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Improved Enantioselectivity in the Epoxidation of Cinnamic Acid Derivatives with Dioxiranes from Keto Bile Acids

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The asymmetric epoxidation of substituted cinnamic acids has been obtained in the presence of different keto bile acid derivatives as optically active carbonyl inducers and Oxone as oxygen source. Predominant or almost exclusive formation of both enantiomeric epoxides is obtained (ee up to 95%) depending on the specific substitution at carbons C(7) and C(12) of the bile acid.

Over the past 20 years, dioxiranes have been shown to be powerful, versatile, and selective oxygen-transfer reagents for a variety of oxyfunctionalization reactions.[1] In this context, the use of chiral dioxiranes, generated by the action of potassium monoperoxysulfate $KHSO_5$ (Oxone) on appropriate optically active ketones for asymmetric epoxidations,[2,3] is unquestionably the most important transformation for synthetic purposes. Since the first report by Curci and co-workers[2a] in 1984 (up to 20% ee for *trans*-β-methylstyrene epoxide), an intensive research effort has been focused on the engineering of suitable chiral ketones stable to oxidative degeneration, kinetically competent and highly stereoselective. Toward this end, important breakthroughs (ee >98%) have been achieved by Yang et al.,[2d] employing a C_2-symmetric binaphthyl-based type **1** ketone, and by Shi et al.,[3c] using D-fructose-derived type **2a** or (−)-quinic acid derived type **2b** ketones (Chart 1).

The ester groups of type **1** ketones seem to be essential to ensure rigidity and C_2 symmetry.[2d,i] On the other hand, for precursors **2a**,**b,** the catalyst architecture was based on derivatives having stereogenic centers in the vicinity of the ketone moiety and fused ring(s) or quaternary carbon α to the reacting carbonyl groups, to ensure efficient stereochemical communication between the substrate and the oxidant.[2k] Many efforts have been made to improve the catalytic performances of fructose-derived ketone **2a** through modification of the protecting groups of the alcohol functions; however, **2a** remains the most effective catalyst for the dioxirane-mediated asymmetric oxyfunctionalization of different classes of organic molecules and, consequently, the most frequently used.[1e]

We recently reported[3f] an efficient epoxidation protocol based on the use of dehydrocholic acid (3,7,12-triketo-5β-cholan-24-oic acid), a cheap and commercially available bile acid, as optically active carbonyl inducer for the enantioselective oxidation of different cinnamic acid derivatives with enantiomeric excesses up to 75%. The structure of dehydrocholic acid, depicted as compound **7** in Chart 2, as well as that of many other bile acids, is characterized by a notable robustness and rigidity of the steroid skeleton that prevents any distortion, thus maintaining unaltered the chiral elements of the ketone function also after its conversion into dioxirane. The structural durability of **7** and related derivatives is also demonstrated by their insignificant loss in the times required to complete the oxidations.[3f,4] Moreover, the carboxylic moiety on the lateral chain allows solubility in almost all solvents, including aqueous media. Due to these peculiar characteristics, our investigation has been

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(3) For asymmetric epoxidations that include electron-poor olefins, see: (a) Wang, Z. X.; Shi, Y. *J. Org. Chem.* **1997**, *62*, 8622. (b) Amstrong, A.; Hayter, B. R. *Chem. Commun.* **1998**, 621. (c) Wang, Z.-X.; Miller, S. M.; Anderson, O. P.; Shi, Y. J. *J. Org. Chem.* **1999**, *64*, 6443. (d) Solladié-Cavallo, A.; Bouérat, L. *Org. Lett.* **2000**, *2*, 3531. (e) Porter, M. J.; Skidmore, J. *Chem. Commun.* **2000**, 1215. (f) Bortolini, O.; Fogagnolo, M.; Fantin, G.; Maietti, S.; Medici, A. *Tetrahedron: Asymmetry* **2001**, *12*, 1113.

(4) A consistent modification (ca. 20%) of the bile acid due to over-oxidation (Baeyer−Villiger?) or ketone inactivation is observed only after 24 h. See also ref 2k.

