

# The effect of Cytochalasans on the Actin Cytoskeleton of Eukaryotic Cells and Preliminary Structure-Activity Relationships

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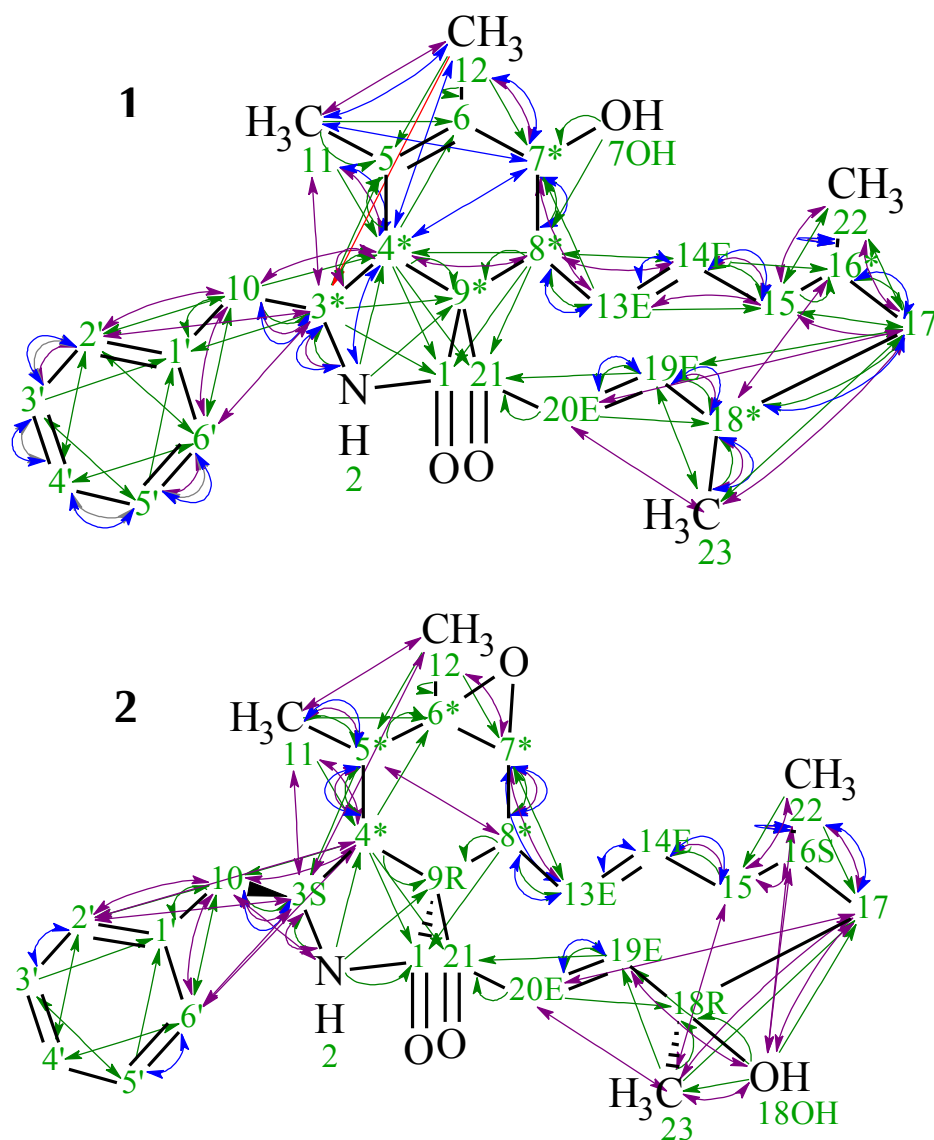
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-----Supporting Information-----



Figures S1 & S2. COSY and TOCSY (blue arrows), HMBC (green arrows) and ROESY (violet arrows) correlations indicating the structures of **1** and **2**.

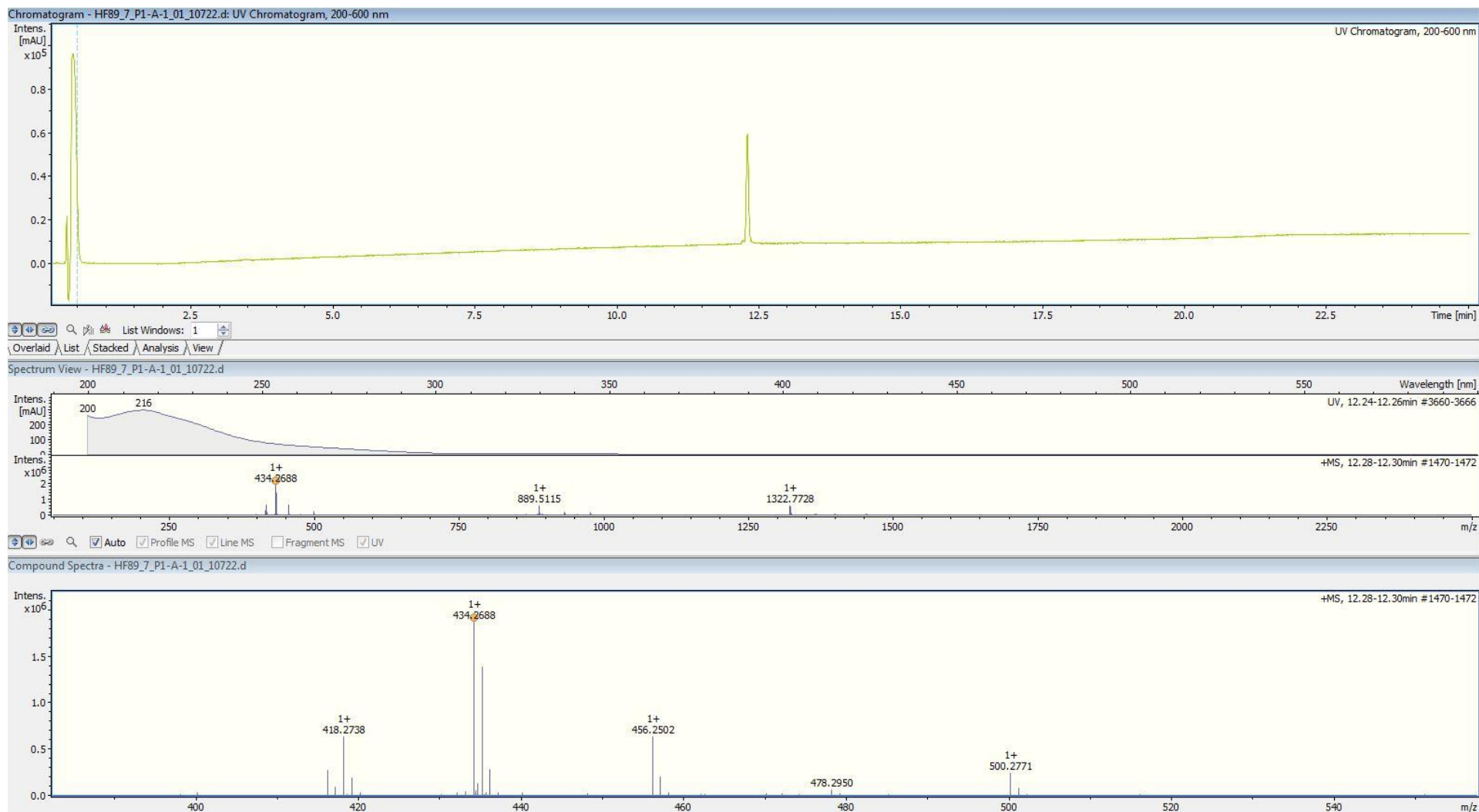


Figure S3. HPLC-HRESIMS data of **1**.

