Synthesis of Analogues of Squalene as Potential Anti-Fungal Agents

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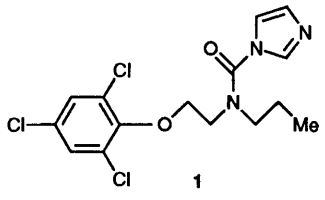
Squalene was selectively degraded to tris-norsqualene, and this was converted into 1-fluoro, 1,1-difluoro, and 2-trimethylsilylmethyl analogues of squalene, and also into 2-cyanonorsqualene. These compounds were designed as potential inhibitors of fungal squalene epoxidase/epoxysqualene cyclase, but biological evaluation using five fungal pathogens revealed that none exhibited more than modest antifungal activity.

Around 30% of potential food production is lost annually due to the ravages caused by insects, weeds and fungi. Numerous effective and relatively safe insecticides and herbicides are available, but the number of broad spectrum antifungal products is limited. This is due in part to the paucity of information available on the biochemical pathways operating in fungi. One area in which some progress has been made is in the control of fungal sterol biosynthesis. Azole-type fungicides, e.g. **1** are



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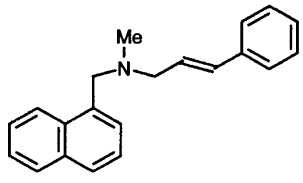


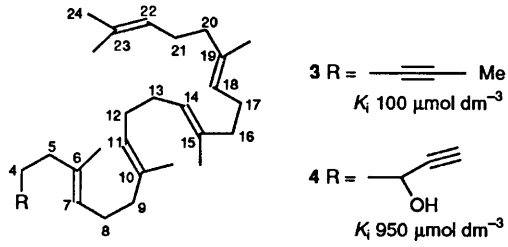
2

believed to exert their effects through inhibition of the cytochrome P_{450} dependent 14-alpha-demethylase,[1] whilst the allylamines, e.g. **2** are believed to inhibit squalene epoxidase.[2] Two key steps on the biosynthetic pathway from squalene to sterols, shown in Fig. 1, can thus be specifically inhibited.

The two enzymes squalene epoxidase and epoxysqualene cyclase are believed to be part of a multi-enzyme complex that catalyses the early stages of this pathway,[3] and a number of squalene analogues have been prepared as potential inhibitors of one or both enzymes. The analogues **3**–**5**[4–6] exhibit a range of potencies against mammalian squalene epoxidase (note K_i values), but their structures reveal little about the mechanism of epoxidation.

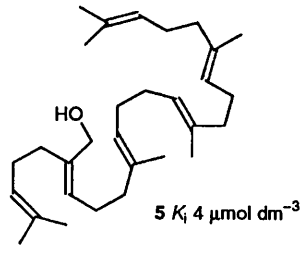
Little is known about the enzyme, save that it requires a flavin cofactor, and it would be interesting to determine the electronic requirements with regard to the terminal double bond of squalene. Our aim was thus to prepare structural analogues of squalene in which the electronic properties of the double bond would be significantly changed. This could have the effect of inhibiting epoxidation, or the resultant epoxide analogue could be less susceptible to the nucleophilic attack envisaged in the process catalysed by the epoxycyclase.†

The overall strategy for our syntheses (a preliminary account of this work has been published)[7] involved selective cleavage of the terminal double bond of squalene and then construction of



3 R = —≡— Me
K_i 100 μmol dm^{-3}

4 R = —≡ OH
K_i 950 μmol dm^{-3}



5 K_i 4 μmol dm^{-3}

the novel analogues *via* Wittig reactions. The selective oxidative cleavage was achieved through a modification of the literature methods[8] and involved initial formation of the terminal epoxide [*N*-bromosuccinimide in wet tetrahydrofuran (THF), then K_2CO_3 in MeOH] followed by reaction with periodic acid. The overall yield of the trisnorsqualene aldehyde **6** was 35% on a 40 g scale. Reaction of the aldehyde with ethoxycarbonylethylidene(triphenyl)phosphorane (in dichloromethane at room temp.) provided the ester **7** and thence the alcohol **8** following reduction with $LiAlH_4$ (in ether at 0 °C) in an overall yield of 90%. The chemical shifts observed for the terminal alkene hydrogen in **7** (6.74 ppm) and **8** (5.42 ppm) were fully consistent with the *trans*-stereochemistry.

