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Synthesis of Dimeric Steroids as Components of Lipid Membranes

Jacek W. Morzycki*, Sławomir Kalinowski, Zenon Łotowski, and Joanna Rabiczko

Institute of Chemistry, University of Warsaw, Bialystok Branch, Al. Pilsudskiego 11/4,15-443 Bialystok, Poland

Abstract: The synthesis of three dimeric steroids **1**,**2**, and **3**, as components of artificial lipid bilayer membranes, is described. Di(3β-hydroxyfurost-5-en-26-yl) (**1**) was obtained from diosgenin by reductive fission of a ring F, substitution of -OH by -I, and the Wurtz reaction. Two other dimers **2** and **3** were synthesised from the pregnanoic ester **10** by an "alkylation-reduction" procedure.

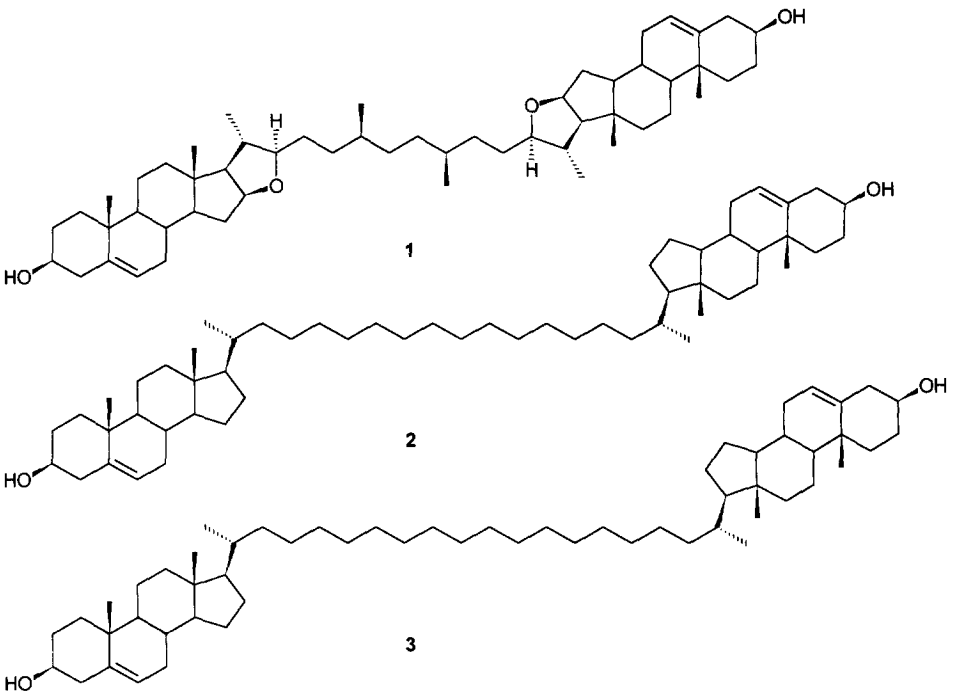
The favoured structure for most phospholipids and glycolipids in aqueous media is a bimolecular sheet, which is also called a lipid bilayer.[1,2] The preference for a bilayer structure is of critical biological importance. Phospholipids and glycolipids are key membrane constituents because they readily form extensive bimolecular sheets. Furthermore, these sheets serve as permeability barriers, yet they are quite fluid.

The formation of lipid bilayers is a self-assembly process.[3] Hydrophobic interactions are the major driving force for the formation of lipid bilayers.[4] The van der Waals attractive forces favour close packing of the hydrocarbon tails. There are also favourable electrostatic and hydrogen-bonding interactions between the polar head groups and water molecules. Thus, lipid bilayers are stabilized by the full array of forces that mediate molecular interactions in biological systems.

An important regulator of membrane fluidity in *Eucaryotes* is cholesterol.[5] This compound prevents the crystallization of lipids by fitting between the fatty acyl chains. Another effect of cholesterol is to sterically block large motions of fatty acyl chains and thereby make the membrane less fluid.

A main obstacle in a common use of artificial lipid membranes is their low stability and a relatively long formation time. A number of attempts have been undertaken to increase the stability of the artificial lipid membranes, eg. by polymerization inside a bilayer, with a limited success only.[6,7] On the other hand, Nature has produced some exceptionally stable membranes, resistant to high temperature and acids. Microorganisms, such as *Thermoplasma acidophilum*, are adjusted to live in boiling water of hot springs and geysers due to the presence in their cell membranes some bilayer-bridging bolaamphiphilic lipids containing two polar groups separated by a long hydrocarbon chain.[2] These compounds are placed across the membrane and bind its monolayers together. Artificial lipids of a similar structure were also described.[8]

We expected that a dimer of cholesterol, obtained by coupling the side chains of two cholesterol molecules with a strong C-C covalent bond, would be an excellent binder of a lipid bilayer. The aims of using such a compound as a stabilizing agent during a lipid membrane formation are the following: increase of the membrane mechanical stability and its life-time, resistance to an electric breakdown, increase of the bilayer formation rate, improvement of quality of the membrane.[9]

