

Supplementary Materials

Modulation of glial responses by furanocembranolides: leptolide diminishes microglial inflammation in vitro and ameliorates gliosis in vivo in a mouse model of obesity and insulin resistance

Miriam Corraliza-Gómez ^{1,†}, Amalia B. Gallardo ^{2,3,†}, Ana R. Díaz-Marrero ^{2,‡}, José M. de la Rosa ^{2,‡}, Luis D'Croz ^{4,5}, José Darías ², Eduardo Arranz ¹, Irene Cózar-Castellano ^{1,6}, María D. Ganfornina ^{1,*} and Mercedes Cueto ^{2,*}

¹ Instituto de Biología y Genética Molecular, Universidad de Valladolid-CSIC, 47003 Valladolid, Spain

² Instituto de Productos Naturales y Agrobiología (IPNA-CSIC). Avenida Astrofísico F. Sánchez, 3, 38206 La Laguna, Tenerife, Spain

³ Facultad de Ciencias, Universidad de Magallanes, Departamento de Ciencias y Recursos Naturales, Avenida Bulnes 01855, Punta Arenas, Chile

⁴ Departamento de Biología Marina y Limnología, Universidad de Panamá, Panama

⁵ Smithsonian Tropical Research Institute, STRI, Box 0843-03092, Balboa, Panama

⁶ Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Madrid, Spain

* Correspondence: opabinia@ibgm.uva.es; Tel.: +34-983-184-814; mcueto@ipna.csic.es; Tel.: +34-922-250-144

† These authors contributed equally to this work

‡ Present address: Instituto Universitario de Bio-Organica Antonio González (IUBO AG), Universidad de La Laguna (ULL), Avda. Astrofísico F. Sánchez 2, 38206 La Laguna, Tenerife, Spain[‡] Present address: Departamento de Bioquímica, Microbiología, Biología Celular and Genética, Facultad de Farmacia, Universidad de La Laguna (ULL), 38206 La Laguna, Tenerife, Spain

TABLE OF CONTENTS.

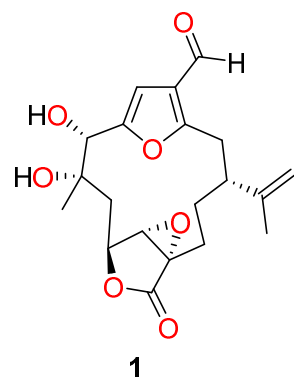
Figure S1. ¹ H NMR spectrum of 1 in CDCl ₃	S2
Figure S2. ¹³ C NMR spectrum of 1 in CDCl ₃	S3
Figure S3. ¹ H NMR spectrum of 2 in CDCl ₃	S4
Figure S4. ¹³ C NMR spectrum of 2 in CDCl ₃	S5
Figure S5. ¹ H NMR spectrum of 3 in CDCl ₃	S6
Figure S6. ¹³ C NMR spectrum of 3 in CDCl ₃	S7
Figure S7. ¹ H NMR spectrum of 4 in CDCl ₃	S8
Figure S8. ¹³ C NMR spectrum of 4 in CDCl ₃	S9
Figure S9. ¹ H NMR spectrum of 5 in CDCl ₃	S10
Figure S10. ¹³ C NMR spectrum of 5 in CDCl ₃	S11
Figure S11. ¹ H NMR spectrum of 6 in CDCl ₃	S12
Figure S12. ¹³ C NMR spectrum of 6 in CDCl ₃	S13
Figure S13. Minimized structures and selected NOE effects of 2 , 4 and 6 .	S14
Figure S14. Effects of furanocembranolides on microglial cell viability and inflammation.	S15

Chemical structure of compound **1** is shown in the top left corner. The ^1H NMR spectrum (CDCl₃) is displayed below, with the x-axis labeled "f1 (ppm)" ranging from 1.0 to 10.0. The spectrum shows several peaks, with integration values indicated above them.

Key peaks and integration values:

- 9.85 (s, 1H, integration 0.02)
- 6.69 (s, 1H, integration 0.02)
- 5.28 (d, 1H, integration 0.02)
- 5.10 (d, 1H, integration 0.02)
- 4.97 (d, 1H, integration 0.02)
- 4.86 (d, 1H, integration 0.02)
- 4.85 (d, 1H, integration 0.02)
- 4.84 (d, 1H, integration 0.02)
- 4.83 (d, 1H, integration 0.02)
- 3.70 (m, 1H, integration 0.02)
- 3.27 (m, 1H, integration 0.02)
- 3.24 (m, 1H, integration 0.02)
- 3.22 (m, 1H, integration 0.02)
- 3.05 (m, 1H, integration 0.02)
- 3.02 (m, 1H, integration 0.02)
- 3.00 (m, 1H, integration 0.02)
- 2.97 (m, 1H, integration 0.02)
- 2.96 (m, 1H, integration 0.02)
- 2.52 (m, 1H, integration 0.02)
- 2.50 (m, 1H, integration 0.02)
- 2.49 (m, 1H, integration 0.02)
- 2.47 (m, 1H, integration 0.02)
- 1.88 (m, 1H, integration 0.02)
- 1.87 (m, 1H, integration 0.02)
- 1.85 (m, 1H, integration 0.02)
- 1.84 (m, 1H, integration 0.02)
- 1.77 (m, 1H, integration 0.02)
- 1.54 (m, 1H, integration 0.02)
- 1.52 (m, 1H, integration 0.02)
- 1.51 (m, 1H, integration 0.02)
- 1.47 (m, 1H, integration 0.02)
- 1.45 (m, 1H, integration 0.02)
- 1.41 (m, 1H, integration 0.02)
- 1.40 (m, 1H, integration 0.02)
- 1.39 (m, 1H, integration 0.02)
- 1.37 (m, 1H, integration 0.02)
- 1.34 (m, 1H, integration 0.02)
- 1.31 (m, 1H, integration 0.02)
- 1.28 (m, 1H, integration 0.02)
- 1.25 (m, 1H, integration 0.02)

