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Efficient routes to Epimerically-Pure Side-chain Derivatives of Lanosterol

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Abstract

A technically simple route is described to individual epimers of side-chain derivatives of lanosterol (3ύ-hydroxy-5′ϒ-lanosta-8,24-diene). Epimerically pure 24,25-epoxy-, 24,25-dihydroxy- and 24-bromo-25-hydroxy-lanosterol have been prepared in good yield from commercial (50-60%) lanosterol. Hypophosphorous acid was used as a catalyst for the cohalogenation of the $\Delta^{24(25)}$ bond and also for the efficient conversion of 24,25-epoxy- and 24-bromo-25-hydroxylanosterol to epimerically pure 24(*R*) or 24(*S*)-24,25-dihydroxylanosterols.

Introduction

Side chain derivatives of lanosterol (3ύ-hydroxy-5′ϒ-lanosta-8,24-diene, **1a**) have been shown to act as inhibitors of $\Delta^{24(25)}$ sterol methyl transferase, which is an essential enzyme in the sterol biosynthesis pathway of protozoa, fungi and plants[1]. Because of this activity these compounds have potential therapeutic applications. 24,25-Epoxylanosterol is a

potent inhibitor of 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA), which is a key regulatory enzyme in the biosynthesis of sterols in humans[2]. There is an association between incidence of cardiovascular disease and levels of serum cholesterol[3], and for hypercholesterolemic individuals, who cannot control serum cholesterol by dietary modification, some other kind of intervention is indicated. For this reason there is intense interest in the development of inhibitors of cholesterol or sterol biosynthesis[4].

Reaction of the $\Delta^{24(25)}$ bond in general leads to the production of a mixture of C-24 epimers. It is probable that, where a mixture of epimers has been used in a bioactivity assay[5,6], the activity resides principally in one epimer and it is thus not possible accurately to gauge this activity. Differential bioactivity of 24(*R*)- and 24(*S*)-epoxylanosterols has been demonstrated[7]. Early reports of the separation of the epimers of 24,25-epoxylanosterol acetate by crystallization[8] have not been amenable to emulation more recently[7,9] and instead separation of the isomers was achieved by repetitive HPLC. No other separations of epimeric C-24 derivatives of lanosterol have been reported.

We here report a technically simple method for the production of individual C-24 epimers of lanosterol derivatives in high yield from commercial (50-60%) lanosterol, without recourse to highly toxic reagents, or tedious chromatography.

Experimental Procedure

Materials: Lanosterol (50-60%) was purchased from Sigma and used without any further purification. Hypophosphorous acid (50 wt.% in water) and all other reagents were purchased from Aldrich.

General Methods: Column chromatography was carried out on Merck silica gel (70-230 mesh) using the eluants indicated. TLC was carried out using Alugram® Sil G/UV_{254} plates.

Elemental analysis was carried out by the Chemistry Department of the University of Otago, New Zealand. All melting points were obtained on a Reichert-Jung micro-melting point determination apparatus and are uncorrected. Optical rotations were measured on a Ramsey AA-5 polarimeter. IR spectra were obtained using a Perkin Elmer M-1600 IR spectrophotometer. Compounds described as epimerically pure were ~97% based on NMR methods.

Gas Chromatography: Gas chromatography with flame ionization detection (GC-FID) was carried out using a Hewlett-Packard Ultra 2 (25m x 0.33mm) cross-linked phenyl methyl silicone fused silica capillary column. Conditions used were 55°C(1 min), 30°C min^{-1} to 200°C, 4°C min^{-1} to 320°C.

Nuclear Magnetic Resonance Spectrometry (NMR): NMR spectra were obtained using a Bruker ADV DRX 400 MHz spectrometer, in chloroform-d_1 and referenced to chloroform. ^{1}H, ^{13}C, DEPT135, H,H-Cosy, HSQC, HMBC and NOE difference experiments were carried out. For HSQC a *J* value of 147 Hz was used and for HMBC a delay value of 65 ms was used for evolution of long range couplings.

X-ray crystallography: X-ray data were obtained with a Bruker CCD diffractometer using Mo-Kα X-rays (λ=0.71073Å). Data were corrected for absorption and other effects using an empirical method (SADABS)[10] and the structures were solved and refined (based on F_o^2) using the SHELX programs[11]. All hydrogen atoms were included in calculated positions using a riding model. Absolute configuration was determined X-ray crystallographically from the anomolous dispersion for the hydroxy-bromides, and by reference to the known configuration of the main skeleton for the epoxide and diol. Full details of the structures have been lodged with the Cambridge Crystallographic Database, CCDC No XXXXXXX.

Acetylation of Lanosterol: To a solution of commercial lanosterol (a mixture of **1a** and **2a**, Scheme 1) (80 g, 0.187 mol, 50-60% pure), dimethylaminopyridine[5,12] (1.6 g, 0.013mol) and pyridine (120 mL) in CH_2Cl_2 (700 mL) was added acetic anhydride (30 mL) and the resulting solution was stirred (6h) at room temperature. The reaction mixture was poured into 300 mL of CH_2Cl_2 and washed successively with 5% HCl, 10% aqueous $NaHCO_3$ and water. After evaporation of the CH_2Cl_2 *in vacuo*, the residue was recrystallized from acetone/methanol to afford a mixture of lanosterol acetate, 3β-acetoxy-5α-lanosta-8,24-diene (**1b**) and dihydrolanosterol acetate (**2b**) as a colourless solid (72 g), mp 125-129°C (Lit.[13] 125-130°C). GC-FID indicates the product is *ca* 61:39 of **1b**:**2b**.