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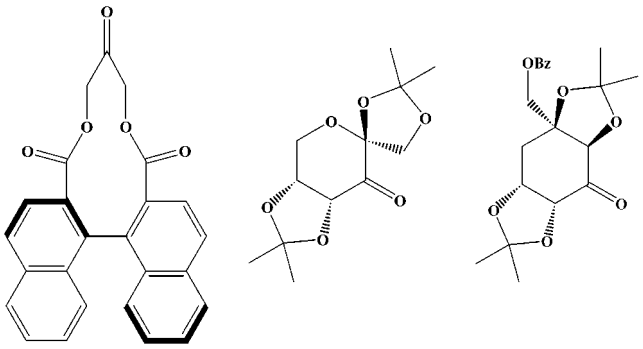
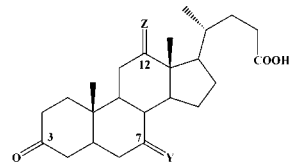
CHART 1

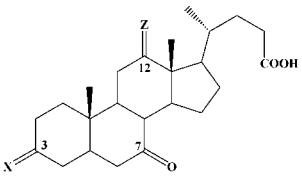


1 **2a** **2b**

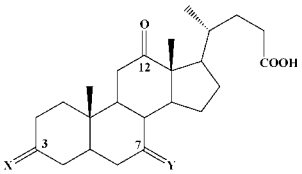
CHART 2. 5β-Cholan-24-oic Acids Investigated in This Study (Succ = $CO(CH_2)_2COOH$)



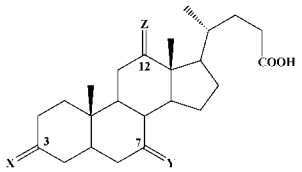
	3-keto bile acids
3a	Y=Z=H,H
3b	Y=α-OH,H; Z=H,H
3c	Y=β-OH,H; Z=H,H
3d	Y=α-OSucc,H; Z=H,H
3e	Y=β-OSucc,H; Z=H,H
3f	Y=H,H; Z=α-OH,H
3g	Y=Z=α-OH,H
3h	Y=Z=α-OAc,H



	7-keto bile acids
4a	X=Z=H,H
4b	X=α-OH,H; Z=H,H
4c	X=Z=α-OH,H



	12-keto bile acids
5a	X=Y=H,H
5b	X=α-OH,H; Y=H,H
5c	X=Y=α-OH,H



	di-keto and tri-keto bile acids
6a	X=Y=O; Z=H,H
6b	X=Z=O; Y=H,H
6c	X=H,H; Y=Z=O
6d	X=Y=O; Z=α-OH,H
6e	X=Z=O; Y=α-OH,H
6f	X=α-OH,H; Y=Z=O
7	X=Y=Z=O

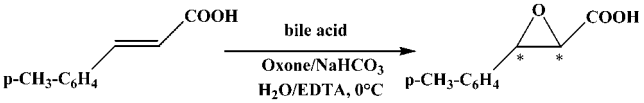
extended to the almost entire class of 5β-cholan-24-oic acids,[5] Chart 2, using the enantioselective epoxidation of electron-poor olefins as reaction probe.

As shown in Chart 2, the bile acids have been grouped according to the number and position of the carbonyl function(s), as well as on the basis of the hydrogen, hydroxy, acetoxy, and hemisuccinyloxy substituents at carbons C(3), C(7), and C(12).

The major intents of this investigation have been the following: (i) the study of the specific reactivity of the different carbonyl moieties pertaining to the chiral ketone; (ii) the evaluation of the role played by the different carbonyls with respect to their position and relationship with the other substituents of the bile acid; (iii) the effect of the C(3), C(7), and C(12) substituents,

(5) *The Bile Acids. Chemistry, Physiology and Metabolism*; Nair, P. P., Kritchevsky, D., Eds.; Plenum Press: New York, 1971; Vol. 1.

