

**Supplementary Materials for**

**Thuwalallenes A–E and Thuwalenynes A–C: New C<sub>15</sub>  
Acetogenins with Anti-Inflammatory Activity from a  
Saudi Arabian Red Sea *Laurencia* sp.**

**Aikaterini Koutsaviti**<sup>1</sup>, **Maria G. Daskalaki**<sup>2</sup>, **Susana R. Agusti**<sup>3</sup>, **Sotirios C. Kampranis**<sup>2,4</sup>, **Christos Tsatsanis**<sup>2,5</sup>, **Carlos M. Duarte**<sup>3</sup>, **Vassilios Roussis**<sup>1</sup> and **Efstathia Ioannou**<sup>1,\*</sup>

<sup>1</sup> Section of Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis Zografou, Athens 15771, Greece; [kkoutsaviti@pharm.uoa.gr](mailto:kkoutsaviti@pharm.uoa.gr) (A.K.); [roussis@pharm.uoa.gr](mailto:roussis@pharm.uoa.gr) (V.R.)

<sup>2</sup> Laboratory of Clinical Chemistry, School of Medicine, University of Crete, Heraklion 70013, Greece; [m.daskalaki@med.uoc.gr](mailto:m.daskalaki@med.uoc.gr) (M.G.D.); [soka@plen.ku.dk](mailto:soka@plen.ku.dk) (S.C.K.); [tsatsani@uoc.gr](mailto:tsatsani@uoc.gr) (C.T.)

<sup>3</sup> Red Sea Research Center, King Abdullah University of Science and Technology, Thuwal, 23955-6900, Saudi Arabia; [susana.agusti@kaust.edu.sa](mailto:susana.agusti@kaust.edu.sa) (S.R.A.); [carlos.duarte@kaust.edu.sa](mailto:carlos.duarte@kaust.edu.sa) (C.M.D.)

<sup>4</sup> Present address: Section of Plant Biochemistry, Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark

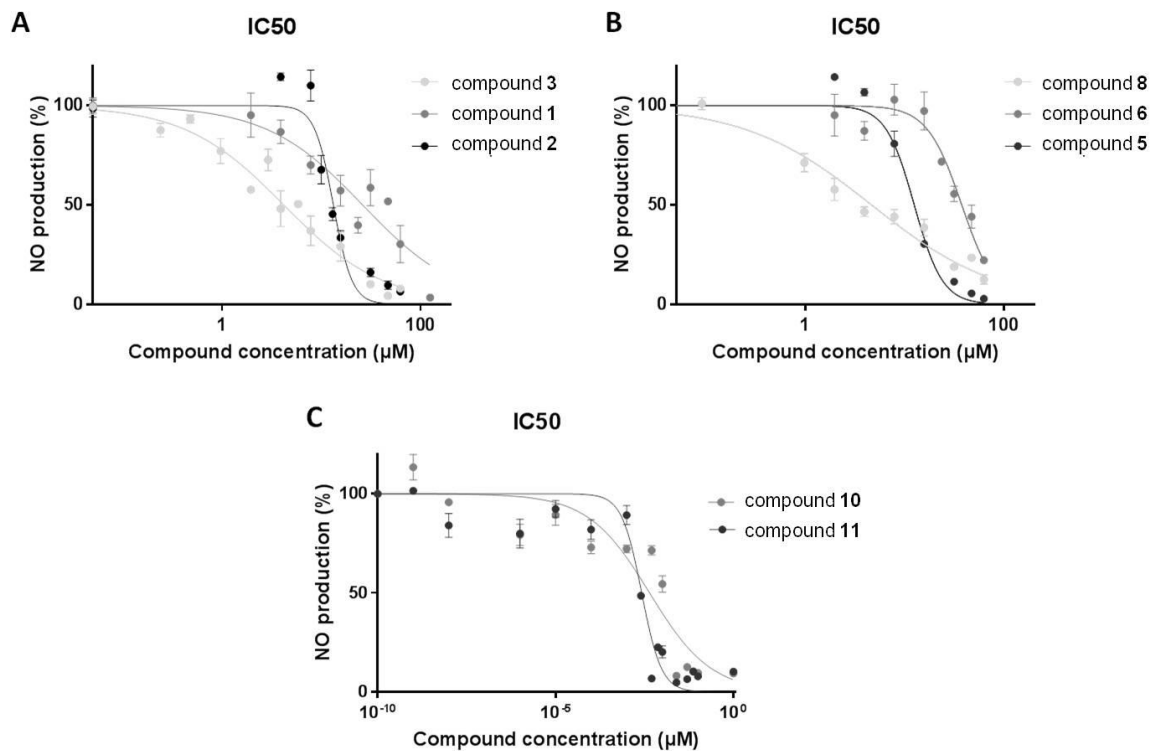
<sup>5</sup> Institute of Molecular Biology and Biotechnology, FORTH, Heraklion 71110, Greece

\* Correspondence: [eioannou@pharm.uoa.gr](mailto:eioannou@pharm.uoa.gr) (E.I.); Tel.: +30 210 727 4913

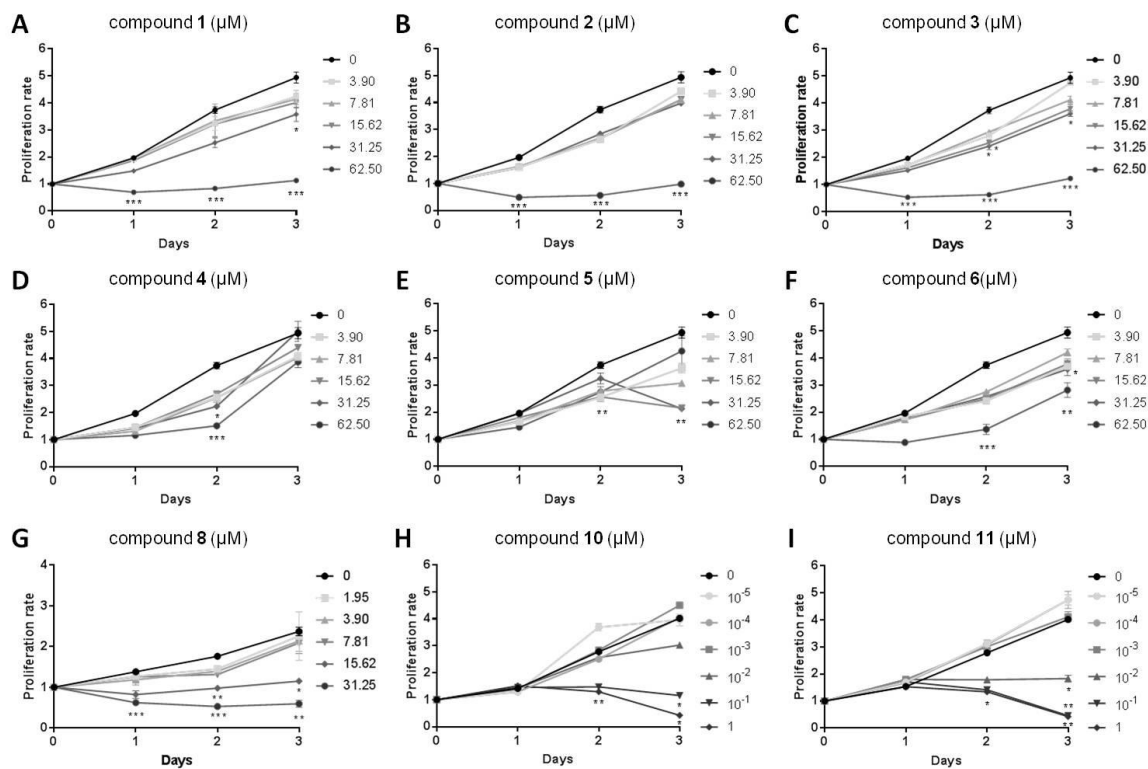
## Table of Contents

<b>Figure S1. (A-C)</b> Determination of concentration inducing 50% inhibition of NO production using LPS-treated RAW 264.7 for compounds <b>1–3, 5, 6, 8, 10</b> and <b>11</b> .	S4
<b>Figure S2. (A-I)</b> Evaluation of the cytotoxic activity of compounds <b>1–6, 8, 10</b> and <b>11</b> by measuring proliferation rate of RAW 264.7 cells.	S5
<b>Figure S3.</b> <sup>1</sup> H NMR spectrum of thuwalallene <b>A (1)</b> in CDCl <sub>3</sub> .	S6
<b>Figure S4.</b> HSQC spectrum of thuwalallene <b>A (1)</b> in CDCl <sub>3</sub> .	S6
<b>Figure S5.</b> HMBC spectrum of thuwalallene <b>A (1)</b> in CDCl <sub>3</sub> .	S7
<b>Figure S6.</b> COSY spectrum of thuwalallene <b>A (1)</b> in CDCl <sub>3</sub> .	S7
<b>Figure S7.</b> NOESY spectrum of thuwalallene <b>A (1)</b> in CDCl <sub>3</sub> .	S8
<b>Figure S8.</b> HR-APCIMS spectrum of thuwalallene <b>A (1)</b> .	S8
<b>Figure S9.</b> <sup>1</sup> H NMR spectrum of thuwalallene <b>B (2)</b> in CDCl <sub>3</sub> .	S9
<b>Figure S10.</b> HSQC spectrum of thuwalallene <b>B (2)</b> in CDCl <sub>3</sub> .	S9
<b>Figure S11.</b> HMBC spectrum of thuwalallene <b>B (2)</b> in CDCl <sub>3</sub> .	S10
<b>Figure S12.</b> COSY spectrum of thuwalallene <b>B (2)</b> in CDCl <sub>3</sub> .	S10
<b>Figure S13.</b> NOESY spectrum of thuwalallene <b>B (2)</b> in CDCl <sub>3</sub> .	S11
<b>Figure S14.</b> HR-APCIMS spectrum of thuwalallene <b>B (2)</b> .	S11
<b>Figure S15.</b> <sup>1</sup> H NMR spectrum of thuwalallene <b>C (3)</b> in CDCl <sub>3</sub> .	S12
<b>Figure S16.</b> <sup>13</sup> C NMR spectrum of thuwalallene <b>C (3)</b> in CDCl <sub>3</sub> .	S12
<b>Figure S17.</b> HSQC spectrum of thuwalallene <b>C (3)</b> in CDCl <sub>3</sub> .	S13
<b>Figure S18.</b> HMBC spectrum of thuwalallene <b>C (3)</b> in CDCl <sub>3</sub> .	S13
<b>Figure S19.</b> COSY spectrum of thuwalallene <b>C (3)</b> in CDCl <sub>3</sub> .	S14
<b>Figure S20.</b> NOESY spectrum of thuwalallene <b>C (3)</b> in CDCl <sub>3</sub> .	S14
<b>Figure S21.</b> HR-APCIMS spectrum of thuwalallene <b>C (3)</b> .	S15
<b>Figure S22.</b> <sup>1</sup> H NMR spectrum of thuwalenyne <b>A (4)</b> in CDCl <sub>3</sub> .	S16
<b>Figure S23.</b> HSQC spectrum of thuwalenyne <b>A (4)</b> in CDCl <sub>3</sub> .	S16
<b>Figure S24.</b> HMBC spectrum of thuwalenyne <b>A (4)</b> in CDCl <sub>3</sub> .	S17
<b>Figure S25.</b> COSY spectrum of thuwalenyne <b>A (4)</b> in CDCl <sub>3</sub> .	S17
<b>Figure S26.</b> NOESY spectrum of thuwalenyne <b>A (4)</b> in CDCl <sub>3</sub> .	S18
<b>Figure S27.</b> HR-APCIMS spectrum of thuwalenyne <b>A (4)</b> .	S18
<b>Figure S28.</b> <sup>1</sup> H NMR spectrum of thuwalallene <b>D (5)</b> in CDCl <sub>3</sub> .	S19
<b>Figure S29.</b> HSQC spectrum of thuwalallene <b>D (5)</b> in CDCl <sub>3</sub> .	S19
<b>Figure S30.</b> HMBC spectrum of thuwalallene <b>D (5)</b> in CDCl <sub>3</sub> .	S20
<b>Figure S31.</b> COSY spectrum of thuwalallene <b>D (5)</b> in CDCl <sub>3</sub> .	S20
<b>Figure S32.</b> NOESY spectrum of thuwalallene <b>D (5)</b> in CDCl <sub>3</sub> .	S21
<b>Figure S33.</b> HR-APCIMS spectrum of thuwalallene <b>D (5)</b> .	S21
<b>Figure S34.</b> <sup>1</sup> H NMR spectrum of thuwalenyne <b>B (6)</b> in CDCl <sub>3</sub> .	S22
<b>Figure S35.</b> HSQC spectrum of thuwalenyne <b>B (6)</b> in CDCl <sub>3</sub> .	S22
<b>Figure S36.</b> HMBC spectrum of thuwalenyne <b>B (6)</b> in CDCl <sub>3</sub> .	S23
<b>Figure S37.</b> COSY spectrum of thuwalenyne <b>B (6)</b> in CDCl <sub>3</sub> .	S23
<b>Figure S38.</b> NOESY spectrum of thuwalenyne <b>B (6)</b> in CDCl <sub>3</sub> .	S24
<b>Figure S39.</b> HR-APCIMS spectrum of thuwalenyne <b>B (6)</b> .	S24
<b>Figure S40.</b> <sup>1</sup> H NMR spectrum of thuwalenyne <b>C (7)</b> in CDCl <sub>3</sub> .	S25
<b>Figure S41.</b> HSQC spectrum of thuwalenyne <b>C (7)</b> in CDCl <sub>3</sub> .	S25
<b>Figure S42.</b> HMBC spectrum of thuwalenyne <b>C (7)</b> in CDCl <sub>3</sub> .	S26
<b>Figure S43.</b> COSY spectrum of thuwalenyne <b>C (7)</b> in CDCl <sub>3</sub> .	S26
<b>Figure S44.</b> NOESY spectrum of thuwalenyne <b>C (7)</b> in CDCl <sub>3</sub> .	S27
<b>Figure S45.</b> HR-APCIMS spectrum of thuwalenyne <b>C (7)</b> .	S27
<b>Figure S46.</b> <sup>1</sup> H NMR spectrum of thuwalallene <b>E (8)</b> in CDCl <sub>3</sub> .	S28
<b>Figure S47.</b> <sup>13</sup> C NMR spectrum of thuwalallene <b>E (8)</b> in CDCl <sub>3</sub> .	S28
<b>Figure S48.</b> HSQC spectrum of thuwalallene <b>E (8)</b> in CDCl <sub>3</sub> .	S29
<b>Figure S49.</b> HMBC spectrum of thuwalallene <b>E (8)</b> in CDCl <sub>3</sub> .	S29
<b>Figure S50.</b> COSY spectrum of thuwalallene <b>E (8)</b> in CDCl <sub>3</sub> .	S30
<b>Figure S51.</b> NOESY spectrum of thuwalallene <b>E (8)</b> in CDCl <sub>3</sub> .	S30
<b>Figure S52.</b> HR-APCIMS spectrum of thuwalallene <b>E (8)</b> .	S31

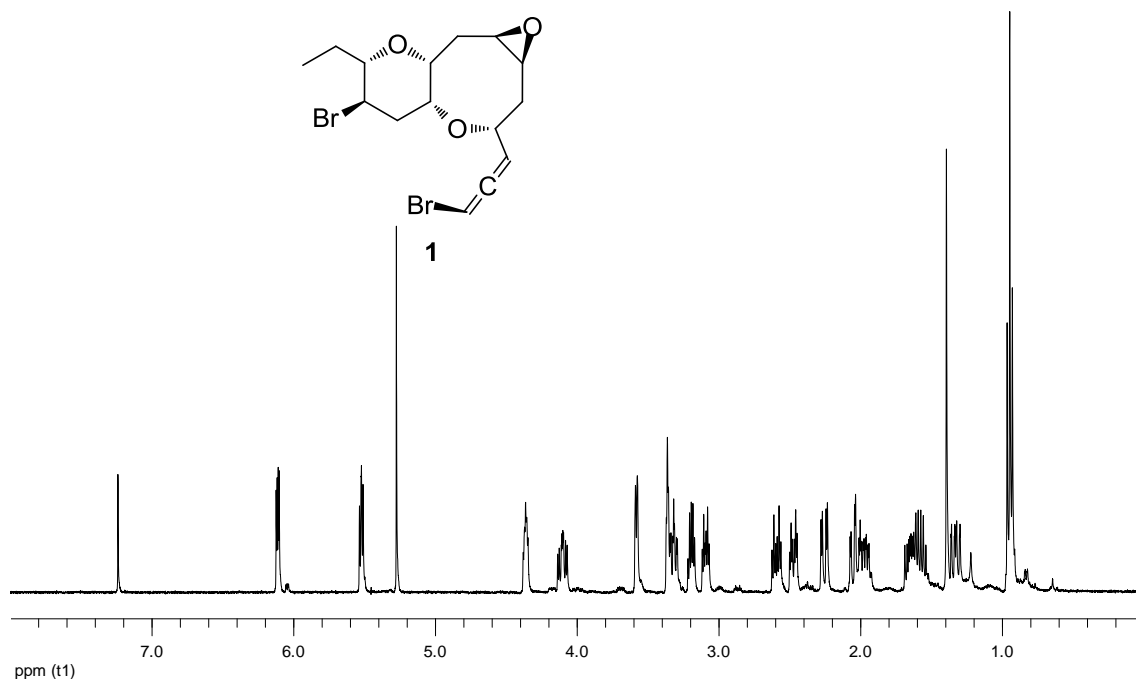
<b>Figure S53.</b> $^1\text{H}$ NMR spectrum of <i>cis</i> -maneonene D ( <b>9</b> ) in $\text{CDCl}_3$ .	S32
<b>Figure S54.</b> $^1\text{H}$ NMR spectrum of thysiferol ( <b>10</b> ) in $\text{CDCl}_3$ .	S32
<b>Figure S55.</b> $^1\text{H}$ NMR spectrum of 23-acetyl-thysiferol ( <b>11</b> ) in $\text{CDCl}_3$ .	S33



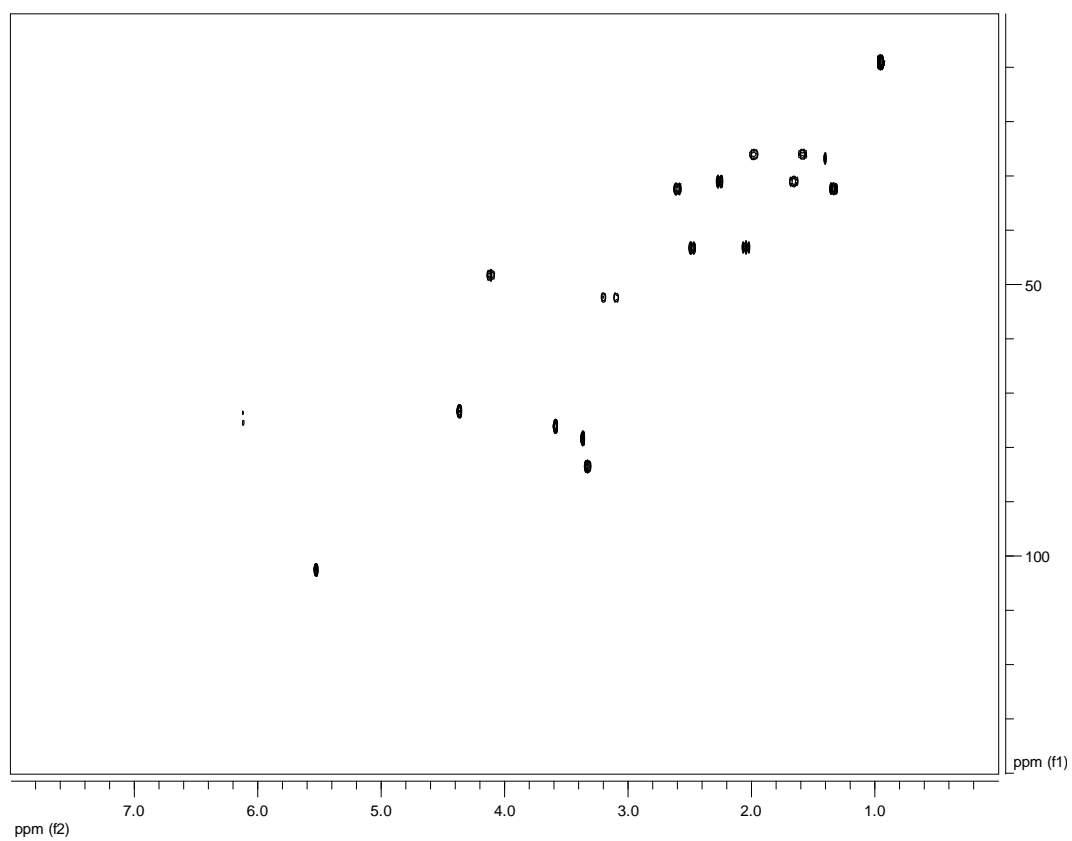
**Figure S1.** (A-C) Determination of concentration inducing 50% inhibition of NO production using LPS-treated RAW 264.7 for compounds 1–3, 5, 6, 8, 10 and 11. Inhibition of NO production was compared to that of cells treated with Carbowax 400 0.1% v/v.



