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A highly stereoselective synthesis of C-24 and C-25 oxysterols from desmosterol

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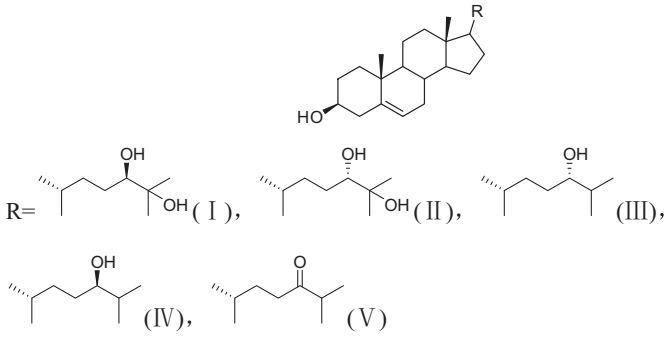
ABSTRACT

A new highly stereoselective construction of the side chain of the C-24 and C-25 oxysterols has been achieved by using desmosterol acetate as the starting material and an improved Sharpless catalytic asymmetric dihydroxylation with 100% d.e. (diastereomeric excess) as the key step. The result is much better than the usual asymmetric dihydroxylation procedure. *t*-Butyl nitrite/2,2,6,6-tetramethylpiperidine N-oxyl radical/$FeCl_3$ catalyst system was developed to activate molecular oxygen for the aerobic oxidation of 24-hydroxycholesterol and the 24-ketocholesterol was obtained in 86.2% yield. The oxidation system has never been reported before. The mechanism for the catalytic aerobic oxidation was also proposed.

1. Introduction

Oxysterols are oxygenated derivatives of cholesterol having a second oxygen function in addition to that at C-3 in the steroids nucleus and bearing an *i*-octyl or modified *i*-octyl side chain at C-17, which are intermediates in bile acid biosynthesis in the hepatocyte and also serve to transport cholesterol from brain to liver for conversion to bile acids [1]. The recent discovery of various biologically active steroids, such as vitamin D metabolites, ecdysteroids, brassinolide and bile alcohols, has led to a marked growth of the interest in oxysterols. The oxidative transformation of cholesterol has become a distinguishing feature of the current stage of steroid studies [2].

Oxysterols have a variety of biological properties such as cytotoxicity, carcinogenicity and mutagenicity. Meanwhile, oxysterols are suggested to play an active role in plaque development. Derivatives of the (I)–(V) type with cholesterol cyclic moiety and a side chain containing a 24- and 25-functional group occupy an important place among oxysterols [3–10].



For example, cerebrosterol (24(S)-hydroxycholesterol, III) formed in small amounts in human and animal brain from cholesterol is important for cholesterol homeostasis. Alzheimer's disease (AD) and vascular demented patients appear to have higher circulating levels of cerebrosterol. Hence it might be used as an early biochemical marker for diagnosis of AD and vascular demented diseases. Moreover, cerebrosterol can significantly activate LXRα and LXRβ, two orphan members of the nuclear receptor superfamily, and play a critical role in controlling the metabolism and

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reverse transportation of cholesterol in the body. As one of the most potent agonists of LXRs, cerebrosterol can be a potential drug for treatment for atherosclerosis [6–10].

The study of biological properties of oxysterols implies the availability of these compounds as standards. In view of this mounting evidence of biochemical importance of oxysterols, it was desired to have access to substantial quantities of these compounds. Up to now, 24- and 25-functionalized oxysterols have remained rather inaccessible. We have already reported methods for the introduction of hydroxyl-groups at C-25 [11,12]. In our continuing efforts to synthesize oxysterol analogs having useful specific and/or enhanced activities, we have now addressed a new highly stereoselective method for introduction of hydroxyl-groups into the 24- and 25-positions of the cholesterol side chain starting from desmosterol. This method was developed for the vitamin D_3 metabolites, but which should also be applicable to the synthesis of other natural products.

2. Experimental

Melting points were determined using WRR melting point apparatus (China). ^{1}H and ^{13}C NMR spectra were recorded on Bruker AV-400 spectrometer (Bruker Corporation, Germany) at working frequencies 400 and 100 MHz. respectively in $CDCl_3$ and with TMS as the internal standard. Chemical shifts are expressed in ppm downfield from TMS and observed coupling constants (*J*) are given in Hertz (Hz). IR spectra were recorded with FT-IR apparatus (Bruker Corporation, Germany). Mass spectra were recorded by EI methods (Bruker Corporation, Germany). Starting materials and solvents were commercially purchased (Sigma–Aldrich, America). The progress of the reactions was monitored by thin-layer chromatography (TLC) using silica gel plates (200 microns) containing a fluorescent indicator (silica gel 60 F_{254}). Detection was performed by spraying with phosphomolybdic acid (5%) and heating at 120 °C. Column chromatography was performed using silica gel, 200–300 mesh, and elution was performed with *n*-hexane/ethyl acetate.