TABLE 1. Asymmetric Epoxidation of *p*-Methylcinnamic Acid as a Function of Bile Acid 3−7



entry	bile acid	reaction time (h)	olefin convn[a] (%)	epoxide yield[b] (%)	opt. rot. sign[c]	ee[a] (%)
1[d]	**3a**	24	18	15	(+)	32
2	**3b**	2	50	45	(−)	26
3	**3c**	2	99	91	(+)	16
4	**3d**	2	99	90	(−)	50
5	**3e**	2	99	93	(+)	49
6	**3f**	2	99	94	(+)	95
7	**3g**	0.5	40	35	(+)	38
8	**3h**	0.5	42	35	(+)	18
9	**4a**	2	33	25	(+)	5
10	**4b**	24	10	6	(+)	6
11	**4c**	0.5	9	6	(+)	41
12	**5a**	24	6			
13	**5b**	24	5			
14	**5c**	24	5			
15	**6a**	0.5	99	92	(+)	36
16	**6b**	0.2	99	93	(+)	74
17	**6c**	24	45	38	(+)	8
18	**6d**	0.2	99	89	(+)	87
19	**6e**	0.5	99	91	(+)	48
20	**6f**	2	47	41	(+)	15
21	**7**	0.5	99	94	(+)	75

[a] Obtained by GC, on the corresponding methyl esters, using a chiral column. [b] Based on material isolated after chromatography. [c] Solvent $CHCl_3$. [d] In the presence of sodium cholate 50 mmol.

in terms of oxidation status (i.e., H, OH, OAc, OSucc, carbonyl), $\alpha-\beta$ (axial−equatorial) positional stereochemistry and bulkiness on the enantiomeric excess and configuration of the epoxidation products. For this latter purpose, we provide preliminary results, based on molecular mechanics calculations, for a preferential approach of the olefin to the lipophilic face of the bile acid dioxirane derivative.

Results and Discussion

Ketones **3**−**7**, the majority commercially available, are readily prepared from common bile acids by adapting the reported procedures (see the Experimental Section for details). Their oxidative efficiency was tested in the epoxidation of the model substrate *p*-methylcinnamic acid, using the optimized conditions reported in our previous study.[3f] The reaction is conveniently performed in water−$NaHCO_3$ at 0 °C using equimolar concentrations of olefin and bile acid inducer **3**−**7**, in the presence of an excess of Oxone (3 equiv). The results, collected in Table 1, show that the different chiral ketones mediate the epoxidation of the selected olefin with varying degrees of efficiency and selectivity.

Of particular interest is the role played by the carbonyl group at carbon C(3), poorly effective if present as the sole function within the molecule (Table 1, entry 1), highly efficient when in the presence of different functions at C(7) and/or C(12) of the bile acid framework (Table 1, entries 3−6, 15, 16, 18, 19, and 21). The apparent low capability of the 3-keto bile acid **3a** to mediate the oxygen-transfer process, however, is related to its scarce solubility in the reaction medium, insolubility that represents the major and striking physiochemical difference with the other bile acids of the series.[5] A partial indication of its reactivity has been obtained by using

slightly modified reaction conditions such as the addition of sodium cholate to the solution,[5] an additive known to increase the solubility of **3a** and to which the results of Table 1 refer. Despite the difficulties in obtaining a real measure of the oxidation activity of the 3-keto function unaffected from other contributions, however, several pieces of evidence support the preferential activation of the C(3) carbonyl group by Oxone to form the bile acid dioxirane intermediate. As shown in Table 1, in fact, the almost complete conversion of the substrate, in short reaction times, is obtained only in the presence of 3-keto-substituted bile acids (Table 1, entries 3−6 and others). Nevertheless, a moderate, although clear, oxidation activity is observed also with the 7-keto **4a** and the 7,-12-diketo-3-hydroxy bile acid **6f**, both lacking of C(3) carbonyl groups. Since the 12-keto derivative **5a** has no effect on the epoxidation process (Table 1, entry 12), the observed reactivity must be ascribed to the carbonyl function at C(7) that, although in much lower extent compared to C(3), may be activated by $KHSO_5$.

