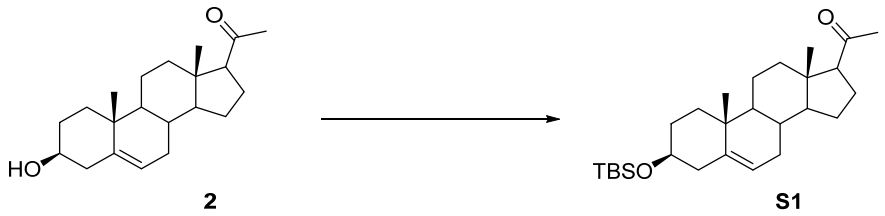
Supporting Information
for

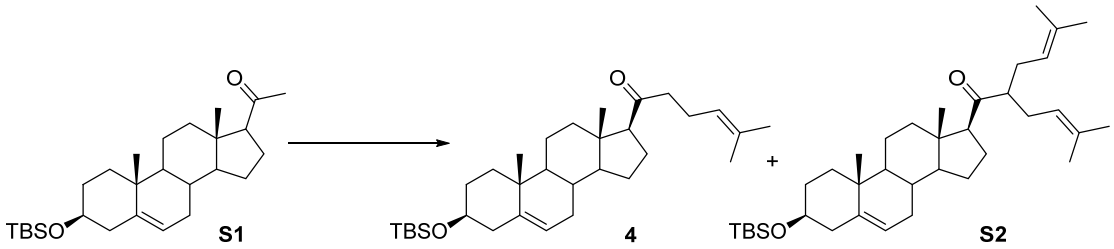
A novel sterol from a plant used by Mayan traditional healers is effective in treatment of visceral leishmaniasis caused by *Leishmania donovani*

Gupta G; Peine KJ; Abdelhamid D; Snider H; Shelton AB; Rao L; Kotha SR; Huntsman A; Varikuti S; Oghumu S; Naman CB; Pan L; Parinandi NL; Papenfuss TL; Kinghorn AD; Bachelder EM; Ainslie KM; Fuchs JR; Satoskar AR*

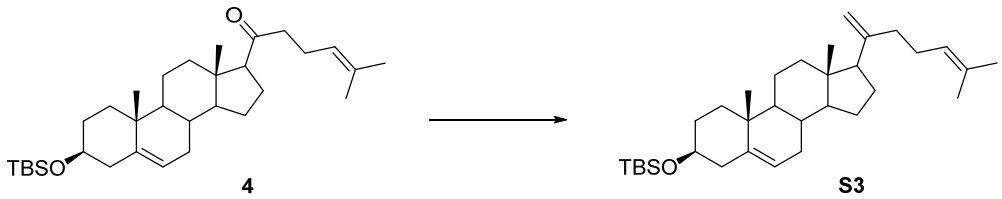
Table of Contents



3β-((*tert*-Butyldimethylsilyl)oxy)-5-pregnen-20-one (S1). Pregnenolone (3β-hydroxy-5-pregnen-20-one, **2**) (3.23 g, 10 mmol) was dissolved in DMF with stirring. Imidazole (0.694 g, 10.2 mmol) was added, followed by TBSCl (1.522 g, 10.1 mmol) was added and stirring was continued at room temperature for 16 h during which time a white precipitate was formed. The reaction mixture was partitioned between water and ether and extracted three times with ether. The combined organic layers were dried over sodium sulfate, concentrated, and purified by silica gel column chromatography (EtOAc-hexanes, 1:9) to give silyl ether **S1** (2.92 g, 68%) as a white crystalline solid: mp 163 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.31 (d, J = 5.1 Hz, 1H), 3.47 (m, 1H), 2.52 (m, 1H), 2.30 – 1.95 (m, 5H), 2.11 (s, 3H), 1.85 – 1.40 (m, 10H), 1.28 – 0.90 (m, 9H), 0.99 (s, 3H), 0.88 (s, 9H), 0.62 (s, 3H), 0.05 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 209.5, 141.5, 120.8, 72.5, 63.7, 56.9, 50.0, 43.9, 42.7, 38.8, 37.3, 36.5, 32.0, 31.8, 31.7, 31.5, 25.9, 24.4, 22.7, 21.0, 19.4, 18.2, 13.2, -4.6; IR (film): 1701 cm^{-1}; HRMS-TOF (*m/z*): $(M + Na)^{+}$ calcd for $C_{27}H_{46}O_2Si$, 453.3165; found, 453.3165.

