Electronic Supplementary Information

One new unusual sesterterpenoid and four new sesquiterpene dimers from *Inula britannica* †

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1 General experimental procedures

Optical rotations were obtained with a JASCO P-2000 polarimeter. IR spectra were obtained with a Bruker FTIR Vector 22 spectrometer. 1D and 2D NMR spectra were recorded on a Bruker 400 spectrometer for ^{1}H-NMR at 400 MHz and ^{13}C-NMR at 100 MHz. ESIMS spectra were recorded on an Agilent LC/MSD Trap XCT spectrometer (Waters, USA), and HR–ESIMS on a Q-TOF micro mass spectrometer (Waters, USA). The normal phase silica gel (100–200, 200–300 mesh, Yantai), MCI gel (CHP20P75–150 lm, Mitsubishi Chemical Co.), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden) were used for column chromatography, and precoated silica $HSGF_{254}$ (10–40 μm, Yantai) plates were used for TLC analysis. HPLC and preparative HPLC were performed with SHIMADZU LC 2010AHT, Agilent Technologies 1200 series and SHIMADZULPD-20A. Optical density was measured using a multifunctional microplate reader Spectromax M5 (Molecular Devices, American) in anti-inflammatory activities assay.

2 Isolation procedure of compounds 1–14

The aerial parts of *I. britannica* were collected in Heilongjiang province PR China, in September 2012, and were authenticated by Professor Hanming Zhang, Department of Pharmacognosy, School of Pharmacy, Second Military Medical University. A voucher specimen (No. IB201209) was deposited at School of Pharmacy, Shanghai Jiao Tong University.

The air-dried and powdered aerial parts of *I. britannica* (15.0 kg) were percolated with 95% EtOH three times (each for 24 h) at room temperature. The combined extracts were concentrated under reduced pressure to afford a residue extract (1.8 kg), which was further suspended in H_2O and partitioned with petroleum ether (PE), CH_2Cl_2 and ethyl acetate (EA), successively. The CH_2Cl_2 fraction (113.8 g) was chromatographed on a silica gel column eluting with a CH_2Cl_2/MeOH (100:0 to 1:1 v/v) gradient to give eleven fractions (Fr.1−Fr.11). Fr.4 (6.0 g) was subjected to CC over

macroporous resin MCI, Sephadex LH-20 and silica gel to give **8** (52.6 mg). Fr.5 (22.0 g) was subjected to a silica gel CC with mixtures of CH_2Cl_2/MeOH (100:0 to 1:1 v/v) as eluents in a stepwise gradient mode to obtain subfractions 5a−5j. From Fr.5a, compounds **1** (8.0 mg) and **4** (10.5 mg) were isolated after CC over macroporous resin MCI followed by preparative HPLC (50% MeOH). From Fr.5d, compound **6** (16.0 mg) was obtained after CC over Sephadex LH-20 and preparative HPLC (65% MeOH). Fr.6 (12.0 g) was subjected to CC over macroporous resin MCI and silica gel eluted with CH_2Cl_2/MeOH (100:0 to 1:1 v/v) to give subfractions 6a−6g. Fr.6a was chromatographed on Sephadex LH-20 and preparative HPLC (55% CH_3CN) to afford **2** (60.0 mg) and **3** (6.0 mg). Fr.6b were further purified by preparative HPLC to give **5** (40% CH3CN, 6.6 mg). Fr.7 (15.0 g) was subjected to CC over macroporous resin MCI and silica gel eluted with CH_2Cl_2/MeOH (100:0 to 1:1 v/v) to yield subfractions 7a−7i. Fr.7d was subjected to Sephadex LH-20 and purified by preparative HPLC (50% MeOH) to obtain compounds **11** (18.0 mg) and **12** (10.8 mg). Using the same procedures, Fr.7e, 7f and 7g were subjected to preparative HPLC (35% MeOH) to afford compounds **7** (30.0 mg), **9** (14.1 mg) and **10** (13.8 mg), respectively. Fr.8 (10.0 g) was subjected to CC over macroporous resin MCI and silica gel eluted with CH_2Cl_2/MeOH (100:0 to 1:1 v/v) to give eight subfractions 8a−8h. **13** (11.0 mg) and **14** (21.0 mg) were obtained after the purification of the Fr.8c (7.5 g) by preparative HPLC (35% CH_3CN).

3 Crystallographic data of compound 1

MeOH, M = 484.57, orthorhombic, space group P2 (1)2(1)2(1), a = 8.7072(2) Å, α= 90°; b = 16.5203(4) Å, β = 90°; c = 17.3249(4) Å , γ = 90°; Z = 4, V = 2492.11(10) Å^3, D_{calcd} = 1.292 mg/m^3, crystal size 0.230 x 0.160 x 0.100 mm^3, Cu Kα (λ =1.54178 Å). F (000) = 1040, T = 140(2) K. The final R values were R_1 = 0.0442, wR_2 = 0.1292, for 10801 observed reflections [I > 2σ (I)]. The absolute structure parameter was -0.05(8).

4 Spectroscopic data of compounds 1–5

Dibritannilactone A (**1**):

Orthorhombic crystals (MeOH); [α]20 D –203.8 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3422, 1765, 1736, 1618, 1460, 1382, 1236, 1040 cm^{-1}; ESIMS *m/z* 475 [M + Na]$^+$, 451 [M – H]$^-$; HRESIMS (positive) *m/z* 453.2292 [M + H]$^+$ (calcd for $C_{27}H_{33}O_6$, 453.2272); ^{1}H and ^{13}C NMR data, see **Tables S1** and **S2**.

Dibritannilactone B (2):

White amorphous powder; [α]20 D +79.0 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3440, 2965, 1766, 1634, 1459,

1384, 1079 cm^{-1}; ESIMS *m/z* 621 $[M + Na]^+$; 597 $[M - H]^-$; HRESIMS (positive) *m/z* 599.3187 $[M + H]^+$ (calcd. for $C_{34}H_{47}O_9$, 599.3215); ^1H and ^{13}C NMR data, see **Tables S1** and **S2**.

Dibritannilactone C (**3**):

White amorphous powder ; [α]20 D +116.8 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3439, 2936, 1762, 1625, 1383, 1241, 1208, 1024, 986 cm^{-1}; ESIMS *m/z* 579 $[M + Na]^+$; 555 $[M - H]^-$; HRESIMS (positive) *m/z* 579.2719 $[M + Na]^+$ (calcd for $C_{32}H_{45}O_8Na$, 579.2723); ^1H and ^{13}C NMR data, see **Tables S1** and **S2**.

Dibritannilactone D (**4**):

White amorphous powder; [α]20 D +67.2 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3440, 2935, 1762, 1630, 1460, 1383, 1239, 1206, 1007, 985 cm^{-1}; ESIMS *m/z* 563 $[M + Na]^+$; 539 $[M - H]^-$; HRESIMS (positive) *m/z* 563.2989 $[M + Na]^+$ (calcd for $C_{32}H_{45}O_7Na$, 563.2985); ^1H and ^{13}C NMR data, see **Tables S1** and **S2**.

Dibritannilactone E (**5**):

White amorphous powder; [α]20 D +58.3 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3438, 2935, 1762, 1632, 1445, 1234 cm^{-1}; ESIMS *m/z* 579 $[M + Na]^+$; 555 $[M - H]^-$; HRESIMS (positive) *m/z* 579.2936 $[M + Na]^+$ (calcd for $C_{32}H_{44}O_8Na$, 579.2934); ^1H and ^{13}C NMR data, see **Tables S1** and **S2**.

Table S1. [1]H NMR data for compounds **1–5**

No.	1[a]	2[b]	3[b]	4[b]	5[b]
1		4.0 0 m; 3.95 m	3.97 m; 3.95 m	3.97 m; 3.95 m	3.49 t (6.3)
2	6.65 s	1.55 m; 1.35 m	1.54 m; 1.35 m	1.50 m; 1.32 m	1.45 m
3		1.33 m; 1.09 m	1.35 m; 1.09 m	1.34 m; 1.05 m	1.48 m
4		2.73 m	2.74 m	2.72 m	2.45 m
5	6.48 d (7.6)				
6	6.61 d (7.6)	4.23 s	4.25 s	4.25 s	5.51 d (1.4)
7	2.25 s	2.74 m	3.21 d (6.3)	2.52 d (6.0)	2.98 m
8		5.07 m	5.10 m	5.10 m	4.96 m
9	6.25 s	2.46 m; 2.42 m	2.46 m; 2.42 m	2.46 d (2.9); 2.42 d (2.8)	2.43 m; 1.96 dd (15.6, 2.4)
10	2.86 dd (12.8, 4.1); 1.55 m				
13		2.08 m; 1.88 m	2.00 m; 1.71 m	1.85 brs; 1.80 m	2.32 m; 1.88 m
14		1.74 s	1.74 s	1.75 s	1.32 s
15		1.13 d (7.0)	1.11 d (7.0)	1.11 d (7.0)	1.10 d (6.8)
1′					
2′	4.58 s	4.52 brs	3.50 brs		4.61 s
3′	2.75 m	2.93 d (1.2)	2.70 m	2.86 brs	2.99 d (1.6)
4′				2.67 m; 2.15 m	

6′	2.48 brd (16.0); 1.90 m	2.72 m; 2.15 m	2.70 m; 2.10 m	2.67 m; 2.15 m	2.76 m; 2.73 m
7′	0.58 m	2.41 m	2.41 m	2.40 m	2.35 m
8′	4.10 dt (11.5, 3.3)	4.57 dt (11.4, 3.6)	4.55 dt (11.2, 3.2)	4.58 dt (11.5, 3.7)	4.55 dt (11.5, 3.7)
9′	2.00 m; 1.50 m	2.33 m; 1.88 m	2.33 m; 1.85 dt (12.6, 3.0)	2.33 m; 1.95m	2.33 m; 1.88m
10′	2.55 m	2.17 m	2.24 m	2.15 m	2.13 m
11′	2.20 m	2.20 m	2.70 m	2.65 m	2.70 m
12′					
13′	1.05 d (7.8)	1.19 d (7.8)	1.19 d (7.8)	1.20 d (7.8)	1.21 d (7.8)
14′	0.97 d (7.1)	1.06 d (7.3)	1.08 d (7.3)	1.14 d (7.3)	1.06 d (7.4)
15′	1.88 s	1.58 d (0.92)	1.54 d (1.3)	1.54 d (1.2)	1.61 d (1.1)
2″	2.11 s	2.12 s	1.96 s	1.97 s	2.02 s
2‴		1.96 s			

[a] Measured at 400 MHz in $CDCl_3$; [b] Measured at 400 MHz in CD_3OD; δ in ppm; J in Hz within parentheses.

