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J-Based Analysis and DFT–NMR Assignments of Natural Complex Molecules: Application to 3β,7-Dihydroxy-5,6-epoxycholestanes

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In order to reproduce the stereochemical dispositions of the epoxy and hydroxy functionalities, four 3β,7-hydroxy-5,6-epoxycholestanes were easily prepared from cholesterol, and their NMR spectroscopic data were experimentally obtained from 1D and 2D NMR experiments. An exhaustive QM-*J*-based analysis was then performed to replicate the experimental H–H and C–H coupling constants as well as the ^{13}C NMR chemical shifts. The B3LYP GIAO methodology with the 6-311-G(d,p) basis set was chosen and showed that the data obtained from rings A and B were sufficient to calculate the correct stereochemistry of the 5,6-epoxy and 7-hydroxy groups.

Introduction

Researchers are always looking for fast and reliable methods to elucidate the three-dimensional structure of new organic entities. The assignment of their relative or absolute configurations is a crucial step in the structural elucidation of a new compound. In addition to traditional structural parameters (chemical shifts, NOE/ROE data, and coupling constants), distances and angular data can now be obtained from cross-correlation relaxation interferences, chemical shift anisotropies, and residual dipolar couplings (RCDs).[1] The magnetically different local environments of various stereoisomers make chemical shifts and coupling constants important data to distinguish between them. In order to make this process possible, NMR assignments can be made by using several strategies, for example, by comparison of chemical shift data with those of similar compounds, the prediction of such values through additivity rules using specialized software,[2] or by comparison of NMR spectroscopic data with those of various closely related stereoisomers from specific databases. However, the absence of appropriate models for making such comparisons and the limited number of specific databases, such as Kishi's "universal NMR spectroscopic database",[3] which requires powerful synthetic libraries, justify the interest in developing other tools to perform such assignments.

In the last 10 years particular emphasis has been placed on the modeling of organic molecules through the calculation of NMR chemical shifts and coupling constants by density functional theory (DFT) methods, an approach that has become a powerful strategy for the interpretation of experimental NMR spectroscopic data in structure elucidation.[4] The prediction of NMR spectroscopic data by DFT can facilitate the interpretation and assignment of different diastereoisomers. Even though important advances have been made towards the goal of calculating accurate magnetic shielding tensors for a number of nuclei, attention needs to be paid to the corroboration and verification of the proposed DFT–NMR methods.[5]

Continuing with our investigations into the elucidation of the relative stereochemistry of organic compounds by DFT–NMR methods,[6] we wanted to apply this strategy to the correct assignment of the relative configuration of 3β,7-dihydroxy-5,6-epoxycholestanols. The planar disposition of the proton attached to the epoxy group makes it very difficult to assign the relative stereochemistry of such a functional group by NOE correlations or by inspection of the proton coupling constants.[7] So far, the stereochemistry of 5,6-epoxysteroids has been elucidated, in most cases, by comparison of their 1D NMR spectroscopic data with those of similar compounds obtained or isolated a long time ago.[8] Natural steroids bearing these substituents show potential pharmacological activities. Some examples reported in the literature are 5β,6β-epoxygorgostane-3β,7α,11α,12β-tetrol 12-acetate isolated from the gorgonian *Isis hippuris*, which showed reversal of multidrug resistance (MDR) with cancer cells expressing P-glycoprotein,[9] 5β,6β-epoxycholestane-24-ene-3β,7β-diol isolated from the soft coral *Gersemia fructicosa* induced an apoptosis of K662 cells with characteristic internucleosomal DNA degradation,[10] and 5β,6β-epoxyergost-24(28)-ene-3β,7β-diol isolated from the gorgonian *Plexaurella grisea* exhibited selective activity against the HT 29 tumor cell line.[11] Further-

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more, the 5,6-epoxy moiety opens the door to the introduction of additional functional groups at these positions, and these are therefore key intermediates in the synthesis of steroids.[12,13] For this reason, the correct stereochemical assignment is a crucial stage in the subsequent reaction steps.

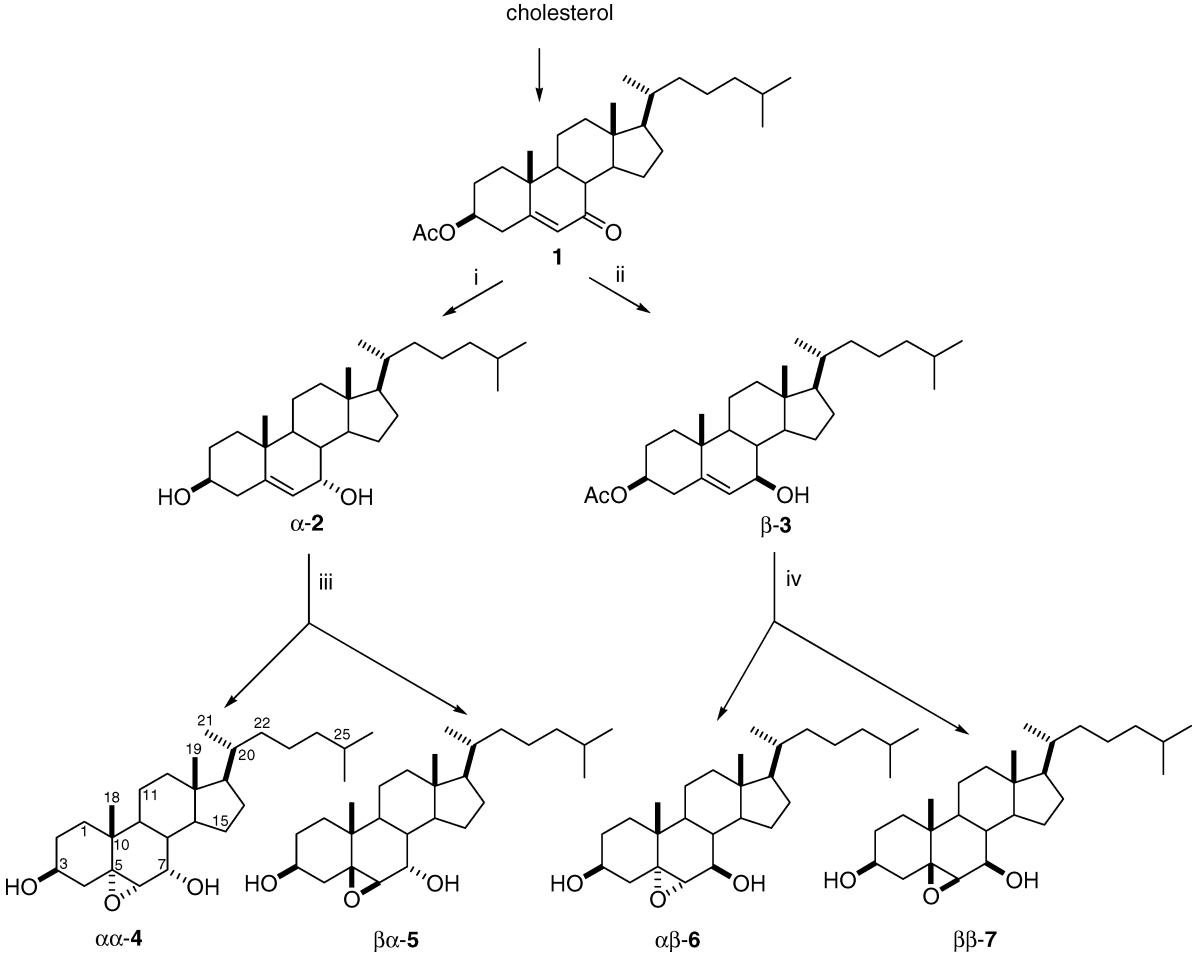
In this paper we wish to present the synthesis of the four possible 3β,7-dihydroxy-5,6-epoxycholestane diastereoisomers, the complete ^1H and ^{13}C NMR assignment by 1D and 2D experiments, the identification of some diagnostic signals, and the use of an exhaustive QM-*J*-based analysis to reproduce the stereochemical dispositions of the epoxy and hydroxy functionalities at these three chiral centers. Furthermore, we also wished to calculate by DFT the chemical shift values for models to replicate the experimental NMR spectroscopic data for this type of compounds.

