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Oxyfunctionalization of (5β)-Bile Acids by Dimethyldioxirane: Hydroxylation at C-5, C-14, and C-17

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Abstract: Dimethyldioxirane in chloroform solution is an efficient reagent for the tertiary hydroxylation of a series of methyl ester peracetate (5β)-bile acid derivatives. 5β-Hydroxylation was observed in all cases, but the presence of a 7-acetyloxy substituent reduced reactivity of the 5β position allowing competitive 14α- and 17α-functionalization subject to steric and electronic constraints. Copyright © 1996 Elsevier Science Ltd

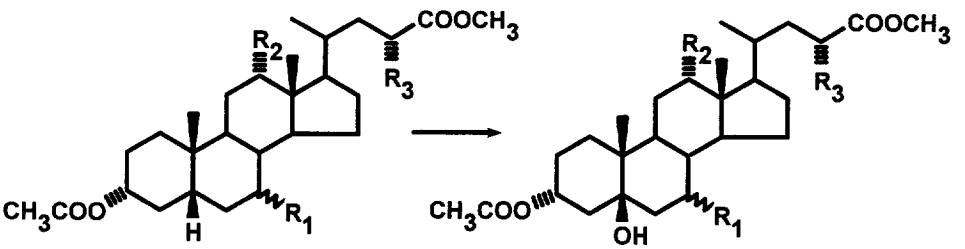
Bile acids are the end products of cholesterol metabolism in most vertebrates. In the small intestine, bile acids function as detergents to solubilize and thereby promote the absorption of dietary lipids. They also serve as an excretable form of cholesterol, necessary for the maintenance of cholesterol homeostasis at the level of the whole organism[2]. In most mammals, cholesterol is converted to 3α,7α-dihydroxy-(5β)-cholan-24-oic acid, *chenodeoxycholic acid* and to a trihydroxy- bile acid in which a hydroxyl group (in addition to 3α and 7α) is inserted on a secondary carbon of the steroidal nucleus or the side chain, for example, at C-6, C-12, C-16 or C-23. We have recently reported that the hamster liver is capable of introducing a tertiary hydroxyl group in the nucleus of *nor-chenodeoxycholic acid* and *nor-ursodeoxycholic acid* (parent compounds of **7** and **8**, respectively) to give the corresponding 5β-hydroxylated derivatives[3]. Hydroxylation by the mammalian liver at a steroidal tertiary carbon is extremely rare, whereas it is common in bacterial biotransformation products and in plants. In order to investigate whether 5β-hydroxy bile acids occur naturally in vertebrates, we required a series of reference compounds for GC-MS studies of natural biliary bile acids.

Three groups have reported the 5β-hydroxylation of methyl ester peracetate derivatives of *lithocholic acid*, **1**, and *deoxycholic acid*, **2**, with dimethyldioxirane (DMDO)[4,5], methyl(trifluoromethyl)-dioxirane (MTFDO)[4], and with perfluorodialkyloxaziridines[6]. Unlike bile acids synthesized by the liver, which always contain 3α- and 7-hydroxyl groups, these two model compounds are produced in nature by bacterial 7-dehydroxylation in the large intestine or the cecum and lack the 7-hydroxyl group. We describe here the regio- and stereoselectivity of the DMDO C-H insertion in a series of 7-hydroxylated (5β)-bile acid derivatives with different side chain length, and some practical aspects of the use of this reagent for preparative purposes.

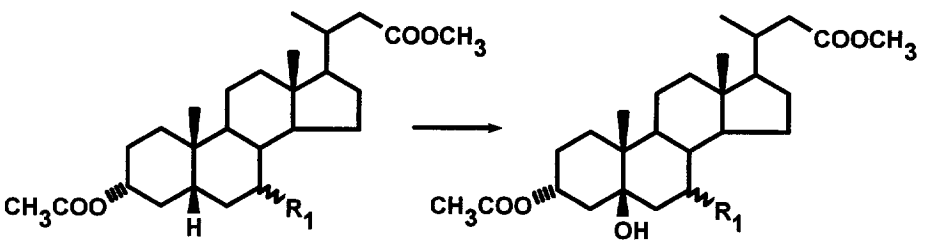
RESULTS

Reaction of the methyl ester peracetate bile acid derivatives **1** and **2** with 2 eq. DMDO in dichloromethane-acetone 2:1 at room temperature gave the 5β-hydroxy derivatives **1a** and **2a** as main products with 35-40% conversions as reported (Scheme I). However, test runs with **3** and **5** proceeded sluggishly, with low conversions even after 48 h. With the more powerful MTFDO in dichloromethane-trifluoroacetone, conversions were high in short times (0.5 - 2 h) in all cases; however, this reagent is relatively expensive and more difficult to generate and handle because of its low boiling point.

Scheme I



1 - 6 **1a - 6a**



7 - 8 **7a - 8a**

	Parent bile acid	R_1	R_2	R_3
1	*Lithocholic*	H	H	H
2	*Deoxycholic*	H	$OCOCH_3$	H
3	*Chenodeoxycholic*	α-$OCOCH_3$	H	H
4	*Ursodeoxycholic*	β-$OCOCH_3$	H	H
5	*Cholic*	α-$OCOCH_3$	$OCOCH_3$	H
6	*Phocaecholic*	α-$OCOCH_3$	H	$OCOCH_3$
7	*Nor-chenodeoxycholic*	α-$OCOCH_3$		
8	*Nor-ursodeoxycholic*	β-$OCOCH_3$		

The rate of DMDO C-H insertion in *cis*-1,2-dimethylcyclohexane is accelerated when the solvent is changed from pure acetone to a 1:1 mixture with CH_2Cl_2 or with $CHCl_3$ by a factor of 2.25 and 3.24, respectively[7]. However, addition of any of these cosolvents to a DMDO acetone solution will cause dilution of both reagent and substrate thus offsetting any reaction rate gain. Indeed, when **1** and **2** were treated with DMDO acetone solution alone (2 eq) the conversions at 24 h were similar to those observed using the solvent mixture.

