

# Supplementary Material

## **(±) Gancochlearols A and B: Cytotoxic and COX-2 inhibitory meroterpenoids from *Ganoderma cochlear***

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## Abstract

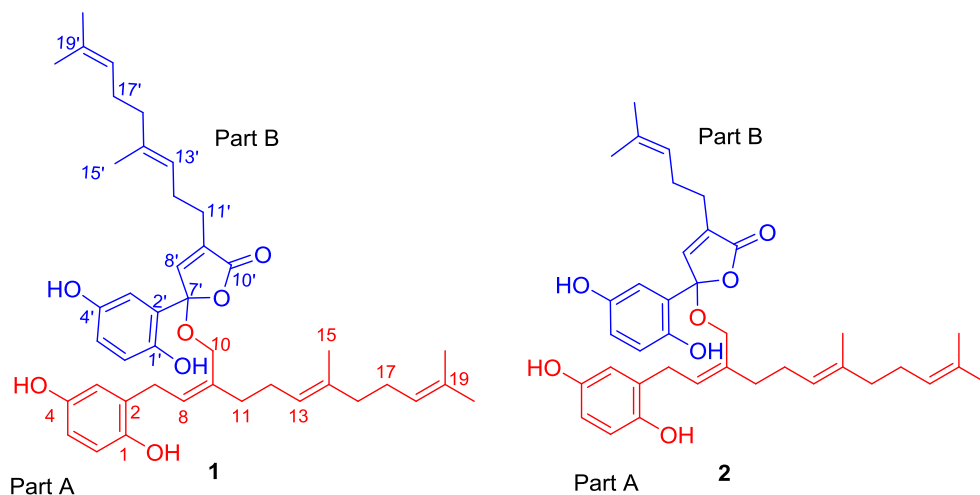
(+)- and (-)-gancochlearols A (**1**) and B (**2**), two pairs of dimeric meroterpenoid enantiomers were isolated from the fruiting bodies of *Ganoderma cochlear*. Their structures were identified by spectroscopic methods. Biological assessments show that the enantiomers of **1** and **2** are cytotoxic against three human cancer cell lines (A549, K562, Huh-7) and could inhibit COX-2 expression with IC<sub>50</sub> values less than 10  $\mu$ M.

**Keywords:** *Ganoderma cochlear*; meroterpenoids; cytotoxic activity; COX-2

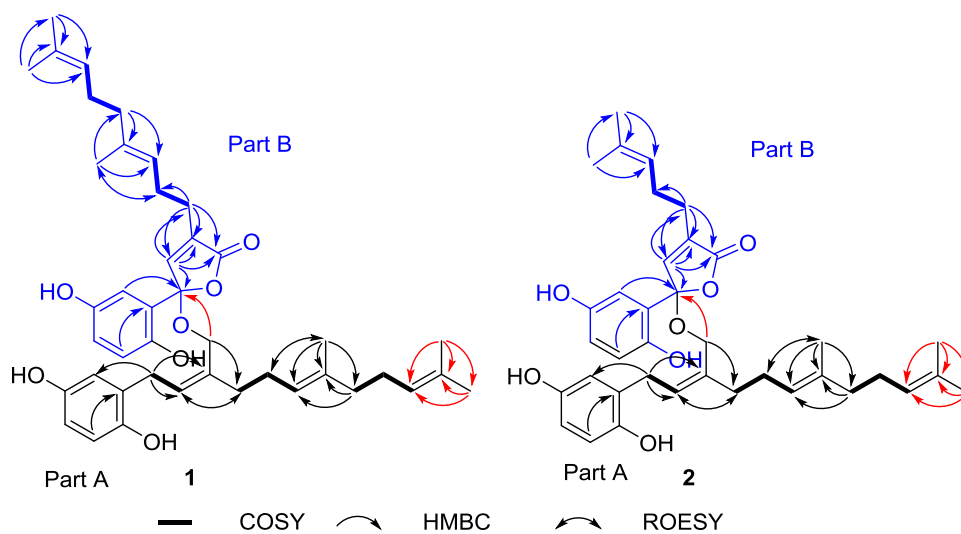
<b>Figure S1.</b> The structures of compounds <b>1</b> and <b>2</b>
<b>Figure S2.</b> Key HMBC , <sup>1</sup> H- <sup>1</sup> H COSY and ROESY correlations of compounds <b>1</b> and <b>2</b>
<b>Table S1.</b> <sup>1</sup> H (600 MHz) and <sup>13</sup> C NMR (150 MHz) data of <b>1</b> ( $\delta$ in ppm, <i>J</i> in Hz, Methanol- <i>d</i> <sub>4</sub> )
<b>Table S2.</b> <sup>1</sup> H (600 MHz) and <sup>13</sup> C NMR (150 MHz) data of <b>2</b> ( $\delta$ in ppm, <i>J</i> in Hz, CDCl <sub>3</sub> )
<b>Table S3.</b> Cytotoxic and COX-2 inhibitory activities of the isolates
<b>Figure S3.</b> <sup>1</sup> H NMR spectrum of <b>1</b> in methanol- <i>d</i> <sub>4</sub>
<b>Figure S4.</b> <sup>13</sup> C NMR and DEPT spectra of <b>1</b> in methanol- <i>d</i> <sub>4</sub>
<b>Figure S5.</b> HSQC spectrum of <b>1</b> in methanol- <i>d</i> <sub>4</sub>
<b>Figure S6.</b> HMBC spectrum of <b>1</b> in methanol- <i>d</i> <sub>4</sub>
<b>Figure S7.</b> <sup>1</sup> H- <sup>1</sup> H COSY spectrum of <b>1</b> in methanol- <i>d</i> <sub>4</sub>
<b>Figure S8.</b> ROESY spectrum of <b>1</b> in methanol- <i>d</i> <sub>4</sub>
<b>Figure S9.</b> HRESIMS of <b>1</b>
<b>Figure S10.</b> Optical rotatory data of (+)- <b>1</b>
<b>Figure S11.</b> Optical rotatory data of (–)- <b>1</b>
<b>Figure S12.</b> CD spectrum of (+)- <b>1</b>
<b>Figure S13.</b> CD spectrum of (–)- <b>1</b>
<b>Figure S14.</b> <sup>1</sup> H NMR spectrum of <b>2</b> in CDCl <sub>3</sub>
<b>Figure S15.</b> <sup>13</sup> C NMR and DEPT spectra of <b>2</b> in CDCl <sub>3</sub>
<b>Figure S16.</b> HSQC spectrum of <b>2</b> in CDCl <sub>3</sub>
<b>Figure S17.</b> HMBC spectrum of <b>2</b> in CDCl <sub>3</sub>
<b>Figure S18.</b> <sup>1</sup> H- <sup>1</sup> H COSY spectrum of <b>2</b> in CDCl <sub>3</sub>
<b>Figure S19.</b> ROESY spectrum of <b>2</b> in CDCl <sub>3</sub>
<b>Figure S20.</b> HRESIMS of <b>2</b>

**Figure S21.** Optical rotatory data of (-)-**2**

**Figure S22.** Optical rotatory data of (+)-**2**



**Figure S1.** The structures of compounds **1** and **2**



**Figure S2.** Key HMBC ,  $^1\text{H}$ - $^1\text{H}$  COSY and ROESY correlations of compounds **1** and **2**

**Table S1.**  $^1\text{H}$  (600 MHz) and  $^{13}\text{C}$  NMR (150 MHz) data of **1** ( $\delta$  in ppm,  $J$  in Hz, Methanol- $d_4$ ).

Position	$\delta_{\text{H}}$ ( $J$ in Hz)	$\delta_{\text{C}}$	Position	$\delta_{\text{H}}$ ( $J$ in Hz)	$\delta_{\text{C}}$
1		149.0 s	1'		149.5 s
2		118.0 s	2'		123.7 s
3	6.48 (d, 2.8)	117.4 d	3'	6.97 (d, 2.0)	114.7 d
4		151.05 s	4'		151.06 s
5	6.43 (dd, 8.5, 2.8)	114.2 d	5'	6.68 (overlap)	118.0 s
6	6.80 (d, 8.5)	116.5 d	6'	6.68 (overlap)	118.3 d
7	3.25 (m)	29.2 t	7'		108.6 s
8	5.48 (t)	129.6 s	8'	7.74 (s)	148.6 d
9		136.6 s	9'		135.5 s
10	4.18 (d, 10.6)	63.1 t	10'		173.7 s
	4.04 (d, 10.6)				
11	2.15 (m)	36.6 t	11'	2.33 (m)	26.0 t
12	2.10 (dd, 13.6, 6.3)	27.7 t	12'	2.26 (m)	26.7 t
13	5.08 (t, 6.0)	125.4 d	13'	5.07 (overlap)	123.7 d
14		137.9 s	14'		136.2 s
15	1.54 (s)	16.2 q	15'	1.49 (s)	16.2 q
16	1.88 (m)	40.8 t	16'	1.92 (m)	40.8 t
17	1.95 (m)	27.7 t	17'	2.02 (m)	27.7 t
18	5.03 (t, 7.1)	125.5 d	18'	5.06 (overlap)	125.6 d
19		132.1 s	19'		132.0 s
20	1.56 (s)	17.8 q	20'	1.57 (s)	17.8 q
21	1.64 (s)	25.9 q	21'	1.64 (s)	25.9 q

