Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Vlasoulides A and B, a pair of neuroprotective C_{32} dimeric sesquiterpenes with hexacyclic 5/7/5/5/(5)/7 carbon skeleton from the roots of *Vladimiria souliei*

Zhi-li Wu [a,b], Qun Wang [b], Lu Fu [b], Ri-xin Su [b], Ze-shi Sun [b], Hui-liang Li [a,b] *

Affiliation

[a] School of Traditional Chinese Pharmacy, China Pharmaceutical University, Nanjing, Jiangsu 211198, P.R. China

[b] School of Pharmacy, Second Military Medical University, Shanghai 200433, P.R. China

Contents

Table S1.^{1}H NMR (600 MHz $CDCl_3$ *J* in Hz) and ^{13}C NMR (150 MHz, $CDCl_3$)

Spectroscopic Data for 1 and 2

Position	1[a]		2[a]	
	δ_H mult (*J* in Hz)	δ_C	δ_H mult (*J* in Hz)	δ_C

1	2.96, m	44.9	2.96, m	44.8
2	2.20, 1.78, m	29.3	2.20, 1.78, m	29.3
3	2.24, 1.76, m	35.8	2.24, 1.78, m	35.7
4		54.3		54.2
5	2.78[c], dd (6.74, 6.52)	54.1	2.77[c] dd (6.65, 6.93)	54.2
6	4.12, t (9.8)	78.8	4.11, t (9.7)	79.0
7	2.86[c], m	57.5	2.85[c], m	56.9
8	2.11, 1.50, m	31.9	2.11, 1.51, m	31.8
9	2.47, 2.15, m	34.1	2.47, 2.12, m	34.0
10		150.1		150.1
11		180.2		180.1
12	1.87, 1.43, m	30.5	1.82, 1.45, m	30.3
13	4.99, s, 4.81, s	114.2	4.98, s, 4.80, s	114.1
14		211.6		211.7
15	2.67, 2.66, d (8.5)	50.5	2.63, 2.62, d (8.7)	51.6
16	4.24, m	63.9	4.25, m	63.5
17	1.21, d (6.3)	22.5	1.20, d (6.4)	22.2
1′	2.89 [c], m	47.4	2.88[c], m	47.4
2′	1.96, 1.82, m	30.0	1.97, 1.82, m	30.0

3′	2.55, 2.44, m	32.2	2.55, 2.44, m	32.2
4′		151.3		151.3
5′	2.74 [c]	52.0	2.73 [c]	51.9
6′	4.21, t (9.5)	84.0	4.22, t (9.6)	84.0
7′	2.09, m	48.7	2.07, m	48.7
8′	1.85[c], 1.64[c], m	25.3	1.83[c], 1.64[c], m	25.3
9′	2.49[c], 2.04[c], m	36.0	2.49[c], 2.05[c], m	36.0
10′		149.6		149.6
11′		76.7		76.7
12′		176.8		176.8
13′	1.81[c], 1.69[c], m	30.8	1.81[c], 1.66[c], m	30.9
14′	4.89,s, 4.80, s	112.2	4.89,s, 4.79, s	112.2
15′	5.19, d (0.8), 5.06, d (1.0)	109.7	5.19, d (1.1), 5.05, d (1.1)	109.7

[a] ^{1}H NMR Spectroscopic Data at 600 MHz and ^{13}C NMR Data at 150 MHz

Materials and methods

***General experimental procedures*.** Column chromatography (CC): silica gel H (10–40 μm; *Marine Chemical Factory*, Qingdao, P. R. China); MCI gel CHP-20P; Sephadex LH-20 (*Pharmacia Fine Chemicals*, Piscataway, NJ, USA); RP-C_{18} gel (40−63 μm; Daiso, Co, Japan) were used for column chromatography. TLC: silica gel plates, visualization by spraying with 10 % H_2SO_4 in EtOH and dragendorff's reagent. Semi-preparative HPLC: Agilent 1260 series with a Zorbax SB-C_{18} (5 μM, 9.4 mm × 25 cm) column. Melting point: X-4B apparatus and was uncorrected. IR spectra: Bruker Vector 22 (KBr pellets). Optical rotation: Autopol VI (serial No. 90079, manufactured by Rudolph Research Analytical, Hackettstown, NJ). UV spectra were obtained by the DAD detector of HLPC (Agilent 1260). NMR Spectra: Bruker Ascend-500 spectrometer (500 MHz); δ in ppm with $SiMe_4$ as internal standard. MS: Agilent *MSD-Trap-XCT* (for ESI) and *Q-Tof* micro mass spectrometer (for HR-ESI).

***Plant Material*.** The whole plants of *Vladimiria souliei* was collected at daJin, SiChuan province of China, in October, 2019, and authenticated by Prof. Bao-Kang Huang of Second Medical Military University. Currently A voucher specimen (No. 20191001) is deposited in School of Pharmacy, Second Military Medical University.

***Extraction and Isolation*.** The dried and chipped roots of *V. souliei* (50.0 kg) were extracted by maceration with 95% ethanol overnight at room temperature (3 × 60 L). After remove of solvent, the ethanol extract (5.60 kg) was partitioned between water and petroleum ether (PE)/ethyl acetate (EtOAc), successively, to give PE, EtOAc and water extracts. EtOAc extract (1.15 Kg) was segmented by MCI column chromatography (MeOH/H_2O, 30:70 to 100:0) to give 8 fractions (Fr. 1-8). Fraction 5 (52.5 g) was further isolated by ODS column chromatography (MeOH/H_2O, 30:70 to 90:10) to obtain 8 subfractions (Fr. 5.1-5.8). Subfraction 5.4 (5.5 g) was further purified by Sephadex LH-20 column chromatography (PE: EtOAc: MeOH, 10:10:1) to give 6 subfractions (Fr.

5.5.1-5.5.6). Subfraction 5.5.4 (252 mg) was purified by semi-preparative RP-C_{18} HPLC (CH_3CN/H_2O, 70:30) to produce compounds **1** (7.2 mg) and **2** (6.5 mg)**.** Above all, compounds **1** (7.2 mg) and **2** (6.5 mg) were obtained.

Compound characterization of 1 and 2

*Vlasoulide A (**1**)*

White powder; [α]25 D -1.00 (*c* 0.08, CH_3OH); UV (CH_3CN/H_2O) λ_{max} 210; IR (KBr) v_{max} 3438, 3079, 2933, 2869, 1770, 1710, 1639, 1448, 1382, 1351, 1268, 1222, 1153, 1272, 1020, 995, 896, cm^{-1}; ^{1}H- and ^{13}C-NMR data (600 MHz/150 MHz), see Table S1; ESIMS *m/z* 561.4 ([M+Na]$^+$); positive HRESIMS *m/z* 561.2828 ([M+Na]$^+$, calcd 561.2823).

*Vlasoulide B (**2**)*

White powder; [α]25 D 0.00 (*c* 0.05, CH_3OH); UV (CH_3CN/H_2O) λ_{max} 210; IR (KBr) v_{max} 3440, 3079, 2929, 2869, 1772, 1752, 1639, 1448, 1400, 1382, 1353, 1282, 1257, 1224, 1068, 1018, 995, 896 cm^{-1}; ^{1}H- and ^{13}C-NMR data (600 MHz/150 MHz), see Table S1; ESIMS *m/z* 561.5 ([M+Na]$^+$); positive HRESIMS *m/z* 561.2829 ([M+Na]$^+$, calcd 561.2823).

(*R*)-and (*S*)-MTPA Esters of compounds 1 and 2

To each compounds **1** and **2** (each 1.5 mg) in pyridine-d_5 (130 μL) was separately added (*R*)-(-)-MTPA (5 μL) and (*S*)-(+)-MTPA (5 μL) at room temperature, followed by stirring at 40℃ for 8 h, and each reaction mixture was transferred into a 1.7 mm NMR tube.

*(R)-MTPA ester of **1***

^{1}H NMR (pyridine-d_5, 600MHz): δ_H 1.20 (3H, d, H_3-17), 2.88 (1H, m H-15a), 2.91 (1H, m, H-15b),

4.30 (1H, t, H-6), 2.91 (1H, m, H-7), 2.21 (1H, m, H-8a), 1.58 (1H, m, H-8b).

*(S)-MTPA ester of **1***

^{1}H NMR (pyridine-d_5, 600MHz): δ_H 1.32 (3H, d, H_3-17), 2.83 (1H, m H-15a), 2.85 (1H, m, H-15b), 4.20 (1H, t, H-6), 2.88 (1H, m, H-7), 2.19 (1H, m, H-8a) 1.56 (1H, m, H-8b).

*(R)-MTPA ester of **2***

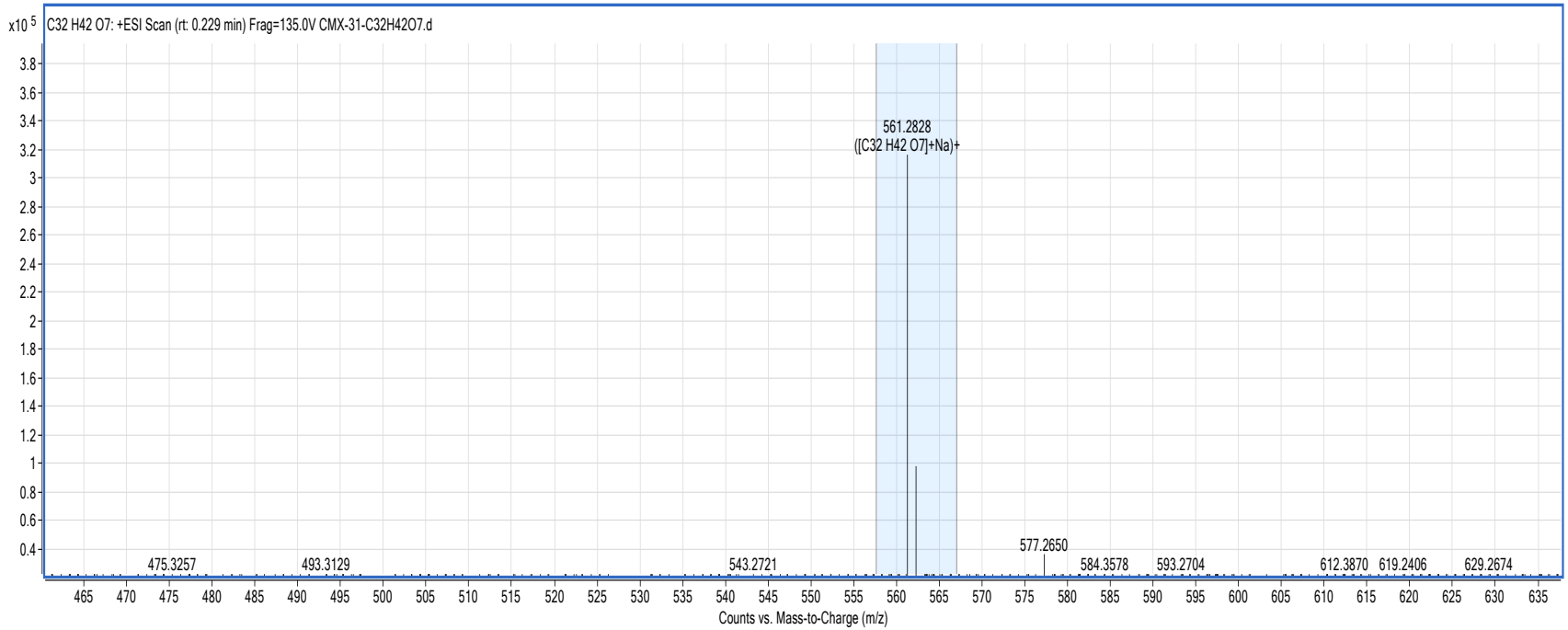
^{1}H NMR (pyridine-d_5, 600MHz): δ_H 1.32 (3H, d, H_3-17), 2.71 (1H, m H-15a), 2.74 (1H, m, H-15b), 4.21 (1H, t, H-6), 2.93 (1H, m, H-7), 2.14 (H, m, H-8a) 1.54 (1H, m, H-8b).

*(S)-MTPA ester of **2***

^{1}H NMR (pyridine-d_5, 600MHz): δ_H 1.22 (3H, d, H_3-17), 2.77 (1H, m H-15a), 2.78 (1H, m, H-15b), 4.27 (1H, t, H-6), 2.98 (1H, m, H-7), 2.16 (1H, m, H-8a), 1.55 (1H, m, H-8b).

Neuroprotection Assay

P-12 cells were seeded in 96-well culture plates at 8 × 10^3 cells/mL at 37°C for 12 h. Then the cells were incubated with glutamate for an additional 24 h and drugs were pretreated for 1 h before treated with glutamate. Cell viability was determined by the cck8 assay, after treatment, 10 μL of cck8 were added to each well and incubated at 37℃ for 4 h. The optical density (OD) was spectrophotometrically measured at 450 nm (cck8) using a microplate reader, respectively (BioTek Instruments, Inc.)