Figure S2. ^{13}C NMR spectrum of leptogorgodiol A **1** in CDCl_3



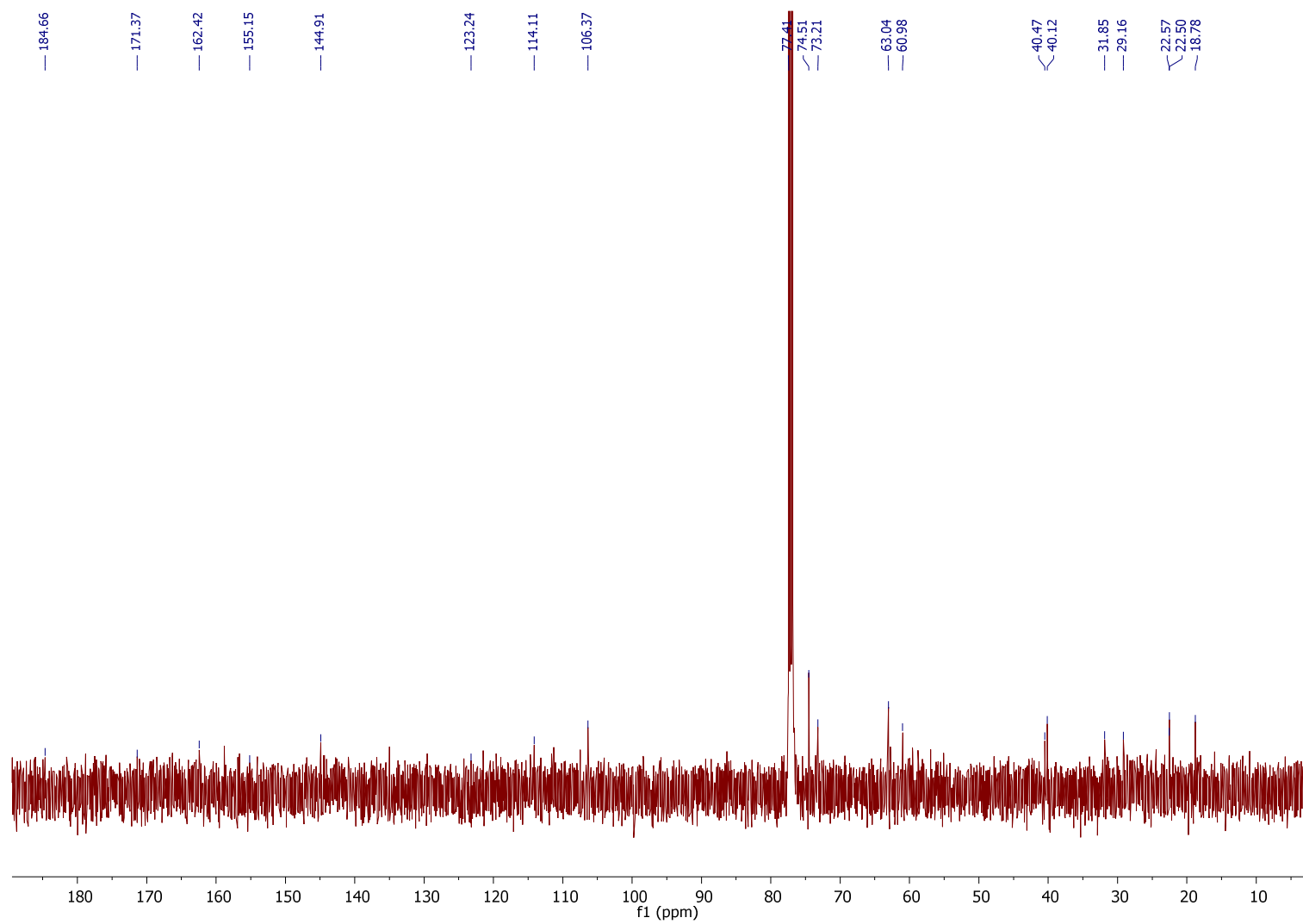


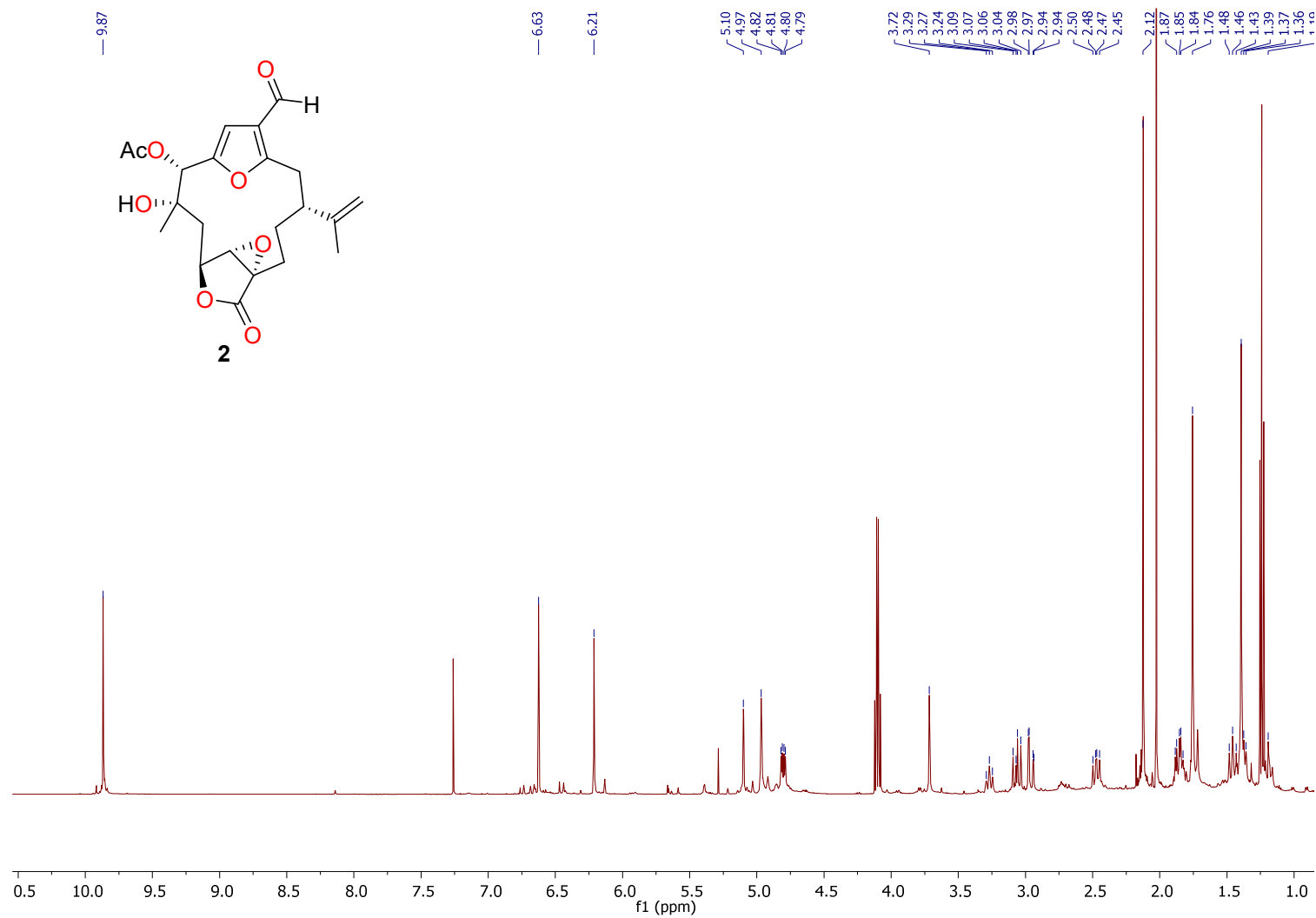
Figure S3. ^1H NMR of 7-acetate-leptogorgodiol A **2** in CDCl_3 