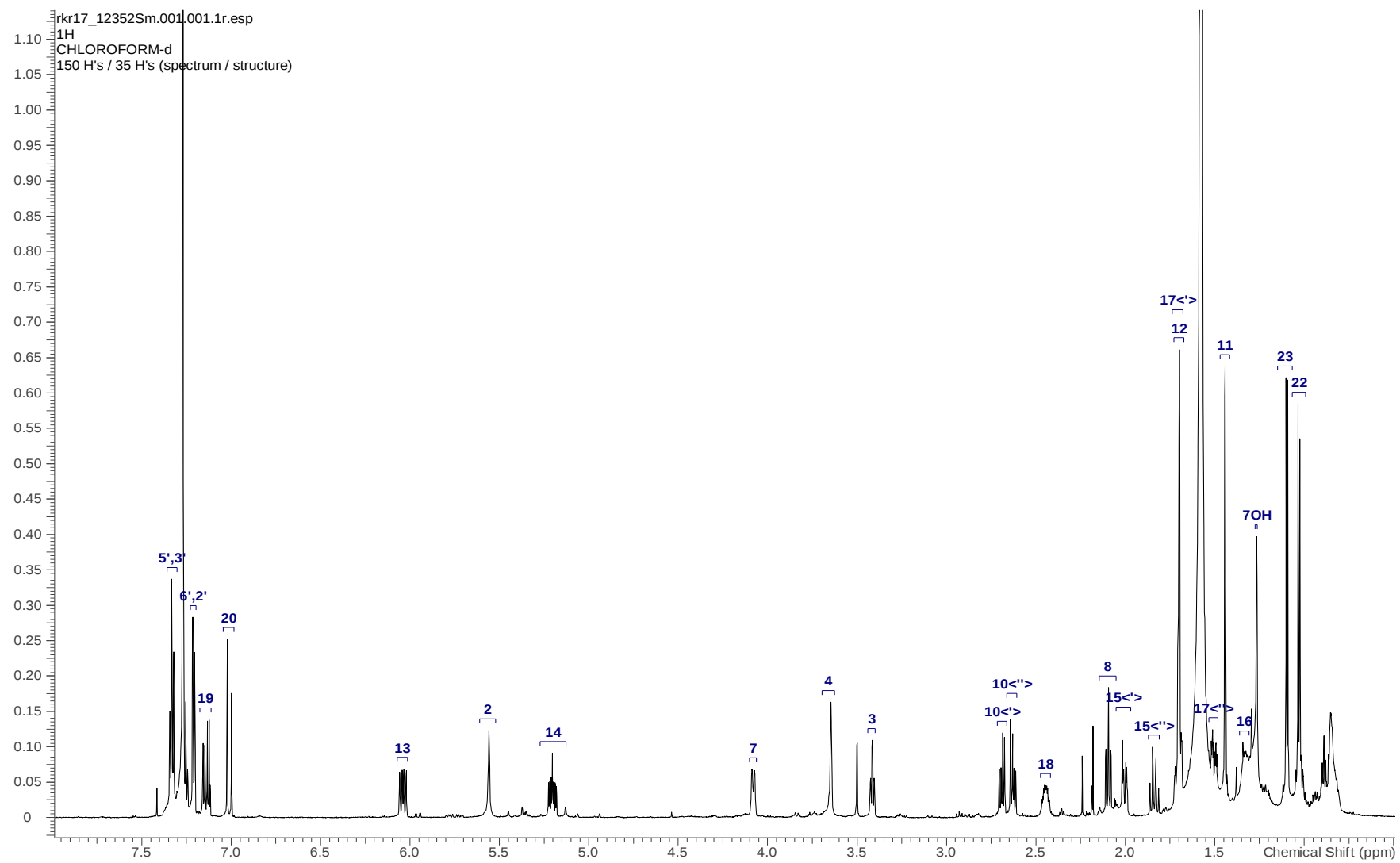
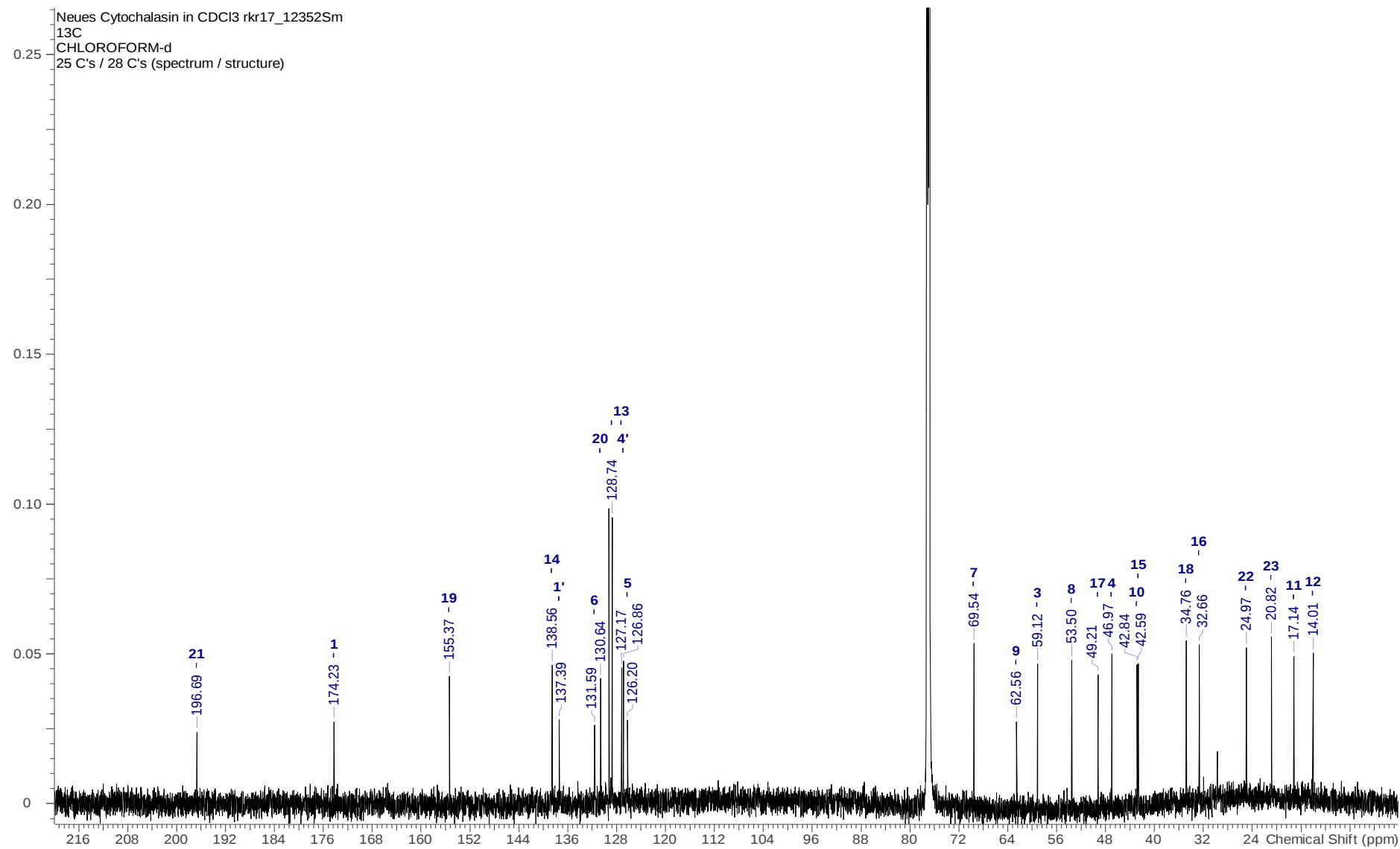
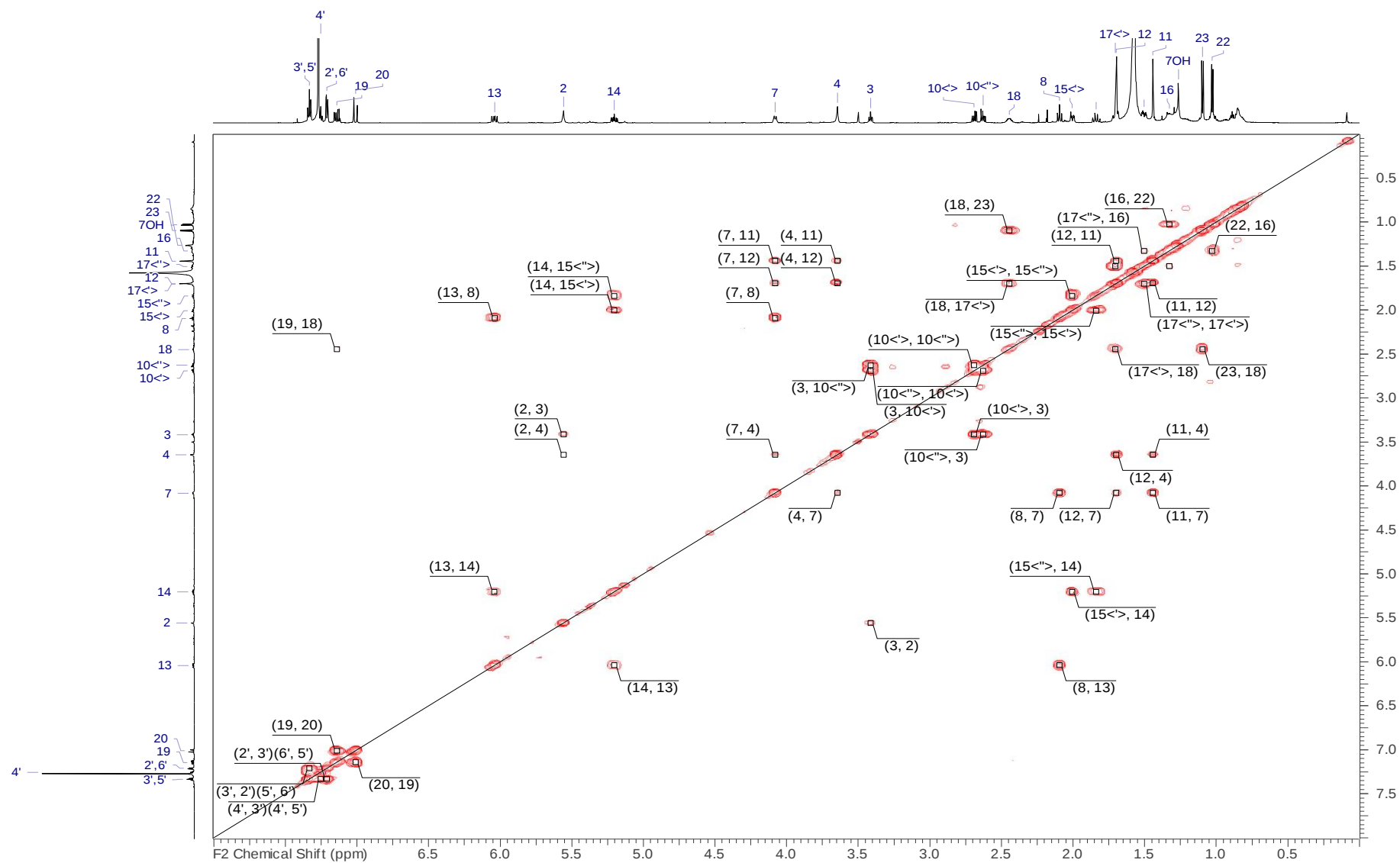
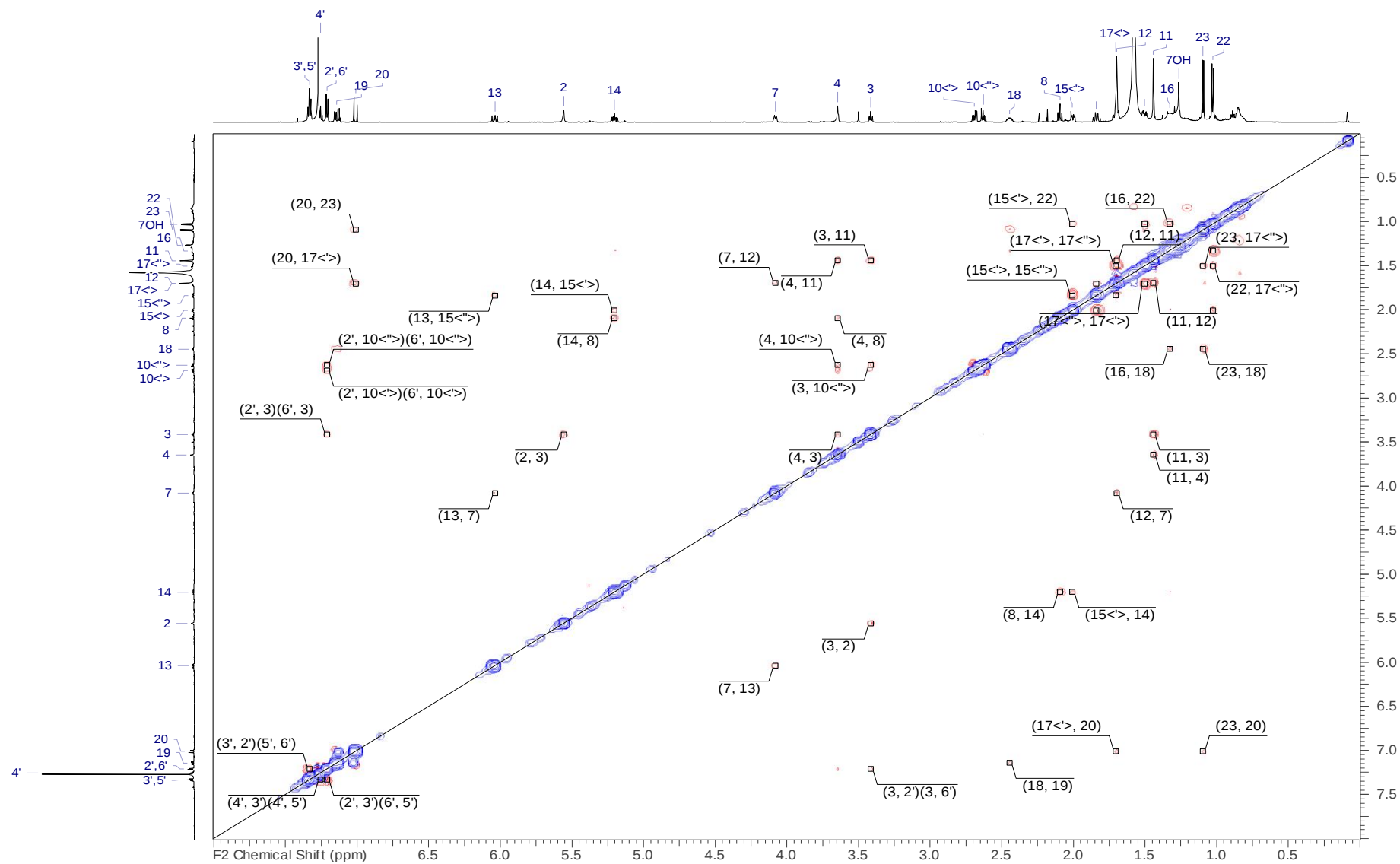
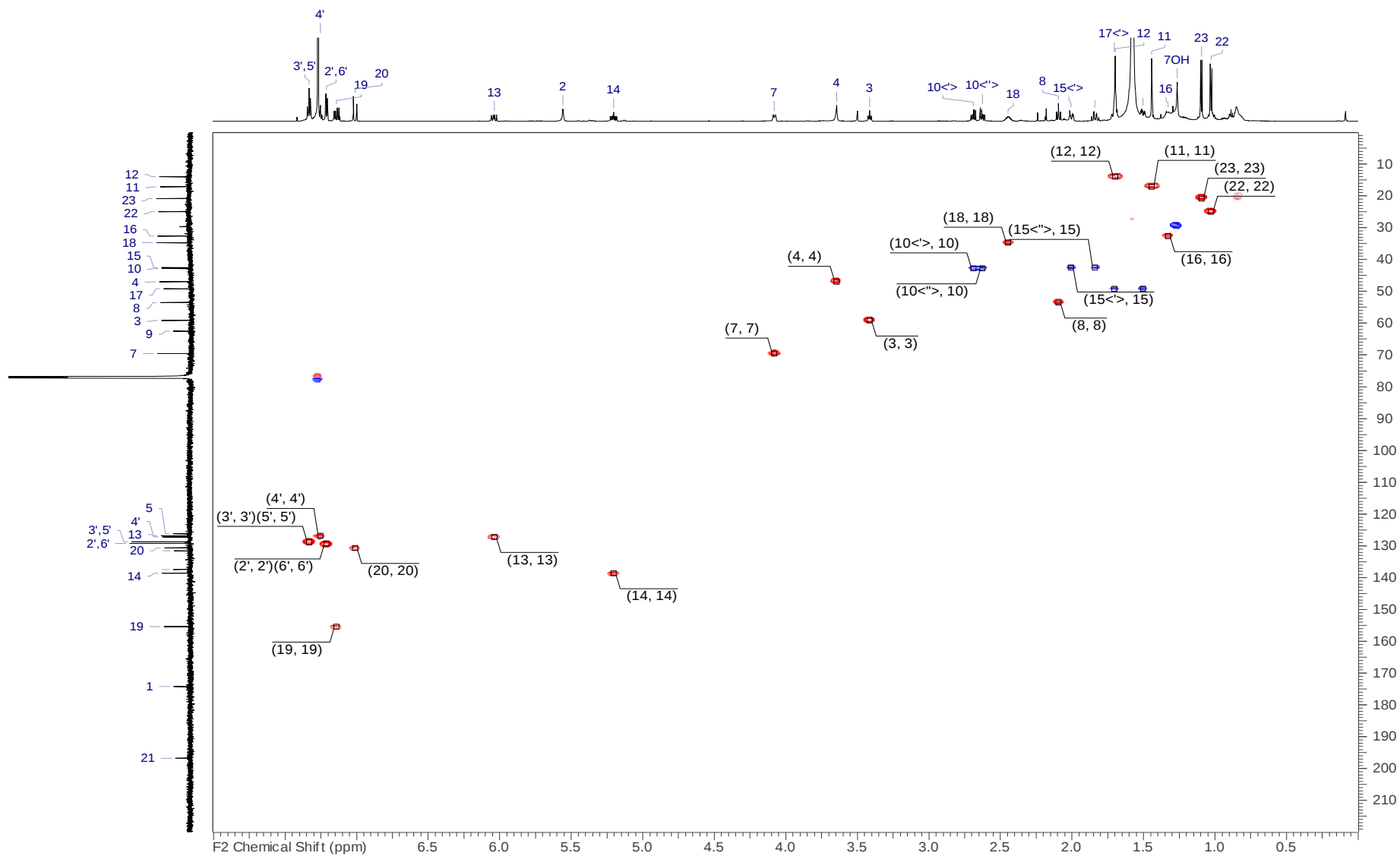


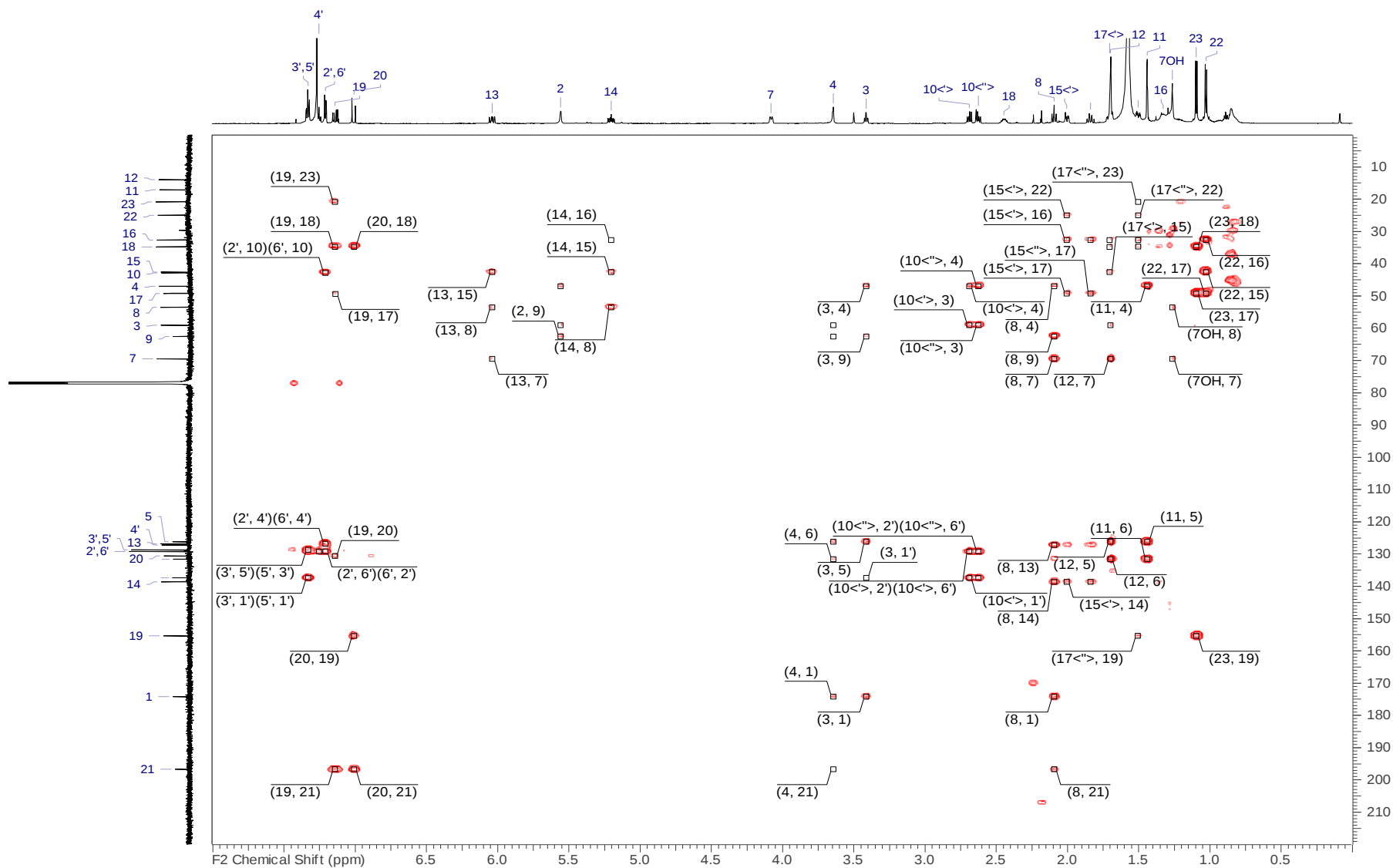
Figure S4.  $^1\text{H}$  NMR spectrum (700 MHz,  $\text{CDCl}_3$ ) of **1**.

