Supporting Information

Diterpenoids with unprecedented ring system from *Euphorbia peplus* and their activities in lysosomal-autophagy pathway

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Contents of Supporting Information

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Experimental section

General Experimental Procedures

IR spectra were recorded on a NICOLET iS107 Mid-infrared spectrometer. NMR spectra were measured on Bruker AVANCE III 500 MHz and AV 600 MHz NMR spectrometers with TMS as the internal standard. Optical rotation measurements were conducted with a Jasco P-1020 automatic polarimeter. ECD spectra were determined on the Applied Photophysics circular dichroism spectrometer (Applied Photophysics, Leatherhead, Surrey, UK). High-resolution MS data were performed on an Agilent 1290 UPLC/6540 Q-TOF mass spectrometer in positive mode. An Agilent 1260 series instrument equipped with a SunFire-C_{18} column (5 μm, 10 mm × 250 mm) and XSelect HSS T3 (5 μm, 10 mm × 150 mm) were used for high-performance liquid chromatography (HPLC). Silica gel (100−200, 200−300, 300−400) mesh (Qingdao Marine Chemical, Inc), NH MB 100-40/75 Silica gel (FUJI SILYSIA CHEMICAL LTD), Lichroprep RP-18 (40−63 μm, Fuji), and Sephadex LH-20 (20−150 μm, Pharmacia) was used for CC.

Plant Material

The seeds of *Euphorbia peplus* were collected in August 2018 from Kunming Botanical Garden, Yunnan Province, People's Republic of China. The plant was identified by Prof. Hu Shi-Jun (Southwest Forestry University). A voucher specimen (no. kep-09-14) has been deposited in the herbarium of the Kunming Institute of Botany, Chinese Academy of Science.

Extraction and Isolation

The air-dried seeds of plant of *E. peplus* (24 kg) were extracted with methanol (room temperature) thrice. The crude extract was obtained by reflux. After suspension in water, the combined extract was successively partitioned with petroleum ether, and ethyl acetate. The ethyl acetate extract (800 g) was then subjected to a silica gel column and eluted with a gradient of petroleum ether/ethyl acetate (100:0 to 0:100, v/v) to obtain 10 fractions, F1−F10, in which diterpenes are mainly concentrated in F6, F7 and

F8. The fraction F7 (58 g) was further subjected to MCI gel with MeOH-H_2O (40:60 to 100:0, v/v), Sephadex LH-20 (MeOH/CH_2Cl_2 50:50, v/v), silica gel column chromatography with petroleum ether/ethyl acetate (20:1 to 1:1, v/v), and preparative HPLC separations (CH_3CN/H_2O, 45:55, v/v), successively. Finally compounds **1** (7.7 mg), **2** (20.4 mg), **3** (8.0 mg) were obtained.

*Cyclojatrophane A (**1**):* a colorless acicular crystal (acetone / H_2O, 20 : 1); mp 181–184 ℃; $[\alpha]^{25}_D$ +30.6 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 195 (4.10), 229 (3.57) nm; IR (KBr) ν_{max} 3459, 2970, 1717, 1698, 1639, 1453, 1379, 1270, 1096 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; (+)-HRESIMS *m/z* $[M + Na]^+$ 491.2050 (calcd for $C_{27}H_{32}O_7Na$, 491.2040).

*Cyclojatrophane B (**2**):* a white solid; mp 218–221 ℃; $[\alpha]^{25}_D$ +25.0 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 195 (4.88), 230 (4.37) nm; IR (KBr) ν_{max} 3436, 2975, 1718, 1630, 1452, 1375, 1272, 1107 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; (+)-HRESIMS *m/z* $[M + Na]^+$ 549.2090 (calcd for $C_{29}H_{34}O_9Na$, 549.2095).

*Cyclojatrophane C (**3**):* a white solid; mp 175–178 ℃; $[\alpha]^{25}_D$ +51.6 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 195 (3.60), 285 (2.42) nm; IR (KBr) ν_{max} 3401, 2938, 1711, 1703, 1628, 1453, 1370, 1272, 1105 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; (+)-HRESIMS *m/z* $[M + Na]^+$ 445.1831 (calcd for $C_{22}H_{30}O_8Na$, 445.1833).

X-ray Crystallographic Analyses

Suitable crystals of **1** were collected on a Bruker APEX DUO diffractometer equipped with Cu Kα radiation (λ = 1.54178 Å). The structures and absolute configurations of **1** were solved and determined with the ShelXT 28 program using Intrinsic Phasing. Crystallographic data for **1** has been deposited at the Cambridge Crystallographic Data Center (heeps://ccdc.cam.ac.uk) with the deposition number of 2043474.

Crystallographic data for compound **1**. $C_{27}H_{32}O_7$, M = 468.52, a = 6.1764(2) Å, b = 22.3986(6) Å, c = 16.9215(5) Å, α = 90°, β = 94.5630(10)°, γ = 90°, V = 2333.55(12) $Å^3$, T = 100.(2) K, space group $P1211$, Z = 4, μ(Cu Kα) = 0.784 mm^{-1}, 40562 reflections measured, 9202 independent reflections (R_{int} = 0.0401). The final R_1 values were 0.0285

($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0709 ($I > 2\sigma(I)$). The final R_1 values were 0.0288 (all data). The final $wR(F^2)$ values were 0.0711 (all data). The goodness of fit on F^2 was 1.034. Flack parameter = 0.00(3).

Chemical transformation of compound 3 into 2

A solution of **3** (2.3 mg, 5.4 μmol) in dry dichloromethane (5 mL) was added 4-dimethylaminopyridine (DMAP, 2.6 mg, 21.3 μmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 2.1 mg, 10.9 μmol), and benzoic acid (1.3 mg, 10.6 μmol) at room temperature. The mixture was stirred at room temperature for 2 h. The crude product was purified by column chromatography on silica gel to give **2** (1.1 mg).

Cell Culture, Transfection and Reagents

HeLa cell line was cultured at 37°C with 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (HyClone), 100 U/mL penicillin and 100 mg/mL streptomycin. The HeLa cells were transient transfected with GFP-LC3 vector using lipotransfectamine 2000 according to the manufacture's protocol.

Screening for Compounds that Induce Lysosomal Biogenesis

HeLa cells with 85% cell density in 96-well plates were treated with compounds **1**−**3** at 20 μM in triplicate. DMSO treatment was used as the negative control, whereas Hep-14 was used as the positive control. Three hours later, cells were grown in fresh medium containing LysoTracker Red DND-99 (0.2 μM) for 30 min. Then medium was changed again to LysoTracker-free medium and images were taken with ArrayScan Infinity (Cellomics, ArrayScan VTI HCS). Compounds were subjected to validation by treating HeLa cells with indicated concentrations in triplicate and staining with LysoTracker Red DND-99.

Confocal Microscopy

GFP-LC3 expressing HeLa cells were treated with indicated compounds and

images were collected by confocal microscopy. For live-cell imaging, cells grown on glass-bottom dishes (In Vitro Scientific) were observed directly. All samples were examined with an inverted Olympus FV1000 confocal microscope. Images were analyzed with FV10-ASW 4.0a Viewer.

Quantitative Real-time PCR with Reverse Transcription (RT-qPCR).

RNA was isolated from HeLa cells using TRIzol Reagent (Invitrogen) as recommended by the manufacturer. A reverse-transcription kit (Promega) was used to reverse transcribe RNA (1 mg) in a 20 μL reaction mixture. Quantification of gene expression was performed using a real-time PCR system (7900HT Fast; Applied Biosystems) in triplicate. Amplification of the sequence of interest was normalized with the reference endogenous gene β-actin.

Statistics and Reproducibility

Data analysis were carried out using GraphPad Prism 7, and Student's *t*-test were employed for statistical analyses with a level of significance of $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

