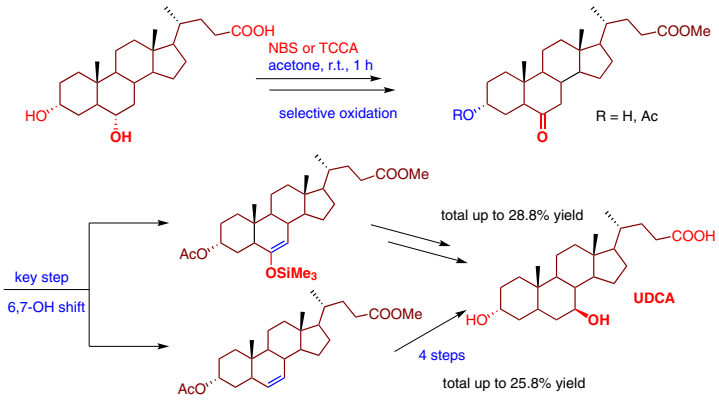
Synthesis Q. Dou, Z. Jiang Paper

A Facile Route to Ursodeoxycholic Acid Based on Stereocontrolled Conversion and Aggregation Behavior Research

Qian Dou
Zhongliang Jiang*

Department of Chemistry, Shanghai Key Laboratory of Chemical Assessment and Sustainability, Tongji University, Shanghai 200092, P. R. of China
zl_jiang@tongji.edu.cn



Received: 03.09.2015
Accepted after revision: 13.11.2015
Published online: 15.12.2015
DOI: 10.1055/s-0035-1560388; Art ID: ss-2015-h0518-op

Abstract A facile route to ursodeoxycholic acid (UDCA) and its aggregation behavior in aqueous phase solution, which is rarely known, are reported. The starting material, hyodeoxycholic acid (HDCA), is a relatively less expensive material and more easily obtained compared with chenodeoxycholic acid (CDCA). A facile route was developed to synthesize UDCA from HDCA with a Shapiro reaction as the key step and in 26% overall yield. A new strategy using organosilane reagent considering its stability, nontoxicity, and abundance in nature was carried out for a more rapid route and higher yield. It was found that the critical micelle concentration value, which is a critical value for surfactants of bile salts, was influenced by the number of hydroxyl groups.

Key words ursodeoxycholic acid, hyodeoxycholic acid, critical micelle concentration, facile route, Shapiro reaction, Clemmensen reaction

Among the bile acids (BAs, Figure 1), which are natural products and the fundamental component of bile,[1] ursodeoxycholic acid (UDCA) has an important medical application due to its ability to resolve cholesterol gallstones.[2,3] UDCA plays an important role in immunoregulation, promotes the secretion of endogenous BAs, and protect the epicytes of liver cells. Most of UDCA is prepared on a large scale from raw materials with high bile content, such as goose and bovine bile, of which the major component is cholic acid that is frequently used as the starting material to synthesize chenodeoxycholic acid (CDCA).[4–7] Recently, numerous routes have been developed for the synthesis of UDCA by employing CDCA as the starting material, but HDCA was not frequently used because a few extra steps would be required for the conversion between 6-OH and 7-OH. The transformation from other BAs to UDCA has been reported. Zhou et al.[8] described a 7-step sequence to synthesize UDCA in 15% total yield. More recently, Pedrini et al.[9,10] reported the synthesis of UDCA with cholic acid and CDCA as the starting materials using an enzyme as catalyst, which is also applied in the synthesis of UCA and 6-FUDCA.[11]

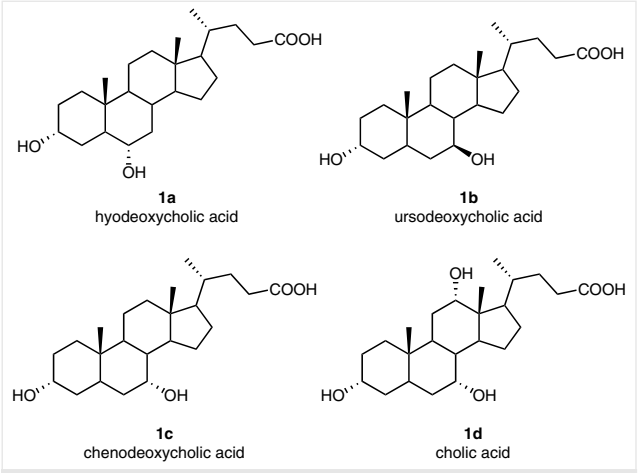


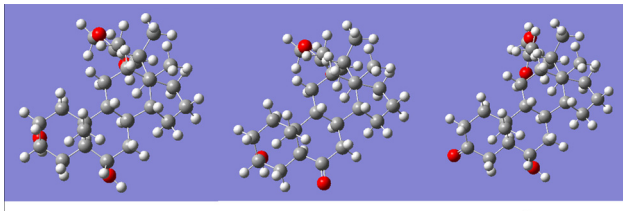
Figure 1 Different types of bile acids

Computation chemistry has been developed for many years to predict the potential property and reactivity of compounds based on the density functional theory.[12–16] Accurate quantum chemical calculation was performed to predict the enthalpy change between various products in selective reactions. Moreover, according to a previous study on avicholic acid,[17] a major constituent of the bile of several avian species, research on critical micelle concentration (CMC) as a reflection of aggregation behavior, which indicates the capability of surfactants, has also been covered in this paper.[18,19]

Path Finding Studies

We discuss here the oxidative reaction of hyodeoxycholic acid methyl ester (**2**) with three potential oxidizing agents to develop a facile route to synthesize UDCA by employing environment-friendly reagents and HDCA. Selective oxidation of 6-OH could be achieved with NBS, NCS, or TCCA (trichloroisocyanuric acid) as the oxidant. The potential result of oxidation was predicted by computations (Table 1) that were accomplished using the density functional theory method on potential products **3** and **3a** performed with the Gaussian 09 program,[20–22] the B3LYP functional and the 6-31+G(d,p) basis for carbon and oxygen atoms. The reaction with NBS was carried out to obtain the best result; the ratio of **3**/**3a** was 8:1 and the yield 85% (Scheme 1).

