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An improved synthesis of 6α-ethylchenodeoxycholic acid (6ECDCA), a potent and selective agonist for the Farnesoid X Receptor (FXR)

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ABSTRACT

The active, potent, and selective Farnesoid X Receptor (FXR) agonist 6α-ethylchenodeoxycholic acid (6ECDCA) has been synthesized in improved yield compared to the published methodologies. The synthesis employed selective oxidation of one of the two hydroxyls of the readily-available starting material chenodeoxycholic acid (CDCA) as a key step. After protection of the remaining hydroxyl, LDA/HMPA/EtI/PPTS provided an efficient deprotonation/ethylation/deprotection sequence. The two synthetic improvements that allow a productive yield are the use of PCC in the oxidation step, and the use of HMPA/ethyl iodide in the stereoselective alkylation step. This synthesis offers an economical and efficient strategy which provides a simple and cost-effective procedure for potential large-scale production of this promising FXR agonist, which is a research tool and potential drug substance of current interest.

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1. Introduction

The American Heart Association reports that approximately 500,000 Americans die each year from heart disease [1]. Since elevated blood cholesterol is a major risk factor in developing atherosclerotic heart disease, there is considerable interest in compounds that can regulate cholesterol homeostasis. Recent work from several labs has shown that the Farnesoid X Receptor (FXR or NRIH4) is a nuclear hormone receptor that regulates gene expression in response to bile acids. Forman et al. [2] originally proposed that FXR was a receptor for the intermediary metabolite farnesol. However, farnesol does not bind to FXR, and more recent work by the Forman lab and two other groups has demonstrated that bile acids are the physiological ligands, endogenous signals and activators of FXR [3–5].

Activation of FXR by bile acids increases high-density lipoprotein (HDL) cholesterol and lowers plasma triglycerides. Interestingly, recent reports indicated that FXR is an emerging therapeutic target for treating atheorsclerosis [6]. Bile acids are negative regulators of human apoA-I expression via activation of the FXR [7]. As these effects are anti-atherogenic, the development of FXR agonists could be useful chemical tools for studying the role of FXR in anti-atherogenic disease.

FXR is highly expressed in the liver and gut. In the liver, FXR regulates bile acid, cholesterol, triglyceride metabolism and importantly, glucose homeostasis. FXR is thought to regulate the expression of cholesterol 7α-hydroxylase, the rate-limiting enzyme in bile acid synthesis, as well as the expression of genes encoding I-BABP, BSEP, and cMOAT [8]. FXR shares a common modular architecture with the other members of the nuclear receptor superfamily, comprising a highly-conserved DNA-binding domain and a carboxy-terminal ligand-binding domain (LBD). When agonists bind to the LBD of FXR, a conformation change occurs in its LBD, which leads to transcriptional activation. The importance of FXR for normal cholesterol homeostasis has been demonstrated in the FXR-null mouse model [9]. Compared with wild-type, FXR-null mice show increased HDL and non-HDL cholesterol and triglycerides, and reduced plasma HDL cholesterol ester clearance.

FXR agonists offer a rational approach to the potential treatment of atherosclerotic disease. Chenodeoxycholic acid (CDCA, Fig. 1) is a primary bile acid and among the most potent natural ligands of FXR, with EC_{50} values around 10–50 μM [12]. Other bile acids, such as lithocholic acid (LCA) and deoxycholic acid (DCA) also activate FXR, while ursodeoxycholic acid (UDCA) binds FXR but does not activate transcription. A potent synthetic non-steroidal FXR agonist, GW4064, has been identified recently through the use of high-throughput screening and combinatorial chemistry [10]. GW4064 activates FXR, increases HDL-cholesterol, and reduces plasma triglycerides in vivo. Unfortunately, GW4064 is not useful therapeutically because of its poor bioavailability.