Results and Discussion

The four possible 3β,7-dihydroxy-5,6-epoxycholestane diastereoisomers **4**–**7** were easily prepared from commercially available cholesterol. The α,β-unsaturated ketone **1**, obtained in high yield from cholesterol,[14] was selectively reduced with L-Selectride[15] or $CeCl_3/NaBH_4$ to 3β,7α-dihydroxycholestane (**2**)[16] and 3β-acetoxy-7β-hydroxy-4α-cholestan (**3**),[17] respectively. The epoxide functionality was then introduced into **2** and **3** by means of standard Prilezhaev oxidation with MCPBA. The hydrogen-bonding interaction between the peracid and the allylic hydroxy group led to epoxidation of the alkene from the same face as the hydroxy group at C-7 to give the major diastereoisomer, along with some epoxidation from the opposite face to give the minor product.[18] Compounds **4** and **5** were purified by flash chromatography, whereas compounds **6** and **7** were separated, after removal of the acetate group, by reversed-phase HPLC (Scheme 1).

With the four diastereoisomers in hand, in order to reproduce the stereochemical dispositions of the epoxy and hydroxy functionalities, complete signal assignment for each proton and carbon atom was carried out by 2D-NMR spectroscopy by using ^1H–^1H COSY, TOCSY, edited HSQC, HSQC–TOCSY, and HMBC experiments (see Tables 1 and 2).

Some key chemical shift signals can be deduced from a study of the ^1H and ^{13}C NMR spectroscopic data of compounds **4**–**7**. The ^1H NMR chemical shift values suggested that only 3-H may give a signal suitable for distinguishing



Scheme 1. Synthesis of 3β,7-dihydroxy-5,6-epoxycholestanes from cholesterol. Reagents and conditions: (i) L-Selectride, THF (95%); (ii) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, THF (90%); (iii) (a) MCPBA, $CHCl_3$ (88%), (b) silica gel column chromatography (hexane/ethyl acetate, 9:1); (iv) (a) MCPBA, $CHCl_3$ (82%), (b) 5% KOH/MeOH (90%), (c) RP-HPLC [MeOH/H_2O (96:4)].

Table 1. ^{1}H NMR chemical shift assignments for compounds **4**–**7**.

Proton	δ_H [ppm]			
	αα-**4**	βα-**5**	αβ-**6**	ββ-**7**
1a	1.71, m	1.96, m	1.45, m	2.03, m
1b	1.37, m	1.34, m	0.92, m	1.23, m
2a	1.95, m	1.82, m	2.06, m	1.42, m
2b	1.62, m	1.32, m	1.61, m	1.84, m
3	3.92, m	3.74, m	3.92, m	3.76, m
4a	2.11, t	2.08, m	1.99, m	2.06, m
	J = 12.4 Hz			
4b	1.38, m	1.45, m	1.11, m	1.48, m
5	–	–	–	–
6	3.24, d	3.11, d	2.91, s	3.16, d
	J = 4.8 Hz	J = 2.8 Hz		J = 1.5 Hz
7	3.87, t	4.07, t	3.72, d	3.54, dd
	J = 4.8 Hz	J = 2.8 Hz	J = 6.3 Hz	J = 8.0 and 1.5 Hz
8	2.05, m	1.57, m	1.29, m	1.42, m
9	1.24, m	1.13, m		0.74, m
10	–	–	–	–
11a	1.40, m	1.42, m	1.45, m	1.43, m
11b	1.29, m	0.89, m	1.24, m	0.89, m
12a	2.12, m	1.97, m	1.96, m	2.00, m
12b	1.40, m	1.13, m	1.13, m	1.15, m
13	–	–	–	–
14	1.36, m	1.24, m	1.06, m	1.06, m
15a	1.81, m	1.63, m	1.76, m	1.94, m
15b	1.07, m	1.14, m	0.97, m	1.39, m
16a	1.88, m	1.87, m	1.93, m	1.88, m
16b	1.25, m	1.30, m	1.25, m	1.30, m
17	1.11, m	1.13, m	1.03, m	1.05, m
18	0.64, s	0.67, m	0.67, s	0.67,s
19	1.09, s	1.01, m	1.13, s	1.01,s
20	1.36, m			
21	0.92, d	0.91, d	0.92, d	0.92, d
	J = 6.5 Hz	J = 6.5 Hz	J = 6.5 Hz	J = 6.5 Hz
22a	1.34, m	1.96, m	1.45, m	1.36, m
22b	1.01, m	1.36, m	0.96, m	1.24, m
23	1.29, m	1.34, m	1.32, m	2.01, m
24	1.11, m	1.12, m	1.17, m	1.07, m
25	1.34, m	1.53, m	1.28, m	1.53, m
26/27	0.88, d	0.87, d	0.88, d	0.88, d
	J = 6.5 Hz	J = 6.5 Hz	J = 6.5 Hz	J = 6.5 Hz

Table 2. ^{13}C NMR chemical shift (ppm) assignments for compounds **4**–**7**.

Carbon	Mult.	δ_C [ppm]			
		αα-**4**	βα-**5**	αβ-**6**	ββ-**7**
1	CH_2	32.5	36.8	32.4	36.9
2	CH_2	31.0	30.8	31.0	31.0
3	CH	68.7	69.2	68.7	69.1
4	CH_2	39.5	41.8	39.2	41.8
5	C	69.3	63.9	66.0	67.2
6	CH	62.7	65.0	62.6	67.5
7	CH	64.9	67.4	70.5	74.8
8	CH	37.1	34.4	40.3	38.4
9	CH	36.9	42.0	39.7	49.7
10	C	35.4	42.2	34.9	34.2
11	CH_2	20.4	21.9	20.5	22.0
12	CH_2	38.7	39.5	39.5	39.6
13	C	41.9	39.4	42.7	43.0
14	CH	48.8	49.4	55.6	55.3
15	CH_2	24.4	23.7	25.2	27.3
16	CH_2	28.1	28.2	28.3	28.6
17	CH	55.6	56.1	55.8	55.4
18	CH_3	11.7	11.6	11.8	11.7
19	CH_3	16.0	17.2	16.2	16.8
20	CH	35.7	34.4	32.4	35.7
21	CH_3	18.7	18.6	18.7	18.8
22	CH_2	36.1	36.9	36.2	37.0
23	CH_2	23.7	23.6	23.8	23.8
24	CH_2	39.5	39.4	39.4	39.5
25	CH	28.0	28.0	28.0	28.0
26/27	CH_3	22.8/22.6	22.8/22.6	22.8/22.6	22.8/22.6

an α-epoxide (higher frequency at $\delta \approx 3.9$ ppm) from the β-configured system ($\delta \approx 3.7$ ppm). The $J_{H6,H7}$ and $J_{H7,H8}$ coupling constants can be helpful in assigning the stereochemistry at the C-7 position: Small/medium coupling constants can be measured in the case of a 7α-OH disposition (4.8 and 2.8 Hz for $^3J_{H6,H7}$ and 0 for $J_{H7,H8}$ in αα-**4**/βα-**5**, respectively) and small/large values in the case of a 7β-OH stereocenter (0 and 1.5 Hz for $^3J_{H7,H8}$ and 6.3 and 8.0 Hz for $J_{H7,H8}$ in αβ-**6**/ββ-**7**, respectively).

