

## Supporting Information

*Rec. Nat. Prod.* X:X (201X) XX-XX

### A new ursane-type triterpene from the roots of *Salvia miltiorrhiza* Bunge

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## **S1:** General procedures

Optical rotations were measured with a DIP-360 digital polarimeter (JASCO, Easton, USA). NMR spectra were recorded on a JEOL ECX 400 FT-NMR spectrometer (JEOL, Japan) and Bruker Avance 500 NMR spectrometer (BrukerSpin, Germany) at room temperature using standard pulse program, with tetramethylsilane as the internal standard and chemical shift values were expressed in  $\delta$  (ppm). ESI-MS experiments employed an Agilent 1260 TripleQuad-6420 LC-MS/MS (Agilent Technologies, USA). HR-ESI-MS experiments employed a JEOL AccuTOF™ LC 1100 mass spectrometer (JEOL, Tokyo, Japan). Column chromatography was performed on silica gel 60 (230–400 mesh, Nacalai Tesque Inc., Kyoto, Japan) and YMC ODS-A gel (50  $\mu\text{m}$ , YMC Co. Ltd., Kyoto, Japan). TLC was performed on Kieselgel 60 F<sub>254</sub> and TLC Silica gel 60 RP-18 F<sub>254S</sub> (Merck, Damstadt, Germany) plates. Spots were visualized by spraying with 1% Ce(SO<sub>4</sub>)<sub>2</sub>-10% aqueous H<sub>2</sub>SO<sub>4</sub> solution, followed by heating.

## S2: Extraction and isolation

The air-dried danshen roots (550 g) were sliced and then extracted with EtOH-H<sub>2</sub>O (80:20, v/v) (1.5 L × 3 times) at 40 °C under sonication. After removal of solvent, the obtained residue (122.3 g) was suspended in water (500 mL) and successively partitioned with hexane, EtOAc, and *n*-BuOH (each 500 mL × 3) to obtain soluble fractions of hexane (8.5 g), EtOAc (35.8 g), and BuOH (26.2 g).

The EtOAc portion was subjected to a silica gel column (Φ85 mm × 90 mm) with a stepwise gradient of hexane-EtOAc (5:1→0:1, v/v) to give seven fractions (F1 ~ F7). The fraction F5 (8.3 g) was further chromatographed over a silica gel column (Φ50 mm × 350 mm) with the eluent of CHCl<sub>3</sub>-MeOH (15:1, v/v) to afford six sub fractions (F5.1~F5.6). The fraction F5.5 (210 mg) was then chromatographed on a reversed-phase C<sub>18</sub> column (Φ20 mm × 400 mm) with MeOH-H<sub>2</sub>O (7:2, v/v) to furnish **1** (11 mg). Likewise, the fraction F5.6 (370 mg) was chromatographed on a reversed-phase C<sub>18</sub> column (Φ30 mm × 400 mm) with MeOH-H<sub>2</sub>O (1:1, v/v) to yield **2** (9 mg).

### **S3: Antiproliferative assay**

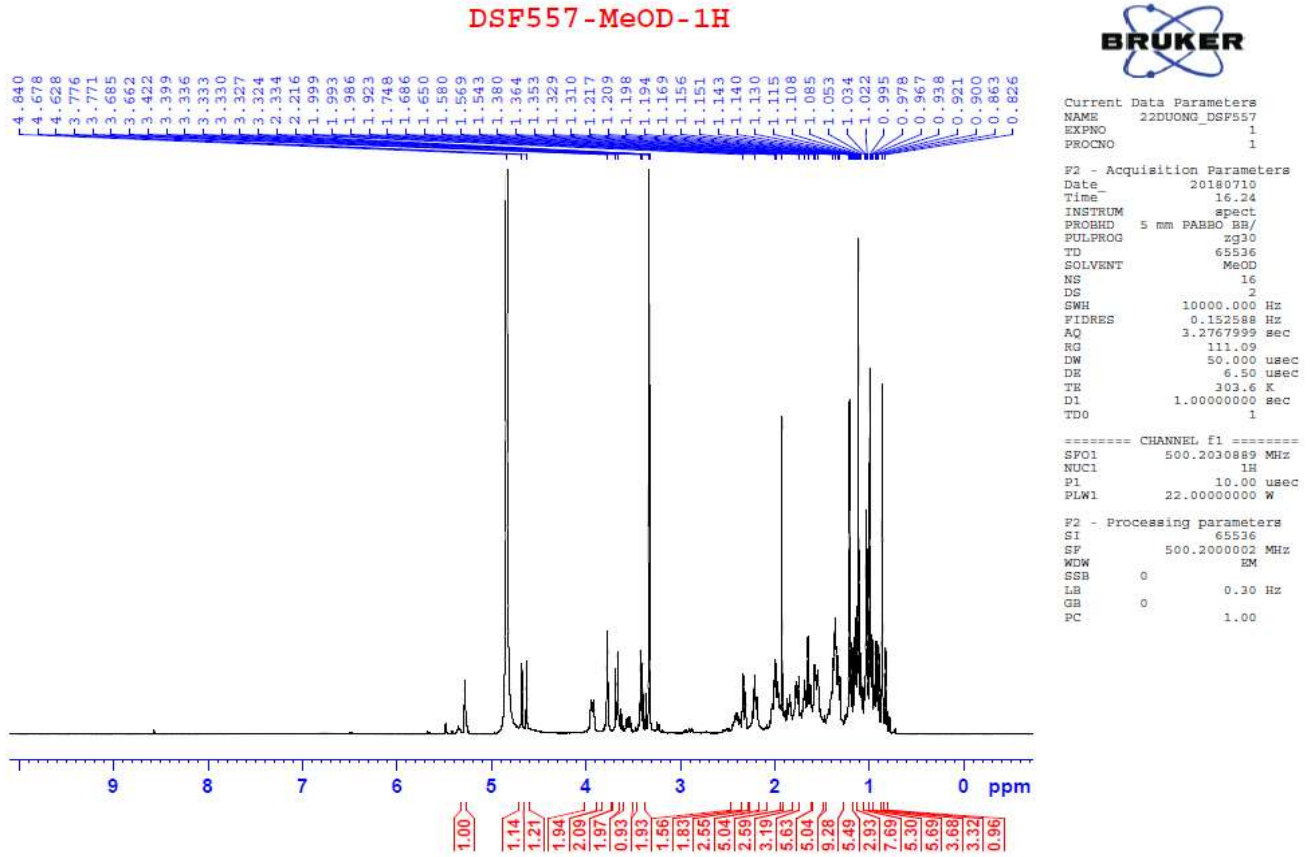
#### *Cell culture and sample treatment*

