

Supporting Information

Isatin-based spiro indolenine alkaloids from *Isatis indigotica* Fortune with anti-neuroinflammatory and acetylcholinesterase inhibitor effects

Ming Bai^{a,1}, Yu-Fei Xi^{a,1}, Si-Hui Mi^a, Pei-Yuan Yang^a, Li-Li Lou^{a,*}, Tian-Ming Lv^a, Xin Zhang^a, Guo-Dong Yao^a, Bin Lin^b, Xiao-Xiao Huang^{a,c*}, Shao-Jiang Song^{a,*}

^a Key Laboratory of Computational Chemistry-Based Natural Antitumor Drug Research & Development, Liaoning Province; Engineering Research Center of Natural Medicine Active Molecule Research & Development, Liaoning Province; Key Laboratory of Natural Bioactive Compounds Discovery & Modification, Shenyang; School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, Liaoning 110016, China

^b Wuya College of Innovation, Shenyang Pharmaceutical University, Shenyang, 110016, People's Republic of China

^c State Key Laboratory of New-tech for Chinese Medicine Pharmaceutical Process, Jiangsu Kanion Pharmaceutical Co., Ltd., Lianyungang, Jiangsu 222001, China

*Corresponding author.

E-mail: lou_li_li@163.com (L. L. Lou); xiaoxiao270@163.com (X. X. Huang); songsj99@163.com (S. J. Song)

¹ The two authors contributed equally to this work

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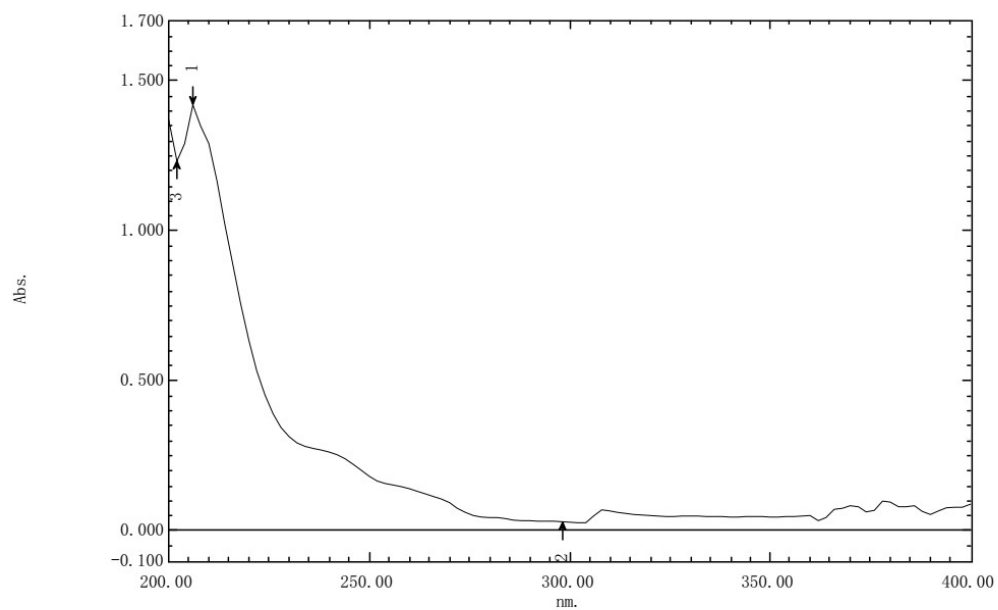
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Table S1. ¹H (400 MHz) and ¹³C NMR (100 MHz) spectroscopic data of **1-3** in DMSO-*d*₆.

Position	1		2		3	
	δ_{H} (multi, <i>J</i> in Hz)	δ_{C}	δ_{H} (multi, <i>J</i> in Hz)	δ_{C}	δ_{H} (multi, <i>J</i> in Hz)	δ_{C}
1	10.28, s	–	7.74, s	–	10.95, s	–
2	–	173.1	–	90.1	–	124.6
3	–	83.6	–	193.3	–	134.0
3a	–	128.1	–	121.4	–	120.3
4	7.97, d (7.7)	128.6	7.61, d (8.0)	124.4	7.69, d (7.6)	117.8
5	7.00, t (7.7)	121.2	7.29, t (8.0)	124.9	6.97, t (7.6)	118.6
6	7.18, t (7.7)	129.4	7.80, t (8.0)	137.7	7.07, t (7.6)	121.7
7	6.58, d (7.7)	109.5	8.37, d (8.0)	116.4	7.27, d (7.6)	111.4
7a	–	142.1	–	150.7	–	133.0
8	–	–	–	167.7	–	–
9	–	–	–	83.7	–	–
10	–	–	4.99, s	85.6	–	–
11	–	–	4.23, d (4.4)	78.9	–	–
12	–	–	3.82, t (6.6)	89.4	–	–
13	–	–	3.42, overlapped	62.1	–	–
1'	3.47, d (12.2)	49.2	7.02, s	–	4.51, d (7.1)	108.2
2'	3.44, overlapped	48.8	–	68.1	4.58, overlapped	70.5
3'	2.79, m	66.4	–	196.6	–	172.1
3'a	–	–	–	123.2	–	–
4'	1.91, m; 1.68, m	24.4	7.51, d (7.6)	123.3	5.01, d (8.2)	76.5
5'	1.79, m	17.5	6.83, overlapped	119.0	3.20, m	78.3

6'	3.10, dd (6.8, 8.2); 2.58, m	52.1	7.39, t (7.6)	136.6	3.71, m; 3.62, m	61.0
7'	–	171.6	6.83, overlapped	112.9	6.06, s	112.4
7'a	–	–	–	160.4	–	–
8'	–	–	–	–	–	172.1
1"	–	124.6	–	–	4.59, overlapped	63.3
2",6"	6.00, s	106.7	–	–	–	–
3",5"	–	147.0	–	–	–	–
4"	–	134.5	–	–	–	–
9-OH	–	–	6.57 (1H, s)	–	–	–
11-OH	–	–	7.07, d (4.6)	–	–	–
13-OH	–	–	4.83, t (4.8)	–	–	–
2'-OH	–	–	–	–	6.76, d (5.9)	–
6'-OH	–	–	–	–	5.06, t (5.5)	–
7'-OCH ₃	3.46, s	51.7	–	–	–	–
1"-OCH ₃	–	–	–	–	3.28, s	57.2
3",5"-OCH ₃	3.43, s	55.7	–	–	–	–
4"-OH	8.17, s	–	–	–	–	–



测定属性
 波长范围 (nm.): 200.00到400.00
 扫描速度: 高速
 采样间隔: 2.0
 自动采样间隔: 停用
 扫描模式: 单一的

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2	⬇	298.00	.031	
3	⬇	202.00	1.232	

Fig. S1 UV spectrum of compound 1.

Mass Spectrum SmartFormula Report

Analysis Info

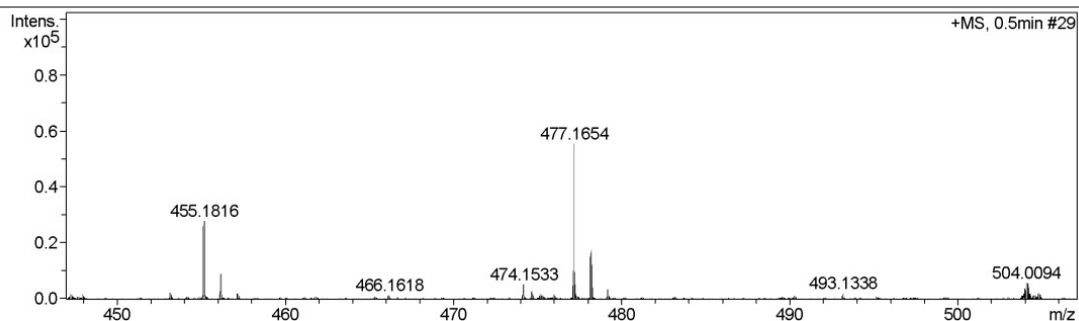
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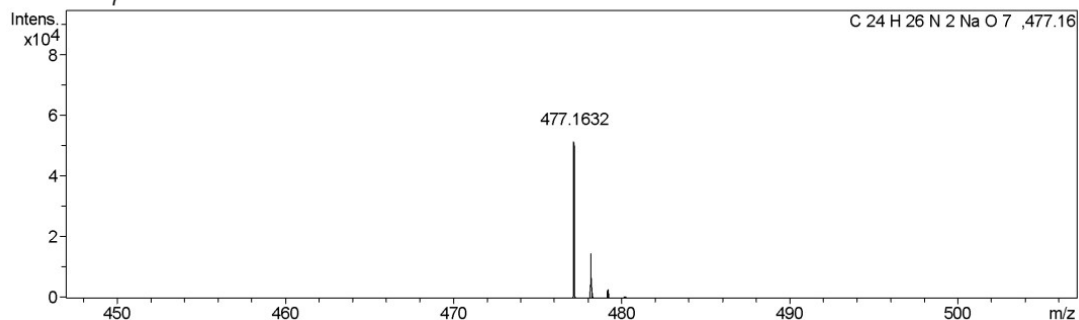
Operator Bruker Customer
 Instrument / Ser# micrOTOF-Q 125

Acquisition Parameter

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Scan End	1500 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source



Meas. #	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e ⁻ Conf	mSigma	Std I	Std Mean	Std VarNo	Std m/z	Std Comb
477.16													
54													
1	C ₂₄ H ₂₆ N ₂ NaO ₇	477.1632	-4.5	-0.9	12.5	ok	even	24.99	0.0450	0.0028	0.0142	0.0059	0.8427



Meas. #	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e ⁻ Conf	mSigma	Std I	Std Mean	Std VarNo	Std m/z	Std Comb

Fig. S2 HRESIMS spectrum of compound **1**.