From the ^{13}C NMR chemical shifts, we noted that the C-1 signal is a diagnostic one because it is shifted to a lower frequency (at $\delta \approx 32$ ppm) for the α-epoxy disposition compared with the β compound ($\delta \approx 36$ ppm). On the other hand, C-7 resonates at a lower frequency in the 7α-hydroxy (δ = 64.9 and 67.4 ppm) compared with the 7β-hydroxy (δ = 70.5 and 74.8 ppm) isomers. Although some differences in chemical shifts were observed, these diagnostic NMR signals can only be applied when additional substituents are not present in the A and B rings. For this reason, we used another approach to assign the correct stereochemistry of similar systems.

Configurational Analysis by a Combined QM and *J*-Based Approach (QM-JCH)

The *J*-based configurational analysis was conducted on **4**–**7** by using *J*-HMBC[19] and HETLOC[20] NMR experiments to calculate the C–H coupling constants for the key A and B rings. Experimental values were then compared with the calculated $^{2,3}J$(CH) values by state-of-the-art density functional theoretical methods. This methodology, known as the QM-*J*-based approach, has become very popular since its introduction by Bifulco and co-workers for the elucidation of the relative configurations of some natural products.[21]

In the interest of computational economy and to circumvent the degree of freedom of a flexible system, four 7-hydroxy-5,6-epoxycholestan-3β-ols without a side-chain (models **8**–**11**) were subjected to a conformational search using the simulated GMMX procedure (PCModel package). Just one global minimum structure was found for every epoxide by using the MMX force-field method.[22] Each geometry was then fully optimized by DFT at the B3LYP/6-31 or 6-311G(d) levels of theory implemented in the Gaussian 03 program package.[23] After the minimized geometries for each diastereoisomer had been obtained, coupling constants and ^{13}C NMR chemical shifts were calculated for each of them at the GIAO-B3LYP or MPW1PW91/6-311G(d,p) DFT level of theory.[24]

Table 3. Experimental *J* values for compounds **1**–**4** versus DFT-calculated values for models **8**–**11**.

	Exp. αα–**4**	DFT αα–**8**	DFT βα–**9**	DFT αβ–**10**	DFT ββ–**11**	$\Delta^{\alpha\alpha\text{-DFT}\alpha\alpha}$	$\Delta^{\alpha\alpha\text{-DFT}\beta\alpha}$	$\Delta^{\alpha\alpha\text{-DFT}\alpha\beta}$	$\Delta^{\alpha\alpha\text{-DFT}\beta\beta}$
^3J (H6-H7)	4.8	4.8	2.7	0.6	2.3	0.0	2.1	4.2	2.5
^3J (H7-H8)	4.8	5.6	3.3	6.0	7.1	0.8	1.5	1.2	2.3
^2J (C5-H4α)	-0.5	-0.8	-6.2	-1.0	-6.3	0.3	5.7	0.5	5.8
^2J (C5-H6)	0.5	-0.6	-0.7	-1.1	-0.6	1.1	1.2	1.6	1.1
^3J (C5-H19)	5.2	5.3	3.9	5.3	3.9	0.1	1.3	0.1	1.3
^2J (C6-H7)	0.7	0.6	-1.4	-3.5	1.8	0.1	2.1	4.2	1.1
^3J (C6-H8)	2.9	0.1	0.5	0.3	1.0	2.8	2.4	2.6	1.9
^2J (C7-H6)	4.7	4.7	4.4	2.7	3.4	0.0	0.3	2.0	1.3
^2J (C7-H8)	-3.2	0.1	-0.5	-5.9	-5.0	3.3	2.7	2.7	1.8
^3J (C8-H6)	4.5	4.3	3.7	3.5	3.8	0.2	0.8	1.0	0.7
					Σ	**8.7**	20.0	20.2	19.7
					MAD	**0.9**	2.0	2.0	2.0

	Exp. βα–**5**	DFT αα–**8**	DFT βα–**9**	DFT αβ–**10**	DFT ββ–**11**	$\Delta^{\beta\alpha\text{-DFT}\alpha\alpha}$	$\Delta^{\beta\alpha\text{-DFT}\beta\alpha}$	$\Delta^{\beta\alpha\text{-DFT}\alpha\beta}$	$\Delta^{\beta\alpha\text{-DFT}\beta\beta}$
^3J (H6-H7)	2.8	4.8	2.7	0.6	2.3	2.0	0.1	2.2	0.5
^3J (H7-H8)	2.8	5.6	3.3	6.0	7.1	2.8	0.5	3.2	4.3
^2J (C5-H4α)	-7.5	-0.8	-6.2	-1.0	-6.3	6.7	1.3	6.5	1.2
^2J (C5-H6)	0.0	-0.6	-0.7	-1.1	-0.6	0.6	0.7	1.1	0.6
^3J (C5-H7)	6.8	0.5	4.7	5.6	-0.1	6.4	2.1	1.2	6.9
^2J (C6-H7)	-4.0	0.6	-1.4	-3.5	1.8	4.6	2.6	0.5	5.8
^2J (C7-H6)	3.4	4.7	4.4	2.7	3.4	1.3	1.0	0.7	0.0
^2J (C7-H8)	-1.0	0.1	-0.5	-5.9	-5.0	1.1	0.5	4.9	4.0
^3J (C8-H6)	4.2	4.3	3.7	3.5	3.8	0.1	0.5	0.7	0.4
^3J (C9-H7)	5.7	5.8	4.2	0.1	0.4	0.0	1.5	5.6	5.3
					Σ	25.6	**10.8**	26.6	29.0
					MAD	2.6	**1.1**	2.7	2.9

	Exp. αβ–**6**	DFT αα–**8**	DFT βα–**9**	DFT αβ–**10**	DFT ββ–**11**	$\Delta^{\alpha\beta\text{-DFT}\alpha\alpha}$	$\Delta^{\alpha\beta\text{-DFT}\beta\alpha}$	$\Delta^{\alpha\beta\text{-DFT}\alpha\beta}$	$\Delta^{\alpha\beta\text{-DFT}\beta\beta}$
^3J (H6-H7)	0.0	4.8	2.7	0.0	2.3	4.8	2.7	0.0	2.3
^3J (H7-H8)	6.3	5.6	3.3	6.0	7.1	0.7	3.0	0.3	0.8
^2J (C5-H6)	-1.6	-0.6	-0.7	-1.1	-0.6	1.0	0.9	0.5	1.1
^2J (C5-H4α)	-1.0	-0.8	-6.2	-1.0	-6.3	0.2	5.2	0.0	5.3
^3J (C5-H7)	5.8	0.5	4.7	5.6	-0.1	5.4	1.1	0.2	5.9
^3J (C5-H19)	5.3	5.3	3.9	5.3	3.9	0.0	1.4	0.0	1.4
^2J (C6-H7)	-5.2	0.6	-1.4	-3.5	1.8	5.8	3.8	1.7	7.0
^2J (C7-H6)	4.1	4.7	4.4	2.7	3.4	0.6	0.3	1.4	0.7
^2J (C7-H8)	-5.9	0.1	-0.5	-5.9	-5.0	6.0	5.4	0.0	0.9
^3J (C8-H6)	4.0	4.3	3.7	3.5	3.8	0.3	0.3	0.5	0.2
^3J (C9-H7)	0.5	5.8	4.2	0.1	0.4	5.3	3.7	0.4	0.1
^3J (C14-H7)	6.5	0.6	1.5	5.3	4.1	5.9	5.0	1.2	2.5
					Σ	35.9	32.6	**6.2**	28.0
					MAD	3.0	2.7	**0.5**	2.3