24(*R*)-3β-acetoxy-24,25-epoxy-5α-lanost-8-ene, (3b*R*), and 24(*S*)-3β-acetoxy-24,25-epoxy-5α-lanost-8-ene, (3b*S*): To a solution of the impure lanosterol acetate from above (**1b** and **2b**) (20 g, 0.028 mol) in dichloromethane (600 mL) a mixture of *m*-chloroperbenzoic acid (70%, 6.5 g, 0.03 mol) and sodium hydrogen carbonate (2.2 g, 0.05 mol) was added in the following manner: half was added at room temperature over 3 hours and the remainder at 0ºC (ice bath), also over 3 hours. The mixture was stirred vigorously (1 hour) and left in a refrigerator overnight. The mixture was washed with water, dried ($MgSO_4$) and after evaporation of the solvent the crude residue was purified by flash column chromatography (50% dichloromethane in light petroleum) giving unreacted dihydrolanosterol acetate (**2b**) (7.4 g, 96% recovery) as a colourless solid. Further elution with dichloromethane afforded 24(*R*,*S*)-3β-acetoxy-24,25-epoxy-5α-lanost-8-ene, (**3b**) as a colourless solid (10.8g, 85% based on **1b** used), mp 172-180ºC. The mixed epoxides were crystallized from hexane (30

mL) at room temperature to give 24(*R*)-3β-acetoxy-24,25-epoxy-5α-lanost-8-ene, **3b*R*** (5.4 g). This product was recrystallised twice from acetone to give pure **3b*R*** (3.9 g) mp 194-196°C (Lit[8,9] 194-197°C or 194-195.5°C) ; $[\alpha]_D^{20}$ +63.7° (*c*1.6, $CHCl_3$); (Found: C, 79.06; H, 10.57. $C_{32}H_{52}O_3$ requires C, 79.35; H, 10.73%). The filtrate from the first crystallisation was evaporated and the residue containing crude 24(*S*)-3β-acetoxy-24,25-epoxy-5α-lanost-8-ene, **3b*S***, (5.2 g) was purified by fractional crystallization from hexane and recrystallization from acetonitrile which afforded pure **3b*S*** (3.6 g), mp 137-139°C (Lit.[8,9] 137-139°C or 140-141.5°C); $[\alpha]_D^{20}$ +56.22° (*c*1.6,$CHCl_3$); (Found: C, 79.22; H, 10.66%. $C_{32}H_{52}O_3$ requires C, 79.35; H, 10.73%). The absolute configurations of **3b*R*** and **3b*S*** were determined by X-ray crystallography of **3b*R***.

24(*R*)-3β-hydroxy-24,25-epoxy-5α-lanost-8-ene (3a*R*) and 24(*S*)-3β-hydroxy-24,25-epoxy-5α-lanost-8-ene (3a*S*): The acetate **3b*S*** (1.2 g, 2.1 mmol) was treated with dry ethanolic 10% KOH with stirring at 60°C for 2 hours. Precipitation with water, extraction into ether and crystallization from hexane/ethyl acetate afforded stereochemically pure 24(*S*)-3β-hydroxy-24,25-epoxy-5α-lanost-8-ene, **3a*S*** (0.86 g, 92%), mp123-124°C (Lit.[7] 120-123°C); $[\alpha]_D^{20}$ +51.2 (c 1.6, $CHCl_3$); (Found: C, 81.21; H, 11.33%. $C_{30}H_{50}O_2$ requires C, 81.46; H, 11.30%). Similar treatment of **3b*R*** (1.2 g, 2.1 mmol) afforded stereochemically pure 24(*R*)-3β-hydroxy-24,25-epoxy-5α-lanost-8-ene **3a*R*** (0.83 g, 89%), mp 130-131°C (Lit.[7,8] 131-134°C); $[\alpha]_D$ +67.5 (c 1.6, $CHCI_3$). (Found: C, 81.19; H, 11.13%. $C_{30}H_{50}O_2$ requires C, 81.46; H, 11.30%).

Conversion of the epoxy acetates, 3b*R* and 3b*S*, to the corresponding diols, 4b*R* and 4b*S*: To a solution of the epoxy acetate, **3b*S*** (5.0 g, 0.01 mol) in 2-propanol (125 mL) was added water (50 mL) and hypophosphorous acid (9 mL, 50% in water). The mixture was

heated to reflux (3h), diluted with water, filtered and washed. After drying, the residue was purified by column chromatography (CH_2Cl_2:EtOAc, 2:1) to afford **4b*S*** (4.82 g, 93%), mp 176-177°C; $[\alpha]_D^{20}$ +26.2 (*c* 1.6, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3540, 3410, 1723, 1468, 1371, 1259, 1164. (Found: C, 76.32; H, 10.75%. $C_{32}H_{54}O_4$ requires C, 76.44; H, 10.83%).

Similar treatment of **3b*R*** afforded **4b*R*** (4.92 g, 95%), mp 186-187°C; $[\alpha]_D^{20}$ +40.0 (*c* 1.6, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3540, 3410, 1723, 1468, 1372, 1257, 1162. (Found: C, 76.10; H, 10.76%. $C_{32}H_{54}O_4$ requires C, 76.44; H, 10.83%). The absolute configurations at C-24 of **4b*R*** and **4b*S*** were confirmed by X-ray crystallography on crystals of the ***S*** epimer.