Attempted fluorination of the alcohol with diethylaminosulfur trifluoride (DAST) (in dichoromethane at −78 °C) produced a mixture of the desired analogue—1-fluorosqualene **9**—but also the not unexpected rearrangement product **10** (ratio 3:7, yield 45%). The terminal CH_2F group exhibited a 2H NMR signal at δ 4.73 (d, J 48 Hz) whilst the CHF group of compound **10** exhibited a 1H signal at δ 4.78 (dd, J 48.5 Hz). In 1975, Middleton showed that such allylic rearrangement could be minimised if DAST was first treated with dimethylaminotrimethylsilane to form diethylamino(dimethylamino)sulfur difluoride.[9] This strategy was effective and upon reaction of

† During the course of our work, the terminal difluorobis(noranalogue) of squalene was prepared, and shown to have a K_i of 4 μmol dm^{-3} against mammalian squalene epoxidase, thus supporting our strategy. W. R. Moore, G. L. Schatzman, E. T. Jarvi, R. S. Gross and J. R. McCarthy. *J. Am. Chem. Soc.*, 1992, **114**, 360

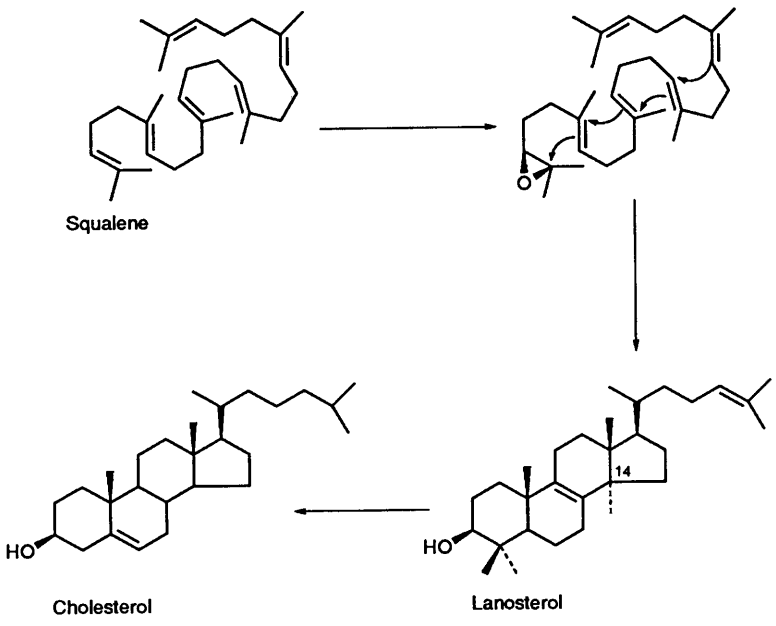
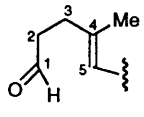


Fig. 1 The biosynthetic pathway from squalene to steroids

alcohol **8** with this reagent (trichlorofluoromethane at −78 °C) the desired fluoro analogue was produced as the sole product in 45% isolated yield.

Oxidation of the alcohol **8** with manganese dioxide provided the aldehyde **11** (95% isolated yield) and treatment of this with neat DAST (40 °C, 48 h) yielded the expected analogue of squalene **12** (45%). The ^{1}H NMR signal for the hydrogen of the CHF_2 group appeared as a triplet at 5.88 ppm (J 57 Hz).



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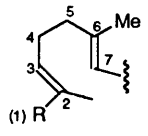
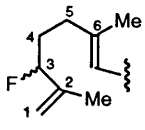
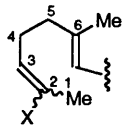


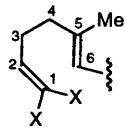
7 R = CO_2Et
8 R = CH_2OH
9 R = CH_2F
11 R = CHO
12 R = CHF_2