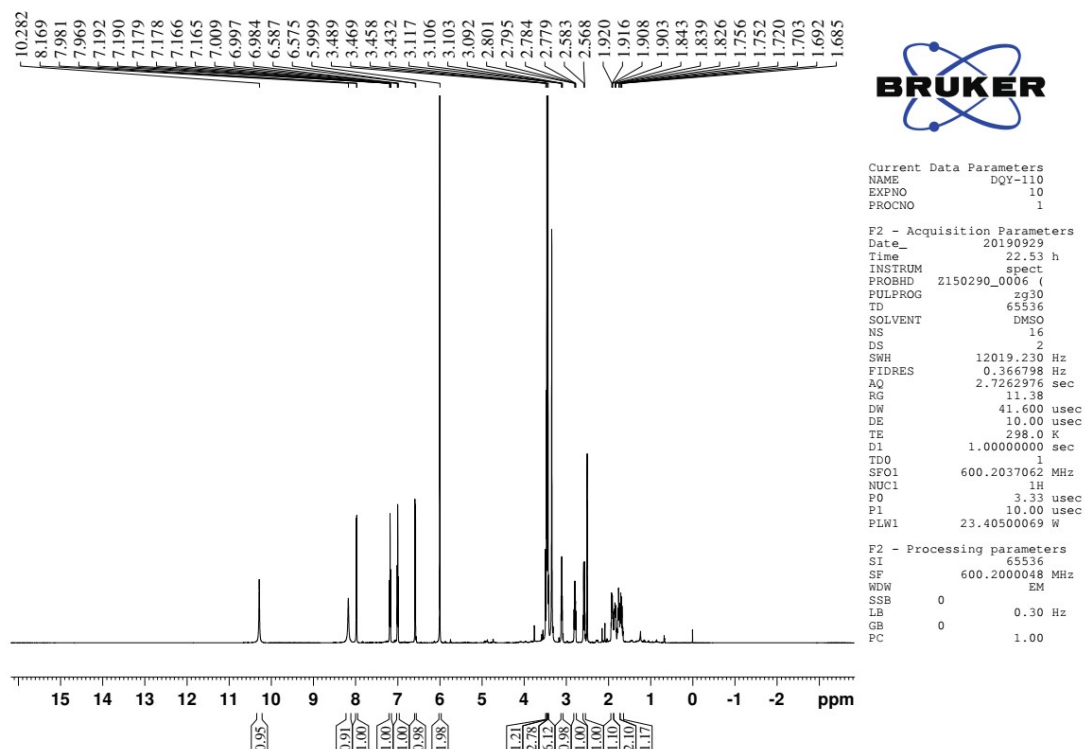


Fig. S3 ^1H NMR spectrum (400 MHz, $\text{DMSO-}d_6$) of compound 1.

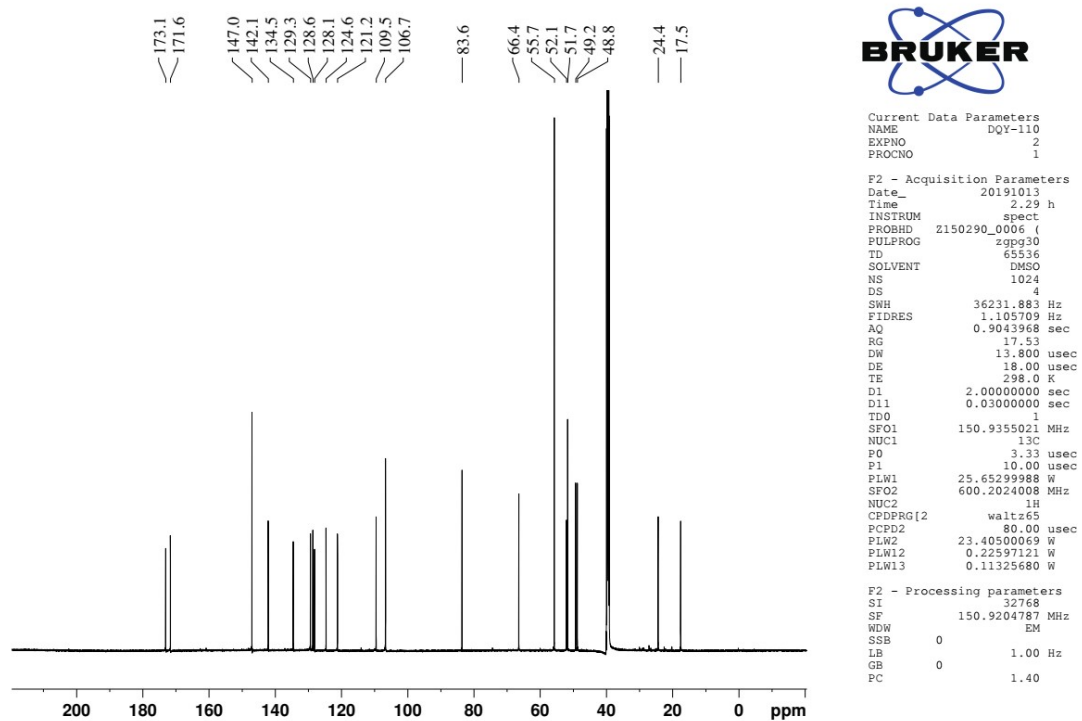


Fig. S4 ^{13}C NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound 1.

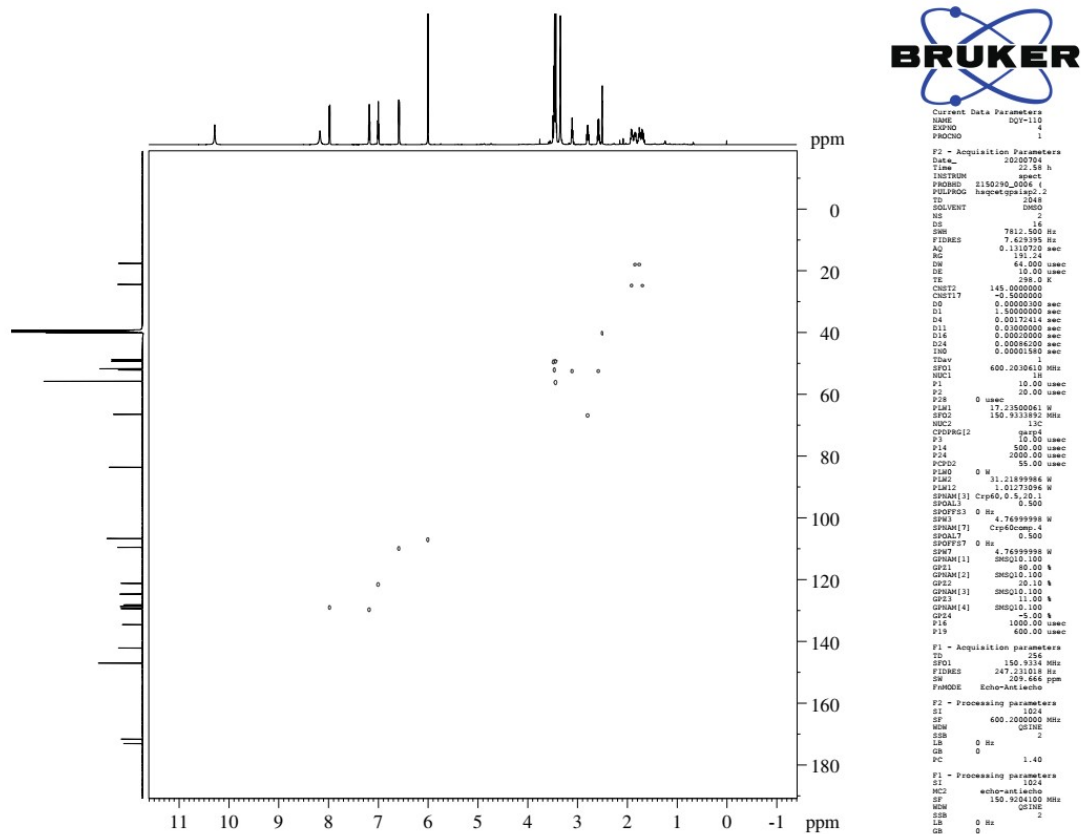


Fig. S5. HSQC spectrum (600 MHz, DMSO- d_6) of compound 1.

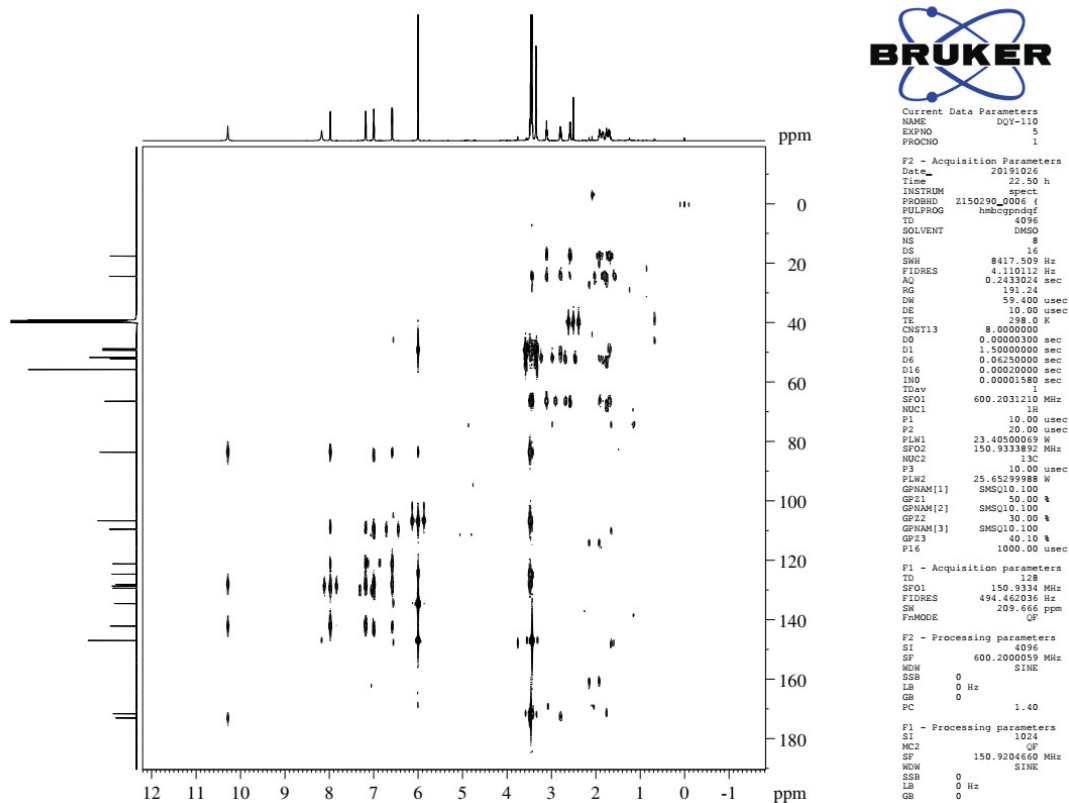


Fig. S6. HMBC spectrum (600 MHz, DMSO- d_6) of compound 1.