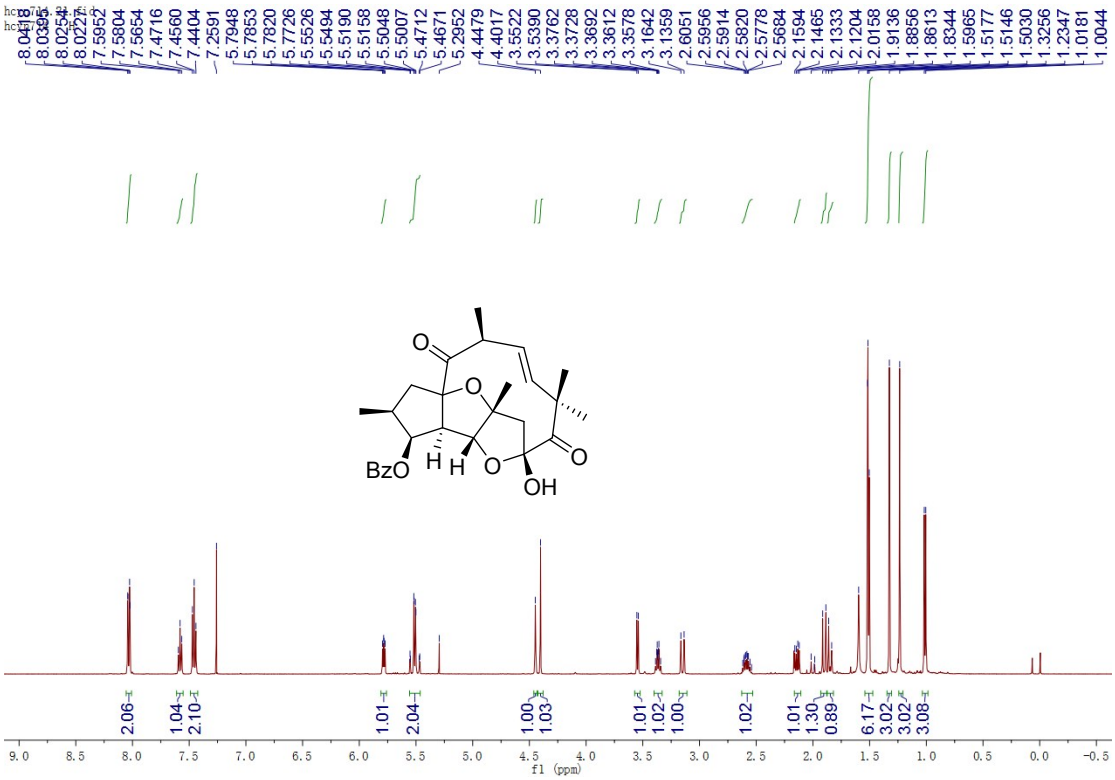


Figure S1. ^{1}H NMR ($CDCl_3$) spectrum of compound **1**

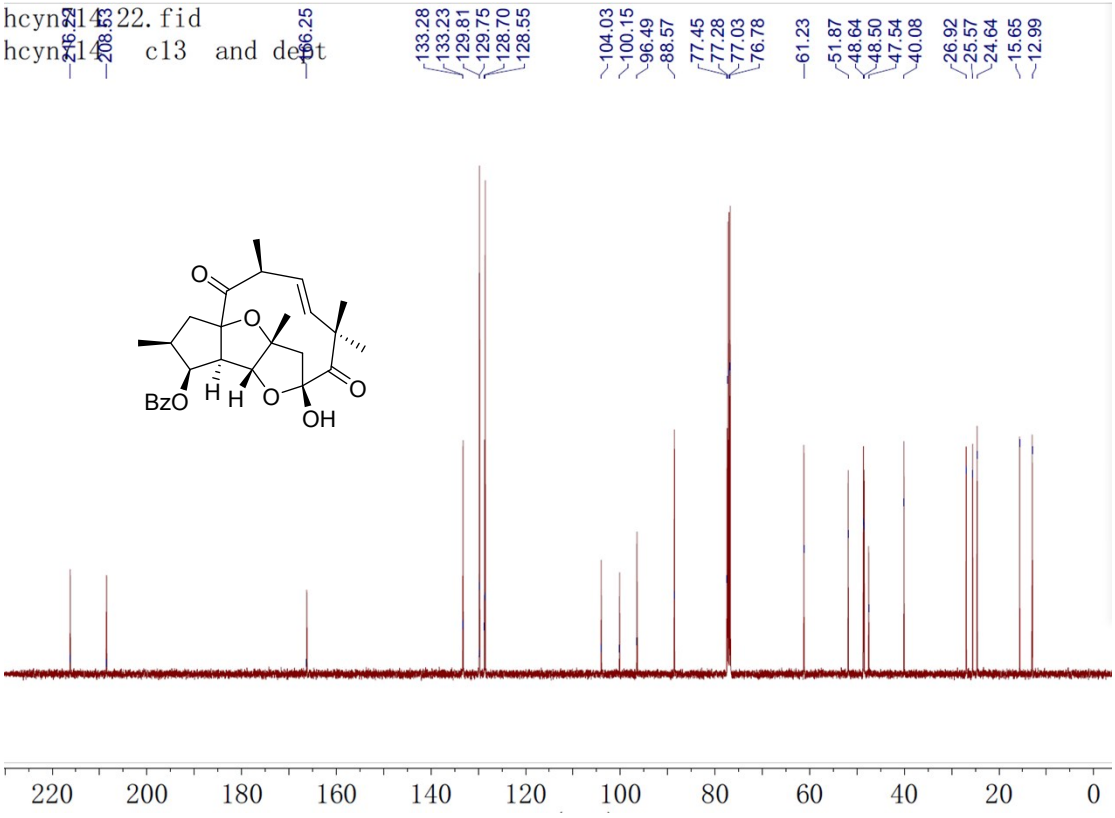


Figure S2. ^{13}C NMR ($CDCl_3$) spectrum of compound **1**

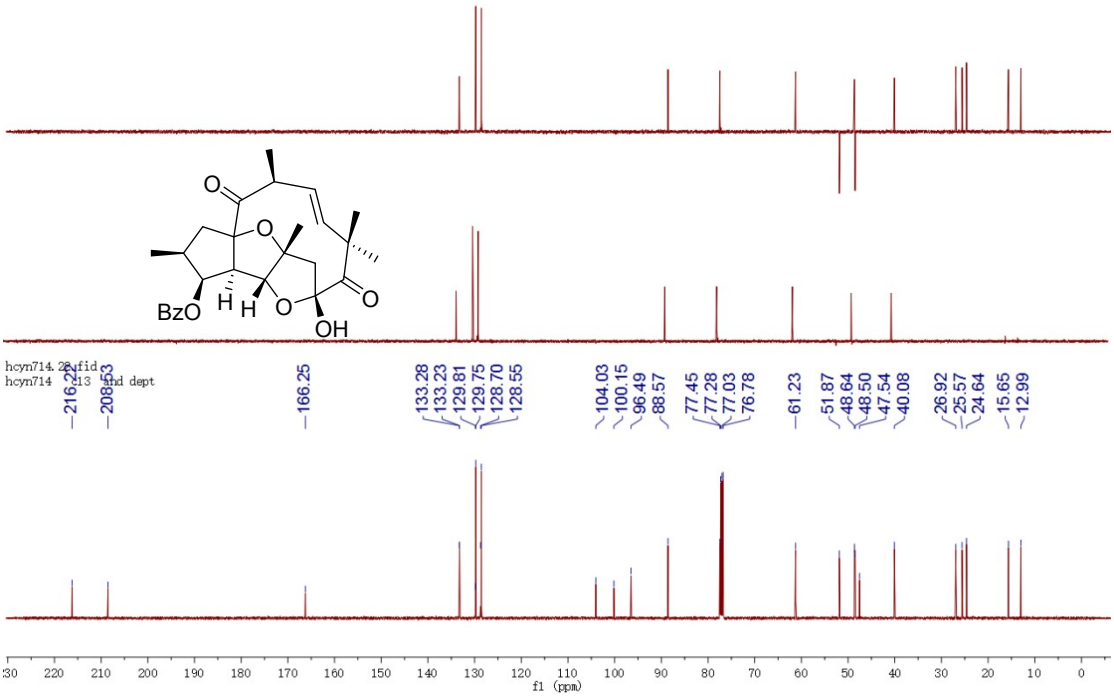


Figure S3. DEPT NMR ($CDCl_3$) spectrum of compound **1**

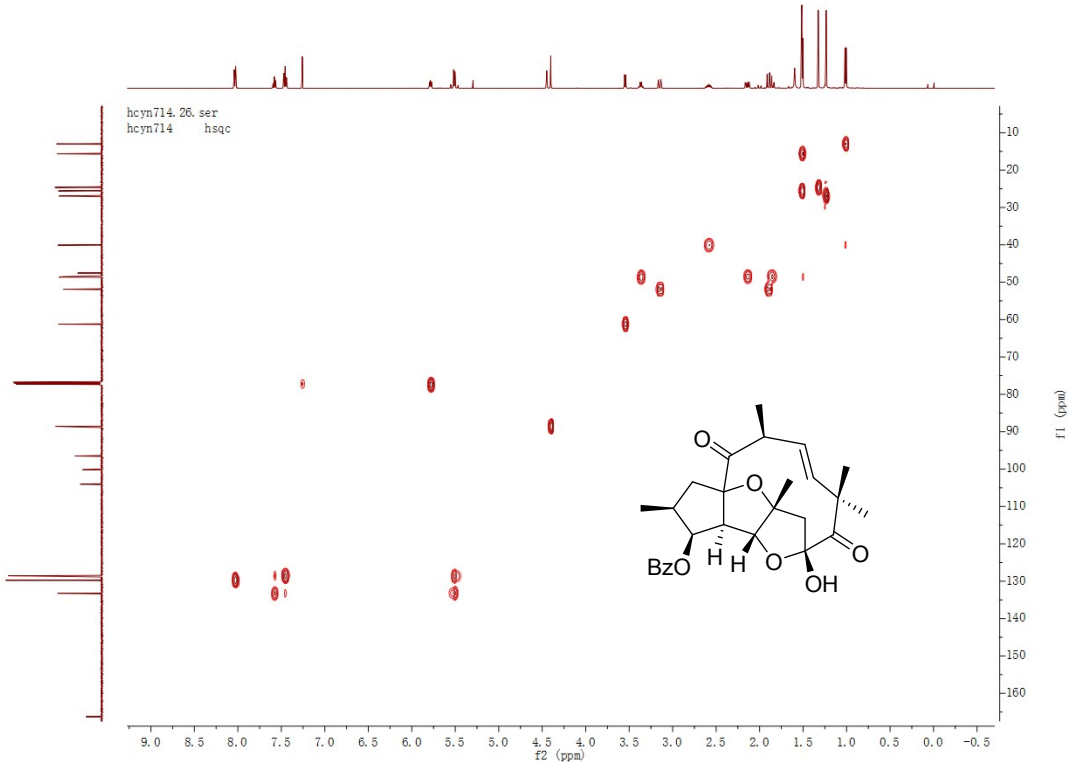


Figure S4. HSQC ($CDCl_3$) spectrum of compound **1**

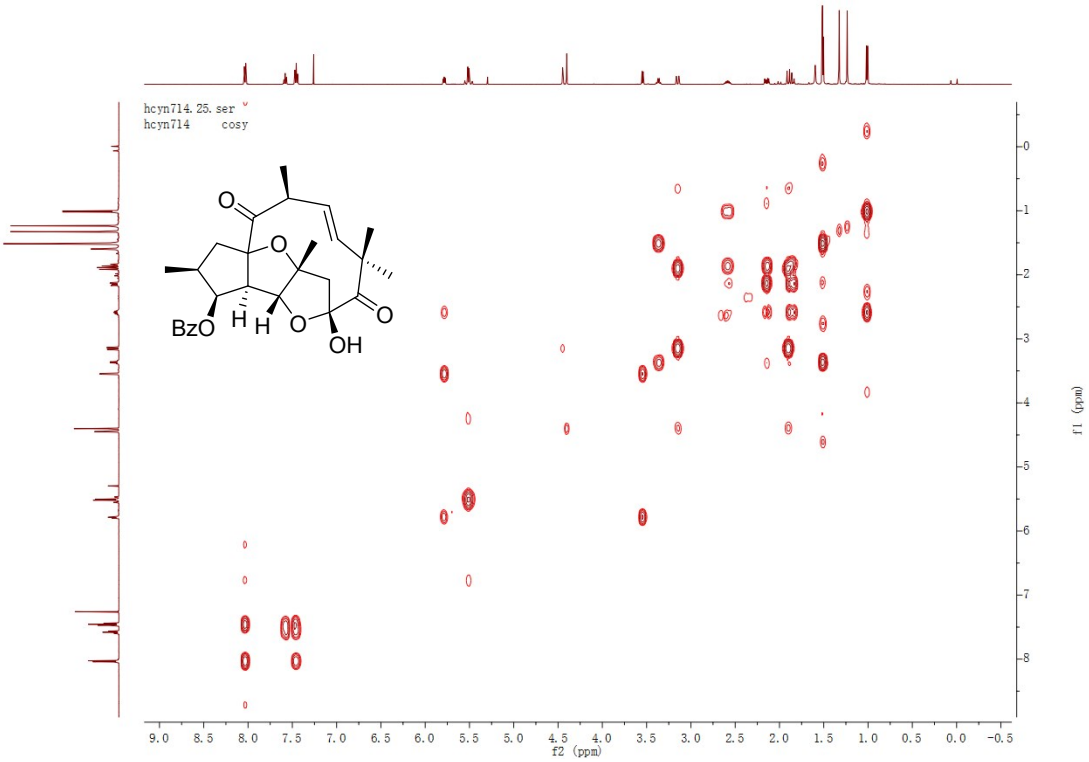


Figure S5. ^{1}H-^{1}H COSY ($CDCl_3$) spectrum of compound **1**

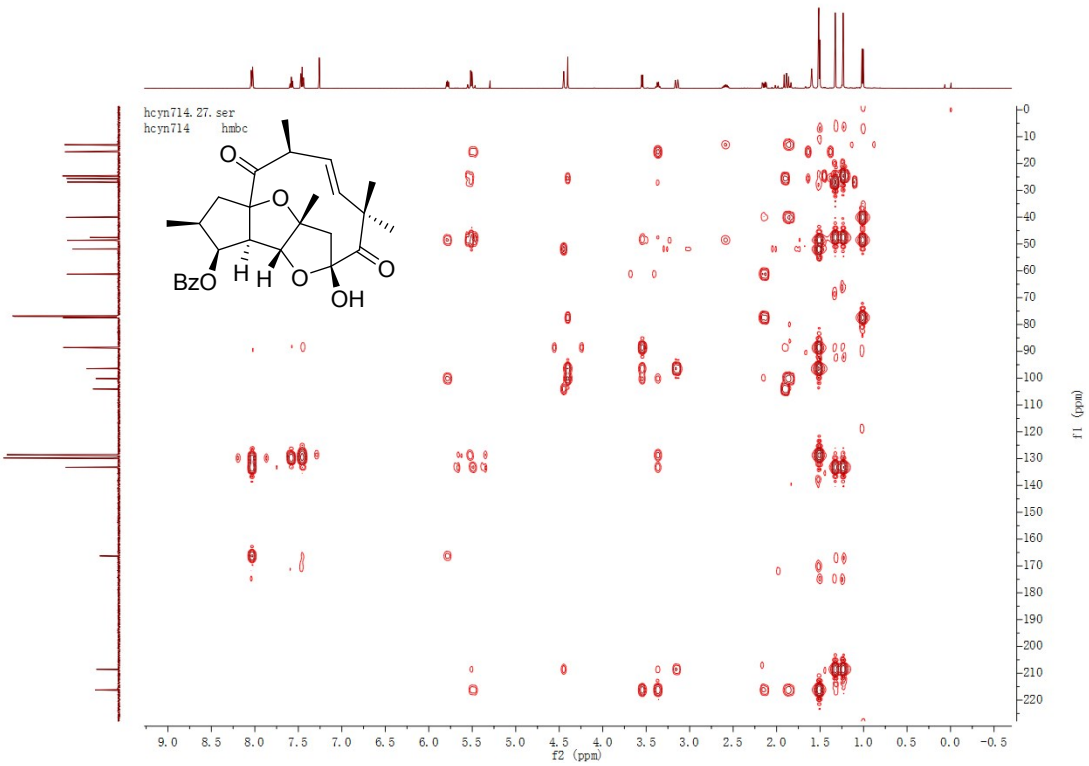


Figure S6. HMBC ($CDCl_3$) spectrum of compound **1**

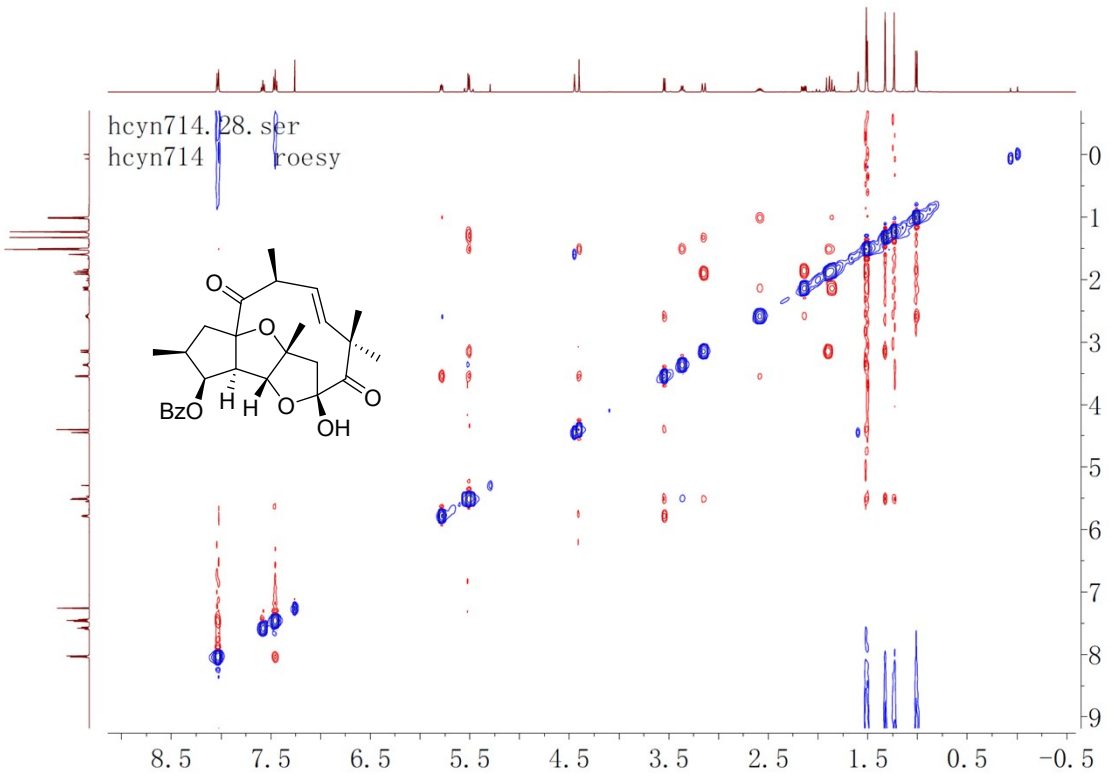
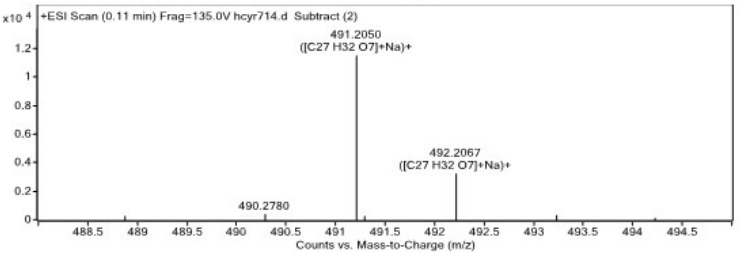


Figure S7. ROESY ($CDCl_3$) spectrum of compound **1**

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	ESI



Peak List

m/z	z	Abund	Formula	Ion
437.1929	1	6975.02		
451.2114	1	6403.15		
453.1682	1	7169.18		
460.2708	1	2565.69		
483.2518	1	2580.63		
491.205	1	11559.79	C27 H32 O7	(M+Na)+
492.2067	1	3304.72	C27 H32 O7	(M+Na)+
513.1851	1	3061.23		
514.2785	1	3175.67		
959.4207	1	2278.08		

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	30

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C27 H32 O7	468.2148	491.2040	491.2050	-1.00	-2.04	12.0000

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Figure S8. HRESIMS spectrum of compound **1**

