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Studies on stereocontrolled epoxidations of bis-alicyclic alcohols in steroidal skeletons: preparation of eight diastereomerically pure epoxides from cholest-4-en-3β,6β-; -3β,6α-; -3α,6β- and -3α,6α-diols

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Abstract—Eight diastereomerically pure epoxides have been prepared from cholest-4-en-3β,6β; -3β,6α-; -3α,6β- and -3α,6α-diols via a combination of steric, protecting group and oxidant effects on stereocontrolled epoxidations of a bis-alicyclic alcohol system within the steroidal skeleton.

1. Introduction

In recent years, oxysterols have attracted a great deal of interest from biological scientists due to their effects on gene regulation,[1] cytotoxicity,[2] atherogenesis,[3] mutagenesis,[4] etc. It is generally believed that the activities are attributed to the numbers and configurations (steric effects) of the oxygenated functional groups (such as hydroxyl and ketone) on the rings as well as on the side chain. Because the availability of oxysterols is limited, both in number and quantity, studies in this field have been seriously hampered. As part of our project toward the development of robust synthetic methods for the preparation of polyhydroxylsterols, we have undertaken the synthesis of all possible stereoisomers of cholestan-3,4,5,6-tetrols, after our discovery of the potent activity of cholestan-3β,5α,6β,7β-tetrol against a number of cancer cell lines.[5] We have developed an efficient preparative syntheses of the cholest-4-en-3β,6β-; -3β,6α-; -3α,6β- and -3α,6α-diols **1**, **6**, **10** and **16**[6] (Table 1). Studies of stereocontrolled epoxidations to gain the eight diastereomerically pure epoxides are described herein.

The *syn*-directing effect of the hydroxyl of allylic and alicyclic alcohols is well known,[7] and the removal of this effect after alkylation to form ethers[8] or acylation to esters is also well documented.[9] In contrast to allylic alcohols, there have only been sporadic reports of epoxidations of bis-allylic and bis-alicyclic alcohols with examples of *cis*- or *trans*-selectivity. Henbest[10] reported the epoxidation of 3β, 6β-dihydroxy-cholest-4-ene **1** with a peracid, showing that the *syn*-directing effect of the hydroxyl functions is significant enough to drive the epoxidation to occur predominantly from the β-side. However, no investigation of the coordination effect of the dihydroxy substrates in bis-allylic and bis-alicyclic alcohol systems and of the effects of hydroxyl protecting groups and oxidants on the stereocontrol of such epoxidations has been reported. Herein we describe such an investigation into the epoxidation of the bis-alicyclic alcohols **1**, **6**, **10** and **16** and their acylated derivatives.

2. Results and discussion

The preparations of cholest-4-en-3,6-diols **1**, **6**, **10** and **16**, the diacetate **8** and the monoacetate **17** have been reported in our preceding paper.[6] The monoacetates **2**, **3**, **7** and **11** and the benzoyl esters **5**, **9**, **14**, **15**, **19** and **20** used in this paper were prepared either by the partial hydrolysis of the appropriate diacetates **4**, **8**, **13** and **18** or by reaction of benzoyl chloride or acetic anhydride with the appropriate alcohols in pyridine at room temperature.

Three typical epoxidation conditions, *m*-CPBA,[11] $VO(acac)_2$/TBHP[12] and Sharpless,[13] were investigated with cholest-4-en-diols **1**, **6**, **10** and **16** and their acetates **2**, **3**, **4**, **7**, **8**, **11**, **12**, **13**, **17** and **18**, benzoates **5**, **9** and **20** and acetate-benzoates **14**, **15** and **19** (Scheme 1). The results are summarised in Table 1.

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Table 1. The summary of the results of the epoxidations[a]

Substrate	3R	6R′	**i** *m*CPBA			**ii** $VO(acac)_2$/TBHP			**iii** $Ti(OiPr)_4$/TBHP/(−)DET		**iv** $Ti(OiPr)_4$ /TBHP/(+)DET		
			α/β Ratio[c]	Yield[b] (%)	Time (h)	α/β Ratio[c]	Yield[b] (%)	Time (h)	α/β Ratio[c]	Yield[b] (%)	α/β Ratio[c]	Yield[b] (%)	Time (h)
1	β-OH	β-OH	**0:100**	98	2	0:100	95	3	–[d]		–[d]		
2	β-OAc	β-OH	7:93	94	4	0:100	90	5	–[e]		–[e]		
3	β-OH	β-OAc	2:98	94	4	0:100	90	5	–[e]		–[e]		
4	β-OAc	β-OAc	**82:18**	71	288	–[d]			–[e]		–[e]		
5	β-OBz	β-OBz	75:25	75	96	–[d]			–[e]		–[e]		
6	α-OH	β-OH	38:62	70	1	**33:67**	93	0.5	–[d]		–[d]		
7	α-OH	β-OAc	91:9	84	5	**100:0**	90	1	–[e]		–[e]		
8	α-OAc	β-OAc	76:34	73	120	–[d]			–[e]		–[e]		
9	α-OBz	β-OBz	50:50	80	48	–[d]			–[e]		–[e]		
10	β-OH	α-OH	12:88	69	4	**0:100**	98	2	0:100	52	94:6	60	12
11	β-OAc	α-OH	42:58	88	11	13:87	82	5	–[d]		–[d]		
12	β-OH	α-OAc	2:98	78	11	0:100	86	5	–[e]		–[e]		
13	β-OAc	α-OAc	73:27	74	120	–[d]			–[e]		–[e]		
14	β-OAc	α-OBz	**95:5**	77	48	–[d]			–[e]		–[e]		
15	β-OBz	α-OBz	87:13	81	48	–[d]			–[e]		–[e]		
16	α-OH	α-OH	**100:0**	98	2	100:0	82	3	100:0	43	100:0	55	12
17	α-OAc	α-OH	27:73	75	4	**0:100**	95	5	–[d]		–[d]		
18	α-OAc	α-OAc	25:75	80	72	–[d]			–[e]		–[e]		
19	α-OAc	α-OBz	52:48	83	48	–[d]			–[e]		–[e]		
20	α-OBz	α-OBz	75:25	84	48	–[d]			–[e]		–[e]		

