Diacetylenic sterols: new potentially photopolymerizable lipids

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Cholesterol analogues with a diacetylenic moiety in their side chains have been prepared from bisnorcholenic acid, using 1,4-bis(trimethylsilyl)buta-1,3-diyne as the synthon to introduce the polymerizable group. A preliminary study of their potential as polymerizable sterols has been carried out by exposing aqueous dispersions (with or without phospholipids) to UV light at 5 °C.

The usefulness of photopolymerizable lipids is well illustrated by the wide range of their applications, from the biomedical to the microelectronic field.[1] Usually, polymerizable lipids are composed of one or several polymerizable moieties present in a fatty acid[2] or in a phospholipid.[3] These moieties include diacetylene, vinyl, methacrylate, styryl, acetylene, sorbate, disulfide or thiol groups.[4] Their positions in different regions of the lipid skeleton have been varied (*e.g.* they can be placed in the head group or in the acyl chain of a phospholipid) in order to modify the physical properties of the bilayers they form in aqueous dispersion.[5]

In this report, we extend this concept by introducing a polymerizable moiety into another important lipid of the cell membrane: cholesterol. The role of cholesterol as a membrane bilayer stabilizer might be enhanced by forming, under irradiation, cross-links between molecules of modified cholesterol (homopolymerization) or between a polymerizable sterol and a polymerizable phospholipid (copolymerization). Here we report the synthesis of the first diacetylenic sterols and initial observations of their ability to undergo photopolymerization.

A previous study showed that a cholesterol derivative having a methacrylate group esterified to the 3β-hydroxy group underwent homopolymerization *via* UV irradiation and free-radical irradiation.[6] We decided to introduce the polymerizable moiety into the sterol side chain since the 3β-hydroxy group of cholesterol must remain free to keep its amphiphilic function. In the present work, the diacetylene group was chosen as the photopolymerizable moiety to be placed in the sterol side chain. Numerous methods have been described to introduce a diacetylene group into a molecule.[7] A common route is to couple a 1-halogenoalkyne to a terminal alkyne.[8] We have previously reported the use of 1,4-bis(trimethylsilyl)buta-1,3-diyne as a convenient synthon to prepare diacetylenic fatty acids.[9] Application of this procedure to a suitable sterol analogue **2** afforded the protected sterol **3** bearing a side chain containing a terminal diacetylenic group. Coupling of **3** with 1-iodoalkanes gave the sterol analogues **4b** and **4c**, which have a nonterminal diacetylene in their side chain.