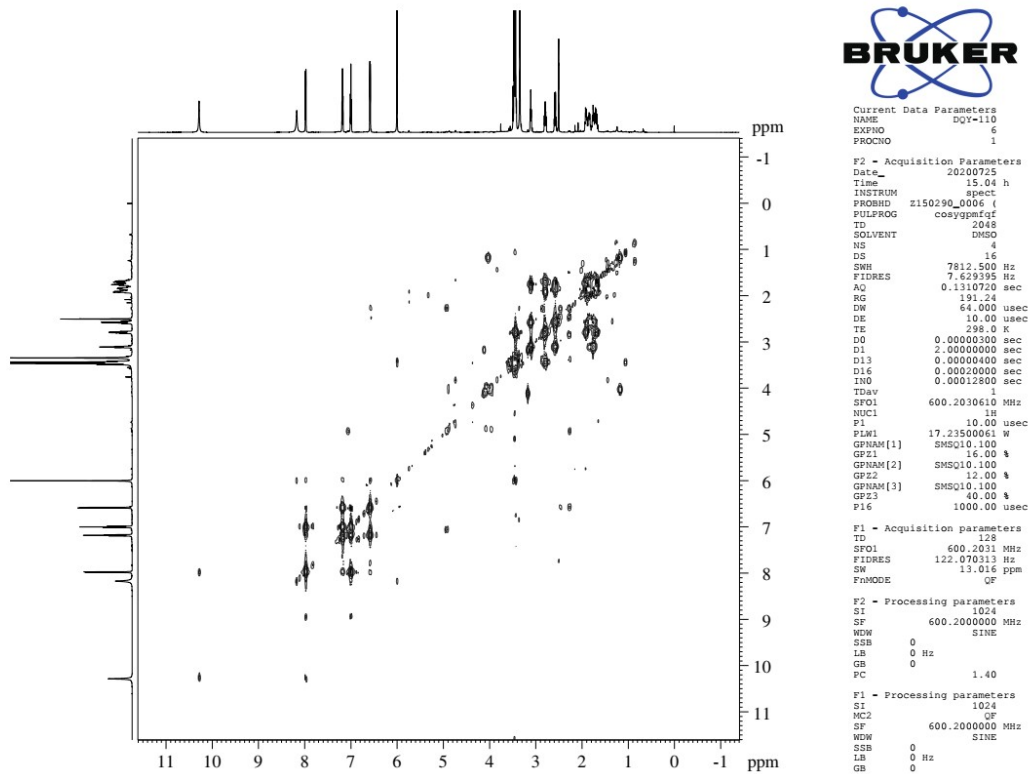


Fig. S7. ^1H - ^1H COSY spectrum (600 MHz, $\text{DMSO}-d_6$) of compound 1.

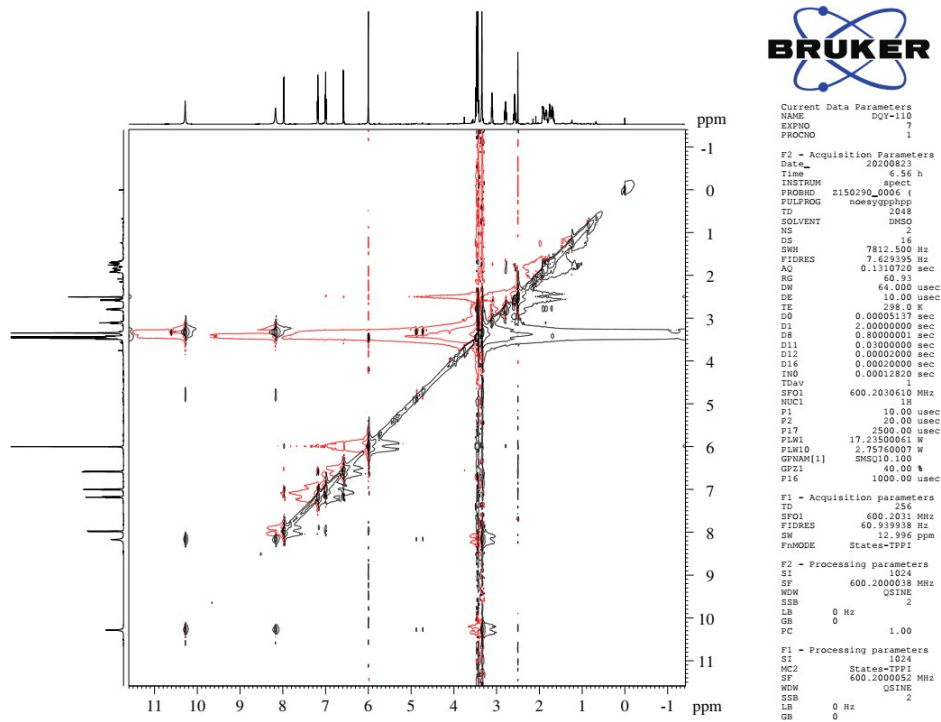


Fig. S8. NOESY spectrum (600 MHz, $\text{DMSO}-d_6$) of compound 1.

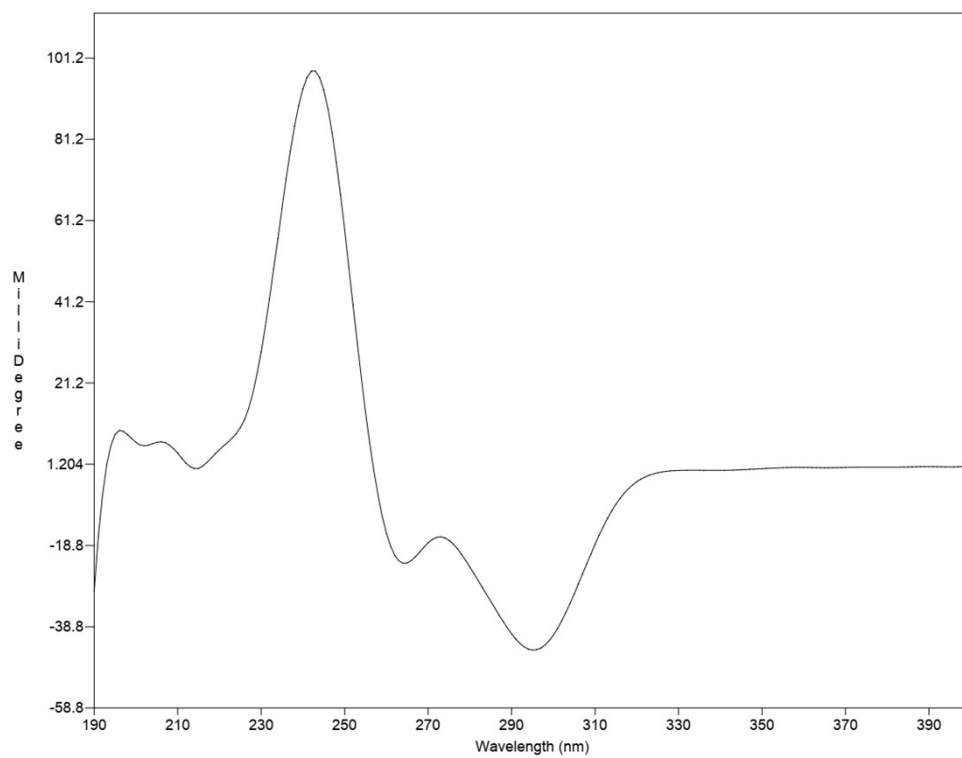


Fig. S9. Experimental ECD spectra of **1a**.

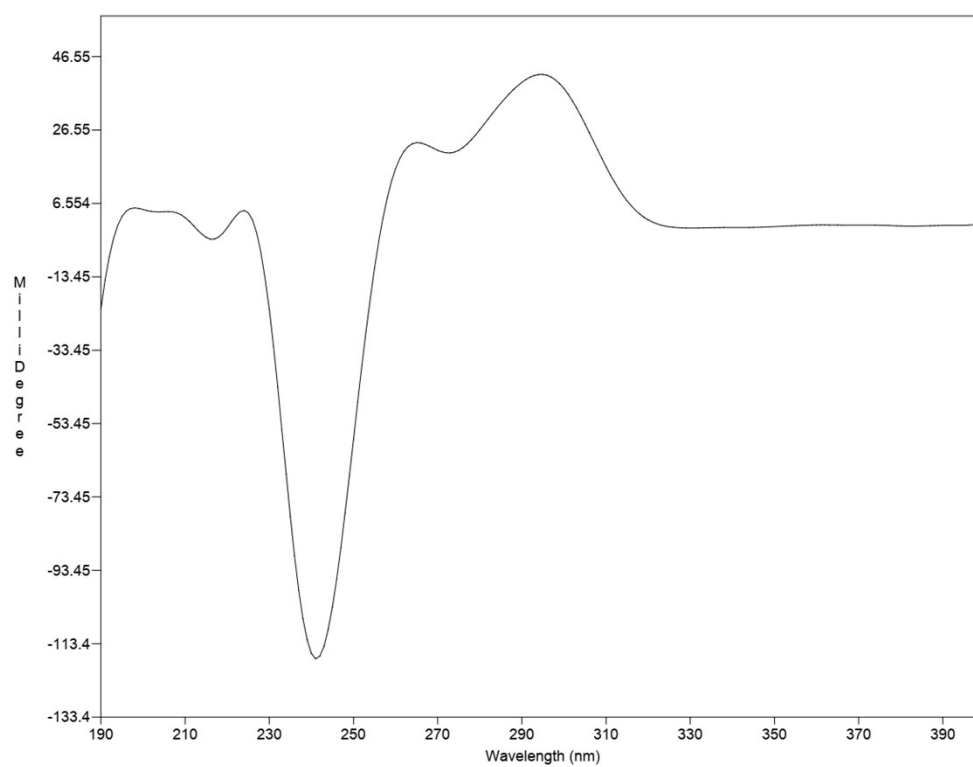


Fig. S10. Experimental ECD spectra of **1b**.

