Synthesis and structural confirmation of calibagenin and saxosterol

Bunta Watanabe, Katsunori Makino, Masaharu Mizutani, Hikaru Takaya

PII: S0040-4020(21)00392-6

DOI: https://doi.org/10.1016/j.tet.2021.132194

Reference: TET 132194

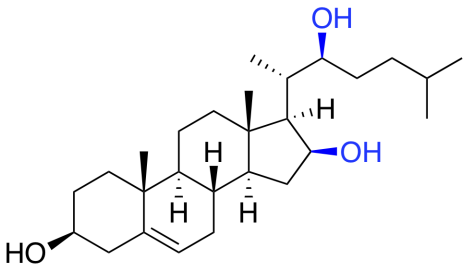
To appear in: *Tetrahedron*

Received Date: 9 February 2021

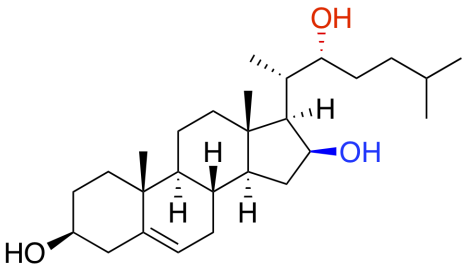
Revised Date: 14 April 2021

Accepted Date: 28 April 2021

Please cite this article as: Watanabe B, Makino K, Mizutani M, Takaya H, Synthesis and structural confirmation of calibagenin and saxosterol, *Tetrahedron*, https://doi.org/10.1016/j.tet.2021.132194.



calibagenin
(reassigned structure)



saxosterol
(confirmed structure)

Synthesis and structural confirmation of calibagenin and saxosterol

Bunta Watanabe,[a, *] Katsunori Makino,[a] Masaharu Mizutani,[b] and Hikaru Takaya[a]

[a] Institute for Chemical Research, Kyoto University, Gokasho, Uji, Kyoto 611-0011, Japan
[b] Graduate School of Agricultural Science, Kobe University, Rokkodai, Nada, Kobe 657-8501, Japan

*To whom correspondence should be addressed. Tel: +81-774-38-3233; Fax: +81-774-38-3229; E-mail: bunta@scl.kyoto-u.ac.jp

Manuscript type: Article

Abstract

Calibagenin and saxosterol are cholesterol-based plant steroids isolated from *Calibanus hookerii* and *Narthecium ossifragum*, respectively. To date, the configurations of their 16- and 22-hydroxy groups have not yet been determined. In this study, all the four 16,22-stereoisomers were chemically synthesized. The ^{1}H and ^{13}C NMR spectra were fully assigned using 2D NMR techniques, and the structures were determined unambiguously using X-ray crystallography. The H-18 and H-22 signals in the NMR spectra of the products are diagnostic for determining the configuration of the 16- and 22-hydroxy groups. A comparison of the NMR, $[\alpha]_D$, and mp data of the four isomers with those of natural calibagenin and saxosterol confirmed that the configurations of the 16- and 22-hydroxy groups of the former and the later are identical to 16β,22*S* and 16β,22*R*, respectively.

Keywords: calibagenin, saxosterol, 2D NMR, X-ray crystallography

1. Introduction

Calibagenin was isolated from *Calibanus hookerii*, a plant native to Tamaulipas in Mexico, in 1975, and its structure was proposed to be one of the four isomers of cholest-5-en-3β,16,22-triol (**1**, Figure 1), while the configuration of the hydroxy groups at C-16 and C-22 remained undetermined.[1] In 1988, saxosterol was isolated from *Narthecium ossifragum*, a plant of Western Europe, and its structure was also proposed to be one of the four isomers of **1**.[2] Stabursvik and Holen deduced the absolute structure of saxosterol to be 16β,22*R*-**1** based on the observation that the complete acetylation of saxosterol required a more reactive reagent than that of calibagenin, presumably due to the steric hindrance of the 16-hydroxy group (β vs. α), and the fact that 22*R*-hydroxycholesterol has been isolated from *N. ossifragum*.[2] They also speculated that the absolute structure of calibagenin should be 16α,22*R*-**1**, albeit that the absolute structures of calibagenin and saxosterol have not yet been determined unequivocally.

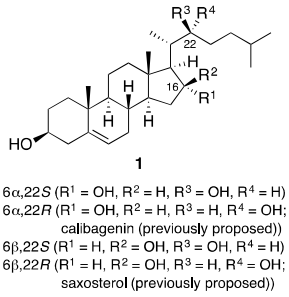


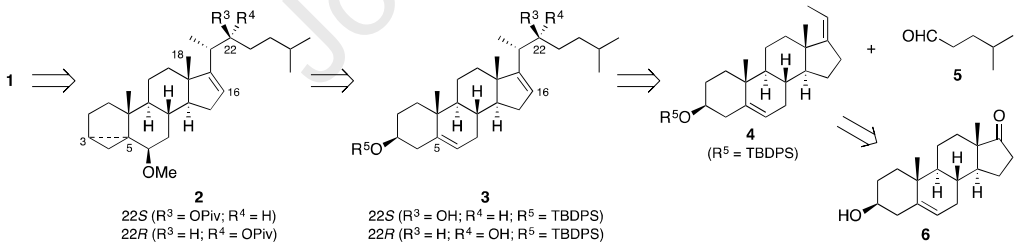
Figure 1. Structures of the four 16,22-stereoisomers of cholest-5-en-3β,16,22-triol (**1**). The previously proposed structures of calibagenin (16α,22*R*-**1**) and saxosterol (16β,22*R*-**1**) are also indicated.

In contrast to the study of Stabursvik and Holen, 16β,22*S*-**1** is a frequently encountered structure in plants. For example, 16β,22*S*-**1** is a common aglycone found in schubertosides B and C from *Allium schubertii*,[3] one of the cholestane bisdesmosides from *Galtonia candicans*,[4] spongioside A from *Dioscorea spongiosa*,[5] and karataviosides J and K from *Allium karataviense*.[6] However, only the mp, $[\alpha]_D$, and limited ^{1}H NMR data have been reported in the cases of calibagenin and saxosterol, and these data were not provided completely in the report of 16β,22*S*-**1** mentioned above. As a result, a structural assignment of calibagenin/saxosterol and 16β,22*S*-**1** has not been yet accomplished. Recently, 16β,22*S*-**1** has been identified as an enzymatic reaction product of cytochromes P450 involved in the biosynthesis of plant steroid diosgenin.[7] Therefore, the synthesis of all the four 16,22-stereoisomers of **1** may not only lead to the absolute structure determination of calibagenin and saxosterol, but also provide a useful tool for the biosynthetic study of plant steroids such as diosgenin.

In this study, we have developed a synthetic route to the four 16,22-stereoisomers of **1** with reliable configurations, and their absolute structures were unambiguously determined using X-ray crystallography. Moreover, we fully assigned their ^{1}H and ^{13}C NMR spectra and established diagnostic chemical shifts that allow differentiating these isomers. Furthermore, a comparison of the NMR, $[\alpha]_D$, and mp data of synthetic **1** with those of calibagenin and saxosterol confirmed the absolute structures of these two natural products.

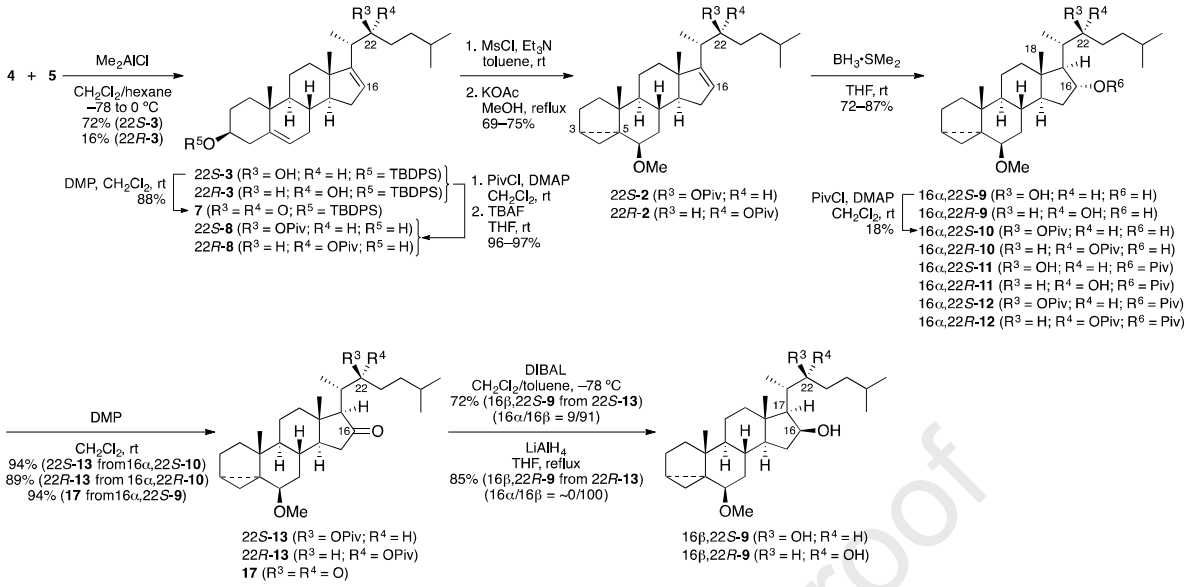
2. Results and Discussion

Our retrosynthetic strategy is outlined in Scheme 1. The introduction of the oxygen functionality at C-16 of **1** was achieved by a hydroboration of the Δ^{16} double bond of **2**. The presence of the C-18 methyl group on the β-face of the steroidal skeleton can be expected to force the borane to approach from the opposite α-face. In fact, the hydroboration of Δ^{16} double bonds has been reported to produce 16α-alcohols.[8-12] Similarly, the reduction of C-16 carbonyl groups is known to afford 16β-alcohols as the major product.[8,9,12] The 3α,5-cyclosteroidal structure of **2** is a protected form of the Δ^{5} double bond of **3** during hydroboration. The side chain and the Δ^{16} double bond of **3** could be constructed by the ene reaction between the (*Z*)-olefin **4**, which is readily prepared in two steps from commercially available dehydroepiandrosterone (**6**),[14] and 4-methylpentanal (**5**). Houston and co-workers previously obtained (22*S*)-3β,22-diacetoxycholest-5,16-diene, which exhibits configurations identical to those of 22*S*-**3**, being the predominant product from a Me_2AlCl-mediated ene reaction between a (*Z*)-olefin similar to **4** (R^5 = Ac) and **5**, followed by acetylation.[13]



Scheme 1. Retrosynthetic strategy.