Figure S5. <sup>13</sup>C NMR spectrum (175 MHz, CDCl<sub>3</sub>) of **1**.

Figure S6. COSY NMR spectrum (700 MHz, CDCl<sub>3</sub>) 1.

Figure S7. ROESY NMR spectrum (700 MHz, CDCl<sub>3</sub>) **1**.

Figure S8. HSQC NMR spectrum (700 MHz,  $\text{CDCl}_3$ ) **1**.

Figure S9. HMBC NMR spectrum (700 MHz, CDCl<sub>3</sub>) **1**.

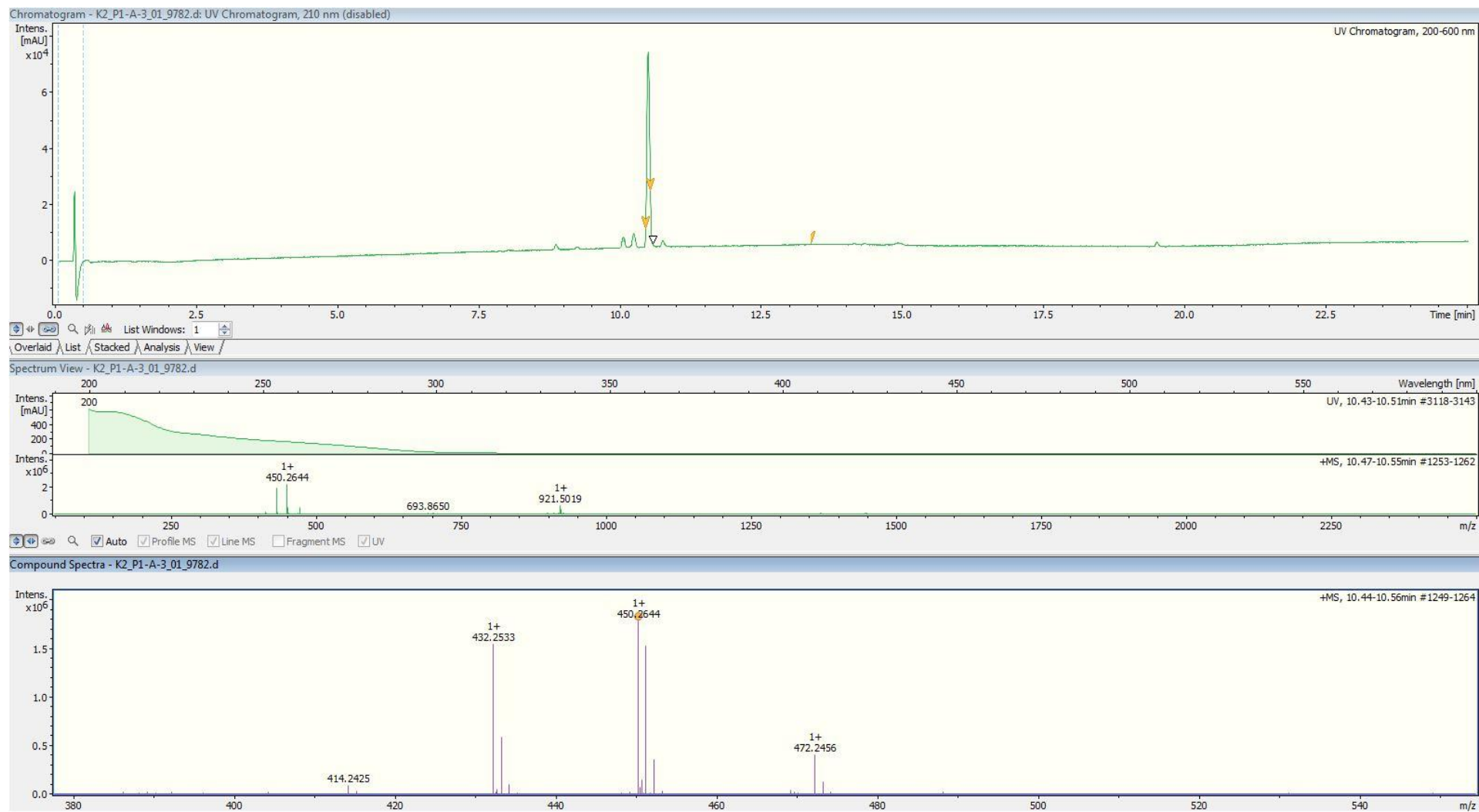


Figure S10. HPLC-HRESIMS data of of 2.

