

## SUPPORTING INFORMATION

### **Higginsianins D and E, Cytotoxic Diterpenoids Produced by *Colletotrichum higginsianum***

Marco Masi,<sup>†</sup> Alessio Cimmino,<sup>†</sup> Flora Salzano<sup>‡</sup>, Roberta Di Lecce,<sup>†</sup> Marcin Górecki,<sup>§,‡</sup> Viola Calabrò,<sup>‡</sup> Gennaro Pescitelli,<sup>\*,§</sup> and Antonio Evidente<sup>\*,†</sup>

<sup>†</sup>Dipartimento di Scienze Chimiche, and <sup>‡</sup>Dipartimento di Biologia, Università di Napoli Federico II, Complesso Universitario Monte Sant'Angelo, Via Cintia 4, 80126, Napoli, Italy

<sup>§</sup>Dipartimento di Chimica e Chimica Industriale, Università di Pisa, Via Moruzzi 3, 56124 Pisa, Italy

<sup>\*</sup>Institute of Organic Chemistry, Polish Academy of Sciences, Kasprzaka 44/52 St., 01-224, Warsaw, Poland

### **Supporting Information List**

Page 3: **Spectra 1.** <sup>1</sup>H NMR spectrum of higginsianin D (1) (CDCl<sub>3</sub>, 400 MHz).

Page 3: **Spectra 2.**  $^{13}\text{C}$  NMR spectrum of higginsianin D (**1**) ( $\text{CDCl}_3$ , 100 MHz).

Page 4: **Spectra 3.** HSQC spectrum of higginsianin D (**1**) ( $\text{CDCl}_3$ , 400/100 MHz).

Page 4: **Spectra 4.** HMBC spectrum of higginsianin D (**1**) ( $\text{CDCl}_3$ , 400/100 MHz).

Page 5: **Spectra 5.** COSY spectrum of higginsianin D (**1**) ( $\text{CDCl}_3$ , 400 MHz).

Page 5: **Spectra 6.** NOESY spectrum of higginsianin D (**1**) ( $\text{CDCl}_3$ , 400 MHz).

Page 6: **Spectra 7.** HR ESIMS of higginsianin D (**1**), recorded in positive modality.

Page 6: **Spectra 8.**  $^1\text{H}$  NMR spectrum of higginsianin E (**2**) ( $\text{CDCl}_3$ , 400 MHz).

Page 7: **Spectra 9.**  $^{13}\text{C}$  NMR spectrum of higginsianin E (**2**) ( $\text{CDCl}_3$ , 100 MHz).

Page 7: **Spectra 10.** HSQC spectrum of higginsianin E (**2**) ( $\text{CDCl}_3$ , 400/100 MHz).

Page 8: **Spectra 11.** HMBC spectrum of higginsianin E (**2**) ( $\text{CDCl}_3$ , 400/100 MHz).

Page 8: **Spectra 12.** COSY spectrum of higginsianin E (**2**) ( $\text{CDCl}_3$ , 400 MHz).

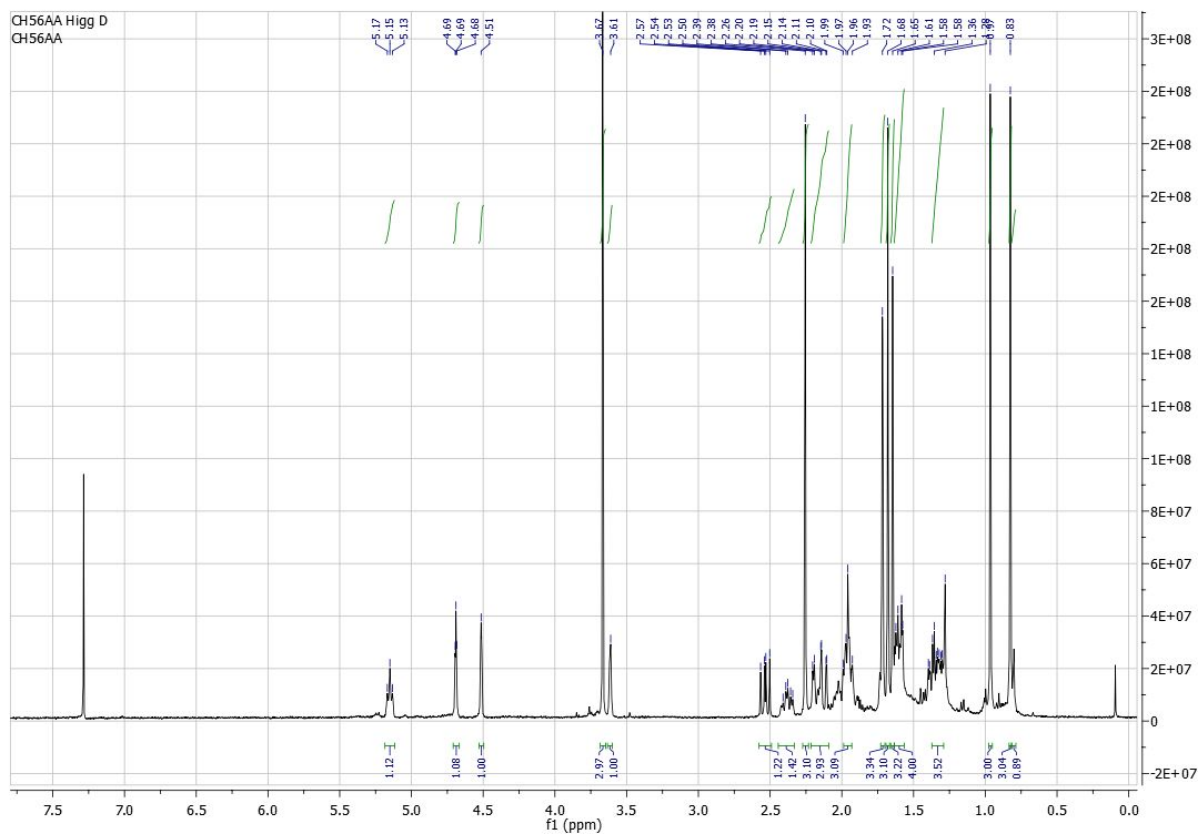
Page 9: **Spectra 13.** NOESY spectrum of higginsianin E (**2**) ( $\text{CDCl}_3$ , 400 MHz).

Page 9: **Spectra 14.** HR ESIMS of higginsianin E (**2**), recorded in positive modality.