As DMDO solutions in acetone cannot be concentrated beyond 0.11 M by distillation[8], we attempted to prepare a solution of DMDO in $CHCl_3$ of higher concentration by aqueous extraction of acetone. This procedure has been applied successfully in the case of MTFDO because trifluoroacetone is easily extracted as the hydrate[9]. When 3 volumes of DMDO solution (0.09 M) in acetone were added to one volume of $CHCl_3$ and the organic phase was extracted repeatedly with water at 4 °C, a 0.15 M solution in $CHCl_3$-acetone 3:1 was obtained with good recovery of DMDO. This solution can be kept at -40 °C for many weeks with minimal decrease in concentration (<5%).

Treating the substrate with the required volume of DMDO solution in $CHCl_3$ resulted in conversion rates increased by a factor of 1.7 relative to DMDO solution in pure acetone (Table 1), an improvement sufficient for the practical preparation of the hydroxy bile acid derivatives within reasonable times.

Table 1: Effect of DMDO Concentration and Solvent on Hydroxylation Rate[a].

[DMDO]/solvent	% starting material converted[b]		
	1	**3**	**5**
0.150 M/$CHCl_3$:acetone (3:1)	64	45	25
0.090 M/acetone	38	27	15

[a]2 eq DMDO, 16 h, 23°C. [b]Conversion determined by GC.

For all the bile acid derivatives tested, the reagents DMDO in acetone, DMDO in $CHCl_3$, or MTFDO in trifluoroacetone-CH_2Cl_2 gave the same mixture of products. We carried out all reactions with DMDO solution in $CHCl_3$ in one or two steps as required (see Experimental).

Bile acid derivatives **1**, **2** and **5** gave **1a**, **2a** and **5a**, respectively, as main but not exclusive reaction products. The reaction mixture contained small amounts of numerous other products, the main ones resulting from further hydroxylation of the primary products. In order to optimize the yield of the desired products we adjusted the extent of conversion to achieve a trade off between yield and ease of purification. In spite of the complexity of the reaction mixtures, adsorption chromatography using one or two silica gel columns provided pure compounds.

^{13}C-NMR data for these and other 5β-hydroxy bile acid derivatives described here are presented in Table 2. The assignment of resonances was based on the spectra of the parent compounds[10], APT editing, and shift parameters, as previously discussed for the structure determination of the 5β-hydroxy nor-bile acid metabolites produced by the hamster liver[3] and by Arnone[6].

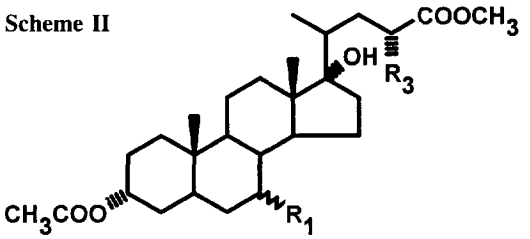
Table 2: Chemical Shifts of Bile Acid Derivatives in $CDCl_3$.