	Exp. ββ–**7**	DFT αα–**8**	DFT βα–**9**	DFT αβ–**10**	DFT ββ–**11**	$\Delta^{\beta\beta\text{-DFT}\alpha\alpha}$	$\Delta^{\beta\beta\text{-DFT}\beta\alpha}$	$\Delta^{\beta\beta\text{-DFT}\alpha\beta}$	$\Delta^{\beta\beta\text{-DFT}\beta\beta}$
^3J (H6-H7)	1.5	4.8	2.7	0.6	2.3	3.3	1.2	0.9	0.8
^3J (H7-H8)	8.0	5.6	3.3	6.0	7.1	2.4	4.7	2.0	0.9
^2J (C5-H4α)	-7.2	-0.8	-6.2	-1.0	-6.3	6.4	1.0	6.2	0.9
^2J (C5-H6)	0.0	-0.6	-0.7	-1.1	-0.6	0.6	0.7	1.1	0.6
^2J (C6-H7)	-1.5	0.6	-1.4	-3.5	1.8	2.1	0.1	2.0	3.3
^3J (C6-H19)	4.0	-0.1	0.0	-0.3	0.4	4.1	4.0	4.3	3.7
^2J (C7-H6)	3.1	4.7	4.4	2.7	3.4	1.6	1.3	0.4	0.3
^2J (C7-H8)	-5.9	0.1	-0.5	-5.9	-5.0	6.0	5.4	0.0	0.9
^3J (C8-H6)	4.6	4.3	3.7	3.5	3.8	0.3	0.9	1.1	0.8
^3J (C7-H9)	3.7	2.2	2.7	1.6	3.4	1.5	1.0	2.1	0.3
					Σ	28.2	20.3	20.0	**12.4**
					MAD	2.8	2.0	2.0	**1.2**

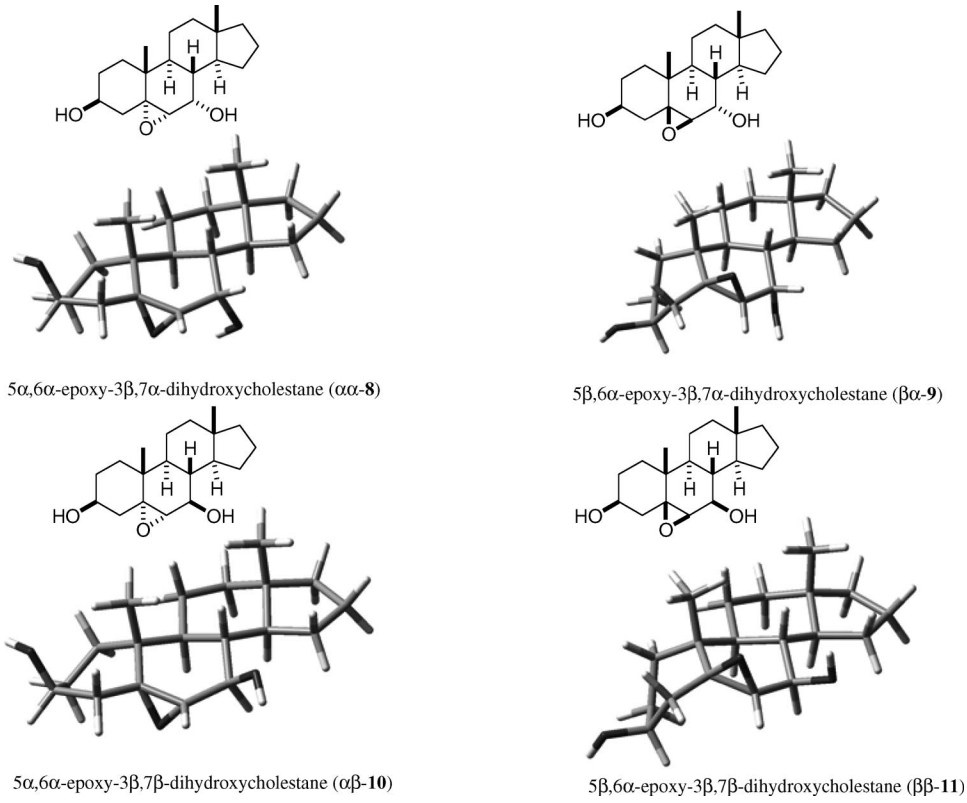


Figure 1. DFT models used for the B3LYP/6-311G(d) optimizations and the B3LYP/6-311G(d,p) NMR calculations.

Several statistical parameters are available to judge the accuracy of computed NMR spectroscopic data: Linear regressions, correlation coefficients, or absolute errors.[25] In this case, the mean absolute error deviation (MAD), defined as $\Sigma[(^nJ_{CH}\text{experimental} - {}^nJ_{CH}\text{calculated})/\text{number of comparisons}]$, was computed.

The correlation matrices between the theoretical and experimental coupling constants are shown in Table 3 for all compounds. The data for each pair (**4/8**, **5/9**, **6/10**, and **7/11**) indicate that the B3LYP method is convincingly able to distinguish the relative configurations for all four pairs. Ten experimental values for αα-**4** were compared with those calculated for αα-**8**, βα-**9**, αβ-**10**, and ββ-**11**. After the absolute differences had been computed, the values of αα-**4** and αα-**8** showed the smallest mean absolute deviation of 0.9. Similar MAD values were found for 10 comparisons between βα-**5**/βα-**9** (1.1), 12 comparisons for αβ-**6**/αβ-**10** (0.5), and 10 comparisons for ββ-**7**/βα-**11** (1.2) and these strongly favor the expected stereochemistry for each compound model pair.

No significant differences were found between the B3LYP and MPW1PW91 functionals, even when the solvent contribution was taken into account by using the integral equation-formalism polarizable continuum model (IEF-PCM) and chloroform as solvent.[26]

The study of the C–H coupling constants showed some differences: $^2J_{C7,H6}$ in the β-epoxides displayed values of around 3.3 Hz, whereas values of around 4.5 Hz were measured for the α-epoxides. Surprisingly, $^2J_{C5,H4\alpha}$ showed a larger absolute value (–7.5 and 7.2 Hz for **5** and **7**, respectively) in the case of the β-epoxide in comparison to the very small values (–0.5 and –1.0 Hz for **4** and **6**, respectively) measured for the α-epoxide. This difference can be explained by the twisted half chair-boat conformation of the A/B rings observed in the α-epoxy DFT models (see Figure 1).

At this stage we have shown that comparison of the experimental and calculated homo- and heteronuclear coupling constants enables the correct stereochemistry at C5–C7 to be determined in these 3β,7-dihydroxy-5,6-epoxycholestanes.

Configurational Analysis by ^{13}C NMR GIAO Chemical Shift Prediction of DFT Geometries (DFT–Chemical-Shift–NMR)

We wanted to test the efficiency of the GIAO methods in discriminating the four different stereoisomers through their ^{13}C NMR chemical shifts. It is well known that the ^{13}C NMR chemical shifts can be predicted with a sufficient degree of precision to discriminate between trial stereostructures that have previously been optimized. In addition, the δ_C values are spread over a large spectral window and are less sensitive to solvent effects. Therefore, a set of shielding tensors for each DFT-minimized geometry was computed by using the B3LYP/6-311G(d,p) functional and comparisons were made between the calculated NMR chemical shifts and the experimental values. We observed

that models with a steroidal side-chain increased the computational time by a factor of two or three in comparison to models **8**–**11**. Therefore, for computational time economy, we again focused our efforts on rings A and B of the steroidal skeleton without a side-chain in order to compare calculated chemical shifts with experimental values for **4**–**7**. We conducted two different approximations: A direct comparison between the C1–C10 framework skeleton and a second methodology that examined the differences in the chemical shifts around key oxygenated carbon atoms (Figure 2 and Table 4).