Height (Calc)	Height Sum% (Calc)	Height % (Calc)	m/z (Calc)	Diff (mDa)	Height	Height %	Height Sum %	m/z
304244.4	69.4	100	561.2823	-0.5	316121.1	100	72.1	561.2828

Best	ID Source	Formula	Mass (MFG)	Species	m/z	Score	Diff (ppm)	Score (MFG)
TRUE	MFG	C32 H42 O7	538.2931	(M+Na)+	561.2828	96.74	-0.76	96.74

Figure S1. HRESIMS spectrum of Vlasoulide A (1)

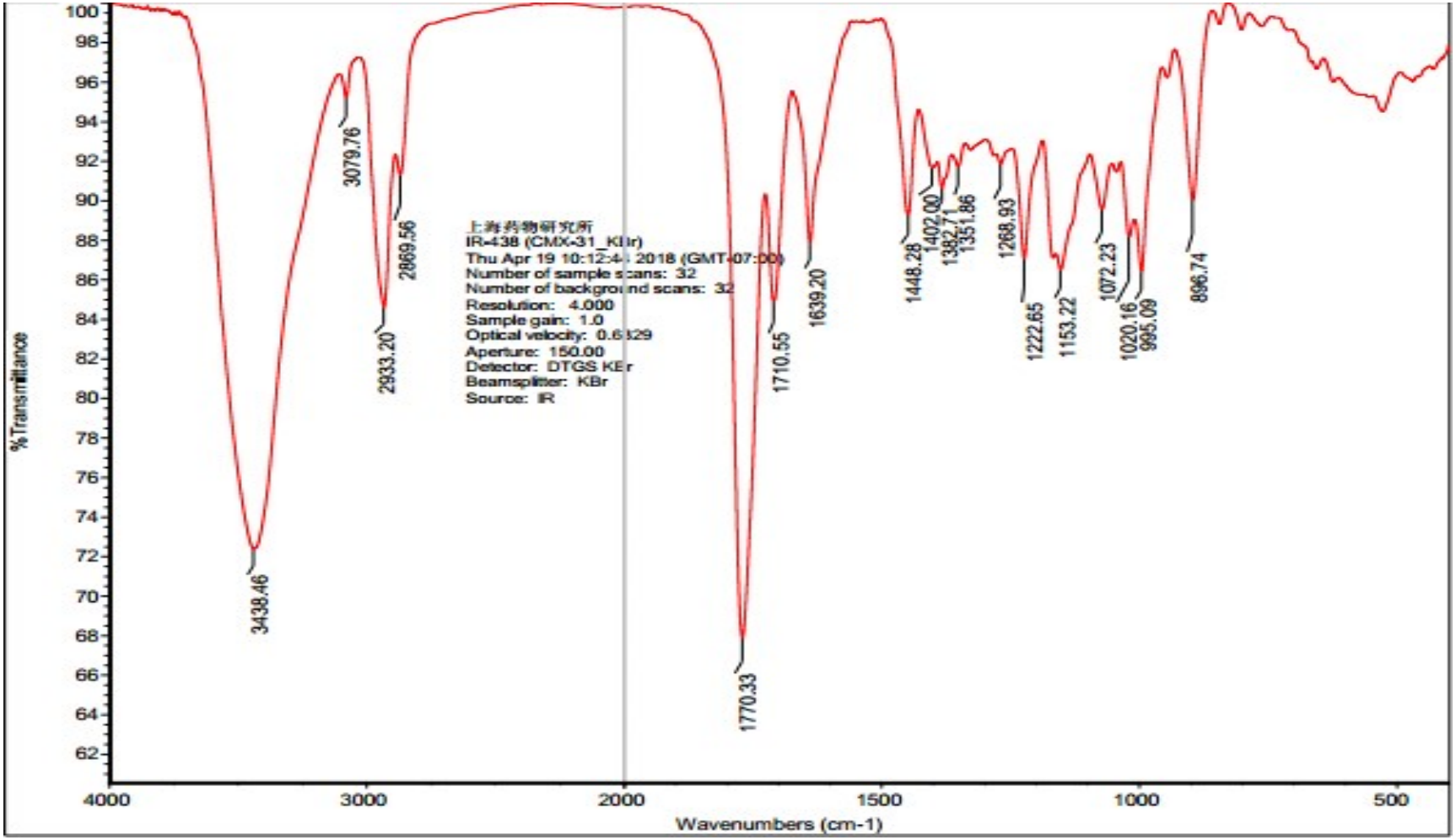


Figure S2. IR spectrum of Vlasoulide A (1)

Rudolph Research Analytical

Friday, 04/20/2018

This sample was measured on an Autopol VI, serial number 90079,
manufactured by Rudolph Research Analytical,Hackettstown,NJ.

LotID : CMX-31
Set Temperature : 20.0
Temp Corr : OFF

n	Average	Std.Dev.	Maximum	Minimum
6	-1.000	1.0000	0.000	-2.000

S.No	Sample ID	Time	Result	Scale	OR °Arc	WLG	Lg.mm	Conc.	Temp.	Comment
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2	CMX-31	04:22:10 PM	-2.000	SR	-0.001	589	100.00	0.050	19.9	
3	CMX-31	04:22:18 PM	-2.000	SR	-0.001	589	100.00	0.050	19.9	
4	CMX-31	04:22:26 PM	-2.000	SR	-0.001	589	100.00	0.050	19.9	
5	CMX-31	04:22:34 PM	0.000	SR	0.000	589	100.00	0.050	19.9	
6	CMX-31	04:22:43 PM	0.000	SR	0.000	589	100.00	0.050	19.9	

_____
Signature

Figure S3. OR Value of Vlasoulide A (1) in CH_3OH

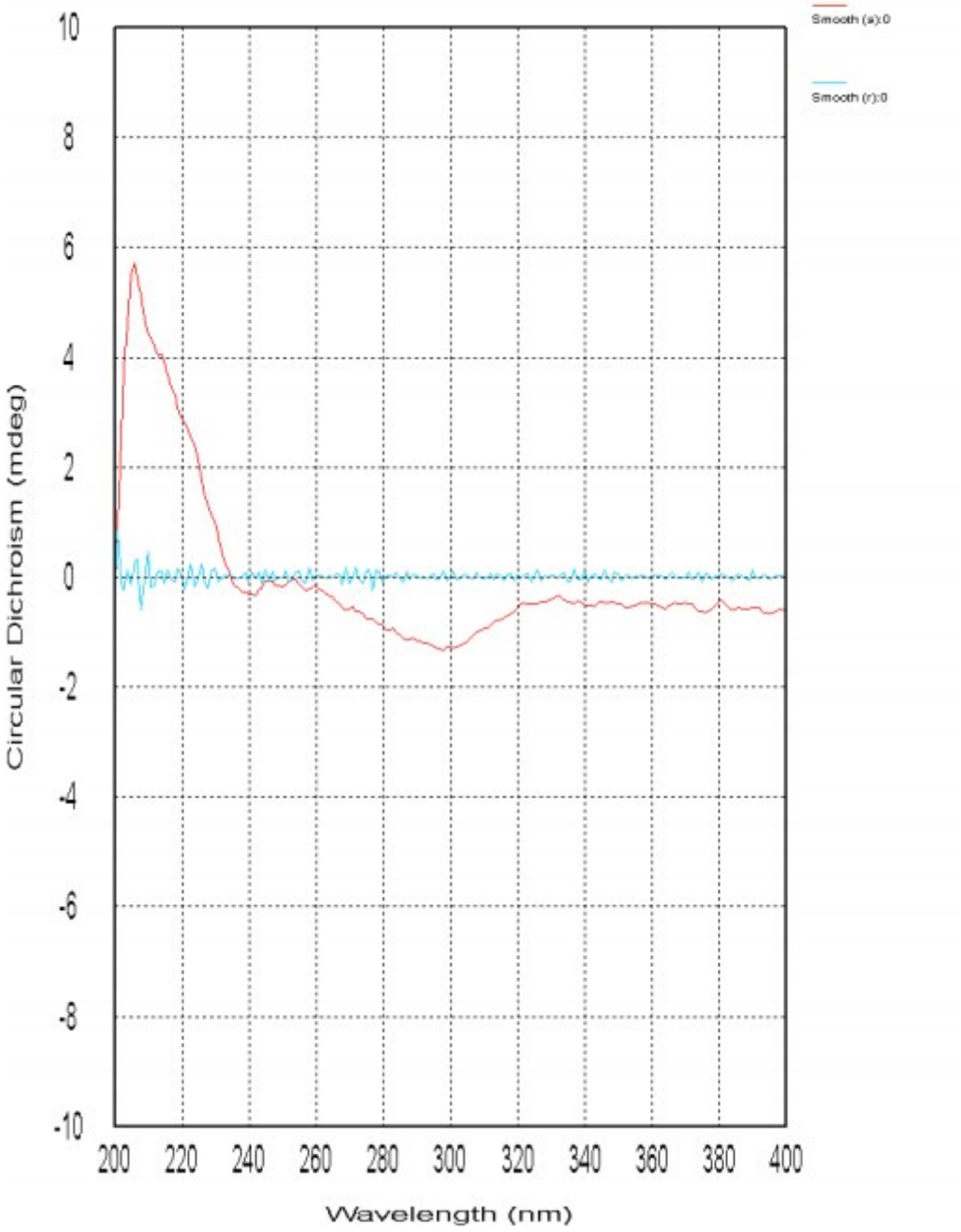


Figure S4. CD Value of Vlasoulide A (1) in CH_3OH

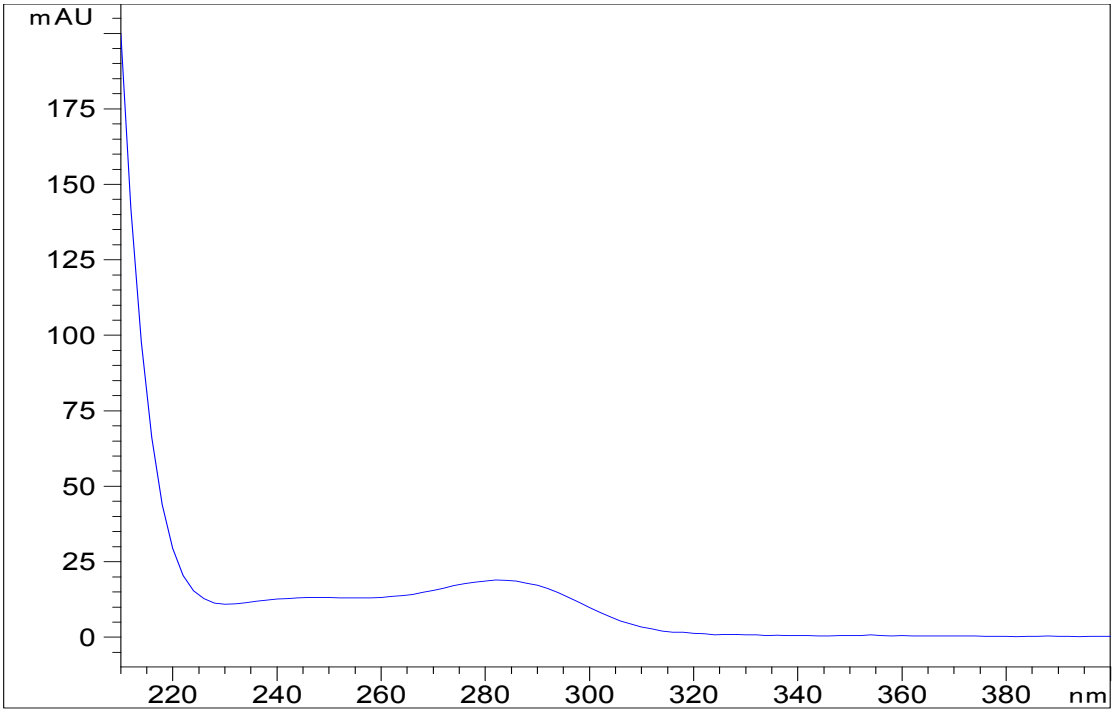
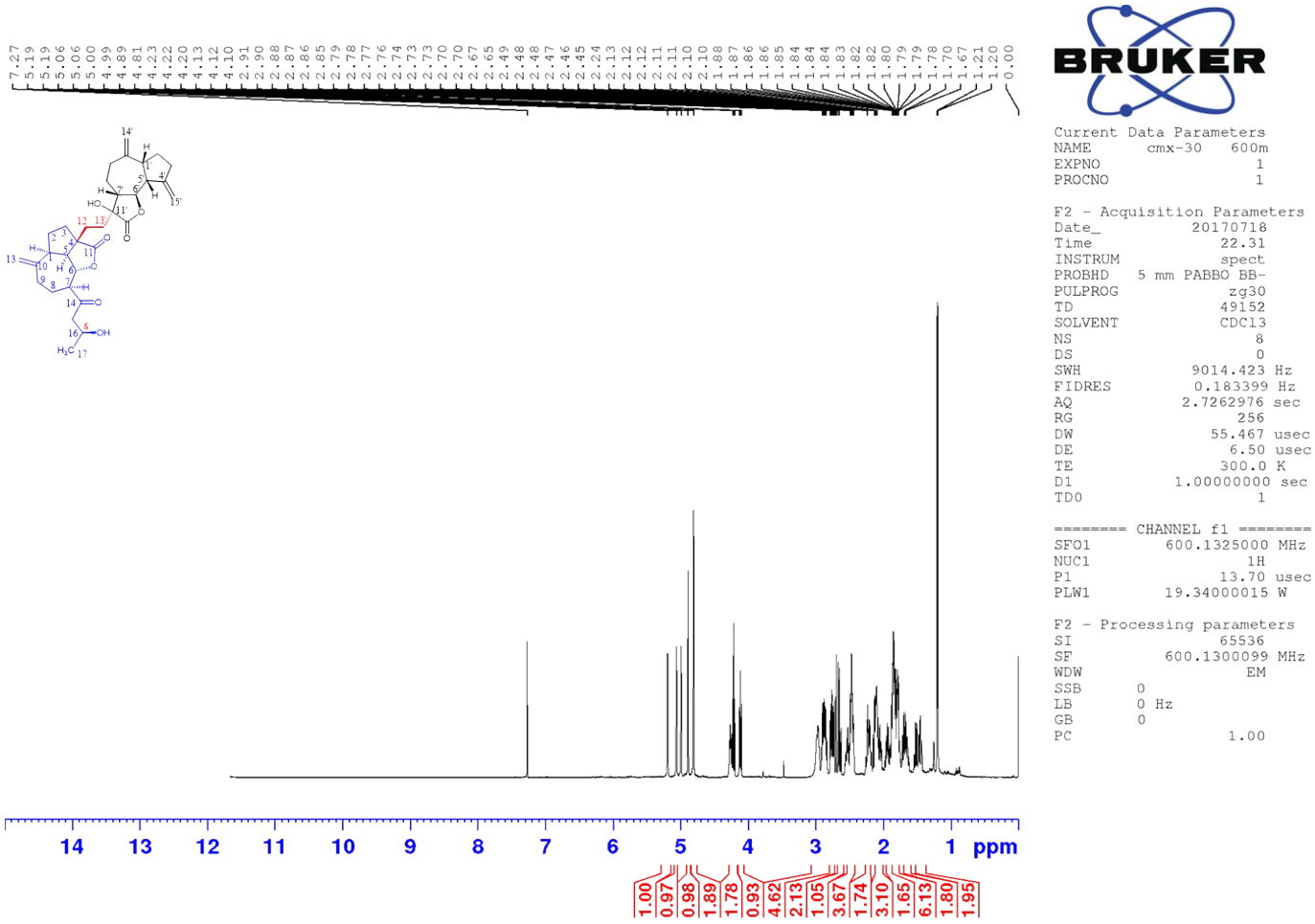


