Formal Synthesis of Squalamine from Desmosterol

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The key intermediate to squalamine, (5α,7α,24*R*)-7,24-dihydroxy-cholestan-3-one, was synthesized from the 3-*O*-acetyl-24*R*,25-dihydroxy derivative of desmosterol *via* 10 steps in 16% overall yield and squalamine was also prepared *via* two further steps in 7.4% total yield from the desmosterol derivative.

Key words sterol; squalamine; antibacterial; natural product

Squalamine (**1**) was isolated from the stomach of the dogfish shark *Squala acanthias* by More and coworkers in 1993.[1] Since then, it has been demonstrated that squalamine has potent antimicrobial activity against gram-negative bacteria, gram-positive bacteria, and fungi. However, it was the discovery of its potent antiangiogenic activity[2] that made squalamine highly attractive to researchers around the world. More's group found that squalamine inhibited endothelial cell function and affected the growth of solid tumors.[1]

Several synthetic routes have been established to prepare squalamine on a large scale to supply it for clinical trials.[3—7] A practical way was found by Kinney and coworkers to prepare it from stigmasterol in 15 steps.[8—10] Very recently, a dramatically shortened synthetic route (10 steps) *via* microbial 7α-hydroxylation of 22-hydroxy-23,24-bisnorchol-4-en-3-one was reported.[11] Other groups also developed their own synthetic route of squalamine. Kim and coworkers prepared it from 22-hydroxy-23,24-bisnorchol-4-en-3-one, *via* 12 steps.[12] Zhou and coworkers reported a short synthetic route (nine steps) to the key squalamine precurcor, (5α,7α,24*R*)-7,24-dihydroxycholestan-3-one (**13**) which was demonstrated to result in the 24-sulfate derivative (**14**), the direct precursor of squalamine, in good yield (Chart 1).[13,14] The starting material, methyl 3-oxo-5α-chenodeoxycholanate, however, was prepared from methyl chenodeoxycholanate *via* five steps.[15]

When the authors began a project to examine the clinical effects of squalamine in 1998, the method starting from stigmasterol was the only practical and reliable way to obtain the compound on a large scale. Although we confirmed that the method is an excellent way to obtain squalamine on a practical scale, it includes somewhat laborious steps such as a cleavage and reconstruction of the side chain and a stoichiometric asymmetric reduction of the C-24 carbonyl group. To solve these problems, we tried to synthesize squalamine starting from desmosterol. Since desmosterol has all the skeletal carbons required for squalamine, it is not necessary to employ a carbon–carbon bond-forming reaction for construction of the side chain. In addition, a catalytic asymmetric dihydroxylation of the double bond at C-24 and C-25[16] (the second step in Chart 1) and regioselective elimination of the hydroxyl group at the C-25 position[17] (the next three steps in Chart 1) have already been established by the groups of Spencer[16] and Fujimoto,[17] respectively. Therefore squalamine is considered to be prepared conveniently, since the stereoselective modification of the remaining A and B rings is not so difficult employing the established method.[16]

Results and Discussion

Desmosterol has been considered to be inadequate as a starting material because of its high price and synthetic inaccessibility.[18] However, Fujimoto and his coworkers at Yoshikawa Seiyu Co. Ltd. discovered that desmosterol was included in a filtrate of recrystallization of crude cholesterol which was made from lanolin alcohol obtained by saponification of wool grease, a washing waste of wool, and the content of desmosterol reached 10—25%. Desmosterol in the crude mixture of the filtrate was directly acetylated at C-3-OH and then dihydroxylated at C-24 and C-25 in the Sharpless asymmetric reaction with the use of AD-mix-β. The 3-*O*-acetyl 24*R*,25-dihydroxy compound (**3**) thus obtained was isolated by silica gel column chromatography.[17] We were offered a sample of the (24*R*)-compound **3** (diastereomeric ratio, 24*R* : 24*S* = 96 : 4, as revealed by the signal intensity of 0.66 (24*R*) and 0.62 (24*S*) ppm of compound **4** in ^{1}H-NMR analysis[19—21]) from the company and started the synthesis from the compound to obtain the key intemediate (**13**) *via* nine steps. Compound **13** was further converted into squalamine **1** in high yield by modified procedures of Kinney's method[9] *via* two further steps. The route of synthesis is shown in Chart 1.

Our initial stage of the synthesis was focused on establishing a reliable method to deoxygenate the redundant 25-hydroxy group. The choice of a protective group at C-24-OH was crucial for this purpose, because the group had to tolerate all the steps to the target compound **13**. In particular, the next step was regioselective dehydration between C-25 and C-26 with $POCl_3$ and pyridine, which appeared to be vigorous conditions for the protective group. Therefore we examined protecting agents for the two steps and the results are shown in Table 1.

As can be seen in Table 1, pivaloyl was the best protective group for the two steps and gave the highest yields. However, the pivaloyl group is usually deprotected by $NaBH_4$ or $LiAlH_4$ and the method requires protection of the C-3 carbonyl group and removal of the protective group. In addition, attempts to remove the pivaloyl group of the 7-*O*-benzoyl-24-*O*-pivaloyl analogue of compound **12**[22] under the same conditions of those in the step from **12** to **13** resulted in deprotection of only the 7-*O*-benzoyl group while the pivaloyl group remained intact. Therefore we chose the benzoyl group