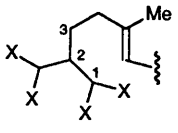
10



13 X = CN
14 X = CH_2SiMe_3



15 X = CN
16 X = CO_2Et



17 X = CO_2Et

The aldehyde **6** was also treated with the ylide cyanoethylidene triphenylphosphorane to produce 2-cyanonorsqualene **13** as a mixture of isomers (E:Z, 72:28) in an isolated yield of 90%. Reaction with the ylide from 1-trimethylsilylpropan-2-ylphosphonium iodide[10] provided the allyl silanes **14** as a mixture of isomers (*ca.* 1:1) in low yield (*ca.* 20%). Several analogues were also prepared using the Knoevenagel reaction, thus reaction of the aldehyde **6** with malononitrile provided the dicyano-bisnorsqualene analogue **15** (46%), while reaction with diethyl malonate yielded the expected analogue **16** (29%) together with the compound **17** (14%). This latter compound is clearly the product of a Michael reaction between diethyl malonate and the Knoevenagel product **16**.

All of these compounds were screened for antifungal activity against five fungal pathogens: *Erysiphe graminis* (powdery mildew of wheat), *Puccinia recondita* (brown rust of wheat), *Septoria nodorum*, *Plasmopara viticola* (grape downy mildew), and *Pyricularia oryzae* (rice blast); but none of them showed any significant activity. In addition, compounds **9**, **12**, **13** and **14** were incubated with a crude squalene epoxidase preparation from the yeast *Saccharomyces cerevisiae*,[11] but none of them showed any inhibitory activity.

These results were rather disappointing, especially since it was anticipated that all of the analogues would possess terminal double bonds with dramatically reduced electron density. The analogue **14** should have exerted a hyperconjugative effect on this double bond. These poor results may be due to a lack of penetration of these highly lipophilic squalene analogues to the active site(s) of the multi-enzyme complex, and experiments are underway to prepare similar analogues of less lipophilic molecules like farnesol and geraniol. In addition, given the potency of many of the known analogues against the mammalian enzymes, we are hoping to evaluate our compounds against these enzymes.

Despite these poor biological results, the methods described in this paper do allow ready access to a range of terminally functionalised analogues of squalene. They may be of more general use for the construction of other similarly functionalised isoprenoids.

Experimental

IR Spectra were recorded on a Perkin-Elmer double grating spectrophotometer; NMR Spectra were recorded with a Perkin-Elmer R34 (220 MHz) instrument, using tetramethylsilane as internal standard; J values are given in Hz. Flash chromatography was performed using Crosfield Sorbsil C60 (40–60 mm); solvents were purified according to standard procedures.[12] All non aqueous reactions were carried out under an atmosphere of

nitrogen. Light petroleum refers to that b.p. 40–60 °C and ether refers to diethyl ether. All compounds were homogenous by TLC in three solvent systems. High resolution mass spectra were obtained by the SERC service at Swansea.

(4E,8E,12E,16E,20E)-4,8,13,17,21-*Pentamethyldocosa*-4,8,12 16,20-*pentaen*-1-*al* **6**.—Squalene (41.1 g, 0.10 mol) was dissolved in tetrahydrofuran (THF) (250 cm^3) and sufficient water (*ca.* 50 cm^3) was added to saturate the solution. The solution was cooled to 0 °C and *N*-bromosuccinimide (NBS) (21.4 g, 0.12 mmol) was then added to it. After 90 min the reaction mixture was extracted with light petroleum (3 × 150 cm^3). The combined extracts were washed with brine (2 × 150 cm^3), dried and concentrated under reduced pressure. The product was purified by flash chromatography to afford squalene 2,3-bromohydrin (17.8 g, 35%), which was then added to a solution of potassium carbonate (9.6 g, 0.070 mmol) in methanol (500 cm^3). After the mixture had been stirred for 1 h at room temp. it was concentrated under reduced pressure to *ca.* 200 cm^3 then diluted with water (200 cm^3). The product was extracted with ether (3 × 100 cm^3), and the extracts were dried and concentrated under reduced pressure to afford squalene 2,3-epoxide (14.0 g, 95%). This epoxide (14.0 g, 0.033 mol) was then added to a vigorously stirred solution of periodic acid (11.2 g, 49.3 mmol) in ether (500 cm^3) at room temp. After 15 min the reaction mixture was washed with saturated brine (3 × 100 cm^3), dried and concentrated under reduced pressure to yield the trisnorsqualene aldehyde **6** (11.7 g, 90%) (30% overall yield from squalene); R_f 0.58 (light petroleum–ether, 4:1); ν_{max}/cm^{-1} 1716; $\delta(CDCl_3)$ 1.64 (m, 18 H, allylic CH_3), 2.0 (m, 18 H, allylic CH_2), 2.40 (t, 2 H, *J*8, 2-CH_2), 4.93 (m, 5 H, vinylic H) and 9.73 (s, 1 H, C*H*O).

