SUPPLEMENTARY MATERIAL

Synthesis and *h*LDHA Inhibitory Activity of New Stiripentol-Related Compounds of Potential Use in Primary Hyperoxaluria

Mario Rico-Molina [1], Juan Ortega-Vidal [1,2], Juan Molina-Canteras [1], Justo Cobo [1], Joaquín Altarejos [1,*] and Sofía Salido [1]

[1] Department of Inorganic and Organic Chemistry, Faculty of Experimental Sciences, University of Jaén, Campus of International Excellence in Agri-Food (ceiA3), 23071 Jaén, Spain; mrico@ujaen.es (M.R.-M.); jovidal@ujaen.es (J.O.-V.); jcantera@ujaen.es (J.M.-C.); jcobo@ujaen.es (J.C.); ssalido@ujaen.es (S.S.)

[2] School of Biological and Chemical Sciences, Ryan Institute, University of Galway, H91TK33 Galway, Ireland; juan.ortegavidal@universityofgalway.ie (J.O.-V.)

* Correspondence: jaltare@ujaen.es

Table of contents

1. **NMR spectra** **p. 2**

2. **High resolution mass spectra** **p. 30**

3. **HPLC chromatograms** **p. 37**

4. **Quiral HPLC chromatograms** **p. 39**

5. **DFT-optimized lowest energy conformers** **p. 42**

6. **Dose response curves against *h*LDHA** **p. 44**

1. NMR spectra

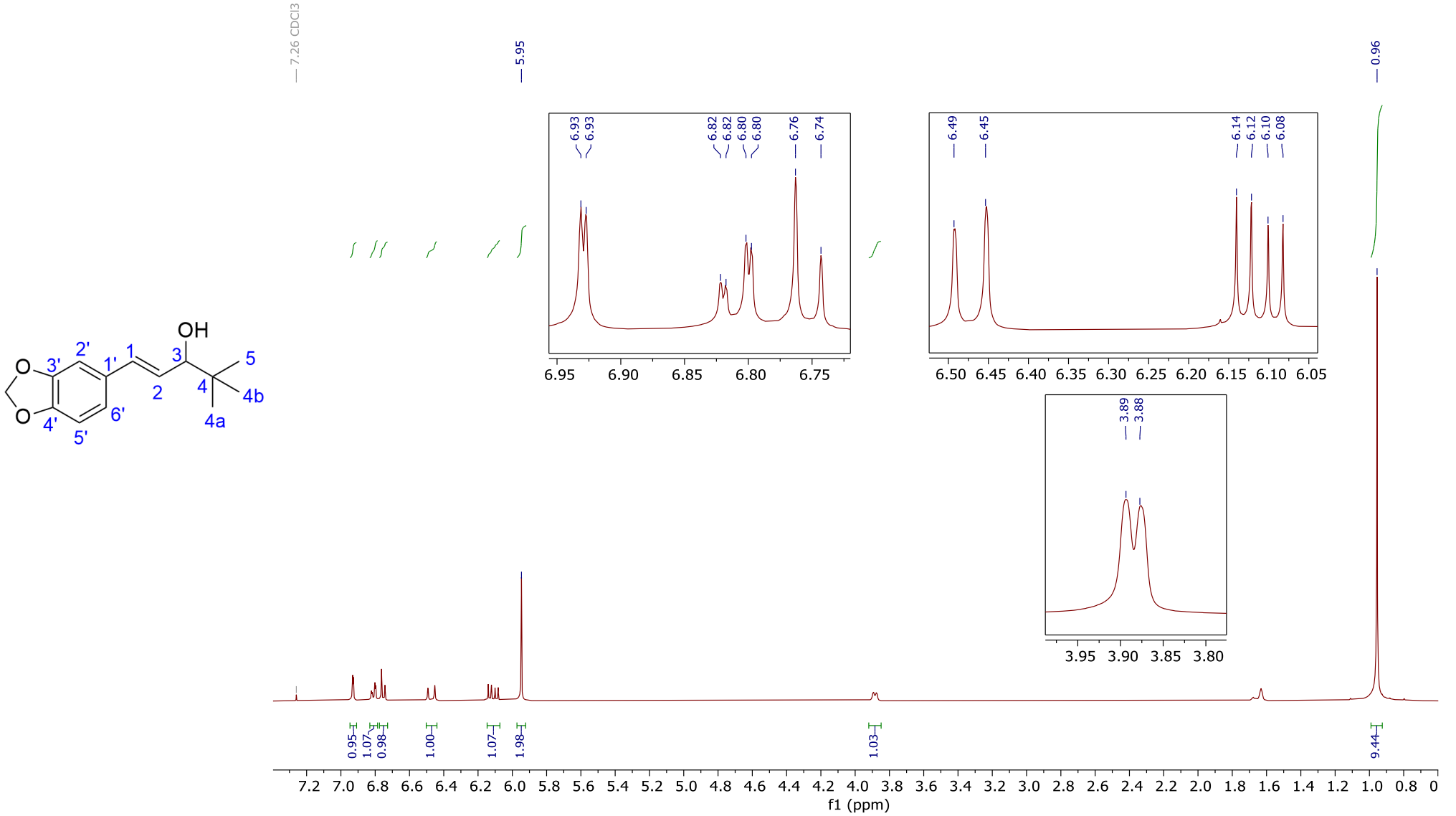


Figure S1. ^{1}H NMR spectrum (400 MHz) of compound **1** in $CDCl_3$.

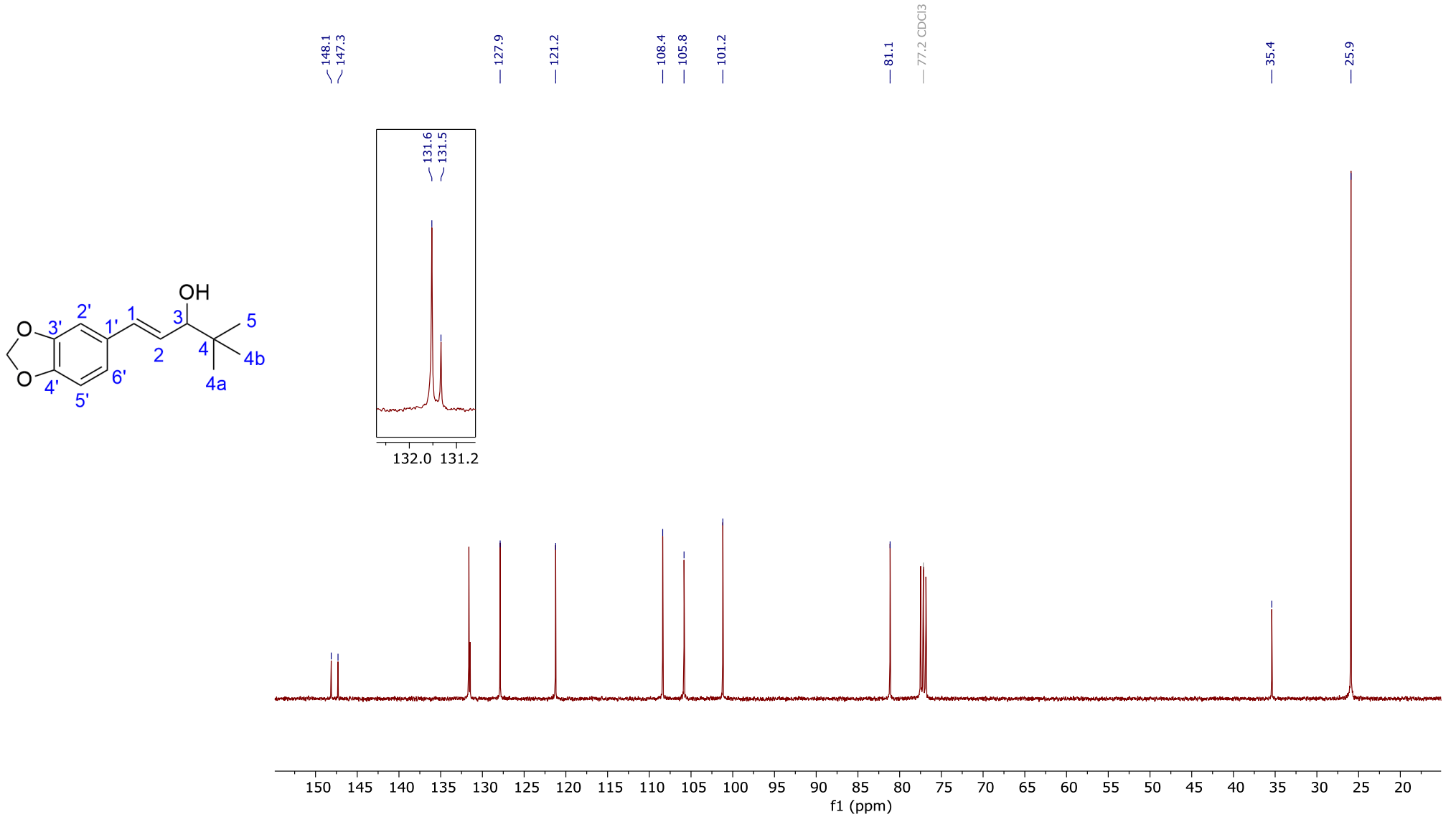


Figure S2. ^{13}C NMR spectrum (100 MHz) of compound **1** in $CDCl_3$.

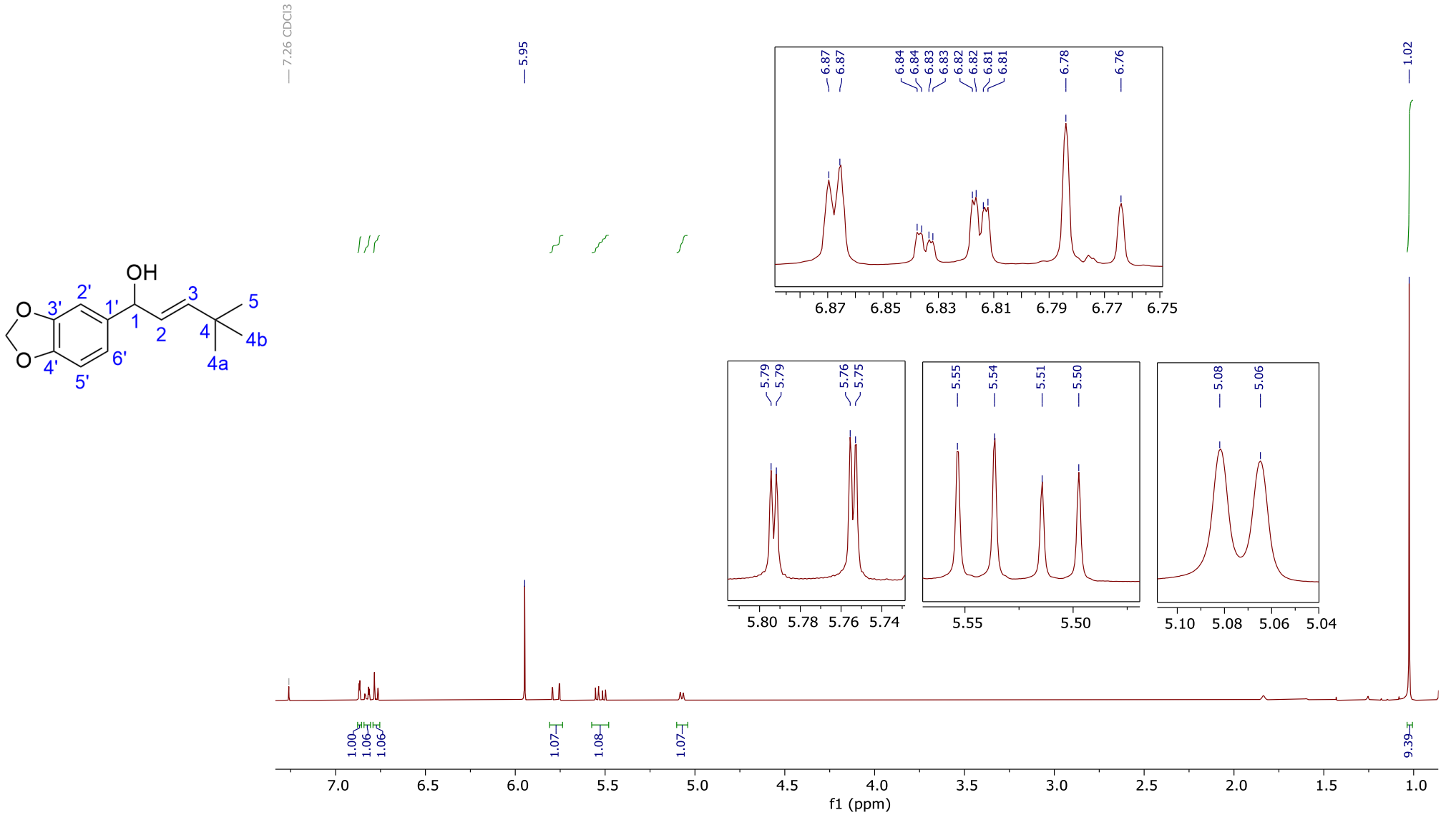


Figure S3. ^{1}H NMR spectrum (400 MHz) of compound **2** in $CDCl_3$.

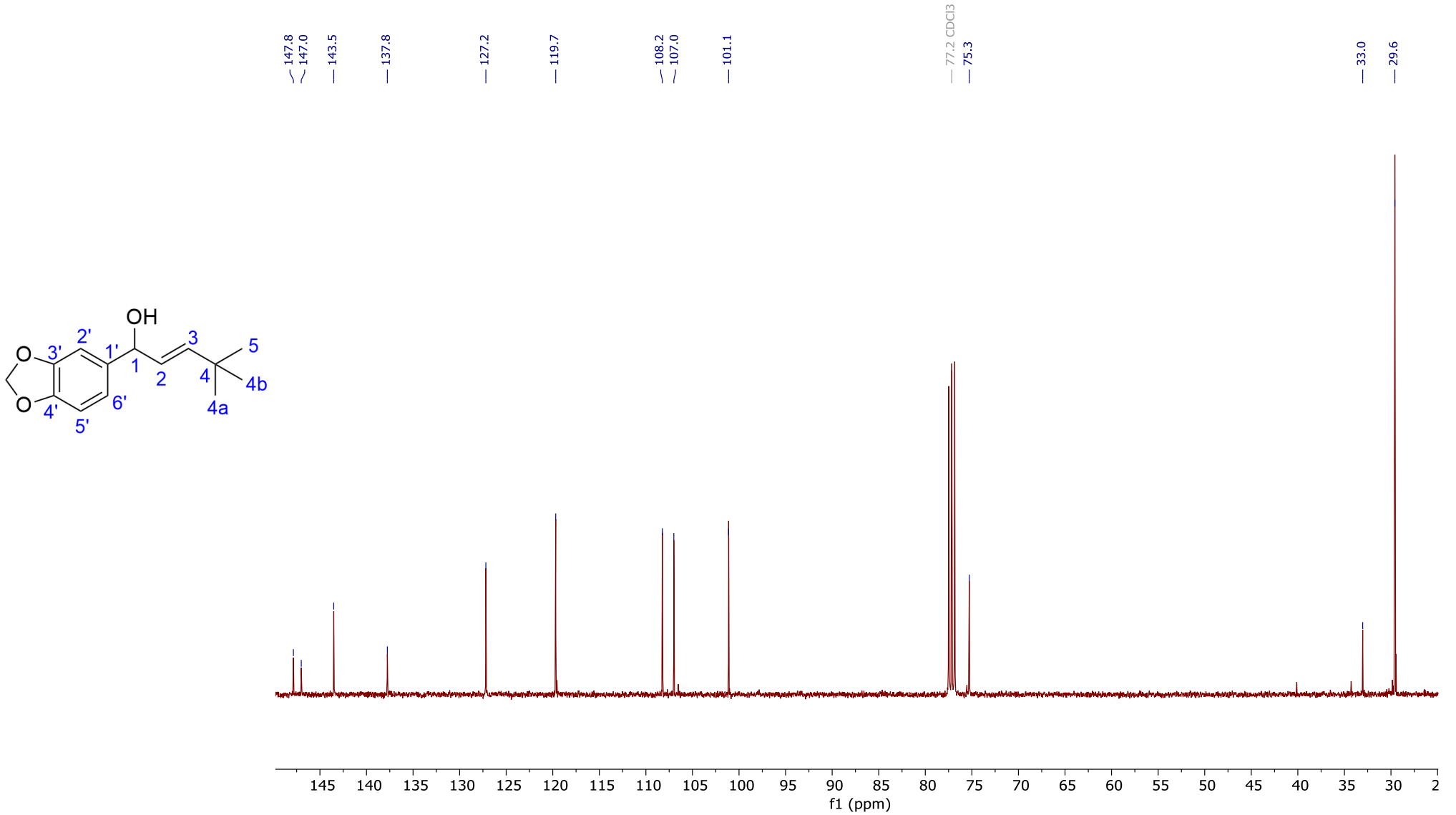


Figure S4. ^{13}C NMR spectrum (100 MHz) of compound **2** in $CDCl_3$.

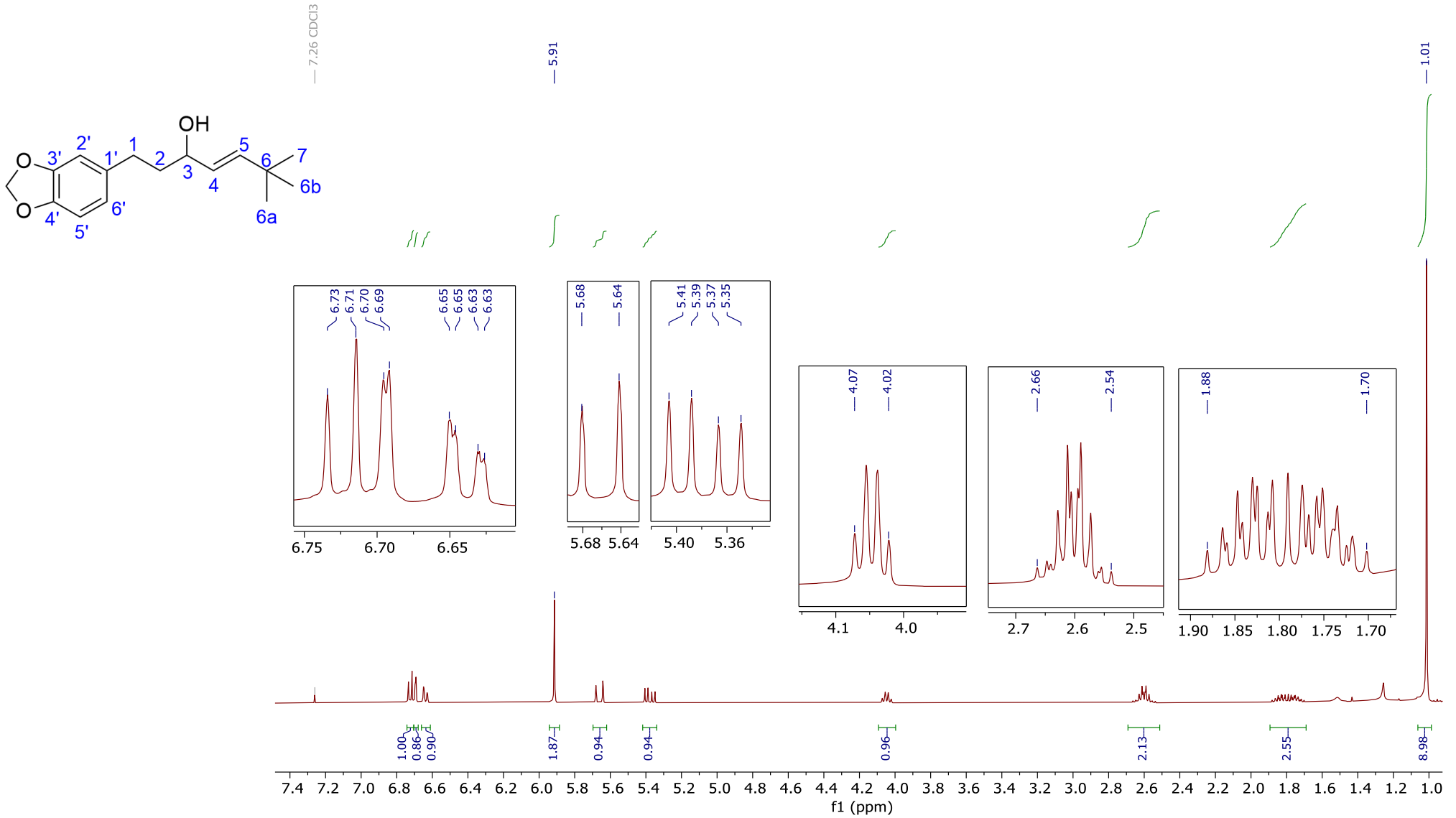


Figure S5. ^{1}H NMR spectrum (400 MHz) of compound **3** in $CDCl_3$.

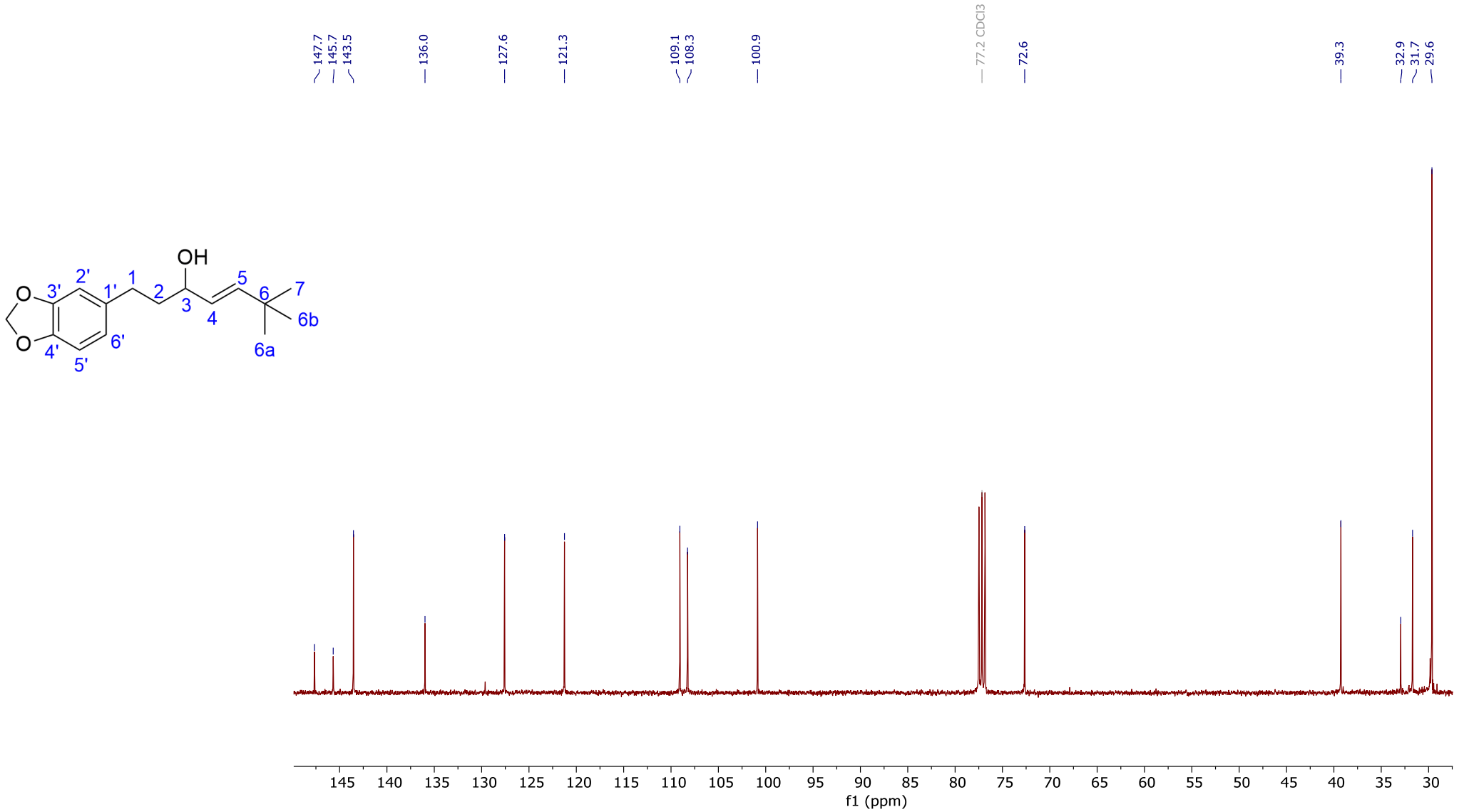


Figure S6. ^{13}C NMR spectrum (100 MHz) of compound **3** in $CDCl_3$.

