Chemical Synthesis of (22*E*)-3α,6α,7α,12α-Tetrahydroxy-5β-chol-22-en-24-oic Acid and Its *N*-Acylamidated Conjugates with Glycine or Taurine: Precursors of the [22,23-^{3}H] Labelled Tracers

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(22*E*)-3α,6α,7α,12α-Tetrahydroxy-5β-chol-22-en-24-oic acid and its *N*-acylamidated conjugates with glycine or taurine were synthesized from cholic acid. The key reactions employed are: 1) degradation of the side chain in intermediary C_{24} 3α,6α,7α,12α-tetrahydroxylated bile acid to the corresponding C_{22} 23,24-dinor-aldehyde, followed by Wittig reaction with methyl (triphenylphosphoranylidene)acetate and 2) *N*-acylamidation of the unconjugated tetrahydroxy-Δ^{22}-5β-cholenoic acid with glycine (or taurine) in the presence of diethylphosphorocyanide and triethylamine as coupling reagents.

Key words bile acid; tetrahydroxy-Δ^{22}-5β-cholenoic acid; gycine conjugate; taurine conjugate; Wittig reaction; *N*-acylamidation

Bile acids in the form of their *N*-acylamidate conjugates (bile salts) with glycine or taurine are amphipathic end products of cholesterol metabolism with multiple physiological functions.[1—3] In addition to conventional bile acids, unusual bile acids, particularly 3,6,7-trihydroxycholanoic and 3,6,7,12-tetrahydroxycholanoic acids having a 6,7-vicinal glycol structure in the 5β-steroid nucleus (*cis* A/B-ring juncture), are of keen interest in biosynthetic and metabolic studies.

It has recently been reported that tetrahydroxylated bile salts are uncommon, nevertheless secreted in large amounts into the bile of bile salt export pump (BSEP; *Abcb11*) knockout mice.[4,5] These hydrophilic bile salts are postulated to neutralize the toxic effect of accumulated hydrophobic bile salts in the liver, thus explaining the lower severity of cholestasis in BSEP knockout mice. Furthermore, a significant amount of tetrahydroxylated bile salts like 3α,6α,7α,12α-tetrahydroxy-5β-cholyl taurine (6α-OH-TC) are detected in the urine of cholestatic humans, amniotic fluids and neonatal urine.[6—10] An unidentified alternative canalicular non-BSEP-mediated transform system is, therefore, proposed to efflux the 6α-OH-TC.

We have recently postulated that murine multidrug resistance-associated protein 2 (mMRP2; *Abcc2*) can transport the hydrophilic 6α-OH-TC found under cholestatic conditions in both humans and BSEP null mice.[11] In addition, we had a good expression system for mMRP2 and could characterize transport of the radiolabeled 3α,6α,7α,12α-tetrahydroxy bile acid. If we are available us with (22*E*)-3α,6α,7α,12α-tetrahydroxy-5β-chol-22-en-24-oic acid [6α-OH-Δ^{22}-CA] for subsequent tritiation, we can test this hypothesis more directly and precisely. Thus, reductive-tritiation of the 6α-OH-Δ^{22}-CA, together with its glycine and taurine conjugates, with carrier-free tritium gas would be expected to obtain the corresponding tritium labeled [22,23-^{3}H]-6α-OH-CAs as tracers.

During the course of our continuous investigation on mMRP2 as a possible alternative transporter for 6α-OH-TC, we urgently needed tritium labeled 22,23-^{3}H-3α,6α,7α,12α-tetrahydroxy-5β-cholan-24-oic acid ([^{3}H]6α-OH-TC) and its conjugates. A particular interest in the topic prompted us to prepare the authentic specimens of the unconjugated and conjugated 6α-OH-Δ^{22}-CAs (**1a**—**c**; Fig. 1).