[a] Epoxidations were performed using the standard protocols.
[b] Yields of α+β.
[c] Calculated from ^{1}H NMR spectra.
[d] No reaction after 3 days.
[e] Did not test.

In this particular group of substrates, MCPBA showed superior reactivity to $VO(acac)_2$/TBHP and Sharpless conditions, and induced satisfactory stereoselectivity. MCPBA effected epoxidation of all substrates with good to excellent yields. The reaction rates with diacetate substrates **4**, **5**, **8**, **9**, **13**, **14**, **15**, **18**, **19** and **20** were dramatically lower because of increased steric hindrance and lack of coordination between MCPBA and the free hydroxyl group. The $VO(acac)_2$/TBHP oxidant generally gave better stereoselectivity than MCPBA with substrates **1**, **2**, **3**, **6**, **7**, **10**, **11**, **12**, **16** and **17**, which bear one or two free hydroxyl groups but failed to react with the diacetates **4**, **5**, **8**, **9**, **13**, **14**, **15**, **18**, **19** and **20.** This demonstrates the importance of coordination between the oxidant and the hydroxyl group in the $VO(acac)_2$/TBHP epoxidation reactions. Sharpless conditions effected epoxidations of **10** and **16** only, possibly due to steric hindrance of the steroidal skeletons blocking the approach of the oxidant complex to the double bond.

When the 3- and 6-hydroxyl groups are of the same configuration (both β or α), **1** and **16**, MCPBA and $VO(acac)_2$/TBHP afforded 100% *cis*-epoxidation products, resulting from an enhanced *syn* directing effect, implying that when the two hydroxyl groups are of the same configuration they are involved in anchoring the active oxygen of MCPBA or $VO(acac)_2$/TBHP to approach the C-C=C plane from the same side (if this was not so there should be less *cis*-epoxidation because of increased *cis*-side congestion).

When the 3- and 6-hydroxyl groups are opposite in configuration the epoxidation with all three oxidants occurred with surprisingly high stereoselectivity. Thus, epoxidation of **6** furnished a 1:2 mixture of α-isomer:β-isomer with both MCPBA and $VO(acac)_2$/TBHP. Epoxidation of **10** with MCPBA gave rise to 88% β-isomer and with $VO(acac)_2$/TBHP the β-isomer was formed with 100% selectivity. This result indicates that the α configured hydroxyl group exerts less *syn* directing effect than the β hydroxyl. In particular, the influence of the 6α-hydroxyl group on the reaction is minimal. This is reminiscent of Lavie's findings that both 4α-hydroxycholest-5-ene and 6β-hydroxycholest-4-ene underwent *cis*-epoxidation with a peracid, but 6α-hydroxycholest-4-ene gave a mixture of products.[14]

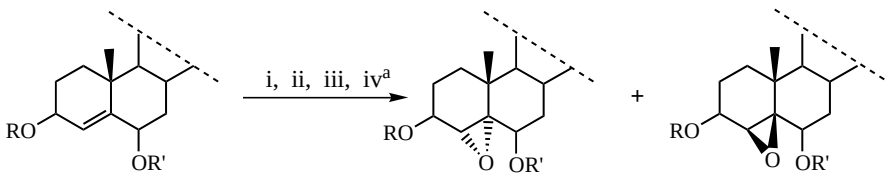
It has been observed that acylation of the hydroxyl group of alicyclic alcohols, cyclohexenol[10] and oxysteroids,[14] resulted in a reorientation of peracid epoxidations from *cis*- to *trans*-stereoselectivity. This is generally attributed to steric hindrance created on the normally favoured *cis*-side by the acetate group, and to prevention of coordination between the oxidant and the hydroxyl group of the substrate.[15]

However, in this study we found that the effect of acylation is much more complicated. When the 3- and 6-hydroxyl groups are β oriented, epoxidation of monoacetates **2** and **3** led to only slightly reduced stereoselectivity in reactions with MCPBA. However, when the 3- and 6-hydroxyl groups are α configured, reaction of a monoacylated substrate with $VO(acac)_2$/TBHP surprisingly led to a total reorientation of stereoselectivity from α-side to β-side, furnishing a single β-isomer in the reaction of **17**. In the cases of the 3α- and 6β-hydroxyl substrates, e.g. **7**, after acylation of the 6β-hydroxyl group, the 3α-OH directed *cis*-epoxidation product dominated; with MCPBA the α-epoxide formed in 91% yield and with $VO(acac)_2$/TBHP the α-epoxide formed with 100% stereoselectivity. In contrast, in the substrates with 3β- and 6α-OH, acylation of the 3β-OH, (compound **11**) failed to increase α-side stereoselectivity. Again, this demonstrated that the 6α-OH exerts little α-side directing effect. In the epoxidation of the 6α-*O*-acylated substrate **12**, β-side epoxidation predominated, a result of the directing influence of the 3β-OH.