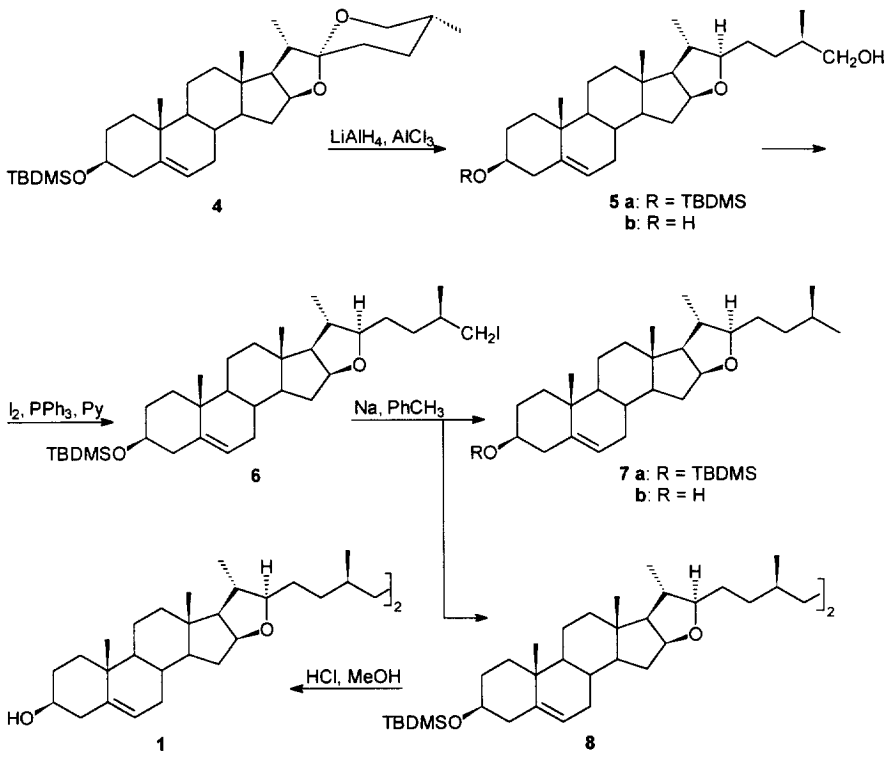


Scheme 1

There is growing interest in the construction of extended molecular frameworks with desired functionality, for application in the fields of biomimetic and molecular recognition chemistry.[10] Some of the most interesting challenges of this type involve the assembly of quite elaborate, extended structures in which spatially separated elements combine to achieve an overall effect. The steroid nucleus has been used for many years as an appendage or a rigid spacer, among others, in the study of hydrophobic aggregates such as lipid membranes.[11] The membrane-spanning porphyrin with four 3β-hydroxy-5-cholenic acid moieties attached has been also reported.[12] Some dimeric steroids and steroid-derived macrocycles (cholaphanes) have recently been synthesized in the hope of mimicing nature by constructing synthetic analogues of enzymes and receptors.[13-16] The strategy is based on the use of steroid-derived frameworks to organize functional group arrays.

Contrary to the highly functionalized steroid structures that serve for molecular recognition, the dimeric steroids designed as lipid bilayer binders are hydrophobic in the central part of molecule. In the present paper

a synthesis of three dimeric steroids **1**, **2** and **3** (Scheme 1) is described. These compounds, chosen by analysis of lipid bilayer dimensions in the natural membranes, differ in hydrocarbon chain length and polarity. The shortest in size, dimer **1**, is maintained in an extended conformation by an oxygen bridge, which also increases the polarity of this compound in comparison with the other dimers **2** and **3**. Of course, all these compounds (**1**, **2**, and **3**) are chiral, their optical rotations ($[\alpha]_D^{24}$) being -42.5°, -26.1°, and -46.5°, respectively. The only symmetry element existing in these systems is a two-fold axis (C_2) that is insufficient to ensure identity of compounds **1**, **2**, and **3** with their mirror images.