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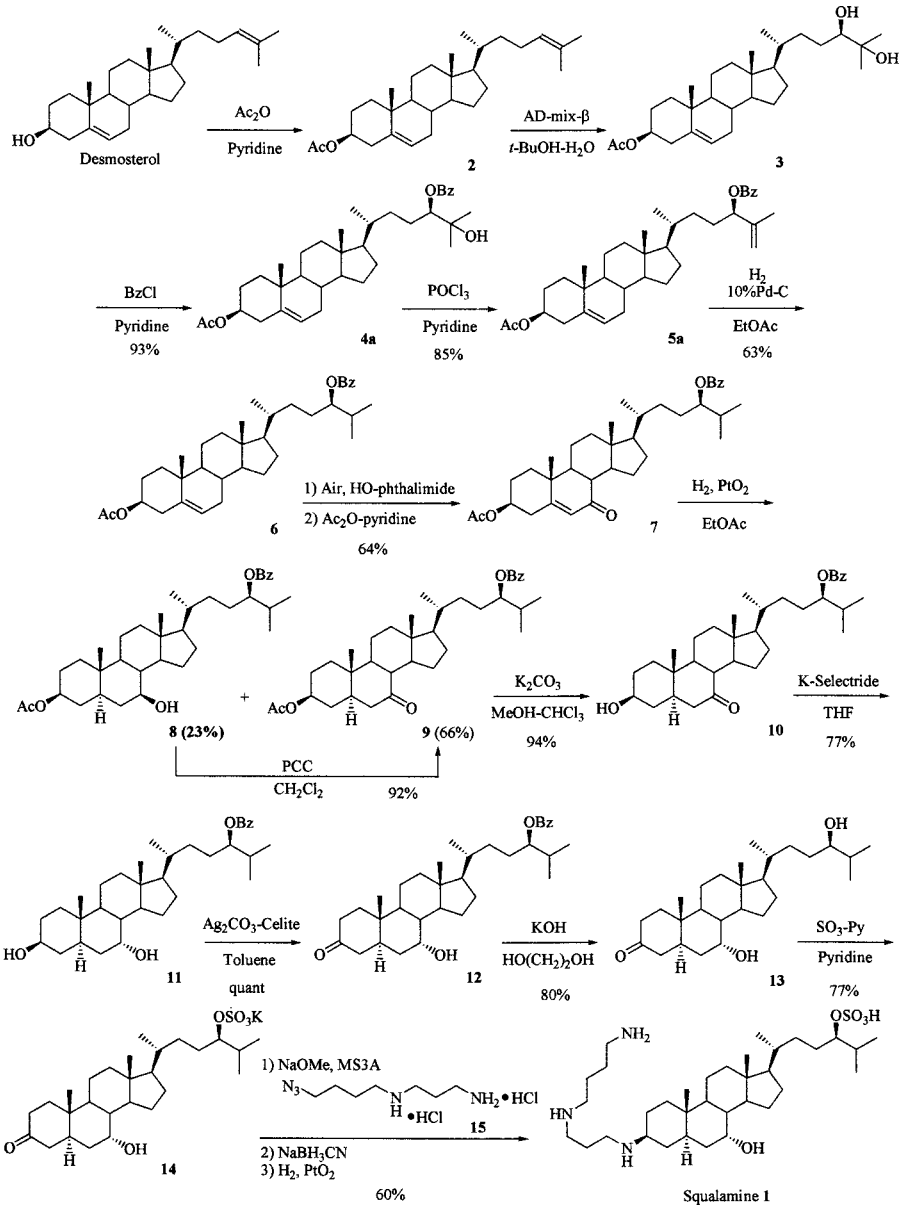


Chart 1

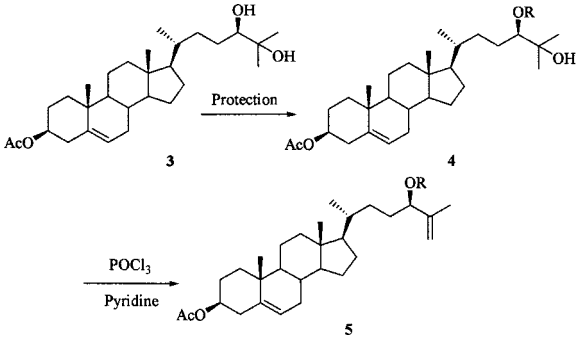
as the protective group.

The double bond between C-25 and C-26 of the Δ^{25} compound (**5a**) was selectively hydrogenated over 10% palladium on charcoal to give the saturated (24*R*)-compound (**6**) in 63% yield because of lower steric hindrance of the double bond compared with the inner double bond between C-5 and C-6. The diastereomeric ratio of the product, that is, (24*R*)-*O*-benzoyl compound **6** and its epimer (24*S*)-*O*-benzoyl,[23,24] was determined to be higher than 95 : 5 based on ^{13}C-NMR spectra. Fujimoto and coworkers reported that the chemical shift of C-24 in ^{13}C-NMR spectra of steroids that bear epimeric 24-hydroxyl groups differs by about 0.4 ppm from the signals for (24*R*)-epimers appearing at higher field.[19—21]

Peaks due to C-24 of the (24*R*)-*O*-benzoyl and (24*S*)-*O*-benzoyl compounds were observed at 79.4 ppm and 79.8 ppm, respectively. The ^{13}C-NMR spectra were measured by taking complete ^{13}C-NMR and inverse gated ^{13}C-NMR to obtain correct integration values of the peaks. The ratio of the (24*R*)-*O*-benzoyl and (24*S*)-*O*-benzoyl compounds was almost the same as that of compound **4a**, thus implying that the C-24 stereochemistry was retained during the conversion of **4a** to **5a**.

The allylic oxidation at C-7 of compound **6** was successfully carried out provide the 7-oxo compound (**7**) with using the hydroxyphthalimide-catalyzed air oxidation method.[8—10] However, since the 3-*O*-acetyl group was partially hydrolyzed during the reaction, the crude product was reacetylated before purification. Hydrogenation of compound 7 using Adams catalyst under an atmospheric pressure of hydrogen resulted in the formation of 7-keto compound **9** in 66% yield together with 7β-alcohol **8** in 23% yield under the influence of steric hindrance of the C-19 methyl group.[25,26] The latter was easily transformed into the former by PCC oxidation in 92% yield. After hydrolysis of the 3-*O*-acetyl group of compound **9**, stereoselective reduction of the 7-keto group resulted in the formation of the desired 7α-alcohol **11** in 77% yield using K-selectride, the stereoselectivity of which was reported by Göndös and Orr.[27] The 3β-hydroxyl

Table 1. Protection of 24-OH and Dehydration at C-25 and C-26



Compound	R[a]	Yield (%)[b]	
		3→4	4→5
a	Bz	93	85
b	Piv	90	92
c	THP	26	48
d	MOM	25	66
e	TBS	78	58

a) Bz, benzoyl; Piv, pivaloyl; THP, tetrahydropyranyl; MOM, methoxymethyl; TBS, *t*-butyldimethylsilyl. *b*) Isolated yield.

group of compound **11** was selectively oxidized with silver carbonate on Celite to give the 3-oxo-7α-hydroxy compound (**12**) almost quantitatively. The 24-*O*-benzoyl group of compound **12** was saponified with KOH in ethylene glycol at 120 °C to provide the desired 3-oxo-7α,24*R*-dihydroxy compound **13** in 80% yield. Compound **13** was converted into the 24-*O*-sulfate derivative (**14**) in 77% yield and then coupled with the spermidine precursor (**15**) to afford squalamine **1** according to the known procedures.[28] However, we found that we could only obtain the reported yield (60%) of squalamine when sodium cyanoborohydride was used in the reductive amination procedure instead of sodium borohydride.