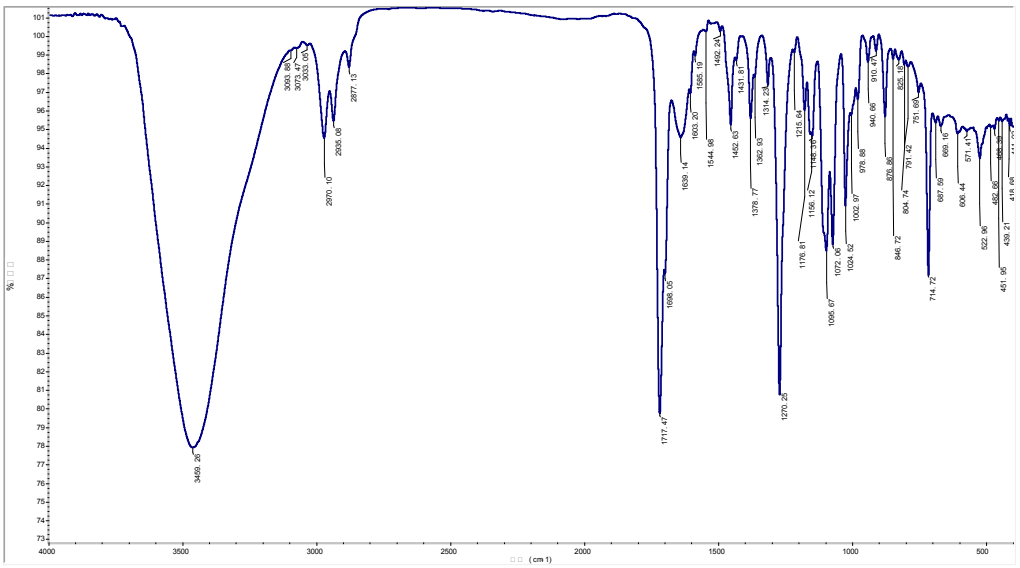


Figure S9. IR spectrum of compound **1**

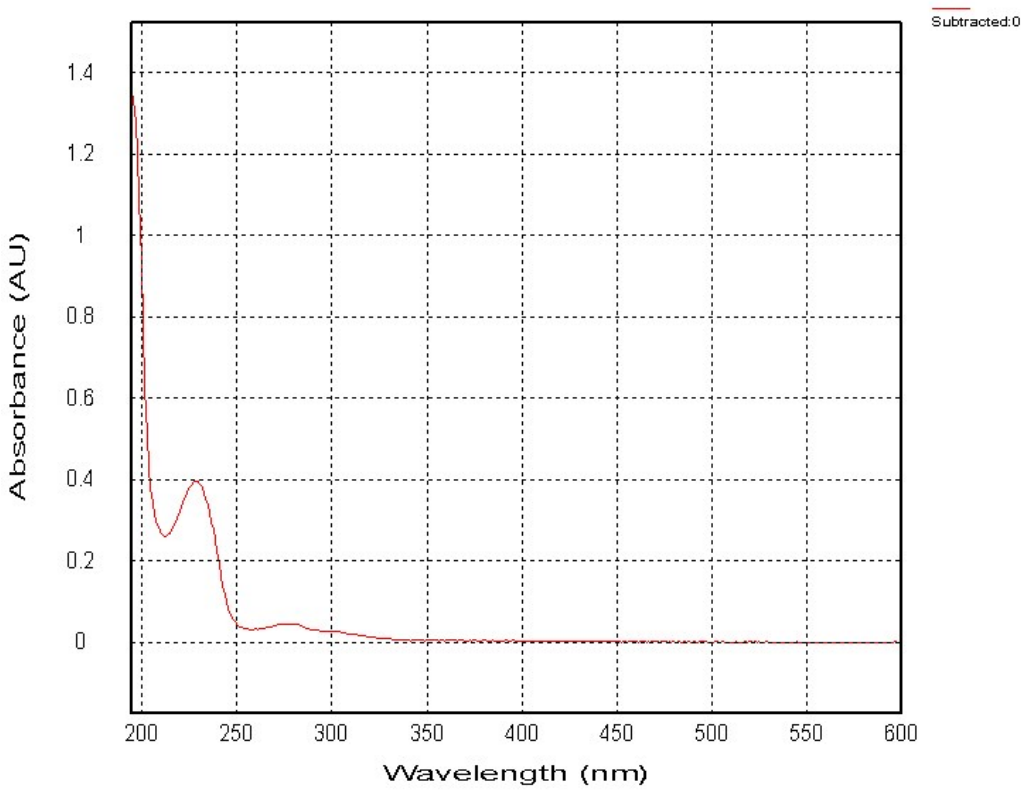


Figure S10. UV spectrum of compound **1**

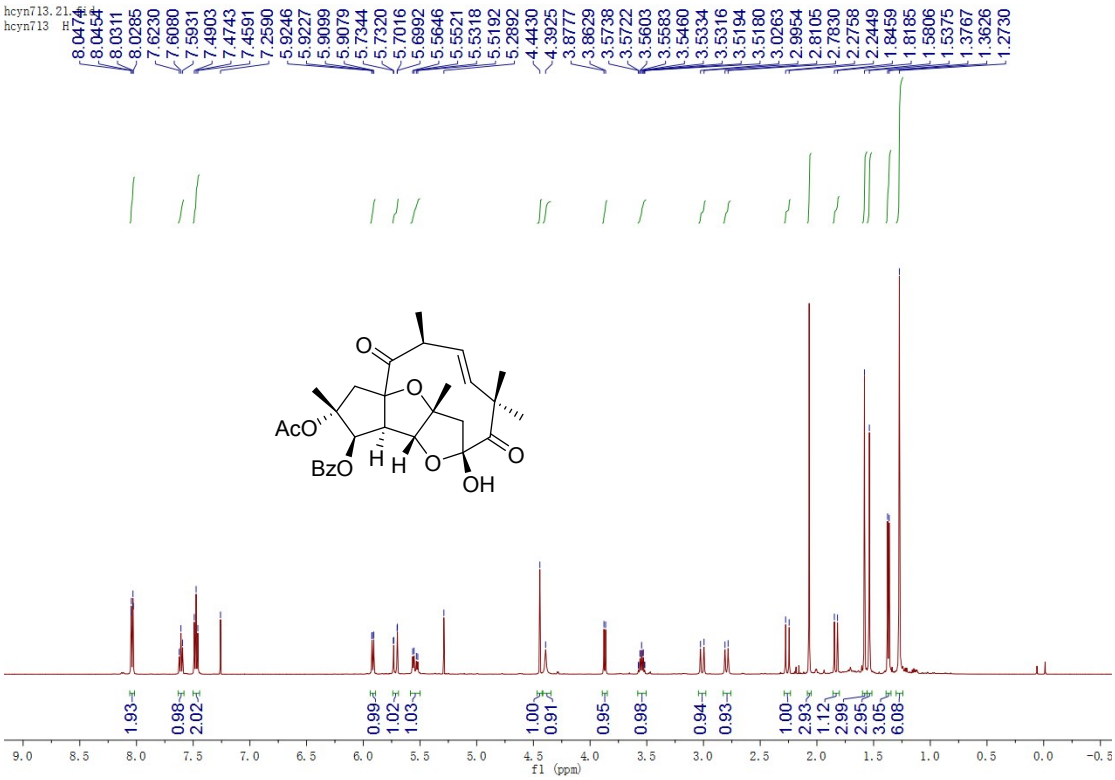


Figure S11. ^{1}H NMR ($CDCl_3$) spectrum of compound **2**

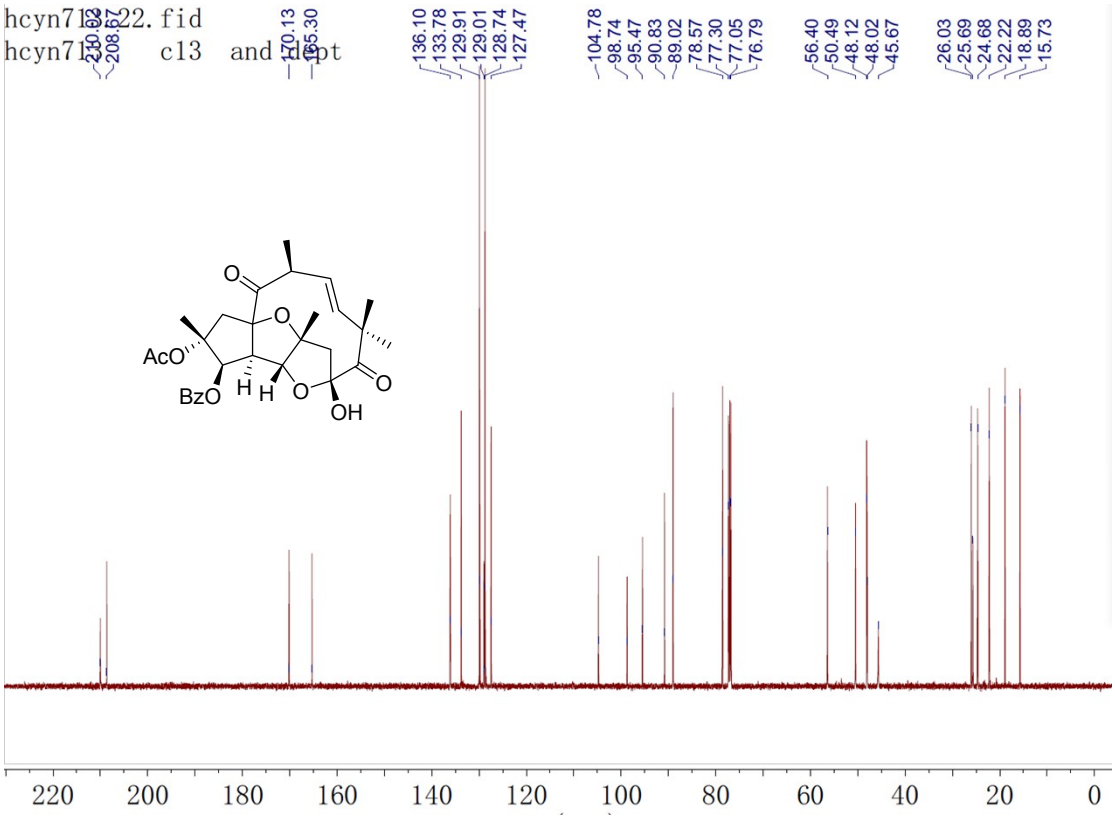


Figure S12. ^{13}C NMR ($CDCl_3$) spectrum of compound **2**

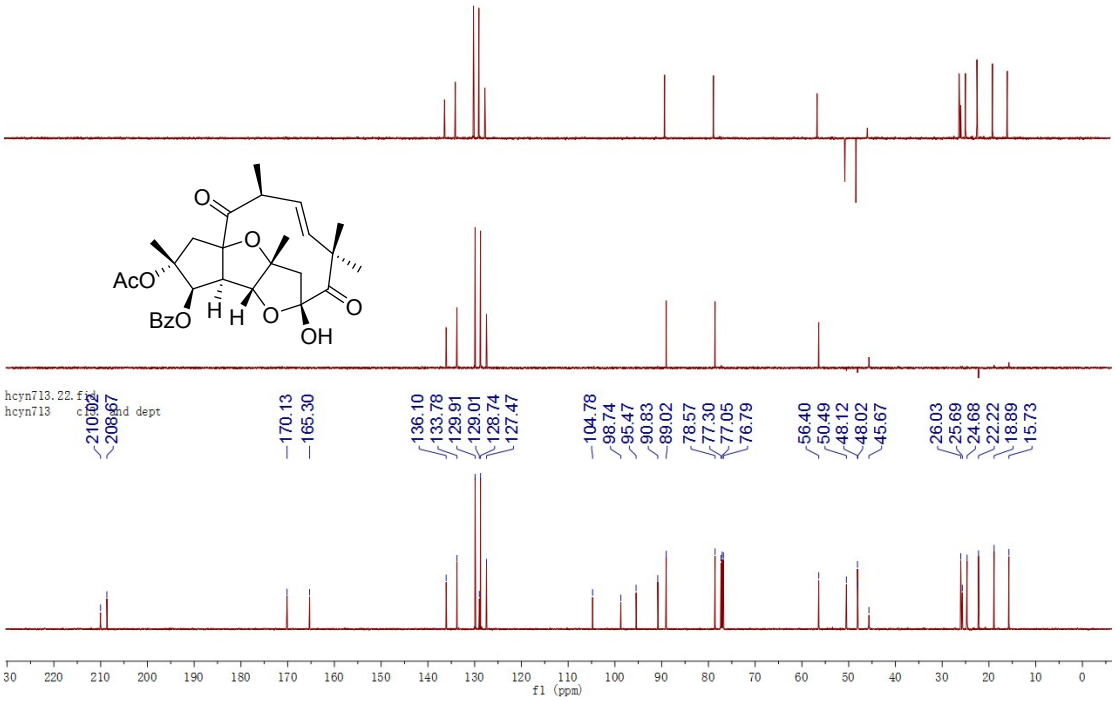


