z:	757.4274	Result type:	Positive ions	Species:	[M+NH4] <sup>+</sup>	
<b>Elements:</b>		C (0-80); H (0-120); O (0-30); N(0-10)				
<b>Ion Formula</b>		Calculated m/z			PPM Error	
C41H61N2O11		757.4270			-0.54	

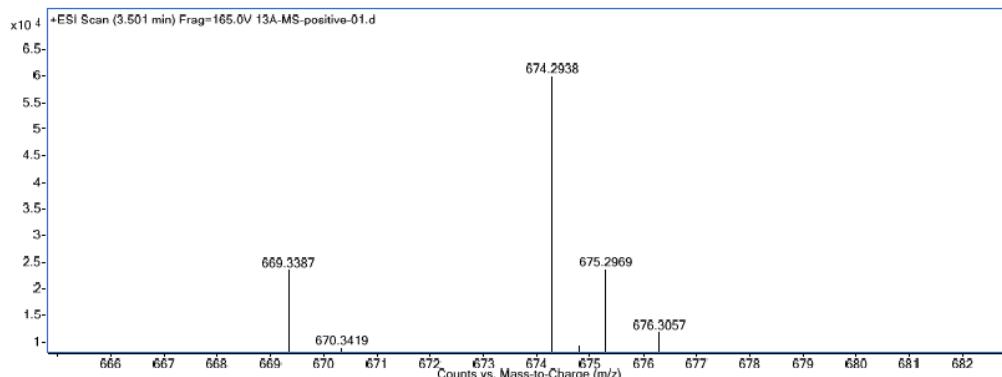
#### HRMS for 12



### Elemental Composition Calculator

Target m/z:	681.3034	Result type:	Positive ions	Species:	[M+Na] <sup>+</sup>
Elements:	C (0-80); H (0-120); O (0-30); Na (0-5) ;				
Ion Formula	Calculated m/z			PPM Error	
C39H46NaO9	681.3034			-0.02	

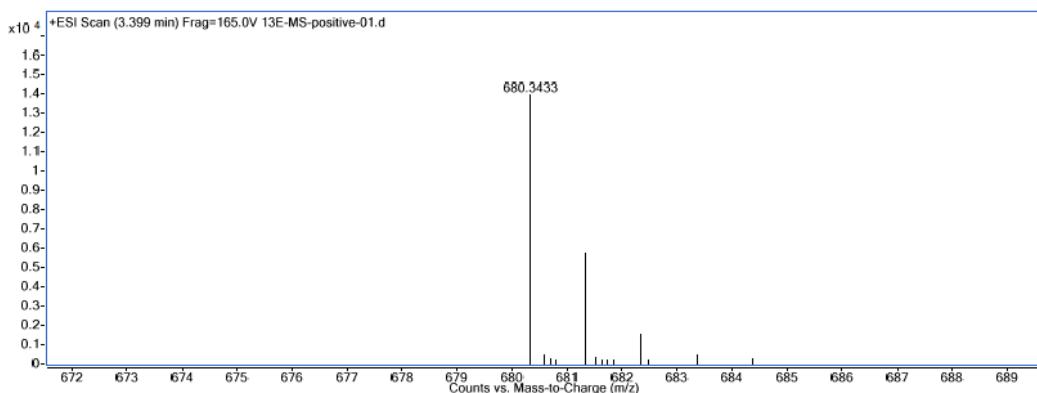
### HRMS for 13a



### Elemental Composition Calculator

Target m/z:	674.2938	Result type:	Positive ions	Species:	[M+Na] <sup>+</sup>
Elements:	C (0-80); H (0-120); O (0-30); Na (0-5) ;N(0-5)				
Ion Formula	Calculated m/z			PPM Error	
C36H45NNaO10	674.2936			-0.28	

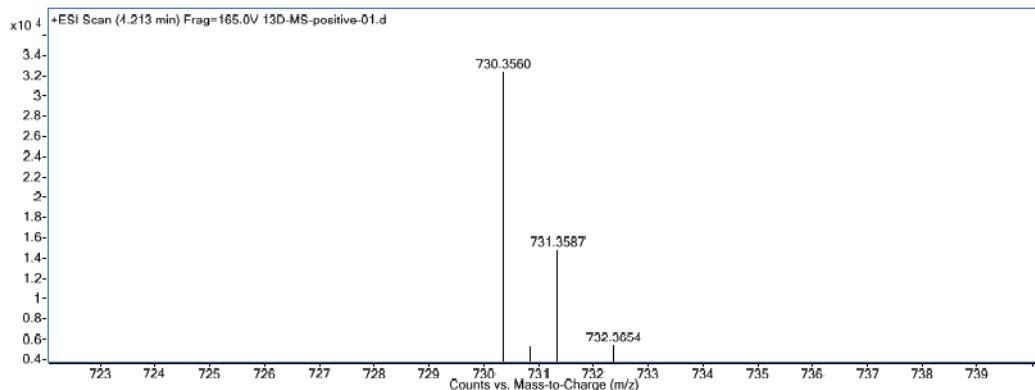
### HRMS for 13b



### Elemental Composition Calculator

Target m/z:	680.3433	Result type:	Positive ions	Species:	[M+H] <sup>+</sup>
Elements:	C (0-80); H (0-120); O (0-30); N(0-10)				
Ion Formula	Calculated m/z			PPM Error	
C38H50NO10	680.3429			-0.5	

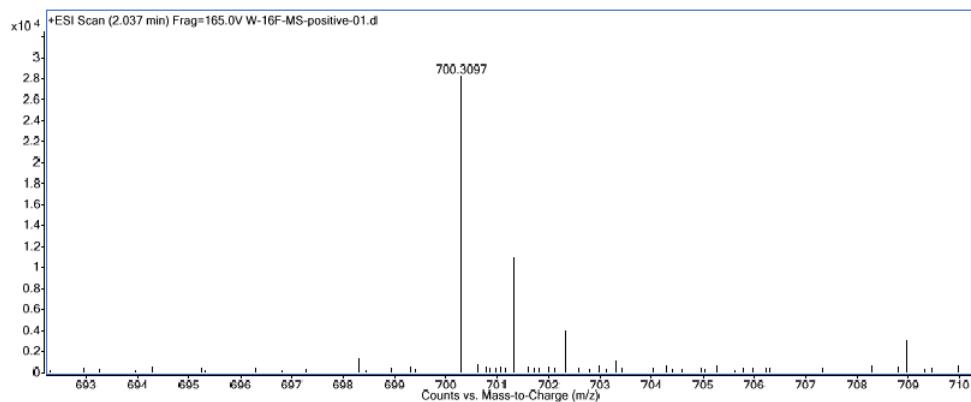
### HRMS for 13c



### Elemental Composition Calculator

Target m/z:	730.3560	Result type:	Positive ions	Species:	[M+Na] <sup>+</sup>
Elements:	C (0-80); H (0-120); O (0-30); Na (0-5) ;N(0-5)				
Ion Formula	Calculated m/z			PPM Error	
C40H53NNaO10	730.3562			0.2	

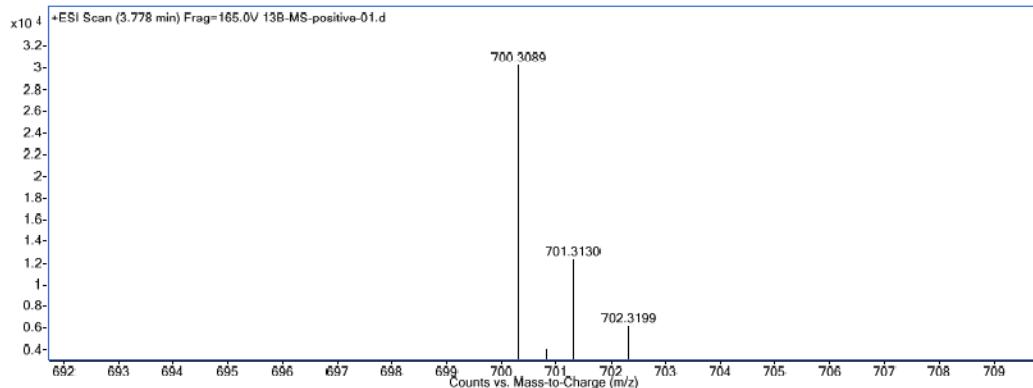
### HRMS for 13d



#### Elemental Composition Calculator

Target $m/z$ :	700.3097	Result type:	Positive ions	Species:	$[M+Na]^+$
<b>Elements:</b>		C (0-80); H (0-120); O (0-30); N(0-5);Na (0-5)			
<b>Ion Formula</b>		<b>Calculated <math>m/z</math></b>			<b>PPM Error</b>
C <sub>38</sub> H <sub>47</sub> NNaO <sub>10</sub>		700.3092			-0.68