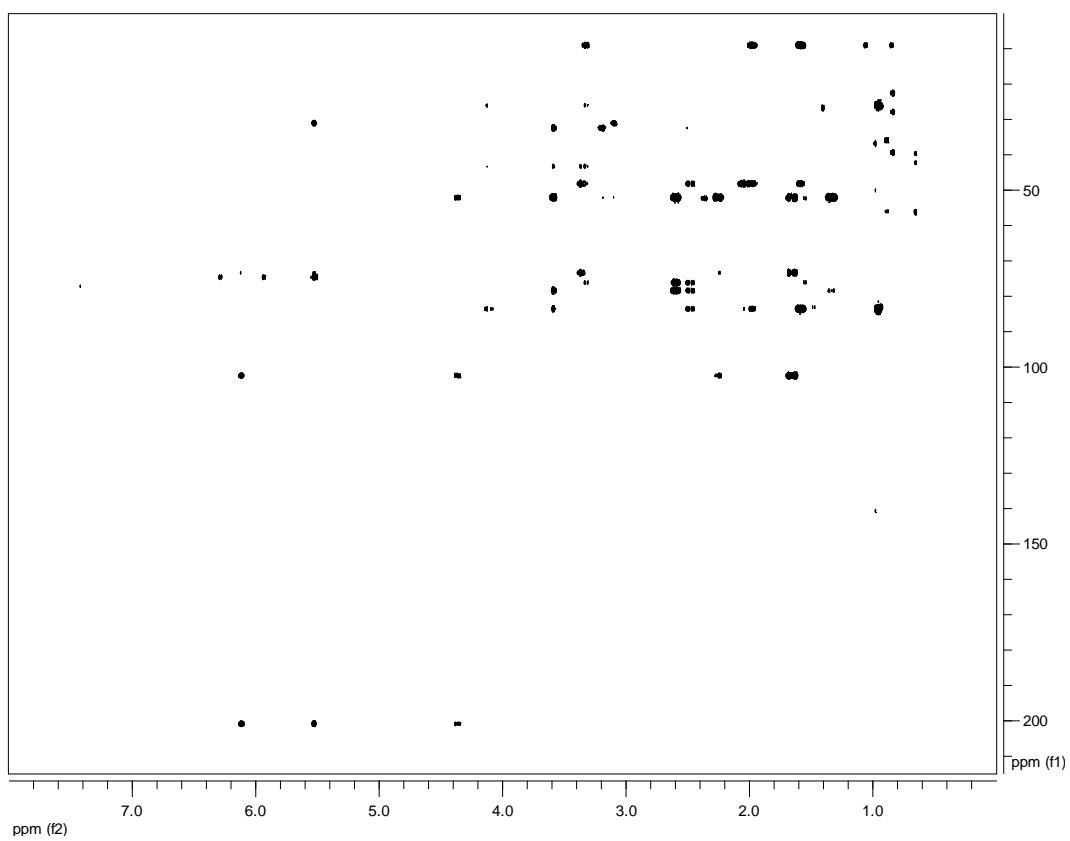
**Figure S2.** (A-I) Evaluation of the cytotoxic activity of compounds 1–6, 8, 10 and 11 by measuring proliferation rate of RAW 264.7 cells. Proliferation rate was established using MTT treatment for 72 h and normalized to measurement of the initial cells plated and compared to that of cells treated with Carbowax 400 0.1% v/v. Statistical analysis was performed using Kruskal-Wallis non-parametric test in Graphpad Prism 7.0. Graphs represent mean  $\pm$  SEM (\* indicates  $P < 0.05$ , \*\* indicates  $P < 0.01$ , \*\*\* indicates  $P < 0.001$ ).



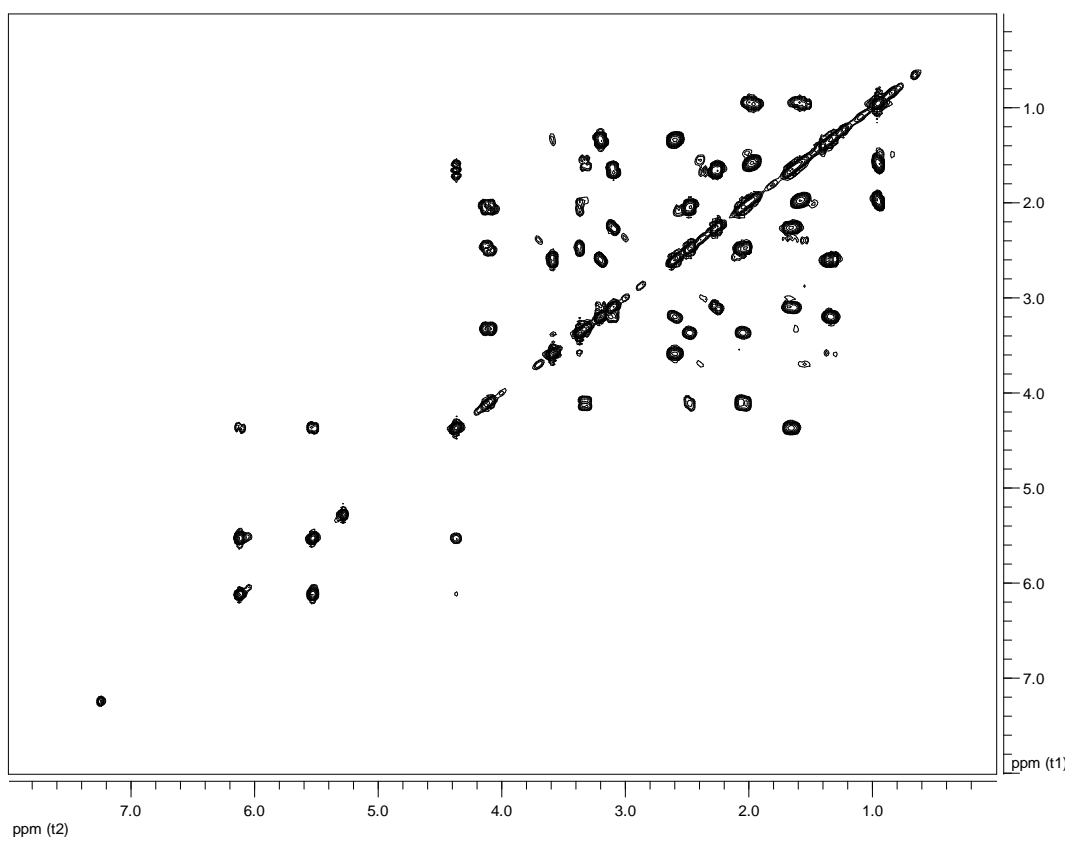
**Figure S3.** <sup>1</sup>H NMR spectrum of thuwalallene A (1) in CDCl<sub>3</sub>.



**Figure S4.** HSQC spectrum of thuwalallene A (1) in CDCl<sub>3</sub>.



**Figure S5.** HMBC spectrum of thuwalallene A (**1**) in  $\text{CDCl}_3$ .



**Figure S6.** COSY spectrum of thuwalallene A (**1**) in  $\text{CDCl}_3$ .

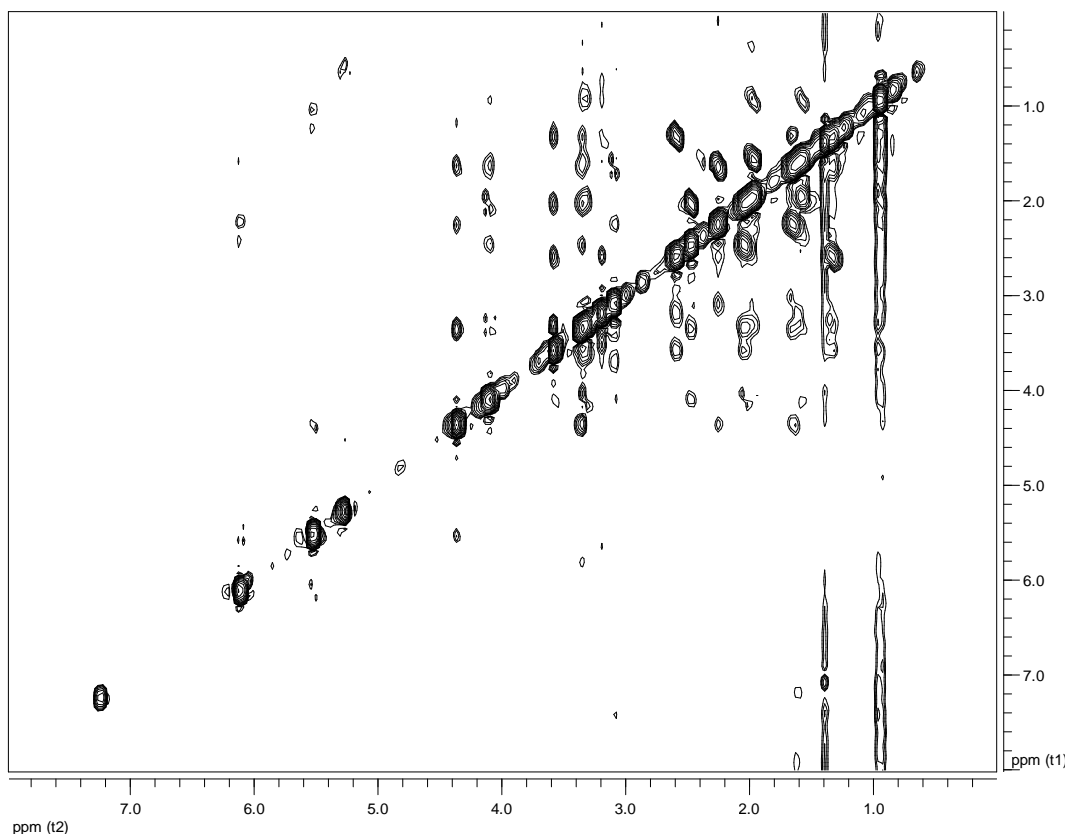


Figure S7. NOESY spectrum of thuwalallene A (1) in CDCl<sub>3</sub>.

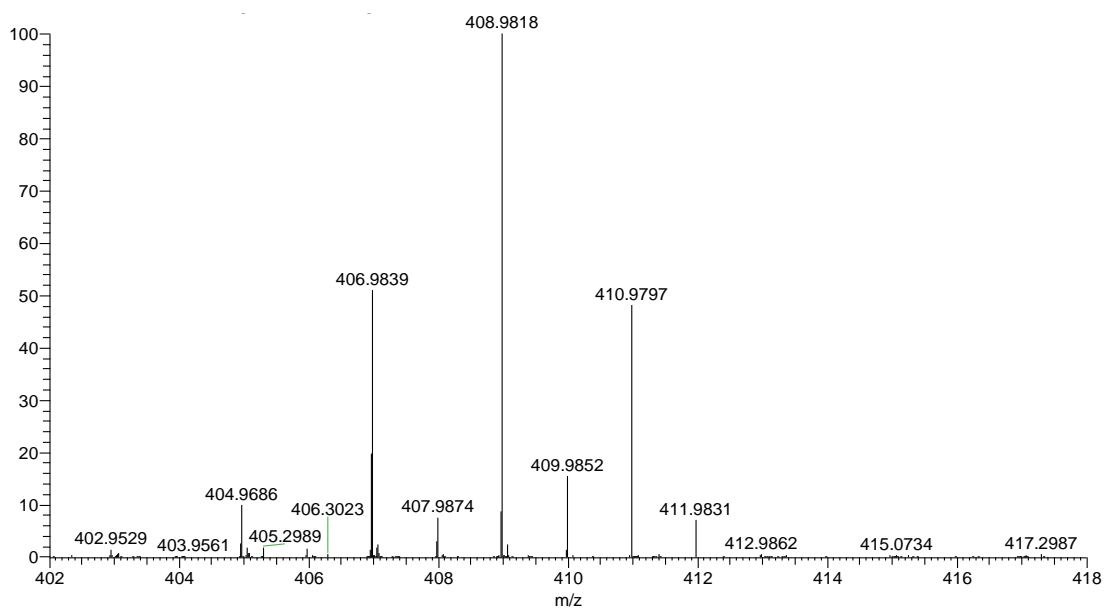
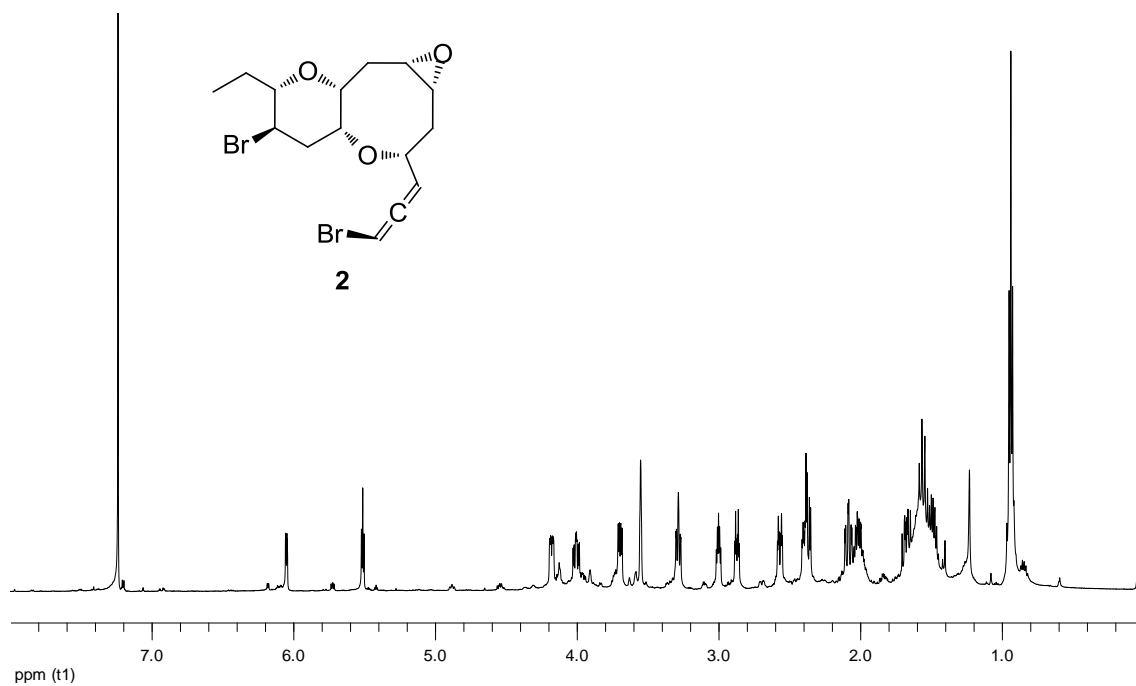
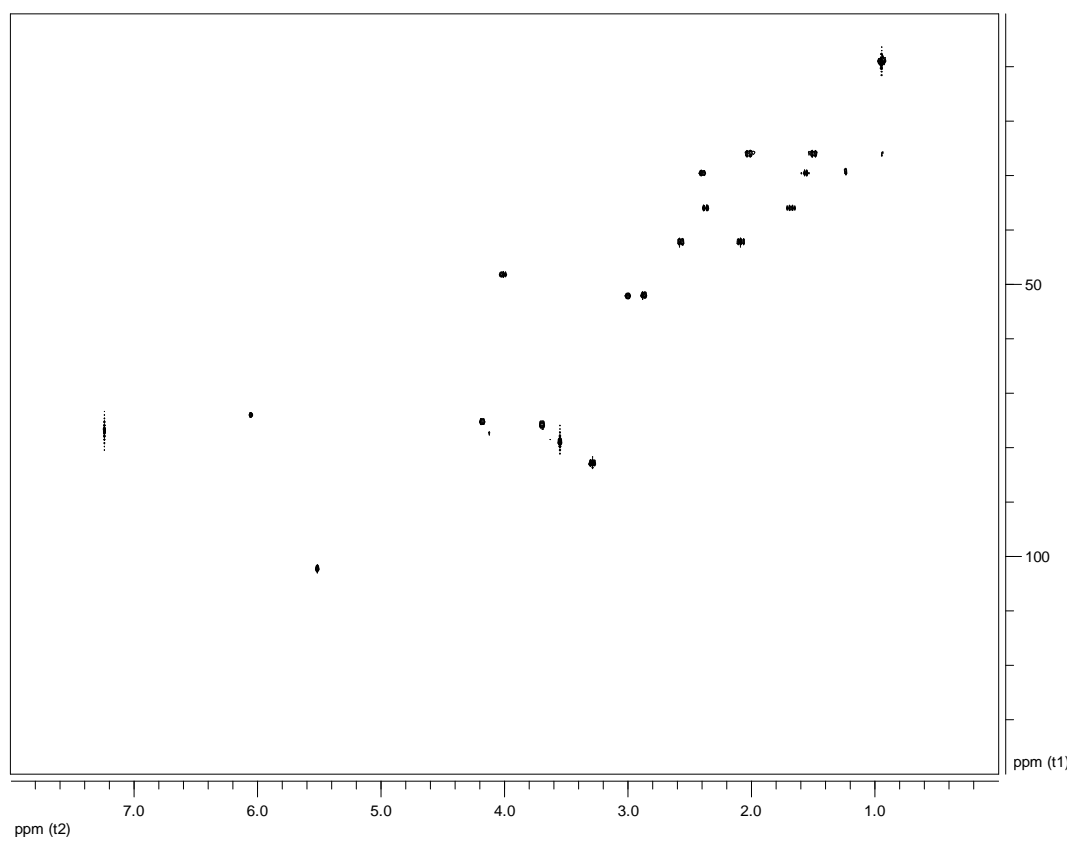


Figure S8. HR-APCIMS spectrum of thuwalallene A (1).