2.1. Δ^5-3β,24(R),25-Trihydroxycholestene-3-acetate (**2**)

To a mixture of $K_3Fe(CN)_6$ (4.94 g, 15 mmol), K_2CO_3 (2.07 g, 15 mmol), $CH_3SO_2NH_2$ (0.48 g, 5 mmol), $(DHQD)_2PHAL$ (0.198 g, 0.25 mmol) and $K_2OsO_2(OH)_4$ (0.01 g, 0.025 mmol) in *t*-butanol-water (1:1, 80 mL) was cooled to 0 °C. A solution of desmosterol acetate **(1)** (2.14 g, 5 mmol) in ethyl acetate (50 mL) was added dropwise. Additional $K_2OsO_2(OH)_4$ (0.01 g, 0.025 mmol) was added 3 h after the beginning of the reaction. The resulting mixture was stirred vigorously for another 3 h (TLC monitoring). The reaction was quenched at 0 °C with sodium sulfite (10.00 g). Stirring was continued for another hour. The organic layers were washed with 2 M KOH, 10% HCl, an aq. saturated solution of $NaHCO_3$, brine, dried over anhydrous sodium sulfate, and evaporated. The final products (2.18 g, 95%) were isolated using column chromatography (absorbent: silica gel, mobile phase: *n*-hexane/EtOAc (2:1, v/v)). **2** [9]: mp: 158.2–159.7 °C (lit. mp: 158–159 °C). $[\alpha]_D^{20}$ = −25.8 (*c*, 0.35, $CHCl_3$). MS-EI (*m*/*z*): 400 (M^+-HOAc), 382 (M^+-HOAc-H_2O). IR (cm^{-1}): 3445 (OH), 1735 (CH_3CO_2). ^{1}H NMR ($CDCl_3$, 400 MHz):δ5.31 (d, *J* = 3.8 Hz, 1H), 4.53 (m, 1H), 3.26 (m, 1H), 2.25 (d, *J* = 7.2 Hz, 2H), 1.96 (s, 3H), 1.14 (s, 3H), 1.09 (s, 3H), 0.95 (s, 3H), 0.87 (d, *J* = 6.2 Hz, 3H), 0.62 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz):δ 169.58, 138.63, 121.61, 77.77, 72.99, 72.15, 55.64, 55.05, 48.99, 41.34, 38.71, 37.09, 35.97, 35.57, 34.59, 31.84, 30.84, 27.27, 27.10, 26.75, 25.54 (X2), 23.25, 22.26, 20.42, 20.01, 18.29, 17.55, 10.87.

2.2. Δ^5-3β,24(S),25-Trihydroxycholestene-3-acetate (epi-**2**)

Δ^5-3β,24(S),25-Trihydroxycholestene-3-acetate (epi-**2**) was synthesized by the procedure described above for **2** except that $(DHQ)_2PHAL$ was used as the chiral ligand in the asymmetric dihydroxylation protocol. epi-**2** [10]: yield: 95%; mp: 132 °C (lit. mp: 135–136 °C). $[\alpha]_D^{20}$ = −27.2 (*c*, 0.9, $CHCl_3$). MS-EI (*m*/*z*): 400 (M^+-HOAc), 382 (M^+-HOAc-H_2O). IR (cm^{-1}): 3445 (OH), 1735 (CH_3CO_2). ^{1}H NMR ($CDCl_3$, 400 MHz):δ5.30 (d, *J* = 3.9 Hz, 1H), 4.53(m, 1H), 3.21 (m, 1H), 2.25 (d, *J* = 7.4 Hz, 2H), 1.96 (s, 3H), 1.14 (s, 3H), 1.09 (s, 3H), 0.95 (s, 3H), 0.87 (d, *J* = 6.4 Hz, 3H), 0.61 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz):δ169.57, 138.66, 121.61, 78.60, 73.00, 72.21, 55.66, 54.96, 49.03, 41.35, 38.73, 37.11, 36.00, 35.59, 34.97, 32.27, 30.87, 27.33, 27.19, 26.77, 25.53 (X2), 23.26, 22.23, 20.43, 20.03, 18.30, 17.84, 10.88.

2.3. Δ^5-3β,24(R),25-Trihydroxycholestene-3,24-diacetate (**3**)

A solution of **2** (4.6 g, 10 mmol) in acetic anhydride (2.04 g, 20 mmol) and pyridine (50 mL) was stirred at room temperature for 4 d. The reaction mixture was rotary-evaporated. The residue was dissolved in dichloromethane (80 mL) and successively washed with 10% HCl, an aq. saturated solution of $NaHCO_3$, brine, dried over anhydrous sodium sulfate, and evaporated. The final products (4.75 g, 95%) were isolated using column chromatography (absorbent: silica gel, mobile phase: *n*-hexane/EtOAc (4:1, v/v)). **3**: $[\alpha]_D^{20}$ = −40.0 (*c*, 0.5, $CHCl_3$). MS-EI (*m*/*z*): 485 (M^++1-H_2O), 442 (M^+-CH_3COOH), 425 (M^++1-CH_3COOH-H_2O). IR (cm^{-1}): 3470 (OH), 1735 (CH_3CO_2), 1713 (CH_3CO_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ5.30 (d, *J* = 4.0 Hz, 1H), 4.71 (dd, *J* = 10.1, 2.5 Hz, 1H), 4.53 (m, 1H), 2.04 (s, 3H), 1.96 (s, 3H), 1.13 (s, 3H), 1.12 (s, 3H), 0.95 (s, 3H), 0.85 (d, *J* = 6.5 Hz, 3H), 0.61 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz):δ170.25, 169.53, 138.65, 121.59, 79.04, 72.97, 71.51, 55.66, 54.78, 48.99, 41.32, 37.10 (X2), 35.98, 35.57, 34.19, 31.03, 30.84, 27.04, 26.75, 25.81 (X2), 24.64, 23.89, 23.21, 20.41, 20.05, 20.00, 18.29, 17.46, 10.84.