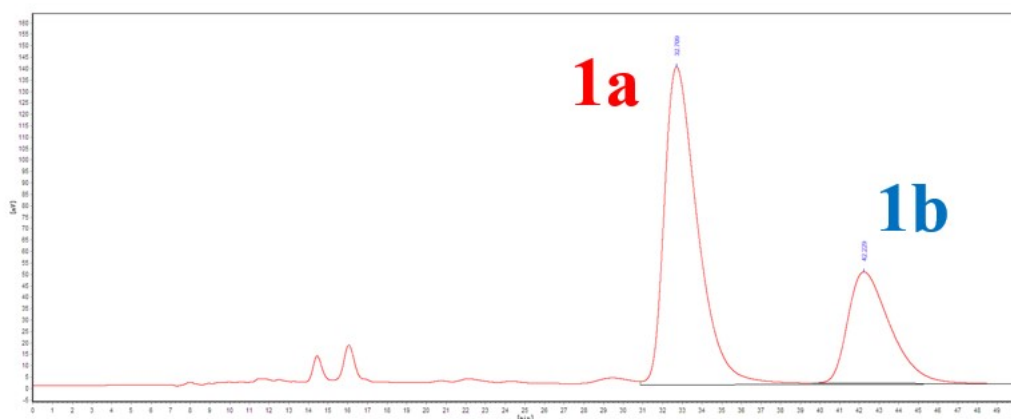
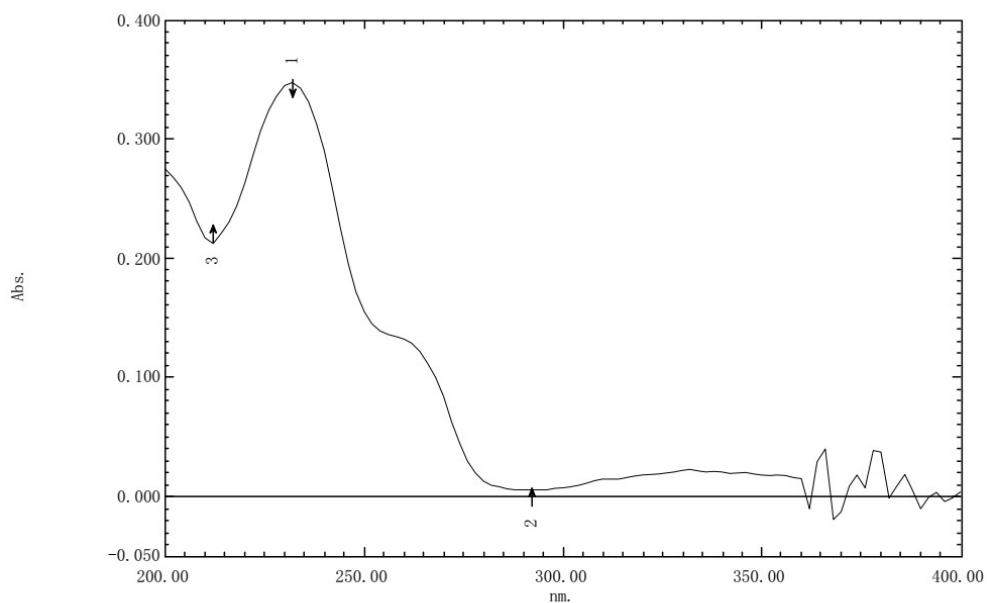


Fig. S11. The chiral HPLC chromatogram of compounds **1a** and **1b**.



测定属性
 波长范围 (nm.): 200.00到400.00
 扫描速度: 高速
 采样间隔: 2.0
 自动采样间隔: 停用
 扫描模式: 单一的

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1	⬆	232.00	.335	
2	⬆	292.00	.006	
3	⬆	212.00	.228	

Fig. S12 UV spectrum of compound **2**.

Mass Spectrum SmartFormula Report

Analysis Info

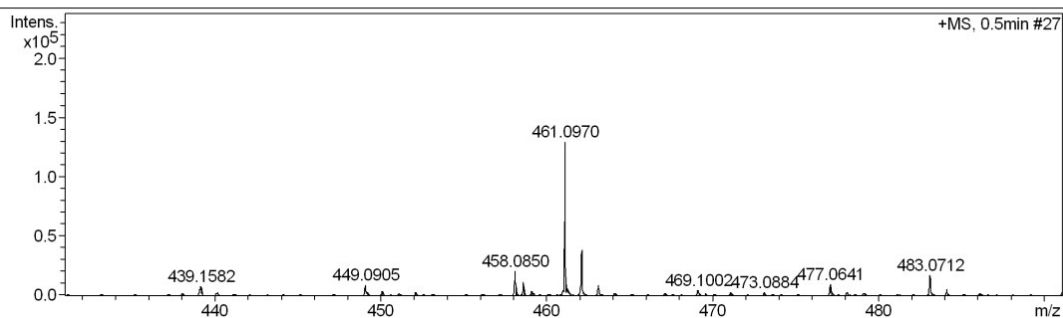
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 Comment

Acquisition Date 6/1/2017 2:17:51 PM

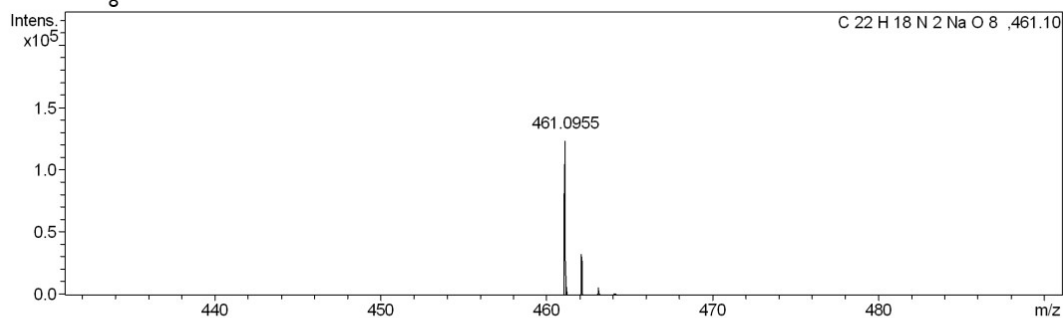
Operator Bruker Customer
 Instrument / Ser# micrOTOF-Q 125

Acquisition Parameter

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Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source



Meas. #	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e ⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNo	Std m/z Diff	Std Comb Dev
461.0970	1 C ₂₂ H ₁₈ N ₂ NaO ₈	461.0955	-3.2	0.6	14.5	ok	even	30.06	0.0584	0.0029	0.0189	0.0062	0.8427



Meas. #	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e ⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNo	Std m/z Diff	Std Comb Dev
461.0970	1 C ₂₂ H ₁₈ N ₂ NaO ₈	461.0955	-3.2	0.6	14.5	ok	even	30.06	0.0584	0.0029	0.0189	0.0062	0.8427

Fig. S13 HRESIMS spectrum of compound 2.

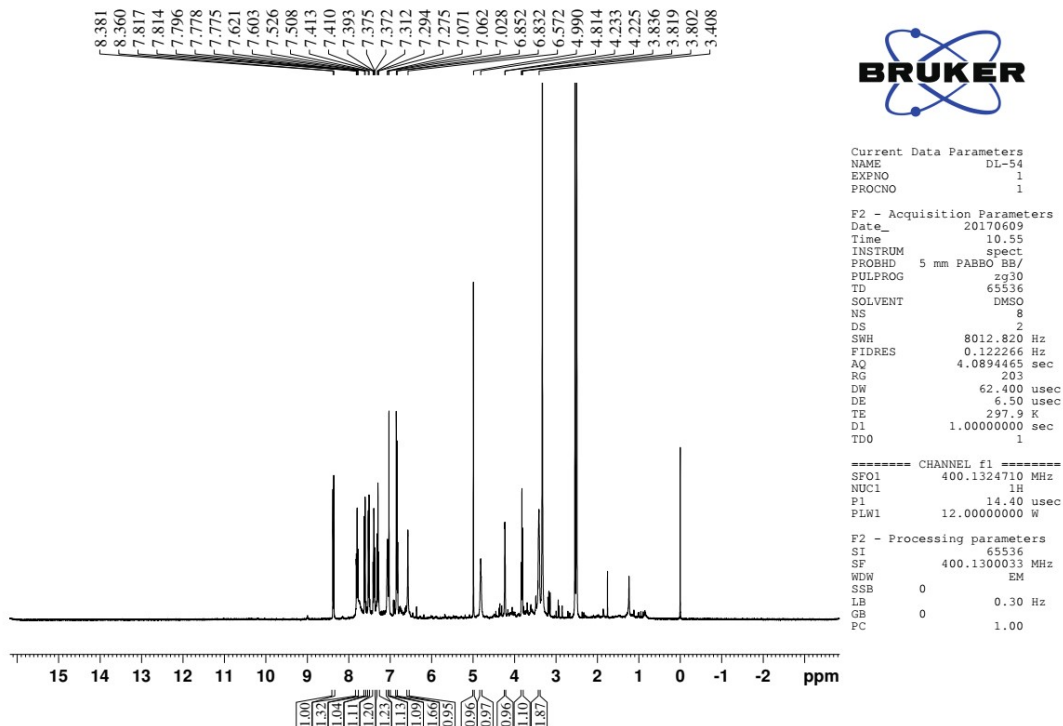


Fig. S14 ^1H NMR spectrum (400 MHz, $\text{DMSO-}d_6$) of compound 2.

