ELECTRONIC SUPPORTING INFORMATION

Phytosterol recognition *via* rationally designed molecularly imprinted polymers

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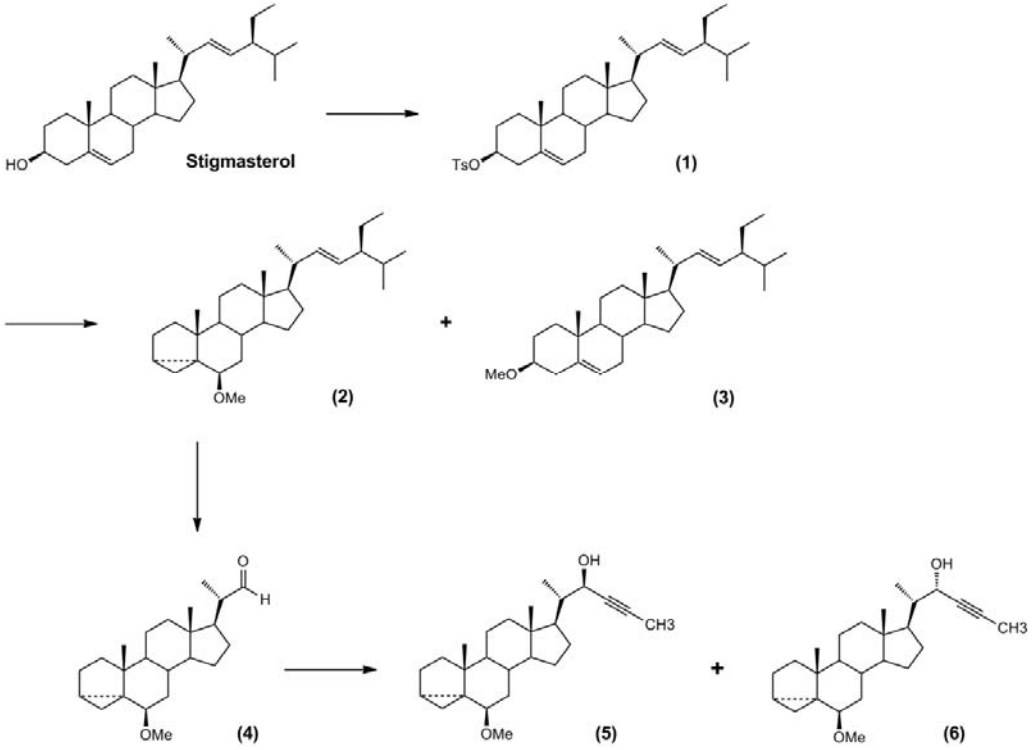
S1. GENERAL EXPERIMENTAL PROCEDURES

The syntheses of campesterol and brassicasterol (Schemes 1-3) were based on the conversion of stigmasterol in high yield to the reactive aldehyde (20*S*)-20-formyl-6β-methoxy-3α,5-cyclo-5α-pregnane (**4**) (Scheme 1). The aldehyde (**4**) was employed as the synthetic precursor to prepare both campesterol (**12**) (Scheme 2) and brassicasterol (**17**) (Scheme 3) *via* strategies adapted from those reported for the preparation of cholesterol and cholestadiene/cholestatriene derivatives [1-5]. Stigmasterol (95%) and anhydrous solvents were purchased from Sigma Aldrich (Sydney, Australia) and Acros Organics (New Jersey, USA) and were used without further purification.

Proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectroscopy were recorded as solutions in deuterated chloroform ($CDCl_3$) or base-washed $CDCl_3$ using a Bruker Avance 400, Ultrashield Plus 400, Varian Unity Inova 500 or Avance III 600 spectrometer at 400, 500 and 600 MHz, respectively. Base-washed $CDCl_3$ was prepared by stirring $CDCl_3$ in anhydrous potassium carbonate followed by filtration. ^{1}H NMR spectra were measured as chemical shifts quoted in parts per million (ppm) from tetramethylsilane, followed by multiplicity, number of equivalent nuclei, coupling constant(s) (where applicable), and assignment for ^{1}H spectra, or the chemical shift was followed by assignment for ^{13}C NMR spectra. The residual peak for chloroform at δ7.26 ppm for ^{1}H NMR and δ77.16 for ^{13}C NMR spectra were used as internal references. The abbreviations s for singlet, d for doublet, t for triplet, q for quartet and br for broad were used in the assignments of multiplicity. A value approximating the centre of the multiplet is quoted. Electrospray ionization mass spectra (ESI-MS) were recorded on a Micromass Platform mass spectrometer.

Melting points (quoted in Celsius) were determined using a Stuart or a Büchi B-545 melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was carried out using Merck Silica 60 F_{254} silica sheets, and stained with a *p*-anisaldehyde solution (made up of *p*-anisaldehyde, acetic acid, sulphuric acid and 96% ethanol (18.4:3.75:12.5:3.8)) or a phosphomolybdic acid solution (2.5% in 96% ethanol) with heating. Column chromatography was carried out using either Merck or Scharlau Silica gel 60 (0.04-0.06 mm, 230-400 mesh ASTM), Merck Alumina 90 (neutral, activity 1) or Acros Organics or ChemSupply Florisil® (60-100 mesh ASTM). Ozonization was carried out using an OzoneLab™ OL100/T generator at a flow rate of 0.75 LPM which is equivalent to 2.2% O_3 by weight.

S2. EXPERIMENTAL DETAILS



Scheme 1: Synthesis of (22*R*)-6β-Methoxy-3α,5-cyclo-26,27-dinor-5α-cholest-23-yn-22-ol **(5)** and (22*S*)-6β-methoxy-3α,5-cyclo-26,27-dinor-5α-cholest-23-yn-22-ol **(6)** from stigmasterol *via* (20*S*)-20-formyl-6β-methoxy-3,5α-cyclo-5α-pregnane **(4)**.

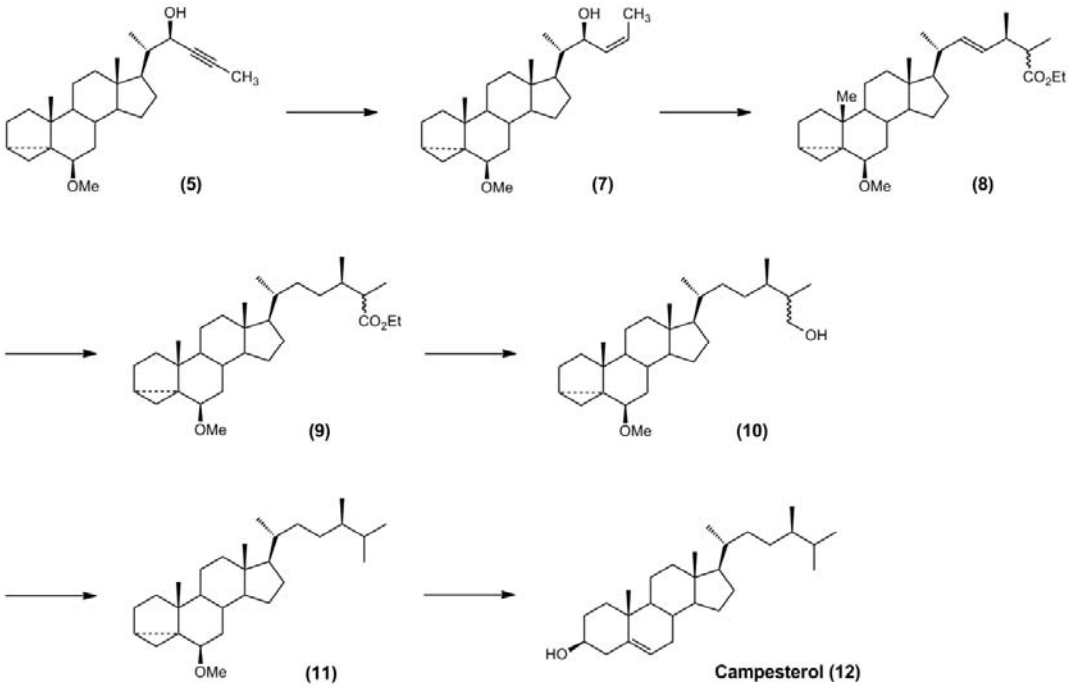
Stigmasteryl tosylate (1) A mixture of stigmasterol (44.73 g, 108.4 mmol) and *p*-toluenesulfonyl chloride (41.55 g, 218.0 mmol) in pyridine (350 mL) was stirred at room temperature for 72 h and then poured into saturated aqueous sodium bicarbonate. The suspension was filtered, the white residue washed copiously with water and dried to

