BEILSTEIN JOURNAL OF ORGANIC CHEMISTRY

Supporting Information

for

Synthesis and biological evaluation of new brassinosteroid analogs with C-22 benzoate function

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NMR spectra of compounds, biological bioassays and protein–ligand interactions (molecular docking)

Table of contents

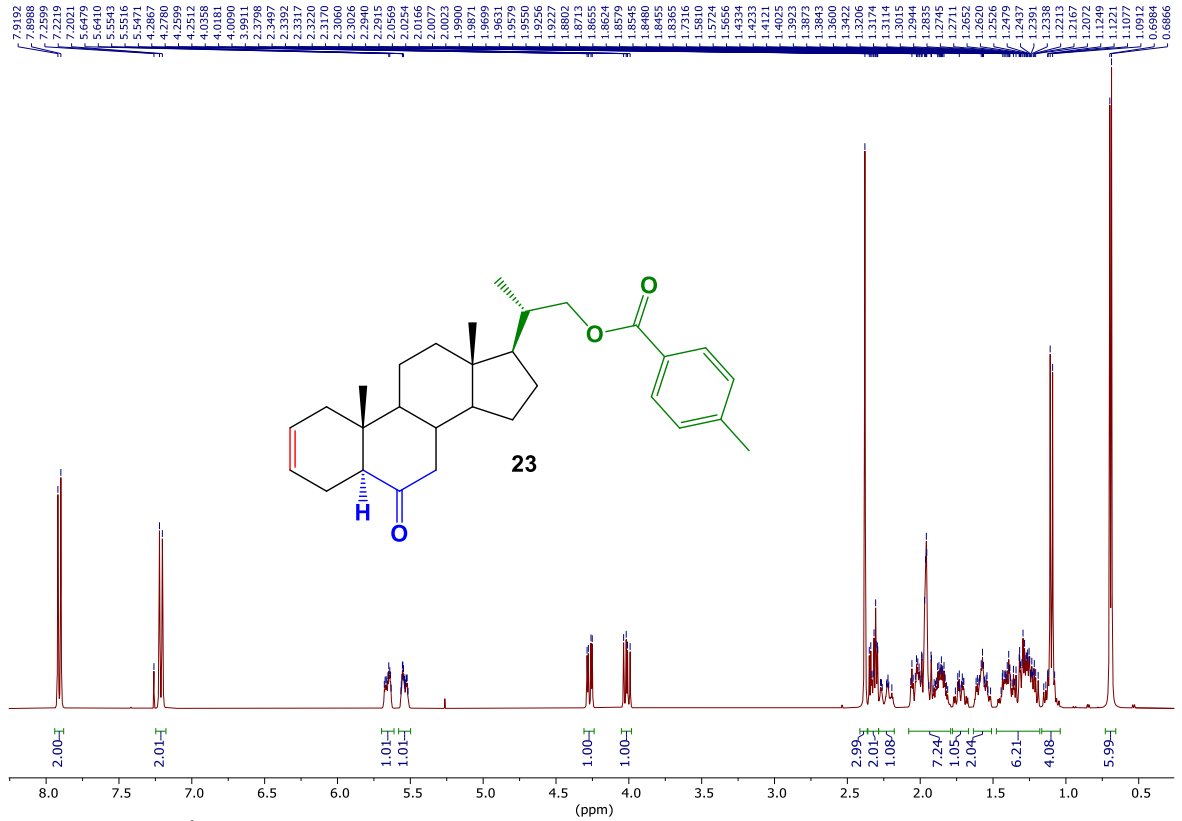


Figure S1. ^{1}H NMR spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-methylbenzoate (**23**).

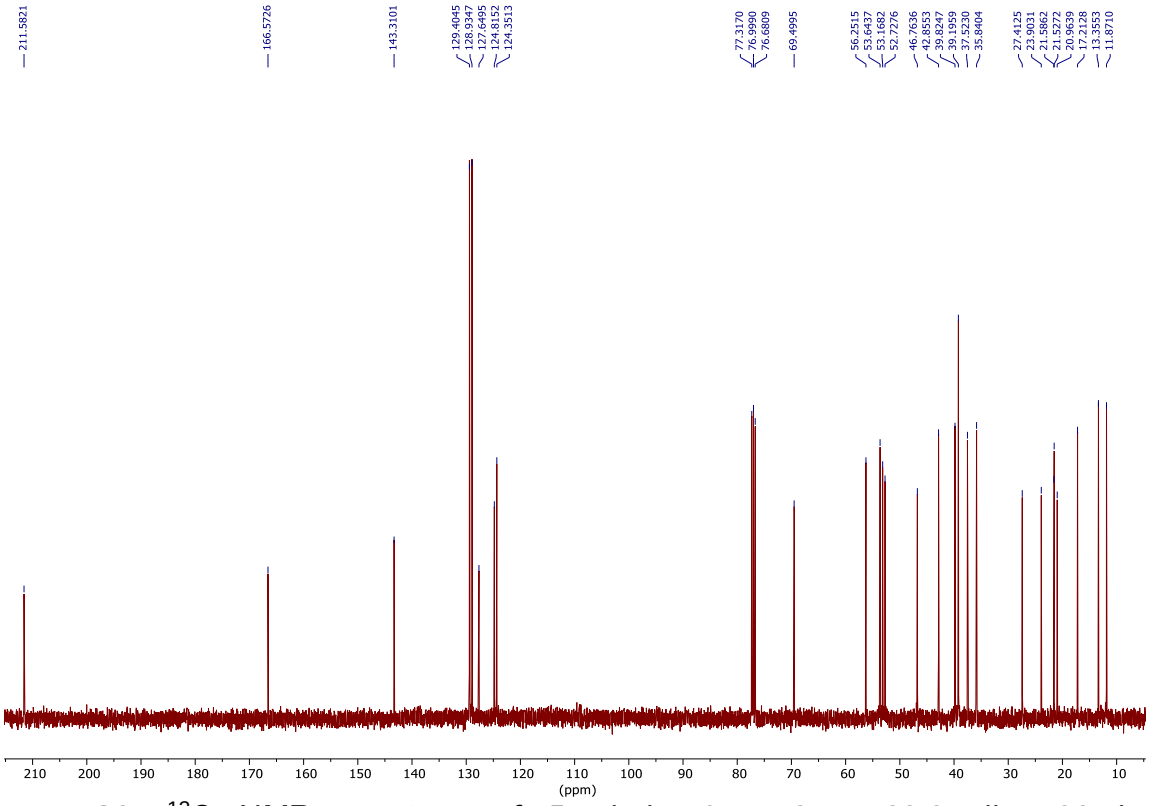


Figure S2. ^{13}C NMR spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-methylbenzoate (**23**).

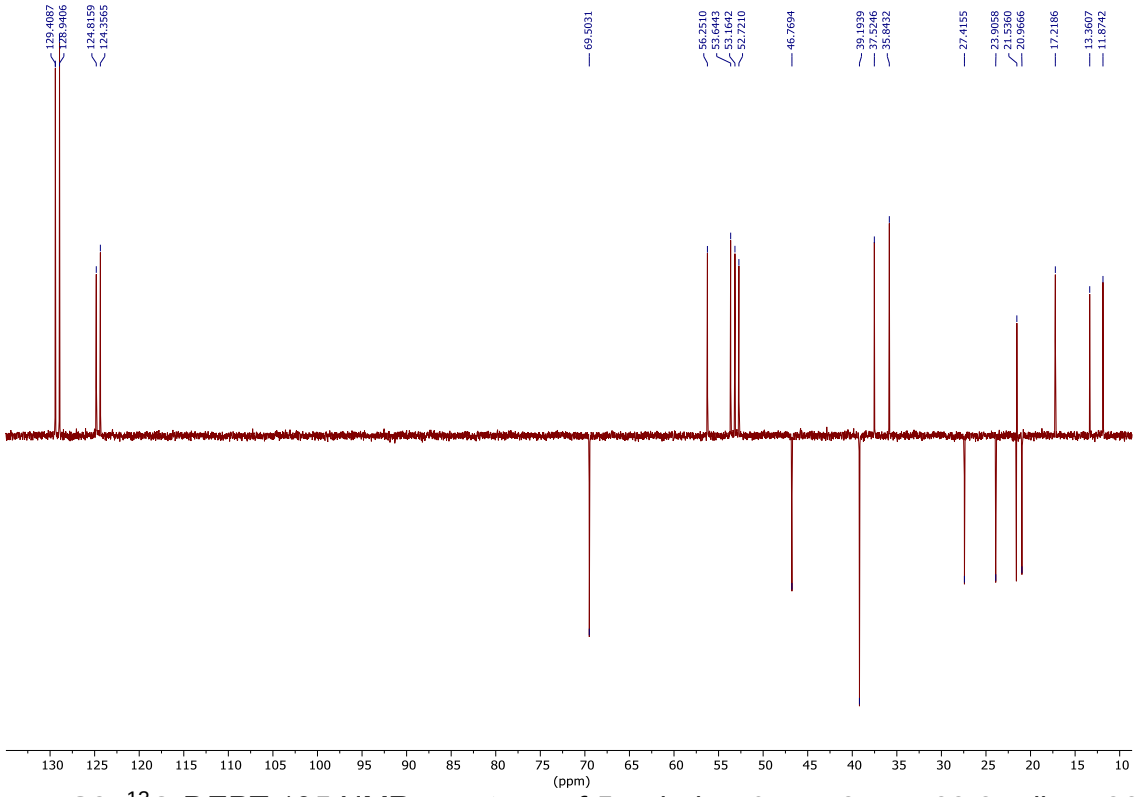


Figure S3. ^{13}C-DEPT 135 NMR spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-methylbenzoate (**23**).

Figure S4. ^{1}H-^{13}C HSQC-ed. spectrum 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-methylbenzoate (**23**).

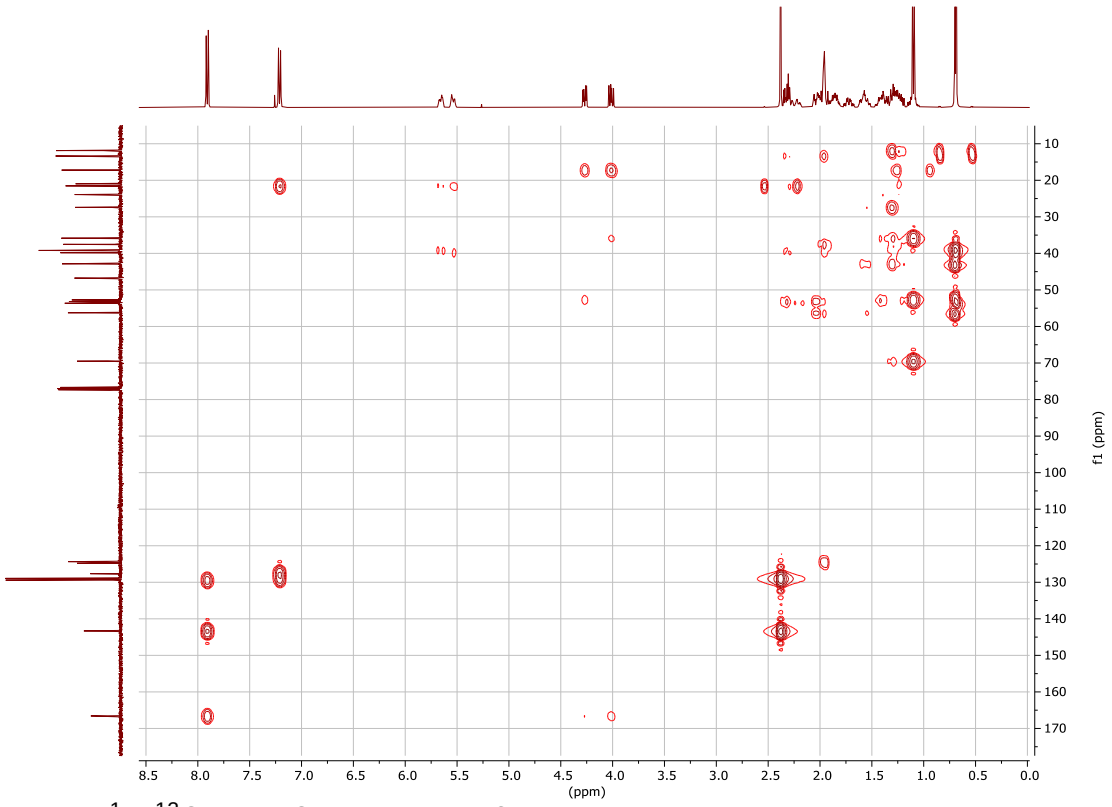


Figure S5. ^{1}H-^{13}C HMBC spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-methylbenzoate (**23**).

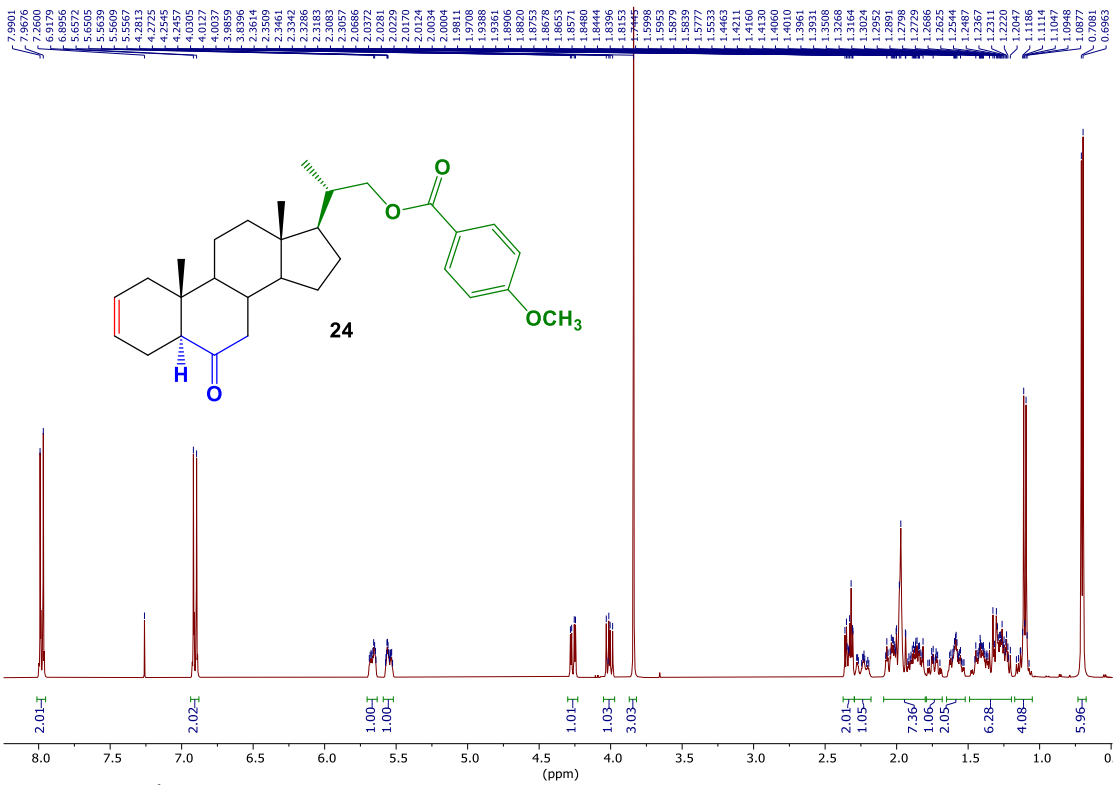


Figure S6. ^{1}H NMR spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-methoxybenzoate (**24**).

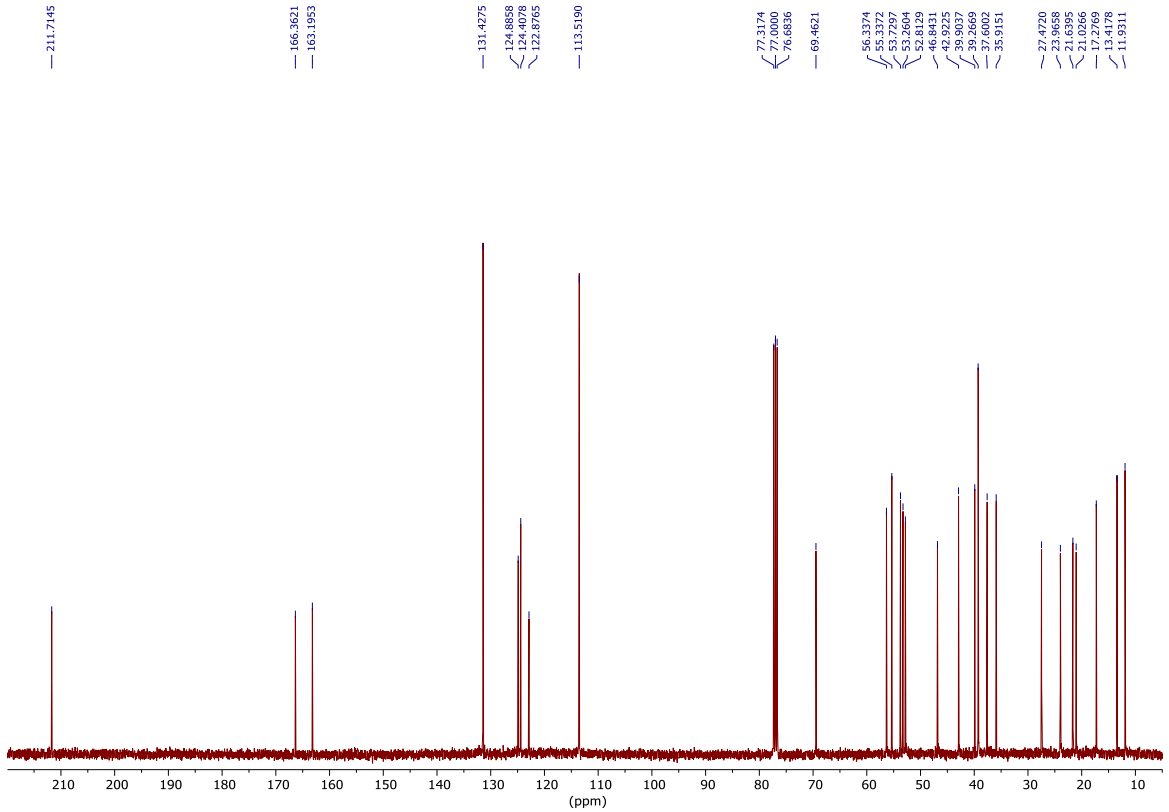


Figure S7. ^{13}C NMR spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-methoxybenzoate (**24**).

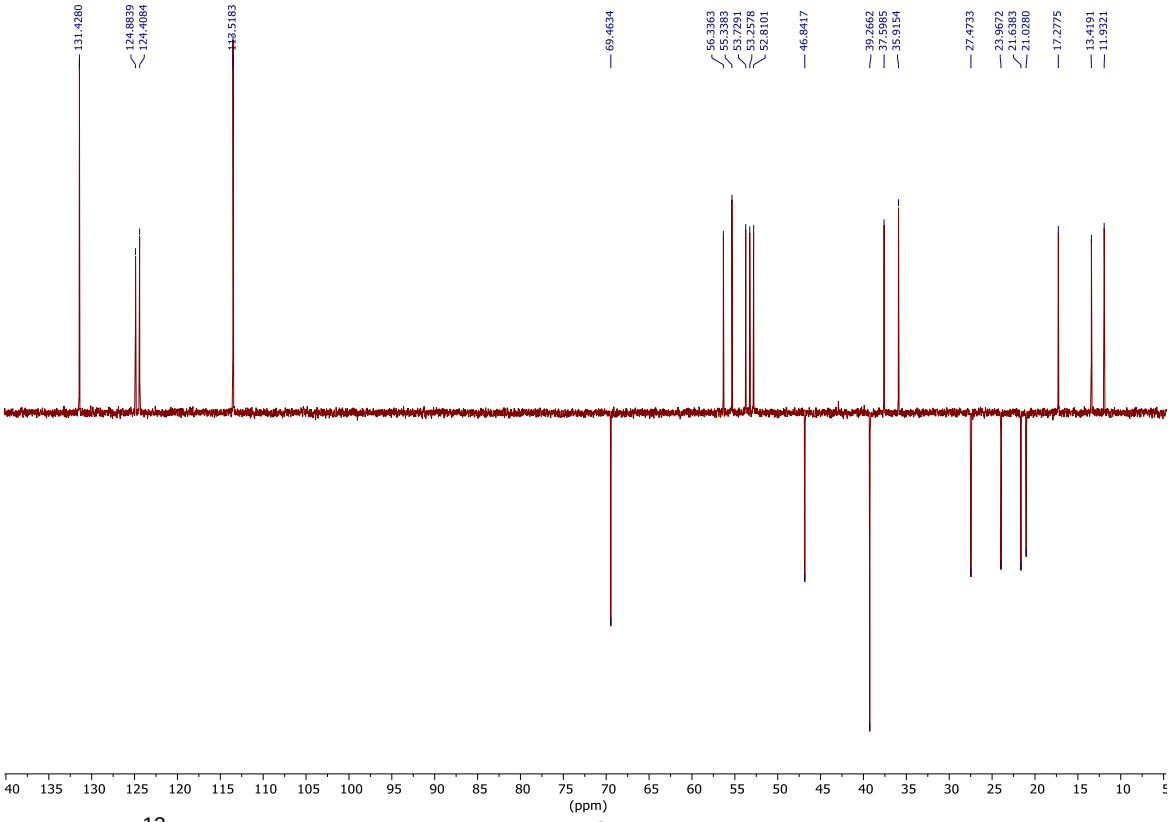


Figure S8. ^{13}C-DEPT 135 NMR spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-methoxybenzoate (**24**).

Figure S9. ^{1}H-^{13}C HSQC-ed. spectrum 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-methoxybenzoate (**24**).

Figure S10. ^{1}H-^{13}C HMBC spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-methoxybenzoate (**24**).

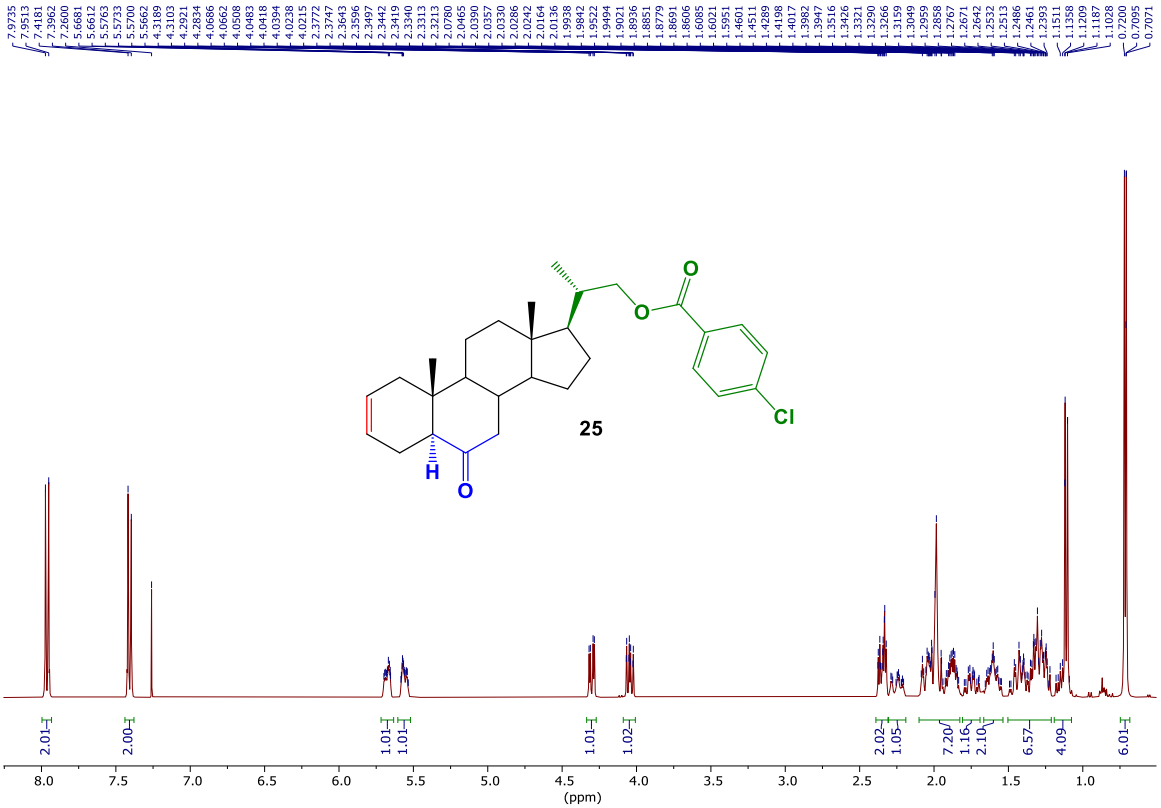


Figure S11. ^{1}H NMR spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-chlorobenzoate (**25**).