Figure S13. DEPT NMR ($CDCl_3$) spectrum of compound **2**

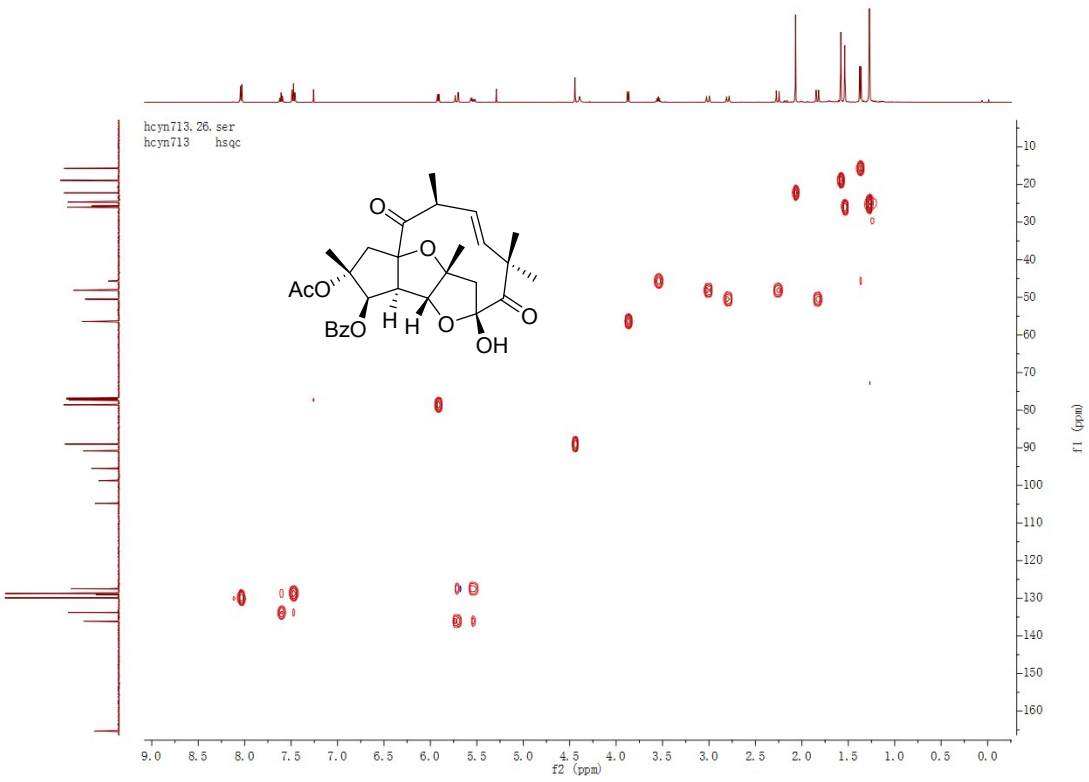


Figure S14. HSQC ($CDCl_3$) spectrum of compound **2**

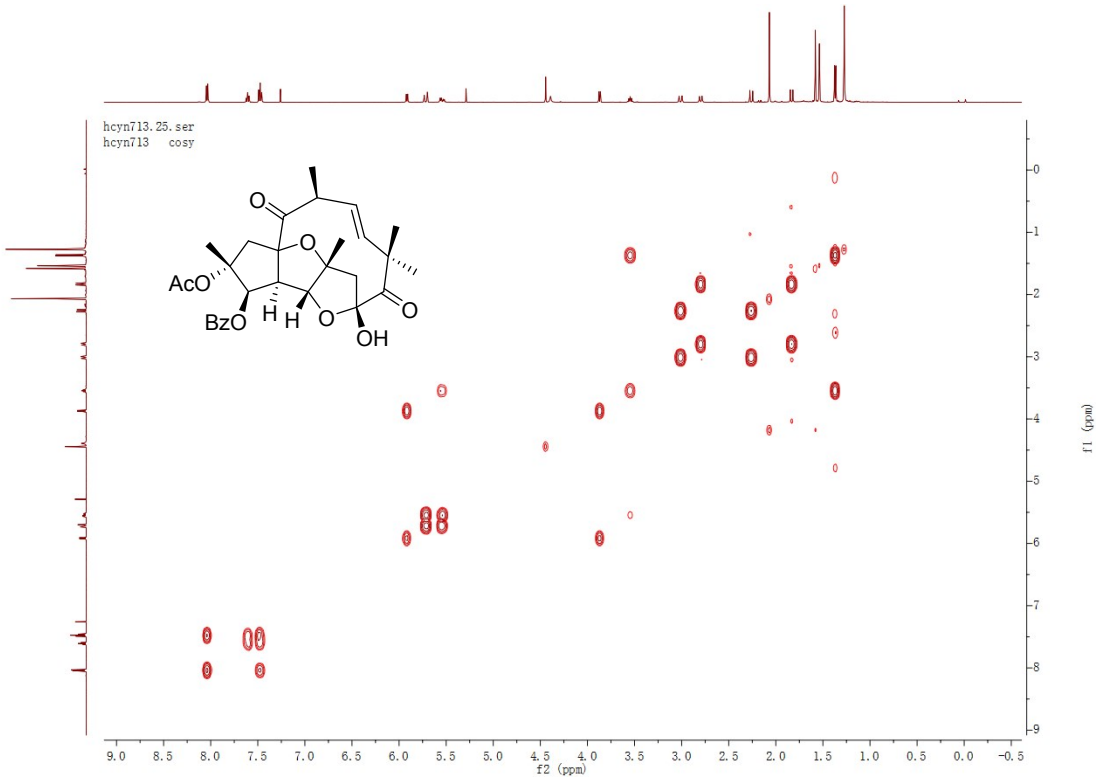


Figure S15. ^{1}H-^{1}H COSY ($CDCl_3$) spectrum of compound **2**

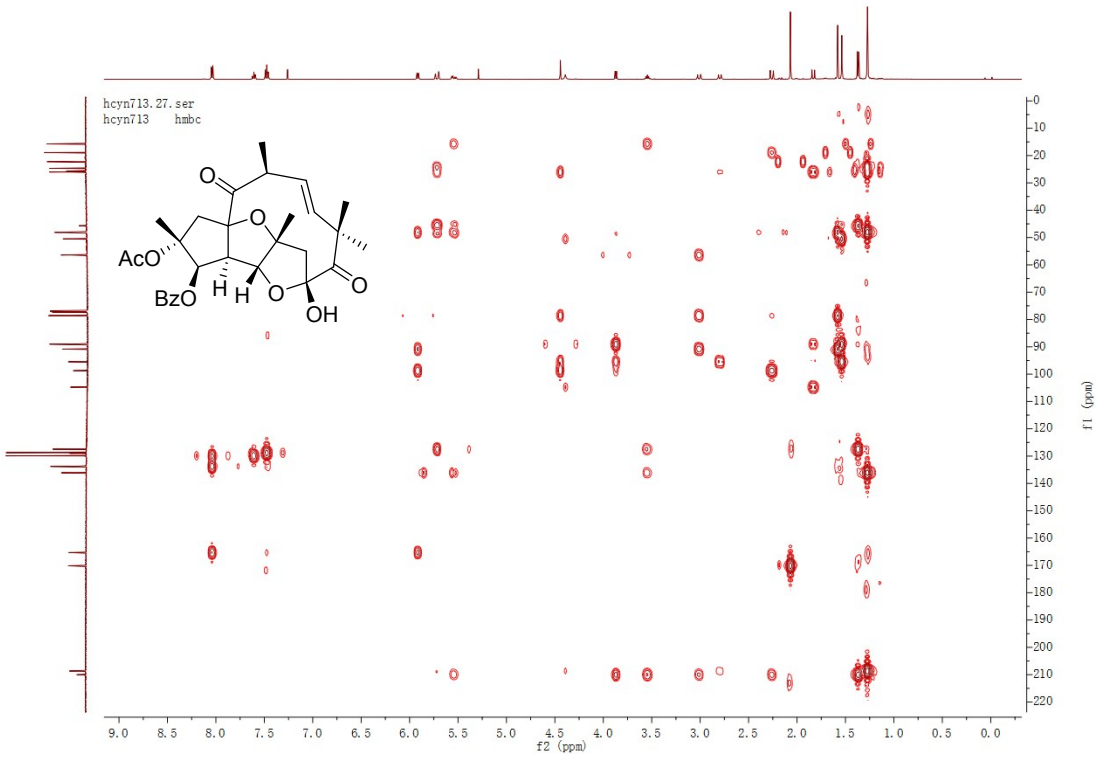


Figure S16. HMBC ($CDCl_3$) spectrum of compound **2**

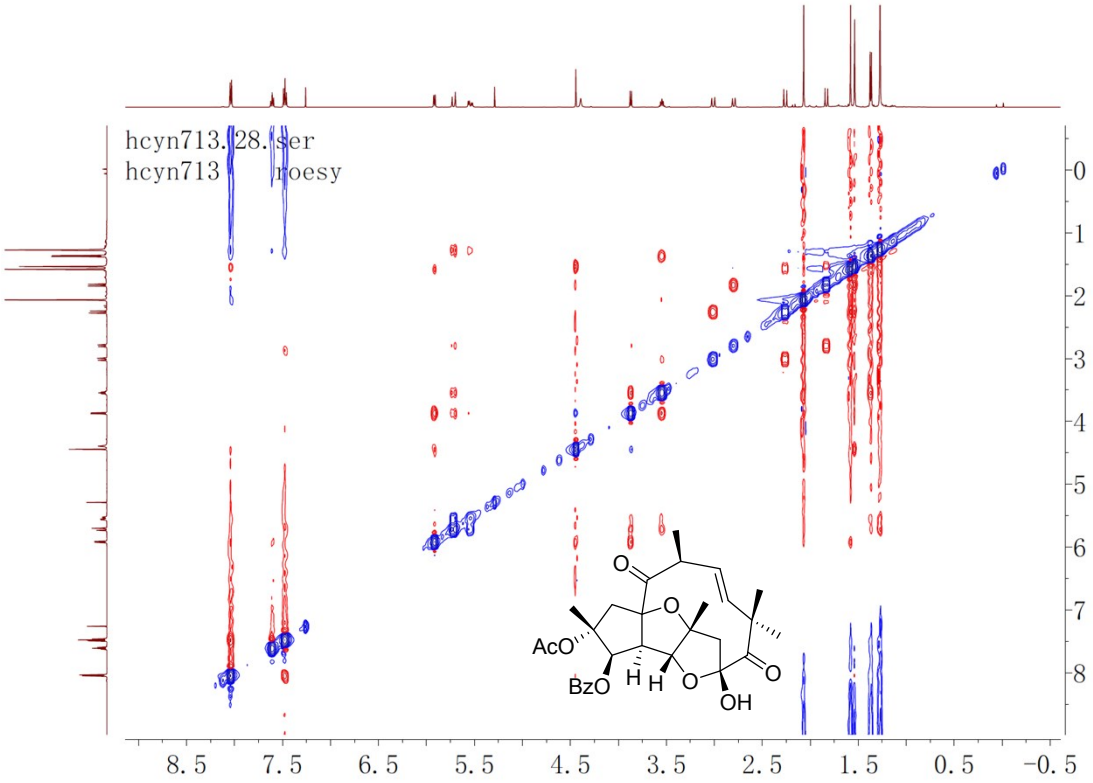
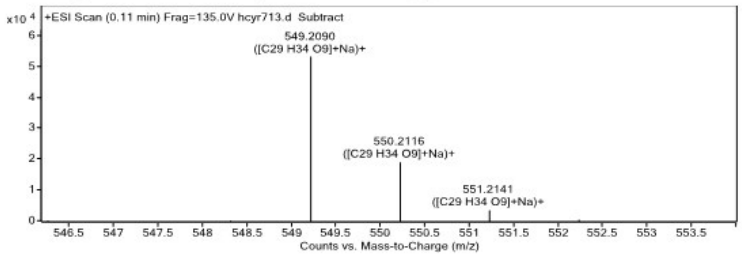