Results and Discussion

The starting compound for our synthesis is the 6α-hydroxylated derivative (3α,6α,7α,12α-tetrahydroxy-5β-cholanoic acid; **2**) of cholic acid (CA), which was prepared in seven steps from CA according to the procedures reported previously.[12,13] The desired 6α-OH-Δ^{22}-CA (**1a**) and its glycine (**1b**) and taurine (**1c**) conjugates were prepared in six steps from the corresponding saturated acid **2** by the routes shown in Fig. 2. Although the synthetic pathways as well as the procedures are essentially application for the preparation of (22*E*)-3α,6β,7β-trihydroxy-5β-chol-22-en-24-oic acid and its conjugates which are major bile acids in the rat,[14] the targeted compounds in this study are especially useful for studies on the hepatobiliary dispoisition of these bile acids as substrates for multiple canalicular transporters as mentioned above.

A preliminary experiment revealed that all the reactions proceeds cleanly, when the hydroxy groups in **2** were protected by the formyl groups. Thus, treatment of **2** with formic acid and perchloric acid resulted in the formation of the corresponding tetraformate **3**, which in turn was converted to the C_{23} 24-nor-22-ene **4** by the side chain degradation with lead tetraacetate and cuprous acetate[15] in boiling pyridine for

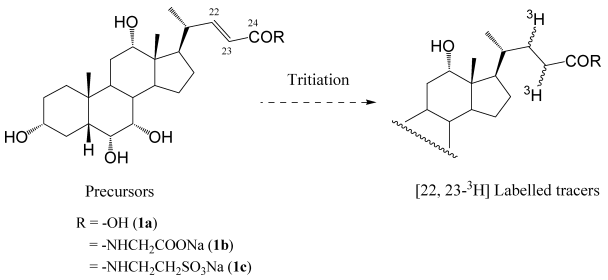

Fig. 1. Structure of (22*E*)-3α,6α,7α,12α-Tetrahydroxy-5β-chol-22-en-24-oic Acid and Its Glycine and Taurine Conjugates (**1a**—**c**)

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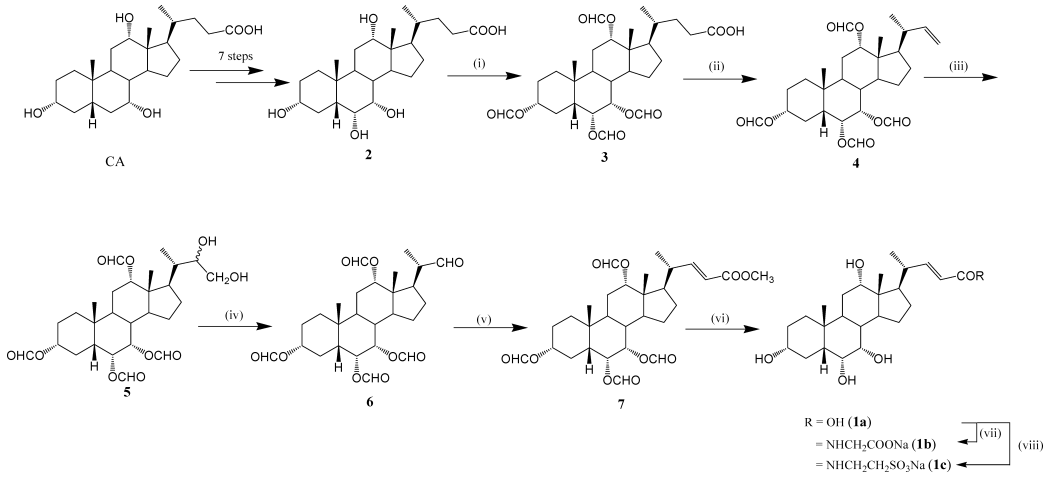

***Reagents and conditions*:** i) HCOOH/ $HClO_4$, r.t. for 2 h. ii) $(AcO)_4Pb$ /$(AcO)_2Cu$ / py./ benzene, reflux for 16 h. iii) OsO_4/ NMO /THF / *tert*-BuOH/ H_2O, r.t. for 12 h. iv) $NaIO_4$/ MeOH/ H_2O, r.t. for 12 h. v) methyl (triphenylphosphoranylidene)acetate/ benzene, reflux for 12 h. vi) 5% KOH/ MeOH, reflux for 6 h. vii) glycine methyl ester hydrochloride/ DEPC/ Et_3N/ DMF, r.t., for 3 h and then 1M NaOH, 50 °C for 1 h. viii) taurine/ DEPC/ Et_3N/ DMF, r.t., for 1.5 h and then 1M NaOH.