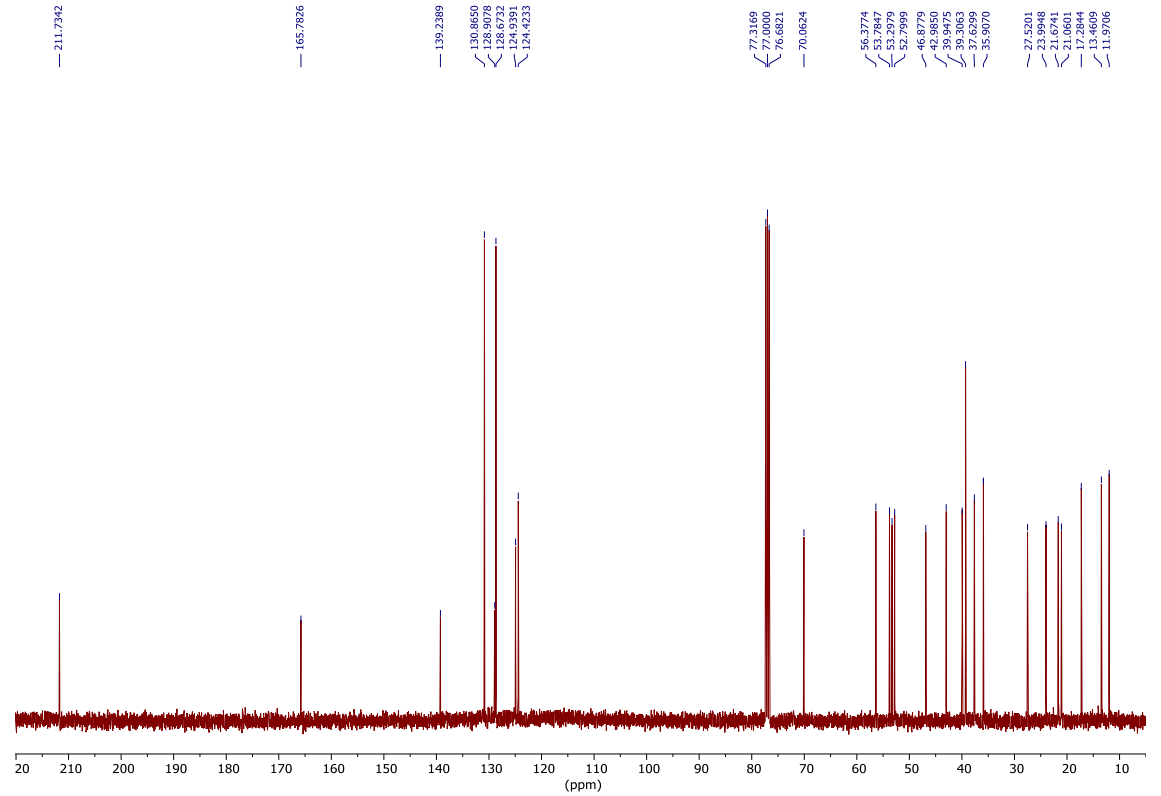


Figure S12. ^{13}C NMR spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-chlorobenzoate (**25**).

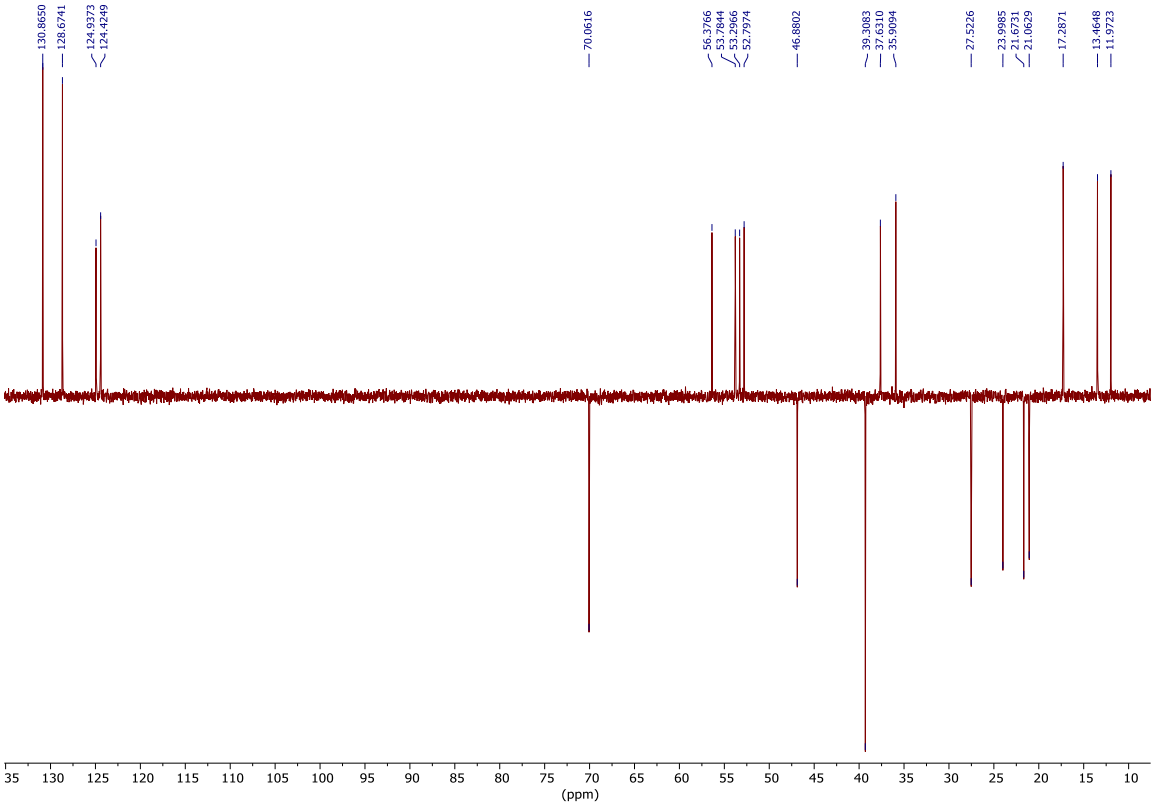


Figure S13. ^{13}C-DEPT 135 NMR spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-chlorobenzoate (**25**).

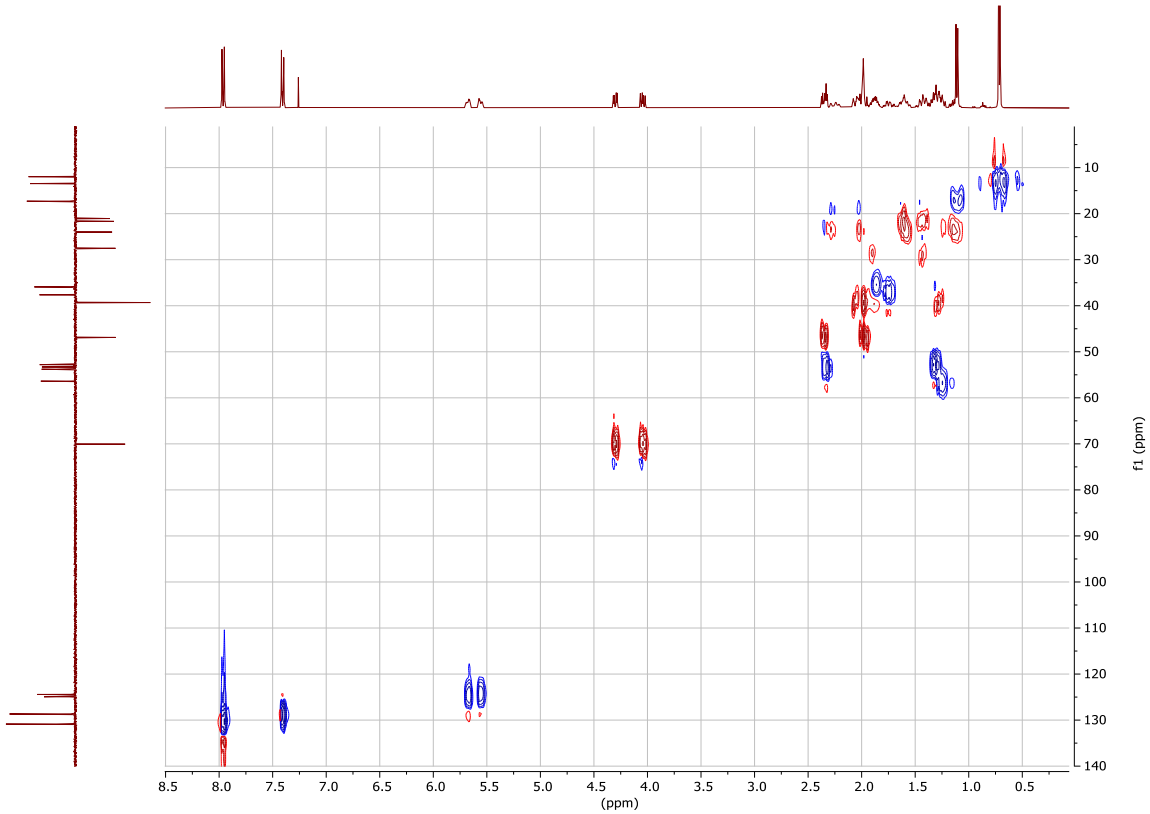


Figure S14. ^{1}H-^{13}C HSQC-ed. spectrum 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-chlorobenzoate (**25**).

Figure S15. ^{1}H-^{13}C HMBC spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-chlorobenzoate (**25**).

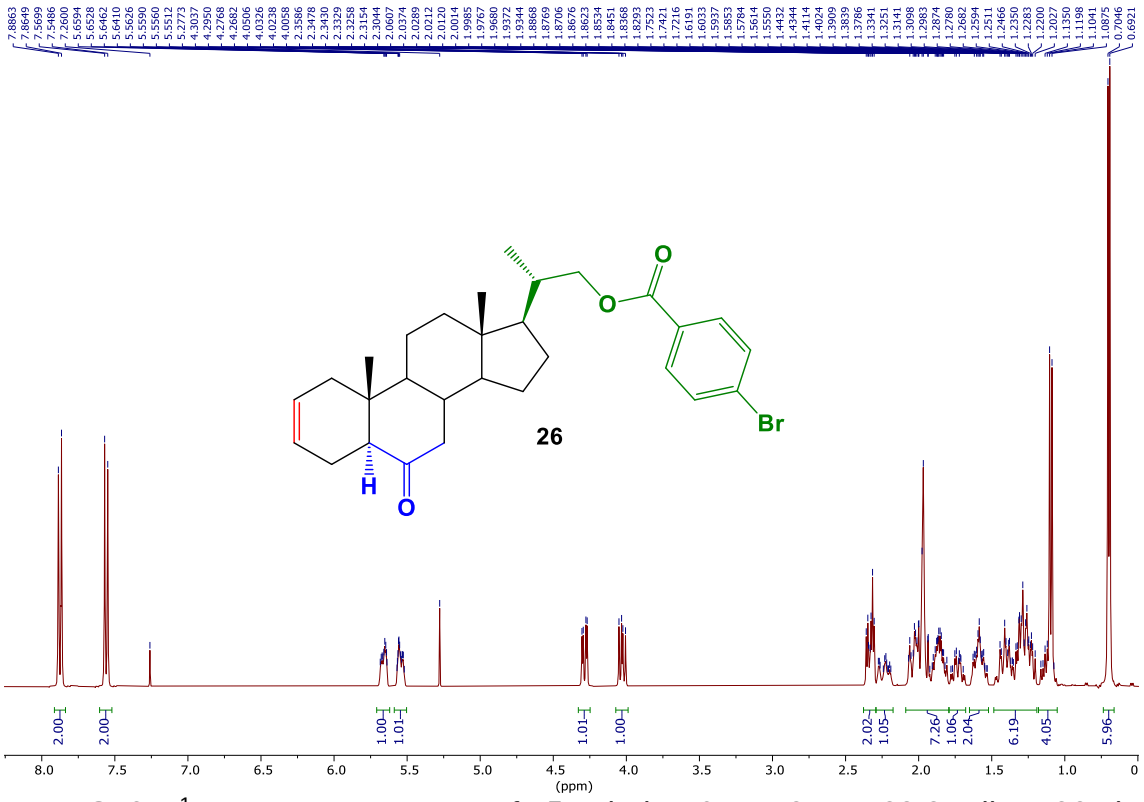


Figure S16. ^{1}H NMR spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-bromobenzoate (**26**).

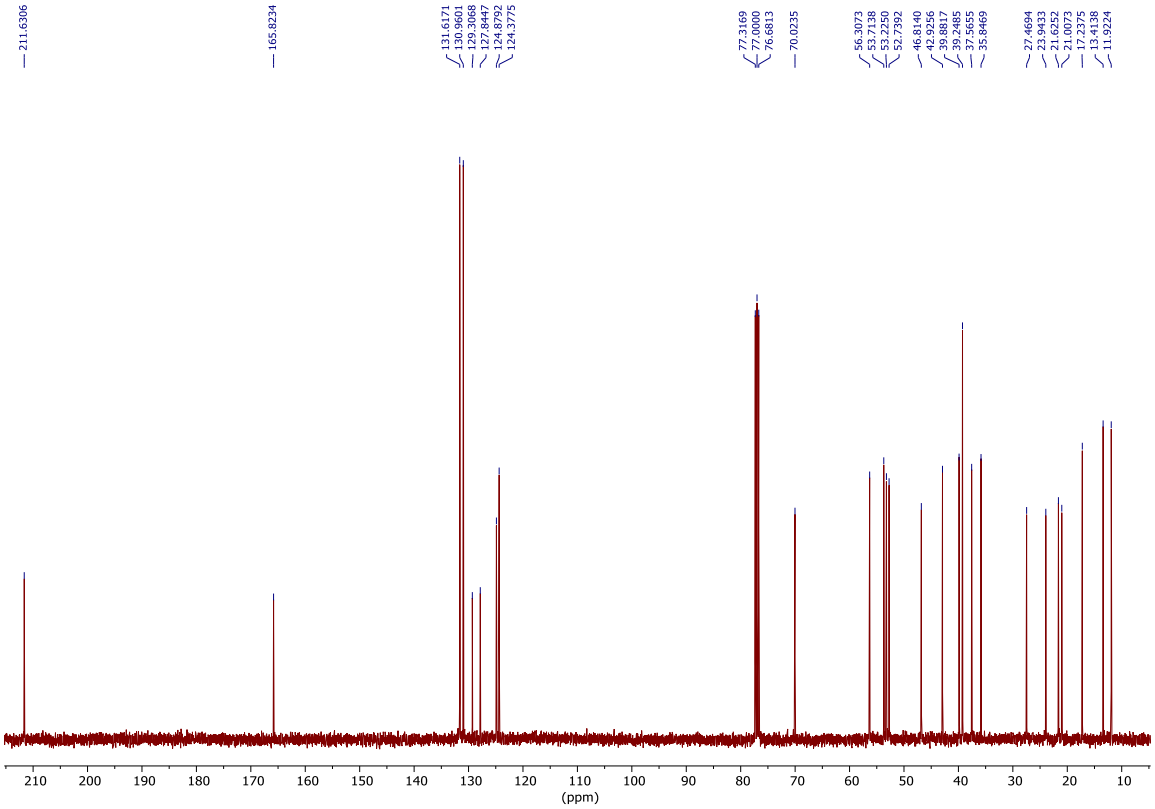


Figure S17. ^{13}C NMR spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-bromobenzoate (**26**).

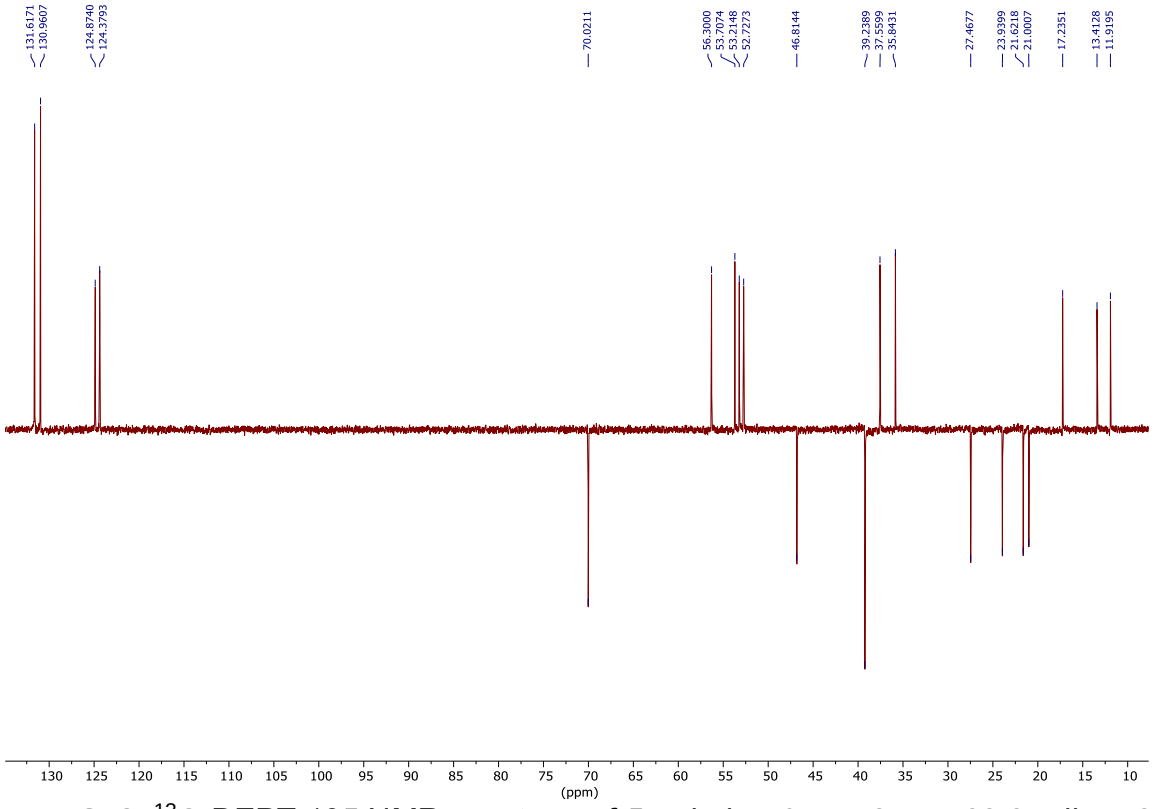


Figure S18. ^{13}C-DEPT 135 NMR spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-bromobenzoate (**26**).

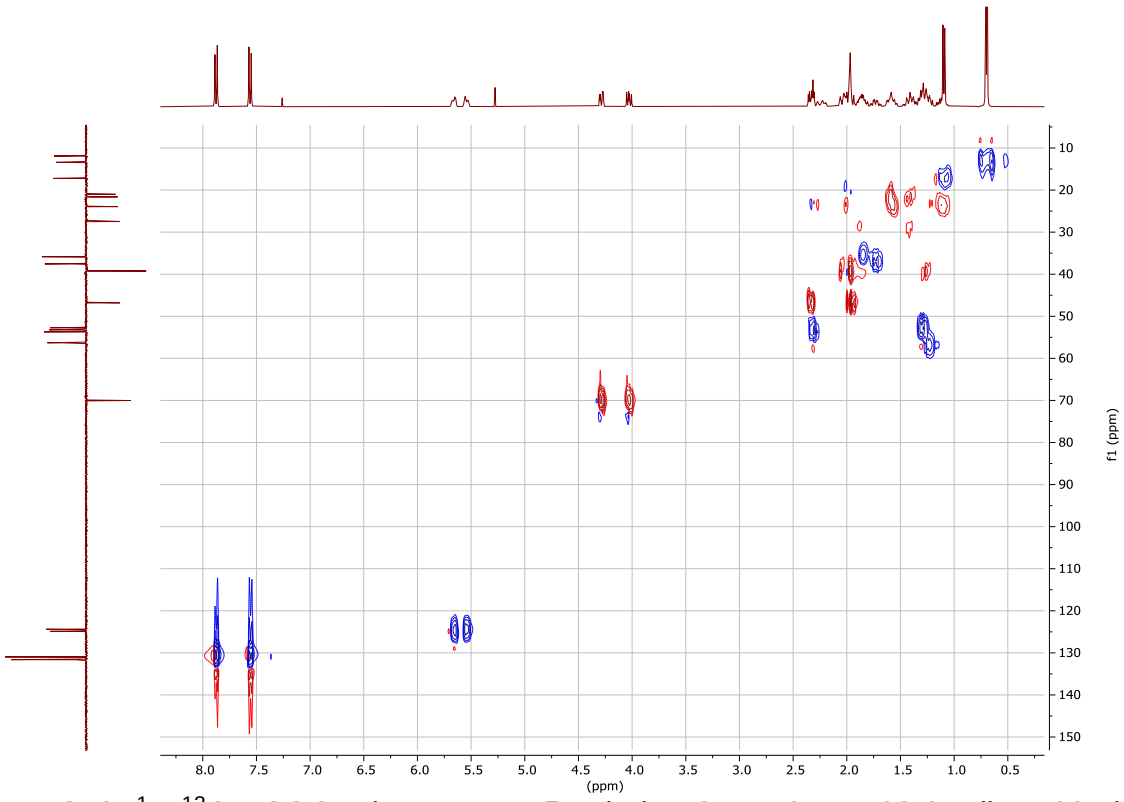


Figure S19. ^{1}H-^{13}C HSQC-ed. spectrum 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-bromobenzoate (**26**).

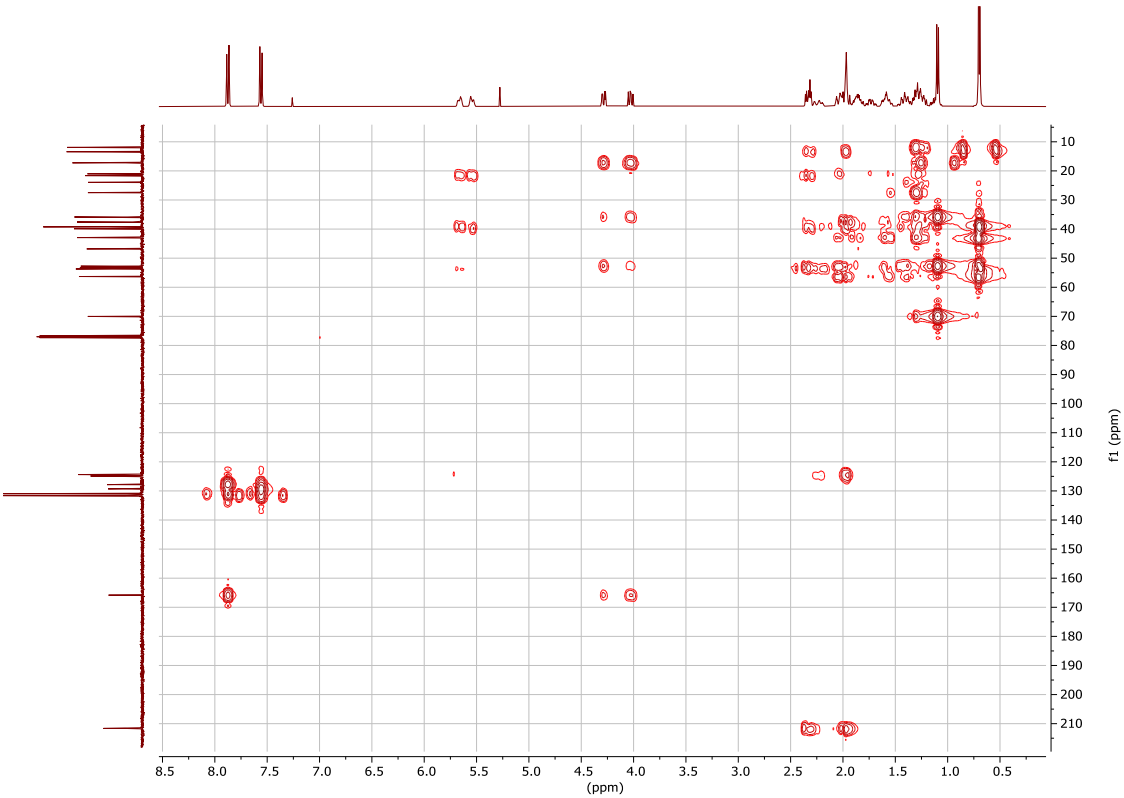


Figure S20. ^{1}H-^{13}C HMBC spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-bromobenzoate (**26**).