By direct comparison, the ^{13}C chemical shift calculated for all four diastereoisomers yielded results that are in good agreement with the experimental data. The linear fitting correlation coefficient index R^2, which shows the significance of the correlations between the experimental and theoretical values, varied from 0.984 to 0.972. For this purpose, the experimental values for αα-**4** were compared with those extracted from the NMR–DFT data obtained for αα-**8**, βα-**9**, αβ-**10**, and ββ-**11**. As expected, the best R^2 value corresponded to that of αα-**8**. Similar agreement was found for βα-**5**, αβ-**6**, and ββ-**7** when compared with the models βα-**9**, αβ-**10**, and ββ-**11**, respectively (see Figure 2).

A second approximation was used in order to remove possible inaccuracies in the DFT calculations. By examining the differences in the chemical shifts around the key oxygenated carbon atoms (C3, C5, C6, and C7), the absolute values of subtractions C3–C5, C3–C6, C3–C7, C5–C6, C5–C7, and C6–C7 for the synthetic compounds **4**–**7** ($\Delta^{\alpha\alpha\text{-}4}$, $\Delta^{\beta\alpha\text{-}5}$, $\Delta^{\alpha\beta\text{-}6}$, and $\Delta^{\beta\beta\text{-}7}$) were compared with those calculated by the B3LYP/6-311G(d,p) functional theory for models **8**–**11** ($\Delta^{\alpha\alpha\text{-}8}$, $\Delta^{\beta\alpha\text{-}9}$, $\Delta^{\alpha\beta\text{-}10}$, and $\Delta^{\beta\beta\text{-}11}$). As shown in Table 4, MAD values, defined as meaning the absolute deviation of such differences (eg., $\Delta^{\alpha\alpha\text{-}4} - \Delta^{\alpha\alpha\text{-}8}$) were calculated for all possible experimental/calculated pairs (six comparisons). Once again, a good level of agreement was reached with this methodology, with the expected experimental/theoretical pairs with the best fitting showing the smallest MAD values.

As the experimental data were recorded in $CDCl_3$, we also optimized the structures in chloroform at the B3LYP/6-311G(d,p) level. We did not observe any solvent effect in the minimized structure because the theoretical data were indistinguishable from those obtained by calculations in vacuo.

In order to validate our methodology for different 5,6-epoxycholestanols, we have applied our approximation to 1α,3β-dihydroxy-5α,6α-epoxycholestane (**12**), which again was compared with the DFT B3LYP/6-311G(d) energy-minimized models **13**–**16** (Figure 3) without side-chains. As expected, the DFT B3LYP/6-311G(d,p) ^{13}C NMR theoretical data of αα-**13** showed the best linear fitting correlation coefficient index R^2 (0.917, see Table 1 in the Supporting Information).

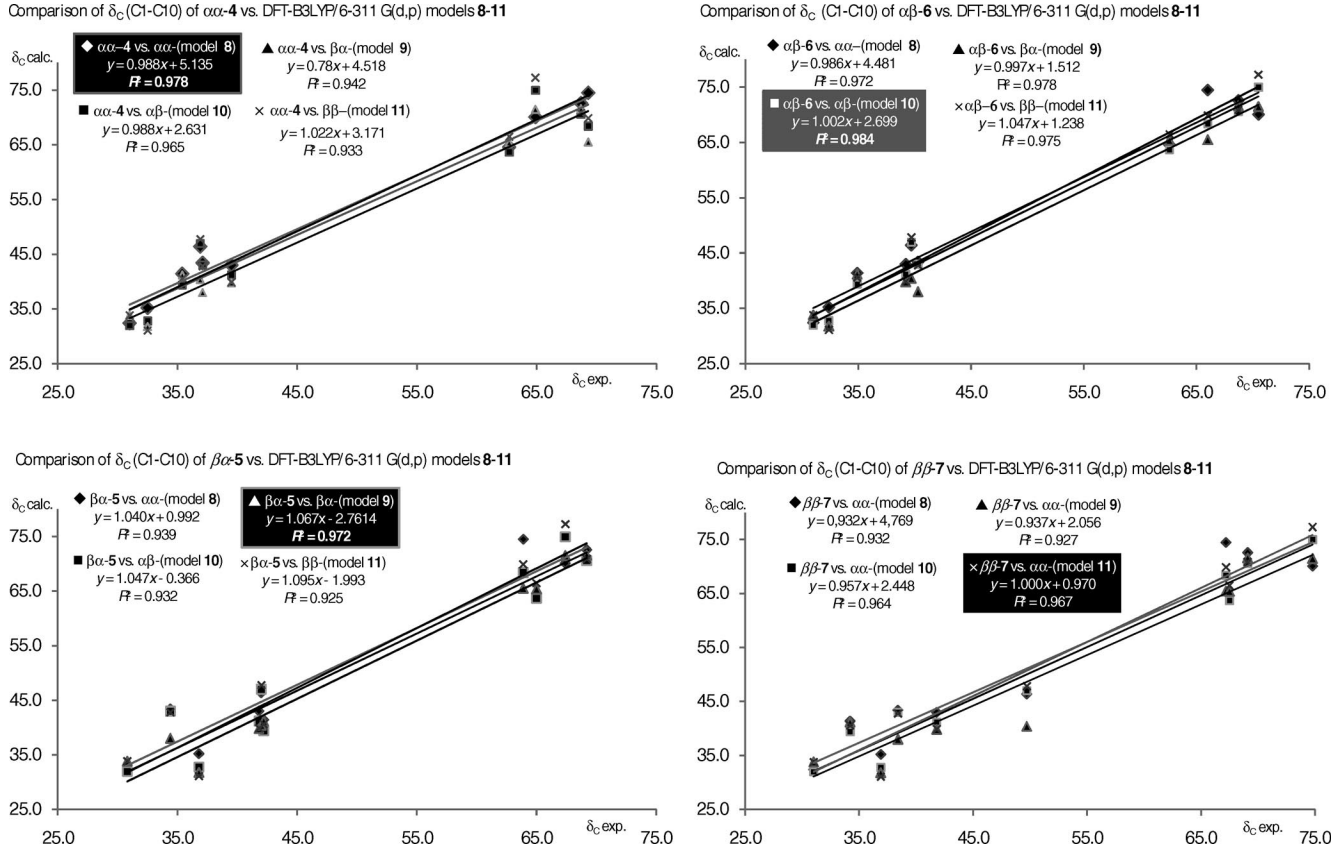


Figure 2. Comparison of the ^{13}C chemical shifts for C1–C10 between the experimental values for **4**–**7** and the values calculated for models **8**–**11**.