In general, when both 3-OH and 6-OH were acetylated, epoxidation occurred selectively from the opposite side to that of its free hydroxy congener, such as the epoxidations of **4**, **8**, **13** and **18**, which were opposite to **1**, **6**, **10** and **16**.

The effect of replacement of the acetyl group with the larger Bz was investigated with the expectation maximising reorientation and selectivity in the epoxidation reactions. The experiments showed Bz apparently exerted less steric hindrance than acetate, e.g. **5**, **9** and **20** furnished less of the re-oriented epoxides compared to **4**, **8** and **18**. Surprisingly, epoxidation of the mixed esters **14** and **19** (with the 3- and 6-OH protected as Ac and Bz esters) resulted in higher levels of the re-oriented epoxides. In particular **14** afforded almost a single isomer on oxidation with MCPBA.

The importance of transition state geometries of allylic[7] and alicyclic[17] alcohols, and in particular, the torsion



1-20

Scheme 1. Epoxidations. [a] See Table 1.

(dihedral) angle of O-C-C=C in the determination of the orientations of epoxidation products has been demonstrated with peracids[16] and $VO(acac)_2$/TBHP.[7] Whitham[16] first proposed a transition state geometry for peracid epoxidations of alicyclic alcohols. In his cyclohexenol model, the optimal arrangement for high *cis*-stereoselectivity and a faster reaction is that the OH occupies a pseudo-equatorial conformation, with a torsion angle of about 150°.[17] Later with the same group of compounds he found a pseudo-axial OH, with a torsion angle of approximately 90°[17] to be responsible for rapid *cis*-selective epoxidation with $VO(acac)_2$/TBHP.[18] A number of epoxidations of the allylic fragments of steroids have been reported, some of which were clearly *cis*-stereoselective,[10] while some were not.[14]

We calculated torsion angles of O-C(3)-C(4)=C(5) and O-C(6)-C(5)=C(4) fragments in the substrates **1**, **6**, **10**, **16** and some of their acetates (Fig. 1). The torsion angle of the 3α-OH is about 108°, while 3β is about −140° (on the opposite side). The torsion angle of the 6α-OH is very small (about 5°), implying that O-C(6)-C(5)=C(4) are almost in the same plane; while 6β is about −105°. Mono- or diacylation has little effect on the torsion angles. None of these torsion angles match those for *cis*-stereoselectivity proposed above, however, the reason for the behaviour of the 6α-OH substrates is clear: Because the oxygen of the 6α-OH is in the same plane with the C(6)-C(5)=C(4) fragment, its transition state geometry is out of line with all predictions. Still, its predominant *trans*-directing effect in the $VO(acac)_2$/TBHP epoxidations of **11** and **17** remains a question, because, in this case, the transition state geometry is perfect for oxidation of an alicyclic alcohol into its enone form according to Ternaishi's model.[17] The dominant *cis*-directing effect of the 3α, 3β and 6β-OH in the epoxidations of this group of substrates with MCPBA and $VO(acac)_2$/TBHP means the torsion angles of their transition states are not that restrictive.

Other epoxidation conditions including chloral hydrate/hydrogen peroxide[19] and dioxarine/oxone/acetone,[20] were also tested on this bis-alicyclic alcohol system, but were found to be ineffective.

In summary, the eight target epoxides have been synthesised in a diastereomerically pure form from the free alcohols **1**, **7**, **10** and **16**, the monoacetate **17** and the di-acetate **14**. The remaining two were formed indirectly; 4,5α-epoxycholest-3β,6β-diol from **4** after hydrolysis and recrystallisation from MeOH, and 4,5β-epoxycholest-3α,6β-diol from the crude product of **6** via di-acetylation, recrystallisation and deprotection. The results show that in this particular group of bis-alicyclic steroidal alcohols, the 3α, 3β and 6β-OH exert *syn* directing effects with MCPBA and $VO(acac)_2$/TBHP, and the enhanced *syn* directing effect was observed when two hydroxyl groups are of the same configuration. In directing the orientation of epoxidations, the 6α-OH behaves differently, *trans*-directing with $VO(acac)_2$/TBHP in particular, e.g. **11** and **17**. Overall, the study shows that by acylation of the hydroxyl functions, the directing effects of the substituents can be manipulated and with careful selection of oxidant high stereoselectivities can be achieved in the epoxidation reactions of these steroid substrates.