24(*R*)-3β-acetoxy-24-bromo-25-hydroxy-5α-lanost-8-ene (5b*R*) and 24(*S*)-3β-acetoxy-24-bromo-25-hydroxy-5α-lanost-8-ene (5b*S*) : To a solution of lanosterol acetate (**1b**) (10 g, 61% **1b**, 0.014 mol) in acetone (900 mL), water (15 mL) and hypophosphorous acid (2.7 mL, 50% aqueous) was added *N*-bromosuccinimide (3.04 g, 0.017 mol). The reaction mixture was stirred at room temperature (5 min) and diluted with water. The product was extracted into ether, washed thoroughly with saturated aqueous sodium thiosulphate, dried ($MgSO_4$) and evaporated under reduced pressure to give a crude mixture of unreacted dihydrolanosterol acetate (**2b**) and 24(*R*,*S*)-3β-acetoxy-24-bromo-25-hydroxy-5α-lanost-8-ene (**5b**) (11.7 g), which was fractionated by flash column chromatography. Elution with hexane/ether (5:1) gave dihydrolanosterol acetate (**2b**) (3.7 g, 95% recovery) with a minor by-product. Further elution with hexane/ether gave **5b** (5.12 g, 84%).

Two-fold recrystallisation from hexane (60 mL) afforded crude 24(*S*)-3β-acetoxy-24-bromo-25-hydroxy-5α-lanost-8-ene (**5b*S***, 2.52 g). This crude product was recrystallized twice from hexane to give pure **5b*S*** (2.1 g), mp 163-165°C; $[\alpha]_D^{20}$ +23.1° (*c*1.6, $CHCl_3$). (Found: C,

68.14; H, 9.21; Br,14.20%. $C_{32}H_{53}O_3Br$ requires C, 68.00; H, 9.38; Br,14.14%). The configuration at C-24 was assigned by X-ray crystallography of both a monoclinic and an orthorhombic polymorph.

The combined filtrates from the first recrystallisations were evaporated under reduced pressure and purified by fractional crystallization from hexane followed by two-fold crystallization from hexane/methyl acetate to afford 24(*R*)-3β-acetoxy-24-bromo-25-hydroxy-5α-lanost-8-ene (**5b*R***, 2.1 g), mp 157-159°C; $[\alpha]_D^{20}$ +66.8° (*c*1.6, $CHCl_3$). (Found: C, 68.28; H, 9.64; Br,14.01%. $C_{32}H_{53}O_3Br$ requires C, 68.00; H, 9.38; Br,14.14%). This form was also fully characterised by an X-ray structure determination.

Conversion of hydroxybromides, 5b*S* and 5b*R*, to the corresponding diols, 4b*R* and 4b*S*:

A solution of **5b*S*** (2.0 g, 3.5 mmol) in dry 2-propanol (100 mL) and anhydrous potassium acetate (1.0 g, 10.2 mmol) was heated under reflux (2h). The reaction mixture was diluted with hot water (6 mL), hypophosphorous acid (60 μL, 50% in water) was added and the mixture further refluxed (2h). The reaction mixture was cooled, diluted with water and extracted into EtOAc. The EtOAc solution was washed successively with water, $NaHCO_3$ (5%) and saturated NaCl solution and then dried and evaporated to a white residue. Column chromatography (CH_2Cl_2:EtOAc, 2:1) afforded **4b*R*** (1.48 g, 84%), identified by NMR spectroscopy.

Similar treatment of **5b*R*** afforded **4b*S*** (1.43g, 81%).

X-ray crystallography:

Structure of the R-epoxide, **3bR:** *Crystal data*: $C_{32}H_{52}O_3$, M = 484.74, orthorhombic, a = 10.6022(1), b = 36.3388(3), c = 7.4573(1) Å, U 2873.08(5) $Å^3$, T -123°C, space group $P2_12_12_1$, Z = 4, F(000) = 1072, μ(Mo-K_α) = 0.069 mm^{-1}, 16833 reflections collected, 5810

unique (R_{int} 0.0271) in the range 1°< θ < 26.5°, corrected for absorption ($T_{max, min}$ 0.993, 0.973). Crystal dimensions 0.40 x 0.38 x 0.10 mm^3. Refinement gave R_1 0.0473 [5076 data with I > 2σ (I)] and wR_2 0.1192 (all data), GoF 1.153.

Structure of the S-diol, **4bS:** *Crystal data:* $C_{32}H_{54}O_4$, M 502.75, orthorhombic, a = 6.0576(4), b = 9.5444(6), c = 50.937(3) Å, U = 2945.0(3) $Å^3$, T 164 K, space group $P2_12_12_1$, Z = 4, F(000) 1112, μ(Mo-Kα) 0.07 mm^{-1}, 17278 reflections collected, 5906 unique (R_{int} 0.0303), in the range 1.5° < 2θ < 26.2°, corrected for absorption ($T_{max,min}$ 0.982, 0.978). Crystal dimensions 0.32 x 0.30 x 0.26 mm^3. Refinement gave R_1 0.0474 [I > 2σ (I) data] and wR_2 0.1218 (all data), GoF 1.076.

Structure of the S-hydroxy bromide, **5bS** (monoclinic form)**:** *Crystal data*: $C_{32}H_{53}O_3Br$, M = 565.65, monoclinic, a = 7.4488(1), b = 19.5340(1), c = 20.8918(3) Å, β = 91.287(1)°, U 3039.09(6) $Å^3$, T -123°C, space group $P2_1$, Z = 4, F(000) = 1216, μ(Mo-K_α) = 1.38 mm^{-1}, 18108 reflections collected, 10970 unique (R_{int} 0.0179) in the range 1°< θ < 26.5°, corrected for absorption ($T_{max, min}$ 0.643, 0.424). Crystal dimensions 0.75 x 0.40 x 0.35 mm^3. Refinement gave R_1 0.0272 [9979 data with I > 2σ (I)] and wR_2 0.0658 (all data), GoF 1.011, absolute structure Flack x parameter 0.004(4).