Carbon	4	5	1a	2a	3a	4a	5a	6a	7a	8a	3b	Hα	Hβ	4b	6b	4c	Hα	Hβ
1	34.4	34.6[a]	29.2	29.0	29.3	29.0	29.1	29.2	29.2	29.0	34.8	1.88	1.08	34.4	34.8	34.5	1.82	1.10
2	26.2	26.7	26.0	26.0[a]	26.2	25.8	26.2	26.2	26.1	25.8[a]	26.6	1.45	1.72	26.2	26.6	26.1	1.44	1.70
3	73.5	74.0	71.4	71.2	70.9	70.6	70.8[a]	70.9	70.9	70.6	74.1		4.59	73.7[a]	74.0	73.5		4.67
4	32.7	34.5[a]	38.0	38.0	40.5[a]	39.0	40.6[b]	40.5[a]	40.5[a]	39.0	34.5	2.08	1.59	32.7	34.4	32.7	1.80	1.76
5	41.9	40.8	75.3	75.1	74.4	74.4	74.2	74.3	74.4	74.3	40.9		1.48	41.9	40.8	41.9		1.55
6	32.7	31.1	36.7	36.5	40.8[a]	41.9	40.7[b]	40.8[a]	40.8[a]	41.8	31.2	1.61	1.96	32.7	31.2	32.9	1.65	1.84
7	73.5	70.6	28.5	28.1	70.9	73.9	70.5[a]	70.9	70.9	73.8	71.0		4.91	73.4[a]	71.0	69.4	5.15	
8	39.8	37.6	34.8	34.5	36.9[b]	39.2	36.9	36.9[b]	36.8[b]	39.1	38.1		1.62	40.0	38.1	43.3		1.99
9	39.2	28.8	43.1	37.0	37.1[b]	41.3	31.7	37.1[b]	37.1[b]	41.2	33.8	1.86		38.9	33.8	32.1	2.14	
10	33.8	34.2	39.5	39.0	39.5	39.0	39.2	39.7	39.7	38.9	34.7			33.8	34.6	34.0		
11	21.1	25.4	21.0	27.1	20.7	21.2	25.7	20.7	20.7	21.1	20.4	1.57	1.29	20.8	20.3	19.9	1.41	1.32
12	39.8	75.3	39.7	75.5	39.1	39.6	75.0	39.0	38.9	39.4	32.0	1.62	1.67	32.2[b]	32.0	31.8	1.75	1.65
13	43.5	44.9	42.4	44.7	42.4	43.3	44.8	42.5	42.5	43.3	47.6			48.4	47.7	47.3		
14	55.1	43.3	56.4	49.3	50.2	55.3	43.0	50.1	50.1	55.2	44.9	2.09		49.8	45.0	83.9		
15	25.5	22.7	24.0	23.3	23.5	25.5	22.7	23.4	23.4	25.4[a]	22.8	1.48[d]	1.10[d]	24.7	22.8	34.8	1.23[d]	1.77[d]
16	28.2	27.0	28.0	25.8[a]	27.8	28.3	27.0	27.9	27.9	28.4	37.8	1.68	1.87	38.4	37.6	27.2	1.96	1.36
17	54.9	47.2	55.6	47.5	55.6	54.9	47.2	55.9	55.6	54.9	86.0			84.9	85.8	49.4	1.71	
18	11.9	12.1	11.8	12.2	11.5	11.9	12.0	11.5	11.5	11.9	14.0	0.73		14.4	14.0	15.5	0.79	
19	23.1	22.4	16.1	15.9	15.7	16.1	15.6	15.7	15.7	16.1	22.6	0.94		23.0	22.6	22.7	0.99	
20	35.1	34.5	35.2	34.7	35.2	35.1	34.5	32.4	33.6	33.5	39.1	1.69		38.9	35.9	34.9	1.44	
21	18.2	17.3	18.1	17.3	18.1	18.2	17.3	18.0	19.4	19.5	13.4	0.91		13.4	13.3	18.2	0.90	
22	30.9[a]	30.7[b]	30.9[a]	30.8[b]	30.9[c]	30.9[a]	30.8[c]	37.1	41.3	41.3	27.3	1.47, 1.88		27.4	33.9	30.9	1.36, 1.83	
23	30.8[a]	30.6[b]	30.8[a]	30.7[b]	30.8[c]	30.8[a]	30.7[c]	70.1	174.0	174.1	32.0	2.25, 2.43		32.0[b]	70.5	31.0	2.25, 2.39	
24	174.8	174.6	174.9	174.7	174.8	174.8	174.7	171.6			174.5			174.5	171.4	174.8		
O<u>C</u>H$_3$	51.4	51.4	51.4	51.4	51.4	51.4	51.5	52.2	51.3	51.3	51.4	3.67		51.4	52.2	51.4	3.67	
CH$_3$<u>C</u>OO	170.7	170.6	170.6	170.5	170.7	170.6	170.7	170.9	170.7	170.7	170.8			170.7	170.9	170.8		
	170.7	170.6		170.6	170.3	170.6	170.6	170.7	170.3	170.6	170.6			170.6	170.8	170.9		
		170.5					170.3	170.3							170.6			
<u>C</u>H$_3$COO	21.7	21.5	21.3	21.3	21.3	21.6	21.5	21.3	21.4	21.6	21.5	2.06		21.7	21.5	21.3	2.03	
	21.3	21.4		21.2	21.3	21.2	21.4	21.3	21.4	21.3	21.3	2.03		21.3	21.3	21.8	2.00	
		21.3					21.3	20.6							20.6			

[a,b,c] Assignments can be exchanged in each column.
[d] Assignments for H-15α and H-15β are tentative.

The methyl ester diacetate of *chenodeoxycholic acid* **3**, which has a 7α-acetyloxy group, gave the expected 5β-hydroxy derivative **3a** (40%) and a second product **3b** (21%) (Scheme II), in addition to overoxidation and other minor products.

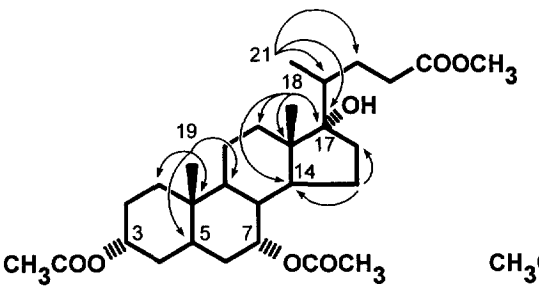
Scheme II



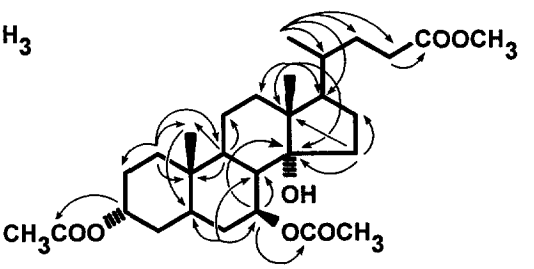
	R_1	R_3
3b	α-$OCOCH_3$	H
4b	β-$OCOCH_3$	H
6b	α-$OCOCH_3$	$OCOCH_3$