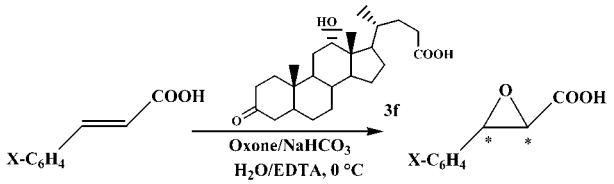
A possible explanation of the preferential activation of the C(3) carbonyl group in 5β-cholan-24-oic acids by Oxone, to afford the corresponding dioxirane, might be found in the known peculiar tendency of this carbon atom to change its hybridization from sp^2 to sp^3. The observation that 3-keto functions of bile acids are prone to ketalize in methanol, in the absence of catalyst, to afford the corresponding (3,3)-dimethoxy-5β-cholan-24-oic acid derivatives supports to this hypothesis.[6] In this specific case, the degree of ketalization follows the sequence 3,-12-diketo **6b** >3-keto **3a** > 3,7,12-triketo **7** > 3,7-diketo **6a**. The ketalization of the C(7) and/or C(12) carbonyl groups was not observed in any case.

One of the most outstanding aspects of the data collected in Table 1 is the control on the enantioselectivity operated by the substituents at carbons C(7) and C(12). Entries 6 and 16, for example, clearly demonstrate that α-hydroxy substitution at C(12) addresses the enantioselectivity of the reaction toward the predominant or almost exclusive formation of the (+) enantiomer (ee 95% using the 3-keto-12-hydroxy derivative **3f**). Noteworthy is the fact that α-hydroxy substitution at C(7) directs the epoxidation toward the formation of the opposite enantiomer, entry 2 (Table 1). For this specific position, two are the major factors that govern the optical yield and sign of the resulting epoxide: the α−β (axial−equatorial) stereochemistry of the C(7) substituent and the bulkiness of the group (hydroxy vs hemisuccinyloxy). As proved by the oxidation reactions reported in entries 3 and 5 of Table 1, mediated by the 3-keto-7β-hydroxy **3c** and 3-keto-7β-hemisuccinyloxy **3e** bile acids, β-substitution at C(7) redirects the stereochemical course of the epoxidation toward the formation of the (+) enantiomer, obtained in excellent chemical yields and ee's ranging from moderate to low. Therefore the α-stereochemistry of the substituent at this specific position appears to be fundamental for the synthesis of the (−) enantiomer. Furthermore, the role played by the bulkiness of the C(7) substituent is well evident by comparing the data of entries 2 vs 4 and 3 vs 6 of Table 1. A general increase of the enantiomeric excess is observed by replacing the hydroxy substituent with the larger succinyloxy group, from 26 to 50% and from 16 to 49%, respectively, thus confirming that steric as well as long-range effects contribute to guide the course of the epoxidation. Long-range effects of different substituents in steroids have been observed by Blickenstaff et al.[7,8] during the acetylation of methyl cholates.

To further reveal the peculiar features of the bile acids as chiral inducers, the two most reactive and of opposite stereochemical outcome, 3-keto-12-α-hydroxy **3f** and 3-keto-7α-hemisuccinyloxy **3d** ketones, were chosen as representatives to explore the epoxidation of different cinnamic acid derivatives. The pertinent results are collected in Tables 2 and 3.

A significant increase of the enantiomeric excess is observed in all cases if compared with the data obtained using dehydrocholic acid[3f] **7** (Table 2) and the less hindered 3-keto-7α-hydroxy-5β-cholan-24-oic acid **3b** (Table 3) with few noticeable features. Independently from the cinnamic acid derivative, α-hydroxy substitution at C(12) was confirmed to be the leading factor in order to obtain the predominant or almost exclusive formation of the (+) enantiomer. On the other hand, α-hydroxy or α-hemisuccinyloxy substitution at C(7) affords the op-

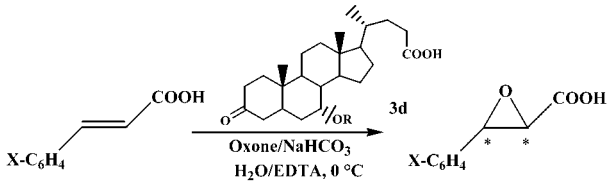
TABLE 2. Asymmetric Epoxidation of Cinnamic Acid Derivatives Using 3f as Chiral Ketone



entry	X	reaction time (h)	epoxide yield[a] (%)	opt. rot. sign[b]	ee[a] (%)
1	*p*-CH_3	2	99	(+)	95 (73)[c]
2	H	2	80	(+)[d]	70 (40)[c]
3	*m*-F	2	57	(+)	63 (46)[c]
4	*o*-Br	2	75	(+)	82 (55)[c]

[a] Obtained by GC on a chiral column. [b] In $CHCl_3$. [c] Enantiomeric excess obtained using dehydrocholic acid **7** as chiral ketone. [d] Absolute configuration (2*S*,3*R*).