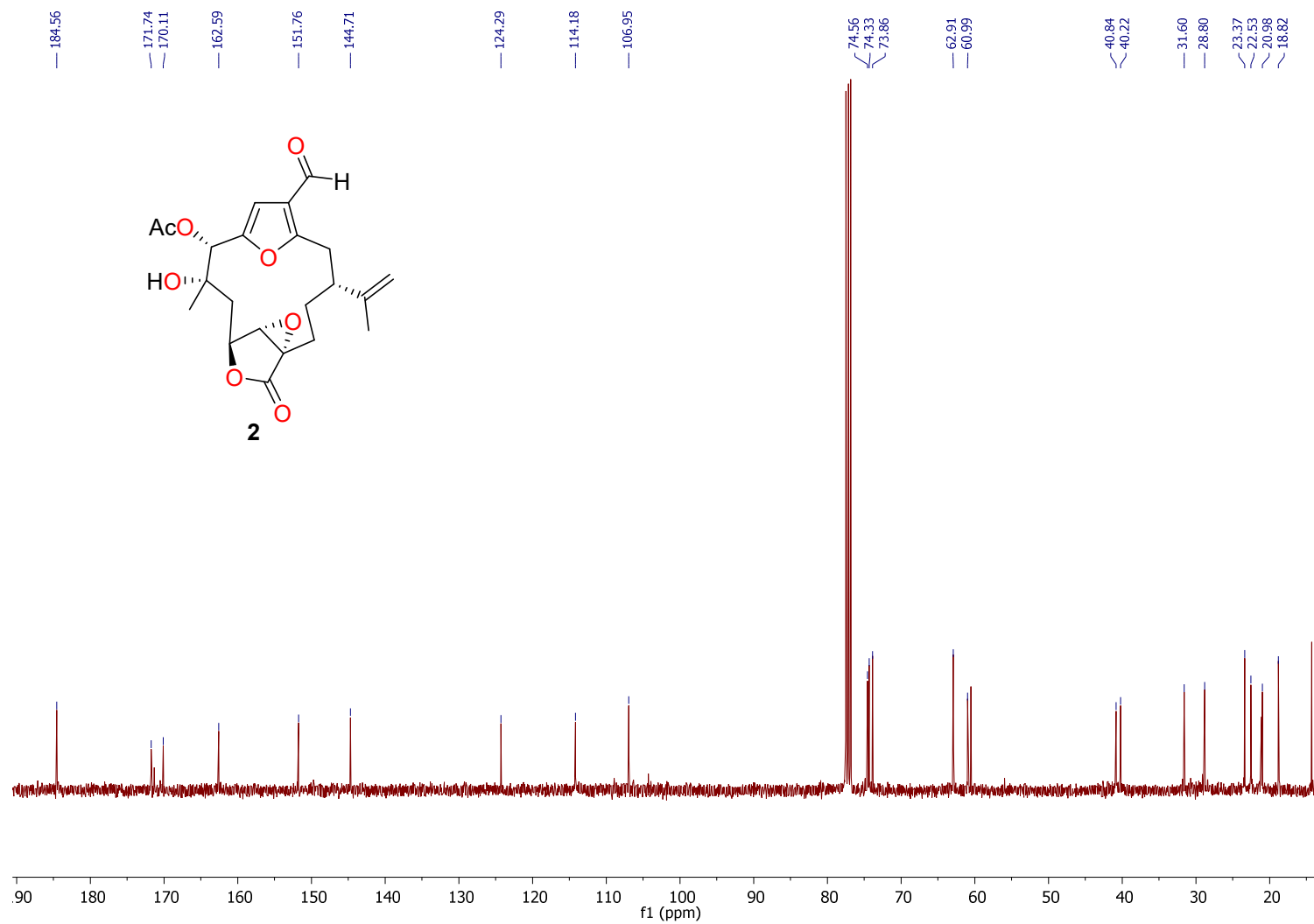
Figure S4. ^{13}C NMR spectrum of 7-acetate-leptogorgodiol A **2** in CDCl_3 

