Lipids
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METHODS

Chemical Synthesis of the Epimeric (23*R*)- and (23*S*)-Fluoro Derivatives of Bile Acids via Horner–Wadsworth–Emmons Reaction

Kaoru Omura[1] · Yuuki Adachi[1] · Yuuki Kobayashi[1] · Shoutaro Sekiguchi[1] · Biao Zhou[1] · Takashi Iida[1]

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Abstract A method for the synthesis of two (23*R*)- and (23*S*)-epimeric pairs of 23-fluoro-3α,7α,12α-trihydroxy-5β-cholan-24-oic acid and 23-fluoro-3α,7α-dihydroxy-5β-cholan-24-oic acid is described. The key intermediates, 23,24-dinor-22-aldehyde peracetates were prepared from cholic and chenodeoxycholic acids via the 24-nor-22-ene, 24-nor-22ξ,23-epoxy, and 23,24-dinor-22-aldehyde derivatives. The Horner–Wadsworth–Emmons reaction of the 23,24-dinor-22-aldehydes using triethyl 2-fluoro-2-phosphonoacetate in the presence of LiCl and 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU), and subsequent hydrogenation of the resulting 23ξ-fluoro-22-ene ethyl esters, followed by hydrolysis, gave a mixture of the epimeric (23*R*)- and (23*S*)-fluorinated bile acids which were resolved efficiently by preparative RP-HPLC. The stereochemical configuration of the fluorine atom at C-23 in the newly synthesized compounds was confirmed directly by the X-ray crystallographic data. The ^{1}H and ^{13}C NMR spectral differences between the (23*R*)- and (23*S*)-epimers were also discussed.

Keywords Bile acid · Epimeric (23*R*)-/(23*S*)-fluoro-bile acids · Horner–Wadsworth–Emmons reaction · α-Fluoro-α,β-unsaturated esterification

Abbreviations

MMO 4-Methylmorpholine *N*-oxide
DBU 1,8-Diazabicyclo[5,4,0]undec-7-ene
NMR Nuclear magnetic resonance
HPLC High-performance liquid chromatography

✉ Takashi Iida
takaiida@chs.nihon-u.ac.jp

[1] Department of Chemistry, College of Humanities and Sciences, Nihon University, Sakurajousui, Setagaya, Tokyo 156-8550, Japan

Introduction

The incorporation of a fluorine atom into an advanced drug candidate is a common strategy in synthetic organic chemistry as well as medicinal chemistry. Extensive research has established that introducing fluorine into a target molecule can effectively modify its physicochemical and drug-like properties by blocking undesired metabolism at a specific site, increasing lipophilicity or binding affinity, or altering drug absorption, distribution, or excretion [1–4].

Considerable effort has been devoted to the development of methods for the introduction of a fluorine atom into a steroid molecule. It has been reported that a 9α-fluoro substituent adjacent to the 11-oxygen function in the adrenal cortical hormones markedly increases biological activity such as a topical anti-inflammatory one [5]. For example, the commercially available triamcinolone, dexamethasone, and betamethasone are the 9α-fluoro derivatives of cortisol (cortisone and hydrocortisone) that have potent topical anti-inflammatory properties. This finding substantiated the concept that a fluorine substituent could strongly influence the biological properties of a steroid. Therefore, the synthesis of fluorinated steroids with stereo control is of major importance in many areas including synthetic organic chemistry, pharmaceuticals, and biochemistry.

Although there have been many reports on the introduction of a fluorine atom in 5α- and 5β-steroid nuclei [6–13], that into the aliphatic cholestane (iso-octane) and cholane (iso-pentane) side chain has not yet been reported. During the course of our synthetic study of new and scarce steroids, we herein report the preparation of two (23*R*)- and

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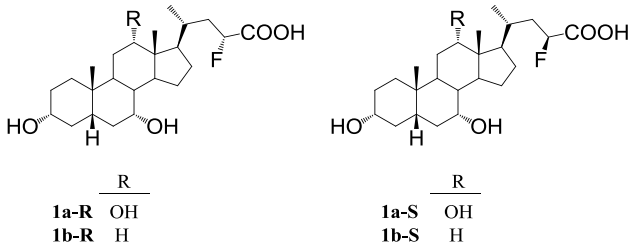


Fig. 1 Structure of epimeric (23*R*)- and (23*S*)-fluoro derivatives of CA and CDCA

(23*S*)-epimeric pairs of 23-fluorinated bile acids, starting from primary bile acids, chenodeoxycholic acid (CDCA; **2b**) and cholic acid (CA; **2a**), which are common in mammals. Thus, the root bile acid should be **2b**, as every bile acid must a hydroxyl group at C-3 and a hydroxyl group at C-7, as 7α-hydroxylation of cholesterol is the rate limiting step in the pathway of bile acid synthesis. The most frequent site of additional hydroxylation of **2b** is at C-12 in **2a**.

We applied herein the Horner-Wadsworth-Emmons reaction of triethyl 2-fluoro-2-phosphonoacetate in the presence of LiCl and 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) as base sensitive reagents on the 23,24-dinor-22-aldehyde derivatives (**6a** and **6b**) of bile acids as the key intermediates. The novel 23-fluorinated bile acids prepared by the method are shown in Fig. 1.

