Cytotoxic Diterpenoids from *Sapium insigne*

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S1. SRB assay for A-549 cell Line.

Inhibition on the A-549 cell growth of the tested compounds was measured using the SRB (sulforhodamine B) assay. Briefly, optimal amount of A-549 cells in 90 μL of culture medium were seeded in triplicate into 96-well plates (Falcon, CA) and allowed 24 h to adhere (culture medium only wells as blank). The cells were then treated with 10 μL of grade concentrations of tested compounds for 72 h at 37°C in 5% CO_2 in culture incubator. The medium was then removed and the cells adhered to the plate were then fixed with 10% trichloroacetic acid in 4 °C for 1 h and washed for 5 times before stained with 4 mg/mL sulforhodamine B (Sigma) in 1% acetum for 15 min. After 5 washings using 1% acetum and dried in air, sulforhodamine B was dissolved in 150 μL buffer containing 10 mM Tris-base. The OD values were measured at 560 nm using a multi-well spectrophotometer (VERSAmax, Molecular Devices, Sunnyvale, CA). Average values determined from triplicate readings were used for the inhibitory rate calculation by the formula: $(OD_{\text{control well}} - OD_{\text{treated well}})/OD_{\text{control well}}\text{-}OD_{\text{blank well}} \times 100\%$. The IC_{50} was calculated using Logistic regression from three independent tests.

S2. MTT assay for HL-60 cell Line.

The growth inhibitory effect of tested compounds on the HL-60 cell line was evaluated by MTT assay (microculture tetrazolium 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Briefly, optimal amount of cells in 90 μL of culture medium were seeded in triplicate into 96-well plates (Falcon, CA) and cultured for 24 h (culture medium only wells as blank), followed by the addition of 10 μL of grade concentrations of tested compounds. The plates were then incubated for 72 h at 37°C in 5% CO_2, and a 20 μL aliquot of MTT solution (5 mg/mL in saline solution) was subsequently added to all the appropriate wells. After another 4 h incubation, 100 μL of "triplex solution" (10% SDS/5% isobutanol/10 mM HCl) was added, and the plates were incubated at 37°C in 5% CO_2 overnight. The OD values were measured by a plate reader at 570 nm (VERSA Max, Molecular Devices). Average values determined from triplicate readings were used for the inhibitory rate calculation by the formula: $(OD_{\text{control well}} - OD_{\text{treated well}})/OD_{\text{control well}}\text{-}OD_{\text{blank well}} \times 100\%$. The IC_{50} was calculated using Logistic regression from three independent tests.

S3. C_{18} HPLC-DAD (254 nm) chromatograms of compounds **1** and **2**, and their co-injection (1.0 mL/min, 50–80% MeCN/H_2O over 15 min)