Figure S5. ^1H NMR of leptogorgodiol B **3** in CDCl_3

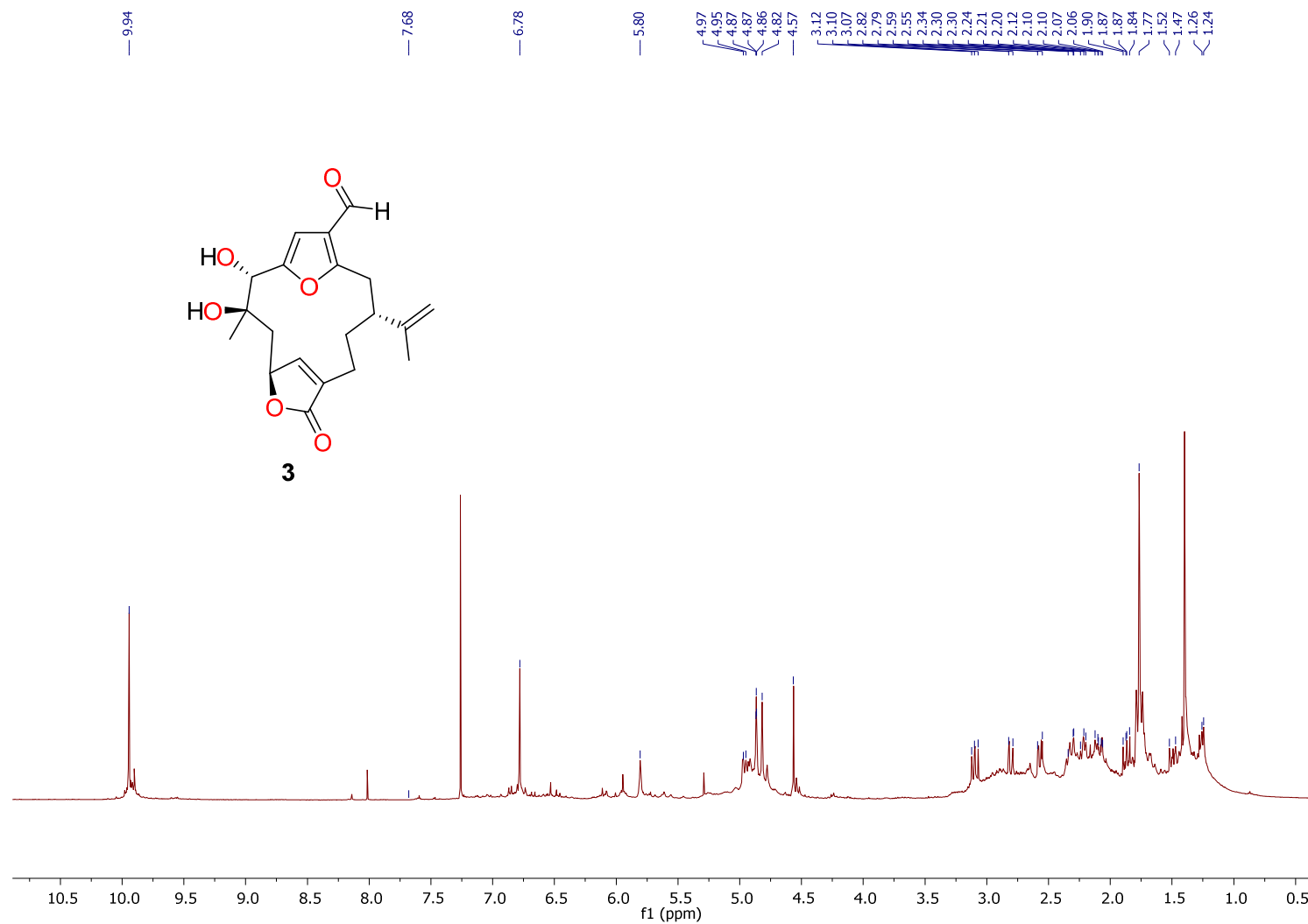


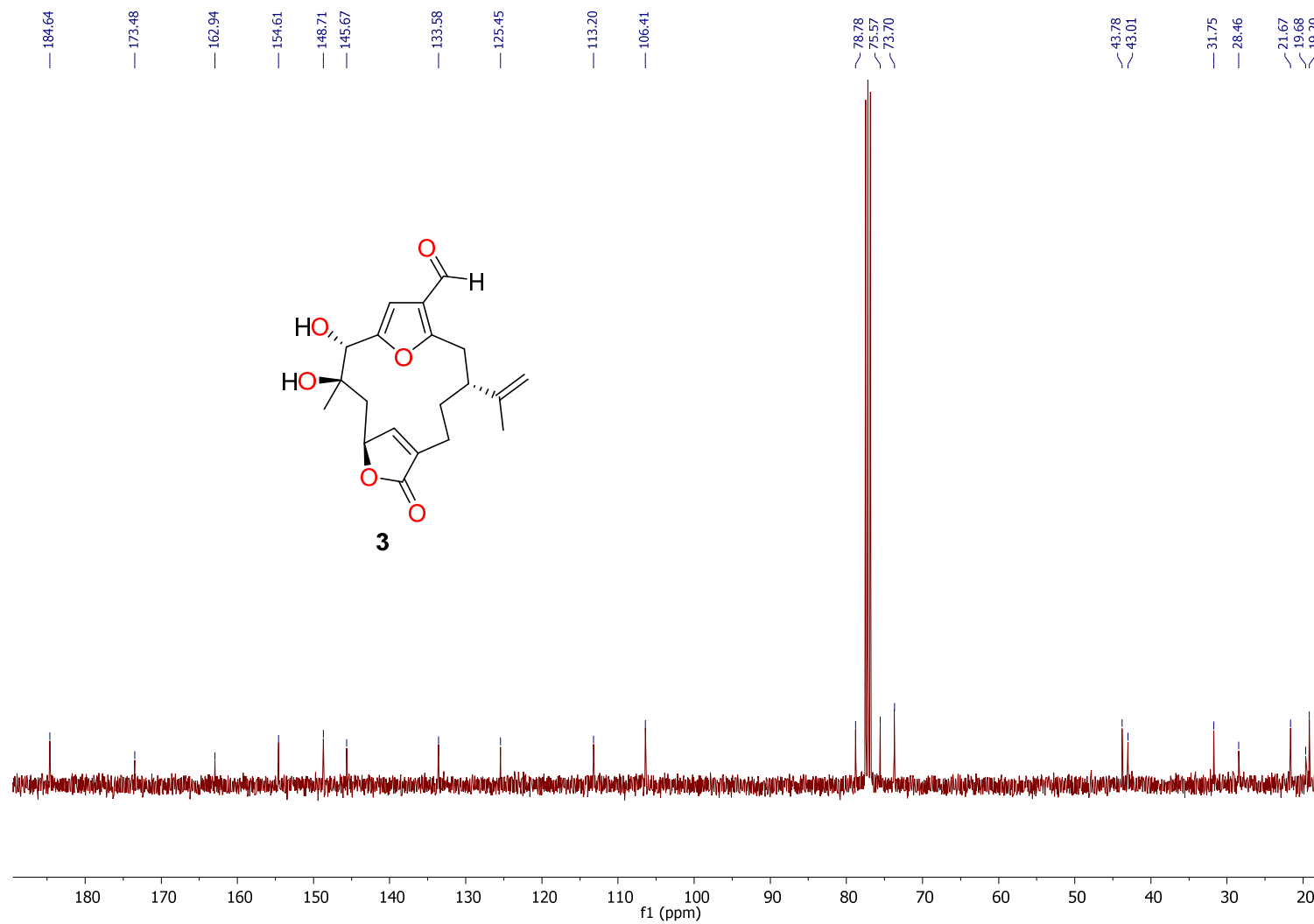
Figure S6. ^{13}C NMR spectrum of leptogorgodiol B **3** in CDCl_3 

