Deuterated squalene and sterols from modified *Saccharomyces cerevisiae*

SUPPORTING INFORMATION: EXPERIMENTAL METHODS

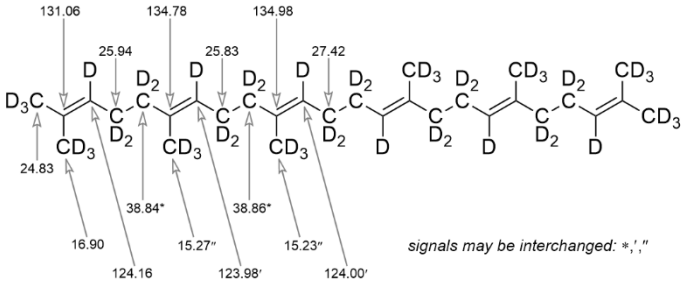
Carl Recsei,[†] Robert Russell, [†] Marina Cagnes[†] & Tamim Darwish[†]

[†]Australian Nuclear Science and Technology Organisation
National Deuteration Facility
New Illawarra Rd, Lucas Heights, New South Wales, Australia 2234

1. General experimental

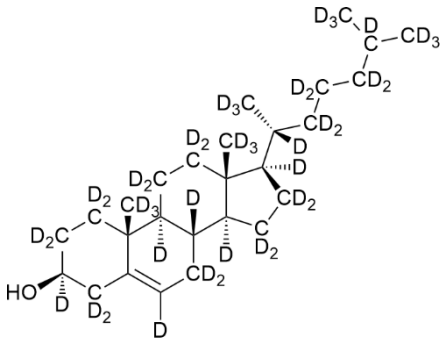
D_2O (99.8%) was supplied by Merck. Dichloromethane was distilled prior to use. Alumina and Florisil were activated prior to use by stirring at 120°C under a vacuum of 1 mBar for 16 hours. All other solvents and reagents were used as received from commercial vendors. Silver-ion chromatography was performed using SiliaSep Flash Cartridges, Silver Nitrate ($AgNO_3$), 40 - 63 µm, 60 Å (FLH-R23530B), obtained from SiliCycle Inc. Bioreactor cultures were cultivated in a Minifors 2 bioreactor (Infors, Switzerland). Growth media were sterilised by vacuum filtration (0.22 µm, PES) prior to use. Optical density (OD) was measured at 600 nm using an Eppendorf D30 Biophotometer. Bead beating was performed with a BeadBeater, Biospec Products, USA. Analytical thin-layer chromatography (TLC) was performed using Merck aluminium backed silica gel 60 F_{254} (0.2 mm) plates, which were visualised with shortwave (254 nm) ultraviolet light or with Hanessian's stain. Column chromatography was performed using a Buchi Pure Chromatography System. Infrared absorption spectra were recorded on a Thermo Scientific Nicolet^{TM} iSTM10 FTIR spectrometer using neat compound, and the data are reported as wavenumbers (cm^{-1}). Nuclear magnetic resonance spectra were recorded at 300 K using a Bruker AVANCE DRX400 (400 MHz) spectrometer equipped with a 5 mm PABBO BB H/D z-gradient probe. ^1H chemical shifts are expressed as parts per million (ppm) with residual chloroform (δ 7.26), as reference and are reported as chemical shift (δ), relative integral, assignment, multiplicity and coupling constants (*J*) reported in Hz – for example: 4.65 (1 H, CH_2, d, *J* 1.8). ^{13}C chemical shifts are expressed as parts per million (ppm) with residual chloroform (δ 77.16) as reference and reported as chemical shift (δ); multiplicity – for example: 38.23 (CD_2). ^2H chemical shifts are reported as parts per million (ppm) and are reported as chemical shift (δ); multiplicity – for example: 4.63 (2 D). ^{13}C resonances attached to deuterium appear as multiplets when only the proton nucleus is decoupled ^{13}C $\{^1H\}$ and resolve to singlets when both proton and deuterium nuclei are decoupled (*i.e.*, ^{13}C $\{^1H,^2H\}$). Assignments in ^{13}C NMR are described as CD_n or CH_n where all carbon atoms bearing measured deuterium enrichment are designated CD_n and natively substituted carbon atoms described as CH_n. Assignments in ^1H NMR are quoted as residual H except where the carbon is fully protiated. Low-resolution mass spectrometry (LRMS) was recorded using electrospray ionisation (ESI) on a 4000 QTrap mass spectrometer (AB Sciex) or atmospheric-pressure chemical ionization (APCI) on an Advion expression Compact Mass Spectrometer. The overall percentage deuteration of the molecules were calculated by mass spectrometry using the isotope distribution analysis of the different isotopologues, including correction factors calculated for the isotopic distribution of non-hydrogen atoms. Gas chromatographic analysis of squalene was performed on a Varian CP-3800 GC attached to a Varian 1200 Quadrupole MS, using electron ionisation (EI) as detection methods. Samples were injected onto a 30 m capillary column (Vf5-ms, Agilent) at 50°C for 2 min, followed by temperature ramp at 20°C·min^{-1} to 280°C. Squalene-d_{50} (81%-*d*) peak appeared at 16.7 minutes, while the native squalene reference peak appeared at 17.0 minutes.