Table 4. Differences between the chemical shifts of the oxygenated carbon atoms (C-3, C-5, and C-7) in **4**–**7**[a] and the models **8**–**11**.[b]

	αα-**4**[a]	βα-**5**[a]	αβ-**6**[a]	ββ-**7**[b]	αα-**8**[b]	βα-**9**[b]	αβ-**10**[b]	ββ-**11**[b]
C3	68.7	69.2	68.7	69.1	72.6	71.6	70.6	70.9
C5	69.3	63.9	66.0	67.2	74.5	65.5	68.4	69.9
C6	62.7	65.0	62.6	67.5	64.6	65.4	63.7	66.5
C7	64.9	67.4	70.5	74.8	70.1	71.5	75.0	77.3
	$\Delta^{\alpha\alpha\text{-}4}$[a]	$\Delta^{\beta\alpha\text{-}5}$[a]	$\Delta^{\alpha\beta\text{-}6}$[a]	$\Delta^{\beta\beta\text{-}7}$[a]	$\Delta^{\alpha\alpha\text{-}8}$[b]	$\Delta^{\alpha\beta\text{-}9}$[b]	$\Delta^{\beta\alpha\text{-}10}$[b]	$\Delta^{\beta\beta\text{-}11}$[b]
C3-C5	-0.6	5.3	2.7	1.9	-1.9	2.2	6.1	1.0
C3-C6	6.0	4.2	6.1	1.6	8.0	6.9	6.2	4.4
C3-C7	3.8	1.8	-1.8	-5.7	2.5	-4.4	0.1	-6.4
C5-C6	6.6	-1.1	3.4	-0.3	9.9	4.7	0.1	3.4
C5-C7	4.4	-3.5	-4.5	-7.6	4.4	-6.6	-6.0	-7.4
C6-C7	-2.2	-2.4	-7.9	-7.3	-5.5	-11.3	-6.1	-10.8
	$\Delta^{\alpha\alpha\text{-}4} - \Delta^{\alpha\alpha\text{-}8}$		$\Delta^{\alpha\alpha\text{-}4} - \Delta^{\beta\alpha\text{-}9}$		$\Delta^{\alpha\alpha\text{-}4} - \Delta^{\alpha\beta\text{-}10}$		$\Delta^{\alpha\alpha\text{-}4} - \Delta^{\beta\beta\text{-}11}$	
C3-C5	1.3		6.7		2.8		1.6	
C3-C6	2.0		0.2		0.9		1.6	
C3-C7	1.3		3.7		8.2		10.2	
C5-C6	3.3		6.5		1.9		3.2	
C5-C7	0.0		10.4		11.0		11.8	
C6-C7	3.3		3.9		9.1		8.6	
Σ	**11.2**		31.4		33.9		37.0	
MAD[c]	**1.9**		5.2		5.6		6.2	
	$\Delta^{\beta\alpha\text{-}5} - \Delta^{\alpha\alpha\text{-}8}$		$\Delta^{\beta\alpha\text{-}5} - \Delta^{\beta\alpha\text{-}9}$		$\Delta^{\beta\alpha\text{-}5} - \Delta^{\alpha\beta\text{-}10}$		$\Delta^{\beta\alpha\text{-}5} - \Delta^{\beta\beta\text{-}11}$	
C3-C5	7.2		0.8		3.1		4.3	
C3-C6	3.8		2.0		2.7		0.2	
C3-C7	0.7		1.7		6.2		8.2	
C5-C6	11.0		1.2		5.8		4.5	
C5-C7	7.9		2.5		3.1		3.9	
C6-C7	3.1		3.7		8.9		8.4	
Σ	33.7		**11.9**		29.8		29.5	
MAD[c]	5.6		**2.0**		5.0		4.9	
	$\Delta^{\alpha\beta\text{-}6} - \Delta^{\alpha\alpha\text{-}8}$		$\Delta^{\alpha\beta\text{-}6} - \Delta^{\beta\alpha\text{-}9}$		$\Delta^{\alpha\beta\text{-}6} - \Delta^{\alpha\beta\text{-}10}$		$\Delta^{\alpha\beta\text{-}6} - \Delta^{\beta\beta\text{-}11}$	
C3-C5	4.6		3.4		0.5		1.7	
C3-C6	1.9		0.1		0.8		1.7	
C3-C7	4.3		1.9		2.6		4.6	
C5-C6	6.5		3.3		1.3		0.0	
C5-C7	8.9		1.5		2.1		2.9	
C6-C7	2.4		1.8		3.4		2.9	
Σ	28.6		12.0		**10.7**		13.8	
MAD[c]	4.8		2.0		**1.8**		2.3	
	$\Delta^{\beta\beta\text{-}7} - \Delta^{\alpha\alpha\text{-}8}$		$\Delta^{\beta\beta\text{-}7} - \Delta^{\beta\alpha\text{-}9}$		$\Delta^{\beta\beta\text{-}7} - \Delta^{\alpha\beta\text{-}10}$		$\Delta^{\beta\beta\text{-}7} - \Delta^{\beta\beta\text{-}11}$	
C3-C5	3.8		0.3		4.2		0.9	
C3-C6	6.4		5.3		4.6		2.8	
C3-C7	8.2		1.3		5.8		0.7	
C5-C6	10.2		5.0		0.4		3.7	
C5-C7	12.0		1.0		1.6		0.2	
C6-C7	1.8		4.0		1.2		3.5	
Σ	42.4		16.9		17.8		**11.8**	
MAD[c]	7.1		2.8		3.0		**2.0**	

[a] Experimental ^{13}C chemical shifts. [b] DFT B3LYP/6-311G(d,p)-calculated chemical shifts. [c] MAD (mean absolute deviation) = Σ/6.

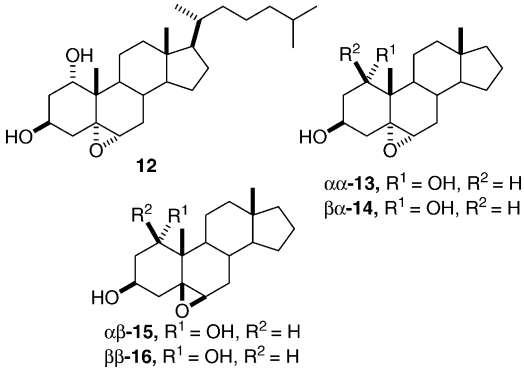


Figure 3. Validation of the DFT–NMR methodology for 1α,3β-dihydroxy-5,6-epoxycholestanes.

Conclusions

Four 3,7-dihydroxy-5,6-epoxycholestanols (**4**–**7**) have been synthesized from cholesterol. With these compounds we conducted a detailed NMR analysis by using 1D and 2D experiments: Chemical shifts and the $^{3}J_{H,H}$ and $^{2,3}J_{C,H}$ values were determined from ^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, edited HSQC, HMBC, *J*-HMBC, and HETLOC experiments. The same parameters were accurately predicted by applying DFT methodologies using the B3LYP and MPW1PW91 functionals to the theoretical structural models **8**–**11** without a side-chain. We have demonstrated the ability of DFT–NMR calculations to assist in identifying the four possible diastereoisomers at the C-5, C-6, and C-7 positions. The comparisons between the synthesized (**4**–**7**) and model (**8**–**11**) compounds gave consistent results for

rings A and B. The calculations confirmed that the hybrid QM-*J*-based methodology is the best tool for identifying each diastereoisomer.

Experimental Section

General: Most reactions were run under argon with strict exclusion of moisture and in oven-dried vessels. Solvents were distilled prior to use. TLC was carried out on precoated sheets (silica gel 60 F254, Merck). Spot visualization was achieved by UV light and/or phosphomolybdic acid solution. Flash chromatography was performed by using the indicated solvents on Merck silica gel 60 (0.04–0.063 mm). NMR spectra were recorded with a Bruker Avance 300 MHz or 500 MHz spectrometer. Carbon–proton coupling constants were calculated by using *J*-HMBC and HETLOC sequences. For *J*-HMBC, 16 scans (2K data points) and 128 experiments were run and transformed by applying QSINE functions in both dimensions and zero-filling at F1. For the HETLOC experiments, 32 scans (4K) and 256 experiments were obtained with zero-filling at F1 to 4 K. A mixing time of 40–75 ms was used. Geometries for models **5**–**7** were obtained by quantum chemical calculations in vacuo with the density functional theory methodology (DFT). Each model was first submitted to a GMMX structural search by using the PCModel package. All models presented just one representative conformer, which was preoptimized at the AM1 level and further optimized at the B3LYP/6-31G(d) level implemented in the Gaussian 03 V.3.0 package. After energy minimization, ^{1}H–^{1}H, ^{13}C–^{1}H coupling constants and ^{13}C chemical shifts were calculated in vacuo with the B3LYP or MPW1PW91 functional using the 6-311G(d,p) basis set. The calculated magnetic shieldings were converted into the ^{13}C chemical shifts by calculation of the absolute δ_C of TMS at the same level of theory. In order to account for solvent effects, we adopted the integral equation-formalism polarizable continuum model (IEF-PCM) method.