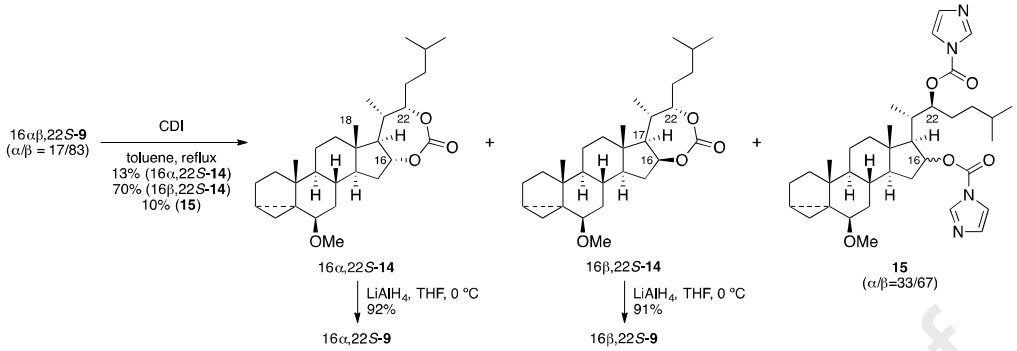
Our synthesis started with the ene reaction between **4**[14] and aldehyde **5**[15] to provide 22*S*-**3** and 22*R*-**3** in 72% and 16% yield, respectively (Scheme 2). To convert the major product 22*S*-**3** into the minor product 22*R*-**3**, 22*S*-**3** was oxidized with Dess-Martin periodinane (DMP), and the resulting ketone **7** was reduced using Li(*sec*-Bu)$_3$BH to furnish 22*R*-**3** in 89% yield. Pivaloylation of the 22-hydroxy group of **3** and hydrolysis of the TBDPS protecting group afforded **8** in 96–97% yield. The obtained **8** was converted into 3α,5-cyclosteroid **2** in 69–75% yield by methanolysis of the homoallylic mesylate in the presence of KOAc.[16]



Scheme 2. Ene reaction and subsequent introduction of the oxygen functionality at C-16.

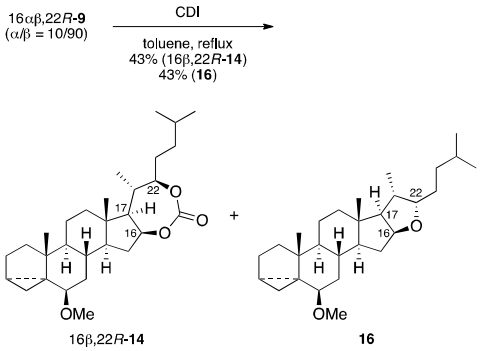
Excess BH_3•SMe_2 (5 equiv.) was necessary to complete the hydroboration of 22*S*-**2**, and 16α,22*S*-**9** was obtained in 87% yield. In the hydroboration of 22*R*-**2** with five equivalents of BH_3•SMe_2, 16α,22*R*-**9** was obtained in 72% yield, and 16α,22*R*-**10**, in which the pivaloyl group remained on the 22-hydroxy group, was obtained in 5.6% yield. The NOE correlation between H-18 and H-16 confirmed the configuration of the 16-hydroxy groups of the hydroboration products to be α. Prior to the inversion of the configuration of the 16-hydroxy group from α to β, the 22-hydroxy group of 16α,22*S*-**9** was re-pivaloylated to give 16α,22*S*-**10** in 18% yield using 1.1 equiv. of PivCl and 1.5 equiv. of DMAP. The remaining products were a mixture of 16α,22*S*-**11**, in which the pivaloyl group was introduced on the 16α-hydroxy group, and bis-pivaloylated 16α,22*S*-**12** (**11**/**12** = 88/12, 79%). When 16α,22*R*-**9** was treated with PivCl (2.7 equiv.) and DMAP (3.2 equiv.), 16α,22*R*-**10** (45%), 16α,22*R*-**11** (5.3%), and 16α,22*R*-**12** (38%) were isolated. 16α,22*S*-**10** and 16α,22*R*-**10** were oxidized with DMP to give 22*S*-**13** and 22*R*-**13** in 89–94% yield (Scheme 2). A reduction of the carbonyl group of 22*S*-**13** with excess DIBAL at –78 °C provided 16αβ,22*S*-**9** (α/β = 9/91) in 72% yield (Scheme 2). During the reaction, the pivaloyl group on the 22-hydroxy group was also removed. While it is difficult to separate 16α,22*S*-**9** and 16β,22*S*-**9** by chromatography on silica gel, their derivatization into the corresponding cyclic carbonates 16α,22*S*-**14** and 16β,22*S*-**14** made their chromatographic separation manageable. When 16αβ,22*S*-**9** (α/β = 17/83) was treated with 1,1′-carbonyldiimidazole (CDI), 16α,22*S*-**14** and 16β,22*S*-**14** were obtained in 13% and 70% yield, respectively, and bis-imidazolylcarbonylated **15** was also isolated in 10% yield (Scheme 3). The NOE correlation between H-18 and H-16 in 16α,22*S*-**14** as well as that between H-17 and H-16 in 16β,22*S*-**14** confirmed their configuration of the oxygen

functionality at C-16. The cyclic carbonates of 16α,22*S*-**14** and 16β,22*S*-**14** were reductively cleaved with $LiAlH_4$ to produce 16α,22*S*-**9** and 16β,22*S*-**9** in 91–92% yield (Scheme 3).



Scheme 3. Separation of 16α,22*S*-**9** and 16β,22*S*-**9** via cyclic carbonates **14**.

In the reduction of the carbonyl group of 22*R*-**13**, treatment with $LiAlH_4$ in refluxing THF gave 16β,22*R*-**9** in 85% yield (Scheme 1). The configuration of the 16-hydroxy group of 16β,22*R*-**9** was confirmed by the NOE correlation between H-17 and H-16. When 22*R*-**13** was treated with DIBAL at –78 °C, β selectivity was somewhat lower (α/β = 6/94, 88%). Similar to 16α,22*S*-**9** and 16β,22*S*-**9**, 16α,22*R*-**9** and 16β,22*R*-**9** are also difficult to separate by chromatography on silica gel. Thus, derivatization into the cyclic carbonates was examined (Scheme 4). When 16αβ,22*R*-**9** (α/β = 10/90) was treated with CDI, cyclic carbonate 16β,22*R*-**14** was obtained in 43% yield together with tetrahydrofuran **16** (43%). In this operation, no attempt was made to isolate the cyclic carbonate from the minor component 16α,22*R*-**9**. The NOE correlation between H-17 and H-16 confirmed the configuration of the oxygen functionality at C-16 of 16β,22*R*-**14** and **16**.



Scheme 4. Formation of 16β,22*R*-**14** and **16**.

In order to improve access to 16β,22*S*-**9** and 16β,22*R*-**9**, we investigated the reduction of diketone **17**, which was derived from 16α,22*S*-**9** in 94% yield (Scheme 2). The reduction of **17** with $LiAlH_4$ at 0 ºC provided 16αβ,22*S*-**9** (α/β = 12/88, 56%) and 16β,22*R*-**9** (26%). 16αβ,22*S*-**9** and 16β,22*R*-**9**

can be separated chromatographically, and the two isomers in 16αβ,22*S*-**9** can be separated via the cyclic carbonates as described above. Therefore, the oxidation–reduction of 16α,22*S*-**9**, which can be easily obtained from the major ene-adduct 22*S*-**3**, can provide 16β,22*S*-**9** and 16β,22*R*-**9** without the involvement of less accessible 16-monoketones such as 22*S*-**13** and 22*R*-**13**.

The final step of the synthesis of **1** was the acid hydrolysis of **9**. Each 16,22-stereoisomer of **9** was treated with *p*-TsOH•H_2O in refluxing aqueous 1,4-dioxane to provide **1** in more than 94% yield. By choosing an appropriate solvent system for each stereoisomer of **1**, single crystals suitable for X-ray crystallography were obtained for all the four 16,22-stereoisomers of **1**. As shown in Figure 2, the absolute structures of synthetic **1** were confirmed unequivocally.