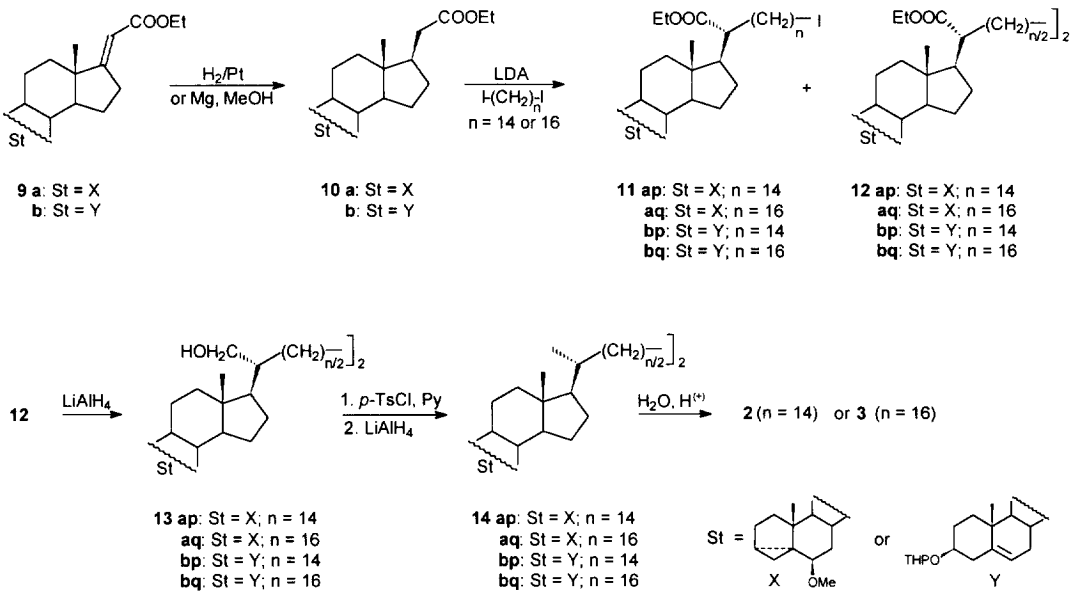


Scheme 2

The synthesis of difurost-5-en-3β-ol (compound **1**) is depicted in Scheme 2. The starting diosgenin *t*-butyldimethyl silyl ether (**4**) was subjected to $LiAlH_4/AlCl_3$ reduction.[17,18] The dihydro (22R)-derivative **5a** which results from the stereoselective opening of ring F, i.e., cleavage of the C(22)-O bond, was accompanied by the minor product - diol **5b**. The primary hydroxyl group in compound **5a** was directly transformed into the iodide **6** with $I_2/PPh_3/Py$ in a high yield.[19] The key step in the whole synthesis was the Wurtz reaction. This reaction is hardly ever used for the large organic fragments coupling.[20] However, the reaction of **6** with sodium, performed in a concentrated toluene solution, was quite successful. The dimer **8** was obtained in a moderate

yield (16%) in addition to furost-5-en-3β-ol *t*-butyldimethyl silyl ether (**7a**; 36%). The $\Delta^{26(27)}$-olefine, that could be expected from the known mechanism of the Wurtz reaction was not found among the products. Finally, the deprotection of a hydroxyl group in **8** afforded difurost-5-en-3β-ol (**1**), as colourless, crystalline material, sparingly soluble in organic solvents and relatively polar (TLC) in comparison with the monomeric compound **7b**.

Dimers **2** and **3** were obtained from the pregnanoic ester **10** by the Wicha and Bal "alkylation-reduction" procedure (Scheme 3).[21,22] In the first series of experiments the functional groups in ring A and B (3β-OH and Δ^5) were protected as *i*-steroid methyl ether (St = X). This was necessary to avoid the Δ^5 double bond



Scheme 3

hydrogenation during the preparation of the pregnanoic ester **10** from its $\Delta^{17(20)}$ precursor **9**. A new method of a conjugate double bond reduction in the α,β-unsaturated ester **9b**, with magnesium in methanol, was recently described.[23] Thanks to this method, a protection of the Δ^5 double bond during the reduction step was no longer needed. Since further transformations of **10** to the dimeric products did not require protection of the double bond either, the next series of experiments was performed with only the 3β-hydroxyl group protected as a THP ether (St = Y). The reactions of **10** with 1,ω-diiodoalkanes afforded the mixtures of the steroid iodides **11** (30-35%) and the dimers **12** (25-30%). The latter compounds were subjected to the routine procedure aiming at the conversion of -COOEt into the methyl group. Thus, the dimeric esters **12** were reduced with lithium aluminum hydride to the primary alcohols **13**, which in turn, were transformed to the corresponding tosylates and again reduced with $LiAlH_4$. The overall yield of the above procedure was very high (about 85%).

The protective groups of dimers **14** were removed and the desired dimers **2** or **3** were obtained. The dimers **2** and **3** appeared to be colourless, crystalline compounds, much less mobile by TLC than cholesterol. Noteworthy is the low solubility of the dimers in chloroform, benzene and acetone compared with cholesterol.

The described dimers **1**, **2** and **3** were used to the formation of phospholipid membranes. The physicochemical properties of these membranes were examined by various electroanalytical techniques. The results of these studies are very promising and will be published elsewhere.

EXPERIMENTAL

Melting points were determined on a Köffler apparatus of the Boetius type and were uncorrected. NMR spectra were taken with a Bruker AC 200F spectrometer using $CDCl_3$ solutions with TMS as the internal standard. Infrared spectra were recorded on a Nicolet series II Magna-IR 550 FT-IR spectrometer as chloroform solutions unless otherwise stated. Mass spectra were obtained at 70 eV with an AMD-604 spectrometer. Elemental analyses were performed at the Institute of Organic Chemistry, Polish Academy of Sciences. The reaction products were isolated by column chromatography performed on 70-230 or 230-400 mesh silica gel (Merck). Thin-layer chromatograms were developed on aluminum TLC sheets precoated with silica gel F_{254} and visualized with 50% sulfuric acid after heating. All solvents were dried and freshly distilled prior to use.

*Reductive ring F fission of diosgenin TBDMS ether **4***

To a stirred ice-cold solution of aluminum chloride (44.8 g; 0.33 mol) in ether was carefully added lithium aluminum hydride (3.15 g; 0.08 mmol). After 30 minutes a solution of diosgenin TBDMS ether **4** (4.41 g; 8.4 mmol) in 150 ml of ether was added to the reducing mixture and stirring was continued for 18 hours. The excess hydride was decomposed with acetone, then aqueous solution of ammonium chloride was added, and the reaction mixture was extracted with chloroform. Removal of solvent from the dried extract afforded an oily residue, which was subjected to column chromatography. The major product **5a** (2.65 g; 60%) was eluted with benzene - ethyl acetate (9:1). Further elution with benzene - ethyl acetate (3:1) gave desilylated product **5b** (mp 166-169°C ; 1.22 g; 35%).