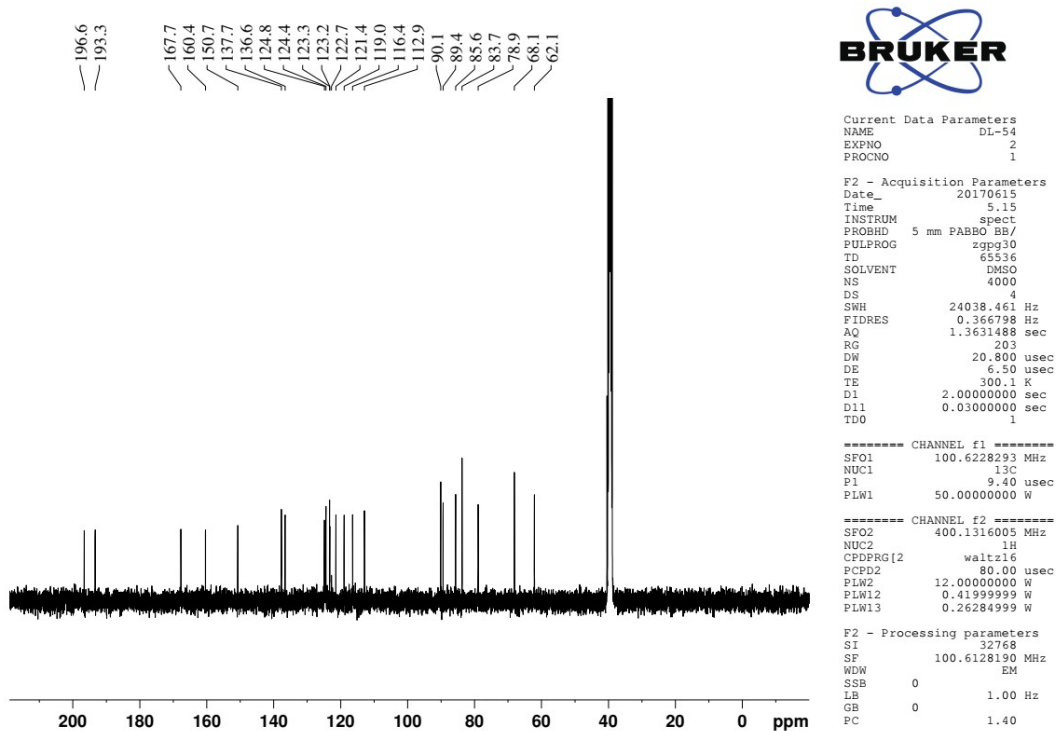
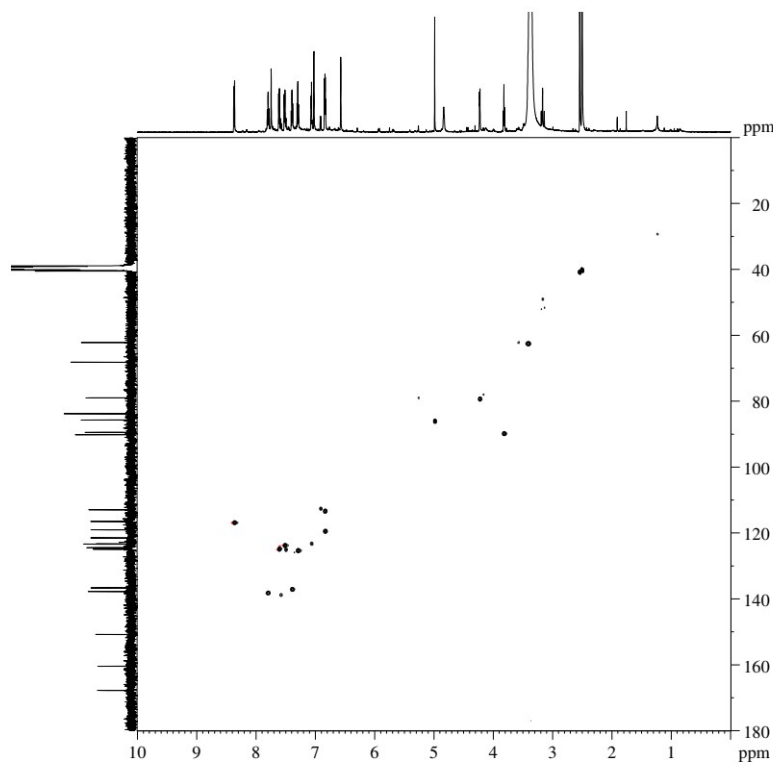


Fig. S15 ^{13}C NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound 2.



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PROCNO       1

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TD           1024
SOLVENT      DMSO
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DS           16
SWH          6009.615 Hz
FIDRES      0.868765 Hz
AQ           0.0851968 sec
RG           251.00
DE           83.200 usec
TE           296.2 K
CHST2       145.000000
D0           0.0000100 sec
D1           1.5000000 sec
D4           0.0017244 sec
D11          0.0000000 sec
D13          0.0000000 sec
D14          0.0000000 sec
D24          0.0011000 sec
IN0          0.0000100 sec
ZGPGPNS

===== CHANNEL f1 =====
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P2           22.20 usec
P28          2500.00 usec
PL1          4.00 dB
PL1W         34.70245579 MHz
SFO1         600.1300000 MHz

===== CHANNEL f2 =====
CPDPRG2      9389
NUC2         13C
P3           9.53 usec
P4           19.06 usec
PCPD2       81.00 usec
PL2          1.00 dB
PL2W        83.20243835 MHz
PL1W        83.20243835 MHz
P12W        1.1809545 MHz
SFO2        150.9143903 MHz

===== GRADIENT CHANNEL =====
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GFC3         40.10 %
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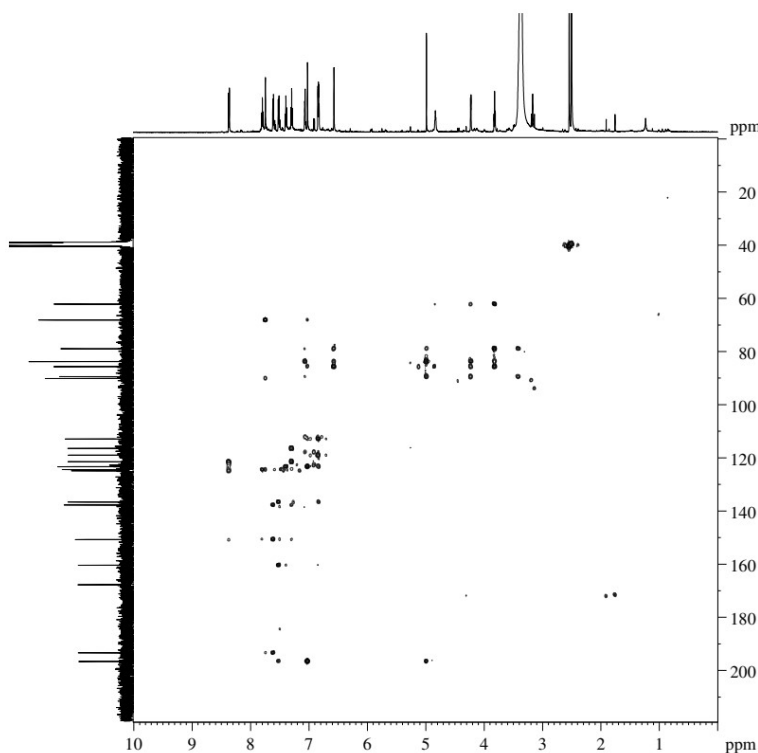
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FIDRES      212.226181 Hz
SR          180.000 ppm
FUNMODE     Echo-Antiecho

F2 - Processing parameters
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SF           600.1300000 MHz
WDW          SINC
SSB          0 Hz
GB           0
PC           1.40

F1 - Processing parameters
SI           1024
MC2         echo-antiecho
SF           150.9028870 MHz
WDW          SINC
SSB          0 Hz
GB           0
PC           1.40

```

Fig. S16. HSQC spectrum (600 MHz, DMSO-*d*₆) of compound 2.



```

Current Data Parameters
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EXPNO        9
PROCNO       1

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TD           1024
SOLVENT      DMSO
NS           16
DS           16
SWH          6009.615 Hz
FIDRES      0.868765 Hz
AQ           0.0851968 sec
RG           251.00
DE           83.200 usec
TE           296.2 K
CHST3       145.000000
D0           0.0000000 sec
D1           1.5000000 sec
D4           0.0017244 sec
D11          0.0000000 sec
D13          0.0000000 sec
D14          0.0000000 sec
D24          0.0001505 sec
IN0          0.0000100 sec
ZGPGPNS

===== CHANNEL f1 =====
NUC1         13C
P1           11.10 usec
P2           22.20 usec
P28          2500.00 usec
PL1          4.00 dB
PL1W         34.70245579 MHz
SFO1         600.1300000 MHz

===== CHANNEL f2 =====
CPDPRG2      9389
NUC2         13C
P3           9.53 usec
P4           19.06 usec
PCPD2       81.00 usec
PL2          1.00 dB
PL2W        83.20243835 MHz
PL1W        83.20243835 MHz
P12W        1.1809545 MHz
SFO2        150.9143903 MHz

===== GRADIENT CHANNEL =====
GPNAM[1]     SINE.100
GPNAM[2]     SINE.100
GFC1         50.00 %
GFC2         10.00 %
GFC3         40.10 %
G14          1000.00 usec

F1 - Acquisition parameters
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SFO1         150.9144 MHz
FIDRES      265.618164 Hz
SR          220.000 ppm
FUNMODE     QF

F2 - Processing parameters
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SF           600.1299970 MHz
WDW          SINC
SSB          0 Hz
GB           0
PC           1.40

F1 - Processing parameters
SI           1024
MC2         QF
SF           150.9028870 MHz
WDW          SINC
SSB          0 Hz
GB           0
PC           1.40

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Fig. S17. HMBC spectrum (600 MHz, DMSO-*d*₆) of compound 2.