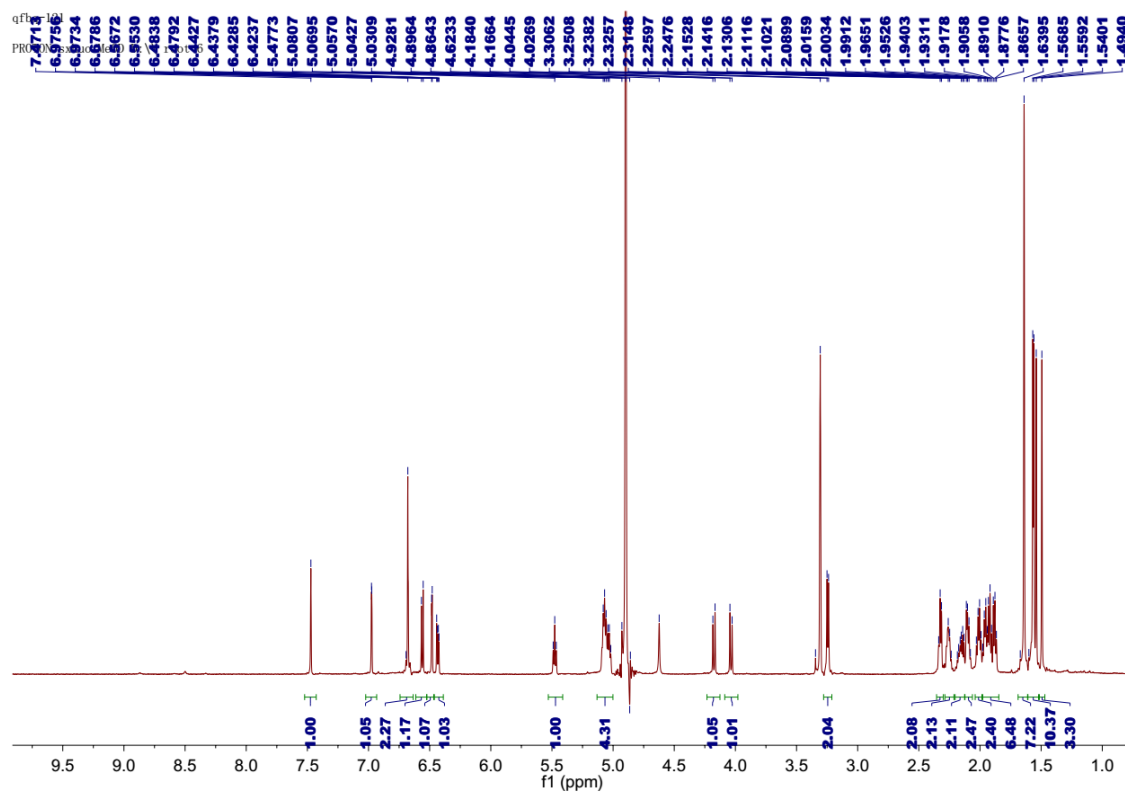
**Table S2.**  $^1\text{H}$  (600 MHz) and  $^{13}\text{C}$  NMR (150 MHz) data of **2** ( $\delta$  in ppm,  $J$  in Hz,  $\text{CDCl}_3$ ).

Position	$\delta_{\text{H}}$ ( $J$ in Hz)	$\delta_{\text{C}}$	Position	$\delta_{\text{H}}$ ( $J$ in Hz)	$\delta_{\text{C}}$
1		147.5 s	1'		148.2 s
2		127.2 s	2'		121.4 s
3	6.60 (d, 2.8)	116.9 d	3'	6.77 (d, 2.6)	112.9 d
4		149.5 s	4'		149.1 s
5	6.58 (d, 8.6, 2.8)	114.1 d	5'	6.79 (dd, 8.6, 2.8)	118.4 s
6	6.65 (d, 8.5)	116.7 d	6'	6.83 (d, 8.6)	118.9 d
7	3.26 (m)	29.1 t	7'		106.6 s
8	5.55 (t)	128.8 s	8'	7.12 (s)	145.6 s
9		135.2 s	9'		135.3 s
10	4.25 (d, 10.6)	62.7 t	10'		171.2 s
	4.12 (d, 10.6)				
11	2.12 (m)	35.6 t	11'	2.33 (m)	25.1 t
12	2.09 (m)	26.4 t	12'	2.26 (m)	25.7 t
13	5.07 (t, 6.9)	123.2 d	13'	5.03 (t, 7.1)	122.2 d
14		135.7 s	14'		133.6 s
15	1.54 (s)	16.0 q	15'	1.53 (s)	17.7 q
16	1.94 (m)	39.6 t	16'	1.63 (s)	25.6 t
17	2.02 (m)	26.4 t			
18	5.04 (t, 7.1)	124.3 d			
19		131.4 s			
20	1.59 (s)	17.7 q			
21	1.67 (s)	25.6 q			

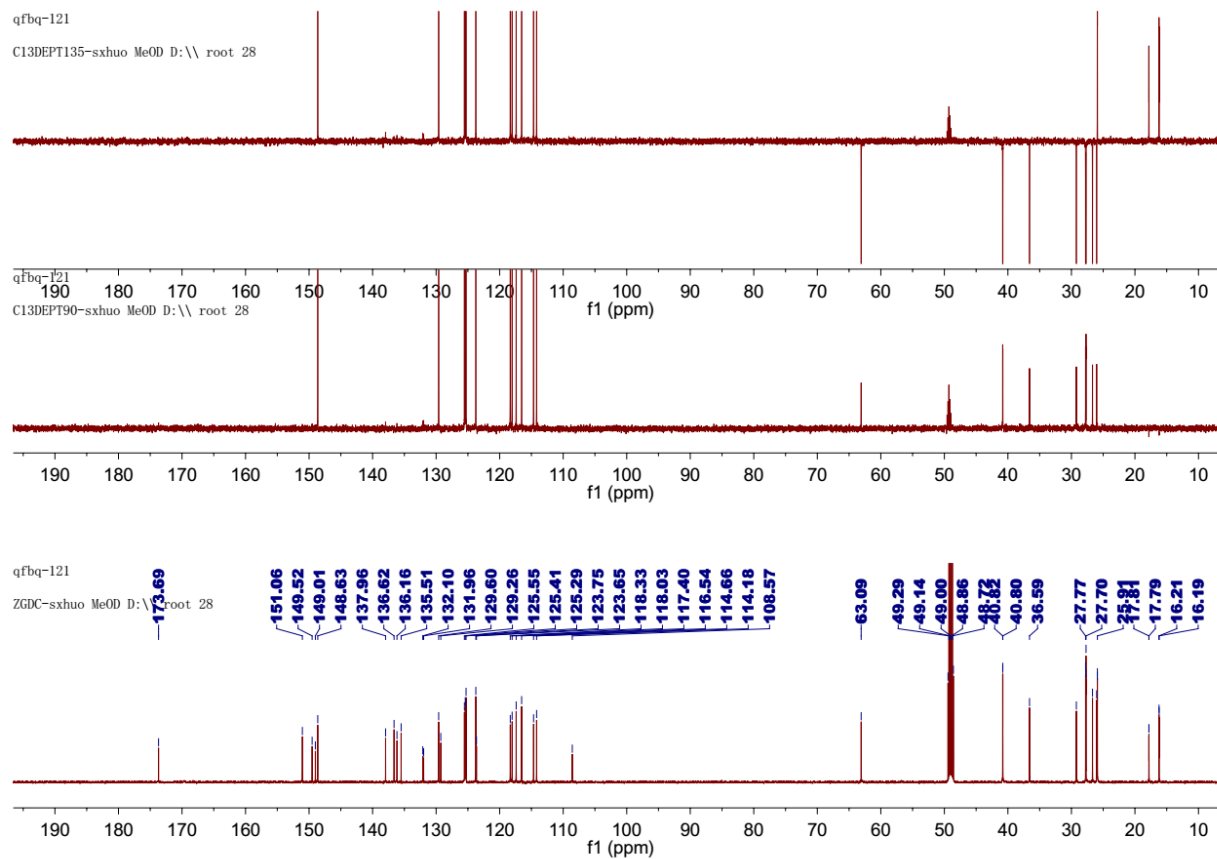
**Table S3.**  
Cytotoxic and COX-2 inhibitory activities of the isolates

Compound	IC <sub>50</sub> (μM)			
	COX-2	A549	K562	Huh-7
(+)- <b>1</b>	2.18	9.59	10.9	9.88
(-)- <b>1</b>	4.24	8.86	12.8	18.4
(+)- <b>2</b>	4.80	10.2	15.2	19.0
(-)- <b>2</b>	4.86	10.4	13.1	7.60
Positive cotontrol <sup>a</sup>	0.0171	0.00172	0.00324	0.00706

<sup>a</sup> Taxol was used as the positive control in the cytotoxic bioassays; Celecoxib was used as the positive control against COX-2.