Figure S17. ROESY ($CDCl_3$) spectrum of compound **2**

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	ESI



Peak List

m/z	z	Abund	Formula	Ion
343.2231	1	7072.08		
449.1952	1	10056.58		
549.209	1	53282.57	C29 H34 O9	(M+Na)+
550.2116	1	19316.61	C29 H34 O9	(M+Na)+
565.1822	1	8849.11		
572.2851	1	9159.15		
651.2408	1	7948.07		
1075.4302	1	75519.15		
1076.4336	1	48138.47		
1077.4361	1	18466.59		

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	30

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C29 H34 O9	526.2203	549.2095	549.2090	0.50	0.91	13.0000

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Figure S18. HRESIMS spectrum of compound **2**

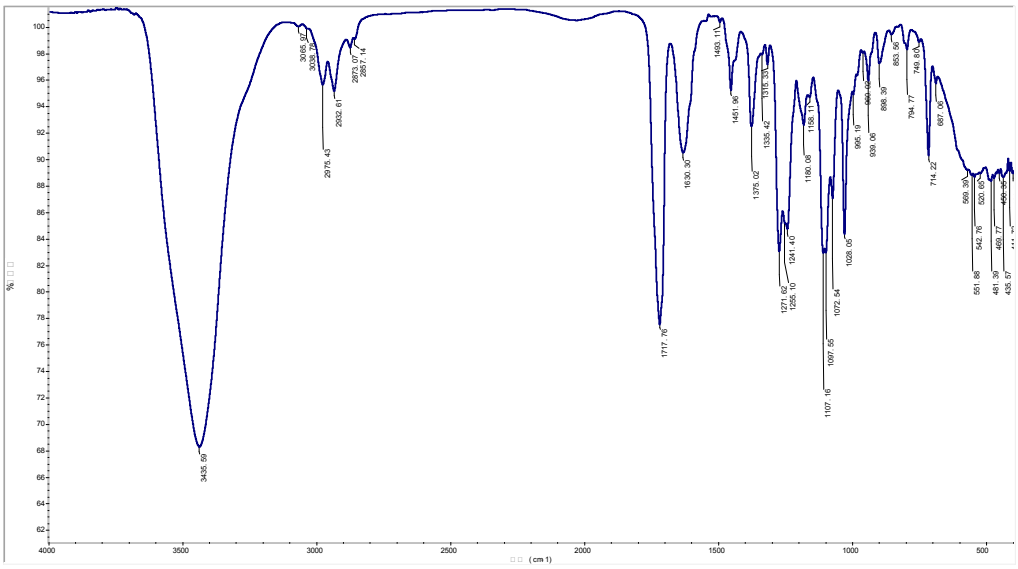


Figure S19. IR spectrum of compound **2**

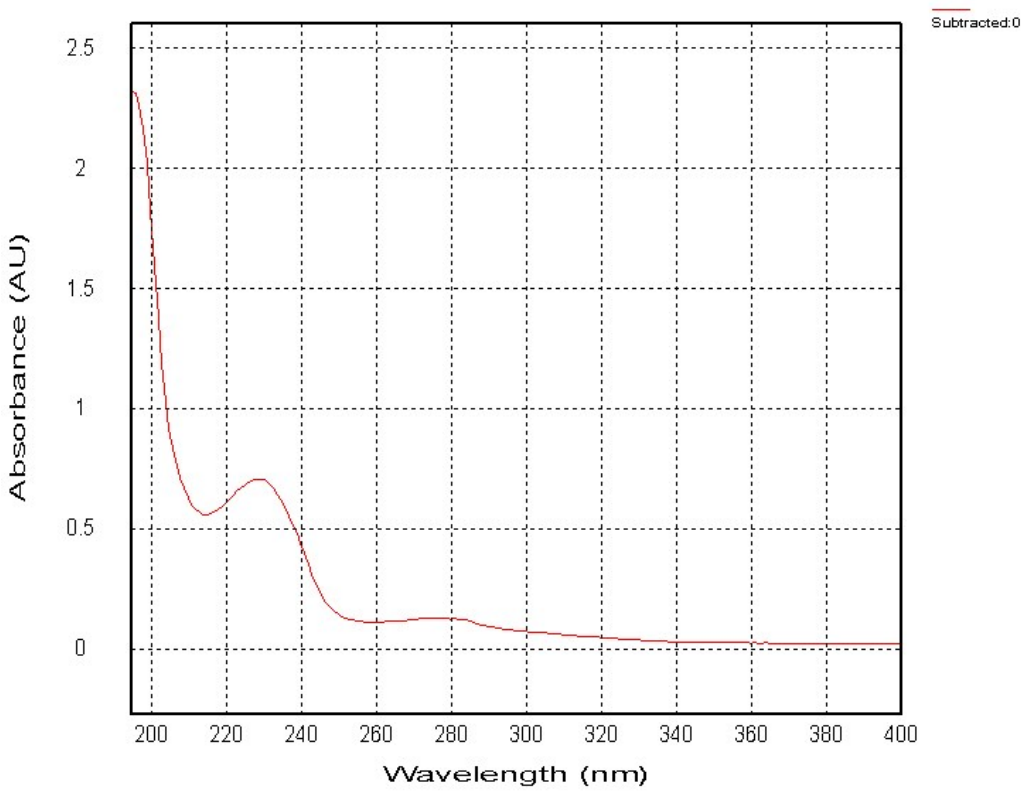


Figure S20. UV spectrum of compound **2**

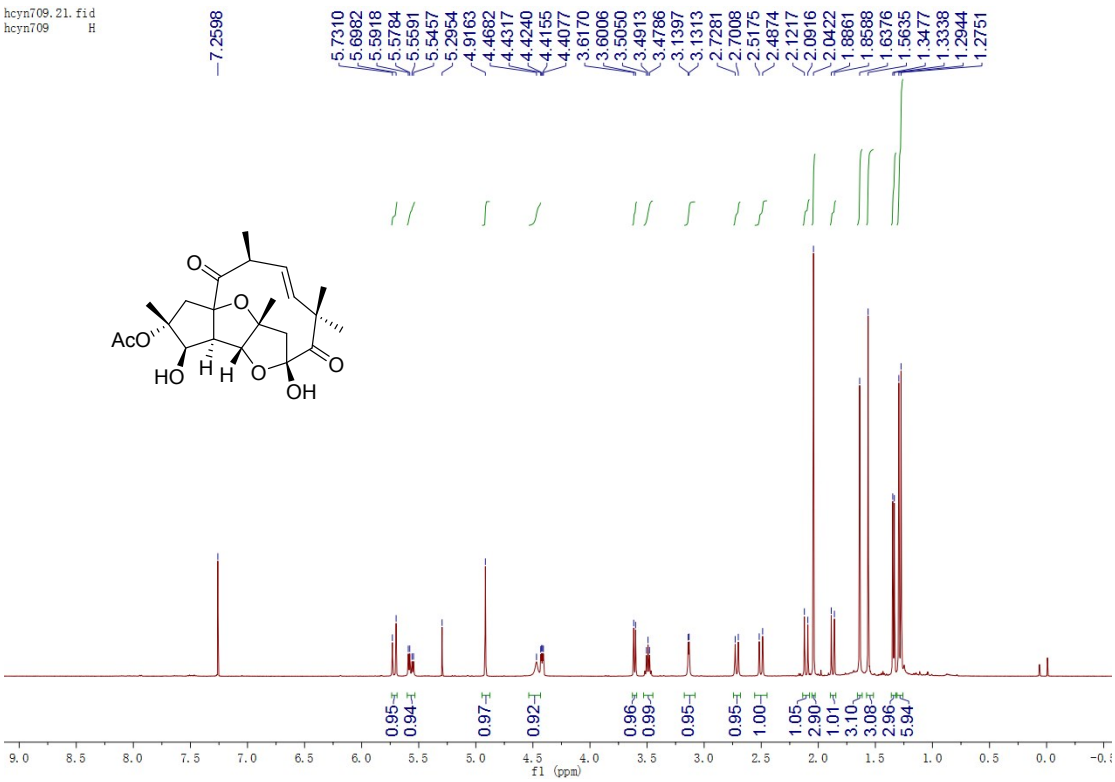


Figure S21. ^{1}H NMR ($CDCl_3$) spectrum of compound **3**

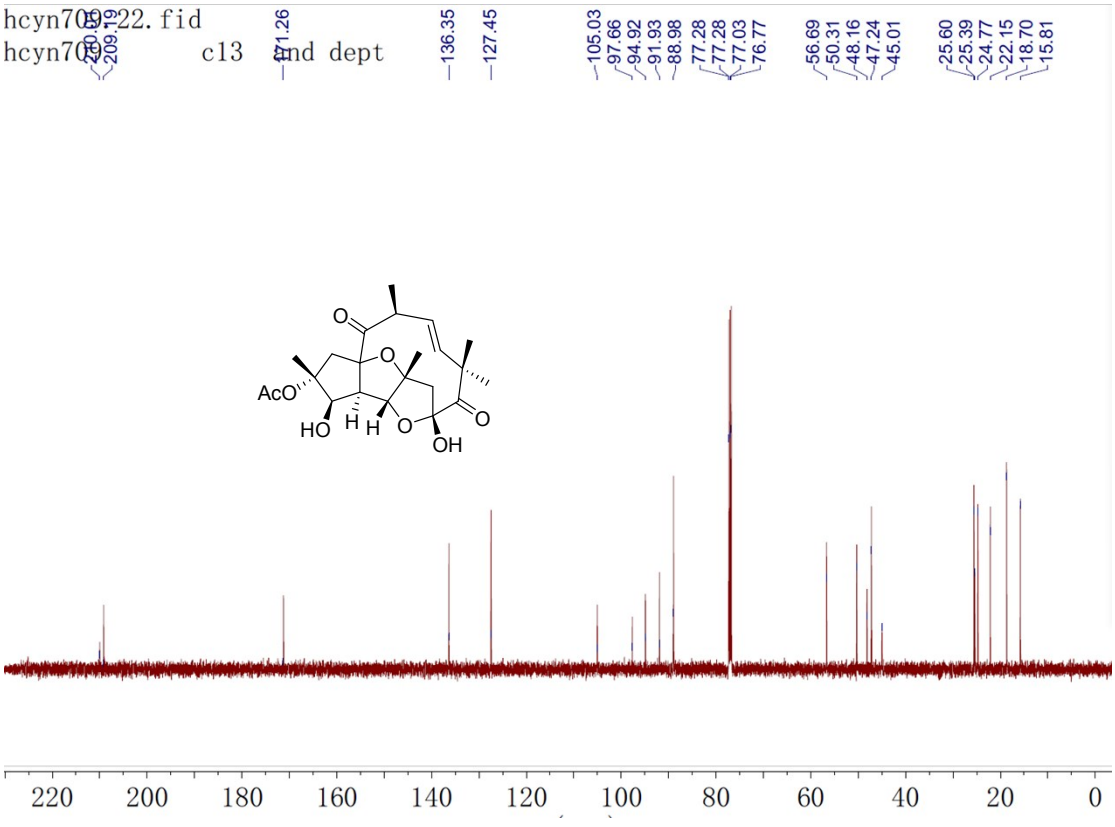


Figure S22. ^{13}C NMR ($CDCl_3$) spectrum of compound **3**

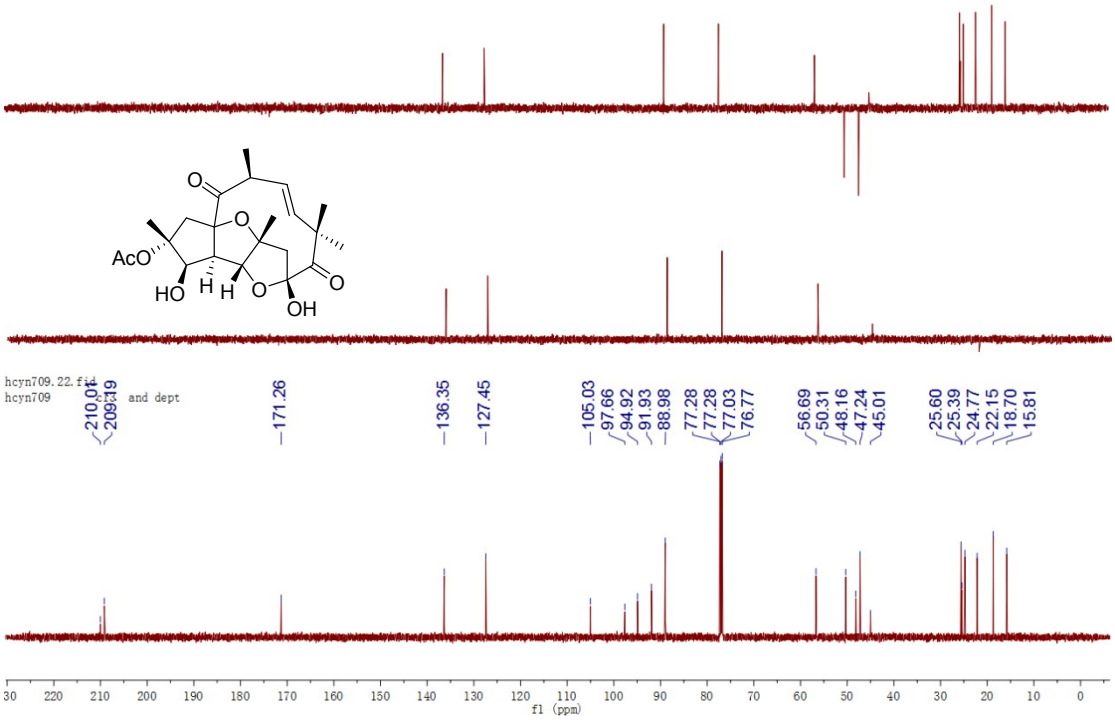


Figure S23. DEPT NMR ($CDCl_3$) spectrum of compound **3**

