

Synthesis and in vitro cholesterol dissolution by 23- and 24-phosphonobile acids

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Abstract

A new class of 23- and 24-phosphonobile acids have been synthesized from bile acid and their in vitro cholesterol-dissolving efficiency have been estimated. 24-Phosphonobile salts (PBSs) are slightly more efficient in solubilizing cholesterol than 23-PBSs and natural bile salts. The cholesterol solubilizing power is influenced by the structure of PBSs, and is considerably reduced with an increase in the bulk pH.

Keywords: Bile acids; Cholesterol dissolution; Gallstone dissolution; Phosphonobile acids

1. Introduction

Bile acids, which are essential for many physiological functions, are end-products of cholesterol metabolism. The physiological functions of bile salts result from their interesting physico-chemical properties, which have been studied extensively [1]. The glycine/taurine conjugates of bile acids form mixed micelles with phospholipids [2,3], which solubilizes cholesterol. Impeding this function may cause the over saturation of cholesterol in the bile leading to its precipitation. The accumulation of cholesterol micro-crystals in the presence of glycoproteins leads to gallstone formation or cholelithiasis [4]. Chenodeoxy [5] and ursodeoxycholic acids [6] have been used with success for the dissolution of cholesterol gallstones. Two of the limitations of bile acid therapy are: (a) they undergo 7-dehydroxylation giving rise to hepatotoxic lithocholic acid and (b) low efficacy and prolonged treatment periods.

There is growing interest in recent years to find a better gallstone-dissolving agent, which would be resistant to 7-dehydroxylation. The design of a few synthetic analogs of bile acids described in the literature has addressed this issue. *N*-methylated (sarcosine) glycocholate has been shown to possess therapeutic value, and is resistant to deconjugation and dehydroxylation [7]. The 7-methylated chenodeoxy and ursodeoxy derivatives were shown to be completely resistant to 7-dehydroxylation in hamsters [8]. Sulfonobile salts, side chain-modified analogs of natural bile salts, are resistant to 7-dehydroxylation, and do not interfere with endogenous bile acid synthesis [9,10]. We have recently reported the synthesis of novel 23-phosphonobile acids [11]. In this paper, we report the synthesis of new 23- and 24-phosphonobile acids and a study of their in vitro cholesterol solubilizing efficiency as a model study for gallstone dissolution.

2. Experimental

2.1. Materials and methods

Cholic, deoxycholic, chenodeoxycholic and lithocholic acids were purchased from Aldrich and were used as such. Ursodeoxycholic acid was also a commercial sample. Precoated silica gel glass plates with UV indicator on (0.25 mm thick-

Abbreviations: PBS, phosphonobile salt; PC, phosphocholate; PCDC, phosphonochenodeoxycholate; PDC, phosphonodeoxycholate; PLC, phosphonolithocholate; PUDC, phosphonoursodeoxycholate

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ness, Sigma–Aldrich) were used for thin layer chromatography and Liebermann–Burchard reagent was used as the spray reagent to visualize the steroids. Acme 100–200 mesh silica gel was used for gravity column chromatography. All the solvents used were of laboratory grade and distilled prior to use. Dichloromethane and carbon tetrachloride were dried over CaH₂. Melting points were recorded in Buchi B-540 melting point instrument. All optical rotation values were recorded on Jasco-DIP-370 polarimeter at 25 °C. Infrared spectra were recorded on a Jasco-70 FTIR spectrometer. Proton and carbon NMR spectra were recorded on a Jeol Lambda 300 MHz and Bruker AMX 400 MHz spectrometers. Phosphorous NMR was recorded on a Bruker AMX 400 MHz spectrometer.

2.1.1. A general procedure for the synthesis of formylated bile acids

Formic acid (20 mL, 517.4 mmol) was added to bile acid (12.7 mmol) and stirred at 55 °C for 20 h. Volatiles were removed under reduced pressure. The free flowing solid was crystallized from EtOH/H₂O (1:1.3, v/v) and dried to give formylated bile acids [12].

2.1.1.1. 3 α , 7 α , 12 α -triformyloxy-5 β -cholan-24-oic acid. Yield 82%; mp: 202–204 °C; ¹H NMR (300 MHz, CDCl₃) δ : 8.165 (s, 1H), 8.11 (s, 1H), 8.02 (s, 1H), 5.27 (bs, 1H), 5.07 (bs, 1H), 4.76 (m, 1H), 2.44–1.27 (m), 1.18–1.05 (m, 2H), 0.94 (s, 3H), 0.85 (d, J = 6.6 Hz, 3H), 0.76 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ : 179.78, 160.59, 160.50, 75.24, 73.72, 70.66, 47.18, 44.99, 42.94, 40.76, 37.69, 34.68, 34.49, 34.41, 34.25, 31.30, 30.80, 30.37, 28.52, 27.12, 26.54, 25.51, 22.74, 22.31, 17.42, 12.12.

2.1.1.2. 3 α , 12 α -diformyloxy-5 β -cholan-24-oic acid. Yield 89%; mp: 189–192 °C; ¹H NMR (300 MHz, CDCl₃) δ : 8.11 (s, 1H), 8.01 (s, 1H), 5.23 (s, 1H), 4.81 (m, 1H), 2.36 (m, 1H), 1.92–0.97 (m), 0.90 (s, 3H), 0.82 (d, J = 6.0 Hz, 3H), 0.73 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ : 180.18, 160.67, 160.53, 75.91, 74.04, 49.18, 47.28, 44.93, 41.65, 35.52, 34.70, 34.58, 34.12, 33.94, 32.01, 30.89, 30.40, 27.27, 26.70, 26.39, 25.83, 25.66, 25.36, 22.86, 17.36, 12.26.

2.1.1.3. 3 α , 7 α -diformyloxy-5 β -cholan-24-oic acid. Yield 95%; mp: 184–185 °C; ¹H NMR (400 MHz, CDCl₃) δ : 8.06 (s, 1H), 8.01 (s, 1H), 5.02 (s, 1H), 4.74–4.69 (m, 1H), 2.41–2.33 (m, 1H), 2.28–2.10 (m, 1H), 2.0–1.1 (m), 0.93 (s, 3H), 0.92 (d, J = 6.0 Hz, 3H), 0.64 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ : 179.93, 160.73, 74.07, 71.42, 55.78, 50.14, 42.75, 41.04, 39.46, 37.96, 35.24, 34.81, 34.62, 34.03, 31.51, 30.96, 30.73, 27.93, 26.77, 23.49, 22.64, 20.65, 18.27, 11.74.

2.1.1.4. 3 α , 7 β -diformyloxy-5 β -cholan-24-oic acid. Yield 96%; mp: 170–171 °C; ¹H NMR (400 MHz, CDCl₃) δ : 8.00 (s, 1H), 7.96 (s, 1H), 4.92–4.85 (m, 1H), 4.83–4.75 (m, 1H), 2.41–2.33 (m, 1H), 2.27–2.19 (m, 1H), 1.86–1.0 (m), 0.97 (s, 3H), 0.91 (d, J = 6.4 Hz, 3H), 0.67 (s, 3H); ¹³C NMR

(100 MHz, CDCl₃) δ : 179.90, 161.00, 160.61, 73.69, 73.44, 53.37, 55.04, 43.68, 42.11, 39.91, 39.50, 35.17, 34.42, 34.00, 32.92, 32.82, 31.03, 30.76, 28.30, 26.42, 25.84, 23.20, 21.26, 18.36, 12.09.

