SUPPLEMENTARY MATERIAL

Stixilamides A and B, two new phenolic amides from the leaves of *Stixis suaveolens*

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Abstract

The ethyl acetate extracts produced from the leaves of *Stixis suaveolens* (Roxb.) was characterized on the basis of NMR spectra combined with extensive mass spectroscopic techniques. The chemical characterization revealed presence of two new phenolic amides which were named as stixilamides A and B.

Keywords: *Stixis suaveolens*, Phenolic amide, Stixilamide

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EXPERIMENTAL SECTION

General experimental procedures.

NMR spectra were recorded on a BRUKER - 500 spectrometer, operating at 125.76 MHz for ^{13}C NMR and at 500.13 MHz for ^{1}H-NMR. ^{1}H chemical shifts were referenced to CD_3OD at 3.31 ppm, while the ^{13}C chemical shifts were referenced to the central peak at 49.0 (CD_3OD). For HMBC experiments the delay (1/2J) was 70 ms. The HR-ESI-MS spectra were measured on a SCIEX X500R-QTOF mass spectrometer. Silica gel plate (Merck F254) was used for analytical TLC and for flash column chromatography, respectively. All solvents used throughout the experiments were obtained from Sigma-Aldrich company.

Assay for NO inhibitory effect using RAW 264.7 cells.

RAW 264.7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with penicillin G sodium (100 units/mL), streptomycin sulfate (100 µg/mL), amphotericin B (0.25 µg/mL), and 10% fetal bovine serum (FBS). The cells were seeded in 96-well culture plates with 1 × 105 cells/well and incubated for 24 h at 37 °C in a humidified atmosphere containing 5% CO_2. The cells were treated with samples dissolved in phenol red-free DMEM for 30 min followed by 1 µg/mL of LPS treatment for 20 h. The amount of NO in the cultured medium was measured by the Griess reagent. The standard curve was created by using known concentrations of sodium nitrite, and the absorbance was measured at 540 nm. To evaluate the cytotoxic effect of samples in RAW 264.7 cells in the assay condition, SRB assay was performed. Briefly, after the fixation with 10% trichloroacetic acid (TCA), cells were stained with 0.4% SRB solution in 1% acetic acid followed by dissolving bound SRB in 10 mM Tris-buffer. The optical density was determined at 515 nm.

Plant Materials

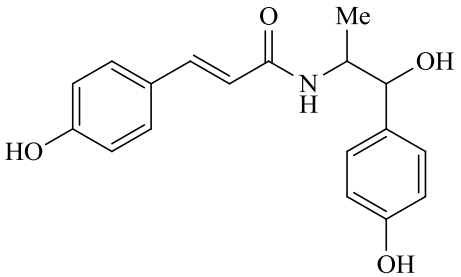
The leaves of *Stixis suaveolens* were collected in Ha Giang province in December 2016, and identified by Dr Nghiem Duc Trong, Hanoi University of Pharmacy. A voucher specimen (HNIP-18481) was deposited at the Herbarium of Medicinal Plants in Vietnam - Hanoi University of Pharmacy.

Extraction and Isolation

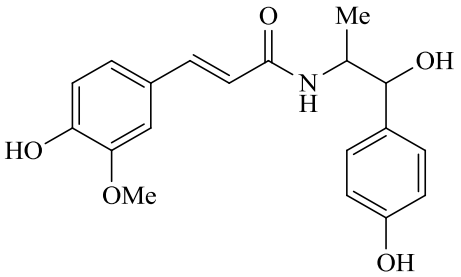
The dried plant sample (5.0 kg) was ground into powder and extracted with MeOH three times (5L each time) in sonication bath at room temperature. After solvent evaporation, (220 g) crude methanol extract was suspended in water and partitioned with dichloromethane, ethyl acetate. The obtained ethyl acetate layer was evaporated to remove solvent under reduced pressure to give a crude ethyl acetate part (13 g). This residue was subjected to reserve phase C-18 (RP-18) resin column and eluted with methanol/water (3/1, v/v) to give four fractions E1-E4. The E2 fraction was continued to subject to silica gel column chromatography with solvent system hexane/ethyl acetate/methanol (1/1/0.1, v/v/v) to give six smaller fractions E2A-E2F. Compounds **1** (7.5 mg) and **2** (3.0 mg) were isolated from fraction E2E using RP-18 column and eluted with acetone/water (1/1.5, v/v).

(1) *(E)-N-*(*erythro*-1-hydroxy-1-(4-hydroxyphenyl)propan-2-yl)-3-(4-hydroxyphenyl) acrylamide : Colorless amorphous powder, $[\alpha]_D^{25}$ (c 0.1, MeOH) = +8.5; HR-ESI-MS: *m/z* 336.1199 $[M+Na]^+$ (Calcd: $C_{18}H_{19}NO_4Na^+$ *m/z* 336.1206); ^1H (CD_3OD, 500 MHz) and ^{13}C (CD_3OD,125 MHz), see table S1.

(2) *(E)-N-*(*erythro*-1-hydroxy-1-(4-hydroxyphenyl)propan-2-yl)-3-(4-hydroxy-3-methoxyphenyl)acrylamide : Colorless amorphous powder, $[\alpha]_D^{25}$ (c 0.1, MeOH) = +10.0; HR-ESI-MS: *m/z* 366.1305 $[M+Na]^+$ (Calcd: $C_{19}H_{21}NO_5Na^+$ *m/z* 366.1312); ^1H (CD_3OD, 500 MHz) and ^{13}C (CD_3OD,125 MHz), see table S1.



(E)-N-(*erythro*-1-hydroxy-1-(4-hydroxyphenyl)propan-2-yl)-3-(4-hydroxyphenyl)acrylamide



(E)-N-(*erythro*-1-hydroxy-1-(4-hydroxyphenyl)propan-2-yl)-3-(4-hydroxy-3-methoxyphenyl)acrylamide

Figure S1: Chemical structures of compounds **1** and **2** isolated from *Stixis suaveolens*.

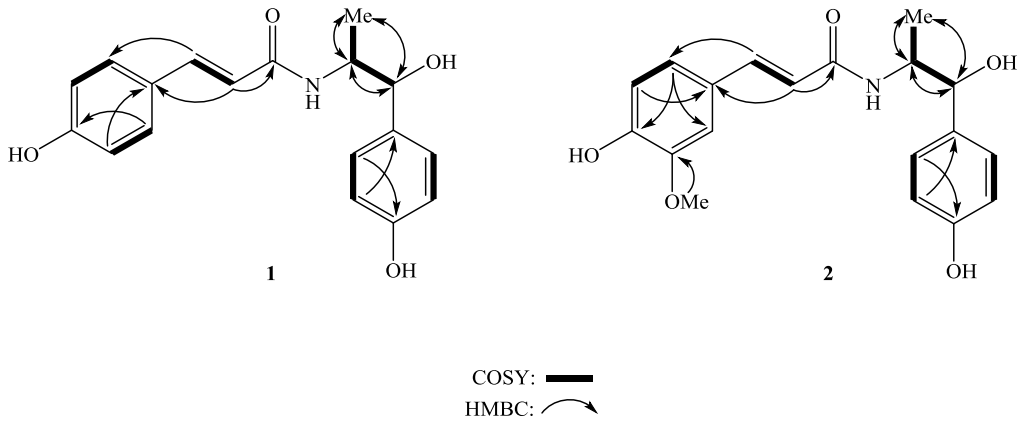


Figure S2: Important COSY and HMBC correlations in compound **1** and **2**.

Table S1: ^{1}H- and ^{13}C-NMR data for compounds **1**, **2**.

No.	**1**		**2**	
	[a,b] δ_C	**[a,c] δ_H (mult., *J* = Hz)**	**[a,b] δ_C**	**[a,c] δ_H (mult., *J* = Hz)**
1	127.8	-	128.4	-
2	130.5	7.41 (d, 8.5)	111.6	7.01 (d, 2.0)
3	116.7	6.80 (d, 8.5)	149.3	-
4	160.5	-	149.8	-
5	116.7	6.80 (d, 8.5)	116.5	6.69 (d, 8.5)
6	130.5	7.41 (d, 8.5)	123.2	6.92 (dd, 2.0, 8.5)
7	141.8	7.43 (d, 15.5)	142.1	7.31 (d, 16.0)
8	118.6	6.42 (d, 15.5)	118.9	6.33 (d, 16.0)
9	168.6	-	168.6	-
1′	134.4	-	134.4	-
2′	128.6	7.24 (d, 8.5)	128.6	7.12 (d, 8.5)
3′	115.8	6.76 (d, 8.5)	115.8	6.65 (d, 8.5)
4′	157.7	-	157.7	-
5′	115.8	6.76 (d, 8.5)	115.8	6.65 (d, 8.5)
6′	128.6	7.24 (d, 8.5)	128.6	7.12 (d, 8.5)
7′	76.8	4.70 (d, 4.5)	76.9	4.58 (d, 4.5)
8′	52.3	4.23 (dq, 4.5, 6.5)	52.3	4.12 (dq, 4.5, 6.5)
9′	14.9	1.13 (d, 6.5)	15.0	1.01 (d, 6.5)
OMe-3′	-		56.4	3.78 (s)