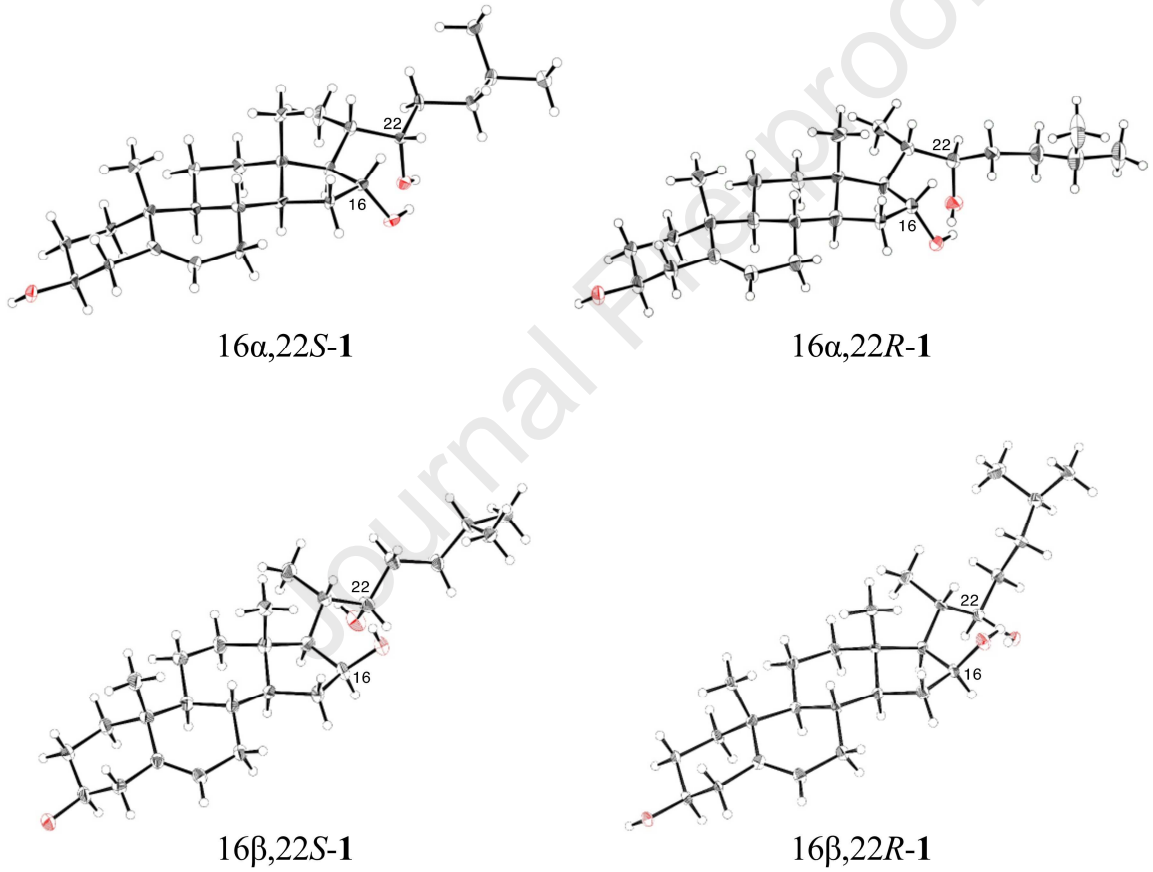


Figure 2. Molecular structures of the 16,22-stereoisomers of **1** with thermal ellipsoids at 30% probability. Solvent molecules are omitted for clarity and carbon atoms 16 and 22 are labelled (*cf.* Figure 1).

^{1}H and ^{13}C NMR spectra of **1** were analyzed in both $CDCl_3$ and C_5D_5N. The results are summarized in Tables S1 and S2. The H-18 signals of the 16α-isomers (0.70–0.74 ppm in $CDCl_3$; 0.79 ppm in C_5D_5N) were observed at considerably higher field than those of the 16β-isomers (0.92–0.94 ppm in $CDCl_3$; 1.20–1.21 ppm in C_5D_5N), which is probably due to deshielding of the 16β-hydroxy group.

This tendency has also been observed in 16-hydroxylated steroids bearing shorter side chains in which stereogenic centers are not present.[8,9,12] Noteworthily, the effect of the configuration of the 22-hydroxy group on the chemical shift of H-18 proved to be marginal. The H-16α signal (4.16–4.23 ppm in $CDCl_3$; 4.41–4.53 ppm in C_5D_5N) also appeared further upfield than that of H-16β (4.35 ppm in $CDCl_3$; 4.66–4.79 ppm in C_5D_5N), while the C-16 signals of the 16α-isomers (75.7–76.6 ppm in $CDCl_3$; 74.4–75.5 ppm in C_5D_5N) were observed at lower field than those of the 16β-isomers (72.0–72.6 ppm in $CDCl_3$; 70.7–70.8 ppm in C_5D_5N). The chemical shift of H-22 did not reflect the configuration of the 22-hydroxy group in $CDCl_3$ (3.63–3.85 ppm for the 22*S*-isomers; 3.65–3.68 ppm for the 22*R*-isomers). However, in C_5D_5N, the H-22 signals of the 22*S*-isomers (4.19–4.26 ppm) appeared at higher field than those of the 22*R*-isomers (4.42–4.45 ppm). This observation is consistent with the report by Challinor and co-workers, in which the H-22 signals of helosides and bethosides with 22*S*-hydroxy groups were observed at 4.15–4.21 ppm, whereas the H-22 signal of a closely related compound with a 22*R*-isomer was observed at 4.48 ppm.[17] Since all these previously reported compounds bear 16β-hydroxy groups, we revealed in this study that the configuration of the 16-hydroxy group only marginally affects the chemical shift of H-22. The advanced Mosher method was required to confirm the absolute configurations of the 22-hydroxy groups of steroidal saponins from *A. schubertii*[3] and *A. karataviense*.[6] The chemical shift of H-22 in C_5D_5N is thus a useful marker for the determination of the configuration of 22-hydroxy group of aglycones similar to **1** in steroidal saponins.

The ^1H and ^{13}C spectra of synthetic 16β,22*S*-**1** in $CDCl_3$ were, bar a few slight exceptions, in good accordance with those of enzymatically prepared 16β,22*S*-**1**[7] (Table S3). In C_5D_5N, however, the ^1H and ^{13}C spectra of synthetic 16β,22*S*-**1** did not agree with those of 16β,22*S*-**1** chemically derivatized from naturally occurring karataviosides J and K, which was reported by Kuroda and co-workers.[6] Their spectra also did not agree well with those of synthetic 16β,22*R*-**1**, 16α,22*S*-**1**, and 16α,22*R*-**1** (Tables S4 and S5). Even though the reasons for these discrepancies are unclear at this point, the spectra of 16β,22*S*-**1** provided by Kuroda and co-workers[6] should be treated carefully.

We also compared the ^1H and ^{13}C spectra of synthetic 16β,22*S*-**1** in C_5D_5N to those of the natural steroidal saponins in which the hydroxy groups at C-3 and/or C-16 of 16β,22*S*-**1** are glycosylated (Figure S1).[3–6] As shown in Tables S6 and S7, glycosylation at the 3-hydroxy group shifts the H-6 and H-19 signals upfield (0.04–0.24 ppm), whereas the C-3 signals are shifted downfield (7.6–8.0 ppm). Similarly, glycosylation at the 16-hydroxy group shifts the H-16 and H-18 signals upfield (0.22–0.30 ppm), whereas the C-16 signals are shifted downfield (11.7–12.2 ppm). With respect to the chemical shift of H-22, which was proven to be a diagnostic marker for determining the configuration of the 22-hydroxy group by this study, a consistent trend could not be established. Therefore, a direct determination of the configuration of the 22-hydroxy group of steroidal saponins

bearing aglycones similar to **1** based on their NMR spectra is difficult at this moment.

The diagnostic data differentiating calibagenin, saxosterol, and synthetic **1** are summarized in Table 1. Based on the lower chemical shifts of the H-18 and H-16 signals of calibagenin and saxosterol, the configurations of their 16-hydroxy groups were determined to be β. Although the H-22 chemical shifts of calibagenin and saxosterol do not reflect their configurations, they are comparable to those of 16β,22*S*-**1** and 16β,22*R*-**1**. Fortunately, the $[\alpha]_D$ value of calibagenin is about twice that of saxosterol, and their values are in accordance with those of 16β,22*S*-**1** and 16β,22*R*-**1**, respectively. The mp of calibagenin and saxosterol are also comparable to those of 16β,22*S*-**1** and 16β,22*R*-**1**. Therefore, it can be concluded that calibagenin is 16β,22*S*-**1**, which is identical to the aglycone of the above mentioned steroidal saponins.[3-6] We also confirmed that the absolute structure of saxosterol is 16β,22*R*-**1**, which is in agreement with the work of Stabursvik and Holen.[2]

Table 1. Comparison of the ^{1}H NMR, $[\alpha]_D$, and mp data of calibagenin, saxosterol, and **1**.