Compound **5a**: mp 134-136°C (acetone - hexane); IR, ν_{max} 3660, 3440, 1090 cm^{-1}; ^1H NMR, δ 5.35 (m, 1H, 6-H), 4.30 (m, 1H, 16α-H), 3.47 (m, 3H, 3α-H i 26-H), 3.32 (m, 1H, 22-H), 1.01 (s, 3H, 18-H), 1.00 (d, J = 8.8 Hz, 3H, 21-H), 0.92 (d, J = 6.7 Hz, 3H, 27-H), 0.89 (s, 9H, t-Bu-Si), 0.81 (s, 3H, 19-H), 0.06 (s, 6H, Me-Si); ^{13}C NMR, δ 141.6 (C), 120.9 (CH), 90.4 (CH), 83.2 (CH), 72.6 (CH), 68.1 (CH_2), 65.1 (CH), 57.0 (CH), 50.2 (CH), 42.8 (CH_2), 40.7 (C), 39.5 (CH_2), 37.9 (CH), 37.4 (CH_2), 36.7 (C), 35.8 (CH), 32.2 (CH_2), 32.1 (2 x CH_2), 31.6 (CH), 30.5 (CH_2), 30.2 (CH_2), 25.9 (3 x CH_3), 20.7 (CH_2), 19.4 (CH_3), 18.9 (CH_3), 18.3 (C), 16.6 (CH_3), 16.4 (CH_3), -4.6 (2 x CH_3).

*Iodination of (25R)-furost-5-en-3β,26-diol 3-TBDMS ether (**5a**)*

Iodine (333 mg; 1.3 mmol) and triphenylphosphine (300 mg; 1.14 mmol) were dissolved in 30 ml of benzene and 1.1 ml of pyridine. The reaction mixture was magnetically stirred 15 minutes, compound **5a** (200 mg; 0.38 mmol) was added, and reaction was continued 2 hours at room temperature. Solvent was evaporated and the residue was chromatographed on a silica gel column. Benzene elution yielded steroid iodide **6** (mp 108-110°C; 230 mg; 96%); IR, ν_{max} 2920, 1090 cm^{-1}; ^1H NMR, δ 5.31 (m, 1H, 6-H), 4.31 (m, 1H, 16α-H), 3.49 (m, 1H, 3α-H), 3.29 (m, 1H, 22-H), 3.22 (m, ABX system, J_{AB} = 9.7 Hz, J_{AX} = 5.4 Hz, J_{BX} = 4.0 Hz, 2H, 26-H), 1.01 (s, 3H, 18-H), 0.96-1.01 (m, 6H, 21-H and 27-H), 0.89 (s, 9H, t-Bu-Si), 0.80 (s, 3H, 19-H), 0.05 (s, 6H, Me-Si); ^{13}C NMR, δ 141.6 (C), 120.9 (CH), 90.0 (CH), 83.2 (CH), 72.5 (CH), 65.1 (CH), 57.0 (CH), 50.2 (CH), 42.8 (CH_2), 40.7 (C), 39.4 (CH_2), 37.9 (CH), 37.4 (CH_2), 36.7 (C), 34.8 (CH), 33.6 (CH_2), 32.2 (CH_2), 32.0 (2 x CH_2), 31.6 (CH), 30.9 (CH_2), 25.9 (3 x CH_3), 20.7 (CH_2), 20.5 (CH_3), 19.4 (CH_3), 19.0 (CH_3), 18.2 (C), 17.8 (CH_2), 16.4 (CH_3), -4.6 (2 x CH_3).

*Wurtz reaction of iodide **6***

Steroid iodide **6** (160 mg; 0.25 mmol) was dissolved in anhydrous toluene (3 ml) and treated with sodium (30 mg; 1.3 mmol). The reaction mixture was refluxed for 20 hours, diluted with benzene, washed with water, dried and evaporated *in vacuo*. The products were subjected to chromatographic separation. Elution with benzene - hexane (1:1) afforded furost-5-en-3β-ol TBDMS ether **7a** (46 mg; 36%). The dimer **8** (20 mg; 16%) was eluted with benzene - hexane (3:1).

Compound **7a**: mp 100-102°C (methanol - hexane); $[\alpha]_D^{24}$ -50.0° (c = 1.0 in $CHCl_3$); IR, ν_{max} 2920, 1090 cm^{-1}; ^1H NMR, δ 5.31 (m, 1H, 6-H), 4.31 (m, 1H, 16α-H), 3.48 (m, 1H, 3α-H), 3.31 (m, 1H, 22-H), 1.01 (s, 3H, 18-H), 0.99 (d, J = 7.0 Hz, 3H, 21-H), 0.89 (s, 9H, t-Bu-Si), 0.88 (d, J = 6.3 Hz, 6H, 26-H and 27-H), 0.81 (s, 3H, 19-H), 0.06 (s, 6H, Me-Si); ^{13}C NMR, δ 141.6 (C), 120.9 (CH), 90.5 (CH), 83.1 (CH), 72.6 (CH), 65.3 (CH), 57.0 (CH), 50.2 (CH), 42.8 (CH_2), 40.7 (C), 39.5 (CH_2), 37.9 (CH), 37.4 (CH_2), 36.7 (C), 35.9 (CH_2), 32.3 (CH_2), 32.1 (2 x CH_2), 31.6 (CH), 31.4 (CH_2), 28.3 (CH), 25.9 (3 x CH_3), 22.6 (CH_3), 22.5 (CH_3), 20.7 (CH_2), 19.5 (CH_3), 19.1 (CH_3), 18.3 (C), 16.4 (CH_3), -4.6 (2 x CH_3); MS, m/z 514 (M^+, <1), 499 (1.5), 457 (100).