TABLE 3. Asymmetric Epoxidation of Cinnamic Acid Derivatives Using 3d as Chiral Ketone



entry	X	reaction time (h)	epoxide yield %[a]	opt. rot. sign[b]	ee (%)[a]
1	*p*-CH_3	2	98	(−)	50 (40)[c]
2	H	2	79	(−)[d]	48 (40)[c]
3	*m*-F	2	70	(−)	40 (23)[c]
4	*o*-Br	2	78	(−)	57 (47)[c]

[a] Obtained by GC on a chiral column. [b] In $CHCl_3$. [c] Enantiomeric excess obtained using 3-keto-7α-hydroxy-5β-cholan-24-oic acid **3b** as chiral ketone. [d] Absolute configuration (2*R*,3*S*).

(6) Fantin, G.; Fogagnolo, M.; Medici, A.; Pedrini, P.; Cova, U. *Tetrahedron Lett.* **1992**, *22*, 3235.

(7) Blickenstaff, R. T.; Sophsan, K. *Tetrahedron* **1972**, *28*, 1945.
(8) Blickenstaff, R. T.; Orwig, B. *J. Org. Chem.* **1969**, *34*, 1377.

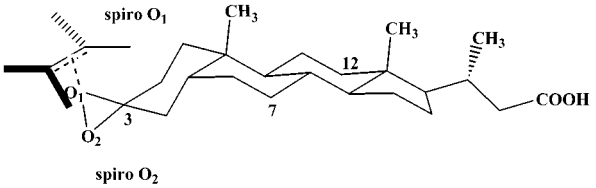


FIGURE 1. Face selectivity of the spiro transition states.

posite (−) enantiomer in good optical and chemical yields, Table 3. Also in this latter case, a moderate although significant increase of the enantiomeric excess is observed, up to 57%, by replacing the α-hydroxy substituent of derivative **3b**, data in parentheses, with the larger α-hemisuccinyloxy group of bile acid **3d**. The poor substrate conversion (3−5% in 2 h) observed in the absence of **3**−**7** with all olefins confirmed that the bile acid is essential for the progress of the reaction.

The obtained opposite enantioselectivity as a function of the chiral carbonyl inducer represent the most peculiar aspect of this study. A possible explanation of the observed selectivity has been addressed by carrying out molecular mechanics calculations.[9] It has been shown by many authors that the epoxidation with dioxirane proceeds mainly via a spiro transition state,[2d−e,10] orientation that could benefit of a stabilizing interaction of an oxygen lone pair with the π^* orbital of the alkene.[10a] In principle, the olefin can be oxidized by both O_1 and O_2 dioxirane oxygen atoms, and consequently, two series of four transition states can be hypothesized depending on the geometry of the double bond spiro O_1 or spiro O_2 approach, Figure 1.

We have evaluated the minimized dioxirane−alkene adducts of the two sets of transition states on the basis of the following parameters: (i) overall change of the distance between the O_1 or O_2 dioxirane oxygen and the double-bond carbon atoms; (ii) preservation of the spiro transition state; (iii) total steric energy. In this frame the qualitative analysis of the calculations indicate, for the studied cases, a remarkable face selectivity toward the more lipophilic side[5] of the bile acid, approach spiro O_1, with the carboxylic function of the alkene likely acting as dipolar opponent of the van der Waals repulsion.[11] The stereochemistry of the resulting epoxide, obtained as minimum value of the different i−iii parameters, is fully consistent with the experimental results. A refining of these calculations using hybrid QM-MM methods[9,12] as well as the extension of the epoxidation protocol to electron-poor cis olefins are currently in progress.

In conclusion, we have demonstrated that the use of bile acid inducers having a carbonyl function at C(3) as well as specific and stereochemically appropriate C(7) and C(12) substitutions has a large effect on the reactivity and selectivity during asymmetric epoxidations with Oxone. In the case of cinnamic acid derivatives, the predominant or almost exclusive formation of the (+) or (−) epoxide may be obtained by selecting α-hydroxy C(12) or α-hemisuccinyloxy C(7) substitution, respectively. Although the assignment of a precise stereochemical model for the transition state of this reaction may still be premature, we provide evidence for a preferential approach of the olefin to the lipophilic face of the bile acid dioxirane derivative.