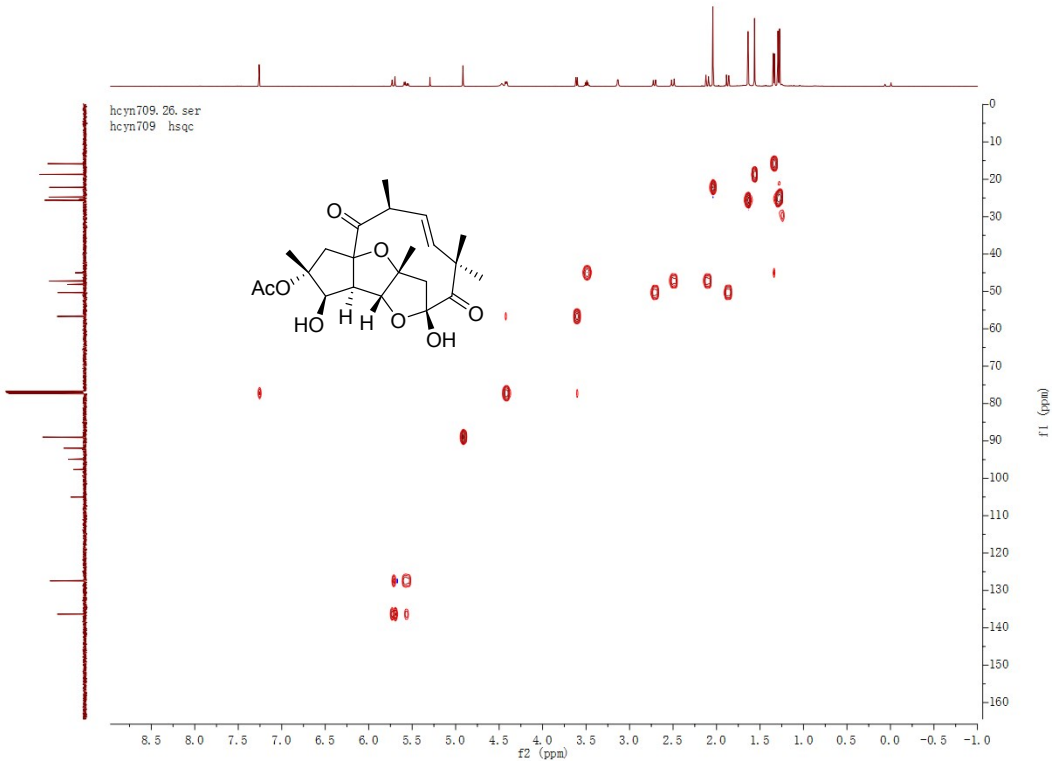


Figure S24. HSQC ($CDCl_3$) spectrum of compound **3**

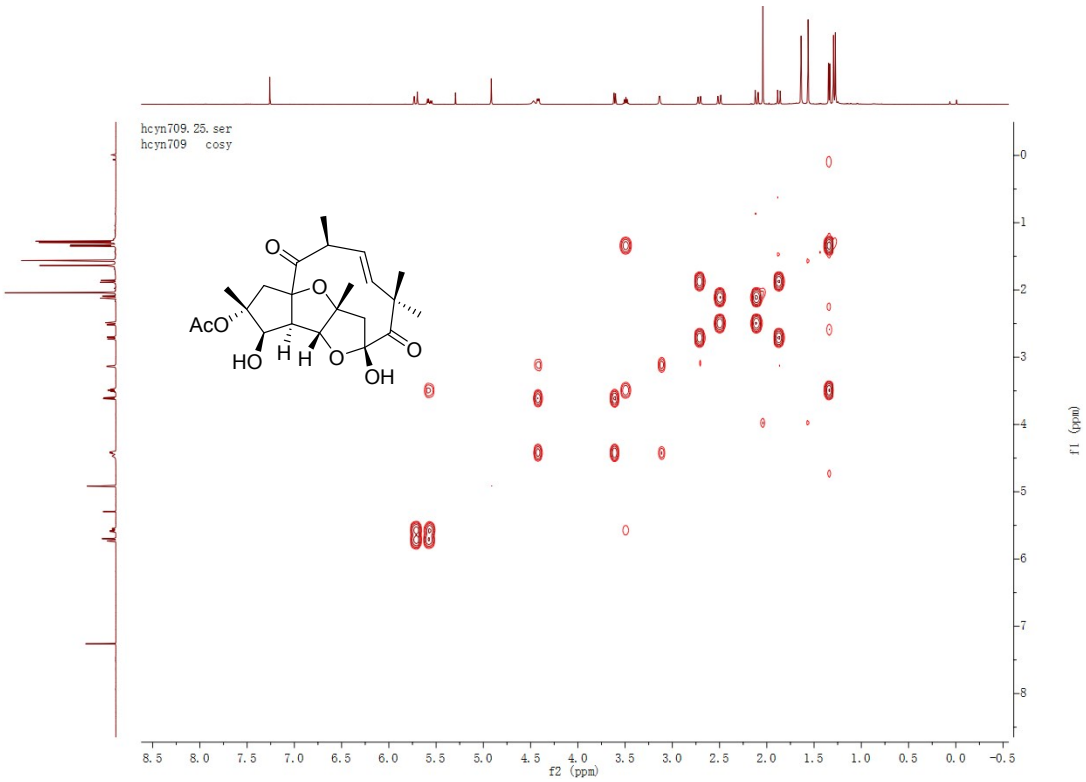


Figure S25. ^{1}H-^{1}H COSY ($CDCl_3$) spectrum of compound **3**

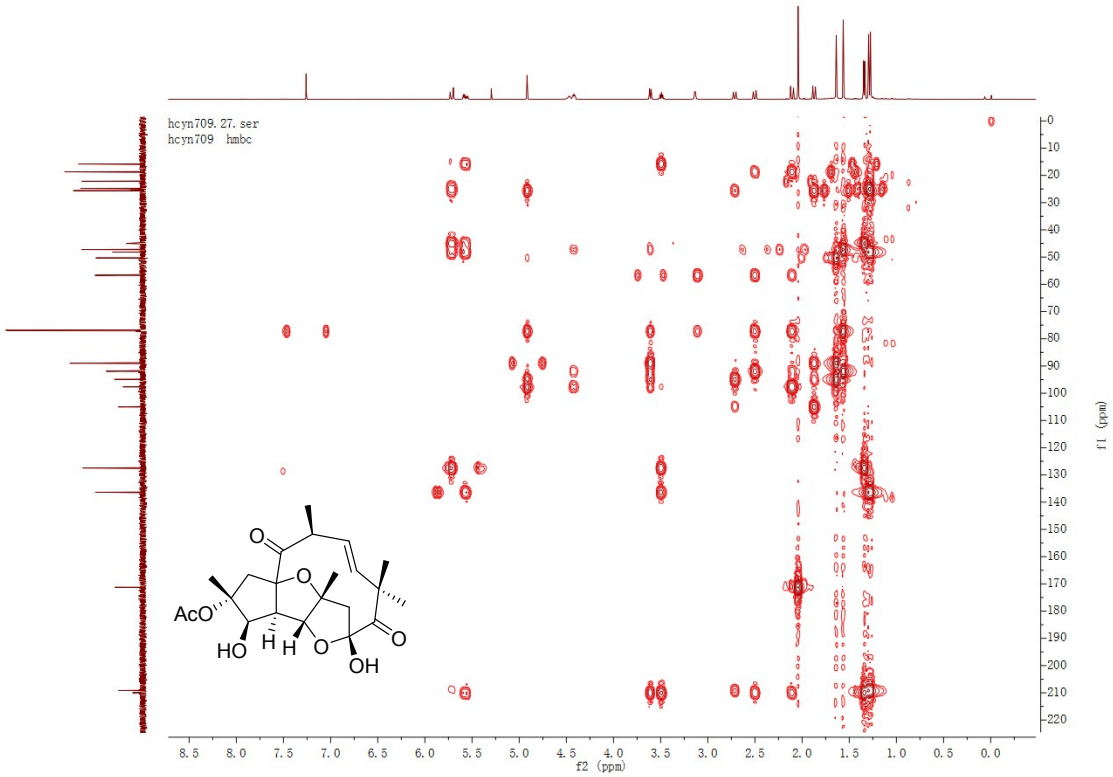


Figure S26. HMBC ($CDCl_3$) spectrum of compound **3**

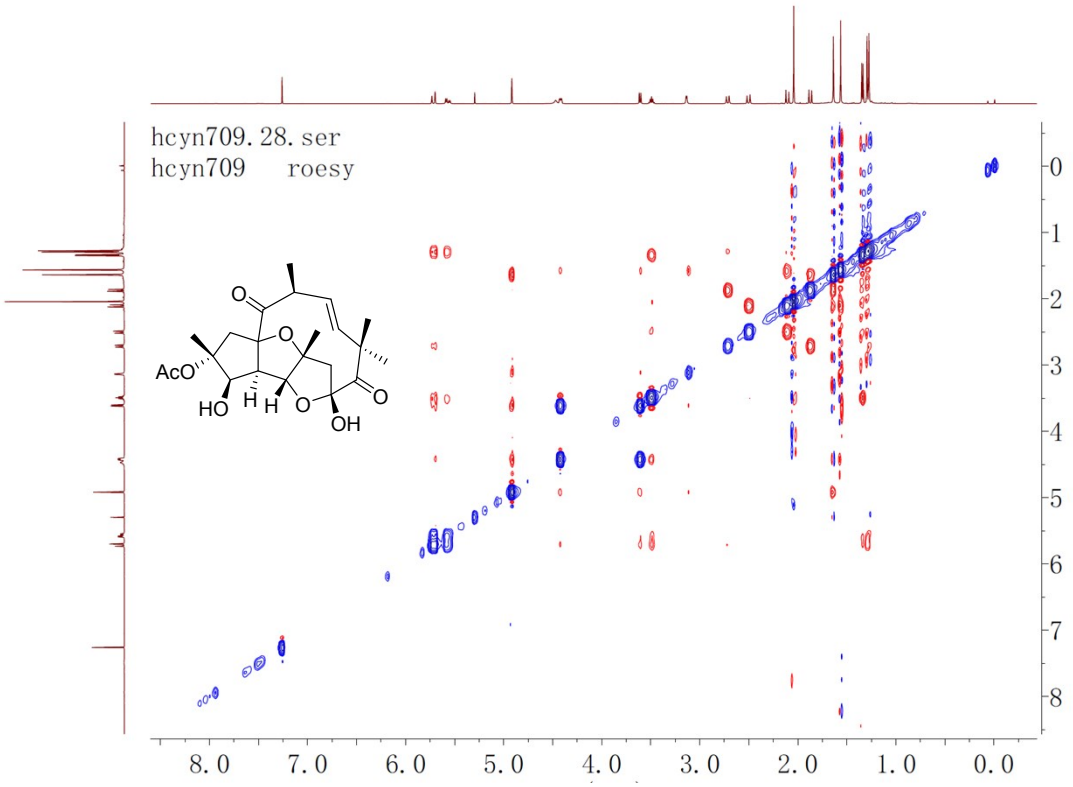
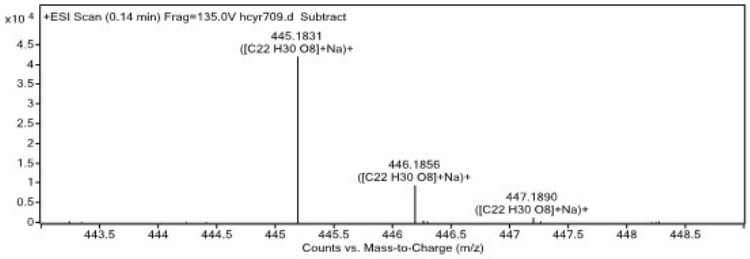


Figure S27. ROESY ($CDCl_3$) spectrum of compound **3**

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	ESI



Peak List

m/z	z	Abund	Formula	Ion
318.2998		5921.19		
437.1931	1	29388.44		
438.197	1	7898.17		
445.1831	1	42073.91	C22 H30 O8	(M+Na)+
446.1856	1	9563.43	C22 H30 O8	(M+Na)+
453.168	1	13768.73		
467.1642	1	6033.7		
577.2408	1	9564.04		
867.3772	1	33312.68		
868.3789	1	14309.04		

Formula Calculator Element Limits

Element	Min	Max
C	3	60
H	0	120
O	0	30

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C22 H30 O8	422.1941	445.1833	445.1831	0.20	0.45	8.0000

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Figure S28. HRESIMS spectrum of compound **3**

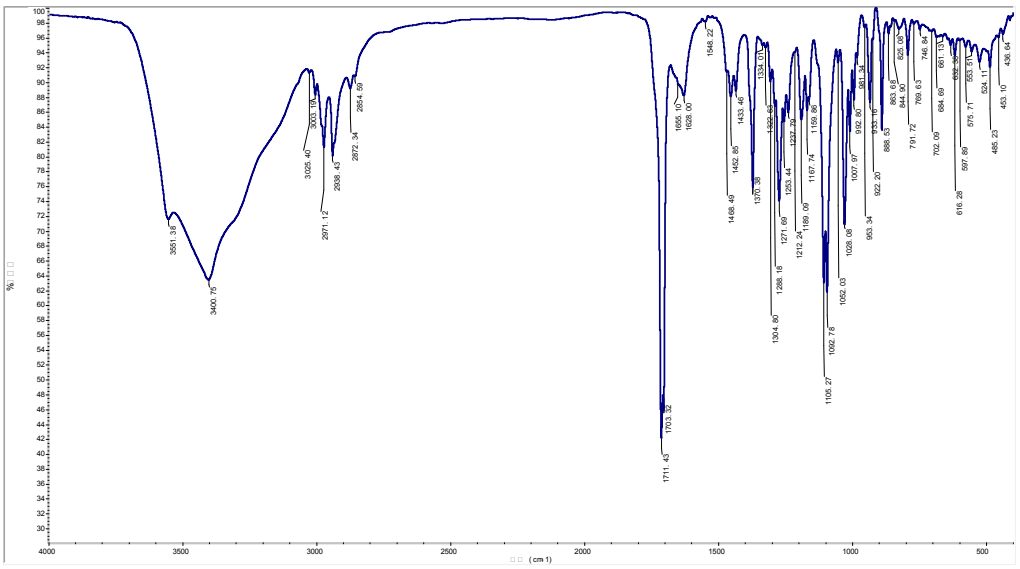

Figure S29. IR spectrum of compound **3**

