Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Synthesis of Obtusifoliol and Analogues as CYP51 Substrates

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Electronic Supplementary Information

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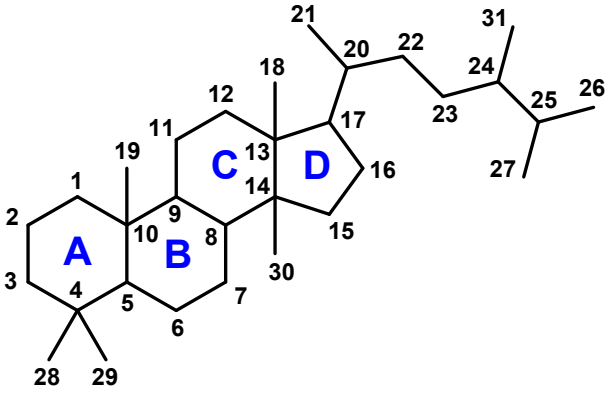


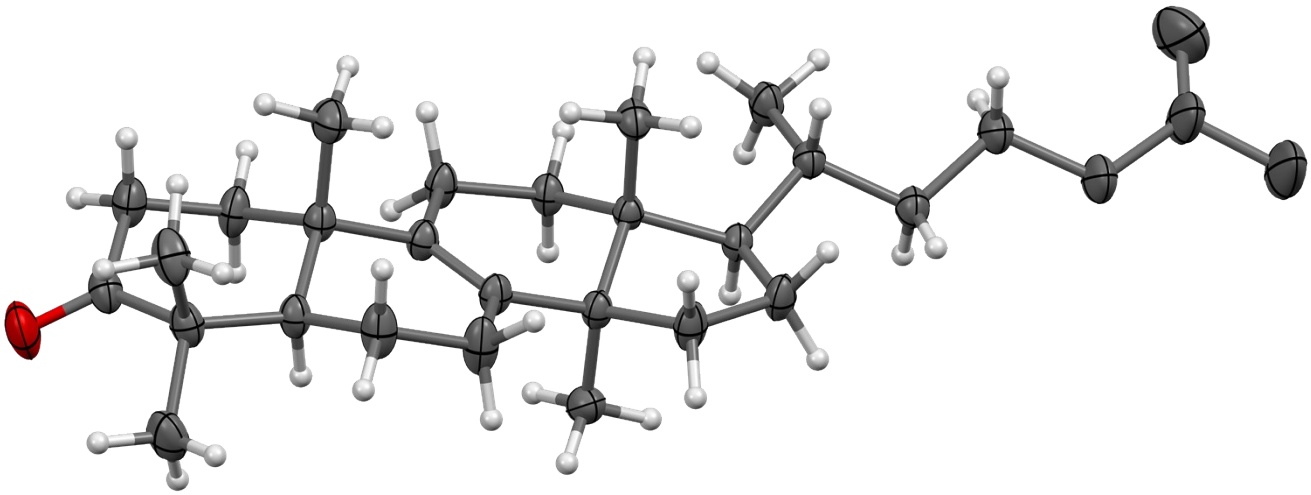
Steroidal structures in this work were numbered using common nomenclature shown above.

1. X-ray data collection and structure determinations

Crystals suitable for X-ray analysis were grown via the slow evaporation of a methanol/water solution. Data were collected on an Oxford Diffraction Gemini CCD diffractometer employing Cu-Kα radiation (1.54184 A) and operating within the range $2 < 2\theta < 125°$. The crystal was cooled to 190 K with an Oxford Cryosystems Desk-top Cooler. Data reduction and empirical absorption corrections (multi-scan) were performed with Oxford Diffraction CrysAlisPro software (Oxford Diffraction). The structure was solved by direct methods with SHELXS and re-fined by full-matrix least-squares analysis with SHELXL.[1] All non-H atoms were refined with anisotropic thermal parameters. The molecular structure diagram was produced with Mercury.[2]

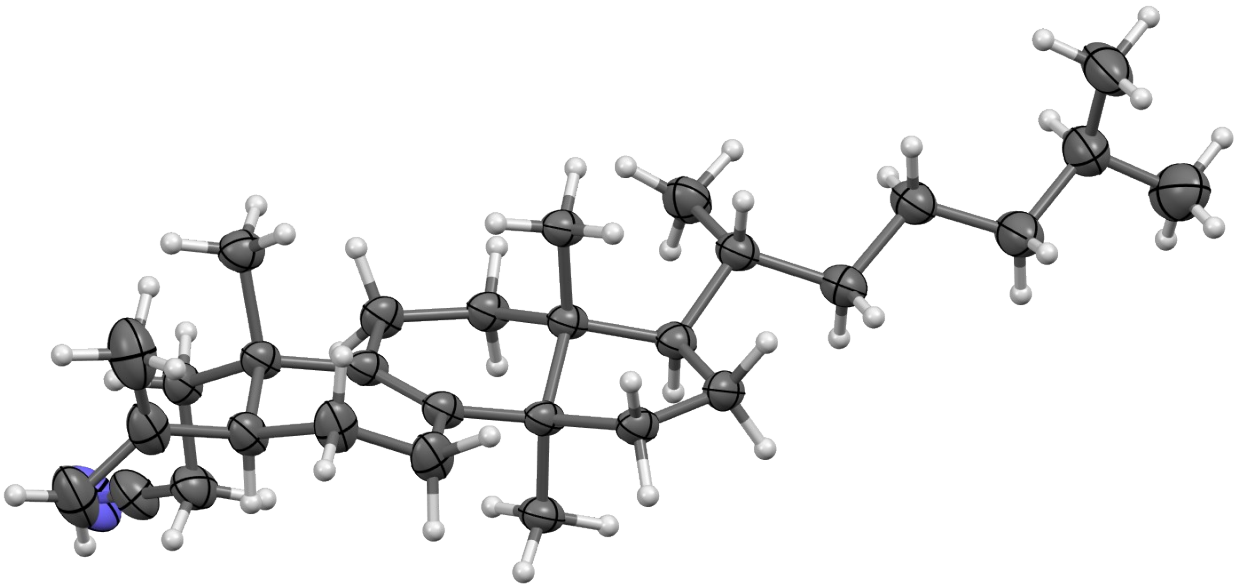
2. Crystal structures

Crystal structure of lanosten-3-one (5a)



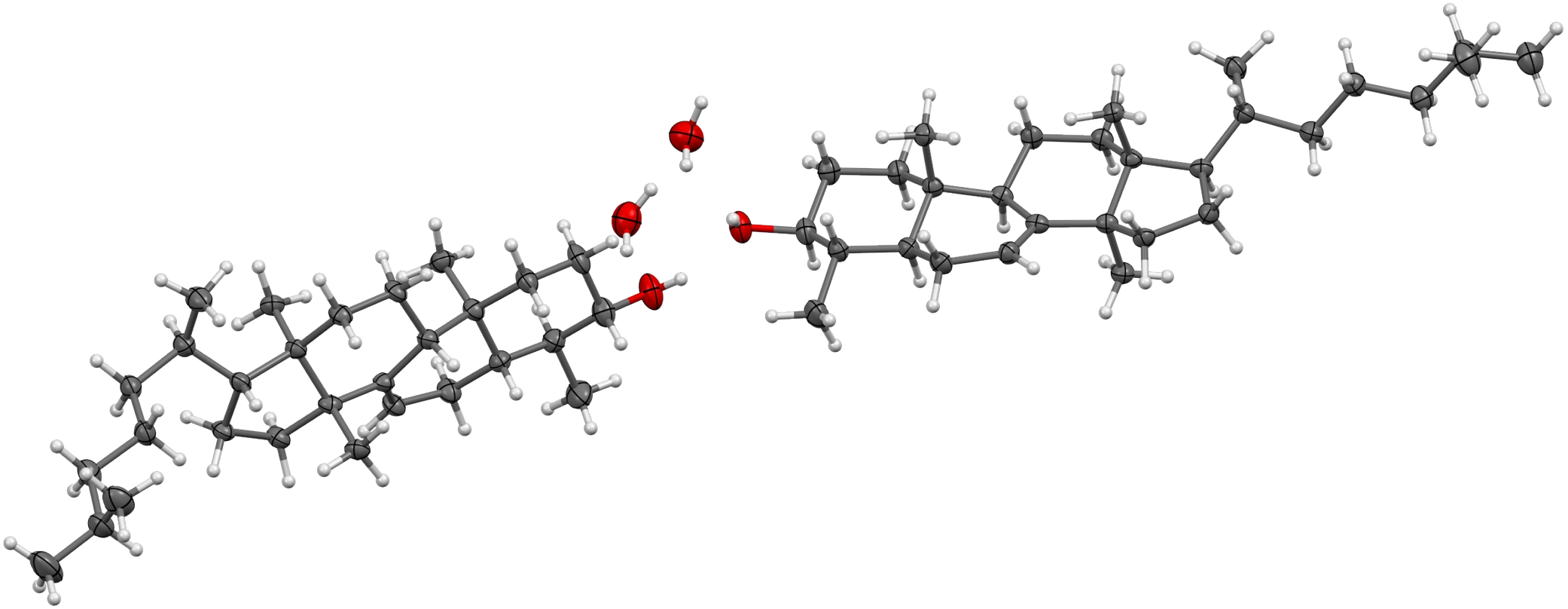
Crystal Data for $C_{30}H_{48.8}O$ (*M* =425.49 g/mol), space group $P2_1$ (no. 4), *a* = 7.5487(3) Å, *b* = 9.0604(3) Å, *c* = 19.9497(8) Å, *β* = 99.087(4)°, *V* = 1347.32(9) Å^3, *Z* = 2, *T* = 190(2) K, μ(CuKα) = 0.45 mm^{-1}, *Dcalc* = 1.049 g/cm^3, 10845 reflections measured (8.978° ≤ 2Θ ≤ 123.378°), 4122 unique (R_{int} = 0.0377, R_{sigma} = 0.026) which were used in all calculations. The final R_1 was 0.045 (I > 2σ(I)) and wR_2 was 0.12 (all data).