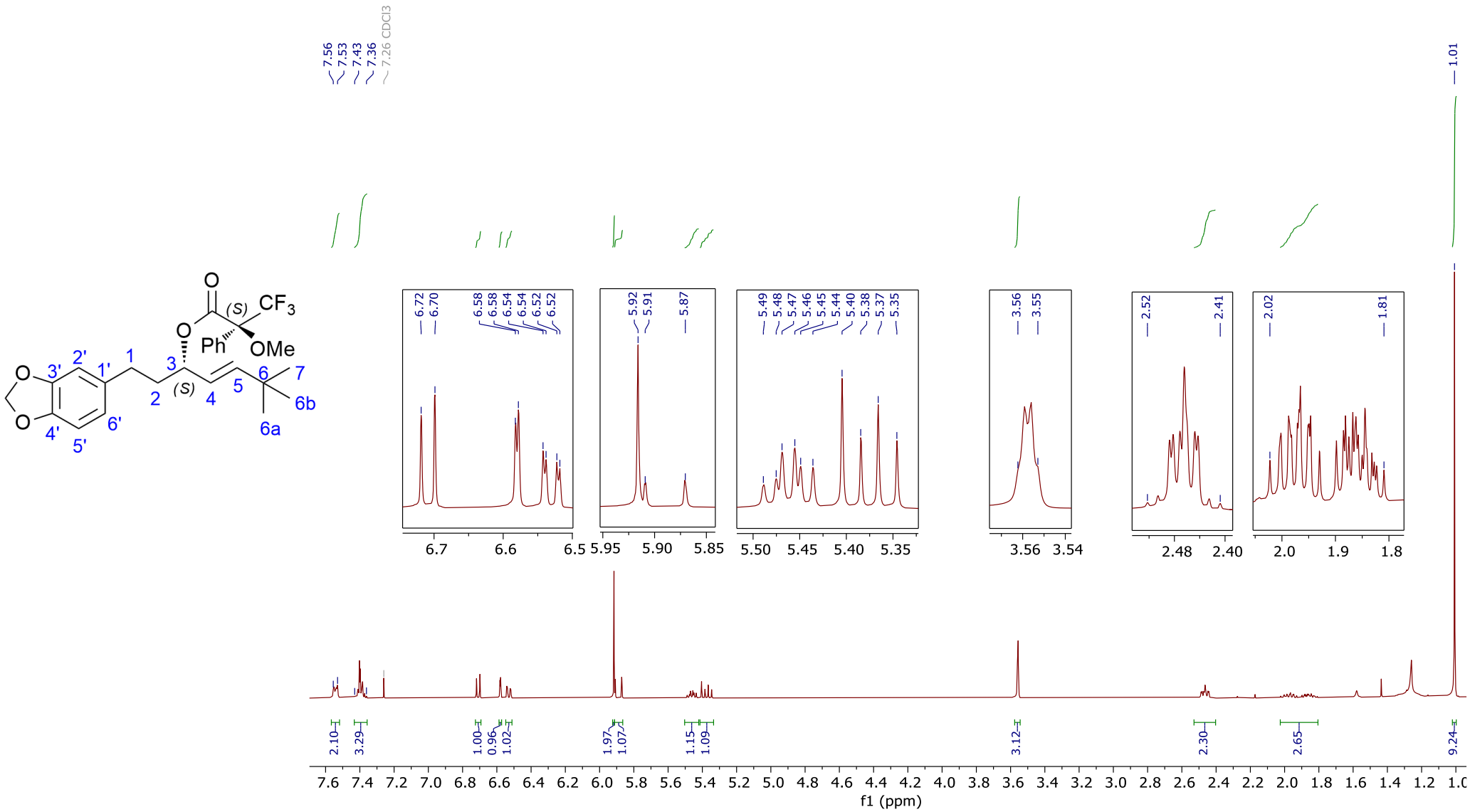


Figure S7. ^{1}H NMR spectrum (400 MHz) of compound **3a** in $CDCl_3$.

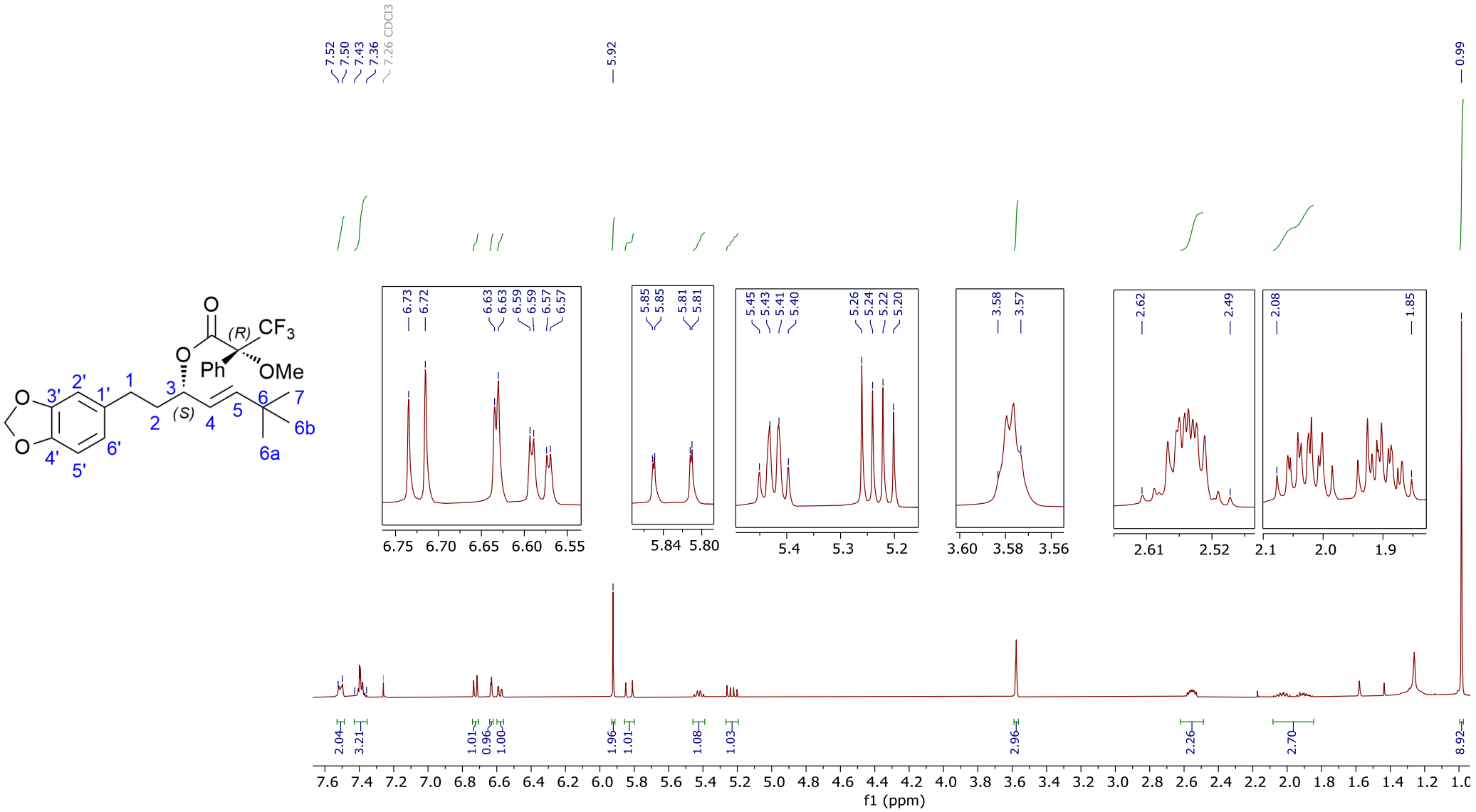


Figure S8. [1]H NMR spectrum (400 MHz) of compound **3b** in $CDCl_3$.

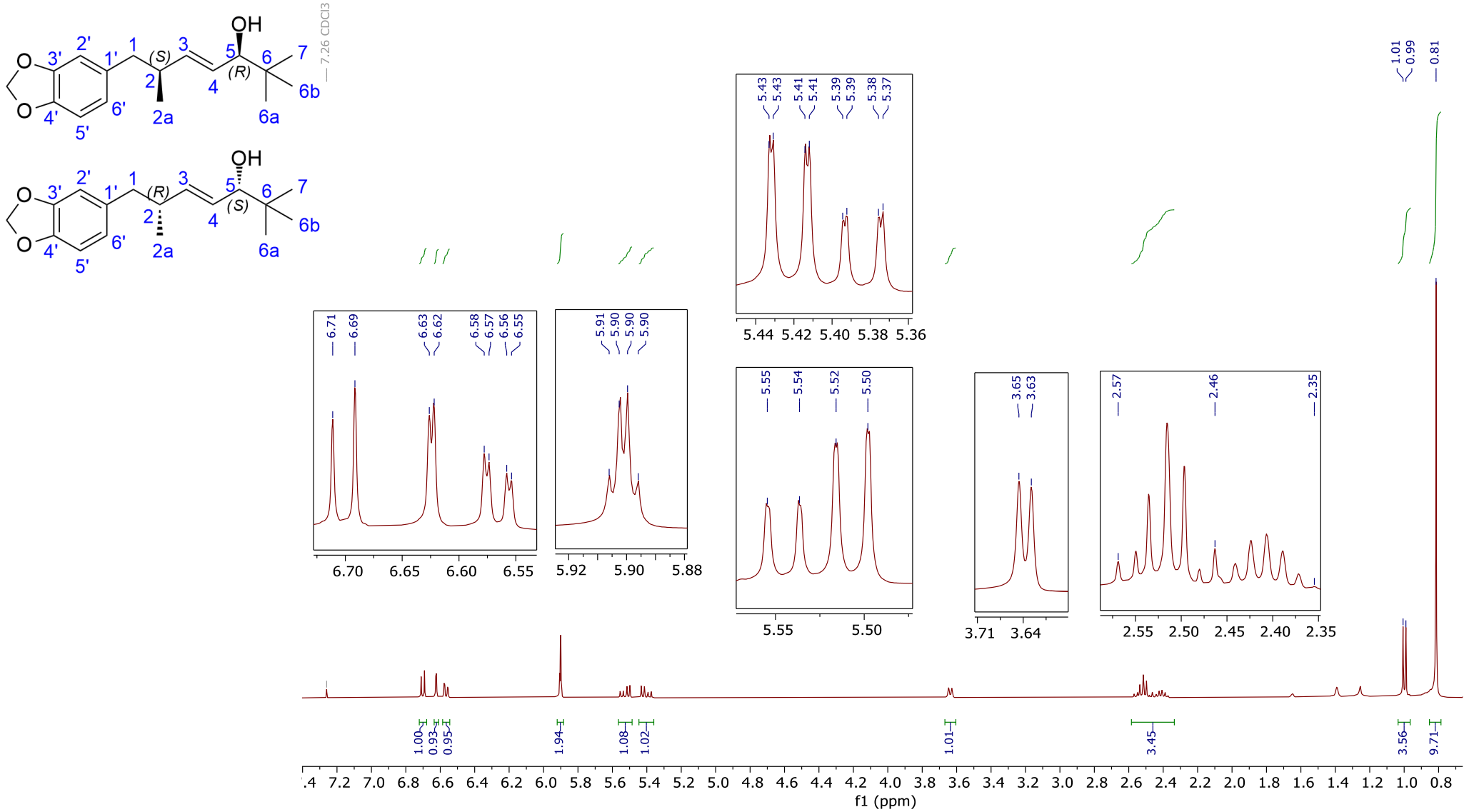


Figure S9. ^{1}H NMR spectrum (400 MHz) of compound (*2S*,5R**)-**4** in $CDCl_3$.

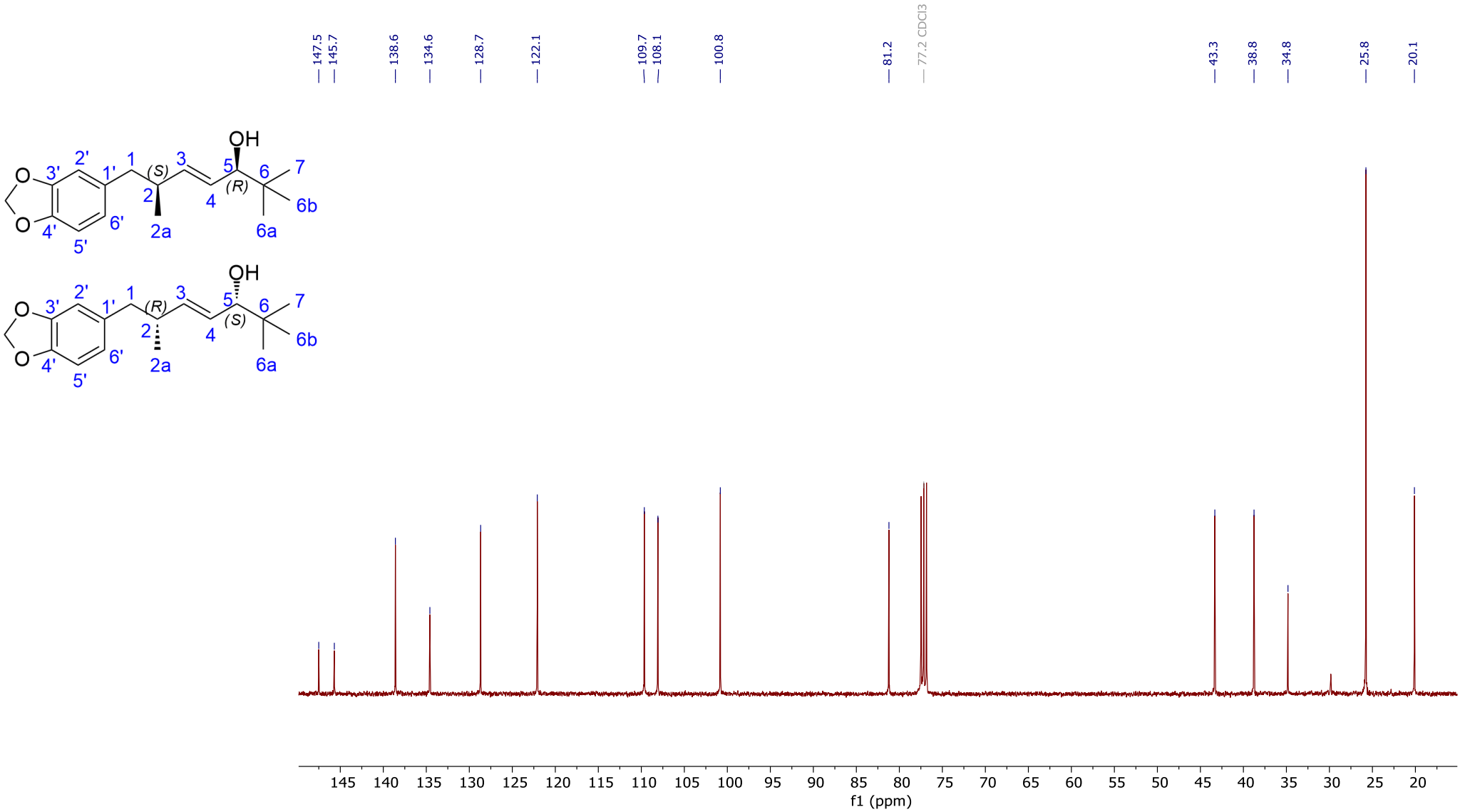


Figure S10. ^{13}C NMR spectrum (100 MHz) of compound (*2S*,5R**)-**4** in $CDCl_3$.

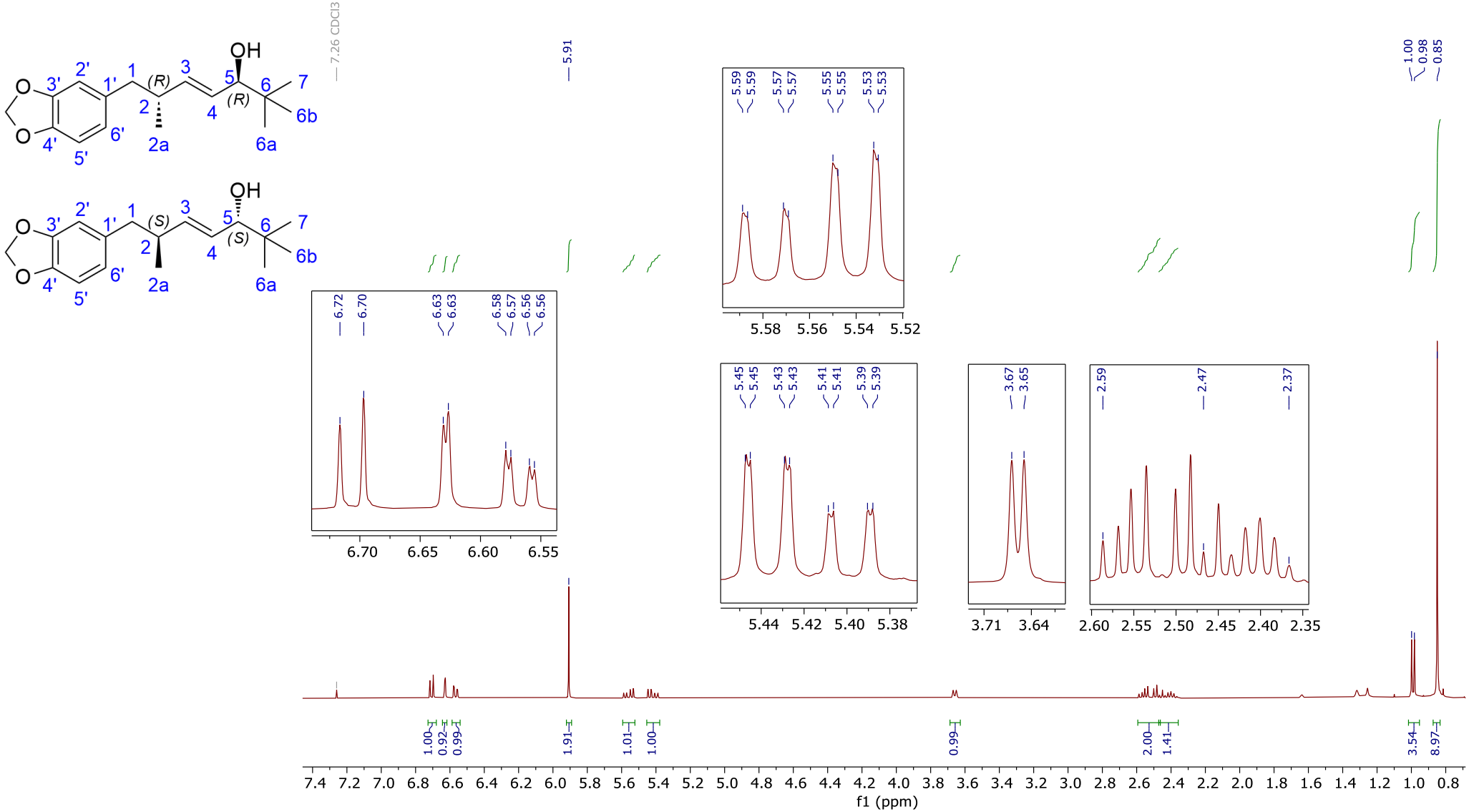


Figure S11. ^{1}H NMR spectrum (400 MHz) of compound (*2R*,5R**)-**4** in $CDCl_3$.

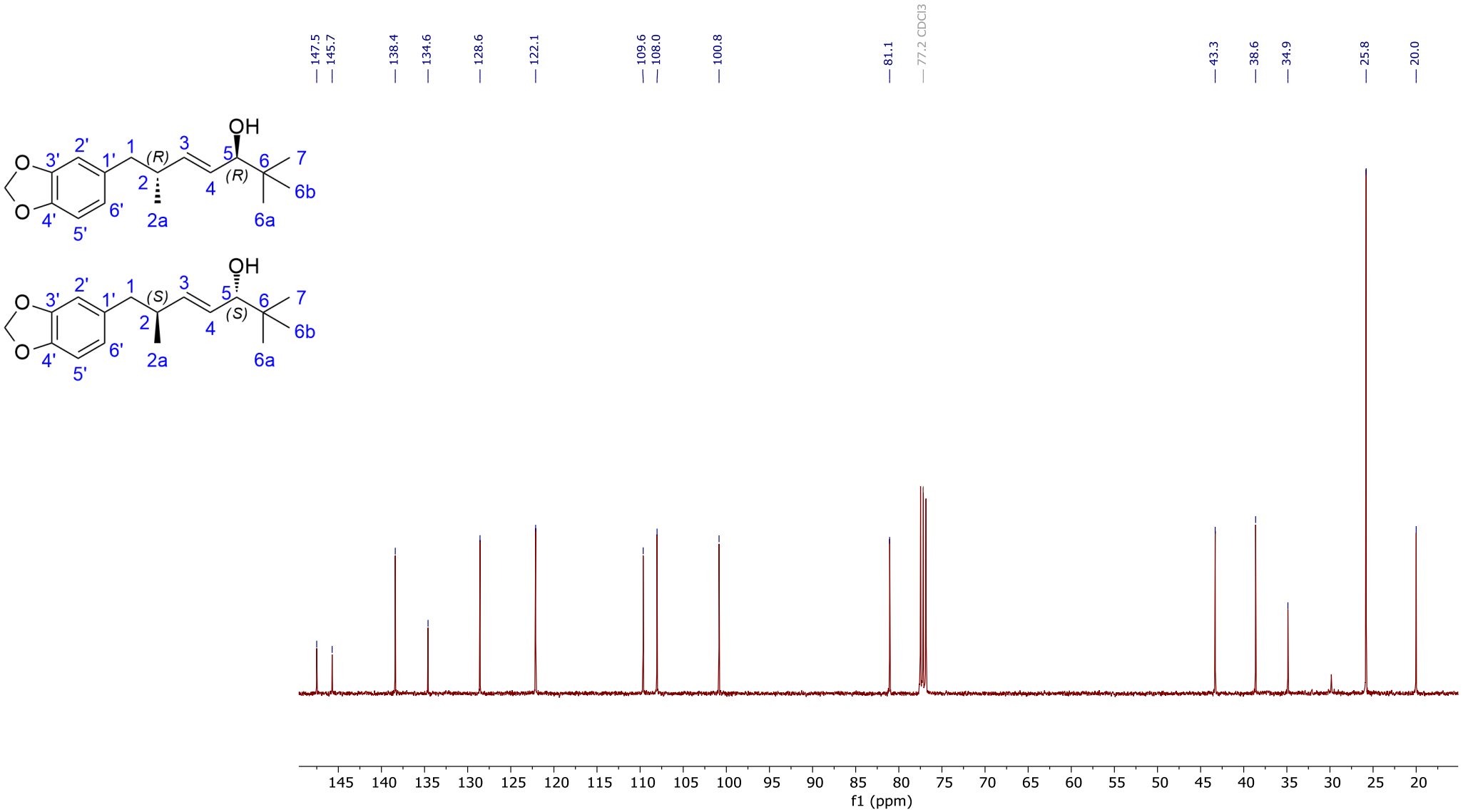


Figure S12. ^{13}C NMR spectrum (100 MHz) of compound (*2R*,5R**)-**4** in $CDCl_3$.

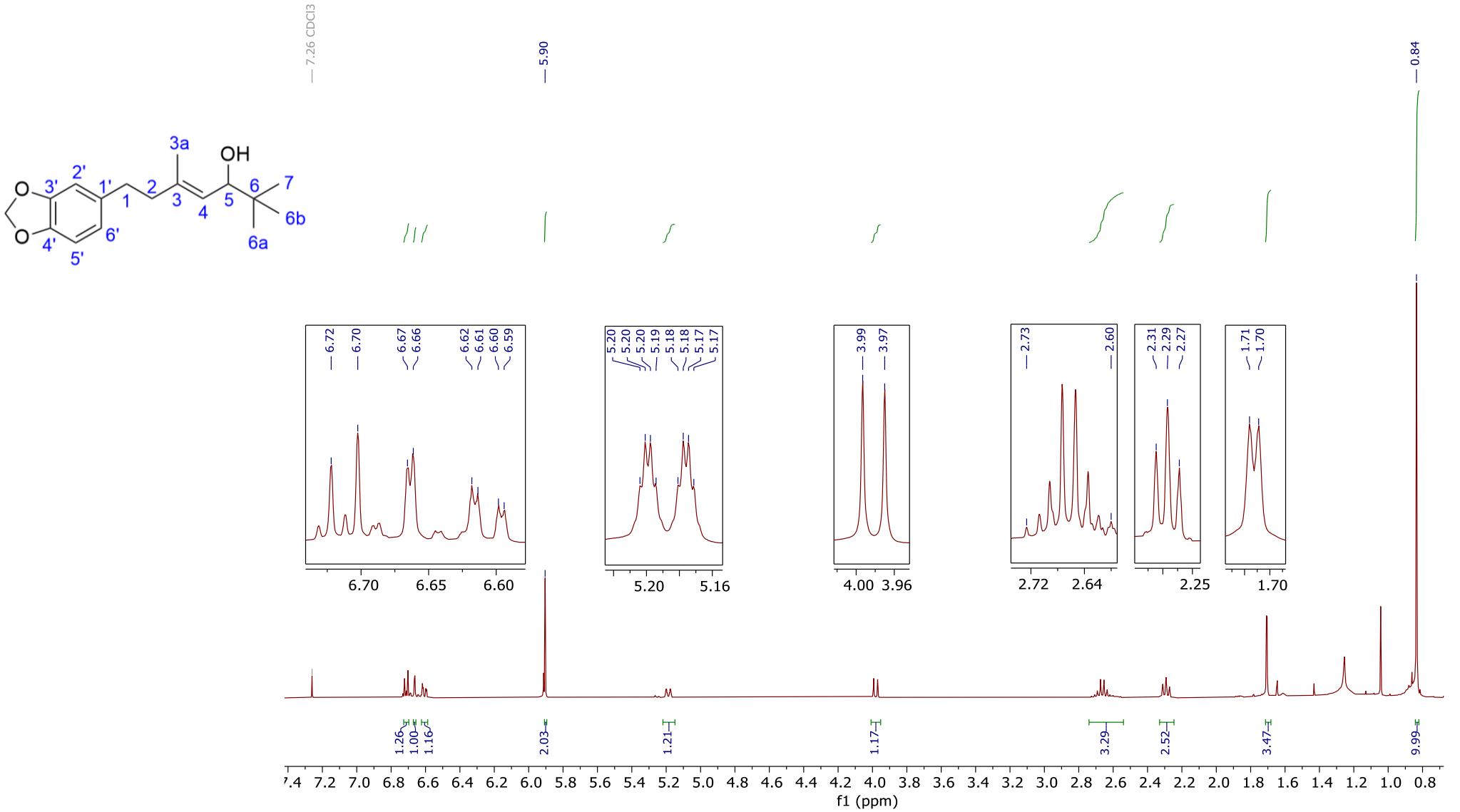


Figure S13. ^{1}H NMR spectrum (400 MHz) of compound **5** in $CDCl_3$.