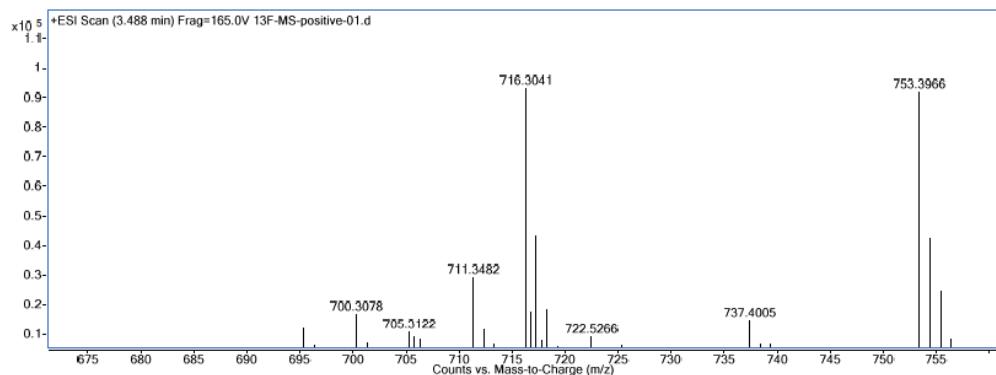
#### HRMS for 13e



#### Elemental Composition Calculator

Target $m/z$ :	700.3089	Result type:	Positive ions	Species:	$[M+Na]^+$
<b>Elements:</b>		C (0-80); H (0-120); O (0-30); Na (0-5) ;N(0-5)			
<b>Ion Formula</b>		<b>Calculated <math>m/z</math></b>			<b>PPM Error</b>
C <sub>38</sub> H <sub>47</sub> NNaO <sub>10</sub>		700.3092			0.52

#### HRMS for 13f



### Elemental Composition Calculator

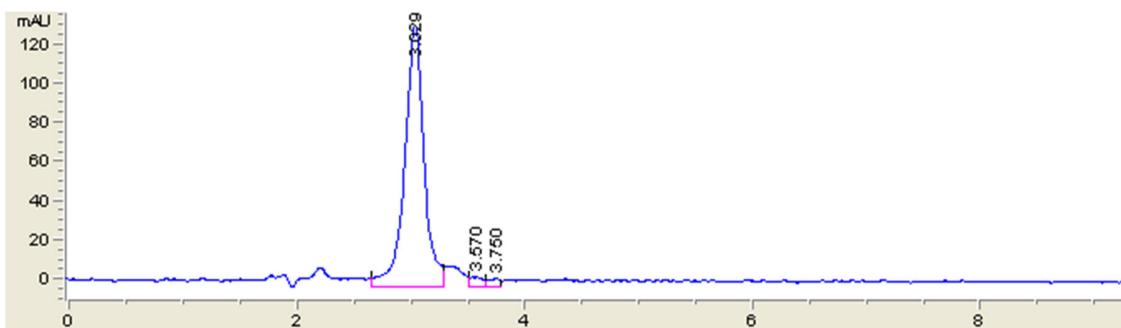
Target m/z:	711.3482	Result type:	Positive ions	Species:	$[M+NH_4]^+$
Elements:	C (0-80); H (0-120); O (0-30); N(0-10)				
Ion Formula	Calculated m/z			PPM Error	
C <sub>38</sub> H <sub>51</sub> N <sub>2</sub> O <sub>11</sub>	711.3487			0.71	

### HPLC for compound **1**

#### Analysis conditions

Sample name:	<b>1</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram



### Peak Results

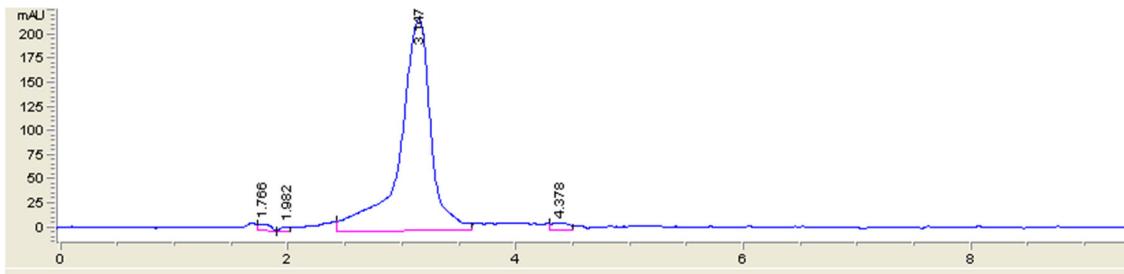
	Retention time (min)	Area	%Area	width
1	3.029	1585.5	95.777	0.1765
2	3.570	38.8	2.343	0.099
3	3.750	31.1	1.880	0.0997

### HPLC for compound **2a**

#### Analysis conditions

Sample name:	<b>2a</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Gradient Conditions	MeOH/H <sub>2</sub> O = 40/60-80/20(10min)

### Chromatogram



### Peak Results

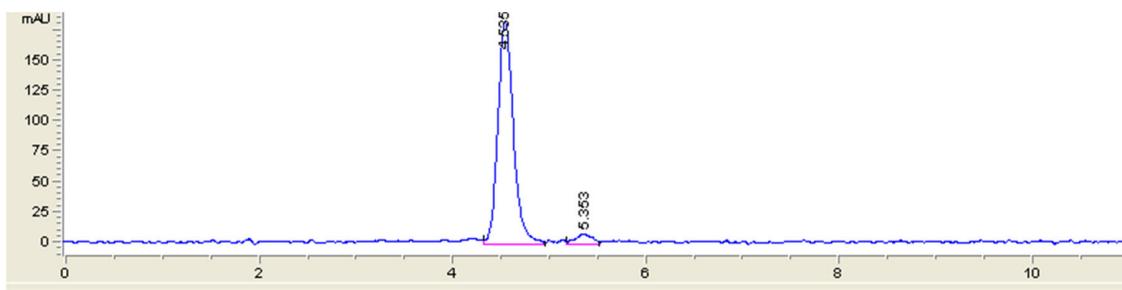
	Retention time (min)	Area	%Area	width
1	1.766	52.2	1.195	0.0904
2	1.982	28.8	0.660	0.0764
3	3.147	4197.3	96.159	0.282
4	4.378	86.7	1.987	0.1364

### HPLC for compound **2b**

Analysis conditions

Sample name:	<b>2b</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Gradient Conditions	MeOH/H <sub>2</sub> O = 40/60-80/20(10min)

### Chromatogram



### Peak Results

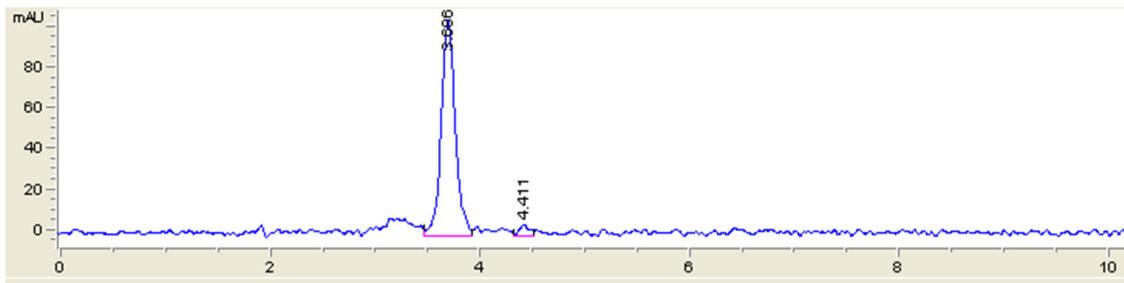
	Retention time (min)	Area	%Area	width
1	4.535	2069.7	95.153	0.1717
2	5.353	105.4	4.847	0.1574

### HPLC for compound **2c**

#### Analysis conditions

Sample name:	<b>2c</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram



### Peak Results

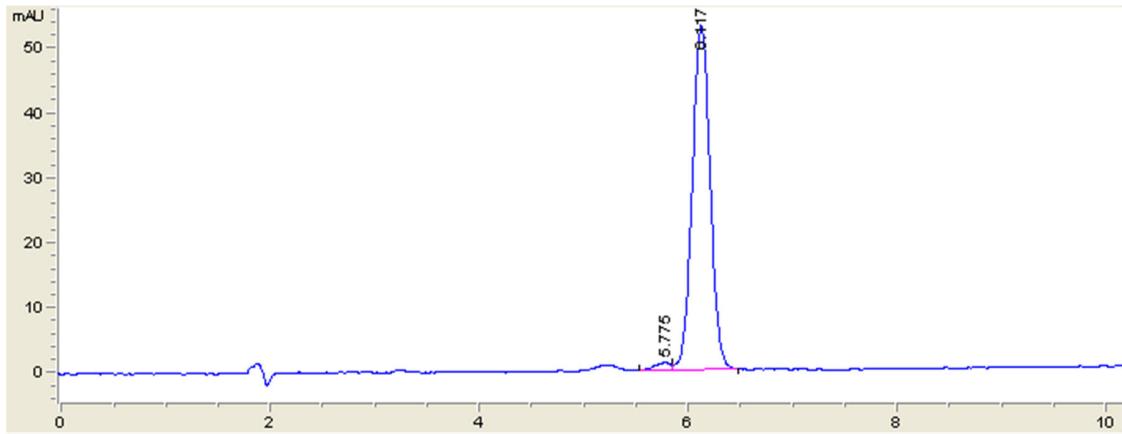
	Retention time (min)	Area	%Area	width
1	3.636	1013.1	95.779	0.1465
2	4.411	44.7	4.221	0.1003