3. Experimental

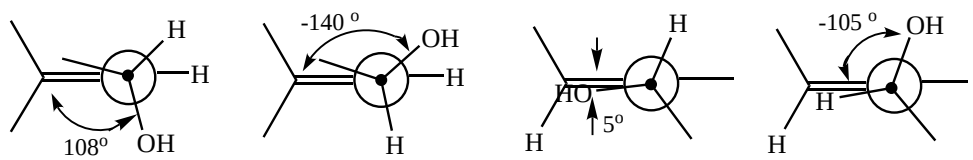
3.1. General

Melting points were obtained on a Reichert-Jung Microthermal apparatus, and are uncorrected. IR spectra were recorded on a Mattson 3000 instrument and NMR spectra at 250 MHz on a Bruker AC-250. Mass spectra (MS) were obtained on a HP G1034C GC/LC-MS Chemstation using atmospheric chemical-ionisation (APCI) method. High-resolution mass spectra (HRMS) were measured on Finnigan MAT 900 XLT high-resolution double-focusing mass spectrometer using electrospray method. Flash chromatography was performed on 200–400 mesh silica-gel and thin-layer chromatography (TLC) was performed using 0.25 mm Merck Kieselgel 60 F254 precoated silica gel plates.

The semi-empirical program MOPAC (ChemOffice Ver. 4.0) was used for the calculations throughout this work. The structure optimisation of compound **10** was also done by ab initio methods at RHF 3-21G level by using the program Gamess (Ver. 6.0) on a PC.

3.2. General procedure of MCPBA epoxidations

The cholest-4-en-3,6-diol or its derivative (1 mmol) and MCPBA (207 mg, 1.2 mmol) were dissolved in DCM (10 mL) and the mixture was stirred at room temperature for a given period (see Table 1). The resulting mixture was washed with 10% aqueous sodium hydroxide and water, then dried over sodium sulphate. Removal of the solvent gave the crude product for further purification (see individual compounds below for details).



3α-OH, O-C_3-C_4=C_5 3β-OH, O-C_3-C_4=C_5 6α-OH, O-C_6-C_5=C_4 6β-OH, O-C_6-C_5=C_4

Figure 1. Torsion angles of 3α-, 3β-,6α- and 6β-OH of cholest-4-en-3,6-diols.

3.3. General procedure of VO(acac2)$_2$/TBHP epoxidations

To a solution of the cholest-4-en-3,6-diol or its derivative (1 mmol) and vanadyl acetylacetonate (5.3 mg) in DCM (10 mL) was added dropwise the TBHP/toluene solution (4 M, 0.75 mL, 3.0 mmol). The resulting homogeneous solution was stirred at room temperature for a given period (Table 1). When TLC showed completion of the reaction, the mixture was washed with saturated sodium bicarbonate (2×20 mL) and brine (2×20 mL), then dried over $MgSO_4$. After removal of the solvent, the residue was subjected to further purification (see individual compounds below for details).

3.4. General procedure of Sharpless epoxidations

Freshly distilled titanium tetra-*iso*-propoxide (426 mg, 1.5 mmol) was added to DCM (2 mL, with the appropriate amount of pre-activated and powdered molecular sieves). The resulting mixture was cooled to −20°C (dry ice/acetone). Freshly distilled (*S*,*S*)-(−)-di-*iso*-propyl tartrate (DIPT) or (*R*,*R*)-(+)-DIPT (352 mg, 1.5 mmol) was then added. The resulting mixture was stirred for 15 min, then a solution of the cholest-4-en-3,6-diol or its derivative (1.0 mmol) in DCM (10 mL) was added. After 10 min of stirring, *tert*-butyl hydroperoxide solution (3.0 mmol) was added dropwise, and the reaction mixture was then stirred at a temperature between −10 and 0°C for 48 h. The resulting mixture was poured into a pre-cooled (0°C) aqueous solution of tartaric acid (10% w/v) (20 mL), then the mixture was vigorously stirred for 30 min, followed by dilution with diethyl ether (50 mL), washing with 10% aqueous tartaric acid (2×25 mL) and brine (2×40 mL), and dry over ($MgSO_4$). After the solvent was removed, the resulting crude epoxide was subjected to further purification (see individual compounds below for details).

3.5. 4α,5-Epoxy-5α-cholestane-3β,6β-diol[21]

4α,5-Epoxy-5α-cholestane-3β,6β-diol 3,6-diacetate (2.0 g, 4.0 mmol) was dissolved in DCM (15 mL) and ethanol (50 mL). 15% Aqueous NaOH solution (8 mL) was added with stirring. The solution was stirred at room temperature for 1.5 h, then poured into water (40 mL). The DCM layer was separated and washed with water then dried over sodium sulphate. The solvent was removed under reduced pressure to give a solid product, which was recrystallised from ether/petroleum ether (yield: 70 g, 91%). Mp 166–167°C; $[\alpha]_D^{15}=+23$ (*c* 10.0, $CHCl_3$); IR: ν_{max} 3500–3300, 2949, 2865, 1469, 1385 and 1051; ^{1}H NMR ($CDCl_3$): δ_H 4.02 (dd, $J=8.0$ and 0.5 Hz, 1H, H-3), 3.22 (s, 1H, H-6), 2.93 (s, 1H, H-4), 1.26 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C 12.0, 17.7, 18.6, 22.5 and 22.7, (5×CH_3), 20.6, 23.8, 24.1, 26.5, 28.1, 29.4, 35.9, 36.1, 39.4 and 39.5 (10×CH_2), 27.9, 30.4, 35.7, 49.7, 55.4, 56.1, 63.6, 64.6 and 73.5 (9×CH), 34.7, 42.5 and 65.9 (3×C); MS (ES$^+$): *m*/*z* 441 (M+Na$^+$, 100).