Experimental

Materials

CA (**2a**) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). CDCA (**2b**) was supplied from Tanabe Mitsubishi Pharmaceutical Co. (Tokyo, Japan). All other chemicals and solvents were of analytical reagent grade and available from commercial sources. All compounds were dried by azeotropic distillation before use in reactions.

Instruments

All melting points (mp) were determined on a micro hot stage apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained with a JEOL ECA 500 FT instrument operated at 500 and 125 MHz, respectively, with $CDCl_3$ or CD_3OD containing 0.1 % Me_4Si as the solvent; chemical shifts were expressed in δ (ppm) relative to Me_4Si (0 ppm). ^{19}F NMR was measured on the same instrument operated at 470 MHz with $CDCl_3$ or CD_3OD containing 0.1 % C_6F_6 as an internal reference compound; chemical shifts were expressed in δ (ppm) relative to C_6F_6 (−162.9 ppm). The ^{13}C distortionless enhancement by polarization transfer (DEPT; 135°, 90°, and 45°) spectra were measured to determine the exact ^{13}C signal multiplicity and to differentiate among CH_3, CH_2, CH, and C based on their proton environments. In order to further confirm the ^{1}H and ^{13}C signal assignments for some of compounds, two-dimensional (2D) ^{1}H detected heteronuclear multiple quantum (HMQC; ^{1}H–^{13}C coupling) and ^{1}H detected heteronuclear multiple bond correlation (HMBC; long-range ^{1}H–^{13}C coupling) experiments were also performed. High-resolution mass spectra by electrospray ionization (HR-ESI–MS) were obtained using a JEOL AccuTOF JMS-T100LC liquid chromatography-mass spectrometer equipped with an ESI source and coupled to an Agilent 1200 series binary pump (Agilent Technologies Inc., Santa Clara, CA, USA) operated in the negative ion mode or positive ion mode.

Normal-phase TLC was performed on pre-coated Kieselgel $60F_{254}$ plates (E. Merck, Darmstadt, Germany) using EtOAc-hexane mixtures as the developing solvent. Reversed-phase (RP)-TLC was performed on pre-coated RP-$18F_{254s}$ plates (E. Merck) using methanol–water mixtures as the developing solvents.

The analytical RP-HPLC apparatus used was a Jasco LC-2000 plus HPLC system, which consisted of two PU-2085 high-pressure pumps, an MX-2080-32 solvent mixing module, and a CO-2060 column heater equipped with a Chrom NAV data processing system (Tokyo, Japan). A Capcell pack type AQ RP-C_{18} column (3.0 × 150 mm I.D.; particle size, 3 μm; Shiseido, Tokyo, Japan) was employed and kept at 37 °C. An Alltech 2000ES evaporative light-scattering detector (ELSD; Deerfield, IL, USA) was used under the following conditions: the flow rate of purified compressed air used as the nebulizing gas was 2.2 L/min, and the temperature of the heated drift was 79.3 °C. The mobile phase used was a mixture of 15 mM ammonium acetate/acetic acid buffer solution (pH 5.4) and methanol (35:65 or 33:67, v/v); the flow rate was kept at 400 μL/min during the analysis.

The preparative RP-HPLC consisted of a Hitachi L-7100 pump (Tokyo, Japan) and was carried out by isocratic elution using a Capcell Pack type MG RP-C_{18} column (20 × 250 mm I.D.; particle size, 5 μm). A mixture of 15 mM ammonium acetate/acetic acid buffer solution (pH 5.4) and methanol (35:65 or 33:67, v/v) was used as the mobile phase; the flow rate was kept at 9.99 mL/min.

X-Ray Crystal Structure Determination of 1b-S

A high quality single crystal of **1b-S** suitable for X-ray diffraction studies was grown by evaporation of a solution of

methanol at room temperature. The X-ray diffraction data were collected on a Rigaku AFC-8 diffractometer equipped with a Mercury CCD detector using monochromated Mo (Kα) radiation (λ = 0.71075 Å) at 173 K. The structure was solved using direct methods (SHELXS-97) and refined by a full-matrix least-squares procedure based on F^2 using the CrystalStructure crystallographic software package. The crystal structures were refined with anisotropic temperature factors for all non-hydrogen atoms. The positions of hydrogen atoms were calculated geometrically, and were refined using the riding model. The crystal data and refinement details for **1b-S** are given in Tables 1 and 2.

Table 1 Crystallographic details for **1b-S**

Formula	$C_{24}H_{39}FO_4 \cdot 1.5H_2O$
M	434.57
Crystal system	Monoclinic
Space group	$P2_1$
a (Å)	10.482(3)
b (Å)	7.582(2)
c (Å)	15.134(4)
α (°)	90
β (°)	100.563(6)
γ (°)	90
V (Å^3)	1182.3(6)
Z value	2
D_c/g cm^{-3}	1.221
F(000)	472
Radiation type	Mo Kα
μ(Mo-K_α)/cm^{-1}	0.090
Crystal description	Block
Crystal size (mm)	0.20 × 0.20 × 0.20
No of reflns measured	25770
No of unique reflns	5405
No. observed ($I > 2\sigma$)	4530
R_1 ($I > 2\sigma$)	0.058
wR_2 ($I > 2\sigma$)	0.164
Goodness of fit	1.123

