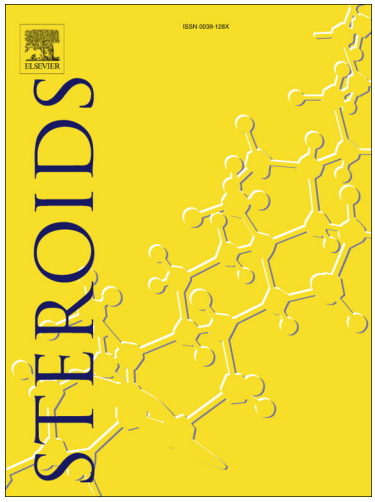

Chemical synthesis of 7α-hydroxycholest-4-en-3-one, a biomarker for irritable bowel syndrome and bile acid malabsorption

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Chemical Synthesis of 7α-hydroxycholest-4-en-3-one, a biomarker for irritable bowel syndrome and bile acid malabsorption

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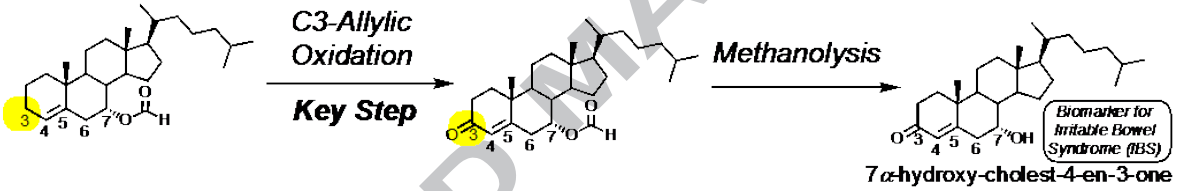
Abstract: 7α-Hydroxy-cholest-4-en-3-one is a biomarker for bile acid loss, irritable bowel syndrome, and other diseases associated with defective bile acid biosynthesis. Furthermore, 7α-hydroxy-cholest-4-en-3-one is the physiological substrate for cytochrome P450 8B1 (P450 8B1 or CYP8B1), the oxysterol 12α-hydroxylase enzyme implicated in obesity and cardiovascular health. We report the chemical synthesis of this physiologically important oxysterol beginning with cholesterol. The key feature of this synthesis involves a regioselective C3-allylic oxidation of a 3-desoxy-Δ^4-7α-formate steroid precursor to form 7α-formyloxy-cholest-4-en-3-one, which was saponified to yield 7α-hydroxy-cholest-4-en-3-one.

Abstract Figure:



Keywords: regioselective oxidation; cytochrome P450; steroids; irritable bowel syndrome; oxysterols

Introduction

7α-Hydroxycholest-4-en-3-one (**1**) is the oxysterol metabolite that has been used as a biomarker for bile acid malabsorption [1, 2], bile acid diarrhea [3], irritable bowel syndrome [4], and cerebrotendinous xanthomatosis [5] in clinical studies. In addition, cytochrome P450 8B1 (P450 8B1) is the liver enzyme that converts 7α-hydroxy-cholest-4-en-3-one (**1**) to 7α-,12α-dihydroxy-cholest-4-en-3-one (**2**) [6, 7] (Figure 1, **1** to **2**). This activity ultimately results in the formation of cholic acid, the bile acid that has enhanced cholesterol absorption properties [8] (Figure 1, **2** to **3**). Furthermore, 7α-hydroxycholest-4-en-3-one (**1**) has been shown to be increased in the blood plasma in mice that lack the gene that expresses cytochrome P450 27A1 (P450 27A1) [9]. A reasonable explanation for this observation is that 7α-hydroxycholest-4-en-3-one (**1**) is found relatively upstream in the biosynthetic pathway to bile acids (e.g. chenodeoxycholic acid). The first step is the incorporation of the 7α-hydroxy group onto cholesterol by cytochrome P450 7A1, and the second step is the oxidation and isomerization of the 3-hydroxy group and the $\Delta^{5,6}$-double bond by 3β-hydroxy steroid dehydrogenase to yield 7α-hydroxycholest-4-en-3-one (**1**) [10]. The deletion of the gene that expresses P450 27A1, which is found downstream in the bile acid pathway, results in the accumulation of the precursor, 7α-hydroxycholest-4-en-3-one (**1**). Moreover, 7α-hydroxycholest-4-en-3-one (**1**) has been shown to be a pregnane X receptor (PXR) agonist [9, 11].

The important roles of 7α-hydroxy-cholest-4-en-3-one (**1**) in diseases associated with bile acid biosynthesis and as a potential oxysterol receptor ligand led to our interest in establishing a synthetic route to 7α-hydroxy-cholest-4-en-3-one (Figure 1, **1**). Although 7α-hydroxy-cholest-4-en-3-one (**1**) is commercially available, it is expensive. A convenient synthesis will allow us to potentially synthesize other steroid analogs with the similar 7-oxygenated 3-keto Δ^4 steroid backbone and further test their biological activity.

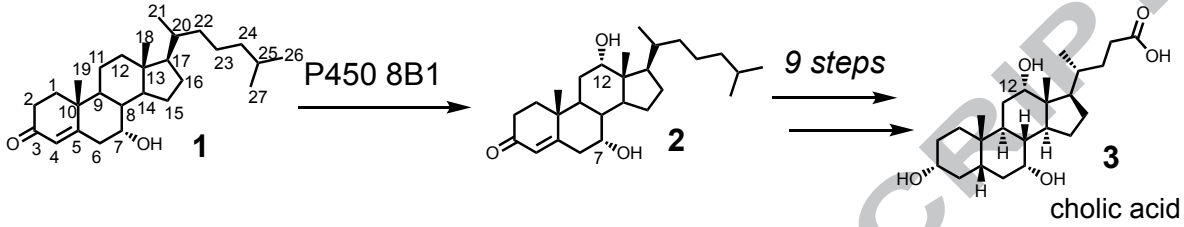


Figure 1. The biochemical reaction catalyzed by P450 8B1 (12α-hydroxylation of **1** to form **2**). Subsequent nine steps result in the formation of the bile acid, cholic acid [10].

The original chemical synthesis of 7α-hydroxy-cholest-4-en-3-one (**1**) was reported in 1961, which involved the MnO_2-mediated oxidation of an allylic alcohol precursor to afford the 3-keto Δ^4 steroid backbone [12]. However, this original report using MnO_2 to introduce the 3-keto-Δ^4 steroid gave only ~4-5% yield in its key step. Another procedure, reported in 1977, for the synthesis of 7α-hydroxy-cholest-4-en-3-one involved the use of cholesta-1,4,6-trien-3-one as the starting material, which was epoxidized at the 6,7-position [13]. The epoxide was opened with HBr to yield a bromohydrin across the 6,7-position, which was reduced with Zn in EtOH. Furthermore, these previously published reports lacked any NMR spectral characterization of the synthesized compounds [12, 13]. Subsequently, an enzymatic oxidation of 7α-hydroxycholesterol (**5**) using cholesterol oxidase as the enzyme (Figure 2A, **5** to **1**) [14] was reported in 1995. In addition, a second chemical synthesis of 7α-hydroxy-cholest-4-en-3-one (**1**) was achieved from a desaturation reaction of a saturated 3-keto steroid (**7**) promoted by hypervalent iodine [15] (Figure 2B, **7** to **8**). However, the starting material used in the previously reported synthesis [15] was the saturated bile acid, chenodeoxycholic acid (**6**), which contains an extended side chain at the C17-position. Moreover, it has been suggested that oxidation of a steroid with the 4,6-dien-3-one backbone could be oxidized to the 6α,7α-epoxide with mCPBA and reacted with $Pd_2(dba)_3$, PPh_3, HCOOH, and Et_3N to yield the 7α-hydroxy 3-keto-Δ^4 steroid product at 80 °C. However, during the final reduction conditions with palladium, the double bond is reduced in the 5β-orientation [16].When an alternative set of these reduction conditions with palladium was carried out at room temperature, the alkene at the C4-position remained intact [17].

We were interested in beginning with a 3β-hydroxy-$\Delta^{5,6}$-steroid backbone, which would later allow us to vary the side chain on the C17-position from our known syntheses of dehydroepiandrosterone derivatives [18]. Our initial studies in converting the 3β-hydroxy-$\Delta^{5,6}$-steroid to the 3-keto-7α-hydroxy-Δ^4-steroid begins with cholesterol (**4**) as the feedstock. The key step in this report is the allylic C3-oxidation of a 3-desoxy Δ^4-steroid precursor **9** (Figure 2C, **9** to **8**). The key C3-allylic oxidation was achieved with 56% yield (Scheme 3, **9** to **8**) using classical C7-allylic oxidation conditions with CrO_3 and 3,5-dimethylpyrazole [19]. To the best of our knowledge, this approach of the C3-allylic oxidation of a 3-desoxy Δ^4 precursor (**9**) to afford a 3-keto-Δ^4 7-oxygenated steroid backbone (**8**) is unprecedented.