Table S2. ^{13}C NMR data for compounds **1–5**

No.	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[b]

1	139.9 s	65.6 t	65.6 t	65.6 s	63.2 t
2	111.7 d	28.2 t	28.1 t	28.0 t	31.6 t
3	158.1 s	33.4 t	33.5 t	33.4 t	36.1 t
4	131.0 s	35.4 d	35.3 d	35.4 d	33.9 d
5	125.2 d	138.1 s	138.0 s	137.4 s	151.3 s
6	121.7 d	64.7 d	64.7 d	64.9 d	118.4 d
7	21.5 q	52.9 d	52.2 d	55.9 d	43.8 d
8	59.7 s	78.5 d	78.6 d	78.3 d	76.9 d
9	104.0 d	35.1 t	35.4 t	35.1 t	40.6 t
10	35.5 t	131.3s	131.4 s	131.2 s	68.7 s
11		56.5 s	56.7 s	57.6 s	59.4 s
12		181.3 s	182.3 s	181.5 s	181.4 s
13		37.9 t	37.3 t	40.3 t	37.6 t
14		20.7 q	20.5 q	20.5 q	28.2 q
15		20.0 q	19.7 q	19.7 q	23.3 q
1′	68.5 s	63.9 s	65.3 s	65.6 s	63.6 s
2′	84.7 d	83.6 d	83.8 d	31.2 t	82.9 d
3′	47.3 d	59.3 d	61.8 d	59.6 d	56.8 d
4′	139.2 s	134.4 s	134.3 s	137.1 s	134.3 s
5′	135.1 s	138.9 s	139.1 s	140.0 s	139.1 s
6′	24.5 t	25.5 t	25.6 t	25.2 t	25.4 t
7′	41.8 d	44.3 d	44.5 d	44.3 d	44.7 d
8′	80.8 d	82.9 t	84.1 d	83.7 d	83.6 d
9′	35.6 t	37.3 t	37.3 t	38.0 t	37.2 t

10′	26.6 d	31.1 d	31.0 d	35.7 d	31.1 d
11′	39.9 d	41.8 d	41.8 d	41.8 d	41.7 d
12′	179.6 s	182.4 s	182.7 s	182.6 s	182.3 s
13′	9.7 q	10.1 q	10.0 q	10.0 q	10.0 q
14′	19.5 q	17.3 q	17.1 q	17.9 q	17.2 q
15′	13.4 q	14.3 q	14.3 q	14.3 q	14.0 q
1″	170.3 s	172.2 s	172.7 s	172.8s	171.7 s
2″	21.2 q	21.3 q	20.8 q	20.9 q	21.0 q
1‴		172.7 s			
2‴		20.9 q			

[a] Measured at 100 MHz in $CDCl_3$; [b] Measured at 100 MHz in CD_3OD; δ in ppm; J in Hz within parentheses.

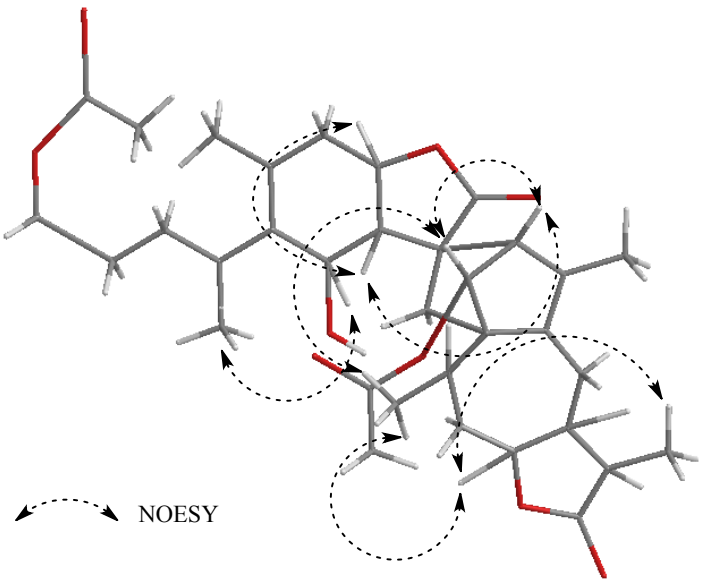

Fig. S1. Key NOESY correlations of compound **2**

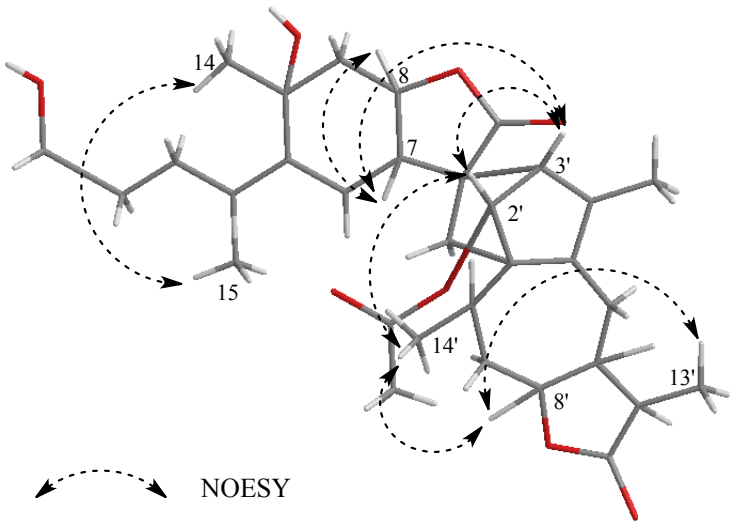

Fig. S2. Key NOESY correlations of compound **5**

5 Nitrite Assay

Inhibitory activities against LPS-induced NO production in RAW 264.7 macrophages[1] were selected as the anti-inflammatory screening method. RAW264.7 cells grown on 100 mm culture dish were harvested and seeded in 96-well plates (1 × 105 cells/well) for NO production. The plates were pretreated with various concentrations (50.0, 10.0, 2.0, and 0.4 μM) of samples for 30 min and incubated with LPS (1 μg/ml) for 24 h. The amount of NO was determined by the nitrite concentration in the cultured RAW264.7 macrophage supernatants with the Griess reagent.