Table 2 Selected bond lengths (Å) and angles (°) for **1b-S**

O1–C3	1.415
O2–C7	1.450
F1–C23	1.397
O3–C24	1.302
O4–C24	1.208
F1–C23–C22	110.2
F1–C23–C24	107.0
C22–C23–C24	111.5

Chemical Synthesis of 23-Fluorinated Bile Acids

*Ethyl (22E)- 23-Fluoro-3α,7α,12α-triacetoxy-5β-chol-22-en-24-oate (**7a**)*

Triethyl 2-fluoro-2-phosphonoacetate (800 mg) and 1,8-diazabicyclo[5,4,0]-7-undecene (DBU, 350 mg) were added gradually to a stirred solution of LiCl (120 mg) in anhydrous CH_3CN (40 mL). To the solution, the 23,24-dinor-22-aldehyde triacetate **6a** (1.2 g, 2.4 mmol) was then added, and the mixture was further stirred under N_2 at room temperature for 3 h. After adding an ice-cold ammonium chloride solution, the reaction product was extracted with EtOAc. The combined extract was washed with saturated brine, dried over Drierite, and evaporated under reduced pressure. The oily residue was chromatographed on a column of silica gel (40 g) and elution with hexane–EtOAc (80:20, v/v) afforded the desired Δ^{22}-23-fluoro-24-carboxylate (**7a**) which crystallized from acetone-Et_2O as colorless prisms: yield, 990 mg (70 %); mp, 98–99 °C. ^{1}H NMR ($CDCl_3$) δ: 0.80 (3H, s, 18-CH_3), 0.93 (3H, s, 19-CH_3), 0.96 (3H, d, J = 6.3 Hz, 21-CH_3), 1.26 (3H, t, J = 6.9 Hz, –$COOCH_2\underline{CH_3}$), 2.05 (3H, s, 3α-$OCOCH_3$), 2.08 (3H, s, 7α-$OCOCH_3$), 2.16 (3H, s, 12α-$OCOCH_3$), 4.13 (2H, m, –$COO\underline{CH_2}CH_3$), 4.58 (1H, brm, 3β-H), 4.91 (1H, brs, 7β-H), 5.10 (1H, brs, 12β-H), 5.68 (1H, m, 22-H). ^{13}C NMR ($CDCl_3$) δ: 34.5 (C-1), 25.4 (C-2), 73.8 (C-3), 34.5 (C-4), 40.7 (C-5), 31.0 (C-6), 70.4 (C-7), 37.6 (C-8), 28.8 (C-9), 34.2 (C-10), 26.4 (C-11), 75.0 (C-12), 45.0 (C-13), 43.3 (C-14), 22.6 (C-15), 26.7 (C-16), 47.5 (C-17), 12.4 (C-18), 22.4 (C-19), 31.8 (C-20), 19.5 (C-21), 128.5 (d, J = 15.5 Hz, C-22), 145.4 (d, J = 250.4 Hz, C-23), 160.9 (d, J = 36.0 Hz, C-24), 170.3, 170.5, 170.5 (each $O\underline{C}OCH_3$), 21.2, 21.3, 21.4 (each $OCO\underline{C}H_3$), 13.9 ($CH_2\underline{C}H_3$), 61.2 ($\underline{C}H_2CH_3$). ^{19}F NMR ($CDCl_3$) δ: −131.8 (1F, t, J = 29.3 Hz, 23-F). LC–ESI–MS: Calcd. for $C_{32}H_{47}FNaO_8$ $[M+Na]^+$, 601.3153; found, *m/z* 601.3151.

*Ethyl (22E)-23-Fluoro-3α,7α-diacetoxy-5β-chol-22-en-24-oate (**7b**)*

The 23,24-dinor-22-aldehyde diacetate **6b** (1.2 g, 2.3 mmol) was subjected to the Horner-Wadsworth-Emmons reaction with triethyl 2-fluoro-2-phosphonoacetate and DBU and LiCl processed as described for the preparation of **7a**. Recrystallization of the product from methanol-$CHCl_3$ gave the desired Δ^{22}-23-fluoro-24-carboxylate (**7b**) as colorless prisms: yield, 870 mg (72 %); mp, 96–98 °C. ^{1}H NMR ($CDCl_3$) δ: 0.73 (3H, s, 18-CH_3), 0.94 (3H, s, 19-CH_3), 1.04 (3H, d, J = 6.8 Hz, 21-CH_3), 1.20 (3H, t, J = 6.9 Hz, –$COOCH_2\underline{CH_3}$), 2.05 (6H, s, 3α- and 7α-$OCO\underline{CH_3}$), 4.13 (2H, m, –$COO\underline{CH_2}CH_3$), 4.58 (1H,