### HPLC for compound 3

#### Analysis conditions

Sample name:	<b>3</b>
Column Details:	C-18 (4.6mm×150mm, 5µm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10µL
Gradient Conditions	MeOH/H <sub>2</sub> O = 60/40-100/0(15min)

### Chromatogram



### Peak Results

	Retention time (min)	Area	%Area	width
1	5.775	13.5	2.088	0.1423
2	6.117	633.5	97.912	0.1853

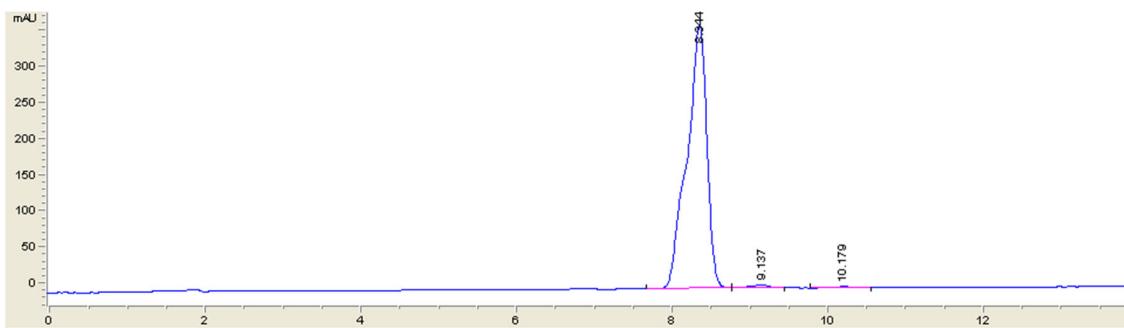
### HPLC for compound 4

#### Analysis conditions

Sample name:	4
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Gradient Conditions	MeOH/H <sub>2</sub> O = 60/40-100/0(15min)

### Chromatogram

S80



### Peak Results

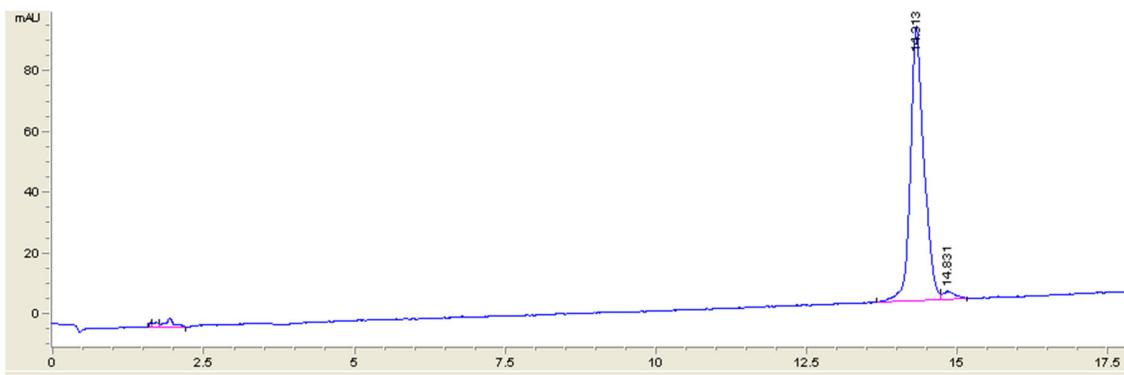
	Retention time (min)	Area	%Area	width
1	8.344	6483.5	98.202	0.2576
2	9.137	83.8	1.269	0.2492
3	10.179	34.9	0.528	0.2231

### HPLC for compound 5

#### Analysis conditions

Sample name:	<b>5</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Gradient Conditions	MeOH/H <sub>2</sub> O = 60/40-100/0(20min)

### Chromatogram



### Peak Results

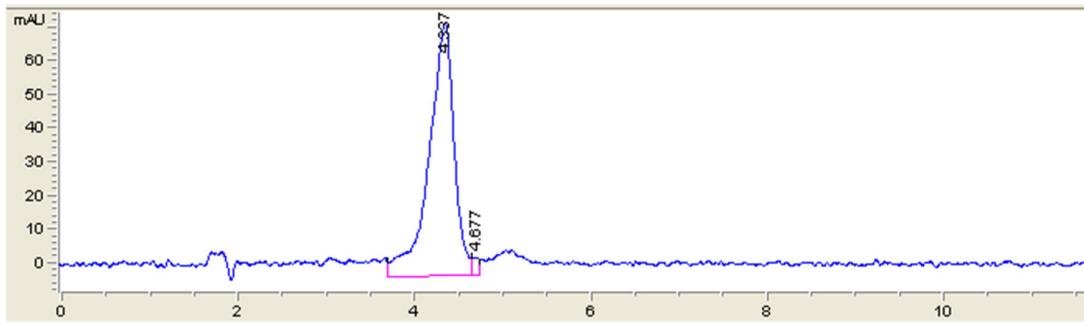
	Retention time (min)	Area	%Area	width
1	14.313	1457.9	97.522	0.2259
2	14.831	37.0	2.478	0.1692

### HPLC for compound **6**

Analysis conditions

Sample name:	<b>6</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram



### Peak Results

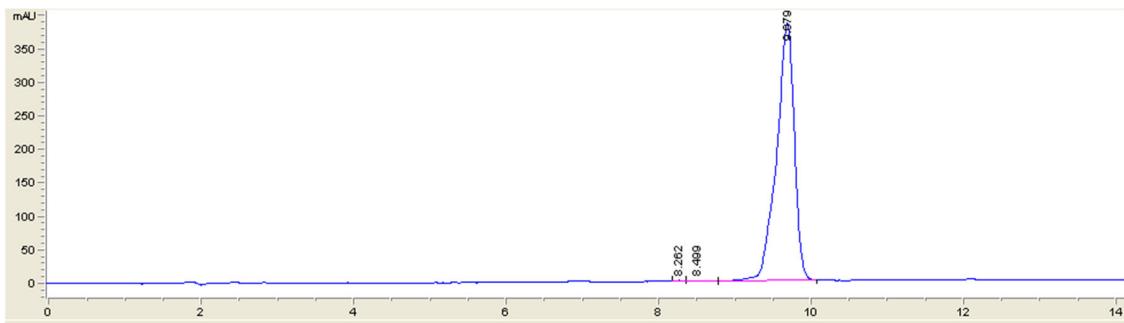
	Retention time (min)	Area	%Area	width
1	4.337	1511.9	98.197	0.2672
2	4.677	27.8	1.803	0.0666

### HPLC for compound 7a

#### Analysis conditions

Sample name:	<b>7a</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Gradient Conditions	MeOH/H <sub>2</sub> O = 60/40-100/0(15min)

### Chromatogram



### Peak Results

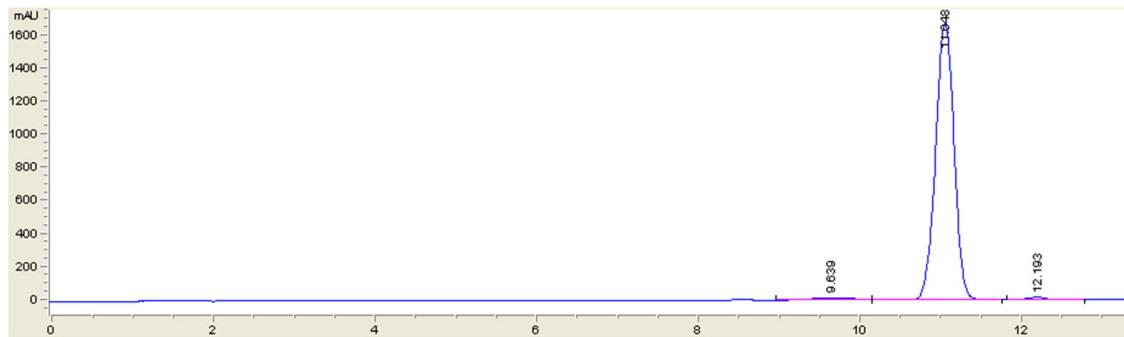
	Retention time (min)	Area	%Area	width
1	8.262	16.9	0.267	0.1228
2	8.499	25	0.394	0.1945
3	9.679	6295.8	99.339	0.2404

### HPLC for compound **7b**

Analysis conditions

Sample name:	<b>7b</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Gradient Conditions	MeOH/H <sub>2</sub> O = 60/40-100/0(15min)