Fig. 2. Synthetic Route to the Unconjugate (**1a**) and Glycine and Taurine Conjugates (**1b**, **1c**) of (22*E*)-3α,6α,7α,12α-Tetrahydroxy-5β-chol-22-en-24-oic Acid

16 h. The compound **4** was isolated in 71% yield, after chromatographic purification of the product on a column of silica gel. Subsequent dihydroxylation of **4** with osmium tetroxide and *N*-methylmorpholine *N*-oxide[14] in a mixture of tetrahydrofuran (THF), *tert*-butyl alcohol (*t*-BuOH) and water at room temperature for 12 h afforded the C-22 epimeric mixture (22*R* and 22*S*) of the C_{23} 24-nor-22ξ,23-diol **5** in 76% yield. Without isolation of each of the 22-epimers, the mixture was subjected to the subsequent reaction.

Oxidation of the 24-nor-22ξ,23-diol **5** with sodium periodate ($NaIO_4$)[14,16] in methanol and water mixture for 12 h at room temperature proceeded satisfactorily to give exclusively the corresponding C_{22} 23,24-dinor-aldehyde **6** in good isolated yield (89%). The resulting aldehyde **6**, when subjected to coupling reaction with methyl (triphenylphosphoranylidene)acetate[14,16] in refluxing benzene for 12 h, underwent Wittig reaction to afford the C_{24} (22*E*)-tetraformyl-22-ene ester **7** in isolated yield of 50%. Finally, elimination of the protecting groups in **7** was carried out by treatment with methanolic KOH, yielding the crude 6α-OH-Δ^{22}-CA **1a**. To purify the polar, hydrophilic product **1a**, it was purified by using a Sep-Pak Vac tC_{18} cartridge for reversed-phase solid phase extraction. After the cartridge was washed with water to remove excess reagents and inorganic salts, elution with 60% acetone gave a homogeneous effluent, which was characterized as the analytically pure **1a** in fairly low isolated yield of 42%. Thus, the overall yield of good quality of **1a** from **2** was at least 7.5%.

The unconjugated 6α-OH-Δ^{22}-CA **1a** was then transformed into the *N*-acylamidated conjugate with glycine (**1b**). As expected, *N*-acylamidation of **1a** with glycine methyl ester hydrochloride in the presence of diethyl phosphorocyanidate (DEPC) and triethylamine (Et_3N)[17] as coupling reagents in *N*,*N*-dimethylformamide (DMF) under mild experimental conditions yielded the corresponding 24-glycine methyl ester derivative of **1a** as the major product. The resulting methyl ester intermediate was hydrolyzed with NaOH. After being adjusting pH of the solution at 8, the crude hydrolysis product was passed through on a Sep-Pak Vac tC_{18} cartridge and elution with 60% methanol yielded the analytically pure glycine conjugate **1b** (as the sodium salt). In a similar manner, the taurine conjugate **1c** was also prepared by the *N*-acylamidation of **1a** with taurine, DEPC, and Et_3N, followed by purification by a Sep-Pak Vac tC_{18} cartridge.

Table 1 shows the ^{13}C chemical shits for the **1a**—**c**. The ^{1}H- and ^{13}C-NMR data provided confirmatory evidence for the structure of the **1a**—**c**. The ^{13}C-NMR signal assignments were exclusively based on the data for the distortionless enhancement by polarization transfer (DEPT) experiments. The chemical shifts of the skeletal ^{1}H and ^{13}C resonance assignments (C-1—C-19) in **1a**—**c** were in good agreement to those of **2** reported previously,[18] thus confirming the 6α equatorial-7α-axial *cis*-glycol structure. The *E*-configuration of the Δ^{22}-bond in **1a**—**c** was also conclusively determined by a doublet signal at 23-H appearing at 5.72—5.90 ppm with a relatively large coupling constant (*J*, 15.0—15.7 Hz).[14] In addition, the ^{13}C signals arising from C-22 and C-23 resonated at 155.3 and 118.9 ppm in **1a** and 150.0 ppm and 121.1—121.4 ppm in **1b** and **1c**, respectively.