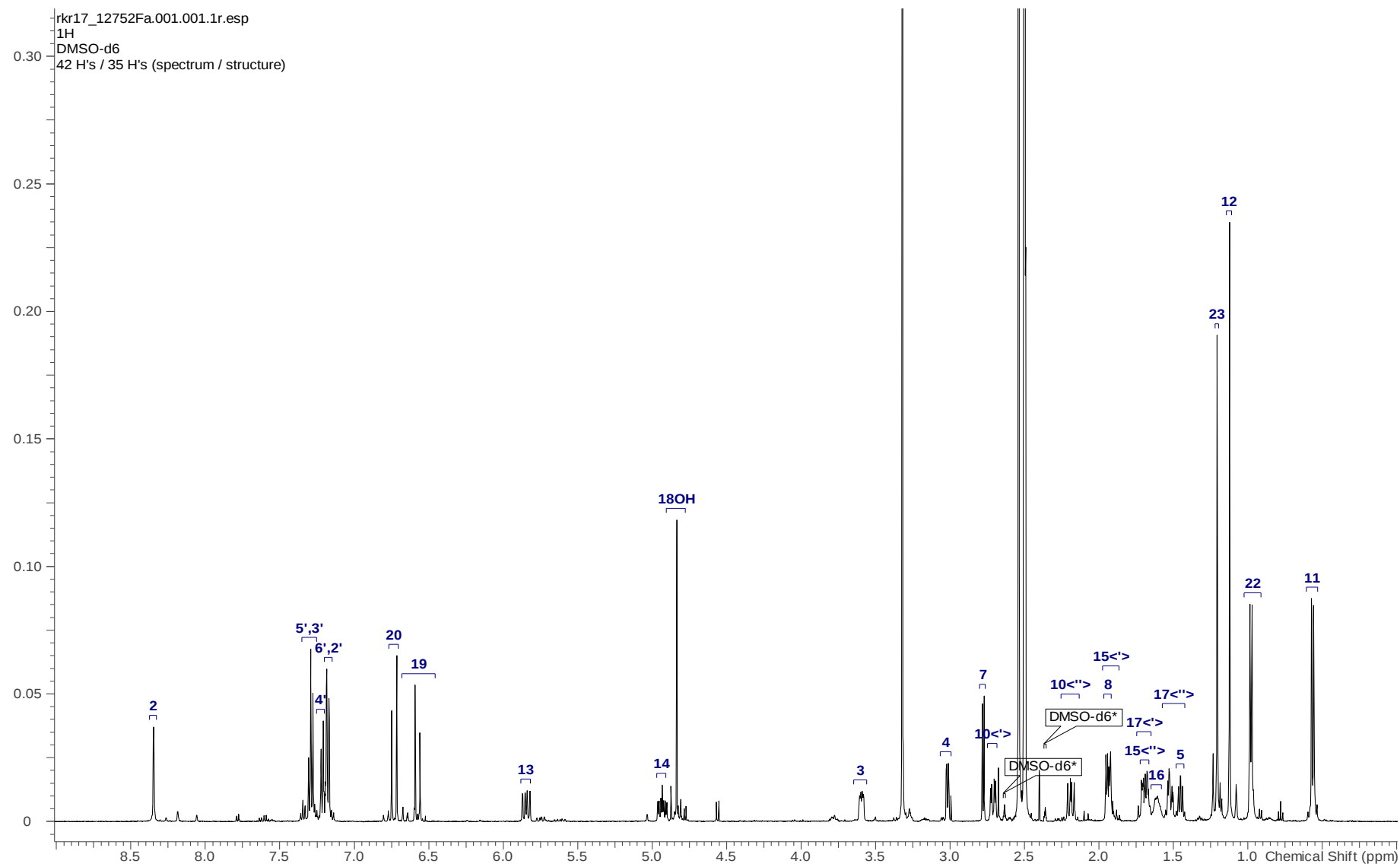
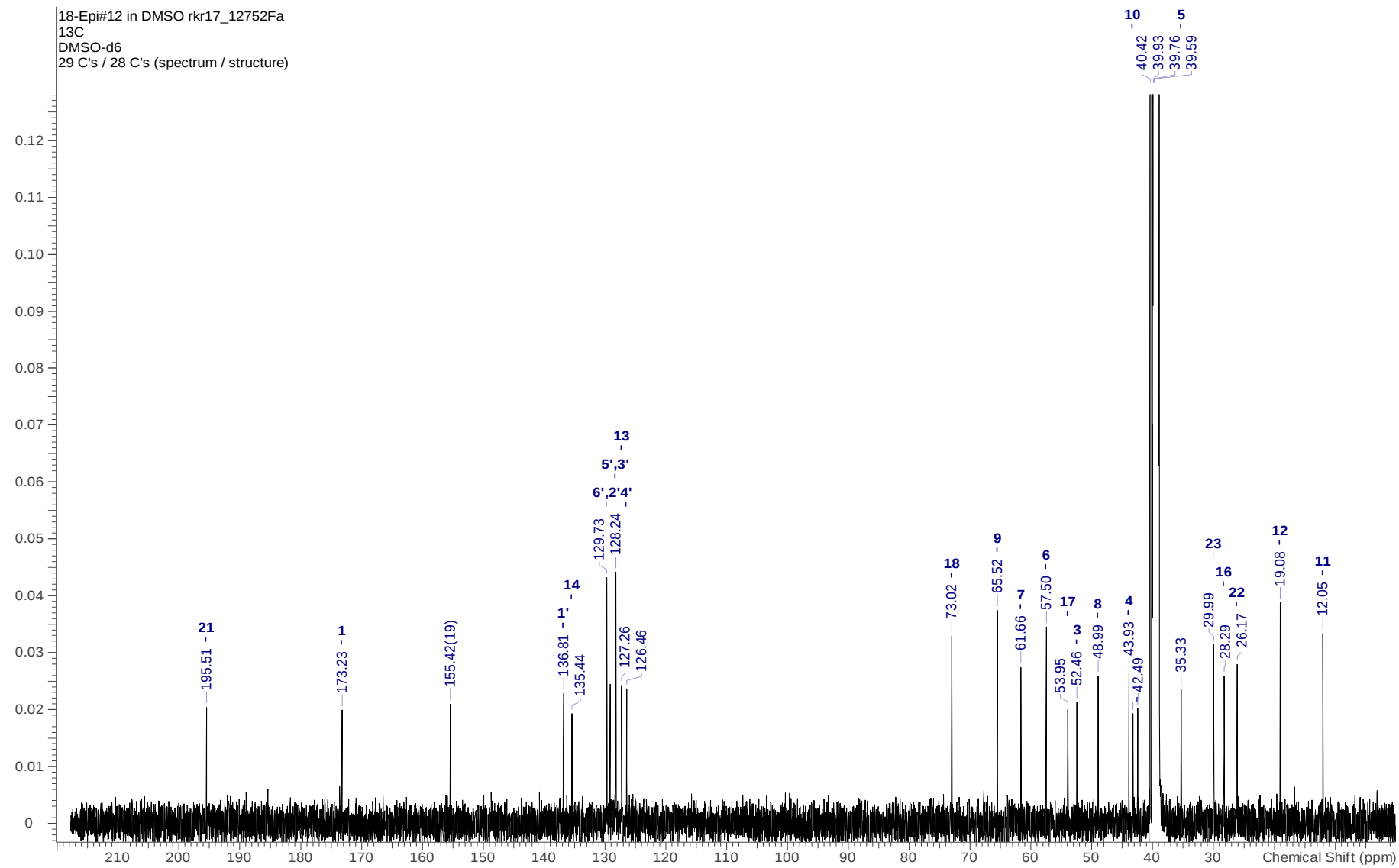
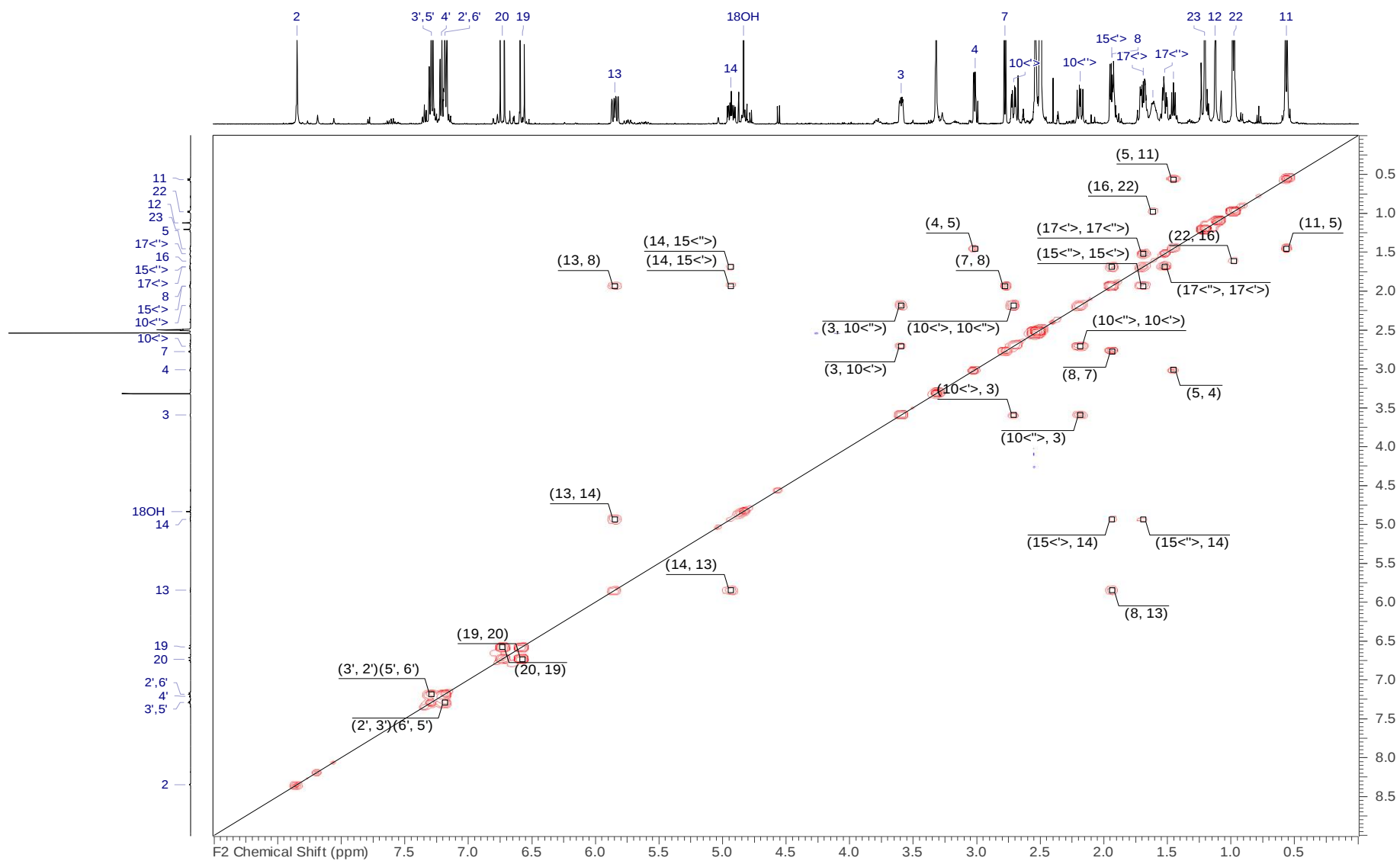
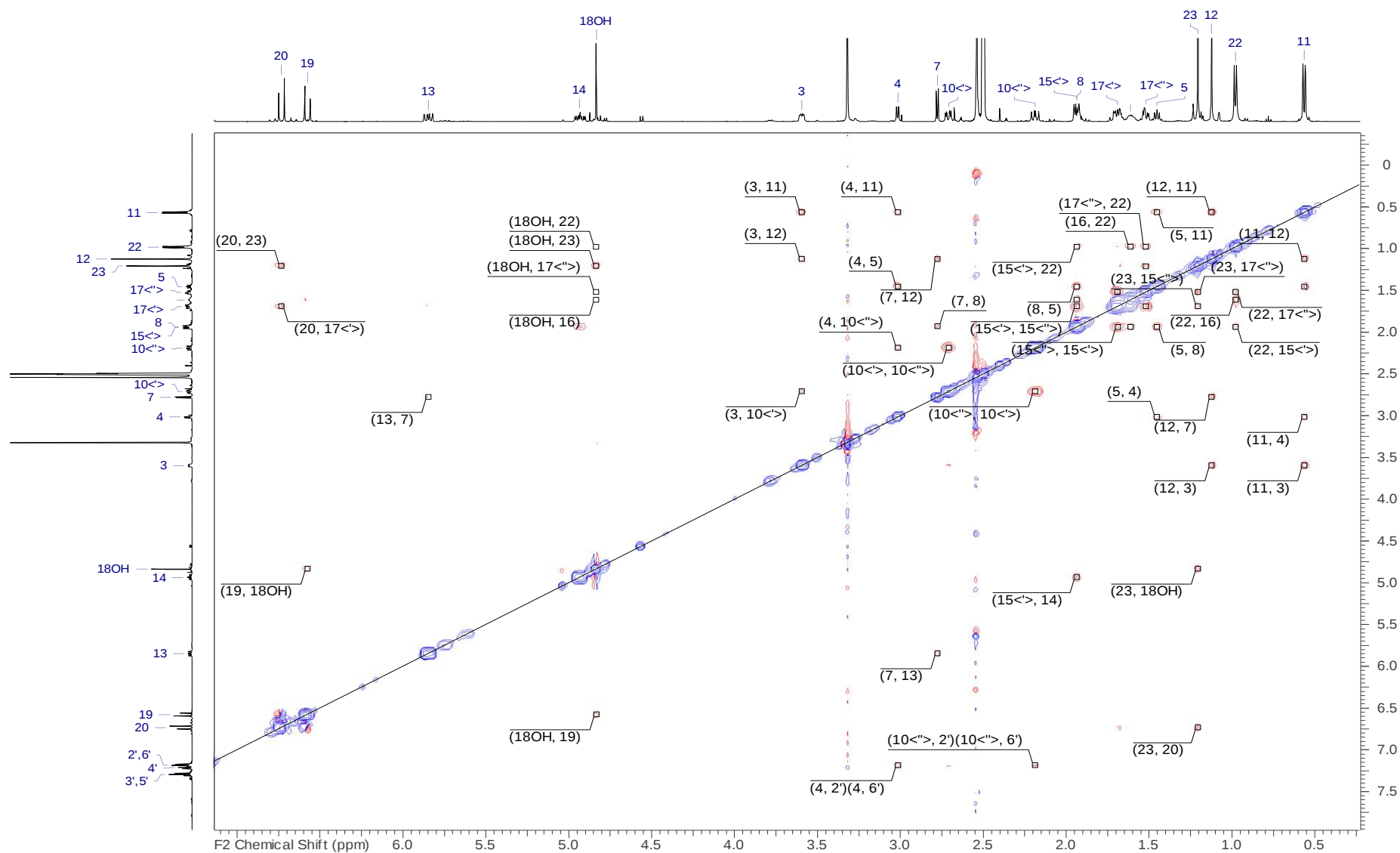
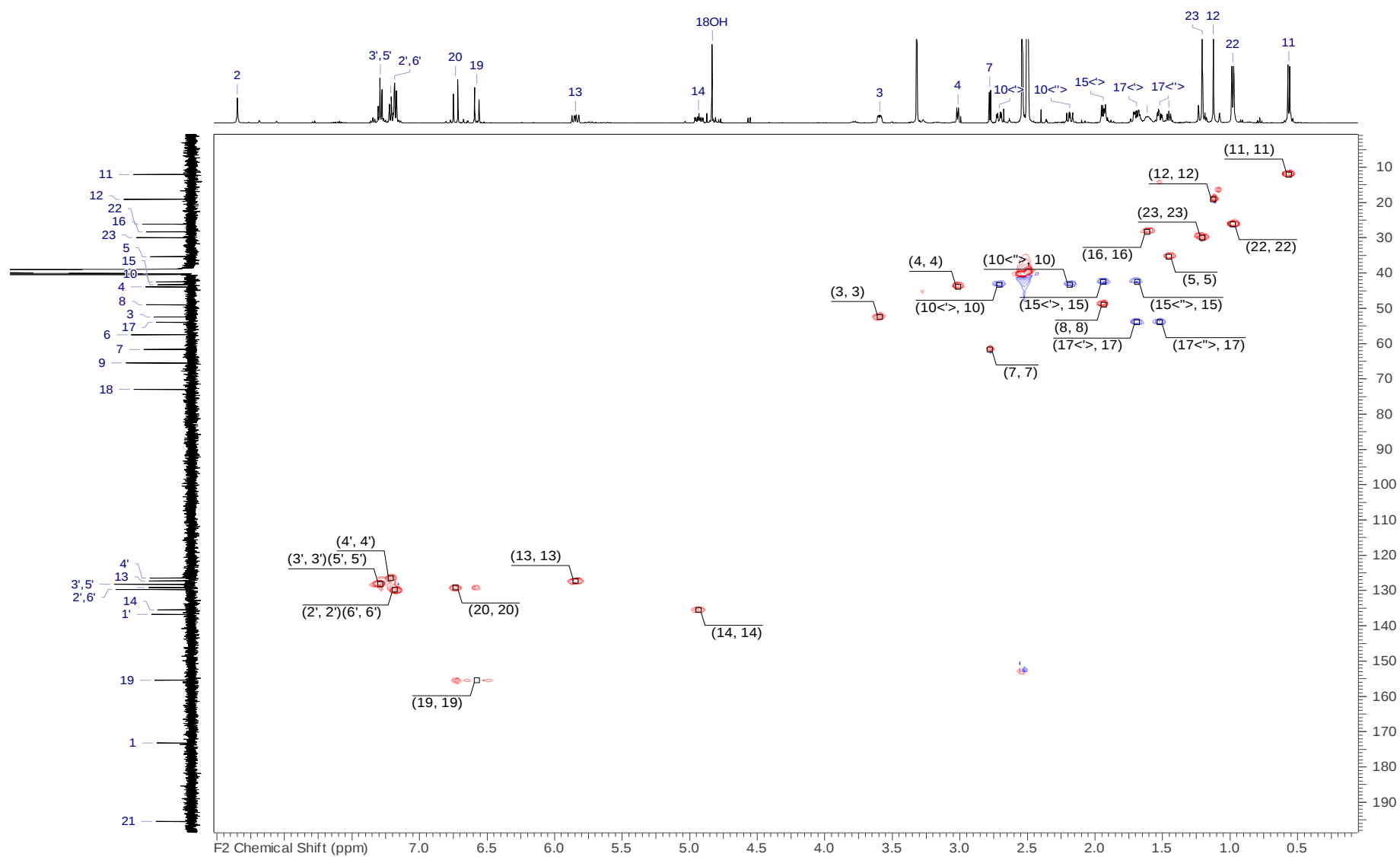


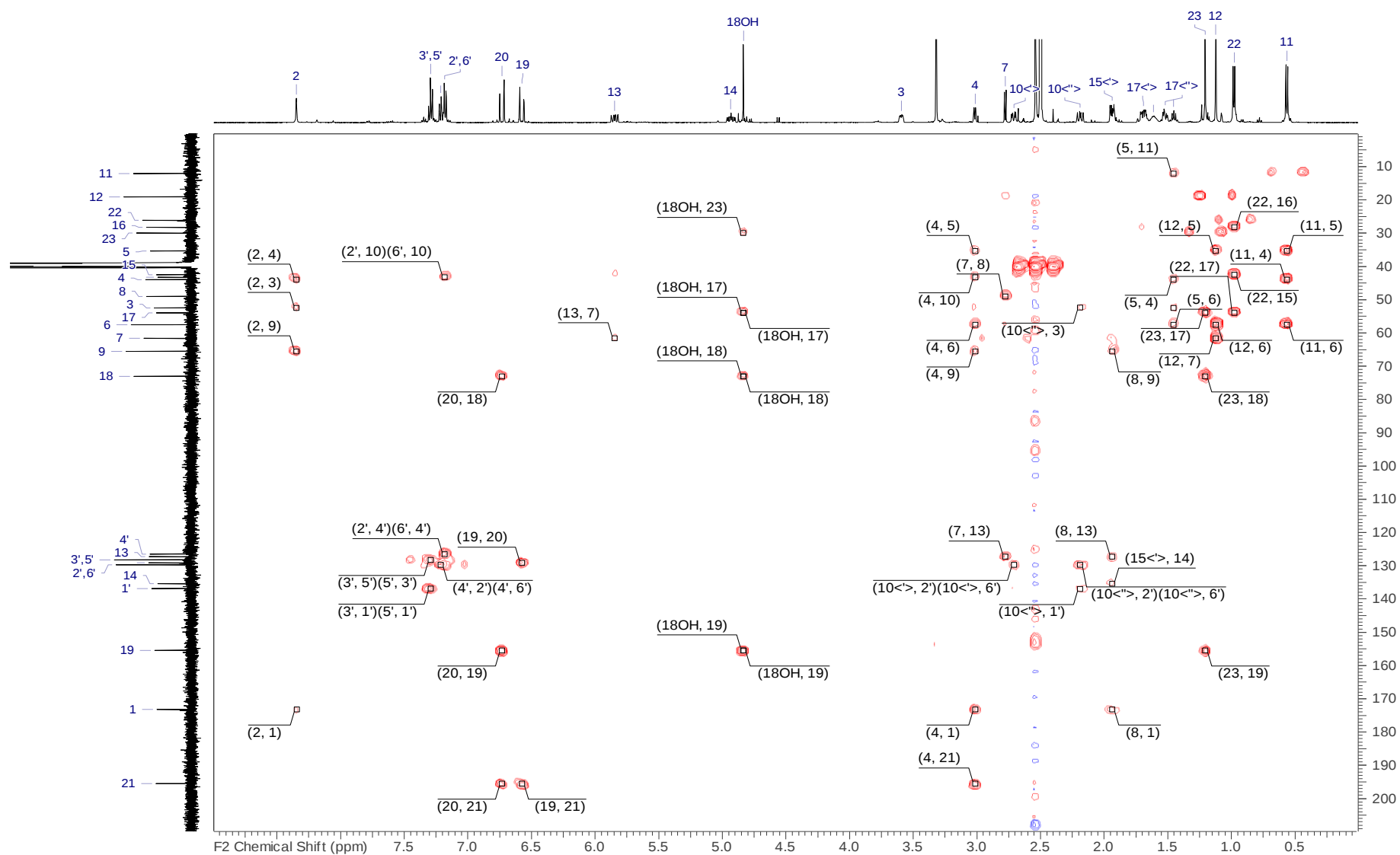
Figure S11.  $^1\text{H}$  NMR spectrum (500 MHz,  $\text{DMSO-d}_6$ ) of **2**.

Figure S12.  $^{13}\text{C}$  NMR spectrum (125 MHz,  $\text{DMSO}-d_6$ ) of 2.

Figure S13. COSY NMR spectrum (500 MHz, DMSO-*d*<sub>6</sub>) of 2.

Figure S14. ROESY NMR spectrum (500 MHz, DMSO- $d_6$ ) of 2.