2.4. Δ^5-3β,24(S),25-Trihydroxycholestene-3,24-diacetate (epi-**3**)

Δ^5-3β,24(S),25-Trihydroxycholestene-3,24-diacetate **(epi-3)** was obtained from Δ^5-3β,24(S),25-trihydroxycholestene-3-acetate (epi-**2)** in 95% yield according to the procedure described above for **3**; epi-**3** [10]: mp: 173 °C (lit. mp: 175–176 °C). $[\alpha]_D^{20}$ = −31.5 (*c*, 0.5, $CHCl_3$). MS-EI (*m*/*z*): 485 (M^++1-H_2O), 442 (M^+-CH_3COOH), 425 (M^++1-CH_3COOH-H_2O). IR (cm^{-1}): 3470 (OH), 1735 (CH_3CO_2), 1713 (CH_3CO_2). ^{1}H NMR ($CDCl_3$, 400 MHz):δ5.30 (d, *J* = 3.7 Hz, 1H), 4.67 (m, 1H), 4.53 (m, 1H), 2.03 (s, 3H), 1.96 (s, 3H), 1.13 (s, 3H), 1.12 (s, 3H), 0.95 (s, 3H), 0.86 (d, *J* = 6.4 Hz, 3H), 0.60 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz):δ170.26, 169.53, 138.63, 121.58, 79.77, 72.95, 71.49, 55.59, 54.67, 48.96, 41.29, 37.09 (X2), 35.96, 35.56, 34.78, 31.40, 30.83, 27.07, 26.74, 25.74 (X2), 24.94, 23.97, 23.22, 20.42, 20.05, 19.98, 18.28, 17.79, 10.83.

2.5. $\Delta^{5,25}$-3β,24(R)-Dihydroxycholestadiene-3,24-diacetate (**4**)

To a solution of **3** (5.02 g, 10 mmol) in pyridine (50 mL) at 0 °C was added dropwise an excess (2.38 g, 20 mmol) of distilled thionyl chloride. The reaction mixture was stirred at 0 °C for 10 h. The reaction mixture was rotary-evaporated. The residue was dissolved in dichloromethane (80 mL) and successively washed with 10% HCl, an aq. saturated solution of $NaHCO_3$, brine, dried over anhydrous sodium sulfate, and evaporated. The final products (4.25 g, 88%) were isolated using column chromatography (absorbent: silica gel, mobile phase: *n*-hexane/EtOAc (16:1, v/v)). **4**: mp: 128.1 °C. $[\alpha]_D^{20}$ = −49.8 (*c*, 0.15, $CHCl_3$). MS-EI (*m*/*z*): 424 (M^+-CH_3COOH), 364 (M^+-2CH_3COOH). IR (cm^{-1}): 3080 (C=CH_2),

1733 (CH_3CO_2), 1654 (C=CH_2) 904 (C=CH_2). ^{1}H NMR ($CDCl_3$, 400 MHz):δ5.30 (d, J = 3.9 Hz, 1H), 5.05 (m, 1H), 4.86 (s, 1H), 4.80 (s, 1H), 4.53(m, 1H), 1.99 (s, 3H), 1.96 (s, 3H), 1.65 (s, 3H), 0.95 (s, 3H), 0.86 (d, J = 6.5 Hz, 3H), 0.60 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz):δ169.50, 169.36, 142.41, 138.65, 121.58, 111.46, 76.59, 72.95, 55.66, 54.73, 48.99, 41.30, 37.10 (X2), 35.98, 35.57, 34.32, 30.85, 30.83, 30.28, 28.02, 27.08, 26.76, 23.22, 20.41, 20.21, 20.00, 18.29, 17.63, 17.17, 10.83.

2.6. $\Delta^{5,25}$-3β,24(S)-Dihydroxycholestadiene-3,24-diacetate (epi-**4**)

$\Delta^{5,25}$-3β,24(S)-dihydroxycholestadiene-3,24-diacetate **(**epi-**4)** was obtained from Δ^5-3β,24(S),25-trihydroxycholestene-3,24-diacetate (epi-**3)** in 88% yield according to the procedure described above for **4**; epi-**4** [10]: mp: 100 °C (lit. mp: 95–95 °C). $[\alpha]_D^{20}$ = −43.5 (*c*, 0.13, $CHCl_3$). MS-EI (*m*/*z*): 424 (M^+-CH_3COOH), 364 (M^+-2CH_3COOH). IR (cm^{-1}): 3080 (C=CH_2), 1733 (CH_3CO_2), 1654 (C=CH_2) 904 (C = CH_2). ^{1}H NMR ($CDCl_3$, 400 MHz):δ5.30 (d, J = 4.2 Hz, 1H), 5.05 (m 1H), 4.87 (s, 1H), 4.82 (s, 1H), 4.53 (m, 1H), 1.98 (s, 3H), 1.96 (s, 3H), 1.64 (s, 3H), 0.95 (s, 3H), 0.86 (d, J = 6.4 Hz, 3H), 0.60 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz):δ169.52, 169.36, 142.02, 138.64, 121.58, 112.09, 77.06, 72.95, 55.64, 54.78, 48.97, 41.30, 37.09 (X2), 35.97, 35.56, 34.39, 30.85, 30.83, 30.29, 27.79, 27.09, 26.75, 23.22, 20.42, 20.25, 19.99, 18.28, 17.62, 16.87, 10.83