2.1.1.5. 3 α -Formyloxy-5 β -cholan-24-oic acid. Yield 95%; mp: 136–138 °C; ¹H NMR (300 MHz, CDCl₃) δ : 8.03 (s, 1H), 4.88–4.81 (m, 1H), 2.44–2.19 (m, 2H), 1.98–0.99 (m), 0.93 (s, 3H), 0.92 (d, J = 6.6 Hz, 3H), 0.64 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ : 180.29, 160.84, 74.42, 56.43, 55.92, 42.72, 41.88, 40.42, 40.09, 35.76, 35.28, 34.94, 34.55, 32.18, 30.98, 30.73, 28.14, 26.95, 26.61, 26.28, 24.15, 23.29, 20.82, 18.22, 12.03.

2.1.2. General procedure for the synthesis of 24-hydroxy bile alcohols

The formylated bile acid (2.26 mmol) was dissolved in THF (8 mL). To this solution, triethylamine (0.4 mL, 2.9 mmol) was added, followed by the dropwise addition of ethyl chloroformate (0.28 mL, 2.92 mmol). After stirring for 2 h, the reaction mixture was cooled with ice bath then added NaBH₄ (0.4 g) and methanol (10 mL) dropwise. After stirring at 0 °C for 15 min, the reaction mixture was stirred at rt for another 15 min, diluted with water (20 mL), acidified with 0.2 M HCl (10 mL), extracted with ethyl acetate (3 \times 25 mL), washed with water and dried over anhyd. Na₂SO₄. The crude-product was purified on a silica gel column using EtOAc/pet. ether (35–50:65–50, v/v).

2.1.2.1. 3 α , 7 α , 12 α -triformyloxy-5 β -24-hydroxy-cholane. Yield 77%; ¹H NMR (300 MHz, CDCl₃) δ : 8.17 (s, 1H), 8.10 (s, 1H), 8.02 (s, 1H), 5.27 (s, 1H), 5.07 (s, 1H), 4.71 (m, 1H), 3.61 (m, 2H), 2.18–1.0 (m), 0.94 (s, 3H), 0.85 (d, J = 6.3 Hz, 3H), 0.75 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ : 160.53, 75.44, 73.79, 70.76, 63.37, 47.46, 45.05, 43.03, 40.90, 37.84, 35.04, 34.58, 34.34, 31.65, 31.41, 29.33, 28.63, 27.30, 26.64, 25.61, 22.83, 22.35, 17.87, 12.62.

2.1.2.2. 3 α , 12 α -diformyloxy-5 β -24-hydroxy-cholane. Yield 92%; ¹H NMR (300 MHz, CDCl₃) δ : 8.13 (s, 1H), 8.02 (s, 1H), 5.25 (s, 1H), 4.87–4.79 (m, 1H), 3.60 (t, J = 6.3 Hz, 2H), 1.9–1.0 (m), 0.92 (s, 3H), 0.83 (d, J = 5.7 Hz, 3H), 0.74 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ : 160.60, 160.48, 75.94, 73.96, 62.79, 49.18, 47.51, 44.90, 41.67, 35.54, 34.96, 34.61, 34.13, 33.91, 32.05, 31.66, 29.12, 27.34, 26.74, 26.37, 25.851, 25.64, 23.38, 22.81, 17.73, 12.22.

2.1.2.3. 3 α , 7 α -diformyloxy-5 β -24-hydroxy-cholane. Yield 66%; ¹H NMR (300 MHz, CDCl₃) δ : 8.08 (s, 1H), 8.03 (s, 1H), 5.03 (s, 1H), 4.76–4.69 (m, 1H), 3.63–3.59 (m, 2H), 2.1–1.0 (m), 0.96 (s, 3H), 0.96–0.92 (unresolved d, 3H), 0.65 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ : 160.73, 160.70, 73.98, 71.35, 63.39, 55.82, 55.02, 42.56, 40.88, 39.34, 37.79, 35.44, 34.69, 34.48, 33.89, 31.69, 31.39, 29.20, 28.00, 26.64, 23.42, 22.57, 20.53, 1.55, 11.64.

2.1.2.4. *3 α , 7 β -diformyloxy-5 β -24-hydroxy-cholane*. Yield 82%; ¹H NMR (300 MHz, CDCl₃) δ : 8.03 (s, 1H), 7.99 (s, 1H), 4.94–4.79 (m, 2H), 3.60 (t, *J* = 6.6 Hz, 2H), 1.88–1.04 (m), 0.99 (s, 3H), 0.93 (d, *J* = 6.3 Hz, 3H), 0.69 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ : 161.01, 160.61, 73.60, 73.35, 63.33, 55.25, 55.06, 43.48, 41.94, 39.78, 39.73, 39.34, 35.35, 34.27, 33.85, 32.78, 32.65, 31.73, 29.22, 28.36, 26.27, 25.76, 23.11, 21.13, 18.63, 11.96.

2.1.2.5. *3 α -Formyloxy-5 β -24-hydroxy-cholane*. Yield 70%; ¹H NMR (300 MHz, CDCl₃) δ : 8.04 (s, 1H), 4.89–4.80 (m, 1H), 3.61 (t, *J* = 5.1 Hz, 2H), 2.0–0.98 (m), 0.94 (s, 3H), 0.93 (d, *J* = 6.6 Hz, 3H), 0.65 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ : 160.82, 74.42, 63.58, 56.47, 56.16, 42.69, 41.90, 40.44, 40.12, 35.76, 35.55, 34.94, 34.56, 32.19, 31.82, 29.38, 28.28, 26.98, 26.62, 26.31, 24.18, 23.31, 20.83, 18.62, 12.03.

2.1.3. General procedure for the preparation of formyl-23-iodo-24-nor-cholanes

In a 100 mL two-necked rb flask fitted with a reflux condenser and a pressure equalizing dropping funnel, formylated bile acid (5.36 mmol) and lead tetraacetate (5.0 g, 11.4 mmol) were suspended in CCl₄ (15 mL). The mixture was refluxed and irradiated with two 100 W tungsten lamps. After 5 min, I₂ (2.7 g, 10.6 mmol) in CCl₄ (60 mL) was added through the dropping funnel until violet color persisted. Heating and irradiation was continued for an additional 1 h. The reaction mixture was filtered through celite, washed with 10% sodium thiosulphate solution (50 mL), water (20 mL) and brine (25 mL), dried over anhyd. Na₂SO₄ and concentrated. The crude-product was purified by column chromatography on silica gel (2.8 cm \times 19.5 cm) using EtOAc/CHCl₃ (2–5:98–95, v/v) to yield 23-iodo derivatives.

2.1.3.1. *3 α , 7 α , 12 α -triformyloxy-5 β -23-iodo-24-nor-cholane*. Yield 60%; ¹H NMR (300 MHz, CDCl₃) δ : 8.16 (s, 1H), 8.10 (s, 1H), 8.02 (s, 1H), 5.28 (bs, 1H), 5.07 (bs, 1H), 4.76–4.68 (m, 1H), 3.33–3.26 (m, 1H), 3.07 (dd, *J* = 8.4 Hz, *J* = 7.8 Hz, 1H), 2.20–1.24 (m, 23H), 1.194–1.090 (m, 2H), 0.952 (s, 3H), 0.844 (d, *J* = 6.0 Hz, 3H), 0.782 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ : 160.49, 160.47, 160.39, 75.13, 73.63, 70.57, 47.02, 45.05, 42.88, 40.72, 39.74, 37.64, 36.42, 34.45, 34.38, 34.22, 31.27, 28.49, 27.13, 26.52, 25.49, 22.29, 16.94, 12.10, 4.69.

2.1.3.2. *3 α , 12 α -diformyloxy-5 β -23-iodo-24-nor-cholane*. Yield 79%; ¹H NMR (300 MHz, CDCl₃) δ : 8.12 (s, 1H), 8.03 (s, 1H), 5.25 (s, 1H), 4.83 (m, 1H), 3.30 (m, 1H), 3.05 (m, 2H), 2.05–0.95 (m), 0.92 (s, 3H), 0.83 (d, *J* = 6.3 Hz, 3H), 0.76 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ : 160.61, 160.47, 75.91, 74.04, 49.23, 47.20, 45.06, 41.68, 39.91, 36.55, 35.56, 34.63, 33.99, 32.06, 27.36, 26.73, 26.44, 25.86, 25.70, 23.37, 22.91, 16.97, 12.31, 4.85.