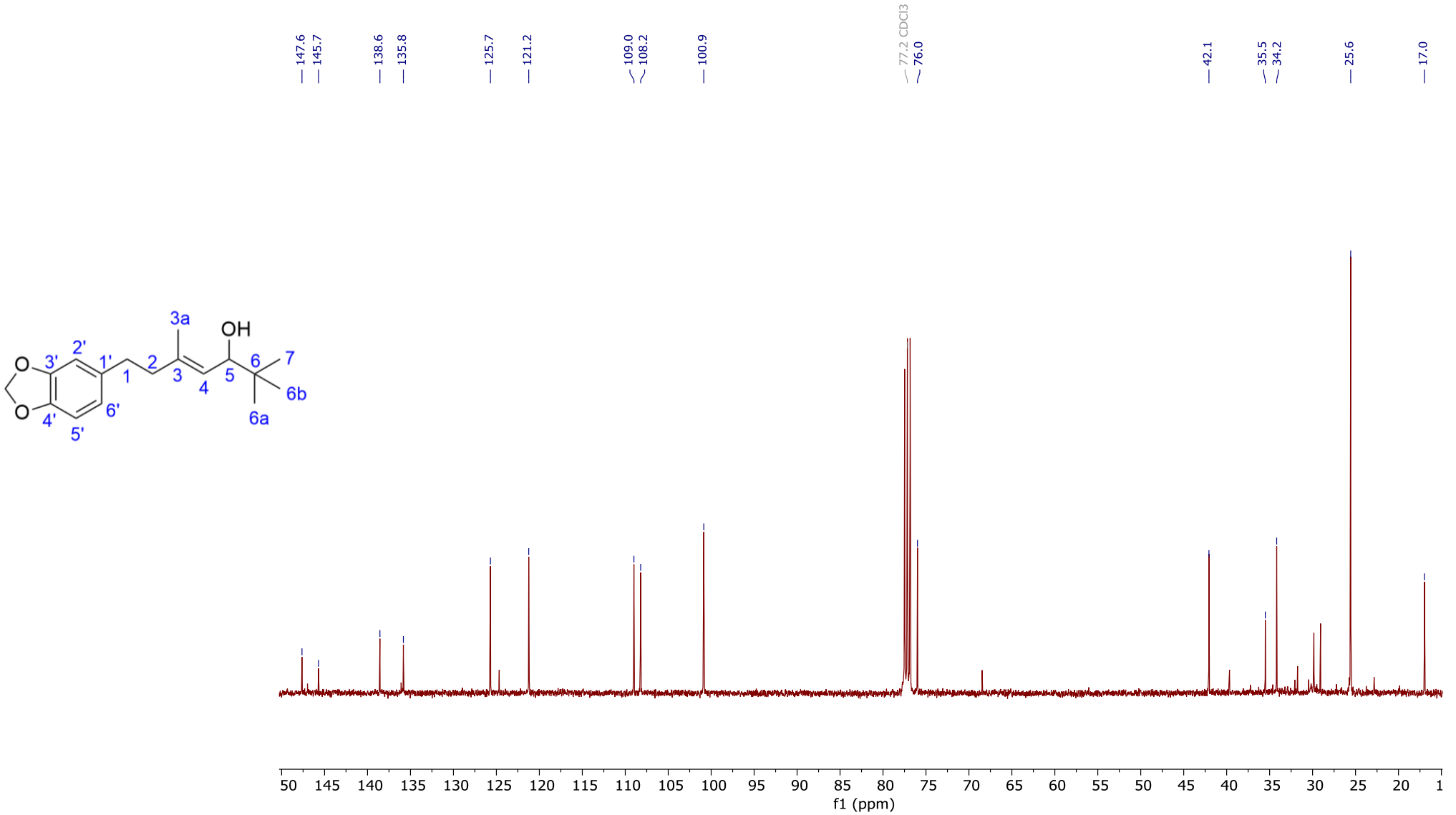


Figure S14. ^{13}C NMR spectrum (100 MHz) of compound **5** in $CDCl_3$.

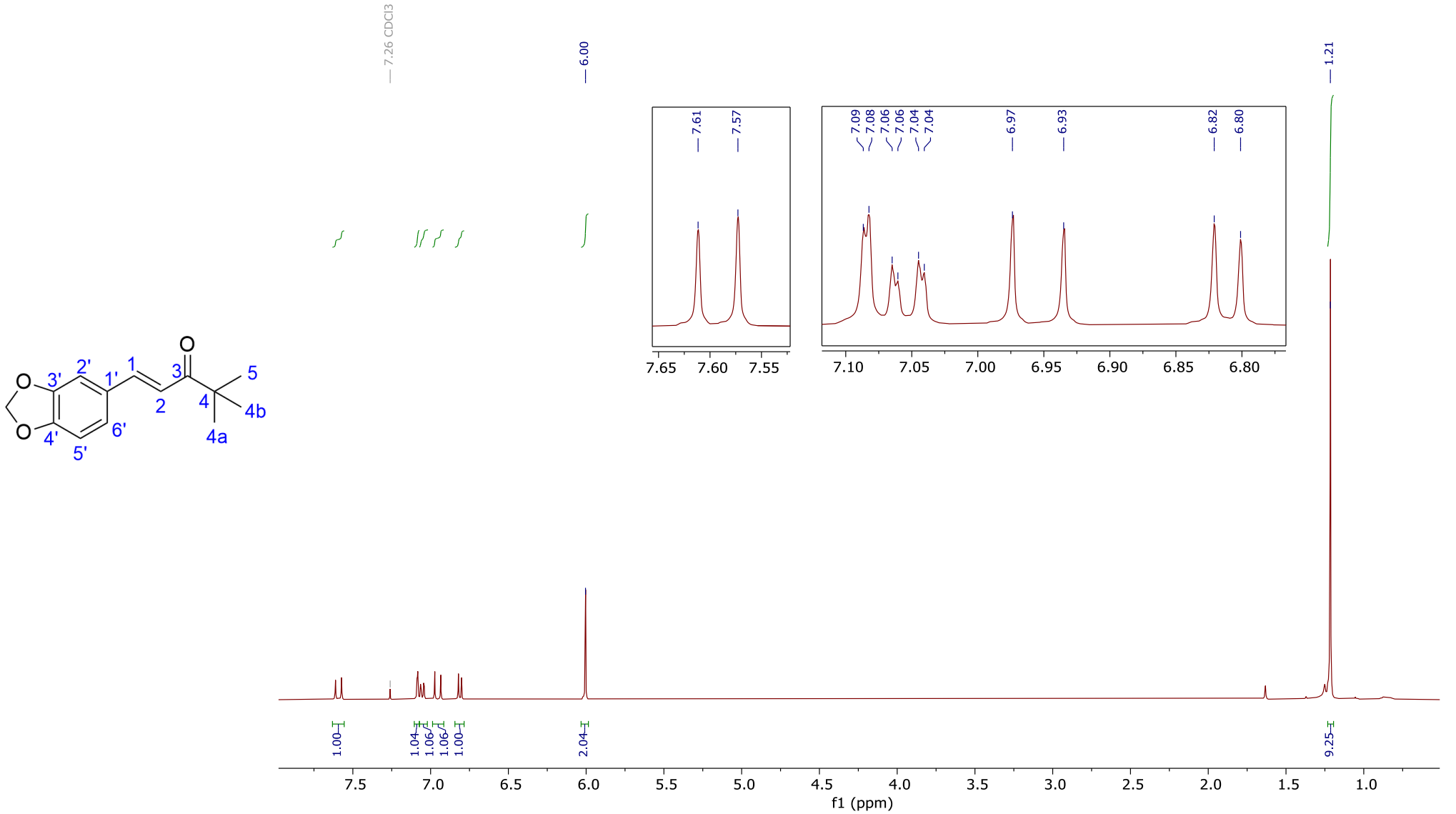


Figure S15. ^{1}H NMR spectrum (400 MHz) of compound **8** in $CDCl_3$.

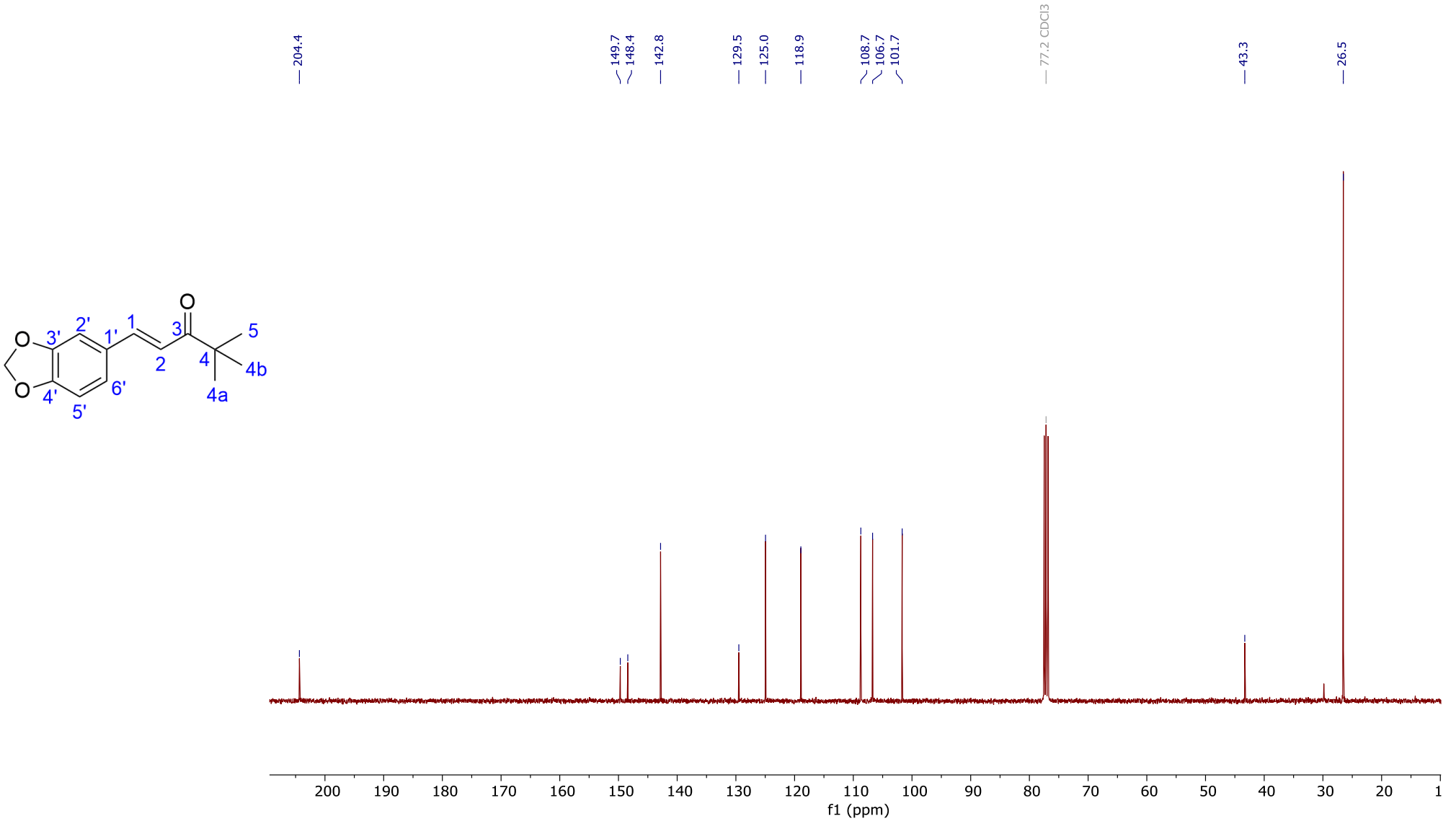


Figure S16. ^{13}C NMR spectrum (100 MHz) of compound **8** in $CDCl_3$.

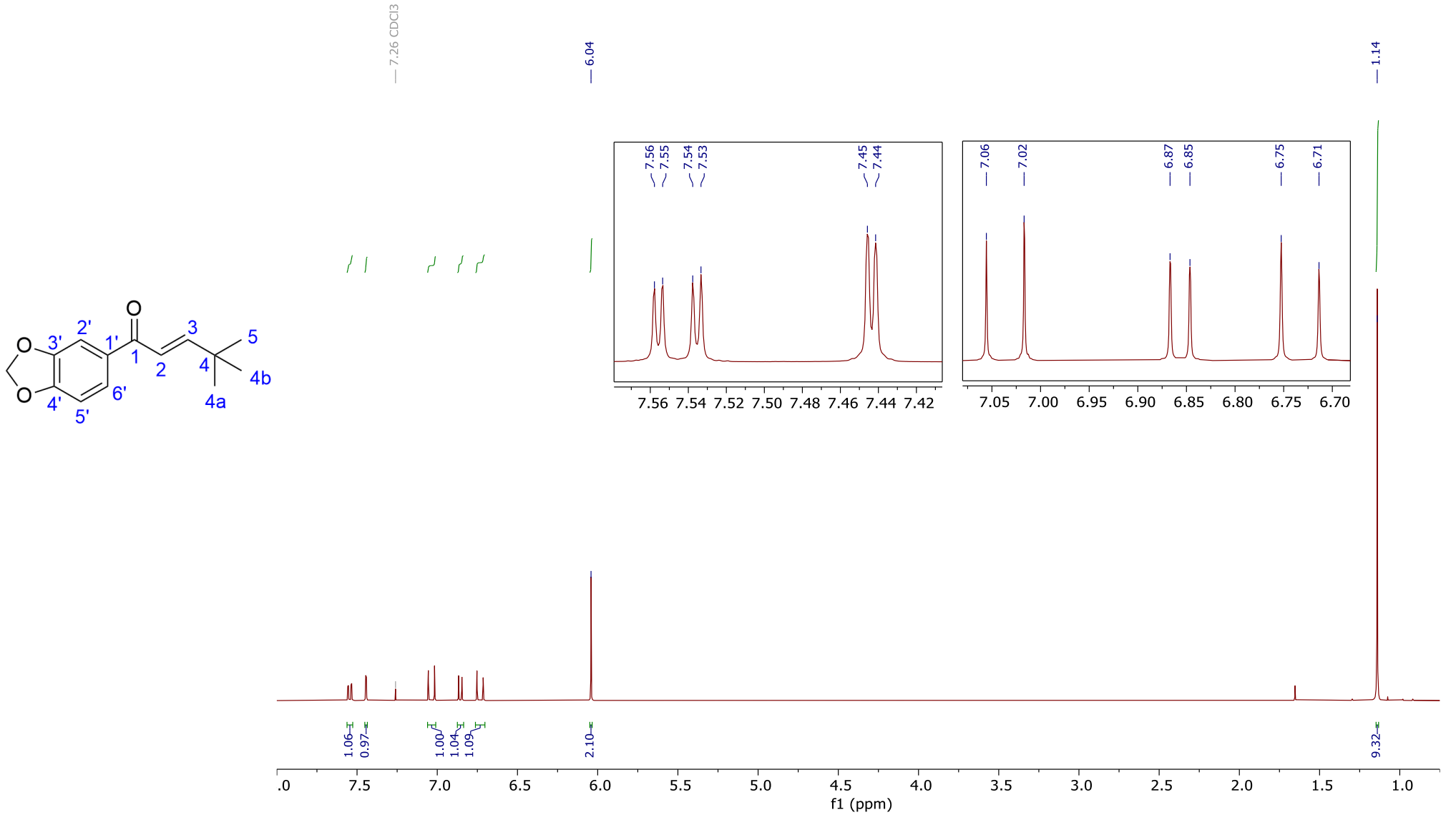


Figure S17. ^{1}H NMR spectrum (400 MHz) of compound **11** in $CDCl_3$.

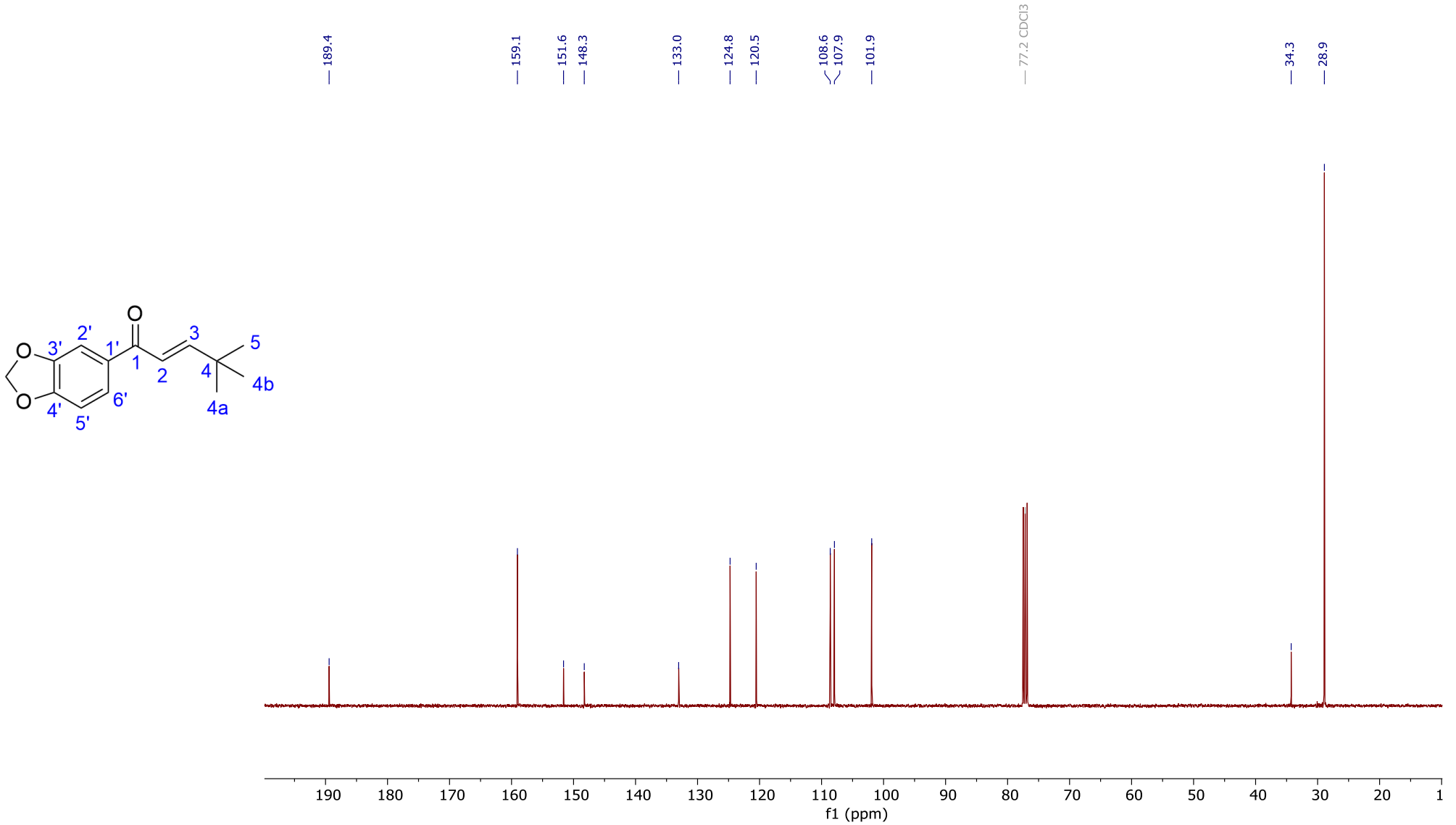


Figure S18. ^{13}C NMR spectrum (100 MHz) of compound **11** in $CDCl_3$.

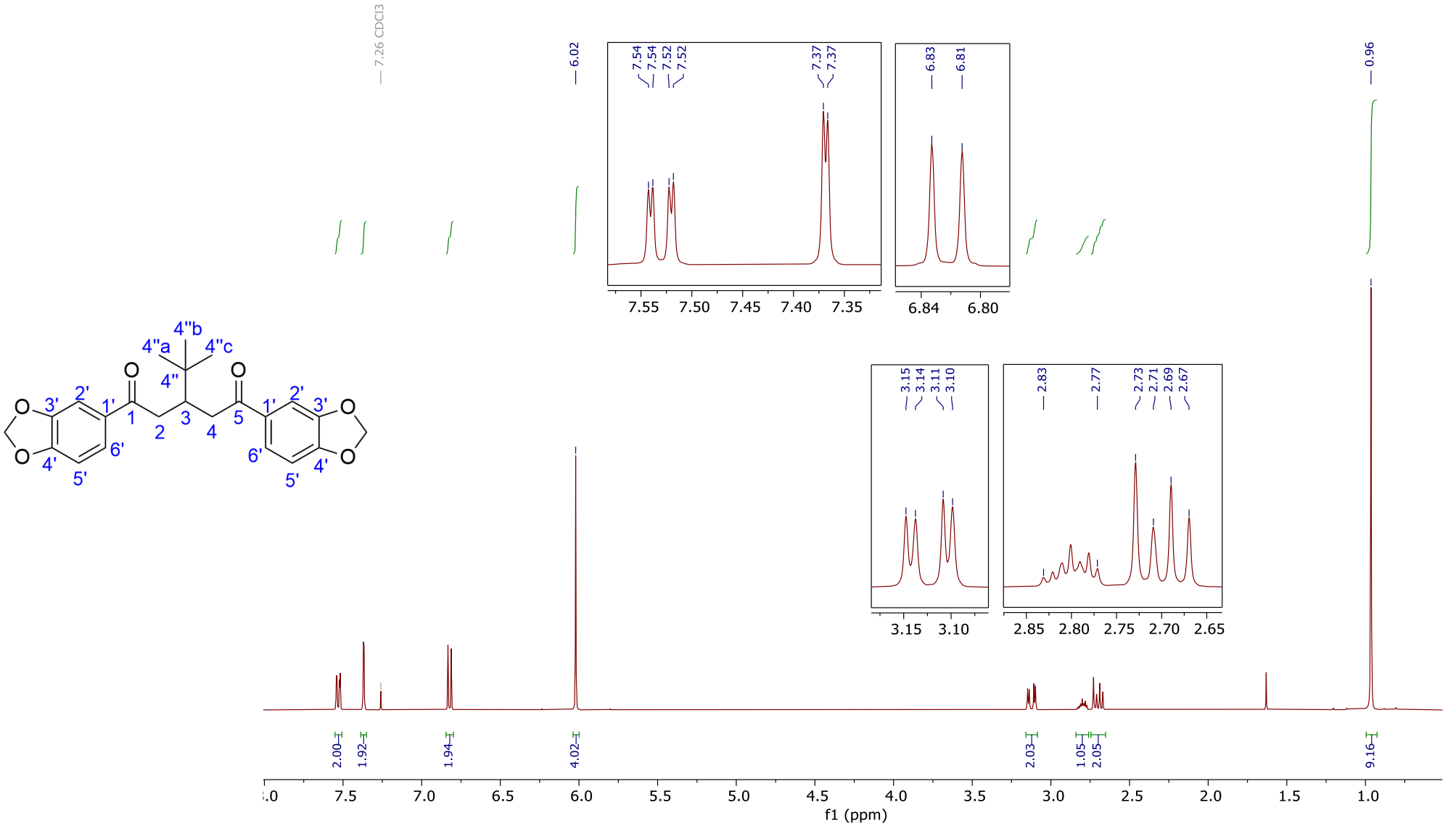


Figure S19. ^{1}H NMR spectrum (400 MHz) of compound **12** in $CDCl_3$.

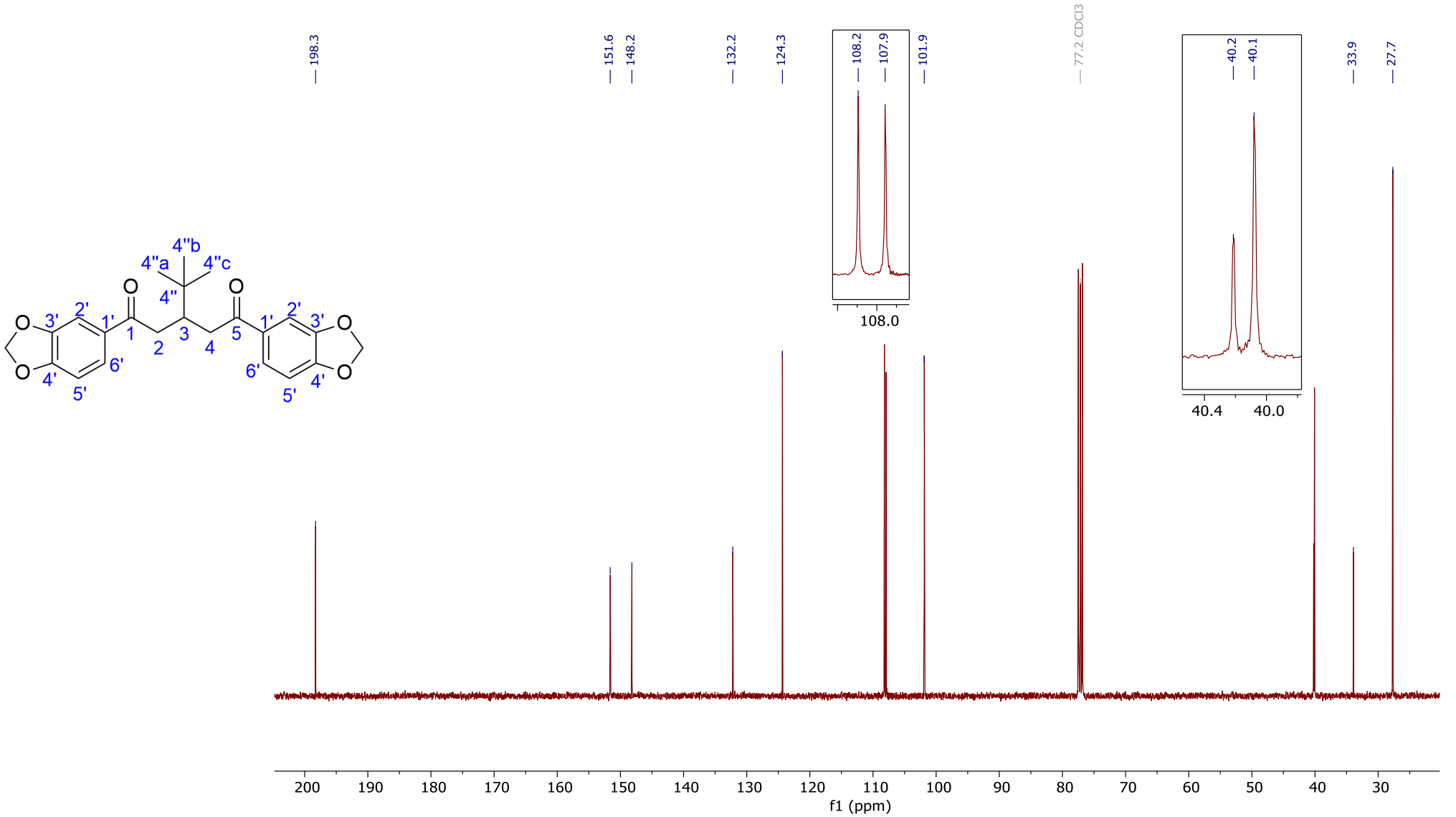


Figure S20. ^{13}C NMR spectrum (100 MHz) of compound **12** in $CDCl_3$.