Results and discussion

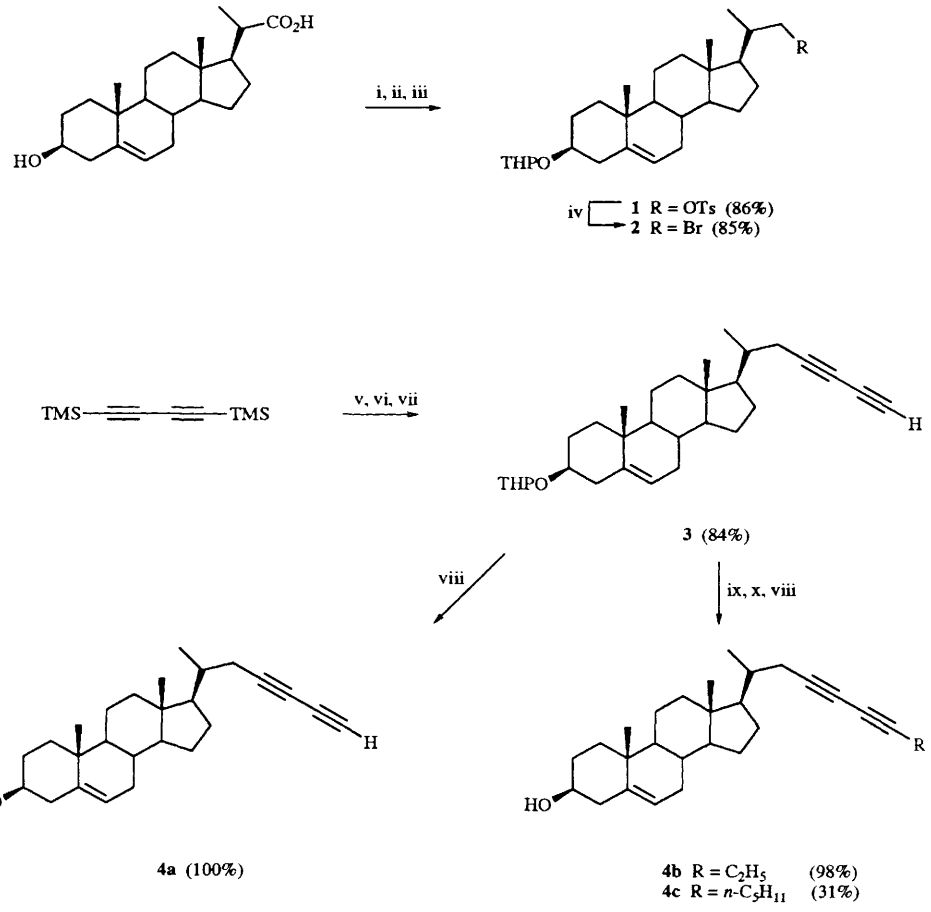
The starting material used to prepare the series of photopolymerizable cholesterol analogues was 3β-(tetrahydropyran-2-yloxy)-20-tosyloxymethylpregn-5-ene **1**, obtained from the commercially available bisnorcholenic acid (3β-hydroxypregn-5-ene-20-carboxylic acid) (Scheme 1). Displacement of the tosylate group by bromide ion, using anhydrous lithium bromide in acetone at reflux for 2 h, gave 20-bromomethyl-3β-(tetrahydropyran-2-yloxy)pregn-5-ene **2**. Monodesilylation of 1,4-bis(trimethylsilyl)buta-1,3-diyne with methyllithium as a complex with lithium bromide, and then coupling with 0.8 equiv. of **2** in hexamethylphosphoramide (HMPA)–tetrahydrofuran (THF) (4:1), led to a sterol side chain containing a terminal 1-trimethylsilylbuta-1,3-diyne moiety. After deprotection of the trimethysilyl group by using $KF\cdot 2H_2O$, the key intermediate **3** was obtained in 84% yield. Deprotection of the tetrahydropyranyl (THP) ether with concentrated hydrochloric acid in methanol, at reflux, afforded **4a** in quantitative yield. Coupling of the lithium salt of **3** with alkyl iodides (iodoethane and 1-iodopentane), and then THP deprotection, gave diynes **4b** and **4c** in 98 and 31% yield, respectively. With this procedure, other sterol analogues having a photopolymerizable moiety in various positions along their side chain may be obtained by using other iodoalkanes, or by using a different starting material, such as cholenic acid, that contains an altered side chain.

The polymerizability of these sterols was estimated from the colour changes of pure sterol monolayers and multilayers with phospholipid exposed to UV light (254 nm) at 5 °C, as described previously.[1a,10] The results are shown in Table 1. 1,2-Bis(tricosa-10,12-diynoyl)-*sn*-glycero-3-phosphocholine ($DC_{8,9}PC$) is a well-known polymerizable phospholipid containing a diacetylenic group in each of its acyl chains.[11] Under the conditions we used, $DC_{8,9}PC$ underwent homopolymerization over a period of 50 min, as indicated by the red colour of the suspension. Sterol **4a** appeared to crosslink with $DC_{8,9}PC$ as judged by TLC. TLC analysis of liposomes prepared from sterol **4a** and $DC_{8,9}PC$ (1:2 mol/mol), after UV exposure for 150 min at 5 °C, showed that no free **4a** remained (elution with hexane–ethyl acetate 4:1; R_f of free sterol, 0.36; R_f of polymer, 0). UV irradiation of sterol **4a** as both a monolayer and a bilayer with 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) afforded yellow and orange suspensions, respectively (Table 1), suggesting the formation of short polydiacetylenes. Sterol **4a** also underwent homopolymerization when heated at 130 °C for several minutes, affording a red–brown solid. This thermochromism effect has been previously described for other diacetylene molecules.[12] However, sterols **4b** and **4c** did not undergo homopolymerization or crosslinking with $DC_{8,9}PC$ in liposomes.