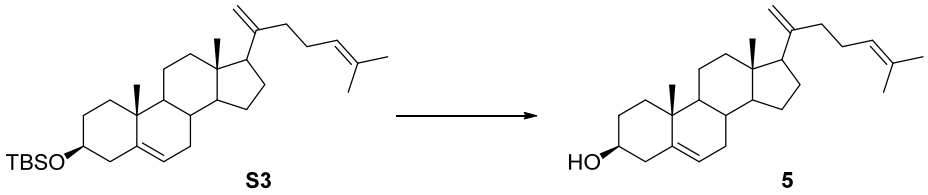


Alkylation of methyl ketone S1. HMPA (522 µl, 3 mmol) was added to a solution of methyl ketone **S1** (1.29 g, 3 mmol) in 15 mL THF. The resulting mixture was added dropwise to a solution of LDA (1.65 ml, 3.3 mmol) in 15 mL of THF at -78 °C. Stirring was continued at that temperature for 3 hours and then 3,3-dimethylallyl bromide **3** (450 µL, 3.9 mmol) was added. The solution was allowed to warm gradually to rt overnight. Saturated aqueous NH_4Cl was then added and stirred for 10 min. The mixture was extracted three times with dichloromethane (DCM). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. Flash chromatography (SiO_2, DCM-hexanes, 1:5) provided the monoalkylated product **4** and dialkylated product **S2** as white solids in a 13:1 ratio. Monoalklyation product **4** (1.17 g, 78 %): m.p. 120-121 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.30 (br s, 1H), 5.06 (m, 1H), 3.47 (m, 1H), 2.50 (m, 1H), 2.38 (m, 2H), 2.30 – 2.14 (m, 5H), 2.05 – 1.95 (m, 2H), 1.85 – 1.00 (m, 14H), 1.66 (s, 3H), 1.60 (s, 3H), 0.99 (s, 3H), 0.88 (s, 9H), 0.60 (s, 3H), 0.05 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 211.2, 141.5, 132.4, 123.1, 120.8, 72.5, 62.9, 56.9, 50.0, 44.3, 44.2, 42.7, 38.9, 37.3, 36.5, 32.0, 31.8 (2), 25.9, 24.6, 22.9, 22.4, 21.0, 19.4, 18.2, 17.6, 13.3, -4.6; IR (film): 1704 cm^{-1}; HRMS-TOF (*m/z*): $(M + Na)^+$ calcd for $C_{32}H_{54}O_2Si$, 521.3791; found, 521.3814. Dialkylation product **S2** (0.10 g, 7%): m.p. 92-93 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.30 (br d, *J* = 5.0 Hz, 1H), 5.04 (m, 2H), 3.46 (m, 1H), 2.59 (m, 2H), 2.60 – 1.00 (m, 23H), 1.67 (s, 3H), 1.64 (s, 3H), 1.59 (s, 3H), 1.55 (s, 3H), 0.98 (s, 3H), 0.88 (s, 9H), 0.59 (s, 3H), 0.05 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 215.4, 141.9, 133.9, 133.2, 123.2, 121.8, 121.3, 72.9, 63.4, 57.5, 53.8, 50.5, 45.1, 43.1, 39.2, 37.8, 37.0, 32.4, 32.2 (2), 32.0, 31.5, 26.3, 26.2, 26.1, 24.9, 23.8, 23.1, 21.4, 18.6, 18.1 (2), 13.6, -4.1; IR (film): 1703 cm^{-1}; HRMS-TOF (*m/z*): $(M + Na)^+$ calcd for $C_{37}H_{62}O_2Si$, 589.4417; found, 589.4418.