Structural evidence for the side chain (C-20—C-26) was made by the appearance of following ^{1}H and ^{13}C signals: the 22-H signal in **1a**, occurred at 6.84 ppm as a double doublet (*J*, 15.7, 8.9 Hz), was shielded to up-field by 0.17—0.18 ppm and resonated at 6.66—6.67 ppm in both the conjugates **1b** and **1c**, owing to the *N*-acylamidation at C-24. Furthermore, the 25-H_2 in **1b** was observed at 3.84 ppm as a broad singlet, while in **1c**, the 26-H_2 signal appeared at 2.93 ppm as a triplet (*J*, 6.9 Hz), together with the 25-H_2 signal at 3.61 ppm as a multiplet. On the other hand, the ^{13}C-NMR exhibited characteristic signals at 42.6 (C-25) and 174.3 (C-26) ppm in **1b** and those at 35.6 (C-25) and 50.5 (C-26) ppm in **1c**, suggesting the formation of *N*-acylamide linkage. The C-22 (155.3 ppm) signal in **1a** was shielded by 5.3 ppm appeared at 150.0 ppm in **1b** and **1c**; the C-23 (118.9 ppm) signal in **1a** was shifted to down-field by 2.2—2.5 ppm and resonated at

Table 1. Complete ^{13}C Chemical Shifts of Unconjugate and *N*-Acylamidate Conjugates (**1a**—**c**)[a)]

Carbon no.	Compound **1a**		Compound **1b**		Compound **1c**	
	Type	^{13}C	Type	^{13}C	Type	^{13}C
1	CH_2	35.4	CH_2	35.4	CH_2	35.7
2	CH_2	29.9	CH_2	29.9	CH_2	30.3
3	CH	71.4	CH	71.4	CH	71.4
4	CH_2	31.9	CH_2	31.9	CH_2	32.4
5	CH	48.3	CH	48.3	CH	48.2
6	CH	69.4	CH	69.4	CH	69.3
7	CH	71.5	CH	71.5	CH	71.5
8	CH	39.0	CH	39.0	CH	39.1
9	CH	26.5	CH	26.5	CH	26.7
10	C	35.4	C	35.4	C	35.6
11	CH_2	28.4	CH_2	28.3	CH_2	28.7
12	CH	72.2	CH	72.2	CH	71.8
13	C	46.5	C	46.5	C	46.5
14	CH	41.5	CH	41.5	CH	41.7
15	CH_2	22.8	CH_2	22.8	CH_2	23.0
16	CH_2	27.2	CH_2	27.2	CH_2	27.5
17	CH	45.9	CH	46.1	CH	46.2
18	CH_3	11.9	CH_3	11.9	CH_3	12.2
19	CH_3	22.1	CH_3	22.1	CH_3	22.6
20	CH	39.7	CH	39.6	CH	39.6
21	CH_3	17.5	CH_3	17.7	CH_3	18.1
22	CH	155.3	CH	150.0	CH	150.0
23	CH	118.9	CH	121.1	CH	121.4
24	C	170.5	C	167.4	C	167.3
25			CH_2	42.6	CH_2	35.6
26			C	174.3	CH_2	50.5

a) Measured in CD_3OD at 125.8 MHz in ^{13}C-NMR; chemical shifts were expressed as δ ppm relative to Me_4Si.

121.1—121.4 ppm in **1b** and **1c**. Similarly, the C-24 signal appearing at 170.5 ppm in **1a** was also shielded by 3.1—3.2 ppm and resonated at 167.3—167.4 ppm in **1b** and **1c**.

Experimental

Materials CA was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Sep-Pak Vac tC_{18} cartridges® (adsorbent weight, 5 g) were purchased from Waters Co. (Milford, MS, U.S.A.) and successively conditioned by washing with methanol and water prior to use. All other chemicals and solvents were of analytical reagent grade and available from commercial sources. All compounds were dried by azeotropic distillation (benzene/methanol or CH_2Cl_2/methanol) before use in reaction.