3.6. 4α,5-Epoxy-5α-cholestane-3β,6β-diol, 3,6-diacetate

Obtained via recrystallisation from methanol as white needles. Mp 157–158°C (lit.[22] 158–160°C); $[\alpha]_D^{15}=+18$ (*c* 10.0, $CHCl_3$); IR: ν_{max} 2954, 2850, 1743, 1465, 1367, 1240 and 1027; ^{1}H NMR ($CDCl_3$): δ_H 4.93 (dd, $J=8.2$ and 0.6 Hz, 1H, H-3), 4.28 (m, 1H, H-6), 3.16 (s, 1H, H-4), 1.19 (s, 3H, CH_3-19), 0.70 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C 11.9, 17.0, 18.5, 20.4, 20.9, 22.4 and 22.6 (7×CH_3), 20.4, 22.8, 23.7, 23.9, 28.0, 29.3, 33.6, 36.0, 39.3 and 39.4 (10×CH_2), 27.8, 30.8, 35.6, 49.4, 55.2, 56.0, 62.2, 66.9 and 74.2 (9×CH), 34.5, 42.4, 63.2, 169.5 and 169.7 (5×C); MS (ES$^+$): *m*/*z* 503 (M+H$^+$, 40), 525 (M+Na$^+$, 100).

3.7. 4β,5-Epoxy-5β-cholestane-3β,6β-diol

Obtained directly from the reaction without further purification. Mp 162–163°C (lit.[23] 164–165°C); $[\alpha]_D^{15}=-7$ (*c* 10.0, $CHCl_3$, lit.[23] $[\alpha]_D^{20}=-7.5$, *c* 0.6, $CHCl_3$); IR: ν_{max} 3500–3300, 2960, 2848, 1463, 1380 and 1059; ^{1}H NMR ($CDCl_3$): δ_H 3.96 (m, 1H, H-3), 3.32 (m, 1H, H-6), 3.22 (d, $J=3.2$ Hz, 1H, H-4), 1.16 (s, 3H, CH_3-19), 0.70 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C 11.9, 18.5, 19.6, 22.5 and 22.7 (5×CH_3), 21.3, 23.7, 24.1, 25.5, 28.7, 32.5, 36.0, 36.8, 39.4 and 39.7 (10×CH_2), 27.9, 29.2, 35.6, 50.6, 55.9, 56.1, 65.1, 66.1 and 74.0 (9×CH), 35.1, 42.4 and 69.4 (3×C); MS (ES$^+$): *m*/*z* 441 (M+Na$^+$, 100).

3.8. 4β,5-Epoxy-5β-cholestane-3β,6β-diol, 3,6-diacetate

Obtained via recrystallisation from methanol as off-white needles. Mp 160–161°C (lit.[23] 154–155°C); $[\alpha]_D^{15}=-60$ (*c* 10.0, $CHCl_3$; lit.[23] $[\alpha]_D^{19}=-58.5$, *c* 1.0, $CHCl_3$); IR: ν_{max} 2948, 2871, 1743, 1463, 1369, 1239 and 1027; ^{1}H NMR ($CDCl_3$): δ_H 5.01 (m, 1H, H-3), 4.51 (m, 1H, H-6), 3.27 (d, $J=3.0$ Hz, 1H, H-4), 1.14 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C 11.8, 18.4, 18.9, 20.7, 21.0, 22.4 and 22.6 (7×CH_3), 21.9, 22.3, 23.6, 24.0, 27.9, 32.2, 35.2, 35.9, 39.2 and 39.4 (10×CH_2), 27.7, 29.9, 35.5, 50.0, 55.4, 55.9, 60.8, 68.6 and 75.4 (9×CH), 35.6, 42.3, 64.8, 169.3 and 170.1 (5×C); MS (ES$^+$): *m*/*z* 525 (M+Na$^+$, 100).

3.9. 4α,5-Epoxy-5α-cholestane-3α,6β-diol

Obtained via hydrolysis of its 6β-acetate, then recrystallisation from chloroform/hexane as granular crystals. Mp 152–154°C; $[\alpha]_D^{20}=+57$ (*c* 10.0, $CHCl_3$); HRMS (ES$^+$): *m*/*z* 419.3528 (M+H$^+$, $C_{24}H_{47}O_3$ requires 419.3525); IR: ν_{max} 3500–3300, 2939, 2865, 1469, 1380 and 1034; ^{1}H NMR ($CDCl_3$): δ_H 4.06 (m, 1H, H-3), 3.26 (m, 2H, H-6, H-4), 1.18 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C 12.0, 17.3, 18.5, 22.5 and 22.7 (5×CH_3), 20.6, 23.8, 24.1, 26.8, 28.1, 29.5, 35.7, 36.0, 39.4 and 39.6 (10×CH_2), 27.9, 30.3, 35.7, 50.2, 55.5, 56.1, 62.8, 63.3 and 73.8 (9×CH), 34.6, 42.5 and 67.7 (3×C); MS (ES$^+$): *m*/*z* 436 (M+NH_4^+, 55), 236 (50), 214 (100).