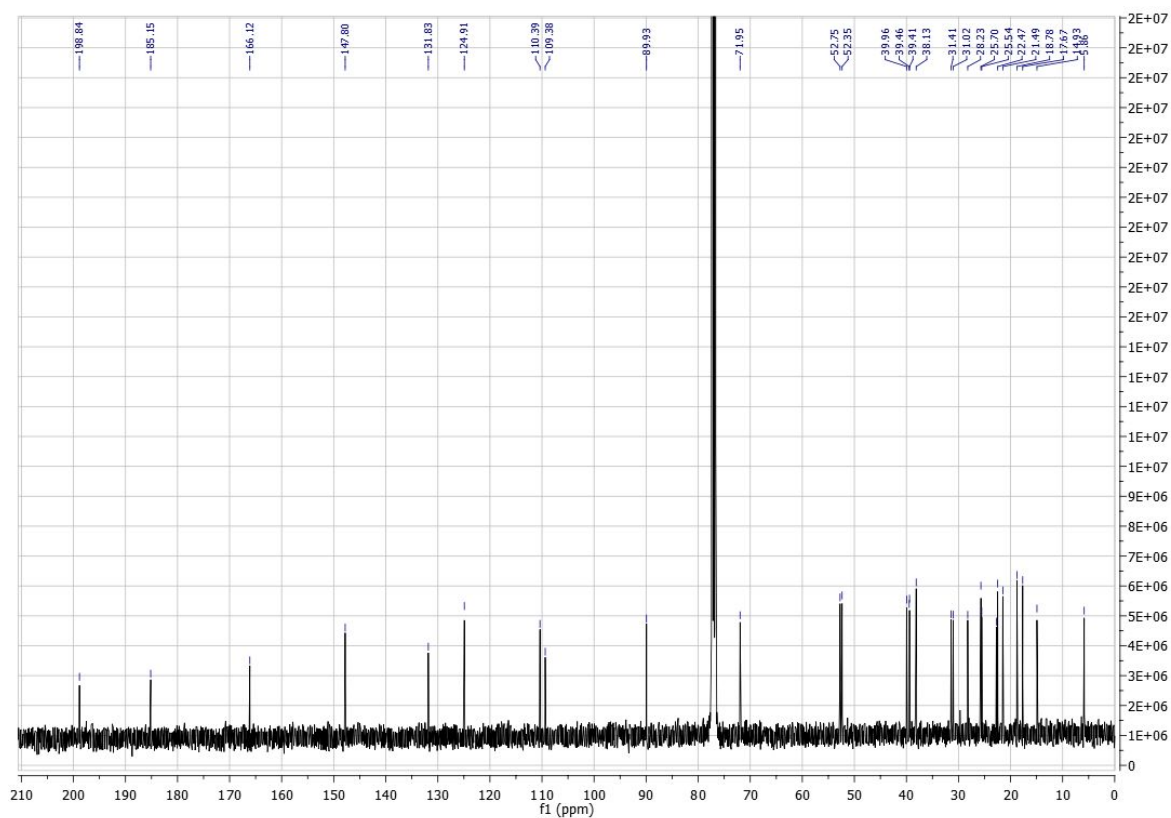
Page 10: **Table S1.** The first nine low-energy structures of the truncated model of higginsianin D (*4R,5R,8R,9S,10R,21S*)-**1** with energies and populations estimated at  $\omega\text{B97X-D/def2-TZVP/PCM}$  level.

Page 11: **Table S2.** The first nine low-energy structures of the truncated model of higginsianin E (*4R,5R,8R,9S,10R,21R*)-**2** with energies and populations estimated at  $\omega\text{B97X-D/def2-TZVP/PCM}$  level.

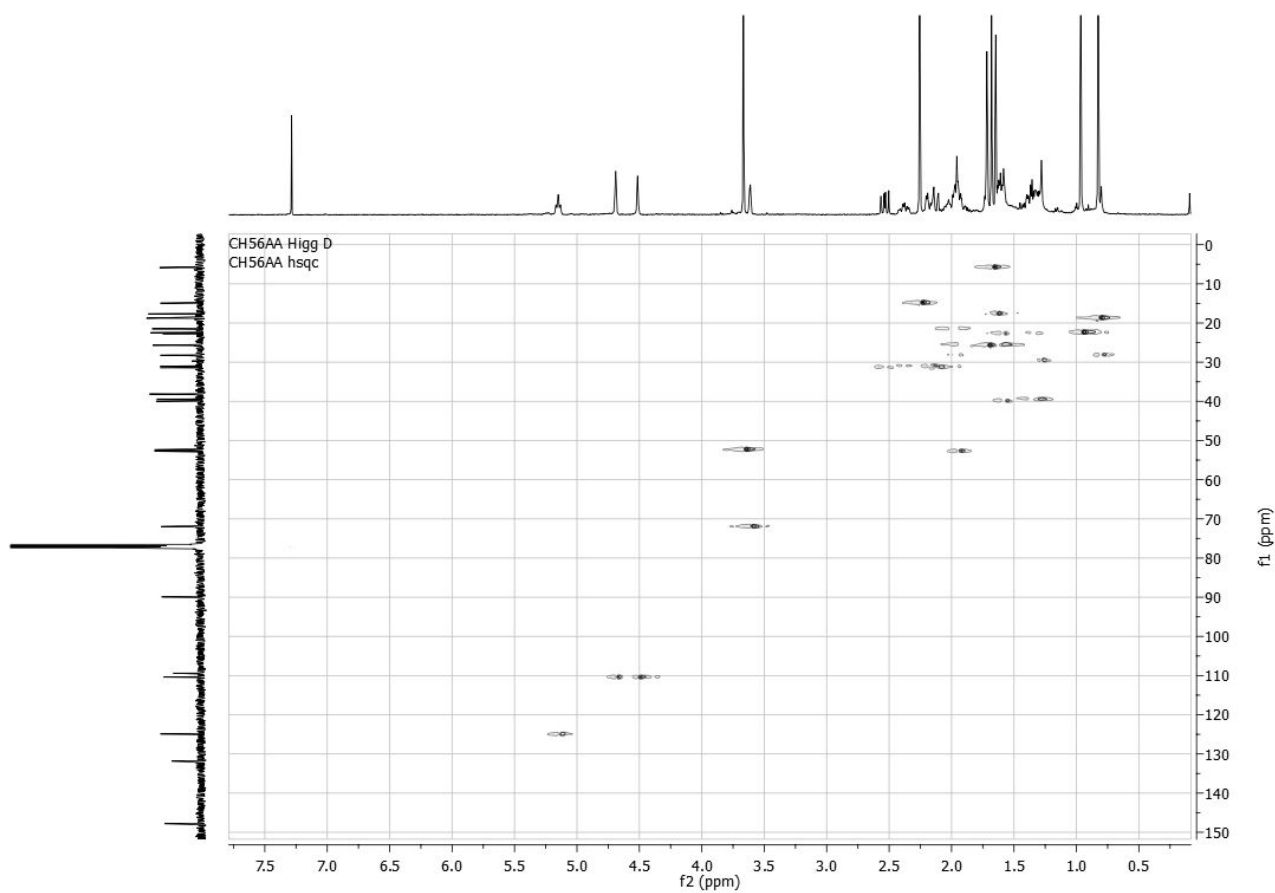
Page 12: **Figure S1.** Comparison between experimental  $^{13}\text{C}$  chemical shifts of higginsianin D and E measured in  $\text{CDCl}_3$  and the shifts calculated for the truncated models of (*4R,5R,8R,9S,10R,21S*)-**1** and (*4R,5R,8R,9S,10R,21R*)-**2** using the procedure described in the main text with final  $\omega\text{B97X-V/6-311+G(2df,2p)}/\omega\text{B97X-D/6-31G(d)}$  energy estimation and geometry optimization and  $\omega\text{B97X-D/6-31G(d)}$  shielding calculations. The comparison is restricted to the dihydrofuran-2-one moiety (i.e. carbon atoms from C-20 to C-27, plus the ester group).