brm, 3β-H), 4.91 (1H, brs, 7β-H), 5.73 (1H, m, 22-H). ^{13}C NMR ($CDCl_3$) δ: 34.6 (C-1), 26.8 (C-2), 74.1 (C-3), 34.9 (C-4), 40.9 (C-5), 31.3 (C-6), 71.2 (C-7), 37.9 (C-8), 34.1 (C-9), 34.8 (C-10), 20.6 (C-11), 39.4 (C-12), 42.9 (C-13), 50.4 (C-14), 23.6 (C-15), 27.4 (C-16), 56.0 (C-17), 12.0 (C-18), 22.7 (C-19), 34.1 (C-20), 20.2 (C-21), 129.0 (d, $J = 14.8$ Hz, C-22), 145.6 (d, $J = 250.4$ Hz, C-23), 161.0 (d, $J = 35.8$ Hz, C-24), 170.4, 170.6 (each O<u>C</u>OCH$_3$), 21.5, 21.6 (each OCO<u>C</u>H$_3$), 14.1 (CH$_2$<u>C</u>H$_3$), 61.3 (<u>C</u>H$_2$CH$_3$). ^{19}F NMR ($CDCl_3$) δ: −131.0 (1F, t, $J = 29.3$ Hz, 23-F). LC–ESI–MS: Calcd. for $C_{30}H_{42}FNaO_6$ $[M+Na]^+$, 543.3098; found, *m/z* 543.3110.

*An Epimeric Mixture of Ethyl (23R)/(23S)-23-Fluoro-3α,7α,12α-triacetoxy-5β-cholan-24-oates (**8a**)*

The 23-fluoro-22-ene ethyl ester **7a** (1.0 g, 1.7 mmol) dissolved in dry THF (15 mL) was catalytically hydrogenated with 10 % Pd/C (0.6 g) at a slight positive pressure of H_2. After stirring at room temperature overnight, the catalyst was filtered off, and the solvent was evaporated under reduced pressure. The oily residue was chromatographed on a silica gel column (30 g) and eluted with hexane–EtOAc (50:50, v/v). The combined homogeneous fraction showed a single spot on TLC, but it was found to be an epimeric mixture (**8a**) of (23*R*)- and (23*S*)-23-fluoro esters by RP-HPLC: yield, 910 mg (91 %). ^{1}H NMR ($CDCl_3$) δ: 0.75 (3H, s, 18-CH$_3$), 0.92 (3H, s, 19-CH$_3$), 0.94 (3H, d, $J = 6.3$ Hz, 21-CH$_3$), 1.29 (3H, t, $J = 7.4$ Hz, –OCH$_2$<u>CH$_3$</u>), 2.05 (3H, s, 3α-OCO<u>CH$_3$</u>), 2.09 (3H, s, 7α-OCO<u>CH$_3$</u>), 2.14 (3H, s, 12α-OCO<u>CH$_3$</u>), 4.13 (2H, m, –O<u>CH$_2$</u>CH$_3$), 4.58 (1H, brm, 3β-H), 4.91 (1H, brs, 7β-H), 4.92 (1H, d, $J = 49.2$ Hz, 23-H), 5.10 (1H, brs, 12β-H). ^{19}F NMR ($CDCl_3$) δ: −188.9 (1F, t, $J = 29.3$ Hz, 23-F) and −192.9 (1F, m, 23-F). LC–ESI–MS: Calcd. for $C_{32}H_{49}FNaO_8$ $[M+Na]^+$, 603.3309; found, *m/z* 603.3311.

*An Epimeric Mixture of Ethyl (23R)/(23S)-23-Fluoro-3α,7α-diacetoxy-5β-cholan-24-oates (**8b**)*

The 23-fluoro-22-ene ethyl ester **7b** (1.0 g, 1.9 mmol), hydrogenated with 10 % Pd/C and processed as described for the preparation of **8a** yielded an epimeric mixture (**8b**) of (23*R*)- and (24*S*)-24-fluoro esters: yield, 920 mg (92 %). ^{1}H NMR ($CDCl_3$) δ: 0.62 (3H, s, 18-CH$_3$), 0.83 (3H, s, 19-CH$_3$), 1.05 (3H, d, $J = 6.3$ Hz, 21-CH$_3$), 1.27 (3H, m, –OCH$_2$<u>CH$_3$</u>), 1.98 (3H, s, 3α-OCOCH$_3$), 2.00 (3H, s, 7α-OCOCH$_3$), 4.20 (2H, m, –O<u>CH$_2$</u>CH$_3$), 4.53 (1H, brm, 3β-H), 4.83 (1H, brs, 7β-H), 4.91 (1H, d, $J = 50.4$ Hz, 23-H). ^{19}F NMR ($CDCl_3$) δ: -188.2 (1F, t, $J = 29.3$ Hz, 23-F) and −192.9 (1F, m, 23-F). LC–ESI–MS: Calcd. for $C_{30}H_{47}FNaO_6$ $[M+Na]^+$, 545.3254; found, *m/z* 545.3253.

*(23R)- and (23S)-23-Fluoro-3α,7α,12α-trihydroxy-5β-cholan-24-oic acids (**1a-R** and **1a-S**)*

A solution of a mixture (**8a**) of the epimeric 23-fluorotriacetoxy esters (100 mg, 0.17 mmol) dissolved in methanol (1 mL) and 20 % NaOH (1.5 mL) was left stand at room temperature for 12 h. After evaporation of the solvents under reduced pressure, the residual oily product was dissolved in water and then acidified with 10 % H_2SO_4 under ice-bath cooling. The precipitated solid was filtered, washed with water, and dried: yield, 70 mg (96 %). The crude product consisted of an epimeric mixture of (23*R*)- and (23*S*)-23-fluorinated epimers (ratio of 9:1) of CA (**2a**) by RP-HPLC. Preparative RP-HPLC of a part of the mixture resulted in separation of the two epimers.