Table 1 Calculated Energy of **2**, **3**, **3a**



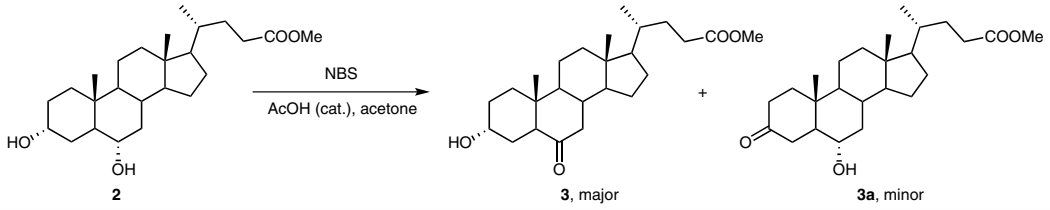
2 3 3a

	2	3	3a
Relative energy (kcal/mol)	0	–27.41	–75.55

After the protection of 3-OH using a traditional reaction with Ac_2O and Na_2CO_3, the substrate **4** was used in the Shapiro reaction as the key step of this route. The reaction between *p*-toluenesulfonyl hydrazide and **4** was carried out in refluxing ethanol for one hour and the product **5** was isolated without further purification. The crucial product **5** was washed with cold ethanol and the yield was >95% over all the operations. From the literature[23] we discovered that the base most frequently used in the Shapiro reaction was *n*-BuLi or LDA. The first example of Shapiro reaction with LiH as the base and toluene as solvent was developed in our present route providing a yield of 74% of the olefin **6**. NaH could be used in this reaction as well, but the yield was lower at 37%. TMEDA played a very important role in this reaction. With its unique coordination with Li, the C=C bond was more easily formed. The ratio of **6**/**6a** was 15:1 (Scheme 2).

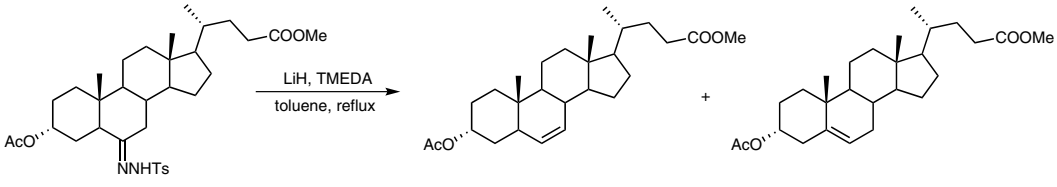
The oxidation reaction of C=C bond to give **7** with *m*-CPBA is a traditional method with mild conditions and high yield (Scheme 3). Enantioselective ring opening of epoxides required Pd/C and H_2 to achieve the selective reduction of **7** based on the higher stability of 7α-OH. *N*-Chlorosuccinimide was used to oxidize **8**, and the last step of subsequent radical reduction to form 7β-OH was accomplished using sodium in *n*-butanol or lithium in liquid ammonia.[24] With compound **9** available, the stereochemical course of nucleophilic attack at C=O bond was controlled by the solvent and reductant.[25,26]

Considering the lower total yield of this route, another route with higher yield was developed using organosilane reagents. Because carbonyl group could be transformed to enol with a highly reactive OH, **10** was obtained using **4** as the substrate and LiH as the base at reflux in redistilled THF with TMSCl overnight. Versatile oxidation of C=C bond required the combined use of *m*-CPBA and CH_2Cl_2 to obtain product **11**. The key step in this route is the selective reduction of **11** with two ester groups and one carbonyl group, under precise control of the conditions (Schemes 4 and 5). However, zinc amalgam, which is mostly used in Clemmensen reduction, was not used in our total synthesis of UDCA. We first developed a new method without using



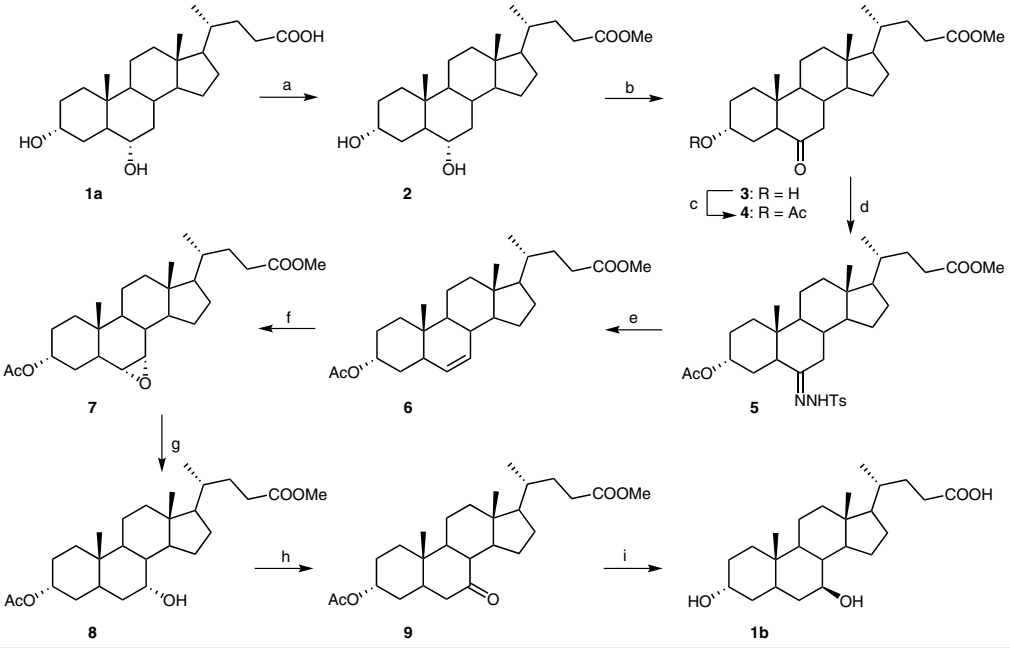
2 **3**, major **3a**, minor

Scheme 1 Selective oxidation of **2** with NBS



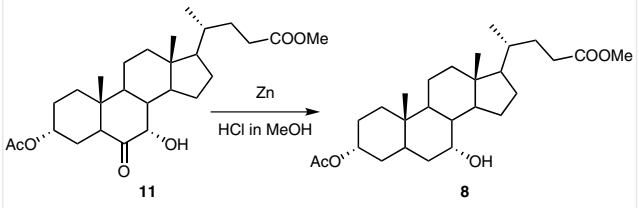
5 **6**, major **6a**, minor

Scheme 2 Shapiro reaction of **5**



Scheme 3 Path I. *Reagents and conditions*: a) MeOH, H_2SO_4, r.t., 8 h, 99%; b) NBS, AcOH, acetone, H_2O, r.t., 1 h, 85%; c) Ac_2O, Na_2CO_3, CH_2Cl_2, r.t., 8 h, 96%; d) $TsNHNH_2$, EtOH, reflux, 1 h, 87%; e) LiH, TMEDA, toluene, reflux, 18 h, 74%; f) *m*-CPBA, CH_2Cl_2, 0 °C, 1 h, 83%; g) Pd/C (10 mol%), H_2, 50 psi, 40 °C, 24 h, 62%; h) NCS, AcOH, acetone, r.t., 40 min, 96%; i) Na, *n*-BuOH, 60 °C, 2 h, 62%.