### Chromatogram



### Peak Results

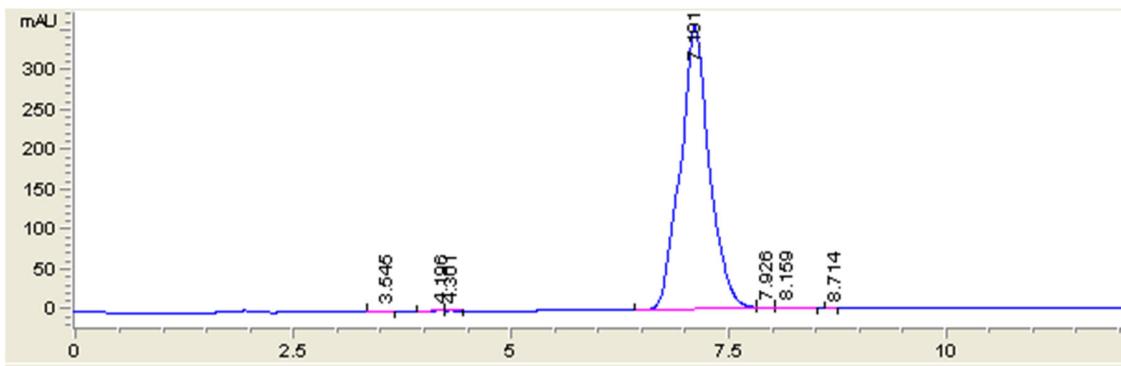
	Retention time (min)	Area	%Area	width
1	9.639	556.3	2.005	0.4354
2	11.048	26806.2	96.855	0.2529
3	12.193	316.5	1.141	0.2623

### HPLC for compound **8a**

#### Analysis conditions

Sample name:	<b>8a</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram



### Peak Results

	Retention time (min)	Area	%Area	width
1	3.545	16	0.190	0.172
2	4.196	17.2	0.204	0.162
3	4.301	11.9	0.140	0.1192
4	7.101	8310.3	98.361	0.3302
5	7.926	32.9	0.389	0.1504
6	8.159	52.3	0.619	0.2308
7	8.714	8.1	0.096	0.1003

### HPLC for compound **8b**

#### Analysis conditions

Sample name:	<b>8b</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram



### Peak Results

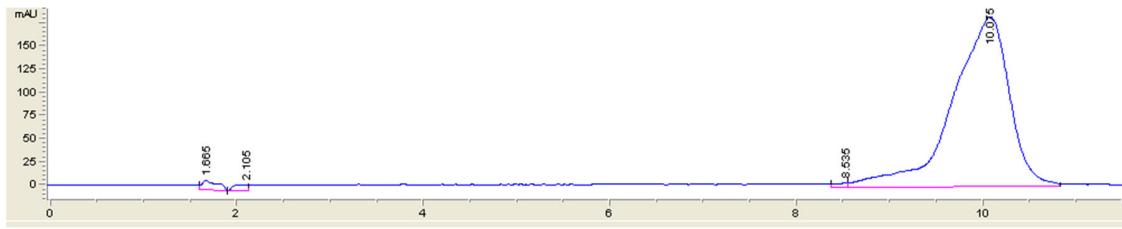
	Retention time (min)	Area	%Area	width
1	7.645	6085	95.252	0.4964
2	9.047	26.5	0.414	0.0563
3	9.17	52.7	0.824	0.1114
4	9.412	224.2	3.510	0.3213

### HPLC for compound **8c**

#### Analysis conditions

Sample name:	<b>8c</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram



### Peak Results

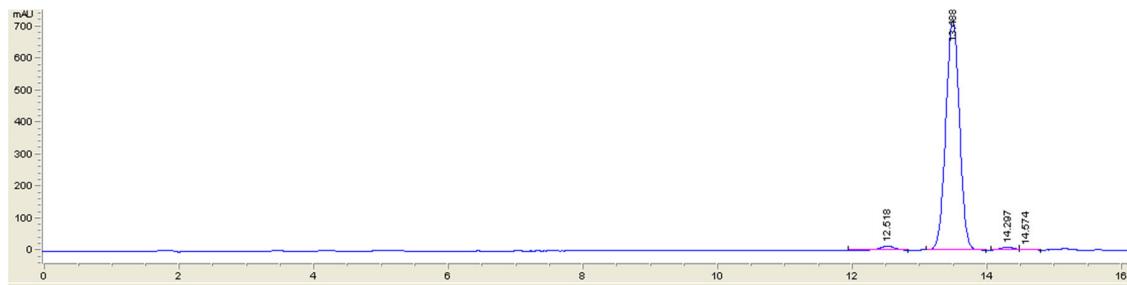
	Retention time (min)	Area	%Area	width
1	1.665	125.9	1.565	0.1466
2	2.105	70.3	0.874	0.1303
3	8.535	48.4	0.602	0.1189
4	10.075	7798.8	96.960	0.5758

### HPLC for compound **8d**

#### Analysis conditions

Sample name:	<b>8d</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Gradient Conditions	MeOH/H <sub>2</sub> O = 60/40-100/0(15min)

### Chromatogram



### Peak Results

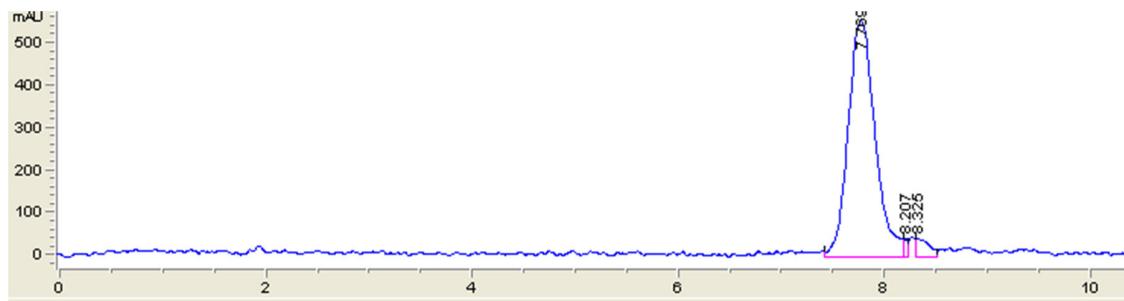
	Retention time (min)	Area	%Area	width
1	12.518	225	2.112	0.2581
2	13.488	10239.8	96.127	0.2262
3	14.297	137.4	1.290	0.2094
4	14.574	50.1	0.471	0.1726

### HPLC for compound **8e**

#### Analysis conditions

Sample name:	<b>8e</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram



### Peak Results

	Retention time (min)	Area	%Area	width
1	7.769	10205.3	95.427	0.2253
2	8.207	84.6	0.791	0.0292
3	8.325	404.5	3.782	0.1564

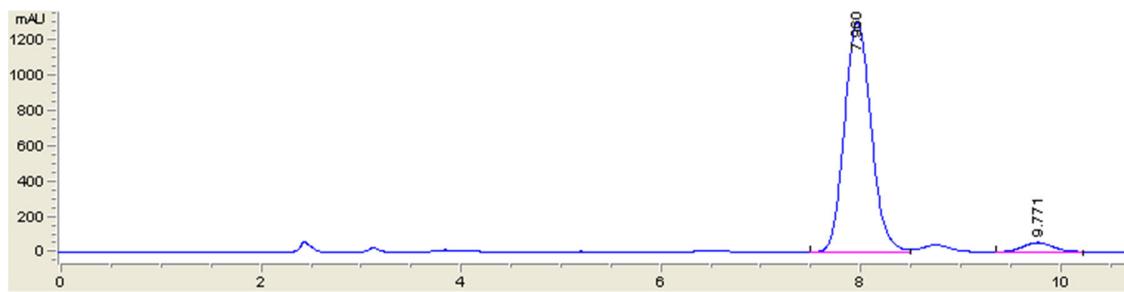
### HPLC for compound **8f**

#### Analysis conditions

Sample name:	<b>8f</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram

S90



### Peak Results

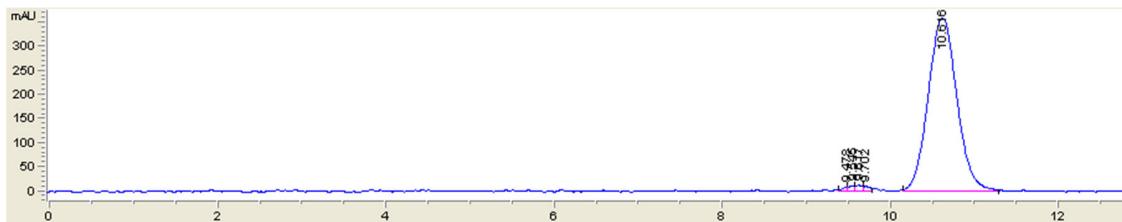
	Retention time (min)	Area	%Area	width
1	7.960	24298.4	95.081	0.2940
2	9.771	1257.2	4.919	0.2895