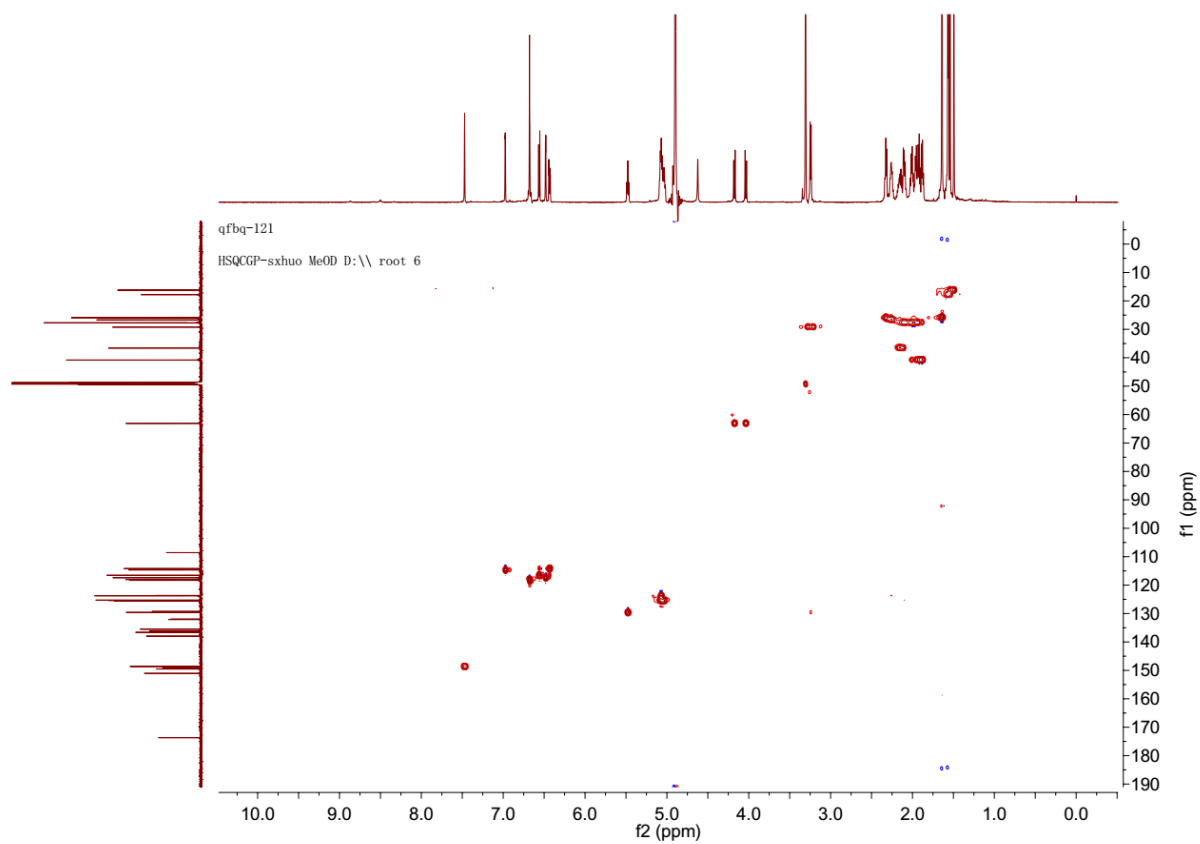


**Figure S3.**  $^1\text{H}$  NMR spectrum of **1** in methanol- $d_4$

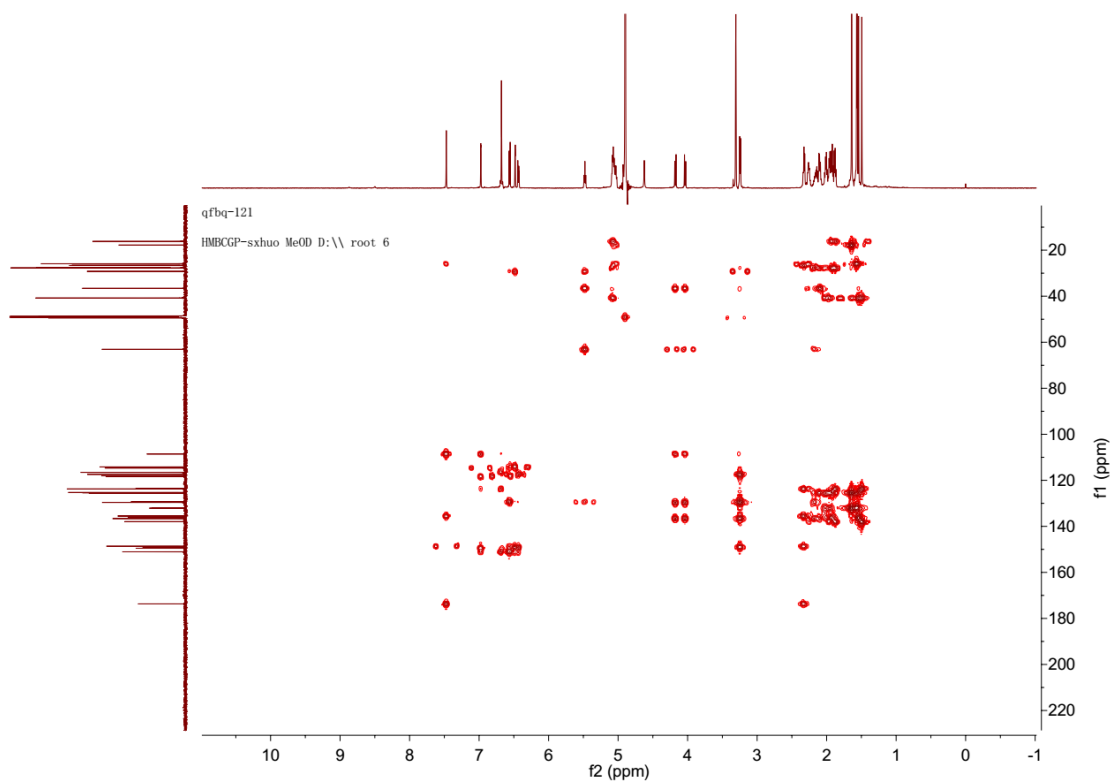


**Figure S4.**  $^{13}\text{C}$  NMR and DEPT spectra of **1** in methanol- $d_4$

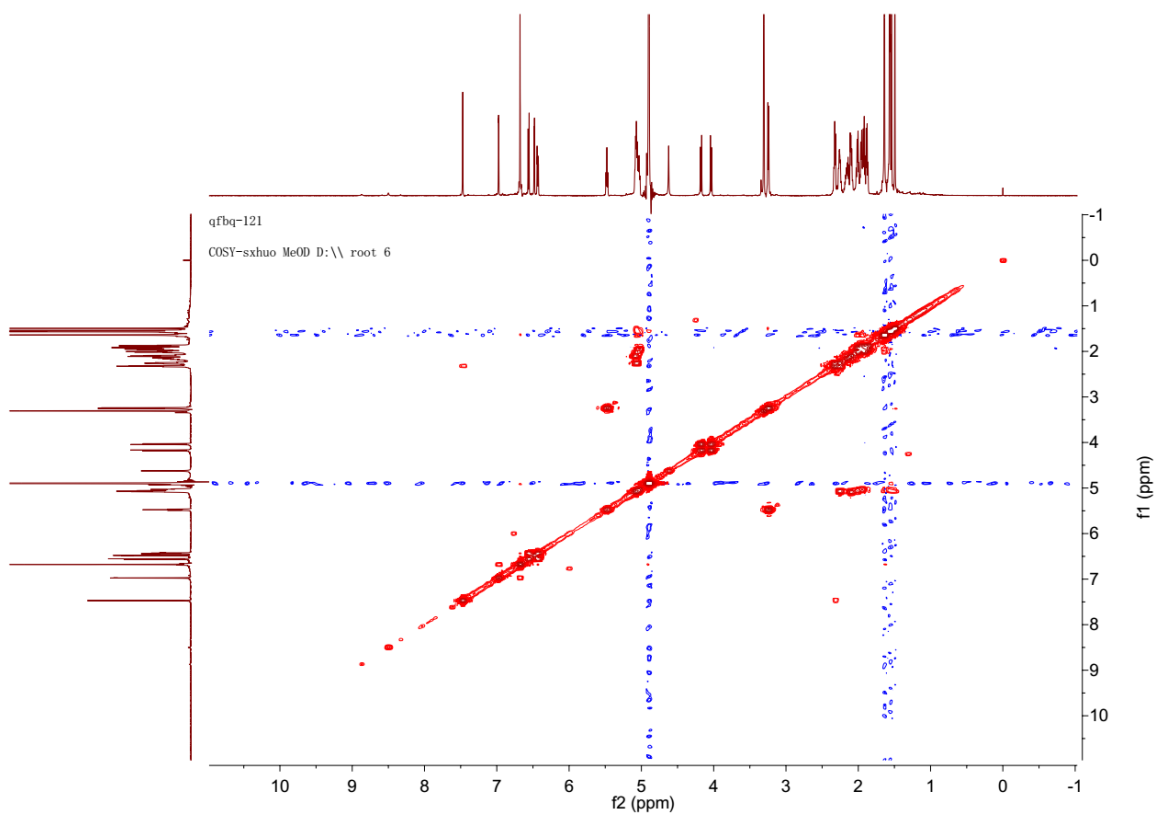




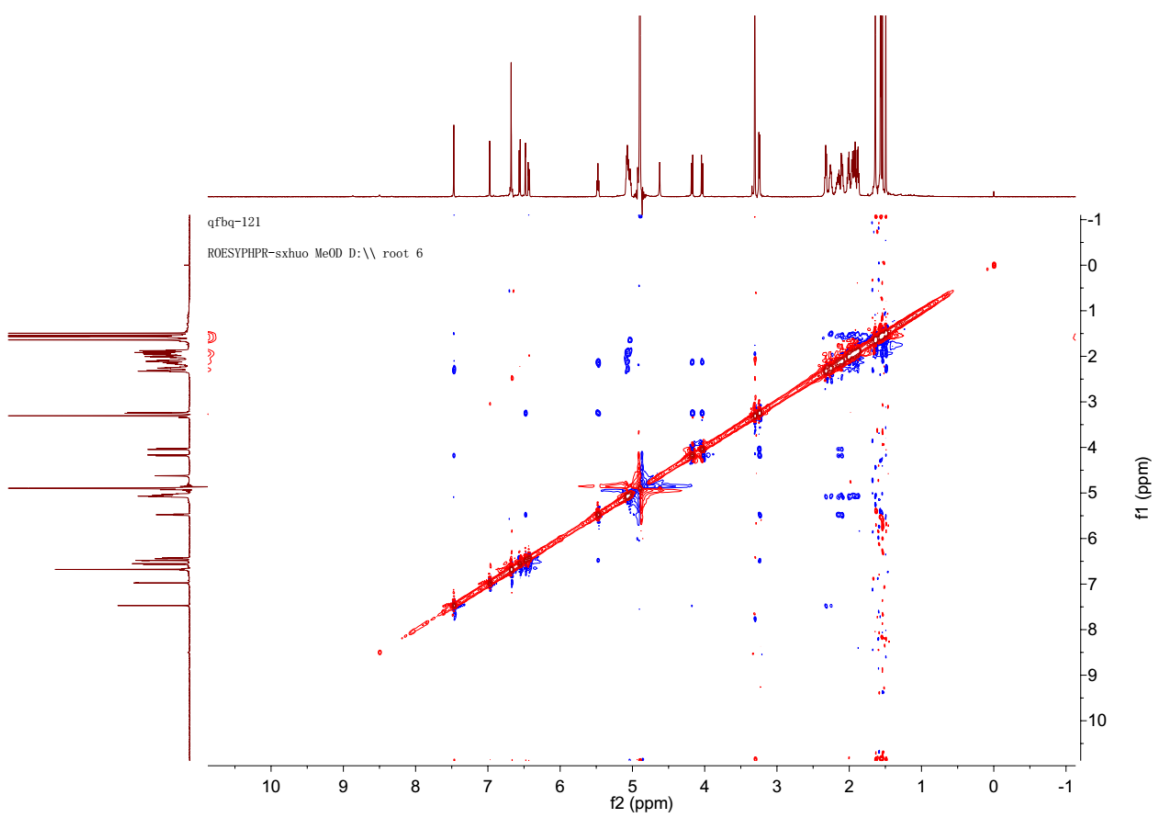
**Figure S5.** HSQC spectrum of **1** in methanol- $d_4$



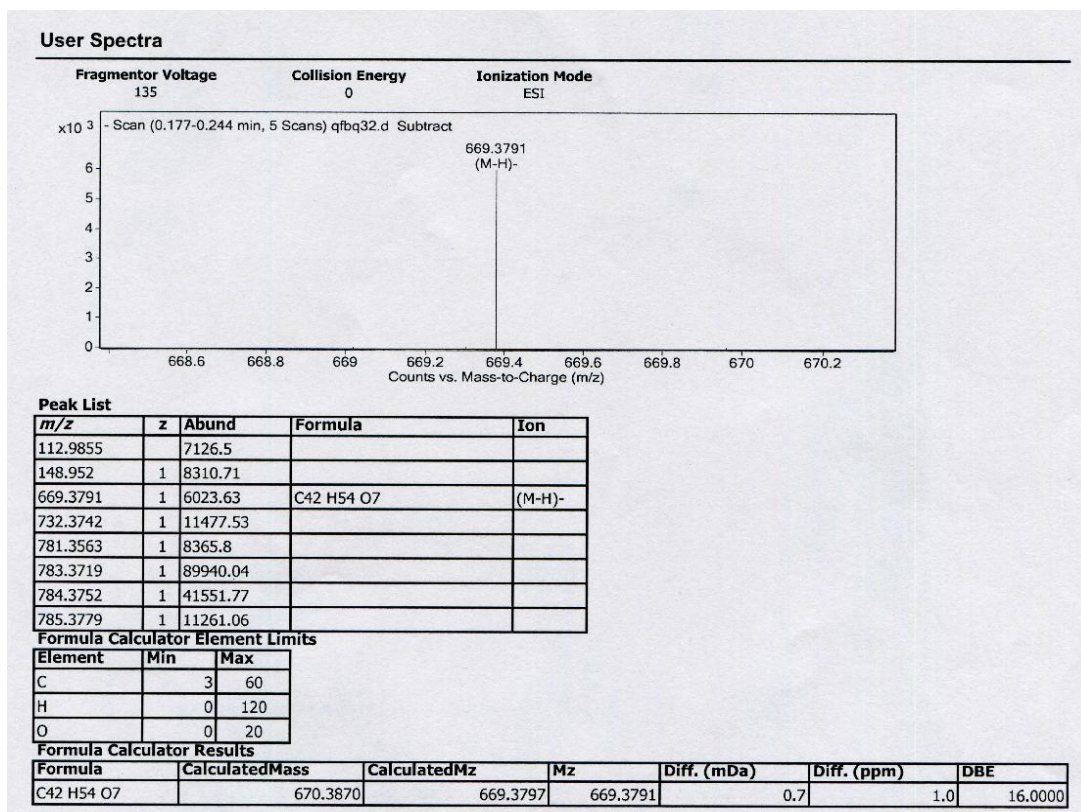