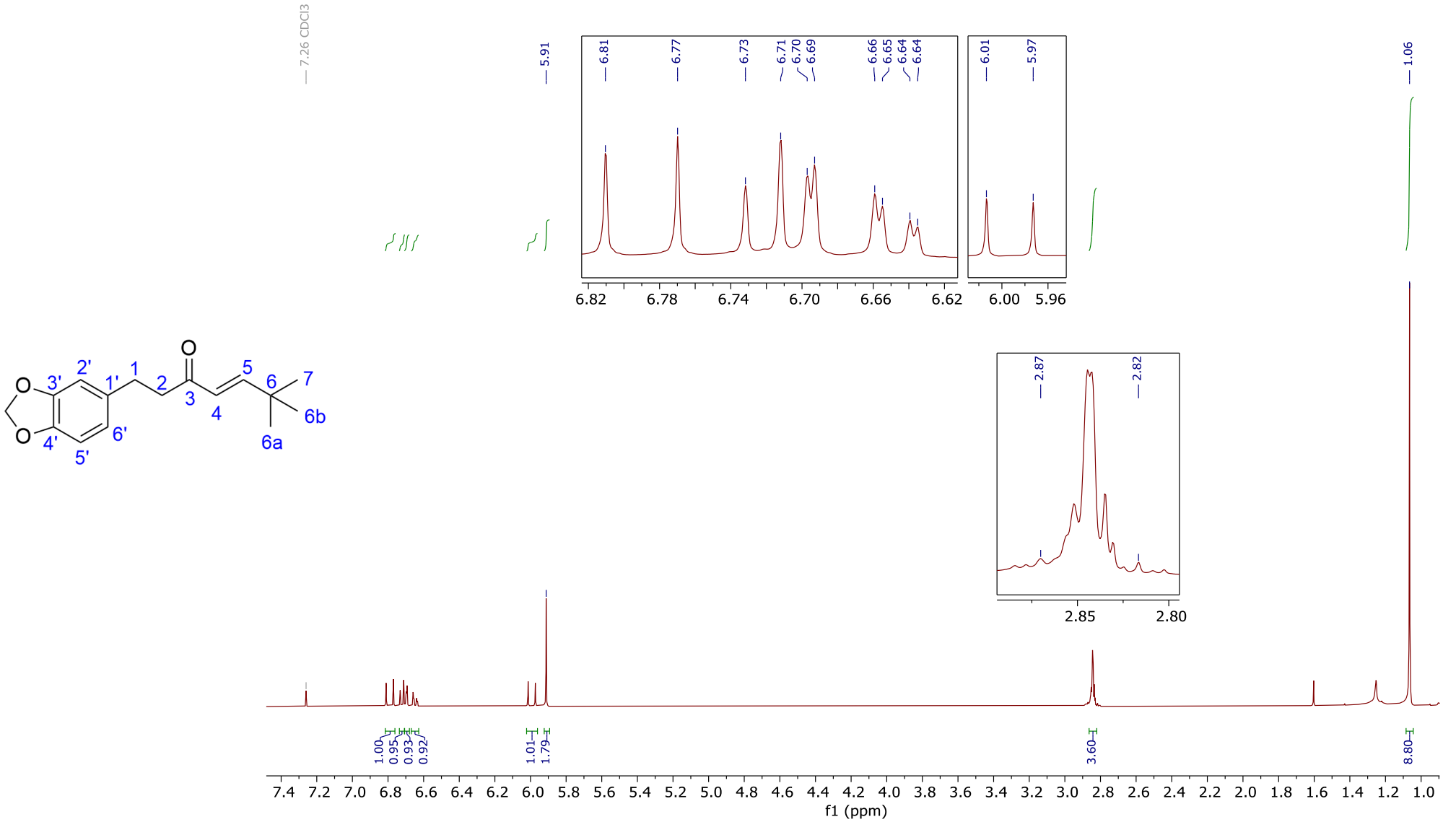


Figure S21. ^{1}H NMR spectrum (400 MHz) of compound **14** in $CDCl_3$.

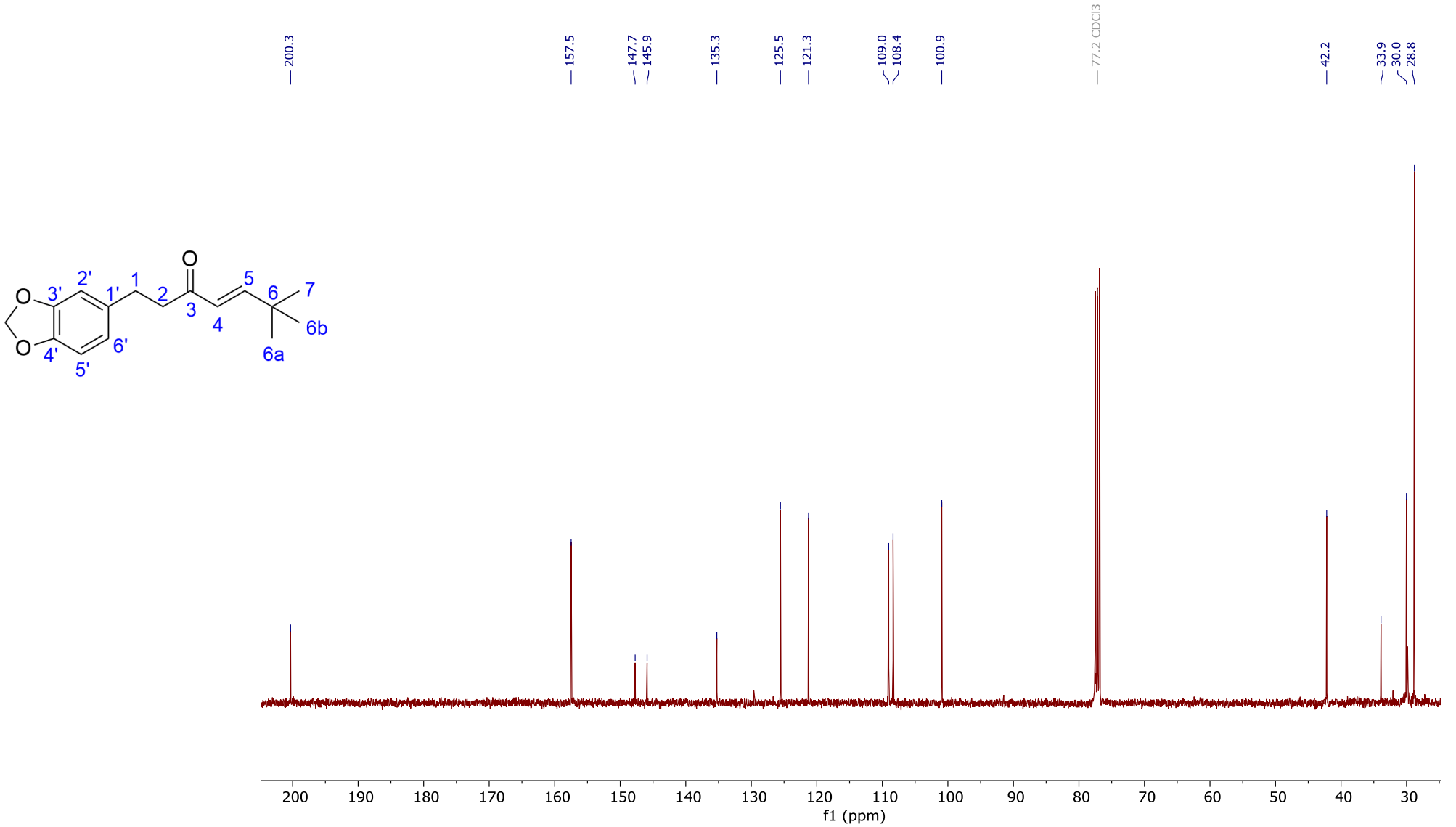


Figure S22. ^{13}C NMR spectrum (100 MHz) of compound **14** in $CDCl_3$.

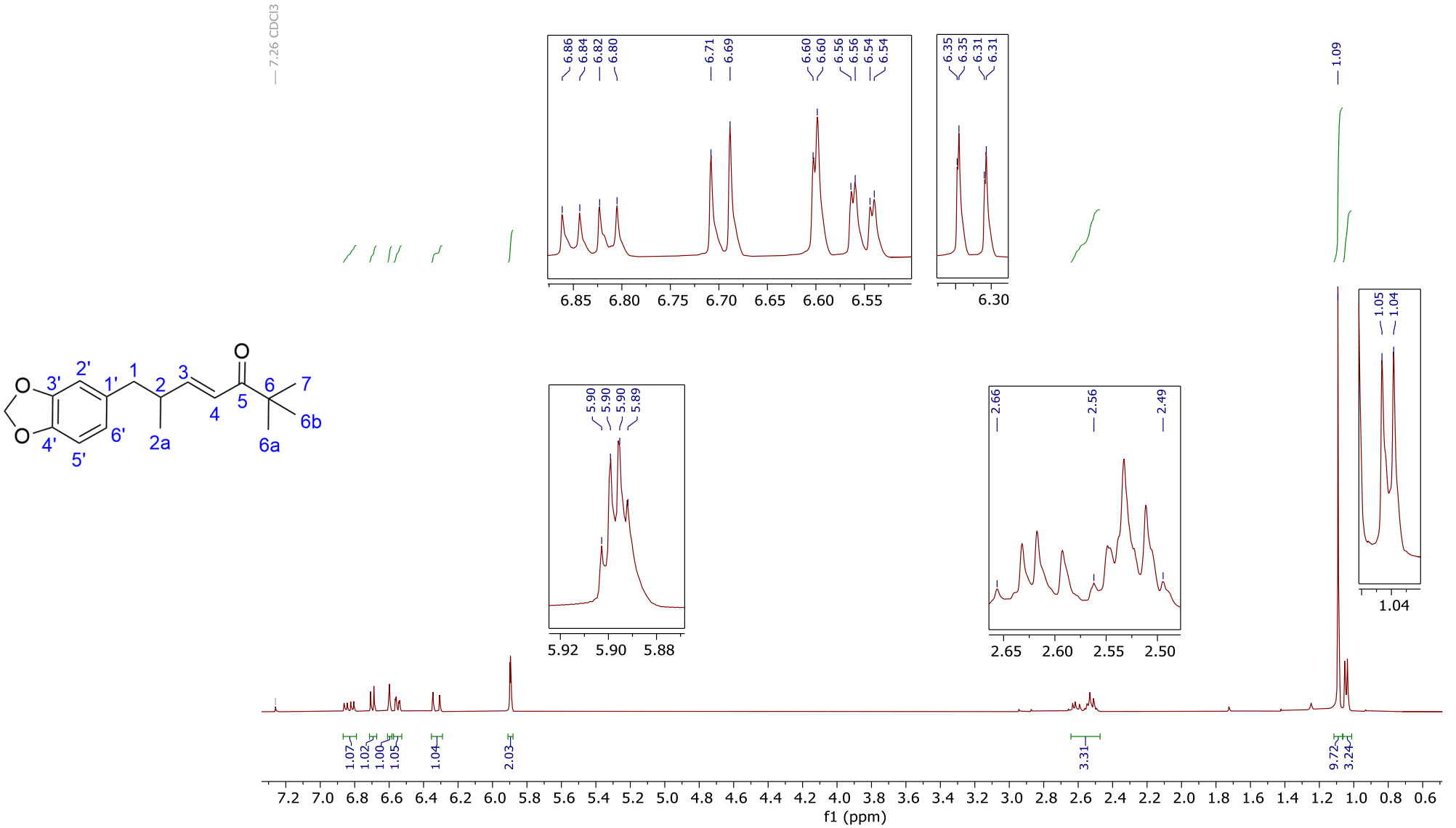


Figure S23. ^{1}H NMR spectrum (400 MHz) of compound **16** in $CDCl_3$.

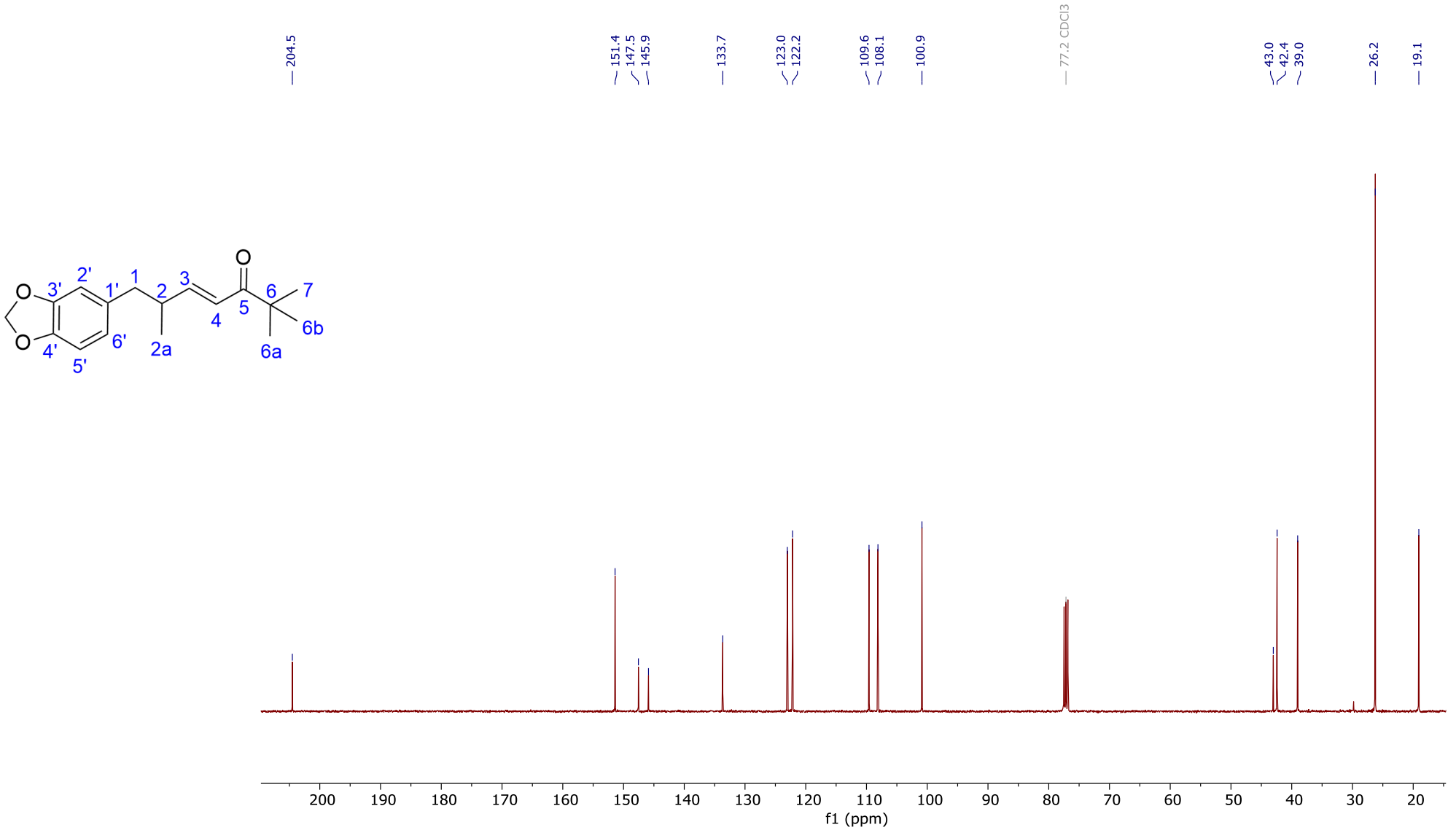


Figure S24. ^{13}C NMR spectrum (100 MHz) of compound **16** in $CDCl_3$.

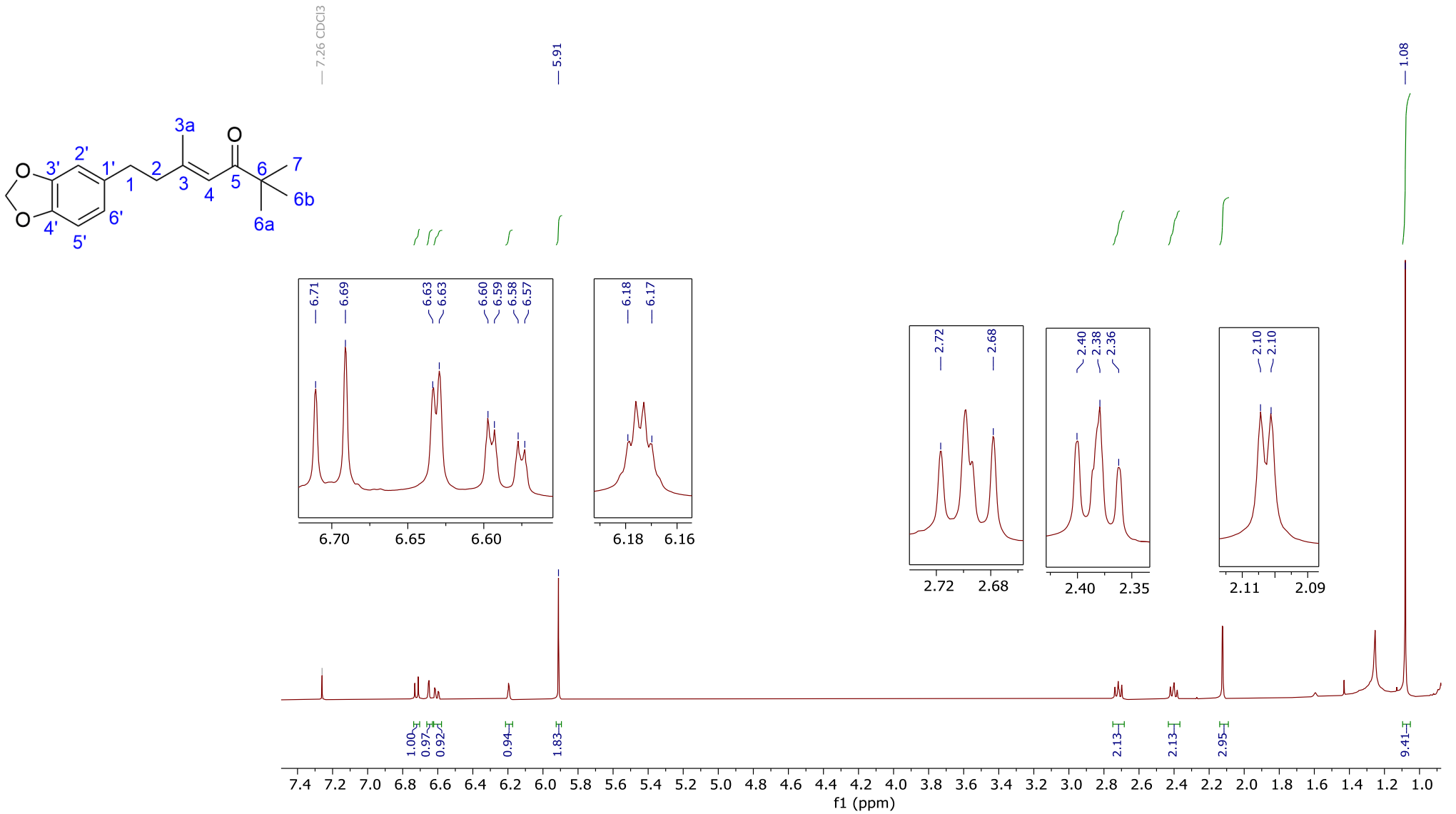


Figure S25. ^{1}H NMR spectrum (400 MHz) of compound **17** in $CDCl_3$.

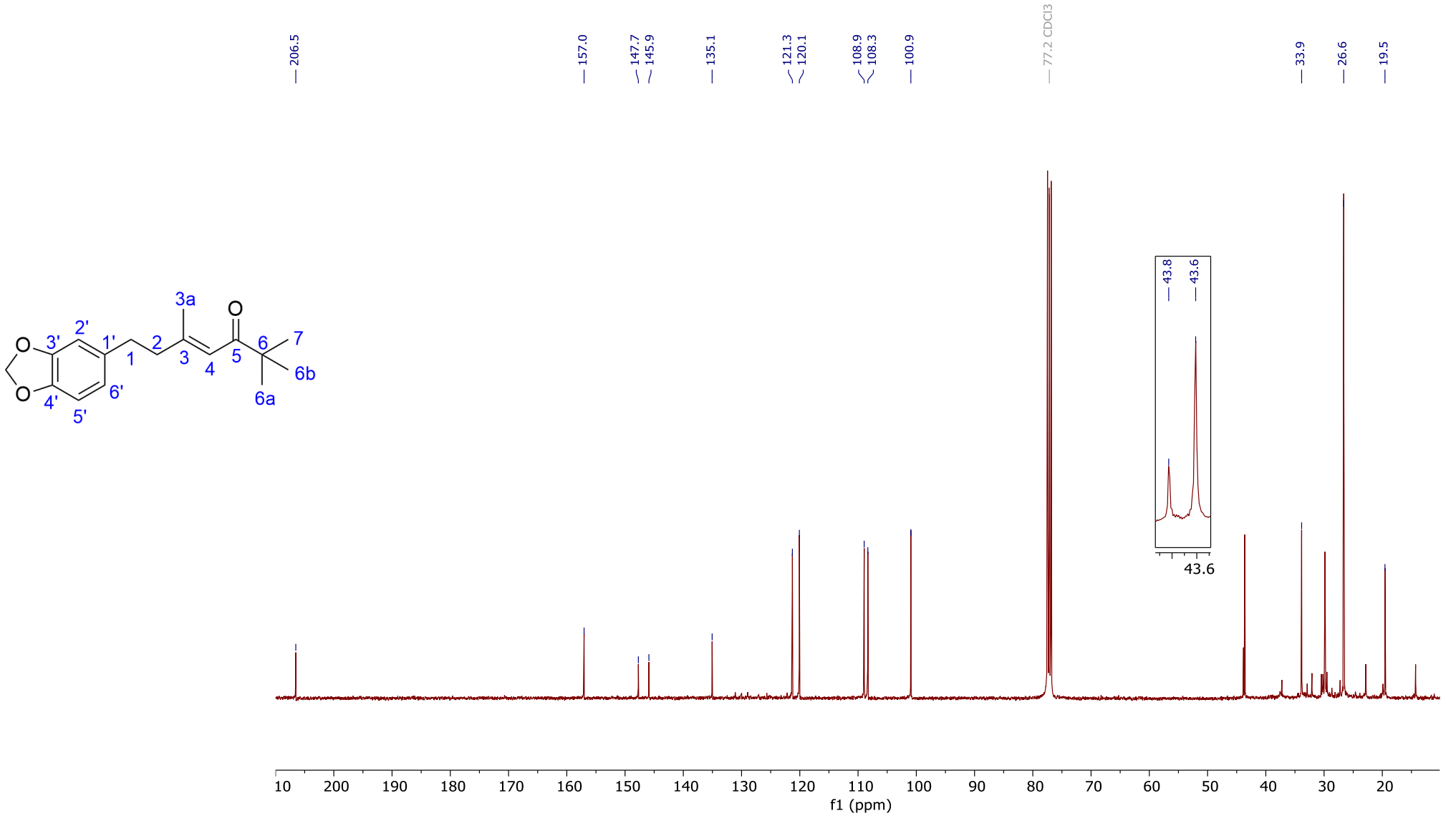


Figure S26. ^{13}C NMR spectrum (100 MHz) of compound **17** in $CDCl_3$.

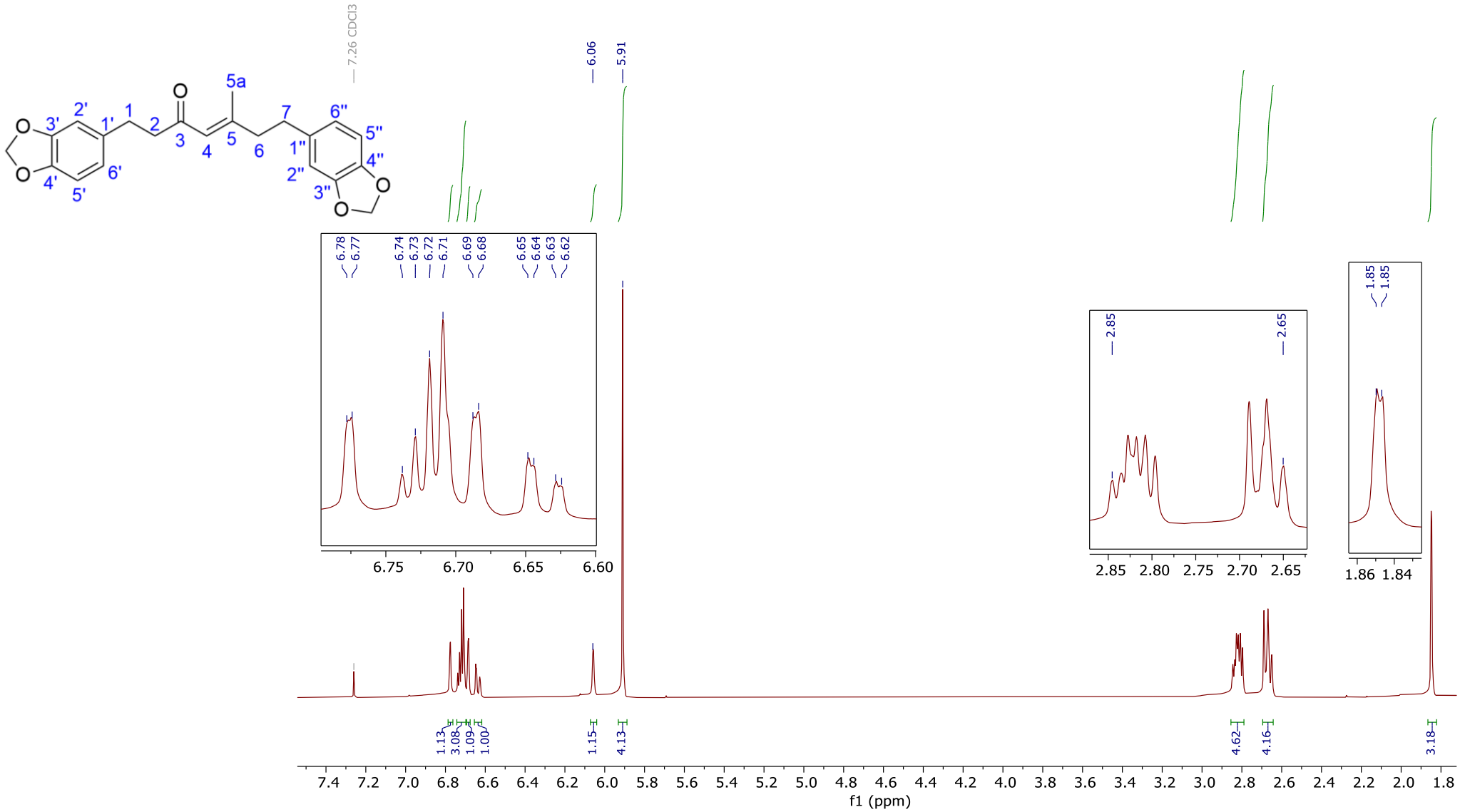


Figure S27. ^{1}H NMR spectrum (400 MHz) of compound **18** in $CDCl_3$.

