

Supplementary Materials for

Production and Structural Diversification of Withanolides by Aeroponic Cultivation of Plants of Solanaceae: Cytotoxic and other Withanolides from Aeroponically Grown *Physalis coztomatl*

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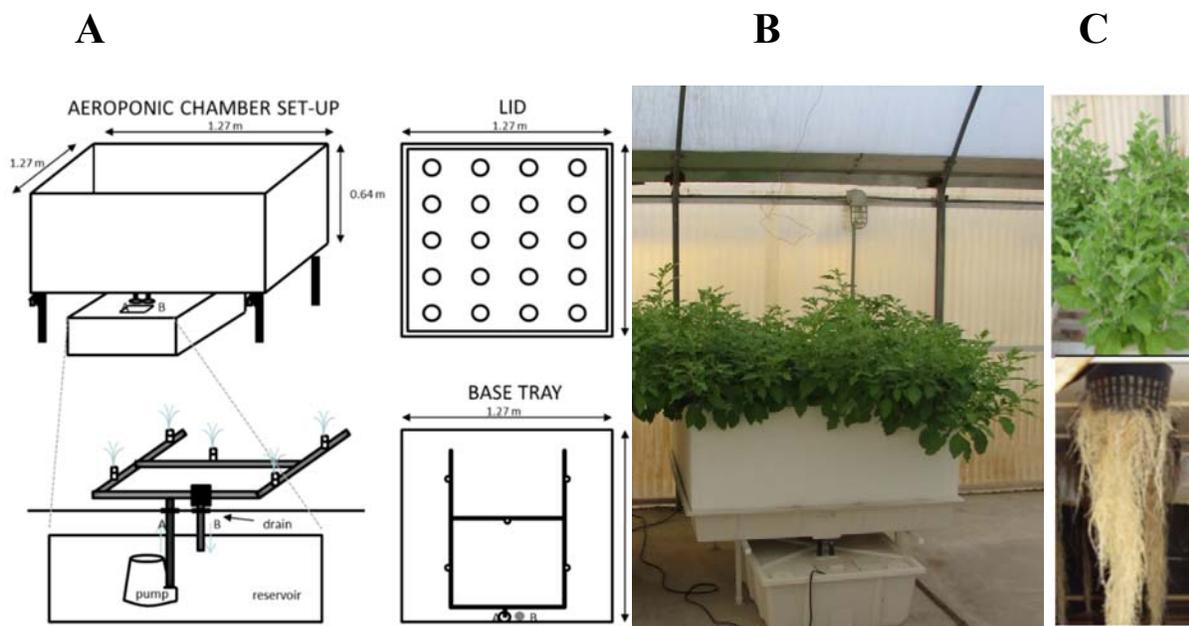
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Figure S1. Aeroponic cultivation of plants of Solanaceae. (A) Schematic of an aeroponic cultivation set-up (for details, see description below); (B) images showing *Withania somnifera* growing under aeroponic conditions in a greenhouse, and (C) roots of *W. somnifera* hanging inside the aeroponic chamber which are sprayed intermittently with the nutrient medium (see Table S1, below).



Procedure: For aeroponic cultivation, plants were grown on top of chambers which measured 1.27 m x 1.27 m x 0.64 m (W x L x H), and equipped with five nozzles powered by a pump to spray nutrient solution every 4 min for a period of 1 min (**Figure S1A**). A reservoir of 150 L of nutrient solution was maintained under each chamber accommodating 20 plants. This nutrient solution was prepared according to a modified Hoagland hydroponic recipe having a pH of 5.8 – 6.0 (**Table S1**).

Table S1. Constitution of the modified Hoagland's nutrient medium providing macro- and micro-nutrients for aeroponic plant cultivation.

Macronutrients	g/L	Required Elements	Micronutrients	g/L	Required Elements
Ca(NO ₃) ₂ 4H ₂ O	0.579	Calcium/Nitrogen	H ₃ BO ₃	0.003	Boron
KNO ₃ ^a	0.515	Potassium/Nitrogen	CuSO ₄ 20%	0.003	Copper
KH ₂ PO ₄	0.204	Potassium/Phosphorous	MnSO ₄ H ₂ O 20%	0.004	Manganese
K ₂ SO ₄	0.193	Potassium/Sulfur	Na ₂ MoO ₄ 2H ₂ O	0.001	Molybdenum
MgSO ₄ 7H ₂ O	0.600	Magnesium/Sulfur	ZnSO ₄ 7H ₂ O 20%	0.004	Zinc
CaCl ₂ 6H ₂ O	0.278	Calcium/Chlorine			
Fe-EDDHA 10%	0.030	Iron			

^aoptional supplement used during plant reproductive phase.

Figure S2. Aeroponic cultivation of *Withania somnifera*. (A) Aeroponically-grown *W. somnifera* (Solanaceae) showing improved plant growth compared to (B) soil-grown *W. somnifera* provided with the same nutrient medium and grown for the same length of time. (C) Comparison of leaf size of *W. somnifera* showing that the leaves of aeroponically grown plants are about five times as large as those of soil grown plants. (D) Unusual withanolides, 3 α -(uracil-1-yl)-2,3-dihydrowithaferin A and 3 β -(adenin-9-yl)-2,3-dihydrowithaferin, isolated from aeroponically-grown *W. somnifera*.

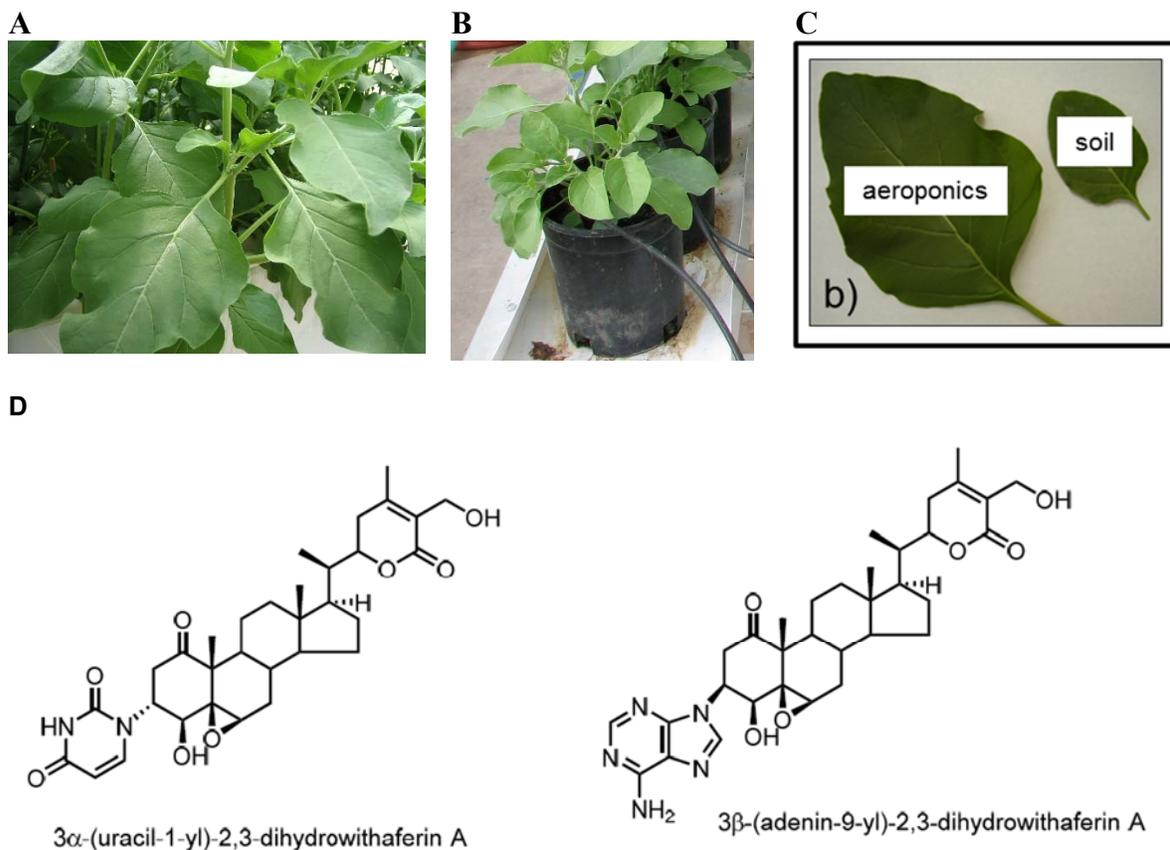


Figure S3. Aeroponic cultivation of *Physalis crassifolia*. **(A)** Aeroponically-grown *P. crassifolia* (Solanaceae) showing improved plant growth compared to **(B)** soil-grown *P. crassifolia* provided with the same nutrient medium and grown for the same length of time in the same greenhouse. **(C)** Comparison of HPLC profiles of withanolide containing extracts of soil-grown and aeroponically-grown *P. crassifolia* (red arrows indicate additional HPLC peaks present in aeroponically-grown plant). **(D)** Structures of withanolides occurring in wild-crafted/soil-grown *P. crassifolia*, and **(E)** structures of 14 additional withanolides, including 11 new withanolides (names in red color) together with 4 withanolides also encountered in wild-crafted soil-grown plant (names in blue color), and 3 previously known withanolides (names in black color) occurring in aeroponically-grown *P. crassifolia*.

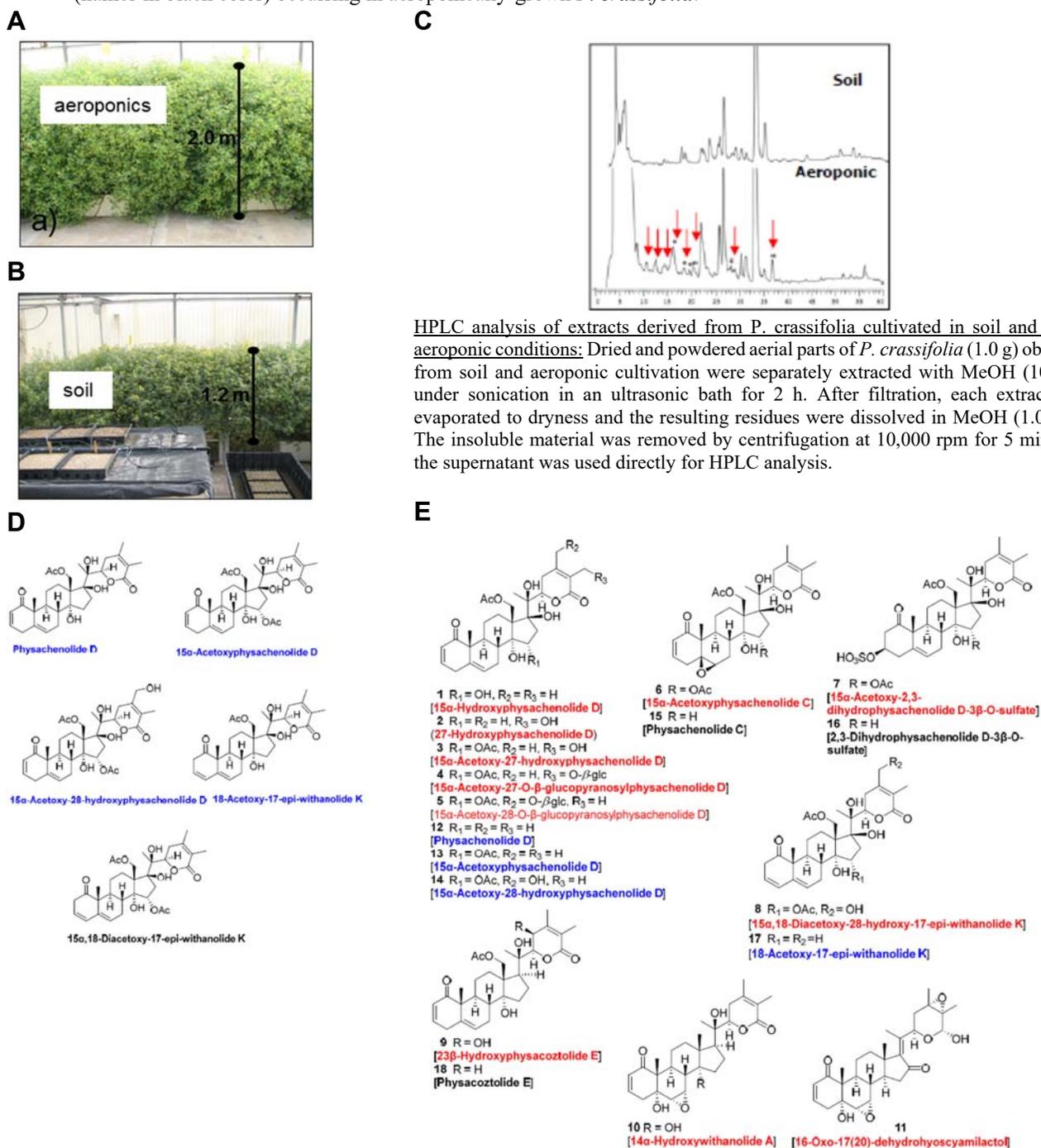


Figure S4. ^1H NMR spectrum (400 MHz) of physacoctolide I (**9**) in $\text{CDCl}_3/\text{CD}_3\text{OD}$ (100:1)

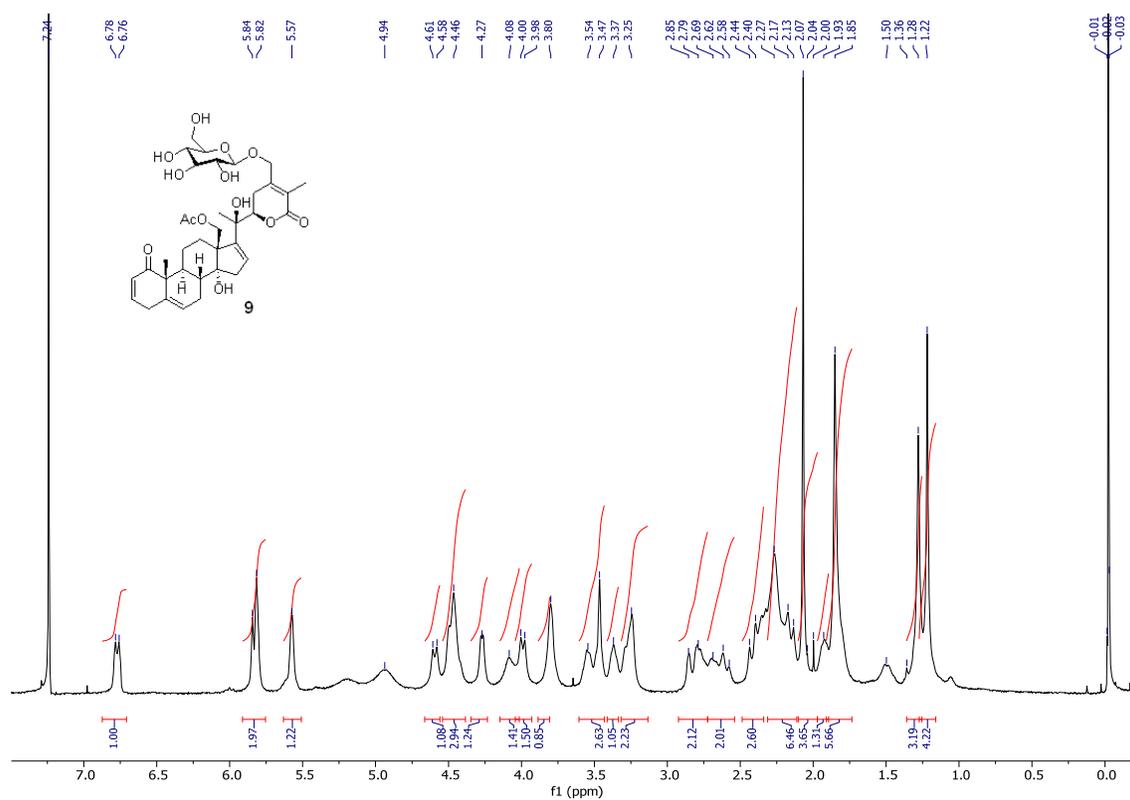


Figure S5. ^{13}C NMR spectrum (100 MHz) of physacoctolide I (**9**) in $\text{CDCl}_3/\text{CD}_3\text{OD}$ (100:1)

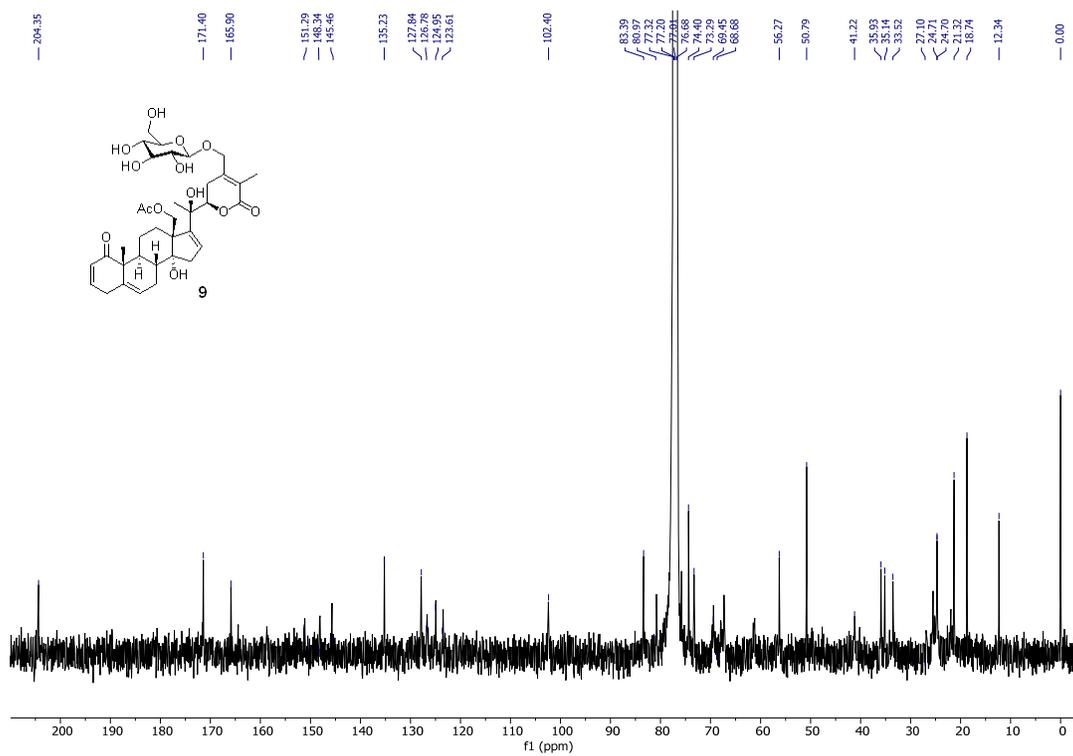


Figure S6. HSQC spectrum (400 MHz) of physacoctolide I (**9**) in CDCl₃/CD₃OD (100:1)

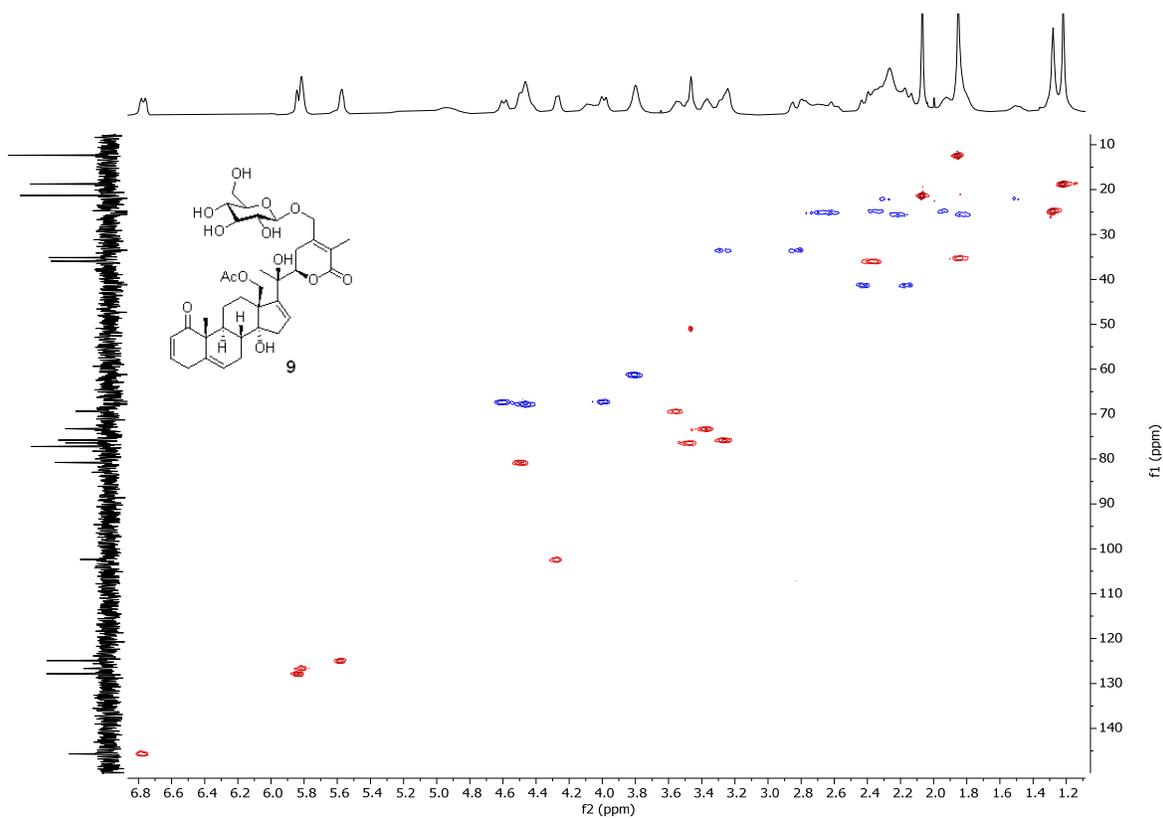


Figure S7. HMBC spectrum (400 MHz) of physacoctolide I (**9**) in CDCl₃/CD₃OD (100:1)