**Figure S6.** HMBC spectrum of **1** in methanol- $d_4$



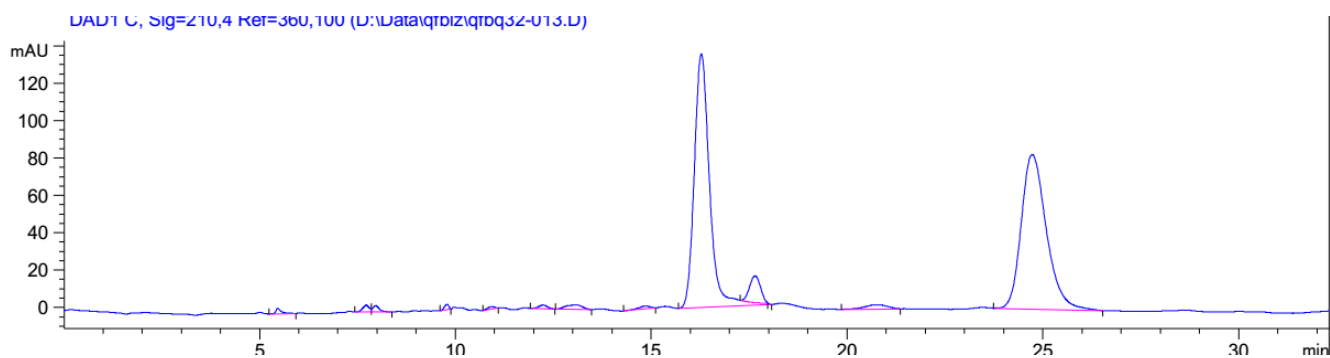
**Figure S7.**  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of **1** in methanol- $d_4$



**Figure S8.** ROESY spectrum of **1** in methanol- $d_4$



**Figure S9. HRESIMS of 1**



**Figure S10. The chiral HPLC chromatogram of 1**

Optical rotation measurement

Model : P-1020 (A060460638)