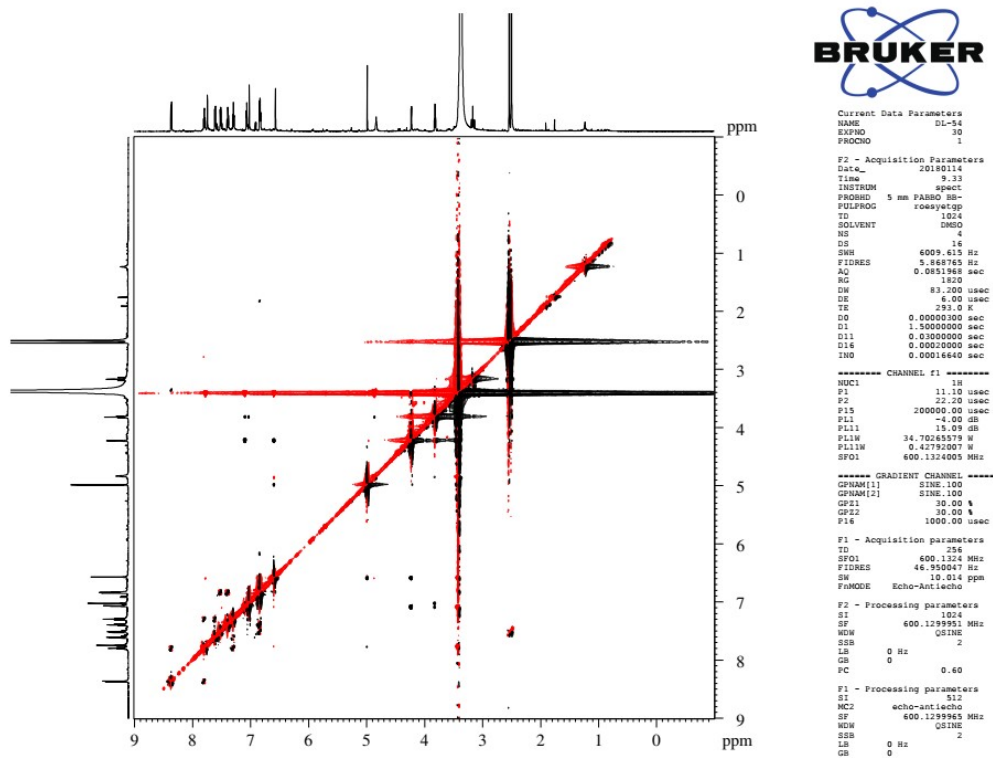


Fig. S18. ROESY spectrum (600 MHz, DMSO- d_6) of compound 2.

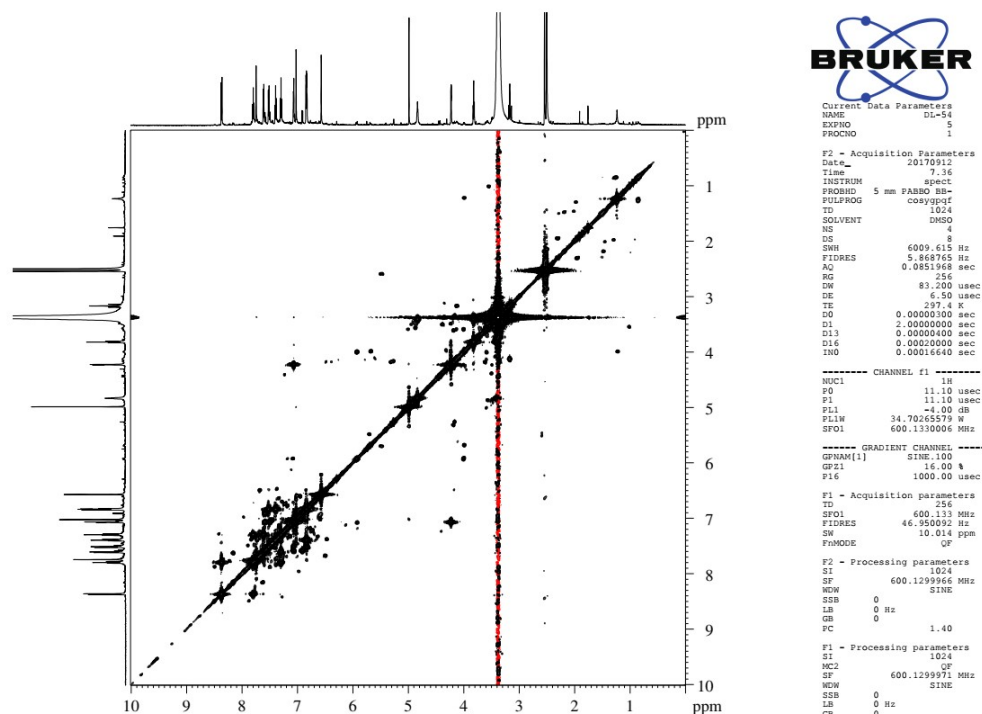


Fig. S19. ^1H - ^1H COSY spectrum (600 MHz, DMSO- d_6) of compound 2.

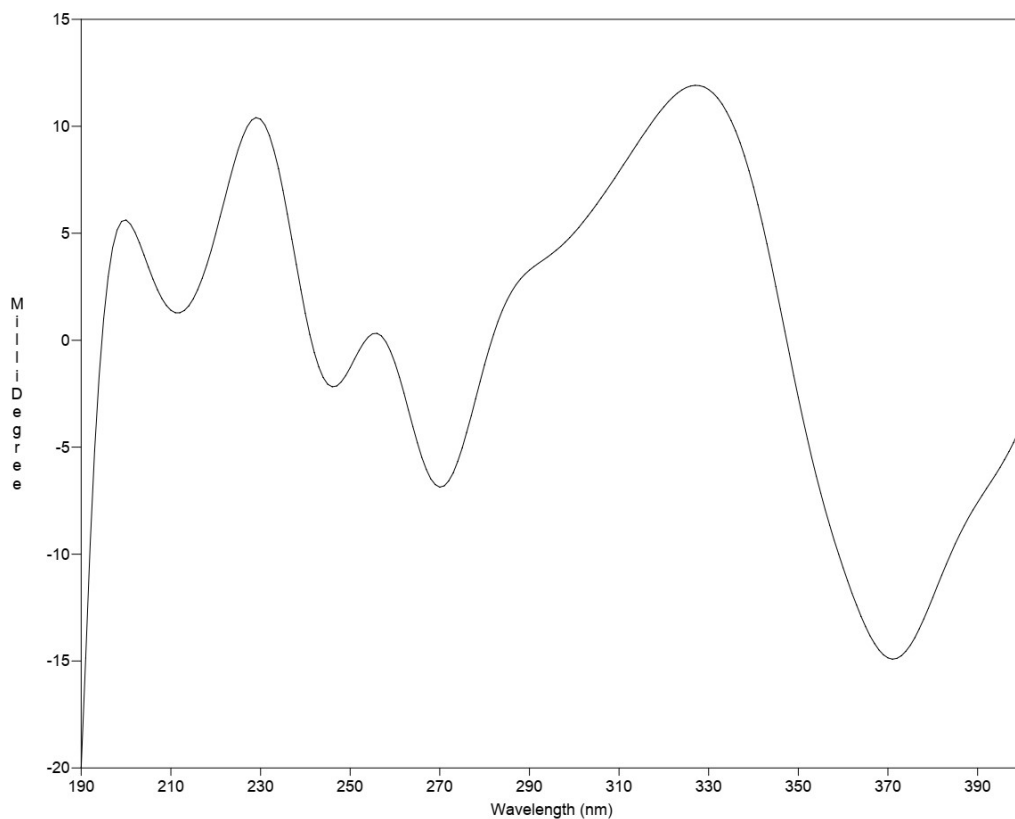
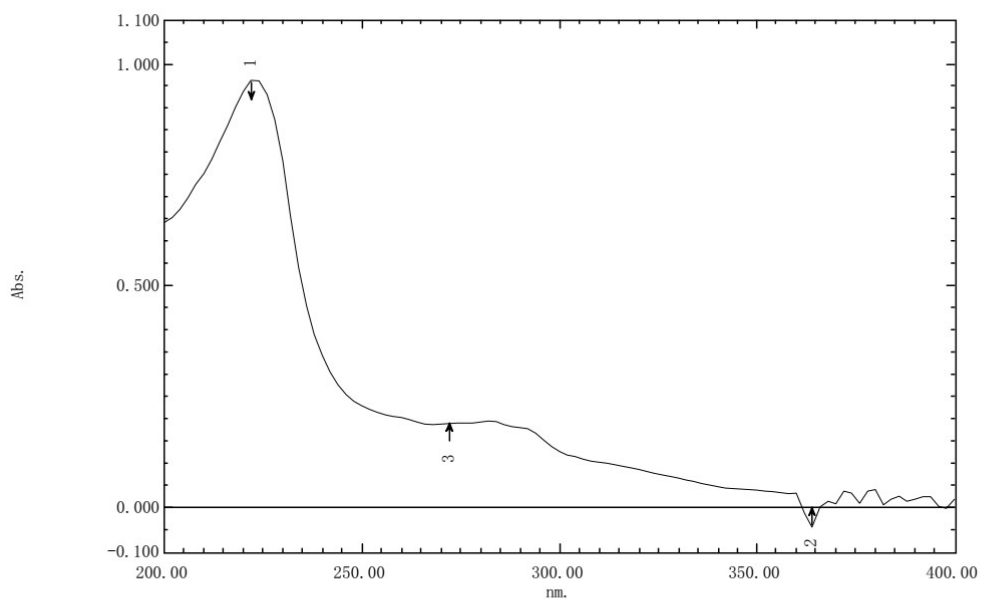


Fig. S20. Experimental ECD spectra of **2**.