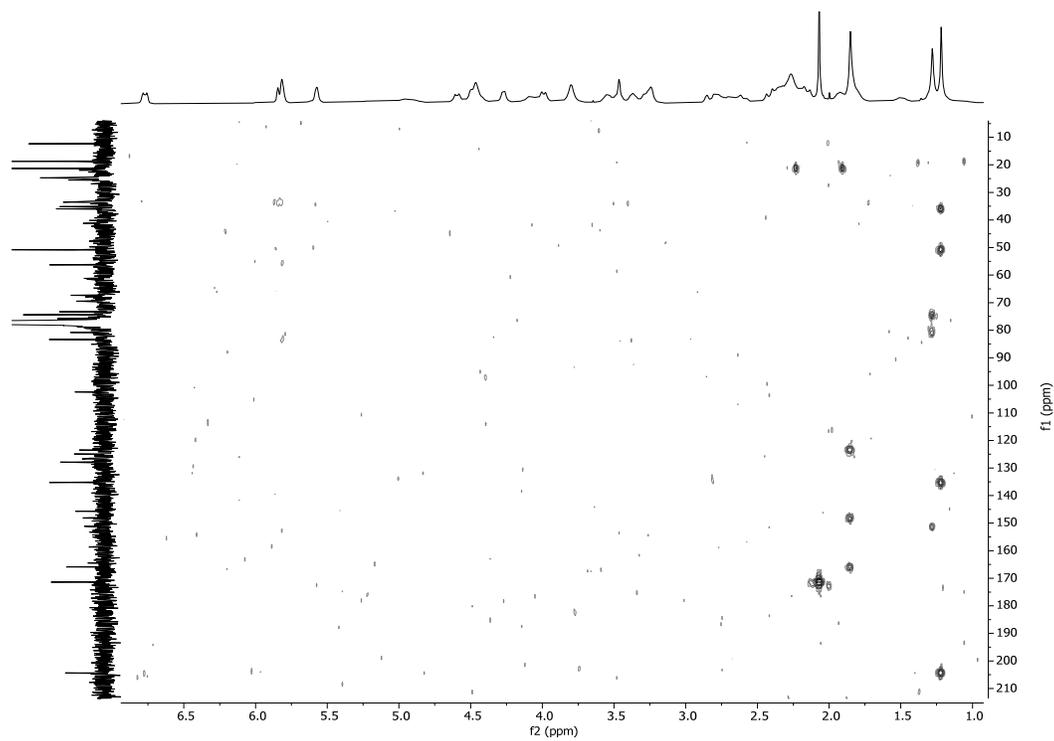


Figure S8. ¹H NMR spectrum (400 MHz) of physacoztolide J (**10**) in CDCl₃/CD₃OD (100:1)

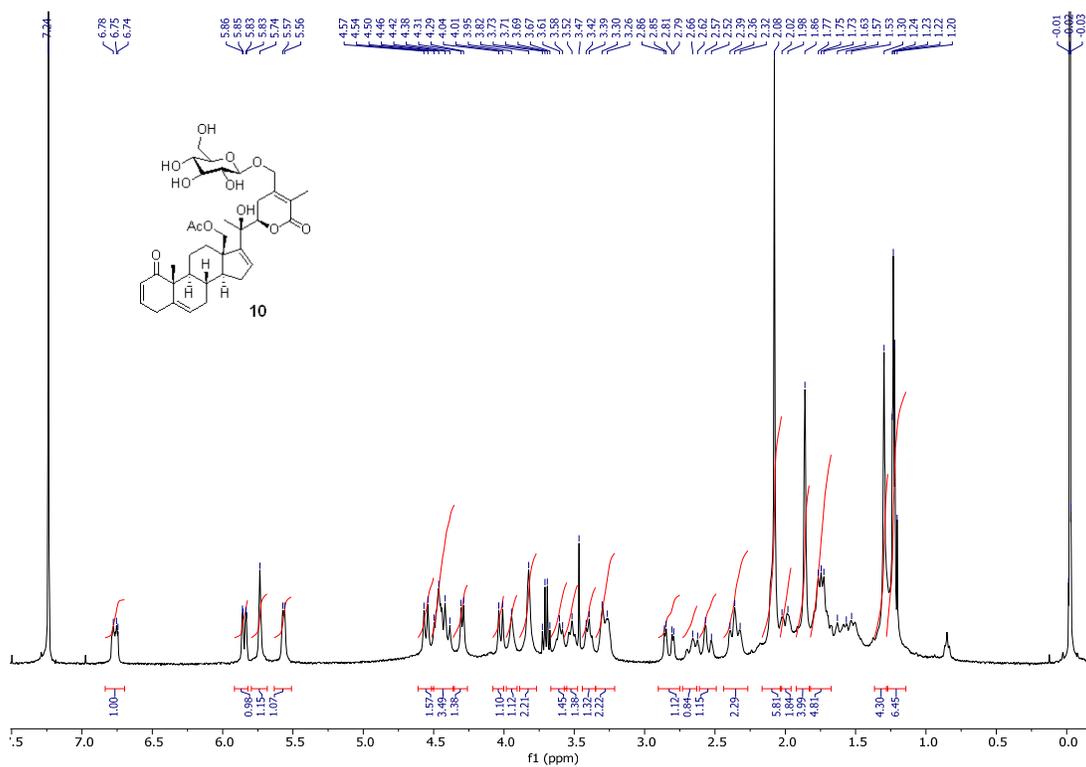


Figure S9. ¹³C NMR spectrum (100 MHz) of physacoztolide J (**10**) in CDCl₃/CD₃OD (100:1)

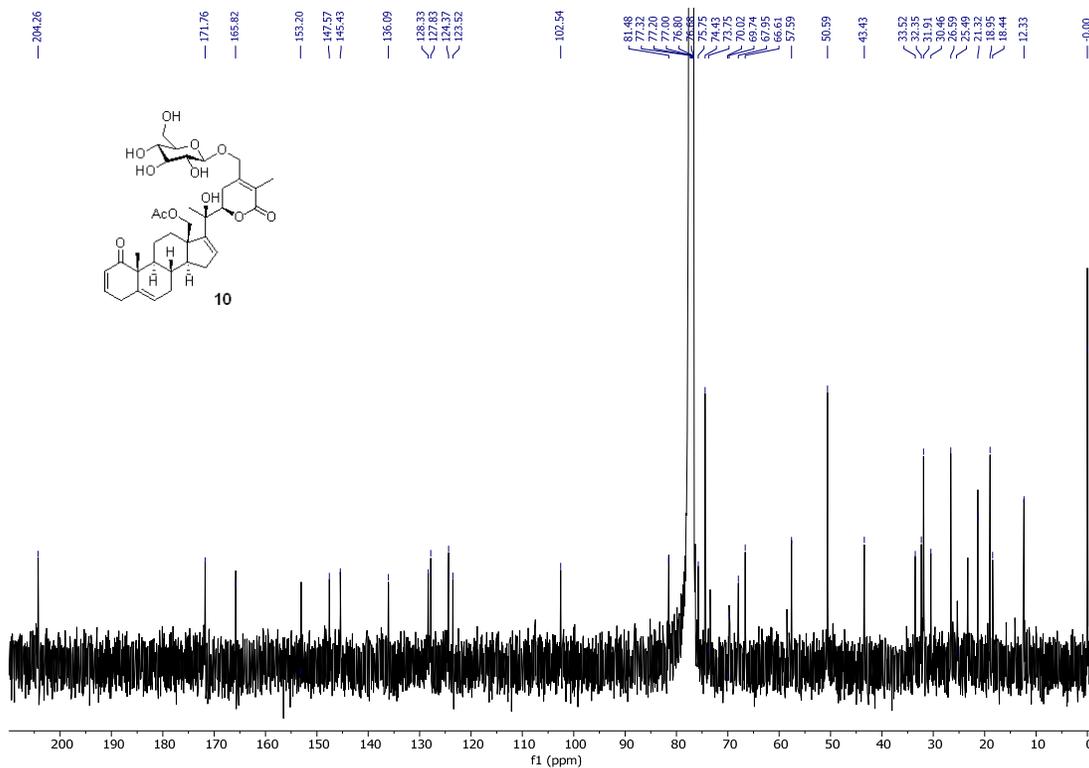


Figure S10. HSQC spectrum (400 MHz) of physacoctolide J (**10**) in CDCl₃/CD₃OD (100:1)

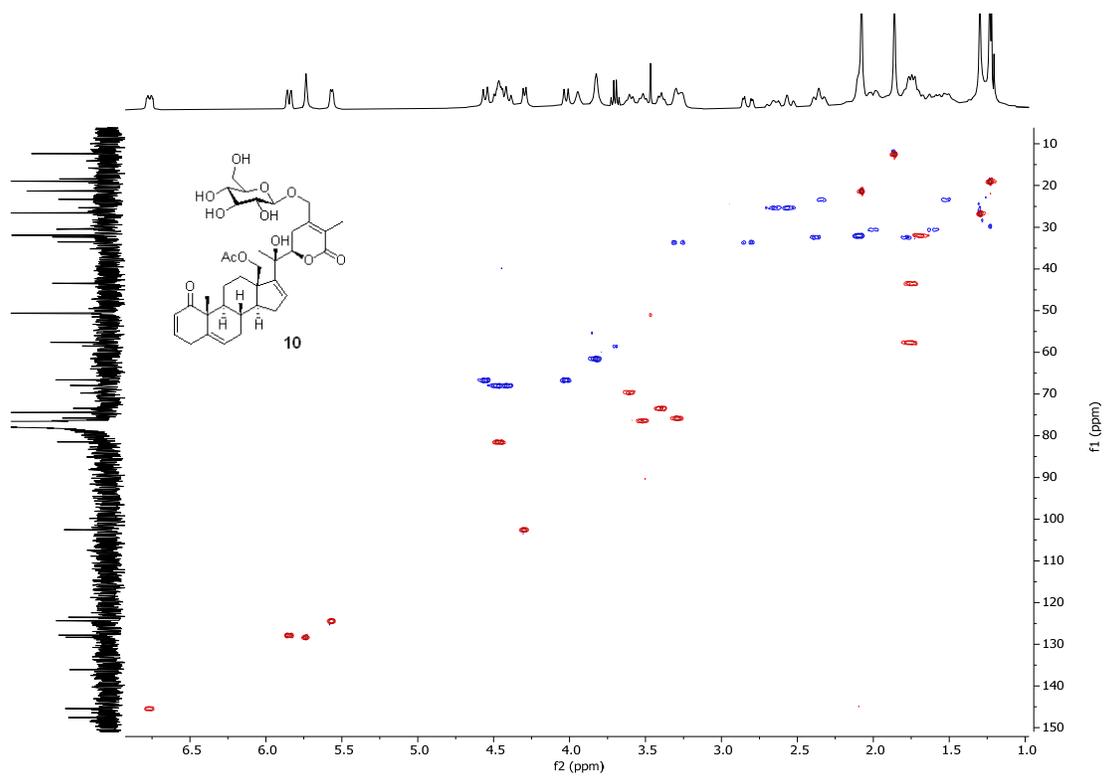


Figure S11. HMBC spectrum (400 MHz) of physacoctolide J (**10**) in CDCl₃/CD₃OD (100:1)