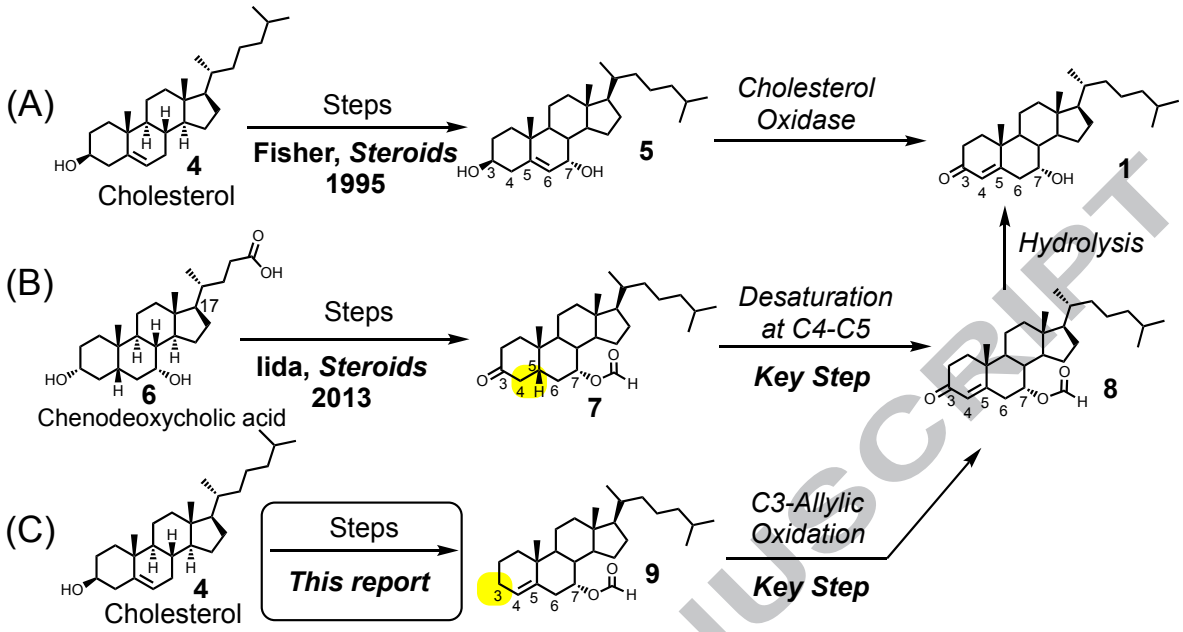


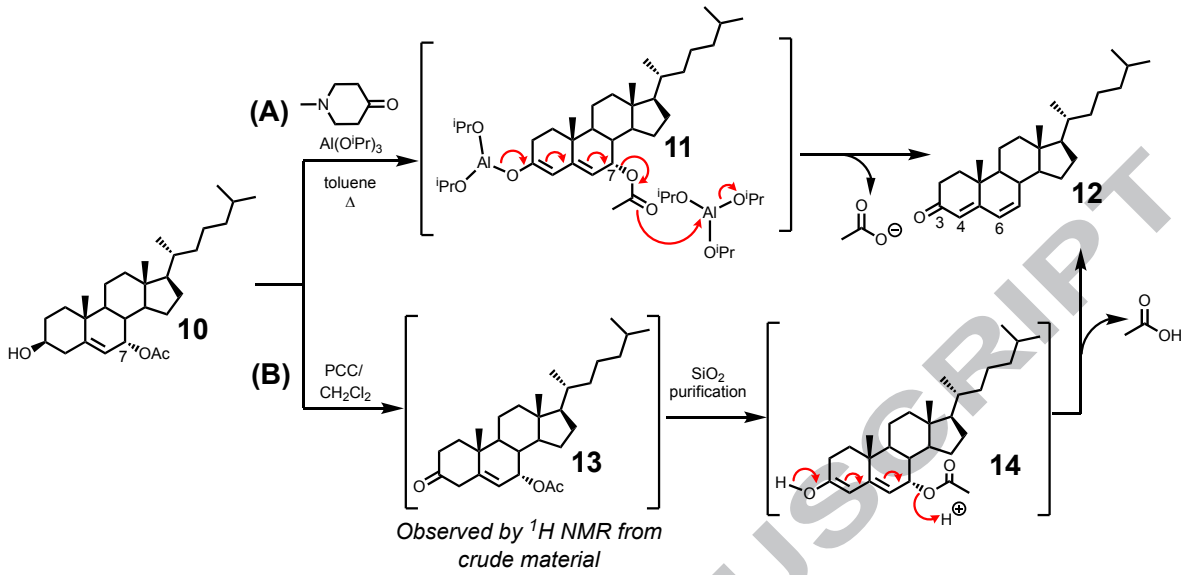
Figure 2. (A) A previous chemoenzymatic synthesis of 7α-hydroxycholest-4-en-3-one (**1**) from cholesterol (**4**) where 7α-hydroxycholesterol (**5**) was regioselecctively oxidized at the C3-position by cholesterol oxidase to yield the desired product. (B) A previous chemical synthesis of 7α-hydroxycholest-4-en-3-one (**1**) from chenodeoxycholic acid (**6**). (B) Chemical synthesis of 7α-hydroxycholest-4-en-3-one (**1**) from cholesterol (**4**) reported in this paper.

Results and Discussion

*The formation of the undesired cholesta-4,6-dien-3-one (**12**)*

The choice of the protecting groups at the C3- and C7- positions were important in obtaining the C3-desoxy precursor. Originally, a C7-acetate protecting group (**10**) was used (Scheme 1). However, during the oxidation of the C3-hydroxy group of the 7-acetate intermediate (**10**) with Oppenauer conditions, the compound would deacetylate at the C7-position and yield the 4,6-dien-3-one product (**12**) (Scheme 1, **10** to **12**). Moreover, when pyridinium chlorochromate was used to oxidize the C3-hydroxy group of intermediate **10** (Scheme 1B), the chromium reagent had precipitated out and the solvent was decanted out of the reaction mixture. The crude reaction mixture was analyzed by ^{1}H NMR spectroscopy to show the isolation of the 3-keto-$\Delta^{5,6}$-steroid product (**13**) (Scheme 1, **13**, see Supporting Information for ^{1}H NMR spectrum). However, upon purification by silica gel column chromatography, only the dienone (**12**) was recovered due to the acidity of the silica gel (Scheme 1, **13** to **14** to **12**).

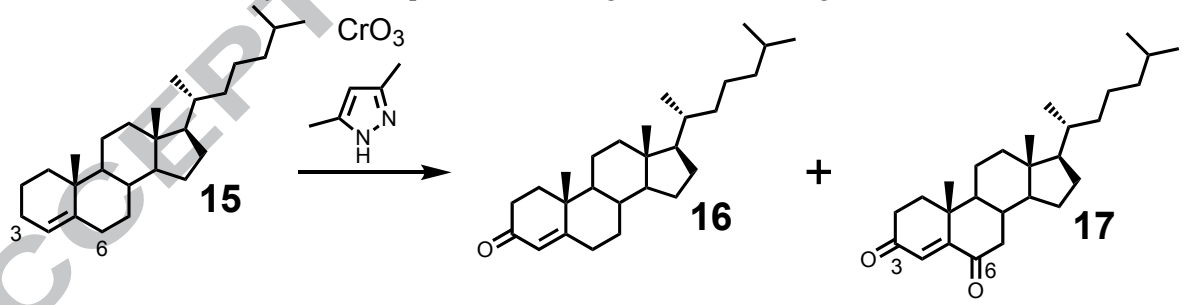
Because of the labile nature of the C7-acetate during the oxidation of the C3-hydroxy group (Scheme 1, **10** to **12**), the C7α-hydroxy group was protected as the TBDMS ether **21** (Scheme 3, **21**). The formation of the undesired dienone **12** from the dehydration of a C7-oxygenated substituent under acidic conditions had been a previously reported problem with the synthesis of 7-hydroxycholest-4-en-3-one [12, 15, 20]. The presence of the conjugated 3-keto Δ^4 moiety may promote the loss of the C7-substituent to form the dienone (**12**). A similar formation of a 4,6-dien-7-one system in the presence of an acid has been reported in a different system [21]. Furthermore, it has been reported that the deprotection of a 3-keto Δ^4-steroid containing a C7-acetate through alkaline hydrolysis conditions yielded the undesired 4,6-dien-3-one product (similar to the structure of compound **12** in Scheme 1) [15]. Therefore, in order to overcome this undesired elimination, we endeavored to synthesize a 3-desoxy Δ^4 steroid backbone containing a C7-formate, which would allow us to oxidize the C3-position and convert the C7-formate to the desired hydroxy group under mild conditions.



Scheme 1. Preliminary studies with a C7-acetate substituent (**10**) resulted in formation of the 4,6-dien-3-one product (**12**) during oxidation conditions with (A) Oppenauer oxidation protocol and (B) PCC as the oxidant.

*The regioselective oxidation at the C3-position of a C3-desoxy Δ⁴ steroid precursor (**15**)*

In our pilot studies to determine the regioselective oxidation at the C3-position of a 3-desoxy-Δ⁴-sterol precursor, we converted cholest-4-en-3-one to its 3-desoxy counterpart **15** (Scheme 2). The subsequent oxidation with CrO_3 (10 mol equivalents) and 3,5-dimethylpyrazole (10 mol equivalents) yielded a mixture of the desired cholest-4-en-3-one product **16** and also the overoxidized cholest-4-en-3,6-dione **17** (Scheme 2). With these promising preliminary findings, we decided to embark on synthesizing 7α-hydroxycholest-4-en-3-one (**1**) through a C3-allylic oxidation of a 3-desoxy Δ⁴-steroid precursor bearing a C7-formate (Figure 2, **9**).



Scheme 2. In our model studies with cholest-4-ene (**15**) as the starting material, both the C3- and C6-positions were oxidized to yield the desired ketone **16** and diketone **17** when treated with CrO_3 and 3,5-dimethylpyrazole.