2.7. Δ^5-3β,24(R)-Dihydroxycholestene (***5***)

A solution of **4** (4.84 g, 10 mmol) in ethyl acetate (100 mL) was stirred at room temperature in the presence of 10% Pd-C (0.5 g) under an atmospheric pressure of hydrogen. After 48 h, the reaction mixture was filtered, and the filtrate was concentrated in *vacuo*. The crude product was dissolved in methanol (200 mL) and 10% KOH and refluxed for 3 h. The reaction mixture was rotary-evaporated. The residue was dissolved in dichloromethane (80 mL) and successively washed with 10% HCl, an aq. saturated solution of $NaHCO_3$, brine, dried over anhydrous sodium sulfate, and evaporated. The final products (3.40 g, 85%) were isolated using column chromatography (absorbent: silica gel, mobile phase: *n*-hexane/EtOAc (4:1, v/v)). **5** [6]: mp: 175–176 °C (lit. mp: 177–180 °C). $[\alpha]_D^{20}$ = −27.0 (*c*, 0.7, $CHCl_3$). MS-EI (*m*/*z*): 402 (M^+), 384 (M^+-H_2O), 369 (M^+-H_2O-Me), 351 (M^+-2H_2O-Me). IR (cm^{-1}): 3369 (OH). ^{1}H NMR ($CDCl_3$, 400 MHz):δ5.28 (d, J = 4.8 Hz, 1H), 3.47 (m, 1H), 3.25 (m, 1H), 0.94 (s, 3H), 0.85 (m, 9H), 0.62 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz):δ139.76, 120.68, 76.08, 70.78, 55.75, 55.03, 49.12, 41.34, 41.28, 38.77, 36.25, 35.49, 34.69, 32.55, 31.05 (X2), 30.89, 30.64, 29.57, 27.26, 23.27, 20.07, 18.38, 17.89, 17.65, 16.21, 10.87.

2.8. Cerebrosterol (epi-***5***)

Cerebrosterol **(**epi-**5)** was obtained from epi-**4** in 85% yield according to the procedure described above for **5**; epi-**5** [10]: mp: 178–179 °C (lit. mp: 181–182 °C). $[\alpha]_D^{20}$ = −49.0 (*c*, 0.03, $CHCl_3$). MS-EI (*m*/*z*): 402 (M^+), 384 (M^+-H_2O), 369 (M^+-H_2O-Me), 351 (M^+-2H_2O-Me). IR (cm^{-1}): 3369 (OH). ^{1}H NMR ($CDCl_3$, 400 MHz):δ5.28 (d, J = 5.0 Hz, 1H), 3.45 (m, 1H), 3.25 (m, 1H), 0.94 (s, 3H), 0.85 (m, 9H), 0.61 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz):δ139.76, 120.67, 76.42, 70.77, 55.73, 54.94, 49.11, 41.33, 41.28, 38.76, 36.25, 35.49, 34.93, 32.12, 31.19 (X2), 30.89, 30.64, 29.71, 27.19, 23.27, 20.07, 18.38, 18.05, 17.81, 15.69, 10.86.

2.9. Δ^5-3β,24-Dihydroxycholestene-3-acetate (***6***)

A solution of **5** (4.02 g, 10 mmol) in acetic anhydride (2.04 g, 20 mmol), dichloromethane (80 mL) and triethylamine (50.60 g, 20 mmol) was stirred at room temperature for 4 d. The reaction mixture was successively washed with 10% HCl, an aq. saturated solution of $NaHCO_3$, brine, dried over anhydrous sodium sulfate, and evaporated. The final products (4.20 g, 95%) were isolated using column chromatography (absorbent: silica gel, mobile phase: *n*-hexane/EtOAc (8:1, v/v)). **6** [13]: mp: 128 °C (lit. mp: 130–141 °C). MS-EI (*m*/*z*): 467 (M^++Na), 385 (M^+-CH_3COOH). IR (cm^{-1}): 3348 (OH), 1737($COCH_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ5.30 (d, J = 4.4 Hz, 1H), 4.53 (m, 1H), 3.25 (d, J = 4.0 Hz, 1H), 2.25 (d, J = 7.3 Hz, 2H), 1.96 (s, 3H), 0.95 (s, 3H), 0.85 (m, 9H), 0.61 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz):δ169.55, 138.64, 121.61, 76.06, 72.99, 55.66, 55.02, 49.01, 41.33, 38.72, 37.11 (X2), 35.98, 35.58, 34.69, 32.54, 31.04, 30.85, 29.58, 27.25, 26.76, 23.26, 20.41, 20.01, 18.29, 17.89, 17.65, 16.22, 10.86.

