**Organic & Biomolecular Chemistry**

**Supporting information**

# Sulfurized diterpenoids in amber as diagenetic indicators for sulfate-reducing processes in past depositional environments

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# 1. Materials and Methods

## 1.1. Sample

The amber sample used to isolate compounds **I-III** was collected from the lowermost bed exposed at the locality Saint-Louis in the city of Piolenc, Vaucluse, SE France. This bed is dated as Santonian in age (86.3-83.6 Ma).[1]

## 1.2 Extraction and fractionation of the Piolenc amber sample

The amber sample was extracted and methylated using *N*,*N*-dimethylformamide dimethyl acetal (DMA/DMF) according to the procedure described by De Lama et al. (2022).[2]

*Extraction*

Typically, 1.00 g of amber from Piolenc was finely ground in an agate mortar and the resulting powder was extracted by sonication (20 min, 4 x) with dichloromethane (DCM; 120 mL) at room temperature. The organic phases recovered after centrifugation (2800 rpm, 10 min) were combined and the solvent removed under reduced pressure, yielding the crude organic extract (286 mg).

*Methylation of the amber extract using N,N-dimethylformamide dimethylacetal*

DMA/DMF in excess (2.00 mL) was added to the amber extract (286 mg) dissolved in toluene (26 mL). After heating at 70 °C for 3 h, the solvent and the reagent were removed under reduced pressure.

*Fractionation*

For analytical purposes, a small aliquot of the methylated extract was fractionated on a silica gel column. The apolar fraction $F_A$ obtained by elution with DCM/ethyl acetate (EtOAc; 8:2 v/v; 3 dead volumes -$D_v$-) was analysed using gas chromatography-mass spectrometry (GC-MS).

The derivatized extract (286 mg) was fractioned into 28 fractions on a silica gel column. The first fraction (F1) was obtained by elution with cyclohexane/DCM (6:4 v/v, 1.5 $D_v$) and four additional fractions (F2-F5) were recovered by eluting with 0.75 $D_v$ of the same eluent. Twelve further fractions (F6 to F17) were then obtained by eluting with 3 $D_v$ of cyclohexane/DCM (1:1 v/v) and nine fractions (F18 to F26) by eluting with 3 $D_v$ of DCM. Finally, two last fractions (F27 and F28) were recovered using, respectively, DCM/EtOAc (8:2 v/v; 2 $D_v$) and DCM/MeOH (4:1 v/v; 2 $D_v$). The presence of compounds **I-III** in the recovered fractions was checked using GC-MS. The latter were shown to be present in fractions F15 to F18 (21.5 mg in total).

*Isolation of compounds **I-III***

The fractions F15 to F18 were purified by HPLC (Zorbax Sil; 4,6 x 250 mm; 5 µm; *n*-Heptane/EtOAc 85:15 v/v, 1 mL $min^{-1}$), yielding compounds **I** and **II** (1.4 mg and 0.5 mg, respectively) with a purity sufficient for structural elucidation using NMR (94 % and 91 % determined by GC-FID for **I** and **II**). Compound **III** was obtained in too low quantities (<0.2 mg) for NMR studies.

## 1.3 Analytical measurements

*GC-MS*

GC-MS analyses were carried out on a Trace GC Ultra gas chromatograph (Thermo Scientific) coupled to a TSQ Quantum mass spectrometer (Thermo Scientific) equipped with a programmed temperature vaporizing (PTV) injector and operating in the electron impact mode (70 eV). Chromatographic separations were performed on a HP5-MS column (30 m x 0.25 mm; 0.1 µm film thickness) using He as carrier gas (constant flow mode, 1.1 mL $min^{-1}$) and the following temperature program: 70 °C (5 min), 70-240 °C (4 °C $min^{-1}$), 240 °C-300 °C (10 °C $min^{-1}$), isothermal at 300 °C.

*High performance liquid chromatography*

Isolation of compounds **I-III** was performed on a HPLC device comprising a Waters 590 pump, a Rheodyne injector and a Waters R401 differential refractometer detector. Compounds were detected on a Kipp & Zonen BD40 recorder, and were collected manually.

*High resolution mass spectrometry*

High resolution mass spectrometry analyses were performed on a micrOTOF-Q II™ ESI-Qq-TOF in positive electrospray ionization mode.

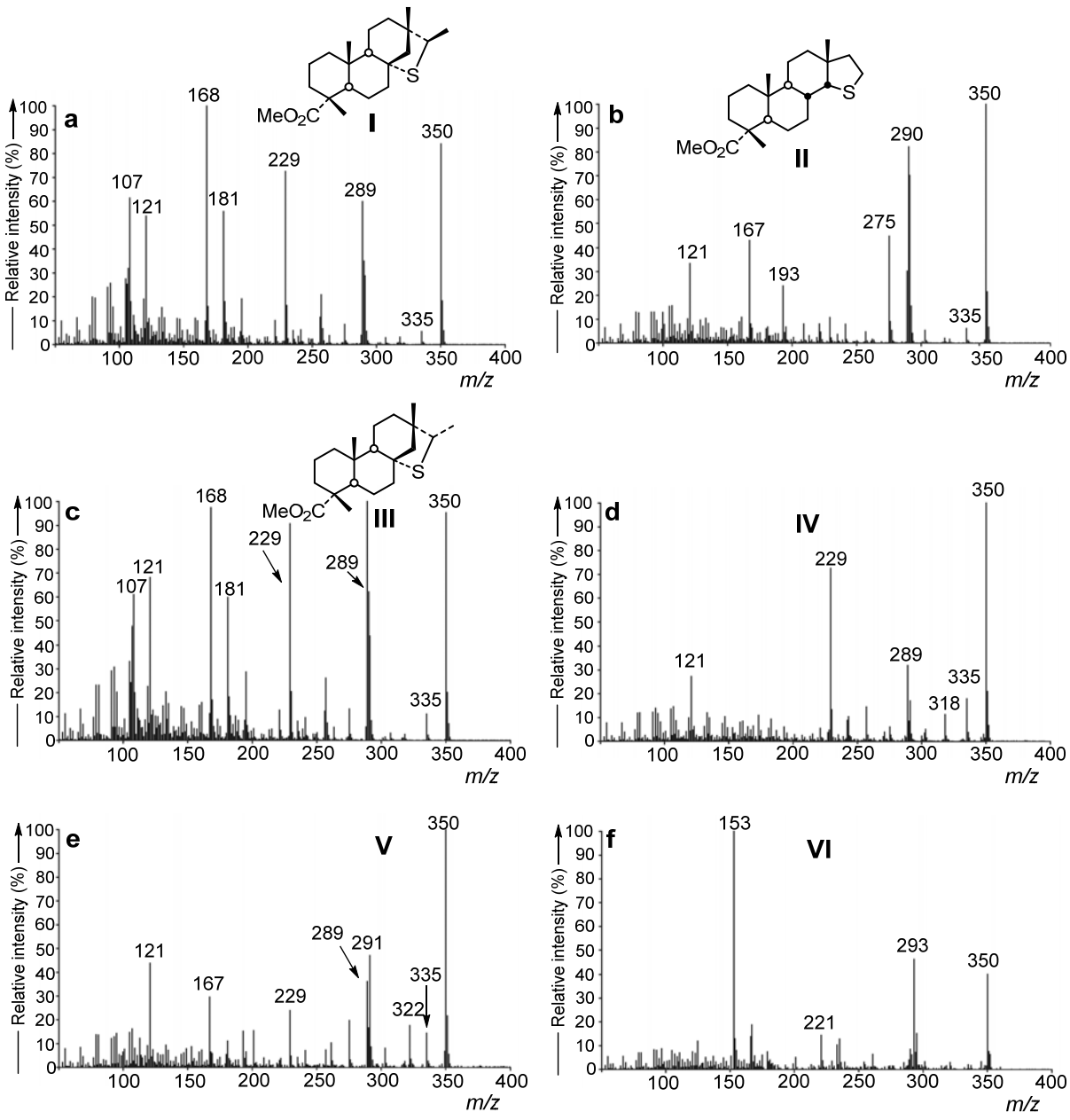
Compound **I**: *m/z* 351,2351 $[M+H]^+$ (calculated for $C_{21}H_{35}O_2S$: 351.2352).