Synthesis of 7α-hydroxy-cholest-4-en-3-one (1) from cholesterol acetate (18)

To begin, the C7-position of cholesterol acetate (**18**) was oxidized with CrO_3 and 3,5-dimethylpyrazole [18, 19, 22] to yield the ketone (**19**) (Scheme 3). As summarized in Table 1, various conditions were tested to optimize the C7-oxidation of cholesterol acetate (**18**) to yield enone **19**. For instance, $Co(OAc)_2$ [23], $Rh_2(cap)_4$ [24], PCC [25], and CuI [26] have all been reported to oxidize the C7-position of Δ⁵ steroids.

Table 1. Summary of C7-oxidation conditions tested. For entries 2-6: the metal was added to the solution of the starting material (**18**, 300 mg, 0.7 mmol) in indicated solvent (5-6 ml) in a 50 ml screw cap vial fitted with a rubber stopper. The reaction mixture was evacuated and backfilled

with nitrogen. For entries 4-6: 1 mg of metal was used. For entry 3: 30 mg of $Co(OAc)_2$ tetrahydrate (0.12 mmol, 0.17 mol eq) was used. For entry 2: 1.8 g of PCC (8.4 mmol, 12 mol eq) and 3.52 g of celite (58 mmol) were used (and stirred at reflux). Entries 3-6: the reaction temperature was 40 °C, 1.5 ml (70% in water, v/v) of *tert*-butylhydroperoxide (11 mmol, 15 mol eq) was used.

Entry	time	Metal	Solvent	Isolated yield of **19**
1	12 h	CrO_3/3,5-DMP	CH_2Cl_2	79%
2	36 h	PCC	toluene	27% (recovered SM)
3	20 h	$Co(OAc)_2$	CH_3CN	68%
4	24 h	$Rh_2(cap)_4$	$Cl(CH)_2Cl$	71% (recovered 60 mg of SM)
5	12 h	$Rh_2(cap)_4$	$Cl(CH)_2Cl$	45% (recovered 100 mg of SM)
6	20 h	CuI	CH_3CN	53% (recovered 56 mg of SM)

The resulting C7-ketone (**19**) was stereoselectively reduced with L-Selectride to yield the 7α-hydroxy epimer (**20**) (9:1 diastereomeric ratio between 7α-hydroxy:7β-hydroxy epimers). Interestingly, in a previously similar reduction of C7-keto cholesterol-3-benzoate, the epimeric mixture obtained was 4:1 C7α- to C7β- [27], which is explained by the difference in protecting group on the C3-position. The resulting C7-hydroxy group was protected as the TBDMS ether (**21**) with TBDMSCl and imidazole in acetonitrile. The modest yield (Scheme 3, **20** to **21**, 64%) prompted us to use iodine to facilitate the TBDMS protection at the sterically hindered C7-hydroxy group [28]. However, the iodine additive (5 mol eq) did not improve the yield of our procedure. We also attempted changing the solvent to CH_2Cl_2 – however, the compound had decomposed to an unknown mixture. Methanolysis of the C3-acetate of compound **21** with K_2CO_3 in CH_3OH yielded the 3-hydroxy intermediate **22**. Subsequent Oppenauer oxidation afforded the 7α-O-*tert*-butyldimethylsilyloxy-3-keto-Δ^4-steroid intermediate **23**. An alternative oxidation with Dess Martin periodinane in CH_2Cl_2 resulted in the elimination of the OTBDMS group to yield the 4,6-dien-3-one (**12**) (data not shown). This elimination most likely occurred due to the slightly acidic conditions that resulted during the oxidation by Dess Martin periodinane. In addition, we also attempted the Swern conditions with oxalyl chloride, DMSO, and triethylamine in CH_2Cl_2 at -78 °C, which furnished the 3-keto $\Delta^{5,6}$ steroid product as the crude product. We attempted to isomerize the double bond to the conjugated system ($\Delta^{5,6}$ to Δ^4) by heating the material in toluene. However, the only observed product was the undesired dienone **12**. This elimination to the dienone **12** was probably entropically favored in the presence of heat. Additionally, the same 3-keto $\Delta^{5,6}$-isomer was loaded directly on the silica gel column, which resulted in the isolation of the dienone **12**. Furthermore, attempt to convert 7-OTBDMS ether (**23**) to the desired final compound, 7α-hydroxy-cholest-4-en-3-one (**1**) using TBAF in THF resulted in the formation of the dienone (**12**) (Scheme 3, **23** to **12**, and Supporting Information). These observations coupled with the previously mentioned labile nature of the C7-acetate (Scheme 1) led us to synthesize the 3-desoxy steroid intermediate (**25** in Scheme 3) bearing a C7-OTBDMS ether, which could be deprotected smoothly under harsh acidic conditions and reprotected as the C7-formate.

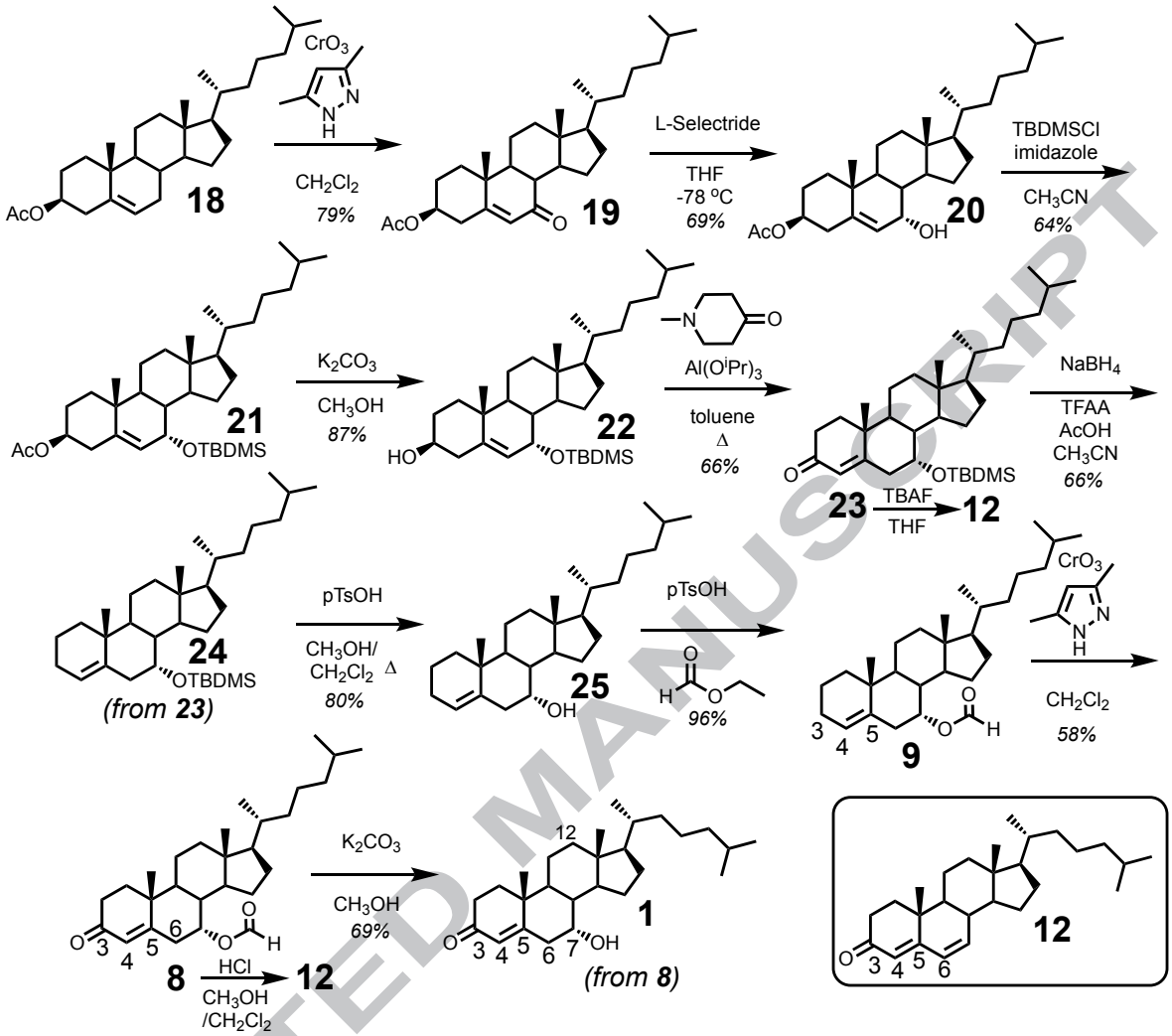
Subsequently, the 3-keto group of TBDMS ether **23** was reduced by treating with $NaBH_4$ in trifluoroacetic acid/acetic acid/CH_3CN (Scheme 3) to yield the 3-desoxy intermediate **24**. Treatment with pTsOH in CH_3OH/CH_2Cl_2 resulted in conversion to the 7α-hydroxy intermediate **25**, which was protected as the formate **9** with ethyl formate and p-TsOH. Gratifyingly, the C3-allylic oxidation with CrO_3 furnished the desired 3-ketone (**8**). On the contrary, in our pilot studies (Scheme 2), treatment with excess of the oxidant CrO_3 (10 mol equivalents) and 3,5-dimethylpyrazole (10 mol equivalents) yielded the overoxidized 3,6-diketone product (**17**) and the 3-ketone (**16**). It is likely that the electron withdrawing nature of

the C7-formate substituent of intermediate **9** deactivates the C6-allylic position and prevents oxidation in the B-ring at C6.