### HPLC for compound **8g**

Analysis conditions

Sample name:	<b>8g</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram



### Peak Results

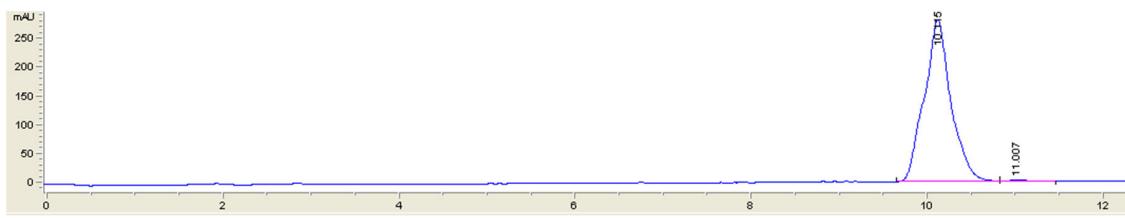
	Retention time (min)	Area	%Area	width
1	9.478	54.3	0.609	0.0645
2	9.545	59.7	0.670	0.0608
3	9.617	78.1	0.877	0.083
4	9.702	64.7	0.726	0.0766
5	10.616	8655.4	97.118	0.3497

### HPLC for compound **8h**

Analysis conditions

Sample name:	<b>8h</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram



### Peak Results

	Retention time (min)	Area	%Area	width
1	10.115	5922.1	99.385	0.2937
2	11.007	36.6	0.615	0.2339

### HPLC for compound **8i**

#### Analysis conditions

Sample name:	<b>8i</b>
Column Details:	C-18 (4.6mm×150mm, 5µm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10µL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram



### Peak Results

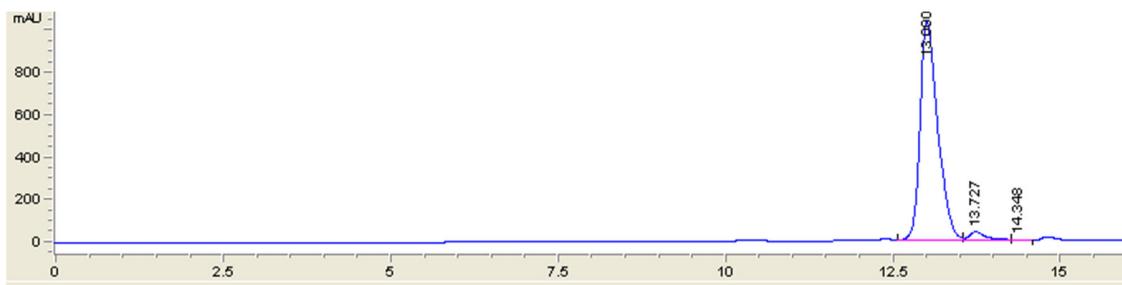
	Retention time (min)	Area	%Area	width
1	13.904	1157.1	97.711	0.2195
2	14.381	9.4	0.790	0.1102
3	14.813	17.7	1.498	0.1664

### HPLC for compound **8j**

#### Analysis conditions

Sample name:	<b>8j</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram



### Peak Results

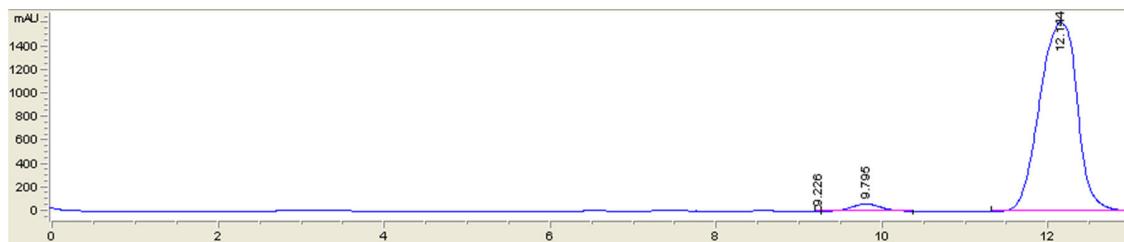
	Retention time (min)	Area	%Area	width
1	13.000	19231.4	95.359	0.2784
2	13.727	868.8	4.308	0.2769
3	14.348	67.2	0.333	0.1674

### HPLC for compound **8k**

#### Analysis conditions

Sample name:	<b>8k</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram



### Peak Results

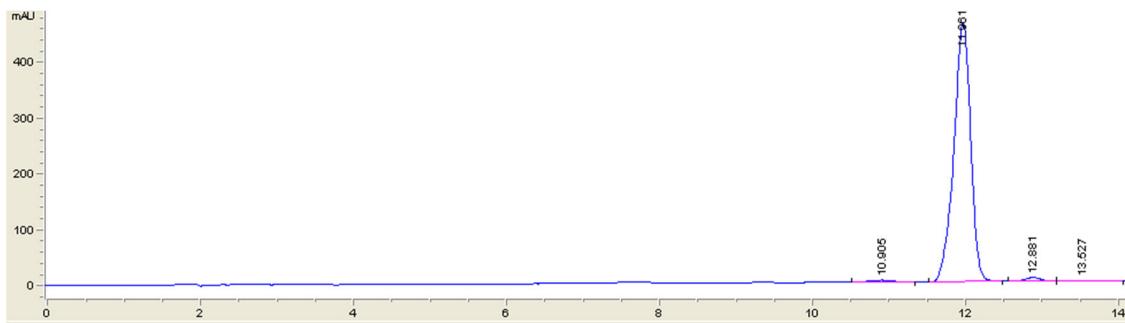
	Retention time (min)	Area	%Area	width
1	9.226	9.7	0.019	0.0596
2	9.795	1607.7	3.088	0.3188
3	12.144	50446	96.893	0.3702

### HPLC for compound **8l**

#### Analysis conditions

Sample name:	<b>8l</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Gradient Conditions	MeOH/H <sub>2</sub> O = 60/40-100/0(15min)

### Chromatogram



### Peak Results

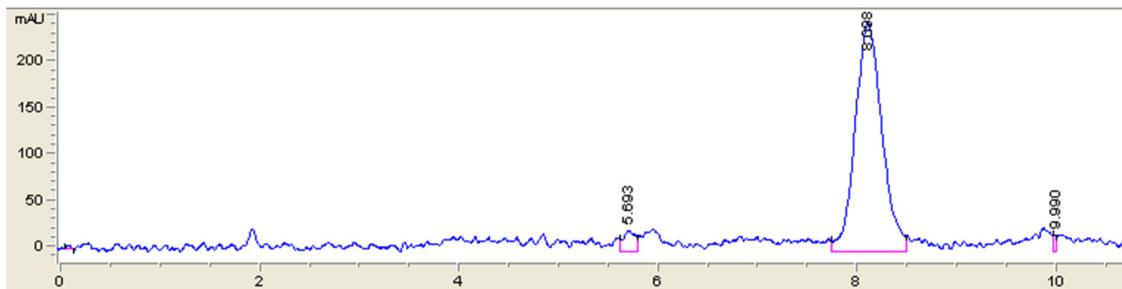
	Retention time (min)	Area	%Area	width
1	10.905	72.1	0.969	0.2565
2	11.961	7130.1	95.772	0.2355
3	12.881	123	1.652	0.2376
4	13.527	49.8	0.669	0.2976

HPLC for compound **9a**

Analysis conditions

Sample name:	<b>9a</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Gradient Conditions	MeOH/H <sub>2</sub> O = 60/40-100/0(15min)

### Chromatogram



### Peak Results

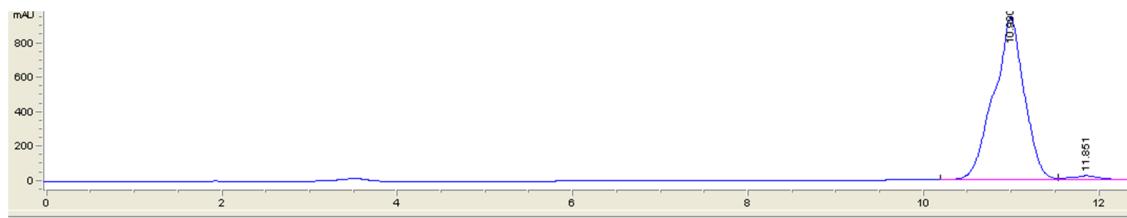
	Retention time (min)	Area	%Area	width
1	5.693	189.3	3.885	0.1039
2	8.098	4642.3	95.262	0.2350
3	9.990	28.4	0.583	0.0261

### HPLC for compound **9b**

#### Analysis conditions

Sample name:	<b>9b</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Gradient Conditions	MeOH/H <sub>2</sub> O = 60/40-100/0(15min)