give stigmasteryl tosylate **(1)** as a white powder (59.22 g, 96%), m.p. 145-148 °C (lit. 148-149 °C [3]; lit. 147-148 °C [1,5]). ^{1}H NMR (500 MHz, base-washed $CDCl_3$): δ 0.67 (s, 3H, H18); 0.79 (d, 3H, *J* 6.5 Hz, H27); 0.80 (t, 3H, *J* 7.5 Hz, H24[1]); 0.84 (d, 3H, *J* 6.5 Hz, H26); 0.96 (s, 3H, H19); 1.01 (d, 3H, *J* 6.5 Hz, H21); 2.45 (s, 3H, Me-OTs); 4.31 (m, 1H, H3α); 5.01 (dd, 1H, *J* 15.5, 8.5 Hz, H23); 5.14 (dd, 1H, *J* 15.3, 8.8 Hz, H22); 5.30 (m, 1H, H6); 7.33 (d, 2H, *J* 8.0 Hz, H3' and H5' -OTs); 7.79 (d, 2H, *J* 8.0 Hz, H2' and H6' -OTs).

(22*E*)-6β-Methoxy-3,5α-cyclo-5α-stigmasta-22-ene (stigmasterol *i*-methyl ether) (2) and 3-*O*-methylstigmasterol (3). Pyridine (2.5 mL, 30.9 mmol) was added to a suspension of stigmasteryl tosylate **(1)** (9.96 g, 17.6 mmol) in anhydrous methanol (100 mL). The white suspension was stirred at reflux under nitrogen for 6 h and cooled to room temperature. The suspension was concentrated to an oily paste which was diluted with water and extracted three times with ethyl acetate. The combined organic phases were washed with brine, dried over magnesium sulphate, filtered and concentrated to a white paste (7.24 g, 97%). The crude sample was dissolved in ethyl acetate and purified by column chromatography by adsorption onto Florisil® (28 g), followed by evaporation of the solvent. The dry Florisil® mixture was loaded into a column and then eluted under gravity with 100% hexane. This procedure separated **(2)** (R_f ~ 0.38) from **(3)** (R_f ~ 0.29) (95:5 hexane/ethyl acetate), to give (**2**) (5.16 g (69%)) as a colourless oil which semi-crystallized in the freezer at -20 °C. ^{1}H NMR (500 MHz, base-washed $CDCl_3$): **(2)** δ 0.43 (m, 1H, H4α); 0.65 (m, 1H, H4β); 0.73 (s, 3H, H18); 0.79 (d, 3H, *J* 7.0 Hz, H27); 0.80 (d, 3H, *J* 7.0 Hz, H24[2]); 0.85 (d, 3H, *J* 6.5 Hz, H26); 1.01 (d, 3H, *J* 6.5 Hz, H21); 1.02 (s, 3H, H19); 2.77 (brt, 1H, *J* 3.0 Hz, H6α); 3.32 (s, 3H, OMe); 5.01 (dd, 1H, *J* 15.5, 8.5 Hz, H23); 5.14 (dd, 1H, *J* 15.3, 8.8 Hz, H22) **(3)** δ 0.70 (s, 3H, H18); 0.79 (d, 3H, *J* 6.5 Hz, H27); 0.80 (t, 3H, *J* 7.5 Hz, H24[2]); 0.84 (d, 3H, *J* 6.5 Hz, H26); 1.00 (s, 3H, H19); 1.01 (d, 3H, *J* 7.0 Hz, H21); 2.38 (m, 1H, H4β); 3.06 (m, 1H, H3); 3.35 (s, 3H, OMe); 5.01 (dd, 1H, *J* 15.3, 9.0 Hz, H23); 5.15 (dd, 1H, *J* 15.0, 8.5 Hz, H22); 5.35 (m, 1H, H6).

(20*S*)-20-Formyl-6β-methoxy-3α,5-cyclo-5α-pregnane (4). Stigmasterol *i*-methyl ether **(2)** (14.32 g, 33.5 mmol) was dissolved in dichloromethane (180 mL) and pyridine (6.8 mL, 83.7 mmol) and chilled to -78 °C. Ozone was bubbled *via* a sparger into the stirred solution for 74 min after which oxygen was bubbled in for 4 min, followed by

nitrogen for 40 min. Zinc (26.31 g, 402.4 mmol) and glacial acetic acid (25 mL) were added and the cold bath removed. The suspension was stirred at room temperature under nitrogen for 3 h. The reaction mixture was filtered through Celite, which was washed thoroughly with dichloromethane. The filtrate was extracted successively with brine, saturated aqueous sodium bicarbonate and brine, dried over anhydrous magnesium sulfate, filtered and concentrated to give the crude product **(4)** as a pale-yellow oil containing a small amount of a suspended white solid (12.73 g, >100%). The crude product was used directly in the next synthesis step. ^{1}H NMR (500 MHz, base-washed $CDCl_3$): δ 0.44 (m, 1H, H4α); 0.65 (m, 1H, H4β); 0.76 (s, 3H, H18); 1.03 (s, 3H, H19); 1.12 (d, 3H, *J* 7.0 Hz, H21); 2.37 (m, 1H, H20); 2.78 (brt, 1H, *J* 2.5 Hz, H6α); 3.33 (s, 3H, OMe); 9.58 (d, 1H, *J* 3.5 Hz, CHO). ^{13}C NMR (500 MHz, base-washed $CDCl_3$): δ 12.74 (C18); 13.23 (C4); 13.55 (C21); 19.42 (C19); 21.59 (C3); 22.83 (C11); 24.68 (C15); 25.07 (C2); 27.27 (C16); 30.64 (C8); 33.49 (C1); 35.20 (C7); 35.34 (C5); 40.08 (C12); 43.52 (C10 or 13); 43.55 (C13 or C10); 48.15 (C9); 49.68 (C20); 51.31 (C17); 55.88 (OMe); 56.72 (C14); 82.41 (C6); 205.41 (CHO).



Scheme 2: Synthesis of campesterol **(12)** from (22*R*)-6β-methoxy-3α,5-cyclo-26,27-dinor-5α-cholest-23-yn-22-ol **(5)**.