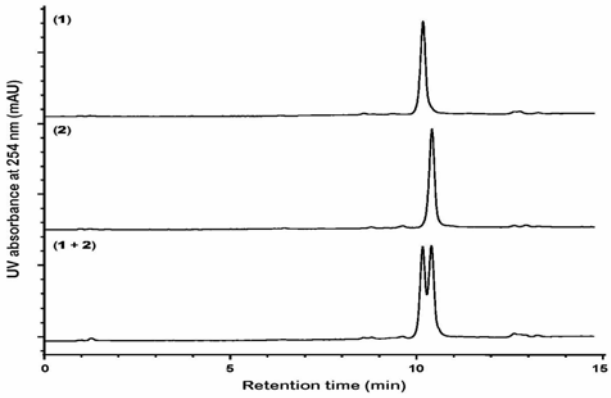


Figure S1. ^{1}H NMR spectrum of **1** in $CDCl_3$

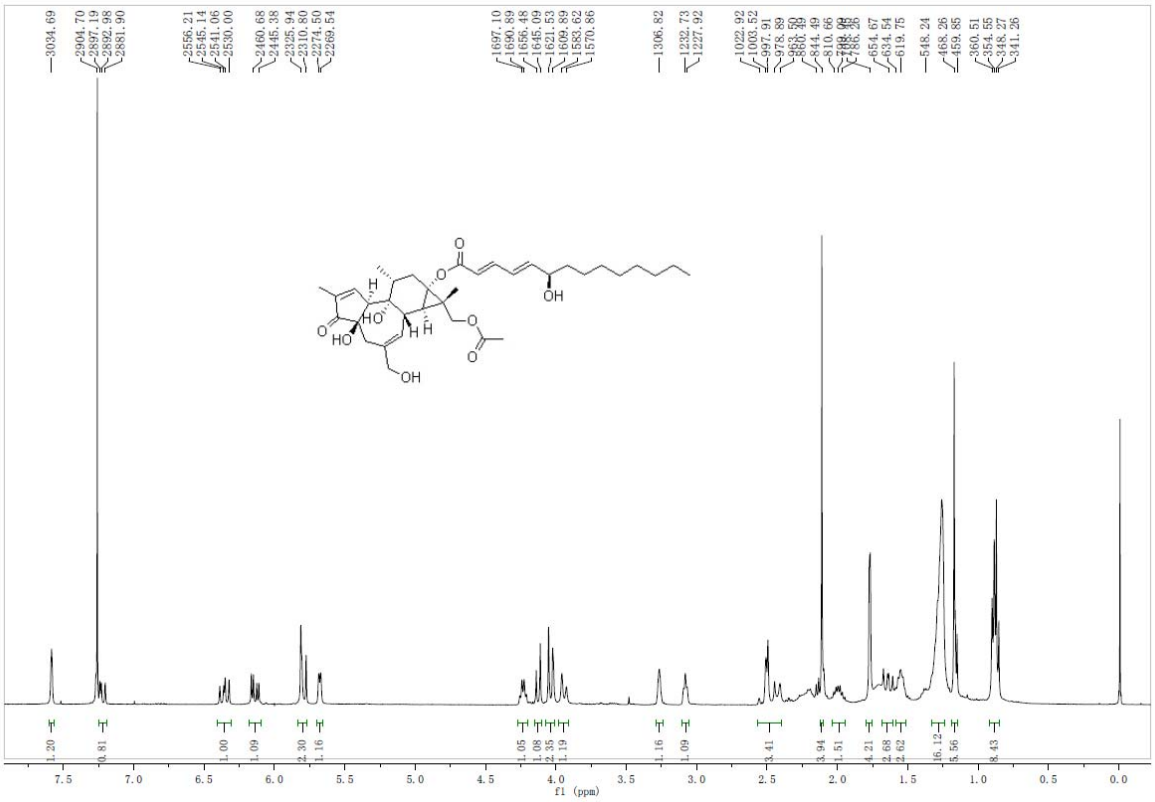


Figure S2. ^{13}C NMR spectrum of **1** in $CDCl_3$

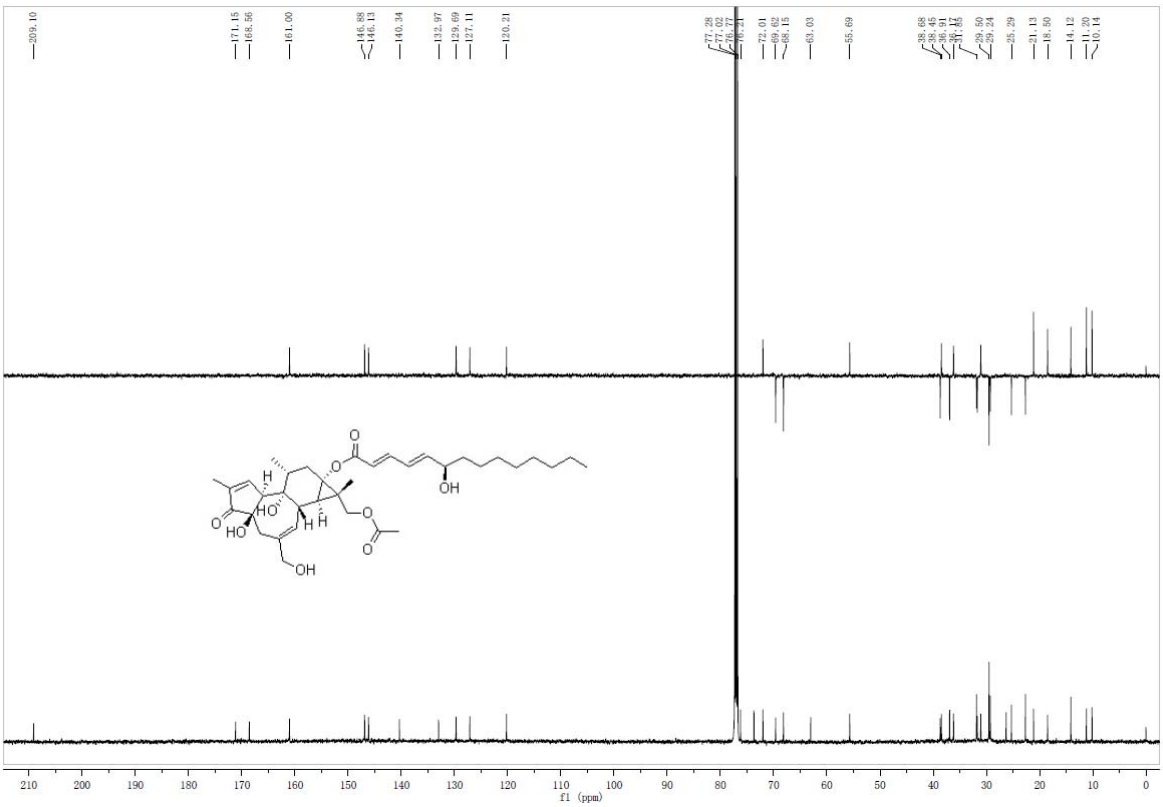


Figure S3. HSQC spectrum of **1** in $CDCl_3$

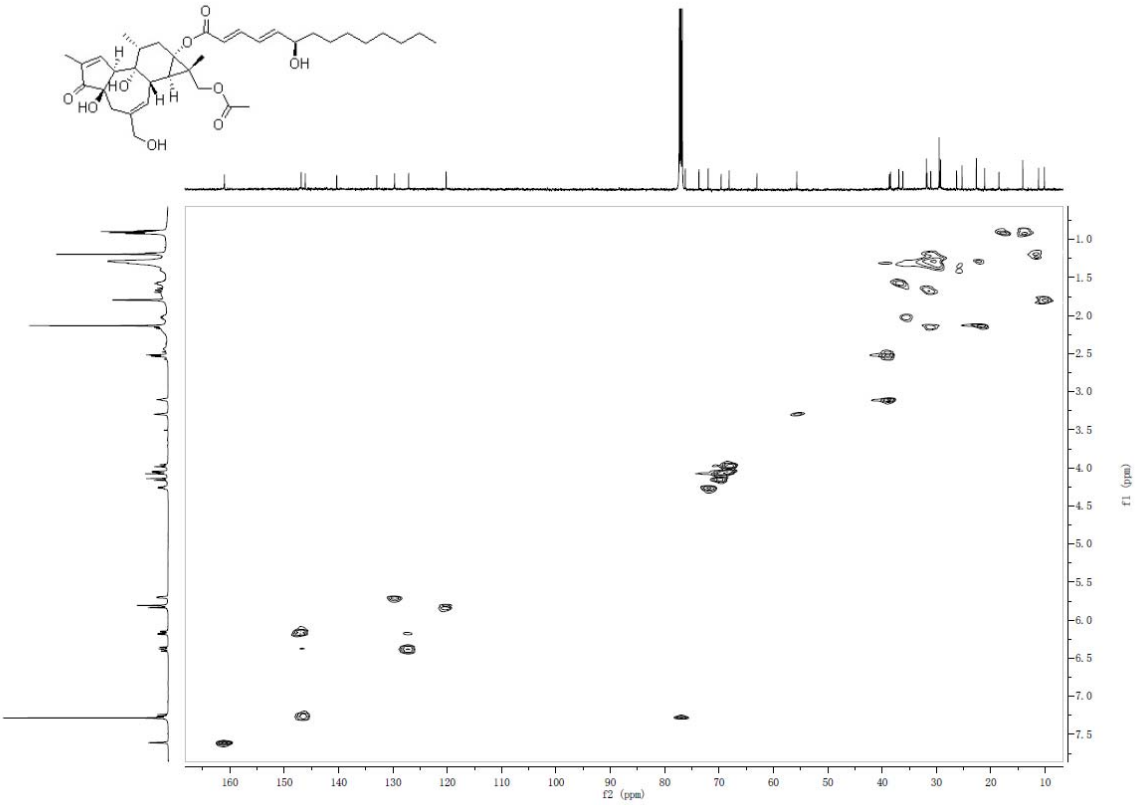


Figure S4. HMBC spectrum of **1** in $CDCl_3$

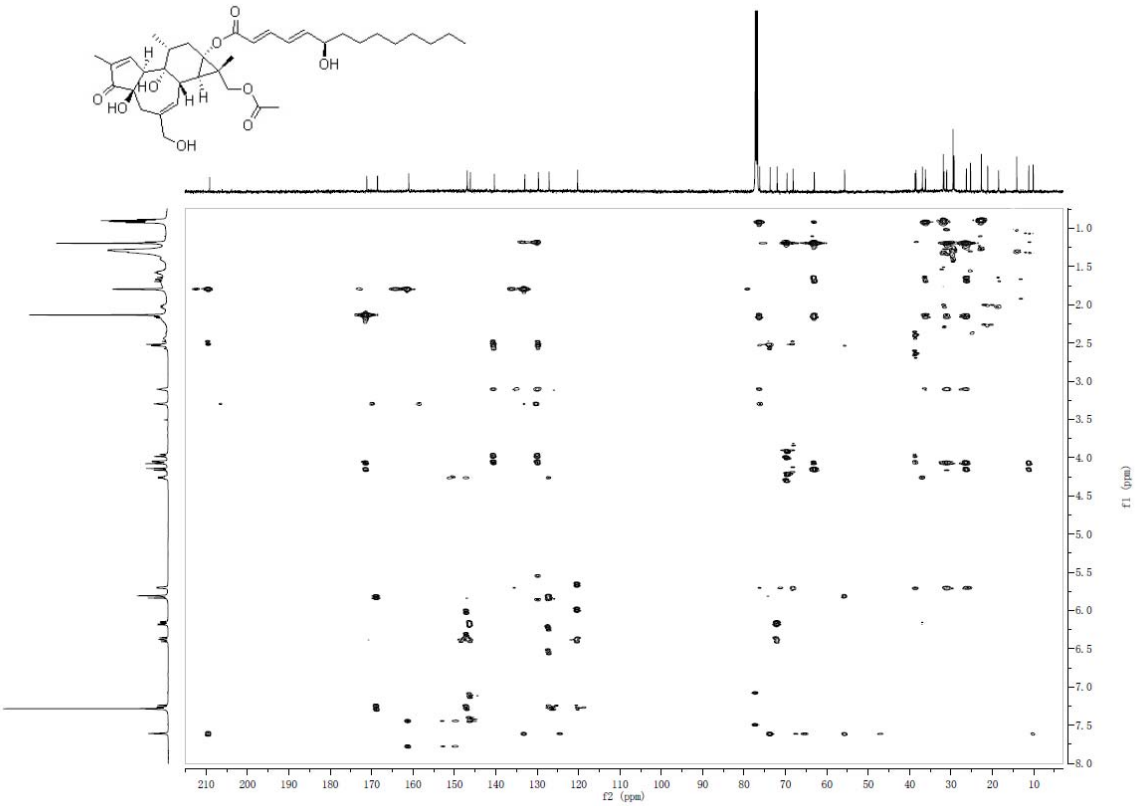


Figure S5. $^{1}H–^{1}H$ COSY spectrum of **1** in $CDCl_3$

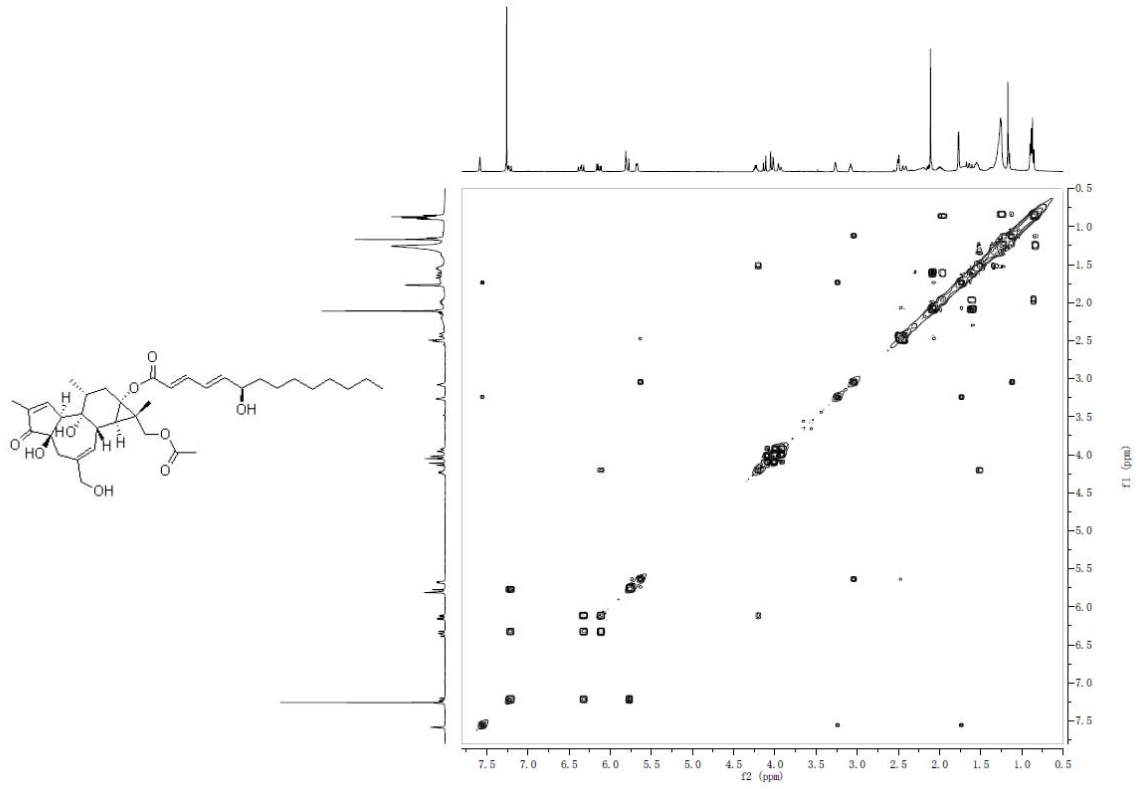


Figure S6. ROESY spectrum of **1** in $CDCl_3$

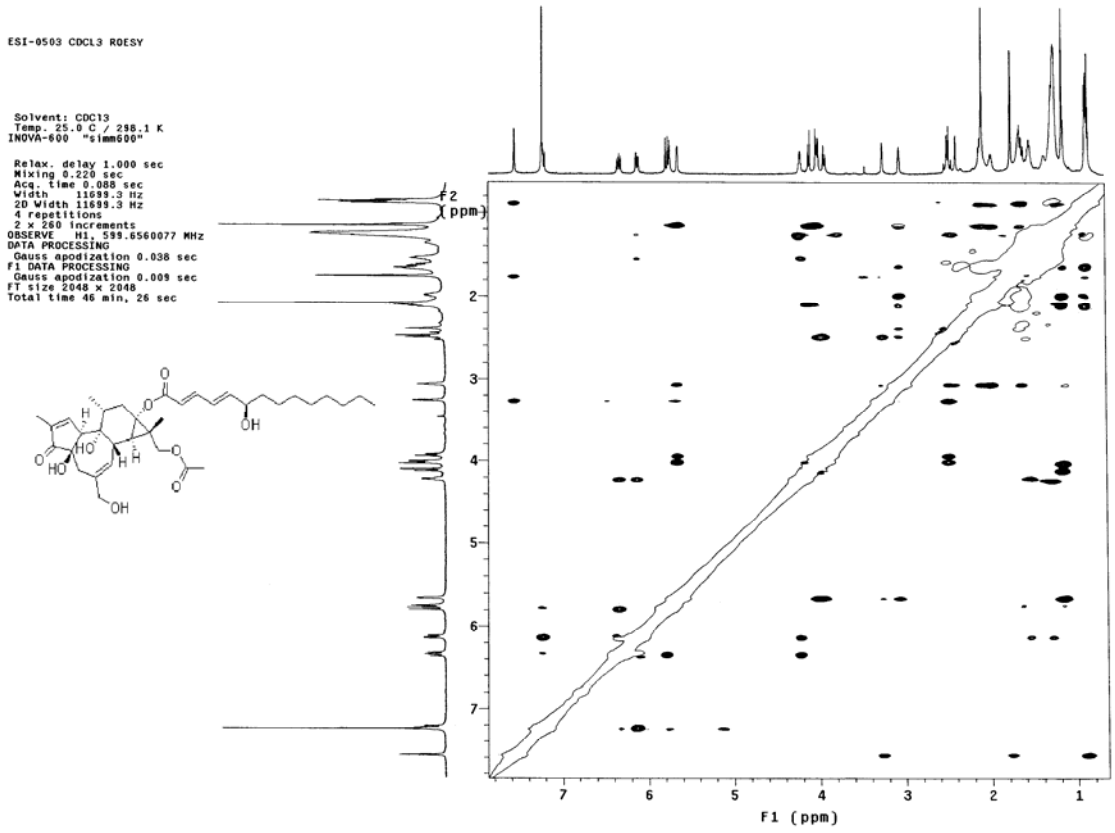


Figure S7. IR spectrum of **1**

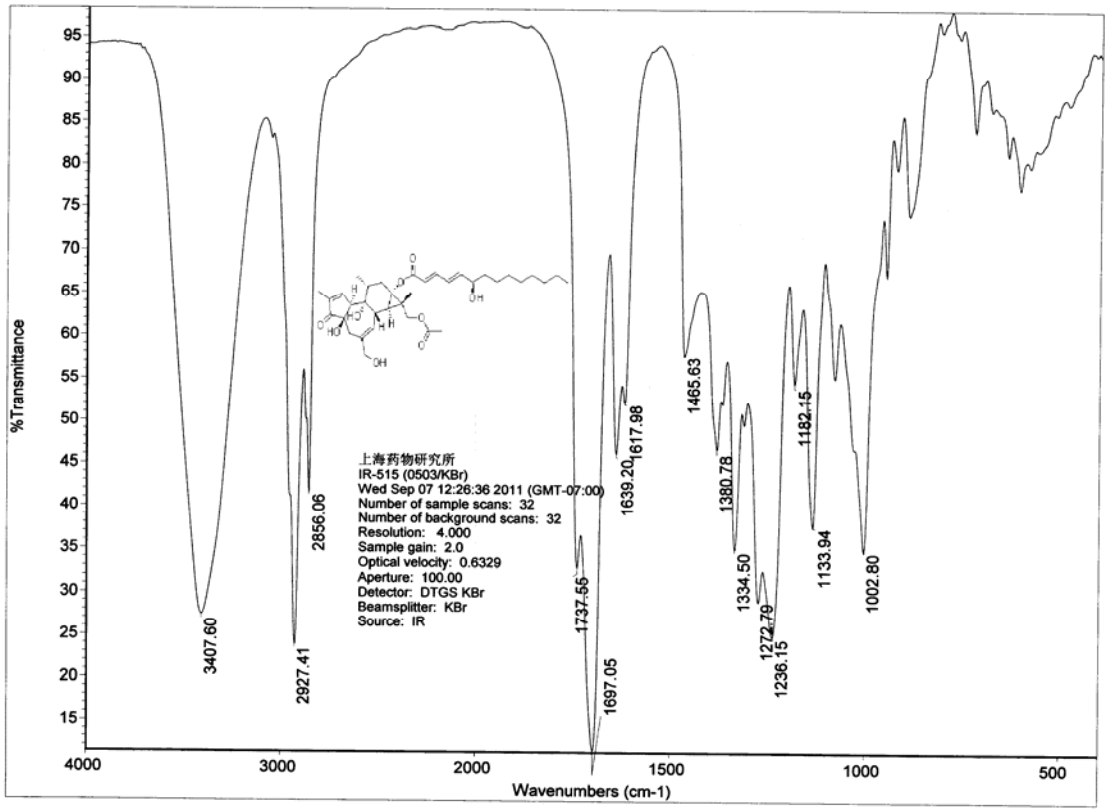


Figure S8. HR-ESI(+)MS spectrum of **1**

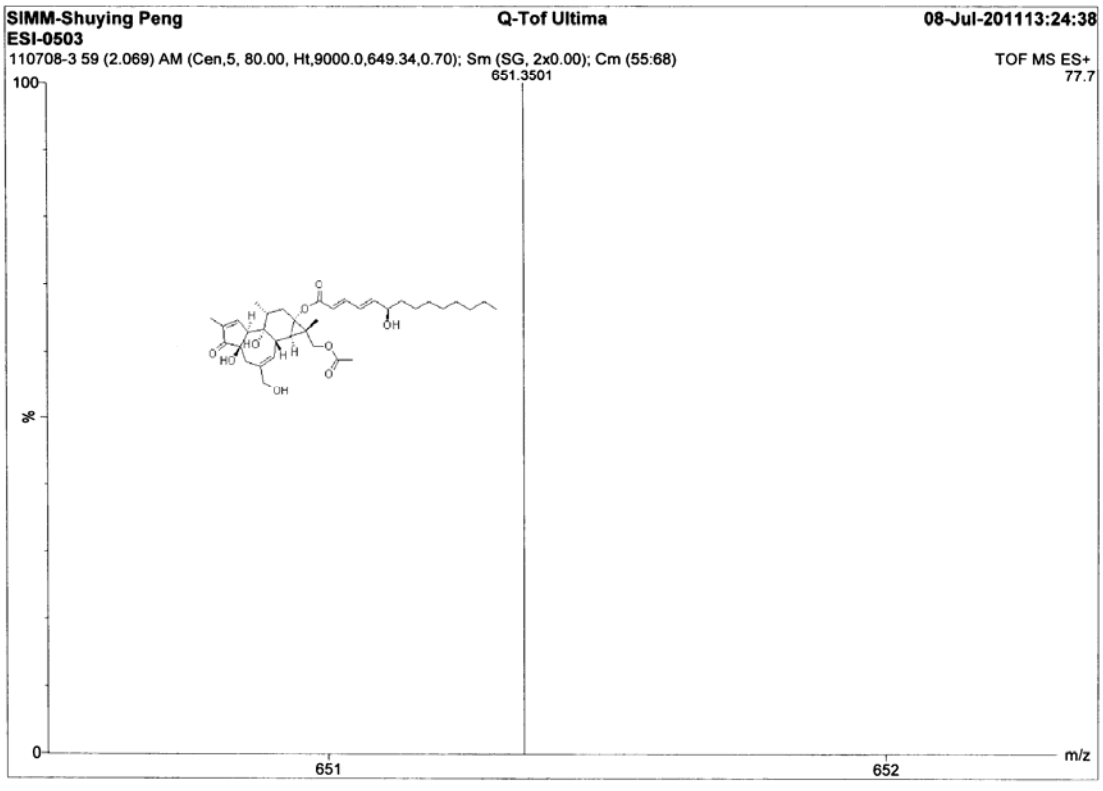


Figure S9. ^{1}H NMR spectrum of **2** in $CDCl_3$

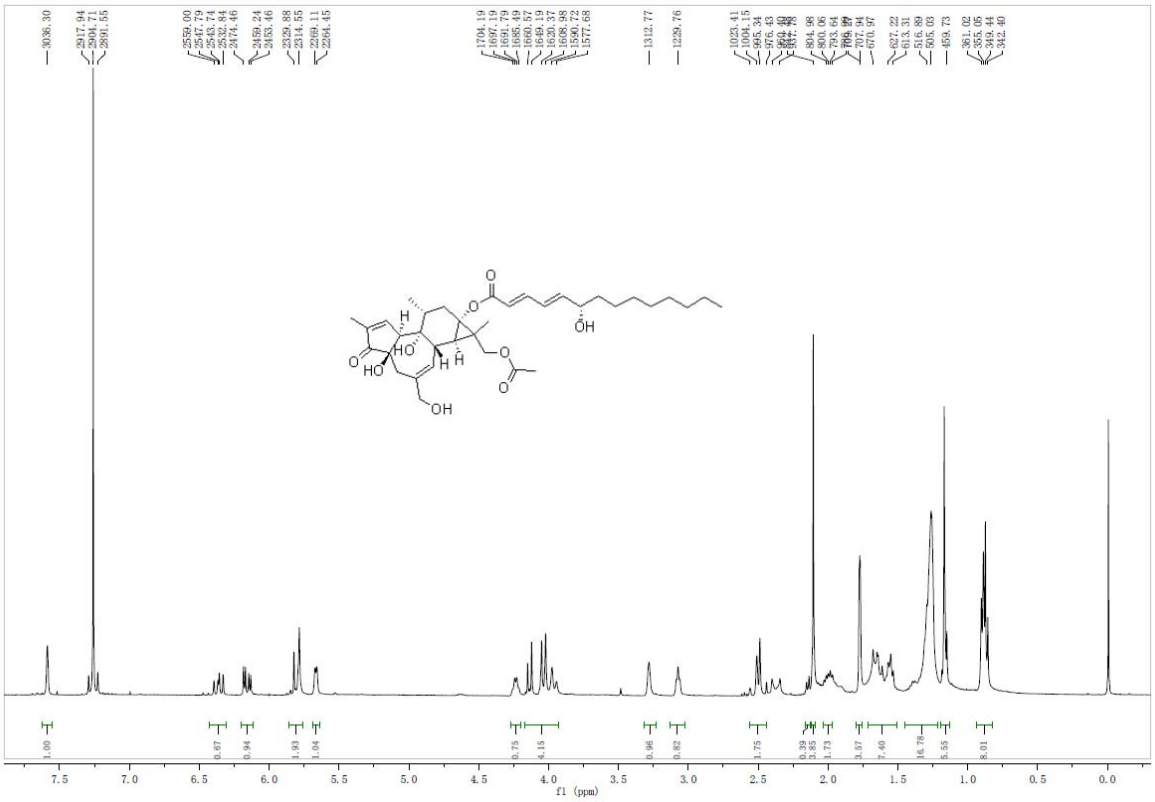


Figure S10. ^{13}C NMR spectrum of **2** in $CDCl_3$

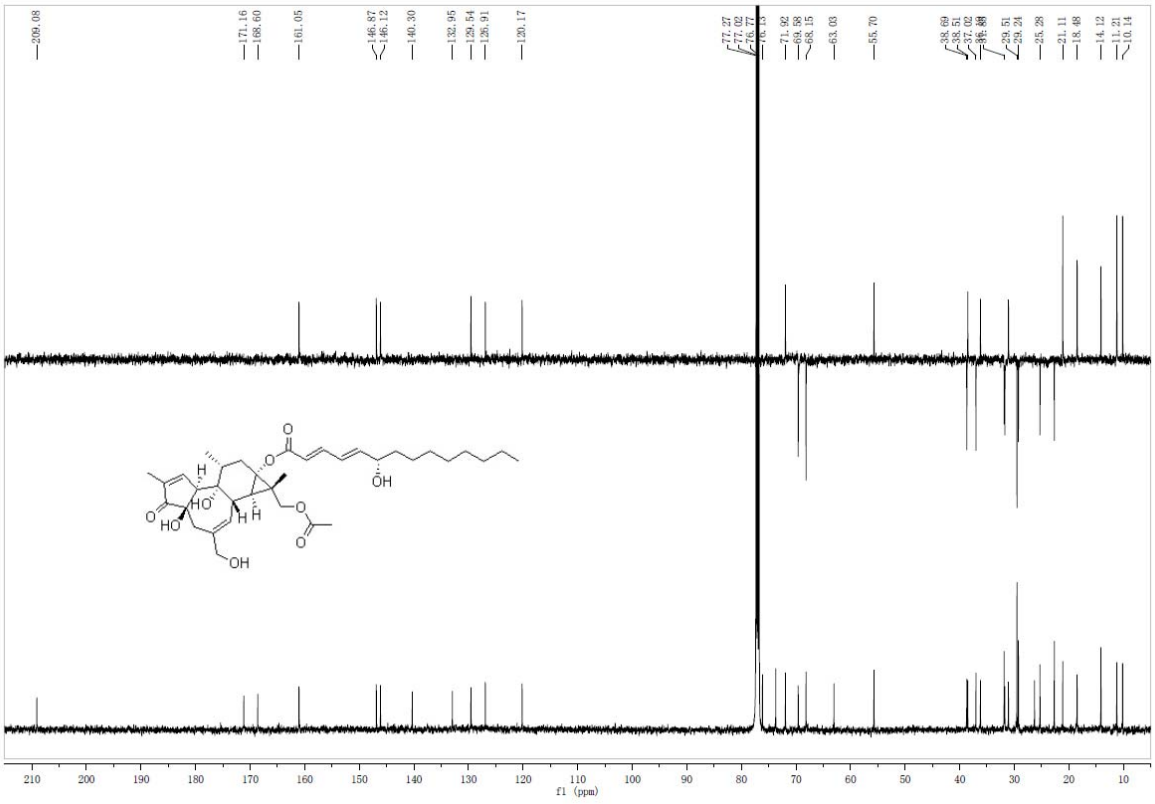


Figure S11. HSQC spectrum of **2** in $CDCl_3$

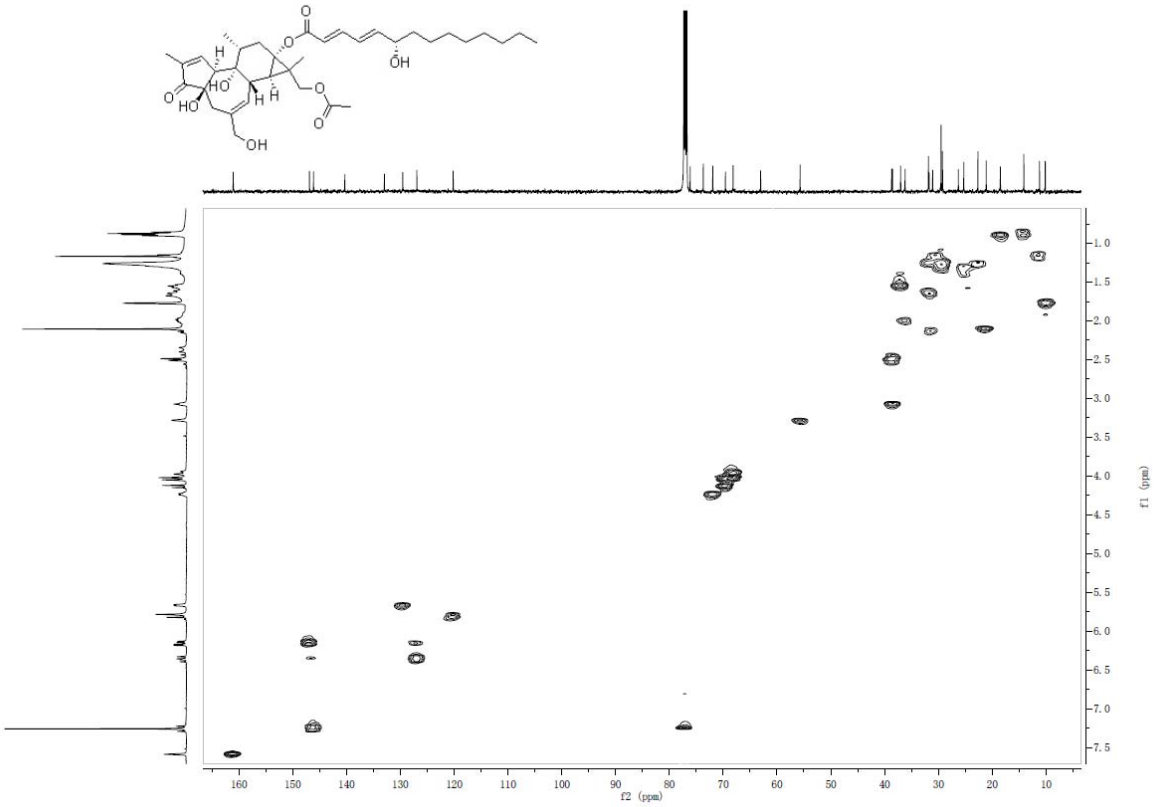


Figure S12. HMBC spectrum of **2** in $CDCl_3$

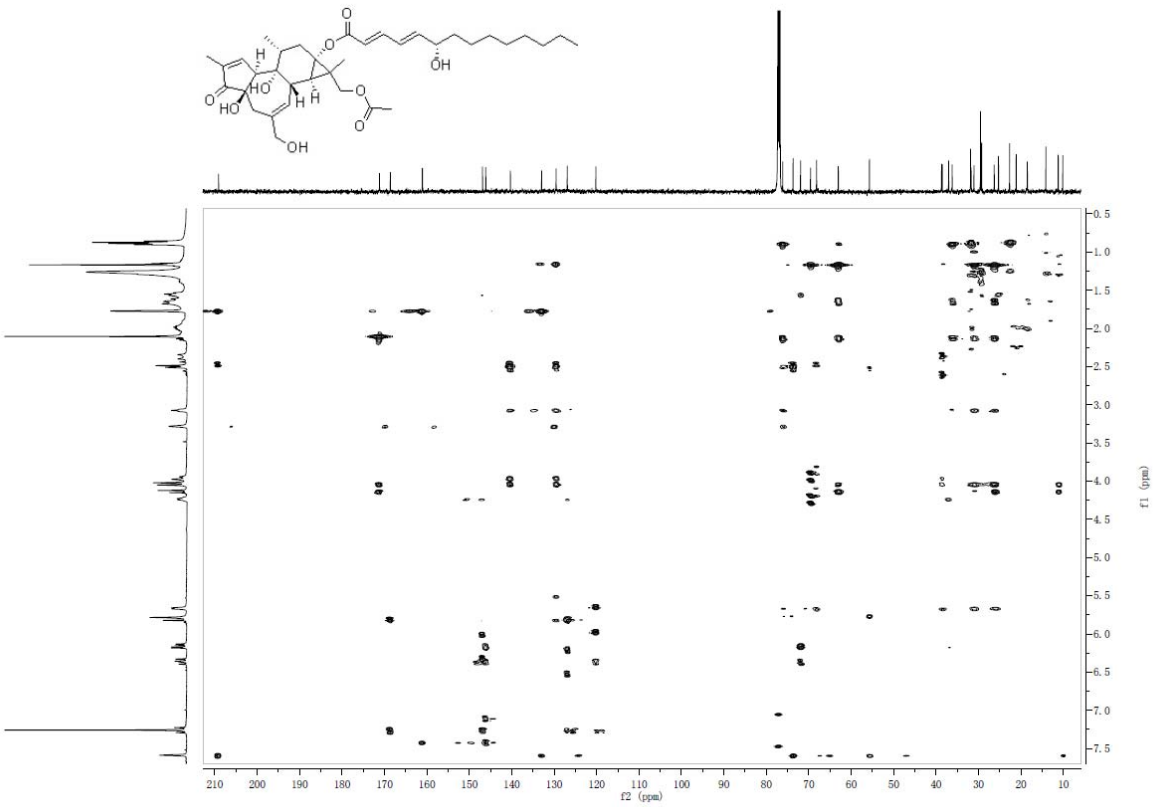


Figure S13. ROESY spectrum of **2** in $CDCl_3$

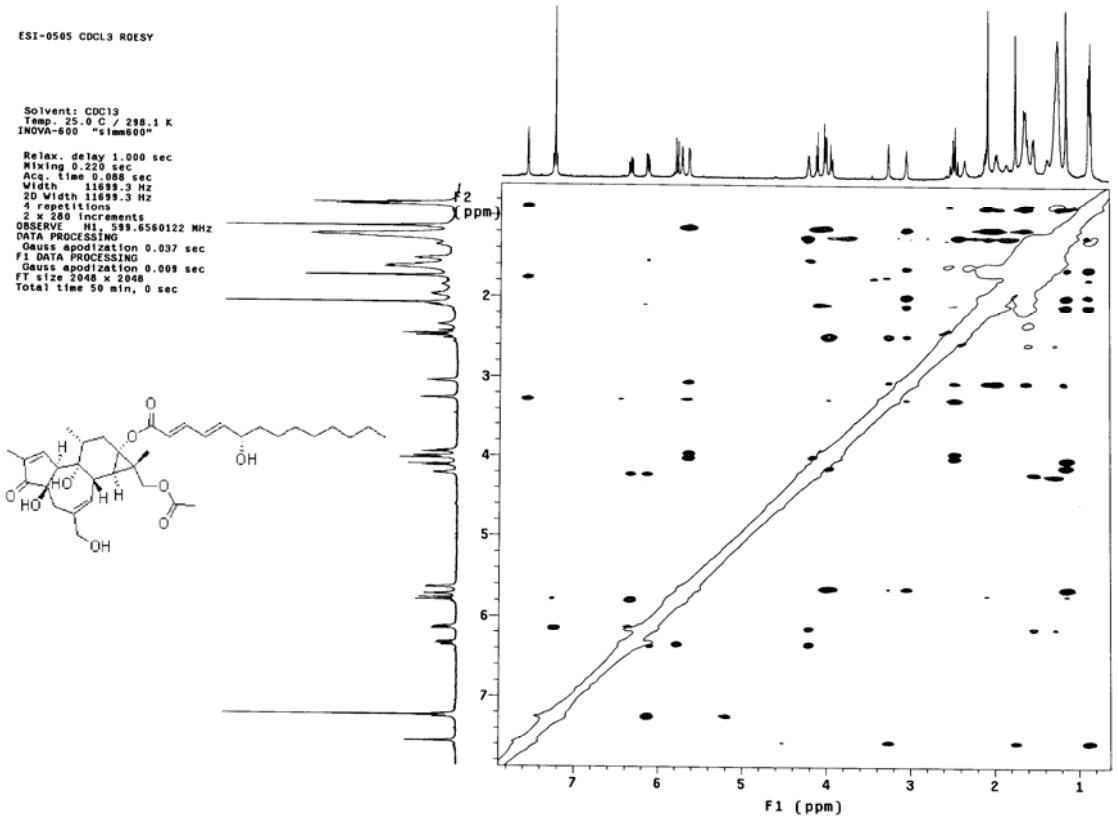


Figure S14. IR spectrum of **2**

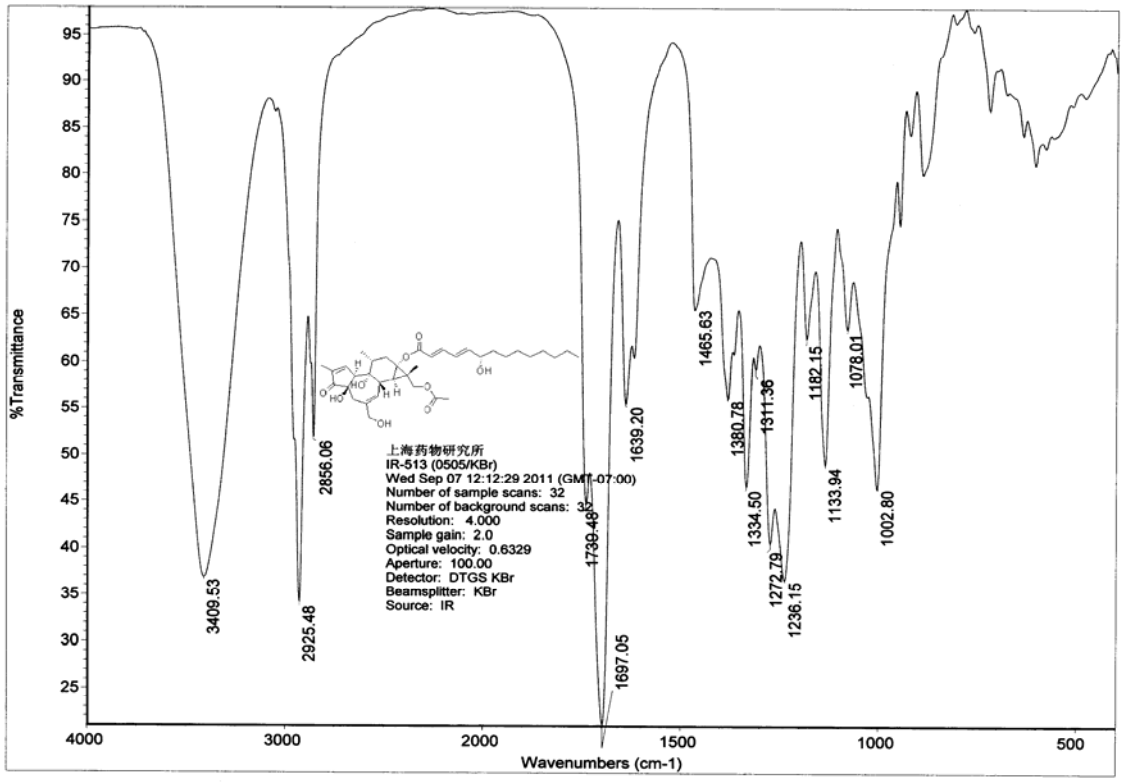


Figure S15. HR-ESI(+)MS spectrum of **2**

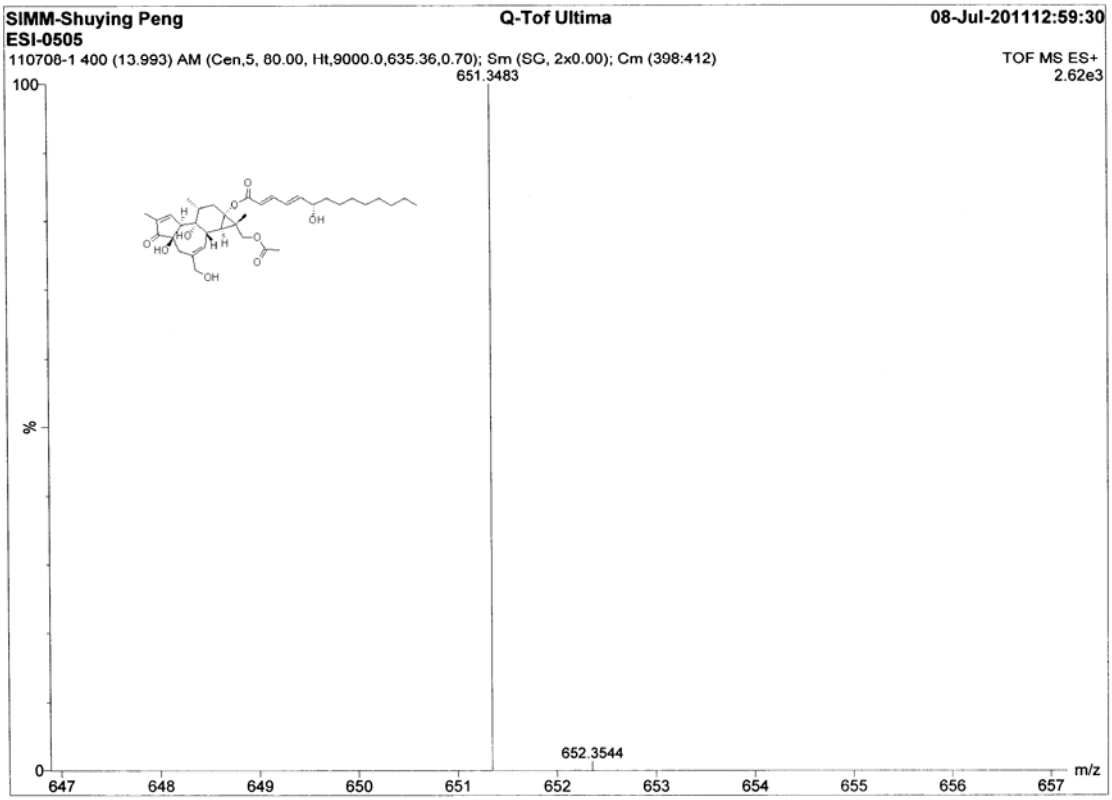


Figure S16. ^{1}H NMR spectrum of **3** in $CDCl_3$

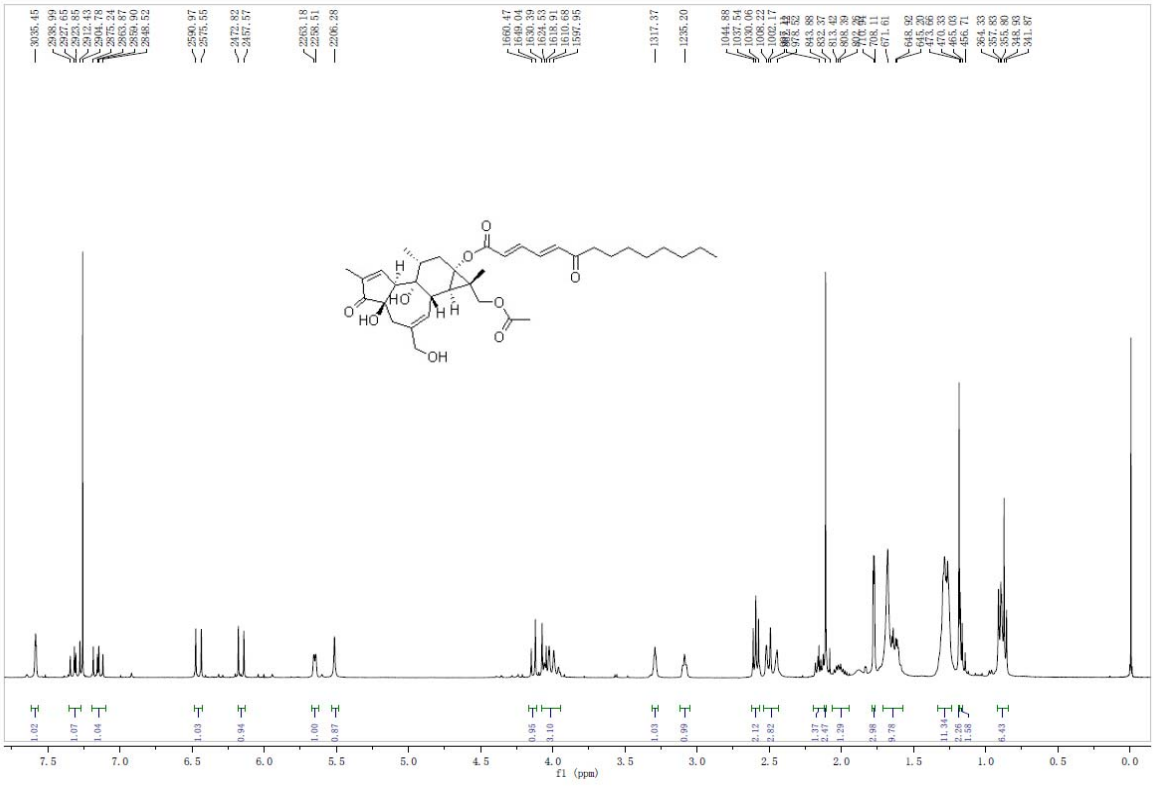


Figure S17. ^{13}C NMR spectrum of **3** in $CDCl_3$

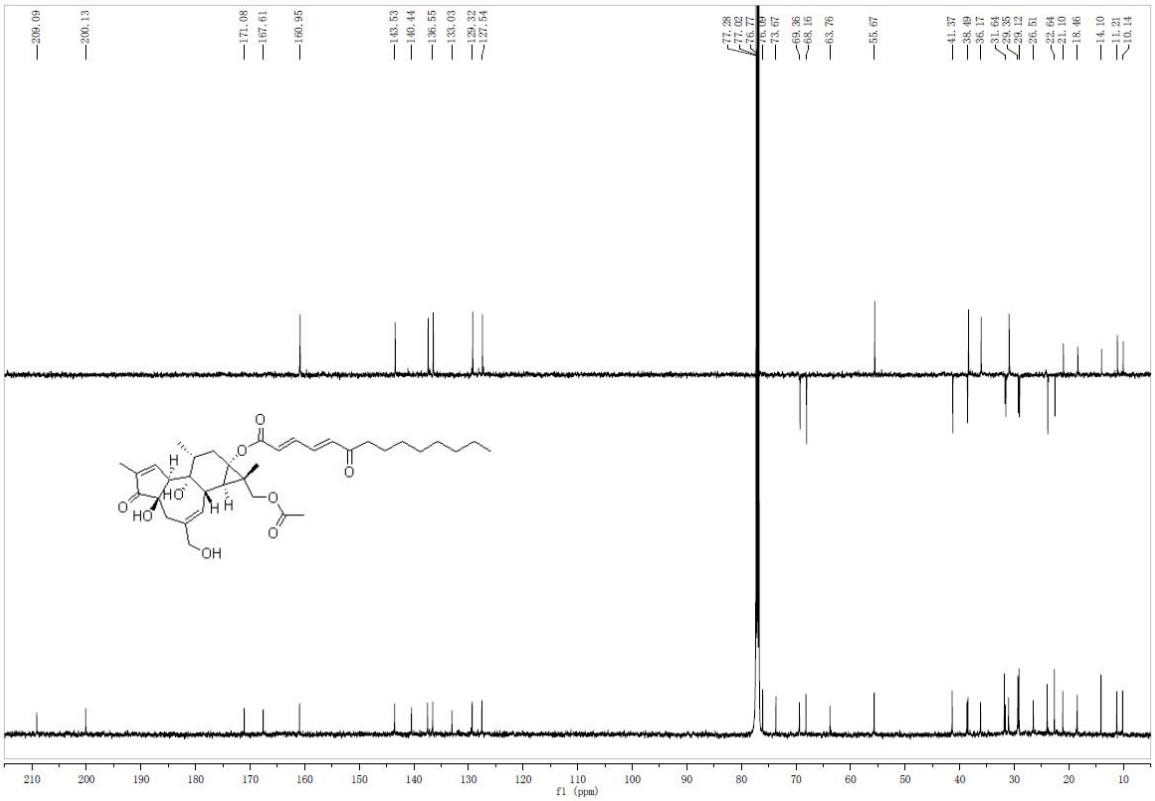


Figure S18. HSQC spectrum of **3** in $CDCl_3$

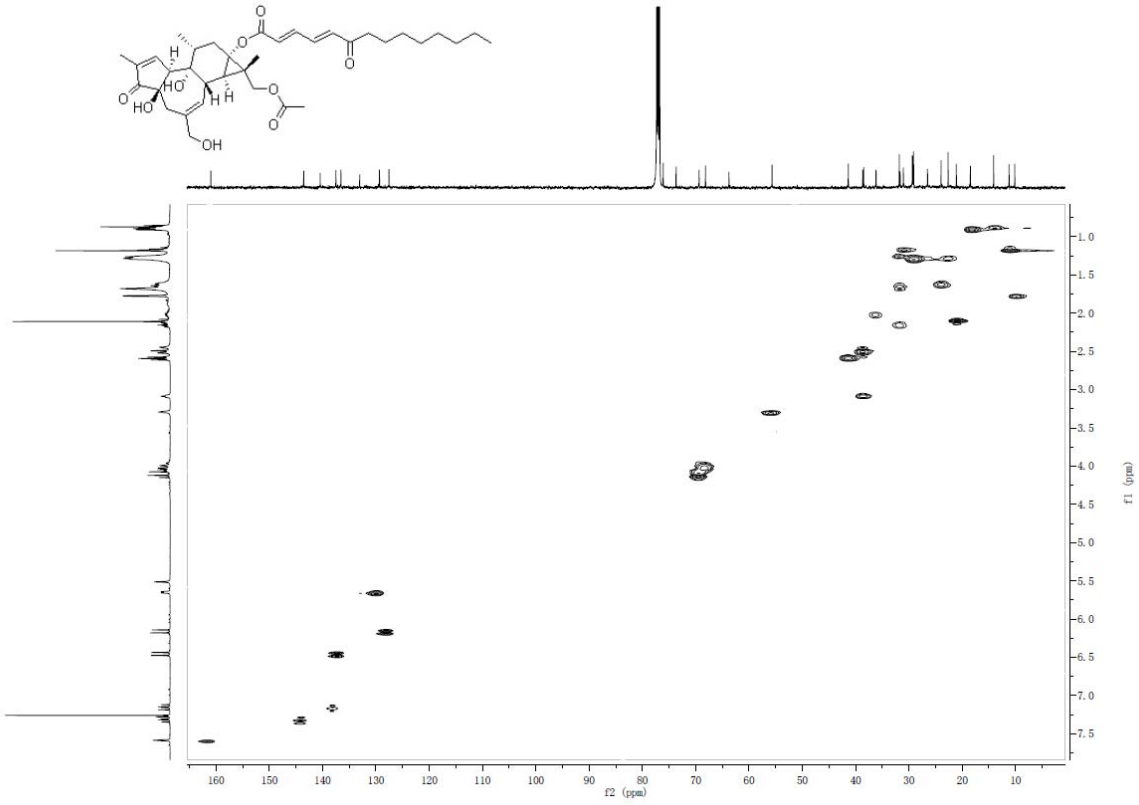


Figure S19. HMBC spectrum of **3** in $CDCl_3$

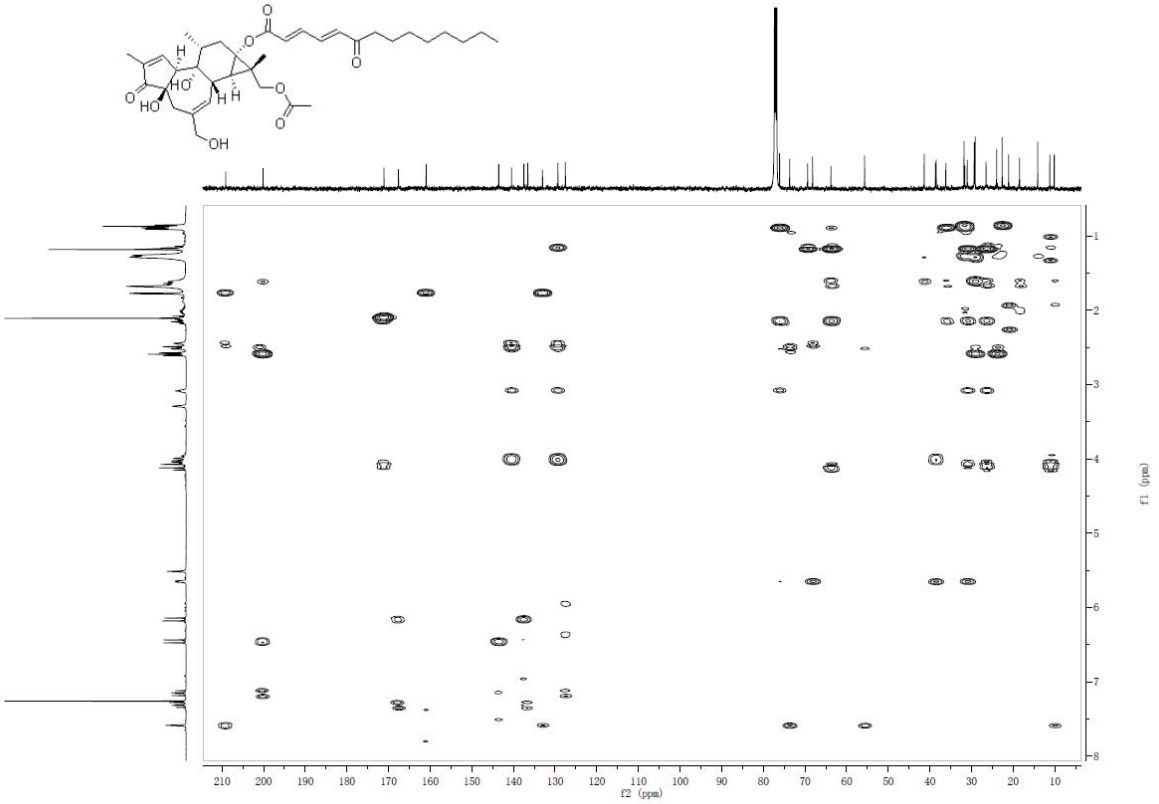


Figure S20. ^{1}H–^{1}H COSY spectrum of **3** in $CDCl_3$

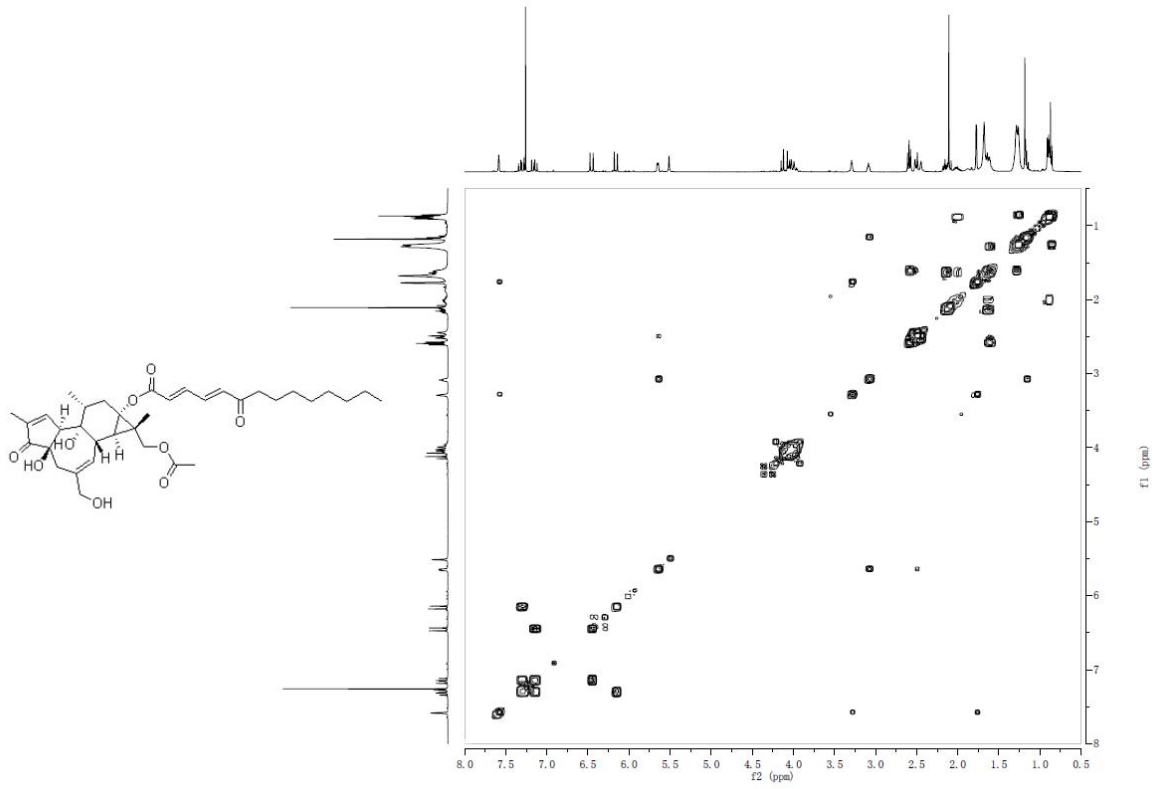


Figure S21. ROESY spectrum of **3** in $CDCl_3$

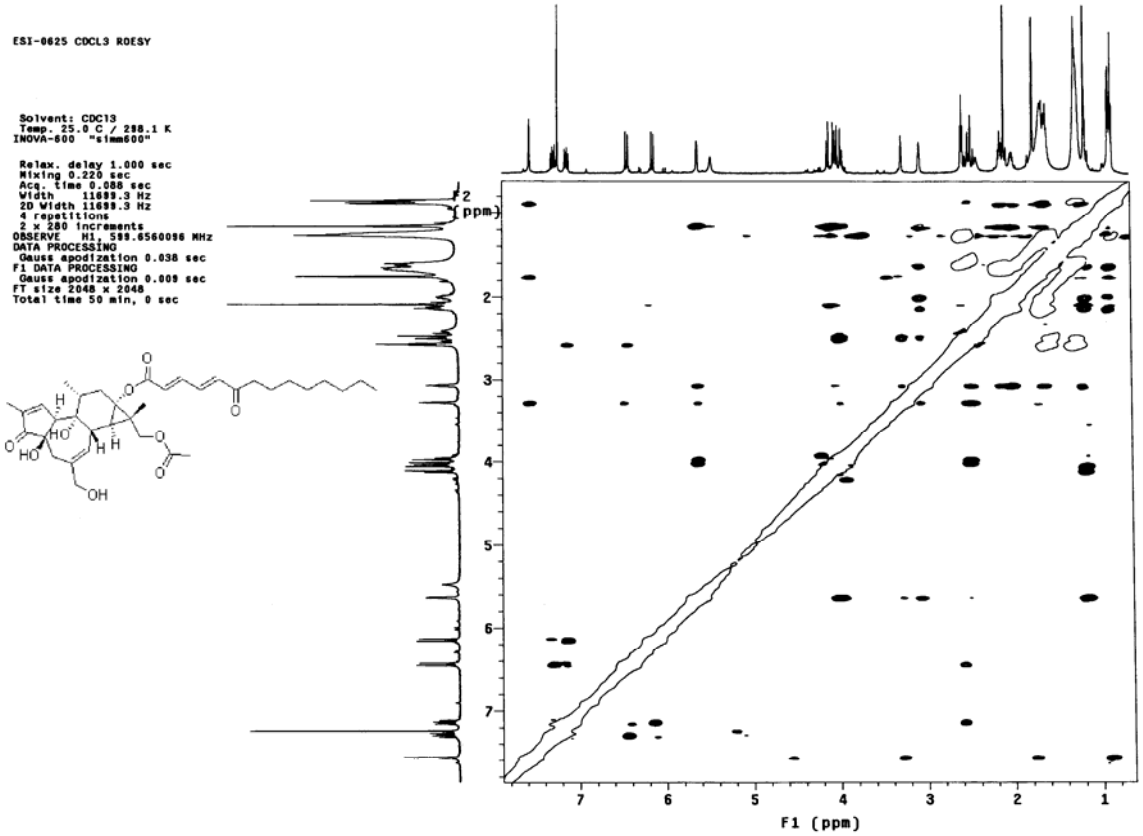


Figure S22. IR spectrum of **3**

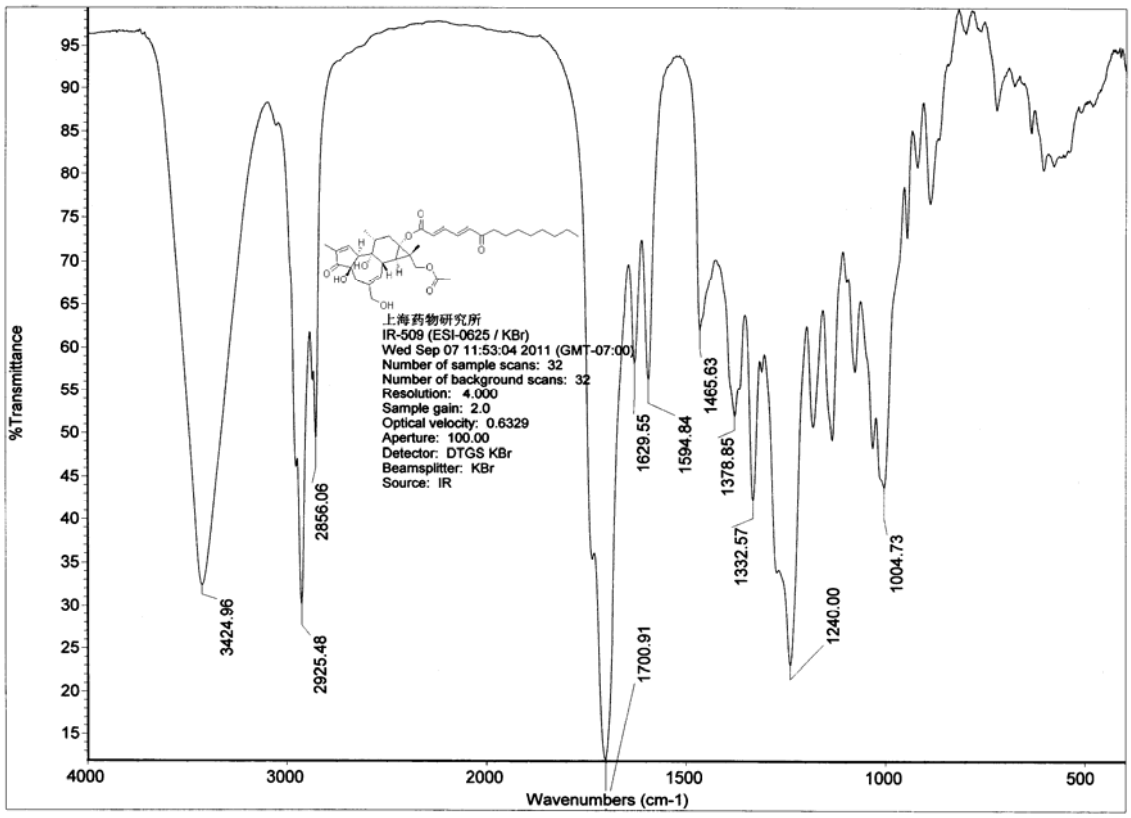


Figure S23. HR-ESI(+)MS spectrum of **3**