The HL-60 cell line was obtained from RIKEN BioResource Center Cell Bank. The cells were maintained in RPMI1640 medium. The medium was supplemented with 10% FBS and 1% penicillin–streptomycin and were then incubated at 37°C under 5% CO<sub>2</sub> in fully humidified conditions. For the cell treatment, DMSO concentrations in the cell culture medium did not exceed 0.2% (v/v) and the controls were always treated with the same amount of DMSO as used in the corresponding experiments.

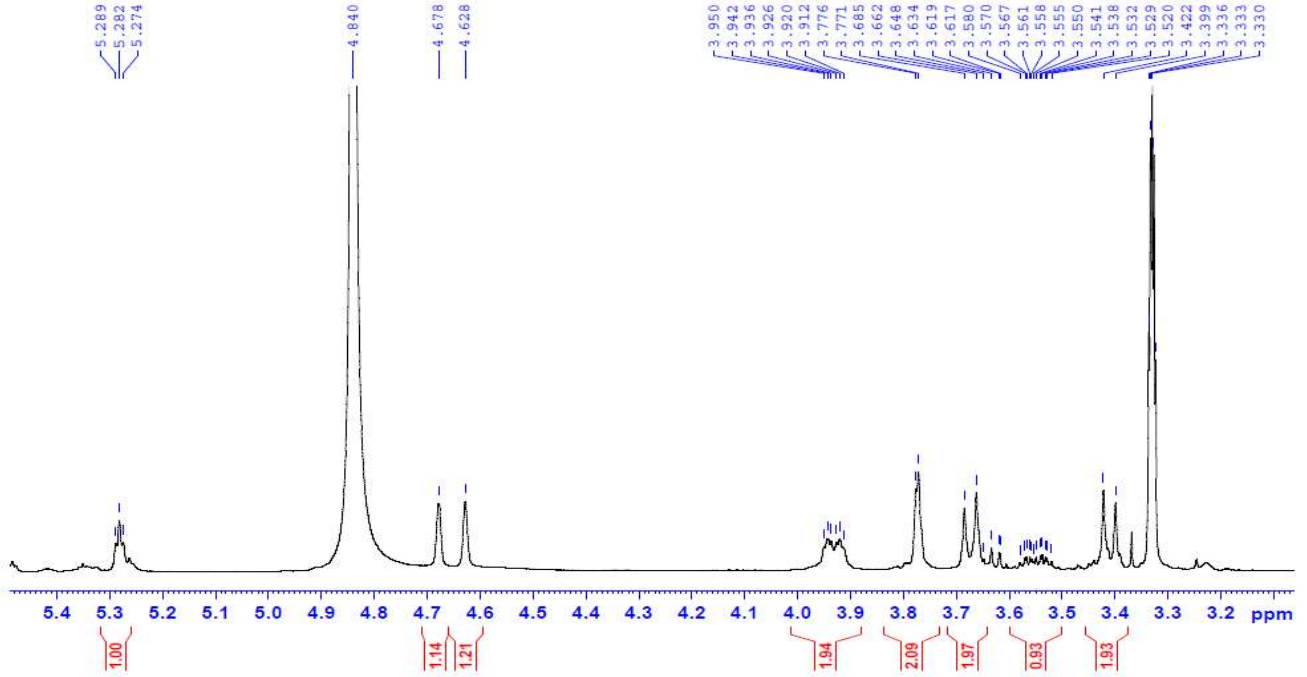
#### *MTT assay*

Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In brief, the cells were seeded in 96-well plates at a density of  $1 \times 10^4$  cells/well for suspension cells. After incubation for 24 h, the cells were treated with each sample at various concentrations for 24 h. At the end of treatment, MTT solution was added to each well, and the cells were incubated for another 4 h. The precipitated MTT-formazan was dissolved with 0.04 N HCl-isopropanol, and the amount of formazan was measured at 595 nm using a microplate reader (iMark, BioRad, Tokyo, Japan). Cell viability was expressed as a percentage of the control culture.