(22*R*)-6β-Methoxy-3α,5-cyclo-26,27-dinor-5α-cholest-23-yn-22-ol (5) and (22*S*)-6β-Methoxy-3α,5-cyclo-26,27-dinor-5α-cholest-23-yn-22-ol (6). A solution of crude

aldehyde **(4)** (12.73 g, 36.9 mmol) in anhydrous tetrahydrofuran (120 mL) was added to a solution of 1-propynylmagnesium bromide (0.5 M in THF, 148 mL) at 0 °C under nitrogen. The mixture was stirred at 0 °C for 1 h after which time the reaction mixture was quenched by slow addition of saturated aqueous ammonium chloride (120 mL). The suspension was poured into water and extracted three times with ether. The combined organic phases were washed with brine, dried over magnesium sulfate, filtered and concentrated to give a crude mixture of the 22*R* product **(5)** and the 22*S* product **(6)** as a cloudy brown oil (11.86 g, 82%). The crude mixture was subjected to flash column chromatography (silica) eluting with 100% hexane then 95:5 hexane/ethyl acetate to give pure **(5)** (2.28 g, 15%) and pure **(6) (**1.00 g, 7%) along with 1.486 g of an overlapping 1:4 mixture of **(5)** and **(6)**.

This mixture was further separated by flash column chromatography (alumina) with elution with 100% hexane, then 9:1 hexane/ethyl acetate to obtain a further crop of pure **(5)** (0.08 g) and pure **(6) (**0.90 g). These second crops were combined with the earlier pure fractions to give a total of 2.36 g (17%) of **(5)** and a total of 1.90 g (13%) of **(6)**. ^{1}H NMR (500 MHz, base-washed $CDCl_3$): **(5)** δ 0.43 (m, 1H, H4α); 0.65 (m, 1H, H4β); 0.73 (s, 3H, H18); 1.02 (s, 3H, H19); 1.11 (d, 3H, *J* 6.5 Hz, H21); 1.85 (d, 3H, *J* 2.0 Hz, H25); 2.77 (brt, 1H, *J* 2.5 Hz, H6α); 3.32 (s, 3H, OMe); 4.41 (m, 1H, H22). **(6)** δ 0.43 (m, 1H, H4α); 0.65 (m, 1H, H4β); 0.74 (s, 3H, H18); 1.02 (s, 3H, H19); 1.03 (d, 3H, *J* 7.0 Hz, H21); 1.85 (d, 3H, *J* 2.0 Hz, H25); 2.77 (brt, 1H, *J* 2.5 Hz, H6α); 3.32 (s, 3H, OMe); 4.41 (m, 1H, H22). ^{13}C NMR (500 MHz, base-washed $CDCl_3$): **(5)** δ 3.71 (C25); 12.35 (C18); 13.20 (C4); 13.28 (C21); 19.43 (C19); 21.66 (C3); 22.89 (C11); 24.32 (C15); 25.10 (C2); 27.80 (C16); 30.69 (C8); 33.49 (C1); 35.15 (C7); 35.42 (C5); 40.17 (C12); 42.48 (C20); 42.82 (C10 or 13); 43.52 (C13 or C10); 48.08 (C9); 52.01 (C17); 56.43 (OMe); 56.70 (C14); 65.86 (C22); 80.56 (C23); 81.30 (C24); 82.53 (C6). **(6)** δ 3.80 (C25); 12.60 (C18); 12.81 (C4); 13.22 (C21); 19.43 (C19); 21.63 (C3); 22.92 (C11); 24.41 (C15); 25.11 (C2); 27.64 (C16); 30.67 (C8); 33.51 (C1); 35.17 (C7); 35.41 (C5); 40.27 (C12); 42.35 (C20); 43.10 (C10 or 13); 45.51 (C13 or C10); 48.17 (C9); 53.12 (C17); 56.22 (C14); 56.71 (OMe); 65.98 (C22); 77.91 (C23); 82.14 (C24); 82.50 (C6).

(22*S*,23*Z*)-6β-Methoxy-3α,5-cyclo-26,27-dinor-5α-cholest-23-en-22-ol (7). A suspension of (22*R*)-6β-methoxy-3α,5-cyclo-26,27-dinor-5α-cholest-23-yn-22-ol **(5)** (3.24

g, 8.42 mmol), 5% palladium on calcium carbonate poisoned with lead (0.66 g) and quinoline (3.28 mL, 27.8 mmol) in ethyl acetate (180 mL) was hydrogenated for 3 h after which it was filtered through Celite, washing through with ethyl acetate. The filtrate was washed successively with 2 M hydrochloric acid, saturated aqueous sodium bicarbonate, water and brine, dried over anhydrous magnesium sulfate, filtered and concentrated to give **(7)** (3.18 g, 98%) as a pale-yellow oil. The product was used as crude starting material in the next step. ^{1}H NMR (500 MHz, base-washed $CDCl_3$): δ 0.43 (m, 1H, H4α); 0.65 (m, 1H, H4β); 0.73 (s, 3H, H18); 0.96 (d, 3H, *J* 6.0 Hz, H21); 1.02 (s, 3H, H19); 1.66 (dd, 3H, *J* 6.5, 1.0 Hz, H25); 2.78 (brt, 1H, *J* 2.5 Hz, H6α); 3.33 (s, 3H, OMe); 4.58 (m, 1H, H22); 5.53 (m, 2H, H23, H24).

(22*E*,24*R*)-Ethyl-6β-methoxy-24-methyl-3*α*,5-cyclo-5*α*-cholest-22-en-26-oate (8). Triethylorthopropionate (8.0 mL, 39.8 mmol) was added to a stirred solution of **(7)** (3.24 g, 8.39 mmol) in dry xylenes (110 mL) under nitrogen. Propionic acid (0.37 mL, 4.96 mmol) was then added. The solution was stirred at reflux under nitrogen for 1 h, cooled to room temperature and then poured into water. The mixture was extracted three times with ethyl acetate. The combined organic phases were washed with water and brine, dried over anhydrous magnesium sulfate, filtered and concentrated to give an epimeric mixture **(8a)** and **(8b)** (4.36 g, >100%) as a pale-yellow oil, which became a sticky white solid on further drying under high vacuum. The crude product contained small amounts of triethyl-orthopropionate and xylenes and was used without further purification. ^{1}H NMR (500 MHz, base-washed $CDCl_3$): δ 0.43 (m, 1H, H4α); 0.65 (m, 1H, H4β); 0.71, 0.72 (s x 2, 3H, H18b, H18a); 0.95, 0.96 (d x 2, 3H, *J* 7.0 Hz, H24^{1}b, H24^{1}a); 0.98, 0.99 (d x 2, 3H, J 7.0 Hz, H21b, H21a); 1.02 (s, 3H, H19); 1.07, 1.08 (d x 2, 3H, *J* 7.0 Hz, H26a, H26b); 1.26 (t, 3H, *J* 7.0 Hz, Me of OEt); 2.77 (brs, 1H, H6α); 3.32 (s, 3H, OMe); 4.12 (m, 2H, CH_2 of OEt); 5.08 (dd, 1H, *J* 15.3, 8.5 Hz, H22 or 23); 5.25 (dd, 1H, *J* 15.3, 8.5 Hz, H23 or 22). ^{13}C NMR (500 MHz, base-washed $CDCl_3$): δ 12.57 (C18); 13.21 (C4); 14.40 (Me-OEt (b)); 14.43 (Me-OEt (a)); 15.26 (C26); 17.40 (C24^{1}); 19.43 (C19a); 19.53 (C19b); 20.83 (C21b); 20.94 (C21a); 21.61 (C3); 22.89 (C11); 24.29 (C15b); 24.35 (C15a); 25.10 (C2); 28.77 (C16b); 29.00 (C16a); 30.61 (C8); 33.48 (C1); 35.20 (C7); 35.39 (C5); 39.57 (C24); 40.30 (C12); 40.38 (C20a); 40.50 (C20b); 42.84 (C13); 43.53 (C10); 45.32 (C25b); 45.70 (C25a); 48.17 (C9); 56.04 (C17); 56.72 (OMe and C14); 60.15 (CH_2 of OEt (b)); 60.19 (CH_2 of OEt (a); 82.54

(C6); 130.78 (C22a); 130.52 (C22b); 137.15 (C23b); 138.00 (C23a); 176.09 (C=O of CO_2Et (b)), 176.57 (C=O of CO_2Et (a)).