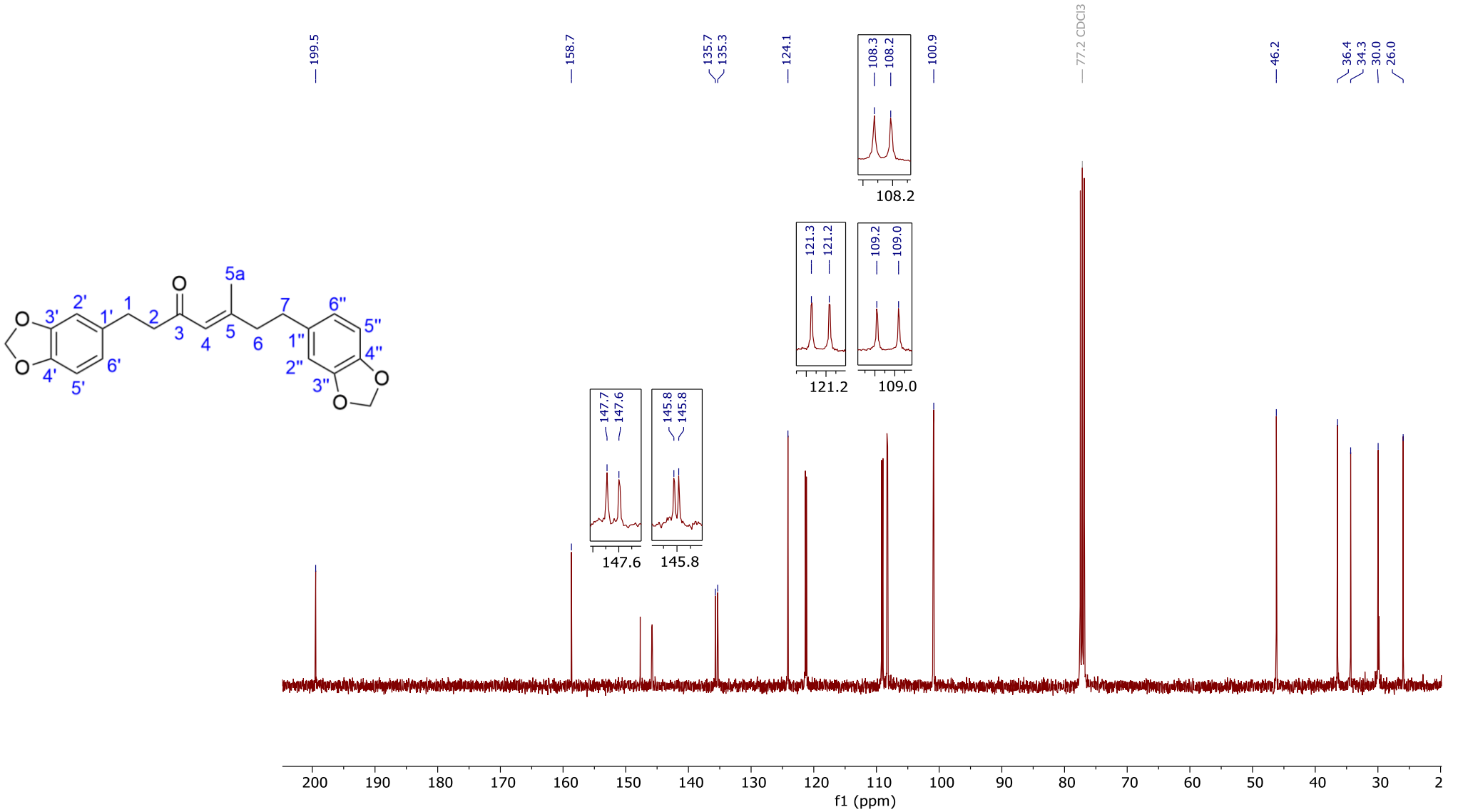


Figure S28. ^{13}C NMR spectrum (100 MHz) of compound **18** in $CDCl_3$.

2. High resolution mass spectra

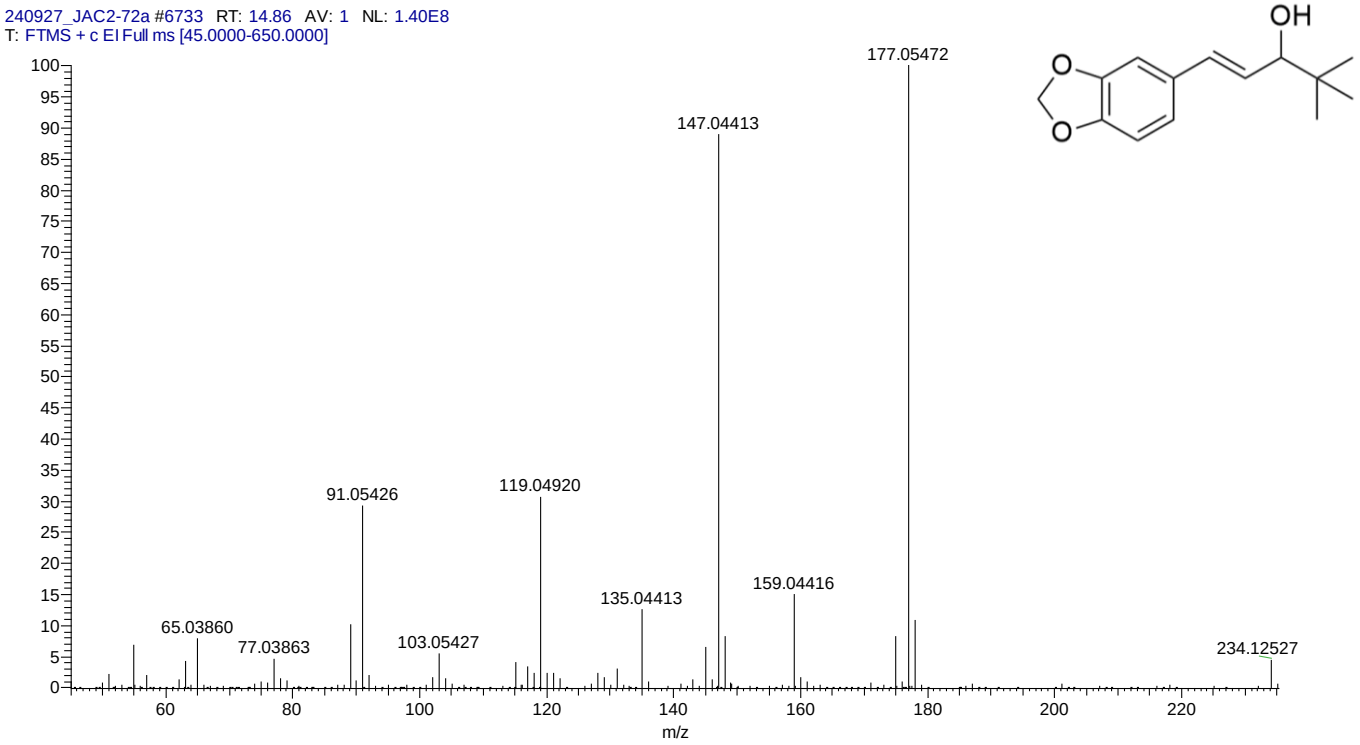


Figure S29. High resolution mass spectrum of compound **1**.

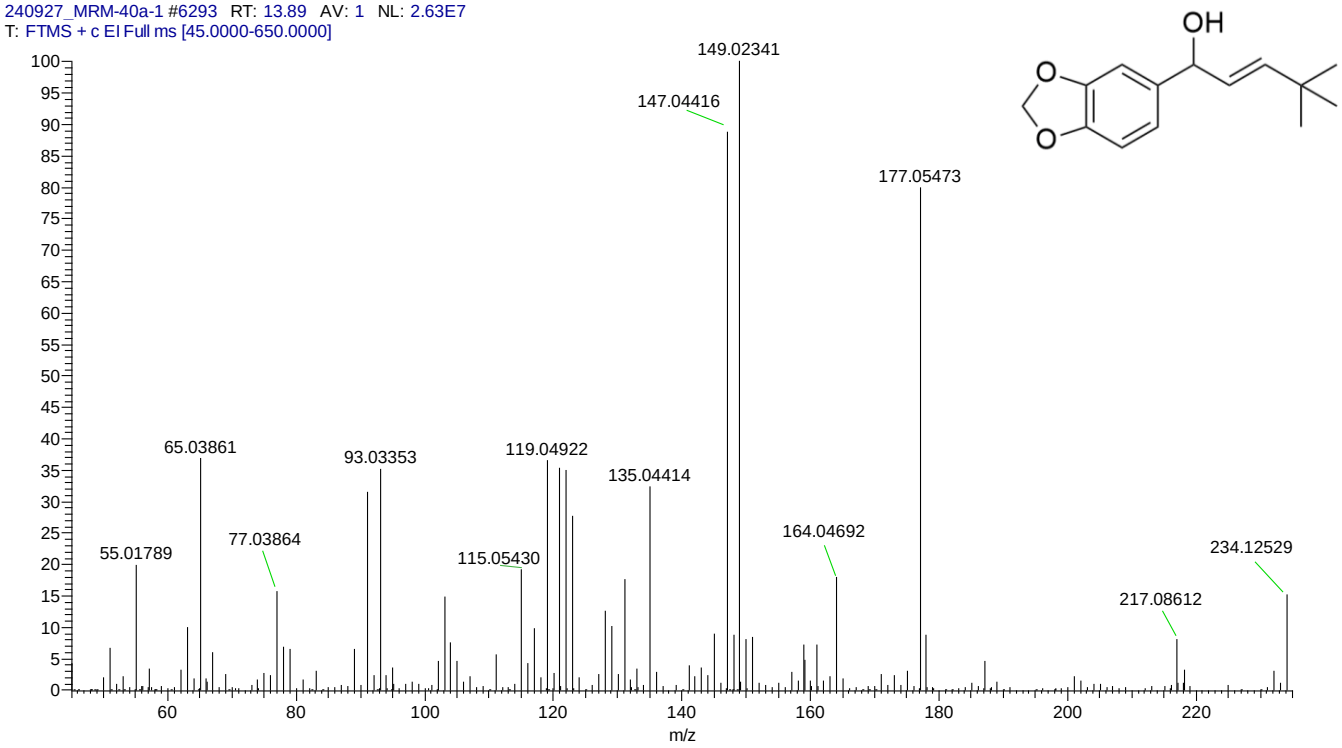


Figure S30. High resolution mass spectrum of compound **2**.

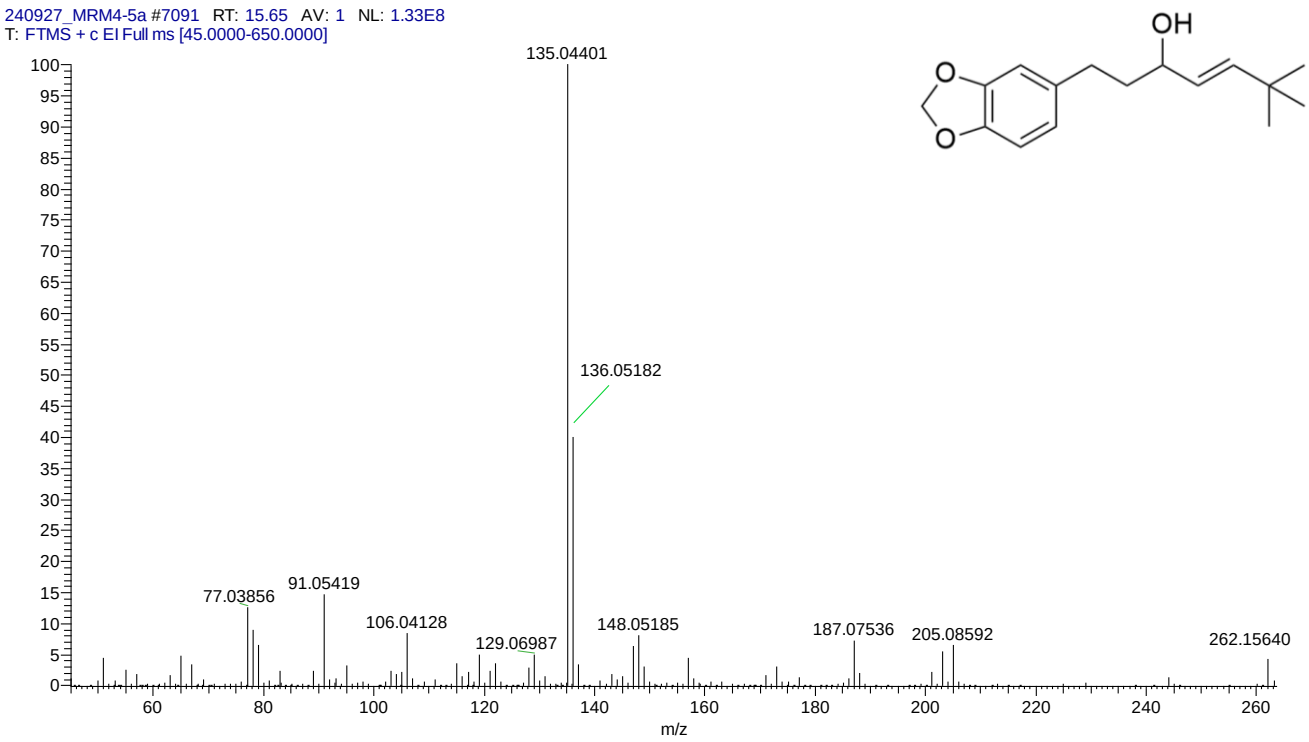


Figure S31. High resolution mass spectrum of compound **3**.

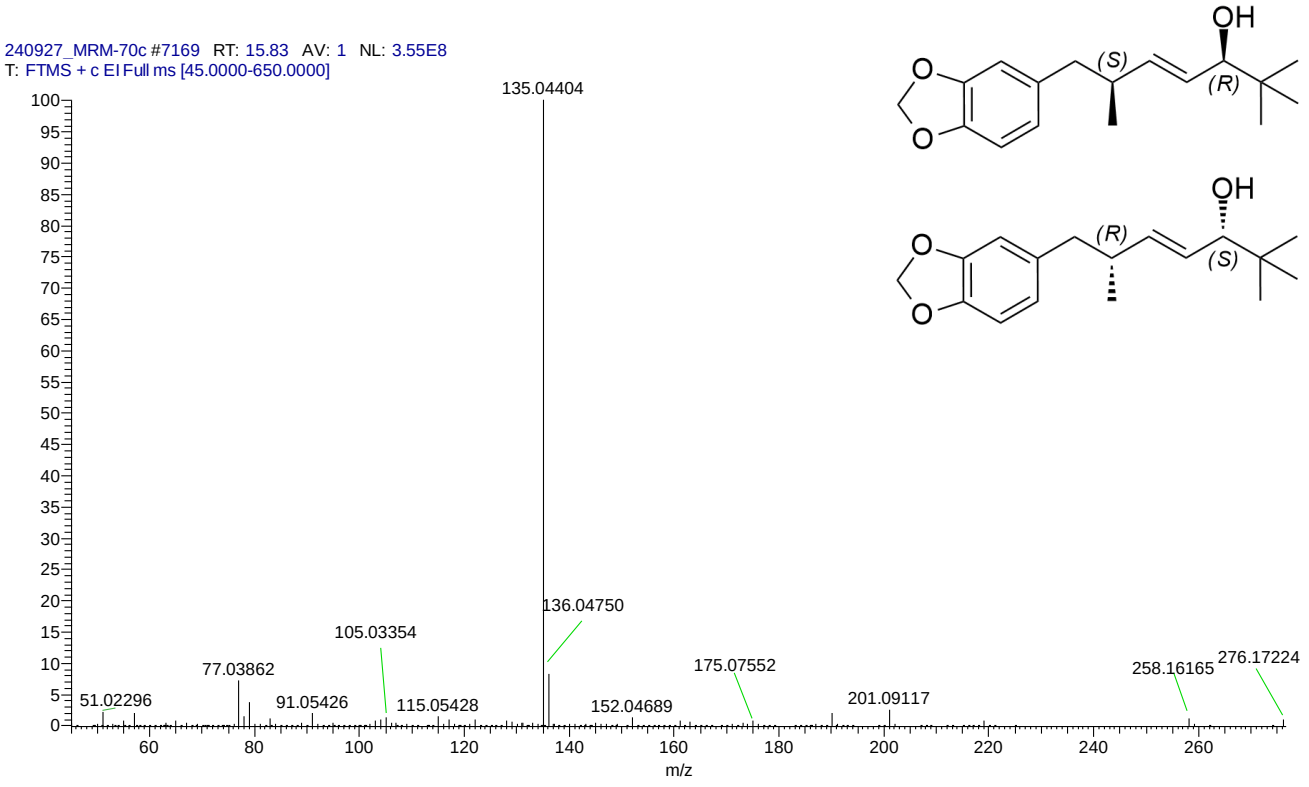


Figure S32. High resolution mass spectrum of compound (*2S*,5R**)-**4**.

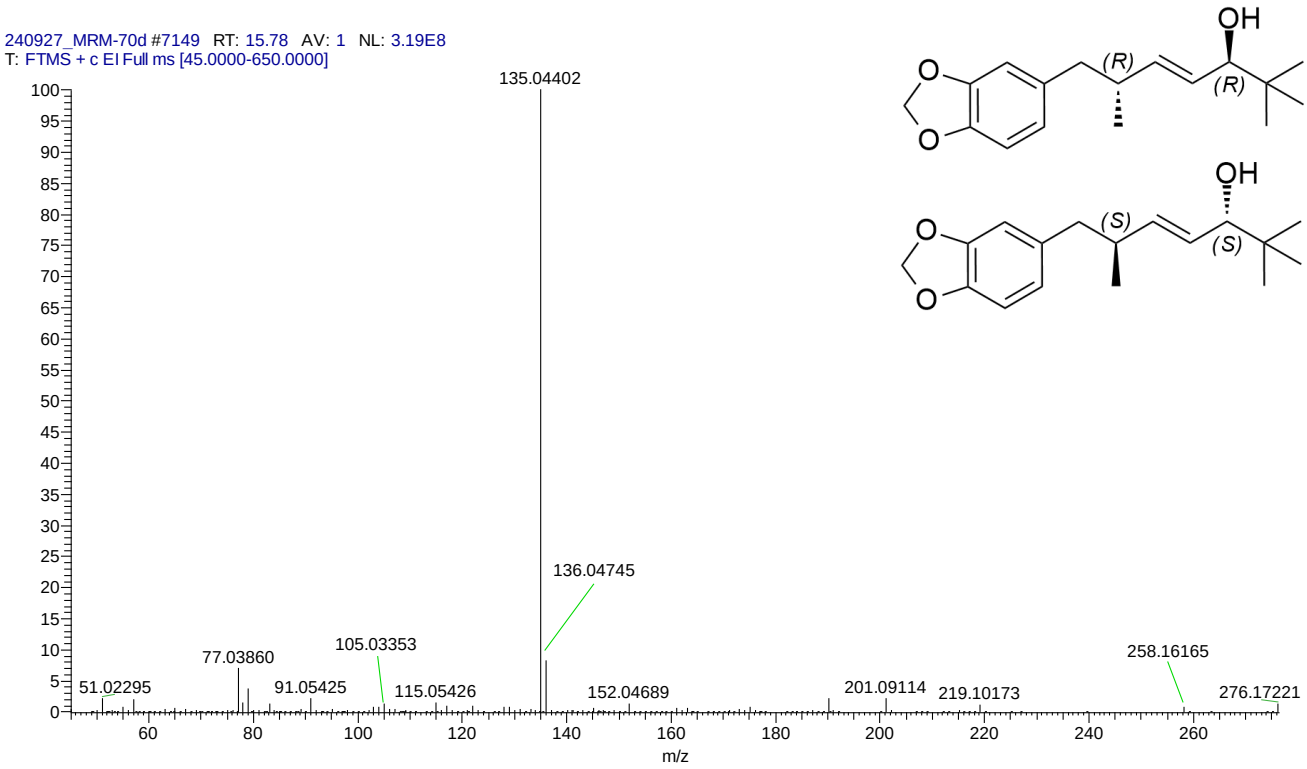


Figure S33. High resolution mass spectrum of compound (*2R*,5R**)-**4**.

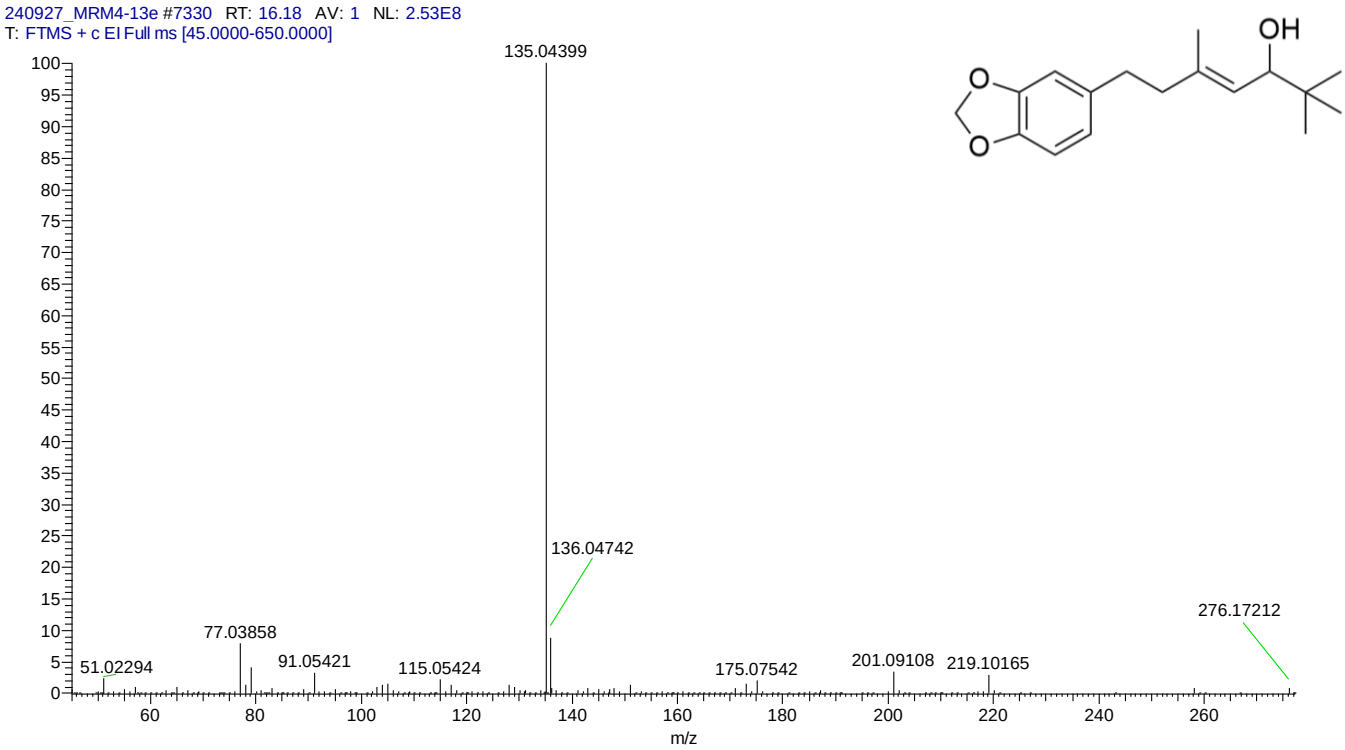


Figure S34. High resolution mass spectrum of compound **5**.