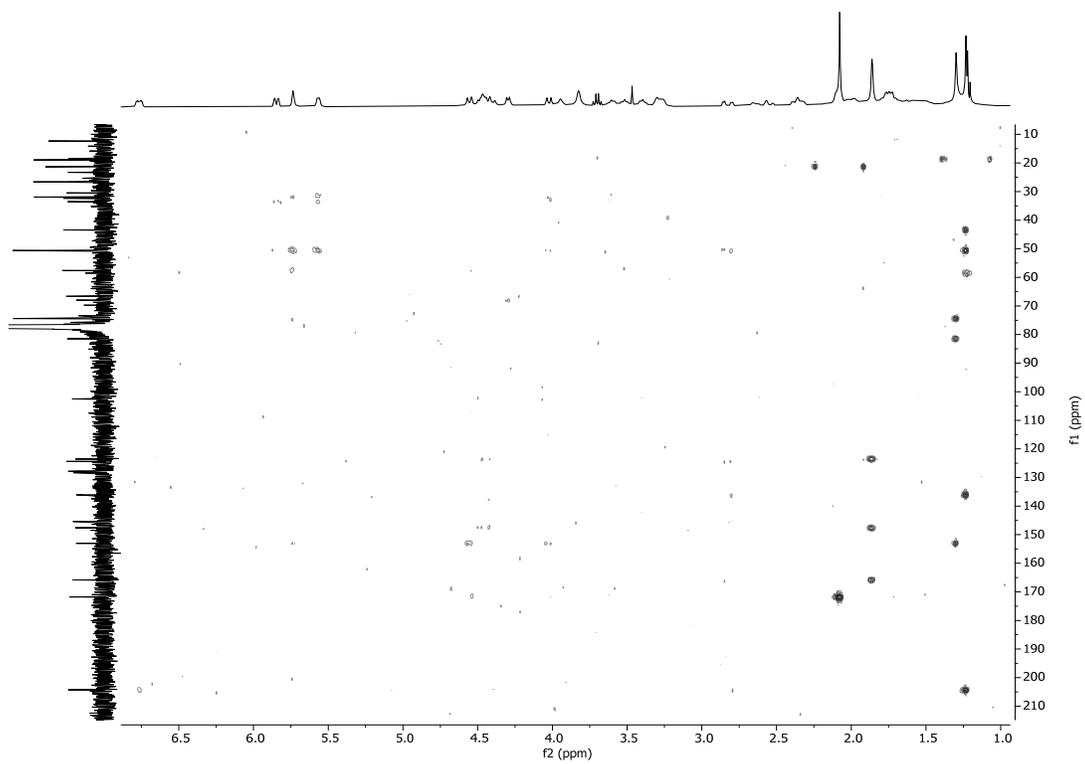


Figure S12. ¹H NMR spectrum (400 MHz) of physacoztolide K (**11**) in CDCl₃

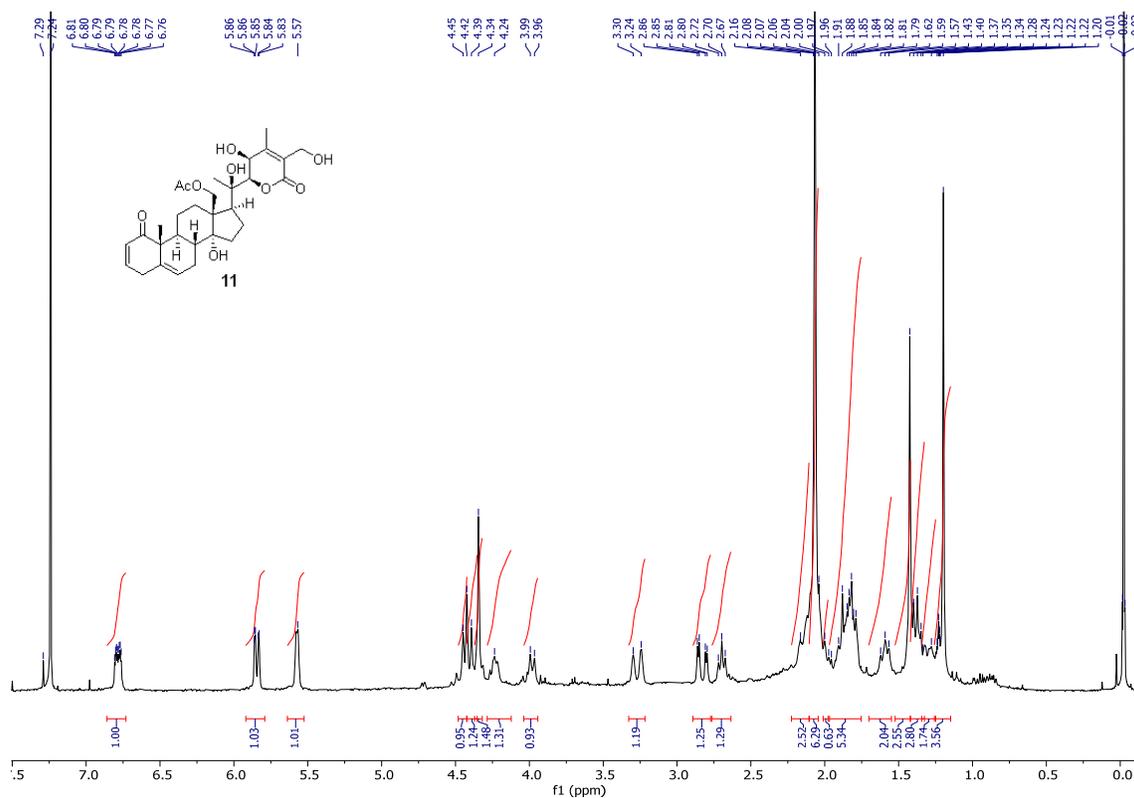


Figure S13. ¹³C NMR spectrum (100 MHz) of physacoztolide K (**11**) in CDCl₃

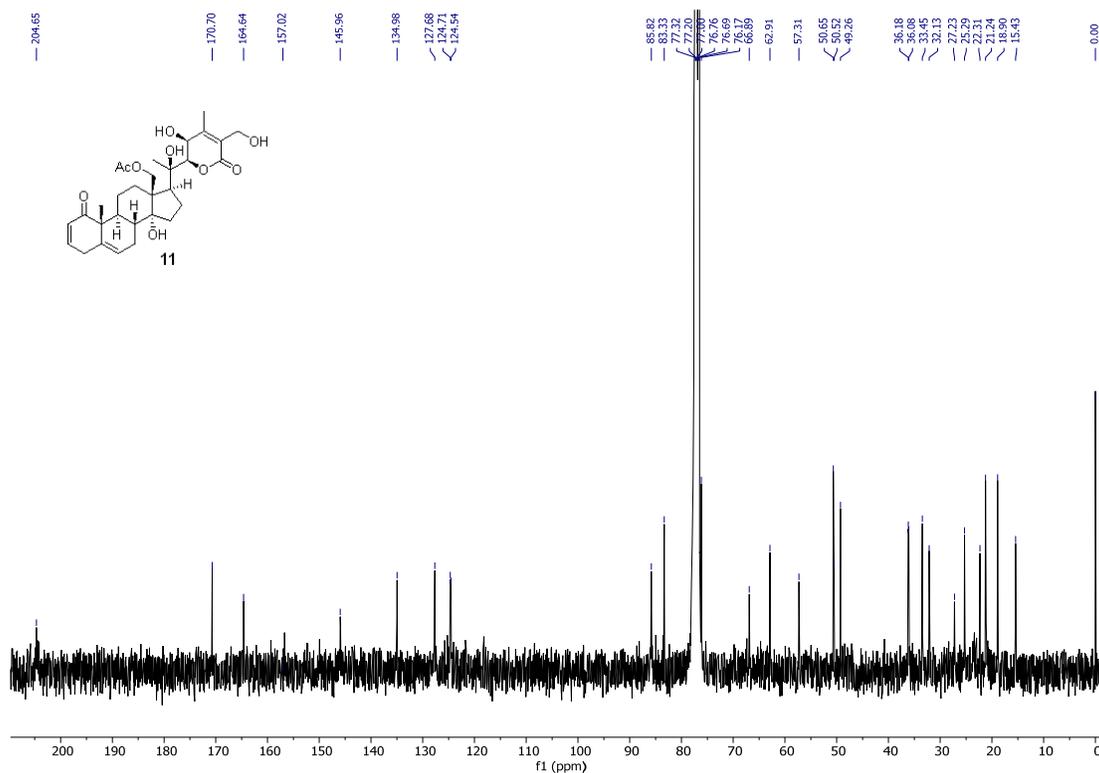


Figure S14. HSQC spectrum (400 MHz) of physacoctolide K (**11**) in CDCl₃

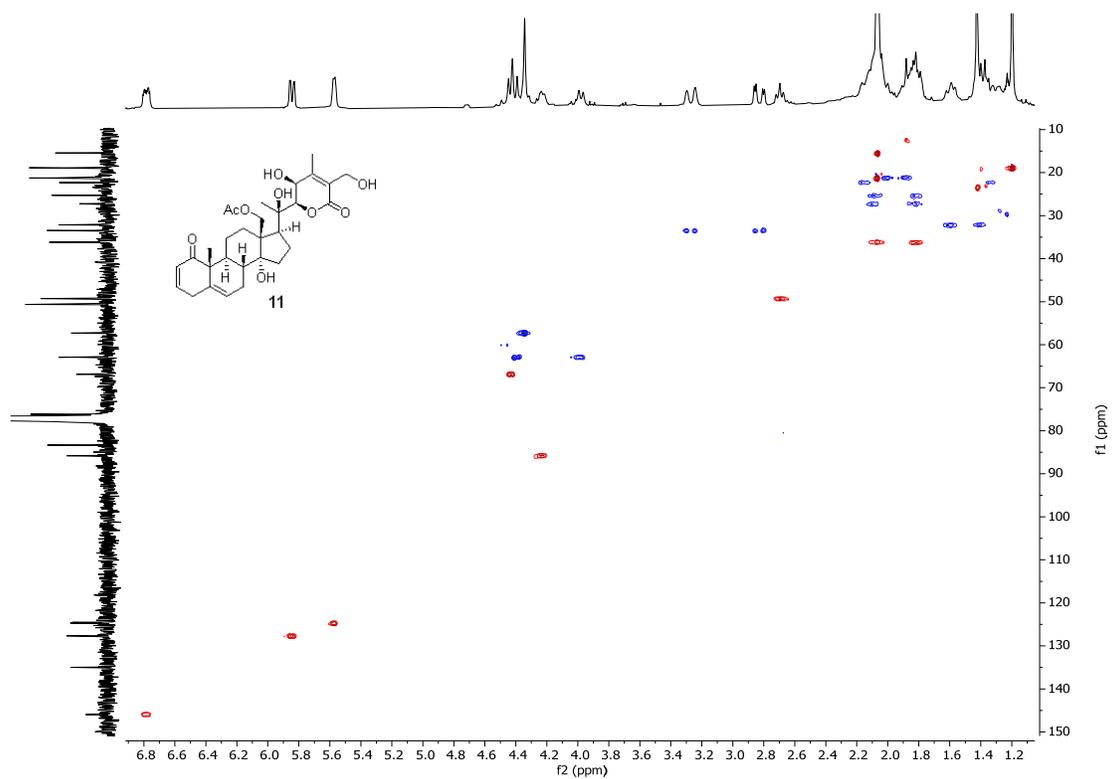


Figure S15. HMBC spectrum (400 MHz) of physacoctolide K (**11**) in CDCl₃

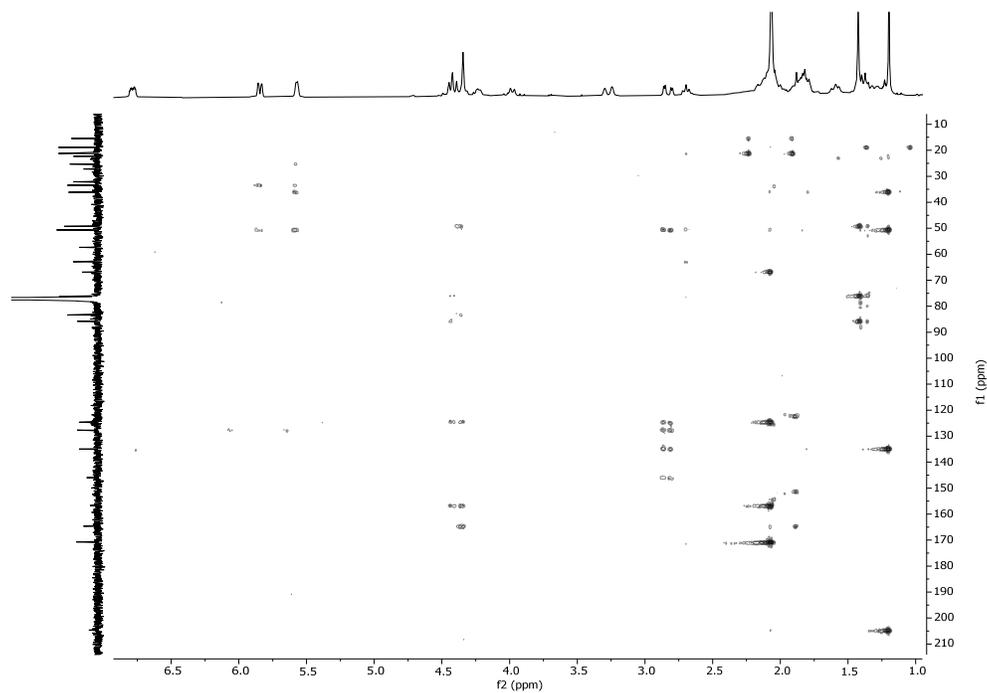


Figure S16. ^1H - ^1H COSY spectrum (400 MHz) of physacoztolide K (**11**) in CDCl_3

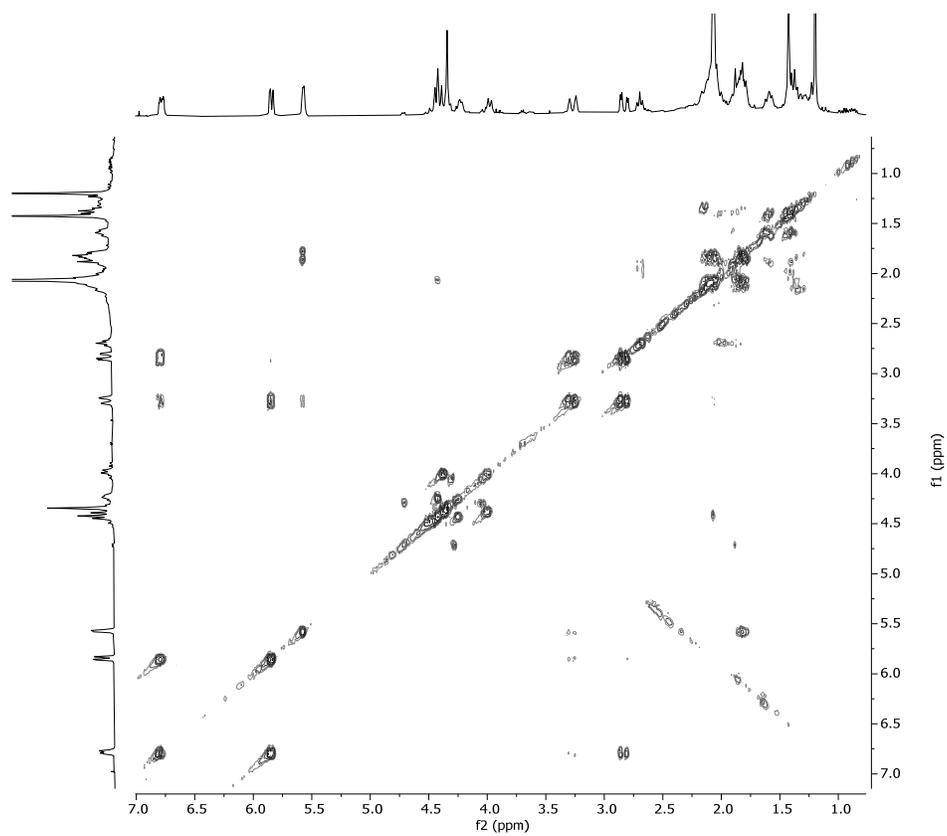


Figure S17. ^1H and 1D NOESY spectra (400 MHz) of physacoztolide K (**11**) in CDCl_3

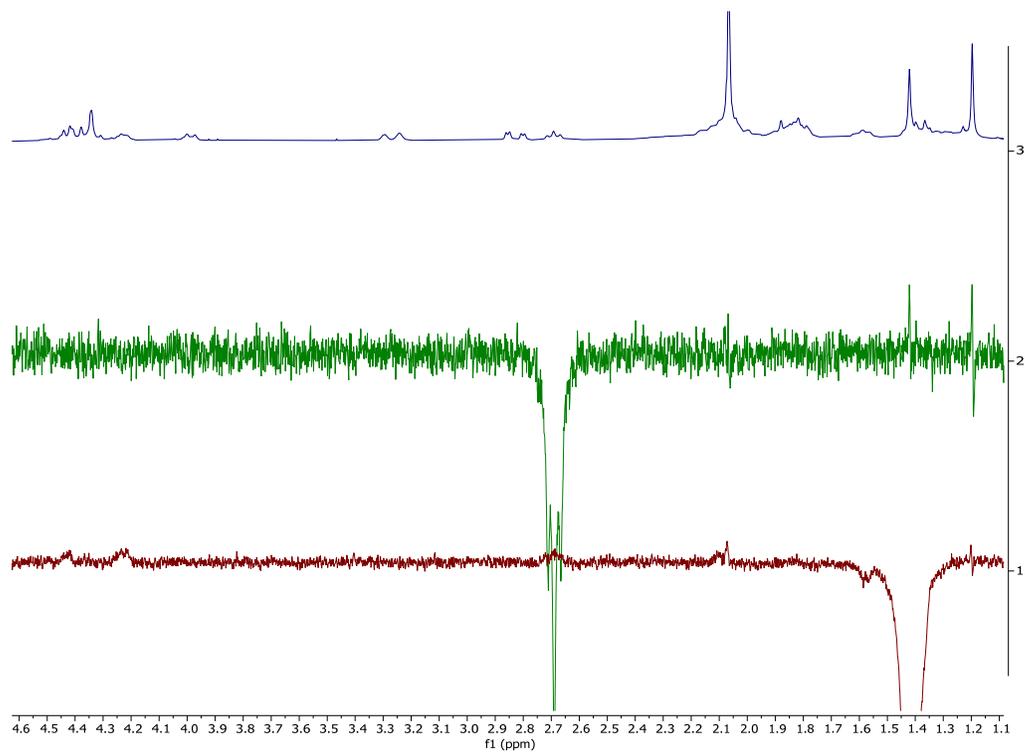


Figure S18. ¹H NMR spectrum (400 MHz) of physacoctolide L (**12**) in CDCl₃

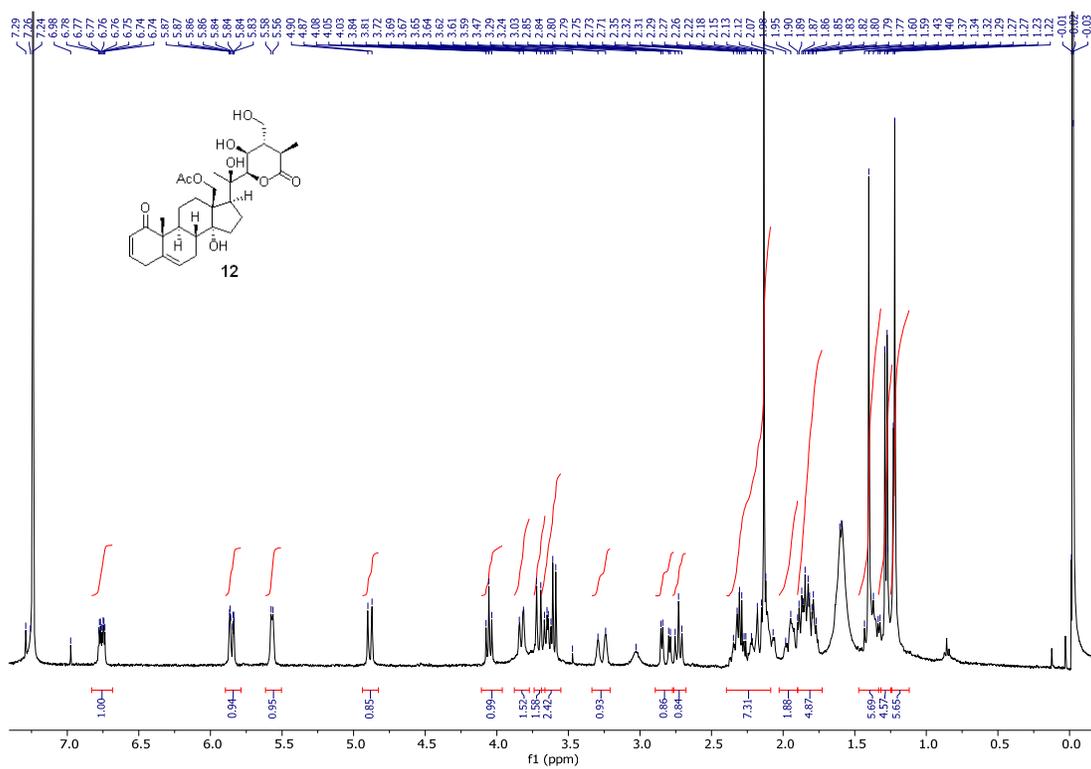


Figure S20. HSQC spectrum (400 MHz) of physacoctolide L (**12**) in CDCl₃

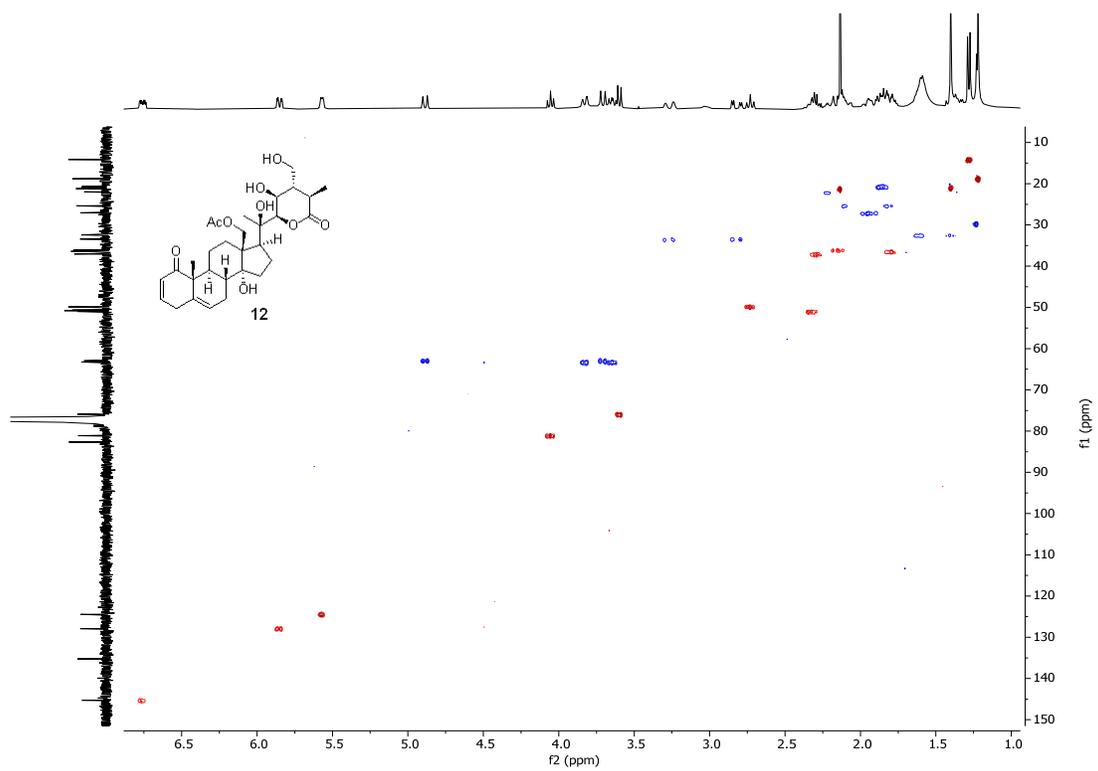


Figure S21. HMBC spectrum (400 MHz) of physacoctolide L (**12**) in CDCl₃

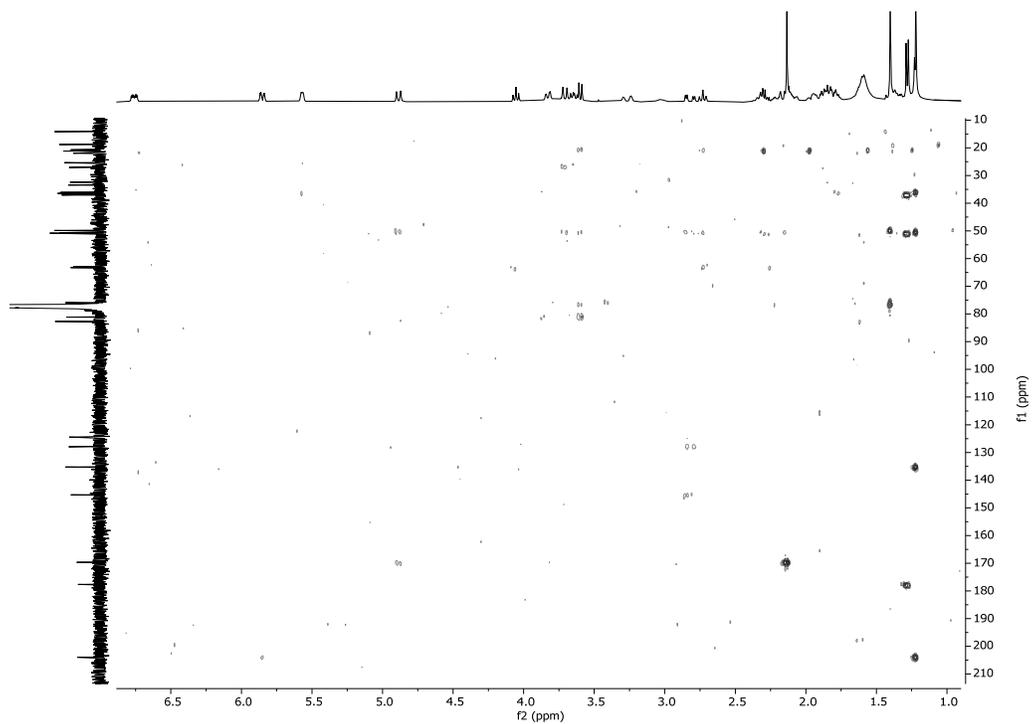


Figure S22. ^1H - ^1H COSY spectrum (400 MHz) of physacoctolide L (**12**) in CDCl_3

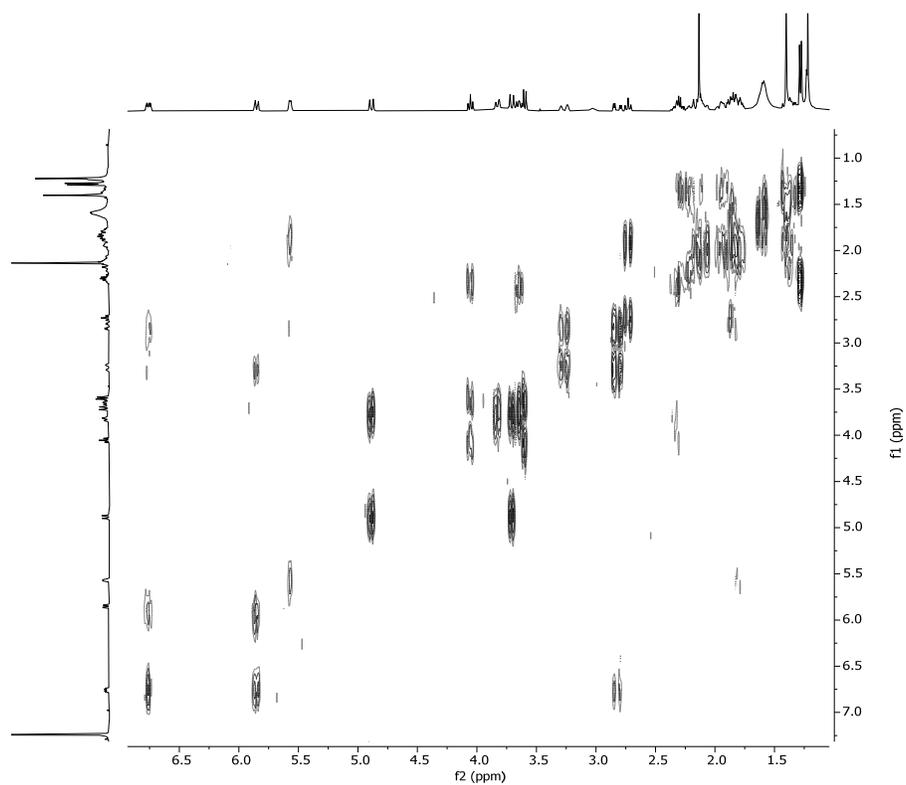


Figure S23. ^1H and 1D NOESY spectra (400 MHz) of physacoctolide L (**12**) in CDCl_3

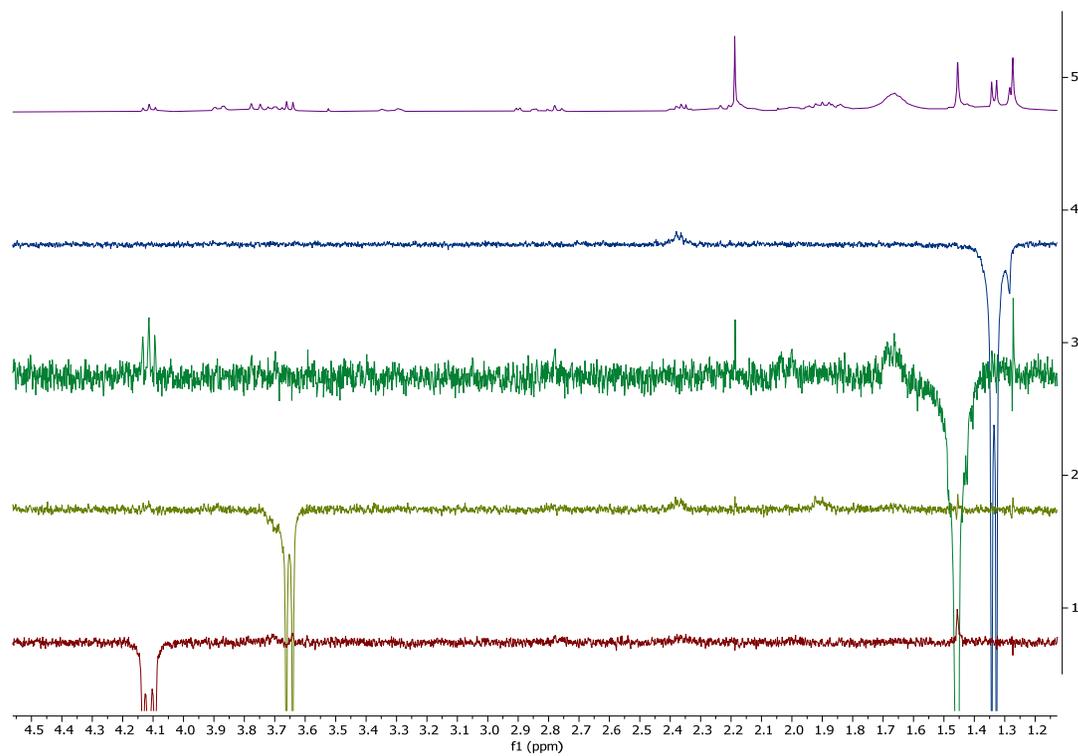


Figure S24. ¹H NMR spectrum (400 MHz) of 28-hydroxyphysachenolide C (**13**) in CD₃OD