(24*R*)-Ethyl-6β-methoxy-24-methyl-3α,5-cyclo-5α-cholestan-26-oate (9). A solution of the epimeric mixture **(8a)** and **(8b)** (6.75 g, 14.3 mmol) and 10% palladium on carbon (0.82 g) in ethyl acetate (250 mL) was hydrogenated for 20 h after which it was filtered through Celite and washed through with ethyl acetate. The filtrate was concentrated to give crude epimers **(9a)** and **(9b)** (8.11 g, >100%) as a colourless oil. The crude reaction product was purified by flash column chromatography (silica) eluting with 100% hexane, followed by 100:1, 98:5 and 95:5 hexane/ethyl acetate to give the epimers **(9a)** and **(9b)** as a colourless oil (6.53 g, 96%). ^{1}H NMR (500 MHz, base-washed $CDCl_3$): δ 0.43 (m, 1H, H4α); 0.65 (m, 1H, H4β); 0.71 (s, 3H, H18); 0.83, 0.88 (d x 2, 3H x 2, *J* 6.5 Hz, H24^{1}b, H24^{1}a); 0.89 (d, 3H, *J* 7.0 Hz, H21); 1.02 (s, 3H, H19); 1.06, 1.10, (d x 2, 3H, *J* 7.0 Hz, H26b, H26a); 1.25, 1.26 (t x 2, 3H, *J* 7.0 Hz, Me-OEt (a, b)); 2.32 (m, 1H, H24 or H25); 2.77 (brs, 1H, H6α); 3.32 (s, 3H, OMe); 4.12 (m, 2H, CH_2 of OEt). ^{13}C NMR (400 MHz, base-washed $CDCl_3$): δ 12.41 (C18a); 12.79 (C26b); 13.22 (C4); 14.00 (C26a); 14.47 (Me-OEt); 15.80 (C24^{1}b); 17.32 (C24^{1}a); 18.73 (C21a); 18.78 (C21b); 19.44 (C19); 21.67 (C3); 22.93 (C11); 24.33 (C15); 25.13 (C2); 28.47 (C16**)**; 29.68 (C23a); 30.64 (C8); 31.27 (C23b); 33.14 (C22); 33.47 (C1b); 33.52 (C1a); 35.21 (C7); 35.47 (C5); 35.85 (C20); 36.223 (C24); 40.45 (C12); 42.95 (C13); 43.55 (C10); 44.87 (C25b); 45.13 (C25a); 48.19 (C9); 56.36 (C17b); 56.44 (C17a); 56.70 (OMe and C14); 60.08 (CH_2 of OEt (a)); 60.14 (CH_2 of OEt (b)); 82.60 (C6); 176.53 (C=O (a)); 176.74 (C=O (b)).

(24*R*)-6β-Methoxy-24-methyl-3α,5-cyclo-5α-cholestan-26-ol (10). The synthetic epimers **(9)** (3.45 g, 7.29 mmol) in anhydrous tetrahydrofuran (50 mL) was added to a stirred suspension of lithium aluminium hydride (0.66 g, 20.6 mmol) in anhydrous tetrahydrofuran (40 mL) under nitrogen. The suspension was stirred at room temperature for 2 h. A mixture of ether/water (90 mL/20 mL) was added slowly, followed by 2 M hydrochloric acid (100 mL), with vigorous stirring. The mixture was poured into water and extracted three times with ether. The organic phases were combined and washed with water and brine, dried over anhydrous magnesium sulfate, filtered and concentrated to give crude **(10)** (3.07 g, 98%) as a pale-yellow oil. This crude product was purified by flash column chromatography (silica) with elution with

100% hexane, then 95:5 and 9:1 hexane/ethyl acetate to give a mixture of epimeric alcohols **(10)** as a viscous colourless oil (4.30 g, 72%). ^{1}H NMR (500 MHz, base-washed $CDCl_3$): δ 0.43 (m, 1H, H4α); 0.65 (m, 1H, H4β); 0.71 (s, 3H) H18; 0.77, 0.82 (d x 2, 3H, *J* 7.0 Hz, H26b, H26a); 0.86, 0.91 (d, 3H, *J* 7.0 Hz, H24^{1} (a, b)); 0.90 (d, 3H, *J* 7.0 Hz, H21); 1.02 (s, 3H, H19); 2.77 (brs, 1H, H6α); 3.32 (s, 3H, OMe); 3.45 (m, 1H, CH_2-OH (Hα-a or Hβ-a)); 3.57, 3.63 (m x 2, 1H, CH_2-OH (Hα-b or Hβ-b, Hβ-a or Hα-a)). ^{13}C NMR (400MHz, base-washed $CDCl_3$): δ 11.75 (C24^{1}b); 12.42 (C18); 13.22 (C4); 13.78 (C24^{1}a); 14.53 (C26b); 17.09 (C26); 18.79 (C21a); 18.88 (C21b); 19.44 (C19); 21.68 (C3); 22.94 (C11); 24.34 (C15); 25.13 (C2); 28.49 (C16); 29.20 (C23a); 30.65 (C8); 31.41 (C23b); 33.52 (C1); 33.87 (C22a); 33.89 (C22b); 34.89 (C24); 35.21 (C7); 35.48 (C5); 36.00 (C20a); 36.04 (C20b); 40.20 (C25b); 40.47 (C12): 41.17 (C25a); 42.95 (C13); 43.55 (C10); 48.20 (C9); 56.41 (C17b); 56.48 (C17a); 56.70 (OMe and C14); 66.41 (CH_2-OH (a)); 67.02 (CH_2-OH (b)); 82.61 (C6).