3β-Acetoxycholest-5-en-7-one (1): Cholesterol (1 g, 2.6 mmol) and acetic anhydride/pyridine (1:1, 10 mL) were stirred at room temperature for 24 h. After removal of the solvents, the residue was dissolved in ethyl acetate (20 mL), and the solution was washed with $NaHCO_3$ (20 mL) and 5% HCl (20 mL) and dried with anhydrous Na_2SO_4. The resulting organic phase was concentrated under vacuum to give 3β-acetoxy-5-cholestene (0.52 g, 94%). *tert*-Butyl hydroperoxide (15.4 mL) was added dropwise to a solution of 3β-acetoxy-5-cholestene (5.5 g, 12.8 mmol) and CuI (0.1 g, 0.5 mmol) in dry benzene (50 mL). The mixture was heated under reflux for 24 h and then cooled. The reaction mixture was added dropwise to a solution of Na_2SO_3 (30 mL). The resulting precipitate was extracted with diethyl ether, and the organic phase was washed (10% HCl, 10% $NaHCO_3$, brine), dried with anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by chromatography (silica gel, hexanes/ethyl acetate, 9:1) to give 3β-acetoxycholest-5-en-7-one (**1**; 2.1 g, 40%). $[\alpha]_D$ = –78.1 (CH_2Cl_2, *c* = 1.0). ^{1}H NMR (200 MHz, $CDCl_3$): δ_H = 5.70 (s, 1 H, 6-H), 4.72 (m, 1 H, 3α-H), 2.53 (m, 1 H, 8-H), 2.06 (s, 3 H, OAc), 1.02 (s, 3 H, 19-H), 0.93 (d, *J* = 6.3 Hz, 3 H, 21-H), 0.87 (d, *J* = 6.6 Hz, 6 H, 26-H, 27-H), 0.67 (s, 3 H, 18-H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ_C = 210.8 (s, C-7), 170.2 (s, OAc), 163.8 (s, C-5), 126.6 (d, C-6), 72.1 (d, C-3), 56.8, 56.0, 50.2, 42.2, 39.6, 39.4, 36.8, 36.2, 35.9, 35.7, 32.0, 31.6, 27.9, 27.7, 25.8, 24.2, 22.7, 22.5, 21.7, 21.3, 20.6, 20.3, 17.2 (q, C-19), 11.9 (q, C-18) ppm. (+)-LRMS (FAB): *m*/*z* (%) = 465 (5) $[M + Na]^+$, 399 (100).

3β,7α-Dihydroxycholest-5-ene (2): L-Selectride (14 mL) was added dropwise over 15 min to a solution of 3β-acetoxycholest-5-en-7-one (**1**; 1.9 g, 4.4 mmol) in dry THF (50 mL). The reaction mixture was stirred at –78 °C for 1 h. The residue was diluted with water (20 mL) and extracted with CH_2Cl_2 (50 mL). The organic phase was washed (10% HCl, 10% $NaHCO_3$, brine), dried with anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by chromatography (silica gel, hexanes/ethyl acetate, 8:2) to give 3β,7α-dihydroxycholest-5-ene (**2**; 1.82 g, 95%). $[\alpha]_D$ = –130.2 (CH_2Cl_2, *c* = 1.0). ^{1}H NMR (200 MHz, $CDCl_3$): δ_H = 5.59 (d, *J* = 4.9 Hz, 1 H, 6-H), 3.84 (m, 1 H, 7β-H), 3.58 (m, 1 H, 3α-H), 0.99 (s, 3 H, 19-H), 0.92 (d, *J* = 6.2 Hz, 3 H, 21-H), 0.85 (d, *J* = 6.6 Hz, 6 H, 26-H, 27-H), 0.68 (s, 3 H, 18-H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ_C = 146.2 (s, C-5), 123.8 (d, C-6), 71.3 (d, C-3), 65.3 (d, C-7), 56.2, 48.6, 42.3, 39.4, 39.2, 37.2, 36.0, 35.8, 35.6, 34.7, 33.3, 31.2, 30.7, 28.0, 27.9, 24.5, 23.7, 22.7, 22.5, 21.4, 20.6, 18.2 (q, C-19), 11.6 (q, C-18) ppm. LRMS (EI, 70eV): *m*/*z* (%) = 402 (4) $[M]^+$, 385 (30) $[M – OH]^+$, 384 (13) $[M – H_2O]^+$, 368 (4) $[M – 2\ OH]^+$, 237 (100).

3β,7α-Dihydroxy-5α,6α-epoxycholestane (αα-4) and 3β,7α-Dihydroxy-5β,6β-epoxycholestane (βα-5): 3β,7α-Dihydroxycholest-5-ene (**2**; 0.1 g, 0.2 mmol) was dissolved in $CHCl_3$ (5 mL) at 0 °C. A solution of *m*-chloroperbenzoic acid (0.1 g, 0.6 mmol) in $CHCl_3$ (5 mL) was added dropwise to the reaction mixture, and the solution was stirred for 22 h. A solution of 5% Na_2SO_3 (10 mL) was added to the mixture with cooling (ice/water bath), and the mixture was kept at this temperature for 6 h. The final aqueous phase was extracted twice with $CHCl_3$ (10 mL), and, after removal of the solvent, the epoxides were separated by flash chromatography (hexane/ethyl acetate, 7:3) to give 3β,7α-dihydroxy-5α,6α-epoxycholestane (αα-**4**; 82 mg) and 3β,7α-dihydroxy-5β 6β-epoxycholestane (βα-**5**; 6 mg) in a yield of 88%.

3β,7α-Dihydroxy-5α,6α-epoxycholestane (αα-4): LRMS (EI, 70 eV): *m*/*z* (%) = 418 (3) $[M]^+$, 400 (15) $[M – H_2O]^+$, 382 (25) $[M – 2\ H_2O]^+$, 95 (100). LRMS (FAB, positive ion, 3-NBA): *m*/*z* (%) = 419 (7) $[M + H]^+$, 401 (100) $[M + H – H_2O]^+$. HRMS (ESI): *m*/*z* (%) = 401.3433 (100) $[M – H_2O]^+$, 419.3530 (27) $[M]^+$, 441.3353 (30) $[M + Na]^+$. $[\alpha]_D$ = –57.4 (*c* = 0.5, CH_2Cl_2).

3β,7α-Dihydroxy-5β,6β-epoxycholestane (βα-5): LRMS (FAB, positive ion, thioglycerol): *m*/*z* (%) = 431 (7) $[M + Na]^+$, 401 (100) $[M + H – H_2O]^+$. LRMS (EI, 70 eV): *m*/*z* (%) = 400 (8) $[M – H_2O]^+$, 95 (100). HRMS (ESI): *m*/*z* (%) = 401.3432 (100) $[M – H_2O]^+$, 419.3531 (27) $[M]^+$, 441.3350 (30) $[M + Na]^+$. $[\alpha]_D$ = –9.4 (*c* = 0.5, CH_2Cl_2).