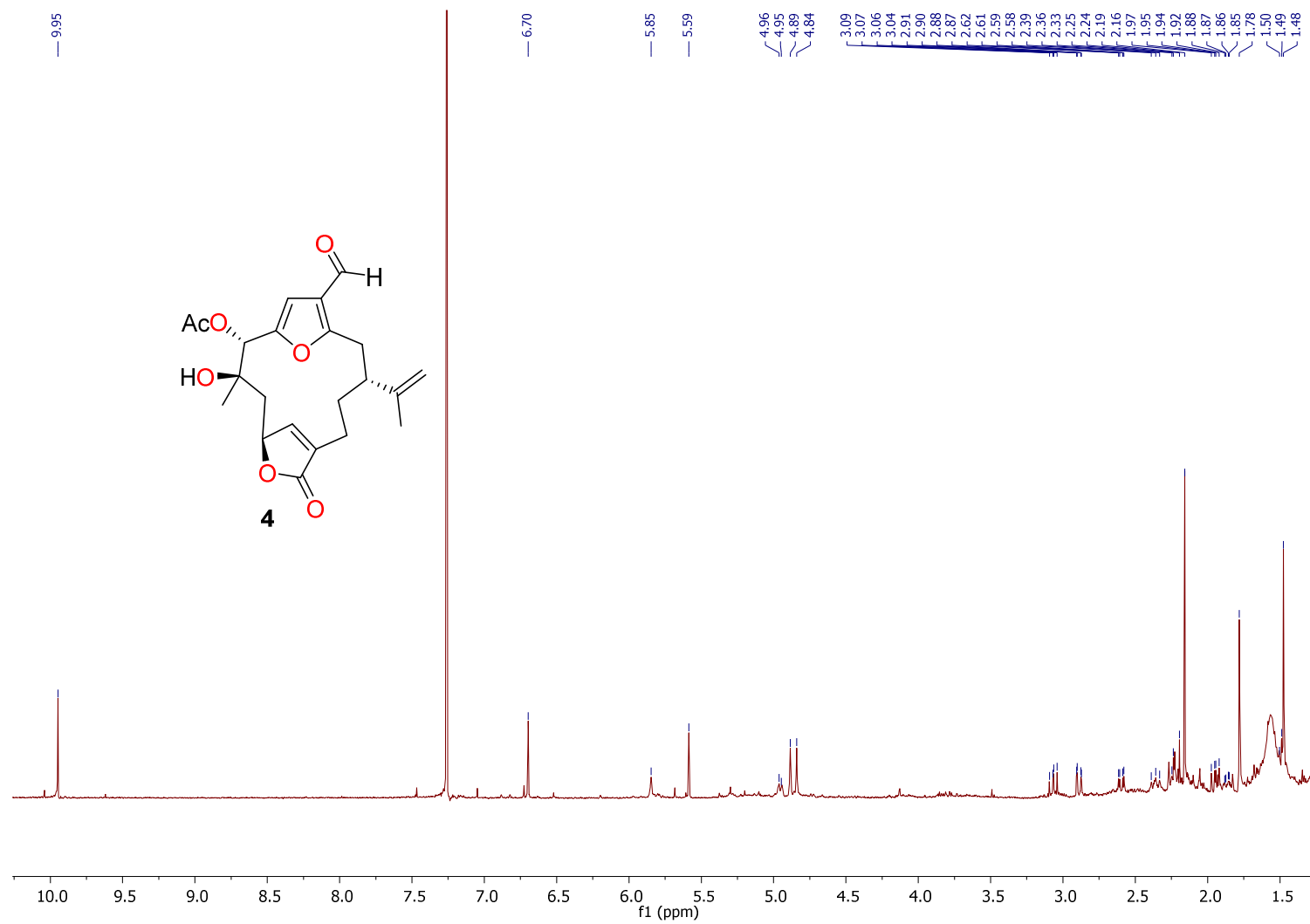
Figure 7. ^1H NMR of 7-acetate-leptogorgodiol B **4** in CDCl_3 .

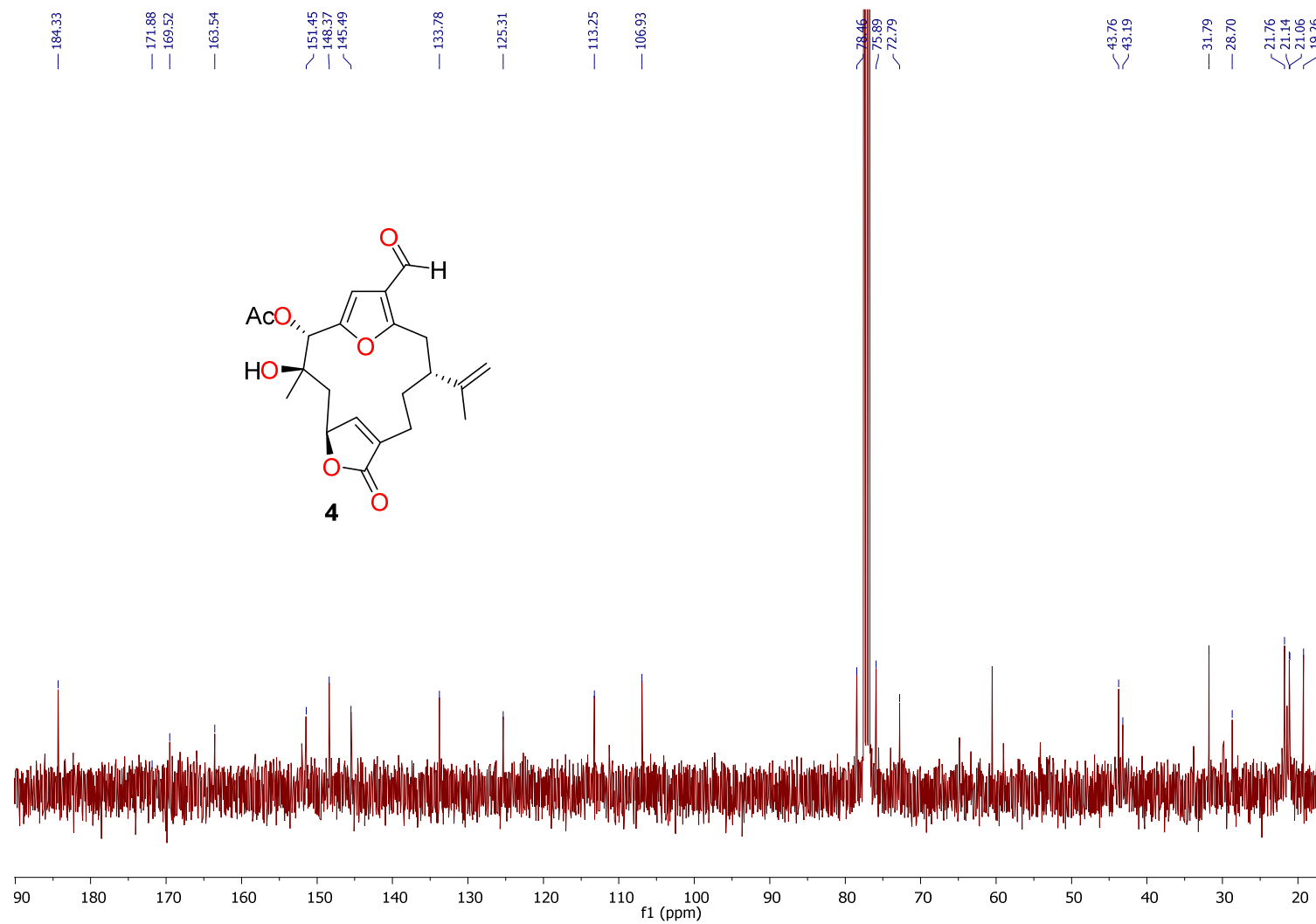
Figure S8. ^{13}C NMR spectrum of 7-acetate-leptogorgodiol B **4** in CDCl_3 