Figure S5. UV spectrum of Vlasoulide A (1) in CH_3CN/H_2O

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EXPNO                   1
PROCNO                  1

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PULPROG             zg30
TD                 49152
SOLVENT            CDCl3
NS                     8
DS                     0
SWH             9014.423 Hz
FIDRES          0.183399 Hz
AQ              2.7262976 sec
RG                   256
DW                55.467 usec
DE                 6.50 usec
TE                300.0 K
D1             1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         600.1325000 MHz
NUC1                   1H
P1                 13.70 usec
PLW1         19.34000015 W

F2 - Processing parameters
SI                 65536
SF           600.1300099 MHz
WDW                   EM
SSB       0
LB        0 Hz
GB        0
PC                 1.00
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Figure S6. ^{1}H- NMR spectrum of Vlasoulide A (1) in $CDCl_3$

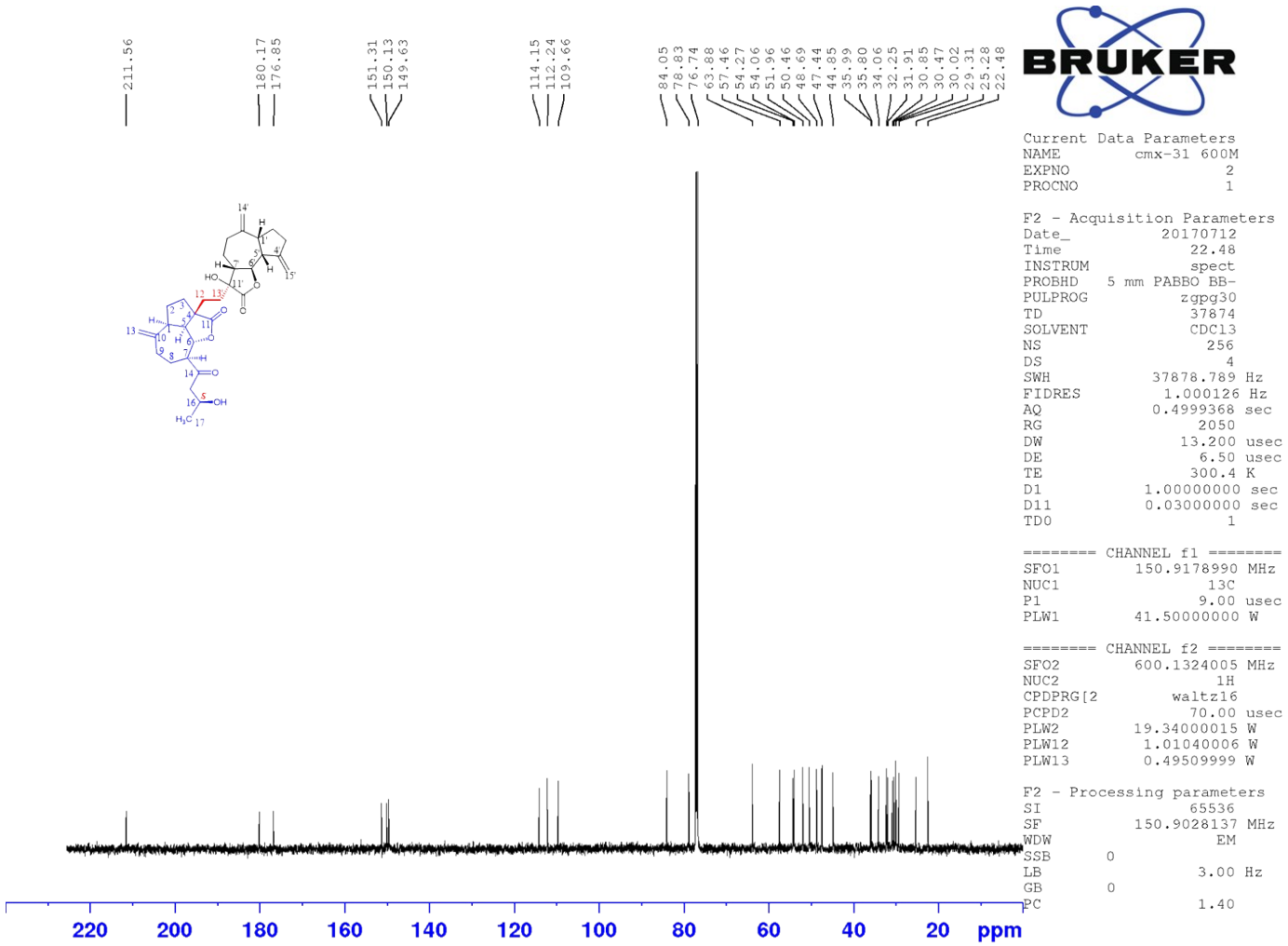


Figure S7. ^{13}C NMR spectrum of Vlasoulide A (1) in $CDCl_3$

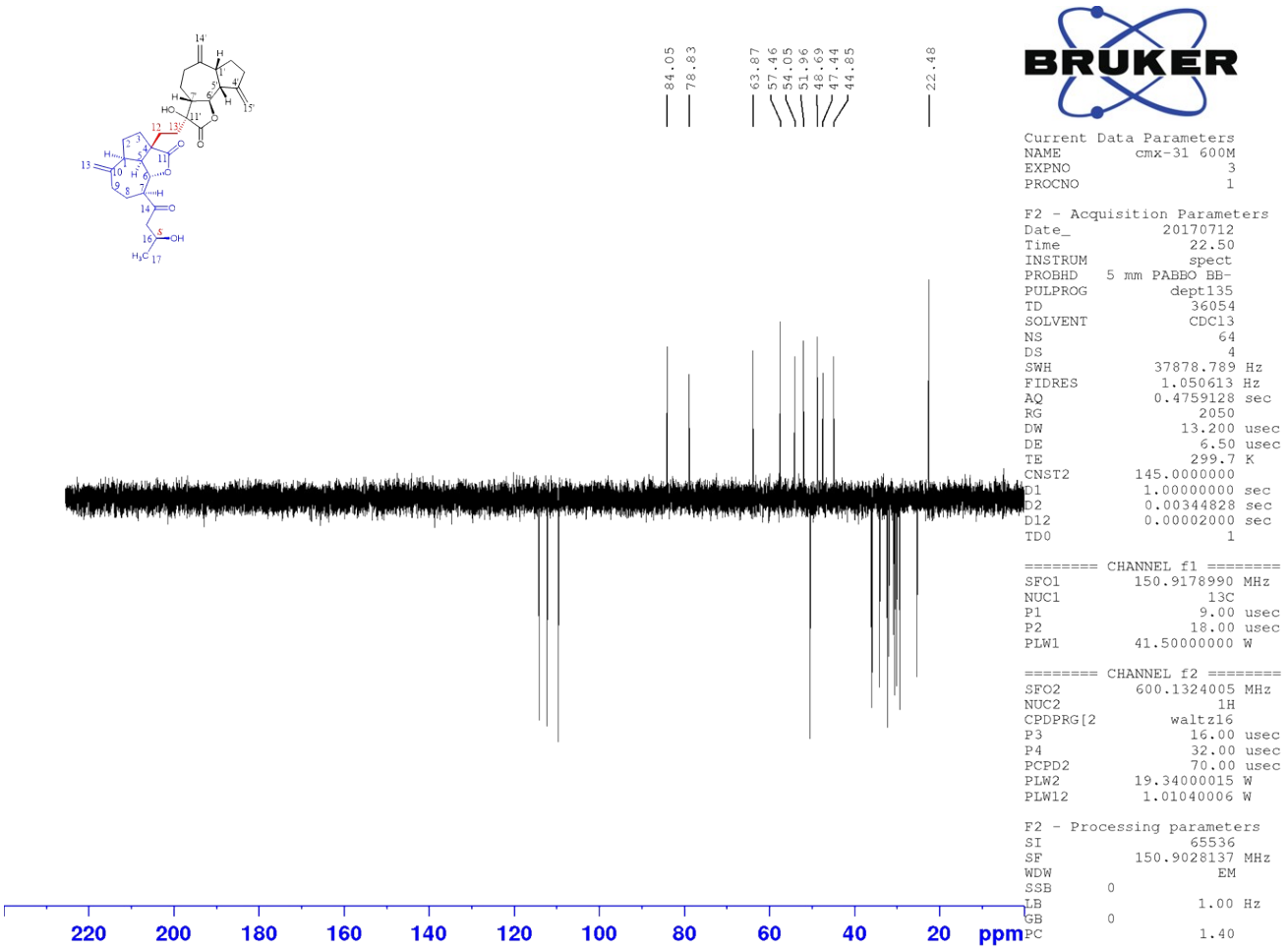
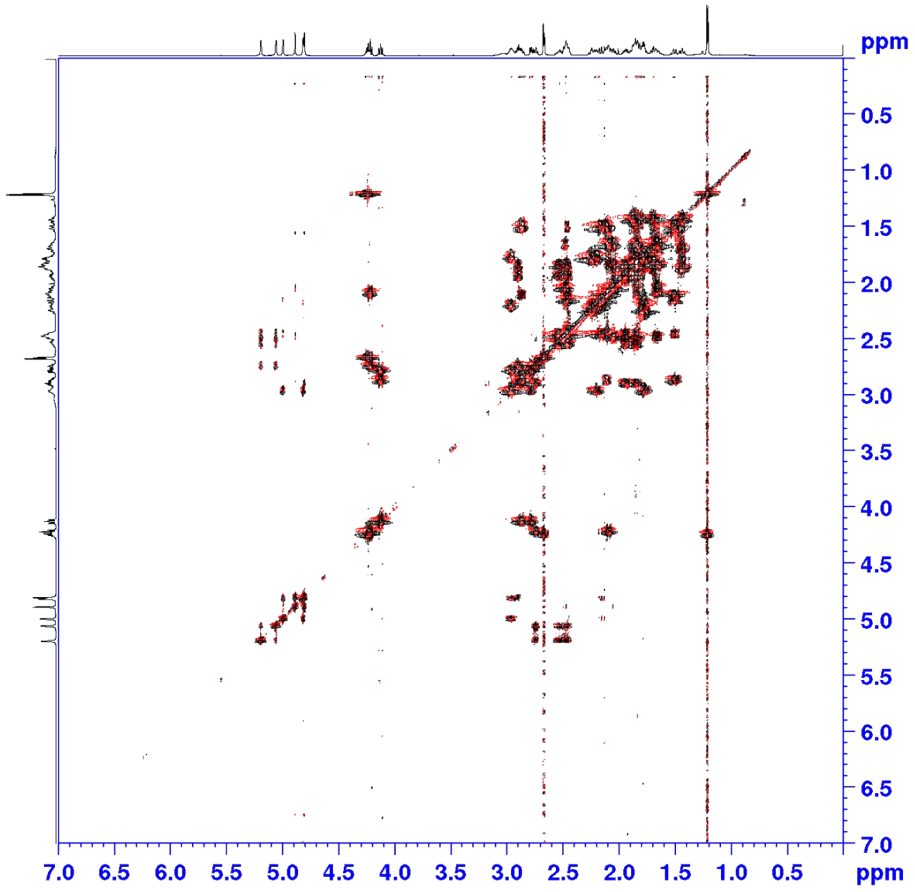