The MS spectra of **3b** showed fragments consistent with the addition of one hydroxyl group which was identified as tertiary by the appearance of a new quaternary carbon at 86.0 ppm in the ^{13}C-NMR and the absence of a new CHOH signal in the ^{1}H-NMR spectrum. In the ^{13}C-NMR spectrum the resonances of C-1 to C-11, and C-19 were unchanged relative to the starting material **3** indicating that the new hydroxyl group was on a tertiary carbon located on ring C or D. This was identified as C-17 by the upfield shifts[11,12] of γ carbons bearing hydrogens 1,3 diaxial to H-17α in the parent compound **3**: C-12 (-7.5 ppm), C-14 (-5.5), C-21 (-4.8) and C-22 (-3.6). C-18 was deshielded by 2.4 ppm as is characteristic for *anti* γ carbons when both intervening carbons (C-17 and C-13) are fully substituted[12]. In the HMQC spectrum of **3b** (Table 2) H-14 appeared deshielded by 0.61 ppm relative to **3** due to its 1,3 diaxial relationship to the new 17α hydroxyl group[12]. In the HMBC spectrum long range correlations were observed (among others) from Me-18 to C-12, C-13, C-14, and C-17, and from Me-21 to C-17, C-20, and C-22 (Scheme III), thus confirming the assignments.

Scheme III



3b



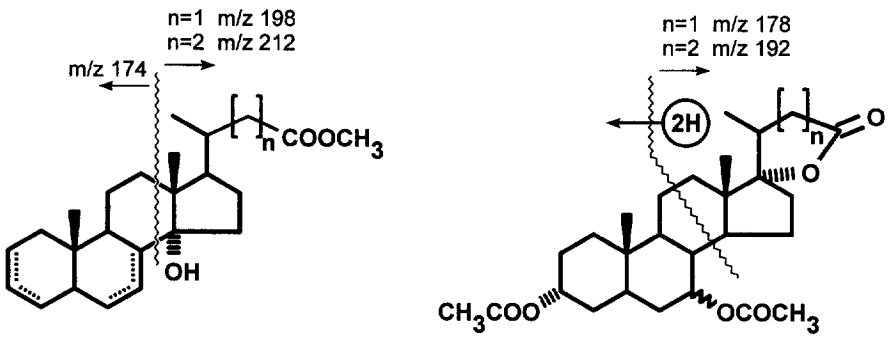
4c

Reaction of the methyl ester acetate derivative of *ursodeoxycholic acid* **4**, with a 7β-acetyloxy-group, gave the 5β-hydroxy derivative **4a** (28%), the 17α-hydroxy derivative **4b** (28%) and a new product **4c** (21%). **4b** was identified by applying the ^{13}C-NMR substituent effects of addition of the 17α hydroxyl determined previously for the case of **3** and **3b** to the values of the starting material **4**. Compound **4c** was also a monohydroxylation product of **4** on a tertiary carbon. In this case the γ carbons exhibiting

shielding relative to **4** were C-7 (-4.9 ppm), C-9 (-7.4), C-12 (-8.3), and C-17 (-5.6) thus locating the new hydroxyl group on C-14(α). C-18 showed a +3.4 ppm deprotection as it is also *anti* γ to the 14α-OH group and both C-14 and C-13 are fully substituted. The HMQC spectrum showed the expected ~0.5 ppm deshielding relative to **4** for H-7, H-9, H-12α, and H-17[13], which are 1,3 diaxial to 14α-OH. The HMBC spectrum showed, among others (Scheme III), long range correlations from Me-18 to C-12, C-13, C-14, and C-17, and from H-7 to C-8 and C-14, which confirmed the location of the new hydroxyl group on 14α.

The presence of a 23-acetate in the side chain did not alter the course of the reaction significantly. The methyl ester peracetate of *phocaecholic acid* **6**, the analogue of **3** with an additional 23R-acetyloxy group, gave the 5β-hydroxy derivative **6a** in 29% yield and the 17α-hydroxy derivative **6b** (11%) as main products. Shortening the side chain by one carbon did not modify the spectrum of products. The methyl peracetyl derivatives of *nor-chenodeoxycholic acid* **7** and *nor-ursodeoxycholic acid* **8**, gave the corresponding 5β-hydroxy derivatives **7a** and **8a** in 25% isolated yield in both cases. Although in this case the amount of material was not sufficient for the isolation of other monohydroxylated compounds in the mixture, GC-MS analysis of the appropriate column fraction indicated the presence of the 14α-hydroxyl derivative of **8** and the 17α-hydroxyl derivatives of **7** and **8**. The mass spectrum of **4c** exhibited two intense peaks at m/z 174 ($C_{13}H_{18}$ by HRMS) and m/z 212 ($C_{12}H_{20}O_3$ by HRMS) which appear to originate from fragmentation through ring C of the molecular ion after the loss of the two acetate groups as shown in Scheme IV. The 14α-hydroxyl derivative of the nor-bile acid derivative **8** showed prominent peaks at m/z 174 and at the corresponding m/z 198.

Scheme IV



Analysis of isolated **3b** and **4b** by GC-MS resulted in one well defined peak in each case. However, their fragmentation pattern was consistent with the products of lactonization of the side chain carboxyl group, most likely by thermal reaction in the injection port (Scheme IV). Their mass spectra showed intense fragments at m/z 414 and 354 originating from the loss of one and two acetic acid molecules from the molecular ion, and a highly diagnostic peak at m/z 192, probably originating from fragmentation through ring C[14]. The 17α-hydroxyl derivatives of **7** and **8** showed the corresponding fragments at m/z 400, 340, and 178 respectively.