Crystal structure of 24,25-dihydro-3,4-secolanost-4-en-2-onitrile (7b)



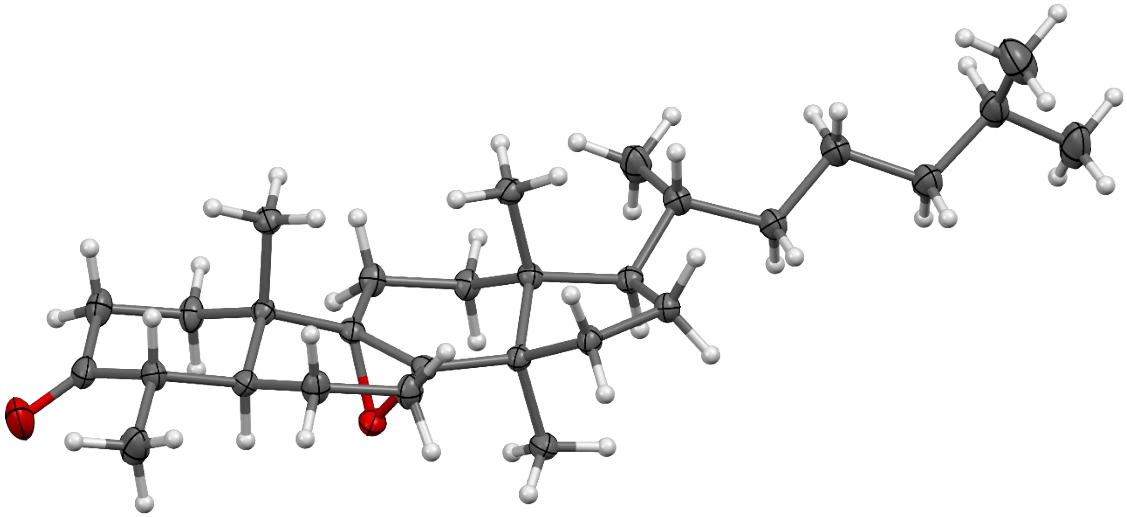
Crystal Data for $C_{30}H_{49}N$ (*M* =423.7 g/mol), space group $P2_12_12_1$ (no. 19), *a* = 7.5911(3) Å, *b* = 15.4627(9) Å, *c* = 22.8695(10) Å, *V* = 2684.4(2) Å^3, *Z* = 4, *T* = 190(2) K, μ(CuKα) = 0.432 mm^{-1}, *Dcalc* = 1.048 g/cm^3, 7994 reflections measured (6.9° ≤ 2Θ ≤ 124.98°), 3871 unique (R_{int} = 0.0273, R_{sigma} = 0.0372) which were used in all calculations. The final R_1 was 0.0538 (I > 2σ(I)) and wR_2 was 0.1519 (all data).

Crystal structure of $\Delta^{7,8}$-4-desmethyl-24,25-dihydrolanosterol (20)



Crystal Data for $C_{29}H_{51.5}O_{1.75}$ (M =428.2 g/mol), space group I2 (no. 5), a = 13.3918(15) Å, b = 6.3858(9) Å, c = 62.756(5) Å, β = 94.619(9)°, V = 5349.3(11) Å^3, Z = 8, T = 190(2) K, μ(CuKα) = 0.475 mm^{-1}, *Dcalc* = 1.063 g/cm^3, 11679 reflections measured (7.57° ≤ 2Θ ≤ 124.986°), 6449 unique (R_{int} = 0.0494, R_{sigma} = 0.0676) which were used in all calculations. The final R_1 was 0.0588 (I > 2σ(I)) and wR_2 was 0.1443 (all data).

Crystal structure of 4-desmethyl-8,9-epoxy-24,25-dihydrolanosta-3-one



Crystal Data for $C_{29}H_{48}O_2$ (*M* =428.67 g/mol), space group $P2_1$ (no. 4), *a* = 13.6644(9) Å, *b* = 6.2847(4) Å, *c* = 15.5138(13) Å, *β* = 101.039(7)°, *V* = 1307.62(17) $Å^3$, *Z* = 2, *T* = 190(2) K, μ(CuKα) = 0.497 mm^{-1}, *Dcalc* = 1.089 g/cm^3, 10322 reflections measured (7.906° ≤ 2Θ ≤ 124.91°), 4120 unique (R_{int} = 0.0621, R_{sigma} = 0.0654) which were used in all calculations. The final R_1 was 0.047 (I > 2σ(I)) and wR_2 was 0.1102 (all data).

3. Additional synthesis

Lanosten-3-one (5a)

Commercial lanosterol (1.64 g, 3.83 mmol, contaminated with approx. 40% C-24,25 dihydro analogue) and *N*-methylmorpholine *N*-oxide monohydrate (782 mg, 5.79 mmol) were added to anhydrous DCM (19 mL). 4Å molecular sieves were added and the mixture was stirred at rt for 1 h. Methyltriphenylphosphonium perruthenate[3] (69 mg, 0.20 mmol) was added and stirring at rt continued for 16 h. The reaction was passed through a pad of silica, which was then washed with DCM (100 mL) and the solvent from the combined eluents evaporated under reduced pressure to give lanosten-3-one (**5a**) as a white powder (1.25 g, 2.94 mmol, 77%), contaminated with approx. 40% 24,25-dihydrolanosten-3-one (**5b**).
Data for **5a**: **^{1}H NMR (500 MHz, $CDCl_3$)**: δ 5.12 (tp, *J* = 7.1, 1.4 Hz, 0.6H, H24), 2.58 (ddd, *J* = 15.7, 11.2, 7.1 Hz, 1H, H2a), 2.40 (ddd, *J* = 15.7, 6.8, 3.6 Hz, 1H, H2b), 1.68 (s, 3H, H26), 1.60 (s, 3H, H27), 1.12 (s, 3H, H19), 1.09 (s, 3H, H28), 1.07 (s, 3H, H29), 0.92 (d, *J* = 6.5 Hz, 3H, H21), 0.89 (s, 3H, H30), 0.71 (s, 3H, H18). **^{13}C NMR (125 MHz, $CDCl_3$)**: δ 217.9 (C3), 135.3 (C8), 133.1 (C9), 131.0 (C25), 125.2 (C24). **IR (cm^{-1}):** 1709 (C=O). **HRMS** ($C_{30}H_{49}O$): calc'd 425.3778; found 425.3767. **GCMS EI m/z (%):** 424 (40, $M^{+\cdot}$), 409 (83, $M^{+\cdot}$-CH_3), 109 (53), 69 (100, $C_5H_9^+$), 55 (54, $C_4H_7^+$), 41 (58).
Key NMR shifts reported for this compound due to extensive overlap with 24,25-dihydro analogue.

Lanosten-3-oxime (**6a**)
To a solution of lanosten-3-one (**5a**) (1.13 g, 2.65 mmol, contaminated with approx. 40% C-24,25 dihydro analogue **5b**) in EtOH (150 mL), anhydrous NaOAc (552 mg, 6.73 mmol) and hydroxylamine hydrochloride (343 mg, 4.94 mmol) were added. The mixture was stirred and heated at reflux for 24 h. The reaction was then diluted with water (200 mL) and extracted with $CHCl_3$ (3 x 150 mL) before the combined organic extracts were dried ($MgSO_4$), filtered and solvent removed under reduced pressure to give lanosten-3-oxime (**6a**) as a flaky white solid (1.16 g, 2.63 mmol, 99%), contaminated with approx. 40% 24,25-dihydrolanosten-3-oxime (**6b**).
Data for **6a**: **^{1}H NMR (500 MHz, $CDCl_3$)**: δ 5.12 (tp, *J* = 7.1, 1.4 Hz, 0.6H, H24), 3.12 (ddd, *J* = 15.3, 5.3, 3.5 Hz, 1H, H2a), 2.20 (ddd, *J* = 15.3, 12.7, 5.7, 1H, H2b), 1.68 (s, 3H, H26), 1.60 (s, 3H, H27), 1.17 (s, 3H, H28), 1.10 (s, 3H, H19), 1.09 (s, 3H, H29), 0.92 (d, *J* = 6.6 Hz, 3H, H21), 0.86 (s, 3H, H30), 0.70 (s, 3H, H18). **^{13}C NMR (125 MHz, $CDCl_3$)**: δ 167.4 (C3), 134.9 (C8), 133.8 (C9), 131.0 (C25), 125.2 (C24). **IR (cm^{-1}):** 3269 (O-H), 1671 (C=N), 932 (N-O). **HRMS** ($C_{30}H_{50}NO$): calc'd 440.3887; found 440.3878. **GCMS EI m/z (%):** 421 (22, $M^{+\cdot}$-H_2O), 207 (52), 95 (28), 69 (100, $C_5H_9^+$), 55 (50, $C_4H_7^+$), 41 (69).
Key NMR shifts reported for this compound due to extensive overlap with 24,25-dihydro analogue.

3,4-secolanost-4-en-2-onitrile (**7a**)
To a solution of lanosten-3-oxime (**6a**) (1.54 g, 3.49 mmol, contaminated with approx. 40% C-24,25 dihydro analogue **6b**) in anhydrous pyridine (25 mL), *p*-toluenesulfonyl chloride (2.13 g, 11.16 mmol) was added. The mixture was stirred and heated at reflux for 3 h. The mixture was allowed to cool to room temperature and acidified with 2M aqueous HCl (50

mL). The product was extracted with Et_2O (3 x 50 mL) and the combined organic layers dried ($MgSO_4$), filtered and solvent removed under reduced pressure to give a yellow gum. The crude product was subject to column chromatography (5% EtOAc in petroleum spirit 40-60) to give 3,4-secolanost-4-en-3-onitrile (**7a**) as a hard white solid (0.81 g, 1.92 mmol, 55%), contaminated with approx. 40% 24,25-dihydro-3,4-secolanost-4-en-2-onitrile (**7b**).
Data for **8**: **^{1}H NMR (500 MHz, $CDCl_3$)**: δ 5.10 (tp, *J* = 7.1, 1.4 Hz, 0.6H, H24), 4.93 (br p, *J* = 1.7 Hz, 1H, H28a), 4.67 (br s, 1H, H28b), 1.76 (s, 3H, H29), 1.68 (s, 3H, H26), 1.60 (s, 3H, H27), 0.96 (s, 3H, H19), 0.92 (s, 3H, H30), 0.91 (d, *J* = 6.6 Hz, 3H, H21), 0.73 (s, 3H, H18). **^{13}C NMR (125 MHz, $CDCl_3$)**: δ 146.8 (C4), 140.9 (C8), 131.0 (C25), 128.0 (C9), 125.2 (C24), 120.6 (C3), 114.4 (C28). **IR (cm^{-1}):** 2247 (C≡N), 1636 (C=C). **HRMS** ($C_{30}H_{48}N$): calc'd 422.3781; found 422.3763. **GCMS EI m/z (%):** 421 (35, $M^{+\cdot}$), 406 (20, $M^{+\cdot}$-CH_3), 95 (31), 69 (100, $C_5H_9^+$), 55 (48, $C_4H_7^+$), 41 (65).
Key NMR shifts reported for this compound due to extensive overlap with 24,25-dihydro analogue.