2.10. Δ^5-3β-Hydroxycholestene-24-one-3-acetate (***7a***)

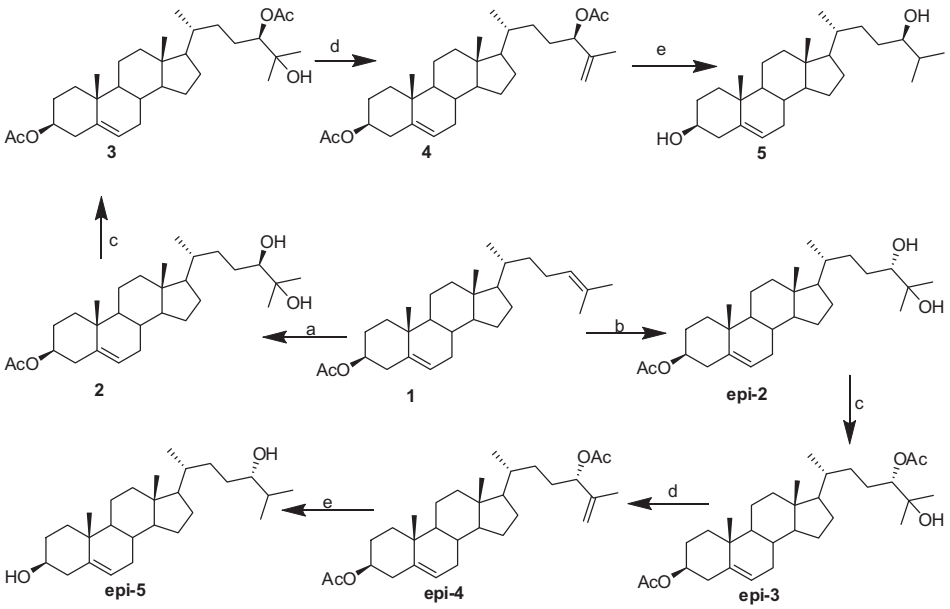
To a stainless steel autoclave was added **6** (2.22 g, 5 mmol), TEMPO (0.04 g, 0.25 mmol), *t*-Butyl nitrite (0.02 g, 0.2 mmol), $FeCl_3$ (0.04 g, 0.25 mmol) and dichloroethane (20 mL). Then the autoclave was closed and charged with oxygen to 0.4 MPa. The reaction mixture was then vigorously stirred at room temperature for 8 h. The barometer dropped to 0.38 MPa, which indicated that the reaction was finished. Carefully depressurized, the reaction mixture was rotary-evaporated. The final products (1.88 g, 86.2%) were isolated using column chromatography (absorbent: silica gel, mobile phase: *n*-hexane/EtOAc (8:1, v/v)). **7a** [1]: mp: 126–128 °C (lit. mp: 128–130 °C). MS-EI (*m*/*z*): 465 (M^++Na), 383 (M^+-CH_3COOH). IR (cm^{-1}): 1730($COCH_3$), 1703(C=O). ^{1}H NMR ($CDCl_3$, 400 MHz): δ5.30 (d, J = 4.2 Hz, 1H), 4.53 (m, 1H), 1.96 (s, 3H), 1.03 (s, 3H), 1.01 (s, 3H), 0.95 (s, 3H), 0.85 (d, J = 6.5 Hz, 3H), 0.61 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz):δ214.41, 169.51, 138.63, 121.59, 72.95, 55.65, 54.87, 48.99, 41.35, 39.82, 38.70, 37.10 (X2), 36.19, 35.98, 35.57, 34.35, 30.85, 28.83, 27.10, 26.76, 23.24, 20.41, 20.01, 18.29, 17.50, 17.36, 17.29, 10.86.

2.11. Δ^5-3β-Hydroxycholestene-24-one (***7***)

Alkaline hydrolysis of **7a** with 10% methanolic KOH, as described for the preparation of **5**, gave Δ^5-3β-hydroxycholestene-24-one (**7**), which recrystallized from methanol as colorless prisms. **7** [1]: mp: 129–130 °C (lit. mp: 131–133 °C). MS-EI (*m*/*z*): 400 (M^+), 385 (M^+-CH_3), 382(M^+-H_2O), 367(M^+-CH_3-H_2O). IR (cm^{-1}): 3381(OH), 1714(C=O). ^{1}H NMR ($CDCl_3$, 400 MHz): δ5.28 (d, J = 4.7 Hz, 1H), 3.45 (m, 1H), 1.03 (s, 3H), 1.01 (s, 3H), 0.94 (s, 3H), 0.85 (d, J = 6.5 Hz, 3H), 0.61 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz):δ214.52, 139.73, 120.67, 70.78, 55.72, 54.89, 49.09, 41.35, 41.27, 39.82, 38.75, 36.24 (X2), 35.48, 34.38, 30.88 (X2), 30.63, 28.84, 27.12, 23.25, 20.06, 18.37, 17.49, 17.36, 17.29, 10.86.