The C7-formate of **8** (Scheme 3) was deprotected with NaOH in THF to afford the final desired product (**1**) with a yield of 39% (Figure 3, crystal structure of compound **1**). However, the previous report in 2013 using NaOH in THF on the same starting material reported 78% [15]. This previous report used 0.8% NaOH (aqueous, w/v) in THF for 30 min while we used 6% NaOH (aqueous, w/v) in THF for 30 min. In our hands, when attempting to repeat the previously reported condition of 0.8% NaOH, no reaction occurred. Therefore, it was necessary to increase the amount of base to 6% NaOH (w/v), which resulted in the final desired product (**1**) in addition to the 4,6-dien-3-one **12**. Interestingly, when the C7-formate intermediate **8** was subjected to methanolysis using HCl in CH_3OH/CH_2Cl_2, the formate was eliminated to yield solely the dienone product **12**. Table 2 summarizes the optimization process for the deprotection of the C7-formate to yield the final product (**8** to **1**). It was found that $KHCO_3$ in CH_3OH/CH_2Cl_2 and K_2CO_3 in CH_3OH/CH_2Cl_2 (Table 2, entries 4 and 5) yielded the most amount of the desired product, 7α-hydroxycholest-4-en-3-one (compound 1) when the ^{1}H NMR spectra of the crude reaction products were taken. Furthermore, both $KHCO_3$ and Et_3N as the base required the addition of a second portion of base to promote conversion to the product.

Table 2. Summary of reaction conditions to deprotect the C7-formate to give the final product. a: ratio of 7α-formate starting material to 7α-hydroxy product to dienone (**8**:**1**:**12**) determined by integration of the formate C-H proton at δ 8.1 ppm (for compound **8**), C7-hydroxymethine proton at ~ δ 4.0 ppm (for compound **1**), and the C6- and C7- vinyl protons at ~ δ 6.1 ppm (for compound **12**), respectively. The ratios were determined by integrating the diagnostic protons of the crude reaction mixtures. All reactions were stirred for 12 hours. For each entry, 10 mg of 7α-formate (compound **8**) starting material was used, 0.5 ml of solvents were used. For the base, 5 mg of $KHCO_3$ or 5 mg of K_2CO_3 or 0.05 ml of Et_3N were used (entries 3-8). In the case of $KHCO_3$ and Et_3N, a second equal portion of base was added after 2 hours based on the amount of starting material present determined by TLC and ^{1}H NMR. NMR data shown in Supporting Information.

Entry	Conditions	Ratio of formate to hydroxy to dienone (**8**:**1**:**12**)[a]
1	$H_2O/CH_3OH/CH_2Cl_2$	No products detected
2	HCl in CH_3OH/CH_2Cl_2	Only dienone **12** detected
3	Et_3N in CH_3OH/CH_2Cl_2	1 : 2.45 : 2.67
4	$KHCO_3$ in CH_3OH/CH_2Cl_2	0 : 2.0 : 1.0
5	K_2CO_3 in CH_3OH/CH_2Cl_2	0 : 1.8 : 1.0
6	Et_3N in CH_3OH/H_2O	1.0 : 2.7 : 2.8
7	$KHCO_3$ in CH_3OH/H_2O	No significant amount of products detected
8	K_2CO_3 in $CH_3OH/H_2O/CH_2Cl_2$	No significant amount of products detected



Scheme 3. Synthesis of 7α-hydroxycholest-4-en-3-one (**1**) from cholesterol acetate (**18**) through a C3-allylic oxidation of a 3-desoxy precursor (**9**) to introduce the C3-ketone (**8**) product as the key step.

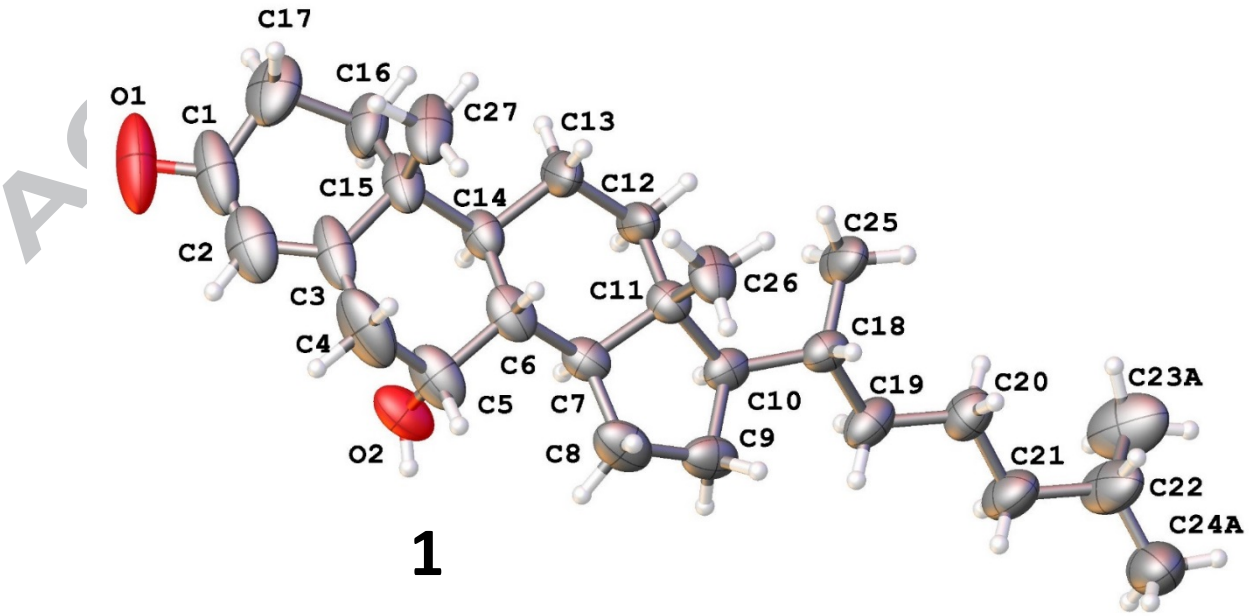
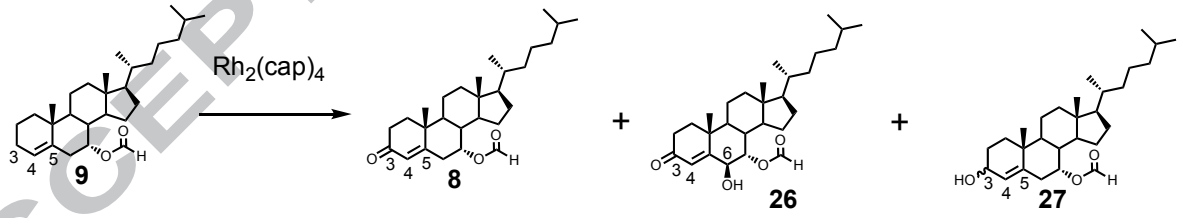


Figure 3. Crystal structure of 7α-hydroxycholest-4-en-3-one (**1**).

Optimization of C3-allylic oxidation and an unexpected C6-allylic oxidation product on substrate **9**

Similar to the various allylic oxidation conditions tested for the conversion of cholesterol acetate (**17**) to enone **18** (see above and Table 1), the different allylic oxidation protocols were also used to find the most efficient way to access enone **8** from 3-desoxy Δ^4 steroid **9**. Interestingly, both rhodium [29] and cobalt conditions yielded an additional product (characterized by ^{1}H NMR spectroscopy) – rhodium gave more of the new product compared to cobalt. This new product was assigned as the 6β-hydroxy product (Scheme 4, **26**). The 6β-hydroxy orientation was assigned through the similar coupling constant (J = 3.7 Hz) and splitting pattern (doublet) of a 6β-,7α-dihydroxy steroid compound [30]. Furthermore, for the allylic oxidation using the rhodium conditions, the stoichiometry of *tert*-butylhydroperoxide was important. In the previous study of C7-oxidation of cholesterol, 5 mol equivalents of *tert*-butylhydroperoxide was used to afford the highest yielding C7-ketone product. When 5 mol equivalents of *tert*-butylhydroperoxide was used in our system, the overoxidation product to the 6β-hydroxy product (**26**) resulted. When the amount of *tert*-butylhydroperoxide was lowered to ~3.5 mol eq, a major side product was isolated and the structure was determined to be the diastereomeric mixture of the C3-allylic alcohol product (Scheme 4, **27**). The structure of the C3-allylic alcohol was confirmed by treatment with Dess-Martin periodinane to yield the enone **8** (see Supporting Information). A similar allylic alcohol product was observed when the stoichiometry was lowered to ~2 mol equivalents in the case of the oxidation of cholesterol at the C7-position [24]. The oxidation of the allylic alcohol to the enone in the conditions using dirhodium catalyst and *tert*-butylhydroperoxide has been reported to be a slow process [24]. The formation of the 6β-hydroxy product (**26**) probably arose from the C6-allylic oxidation of the 3-keto Δ^4 compound (**8**) because when compound 8 was treated in the same conditions (Table 3, entry 3), the 6β-hydroxy product (**26**) was observed in the ^{1}H NMR spectrum of the crude reaction mixture.



t-BuOOH (amount)	*time*	*Ratio of Products (**8**:**26**:**27**)*				
~5.0 mol eq	24 h	33%	:	21%	:	Not isolated
~3.5 mol eq	17 h	37%	:	Not isolated	:	6%

Scheme 4. Alternative allylic oxidation products (**8** or **26** or **27**) observed depending on different amounts of *tert*-butylhydroperoxide used (5.0 mol eq or 3.5 mol eq) with the $Rh_2(cap)_4$ catalyst for 24 h and 17 h (Table 3, entries 3 and 4). Isolated percent yields reported.

As summarized in Table 3, CrO_3 and 3,5-dimethylpyrazole gave about the same yield of the desired product (**8**) compared to $Co(OAc)_2$ and CuI (Table 3, Entries 2, 3, and 5).