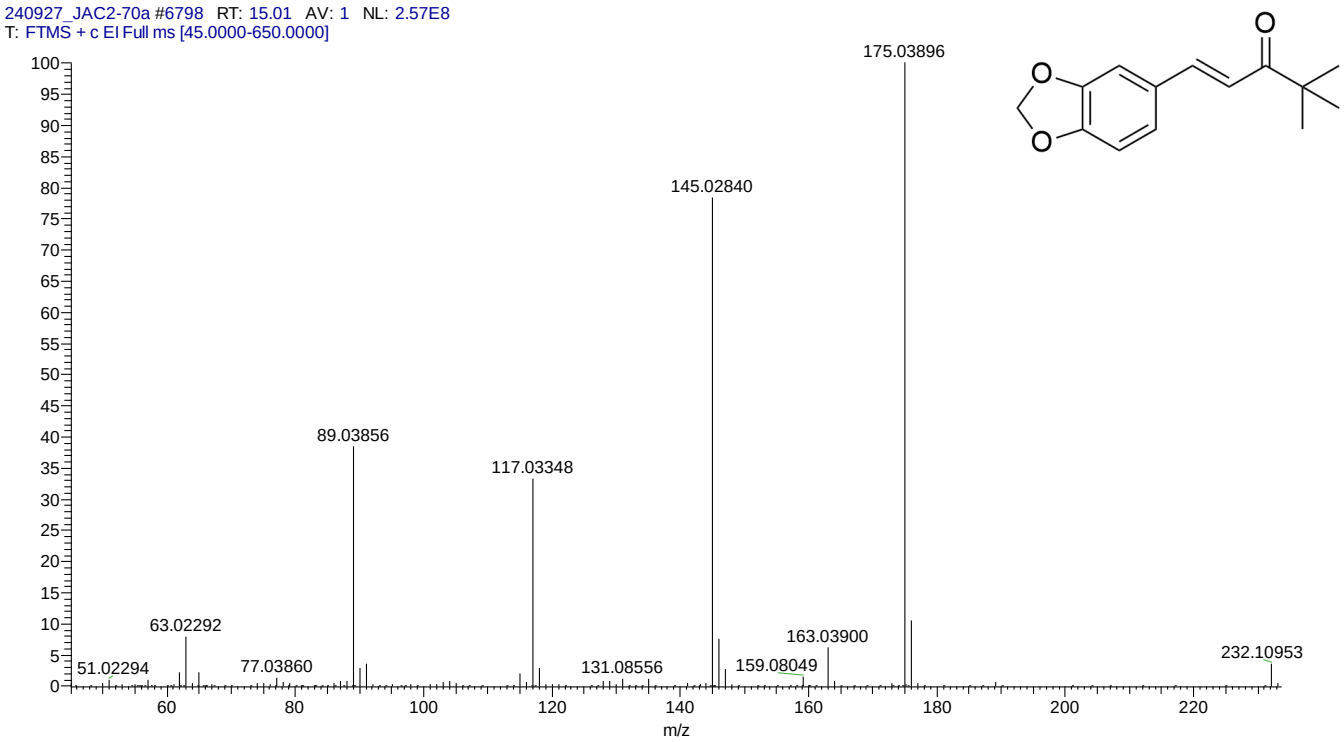


Figure S35. High resolution mass spectrum of compound **8**.

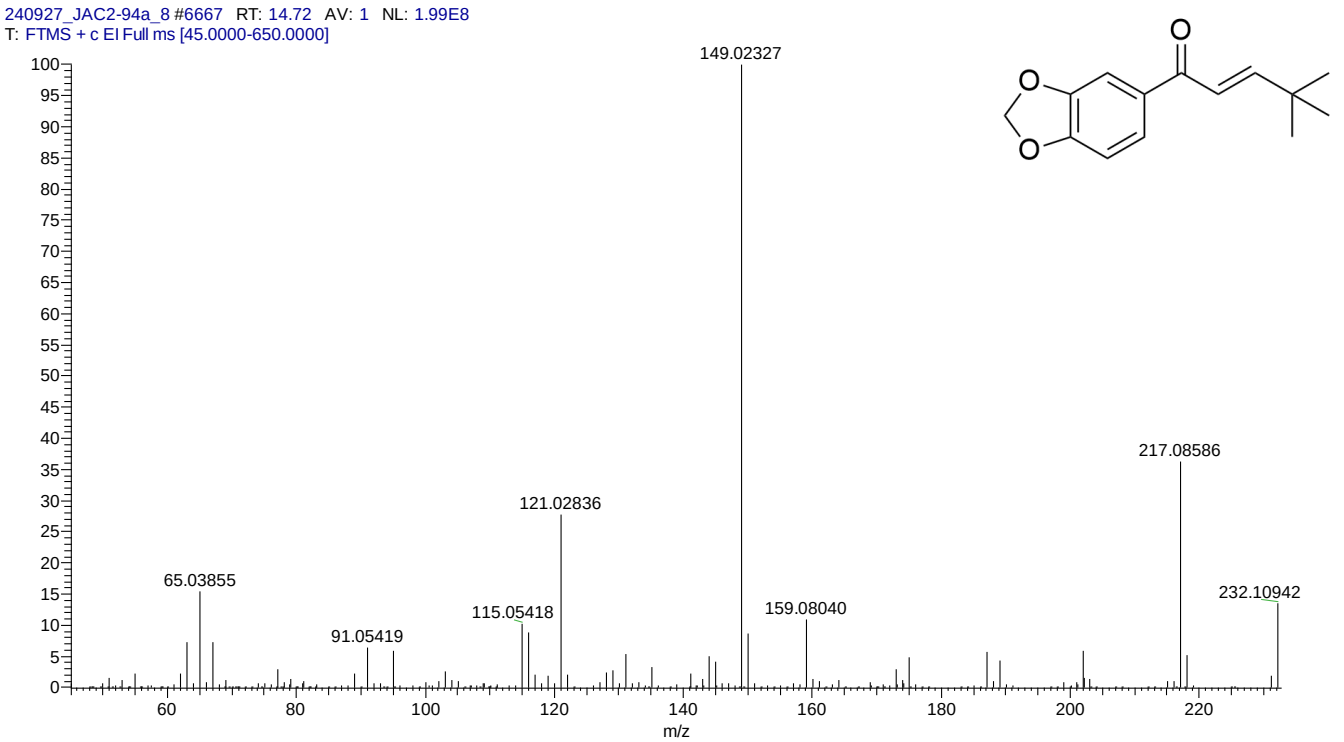


Figure S36. High resolution mass spectrum of compound **11**.

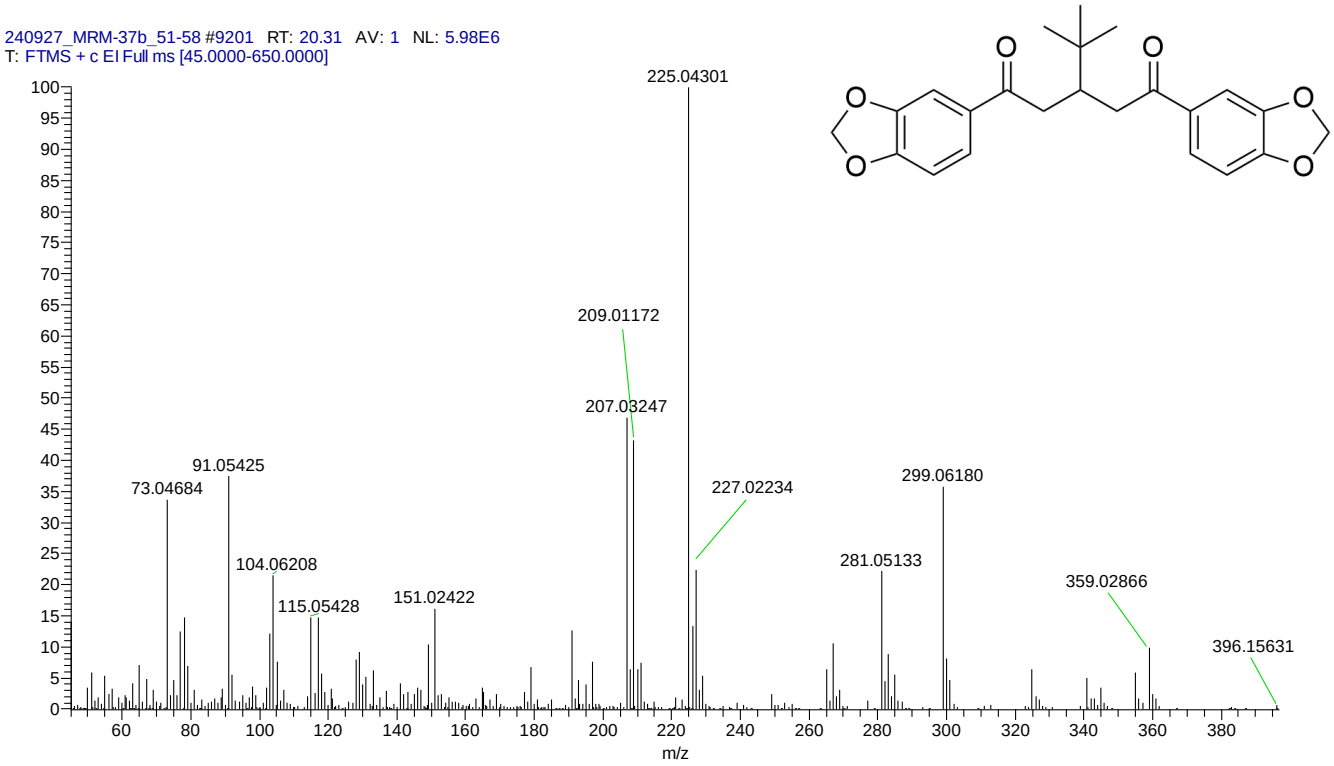


Figure S37. High resolution mass spectrum of compound **12**.

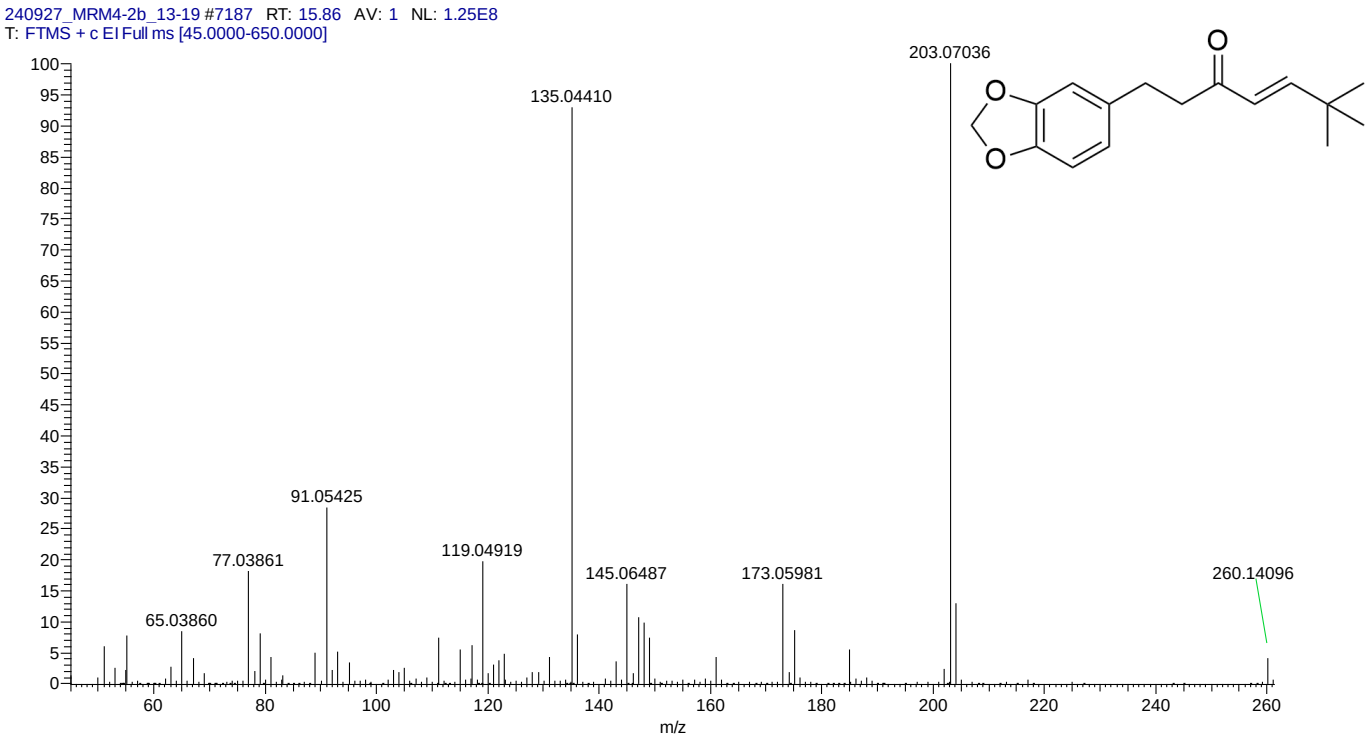


Figure S38. High resolution mass spectrum of compound **14**.

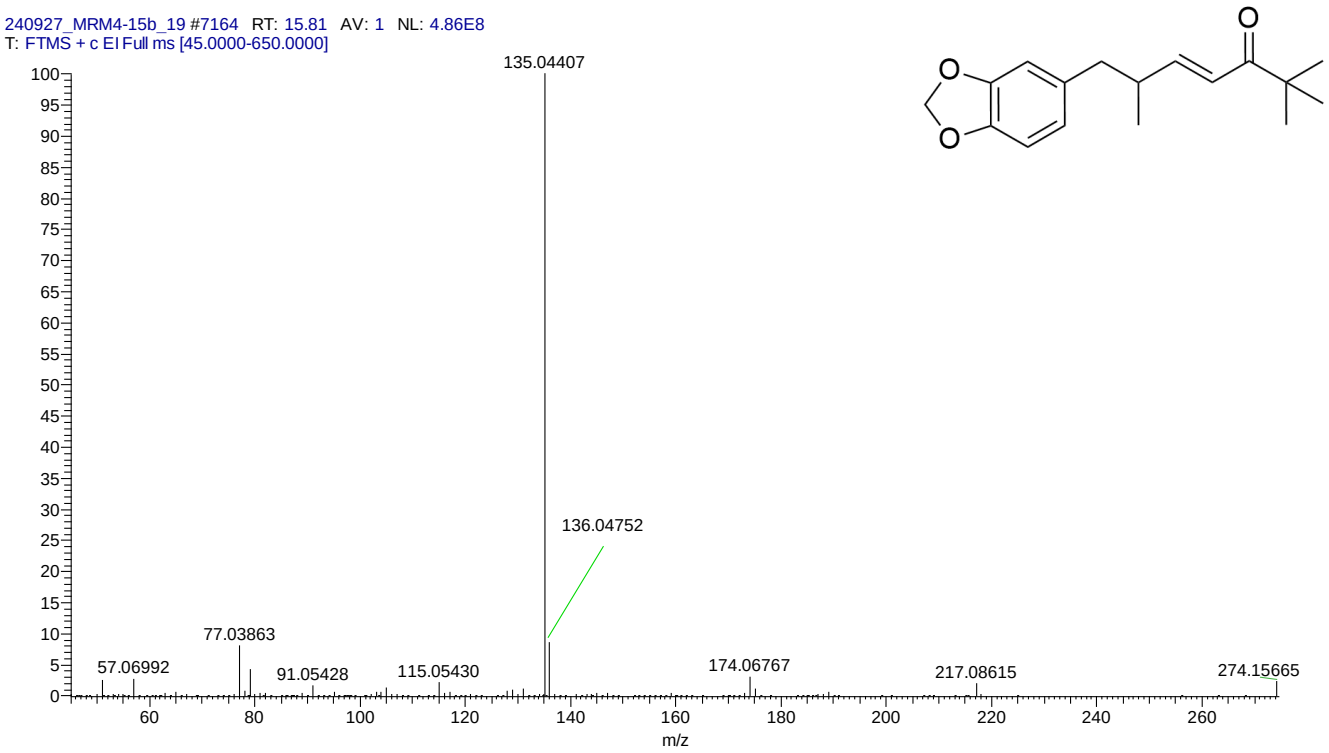


Figure S39. High resolution mass spectrum of compound **16**.

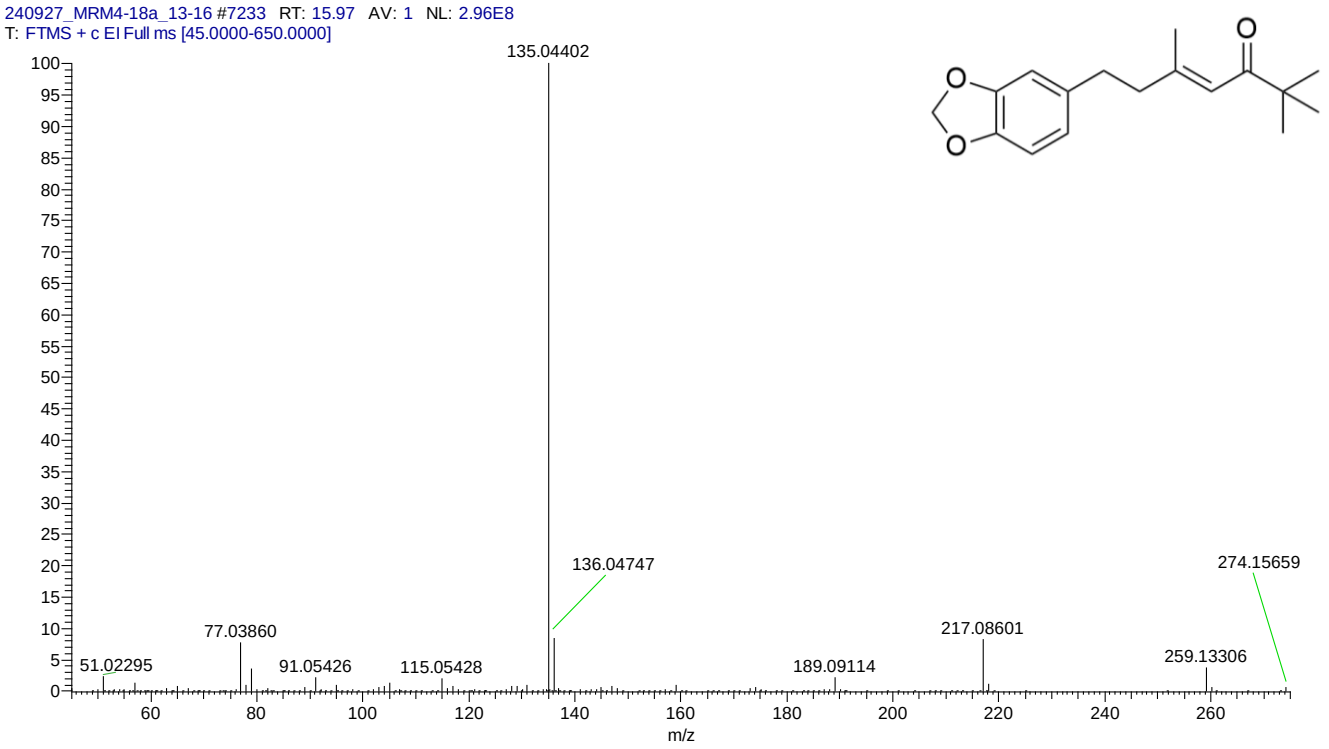


Figure S40. High resolution mass spectrum of compound **17**.

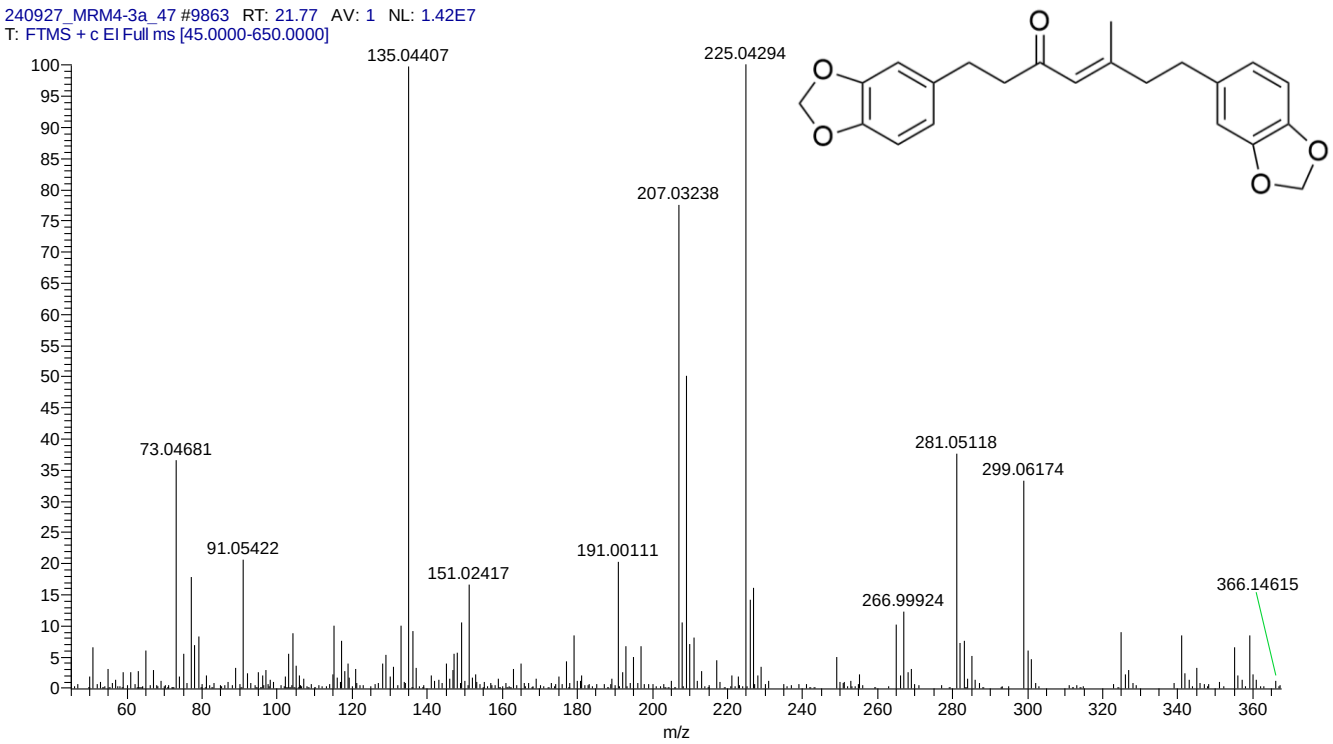


Figure S41. High resolution mass spectrum of compound **18**.

3. HPLC chromatograms

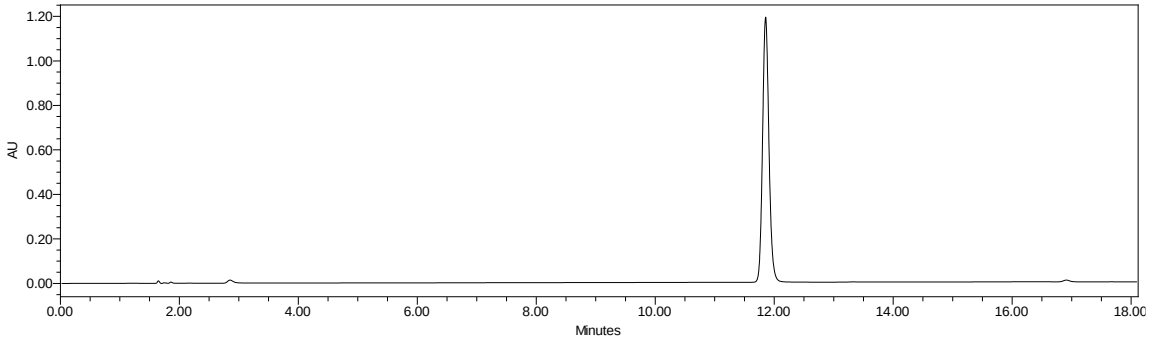


Figure S42. HPLC chromatogram at 254 nm of compound **1** (t_R = 11.9 min). Purity: 99%.

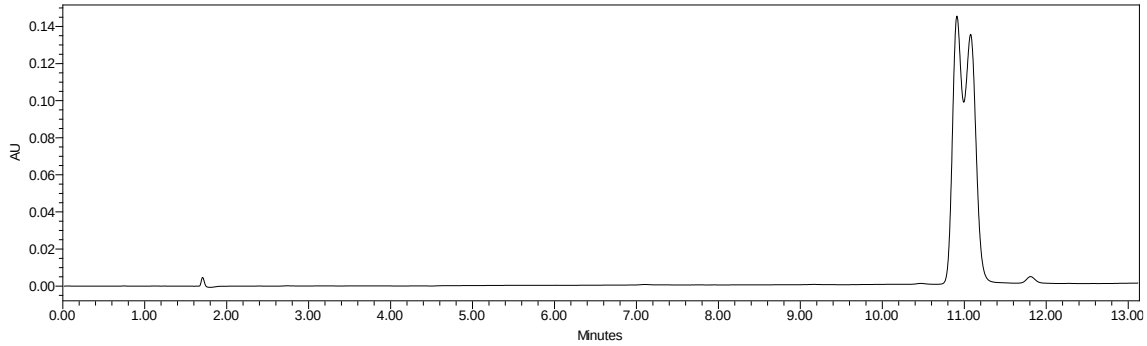


Figure S43. HPLC chromatogram at 254 nm of compound **2** (t_R = 10.9 min). The peak at t_R = 11.1 min corresponds to stiripentol (**1**).

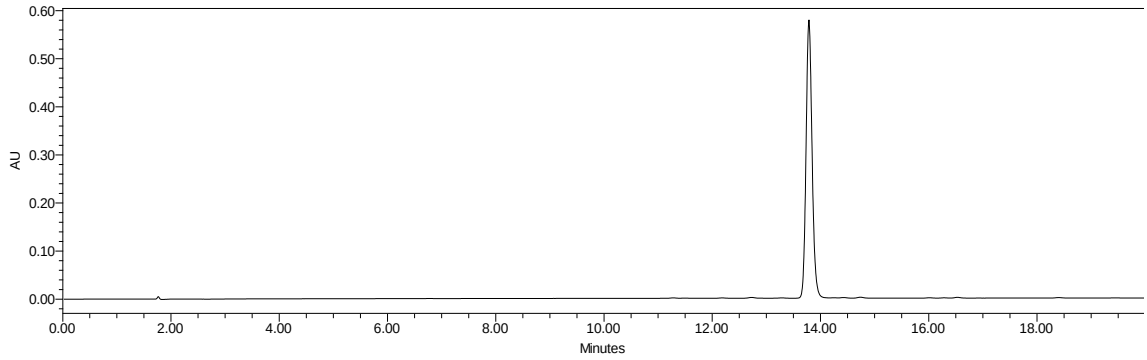


Figure S44. HPLC chromatogram at 285 nm of compound **3** (t_R = 13.8 min). Purity: >99%.