Structure of the S-hydroxy bromide, **5bS** (orthorhombic form)**:** *Crystal data*: $C_{32}H_{53}O_3Br$, M = 565.65, orthorhombic, a = 7.5434(1), b = 11.4508(2), c = 35.3936(5) Å, U 3057.23(8) $Å^3$, T -123°C, space group $P2_12_12_1$, Z = 4, F(000) = 1216, μ(Mo-K_α) = 1.37 mm^{-1}, 17512 reflections collected, 5752 unique (R_{int} 0.0284) in the range 1°< θ < 25.5°, corrected for absorption ($T_{max, min}$ 0.885, 0.578). Crystal dimensions 0.72 x 0.40 x 0.12 mm^3. Refinement gave R_1 0.0304 [5300 data with I > 2σ (I)] and wR_2 0.0763 (all data), GoF 1.048, absolute structure Flack x parameter 0.022(6).

Structure of the R-hydroxy bromide, **5bR:** *Crystal data*: $C_{32}H_{53}O_3Br$, M = 565.65, monoclinic, a = 12.7619(1), b = 6.7924(1), c = 17.3796(1) Å, β = 101.73(1)°, U 1475.06(3) Å3, T -123°C, space group $P2_1$, Z = 2, F(000) = 608, μ(Mo-K_α) = 1.42 mm-1, 8721 reflections collected, 5292 unique (R_{int} 0.0266) in the range 1.5°< θ < 26.5°, corrected for absorption ($T_{max, min}$ 0.919, 0.458). Crystal dimensions 0.65 x 0.08 x 0.06 mm^3. Refinement gave R_1 0.0341 [4565 data with I > 2σ (I)] and wR_2 0.0667 (all data), GoF 0.953, absolute structure Flack x parameter 0.019(6).

Results and Discussion

Epoxidation of acetylated commercial lanosterol (mixture of **1b** and **2b** in the approximate ratio 61:39) afforded a mixture of the epimeric 24,25-epoxylanosterol acetates (**3b*R*** and **3b*S***) (Scheme 1). After column chromatography to remove **2b**, the epoxides were separated by fractional crystallization from hexane, making use of the much higher solubility of the ***S*** epoxide in hexane. This procedure was based on one previously reported[8] but the separation method has been improved, since the earlier one was apparently not reproducible[7,9]. (A similar method has been applied very recently for obtaining pure samples of **2b**, but the epoxides were not further separated[10]). The individual epimeric acetates **3b*R*** and **3b** (and the de-acetylated alcohols **3a*R*** and **3a*S***) were characterized by NMR. Only the signals for the side chain atoms are reported in Table 1, with the full carbon and proton chemical shifts of the acetates and corresponding free sterols listed in Supplementary Tables 1 - 3 respectively since they match literature values, where they are available[14].

The earlier study[8] deduced the absolute stereochemistry of the epoxides by chemical methods via reduction to the corresponding 24-OH species, and we have now confirmed the assignment by an X-ray crystal structure determination (See Fig 1a for the conformation of

the side-chain of **3b*R***). For these epoxides, the separate epimers are best identified by their melting points and by their optical rotation, because there are insufficient differences in the ^{1}H NMR spectra for reliable discrimination, and the ^{13}C spectra can only just distinguish them[9].

The epoxides are useful materials for preparing new derivatives. Upon refluxing in 2-propanol with aqueous hypophosphorous acid, **3b*R*** and **3b*S*** afforded the corresponding epimers of the new diols, 24,25-dihydroxylanosterol acetate, **4b*R*** and **4b*S*** respectively, in excellent yield (Scheme 1). 2-Propanol was the solvent of choice to afford miscibility with water without competing as a nucleophile and thereby forming unwanted ethers, which arose if methanol or ethanol was used instead. It was reported earlier that water-soluble organo-halides could be reduced in high yields using hypophosphorous acid, AIBNA and $NaHCO_3$ in water [15] but there appears to be no precedent for use of hypophosphorous acid for the synthesis of vicinal diols from epoxides. This acid appears to as efficient (and as free from by-products) for the opening of the epoxides as the more more usual perchloric acid[16], without the associated hazards of the latter.

The diols **4b*R*** and **4b*S*** were fully characterized. Selected side-chain carbon and proton NMR chemical shifts are reported in Tables 1 and 2 respectively, with full assignments in the Supplementary information. In this case, the epimers could be clearly distinguished, and the epimeric purity assessed, by the ^{1}H signals from the protons on C-24. For the ***R*** epimer this appears as a pseudo triplet at δ 3.32 (apparent J = *ca* 6 Hz), while the equivalent ***S*** is an apparent doublet (J = 10 Hz) at δ 3.26.

The high yield and the retention of configuration on going from the epoxide to the diol indicates that the reaction proceeds by the predicted[16] nucleophilic attack upon C-25 under these acidic conditions.

The assignment of configuration for the diols **4b** was confirmed by an X-ray crystal structure determination on **4b*S***, the side-chain being that illustrated in Fig 1b. Interestingly the ***R*** and ***S*** diol epimers form isomorphous crystals, with one very long (51 Å) axis so cannot be distinguished on the basis of unit cell and space-group measurements. Because of this they tend to co-crystallise so epimerically-impure samples cannot be readily purified by recrystallisation.