测定属性
 波长范围 (nm.): 200.00到400.00
 扫描速度: 高速
 采样间隔: 2.0
 自动采样间隔: 停用
 扫描模式: 单一的

No.	P/V	Wavelength	Abs.	描述
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2	⬇	364.00	.001	
3	⬆	272.00	.190	

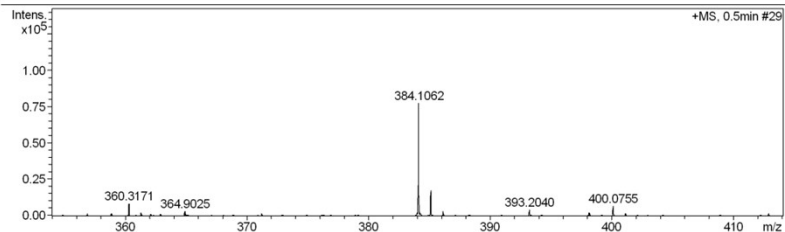
Fig. S21 UV spectrum of compound **3**.

Mass Spectrum SmartFormula Report

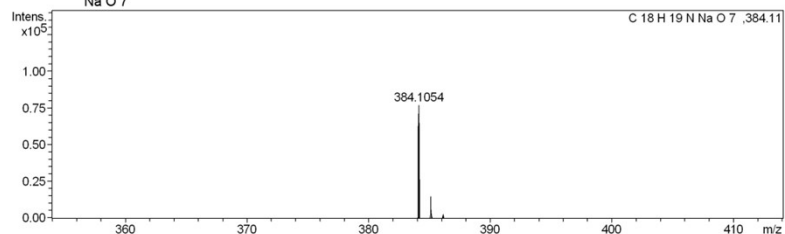
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Sample Name	DL-96			
Comment				

Acquisition Parameter

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Scan End	1500 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source



Meas. #	Formul a	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e ⁻ Conf	mSigma a	Std I	Std Mean m/z	Std I VarNor m	Std m/z Diff	Std Comb Dev
384.1062	1 C 18 H 19 N Na O 7	384.1054	-2.2	1.5	9.5	ok	even	16.25	0.0283	0.0028	0.0121	0.0065	0.8427



Meas. #	Form ula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rul e	e ⁻ Conf	mSig ma	Std I	Std Mean m/z	Std I VarNo rm	Std m/z Diff	Std Comb Dev
384.1054	C 18 H 19 N Na O 7	384.11											

Fig. S22 HRESIMS spectrum of compound 3.

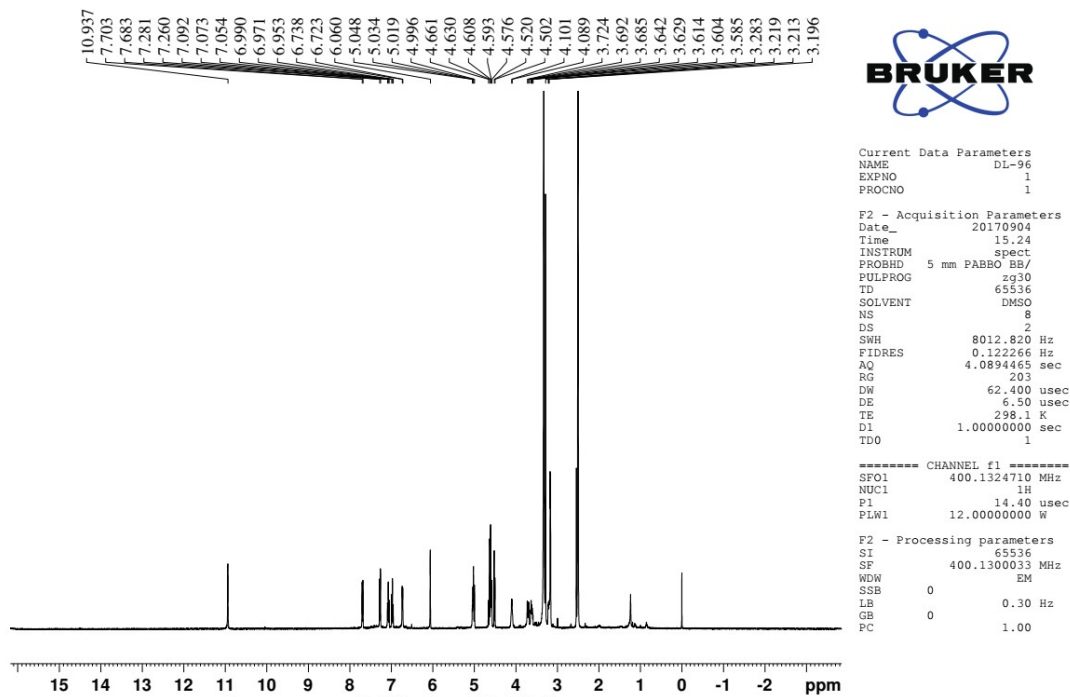
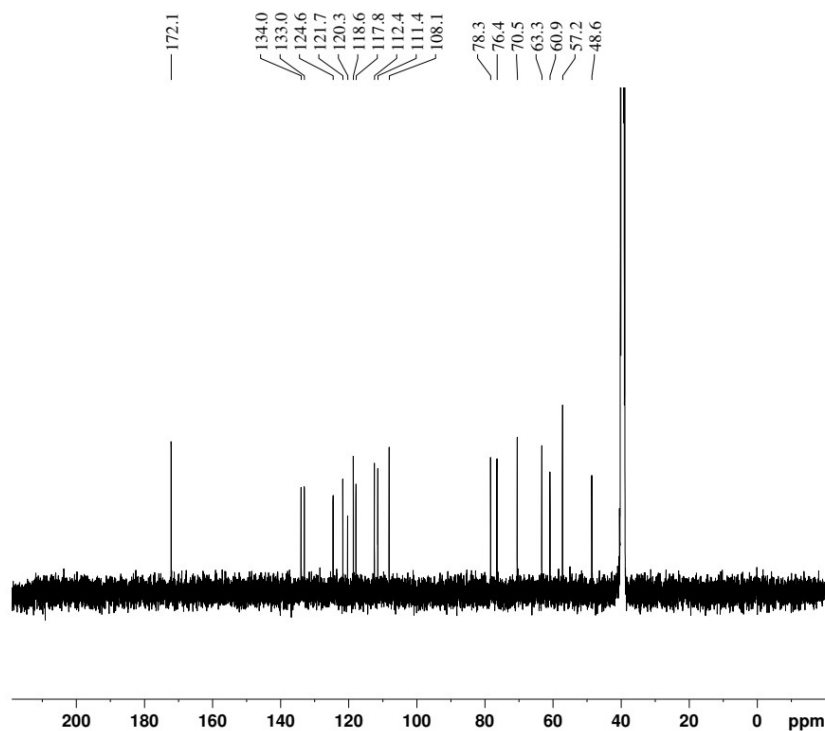


Fig. S23 ¹H NMR spectrum (400 MHz, DMSO-*d*₆) of compound 3.



```

Current Data Parameters
NAME          DL-96
EXPNO         2
PROCNO        1

F2 - Acquisition Parameters
Date_         20170913
Time          9.13
INSTRUM       spect
PROBHD        5 mm PABBO BB/
PULPROG       zgpg30
TD            65536
SOLVENT       DMSO
NS            10000
DS            4
SWH           24038.461 Hz
FIDRES        0.366798 Hz
AQ            1.3631488 sec
RG            203
DW            20.800 usec
DE            6.50 usec
TE            300.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0           1

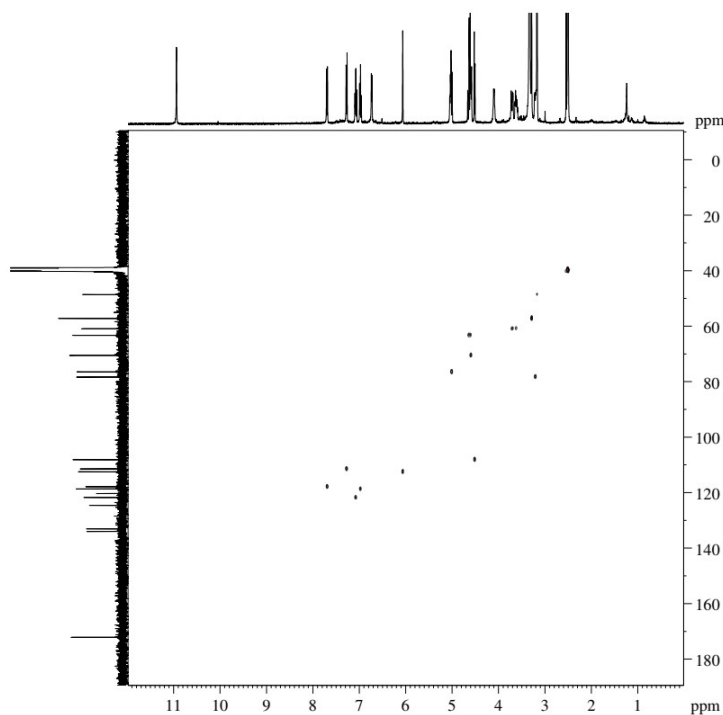
===== CHANNEL f1 =====
SFO1          100.628293 MHz
NUC1          13C
P1            9.40 usec
PLW1          50.0000000 W

===== CHANNEL f2 =====
SFO2          400.1316005 MHz
NUC2          1H
CPDPRG2       waltz16
PCPD2         80.00 usec
PLW2          12.00000000 W
PLW12         0.41999999 W
PLW13         0.26284999 W

F2 - Processing parameters
SI            32768
SF            100.6128185 MHz
WDW           EM
SSB           0
LB            1.00 Hz
GB            0
PC            1.40