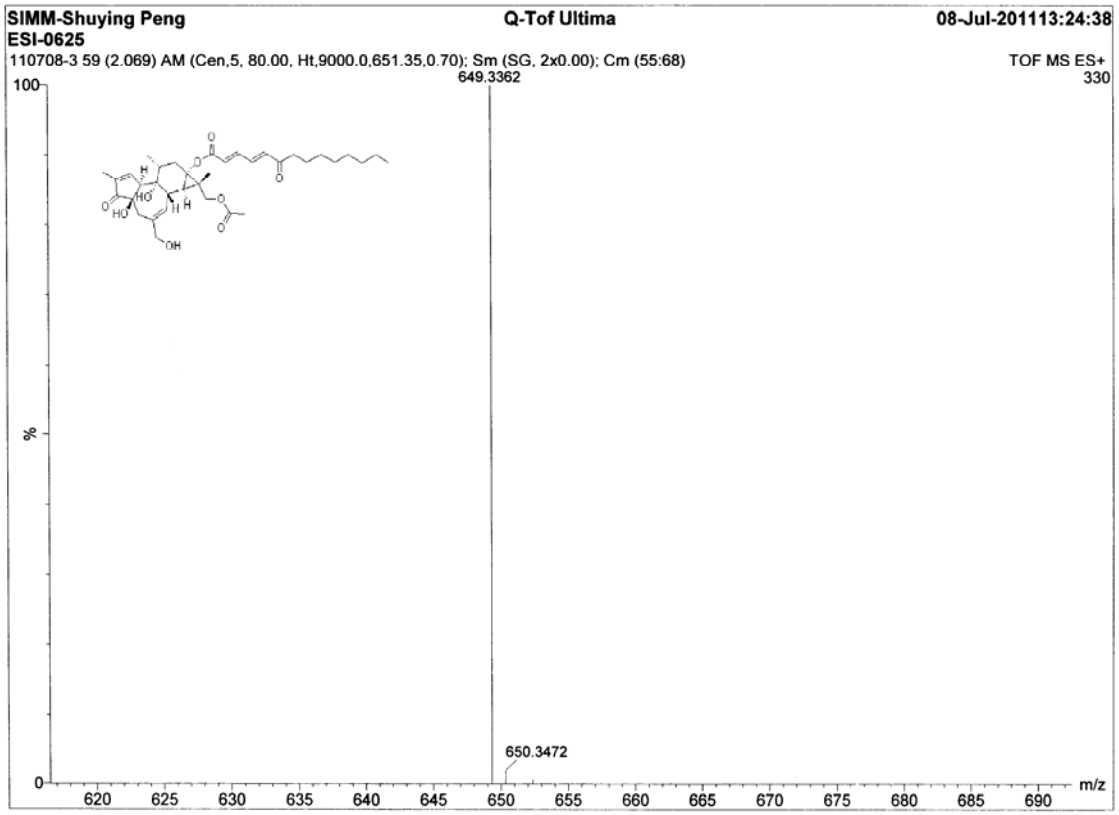


Figure S24. ^{1}H NMR spectrum of **4** in $CDCl_3$

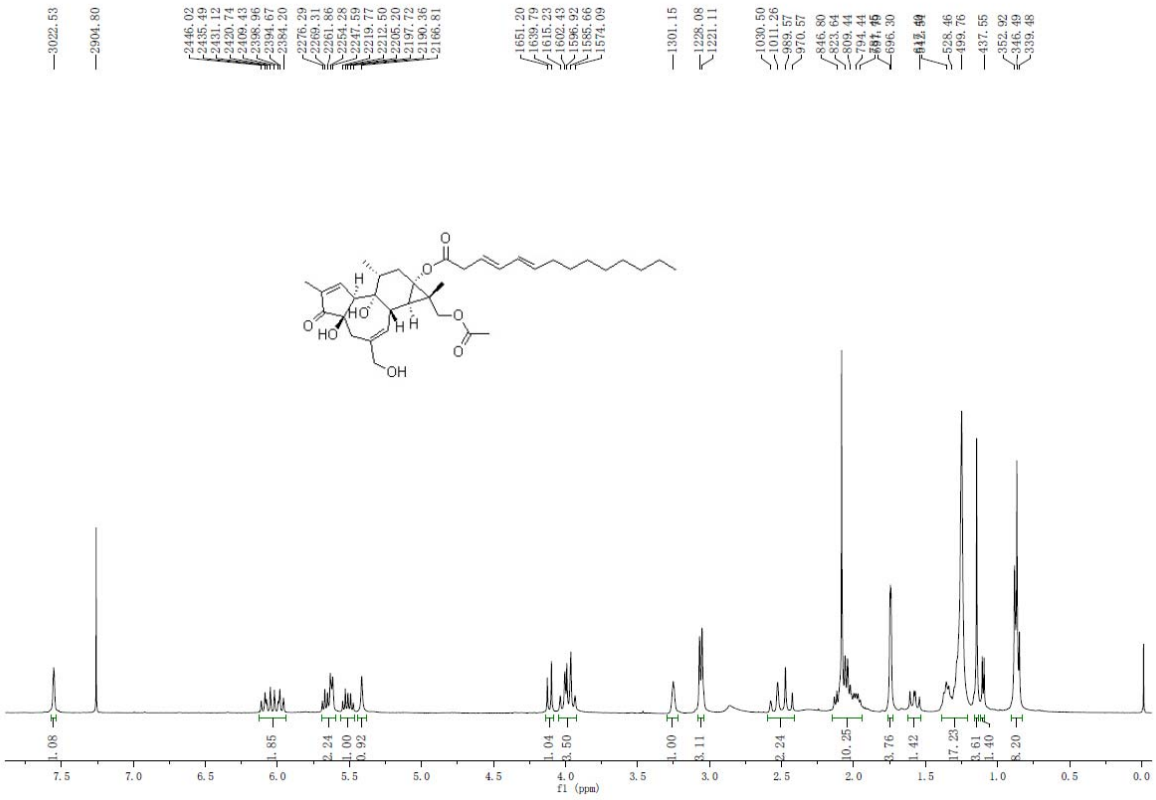


Figure S25. ^{13}C NMR spectrum of **4** in $CDCl_3$

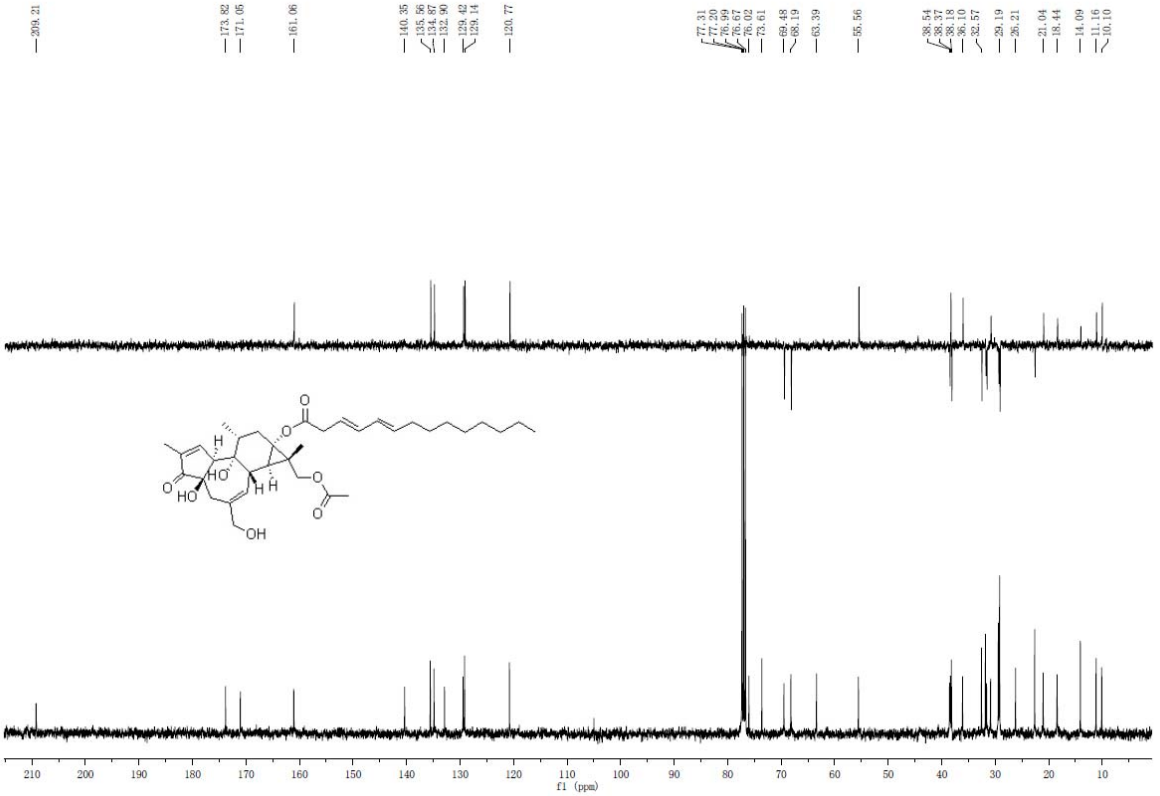


Figure S26. HSQC spectrum of **4** in $CDCl_3$

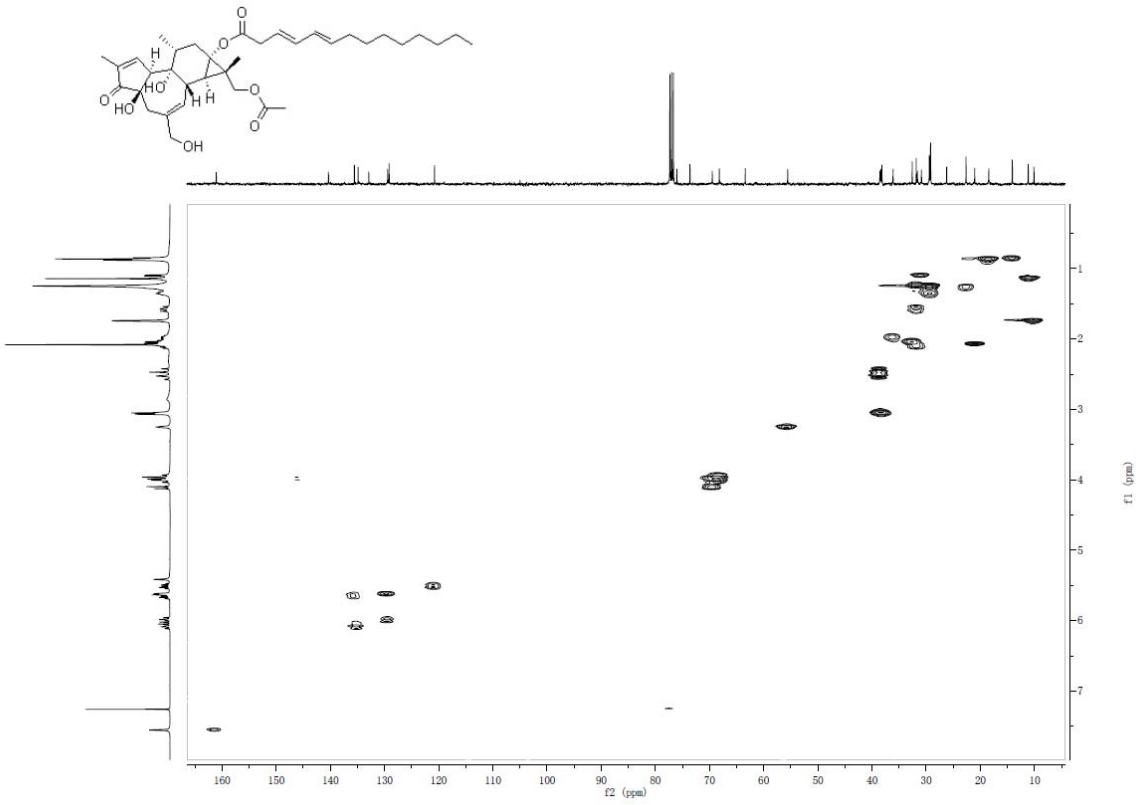


Figure S27. HMBC spectrum of **4** in $CDCl_3$

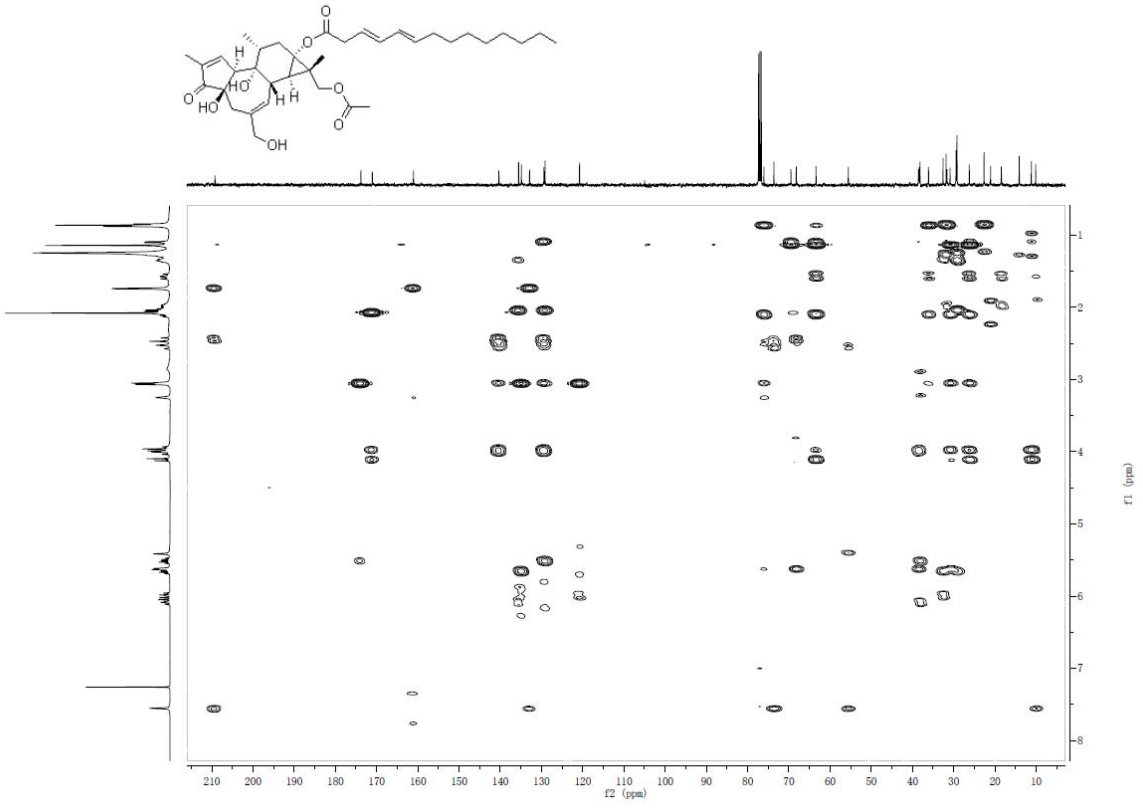


Figure S28. ^{1}H–^{1}H COSY spectrum of **4** in $CDCl_3$

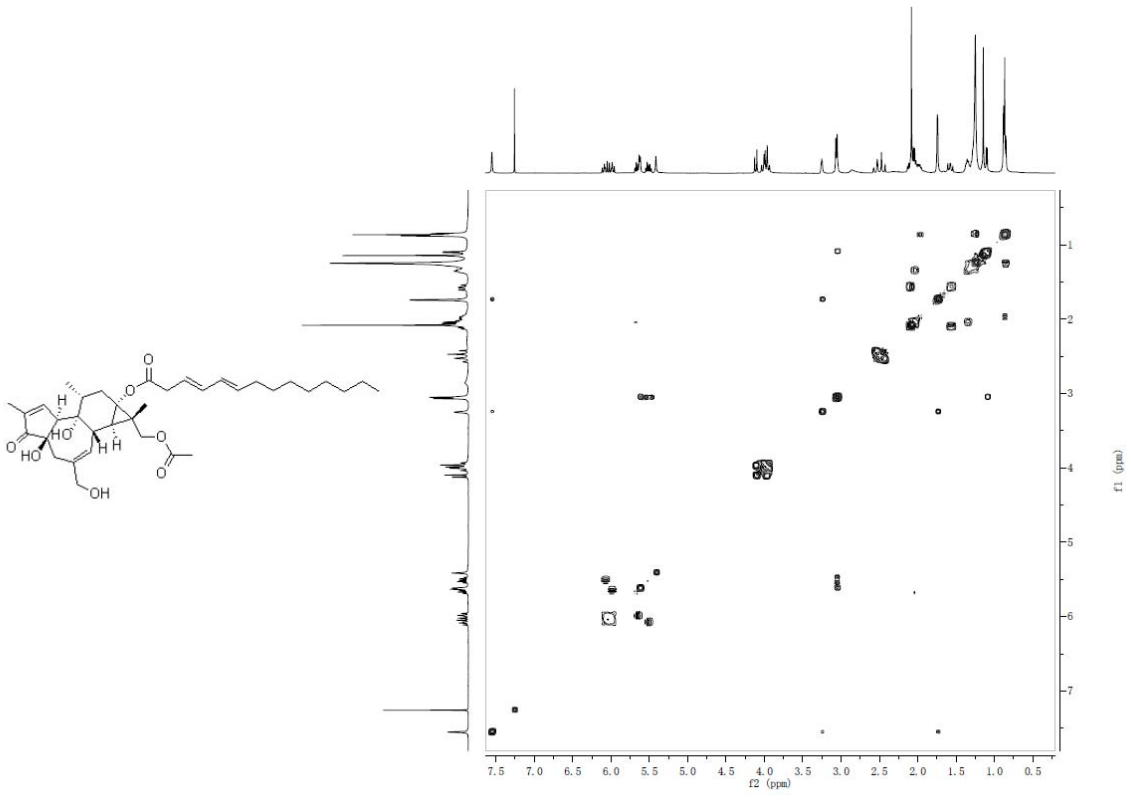


Figure S29. ROESY spectrum of **4** in $CDCl_3$

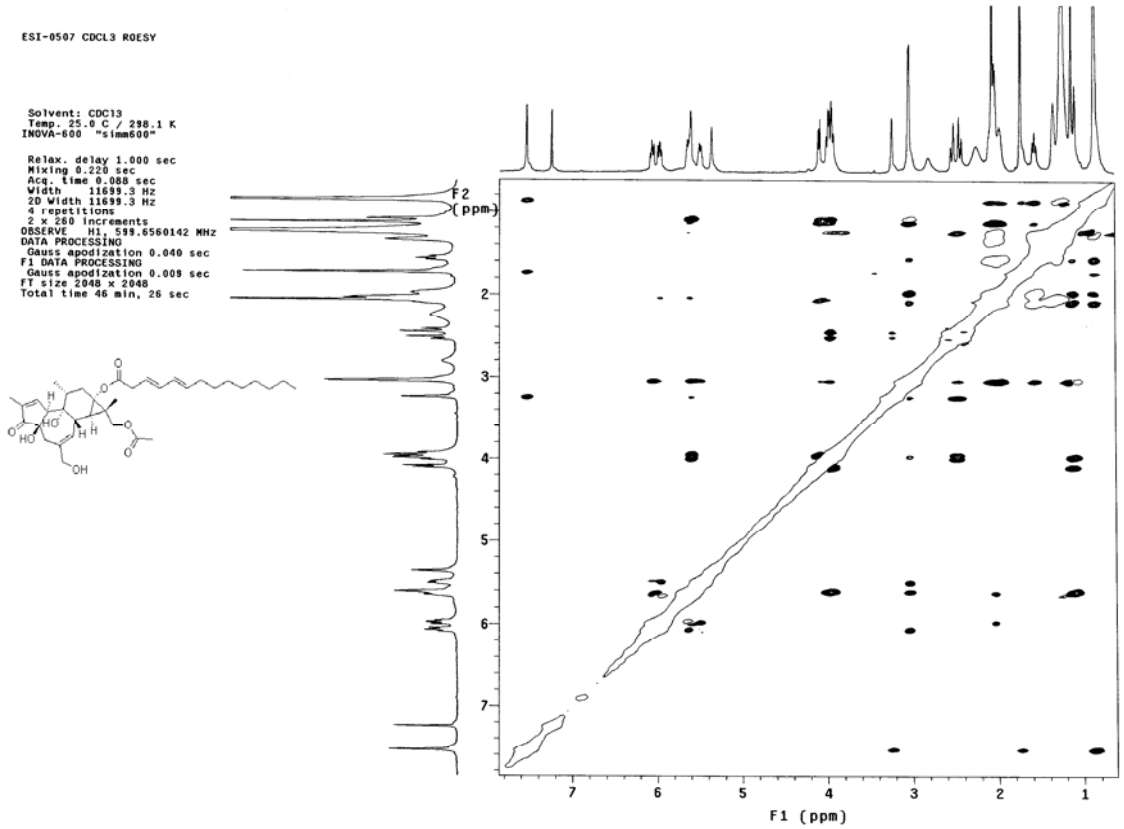


Figure S30. IR spectrum of **4**

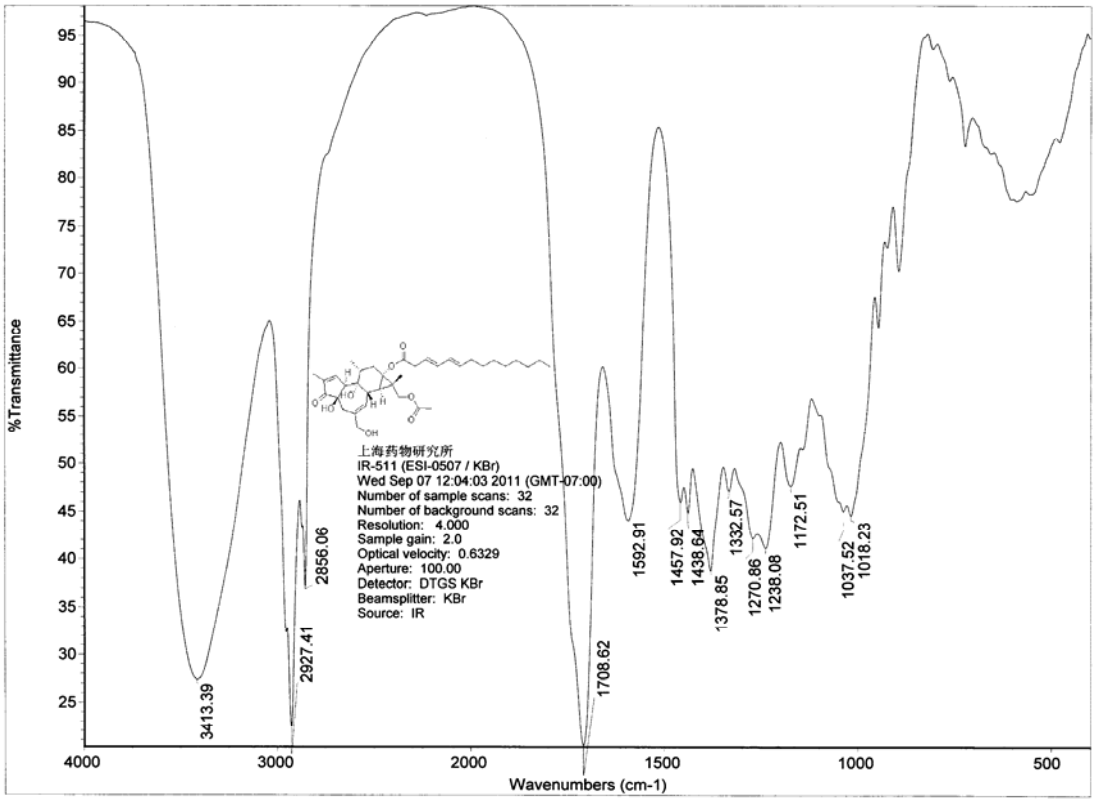


Figure S31. HR-ESI(+)MS spectrum of **4**

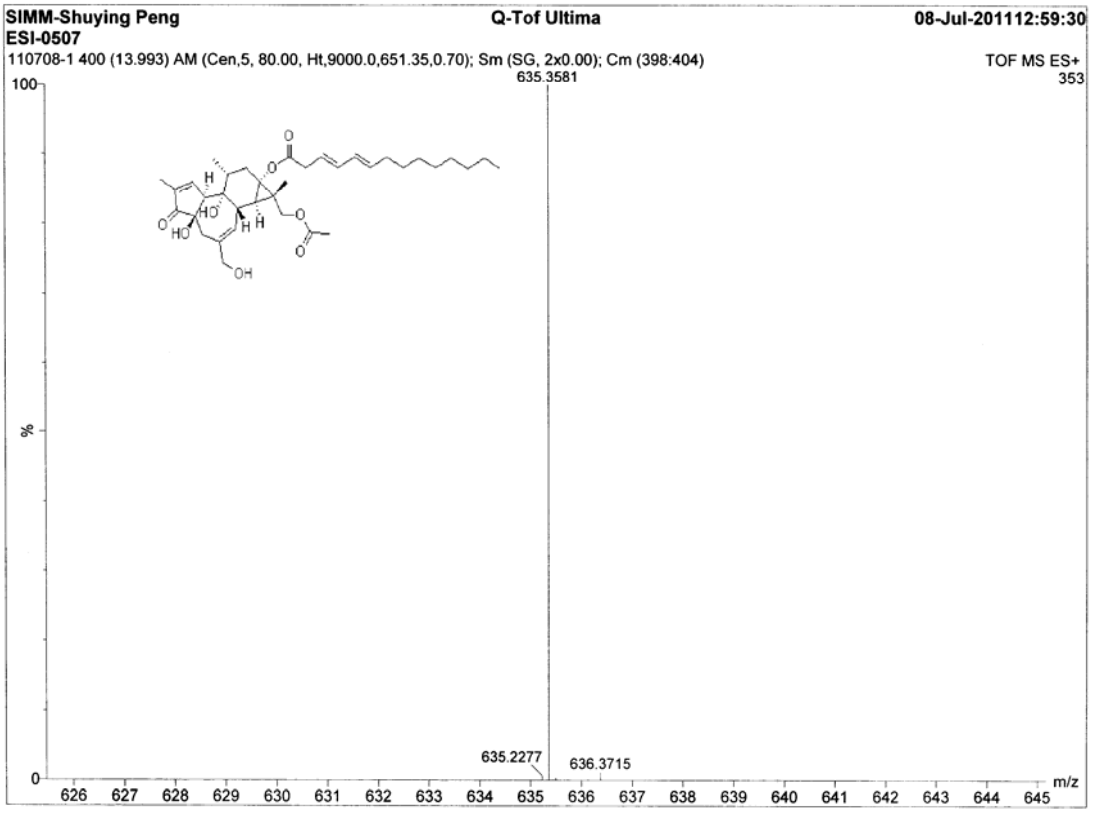


Figure S32. ^{1}H NMR spectrum of **1a** in $CDCl_3$

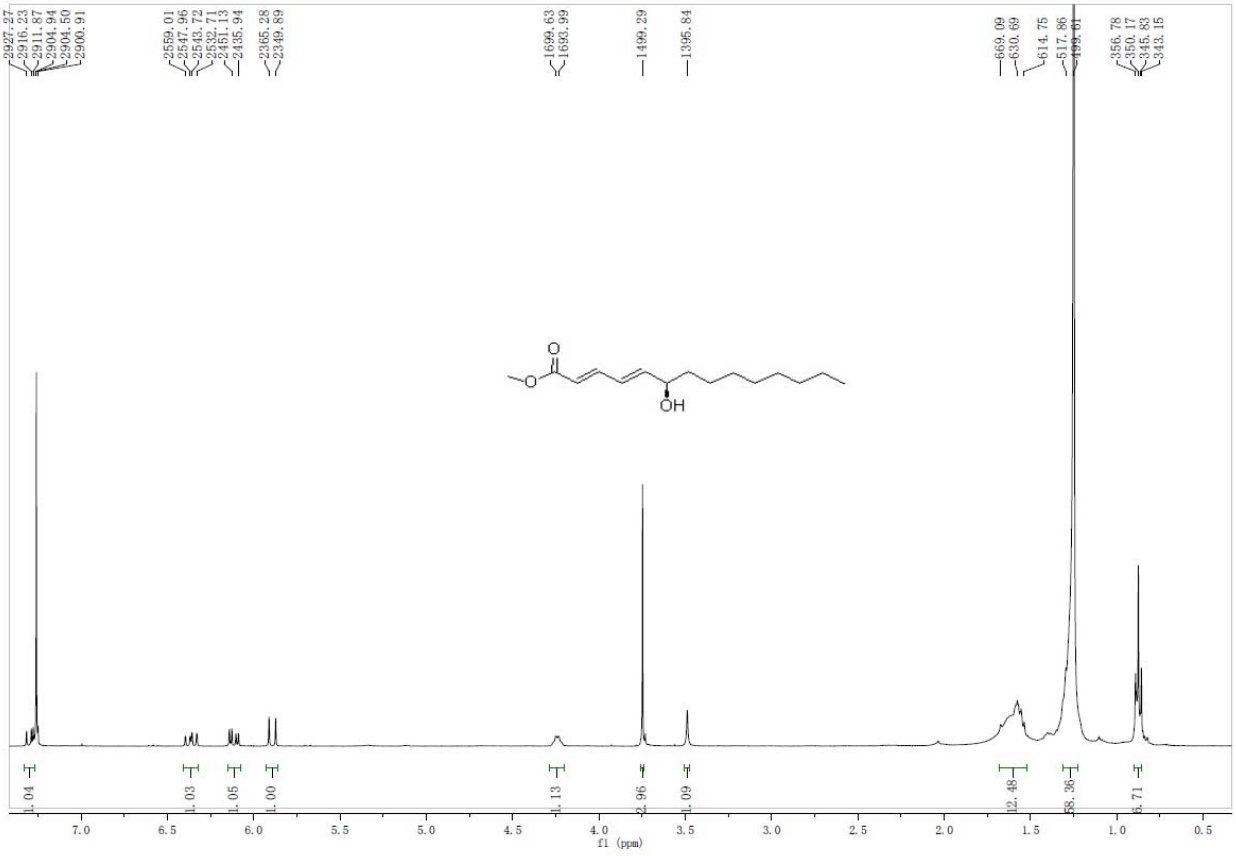


Figure S33. ^{13}C NMR spectrum of **1a** in $CDCl_3$

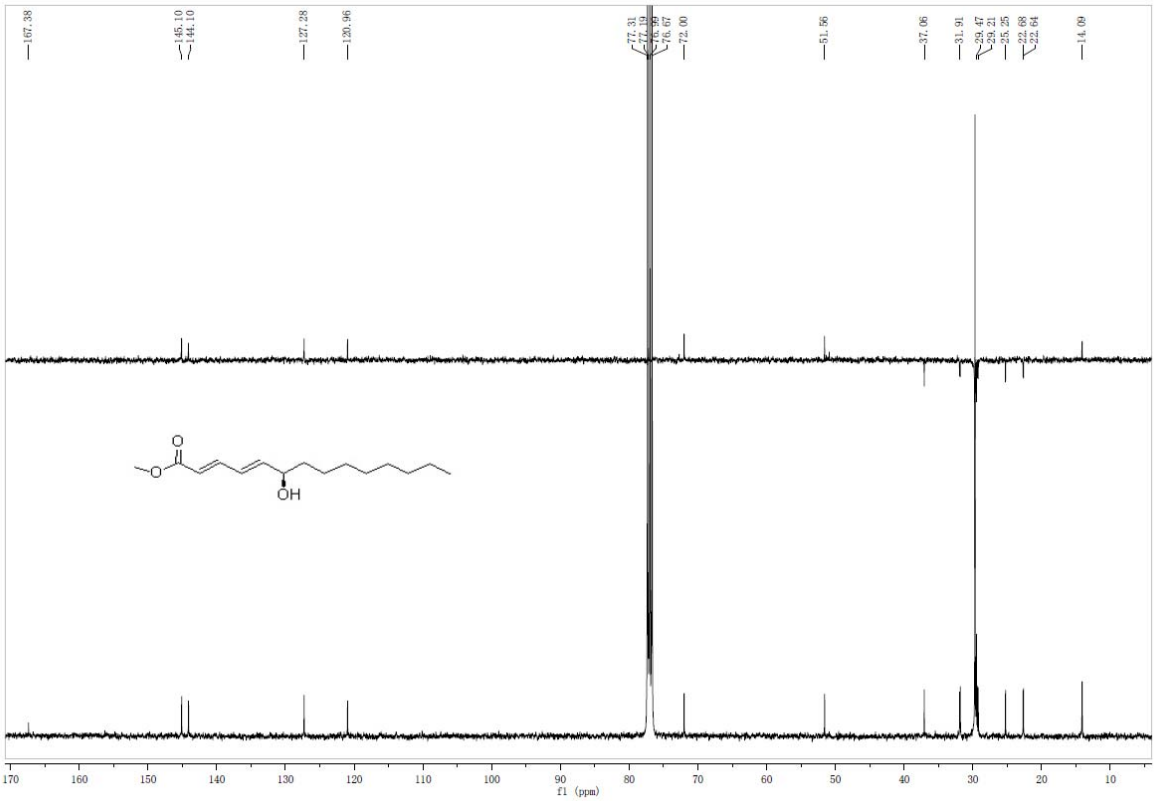


Figure S34. HSQC spectrum of **1a** in $CDCl_3$

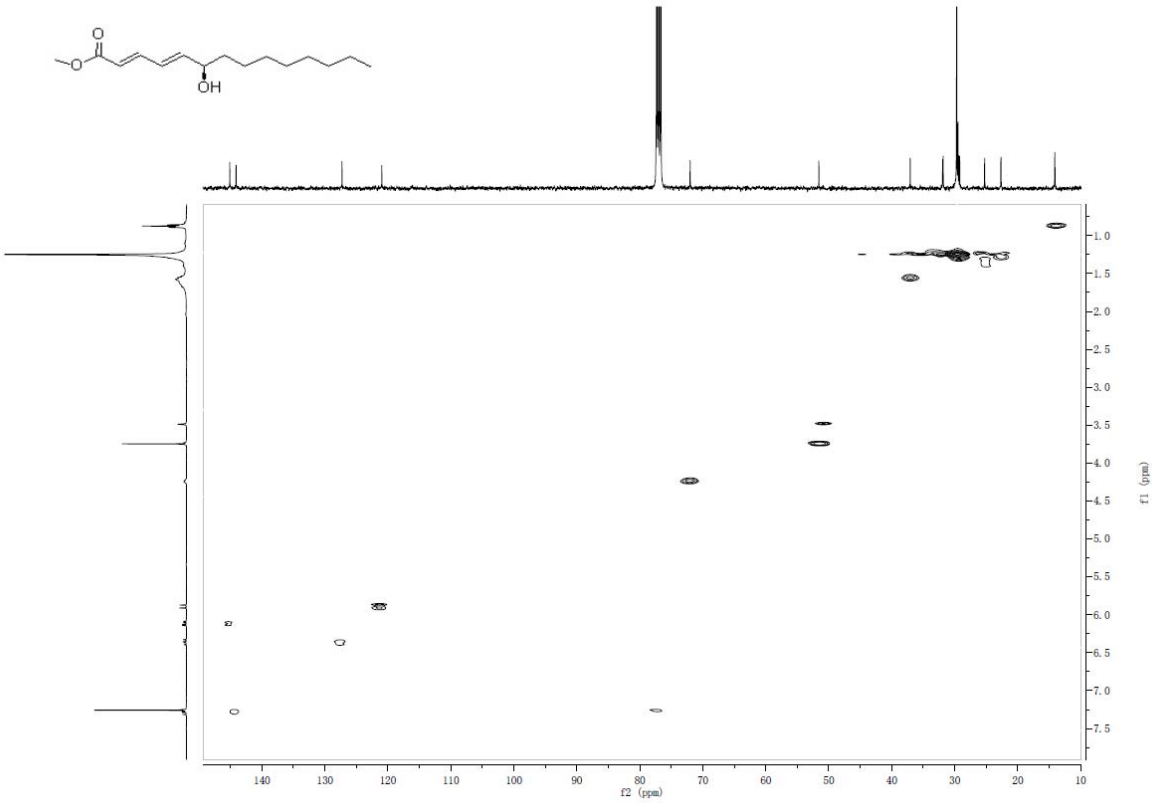


Figure S35. HMBC spectrum of **1a** in $CDCl_3$

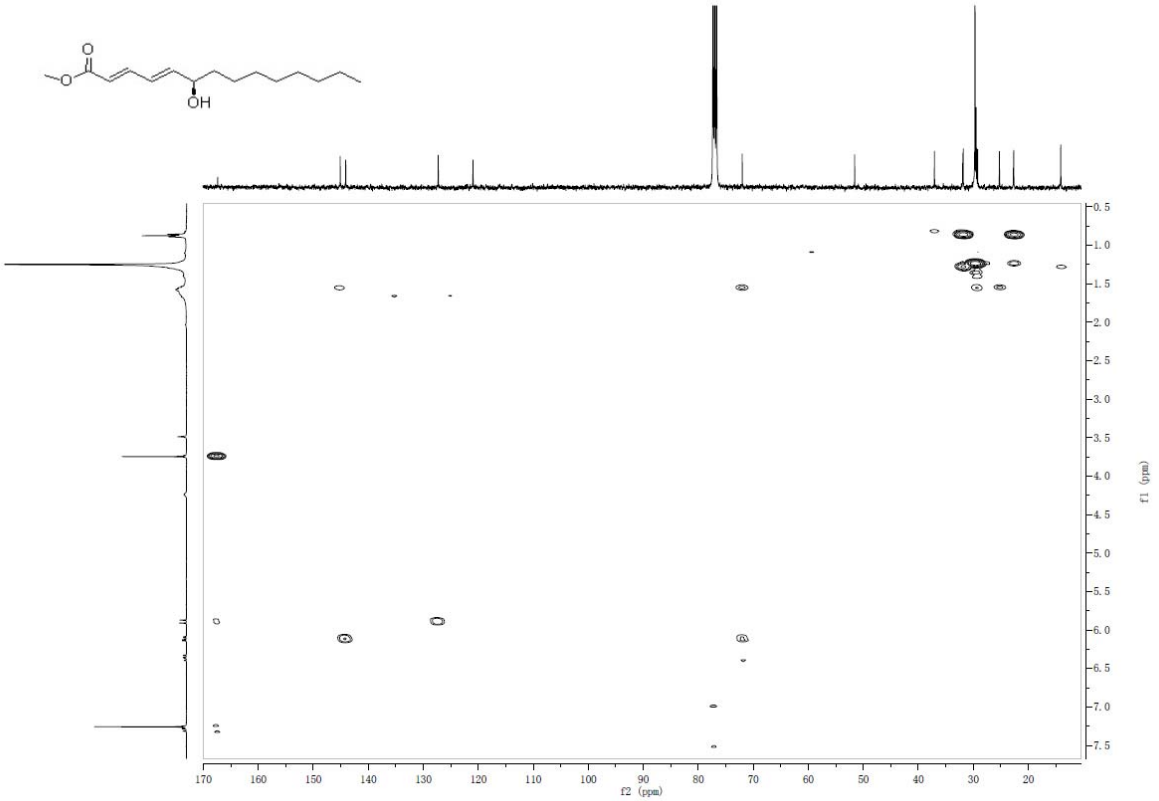


Figure S36. ^{1}H–^{1}H COSY spectrum of **1a** in $CDCl_3$

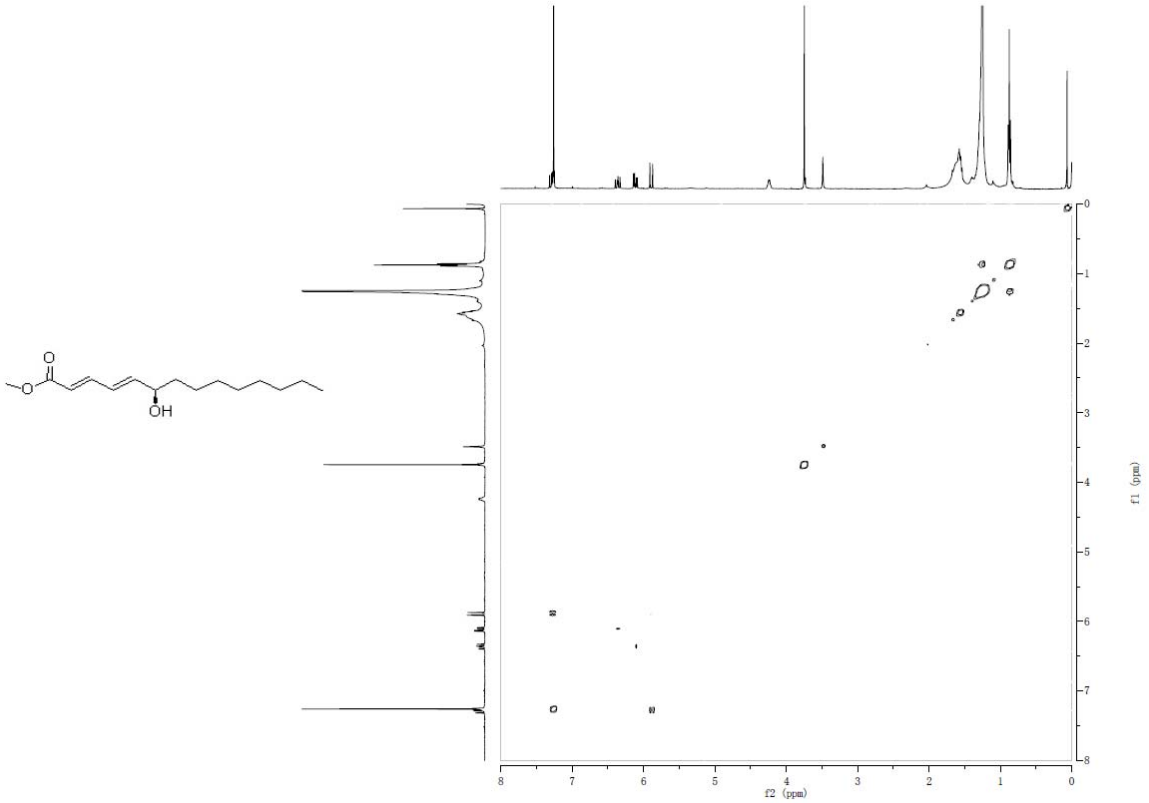


Figure S37. IR spectrum of **1a**

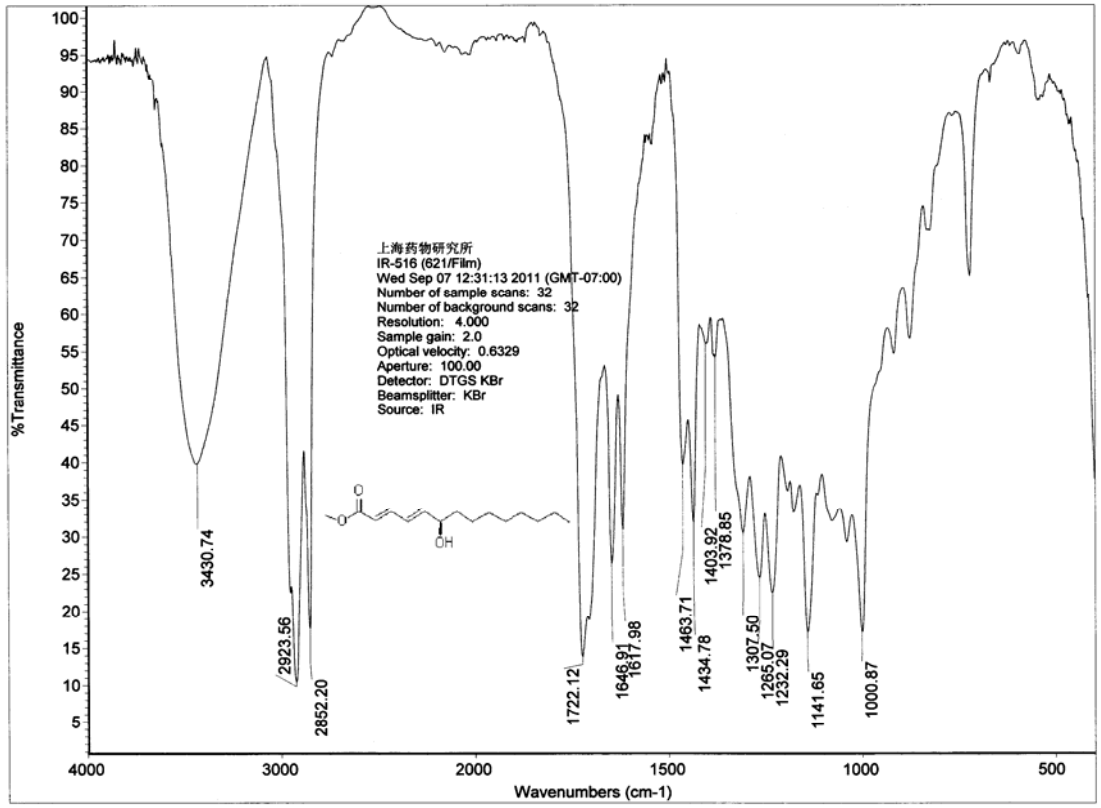


Figure S38. LR-EI-MS spectrum of **1a**

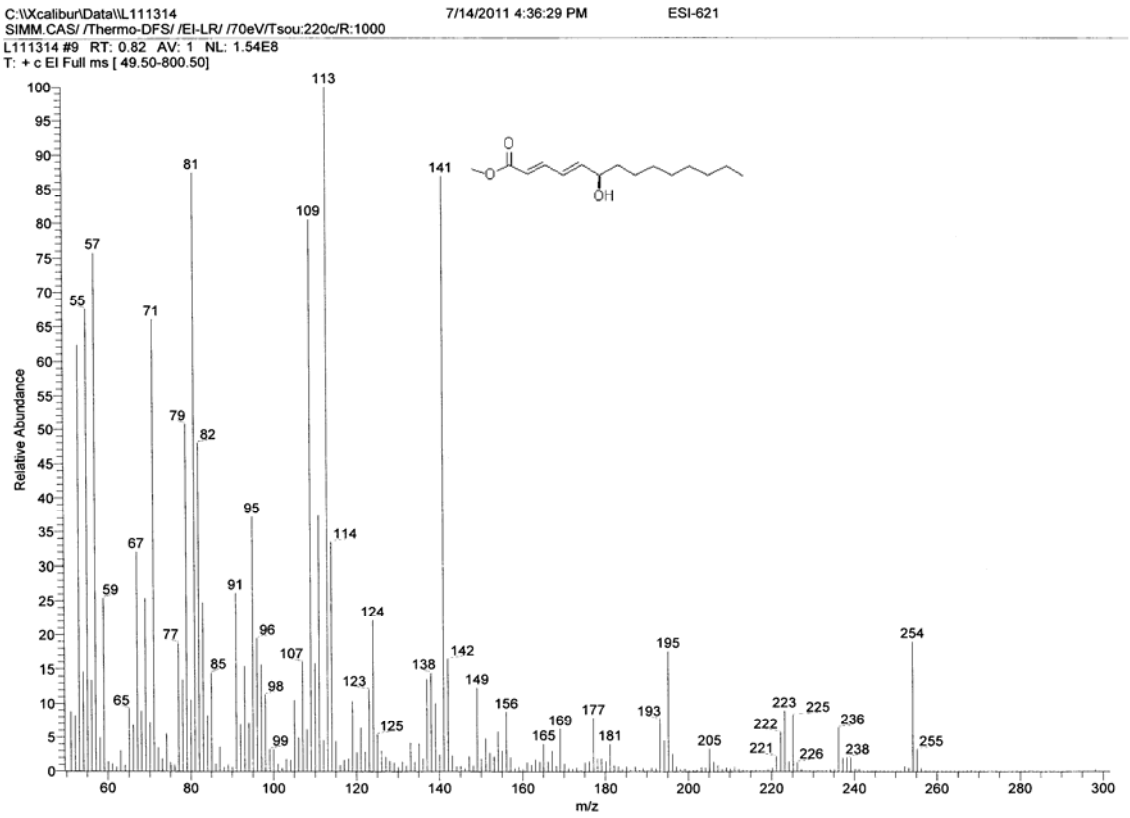


Figure S39. HR-EI-MS spectrum of **1a**

LIST: h110428-c1 15-Jul-11 Elapse: 04:48.1 23
Samp: ESI-621 Start : 10:35:14 27
Comm: Finnigan/MAT95//70eV/Tsou:220c/R:10000
Mode: EI +VE +LMR BSCAN (EXP) UP HR NRM Study : S/N: PT200712-01-01
Oper: WANG_J@SIMM.CAS Inlet :
Limt: (0) . .
: (353) C17.H100.O3
Peak: 1000.00 mmu R+D: -2.0 > 60.0
Data: CMASS : converted

Mass	6264 Intensity		%RA	%RIC	(mmu) Delta	R+D	Composition
71.08631	*	34292	16.42	0.32	-0.2	0.5	C5.H11
77.04053	*	9320	4.46	0.09	-1.4	4.5	C6.H5
78.04808	*	13731	6.58	0.13	-1.1	4.0	C6.H6
79.05561	*	39130	18.74	0.37	-0.8	3.5	C6.H7
81.03365	*	50656	24.26	0.48	0.4	3.5	C5.H5.O
81.06945	*	11170	5.35	0.11	1.0	2.5	C6.H9