Instruments All melting points (mp) were determined on a micro hot-stage apparatus and are uncorrected. ^1H- and ^{13}C-NMR spectra were obtained on a JEOL JNM-EX 270 FT instrument at 270 and 68.8 MHz, respectively, with $CDCl_3$ or CD_3OD containing 0.1% Me_4Si as the solvent; chemical shifts were expressed as δ-ppm relative to Me_4Si. The ^{13}C-NMR spectra were also recorded on a JEOL JNM-ECA 500 FT instrument at 125.8 MHz. The ^{13}C DEPT (135°, 90°, 45°) spectra were measured to determine the ^1H signal multiplicity and to differentiate among CH_3, CH_2, CH, and C based on their proton environments. Low-resolution mass spectra (LR-MS) were recorded on a JEOL JMS-DX 303 mass spectrometer with an electron ionization (EI) source at 70 eV under the positive ion mode (PIM). High-resolution mass spectra (HR-MS) were measured using a JEOL JMS-700 with an EI source under the PIM. For taurine- or glycine-conjugated compounds, LR-MS and HR-MS were also recorded on Shimadzu LCMS-IT-TOF with an electrospray ionization (ESI) source under the negative ion mode (NIM).

Normal-phase TLC for unconjugated compounds was performed on pre-coated silica gel plates (0.25 mm layer thickness; E. Merck, Darmstadt, Germany) using hexane–EtOAc (6 : 4—3 : 7, v/v) mixtures as the developing solvent. Reversed-phase TLC for conjugated compounds was carried out on pre-coated RP-$18F_{254S}$ plates using methanol, water and acetic acid mixtures (7 : 3 : 0.1, v/v/v) as the developing solvent.

Chemical Synthesis. 3α,6α,7α,12α-Tetraformyloxy-5β-cholan-24-oic Acid (3) A solution of 3α,6α,7α,12α-tetrahydroxy-5β-cholanoic acid (**2**; 2.0 g, 4.7 mmol), prepared in seven steps from cholic acid,[12,13] in formic acid (10 ml) containing 60% perchloric acid (one drop) was stirred at room temperature for 2 h. Water was added to the mixture and the reaction product was extracted with EtOAc. The combined extract was washed with saturated brine and water, dried with Drierite, and evaporated to dryness. Although the residual oily product of the tetraformate **3** was found to be homogeneous according to TLC and ^1H-NMR, it resisted crystallization attempts: yield, 1.9 g (74%). ^1H-NMR ($CDCl_3$) δ: 0.77 (3H, s, 18-CH_3), 0.83 (3H, d, J=4.9 Hz, 21-CH_3), 1.03 (3H, s, 19-CH_3), 4.70 (1H, m, 3β-H), 5.27 (1H, m, 7β-H), 5.30 (1H, m, 6β-H), 5.40 (1H, br s, 12β-H), 7.96, 8.03 (each 1H, s, OCHO), 8.16 (2H, s, OCHO). LR-MS (ESI-NIM) *m/z*: 535 (M−H, 44%), 507 (27%), 87 (100%). HR-MS (ESI-NIM): Calcd for $C_{28}H_{40}O_{10}$ $[M-H]^-$: 535.2543; Found *m/z*: 535.2557.