	^{1}H NMR[a]			$[\alpha]_D$[b]	Mp	
	H-18	H-16	H-22		(°C)	Solvent[c]
calibagenin	0.91 (s)	4.36 (m)[d]	3.56 (m)[e]	–56[f]	195–196	MeOH/H_2O
saxosterol	0.92 (s)	4.35 (m)	3.65 (m)	–27[f]	182–183	MeOH
16α,22*S*-**1**	0.70 (s)	4.23 (ddd, 8.0, 6.7, 1.3)	3.85 (ddd, 8.2, 4.5, 2.4)	–53.1[g]	230	MeOH
16α,22*R*-**1**	0.74 (s)	4.16 (m)	3.68 (ddd, 9.0, 4.5, 2.4)	–56.4[h]	118–119	MeOH
16β,22*S*-**1**	0.94 (s)	4.35 (ddd, 8.0, 6.9, 4.6)	3.63 (ddd, 9.7, 2.3, 2.3)	–55.8[i]	189	MeOH/H_2O
16β,22*R*-**1**	0.92 (s)	4.35 (ddd, 8.0, 7.2, 5.0)	3.65 (ddd, 8.6, 6.1, 6.1)	–32.6[i]	185–186	MeOH

[a] Recorded in $CDCl_3$ at 60 MHz (calibagenin), 270 MHz (saxosterol), or 600 MHz (**1**); [b] Measured in $CHCl_3$ (*c* 0.2 g/dL). [c] Solvent used for recrystallization. [d] Assigned as H-22 in the original literature. [e] Assigned as H-16 in the original literature. [f] Measured at 20 °C. [g] Measured at 19 °C. [h] Measured at 17 °C. [i] Measured at 18 °C.

3. Conclusions

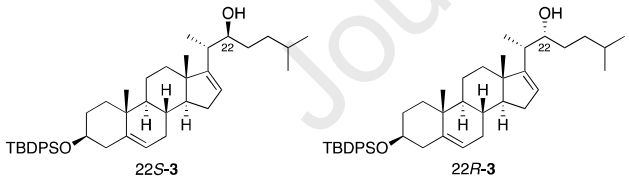
In this study, the four 16,22-stereoisomers of **1** were synthesized. During the synthesis, easy separation of the 16α- and 16β-isomer of the 16,22-diols **9** was achieved via their derivatization into cyclic carbonates **14**, and a simple route to 16β,22*S*- and 16β,22*R*-isomers via the reduction of the 16,22-diketone **17** was developed. X-ray crystallographic data unambiguously determined the structure of the isomers of synthetic **1**. Consequently, the absolute structures of calibagenin and saxosterol were determined to be 16β,22*S*-**1** and 16β,22*R*-**1**, respectively. The spectroscopic data presented in this study can thus be expected to serve as useful criteria for the configurational determination of steroidal saponins bearing hydroxy groups at C-16 and C-22.

4. Materials and Methods

4.1. General.

Melting points (mp) were measured on an AS ONE ATM-01 melting point apparatus and are uncorrected. Optical rotations were measured using a HORIBA SEPA-500 polarimeter. NMR spectra were obtained using a Bruker AVANCEIII 600 or JEOL JNM-ECA600 spectrometer (600 MHz for ^{1}H; 151 MHz for ^{13}C). Chemical shifts are reported in parts per million relative to the internal standards [tetramethylsilane (0.00 ppm) for ^{1}H; the solvents $CDCl_3$ (77.0 ppm) and C_5D_5N (149.2 ppm) for ^{13}C]. Assignment of the ^{1}H and ^{13}C NMR signals was based on 2D NMR measurements (^{1}H-^{1}H COSY, NOESY, HSQC, and HMBC). HRMS were recorded on a JEOL JMS-700 spectrometer. Reagents were used as received from commercial suppliers unless otherwise stated; **4**[14] and **5**[15] were prepared according to previously reported procedures. Flash column chromatography on silica gel was carried out using a Biotage Isolera One chromatograph with SNAP Ultra cartridges (silica gel, 25 µm) or a Yamazen W-prep 2XY chromatograph with UNIVERSAL Premium columns (silica gel, 30 µm). Single-crystal X-ray crystallographic analyses were performed at the BL40XU and BL02B1 beam lines of SPring-8 using Si (111) monochromated synchrotron radiation (0.78229 and 0.70060 Å) with a Rigaku Saturn 724+ CCD detector.

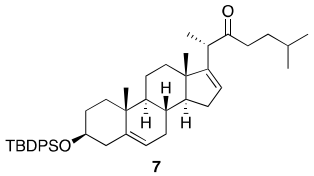
4.2. (22*S*)- and (22*R*)-3β-(*tert*-butyldiphenylsiloxy)cholesta-5,16-dien-22-ol (22*S*- and 22*R*-**3**).



A Me_2AlCl solution (1.0 M in hexane, 100 mL, 100 mmol) was added to anhydrous CH_2Cl_2 (200 mL) at –78 °C under an argon atmosphere. This mixture was treated dropwise with 4-methylpentanal **5** (10.2 g, 102 mmol) dissolved in anhydrous CH_2Cl_2 (50 mL), followed by stirring for 30 min at the same temperature. The resulting mixture was treated dropwise with **4** (27.5 g, 51.0 mmol) dissolved in anhydrous CH_2Cl_2 (100 mL) at –78 °C; the mixture was gradually warmed to 0 °C for 5 h under constant stirring. The reaction was quenched by dropwise addition of MeOH/H_2O (1/1, v/v, 50 mL), and subsequently diluted with $CHCl_3$ (100 mL). The organic layer was washed successively with 1 M HCl, water, a saturated aqueous solution of $NaHCO_3$, and brine (100 mL each), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 95/5–85/15, v/v). 22*S*-**3** (23.4 g, 72%) eluted after 22*R*-**3** (5.37 g, 16%). 22*S*-**3**. Colorless oil. $[\alpha]_D^{26}$ –46.9 (*c* 1.71 g/dL,

$CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$, δ): 0.84 (3H, s, H_3-18), 0.86 (1H, m, H-1α), 0.88 (3H, d, *J* = 6.6 Hz, H_3-26), 0.89 (1H, m, H-9α), 0.89 (3H, d, *J* = 6.6 Hz, H_3-27), 1.00 (3H, d, *J* = 7.0 Hz, H_3-21), 1.02 (3H, s, H_3-19), 1.06 (9H, s, *t*-Bu), 1.17 (1H, m, H_a-24), 1.26 (1H, m, H-14α), 1.28 (1H, ddd, *J* = 12.1, 12.1, 4.8 Hz, H-12α), 1.34 (1H, m, H_b-24), 1.43 (1H, m, H_a-23), 1.50 (1H, m, H_b-23), 1.51 (2H, m, H-7α and H-11β), 1.54 (2H, m, H-11α and H-25), 1.61 (1H, m, H-2β), 1.63 (1H, m, H-8β), 1.68 (1H, m, H-2α), 1.69 (1H, m, H-1β), 1.74 (1H, ddd, *J* = 12.1, 4.4, 2.6 Hz, H-12β), 1.86 (1H, dddd, *J* = 14.9, 11.5, 1.7, 1.7 Hz, H-15β), 1.93 (1H, dddd, *J* = 17.4, 5.2, 5.2, 2.7 Hz, H-7β), 2.05 (1H, ddd, *J* = 14.9, 6.3, 3.2 Hz, H-15α), 2.15 (1H, ddd, *J* = 13.3, 4.7, 2.1 Hz, H-4α), 2.26 (1H, m, H-20), 2.34 (1H, m, H-4β), 3.54 (1H, dddd, *J* = 11.0, 11.0, 4.7, 4.7 Hz, H-3α), 3.61 (1H, m, H-22), 5.14 (1H, m, H-6), 5.47 (1H, m, H-16), 7.36 (4H, m, Ph-3/5), 7.41 (2H, m, Ph-4), 7.68 (4H, m, Ph-2/6). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 14.1 (C-21), 16.7 (C-18), 19.1 (*t*-Bu), 19.3 (C-19), 20.6 (C-11), 22.58 (C-26), 22.64 (C-27), 27.0 (3C, *t*-Bu), 28.1 (C-25), 30.3 (C-8), 31.2 (C-15), 31.5 (C-7), 31.8 (C-2), 32.4 (C-23), 34.7 (C-12), 35.5 (C-24), 36.7 (C-10), 37.1 (C-1), 37.7 (C-20), 42.5 (C-4), 46.8 (C-13), 50.5 (C-9), 57.8 (C-14), 73.0 (C-22), 73.2 (C-3), 120.9 (C-6), 124.3 (C-16), 127.42 (2C, Ph-3/5), 127.44 (2C, Ph-3/5), 129.40 (Ph-4), 129.43 (Ph-4), 134.7 (Ph-1), 134.8 (Ph-1), 135.7 (2C, Ph-2/6), 135.8 (2C, Ph-2/6), 141.5 (C-5), 158.6 (C-17). HRMS-FAB-NBA (*m*/*z*): $[M–H]^+$ calcd for $C_{43}H_{61}O_2Si$, 637.4441; found, 637.4431. 22*R*-**3**. Colorless oil. $[\alpha]_D^{27}$ –46.6 (*c* 1.25 g/dL, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$, δ): 0.79 (3H, s, H_3-18), 0.85 (1H, ddd, *J* = 14.2, 14.2, 4.3 Hz, H-1α), 0.89 (3H, d, *J* = 6.5 Hz, H_3-26), 0.90 (3H, d, *J* = 6.5 Hz, H_3-27), 0.90 (1H, m, H-9α), 0.97 (3H, d, *J* = 7.0 Hz, H_3-21), 1.02 (3H, s, H_3-19), 1.06 (9H, s, *t*-Bu), 1.26 (1H, m, H_a-24), 1.29 (1H, m, H-14α), 1.30 (1H, m, H_a-23), 1.36 (1H, ddd, *J* = 12.1, 12.1, 5.0 Hz, H-12α), 1.42 (1H, m, H_b-24), 1.50 (1H, m, H-11β), 1.51 (1H, m, H-7α), 1.54 (1H, m, H-11α), 1.55 (1H, m, H-25), 1.59 (1H, m, H-2β), 1.61 (1H, m, H-8β), 1.64 (1H, m, H_b-23), 1.68 (1H, m, H-2α), 1.69 (1H, m, H-1β), 1.75 (1H, ddd, *J* = 12.1, 4.2, 2.6 Hz, H-12β), 1.87 (1H, m, H-15β), 1.93 (1H, dddd, *J* = 17.2, 5.2, 5.2, 2.8 Hz, H-7β), 2.07 (1H, ddd, *J* = 15.0, 6.6, 3.2 Hz, H-15α), 2.14 (1H, m, H-4α), 2.15 (1H, m, H-20), 2.34 (1H, m, H-4β), 3.54 (1H, dddd, *J* = 11.1, 11.1, 4.5, 4.5 Hz, H-3α), 3.55 (1H, m, H-22), 5.14 (1H, m, H-6), 5.47 (1H, m, H-16), 7.36 (4H, m, Ph-3/5), 7.41 (2H, m, Ph-4), 7.68 (4H, m, Ph-2/6). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 16.2 (C-18), 18.0 (C-21), 19.1 (*t*-Bu), 19.3 (C-19), 20.7 (C-11), 22.4 (C-26), 22.9 (C-27), 27.0 (3C, *t*-Bu), 28.2 (C-25), 30.5 (C-8), 31.20 (C-15), 31.21 (C-23), 31.5 (C-7), 31.8 (C-2), 34.7 (C-12), 34.8 (C-24), 36.7 (C-10), 37.1 (C-1), 40.1 (C-20), 42.5 (C-4), 47.1 (C-13), 50.6 (C-9), 57.4 (C-14), 73.1 (C-3), 73.9 (C-22), 120.9 (C-6), 123.0 (C-16), 127.42 (2C, Ph-3/5), 127.45 (2C, Ph-3/5), 129.40 (Ph-4), 129.43 (Ph-4), 134.76 (Ph-1), 134.78 (Ph-1), 135.7 (2C, Ph-2/6), 135.8 (2C, Ph-2/6), 141.5 (C-5), 158.5 (C-17). HRMS-FAB-NBA (*m*/*z*): $[M–H]^+$ calcd for $C_{43}H_{61}O_2Si$, 637.4441; found, 637.4442.