82.04066	*	10743	5.14	0.10	1.2	3.0	C5.H6.O
82.07815	*	7755	3.71	0.07	0.1	2.0	C6.H10
83.08576	*	6616	3.17	0.06	0.3	1.5	C6.H11
85.10143	*	14869	7.12	0.14	0.3	0.5	C6.H13
91.05536	*	22695	10.87	0.21	-0.6	4.5	C7.H7
93.07155	*	6758	3.24	0.06	-1.1	3.5	C7.H9
95.05002	*	14869	7.12	0.14	-0.3	3.5	C6.H7.O
95.08676	*	7114	3.41	0.07	-0.7	2.5	C7.H11
96.05701	*	7257	3.48	0.07	0.5	3.0	C6.H8.O
105.0672	*	8324	3.99	0.08			
107.0470	*	10600	5.08	0.10	2.7	4.5	C7.H7.O
109.0263	*	36285	17.38	0.34	2.7	4.5	C6.H5.O2
111.0424	*	20205	9.68	0.19	2.2	3.5	C6.H7.O2
113.0588	*	208817	100.00	1.98	1.5	2.5	C6.H9.O2
114.0634	*	12308	5.89	0.12			
119.0494	*	10245	4.91	0.10	0.3	5.5	C8.H7.O
124.0507	*	9604	4.60	0.09	1.7	4.0	C7.H8.O2
124.1238	*	6403	3.07	0.06	1.4	2.0	C9.H16
137.0594	*	9462	4.53	0.09	0.8	4.5	C8.H9.O2
138.0676	*	20134	9.64	0.19	0.5	4.0	C8.H10.O2
139.0398	*	7897	3.78	0.07	-0.2	4.5	C7.H7.O3
141.0554	*	28530	13.66	0.27	-0.3	3.5	C7.H9.O3
141.1282	*	81961	39.25	0.78	-0.3	1.5	C9.H17.O
149.0236	*	17786	8.52	0.17	0.3	6.5	C8.H5.O3
154.0644	*	8537	4.09	0.08	-1.4	4.0	C8.H10.O3
195.1754	*	7826	3.75	0.07	-0.5	2.5	C13.H23.O
236.1772	*	16292	7.80	0.15	0.5	4.0	C15.H24.O2
254.1875	*	12735	6.10	0.12	0.7	3.0	C15.H26.O3
378.9728	*	12521	6.00	0.12			
381.9852	*	10031	4.80	0.10			

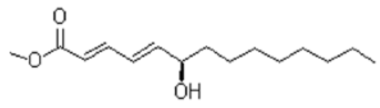


Figure S40. ^{1}H NMR spectrum of **2a** in $CDCl_3$

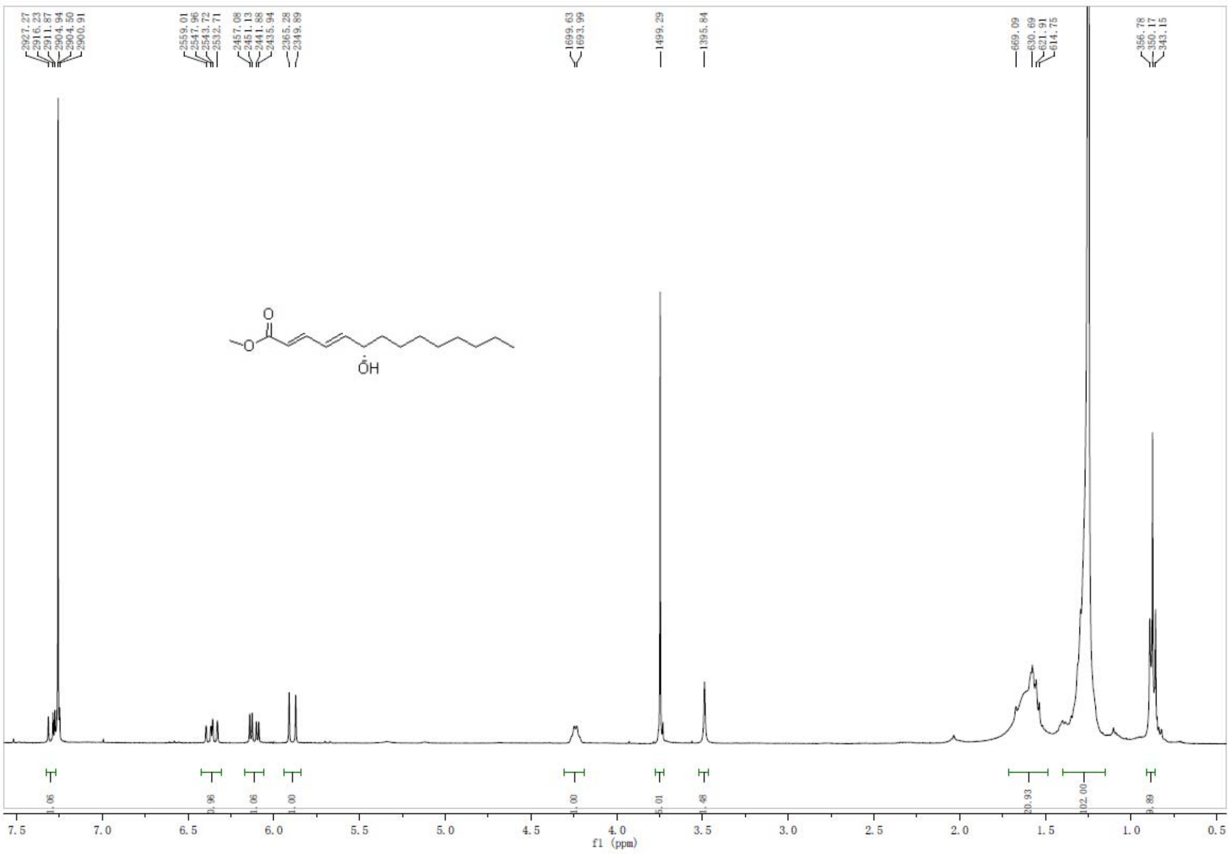


Figure S41. LR-EI-MS spectrum of **2a**

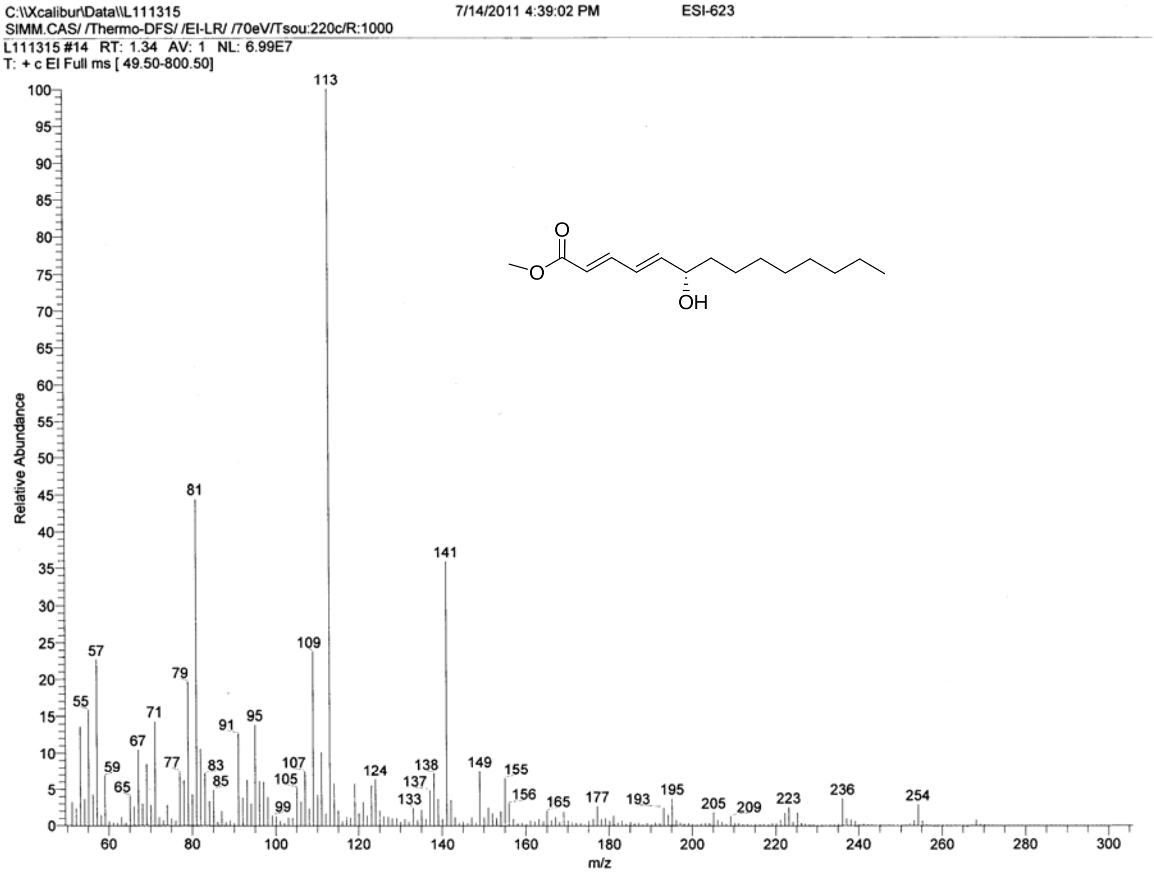
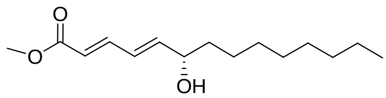


Figure S42. HR-EI-MS spectrum of **2a**




```
LIST:  h110429-c1                                15-Jul-11   Elapse:   05:23.4      25
Samp:  ESI-623                                               Start :   10:46:01     25
Comm:  Finnigan/MAT95//70eV/Tsou:220c/R:10000
Mode:  EI +VE +LMR  BSCAN (EXP) UP HR NRM                    Study :   S/N: PT200712-01-01
Oper:  WANG_J@SIMM.CAS                                       Inlet :
Limt:  (   0)     .    .
    :  ( 353 )  C17.H100.O3
Peak:  1000.00  mmu     R+D:  -2.0 > 60.0
Data:  CMASS : converted