2.1.3.3. *3 α , 7 α -diformyloxy-5 β -23-iodo-24-nor-cholane*. Yield 99%; ¹H NMR (300 MHz, CDCl₃) δ : 8.07 (s, 1H), 8.02 (s, 1H), 5.03 (s, 1H), 4.76–4.68 (m, 1H), 3.32–3.27 (m, 1H), 3.12–3.06 (m, 1H), 2.18–1.01 (m), 0.95 (s, 3H), 0.93 (d, *J* = 6.0 Hz, 3H), 0.67 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ : 160.66, 74.05, 71.38, 55.68, 50.15, 42.84, 41.03, 40.25, 39.46, 37.95, 37.09, 34.83, 34.63, 34.05, 31.51, 27.98, 26.79, 23.49, 22.67, 20.66, 17.87, 11.76, 4.83.

2.1.3.4. *3 α , 7 β -diformyloxy-5 β -23-iodo-24-nor-cholane*. Yield 91%; ¹H NMR (400 MHz, CDCl₃) δ : 8.02 (s, 1H), 7.98 (s, 1H), 4.93–4.86 (m, 1H), 4.85–4.77 (m, 1H), 3.33–3.27 (m, 1H), 3.12–3.06 (m, 1H), 2.03–1.98 (m, 2H), 1.87–1.07 (m), 0.99 (s, 3H), 0.92 (d, *J* = 6.4 Hz, 3H), 0.70 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ : 160.92, 160.53, 73.62, 73.42, 55.38, 54.96, 43.78, 42.12, 40.25, 39.92, 39.52, 36.97, 34.44, 34.01, 32.93, 32.85, 28.31, 26.43, 25.82, 23.22, 21.25, 17.97, 12.01, 5.1.

2.1.3.5. *3 α -Formyloxy-5 β -23-iodo-24-nor-cholane*. Yield 76%; ¹H NMR (300 MHz, CDCl₃) δ : 8.02 (s, 1H), 4.87–4.80 (m, 1H), 3.32–3.25 (m, 1H), 3.12–3.04 (m, 1H), 2.06–1.05 (m), 0.92 (s, 3H), 0.90 (d, *J* = 6.0 Hz, 3H), 0.65 (s, 1H); ¹³C NMR (75 MHz, CDCl₃) δ : 160.73, 74.33, 56.41, 55.82, 42.79, 41.84, 40.39, 40.28, 40.07, 37.15, 35.72, 34.91, 34.54, 32.17, 28.15, 26.93, 26.60, 26.26, 24.11, 23.30, 20.79, 17.81, 12.03, 5.16.

2.1.4. General procedure for the synthesis of formyl 24-iodo cholanes

To a solution of PPh₃ (0.59 g, 2.3 mmol) and imidazole (0.16 g, 2.3 mmol) in dichloromethane (10 mL) at 0 °C, iodine (0.58 g, 2.3 mmol) was added in portions over 20 min. The formylated cholanol (0.72 g, 1.5 mmol) in dichloromethane (5 mL) was added and warmed to rt. After 36 h, the reaction mixture was diluted with CHCl₃ (30 mL), washed with 10% aq. sodium thiosulphate solution (30 mL), and water (20 mL) dried over anhyd. Na₂SO₄. The crude-product was purified on silica gel column using EtOAc/CHCl₃ (2–5:98–95, v/v).

2.1.4.1. *3 α , 7 α , 12 α -triformyloxy-5 β -24-iodo-cholane*. Yield 95%; ¹H NMR (400 MHz, CDCl₃) δ : 8.16 (s, 1H), 8.10 (s, 1H), 8.02 (s, 1H), 5.26 (s, 1H), 5.08 (s, 1H), 4.75–4.68 (m, 1H), 3.21–3.08 (m, 2H), 2.18–1.05 (m), 0.95 (s, 3H), 0.85 (d, *J* = 6.4 Hz, 3H), 0.76 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ : 160.49, 75.35, 73.76, 70.21, 47.36, 45.08, 43.02, 40.90, 37.84, 36.59, 34.53, 34.34, 31.41, 30.20, 28.63, 27.29, 26.64, 25.60, 22.82, 22.35, 17.97, 12.16, 7.32.

2.1.4.2. *3 α , 12 α -diformyloxy-5 β -24-iodo-cholane*. Yield 73%; ¹H NMR (300 MHz, CDCl₃) δ : 8.14 (s, 1H), 8.03 (s, 1H), 5.25 (s, 1H), 4.87–4.80 (m, 1H), 3.23–3.08 (m, 2H), 1.90–1.03 (m), 0.93 (s, 3H), 0.84 (d, *J* = 6.3 Hz, 3H), 0.75 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ : 160.57, 160.48, 76.04, 74.10, 49.31, 47.54, 45.08, 42.09, 36.64, 35.69, 34.74,

34.59, 34.29, 34.08, 32.19, 30.29, 27.50, 26.85, 26.54, 25.96, 25.80, 23.50, 22.96, 12.38, 7.50.

2.1.4.3. *3 α , 7 α -diformyloxy-5 β -24-iodo-cholane*. Yield 87%; ^1H NMR (300 MHz, CDCl_3) δ : 8.08 (s, 1H), 8.03 (s, 1H), 5.03 (s, 1H), 4.77–4.69 (m, 1H), 3.23–3.08 (m, 2H), 2.1–1.0 (m), 0.95 (s, 3H), 0.93 (d, $J=6.6$ Hz), 0.66 (s, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ : 160.70, 73.99, 71.32, 55.70, 50.06, 42.62, 40.91, 39.35, 37.82, 36.75, 34.97, 34.72, 34.51, 33.92, 31.42, 30.22, 28.05, 26.68, 23.46, 22.62, 20.54, 18.67, 11.68, 7.78.

2.1.4.4. *3 α , 7 β -diformyloxy-5 β -24-iodo-cholane*. Yield 86%; ^1H NMR (300 MHz, CDCl_3) δ : 8.03 (s, 1H), 7.98 (s, 1H), 4.94–4.79 (m, 2H), 3.23–3.08 (m, 2H), 2.0–1.0 (m), 0.99 (s, 3H), 0.92 (d, $J=6.6$ Hz), 0.69 (s, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ : 160.86, 160.48, 73.45, 73.23, 55.17, 54.84, 43.46, 41.88, 39.68, 39.28, 36.65, 34.79, 34.23, 33.80, 32.73, 32.62, 30.18, 28.34, 26.24, 25.71, 23.09, 21.09, 18.67, 11.94, 7.70.

2.1.4.5. *3 α -Formyloxy-5 β -24-iodo-cholane*. Yield 94%; ^1H NMR (300 MHz, CDCl_3) δ : 8.04 (s, 1H), 4.9–4.8 (m, 1H), 3.24–3.1 (m, 2H), 2.0–1.0 (m), 0.94 (s, 3H), 0.92 (d, $J=6.6$ Hz, 3H), 0.65 (s, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ : 160.80, 74.40, 56.46, 56.03, 42.71, 41.90, 40.44, 40.09, 36.83, 35.76, 35.07, 34.94, 34.56, 32.19, 30.35, 28.28, 26.96, 26.62, 26.31, 24.18, 23.31, 20.83, 18.71, 12.03, 7.91.