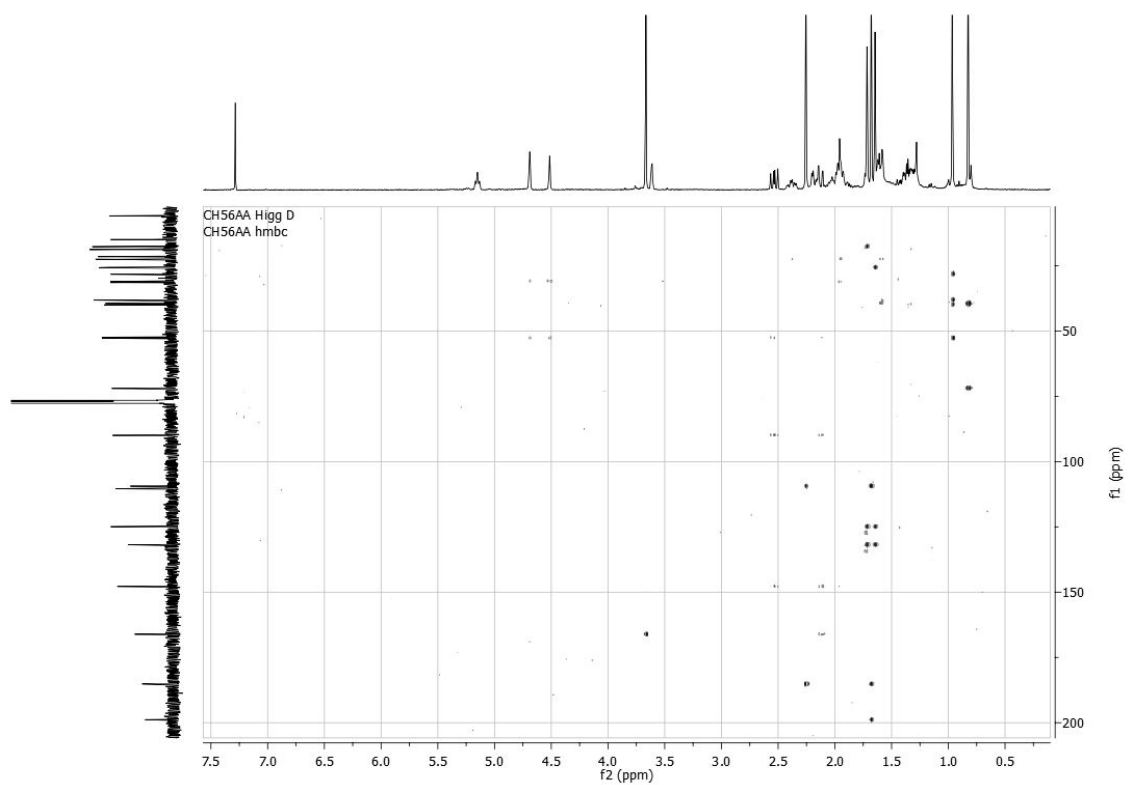
**Spectra 1.**  $^1\text{H}$  NMR spectrum of higginsianin D (**1**) ( $\text{CDCl}_3$ , 400 MHz).



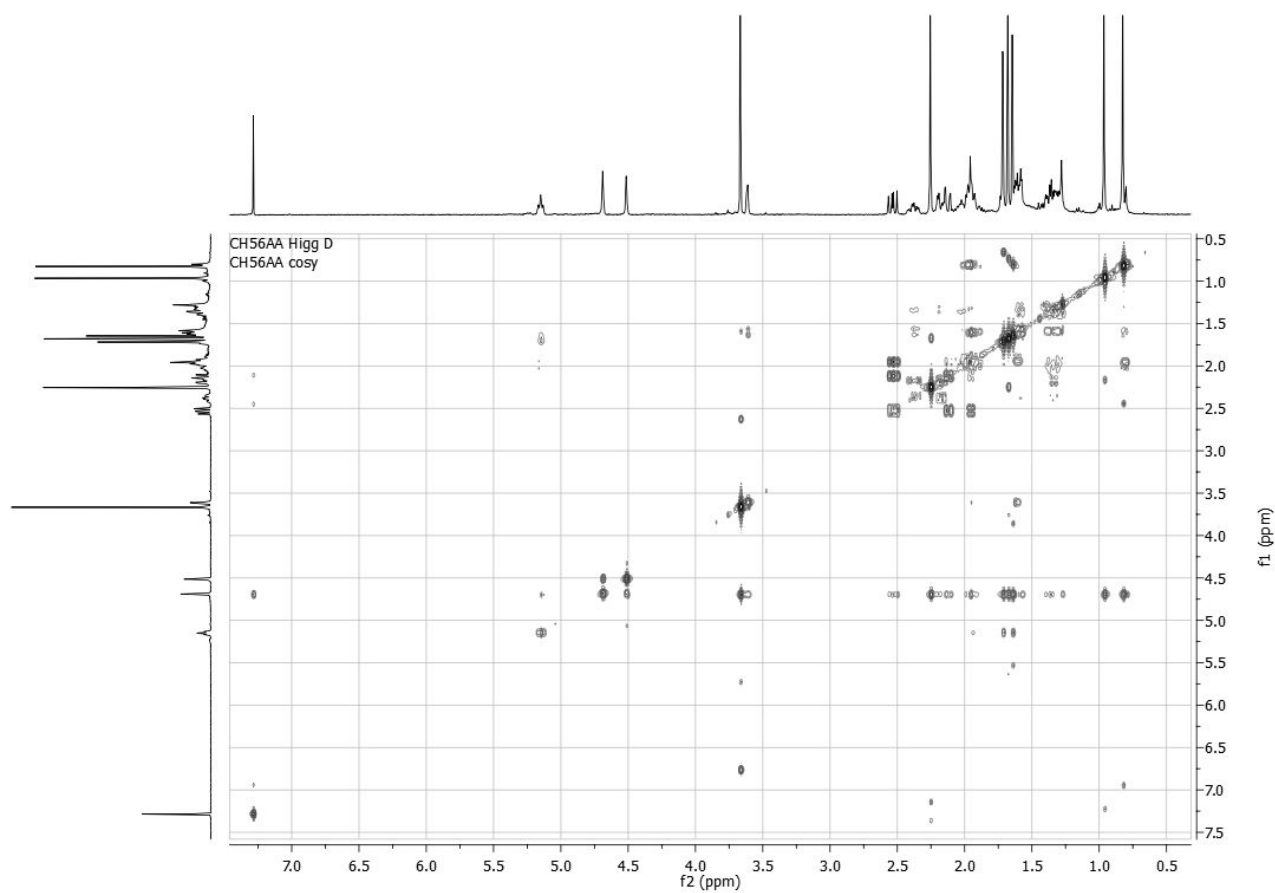
**Spectra 2.**  $^{13}\text{C}$  NMR spectrum of higginsianin D (**1**) ( $\text{CDCl}_3$ , 100 MHz).