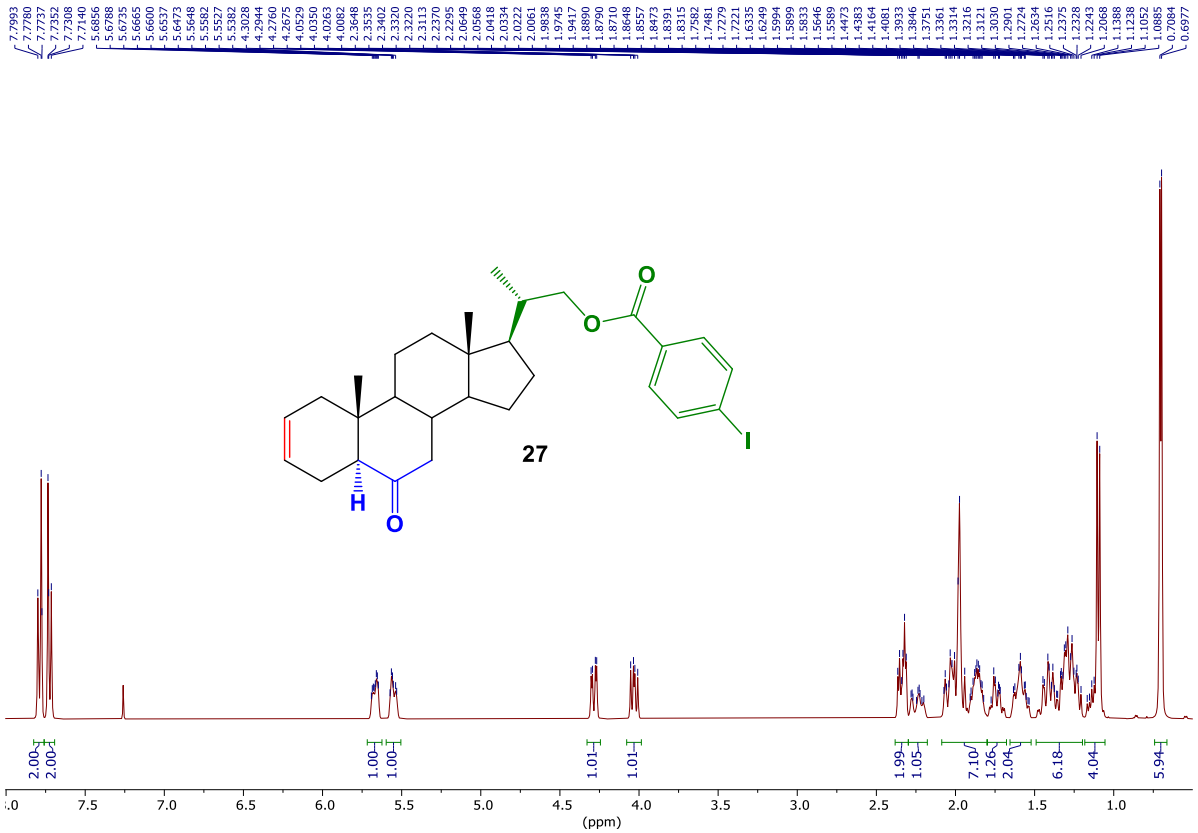


Figure S21. ^{1}H NMR spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-iodobenzoate (**27**).

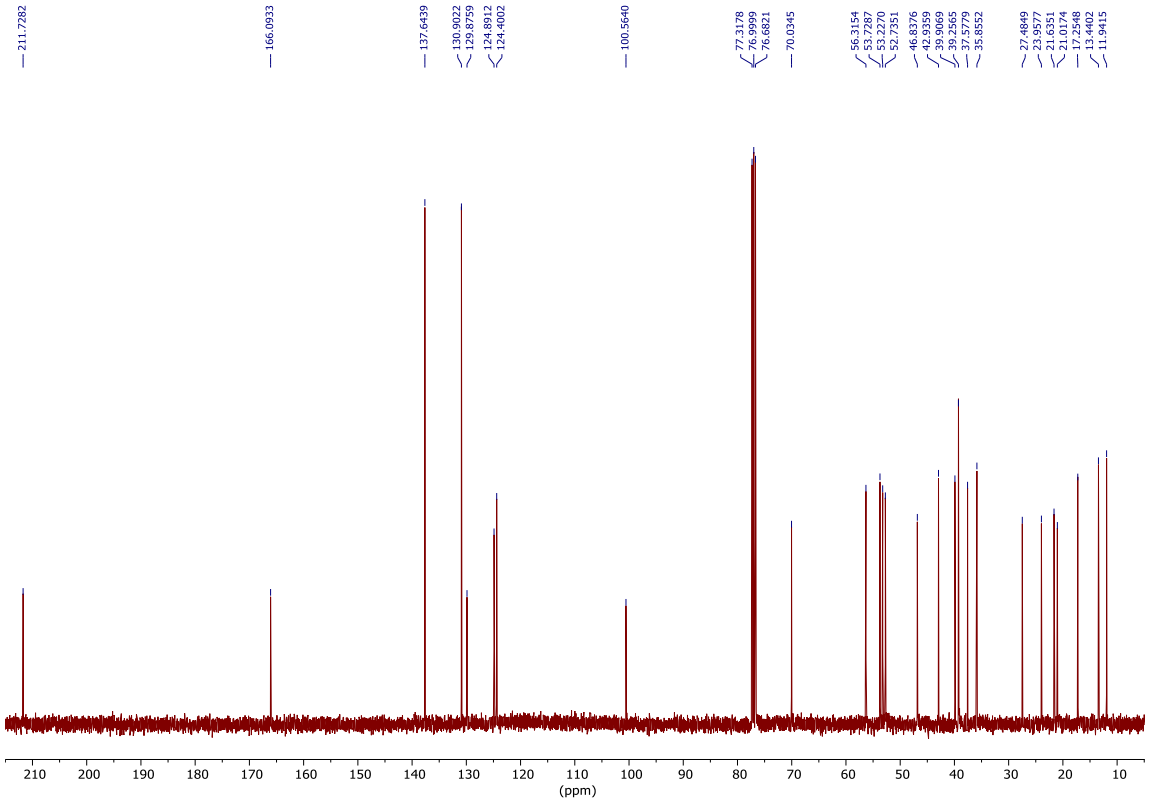


Figure S22. ^{13}C NMR spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-iodobenzoate (**27**).

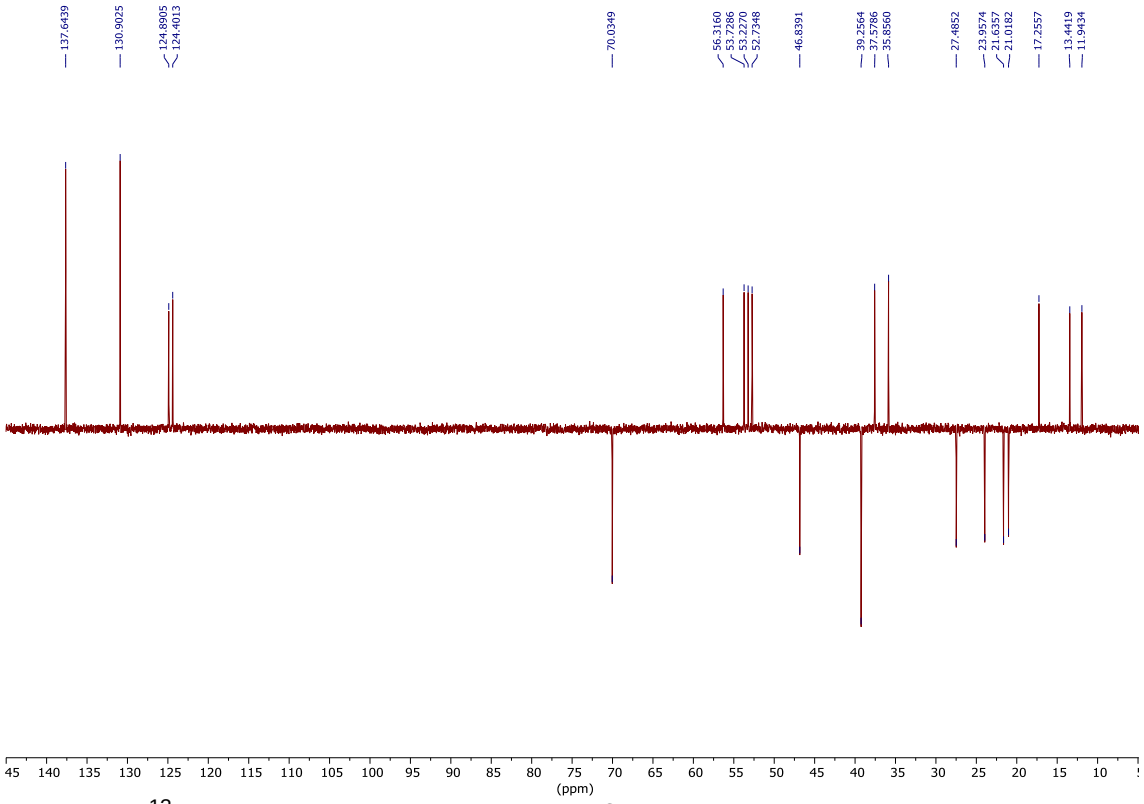


Figure S23. ^{13}C-DEPT 135 NMR spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-iodobenzoate (**27**).

Figure S24. ^{1}H-^{13}C HSQC-ed. spectrum 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-iodinebenzoate (**27**).

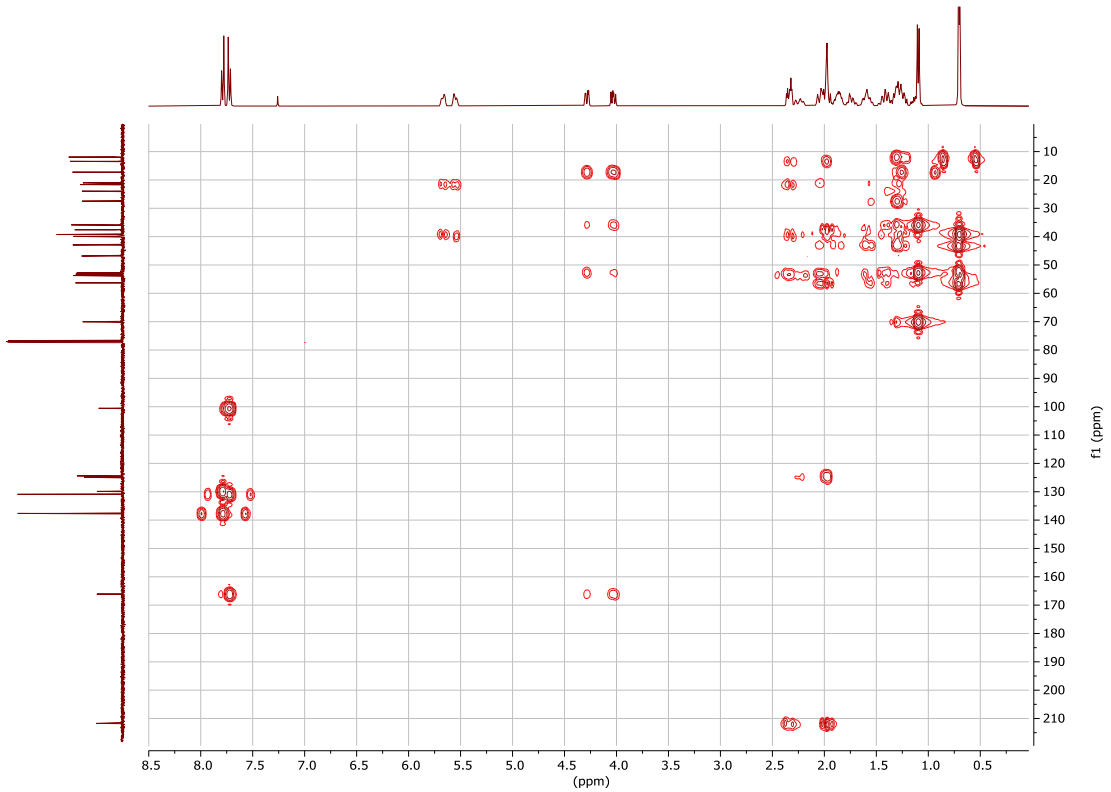


Figure S25. ^{1}H-^{13}C HMBC spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-iodobenzoate (**27**).

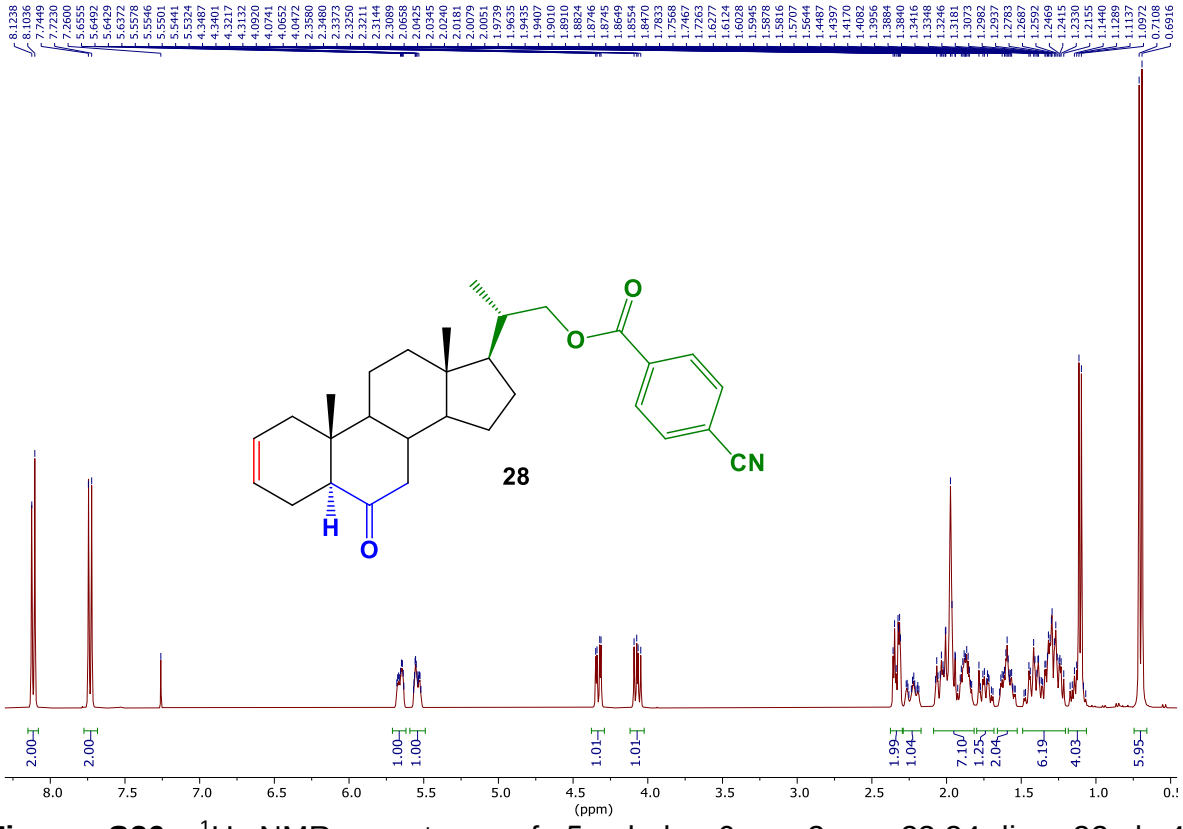


Figure S26. ^{1}H NMR spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-cyanobenzoate (**28**).

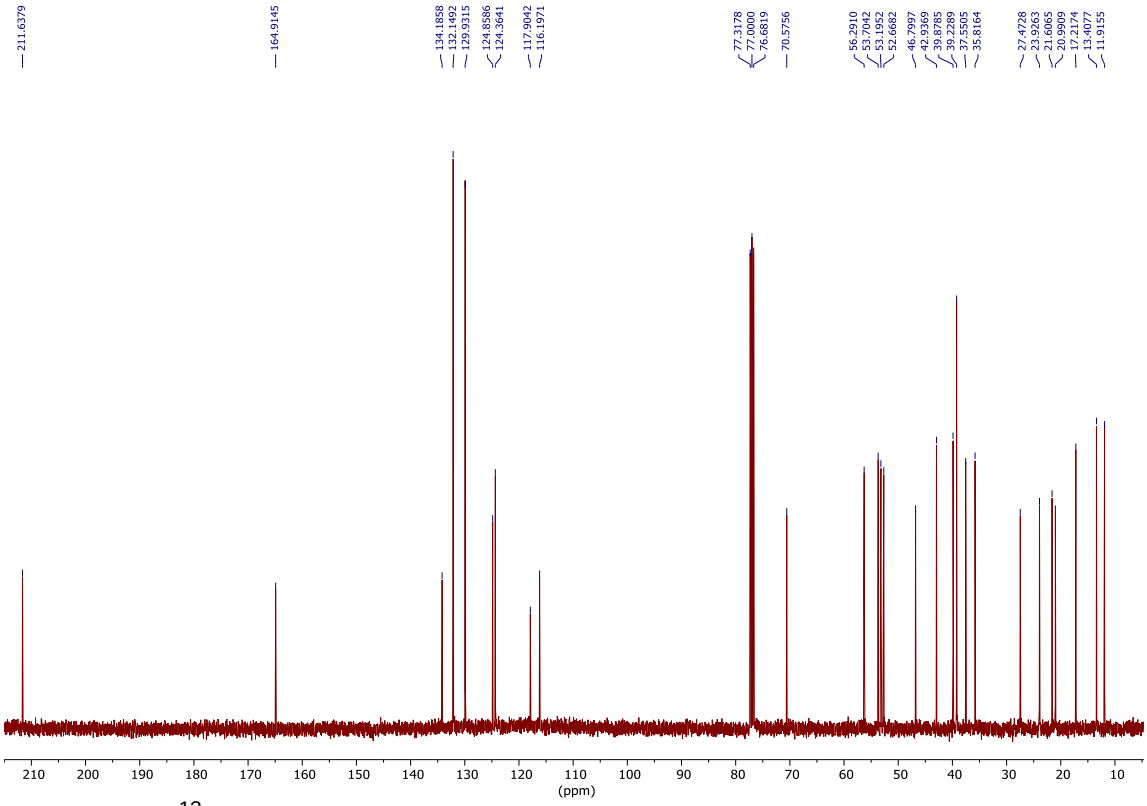


Figure S27. ^{13}C NMR spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-cyanobenzoate (**28**).

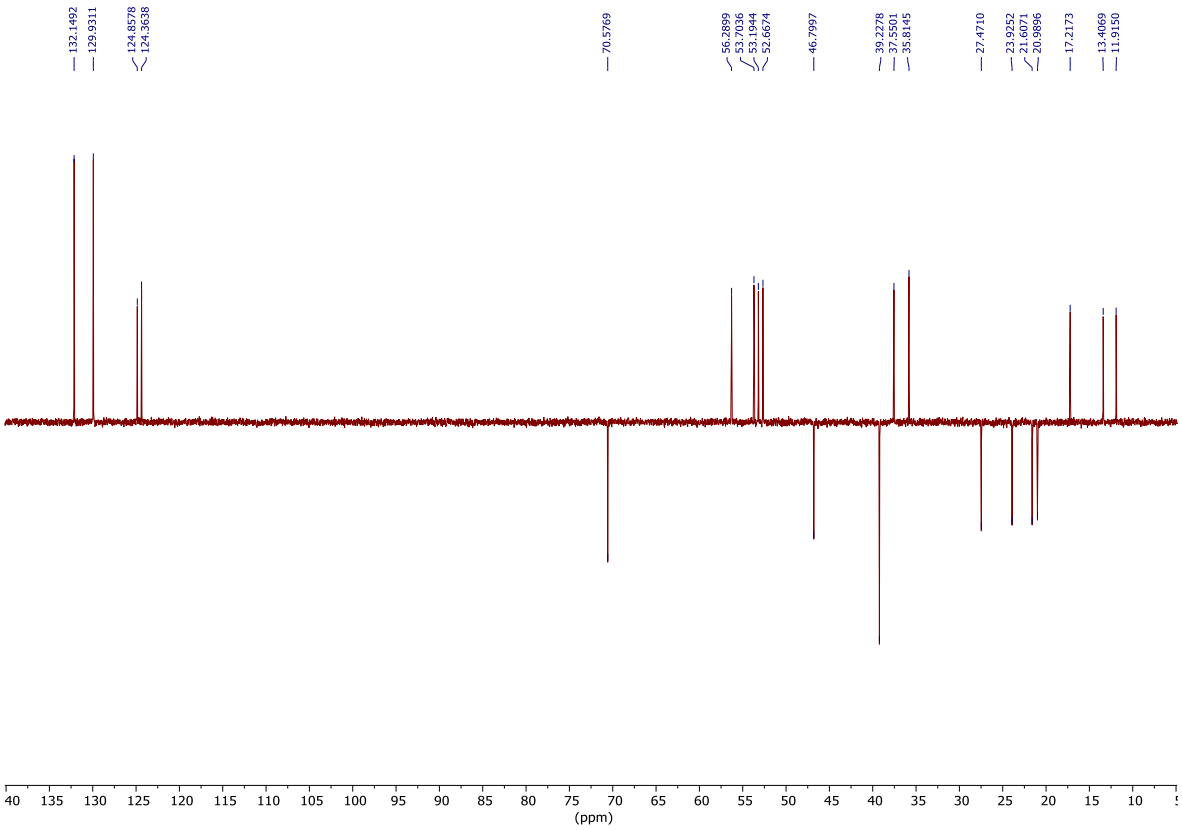


Figure S28. ^{13}C-DEPT 135 NMR spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-cyanobenzoate (**28**).

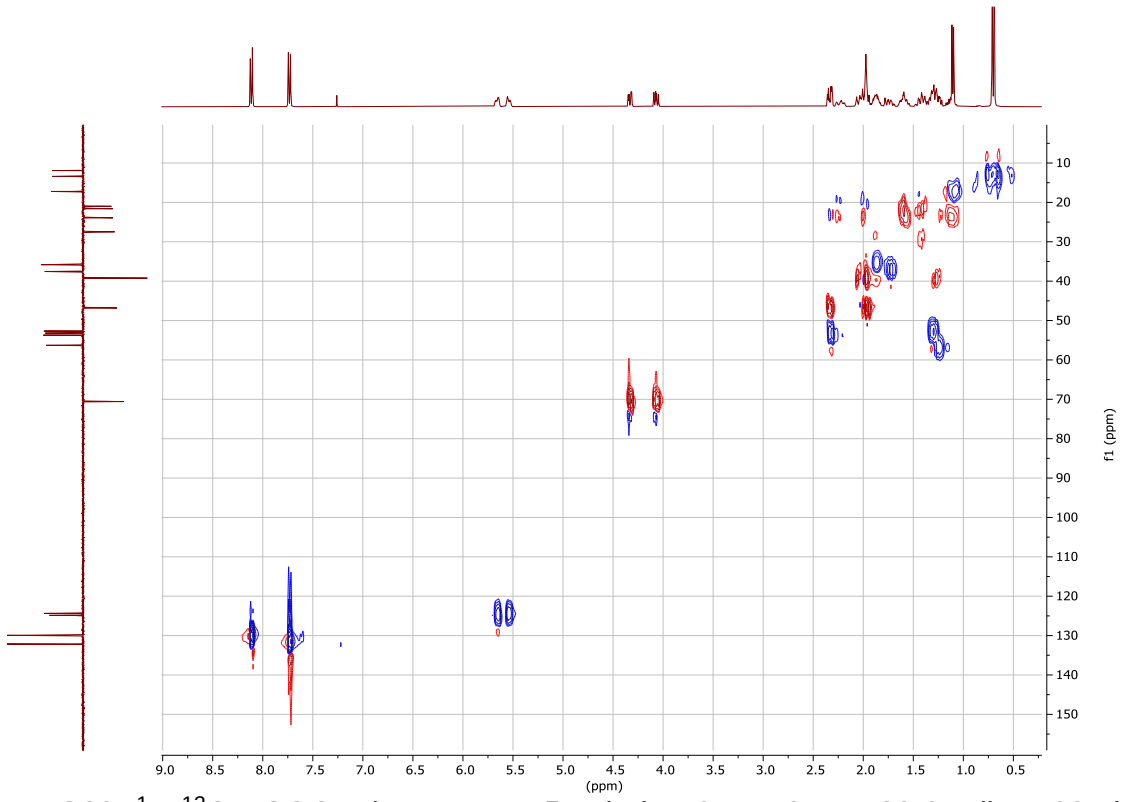


Figure S29. ^{1}H-^{13}C HSQC-ed. spectrum 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-cyanobenzoate (**28**).