2.1.5. General procedure for the synthesis of diethyl 23- and 24-phosphonocholanes

In a 50 mL rb flask fitted with a reflux condenser, formylated iodocholeane (1.9 mmol) in $\text{P}(\text{OEt})_3$ (1.6 mL, 9.5 mmol) was heated at 160 °C under nitrogen. After 6 h, excess $\text{P}(\text{OEt})_3$ and other volatiles were removed under vacuum. The product was purified by column chromatography on silica gel using $\text{EtOAc}/\text{CHCl}_3$ (30:70, v/v), yielding phosphonocholane a viscous liquid.

2.1.5.1. *Diethyl 3 α , 7 α , 12 α -triformyloxy-24-nor-5 β -cholane-23-phosphonate*. Yield 71%; ^1H NMR (300 MHz, CDCl_3) δ : 8.12 (s, 1H), 8.06 (s, 1H), 7.98 (s, 1H), 5.22 (s, 1H), 5.03 (s, 1H), 4.71–4.63 (m, 1H), 4.08–4.00 (m, 4H), 2.15–1.39 (m), 1.33–1.24 (m, 6H), 1.21–1.0 (m), 0.90 (s, 3H), 0.80 (d, $J=6.3$ Hz, 3H), 0.71 (s, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ : 160.46, 160.38, 75.06, 73.58, 61.45 (d, $J=6$ Hz), 46.63, 44.84, 42.81, 40.65, 37.57, 35.27 (d, $J=17$ Hz), 34.38, 34.30, 34.14, 31.19, 28.40, 27.73, 26.92, 26.43, 25.40, 22.61, 22.19, 21.80 (d, $J=140$ Hz), 17.11, 16.33 (d, $J=5$ Hz), 12.02; ^{31}P NMR (162 MHz, CDCl_3) δ : 32.74; MALDI-TOF MS: calc. for $M+\text{Na}^+$, $M+\text{K}^+$: 607.6, 623.8; obsd. 607.7, 623.8.

2.1.5.2. *Diethyl 3 α , 12 α -diformyloxy-24-nor-5 β -cholane-23-phosphonate*. Yield 80%; ^1H NMR (300 MHz, CDCl_3) δ : 8.13 (s, 1H), 8.04 (s, 1H), 5.24 (bs, 1H), 4.87–4.80 (m, 1H),

3.735 (d, $J=9.0$ Hz, 6H), 1.9–1.0 (m, 26 H), 0.93 (s, 3H), 0.84 (d, $J=6.6$ Hz, 3H), 0.75 (s, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ : 160.45, 160.33, 75.69, 73.81, 61.27 (d, $J=7$ Hz), 49.03, 46.76, 44.79, 44.54, 35.41, 35.32 (d, $J=14$ Hz), 34.49, 34.00, 33.83, 31.90, 27.78, 27.72, 27.17, 26.60, 26.28, 25.72, 25.54, 23.24, 22.15, 21.70 (d, $J=135$ Hz), 17.05, 16.30 (d, $J=6$ Hz), 12.19; ^{31}P NMR (162 MHz, CDCl_3) δ : 32.81; MALDI-TOF MS: calc. for $M+\text{Na}^+$, $M+\text{K}^+$: 564.0, 579.9; obsd. 563.7, 579.8.

2.1.5.3. *Diethyl 3 α , 7 α -diformyloxy-24-nor-5 β -cholane-23-phosphonate*. Yield 75%; ^1H NMR (300 MHz, CDCl_3) δ : 8.09 (s, 1H), 8.03 (s, 1H), 5.03 (s, 1H), 4.72 (m, 1H), 4.14–4.02 (m, 4H), 2.18–1.00 (m), 0.95 (bs, 6H), 0.68 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ : 160.56, 73.87, 71.18, 61.39 (d, $J=5.0$ Hz), 55.18, 50.00, 42.59, 40.91, 39.31, 37.83, 35.83 (d, $J=15.9$ Hz), 34.70, 34.51, 33.90, 31.40, 27.96, 27.78, 26.67, 23.37, 22.55, 21.89 (d, $J=133.5$ Hz), 20.53, 17.98, 16.38 (d, $J=4.3$ Hz), 11.66; ^{31}P NMR (162 MHz, CDCl_3) δ : 32.84.

2.1.5.4. *Diethyl 3 α , 7 β -diformyloxy-24-nor-5 β -cholane-23-phosphonate*. Yield 88%; ^1H NMR (300 MHz, CDCl_3) δ : 8.02 (s, 1H), 7.97 (s, 1H), 4.94–4.80 (m, 2H), 4.12–4.05 (m, 4H), 1.87–1.02 (m), 0.98 (s, 3H), 0.91 (d, $J=6.0$ Hz, 3H), 0.68 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ : 160.88, 160.51, 73.53, 73.33, 61.49 (d, $J=5.3$ Hz), 55.27, 54.50, 43.56, 42.02, 39.82, 39.40, 35.83 (d, $J=16.1$ Hz), 34.35, 33.91, 32.85, 32.75, 28.18, 28.02, 26.35, 25.76, 23.13, 22.03 (d, $J=139.7$ Hz), 21.18, 18.09, 16.39 (d, $J=4.2$ Hz), 12.04; ^{31}P NMR (162 MHz, CDCl_3) δ : 32.92; LRMS-ESI: calc. for $M+\text{Na}^+$: 563.3; obsd. 563.7.

2.1.5.5. *Diethyl 3 α -formyloxy-24-nor-5 β -cholane-23-phosphonate*. Yield 75%; ^1H NMR (300 MHz, CDCl_3) δ : 8.00 (s, 1H), 4.81–4.78 (m, 1H), 4.11–3.96 (m, 4H), 1.94–1.04 (m), 0.90 (s, 3H), 0.88 (d, $J=9.0$ Hz, 3H), 0.61 (s, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ : 160.89, 74.49, 61.58 (d, $J=7$ Hz), 56.53, 55.61, 42.81, 41.00, 40.53, 40.18, 36.15 (d, $J=17$ Hz), 35.88, 35.15, 34.67, 32.30, 28.17, 28.13, 27.07, 26.72, 26.39, 24.25, 23.41, 22.17 (d, $J=139$ Hz), 20.92, 18.13, 16.57 (d, $J=6.2$ Hz), 12.17; ^{31}P NMR (162 MHz, CDCl_3) δ : 33.35; MALDI-TOF MS: calc. for $M+\text{Na}^+$: 519.3; obsd. 519.7.

2.1.5.6. *Diethyl 3 α , 7 α , 12 α -triformyloxy-5 β -cholane-24-phosphonate*. Yield 97%; ^1H NMR (300 MHz, CDCl_3) δ : 8.01 (s, 1H), 7.96 (s, 1H), 7.87 (s, 1H), 5.11 (s, 1H), 4.92 (s, 1H), 4.56 (m, 1H), 3.95–3.91 (m, 4H), 2.04–0.91 (m), 0.80 (s, 3H), 0.70 (d, $J=6.4$ Hz, 3H), 0.61 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ : 160.44, 75.24, 73.64, 70.58, 61.27 (d, $J=5.8$ Hz), 47.22, 44.94, 42.88, 40.76, 37.69, 36.59 (d, $J=15.9$ Hz), 34.82, 34.47, 34.41, 34.21, 31.29, 28.48, 27.17, 26.52, 25.91 (d, $J=139.7$ Hz), 25.47, 22.69, 22.24, 18.99, 18.94, 17.63, 16.37 (d, $J=4.9$ Hz), 12.03; ^{31}P NMR

(162 MHz, CDCl₃) δ : 31.69; LRMS-ESI: calc. for $M+Na^+$, $M+K^+$: 621.3, 637.3; obsd. 621, 637.