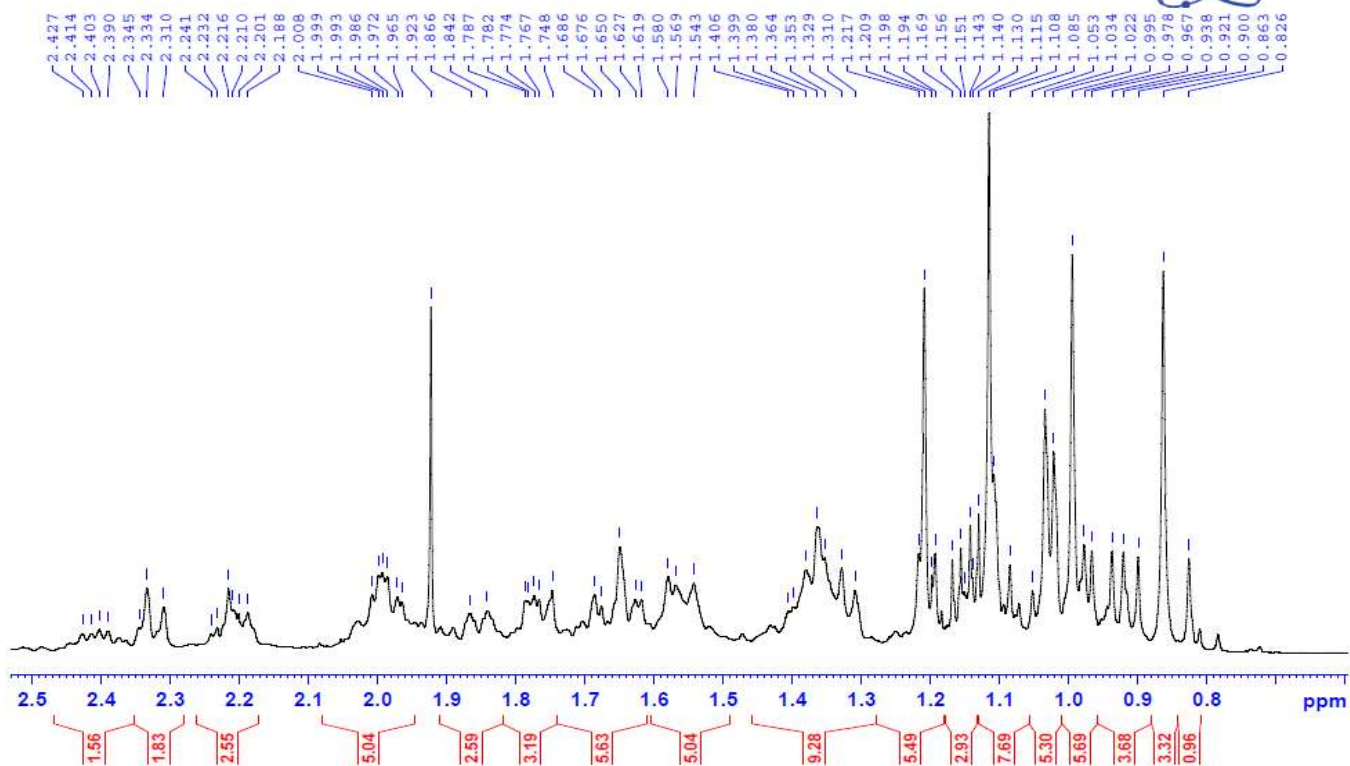
S4: The  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ ) spectrum of 2