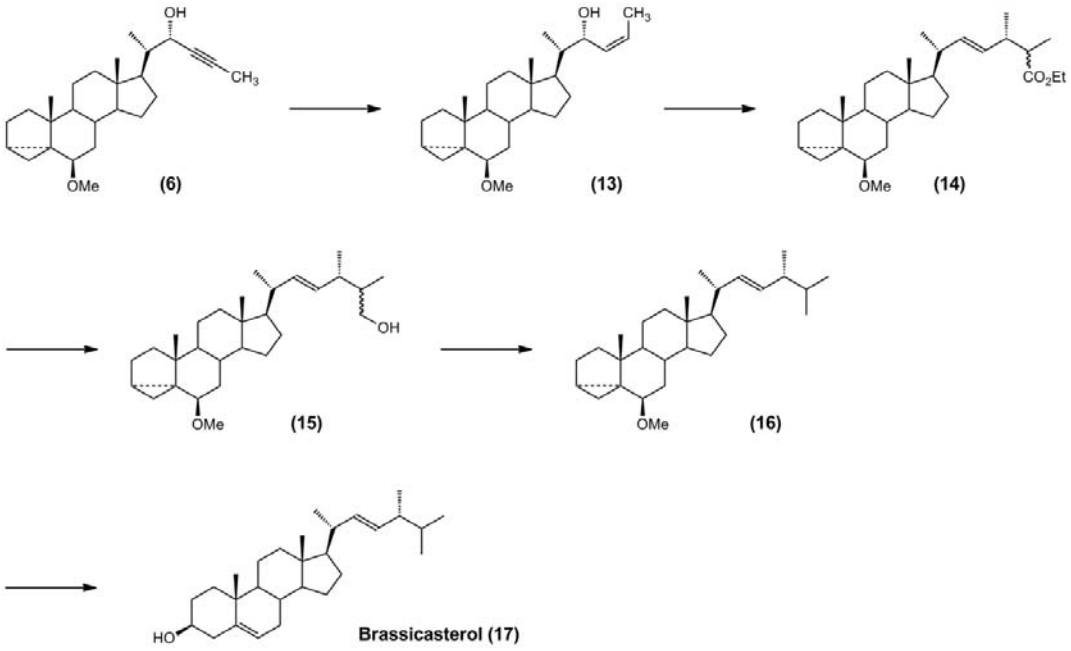
(24*R*)-6β-Methoxy-24-methyl-3α,5-cyclo-5α-cholestane (campesterol *i*-methyl ether) (11). Mesyl chloride (2.32 mL, 30.0 mmol) was added to a stirred solution of **(10)** (4.30 g, 9.98 mmol) in pyridine (45 mL). The mixture was stirred at room temperature (reaction flask equipped with calcium chloride drying tube) for 2 h and poured into water. The mixture was extracted three times with ether. The combined organic phases were washed with saturated aqueous sodium bicarbonate, water and brine, dried over magnesium sulfate, filtered and concentrated to give the intermediate mesylate as a pale-yellow oil. which became a foamy white solid (4.89 g, 96%) upon further evaporation under vacuum. The crude mesylate was purified by flash column chromatography (silica) eluting with 100% hexane, followed by 95:5 then 8:2 hexane/ethyl acetate to give the intermediate mesylate as a sticky white solid on further drying (4.77 g, 94%). ^{1}H NMR (500 MHz, base-washed $CDCl_3$): δ 0.43 (m, 1H, H4α); 0.65 (m, 1H, H4β); 0.71 (s, 3H, H18); 0.89 (m, 6H, H26, H28); 0.96 (d, 3H *J* 7.0 Hz, H21); 1.02 (s, 3H, H19); 2.77 (brs, 1H, H6α); 3.004, 3.006 (s x 2, 3H, Me-OMs (a, b)); 3.32 (s, 3H, OMe); 4.04 (m, 1H, CH_2-OH α or β, (a, b)); 4.15, 4.20 (m x 2, 1H, (CH_2-OH) β or α, (a, b)). ^{13}C NMR (500 MHz, base-washed $CDCl_3$): δ 12.42 (C18); 13.22 (C4); 15.55 (C24^{1}); 18.42 (C26 or C27); 18.40 (C21); 19.45 (C19); 20.36 (C27 or C26); 21.68 (C3); 22.95 (C11); 24.35 (C15); 25.14 (C2); 28.49 (C16); 30.49 (C23); 30.66 (C8); 32.58 (C25); 33.53 (C1); 33.86 (C22); 35.22 (C7); 35.49 (C5); 30.07 (C20); 39.01

(C24); 40.48 (C12); 42.95 (C13 or C10); 43.56 (C10 or C13); 48.22 (C9); 56.37 (C17); 56.71 (C14 and OMe); 82.63 (C6). ESI-MS: *m/z* 415.2 $[M+H]^+$; 383.4 $[M+H\text{-}MeOH]^+$, 437.3 $[M+Na]^+$.

The purified mesylate was dissolved in anhydrous tetrahydrofuran (55 mL) and the solution added to a stirred suspension of lithium aluminium hydride (0.61 g, 19.1 mmol) in anhydrous tetrahydrofuran (55 mL) under nitrogen. The mixture was stirred at room temperature under nitrogen for 2 h after which more lithium aluminium hydride was added in ~ 2 equivalent portions at hourly intervals as follows: 0.61 g (19.1 mmol); 0.62 g (19.4 mmol); 0.61 g (19.9 mmol); 0.61 g (19.0 mmol). The suspension was then left to stir overnight (19h) after which time a mixture of ether/water (100 mL/10 mL) was added slowly with vigorous stirring, followed by 2M hydrochloric acid (110 mL). The mixture was poured into water and extracted three times with ether. The combined organic phases were washed twice with water and once with brine, dried over anhydrous magnesium sulfate, filtered and concentrated to give **(2)** as a pale-yellow oil, which became a sticky white solid on further drying (4.21 g, > 100%). The crude product was purified by flash column chromatography (silica) eluting with 100% hexane followed by 98:5 then 95:5 hexane/ethyl acetate to give **(11)** (3.90 g, 94%) as a colourless oil which became a sticky white solid on further drying. ^{1}H NMR (500 MHz, base-washed $CDCl_3$): δ 0.43 (m, 1H, H4α); 0.65 (m, 1H, H4β); 0.71 (s, 3H, H18); 0.77 (d, 3H, *J* 6.5 Hz, H26 or H27); 0.80 (d, 3H, *J* 7.0 Hz, H27 or H26); 0.85 (d, 3H, *J* 6.5 Hz, H24^1); 0.90 (d, 3H, *J* 6.5 Hz, H21); 1.02 (s, 3H, H19); 2.77 (brt, 1H, *J* 2.5 Hz, H6α); 3.32 (s, 3H, OMe). ^{13}C NMR (500 MHz, base-washed $CDCl_3$): δ 12.42 (C18); 13.22 (C4); 15.54 (C24^1); 18.42 (C26 or C27); 18.84 (C21); 19.45 (C19); 20.36 (C27 or 26); 21.69 (C3); 22.95 (C11); 24.35 (C15); 25.14 (C2); 28.49 (C16); 30.49 (C23); 30.66 (C8); 32.58 (C25); 33.53 (C1); 33.86 (C22); 35.22 (C7); 35.49 (C5); 36.07 (C20); 39.01 (C24); 40.48 (C12); 42.95 (C13); 43.56 (C10); 48.22 (C9); 56.37 (C17); 56.71 (OMe and C14); 82.63 (C6).