An alternative route for epimeric separation of lanosterol side-chain species was developed using novel hydroxy-bromide derivatives. Thus treatment of acetylated commercial lanosterol with *N*-bromosuccinimide in acetone, water and in the presence of a catalytic amount of hypophosphorous acid, (Scheme 1), afforded the epimeric 24-bromo-25-hydroxylanosterol acetates (**5b*R*** and **5b*S***), which were separated from unreacted **2b**, by flash column chromatography and from each other by crystallisation. It is significant that this procedure gives only 24-Br, 25-OH derivatives regiospecifically, whereas mixtures of isomers are more normally obtained. Solvent choice is critical since the same reaction in CH_2Cl_2 lacks this specificity.

In the NMR spectra of **5b*R*** and **5b*S*** the ^{1}H patterns are again sufficiently different to distinguish the epimeric forms, through the signals of the protons attached to C-22, C-23 and C24 (see Table 2). The absolute configuration of C-24 was assigned for both epimers by X-ray crystallography. The structures of the side-chains of **5b*R*** and **5b*S*** are shown in Figures 1c and 1d.

The hydroxy-bromides, **5b**, could also be converted to the diols, **4b**, using a one-pot procedure. When the (*S*)-hydroxybromide **5b*S*** was used as the starting material the (*R*)-diol **4b*R*** resulted, showing inversion of configuration at C-24 with high stereospecificity . This can be rationalised assuming there is initial conversion of **5b*S*** to the epoxide **3b*R*** with

inversion of configuration and subsequent *in situ* conversion to the diol with retention of configuration.

The crystal structure determinations were carried out to provide unambiguous correlation between absolute configuration and physical properties. For the most part the structures were unremarkable. The epoxide **3b*R*** packs in the crystal with only weak C-H...O interactions, as expected. All of the others have structures that are controlled by hydrogen-bonding interactions. The diol **4b*S*** forms chains of molecules linked by hydrogen-bonding from O(3)-H...O(4')-H, with the arrangement of molecules such that the opposite configuration at C-24 can be accommodated with little rearrangment, which explains the isomorphism/ co-crystallisation of the two forms. For the S-hydroxy-bromide **5b*S*** there are two crystal polymorphs. The monoclinic form has two independent molecules which are linked as H-bonded trimers -O(3)-H...O(3')-H...O(12")=C<, whereas the orthorhombic form has simpler packing involving linear chains of molecules packed head-to-tail with O(3)-H...O(12')=C< interactions. For the R-hydroxybromide **5b*R*** the same O(3)-H...O(12')=C< link is used but the molecules are now stacked side-to-side to generate a zig-zag chain.

Conclusion.

In conclusion, we report here (i) an improved route to separated epimers of the 25, 25-epoxide of lanosterol; (ii) the conversion of the R and S epoxides to the corresponding 24, 25-diols with retention of configuration; (iii) a route to the pure epimers of novel 24-Br, 25-OH derivatives of lanosterol; and (iv) conversion of the R and S bromo-hydroxides to the S and R diols respectively. In each case the transformation and separation of the epimers are experimentally straightforward with good yields. This work also establishes a new procedure using hypophosphorous acid as an efficient reagent for regioselective hydroxy-bromination of olefins, and for ring-opening reactions of epoxides.

The absolute configurations were established unambiguously via X-ray structure determinations. These species should prove to be useful for preparing other epimerically pure side-chain compounds for biological use.