Compound **II**: *m/z* 351,2354 $[M+H]^+$ (calculated for $C_{21}H_{35}O_2S$: 351,2352).

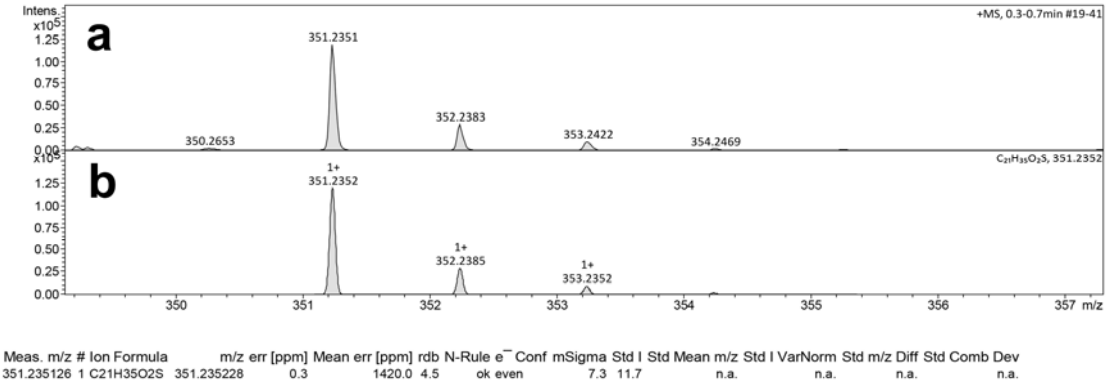
*Nuclear magnetic resonance spectroscopy*

NMR analyses were performed on a Bruker Avance I – 500 MHz spectrometer operating at an observation frequency of 500 MHz ($^1H$) and 125 MHz ($^{13}C$). The chemical shifts are reported in ppm relative to tetramethylsilane with the solvent used as internal standard ($CDCl_3$: $\delta^1H$ 7.24 ppm; $\delta^{13}C$ 77.0 ppm). NMR analyses comprised 1D ($^1H$, $^{13}C$, DEPT) as well as 2D homo- ($^1H$-$^1H$ COSY, $^1H$-$^1H$-NOESY) and heteronuclear ($^1H$-$^{13}C$-HSQC, and $^1H$-$^{13}C$-HMBC) experiments.

## 2. Mass spectrometric data



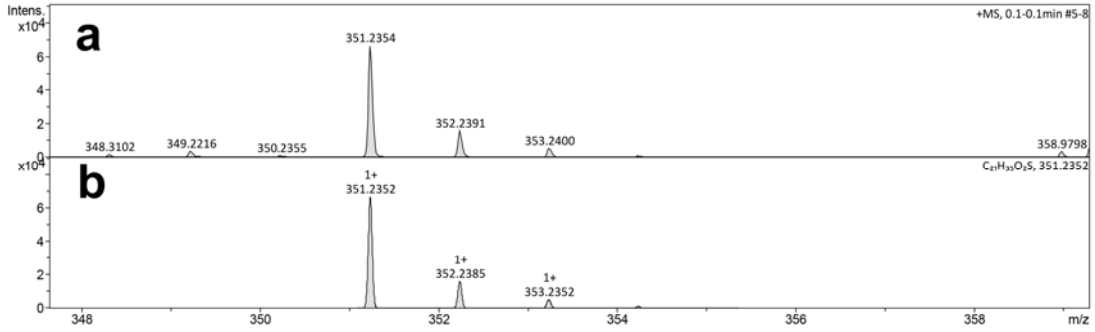

**Supplementary figure S1 a-f:** Mass spectra (GC-MS, EI, 70 eV) of compounds **I-VI** (cf. Figure 2) belonging to the series of compounds having a molecular mass of 350 Da present in the apolar fraction $F_A$ isolated from the methylated extract of the Piolenc amber sample.




| Meas. m/z | # | Ion Formula | m/z err [ppm] | Mean err [ppm] | rdb | N-Rule | e¯ Conf | mSigma | Std I | Std Mean m/z | Std I | VarNorm | Std m/z | Diff Std Comb | Dev |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 351.235126 | 1 | C21H35O2S | 351.235228 | 0.3 | 1420.0 | 4.5 | ok | even | 7.3 | 11.7 | n.a. | n.a. | n.a. | n.a. | |

**Supplementary figure S2** : High resolution mass spectrum (ESI-Qq-TOF) of compound **I**.

(a) measured spectrum; (b) calculated spectrum for the formula $C_{21}H_{35}O_2S$.