Compound **8**: mp 192-194°C (acetone - hexane); $[\alpha]_D^{24}$ -30.6° (c = 1.0 in $CHCl_3$); IR, ν_{max} 2920, 1090 cm^{-1}; ^1H NMR, δ 5.31 (m, 1H, 6-H), 4.30 (m, 1H, 16α-H), 3.46 (m, 1H, 3α-H), 3.30 (m, 1H, 22-H), 1.01 (s, 3H, 18-H), 1.00 (d, J = 7.4 Hz, 3H, 21-H), 0.89 (s, 9H, t-Bu-Si), 0.85 (d, J = 5.5 Hz, 3H, 27-H), 0.80 (s, 3H, 19-H), 0.06 (s, 6H, Me-Si); MS m/z 1026 (M^+, 66), 1011 (18), 969 (50), 894 (40), 763 (100), 745 (44).

Hydrolysis of TBDMS ether - dimer 1

Compound **8** (210 mg; 0.205 mmol) was dissolved in chloroform (2 ml) and methanol (2 ml), then 1 ml of methanol - water - hydrochloric acid (0.5:0.25:0.25) mixture, was added. The reaction mixture was allowed to stand at room temperature for 15 minutes, poured into diluted sodium bicarbonate solution and extracted with chloroform. The crude product was purified by a silica gel column chromatography (elution with benzene - ethyl acetate 3:1). Yield of dimer **1** - 142 mg (87%); mp 123-126°C (isopropanol); $[\alpha]_D^{24}$ -46.5° (c = 0.75 in $CHCl_3$); IR, ν_{max} 3599, 3389, 1096, 1046 cm^{-1}; ^1H NMR, δ 5.34 (m, 1H, 6-H), 4.30 (m, 1H, 16α-H), 3.52 (m, 1H, 3α-H), 3.30 (m, 1H, 22-H), 1.02 (s, 3H, 18-H), 1.00 (d, J = 6.8 Hz, 3H, 21-H), 0.85 (d, J = 5.5 Hz, 3H, 27-H), 0.81 (s, 3H, 19-H); ^{13}C NMR, δ 140.7 (C), 121.5 (CH), 90.5 (CH), 83.1 (CH), 71.7 (CH), 65.2 (CH), 57.0 (CH), 50.1 (CH), 42.2 (CH_2), 40.7 (C), 39.4 (CH_2), 37.9 (CH), 37.2 (CH_2), 36.6 (C), 34.1 (CH_2), 34.0 (CH_2), 33.3 (CH), 32.3 (CH_2), 32.0 (CH_2), 31.58 (CH), 31.57 (CH_2), 31.1 (CH_2), 20.7 (CH_2), 19.5 (CH_3), 19.4 (CH_3), 19.1 (CH_3), 16.4 (CH_3); MS, m/z 798 (M^+, 50), 783 (26), 780 (56), 763 (27), 747 (10), 355 (22), 271 (100), 253 (72).

*Alkylation of pregnanoic ester **10a** with 1,14-diiodotetradecane.*

A solution of pregnanoic ester **10a** (552 mg; 1.48 mmol) in anhydrous THF (1.5 ml) was cooled to -20°C and treated under argon with 2 M solution of LDA in THF/ethylbenzene/heptane (Aldrich; 0.8 ml). The reaction mixture was stirred for 15 minutes before dropwise addition of 1,14-diiodotetradecane (332 mg; 0.74 mmol) dissolved in 1 ml of THF. The reaction was maintained at -20°C for 2.5 hours, quenched with aqueous ethanol, and extracted with chloroform. The extract was dried, evaporated *in vacuo*, and the residue was chromtographed on a silica gel column. Elution with a heptane - ethyl acetate (98:2) mixture afforded a steroid iodide **11ap** (158 mg; 31%). The unreacted starting ester **10a** (121 mg; 22%) was recovered by heptane - ethyl acetate (97:3) elution. The dimeric product **12ap** (180 mg; 26%) was eluted with 5% ethyl acetate in heptane.

Compound **11ap**: an oil; IR, ν_{max} 1720, 1092 cm^{-1}; ^1H NMR, δ 4.10 (q, J = 7.1 Hz, 2H, O-C<u>H</u>$_2$CH$_3$), 3.32 (s, 3H, O-CH_3), 3.19 (t, J = 7.0 Hz, 2H, CH_2I), 2.76 (m, 1H, 6α-H), 2.25 (m, 1H, 20-H), 1.26 (t, J = 7.1 Hz, 3H, O-CH_2C<u>H</u>$_3$), 1.24 (narrow m, side chain methylene protons), 1.01 (s, 3H, 19-H), 0.75 (s, 3H, 18-H), 0.64 and 0.43 (2 x m, 2 x 1H, cyclopropane protons); ^{13}C NMR, δ 176.3 (C), 82.3 (CH), 59.6 (CH_2), 56.5 (CH_3), 55.8 (CH), 52.8 (CH), 48.0 (CH), 47.4 (CH), 43.4 (C), 42.3 (C), 37.9 (CH_2), 35.2 (C), 35.0 (CH_2), 33.5 (CH_2), 33.3 (CH_2), 32.0 (CH_2), 30.5 (CH_2), 30.4 (CH), 29.4 - 29.6 (8 x CH_2), 28.5 (CH_2), 27.2 (CH_2), 27.1 (CH_2), 24.9 (CH_2), 23.7 (CH_2), 22.6 (CH_2), 21.4 (CH), 19.2 (CH_3), 14.2 (CH_3), 13.1 (CH_2), 12.4 (CH_3), 7.2 (CH_2); MS, m/z 696 (M^+, 69), 681 (52), 664 (100), 641 (65); exact mass calcd for $C_{38}H_{65}O_3I$: 696.3978; found: 696.3981.