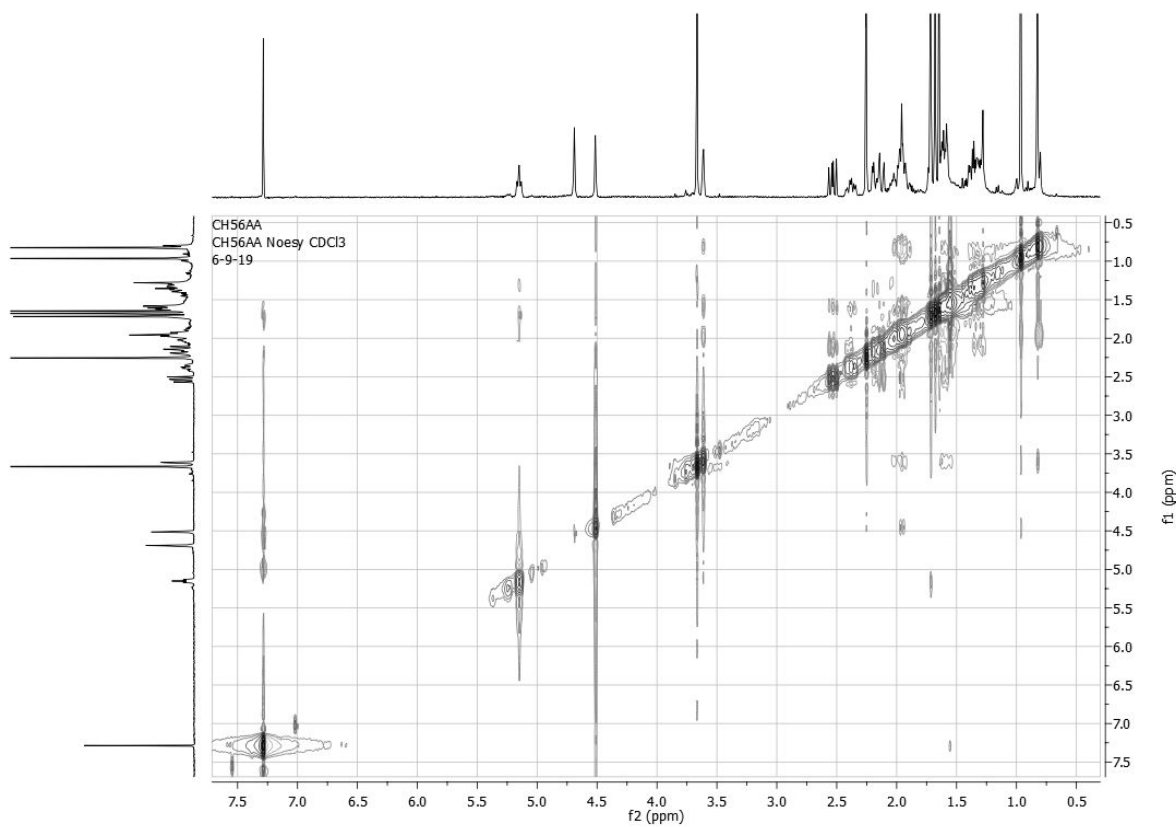
Spectra 3. HSQC spectrum of higginsianin D (1) (CDCl<sub>3</sub>, 400/100 MHz).



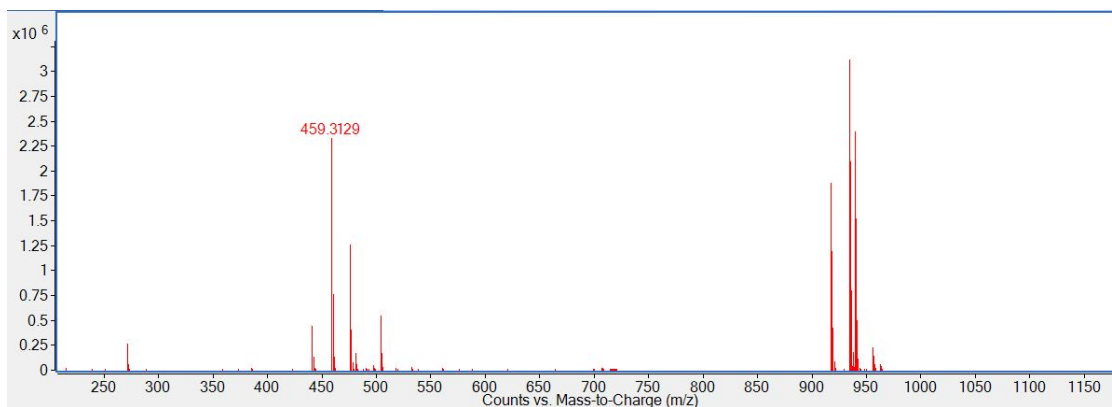
Spectra 4. HMBC spectrum of higginsianin D (1) (CDCl<sub>3</sub>, 400/100 MHz).



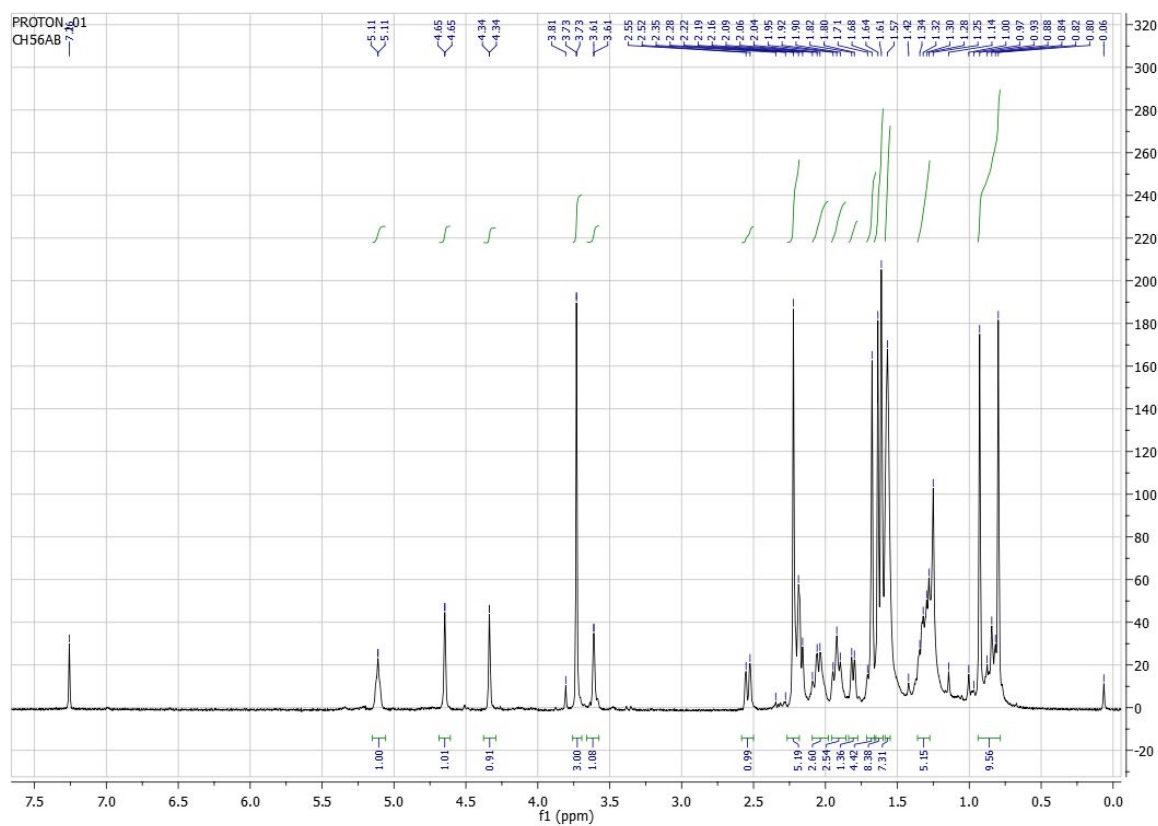
**Spectra 5.** COSY spectrum of higginsianin D (**1**) (CDCl<sub>3</sub>, 400 MHz).