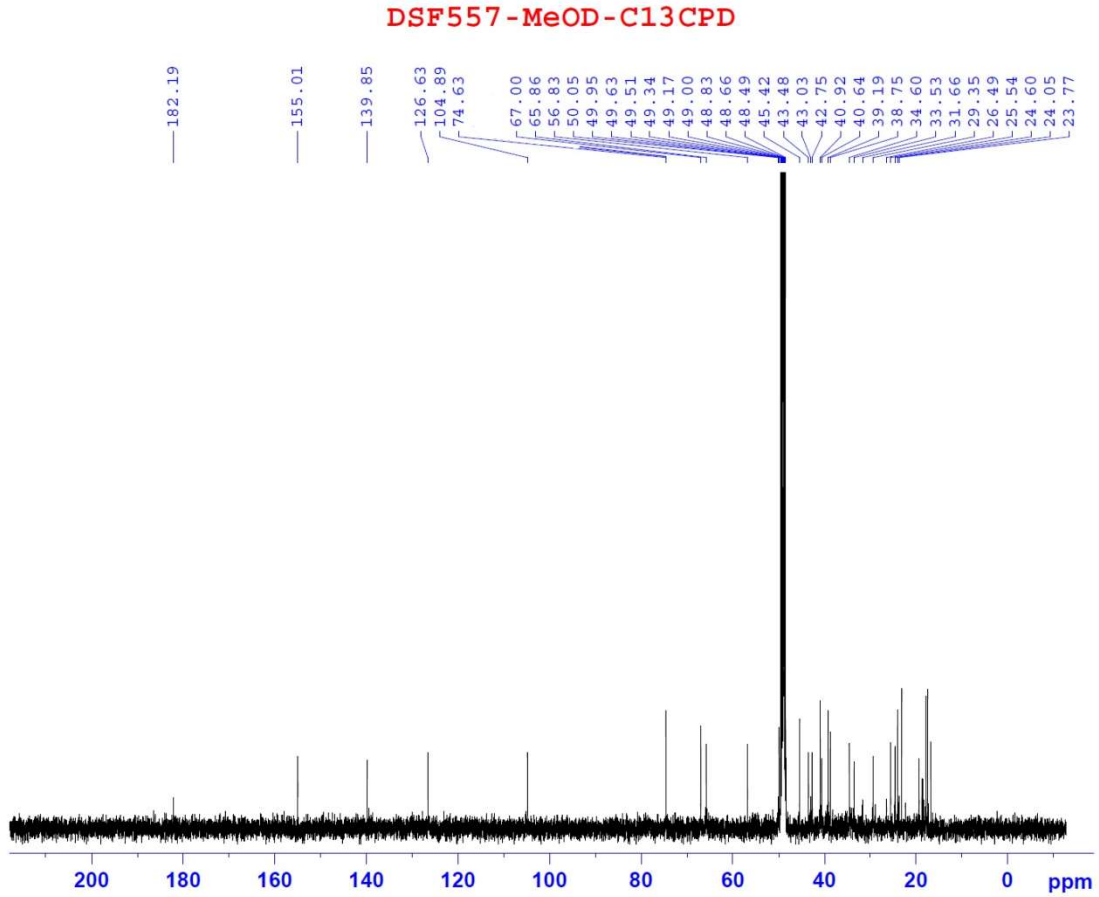
DSF557-MeOD-1H



DSF557-MeOD-1H



S5: The  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ ) spectrum of 2



```

Current Data Parameters
NAME      22DUONG_DS557
EXPNO     2
PROCNO    1

F2 - Acquisition Parameters
Date_     20180712
Time      14.38
INSTRUM   spect
PROBHD    5 mm PABBO BB/
PULPROG   zgpg30
TD         65536
SOLVENT   MeOD
NS         2048
DS         4
SWH        31250.000 Hz
FIDRES     0.476837 Hz
AQ         1.0485760 sec
RG         198.57
DW         16.000 usec
DE         6.50 usec
TE         304.2 K
D1         2.00000000 sec
D11        0.03000000 sec
TD0        1

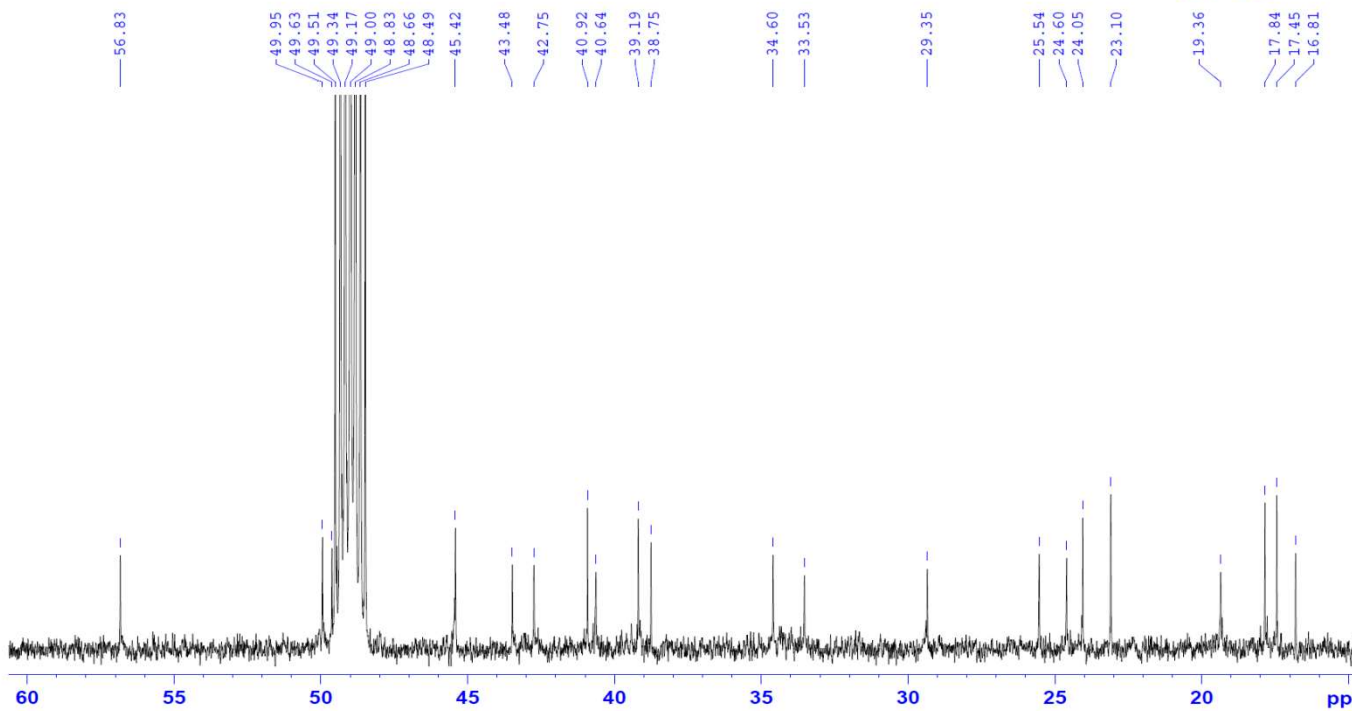
===== CHANNEL f1 =====
SFO1      125.7892253 MHz
NUC1       13C
P1         10.00 usec
PLW1      88.00000000 W

===== CHANNEL f2 =====
SFO2      500.2020008 MHz
NUC2       1H
CPDPRG[2] waltz16
PCPD2     80.00 usec
PLW2      22.00000000 W
PLW12     0.34375000 W
PLW13     0.22000000 W

F2 - Processing parameters
SI         32768
SF         125.7752143 MHz
WDW        EM
SSB        0
LB         1.00 Hz
GB         0
PC         1.40
    
```