Compound **12ap**: an oil; IR, ν_{max} 1720, 1093 cm^{-1}; ^1H NMR, δ 4.10 (q, J = 7.1 Hz, 4H, O-C<u>H</u>$_2$CH$_3$), 3.32 (s, 6H, O-CH_3), 2.76 (m, 2H, 6α-H), 2.25 (m, 2H, 20-H), 1.26 (t, J = 7.1 Hz, 6H, O-CH_2C<u>H</u>$_3$), 1.24 (narrow m, side chain methylene protons), 1.01 (s, 6H, 19-H), 0.75 (s, 6H, 18-H), 0.64 and 0.43 (2 x m, 2 x 2H,

cyclopropane protons); ^{13}C NMR, δ 176.3 (C), 82.3 (CH), 59.6 (CH_2), 56.5 (CH_3), 55.8 (CH), 52.8 (CH), 48.0 (CH), 47.4 (CH), 43.4 (C), 42.4 (C), 38.0 (CH_2), 35.2 (C), 35.0 (CH_2), 33.3 (CH_2), 32.1 (CH_2), 30.5 (CH), 29.5 - 29.6 (5 x CH_2), 27.3 (CH_2), 27.2 (CH_2), 24.9 (CH_2), 23.7 (CH_2), 22.6 (CH_2), 21.5 (CH), 19.3 (CH_3), 14.2 (CH_3), 13.1 (CH_2), 12.4 (CH_3); MS, m/z 942 (M^+, 18), 927 (33), 910 (87), 895 (41), 878 (58), 855 (23), 832 (21), 804 (100), 789 (33).

A similar reaction of **10a** with 1,16-diiodotetradecane afforded iodide **11aq** (32%), unreacted ester **10a** (22%), and the dimeric compound **12aq** (27%). The steroid iodides **11ap** and **11aq** may be used as alkylating agents in the separate reactions.

*Reduction of the dimeric ester **12ap***

To a solution of the dimeric ester **12ap** (175 mg; 0.19 mmol) in THF (7 ml), lithium aluminum hydride (60 mg; 1.58 mmol) was added, and the reaction mixture was stirred at room temperature overnight. The excess hydride was decomposed with a drop of water, anhydrous $MgSO_4$ was added, and all the inorganic material was filtered off. Evaporation of solvent *in vacuo* afforded crude product **13ap**, which was purified by column chromatography. Elution with a petroleum ether - ethyl acetate mixture (8:2) yielded an oily alcohol **13 ap** (142 mg: 89%); IR, ν_{max} 3627, 3430, 1094, 1076 cm^{-1}; ^{1}H NMR, δ 3.69 (m, 4H, CH_2OH), 3.33 (s, 6H, O-CH_3), 2.77 (m, 2H, 6α-H), 1.25 (narrow m, side chain methylene protons), 1.02 (s, 6H, 19-H), 0.74 (s, 6H, 18-H), 0.65 and 0.43 (2 x m, 2 x 2H, cyclopropane protons); ^{13}C NMR, δ 82.4 (CH), 62.7 (CH_2), 56.5 (CH_3), 56.4 (CH), 50.5 (CH), 48.0 (CH), 43.4 (C), 42.5 (C), 42.4 (CH), 39.7 (CH_2), 35.2 (C), 35.0 (CH_2), 33.3 (CH_2), 30.5 (CH), 30.2 (CH_2), 29.7 (CH_2), 29.6 (3 x CH_2), 29.4 (CH_2), 27.7 (CH_2), 26.4 (CH_2), 24.9 (CH_2), 24.0 (CH_2), 22.7 (CH_2), 21.5 (CH), 19.3 (CH_3), 13.1 (CH_2), 12.5 (CH_3); MS, m/z 858 (M^+, 2), 843 (8), 826 (15), 811 (12), 794 (11), 776 (10), 761 (6), 44 (100).

Analogous reduction of the dimeric ester **12aq** afforded alcohol **13aq** (93%).

*Deoxygenation of the dimeric alcohol **13ap***

To a solution of the compound **13ap** (140 mg; 0.16 mmol) in 10 ml of dichloromethane and 0.7 ml of anhydrous pyridine *p*-toluenesulfonyl chloride (255 mg; 1.34 mmol) was added. The reaction mixture was allowed to stand overnight, poured into iced water, and the crude tosylate was extracted with chloroform. The flash chromatography afforded pure tosylate (182 mg; 96%);

The above tosylate (182 mg; 0.16 mmol) was dissolved in 10 ml of anhydrous THF, lithium aluminum hydride (65 mg; 1.71 mmol) was added and the reaction mixture was stirred at room temperature overnight. The excess hydride was decomposed with a drop of water, anhydrous $MgSO_4$ was added and all the inorganic material was filtered off. The crude product obtained by evaporation of solvent *in vacuo* was purified by a silica gel column chromatography. Elution with heptane - ethyl acetate (95:5) mixture afforded the compound **14ap** (103 mg; 80%) as a colourless oil; IR, ν_{max} 1093, 1076 cm^{-1}; ^{1}H NMR, δ 3.32 (s, 6H, O-CH_3), 2.77 (m, 2H,

6α-H), 1.26 (narrow m, side chain methylene protons), 1.02 (s, 6H, 19-H), 0.90 (d, J = 6.4 Hz, 6H, 21-H), 0.71 (s, 6H, 18-H), 0.64 and 0.43 (2 x m, 2 x 2H, cyclopropane protons); ^{13}C NMR, δ 82.4 (CH), 56.5 (CH and CH_3), 56.3 (CH), 48.0 (CH), 43.4 (C), 42.8 (C), 40.3 (CH_2), 35.9 (CH_2), 35.8 (CH), 35.3 (C), 35.0 (CH_2), 33.3 (CH_2), 30.5 (CH), 30.2 (CH_2), 29.8 (CH_2), 29.7 (3 x CH_2), 28.3 (CH_2), 26.1 (CH_2), 25.0 (CH_2), 24.2 (CH_2), 22.8 (CH_2), 21.5 (CH), 19.3 (CH_3), 18.7 (CH_3), 13.0 (CH_2), 12.2 (CH_3).