24,25-epoxy-3,4-secolanost-4-en-2-onitrile (**8**)
A solution of 3,4-secolanost-4-en-2-onitrile (**7a**) (1.37 g, approx. 1.94 mmol (60%) C-24,25 alkene, 1.29 mmol (40%) C-24,25 dihydro **7b**) in anhydrous DCM (80 mL) was cooled to 0°C. *Meta*-chloroperoxybenzoic acid (480 mg, 2.14 mmol) was added and the mixture was stirred at 0°C under N_2 for 1 h. The mixture was then washed with a saturated aqueous solution of $NaHCO_3$ (3 x 100 mL), water (2 x 100 mL), brine (1 x 100 mL) and the organic layer dried ($MgSO_4$), filtered and solvent removed under reduced pressure. The crude product was subject to column chromatography (5% EtOAc in hexanes) to give **7b**, and further elution gave (24*R,S*)-24,25-epoxy-3,4-secolanost-4-en-2-onitrile (**8**) (0.373 g, 0.852 mmol, 44%) as a white solid. **^{1}H NMR (500 MHz, $CDCl_3$)**: δ 4.93 (br p, *J* = 1.7 Hz, 1H, H28a), 4.67 (br s, 1H, H28b), 2.68 (t, *J* = 6.3 Hz, 1H, H24), 2.32 (ddd, *J* = 16.8, 11.6, 5.6 Hz, 1H, H2a), 2.20 - 2.10 (m, 2H, H11), 2.07 - 2.00 (m, 4H, H2b, H5, H7), 1.99 - 1.88 (m, 2H, H1a, H16a), 1.86 - 1.72 (m, 4H, H1b, H6a, H12), 1.76 (s, 3H, H29), 1.65 - 1.35 (m, 8H, H6b, H15a, H16b, H17, H20, H22a, H23), 1.31 (s, 3H, H26), 1.27 (s, 3H, H27), 1.25 - 1.10 (m, 2H, H15b, H22b), 0.97 (s, 3H, H19), 0.93 (d, *J* = 6.6 Hz, 3H, H21), 0.92 (s, 3H, H30), 0.74 (s, 3H, H18). **^{13}C NMR (125 MHz, $CDCl_3$)**: δ 146.7 (C4), 140.8 (C8), 128.0 (C9), 120.6 (C3), 114.4 (C28), 64.9 (C24), 64.7 (C24), 58.4 (C25), 58.1 (C25), 50.8 (C14), 50.3 (C17), 50.2 (C17), 46.8 (C5), 44.3 (C13), 40.7 (C10), 36.4 (C20), 36.2 (C20), 33.1 (C1), 32.8 (C22), 32.6 (C22), 30.9 (C15), 30.9 (C12), 28.0 (C16), 26.0 (C7), 25.9 (C23), 25.6 (C23), 25.3 (C30), 24.9 (C26), 23.7 (C6), 22.6 (C29), 22.0 (C19), 21.9 (C11), 18.7 (C27), 18.6 (C21), 15.9 (C18), 12.2 (C2). **IR (cm^{-1}):** 2247 (C≡N), 1638 (C=C). **HRMS** ($C_{30}H_{48}NO$): calc'd 438.3735; found 438.3728. NMR data was in agreement with values from literature.[4] Assignments were made through the use of complimentary 2D NMR techniques: COSY, HSQC, HMBC.

24,25-dihydro-3,4-secolanost-4-en-2-onitrile (**7b**) (0.522 g, 1.23 mmol, 95% recovered yield, 39% overall from **1b**) was recovered as a white solid. **M.P.:** 91-92°C. **^{1}H NMR (500 MHz, $CDCl_3$)**: δ 4.93 (br p, *J* = 1.7 Hz, 1H, H28a), 4.67 (br s, 1H, H28b), 2.31 (ddd, *J* = 16.9, 11.6, 5.6 Hz, 1H, H2a), 2.20 - 2.10 (m, 1H, H11a), 2.04 (dd, *J* = 8.6, 2.8 Hz, 1H, H5), 2.04 - 1.97 (m, 3H, H2b, H7), 1.96 - 1.87 (m, 2H, H1a, H16a), 1.85 - 1.77 (m, 3H, H1b, H12), 1.76 (s, 3H, H29), 1.75 - 1.69 (m, 1H, H6a), 1.60 - 1.47 (m, 4H, H6b, H15a, H17, H25), 1.40 - 1.29 (4H, H16b, H20, H23), 1.23 - 1.09 (m, 3H, H15b, H24), 1.03 - 0.97 (m, 2H, H22), 0.96 (s, 3H, H19), 0.92 (s, 3H, H30), 0.90 (d, *J* = 6.6 Hz, 3H, H21), 0.872 (d, *J* = 6.6 Hz, 3H, H27), 0.867 (d, *J* = 6.6 Hz, 3H,

H26), 0.73 (s, 3H, H18). **^{13}C NMR (125 MHz, $CDCl_3$)**: *δ* 146.7 (C4), 140.9 (C8), 128.0 (C9), 120.6 (C3), 114.3 (C28), 50.8 (C14), 50.4 (C17), 46.8 (C5), 44.3 (C13), 40.7 (C10), 39.5 (C24), 36.5 (C20), 36.4 (C22), 33.1 (C1), 31.0 (C15), 31.0 (C12), 28.0 (C16), 28.0 (C25), 25.9 (C7), 25.3 (C30), 24.1 (C23), 23.7 (C6), 22.8 (C27), 22.6 (C29), 22.5 (C26), 22.0 (C19), 21.9 (C11), 18.7 (C21), 15.9 (C18), 12.2 (C2). **IR (cm^{-1}):** 2243 (C≡N), 1638 (C=C). **HRMS** ($C_{30}H_{50}N$): calc'd 424.3943; found 424.3956.

4. References

1 G. M. Sheldrick, *Acta Crystallogr. Sect. A Found. Crystallogr.*, 2008, **64**, 112–122.

2 C. F. Macrae, I. Sovago, S. J. Cottrell, P. T. A. Galek, P. McCabe, E. Pidcock, M. Platings, G. P. Shields, J. S. Stevens, M. Towler and P. A. Wood, *J. Appl. Crystallogr.*, 2020, **53**, 226–235.

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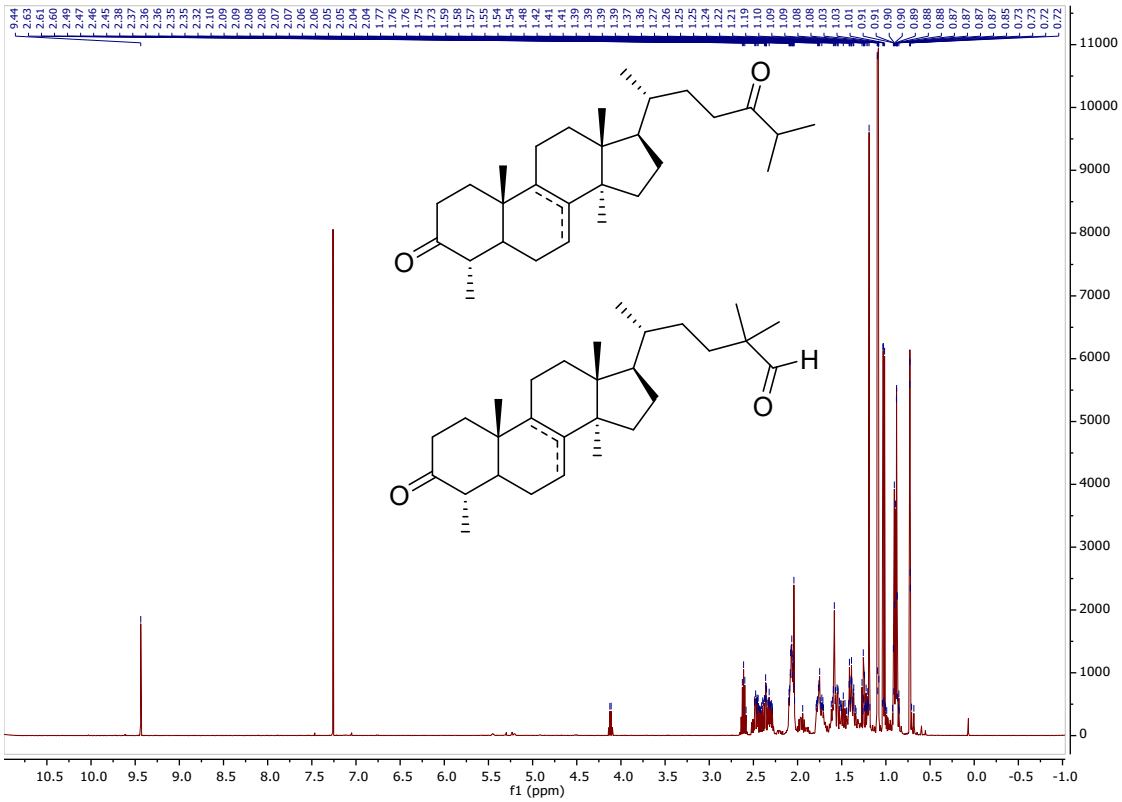
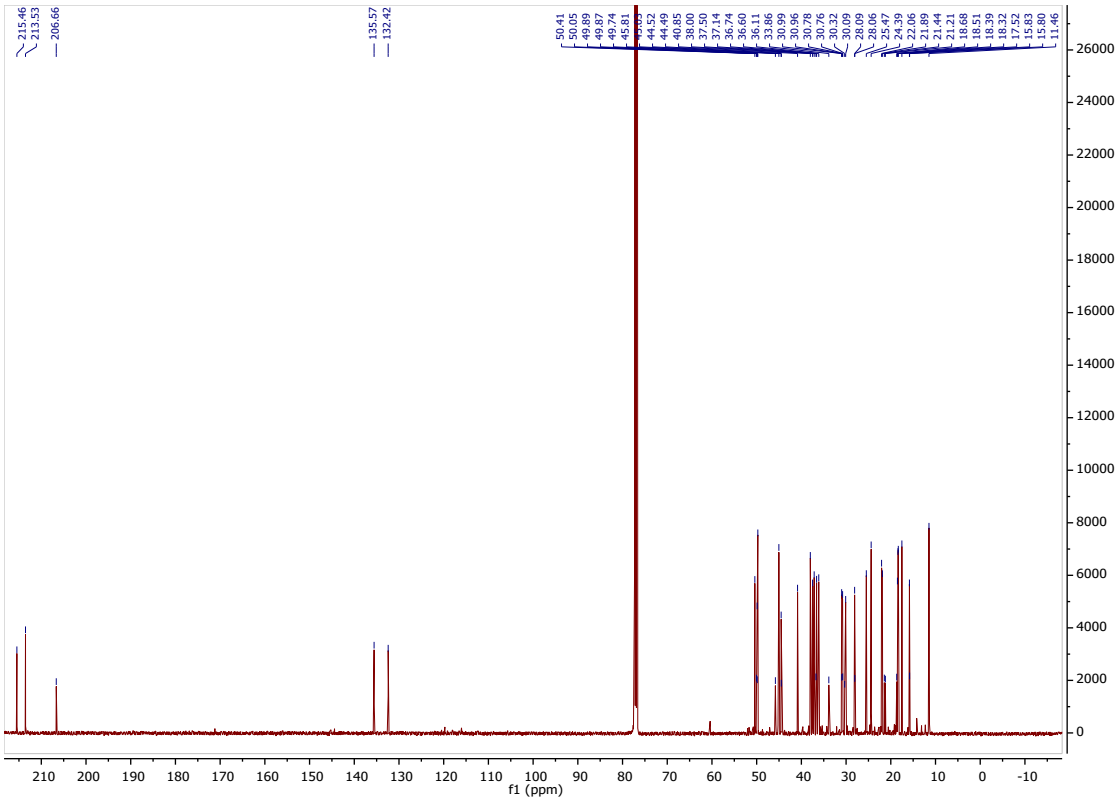
5. Spectra

4-desmethyllanosten-3,24-dione (10) and aldehyde (11)