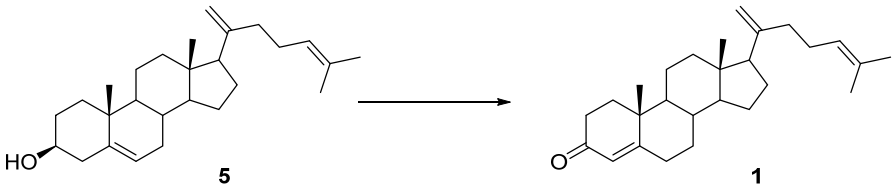
Olefination of ketone 4. Tebbe reagent (6 mL, 3 mmol, 0.5 M in toluene) was added slowly to a flame dried flask containing dry THF (3 mL) and dry toluene (3 mL). Ketone **4** (498 mg, 1 mmol) was dissolved in 10 mL THF and added dropwise to the solution of Tebbe reagent at room temperature and stirred for 16 h. The reaction was cooled to 0 °C

before being quenched cautiously with 1 M NaOH and stirring for an additional 10 minutes. The reaction mixture was filtered through Celite and the filtrate was extracted three times with DCM. The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. Flash chromatography (SiO_2, DCM-hexanes, 1:9) afforded **S3** (277 mg, 56 %) as a white solid: m.p. 86 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.31 (d, *J* = 5.1 Hz, 1H), 5.10 (m, 1H), 4.87 (s, 1H), 4.78 (s, 1H), 3.48 (m, 1H), 2.27 – 0.80 (m, 24H), 1.68 (s, 3H), 1.61 (s, 3H), 0.99 (s, 3H), 0.88 (s, 9H), 0.57 (s, 3H), 0.05 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 149.3, 141.6, 131.4, 124.4, 121.0, 109.4, 72.5, 56.6, 55.9, 50.3, 43.0, 42.7, 38.7, 37.6, 37.3, 36.6, 32.3, 32.1, 31.8, 27.1, 25.9, 25.8, 25.7, 24.2, 21.1, 19.5, 18.3, 17.7, 12.7, -4.5; IR (film): 2928 cm^{-1}.



S3 **5**

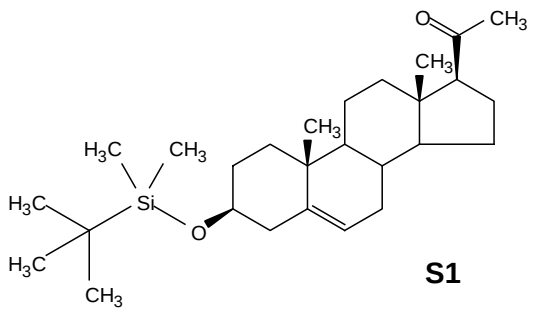
Desilylation of S3. Tetrabutylammonium fluoride (TBAF) (0.5 ml, 0.5 mmol, 1 M in THF) was added to a solution of silyl ether **S3** (124 mg, 0.25 mmol) in 1.25 ml of dry THF. After stirring overnight, the mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc-DCM-hexanes, 1:4:5) to give **5** (94 mg, 94%) as a white solid: m.p. 89-91 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.35 (d, *J* = 5.0 Hz, 1H), 5.11 (m, 1H), 4.87 (s, 1H), 4.78 (s, 1H), 3.52 (m, 1H), 2.30 – 0.80 (m, 24H), 1.68 (s, 3H), 1.60 (s, 3H), 1.00 (s, 3H), 0.57 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 149.2, 140.8, 131.4, 124.3, 121.1, 109.3, 71.8, 56.6, 55.9, 50.2, 43.0, 42.3, 38.6, 37.7, 37.2, 36.5, 32.2, 31.8, 31.6, 27.1, 25.8, 25.7, 24.2, 21.1, 19.4, 17.7, 12.7; HRMS-TOF (*m/z*): $(M + Na)^+$ calcd for $C_{27}H_{42}O$, 405.3133; found, 405.3151.