Campesterol (12). *p*-Toluenesulfonic acid (0.32 g, 1.69 mmol) was added to a suspension of **(11)** (3.52 g, 8.49 mmol) in dioxane/water (180 mL/60 mL). The mixture was stirred at reflux under nitrogen for 1 h and cooled to room temperature. The suspension was poured into water and extracted three times with ether. The combined organic phases were washed with water and brine, dried over anhydrous magnesium

sulfate, filtered and concentrated to a white solid (2.95 g, 87%). This crude product was purified by flash column chromatography (silica), eluting with 100% hexane then 95:5 followed by 9:1 then 8:2 hexane/ethyl acetate to return pure campesterol **(12)** as a white solid (2.94 g, 87%), m.p. 161.0-161.8 °C (157.5-159.5 °C [4]). ^{1}H NMR (500 MHz, base-washed $CDCl_3$): δ 0.68 (s, 3H, H18); 0.77 (d, 3H, *J* 6.5 Hz, H26 or H27); 0.80 (d, 3H, *J* 7.0 Hz, H27 or H26); 0.85 (d, 3H, *J* 7.0 Hz, H24[1]); 0.91 (d, 3H, *J* 6.5 Hz, H21); 1.01 (s, 3H, H19); 3.52 (m, 1H, H3); 5.35 (m, 1H, *J* 3.5 Hz, H6). ^{13}C NMR (500 MHz, base-washed $CDCl_3$): δ 12.01 (C18); 15.51 (C24[1]); 18.39 (C26 or C27); 18.84 (C21); 19.55 (C19); 20.35 (C27 or C26); 21.22 (C11); 24.44 (C15); 28.38 (C16); 30.39 (C23); 31.80 (C2); 32.05 (C7 and C8); 32.56 (C25); 33.84 (C22); 36.02 (C20); 36.64 (C10); 37.39 (C1); 38.96 (C24); 39.91 (C12); 42.45 (C4 and C13); 50.25 (C9); 56.23 (C17); 56.90 (C14); 71.96 (C3); 121.86 (C6); 140.89 (C5). ESI-MS: *m/z* 383.4 $[M+H-H_2O]^+$.



Scheme 3: Synthesis of brassicasterol **(17)** from (22*S*)-6β-methoxy-3α,5-cyclo-26,27-dinor-5α-cholest-23-yn-22-ol **(6)**.

(22*R*,23*Z*)-6β-Methoxy-3α,5-cyclo-26,27-dinor-5α-cholest-23-en-22-ol (13). A suspension of (22*S*)-6*β*-methoxy-3*α*,5-cyclo-26,27-dinor-5*α*-cholest-23-yn-22-ol **(6)** (1.90 g, 4.93 mmol), 5% palladium on calcium carbonate poisoned with lead (0.39 g) and quinoline (1.92 mL, 16.3 mmol) in ethyl acetate (120 mL) was hydrogenated for 3 h after which it was filtered through Celite, washing through with ethyl acetate. The

filtrate was washed successively with 2 M hydrochloric acid, saturated sodium bicarbonate, water and brine, dried over anhydrous magnesium sulfate, filtered and concentrated to give **(13)** (1.88 g, 99%) as a foamy white solid, which was used directly in the next step. ^{1}H NMR (500 MHz, base-washed $CDCl_3$): δ 0.43 (m, 1H, H4α); 0.65 (m, 1H, H4β); 0.75 (s, 3H, H18); 1.01 (d, 3H, *J* 7.0 Hz, H21); 1.02 (s, 3H, H19); 1.73 (dd, 3H, *J* 7.0, 1.5 Hz, H25); 2.76 (brt, 1H, *J* 2.5 Hz, H6α); 3.32 (s, 3H, OMe); 4.52 (m, 1H, H22); 5.49 (ddq, 1H, *J* 11.6, 9.5, 1.5 Hz, H23); 5.68 (m, 1H, H24).

(22*E*,24*S*)-Ethyl-6β-methoxy-24-methyl-3α,5-cyclo-5α-cholest-22-en-26-oate (14). Triethyl-orthopropionate (4.15 mL, 20.6 mmol) was added to a stirred solution of **(13)** (1.66 g, 4.39 mmol) in dry xylenes (50 mL) under nitrogen. Propionic acid (0.20 mL, 2.63 mmol) was added. The solution was stirred at reflux under nitrogen for 1h and cooled to room temperature before pouring into water. The mixture was extracted three times with ethyl acetate. The combined organic phases were washed with water and brine, dried over anhydrous magnesium sulfate, filtered and concentrated to give **(14)** as an epimeric mixture **(14a)** and **(14b)** (2.08 g, ~100%) as a pale-yellow oil which became a sticky white solid on further drying under high vacuum. The crude product contained small amounts of triethylorthopropionate and xylenes and was used without further purification in the next step. ^{1}H NMR (500 MHz, base-washed $CDCl_3$): δ 0.43 (m, 1H, H4α); 0.65 (m, 1H, H4β); 0.71, 0.72 (s x 2, 3H, H18b, H18a); 0.96, 0.97 (d x 2, 3H, *J* 6.8 Hz, H24^{1}b, H24^{1}a); 0.98, 0.99 (d x 2, 3H, *J* 6.8 Hz, H21b, H21a); 1.02 (s, 3H, H19); 1.07, 1.08 (d x 2, 2H x1, 1H x 1, *J* 6.8 Hz, H26a, H26b); 1.26 (t, 3H, *J* 7.0 Hz, Me of OEt); 2.77 (brt, 1H, *J* 2.5 Hz, H6α); 3.32 (s, 3H, OMe); 4.12 (m x 2, 2H, CH_2-CO_2Et); 5.10 (dd, 1H, *J* 15.0, 8.0 Hz, H22 or 23); 5.25 (dd, 1H, *J* 15.0, 8.5 Hz, H23 or 22). ^{13}C NMR (400 MHz, base-washed $CDCl_3$): δ 12.62 (C18); 13.24 (C4); 14.41 (Me-OEt (b)); 14.45 (Me-OEt (a)); 14.82 (C26); 17.22 (C24^{1}); 19.30 (C19b); 19.44 (C19a); 20.89 (C21b); 20.94 (C21a); 21.65 C3); 22.91 (C11); 24.32 (C15); 25.13 (C2); 28.70 (C16); 30.65 (C8); 33.53 (C1); 35.24 (C7); 35.40 (C5); 39.74 (C24); 40.22 (C20b); 40.24 (C20a); 40.34 (C12); 42.89 (C13); 43.57 (C10); 45.48 (C25b); 45.64 (C25a); 48.24 (C9); 56.15 (C17b); 56.17 (C17a); 56.72 (OMe or C14); 56.75 (C14 or OMe); 60.08 (CH_2 of OEt (b)), 60.19 (CH_2 of OEt (a)); 82.59 (C6); 129.82 (C22a); 130.63 (C22b); 137.01 (C23b); 137.88 (C23a); 176.47 (C=O of CO_2Et).

(22*E*,24*S*)-6β-Methoxy-24-methyl-3α,5-cyclo-5α-cholest-22-en-26-ol (15). A solution