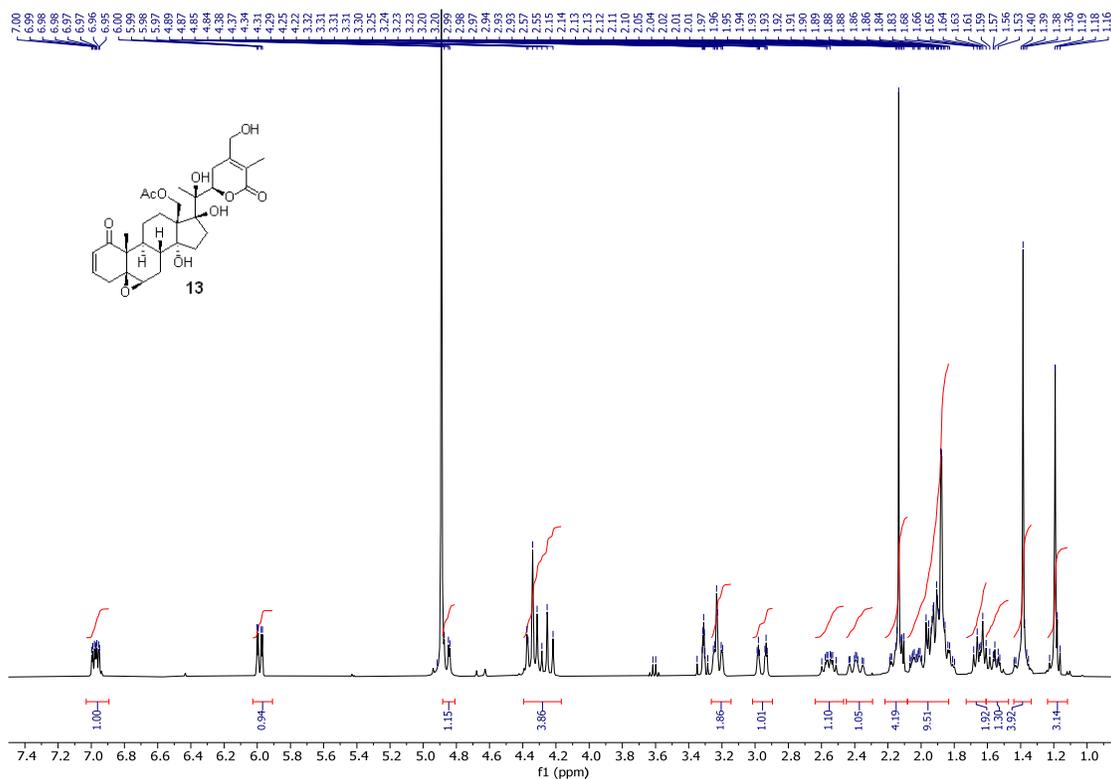


Figure S25. ¹³C NMR spectrum (100 MHz) of 28-hydroxyphysachenolide C (**13**) in CD₃OD

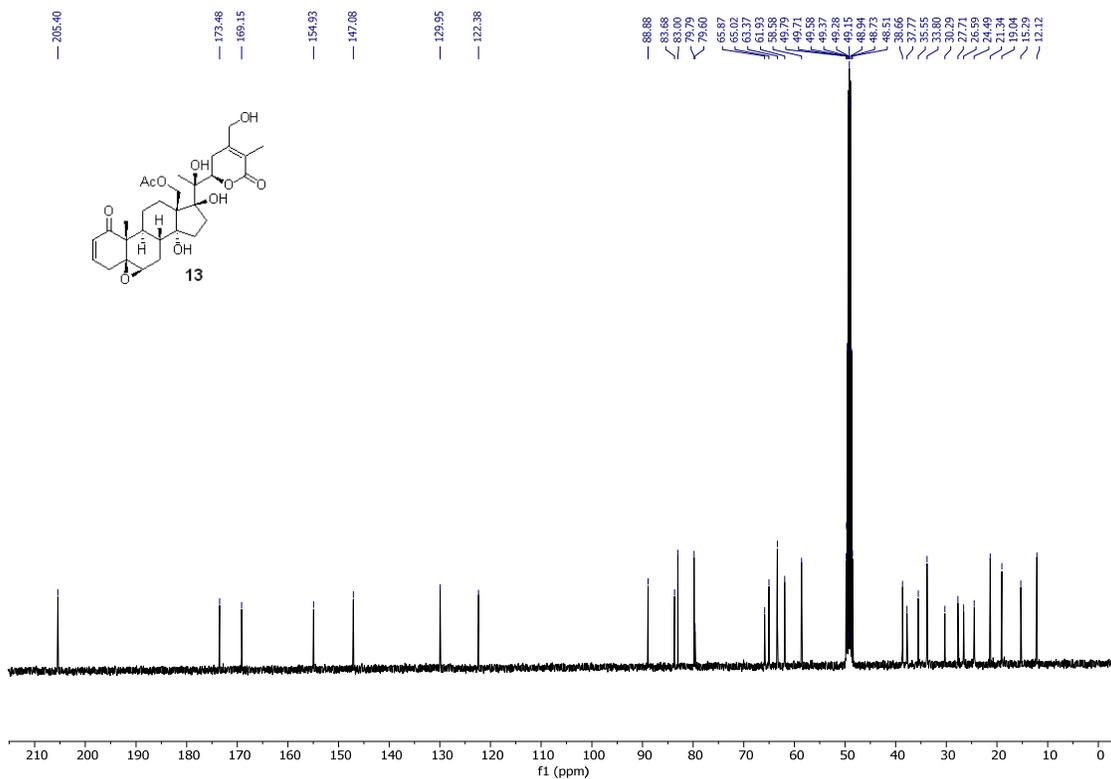


Figure S26. HSQC spectrum (400 MHz) of 28-hydroxyphysachenolide C (**13**) in CD₃OD

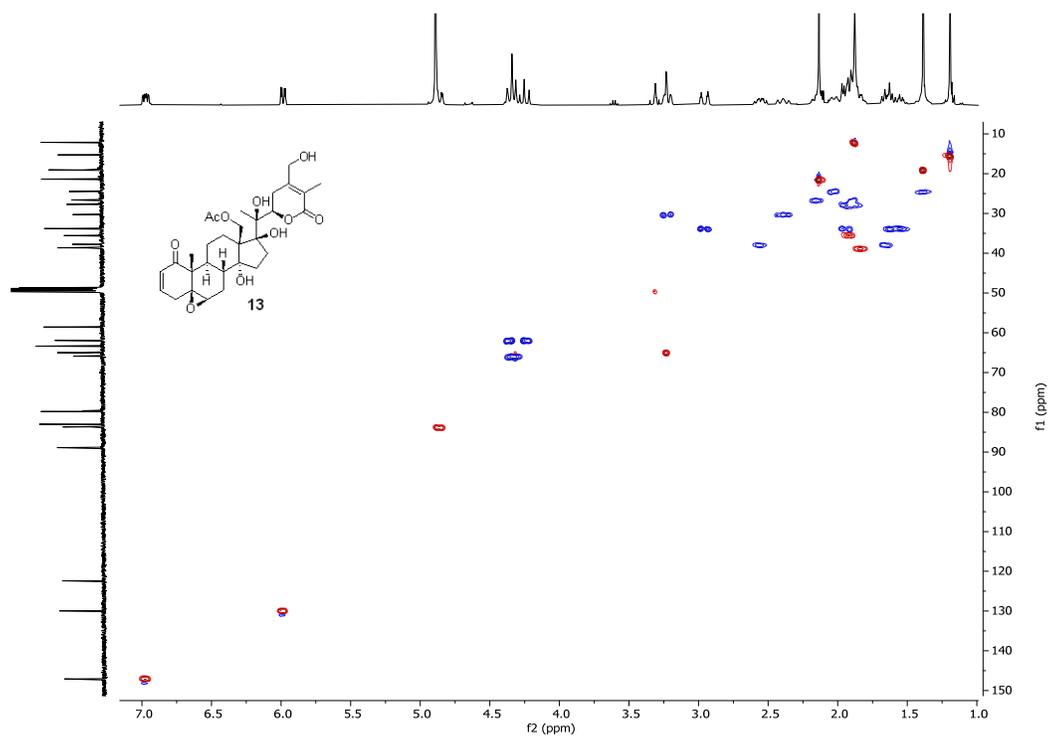


Figure S27. HMBC spectrum (400 MHz) of 28-hydroxyphysachenolide C (**13**) in CD₃OD

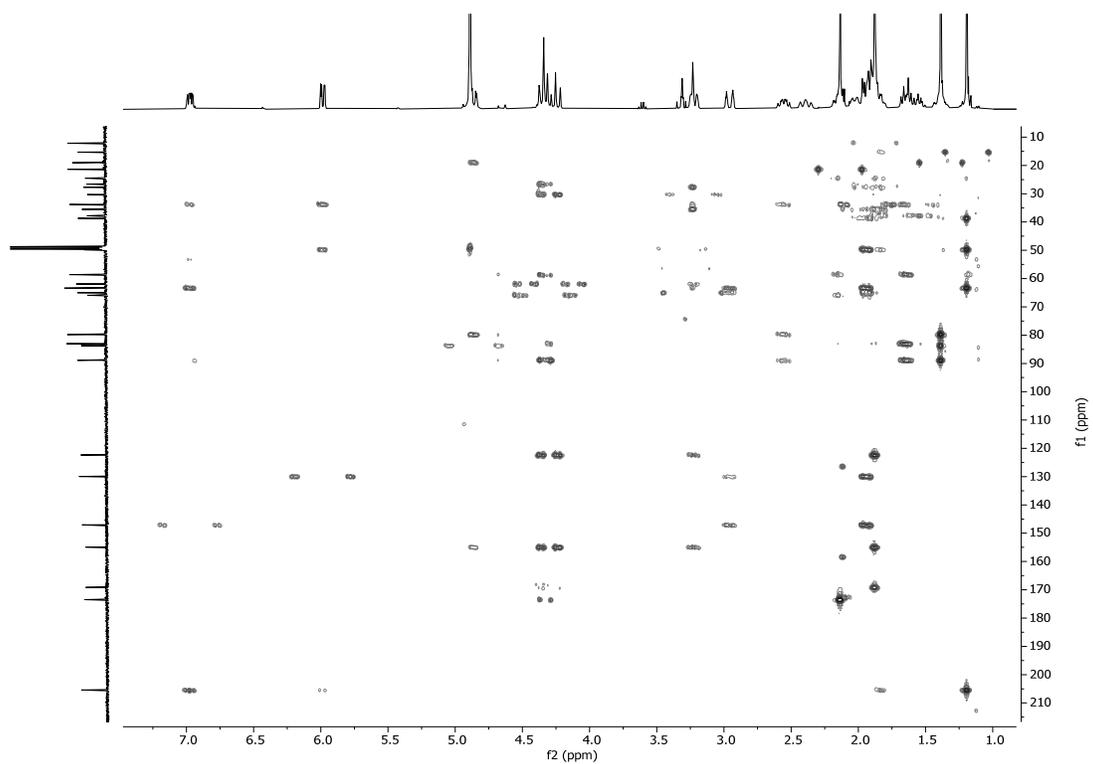


Figure S28. ^1H NMR spectrum (400 MHz) of 15α -acetoxy-28-hydroxyphysachenolide C (**14**) in CD_3OD

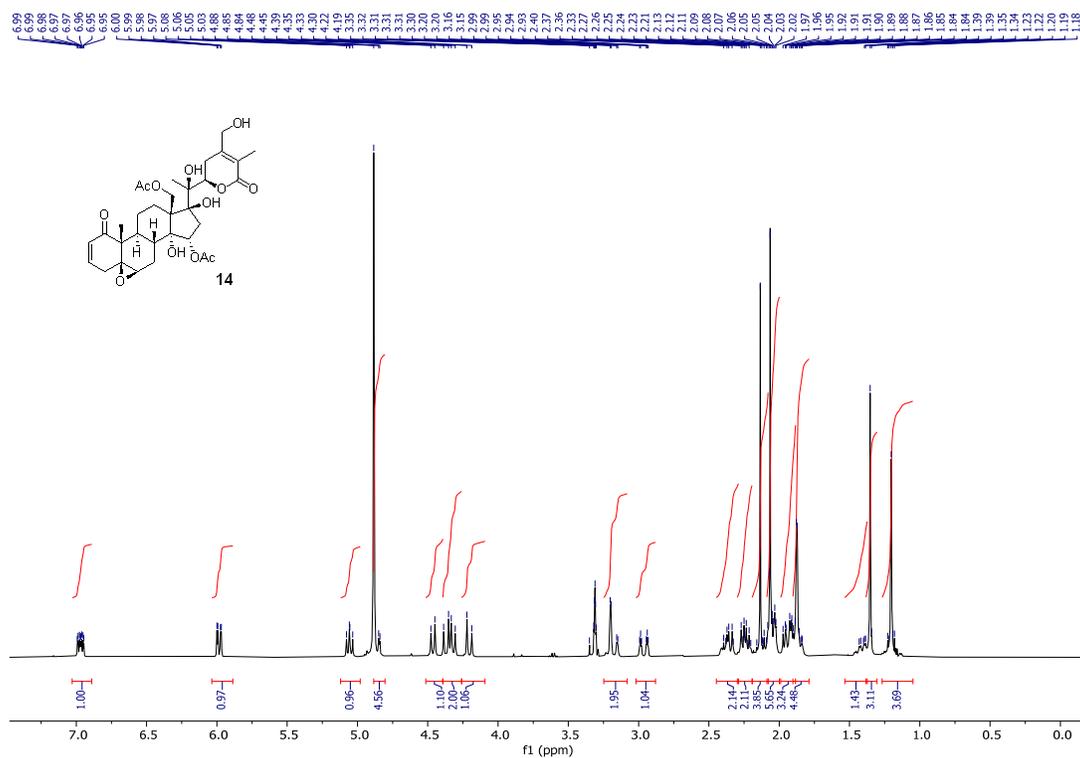


Figure S29. ^{13}C NMR spectrum (100 MHz) of 15α -acetoxy-28-hydroxyphysachenolide C (**14**) in CD_3OD

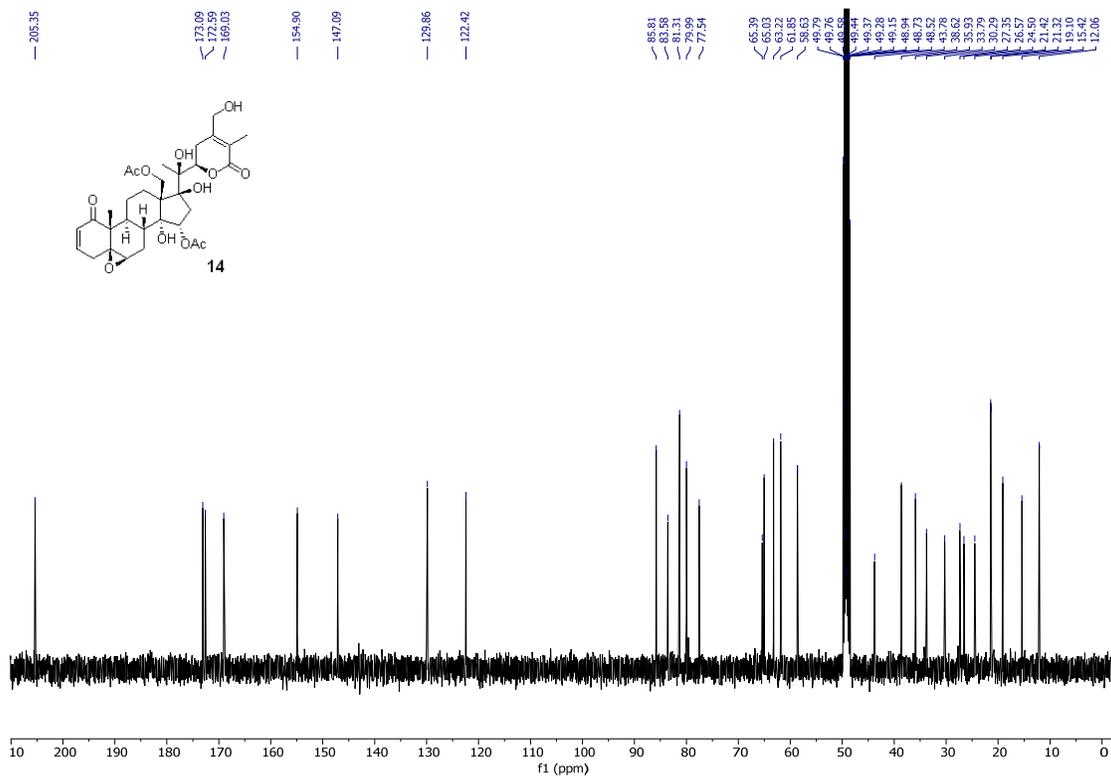


Figure S30. HSQC spectrum (400 MHz) of 15 α -acetoxy-28-hydroxyphysachenolide C (**14**) in CD₃OD

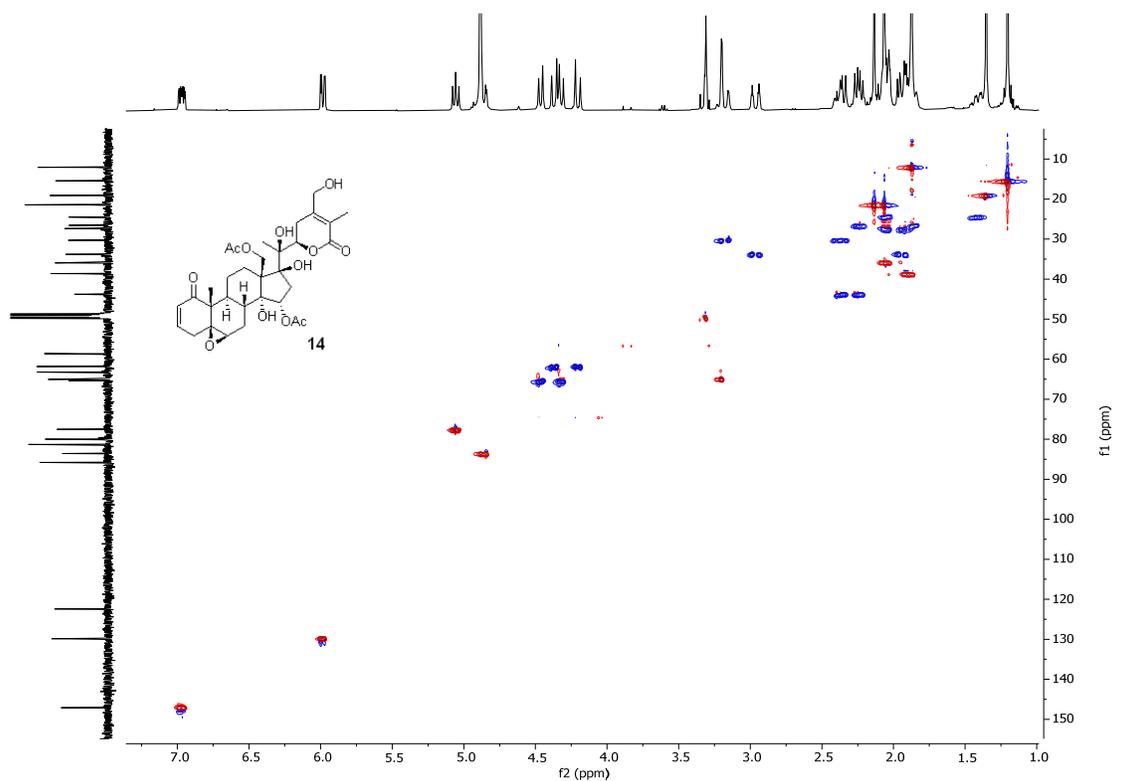


Figure S31. HMBC spectrum (400 MHz) of 15 α -acetoxy-28-hydroxyphysachenolide C (**14**) in CD₃OD