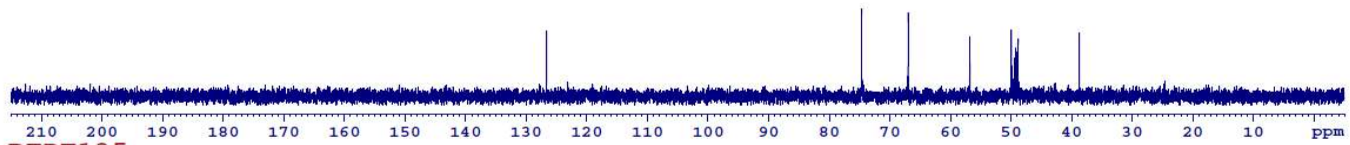
DSF557-MeOD-C13CPD



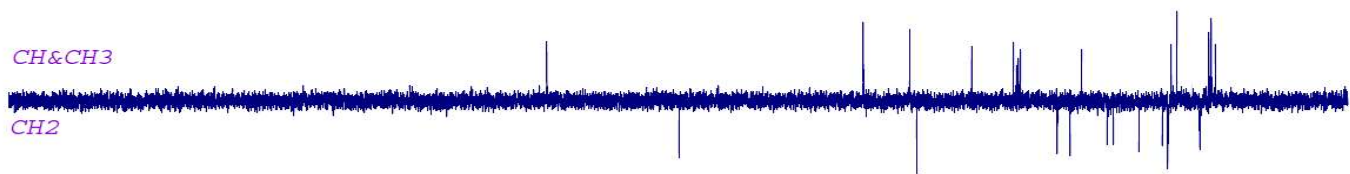
S6: The DEPT spectrum of 2

DSF557 - MeOD - C13CPD&DEPT

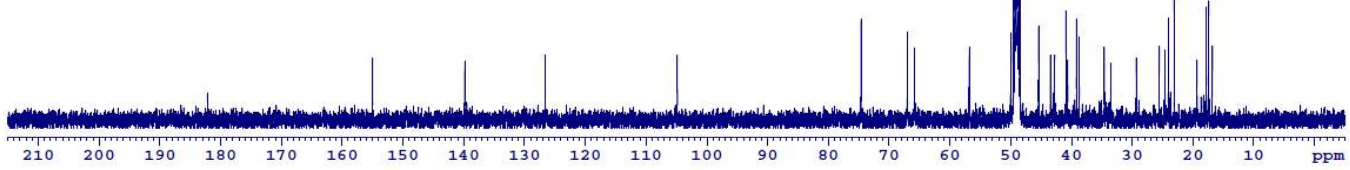
DEPT90



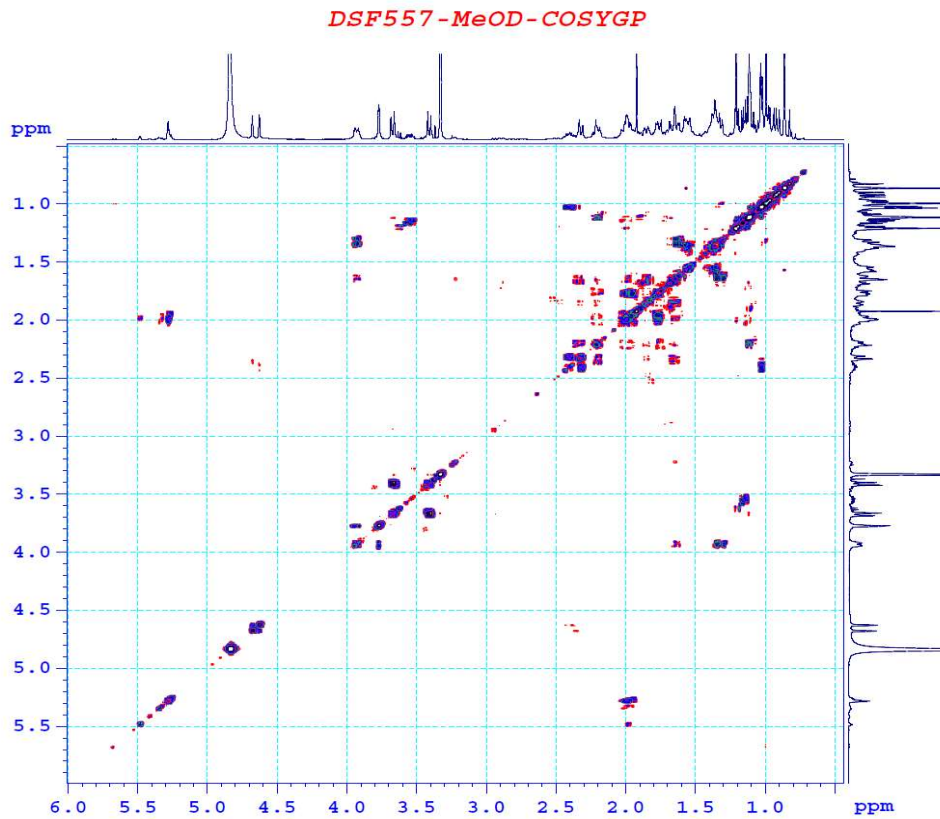
DEPT135



C13CPD



S7: The H-H COSY spectrum of 2



```

Current Data Parameters
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EXPNO     7
PROCNO    1

F2 - Acquisition Parameters
Date_     20180714
Time      1.44
INSTRUM   spect
PROBHD    5 mm PABBO BB/
PULPROG   cosygpgpgf
TD         2048
SOLVENT    MeOD
NS         2
DS         8