Table 3. Summary of C3-oxidation conditions tested to convert 3-desoxy-,7α-formyloxy Δ^4 steroid to the 3-keto-,7α-formyloxy Δ^4 steroid (**9** to **8**). For entries 2-5: *tert*-butylhydroperoxide was used (0.15 ml, 1.1 mmol), Reaction temperature was 40 °C, 5 ml of solvent was used. Entry 2: 105 mg of starting material (0.25 mmol), 10 mg of $Co(OAc)_2$ (0.04 mmol), gave 66 mg of product (0.15 mmol, 60%). Entry 3: 92 mg of starting material (0.22 mmol), 1 mg of $Rh_2(cap)_4$ used gave 31 mg of product (0.072 mmol, 33%), 21 mg of **26** (0.046 mmol, 21%). Entry 4: 130 mg of starting material (0.31 mmol), 1 mg of $Rh_2(cap)_4$ used gave 50 mg of product (0.12 mmol, 37%) and 8 mg of **27** (0.019 mmol, 6%). Entry 5: 83 mg of starting material (0.2 mmol), 1 mg of CuI used gave 50 mg of product isolated (0.12 mmol, 58%). 3,5-DMP: 3,5-dimethylpyrazole.

Entry	time	Metal	Solvent	Isolated yield of **8**
1	12 h	CrO_3/3,5-DMP	CH_2Cl_2	56%
2	20 h	$Co(OAc)_2$	CH_2Cl_2	60%
3	24 h	$Rh_2(cap)_4$	$Cl(CH)_2Cl$	33%
4	17 h	$Rh_2(cap)_4$	$Cl(CH)_2Cl$	37%
5	20 h	CuI	CH_2Cl_2	58%

Conclusion

In conclusion, we report a new approach to access 7α-hydroxy-cholest-4-en-3-one, the classical substrate of P450 8B1 and a biomarker for irritable bowel syndrome and bile acid malabsorption, through a C3-allylic oxidation of a C3-desoxy Δ^4-steroid precursor. The choice of protecting groups to mask the C7-oxy substituent during the route was critical in avoiding elimination to yield the 4,6-dien-3-one (Scheme 1 and Scheme 3, formation of **12**). The key C3-allylic oxidation using CrO_3 and 3,5-dimethylpyrazole was achieved with a respectable yield of 56% (Scheme 3, **9** to **8**). This fully synthetic approach to access 7α-hydroxycholest-4-en-3-one (**1**) from cholesterol acetate (**18**) required 10 steps (Scheme 3). Similarly, the previously reported approach by Iida and co-workers have accessed the title compound in 11 steps from chenodeoxyholic acid [15]. However, this new approach from cholesterol may be beneficial in that it provides a route to access other physiologically relevant oxysterol analogs such as 7β-hydroxy-cholest-4-en-3-one (see references [22] and [18] for converting the C7-ketone to the C7β-hydroxy stereochemistry with $NaBH_4$). 7β-Hydroxy-cholest-4-en-3-one would be biosynthetically derived from the 3β-hydroxysteroid dehydrogenase-catalyzed [31] conversion of 7β-hydroxycholesterol, a biomarker for oxidative stress [32]. Thus, this method will be used to enable the exploration of the biological activity of other oxysterol analogs.

Materials and Methods

Melting points were obtained from an Optimelt MPA100 automated melting point apparatus (Stanford Research Systems, Sunnyvale, CA). NMR spectra were obtained from a Bruker (Billerica, MA) NMR spectrometer (300 MHz or 500 MHz). Deuterochloroform ($CDCl_3$) was used as the solvent for acquiring NMR spectra and chloroform chemical shift was referenced to δ 7.26 ppm and δ 77.16 ppm in the proton and carbon NMR spectra, respectively. A Waters Acquity UPLC (Waters, Milford, MA) connected to an LTQ Orbitrap XL high resolution mass spectrometer (Thermo Fisher Scientific, Waltham, MA) was used to analyze the high resolution mass spectra for each intermediate. The mass spectrometer was tuned with Pierce LTQ ESI positive mode solution (Thermo Fisher Scientific, Waltham, MA) and each run was ionized with electrospray ionization positive mode. Optical rotations were calculated by the following formula: $[\alpha]_D^{20} = \alpha/l*c$, where α was defined as: [(the measured angle)- (the calibrated angle from the blank solution)]. The calibrated angle from the blank solution for chloroform (CH_3Cl) was: 78.5. The value of *l*, the length of the cell, was 1 dm, c = concentration with units of mg/ml. The specific rotation of the final compound (compound **1**) was measured on an Autopol IV instrument (Automatic Polarimeter) from Rudolph Research (Hackettstown, NJ).

3β-Acetoxycholest-5-en-7-one (Compound **19***)*

Chromium trioxide (47 g, 470 mmol, 12 mol eq) and 3,5-dimethylpyrazole (47 g, 490 mmol, 12 mol eq) was added sequentially at an interval of 45 minutes to a stirring solution of dry dichloromethane (700 ml) at -78 °C. Cholesterol-3-acetate **18** (17 g, 40 mmol, 1 mol eq) was dissolved in 100 ml dry dichloromethane and added to the stirring solution. The reaction was stirred for 1 h at -78 °C then the dry ice bath was removed and the reaction was stirred at room temperature for another 12 h. The resulting black crude solution was loaded directly on a column packed with silica gel in hexanes. Purification was done by eluting (isocratic 20% ethyl acetate in hexanes) to afford a white solid as product **19** (14 g, 32 mmol, 79%). R_f: 0.49 (4:1, hexanes:ethyl acetate, v/v); $[\alpha]_D^{20}$ - 15° [c = 1 mg/ml in $CHCl_3$; IR (ATR) 2940.11, 2868.62, 1730.92, 1711.77, 1669.97, 1466.25, 1366.41, 1237.69 cm-1; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.70 (s, 1H), 4.71 (m, 1H), 2.60 - 2.31 (m, 3H), 2.22 (t, J = 9.9 Hz, 1H), 2.04 (s, 3H), 2.02 - 0.97 (m, 22H), 1.02 (s, 3H), 0.91 (d, J = 6.66 Hz, 3H), 0.86 (dd, J = 6, 1 Hz, 6H), 0.68 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.12, 170.43, 163.99, 126.85, 72.35, 54.91, 50.09, 49.95, 45.56, 43.25, 39.61, 38.80, 38.45, 37.88, 36.31, 36.13, 35.85, 28.67, 28.13, 27.49, 26.44, 23.96, 22.94, 22.69, 21.40, 21.30, 19.00, 17.39, 12.10. mp, 156-158 °C (literature value: mp 159 – 161 °C [33]). HRMS (*m/z*, ESI+) calculated for: $[M+H]^+$, $C_{29}H_{47}O_3^+$, 443.3520; found 443.3523 (Δ 0.7 ppm).

3β-Acetoxycholest-5-en-7α-ol (Compound **20***)*

An oven dried 250 ml round bottom flask containing a magnetic stirrer was sealed with a double septum. The round bottom flask was repeatedly degassed and backed-filled (×3) with N_2 gas. The ketone substrate **19** (3.0 g, 6.77 mmol, 1 mol eq) was dissolved in THF (150 ml) and transferred into the already sealed round bottom via cannula. The solution was cooled to -78 °C and L-Selectride (6.77 ml, 6.77 mmol, 1 mol eq) was introduced into the reaction flask via a syringe. The reaction was stirred for 3 h and then quenched at -78 °C by adding deionized water (50 ml). The resulting solution was extracted (×3) with ethyl acetate and concentrated under reduced pressure to form crude colorless oil. The oil was purified by silica gel column chromatography (isocratic 20% ethyl acetate in hexanes) to afford a white waxy solid as product **20** (2.1 g, 4.72 mmol, 69%). R_f: 0.36 (hexanes:ethyl acetate, 4:1, v/v); $[\alpha]_D^{20}$ - 23° [c = 0.2 mg/ml in $CHCl_3$; IR (ATR) 3414.05, 2932.82, 2865.96, 1732.18, 1466.04, 1374.06, 1237.80 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.63 (d, J = 5 Hz, 1H), 4.64 (m, 1H), 3.87 (m, 1H), 2.35 (m, 2H), 2.03 (s, 3H), 1.94 -1.05 (m, 25H), 1.00 (s, 3H), 0.92 (d, J = 7.89 Hz, 3H), 0.86 (dd, J = 6, 1 Hz, 6H), 0.68 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.55, 145.33, 124.89, 73.52, 65.38, 55.98, 49.53, 42.34, 42.25, 36.66, 39.28, 38.05, 37.62, 36.89, 36.30, 35.92, 28.41, 28.15, 27.64, 24.39, 23.85, 22.94, 22.70, 21.51, 20.80, 18.88, 18.31, 11.77; mp, 131-133 °C; (literature value: mp 138 - 139 °C [34]); HRMS (*m/z*, ESI+); calculated for: $[M+H]^+$, $C_{29}H_{49}O_3^+$, 445.3676; found 445.3681 (Δ 1.1 ppm).