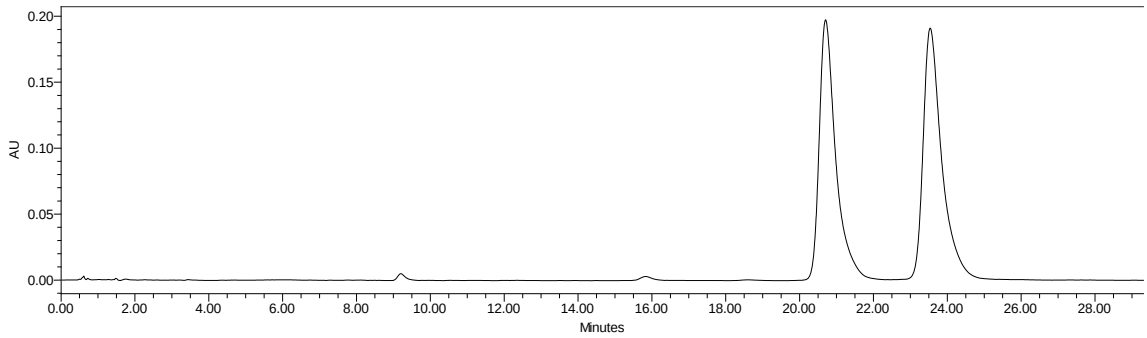


Figure S45. HPLC chromatogram at 285 nm of compound **4**. The peak at t_R = 20.7 min corresponds to diastereomer (*2S*,5R**)-**4**. The peak at t_R = 23.5 min corresponds to diastereomer (*2R*,5R**)-**4**.

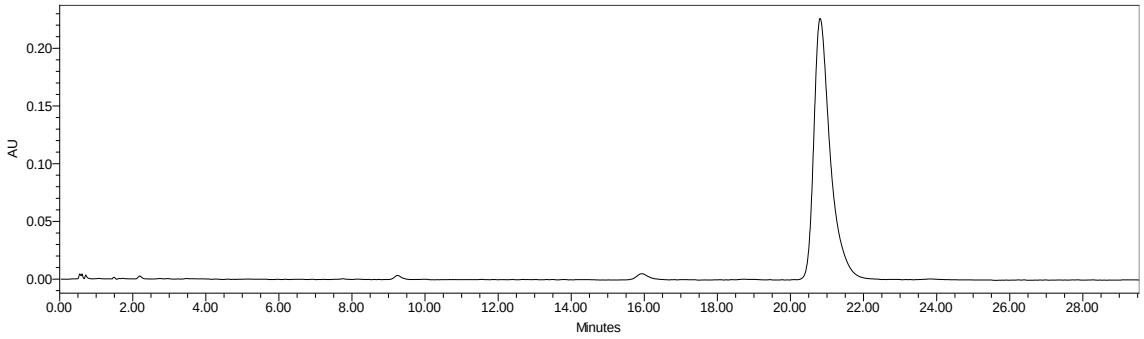


Figure S46. HPLC chromatogram at 285 nm of compound (*2S*,5R**)-**4**. Purity: 98%.

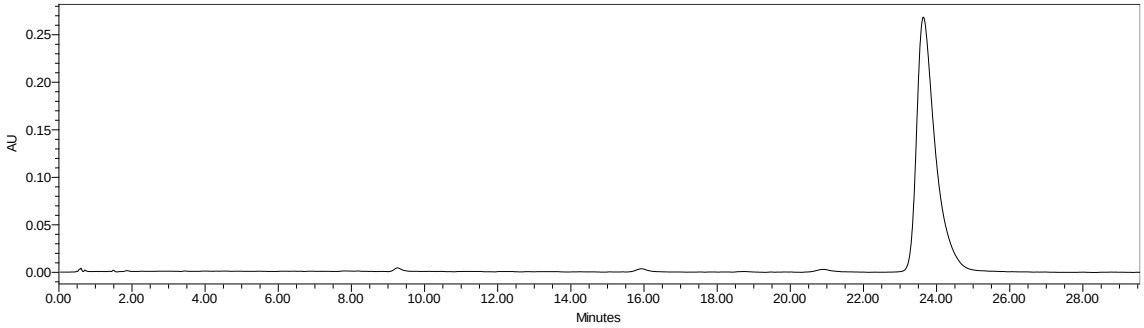


Figure S47. HPLC chromatogram at 285 nm of compound (*2R*,5R**)-**4**. Purity: 99%.

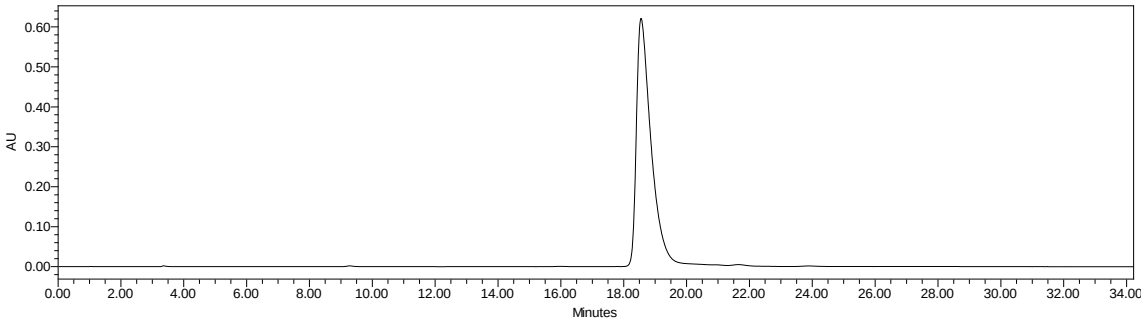


Figure S48. HPLC chromatogram at 285 nm of compound **5** (t_R = 18.6 min). Purity: >99%.

4. Chiral HPLC chromatograms.

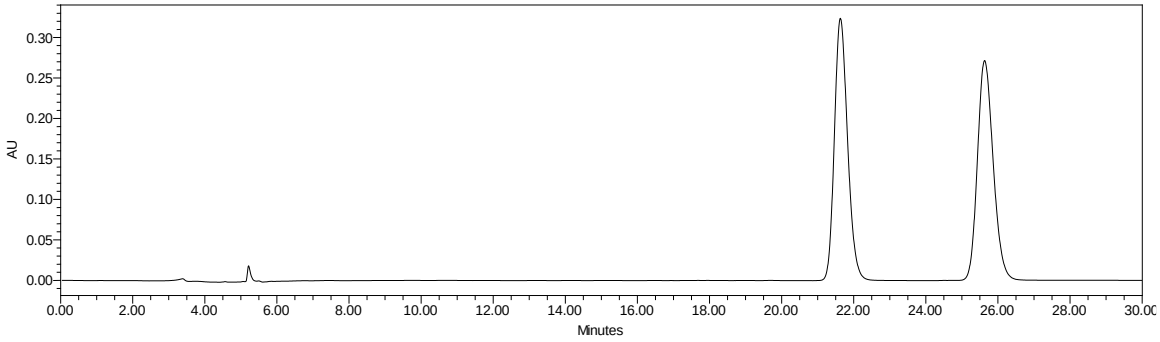


Figure S49. Chiral HPLC chromatogram at 254 nm of compound (±)-**1**. The peak at t_R = 21.6 min corresponds to enantiomer **(–)**-(*S*)-**1**. The peak at t_R = 25.6 min corresponds to enantiomer (+)-(*R*)-**1**.

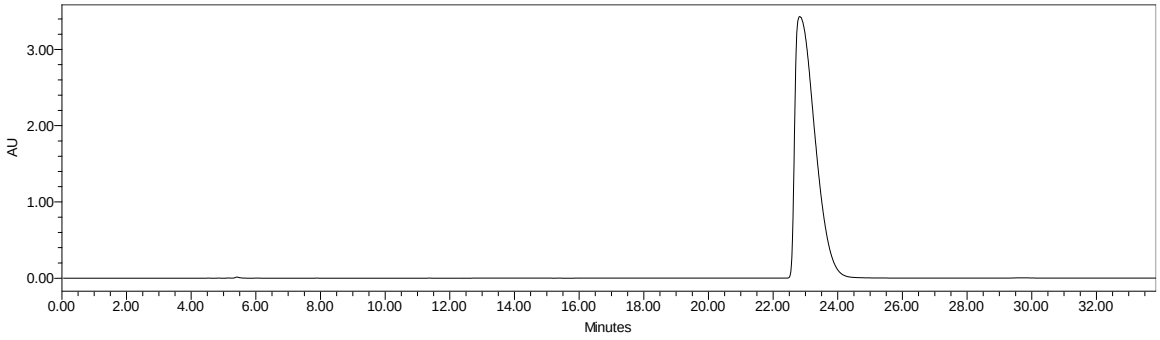


Figure S50. Chiral HPLC chromatogram at 254 nm of compound **(–)**-(*S*)-**1**. Purity: >99%.

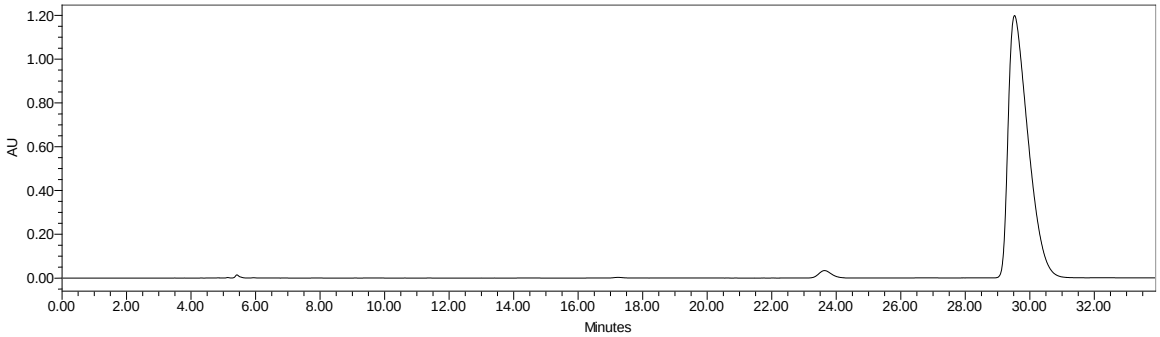


Figure S51. Chiral HPLC chromatogram at 254 nm of compound (+)-(*R*)-**1.** Purity: 98%.

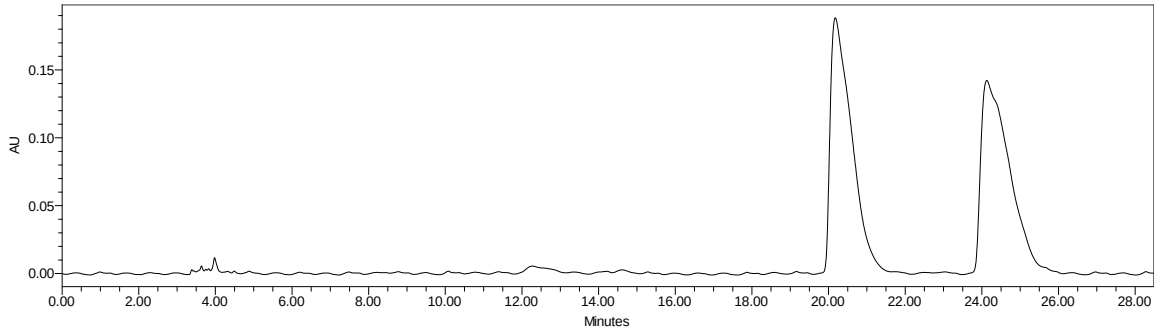


Figure S52. Chiral HPLC chromatogram at 285 nm of compound (±)-**3**. The peak at t_R = 20.2 min corresponds to enantiomer **(–)**-(*S*)-**3**. The peak at t_R = 24.1 min corresponds to enantiomer (+)-(*R*)-**3**.

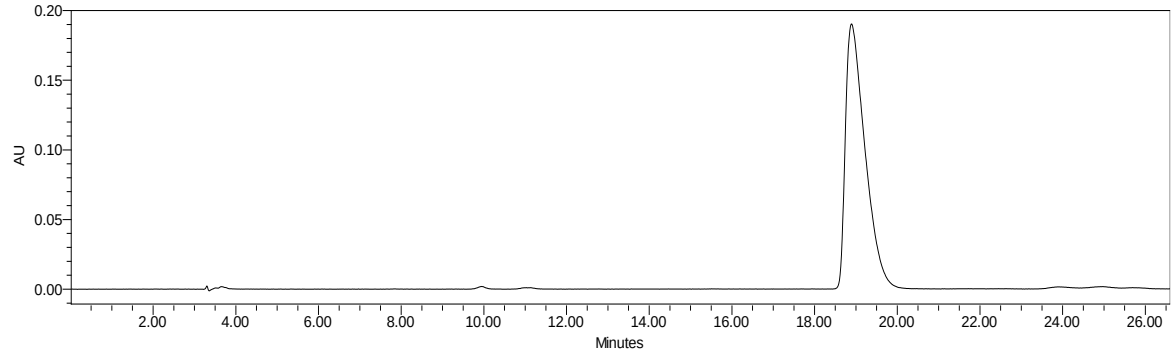


Figure S53. Chiral HPLC chromatogram at 285 nm of compound **(–)**-(*S*)-**3**. Purity: >99%.

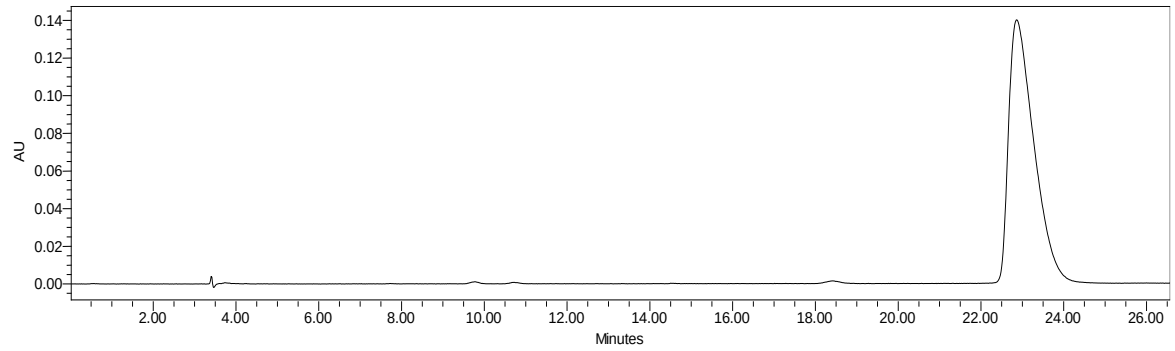


Figure S54. Chiral HPLC chromatogram at 285 nm of compound (+)-(*R*)-**3**. Purity: >99%.

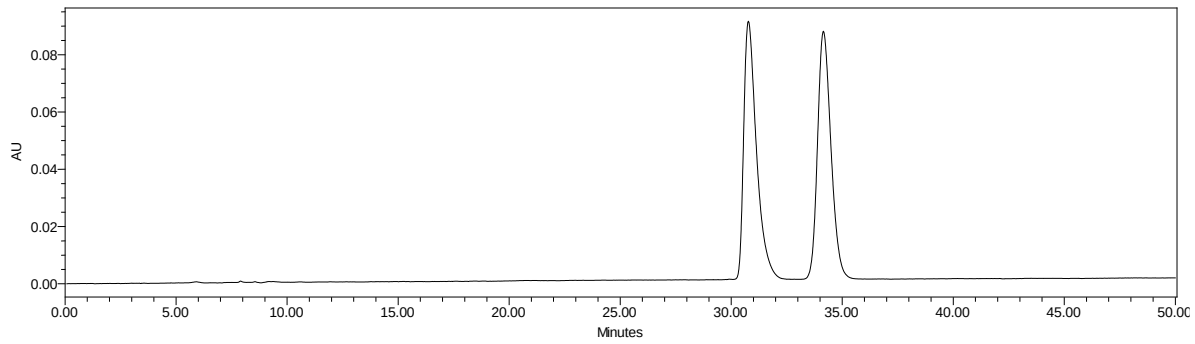


Figure S55. Chiral HPLC chromatogram at 285 nm of compound (±)-(*2S*,5R**)-**4**. The peak at t_R = 30.8 min corresponds to enantiomer **(–)**-(*2R,5S*)-**4**. The peak at t_R = 34.2 min corresponds to enantiomer (+)-(*2S,5R*)-**4**.

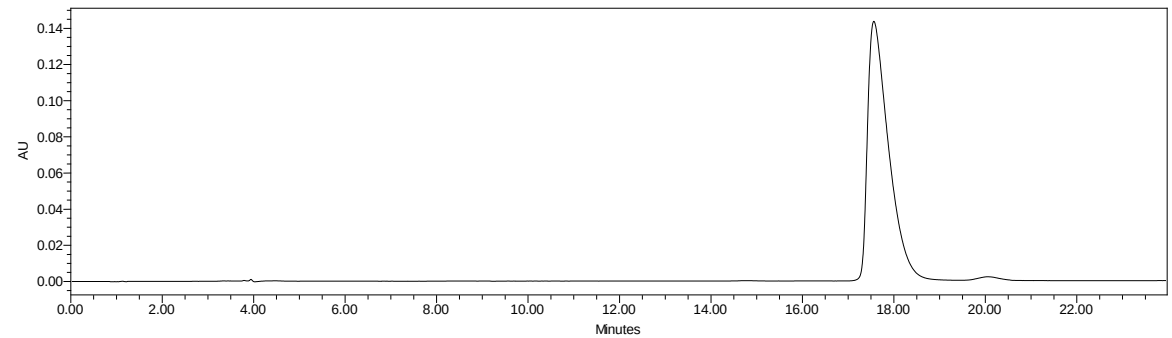


Figure S56. Chiral HPLC chromatogram at 285 nm of compound **(–)**-(*2R,5S*)-**4**. Purity: 98%.

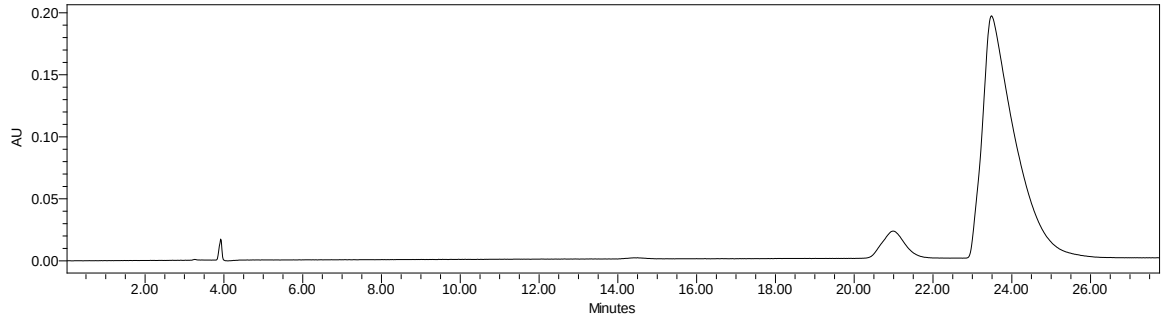


Figure S57. Chiral HPLC chromatogram at 285 nm of compound (+)-(*2S,5R*)-**4**. Purity: 95%.

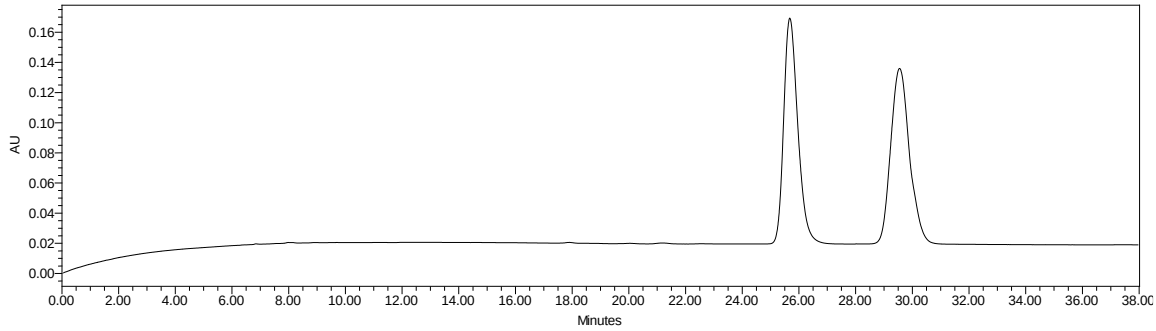


Figure S58. Chiral HPLC chromatogram at 285 nm of compound (±)-(*2R*,5R**)-**4**. The peak at t_R = 25.7 min corresponds to enantiomer (+)-(*2R,5R*)-**4**. The peak at t_R = 29.6 min corresponds to enantiomer (**–**)-(*2S,5S*)-**4**.

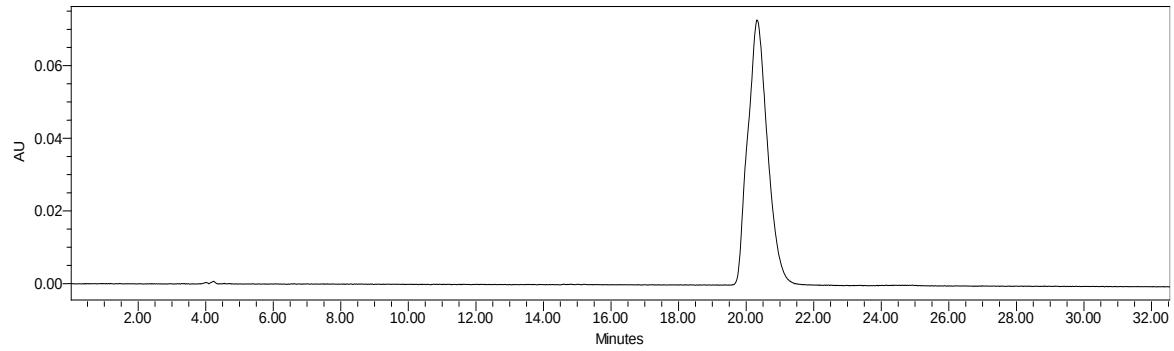


Figure S59. Chiral HPLC chromatogram at 285 nm of compound (+)-(*2R,5R*)-**4**. Purity: 99%.

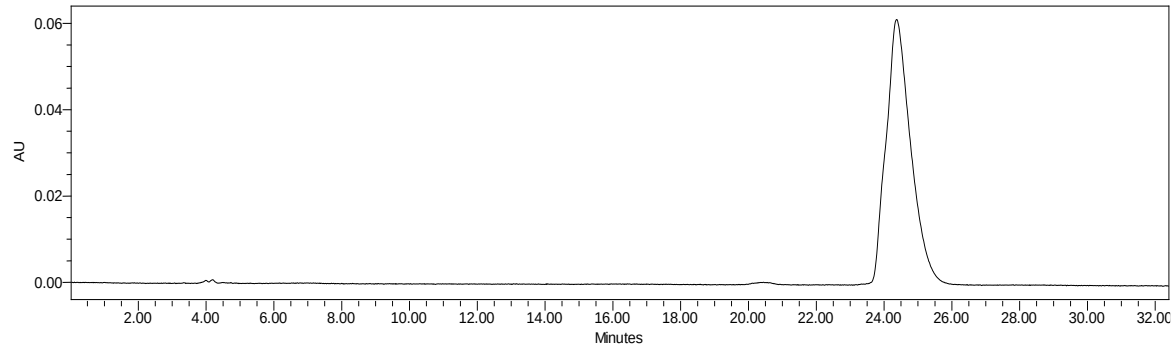


Figure S60. Chiral HPLC chromatogram at 285 nm of compound (**–**)-(*2S,5S*)-**4**. Purity: 99%.

5. DFT-optimized lowest energy conformers

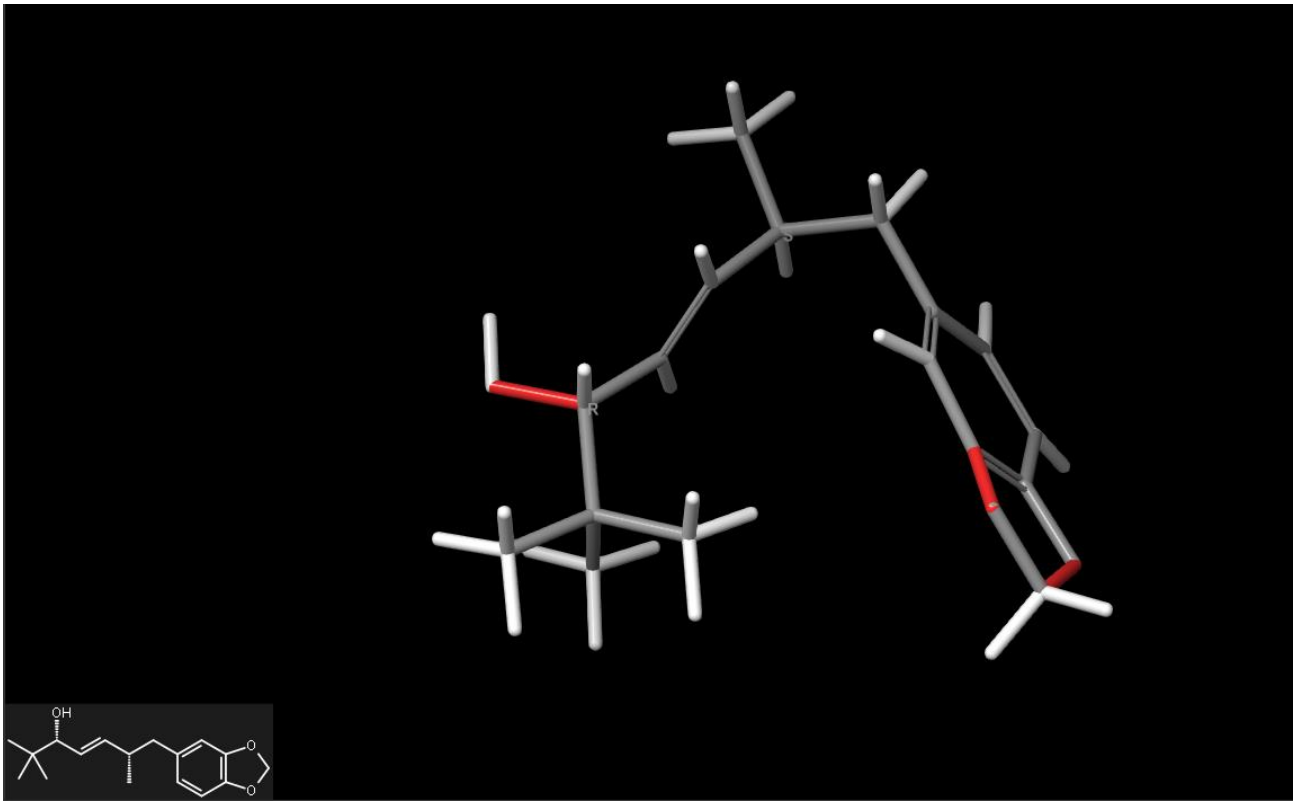


Figure S61. DFT-optimized lowest energy conformer of (*2R,5S*)-**4**.