2.1.5.7. Diethyl 3 α , 12 α -diformyloxy-5 β -cholane-24-phosphonate. Yield 83%; ¹H NMR (400 MHz, CDCl₃) δ : 8.13 (s, 1H), 8.03 (s, 1H), 5.24 (s, 1H), 4.87–4.81 (m, 1H), 4.14–4.04 (m, 4H), 1.91–1.4 (m), 1.34–1.30 (m, 6H), 1.0–0.90 (m), 0.92 (s, 3H), 0.84 (d, $J=6.4$ Hz), 0.74 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ : 160.55, 160.47, 76.01, 74.04, 61.34 (d, $J=5.9$ Hz), 49.21, 47.45, 44.97, 41.73, 36.73 (d, $J=16.9$ Hz), 35.60, 34.65, 34.18, 33.98, 32.09, 27.39, 26.75, 26.44, 26.00 (d, $J=140.9$ Hz), 25.86, 25.70, 23.38, 22.86, 19.08 (d, $J=3.3$ Hz), 17.67, 16.40 (d, $J=5.0$ Hz), 12.26; ³¹P NMR (162 MHz, CDCl₃) δ : 30.57; LRMS-ESI: calc. for $M+Na^+$: 577.3; obsd. 578.

2.1.5.8. Diethyl 3 α , 7 α -diformyloxy-5 β -cholane-24-phosphonate. Yield 98%; ¹H NMR (400 MHz, CDCl₃) δ : 8.08 (s, 1H), 8.03 (s, 1H), 5.03 (s, 1H), 4.77–4.69 (m, 1H), 4.12–4.06 (m, 4H), 2.1–1.38 (m), 1.33 (t, $J=7.2$ Hz, 6H), 1.25–1.0 (m), 0.95 (s, 3H), 0.93 (d, $J=6.0$ Hz, 3H), 0.65 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ : 160.59, 74.02, 71.37, 61.34 (d, $J=5.9$ Hz), 55.84, 50.11, 42.68, 41.02, 39.43, 37.95, 36.94 (d, $J=16.1$ Hz), 35.39, 34.79, 34.62, 34.01, 31.50, 28.02, 26.75, 26.12 (d, $J=138.4$ Hz), 26.75, 23.46, 22.61, 20.61, 19.07 (d, $J=4.0$ Hz), 18.50, 16.43 (d, $J=5.0$ Hz), 11.66; ³¹P NMR (162 MHz, CDCl₃) δ : 31.93; LRMS-ESI: calc. for $M+Na^+$: 577.3; obsd. 578.

2.1.5.9. Diethyl 3 α , 7 β -diformyloxy-5 β -cholane-24-phosphonate. Yield 93%; ¹H NMR (300 MHz, CDCl₃) δ : 8.03 (s, 1H), 7.99 (s, 1H), 4.94–4.79 (m, 2H), 4.12–4.06 (m, 4H), 2.1–1.37 (m), 1.33 (t, $J=7.2$ Hz, 6H), 1.25–1.0 (m), 0.99 (s, 3H), 0.93 (d, $J=6.6$ Hz, 3H), 0.68 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ : 160.67, 160.27, 73.34, 73.10, 61.12 (d, $J=5.9$ Hz), 55.10, 54.81, 43.34, 41.82, 39.62, 39.21, 36.69 (d, $J=17.0$ Hz), 35.06, 34.14, 33.70, 32.66, 32.53, 28.17, 26.14, 25.80 (d, $J=138.7$ Hz), 25.57, 22.93, 20.97, 18.86 (d, $J=3.5$ Hz), 18.34, 16.21 (d, $J=5.2$ Hz), 11.77; ³¹P NMR (162 MHz, CDCl₃) δ : 31.87; LRMS-ESI: calc. for $M+Na^+$: 577.3; obsd. 577.7.

2.1.5.10. Diethyl 3 α -formyloxy-5 β -cholane-24-phosphonate. Yield 95%; ¹H NMR (400 MHz, CDCl₃) δ : 8.04 (s, 1H), 4.85 (m, 1H), 4.12–4.07 (m, 4H), 2.0–1.4 (m), 1.35–1.30 (m, 6H), 1.29–1.04 (m), 0.93–0.9 (m, 6H), 0.64 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ : 160.67, 74.39, 61.49 (d, $J=4.7$ Hz), 56.48, 56.14, 41.95, 40.50, 40.14, 36.97 (d, $J=16.6$ Hz), 35.81, 35.41, 34.97, 32.25, 28.21, 26.98, 26.64, 26.28, 26.04 (d, $J=139.2$ Hz), 24.14, 23.25, 20.83, 19.06, 18.46, 16.37 (d, $J=3.7$ Hz), 11.98; ³¹P NMR (162 MHz, CDCl₃) δ : 32.24; LRMS-ESI: calc. for $M+Na^+$: 533.3; obsd. 534.

2.1.6. General procedure for the synthesis of 23- and 24-phosphonobile acids

Diethyl phosphonate ester (0.26 g, 0.44 mmol) was dissolved in dry dichloromethane (3 mL) under nitrogen and cooled in an ice-water bath. Iodotrimethylsilane (0.14 mL, 0.98 mmol) was added dropwise, while maintaining the temperature 0–5 °C, and then warmed to room temperature. After 24 h, the reaction mixture was evaporated in vacuo and the brownish residue was stirred with aq. NaOH solution (4 mL of 0.8 M, 3.2 mmol). After 4 h, the basic solution was acidified with conc. HCl and the precipitated pale yellow product was crystallized from MeOH/acetone (1:2) to yield phosphonobile acid as a white solid.

2.1.6.1. 3 α , 7 α , 12 α -trihydroxy-24-nor-5 β -cholane-23-phosphonic acid (23-PCA, 1). Yield 65%; mp: 257.4–259 °C; $[\alpha]_D^{25}$ (c 1.85 in MeOH) = +27; FTIR (KBr): 3426 cm⁻¹ (hydroxyl); ¹H NMR (300 MHz, DMSO-d₆) δ : 3.77 (s, 1H), 3.60 (s, 1H), 3.16 (bs, 1H), 1.8–1.13 (m), 0.91 (d, $J=6$ Hz, 3H), 0.81 (s, 3H), 0.59 (s, 3H); ¹³C NMR (100 MHz, DMSO-d₆) δ : 71.00, 70.43, 66.26, 45.66, 41.50, 41.36, 35.79 (d, $J=15$ Hz), 35.29, 34.84, 34.36, 30.38, 28.68, 28.52, 27.26, 26.21, 23.99 (d, $J=136$ Hz), 22.78, 22.60, 16.81, 12.35; ³¹P NMR (162 MHz, DMSO-d₆) δ : 29.55; HRMS Q-TOF: calc. for $M+Na^+$: 467.2538; obsd. 467.2505.

2.1.6.2. 3 α , 12 α -dihydroxy-24-nor-5 β -cholane-23-phosphonic acid (23-PDCA, 2). Yield 70%; mp: 262–263 °C; $[\alpha]_D^{25}$ (c 1.05 in MeOH) = +44; FTIR (KBr): 3400 cm⁻¹ (hydroxyl); ¹H NMR (300 MHz, DMSO-d₆) δ : 3.77 (s, 1H), 3.36 (m, 1H), 1.90–1.0 (m), 0.90 (d, $J=6.0$ Hz, 3H), 0.83 (s, 3H), 0.59 (s, 3H); ¹³C NMR (100 MHz, DMSO-d₆) δ : 71.02, 69.95, 47.44, 45.95, 45.76, 41.61, 36.27, 35.72 (d, $J=15$ Hz), 35.14, 33.80, 32.93, 30.20, 28.59, 27.16, 26.97, 26.09, 23.99 (d, $J=135$ Hz), 23.48, 23.07, 16.79, 12.46; ³¹P NMR (162 MHz, DMSO-d₆) δ : 29.63; HRMS Q-TOF: calc. for $M+Na^+$: 451.2589; obsd. 451.2577.