Recorded in [a] CD_3OD, [b] 125 MHz, [c] 500 MHz

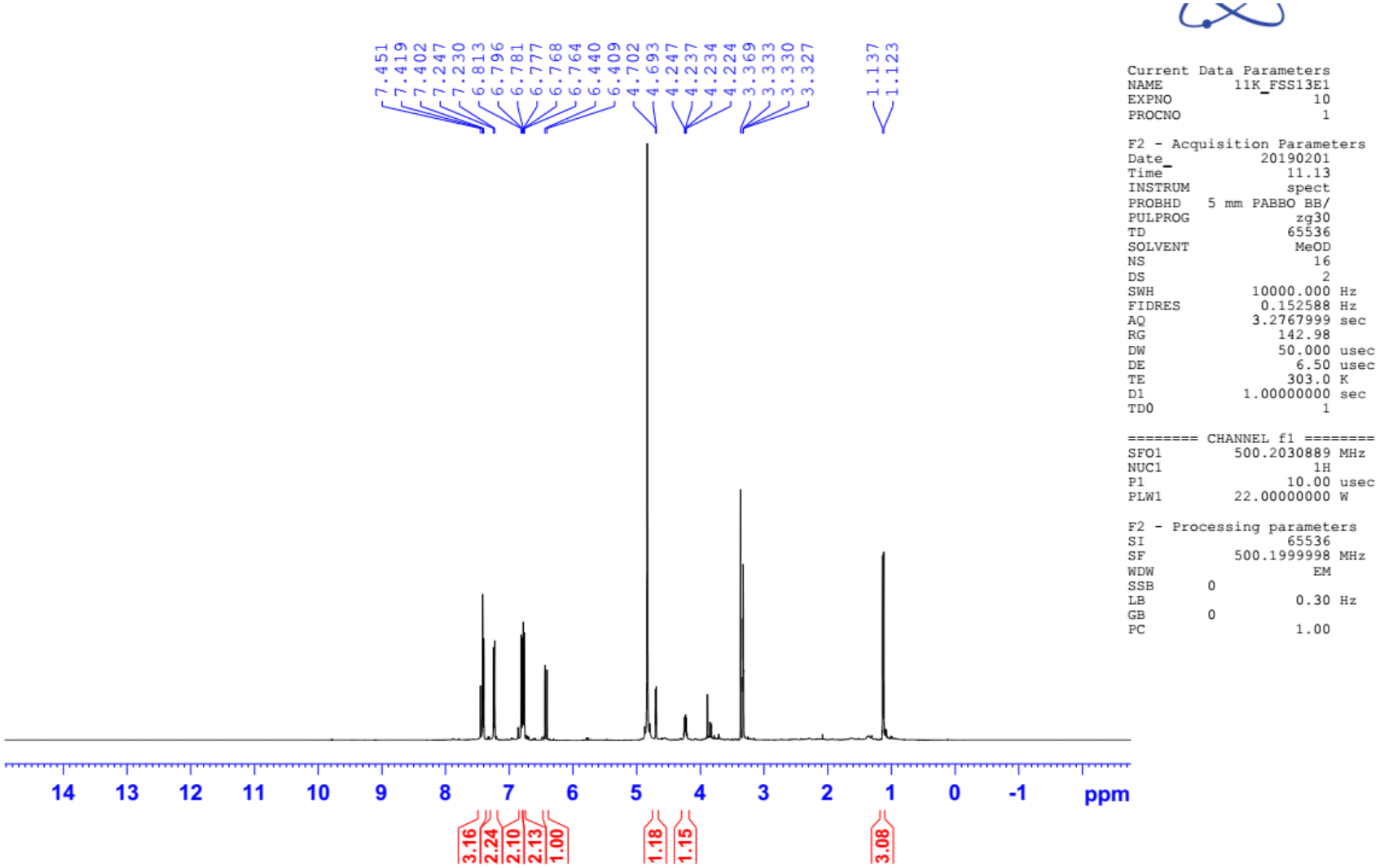


Figure S3.[1]H spectrum of compound **1**

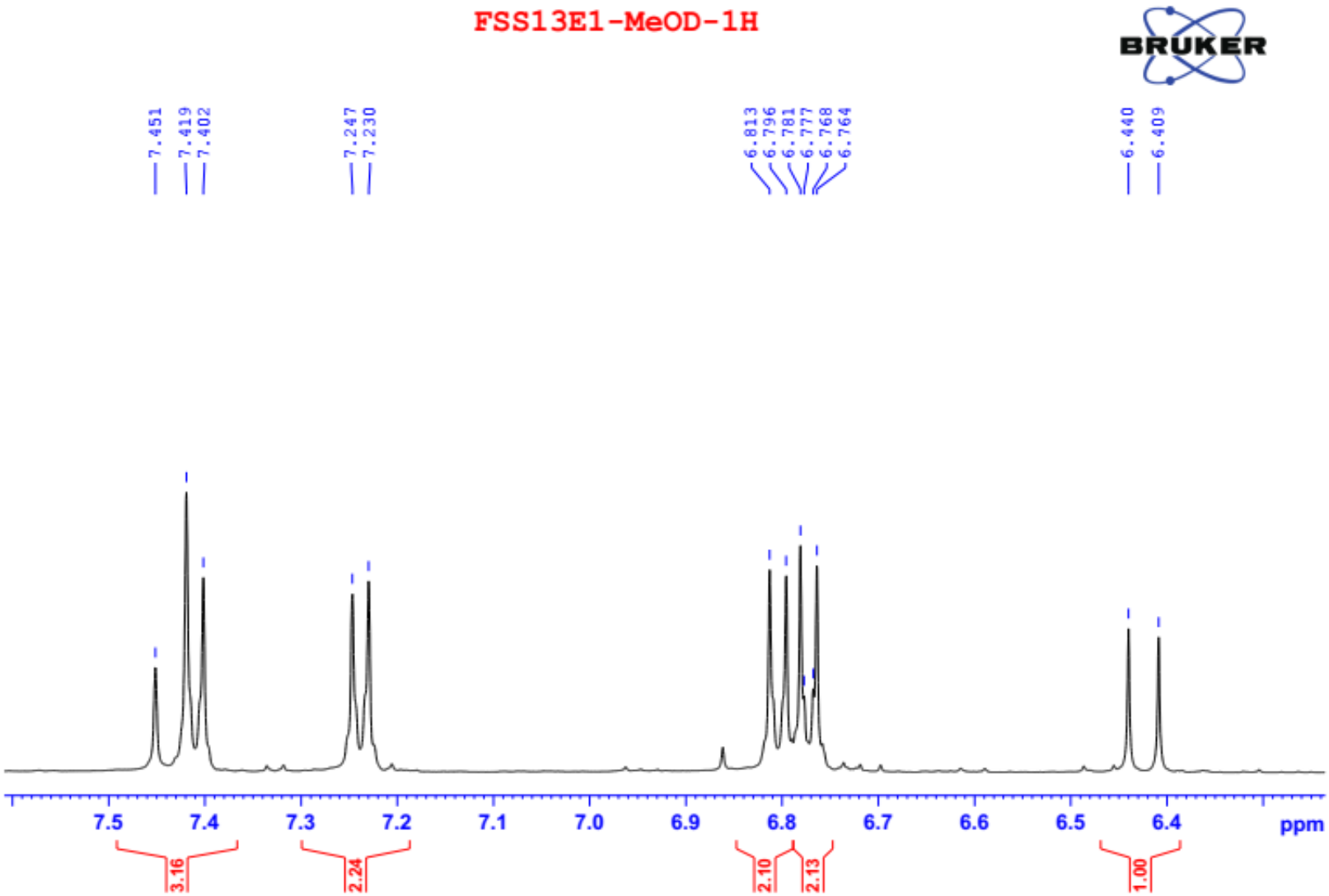


Figure S3.1.[1]H spectrum of compound **1**

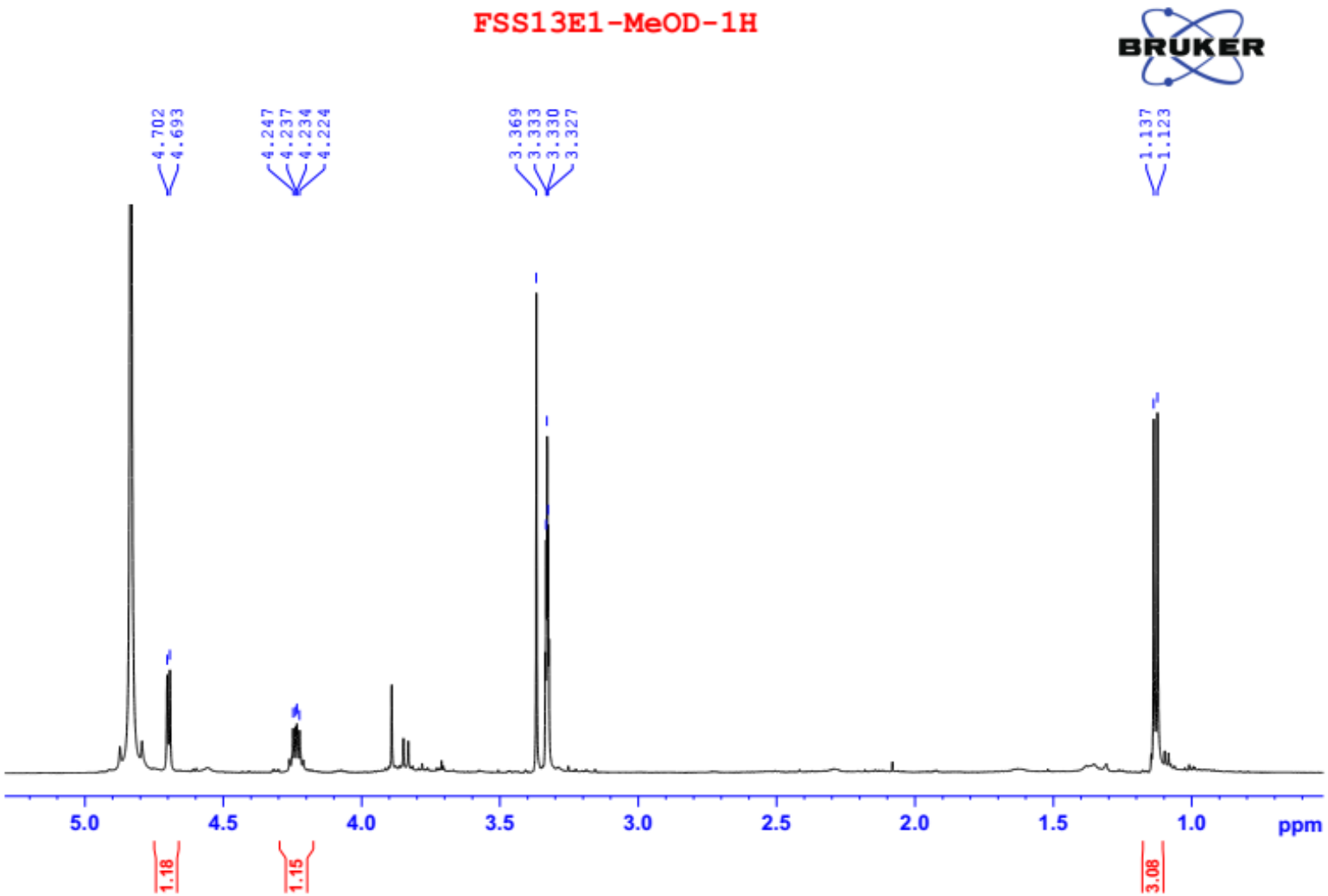
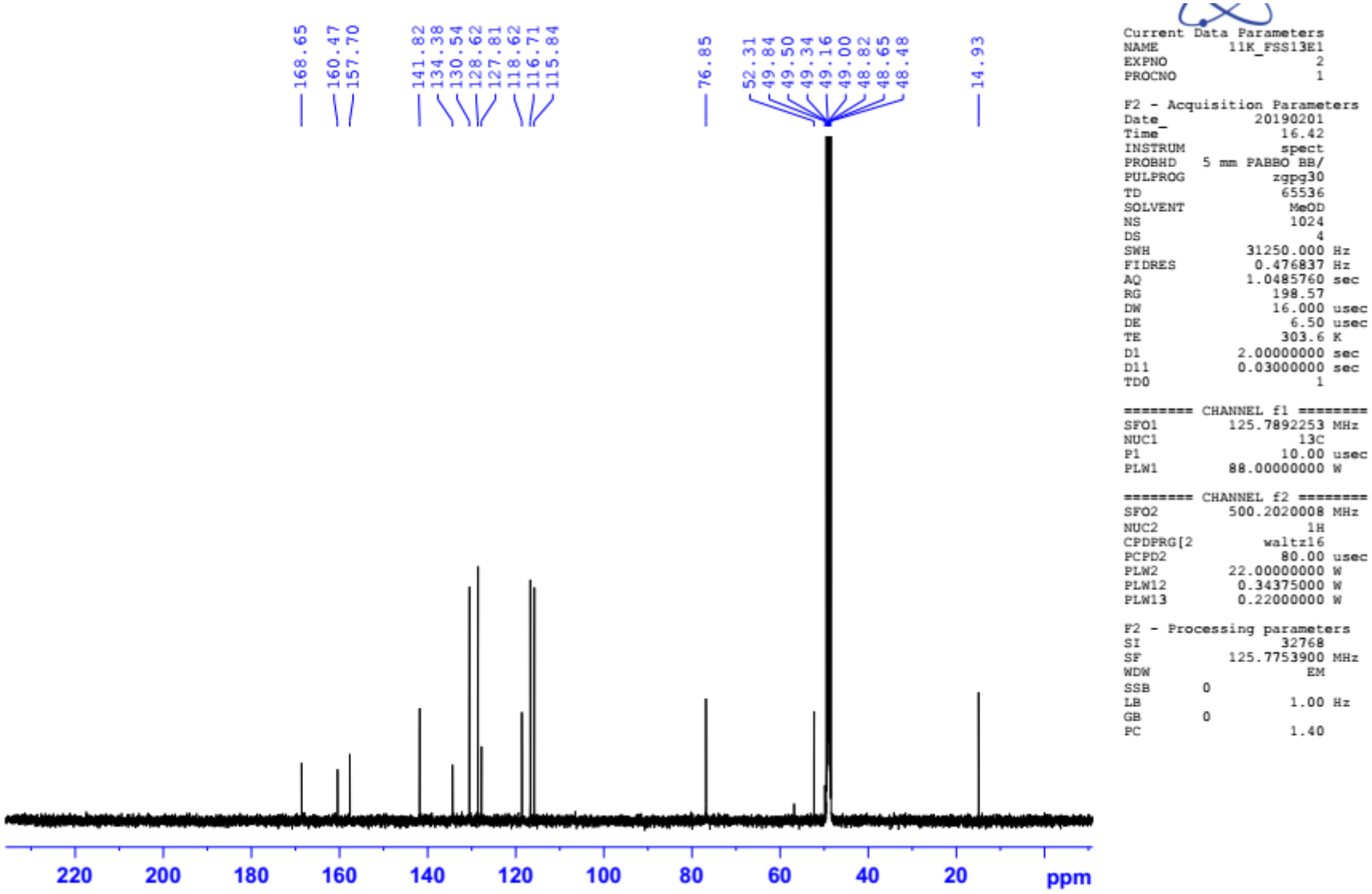


Figure S3.2.[1]H spectrum of compound **1**

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PROCNO                  1

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SOLVENT            MeOD
NS                 1024
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              303.6 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
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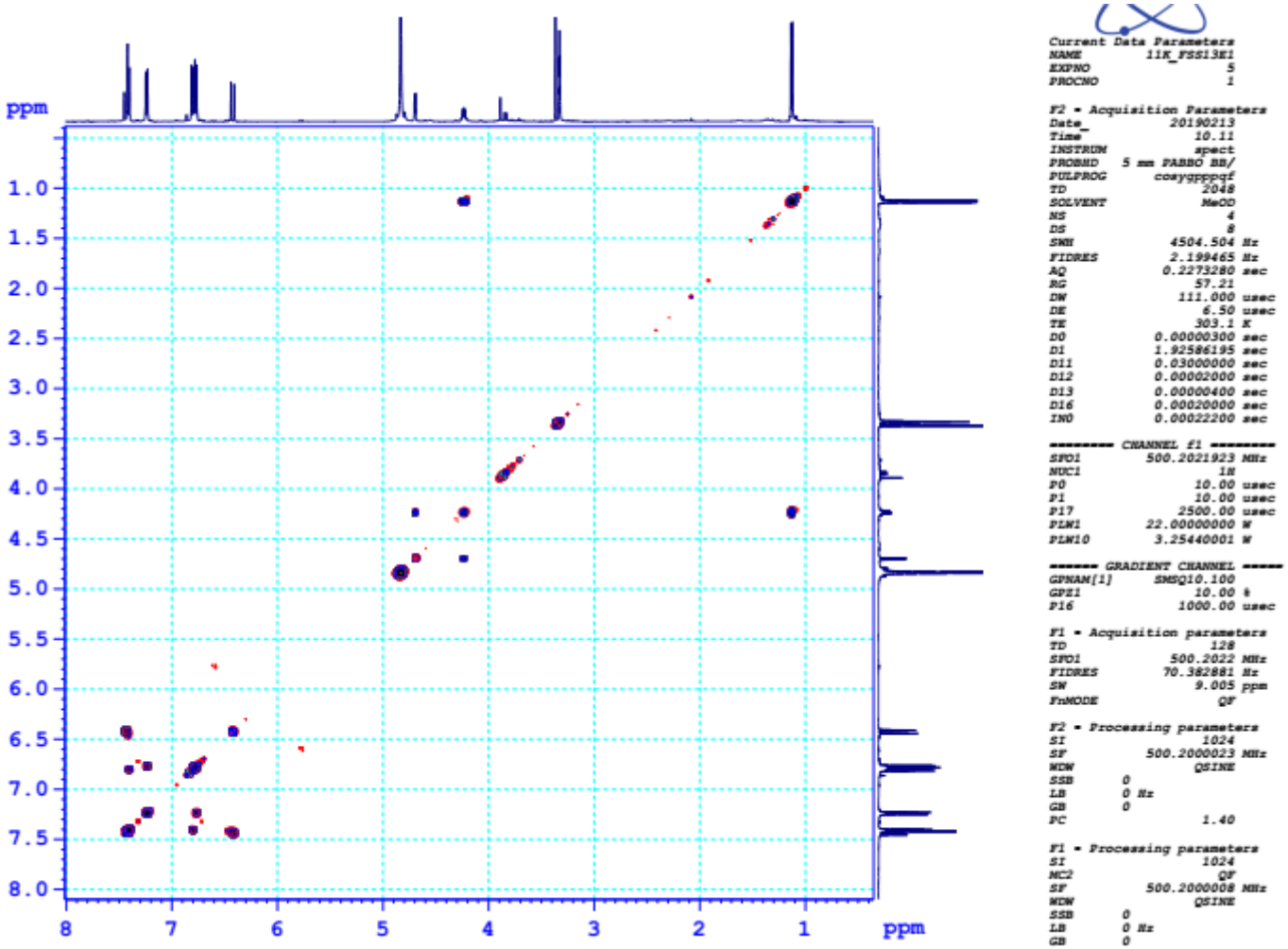
Figure S4. ^{13}C spectrum of Compound **1**

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PROCNO                  1

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PULPROG       cosygpppqf
TD                  2048
SOLVENT             MeOD
NS                     4
DS                     8
SWH           4504.504 Hz
FIDRES        2.199465 Hz
AQ            0.2273280 sec
RG                 57.21
DW               111.000 usec
DE                 6.50 usec
TE                303.1 K
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D1            1.92586195 sec
D11           0.03000000 sec
D12           0.00002000 sec
D13           0.00000400 sec
D16           0.00020000 sec
IN0           0.00022200 sec

======== CHANNEL f1 ========
SFO1         500.2021923 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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GPZ1               10.00 %
P16              1000.00 usec

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FIDRES       70.382881 Hz
SW             9.005 ppm
FnMODE                QF

F2 - Processing parameters
SI                  1024
SF           500.2000023 MHz
WDW                QSINE
SSB        0
LB         0 Hz
GB         0
PC                   1.40

F1 - Processing parameters
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MC2                   QF
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WDW                QSINE
SSB        0
LB         0 Hz
GB         0
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Figure S5. COSY spectrum of compound **1**