^{1}H NMR

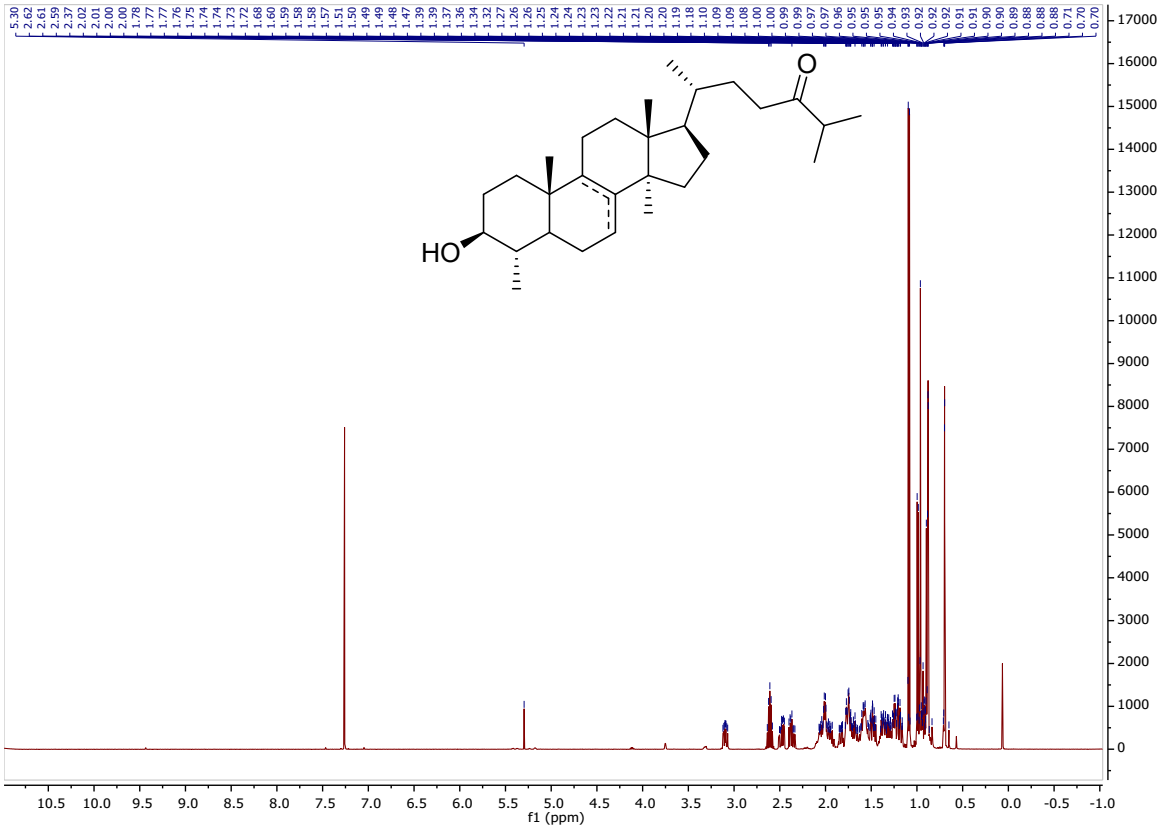


^{13}C NMR

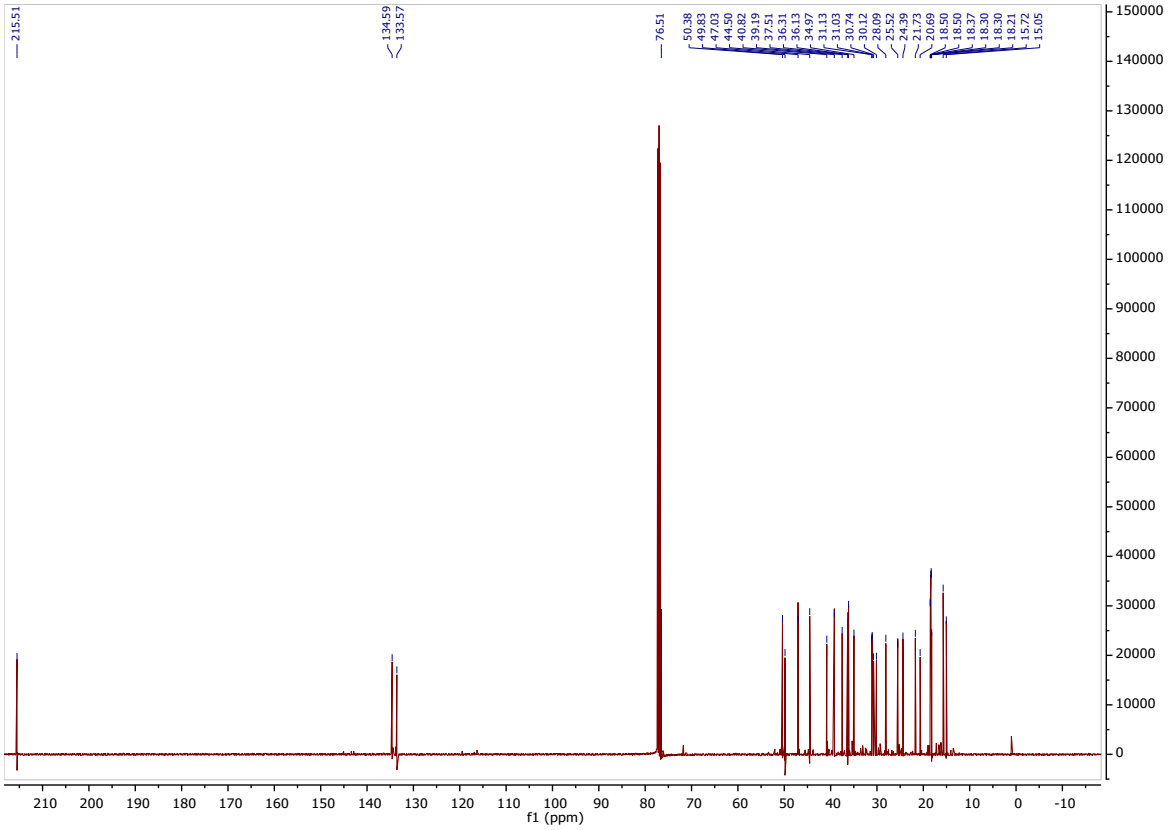


(3*S*)-4-desmethyl-24-oxolanosterol (3*S*-12)

^{1}H NMR



^{13}C NMR



(3*R*)-4-demethyl-24-oxolanosterol (3*R*-12)