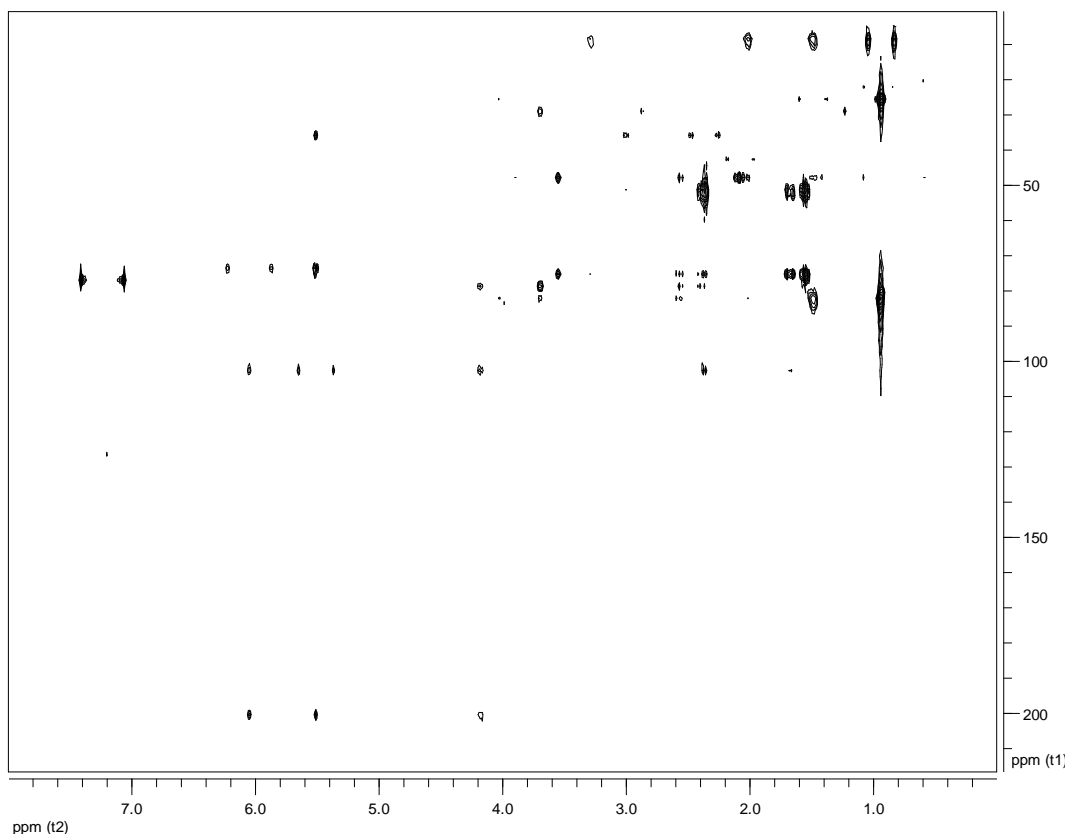




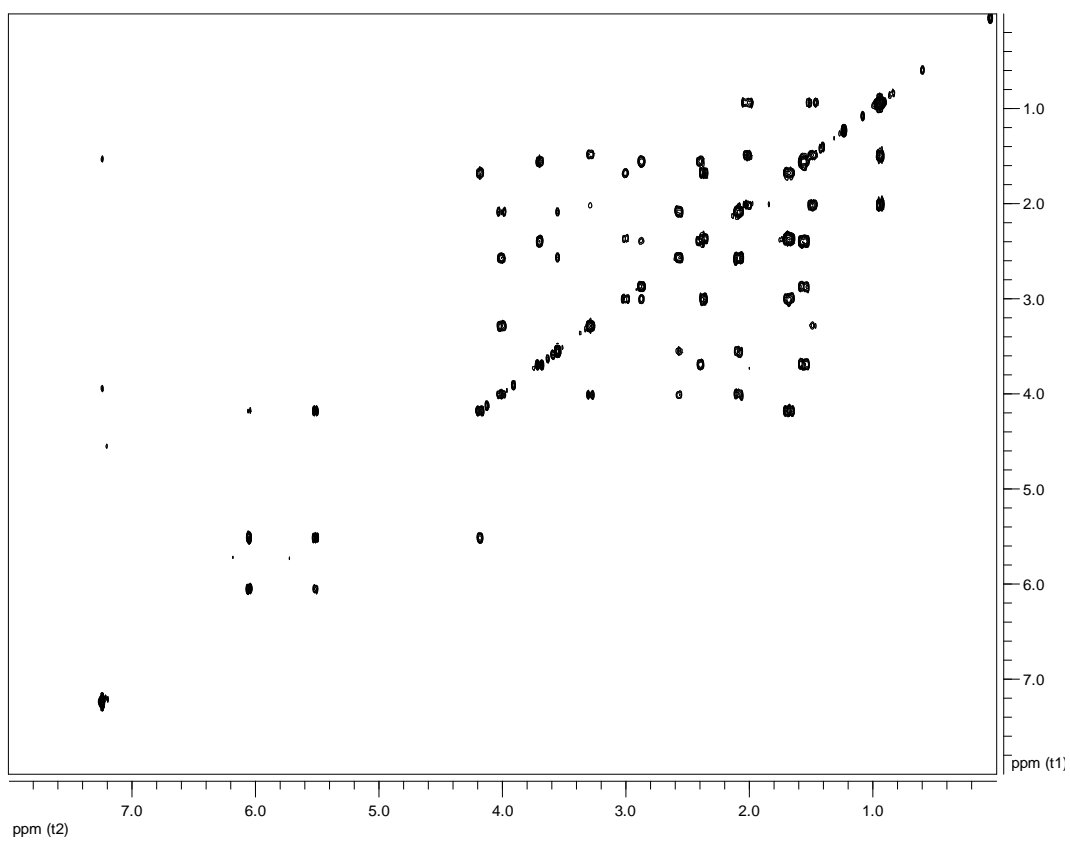
**Figure S9.** <sup>1</sup>H NMR spectrum of thuwalallene B (**2**) in CDCl<sub>3</sub>.



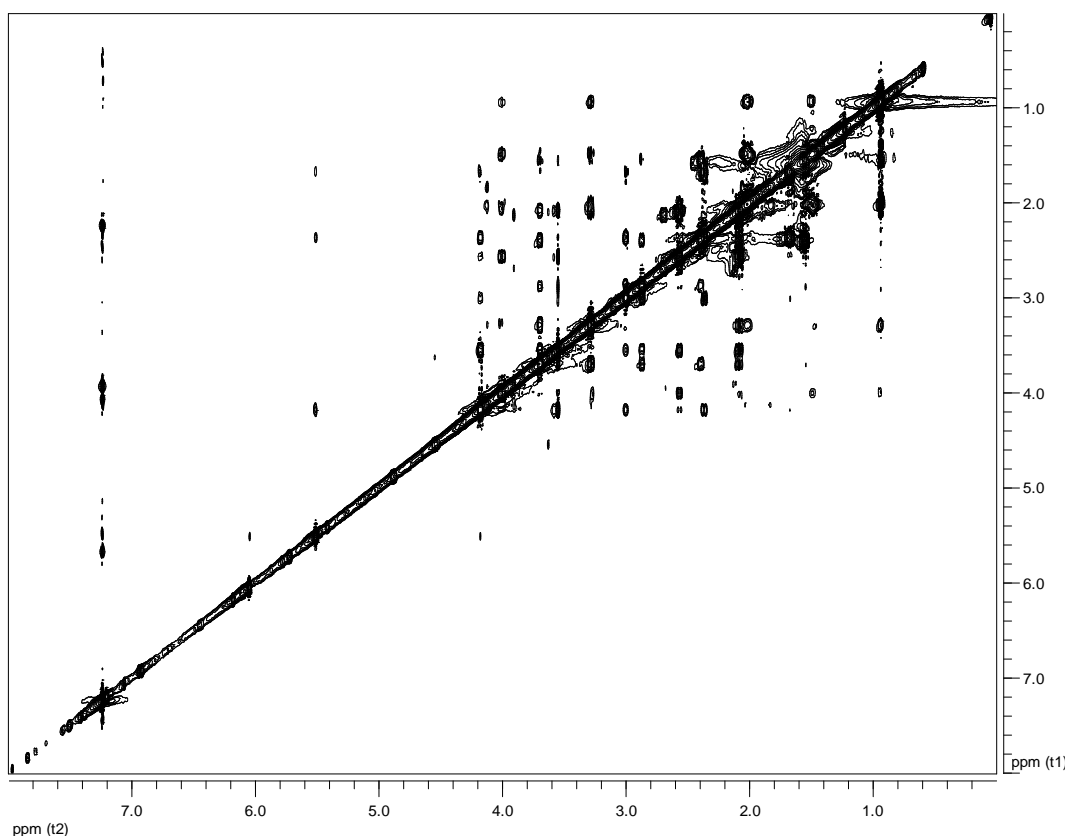
**Figure S10.** HSQC spectrum of thuwalallene B (**2**) in CDCl<sub>3</sub>.



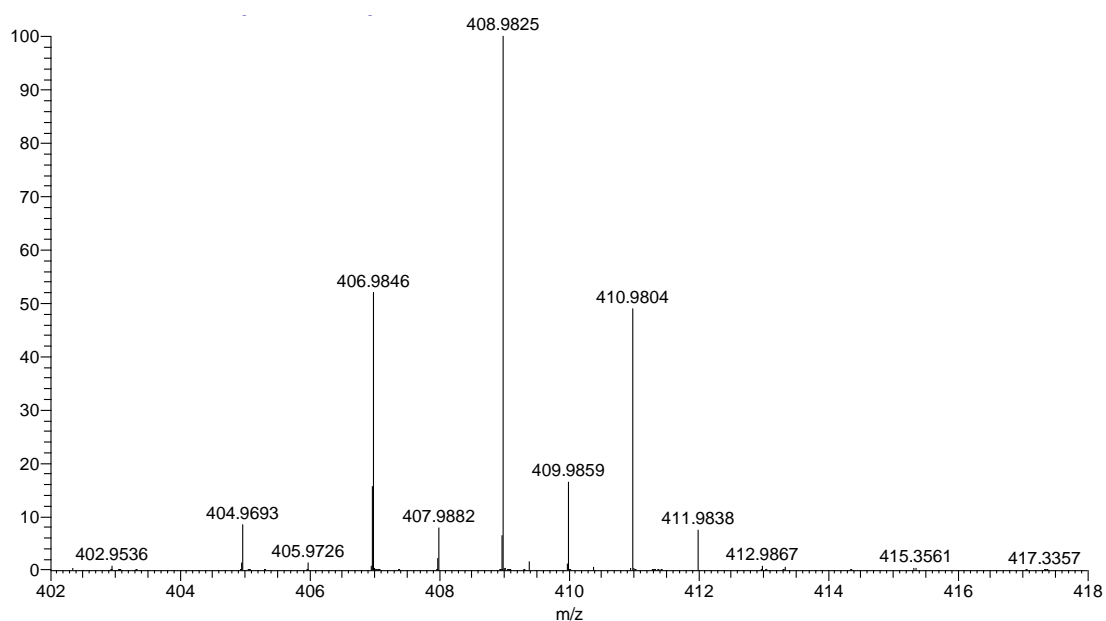
**Figure S11.** HMBC spectrum of thuwalallene B (**2**) in CDCl<sub>3</sub>.



**Figure S12.** COSY spectrum of thuwalallene B (**2**) in CDCl<sub>3</sub>.



**Figure S13.** NOESY spectrum of thuwalallene B (2) in  $\text{CDCl}_3$ .



**Figure S14.** HR-APCIMS spectrum of thuwalallene B (2).

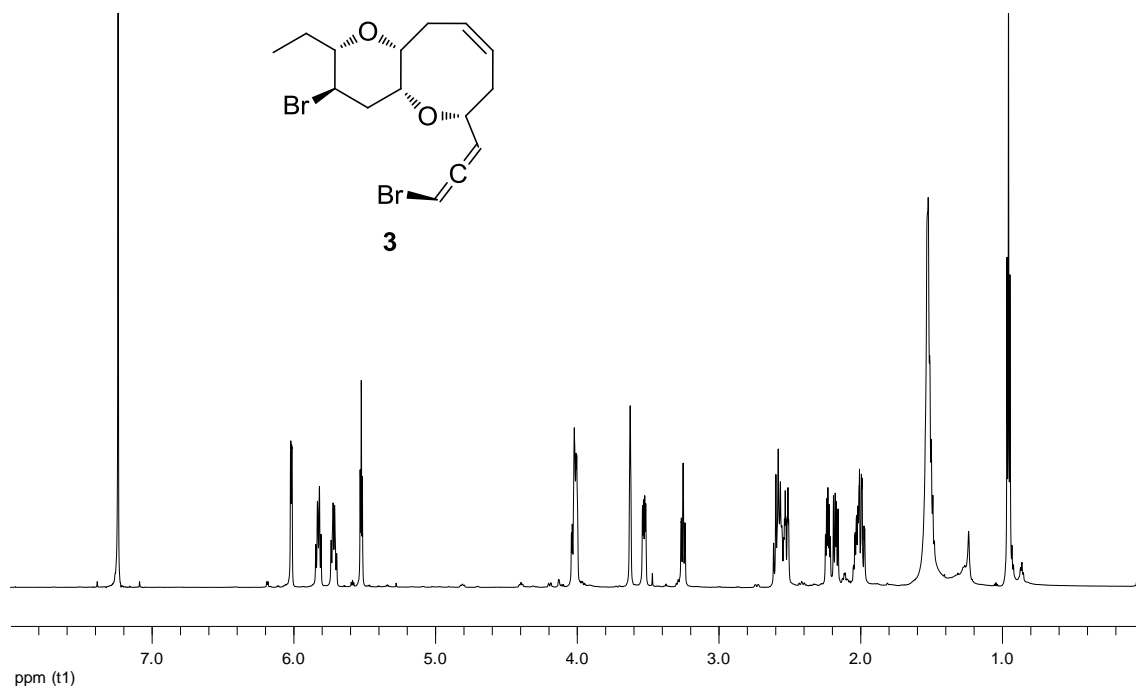


Figure S15. <sup>1</sup>H NMR spectrum of thuwalallene C (3) in CDCl<sub>3</sub>.

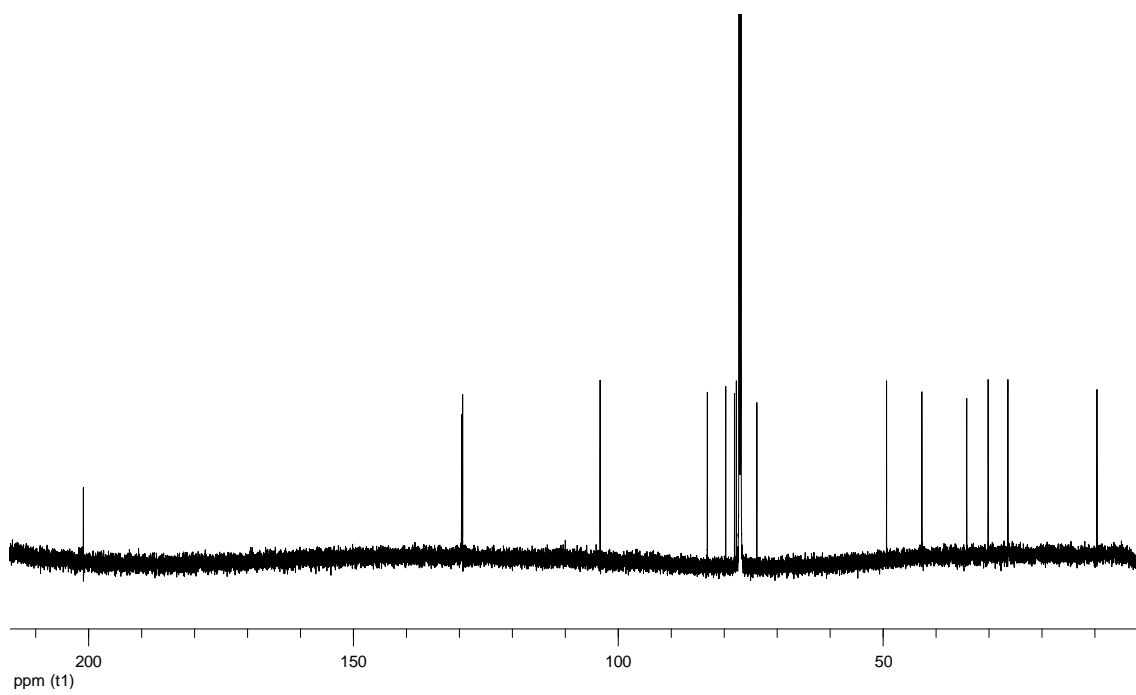
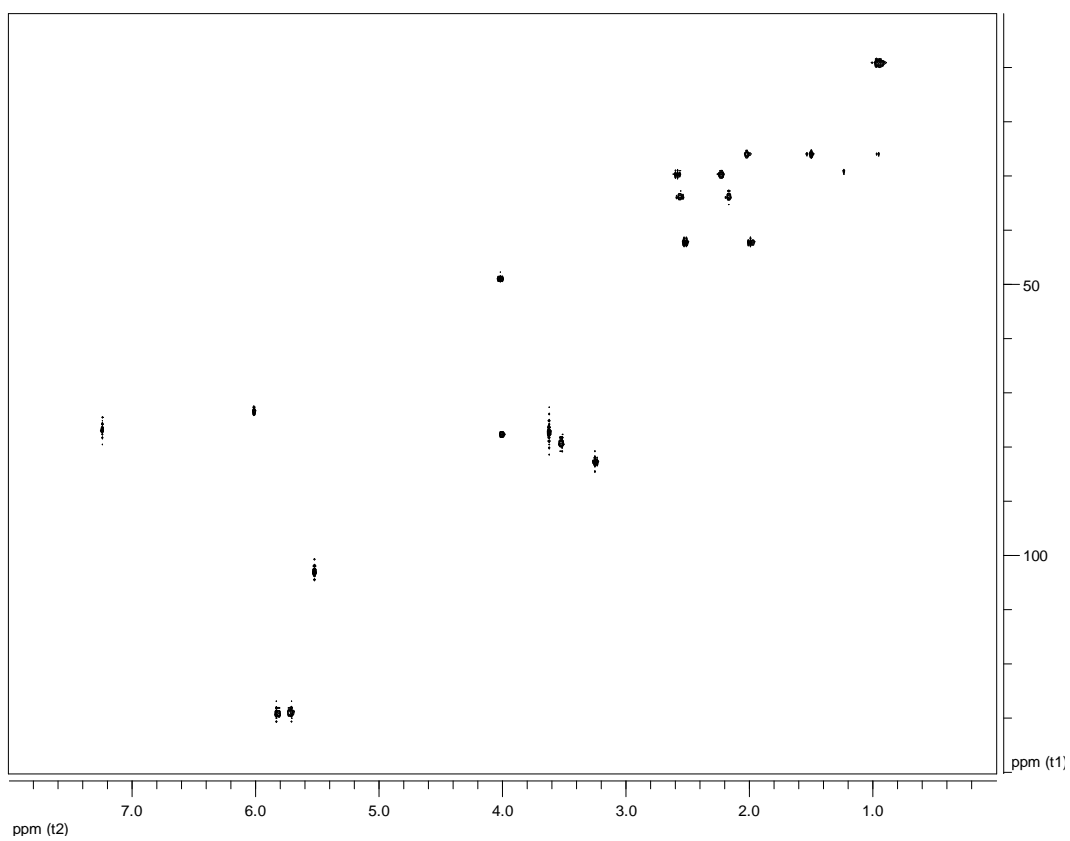
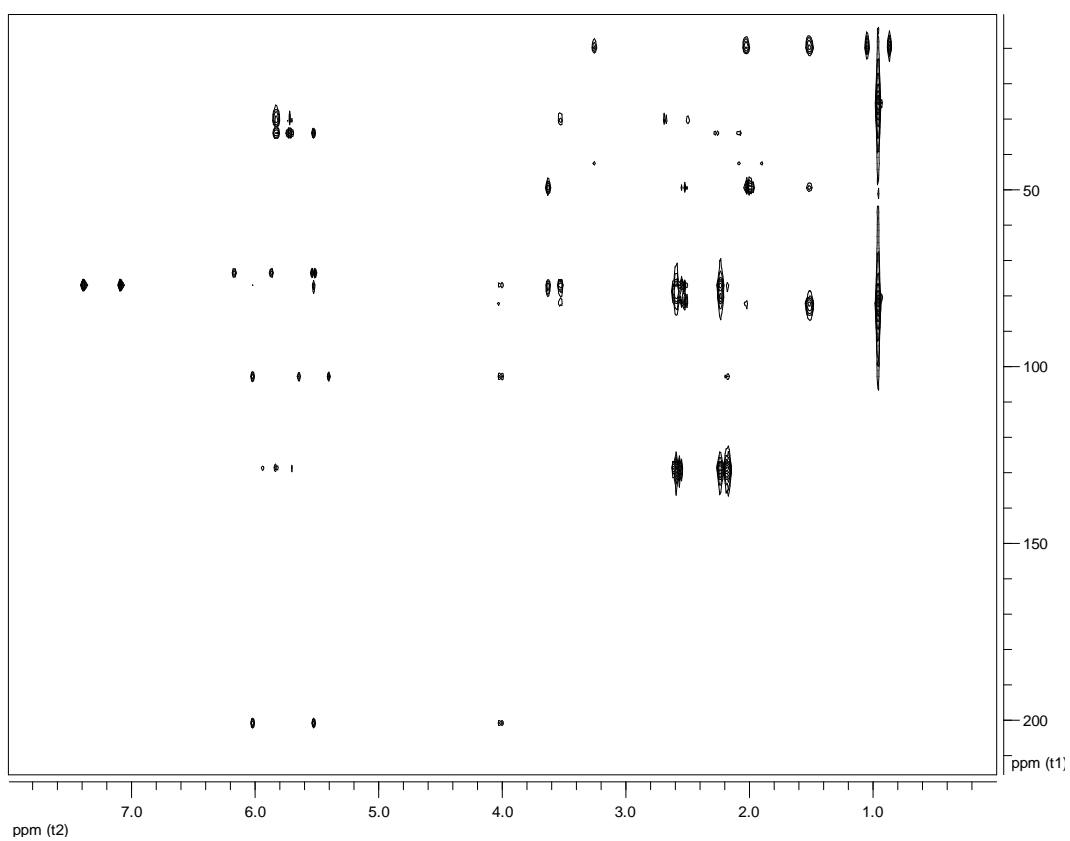


Figure S16. <sup>13</sup>C NMR spectrum of thuwalallene C (3) in CDCl<sub>3</sub>.