In summary, we have prepared diacetylenic sterols that have a potential for polymerization. Varying the position of the diacetylenic group in the side chain by increasing the number of carbon atoms between the polymerizable group and the cyclic skeleton may make the polymerizable moiety more available for homo- or co-polymerization.

Experimental

Solvents were dried by distillation as follows and then stored over 3 Å molecular sieves: THF, from lithium aluminium



Scheme 1 *Reagents and conditions:* i, dihydropyran (DHP), *p*-TsOH, benzene, 1 h, RT; ii, $LiAlH_4$, THF, 16 h, RT; iii, *p*-TsCl, py, 16 h, RT; iv, LiBr, acetone, 2 h, reflux; v, MeLi–LiBr, THF, 3.5 h, −78 °C to RT; vi, **2**, HMPA–THF (4:1), 4 h, −78 °C to RT; vii, $KF{\cdot}2H_2O$, DMF, 4 h, RT; viii, HCl, MeOH, 15 min, reflux; ix, BuLi, THF, 1 h, −23 °C; x, RI, HMPA, 5 h, −23 °C to RT

Table 1 Effect of UV light on monolayers of sterol **4a** and bilayers of PC–**4a** at 5 °C[a]

Time of exposure (min)	**4a**	$DC_{8,9}PC$	$DC_{8,9}PC$–**4a**	$DC_{8,9}PC$–**4b**	$DC_{8,9}PC$–**4c**	DPPC–**4a**
30	Yellow	Orange	Red	Orange	Orange	Orange
150	Yellow	Red	Red	Orange–red	Orange–red	Orange

[a] Sterols **4b** and **4c** as monolayers or bilayers with DPPC stayed as a colourless suspension under these conditions. Incorporation of cholesterol did not affect the time course of colour change of $DC_{8,9}PC$ liposomes, suggesting that lateral phase separation into sterol-rich and sterol-poor domains may take place.

hydride; acetone, from phosphorus pentoxide; *N*,*N*-dimethylformamide (DMF) and hexamethylphosphoramide (HMPA) from calcium hydride. Bisnorcholenic acid (pregn-5-ene-20-carboxylic acid) was purchased from Steraloids Inc., Wilton, NH, USA. ^1H and ^{13}C NMR spectra were recorded on an IBM-Bruker 200 MHz spectrometer in deuteriochloroform. Chemical shifts are given in ppm from tetramethylsilane as internal standard; *J* values are given in Hz. IR spectra were recorded on a Perkin-Elmer 1600 FT-IR spectrophotometer. GC-MS spectra were recorded on a Hewlett Packard 5988 A GC-quadrupole MS with a H-P1000 data system. Silica gel G TLC plates of 0.25 mm thickness (Analtech, Newark, DE) were used to monitor reactions, with 10% sulfuric acid in ethanol and short wavelength UV light to visualize the spots. E. Merck silica gel 60 (230–400 ASTM mesh) was used for flash chromatography.