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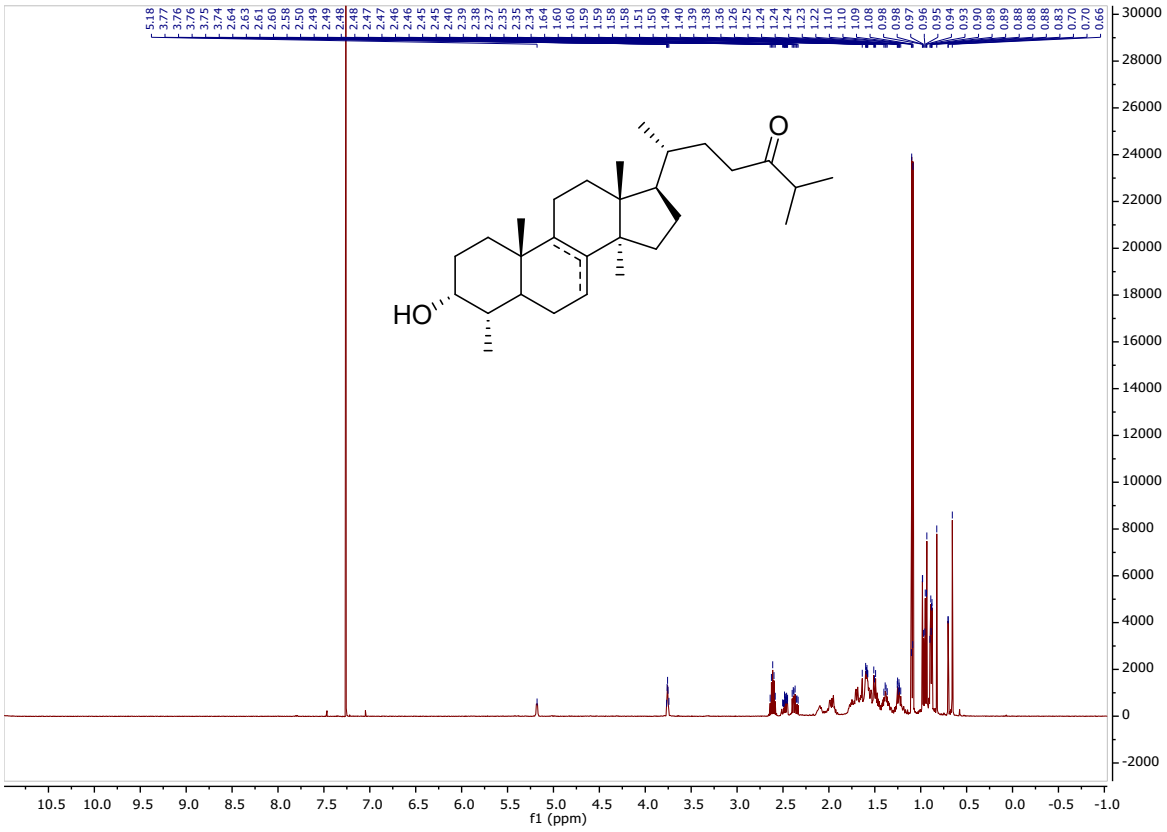
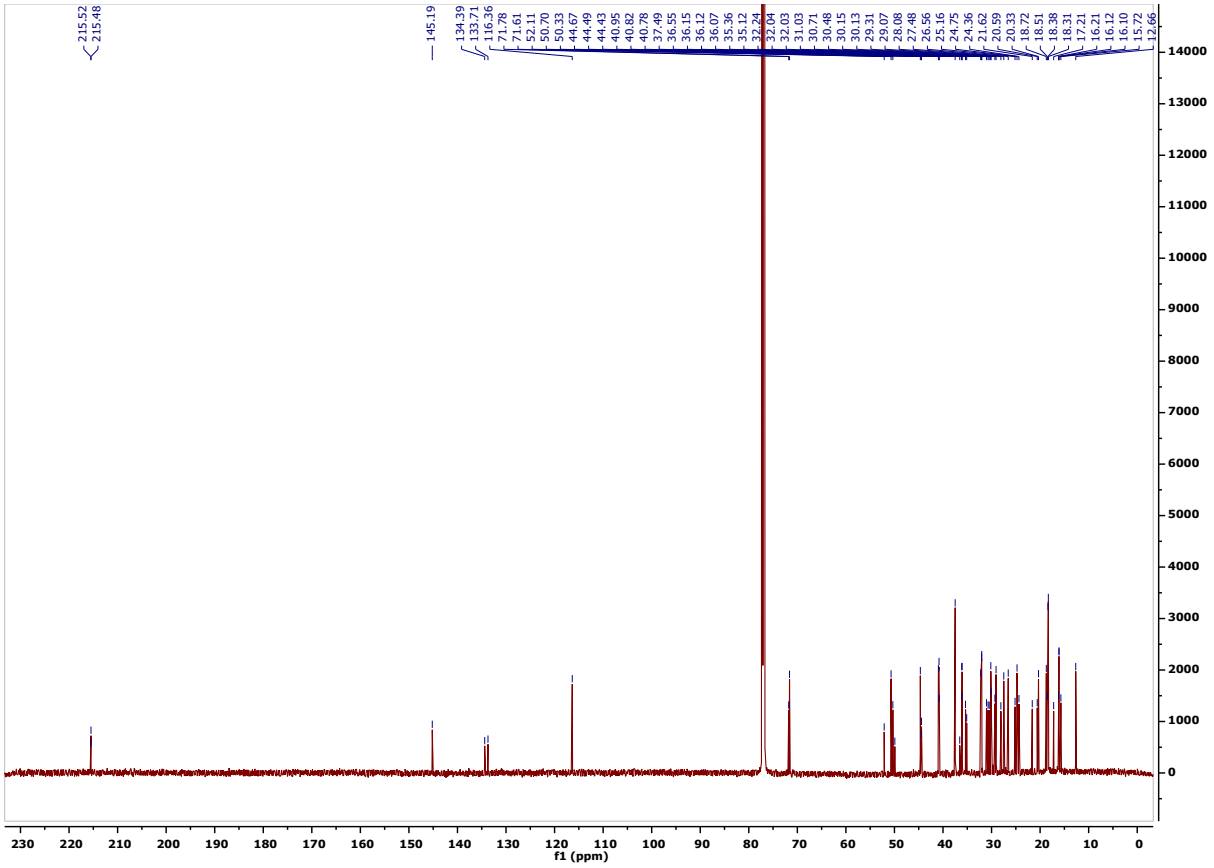
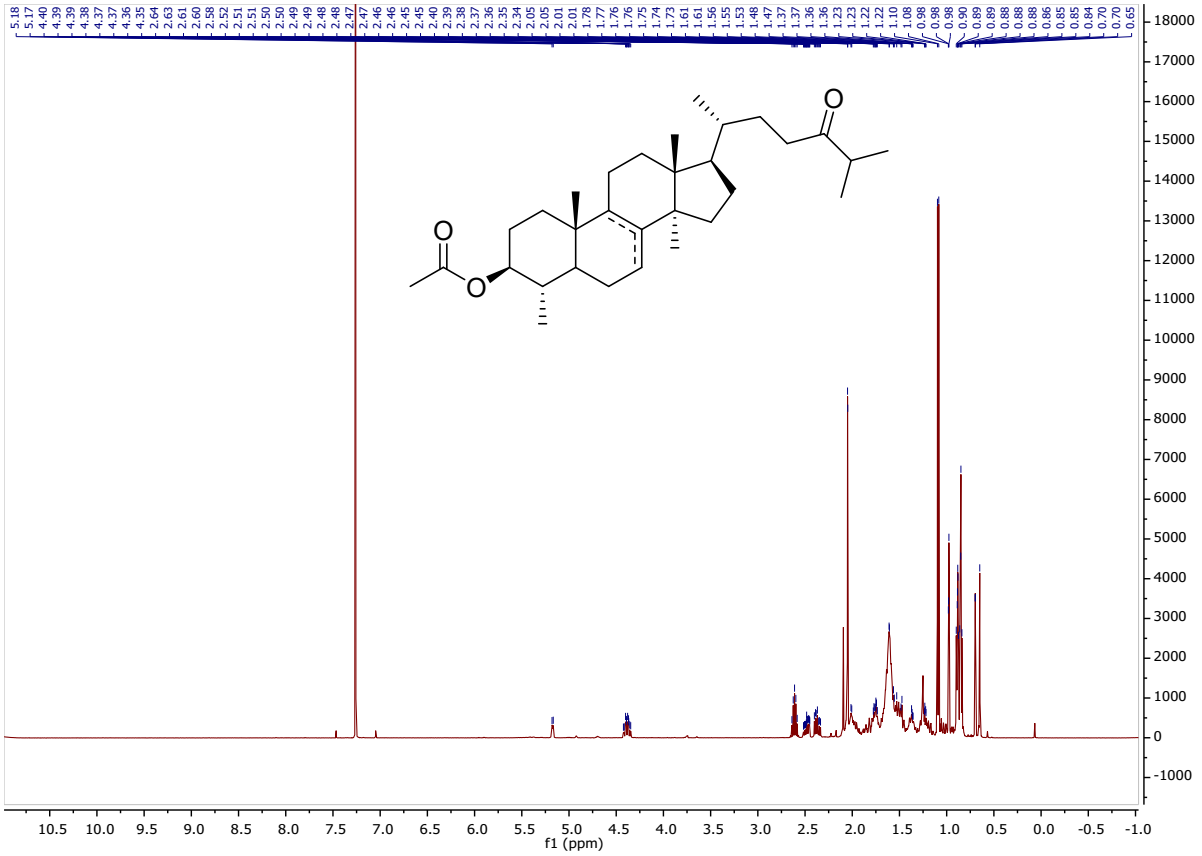




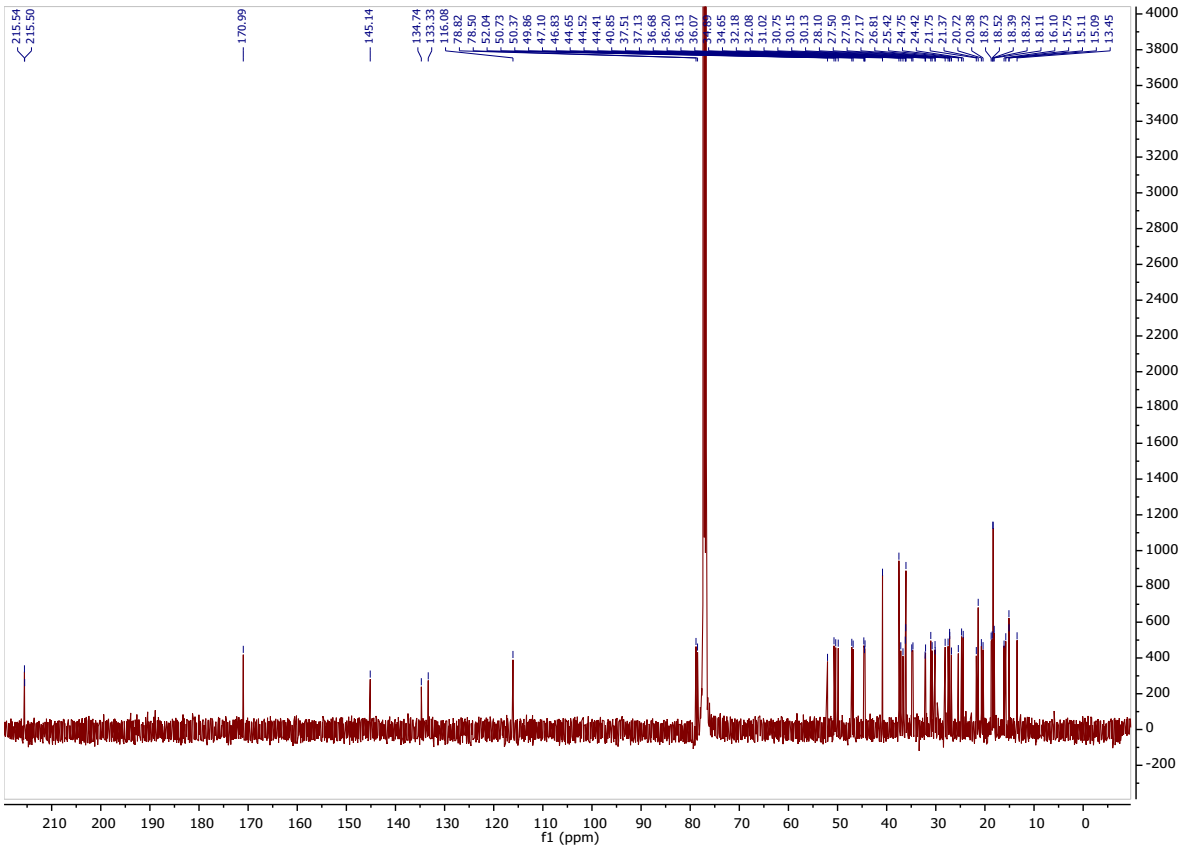
^{13}C NMR (175 MHz)

4-desmethyl-24-oxolanosteryl-3-acetate (14)