In conclusion, the key intermediate **13** for squalamine synthesis was prepared from the desmosterol derivative, 3-*O*-acetyl 24*R*,25-dihydroxy compound **3**, *via* 10 steps in 16% overall yield. Squalamine **1** was also prepared *via* two further steps in 7.4% total yield from **3**. Since the starting material was obtained from the washing waste of wool and the reactions used for each step were all well established, this method may provide a practical way to synthesize squalamine and its derivatives.

Experimental

General The melting points were determined using a Yanagimoto micromelting point apparatus and were uncorrected. The IR spectra were recorded on a Perkin-Elmer 1720-X FT-IR spectrometer. The ^{1}H- and ^{13}C-NMR spectra were obtained on a JEOL JNM-A 400 spectrometer in $CDCl_3$ with tetramethylsilane as an internal standard. The optical rotations were measured with a JASCO DIP-4 polarimeter. Elemental analyses were performed at the Laboratory for Organic Elemental Microanalysis, Faculty of Pharmaceutical Sciences, Kyoto University. Thin-layer chromatography was run on Merck silica gel 60 F_{254}, and column chromatography was performed using Wakogel C-300. Silver carbonate on Celite was prepared using Kinney's method.[3]

(24*R*)-3β-Acetoxy-24-benzoyloxy-25-hydroxycholest-5-ene (4a) To a solution of 3-*O*-acetyl 24*R*,25-dihydroxy compound **3** (11.60 g, 25.34 mmol) in pyridine (35 ml) benzoyl chloride (9.34 ml, 81.1 mmol) was added at 0 °C. The mixture was stirred overnight at room temperature. The reaction mixture was diluted with EtOAc (50 ml) and washed with 1 N hydrochloric acid (15 ml×2), saturated aqueous $NaHCO_3$ (15 ml×2), and brine (15 ml×2). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified on a silica gel column (hexane : EtOAc=3 : 1) to afford 24-*O*-benzoate **4a** as a colorless syrup (13.35 g, 93% yield): $[\alpha]_D^{26}$ −22.2° (*c*=1.08, $CHCl_3$); IR (neat) 3475, 2941, 1718, 1451 cm^{-1}; ^{1}H-NMR δ: 0.66 (s, 3H), 0.93 (d, 3H, *J*=6.8 Hz), 1.00 (s, 3H), 1.27 (s, 6H), 2.02 (s, 3H, –C(O)C$\underline{H}_3$), 0.89—2.16 (m, 24H), 2.30—2.32 (m, 2H), 4.58—4.60 (m, 1H, C-3H), 5.0—5.07 (m, 1H, C-24H), 5.36 (d, 1H, olefin-H, *J*=5.1 Hz), 7.43—7.59 (m, 3H, Ar-$\underline{H}$), 8.05—8.07 (m, 2H, Ar-$\underline{H}$); ^{13}C-NMR δ: 14.0, 18.3, 19.1, 20.8, 21.2, 24.0, 25.3, 25.6, 26.1, 27.5, 27.8, 31.6, 31.9, 35.0, 36.3, 36.8, 37.9, 39.5, 42.1, 49.8, 55.5, 56.4, 60.1, 72.3, 73.7, 80.4, 122.4, 128.2 (×2), 129.5 (×2), 130.1, 132.7, 139.4, 166.4, 170.3. *Anal.* Calcd for $C_{36}H_{52}O_5 \cdot 0.5H_2O$: C, 75.36; H, 9.31. Found: C, 75.78; H, 9.20.

(24*R*)-3β-Acetoxy-24-pivaloyloxy-25-hydroxycholest-5-ene (4b) To a solution of 3-*O*-acetyl 24*R*,25-dihydroxy compound **3** (500 mg, 1.092 mmol) in pyridine (10 ml) pivaloyl chloride (0.426 ml, 3.495 mmol) was added at 0 °C. The mixture was stirred overnight at room temperature. The reaction mixture was diluted with EtOAc (10 ml) and washed with 1 N hydrochloric acid (5 ml×2), saturated aqueous $NaHCO_3$ (5 ml×2), and brine (5 ml×2). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified on a silica gel column (hexane : EtOAc=4 : 1) to afford 24-*O*-pivaloate **4b** as a white solid (533 mg, 90% yield): mp 139—140.5 °C; $[\alpha]_D^{26}$ −27.3° (*c*=1.00, $CHCl_3$); IR (KBr) 3507, 2968, 2852, 1729, 1471 cm^{-1}; ^{1}H-NMR δ: 0.67 (s, 3H), 0.92 (d, 3H, *J*=6.4 Hz), 1.02 (s, 3H), 1.18 (s, 3H), 1.19 (s, 3H), 1.24 (s, 9H), 2.03 (s, 3H, –C(O)C$\underline{H}_3$), 0.91—2.03 (m, 24H), 2.31—2.33 (m, 2H), 4.59—4.61 (m, 1H, C-3H), 4.75—4.78 (m, 1H, C-24H), 5.37 (d, 1H, olefin-H, *J*=4.8 Hz); ^{13}C-NMR δ: 11.8, 18.6, 19.3, 21.0, 21.4, 24.2, 25.1, 25.6, 26.5, 27.3 (×3), 27.8, 28.1, 31.9 (×2), 32.1, 35.1, 36.6, 37.0, 38.1, 39.0, 39.7, 42.3, 50.0, 55.8, 56.7, 72.6, 74.0, 79.6, 122.6, 139.6, 170.5, 178.4.