2. Squalene-d_{50}



Squalene-d_{50} (81%-*d*) was produced using *Saccharomyces cerevisiae* strain Y2805 according to the method of Choi and coworkers. [1] In this work, yeast growth medium (YPD) comprised 0.5% yeast extract, 1% peptone, 2% dextrose per litre in deuterium oxide (99.8%-*d*). A single colony was picked from a uracil dropout agar plate and inoculated into 10 mL YPD medium and incubated at 30°C and 200 rpm orbital shaking. 1 mL of turbid culture (Optical density (OD) = 1-2) was inoculated into 10 mL of 50%-*d* D_2O (1:1 D_2O: H_2O) YPD and incubated as above. This adaptation was repeated in 90% and 99.8%-*d* D_2O YPD, then a seed culture was prepared by inoculating into 100 mL 99.8%-*d* D_2O YPD (10% v/v). At OD = 1-2 the seed culture was inoculated into 900 mL of 99.8%-*d* D_2O YPD and cultivated in a Minifors 2 bioreactor, until a rise in dissolved oxygen was observed. A feed solution containing 50 $g \cdot L^{-1}$ galactose and 5 $g \cdot L^{-1}$ glucose was commenced to induce squalene synthesis. Terbinafine was added at 10 $mg \cdot L^{-1}$. Cells were harvested by centrifugation, washed in 20 mM TRIS-HCl buffer, and lysed by vigorous vortexing with glass beads (5 x 1 min with 1 min rest intervals). The lysed cell suspension was then saponified in a solution of 8 M KOH in methanol, with 0.125% pyrogallol (w/v). After refluxing for 2 hours, the mixture was extracted three times with *n*-hexane. The solvent was removed *in vacuo*, and the residue purified by silica column chromatography (*n*-hexane) to give squalene-d_{50} (81%-*d*, 800 mg); >98% purity (GC); ^{1}H NMR (400 MHz, $CDCl_3$) 5.07 – 5.16 (6 residual H), 2.00 – 2.08 (8 residual H), 1.92 – 2.00 (12 residual H) 1.63 – 1.68 (6 residual H), 1.52 – 1.62 (18 residual H); ^{2}H NMR (61 MHz, $CDCl_3$) 4.96 – 5.33 (6 D, m), 1.76 – 2.21 (20 D, m), 1.45 – 1.76 (24 D, m); $^{13}C\{^1H,^2H\}$ NMR (101 MHz, 400 MHz, $CDCl_3$) 134.98 (2 C), 134.78 (2 C), 131.06 (2 C), 124.16 (2 CD), 124.00 (2 CD), 123.98 (2 CD), 38.86 (2 CD_2), 38.84 (2 CD_2), 27.42 (2 CD_2), 25.94 (2 CD_2), 25.83 (2 CD_2), 24.83 (2 CD_3), 16.90 (2 CD_3), 15.27 (2 CD_3), 15.23 (2 CD_3); $^{13}C\{^1H\}$ NMR (101 MHz, 400 MHz, $CDCl_3$) 134.5 – 135.1 (4 C), 130.8 – 131.1 (2 C), 123.5 – 124.5 (6 CD), 38.5 – 39.6 (4 CD_2), 27.1 – 28.1 (2 CD_2), 24.5 – 26.6 (4 CD_2 + 2 CD_3), 16.3 – 17.8 (2 CD_3), 14.8 – 16.0 (4 CD_3); IR (neat, cm^{-1}) 2908, 2222, 2191, 2110, 1280, 1264, 1047; MS ($APCI^+$, neat), quoted as: mass (relative intensity): 460 ($M^{\cdot+}$ = $[C_{30}D_{50}]^{\cdot+}$, 5%), 459 (10), 458 (20), 457 (35), 456 (52), 455 (73), 454 (88), 453 (95), 452 (99), 451 (100), 450 (92), 449 (81), 448 (66), 447 (53), 446 (38), 445 (26), 444 (20), 443 (11), 442 (5), 441 (3).