3.10. 4α,5-Epoxy-5α-cholestane-3α,6β-diol, 3,6-diacetate

Obtained via acetylation of the crude 6β-acetate with acetic anhydride and pyridine in toluene at reflux for 2 h, subsequent recrystallisation from methanol afforded the product as white crystals. Mp 98–99°C; $[\alpha]_D^{20}=+60$ (*c* 10.0, $CHCl_3$); HRMS (ES^+): *m/z* 520.4002 ($M+NH_4^+$, $C_{31}H_{54}NO_5$ requires 520.4002); IR: ν_{max} 2942, 1753, 1467, 1383, 1220 and 1026; ^1H NMR ($CDCl_3$): δ_H 5.15 (m, 1H, H-3), 4.30 (m, 1H, H-6), 3.44 (d, $J=3.5$ Hz, 1H, H-4), 1.09 (s, 3H, CH_3-19), 0.70 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C 12.0, 16.8, 18.5, 21.0, 21.3, 22.5 and 22.7 (7×CH_3), 20.5, 23.6, 23.7, 24.0, 28.0, 30.0, 33.8, 36.0, 39.4 and 39.5 (10×CH_2), 27.9, 30.8, 35.7, 49.9, 55.3, 55.9, 60.4, 66.4 and 74.8 (9×CH), 34.8, 42.5, 63.8, 169.8 and 170.5 (5×C); MS (ES^+): *m/z* 525 ($M+Na^+$, 100).

3.11. 4β,5-Epoxy-5β-cholestane-3α,6β-diol

Obtained via hydrolysis of its 3,6-diacetate and recrystallisation from DCM/hexane which afforded the product as a white solid. Mp 137–138°C; $[\alpha]_D^{20}=+10$ (*c* 1.0, $CHCl_3$); HRMS (ES^+): *m/z* 419.3517 ($M+H^+$, $C_{24}H_{47}O_3$ requires 419.3525); IR: ν_{max} 3500–3300, 2942, 2865, 1463, 1378 and 1058; ^1H NMR ($CDCl_3$): δ_H 3.94 (dd, $J=9.1$ and 2.6 Hz, 1H and H-3), 3.35 (m, 1H, H-6), 2.93 (s, 1H, H-4), 1.14 (s, 3H, CH_3-19), 0.69 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C 11.9, 18.5, 20.6, 22.5 and 22.7 (5×CH_3); 21.0, 23.8, 24.2, 25.3, 27.3, 28.1, 35.8, 36.0, 39.4 and 39.6 (10×CH_2), 7.9, 29.4, 35.7, 46.4, 55.9, 56.1, 65.8, 66.1 and 74.3 (9×CH_2), 35.8, 42.5 and 67.6 (3×C); MS (ES^+): *m/z* 441 ($M+Na^+$, 100), 419 ($M+H^+$, 39), 236 (61), 214 (37).

3.12. 4β,5-Epoxy-5β-cholestane-3α,6β-diol, 3,6-diacetate

Obtained via recrystallisation from methanol as small white plates. Mp 72–73°C; HRMS (ES^+): *m/z* 520.3988 ($M+NH_4^+$, $C_{31}H_{54}NO_5$ requires 520.4002); $[\alpha]_D^{20}=-24$ (*c* 8.0, $CHCl_3$); IR: ν_{max} 2946, 2871, 1745, 1463, 1365, 1236 and 1034; ^1H NMR ($CDCl_3$): δ_H 4.85 (dd, $J=9.8$ and 2.0 Hz, 1H, H-3), 4.52 (m, 1H, H-6), 2.92 (s, 1H, H-4), 1.11 (s, 3H, CH_3-19), 0.70 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C 11.9, 18.5, 20.0, 21.0, 21.3, 22.4 and 22.7 (7×CH_3), 20.9, 21.8, 23.6, 24.1, 26.6, 28.0, 35.1, 36.0, 39.3 and 39.4 (10×CH_2), 27.9, 30.1, 35.6, 46.1, 55.6, 56.0, 62.1, 68.0 and 75.7 (9×CH), 36.0, 42.5, 64.2, 169.9 and 170.1 (5×C); MS (ES^+): *m/z* 525 ($M+Na^+$, 100).

3.13. 4α,5-Epoxy-5α-cholestane-3β,6α-diol

Obtained via hydrolysis of 3β-acetoxy-6α-benzoxy-4α,5-epoxy-5α-cholestane as white solid. Mp 180–181°C; $[\alpha]_D^{20}=+49$ (*c* 1.0, $CHCl_3$); HRMS (ES^+): *m/z* 436.3788 ($M+NH_4^+$, $C_{24}H_{50}NO_3$ requires 436.3791); IR: ν_{max} 3500–3300, 2937, 1469, 1382 and 1074; ^1H NMR ($CDCl_3$): δ_H 3.95 (m, 2H, H-3, H-6), 3.39 (s, 1H, H-4), 1.07 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C 11.9, 17.1, 18.5, 22.5 and 22.7 (5×CH_3), 20.7, 23.9, 24.1, 25.7, 28.1, 29.0, 36.0, 38.1, 39.4 and 39.5 (10×CH_2), 27.9, 34.3, 35.7, 49.3, 55.4, 56.2, 60.1, 64.9 and 65.4 (9×CH), 36.2, 42.6 and 69.3 (3×C); MS (ES^+): *m/z* 441 ($M+Na^+$, 100).

