Synthesis of an Olefinic Sterol Derivative for use as a Membrane Probe

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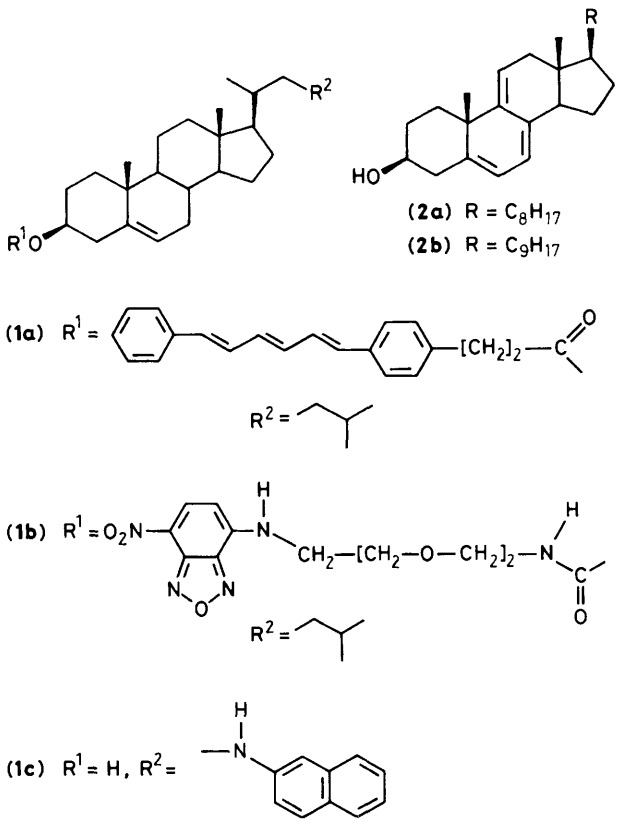
The synthesis and characterization of a sterol with the same structural features as cholesterol and possessing a hydrophobic fluorescent side-chain suitable for membrane fluidity studies is reported.

Aromatic olefins, particularly diphenylhexatriene (DPH), have been used extensively as fluorescent probes of membrane fluidity.[1] Such molecules, however are not natural membrane constituents and can only provide indirect insight into protein–lipid and lipid–lipid interactions.

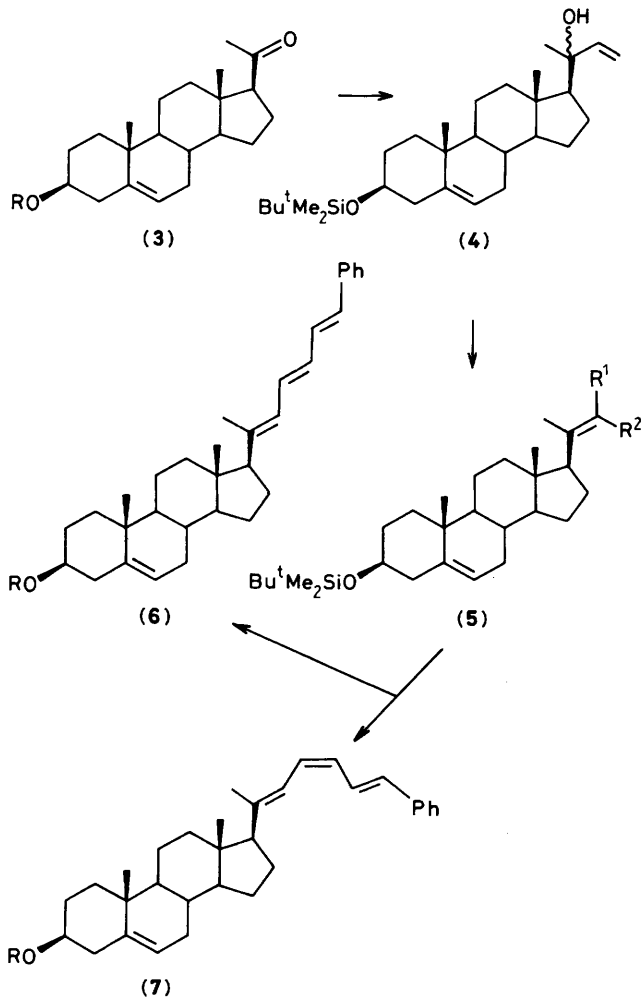
While cholesterol is an important lipid component, its role in influencing membrane structure and dynamics is poorly understood. So, it was decided to focus on the synthesis of a suitable fluorescent derivative of this compound. Some cholesterol-type molecules in which the C-3 alcohol function has been derivatised[2] (**1a** and **b**) have previously been synthesized. Apart from loss of the amphipathic nature of the cholesterol moiety in some of these derivatives [*e.g.* (**1a**)], in all cases the C-3 hydroxy function has been drastically altered. Other probes have been synthesized[3] in which the C-3 hydroxy group is intact (**2a** and **b**) but instead have a very different molecular geometry from cholesterol owing to extra unsaturation in the ring system. Such probes would be expected to pack differently in membranes and the lipid–probe interactions would be different from those of lipid–cholesterol.