3. Cholesterol-d_{45} (79%-*d*)



Cholesterol-d_{45} (79%-*d*) was produced using *Saccharomyces cerevisiae* strain RH6829. **G**rowth media consisted of 7 g·L^{-1} yeast nitrogen base with amino acids, 5 g·L^{-1} yeast extract, 20 g·L^{-1} D-(+)-glucose, 30 mg·L^{-1} uracil and 30 mg·L^{-1} L-leucine.

H_2O growth media (10 mL) was inoculated with a single colony from RH6829 plate culture. The culture was incubated (30°C, 220 rpm) until the stationary phase (OD ~ 20) was reached. 50%-*d* D_2O growth media (10 mL) was inoculated with 0.1 mL of the preceding H_2O culture, then incubated until the stationary phase was reached (OD ~ 20). 94%-*d* D_2O growth media (100 mL) was inoculated with 1 mL of the preceding 50%-*d* D_2O culture, then incubated until the stationary phase was reached (OD ~ 10).

94%-*d* D_2O yeast media (3 L) was inoculated with the preceding 94%-*d* D_2O culture in a 4 L bioreactor. A drop of Antifoam 204 was added. The bioreactor was run at 30°C with stirring at 500 rpm, airflow 0.5 L·min^{-1}, minimum pO_2 at 30% and minimum pH at 5.5. 2.5 M NaOH was used for pH adjustment. Once a stationary phase was observed (accompanied by a sharp rise in pO_2), a feed solution (250 mL) (consisting of 2.4 g·L^{-1} yeast nitrogen base, 2 g·L^{-1} yeast extract, 300 g·L^{-1} D-glucose, 180 mg·L^{-1} uracil and 180 mg·L^{-1} L-leucine in 94%-*d* D_2O and filter-sterilised) was added dropwise to the bioreactor culture. At the subsequent stationary phase, further feed solution (250 mL) was added.

When the stationary phase was again reached, the culture was harvested by centrifugation (4000 x *g*, 25 °C, 30 minutes). The resulting wet cell pellet (70 g) was lysed by vigorous vortexing with glass beads† for 5 × 1 minutes with 1 minute rest intervals, before resuspending in 100 mL of saponification solution consisting of 450 g·L^{-1} potassium hydroxide, 3 g·L^{-1} pyrogallol and 140 mL·L^{-1} water in methanol. The mixture was stirred at 90°C for 5 hours, then diluted with water (50 mL) and extracted into 10% toluene/hexane (3 x 150 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated to provide a cream-coloured solid (578 mg). The solid was purified by automated flash chromatography (hexanes → hexanes/EtOAc : 9:1) to provide cholesterol-d_{45} (343 mg (or 98 mg·L^{-1}), 79±2%-*d*) as a colourless powder; R_f 0.15 (hexanes/EtOAc : 9:1); ^{1}H NMR (400 MHz, $CDCl_3$) 5.29 – 5.34 (1 residual H, m), 3.45 – 3.52 (1 residual H, m), 2.19 (1 OH), 0.59-2.30 (45 residual H); ^{2}H NMR (61 MHz, $CDCl_3$) 5.33 (1 D, br), 3.44 (1 D, br), 0.41 – 2.36 (43 D, m); ^{13}C{^{1}H,^{2}H} NMR (101 MHz, 400 MHz, $CDCl_3$) 140.93 (C), 121.21 (CD), 71.06 (CD), 55.88 (CD), 55.27 (CD), 49.20 (CD), 41.86 (C), 41.34 (CD_2), 38.68 (CD_2), 38.23 (CD_2), 36.29 (CD_2), 35.99 (C), 35.04 (CD_2), 34.77 (CD), 31.09 (CD_2), 30.90 (CD), 30.54 (CD_2), 27.24 (CD_2), 26.88 (CD), 23.32 (CD_2), 22.71 (CD_2), 21.73 (CD_3), 21.49 (CD_3), 20.15 (CD_2), 18.51 (CD_3), 17.72 (CD_3), 10.99 (CD_3); ^{13}C{^{1}H} NMR (101 MHz, 400 MHz, $CDCl_3$)‡ 140.94 (C), 120.75 – 121.81 (CD), 70.65 – 71.89 (CD), 54.89 – 56.82 (CD), 48.91 – 50.53 (2 CD), 48.83 – 50.13 (CD), 41.10 – 42.53 (C + CD_2), 37.73 – 39.82 (2 CD_2), 34.41 – 37.39(C + CD + CD_2), 30.13 – 31.95 (2 CD + CD_2), 26.59 – 28.47 (CD + CD_2), 19.72 – 24.24 (3 CD_2 + 2 CD_3), 17.07 – 19.70 (2 CD_3), 10.54 – 11.96 (CD_3); IR (neat, cm^{-1}) 3425, 2877, 2210, 2102, 1290, 1082, 1052, 943; MS (ESI$^+$, MeOH/$CHCl_3$/HCOOH), quoted as: mass (relative intensity): 414 ([M−H_2O]$^+$ = [$C_{27}D_{45}$]$^+$, 1%), 413 (3), 412 (5), 411 (12), 410