6 NMR spectra for compounds 1–5

Fig. S3 ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of compound **1**

Fig. S4 ^{13}C NMR spectrum ($CDCl_3$, 100 MHz) of compound **1**

Fig. S5 DEPT spectrum ($CDCl_3$, 100 MHz) of compound **1**

Fig. S6 HMQC spectrum ($CDCl_3$, 400 MHz) of compound **1**

Fig. S7 ^{1}H–^{1}H COSY spectrum ($CDCl_3$, 400 MHz) of compound **1**

Fig. S8 HMBC spectrum ($CDCl_3$, 400 MHz) of compound **1**

Fig. S9 NOESY spectrum ($CDCl_3$, 400 MHz) of compound **1**

Fig. S10 ^{1}H NMR spectrum (CD_3OD, 400 MHz) of compound **2**

Fig. S36 NOESY spectrum (CD_3OD, 400 MHz) of compound **5**

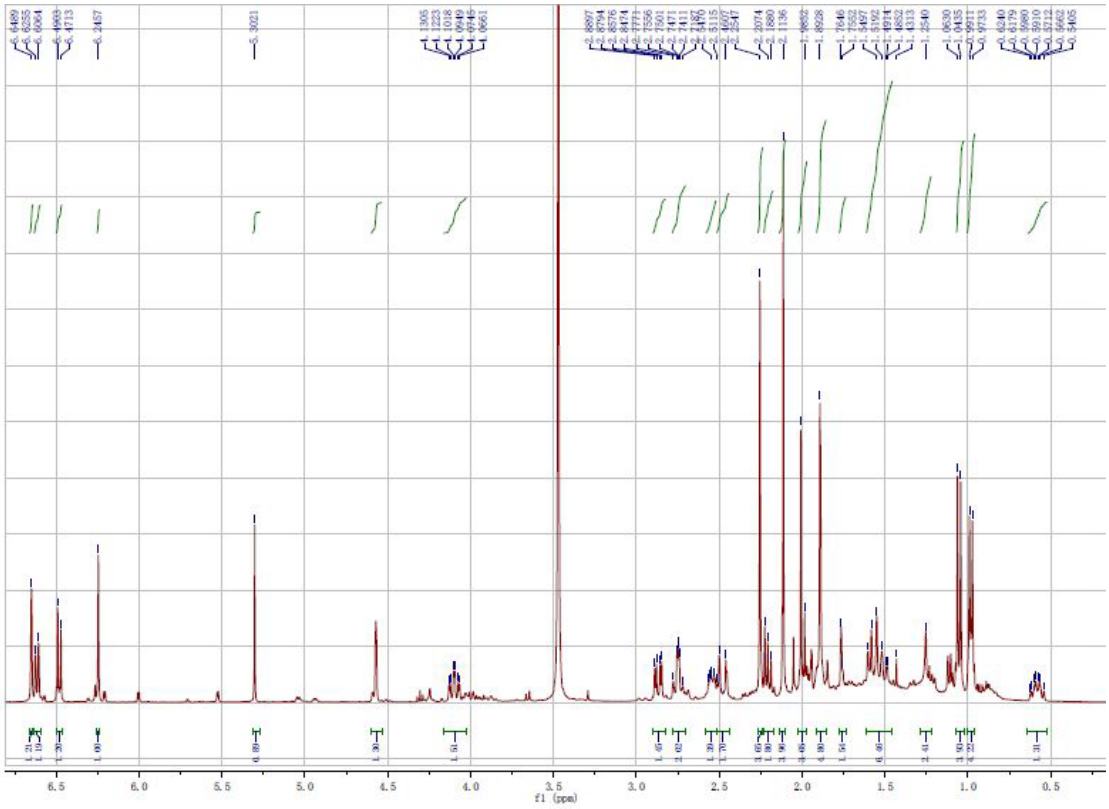

Fig. S3 ^1H NMR spectrum ($CDCl_3$, 400 MHz) of compound **1**

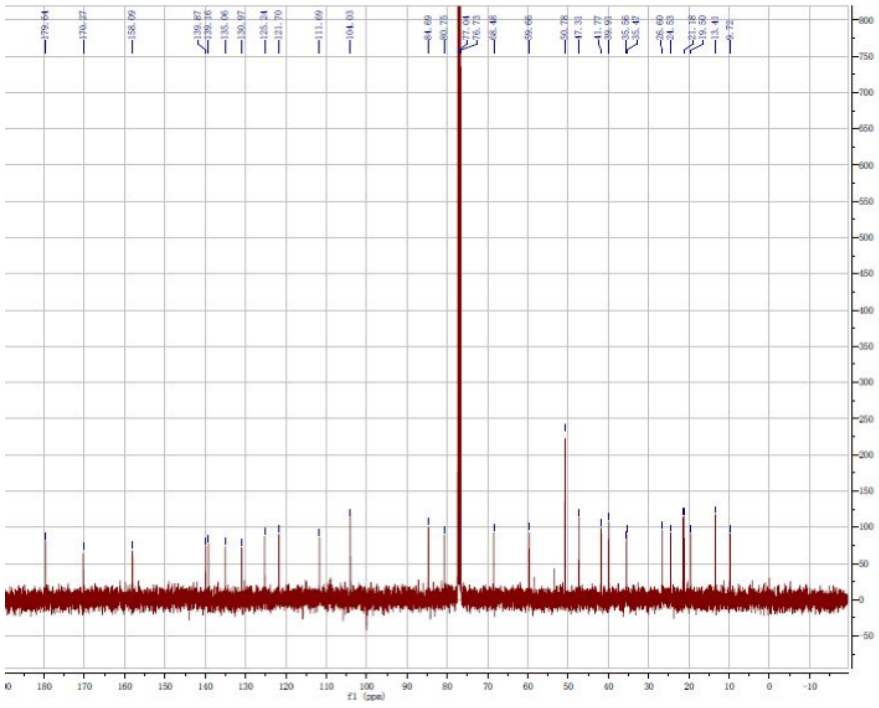