4.3. 3β-(*tert*-Butyldiphenylsiloxy)cholesta-5,16-dien-22-one (**7**).



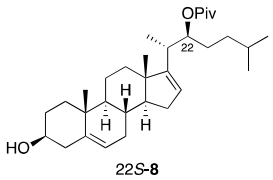
7

A solution of 22*S*-**3** (11.3 g, 17.7 mmol) in anhydrous CH_2Cl_2 (100 mL) was treated with DMP (8.38 g, 19.8 mmol) at 0 °C, and the mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with $CHCl_3$ (400 mL), washed successively with a saturated aqueous solution of $NaHCO_3$ and brine (200 mL each), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 98/2, v/v) to give **7** (9.92 g, 88%) as a colorless oil. $[\alpha]_D^{26}$ +6.5 (*c* 1.55 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.82 (3H, s), 0.85 (3H, d, *J* = 6.5 Hz), 0.86 (3H, d, *J* = 6.5 Hz), 1.02 (3H, s), 1.06 (9H, s), 1.13 (3H, d, *J* = 6.8 Hz), 3.17 (1H, q, *J* = 6.8 Hz), 3.54 (1H, dddd, *J* = 10.9, 10.9, 4.5, 4.5 Hz), 5.12–5.14 (1H, m), 5.34–5.35 (1H, m), 7.34–7.37 (4H, m), 7.39–7.42 (2H, m), 7.66–7.69 (4H, m). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 16.2, 16.8, 19.1, 19.3, 20.7, 22.2, 22.5, 27.0 (3C), 27.6, 30.5, 31.2, 31.5, 31.8, 33.0, 34.6, 36.7, 37.1, 38.2, 42.5, 45.7, 47.3, 50.5, 57.1, 73.2, 120.8, 125.1, 127.42 (2C), 127.44 (2C), 129.40, 129.43, 134.76, 134.77, 135.7 (4C), 141.5, 154.3, 211.6. HRMS-FAB-NBA (*m*/*z*): $[M–H]^+$ calcd for $C_{43}H_{59}O_2Si$, 635.4284; found, 635.4287.

4.4. Reduction of **7**.

A solution of **7** (9.00 g, 14.1 mmol) in anhydrous THF (150 mL) at –78 °C under an argon atmosphere was treated with a solution of Li(*sec*-Bu)$_3$BH (1.0 M in THF, 72 mL, 72 mmol). After 5 h of stirring at –78 °C, Li(*sec*-Bu)$_3$BH (1.0 M in THF, 23 mL, 23 mmol) was added dropwise to the mixture, and stirring was continued for a further 2 h at –78 °C. Then, the mixture was treated dropwise at –78 °C with MeOH (20 mL), and at 0 °C with a 10% aqueous solution of NaOH (100 mL) and a 30% aqueous solution of H_2O_2 (100 mL). The mixture was stirred for 1 h at 0 °C and then diluted with water (300 mL). The aqueous layer was extracted with EtOAc (1 × 300 mL, 2 × 150 mL), and the combined organic layers were washed with brine (150 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 95/5, v/v) to give 22*R*-**3** (8.03 g, 89%) as a colorless oil.

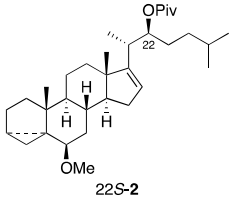
4.5. (22*S*)-22-(2,2-Dimethylpropanoyloxy)cholesta-5,16-dien-3β-ol (22*S*-**8**).



22*S*-**8**

An ice-cold mixture of 22*S*-**3** (8.71 g, 13.6 mmol) and DMAP (4.20 g, 34.4 mmol) in anhydrous CH_2Cl_2 (100 mL) was treated with PivCl (3.4 mL, 28 mmol), and the mixture was stirred for 21 h at room temperature. The solvent was evaporated, and 1 M HCl (150 mL) was added to the residue. The aqueous layer was extracted with toluene (1 × 150 mL, 2 × 50 mL), and the combined organic layers were washed successively with water, a saturated aqueous solution of $NaHCO_3$, and brine (75 mL each), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residual colorless oil (10.3 g) was dissolved in anhydrous THF (100 mL) and treated with a TBAF solution (1 M in THF, 18 mL, 18 mmol). After 22 h of stirring at room temperature under an argon atmosphere, additional TBAF solution (1 M in THF, 18 mL, 18 mmol) was added to the mixture, before stirring was continued for a further 2 days. The reaction was quenched with AcOH (2.0 mL, 35 mmol) and the solvent was evaporated. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 80/20–60/40, v/v) to give 22*S*-**8** (6.37 g, 96%) as a colorless solid. Mp 109 °C. $[\alpha]_D^{26}$ –44.4 (*c* 1.18 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.82 (3H, s), 0.83 (3H, d, *J* = 6.2 Hz), 0.84 (3H, d, *J* = 6.2 Hz), 0.99 (3H, d, *J* = 6.9 Hz), 1.05 (3H, s), 1.21 (9H, s), 3.53 (1H, dddd, *J* = 11.2, 11.2, 4.5, 4.5 Hz), 4.99 (1H, ddd, *J* = 8.0, 8.0, 4.0 Hz), 5.36–5.38 (1H, m), 5.40–5.41 (1H, m). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 16.2, 17.6, 19.3, 20.7, 22.3, 22.7, 27.3 (3C), 27.8, 30.46, 30.48, 31.3, 31.5, 31.6, 34.3, 34.9, 36.4, 36.7, 37.2, 38.9, 42.3, 47.1, 50.7, 57.3, 71.7, 76.3, 121.5, 123.2, 141.0, 157.2, 178.1. HRMS-FAB-NBA (*m*/*z*): $[M+Na]^+$ calcd for $C_{32}H_{52}O_3Na$, 507.3814; found, 507.3812.

4.6. (22*S*)-22-(2,2-Dimethylpropanoyloxy)-6β-methoxy-3α,5-cyclocholest-16-ene (22*S*-**2**).