Figure S8. DEPT-135 NMR spectrum of Vlasoulide A (1) in $CDCl_3$

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PROCNO                1

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PULPROG    cosygpmfphpp
TD                3072
SOLVENT           CDCl3
NS                   1
DS                  16
SWH           4795.396 Hz
FIDRES        1.561001 Hz
AQ            0.3203072 sec
RG                2050
DW             104.267 usec
DE               6.50 usec
TE              299.3 K
D0          0.00008686 sec
D1          1.50000000 sec
D11         0.03000000 sec
D12         0.00002000 sec
D16         0.00020000 sec
IN0         0.00020860 sec

======== CHANNEL f1 ========
SFO1        600.1325000 MHz
NUC1                1H
P1                13.70 usec
P2                27.40 usec
P17             2500.00 usec
PLW1        19.34000015 W
PLW10        5.36969995 W

====== GRADIENT CHANNEL =====
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GPZ2              20.00 %
P16             1000.00 usec

F1 - Acquisition parameters
TD                 512
SFO1          600.1325 MHz
FIDRES        18.726030 Hz
SW             7.988 ppm
FnMODE       States-TPPI

F2 - Processing parameters
SI                2048
SF          600.1300107 MHz
WDW               QSINE
SSB                   2
LB        0 Hz
GB        0
PC                1.40

F1 - Processing parameters
SI                2048
MC2          States-TPPI
SF          600.1300107 MHz
WDW               QSINE
SSB                   2
LB        0 Hz
GB        0
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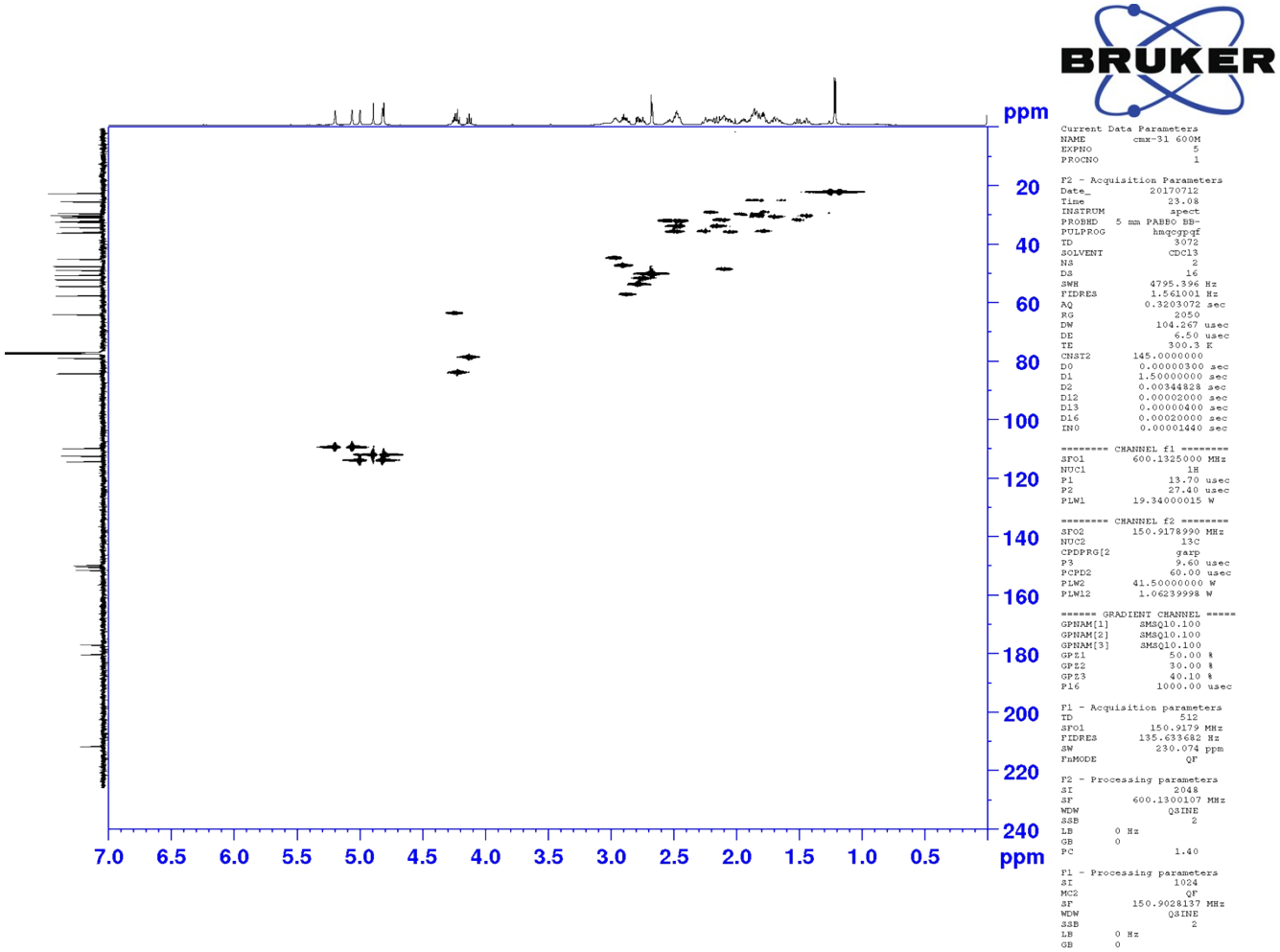
Figure S9. ^{1}H-^{1}H COSY spectrum of Vlasoulide A (1) in $CDCl_3$



BRUKER

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EXPNO                   5
PROCNO                  1

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TD                3072
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NS                   2
DS                  16
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FIDRES         1.561001 Hz
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DW            104.267 usec
DE              6.50 usec
TE               300.3 K
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D1          1.50000000 sec
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======== CHANNEL f1 ========
SFO1        600.1325000 MHz
NUC1                1H
P1                13.70 usec
P2                27.40 usec
PLW1       19.34000015 W

======== CHANNEL f2 ========
SFO2        150.9178990 MHz
NUC2                13C
CPDPRG[2           garp
P3                 9.60 usec
PCPD2             60.00 usec
PLW2       41.50000000 W
PLW12       1.06239998 W

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GPNAM[2]      SMSQ10.100
GPNAM[3]      SMSQ10.100
GPZ1               50.00 %
GPZ2               30.00 %
GPZ3               40.10 %
P16              1000.00 usec

F1 - Acquisition parameters
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SFO1        150.9179 MHz
FIDRES      135.633682 Hz
SW          230.074 ppm
FnMODE              QF

F2 - Processing parameters
SI                 2048
SF          600.1300107 MHz
WDW              QSINE
SSB                 2
LB        0 Hz
GB        0
PC                1.40

F1 - Processing parameters
SI                 1024
MC2                QF
SF          150.9028137 MHz
WDW              QSINE
SSB                 2
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Figure S10. HSQC spectrum of Vlasoulide A (1) in $CDCl_3$

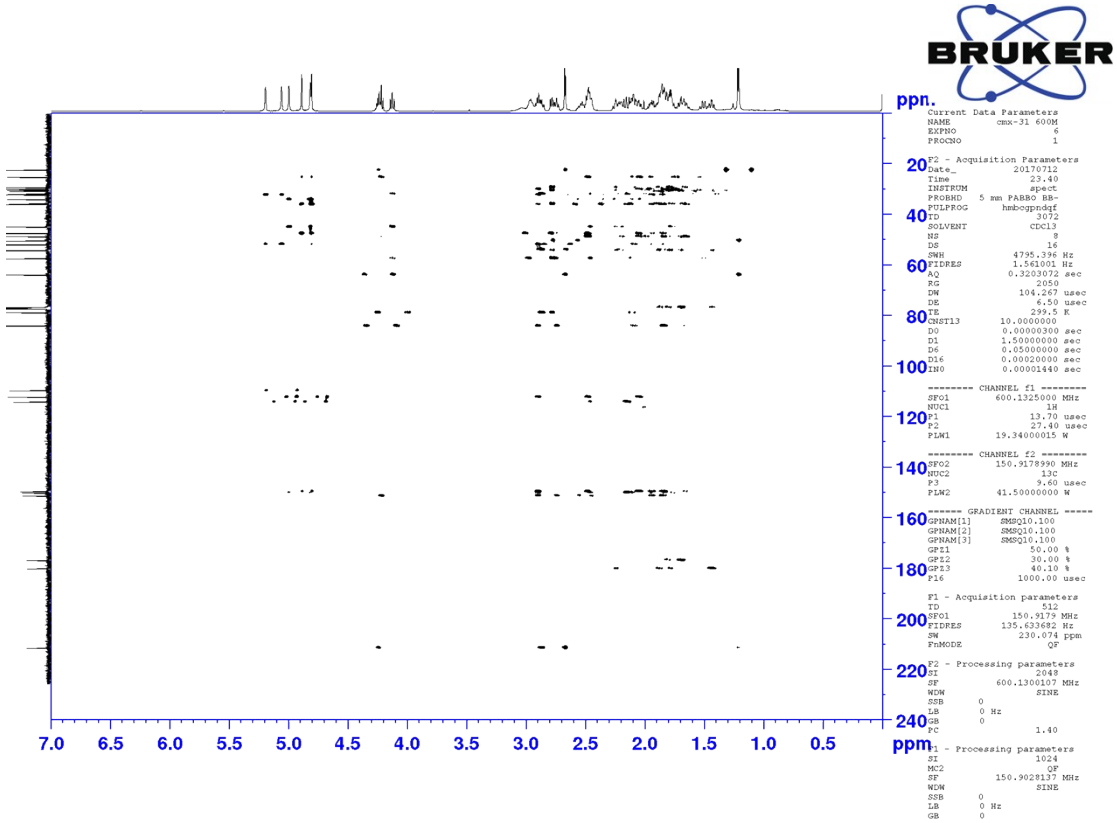
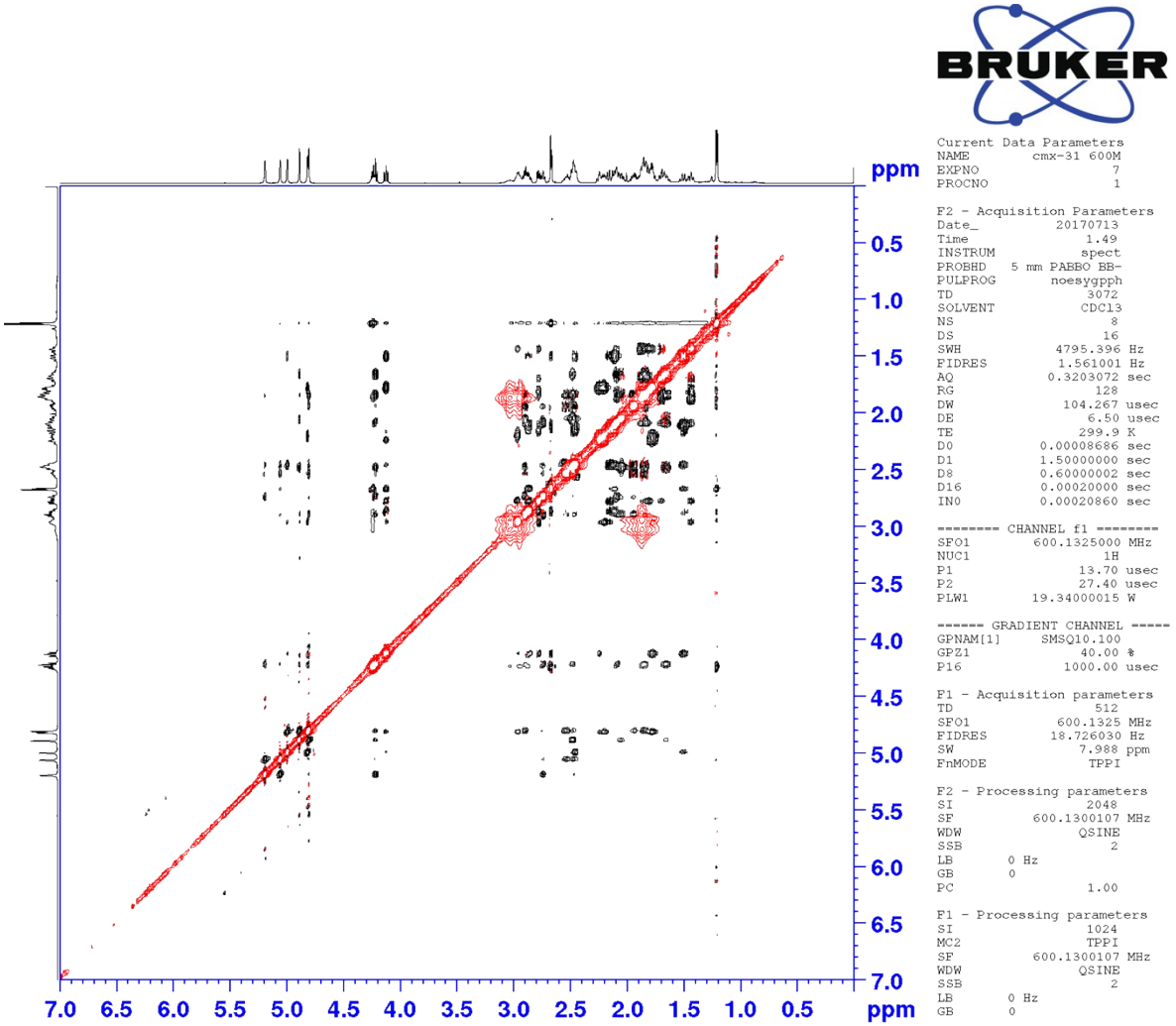