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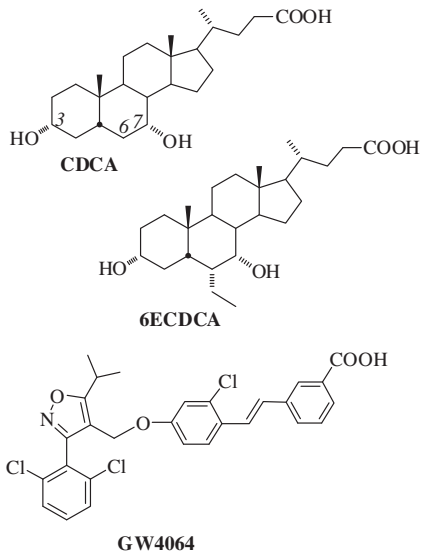

Fig. 1. Natural and synthetic ligands of FXR.

Using traditional medicinal chemistry methods, the synthetic steroid 6α-ethylchenodeoxycholic acid (6ECDCA) has been found to be a more potent FXR agonist than CDCA, with EC_{50} values around 100 nM. This suggests the existence of a hydrophobic pocket in the FXR LBD corresponding to the 6α position of the bile acid [11]. A bile acid–based FXR ligand like 6ECDCA may be an appropriate starting point for medicinal chemical modulation. 6ECDCA could thus be an ideal chemical tool to study the function of FXR.

Since the reported synthesis of 6ECDCA starts with the expensive 7-keto-lithocholic acid (**1**) and gives only a 2–3% overall yield [12], there is no doubt that a more efficient and less expensive synthetic methodology for the production of 6ECDCA is needed. Herein we report an improved, cost-effective, and efficient highly stereoselective synthesis of 6ECDCA that may facilitate studies of the function of FXR.

The published method [12] for preparation of 6ECDCA begins with protection of the hydroxyl of **1**. The protected product's ketone is α-alkylated with LDA/ethyl bromide, followed by Fischer esterification of the acid group. Selective reduction of the resulting methyl ester's keto group with sodium borohydride and subsequent saponification with NaOH in methanol gives 6ECDCA in about 2–3% overall yield.

Our procedure began with the less expensive, readily available starting material CDCA. We performed regioselective oxidation of the 7α-hydroxyl of CDCA with pyridinium chlorochromate (PCC) in $CHCl_3/CH_2Cl_2$ at room temperature to obtain the desired 7-keto **1** in 82% yield (Fig. 2). The survival of the 3α-hydroxyl may seem surprising at first. However, it is known that axial hydroxyl groups are oxidized by chromic acid oxidants about three times faster than equatorial hydroxyls [13]. The MM2-minimized structure of CDCA in Fig. 3 shows the axial 7α-hydroxyl and the equatorial 3α-hydroxyl.

The slow step of the mechanism is removal of the carbinol hydrogen (after fast formation of the chromate ester) as shown in Fig. 4. During the removal of the chromate ester's carbinol hydrogen, 1,3-diaxial strain is relieved for an axial chromate ester, but not for an equatorial ester. If one of the 1,3-diaxial bonds connects to an alkyl group, the additional strain relief gives a more than 200-fold advantage to oxidation of an axial hydroxyl [13,14]. In the case of CDCA, not only does carbon 4 provide this additional strain, but the axial hydrogen from carbon 14 also contributes (Fig. 4).

Although initial oxidation favored the formation of the desired 7-keto-lithocholic acid **1**, after 15–30 min some oxidation of the 3-hydroxyl group could also be observed, resulting in the formation of trace amounts of the 3,7-diketo product.

Partial oxidation of CDCA had been accomplished by Piatkowski et al. [15], but the method was complicated and restricted. Fieser et al. [16] reported the partial oxidation of CDCA in 60% yield using a tedious potassium chromate procedure; a similar report was made by Hsia et al. [17]. The current method is simpler to perform and gives higher yields.

Following a methodology similar to that previously reported [12], the 3-hydroxyl of **1** was protected by treatment with 3,4-dihydro-2H-pyrane and a catalytic amount of *p*-toluenesulfonic acid in $CHCl_3/Cl_2CH_2$ to give the corresponding THP ether **2**. α-Alkylation of **2** using LDA/EtBr gave an unsatisfactory yield in our hands, but the alternative of LDA/EtI worked well. Neutralization of the carboxyl hydrogen of **2** with BuLi, enolate formation with LDA/HMPA, alkylation with iodoethane, and removal of the THP group with pyridinium *p*-toluenesulfonate (PPTS) [18] led to the ethylated intermediate **3**. Protection of the carboxyl prior to the next step was not necessary. Stereoselective reduction of **3** with sodium borohydride from the less-hindered face gave desired product 6ECDCA in 20% yield over 4 steps. Purification by flash column chromatography ($CHCl_3$:CH_3OH 9:1) gave 6ECDCA as a white solid, mp 120.7 °C.