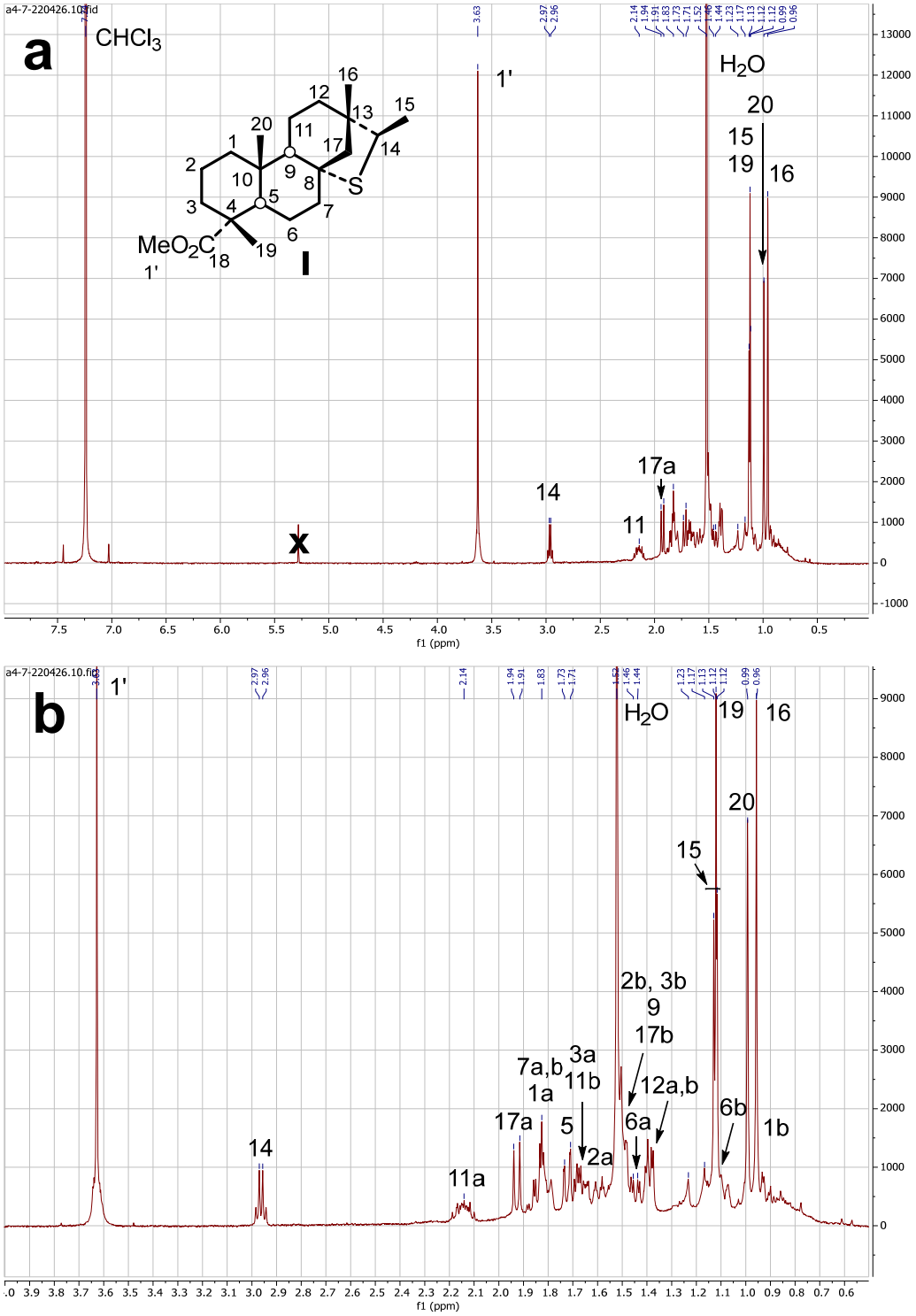



| Meas. m/z | # | Ion Formula | m/z err [ppm] | Mean err [ppm] | rdb | N-Rule | e¯ Conf | mSigma | Std I | Std Mean m/z | Std I | VarNorm | Std m/z | Diff Std Comb | Dev |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 351.235432 | 1 | C21H35O2S | 351.235228 | -0.6 | 7.7 | 4.5 | ok | even | 2.5 | 3.7 | n.a. | n.a. | n.a. | n.a. | |

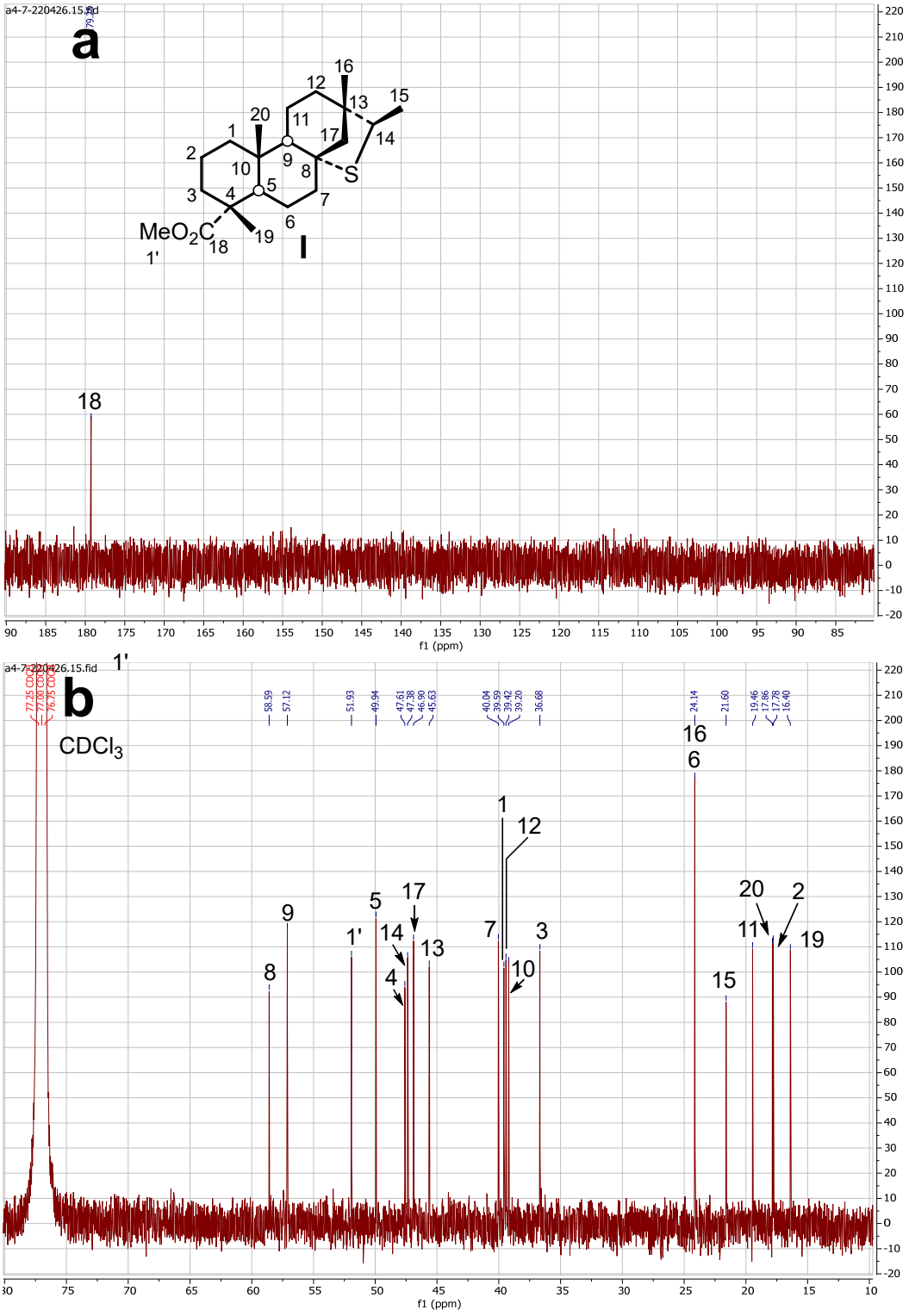
**Supplementary figure S3** : High resolution mass spectrum (ESI-Qq-TOF) of compound **II**.