### Chromatogram



### Peak Results

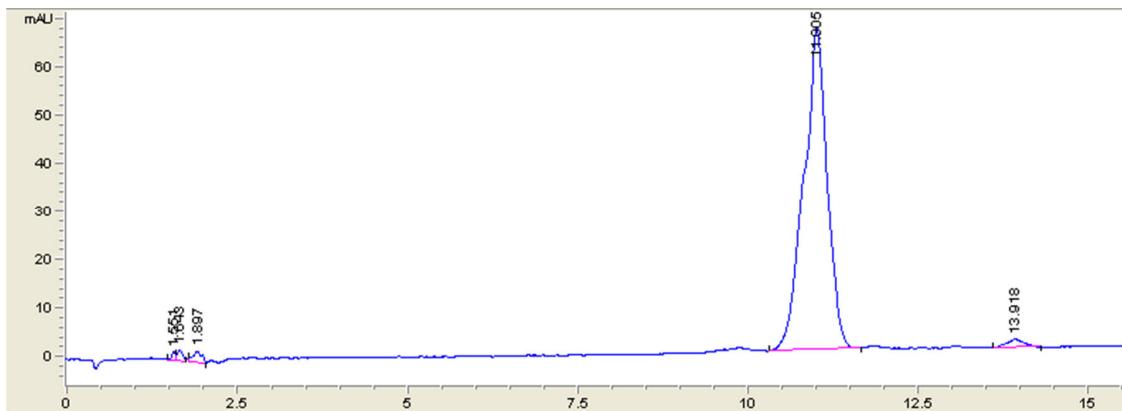
	Retention time (min)	Area	%Area	width
1	10.990	23105.7	97.090	0.3347
2	11.851	692.6	2.910	0.3494

### HPLC for compound **9c**

Analysis conditions

Sample name:	<b>9c</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Gradient Conditions	MeOH/H <sub>2</sub> O = 60/40-100/0(15min)

### Chromatogram



### Peak Results

	Retention time (min)	Area	%Area	width
1	1.551	7.9	0.477	0.0624
2	1.643	12.8	0.773	0.0768
3	1.897	21.4	1.289	0.1296
4	11.005	1587.6	95.662	0.3288
5	13.918	29.9	1.799	0.2177

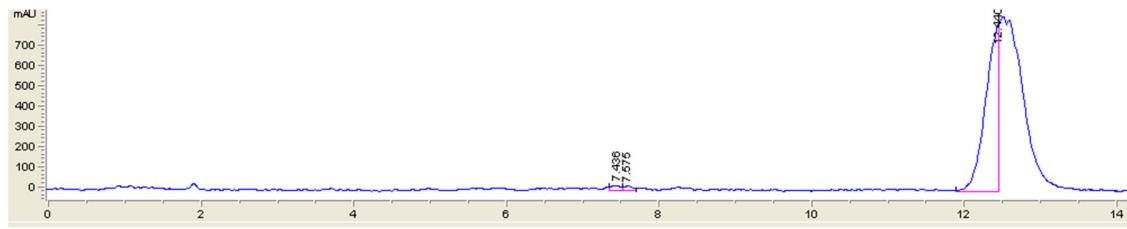
### HPLC for compound **9d**

Analysis conditions

Sample name:	<b>9d</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Gradient Conditions	MeOH/H <sub>2</sub> O = 60/40-100/0(15min)

### Chromatogram

S100



### Peak Results

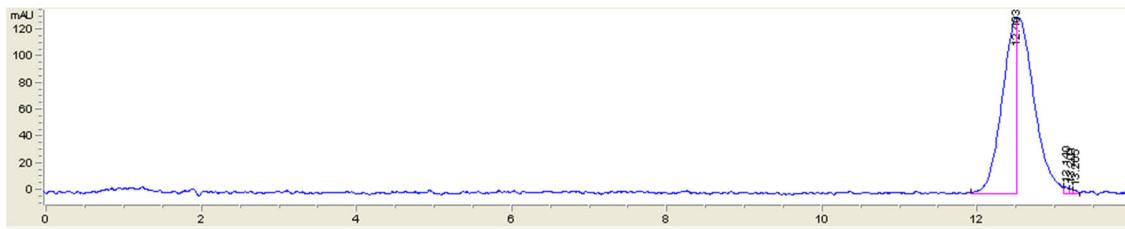
	Retention time (min)	Area	%Area	width
1	7.436	276.3	2.670	0.1246
2	7.575	215.9	2.086	0.1054
3	12.440	9857.8	95.244	0.1508

### HPLC for compound **9e**

#### Analysis conditions

Sample name:	<b>9e</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Gradient Conditions	MeOH/H <sub>2</sub> O = 60/40-100/0(15min)

### Chromatogram



### Peak Results

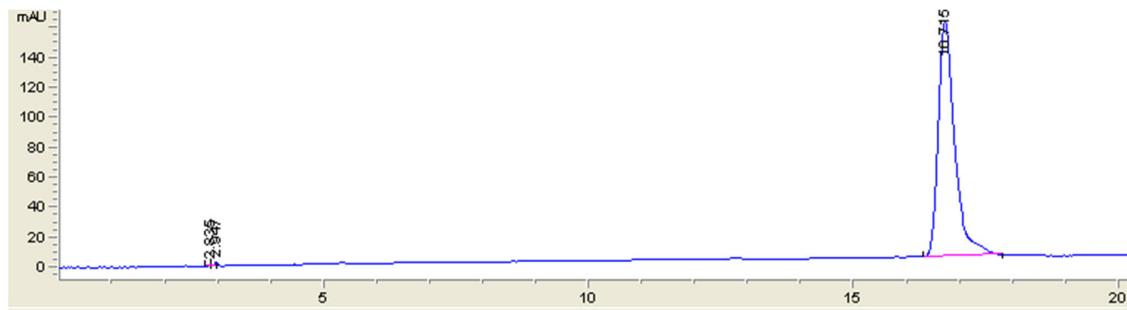
	Retention time (min)	Area	%Area	width
1	12.493	1777.6	97.483	0.1708
2	13.14	21.9	1.202	0.0572
3	13.201	11.7	0.644	0.043
4	13.265	12.2	0.671	0.0559

HPLC for compound **9f**

Analysis conditions

Sample name:	<b>9f</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Gradient Conditions	MeOH/H <sub>2</sub> O = 60/40-100/0(20min)

### Chromatogram



### Peak Results

	Retention time (min)	Area	%Area	width
1	2.835	5	0.152	0.0643
2	2.974	11.7	0.351	0.0664
3	16.715	3300.9	99.497	0.3115

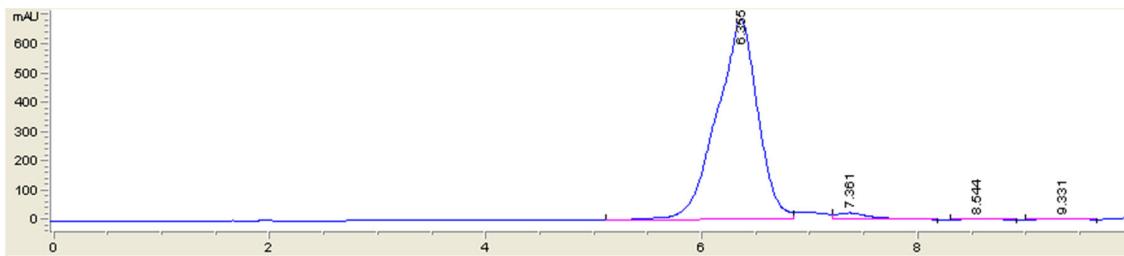
### HPLC for compound **10a**

Analysis conditions

Sample name:	<b>10a</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram

S103



### Peak Results

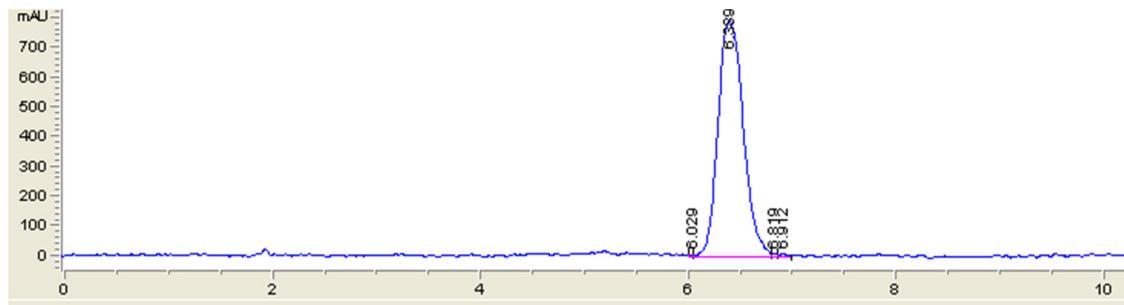
	Retention time (min)	Area	%Area	width
1	6.355	18279.7	96.628	0.3659
2	7.361	556.3	2.940	0.3227
3	8.544	37	0.196	0.2676
4	9.331	44.7	0.236	0.2323

### HPLC for compound **10b**

Analysis conditions

Sample name:	<b>10b</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram



### Peak Results

	Retention time (min)	Area	%Area	width
1	6.029	26.5	0.192	0.0386
2	6.389	13650.9	98.880	0.2251
3	6.819	42.9	0.311	0.0388
4	6.912	85.3	0.618	0.0804