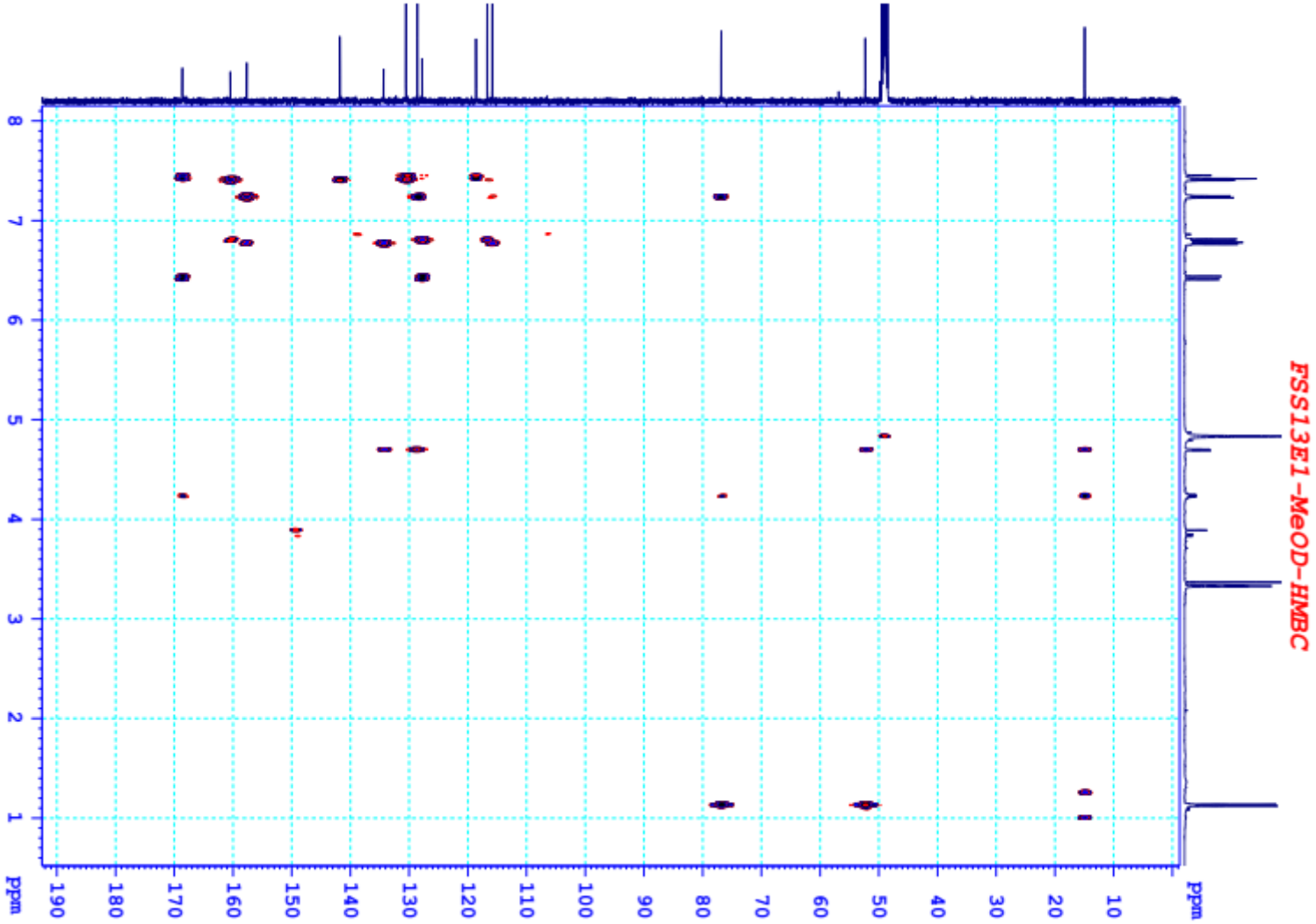


Figure S6. HMBC spectrum of compound **1**

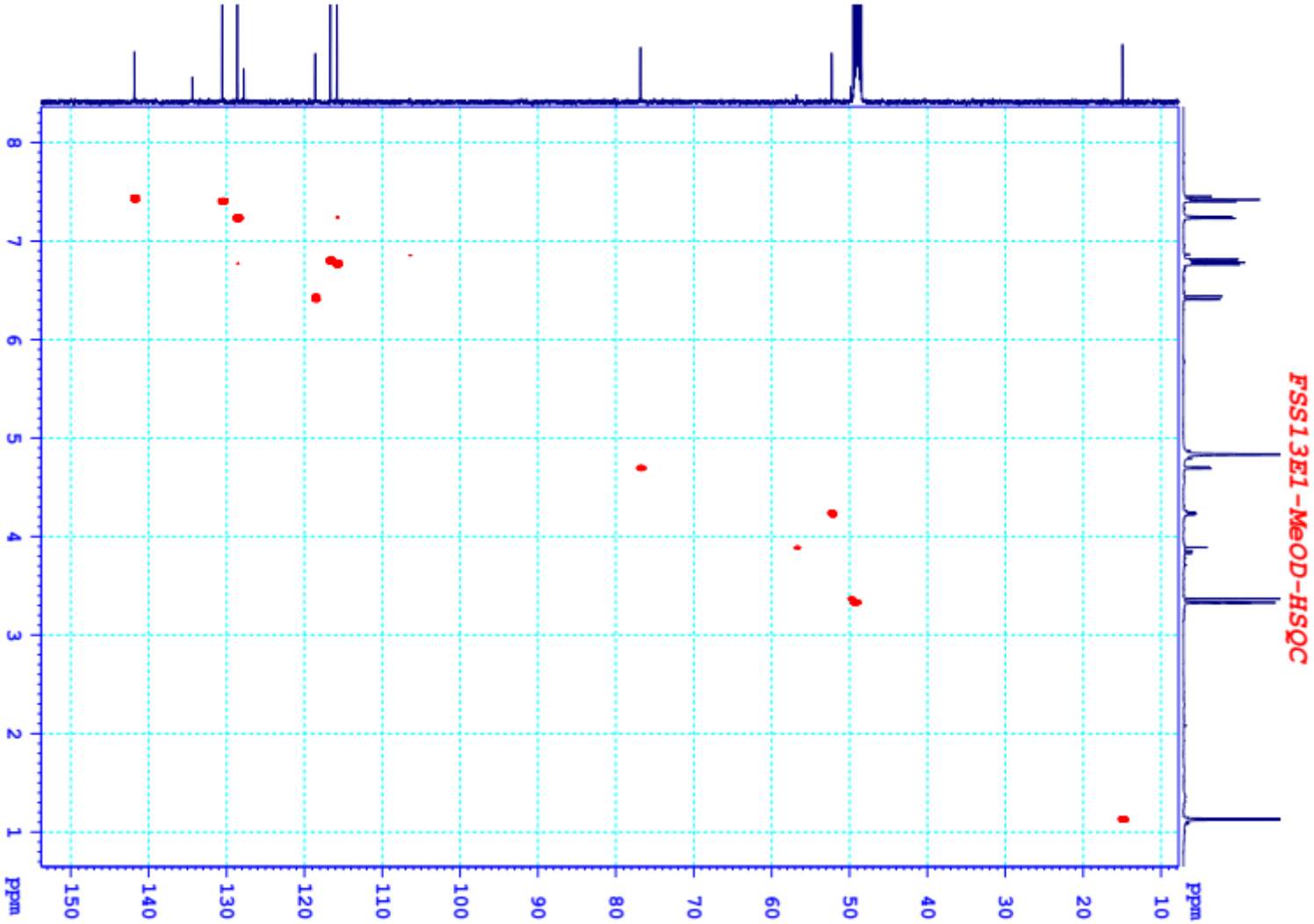
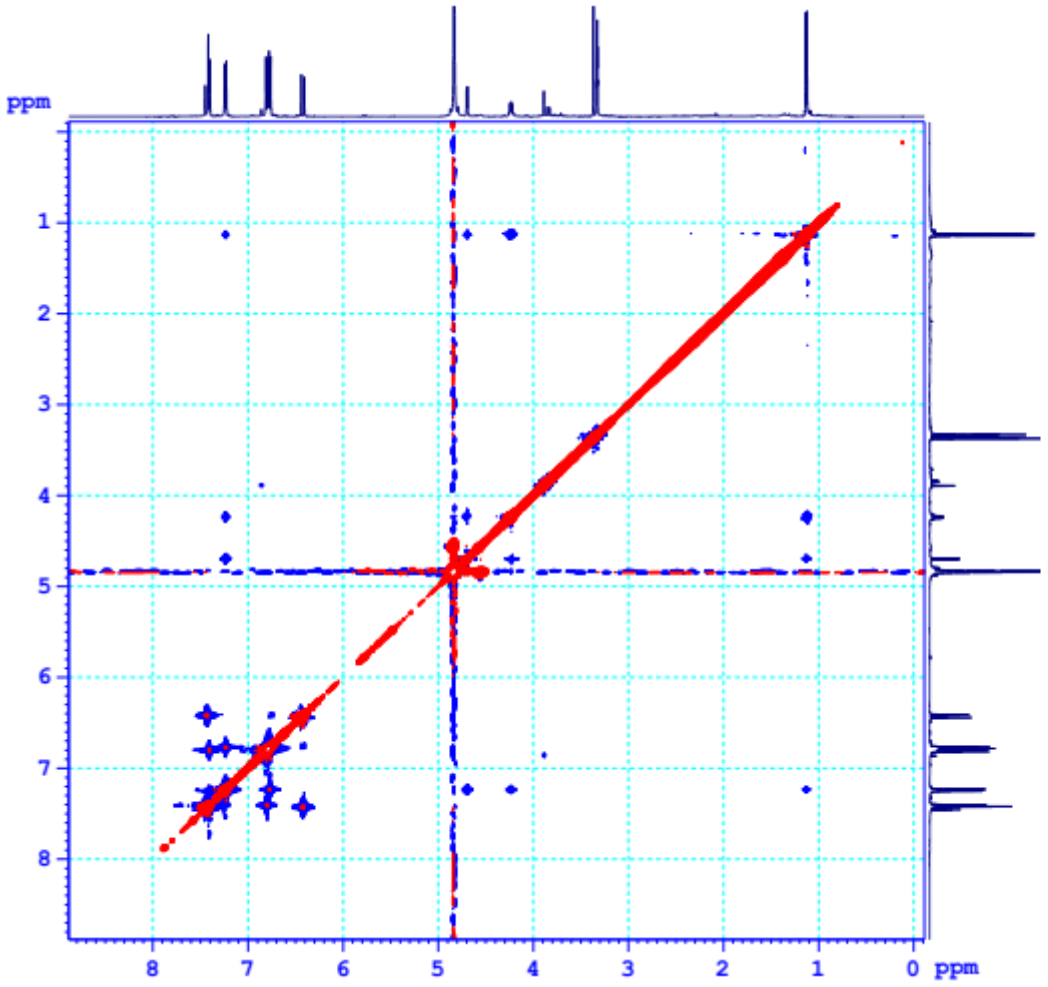