3. Results and discussion

Our initial step of the synthesis was focused on establishing a reliable method to generate a specific chiral center on the 24-position of the cholesterol side chain. In the past, the synthesis of 24-functionalized oxysterols was performed by benzoylation of a mixture of 24R- and 24S-hydroxycholesterol, followed by either resolution via recrystallization or chromatography and hydrolysis [10]. Recently, it is reported that the fundamental strategy adopted for stereoselective generation of the 24-functionalized oxysterols was to apply Sharpless asymmetric dihydroxylation to an appropriate derivative of desmosterol, such as acetate **1**, to produce 24 (R)-hydroxycholesterol and it epimer [epi's]. The starting material, desmosterol acetate **1**, was prepared according to the literature [11]. As depicted in Scheme 1, with adequate quantities of starting



Scheme 1. The synthesis routes of C-24 and C-25 oxysterols from desmosterol. Reagents and conditions: (a) $K_3Fe(CN)_6$, K_2CO_3, $CH_3SO_2NH_2$, $(DHQD)_2PHAL$, $K_2OsO_2(OH)_4$, *t*-butanol-water-ethyl acetate (1:1:1.2), 0 °C. (b) $K_3Fe(CN)_6$, K_2CO_3, $CH_3SO_2NH_2$, $(DHQ)_2PHAL$, $K_2OsO_2(OH)_4$, *t*-butanol-water-ethyl acetate (1:1:1.2), 0 °C. (c) Ac_2O, Py, rt. (d) $SOCl_2$, Py, 0 °C. (e) 10% Pd-C, EtOAc, rt, then KOH, MeOH, reflux.

material available, its asymmetric dihydroxylation by the Sharpless procedure was explored.

In our systematic study of Sharpless catalytic asymmetric dihydroxylation of Δ^{24}-sterols, we found that the key factor influencing efficiency of the reaction is the solubility of steroids in Sharpless catalytic asymmetric dihydroxylation solvent system (*t*-butanol-water/1:1). The low solubility of steroids in solvent system led to the lower value of % *d.e.* and the longer reaction time [9,14]. Thus the aim for using desmosterol acetate is to increase the solubility of the substrate in the solvent system and to enhance the efficiency of Sharpless catalytic asymmetric dihydroxylation. However, the solubility of desmosterol acetate is still low. Fortunately, we found that the efficiency of Sharpless catalytic asymmetric dihydroxylation can be promoted by means of enhancement of the volume of the solvent and addition of suitable auxiliary solvent. Different auxiliary solvent were screened and the results were summarized in Table 1.

Among the screened solvents, EtOAc was found to be the best solvent (Table 1, entry 4). This solvent system has the advantages over the reported solvent system (1:1 *t*-BuOH-H_2O [9] and 1:1:1.2 *t*-BuOH-H_2O-MTBE [10]) in the asymmetric dihydroxylation reaction in that it can greatly shorten the reaction time and increase the diastereoselectivity due to the large solubility of steroids therein. Meanwhile, we found that the Sharpless catalytic asymmetric dihydroxylation proceeded more efficiently when $K_2OsO_2(OH)_4$ was added to the reaction mixture in portions, which had never been reported before on steroidal substrates. Thus, using an improved Sharpless catalytic asymmetric dihydroxylation, **1** was dihydroxylated with $(DHQD)_2PHAL$ and $K_2OsO_2(OH)_4$ in *t*-BuOH-H_2O-EtOAc (1:1:1.2) within 6 h to give **2** in 100.0% *d.e.* and 95% yield. No dihydroxylation of the $\Delta^{5,6}$-double bond or formation of 24(S)-diol (epi-**2**) could be detected by ^1H NMR and ^{13}C NMR. The result is much better than the usual asymmetric dihydroxylation reaction procedure in terms of *d.e.* value and reaction time. This reaction was regio- and stereospecific. Thereby 24(R)-hydroxyl group was successfully introduced with the complete stereoselectivity. Δ^5-3β,24(S),25-Trihydroxycholestene-3-acetate (epi-**2**) was also prepared by a parallel route by use of $(DHQ)_2PHAL$ as catalyst in the asymmetric dihydroxylation step.

In the next step, compound **2** was selectively acetylated by using acetic anhydride and pyridine to give the acetate **3** in 95% yield. Dehydration of the 25-*tert*-hydroxy group in **3** with thionyl chloride and pyridine gave the compound **4** in 88% yield. This result is much better than that obtained from the methanesulfonyl chloride and triethylamine, as described in the preliminary literature [6,10,15,16]. However, when $POCl_3$ and pyridine was used, no product was detected within 24 h.

Fujimoto 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 [14,17,18]. Peaks due to C-24 of 24(R)-*O*-acetyl and 24(S)-*O*-acetyl compounds were observed at 76.59 ppm and 77.06 ppm, respectively. The configuration of 24(R)-*O*-acetyl compound **4** and 24(S)-*O*-acetyl compounds epi-**4** was the same as that of the starting material **3**, thus implying that the C-24 stereochemistry was retained during the course of dehydration.