```

Mass		Intensity (17705)	%RA	%RIC	Delta (mmu)	R+D	Composition
71.05041	*	24688	2.79	0.17	-0.7	1.5	C4.H7.O
71.08650	*	113550	12.83	0.78	-0.4	0.5	C5.H11
77.04037	*	23834	2.69	0.16	-1.2	4.5	C6.H5
79.05618	*	76483	8.64	0.53	-1.4	3.5	C6.H7
81.03432	*	283023	31.97	1.95	-0.3	3.5	C5.H5.O
81.07026	*	60617	6.85	0.42	0.2	2.5	C6.H9
82.04105	*	55850	6.31	0.39	0.8	3.0	C5.H6.O
82.07797	*	18071	2.04	0.12	0.3	2.0	C6.H10
83.04951	*	25328	2.86	0.17	0.2	2.5	C5.H7.O
83.08592	*	32016	3.62	0.22	0.2	1.5	C6.H11
85.10069	*	26609	3.01	0.18	1.0	0.5	C6.H13
91.05431	*	47241	5.34	0.33	0.5	4.5	C7.H7
93.07041	*	29526	3.34	0.20	0.0	3.5	C7.H9
95.04939	*	38419	4.34	0.27	0.3	3.5	C6.H7.O
95.08527	*	37921	4.28	0.26	0.8	2.5	C7.H11
96.05650	*	29170	3.29	0.20	1.0	3.0	C6.H8.O
97.10100	*	19352	2.19	0.13	0.7	1.5	C7.H13
98.03636	*	20490	2.31	0.14	0.4	3.0	C5.H6.O2
105.0689	*	20988	2.37	0.14	1.6	4.5	C8.H9
107.0475	*	20632	2.33	0.14	2.2	4.5	C7.H7.O
109.0271	*	177156	20.01	1.22	1.9	4.5	C6.H5.O2
110.0337	*	22197	2.51	0.15			
111.0428	*	35787	4.04	0.25	1.8	3.5	C6.H7.O2
113.0594	*	885284	100.00	6.11	0.8	2.5	C6.H9.O2
114.0634	*	48593	5.49	0.34			
117.0700	*	22340	2.52	0.15	0.4	5.5	C9.H9
123.1179	*	19636	2.22	0.14	-0.5	2.5	C9.H15
124.0521	*	37921	4.28	0.26	0.3	4.0	C7.H8.O2
124.1241	*	21486	2.43	0.15	1.1	2.0	C9.H16
131.0486	*	21628	2.44	0.15	1.1	6.5	C9.H7.O
137.0610	*	19565	2.21	0.14	-0.7	4.5	C8.H9.O2
138.0678	*	29241	3.30	0.20	0.3	4.0	C8.H10.O2
141.0553	*	133685	15.10	0.92	-0.1	3.5	C7.H9.O3
141.1280	*	329980	37.27	2.28	-0.1	1.5	C9.H17.O
142.1322	*	30308	3.42	0.21			
156.0775	*	26395	2.98	0.18	1.2	3.0	C8.H12.O3
163.0735	*	35644	4.03	0.25	2.4	5.5	C10.H11.O2
177.1644	*	23478	2.65	0.16	-0.1	3.5	C13.H21
195.1751	*	36000	4.07	0.25	-0.2	2.5	C13.H23.O
204.0785	*	38134	4.31	0.26	0.1	7.0	C12.H12.O3
236.1780	*	21842	2.47	0.15	-0.4	4.0	C15.H24.O2
254.1880	*	48308	5.46	0.33	0.2	3.0	C15.H26.O3
257.1906	*	55637	6.28	0.38			
288.2094	*	23407	2.64	0.16			
289.2178	*	53218	6.01	0.37			
316.2056	*	74135	8.37	0.51			
317.2108	*	21771	2.46	0.15			
345.2056	*	42688	4.82	0.29			
377.2374	*	28885	3.26	0.20			