3α,6α,7α,12α-Tetraformyloxy-24-nor-5β-chol-22-ene (4) To a solution of the tetraformate **3** (1.3 g, 2.4 mmol) in dry benzene (20 ml) was added successively lead tetraacetate (2.0 g, 4.5 mmol), cuprous acetate (0.2 g, 0.11 mmol) and dry pyridine (0.1 ml), and the mixture was refluxed for 16 h. Most of the solvent was evaporated under reduced pressure, and the residual oily product was poured onto a column of silica gel (50 g). Elution with hexane–EtOAc (3 : 1—1 : 1, v/v) afforded a homogeneous effluent. After evaporation of the solvent, the residue was recrystallized from acetone–hexane to give the 24-nor-22-ene **4** as colorless needles: yield, 850 mg (71%); mp 197—198 °C. ^1H-NMR ($CDCl_3$) δ: 0.79 (3H, s, 18-CH_3), 0.95 (3H, d, J=6.8 Hz, 21-CH_3), 1.04 (3H, s, 19-CH_3), 4.70 (1H, m, 3β-H), 4.86 (1H, m, 23-CH_2), 5.27 (1H, m, 7β-H), 5.30 (1H, m, 6β-H), 5.40 (1H, br s, 12β-H), 5.60 (1H, br m, 22-CH), 7.96, 8.03, 8.16, 8.17 (each 1H, s, OCHO). LR-MS (EI-PIM) *m/z*: 416 (M−C_2H_4−HCOOH, 5%), 370 (M−C_2H_4−2HCOOH, 49%), 324 (M−C_2H_4−3HCOOH, 81%), 269 (100%), 251 (M−4HCOOH-side chain (S.C.), 57%). HR-MS (EI-PIM): Calcd for $C_{26}H_{36}O_6$ $[M-HCOOH]^+$, 444.2512; Found *m/z*, 444.2489.

3α,6α,7α,12α-Tetraformyloxy-24-nor-5β-cholan-22ξ,23-diol (5) To a solution of the 24-nor-22-ene **4** (500 mg, 1.0 mmol) in THF (3.5 ml), *t*-BuOH (10 ml) and water (3 ml) was added *N*-methylmorpholine *N*-oxide (1 ml) and OsO_4 (10 mg, 40 μmol). The mixture was stirred at room temperature for 12 h. The reaction product was extracted with EtOAc, and the combined extract was washed with saturated brine and water, dried with Drierite, and evaporated to dryness. Chromatography of the oily residue on a column of silica gel (30 g) and elution with benzene–EtOAc (1 : 4—1 : 9, v/v) afforded a C-22 epimeric mixture of the 24-nor-22ξ,23-diol **5**, which recrystallized from acetone–hexane as colorless amorphous solids: yield, 410 mg (76%); mp 202—204 °C. ^1H-NMR ($CDCl_3$) δ: 0.81 (3H, s, 18-CH_3), 0.84 (3H, d, J=8.1 Hz, 21-CH_3), 1.06 (3H, s, 19-CH_3), 3.25—3.44 (3H, m, 22ξ-H, 23-H_2), 4.64 (1H, m, 3β-H), 5.22 (1H, m, 7β-H), 5.33 (1H, m, 6β-H), 5.35 (1H, br s, 12β-H), 7.95, 7.98, 8.12, 8.14 (each 1H, s, OCHO). LR-MS (EI-PIM) *m/z*: 478 (M−HCOOH, 2%), 404 (38%), 386 (M−3HCOOH, 42%), 358 (65%), 297 (M−3HCOOH-S.C., 59%), 269 (100%), 251 (M−4HCOOH-S.C., 57%). HR-MS (EI-PIM): Calcd for $C_{26}H_{38}O_8$ $[M-HCOOH]^+$, 478.2567; Found *m/z*, 478.2538.

3α,6α,7α,12α-Tetraformyloxy-23,24-dinor-5β-cholan-22-al (6) To a solution of the 24-nor-22ξ,23-diol **5** (120 mg, 0.23 mmol) in methanol (3 ml) was added a solution of $NaIO_4$ (250 mg, 1.2 mmol) dissolved in water (1.2 ml) and methanol (3 ml), and the mixture was stirred at room temperature for 12 h. The reaction product was extracted with EtOAc, and the combined extract was washed with saturated brine and water, dried with Drierite, and evaporated to dryness. Recrystallization of the oily residue from methanol–water gave the 23,24-dinor-aldehyde **6** as colorless amorphous solids: yield, 120 mg (89%); mp 175—176 °C. ^1H-NMR ($CDCl_3$) δ: 0.81 (3H, s, 18-CH_3), 1.04 (3H, d, J=8.1 Hz, 21-CH_3), 1.04 (3H, s, 19-CH_3), 4.71 (1H, m, 3β-H), 5.25 (1H, m, 7β-H), 5.31 (1H, m, 6β-H), 5.42 (1H, br s, 12β-H), 7.96, 8.04, (each 1H, s, OCHO), 8.17 (2H, s, OCHO), 9.54 (1H, d, J=2.7 Hz, 22-H). LR-MS (EI-PIM) *m/z*: 446 (M−HCOOH, 5%), 400 (M−2HCOOH, 5%), 354 (M−3HCOOH, 26%), 326 (77%), 308 (M−4HCOOH, 13%), 298 (100%), 280 (20%), 252 (15%). HR-MS (EI-PIM): Calcd for $C_{25}H_{34}O_7$ $[M-HCOOH]^+$, 446.2305; Found *m/z*, 446.2300.