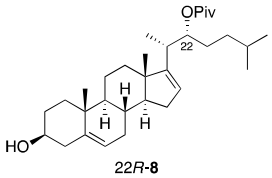


22*S*-**2**

An ice-cold solution of 22*S*-**8** (6.25 g, 12.9 mmol) and Et_3N (2.60 g, 25.7 mmol) in toluene (100 mL) was treated with MsCl (1.50 mL, 19.4 mmol), before the mixture was stirred for 1 h at room temperature. The reaction mixture was diluted with toluene (100 mL) and washed successively with water (2 × 50 mL), 2 M HCl (50 mL), water (50 mL), a saturated aqueous solution of $NaHCO_3$ (50 mL), and brine (50 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. A mixture of the residual colorless solid (6.55 g) and KOAc (6.45 g, 65.7

mmol) in MeOH (200 mL) was stirred under reflux for 1 h and then cooled to room temperature. The solvent was evaporated, and toluene (200 mL) was added to the residue. The organic layer was washed successively with water (100 mL) and brine (50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 98/2–90/10, v/v) to give 22*S*-**2** (4.42 g, 69%) as a colorless oil. $[\alpha]_D^{18}$ +34.7 (*c* 1.91 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.45 (1H, dd, *J* = 8.0, 5.0 Hz, H-4α), 0.66 (1H, dd, *J* = 5.0, 3.9 Hz, H-4β), 0.83 (3H, d, *J* = 6.5 Hz, H_3-26), 0.84 (3H, d, *J* = 6.5 Hz, H_3-27), 0.85 (3H, s, H_3-18), 0.86 (1H, m, H-1β), 0.90 (2H, m, H-3 and H-9α), 0.99 (3H, d, *J* = 6.9 Hz, H_3-21), 1.06 (3H, s, H_3-19), 1.13 (1H, m, H_a-24), 1.14 (1H, m, H_b-24), 1.15 (1H, m, H-7α), 1.21 (9H, s, Piv), 1.35 (1H, m, H-14α), 1.37 (1H, m, H-12α), 1.43 (1H, m, H_a-23), 1.48 (1H, m, H-25), 1.50 (1H, m, H-11β), 1.52 (1H, m, H-1α), 1.53 (1H, m, H-2β), 1.54 (1H, m, H-11α), 1.66 (1H, m, H_b-23), 1.75 (1H, m, H-2α), 1.76 (1H, m, H-12β), 1.90 (1H, m, H-15β), 1.93 (1H, m, H-7β), 1.96 (1H, m, H-8β), 2.08 (1H, ddd, *J* = 14.8, 6.5, 3.2 Hz, H-15α), 2.32 (1H, m, H-20), 2.79 (1H, dd, *J* = 2.8, 2.3 Hz, H-6α), 3.34 (3H, s, OMe), 5.00 (1H, ddd, *J* = 8.1, 8.1, 4.0 Hz, H-22), 5.39 (1H, m, H-16). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 13.0 (C-4), 16.7 (C-18), 17.8 (C-21), 19.2 (C-19), 21.4 (C-3), 22.31 (C-11), 22.32 (C-26), 22.7 (C-27), 24.9 (C-2), 27.3 (3C, Piv), 27.8 (C-25), 29.0 (C-8), 30.4 (C-23), 31.2 (C-15), 33.1 (C-1), 34.3 (C-24), 35.0 (C-7), 35.2 (C-12), 35.4 (C-10), 36.4 (C-20), 38.9 (Piv), 43.6 (C-5), 47.4 (C-13), 48.6 (C-9), 56.6 (OMe), 57.4 (C-14), 76.3 (C-22), 82.3 (C-6), 122.9 (C-16), 157.5 (C-17), 178.1 (Piv). HRMS-FAB-NBA (*m*/*z*): $[M–H]^+$ calcd for $C_{33}H_{53}O_3$, 497.3995; found, 497.3999.

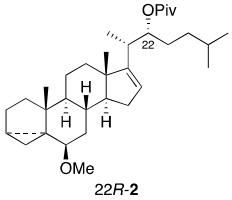
4.7. (22*R*)-22-(2,2-Dimethylpropanoyloxy)-6β-methoxy-3α,5-cyclocholest-16-ene (22*R*-**8**).



22*R*-**8**

Using the same procedure as for the synthesis of 22*S*-**8**, 22*R*-**3** (3.39 g, 5.30 mmol) was converted into 22*R*-**8** (2.49 g, 97%). Colorless oil. $[\alpha]_D^{26}$ –39.5 (*c* 1.06 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.83 (3H, s), 0.851 (3H, d, *J* = 6.5 Hz), 0.853 (3H, d, *J* = 6.5 Hz), 1.00 (3H, d, *J* = 7.2 Hz), 1.04 (3H, s), 1.19 (9H, s), 3.53 (1H, dddd, *J* = 11.2, 11.2, 4.5, 4.5 Hz), 4.98 (1H, ddd, *J* = 8.2, 5.6, 3.7 Hz), 5.36–5.37 (1H, m), 5.46–5.47 (1H, m). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 16.0, 16.4, 19.3, 20.7, 22.4, 22.7, 27.3 (3C), 27.6, 27.9, 30.5, 31.2, 31.58, 31.62, 34.5, 34.7, 35.8, 36.7, 37.1, 38.8, 42.3, 46.9, 50.6, 57.6, 71.7, 75.9, 121.5, 123.4, 141.1, 156.4, 178.3. HRMS-FAB-NBA (*m*/*z*): $[M+Na]^+$ calcd for $C_{32}H_{52}O_3Na$, 507.3814; found, 507.3825.

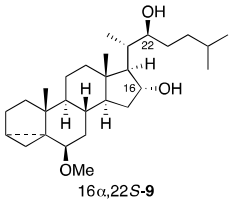
4.8. (22*R*)-22-(2,2-Dimethylpropanoyloxy)-6β-methoxy-3α,5-cyclocholest-16-ene (22*R*-**2**).



22*R*-**2**

Using the same procedure as for the synthesis of 22*S*-**2**, 22*R*-**8** (2.31 g, 4.77 mmol) was converted into 22*R*-**2** (1.79 g, 75%). Colorless oil. $[\alpha]_D^{18}$ +40.2 (*c* 3.19 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.45 (1H, dd, *J* = 8.0, 5.0 Hz, H-4α), 0.66 (1H, dd, *J* = 5.0, 3.9 Hz, H-4β), 0.853 (3H, d, *J* = 6.6 Hz, H_3-27), 0.854 (3H, d, *J* = 6.6 Hz, H_3-26), 0.86 (3H, s, H_3-18), 0.86 (1H, m, H-1β), 0.89 (2H, m, H-3 and H-9α), 1.00 (3H, d, *J* = 7.0 Hz, H_3-21), 1.05 (3H, s, H_3-19), 1.14 (2H, m, H-7α and H_a-24), 1.16 (1H, m, H_b-24), 1.19 (9H, s, Piv), 1.34 (1H, m, H-14α), 1.36 (1H, m, H-12α), 1.49 (1H, m, H-25), 1.50 (1H, m, H-11β), 1.51 (1H, m, H_a-23), 1.52 (2H, m, H-1α and H-2β), 1.54 (1H, m, H-11α), 1.56 (1H, m, H_b-23), 1.75 (1H, m, H-2α), 1.86 (1H, m, H-12β), 1.89 (1H, m, H-15β), 1.92 (1H, m, H-7β), 1.95 (1H, m, H-8β), 2.06 (1H, ddd, *J* = 14.9, 6.5, 3.2 Hz, H-15α), 2.43 (1H, m, H-20), 2.79 (1H, dd, *J* = 2.7, 2.7 Hz, H-6α), 3.35 (3H, s, OMe), 4.99 (1H, ddd, *J* = 8.4, 6.0, 3.4 Hz, H-22), 5.44 (1H, m, H-16). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 13.1 (C-4), 16.4 (C-21), 16.7 (C-18), 19.2 (C-19), 21.4 (C-3), 22.3 (C-11), 22.4 (C-26), 22.7 (C-27), 24.9 (C-2), 27.3 (3C, Piv), 27.8 (C-23), 27.9 (C-25), 29.1 (C-8), 31.2 (C-15), 33.1 (C-1), 34.4 (C-24), 35.0 (C-12), 35.1 (C-7), 35.3 (C-10), 35.7 (C-20), 38.8 (Piv), 43.6 (C-5), 47.2 (C-13), 48.6 (C-9), 56.6 (OMe), 57.5 (C-14), 75.9 (C-22), 82.3 (C-6), 123.0 (C-16), 156.6 (C-17), 178.2 (Piv). HRMS-FAB-NBA (*m*/*z*): $[M–H]^+$ calcd for $C_{33}H_{53}O_3$, 497.3995; found, 497.3997.

4.9. (22*S*)-6β-Methoxy-3α,5-cyclocholestane-16α,22-diol (16α,22*S*-**9**).