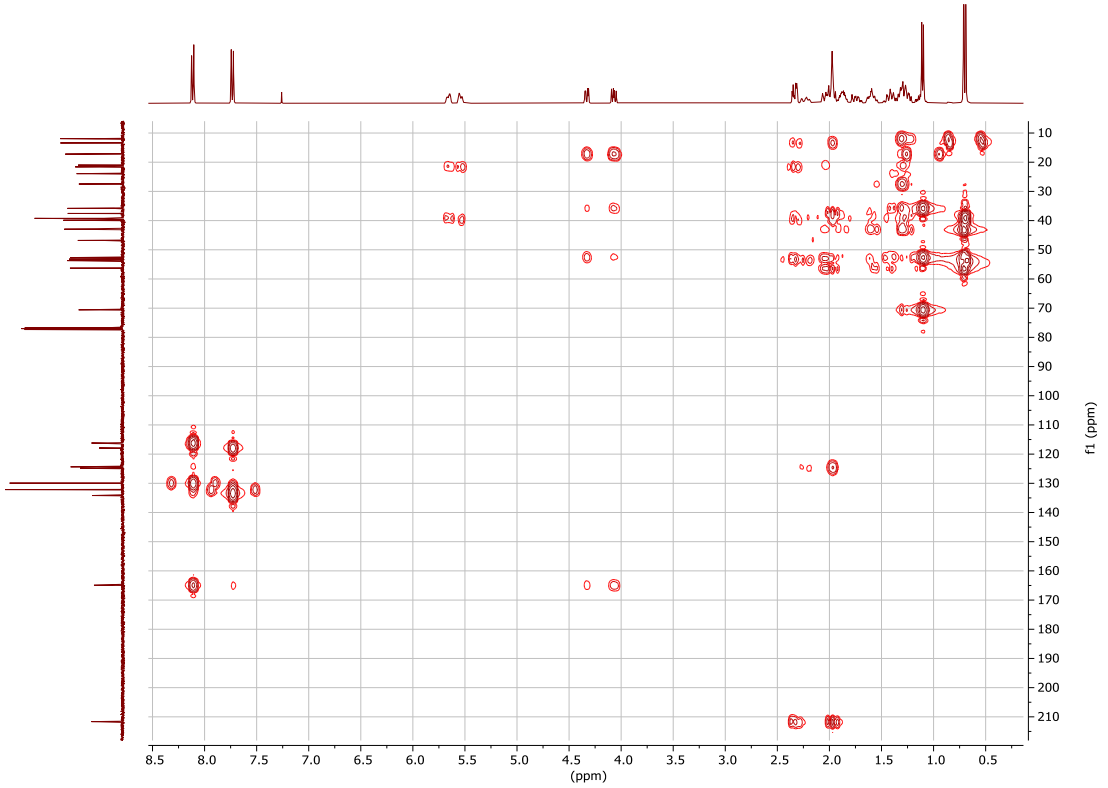


Figure S30. ^{1}H-^{13}C HMBC spectrum of 5α-cholan-6-oxo-2-ene-23,24-dinor-22-yl 4-cyanobenzoate (**28**).

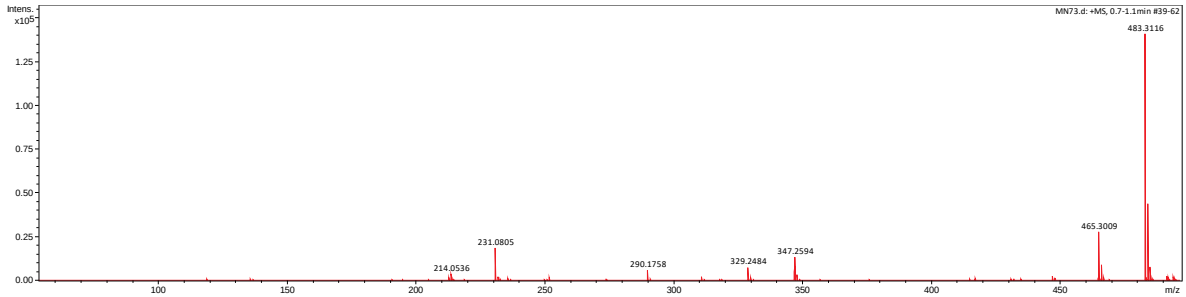


Figure S31. HRMS spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-methylbenzoate (**17**).

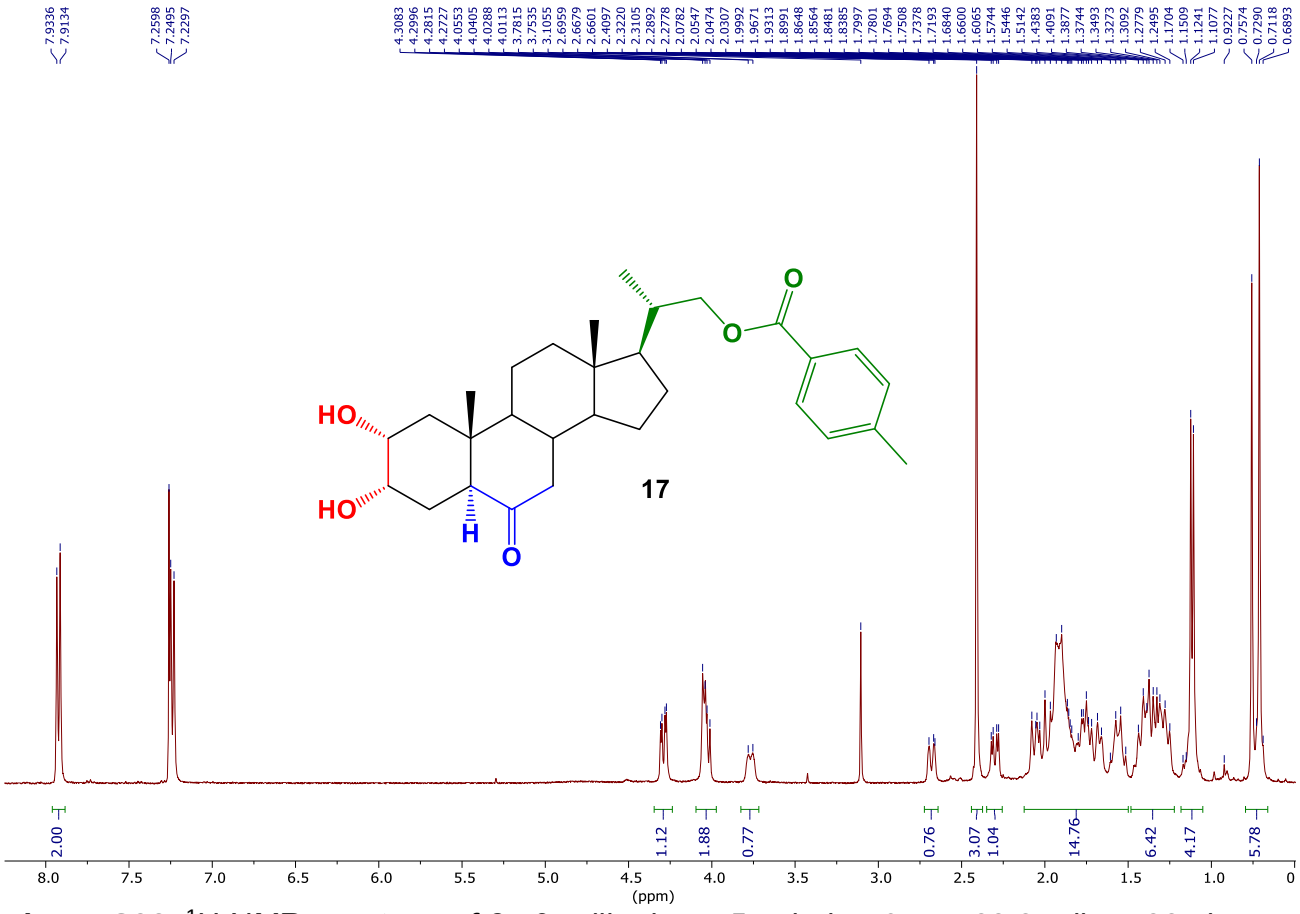


Figure S32. ^{1}H NMR spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-methylbenzoate (**17**).

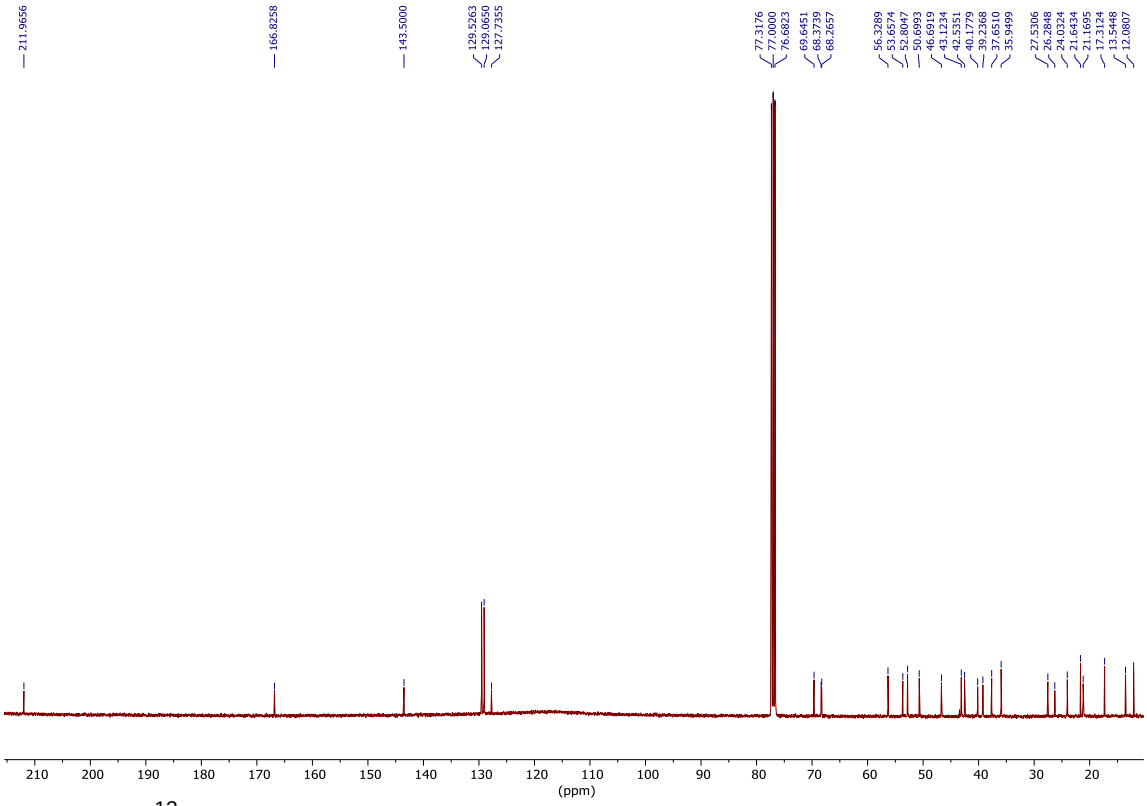


Figure S33. ^{13}C NMR spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-methylbenzoate (**17**).

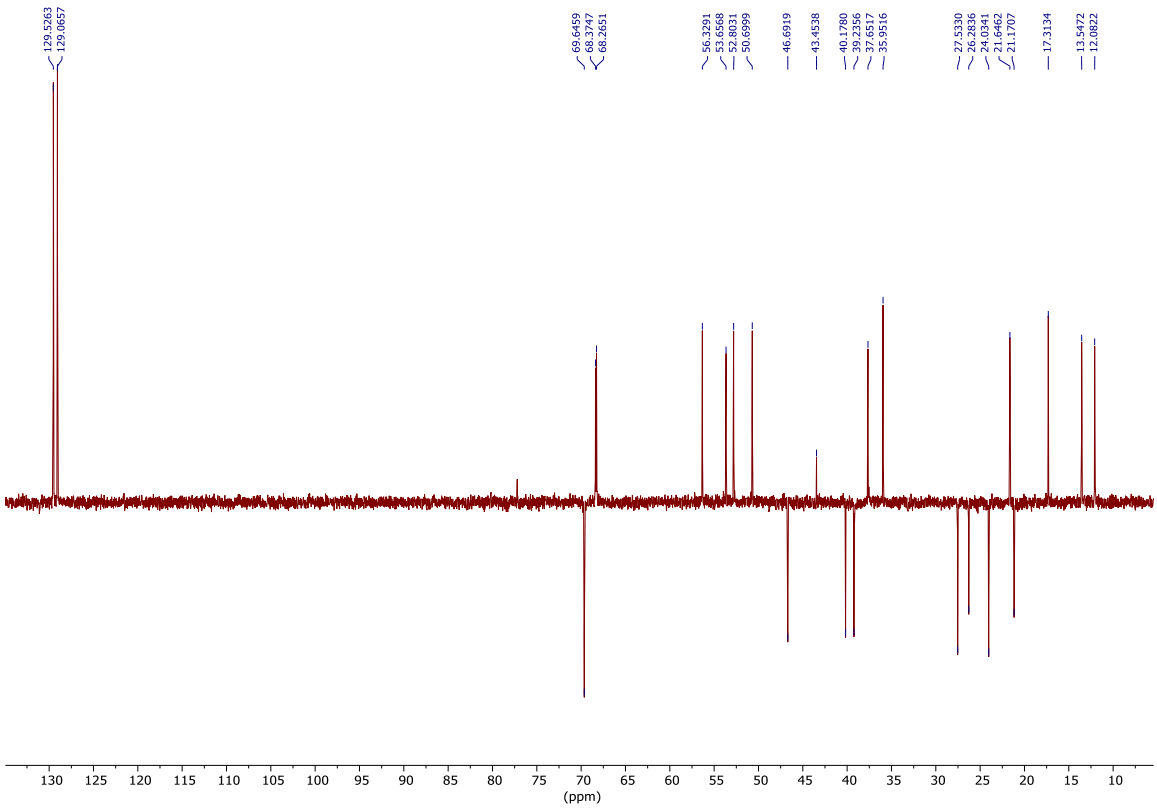


Figure S34. ^{13}C-DEPT 135 NMR spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-methylbenzoate (**17**).

Figure S35. ^{1}H-^{13}C HSQC-ed. spectrum 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-methylbenzoate (**17**).

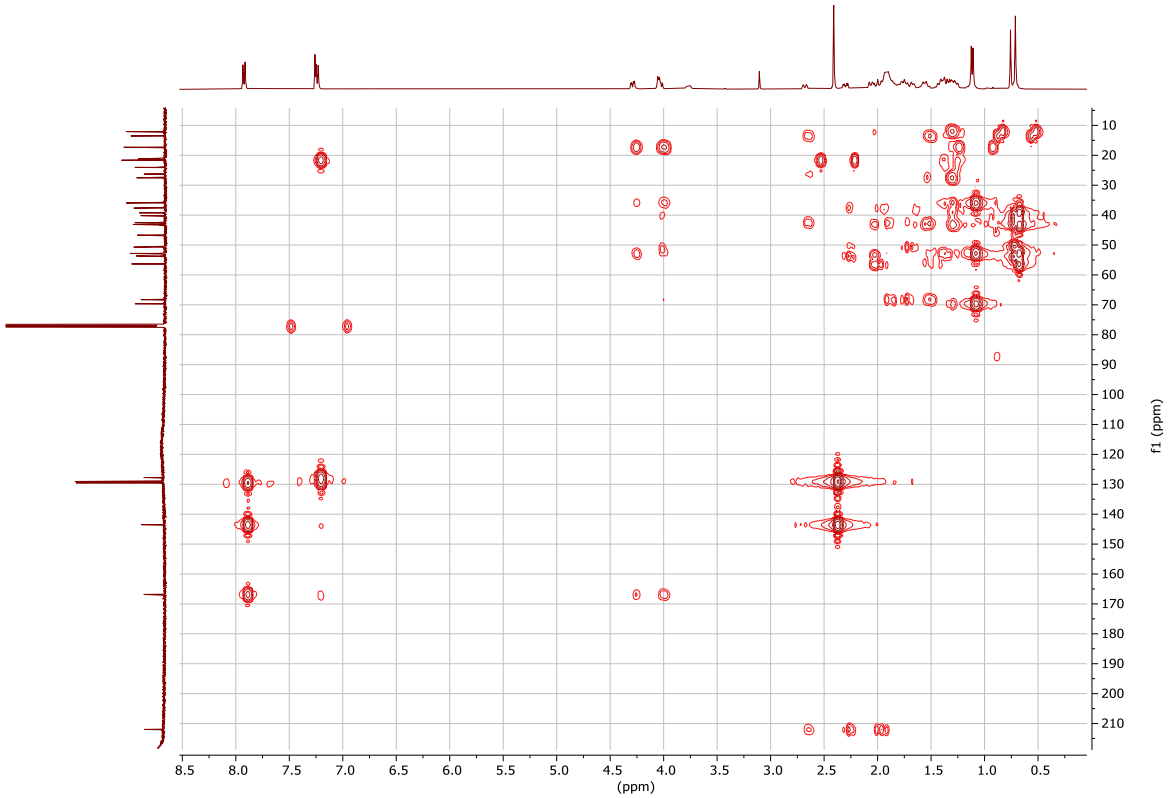


Figure S36. ^{1}H-^{13}C HMBC spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-methylbenzoate (**17**).

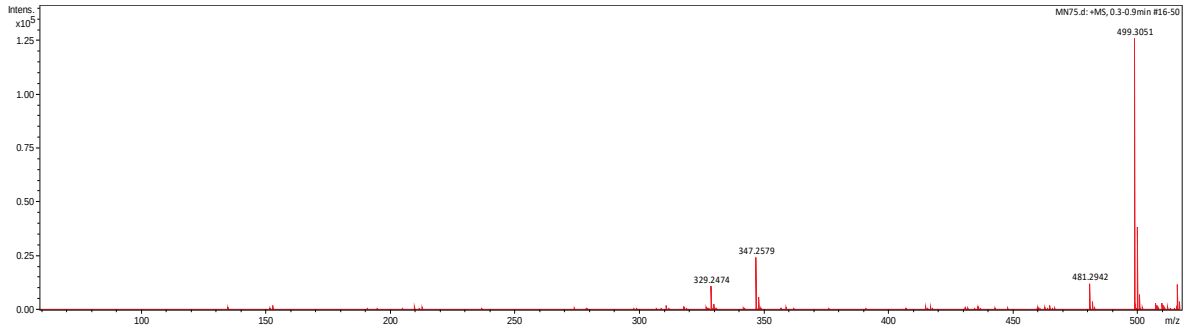


Figure S37. HRMS spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-methoxybenzoate (**18**).

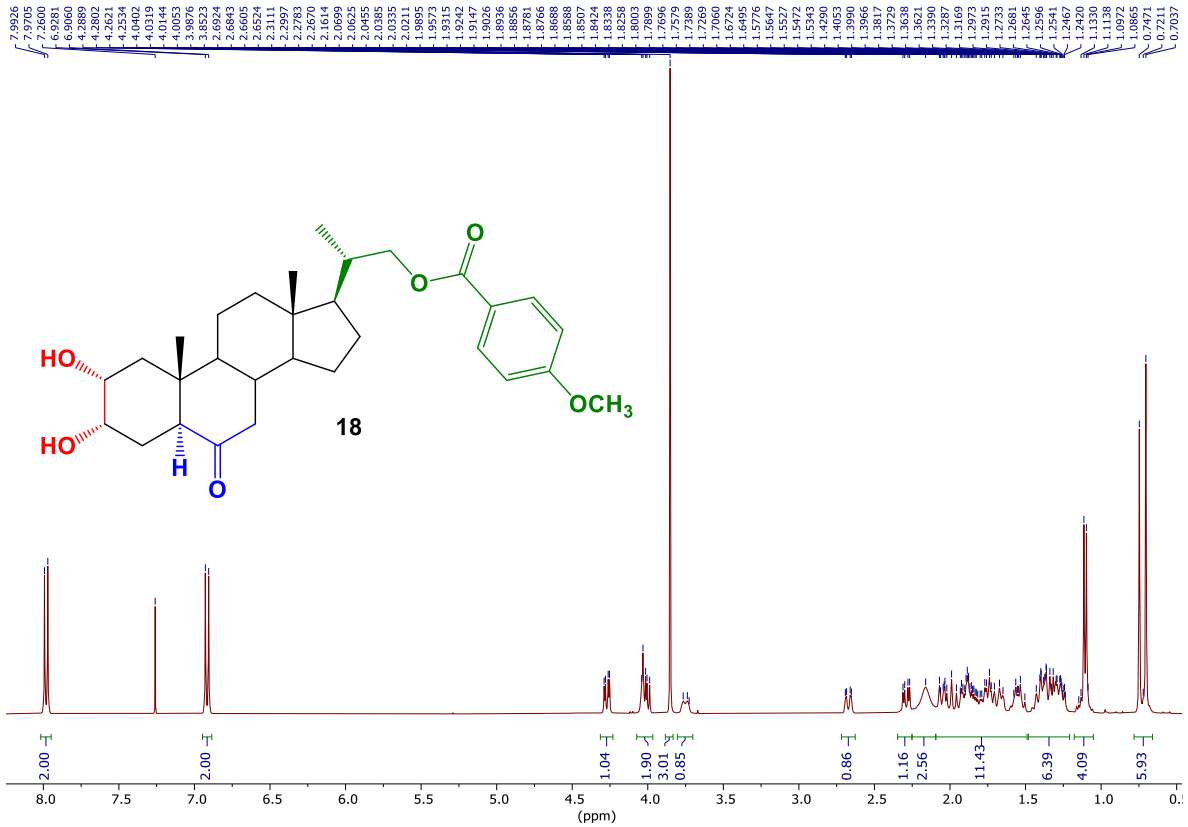


Figure S38. ^{1}H NMR spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-methoxybenzoate (**18**).

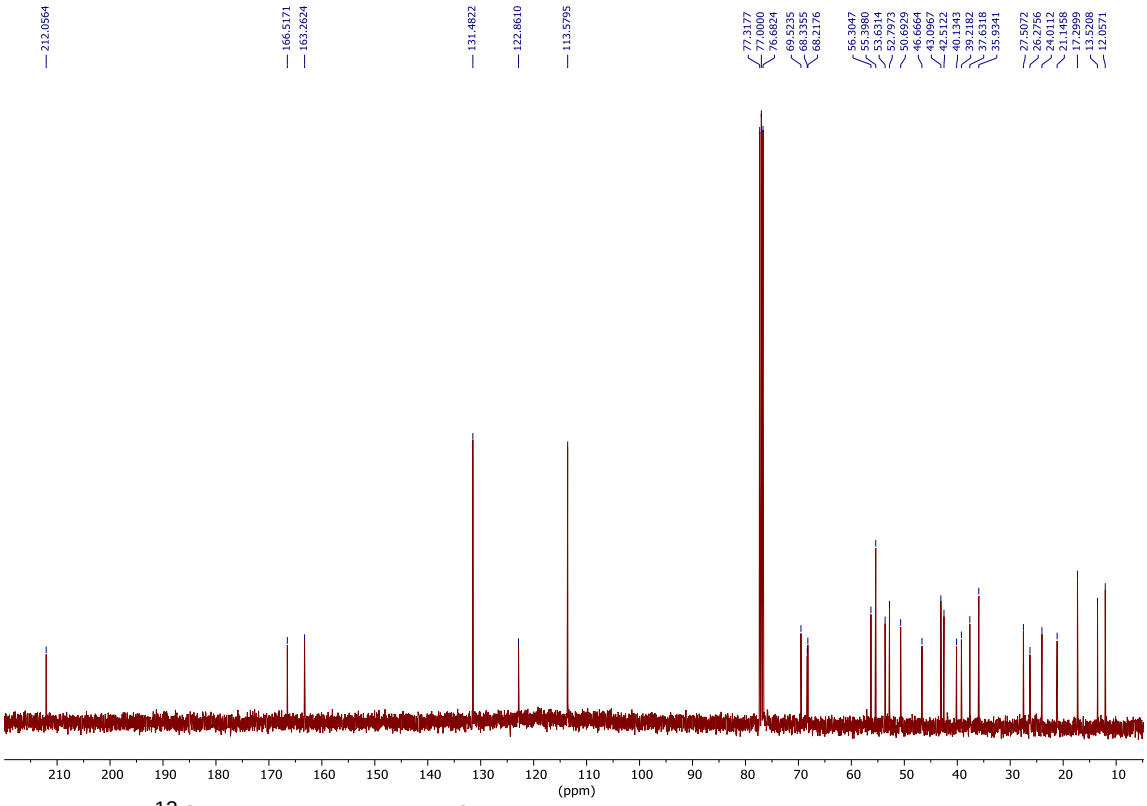


Figure S39. ^{13}C NMR spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-methoxybenzoate (**18**).