Experimental Section

General Methods. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, except where otherwise indicated. Oxidation reactions were monitored via quantitative GC analysis using a Megadex DETTBSβ capillary column. Optical rotations were obtained in $CHCl_3$. Oxone and cinnamic acid derivatives are commercially available compounds used without further purification. Melting points were uncorrected and determined in open-ended capillaries. Computational studies were carried out by minimizing all the molecular models by MM2[9] using Chem3D and comparing the obtained results according to i−iii parameters; see text.

General Procedure for Epoxidation Reactions on an **Analytical Scale.** In a 10 mL volumetric flask were dissolved the cinnamic acid derivative (0.1 mmol) and the chiral ketone **3**−**7** (0.1 mmol) in 5% aqueous $NaHCO_3$ (5 mL, pH ca. 8) in the presence of EDTA (0.1 mL of a solution 4 10^{-4} M) as a sequestrant of trace transition-metal ions.[13] After the mixture was cooled to 0 °C, 0.3 mmol of Oxone was added under stirring. At increasing reaction times, samples of the mixture were taken out, quenched with HCl 5%, extracted with ethyl acetate, converted to the corresponding methyl ester by treatment with diazomethane and monitored by GC analysis on a chiral column to estimate both alkene conversion and the enantiomeric excess.

General Procedure for Epoxidation Reactions on a **Preparative Scale.** In a 250 mL Erlenmeyer flask were dissolved the cinnamic acid derivative (5 mmol) and the chiral ketone **3**−**7** (5 mmol) in 5% aqueous $NaHCO_3$ (100 mL, pH ca. 8) in the presence of EDTA (5 mL of a solution 4×10^{-4} M) as a sequestrant of trace transition-metal ions.[13] After cooling to 0 °C, 15 mmol of Oxone was added under stirring. After the appropriate reaction time, see Table 1, the reaction was treated as described above and the epoxide was separated from the crude mixture by column chromatography over silica gel, characterized by ^{1}H and ^{13}C NMR analysis, and submitted to optical rotation measurement and chiral GC analysis. The absolute configuration of (+)-(2*S*,3*R*)-phenylglycidic acid was obtained from the corresponding methyl ester by comparison with literature values.[14] For all other cases, only the sign of the optical rotations are given ($CHCl_3$).

Materials. The chiral carbonyl inducers **3**−**7** are almost all commercially available products that, alternatively, may be prepared from common bile acids according to the following procedures.

3-Keto- (3a), 3,7-diketo- (6a), 3,12-diketo- (6b), and 3,7,-12-triketo-5β-cholan-24-oic acid (7) were prepared starting from the 3-hydroxy, 3,7-dihydroxy, 3,12-dihydroxy, and 3,7,-12-trihydroxy precursors, respectively, via oxidation with the Jones' reagent.[15] This reagent was obtained by adding 2.2 g of chromium trioxide to 2.8 mL of concentrated sulfuric acid and the solution diluted to 10 mL with water. The hydoxy bile acid precursor (5 mmol) was dissolved in 50 mL of acetone and treated with Jones' reagent until a slight permanent

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orange color was obtained. After the mixture was allowed to stand for 5 min, several drops of isopropyl alcohol were added to destroy the oxidant in excess. The reaction mixture was filtrated over Celite, concentrated under reduced pressure, diluted with 20 mL of water and extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed in a vacuum to give the expected carbonyl derivative in nearly quantitative yield. **3a**: white crystal; mp 138−140 °C (lit.[5] mp 140 °C); ^{1}H NMR[16,17] δ 0.69 (s, 3H), 0.92 (d, J = 5.2 Hz, 3H), 1.01 (s, 3H), 3.62 (s, 3H). **6a**: white crystal; mp 154−155 °C (lit.[18] mp 153−154 °C); ^{1}H NMR[16,17] δ 0.69 (s, 3H), 0.92 (d, J = 5.7 Hz, 3H), 1.27 (s, 3H), 3.65 (s, 3H). **6b**: white crystal; mp 184−186 °C (lit.[5] mp 187 °C); ^{1}H NMR[16,17] δ 0.87 (d, J = 6 Hz, 3H), 1.05 (s, 3H), 1.10 (s, 3H), 3.60 (s, 3H). **7**: white crystal; mp 237−240 °C (lit.[19] mp 238−240 °C); ^{1}H NMR[16,17] δ 0.85 (d, J = 5.2 H, 3H), 1.06 (s, 3H), 1.40 (s, 3H), 3.61 (s, 3H).