(24*R*)-3β-Acetoxy-24-tetrahydropyranyloxy-25-hydroxycholest-5-ene (4c) To a solution of 3-*O*-acetyl 24*R*,25-dihydroxy compound **3** (500 mg, 1.092 mmol) in CH_2Cl_2 (10 ml) 3,4-dihydro-2*H*-pyrane (0.110 ml, 1.201 mmol) and pyridinium *p*-toluenesulfonate (27 mg, 0.107 mmol) were added at room temperature and allowed to stand overnight. The reaction mixture was diluted with ether (10 ml) and washed with 1 N hydrochloric acid (5 ml×2), saturated aqueous $NaHCO_3$ (5 ml×2), and brine (5 ml×2). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified on a silica gel column (hexane : EtOAc=4 : 1) to afford 24-*O*-tetrahydropyranyloxy compound **4c** as a white solid (156 mg, 26% yield): mp 97—98.5 °C; $[\alpha]_D^{26}$ −49.1° (*c*=1.00, $CHCl_3$); IR (KBr) 3425, 2943, 2868, 2851, 1731, 1468 cm^{-1}; ^{1}H-NMR δ: 0.69 (s, 3H), 0.93 (d, 3H, *J*=6.8 Hz), 1.02 (s, 3H), 1.12 (s, 3H), 1.14 (s, 3H), 2.03 (s, 3H, –C(O)C$\underline{H}_3$), 0.92—2.03 (m, 29H), 2.31—2.33 (m, 2H), 3.28—3.30 (m, 1H), 3.48—3.53 (m, 1H, C-3H), 4.00—4.03 (m, 1H), 4.39—4.40 (m, 1H, C-24H), 4.59—4.61 (m, 2H), 5.37 (d, 1H, olefin-H, *J*=4.4 Hz); ^{13}C-NMR δ: 11.8, 18.5, 19.3, 21.0, 21.4, 21.7, 23.5, 24.2, 24.9, 26.5, 27.7, 28.2, 31.5, 31.8 (×3), 32.7, 35.4, 36.5, 37.0, 38.1, 39.7, 42.3, 50.0, 55.9, 56.7, 65.6, 71.6, 73.9, 91.0, 103.4, 122.5, 139.7, 170.4.

(24*R*)-3β-Acetoxy-24-methoxymethyloxy-25-hydroxycholest-5-ene (4d) To a solution of 3-*O*-acetyl 24*R*,25-dihydroxy compound **3** (500 mg, 1.092 mmol) in CH_2Cl_2 (10 ml) chloromethyl methyl ether (0.083 ml, 1.092 mmol) and *N*,*N*-diisopropylethylamine (0.209 ml, 1.201 mmol) were added at 0 °C. The mixture was stirred overnight at room temperature. The reaction mixture was diluted with ether (10 ml) and washed with 1 N hydrochloric acid (5 ml×2), saturated aqueous $NaHCO_3$ (5 ml×2), and brine (5 ml×2). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified on a silica gel column (hexane : EtOAc=3 : 1) to afford 24-*O*-methoxymethyloxy compound **4d** as a white solid (137 mg, 25% yield): mp 87—88.5 °C; $[\alpha]_D^{26}$ −44.1° (*c*=1.00, $CHCl_3$); IR (KBr) 3444, 2940, 2887, 2869, 1731, 1468 cm^{-1}; ^{1}H-NMR δ: 0.68 (s, 3H), 0.93 (d, 3H, *J*=6.3 Hz), 1.02 (s, 3H), 1.16 (s, 6H), 2.03 (s, 3H, –C(O)C$\underline{H}_3$), 0.81—2.03 (m, 24H), 2.31—2.33 (m, 2H), 3.19—3.21 (m, 1H, C-3H), 3.44 (s, 3H), 4.57—4.62 (m, 1H, C-24H), 4.72 (ABq, 2H, *J*=6.4 Hz), 5.37 (d, 1H, olefin-H, *J*=4.4 Hz); ^{13}C-NMR δ: 11.9, 18.5, 19.3, 21.0, 21.4, 23.8, 24.3, 26.5, 27.8, 28.1, 28.3, 29.7, 31.9, 32.7, 35.6, 36.6, 37.0, 38.1, 39.7, 42.4, 50.0, 56.0, 56.1, 56.7, 72.2, 74.0, 90.7, 99.1, 122.6, 139.7, 170.5.

(24*R*)-3β-Acetoxy-24-*t*-butyldimethylsilyloxy-25-hydroxycholest-5-ene (4e) To a solution of 3-*O*-acetyl 24*R*,25-dihydroxy compound **3** (300 mg, 0.655 mmol) in CH_2Cl_2 (10 ml) was added *t*-butyldimethylchlorosilane (296 mg, 1.966 mmol) and imadazole (223 mg, 3.217 mmol) at 0 °C. The mixture was stirred at room temperature for 2 d. The reaction mixture was diluted with ether (10 ml) and washed with 1 N hydrochloric acid (5 ml×2),

saturated aqueous $NaHCO_3$ (5 ml×2), and brine (5 ml×2). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified on a silica gel column (hexane : EtOAc=5 : 1) to afford 24-*O*-*t*-butyldimethylsilyloxy compound **4e** as a white solid (292 mg, 78% yield): mp 93.5—95 °C; $[\alpha]_D^{26}$ −34.4° (*c*=1.00, $CHCl_3$); IR (KBr) 3470, 2937, 1733, 1471 cm^{-1}; ^{1}H-NMR δ: 0.09 (s, 3H), 0.10 (s, 3H), 0.68 (s, 3H), 0.92 (s, 12H), 1.02 (s, 3H), 1.13 (s, 3H), 1.15 (s, 3H), 2.03 (s, 3H, –C(O)C<u>H</u>$_3$), 0.64—2.04 (m, 24H), 2.31—2.33 (m, 2H), 3.35—3.44 (m, 1H, C-3H), 4.56—4.64 (m, 1H, C-24H), 5.37 (d, 1H, olefin-H, *J*=4.8 Hz); ^{13}C-NMR δ: −4.3 (×2), −3.6, 11.8, 18.3, 18.6, 19.3, 21.0, 21.4, 24.2, 24.3, 26.1 (×3), 26.9, 27.8, 28.3, 30.1, 31.9 , 33.1, 36.3, 36.6, 37.0, 38.1, 39.7, 42.3, 50.0, 56.1, 56.7, 73.2, 74.0, 80.3, 122.6, 139.7, 170.5.