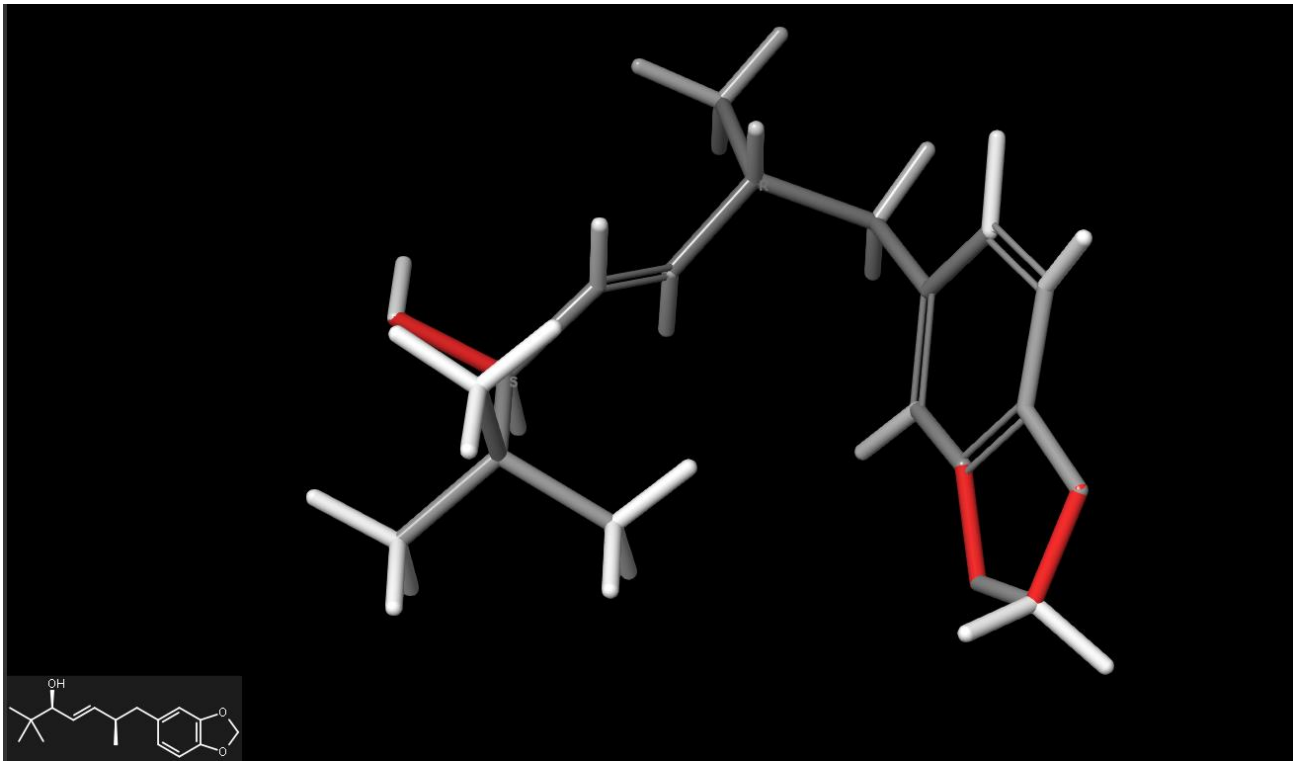


Figure S62. DFT-optimized lowest energy conformer of (*2S,5R*)-**4**.

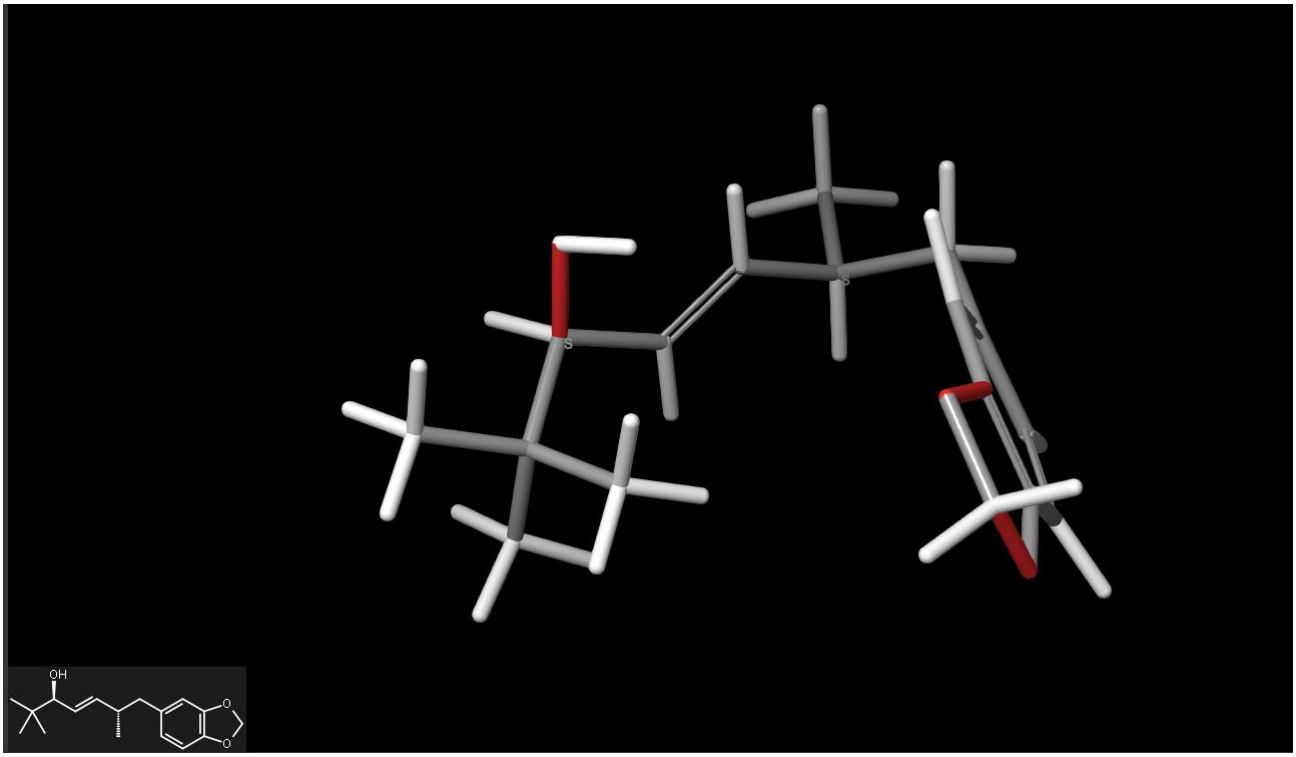


Figure S63. DFT-optimized lowest energy conformer of (2*S*,5*S*)-**4**.

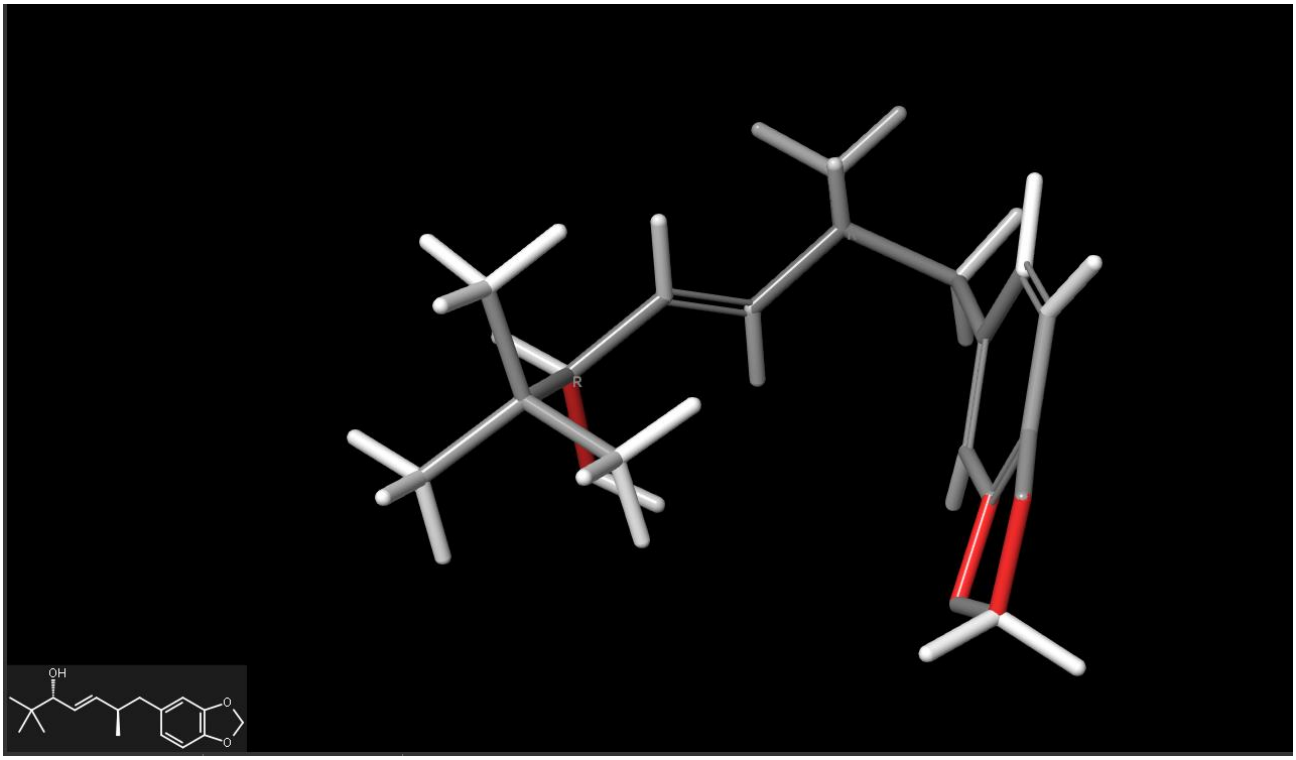


Figure S64. DFT-optimized lowest energy conformer of (2*R*,5*R*)-**4**.

6. Dose response curves against *h*LDHA

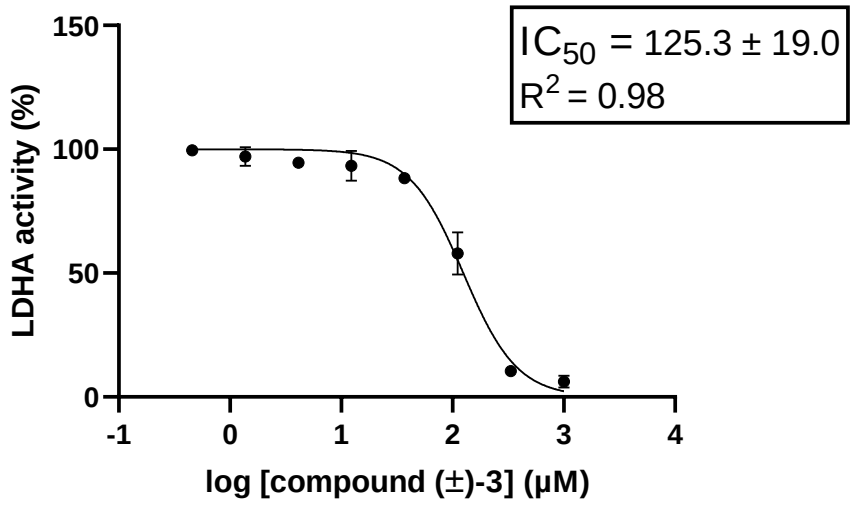


Figure S65. *h*LDHA inhibition curve of compound (±)-**3** (mean ± SD of n = 3 replicates).

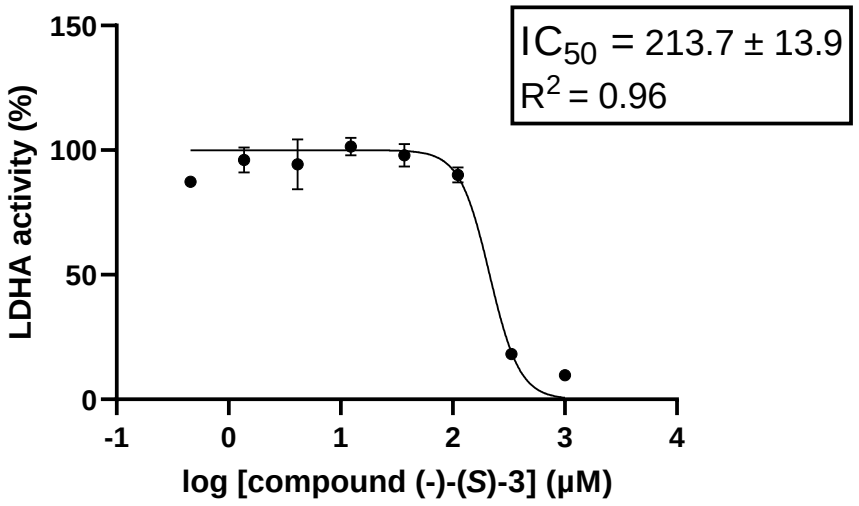


Figure S66. *h*LDHA inhibition curve of compound (**–**)-(*S*)-**3** (mean ± SD of n = 3 replicates).

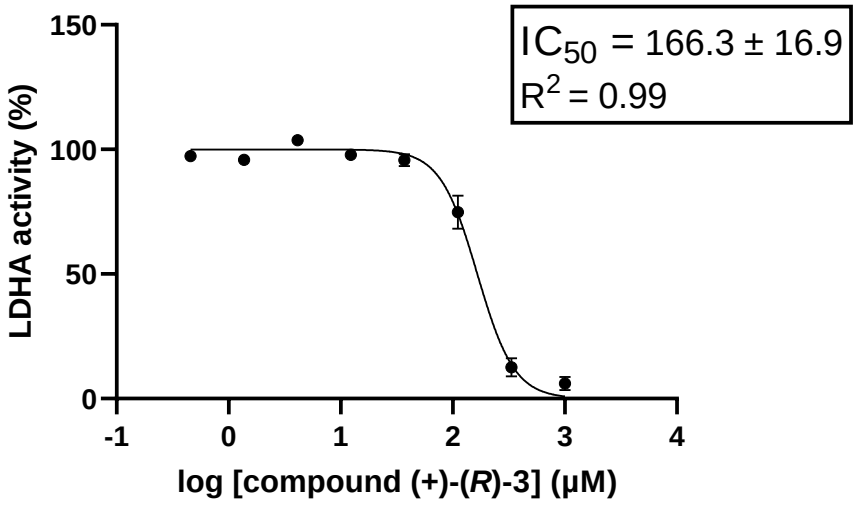


Figure S67. *h*LDHA inhibition curve of compound (+)-(*R*)-**3** (mean ± SD of n = 3 replicates).

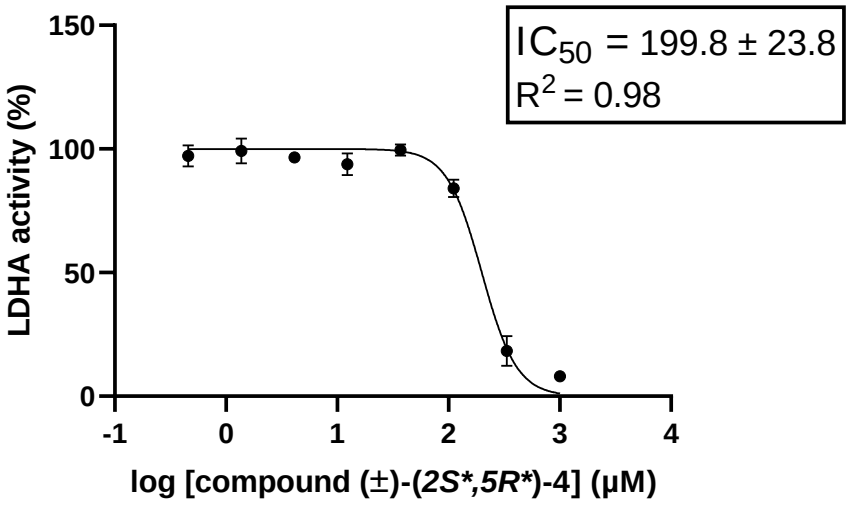


Figure S68. *h*LDHA inhibition curve of compound (±)-(2*S**,5*R**)-**4** (mean ± SD of n = 3 replicates).

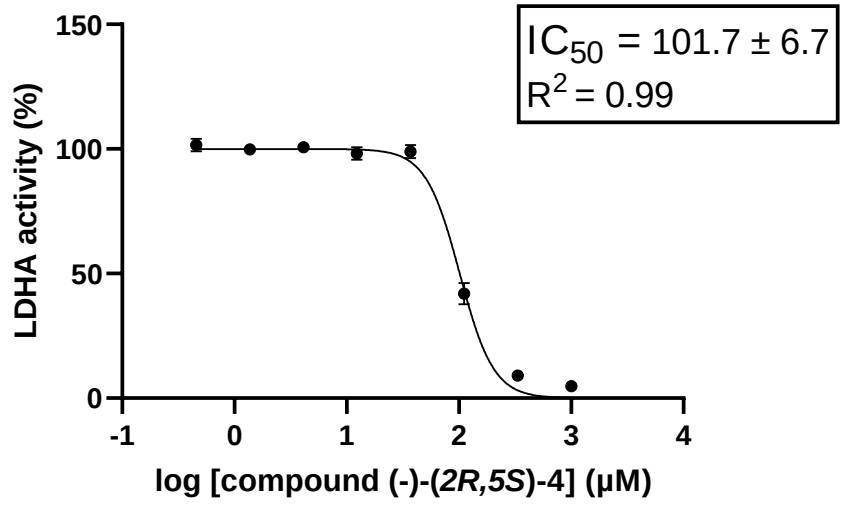


Figure S69. *h*LDHA inhibition curve of compound (–)-(2*R*,5*S*)-**4** (mean ± SD of n = 3 replicates).

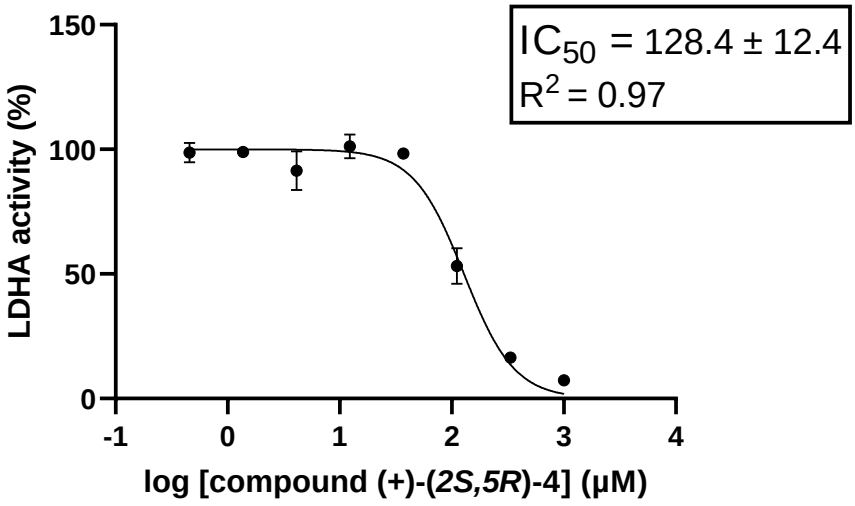


Figure S70. *h*LDHA inhibition curve of compound (+)-(2*S*,5*R*)-**4** (mean ± SD of n = 3 replicates).

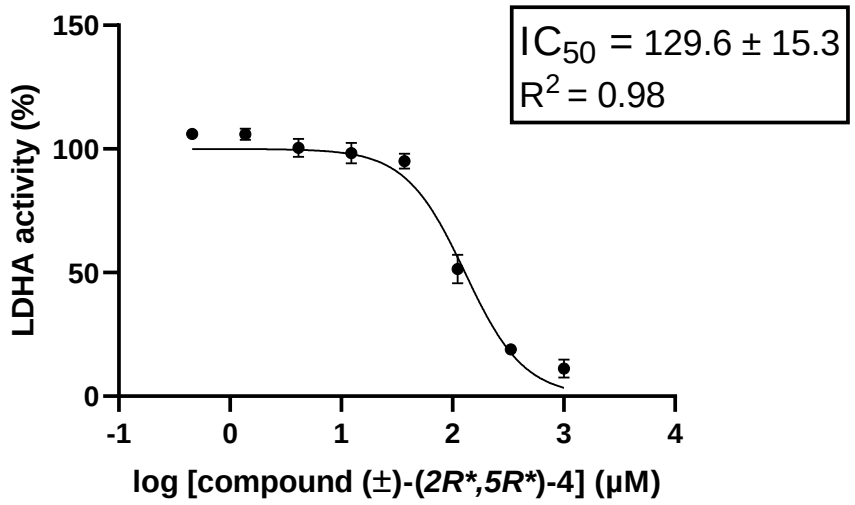


Figure S71. *h*LDHA inhibition curve of compound (±)-(*2R*,5R**)-**4** (mean ± SD of n = 3 replicates).

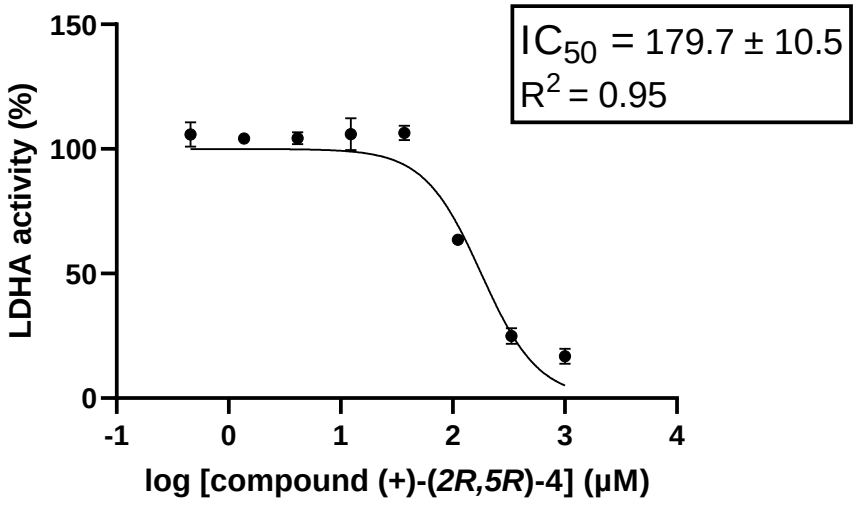


Figure S72. *h*LDHA inhibition curve of compound (+)-(*2R,5R*)-**4** (mean ± SD of n = 3 replicates).

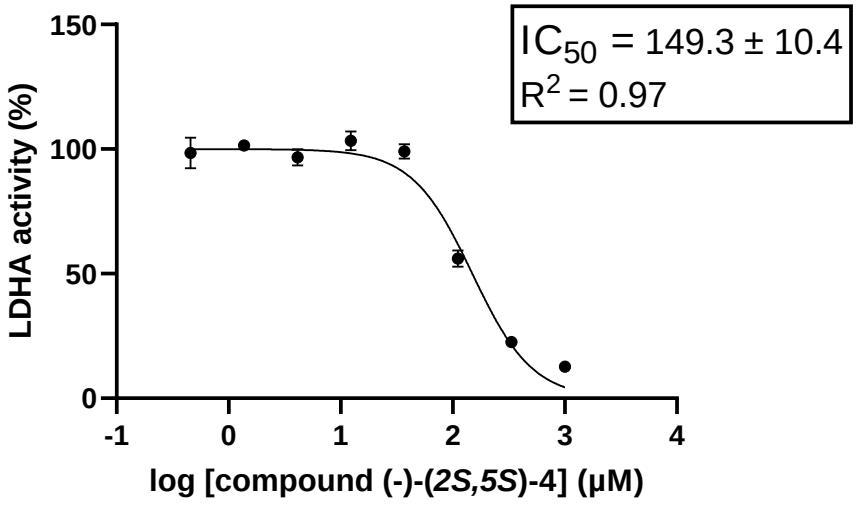


Figure S73. *h*LDHA inhibition curve of compound (–)-(*2S,5S*)-**4** (mean ± SD of n = 3 replicates).

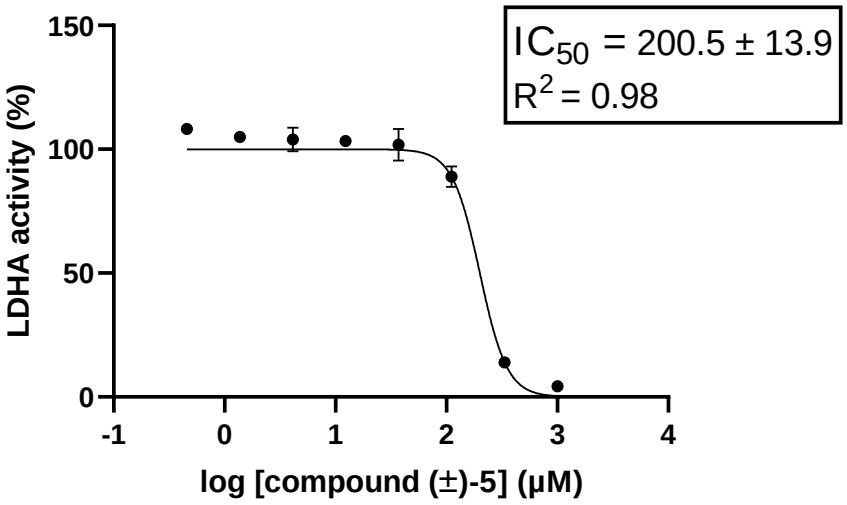


Figure S74. *h*LDHA inhibition curve of compound (±)-**5** (mean ± SD of n = 3 replicates).