(30), 409 (51), 408 (68), 407 (93), 406 (94), 405 (100), 404 (90), 403 (89), 402 (60), 401 (51), 400 (40), 399 (38), 398 (25).

†Omission of the bead-beating step is not deleterious to the yield if the lysis in methanol is performed as written here.

‡This sample of cholesterol-d_{45} (79%-*d*) was highly concentrated (>100 mg in 0.4 mL $CDCl_3$) to furnish sufficient signal to noise for site-specific deuteration calculations; resultant line broadening and chemical shift changes are evident with respect to samples of lower concentration. Reference should additionally be made to the $^{13}C\{^1H,^2H\}$ NMR for of cholesterol-d_{45} (98%-*d*); *see*: page 5 for listed data.

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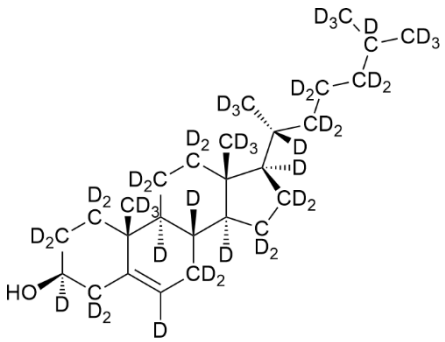
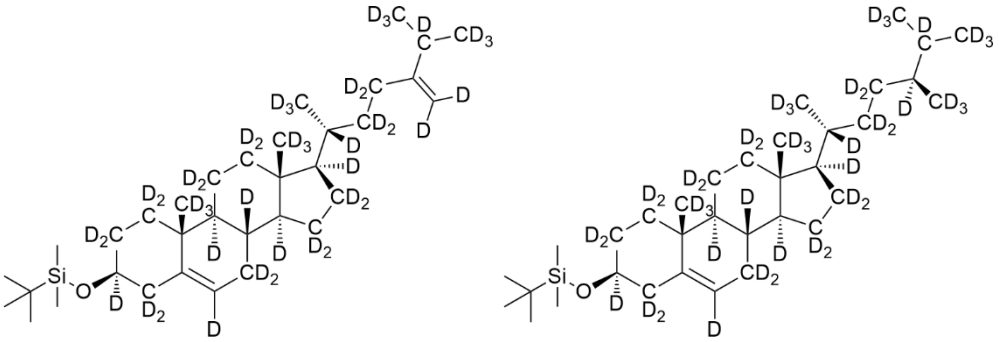
4. Cholesterol-d_{45} (98%-*d*)