Fluorescent chromophores in the side-chain of cholesterol have been reported[4] [*e.g.* (**1c**)] but for our projected studies we wanted to maintain the hydrophobic character of the side-chain. By designing a probe with an olefinic fluorescent chromophore in the side-chain, one would have a molecule that resembled cholesterol more closely in both geometry and amphipathic nature.

The t-butyldimethylsilyl ether† (**3**; R = Me_3CSiMe_2–) of



† All compounds reported have been characterised by complete spectral and analytical data.



readily available pregnenolone (**3**; R = H) was used as starting material. Treatment of this substance with vinylmagnesium bromide gave a quantitative yield of a 90:10 mixture[5] of epimeric C-20 alcohols (determined by integration of the 23-H peaks centred at δ 5.27 and 5.18; δ 5.09 and 5.00). Diastereoisomeric aldehydes were obtained in 88% yield by oxidative rearrangement of the epimeric alcohols (**4**) with pyridinium chlorochromate[6] and NaOAc in CH_2Cl_2 at room temperature for 20 hours. The *E*- and *Z*-aldehydes were assigned on the basis of their 21-CH_3 300 MHz ^{1}H n.m.r. resonances[7] [(**5**; R^1 = CHO, R^2 = H) δ (21-CH_3) 2.20; (**5**; R^1 = H, R^2 = CHO) δ (21-CH_3) 1.99] and were formed in an 80:20 ratio respectively [determined by integration of the 23-H peaks at δ 10.07(*E*) and δ 9.97(*Z*) and the 22-H peaks at δ 6.05(*Z*) and δ 5.94(*E*)]. Separation of the isomers was effected by reversed phase h.p.l.c. (Altex Ultrasphere ODS 10 mm × 25 cm column, 100% MeCN). The overall yield of 70% for the *E*-isomer is an improvement over the methods[5,8] used previously for the analogous C-3 acetoxy compound.

The *E*-aldehyde (**5**; R^1 = CHO, R^2 = H) was treated with *E*-cinnamyltriphenylphosphonium chloride and Bu^{n}Li in tetrahydrofuran giving a 75% mixture of conjugated trienes which were deprotected at C-3 with tetrabutylammonium fluoride.[9] From ^{1}H n.m.r. analysis (^{1}H–^{1}H correlated spectrum or COSY, coupling constants, integration, and computer simulation in the olefinic proton region) the mixture of isomeric alcohols was assigned to a 39:61 ratio of the *EEE* (**6**; R = H) and the *EZE* (**7**; R = H) trienes. The integration experiments focused on the 25-H signal of the *EEE*-isomer (**6**; R = H) centred at δ 6.86 and the 6-H signal from both isomers at δ 5.34. Separation of these isomers was achieved by reversed-phase h.p.l.c. (Altex Ultrasphere ODS 10 mm × 25 cm column, 98:2 MeCN:H_2O). The characteristic n.m.r. signals for the olefinic protons in the side-chain of each of the isomers are given in Table 1.

Table 1. Chemical shifts and coupling constants of olefinic protons in the isomeric conjugated trienes (**6**; R = H) and (**7**; R = H).

	δ (*J* in Hz)	
Proton	*EEE* Isomer (**6**; R = H)	*EZE* Isomer (**7**; R = H)
22	5.98 (11.3)	6.45 (12.3)
23	6.62 (11.3, 14.5)	6.32 (11.2, 11.2)
24	6.30 (14.6, 10.8)	6.08 (11.1, 11.1)
25	6.86 (10.8, 15.6)	7.2—7.3 (under aromatic proton)
26	6.49 (15.5)	6.54 (15.4)

The *EEE*-isomer (**6**; R = H) isolated showed no detectable impurities and exhibited m.p. 171.5—173 °C; $\lambda^{abs.}$ 331 nm, ε 42 000 dm^3 cm^{-1} mol^{-1} (methylcyclohexane); $\lambda^{fl.}$ 390 nm ($\lambda_{ex.}$ 330 nm, methylcyclohexane). Preliminary experiments with this isomer incorporated in model lipid membranes, with and without added cholesterol, show very significant changes in order parameters.[1c] These results confirm the potential usefulness of the *EEE*-isomer (**6**; R = H) as a probe of membrane fluidity and details of these studies will be reported separately.

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