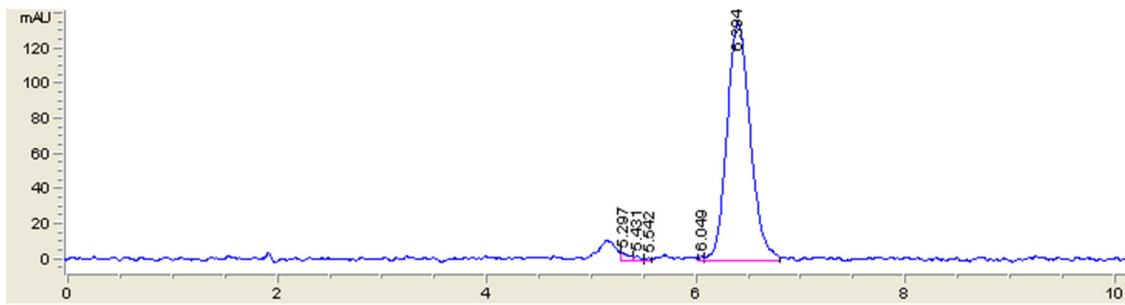
### HPLC for compound **10c**

#### Analysis conditions

Sample name:	<b>10c</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram

S105



### Peak Results

	Retention time (min)	Area	%Area	width
1	5.297	29.9	1.364	0.0767
2	5.431	13	0.592	0.0541
3	5.542	8.5	0.388	0.0496
4	6.049	10.4	0.474	0.0471
5	6.394	2129	97.182	0.2337

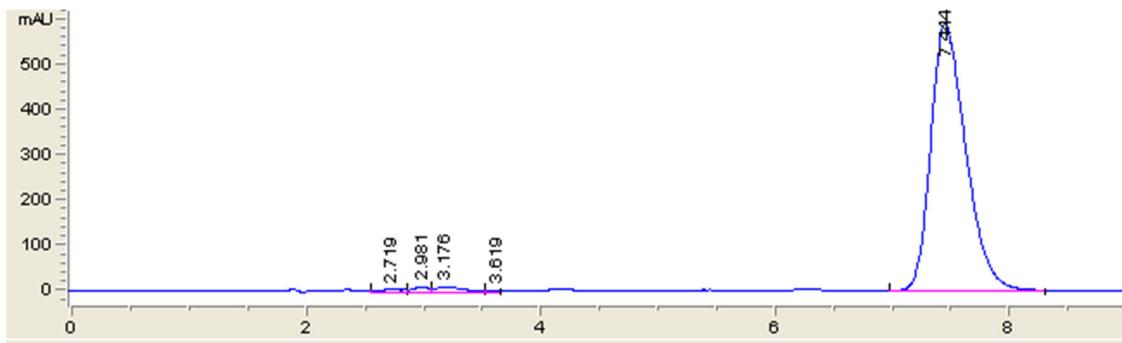
### HPLC for compound **10d**

Analysis conditions

Sample name:	<b>10d</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram

S106



### Peak Results

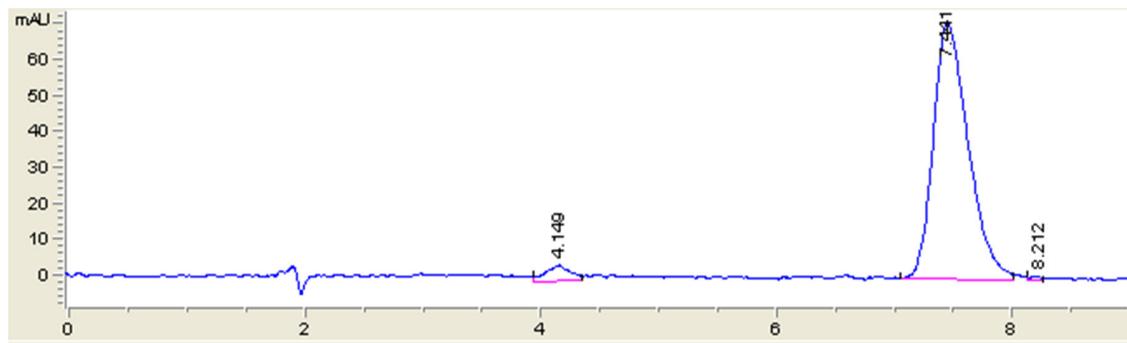
	Retention time (min)	Area	%Area	width
1	2.719	115.8	0.887	0.2014
2	2.981	131.6	1.007	0.1372
3	3.176	231.8	1.775	0.2264
4	3.619	39	0.299	0.0893
5	7.444	12543.3	96.032	0.3182

### HPLC for compound **10e**

Analysis conditions

Sample name:	<b>10e</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram



### Peak Results

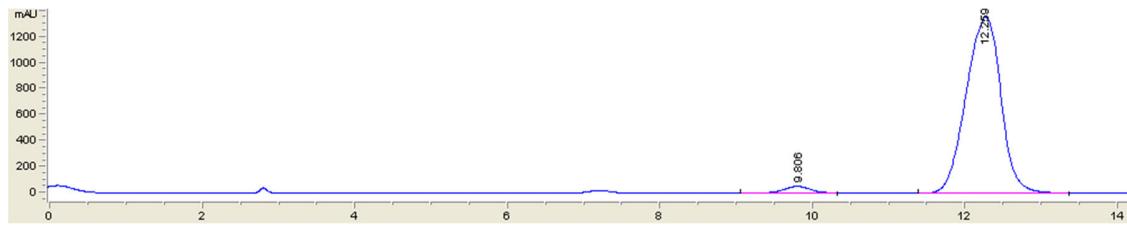
	Retention time (min)	Area	%Area	width
1	4.149	69.4	4.435	0.1884
2	7.441	1487.9	95.125	0.3050
3	8.212	6.9	0.440	0.0818

### HPLC for compound **10f**

#### Analysis conditions

Sample name:	<b>10f</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram



### Peak Results

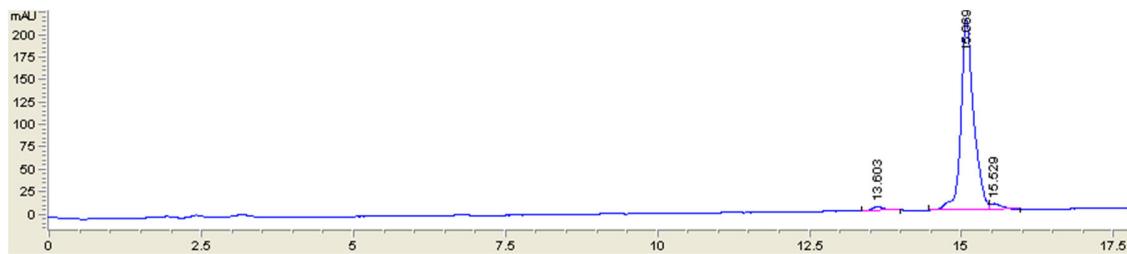
	Retention time (min)	Area	%Area	width
1	9.806	1364.5	3.081	0.3140
2	12.259	42930.5	96.919	0.3814

### HPLC for compound **11**

Analysis conditions

Sample name:	<b>11</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Gradient Conditions	MeOH/H <sub>2</sub> O = 60/40-100/0(20min)

### Chromatogram



### Peak Results

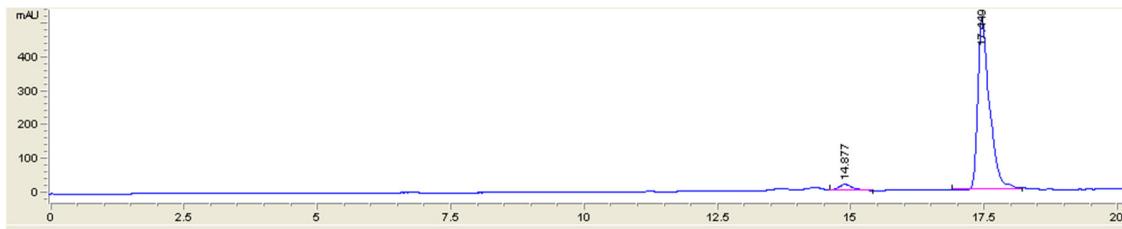
	Retention time (min)	Area	%Area	width
1	13.603	68.9	2.106	0.1984
2	15.069	3115.7	95.165	0.2152
3	15.529	89.3	2.729	0.1833

### HPLC for compound **12**

#### Analysis conditions

Sample name:	<b>12</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Gradient Conditions	MeOH/H <sub>2</sub> O = 60/40-100/0(20min)