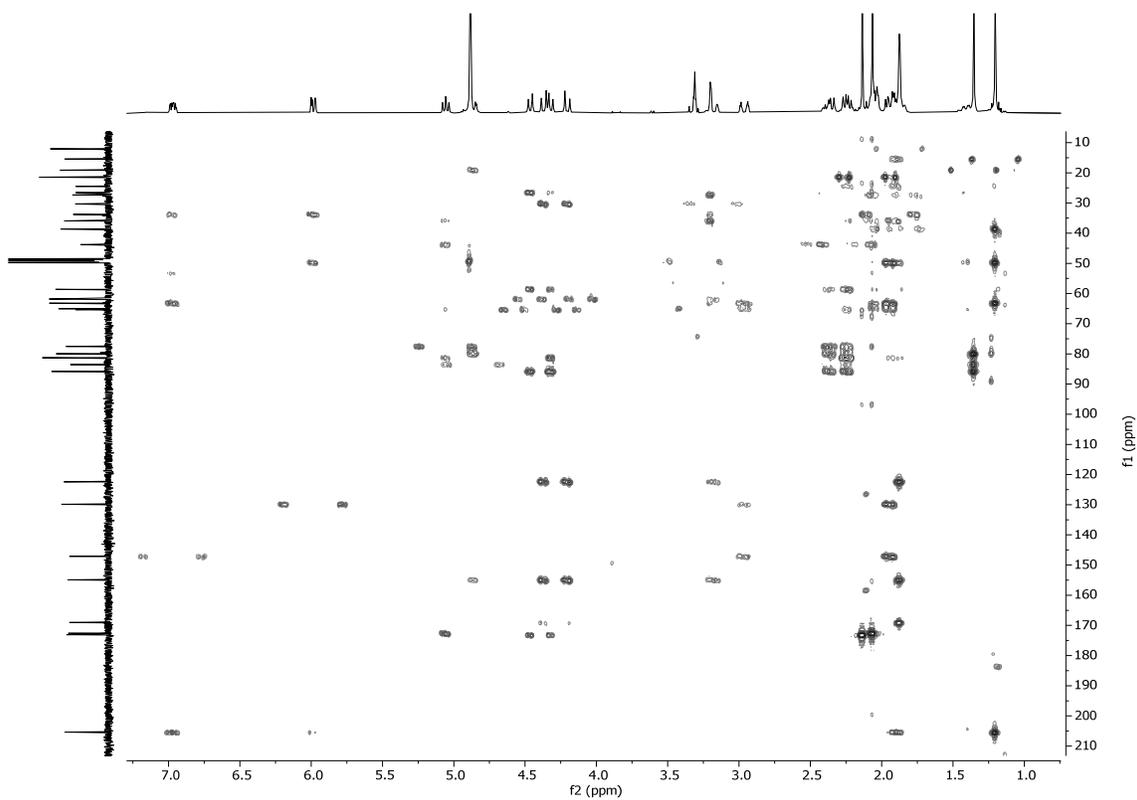


Figure S32. ¹H NMR spectrum (400 MHz) of 28-oxophysachenolide C (**15**) in CDCl₃

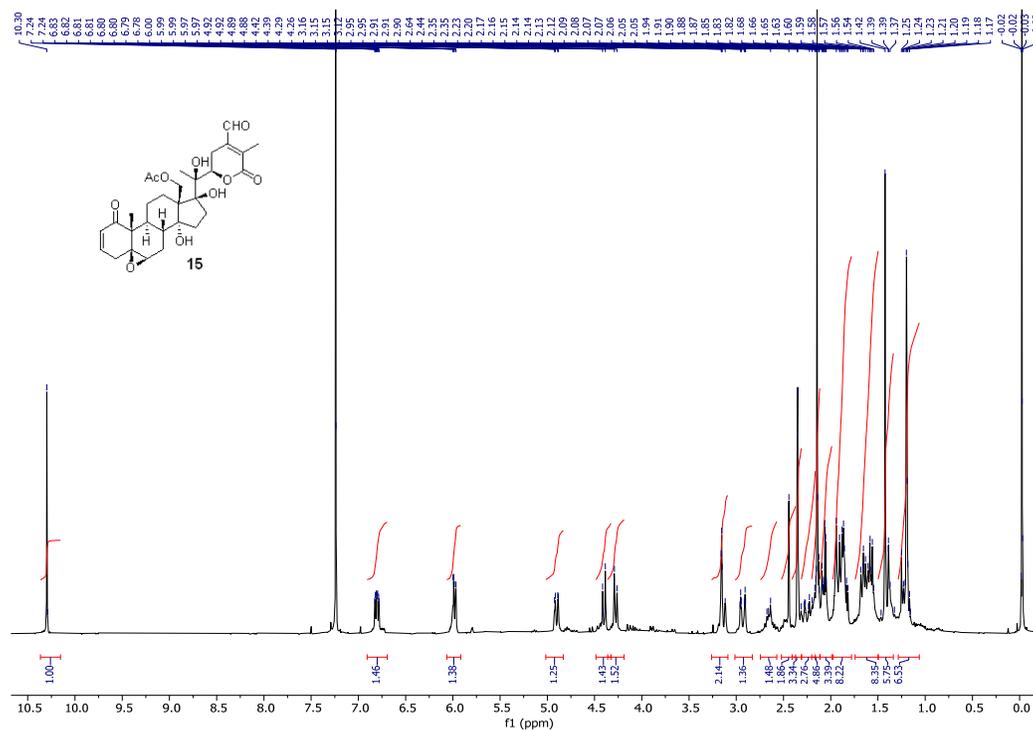


Figure S33. ¹³C NMR spectrum (100 MHz) of 28-oxophysachenolide C (**15**) in CDCl₃

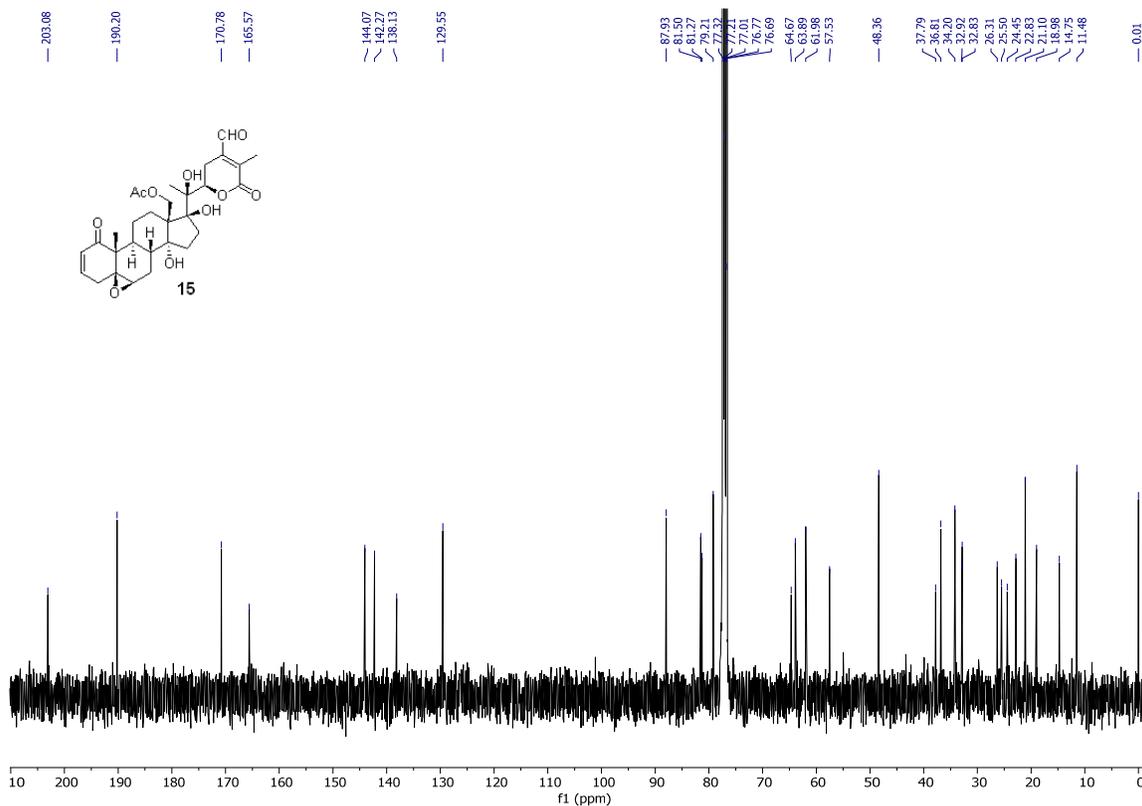


Figure S34. HSQC spectrum (400 MHz) of 28-oxophysachenolide C (**15**) in CDCl₃

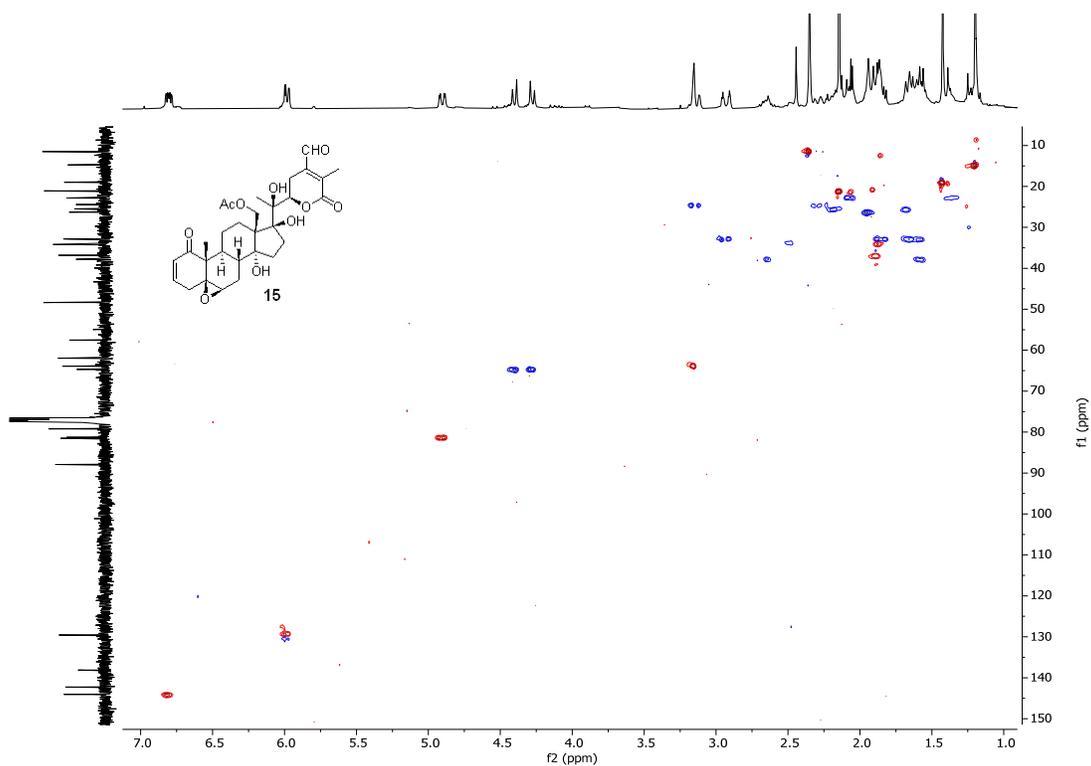


Figure S35. HMBC spectrum (400 MHz) of 28-oxophysachenolide C (**15**) in CDCl₃

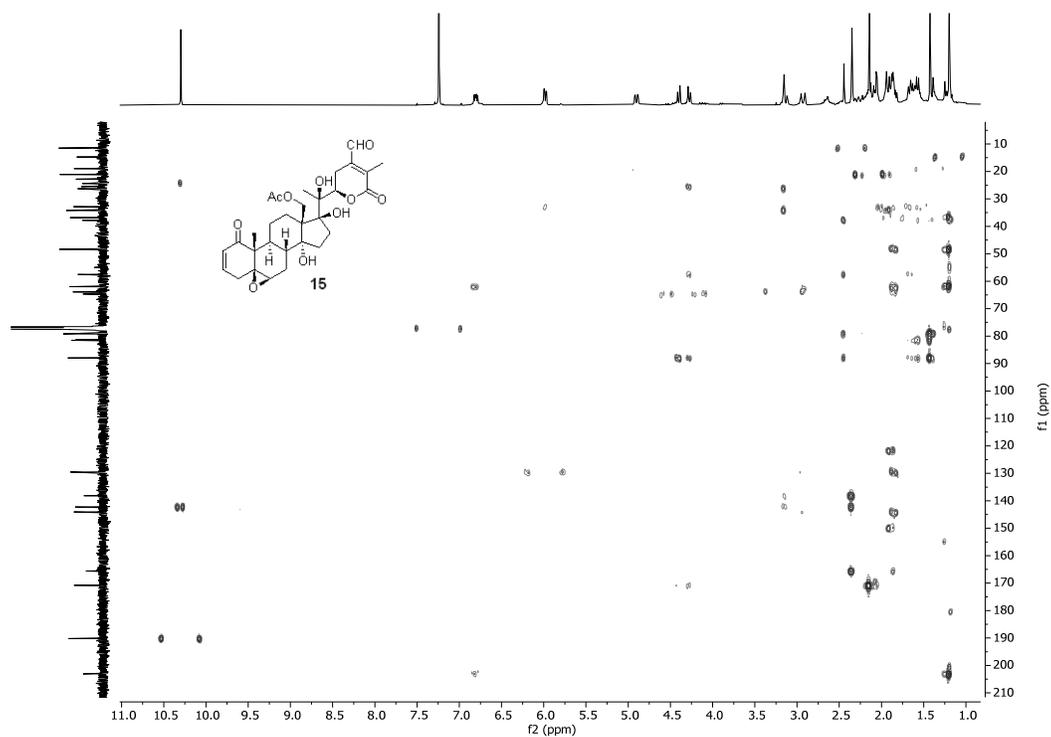


Figure S36. ¹H NMR spectrum (400 MHz) of physacoctolide M (**16**) in CDCl₃/CD₃OD (100:1)

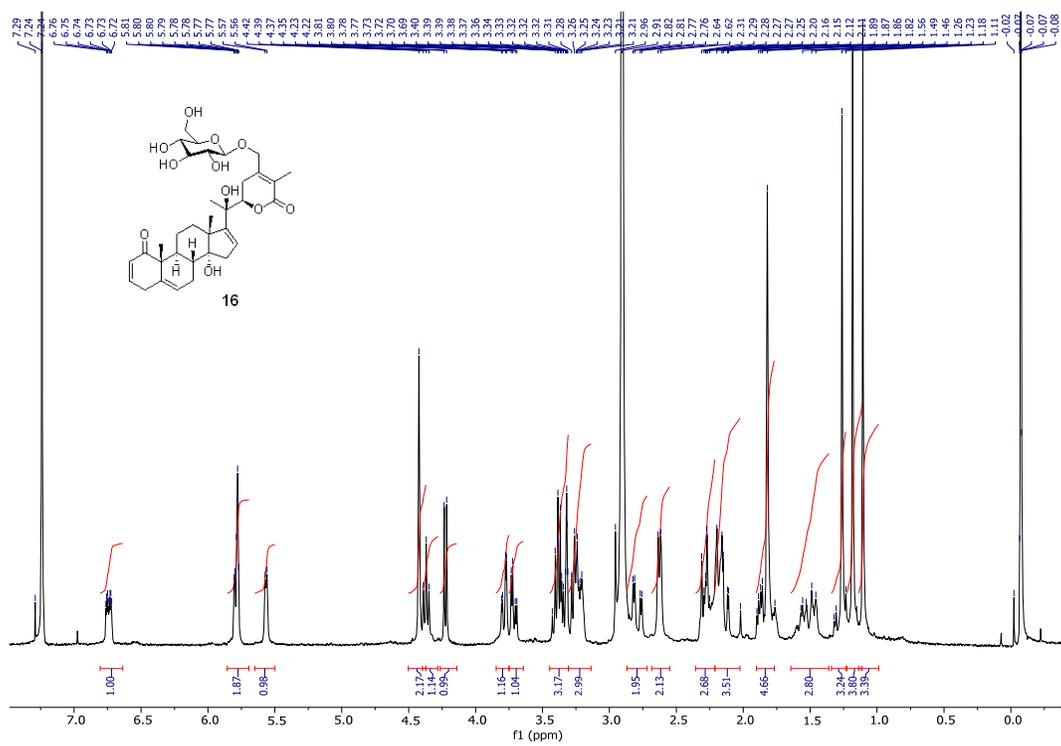


Figure S37. ¹³C NMR spectrum (100 MHz) of physacoctolide M (**16**) in CDCl₃/CD₃OD (100:1)

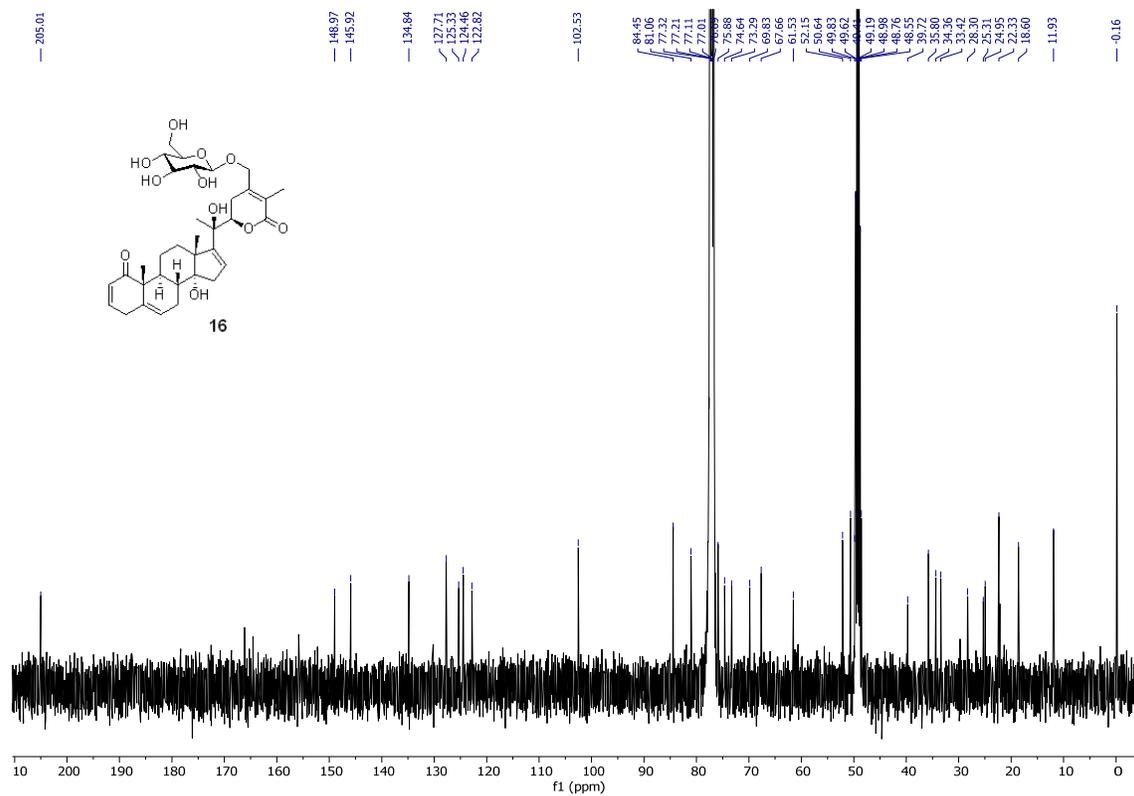


Figure S38. HSQC spectrum (400 MHz) of physacoctolide M (**16**) in CDCl₃/CD₃OD (100:1)

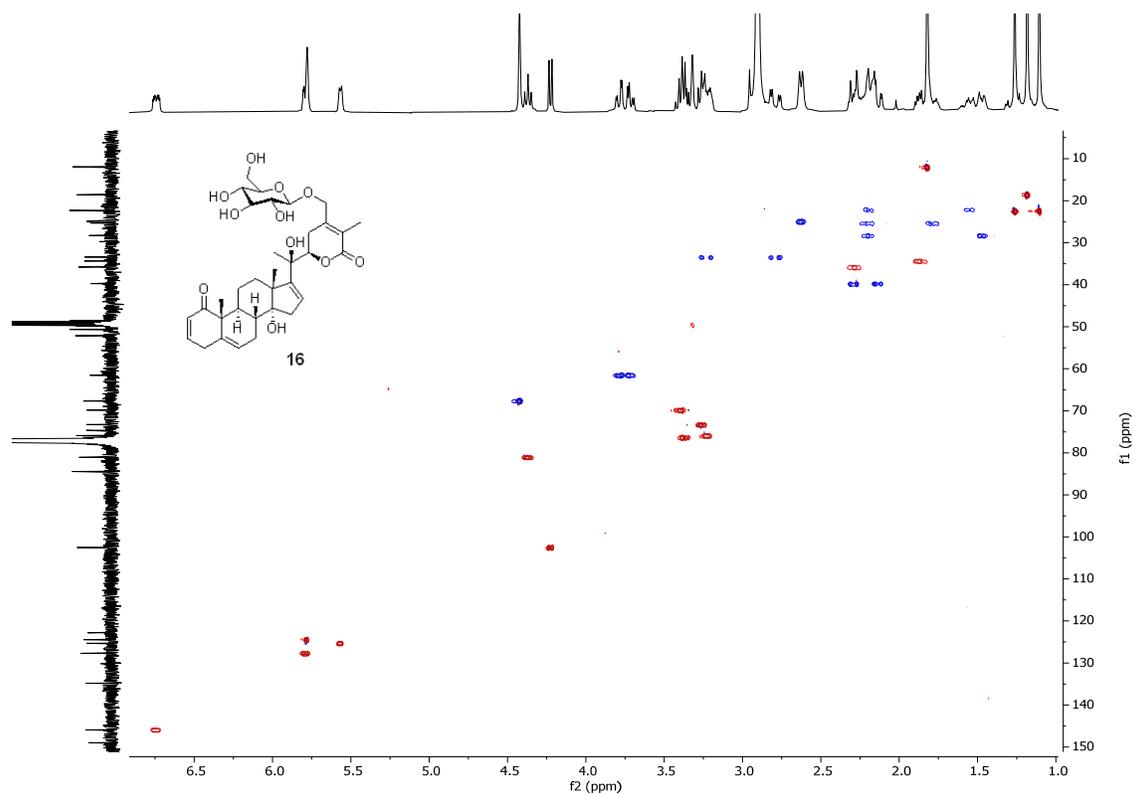


Figure S39. HMBC spectrum (400 MHz) of physacoctolide M (**16**) in CDCl₃/CD₃OD (100:1)