SWH        2732.240 Hz
FIDRES     1.334102 Hz
AQ         0.3747340 sec
RG         57.21
DW         183.000 usec
DE         6.50 usec
TE         303.7 K
D0         0.00000300 sec
D1         1.77840602 sec
D11        0.03000000 sec
D12        0.00002000 sec
D13        0.00000400 sec
D16        0.00020000 sec
IN0        0.00036600 sec

----- CHANNEL f1 -----
SFO1      500.2015076 MHz
NUC1       1H
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P1         10.00 usec
P17        2500.00 usec
PLW1       22.00000000 W
PLW10      3.25440001 W

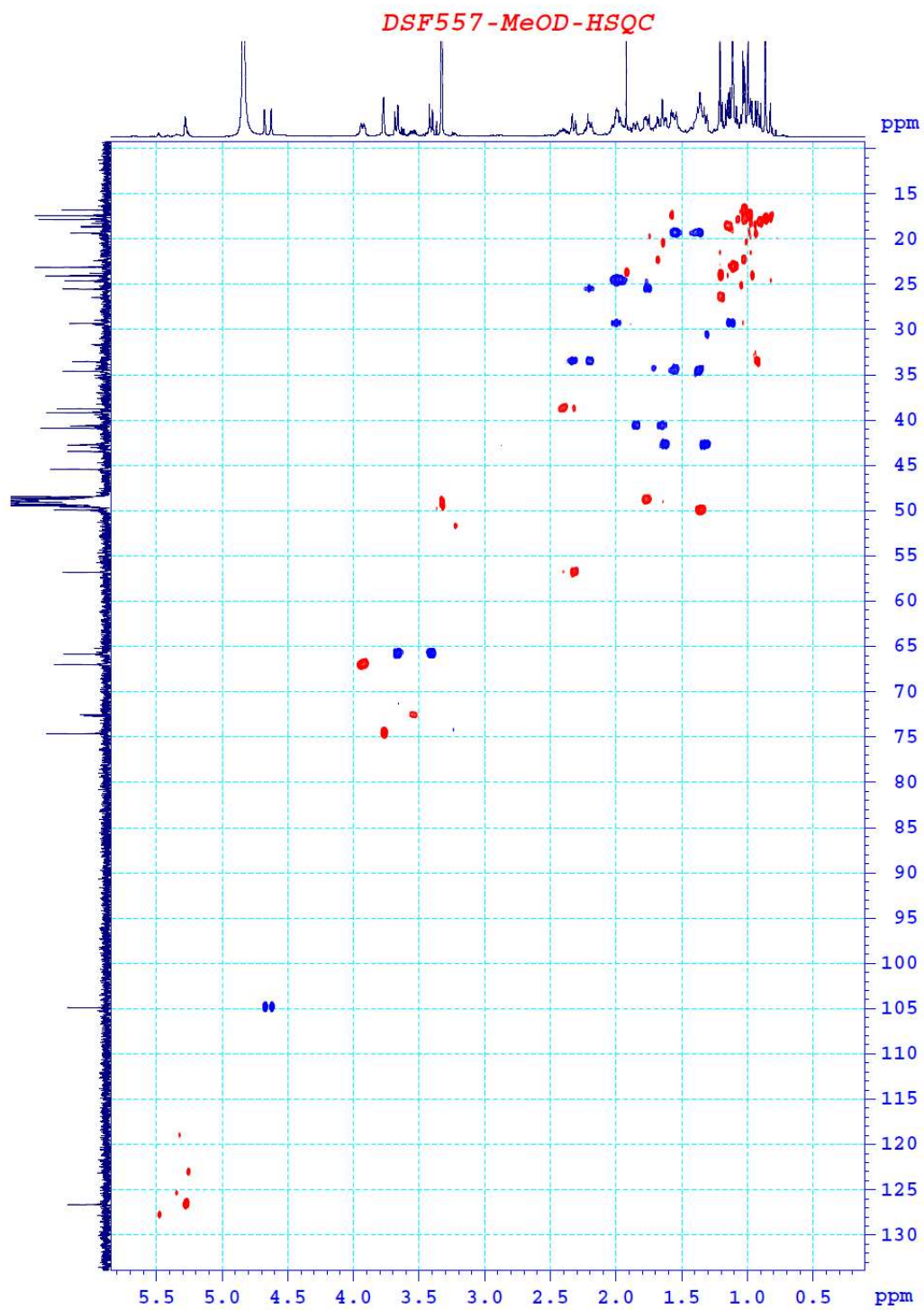