3β-(Tetrahydropyran-2-yloxy)-20-tosyloxymethylpregn-5-ene 1
This compound was prepared from bisnorcholenic acid in 86% yield as described previously.[13]

20-Bromomethyl-3β-(tetrahydropyran-2-yloxy)pregn-5-ene 2
The tosylate **1** (760 mg, 1.33 mmol) was refluxed with anhydrous lithium bromide (1.0 g, 11.5 mmol) in acetone (10 cm^3) for 2 h. The mixture was cooled and evaporated to dryness under reduced pressure, after which the residue was solubilized in water–dichloromethane (1:1), and the aqueous phase was extracted with dichloromethane. The organic extract was dried (Na_2SO_4), filtered, evaporated to dryness under reduced pressure and purified by flash chromatography (elution with hexane–ethyl acetate, 98:2) to afford compound **2** (540 mg, 85%): δ_H(200 MHz, $CDCl_3$) 0.704 (3 H, s, 19-Me), 1.008 (3 H, s, 18-Me), 1.093 (3 H, d, *J* 6.4, 21-Me), 3.3–4.0 (5 H, m, 3α-H, 22-H_2, 6′-H_2 of THP), 4.72 (1 H, m, 2′-H of THP) and 5.35 (1 H, m, 6-H); δ_C(50 MHz, $CDCl_3$) 12.21 (C-18), 18.64 (C-21), 19.34 (C-19), 20.02 (C-4′), 21.03 (C-11), 24.19 (C-15), 25.51 (C-3′), 27.53 (C-16), 29.70 (C-2), 31.31 (C-2′), 31.86 and 31.98 (C-7 and -8), 36.78 (C-10), 37.23 (C-1), 37.83 (C-22), 38.81 (C-4), 39.52 (C-12), 42.41 (C-13), 43.38 (C-20), 50.13 (C-9), 53.81 (C-17), 56.47 (C-14), 62.77 (C-5′), 76.02 (C-3), 96.85 (C-1′), 121.33 (C-6) and 140.99 (C-5).

20(*R*)-(Penta-2′,4′-diynyl)-3β-(tetrahydropyran-2-yloxy)pregn-5-ene 3

To a solution of 1,4-bis(trimethylsilyl)buta-1,3-diyne (275 mg, 1.41 mmol) in THF (2 cm^3) cooled at −78 °C under nitrogen, was added methyllithium (1.5 mol dm^{-3} as a complex with lithium bromide, 0.95 cm^3, 1.42 mmol). The solution was stirred at room temp. for 3.5 h, and then cooled to −78 °C. A solution of **2** (544 mg, 1.13 mmol) in THF (1 cm^3) and HMPA (4 cm^3) was added to the reaction mixture which was then stirred for 4 h at room temperature. After this it was poured into hexane–water and the aqueous phase was separated and extracted with hexane. The combined organic phases were dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. A slurry of $KF·2H_2O$ (285 mg, 3.03 mmol) in DMF (6 cm^3) was added to the residue to give a dark solution, which was stirred for 4 h at room temperature and then poured into 3 mol dm^{-3} aq. hydrochloric acid and extracted with hexane. The combined organic phases were washed with 3 mol dm^{-3} aq. hydrochloric acid, saturated aq. sodium hydrogen carbonate and saturated brine, dried (Na_2SO_4), filtered, evaporated to dryness under reduced pressure and purified by column chromatography (elution with hexane–ethyl acetate 98:2) to give the dialkyne sterol **3** (425 mg, 84%); δ_H(200 MHz, $CDCl_3$) 0.681 (3 H, s, 19-Me), 1.006 (3 H, s, 18-Me), 1.092 (3 H, d, *J* 6.5, 21-Me), 3.4–4.0 (3 H, m, 3α-H and 6′-H_2 of THP), 4.72 (1 H, m, 2′-H of THP) and 5.35 (1 H, m, 6-H); δ_C(50 MHz, $CDCl_3$) 11.95 (C-18), 19.29 (C-21), 19.37 (C-19), 20.01 (C-4′), 21.04 (C-11), 24.26 (C-15), 25.55 (C-3′), 26.39 (C-22), 28.08 (C-16), 29.73 (C-2), 31.33 (C-2′), 31.91 and 31.97 (C-7 and -8), 35.51 (C-20), 36.81 (C-10), 37.27 (C-1), 38.85 (C-4), 39.58 (C-12), 42.43 (C-13), 50.20 (C-9), 55.12 (C-17), 56.67 (C-14), 62.77 (C-5′), 64.18 (2 C from alkyne group), 65.72 (C from alkyne group), 65.79 (C from alkyne group), 76.08 (C-3), 96.88 (C-1′), 121.33 (C-6) and 141.03 (C-5).