of **(14)** (4.36 g, 9.25 mmol) in anhydrous tetrahydrofuran (70 mL) was added to a stirred suspension of lithium aluminium hydride (0.77 g, 24.0 mmol) in anhydrous tetrahydro-furan (50 mL) under nitrogen. The suspension was stirred at room temperature under nitrogen for 2 h after which a mixture of ether/water (13 mL/3 mL) was added slowly, followed by 2 M hydrochloric acid (16 mL), with vigorous stirring. The mixture was poured into water and extracted three times with ether. The combined organic phases were washed with water and brine, dried over anhydrous magnesium sulfate, filtered and concentrated to give **(15)** as an epimeric mixture **(15a)** and **(15b)** (3.90 g, 98%) as a pale-yellow oil which was used directly in the next step. ^{1}H NMR (400 MHz, base-washed $CDCl_3$): δ 0.43 (m, 1H, H4α); 0.65 (m, 1H, H4β); 0.73 (s, 3H, H18); 0.85, 0.89 (d x 2, 2H x 1, 1H x 1, *J* 6.8 Hz, H26a and H26b); 0.95, 0.98 (d x 2, 1H x 1, 2H x 1, *J* 6.8 Hz, H24^{1}b and H24^{1}a); 1.01 (d, 3H, *J* 6.4 Hz, H21); 1.02 (s, 3H, H19); 2.77 (brt, 1H, *J* 2.6 Hz, H6α); 3.32 (s, 3H, OMe); 3.45 (m, 1H, CH_2-OH Hα or Hβ); 3.57 (m, 1H, CH_2-OH Hβ or Hα); 5.24 (m, 2H, H22, H23). ^{13}C NMR (400 MHz, base-washed $CDCl_3$): δ 12.62 (C18); 12.85 (C26a); 13.24 (C4); 14.24 (C26b); 17.56 (C24^{1}b); 18.54 (C24^{1}a); 19.44 (C19); 20.94 (C21b) 21.04 (C21b); 21.65 (C3); 22.91 (C11); 24.35 (C15); 25.13 (C2); 28.80 (C16 a and b); 30.65 (C8); 33.53 (C1); 35.25 (C7); 35.45 (C5); 38.27 (C24a); 39.43 (C24b); 40.25 (C20); 40.35 (C12); 40.96 (C25b), 41.12 (C25a); 42.89 (C13); 43.57 (C10); 48.23 (C9); 56.24 (C17a); 56.25 (C17b); 56.72 (C14); 56.75 (OMe); 67.05 (CH_2-OH (a)); 67.18 (CH_2-OH (b)); 82.59 (C6); 110.26 (C23a); 132.30 (C23b); 136.43 (C22b); 137.19 (C22a).

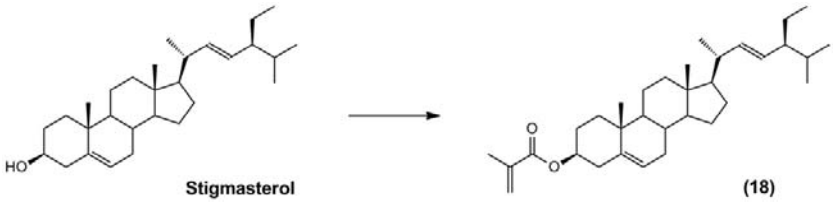
(22*E*,24*R*)-6β-Methoxy-24-methyl-3*α*,5-cyclo-5*α*-cholest-22-ene (brassicasterol *i*-methyl ether) (16). Mesyl chloride (2.75 mL, 35.5 mmol) was added to a stirred solution of **(15)** (5.06 g, 11.8 mmol) in pyridine (50 mL). The mixture was stirred at room temperature (reaction flask equipped with calcium chloride drying tube) for 2 h and poured into water. The mixture was extracted three times with ether. The combined organic phases were washed successively with saturated aqueous sodium bicarbonate, water and brine, dried over anhydrous magnesium sulfate, filtered and concentrated to give the epimeric mesylate intermediate (a) and (b) as a pale yellow (6.26 g, >100%). ^{1}H NMR (500 MHz, base-washed $CDCl_3$): δ 0.43 (m, 1H, H4α); 0.65 (m, 1H, H4β); 0.72 (s, 3H, H18); 0.90 (d, 3H, *J* 7.0 Hz, H24^{1}); 1.00 (d, 3H, *J* 7.0 Hz, H26); 1.01 (d, 3H, *J* 6.5 Hz, H21); 1.02 (s, 3H, H19); 2.77 (brt, 1H, *J* 2.5 Hz, H6α); 2.99, 3.00 (s x 2,

3H, Me-OMs (α or β, b and a)); 3.32 (s, 3H, OMe); 4.00, 4.11, 4,20 (m x 3, 2H, CH_2-OMs (β or α, a and b)); 5.17 (m, 1H, H22 or H23); 5.25 (m, 1H, H23 or H22).

The crude mesylate was dissolved in anhydrous tetrahydrofuran (120 mL) and added to a stirred suspension of lithium aluminium hydride (0.80 g, 25.0 mmol) in anhydrous tetrahydrofuran (130 mL) under nitrogen. The mixture was stirred at room temperature under nitrogen for 2 h after which time more lithium aluminium hydride additions were made in portions at hourly intervals as follows: 0.77 g (24.1 mmol); 0.77 g (24.1 mmol); 0.77 g (24.0 mmol); 0.75 g (23.4 mmol). The suspension was then left to stir overnight (16 h), then a mixture of ether/water (120 mL/10 mL) was added slowly with vigorous stirring, followed by 1 M hydrochloric acid (130 mL). The mixture was poured into water and extracted three times with ether. The combined organic phases were washed twice with water and once with brine, dried over anhydrous magnesium sulfate, filtered and concentrated to give **(16)** as a yellow oil (4.94 g, ~ 100%). The crude product, containing a small amount of tetrahydrofuran, was used without further purification. ^{1}H NMR (500 MHz, base-washed $CDCl_3$): δ 0.43 (m, 1H, H4α); 0.65 (m, 1H, H4β); 0.72 (s, 3H, H18); 0.90 (d, 3H, *J* 7.0 Hz, H24^1); 1.00 (d, 3H, *J* 7.0 Hz, H26); 1.01 (d, 3H, *J* 6.5 Hz, H21); 1.02 (s, 3H, H19); 2.77 (brt, 1H, *J* 2.5 Hz, H6α); 2.99, 3.00 (s x 2, 3H, Me-OMs (α or β, b and a)); 3.32 (s, 3H, OMe); 4.00, 4.11, 4,20 (m x 3, 2H, CH_2-OMs (β or α, a and b)); 5.17 (m, 1H, H22 or H23); 5.25 (m, 1H, H23 or H22).

Brassicasterol (17). *p*-Toluenesulfonic acid (0.467 g, 2.40 mmol) was added to a suspension of **(16)** (4.88 g, 11.8 mmol) in dioxane/water (270 mL/90 mL). The mixture was stirred at reflux under nitrogen for 1h and cooled to room temperature. The suspension was poured into water and extracted three times with ether. The combined organic phases were washed with water and brine, dried over magnesium sulfate, filtered and concentrated to a white solid (4.60 g, 98%). The crude product was purified by flash column chromatography (silica), eluting with 100% hexane then 95:5 followed by 85:15 hexane/ethyl acetate. This gave pure brassicasterol **(17)** as a white solid (3.70 g, 73%), m.p. 148.9 – 150 °C (lit. 145-147 °C [4]). ^{1}H NMR (500 MHz, base-washed $CDCl_3$): δ 0.69 (s, 3H, H18); 0.82 (d, 3H, *J* 7.0 Hz, H26 or H27); 0.83 (d, 3H, *J* 7.0 Hz, H27 or H26); 0.91 (d, 3H, *J* 7.0 Hz, H24^1); 1.00 (d, 3H, *J* 6.0 Hz, H21); 1.01 (s, 3H, H19); 3.52 (m, 1H, H3); 5.18 (m, 2H, H22 and H23); 5.34 (brd, 1H, *J* 3.5 Hz, H6). ^{13}C NMR (500 MHz, base-washed $CDCl_3$): δ 12.22 (C18); 17.77 (C24^1); 19.55 (C19);

19.79 (C26 or 27); 20.11 (C27 or 26); 21.10 (C21); 21.21 (C11); 24.42 (C15); 28.69 (C16); 31.80 (C2); 32.04 (C7, C8); 33.24 (C25); 36.66 (C10); 37.39 (C1); 39.81 (C12); 40.32 (C20); 42.37 (C4); 42.44 (C13); 42.94 (C24); 50.29 (C9); 56.14 (C17); 56.98 (C14); 71.96 (C3); 121.86 (C6); 131.85 (C23); 135.98 (C22); 140.89 (C5). ESI-MS: *m/z* 381.19 $[M+H-H_2O]^+$.