^{1}H NMR

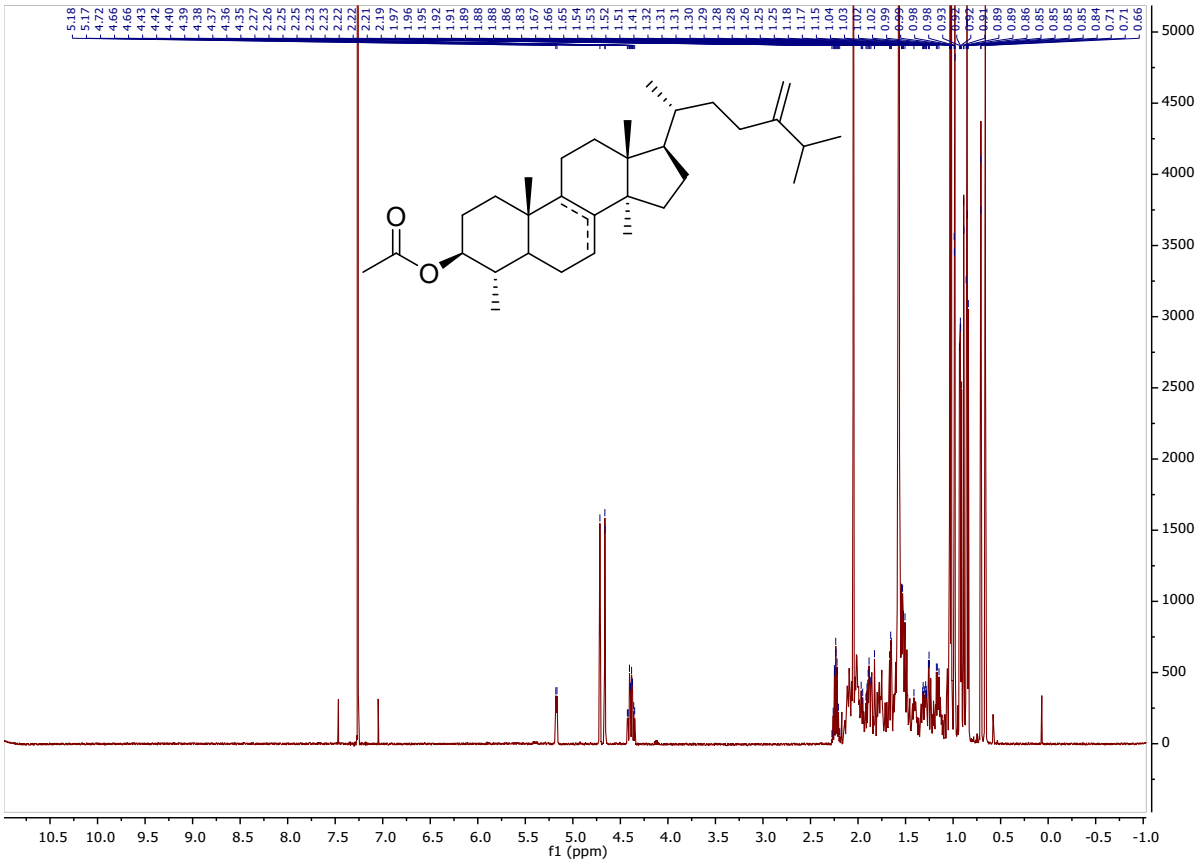


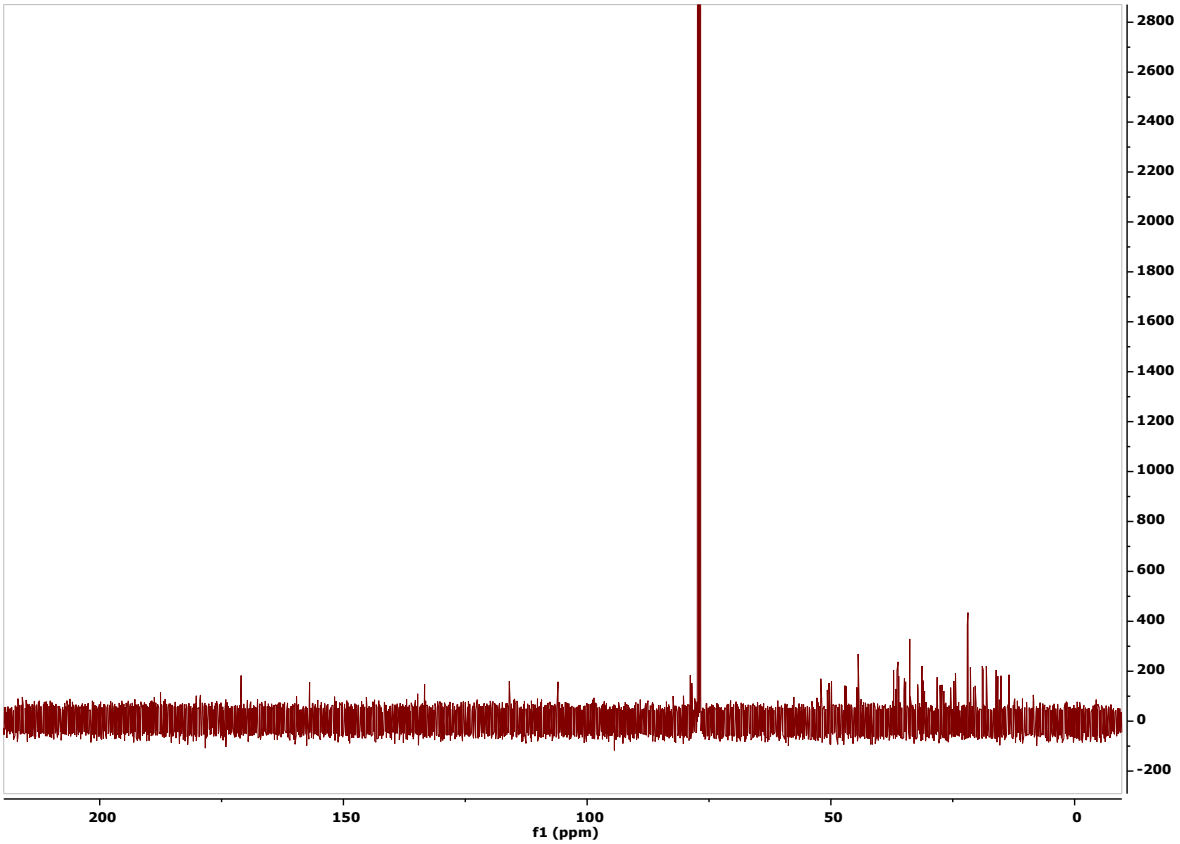
^{13}C NMR



Obtusifoliol-3-acetate (15)

^{1}H NMR

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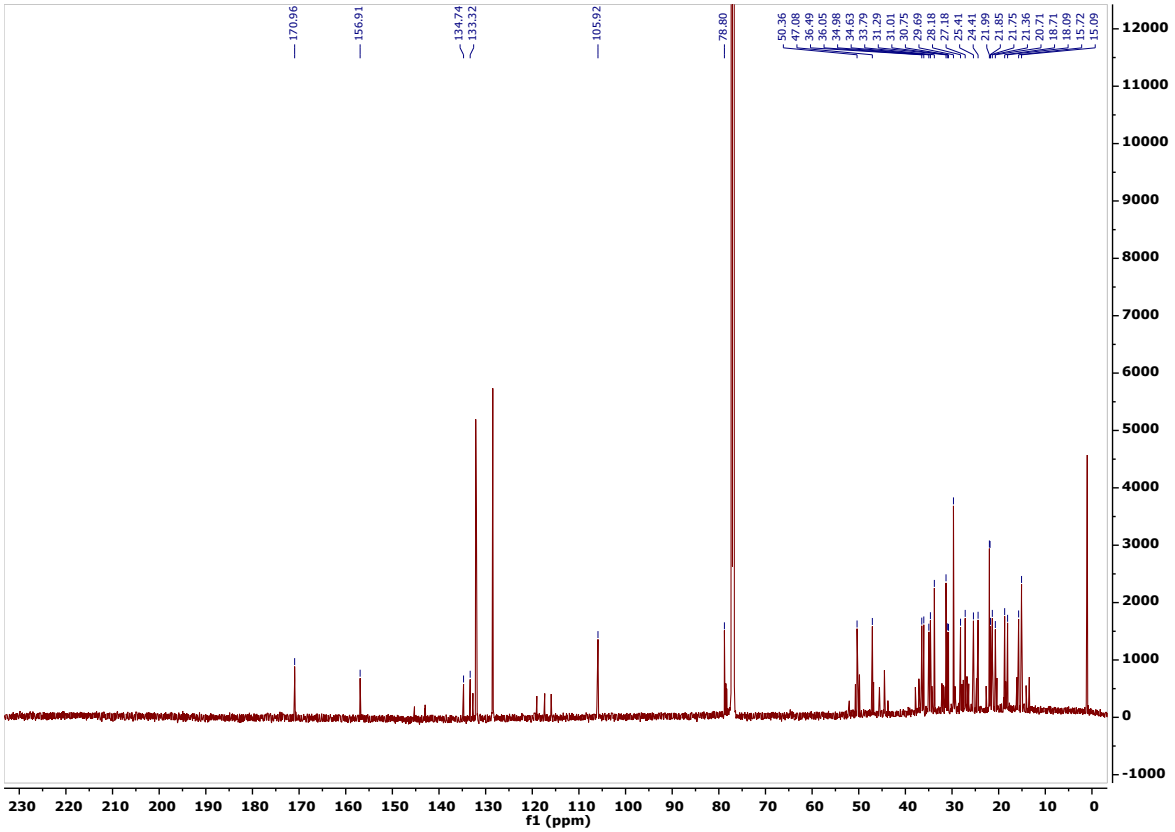
f1 (ppm)