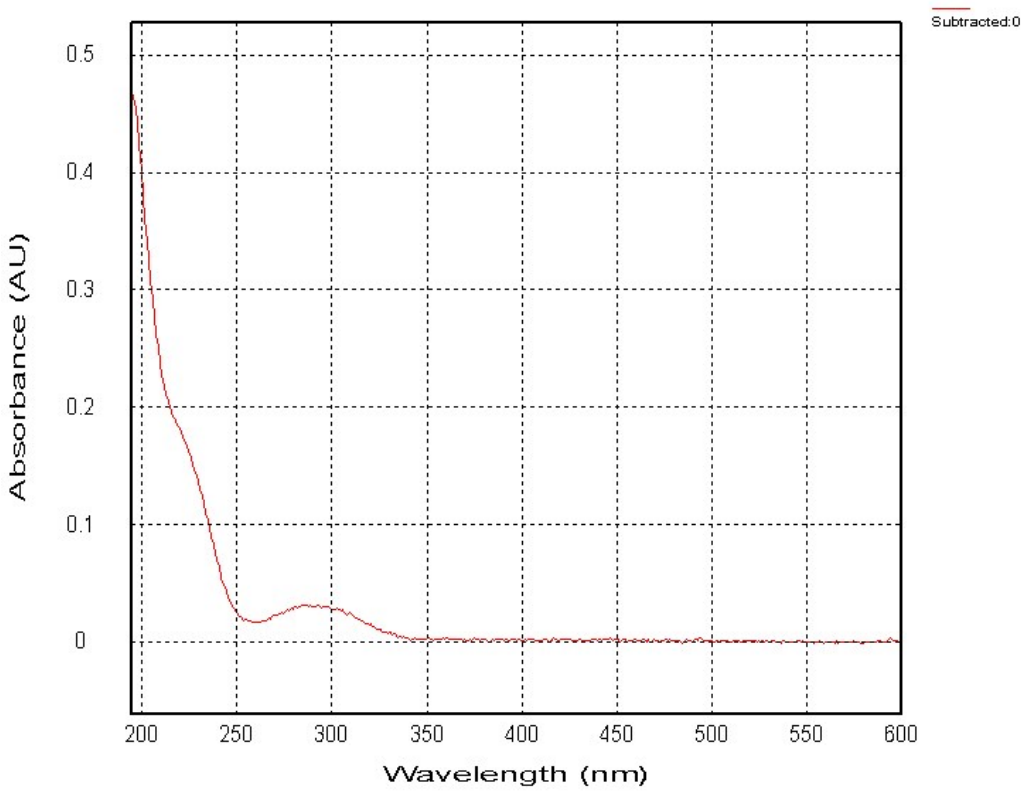

Figure S30. UV spectrum of compound **3**

Table S1. Crystal data and structure refinement for compound **1**

Identification code	global	
Empirical formula	$C_{27}H_{32}O_7$	
Formula weight	468.52	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal system	Monoclinic	
Space group	P 1 21 1	
Unit cell dimensions	a = 6.1764(2) Å	α= 90°.
	b = 22.3986(6) Å	β= 94.5630(10)°.
	c = 16.9215(5) Å	γ = 90°.
Volume	2333.55(12) $Å^3$	
Z	4	
Density (calculated)	1.334 Mg/m^3	
Absorption coefficient	0.784 mm^{-1}	
F(000)	1000	
Crystal size	0.440 x 0.260 x 0.060 mm^3	
Theta range for data collection	2.62 to 72.27°.	
Index ranges	-7<=h<=6, -27<=k<=27, -20<=l<=20	
Reflections collected	40562	
Independent reflections	9202 [R(int) = 0.0401]	
Completeness to theta = 72.27°	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.95 and 0.80	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	9202 / 67 / 643	
Goodness-of-fit on F^2	1.034	
Final R indices [I>2sigma(I)]	R1 = 0.0285, wR2 = 0.0709	
R indices (all data)	R1 = 0.0288, wR2 = 0.0711	
Absolute structure parameter	0.00(3)	
Largest diff. peak and hole	0.274 and -0.293 $e.Å^{-3}$	