(24*R*)-3β-Acetoxy-24-benzoyloxycholesta-5,22-diene (5a) To a solution of 24-*O*-benzoate **4a** (7.80 g, 13.8 mmol) in pyridine (130 ml) $POCl_3$ (5.20 ml, 55.2 mmol) was added at 0 °C, and the mixture was stirred for 12 h at room temperature. EtOAc (50 ml) and water (20 ml) were added to the reaction mixture, and the solution was stirred for 30 min at room temperature, and then the organic layer was washed with 1 N hydrochloric acid (15 ml×2), saturated aqueous $NaHCO_3$ (15 ml×2), and brine (15 ml×2). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give a white crystalline solid, which was recrystallized from hexane–EtOAc to afford Δ^{25} compound **5a** as a white powder (7.55 g, 86% yield). mp 137—143 °C; $[\alpha]_D^{26}$ −53.0° (*c*=1.00, $CHCl_3$); IR (KBr) 3434, 2936, 2871, 1733, 1708, 1654, 1602, 1453 cm^{-1}; ^{1}H-NMR δ: 0.66 (s, 3H), 0.95 (d, 3H, *J*=6.3 Hz), 1.00 (s, 3H), 1.79 (s, 3H), 2.01 (s, 3H, –C(O)C<u>H</u>$_3$), 0.90—2.02 (m, 23H), 2.30—2.33 (m, 2H), 4.58—4.60 (m, 1H, C-3H), 4.90 (s, 1H, one proton of C=C<u>H</u>$_2$), 5.02 (s, 1H, another proton of C=C<u>H</u>$_2$), 5.35—5.39 (m, 2H, olefin-<u>H</u> and C-24H), 7.42 (t, 2H, Ar-<u>H</u>, *J*=7.8 Hz), 7.53 (t, 1H, Ar-<u>H</u>, *J*=6.8 Hz), 8.05 (d, 2H, Ar-<u>H</u>, *J*=8.3 Hz); ^{13}C-NMR δ: 11.8, 18.3, 18.7, 19.3, 21.0, 21.4, 24.2, 27.8, 28.1, 29.2, 31.4, 31.9, 35.4, 36.6, 37.0, 38.1, 39.7, 42.3, 50.0, 55.7, 56.7, 74.0, 77.2, 78.2, 112.6, 122.6, 128.3 (×2), 129.5 (×2), 130.7, 132.8, 139.6, 143, 4, 165.8, 170.5. *Anal.* Calcd for $C_{36}H_{50}O_4$: C, 79.08; H, 9.22. Found: C, 79.05; H, 9.28.

(24*R*)-3β-Acetoxy-24-pivaloyloxycholesta-5,22-diene (5b) The pivaloyl compound **5b** (248 mg, 92%) was obtained from **4b** (278 mg, 513 mmol) by a similar procedure to that for the benzoyl analogue **5a**. mp 139—146 °C; $[\alpha]_D^{26}$ −40.1° (*c*=1.00, $CHCl_3$); IR (KBr) 2939, 2906, 2871, 2821, 1731, 1653, 1458 cm^{-1}; ^{1}H-NMR δ: 0.67 (s, 3H), 0.93 (d, 3H, *J*=6.4 Hz), 1.02 (s, 3H), 1.21 (s, 9H), 1.71 (s, 3H), 2.03 (s, 3H, –C(O)C<u>H</u>$_3$), 0.92—2.04 (m, 23H), 2.31—2.33 (m, 2H), 4.59—4.61 (m, 1H, C-3H), 4.85 (s, 1H), 4.92 (s, 1H), 5.06—5.10 (m, 1H, C-24H), 5.37 (d, 1H, olefin-H, *J*=4.8 Hz); ^{13}C-NMR δ: 11.8, 18.3, 18.7, 18.9, 19.3, 21.0, 21.4, 24.2, 27.1, 27.2 (×3), 27.8, 28.1, 29.1, 31.3, 31.9, 35.2, 36.6, 37.0, 38.1, 38.8, 39.7, 42.3, 50.0, 55.8, 56.7, 74.0, 111.8, 122.6, 139.7, 143.8, 170.5, 177.6.

(24*R*)-3β-Acetoxy-24-tetrahydropyranyloxycholesta-5,22-diene (5c) The tetrahydropyranyl compound **5c** (72 mg, 48%) was obtained from **4c** (156 mg, 288 mmol) by a similar procedure to that for the benzoyl analogue **5a**. mp 131—135 °C; $[\alpha]_D^{26}$ −60.7° (*c*=1.00, $CHCl_3$); IR (KBr) 2940, 1732, 1652, 1441 cm^{-1}; ^{1}H-NMR δ 0.68 (s, 3H), 0.92 (d, 3H, *J*=6.4 Hz), 1.02 (s, 3H), 1.09 (d, 3H, *J*=6.8 Hz), 1.73 (s, 3H), 2.03 (s, 3H, –C(O)C<u>H</u>$_3$), 0.91—2.03 (m, 25H), 2.31—2.33 (m, 2H), 3.28—3.30 (m, 1H), 3.40—3.45 (m, 1H, C-3H), 3.82—3.92 (m, 1H), 4.55—4.65 (m, 1H, C-24H), 4.66—4.68 (m, 2H), 4.82 (s, 1H), 4.92 (s, 1H), 5.37 (d, 1H, olefin-H, *J*=4.8 Hz); ^{13}C-NMR δ: 11.8, 17.4, 17.8, 18.7, 19.3, 19.6, 21.0, 21.4, 24.2, 25.5, 27.7, 28.1, 30.9, 31.0, 31.2, 31.8, 35.6, 36.5, 36.9, 38.1, 39.6, 42.2, 49.9, 55.7, 56.6, 62.6, 73.9, 81.3, 97.9, 111.5, 122.6, 139.6, 145.9, 170.5.

(24*R*)-3β-Acetoxy-24-methoxymethyloxycholesta-5,22-diene (5d) The methoxymethyl compound **5d** (86 mg, 66%) was obtained from **4d** (135 mg, 269 mmol) by a similar procedure to that for the benzoyl analogue **5a**. mp 97—103 °C; $[\alpha]_D^{26}$ +4.7° (*c*=1.00, $CHCl_3$); IR (KBr) 2939, 2360, 1731, 1654, 1560, 1458 cm^{-1}; ^{1}H-NMR δ: 0.68 (s, 3H), 0.94 (d, 3H, *J*=6.3 Hz), 1.02 (s, 3H), 1.66 (s, 3H), 2.03 (s, 3H, –C(O)C<u>H</u>$_3$), 0.93—2.03 (m, 25H), 2.31—2.33 (m, 2H), 3.83 (s, 3H), 3.89—3.93 (m, 1H, C-3H), 4.56 (ABq, 2H, *J*=6.8 Hz), 4.59—4.63 (m, 1H, C-24H), 5.37 (d, 1H, olefin-H, *J*=4.9 Hz); ^{13}C-NMR δ: 11.9, 16.8, 18.7, 19.3, 21.0, 21.4, 24.3, 27.8, 28.2, 30.1, 31.9, 32.0, 35.7, 36.6, 37.0, 38.1, 39.7, 42.3, 50.0, 55.5 (×2), 55.9, 56.7, 74.0, 80.6, 93.6, 113.7, 122.6, 139.7, 144.3, 170.5.