3β-Acetoxy-7α-[[(1,1-dimethylethyl)dimethylsilyl]oxy]cholest-5-ene (Compound **21***)*

To 250 ml round bottom flask was added the 7α-hydroxy-cholesterol substrate **20** (10.0 g, 22.5 mmol, 1 mol eq) in 200 ml of acetonitrile. Imidazole (27.5 g, 405 mmol, 18 mol eq) and tert-butyldimethylsilyl chloride (40.7 g, 270 mmol, 12 mol eq) were added sequentially to the stirring solution. The reaction was refluxed for 4 h. Ethyl acetate (200 ml) was added to the reaction solution and with a separatory funnel the resulting was washed with deionized water (3 × 100 ml). The organic layer was dried over magnesium sulfate and concentrated under reduced pressure to form a crude yellow oil. Subsequent purification by silica gel column chromatography (isocratic 10% ethyl acetate in hexanes) afforded a colorless oil as product **21** (8.04 g, 14.4 mmol, 64%). R_f: 0.88 (4:1, hexanes:ethyl acetate, v/v); IR (ATR) 2931.58, 2854.06, 1735.56, 1468.24, 1364.16, 1235.38 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.50 (d, J = 5 Hz, 1H), 4.61 (m, 1 H), 3.83 (apparent s, 1H), 2.40 - 2.27 (m, 2H), 2.03 (s, 3H), 1.98 (apparent d, J = 14, 1H), 1.99

– 1.75 (m, 4H), 1.68 -1.39 (m, 10H), 1.28 – 1.09 (m, 9H) 0.98 (s, 3H), 0.92 (d, J = 5 Hz, 3H), 0.86 (dd, J = 7, 3 Hz, 6H), 0.85 (s, 9H), 0.65 (s, 3H), 0.03 (apparent s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.61, 143.32, 125.86, 73.92, 65.82, 56.40, 49.15, 41.99, 41.68, 39.62, 39.49, 38.30, 38.25, 38.30, 38.25, 37.57, 36.83, 36.39, 36.21, 28.36, 28.11, 27.73, 26.04, 24.30, 24.26, 22.96, 22.68, 21.56, 20.86, 18.90, 18.40, 18.31, 11.84, -2.74, -4.51. HRMS (*m*/*z*, ESI+) calculated for: $[M+H]^+$, $C_{35}H_{63}O_3Si^+$, 559.4541; found 559.4508 (Δ -5.9 ppm).

7α-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-cholest-5-en-3β-ol (Compound **22***)*

Potassium carbonate (1.0 g, 7.24 mmol, 0.4 mol eq) was added to cholesterol-3β-acetoxy-*7α-tert*-butyldimethylsilyl ether **21** substrate (10 g, 17.9 mmol, 1 mol eq) in methanol (50 ml). The reaction was refluxed for 4 h. The solution was cooled to room temperature, diluted with deionized water (100 ml) and extracted with ethyl acetate (300 ml). The ethyl acetate extract was dried over magnesium sulfate and concentrated under reduced pressure to form a crude white solid. The crude solid was purified by silica gel column chromatography (isocratic 10 % ethyl acetate in hexanes) to afford a white solid as product **22** (8.04 g, 15.6 mmol, 87 %). R_f: 0.28 (4:1, hexanes:ethyl acetate, v/v); $[\alpha]_D^{20}$ -20° [c = 0.3 mg/ml in $CHCl_3$];IR (ATR) 3233.36, 2926.80, 2854.16, 2854.16, 1662.37, 1460.31, 1374.57, 1245.96 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.49 (d, J = 5Hz, 1H), 3.84 (apparent s, 1H), 3.45 (m, 1H), 2.28 (d, J = 8Hz, 2H), 1.97 (dt, J = 14, 3 Hz, 1H), 1.86 – 1.77 (m, 1H), 1.68 -1.60 (m, 1H), 1.53 – 1.02 (m, 20H), 0.97 (s, 3H), 0.92 (d, J = 7.8 Hz , 1H), 0.86 (dd, J = 7.77, 3.45 Hz, 6H), 0.85 (s, 9H), 0.66 (s, 3H), 0.02 (d, J = 11. 47 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.21, 125.16, 71.84, 65.92, 56.43, 49.23, 42.45, 42.00, 41.82, 39.62, 39.56, 38.31, 37.45, 37.17, 36.40, 36.21, 31.71, 28.37, 28.11, 26.04, 24.31, 24.27, 22.96, 22.68, 20.94, 18.90, 18.42, 11.84, -2.83, -4.44. mp, 131-133 °C. HRMS (*m*/*z*, ESI+) calculated for: $[M+H]^+$, $C_{33}H_{61}O_2Si^+$, 517.4435; found 517.4439 (Δ 0.7 ppm).

7α-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-cholest-4-en-3-one (Compound **23***)*

To a solution of 3β-hydroxy sterol substrate **22** (3.4 g 6.6 mmol, 1 mol eq) in 300 ml of toluene in a 500 ml round bottom flask was added aluminum isopropoxide (20.2 g, 98 mmol, 15 mol eq) and 1-methyl 4-piperidone (11.4 ml, 98 mmol, 15 mol eq). The reaction was refluxed using a Dean Stark apparatus and a reflux condenser, which was wrapped with aluminum foil with cotton embedded, for 24 h maintaining a constant volume of toluene above the oil bath (250 ml) – the reaction was carefully monitored so that the solvent was not completely evaporated to avoid decomposition of the compound. The progress of the reaction was monitored continuously by TLC and NMR. The reaction solution was cooled to room temperature and diluted with deionized water (200 ml). The resulting solution was extracted with ethyl acetate (3 × 200 ml). The ethyl acetate extract was dried over magnesium sulfate and concentrated under reduced pressure to form a crude orange oil. The crude oil was purified by silica gel chromatography (isocratic 15% ethyl acetate in hexanes) to afford the C3-keto Δ^4 product as a white solid **23** (2.29 g, 4.38 mmol, 66%). R_f: 0.36 (4:1, hexanes:ethyl acetate, v/v); $[\alpha]_D^{20}$ - 32° [c = 0.5 mg/ml in $CHCl_3$; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.69 (s, 1H), 3.93 (s. 1H), 2.51 – 2.30 (m, 4H), 2.02 (m, 2H), 1.87 – 1.69 (m, 2H), 1.65 – 1.57 (m, 2H), 1.53 – 1.47 (m, 3H), 1.46 – 1.39 (m, 1H), 1.38 – 1.31 (m, 4H), 1.29 - 1.21 (m, 2H), 1.17 (s, 3H), 1.15 – 1.05 (m, 5H), 1.01 – 0.94 (m, 1H), 0.91 (d, J = 6.7 Hz, 3H), 0.86 (dd, J = 6.7, 2 Hz, 6H), 0.83 (s. 9H), 0.69 (s, 3H), 0.04 (d, J = 12.77 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.2, 170.0, 126.54, 69. 34, 56.35, 50.20, 44.74, 42.33, 41.66, 41.01, 39.58, 39.46, 38.50, 36.29, 36.10, 35.67, 34.13, 28.21, 28.09, 25.93, 24.22, 23.91, 22.94, 22.66, 20.87, 18.79, 18.34, 17.35, 12.02, -3.43, -4.95; IR (ATR) 2947.44, 2866.75, 1671.50, 1469.65, 1374.81, 1250.28 cm^{-1}; The reaction was scaled up to yield 1.07 g of compound **23**, which was used for the synthesis of compound **21**. mp, 124-126 °C. HRMS (*m*/*z*, ESI+) calculated for: $[M+H]^+$, $C_{33}H_{59}O_2Si^+$, 515.4279; found 515.4279 (Δ 0 ppm).

7α-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-cholest-4-ene (Compound **24***)*

To a cooled solution of acetonitrile (5 ml) in a round bottom flask was added trifluoroacetic acid (5 ml) and glacial acetic acid (5 ml). Sodium borohydride (1.0 g, 20 mmol, 12 mol eq,) was added to the stirring solution at 0 °C and left to stir for 2 minutes. The 3-keto-Δ^4 sterol substrate **23** (1.07 g, 2.08 mmol, 1 mol eq) was dissolved in dichloromethane (2 ml) and added to the stirring solution. The reaction was further stirred for 2 h and then quenched by the addition of deionized water (5 ml). The resulting aqueous solution was extracted with dichloromethane (3 × 20 ml). The dichloromethane extract was concentrated under reduced pressure to give crude colorless oil. Purification through silica gel column chromatography (isocratic 5% ethyl acetate in hexanes, v/v) afforded colorless oil as product **24** (0.7 g 1.39 mmol, 66 %). R_f: 0.94 (100% hexanes); $[\alpha]_D^{20}$ - 19° [c = 0.8 mg/ml in $CHCl_3$; IR (ATR) 2930.04, 2864.59, 1658.91, 1462.52, 1374.64, 1250.80 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.20 (s, 1H), 3.77 (s, 1H), 2.28 (apparent d, J = 17.53 Hz, 1H), 2.01 (dd, J = 14, 4 Hz, 1H), 1.96 – 1.88 (m, 3H), 1.83 – 1.75 (m, 1H), 1.69 (m, 1H), 1.63 – 1.55 (m, 2H), 1.51 – 1.48 (m, 3H), 1.44 – 1.36 (m, 3H), 1.26 – 1.18 (m, 4H), 1.16 – 1.04 (m, 6H), 0.99 (s, 3H), 1.04 -0.93 (m, 3H), 0.90 (d, J = 6.8 Hz, 3H), 0.86 (dd, J = 6, 2.3 Hz, 6H), 0.86 (s, 9H), 0.65 (s, 3H), 0.02 (d, 21.82 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 140.80, 122.07, 69.59, 56.48, 50.36, 45.07, 42.42, 41.23, 41.03, 39.83, 39.61, 37.60, 36.86, 36.37, 36.17, 28.30, 28.13, 26.04, 25.94, 24.27, 24.00, 22.98, 22.69, 22.12, 19.40, 19.30, 18.84, 18.45, 12.04, -3.37, -4.83. HRMS (*m*/*z*, ESI+) calculated for: $[M+H]^+$, $C_{33}H_{61}OSi^+$, 501.4486; found 501.4396 (Δ -17 ppm).