The more polar, major fraction (90 % by RP-HPLC) eluted with a mixture of 15 mM ammonium acetate/acetic acid buffer solution (pH 5.4) and methanol (65:35, v/v) and gave a homogeneous oil which crystallized from methanol as a colorless amorphous solid and was identified as the (23*R*)-fluorinated epimer (**1a-R**) of **2a**. ^{1}H NMR ($CDCl_3$ containing 20 % CD_3OD) δ: 0.71 (3H, s, 18-CH$_3$), 0.89 (3H, s, 19-CH$_3$), 1.12 (3H, d, $J = 5.2$ Hz, 21-CH$_3$), 3.38 (1H, brm, 3β-H), 3.83 (1H, brs, 7β-H), 3.97 (1H, brs, 12β-H), 4.91 (1H, brd, $J = 52.7$ Hz, 23-H). ^{13}C NMR: see Table 3. ^{19}F NMR ($CDCl_3$ containing 20 % CD_3OD) δ: −185.22 (1F, brs, 23-F). LC–ESI–MS: Calcd. for $C_{24}H_{38}FO_5$ $[M-H]^-$, 425.2703; found, *m/z* 425.2684.

The less polar, minor fraction (10 % by RP-HPLC), which was eluted with the same solvent system, gave a homogeneous oil which crystallized from methanol as a colorless amorphous solid and was identified as the (23*S*)-fluorinated epimer (**1a-S**) of **2a**. ^{1}H NMR ($CDCl_3$ containing 20 % CD_3OD) δ: 0.72 (3H, s, 18-CH$_3$), 0.90 (3H, s, 19-CH$_3$), 1.10 (3H, d, $J = 6.3$ Hz, 21-CH$_3$), 3.38 (1H, brm, 3β-H), 3.84 (1H, brs, 7β-H), 3.98 (1H, brs, 12β-H), 4.96 (1H, dd, $J = 50.6$, 10.9 Hz, 23-H). ^{13}C NMR: see Table 3. ^{19}F NMR ($CDCl_3$ containing 20 % CD_3OD) δ: −191.92 (1F, brs, 23-F). LC–ESI–MS: Calcd. for $C_{24}H_{38}FO_5$ $[M-H]^-$, 425.2703; found, *m/z* 425.2691.

*(23R)- and (23S)-23-Fluoro-3α,7α-dihydroxy-5β-cholan-24-oic acids (**1b-R** and **1b-S**)*

The compounds were prepared from a mixture (**8b**) of the epimeric 23-fluorodiacetoxy esters (100 mg, 0.19 mmol) by the procedure described for the preparation of **1a**. The crude product (70 mg, 90 %) consisted of a 23*R*/23*S* ratio of 6:4 by RP-HPLC.

The first fraction (60 % by RP-HPLC) which eluted with a mixture of 15 mM ammonium acetate/acetic acid buffer solution (pH 5.4) and methanol (67:33, v/v) consisted of a

Table 3 ^{13}C NMR spectral data for the epimeric 23-fluoro derivatives of bile acids

Carbon	(23*R*)-23-Fluoro-3α,7α,12α-trihydroxy-5β-cholan-24-oic acid (**1a-R**)	(23*S*)-23-Fluoro-3α,7α,12α-trihydroxy-5β-cholan-24-oic acid (**1a-S**)	(23*R*)-23-Fluoro-3α,7α-dihydroxy-5β-cholan-24-oic acid (**1b-R**)	(23*S*)-23-Fluoro-3α,7α-dihydroxy-5β-cholan-24-oic acid (**1b-S**)
1	35.1	35.0	35.2	35.1
2	29.7	29.6	30.1	30.1
3	71.3	71.3	71.5	71.5
4	39.0	39.0	39.1	39.1
5	41.2	41.2	41.4	41.3
6	34.3	34.3	34.4	34.4
7	68.1	68.1	68.1	68.1
8	39.2	39.2	39.1	39.1
9	26.1	26.1	32.6	32.6
10	34.5	34.5	34.9	34.8
11	27.8	27.8	20.4	20.4
12	72.7	72.7	39.4	39.4
13	46.3	46.3	42.6	42.6
14	41.5	41.5	50.2	50.2
15	23.0	23.0	23.4	23.4
16	27.5	27.3	28.2	28.0
17	47.0	47.1	56.1	56.0
18	12.1	12.2	11.4	11.5
19	22.2	22.2	22.6	22.6
20	33.9	32.0	34.0	32.1
21	18.0	16.7	19.1	17.8
22	38.6 (*J*, 19.2 Hz)	38.5 (*J*, 20.4 Hz)	38.7 (*J*, 19.2 Hz)	38.6 (*J*, 20.4 Hz)
23	89.2 (*J*, 180.0 Hz)	87.2 (*J*, 182.4 Hz)	89.2 (*J*, 183.6 Hz)	86.7 (*J*, 182.4 Hz)
24	174.1 (*J*, 25.2 Hz)	173.2 (*J*, 25.2 Hz)	174.0 (*J*, 20.3 Hz)	173.2 (*J*, 22.8 Hz)