Figure S15. HSQC NMR spectrum (500 MHz, DMSO- $d_6$ ) of 2.

Figure S16. HMBC NMR spectrum (500 MHz, DMSO-*d*<sub>6</sub>) of **2**.

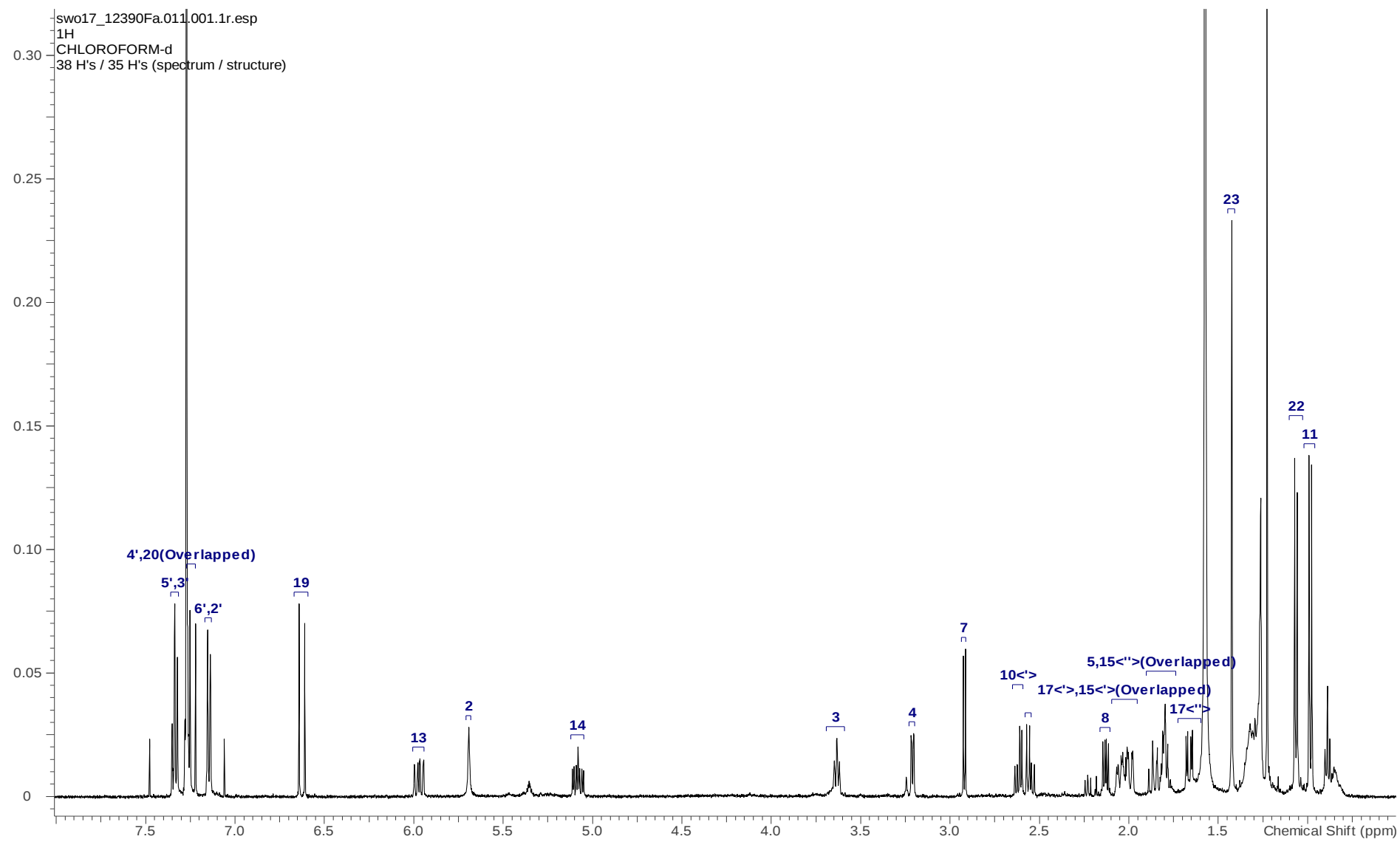
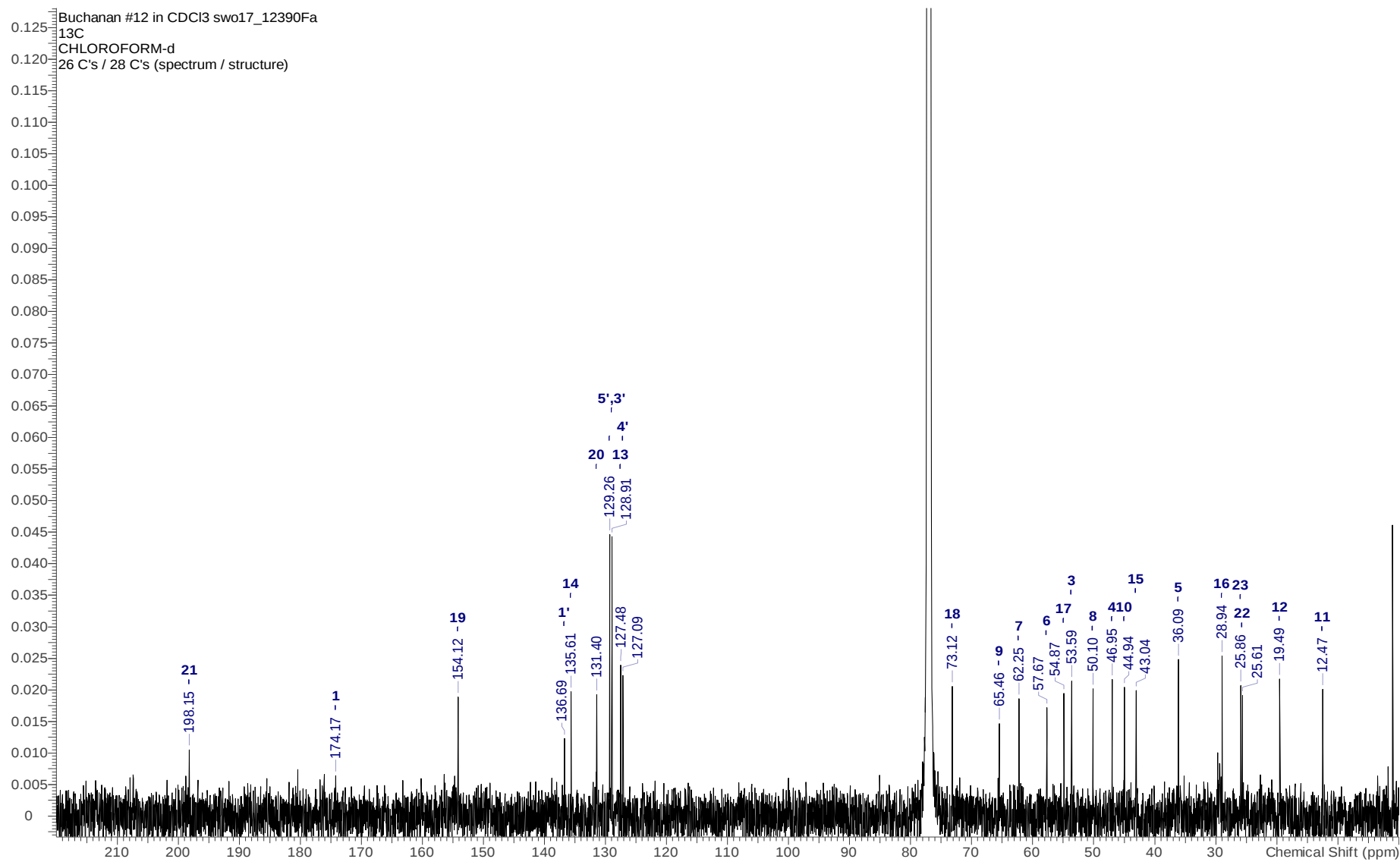


Figure S17.  $^1\text{H}$  NMR spectrum (500 MHz,  $\text{CDCl}_3$ ) of 17.

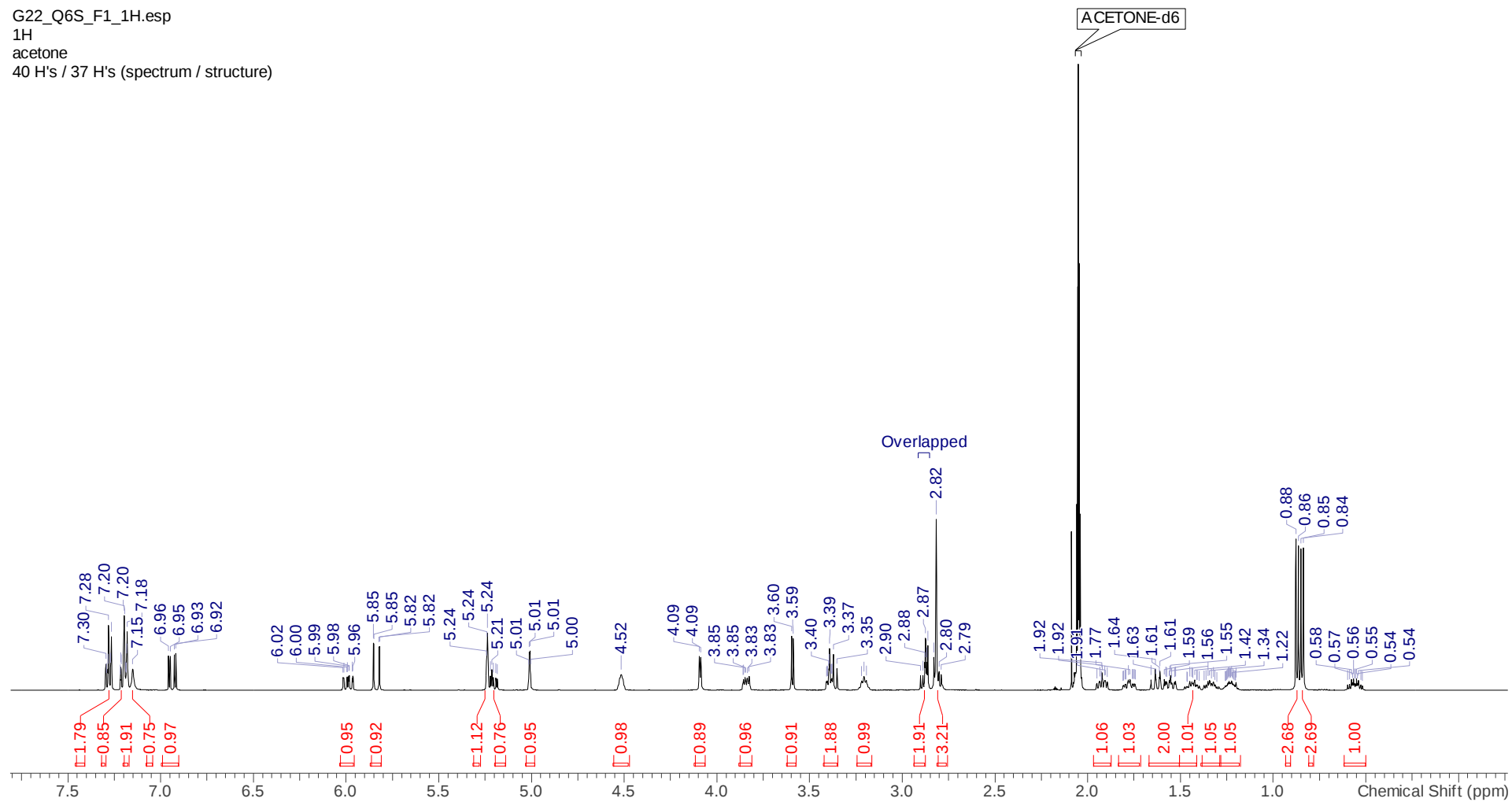
Figure S18. <sup>13</sup>C NMR spectrum (125 MHz, CDCl<sub>3</sub>) of 17.

G22\_Q6S\_F1\_1H.esp

1H

acetone

40 H's / 37 H's (spectrum / structure)

Figure S19. <sup>1</sup>H NMR spectrum (500 MHz, Acetone-d6) of cytochalasin B (4)