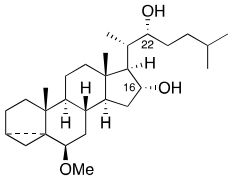


16α,22*S*-**9**

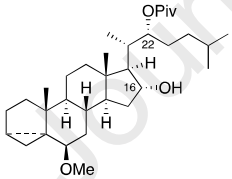
An ice-cold solution of 22*S*-**2** (5.21 g, 10.4 mmol) in anhydrous THF (180 mL) under an argon atmosphere was treated dropwise with a BH_3•THF solution (1.0 M in THF, 54 mL, 54 mmol), before the mixture was stirred for a further 18 h at room temperature. The reaction mixture was treated dropwise at 0 °C with a 10% aqueous solution of NaOH (55 mL) and a 30% aqueous solution of H_2O_2 (55 mL). After 30 min of stirring at the same temperature, the mixture was diluted with water (100 mL) and extracted with EtOAc (1 × 100 mL, 2 × 50 mL). The combined organic layers were washed with brine (100 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc =

85/15–70/30, v/v) to give 16α,22*S*-**9** (3.92 g, 87%) as a colorless solid. Mp 160–161 °C. $[\alpha]_D^{28}$ +34.4 (*c* 1.06 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.45 (1H, dd, *J* = 8.0, 5.0 Hz, H-4α), 0.66 (1H, dd, *J* = 5.0, 4.0 Hz, H-4β), 0.74 (3H, s, H_3-18), 0.86 (1H, ddd, *J* = 13.3, 11.6, 7.7 Hz, H-1β), 0.89 (1H, m, H-9α), 0.90 (6H, d, *J* = 6.7 Hz, H_3-26 and H_3-27), 0.90 (1H, m, H-3), 0.92 (3H, d, *J* = 6.7 Hz, H_3-21), 1.02 (3H, s, H_3-19), 1.15 (1H, m, H_a-24), 1.15 (1H, ddd, *J* = 13.3, 12.1, 2.9 Hz, H-7α), 1.32 (1H, m, H-12α), 1.36 (1H, m, H_b-24), 1.37 (1H, m, H-11β), 1.39 (1H, m, H-17α), 1.40 (1H, m, H_a-23), 1.44 (1H, m, H-11α), 1.50 (2H, m, H-1α and H-14α), 1.53 (1H, m, H_b-23), 1.54 (1H, m, H-2β), 1.56 (1H, m, H-25), 1.58 (1H, m, H-20), 1.60 (1H, m, H-15α), 1.69 (1H, m, H-15β), 1.74 (1H, m, H-8β), 1.76 (1H, m, H-2α), 1.85 (1H, ddd, *J* = 13.3, 2.9, 2.9 Hz, H-7β), 1.93 (1H, ddd, *J* = 11.9, 3.2, 2.8 Hz, H-12β), 2.79 (1H, dd, *J* = 2.9, 2.9 Hz, H-6α), 2.86 (1H, br s, OH), 2.98 (1H, br s, OH), 3.33 (3H, s, OMe), 3.84 (1H, br s, H-22), 4.22 (1H, dd, *J* = 7.2, 7.2 Hz, H-16β). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 12.5 (br, C-21), 13.1 (C-4), 14.3 (C-18), 19.2 (C-19), 21.3 (C-3), 22.46 (C-11), 22.54 (C-26), 22.7 (C-27), 24.9 (C-2), 28.2 (C-25), 30.1 (C-8), 32.0 (br, C-23), 33.2 (C-1), 35.0 (C-5), 35.1 (C-7), 35.8 (C-24), 36.1 (C-15), 37.3 (br, C-20), 40.4 (C-12), 43.3 (C-10), 44.3 (C-13), 47.8 (C-9), 53.5 (C-14), 56.6 (OMe), 63.1 (br, C-17), 73.9 (br, C-22), 75.5 (C-16), 82.2 (C-6). HRMS-FAB-NBA (*m*/*z*): $[M+Na]^+$ calcd for $C_{28}H_{48}O_3Na$, 455.3501; found, 455.3504.

4.10. (22*R*)-6β-Methoxy-3α,5-cyclocholestane-16α,22-diol (16α,22*R*-**9**) and (22*R*)-22-(2,2-dimethylpropanoyloxy)-6β-methoxy-3α,5-cyclocholestan-16α-ol (16α,22*R*-**10**).



16α,22*R*-**9**

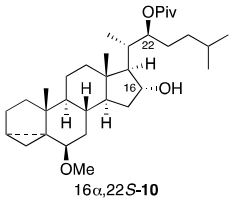


16α,22*R*-**10**

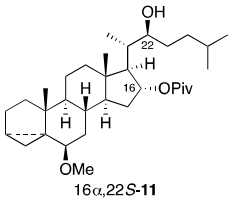
An ice-cold solution of 22*R*-**2** (2.89 g, 5.79 mmol) in anhydrous THF (100 mL) was treated dropwise under an argon atmosphere with a BH_3•THF solution (1.0 M in THF, 30 mL, 30 mmol), before the mixture was stirred for 17 h at room temperature. At 0 °C, the mixture was then successively treated dropwise with a 10% aqueous solution of NaOH (30 mL) and a 30% aqueous solution of H_2O_2 (30 mL), before the resulting mixture was stirred for 30 min at the same temperature. The mixture was diluted with water (60 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 85/15–70/30, v/v) to give 16α,22*R*-**9** (1.81 g, 72%) and 16α,22*R*-**10** (167 mg, 5.6%), respectively, as colorless solids. 16α,22*R*-**9**. Mp 119–120 °C. $[\alpha]_D^{27}$ +28.3 (*c* 1.02 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.44 (1H, dd, *J* = 8.0, 5.1 Hz, H-4α), 0.66 (1H, dd, *J* = 5.1, 3.7 Hz, H-4β), 0.78 (3H, s, H_3-18), 0.86 (1H, m, H-1β), 0.88 (1H, m, H-9α), 0.89 (3H, d, *J* =

6.6 Hz, H_3-26), 0.90 (3H, d, *J* = 6.6 Hz, H_3-27), 0.90 (1H, m, H-3), 0.97 (3H, d, *J* = 6.8 Hz, H_3-21), 1.01 (3H, s, H_3-19), 1.14 (1H, ddd, *J* = 13.4, 12.1, 2.9 Hz, H-7α), 1.21 (1H, dd, *J* = 10.9, 6.0 Hz, H-17α), 1.21 (1H, m, H_a-24), 1.28 (1H, ddd, *J* = 12.4, 12.4, 4.1 Hz, H-12α), 1.36 (1H, m, H_a-23), 1.37 (1H, m, H-11β), 1.38 (1H, m, H_b-24), 1.42 (1H, m, H-11α), 1.44 (1H, m, H-14α), 1.50 (1H, m, H-1α), 1.53 (1H, m, H-2β), 1.55 (1H, m, H-25), 1.59 (1H, m, H-15α), 1.61 (1H, m, H_b-23), 1.72 (1H, m, H-15β), 1.75 (2H, m, H-8β and H-20), 1.76 (1H, m, H-2α), 1.86 (1H, ddd, *J* = 13.4, 2.9, 2.9 Hz, H-7β), 1.98 (1H, ddd, *J* = 12.4, 3.1, 3.1 Hz, H-12β), 2.78 (1H, dd, *J* = 2.9, 2.9 Hz, H-6α), 3.33 (3H, s, OMe), 3.66 (1H, ddd, *J* = 8.9, 4.4, 2.6 Hz, H-22), 4.15 (1H, dd, *J* = 6.9, 6.9 Hz, H-16β). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 13.1 (C-4), 13.7 (C-18), 15.5 (C-21), 19.2 (C-19), 21.3 (C-3), 22.46 (C-11), 22.53 (C-26), 22.8 (C-27), 24.9 (C-2), 28.2 (C-25), 30.0 (C-8), 31.1 (C-23), 33.2 (C-1), 35.0 (C-5), 35.1 (C-7), 35.5 (C-24), 36.7 (C-15), 40.4 (C-12), 40.8 (C-20), 43.3 (C-10), 45.1 (C-13), 47.8 (C-9), 53.2 (C-14), 56.6 (OMe), 63.8 (C-17), 76.6 (C-16), 76.7 (C-22), 82.2 (C-6). HRMS-FAB-NBA (*m*/*z*): $[M+Na]^+$ calcd for $C_{28}H_{48}O_3Na$, 455.3501; found, 455.3503. 16α,22*R*-**10**. Mp 128–129 °C. $[\alpha]_D^{27}$ +36.5 (*c* 0.836 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.44 (1H, dd, *J* = 7.9, 4.9 Hz, H-4α), 0.65 (1H, dd, *J* = 4.9, 4.1 Hz, H-4β), 0.74 (3H, s, H_3-18), 0.857 (3H, d, *J* = 6.6 Hz, H_3-27), 0.860 (3H, d, *J* = 6.6 Hz, H_3-26), 0.86 (1H, m, H-1β), 0.88 (1H, m, H-9α), 0.89 (1H, ddd, *J* = 7.9, 7.9, 4.1 Hz, H-3), 0.93 (3H, d, *J* = 6.8 Hz, H_3-21), 1.01 (3H, s, H_3-19), 1.13 (1H, ddd, *J* = 13.5, 12.1, 2.8 Hz, H-7α), 1.16 (3H, m, H-17α and H_2-24), 1.21 (9H, s, Piv), 1.27 (1H, ddd, *J* = 12.6, 12.6, 4.2 Hz, H-12α), 1.36 (1H, dddd, *J* = 12.6, 12.6, 12.6, 3.2 Hz, H-11β), 1.41 (1H, m, H-11α), 1.45 (2H, m, H-14α and H_a-23), 1.49 (1H, m, H-1α), 1.51 (1H, m, H-25), 1.53 (2H, m, H-2β and H-15α), 1.62 (1H, m, H_b-23), 1.72 (1H, m, H-15β), 1.74 (1H, m, H-8β), 1.75 (1H, m, H-2α), 1.85 (1H, ddd, *J* = 13.5, 2.8, 2.8 Hz, H-7β), 1.86 (1H, qdd, *J* = 6.8, 6.8, 2.7 Hz, H-20), 1.93 (1H, ddd, *J* = 12.6, 3.2, 3.2 Hz, H-12β), 2.78 (1H, dd, *J* = 2.8, 2.8 Hz, H-6α), 3.33 (3H, s, OMe), 4.30 (1H, dd, *J* = 6.8, 6.8 Hz, H-16β), 5.10 (1H, ddd, *J* = 10.9, 2.7, 1.8 Hz, H-22). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 13.0 (C-21), 13.1 (C-4), 13.5 (C-18), 19.2 (C-19), 21.3 (C-3), 22.4 (2C, C-11 and C-26), 22.8 (C-27), 24.896 (C-23), 24.904 (C-2), 27.2 (3C, Piv), 27.8 (C-25), 29.9 (C-8), 33.2 (C-1), 35.0 (C-5), 35.1 (C-7), 35.6 (C-24), 37.5 (C-15), 38.2 (C-20), 38.9 (Piv), 40.3 (C-12), 43.3 (C-10), 44.4 (C-13), 47.9 (C-9), 53.4 (C-14), 56.6 (OMe), 62.1 (C-17), 76.6 (C-16), 77.3 (C-22), 82.2 (C-6), 178.6 (Piv). HRMS-FAB-NBA (*m*/*z*): $[M+Na]^+$ calcd for $C_{33}H_{56}O_4Na$, 539.4076; found, 539.4078.