5 **1**

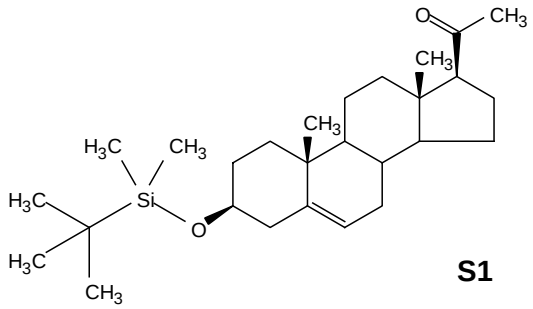
Pentalinonsterol (1). Alcohol **5** (38.2 mg, 0.1 mmol) was dissolved in 2.5 ml of dry toluene. *N*-methylpiperidone (1.0 ml, 8.4 mmol) was added and the solution was refluxed for 15 min. Aluminium isopropoxide (245 mg, 1.2 mmol) was added and heating was continued for 2 h. The reaction mixture was cooled to room temperature and washed 4 times with 1% H_2SO_4 and once with saturated NaCl. The mixture was then dried over sodium sulfate and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography (DCM-hexanes, 3:7) to provide pentalinonsterol (**1**) (33 mg, 88%) as a white solid: m.p. 40 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.72 (s, 1H),

5.10 (br s, 1H), 4.88 (s, 1H), 4.79 (s, 1H), 2.50 – 0.80 (m, 24H), 1.68 (s, 3H), 1.60 (s, 3H), 1.18 (s, 3H), 0.60 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 199.7, 171.6, 149.0, 131.5, 124.2, 123.7, 109.6, 55.8, 55.7, 53.8, 43.0, 38.6, 38.5, 37.7, 36.0, 35.7, 34.0, 32.9, 31.9, 27.1, 25.8, 25.7, 24.1, 21.1, 17.7, 17.4, 12.8; IR (film): 1677 cm^{-1}; HRMS-TOF (*m/z*): $(M + Na)^+$ calcd for $C_{27}H_{40}O$, 403.2977; found, 403.2984.



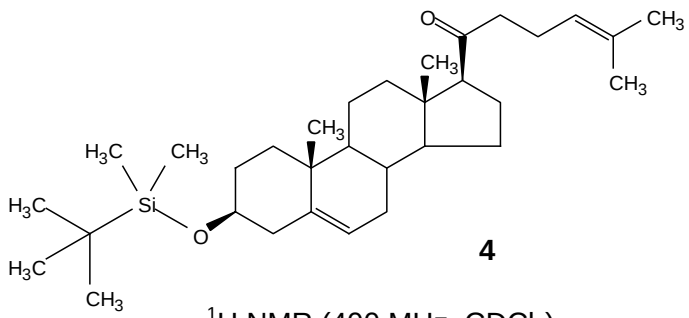
S1

^{1}H NMR (400 MHz, $CDCl_3$)



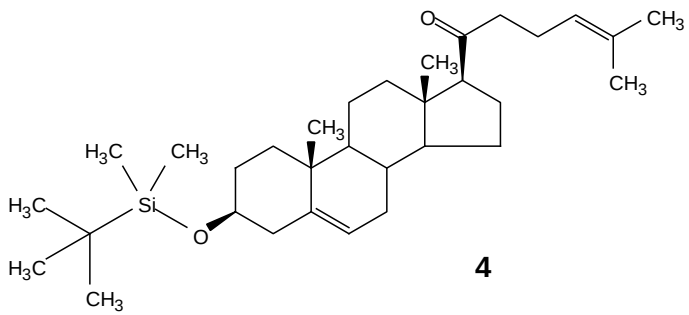
S1

^{13}C NMR (100 MHz, $CDCl_3$)