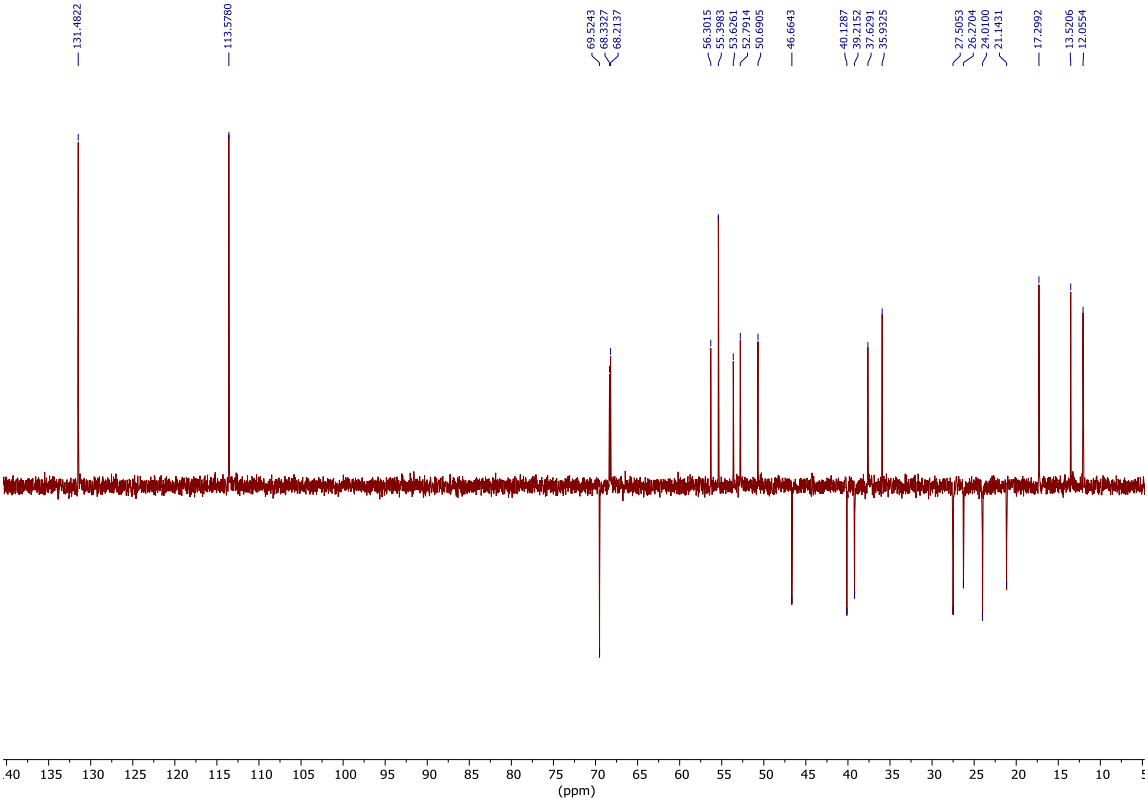


Figure S40. ^{13}C-DEPT 135 NMR spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-methoxybenzoate (**18**).

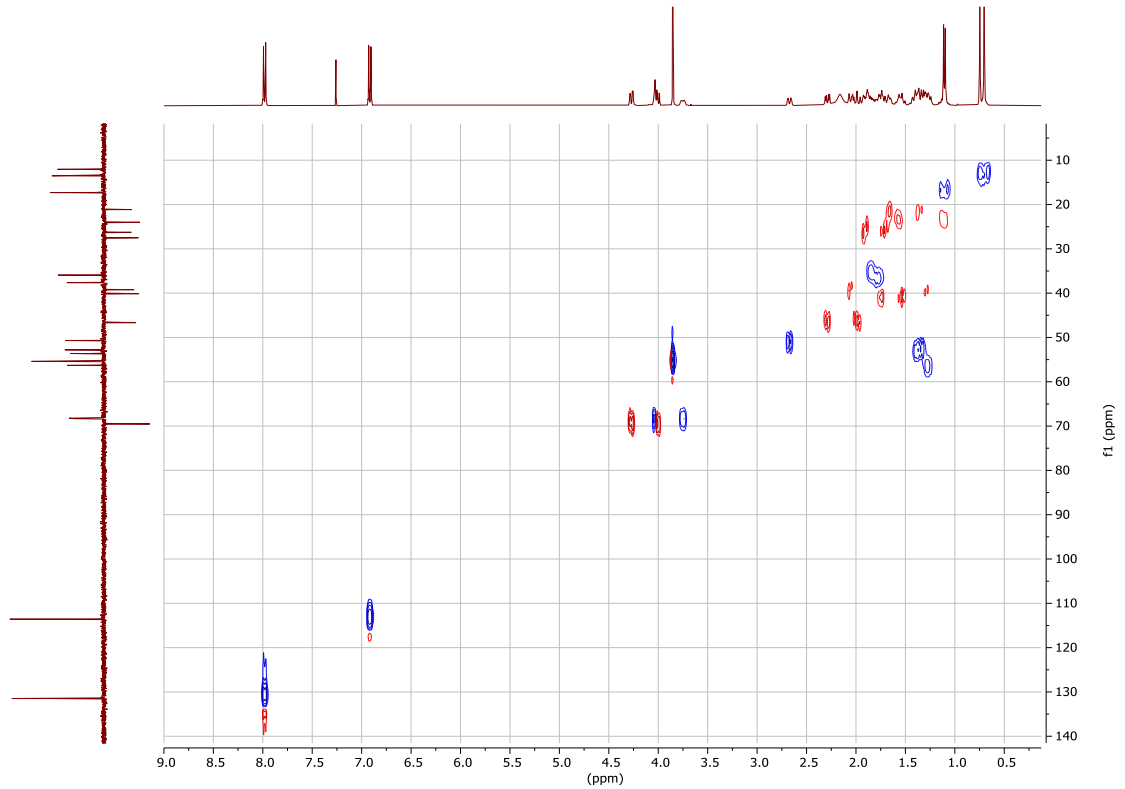


Figure S41. ^{1}H-^{13}C HSQC-ed. spectrum 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-methoxybenzoate (**18**).

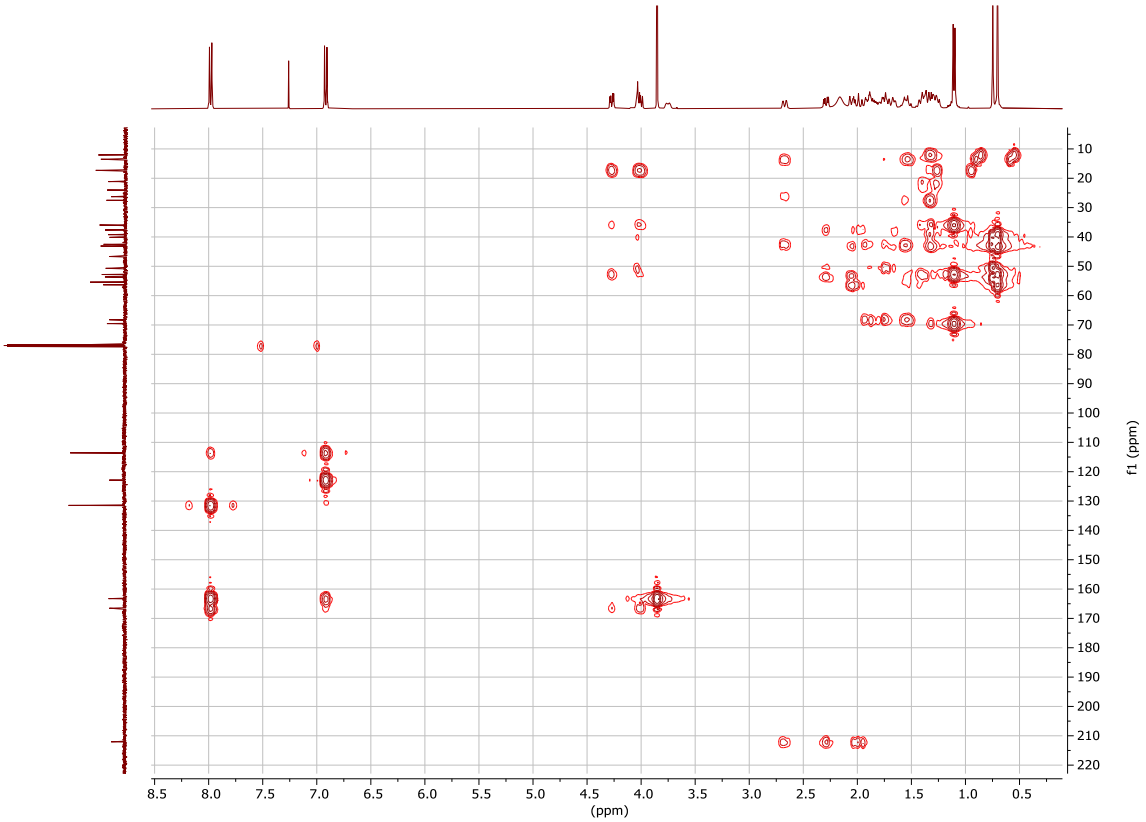


Figure S42. ^{1}H-^{13}C HMBC spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-methoxybenzoate (**18**).

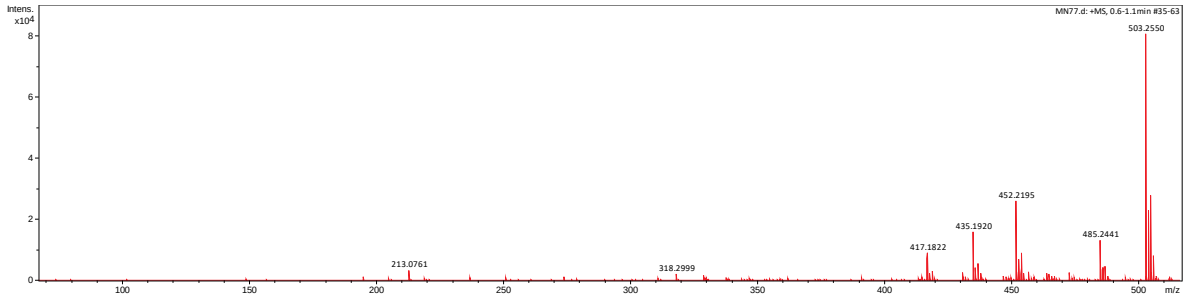


Figure S43. HRMS spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-chlorobenzoate (**19**).

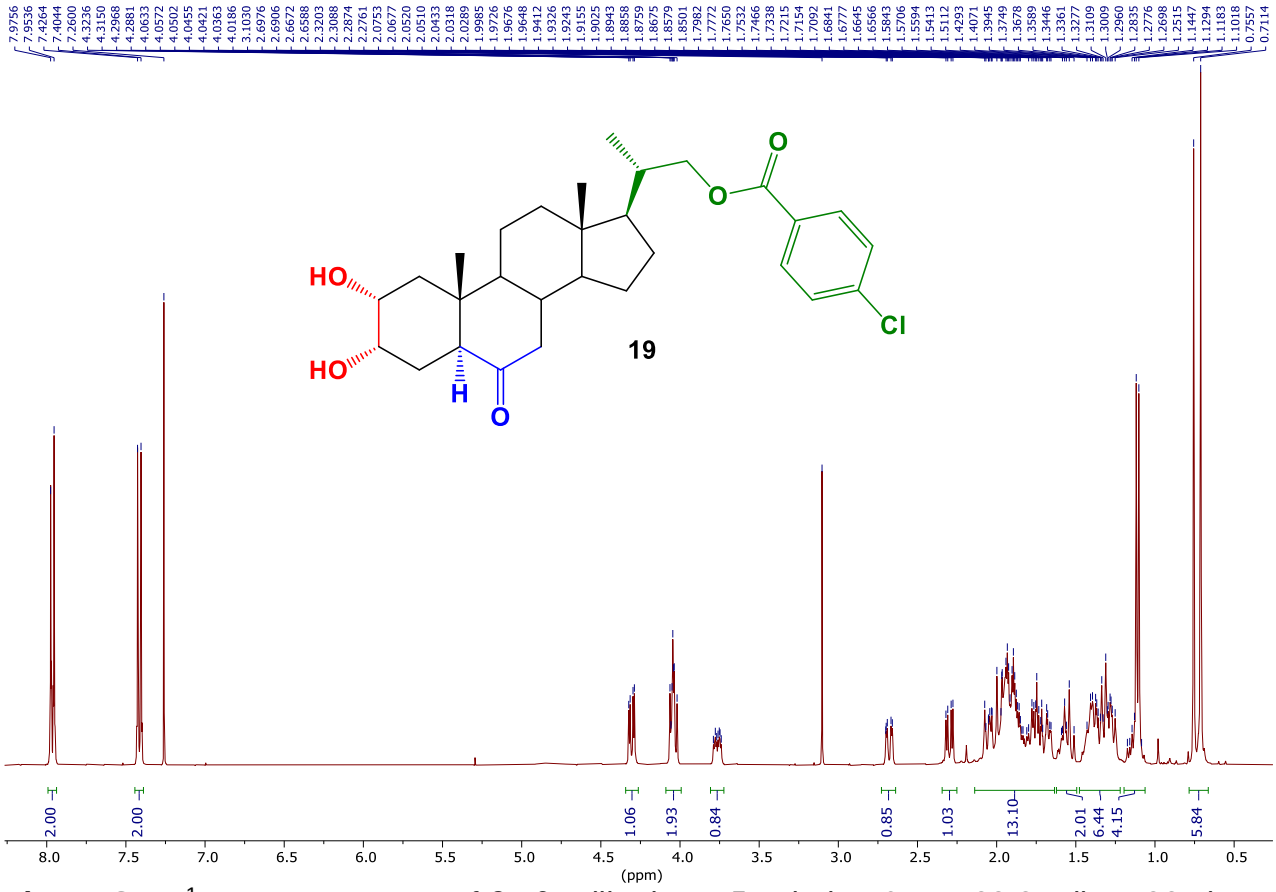


Figure S44. ^{1}H NMR spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-chlorobenzoate (**19**).

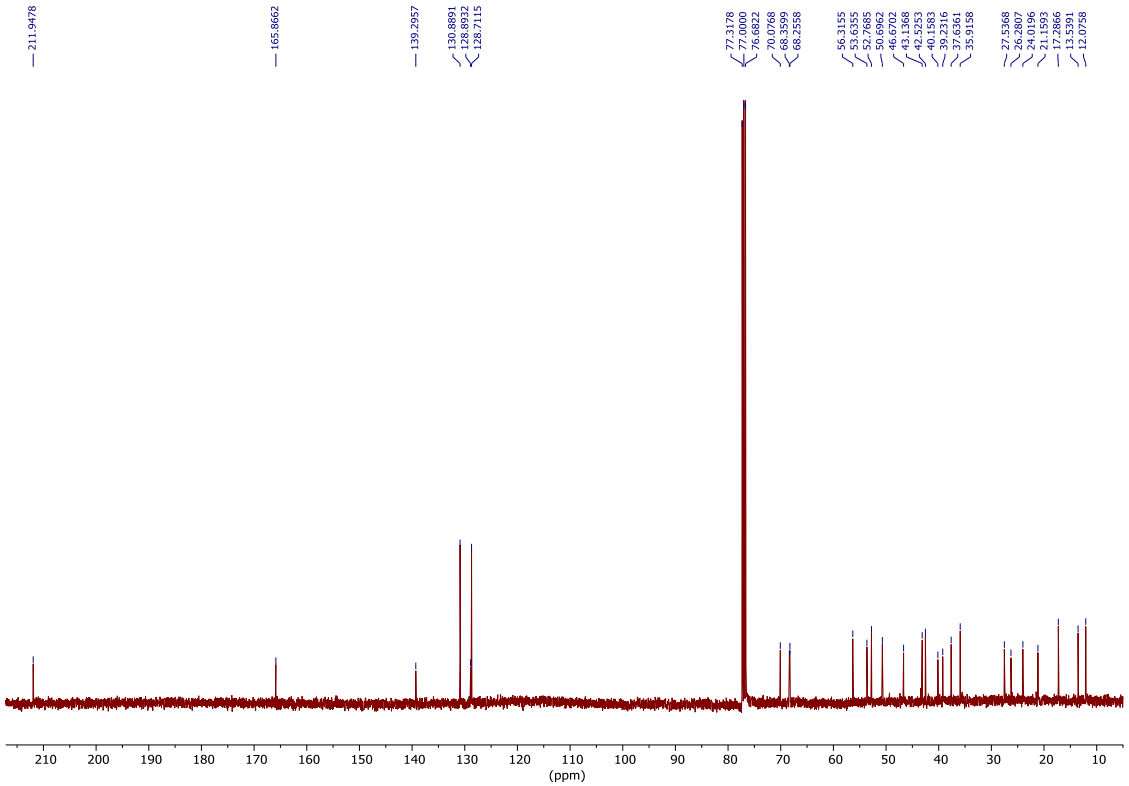


Figure S45. ^{13}C NMR spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-chlorobenzoate (**19**).

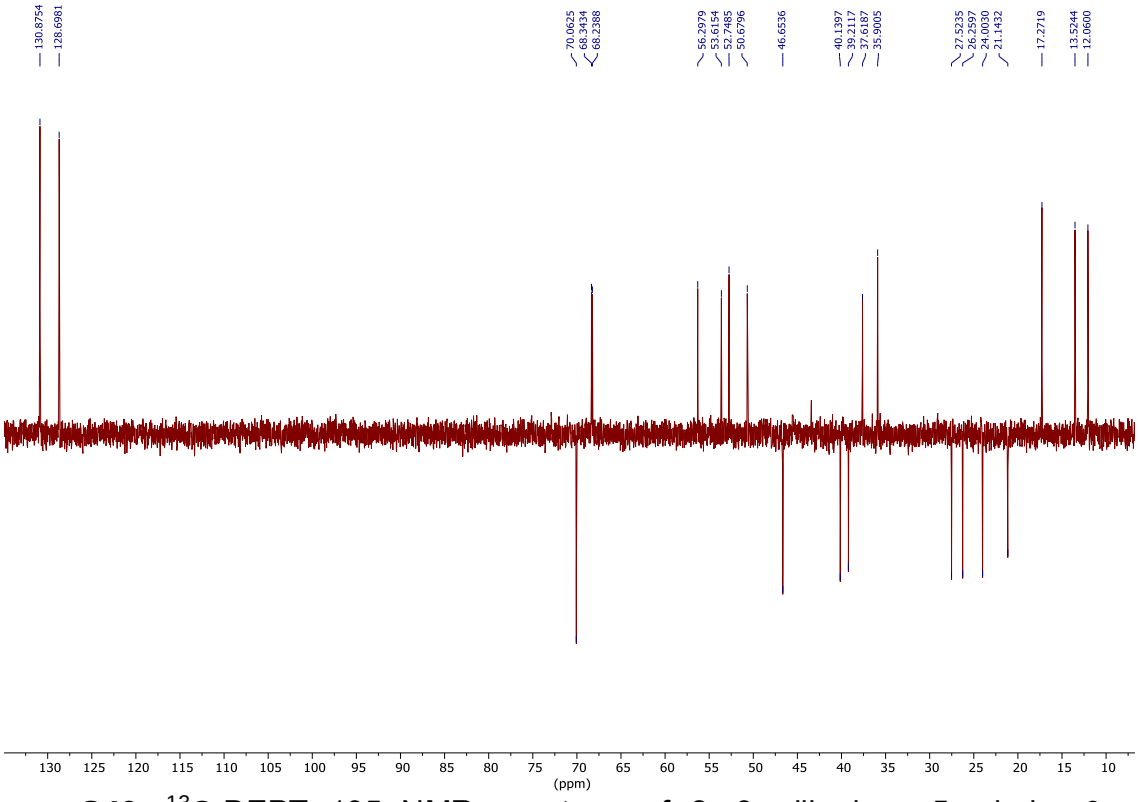


Figure S46. ^{13}C-DEPT 135 NMR spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-chlorobenzoate (**19**).

Figure S47. ^{1}H-^{13}C HSQC-ed. spectrum 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-chlorobenzoate (**19**).

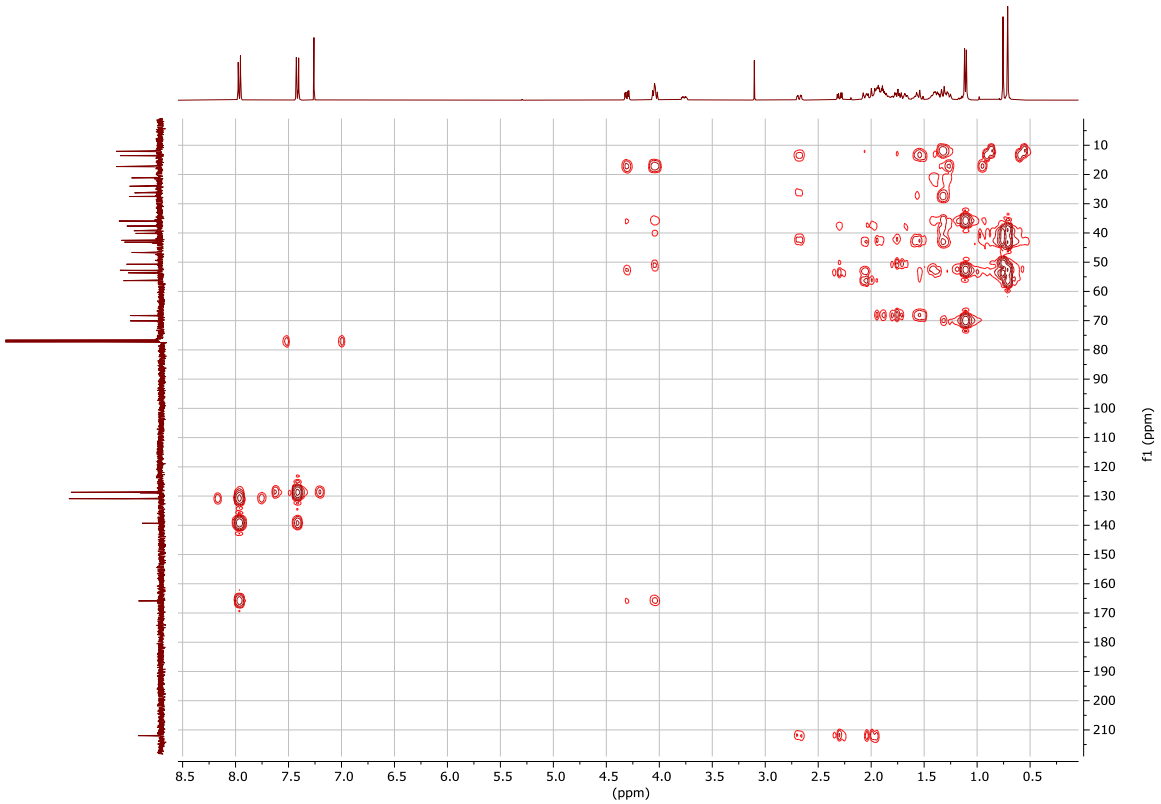


Figure S48. ^{1}H-^{13}C HMBC spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-chlorobenzoate (**19**).

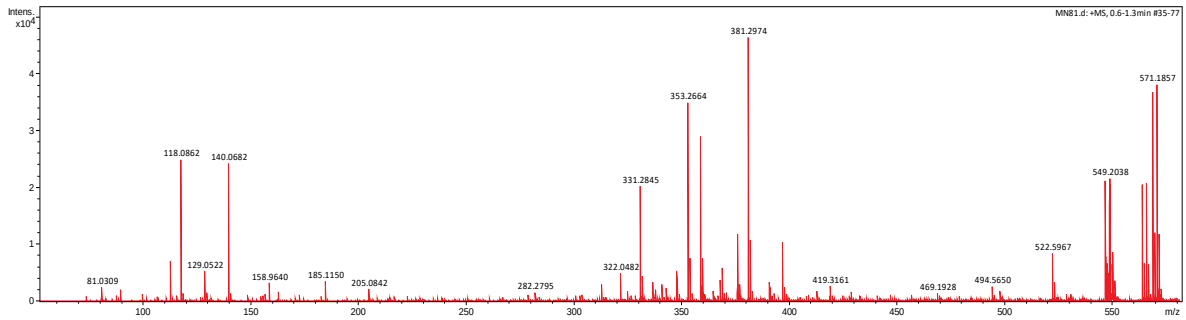


Figure S49. HRMS spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-bromobenzoate (**20**).

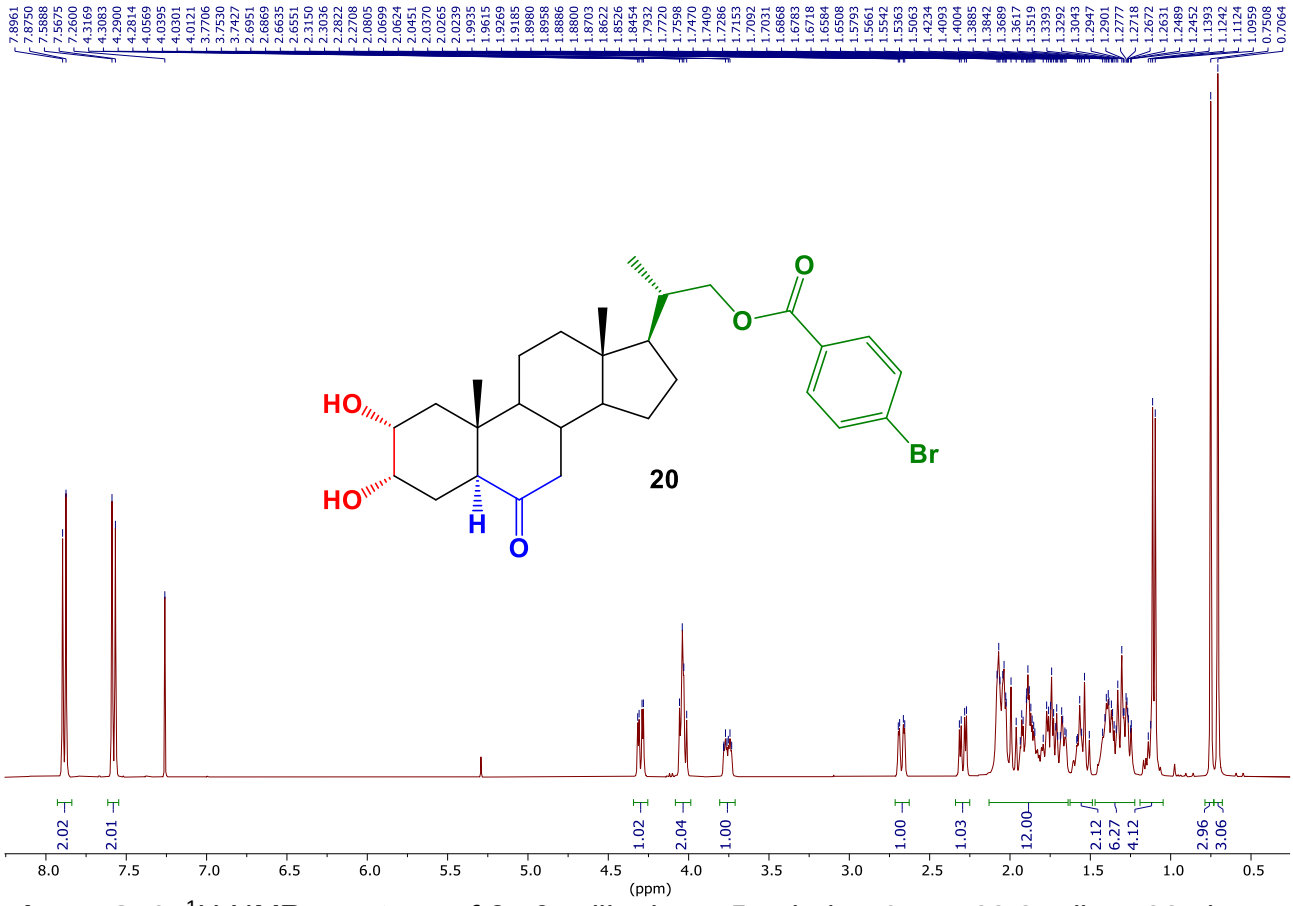


Figure S50. ^{1}H NMR spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-bromobenzoate (**20**).