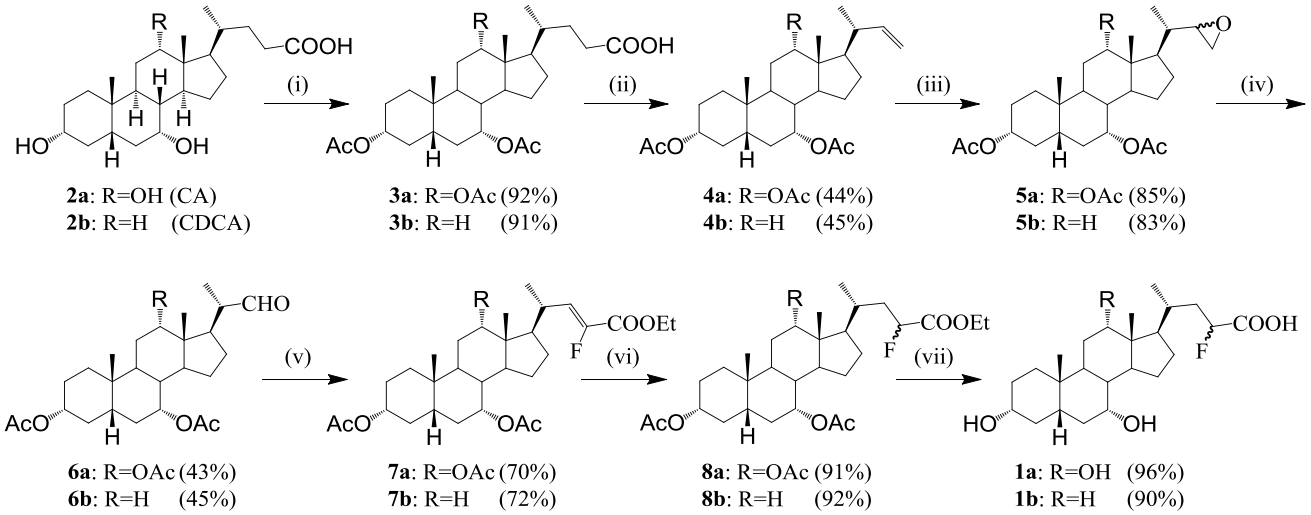
Measured in $CDCl_3$ containing 20 % of CD_3OD

homogenous oil that crystallized from methanol as a colorless amorphous solid and was characterized as the (23*R*)-fluorinated epimer (**1b-R**) of CDCA (**2b**). ^1H NMR ($CDCl_3$ containing 20 % CD_3OD) δ: 0.68 (3H, s, 18-CH_3), 0.91 (3H, s, 19-CH_3), 1.06 (3H, d, $J = 6.3$ Hz, 21-CH_3), 3.41 (1H, brm, 3β-H), 3.83 (1H, brs, 7β-H), 4.90 (1H, brd, $J = 49.0$ Hz, 23-H). ^{13}C NMR: see Table 3. ^{19}F NMR ($CDCl_3$ containing 20 % CD_3OD) δ: −185.85 (1F, brs, 23-F). LC–ESI–MS: Calcd. for $C_{24}H_{38}FO_4$ $[M\text{-}H]^-$, 409.2754; Found, *m/z* 409.2800.

The second fraction (40 % by RP-HPLC), which eluted with the same solvent system, gave an oil that crystallized from methanol as colorless prisms and was characterized as the (23*S*)-fluorinated epimer (**1b-S**) of **2b**. ^1H NMR ($CDCl_3$ containing 20 % CD_3OD) δ: 0.69 (3H, s, 18-CH_3), 0.91 (3H, s, 19-CH_3), 1.04 (3H, d, $J = 6.3$ Hz, 21-CH_3), 3.41 (1H, brm, 3β-H), 3.83 (1H, brs, 7β-H), 4.96 (1H, dd, $J = 51.0$, 9.8 Hz, 23-H). ^{13}C NMR: see Table 3. ^{19}F NMR ($CDCl_3$ containing 20 % CD_3OD) δ: −191.89 (1F, brs, 23-F). LC–ESI–MS: Calcd. for $C_{24}H_{38}FO_4$ $[M-H]^-$, 409.2754; Found, *m/z* 409.2798.

Results and Discussion

As illustrated in Fig. 2, the synthetic routes to key intermediates (**6a** and **6b**) from primary bile acids (**2a** and **2b**) are essentially the same as those reported previously [14]. Thus, treatment of **2a** and **2b** with acetic anhydride and 4-dimethylaminopyridine resulted in the formation of the peracetate derivatives (**3a** and **3b**), which in turn were transformed into the C_{23} 24-nor-22-enes (**4a** and **4b**) by the side chain degradation with lead tetraacetate $[(AcO)_4Pb]$ and cuprous acetate $[(AcO)_2Cu]$ in boiling pyridine [15, 16]. Epoxidation of **4a** and **4b** with *m*-chloroperbenzoic acid (*m*-CPBA) in the presence of $NaHCO_3$ at room temperature [16] gave the 24-nor-22,23-epoxides (**5a** and **5b**) in good isolated yields (85 and 83 %). Subsequent oxidative-cleavage of the 22,23-epoxide ring in **5a** and **5b** with orthoperiodic acid (HIO_4) in THF at room temperature [16] afforded the corresponding C_{22} 23,24-dinor-22-aldehyde derivatives (**6a** and **6b**) in isolated yields of 43 and 45 %, respectively.