(a) measured spectrum; (b) calculated spectrum for the formula $C_{21}H_{35}O_2S$.

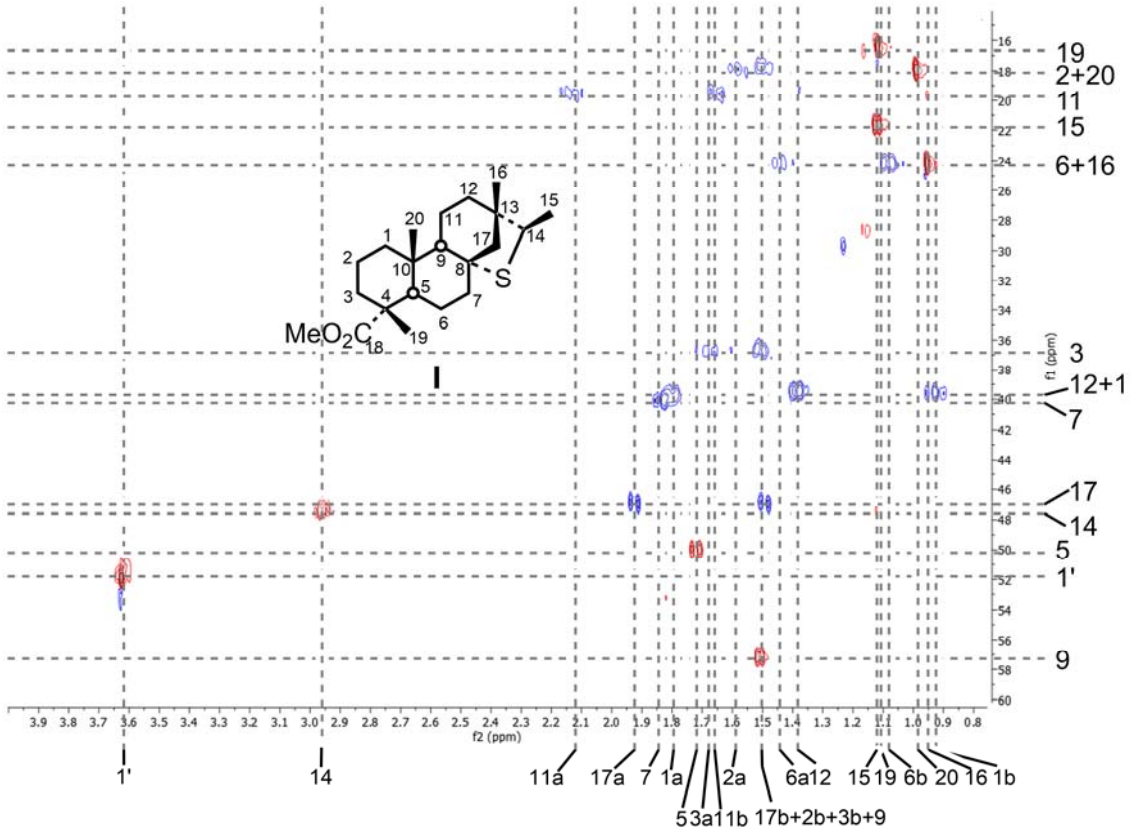
## 3. NMR Data



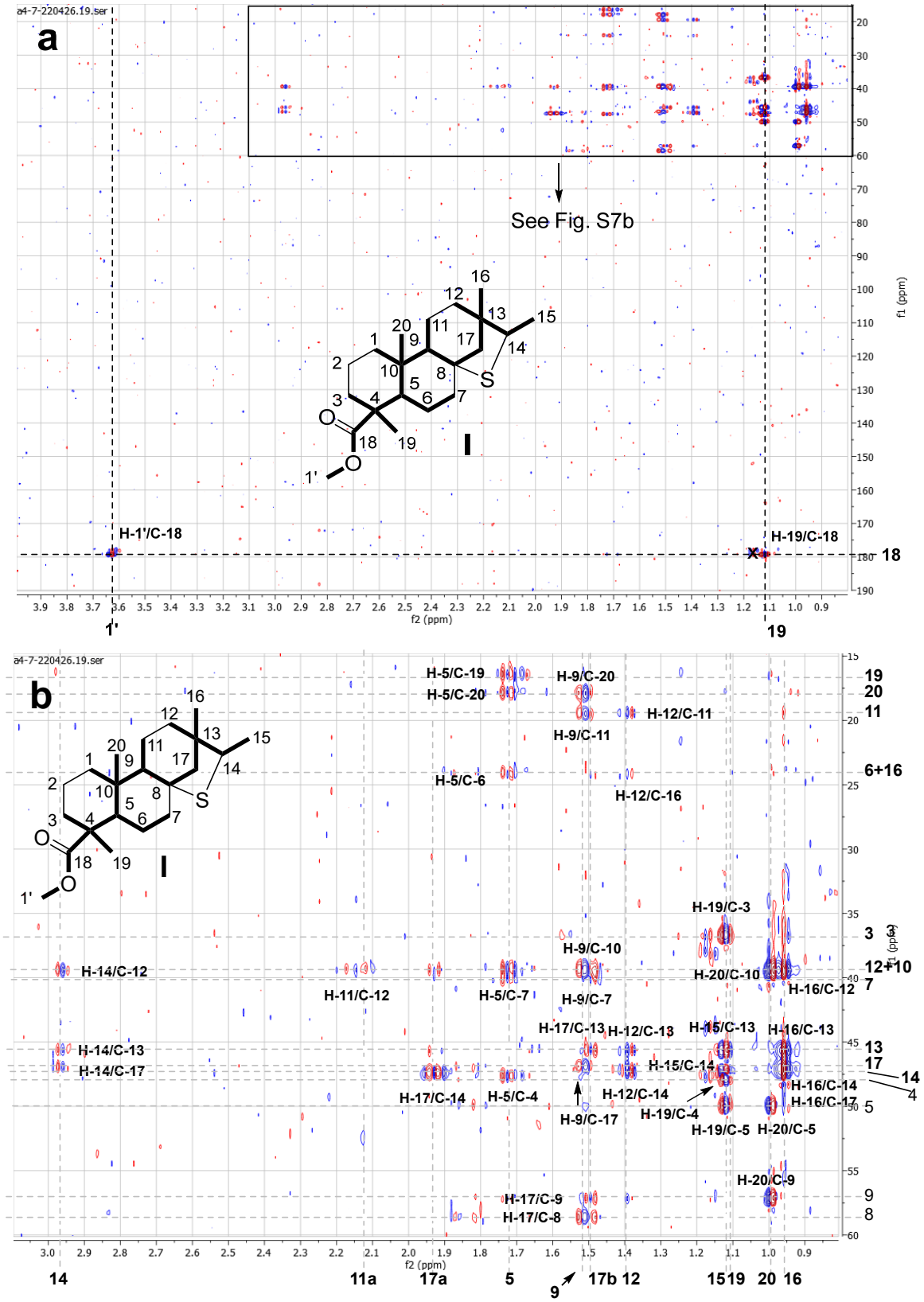

**Supplementary figure S4**: $^{1}$H-NMR spectrum (500 MHz, $CDCl_3$) of