Cholesterol-d_{45} (98%-*d*) was produced using *Saccharomyces cerevisiae* strain (RH6829). Following adaptation to growth on D_2O (*see*: page 3, as for cholesterol-d_{45} (79%-*d*)), *S. cerevisiae* seed culture was grown on a highly deuterated medium comprising 0.1 $g{\cdot}L^{-1}$ yeast nitrogen base, 0.1 $g{\cdot}L^{-1}$ yeast extract, 5 $g{\cdot}L^{-1}$ glucose-d_{12}, and 30 $mg{\cdot}L^{-1}$ each of uracil and L-leucine in deuterium oxide (99.8%-*d*). The seed culture was inoculated into a 1 L D_2O yeast growth medium and cultivated in a Minifors 2 bioreactor until stationary phase was observed (indicated by rise in dissolved oxygen). Cells were harvested by centrifugation and saponified then subjected to extractive workup and purification by flash column chromatography according to the procedure for cholesterol-d_{45} (79%-*d*) to give cholesterol-d_{45} (15 mg, 98%-*d*) as a colourless solid; ^2H NMR (61 MHz, $CDCl_3$) 5.33 (1 D, br), 3.46 (1 D, br), 0.44 – 2.32 (43 D, m); $^{13}C\{^1H,^2H\}$ NMR (101 MHz, 400 MHz, $CDCl_3$) 140.94 (C), 121.38 (CD), 71.26 (CD), 55.94 (CD), 55.28 (CD), 49.24 (CD), 41.86 (C), 41.46 (CD_2), 38.71 (CD_2), 38.29 (CD_2), 36.32 (CD_2), 36.08 (C), 35.06 (CD_2), 34.81 (CD), 31.17 (CD_2), 30.97 (CD), 30.69 (CD_2), 27.29 (CD_2), 26.95 (CD), 23.39 (CD_2), 22.72 (CD_2), 21.78 (CD_3), 21.52 (CD_3), 20.21 (CD_2), 18.56 (CD_3), 17.77 (CD_3), 11.04 (CD_3); MS (ESI^+, MeOH/$CHCl_3$/HCOOH), 414 ($[M-H_2O]^+ = [C_{27}D_{45}]^+$, 100%), 413 (20).

5. 22,23-Dihydrobrassicasterol-d_{47} (87%-*d*) *O-tert*-butyldimethylsilyl ether *and* 24-methylenecholesterol-d_{45} (87%-*d*) *O-tert*-butyldimethylsilyl ether.



Saccharomyces cerevisiae strain RH6827, reported as producing campesterol, was grown under the same conditions as for cholesterol-d_{45} (79%-*d, see*: page 3), using deuterium oxide (98%-*d*) in the growth medium. The crude extract was subjected to flash column chromatography (gradient elution, 0 → 15% EtOAc in hexanes) to give, after removal of solvent in vacuo, a mixture of deuterated sterols (185 mg, *ca.* 0.41 mmol, 1.0 equiv.), which was taken up, under a nitrogen atmosphere, in anhydrous DMF (10 mL). The solution was cooled to –5°C, sparging with nitrogen and stirring, and *tert*-butyldimethylsilyl chloride (79 mg, 0.52 mmol, *ca.* 1.3 equiv.) was added, followed by imidazole (60 mg, 0.88 mmol, *ca.* 2.1 equiv.). Nitrogen sparging was continued for 5 minutes and the mixture allowed to warm slowly to room temperature, then heated to 30°C with stirring (40 h). The resulting suspension was poured onto ice water (100 mL) and this mixture extracted with 10% ether in hexane (3 × 75 mL). The combined ether extracts were washed with aqueous lithium chloride (1 M, 150 mL), brine (150 mL), dried over Na_2SO_4 and concentrated. Flash column chromatography (hexanes) gave a fraction containing a mixture of silylated sterols. To 115 mg of this mixture was added hexane (10 mL, then Florisil (2 g) was added, and volatile components removed *in vacuo*. The resulting free-flowing powder was packed into a solid-loading cartridge and subjected to column chromatography, using a 24 g silver ion column (SiliaSep Flash Cartridges, Silver Nitrate ($AgNO_3$), 40 - 63 µm, 60 Å (FLH-R23530B)). 22,23-Dihydrobrassicasterol-d_{47} *O-tert*-butyldimethylsilyl ether was eluted with hexane and isolated as a colourless solid after removal of solvent *in vacuo* (88 mg, 87.6±2%-*d*)†; R_f 0.2 (hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) 5.30 – 5.32 (1 residual H, m), 3.45 – 3.50 (1 residual H, m), 0.59-2.29 (45 residual H), 0.89 (9 H, s), 0.06 (6 H, s); ^{2}H NMR (61 MHz, $CDCl_3$) 5.30 (1 D), 3.42 (1 D), 0.44 – 2.50 (45 D, m); ^{13}C{^{1}H,^{2}H} NMR (101 MHz, 400 MHz, $CDCl_3$) 141.72 (C), 120.84 (CD), 72.12 (CD), 55.98 (CD), 55.14 (CD), 49.34 (CD), 42.00 (CD_2), 41.87 (C), 38.74 (CD), 38.06 (CD_2), 36.47 (CD_2), 36.18 (C), 35.21 (CD), 32.64 (CD_2), 31.20‡, 31.14‡, 31.03‡, 30.49 (CD), 29.55 (CD_2), 27.27 (CD_2), 26.11 (3 CH_3), 23.41 (CD_2), 20.21 (CD_2), 19.54 (CD_3), 18.60 (CD_3), 18.42 (C), 18.00 (CD_3), 16.66 (CD_3), 14.55 (CD_3), 11.05 (CD_3), −4.41 (2 CH_3); ^{13}C{^{1}H} NMR (101 MHz, 400 MHz, $CDCl_3$) 141.72 (C), 120.33 – 121.40 (1 CD), 71.83 – 72.80 (1 CD), 54.68 – 56.87 (2 CD), 49.02 – 49.78 (1 CD), 41.48 – 43.23 (C + CD_2), 37.52 – 40.01 (CD + CD_2), 34.70 – 37.52 (C + CD + CD_2), 32.19 – 33.76 (CD_2), 28.87 – 32.19 (2 CD, 3 CD_2), 26.55 – 28.27 (CD_2), 26.11 (3 CH_3), 22.75 – 24.51 (CD_2), 18.41 (C), 16.13 – 21.17 (3 CD_3 + CD_2), 14.37 – 15.38 (CD_3), 10.66 – 12.02 (CD_3), −4.41 (2 CH_3); IR (neat, cm^{-1}) 2956, 2919, 2849, 2212, 1728, 1272, 1121, 1071; MS (APCI$^+$, neat), quoted as: mass (relative intensity): 430 ([M−TBSO]$^+$ = [$C_{28}D_{47}$]$^+$, 12%), 429 (33), 428 (54), 427 (73), 426 (92), 425 (93), 424 (100), 423 (86), 422 (79), 421 (52), 420 (66), 419 (33), 418 (22), 417 (13), 416 (9), 415 (4), 414 (3). Further elution (10% ethyl acetate/90% hexane) gave 24-