(24*R*)-3β-Acetoxy-24-*t*-butyldimethylsilyloxycholesta-5,22-diene (5e) The *t*-butyldimethylsilyl compound **5e** (165 mg, 58%) was obtained from **4e** (292 mg, 510 mmol) by a similar procedure to that for the benzoyl analogue **5a**. mp 86—89 °C; $[\alpha]_D^{26}$ −31.1° (*c*=1.00, $CHCl_3$); IR (KBr) 2935, 2886, 2853, 1736, 1675, 1647, 1472 cm^{-1}; ^{1}H-NMR δ: 0.04 (s, 3H), 0.11 (s, 3H), 0.68 (s, 3H), 0.89 (s, 9H), 0.95 (s, 3H), 1.02 (s, 3H), 1.67 (s, 3H), 2.03 (s, 3H, –C(O)C<u>H</u>$_3$), 0.81—2.03 (m, 23H), 2.31—2.33 (m, 2H), 3.95—3.98 (m, 1H, C-3H), 4.59—4.62 (m, 1H, C-24H), 4.74 (s, 1H), 4.84 (s, 1H), 5.37 (d, 1H, olefin-H, *J*=5.2 Hz); ^{13}C-NMR δ: −5.0, −4.7, −3.8, 11.8, 17.3, 18.0, 18.8, 19.3, 21.0, 21.4, 25.9, 26.0 (×3), 27.8, 28.0, 31.6, 31.9, 32.5, 35.6, 36.0, 36.6, 37.0, 38.1, 39.7, 42.3, 50.0, 55.9, 56.6, 74.0, 110.3, 122.6, 139.6, 148.0,170.4.

(24*R*)-3β-Acetoxy-24-benzoyloxycholest-5-ene (6) A solution of Δ^{25} compound **5a** (3.70 g, 6.77 mmol) in EtOAc (100 ml) was stirred at room temperature in the presence of 10% Pd–C (370 mg) under an atmospheric pressure of hydrogen. After 3 h, the reaction mixture was filtered, and the filtrate was concentrated *in vacuo*. The resulting syrup was purified by silica gel column chromatography (hexane : EtOAc=8 : 1) to afford the saturated compound **6** as a white powder (2.34 g, 63% yield): mp 114—121 °C; $[\alpha]_D^{26}$ −30.0° (*c*=1.02, $CHCl_3$); IR (KBr) 3444, 3068, 2934, 2872, 1735, 1708, 1601, 1584, 1467, 1452 cm^{-1}; ^{1}H-NMR δ: 0.66 (s, 3H), 0.92 (d, 3H, *J*=6.8 Hz), 0.96 (d, 3H, *J*=6.8 Hz), 0.98 (d, 3H, *J*=6.8 Hz), 1.01 (s, 3H), 2.02 (s, 3H, –C(O)C<u>H</u>$_3$), 0.93—2.04 (m, 24H), 2.30—2.32 (m, 2H), 4.58—4.61 (m, 1H, C-3H), 4.95—4.99 (m, 1H, C-24H), 5.36 (d, 1H, olefin-<u>H</u>, *J*=4.4 Hz), 7.44 (t, 2H, Ar-<u>H</u>, *J*=7.8 Hz), 7.55 (t, 1H, Ar-<u>H</u>, *J*=7.3 Hz), 8.05 (d, 2H, Ar-<u>H</u>, *J*=8.3 Hz); ^{13}C-NMR δ: 11.8, 17.7, 18.6, 18.7, 19.3, 20.9, 21.4, 24.2, 27.5, 27.7, 28.0, 31.5, 31.6, 31.8, 35.4, 36.6, 37.0, 38.1, 39.7, 42.3, 50.0, 55.7, 56.6, 74.0, 77.2, 79.4, 122.6, 128.3 (×2), 129.5 (×2), 130.9, 132.6, 139.6, 166.4, 170.5. *Anal.* Calcd for $C_{36}H_{52}O_4$: C, 78.79; H, 9.55. Found: C, 78.60; H, 9.62.

(24*R*)-3β-Acetoxy-24-benzoyloxycholest-5-en-7-one (7) The hydrogenated product **6** (3.00 g, 5.47 mmol) and *N*-hydroxylphthalamide (893 mg, 5.58 mmol) were dissolved in EtOAc–acetone (300 ml, 1 : 1 v/v), and benzoyl peroxide (300 mg) was added to the reaction solution at 50—60 °C. Air was bubbled into the reaction solution with stirring for 48 h at 50—60 °C, and then the reaction mixture was concentrated *in vacuo*. Carbon tetrachloride (200 ml) was added to the residual syrup, the suspension was stirred for 10 min at room temperature, and then the insoluble material was filtered off. The filtrate was concentrated *in vacuo*, and a solution of the residue in pyridine (50 ml) was treated with acetic anhydride (5 ml) for 12 h at room temperature. The reaction mixture was concentrated *in vacuo* to give a syrup, which was purified on a silica gel column (hexane : EtOAc=4 : 1) and then the crude crystals were recrystallized from hexane–EtOAc to afford 7-oxo compound **7** as a white powder (1.96 g, 64% yield): mp 137—140 °C; $[\alpha]_D^{26}$ −86.2° (*c*=1.04, $CHCl_3$); IR (KBr) 3456, 2960, 2866, 1736, 1709, 1665, 1632, 1605, 1587, 1454 cm^{-1}; ^{1}H-NMR δ: 0.66 (s, 3H), 0.93 (d, 3H, *J*=6.3 Hz), 0.97 (d, 3H, *J*=6.3 Hz), 0.97 (d, 3H, *J*=6.3 Hz), 1.20 (s, 3H), 2.05 (s, 3H, –C(O)C<u>H</u>$_3$), 0.93—2.56 (m, 24H), 4.68—4.74 (m, 1H, C-3H), 4.95—4.99 (m, 1H, C-24H), 5.69 (d, 1H, olefin-<u>H</u>, *J*=1.5 Hz), 7.44 (t, 2H, Ar-<u>H</u>, *J*=7.3 Hz), 7.55 (t, 1H, Ar-<u>H</u>, *J*=7.3 Hz), 8.05 (d, 2H, Ar-<u>H</u>, *J*=7.3 Hz); ^{13}C-NMR δ: 11.9, 17.2, 17.8, 18.7, 18.8, 21.1, 21.2, 26.2, 27.3, 28.3, 31.4, 31.6, 35.2, 36.0, 37.7, 38.3, 38.6, 43.1, 45.4, 49.8, 49.9, 54.4, 72.2, 77.2, 79.3, 126.7, 128.3 (×2), 129.5 (×2), 130.8, 132.7, 163.8, 166.4, 170.2, 201.8. *Anal.* Calcd for $C_{36}H_{50}O_5 \cdot 0.3H_2O$: C, 76.10; H, 8.97. Found: C, 76.14; H, 9.04.