DISCUSSION

Reaction of DMDO solutions enriched in chloroform with methyl ester acetates of (5β)-bile acids appears to be a general method to obtain 5β-hydroxyl derivatives. In this way, a set of valuable reference compounds, which would have otherwise required a multistep process, has been prepared.

Bile acid derivatives containing a 7-hydroxyl group gave significant amounts of 17α and 14α hydroxylated products, which were not detected among reaction products of the other bile acids. This intriguing result can be rationalized in terms of electronic and steric effects. DMDO inserts relatively fast into the 5β C-H bond of **1** to give only one main product. As shown in Table 1, the addition of successive acetyloxy groups decreases the insertion rate at C-5 relative to **1**[15]. It has recently been shown that the C-H insertion of DMDO in a series of adamantanes is electrophilic in nature[16]. Here, the addition of an electron withdrawing group on C-7 seems to deactivate the 5 position, and surprisingly, an acetyloxy group on the relatively distant C-12 causes further deactivation. Similar effects have been observed in the C-H insertion of DMDO in protonated amines[17], the C-H oxygen insertion by perfluorodialkyloxaziridines[6], and the electrophilic hydroxylation of tertiary carbons by fluorine in acetonitrile[18]. The reduction in reaction rate at C-5 allows competitive insertion at other positions according to their steric availability. DMDO, like most other reagents sensitive to steric hindrance, can approach the 5β steroidal nucleus from the β face on ring A or from the α face on rings C and D. The equatorial 7β-AcO group does not add significant hindrance to an α face approach and therefore **4** and **8** are hydroxylated on C-14α and C-17α, whereas the axial 7α-AcO in **3**, **6**, and **7** prevents attack on C-14α, but not on 17α[19]. However, the 12α-AcO in **2**, and the combination of 7α and 12α AcO groups in **5** effectively mask both the 14α and 17α position and only the 5β hydroxylated product is formed.

It is also possible than in addition to the steric effects, the axial acetyloxy groups destabilize the transition state for DMDO insertion in nearby carbons through unfavorable electrostatic interactions. The interplay of these and perhaps other factors may be more subtle: hydroxylation of C-14 in **4** and **8** requires that the equatorial 7-acetyloxy group causes a larger decrease in reaction rate at C-5 than at C-14, either by decreasing the electronic density on the equatorial C_5-H bond more than it does on the axial C_{14}-H bond, or by destabilizing the transition state leading to insertion on C-5 more than the transition state leading to 14α insertion. It will be interesting to investigate if by manipulation of the functionality on the steroidal nucleus, the hydroxylation on C-17 can be increased at the expense of C-5 insertion, thus providing easy access to functionalization of ring D in steroids.

EXPERIMENTAL

Melting points (Electrothermal capillary apparatus) are uncorrected. IR spectra were recorded in CH_2Cl_2 solution with a 2020 Galaxy Series FT-IR (Mattson Instruments). ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on a Varian Unity 500 spectrometer at 25 °C in $CDCl_3$. Chemical shifts are in ppm relative to Me_4Si. HMQC[20] data, 16 scans per t_1 increment, were collected in a 2048 × 256 matrix with spectral width of 2584 Hz in F_2 and 21894 Hz in F_1 using the TPPI method. The null time after the BIRD pulse was 400 ms and the experiment was optimized for $^1J_{CH}$ = 135 Hz. After gaussian weighting in both domains, data were processed in a 4096 × 1024 matrix in phase sensitive mode. HMBC[21] spectra, 64 scans per t_1 increment, were optimized for a long range coupling constant of 8.3 Hz and were processed in the

absolute mode. Data points and weighting were similar to the HMQC spectra. GC-MS analyses were performed with a Hewlett-Packard 5890 gas chromatograph - 5970 Series Mass Selective Detector on a 30 m × 0.25 mm 35% phenyl methyl silicone column (SPB-35, Supelco, Bellefonte, PA) at 275 °C (isothermal) with injection port at 295 °C, interface at 290 °C and 1 ml/min helium carrier gas. EI-HRMS of **3b**, **4b**, **4c**, and **6b** were obtained with a VG 70-SE mass spectrometer by direct insertion. HRMS molecular formula analyses were obtained on an AUTOSPEC M mass spectrometer (Micromass, U.K.) in the positive electrospray mode. Resolution was set at 5000, and the standard deviation of the measurements was 4 ppm. Microanalyses were performed by Galbraith Laboratories Inc, Knoxville, TN. All separations were carried out by column chromatography using 40 μm silica gel (J.T. Baker, Phillipsburg, NJ). Chloroform for the preparation of DMDO solutions was washed four times with distilled water, all other solvents were HPLC grade and were used without further purification. *Chenodeoxycholic acid* and *ursodeoxycholic acid* were a gift of Diamalt AG, Raubling, Germany. *Phocaecholic acid*, isolated from duck bile, was a generous gift of Dr. M. Jirsa, Prague, Czech Republic. *Nor-chenodeoxycholic* acid and *nor-ursodeoxycholic acid* were prepared as described[22]; methyl ester acetates of bile acids were prepared by standard methods. All other reagents were from Aldrich Chemical Co., Milwaukee, WI. Yields are based on consumed starting material, determined by GC-MS.

Preparation of DMDO solutions in chloroform.