**Figure S17.** HSQC spectrum of thuwalallene C (**3**) in CDCl<sub>3</sub>.



**Figure S18.** HMBC spectrum of thuwalallene C (**3**) in CDCl<sub>3</sub>.

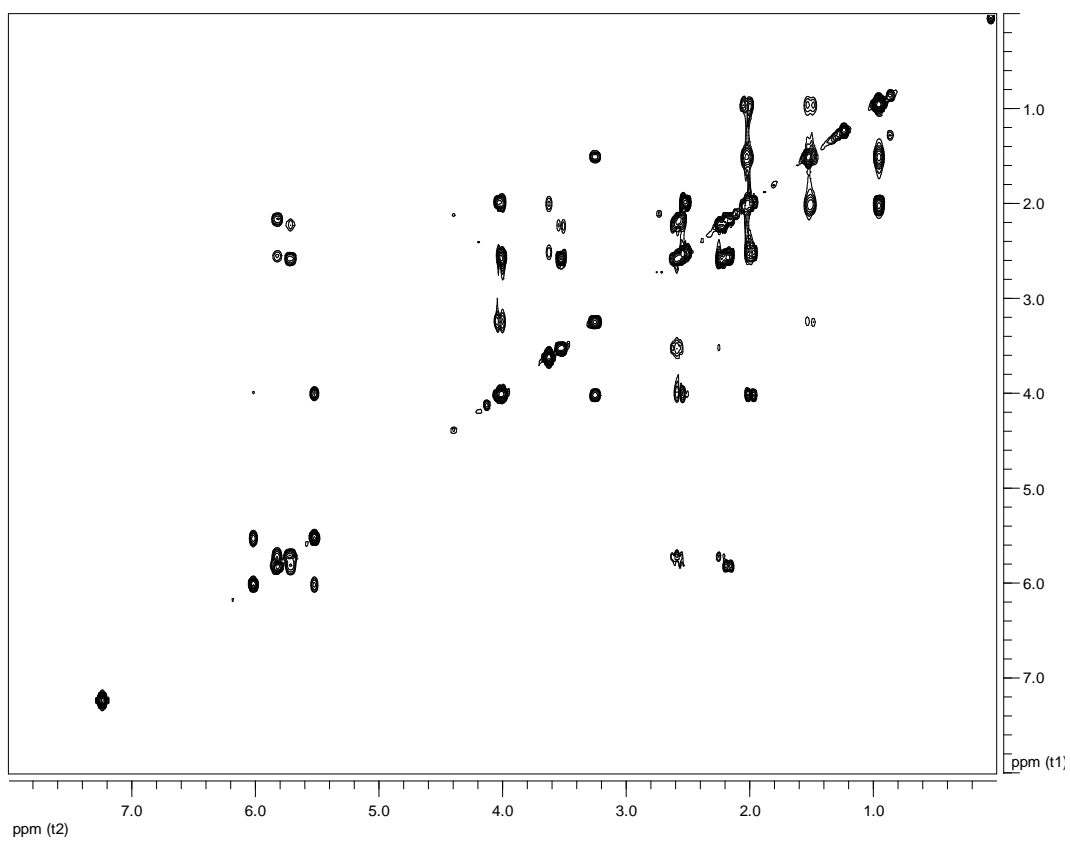


Figure S19. COSY spectrum of thuwalallene C (3) in  $\text{CDCl}_3$ .

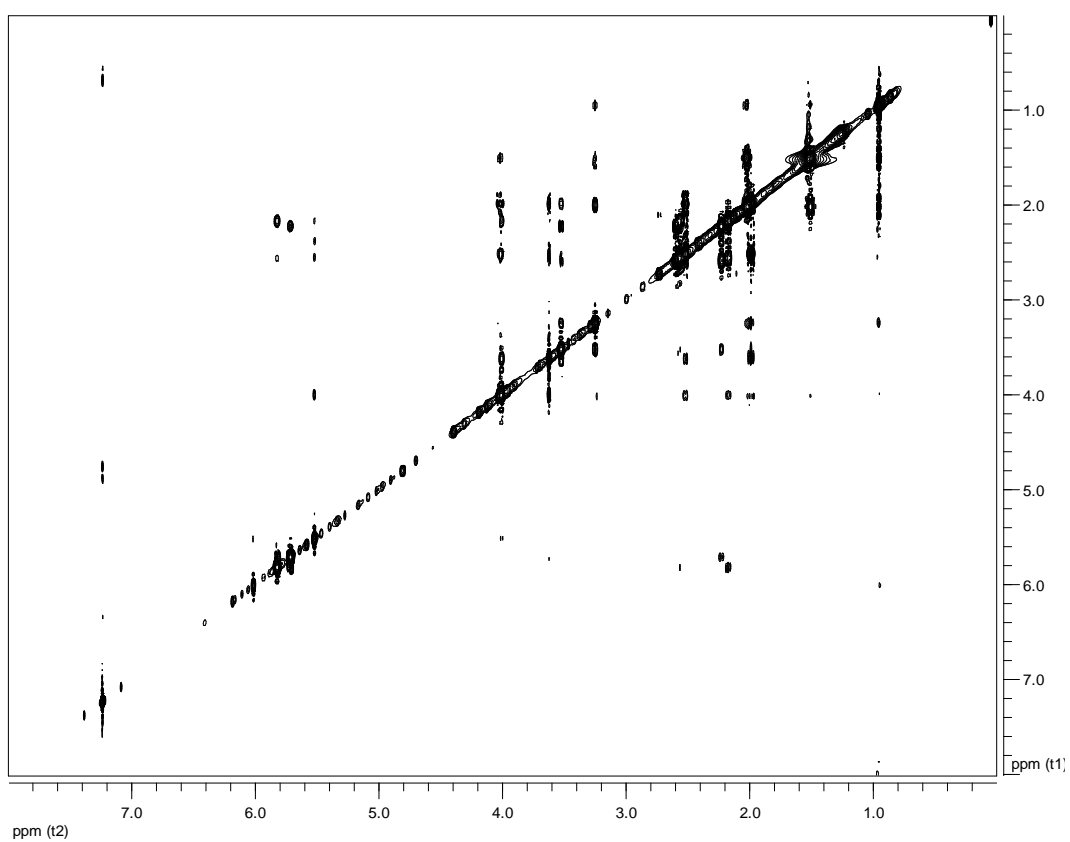
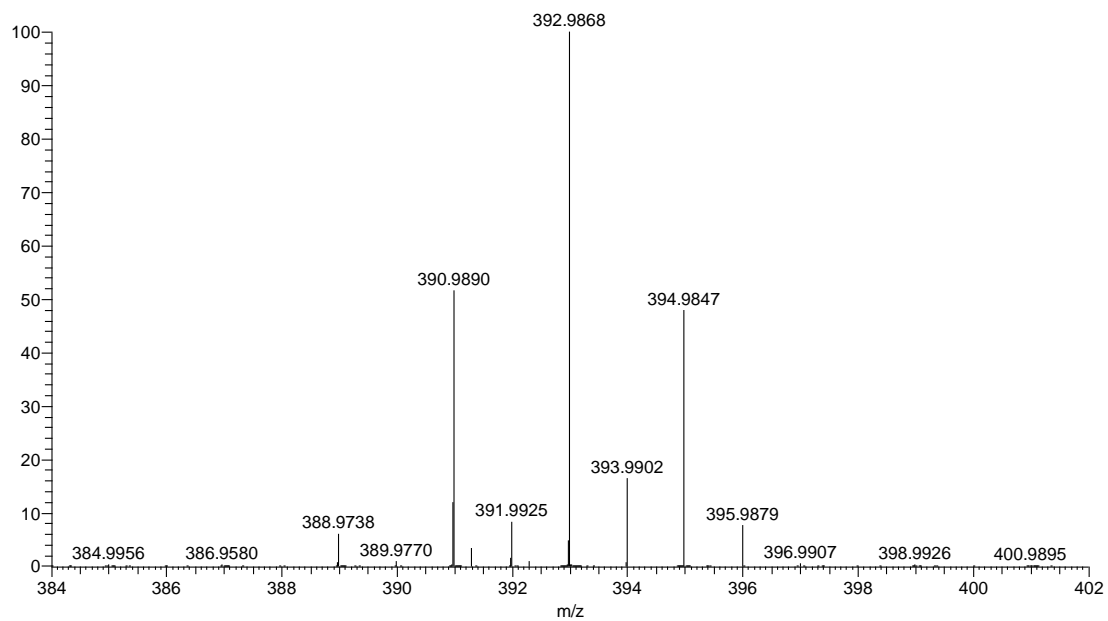


Figure S20. NOESY spectrum of thuwalallene C (3) in  $\text{CDCl}_3$ .



**Figure S21.** HR-APCIMS spectrum of thuwalallene C (3).

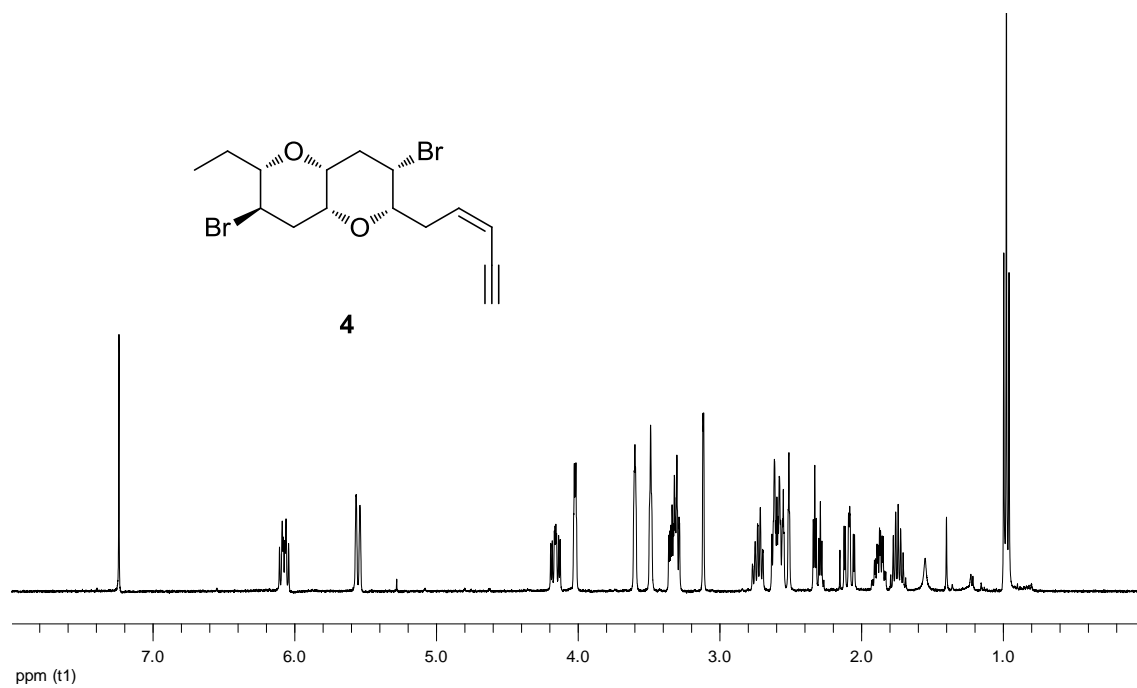


Figure S22. <sup>1</sup>H NMR spectrum of thuwalenyne A (4) in CDCl<sub>3</sub>.

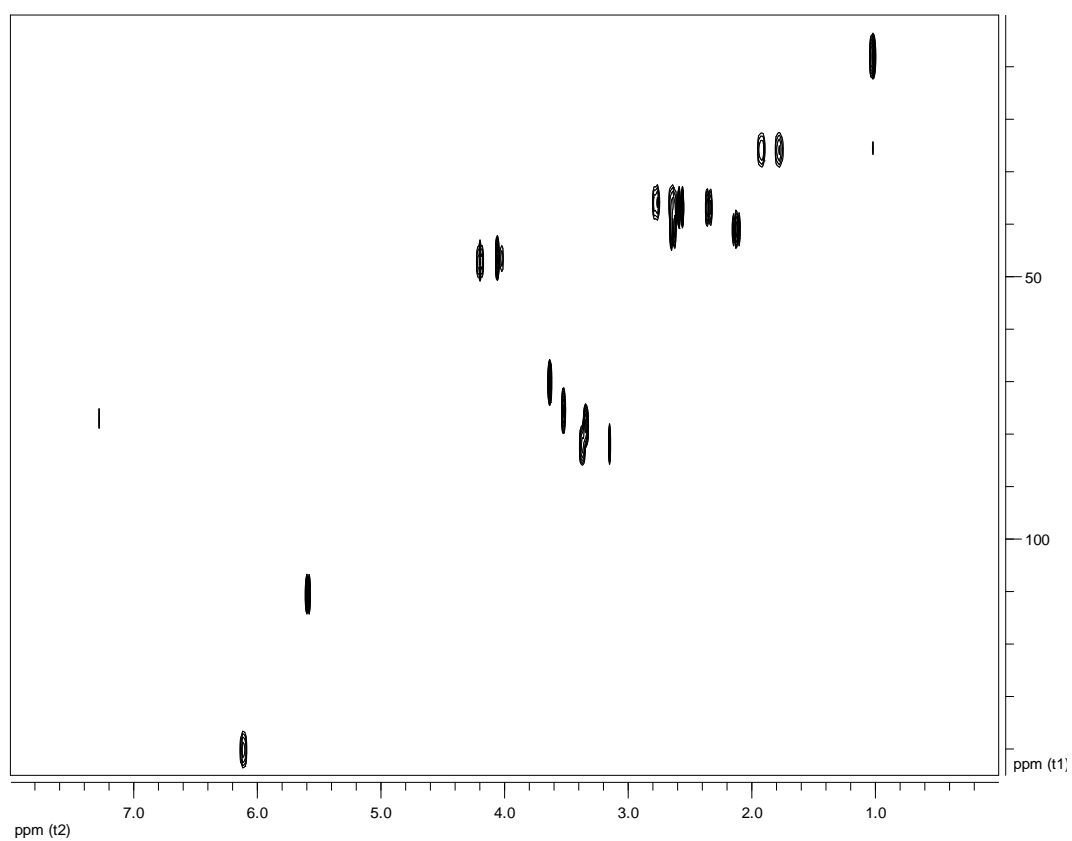


Figure S23. HSQC spectrum of thuwalenyne A (4) in CDCl<sub>3</sub>.



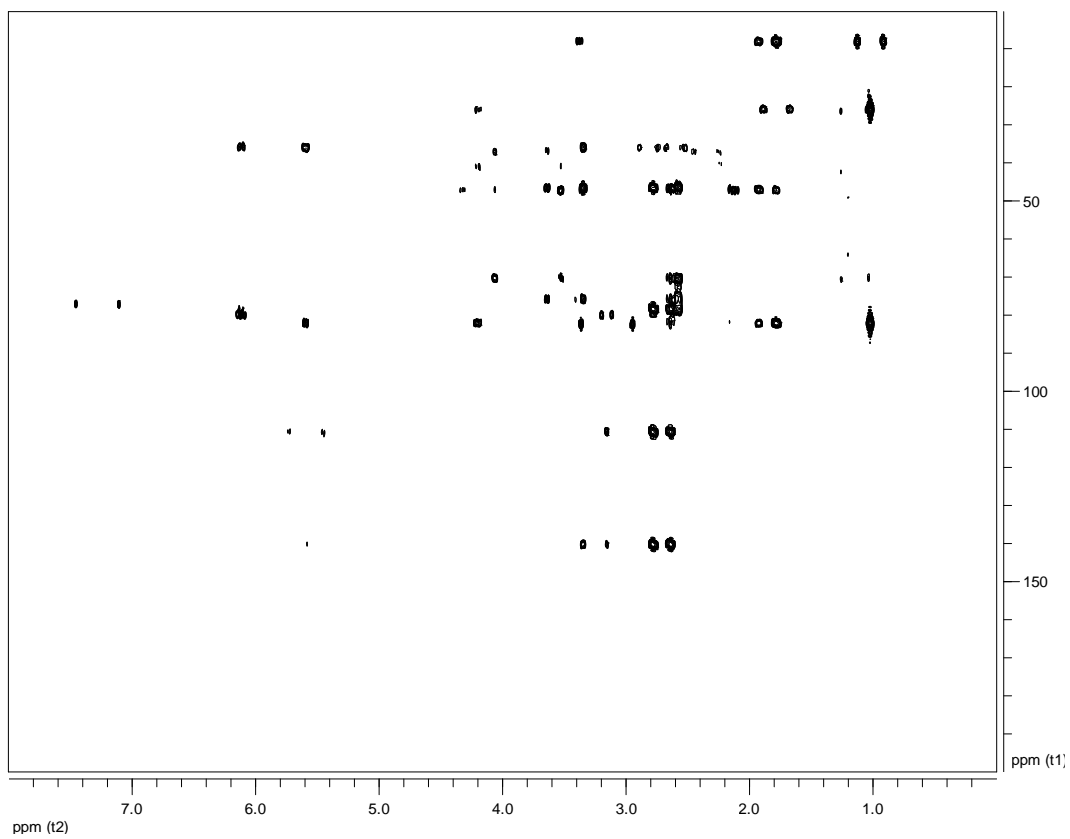


Figure S24. HMBC spectrum of thuwalenyne A (4) in CDCl<sub>3</sub>.

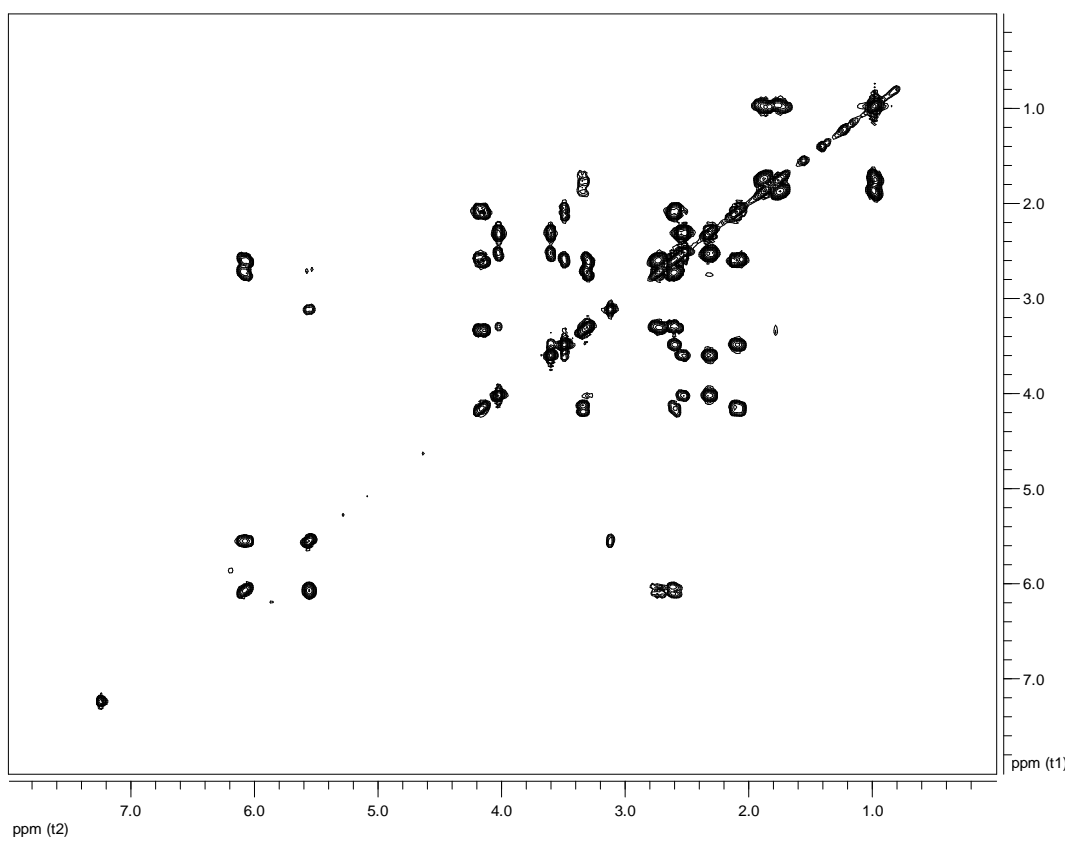


Figure S25. COSY spectrum of thuwalenyne A (4) in CDCl<sub>3</sub>.