7-Keto- (4a), 12-keto- (5a), and 7,12-diketo-5β-cholan-24-oic acid (6c) were prepared starting from the 3-keto-7-hydroxy **3b**, 3-keto-12-hydroxy **3f**, and 3-keto-7,12-dihydroxy derivative **3g**, respectively. Four grams of 85% KOH and 5 mL of 80% hydrazinium hydroxide were added to a solution containing 10 mmol of **3b**, **3f**, or **3g**, 35 mL of methylcellosolve (ethylene glycol monomethyl ether), and 3 mL of H_2O. The solution was heated at 110 °C for 4 h and then distilled to obtain a temperature of the reaction mixture of about 135 °C. This temperature was maintained for an additional 8 h. The reaction mixture was cooled to 20 °C, diluted with 12 mL of water, and acidified to pH 2 using H_2SO_4 (40%).[20] The product was collected by filtration and submitted to the oxidation step using Jones' protocol described for compound **3a**; see the previous paragraph. **4a**: overall yield 70%; white crystal; mp 149−151 °C (methanol/water) (lit.[21] mp 150−151 °C); ^{1}H NMR[16,17] δ 0.65 (s, 3H), 0.90 (d, J = 5.2 Hz, 3H), 1.18 (s, 3H), 3.65 (s, 3H). **5a**: overall yield 75%; white crystal; mp 185−187 °C (ethyl acetate/*n*-hexane) (lit.[22] mp 187−189 °C); ^{1}H NMR[16,17] δ 0.82 (d, J = 6 Hz, 3H), 0.99 (s, 6H), 3.62 (s, 3H). **6c**: overall yield 65%; white crystal; mp 119−121 °C (ethyl acetate/*n*-hexane) (lit.[23] mp 120−122 °C); ^{1}H NMR[16,17] δ 0.83 (d, J = 6 Hz, 3H), 1.02 (s, 3H), 1.27 (s, 3H) 3.62 (s, 3H).

3,7-Diketo-12α-hydroxy-5β-cholan-24-oic acid (6d) was obtained from the 3-keto-7,12-dihydroxy derivative **3g** by oxidation with NBS.[24] A 2.03 g (5 mmol) portion of **3g** was dissolved, upon warming, in 75 mL of tap water containing 2.5 g of sodium bicarbonate. The solution was cooled to 25 °C, treated with 1.11 g (6.25 mmol) of *N*-bromosuccinimide, and shaken occasionally until complete dissolution of the reagent. The yellow solution was allowed to stand at 25 °C for about 24 h, heated on the steam bath for 1 h, cooled to 0 °C, and acidified with 10% aqueous HCl. The keto acid **6d** separate as a white solid that was collected by filtration and crystallized from ethyl acetate: yield 1.5 g (75%); mp 168−170 °C (lit.[25] mp 168−170 °C); ^{1}H NMR (CD_3OD)[25] δ 0.75 (s, 3H), 1.00 (d, J = 6 Hz, 3H), 1.30 (s, 3H), 4.05 (m, 1H).