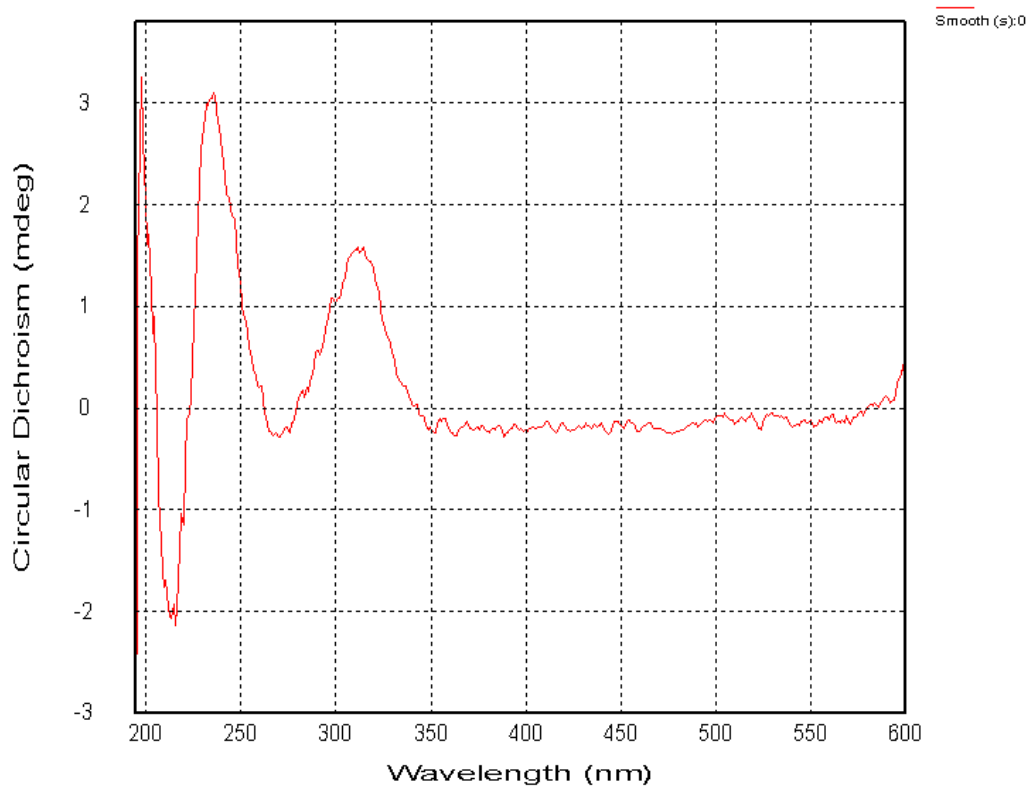
No.	Sample	Mode	Data	Monitor Blank	Temp. Cell Temp Point	Date Comment Sample Name	Light Filter Operator	Cycle Time Integ Time
No.1	1 (1/3)	Sp.Rot	47.5160	0.0153 0.0000	22.7 10.00	Sat Oct 24 11:02:31 2015 0.00322g/mL Acetonitrile QFBQ32A	Na 589nm	2 sec 10 sec
No.2	1 (2/3)	Sp.Rot	48.1370	0.0155 0.0000	22.7 10.00	Sat Oct 24 11:02:44 2015 0.00322g/mL Acetonitrile QFBQ32A	Na 589nm	2 sec 10 sec
No.3	1 (3/3)	Sp.Rot	48.1370	0.0155 0.0000	22.7 10.00	Sat Oct 24 11:02:58 2015 0.00322g/mL Acetonitrile QFBQ32A	Na 589nm	2 sec 10 sec

+47.9596°

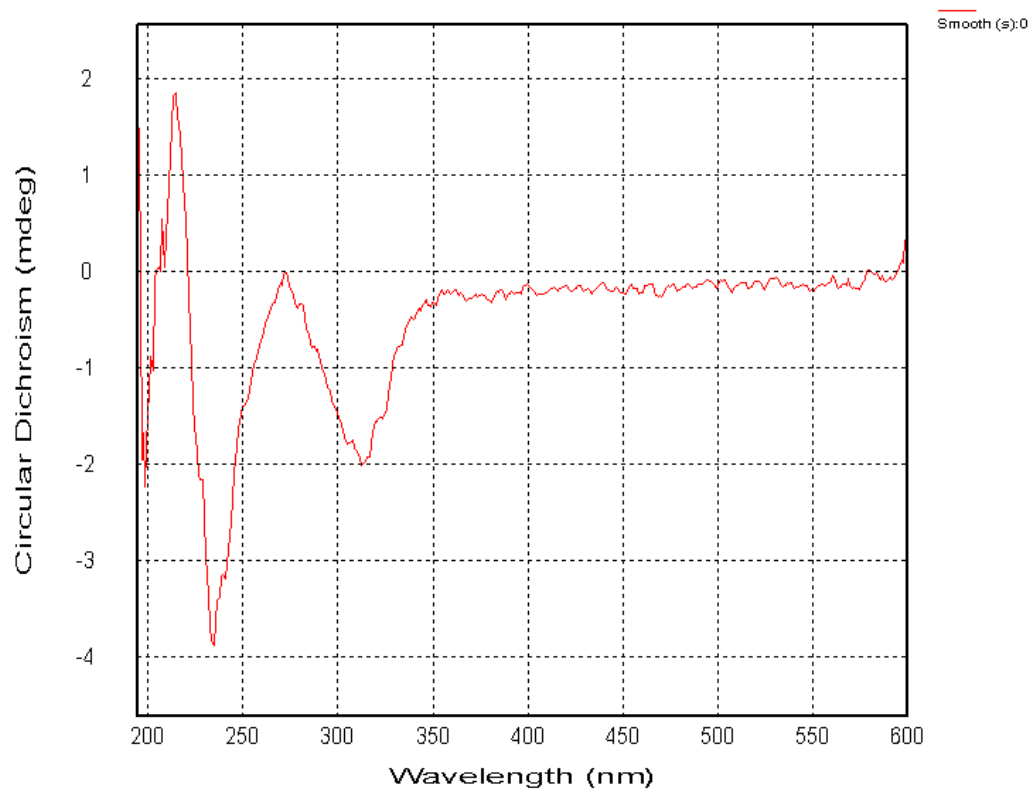
**Figure S11. Optical rotatory data of (+)-1**

Optical rotation measurement									
Model	P-1020 (A060460638)								
No.	Sample	Mode	Data	Monitor Blank	Temp. Cell	Date Comment	Light Filter	Cycle Time	
					Temp Point	Sample Name	Operator	Integ Time	
No.1	2 (1/3)	Sp.Rot	-50.6170	-0.0164 0.0000	23.1 10.00 Cell	Sat Oct 24 11:17:46 2015 0.00324g/mL Acetonitrile QFBQ32B	Na 589nm	2 sec 10 sec	
No.2	2 (2/3)	Sp.Rot	-49.3830	-0.0160 0.0000	23.1 10.00 Cell	Sat Oct 24 11:17:59 2015 0.00324g/mL Acetonitrile QFBQ32B	Na 589nm	2 sec 10 sec	-50.4117
No.3	2 (3/3)	Sp.Rot	-51.2350	-0.0166 0.0000	23.1 10.00 Cell	Sat Oct 24 11:18:12 2015 0.00324g/mL Acetonitrile QFBQ32B	Na 589nm	2 sec 10 sec	

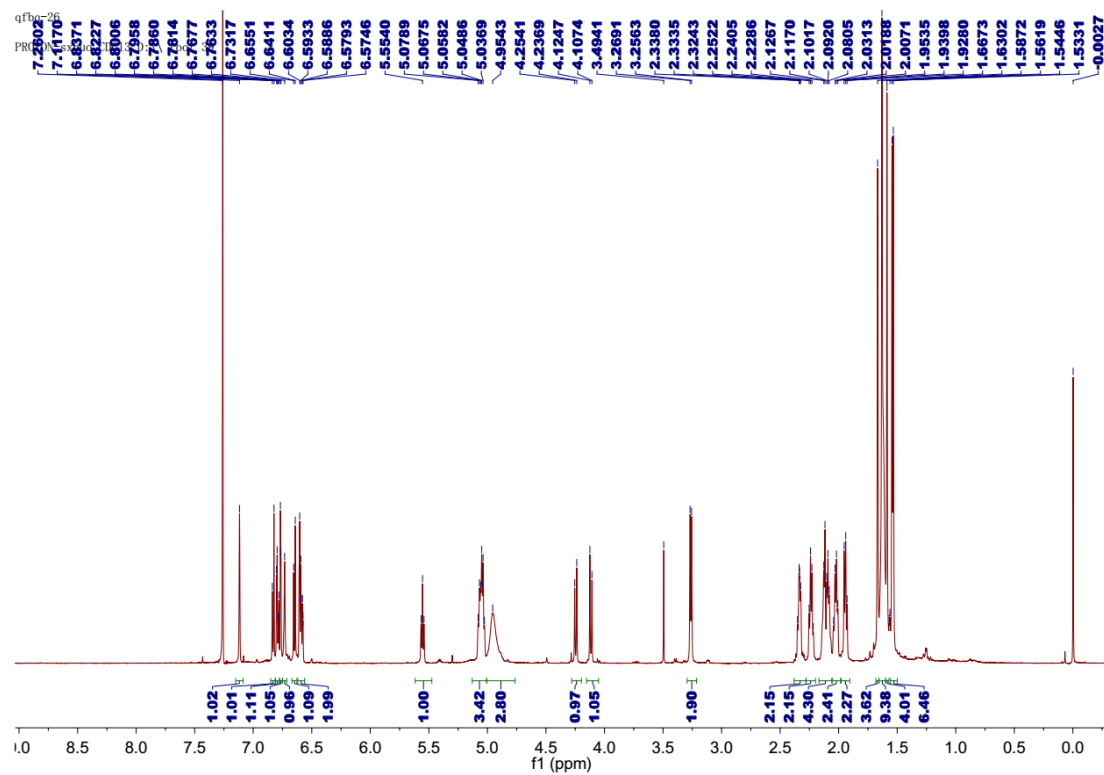
**Figure S12.** Optical rotatory data of (–)-1