Cholest-4-en-7α-ol (Compound **25***)*

Toluene sulfonic acid (0.37 g, 2.14 mmol, 2.44 mol eq) was added to a solution of cholesterol-7*α*-tert-butyldimethylsilyl ether **24** (0.44 g, 0.87 mmol, 1 mol eq) in methanol and dichloromethane (20 ml, 1:1, v/v mixture). The reaction was refluxed for 2 h and carefully monitored by TLC and NMR. The reaction was cooled to room temperature and then quenched by the addition of saturated sodium bicarbonate (20 ml). The resulting solution was extracted with dichloromethane (3 × 20 ml) using a separatory funnel. The dichloromethane extract was concentrated under reduced pressure to afford crude white solids which was then purified by silica gel column chromatography (10 % ethyl acetate in hexanes) to give the product as a white solid **25** (0.27 g, 0.698 mmol, 80%): R_f: 0.43 (9:1, hexanes:ethyl acetate, v/v);); $[\alpha]_D^{20}$ - 180° [c = 0.08 mg/ml in $CHCl_3$; IR (ATR) 3472.38, 2926.00, 2864.77, 1656.94, 1466.92, 1372.77, 1259.61 cm^{-1} ^1H NMR (500 MHz, $CDCl_3$) δ 5.43 (s, 1H), 3.74 (apparent s, 1H), 2.51 (apparent d, J = 17.47 Hz, 1H), 2.08 (dd, J = 13.47, 4.49 Hz, 1H), 1.98 -1.92 (m, 3H), 1.89 – 1.82 (m, 1H), 1.75 – 1.67 (m, 2H), 1.64 – 1.58 (m, 1H), 1.53 – 1.47 (m, 2H), 1.46 – 1.40 (m, 2H), 1.38 – 1.05 (m, 11H), 1.01 (s, 3H), 1.04 – 0.96 (m, 1H), 0.90 (d, J = 5.76 Hz, 3H), 0.86 (dd, J = 6.99, 2.15, 6H), 0.67 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 140.17, 124.43, 68.65, 56.18, 50.90, 46.62, 42.66, 40.64, 40.46, 39.69, 39.65, 37.62, 37.24, 36.28, 35.93, 28.36, 28.15, 26.03, 23.88, 23.83, 22.94, 22.71, 21.41, 19.34, 19.07, 18.80, 12.00. HRMS (*m*/*z*, ESI+) calculated for: $[M+H]^+$, $C_{27}H_{47}O^+$, 387.3621; found 387.3621 (Δ 0 ppm).

7α-Formyloxy-4-cholestene (Compound **9***)*

To the 7*α*-hydroxy-Δ4-cholesterol **25** (0.15 g, 0.388 mmol, 1 mol eq) in ethyl formate (25 ml, 310 mmol, 798 mol eq) in a 100 ml round bottom flask was added toluene sulfonic acid (0.01 g, 0.058 mmol, 0.14 mol eq). The reaction was refluxed for 2 h and cooled to room temperature. The resulting solution concentrated under reduced pressure to afford a crude colorless oil. The crude oil was purified via silica gel column chromatography (isocratic 10% ethyl acetate in hexanes, v/v) to give colorless oil as product **9** (0.16 g, 3.73 mmol, 96%). R_f: 0.83 (17:3, hexanes:ethyl acetate, v/v); IR (ATR) 2929.35, 2866.84, 1714.13, 1465.80, 1375.54, 1182.46 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.12 (s, 1H), 5.27 (apparent s, 1H), 5.00 (apparent s, 1H), 2.42 (m, 1H), 2.26 (m, 1H), 2.01 – 1.88 (m, 3H), 1.88 – 0.94 (m, 23H) 1.03 (s, 3H), 0.90 (d, J = 7 Hz, 3H), 0.85 (dd, J = 6.7, 1.5 Hz, 6H), 0.68 (s. 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.10, 139.43, 122.91, 72.49, 56.20, 50.37, 46.98, 39.63, 39.56, 38.68, 37.47, 37.19, 36.89, 36.25, 35.87, 28.19, 28.16, 25.85, 23.91, 23.72, 22.96, 22.70, 21.36, 19.24, 19.03, 18.79, 11.85; HRMS (*m*/*z*, ESI+) calculated for: $[M+H]^+$, $C_{28}H_{47}O_2^+$, 415.3571; found 415.3576 (Δ 1.2 ppm).

7α-Formyloxy-4-cholesten-3-one (Compound **8***)*

Following the procedure for the synthesis of compound **19**, the desired product **8** was a white solid (0.168 g, 0.393 mmol, 56%) from compound **9** (0.29 g, 0.70 mmol, 1 mol eq) using CrO_3 (0.80 g, 8.1 mmol, 12 mol eq), 3,5-dimethylpyrazole (0.77 g, 8.0 mmol, 12 mol eq) in 10 ml of CH_2Cl_2. R_f: 0.16 (17:3, hexanes:ethyl acetate, v/v); $[\alpha]_D^{20}$ - 48° [c = 0.2 mg/ml in $CHCl_3$]; IR (ATR) 2945.62, 2867.39, 1716.35, 1668.82, 1467.66, 1376.52, 1284.23 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.04 (s, 1H), 5.69 (s, 1H), 5.16 (s, 1H), 2.57 (m, 2H), 2.46 - 2.31 (m, 2H), 2.12 -1.89 (m, 2H), 1.91 – 1.65 (m, 3H), 1.53 – 0.94 (m, 17H), 1.26 (s, 3H), 0.91(d, J = 6.73 Hz, 3H), 0.86 (dd, J = 6, 1 Hz, 6H), 0.71 (s, 3H) ^{13}C NMR (75 MHz $CDCl_3$) 198.93, 166.24, 160.57, 126.67, 71.34, 56.07, 50.16, 46.37, 42.59, 39.60, 39.19, 38.46, 38.39, 37.58, 36.18, 35.81, 35.57, 34.08, 28.14, 23.86, 23.64, 22.93, 22.68, 21.00, 18.76, 17.22, 11.84. mp, 152-154 °C (literature value: mp 152 -155 °C [15]). HRMS (*m/z*, ESI+) calculated for: $[M+H]^+$, $C_{28}H_{45}O_3^+$, 429.3363; found 429.3364 (Δ 0.2 ppm).

7α-Hydroxy-4-cholesten-3-one (Compound **1***)*

To the solution of 7α-formate **8** (90 mg, 0.202 mmol, 1 mol eq) in THF (10 ml) was added aqueous sodium hydroxide (2 ml, 2.85 mmol, 6 % w/v, aqueous). The reaction was stirred for 20 minutes with the deprotection of the C7 formate carefully monitored by TLC. The reaction was diluted with deionized water (10 ml) and extracted over ethyl acetate (3 × 20 ml). The ethyl acetate layer was concentrated under reduced pressure and then flushed (50% ethyl acetate in hexanes) on a short silica gel column pad. The final product **1** was afforded as a white solid (32 mg, 0.079 mmol, 39 %). R_f: 0.13 (4:1, hexanes:ethyl acetate, v/v); $[\alpha]_D^{20}$ + 56° [c = 5 mg/ml in $CHCl_3$, calculation = 0.280/((1 dcm)*(0.005 g/mol))] compared to literature value of $[\alpha]_D^{20}$ + 67° [c = 0.8 in $CHCl_3$] [27]; IR (ATR) 3512.69, 2931.59, 2864.78, 1659.94, 1464.97, 1382.16, 1218.36 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.80 (s, 1H), 3.96 (m, 1H), 2.62 (m, 1H), 2.45 – 2.35 (m, 3H), 2.09 – 1.97 (m, 2H), 1.94 – 1.64 (m, 3H), 1.56 – 0.95 (m, 18H), 1.19 (s, 3H), 0.91 d, J = 6.73 Hz, 3H), 0.84 (dd, J = 6, 1 Hz, 6H), 0.71 (s, 3H); ^{13}C NMR (75 MHz $CDCl_3$) δ 198.95, 167.88, 126.94, 68.64, 56.11, 50.57, 45.35, 42.57, 41.01, 39.90, 39.62, 39.32, 38.61, 36.22, 35.87, 35.55, 34.10, 28.29, 28.14, 23.86, 23.72, 22.93, 22.69, 20.97, 18.76, 17.16, 11.93; Recrystallization of **1** (32 mg) in acetone (1 ml) in an open 1 dram vial, which was left 24 hours in the fumehood, gave colorless needlelike crystals, mp, 182-184 °C (literature value: mp 183 – 184 °C [15, 27]). HRMS (*m/z*, ESI+) calculated for: $[M+H]^+$, $C_{27}H_{45}O_2^+$, 401.3414; found 401.3415 (Δ 0.2 ppm).*

* The yield of this reaction was improved with the following conditions:
K_2CO_3 (20 mg, 0.145 mmol, 0.37) was added to a solution of 7α-formate (168 mg, 0.393 mmol, 1.0 mol eq) in CH_3OH (10 ml). The reaction was stirred for 12 h. The reaction was concentrated by reduced pressure (water bath remained at rt) and the crude material was loaded onto a silica gel column (100% hexanes to 50% ethyl acetate in hexanes) to yield 7α-hydroxy-cholest-4-en-3-one as a white solid (108 mg, 0.27 mmol, 69%) and cholesta-4-,6-dien-3-one (20 mg, 0.05 mmol, 13%).

