Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsyc20

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Published online: 09 Nov 2006.

To cite this article: Takeru Yamaguchi , Ryusei Nakamori , Takashi Iida & Toshio Nambara (2001) 1β-HYDROXYLATION IN 5β-STEROIDS: AN EFFICIENT SYNTHESIS OF 1β,3α-DIHYDROXY-5β-CHOLAN-24-OIC ACID, Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry, 31:8, 1213-1219, DOI: 10.1081/SCC-100104006

To link to this article: http://dx.doi.org/10.1081/SCC-100104006

SYNTHETIC COMMUNICATIONS, 31(8), 1213–1219 (2001)

1β-HYDROXYLATION IN 5β-STEROIDS: AN EFFICIENT SYNTHESIS OF 1β,3α-DIHYDROXY-5β-CHOLAN-24-OIC ACID

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ABSTRACT

An efficient method for the oxyfunctionalization at C-1 in 5β-steroid (A/B-*cis*) is described as exemplified by conversion of lithocholic acid (3α-hydroxy-5β-cholan-24-oic acid) to 1β,3α-dihydroxy-5β-cholan-24-oic acid. The key reactions used are regioselective functionalization at C-2 in the 5β-steroid nucleus, stereoselective epoxidation of intermediary α,β-conjugated ketone with dimethyldioxirane, and subsequent reductive cleavage of the resulting β-epoxy-ketone with PhSeNa.

In continuation of a program of synthesis of biologically and physiologically important bile acid metabolites, the need for a supply of 1β-hydroxylated 5β-bile acids[1–5] as authentic specimens prompted us to examine literature preparations[6,7] of the compounds. The method, reported

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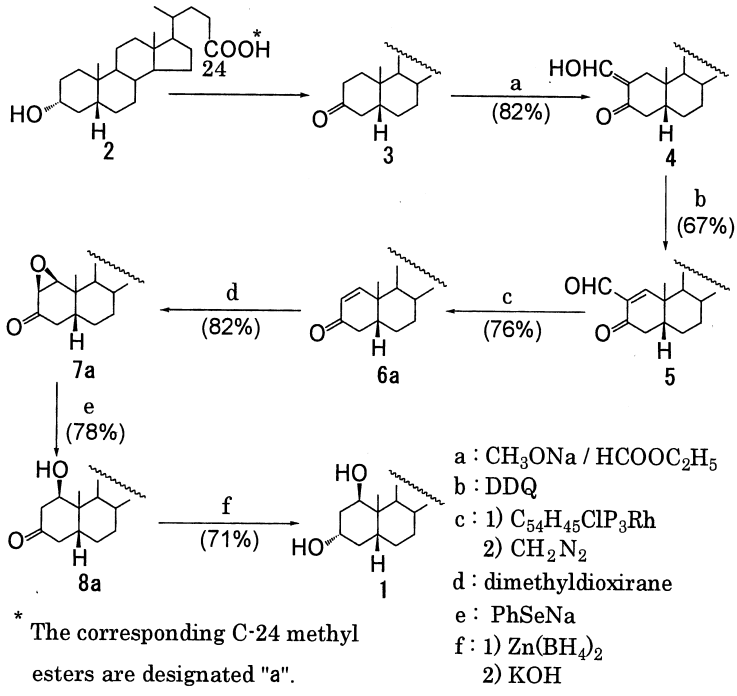
by Thoma *et al.*,[7] which involves selective dehydrobromination of 2,4-dibromo-3-oxo derivatives of 5β-bile acids, epoxidation of the resulting α,β-enones with alkaline hydrogen peroxide, and reductive cleavage of the 1β,2β-epoxy-3-ketones with chromous acetate, is somewhat complicated and inelegant, and renders low yields. The result implies that oxyfunctionalization at C-1 in 5β-steroids (normal series; A/B-*cis*) is less straightforward than in the more stable 5α-steroids (allo series; A/B-*trans*).

We report here a regio- and stereoselective method for the preparation of 1β-hydroxylated 5β-steroids, as exemplified by conversion of lithocholic acid (3α-hydroxy-5β-cholan-24-oic acid; **2**) to 1β,3α-dihydroxy-5β-cholan-24-oic acid (**1**).

As outlined in Scheme 1, the key intermediate is the α,β-conjugated enone ester **6a**, which was prepared regioselectively in three steps from the 3-oxo acid (**3**) of **1**, based on the preferential formylation at C-2 of 3-oxo-5β-steroids with A/B-*cis* junction.[8] Thus, the steps involve: 1) conversion of **3** into the 2-hydroxymethylene derivative **4** with sodium methoxide and ethyl formate; 2) dehydrogenation of **4** with



Scheme 1.