zinc amalgam such that zinc powder was added directly into the reaction mixture, and the reaction time and temperature were accurately controlled to produce **8** in 74% yield for this single step (Scheme 4).



Scheme 4 Clemmensen reduction without zinc amalgam

The 7-OH inversion was chemically accomplished through the regioselective oxidation of the 7α-OH function and reduction with Na in *n*-BuOH (Scheme 5). The overall yield of this route is 29%.

Research on Aggregation Behavior of Bile Salts

Bile salts are a type of surfactants containing a hydrophobic group and a hydrophilic group. Critical micelle concentration (CMC) as a reflection of aggregation behavior indicates the surfactant capability, which is mainly related to the number of hydroxyl groups in steroid compounds. Therefore, our research was focused on determining the CMC index by detecting the fluorescence spectrum with different types of steroid compounds, such as lithocholic acid, HDCA, CDCA, UDCA, and cholic acid (Figure 2). Reports on the fluorescence absorption behavior in aqueous medium are scarce, thus, we decided to research on the micellization in aqueous solution with pyrene as a fluorescent probe to measure the CMC index of bile salts. The signal intensity of two vibronic bands III/I of fluorescence spectrum indicate the amount of fluorescence absorption, namely, the amount of pyrene that dissolved in water together with the surfactants (see Supporting Information).

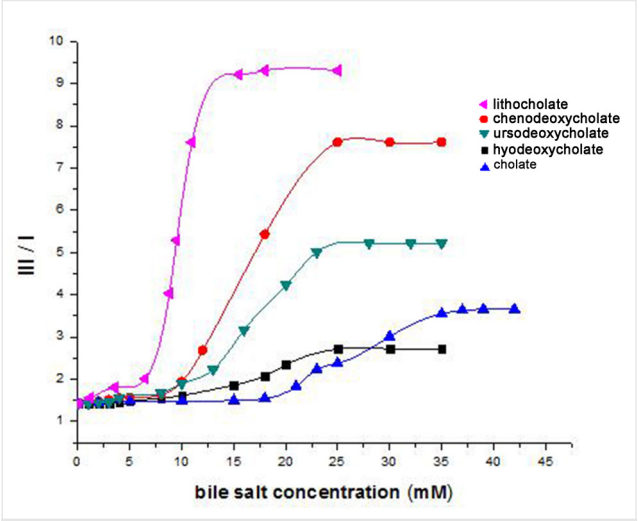
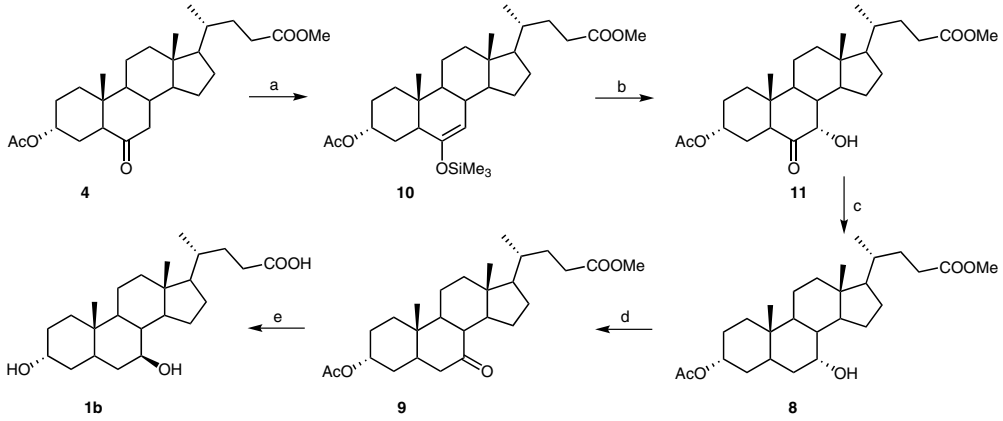


Figure 2 Intensity ratios (III/I) of vibronic bands of pyrene fluorescence as a function of bile salt concentration at room temperature with distilled water as solvent



Scheme 5 Path II. *Reagents and conditions*: a) LiH, TMSCl, THF, 60 °C, 6 h, 89%; b) *m*-CPBA, CH_2Cl_2, r.t., 4 h, 91%; c) Zn, HCl, –20 °C, 45 min, 74%; d) NCS, AcOH, acetone, r.t., 1 h, 96%; e) Na, *n*-BuOH, 60 °C, 2 h, 62%.

The data in the diagram indicate that the value of CMC correlated with the number of hydroxyl groups in bile salts. Compound with fewer hydroxyl groups would have a lower CMC value, which indicates the lower capacity of surfactants. Moreover, the position and space configuration would have tremendous influence on the value of III/I rather than the CMC value, implying that the selective reduction of **11** and the transformation from 6-OH to 7-OH are related to the reactivity of substrates but not to their aggregation behavior.

In summary, a novel, facile, and efficient route to synthesize UDCA was developed with the Shapiro reaction as the key step for the 6,7-OH transformation, and the final product was obtained through the 7-OH inversion employing regioselective oxidation and subsequent radical reduction. Moreover, another route with higher total yield was discovered using organosilane reagents, which was proven stable, nontoxic, and high yielding. In this route, the first Clemmensen reduction performed without zinc amalgam became the yield-controlling step, with precisely controlled conditions and reaction time. Further studies on improved routes and higher yield are underway in our laboratory.