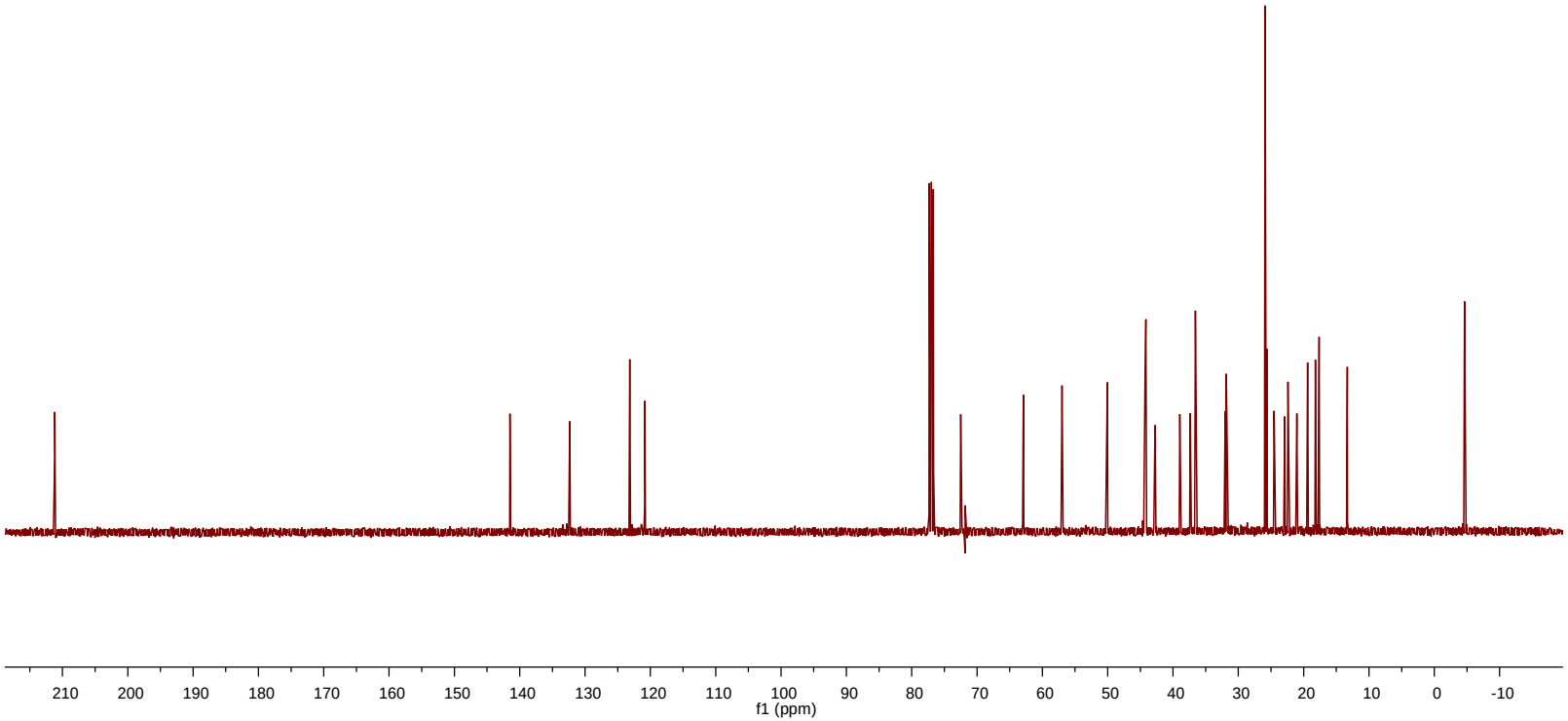
4

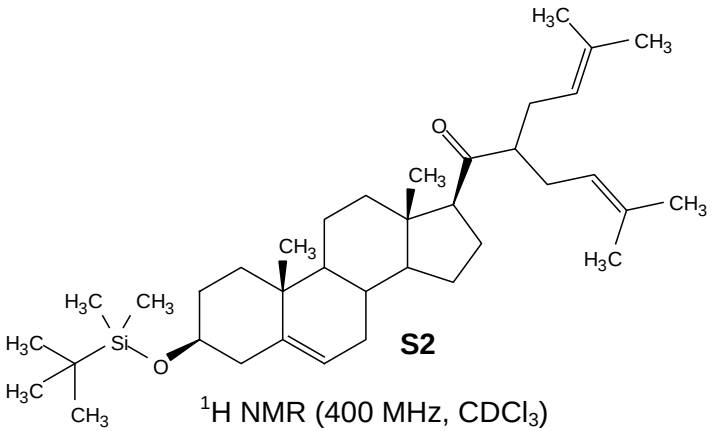
^{1}H NMR (400 MHz, $CDCl_3$)