20(*R*)-(Penta-2′,4′-diynyl)pregn-5-en-3β-ol 4a

Compound **3** (100 mg, 0.22 mmol) was refluxed with methanol (10 cm^3) and conc. hydrochloric acid (33 mm^3) for 15 min after which the reaction mixture was cooled and evaporated under reduced pressure. The residue was dissolved in diethyl ether and the resulting solution was washed with saturated aq. sodium hydrogen carbonate, dried (Na_2SO_4), filtered, evaporated to dryness under reduced pressure and purified by flash chromatography (elution with hexane–ethyl acetate 98:2) to afford the sterol **4a** (81 mg, 100%); *m/z* 364 (M^+, 64%), 159 (34), 145 (56), 131 (46), 119 (51), 105 (83), 91 (100), 79 (69), 67 (47), 55 (68) and 41 (52); $\nu_{max}(CCl_4)/cm^{-1}$ 3606 (OH), 3308 (C≡CH), 2940 (CH_3, CH_2, CH), 2222 (C≡C), 1462 (CH_3), 1382 (CH_3–C), 1262 (C–O) and 1096 (C–O); δ_H(200 MHz, $CDCl_3$) 0.690 (3 H, s, 19-Me), 1.013 (3 H, s, 18-Me), 1.100 (3 H, d, *J* 6.5, 21-Me), 3.53 (1 H, m, 3α-H), 5.37 (1 H, m, 6-H); δ_C(50 MHz, $CDCl_3$) 11.95 (C-18), 19.29 (C-21), 19.37 (C-19), 21.06 (C-11), 24.26 (C-15), 26.39 (C-22), 28.08 (C-16), 31.72 (C-2), 31.87 and 31.98 (C-7 and -8), 35.51 (C-20), 36.54 (C-10), 37.31 (C-1), 39.57 (C-12), 42.38 (C-13 and -4), 50.14 (C-9), 55.10 (C-17), 56.66 (C-14), 64.18 (2 C from alkyne group), 65.72 (C from alkyne group), 65.79 (C from alkyne group), 71.79 (C-3), 121.58 (C-6) and 140.84 (C-5).

Coupling reaction with alkyl iodides

To a solution of **3** (0.13 mmol) in THF (1 cm^3) under nitrogen, was added butyllithium (2.5 mol dm^{-3} solution in hexane, 70 mm^3, 0.17 mmol) at −23 °C. The reaction mixture was stirred for 1 h at −23 °C after which it was treated with a solution of alkyl iodide (ethyl iodide or pentyl iodide, 0.22 mmol) in HMPA (4 cm^3). The reaction mixture was stirred for 1 h at −23 °C and for 4 h at room temperature, after which it was poured into 3 mol dm^{-3} aq. hydrochloric acid and extracted with hexane. The combined organic phases were washed with saturated aq. sodium hydrogen carbonate, dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue was refluxed with methanol (10 cm^3) and conc. hydrochloric acid (33 mm^3) for 15 min, cooled, evaporated to dryness under reduced pressure and solubilized in diethyl ether–aq. sodium hydrogen carbonate (1:1). The ether phase was washed with saturated aq. sodium hydrogen carbonate, dried (Na_2SO_4), filtered, evaporated to dryness under reduced pressure and purified by flash chromatography (elution with hexane–ethyl acetate 98:2).