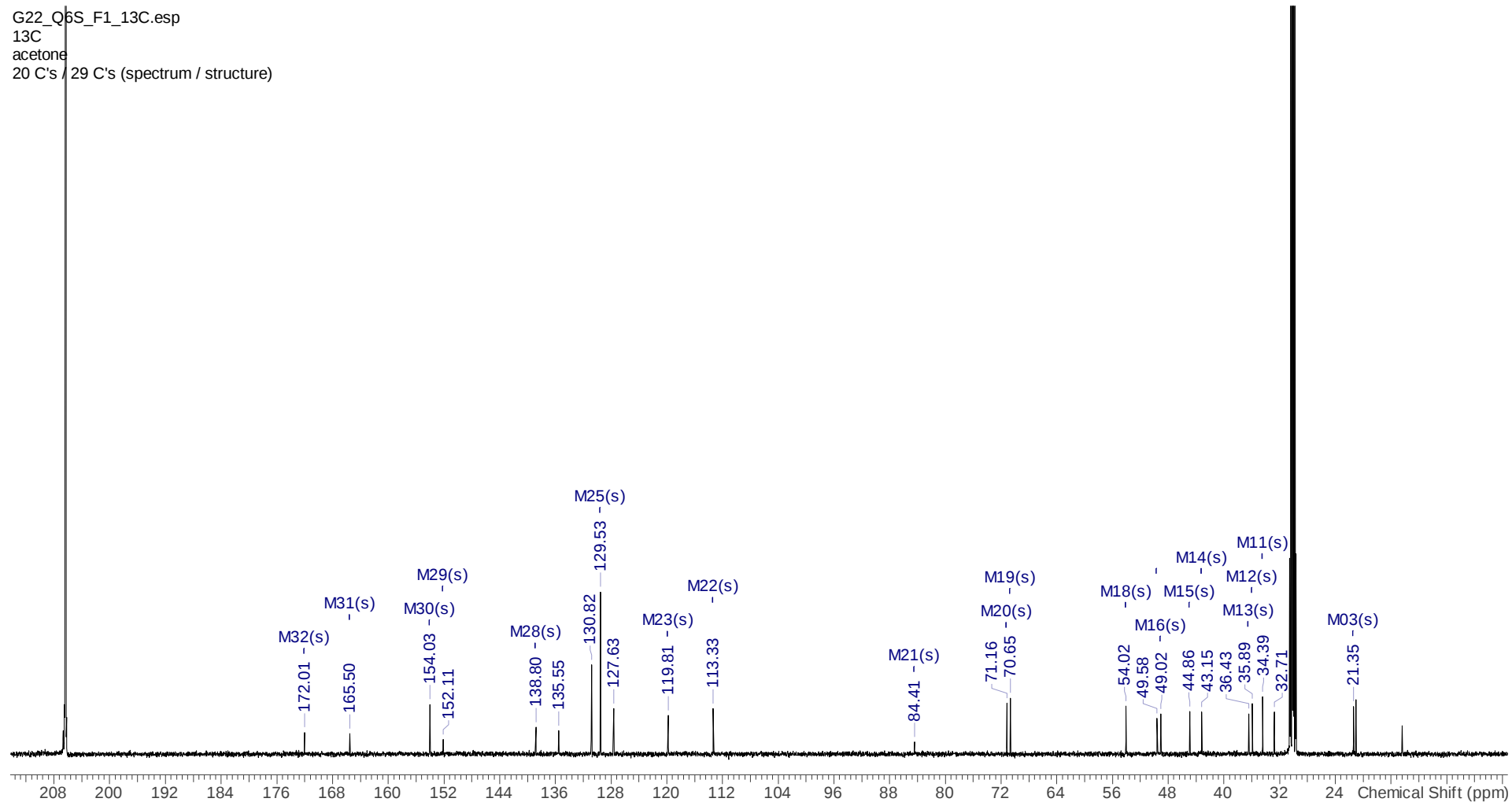
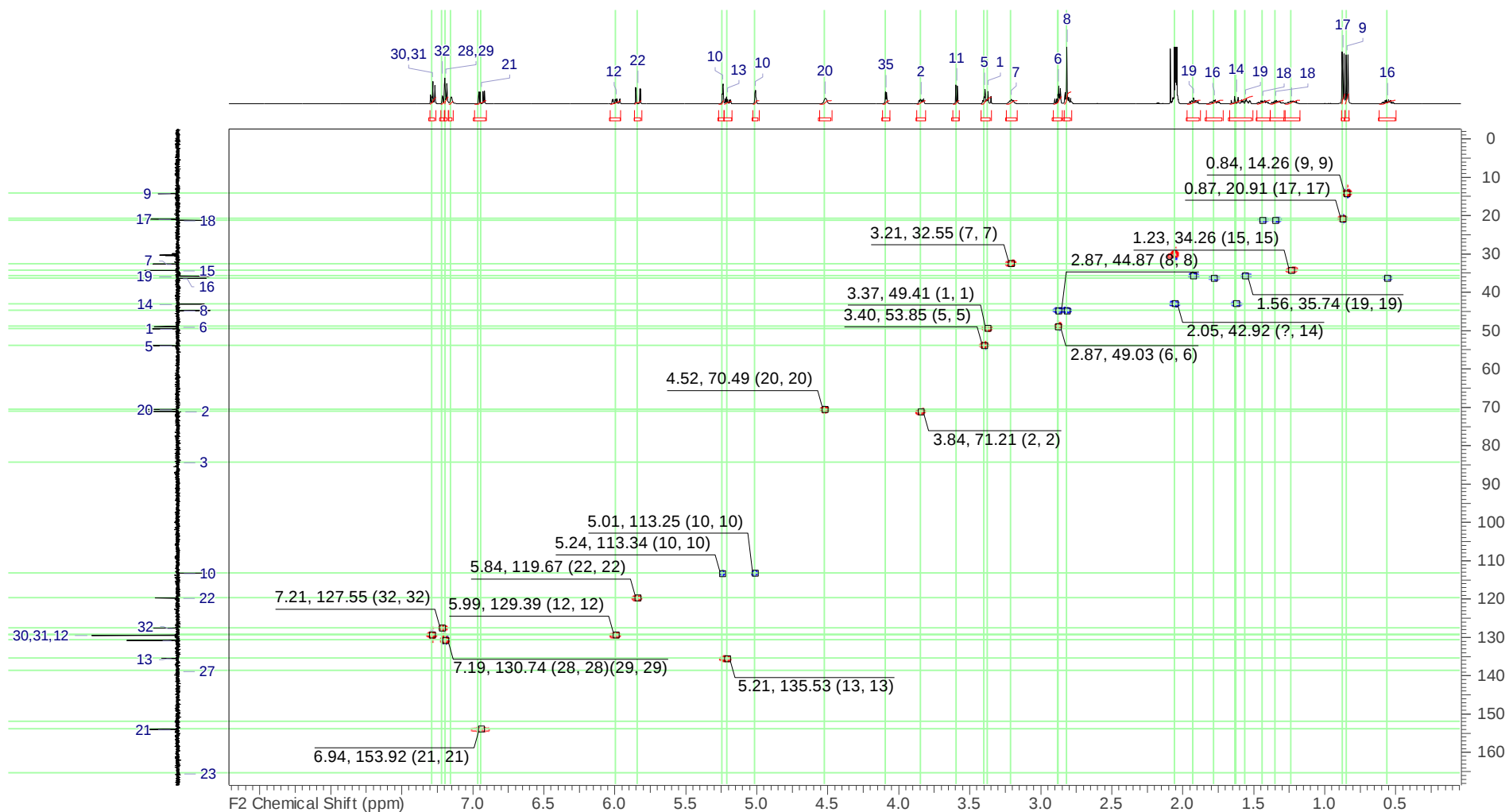


Figure S20.  $^{13}\text{C}$  NMR spectrum (500 MHz, Acetone- $\text{d}_6$ ) of cytochalasin B (**4**).

Figure S21. HSQC NMR spectrum (500 MHz, Acetone-d<sub>6</sub>) of cytochalasin B (4).

G22-F2-1H.esp  
1H  
acetone  
36 H's

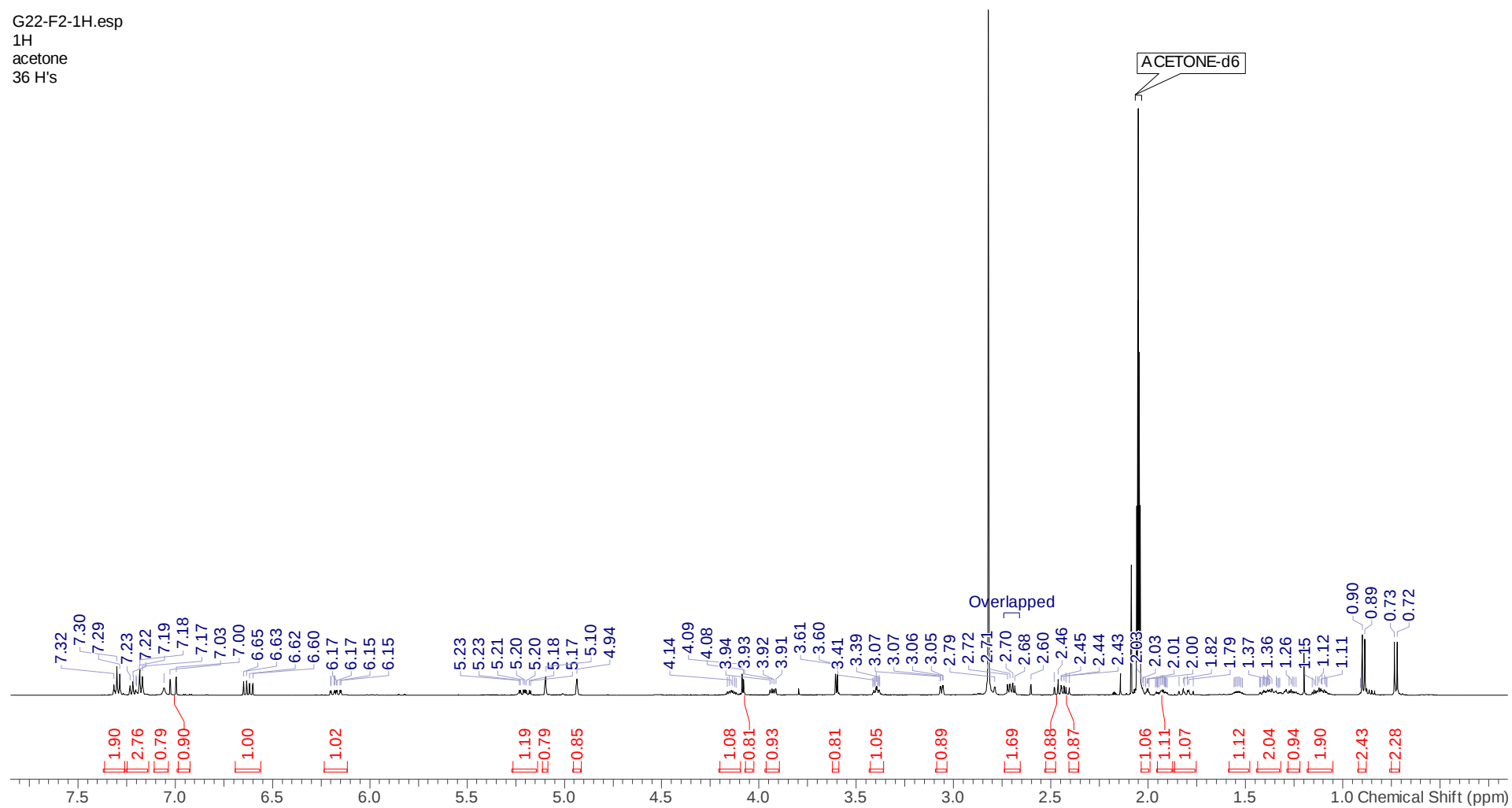


Figure S22.  $^1\text{H}$  NMR spectrum (500 MHz, Acetone- $\text{d}_6$ ) of deoxaphomin (5).

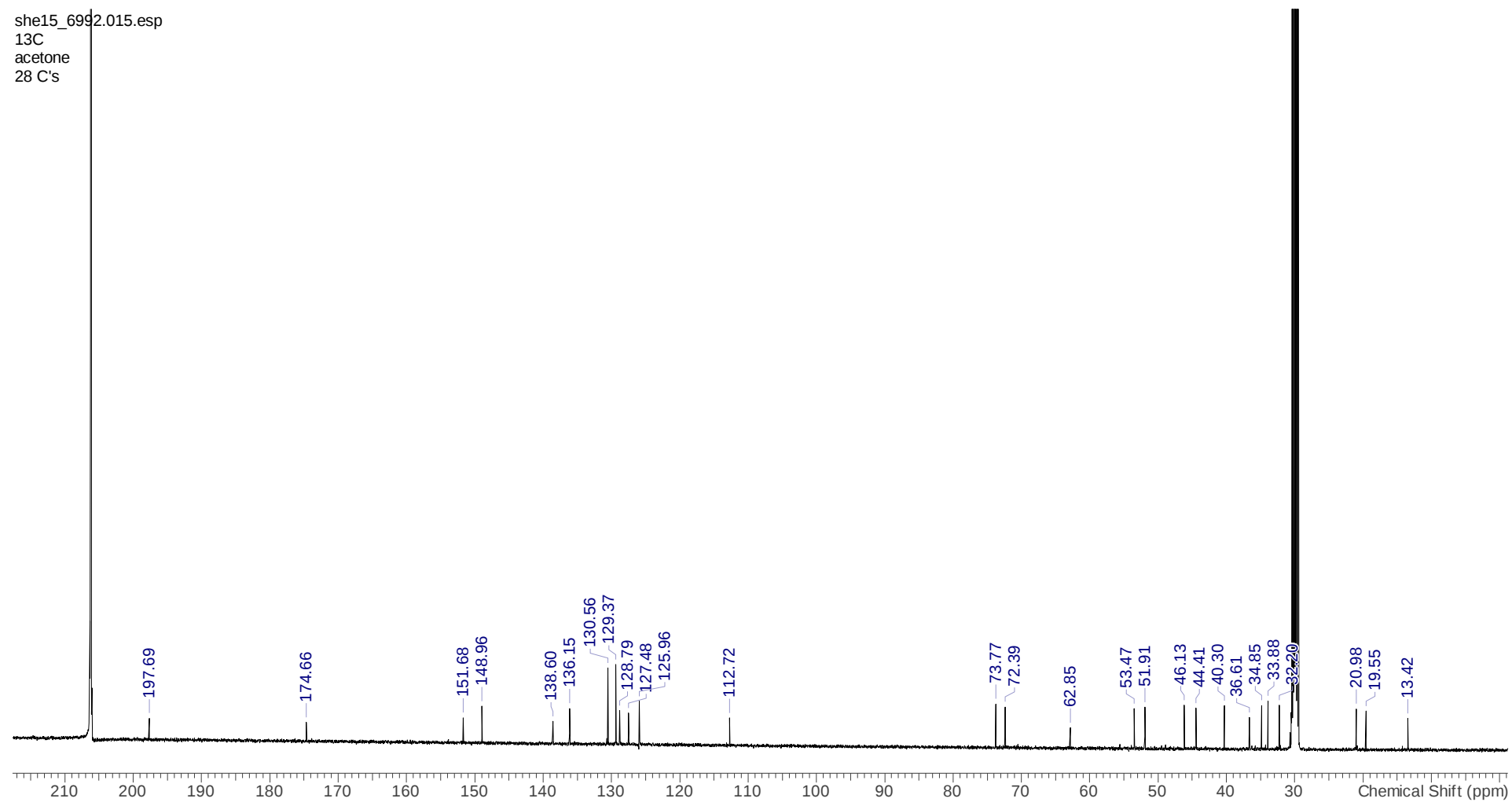


Figure S23.  $^{13}\text{C}$  NMR spectrum (500 MHz, Acetone- $\text{d}_6$ ) of deoxaphomin (**5**).

she15\_10009Sa.012.esp  
1H  
acetone  
44 H's / 37 H's (spectrum / structure)