Finally, the double bond between C-25 and C-26 of the compound **4** was selectively hydrogenated over 10% palladium

Table 1
Sharpless catalytic asymmetric dihydroxylation of desmosterol acetate in different solvents.[a]

Entry	Solvent[b]	Time/h	Yield[c]/%
1	–	72	83.0
2[d]	THF	10	85.5
3[d]	1,2-Dioxane	12	83.8
4	EtOAc	6	95.0
5[e]	EtOAc	12	86.1
6	MTBE	8	90.0
7	CH_2Cl_2	24	–

[a] Reaction conditions: desmosterol acetate (5 mmol), $K_3Fe(CN)_6$ (15 mmol), K_2CO_3 (15 mmol), $CH_3SO_2NH_2$ (5 mmol), $(DHQD)_2PHAL$ (0.25 mmol), $K_2OsO_2(OH)_4$ (0.025 mmol), t-butanol-water (1:1, 80 mL), 0 °C, solvent (50 mL). Additional $K_2OsO_2(OH)_4$ (0.01 g, 0.025 mmol) was added 3 h after the beginning of the reaction.

[b] THF, tetrahydrofuran; EtOAc, ethyl acetate; MTBE, methyl t-butyl ether; CH_2Cl_2, dichloromethane.

[c] Isolated yield with chromatography.

[d] Solvent (100 mL).

[e] $K_2OsO_2(OH)_4$ (0.05 mmol)was added in one portion.

on charcoal to give 24(R)-hydroxycholesterol (**5**) in 85% yield because of lower steric hindrance of the double bond compared with the inner double bond between C-5 and C-6. Taken together, one of the target molecules, 24(R)-hydroxycholesterol (**5**) was synthesized from desmosterol acetate through 4 steps in an overall yield of 67.5% and 100% *d.e.*

With the key intermediate 24-hydroxycholesterol in hand, attention was then turned to develop a facile synthesis of naturally occurring 24-ketocholesterol, which has been isolated from various biological fluids [19–21]. Oxidation of 24-hydroxycholesterol with a variety of chromium (VI) compounds have been used in the synthesis of 24-ketocholesterol (Scheme 2) [1,2,22–26]. However, complications in applying these methods remain inconvenient because of the harsh reaction conditions required and difficult workup and purification procedures. In addition, the accumulations of chromic acid or chromium salt wastes that are the side products of these reactions are of great environmental concern. From economic and environmentally perspectives, there is an urgent need for inexpensive and intrinsically waste-free oxidants and a recyclable catalyst that may be used to perform this transformation. Therefore, the pursuit of using molecular oxygen or air as terminal oxidant is particularly attractive, because molecular oxygen is inexpensive, abundant and water is produced as the only byproduct in principle. However, according to the best of our knowledge, the catalytic molecular-oxygen-driven oxidation of steroidal substrates has seldom been reported in the literature. Hence, taking practical, environmental, and economic factors into accounts, it is necessary to develop cleaner and cheaper aerobic oxidation systems for the 24-functionalized oxysterols.

In the first stage, the 24-hydroxycholesterol acetate (**6**) was synthesized in the presence of acetic anhydride and triethylamine. Because of the high energy barrier for electron transfer from substrate to oxygen, the direct oxidation by molecular oxygen is often kinetically unfavored. In order to effectively activate molecular oxygen, numerous catalytic systems have been developed. Of particular interest are catalytic systems involving stable nitroxyl free radicals, e.g., 2,2,6,6-tetramethyl-piperidyl-1-oxy (TEMPO). The catalytically active species in oxidation is not the nitroxide itself, but its oxidized form, the corresponding *N*-oxo-ammonium salt. In practice, the oxoammonium salt is generated *in situ* by one-electron oxidation using various cooxidants. Thus, the introduction of TEMPO as a catalyst in the cooxidants-based catalytic systems was examined (Table 2).

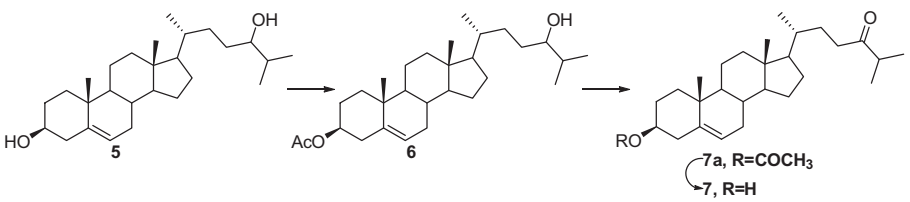
According to the literature [27], the electrode potential (E^0) of TEMPO cation/TEMPO is 0.75 V, while E^0 of N_2O_4/NO is 1.03 V. Therefore, we reckoned that NO_2 can oxidize TEMPO into the TEMPO cation to initiate the oxidation. Many reports in literature [28–31] showed that $NaNO_2$ acts as a cheapest and most convenient source of NO. However, $NaNO_2$ alone showed almost no activity in TEMPO-catalyzed aerobic oxidation. Most of these methods require a co-catalyst to bridge the two catalytic cycles between TEMPO cation/TEMPOH and NO/NO_2. Iron is an extremely abundant metal in the earth's crust and one of its important functions in living systems is responsibility for oxygen transport and electron transport linking the oxidation for substrates to the reduction of oxygen. We were inspired as to whether the combination of Fe and NO source could lead to an efficient catalytic system for 24-functionalized oxysterols oxidation under mild conditions in the presence of TEMPO.