20(*R*)-(Hepta-2′,4′-diynyl)pregn-5-en-3β-ol 4b, 98% Yield, mp 146–147 °C, *m/z* 392 (M^+, 7%), 359 (9), 271 (34), 253 (11), 213 (17), 159 (40), 146 (99), 131 (44), 119 (42), 105 (74), 91 (100), 67 (40), 55 (63), 41 (35); δ_H(200 MHz, $CDCl_3$) 0.683 (3 H, s, 19-Me), 1.010 (3 H, s, 18-Me), 1.085 (3 H, d, *J* 6.5, 21-Me), 1.160 (3 H, t, *J* 7.5, 5′-Me), 3.53 (1 H, m, 3α-H) and 5.36 (1 H, m, 6-H); δ_C(50 MHz, $CDCl_3$) 11.95 (C-18), 12.90 and 13.39 (C-27 and -28), 19.30 (C-21), 19.37 (C-19), 21.07 (C-11), 24.26 (C-15), 26.53 (C-22), 28.05 (C-16), 31.72 (C-2), 31.90 and 31.98 (C-7 and -8), 35.62 (C-20), 36.54 (C-10), 37.31 (C-1), 39.58 (C-12), 42.39 (C-13 and -4), 50.17 (C-9), 55.07 (C-17), 56.66 (C-14), 64.95 (C from alkyne group), 66.30 (C from alkyne group), 71.78 (C-3), 76.53 (C from alkyne group), 78.32 (C from alkyne group), 121.58 (C-6) and 140.84 (C-5).

20(*R*)-(Deca-2′,4′-diynyl)pregn-5-en-3β-ol 4c, 31% Yield, mp 120 °C, *m/z* 434 (M^+, 5%), 271 (45), 253 (15), 188 (74), 173 (35), 159 (47), 146 (55), 131 (47), 119 (54), 105 (96), 91 (100), 79 (65), 67 (39), 55 (55), 41 (31); δ_H(200 MHz, $CDCl_3$) 0.674 (3 H, s, 19-Me), 0.893 (3 H, t, *J* 6.7, 10′-Me), 1.003 (3 H, s, 18-Me), 1.081 (3 H, d, *J* 6.4, 21-Me), 3.50 (1 H, m, 3α-H) and 5.36 (1 H, m, 6-H); δ_C(50 MHz, $CDCl_3$) 11.96 (C-18), 13.86 and 19.23 (C-27 and -31), 19.30 (C-21), 19.37 (C-19), 21.07 (C-11), 22.13 (C-30), 24.27 (C-15), 26.54 (C-22), 28.08 (C-16 and -28), 31.04 (C-29), 31.72 (C-2), 31.88 and 31.90 (C-7 and -8), 35.65 (C-20), 36.54 (C-10), 37.31 (C-1), 39.58 (C-12), 42.40 (C-13 and -4), 50.17 (C-9), 55.09 (C-17), 56.66 (C-14), 65.50 (C from alkyne group), 66.60 (C from alkyne group), 66.38 (C from alkyne group), 71.78 (C-3), 77.25 (C from alkyne group), 121.58 (C-6) and 140.84 (C-5).

Preparation of liposomes

A chloroform solution containing the sterol (1.5 μmol) and the phospholipid (3.0 μmol) was evaporated under a stream of nitrogen to form a lipid film, which was dried under reduced pressure for 2 h. Buffer (0.050 mol dm^{-3} KCl containing 1 mmol dm^{-3} Na_2EDTA and 0.05% NaN_3; 1 cm^3) was pre-warmed to 55 °C and then added to the lipid film. The aqueous suspension was vortexed for 5 min at 55 °C, and then cooled overnight at 4 °C.

Photopolymerization assays

The liposomes were placed on a watch glass and exposed to 254 nm UV light (UVG-11 Mineralight® lamp) for 150 min at 5 °C. The light source was *ca.* 10 mm from the samples.

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