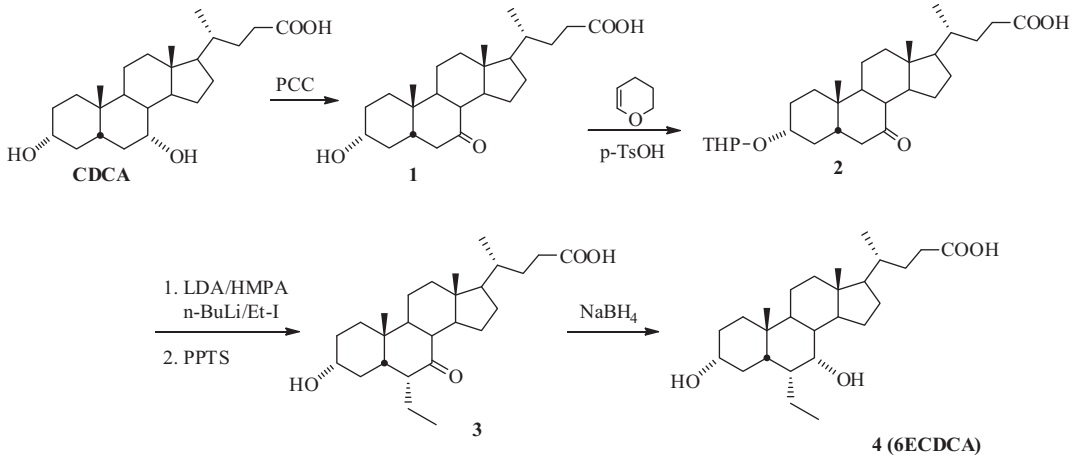

Fig. 2. An improved synthesis of 6ECDCA.

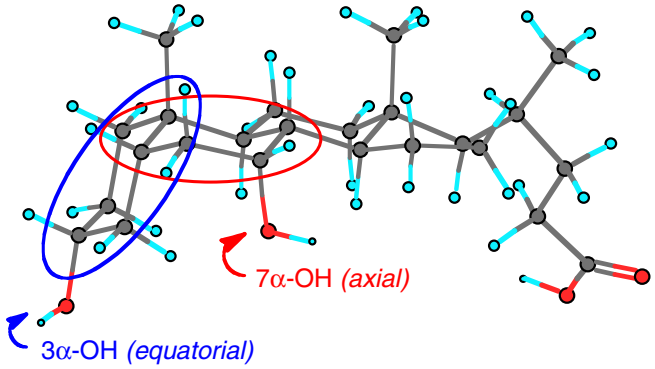

Fig. 3. MM2 structure of CDCA (Chem3D). Ovals indicate pertinent cyclohexane chairs with the equatorial and axial hydroxyls.

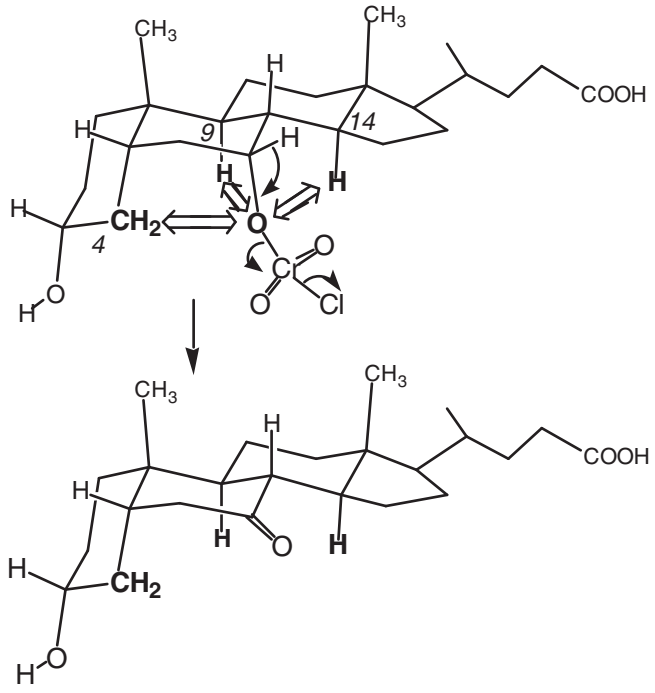

Fig. 4. Oxidation of CDCA with PCC.