Fig. S4 ^{13}C NMR spectrum ($CDCl_3$, 100 MHz) of compound **1**

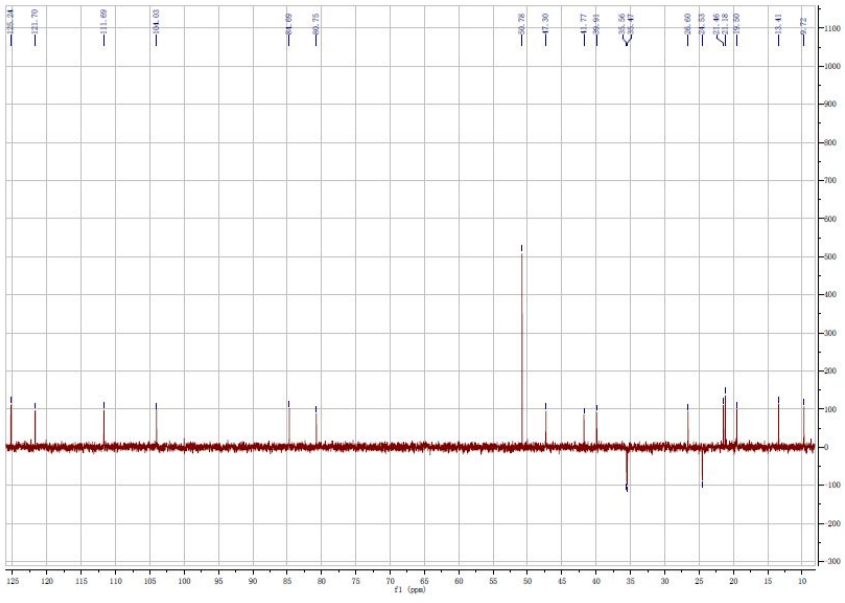

Fig. S5 DEPT spectrum ($CDCl_3$, 100 MHz) of compound **1**

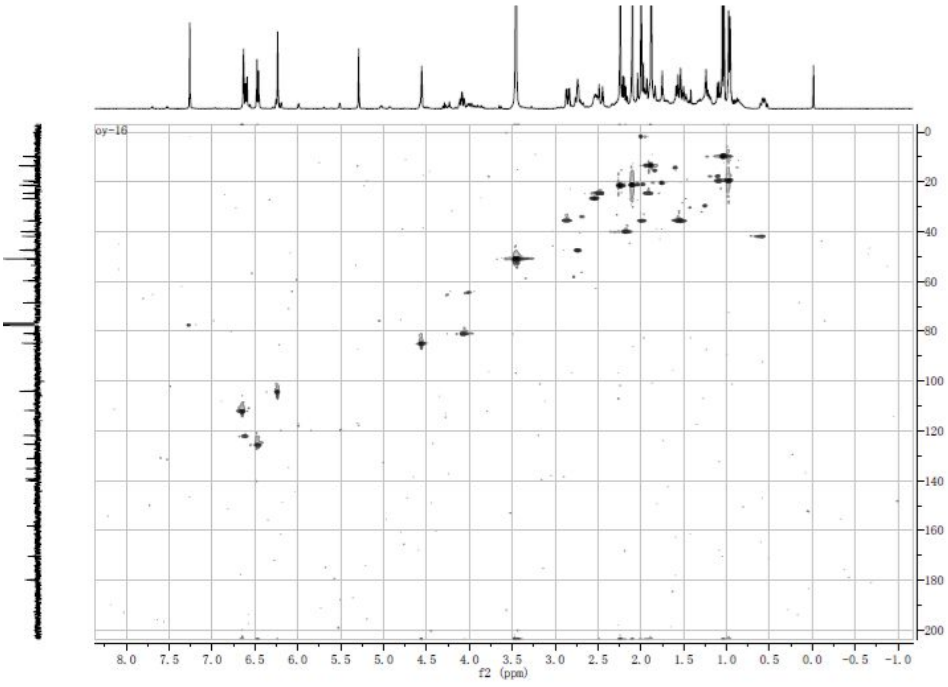

Fig. S6 HMQC spectrum ($CDCl_3$, 400 MHz) of compound **1**

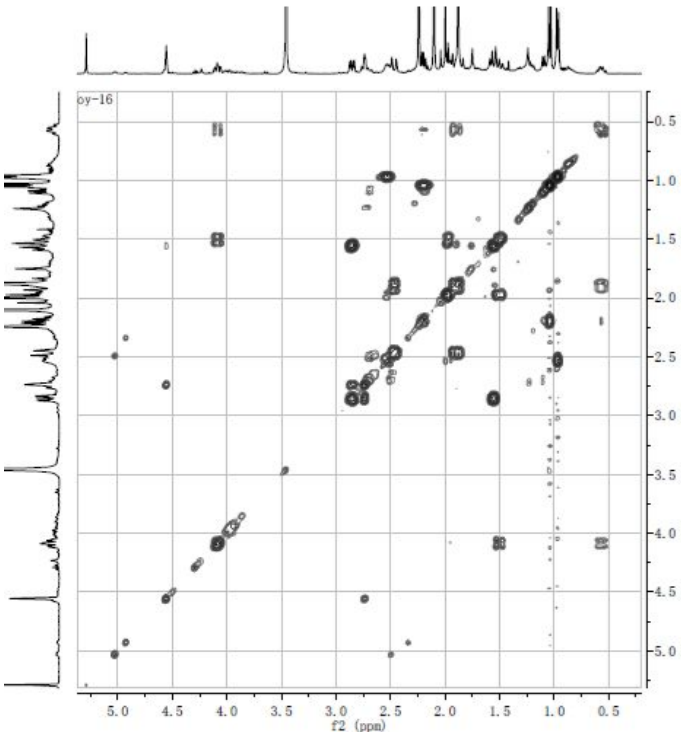

Fig. S7 $^{1}H–^{1}H$ COSY spectrum ($CDCl_3$, 400 MHz) of compound **1**

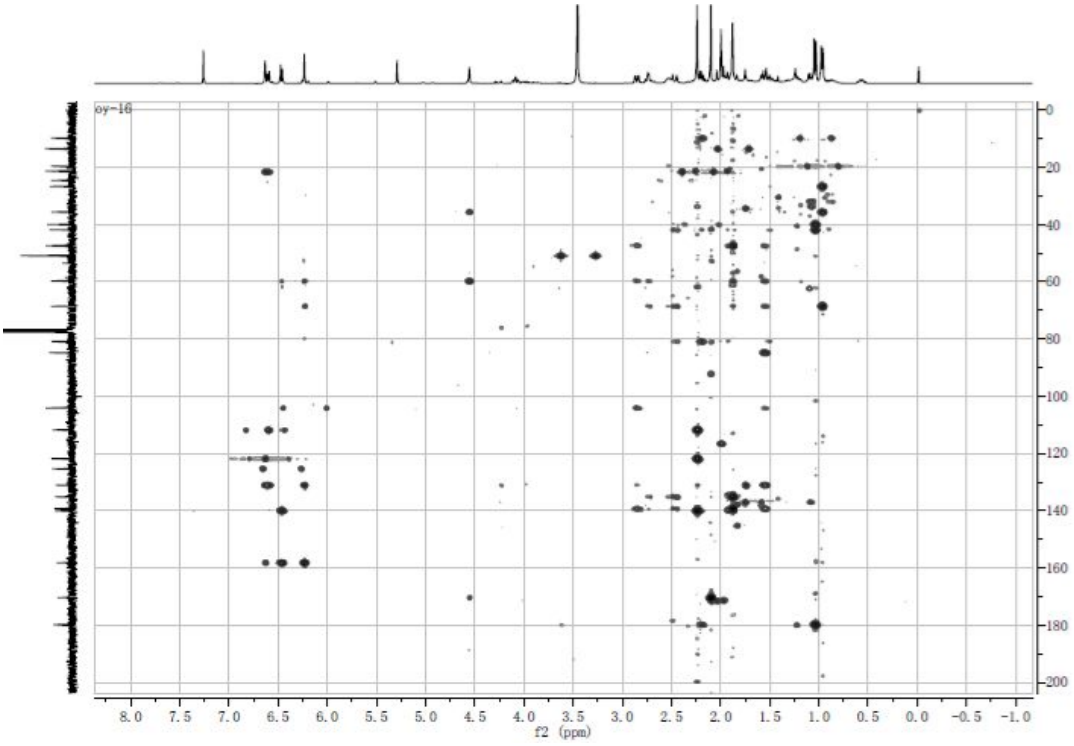

Fig. S8 HMBC spectrum ($CDCl_3$, 400 MHz) of compound **1**

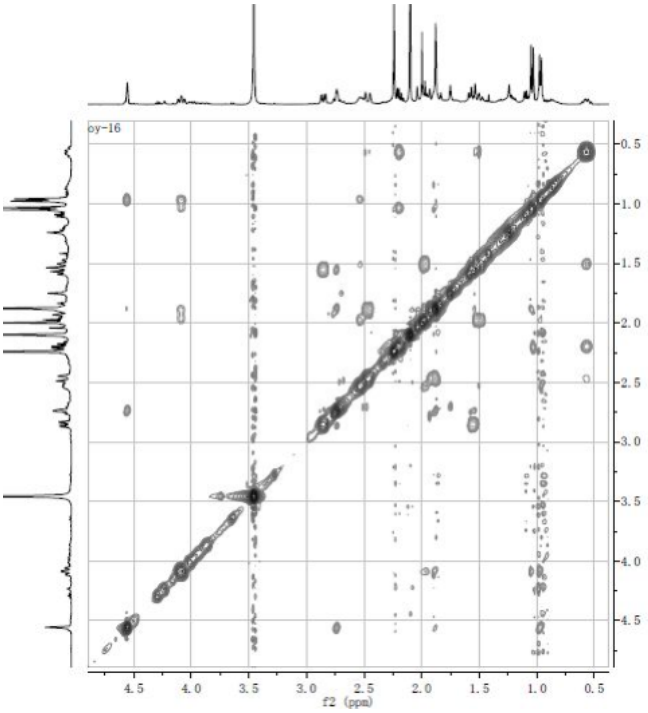

Fig. S9 NOESY spectrum ($CDCl_3$, 400 MHz) of compound **1**

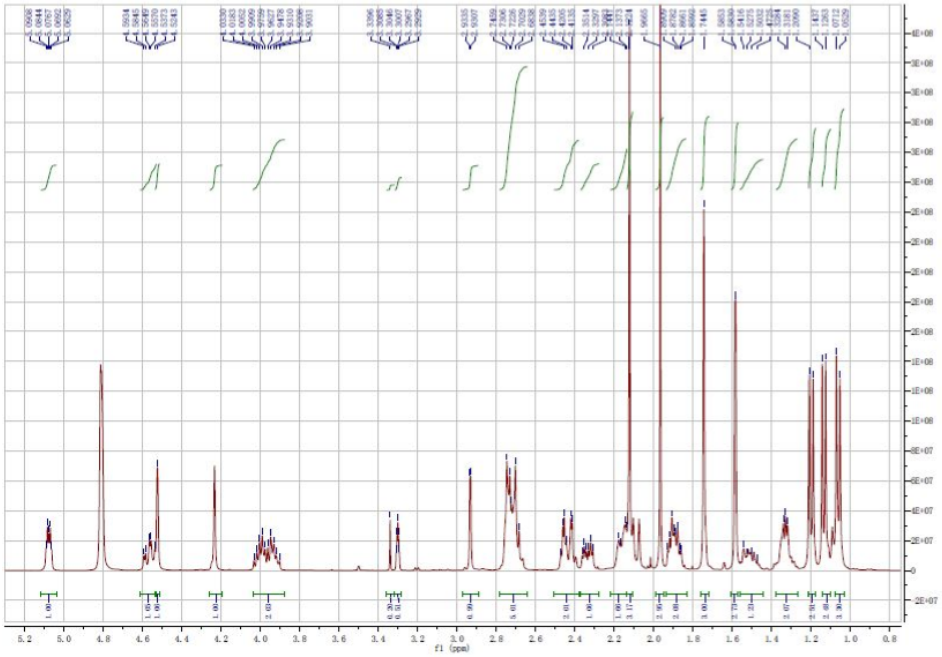

Fig. S10 ^{1}H NMR spectrum (CD_3OD, 400 MHz) of compound **2**

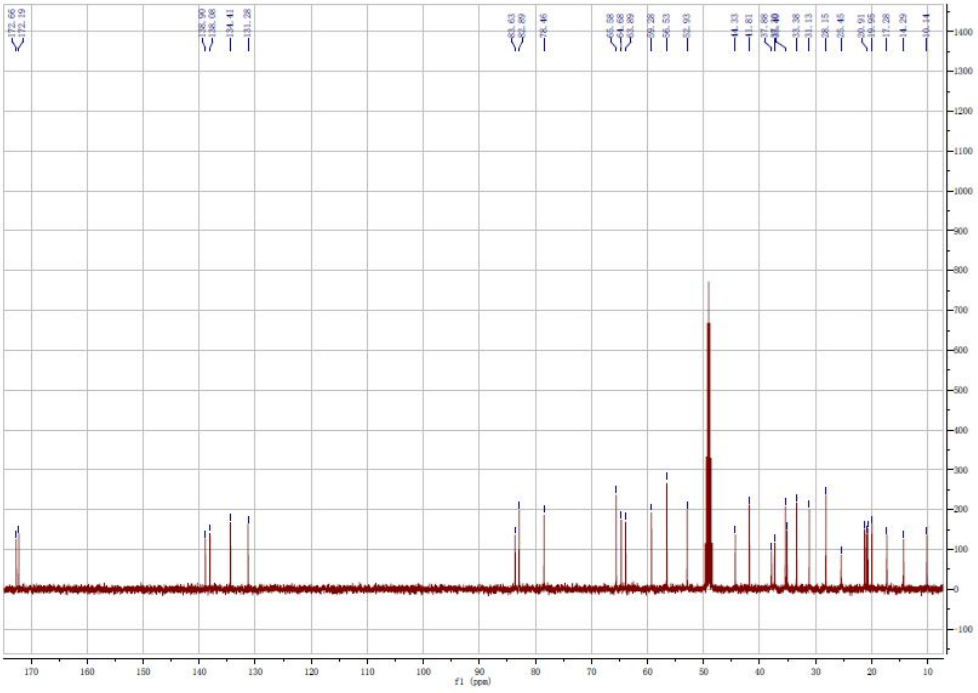