Figure S43. ^{1}H NMR spectrum of **5** in C_5D_5N

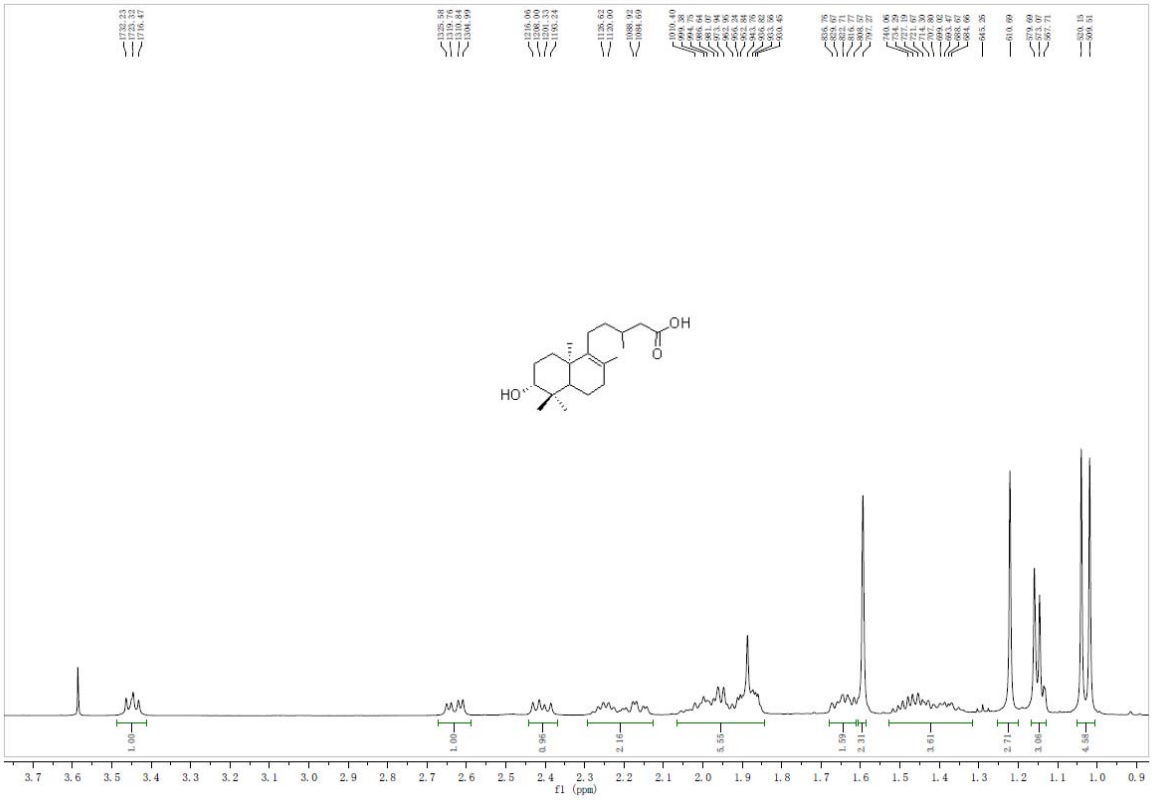


Figure S44. ^{13}C NMR spectrum of **5** in C_5D_5N

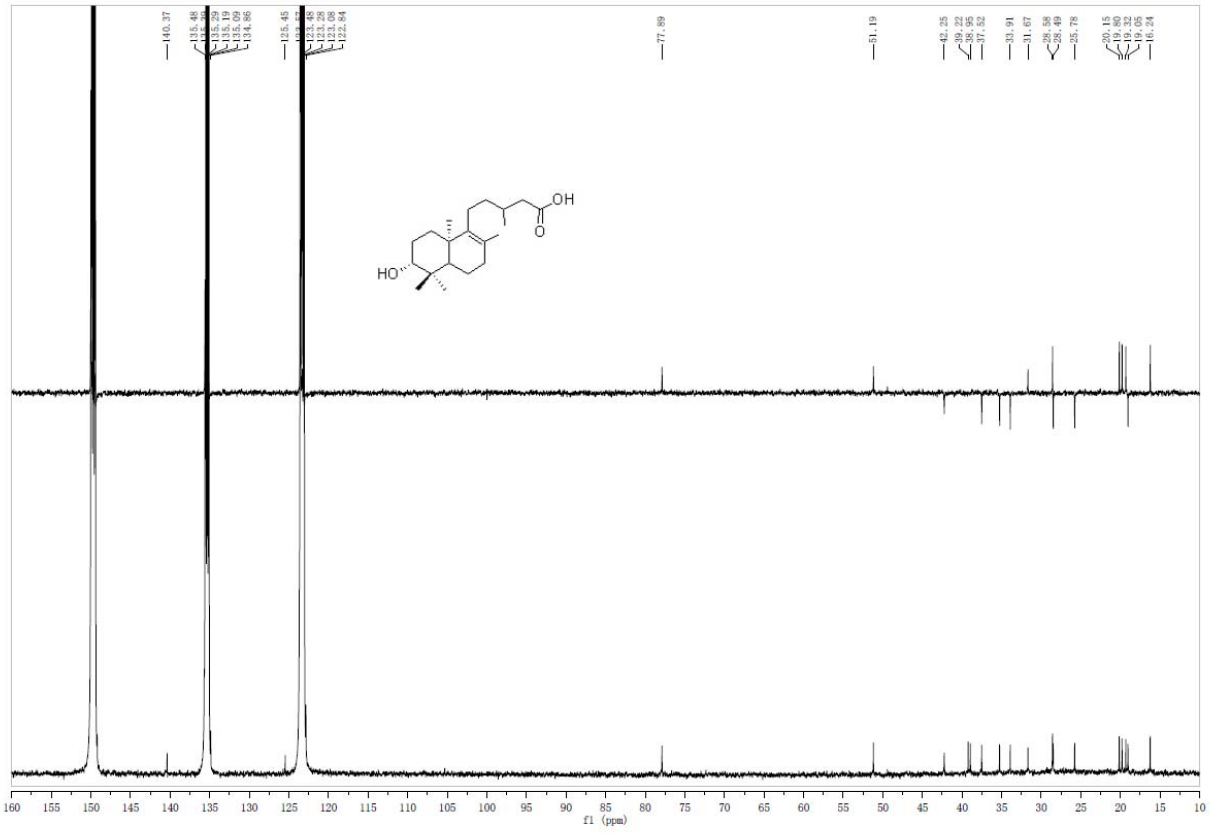


Figure S45. HSQC spectrum of **5** in C_5D_5N

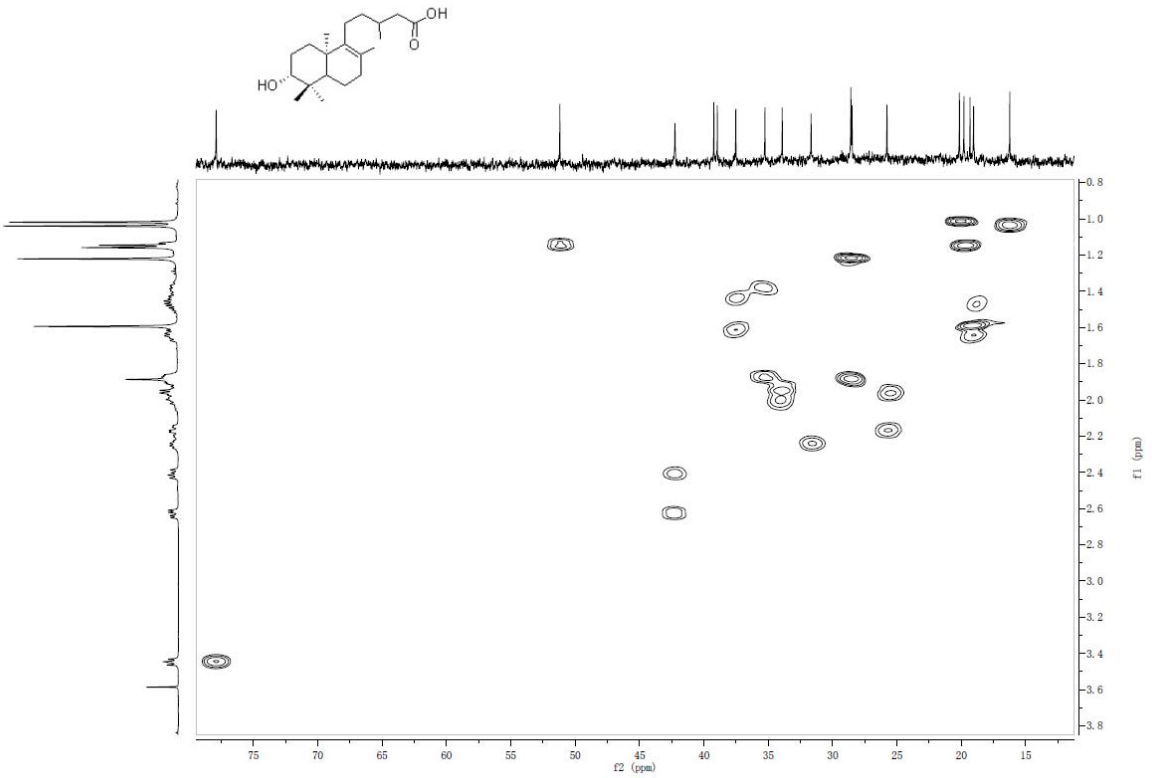


Figure S46. HMBC spectrum of **5** in C_5D_5N

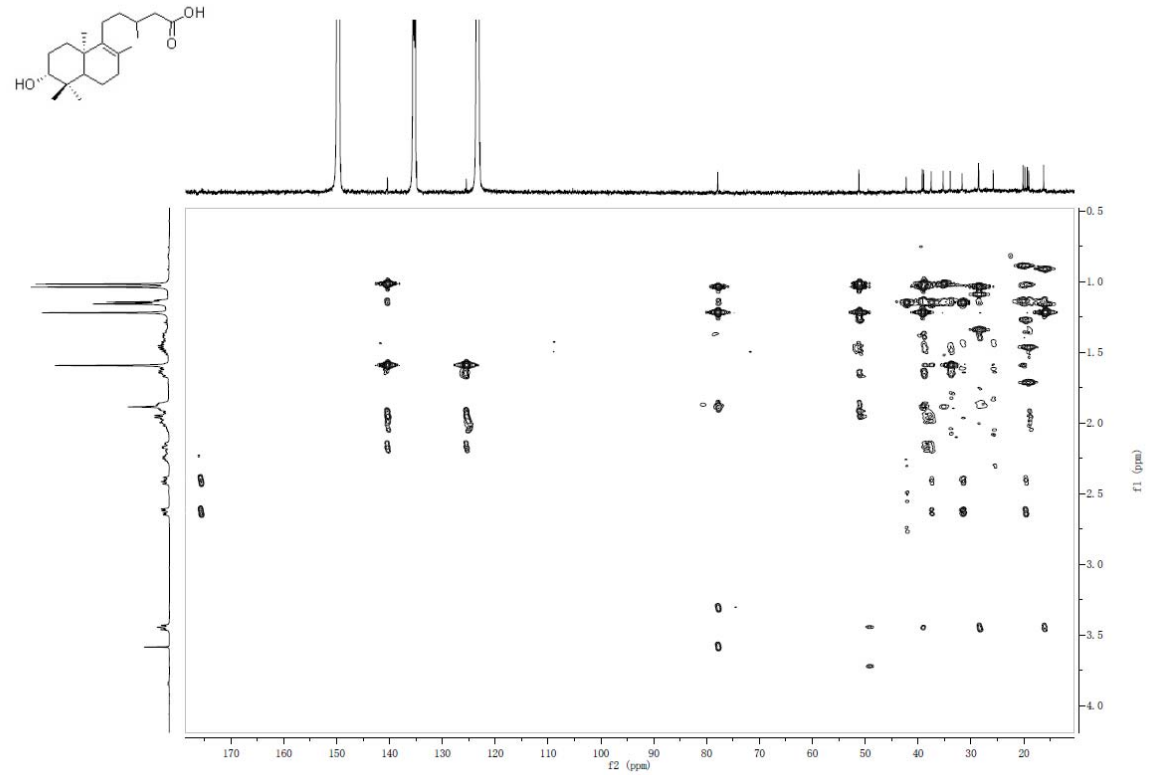


Figure S47. $^{1}H–^{1}H$ COSY spectrum of **5** in C_5D_5N

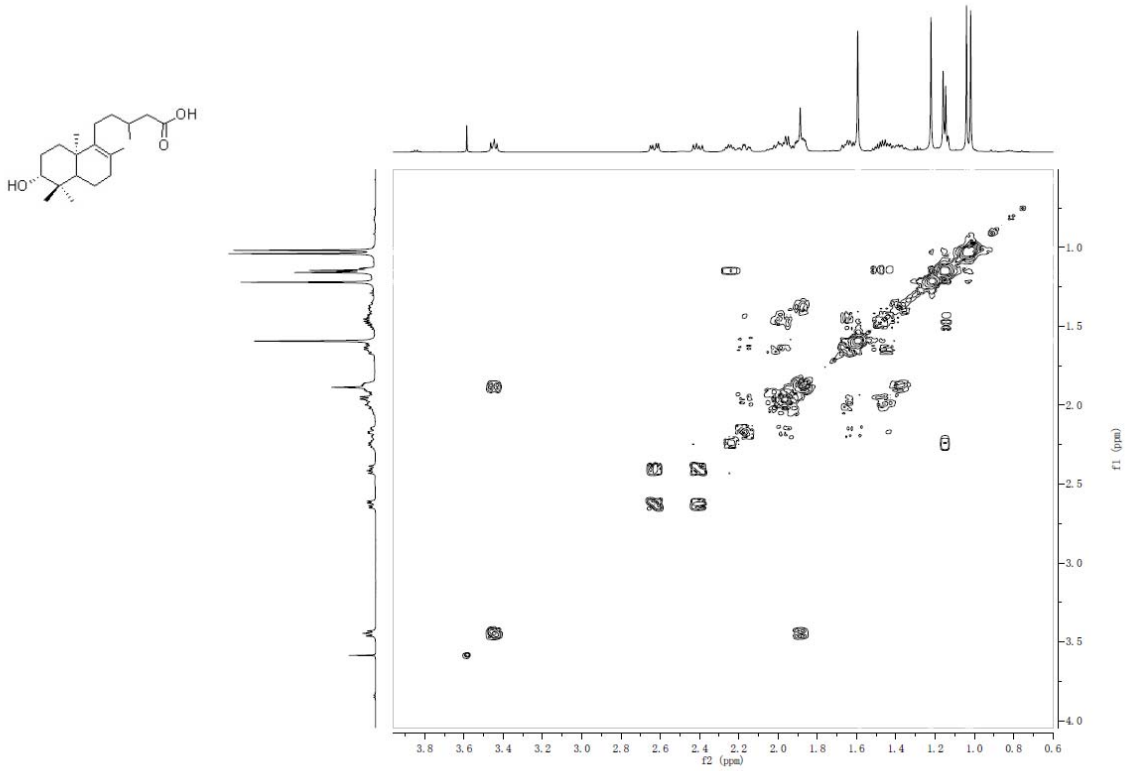


Figure S48. ROESY spectrum of **5** in C_5D_5N

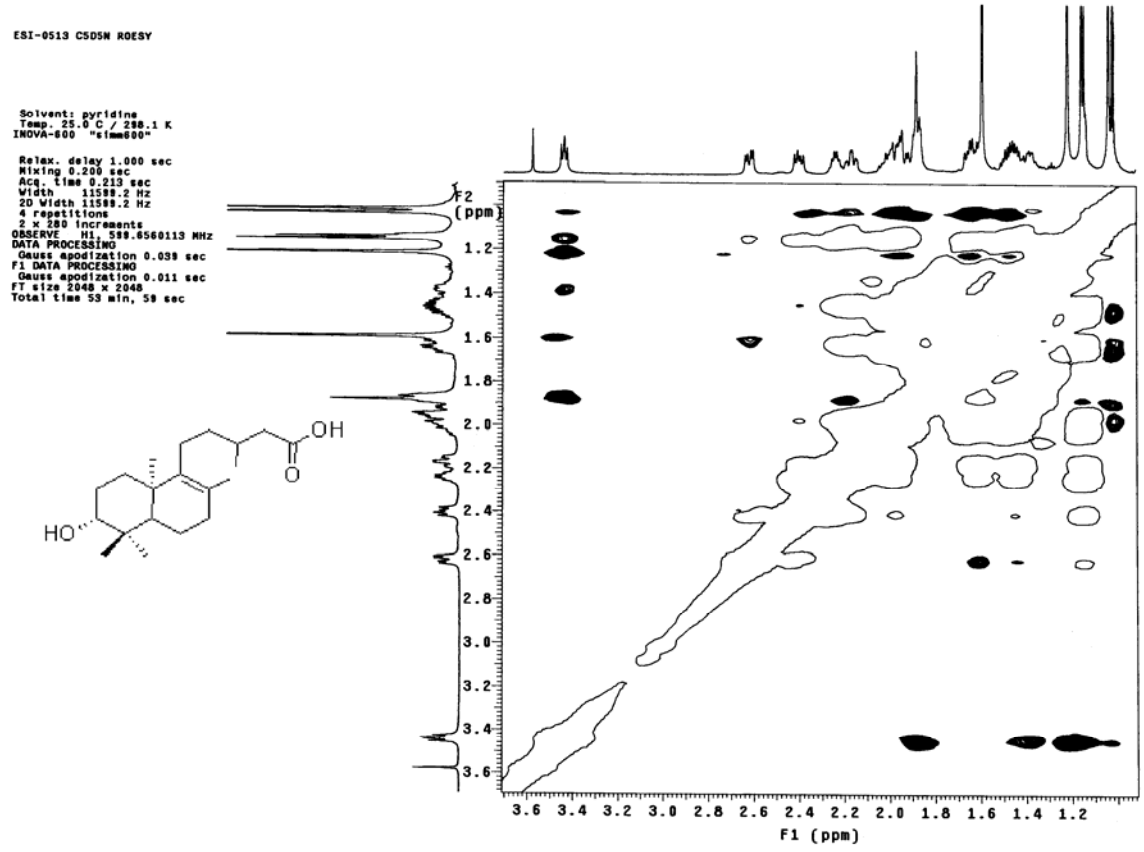


Figure S49. IR spectrum of **5**

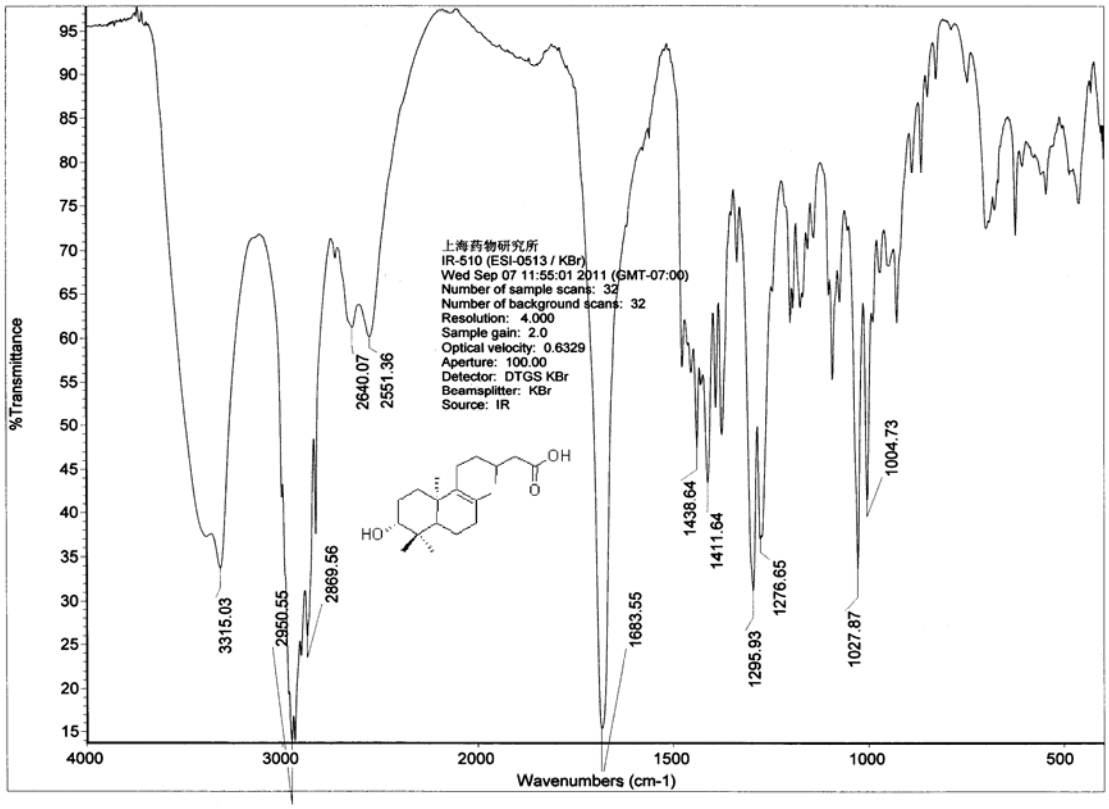


Figure S50. HR-ESI(–)MS spectrum of **5**

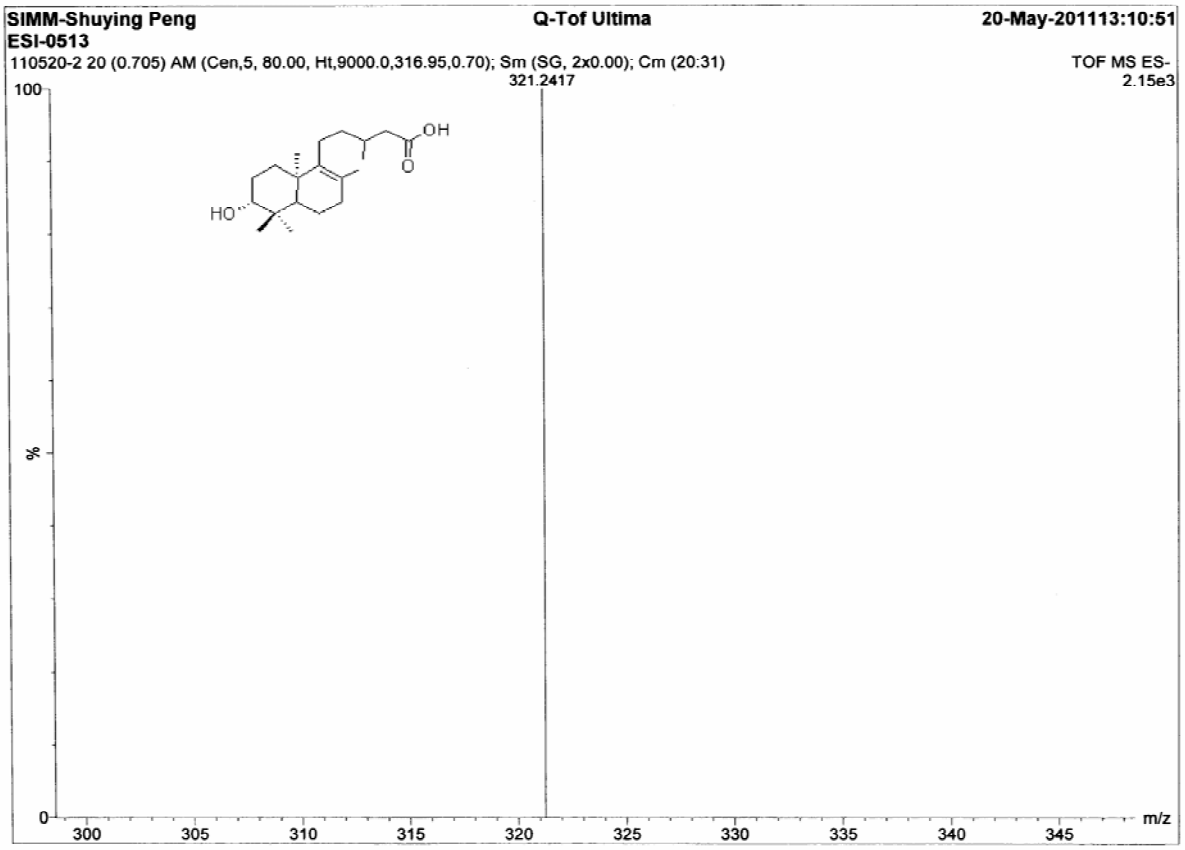


Figure S51. ^{1}H NMR spectrum of **6** in $CDCl_3$

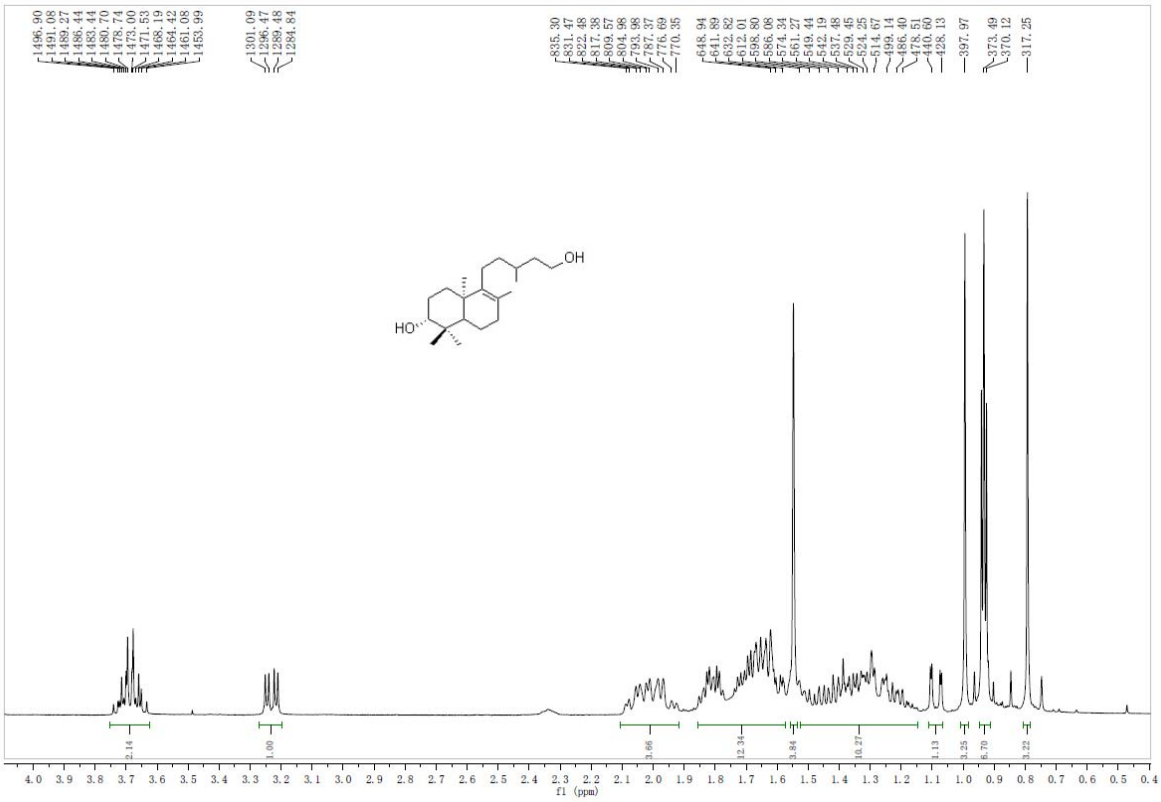


Figure S52. ^{13}C NMR spectrum of **6** in $CDCl_3$

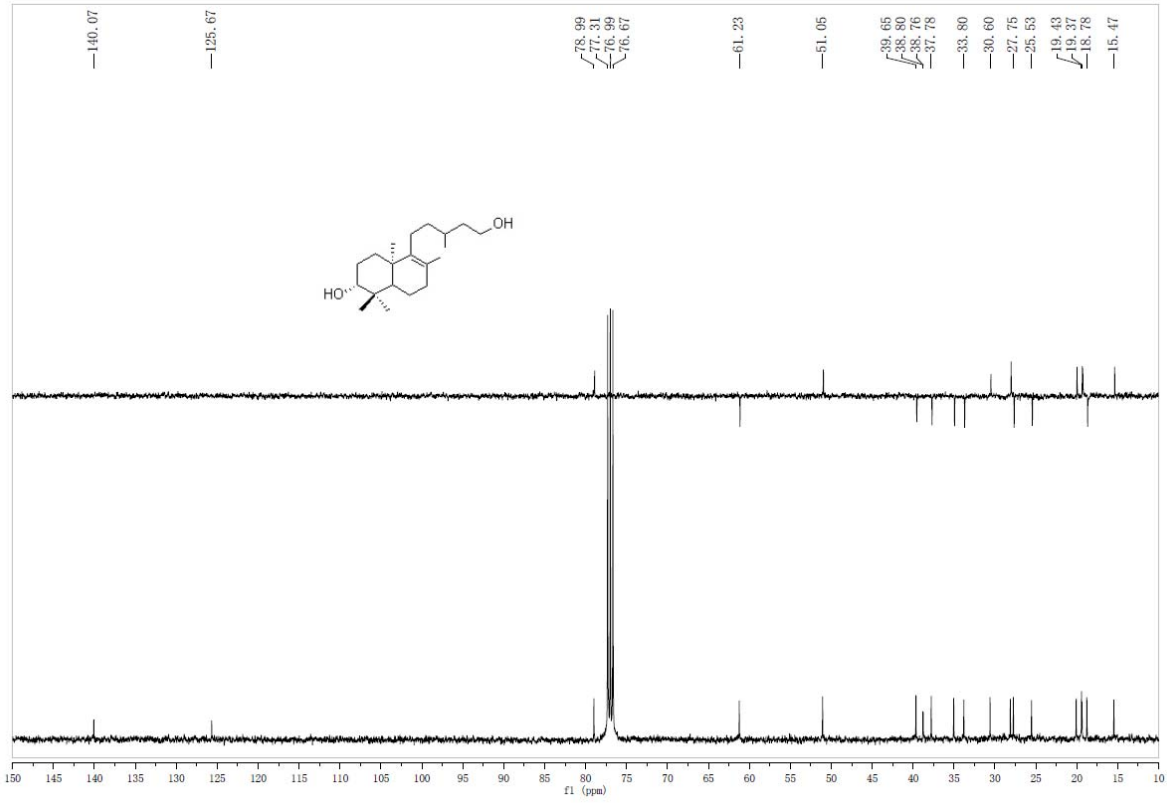


Figure S53. HSQC spectrum of **6** in $CDCl_3$

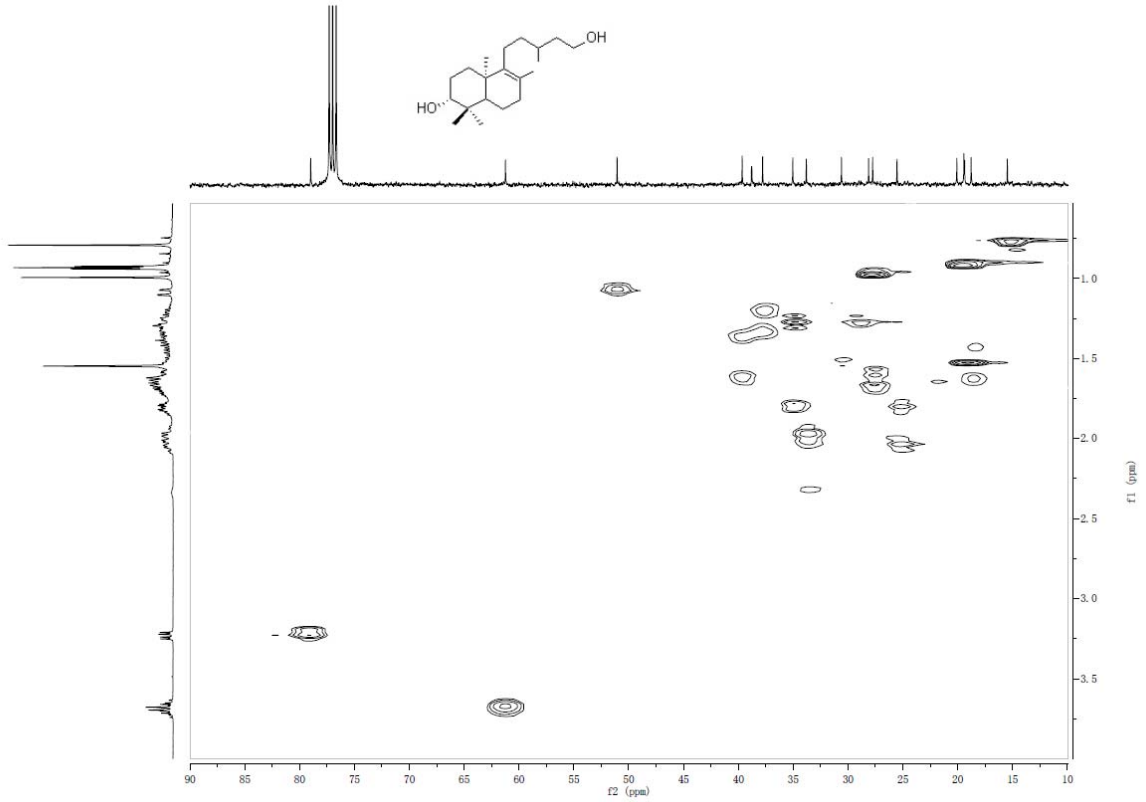


Figure S54. HMBC spectrum of **6** in $CDCl_3$

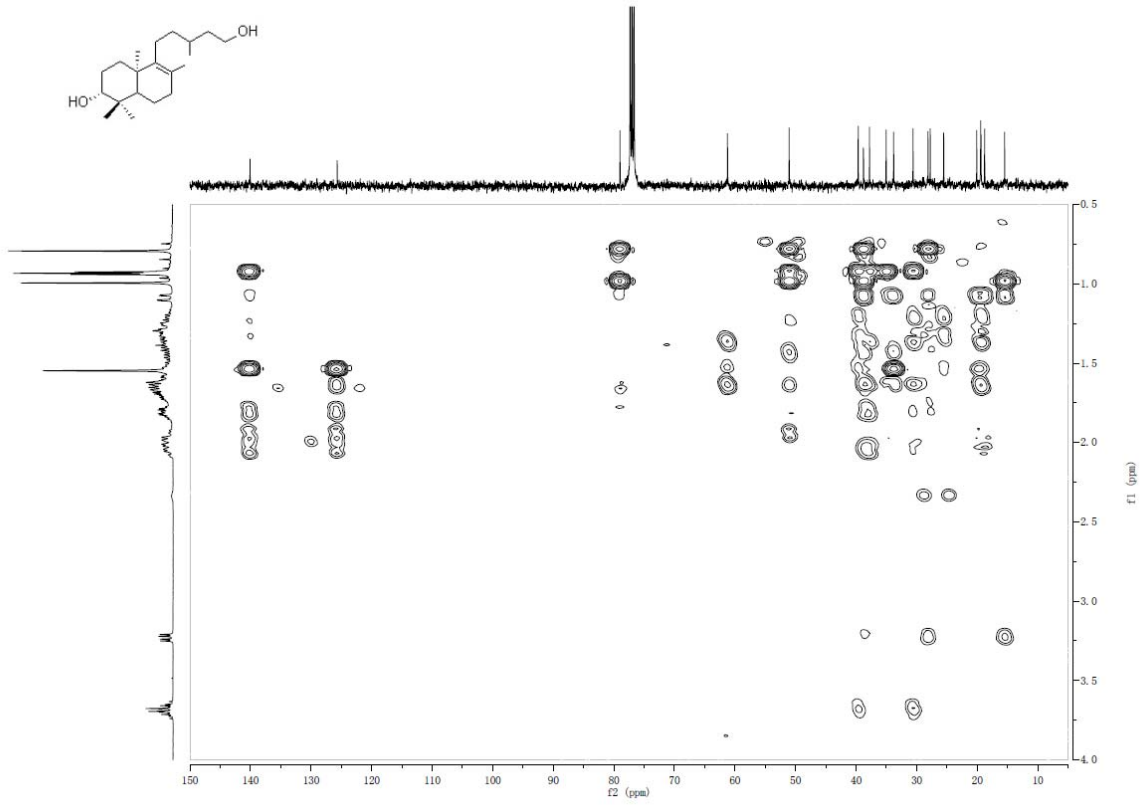


Figure S55. $^{1}H–^{1}H$ COSY spectrum of **6** in $CDCl_3$

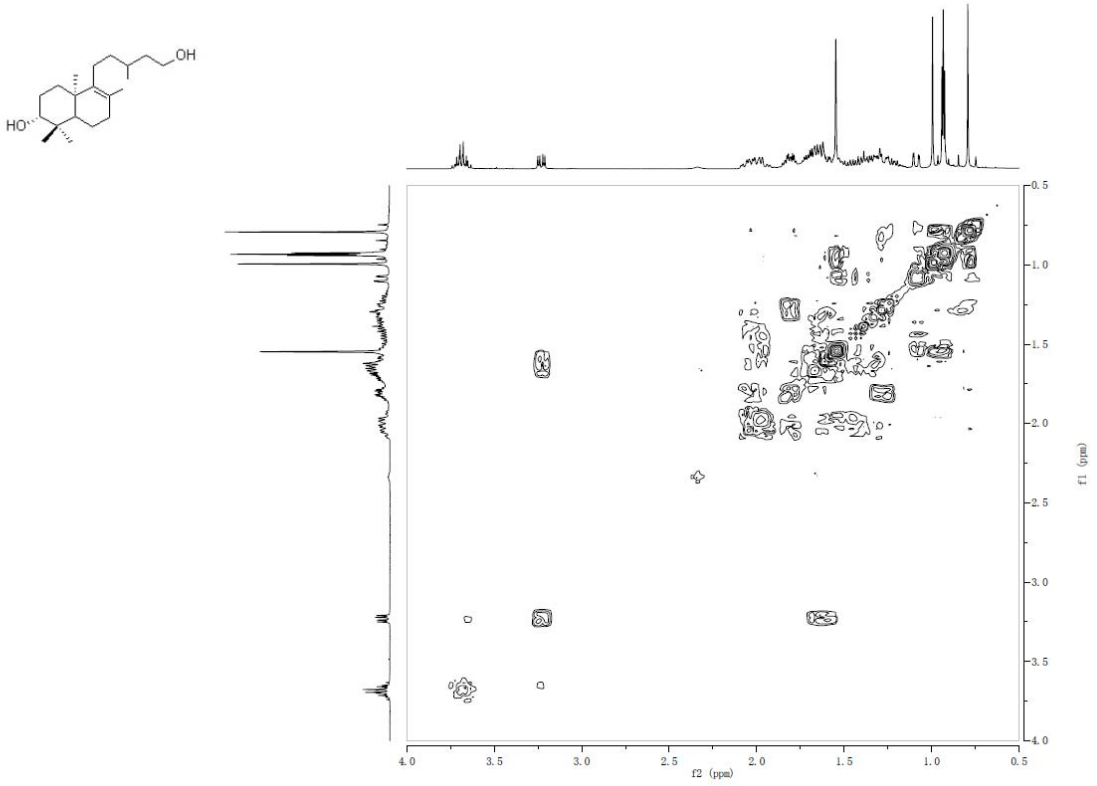


Figure S56. ROESY spectrum of **6** in $CDCl_3$

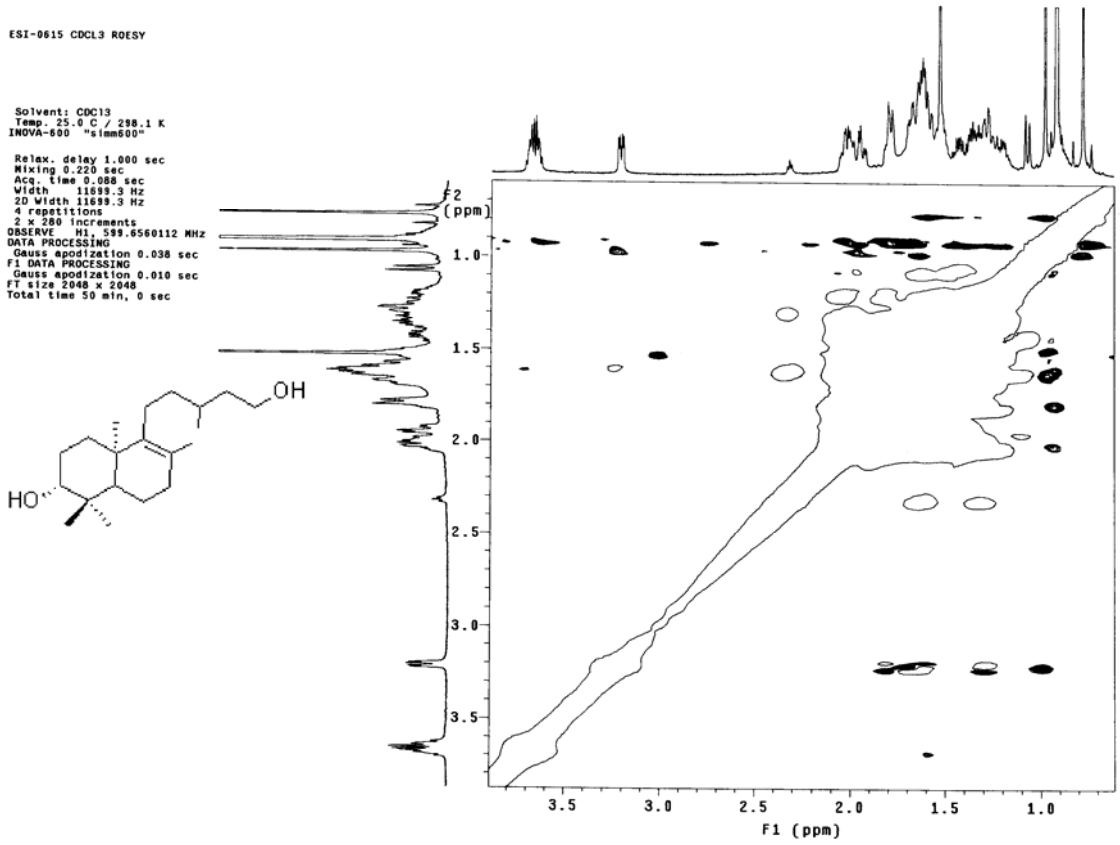


Figure S57. IR spectrum of **6**

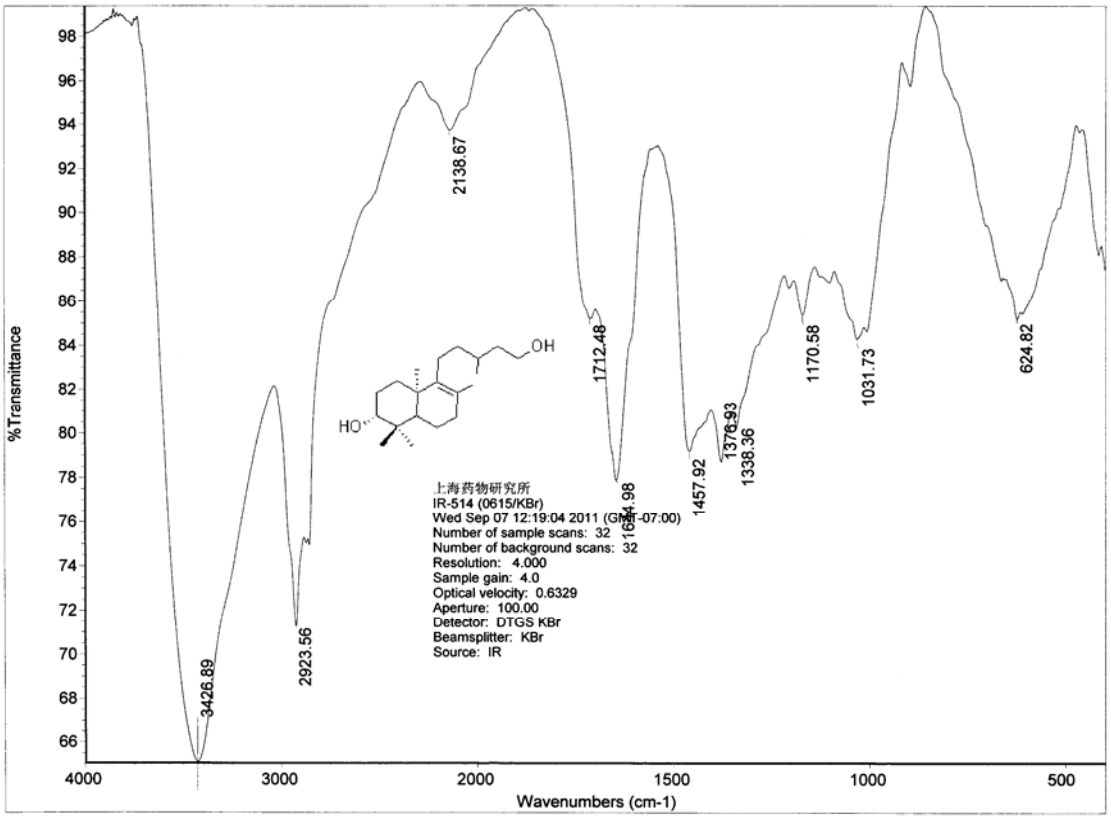


Figure S58. LR-EI-MS spectrum of **6**

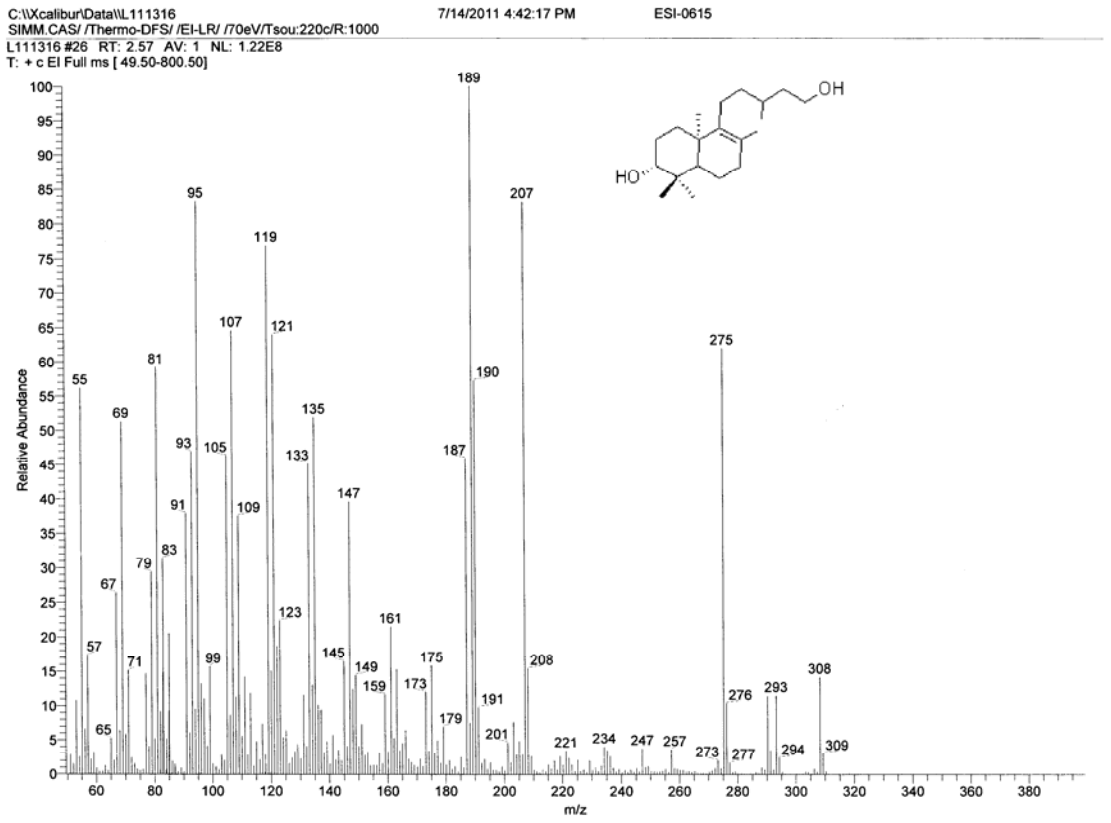
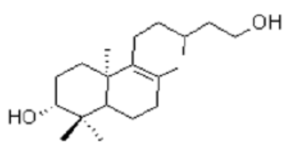


Figure S59. HR-EI-MS spectrum of **6**

```
LIST:  h110430-c1                                15-Jul-11   Elapse:  04:26.8       21
Samp:  ESI-061S                                              Start :  10:53:21      23
Comm:  Finnigan/MAT95//70eV/Tsou:220c/R:10000
Mode:  EI +VE +LMR  BSCAN (EXP) UP HR NRM                    Study :  S/N: PT200712-01-01
Oper:  WANG_J@SIMM.CAS                                       Inlet :
Limt:  (   0 )    .    .
    :  ( 397 )  C22.H100.O2
Peak:  1000.00  mmu     R+D:  -2.0 > 60.0
Data:  CMASS : converted
```



Mass		Intensity (25297)	%RA	%RIC	Delta (mmu)	R+D	Composition
71.03745	*	42688	6.75	0.29			