3.14. 4α,5-Epoxy-5α-cholestane-3β,6α-diol, 3,6-diacetate

Obtained via acetylation of the correspondent diol as a colourless gum. $[\alpha]_D^{20}=+47$ (*c* 2.9, $CHCl_3$); HRMS (ES^+): *m/z* 503.3738 ($M+H^+$, $C_{31}H_{51}O_5$ requires 503.3736);. IR: ν_{max} 2948, 2865, 1733, 1465, 1379, 1246 and 1033; ^1H NMR ($CDCl_3$): δ_H 5.23 (dd, $J=11.9$ and 4.7 Hz, 1H, H-6), 4.93 (m, 1H, H-3), 3.08 (s, 1H, H-4), 1.11 (s, 3H, CH_3-19), 0.60 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C 11.9, 17.0, 18.5, 21.0, 21.2, 22.4, 22.7 (7×CH_3), 20.6, 22.8, 23.7, 23.9, 27.9, 28.4, 34.0, 35.9 and 39.3 (two carbons) (10×CH_2), 27.8, 34.1, 35.6, 49.2, 55.1, 55.9, 57.5, 67.1 and 67.2 (9×CH); 36.4, 42.5, 66.0, 169.2 and 169.6 (5×C); MS (ES^+): *m/z* 525 ($M+Na^+$, 100).

3.15. 4β,5-Epoxy-5β-cholestane-3β,6α-diol

Obtained via flash chromatography (ether as eluent) as white solids. Mp 66–67°C; $[\alpha]_D^{20}=-3$ (*c* 10.0, $CHCl_3$); HRMS (ES^+): *m/z* 436.3787 ($M+NH_4^+$, $C_{24}H_{50}NO_3$ requires 436.3791); IR: ν_{max} 3500–3300, 2938, 2865, 1463, 1378 and 1066; ^1H NMR ($CDCl_3$): δ_H 3.95 (m, 2H, H-3, H-6), 3.65 (s, 1H, H-4), 0.95 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C 11.8, 18.5, 19.0, 22.5 and 22.7 (5×CH_3), 21.3, 23.7, 24.1, 25.3, 27.9, 28.0, 36.0, 37.8, 39.3 and 39.5 (10×CH_2), 27.9, 29.4, 35.7, 46.4, 55.9, 56.1, 65.8, 66.1 and 74.3 (9×CH), 36.4, 42.4 and 71.0 (3×C); MS (ES^+): *m/z* 441 ($M+Na^+$, 100).

3.16. 4β,5-Epoxy-5β-cholestane-3β,6α-diol, 3,6-diacetate

Obtained via acetylation of the corresponding diol. Isolated as a colourless gum. $[\alpha]_D^{20}=-15$ (*c* 10.0, $CHCl_3$); HRMS (ES^+): *m/z* 503.3733 ($M+H^+$, $C_{31}H_{51}O_5$ requires 503.3736); IR: ν_{max} 2948, 2865, 1733, 1465, 1378, 1246 and 1033; ^1H NMR ($CDCl_3$): δ_H 5.20 (dd, $J=12.2$ and 4.8 Hz, 1H, H-6), 5.10 (m, 1H, H-3), 3.54 (d, $J=3.6$ Hz, 1H, H-4), 1.06 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C 11.7, 18.4, 18.6, 20.7, 20.8, 22.3 and 22.6 (7×CH_3), 21.2, 22.2, 23.6, 23.9, 27.9, 28.9, 35.9, 36.0, 39.2 and 39.3 (10×CH_2), 27.7, 33.6, 35.5, 47.8, 54.6, 55.5, 55.9, 66.3 and 66.9 (9×CH), 36.7, 42.4, 66.3, 169.5 and 170.3 (5×C) MS (ES^+): *m/z* 525 ($M+Na^+$, 100).

3.17. 4α,5-Epoxy-5α-cholestane-3α,6α-diol

Obtained via recrystallisation from chloroform/hexane as a white solid. Mp 173–174°C; $[\alpha]_D^{20}=+64$ (*c* 4.0, $CHCl_3$); HRMS (ES^+): *m/z* 436.3790 ($M+NH_4^+$, $C_{24}H_{50}NO_3$ requires 436.3791); IR: ν_{max} 3500–3300, 2943, 1469, 1376 and 1042; ^1H NMR ($CDCl_3$): δ_H 4.04 (m, 1H, H-3), 3.87 (m, 1H, H-6), 3.71 (d, $J=4.4$ Hz, 1H, H-4), 0.98 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C 11.9, 16.6, 18.5, 22.5 and 22.7 (5×CH_3), 20.6, 23.8, 24.1, 26.6, 28.0, 28.1, 36.0, 38.6, 39.4 and 39.5 (10×CH_2), 27.9, 34.3, 35.7, 49.8, 55.4, 56.1, 57.7, 62.5 and 64.8 (9×CH), 36.2, 42.5 and 70.5 (3×C); MS (ES^+): *m/z* 441 ($M+Na^+$, 100), 401 (55), 214 (71), 114 (43).