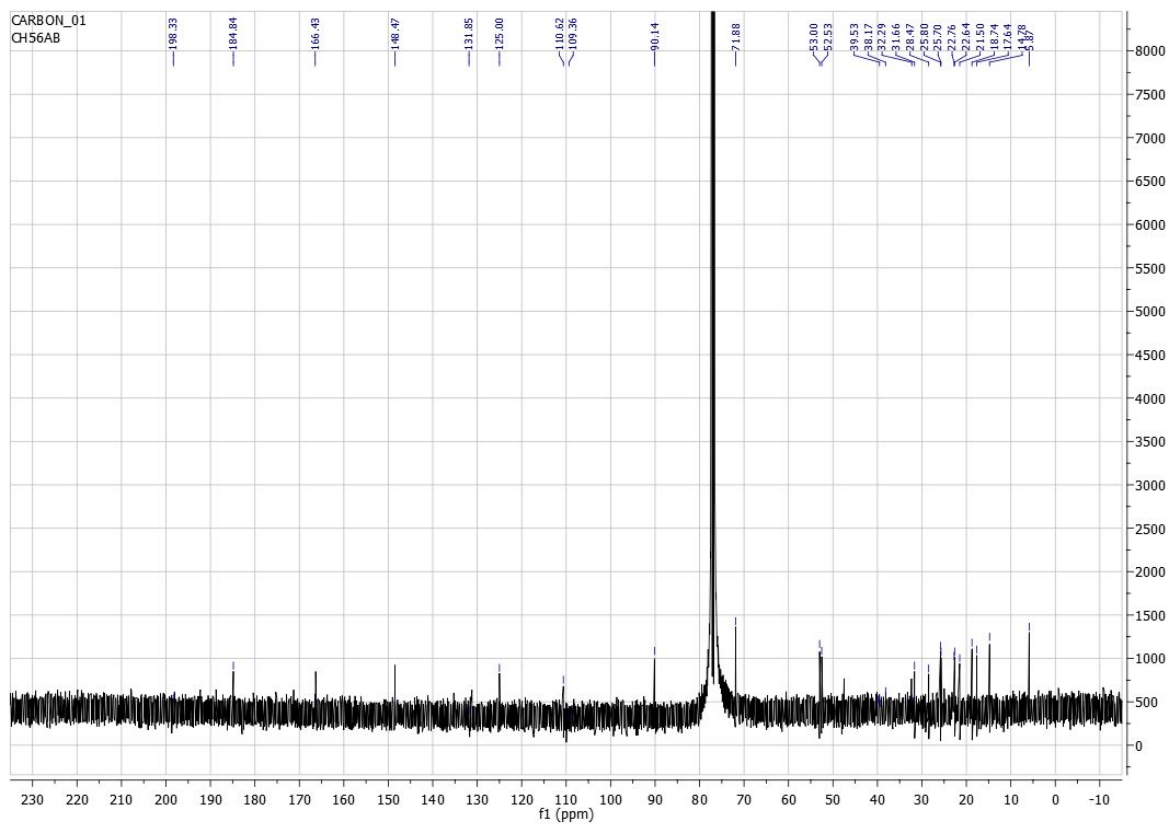
**Spectra 6.** NOESY spectrum of higginsianin D (**1**) (CDCl<sub>3</sub>, 400 MHz).



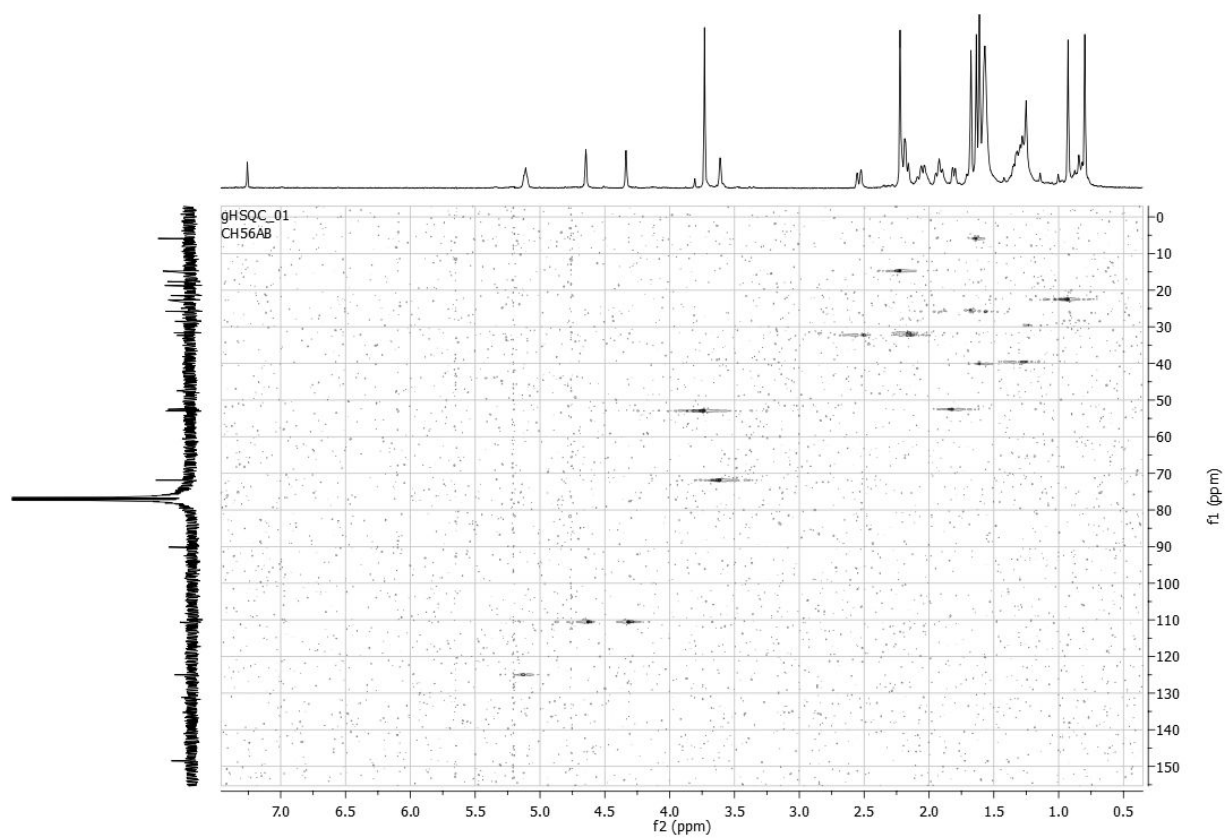
**Spectra 7.** HR ESIMS of higginsianin D (1), recorded in positive modality.