Figure S7. HSQC spectrum of compound **1**

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PROCNO                          1

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INSTRUM                     spect
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PULPROG              noesygpphpp
TD                          2048
SOLVENT                     MeOD
NS                             8
DS                            32
SWH                     4504.504 Hz
FIDRES                  2.199465 Hz
AQ                      0.2273280 sec
RG                          57.21
DW                        111.000 usec
DE                          6.50 usec
TE                         303.0 K
D0                      0.00009827 sec
D1                      1.97747195 sec
D8                      0.30000001 sec
D11                     0.03000000 sec
D12                     0.00002000 sec
D16                     0.00020000 sec
IN0                     0.00022200 sec

======== CHANNEL f1 ========
SFO1                  500.2021923 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
GPZ1                        40.00 %
P16                       1000.00 usec

F1 - Acquisition parameters
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SFO1                  500.2022 MHz
FIDRES                17.595720 Hz
SW                        9.005 ppm
FnMODE                States-TPPI

F2 - Processing parameters
SI                            1024
SF                    500.2000011 MHz
WDW                         QSINE
SSB                             2
LB                  0 Hz
GB                  0
PC                           1.00

F1 - Processing parameters
SI                            1024
MC2                   States-TPPI
SF                    500.1999996 MHz
WDW                         QSINE
SSB                             2
LB                  0 Hz
GB                  0
```

Figure S8. NOESY spectrum of compound **1**

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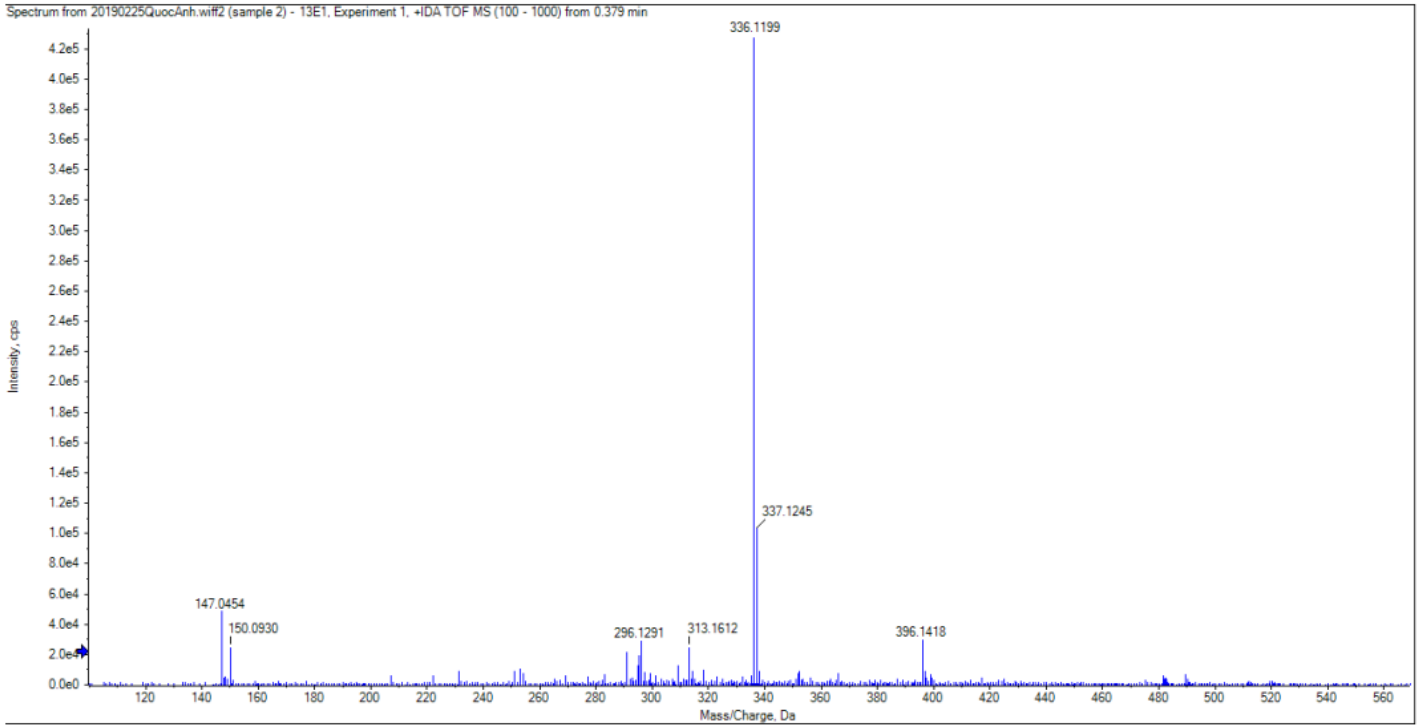
Operator: Le Anh VHH
Method: +IDA TOF MS
Date: 2019.02.25



Hit	Formula	Ion	m/z(Chemdraw)	RDB	ppm	MS Rank
1	C18H19NO4	[M+Na]+	336.1206	10	-6.5	1

Device Model: SCIEX X500 QTOF

Figure S9. ESI-HRMS spectrum of compound **1**

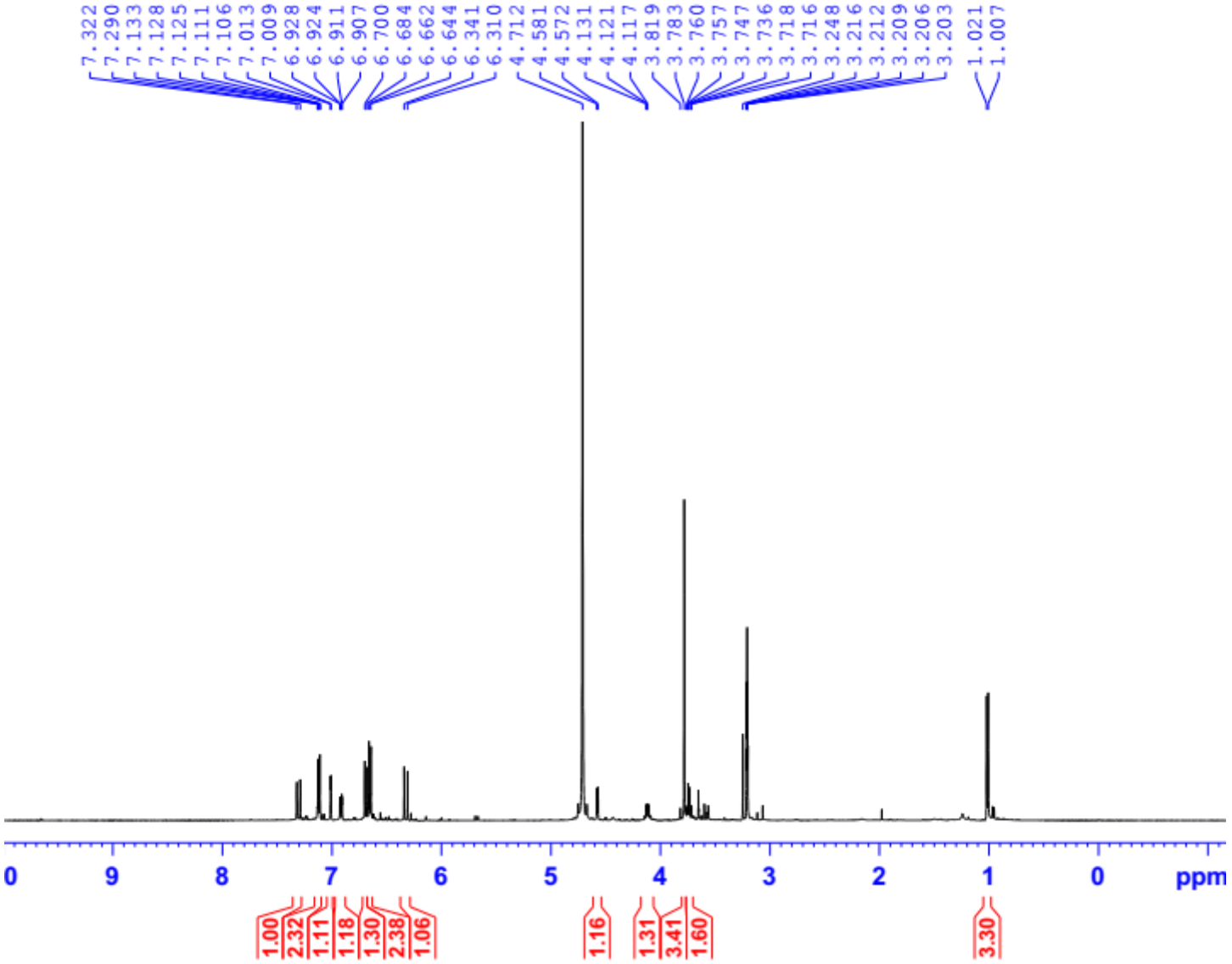


Figure S10. ^{1}H spectrum of compound **2**

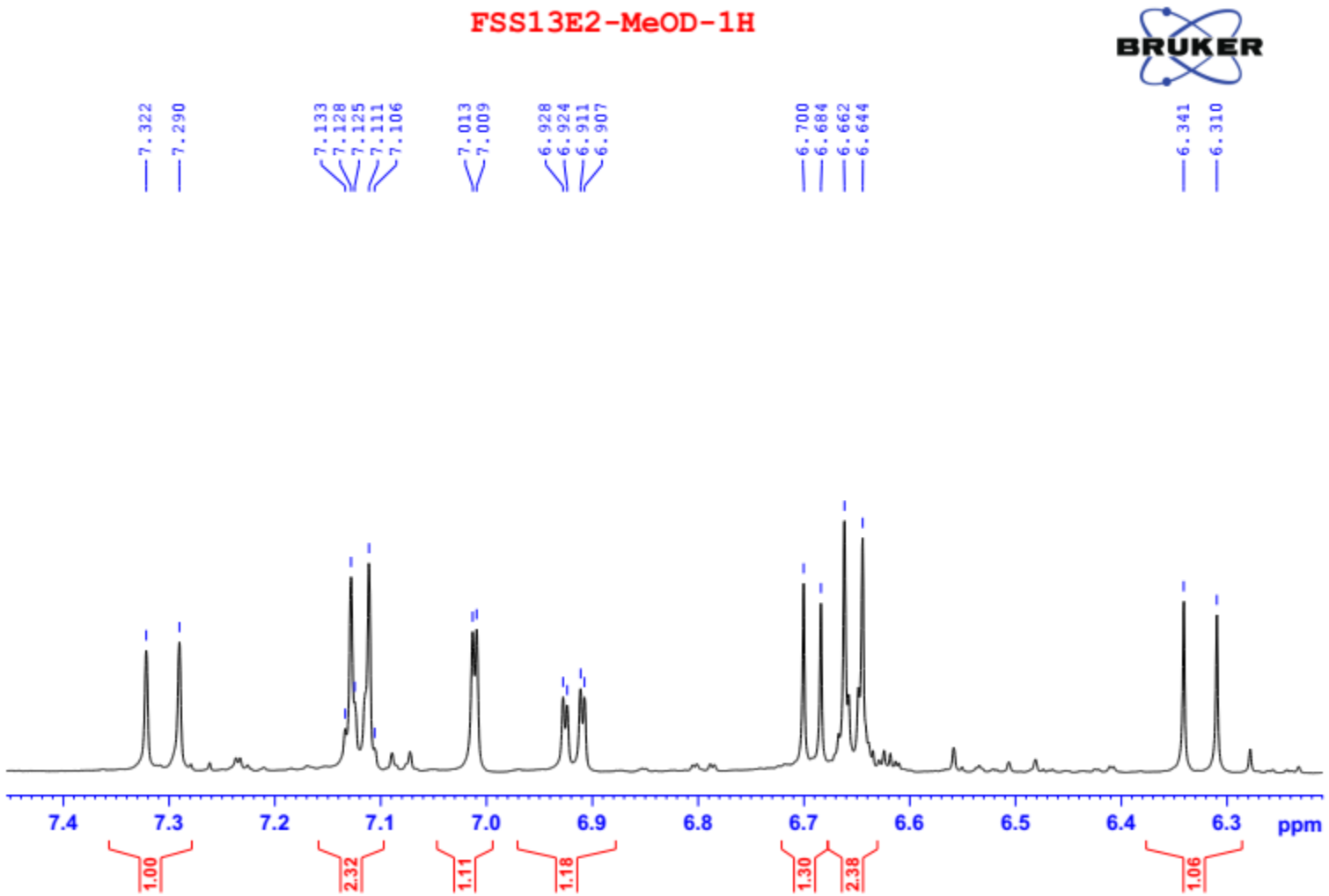


Figure S10.1. ^{1}H spectrum of compound **2**

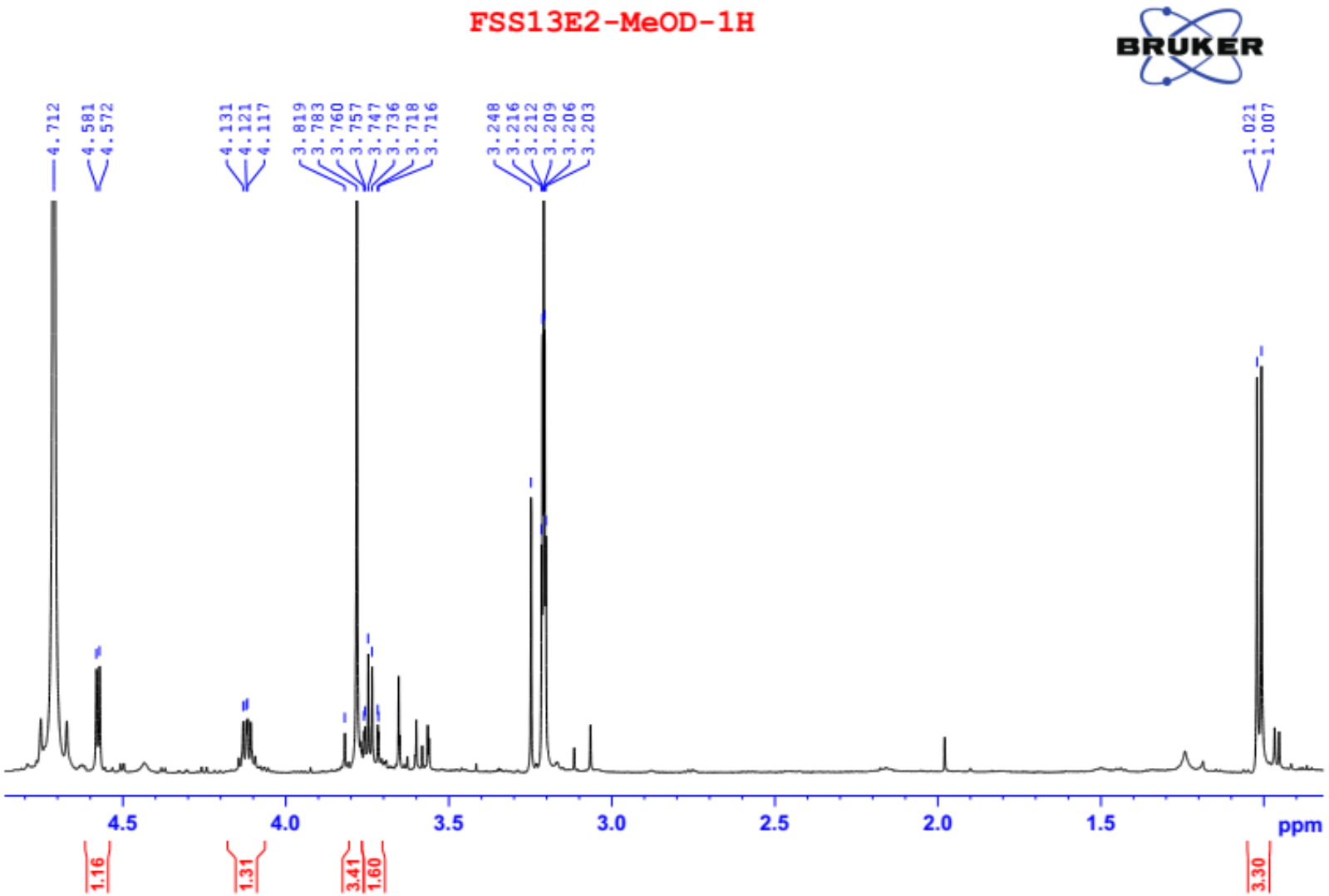