The analogous two-step procedure gave the compound **14aq** from the dimeric alcohol **13aq** (overall yield of **14aq** from **13aq** - 73%).

Deprotection of the functional groups - dimer 2

Compound **14ap** (103 mg; 0.125 mmol) was dissolved in dioxane (10 ml) and a solution of *p*-toluenesulfonic acid (15 mg; 0.08 mmol) in 3 ml of water was added. The reaction mixture was heated at 80°C for 2 hours, then it was poured into water and extracted with chloroform. The extract was dried, evaporated under the reduced pressure and the residue was purified on a silica gel column. Elution with a petroleum ether - ethyl acetate (65:35) mixture afforded dimer **2** (82 mg; 82%); mp 182-185°C (hexane - methylene chloride); $[\alpha]_D^{24}$ -42.5° (c = 0.75 in $CHCl_3$); IR, ν_{max} 3600, 2931, 958 cm^{-1}; ^{1}H NMR, δ 5.35 (m, 2H, 6-H), 3.52 (m, 2H, 3α-H), 1.25 (narrow m, side chain methylene protons), 1.01 (s, 6H, 19-H), 0.91 (d, J = 6.4 Hz, 6H, 21-H), 0.67 (s, 6H, 18-H); ^{13}C NMR, δ 140.7 (C), 121.6 (CH), 71.7 (CH), 56.7 (CH), 56.1 (CH), 50.1 (CH), 42.3 (C and CH_2), 39.7 (CH_2), 37.2 (CH_2), 36.4 (C), 35.9 (CH_2), 35.7 (CH), 31.87 (CH_2), 31.85 (CH), 31.6 (CH_2), 30.1 (CH_2), 29.73 (CH_2), 29.66 (3 x CH_2), 28.2 (CH_2), 26.1 (CH_2), 24.3 (CH_2), 21.0 (CH_2), 19.4 (CH_3), 18.7 (CH_3), 11.8 (CH_3); MS, m/z 798 (M^+, 0.2), 780 (34), 765 (24), 762 (51), 747 (20), 257 (100); anal. calcd for $C_{56}H_{94}O_2$: C, 84.14; H, 11.85; found: C, 83.84; H, 11.78.

Cycloreversion of **14aq** gave compound **3**, identical in all respects with the dimer **3** described in the next section, in 81% yield.

*Alkylation of ester **10b***

A solution of compound **10b** (1680 mg; 3.78 mmol) in 6 ml of anhydrous THF was cooled to -20°C and treated with 2 M LDA (Aldrich; 2.0 ml) under argon. The reaction mixture was stirred for 15 minutes, 1,16-diiodohexadecane (903 mg; 1.89 mmol) dissolved in 3 ml of THF was dropwise added and stirring was continued at -20°C for 2.5 hours. The reaction was quenched with aqueous ethanol and extracted with chloroform. Removal of solvent from the the dried extract afforded an oily residue which was subjected to the silica gel column chromatography. Elution with a petroleum ether - ethyl acetate (96:4) mixture yielded iodide **11bq** (530 mg; 35%) followed by the unreacted starting material **10b** (505 mg; 30%). The dimeric product **12bq** (554 mg; 26%) was eluted with a petroleum ether - ethyl acetate (9:1) mixture.

Compound **11bq**: an oil; IR, ν_{max} 1720, 1023 cm^{-1}; ^1H NMR, δ 5.34 (m, 1H, 6-H), 4.71 (m, 1H, THP acetal H), 4.11 (q, J = 7.1 Hz, 2H, O-C$\underline{H}_2$CH$_3$), 3.90 (m, 1H, THP), 3.50 (m, 2H, THP and 3α-H), 3.19 (t, J = 7.0 Hz, 2H, CH_2I), 1.27 (t, J = 7.1 Hz, 3H, O-CH$_2$C$\underline{H}_3$), 1.24 (narrow m, side chain methylene protons), 0.99 (s, 3H, 19-H), 0.70 (s. 3H, 18-H); MS, m/z 794 (M^+, <1), 692 (44), 566 (13), 85 (100).

Compound **12bq**: an oil; IR, ν_{max} 1720, 1023 cm^{-1}; ^1H NMR, δ 5.34 (m, 2H, 6-H), 4.71 (m, 2H, THP acetal H), 4.11 (q, J = 7.1 Hz, 4H, O-C$\underline{H}_2$CH$_3$), 3.89 (m, 2H, THP), 3.51 (m, 4H, THP and 3α-H), 1.27 (t, J = 7.1 Hz, 6H, O-CH$_2$C$\underline{H}_3$), 1.24 (narrow m, side chain methylene protons), 0.99 (s, 6H, 19-H), 0.70 (s, 6H, 18-H); MS, m/z 925 (4), 907 (22), 892 (6), 861 (15), 833 (93), 818 (38), 657 (34), 254 (100).