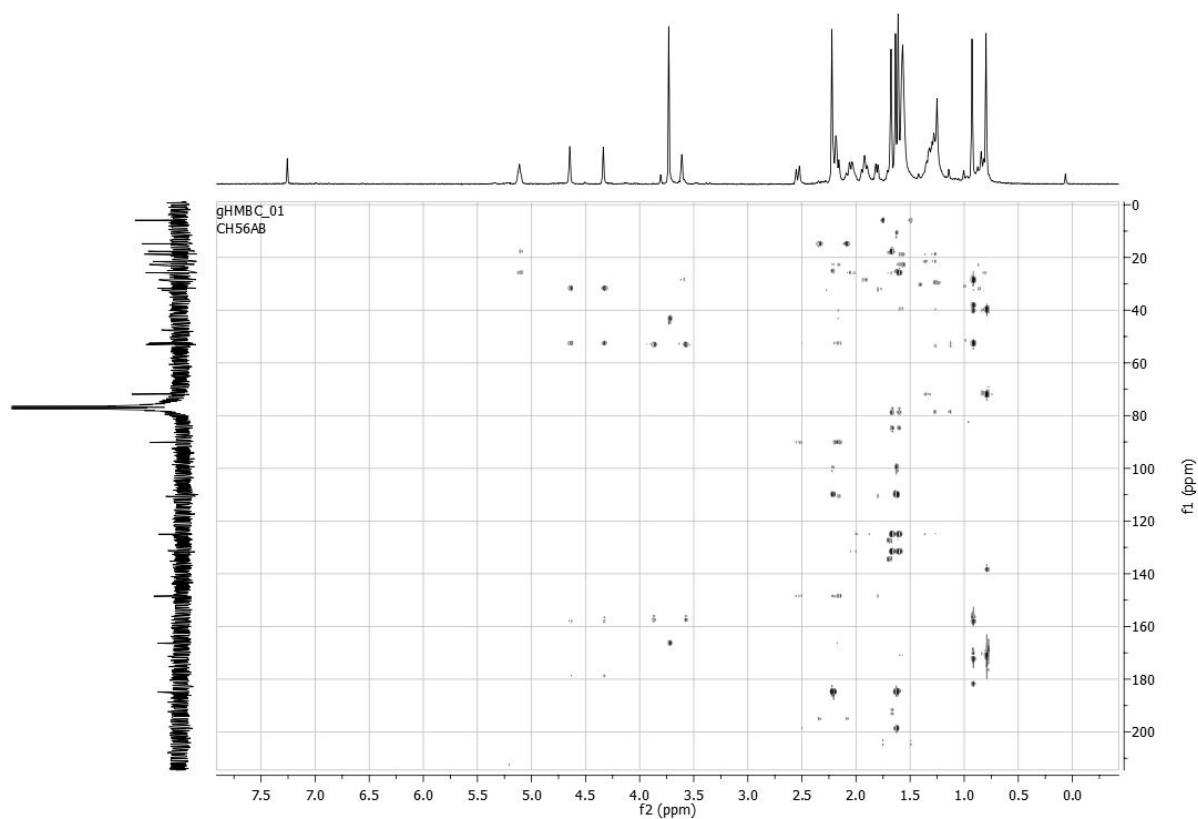
**Spectra 8.**  $^1\text{H}$  NMR spectrum of higginsianin E (2) ( $\text{CDCl}_3$ , 400 MHz).



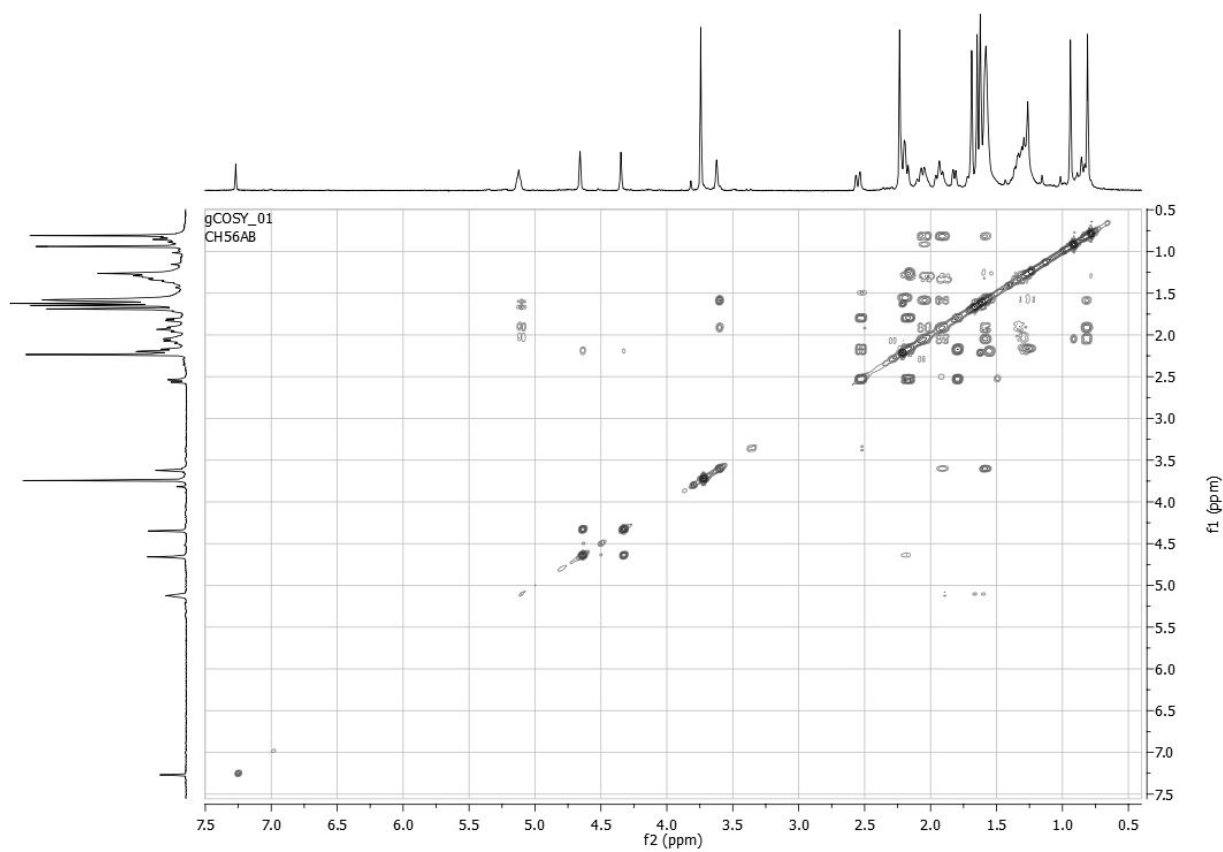
Spectra 9.  $^{13}\text{C}$  NMR spectrum of higginsianin E (2) ( $\text{CDCl}_3$ , 100 MHz).



Spectra 10. HSQC spectrum of higginsianin E (2) ( $\text{CDCl}_3$ , 400/100 MHz).