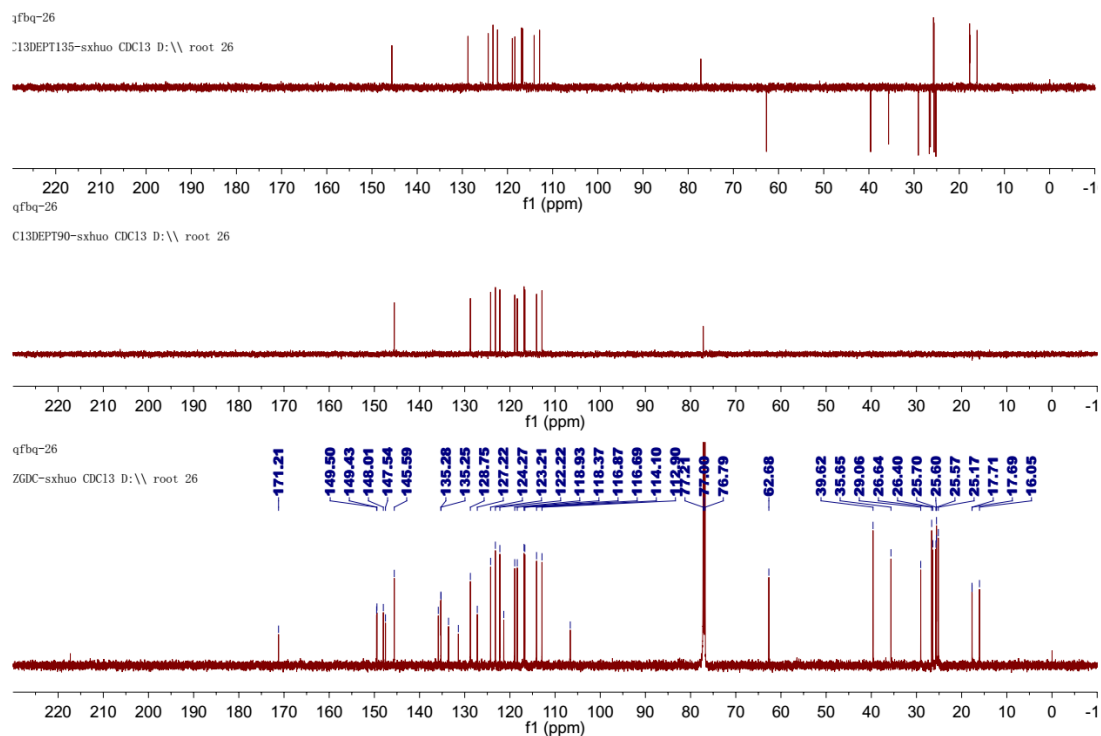
**Figure S13.** CD spectrum of (+)-1



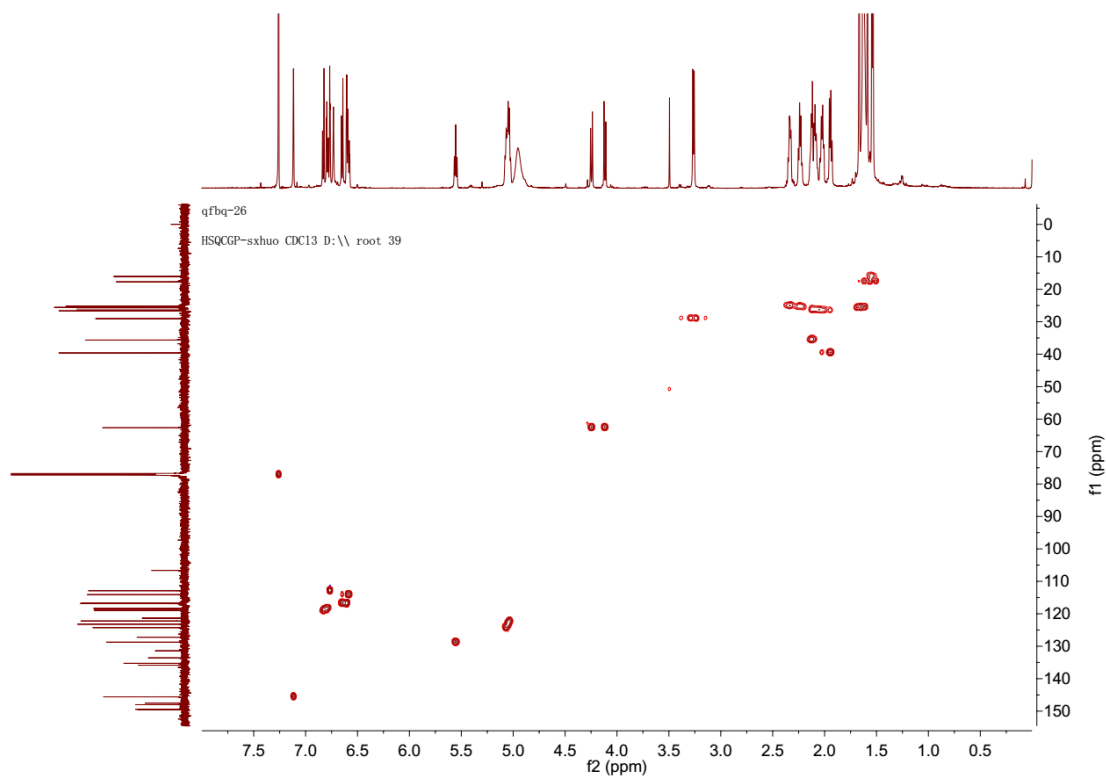
**Figure S14.** CD spectrum of (-)-1



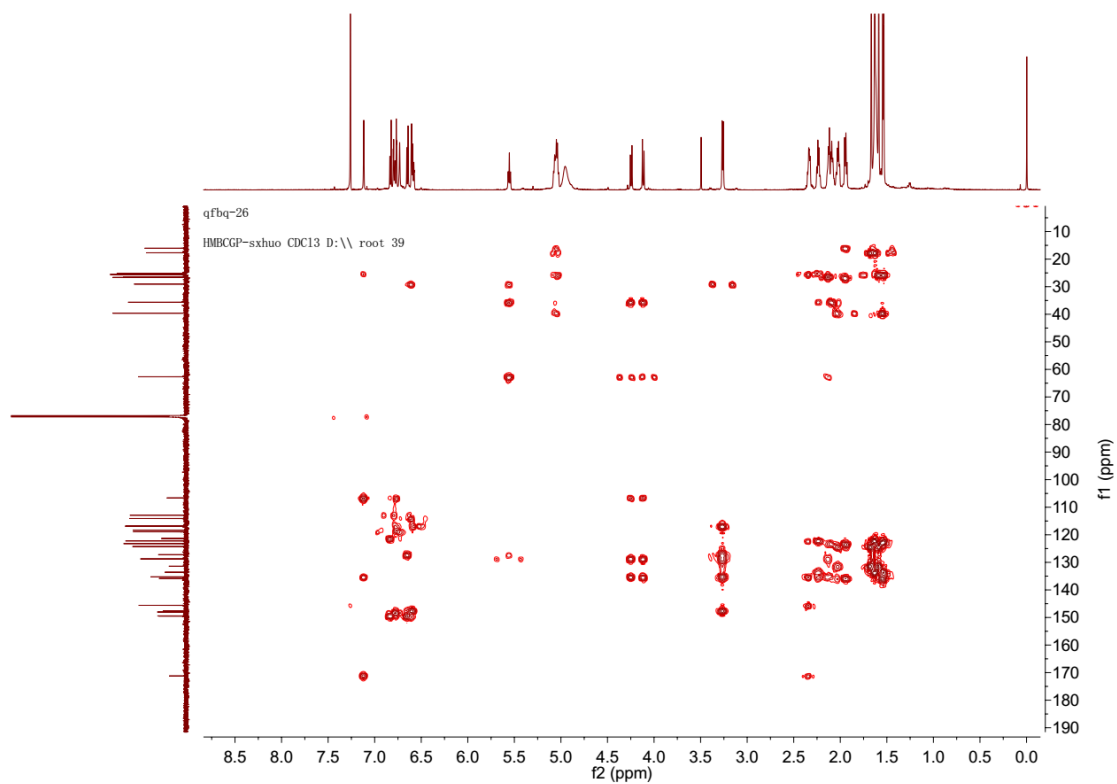
**Figure S15.**  $^1\text{H}$  NMR spectrum of **2** in  $\text{CDCl}_3$