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GFZ1       10.00 %
P16        1000.00 usec

F1 - Acquisition parameters
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SFO1      500.2015 MHz
FIDRES     21.345629 Hz
SW         5.462 ppm
FhMODE     QF

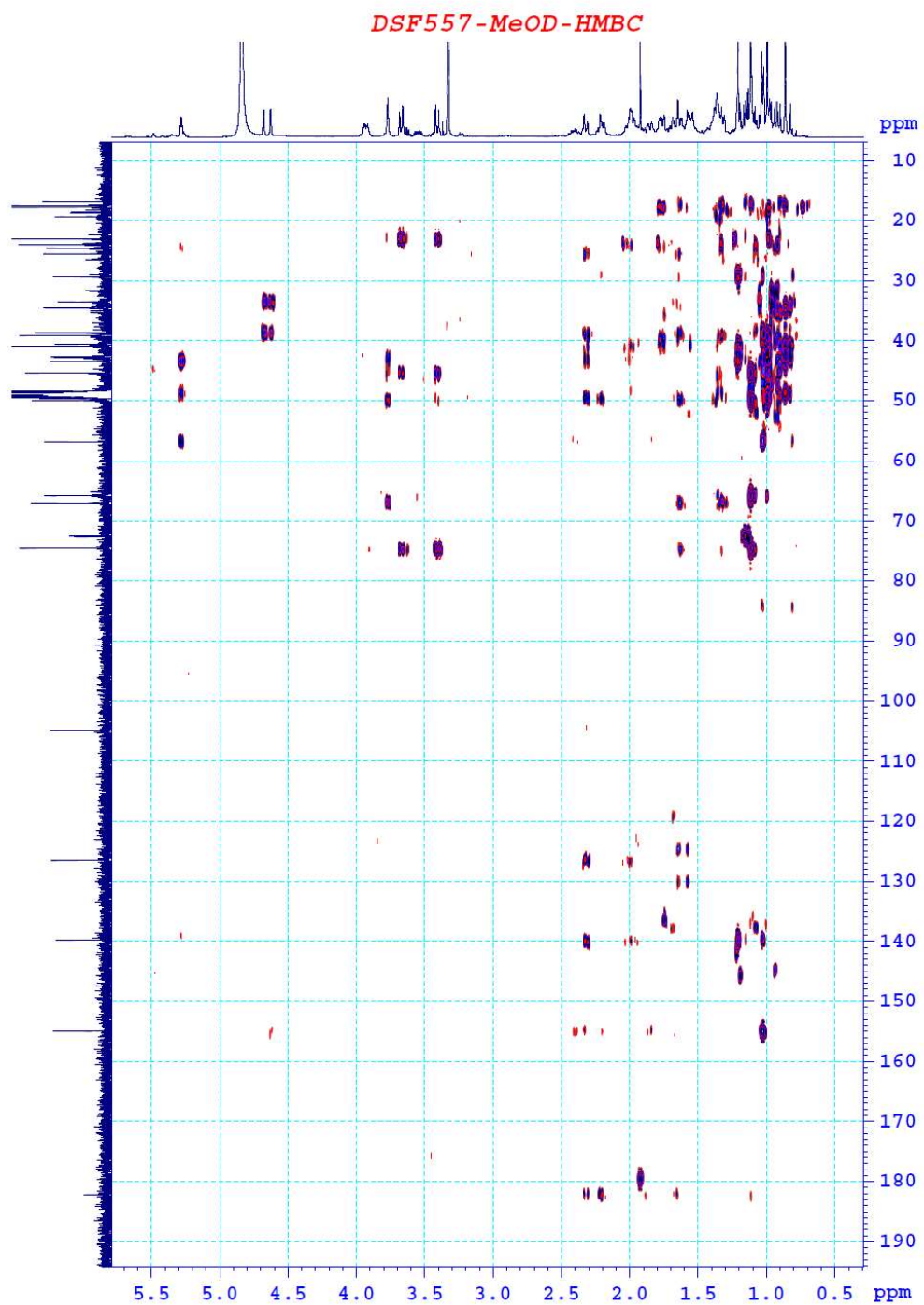
F2 - Processing parameters
SI         1024
SF         500.2000000 MHz
WDW        QSINE
SFB        0
LB         0 Hz
GB         0
PC         1.40

F1 - Processing parameters
SI         1024
SF         500.2000000 MHz
WDW        QSINE
SFB        0
LB         0 Hz
GB         0
    
```