Table 2
Oxidation of 24-hydroxycholesterol acetate.[a]

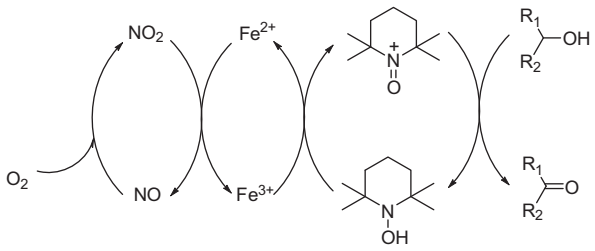
Entry	NO equivalent/mmol	Metal salt/mmol	Yield[b]/%
1	$NaNO_2$	$FeCl_3$	40.7
2	$Fe(NO_3)_3{\cdot}9H_2O$	$FeCl_3$	48.3
3	$Cu(NO_3)_2{\cdot}2.5H_2O$	$FeCl_3$	25.8
4	$Co(NO_3)_2{\cdot}6H_2O$	$FeCl_3$	–
5	$Ce(NO_3)_2{\cdot}6H_2O$	$FeCl_3$	–
6	$Mn(NO_3)_2$	$FeCl_3$	–
7	$Zn(NO_3)_2{\cdot}6H_2O$	$FeCl_3$	–
8	NH_2OH	$FeCl_3$	51.8
9	NH_4NO_3	$FeCl_3$	–
10	TBN	$FeCl_3$	86.2
11	TBN	$Fe(NO_3)_3{\cdot}9H_2O$	38.5
12	TBN	$Fe_2(SO_4)_3{\cdot}6H_2O$	30.0
13	TBN	$FeSO_4{\cdot}7H_2O$	29.8
14	TBN	$CuCl_2$	80.0
15	TBN	–	30.1
16	–	$FeCl_3$	–
17[c]	–	$FeCl_3$	20.5
18[d]	TBN	$FeCl_3$	85.9
19[e]	TBN	$FeCl_3$	–

[a] Reaction conditions: substrate (5 mmol), dichloroethane (20 mL), TEMPO (5%), O_2 (0.4 MPa), NO equivalent (4%), metal salt (5%), room temperature, 8 h.
[b] Isolated yield with chromatography.
[c] $FeCl_3$ (5 mmol).
[d] The reaction was heated to 80 °C.
[e] Without TEMPO.

In our initial experiments, we decided to investigate various NO sources as potential catalytic promoters of these processes. Considering the catalytically active NO and NO_2 will be removed from the reaction mixture while flushing the head space with oxygen, we decide to perform our studies in a stainless steel autoclave to prevent leakage of NO and NO_2. A constant O_2 pressure of 4 bar was used in all experiments. First experiment was performed using **6** as substrate with TEMPO, $FeCl_3$ and $NaNO_2$ in dichloroethane at room temperature according to the literature [28]. However, the result was poor. A more robust system is clearly desirable. One to overcome this inherent limitation is to develop a more efficient NO source. Toward this end, various inorganic and organic nitrites or even aqueous hydroxylamines were examined. The result showed that the *t*-butyl nitrite (TBN), which has been frequently used as the reagents for nonaqueous nitrozation and diazotization, is the most efficient NO source. Further experiments showed that the iron counterion is critical to the reaction with chloride proving to be superior to sulfate and nitrate. The reaction could be accomplished using $CuCl_2$ in place of $FeCl_3$ with a slightly decrease of yield. Interestingly, we found that increasing the temperature has little effect on the yield. In addition, control experiments showed that the catalytic system was ineffective in the absence of TEMPO. When air was used to replace pure oxygen, a slightly lower conversion rate was observed.



Scheme 2. The synthesis of 24-ketocholesterol.



Scheme 3. A plausible mechanism for the catalytic oxidation system.

After systematic optimization of reaction conditions, we successfully developed a high efficient and applicable catalytic system for the oxidation of 24-functionalized oxysterols with oxygen, which has never been achieved before.

A plausible mechanism of this catalytic oxidation system is described below (Scheme 3). Experiment showed that 20% of compound **6** was oxidized in the presence of stoichiometric oxidant $FeCl_3$ and TEMPO. Therefore, TEMPO is envisioned to perform the oxidation of 24-functionalized oxysterols with the help of Fe^{3+} that initiates a series of electron and proton transfer steps. The role of TBN could be the source of NO. The catalytic mechanism involves three tandem reaction cycles: (1) a redox cycle of NO and NO_2 responsible for activating the molecular oxygen; (2) a redox of TEMPO and its oxoammonium cation resulting in the oxidation of 24-functionalized oxysterols to the corresponding carbonyl compounds; (3) the redox cycle of Fe^{2+} and Fe^{3+} bridging between the above two cycles.

4. Conclusion

In summary, there are two key elements in our synthesis of oxysterols (I)–(V). The first one is the mild, efficient and stereoselective introduction of the 24R- and 24S-hydroxy group into the side chain of the desmosterol acetate, via an improved Sharpless catalytic asymmetric dihydroxylation with 100% d.e. The second is the catalytic molecular-oxygen-driven oxidation of 24-hydroxycholesterol, which was never been achieved before. Compared to other pre-existing chromium-mediated methods, this system was proved to be inexpensive, atom-economy and waste-free. $FeCl_3$-TEMPO-TBN system can act as a new, efficient and attractive catalyst for steroidal substrates aerobic oxidation, using commercially available materials.

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