The silica gel used for column chromatography was 300–400 mesh in size. TLC was carried out using commercial silica gel GF254, and plates were developed with CMC (carboxymethylcellulose sodium) The reagents used were of analytical grade, all of the solvents were redistilled before use, and all of the reactions were performed in oven-dried three-necked flasks. Compounds were detected with 10% ethanolic phosphomolybdic acid solution as chromogenic agent. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX 400 spectrometer in $CDCl_3$ or DMSO-d_6 with reference to the residual $CHCl_3$ at 7.26 ppm and DMSO at 1.56 ppm for ^{1}H NMR, and 77.0 ppm for $CHCl_3$ and 39.52 ppm for DMSO for ^{13}C NMR. High-resolution mass spectra were obtained on a Bruker MicroTOF II ESI-TOF mass spectrometer. IR spectra were recorded using KBr discs on Nicolet FT-IR spectrometer, and fluorescence spectra were detected on Hitachi F-7000. Optional rotations were recorded on SG WXG-4 automatic polarimeter.

Methyl 3α,6α-Dihydroxycholan-24-oate (2)

To a solution of hyodeoxycholic acid (**1a**; 6.00 g, 15.3 mmol) in MeOH (50 mL) at 5 °C was added H_2SO_4 (5 drops) dropwise over 10 min with stirring until all solids were dissolved completely. The mixture was then allowed to warm to r.t. and stirred overnight. The reaction was quenched with sat. aq $NaHCO_3$ (10 mL) and evaporated under reduced pressure. After extraction with EtOAc (50 mL), the combined organic layers were washed with sat. aq $NaHCO_3$ (2 × 35 mL) and brine (3 × 50 mL), and dried (Na_2SO_4). Evaporation of the solvent under reduced pressure afforded **2** (6.22 g, 99%) as a white solid, which was recrystallized from toluene; yield: 6.22 g (99%); mp 165–167 °C; $[\alpha]_D^{25}$ +15.7 (*c* 1 g/100 mL EtOH).

IR (KBr): 3350 (O–H), 1743 (C=O), 1123 cm^{-1} (C–O).

^{1}H NMR (400 MHz, $CDCl_3$/TMS): δ = 0.66 (3 H, s, 18-H), 0.94 (6 H, t, *J* = 1.4 Hz), 3.63 (1 H, m, 6α-H), 3.69 (3 H, s), 4.07 (1 H, m, 3α-H).

^{13}C NMR (100 MHz, $CDCl_3$/TMS): δ = 12.05, 18.28, 20.79, 23.56, 24.24, 28.16, 29.27, 30.15, 30.98, 31.10, 34.85, 34.89, 35.39, 35.63, 35.97, 39.89, 39.99, 42.87, 48.47, 51.54, 55.96, 56.21, 68.05, 71.54, 174.79.

HRMS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{25}H_{42}O_4Na$: 429.2975; found: 429.2965.

Methyl 3α-Hydroxy-6-oxocholan-24-oate (3)

Method 1: To a stirred solution of **2** (2.40 g, 5.91 mmol) in acetone (100 mL) containing AcOH (0.40 mL, 7.09 mmol) and H_2O (5 mL) was added NBS (2.10 g, 11.8 mmol) in ten portions at 0 °C. The mixture was then allowed to warm to r.t. after stirring for 10 min. The course of the reaction was monitored by TLC. After 1 h, the reaction was then quenched with sat. aq Na_2SO_3 (20 mL) and the mixture was evaporated under reduced pressure. EtOAc (25 mL) was added to the residue and filtered. The filtrate was washed with sat. aq Na_2SO_3 (2 × 25 mL), sat. aq $NaHCO_3$ (2 × 25 mL), and brine (3 × 15 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo to give the crude product, which was chromatographed on a silica gel column eluting with EtOAc–PE (1:5) to afford **3** as white crystals; yield: 2.0 g (85%).

Method 2: TCCA (225 mg, 0.95 mmol) dissolved in acetone (10 mL) was added dropwise to a stirred solution of **2** (1.00 g, 2.45 mmol) in freshly distilled pyridine (0.225 mL, 2.85 mmol) and acetone (15 mL) at 0 °C, followed by gentle heating to r.t. over 40 min. The reaction was then quenched with sat. aq Na_2SO_3 (15 mL) and the volatiles were evaporated under reduced pressure. After extraction of the residue with EtOAc (15 mL), the separated aqueous layer was extracted with EtOAc (10 mL). The combined organic solutions were washed with aq 1 M HCl (2 × 10 mL), sat. aq $NaHCO_3$ (3 × 25 mL) and brine (2 × 20 mL), dried (Na_2SO_4), and concentrated. The product was purified by silica gel column chromatography (eluent: EtOAc–PE, 1:5) to afford **3** as white crystals; yield: 753 mg (76%); mp 133–135 °C (EtOH); $[\alpha]_D^{25}$ –7.1 (*c* 1 g/100 mL CH_2Cl_2).

IR (KBr): 3330 (O–H), 1743 and 1690 (C=O), 1123 cm^{-1} (C–O).

^1H NMR (400 MHz, $CDCl_3$/TMS): δ = 0.70 (3 H, s, 18-H), 0.94 (3 H, d, *J* = 6.4 Hz, 21-H), 1.03 (3 H, s, 19-H), 3.69 (3 H, s), 4.13 (1 H, m, 3α-H).

^{13}C NMR (100 MHz, $CDCl_3$/TMS): δ = 12.04, 18.26, 21.09, 22.84, 24.15, 28.06, 30.94, 31.04, 34.36, 34.56, 35.31, 36.04, 36.21, 37.05, 37.09, 39.83, 40.27, 42.84, 50.18, 51.48, 55.94, 56.12, 67.62, 174.66, 212.71.

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{25}H_{40}O_4Na$: 427.2819; found: 427.2814.