Methyl (22*E*)-3α,6α,7α,12α-Tetraformyloxy-5β-chol-22-en-24-oate (7) To a solution of the 23,24-dinor-aldehyde **6** (100 mg, 0.20 mmol) in dry benzene (10 ml) was added methyl(triphenylphosphoranylidene)acetate (200 mg, 0.6 mmol), and the mixture was refluxed for 12 h. After evaporation of the solvent, the residual oily product was poured onto a column of silica gel (20 g). Elution with hexane–EtOAc (7 : 3, v/v) gave the tetraformyloxy-22-ene ester **7** as colorless viscous oil. This compound was homogeneous according to TLC and ^1H-NMR, but hard to crystallize: yield, 45 mg (50%). ^1H-NMR ($CDCl_3$) δ: 0.80 (3H, s, 18-CH_3), 1.00 (3H, d, J=6.8 Hz, 21-CH_3), 1.04 (3H, s, 19-CH_3), 3.70 (3H, s, $COOCH_3$), 4.70 (1H, m, 3β-H), 5.27 (1H,

m, 7β-H), 5.30 (1H, m, 6β-H), 5.40 (1H, br s, 12β-H), 5.74 (1H, d, J=15.4 Hz, 23-CH), 6.78 (1H, dd, J=15.7, 8.9 Hz, 22-CH), 7.96, 8.03, 8.15, 8.18 (each 1H, s, OCHO). LR-MS (EI-PIM) m/z: 548 (M^+, 2%), 502 (M−HCOOH, 12%), 428 (60%), 410 (M−3HCOOH, 63%), 382 (47%), 269 (100%), 251 (M−4HCOOH-S.C., 90%), 209 (M−4HCOOH-S.C.-ring D, 15%). HR-MS (EI-PIM): Calcd for $C_{29}H_{40}O_{10}$ $[M]^+$, 548.2621; Found m/z, 548.2612.

(22*E*)-3α,6α,7α,12α-Tetrahydroxy-5β-chol-22-en-24-oic Acid (1a) A solution of the tetraformyloxy-22-ene ester **7** (100 mg, 0.18 mmol) in 5% methanolic KOH (5 ml) was refluxed for 6 h. After evaporation of the solvent, the residue was dissolved in water and acidified by 10% HCl with ice-bath cooling. The precipitated solid was filtered and washed with water. The crude product dissolved a small amount of methanol was loaded onto a Sep-Pak Vac tC_{18} cartridge. After being washed with water, elution with acetone–water (3 : 2, v/v) afforded the desired 6α-OH-Δ^{22}-CA **1a**, which was recrystallized from EtOAc as colorless amorphous solids: yield, 32 mg (42%); mp 164—165 °C. ^{1}H-NMR (CD_3OD) δ: 0.75 (3H, s, 18-CH_3), 0.92 (3H, s, 19-CH_3), 1.16 (3H, d, J=6.7 Hz, 21-CH_3), 3.34 (1H, br m, 3β-H), 3.76 (2H, br m, 7β-H, 6β-H), 3.94 (1H, br s, 12β-H), 5.72 (1H, d, J=15.4 Hz, 23-CH), 6.84 (1H, dd, J=15.7, 8.9 Hz, 22-CH). LR-MS (ESI-NIM) m/z: 421 ($[M-H]^-$, 100%), 377 (M−COOH, 20%), 249 (M−2CH_3−2H_2O-S.C.-part of ring D, 42%), 197 (M−2CH_3−3H_2O-S.C.-ring D, 16%), 155 (S.C., 11%). HR-MS (ESI-NIM): Calcd for $C_{24}H_{38}O_6$ $[M-H]^-$, 421.2590; Found m/z, 421.2615.