Ethyl (2*E*,6*E*,10*E*,14*E*,18*E*,22*E*)-2,6,10,15,19,23-*Hexamethyltetracosa*-2,6,10,14,18,22-*hexaen*-1-*oate* **7**.—Ethoxycarbonyl: ethylidene(triphenyl)phosphorane (518 mg, 1.4 mmol) was dissolved in dry dichloromethane (10 cm^3) and the trisnorsqualene aldehyde **6** (500 mgs, 1.3 mmol) in dichloromethane (5 cm^3) was added to the solution. After 3 h the solvent was removed under reduced pressure. Ether (20 cm^3) was added to the residue and the resulting suspension filtered. The filtrate was then concentrated under reduced pressure and the crude product purified by flash chromatography eluting with light petroleum–ether (19:1) to yield the ester **7** as a pale yellow oil (490 mg, 85%); R_f 0.52 (light petroleum–ether, 9:1); ν_{max}/cm^{-1} 1710, 1666, 1614, 1447, 1270 and 1123; $\delta_H(CDCl_3$, 220 MHz) 1.29 (t, 3 H, *J* 6, C*H*$_3$CH$_2$O), 1,60–1.69 (m, 18 H, allylic CH_3), 1.84 (s, 3 H, 1-CH_3), 2.02 (m, 18 H, allylic CH_2), 2.28 (br q, 2 H, 4-CH_2), 3.20 (q, 2 H, *J* 5, CH_3CH_2O), 5.17 (m, 5 H, vinylic H) and 6.74 (t, 1 H, *J* 8, vinylic 3-CH) (Found: $[M + H]^+$, 486.3967. $C_{32}H_{52}O_2$ + 1 H requires *M*, 486.3967).

(2E,6E,10E,14E,18E,22E)-2,6,10,15,19,23-*Hexamethyltetracosa*-2,6,10,14,18,22-*hexaene*-1-*ol* **8**.— The ester **7** (2.0 mg, 4.3 mmol) was dissolved in ether (30 cm^3) and the solution cooled to 0 °C. Lithium aluminium hydride (320 mg, 8.6 mmol) was added to it and the mixture was stirred for 1.5 h at 0–5 °C. It was then diluted with ice–water (10 cm^3) and saturated aqueous citric acid (10 cm^3). After the mixture had been washed with brine (30 cm^3), the ethereal layer separated, dried and concentrated under reduced pressure to yield the alcohol **8** which did not require further purification (1.67 g, 92%); R_f 0.25 (light-petroleum–ether, 4:1); ν_{max}/cm^{-1} 3341, 2922, 1666, 1447 and 1059; $\delta_H(CDCl_3$, 220 MHz) 1.62 and 1.69 (m, 21 H, allylic CH_3), 1.94–2.20 (m, 20 H, allylic CH_2), 4.02 (s, 2 H, C*H*$_2$OH), 5.06–5.22 (m, 5 H, vinylic H) and 5.42 (br t, 1 H, *J* 7, vinylic H *cis* to CH_2OH) (Found: $[M + NH_4]^+$, 444.4205. $C_{30}H_{50}O + NH_4$ requires *M*, 444.4205).