Methyl 3α-Acetoxy-6-oxocholan-24-oate (4)

Method 1: Compound **3** (1.50 g, 3.71 mmol) dissolved in anhydrous CH_2Cl_2 (50 mL) was reacted with Ac_2O (0.42 mL, 4.45 mmol) in the presence of Na_2CO_3 (39.21 mg, 0.37 mmol). The mixture was allowed to stand for 8 h at r.t. and diluted with sat. aq $NaHCO_3$ (10 mL). The organic layer was then separated and the aqueous phase was extracted with an additional amount of CH_2Cl_2 (15 mL). The combined extracts were successively washed with aq 1 M HCl (2 × 15 mL), sat. aq $NaHCO_3$ (3 × 25 mL), and brine (2 × 25 mL). The organic layer was dried (Na_2SO_4) and filtered. The filtrate was evaporated under reduced pressure to leave **4** as a pale yellow solid, which was used in the next step without purification; yield: 1.59 g (96%).

Method 2: To a stirred solution of **3** (300 mg, 0.74 mmol) in anhydrous CH_2Cl_2 (15 mL) was added sequentially dropwise AcCl (0.06 mL, 0.81 mmol) and Et_3N (0.10 mL, 0.72 mmol). The mixture was stirred at r.t. overnight. After removal of volatiles, the mixture was acidified with aq 1 M HCl (2 × 15 mL), basified with sat. aq $NaHCO_3$ (2 × 10 mL), washed with brine (20 mL), and filtered. The solid residue was purified on a silica gel column (4 cm × 5 cm) using EtOAc–PE (1:3) to afford **4** as a pale yellow solid; yield: 231.0 mg (86%); mp 183–185 °C (EtOH); $[\alpha]_D^{25}$ –9.4 (*c* 1 g/100 mL CH_2Cl_2).

IR (KBr): 1770, 1743 and 1690 (C=O), 1123 cm^{-1} (C–O).

^1H NMR (400 MHz, $CDCl_3$/TMS): δ = 0.70 (3 H, s, 18-H), 0.94 (3 H, d, *J* = 6.4 Hz, 21-H), 1.09 (3 H, s, 19-H), 2.03 (3 H, s), 3.69 (3 H, s), 5.19 (1 H, m, 3α-H).

^{13}C NMR (100 MHz, $CDCl_3$/TMS): δ = 12.02, 18.26, 21.03, 21.20, 22.63, 24.05, 28.04, 30.91, 30.93, 31.02, 34.43, 35.29, 36.31, 36.77, 36.85, 36.89, 39.78, 40.32, 42.88, 47.09, 51.45, 55.92, 56.12, 70.56, 170.24, 174.58, 211.85.

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{27}H_{42}O_5$: 469.2924; found: 469.2915.

Methyl 3α-Acetoxy-6-[2-[(4-methylphenyl)sulfonyl]hydrazinylidene]cholan-24-oate (5)

p-Toluenesulfonyl hydrazone (0.40 g, 2.15 mmol) was added in five portions to a stirred solution of **4** (0.80 g, 1.79 mmol) in anhydrous EtOH (15 mL) over 10 min. The mixture was heated at reflux for 45 min under N_2 atmosphere and then allowed to stir at r.t. for 15 min prior to dilution with Et_2O (10 mL). The course of the reaction was monitored by TLC. The mixture was evaporated under reduced pressure to leave a light yellow solid, which was dissolved in EtOAc (15 mL) and the EtOAc layer was washed with brine (2 × 20 mL). The EtOAc solution was dried (Na_2SO_4) and concentrated. After the crude product was recrystallized from ethanol twice, chromatography of the residue on silica gel (eluent: EtOAc–CH_2Cl_2–PE, 1:1:6) gave **5** as a white solid; yield: 0.96 g (87%); mp 210–211 °C (EtOH); $[\alpha]_D^{25}$ –18.3 (*c* 1 g/100 mL CH_2Cl_2).

IR (KBr): 3389 (N–H), 1743 and 1690 (C=O), 1127 (C–O), 710 and 650 cm^{-1} (C–H).

^1H NMR (400 MHz, $CDCl_3$/TMS): δ = 0.64 (3 H, s, 18-H), 0.90 (3 H, d, *J* = 6.4 Hz, 21-H), 0.96 (3 H, d, *J* = 6.4 Hz, 19-H), 2.04 (3 H, s), 2.43 (3 H, s), 3.66 (3 H, s), 5.14 (1 H, m, 3α-H), 7.31 (2 H, d, *J* = 7.8 Hz), 7.84 (2 H, d, *J* = 7.8 Hz).

^{13}C NMR (100 MHz, $CDCl_3$/TMS): δ = 12.01, 14.22, 18.25, 21.07, 21.44, 21.64, 22.81, 22.96, 28.06, 30.93, 30.97, 31.05, 34.39, 34.45, 35.31, 36.50, 39.76, 42.85, 45.42, 46.70, 51.53, 55.91, 56.05, 56.13, 70.75, 128.00, 128.10, 129.52, 129.57, 135.46, 143.93, 161.99, 170.48, 174.70.

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{34}H_{50}N_2O_6SNa$: 637.3282; found: 637.3273.

Methyl 3α-Acetoxychol-6-en-24-oate (6)

A magnetically stirred solution of **5** (1.30 g, 2.12 mmol) in freshly distilled toluene (100 mL) was treated dropwise with a solution of LiH in freshly distilled toluene (20 mL) over 10 min at 60 °C, and TMEDA (0.22 mL) was then added dropwise. The suspension was warmed to 90 °C at reflux under N_2 atmosphere. The process of the reaction was monitored by TLC. After stirring for 18 h, the reaction was quenched with sat. aq NH_4Cl (12 mL) and filtered through a Celite pad. The filtrate was evaporated under reduced pressure and extracted with EtOAc (25 mL). The combined organic extracts were washed with sat. aq $NaHCO_3$ (2 × 25 mL) and brine (20 mL), dried (Na_2SO_4) and filtered. Evaporation of the solvent gave a brown oil, which was purified chromatographically on a silica gel column (eluent: EtOAc–CH_2Cl_2–PE, 1:5:50) to give the pure product **6** as a white solid; yield: 528 mg (74%); mp 120–123 °C (EtOH); $[\alpha]_D^{25}$ +3.1 (*c*, 1 g/100 mL CH_2Cl_2).

IR (KBr): 3299 (C=C), 1743 and 1700 (C=O), 1127 cm^{-1} (C–O).

^1H NMR (400 MHz, $CDCl_3$/TMS): δ = 0.67 (3 H, s, 18-H), 0.92 (3 H, d, *J* = 6.4 Hz, 21-H), 1.02 (3 H, s, 19-H), 2.07 (3 H, s), 3.67 (3 H, s), 5.14 (1 H, m, 3α-H), 5.68 (1 H, m, 6-H), 5.78 (1 H, m, 7-H).