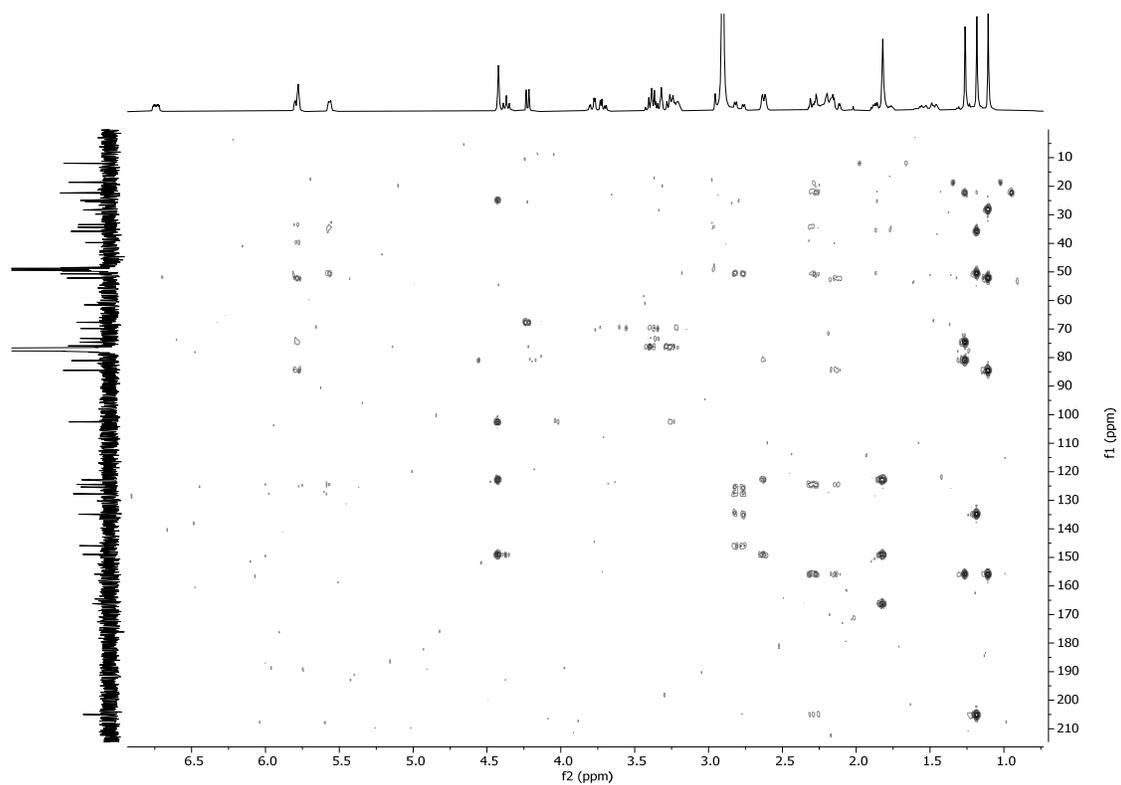


Figure S40. ¹H NMR spectrum (400 MHz) of 5 α -chloro-6 β -hydroxy-5,6-dihydrophysachenolide D (17) in CDCl₃

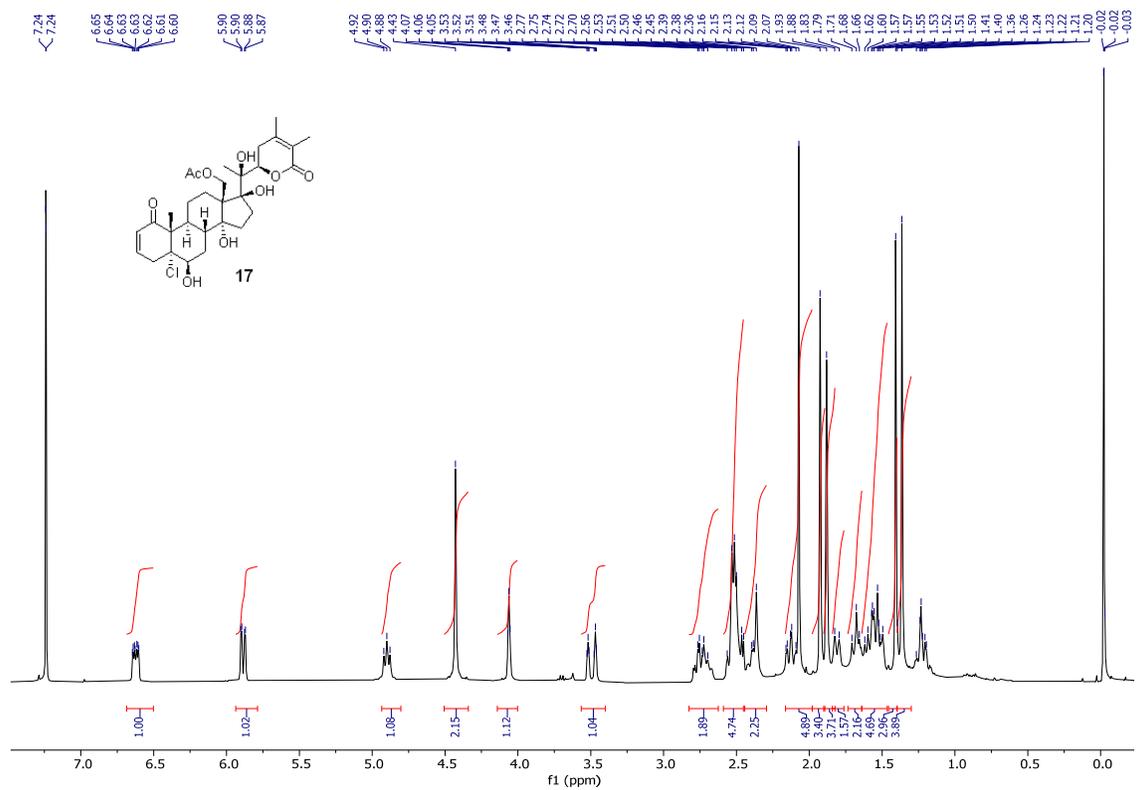


Figure S41. ¹³C NMR spectrum (100 MHz) of 5 α -chloro-6 β -hydroxy-5,6-dihydrophysachenolide D (17) in CDCl₃

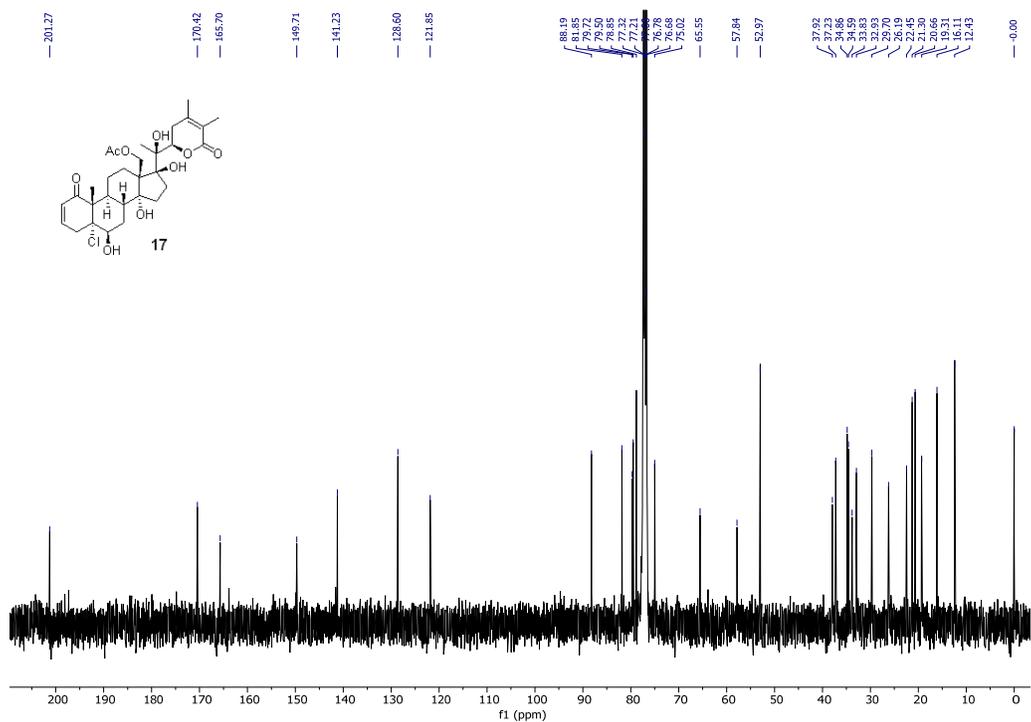


Figure S42. HSQC spectrum (400 MHz) of 5 α -chloro-6 β -hydroxy-5,6-dihydrophysachenolide D (**17**) in CDCl₃

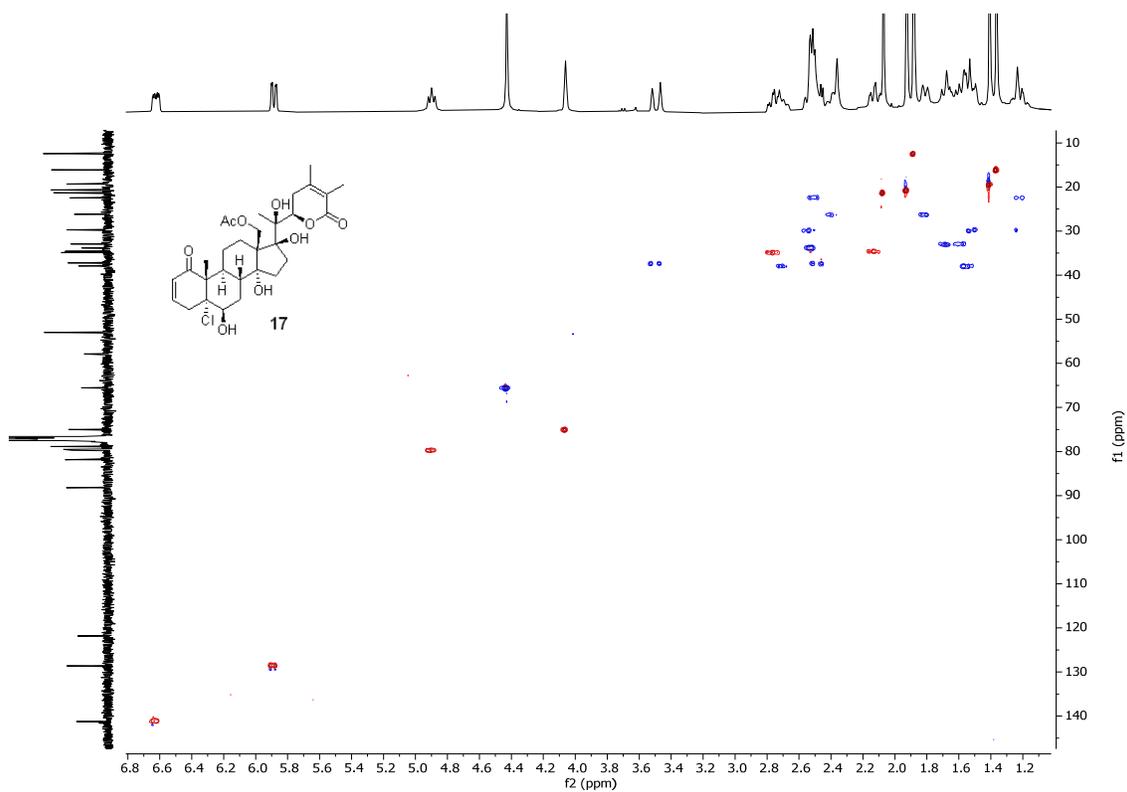


Figure S43. HMBC spectrum (400 MHz) of 5 α -chloro-6 β -hydroxy-5,6-dihydrophysachenolide D (**17**) in CDCl₃

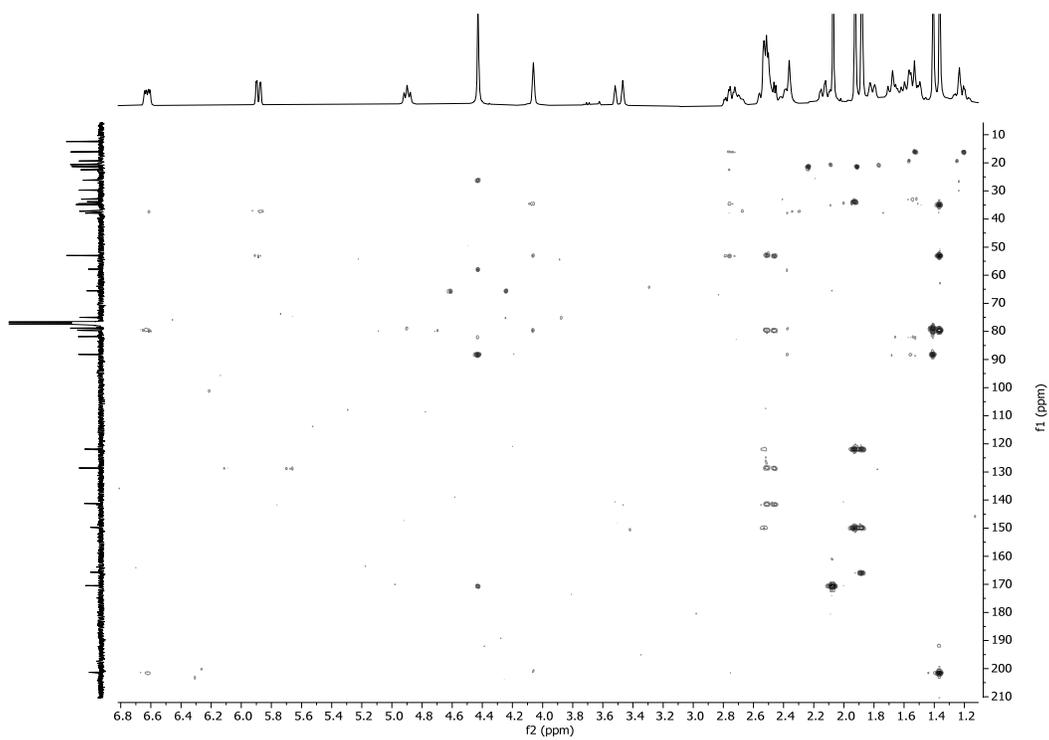


Figure S44. ^1H and 1D NOESY spectra (400 MHz) of 5 α -chloro-6 β -hydroxy-5,6-dihydrophysachenolide D (**17**) in CDCl_3

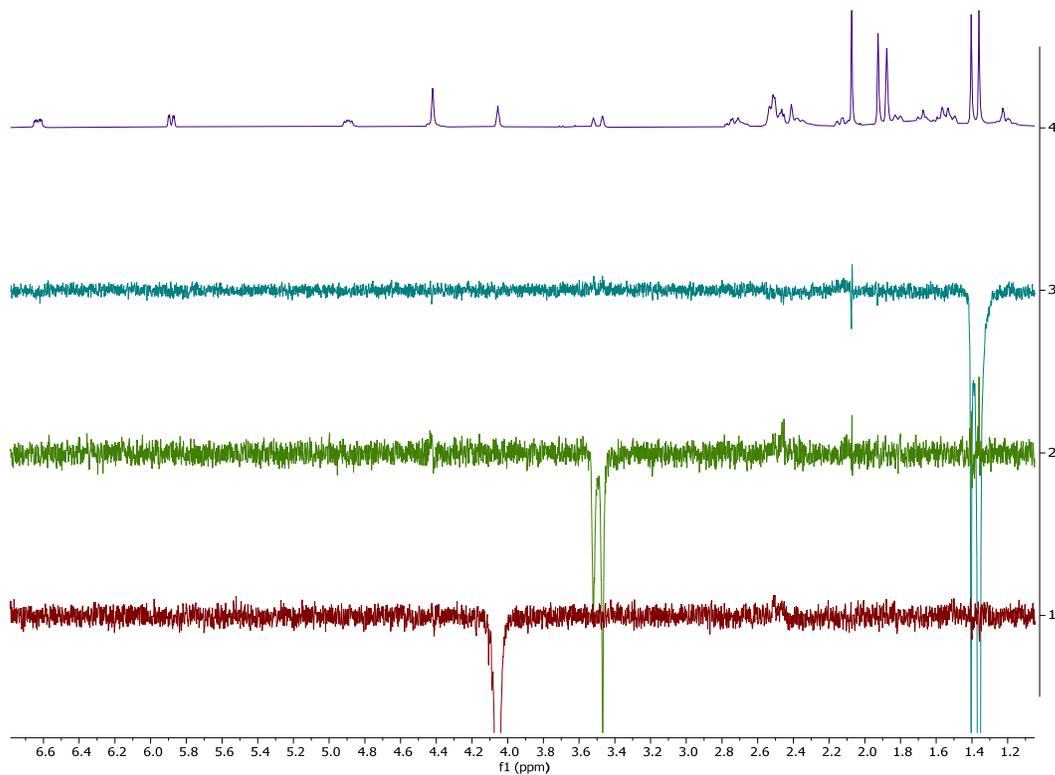


Figure S45. ^1H NMR spectrum (400 MHz) of 15 α -acetoxy-5 α -chloro-6 β -hydroxyphysachenolide C (**18**) in CDCl_3

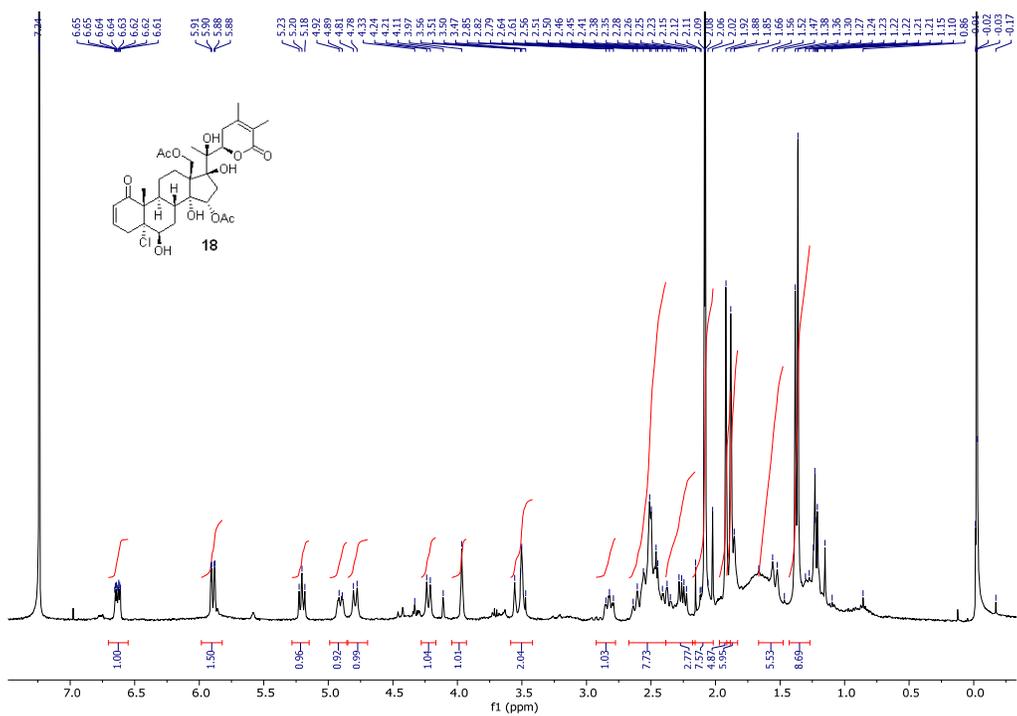


Figure S46. ^{13}C NMR spectrum (100 MHz) of 15 α -acetoxy-5 α -chloro-6 β -hydroxy-5,6-dihydrophysachenolide D (**18**) in CDCl_3

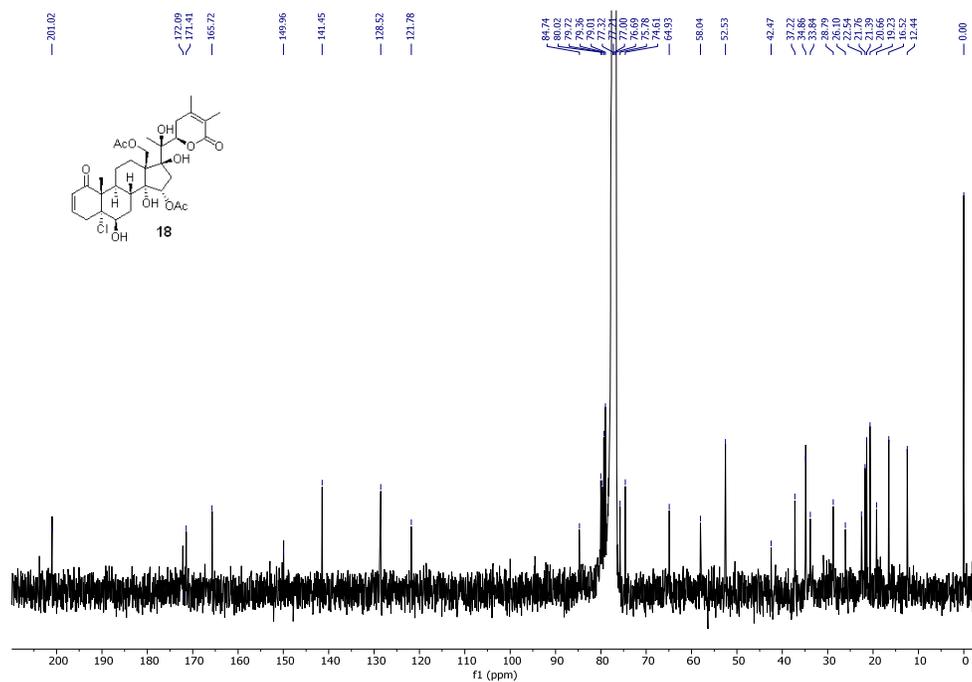


Figure S47. HSQC spectrum (400 MHz) of 15 α -acetoxy-5 α -chloro-6 β -hydroxy-5,6-dihydrophysachenolide D (**18**) in CDCl_3