2,3-dichloro-5,6-dicyano-benzoquinone (DDQ); and 3) subsequent deformylation and methyl esterification of the resulting Δ^1-2-formyl-3-ketone **5** with chlorotris(triphenyl phosphine) rhodium. Each step proceeds through easily prepared, well-defined intermediates, and affords a pure product **6a** in reasonable yields.

Dimethyldioxirane, a new class of powerful organic oxidant and/or oxygen-transfer reagent, has been shown to carry out a variety of synthetically useful transformations.[9,10] Indeed, when the conjugated enone ester **6a** was subjected to the oxidation with dimethyldioxirane in $CHCl_3$ at room temperature for 1 h, the reaction proceeded stereoselectively to give the expected 1β,2β-epoxy-3-oxo ester **7a** in an excellent isolated yield (82%). Although the reagent is not commercially available, mild and neutral conditions, as well as easy work-up after the reaction, are especially attractive, since no unfavorable side reactions such as hydrolysis of the C-24 ester group occur, in contrast with the conventional epoxidation of α,β-enones with alkaline hydrogen peroxide.

Reductive cleavage of the 1β,2β-epoxy-3-ketone **7a** to the corresponding 1β-hydroxy-3-oxo ester **8a** was successfully achieved by organoselenium-mediated reduction with PhSeNa,[11] which is generated by simply mixing diphenyl diselenide and $NaBH_4$ in ethanol in the presence of a catalytic amount of acetic acid, and led to β-hydroxylation at C-1. The organoselenium-mediated reduction is rapid (20 m) and clean, and raised the isolated yield of **8a** to 78%.

Although $LiAlH_4$ and chromous acetate have been traditionally employed for the reductive cleavage of steroidal epoxides, the former reduced the C-24 ester group of **7a**, while the latter produced the desired **8a** in a fairly low yield.

The final step of stereoselective reduction of **8a** to its corresponding C-3 equatorial alcohol (**1a**) initially appeared simply to require reduction by $NaBH_4$, *tert*-butylamine-borane complex or metallic K/*tert*-amyl alcohol, which normally reduce saturated 3-ketones stereoselectively to C-3 equatorial products **2**.[12] However, with **8a**, the reducing reagents unexpectedly yielded the 3β-hydroxy epimer in considerable amounts, probably owing to shielding of the β-face of the molecule by the axial 1β-hydroxy substituent. Ultimately, selective reduction to **1a** was successful when the reagent was $Zn(BH_4)_2$. With this reagent, **8a** underwent stereoselective reduction at C-3, and after hydrolysis of the C-24 ester group, yielded the desired 1β,3α-dihydroxy acid (**1**) in a good isolated yield. Thus, the overall yield of good quality of **1** from **2** was at least ca. 18%.

In conclusion, the highly regio- and stereoselective reactions found in this study suggest their utility for the preparation of other bioactive 1β-hydroxylated steroids and vitamin D analogs in the 5β-series.

EXPERIMENTAL

Melting points (m.p.) were determined on an electric micro hot stage and are uncorrected. IR spectra were obtained on a Bio Rad FTS-7 FT-IR spectrometer (Philadelphia, PA) as KBr disks. UV spectra were measured on a HITACHI U-2000A spectrophotometer in ethanol solution. ^{1}H NMR spectra were obtained on a JEOL JNM-EX 270 instrument at 270 MHz, with $CDCl_3$ containing Me_4Si as the solvent, unless otherwise specified; chemical shifts are expressed in δ (ppm) relative to Me_4Si. Analytical TLC was performed on precoated silica gel plates (20 cm × 20 cm, 0.25 mm layer thickness; Merck, Darmstadt, Germany) using EtOH-hexane-acetic acid (50:50:1∼10:40:2, v/v/v) as the developing solvent. Lithocholic acid was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan); it was converted to the 3-oxo derivative (3) by the usual method.

A solution of dimethyldioxirane in $CHCl_3$ was prepared as described by Singh and Murray[13] and was standardized by iodometric titration; the concentration was 0.25 M.