compound **I** (a) 0.0-8.0 ppm range; (b) 0.5-4.0 ppm range.



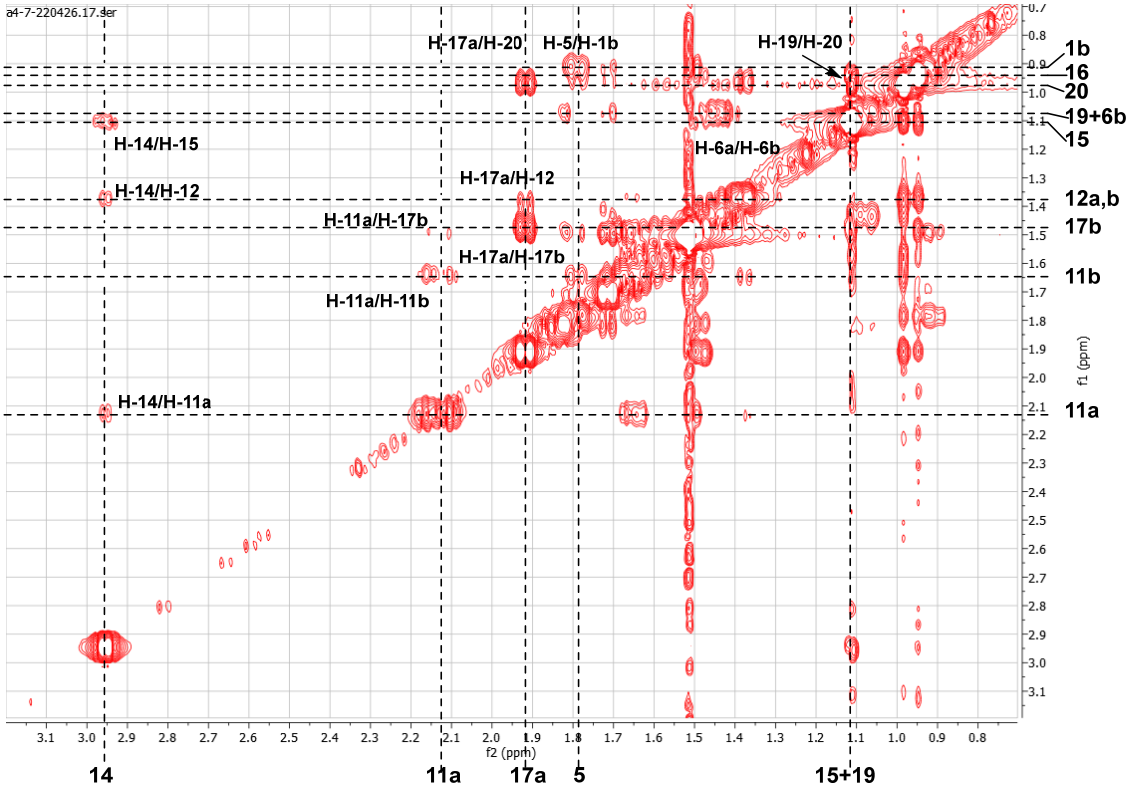

**Supplementary figure S5**: $^{13}$C-NMR spectrum (125 MHz, $CDCl_3$) of compound **I** (a) 80-190 ppm range; (b) 10-80 ppm range.