77.00537	*	44395	7.02	0.30	-2.6	5.5	C5.H.O
79.01763	*	80182	12.68	0.54	0.8	4.5	C5.H3.O
81.03145	*	165844	26.22	1.11	2.6	3.5	C5.H5.O
82.04279	*	28031	4.43	0.19	-0.9	3.0	C5.H6.O
83.05680	*	94270	14.91	0.63			
85.04508	*	74064	11.71	0.50			
91.05183	*	83953	13.27	0.56	2.9	4.5	C7.H7
93.07027	*	133329	21.08	0.90	0.2	3.5	C7.H9
94.07796	*	33439	5.29	0.22	0.3	3.0	C7.H10
95.08600	*	304083	48.08	2.04	0.1	2.5	C7.H11
96.09211	*	44965	7.11	0.30	1.8	2.0	C7.H12
99.08084	*	60190	9.52	0.40	0.2	1.5	C6.H11.O
105.0678	*	111914	17.70	0.75	2.6	4.5	C8.H9
107.0848	*	182777	28.90	1.23	1.3	3.5	C8.H11
108.0908	*	37138	5.87	0.25			
109.0997	*	100175	15.84	0.67	2.0	2.5	C8.H13
111.0787	*	54071	8.55	0.36	2.3	2.5	C7.H11.O
119.0866	*	237275	37.52	1.60	-0.5	4.5	C9.H11
120.0930	*	47810	7.56	0.32	0.9	4.0	C9.H12
121.1014	*	189465	29.96	1.27	0.3	3.5	C9.H13
122.1084	*	58554	9.26	0.39	1.2	3.0	C9.H14
123.1168	*	67732	10.71	0.46	0.6	2.5	C9.H15
131.0858	*	31020	4.90	0.21	0.2	5.5	C10.H11
133.1011	*	137385	21.72	0.92	0.7	4.5	C10.H13
134.1074	*	37850	5.98	0.25	2.2	4.0	C10.H14
135.0803	*	32727	5.17	0.22	0.7	4.5	C9.H11.O
135.1168	*	156096	24.68	1.05	0.6	3.5	C10.H15
136.1235	*	46032	7.28	0.31	1.7	3.0	C10.H16
145.1005	*	47597	7.53	0.32	1.2	5.5	C11.H13
147.1163	*	115187	18.21	0.77	1.1	4.5	C11.H15
148.1242	*	39415	6.23	0.26	1.0	4.0	C11.H16
149.0230	*	27320	4.32	0.18			
149.1321	*	32016	5.06	0.22	0.9	3.5	C11.H17
159.1167	*	33439	5.29	0.22	0.7	5.5	C12.H15
161.1325	*	73210	11.58	0.49	0.6	4.5	C12.H17
163.1485	*	39913	6.31	0.27	0.2	3.5	C12.H19
166.1345	*	29028	4.59	0.20	1.2	3.0	C11.H18.O
173.1317	*	31375	4.96	0.21	1.3	5.5	C13.H17
175.1487	*	54712	8.65	0.37	-0.1	4.5	C13.H19
187.1480	*	160650	25.40	1.08	0.6	5.5	C14.H19
189.1641	*	632427	100.00	4.25	0.2	4.5	C14.H21
190.1690	*	171891	27.18	1.16			
191.1747	*	27249	4.31	0.18			
203.1444	*	49589	7.84	0.33	-0.8	5.5	C14.H19.O
207.1749	*	313047	49.50	2.10	0.0	3.5	C14.H23.O
208.1796	*	46886	7.41	0.32			
275.2386	*	147772	23.37	0.99	-1.2	4.5	C19.H31.O
276.2402	*	26395	4.17	0.18			
290.2595	*	41621	6.58	0.28	1.5	4.0	C20.H34.O
293.2481	*	33154	5.24	0.22	0.0	3.5	C19.H33.O2
308.2708	*	59123	9.35	0.40	0.7	3.0	C20.H36.O2