Fig. S11 ^{13}C NMR spectrum (CD_3OD, 100 MHz) of compound **2**

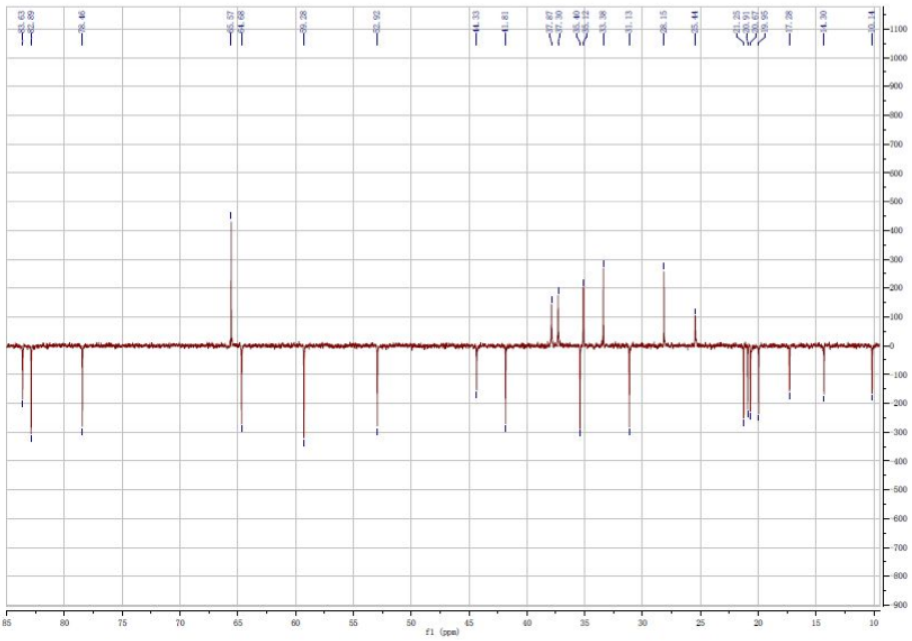

Fig. S12 DEPT spectrum (CD_3OD, 100 MHz) of compound **2**

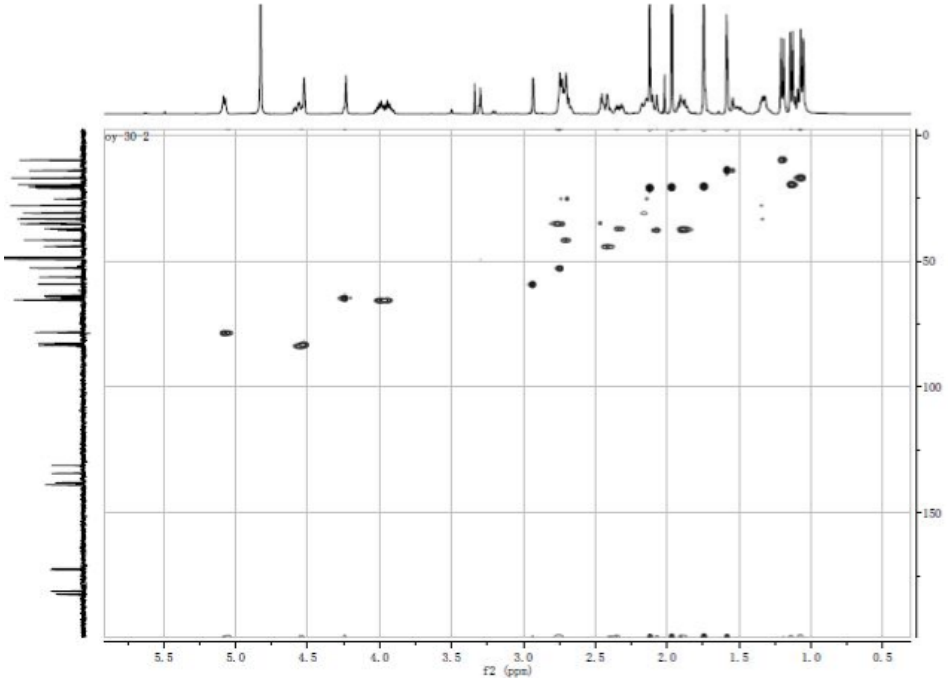

Fig. S13 HMQC spectrum (CD_3OD, 400 MHz) of compound **2**

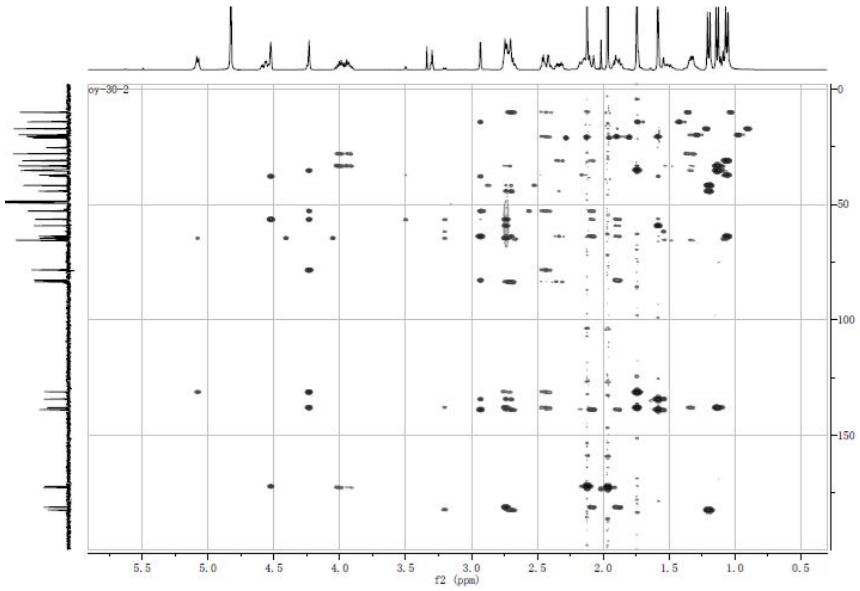

Fig. S14 HMBC spectrum (CD_3OD, 400 MHz) of compound **2**

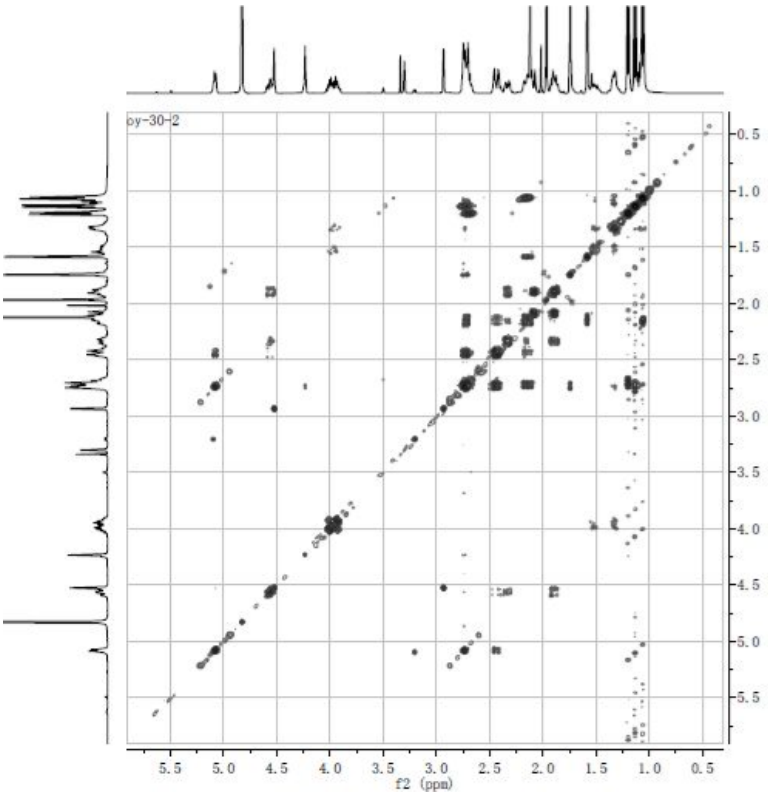

Fig. S15 ^{1}H–^{1}H COSY spectrum (CD_3OD, 400 MHz) of compound **2**

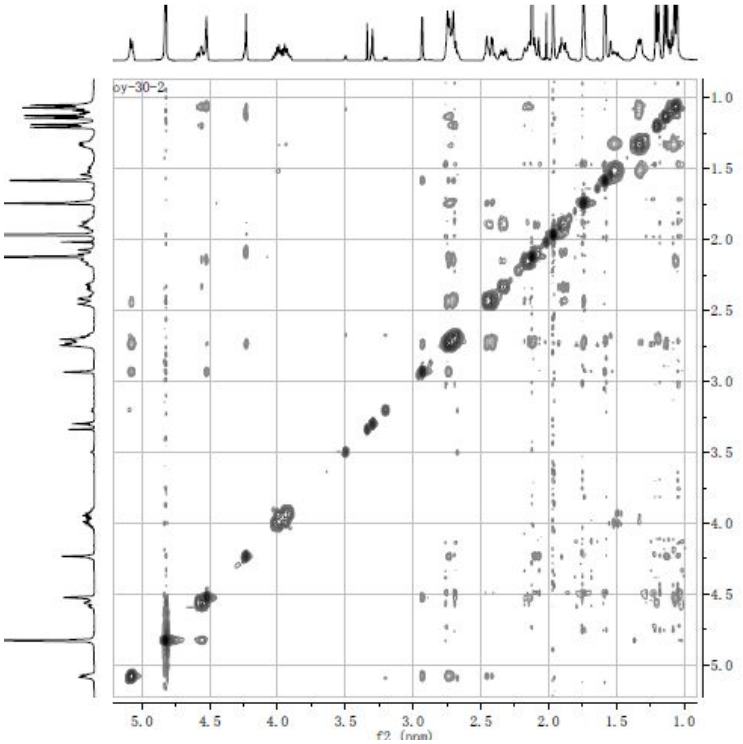

Fig. S16 NOESY spectrum (CD_3OD, 400 MHz) of compound **2**

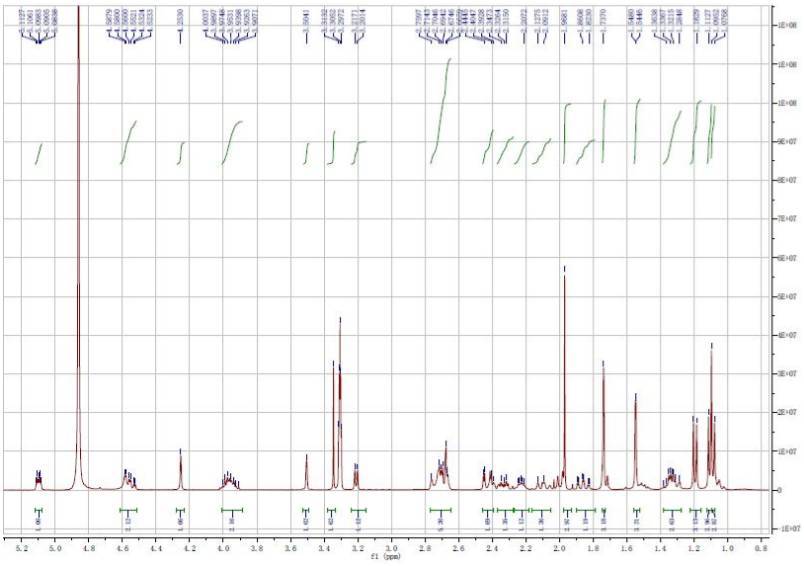

Fig. S17 ^1H NMR spectrum (CD_3OD, 400 MHz) of compound **3**

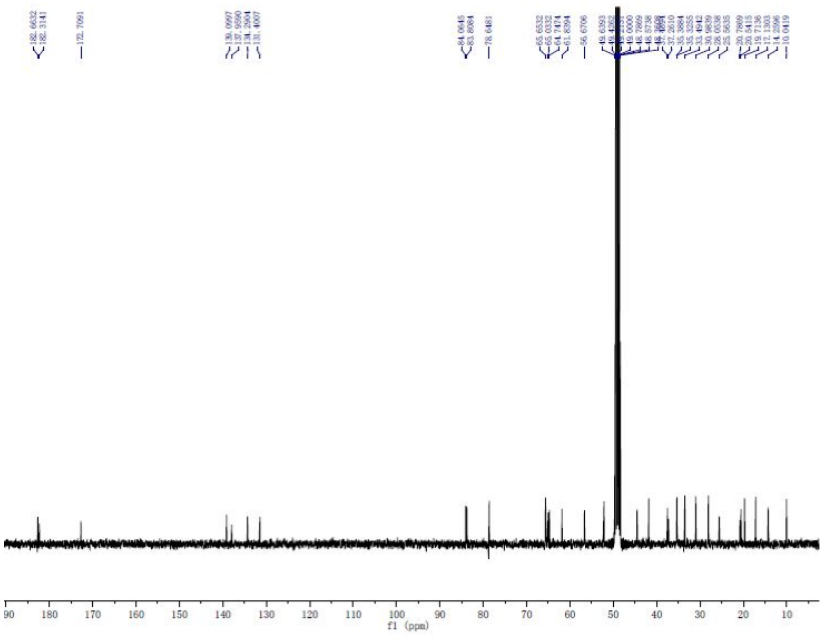