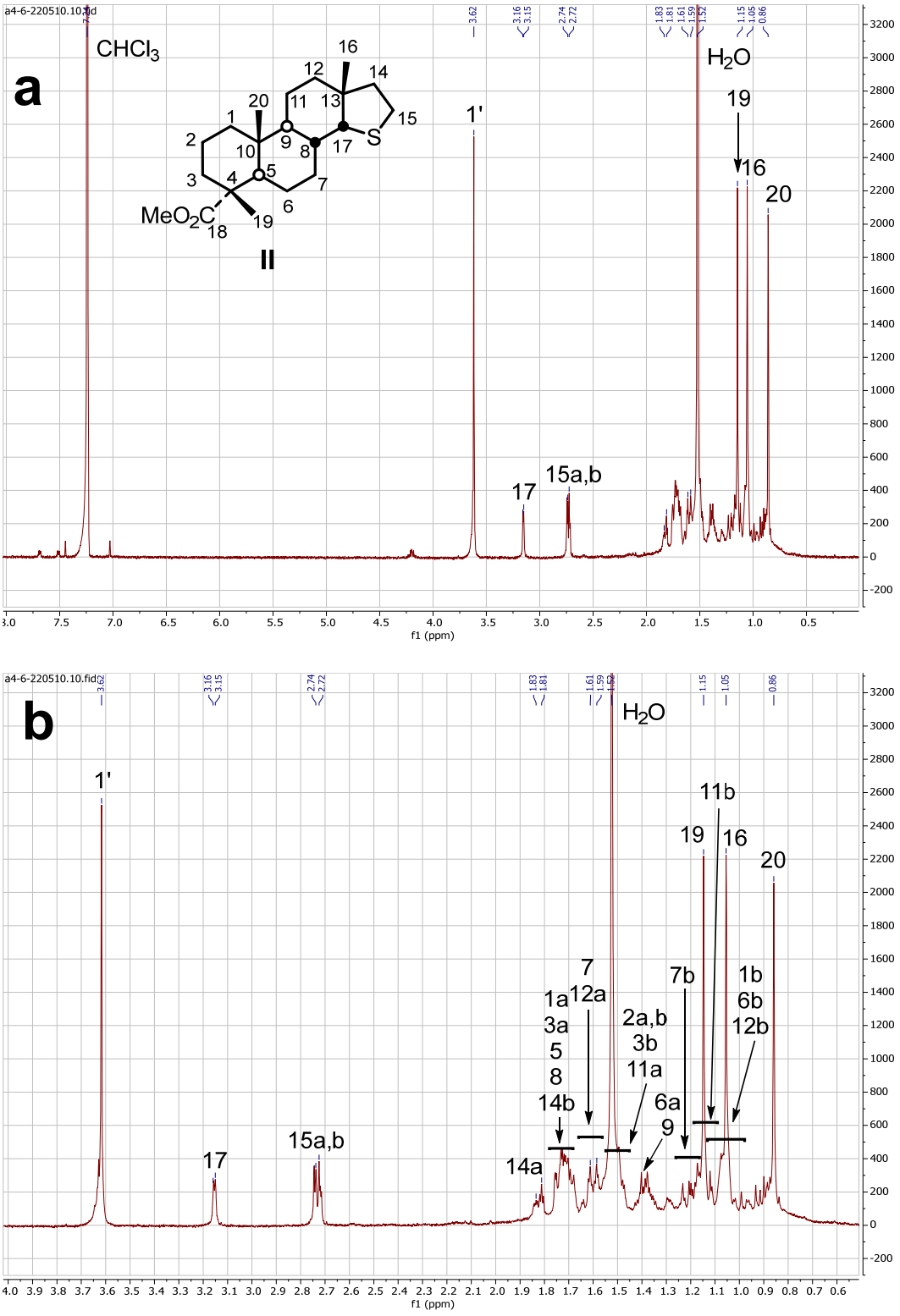

**Supplementary figure S6**: $^{1}$H-$^{13}$C one bond ($^{1}J$) correlation pattern (HSQC, 500 MHz, $CDCl_3$) for compound **I** ($^{1}$H: 0.75-4.00 ppm range/ $^{13}$C: 15-60 ppm range).



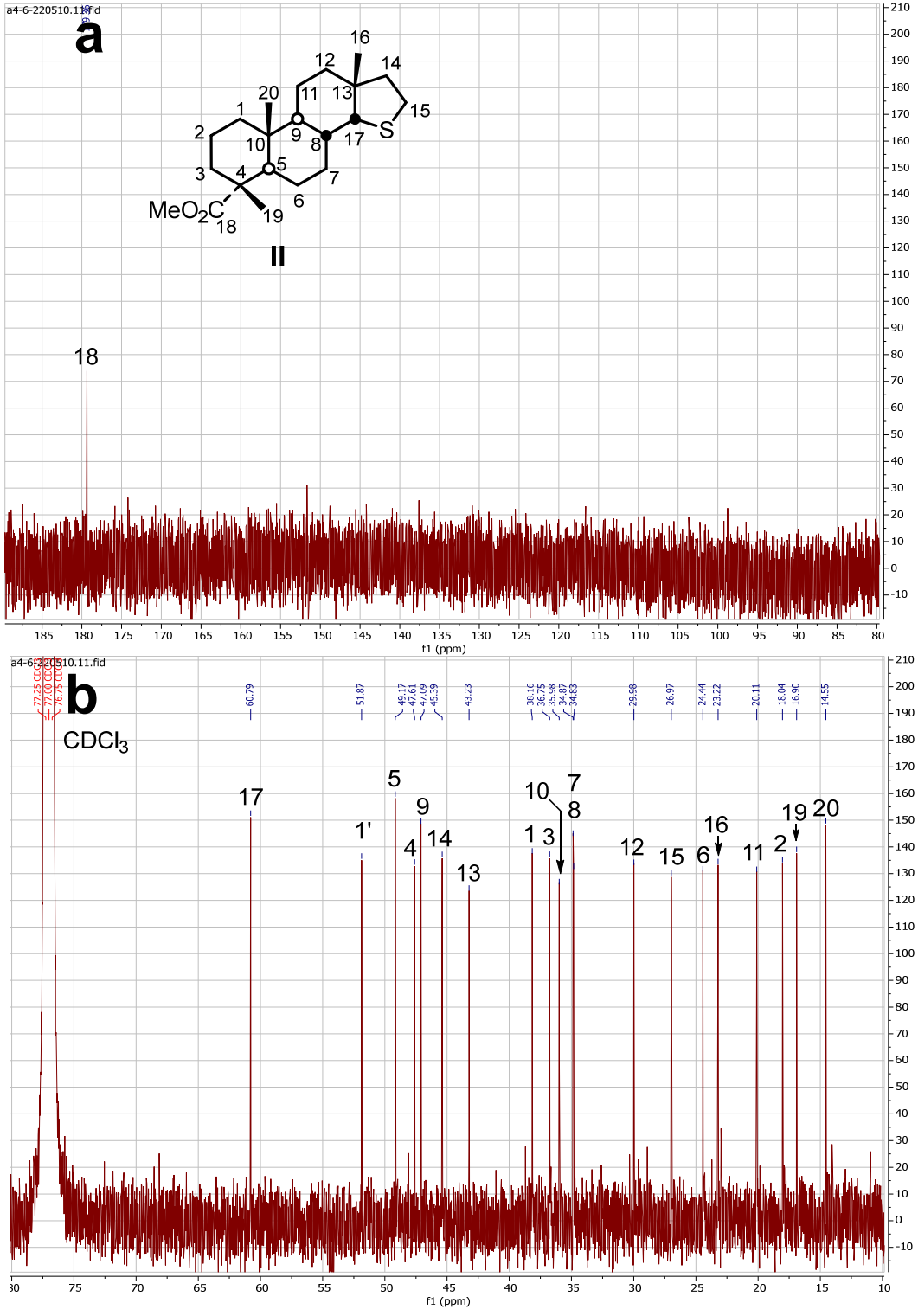

**Supplementary figure S7**: $^{1}$H-$^{13}$C long range ($^{2,3}J$) correlation pattern (HMBC, 500 MHz, $CDCl_3$) for compound **I**. (a) $^{1}$H: 0.8-4.0 ppm range/ $^{13}$C: 15-190 ppm range; (b) $^{1}$H: 0.8-3.1 ppm range/ $^{13}$C: 15-60 ppm range.