Reagents and conditions: i) acetic anhydride/ 4-dimethylaminopyridine/ py., at 40 °C for 2 h. ii) $Pb(OAc)_4$/ $Cu(OAc)_2$/ py./ benzene, reflux for 16 h. iii) *m*-CPBA/ $NaHCO_3$/ CH_2Cl_2, at r.t. for 4 h. (iv) *o*-periodic acid/ THF, at r.t. for 4 h. v) triethyl 2-fluoro-2-phosphonoacetate/ LiCl/ DBU/ CH_3CN, at r.t. for 3 h. vi) H_2/ Pd-C/ THF, at r.t. for 10 h. vii) NaOH/ MeOH/ H_2O, at r.t. for 12 h.

Fig. 2 Synthetic route to epimeric (23*R*)- and (23*S*)-fluoro-bile acids from CA and CDCA

When **6a** and **6b** were subjected to the Horner-Wadsworth-Emmons reaction with triethyl 2-fluoro-2-phosphonoacetate in the presence of LiCl and 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) as base-sensitive reagents [17], the reaction proceeded smoothly to give the more thermodynamically stable (*E*)-isomer of the C_{24} α-fluoro-α,β-unsaturated esters (**7a** and **7b**) in reasonable isolated yields of 70 and 72 %. The stereochemical configuration of the Δ^{22}-bond in **7a** and **7b** was conclusively determined as the *E*-form, on the basis of a multiplet ^{1}H signal at 22-H appearing at 5.68-5.73 ppm in the ^{1}H NMR spectra [18–20]. The characteristic ^{13}C doublet signals occurring at 128.5–129.0 (*J*, 14.8-15.5 Hz), 145.4–145.6 (*J*, 249.0–250.4 Hz), and 160.9–161.0 (*J*, 35.8–36.8 Hz) ppm were also confirmed as C-22, C-23, and C-24, respectively, with a fluoro atom at the C-23 position. Furthermore, the ^{19}F signal was observed as the triplet signal (*J*, 29.3 Hz) at −131.8 ppm in **7a** and at −131.0 ppm in **7b**.

Catalytic hydrogenation of the α-fluoro-α,β-unsaturated esters (**7a** and **7b**) in the presence of Pd/C as a catalyst yielded an epimeric mixture of the (23*R*)- and (23*S*)-23-fluoro-peracetate esters (**8a** and **8b**) nearly quantitatively. Without isolating each of the epimeric 23ξ-fluoro acetate-ethyl esters, the mixtures were hydrolyzed with methanolic NaOH to afford epimeric mixtures of the free 23ξ-fluoro acids (**1a** and **1b**). Figure 3 shows the RP-HPLC of **1a** and **1b**, in which the presence of an axially-oriented 12α-hydroxy group in **1a** appreciably decreased the formation of one (**1a-S**) of the two epimers (ratio of 9:1), probably due to steric hindrance of the hydroxyl group. Careful separation of each of the epimeric mixtures by preparative RP-HPLC provided two well-separated fractions. The more polar fractions of each of the epimeric mixtures eluted with a mixture of 15 mM ammonium acetate/acetic acid buffer solution (pH 5.4) and methanol (35:65 ~ 33:67, v/v) were characterized as the desired (23*R*)-23-fluoro-3α,7α,12α-trihydroxy acid (**1a-R**) and (23*R*)-23-fluoro-3α,7α-dihydroxy analog (**1b-R**) and less polar fractions as the corresponding (23*S*)-epimers (**1a-S** and **1b-S**) as described below.

Table 3 shows the ^{13}C NMR chemical shifts for the isolated **1a-R**, **1a-S**, **1b-R**, and **1b-S**. In the ^{1}H NMR spectra, the two epimeric pairs showed essentially identical spectral patterns and chemical shift values. In the ^{13}C NMR spectra, however, a doublet signal (*J*, 180.0–183.6 Hz) arising from the C-23 (89.2 ppm) in the (23*R*)-epimers (**1a-R** and **1b-R**) resonated at a lower field than the corresponding signal (87.2 and 86.7 ppm) in the (23*S*)-epimers (**1a-S** and **1b-S**), strongly suggesting the presence of a fluoro atom at C-23 and the stereochemical difference. A minor, but consistent chemical shift difference between the two epimeric pairs was observed for the C-20 and C-21 signals. A significantly large, distinct difference was also observed in the ^{19}F NMR spectra, in which the 23-F signals (−185.22 and −185.85 ppm) in the (23*R*)-epimers (**1a-R** and **1b-R**) resonated at an appreciably higher field than the corresponding signals (−191.92 and −191.89 ppm) in the (23*S*)-epimers (**1a-S** and **1b-S**).