(2E,6E,10E,14E,18E,22E)-1-*Fluoro*-2,6,10,15,19,23-*hexamethyltetracosa*-2,6,10,14,18,22-*hexaene* **9**.—A solution of dimethylamino(trimethyl)silane (410 mg, 3.5 mmol) in fluorotrichloromethane (20 cm^3) was cooled to −78 °C and to it was added diethylaminosulfur trifluoride (DAST) (0.55 cm^3, 4.2 mmol). After 10 min at −78 °C the reaction mixture was allowed to warm to room temp. and then recooled to −78 °C. Squalene-1-ol (950 mg, 2.3 mmol) in fluorotrichloromethane (5 cm^3) was then added to the mixture which after 45 min at −78 °C was warmed to room temp., poured into water (30 cm^3) and neutralised with solid sodium hydrogen carbonate. The organic layer was separated and the aqueous phase extracted with dichloromethane (3 × 50 cm^3). The combined extracts were dried and concentrated under reduced pressure. The crude product was purified by flash chromatography (light petroleum–ether, 19:1) to yield the monofluorosqualene **9** (409 mg, 43%); R_f 0.71 (light petroleum–ether, 19:1); ν_{max}/cm^{-1} 2965, 1666, 1449, 1381 and 1248; $\delta_H(CDCl_3$, 220 MHz) 1.64–1.72 (m, 21 H, allylic CH_3), 1.98 (m, 20 H, allylic CH_2), 4.73 (d, 2 H, *J* 48, CH_2F), 5.18 (m, 5 H, vinylic H) and 5.54 (m, 1 H, vinylic H *cis* to CH_2F) (Found: $[M - F]^+$, 409.3834. $C_{30}H_{49}F$ − F requires 409.3834).

(6E,10E,14E,18E,22E)-3-*Fluoro*-2,6,10,15,19,23-*hexamethyltetracosa*-1,6,10,14,18,22-*hexaene* **10**.—To a solution of DAST (0.20 g, 1.3 mmol) in dichloromethane (2 cm^3) cooled to −78 °C was added squalen-1-ol **8** (550 mg, 1.3 mmol) in dichloromethane (2 cm^3). The reaction mixture was maintained at −78 °C for 10 min and then warmed to room temp. and poured into ice–water (5 cm^3). The organic layer was separated and the aqueous layer neutralised with sodium hydrogen carbonate and extracted with dichloromethane (2 × 20 cm^3). The combined extracts were washed with brine, dried and concentrated under reduced pressure. The crude product was purified by flash chromatography eluting with light petroleum–ether (19:1), to afford a mixture of compounds **9** and **10** (29:71) (240 mg, 45%); R_f 0.71 (light petroleum–ether, 19:1); ν_{max}/cm^{-1} 2965, 1666, 1449, 1381 and 1248; $\delta_H(CDCl_3)$ 1.65–1.78 (m, 21 H, allylic CH_3), 2.06 (m, 20 H, allylic CH_2 and 4-, 5-CH_2), 4.78 (dd, 1 H, *J* 48.3 and 7.7, CHF), 4.91 (s, 1 H, *cis* 1-H), 4.97 (s, 1 H, *trans* 1-H) and 5.01–5.21 (m, 5 H, vinylic H) (Found: $[M - F]^+$, 409.3834. $C_{30}H_{49}F$ − F requires 409.3834).

(2E,6E,10E,14E,18E,22E)-2,6,10,15,19,23-*Hexamethyltetracosa*-2,6,10,14,18,22-*hexaenal* **11**.—Squalene-1-ol **8** (521 mg, 1.23 mmol) was dissolved in cyclohexane (30 cm^3) and an excess of manganese dioxide was added to the solution. The reaction mixture was stirred for 24 h at room temp. and then filtered through a Celite pad. The filtrate was concentrated under reduced pressure to give the α,β-unsaturated aldehyde **11** (430 mg, 82%) which required no further purification; R_f 0.63 (light petroleum–ether 4:1); ν_{max}/cm^{-1} 2965, 1666 and 1610; $\delta_H(CDCl_3$, 220 MHz) 1.61–1.64 (m, 18 H, allylic CH_3), 1.76 (s, 3 H, 2-CH_3), 1.90–2.20 (m, 18 H, allylic CH_2), 2.47 (dt, 2 H, *J* 7 and 5, 4-CH_2), 5.16 (m, 5 H, vinylic H), 6.47 (t, 1 H, *J* 6, vinylic 3-CH) and 9.38 (s, 1 H, CHO).