(24*R*)-3β-Acetoxy-24-benzoyloxy-7β-hydroxycholestane (8) and (24*R*)-3β-Acetoxy-24-benzoyloxycholestan-7-one (9) A solution of 7-oxo compound **7** (2.52 g, 4.59 mmol) in EtOAc (200 ml) was stirred at room temperature in the presence of platinum oxide (126 mg) under an atmospheric pressure of hydrogen. After 3 h, the reaction mixture was filtered, and the filtrate was concentrated *in vacuo* to give a syrup, which was purified by silica gel column chromatography (hexane : EtOAc=3 : 1) to yield 5α-7-hydroxy compound **8** as a colorless syrup (585 mg, 23% yield) and 5α-7-oxo compound **9** as a colorless syrup (1.66 g, 66% yield). Compound **8**: $[\alpha]_D^{26}$ −28.5° (*c*=0.96, $CHCl_3$); IR 3417, 2950, 2873, 1737, 1713, 1603, 1451 cm^{-1}; ^{1}H-NMR δ: 0.66 (s, 3H), 0.84 (s, 3H), 0.92 (d, 3H, *J*=6.4 Hz), 0.96 (d, 3H, *J*=6.3 Hz), 0.97 (d, 3H, *J*=6.8 Hz), 2.02 (s, 3H, –C(O)C<u>H</u>$_3$), 0.91—2.35 (m, 28H), 3.35 (m, 1H, C-7H), 4.67 (m, 1H, C-3H), 4.96 (m, 1H, C-24H), 7.44 (t, 2H, Ar-<u>H</u>, *J*=7.8 Hz), 7.55 (t, 1H, Ar-<u>H</u>, *J*=6.8 Hz), 8.05 (d, 2H, Ar-<u>H</u>, *J*=7.3 Hz); ^{13}C-NMR δ: 12.0, 12.2, 14.1, 17.6, 18.6, 21.3, 26.7, 27.3, 27.4, 28.4, 31.4, 31.5, 33.5, 34.8, 35.2, 36.5, 37.9, 39.8, 41.7, 43.2, 43.4, 52.2, 54.7, 55.5, 73.3, 74.7, 77.3, 79.2, 128.2 (×2), 129.4 (×2), 130.7, 132.6, 166.2, 170.5; *Anal.* Calcd for $C_{36}H_{54}O_5 \cdot 0.2H_2O$: C, 75.80; H, 9.61. Found: C, 75.77; H, 9.71. Compound **9**: $[\alpha]_D^{26}$ +29.6° (*c*=1.04, $CHCl_3$); IR (neat) 3452, 2947, 2869, 1718, 1603, 1470, 1452 cm^{-1}; ^{1}H-NMR δ: 0.63 (s, 3H), 0.91 (d, 3H, *J*=6.8 Hz), 0.96 (d, 3H, *J*=6.4 Hz), 0.97 (d, 3H, *J*=6.4 Hz), 1.08 (s, 3H), 2.02 (s, 3H, –C(O)C<u>H</u>$_3$), 0.87—2.35 (m, 27H), 4.67 (m, 1H, C-3H), 4.96 (m, 1H, C-24H), 7.44 (t, 2H, Ar-<u>H</u>, *J*=7.8 Hz), 7.55 (t, 1H, Ar-<u>H</u>, *J*=7.3 Hz), 8.05 (d, 2H, Ar-<u>H</u>, *J*=7.3 Hz); ^{13}C-NMR δ: 11.5, 11.9, 17.6, 18.5, 20.8, 21.1, 21.6, 24.7, 26.9, 27.1, 28.0, 31.2, 31.4, 33.6, 34.9, 35.6, 35.7, 38.5, 42.3, 45.6, 46.3, 48.7, 49.7, 54.4, 54.7, 72.5, 79.0,

128.1 (×2), 129.3 (×2), 130.6, 132.5, 166.1, 170.1, 211.0. *Anal.* Calcd for $C_{36}H_{52}O_5$: C, 76.56; H, 9.28. Found: C, 76.08; H, 9.36.

Oxidation of 7-Hydroxy Compound 8 to 7-Oxo Compound 9 A suspension of 5α-7-hydroxy compound **8** (150 mg, 0.271 mmol), PCC (88 mg, 0.407 mmol), and Celite (150 mg) in CH_2Cl_2 (30 ml) was stirred at room temperature for 12 h. The reaction mixture was filtered, and the filtrate was concentrated *in vacuo* to give a syrup, which was purified by silica gel column chromatography (hexane : EtOAc=3 : 1) to afford 5α-7-oxo compound **9** as a colorless syrup (137 mg, 92% yield).

(24*R*)-24-Benzoyloxy-3β-hydroxycholestan-7-one (10) A mixture of 5α-7-oxo compound **9** (424 mg, 0.770 mmol) and K_2CO_3 (424 mg, 3.07 mmol) in $CHCl_3$–methanol (100 ml, 1 : 1 v/v) was stirred at room temperature. After 12 h, water (50 ml) was added to the reaction mixture and the organic layer was separated. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The resulting syrup was purified by silica gel column chromatography (hexane : EtOAc=1 : 1) to give 3β-hydroxy-7-oxo compound **10** as a colorless syrup (367 mg, 94% yield): $[\alpha]_D^{26}$ −28.4° (c=1.03, $CHCl_3$); IR (neat) 3416, 2942, 2871, 1713, 1602, 1451 cm^{-1}; ^{1}H-NMR δ: 0.63 (s, 3H), 0.91 (d, 3H, J=6.8 Hz), 0.96 (d, 3H, J=6.8 Hz), 0.97 (d, 3H, J=6.3 Hz), 1.07 (s, 3H), 0.90—2.17 (m, 28H), 2.30—2.37 (m, 2H), 3.60 (m, 1H, C-3H); 4.96 (m, 1H, C-24H), 7.44 (t, 2H, Ar-H, J=7.3 Hz), 7.55 (t, 1H, Ar-H, J=7.3 Hz), 8.05 (d, 2H, Ar-H, J=6.8 Hz); ^{13}C-NMR δ: 11.8, 12.0, 17.7, 18.6, 18.7, 21.8, 24.8, 27.3, 28.2, 31.0, 31.3, 31.6, 35.1, 35.9, 36.1, 37.8, 38.7, 42.5, 46.0, 46.8, 48.8, 49.9, 54.6, 55.2, 70.6, 79.3, 128.3 (×2), 129.5 (×2), 130.8, 132.6, 166.4, 212.0. *Anal.* Calcd for $C_{34}H_{50}O_4\cdot 0.2H_2O$: C, 77.58; H, 9.65. Found: C, 77.52; H, 9.81.