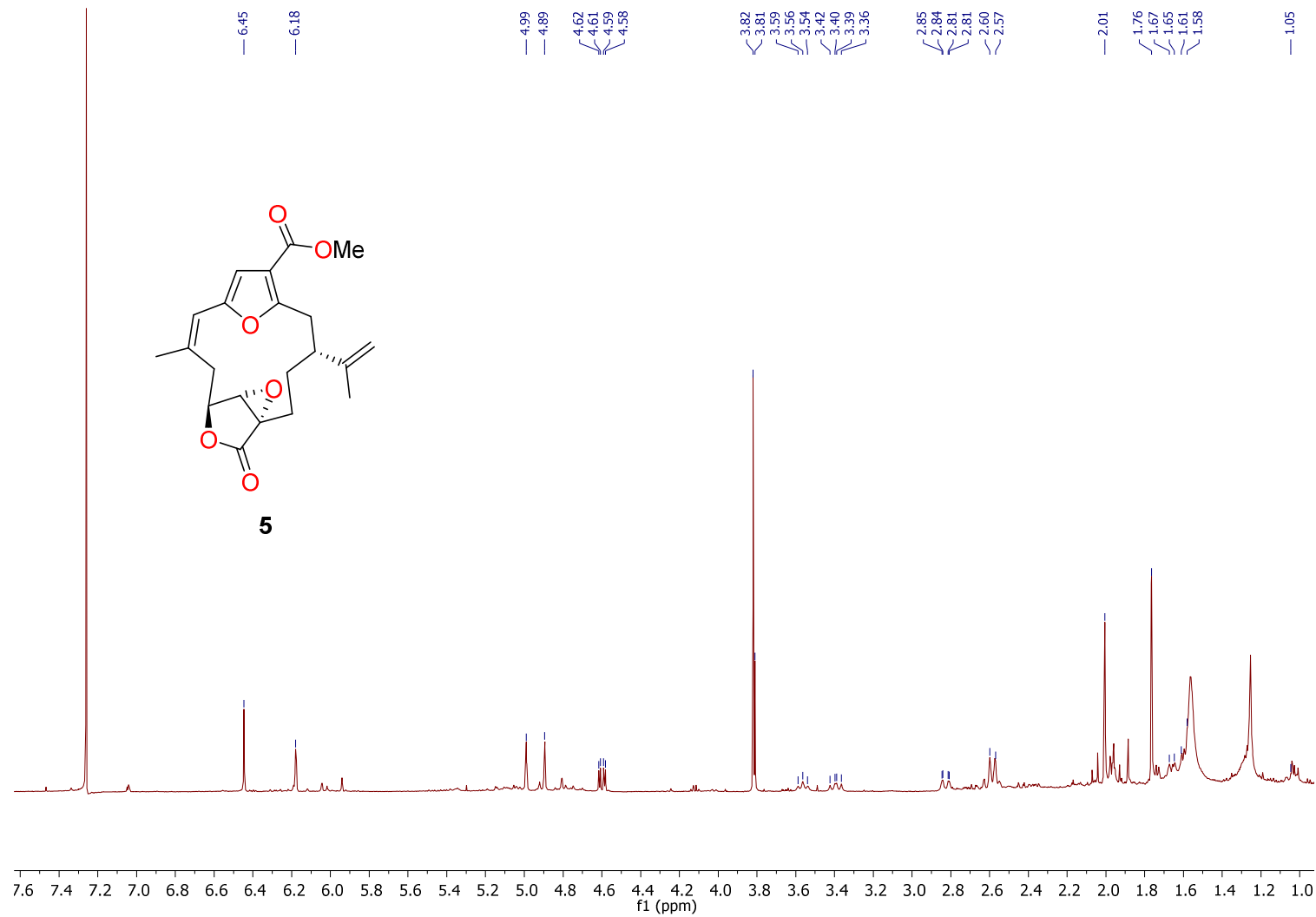
Figure 9. ^1H NMR of Z-isopukalide **5** in CDCl_3 .

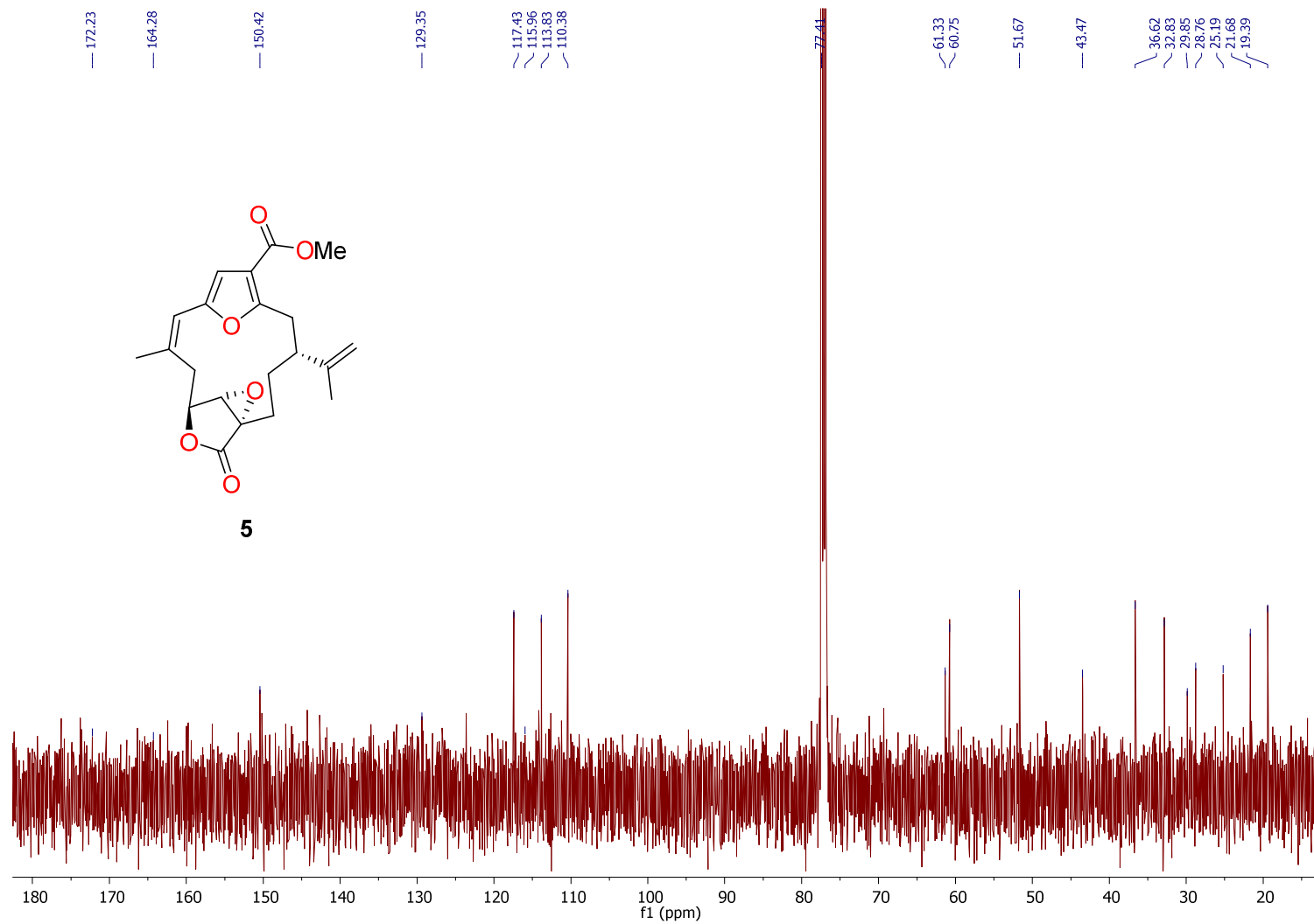
Figure 10. ^{13}C NMR spectrum of Z-isopukalide **5** in CDCl_3 .