2-Hydroxymethylene-3-oxo-5β-cholan-24-oic Acid (4)

To a stirred solution of **3** (1.0 g) (prepared from **2** by the Jones oxidation) and sodium methoxide (600 mg) in dry pyridine (40 mL) was added dropwise ethyl formate (12 mL), and the mixture was stirred at room temperature under N_2 for 8 h. The reaction was quenched by adding an excess of water and then acidified with 10% HCl. The reaction product was extracted with EtOAc. The combined extracts were washed with 10% HCl and saturated brine, dried over Drierite, and evaporated to give the title compound **4**, which was recrystallized from acetone-hexane as a colorless amporphous solid: yield, 886 mg (82%); m.p. 180°∼183°C. IR, ν_{max} cm^{-1}: 1712 (C=O). UV λ_{max}: 288 nm (ε, 7400, EtOH). ^{1}H NMR ($CDCl_3$ + 20% DMSO-d_6) δ: 0.66 (s, 3H, 18-CH_3), 0.90 (d, 3H, J = 6.2 Hz, 21-CH_3), 1.05 (s, 3H, 19-CH_3), 8.29 (s, 1H, 2-CHOH). MS (as the methyl ester), m/z: 416 (8%, M), 398 (14%, M−H_2O), 388 (25%, M−CO), 387 (32%, M−CHO), 318 (72%, M−ringA).

2-Formyl-3-oxo-5β-chol-1-en-24-oic Acid (5)

To a solution of the 2-hydroxymethylene-3-oxo acid **4** (1.0 g) in dry benzene (40 mL) was added DDQ (600 mg), and the resulting mixture was stirred at room temperature for 5 m. The solvent was evaporated under

reduced pressure, and the reaction product was dissolved in CH_2Cl_2 (20 mL). The insoluble material was filtered off, and the mother liquor was evaporated to give a residue. Chromatography of the residue on a column of silica gel (30 g) and elution with CH_2Cl_2-acetone (98:2, v/v) afforded the title compound **5**, which was recrystallized from EtOAc-hexane as a colorless amorphous solid: yield, 660 mg (67%); m.p. 188° ~ 191°C. IR ν_{max} cm^{-1}: 1735, 1704, 1684 (C = O). ^1H NMR δ: 0.71 (s, 3H, 18-CH_3), 0.92 (d, 3H, J = 6.2 Hz, 21-CH_3), 1.30 (s, 3H, 19-CH_3), 7.65 (s, 1H, 1-H), 10.09 (s, 1H, 2-CHO). MS (as the methyl ester), *m/z*: 414 (10%, M), 386 (100%, M−CO), 318 (35%, M−ringA).

Methyl 3-Oxo-5β-chol-1-en-24-oate (6a)

To a solution of the 2-formyl-Δ^1 acid **5** (500 mg) in dry benzene (50 mL) was added freshly prepared chlorotris(triphenylphosphine)-rhodium (1.0 g), and the resulting mixture was refluxed under N_2 for 3 h. The solvent was evaporated, and the reaction product was dissolved in methanol (10 mL). After removal of the insoluble material by filtration, the filtrate was esterified with diazomethane. The methylated product, which was shown by TLC to consist essentially of a single component, was purified by a column of silica gel (20 g). Elution with benzene-EtOAc (95:5, v/v) afforded the title compound **6a**, which was recrystallized from EtOAc-hexane as a colorless thin plate: yield, 365 mg (76%); m.p. 141°~143°C (lit.[7] 144°~145°C). IR ν_{max} cm^{-1}: 1735, 1679 (C = O). ^1H NMR δ: 0.69(s, 3H, 18-CH_3), 0.91 (d, 3H, J = 6.2 Hz, 21-CH_3), 1.19 (s, 3H, 19-CH_3), 3.66 (s, 3H, $COOCH_3$), 5.89 (d, 1H, J = 10.0 Hz, 2-H), 6.83 (d, 1H, J = 10.0 Hz, 1-H). MS, *m/z*: 386 (100%, M), 355 (25%, M−OCH_3), 271 (71%, M−S.C.).