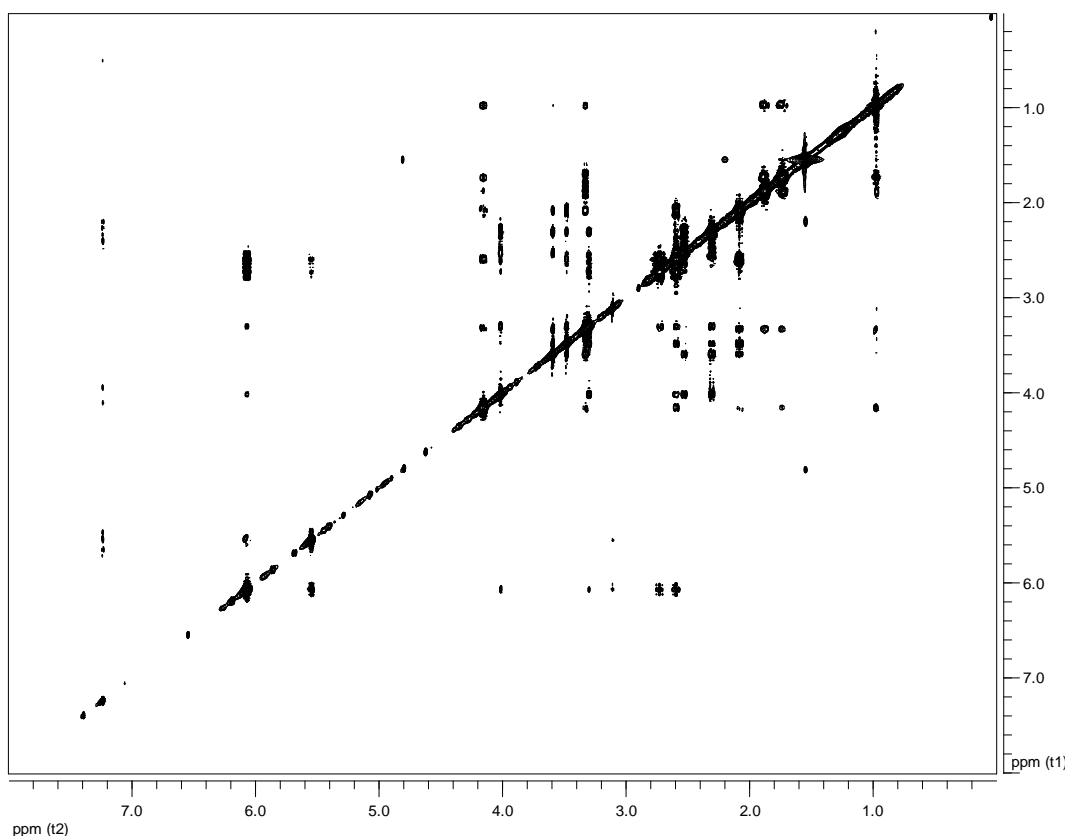


Figure S26. NOESY spectrum of thuwalenyne A (4) in CDCl<sub>3</sub>.

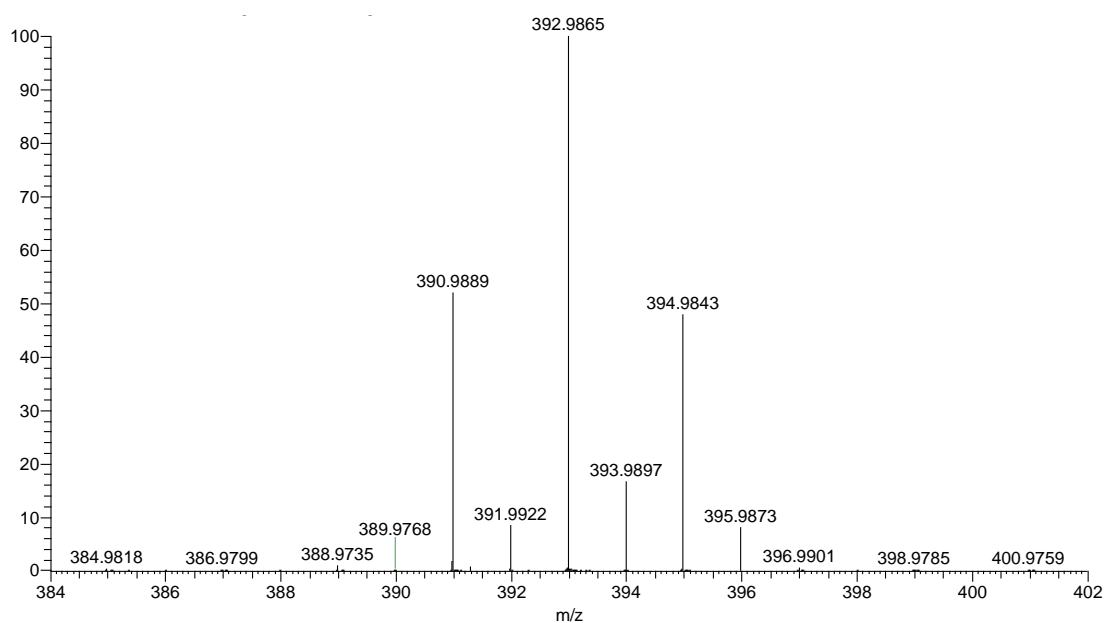


Figure S27. HR-APCIMS spectrum of thuwalenyne A (4).

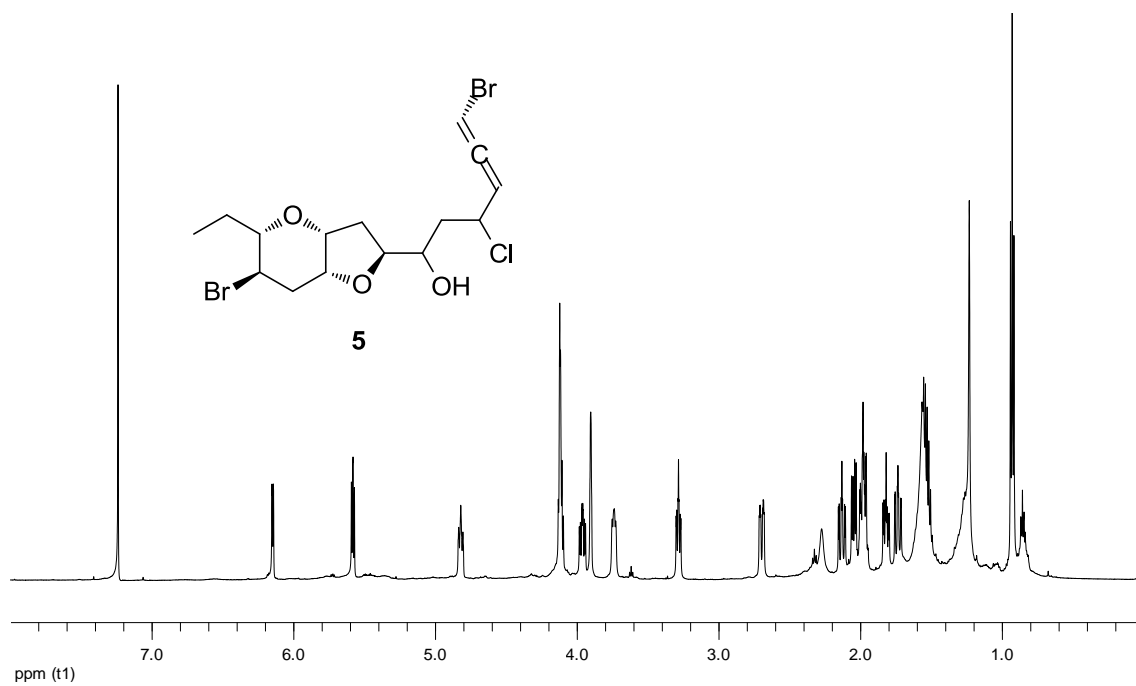


Figure S28.  $^1\text{H}$  NMR spectrum of thuwalallene D (5) in  $\text{CDCl}_3$ .

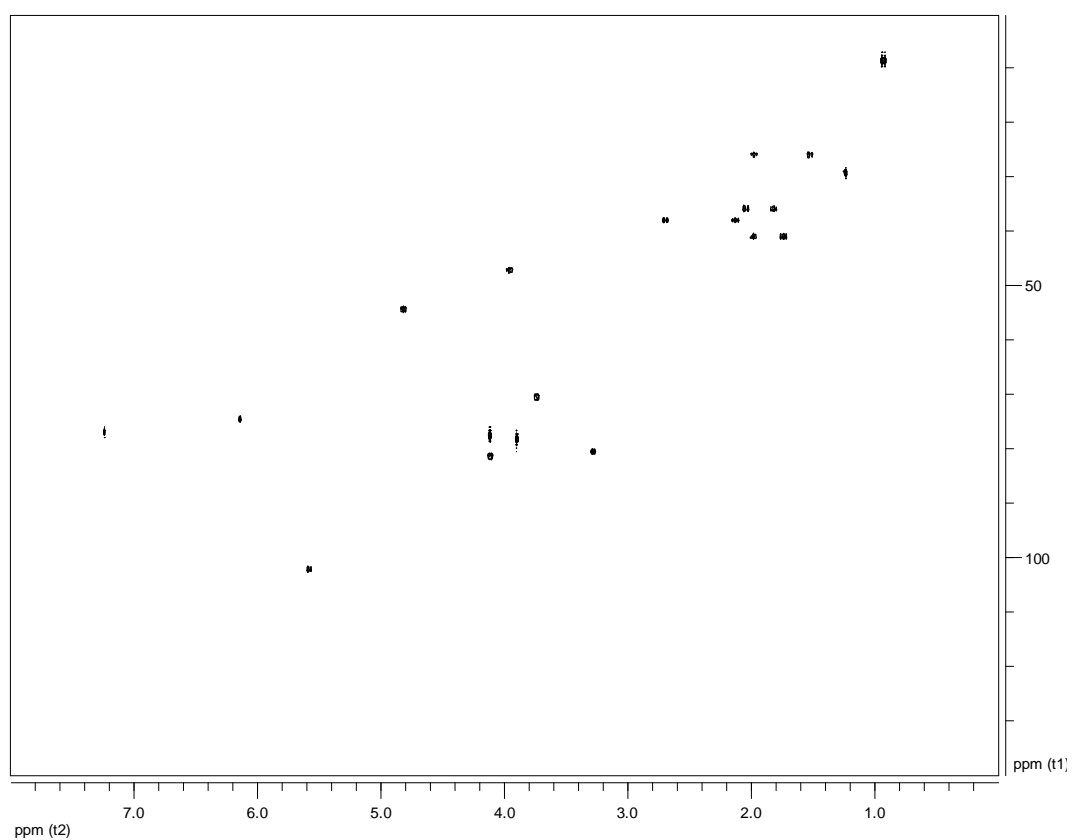


Figure S29. HSQC spectrum of thuwalallene D (5) in  $\text{CDCl}_3$ .

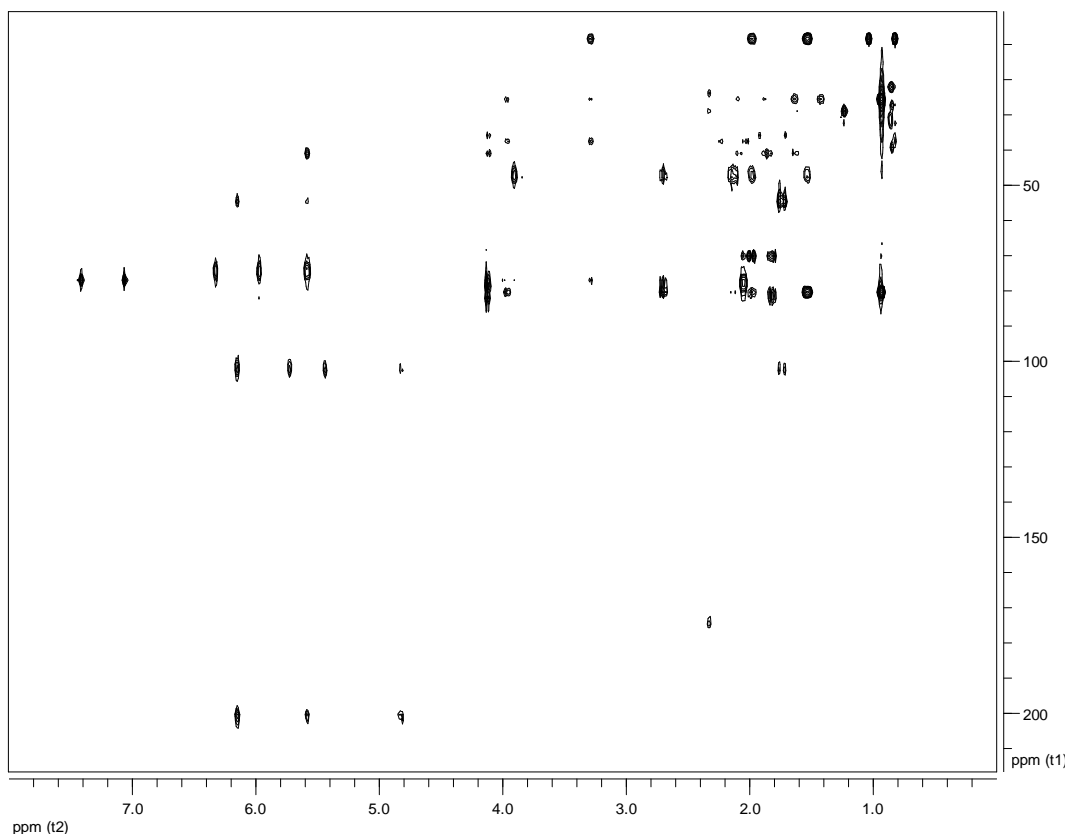


Figure S30. HMBC spectrum of thuwalallene D (5) in CDCl<sub>3</sub>.

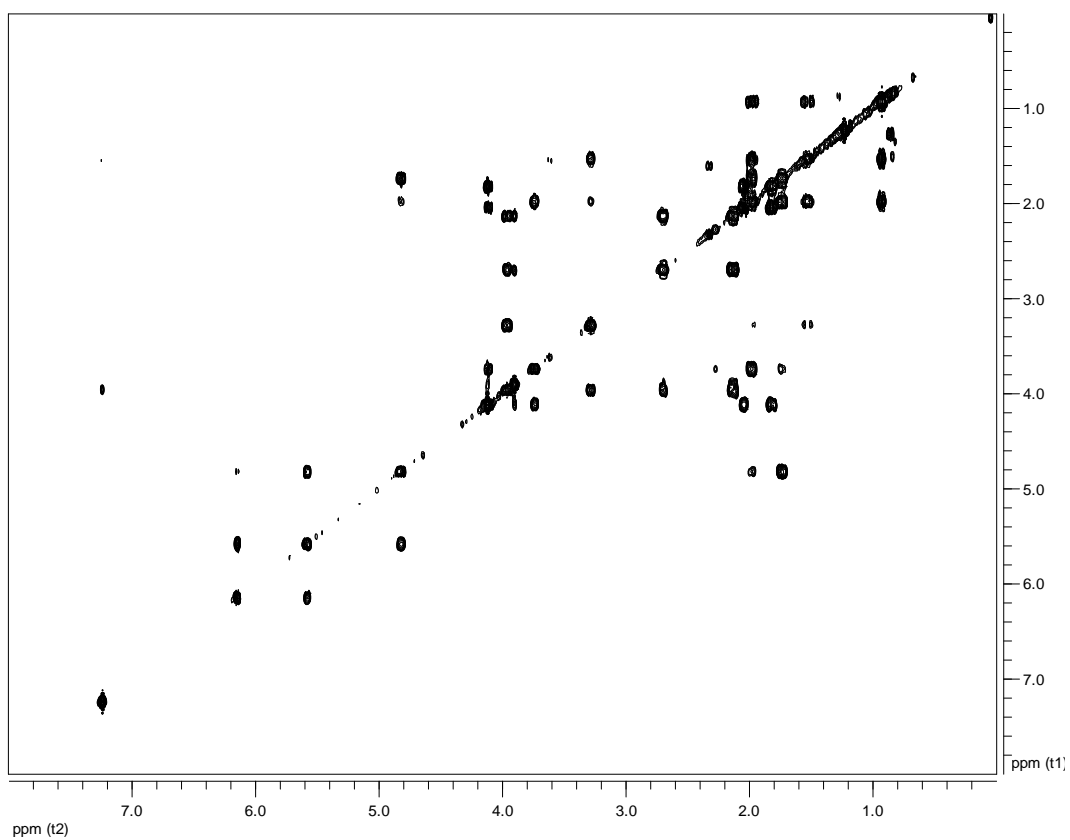


Figure S31. COSY spectrum of thuwalallene D (5) in CDCl<sub>3</sub>.

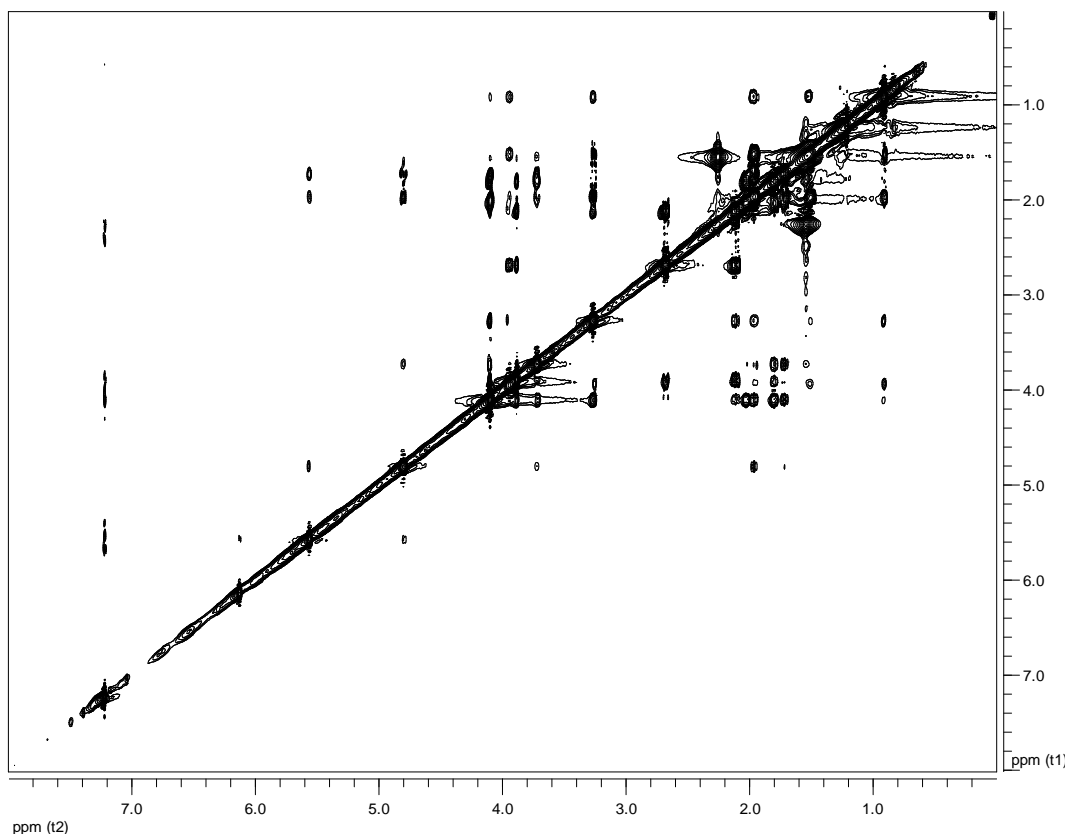


Figure S32. NOESY spectrum of thuwalallene D (5) in  $\text{CDCl}_3$ .