DMDO solutions in acetone were prepared by a simplified procedure based on Adam[8,23]. A 2 l round bottom flask was provided with a strong magnetic stirrer and a straight vacuum adapter closed by a removable stopper. The side arm of the adaptor was connected with Tygon® tubing to the top inlet of a cold finger condenser leading to a 250 ml, 2 neck round bottom flask, both cooled with dry ice-acetone. The receiving round bottom flask was connected to a vacuum pump through 2 cold (-78 °C) traps. The reaction vessel was charged with 37.8 g $NaHCO_3$, 168 ml water, 128 ml acetone, and cooled to 5 °C with an external ice bath. With constant stirring, 88 g of potassium monoperoxy sulfate (Oxone®) was added in 3 portions at 5 min intervals through the top of the vacuum adaptor. After 5 min the product was distilled at 60 mm Hg for 15 min, the ice bath was removed and the distillation was continued for 20 min at 100 mm Hg. Approximately 80-90 ml of 0.088 - 0.093 M (iodometric titration) DMDO solution in acetone was obtained.

DMDO solutions in $CHCl_3$ were prepared by adding cold DMDO acetone solution, 150 ml, to a mixture of 50 ml $CHCl_3$ and 30 g ice while vigorously stirring in an ice bath. The mixture was transferred to a chilled separatory funnel and washed with 200, 120, and 90 ml ice cold water. The organic layer was dried with Na_2SO_4 for 10 min at 4 °C and filtered through glass wool to yield 60 ml of a 0.150 - 0.165 M solution of DMDO in $CHCl_3$-acetone approximately 75:25 v/v (70% recovery of DMDO).

General procedure for hydroxylation of bile acid derivatives.

The bile acid methyl ester acetate derivative was dissolved in the appropriate volume of DMDO solution in chloroform containing 2 eq. of reagent (without cosolvent) and allowed to react for 24 h at room temperature. The solvent was evaporated under a N_2 stream, and if necessary, the reaction was repeated with the second number of equivalents indicated.

Methyl-3α-acetyloxy-5-hydroxy-5β-cholan-24-oate 1a.

1, 486 mg was treated with 2 eq DMDO (82% conversion) and the reaction mixture was chromatographed with $CHCl_3 \rightarrow CHCl_3$-MeOH 98:2, and the fractions containing **1a** rechromatographed with toluene-acetone 95:5 to give 132 mg **1a**[4] (32%). MS m/z: 430 (5), 388 (17), 370 (100), 355 (24), 334 (99), 315 (14), 273 (36), 255 (55), 228 (26), 213 (68).

Methyl-3α,12α-diacetyloxy-5-hydroxy-5β-cholan-24-oate 2a.

2, 650 mg, was treated with 2 + 1 eq DMDO (92% conversion) and the crude reaction mixture was chromatographed with toluene-acetone 95:5 to afford 280 mg (45%) of **2a**[5]. MS m/z: 428 (29), 386 (21), 368 (84), 353 (24), 332 (55), 331 (53), 313 (40), 271 (44), 253 (100), 211 (24).

Reaction of DMDO with 3.

3, 1.0 g was reacted with 2 eq DMDO to 56% conversion and the reaction mixture chromatographed with $CHCl_3$ to give 221 mg **3a** (40%). Fractions containing **3b** were rechromatographed with $CHCl_3$ to give 123 mg **3b** (21%).

Methyl-3α,7α-diacetyloxy-5-hydroxy-5β-cholan-24-oate 3a.

Mp 158-159 °C (Et_2O-hexanes); IR: 3606, 2909, 2873, 1730, 1473, 1366, 1027 cm^{-1}; ^1H-NMR: δ 0.65 (s, 3H, Me-18), 0.91 (s, 3H, Me-19), 0.93 (d, 6.5 Hz, 3H, Me-21), 2.03 and 2.07 (2 × [s, 3H, Ac]), 2.23 (m, 1H, H-23), 2.33 (t, 12.5 Hz, 1H, H-4α), 2.35 (m, 1H, H-23'), 3.67 (s, 3H, -$COOCH_3$), 4.96 (m, 1H, H-7), 5.02 (m, 1H, H-3); MS m/z: 428 (10), 386 (84), 368 (82), 353 (23), 332 (100), 331 (35), 313 (39), 271 (85), 253 (42), 226 (33), 211 (37); anal. calcd for $C_{29}H_{46}O_7$: C, 68.74; H, 9.15; found: C, 68.73; H, 9.39; HRMS calcd for $C_{29}H_{46}O_7Na$ ($M+Na^+$) 529.3141, found 529.3125.

Methyl-3α,7α-diacetyloxy-17α-hydroxy-5β-cholan-24-oate 3b.

Viscous oil; IR: 3606, 2932, 2872, 1729, 1382, 1367, 1069, 1026 cm^{-1}; ^1H-NMR: δ 0.73 (s, 3H, Me-18), 0.91 (d, 6.0 Hz, 3H, Me-21), 0.94 (s, 3H, Me-19), 2.03 and 2.06 (2 × [s, 3H, Ac]), 2.25 (m, 1H, H-23), 2.43 (m, 1H, H-23'), 3.67 (s, 3H, -$COOCH_3$), 4.59 (m, 1H, H-3), 4.91 (m, 1H, H-7); MS (direct insertion) m/z: 446 (5), 428 (15), 413 (17), 386 (6), 373 (13), 368 (16), 353 (34), 313 (41), 288 (17), 281 (31), 271 (6), 253 (100), 228 (56), 215 (20), 213 (18), 171 (50); HRMS calcd for $C_{29}H_{46}O_7Na$ ($M+Na^+$) 529.3141, found 529.3153.