Figure S11. HMBC spectrum of Vlasoulide A (1) in $CDCl_3$



BRUKER

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PROCNO                 1

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PULPROG       noesygpph
TD                 3072
SOLVENT           CDCl3
NS                    8
DS                   16
SWH           4795.396 Hz
FIDRES        1.561001 Hz
AQ            0.3203072 sec
RG                  128
DW              104.267 usec
DE                6.50 usec
TE                299.9 K
D0          0.00008686 sec
D1          1.50000000 sec
D8          0.60000002 sec
D16         0.00020000 sec
IN0         0.00020860 sec

======== CHANNEL f1 ========
SFO1        600.1325000 MHz
NUC1                   1H
P1                 13.70 usec
P2                 27.40 usec
PLW1        19.34000015 W

====== GRADIENT CHANNEL =====
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GPZ1              40.00 %
P16             1000.00 usec

F1 - Acquisition parameters
TD                   512
SFO1          600.1325 MHz
FIDRES       18.726030 Hz
SW               7.988 ppm
FnMODE              TPPI

F2 - Processing parameters
SI                  2048
SF          600.1300107 MHz
WDW                QSINE
SSB                    2
LB        0 Hz
GB        0
PC                  1.00

F1 - Processing parameters
SI                  1024
MC2                 TPPI
SF          600.1300107 MHz
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SSB                    2
LB        0 Hz
GB        0
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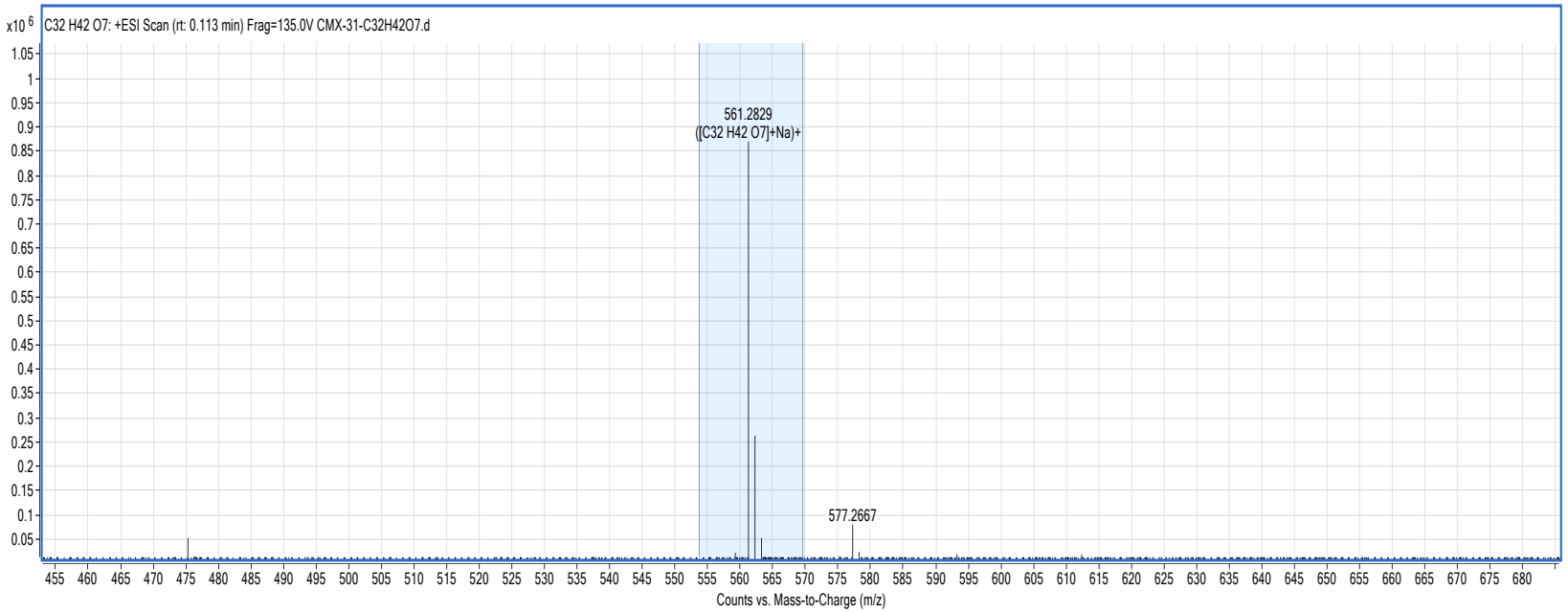
Figure S12. NOESY spectrum of Vlasoulide A (1) in $CDCl_3$



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829908.4	70	100	561.2823	-0.6	870177.4	100	73.4	561.2829

Species	m/z	Score (iso. abund)	Score (mass)	Score (MS)	Score (MFG)	Score (iso. spacing)	Height	Ion Formula
(M+Na)+	561.2829	88.61	98.95	96.12	96.12	99.48	870177.4	C32 H42 Na O7

Figure S13. HRESIMS spectrum of Vlasoulide B (2)

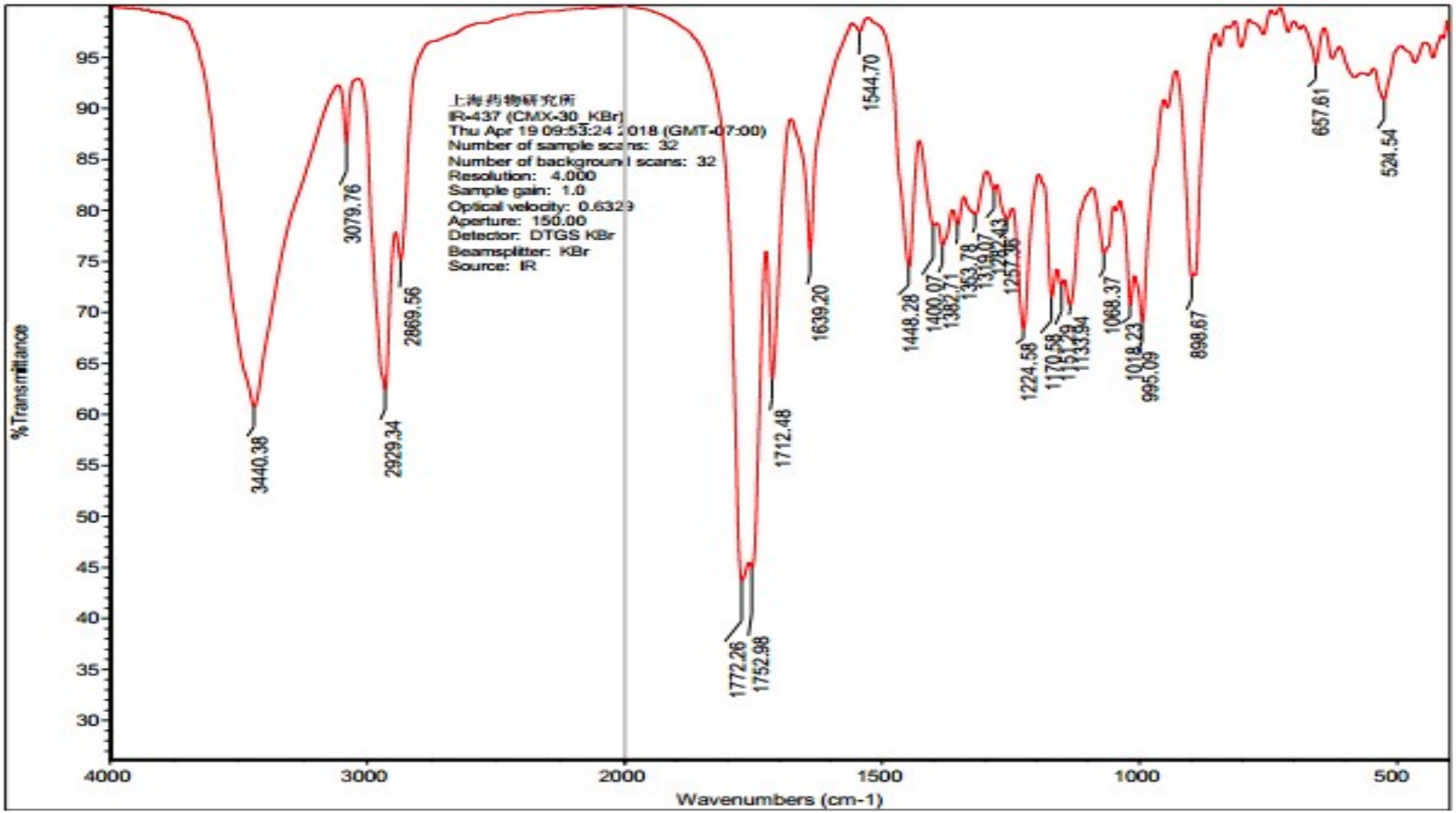

Figure S14. IR spectrum of Vlasoulide B (2)

Rudolph Research Analytical

Friday, 04/20/2018

This sample was measured on an Autopol VI, serial number 90079,
manufactured by Rudolph Research Analytical,Hackettstown,NJ.

LotID : CMX-30
Set Temperature : 20.0
Temp Corr : OFF

n	Average	Std.Dev.	Maximum	Minimum
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S.No	Sample ID	Time	Result	Scale	OR °Arc	WLG	Lg.mm	Conc.	Temp.	Comment
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2	CMX-30	03:58:43 PM	0.000	SR	0.000	589	100.00	0.080	20.2	
3	CMX-30	03:58:51 PM	0.000	SR	0.000	589	100.00	0.080	20.1	
4	CMX-30	03:58:58 PM	0.000	SR	0.000	589	100.00	0.080	20.1	
5	CMX-30	03:59:06 PM	0.000	SR	0.000	589	100.00	0.080	20.1	
6	CMX-30	03:59:14 PM	0.000	SR	0.000	589	100.00	0.080	20.1	