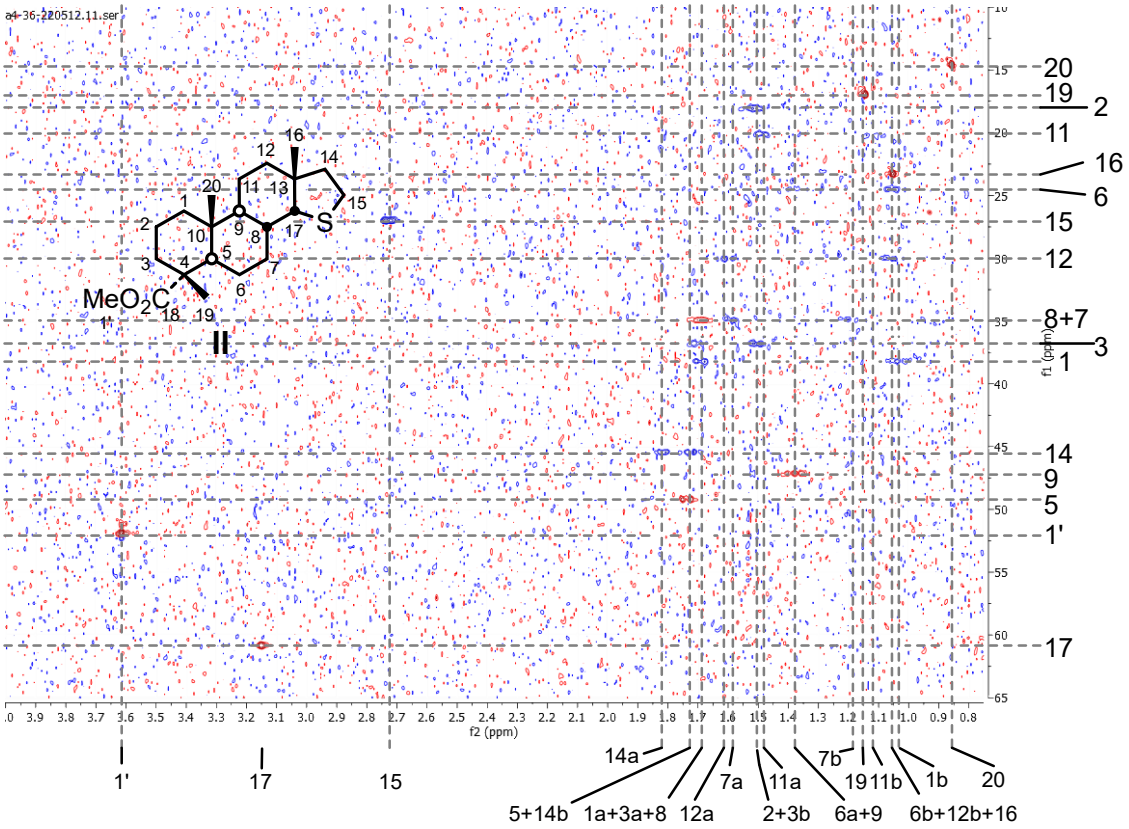

**Supplementary figure S8**: $^{1}$H-$^{1}$H NOESY correlation pattern (500 MHz, $CDCl_3$) for compound **I** (0.7-3.2 ppm range).