^{13}C NMR (100 MHz, $CDCl_3$/TMS): δ = 11.96, 18.28, 21.40, 21.45, 22.25, 22.83, 24.15, 28.07, 31.00, 31.05, 32.79, 33.81, 34.63, 35.26, 35.32, 39.99, 40.25, 42.85, 46.98, 51.49, 55.87, 55.91, 71.86, 125.11, 128.13, 170.58, 174.76.

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{27}H_{42}O_4Na$: 453.2975; found: 453.2946.

Methyl 3α-Acetoxy-6,7-epoxycholan-24-oate (7)

m-CPBA (3-chloroperbenzoic acid, 324 mg, 1.88 mmol) dissolved in anhydrous CH_2Cl_2 (10 mL) was added dropwise at 0 °C to a stirred solution of **6** (675 mg, 1.57 mmol) in anhydrous CH_2Cl_2 (15 mL) over 15 min. The reaction mixture was allowed to reach 35 °C and stirred for 1 h and the course of reaction was monitored by TLC. The reaction was quenched with sat. aq Na_2SO_3 (20 mL) and evaporated under reduced pressure prior to extraction with Et_2O (20 mL). The combined organic phases were washed with sat. aq Na_2SO_3 (20 mL), sat. aq

$NaHCO_3$ (2 × 25 mL), and brine (2 × 20 mL), dried (Na_2SO_4), and concentrated. Purification by chromatography on a silica gel column (eluent: EtOAc–CH_2Cl_2–PE, 1:4:60) resulted in pure **7** as a white solid; yield: 581 mg (83%); mp 158–160 °C (EtOH); $[\alpha]_D^{25}$ +13.1 (*c*, 1 g/100 mL CH_2Cl_2).

IR (KBr): 1749 and 1700 (C=O), 1127 and 1121 cm^{-1} (C–O).

^1H NMR (400 MHz, $CDCl_3$/TMS): δ = 0.66 (3 H, s, 18-H), 0.91 (3 H, s, 19-H), 0.93 (3 H, d, *J* = 1.5 Hz, 21-H), 2.08 (3 H, s), 3.20 (1 H, d, *J* = 4.0 Hz, 6-H), 3.22 (1 H, d, *J* = 3.6 Hz, 7-H), 3.67 (3 H, s), 5.17 (1 H, m, 3α-H).

^{13}C NMR (100 MHz, $CDCl_3$/TMS): δ = 11.92, 18.31, 20.30, 20.75, 21.52, 22.15, 24.15, 28.08, 29.10, 30.98, 31.06, 34.15, 34.23, 35.34, 39.76, 42.04, 42.81, 47.11, 51.10, 51.56, 52.78, 55.94, 55.97, 70.47, 77.25, 170.49, 174.73.

HRMS (ESI): *m*/*z* $[M]^+$ calcd for $C_{27}H_{42}O_5Na$: 469.2924; found: 469.2929.

Methyl 3α-Acetoxy-7α-hydroxycholan-24-oate (8)

Path I: To a pressure bottle containing anhydrous CH_2Cl_2 (15 mL) was added a solution of **7** (2.1 g, 4.71 mmol) in freshly distilled CH_2Cl_2 (60 mL) and anhydrous pyridine (1 mL, 12.44 mmol) in the presence of Pd/C (200 mg, 10 mol%) at r.t. The reaction mixture was heated to 40 °C for 24 h under 50 psi of H_2. H_2O (30 mL) was then added and the catalyst was removed by filtration through a Celite pad. The filtrate was concentrated and the residue was dissolved in EtOAc (30 mL). The organic layer was washed with aq 1 M HCl (15 × 2 mL), sat. $NaHCO_3$ (50 × 3 mL) and brine (25 × 2 mL), and dried (Na_2SO_4). The mixture was concentrated to yield an 8:1 mixture of hydroxy epimers (α/β) as shown by the integration of methine signals connected to hydroxy epimer in the ^1H NMR spectrum. Chromatography of the residue on silica gel column (eluent: EtOAc–CH_2Cl_2–hexane, 1:4:45) afforded pure **8** as a white solid; yield: 1.31 g (62%).

Path II: Compound **11** (0.12 g, 0.26 mmol) was dissolved in MeOH (10 mL) at –20 °C and stirred for 30 min. To the mixture was added concd HCl (5 mol/L, 2.0 mL) dropwise and activated Zn powder (0.12 g, 1.82 mmol) in five portions at intervals of 1 min until the solution began to turn yellow. The reaction was terminated by the addition of sat. aq $NaHCO_3$ (15 mL), filtered, and evaporated under reduced pressure. The residue was dissolved in EtOAc (15 mL) and washed with sat. aq $NaHCO_3$ (25 × 3 mL) and brine (20 × 2 mL), and dried (Na_2SO_4). The residue obtained after evaporation of the solvent was chromatographed on a silica gel column (eluent: EtOAc–CH_2Cl_2–PE, 1:1:25) to afford **8** as a white solid; yield: 86.1 mg (74%); mp 173–176 °C (EtOH); $[\alpha]_D^{25}$ +27 (*c* 1 g/100 mL CH_2Cl_2).

IR (KBr): 3330 (O–H), 1743 and 1720 (C=O), 1123 cm^{-1} (C–O).

^1H NMR (400 MHz, $CDCl_3$/TMS): δ = 0.68 (3 H, s, 18-H), 0.95 (3 H, d, *J* = 3.1 Hz, 21-H), 0.97 (3 H, s, 19-H), 2.09 (3 H, s), 3.54 (1 H, m, 7α-H), 3.70 (3 H, s), 4.91 (1 H, q, *J* = 3.1 Hz, 3α-H).

^{13}C NMR (100 MHz, $CDCl_3$/TMS): δ = 11.72, 18.30, 20.67, 21.65, 22.75, 23.58, 28.05, 29.72, 30.98, 31.02, 31.46, 34.14, 34.77, 35.22, 35.31, 37.96, 38.94, 39.54, 41.14, 42.72, 50.43, 51.52, 55.73, 71.40, 71.83, 170.67, 174.74.