X-Ray Crystallography of 7α-hydroxy-cholest-4-en-3-one (Compound 1)

Single crystals of $C_{27}H_{45}O_2$ (**1**) was prepared by slow evaporation of acetone (see above). Suitable colorless plate-like crystals for compound **1** with dimensions of 0.50 mm × 0.27 mm × 0.17 mm, was mounted in paratone oil onto a nylon loop. All data were collected at 98(2) K, using a Rigaku AFC12 / Saturn 724 CCD fitted with MoKα radiation (λ = 0.71075 Å). Data collection and unit cell refinement were performed using *CrysAlisPro* software [35]. The total number of data were measured in the range 4.1° < 2θ < 50.1, using ω scans. Data processing and

absorption correction, giving minimum and maximum transmission factors (0.707, 1.000) were accomplished with *CrysAlisPro* [35] and *SCALE3 ABSPACK* [36], respectively. The structure, using Olex2 [37] was solved with the ShelXT [36] structure solution program using direct methods and refined (on F^2) with the ShelXL [38] refinement package using full-matrix, least-squares techniques. All non-hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atom positions were determined by geometry and refined by a riding model.

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Reference

1. Camilleri, M., et al., *Measurement of serum 7alpha-hydroxy-4-cholesten-3-one (or 7alphaC4), a surrogate test for bile acid malabsorption in health, ileal disease and irritable bowel syndrome using liquid chromatography-tandem mass spectrometry.* Neurogastroenterol. Motil., 2009. **21**: p. 734-e43.
2. Gothe, F., et al., *Bile acid malabsorption assessed by 7alpha-hydroxy-4-cholesten-3-one in pediatric inflammatory bowel disease: correlation to clinical and laboratory findings.* Journal of Crohn's and Colitis, 2014. **8**: p. 1072-1078.
3. Pattni, S.S., et al., *Fibroblast growth factor 19 and 7alpha-hydroxy-4-cholesten-3-one in the diagnosis of patients with possible bile acid diarrhea.* Clinical and Translational Gastroenterology, 2012. **3**: p. e18.
4. Wong, B.S., et al., *Increased bile acid biosynthesis is associated with irritable bowel syndrome with diarrhea.* Clinical gastroenterology and hepatology, 2012.
5. DeBarber, A.E., et al., *ESI-MS/MS quantification of 7alpha-hydroxy-4-cholesten-3-one facilitates rapid, convenient diagnostic testing for cerebrotendinous xanthomatosis.* Clinica Chimica Acta, 2010. **411**: p. 43-48.
6. Murakami, K., Y. Okada, and K. Okuda, *Purification and Characterization of 7α-Hydroxy-4-cholesten-3-one 12α-Monooxygenase.* J. Biol. Chem., 1982. **257**: p. 8030-8035.
7. H, I., et al., *Purification and Characterization of 7α-Hydroxy-4-cholesten-3-one 12α-Hydroxylase.* J. Biol. Chem., 1992. **267**: p. 21319-21323.
8. Woollett, L.A., et al., *Cholic Acid Supplementation Enhances Cholesterol Absorption in Humans.* Gastroenterology, 2004. **126**: p. 724-731.
9. Griffiths, W.J., et al., *Additional pathways of sterol metabolism: Evidence from analysis of Cyp27a1 -/- mouse brain and plasma.* BBA - Molecular and Cell Biology of Lipids, 2019. **1864**: p. 191-211.

10. Russell, D.W. and K.D.R. Setchell, *Bile Acid Biosynthesis.* Biochemistry, 1992. **31**: p. 4738-4749.
11. Goodwin, B., et al., *Identification of bile acid precursors as endogenous ligands for the nuclear xenobiotic pregnane X receptor.* PNAS, 2003. **100**: p. 223-228.
12. Danielsson, H., *Synthesis and metabolism of delta4-cholestene-7alpha-ol-3-one.* Acta Chemica Scandinavica, 1961. **15**: p. 242-248.
13. Byon, C.-Y., H.L. Kimball, and M. Gut, *Synthesis of 7alpha-hydroxycholesterol-1,2-^{3}H.* Steroids, 1977. **30**: p. 419-423.
14. Alexander, D.L. and J.F. Fisher, *A convenient synthesis of 7alpha-hydorxycholest-4-en-3-one by the hydroxypropyl-beta-cyclodextrin-facilitated cholesterol oxidase oxidation of 3beta,7alpha-cholest-5-ene-3,7-diol.* Steroids, 1995. **60**: p. 290-294.
15. Ogawa, S., et al., *An efficient synthesis of 7alpha,12alpha-dihydroxy-4-cholesten-3-one and its biological precursor 7alpha-hydroxy-4-cholesten-3-one: Key intermediates in bile acid biosynthesis.* Steroids, 2013. **78**: p. 927-937.
16. Roussi, F., et al., *The design and synthesis of new steroidal compounds as potential mimics of taxoids.* Eur. J. Org. Chem., 2005: p. 3952-3961.
17. Selvaraj, F., *Competitive Immunoassay Methods.* WO 2018/011691 A1. International Publication Date: 18 January 2018.
18. Offei, S.D., et al., *Chemical synthesis of 7-oxygenated 12alpha-hydroxy steroid derivatives to enable the biochemical characterization of cytochrome P450 8B1, the oxysterol 12alpha-hydroxylase enzyme implicated in cardiovascular health and obesity.* Steroids, 2018. **140**: p. 185-195.
19. Salmond, W.G., M.A. Barta, and J.L. Havens, *Allylic oxidation with 3,5-dimethylpyrazole. Chromium trioxide complex. Steroidal Δ^{5}-7-ketones.* J. Org. Chem., 1978. **43**: p. 2057-2059.
20. Greenhalgh, C.W., H.B. Henbest, and E.R.H. Jones, *Studies in the sterol group. Part LIII.* The preparation of 7-hydroxy-3-keto-delta4-steroids.* Journal of Chemical Society, 1952: p. 2375-2380.
21. Ghosh, P., et al., *Oxysterols: synthesis and anti-leishmanial activities.* Steroids, 2016. **107**: p. 65-73.
22. Yoshimoto, F.K., et al., *Chemical synthesis of 7alpha-hydroxypregnenolone, a neuroactive steroid that stimulates locomotor activity.* Steroids, 2017. **128**: p. 50-57.
23. Salvador, J.A.R. and J.H. Clark, *The allylic oxidation of unsaturated steroids by tert-butyl hydroperoxide using homogeneous and heterogeneous cobalt acetate.* Chem. Commun., 2001: p. 33-34.
24. Choi, H. and M.P. Doyle, *Optimal TBHP allylic oxidation of Δ^{5}-steroids catalyzed by dirhodium caprolactamate.* Organic Letters, 2007. **9**: p. 5349-5352.
25. Gao, J., et al., *Novel synthesis strategy for the preparation of individual phytosterol oxides.* J. Agric. Food Chem., 2013. **61**: p. 982-988.
26. Salvador, J.A.R., M.L. Sa e Melo, and A.S. Campos Neves, *Copper-catalysed allylic oxidation of Δ^{5}-steroids by t-butyl hydroperoxide.* Tetrahedron Letters, 1997. **38**: p. 119-122.

27. Alexander, D.L. and J.F. Fisher, *A Convenient Synthesis of 7α-Hydroxycholest-4-en-3-one by the Hydroxypropyl-ß-Cyclodextrin-Facilitated Cholesterol Oxidase Oxidation of 3ß,7α-holet-5-ene-3,7-diol.* Steroids, 1995. **60**: p. 290-294.
28. Bartoszewicz, A., et al., *A new reagent system for efficient silylation of alcohols: silyl chloride-N-methylimidazole-iodine.* Synlett, 2008. **1**: p. 37-40.
29. Ratnikov, M.O., et al., *Allylic oxidation catalyzed by dirhodium(II) tetrakis[ε-caprolactamate] of tert-butyldimethylsilyl-protected trans-dehydroandrosterone.* Org. Syn., 2012. **89**: p. 19-33.
30. Tal, D.M., *6,7-Dihydroxy-6,7-dihydrocanrenone isomers: improved synthesis and proton NMR study.* Steroids, 1989. **54**: p. 113-122.
31. SImard, J., et al., *Molecular biology of the 3beta-hydroxysteroid dehydrogenase/delta5 - delta4 isomerase gene family.* Endocrine Reviews, 2005. **26**: p. 525-582.
32. Zarrouk, A., et al., *7beta-Hydroxycholesterol-induced cell death, oxidative stress, and fatty acid metabolism dysfunctions attenuated with sea urchin egg oil.* Biochimie, 2018. **153**: p. 210-219.
33. Kent, G.J. and E.S. Wallis, *Preparation and solvolysis of epi-psi-cholesterol.* The Journal of Organic Chemistry, 1959. **24**: p. 1235-1239.
34. Nickon, A. and J.F. Bagli, *Reactivity and geometry in allylic systems. I. Stereochemistry of photosensitized oxygenation of monoolefins.* J. Am. Chem. Soc., 1961. **83**: p. 1498-1508.
35. 1.171.38.41, C., *Rigaku Oxford Diffraction.* 2015.
36. *SCALE3 ABSPACK - An Oxford Diffraction Program(1.0.4,gui:1.0.3) (C).* Oxford Diffraction Ltd., 2005.
37. Dolomanov, O.V., et al., *OLEX2: a complete structure solution refinement and analysis program.* J. Appl. Cryst., 2009. **42**: p. 339.
38. Sheldrick, G.M., *A short history of SHELX.* Acta Cryst. Section A, 2008. **A64**: p. 112-122.

Highlights

-Regioselective C3-oxidation of a C3-desoxy-Δ^4-7-oxygenated steroid precursor

-X-ray crystal structure of the title compound (7alpha-hydroxy-cholest-4-en-3-one)