The identity of 6ECDCA we synthesized in improved way was secured by comparison of its ^{1}H NMR and ^{13}C NMR with those previously reported. In a reporter assay we demonstrated that the so obtained 6ECDCA is a very potent FXR agonist with EC_{50} values around 100 nM. This result is consistent with previous observation [12].

In summary, we have modified several critical steps in the synthesis of 6ECDCA, resulting in an economical and efficient strategy. The two key synthetic improvements that allow a productive yield are the use of PCC in the oxidation step and the use of HMPA/ethyl iodide in the stereo-selective alkylation step that provides a simple and cost-effective procedure for potential large-scale production of this promising FXR agonist, which is a research tool and potential drug substance of current interest.

2. Experimental section

General procedures: Organic reagents were purchased from commercial suppliers unless otherwise noted and were used without further purification. All solvents were analytical or reagent grade. All reactions were carried out in flame-dried glassware under argon or nitrogen. Melting points were determined and reported automatically by an optoelectronic sensor in open capillary tubes and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were measured at 500 and 125 MHz, respectively, and using $CDCl_3$ or CD_3OD as the solvents and tetramethylsilane (Me_4Si) as the internal standard. Flash column chromatography was performed using Sigma–Aldrich silica gel 60 (200–400 mesh), carried out under moderate pressure by using columns of an appropriate size packed and eluted with appropriate eluents. Silica gel chromatography was performed on a Biotage flash column gradient pump system using 15 cm long columns. All reactions were monitored by TLC on precoated plates (silica gel HLF). TLC spots were visualized either by exposure to iodine vapors or by irradiation with UV light.

2.1. 3α-Hydroxy-7-keto-5β-cholan-24-oic acid (1)

To a suspension of chenodeoxycholic acid (**CDCA**, Sigma–Aldrich, 1.0 g, 2.5 mmol) and silica gel (4 g, 200–400 mesh, Aldrich) in anhydrous $CHCl_3$ (2 mL) was added, portionwise, pyridinium chlorochromate (PCC, 0.61 g, 2.8 mmol) in 25 mL of CH_2Cl_2 and the reaction mixture was stirred at room temperature for 15 min. The mixture was filtered and the filtrate was washed with water (20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4 and concentrated. The resulting crude oil was purified by flash column chromatography (CH_2Cl_2: MeOH 95:5) to afford **1** as a solid (0.79 g, 82% yield), mp 201.1–201.7 °C (lit [11]. mp 201–203 °C). ^{1}H NMR (500 MHz, CD_3OD) δ 3.50 (m, 1H), 2.94 (m, 1H), 2.52 (t, 1H), 2.30 (m, 2H), 2.19 (m, 6H), 1.70 (m, 2H), 1.43 (m, 4H), 1.31 (m, 6H), 1.19 (s, 3H), 1.12 (m, 4H), 0.92 (d, 3H), 0.67 (s, 3H). ^{13}C NMR (125 MHz,CD_3OD) δ 213.7, 176.8, 70.1, 54.8, 49.2, 48.9, 47.7, 46.0, 44.9, 43.0, 42.4, 38.9, 36.8, 35.1, 34.9, 33.7, 31.0, 30.6, 29.2, 27.8, 24.3, 22.0, 21.4, 17.3, 10.5. Anal. Calcd for $C_{24}H_{38}O_4$: C, 73.81; H, 9.81. Found: C, 73.50; H, 9.63.