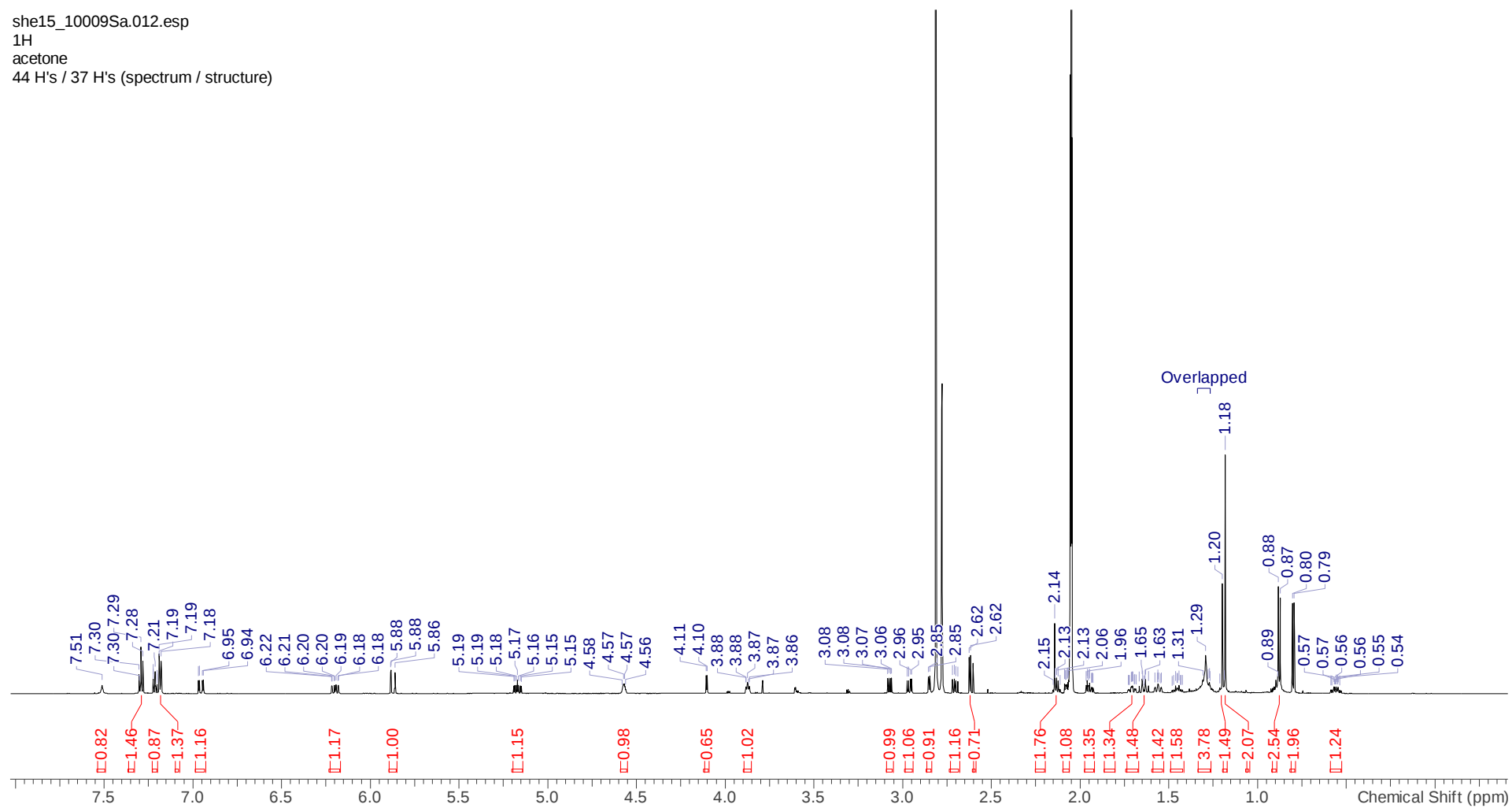


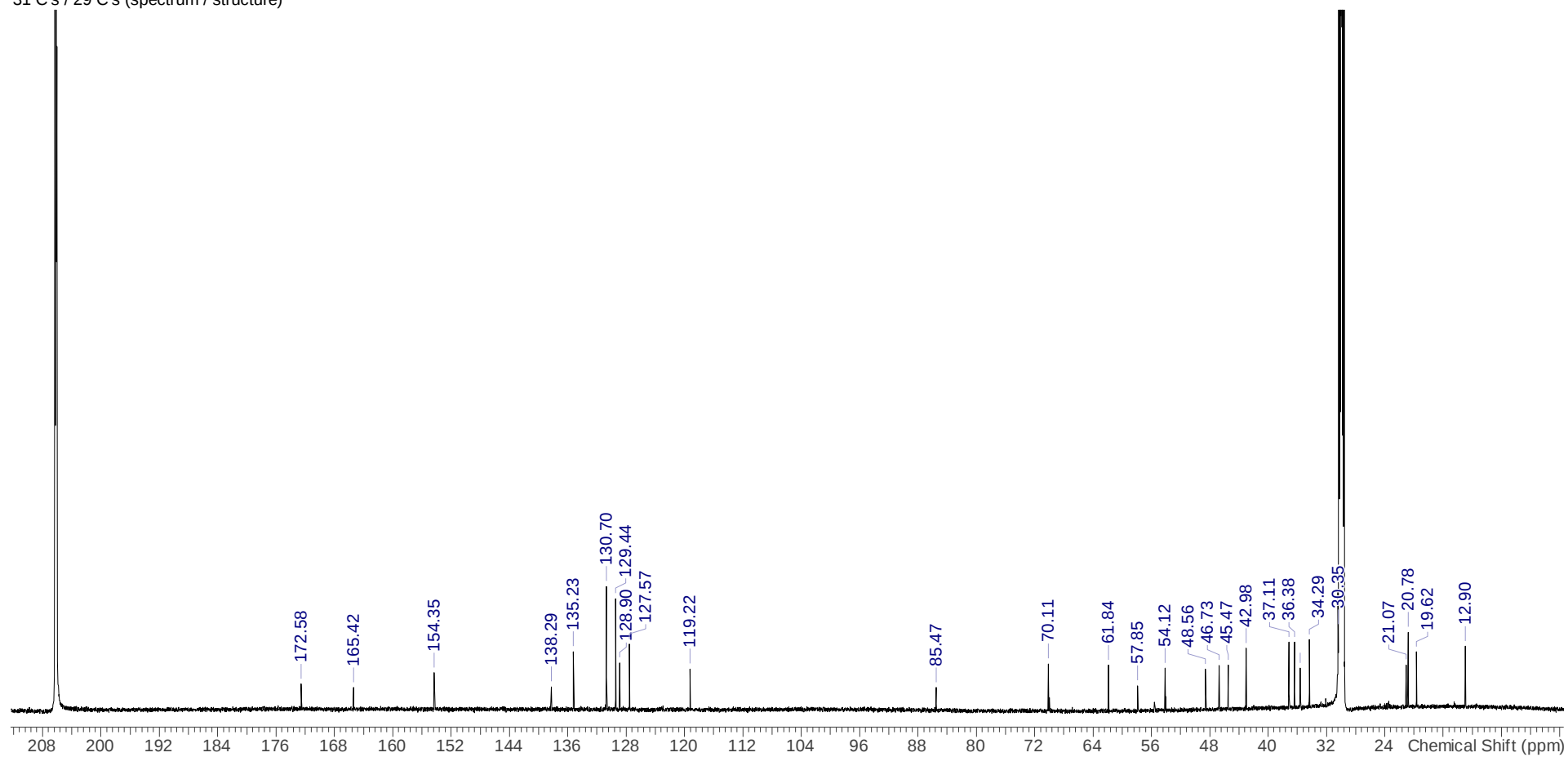
Figure S24.  $^1\text{H}$  NMR spectrum (500 MHz, Acetone- $\text{d}_6$ ) of cytochalasin F (7).

sh-014009Sa.017.esp

13C NMR

acq-017

31 C's / 29 C's (spectrum / structure)

Figure S25.  $^{13}\text{C}$  NMR spectrum (500 MHz, Acetone- $\text{d}_6$ ) of cytochalasin F (7).

MICS7947.010.esp  
1H  
acetone  
33 H's / 37 H's (spectrum / structure)

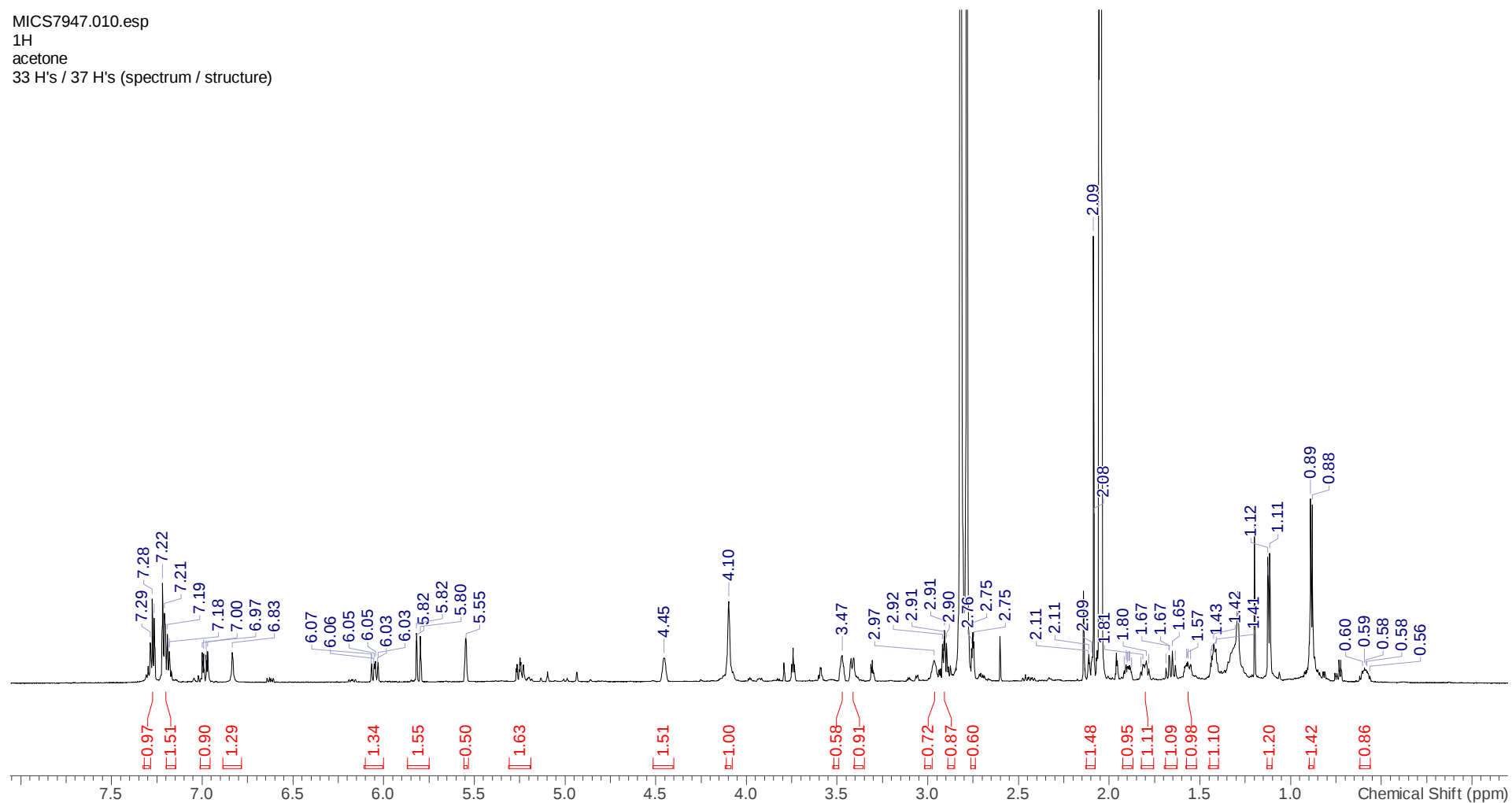


Figure S26. <sup>1</sup>H NMR spectrum (500 MHz, Acetone-d<sub>6</sub>) of cytochalasin Z2 (**11**).