methylenecholesterol-d_{45} *O-tert*-butyldimethylsilyl ether, isolated as a colourless solid after removal of solvent *in vacuo* (18 mg, 87.0±2%-*d*)†; R_f 0.2 (hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) 5.30 – 5.32 (1 residual H, m), 4.71 (1 H, CH_2, dd, *J* 1.8. 0.4), 4.69 (1 residual H, CDH, *J* 0.4), 4.65 (1 H, CH_2, d, *J* 1.8), 4.64 (1 residual H, CHD, s), 3.45 – 3.49 (1 residual H, m), 0.60-2.29 (43 residual H), 0.89 (9 H, s), 0.06 (6 H, s); ^{2}H NMR (61 MHz, $CDCl_3$) 5.29 (1 D), 4.64 (2 D), 3.41 (1 D), 0.44 – 2.44 (41 D); ^{13}C{^{1}H,^{2}H} NMR (101 MHz, 400 MHz, $CDCl_3$) 156.78 (C), 141.74 (C),120.82 (CD), 105.51 (CD_2), 72.11 (CD), 55.98 (CD), 55.13 (CD), 49.32 (CD), 41.98 (CD_2), 41.92 (C), 38.74 (CD_2), 36.45 (CD_2), 36.17 (C), 34.78 (CD), 33.67 (CD_2), 32.91 (CD), 31.19 (CD_2), 31.12‡, 31.00‡, 30.08 (CD_2), 26.11 (3 CH_3), 27.28 (CD_2), 23.39 (CD_2), 21.02 (CD_3), 20.88 (CD_3), 20.20 (CD_2), 18.60 (CD_3), 18.42 (C), 17.77 (CD_3), 11.05 (CD_3), −4.42 (2 CH_3); ^{13}C{^{1}H} NMR (101 MHz, 400 MHz, $CDCl_3$) 156.69 – 157.06 (C), 141.57 – 141.91 (C), 120.28 – 121.20 (CD), 104.78 –106.17 (CD_2), 71.65 – 72.72 (CD), 54.67 – 56.68 (2 CD), 48.92 – 49.92 (CD), 41.40 – 42.77 (C + CD_2), 38.06 – 39.44 (CD_2), 35.81 – 37.25 (C + CD_2), 32.42 – 35.60 (2 CD + CD_2), 29.19 – 31.88 (CD + 2 CD_2), 26.63 –27.95 (CD_2), 26.11 (3 CH_3), 22.49 – 24.10 (CD_2), 19.55 – 22.03 (CD_2 + 2 CD_3), 18.42 (C), 17.04 – 19.34 (2 CD_3), 10.29 – 11.87 (CD_3), −4.42 (2 CH_3); IR (neat, cm^{-1}) 2955, 2928, 2211,1255, 1087, 837; MS (APCI$^+$, neat), quoted as: mass (relative intensity): 426 ([M−TBSO]$^+$ = [$C_{28}D_{45}$]$^+$, 7%), 425 (23), 424 (48), 423 (76), 422 (95), 421 (100), 420 (91), 419 (76), 418 (51), 417 (32), 416 (19), 415 (10), 414 (5).