### Chromatogram



### Peak Results

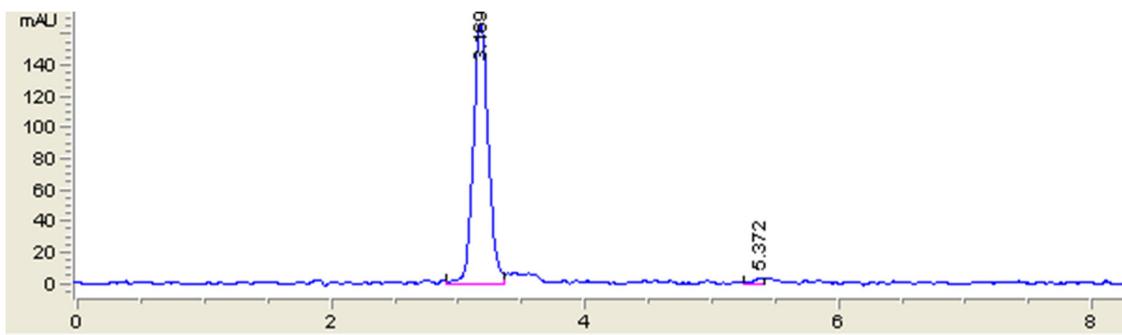
	Retention time (min)	Area	%Area	width
1	14.877	350.7	4.331	0.2629
2	17.449	7746.6	95.669	0.2205

### HPLC for compound **13a**

Analysis conditions

Sample name:	<b>13a</b>
Column Details:	C-18 (4.6mm×150mm, 5µm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10µL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram



### Peak Results

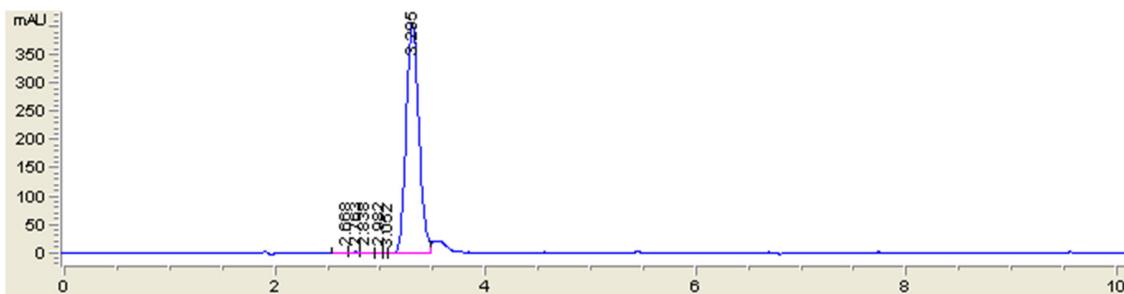
	Retention time (min)	Area	%Area	width
1	3.169	1425.7	2.343	0.1289
2	5.372	34.5	97.657	0.0940

### HPLC for compound **13b**

Analysis conditions

Sample name:	<b>13b</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram



### Peak Results

	Retention time (min)	Area	%Area	width
1	2.668	32.7	0.914	0.1043
2	2.763	24.5	0.685	0.0693
3	2.838	26.1	0.728	0.0943
4	2.982	12.4	0.346	0.0610
5	3.052	6.7	0.188	0.0393
6	3.295	3479.0	97.139	0.1309

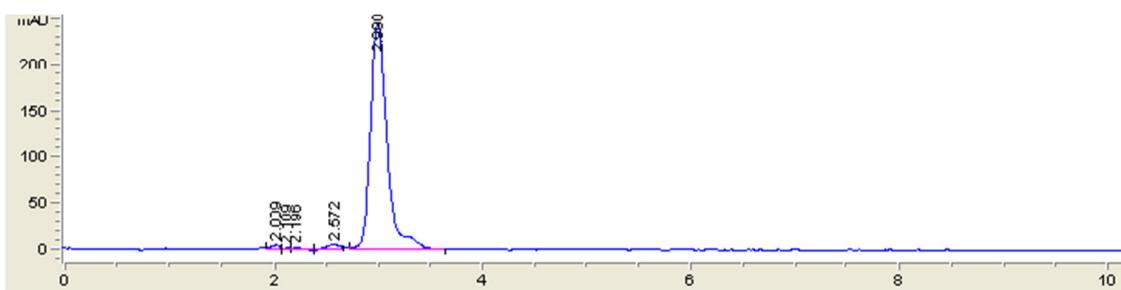
### HPLC for compound **13c**

#### Analysis conditions

Sample name:	<b>13c</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram

S113



### Peak Results

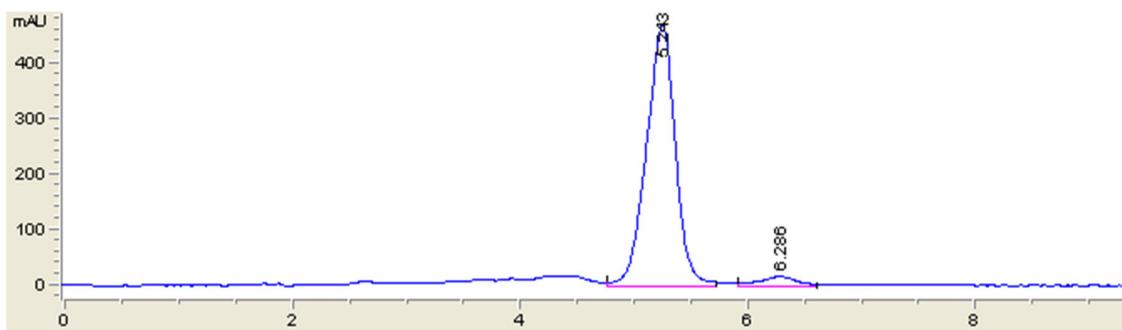
	Retention time (min)	Area	%Area	width
1	2.009	35.3	1.226	0.0937
2	2.109	9.4	0.325	0.0552
3	2.100	10.0	0.052	0.1000
4	2.572	62.7	2.174	0.1395
5	2.990	2755.9	95.623	0.1716

### HPLC for compound **13d**

#### Analysis conditions

Sample name:	<b>13d</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram



### Peak Results

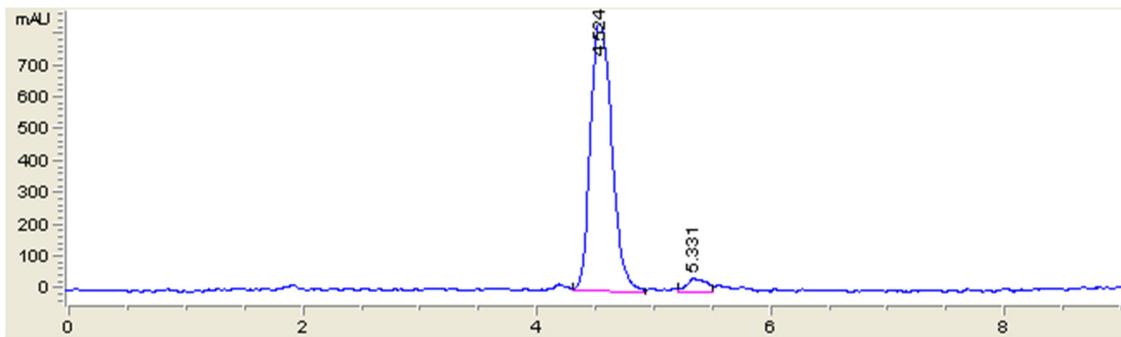
	Retention time (min)	Area	%Area	width
1	5.243	8372	95.214	0.2636
2	6.286	420.8	4.786	0.2927

### HPLC for compound **13e**

Analysis conditions

Sample name:	<b>13e</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram



### Peak Results

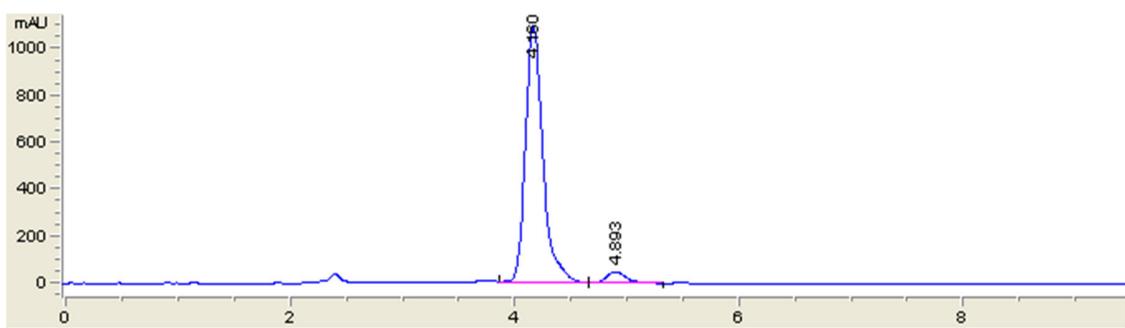
	Retention time (min)	Area	%Area	width
1	4.524	10863.7	95.489	0.2042
2	5.331	513.2	4.511	0.1536

### HPLC for compound **13f**

Analysis conditions

Sample name:	<b>13f</b>
Column Details:	C-18 (4.6mm×150mm, 5μm)
Column Temperature:	30 °C
Flow Rate:	1mL/min
Detector Wavelength:	254 nm
Injection volume:	10μL
Isocratic Conditions	MeOH/H <sub>2</sub> O = 75/25

### Chromatogram



### Peak Results

	Retention time (min)	Area	%Area	width
1	4.160	12195.8	95.835	0.1718
2	4.893	530.2	4.165	0.2099