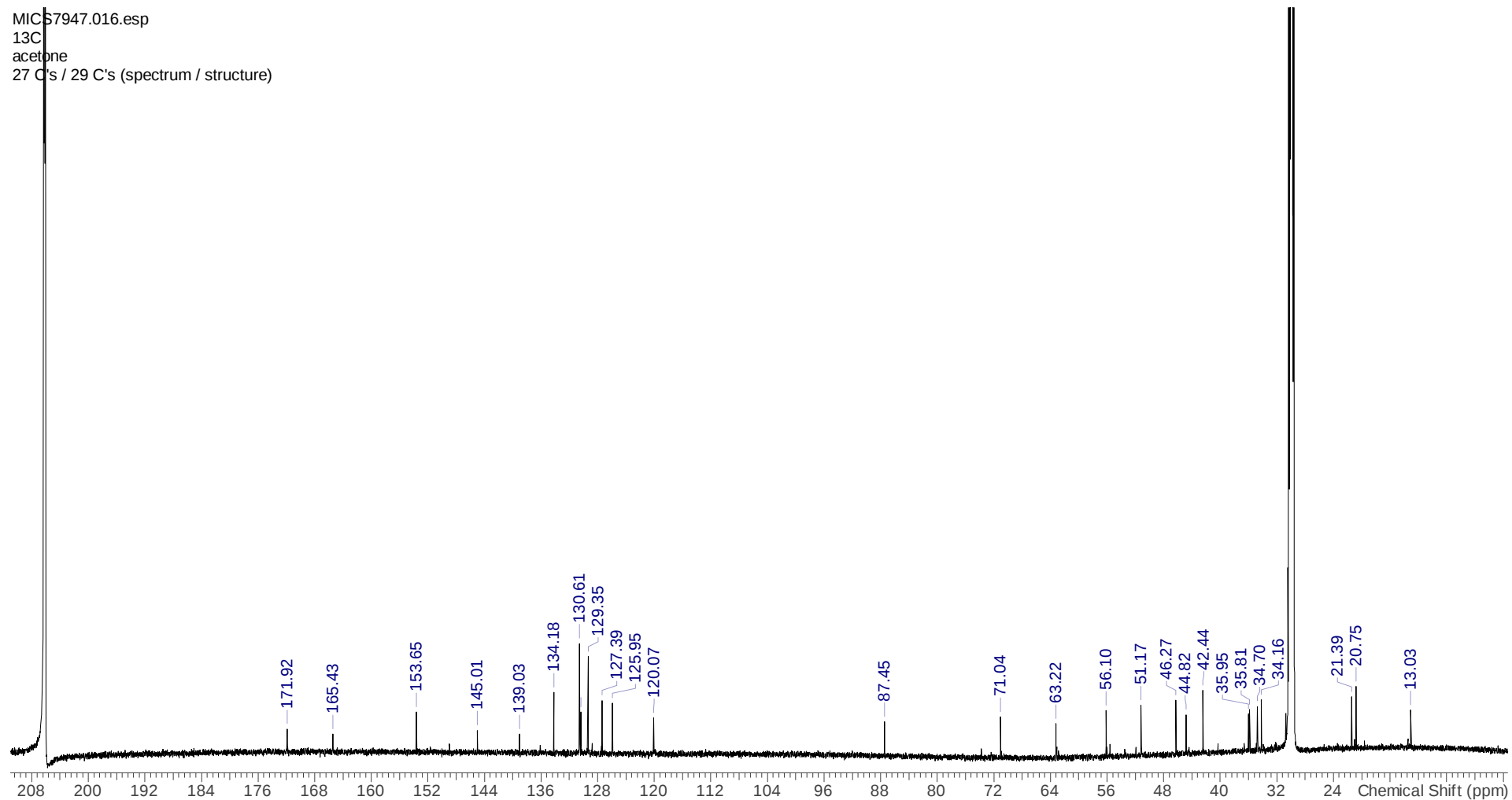
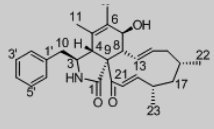
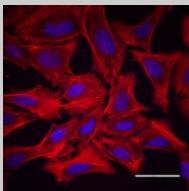
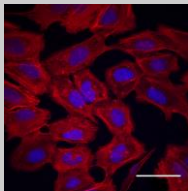
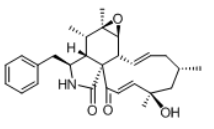
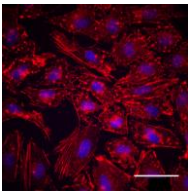
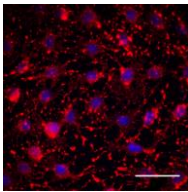
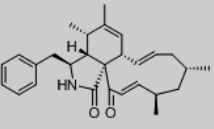
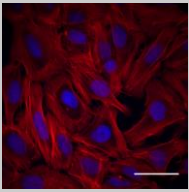
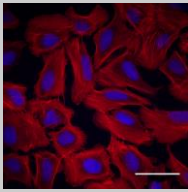
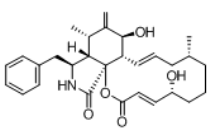
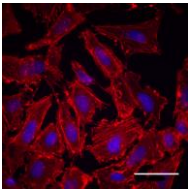
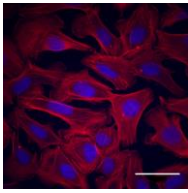
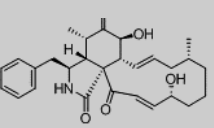
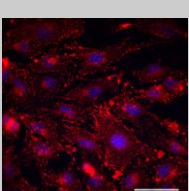
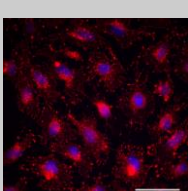
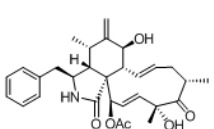
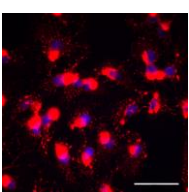
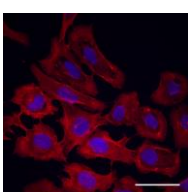
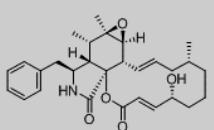
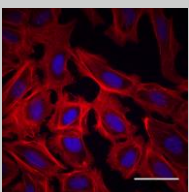
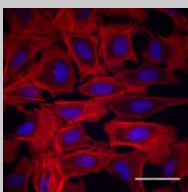
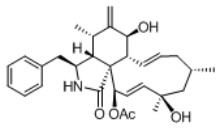
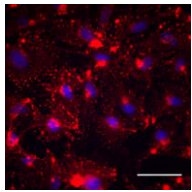
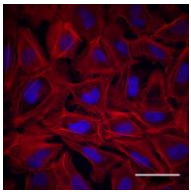
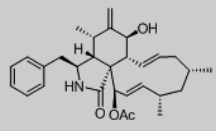
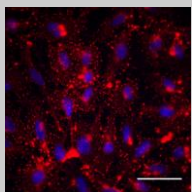
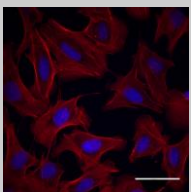
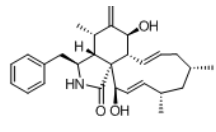
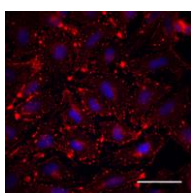
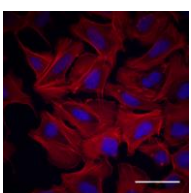
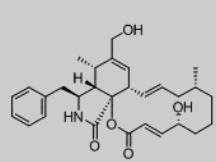
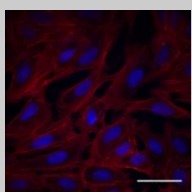
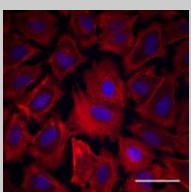
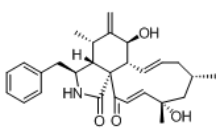
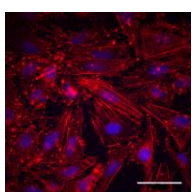
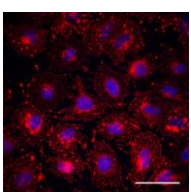
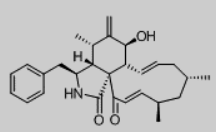
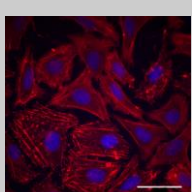
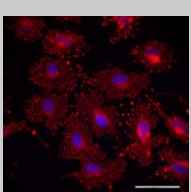
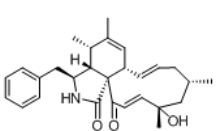
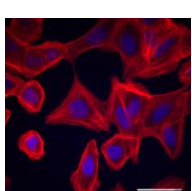
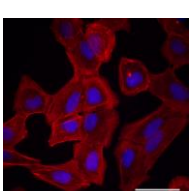
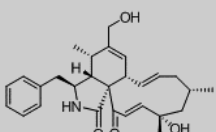
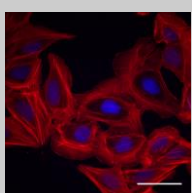
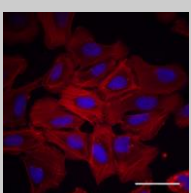
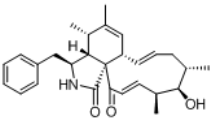
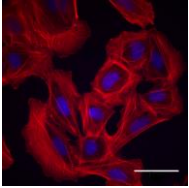
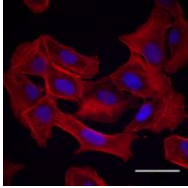
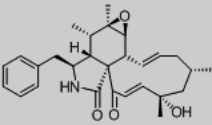
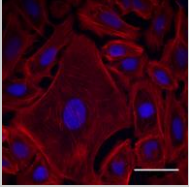
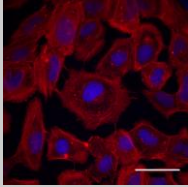
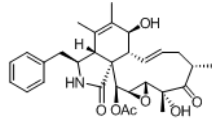
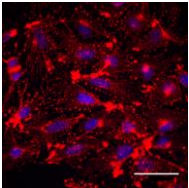
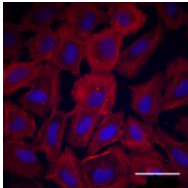
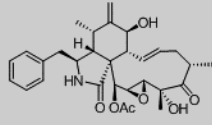
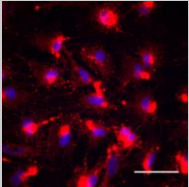
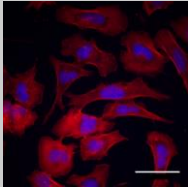
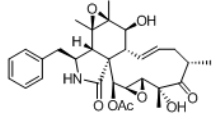
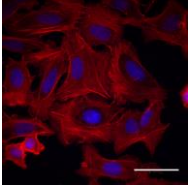
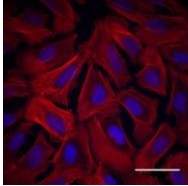
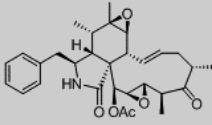
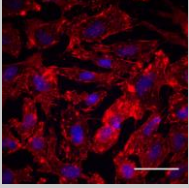
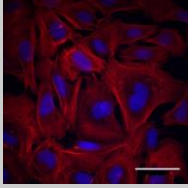
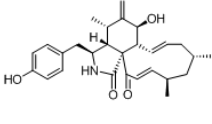
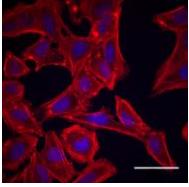
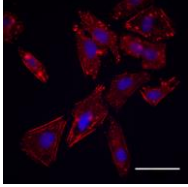
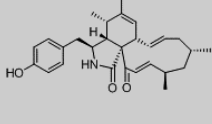
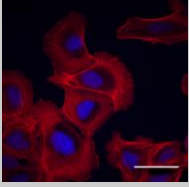
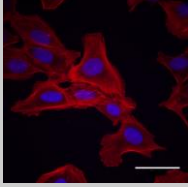


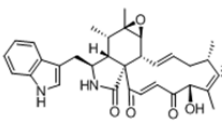
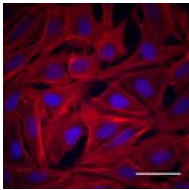
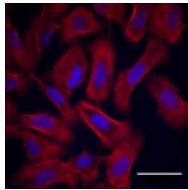
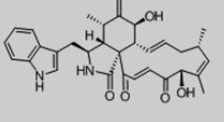
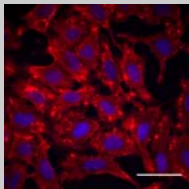
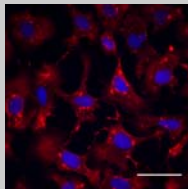
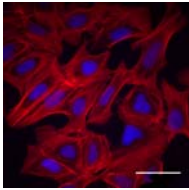
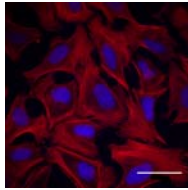
Figure S27.  $^{13}\text{C}$  NMR spectrum (500 MHz, Acetone- $\text{d}_6$ ) of cytochalasin Z2 (**11**).

Table S1. Cytochalasan treatment of U2OS cells

|   | compound                               | structure                                                                           | 1 hour 1 $\mu$ M                                                                    | 1 hour washout                                                                       | effect | reversible |
|---|----------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|--------|------------|
| 1 | Fragiformin C                          |    |    |    | +      | +/-        |
| 2 | Fragiformin D                          |    |    |    | +++    | -          |
| 3 | Saccalasin A                           |    |   |   | -      | n.d.       |
| 4 | Cytochalasin B<br>(reference compound) |  |  |  | ++     | +          |
| 5 | Deoxaphomin                            |  |  |  | +++    | -          |
| 6 | Cytochalasin D                         |  |  |  | +++    | +/-        |
| 7 | Cytochalasin F                         |  |  |  | +      | +          |

|    |                       |                                                                                     |                                                                                     |                                                                                      |     |     |
|----|-----------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|-----|-----|
| 8  | Cytochalasin H        |    |    |    | +++ | +   |
| 9  | L-696,474             |    |    |    | +++ | +   |
| 10 | 21-O-Deacyl-L-696,474 |    |    |    | +++ | +   |
| 11 | Cytochalasin Z2       |   |   |   | +   | +   |
| 12 | 12                    |  |  |  | +++ | -   |
| 13 | 13                    |  |  |  | ++  | -   |
| 14 | 14                    |  |  |  | +   | +/- |
| 15 | 15                    |  |  |  | +   | +/- |

|    |                                   |                                                                                     |                                                                                     |                                                                                      |     |      |
|----|-----------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|-----|------|
| 16 | 16                                |    |    |    | -   | n.d. |
| 17 | 17                                |    |    |    | +   | +/-  |
| 18 | 19,20-Epoxychothalasin C          |    |    |    | +++ | +    |
| 19 | 19,20-Epoxychothalasin D          |   |   |   | +++ | +/-  |
| 20 | 19,20-Epoxychothalasin N          |  |  |  | +   | +    |
| 21 | 18-Deoxy-19,20-Epoxychothalasin D |  |  |  | ++  | +    |
| 22 | Phenochalasin C                   |  |  |  | ++  | -    |
| 23 | Phenochalasin D                   |  |  |  | -   | n.d. |

|    |                           |                                                                                   |                                                                                   |                                                                                    |    |   |
|----|---------------------------|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|------------------------------------------------------------------------------------|----|---|
| 24 | Chaetoglobosin A          |  |  |  | +  | + |
| 25 | Chaetoglobosin D          |  |  |  | ++ | - |
| 26 | DMSO<br>(vehicle control) | -                                                                                 |  |  | /  | / |

**Table S1:** Cytochalasan treatment of U2OS cells. U2OS cells [ATCC HTB-96] were cultivated in Dulbecco's modified minimum essential medium (DMEM, Life Technologies, Carlsbad, CA, USA) with 10% fetal bovine serum, 1% L-glutamine, 1% minimum essential medium nonessential amino acids (MEM NEAA) and 1% sodium pyruvate (Life technologies, Carlsbad, CA, USA) at 36°C and 5% CO<sub>2</sub>. For cytochalasan treatment, cells were seeded on glass coverslips which in turn were placed in a six-well-plate. Prior to cell growth, the coverslips were coated with 25 µg/ml fibronectin in PBS for one hour. Cytochalasans were dissolved in DMSO (Carl Roth GmbH, Karlsruhe, Germany) for treatment. When the cells reached 80% confluency, cytochalasans were applied at a concentration of 1 and 5 µg/ml for 1 h. Wash out experiments were conducted by exchanging the cytochalasan containing medium after incubation time with DMEM followed by incubation for another hour. As vehicle control, DMSO was used at the same concentrations (1 µg/ml, 5 µg/ml) as cytochalasans. After incubation, the cells were fixed by incubation with 4% paraformaldehyde (AppliChem, Darmstadt, Germany) in PBS for 20 min at room temperature. Fixed cells were washed with PBS and permeabilized with 0.1% Triton X-100 (Bio-Rad Laboratories, Hercules, CA, USA) in PBS for 1 min at room temperature. The cells were washed again thrice with PBS and afterwards stained using fluorescently labeled phalloidin ATTO 594 (ATTO-Tec, Siegen, Germany), diluted 1:200 in PBS. The coverslips were mounted in Prolong Diamond antifade mountant with DAPI (inVitrogen, Carlsbad, CA, USA) in order to stain the nucleus. Pictures of the cells were taken using a Axio Vert 135 TV inverse microscope with phase contrast and CoolSnap 4k camera (Zeiss, Oberkochen, Germany). Pictures were processed using the Image J software package (NIH, USA). The treatments were run in duplicates and the experiments repeated always with the same results.