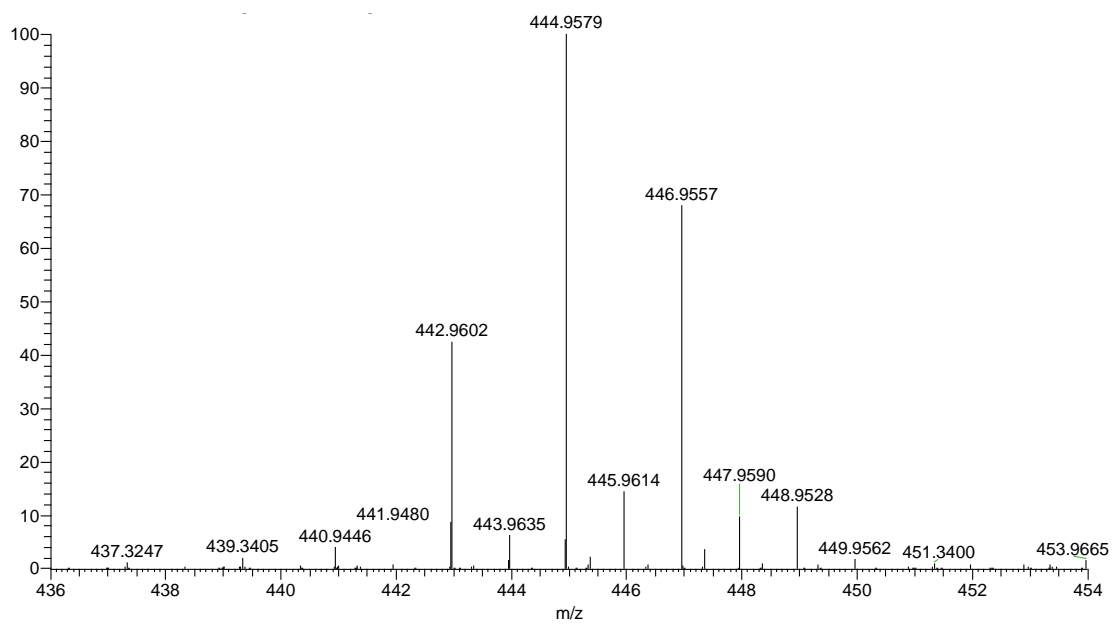
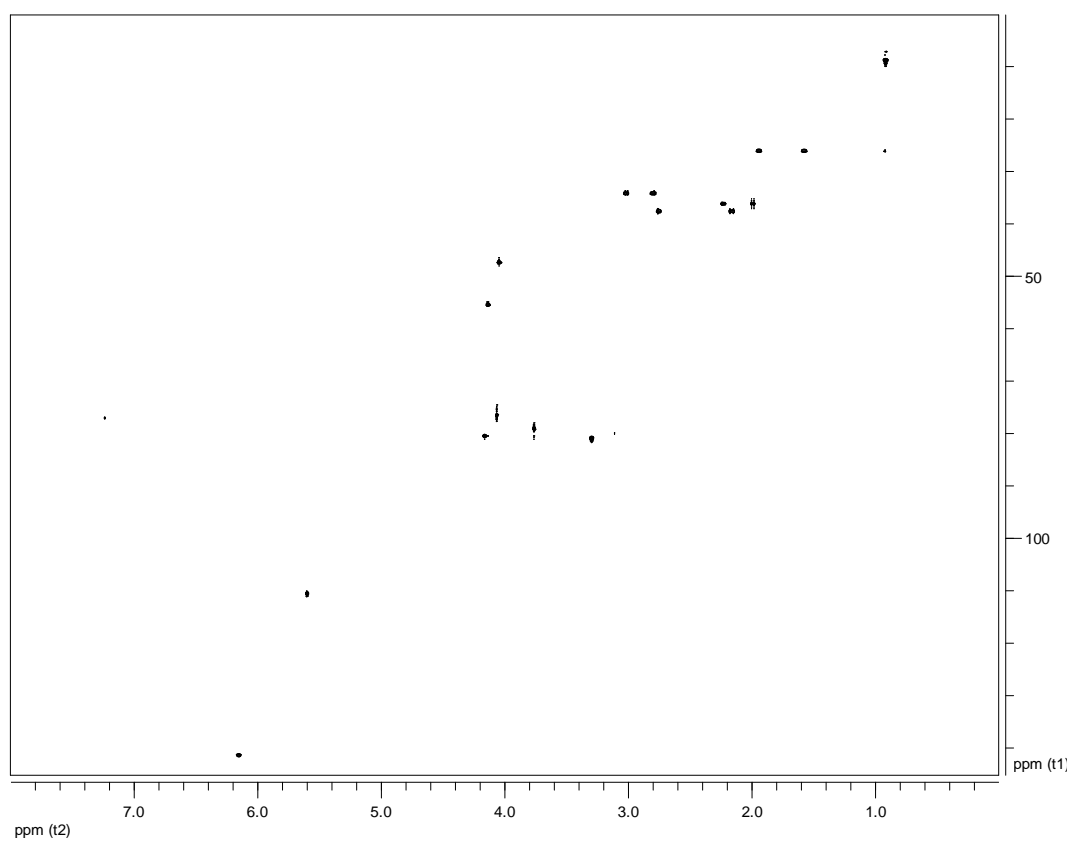
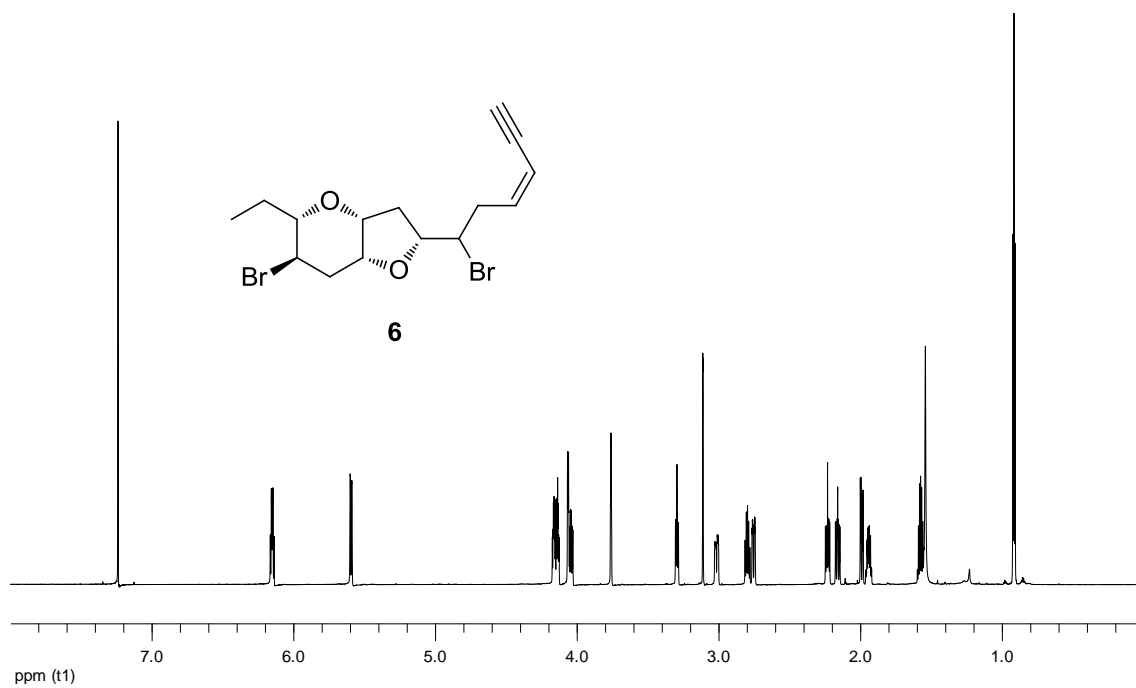


Figure S33. HR-APCIMS spectrum of thuwalallene D (5).



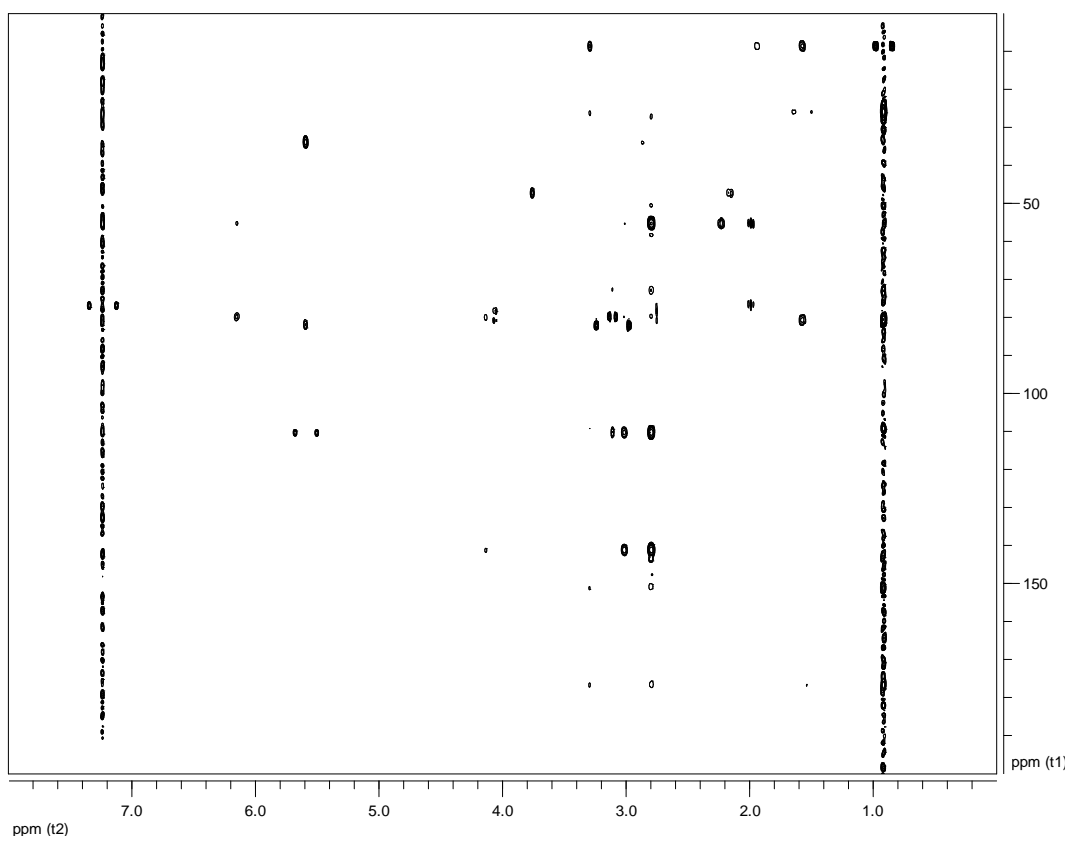


Figure S36. HMBC spectrum of thuwalenyne B (6) in CDCl<sub>3</sub>.

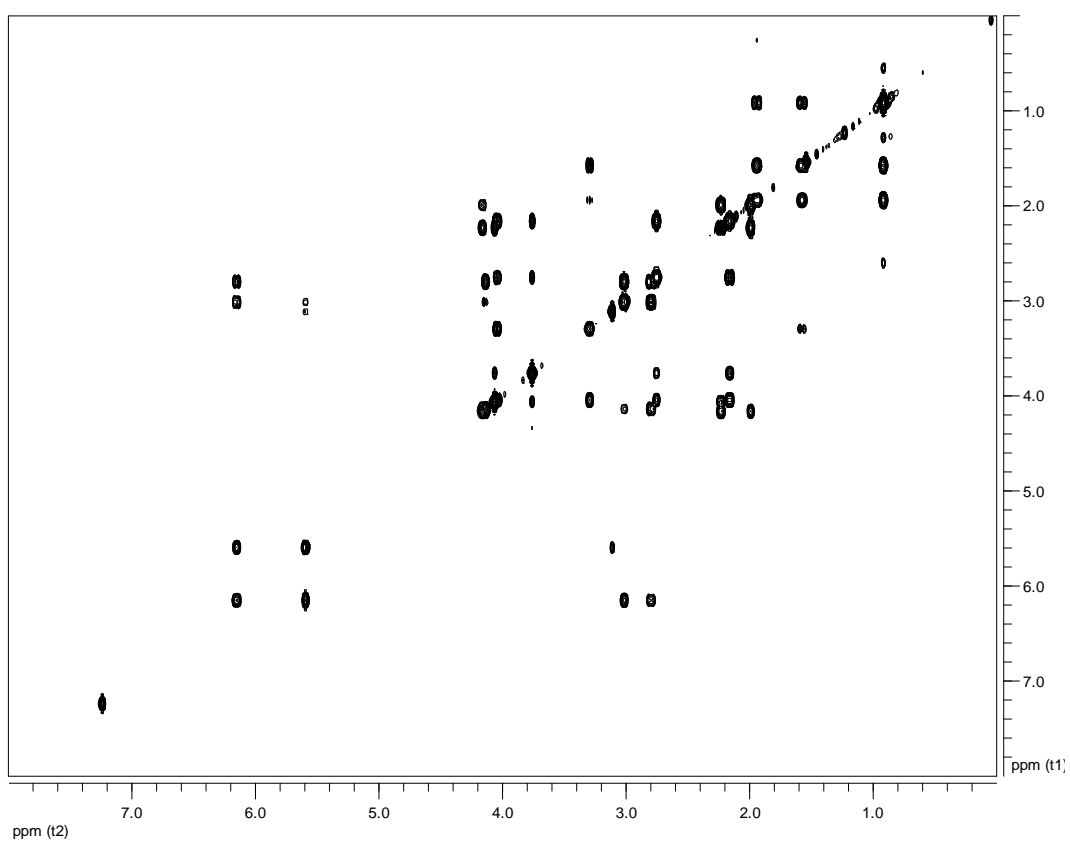


Figure S37. COSY spectrum of thuwalenyne B (6) in CDCl<sub>3</sub>.

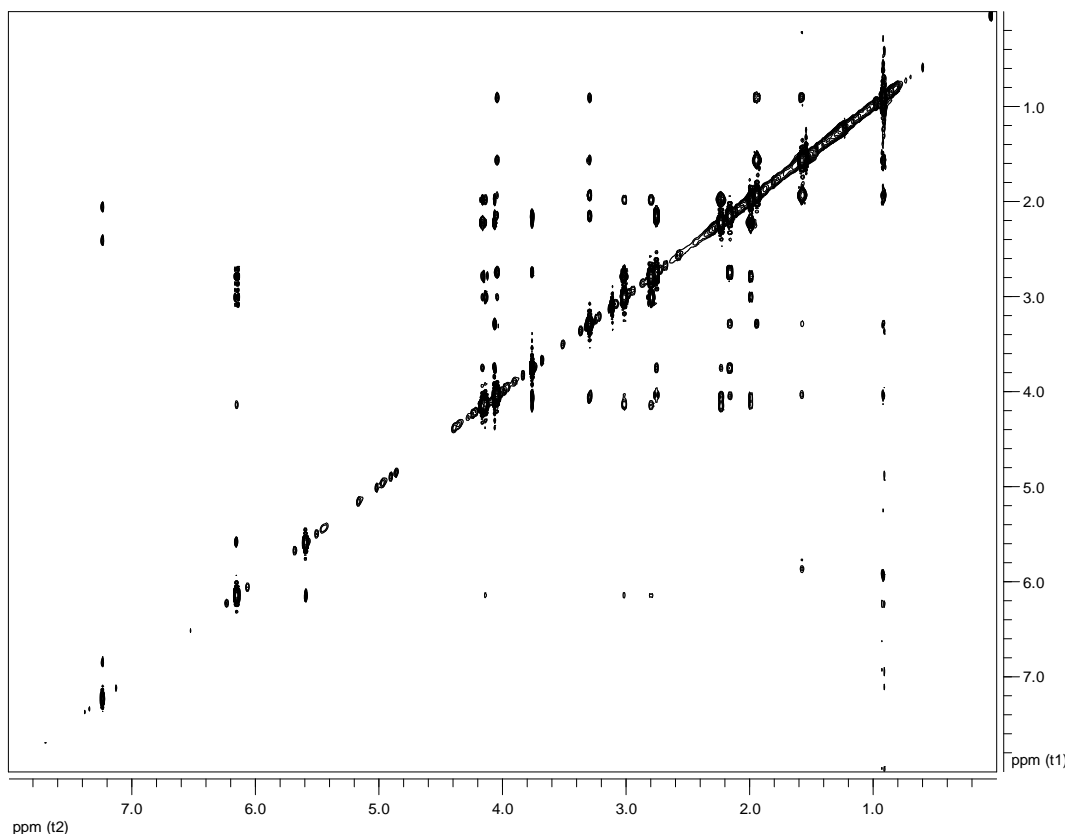


Figure S38. NOESY spectrum of thuwalenyne B (6) in CDCl<sub>3</sub>.

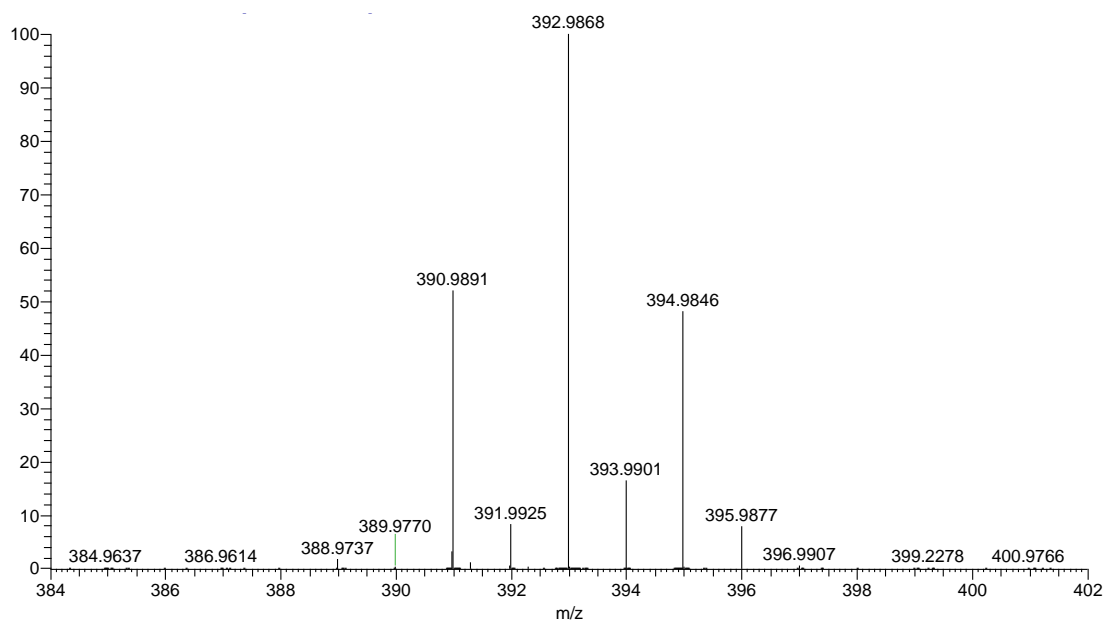


Figure S39. HR-APCIMS spectrum of thuwalenyne B (6).



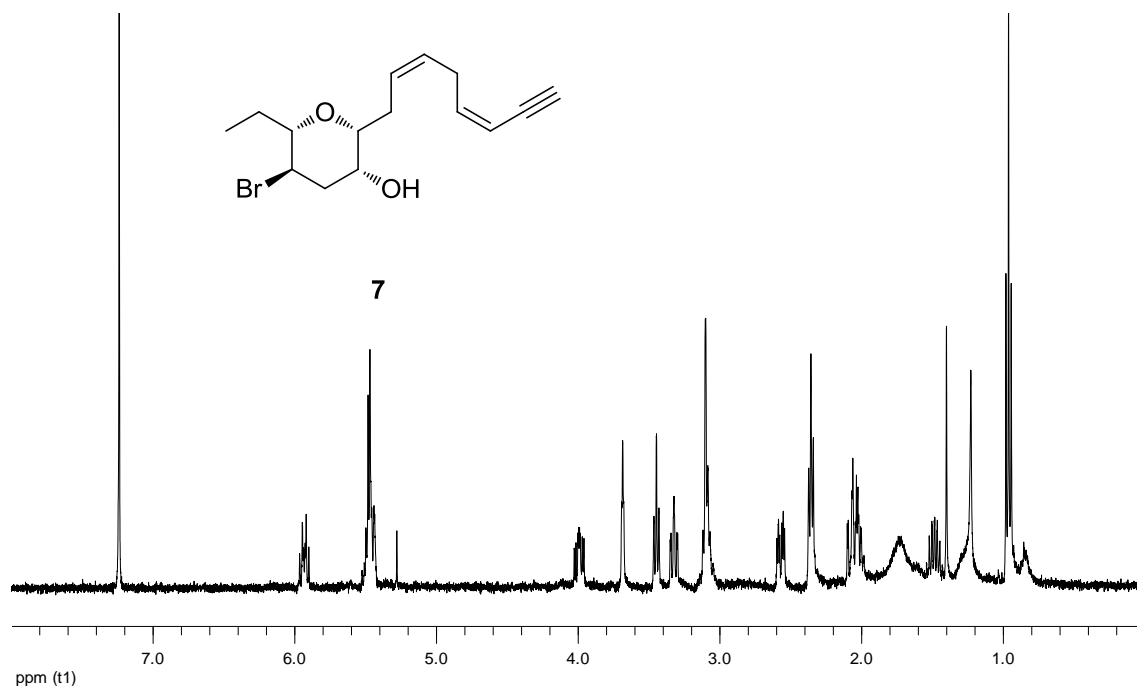


Figure S40. <sup>1</sup>H NMR spectrum of thuwalenyne C (7) in CDCl<sub>3</sub>.