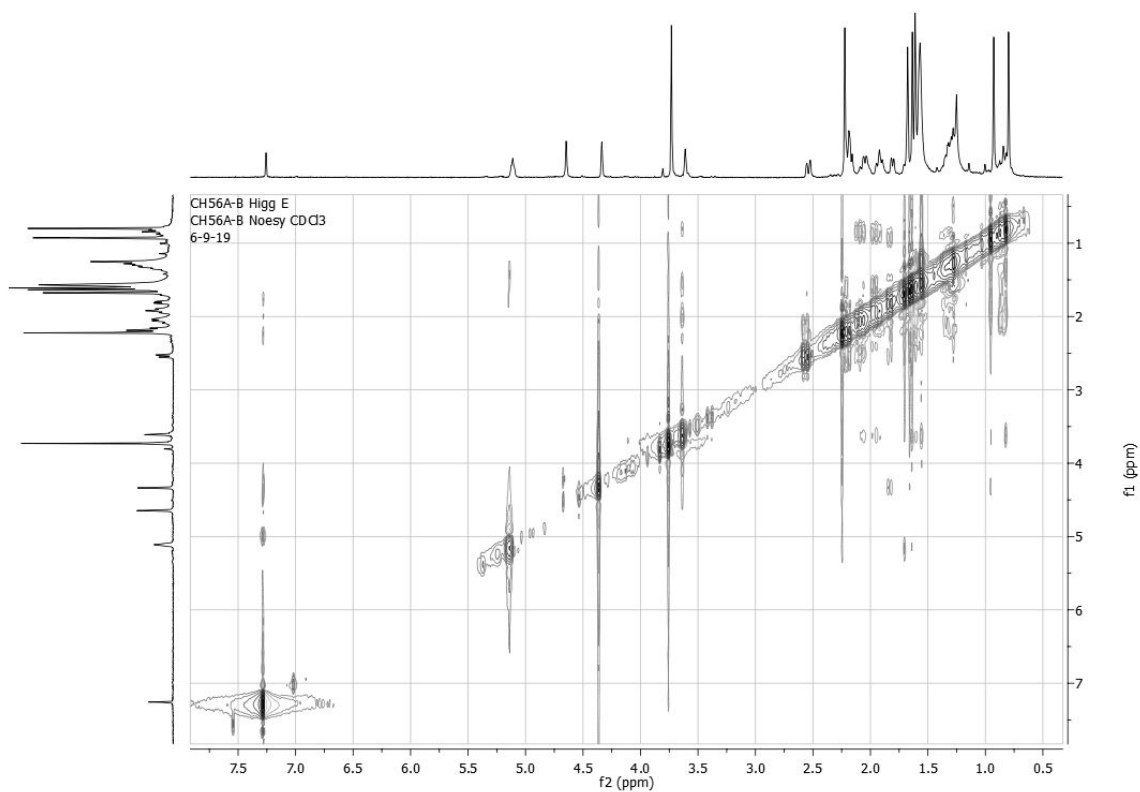


**Spectra 11.** HMBC spectrum of higginsianin E (**2**) (CDCl<sub>3</sub>, 400/100 MHz).

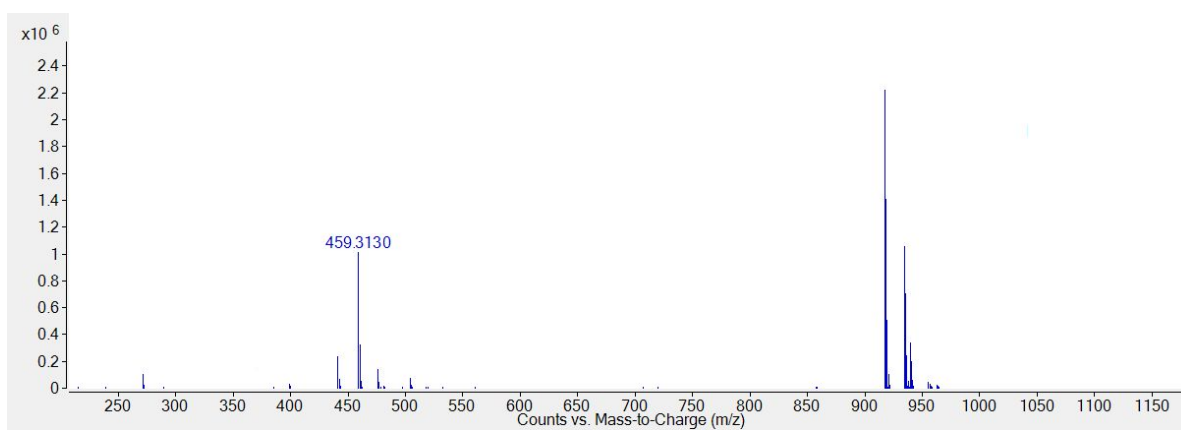


**Spectra 12.** COSY spectrum of higginsianin E (**2**) (CDCl<sub>3</sub>, 400 MHz).



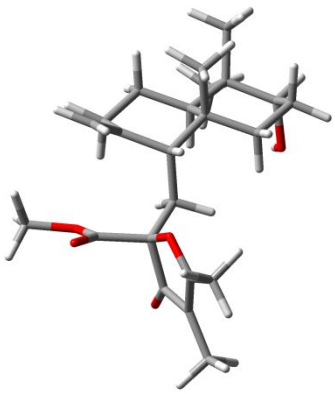
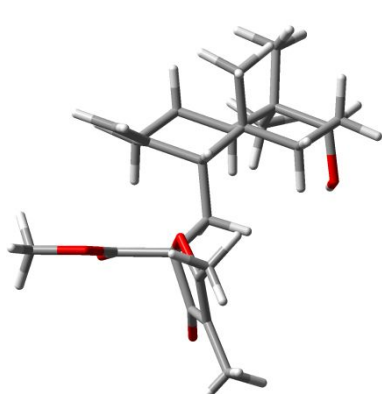
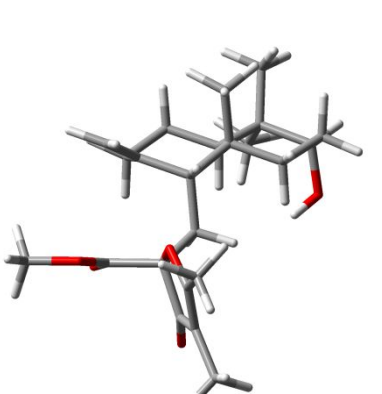
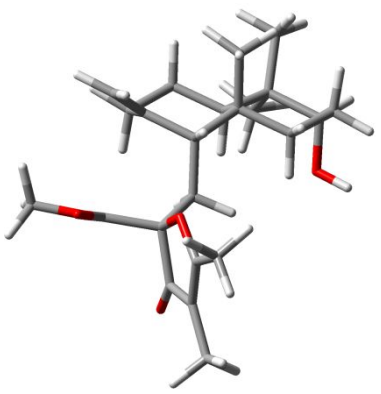
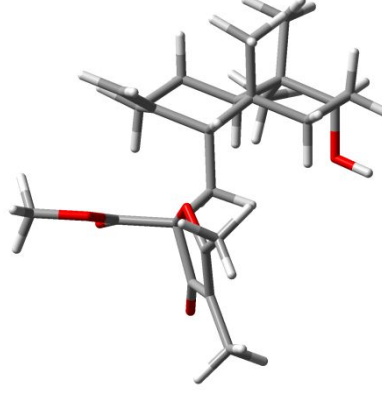
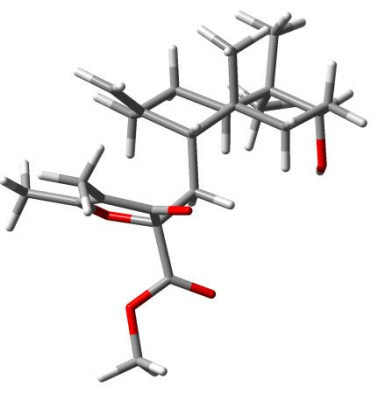
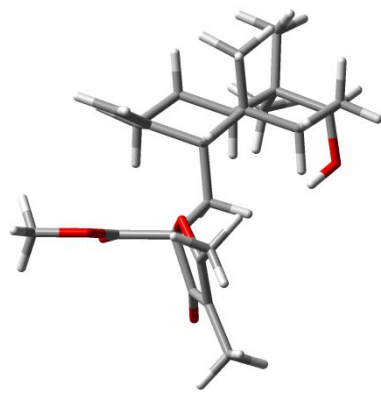
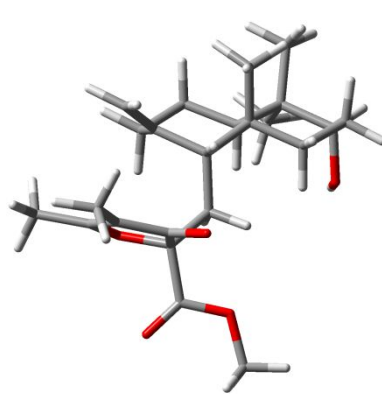
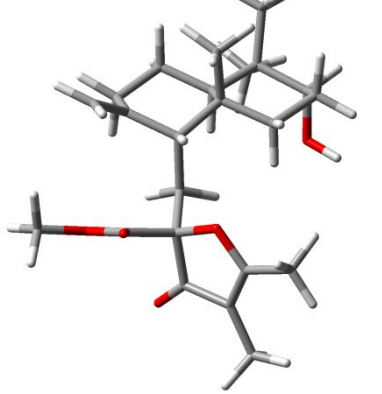