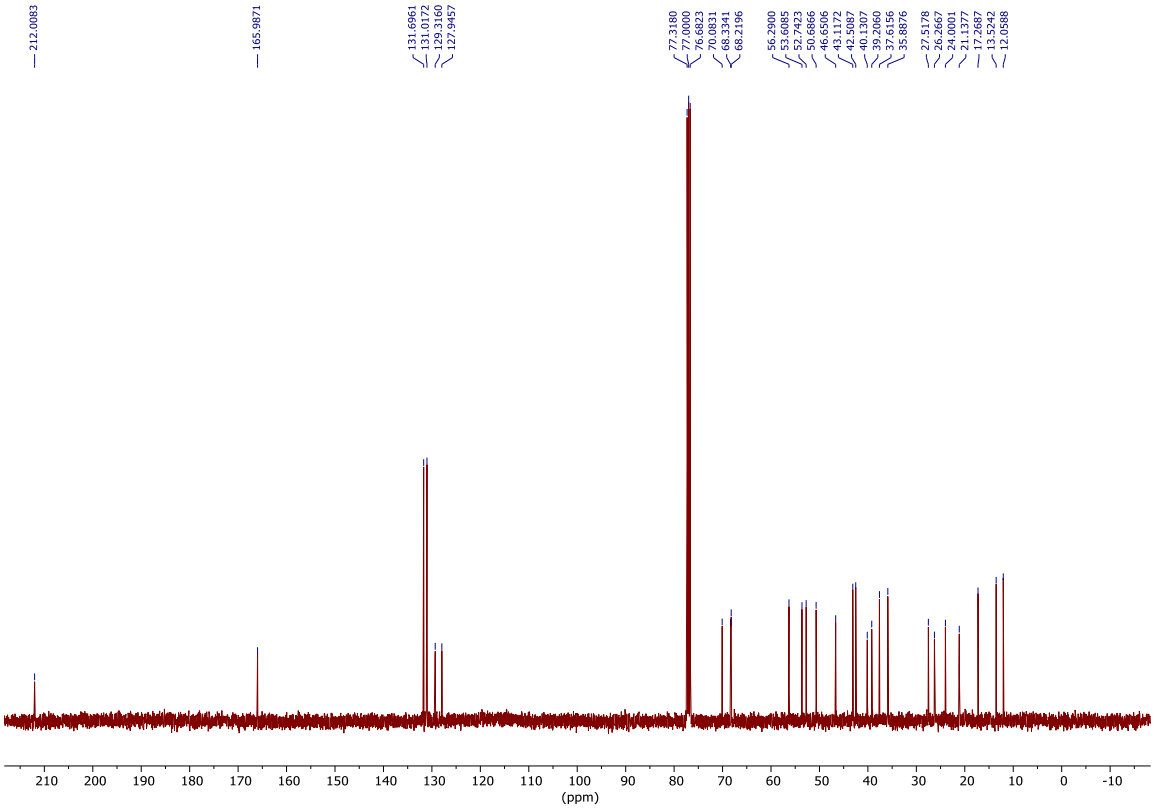


Figure S51. ^{13}C NMR spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-bromobenzoate (**20**).

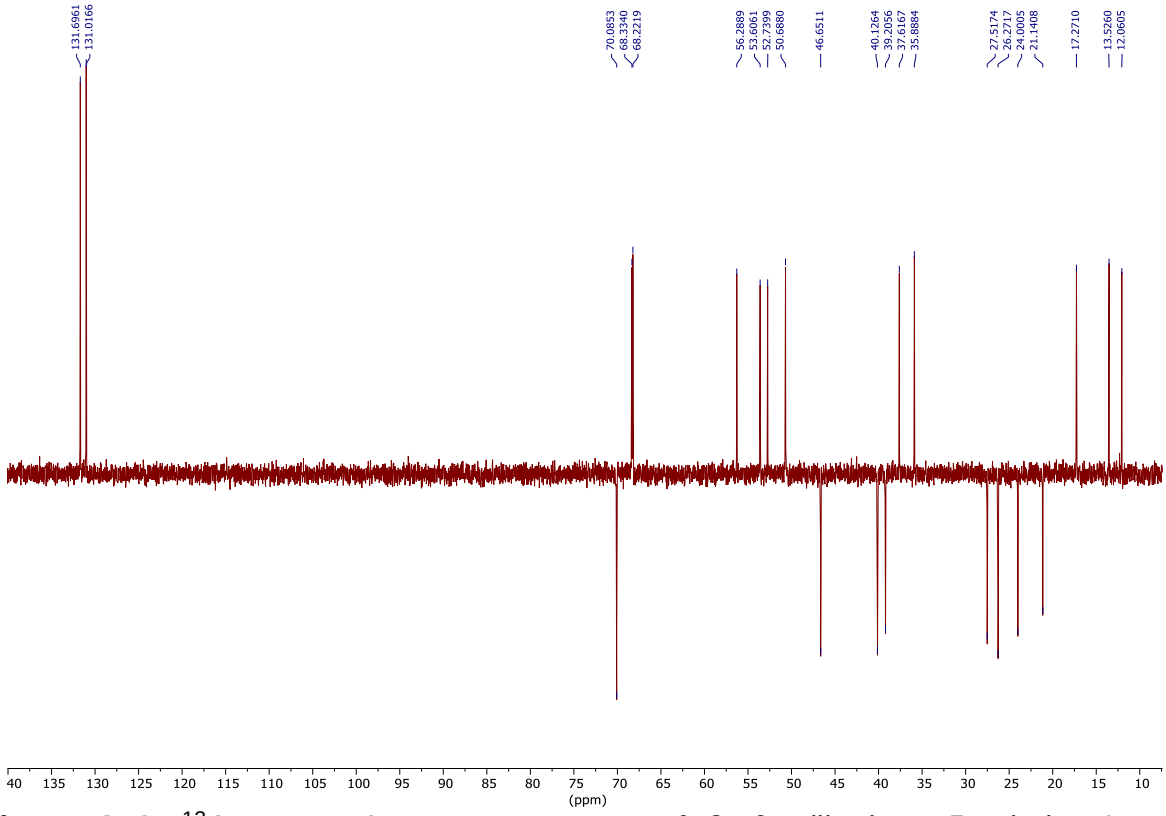


Figure S52. ^{13}C-DEPT 135 NMR spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-bromobenzoate (**20**).

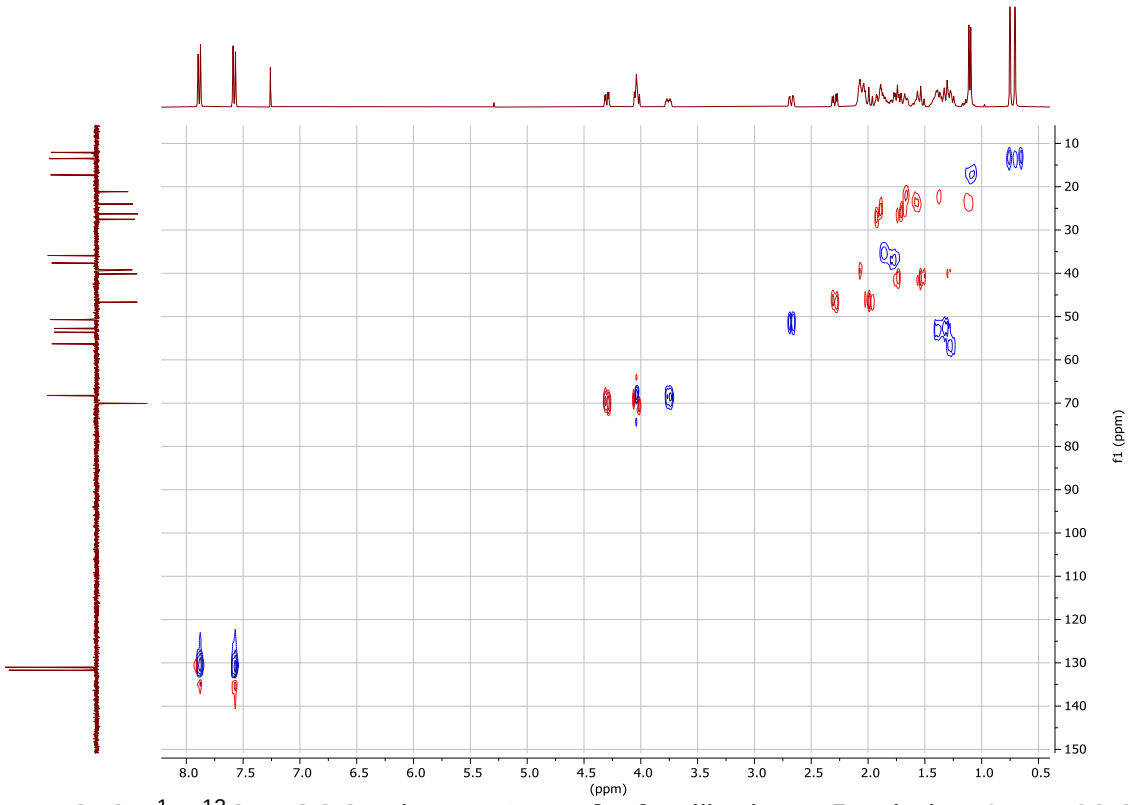


Figure S53. ^{1}H-^{13}C HSQC-ed. spectrum 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-bromobenzoate (**20**).

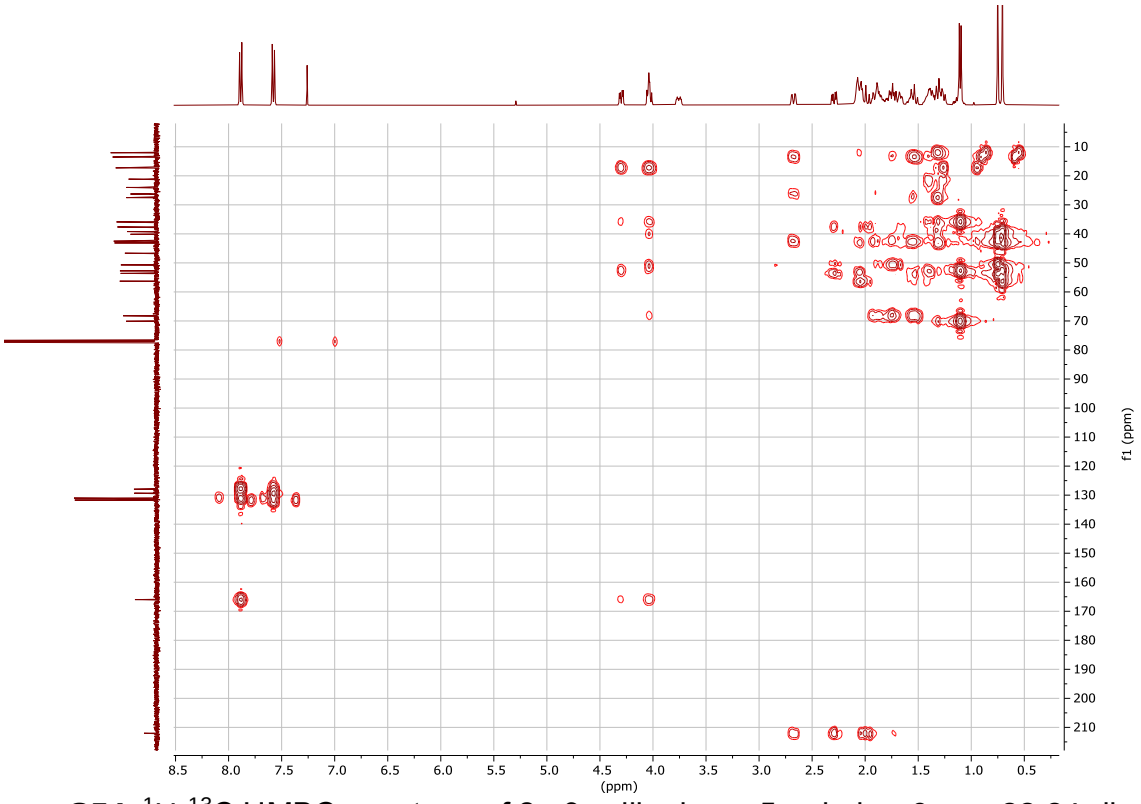


Figure S54. ^{1}H-^{13}C HMBC spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-bromobenzoate (**20**).

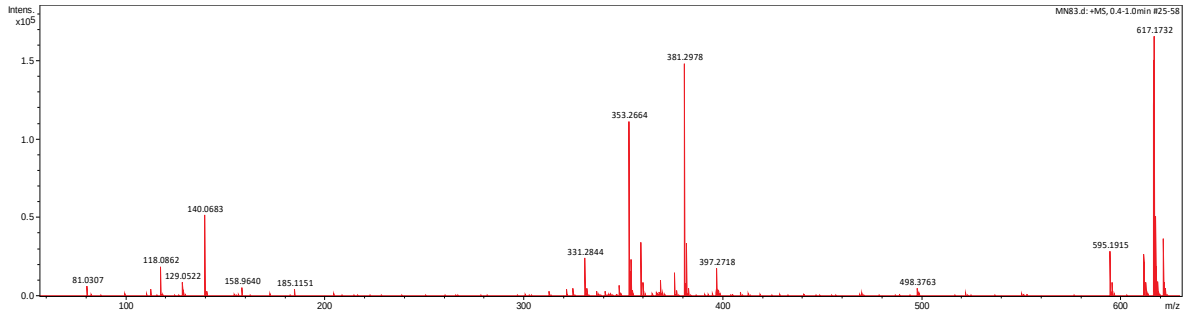


Figure S55. HRMS spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-iodobenzoate (**21**).

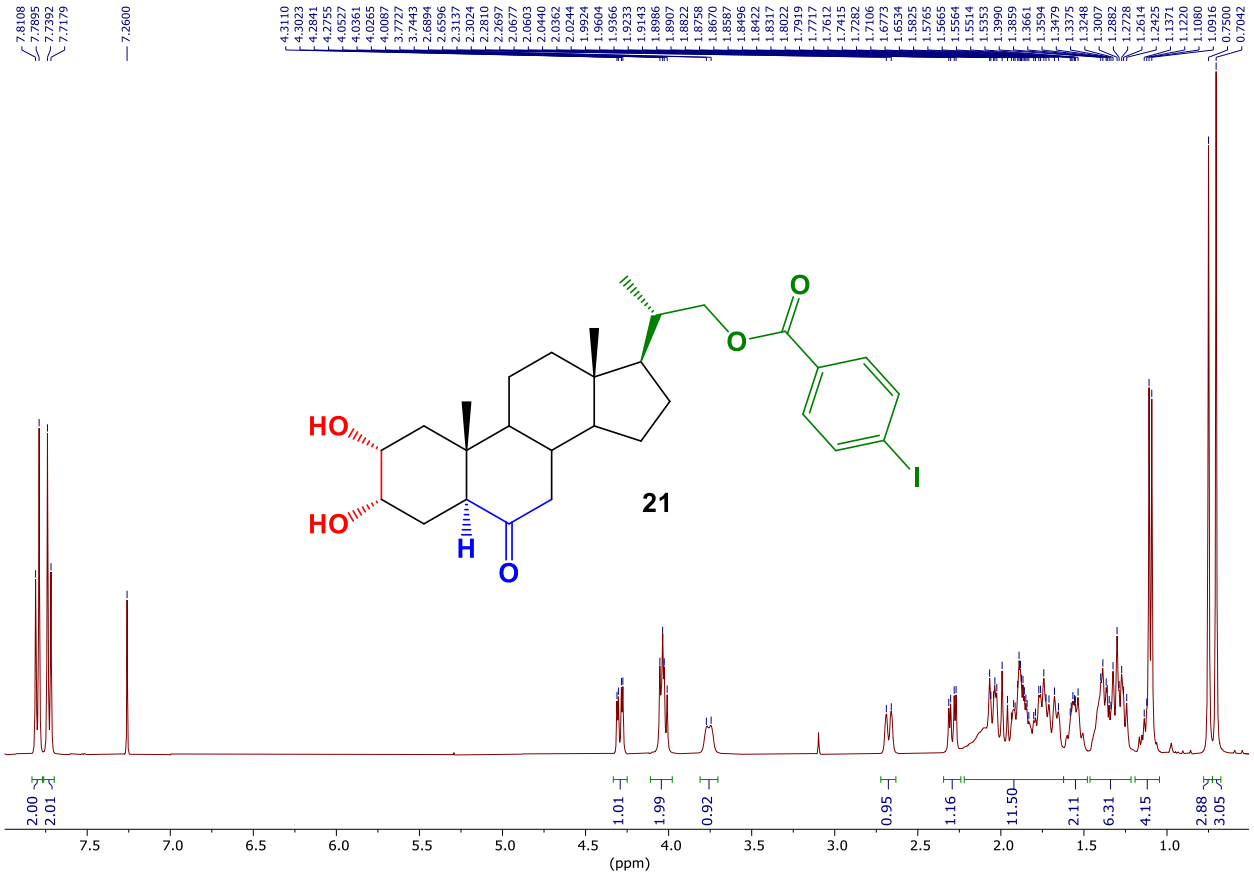


Figure S56. ^{1}H NMR spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-iodobenzoate (**21**).

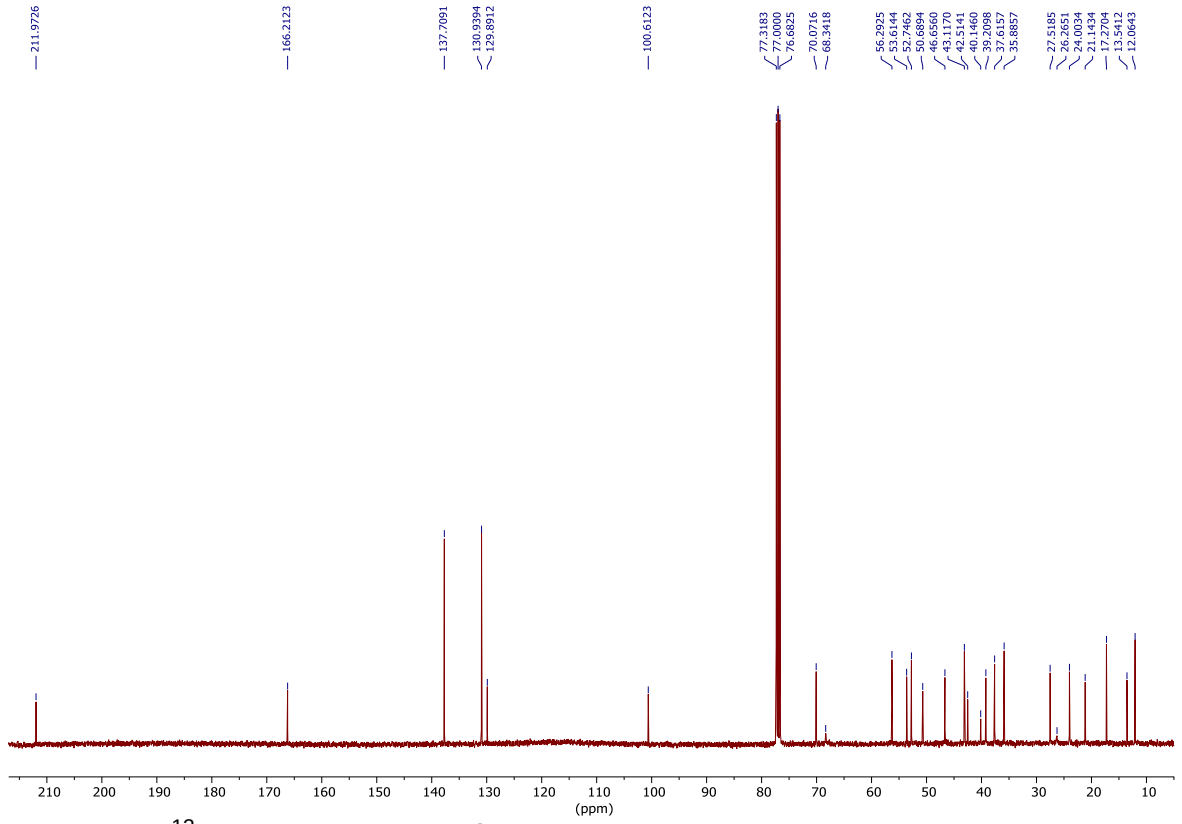


Figure S57. ^{13}C NMR spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-iodobenzoate (**21**).

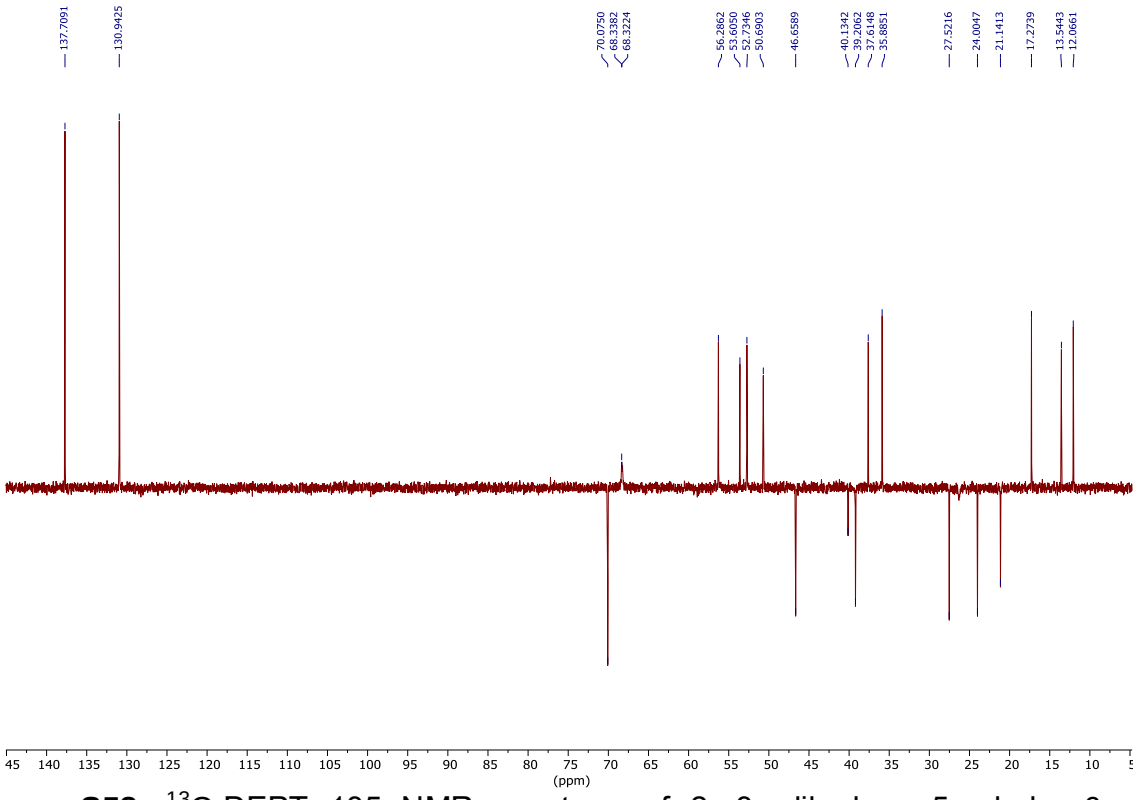


Figure S58. ^{13}C-DEPT 135 NMR spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-iodobenzoate (**21**).