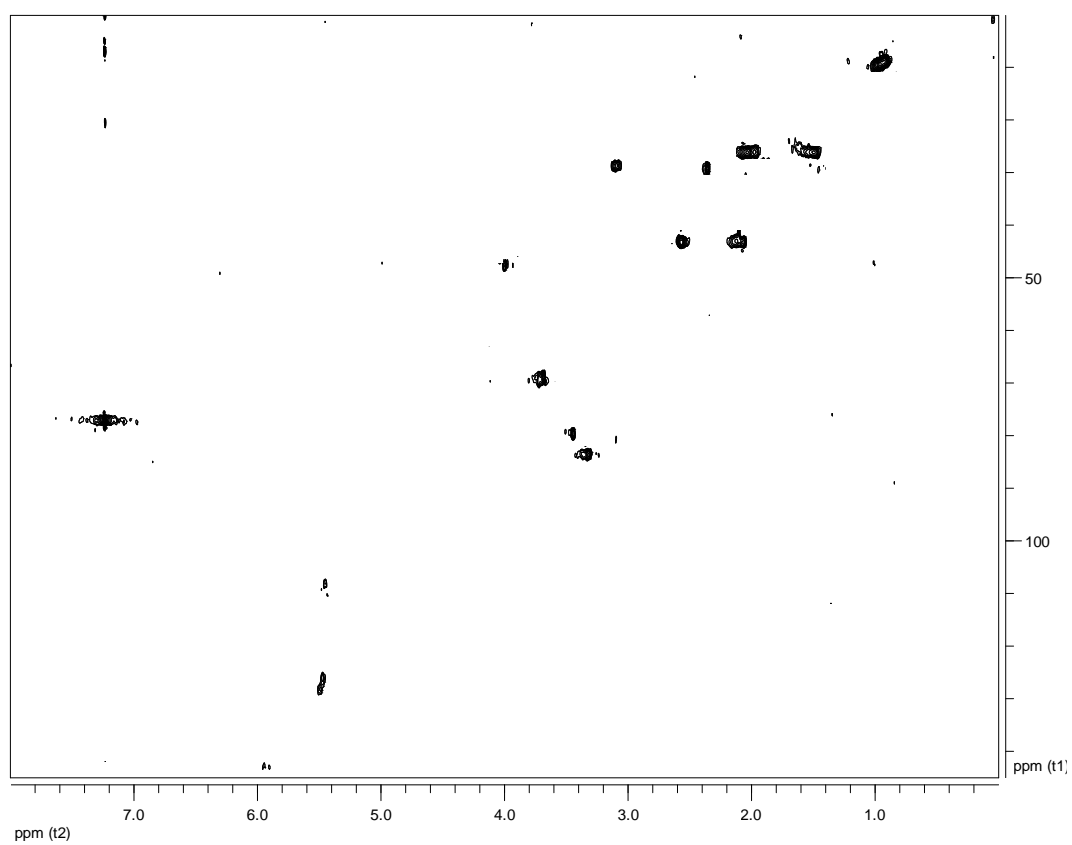


Figure S41. HSQC spectrum of thuwalenyne C (7) in CDCl<sub>3</sub>.

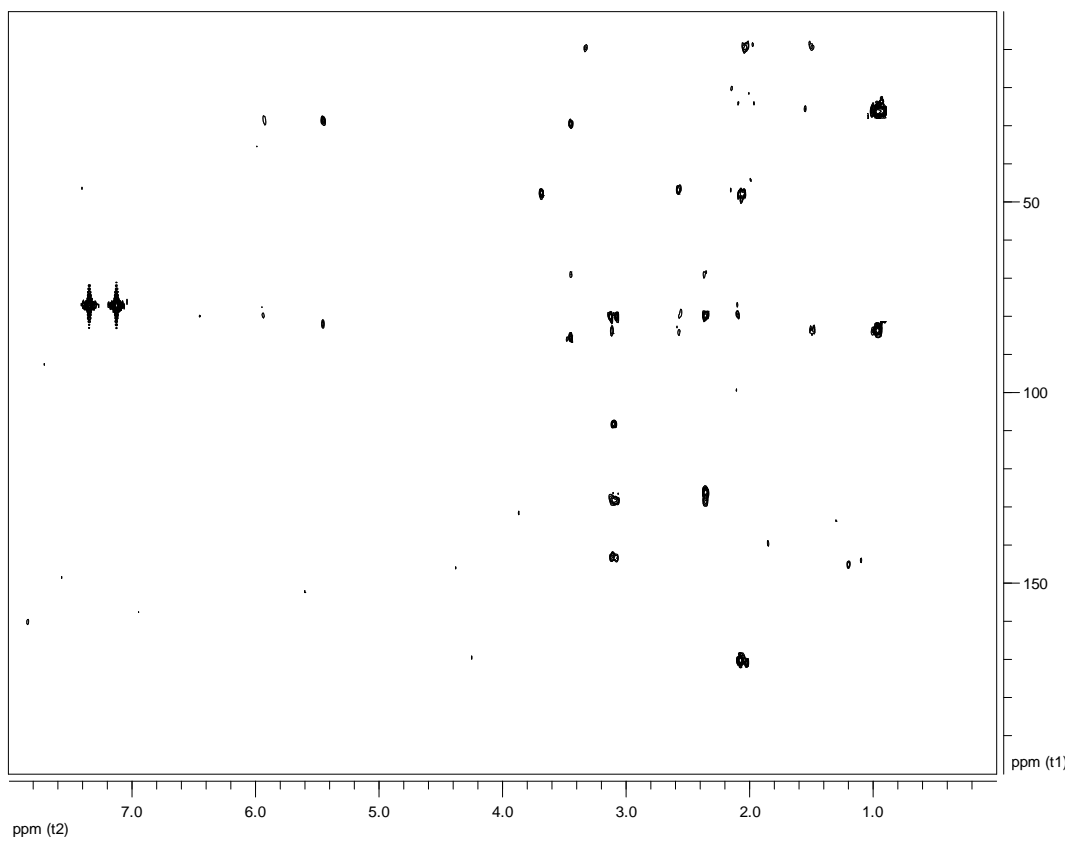


Figure S42. HMBC spectrum of thuwalenyne C (7) in CDCl<sub>3</sub>.

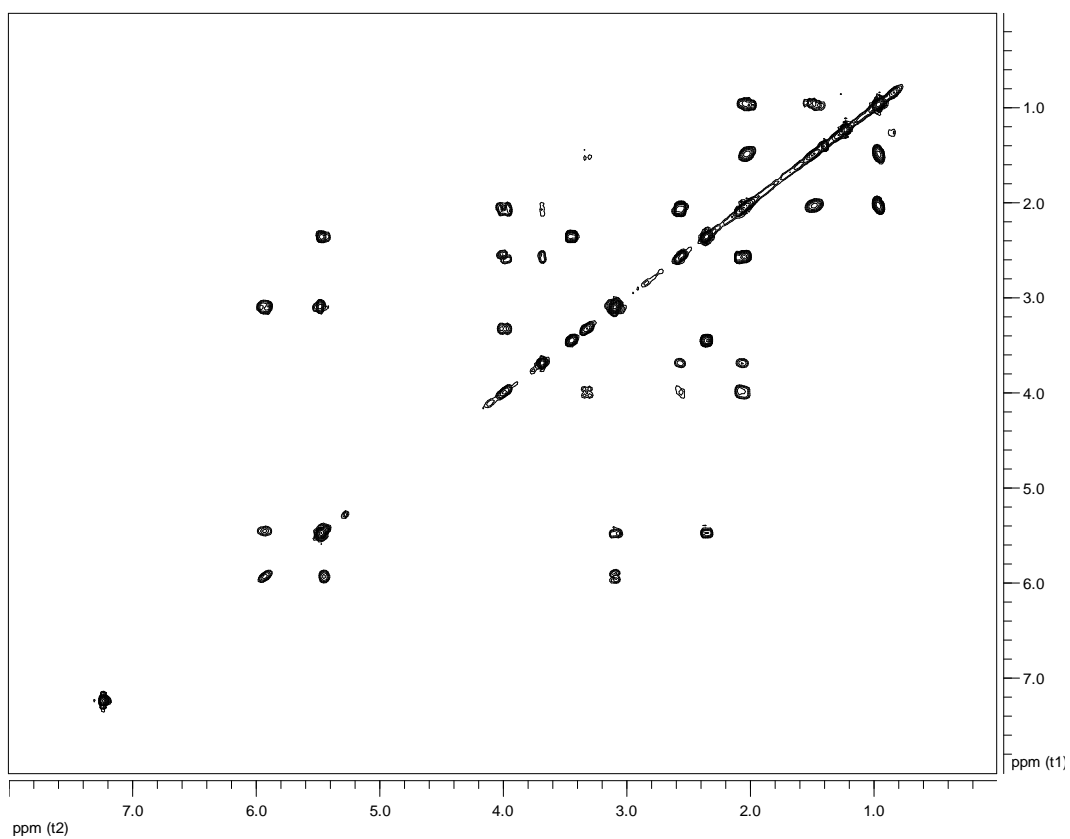


Figure S43. COSY spectrum of thuwalenyne C (7) in CDCl<sub>3</sub>.

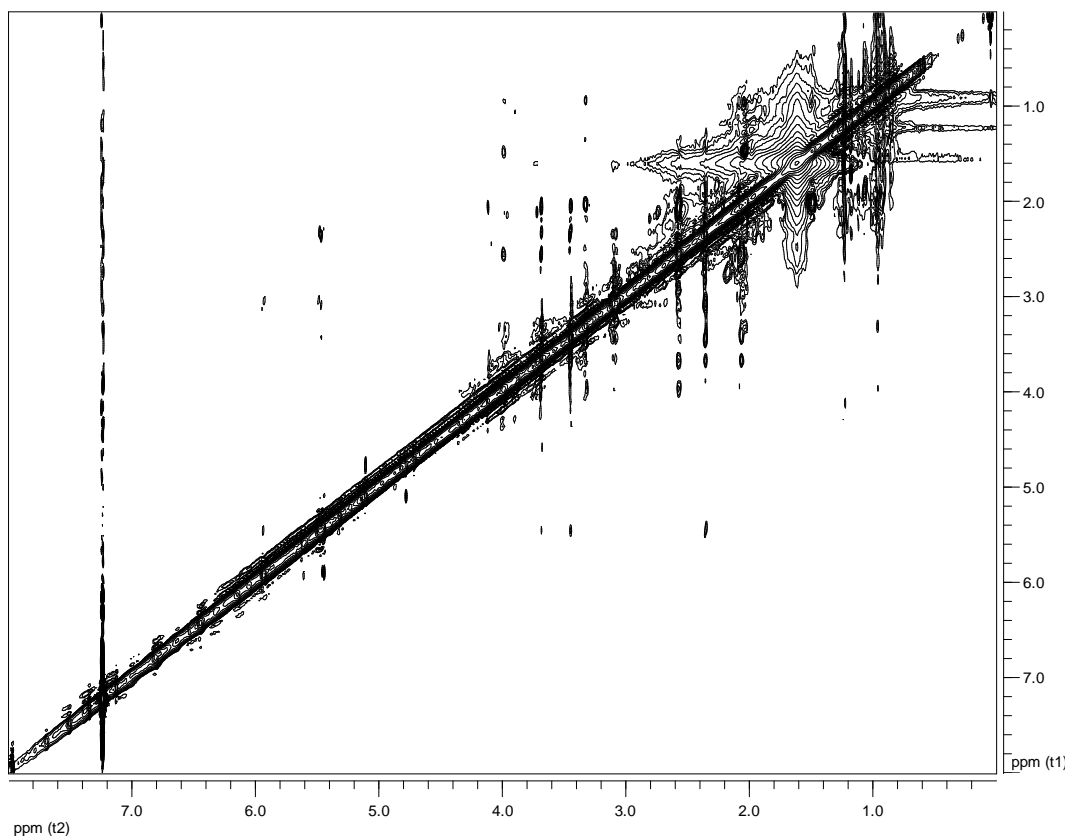


Figure S44. NOESY spectrum of thuwalenyne C (7) in  $\text{CDCl}_3$ .

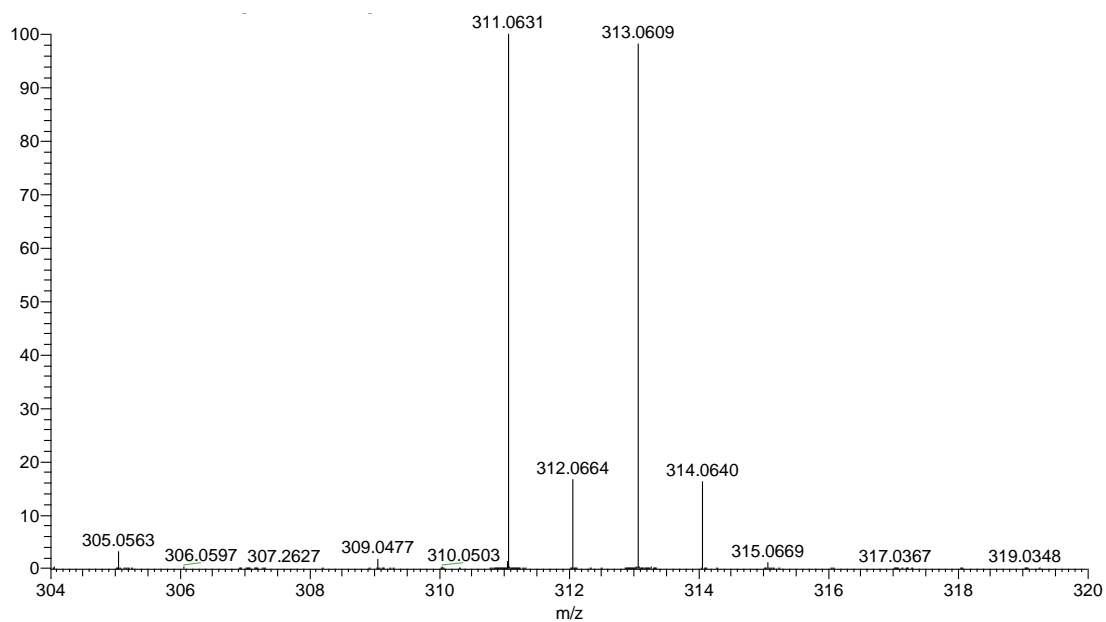


Figure S45. HR-APCIMS spectrum of thuwalenyne C (7).

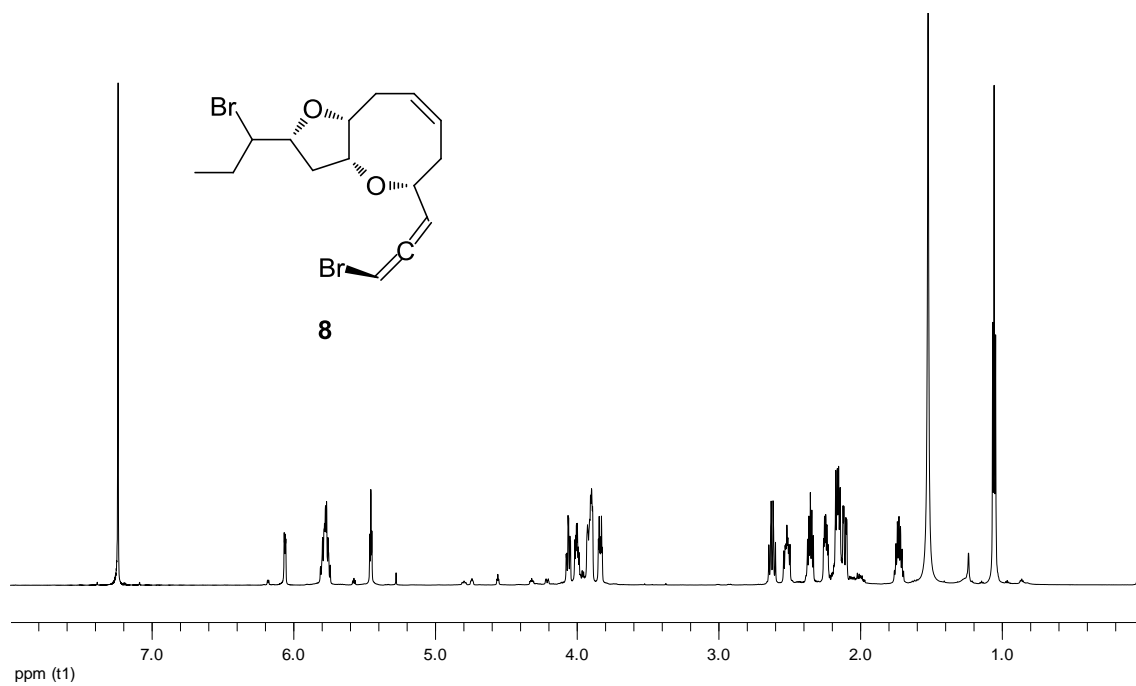


Figure S46.  $^1\text{H}$  NMR spectrum of thuwalallene E (8) in  $\text{CDCl}_3$ .

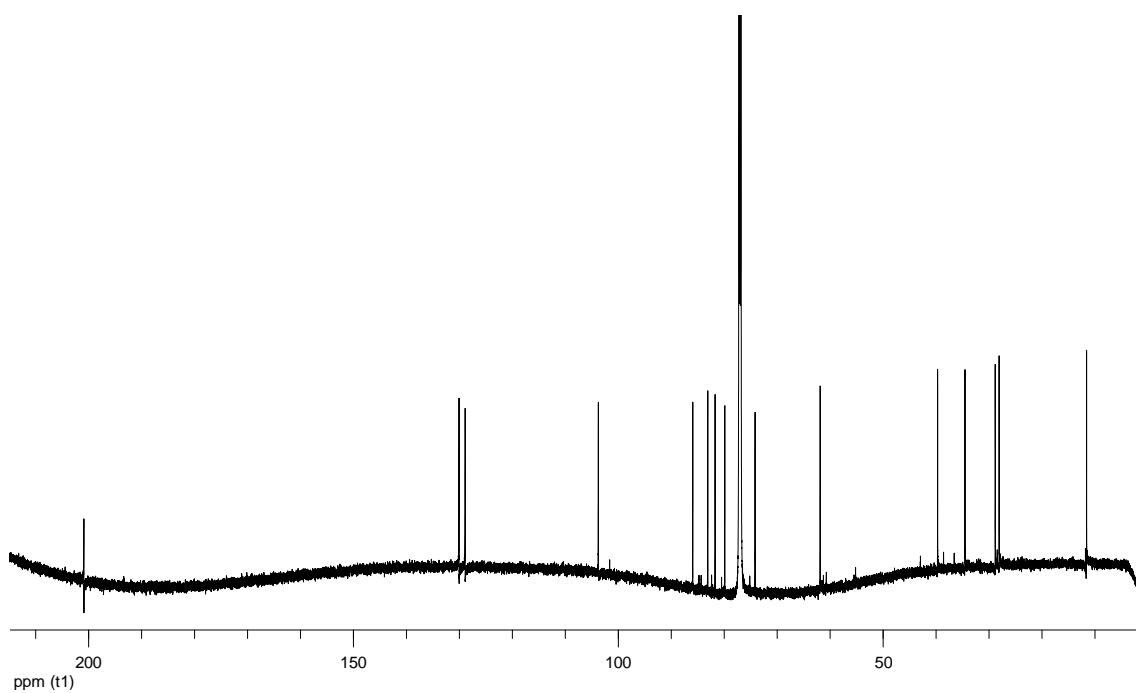


Figure S47.  $^{13}\text{C}$  NMR spectrum of thuwalallene E (8) in  $\text{CDCl}_3$ .

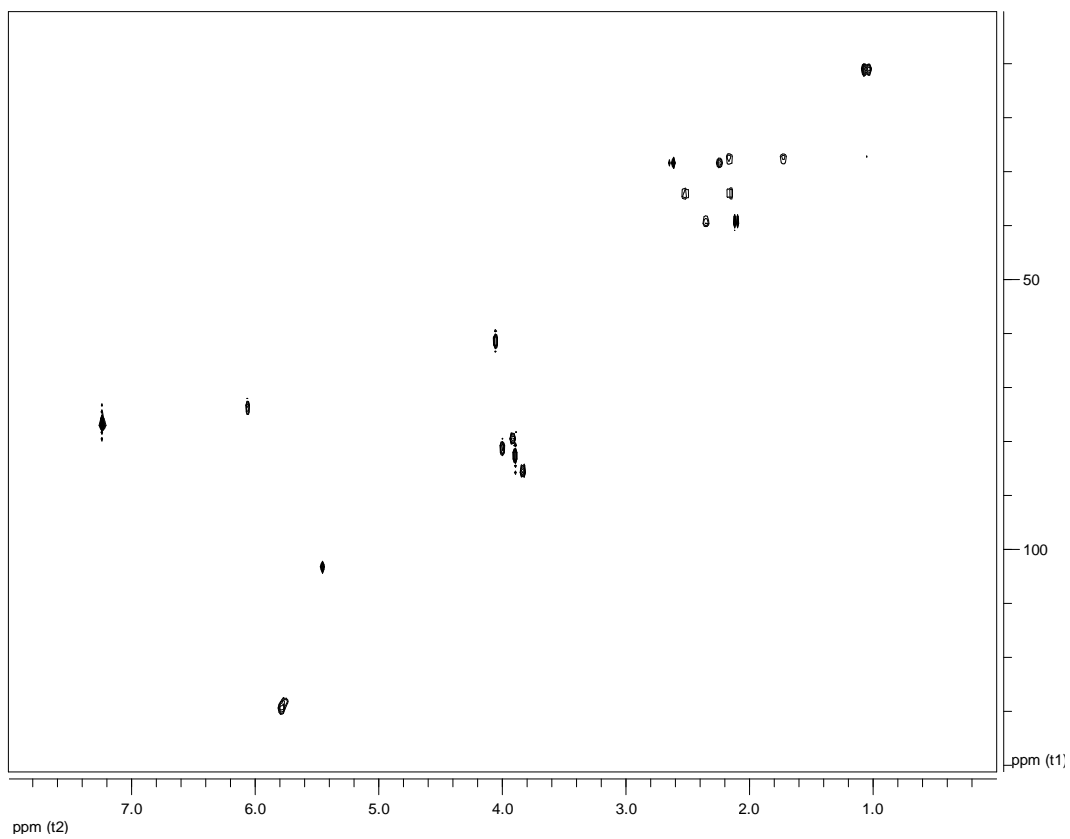


Figure S48. HSQC spectrum of thuwalallene E (8) in CDCl<sub>3</sub>.