3.18. 4α,5-Epoxy-5α-cholestane-3α,6α-diol, 3,6-diacetate

Obtained via acetylation of the corresponding diol. Isolated as a white solid. Mp 160–161°C; $[\alpha]_D^{20}=+94$ (*c* 10.0, $CHCl_3$); HRMS (ES^+): *m*/*z* 520.4000 ($M+NH_4^+$, $C_{31}H_{54}NO_5$ requires 520.4002); IR: ν_{max} 2937, 2861, 1735, 1465, 1379, 1236 and 1033; ^1H NMR ($CDCl_3$): δ_H 5.20 (dd, $J=12.0$ and 4.8 Hz, 1H, H-6), 5.12 (m, 1H, H-3), 3.44 (d, $J=4.0$ Hz, 1H, H-4), 1.06 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C 11.9, 16.7, 18.5, 20.9, 21.0, 22.4 and 22.7 (7×CH_3), 20.5, 23.4, 23.7, 23.9, 28.0, 28.8, 34.2, 36.0, 39.3 and 39.4 (10×CH_2), 27.9, 34.3, 35.6, 49.7, 55.0, 55.2, 56.0, 66.1 and 66.8 (9×CH), 36.8, 42.5, 66.9, 170.3 and 170.5 (5×C); MS (ES^+): *m*/*z* 525 ($M+Na^+$, 100).

3.19. 4β,5-Epoxy-5β-cholestane-3α,6α-diol

Obtained via hydrolysis of the 3-acetate and flash chromatography. Isolated as a white solid. Mp 99–100°C; $[\alpha]_D^{20}=+25$ (*c* 3.0, $CHCl_3$); HRMS (ES^+): *m*/*z* 436.3793 ($M+NH_4^+$, $C_{24}H_{50}NO_3$ requires 436.3791); IR: ν_{max} 3500–3300, 2937, 1467, 1365 and 1078; ^1H NMR ($CDCl_3$): δ_H 4.01 (m, 2H, H-3 and H-6), 3.36 (s, 1H, H-4), 0.96 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C 11.8, 18.5, 18.9, 22.4 and 22.7, (5×CH_3), 21.1, 23.8, 24.2, 24.6, 26.4, 28.1, 36.0, 37.7, 39.4 and 39.5 (10×CH_2), 27.9, 34.0, 35.7, 45.5, 55.6, 56.2, 59.8 and 65.9 (two carbons) (9×CH), 36.0, 42.5 and 69.8 (3×C); MS (ES^+): *m*/*z* 436 ($M+NH_4^+$, 62), 401 (100), 236 (15), 214 (18).

3.20. 4β,5-Epoxy-5β-cholestane-3α,6α-diol, 3,6-diacetate

Obtained via acetylation of the correspondent diol. Isolated as a white solid. Mp 63–64°C; $[\alpha]_D^{20}=+26$ (*c* 1.0, $CHCl_3$); HRMS (ES^+): *m*/*z* 520.4000 ($M+NH_4^+$, $C_{31}H_{54}NO_5$ requires 520.4002); IR: ν_{max} 2952, 2861, 1741, 1465, 1379, 1234 and 1033; ^1H NMR ($CDCl_3$): δ_H 5.21 (dd, $J=12.2$, 4.8 Hz, 1H and H-6), 4.88 (dd, $J=9.2$ and 0.4 Hz, 1H and H-3), 3.19 (s, 1H, H-4), 1.02 (s, 3H, CH_3-19), 0.63 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C 11.8, 18.5, 18.8, 20.9, 21.0, 22.4 and 22.7 (7×CH_3), 21.0, 21.6, 23.6, 24.0, 25.8, 27.9, 35.9, 36.0, 39.3 and 39.4 (10×CH_2) 27.8, 33.9, 35.5, 45.6, 55.6, 56.0, 57.0, 67.2 and 68.1 (9×CH), 37.1, 42.5, 66.1 and 169.8 (two carbons) (5×C); MS (ES^+): *m*/*z* 525 ($M+Na^+$, 100).

3.21. 3β-Acetoxy-6α-benzoxy-4α,5-epoxy-5α-cholestane

Obtained via recrystallisation from acetone. Isolated as white flakes. Mp 145–146°C; $[\alpha]_D^{20}=+82$ (*c* 10.0, $CHCl_3$); HRMS (ES^+): *m*/*z* 582.4160 ($M+NH_4^+$, $C_{36}H_{56}NO_5$ requires 582.4158); IR: ν_{max} 2946, 2869, 1730, 1724, 1447, 1378, 1272, 1240, 1110, 1032 and 715; ^1H NMR ($CDCl_3$): δ_H 5.48 (dd, $J=12.2$ and 4.8 Hz, 1H and H-6), 4.98 (m, 1H, H-3), 3.21 (s, 1H, H-4), 1.20 (s, 3H, CH_3-19), 0.70 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C 11.9, 17.1, 18.6, 21.0, 22.5 and 22.7 (6×CH_3), 20.7, 22.9, 23.8, 24.0, 28.0, 28.6, 34.1, 36.0, 39.3 and 39.4 (10×CH_2), 27.9, 34.3, 35.7, 49.3, 55.2, 56.0, 57.6, 67.3, 67.6, 128.3 (two carbons), 129.6 (two carbons) and 133.0 (14×CH), 36.7, 42.6, 66.7, 129.8, 165.5 and 169.8 (6×C); MS (ES^+): *m*/*z* 587 ($M+Na^+$, 100).

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