2.1.6.3. 3 α , 7 α -dihydroxy-24-nor-5 β -cholane-23-phosphonic acid (23-PCDCA, 3). Yield 66%; mp: 226–227 °C; $[\alpha]_D^{25}$ (c 2.1 in MeOH) = +9; ¹H NMR (400 MHz, DMSO-d₆) δ : 3.64 (s, 1H), 3.22–3.17 (m, 1H), 1.93–0.98 (m), 0.89 (d, $J=6.4$ Hz, 3H), 0.85 (s, 3H), 0.62 (s, 3H); ¹³C NMR (100 MHz, DMSO-d₆) δ : 70.33, 66.17, 55.24, 49.97, 41.89, 41.44, 35.64 (d, $J=15.3$ Hz), 35.31, 34.80, 34.72, 32.30, 30.54, 28.59, 27.72, 23.91 (d, $J=136.5$ Hz), 23.12, 22.69, 20.25, 18.04, 11.69; ³¹P NMR (162 MHz, DMSO-d₆) δ : 30.10; HRMS Q-TOF: calc. for $M+Na^+$: 451.2589; obsd. 451.2655.

2.1.6.4. 3 α , 7 β -dihydroxy-24-nor-5 β -cholane-23-phosphonic acid (23-PUDCA, 4). Yield 76%; mp: 234–235 °C; $[\alpha]_D^{25}$ (c 1.44 in MeOH) = +51; ¹H NMR (400 MHz, DMSO-d₆) δ : 3.34–3.26 (m, 2H), 1.96–1.09 (m), 0.89 (d, $J=4.8$ Hz, 3H), 0.88 (s, 3H), 0.63 (s, 3H); ¹³C NMR (100 MHz,

DMSO- d_6) δ : 69.74, 69.47, 55.83, 54.38, 49.05, 43.02, 42.18, 39.82, 38.75, 37.67, 37.23, 35.56 (d, $J=15.2$ Hz), 34.82, 33.74, 30.20, 28.67, 28.63, 28.10, 26.63, 23.94 (d, $J=132.6$ Hz), 23.28, 20.85, 18.18, 12.07; ^{31}P NMR (162 MHz, DMSO- d_6) δ : 29.82; HRMS Q-TOF: calc. for $M + \text{Na}^+$: 451.2589; obsd. 451.2603.

2.1.6.5. 3α -hydroxy-24-nor-5 β -cholane-23-phosphonic acid (23-PLCA, 5). Yield 75%; mp: 226.6–229 °C; $[\alpha]_{\text{D}}^{25}$ (c 0.65 in EtOH) = +30; FTIR (KBr): 3418 cm^{-1} (hydroxyl); ^1H NMR (300 MHz, DMSO- d_6) δ : 3.2–3.17 (m, 1H), 1.9–1.0 (m), .87 (bs, 6H), 0.63 (s, 3H); ^{13}C NMR (75 MHz, DMSO- d_6) δ : 69.90, 56.06, 55.26, 42.26, 41.57, 36.29, 35.64 (d, $J=17$ Hz), 35.44, 35.19, 34.24, 30.39, 28.69, 27.72, 26.93, 26.21, 24.03 (d, $J=136$ Hz), 23.98, 23.31, 20.45, 18.08, 11.96; ^{31}P NMR (162 MHz, DMSO- d_6) δ : 29.33; HRMS Q-TOF: calc. for $M + \text{Na}^+$; $M + \text{K}^+$: 435.2640, 451.2331; obsd. 435.2653, 451.2379.

2.1.6.6. 3α , 7 α , 12 α -trihydroxy-5 β -cholane-24-phosphonic acid (24-PCA, 6). Yield 60%; mp: 189–190 °C; $[\alpha]_{\text{D}}^{24}$ (c 1.68 in MeOH) = +30; ^1H NMR (400 MHz, DMSO- d_6) δ : 3.79 (s, 1H), 3.61 (s, 1H), 3.21–3.16 (m, 1H), 2.3–2.11 (m, 2H), 2.0–1.1 (m), 0.93 (d, $J=6.4$ Hz, 3H), 0.81 (s, 3H), 0.59 (s, 3H); ^{13}C NMR (100 MHz, DMSO- d_6) δ : 70.91, 70.31, 66.20, 66.15, 45.97, 45.59, 41.40, 41.21, 36.65 (d, $J=5.6$ Hz), 35.17, 34.95, 34.70, 34.25, 30.25, 28.42, 27.94 (d, $J=134.5$ Hz), 27.20, 26.09, 22.62, 22.44, 19.15, 17.12, 12.16; ^{31}P NMR (162 MHz, DMSO- d_6) δ : 28.66; HRMS Q-TOF: calc. for $M + \text{Na}^+$; $M + \text{K}^+$: 481.2695, 497.2434; obsd. 481.2664, 497.2411.

2.1.6.7. 3α , 12 α -dihydroxy-5 β -cholane-24-phosphonic acid (24-PDCA, 7). Yield 55%; mp: 242–243.5 °C; $[\alpha]_{\text{D}}^{24}$ (c 1.3 in MeOH) = +49; ^1H NMR (400 MHz, DMSO- d_6) δ : 3.80 (s, 1H), 3.41–3.34 (m, 1H), 1.85–1.0 (m), 0.94 (d, $J=6.8$ Hz, 3H), 0.86 (s, 3H), 0.61 (s, 3H); ^{13}C NMR (75 MHz, DMSO- d_6) δ : 71.02, 69.93, 47.48, 46.13, 45.95, 41.62, 36.75 (d, $J=17.3$ Hz), 36.29, 35.68, 35.11, 33.84, 32.95, 30.24, 28.11 (d, $J=135.8$ Hz), 28.66, 27.34, 27.01, 26.13, 23.53, 23.13, 19.33, 19.28, 17.24, 12.46; ^{31}P NMR (162 MHz, DMSO- d_6) δ : 28.71; HRMS Q-TOF: calc. for $M + \text{Na}^+$; $M + \text{K}^+$: 465.2746, 481.2485; obsd. 465.2740, 481.2447.

2.1.6.8. 3α , 7 α -dihydroxy-5 β -cholane-24-phosphonic acid (24-PCDCA, 8). Yield 75%; mp: 234–235 °C; $[\alpha]_{\text{D}}^{25}$ (c 1.38 in MeOH) = +15; ^1H NMR (400 MHz, DMSO- d_6) δ : 3.61 (s, 1H), 3.23–3.15 (m, 1H), 1.9–1.0 (m), 0.89 (d, $J=6.4$ Hz, 3H), 0.83 (s, 3H), 0.60 (s, 3H); ^{13}C NMR (100 MHz, DMSO- d_6) δ : 70.20, 66.03, 55.57, 49.86, 41.79, 41.32, 36.53 (d, $J=15.8$ Hz), 35.19, 34.89, 34.65, 34.59, 32.15, 30.39, 27.86 (d, $J=134.9$ Hz), 27.72, 22.99, 22.55, 20.12, 19.16, 18.31, 11.49; ^{31}P NMR (162 MHz, DMSO- d_6) δ : 28.58; HRMS Q-TOF: calc. for $M + \text{Na}^+$; $M + \text{K}^+$: 465.2746, 481.2485; obsd. 465.2729, 481.2477.