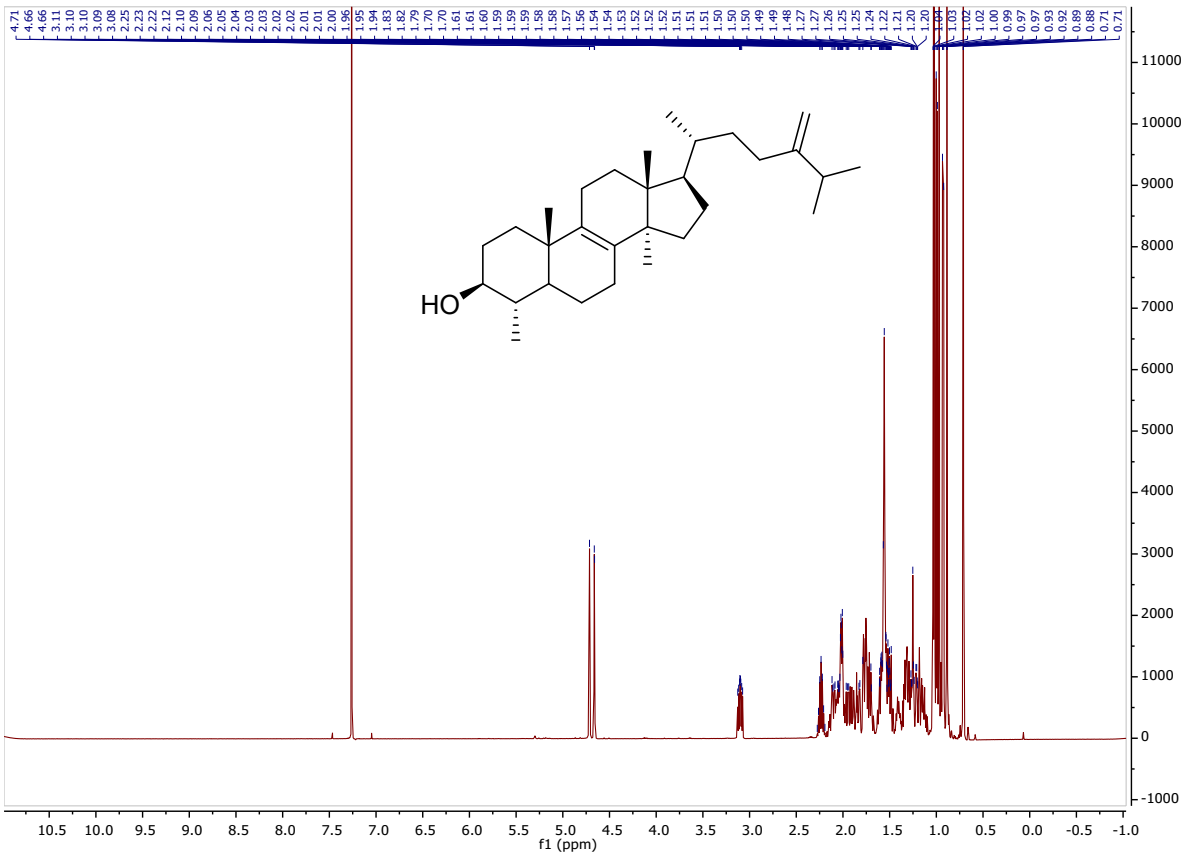
^{13}C NMR



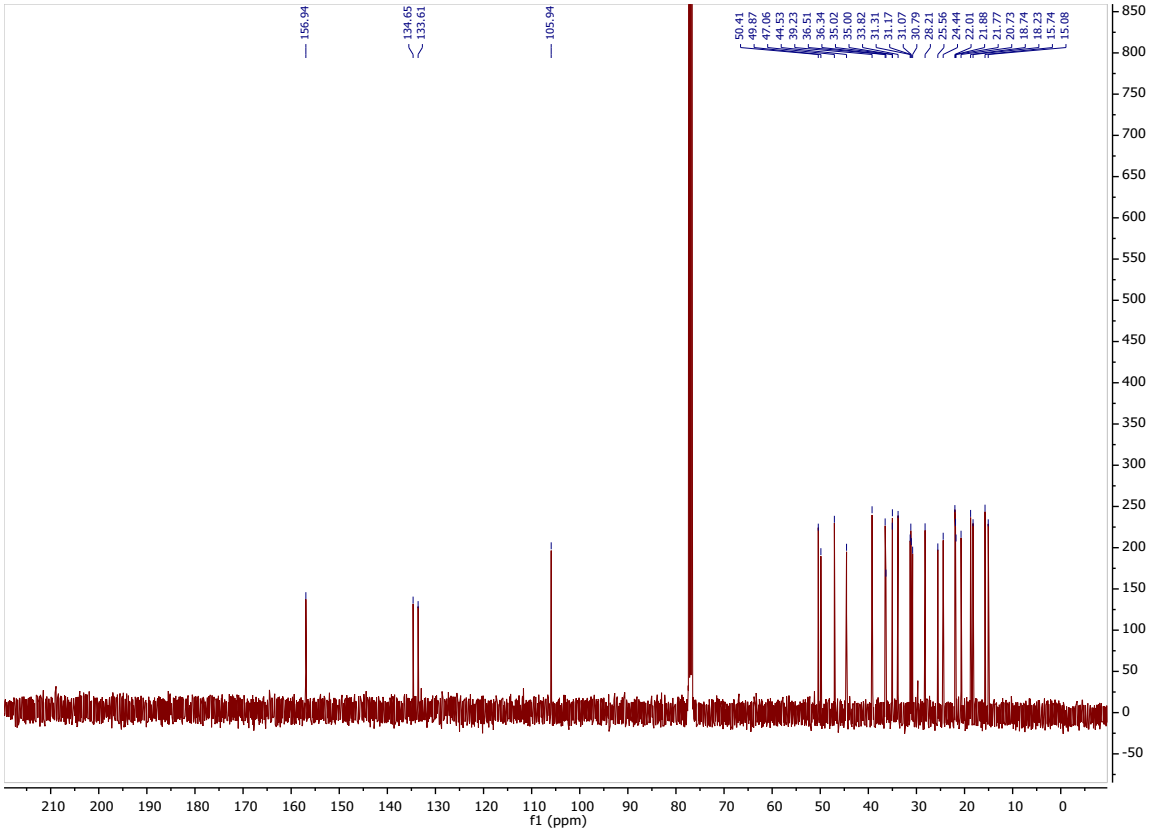
^{13}C NMR (175MHz, another sample)

Obtusifoliol (4)

^{1}H NMR

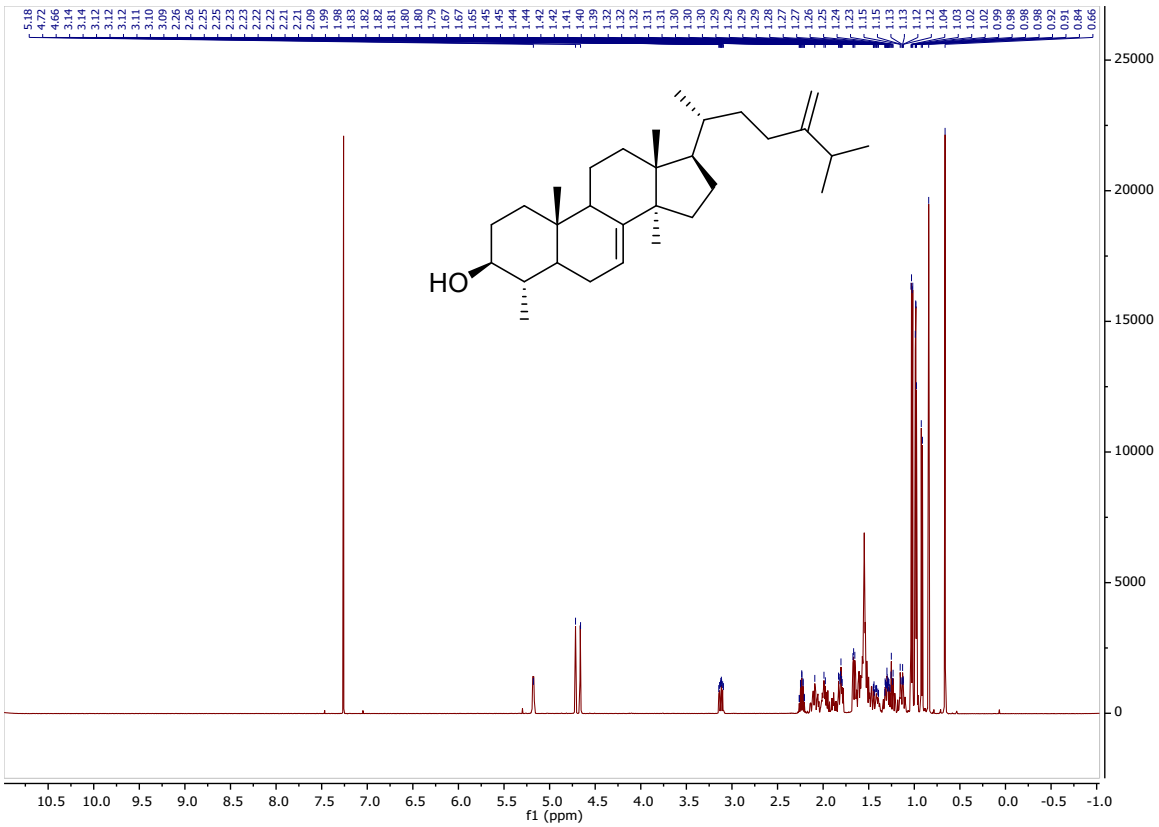


^{13}C NMR

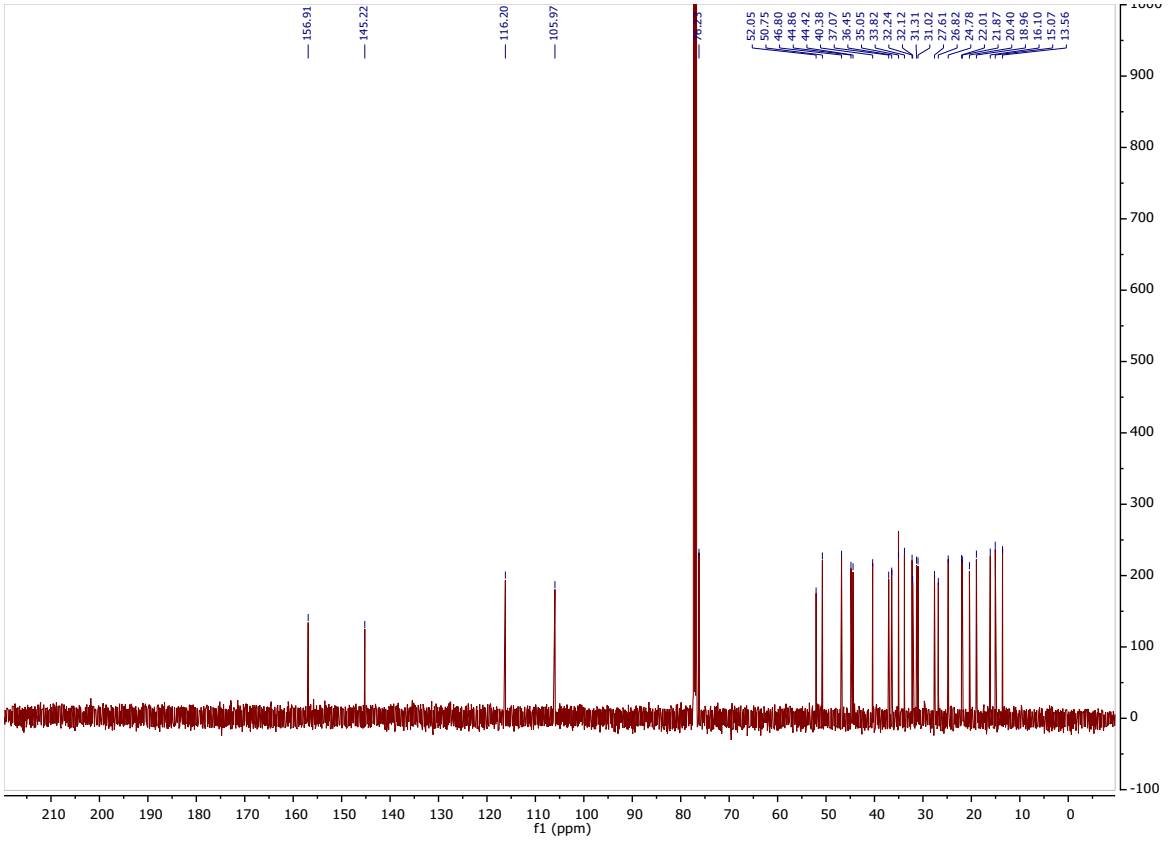


$\Delta^{7,8}$-obtusifoliol (16)