Fig. S18 ^{13}C NMR spectrum (CD_3OD, 100 MHz) of compound **3**

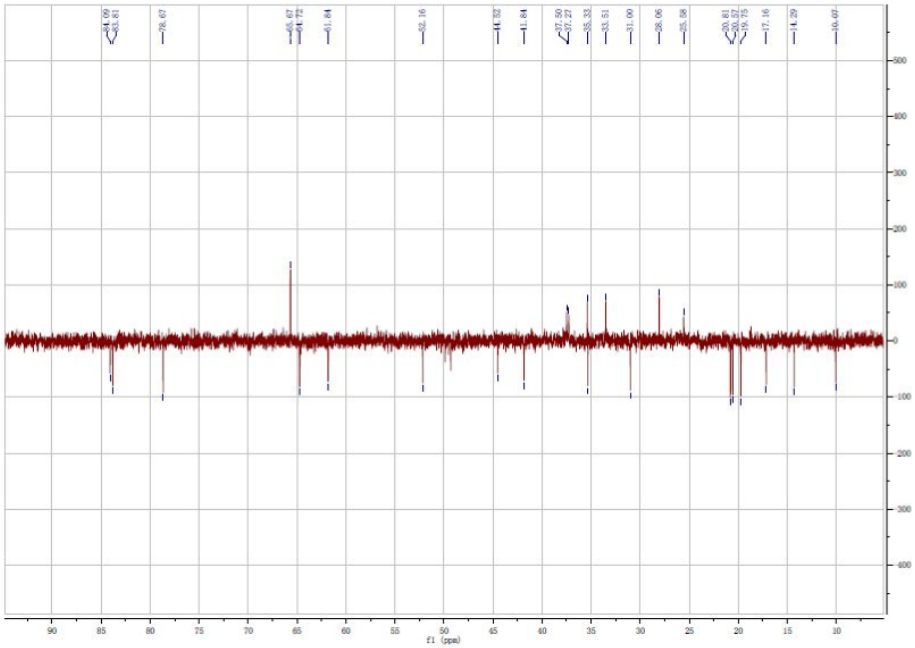

Fig. S19 DEPT spectrum (CD_3OD, 100 MHz) of compound **3**

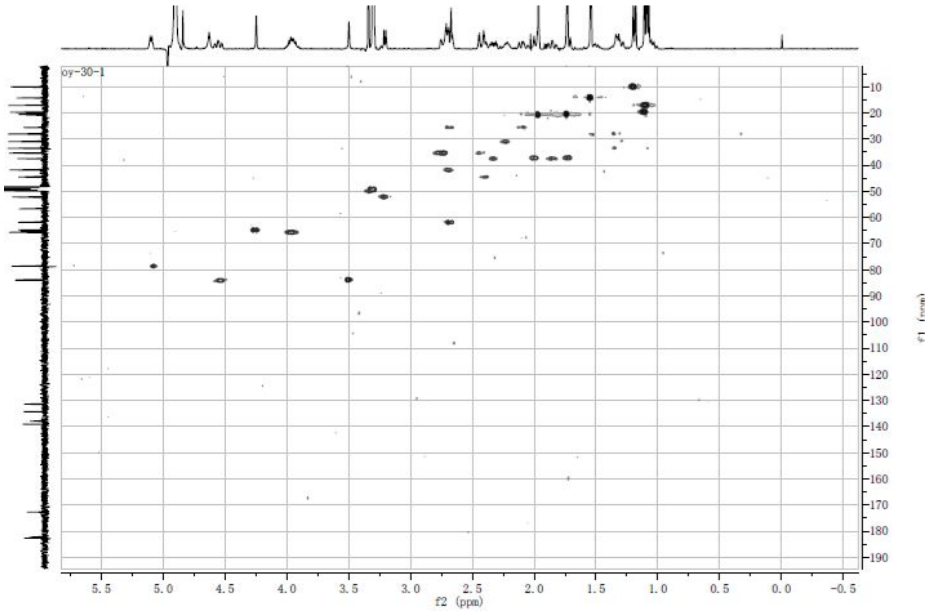

Fig. S20 HMQC spectrum (CD_3OD, 400 MHz) of compound **3**

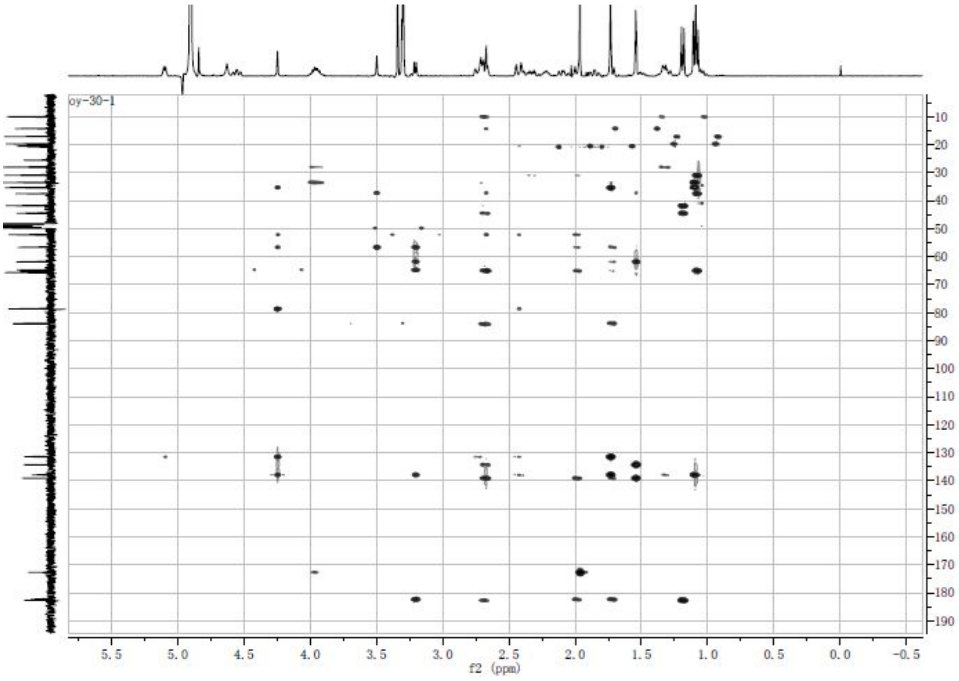

Fig. S21 HMBC spectrum (CD_3OD, 400 MHz) of compound **3**

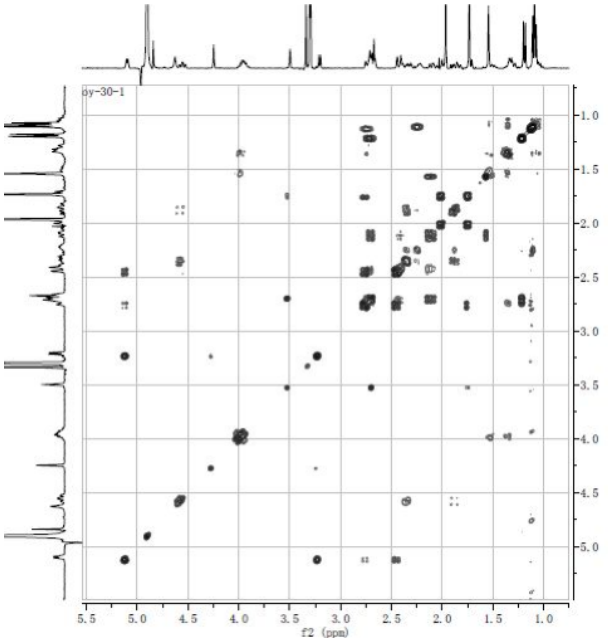

Fig. S22 $^{1}H–^{1}H$ COSY spectrum (CD_3OD, 400 MHz) of compound **3**

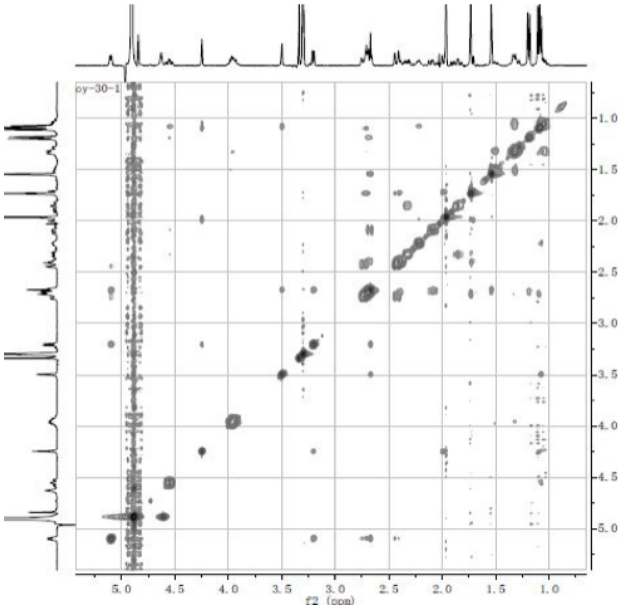

Fig. S23 NOESY spectrum (CD_3OD, 400 MHz) of compound **3**

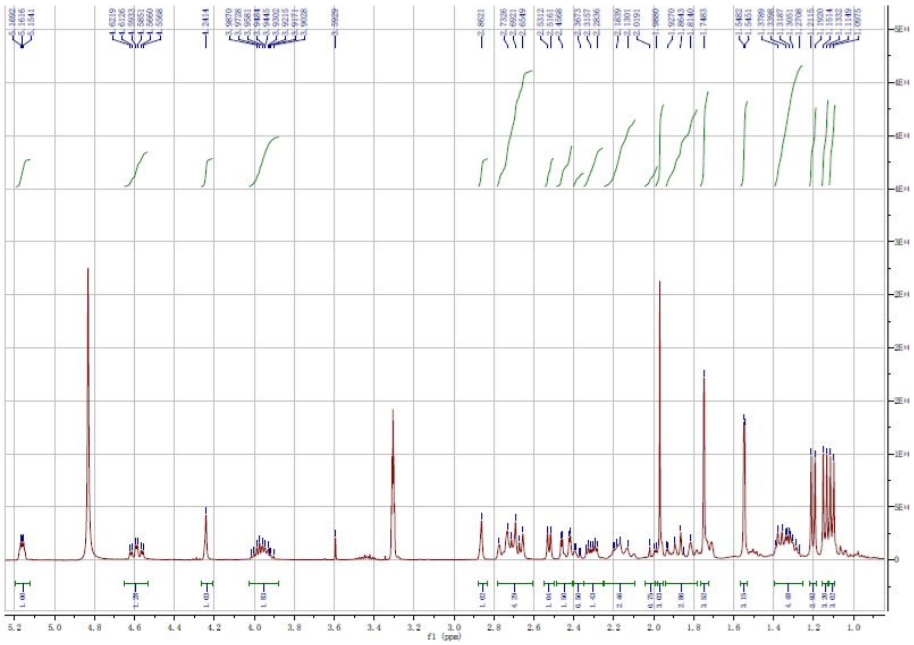

Fig. S24 ^{1}H NMR spectrum (CD_3OD, 400 MHz) of compound **4**

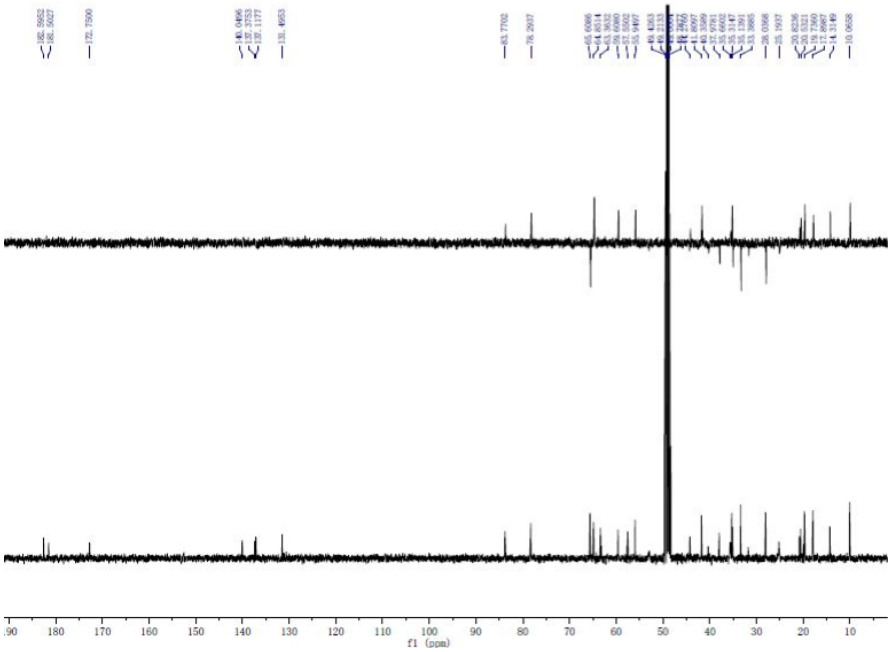