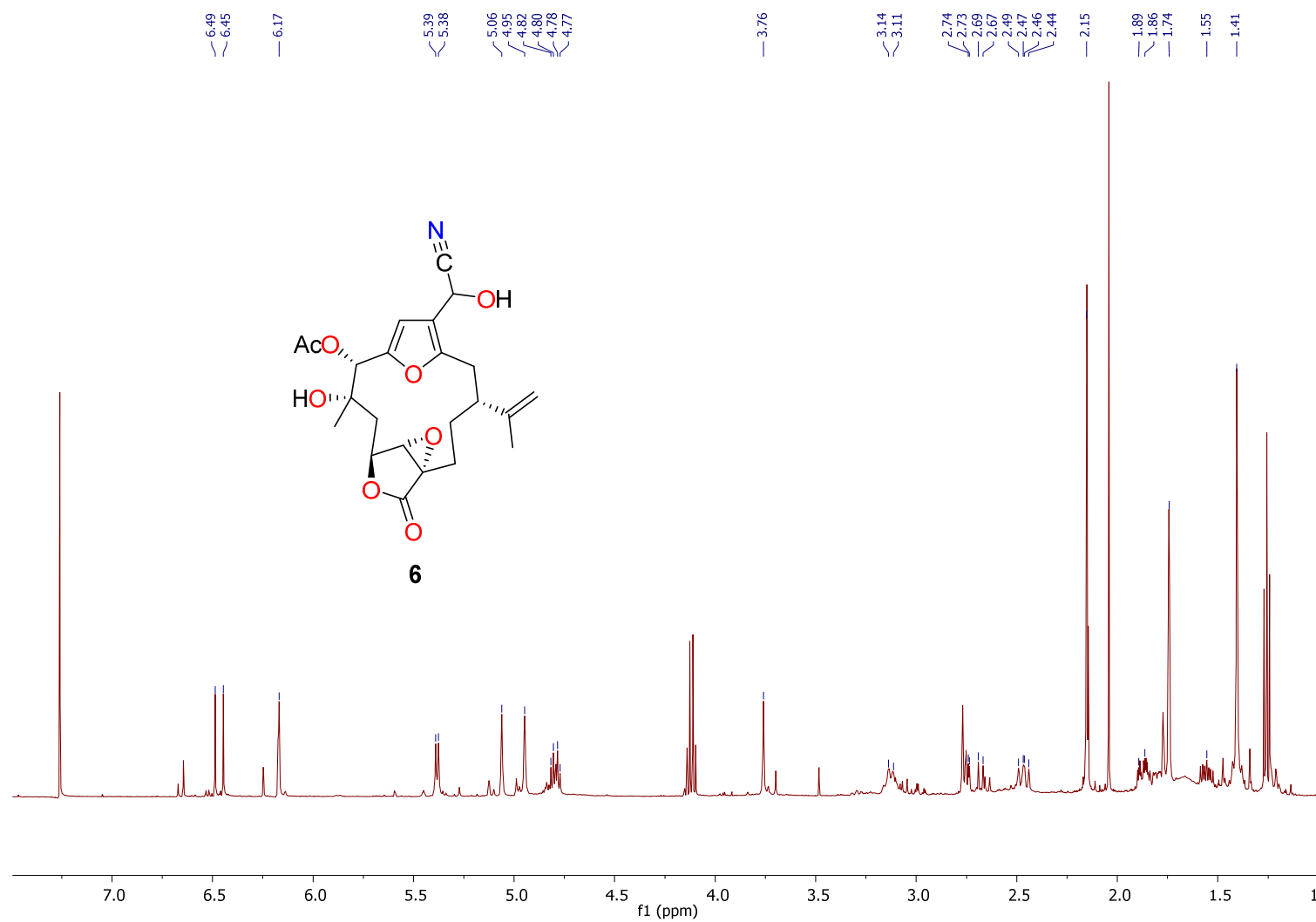
Figure S11. ^1H NMR of **6** in CDCl_3 

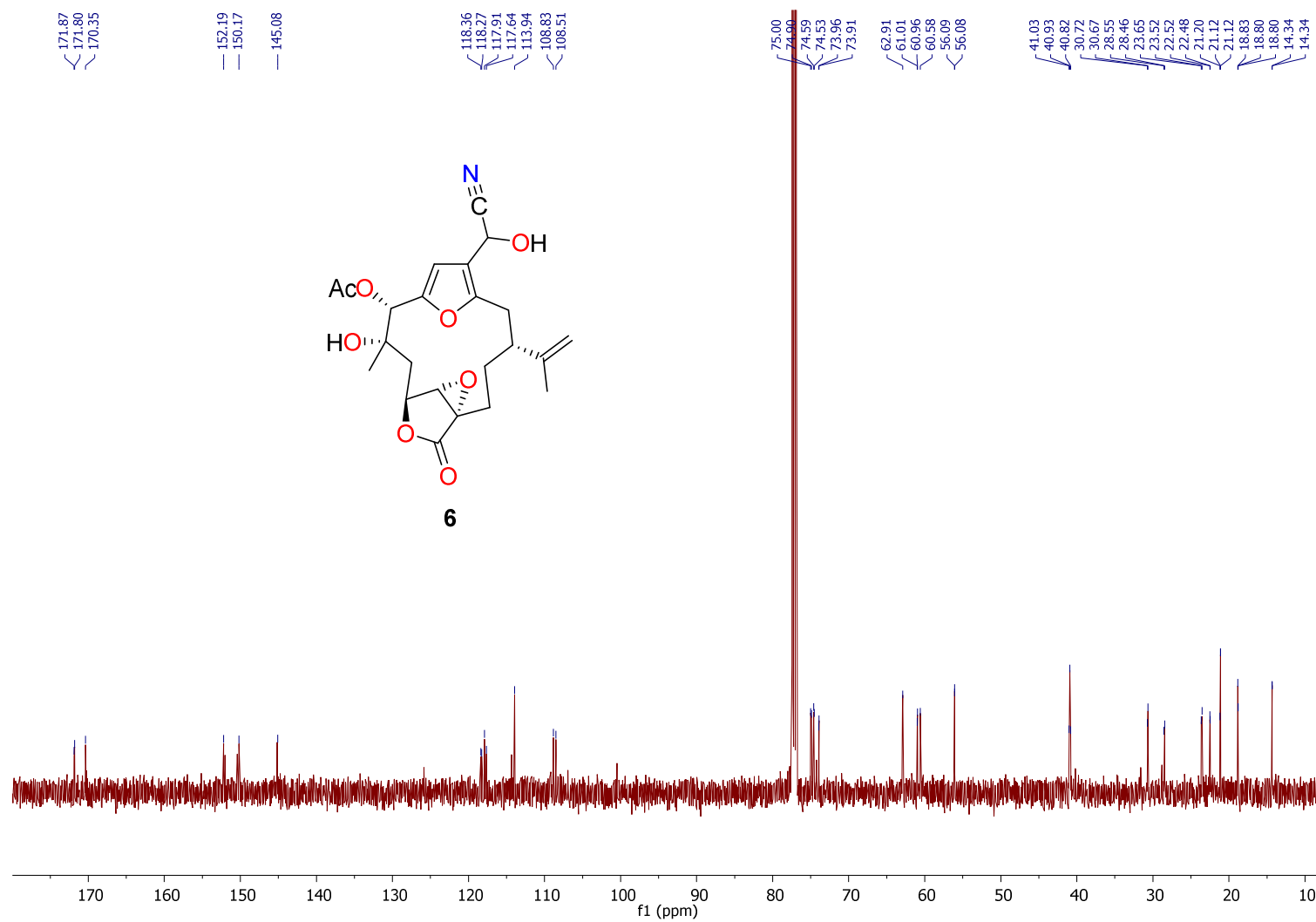
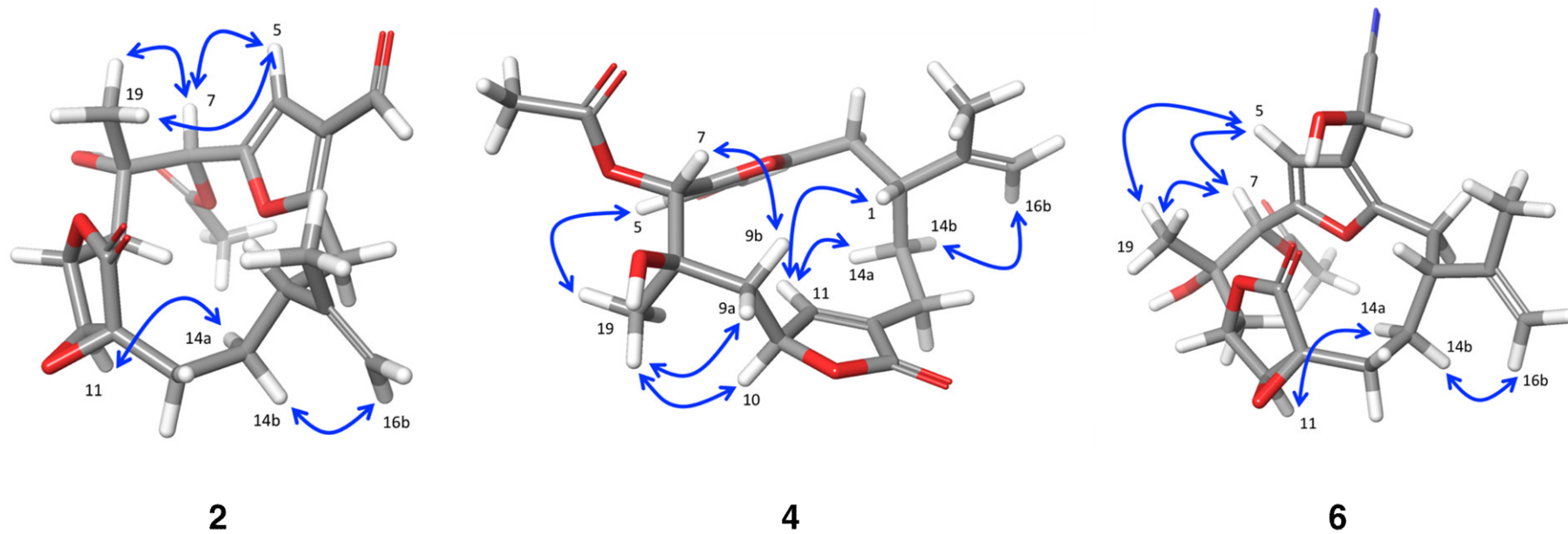
Figure S12. ^{13}C NMR spectrum of **6** in CDCl_3 

Figure 13. Minimized structures and selected NOE effects ($\leftrightarrow$) of 2, 4 and 6.



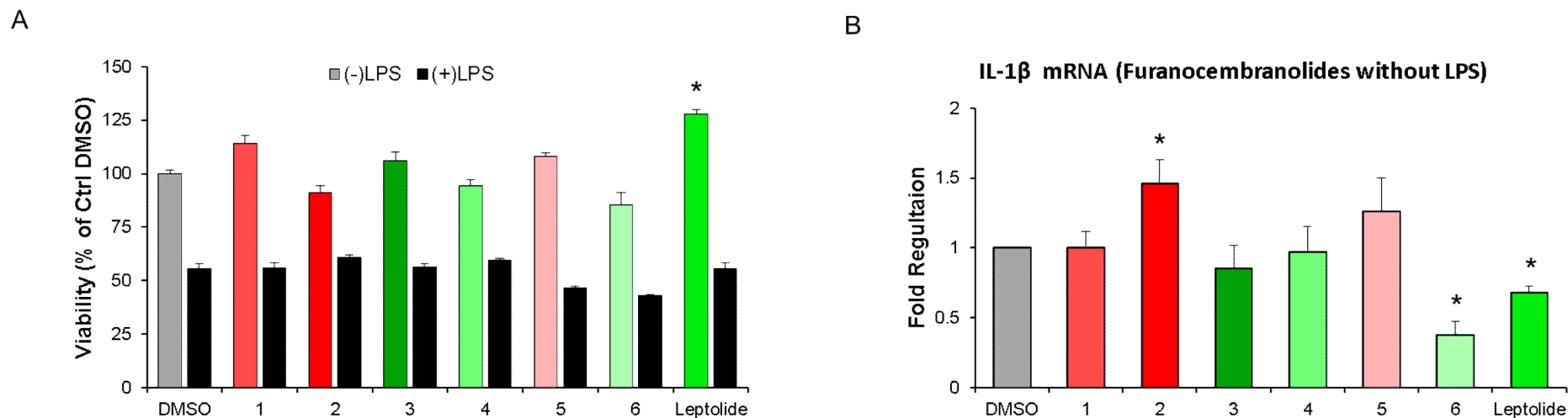


Figure S14. Effects of furanocembranolides on microglial cell viability and inflammation. A) Cell viability results measured by MTT, normalized to the Control DMSO group (termed as 100% viability). The bars show the mean value for quadruplicates, and error bars show the standard error mean. B) Total fold change of IL-1 β mRNA production in BV2 cells treated only with furanocembranolides in the absence of LPS. Statistical differences were analyzed by one-way ANOVA followed by all pairwise multiple comparisons by Bonferroni t-test. Only biologically significant differences are shown.