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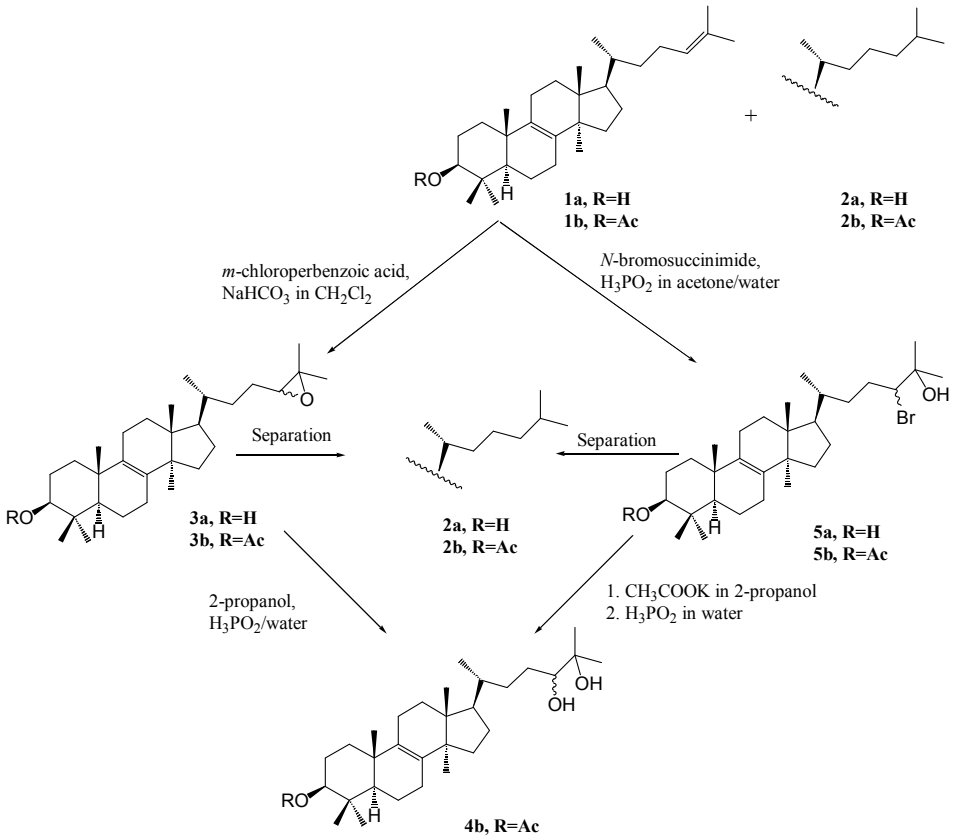
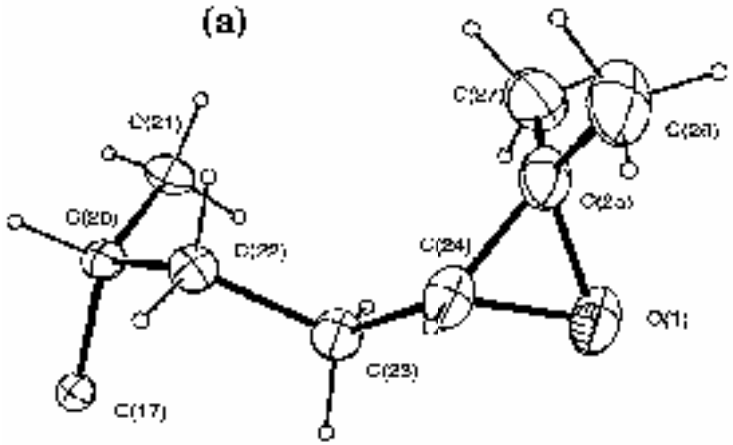
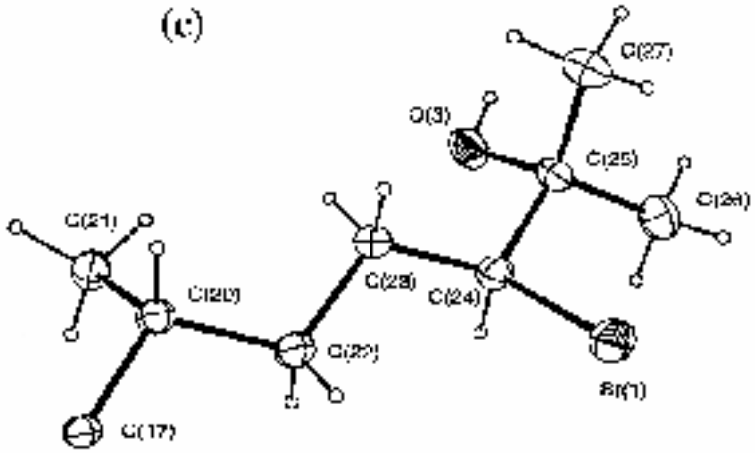
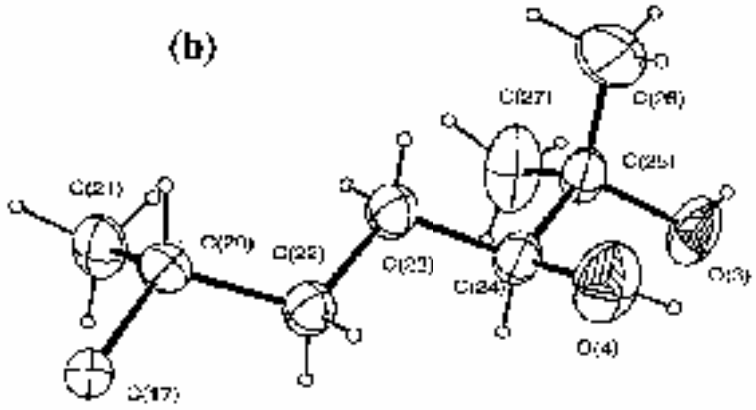

RO
H
1a, R=H
1b, R=Ac
+
2a, R=H
2b, R=Ac
m-chloroperbenzoic acid,
NaHCO3 in CH2Cl2
N-bromosuccinimide,
H3PO2 in acetone/water
O
Separation
Separation
OH
Br
3a, R=H
3b, R=Ac
2a, R=H
2b, R=Ac
5a, R=H
5b, R=Ac
2-propanol,
H3PO2/water
1. CH3COOK in 2-propanol
2. H3PO2 in water
OH
OH
4b, R=Ac

Captions to Figures

Figure 1. The conformations of the side chains for the structures of: (a) the *R*-epoxide **3b*R***; (b) the *S*-diol **4b*S***; (c) the *S*-hydroxybromide **5b*S*** (in the orthorhombic form); and (d) the *R*-hydroxybromide **5b*R***.


(a)
C(21)
C(20)
C(22)
C(24)
C(23)
C(17)
O(1)


(c)
C(27)
O(3)
C(25)
C(26)
C(21)
C(20)
C(22)
C(24)
C(17)
Br(1)


(b)
C(27)
C(26)
C(25)
C(21)
C(20)
C(22)
C(23)
C(24)
O(3)
O(4)
C(17)