Fig. S25 ^{13}C NMR and DEPT spectra (CD_3OD, 100 MHz) of compound **4**

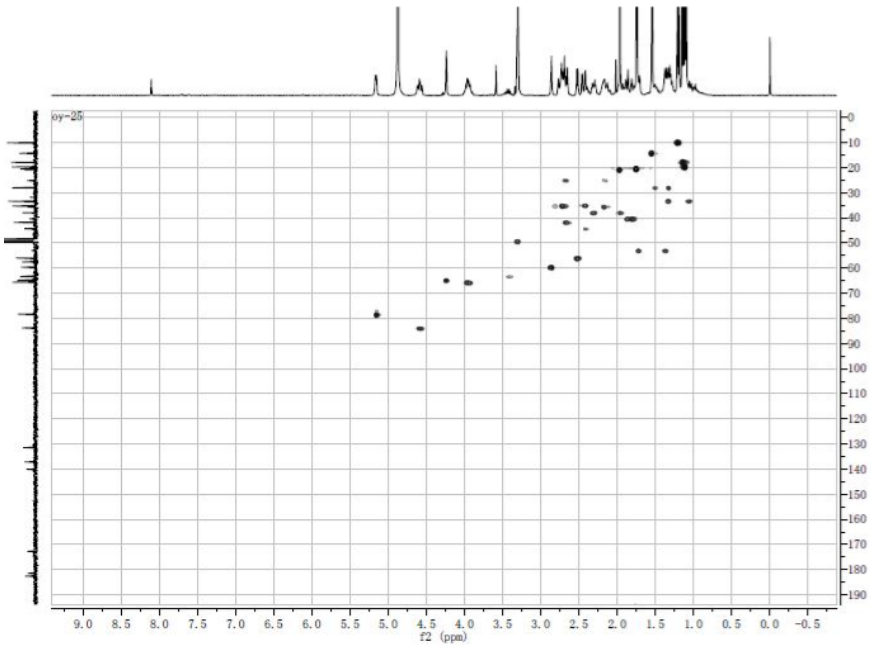

Fig. S26 HMQC spectrum (CD_3OD, 400 MHz) of compound **4**

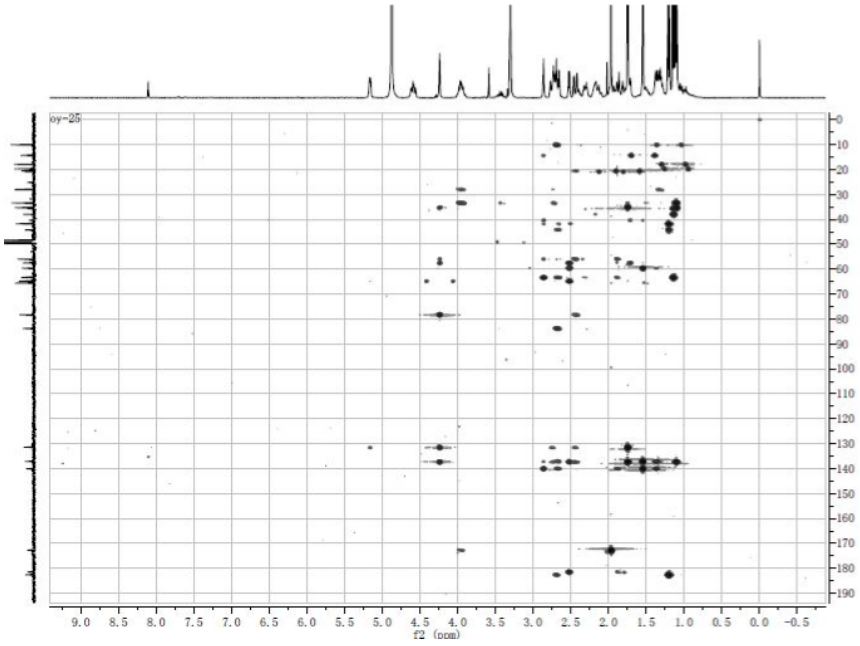

Fig. S27 HMBC spectrum (CD_3OD, 400 MHz) of compound **4**

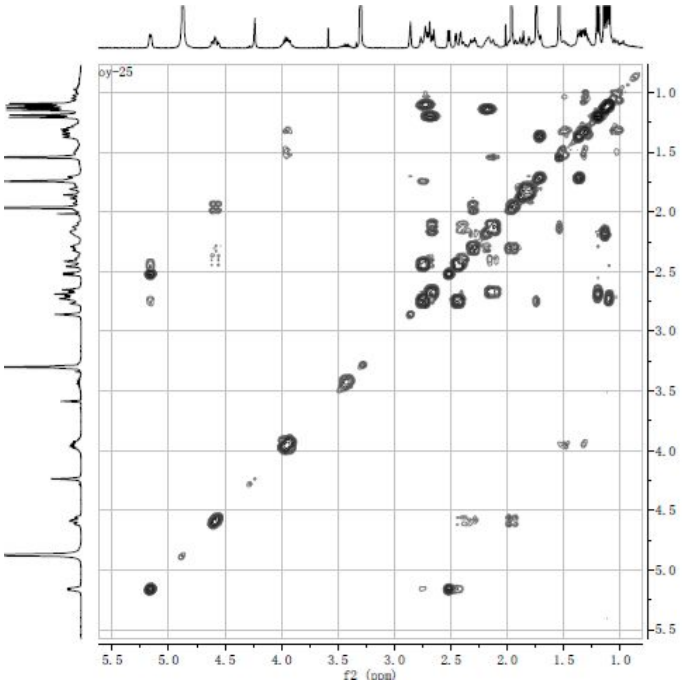

Fig. S28 $^{1}H–^{1}H$ COSY spectrum (CD_3OD, 400 MHz) of compound **4**

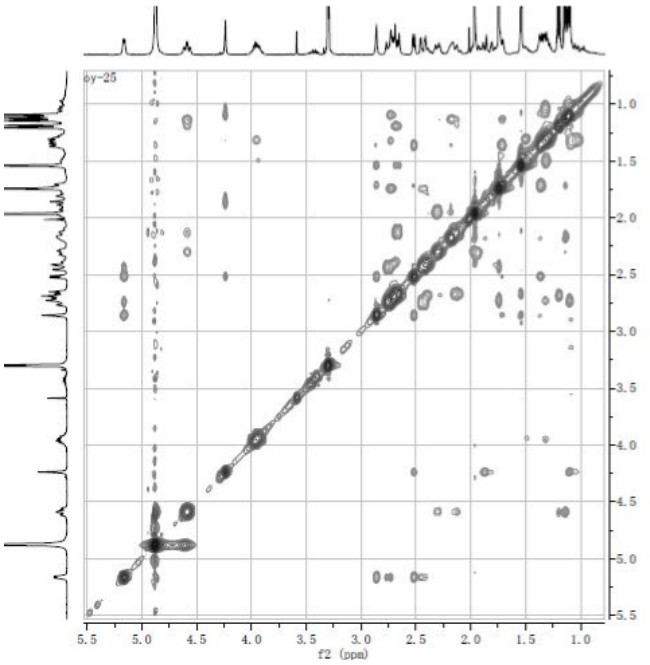

Fig. S29 NOESY spectrum (CD_3OD, 400 MHz) of compound **4**

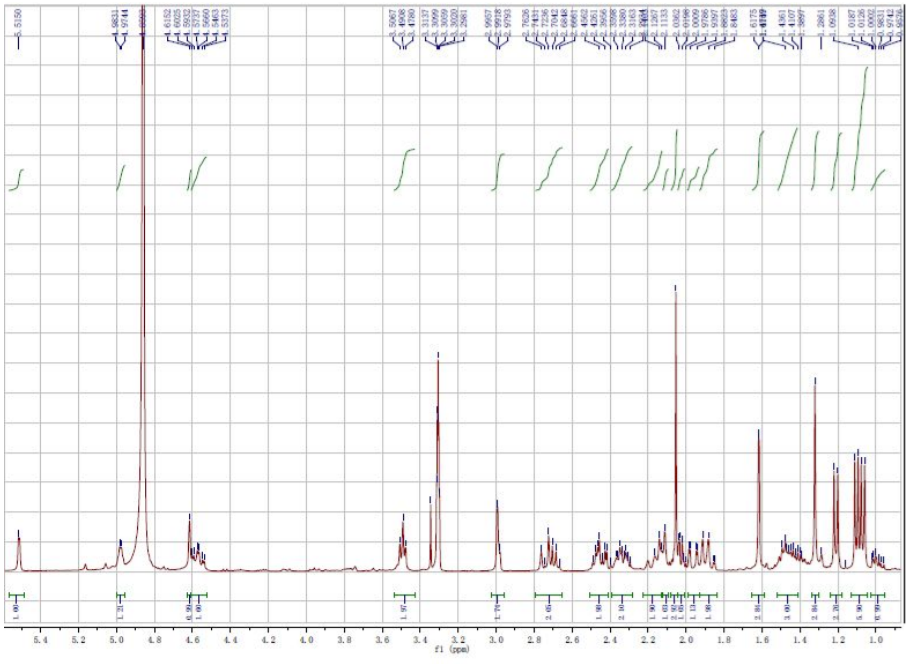

Fig. S30 ^{1}H NMR spectrum (CD_3OD, 400 MHz) of compound **5**

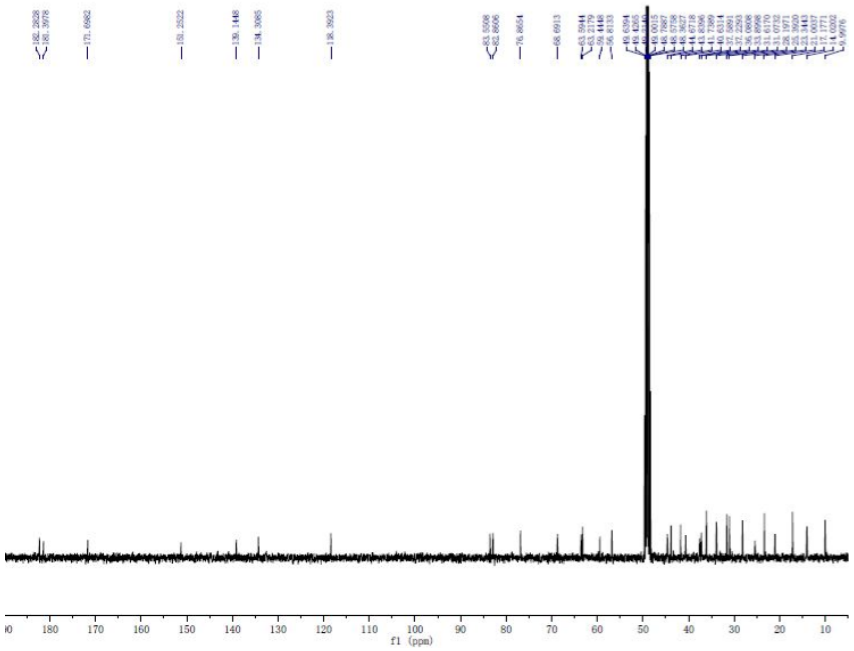

Fig. S31 ^{13}C NMR spectrum (CD_3OD, 100 MHz) of compound **5**

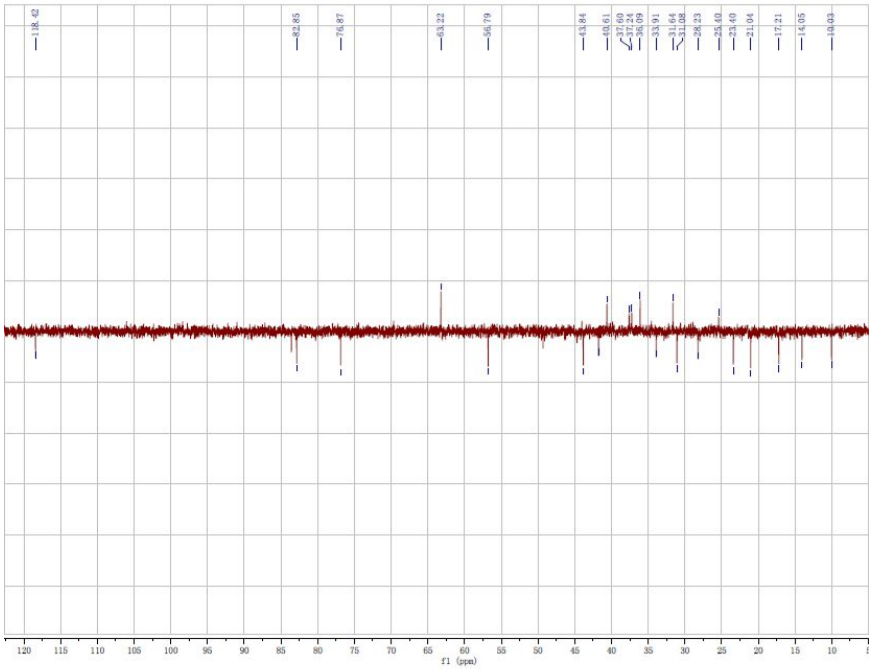