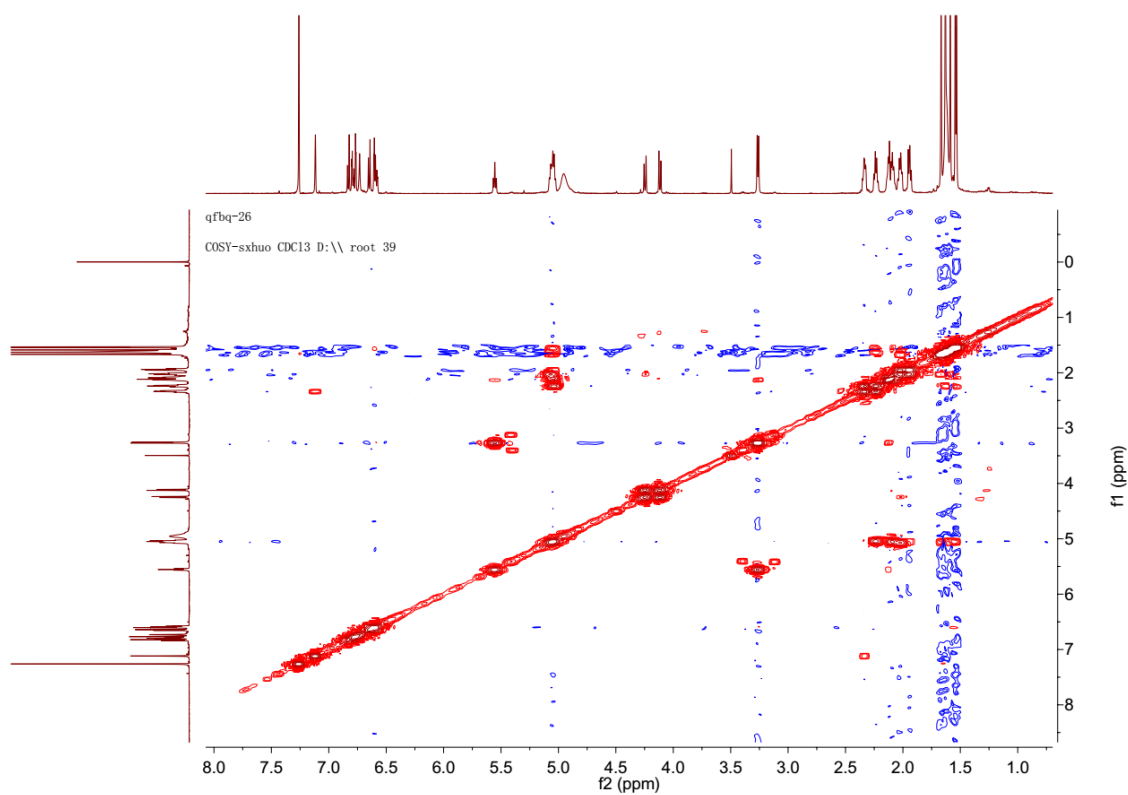
**Figure S16.**  $^{13}\text{C}$  NMR and DEPT spectrum of **2** in  $\text{CDCl}_3$



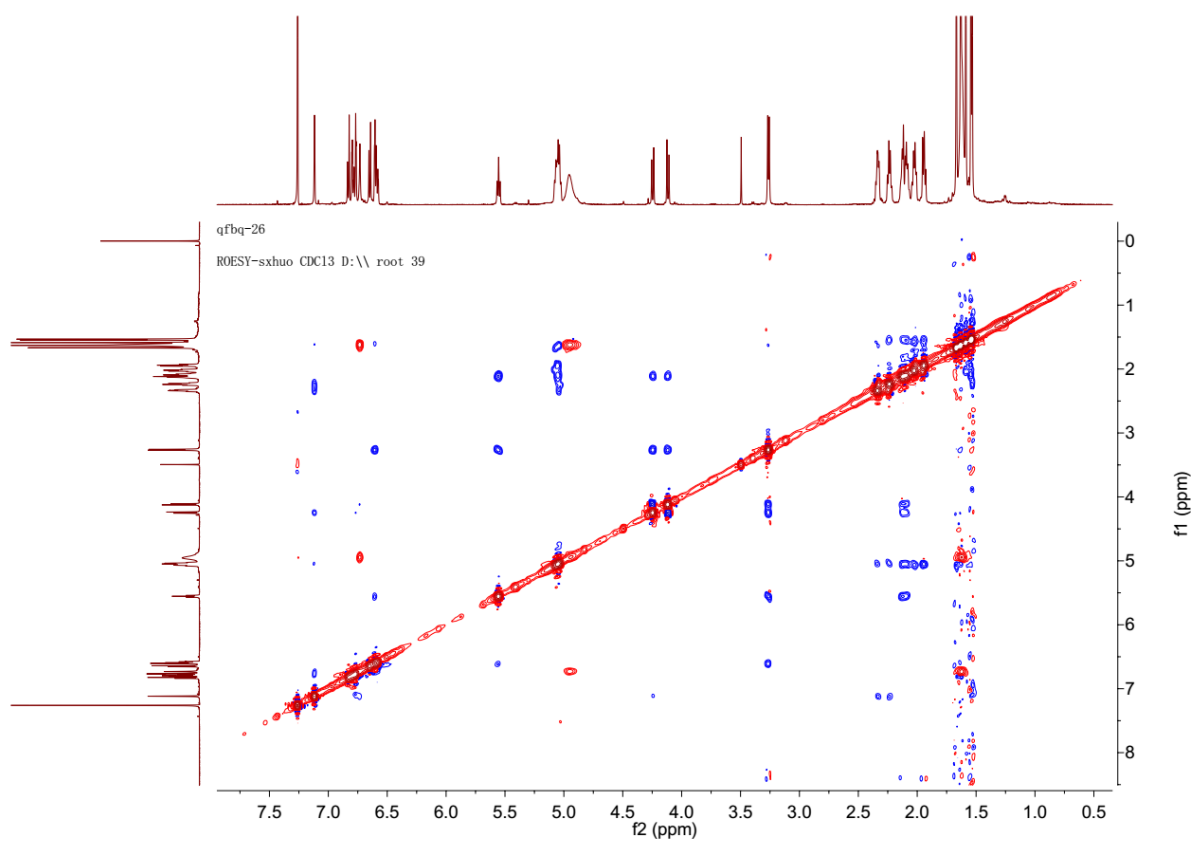
**Figure S17.** HSQC spectrum of **2** in  $\text{CDCl}_3$



**Figure S18.** HMBC spectrum of **2** in  $\text{CDCl}_3$



**Figure S19.**  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of **2** in  $\text{CDCl}_3$



**Figure S20.** ROESY spectrum of **2** in CDCl<sub>3</sub>



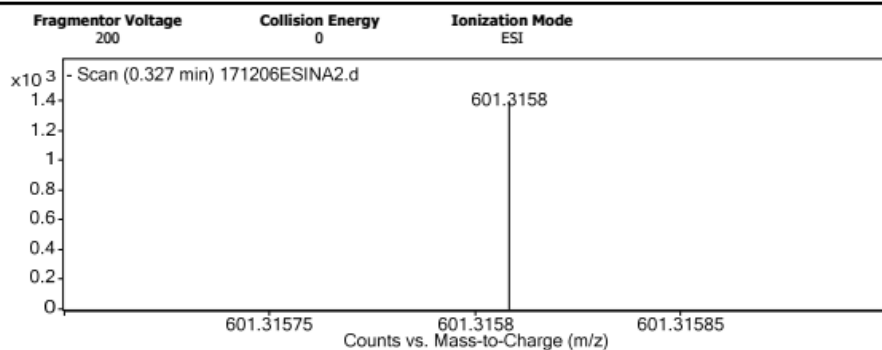
## Qualitative Analysis Report

<b>Data Filename</b>	171206ESINA2.d	<b>Sample Name</b>	qfbq26a
<b>Sample Type</b>	Sample	<b>Position</b>	
<b>Instrument Name</b>	Agilent G6230 TOF MS	<b>User Name</b>	KIB
<b>Acq Method</b>	ESIN.m	<b>Acquired Time</b>	12/6/2017 10:15:38 AM
<b>IRM Calibration Status</b>	Success	<b>DA Method</b>	ESI.m
<b>Comment</b>			

**Sample Group** Info.

**Acquisition SW** 6200 series TOF/6500 series  
**Version** Q-TOF B.05.01 (B5125.2)

### User Spectra



#### Peak List

m/z	z	Abund	Formula	Ion
112.9856		1482.05		
227.1076		858.24		
245.1186		971.9		
271.0972		833.48		
303.124	1	9851.41		
325.1843		1276.8		
339.2001		1125.29		
601.3158	1	1390.31	C37 H45 O7	M-
1033.9881	1	180450.33		
1034.9898	1	21115.04		