(24*R*)-24-Benzoyloxy-3β,7α-dihydroxycholestane (11) To a solution of 3β-hydroxy-7-oxo compound **10** (1.249 g, 2.454 mmol) in THF (120 ml) 1.0 M K-Selectride in THF (5.40 ml, 5.40 mmol) was added dropwise with stirring at −20 °C under an Ar atmosphere. After 3 h, 30% H_2O_2 was added to the reaction mixture until evolution of gas ceased. The reaction mixture was extracted with toluene (30 ml×3) and the combined extracts were washed with 1 N hydrochloric acid (20 ml×2), saturated aqueous $NaHCO_3$ (20 ml×2), and brine (30 ml×3). The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give a yellow syrup, which was purified by silica gel column chromatography (hexane : EtOAc=2 : 3) to give white crystals. Recrystallization from hexane–EtOAc afforded 3β,7α-dihydroxy compound **11** as a white powder (970 mg, 77% yield): mp 97—101 °C; $[\alpha]_D^{26}$ +5.3° (c=0.99, $CHCl_3$); IR (neat) 3396, 2932, 2869, 2361, 1718, 1603, 1586, 1451 cm^{-1}; ^{1}H-NMR δ: 0.62 (s, 3H), 0.64 (s, 3H), 0.91 (d, 3H, J=6.3 Hz), 0.96 (d, 3H, J=6.8 Hz), 0.97 (d, 3H, J=6.3 Hz), 0.91—1.98 (m, 29H), 3.62 (m, 1H, C-3H), 3.81 (br s, 1H, C-7H), 4.94—4.98 (m, 1H, C-24H), 7.44 (t, 2H, Ar-H, J=7.8 Hz), 7.55 (t, 1H, Ar-H, J=7.3 Hz), 8.05 (d, 2H, Ar-H, J=7.3 Hz); ^{13}C-NMR δ: 11.2, 11.8, 17.7, 18.5, 18.7, 21.0, 23.6, 27.4, 28.0, 31.4, 31.6, 35.3, 35.5, 36.3, 36.7, 37.0, 37.7, 39.5 (×2), 42.6, 45.8, 50.5, 55.7, 67.9, 71.1, 76.7, 79.4, 128.3 (×2), 129.5 (×2), 130.8, 132.7, 166.4. *Anal.* Calcd for $C_{34}H_{52}O_4\cdot 0.4H_2O$: C, 76.76; H, 10.00. Found: C, 76.88; H, 9.98.

(24*R*)-24-Benzoyloxy-7α-hydroxycholestan-3-one (12) A suspension of 3β,7α-dihydroxy compound **11** (495 mg, 0.969 mmol) and silver carbonate on Celite (495 mg) in toluene (100 ml) was stirred under reflux for 8 h. The reaction mixture was filtered through Celite and the filtrate was concentrated *in vacuo*. The residual syrup was purified by silica gel column chromatography (hexane–EtOAc=2 : 1) to give crude crystals. The crystals were recrystallized from hexane–EtOAc to afford 3-oxo-7α-hydroxy compound **12** as a white powder (490 mg, quant.): mp 152—154 °C; $[\alpha]_D^{26}$ +20.6° (c=1.03, $CHCl_3$); IR (KBr) 3433, 2938, 2860, 2361, 1716, 1601, 1451 cm^{-1}; ^{1}H-NMR δ: 0.67 (s, 3H), 0.92 (d, 3H, J=6.3 Hz), 0.96 (d, 3H, J=6.8 Hz), 0.97 (d, 3H, J=6.8 Hz), 0.99 (s, 3H), 2.37 (m, 28H), 3.84 (br s, 1H, C-7H), 4.94—4.99 (m, 1H, C-24H), 7.44 (t, 2H, Ar-H, J=7.8 Hz), 7.55 (t, 1H, Ar-H, J=7.8 Hz), 8.05 (d, 2H, Ar-H, J=7.3 Hz); ^{13}C-NMR δ: 10.4, 11.8, 17.7, 18.6, 18.7, 21.2, 23.6, 27.4, 28.0, 31.4, 31.7, 35.3, 35.7, 36.5, 38.1, 39.0, 39.4, 39.5, 42.6, 44.1, 45.2, 50.4, 55.7, 67.5, 76.7, 79.3, 128.3 (×2), 129.5 (×2), 130.9, 132.7, 166.4, 211.6. *Anal.* Calcd for $C_{34}H_{50}O_4$: C, 78.12; H, 9.64. Found: C, 77.90; H, 9.72.

(24*R*)-7α,24-Dihydroxycholestan-3-one (13) A mixture of 3-oxo-7α-hydroxy compound **12** (400 mg, 0.786 mg) and potassium hydroxide (265 mg, 4.72 mmol) in ethylene glycol (100 ml) was stirred at 120 °C. After 3 h, water (50 ml) was added to the reaction mixture, and the reaction mixture was extracted with $CHCl_3$ (30 ml×3). The combined extracts were dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The resulting syrup was purified by silica gel column chromatography (hexane : EtOAc=1 : 1) to give crude crystals. The crystals were recrystallized from hexane–EtOAc to give 3-oxo-7α,24*R*-dihydroxy compound **13** as a white powder (254 mg, 80% yield): mp 151—153 °C (lit. 151—153 °C[11]); $[\alpha]_D^{26}$ +33.1° (c=0.32, $CHCl_3$); IR (neat) 3505, 2943, 2869, 2361, 1745, 1708, 1468, 1444 cm^{-1}; ^{1}H-NMR δ: 0.71 (s, 3H), 0.94 (m, 9H), 1.02 (s, 3H), 1.10—2.50 (m, 29H), 3.33 (m, 1H), 3.88 (m, 1H); ^{13}C-NMR δ: 10.5, 11.9, 17.3, 18.6, 18.9, 21.2, 23.7, 28.3, 30.6, 32.0, 33.6, 35.7, 36.0, 36.6, 38.1, 38.2, 39.1, 39.4, 39.5, 42.7, 44.2, 45.2, 50.5, 56.0, 67.5, 77.4, 211.7. *Anal.* Calcd for $C_{27}H_{46}O_3$: C, 77.46; H, 11.07. Found: C, 77.21; H, 11.26.

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