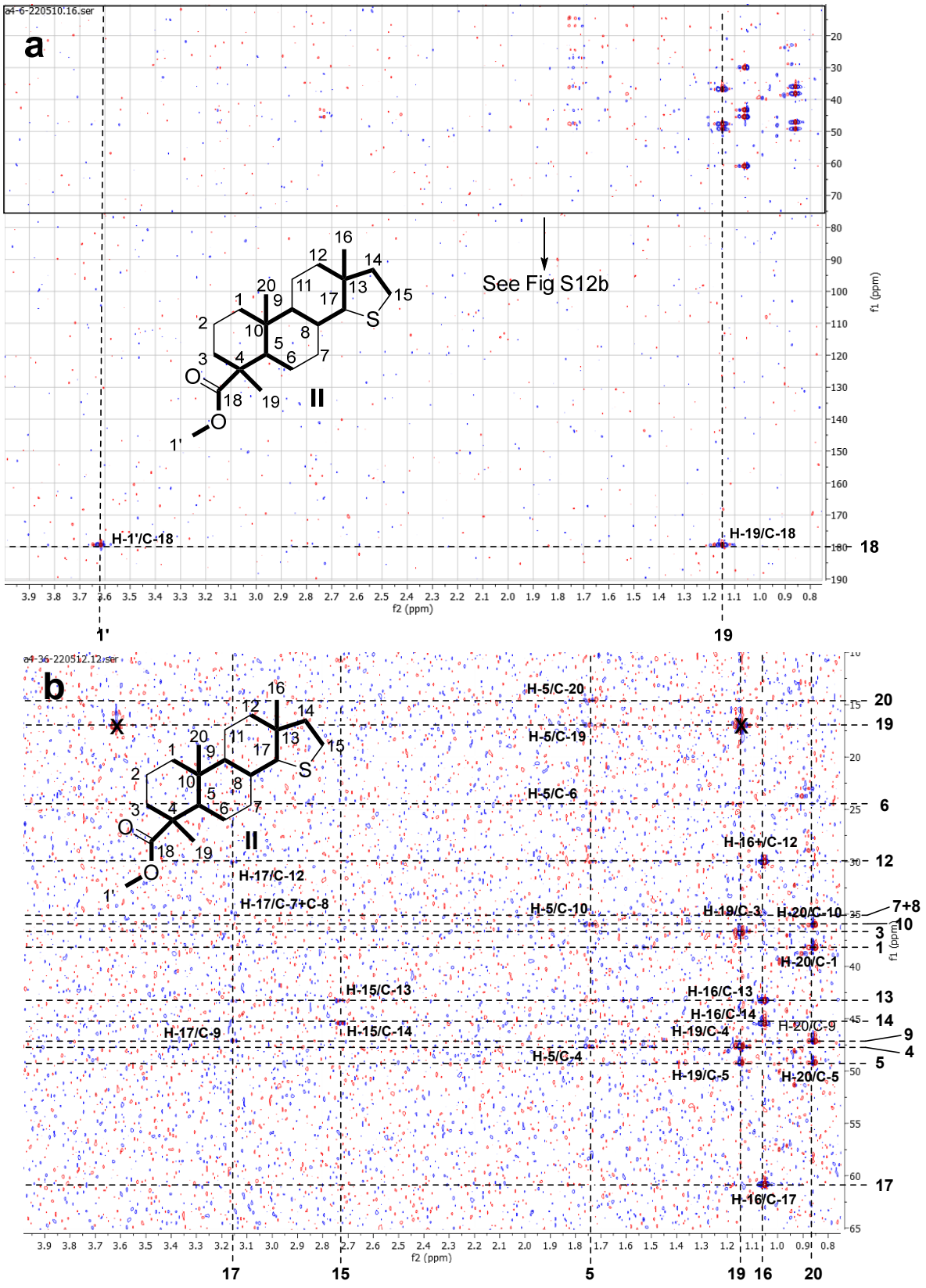

**Supplementary figure S9**: $^{1}$H-NMR spectrum (500 MHz, $CDCl_3$) of compound **II**. (a) 0.0-8.0 ppm range; (b) 0.5-4.0 ppm range.



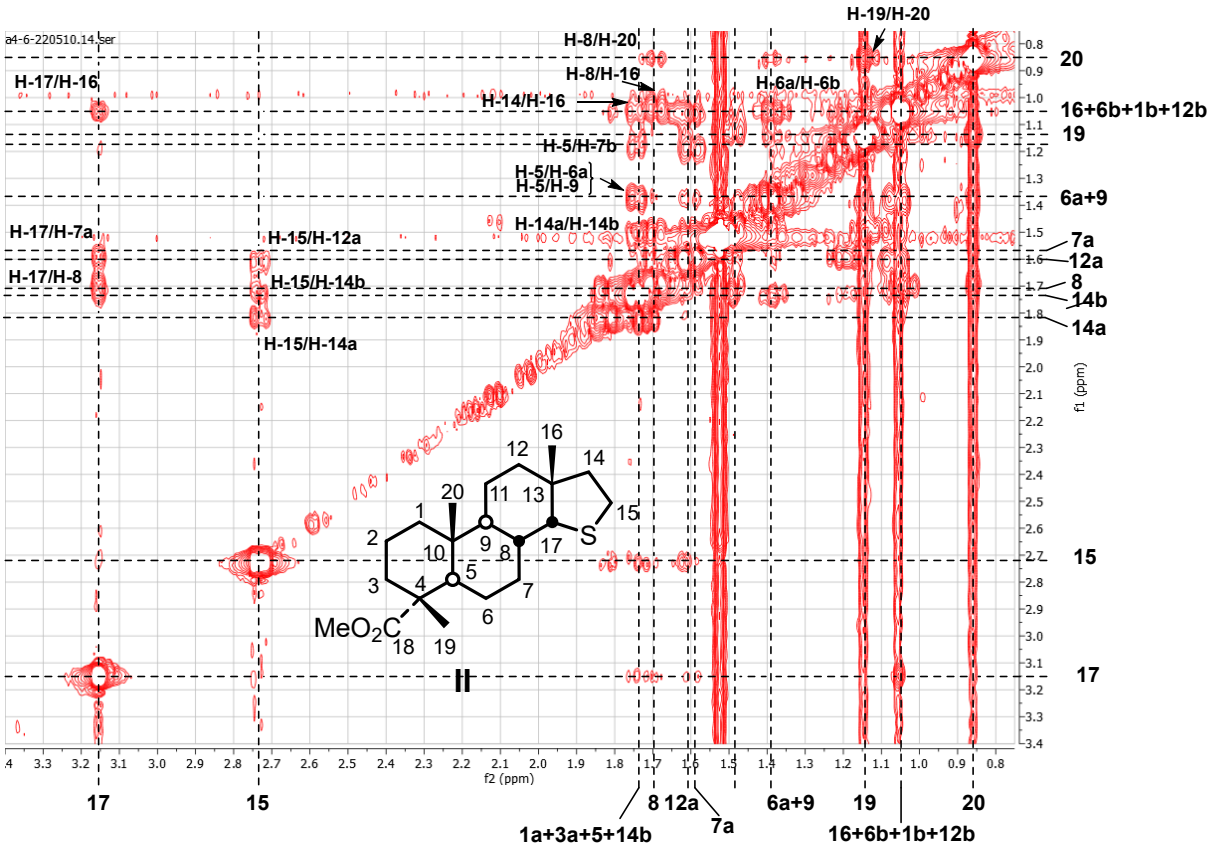

**Supplementary figure S10**: $^{13}$C-NMR spectrum (500 MHz, $CDCl_3$) of compound **II**. (a) 80-195 ppm range; (b) 10-80 ppm range.




**Supplementary figure S11**: $^{1}$H-$^{13}$C one bond ($^{1}J$) correlation pattern (HSQC, 500 MHz, $CDCl_3$) for compound **II** ($^{1}$H: 0.75-4.00 ppm range; $^{13}$C: 10-65 ppm range).




**Supplementary figure S12**: $^{1}$H-$^{13}$C long range ($^{2,3}J$) correlation pattern (HMBC, 500 MHz, $CDCl_3$) for compound **II**. (a) $^{1}$H: 0.75-4.0 ppm range; $^{13}$C: 10-190 ppm range; (b) $^{1}$H: 0.75-4.0 ppm range; $^{13}$C: 10-65 ppm range. **X** : artefacts.




**Supplementary figure S13**: $^{1}$H-$^{1}$H NOESY correlation pattern (500 MHz, $CDCl_3$) for compound **II** (0.75-3.4 ppm range).

## 4. References


1. B. Gomez, G. Barale, D. Saad, V. Perrichot, Santonian Angiosperm-dominated leaf assemblage from Piolenc (Vaucluse, Sud-Est de la France). *C. R. Palevol* 2003, **2**, 197-204.

2. N. de Lama Valderrama, P. Schaeffer, A. Leprince, S. Schmitt, P. Adam, Novel oxygenated fossil nor-diterpenoids from Cretaceous amber (South-Western France) as potential markers from Cupressaceae and/or Cheirolepidiaceae. *Org. Geochem.*, 2022, **167**, 104372.