(22*E*)-3α,6α,7α,12α-Tetrahydroxy-5β-chol-22-en-24-oyl Glycine Sodium Salt (1b) The 6α-OH-Δ^{22}-CA **1a** (10 mg, 24 μmol) in dry DMF (1 ml) was treated successively with glycine methyl ester hydrochloride (10 mg, 80 μmol), DEPC (10 ml) and Et_3N (10 μl). After stirring at room temperature for 3 h, 1 N NaOH (0.5 ml) was added, and the mixture was further stirred at 50 °C for 1 h. The resulting solution was diluted with water (5 ml), adjusted to pH 8 with 10% HCl, and passed through a preconditioned Sep-Pak Vac tC_{18} cartrigde. After being washed with water, elution with 60% methanol gave the glycine-conjugated 6α-OH-Δ^{22}-CA (**1b**; as the sodium salt), which was recrystallized from methanol–EtOAc as colorless amorphous solids: yield, 6 mg (52%); mp 165—167 °C. ^{1}H-NMR (CD_3OD) δ: 0.73 (3H, s, 18-CH_3), 0.90 (3H, s, 19-CH_3), 1.14 (3H, d, J=6.3 Hz, 21-CH_3), 3.32 (1H, br m, 3β-H), 3.75 (2H, br m, 7β-H, 6β-H), 3.84 (2H, br s, $NHCH_2$), 3.93 (1H, br s, 12β-H), 5.90 (1H, d, J=15.0 Hz, 23-CH), 6.67 (1H, dd, J=15.4, 9.2 Hz, 22-CH). LR-MS (ESI-NIM) m/z: 478 ($[M-H]^-$, 100%). HR-MS (ESI-NIM): Calcd for $C_{26}H_{41}NO_7$ $[M-H]^-$, 478.2805; Found m/z, 478.2824.

(22*E*)-3α,6α,7α,12α-Tetrahydroxy-5β-chol-22-en-24-oyl Taurine Sodium Salt (1c) To a solution of the 6α-OH-Δ^{22}-CA **1a** (10 mg, 24 μmol) in dry DMF (1 ml) was successively added powdered taurine (10 mg, 80 μmol), DEPC (1 μl) and Et_3N (10 μl). The mixture was stirred at room temperature for 1.5 h. The reaction mixture was diluted with water (5 ml), adjusted to pH 8 with 1 M NaOH and loaded onto a preconditioned Sep-Pak Vac tC_{18} cartridge, which was washed with water. Elution with 60% methanol afforded the taurine-conjugated 6α-OH-Δ^{22}-CA (**1c**; as the sodium salt), which was recrystallized from methanol–EtOAc in the form of colorless amorphous solids: yield, 4 mg (32%); mp 240—241 °C. ^{1}H-NMR (CD_3OD) δ: 0.74 (3H, s, 18-CH_3), 0.92 (3H, s, 19-CH_3), 1.16 (3H, d, J=5.5 Hz, 21-CH_3), 2.93 (2H, t, J= 6.9 Hz, CH_2S), 3.32 (1H, br m, 3β-H), 3.61 (2H, m, CH_2N), 3.74 (2H, br m, 7β-H, 6β-H), 3.93 (1H, br s, 12β-H), 5.84 (1H, d, J=15.4 Hz, 23-CH), 6.66 (1H, dd, J=15.4, 8.6 Hz, 22-CH). LR-MS (ESI-NIM) m/z: 528 ($[M-H]^-$, 100%). HR-MS (ESI-NIM): Calcd for $C_{26}H_{43}NO_8S$ $[M-H]^-$, 528.2631; Found m/z, 528.2654.

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