Figure S60. ^{1}H NMR spectrum of **7** in $CDCl_3$

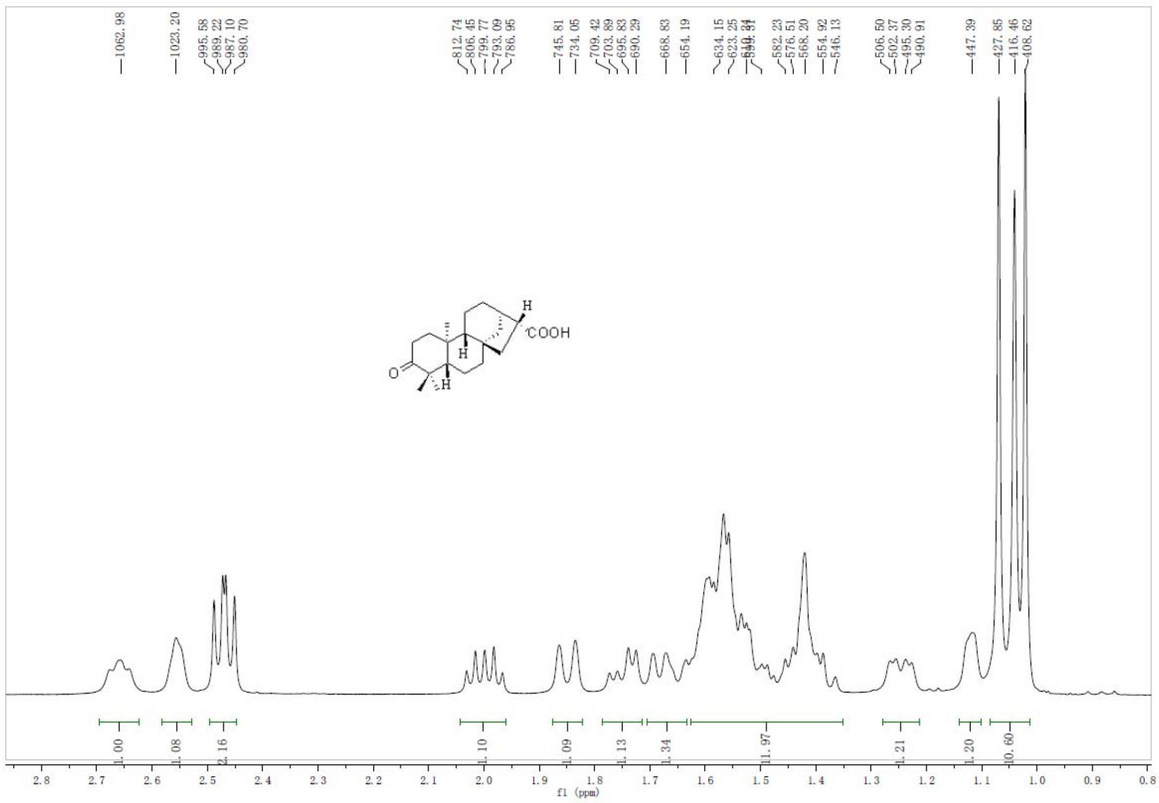


Figure S61. ^{13}C NMR spectrum of **7** in $CDCl_3$

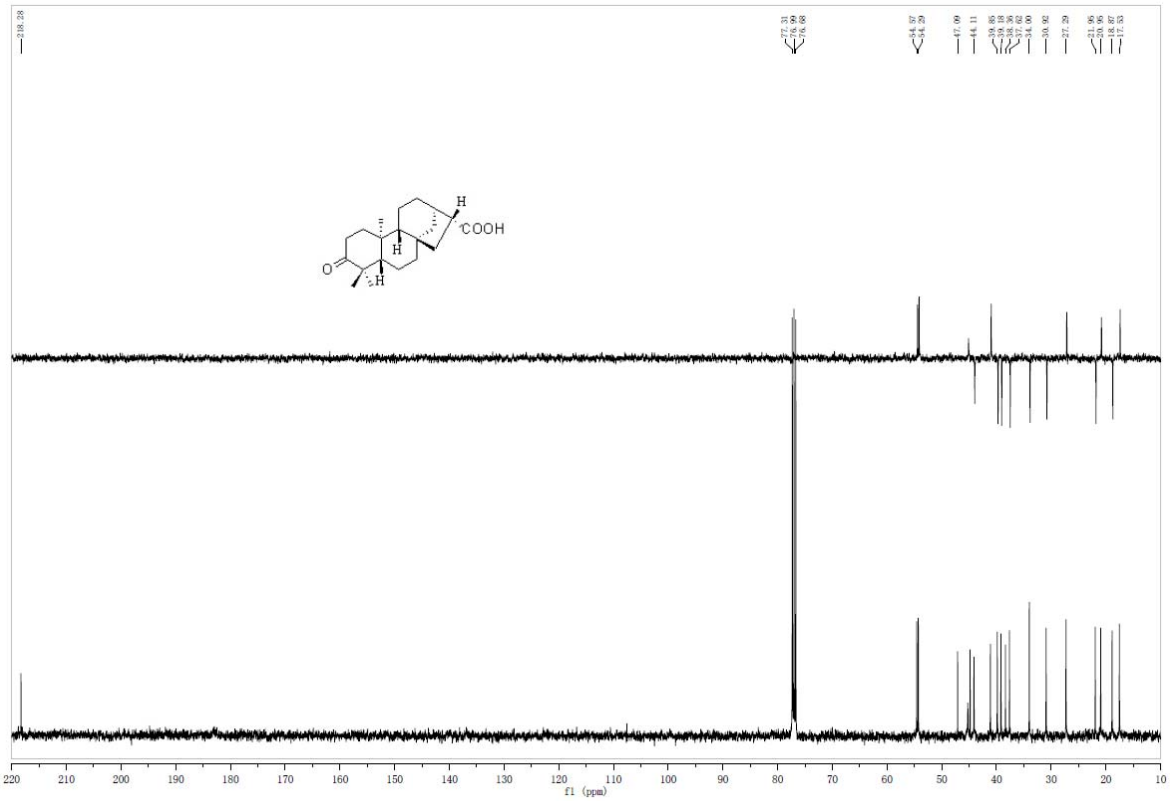


Figure S62. HSQC spectrum of **7** in $CDCl_3$

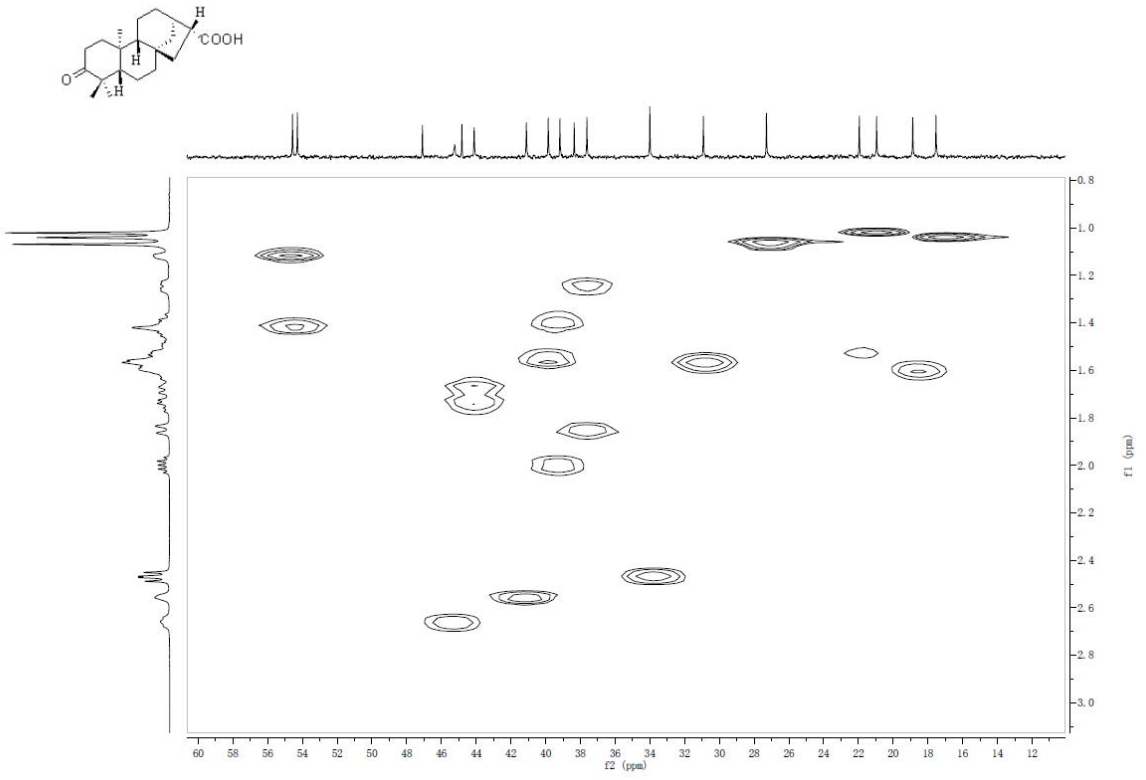


Figure S63. HMBC spectrum of **7** in $CDCl_3$

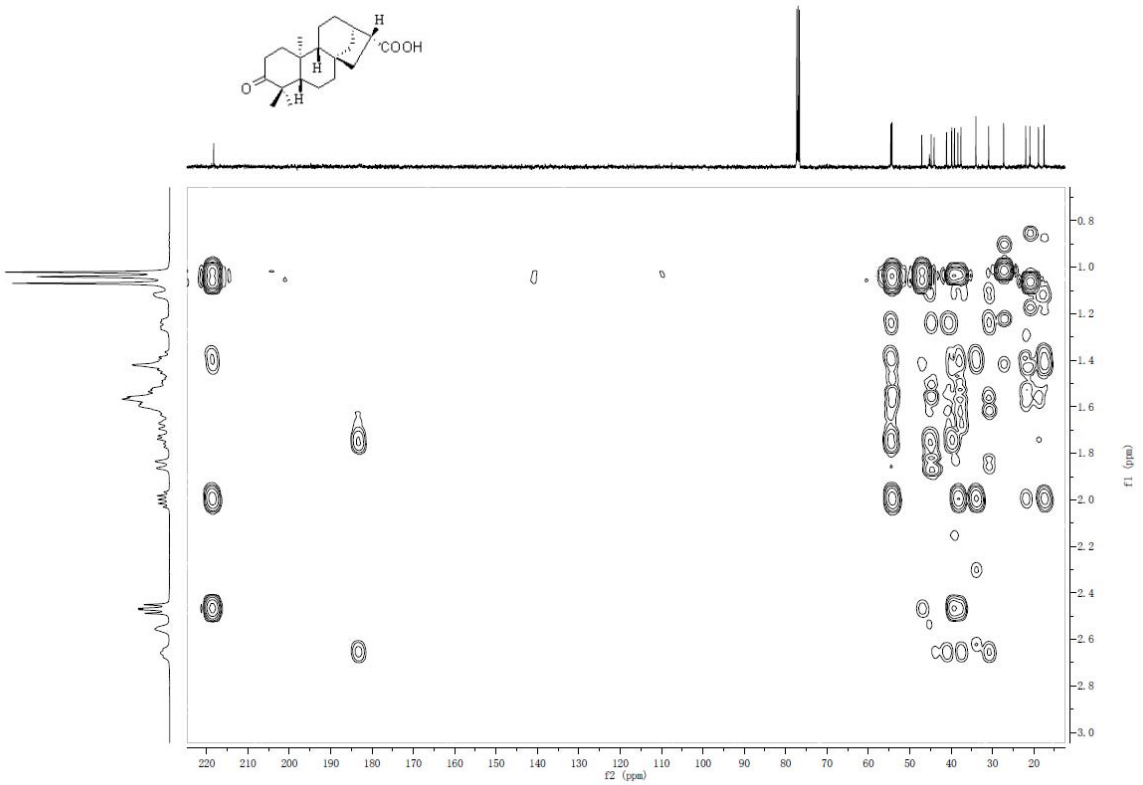


Figure S64. ^{1}H–^{1}H COSY spectrum of **7** in $CDCl_3$

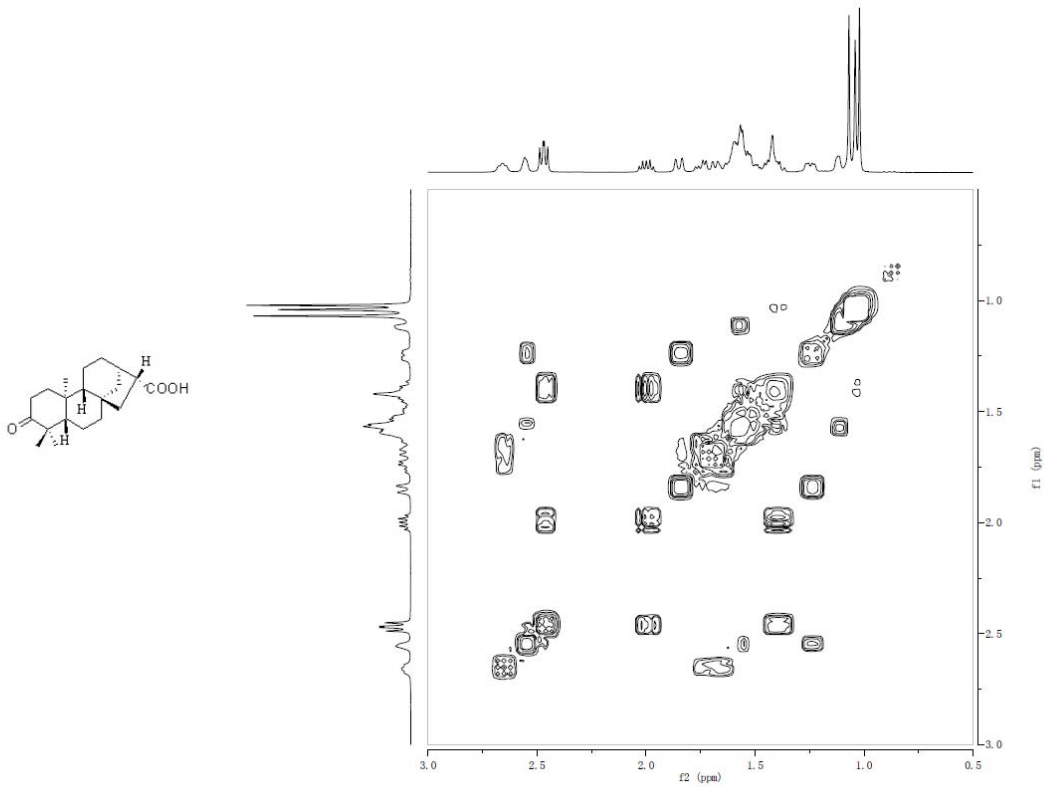


Figure S65. ROESY spectrum of **7** in $CDCl_3$

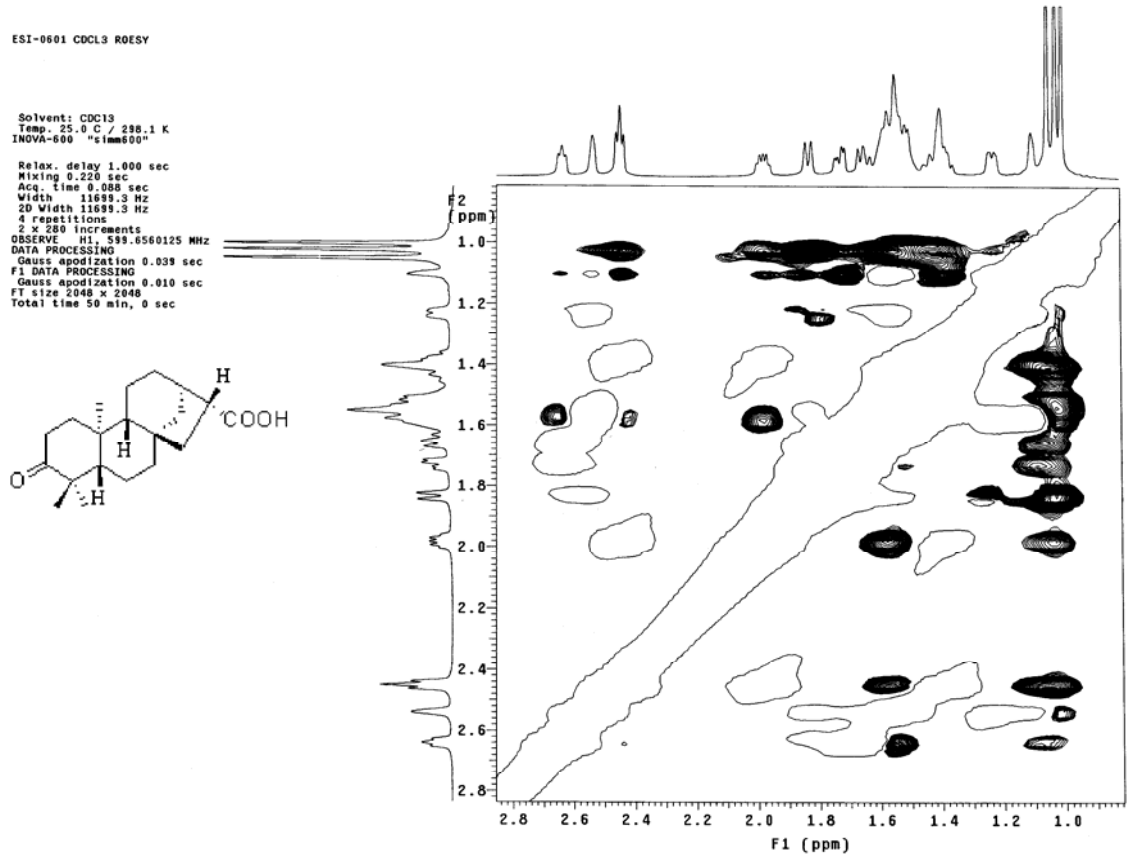


Figure S66. IR spectrum of **7**

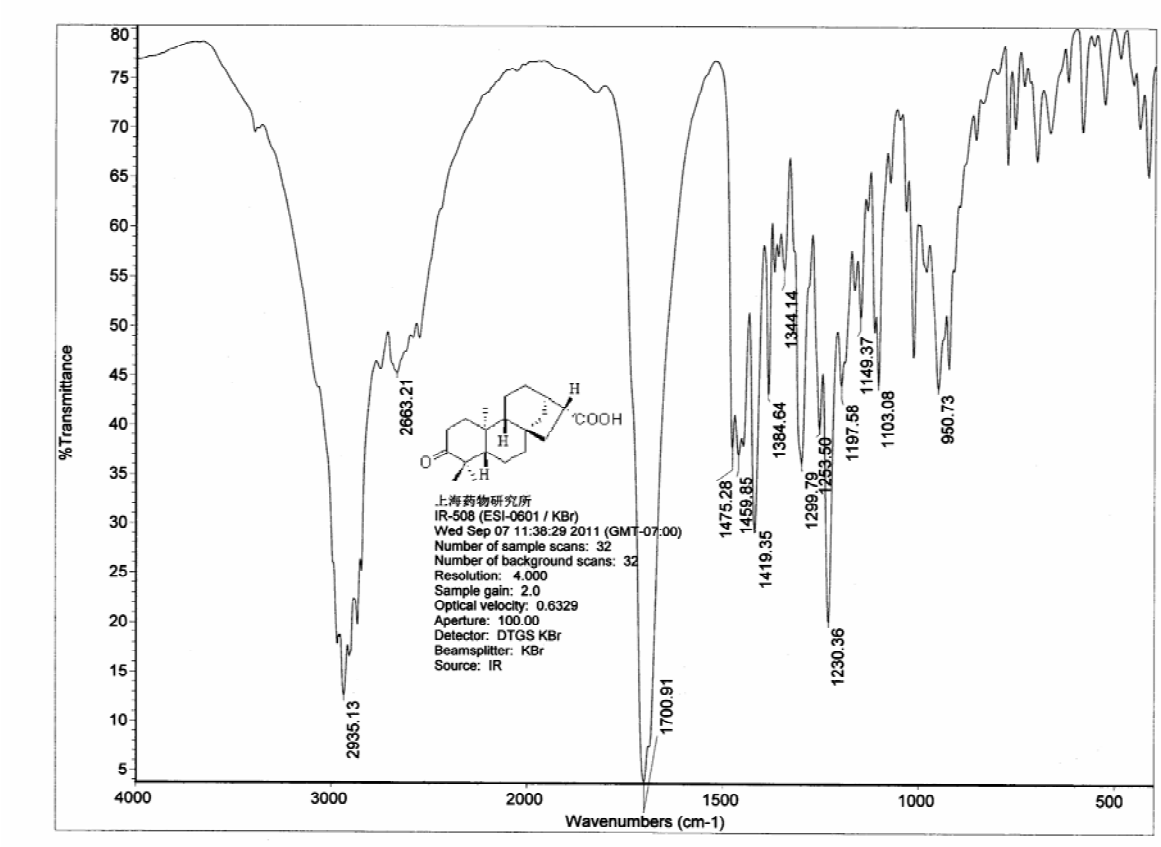


Figure S67. HR-ESI(+)MS spectrum of **7**

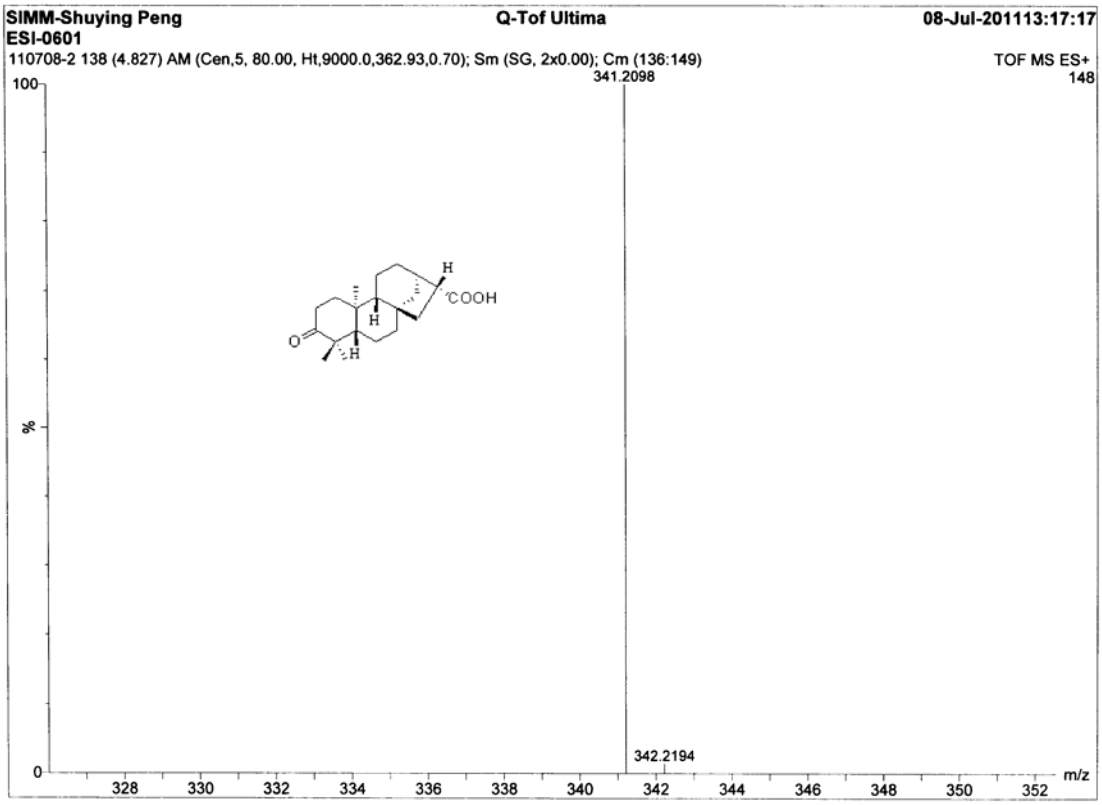


Figure S68. ^{1}H NMR spectrum of (*S*)-MTPA ester of compound **1a**

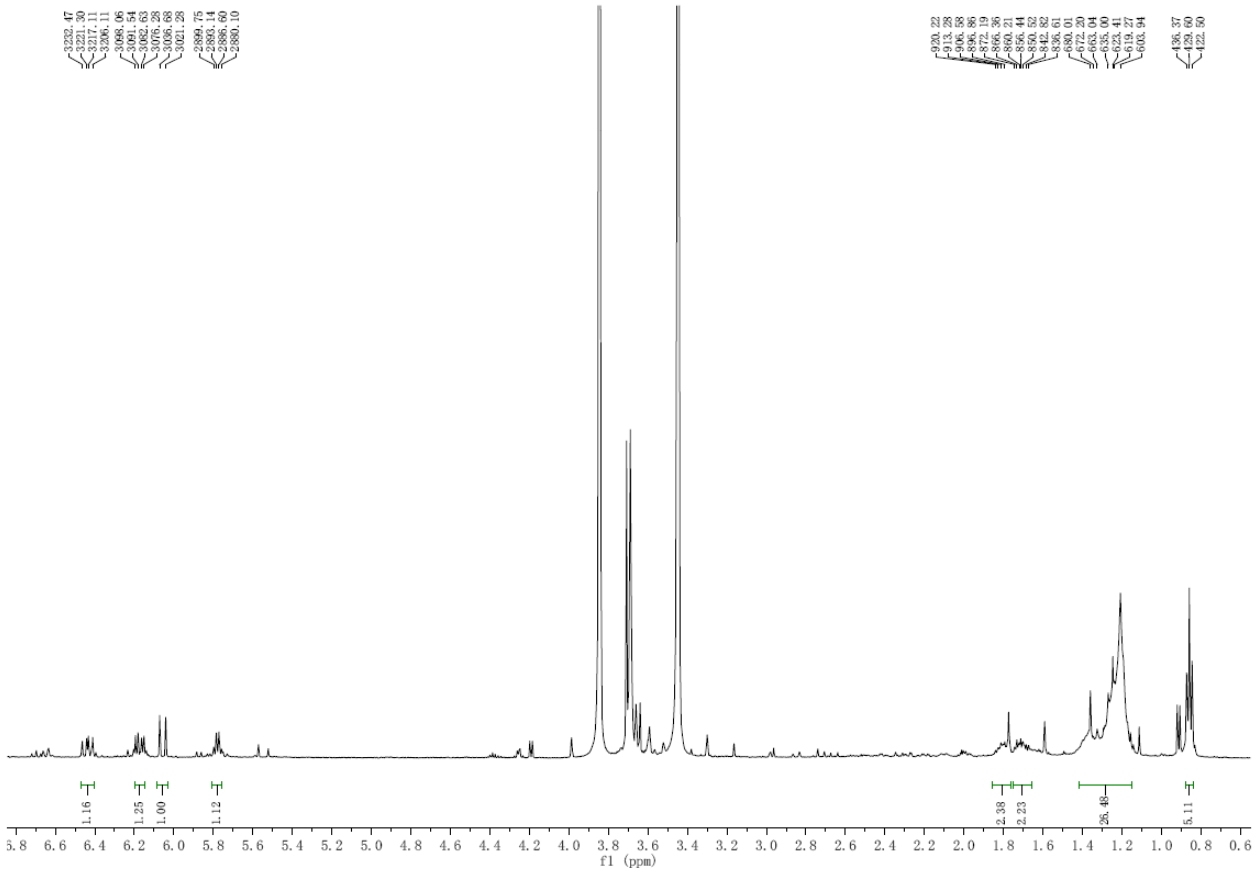


Figure S69. ^{1}H NMR spectrum of (*R*)-MTPA ester of compound **1a**