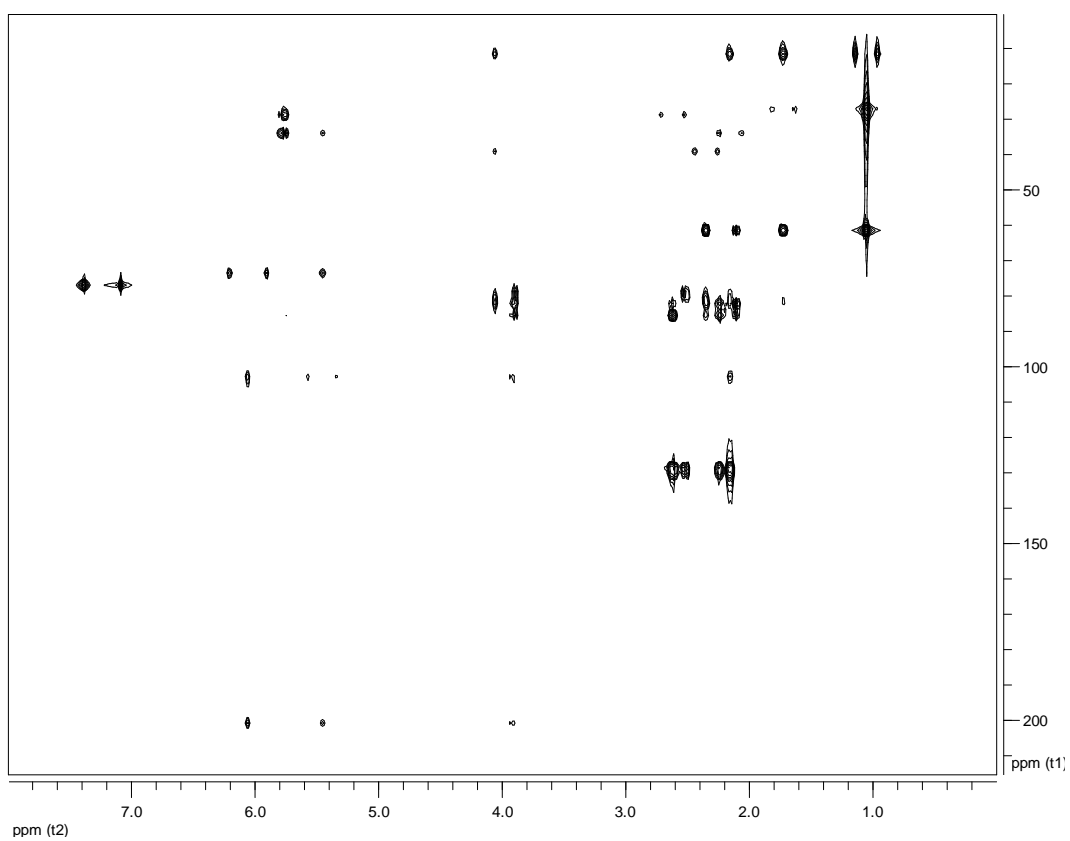


Figure S49. HMBC spectrum of thuwalallene E (8) in CDCl<sub>3</sub>.

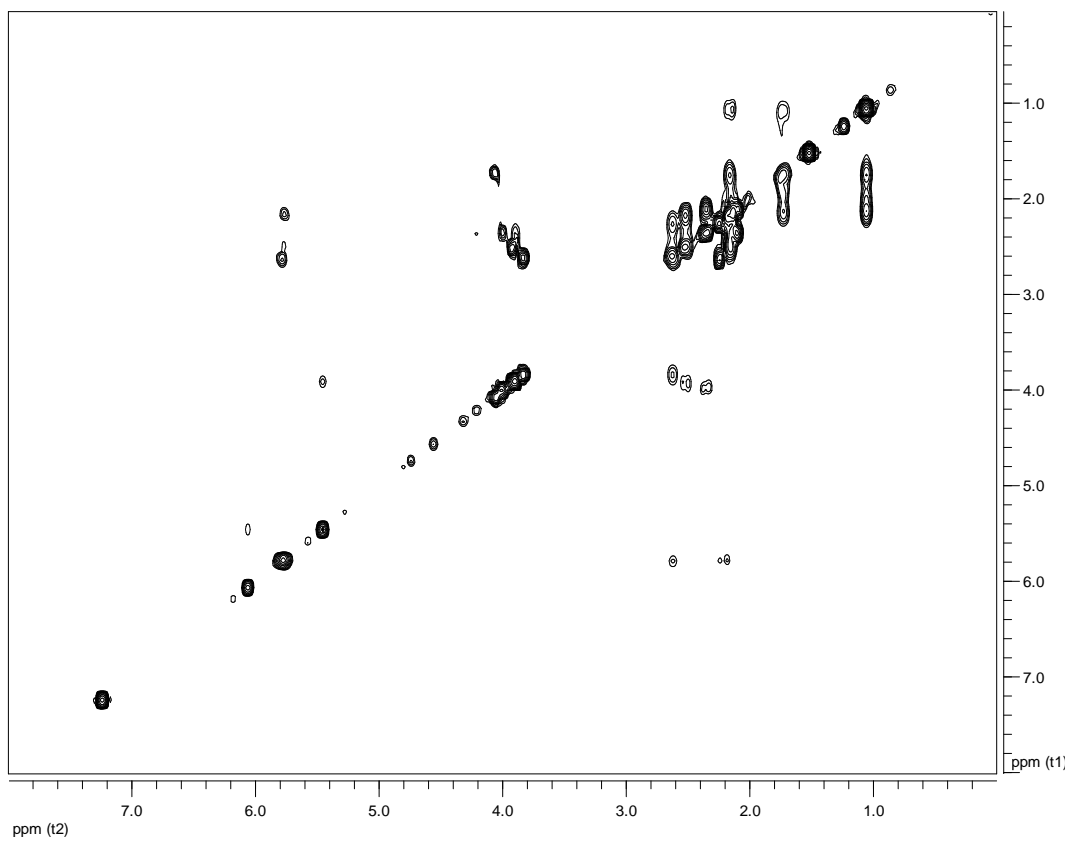


Figure S50. COSY spectrum of thuwalallene E (8) in CDCl<sub>3</sub>.

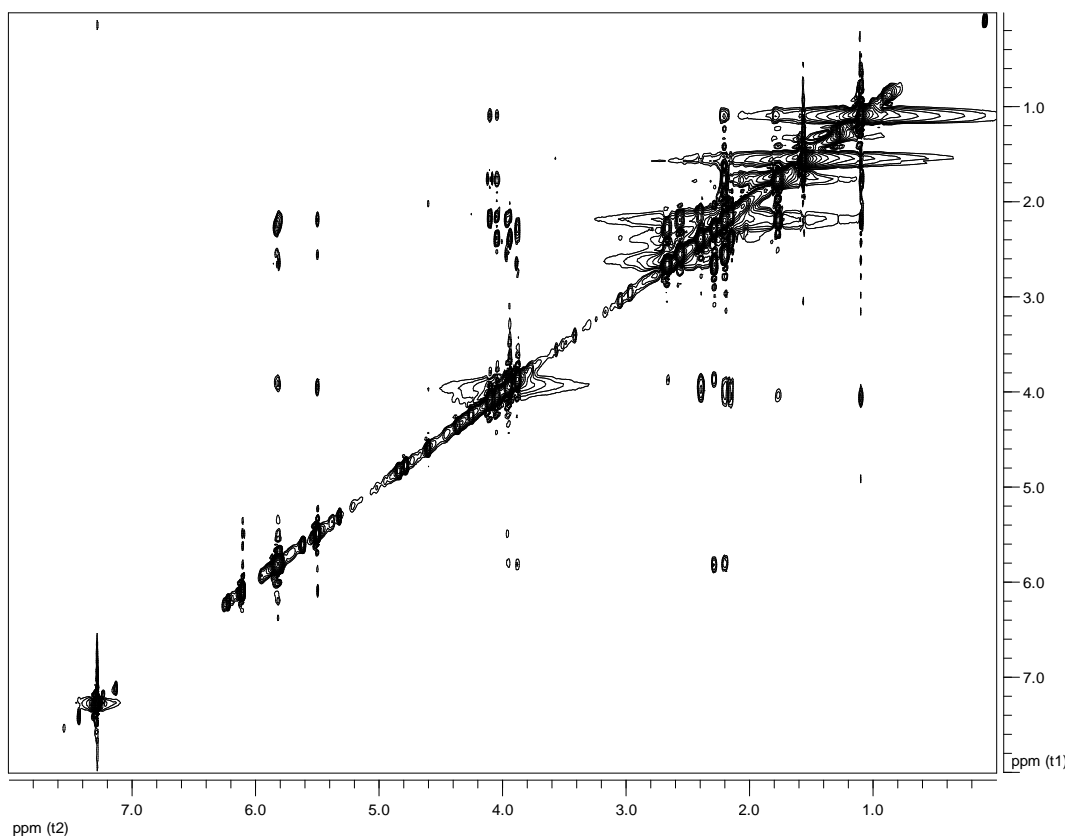


Figure S51. NOESY spectrum of thuwalallene E (8) in CDCl<sub>3</sub>.

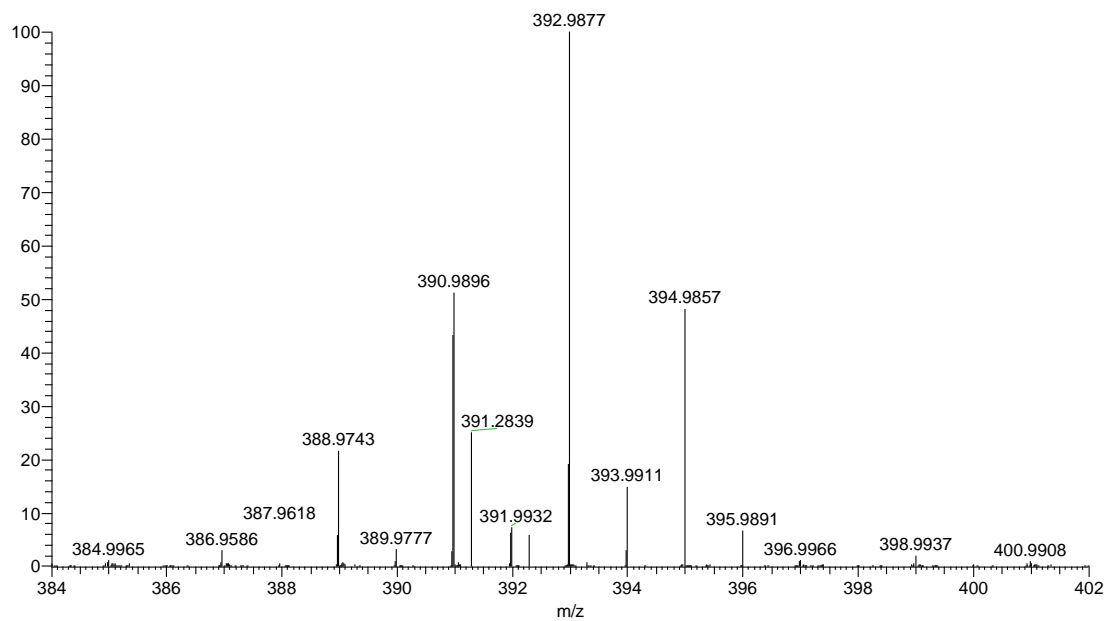


Figure S52. HR-APCIMS spectrum of thuwalallene E (8).

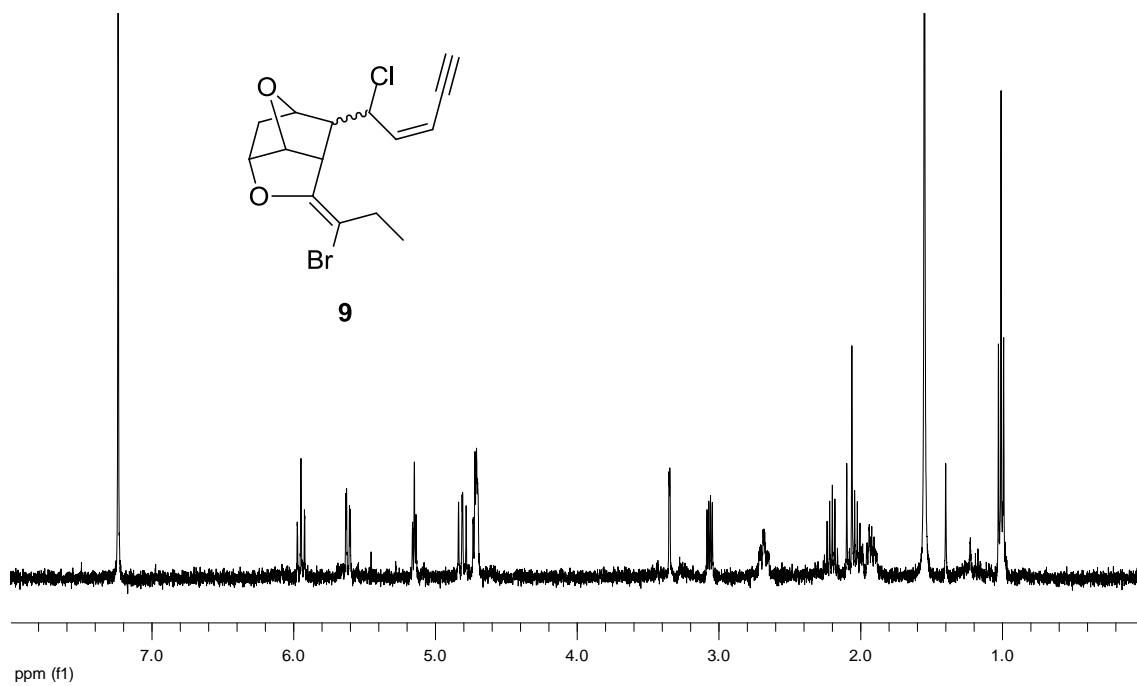


Figure S53.  $^1\text{H}$  NMR spectrum of *cis*-maneone D (9) in  $\text{CDCl}_3$ .

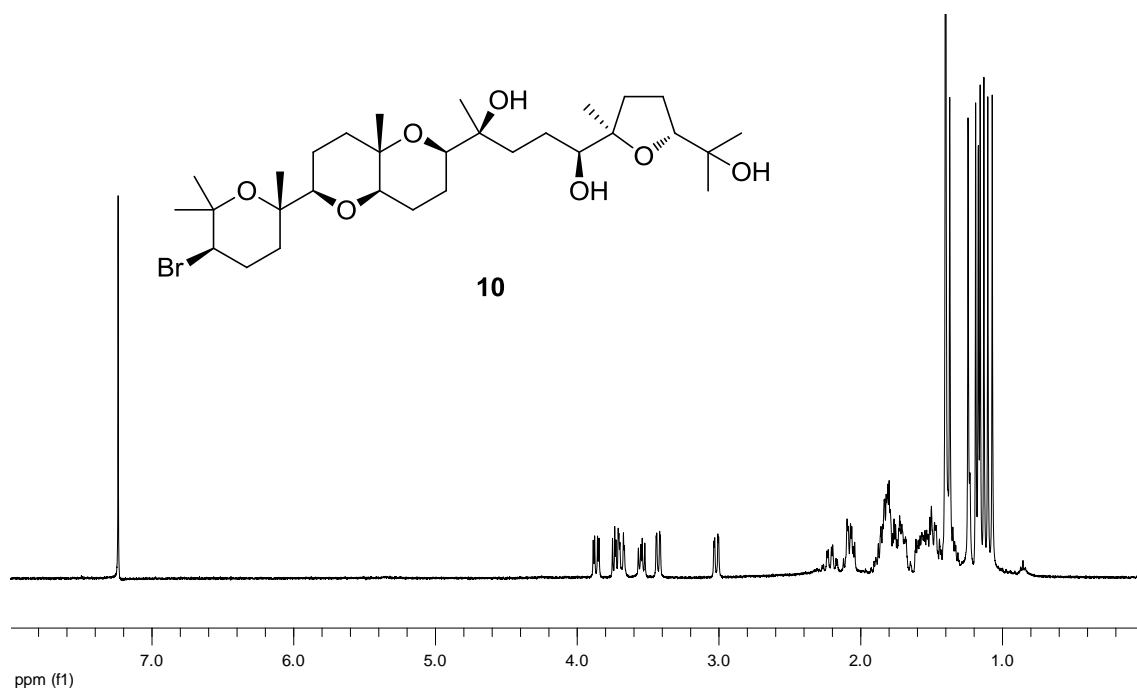


Figure S54.  $^1\text{H}$  NMR spectrum of thysiferol (10) in  $\text{CDCl}_3$ .



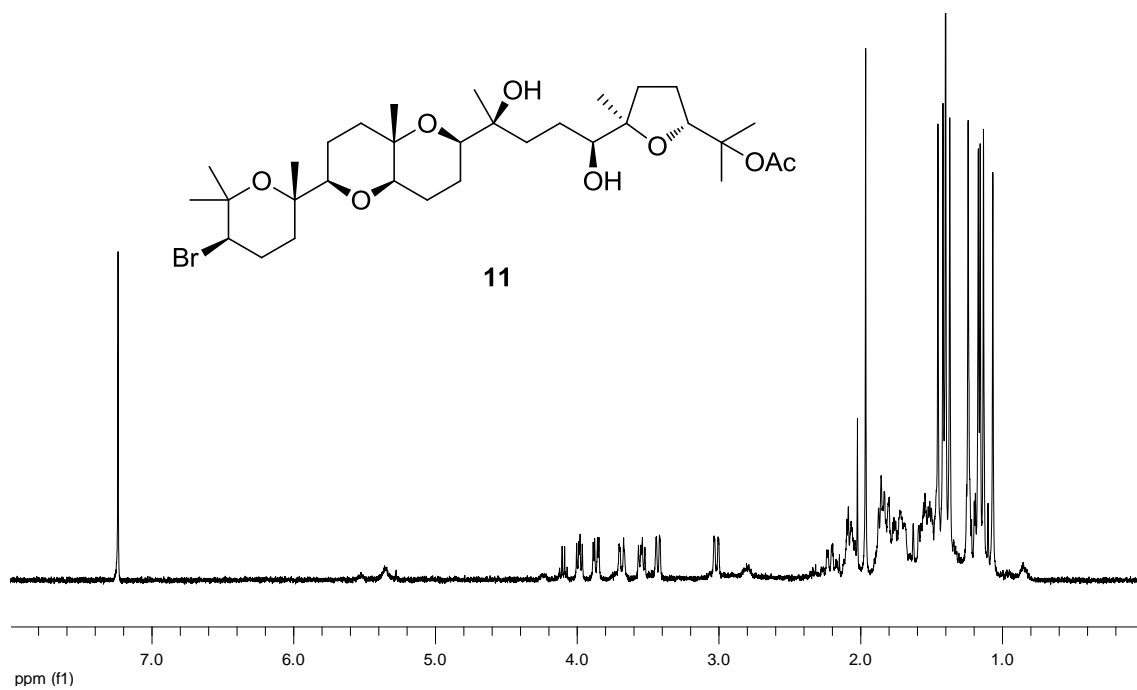


Figure S55. <sup>1</sup>H NMR spectrum of 23-acetyl-thyrsiferol (**11**) in CDCl<sub>3</sub>.