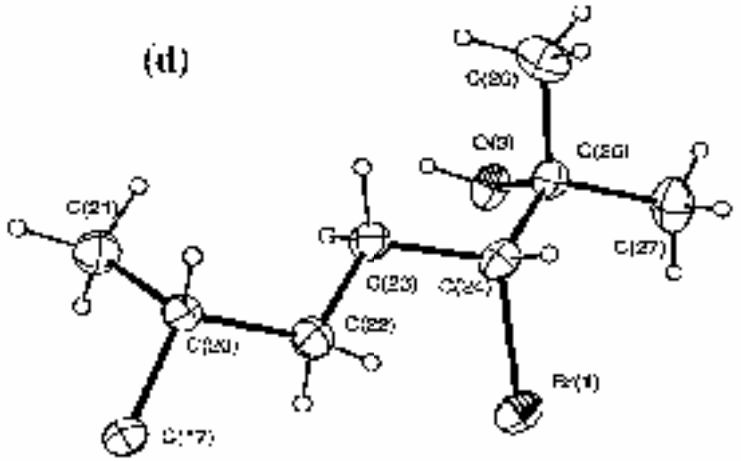

(d)
C(26)
O(3)
C(25)
C(21)
C(27)
C(23)
C(24)
C(22)
C(20)
Br(1)
C(17)

Table 1 ^{13}C and ^{1}H NMR Assignments of Side-chains of Acetates of Lanosterol Derivatives

C	3b*R*	3b*S*	4b*R*	4b*S*	*5bR*	5b*S*
	Carbon Chemical Shift (ppm)					
20	36.27	36.4	36.6	37.06	35.84	36.87
21	18.72	18.62	18.86	19.14	18.71	19.42
22	32.64	32.84	33.45	33.9	35.39	36.01
23	25.67	25.97	28.73	29.03	31.03	31.63
24	64.85	65.00	79.05	79.22	72.5	73.41
25	58.49	58.20	73.45	73.50	72.88	72.94
26	24.99	25.01	26.85	26.82	27.09	27.01
27	18.80	18.70	23.61	23.53	26.11	26.16
28	27.95	27.96	28.22	28.22	28.23	28.23
	Proton Chemical Shift (ppm), Coupling constants (Hz), NOE Enhancement (%)					
20	1.42	1.45	1.40	1.36	1.40	1.41
21	0.91 $J_{21,20}$=6.2Hz	0.91 $J_{21,20}$=5.7Hz	0.89 $J_{21,20}$=6.1Hz	0.89 $J_{21,20}$=6.3 Hz	0.90 $J_{21,20}$=5.7Hz	0.92 $J_{21,20}$=6.3Hz
22	1.20, 1.51	1.61, 1.10	1.47, 1.26	0.95, 1.73	1.40, 1.52	1.05, 1.88
23	1.39, 1.65	1.46, 1.58	1.36	1.10, 1.52	1.79	1.58, 1.99
24	2.69 (6.2 & 6.2Hz)	2.69 (6.1& 6.1Hz)	3.32 (6.1 & 6.1Hz)	3.26 (2.0 & 10.0 Hz)	4.00 (3.8& 9.7Hz)	3.95 (1.8& 11.1Hz)
26[a]	1.30 (6%)	1.30 (5%)	1.19 (2%)	1.19 (5%)	1.36(3%)	1.35 (2.3%)
27	1.26 (0%)	1.26 (0%)	1.14 (0%)	1.13 (0%)	1.33 (1.8%)	1.33 (1.8%)
28	0.88	0.88	0.86	0.86	0.88	0.88
29	0.88	0.88	0.86	0.86	0.88	0.88
30	0.87	0.87	0.86	0.86	0.88	0.88

[a] Assigned on the basis that it displayed the largest NOE enhancement upon irradiation of H-24 (given in parenthesis).

Supplementary ***Table 1:***

^{13}C NMR Spectra of Side-chain Derivatives of Lanosterol Acetate

C	3bR	3bS	4bR	4bS	5bR	5bS
1	35.30	35.30	35.60	35.60	35.61	35.61
2	24.21	24.21	24.49	24.48	24.50	24.50
3	80.97	80.98	81.29	81.29	81.24	81.25
4	37.85	37.85	38.12	38.12	38.14	38.13
5	50.52	50.53	50.83	50.79	50.85	50.85
6	18.16	18.16	18.44	18.44	18.45	18.44
7	26.42	26.42	26.70	26.70	26.72	26.71
8	134.46	134.46	134.79	134.80	134.80	134.78
9	134.31	134.31	134.60	134.60	134.63	134.63
10	36.93	36.93	37.22	37.22	37.24	37.24
11	21.03	21.03	21.31	21.32	21.32	21.31
12	30.98	31.00	31.33	31.33	31.33	31.30
13	44.53	44.52	44.84	44.82	44.87	44.85
14	49.86	49.85	50.15	50.12	50.18	50.14
15	30.84	30.84	31.12	31.12	31.12	31.12
16	28.29	28.24	28.59	28.48	28.54	28.45
17	50.31	50.41	50.91	50.83	50.74	50.73
18	15.81	15.81	16.11	16.11	16.13	16.10
19	19.24	19.24	19.49	19.49	19.51	19.50
20	36.27	36.40	36.60	37.06	35.84	36.87
21	18.72	18.62	18.86	19.14	18.71	19.42
22	32.64	32.84	33.45	33.9	35.39	36.01
23	25.67	25.97	28.73	29.03	31.03	31.63
24	64.85	65.00	79.05	79.22	72.50	73.41
25	58.49	58.20	73.45	73.50	72.88	72.94
26	24.99	25.01	26.85	26.82	27.09	27.01
27	18.80	18.70	23.61	23.53	26.11	26.16
28	27.95	27.96	28.22	28.22	28.23	28.23

CH_3(acetyl)	21.40	21.40	21.60	21.60	21.61	21.61
CO (acetyl)	171.1	171.11	171.34	171.34	171.27	171.28
29	16.59	16.56	16.83	16.83	16.84	16.84
30	24.29	24.28	24.57	24.57	24.57	24.57