(2E,6E,10E,14E,18E,22E)-1,1-*Difluoro*-2,6,10,15,19,23-*hexamethyltetracosa*-2,6,10,14,18,22-*hexaene* **12**.—To a solution of the squalene allylic aldehyde **11** (1.00 g, 2.35 mmol) dissolved in dichloromethane (15 cm^3) was added DAST (2.0 cm^3, 15 mmol). The reaction mixture was refluxed for 48 h, and then poured into ice–water (20 cm^3) and neutralised with saturated aqueous sodium hydrogen carbonate. The organic layer was separated and the aqueous phase extracted with dichloromethane (2 × 50 cm^3). The combined extracts were dried and concentrated under reduced pressure. The crude product was purified by flash chromatography eluting with light petroleum–

ether (19:1) to yield the product **12** as a colourless oil (267 mg, 25%); R_f 0.71 (light petroleum–ether 19:1); δ_H($CDCl_3$, 220 MHz) 1.61–1.69 (m, 21 H, allylic CH_3), 1.97–2.15 (m, 20 H, allylic CH_2), 5.06–5.22 (m, 5 H, vinylic H), 5.58 (m, 1 H, vinylic 3-CH) and 5.88 (t, 1 H, *J* 57, CHF_2).

(2E,6E,10E,14E,18E,22E)-2-*Cyano*-6,10,15,19,23-*pentamethyltetracosa*-2,6,10,14,18,22-*hexaene* **13**.—Trisnorsqualene aldehyde **6** (200 mg, 0.50 mmol) was dissolved in dichloromethane (10 cm^3) and treated with cyanoethan-2-yl triphenylphosphorane (180 mg, 0.55 mmol). After 2 h the solvent was removed under reduced pressure and the residue was purified by flash chromatography eluting with light petroleum–ether (19:1) to yield the desired nitrile **13** (200 mg, 95%) as a mixture of *trans* and *cis* isomers (72:28); R_f 0.33 (light petroleum–ether, 19:1); δ_H($CDCl_3$, 220 MHz) 1.61–1.69 (m, 18 H, allylic CH_3), 1.86 (s, 3 H, 1-CH_3), 1.92–2.18 (m, 18 H, allylic CH_2), 2.26 (m, 2 H, 4-CH_2), 5.06–5.20 (m, 5 H, vinylic H), 6.10 (t, 1 H, *cis* 3-CH) and 6.35 (t, 1 H, *J* 6, *trans* 3-CH) (Found: M^+, 421.3709. $C_{30}H_{47}N$ requires *M*, 421.3709).

(2E,6E,10E,14E,18E,22E)-2,6,10,15,19,23-*Hexamethyl*-1-*trimethylsilyltetracosa*-2,6,10,14,18,22-*hexaene* **14**.—Butyllithium (1.6 mol dm^{-3} in hexane; 1.6 cm^3, 2.6 mmol) was added dropwise over 30 min to a suspension of ethyl(triphenyl)phosphonium bromide (0.96 g, 2.6 mmol) in dry THF at 0 °C. The reaction was warmed to room temp. and stirred for 1 h, recooled to 0 °C and iodomethyl(trimethyl)silane (0.38 cm^3, 2.6 mmol) was slowly added to it. After 1 h, further butyllithium in hexane was added (1.6 cm^3, 2.6 mmol) to the mixture which was then allowed to warm to room temp. It was then stirred for 1 h before being cooled to −78 °C. Trisnorsqualene aldehyde (1.00 g, 2.6 mmol) was added to the ylide over a 15 min period. After a further 30 min at −78 °C the reaction mixture was warmed to room temp., and stirred for 2 h, poured into saturated aqueous ammonium chloride (30 cm^3) and extracted with diethyl ether (3 × 30 cm^3). The combined extracts were dried and concentrated under reduced pressure. The crude product was purified by flash chromatography eluting with light petroleum–ether (9:1) to yield the allylsilane **14** as a colourless oil (200 mg, 16%) as a 50:50 mixture of *cis* and *trans* isomers; R_f 0.71 (light petroleum–ether, 19:1); δ_H($CDCl_3$, 220 MHz) −0.04 [s, 9 H, $(CH_3)_3Si$], 1.42 (*cis*), 1.48 (*trans*) (s, 2 H, $TMSCH_2$), 1.60–1.66 (m, 21 H, allylic CH_3), 1.90–2.1 (m, 20 H, allylic CH_2) and 5.02–5.2 (m, 6 H, vinylic H) (Found: $[M + H]^+$ 483.4306. $C_{33}H_{58}Si$ + H requires *M*, 483.4306).