Signature

Figure S15. OR Value of Vlasoulide B (2) in CH_3OH

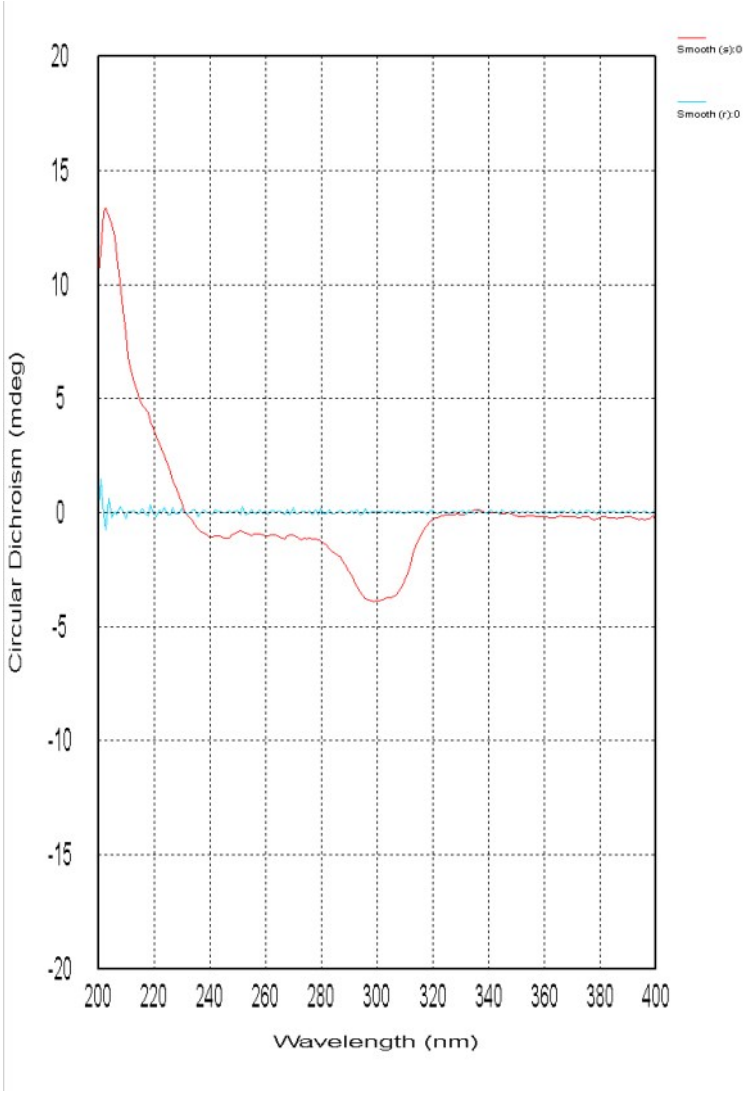


Figure S16. CD Value of Vlasoulide B (2) in CH_3OH

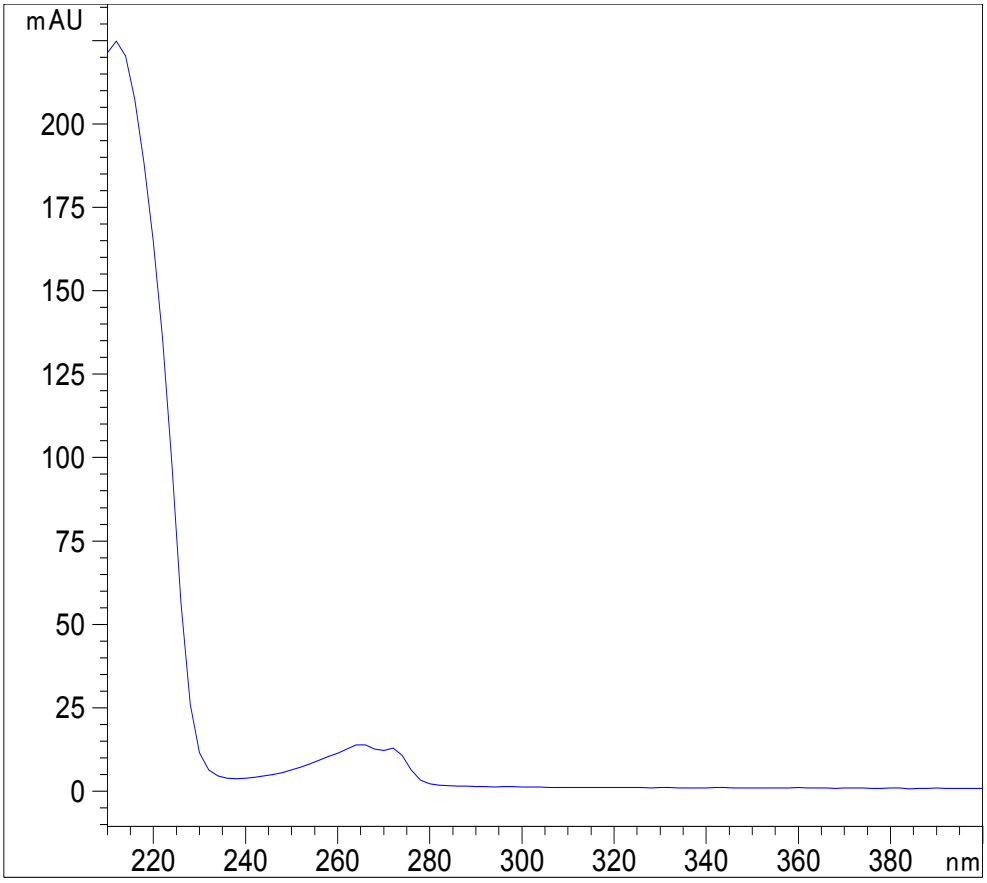


Figure S17. UV spectrum of Vlasoulide B (2) in CH_3CN/H_2O

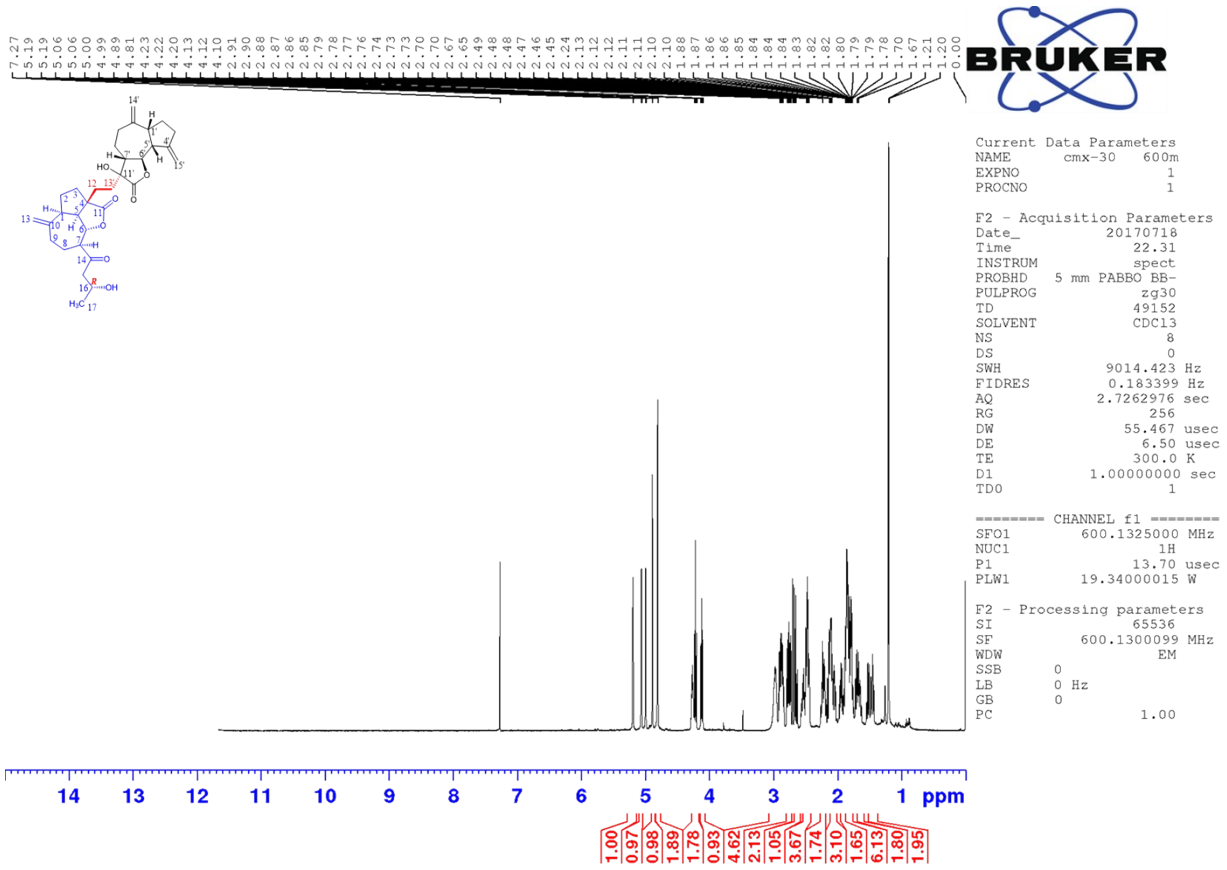


Figure S18. ^{1}H NMR spectrum of Vlasoulide B (2) in $CDCl_3$

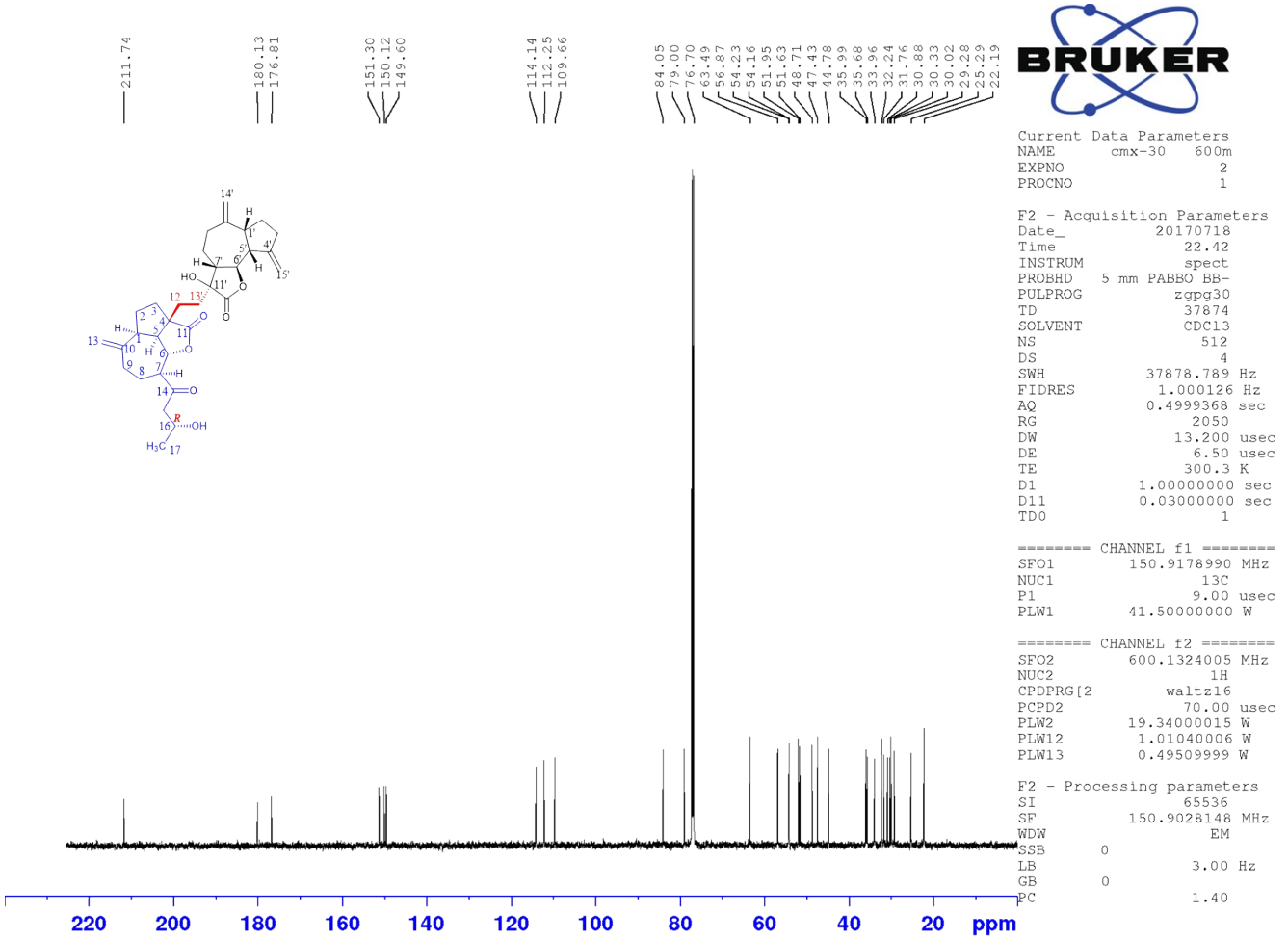


Figure S19. ^{13}C NMR spectrum of Vlasoulide B (2) in $CDCl_3$

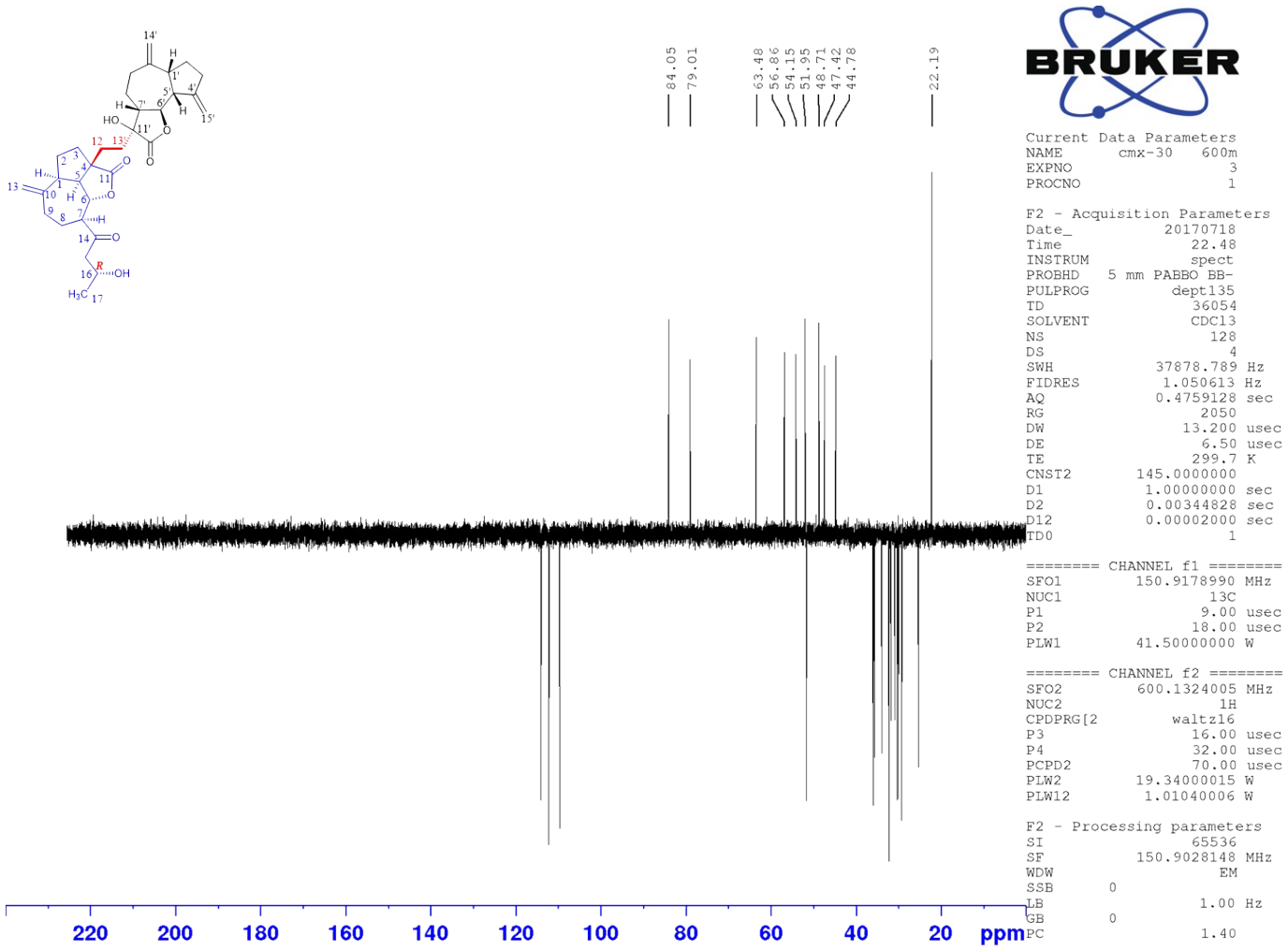
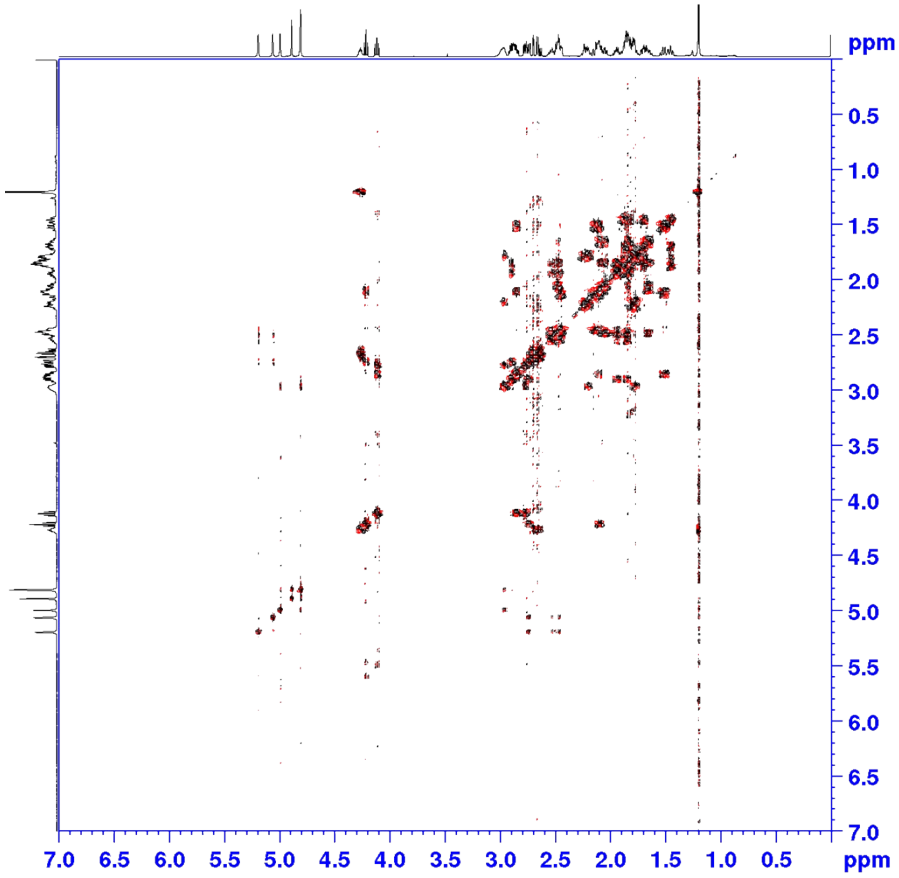


Figure S20. DEPT-135 NMR spectrum of Vlasoulide B (2) in $CDCl_3$



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PROCNO 1

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TD 3072
SOLVENT CDCl3
NS 1
DS 16
SWH 4795.396 Hz
FIDRES 1.561001 Hz
AQ 0.3203072 sec
RG 2050
DW 104.267 usec
DE 6.50 usec
TE 299.3 K
D0 0.00008686 sec
D1 1.50000000 sec
D11 0.03000000 sec
D12 0.00002000 sec
D16 0.00020000 sec
IN0 0.00020860 sec

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NUC1 1H
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P2 27.40 usec
P17 2500.00 usec
PLW1 19.34000015 W
PLW10 5.36969995 W

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GPZ2 20.00 %
P16 1000.00 usec

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FIDRES 18.726030 Hz
SW 7.988 ppm
FnMODE States-TPPI

F2 - Processing parameters
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WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 2048
MC2 States-TPPI
SF 600.1300099 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