Figure S59. ^{1}H-^{13}C HSQC-ed. spectrum 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-iodobenzoate (**21**).

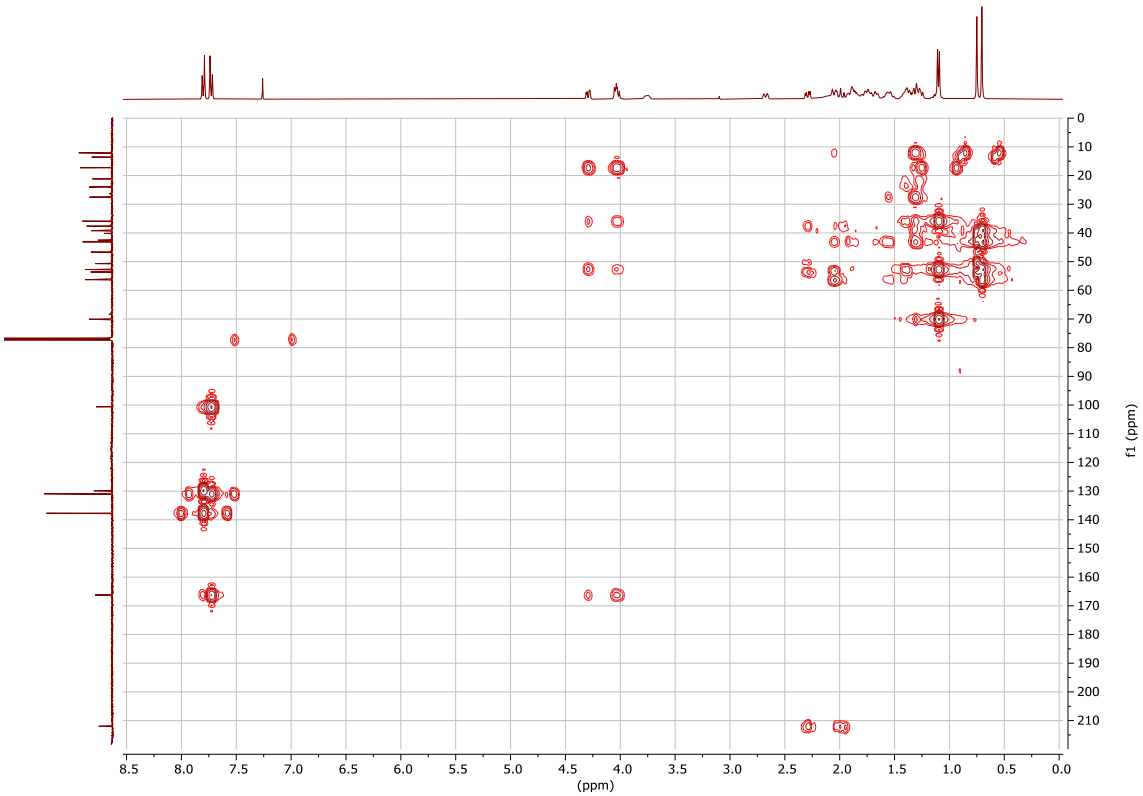


Figure S60. ^{1}H-^{13}C HMBC spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-iodobenzoate (**21**).

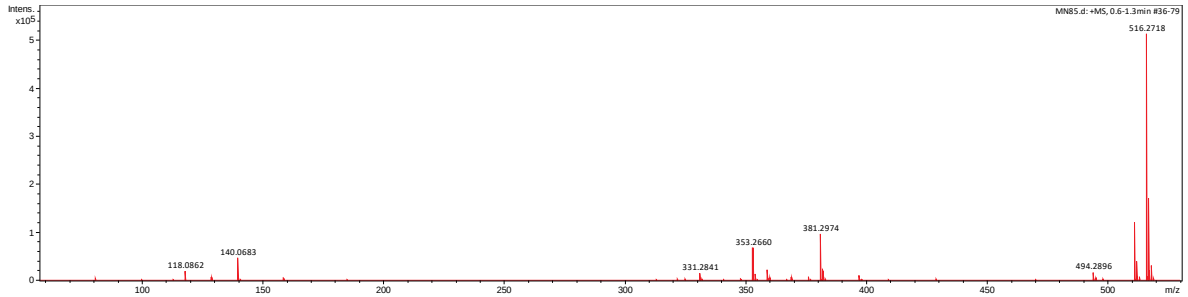


Figure S61. HRMS spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-cyanobenzoate (**22**).

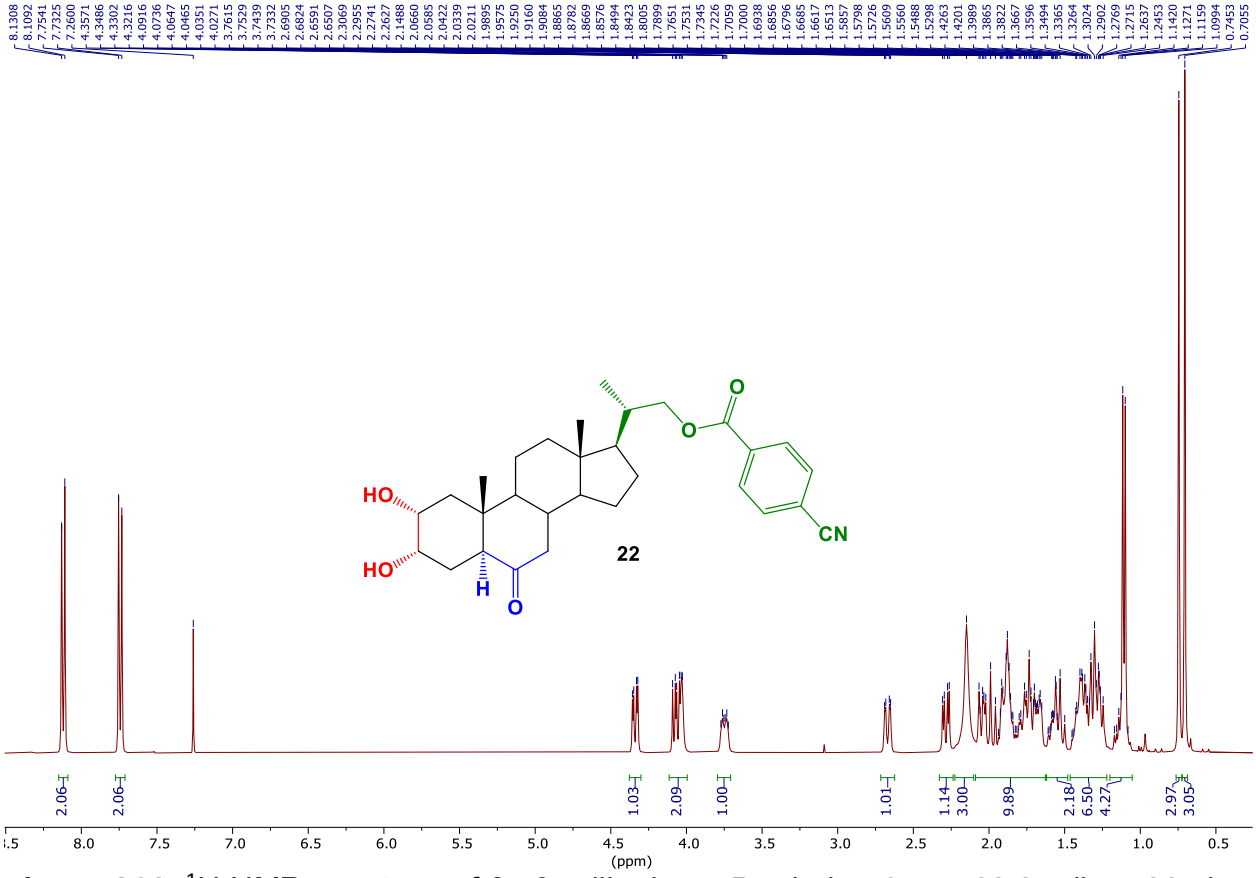


Figure S62. ^{1}H NMR spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-cyanobenzoate (**22**).

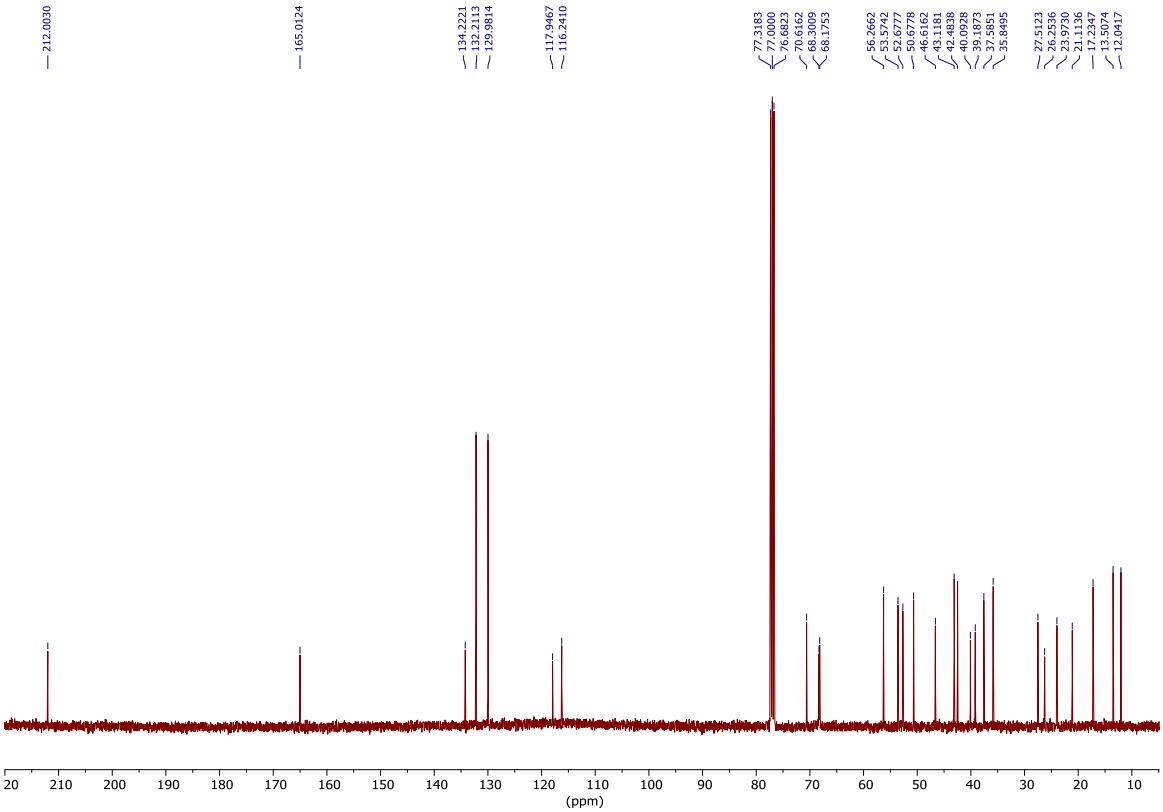


Figure S63. ^{13}C NMR spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-cyanobenzoate (**22**).

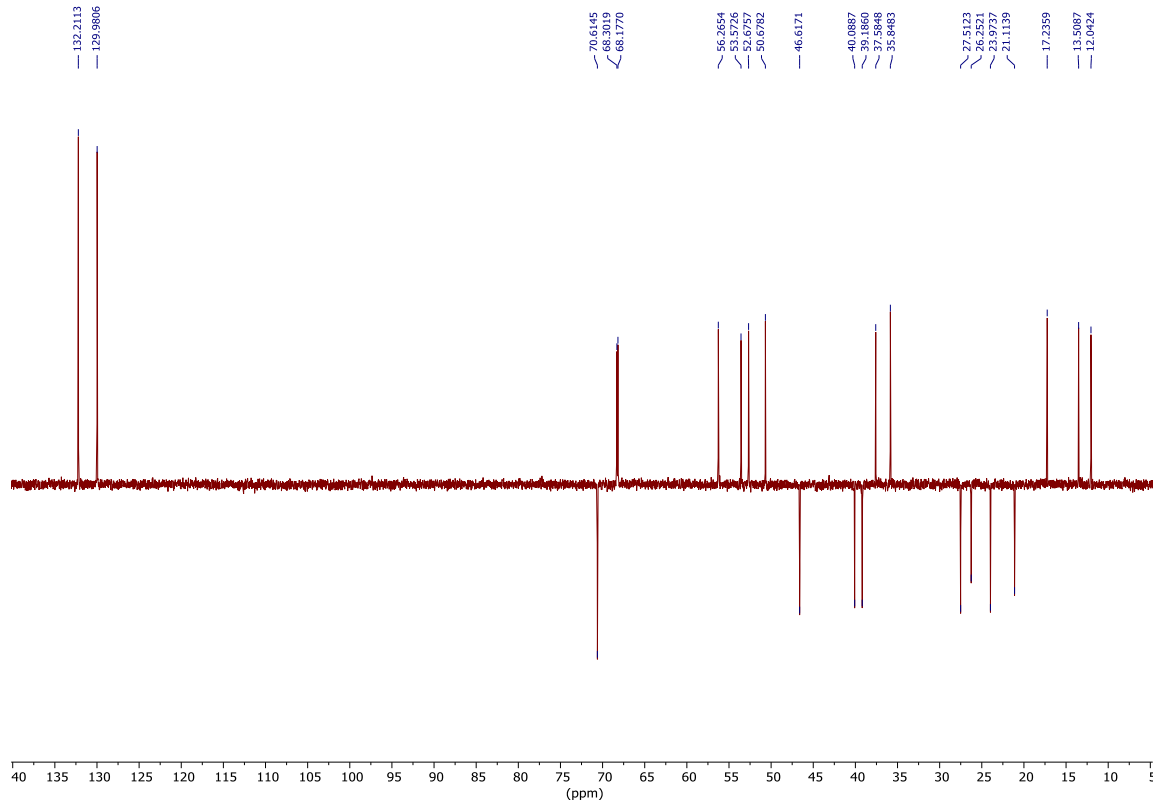


Figure S64. ^{13}C-DEPT 135 NMR spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-cyanobenzoate (**22**).

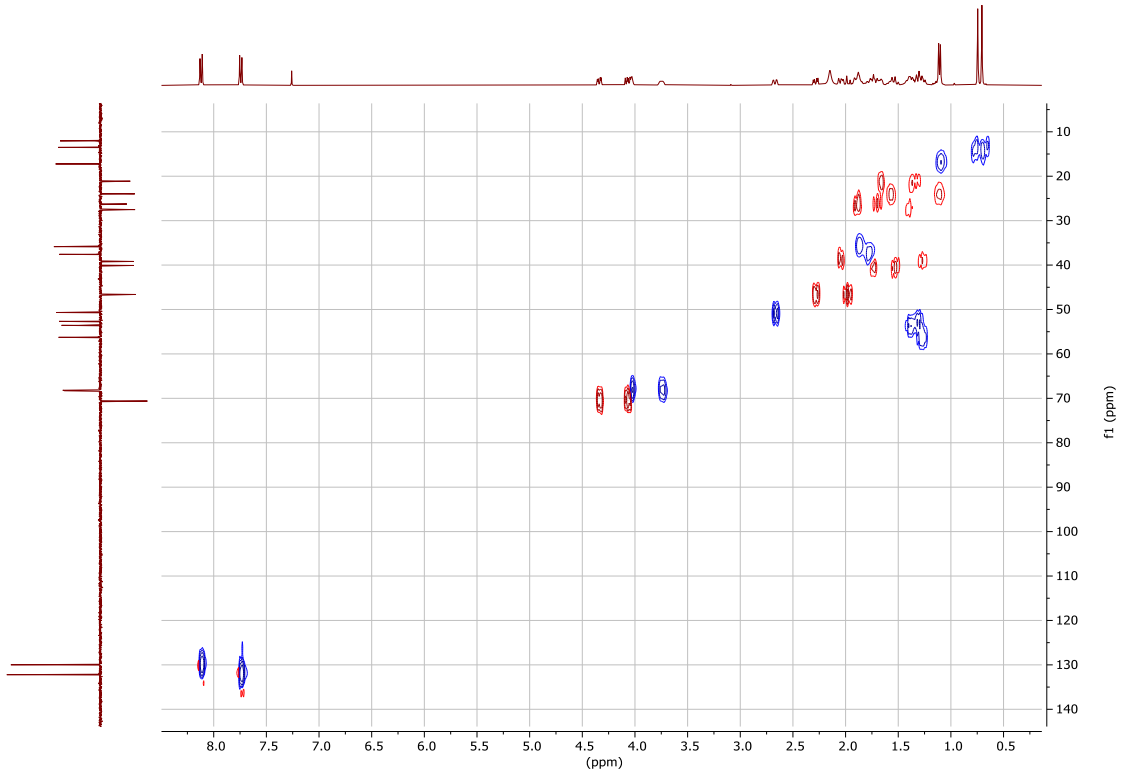


Figure S65. ^{1}H-^{13}C HSQC-ed. spectrum 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-cyanobenzoate (**22**).

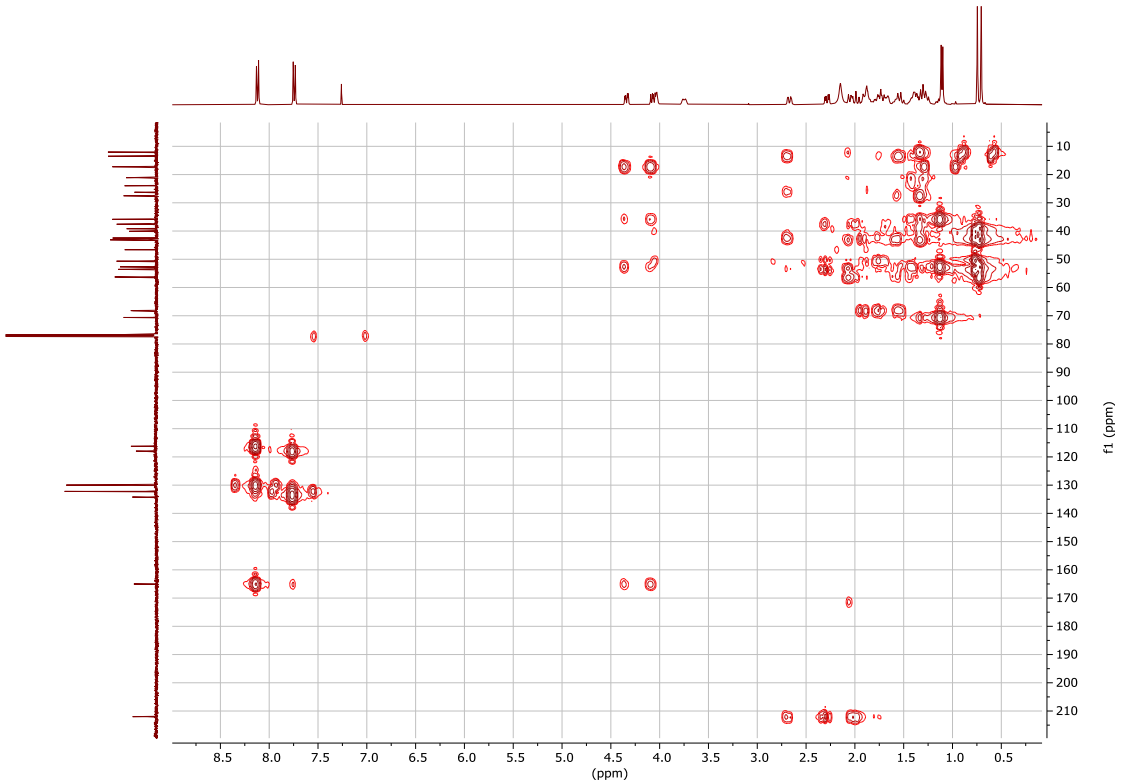


Figure S66. ^{1}H-^{13}C HMBC spectrum of 2α,3α-dihydroxy-5α-cholan-6-oxo-23,24-dinor-22-yl 4-cyanobenzoate (**22**).

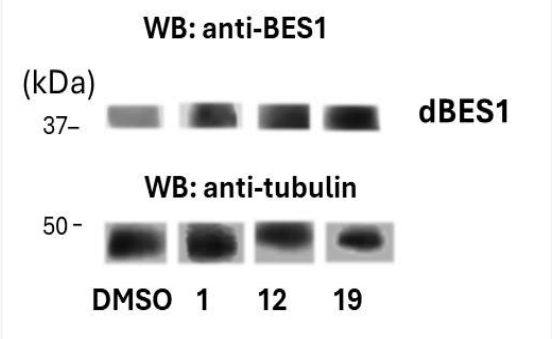


Figure S67. Representative Western blot analysis showing dephosphorylation of BES1 after treatment with active compound **12** and **19**. WB: Western blot; dBES1: dominant Brassinosteroid-Insensitive 1 Suppressor 1; DMSO: Dimethyl sulfoxide; kDa: Kilodalton. Representative images are shown. dBES1, dephosphorylated BES1.

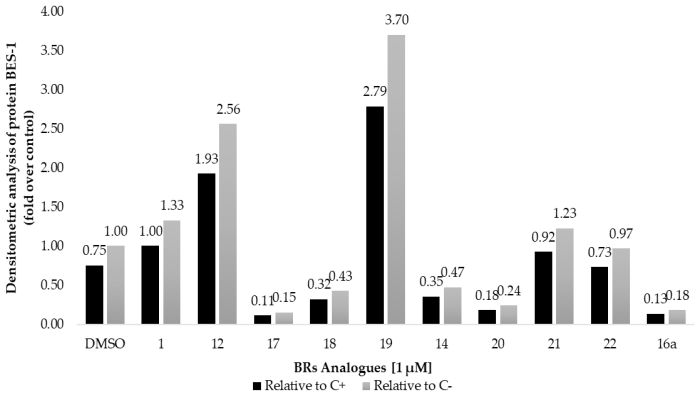
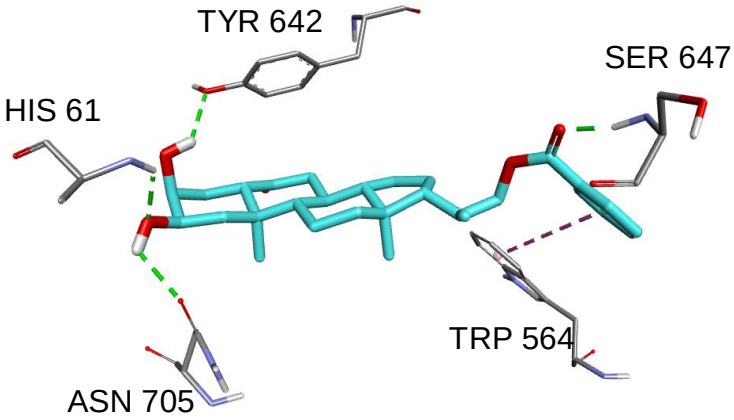
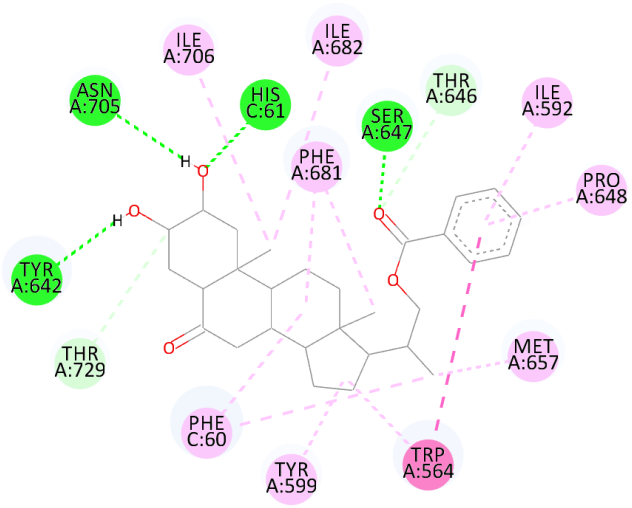


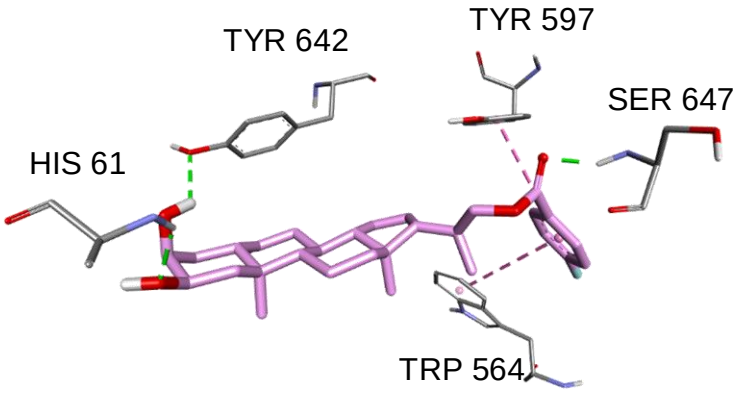
Figure S68: BES1 phosphorylation status tested by immunoblot with α-BES1 antibody in roots of *Arabidopsis thaliana* ecotype Col-0 6-day-old seedlings after analogues of BR treatment. Tubulin detected with α-tubulin antibody was used as a loading control. The graph shows the percentage of dephosphorylated BES1 relative to total BES1 detected in wild-type *Arabidopsis* ecotype (Col-0) treated with brassinolide (**1**) after 6 days.