Stigmasterol (18)

Scheme 4: Synthesis of the polymerizable template stigmasteryl methacrylate **(18)** from stigmasterol

Stigmasteryl methacrylate (18). 4-Dimethylaminopyridine (80 mg, 0.654 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (1.35 g, 6.54 mmol) were dissolved in anhydrous dichloromethane (25 mL). Methacrylic acid (555 μL, 563 mg, 6.54 mmol) was added, and within 3 minutes a solid started to settle out of solution. After a further 5 minutes, stigmasterol (2.06 g, 5.00 mmol) was added and washed in with further dichloromethane (20 mL). The reaction was left to stir overnight at room temperature. [Note: TLC after 3 h and after 18 h looked similar]. The solid dicyclohexylurea (DCU) byproduct was filtered off and washed in the funnel with further dichloromethane (three times). The combined filtrate was diluted with dichloromethane and successively washed with water, 5% aqueous acetic acid, saturated aqueous sodium bicarbonate, brine and water (twice). The organic phase was then dried over anhydrous sodium sulfate, filtered and concentrated to dryness to give 2.85 g of a white powder. This crude product was purified by flash column chromatography (silica) by gradient elution starting with 100% hexane and finishing with 19:1 hexane/EtOAc to give 1.78 g (74.2%) of stigmasteryl methacrylate **(18)** as a white powder, m.p. 145.2 °C. R_f 0.64

(19:1 hexane/EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.70 (s, 3H, H18), 0.83 (m, 3H x 3, H24[2], H26, H27), 1.02 (d, 3H, *J* = 6.8Hz, H19), 1.04 (s, 3H, H21), 1.94 (s, 3H, Me-methacrylate), 2.37 (m, 2H, H4α, H4β), 4.68 (m, 1H, H3α), 5.12 (dd, 1H, *J* = 8.4, 15.2Hz, H23), 5.17 (dd, 1H, H22), 5.38 (d, 1H, H6), 5.53 (brs, 1H, CH_2-methacrylate a); 6.08 (brs, 1H, CH_2-methacrylate b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.39, 12.59, 18.66, 19.34, 19.70, 21.43, 21.57, (7 x CH_3), 21.38, 24.71, 25.75, 28.13, 29.26, 32.23, 32.26, 36.99, 37.37, 38.48, 39.99, 40.84, 42.57, 50.42, 51.59, 56.30, 57.15, 74.56, 122.97, 125.24, 129.64, 137.23, 138.65, 140.05, 167.20; ESI-MS: *m/z* 503 ($[M+Na]^+$.

^{1}H and ^{13}C NMR of stigmasteryl methacrylate (18). ^{1}H and ^{13}C nuclear magnetic resonance spectra were recorded as solutions in deuterated chloroform ($CDCl_3$), or base-washed $CDCl_3$ using a Bruker Avance 400 or Ultrashield Plus 400, Varian Unity Inova 500, or Avance III 600 spectrometer at 400, 500 and 600 MHz, respectively.

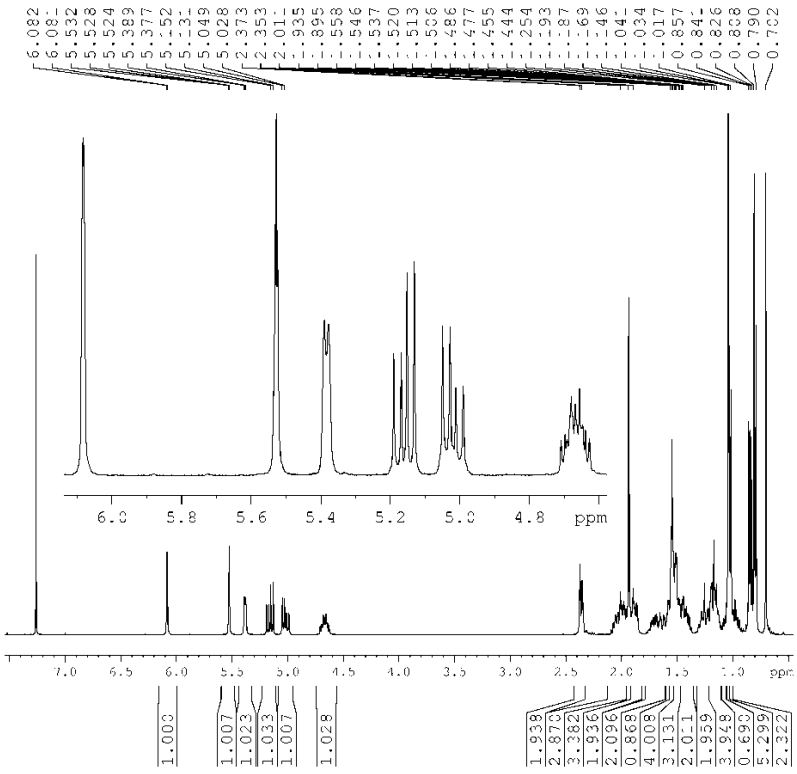


Figure S1: ^{1}H NMR (400 MHz, $CDCl_3$) of stigmasteryl methacrylate (**18**).

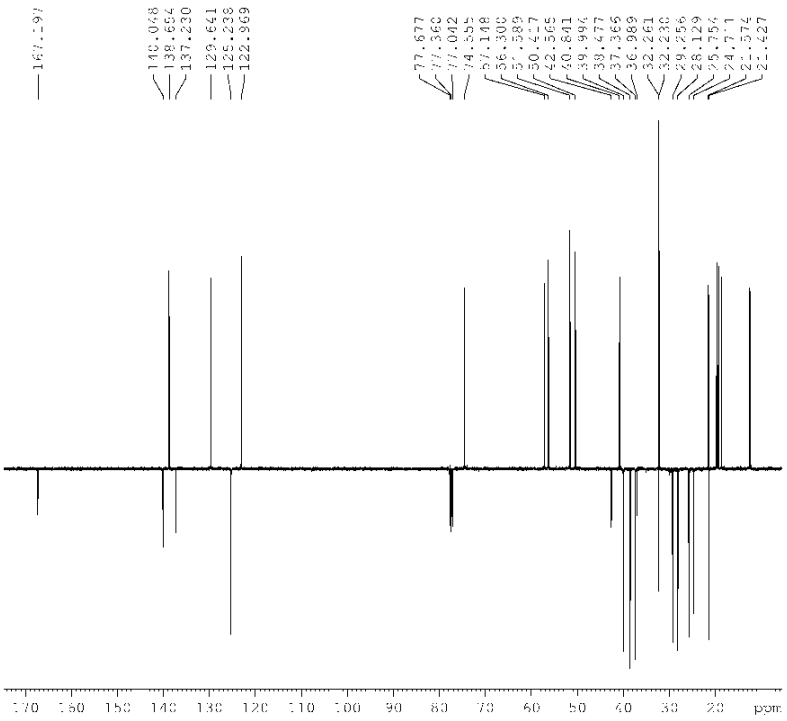


Figure S2: ^{13}C NMR (100 MHz, $CDCl_3$,) of stigmasteryl methacrylate **(18)**.

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