A similar reaction of **10b** with 1,14-diiodohexadecane yielded iodide **11bp** (35%), unreacted ester **10b** (26%), and the dimeric compound **12 bp** (30%).

*Reduction of the dimeric ester **12bq***

To a solution of compound **12bq** (554 mg; 0.5 mmol) in 20 ml of anhydrous THF, lithium aluminum hydride (416 mg; 10.95 mmol) was added, and the reaction mixture was heated under reflux for 45 minutes. After cooling, an excess hydride was decomposed with ethyl acetate and a drop of water, drying agent ($MgSO_4$) was added, and the inorganic material was removed by filtration. Evaporation of solvent *in vacuo* from the filtrate afforded an oily product **13bq** which was purified on a silica gel column. Compound **13bq** (454 mg; 89%) was eluted with petroleum ether - ethyl acetate (8:2); IR, ν_{max} 3661, 3414, 1023 cm^{-1}; ^1H NMR, δ 5.35 (m, 2H, 6-H), 4.71 (m, 2H, THP acetal H), 3.89 (m, 2H, THP), 3.69 (m, 4H, CH_2OH), 3.51 (m, 4H, THP and 3α-H), 1.25 (narrow m, side chain methylene protons), 1.01 (s, 6H, 19-H), 0.69 (s, 6H, 18-H); MS, m/z 822 (0.2), 592 (0.4), 274 (3), 84 (100).

Similar reduction of **12bp** afforded the dimeric alcohol **13bp** in 90% yield.

*Deoxygenation of the dimeric alcohol **13bq***

Compound **13bq** (451 mg; 0.44 mmol) was dissolved in dichloromethane (15 ml) and pyridine (2 ml), *p*-toluenesulfonyl chloride (632 mg; 3.32 mmol) was added, and the reaction mixture was allowed to stand overnight. It was then quenched by pouring into iced water, and extracted with chloroform. Evaporation of solvent from the dried extract afforded crude tosylate which was purified by flash chromatography. Yield: 566 mg (97%).

The above tosylate (563 mg; 0.42 mmol) was dissolved in 20 ml of anhydrous THF, lithium aluminium hydride was added (405 mg; 10.66 mmol), and the reaction was refluxed for 1 hour. Excess hydride was carefully decomposed with ethyl acetate and a drop of water, anhydrous $MgSO_4$ was then added, and all the inorganic material was filtered off. The solvent was removed from the filtrate and the crude product **14bq** was purified by a silica gel column chromatography. Elution with petroleum ether - ethyl acetate (95:5) afforded 413 mg (98.5%) of **14bq**; IR, ν_{max} 1022 cm^{-1}; ^1H NMR, δ 5.35 (m, 2H, 6-H), 4.71 (m, 2H, THP acetal H), 3.90

(m, 2H, THP), 3.51 (m, 4H, THP and 3α-H), 1.25 (narrow m, side chain methylene protons), 1.00 (s, 6H, 19-H), 0.90 (d, J = 6.4 Hz, 6H, 21-H), 0.67 (s, 6H, 18-H); MS, m/z 822 (4), 808 (28), 790 (52), 775 (20), 257 (94), 55(100).

A similar two-step procedure was applied to a deoxygenation of the dimeric alcohol **13bp**. Compound **14bp** was obtained in overall yield of 94%.

Hydrolysis of THP ether - dimer 3

Compound **14bq** (413 mg; 0.42 mmol) was dissolved in 15 ml of THF and 3 ml of water, *p*-toluenesulfonic acid (32 mg; 0.17 mmol) was added, and the solution was heated under reflux for 2 hours. The reaction mixture was diluted with water and extracted with chloroform. The solvent was evaporated *in vacuo* from the dried extract and the residue was purified by flash chromatography with petroleum ether - ethyl acetate (65:35). Yield of dimer **3** - 313 mg (91%); mp 165-168°C (methanol - methylene chloride); $[\alpha]_D^{24}$ -26.1° (c = 1.0 in $CHCl_3$); IR, ν_{max} 3600, 2930, 958 cm^{-1}; ^1H NMR, δ 5.35 (m, 2H, 6-H), 3.52 (m, 2H, 3α-H), 1.25 (narrow m, side chain methylene protons), 1.01 (s, 6H, 19-H), 0.91 (d, J = 6.4 Hz, 6H, 21-H), 0.67 (s, 6H, 18-H); ^{13}C NMR, δ 140.7 (C), 121.7 (CH), 71.8 (CH), 56.7 (CH), 56.1 (CH), 50.1 (CH), 42.3 (C and CH_2), 39.8 (CH_2), 37.2 (CH_2), 36.5 (C), 36.0 (CH_2), 35.7 (CH), 31.90 (CH_2), 31.89 (CH), 31.6 (CH_2), 30.2 (CH_2), 29.75 (CH_2), 29.68 (4 x CH_2), 28.2 (CH_2), 26.1 (CH_2), 24.3 (CH_2), 21.1 (CH_2), 19.4 (CH_3), 18.7 (CH_3), 11.8 (CH_3); MS, m/z 826 (M^+, 2), 808 (30), 793 (21), 790 (39), 775 (15), 257 (100); anal. calcd for $C_{58}H_{98}O_2 \cdot H_2O$: C, 82.40; H, 11.92; found: C, 82.70; H, 11.90.

Dimer **2**, identical in all respects with the compound **2** previously described, was obtained in the same manner from THP ether **14bp**.

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