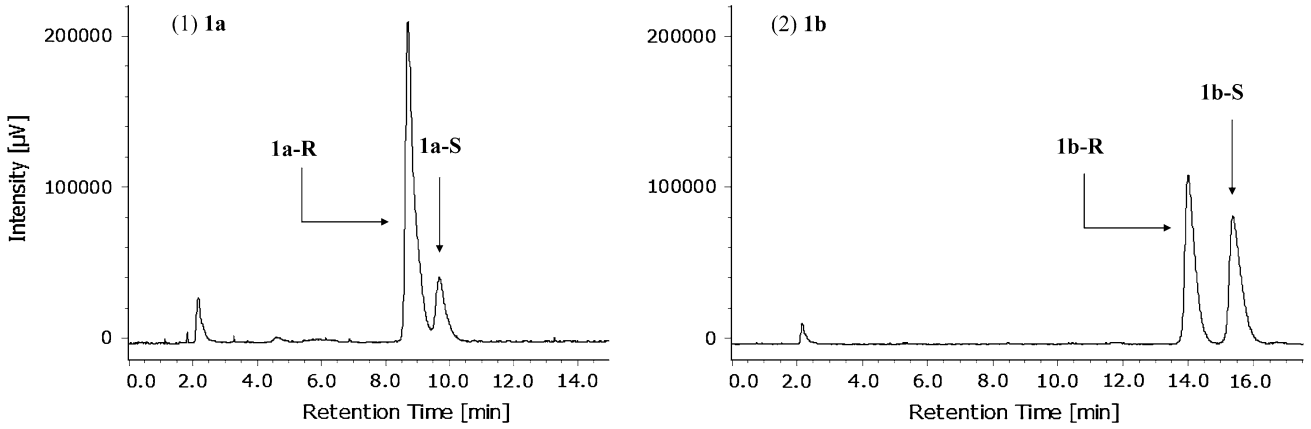


Fig. 3 RP-HPLC profile of the epimeric mixtures of the 23ξ-fluoro derivatives of (*1*) 3α,7α,12α-trihydroxy-5β-cholanoic acid (**1a**), and (*2*) 3α,7α-dihydroxy-5β-cholanoic acid (**1b**)

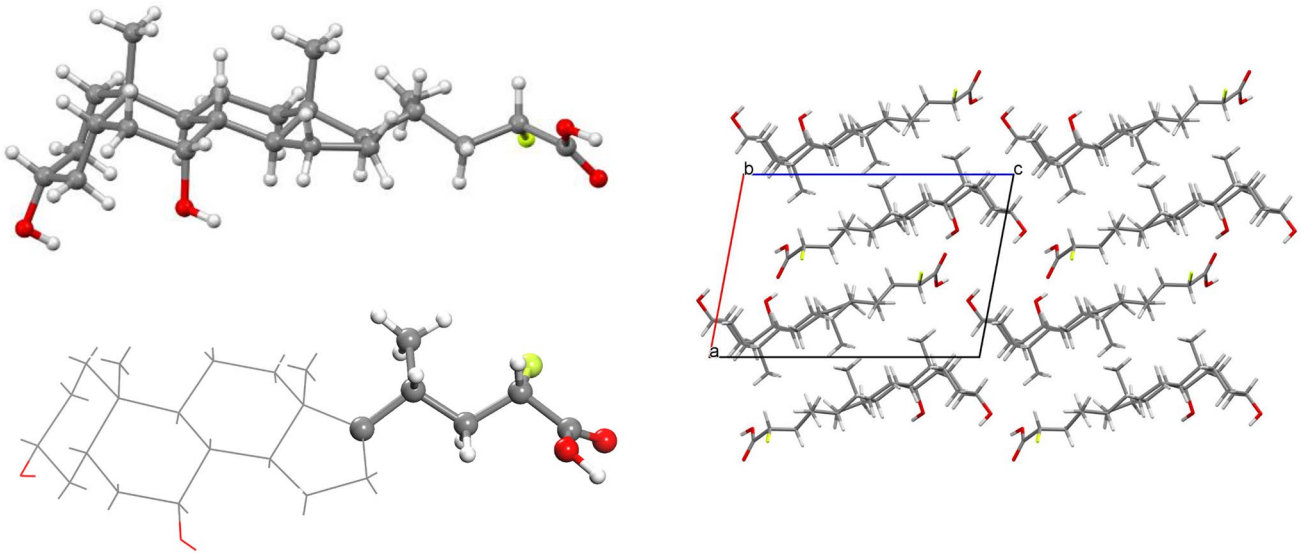


Fig. 4 The molecular and crystal structures of compound **1b-S**

Fractional crystallization of **1b-S** from methanol gave single-crystals suitable for X-ray measurement. Figure 4 shows the molecular and crystal structures of **1b-S** determined by X-ray analysis, respectively. Table 1 shows the crystallographic details for **1b-S**. Selected bond lengths and angles for **1b-S** are also given in Table 2. **1b-S** crystallizes in the monoclinic system with space groups $P2_1$, with one independent molecule in an unit cell. The crystals contain about 1.5 molecule of water per molecule of **1b-S**, likely from methanol used for recrystallization that had not been dried prior to use. Thus, the X-ray analysis provided directly the absolute configuration at C-23, in analogy with hydroxylated derivatives epimeric at C-23, C-25, C-22/C-25 or C-24/C-25 of bile acids and bile alcohols [14, 21–24], and conclusive evidence for the stereochemical assignment of **1b-S** as the (23*S*)-configuration, which was the secondary-eluted peak in Fig. 3 (2); **1b-R** was therefore assigned as (23*R*). Based on the above finding and the ^{13}C and ^{19}F NMR characteristics, analogous **1a-R** and **1a-S** were assigned as (23*R*)- and (23*S*)-configuration, respectively.

The biological evaluation of the synthesized 23-fluorobile acids is now progressing in our laboratory, and the result will be reported elsewhere.

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