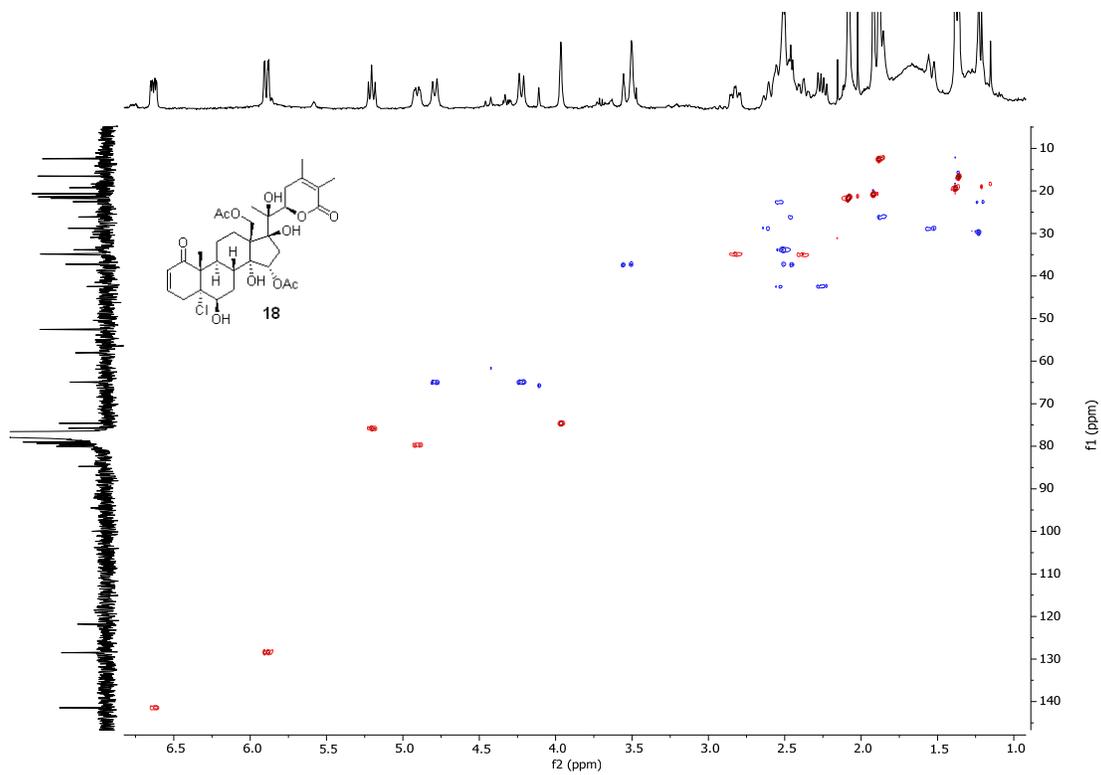


Figure S48. HMBC spectrum (400 MHz) of 15 α -acetoxy-5 α -chloro-6 β -hydroxy-5,6-dihydrophysachenolide D (**18**) in CDCl₃

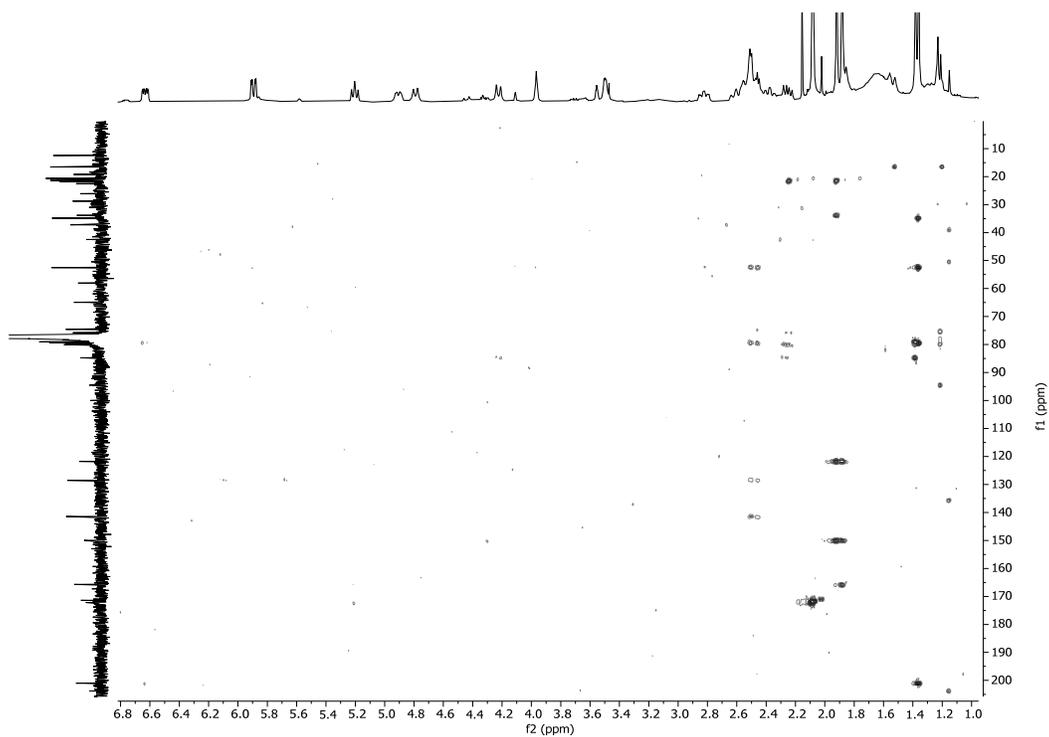


Figure S49. ¹H and 1D NOESY spectra (400 MHz) of 15 α -acetoxy-5 α -chloro-6 β -hydroxy-5,6-dihydrophysachenolide D (**18**) in CDCl₃

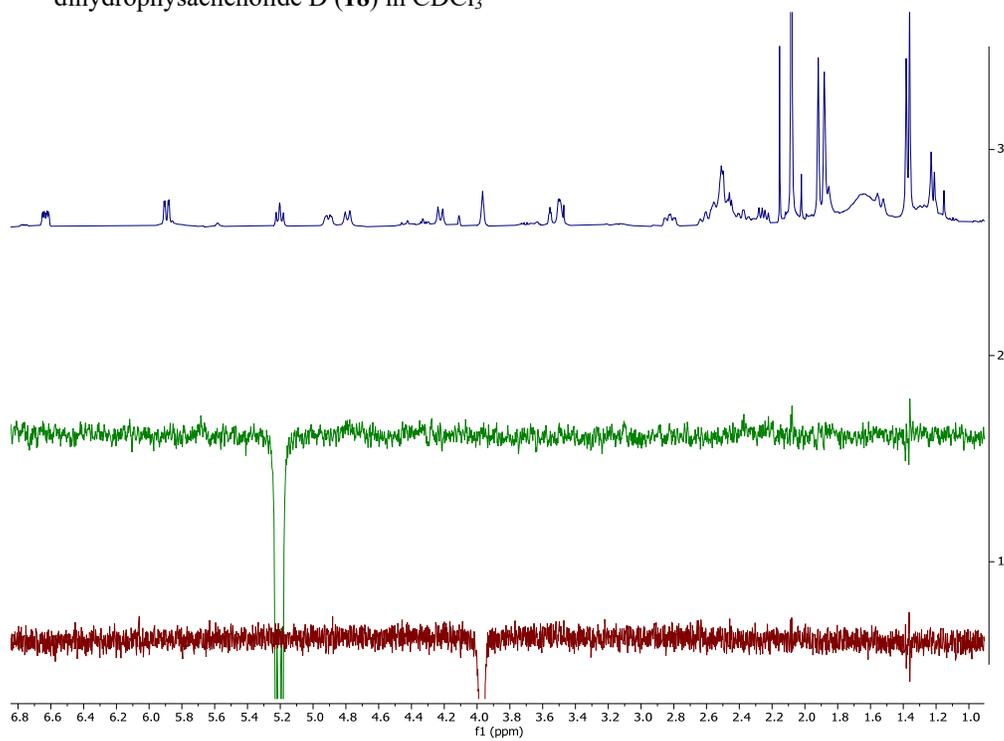


Figure S51. ^{13}C NMR spectrum (100 MHz) of 28-hydroxy-5 α -chloro-6 β -hydroxy-5,6-dihydrophysachenolide D (**19**) in CDCl_3

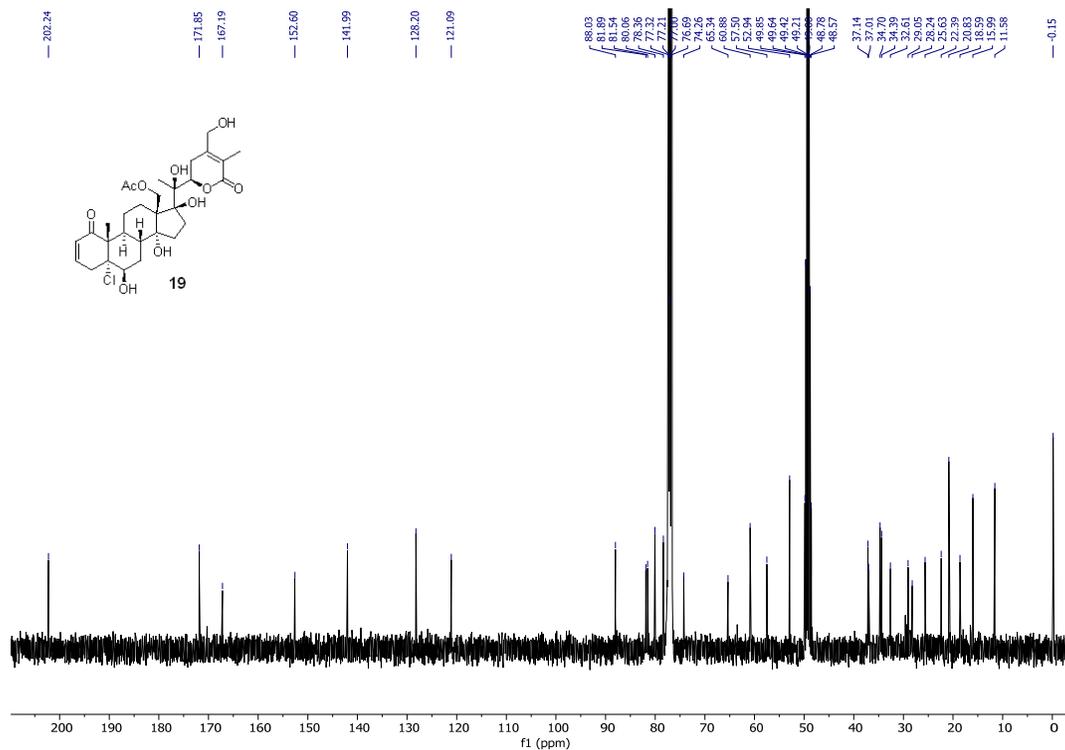


Figure S51a. ^{13}C NMR spectrum (100 MHz) of 28-hydroxy-5 α -chloro-6 β -hydroxy-5,6-dihydrophysachenolide D (**19**) in CD_3OD

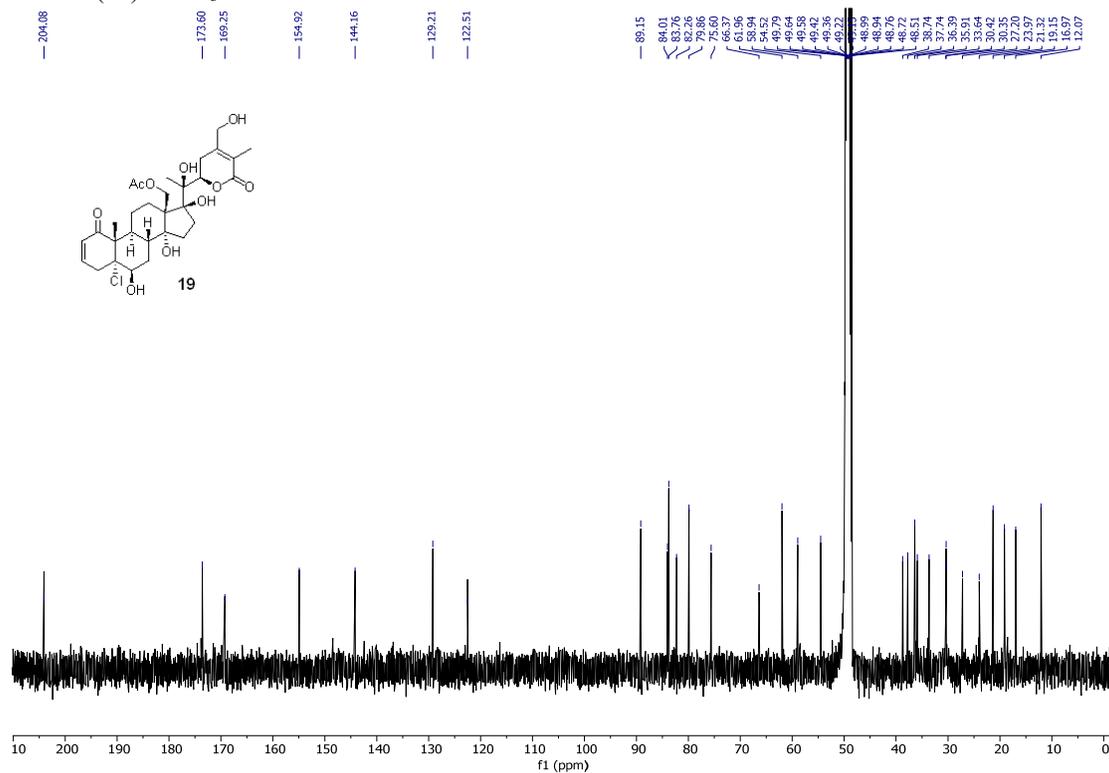


Figure S52. HSQC spectrum (400 MHz) of 28-hydroxy-5 α -chloro-6 β -hydroxy-5,6-dihydrophysachenolide D (**19**) in CD₃OD

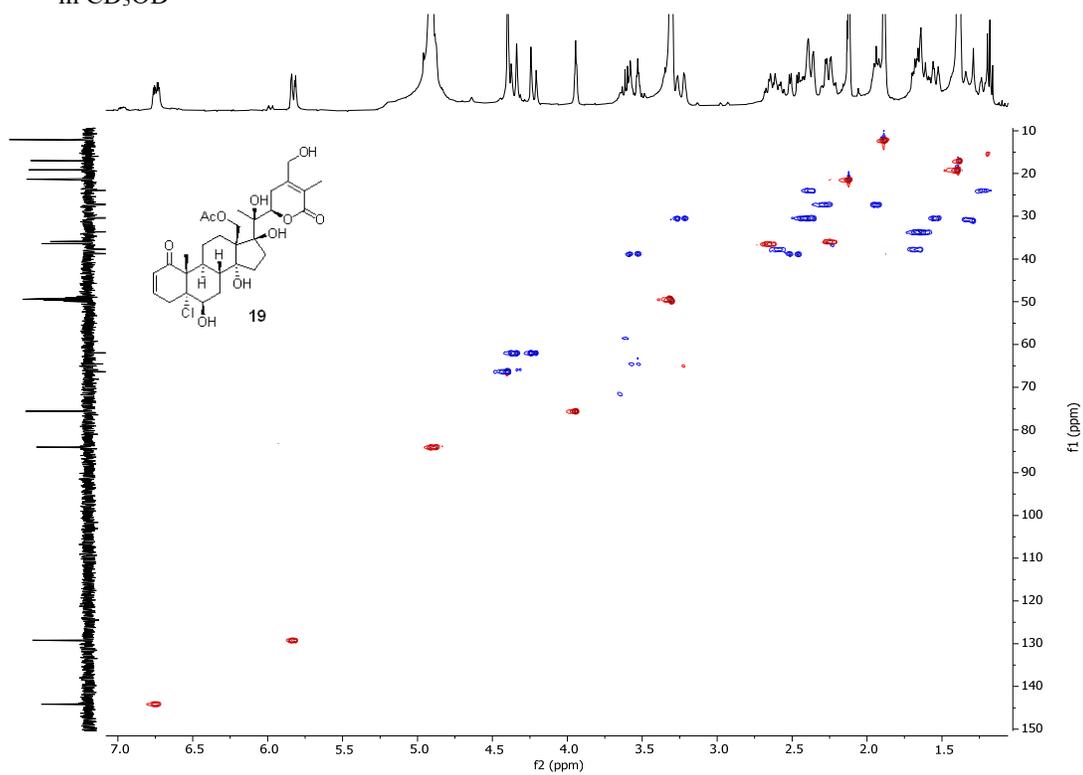


Figure S53. HMBC spectrum (400 MHz) of 28-hydroxy-5 α -chloro-6 β -hydroxy-5,6-dihydrophysachenolide D (**19**) in CD₃OD

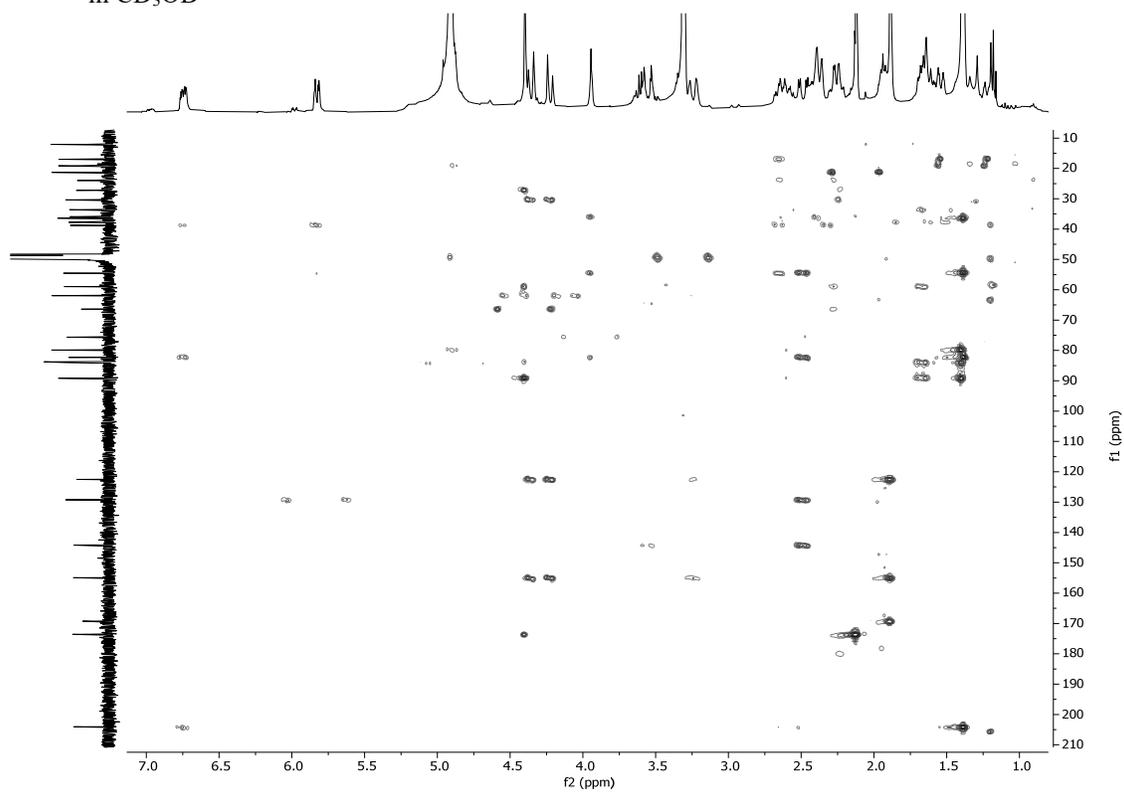


Figure S54. ^1H - ^1H COSY spectrum (400 MHz) of 28-hydroxy-5 α -chloro-6 β -hydroxy-5,6-dihydrophysachenolide D (**19**) in CD_3OD

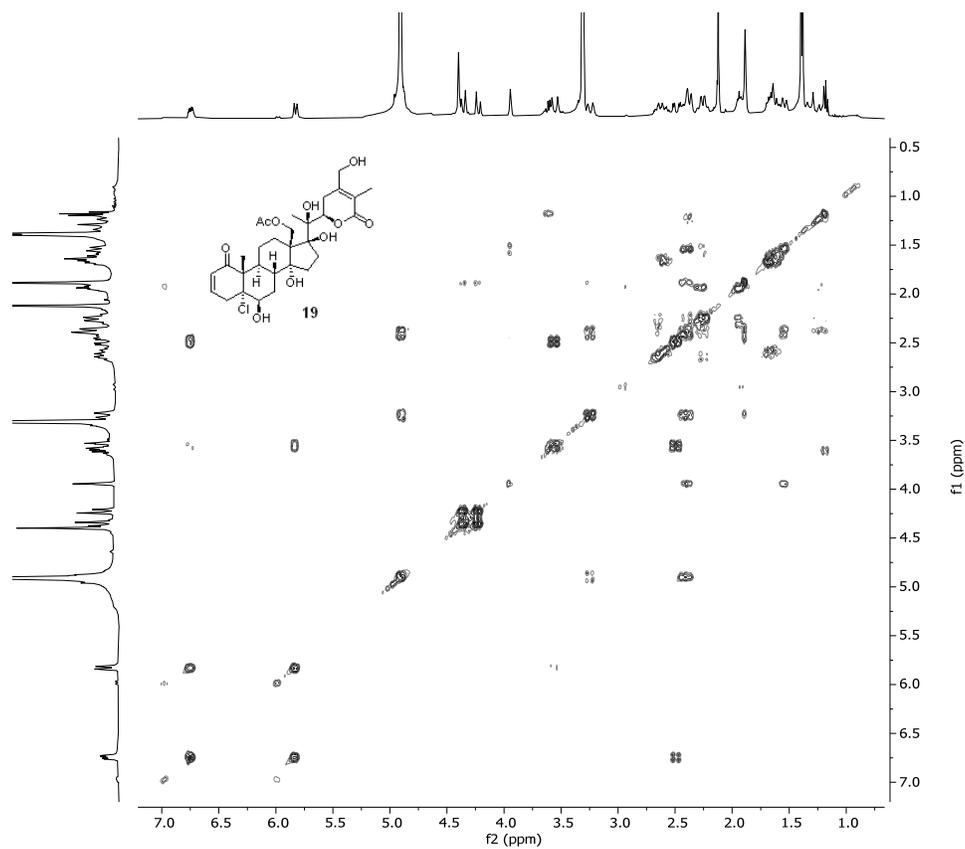


Figure S55. ^1H NMR spectrum (400 MHz) of physachenolide A-5-methyl ether (**20**) in CDCl_3