†These materials have the following values for molecular weight, used to calculate the yield in the subsequent deprotection step and derived from the measured deuteration level:

Compound	*Calculated molecular weight*
22,23-dihydrobrassicasterol-d_{47} *O-tert*-butyldimethylsilyl ether (87.6±2%)	556.38±0.95
24-methylenecholesterol-d_{45} *O-tert*-butyldimethylsilyl ether (87.0±2%)	552.33±0.91

‡multiplicity not assigned due to complex, overlapping signals.

6. General procedure for deprotection of silyl ethers

The silyl ethers were taken up in hexane (1 mL per mg) and added to a suspension of alumina, activated and wetted with 1.5% w/w water according to the procedure of Guerrero and co-workers. [2] The resulting suspension was stirred vigorously (16 h) then volatile components removed *in vacuo*. The resulting free-flowing powder was packed into a solid-loading cartridge and subjected to automated column chromatography (hexanes → hexanes/EtOAc : 9:1), followed by removal of the solvent *in vacuo*, to give the sterol as a colourless solid.

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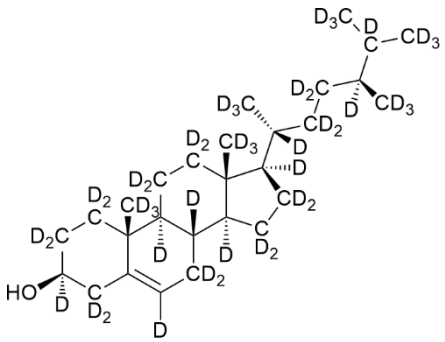
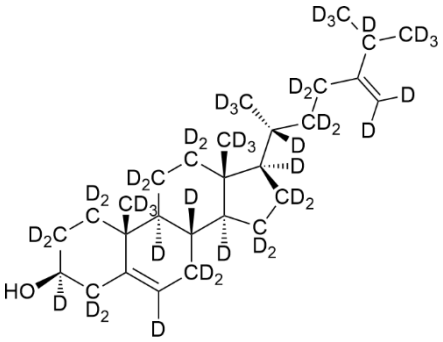
7. 22,23-Dihydrobrassicasterol-d_{47} (87%-*d*)



22,23-Dihydrobrassicasterol-d_{47} *O*-*tert*-butyldimethylsilyl ether (88 mg) was desilylated according to the general procedure to give 22,23-dihydrobrassicasterol-d_{47} (61 mg, 88±1% yield, 86.7±2%-*d*)† as a colourless powder; R_f 0.15 (hexanes/EtOAc : 9:1); ^{1}H NMR (400 MHz, $CDCl_3$) 5.32 – 5.35 (1 residual H, m), 3.47 – 3.54 (1 residual H, m), 0.59-2.29 (45 residual H); ^{2}H NMR (61 MHz, $CDCl_3$) 5.33 (1 D), 3.45 (1 D), 0.38 – 2.55 (45 D, m); ^{13}C{^{1}H,^{2}H} NMR (101 MHz, 400 MHz, $CDCl_3$) 140.92 (C), 121.35 (CD), 71.22 (CD), 55.91 (CD), 55.11 (CD), 49.23 (CD), 41.84 (C), 41.43 (CD_2), 38.68 (CD_2), 38.01 (CD), 36.31 (CD_2), 36.06 (C), 35.18 (CD), 32.60 (CD_2), 31.15 (CD_2), 30.96 (CD), 30.66 (CD_2), 30.44 (CD), 29.52 (CD_2), 27.24 (CD_2), 23.38 (CD_2), 20.20 (CD_2), 19.50 (CD_3), 18.55 (CD_3), 17.93 (CD_3), 16.63 (CD_3), 14.52 (CD_3), 11.05 (CD_3); ^{13}C{^{1}H} NMR (101 MHz, 400 MHz, $CDCl_3$) 141.92 (C), 120.90 – 122.04 (CD), 71.99 – 70.79 (CD), 54.71 – 57.03 (2 CD), 48.91 – 50.53 (CD), 40.94 – 42.59 (C + CD_2), 37.67 – 39.92 (CD + CD_2), 34.86 – 37.45 (C + CD + CD_2), 32.13 – 33.71 (CD_2), 28.91 – 32.13 (2 CD +, 3 CD_2), 26.55 – 28.20 (CD_2), 22.73 – 24.48 (CD_2), 15.99 – 21.13 (4 CD_3 + CD_2), 13.87 – 15.75 (CD_3), 10.51 – 12.12 (CD_3); IR (neat, cm^{-1}) 3396, 2926, 2212, 2100, 1287, 1052; MS (APCI$^+$, neat), quoted as: mass (relative intensity): 430 ([M−OH]$^+$ = $[C_{28}D_{47}]^+$, 13%), 429 (32), 428 (57), 427 (81), 426 (97), 425 (100), 424 (91), 423 (78), 422 (62), 421 (51), 420 (39), 419 (23), 418 (14), 417 (9), 416 (4).