#### Formula Calculator Element Limits

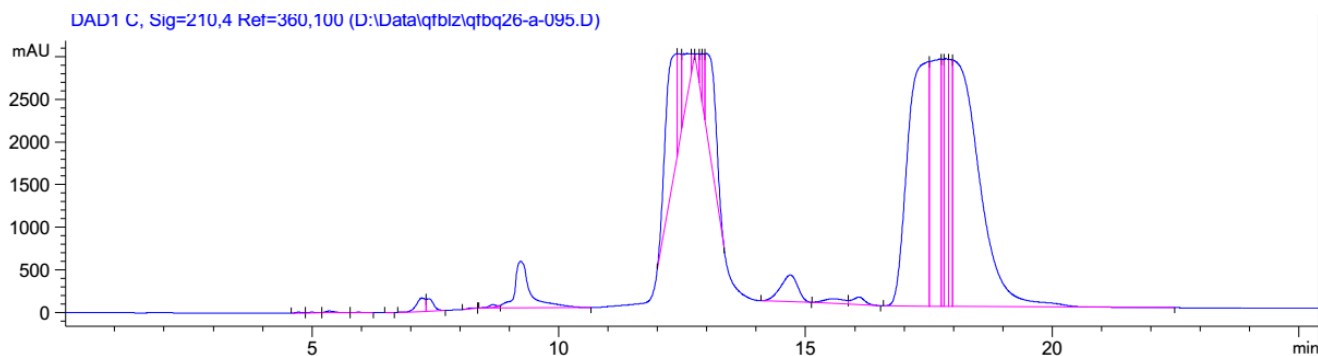
Element	Min	Max
C	0	200
H	0	400
O	3	10

#### Formula Calculator Results

Formula	CalculatedMass	Mz	Diff.(mDa)	Diff. (ppm)	DBE
C37 H45 O7	601.3165	601.3158	0.7	1.2	15.5

--- End Of Report ---

**Figure S21. HRESIMS of 2**



**Figure S22.** The chiral HPLC chromatogram of **2**

Optical rotation measurement

Model : P-1020 (A060460638)

No.	Sample	Mode	Data	Monitor Blank	Temp. Cell Temp Point	Date Comment Sample Name	Light Filter Operator	Cycle Time Integ Time
No.1	6 (1/3)	Sp.Rot	41.6670	0.0175 0.0000	23.2 50.00 Cell	Fri Oct 30 18:07:23 2015 0.00084g/mL MeOH QFBQ26A	Na 589nm	2 sec 10 sec
No.2	6 (2/3)	Sp.Rot	42.8570	0.0180 0.0000	23.3 50.00 Cell	Fri Oct 30 18:07:36 2015 0.00084g/mL MeOH QFBQ26A	Na 589nm	2 sec 10 sec
No.3	6 (3/3)	Sp.Rot	42.3810	0.0178 0.0000	23.3 50.00 Cell	Fri Oct 30 18:07:50 2015 0.00084g/mL MeOH QFBQ26A	Na 589nm	2 sec 10 sec

*+42.3016°*

**Figure S23.** Optical rotatory data of (+)-**2**

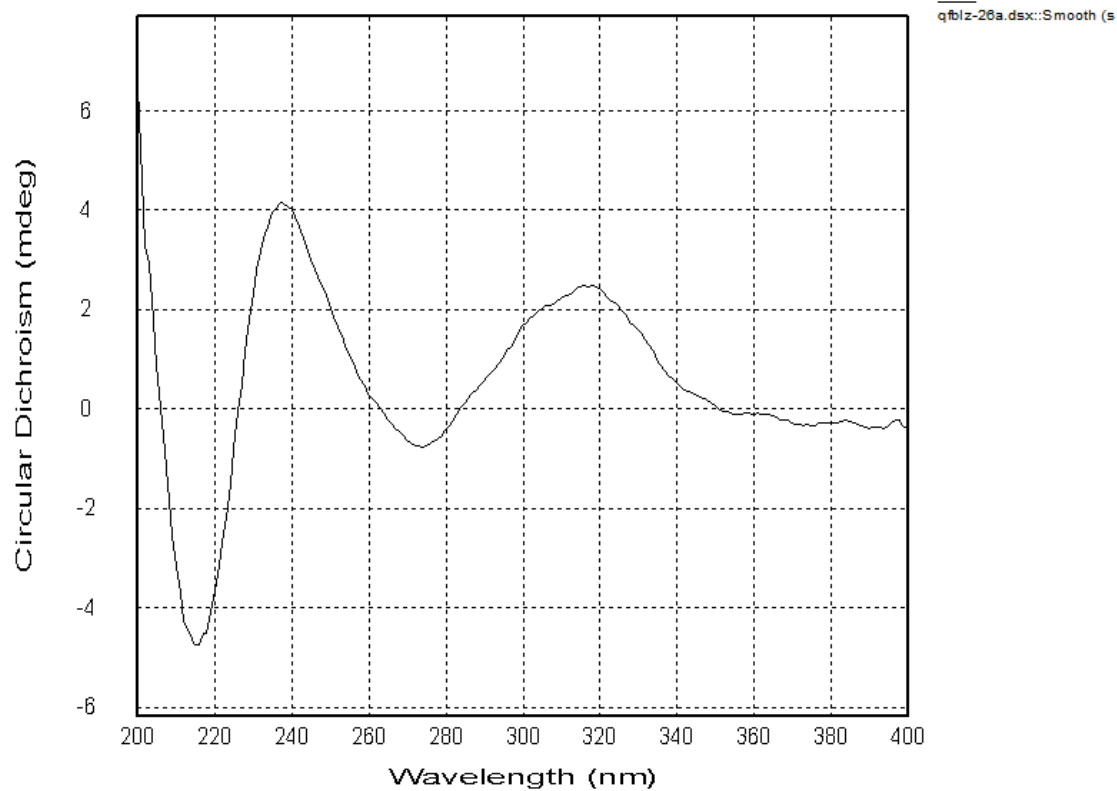
Optical rotation measurement

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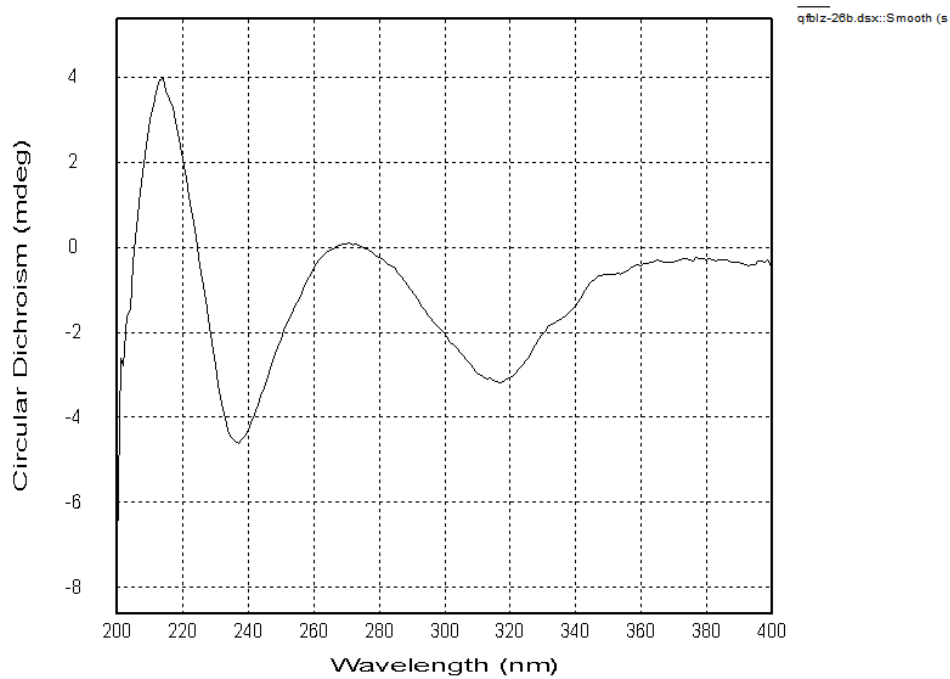
No.	Sample	Mode	Data	Monitor Blank	Temp. Cell Temp Point	Date Comment Sample Name	Light Filter Operator	Cycle Time Integ Time
No.1	8 (1/3)	Sp.Rot	-59.4440	-0.0214 0.0000	23.3 50.00 Cell	Fri Oct 30 18:16:36 2015 0.00072g/mL MeOH QFBQ26B	Na 589nm	2 sec 10 sec
No.2	8 (2/3)	Sp.Rot	-61.1110	-0.0220 0.0000	23.3 50.00 Cell	Fri Oct 30 18:16:50 2015 0.00072g/mL MeOH QFBQ26B	Na 589nm	2 sec 10 sec
No.3	8 (3/3)	Sp.Rot	-61.3890	-0.0221 0.0000	23.3 50.00 Cell	Fri Oct 30 18:17:03 2015 0.00072g/mL MeOH QFBQ26B	Na 589nm	2 sec 10 sec

*-60.6481°*

**Figure S24.** Optical rotatory data of (–)-**2**



**Figure S25.** CD spectrum of (+)-2



**Figure S26.** CD spectrum of (-)-2