3β-Acetoxy-7β-hydroxy-4-cholestene (3): $NaBH_4$ (0.017 g, 0.06 mmol) was added to a solution of 3β-acetoxycholest-5-en-7-one (**1**; 0.1 g, 0.25 mmol) and $CeCl_3 \cdot 7H_2O$ (0.086 g, 0.23 mmol) in a dry mixture of THF/MeOH (1:1, 5 mL) . The reaction mixture was stirred at room temperature for 2 h. The residue was diluted with water (10 mL) and extracted with CH_2Cl_2 (10 mL). The organic phase was washed with 5% HCl and brine, dried with anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by chromatography (silica gel, hexanes/ethyl acetate, 9:1) to give 3β-acetoxy,7β-hydroxy-4-cholestene (**3**; 0.090 g, 90%). $[\alpha]_D$ = –132.6 (*c* = 0.1, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ_H = 5.31 (s, 1 H, 6-H), 4.61 m, 1 H, (3α-H), 3.84 (d, *J* = 8.0 Hz, 1 H, 7α-H), 2.03 (s, 3 H, OAc), 1.06 (s, 3 H, 19-H), 0.89 (d, *J* = 6.3 Hz, 3 H, 21-H), 0.87 (d, *J* = 6.6 Hz, 6 H, 26-H, 27-H), 0.68 (s, 3 H, 18-H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ_C = 170.5 (OAc), 142.3 (s, C-5), 126.2 (d, C-6), 73.4 (d, C-3), 73.1 (d, C-7), 55.8, 55.3, 48.1, 42.8, 40.7, 39.4, 37.5, 36.6, 36.4, 35.7, 35.2, 28.0, 27.5, 27.2, 24.5, 23.8, 23.1, 22.7, 22.5, 21.3, 20.5, 18.6, 18.3 (q, C-19), 11.3 (q, C-18) ppm. LRMS (EI, 70 eV): *m*/*z* (%) = 444 (4) $[M]^+$, 384 (100).

3β,7β-Dihydroxy-5α,6α-epoxycholestane (αβ-6) and 3β,7β-Dihydroxy-5β,6β-epoxycholestane (ββ-7): 3β-Acetoxy,7β-hydroxy-4-cholestene (**3**; 0.10 g, 0.23 mmol) was dissolved in $CHCl_3$ (10 mL) at 0 °C. A solution of *m*-chloroperbenzoic acid (0.11 g, 0.7 mmol) in $CHCl_3$ (10 mL) was added dropwise to the reaction mixture, and the solution was stirred for 24 h. A solution of 5% Na_2SO_3 (10 mL) was added to the mixture with cooling (ice/water bath), and the mixture was kept at this temperature for 6 h. The final aqueous phase was extracted twice with $CHCl_3$ (10 mL) and, after removal of the solvent, the residue was purified by chromatography (hexane/ethyl acetate, 7:3) to give a mixture of 3β-acetoxy-7β-hydroxy-5α,6α-epoxycholestane and 3β-acetoxy-7β-hydroxy-5β,6β-epoxycholestane in a yield of 82%.

3β-Acetoxy-7β-hydroxy-5α,6α-epoxycholestane: $[\alpha]_D$ = +71.0 ($CHCl_3$, *c* = 0.1). ^{1}H NMR (200 MHz, $CDCl_3$): δ_H = 4.75 (m, 1 H, 3α-H), 3.52 (m, 1 H, 7α-H), 2.87 (s, 1 H, 6β-H), 2.03 (s, 3 H, OAc), 1.01 (s, 3 H, 19-H), 0.90 (d, *J* = 6.2 Hz, 3 H, 21-H), 0.86 (d, *J* = 6.2 Hz, 6 H, 26-H, 27-H), 0.64 (s, 3 H, 18-H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ_C = 170.0 (OAc), 74.2 (d, C-7), 70.4 (d, C-3), 66.8 (s, C-5), 66.3 (d, C-6), 54.9, 54.7, 48.9, 42.5, 39.1, 39.0, 27.8, 37.1, 35.8, 35.6, 35.2, 34.3, 33.2, 28.2, 27.6, 26.7, 23.4, 22.2, 22.1, 21.4, 20.8, 18.7, 16.2 (q, C-19), 11.3 (q, C-18) ppm. LRMS (EI, 70 eV): *m*/*z* (%) = 460 (4) $[M]^+$, 84 (100).

3β-Acetoxy-7β-hydroxy-5β,6β-epoxycholestane: $[\alpha]_D$ = +71.0 (*c* = 0.1, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ_H = 4.86 (m, 1 H, 3α-H), 3.71 (m, 1 H, 7α-H), 3.16 (s, 1 H, 6α-H), 2.02 (s, 3 H, OAc), 1.01 (s, 3 H, 19-H), 0.89 (d, *J* = 6.4 Hz, 3 H, 21-H), 0.87 (d, *J* = 6.4 Hz, 6 H, 26-H, 27-H), 0.65 (s, 3 H, 18-H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ_C = 170.0 (OAc), 74.2 (d, C-7), 70.4 (d, C-3), 66.8 (s, C-5), 66.3 (d, C-6), 54.9, 54.7, 48.9, 42.5, 39.1, 39.0, 27.8, 37.1, 35.8, 35.6, 35.2, 34.3, 33.2, 28.2, 27.6, 26.7, 23.4, 22.2, 22.1, 21.4, 20.8, 18.7, 16.2 (q, C-19), 11.3 (q, C-18) ppm. LRMS (EI, 70 eV): *m*/*z* (%) = 460 (4) $[M]^+$, 84 (100).

A mixture of 3β-acetoxy-7β-hydroxy-5α,6α-epoxycholestane and 3β-acetoxy-7β-hydroxy-5β,6β-epoxycholestane (0.1 g, 0.2 mmol) was dissolved in a 5% methanolic potassium hydroxide solution (5 mL) under argon. The mixture was stirred at room temperature for 2 h, and then the solvent was evaporated under reduced pressure. This solution was poured into ice/water (10 g) and the product extracted with ethyl acetate (2 × 10 mL). The combined extracts were washed with saturated brine, dried, and concentrated under reduced pressure. The residue was purified by chromatography (silica gel, hexanes/ethyl acetate, 9:1, 8:2, 7:3) to give 0.090 g of a mixture of 3β,7β-dihydroxy-5α,6α-epoxycholestane (αβ-**6**) and 3β,7β-dihydroxy-5β,6β-epoxycholestane (ββ-**7**) in a yield of 90%. Each epoxide was separated and purified by RP-HPLC [Sharlau C18, flow rate 1 mL/min, MeOH/H_2O, 96:4, retention time: 31–34 min for ββ-**7** and 35–36 min for αβ-**6**].

3β,7β-Dihydroxy-5α,6α-epoxycholestane (αβ-6): LRMS (FAB, positive ion, 3-NBA): *m*/*z* (%) = 419 (8) $[M + H]^+$, 154 (100). HRMS (ESI): *m*/*z* (%) = 401.3413 (100) $[M - H_2O]^+$, 419.3512 (62) $[M]^+$, 441.3330 (38) $[M + Na]^+$. $[\alpha]_D$ = –26.1 (*c* = 0.01, $CHCl_3$).

3β,7β-Dihydroxy-5β,6β-epoxycholestane (ββ-7): LRMS (FAB, positive ion, thioglycerol): *m*/*z* (%) = 419 (23) $[M + H]^+$, 401 (100) $[M + H - H_2O]^+$. LRMS (EI, 70 eV): *m*/*z* (%) = 419 (3) $[M]^+$, 401 (8) $[M - H_2O]^+$, 84 (100). HRMS (ESI): *m*/*z* (%) = 401.3407 (68) $[M - H_2O]^+$, 419.3514 (63) $[M]^+$, 441.3331 (62) $[M + Na]^+$. $[\alpha]_D$ = +15.2 (*c* = 0.01, CH_2Cl_2).

Supporting Information (see footnote on the first page of this article): 1D and 2D NMR spectra for synthetic intermediates and for compounds **4**–**7**.

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