Supplementary Table 2

^{1}H NMR Spectra of Lanosterol Acetate Side-chain Derivatives

C	3b***R***	3b***S***	4b***R***	4b***S***	5b***R***	5b***S***
1	1.32α, 1.73β	1.31α, 1.73β	1.29 α, 1.70β	1.26α, 1.68β	1.31α, 1.73β	1.30α, 1.73β
2	1.62β, 1.70α	1.62β, 1.66α	1.61β, 1.66 α	1.56β, 1.64α	1.63β, 1.69α	1.60β, 1.70α
3	4.49 (11.5 & 4.5Hz)	4.49 (11.5& 4.5Hz)	4.48 (11.5& 4.5Hz)	4.48 (11.5& 4.5Hz)	4.50 (11.6& 4.5Hz)	4.50 (11.5& 4.5Hz)
5	1.14	1.14	1.14	1.10	1.13	1.15
6	1.55α, 1.67β	1.51α, 1.65β	1.49α, 1.65β	1.46α, 1.62β	1.52α, 1.67β	1.51α, 1.67β
7	2.05	2.04	2.02	1.99	2.04	2.04
11	2.04	2.03	1.99	1.96	2.03	2.03
12	1.70, 1.80	1.72, 1.76	1.68, 1.73	1.65, 1.70	1.69, 1.74	1.69, 1.74
15	1.17α, 1.59β	1.17α, 1.60β	1.16, 1.57	1.13, 1.54	1.19α, 1.60β	1.19α, 1.60β
16	1.32β, 1.93α	1.34β, 1.93α	1.94, 1.35	1.31, 1.89	1.34β, 1.97α	1.37β, 1.94α
17	1.50	1.49	1.48	1.45	1.50	1.49
18	0.69	0.69	0.68	0.67	0.70	0.69
19	1.00	1.00	0.99	0.99	1.00	1.00
20	1.42	1.45	1.40	1.36	1.40	1.41
21	0.911 $J_{21,20}$=6.2Hz	0.91 $J_{21,20}$=5.7Hz	0.89 $J_{21,20}$=6.1Hz	0.90 $J_{21,20}$=6.3 Hz	0.90 $J_{21,20}$=5.7Hz	0.92 $J_{21,20}$=6.3Hz
22	1.20, 1.51	1.61, 1.10	1.47, 1.26	0.95, 1.73	1.40, 1.52	1.05, 1.88
23	1.39, 1.65	1.46, 1.58	1.36	1.10, 1.52	1.79	1.58, 1.99
24	2.69 (6.2&6.2Hz)	2.69 (6.1&6.1Hz)	3.32 (6.1&6.1Hz)	3.26 (2.0&10.0 Hz)	4.00 (3.8& 9.7Hz)	3.95 (1.8& 11.1Hz)
26[a]	1.30 (6%)	1.30 (5%)	1.19 (2%)	1.19 (5%)	1.36(3%)	1.36(2.3%)
27	1.26(0%)	1.26 (0%)	1.14 (0%)	1.14 (0%)	1.33(1.8%)	1.33(1.8%)
28	0.88	0.88	0.86	0.86	0.88	0.88
CH_3[b]	2.05	2.05	2.03	2.02	2.04	2.04
29	0.88	0.88	0.86	0.86	0.88	0.88
30	0.87	0.87	0.86	0.86	0.88	0.88

[a] Assigned on the basis that it displayed the largest NOE enhancement upon irradiation of H-24(given in parenthesis).
[b] 3-*O*-acetyl group

Supplementary Table 3

^{13}C and ^{1}H NMR Chemical Shifts of 24(*R*)- and 24(*S*),25-epoxy-5-α-lanost-8-en-3-β-ol.

	3a*R*		**3a*S***	
C	**C**	**H**	**C**	**H**
1	35.65	1.23α, 1.73β	35.65	1.23α, 1.73β
2	27.91	1.55β, 1.65α	27.91	1.58β, 1.65α
3	79.02	3.22 (4.5 & 11.5 Hz)	79.02	3.23 (4.8 & 11.5Hz)
4	38.94	-	38.94	-
5	50.48	1.04	50.48	1.05
6	18.30	1.50α, 1.68β	18.30	1.50α, 1.68β
7	26.55	2.03	26.55	2.04
8	134.42	-	134.41	-
9	134.52	-	134.52	-
10	37.08	-	37.09	-
11	21.04	2.01	21.04	2.02
12	31.04	1.67, 1.73	31.06	1.67, 1.75
13	44.57	-	44.57	-
14	49.88	-	49.87	-
15	30.87	1.18α, 1.60β	30.87	1.18α, 1.60β
16	28.27	1.33β, 1.93α	28.23	1.35β, 1.93α
17	50.35	1.49	50.45	1.48
18	15.81	0.70	15.81	0.70
19	19.18	0.98	19.18	0.98
20	36.26	1.43	36.37	1.46
21	18.71	0.91 $J_{21,20}$=6.1Hz	18.62	0.91 $J_{21,20}$=6.0Hz
22	32.67	1.22, 1.52	32.86	1.09,1.61
23	25.67	1.39, 1.61	25.95	1.45, 1.55
24	64.82	2.70 (6.2&6.2Hz)	64.96	2.68 (6.0&6.0Hz)
25	58.42	-	58.12	-
26	24.96	1.30	24.97	1.30
27	18.78	1.26	18.68	1.26
28	28.00	1.00	28.00	1.00

29	15.44	0.81	15.44	0.81
30	24.28	0.88	24.28	0.88