3-Keto-7α-succinyloxy-5β-cholan-24-oic acid (3d) and **3-keto-7β-succinyloxy-5β-cholan-24-oic acid (3e)** were prepared from the commercially available chenodeoxycholic or ursodeoxycholic acid, respectively. A 5 g (12.7 mmol) portion of chenodeoxycholic or ursodeoxycholic acid was suspended in ethyl acetate (30 mL) in the presence of succinic anidride (6 g, 60 mmol), triethylamine (1.27 mL, 12.7 mmol), and DMAP (0.15 g, 1.27 mmol). The reaction mixture, monitored by TLC (ethyl acetate/cyclohexane/HAc 50/50/1), was refluxed for 40 or 10 h, respectively, depending on the bile acid precursor. After completion, 20 mL of ethyl acetate and 15 mL of water were added to the mixture, and the organic layer was washed with acid water (15 mL, pH ca. 1), dried on anydrous Na_2SO_4, and evaporated to obtain the bis-hemisuccinate. The bis-hemisuccinates were used for the subsequent reaction without further purification. The identity of both intermediates could be validated by ^{1}H NMR: (α-isomer) δ 0.65 (s, 3H), 0.95 (s, 3H), 0.97 (d, J = 6.5 Hz, 3H), 4.60 (m, 1H), 4.92 (m, 1H); (β-isomer) (CD_3OD) δ 0.70 (s, 3H), 0.92 (d, J = 6.5 Hz, 3H), 0.98 (s, 3H), 4.65 (m, 1H), 4.78 (m, 1H). The C(3) hydrolysis of both products was obtained by dissolving the bis-hemisuccinates in MeOH/NaOH 5% (1:1 v/v), following the reaction by TLC (ethyl acetate/cyclohexane/HAc 50:50:1). After 1 h, the solution was acidified with dilute H_2SO_4. Addition of 50 mL of H_2O caused the precipitation of the 3-hydroxy derivative that was recovered by filtration. The 7-hemisuccinates were used for the subsequent reaction without further purification. The identity of both intermediates could be validated by ^{1}H NMR: (α-isomer) ($CDCl_3$/CD_3OD 1:1 v/v) δ 0.60 (s, 3H), 0.88 (s, 3H), 0.90 (d, J = 6.5 Hz), 3.42 (m, 1H), 4.85 (m, 1H); (β-isomer) δ 0.65 (s, 3H), 0.92 (d, J = 6.5 Hz, 3H), 0.95 (s, 3H), 3.58 (m, 1H), 4.75 (m, 1H). The further Jones' oxidation of the C(3) hydroxy function was carried out as described for compound **3a**, see previous paragraph. **3d**: final yield 90%; mp 85−87 °C; ^{1}H NMR δ 0.70 (s, 3H), 0.95 (d, J = 6.5 Hz, 3H), 1.05 (s, 3H), 5.05 (m, 1H). **3e**: final yield 92%; mp 92−95 °C; ^{1}H NMR δ 0.73 (s, 3H), 0.94 (d, J = 6.5 Hz, 3H), 1.08 (s, 3H), 4.85 (m, 1H).

3-Keto-7β-hydroxy-5β-cholan-24-oic Acid (3c). A 4.9 g (10 mmol) portion of the 3-keto-7β-hemisuccinyloxy **3e** was dissolved in 30 mL of NaOH (20%) and the reaction mixture refluxed for 1 h. The solution was cooled and acidified with dilute H_2SO_4, and the precipitate was collected by filtration. The crude product was purified by crystallization (ethyl acetate): yield 95%; mp73 °C (ethyl acetate);[26] ^{1}H NMR (CD_3OD)[26] δ 0.75 (s, 3H), 0.96 (d, J = 6.5 Hz, 3H), 1.05 (s, 3H), 3.50 (m, 1H).

3-Keto-7α-hydroxy- (3b),[27] 3-keto-12α-hydroxy- (3f),[27] 3-keto-7,12α-dihydroxy- (3g),[27] 3-keto-7,12α-dihydroxy-acetate- (3h),[27] 3,12-diketo-7α-hydroxy-5β-cholan-24-oic acid (6e),[27] 3,7α-dihydroxy-12-keto-5β-cholan-24-oic acid (5c),[28] 3α-hydroxy-7-keto-5β-cholan-24-oic acid (4b),[29] 3,-12α-dihydroxy-7-keto-5β-cholan-24-oic acid (4c),[29] 3α-hydroxy-12-keto-5β-cholan-24-oic acid (5b),[30] and 3α-hydroxy-7,12-diketo-5β-cholan-24-oic acid (6f)[30] were prepared and characterized according to recently described procedures.[27−30]

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Supporting Information Available: MM2 total and relative energies. This material is available free of charge via the Internet at http://pubs.acs.org.

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