4

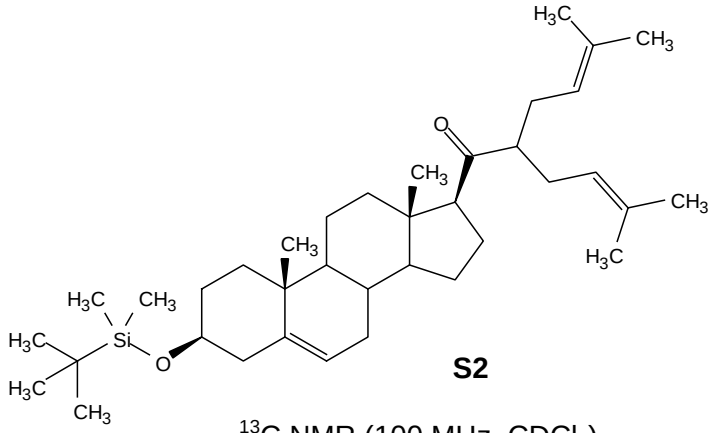
^{13}C NMR (100 MHz, $CDCl_3$)





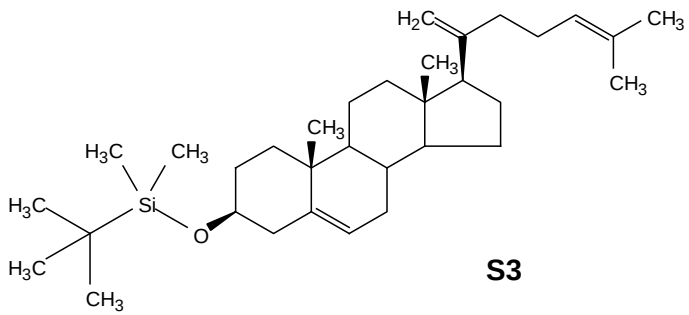
S2

^{1}H NMR (400 MHz, $CDCl_3$)



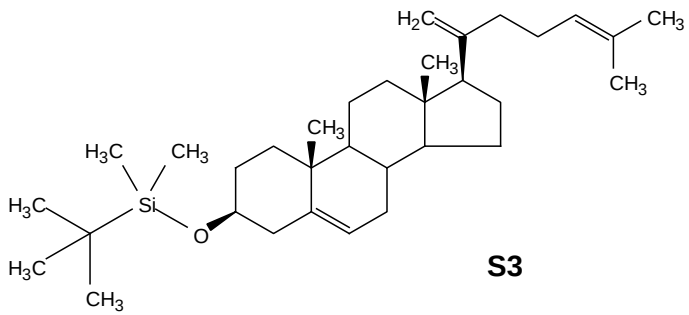
S2

^{13}C NMR (100 MHz, $CDCl_3$)



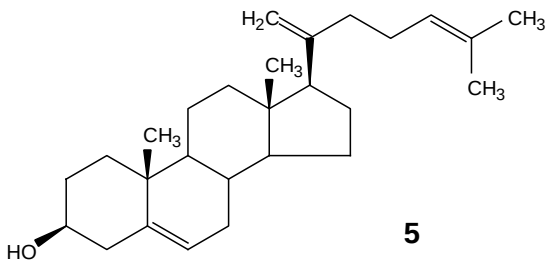
S3

^{1}H NMR (400 MHz, $CDCl_3$)



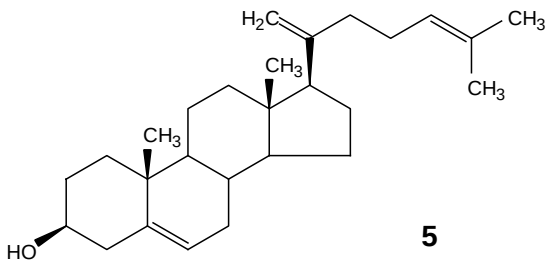
S3

^{13}C NMR (100 MHz, $CDCl_3$)



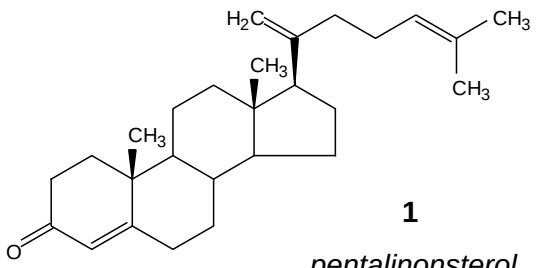
5

^{1}H NMR (400 MHz, $CDCl_3$)