Reaction of DMDO with 4.

4, 1.2 g was reacted with 2 eq DMDO to 42% conversion and the products purified by chromatography with $CHCl_3$ to give 110 mg **4c** (21%), 145 mg **4b** (28%), and 147 mg **4a** (28%).

Methyl-3α,7β-diacetyloxy-5-hydroxy-5β-cholan-24-oate 4a.

Mp 148-149 °C (Et_2O-hexanes); IR: 3600, 2935, 2910, 2874, 1729, 1366, 1027 cm^{-1}; ^1H-NMR: δ 0.68 (s, 1H, Me-18), 0.92 (d, 6.5 Hz, 3H, Me-21), 0.94 (s, 3H, Me-19), 1.99 and 2.02 (2 × [s, 3H, Ac]), 2.23 (m, 1H, H-23), 2.35 (m, 1H, H-23'), 3.67 (s, 3H, -$COOCH_3$), 4.64 (td, 11.0 and 5.5 Hz, 1H, H-7), 5.06 (m, 1H, H-3); MS m/z: 428 (5), 386 (53), 368 (63), 353 (16), 332 (100), 331 (17), 313 (27), 271 (78), 253 (63), 226 (10), 211 (16); anal. calcd for $C_{29}H_{46}O_7$: C, 68.74; H, 9.15; found: C, 68.64; H, 9.47; HRMS calcd for $C_{29}H_{46}O_7Na$ ($M+Na^+$) 529.3141, found 529.3144.

Methyl-3α,7β-diacetyloxy-17α-hydroxy-5β-cholan-24-oate 4b.
Viscous oil; IR: 3607, 2938, 2876, 1729, 1366, 1025 cm^{-1}; ^{1}H-NMR: δ 0.76 (s, 3H, Me-18), 0.90 (d, 6.5 Hz, 3H, Me-21), 0.98 (s, 3H, Me-19), 1.98 and 2.03 (2 × [s, 3H, Ac]), 2.26 (m, 1H, H-23), 2.44 (m, 1H, H-23'), 3.68 (s, 3H, -$COOCH_3$), 4.67 (m, 1H, H-3), 4.81 (td, 10.7 and 5.5 Hz, 1H, H-7); MS (direct insertion) m/z: 488 (11), 446 (40), 428 (8), 413 (15), 386 (34), 368 (23), 353 (27), 313 (73), 288 (31), 281 (14), 271 (24), 253 (59), 228 (100), 215 (27), 213 (24), 171 (76); HRMS calcd for $C_{29}H_{46}O_7Na$ ($M+Na^+$) 529.3141, found 529.3119.

Methyl-3α,7β-diacetyloxy-14α-hydroxy-5β-cholan-24-oate 4c.
Viscous oil; IR: 3609, 2936, 2876, 1729, 1366, 1048, 1027 cm^{-1}; ^{1}H-NMR: δ 0.79 (s, 3H, Me-18), 0.90 (d, 6.5 Hz, 3H, Me-21), 0.99 (s, 3H, Me-19), 2.00 and 2.03 (2 × [s, 3H, Ac]), 2.14 (td, 12.0 and 4.5 Hz, 1H, H-9), 2.25 (m, 1H, H-23), 2.39 (m, 1H, H-23'), 3.67 (s, 3H, -$COOCH_3$), 4.67 (m, 1H, H-3), 5.15 (td, 11.0 and 5.5 Hz, 1H, H-7); MS m/z: 446 (2), 428 (10), 386 (20), 368 (23), 353 (13), 313 (34), 281 (6), 271 (10), 253 (36), 212 (82), 174 (41), 97 (81), 96 (100); HRMS calcd for $C_{29}H_{46}O_7Na$ ($M+Na^+$) 529.3141, found 529.3112.

Methyl-5-hydroxy-3α,7α,12α-triacetyloxy-5β-cholan-24-oate 5a.
5, 1.25 g, was treated with 2 + 1 eq DMDO (58% conversion) and the reaction mixture was chromatographed with $CHCl_3$-MeOH 99:1. The fractions containing the main product were pooled and rechromatographed with dichloromethane-acetone 96:4 → 85:15 to afford 390 mg (53%) of **5a**: mp 87-89 °C (Et_2O-hexanes); IR: 3608, 2921, 2872, 1730, 1379, 1368, 1030 cm^{-1}; ^{1}H-NMR δ 0.73 (s, 3H, Me-18), 0.82 (d, 6.0 Hz, 3H, Me-21), 0.90 (s, 3H, Me-19), 2.05, 2.11, and 2.14 (3 × [s, 3H, Ac]), 2.22 (m, 1H, H-23), 2.24 (t, 13.0 Hz, 1H, H-4α), 2.35 (m, 1H, H-23'), 3.67 (s, 3H, -$COOCH_3$), 4.96 (m, 1H, H-7), 5.02 (m, 1H, H-3), 5.11 (bs, 1H, H-12); MS m/z: 444 (31), 426 (41), 384 (90), 366 (80), 351 (35), 330 (81), 329 (71), 311 (32), 269 (100), 251 (78), 209 (36); HRMS calcd for $C_{31}H_{48}O_9Na$ ($M+Na^+$) 587.3196, found 587.3167.