2.1.6.9. 3α , 7 β -dihydroxy-5 β -cholane-24-phosphonic acid (24-PUDCA, 9). Yield 64%; mp: 182–183 °C; $[\alpha]_{\text{D}}^{25}$ (c 1.7 in MeOH) = +53. ^1H NMR (400 MHz, DMSO- d_6) δ : 3.36–3.26 (m, 2H), 1.9–1.0 (m), 0.90 (d, $J=6.4$ Hz, 3H), 0.88 (s, 3H), 0.64 (s, 3H); ^{13}C NMR (100 MHz, DMSO- d_6) δ : 69.55, 69.29, 55.73, 54.69, 42.67, 42.03, 38.59, 37.54, 37.12, 36.52 (d, $J=16.1$ Hz), 34.79, 34.69, 33.60, 30.07, 27.83 (d, $J=135.9$ Hz), 28.09, 27.15, 26.50, 23.13, 20.69, 19.19, 18.43, 11.85; ^{31}P NMR (162 MHz, DMSO- d_6) δ : 28.67; HRMS Q-TOF: calc. for $M + \text{Na}^+$; $M + \text{K}^+$: 465.2746, 481.2485; obsd. 465.2743, 481.2408.

2.1.6.10. 3α -Hydroxy-5 β -cholane-24-phosphonic acid (24-PLCA, 10). Yield 90%; m. p.: 227–228 °C; $[\alpha]_{\text{D}}^{25}$ (c 1.0 in EtOH) = +32; ^1H NMR (400 MHz, DMSO- d_6) δ : 3.40–3.35 (m, 1H), 1.96–1.0 (m), 0.89 (d, $J=7.2$ Hz, 3H), 0.88 (s, 3H), 0.63 (s, 3H); ^{13}C NMR (100 MHz, DMSO- d_6) δ : 69.80, 56.01, 55.65, 42.19, 41.47, 36.52 (d, $J=16.1$ Hz), 36.18, 35.33, 35.07, 34.85, 34.12, 30.25, 27.88 (d, $J=135.5$ Hz), 27.71, 26.81, 26.06, 23.73, 23.15, 20.33, 19.24, 18.31, 11.58; ^{31}P NMR (162 MHz, DMSO- d_6) δ : 28.72; HRMS Q-TOF: calc. for $M + \text{Na}^+$: 449.2797; obsd. 449.2791.

2.1.7. Estimation of solubilized cholesterol by enzymatic assay

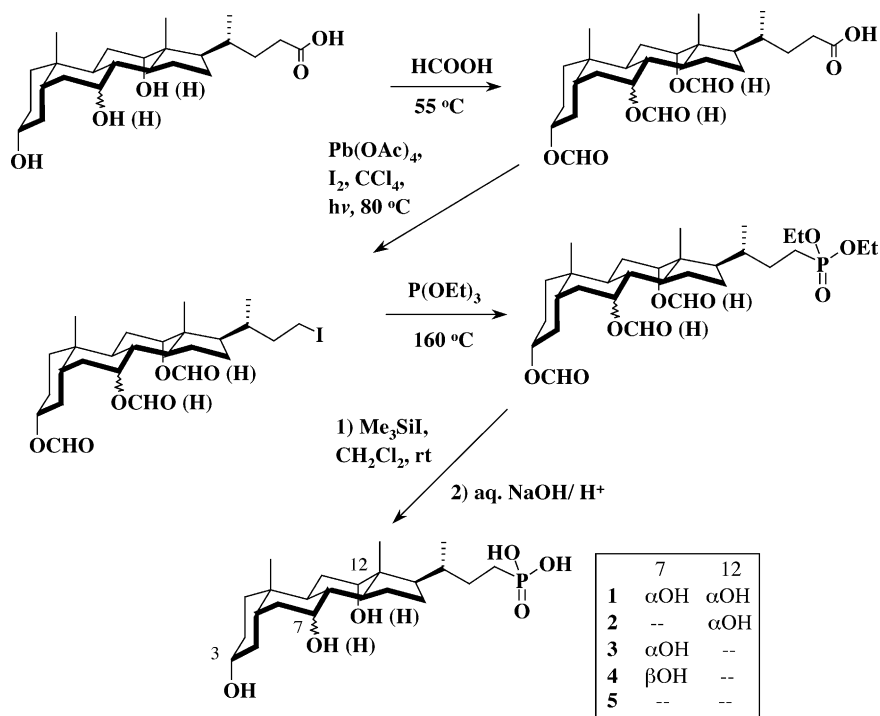
The amount of solubilized cholesterol was estimated by an enzymatic assay [13]. Samples were incubated with a commercially available cholesterol reagent [14] at 37 °C for 10 min, and then absorbance was recorded at 510 nm. The cholesterol converted was estimated by comparing this absorbance with that from a standard sample.

3. Results and discussion

3.1. Synthesis

The syntheses of 23-phosphonobile acids were accomplished as shown in Scheme 1. In addition, the one-carbon homologs were also synthesized starting from bile acids (Scheme 2). Cholic acid (CA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), ursodeoxycholic acid (UDCA) and lithocholic acid (LCA) were converted to their corresponding formyl derivatives by heating with formic acid.

For the syntheses of 23-phosphonobile acids, formyl protected bile acids were subjected to a modified Hunsdiecker reaction (iododecarboxylation) in the presence of $\text{Pb}(\text{OAc})_4/\text{I}_2/h\nu$ to yield the corresponding formylated 24-nor-23-iodo derivatives. The iodo derivatives were refluxed with triethyl phosphite at 160 °C under nitrogen (Arbuzov reaction) to furnish the corresponding formylated diethyl phosphocholanate esters. The cleavage of the ethyl groups was achieved by stirring with Me_3SiI in CH_2Cl_2 at rt. The removal of the TMS ester and the formyl groups were effected in the same pot by alkaline hydrolysis in MeOH. The basic solution

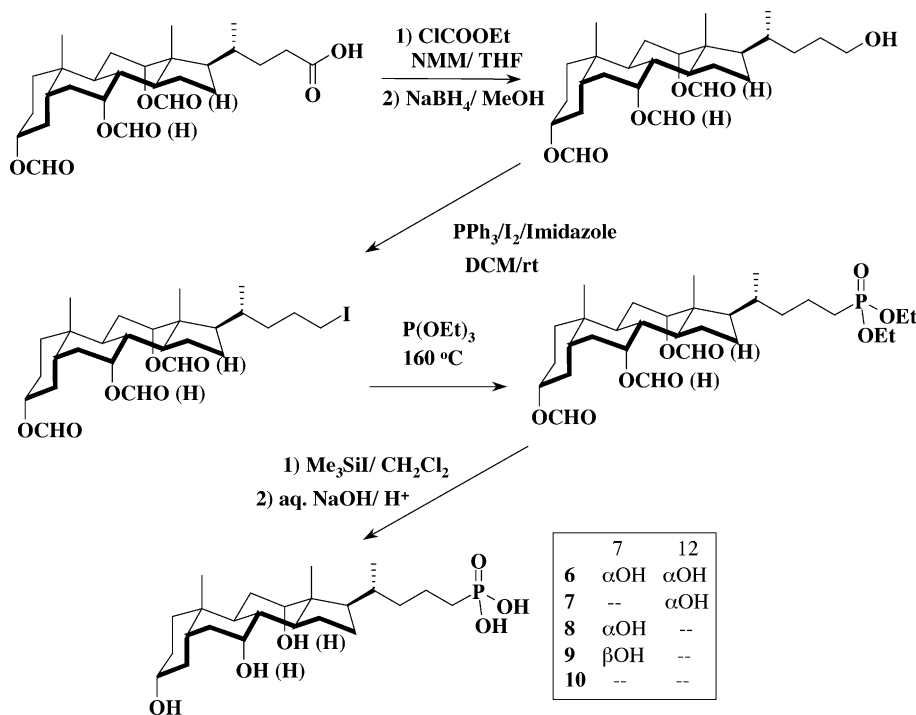


Scheme 1.

containing phosphonobile salt was neutralized (pH ~ 1) and filtered to yield the 23-phosphonobile acids.