^{1}H NMR

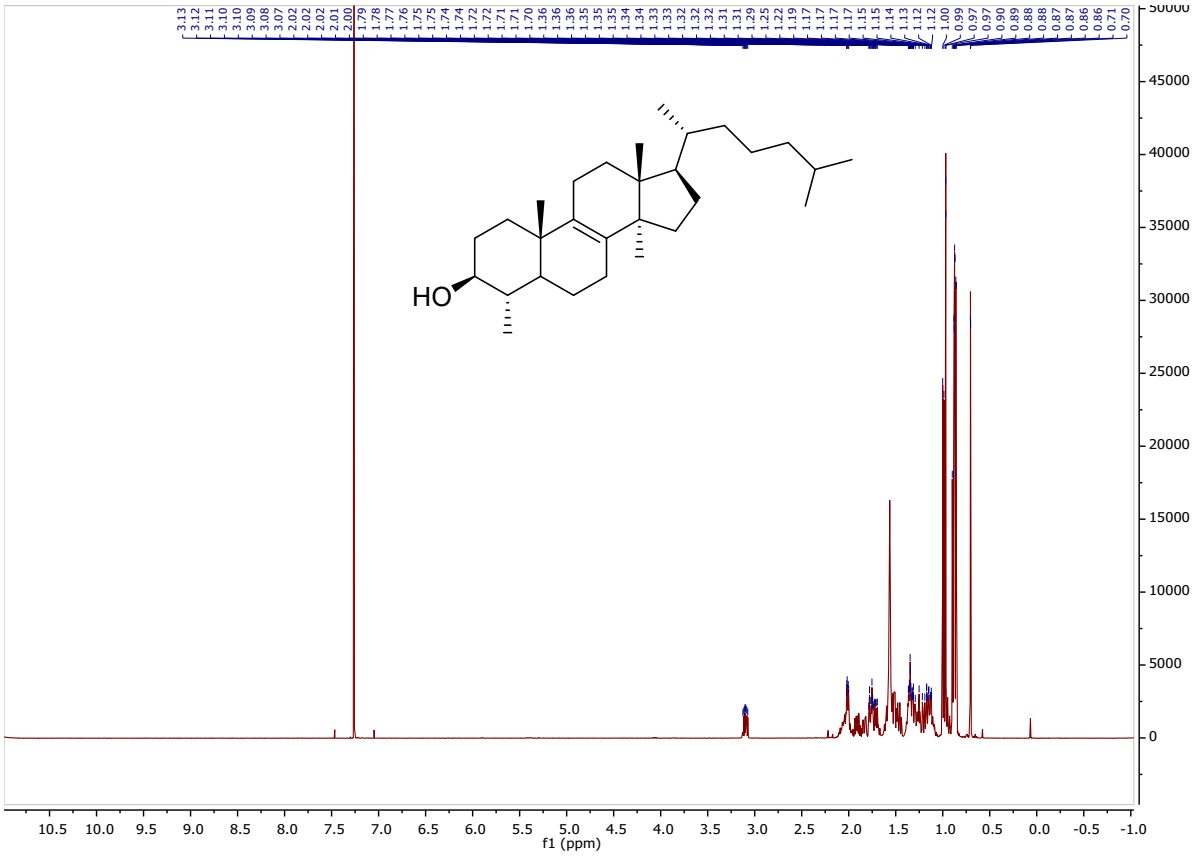


^{13}C NMR

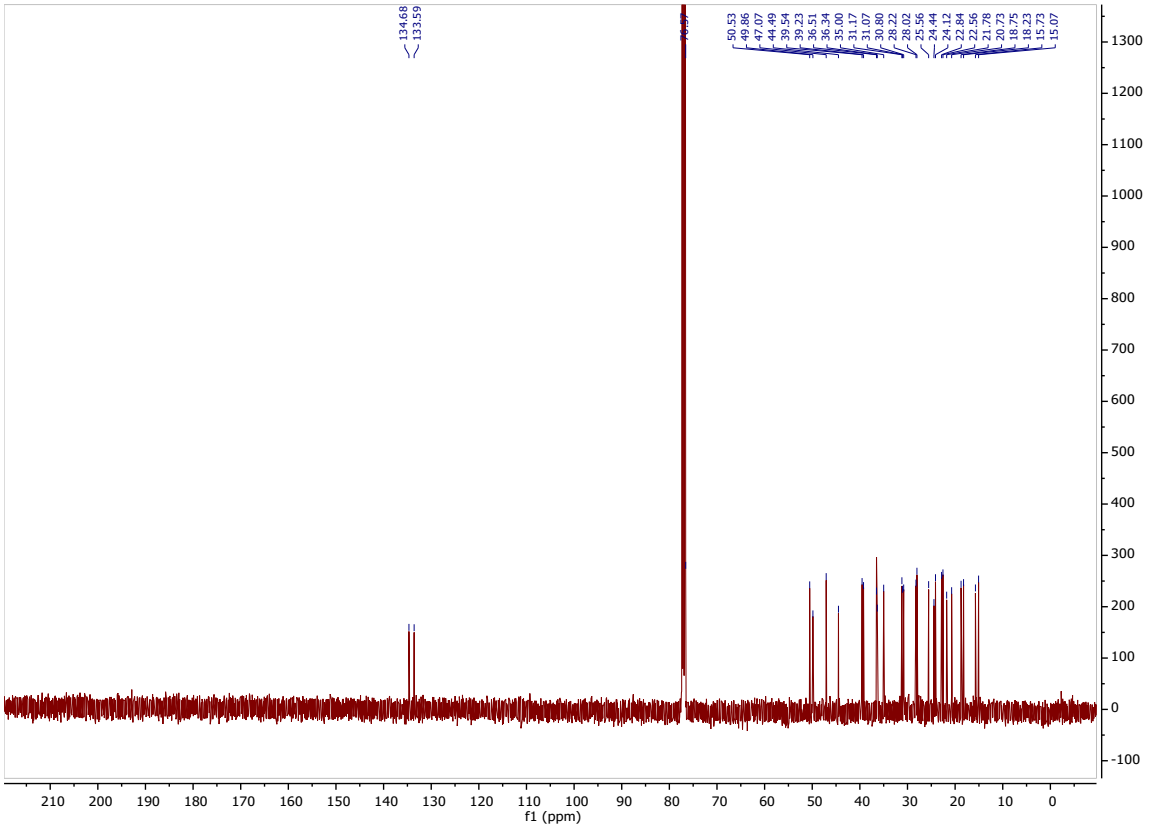


4-desmethyl-24,25-dihydrolanosterol (4b)

^{1}H NMR

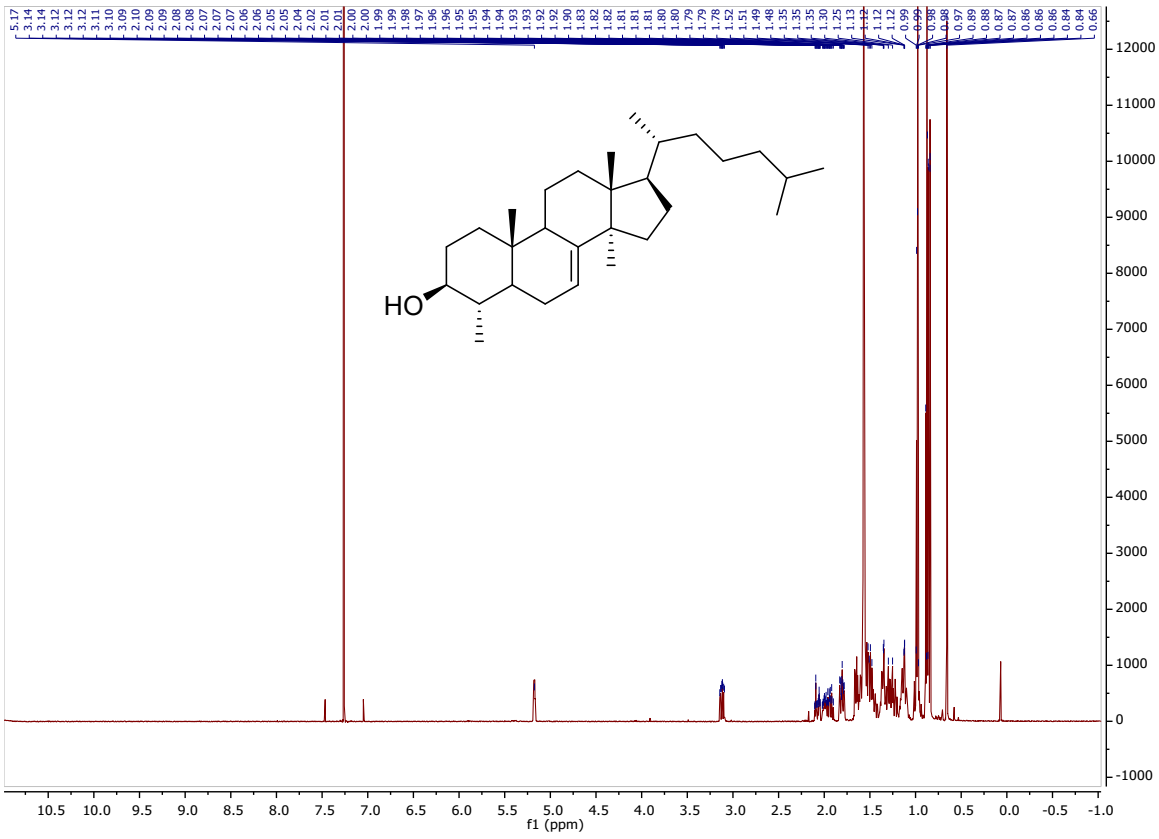


^{13}C NMR

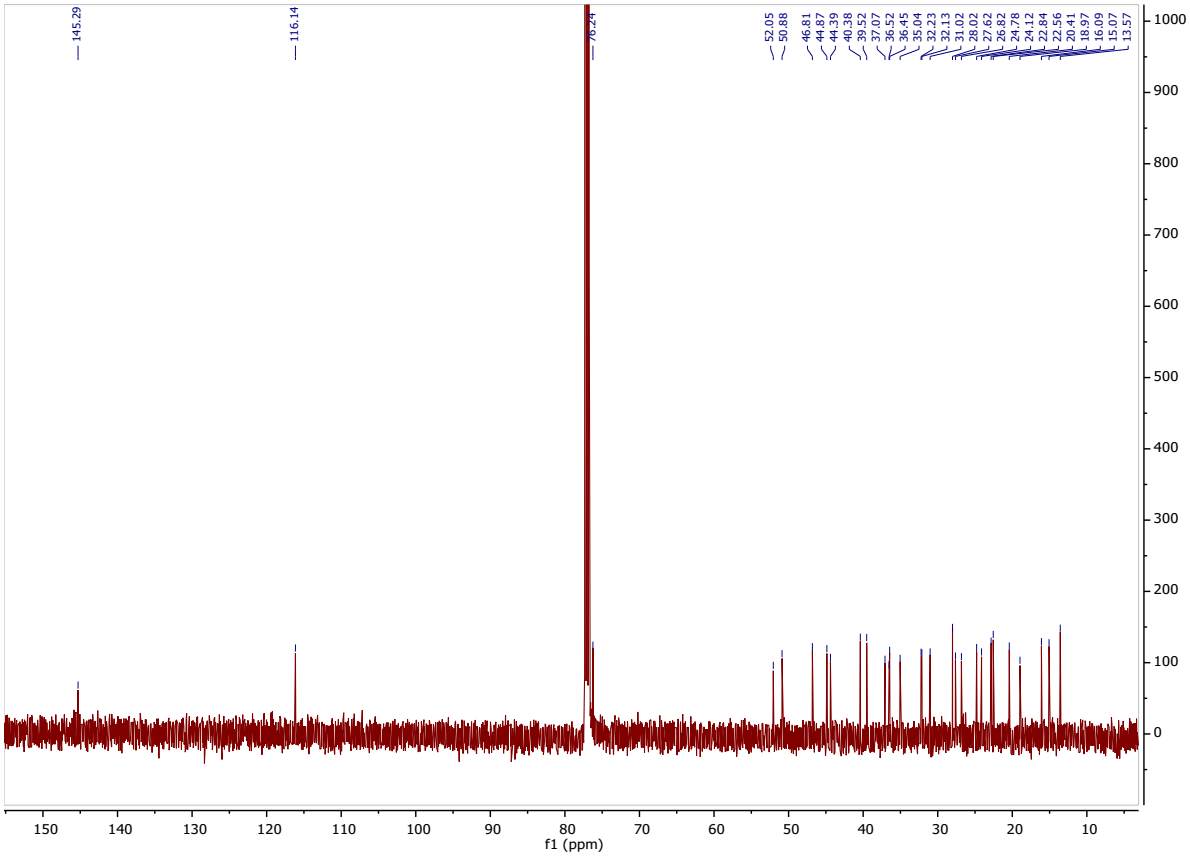


Δ^{7,8}-4-desmethyl-24,25-dihydrolanosterol (20)

^{1}H NMR



^{13}C NMR


145.29
116.14
76.24
52.05
50.88
46.81
44.87
44.39
40.38
39.52
37.07
36.52
36.45
35.04
32.23
32.13
31.02
28.02
27.62
26.82
24.78
24.12
22.84
22.56
20.41
18.97
16.09
15.07
13.57
1000
900
800
700
600
500
400
300
200
100
0
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)