Reaction of DMDO with ***6****.*
6, 390 mg was reacted with 2 eq DMDO to 57% conversion and the reaction mixture was chromatographed with $CHCl_3$ to give 25 mg **6b** (11%) and 66 mg **6a** (29%).

Methyl-5-hydroxy-3α,7α,23(R)-triacetyloxy-5β-cholan-24-oate 6a.
Mp 171-173 °C (Et_2O-hexanes); IR: 3611, 2942, 2913, 2876, 1741, 1732, 1377, 1369, 1078, 1029 cm^{-1}; ^{1}H-NMR: δ 0.67 (s, 3H, Me-18), 0.92 (s, 3H, Me-19), 0.97 (d, 6.5 Hz, 3H, Me-21), 2.03, 2.07, and 2.15 (3 × [s, 3H, Ac]), 2.31 (t, 12.5 Hz, 1H, H-4α), 3.74 (s, 3H, -$COOCH_3$), 4.93 (m, 1H, H-7), 5.02 (m, 1H, H-3), 5.07 (dd, 11.0 and 1.5 Hz, 1H, H-23); MS m/z: 486 (7), 444 (72), 426 (84), 411 (18), 390 (100), 389 (28), 385 (14), 384 (16), 366 (19), 329 (27), 325 (18), 313 (20), 311 (21), 271 (40), 269 (30), 253 (29), 226 (33), 211 (33); HRMS calcd for $C_{31}H_{48}O_9Na$ ($M+Na^+$) 587.3196, found 587.3179.

Methyl-17α-hydroxy-3α,7α,23(R)-triacetyloxy-5β-cholan-24-oate 6b.
Viscous oil; IR: 3603, 2930, 2874, 1729, 1606, 1379, 1366, 1070, 1028 cm^{-1}; ^{1}H-NMR: δ 0.75 (s, 3H, Me-18), 0.95 (s, 3H, Me-19), 0.95 (d, 6.5 Hz, 3H, Me-21), 2.03, 2.06, and 2.16 (3 × [s, 3H, Ac]), 3.74 (s, 3H, -$COOCH_3$), 4.60 (m, 1H, H-3), 4.91 (m, 1H, H-7), 5.02 (dd, 11.5 and 2.5 Hz, 1H, H-23); MS (direct insertion) m/z: 504 (9), 486 (6), 471 (7), 444 (27), 429 (7), 426 (10), 411 (18), 384 (3), 373 (5), 366 (6), 360 (10), 351 (6), 313 (32), 288 (39), 281 (8), 271 (8), 253 (61), 228 (100), 215 (37), 213 (22), 169 (40); HRMS calcd for $C_{31}H_{48}O_9Na$ ($M+Na^+$) 587.3196, found 587.3156.

Methyl-3α,7α-diacetyloxy-5-hydroxy-24-nor-5β-cholan-23-oate 7a.
7, 320 mg, was treated with 2 + 0.7 eq DMDO to 83% conversion and the reaction mixture was chromatographed with $CHCl_3$ to afford 67 mg (25%) of **7a**. Mp 199-201 °C (EtAcO-hexanes); IR: 3606, 2940, 2876, 1730, 1379, 1367, 1029 cm^{-1}; ^{1}H-NMR: δ 0.69 (s, 3H, Me-18), 0.91 (s, 3H, Me-19), 0.99 (d, 6.5 Hz, 3H, Me-21), 2.03 and 2.07 (2 × [s, 3H, Ac]), 2.31 (t, 12.5 Hz, 1H, H-4α), 2.42 (dd, 14.5 and 3.5 Hz, 1H, H-22), 3.67 (s, 3H, -$COOCH_3$), 4.93 (m, 1H, H-7), 5.02 (m, 1H, H-3); MS m/z: 414 (11), 372 (82), 354 (73), 341 (21), 339 (24), 331 (14), 318 (100), 313 (30), 281 (29), 271 (60), 253 (31), 226 (32), 211 (39); HRMS calcd for $C_{28}H_{44}O_7Na$ ($M+Na^+$) 515.2985, found 515.2971.

Methyl-3α,7β-diacetyloxy-5-hydroxy-24-nor-5β-cholan-23-oate 8a.
8, 580 mg, was treated with 2 + 0.7 eq DMDO to 73% conversion and the reaction mixture was chromatographed with $CHCl_3$ to afford 110 mg of **8a** (26%). Mp 126-127 °C (Et_2O-hexanes); IR: 3607, 2943, 2878, 1731, 1368, 1029 cm^{-1}; ^{1}H-NMR: δ 0.72 (s, 3H, Me-18), 0.95 (s, 3H, Me-19), 0.98 (d, 6.5 Hz, 3H, Me-21), 2.00 and 2.03 (2 × [s, 3H, Ac]), 2.42 (dd, 14.5 and 3.5 Hz, 1H, H-22), 3.67 (s, 3H, -$COOCH_3$), 4.64 (td, 11.0 and 5.5 Hz, 1H, H-7), 5.06 (m, 1H, H-3); MS m/z: 372 (46), 354 (43), 341 (13), 339 (13), 331 (7), 318 (100), 313 (13), 299 (20), 281 (61), 271 (29), 253 (22), 226 (9), 211 (15); HRMS calcd for $C_{28}H_{44}O_7Na$ ($M+Na^+$) 515.2985, found 515.3009.

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