**Spectra 13.** NOESY spectrum of higginsianin E (**2**) (CDCl<sub>3</sub>, 400 MHz).

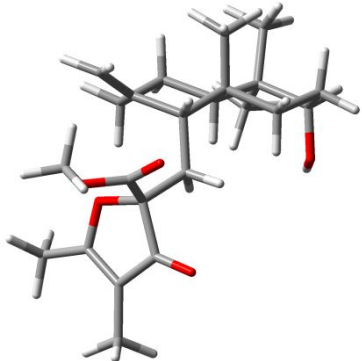
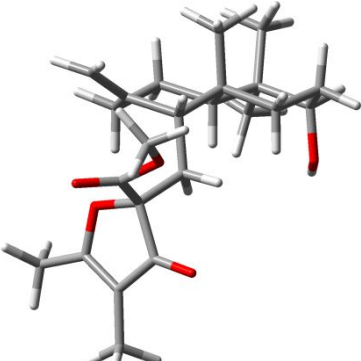
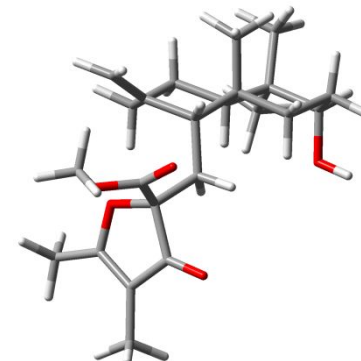
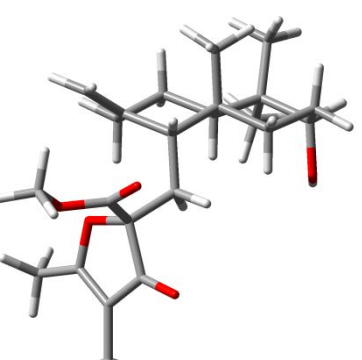
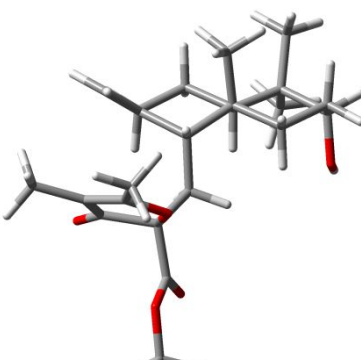
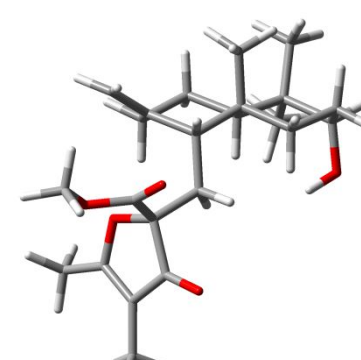
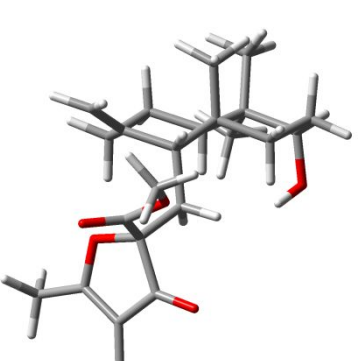
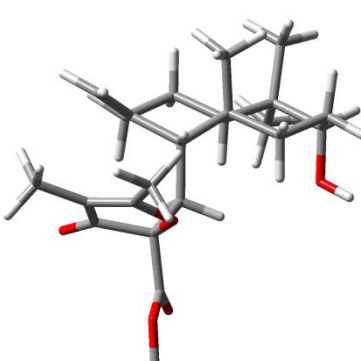



**Spectra 14.** HR ESIMS of higginsianin E (**2**), recorded in positive modality.

**Table S1.** The first nine low-energy structures of the truncated model of higginsianin D (4*R*,5*R*,8*R*,9*S*,10*R*,21*S*)-1 with energies and populations estimated at  $\omega$ B97X-D/def2-TZVP/PCM level.

 <p>Abs. min. (24.5%)</p>	 <p>+0.29 kcal/mol (15.0%)</p>	 <p>+0.45 kcal/mol (11.5%)</p>
 <p>+0.63 kcal/mol (8.6%)</p>	 <p>+0.76 kcal/mol (6.8%)</p>	 <p>+0.79 kcal/mol (6.5%)</p>
 <p>+0.90 kcal/mol (5.5%)</p>	 <p>+0.90 kcal/mol (5.5%)</p>	 <p>+1.11 kcal/mol (3.8%)</p>

**Table S2.** The first nine low-energy structures of the truncated model of higginsianin E (4*R*,5*R*,8*R*,9*S*,10*R*,21*R*)-**2** with energies and populations estimated at  $\omega$ B97X-D/def2-TZVP/PCM level.

 <p>Abs. min. (19.9%)</p>	 <p>+0.11 kcal/mol (16.6%)</p>	 <p>+0.40 kcal/mol (10.2%)</p>
 <p>+0.40 kcal/mol (10.2%)</p>	 <p>+0.43 kcal/mol (9.6%)</p>	 <p>+0.65 kcal/mol (6.7%)</p>
 <p>+0.76 kcal/mol (5.5%)</p>	 <p>+0.94 kcal/mol (4.1%)</p>	 <p>+0.95 kcal/mol (4.0%)</p>

**Figure S1.** Comparison between experimental  $^{13}\text{C}$  chemical shifts of higginsianin D and E measured in  $\text{CDCl}_3$  and the shifts calculated for the truncated models of (4*R*,5*R*,8*R*,9*S*,10*R*,21*S*)-**1** and (4*R*,5*R*,8*R*,9*S*,10*R*,21*R*)-**2** using the procedure described in the main text with final  $\omega\text{B97X-V/6-311+G(2df,2p)}/\omega\text{B97X-D/6-31G(d)}$  energy estimation and geometry optimization and  $\omega\text{B97X-D/6-31G(d)}$  shielding calculations. The comparison is restricted to the dihydrofuran-2-one moiety (i.e. carbon atoms from C-20 to C-27, plus the ester group).