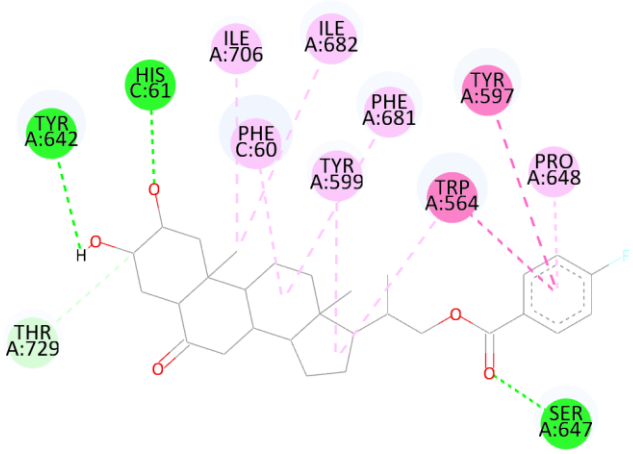
a) Compound **12**



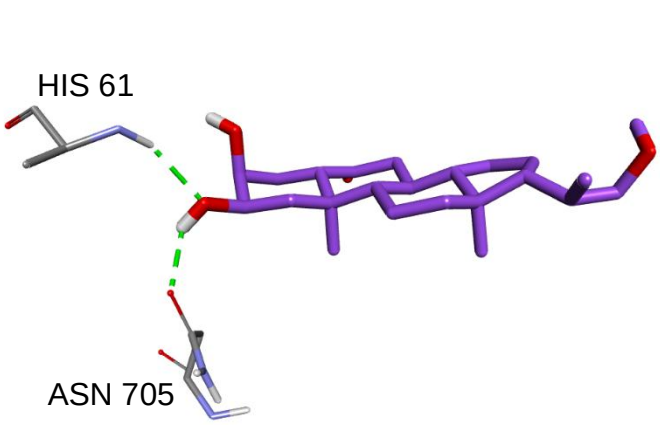


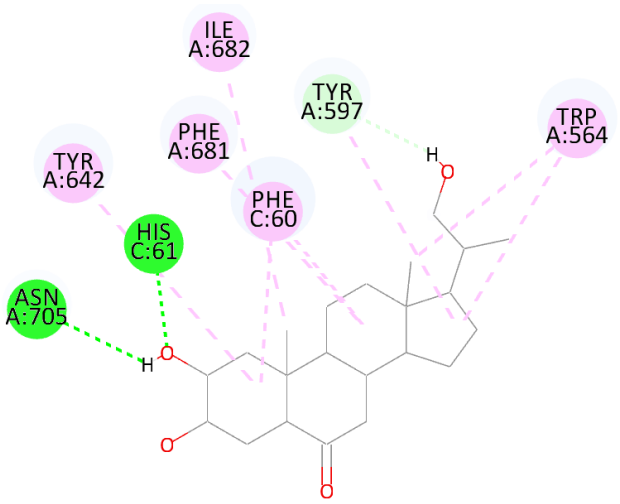
b) Compound **14**



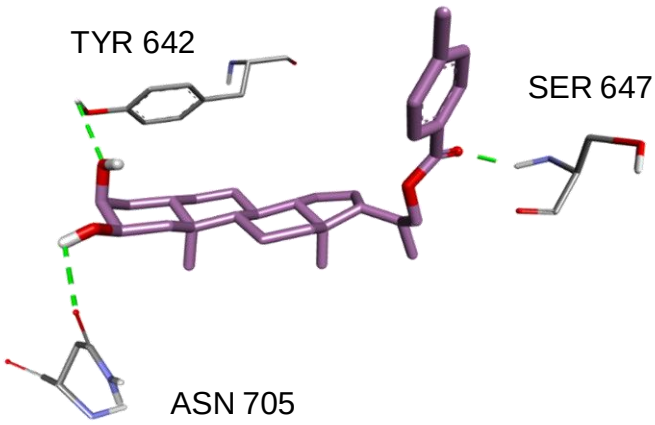


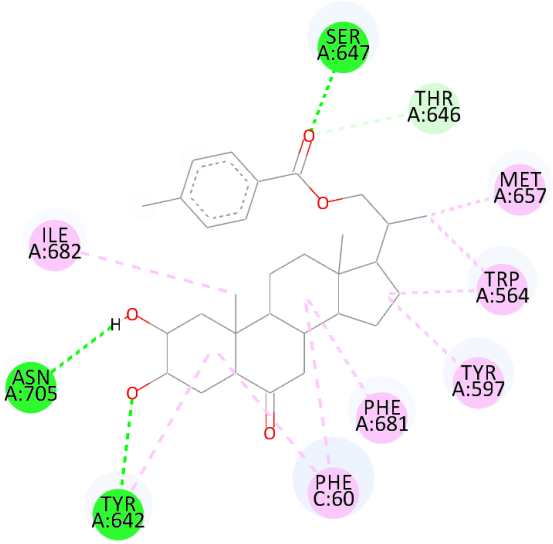
c) Compound **16a**



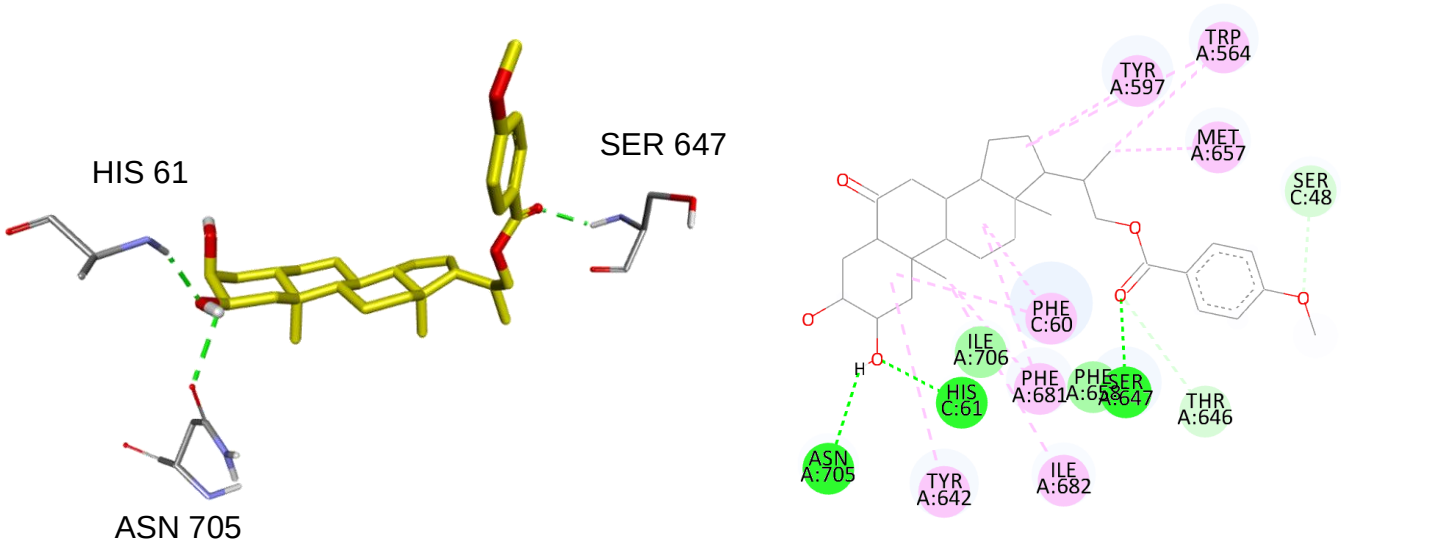


d) Compound **17**

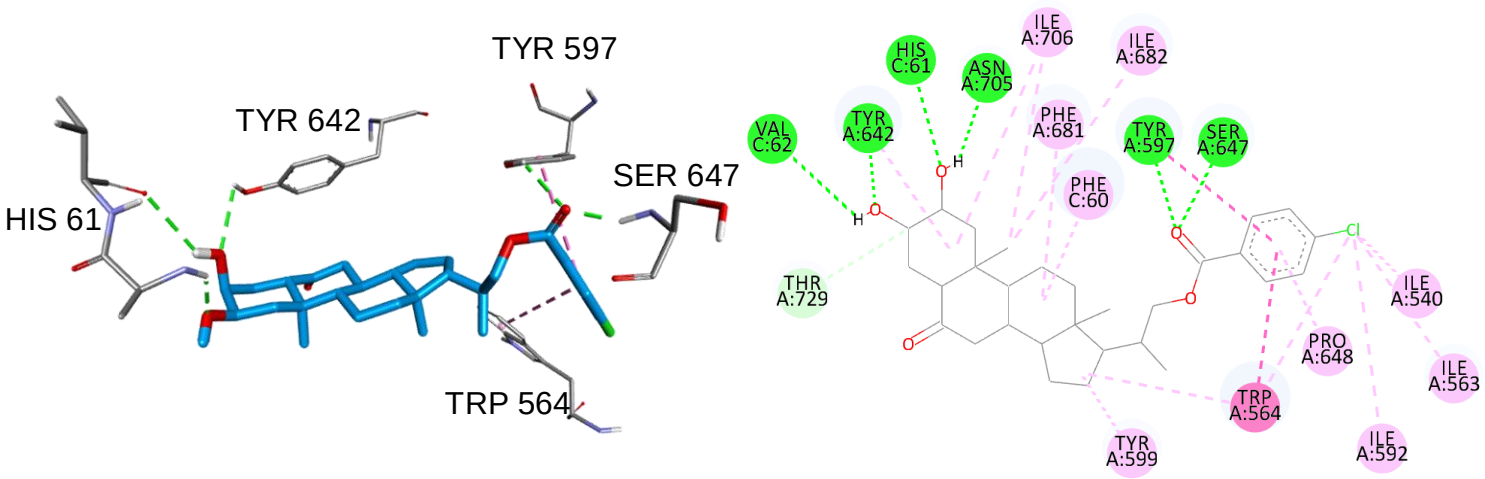




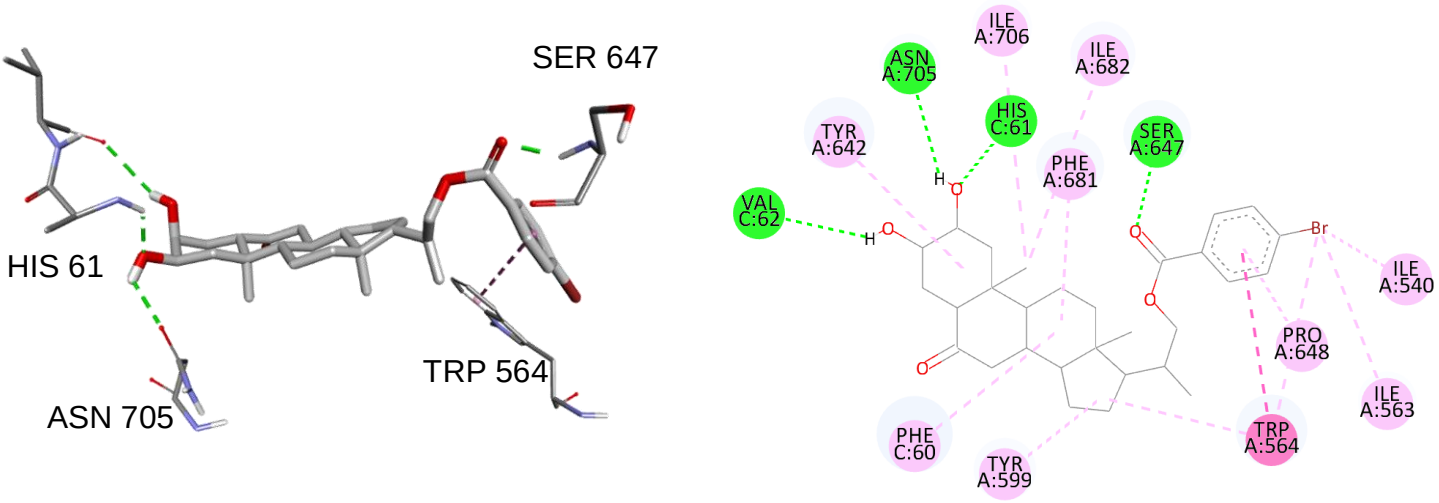
e) Compound **18**



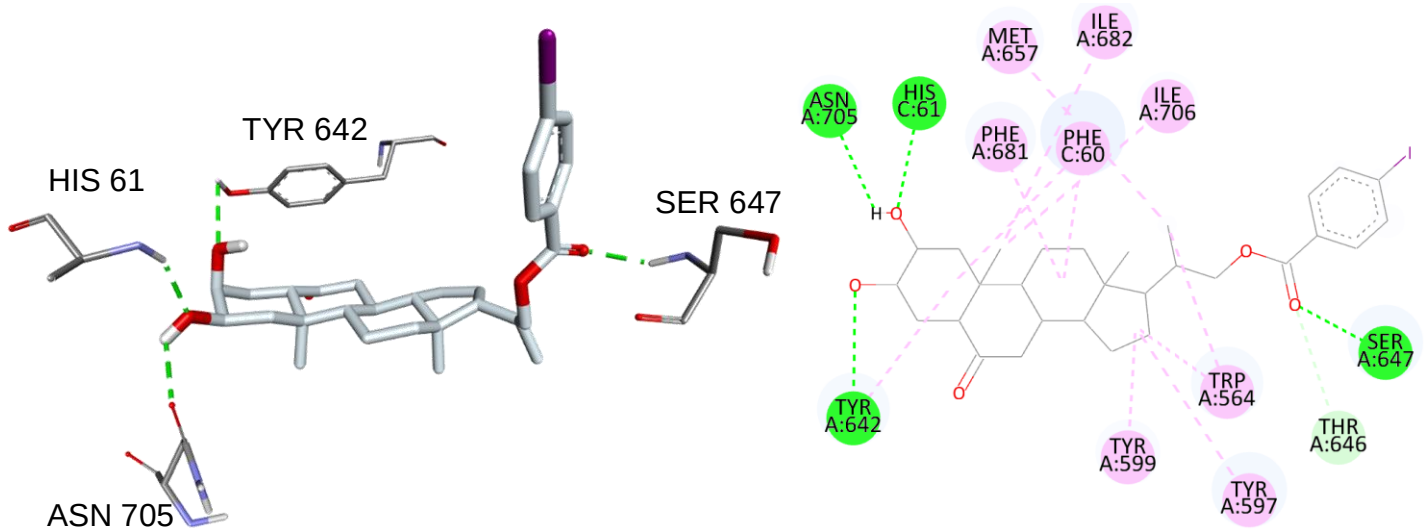
f) Compound **19**



g) Compound **20**



h) Compound **21**



i) Compound **22**

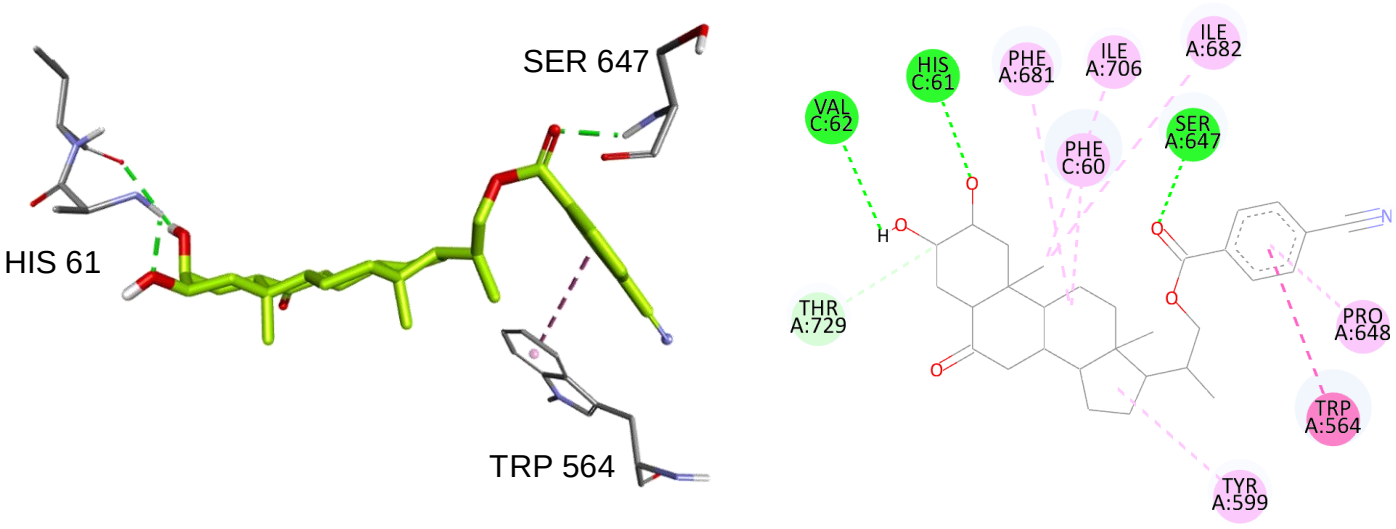
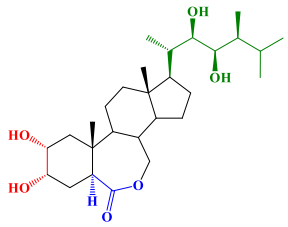
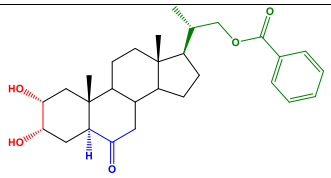
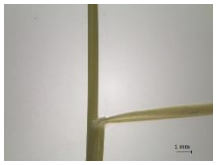
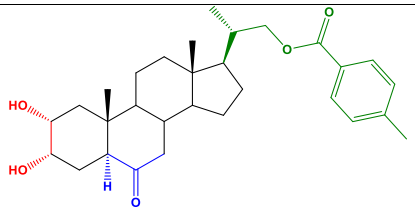
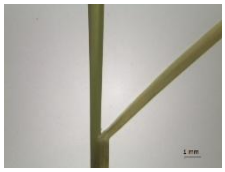
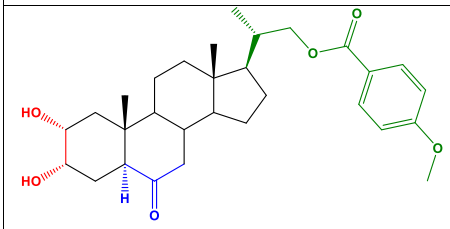
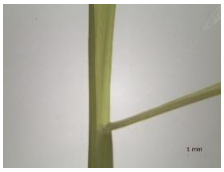
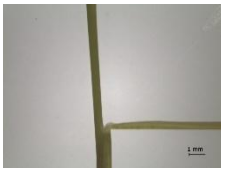
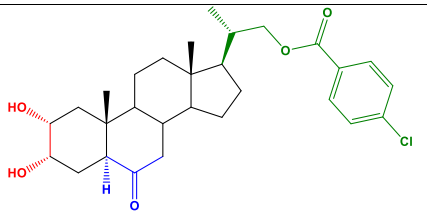
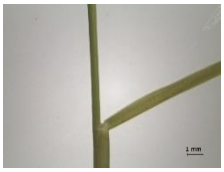
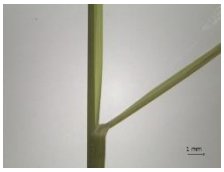
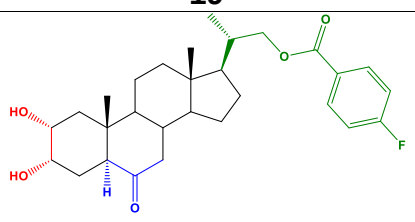
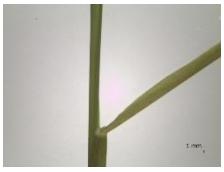


Figure S69. Protein–ligand interactions with a) compound **12** b) compound **14**; c) compound **16a**, d) compound **17**, e) compound **18**, f) compound **19**, g) compound **20**, h) compound **21** and i) compound **22**. Hydrogen bonds are represented in green segmented lines. Π–π stacking are represented in dark pink segmented lines. Hydrophobic interactions are represented in pink segmented lines. Visualization of the docked poses was performed using Discovery Studio Visualizer (BIOVIA, San Diego, CA, USA).

Table S1: Rice lamina assays using the second leaf lamina joints (angle opening, degrees) of excised leaf segments treated with BRs analogs (**1**, **12**, **14** and **17**–**22**) at different concentrations. Brassinolide was used as positive control at the same concentrations.

	RLIT (angle opening, degrees)		
Compounds	1×10^{-8} M	1×10^{-7} M	1×10^{-6} M
 **1**			
 **12**			
 **17**			
 **18**			
 **19**			
 **14**			

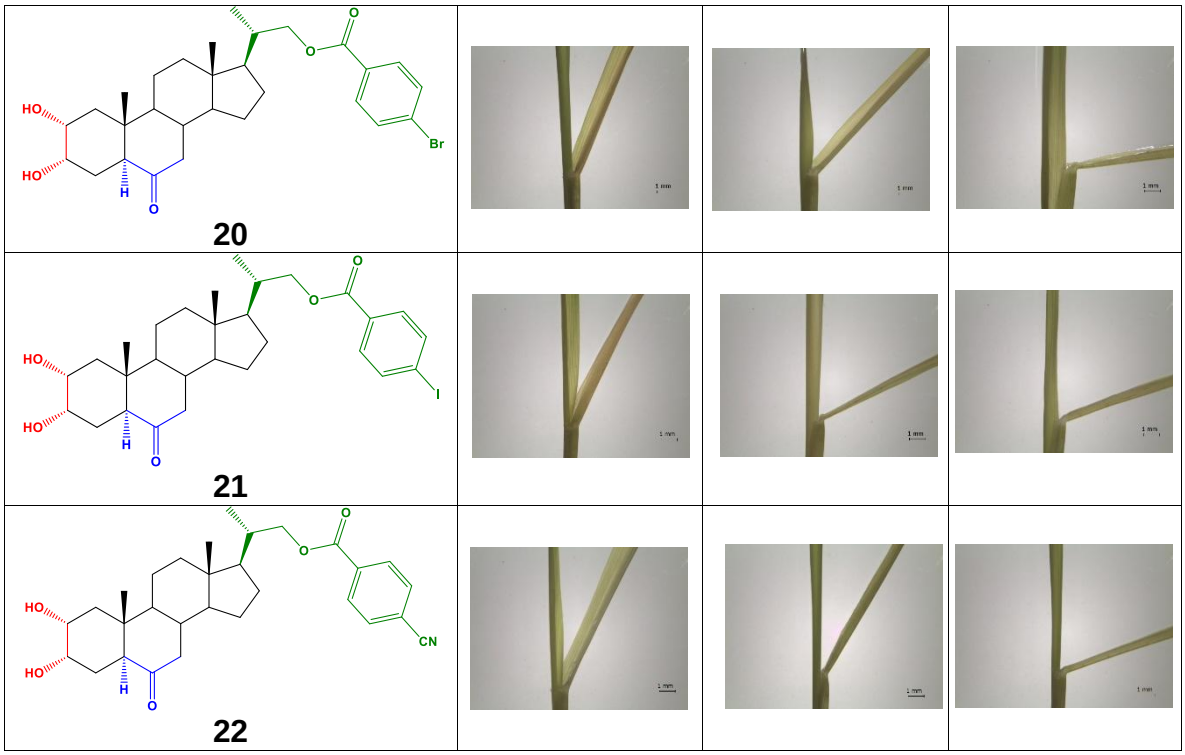


Table S2. Docked compounds–heterodimer protein contacts of synthetic analogs (**12–22**).

Compound	Protein contacts	
	Hydrogen bonds	Non-polar interactions
12	Asn705 His61 Tyr642 Ser647	Ile706, Tyr599, Phe681, Met657, Trp564, Pro648, Ile682, Ile592, Phe60
14	His61 Tyr642 Ser647	Ile706, Tyr597, Trp564, Phe681, Ile682, Phe60, Tyr597, Tyr599, Pro648
16a	Asn705 His61	Trp564, Phe681, Ile682, Phe60, Tyr642, Phe60, Tyr597
17	Asn705 Ser647 Tyr642	Tyr597, Trp564, Met657, Ile682, Phe60, Phe681, Tyr642
18	Ser647 Asn705	Tyr597, Trp564, Met657, Ile682, Phe60, Phe681, Tyr642

	His61	
19	Ser647 Val62 Tyr597 His61 Asn705 Tyr642	Tyr599, Trp564, Ile682, Phe60, Phe681, Tyr642, Ile706, Ile540, Ile563, Pro648
20	Ser647 Val62 His61 Asn705	Tyr599, Trp564, Ile682, Phe60, Phe681, Tyr642, Ile706, Ile540, Ile563, Pro648
21	Ser647 Asn705 Tyr642 His61	Tyr599, Trp564, Ile682, Phe60, Phe681, Tyr642, Ile706, Met657, Tyr597
22	Ser647 Val62 His61	Tyr599, Trp564, Ile682, Pro648, Phe681, Ile706, Phe60