For the synthesis of 24-phosphonobile acids, the formylated bile acids were reduced to the corresponding formyl protected alcohols in two steps. First a mixed anhydride was made with ethyl chloroformate in the presence of triethylamine, which was then selectively reduced by NaBH_4 in

MeOH . The 24-hydroxy compounds were converted to the corresponding 24-iodo derivatives using $\text{PPh}_3/\text{I}_2/\text{imidazole}$ in dichloromethane at rt [15]. The formation of diethyl esters of 24-phosphonobile acids and subsequent deprotection of ethyl and formyl groups were accomplished following



Scheme 2.

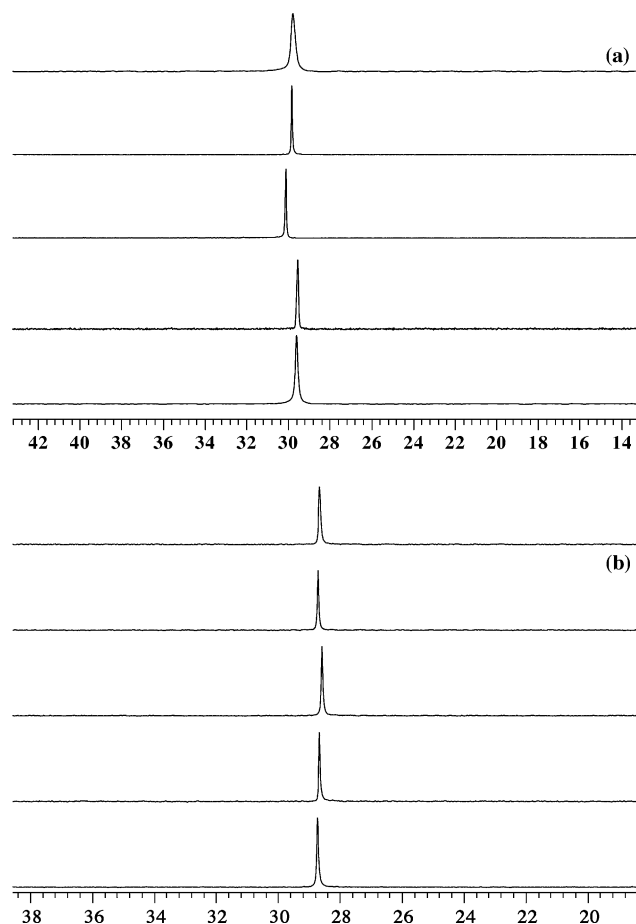


Fig. 1. ^{31}P NMR spectra of 23-PBA (a) and 24-PBA (b) in DMSO-d_6 (162 MHz). Top to bottom: phosphonochoholic, phosphonodeoxychoholic, phosphonochenodeoxychoholic, phosphonoursodeoxychoholic and phosphonolithocholic acid.

the same procedures as described for 23-phosphonobile acids. All the compounds synthesized were characterized spectroscopically. The ^{31}P NMR spectra of 23- and 24-phosphonobile acids are shown in Fig. 1.

3.2. *In vitro* equilibrium cholesterol solubilization by 23- and 24-PBSs

All the equilibrium cholesterol solubilization experiments were done at 37°C in solutions containing varying concentrations of disodium salt of phosphonobile acids and 0.15 M NaCl in pH 6.4 (0.1 M phosphate) buffer solutions. The solubilized cholesterol was analyzed using an enzymatic assay (see Section 2). The equilibrium cholesterol solubility data for 23- and 24-phosphonobile salts at pH 6.4 (0.1 M potassium phosphate), where the phosphonobile acids are predominantly monoionic, are presented in Table 1. In general, the equilibrium solubilizing power (Sp), defined as the ratio of cholesterol:PBS, is slightly higher for 24-PBSs than that of 23-PBSs with the solubilizing power of the corresponding bile salts (at pH 10.0) [16,17] being in between. The equilibrium solubility of cholesterol is greatly influenced by the

Table 1
Comparison of cholesterol solubilization efficiency of 23- and 24-PBSs with those of bile salts

Entry	PBS (50 mM) in pH 6.4 (0.1 M phosphate buffer), 0.15 M NaCl at 37°C	Ratio of cholesterol:bile salt (Sp) ^a		
		23-PBS	24-PBS	BS ^b
1	PC	1:46 (1)	1:23 (6)	1:29
2	PDC	1:19 (2)	1:13.5 (7)	1:15.6
3	PCDC	1:20 (3)	(8) ^c	1:14
4	PUDC	1:238 (4)	1:151 (9)	1:364

^a Molar concentration of dissolved cholesterol = $0.05 \times \text{Sp}$; the lithocholate analogs (5 and 10) were insoluble under identical conditions.

^b The values are taken from ref. [17] at pH 10.

^c Under the experimental conditions, a turbid gel was obtained, so the amount of solubilized cholesterol could not be measured.

structure and number of hydroxyl groups present in the PBSs. For both 23- and 24-PBSs, the solubilizing power decreases in the following order: $\text{PDC} \cong \text{PCDC} > \text{PC} \gg \text{PUDC}$. The same trend of cholesterol solubilization is observed for bile salts, which correlated inversely with hydrophilicity [18]. Interestingly, the 23- and 24-PUDC showed 1.5 and 2.4 times higher solubilization, respectively, compared to that of UDC. Kinetic experiments show a fast initial rate of solubilization, which slows down with time. For 23-PCDC, cholesterol solubilization saturates in about 8 h.

3.3. Effect of pH on equilibrium cholesterol solubility

The effect of pH on the equilibrium cholesterol solubility was examined using 23-PCDC and 23-PUDC as the solubilizing agents. The solubilization experiments were done in three different buffer solutions (0.1 M): pH 6.4 (phosphate), 7.4 (TRIS) and 10.0 (carbonate) and 0.15 M NaCl. The equilibrium cholesterol solubility decreases considerably upon increasing the bulk pH. The same trend was observed for phosphonocheno and phosphonoursodeoxycholates (Fig. 2). An increase in the bulk pH increases the CMC of phospho-

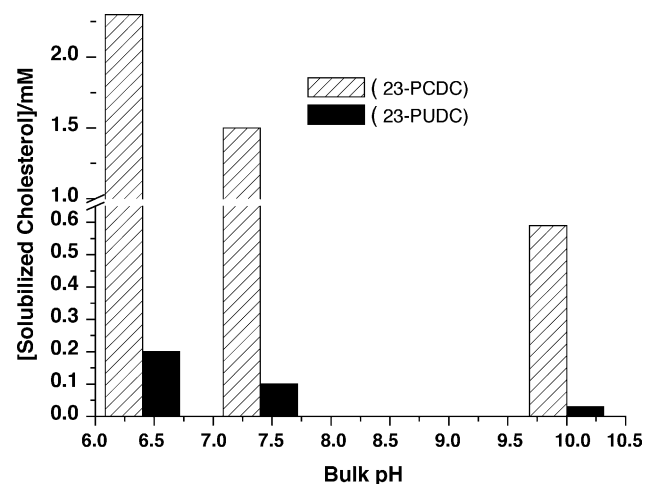


Fig. 2. Amount of solubilized cholesterol (by 23-PCDC and 23-PUDC, 50 mM) as a function of pH 6.4 (phosphate), 7.4 (TRIS) and 10.0 (carbonate).

nobile salts. For example, the CMC of 23-PCDC increased from 2 mM to 7–8 mM when the pH is changed from 6.4 to 10. Hence, the observed decrease in equilibrium solubility is possibly due to an increase in the CMC, which decreases the number of micelles formed at a given total concentration of the bile salt.

4. Conclusions

We synthesized a new class of bile acid analogs. In vitro cholesterol solubilizing efficiency of 23- and 24-phosphonobile salts has been studied. The 24-PBSs solubilize cholesterol slightly better than 23-PBSs and bile salts. We believe that our preliminary results are encouraging to further explore a better medicinal therapy for cholesterol gallstone dissolution. The aggregation and gelation properties of these new compounds will be published elsewhere.

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