Figure S10.2. ^{1}H spectrum of compound **2**

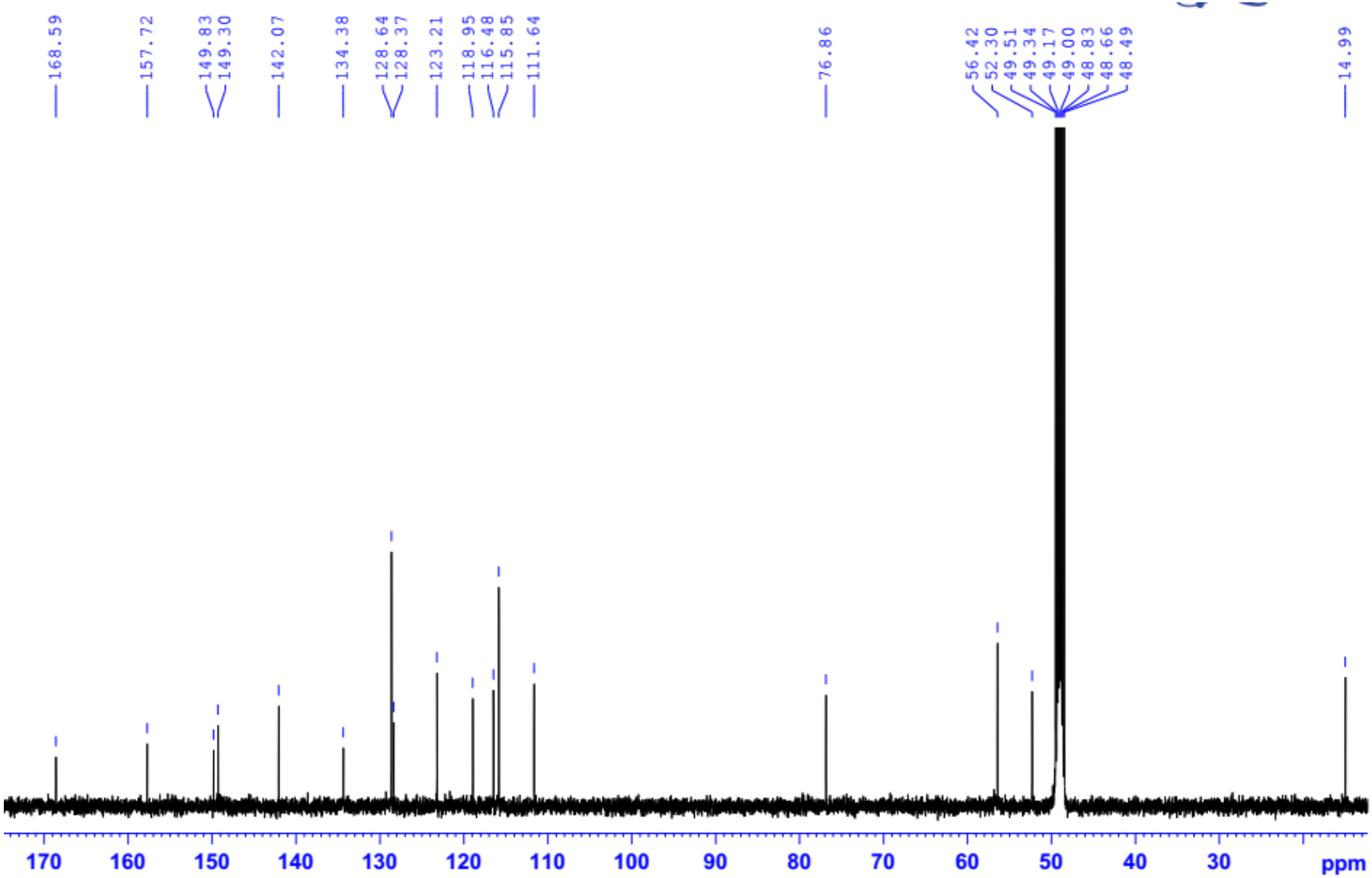


Figure S11. ^{13}C spectrum of compound **2**

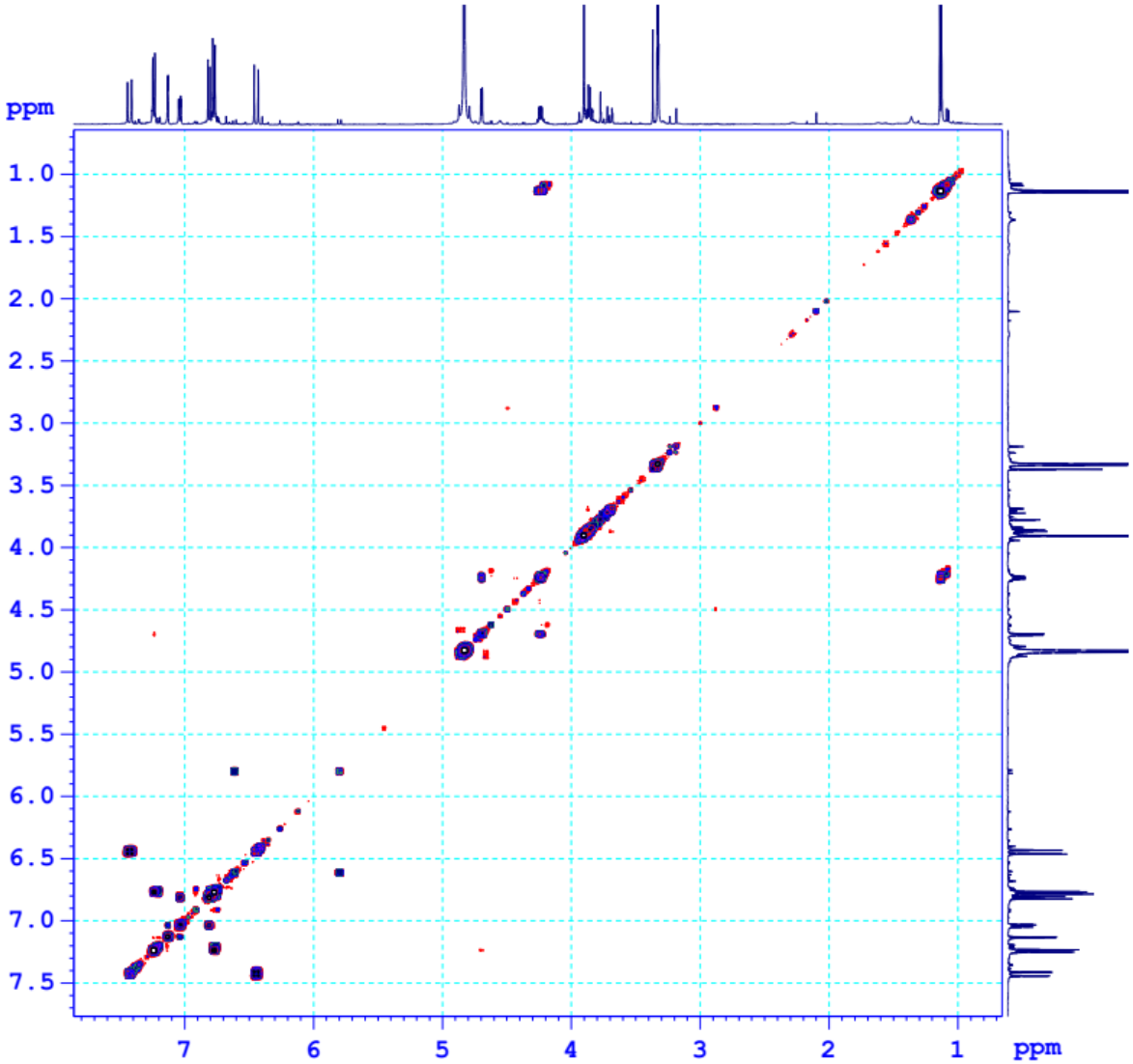


Figure S12. COSY spectrum of compound **2**

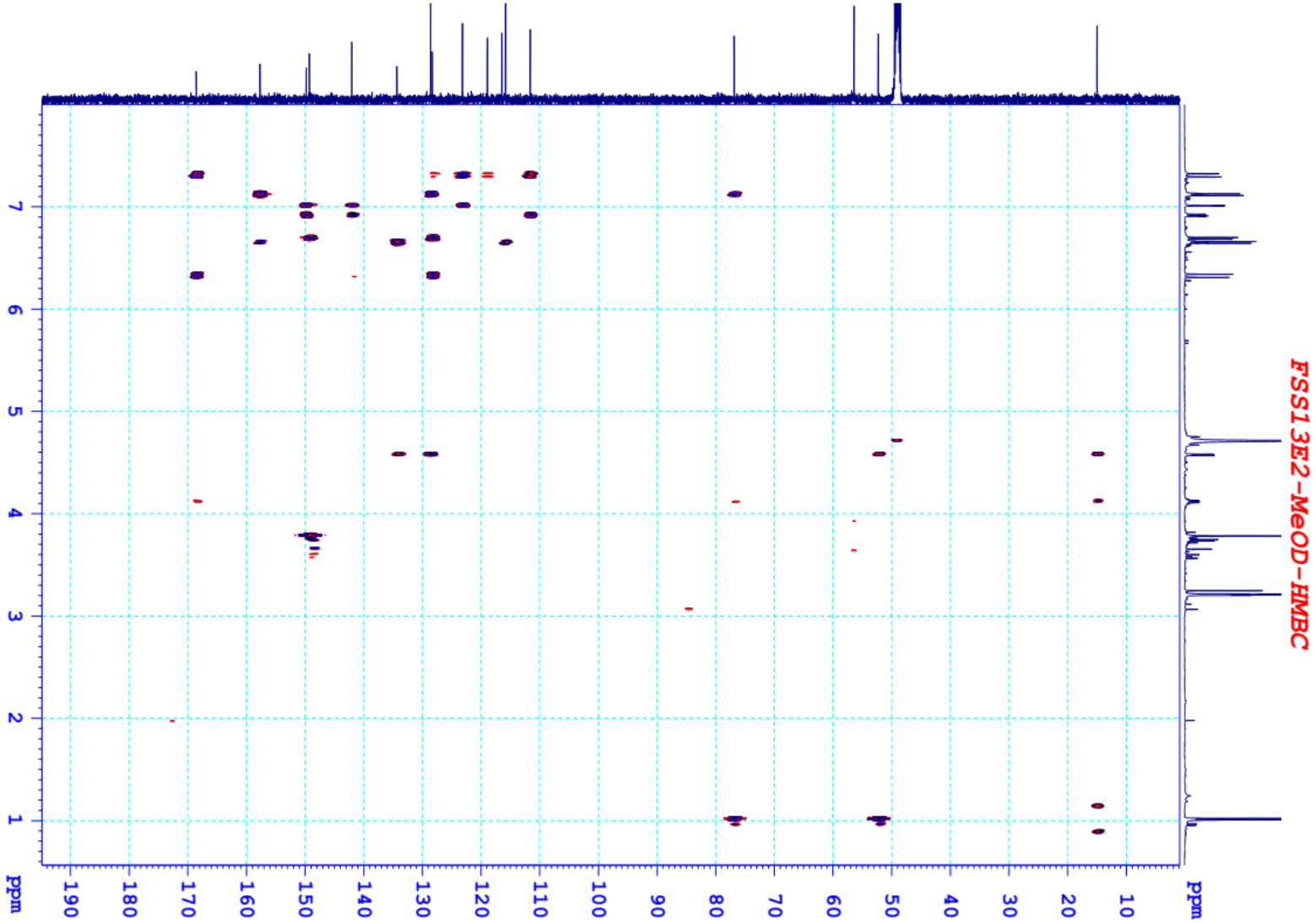


Figure S13. HMBC spectrum of compound **2**

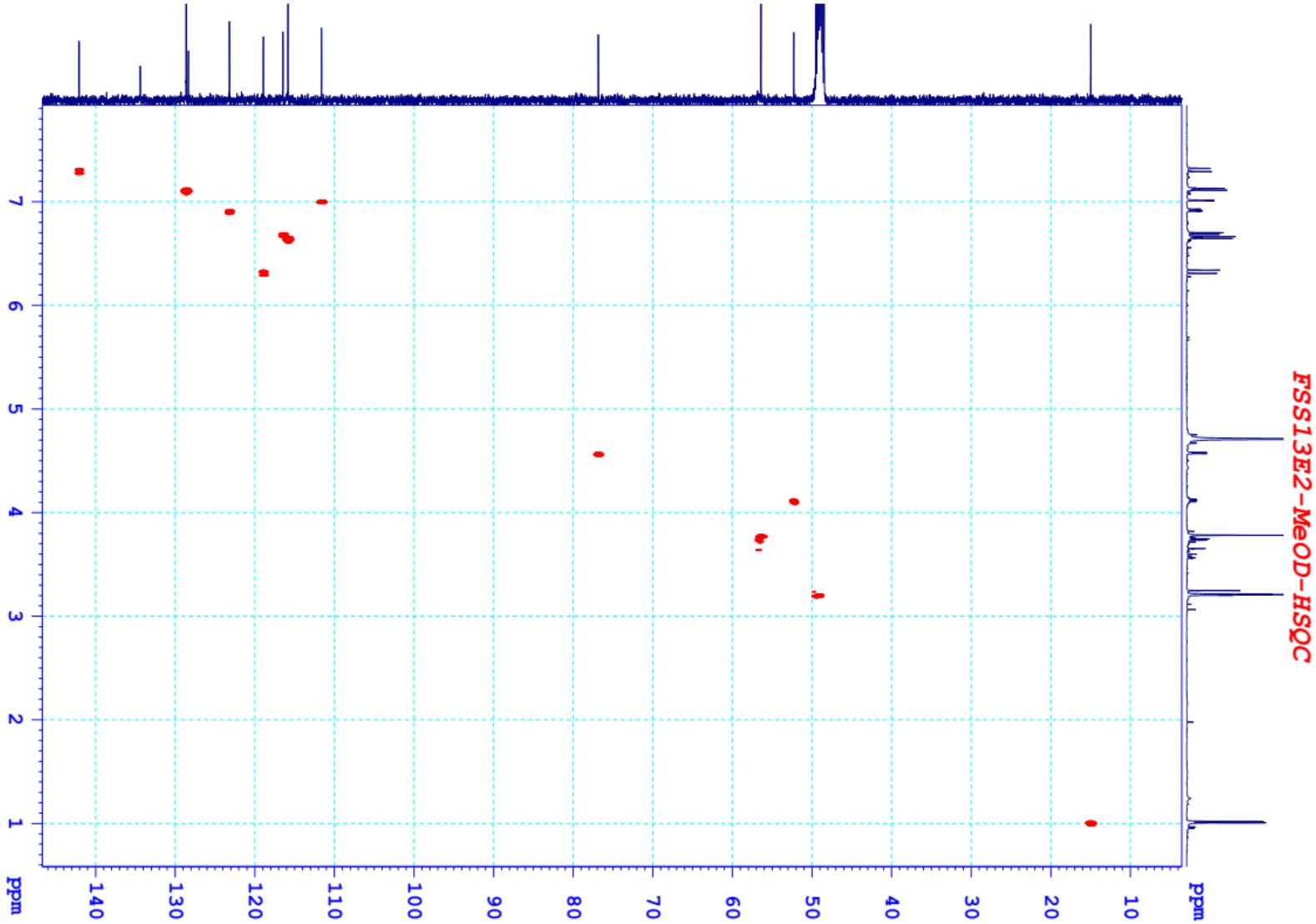


Figure S14. HSQC spectrum of compound **2**

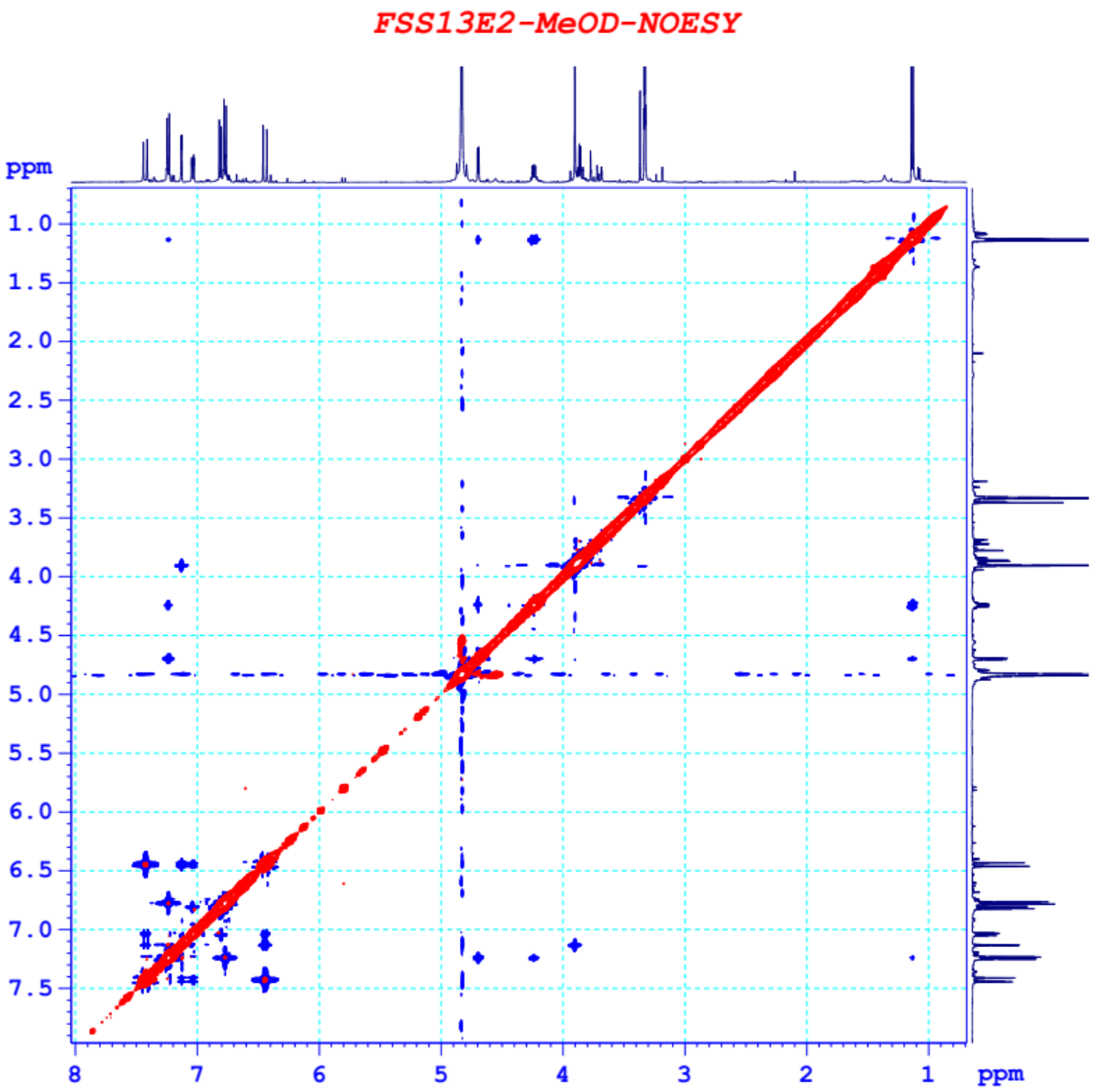


Figure S15. NOESY spectrum of compound **2**

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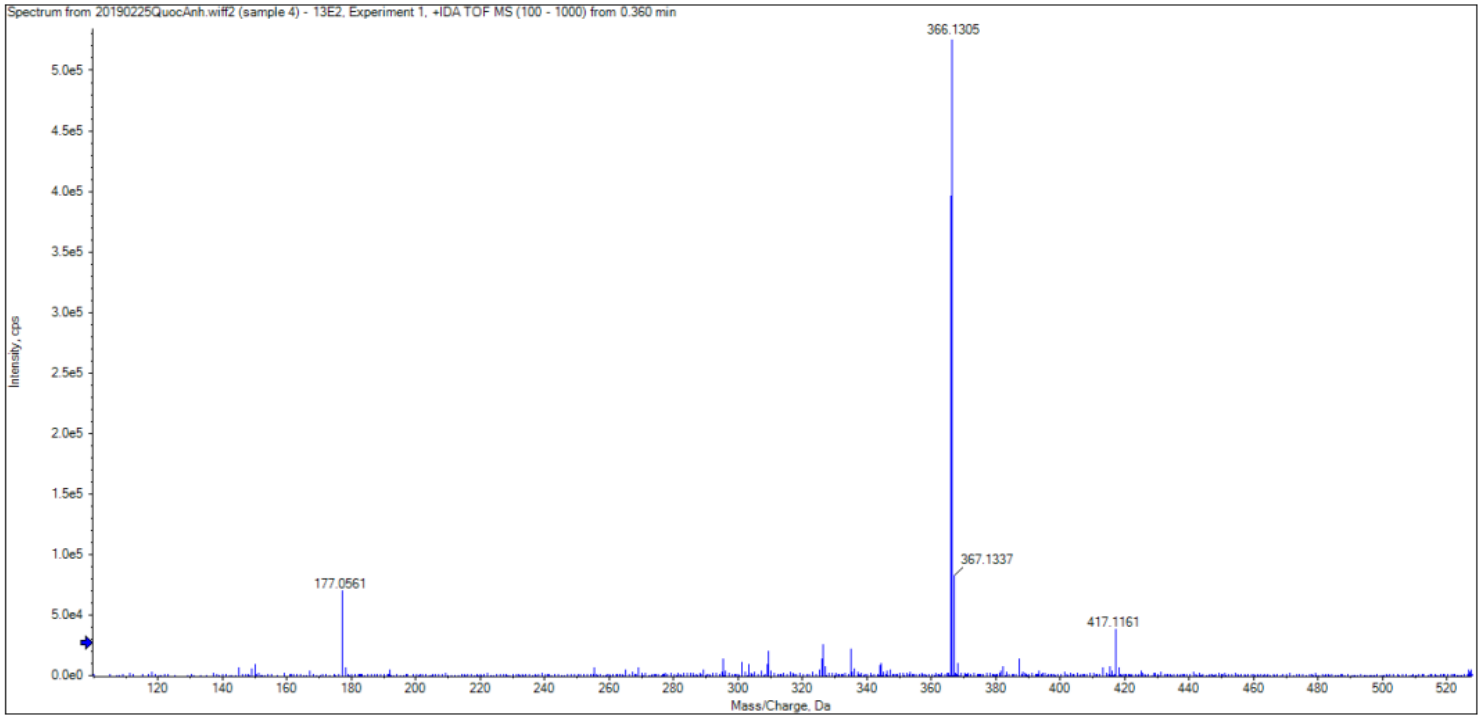
Operator: Le Anh VHH
Method: +IDA TOF MS
Date: 2019.02.25



Hit	Formula	Ion	m/z(Chemdraw)	RDB	ppm	MS Rank
1	C19H21NO5	[M+Na]+	366.1312	10	-9.2	1

Device Model: SCIEX X500 QTOF

Figure S16. ESI-HRMS spectrum of compound **2**