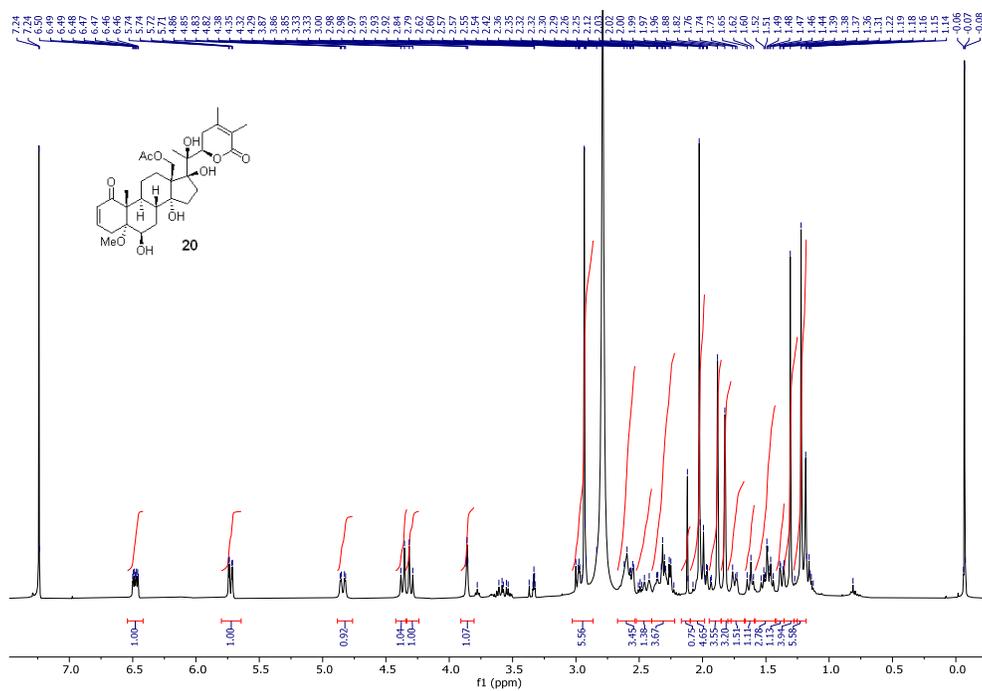


Figure S56. ^{13}C NMR spectrum (100 MHz) of physachenolide A-5-methyl ether (**20**) in $\text{CDCl}_3/\text{CD}_3\text{OD}$ (100:1)

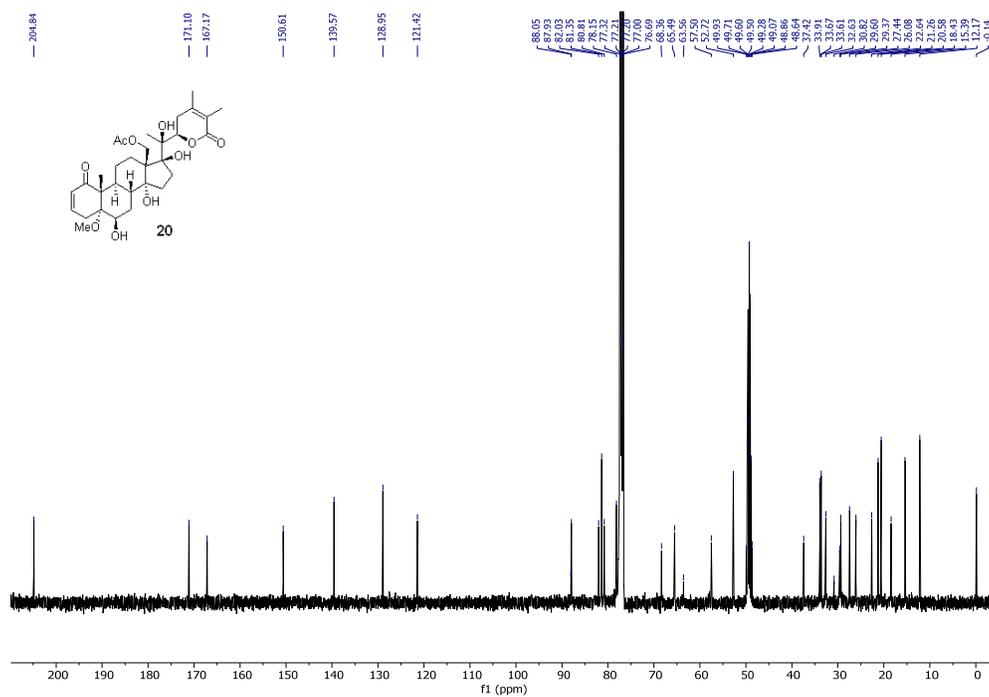


Figure S57. HSQC spectrum (400 MHz) of physachenolide A-5-methyl ether (**20**) in $\text{CDCl}_3/\text{CD}_3\text{OD}$ (100:1)

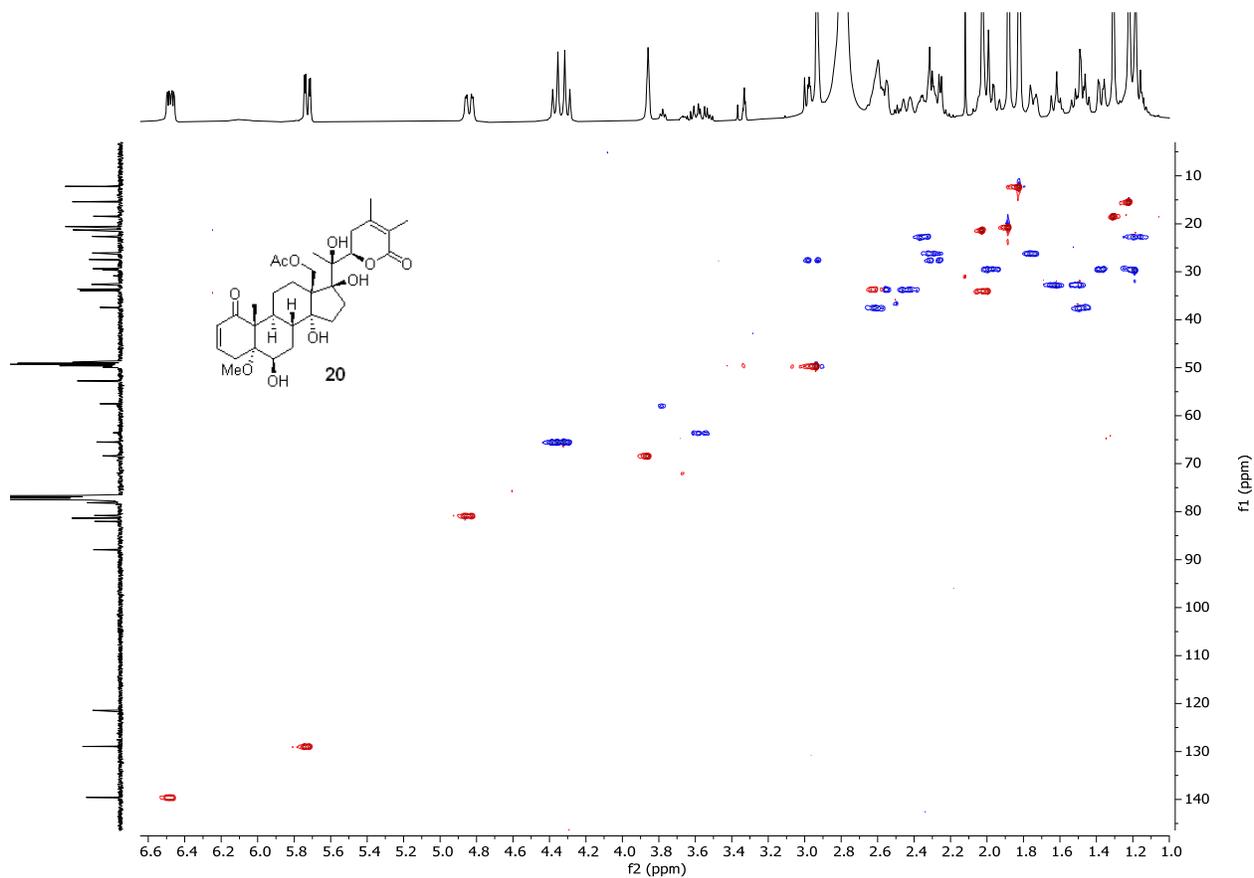


Figure S58. HMBC spectrum (400 MHz) of physachenolide A-5-methyl ether (**20**) in CDCl₃/CD₃OD (100:1)

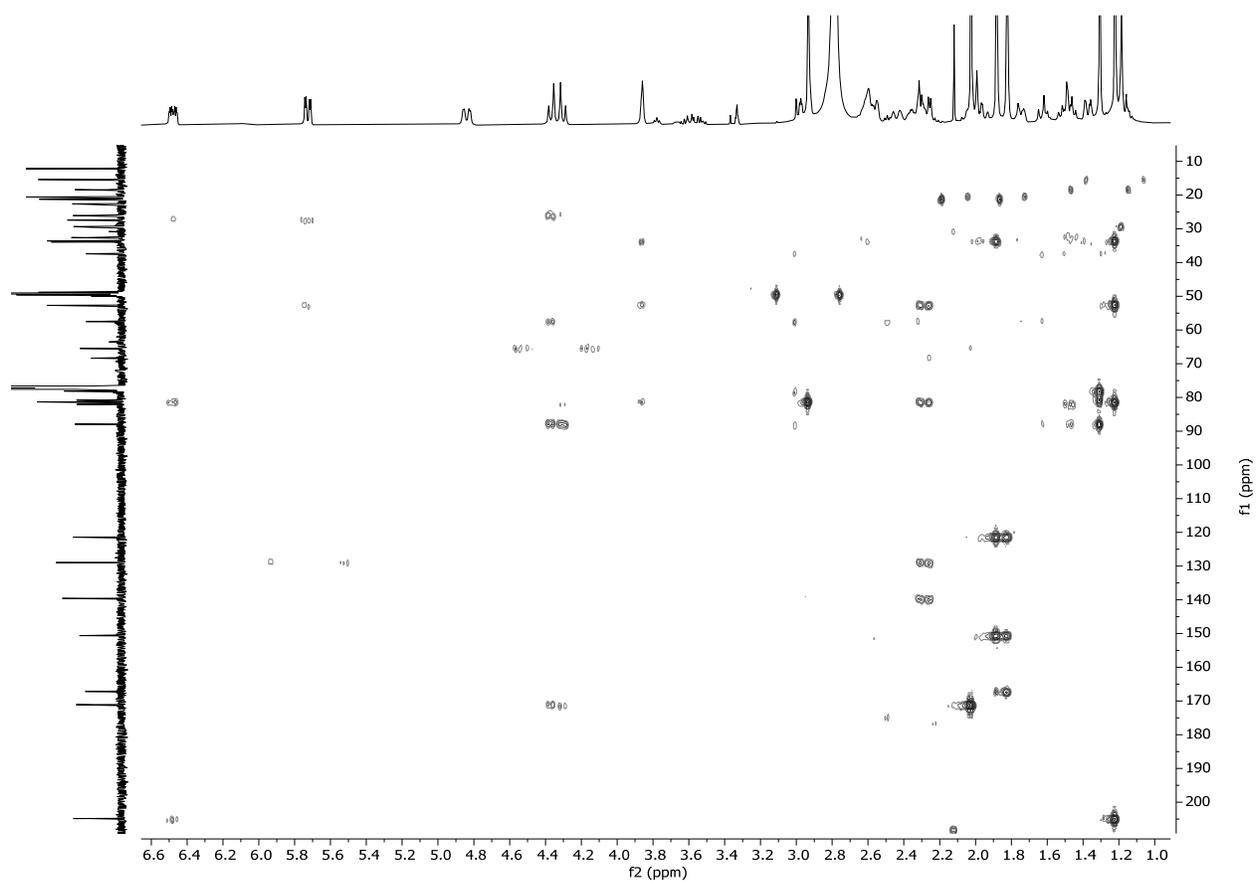


Figure S59. ECD spectra of withanolides 9–20.

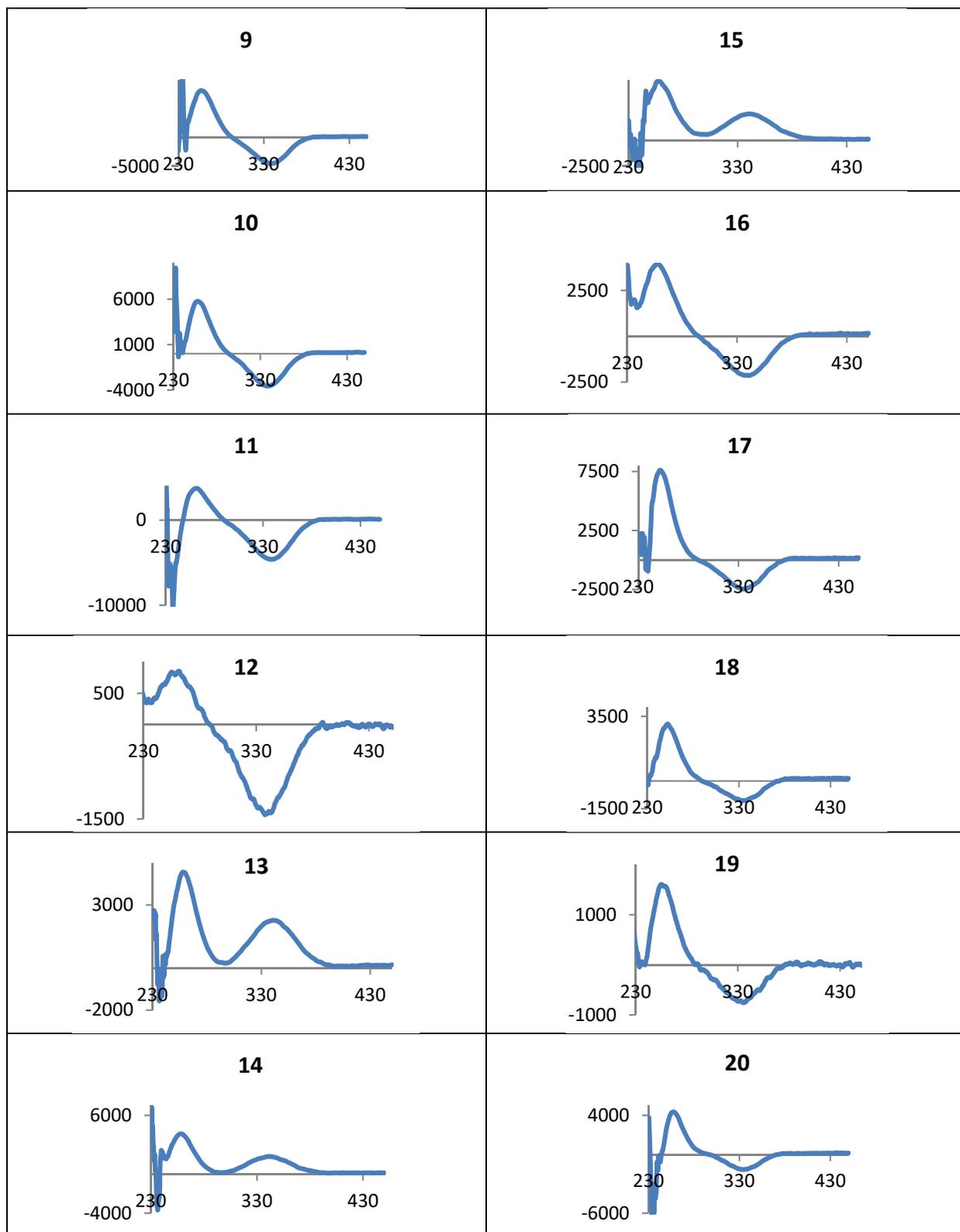


Figure S60. Key HMBC correlations (\rightarrow) of **9–20** and Key ^1H - ^1H COSY correlations (\leftarrow) of **11, 12, 19, and 20**

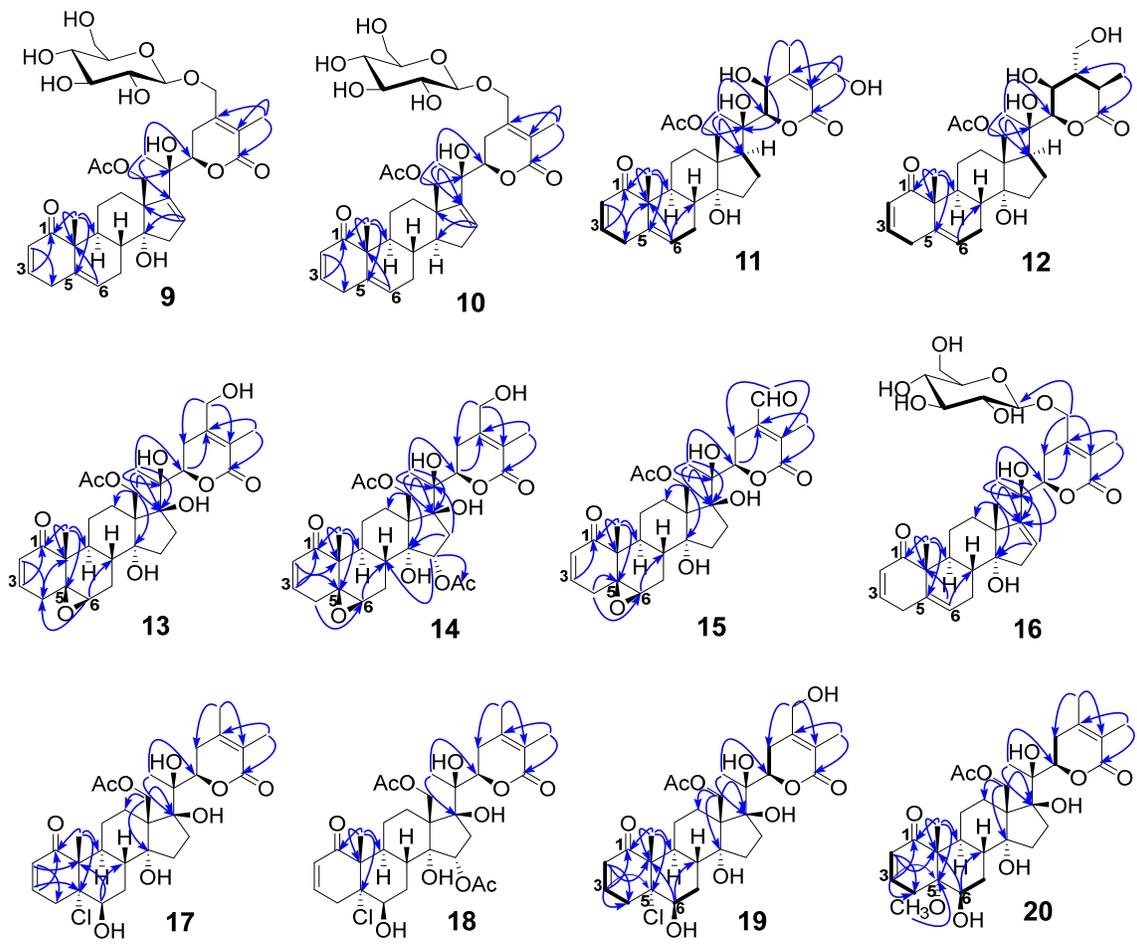


Figure S62. Investigation of products formed on exposure of physachenolide C (**8**) to mild acidic conditions by HPLC. **A:** HPLC trace of **8** in MeOH/H₂O. **B:** HPLC trace of withanolide **8** (0.05% w/v) exposed to 0.2 N HCl in MeOH/H₂O for 4 h at 25 °C showing its conversion to 5 α -chloro-6 β -hydroxy-5,6-dihydrophysachenolide D (**17**), 5 α ,6 β -dihydroxy-5,6-dihydrophysachenolide D (**21**) and 5 α -methoxy-6 β -hydroxy-5,6-dihydrophysachenolide D (**20**), all of which were encountered in *P. coztomatl* extract. Identity of peaks due to **17**, **20**, and **21** were confirmed by the peak enhancement method.

Conditions used for HPLC analysis: 250 x 4.6 mm C-18 HPLC column, flow rate: 0.7 mL/min, Solvent: MeOH-H₂O gradient system by increasing MeOH from 40% to 100% in 30 min, detection: UV 230 nm.