HRMS (ESI): *m*/*z* [M + Na]+ calcd for $C_{27}H_{44}O_5Na$: 471.3081; found: 471.3083.

Methyl 3α-Acetoxy-7-oxocholan-24-oate (9)

To a stirred solution of **8** (460 mg, 1.03 mmol) in a mixture of acetone (30 mL) and AcOH (0.07 mL, 1.24 mmol) was added dropwise a solution of NCS (205 mg, 1.54 mmol) in acetone (10 mL) at 0 °C over 10 min and the reaction mixture was then allowed to rise to r.t. during 40 min with stirring before quenching with sat. aq Na_2SO_3 (15 mL). The course of the reaction was monitored by TLC. The mixture was evaporated under reduced pressure and extracted with EtOAc (20 mL). The combined organic layers were washed with sat. aq $NaHCO_3$ (25 × 2 mL) and brine (25 mL), dried (Na_2SO_4), and concentrated to give **9** as a white solid, which was pure enough to be used directly in the next step; yield: 440 mg (96%); mp 144–147 °C (EtOH); $[\alpha]_D^{25}$ –1 (*c* 1 g/100 mL CH_2Cl_2).

IR (KBr): 3330 (O–H), 1743 and 1720 (C=O), 1123 cm^{-1} (C–O).

^1H NMR (400 MHz, $CDCl_3$/TMS): δ = 0.72 (3 H, s, 18-H), 0.97 (3 H, d, *J* = 6.4 Hz, 21-H), 1.07 (3 H, s, 19-H), 2.08 (3 H, s), 3.03 (1 H, t, *J* = 14.4 Hz), 3.70 (3 H, s), 5.00 (1 H, q, *J* = 3.1 Hz, 3α-H).

^{13}C NMR (100 MHz, $CDCl_3$/TMS): δ = 11.75, 18.32, 21.06, 21.49, 21.89, 23.57, 28.01, 30.95, 31.00, 31.05, 34.93, 35.01, 35.29, 36.58, 36.91, 37.93, 39.41, 42.60, 42.77, 44.76, 50.34, 51.53, 55.74, 71.14, 170.26, 174.67, 212.50.

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{27}H_{42}O_5Na$: 469.2924; found: 469.2917.

Ursodeoxycholic Acid (UDCA, 1b)

Na (86.85 mg, 3.78 mmol), cut into pieces was added in portions to a stirred solution of **9** (281 mg, 0.63 mmol) in freshly distilled *n*-BuOH (15 mL) at r.t. within 15 min. After refluxing the reaction mixture at 60 °C for 2 h under N_2 atmosphere, the mixture was quenched with sat. aq NH_4Cl (15 mL) and evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (12 mL) and the organic layer was washed with sat. aq $NaHCO_3$ (15 mL) and brine (15 × 2 mL), dried (Na_2SO_4), and concentrated to obtain an orange oil. The oil was purified by chromatography on a silica gel column (eluent: MeOH–CH_2Cl_2–PE, 11:4) to furnish **1b** as a white solid; yield: 153 mg (62%); mp 195–197 °C (EtOH); $[\alpha]_D^{25}$ +54 (*c* 1 g/100 mL EtOH).

IR (KBr): 3330 cm^{-1} (O–H).

^1H NMR (400 MHz, DMSO-d_6/TMS): δ = 0.62 (3 H, s, 18-H), 0.90 (6 H, m, 19,21-H), 3.29 (1 H, m, 7β-H), 3.90 (1 H, d, *J* = 8.0 Hz), 4.47 (1 H, d, *J* = 4.5 Hz), 11.97 (1 H, s).

^{13}C NMR (100 MHz, DMSO-d_6/TMS): δ = 12.50, 14.55, 18.77, 19.02, 21.32, 23.78, 27.18, 28.64, 30.72, 31.24, 34.23, 35.31, 37.74, 38.19, 39.19, 42.65, 43.48, 43.56, 55.15, 56.31, 56.50, 69.92, 70.19, 175.35.

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{24}H_{40}O_4Na$: 415.2819; found: 415.2811.

Methyl 3α-Acetoxy-6-trimethylsilyloxychol-6-en-24-oate (10)

A Schlenk tube containing a suspension of LiH (0.27 g, 33.61 mmol) in freshly distilled THF (10 mL) was stirred at 30 °C for 5 min under N_2 atmosphere. To this was added dropwise a solution of TMSCl (5.25 mL, 42.01 mmol) in freshly distilled THF (10 mL). After stirring the mixture for 5 min, a solution of **4** (1.5 g, 3.36 mmol) in freshly distilled THF (15 mL) was added dropwise and the mixture was stirred for 30 min until the solution turned yellow. The mixture was heated to 60 °C and treated dropwise with Et_3N (10.51 mL, 33.61 mmol). After 6 h, sat. aq NH_4Cl (20 mL) was poured into the mixture to quench the reaction, followed by evaporation under reduced pressure. The mixture was extracted with EtOAc (25 mL). The combined organic phases were washed with sat. aq $NaHCO_3$ (2 × 30 mL) and brine (20 mL), dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography (eluent: EtOAc–PE, 1:50) to afford **10** as a colorless oil; yield: 1.55 g (89%); mp 167–170 °C (EtOH); $[\alpha]_D^{25}$ +12.5 (*c* 1 g/100 mL CH_2Cl_2).

IR (KBr): 1743 and 1720 (C=O), 1123 cm^{-1} (C–O).

^1H NMR (400 MHz, $CDCl_3$/TMS): δ = 0.24 (9 H, s), 0.67 (3 H, s, 18-H), 0.94 (3 H, d, *J* = 6.5 Hz, 21-H), 1.01 (3 H, s, 19-H), 2.06 (3 H, s), 3.69 (3 H, s), 4.87 (1 H, s, 7-H), 5.10 (1 H, m, 3α-H).

^{13}C NMR (100 MHz, $CDCl_3$/TMS): δ = 0.33, 0.47, 0.50, 11.94, 18.24, 21.33, 22.21, 24.14, 26.63, 28.07, 30.95, 32.61, 34.29, 34.59, 35.28, 40.04, 42.80, 42.83, 42.89, 46.07, 51.38, 55.84, 55.88, 55.92, 56.03, 71.94, 102.64, 151.00, 170.39, 174.56.