2.2. 3α-Tetrahydropyranyloxy-7-keto-5β-cholan-24-oic acid (2)

To a solution of **1** (0.50 g, 1.3 mmol) in 16 mL of $CHCl_3$:Cl_2CH_2:-Et_2O (1:1:2) were added *p*-toluensulfonic acid (0.06 g, 0.3 mmol) and 3,4-dihydro-2H-pyrane (0.41 g, 4.9 mmol). The reaction mixture was stirred at room temperature for 60 min. Water (10 mL) was added and the mixture was extracted with EtOAc (3 × 30 ml); the combined organic layers were washed with saturated $NaHCO_3$ and brine and concentrated. The resulting crude oil was purified by flash column chromatography (CH_2Cl_2:Et_2O 1:2) to afford **2** as a white solid (0.51 g, 82% yield), mp 160.0–160.8 °C (lit [9]. mp 157–159 °C). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.73 (d, 1H), 3.86 (m, 1H), 3.59 (m, 1H), 3.46 (m, 1H), 2.82 (m, 1H), 1.17 (s, 3H), 0.92 (d, 3H), 0.63 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 212.3, 179.8, 96.4, 62.8, 62.1, 19.8, 18.1, 11.4. Anal. Calcd for $C_{29}H_{46}O_5$: C, 73.38; H, 9.77. Found: C, 73.30; H, 9.76.

2.3. 3α-Hydroxy-6α-ethyl-7-keto-5β-cholan-24-oic acid (3)

To a solution of **2** (0.30 g, 0.63 mmol) in dry THF (20 mL) at −78 °C were added dropwise *n*-butyllithium (1.0 mL, 1.6 M solution in hexane, 1.6 mmol), HMPA (0.7 g, 4 mmoL) and LDA (2.0 mL, 1.8 M in THF/heptane/ethylbenzene, 3.6 mmol). The reaction mixture was stirred for 30 min. Iodoethane (2.0 g, 13 mmol) was slowly added and the reaction mixture was allowed to warm overnight to room temperature. After rotary evaporation, water and ether were added and the aqueous layer was acidified with 10% HCl and extracted with EtOAc (5 × 20 mL). The organic layers were washed with brine, dried over Na_2SO_4, and concentrated to give a yellow oil. After a short column (CH_2Cl_2:Et_2O 1:2), the crude semi-solid was dissolved in ethanol (5 mL) and pyridinium *p*-toluenesulfonate (15 mg, 0.06 mmol) was added. The reaction

mixture was stirred at 55 °C for 3 h. After rotary evaporation, the product was passed through a short column (CH_2Cl_2:Et_2O 1:2) to obtain **3** as a semi-solid (0.08 g, 37%) that was used in the next step without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.57 (br s, 1H), 2.83 (m, 1H), 2.31 (m, 1H), 2.17 (m, 1H), 1.13 (s, 3H), 0.97 (d, 3H), 0.85 (m, 5H), 0.64 (s, 3H).

2.4. 3α,7α-Dihydroxy-6α-ethyl-5β-cholan-24-oic acid (4, 6ECDCA)

To a solution of **3** (0.05 g, 0.12 mmol) in dry MeOH (5 mL) was added $NaBH_4$ (0.03 g, 0. 84 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 3 h. Water (10 mL) was slowly added and the reaction mixture and was partially concentrated by rotary evaporation and extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated to give a solid. Purification by flash column chromatography ($CHCl_3$:CH_3OH 9:1) gave 0.04 g of **6ECDCA** (82% yield), mp 120.7–121.0 °C. ^{1}H NMR (500 MHz, CD_3OD) 3.66 (br s, 1H), 3.31 (m, 1H), 2.33 (m, 1H), 2.20 (m, 1H), 0.97 (d, 3H), 0.89 (m, 8H), 0.69 (s, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 177.0, 71.8, 69.7, 55.9, 50.2, 45.5, 42.3, 41.7, 40.1, 39.6, 35.3, 35.2, 33.1, 33.0, 31.0, 29.8, 27.8, 23.1, 22.3, 22.1, 20.5, 17.3, 11.0, 10.6. Anal. Calcd for $C_{26}H_{44}O_4 \cdot \frac{1}{4}$ H_2O: C, 73.44; H, 10.43. Found: C, 73.24; H, 10.66.

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