Figure S21. ^{1}H-^{1}H COSY spectrum of Vlasoulide B (2) in $CDCl_3$

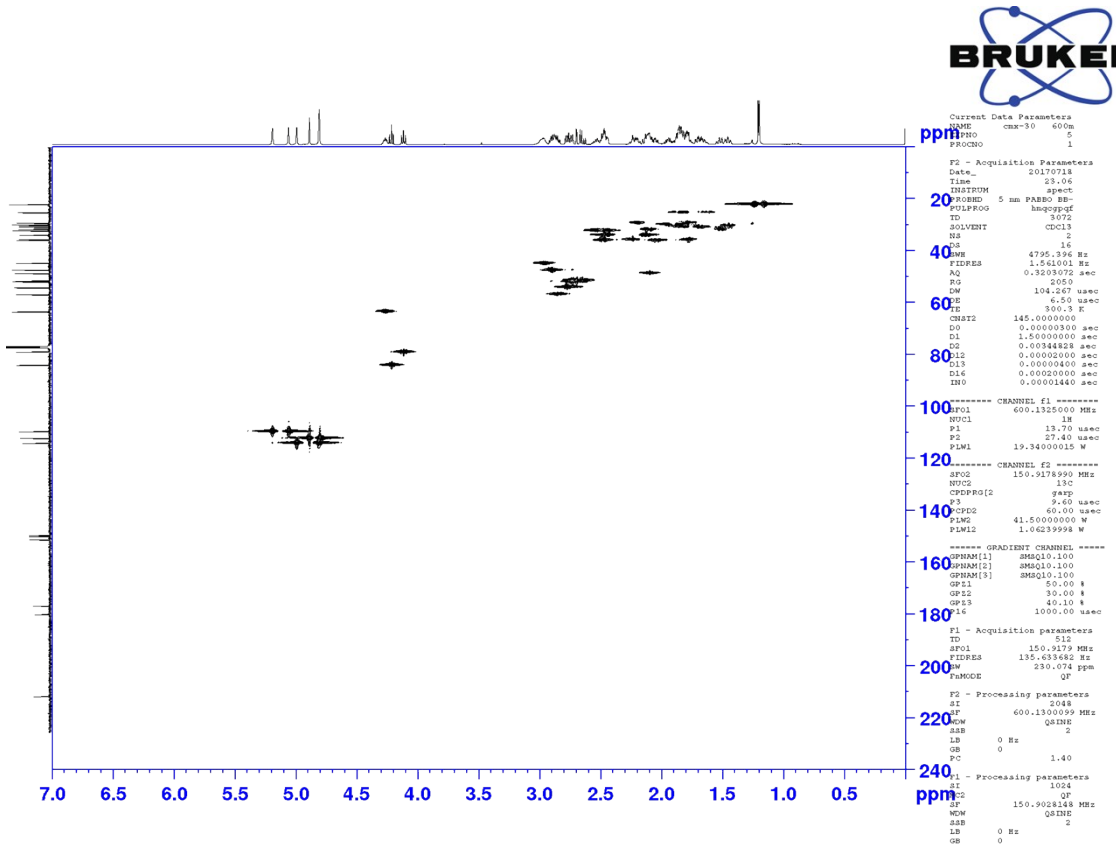


Figure S22. HSQC spectrum of Vlasoulide B (2) in $CDCl_3$

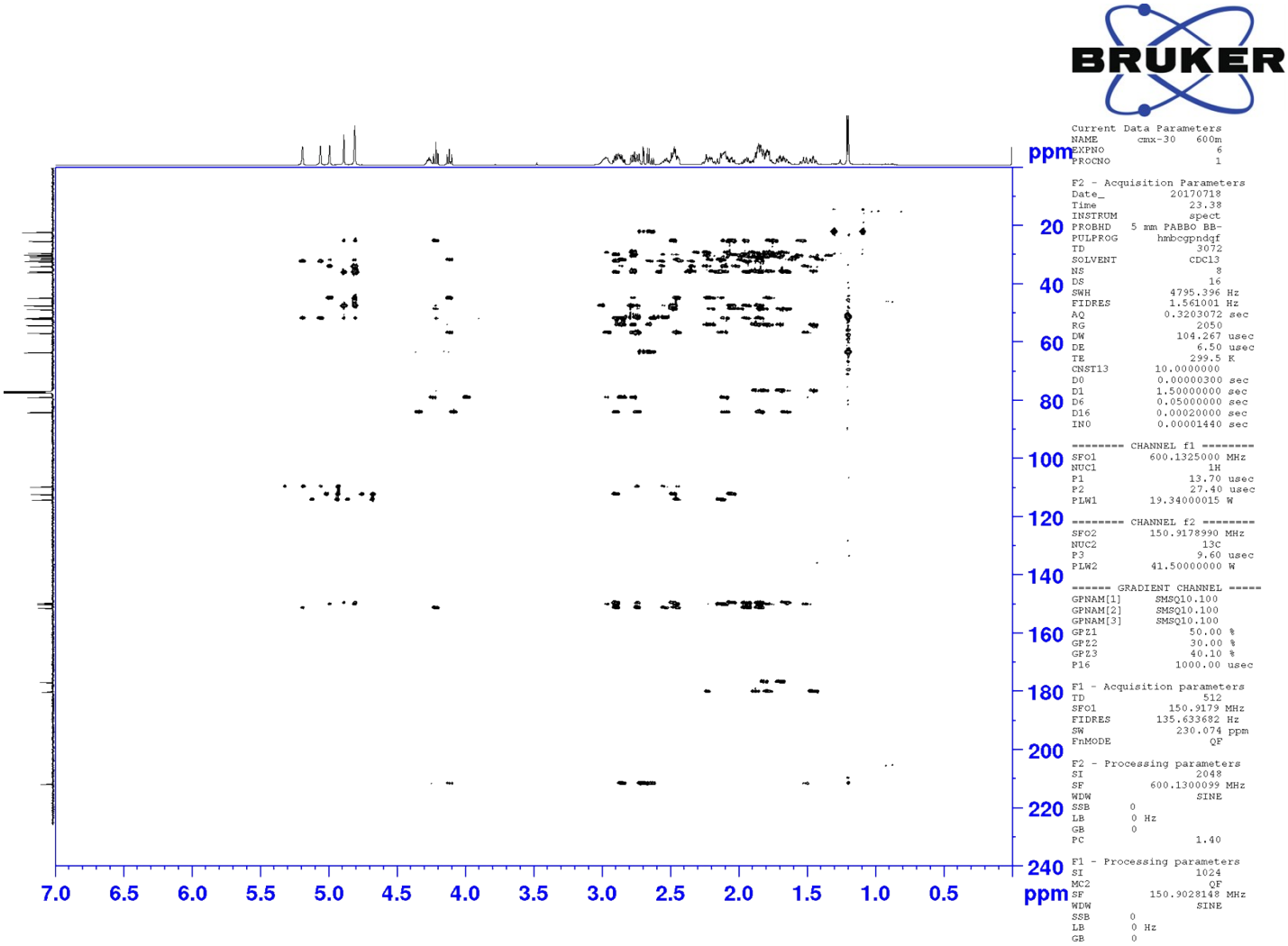
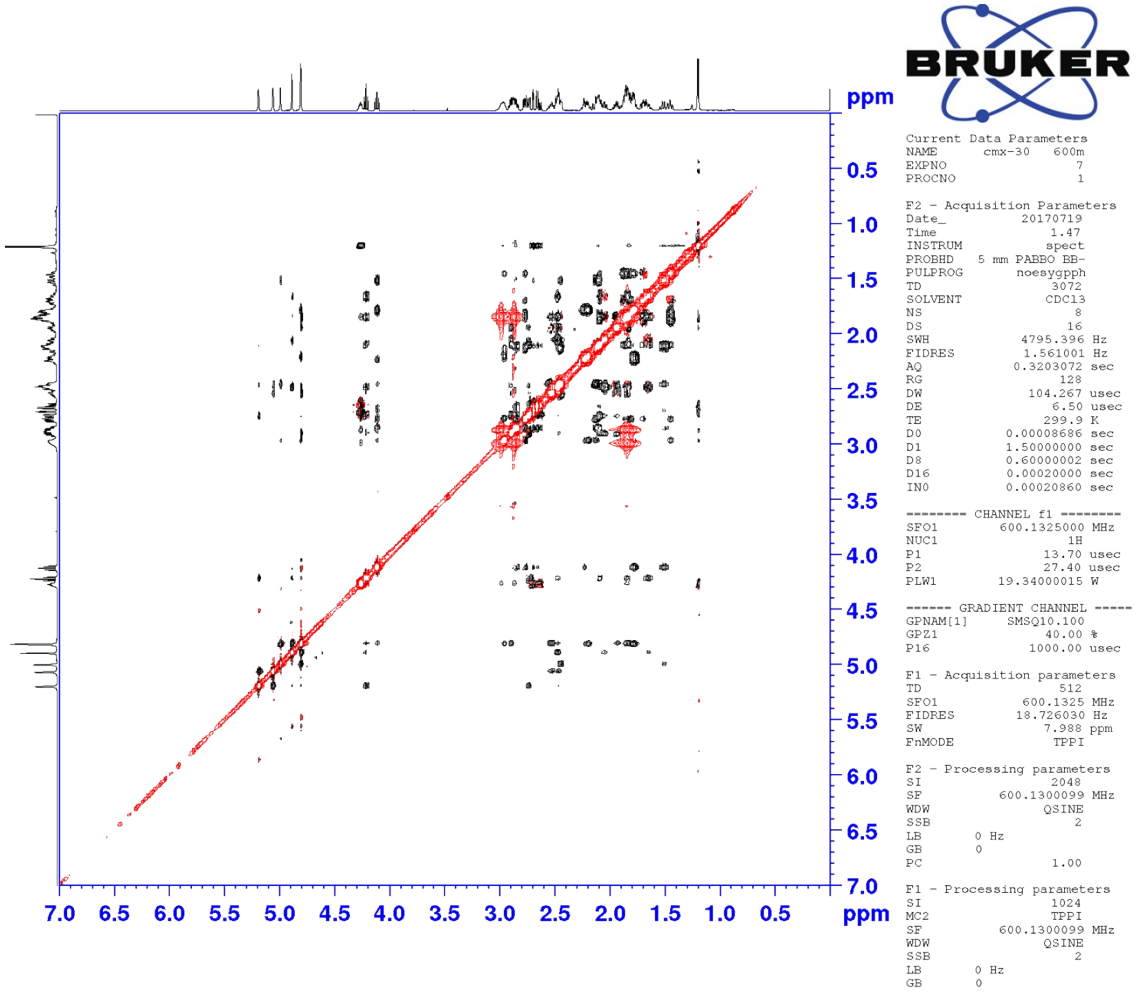


Figure S23. HMBC spectrum of Vlasoulide B (2) in $CDCl_3$



BRUKER

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TD                3072
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NS                   8
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FIDRES        1.561001 Hz
AQ           0.3203072 sec
RG                 128
DW            104.267 usec
DE              6.50 usec
TE              299.9 K
D0          0.00008686 sec
D1          1.50000000 sec
D8          0.60000002 sec
D16         0.00020000 sec
IN0         0.00020860 sec

======== CHANNEL f1 ========
SFO1       600.1325000 MHz
NUC1               1H
P1                13.70 usec
P2                27.40 usec
PLW1       19.34000015 W

====== GRADIENT CHANNEL =====
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GPZ1              40.00 %
P16             1000.00 usec

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SFO1          600.1325 MHz
FIDRES       18.726030 Hz
SW              7.988 ppm
FnMODE            TPPI

F2 - Processing parameters
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WDW               QSINE
SSB                  2
LB        0 Hz
GB        0
PC                1.00

F1 - Processing parameters
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SSB                  2
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GB        0
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Figure S24. NOESY spectrum of Vlasoulide B (2) in $CDCl_3$

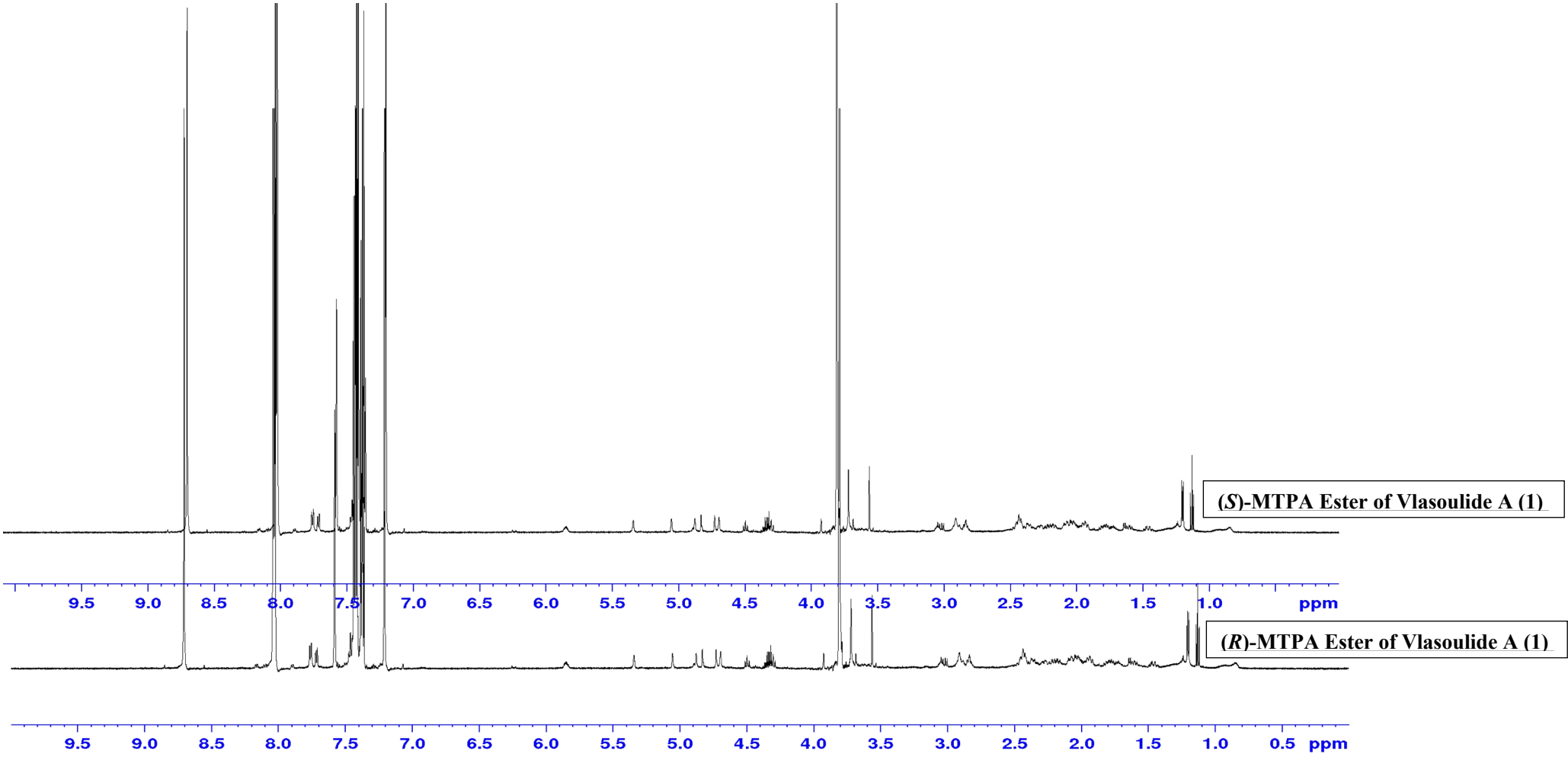

Figure S25. (*R*)and (*S*)-MTPA Esters of Vlasoulide A (1)

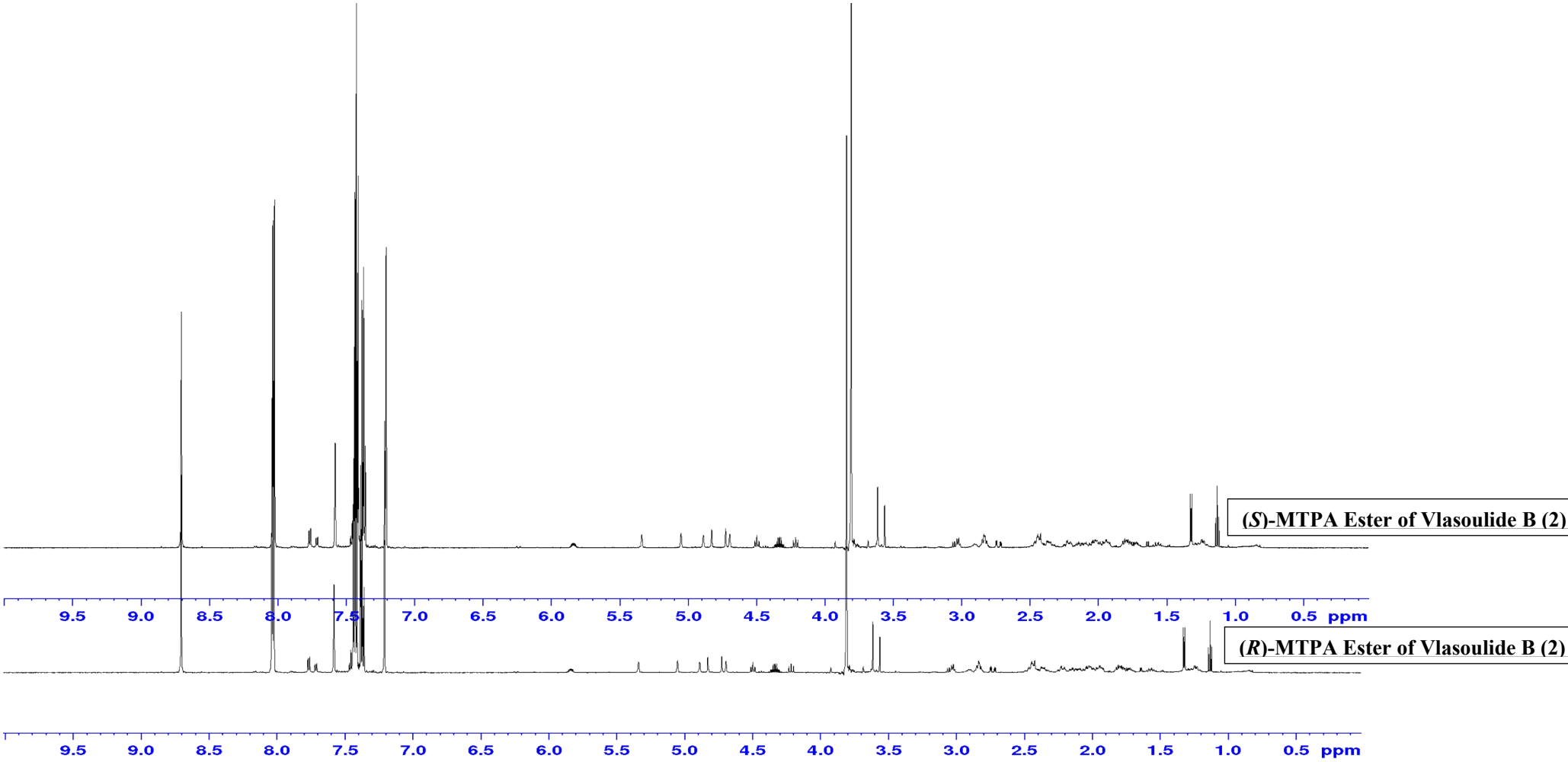

Figure S26. (*R*)and (*S*)-MTPA Esters of Vlasoulide B (2)