(5E,9E,13E,17E,21E)-1,1-*Dicyano*-5,9,14,18,22-*pentamethyltricosa*-1,5,9,13,17,21-*hexaene* **15**.—To a solution of trisnorsqualene aldehyde **6** (500 mg, 1.3 mmol) in toluene (30 cm^3) and acetic acid (5 cm^3) was added ammonium acetate (100 mg, 1.30 mmol) and malononitrile (200 mg, 3.0 mmol). After being stirred for 8 h at room temp., the reaction mixture was washed with water (10 cm^3) and aqueous sodium hydrogen carbonate (2 mol dm^{-3}; 10 cm^3). The organic layer was then dried and concentrated under reduced pressure. The crude product was purified by flash chromatography, eluting with light petroleum–ether (9:1) to yield the nitrile **15** as a colourless oil (250 mg, 46%); R_f 0.21 (light petroleum–ether, 9:1); ν_{max}/cm^{-1} 2921, 1664, 1446 and 1380; δ_H($CDCl_3$, 220 MHz) 1.60–1.68 (m, 18 H, allylic CH_3), 1.95–2.15 (m, 16 H, allylic CH_2), 2.22 (t, 2 H, *J* 8, 4-CH_2), 2.70 (dt, 2 H, *J* 6 and 5,3-CH_2), 5.00–5.25 (m, 5 H, vinylic H), 7.28 (t, 1 H, *J* 8, 2-CH) (Found: $[M + H]^+$ 433.3583. $C_{30}H_{44}N_2$ + 1 H requires *M*, 433.3583).

Diethyl (5E,9E,13E,17E,21E)-5,9,14,18,22-*Pentamethyltricosa*-1,5,9,13,17,21-*hexaene*-1,1-*dicarboxylate* **16** and *Diethyl* 5E,9E,13E,17E,21E-2-(*diethoxycarbonylmethyl*)-5,9,14,18,22-*pentamethyltricosa*-5,9,13,17,21-*pentaene*-1,1-*dicarboxylate* **17**.—To dry THF (20 cm^3) at 0 °C was added titanium tetrachloride (2.5 cm^3; 1 mol dm^{-3} in CH_2Cl_2), trisnorsqualene aldehyde **6** (500 mg, 1.26 mmol) in THF (10 cm^3) and diethyl malonate (200 mg, 1.26 mmol) in THF (cm^3). The reaction was stirred for 1 h at 0 °C and then pyridine was added (0.40 cm^3, 5 mmol) to it. The mixture was allowed to warm to room temperature and stirred for 24 h. It was then diluted with water (20 cm^3) and extracted with ether (3 × 30 cm^3). The combined extracts were dried and concentrated under reduced pressure. The crude product mixture was purified by flash chromatography, eluting with light petroleum–ether (4:1) to afford the diester **16** (190 mg, 29%) and the bis-diester **17** (90 mg, 14%). Characterisation for **16**; R_f 0.42 (light petroleum–ether, 4:1); ν_{max}/cm^{-1} 1730; δ_H($CDCl_3$, 220 MHz) 1.28–1.34 (m, 6 H, CH_3CH_2O), 1.61–1.70 (m, 18 H, allylic CH_3), 2.00–2.20 (m, 18 H, allylic CH_2), 2.42 (q, 2 H, *J* 6, 3-CH_2), 4.25–4.28 (m, 4 H, *J* 6, CH_3CH_2O), 5.1–5.2 (m, 5 H, vinylic H) and 6.98 (t, 1 H, *J* 7, 2-CH) (Found: $[M + H]^+$ 527.4100. $C_{34}H_{54}O_4$ + 1 H requires *M*, 527.4100). Characterisation for **17**; R_f 0.21 (light petrolum–ether, 4:1); ν_{max}/cm^{-1} 1730; δ_H($CDCl_3$, 220 MHz) 1.28 (m, 12 H, CH_3CH_2O), 1.61–1.70 (m, 18 H, allylic CH_3), 1.95–2.20 (m, 18 H, allylic CH_2), 2.98 (t, 2 H, *J* 7, 4-CH_2), 3.36 (m, 1 H, 2-CH), 3.76 (d, 2 H, *J* 7, 1-CH and 1-'CH), 4.20 (m, 8 H, CH_3CH_2O), 4.9–5.2 (m, 5 H, vinyl H) (Found: M^+ 687.4835. $C_{41}H_{67}O_8$ requires *M*, 687.4386).

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