S8: The HSQC spectrum of 2



S9: The HMBC spectrum of 2





S10: The NOESY spectrum of 2

DSF557-MeOD-NOESY



Current Data Parameters  
 NAME 22DUONG\_DS557  
 EXPNO 9  
 PROCNO 1

F2 - Acquisition Parameters  
 Date\_ 20180714  
 Time 1.55  
 INSTRUM spect  
 PROBHD 5 mm PABBO BB/  
 PULPROG noesygpph  
 TD 2048  
 SOLVENT MeOD  
 NS 8  
 DS 32  
 SWH 2732.240 Hz  
 FIDRES 1.334102 Hz  
 AQ 0.3747840 sec  
 RG 57.21  
 DW 183.000 usec  
 DE 6.50 usec  
 TE 303.7 K  
 DO 0.00017027 sec  
 D1 1.83001602 sec  
 D9 0.30000001 sec  
 D11 0.03000000 sec  
 D12 0.00020000 sec  
 D16 0.00020000 sec  
 INO 0.00036600 sec

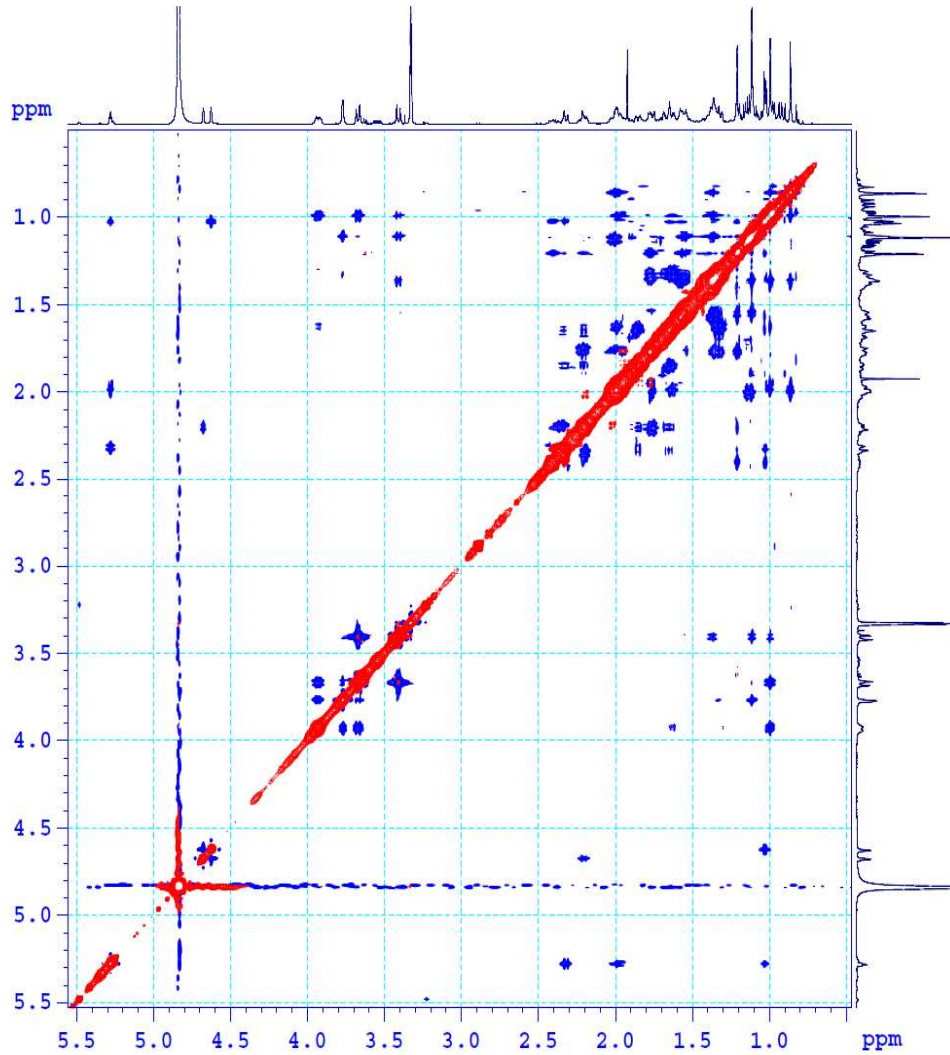
==== CHANNEL f1 =====  
 SFO1 500.2015076 MHz  
 NUC1 1H  
 P1 10.00 usec  
 P2 20.00 usec  
 P17 2500.00 usec  
 PLW1 22.00000000 W  
 PLW10 3.25440001 W

==== GRADIENT CHANNEL =====  
 GPNAM(1) SMSQ10.100  
 GFZ1 40.00 %  
 P16 1000.00 usec

F1 - Acquisition parameters  
 TD 256  
 SFO1 500.2015 MHz  
 FIDRES 21.345629 Hz  
 SW 5.462 ppm  
 FMODE States-TPPI

F2 - Processing parameters  
 SI 1024  
 SF 500.2000001 MHz  
 WDW QSINE  
 SSB 2  
 LB 0 Hz  
 GE 0  
 PC 1.00

F1 - Processing parameters  
 SI 1024  
 MC2 States-TPPI  
 SF 500.2000014 MHz  
 WDW QSINE  
 SSB 2  
 LB 0 Hz  
 GB 0



**S11: The HR-MS spectrum of 2**