Fig. S32 DEPT spectrum (CD_3OD, 100 MHz) of compound **5**

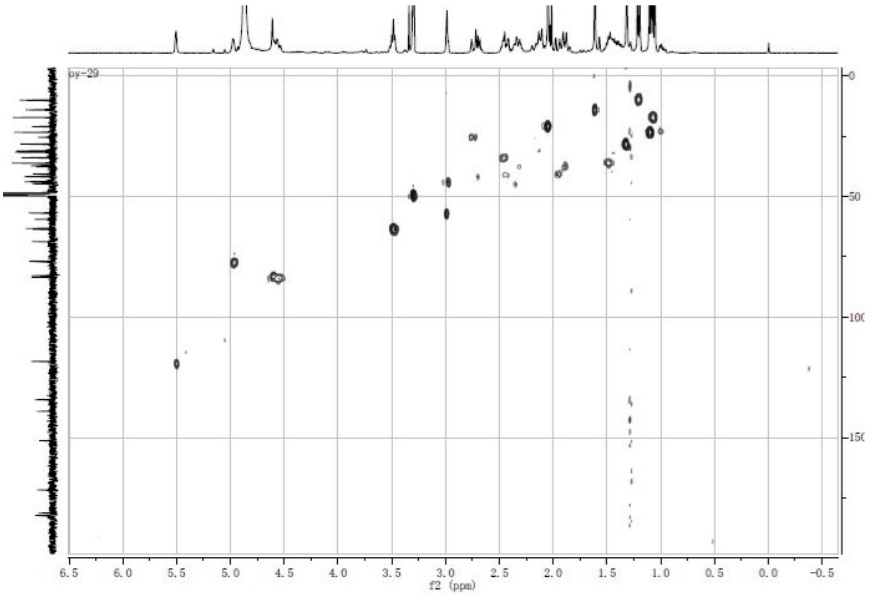

Fig. S33 HMQC spectrum (CD_3OD, 400 MHz) of compound **5**

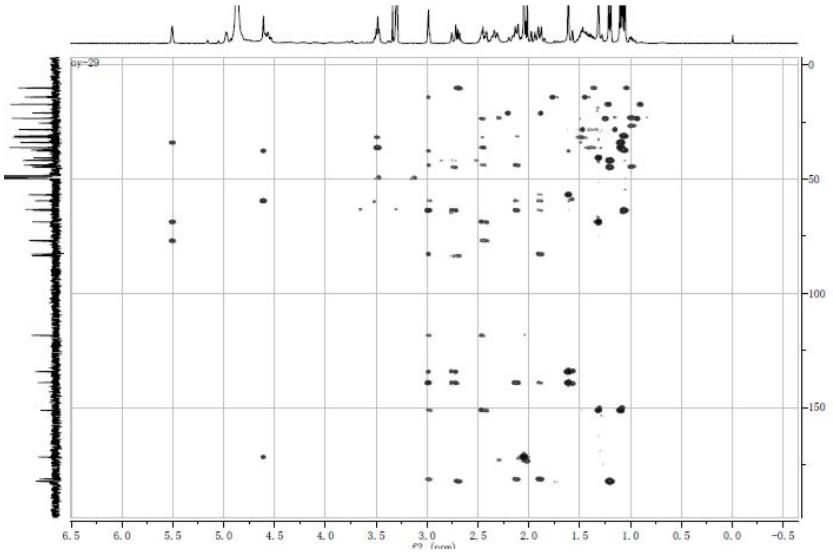

Fig. S34 HMBC spectrum (CD_3OD, 400 MHz) of compound **5**

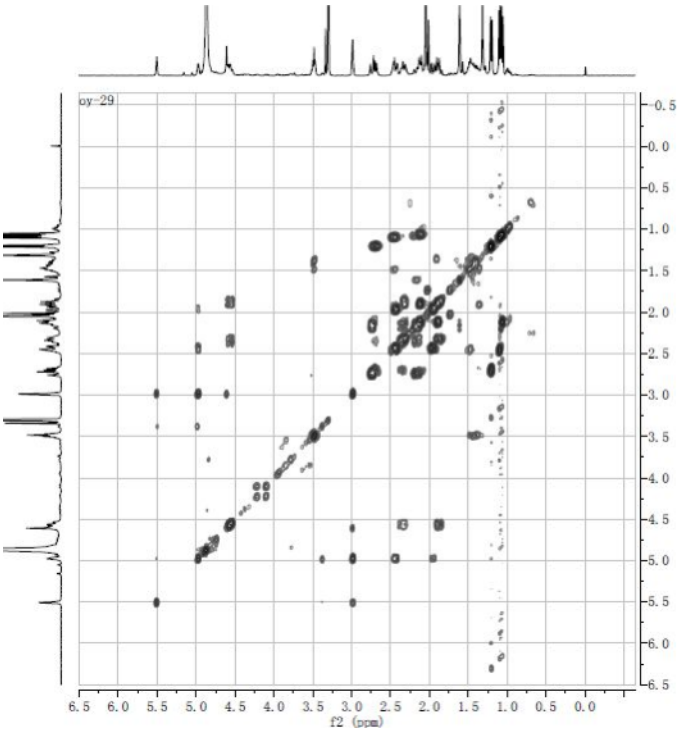

Fig. S35 $^{1}H–^{1}H$ COSY spectrum (CD_3OD, 400 MHz) of compound **5**

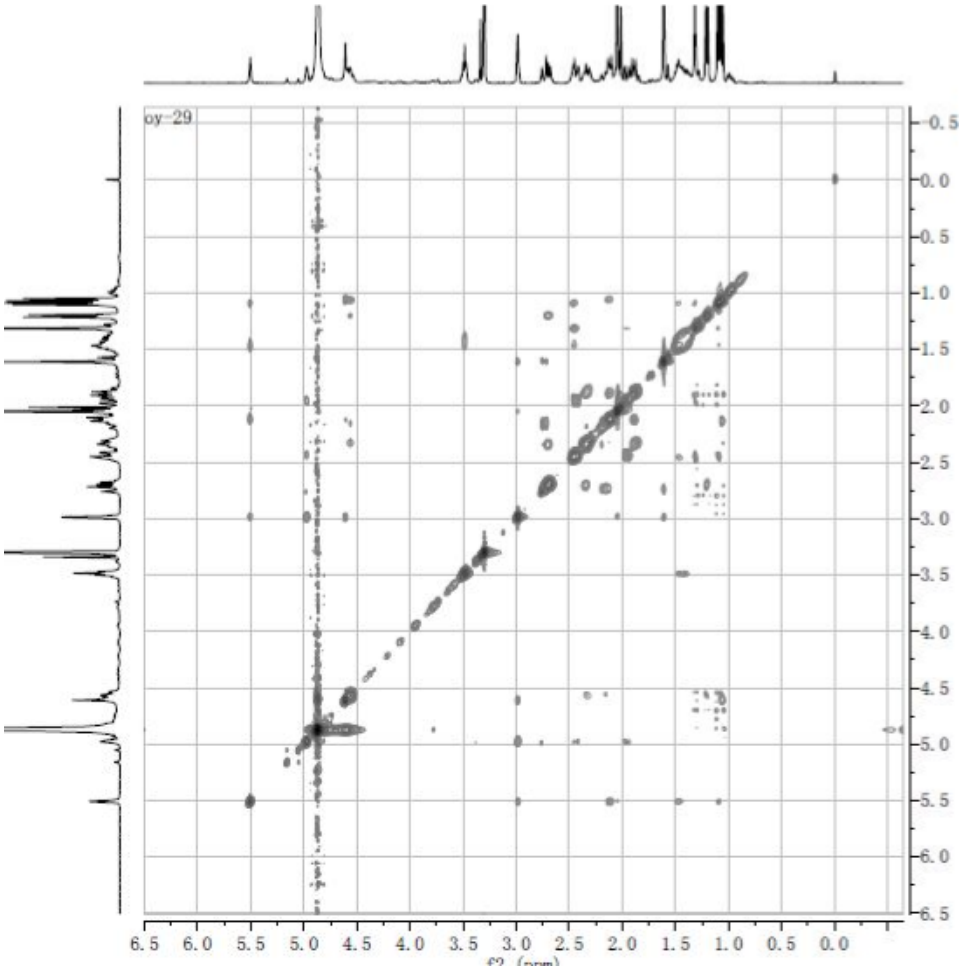

Fig. S36 NOESY spectrum (CD_3OD, 400 MHz) of compound **5**

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1 H. H. H. W. Schmidt, M. Kelm, Determination of nitrite and nitrate by the Griess reaction. In: Feelisch, M., Stramler, J., editors. Methods in Nitrix Oxide research. New York: John Wiley & Sons, Ltd.: London, 1996, **33**, 491−497.