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{30}H_{50}O_5SiNa$: 541.3320; found: 541.3323.

Methyl 3α-Acetoxy-7α-hydroxyl-6-oxocholan-24-oate (11)

To a stirred, chilled (0 °C) solution of **10** (0.76 g, 1.47 mmol) in anhydrous CH_2Cl_2 (30 mL) was added a solution of *m*-CPBA (0.38 g, 2.20 mmol) in anhydrous CH_2Cl_2 (20 mL) dropwise and then the reaction was allowed to warm to r.t. over 4 h. Sat. aq Na_2SO_3 (20 mL) was then introduced to quench the reaction. The organic layer was separated, washed with sat. aq $NaHCO_3$ (2 × 50 mL), brine (25 mL), and dried (Na_2SO_4). The solvent was evaporated under vacuum and the residue was chromatographed on a silica gel column (eluent: EtOAc–PE, 1:12) to afford pure **11** as a white solid; yield: 0.62 g (91%); mp 151–153 °C (EtOH); $[\alpha]_D^{25}$ +51.3 (*c* 1 g/100 mL CH_2Cl_2).

IR (KBr): 1743, 1720, and 1690 (C=O), 1123 cm^{-1} (C–O).

^1H NMR (400 MHz, $CDCl_3$/TMS): δ = 0.71 (3 H, s, 18-H), 0.94 (3 H, d, *J* = 6.4 Hz, 21-H), 1.12 (3 H, s, 19-H), 2.04 (3 H, s), 3.70 (3 H, s), 4.49 (1 H, d, *J* = 9.3 Hz, 7-H), 5.23 (1 H, m, 3α-H).

^{13}C NMR (100 MHz, $CDCl_3$/TMS): δ = 12.06, 14.22, 18.28, 21.34, 21.64, 22.98, 24.04, 28.08, 30.94, 31.04, 31.97, 35.05, 35.31, 38.65, 39.67, 42.60, 42.92, 51.52, 54.96, 55.91, 56.05, 60.40, 71.10, 73.42, 170.53, 174.66, 209.77.

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{27}H_{42}O_6Na$: 485.2874; found: 485.2871.

Acknowledgment

The research was supported by Shanghai Key Laboratory of Chemical Assessment and Sustainability. We would like to thank Dr. Jie Zhao for his help in the preparation of this manuscript.

Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0035-1560388.

References

(1) Kritchevsky, D. *The Bile Acids - Chemistry, Physiology and Metabolism*; Plenum Press: New York, **1971**.
(2) Salen, G.; Colalillo, A.; Verga, D.; Bagan, E.; Tint, G. S.; Shefer, S. *Gastroenterology* **1980**, *78*, 1412.
(3) Crosignani, A.; Setchell, K. D.; Invernizzi, P.; Larghi, A.; Rodrigues, C. M.; Podda, M. *Clin. Pharmacokinet.* **1996**, *30*, 333.
(4) Ren, J.; Wang, Y. C.; Wang, J. L.; Lin, J.; Wei, K.; Huang, R. *Steroids* **2013**, *78*, 53.
(5) Dosa, P. I.; Ward, T.; Castro, R. E.; Rodrigues, C. M.; Steer, C. J. *ChemMedChem* **2013**, *8*, 1002.
(6) Zheng, M. M.; Wang, R. F.; Li, C. X.; Xu, J. H. *Proc. Biochem.* **2015**, *50*, 598.
(7) Eggert, T.; Bakonyi, D.; Hummel, W. *J. Biotechnol.* **2014**, *191*, 11.
(8) Zhou, W. S.; Wang, Z. Q.; Jiang, B. *J. Chem. Soc., Perkin Trans. 1* **1990**, 1.
(9) Pedrini, P.; Andreotti, E.; Guerrini, A.; Dean, M.; Fantin, G.; Giovannini, P. P. *Steroids* **2006**, *71*, 189.
(10) Giovannini, P. P.; Grandini, A.; Perrone, D.; Pedrini, P.; Fantin, G.; Fogagnolo, M. *Steroids* **2008**, *73*, 1385.
(11) Medici, A.; Pedrini, P.; Bianchini, E.; Fantin, G.; Guerrini, A.; Natalini, B.; Pellicciari, R. *Steroids* **2002**, *67*, 51.
(12) Hohenberg, P.; Kohn, W. *Phys. Rev.* **1964**, *136*, 864.
(13) Levy, M. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 6062.
(14) Sousa, A. M.; Coutinho, W. S.; Lima, A. F.; Lalic, M. V. *J. Chem. Phys.* **2015**, *142*, 74703.
(15) Zhou, Q. H.; Li, Y. X. *J. Am. Chem. Soc.* **2015**, *137*, 10182.
(16) Visitsatthawong, S.; Chenprakhon, P.; Chaiyen, P.; Surawatanawong, P. *J. Am. Chem. Soc.* **2015**, *137*, 9363.
(17) Samrat, M.; Uday, M. *Org. Lett.* **2004**, *6*, 31.
(18) Karamanis, P.; Pouchan, C. *J. Phys. Chem. C* **2012**, *116*, 11808.
(19) Wang, Y.; Cheng, L. T. *J. Phys. Chem.* **1992**, *96*, 1530.
(20) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A. *Gaussian 09, Revision A.1*; Gaussian, Inc: Pittsburgh, **2009**.
(21) Wang, Q.; Zhao, J.; Wang, X. F. *J. Phys. Chem. A* **2015**, *119*, 2244.
(22) Dai, Y. F.; Li, Z. Y.; Yang, J. L. *J. Phys. Chem. C* **2014**, *118*, 3313.
(23) Nicolaou, K. C.; Yang, Z.; Liu, J. J.; Ueno, H.; Nantermet, P. G.; Guy, R. K.; Claiborne, C. F.; Renaud, J.; Couladouros, E. A.; Paulvannan, K.; Sorensen, E. J. *Nature* **1994**, *367*, 630.
(24) Guillemette, A.; Francois, A. German Patent DE2950481, **1980**.
(25) Giordano, C.; Perdoncin, G.; Castaldi, G. *Angew. Chem. Int. Ed.* **1985**, *24*, 499.
(26) Castaldi, G.; Perdoncin, G.; Giordano, C.; Minisci, F. *Tetrahedron Lett.* **1983**, *24*, 2487.