Methyl 1β,2β-Epoxy-3-oxo-5β-cholan-24-oate (7a)

To a solution of the 3-oxo-Δ^1 ester **6a** (300 mg) in CH_2Cl_2 (10 mL) was added a solution of dimethyldioxirane (0.25 M, 6 mL; 1.5 mmol) in $CHCl_3$, and the mixture was stirred at room temperature for 1 h. Evaporation of the solvent yielded the 1β,2β-epoxy-3-oxo ester **7a**, which was recrysatllized from methanol as colorless needles: yield, 256 mg (82%); m.p. 111°~115°C (lit.[7] 123°~124°C). IR ν_{max} cm^{-1}: 1740, 1713 (C = O). ^1H NMR δ: 0.85 (s, 3H, 18-CH_3), 0.68 (d, 3H, J = 7.0 Hz, 21-CH_3), 1.27 (s, 3H, 19-CH_3), 3.26 (d, 1H, J = 4.1 Hz, 1α-H), 3.39 (d, 1H, J = 4.1 Hz, 2α-H), 3.64

(s, 3H, $COOCH_3$). MS, *m/z*: 402 (16%, M), 384 (26%, M−H_2O), 371 (10%, M−OCH_3), 353 (40%, M−H_2O−OCH_3), 287 (47%, M−S.C.).

Methyl 1β-Hydroxy-3-oxo-5β-cholan-24-oate (8a)

Acetic acid (36 μL) was added to a solution of PhSeNa, prepared by the reduction of diphenyl diselenide (600 mg) with $NaBH_4$ (185 mg) in ethanol (12 mL),[11] and the mixture was stirred at 0°∼5°C for 30 min. The resulting solution was added at once to a solution of the 1β,2β-epoxy ketone **7a** (200 mg) in ethanol (4 mL) under N_2 and stirred for 20 min at 0°∼5°C. The reaction mixture was diluted with water, neutralized with 10% H_2SO_4, and evaporated. The combined CH_2Cl_2 layer was washed with water, dried with Drierite, and evaporated, and the residue was chromatographed on silica gel (8 g). Elution with benzene-EtOAc (9:1, v/v) and recrystallization of the title compound **8** from EtOAc-hexane as colorless thin plates: yield, 156 mg (78%); m.p. 178°∼180°C (lit.[7] 179°∼181°C). IR ν_{max}. cm^{-1}: 3418 (OH), 1722, 1706 (C=O). ^1H NMR δ: 0.69 (s, 3H, 18-CH_3), 0.91 (d, 3H, J = 6.5 Hz, 21-CH_3), 1.15 (s, 3H, 19-CH_3), 3.67 (s, 3H, $COOCH_3$).4.16 (brm, 1H, J = 2.2 Hz, 1α-H). MS, *m/z*: 386 (100%, M), 355 (25%, M−OCH_3−H_2O), 271 (44%, M−H_2O–S.C.).

1β,3α-Dihydroxy-5β-cholan-24-oic Acid (1)

To a stirred solution of $Zn(BH_4)_2$ in Et_2O (2.5 mL, 4.8 mmol), a solution of 1β-hydroxy-3-oxo ester **8a** (100 mg) in C_6H_6-Et_2O (6 mL, 1:1, v/v) was added dropwise under N_2. After further stirring for 30 min at room temperature, the mixture was poured into water, dried with Drierite, and evaporated to dryness. Crystallization of the residue from EtOAc-hexane gave the 1β,3α-dihydroxy ester (**1a**) as colorless thin plates: yield, 75 mg (75%); m.p. 195°∼198°C (lit.[7] 203°∼205°C). IR ν_{max}. cm^{-1}: 3425 (OH), 1720, (C=O). ^1H NMR δ: 0.65 (s, 3H, 18-CH_3), 0.90 (d, 3H, J = 6.2 Hz, 21-CH_3), 1.05 (s, 3H, 19-CH_3), 3.67 (s, 3H, $COOCH_3$), 3.94 (brm, 1H, 1α-H), 4.13 (m, 1H, J = 5.1 Hz, 3β-H). MS (as the methyl ester-trimethylsilyl ether derivative), *m/z*: 535 (2%, M−CH_3), 460 (85%, M−TMSOH), 370 (6%, M−2TMSOH).

The ester **1a** was hydrolyzed by the usual methanolic potassium hydroxide. Recrystallization of the product, from EtOH-hexane gave the desired acid **1** as a colorless amorphous solid: yield, 94%; m.p. 272°∼275°C (lit.[7] 274°∼278°C). IR ν_{max} cm^{-1}: 3267 (OH), 1713 (C=O). ^1H NMR

($CDCl_3 + 20\%$ DMSO-d_6) δ: 0.64 (s, 3H, 18-CH_3), 0.92 (d, 3H, J = 6.2 Hz, 21-CH_3), 1.03 (s, 3H, 19-CH_3).

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan. Masami Kitamura and Masako Ishibashi contributed able technical assistance.

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Received May 15, 2000