5

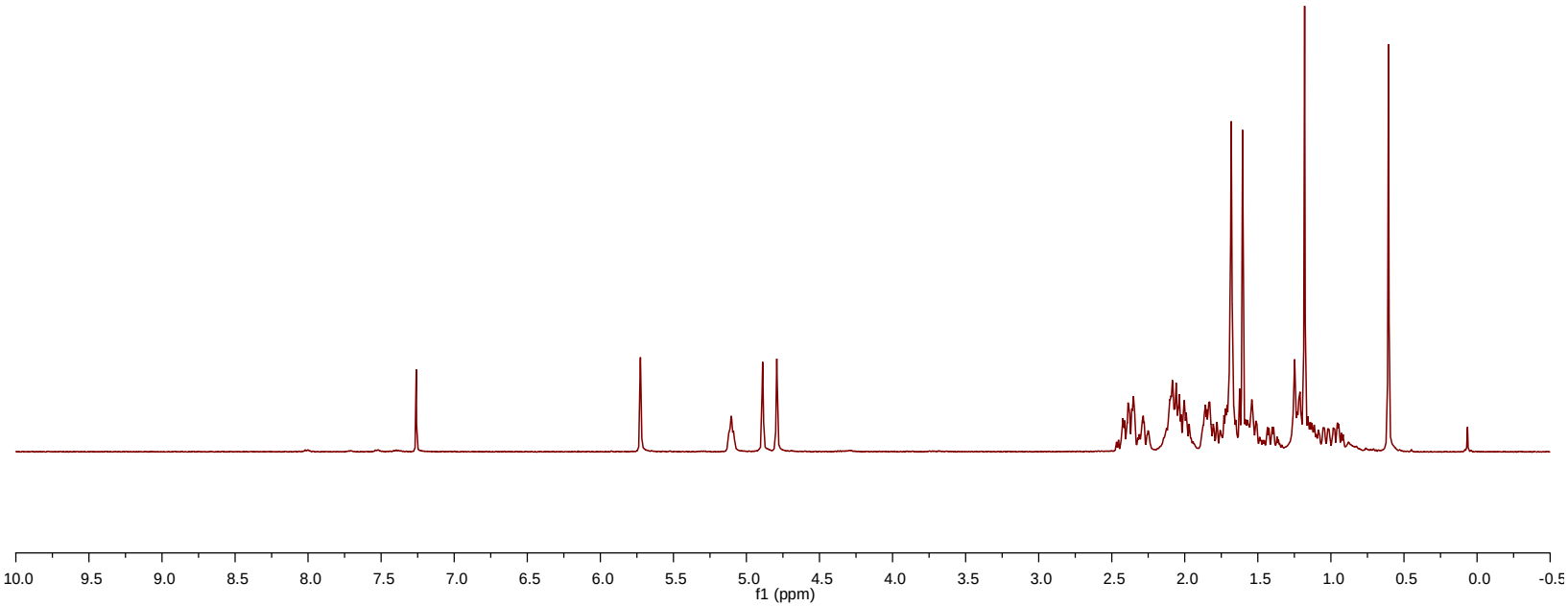
^{13}C NMR (100 MHz, $CDCl_3$)

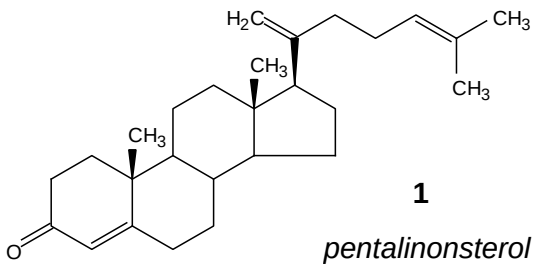


1

pentalinonsterol

^{1}H NMR (400 MHz, $CDCl_3$)





1

pentalinonsterol

^{13}C NMR (100 MHz, $CDCl_3$)