†This sample of 22,23-dihydrobrassicasterol-d_{47} has a molecular weight of 441.69±0.95, used to calculate the yield and derived from the measured deuteration level.

8. 24-Methylenecholesterol-d_{45} (87%-*d*)



24-Methylenecholesterol-d_{45} *O*-*tert*-butyldimethylsilyl ether (18 mg) was desilylated according to the general procedure to give 24-methylenecholesterol-d_{45} (12 mg, 83±4% yield, 87.1±2%-*d*)† as a colourless powder; R_f 0.15 (hexanes/EtOAc : 9:1); ^{1}H NMR (400 MHz, $CDCl_3$) 5.33 – 5.37 (1 residual H, m), 4.70 (1 H, CH_2, dd, *J* 1.8. 0.3), 4.69 (1 residual H, CDH, *J* 0.3), 4.65 (1 H, CH_2, d, *J* 1.9), 4.64 (1 residual H, CHD, s), 3.47 – 3.55 (1 residual H, m), 0.59-2.33 (41 residual H); ^{2}H NMR (61 MHz, $CDCl_3$) 5.32 (1 D), 4.63 (2 D), 3.45 (1 D), 0.45 – 2.44 (41 D, m); ^{13}C{^{1}H,^{2}H} NMR (101 MHz, 400 MHz, $CDCl_3$) 156.79 (C), 140.93 (C), 121.35 (CD), 105.50 (CD_2), 71.24 (CD), 55.92 (CD), 55.11 (CD), 49.23 (CD), 41.45 (CD_2), 41.86 (C), 38.70 (CD_2), 36.31 (CD_2), 36.08 (C), 34.77 (CD), 33.66 (CD_2), 32.89 (CD), 31.17 (CD_2), 30.96 (CD), 30.69 (CD_2), 30.08 (CD_2), 27.27 (CD_2), 23.38 (CD_2), 21.01 (CD_3), 20.87 (CD_3), 20.21 (CD_2), 18.56 (CD_3), 17.76 (CD_3), 11.05 (CD_3); ^{13}C{^{1}H} NMR (101 MHz, 400 MHz, $CDCl_3$) 156.69 – 157.06 (C), 140.94 (C), 120.99 – 122.07 (CD), 104.92 –106.40 (CD_2), 70.88 – 72.03 (CD), 54.84 – 56.26 (2 CD), 48.93 – 49.91 (CD), 41.20 – 42.55 (C + CD_2), 38.16 – 39.47 (CD_2), 35.79 – 37.35 (C + CD_2), 32.41 – 35.79 (2 CD + CD_2), 28.70 – 32.28 (CD + 3 CD_2), 26.71 – 28.13 (CD_2), 22.62 – 24.08 (CD_2), 19.65 – 22.32 (CD_2 + 2 CD_3), 16.98 – 19.65 (2 CD_3), 10.40 – 12.26 (CD_3); IR (neat, cm^{-1}) 3430, 2925, 2213, 2104, 1288, 1137, 1083, 1052; MS (APCI$^+$, neat), quoted as: mass (relative intensity): 426 ([M−OH]$^+$ = $[C_{28}D_{45}]^+$, 6%), 425 (24), 424 (47), 423 (77), 422 (94), 421 (100), 420 (90), 419 (72), 418 (51), 417 (32), 416 (18), 415 (9), 414 (4).

†This sample of 24-methylenecholesterol-d_{45} has a molecular weight of 438.11±0.91, used to calculate the yield and derived from the measured deuteration level.

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9. References

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