```

Fig. S24 ^{13}C NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound 3.



```

Current Data Parameters
NAME          DL-96
EXPNO         2
PROCNO        1

F2 - Acquisition Parameters
Date_         20180928
Time          10.31
INSTRUM       spect
PROBHD        5 mm PABBO BB/
PULPROG       hugocor1
TD            65536
SOLVENT       DMSO
NS            10000
DS            4
SWH           7183.908 Hz
FIDRES        7.015535 Hz
AQ            0.071704 sec
RG            25100
DW            69.400 usec
DE            6.50 usec
TE            298.4 K
===== CHANNEL f1 =====
SFO1          600.136200 MHz
NUC1          13C
P1            11.10 usec
PLW1          22.20 usec
LB            1.00 Hz
GB            0
PC            1.40

===== CHANNEL f2 =====
SFO2          400.1316005 MHz
NUC2          1H
P2            15.00 usec
PLW2          15.00 usec
LB            1.00 Hz
GB            0
PC            1.40

===== GRADIENT CHANNEL =====
GRAMP1[1]    SINE 100
GRAMP1[2]    SINE 100
GRF1         80.00 Hz
GRF2         20.10 Hz
F14          1000.00 usec

F1 - Acquisition parameters
TD           256
SFO1         150.1164 MHz
FIDRES       235.806854 Hz
AQ           100.00000000 sec
FAMODE       Echo-Antiecho

F1 - Processing parameters
SI            65536
SF            600.136200 MHz
WDW           SINE
SSB           0 Hz
LB            1.40
GB            0
PC            1.40

F1 - Processing parameters
SI            65536
SF            400.1316005 MHz
WDW           SINE
SSB           0 Hz
LB            1.40
GB            0
PC            1.40

```

Fig. S25. HSQC spectrum (600 MHz, $\text{DMSO-}d_6$) of compound 3.

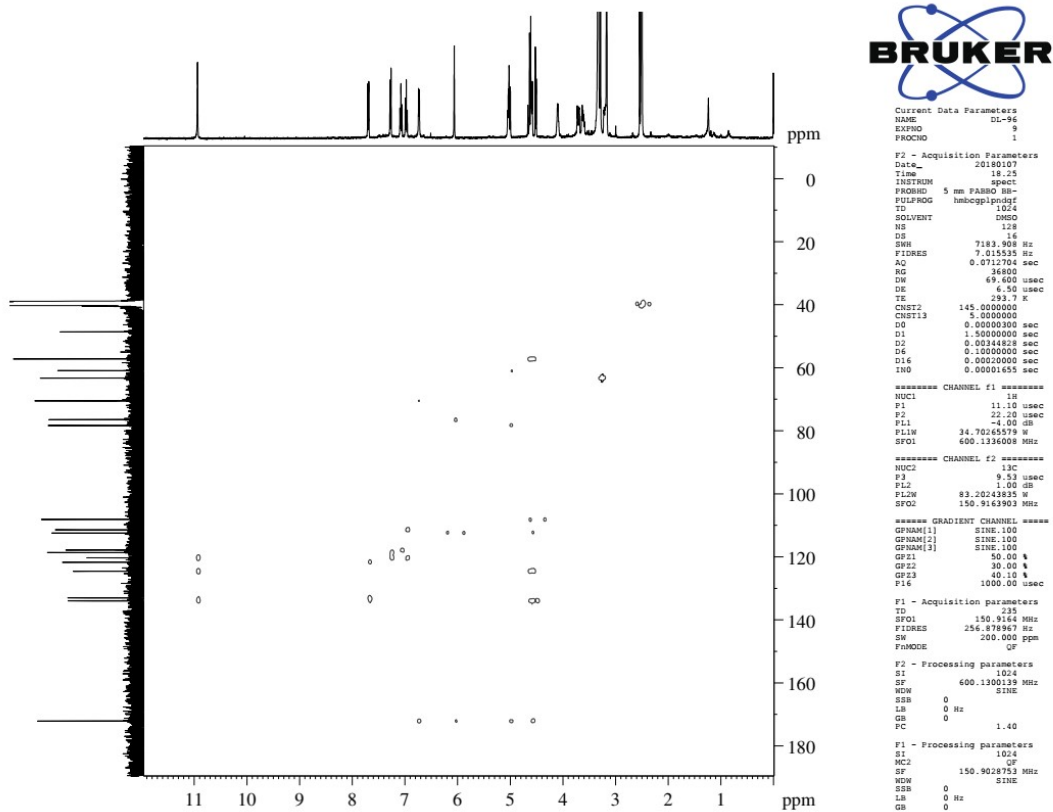


Fig. S26. HMBC spectrum (600 MHz, DMSO- d_6) of compound 3.

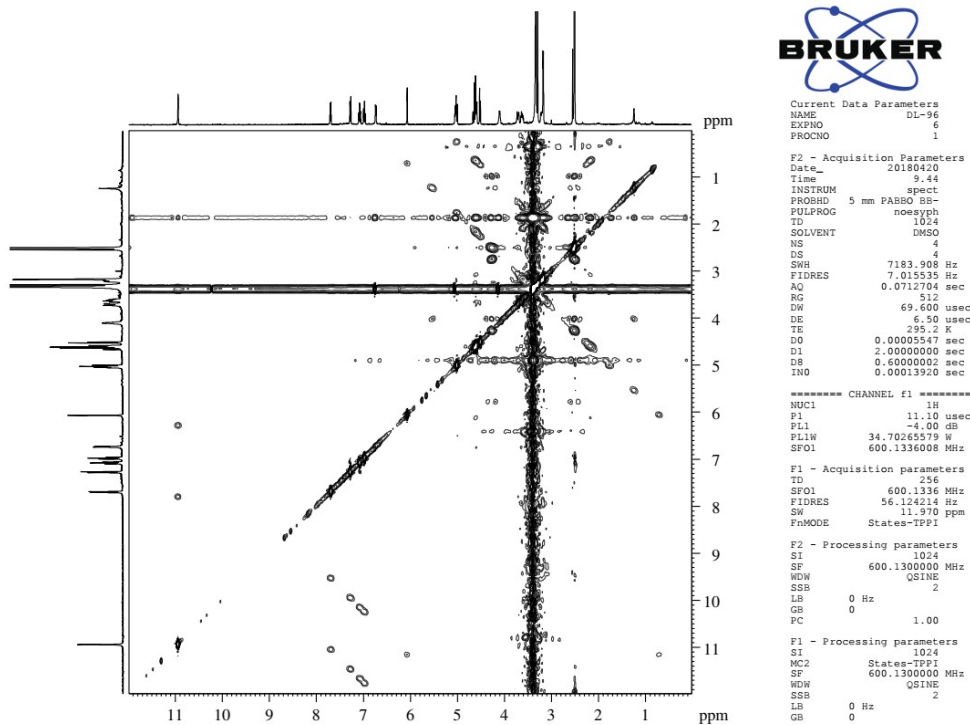


Fig. S27. NOESY spectrum (600 MHz, DMSO- d_6) of compound 3.

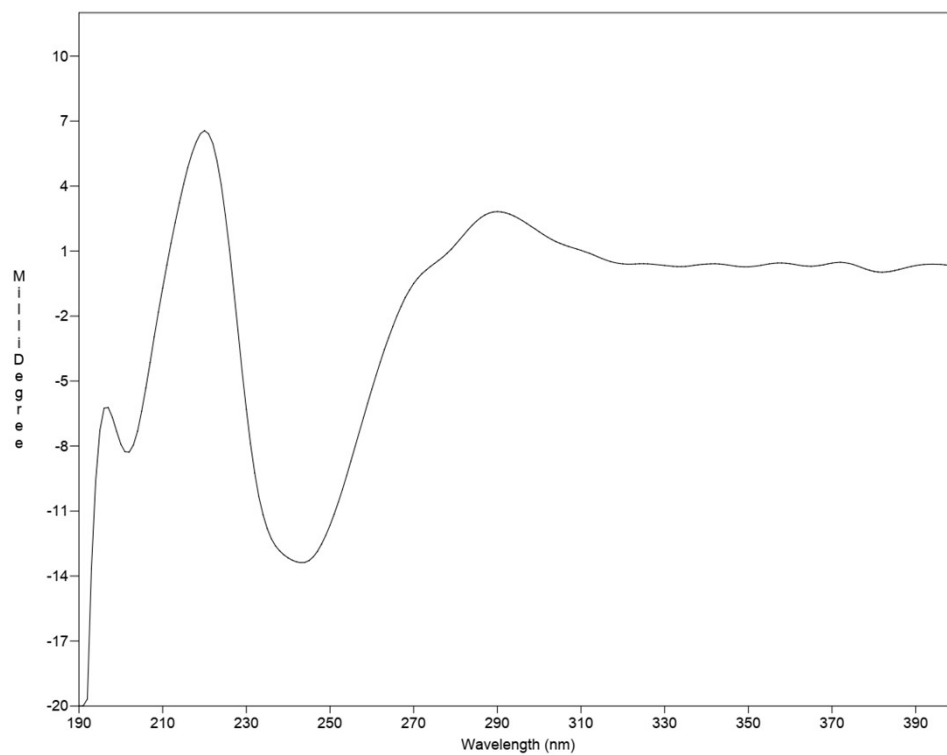


Fig. S28. Experimental ECD spectra of **3**.