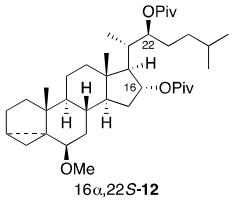
4.11. (22*S*)-22-(2,2-Dimethylpropanoyloxy)-6β-methoxy-3α,5-cyclocholestan-16α-ol (16α,22*S*-**10**), (22*S*)-16α-(2,2-dimethylpropanoyloxy)-6β-methoxy-3α,5-cyclocholestan-22-ol (16α,22*S*-**11**), and (22*S*)-16α,22-bis(2,2-dimethylpropanoyloxy)-6β-methoxy-3α,5-cyclocholestane (16α,22*S*-**12**).



16α,22*S*-**10**



16α,22*S*-**11**

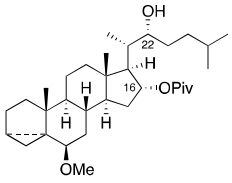


16α,22*S*-**12**

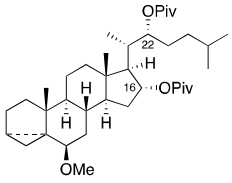
An ice-cold solution of 16α,22*S*-**9** (715 mg, 1.65 mmol) and DMAP (304 mg, 2.49 mmol) in anhydrous CH_2Cl_2 (15 mL) was slowly treated with PivCl (0.22 mL, 1.8 mmol), before the mixture was stirred for 22 h at room temperature. The reaction was quenched by addition of a saturated aqueous solution of $NaHCO_3$ (40 mL). After 20 min of stirring at room temperature, the mixture was extracted with $CHCl_3$ (1 × 30 mL, 2 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 85/15–65/35, v/v) to give 16α,22*S*-**10** (151 mg, 18%) as a colorless oil, which solidified during storage at room temperature. Mp 52–53 °C. $[\alpha]_D^{28}$ +23.4 (*c* 0.707 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.44 (1H, dd, *J* = 8.0, 5.0 Hz, H-4α), 0.65 (1H, dd, *J* = 5.0, 4.0 Hz, H-4β), 0.77 (3H, s, H_3-18), 0.85 (1H, ddd, *J* = 13.2, 11.9, 7.6 Hz, H-1β), 0.879 (3H, d, *J* = 6.6 Hz, H_3-26), 0.880 (3H, d, *J* = 6.6 Hz, H_3-27), 0.88 (1H, m, H-9α), 0.89 (1H, m, H-3), 1.00 (3H, d, *J* = 6.8 Hz, H_3-21), 1.01 (3H, s, H_3-19), 1.10 (1H, dd, *J* = 11.4, 4.5 Hz, H-17α), 1.14 (1H, m, H_a-24), 1.15 (1H, m, H_b-24), 1.17 (1H, ddd, *J* = 13.5, 11.4, 2.9 Hz, H-7α), 1.22 (9H, s, Piv), 1.24 (1H, ddd, *J* = 12.4, 12.4, 4.7 Hz, H-12α), 1.36 (1H, dddd, *J* = 13.7, 13.7, 12.4, 3.3 Hz, H-11β), 1.40 (1H, dddd, *J* = 13.7, 4.7, 4.7, 3.3 Hz, H-11α), 1.48 (2H, m, H-1α and H-14α), 1.49 (1H, m, H_a-23), 1.52 (1H, m, H-2β), 1.53 (1H, m, H-25), 1.64 (1H, m, H-15β), 1.65 (1H, m, H-15α), 1.70 (2H, m, H-20 and H_b-23), 1.75 (1H, m, H-2α), 1.77 (1H, dddd, *J* = 11.4, 11.4, 11.4, 2.9 Hz, H-8β), 1.88 (1H, ddd, *J* = 13.5, 2.9, 2.9 Hz, H-7β), 1.93 (1H, ddd, *J* = 12.4, 3.3, 3.3 Hz, H-12β), 2.78 (1H, dd, *J* = 2.9, 2.9 Hz, H-6α), 3.33 (3H, s, OMe), 3.61 (1H, br s, OH), 3.97 (1H, m, H-16β), 5.04 (1H, dd, *J* = 8.5, 5.3 Hz, H-22). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 13.0 (br, C-21), 13.1 (C-4), 13.4 (C-18), 19.2 (C-19), 21.3 (C-3), 22.49 (C-26), 22.52 (C-11), 22.53 (C-27), 24.9 (C-2), 27.2 (3C, Piv), 27.9 (C-25), 29.8 (C-8), 30.9 (br, C-23), 33.3 (C-1), 34.9 (C-24), 34.99 (C-7), 35.04 (C-5), 36.2 (C-15), 38.7 (C-20), 39.2 (Piv), 40.6 (C-12), 43.3 (C-10), 44.7 (C-13), 47.7 (C-9), 53.3 (C-14), 56.6 (OMe), 63.9 (br, C-17), 76.6 (br, C-22), 76.8 (C-16), 82.3 (C-6), 179.8 (Piv). HRMS-FAB-NBA (*m*/*z*): $[M+Na]^+$ calcd for $C_{33}H_{56}O_4Na$, 539.4076; found, 539.4076. In this experiment, a mixture of 16α,22*S*-**11** and 16α,22*S*-**12** (691 mg, **11**/**12**=88/12, 79%) was also obtained as a colorless oil. A part of them were further separated by flash column

chromatography on silica gel (hexane/EtOAc = 90/10–75/25, v/v) to use the following analysis. 16α,22*S*-**11**. Colorless oil, which solidified during storage at room temperature. Mp 132–135 °C. $[\alpha]_D^{28}$ –29.6 (*c* 0.678 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.43 (1H, dd, *J* = 8.0, 5.0 Hz, H-4α), 0.65 (1H, dd, *J* = 5.0, 4.0 Hz, H-4β), 0.79 (3H, s, H_3-18), 0.87 (1H, m, H-1β), 0.87 (3H, d, *J* = 6.6 Hz, H_3-26), 0.88 (3H, d, *J* = 6.6 Hz, H_3-27), 0.89 (1H, m, H-9α), 0.91 (1H, m, H-3), 0.93 (3H, d, *J* = 6.7 Hz, H_3-21), 1.02 (3H, s, H_3-19), 1.07 (1H, m, H_a-24), 1.10 (1H, m, H-7α), 1.21 (9H, s, Piv), 1.29 (1H, m, H_a-23), 1.31 (1H, m, H_b-24), 1.34 (1H, m, H-12α), 1.39 (1H, m, H-14α), 1.40 (1H, m, H-11β), 1.44 (1H, m, H-15α), 1.46 (1H, m, H-11α), 1.51 (1H, m, H-1α), 1.52 (2H, m, H_b-23 and H-25), 1.54 (1H, m, H-2β), 1.55 (1H, m, H-20), 1.62 (1H, br s, OH), 1.66 (1H, dd, *J* = 11.1, 7.0 Hz, H-17α), 1.75 (1H, m, H-8β), 1.76 (1H, m, H-2α), 1.85 (1H, m, H-15β), 1.86 (1H, m, H-7β), 2.01 (1H, ddd, *J* = 12.1, 3.1, 3.1 Hz, H-12β), 2.76 (1H, dd, *J* = 2.8, 2.8 Hz, H-6α), 3.30 (3H, s, OMe), 3.44 (1H, br d, *J* = 7.7 Hz, H-22), 4.89 (1H, dd, *J* = 7.0, 7.0 Hz, H-16β). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 11.4 (C-21), 12.9 (C-4), 13.6 (C-18), 19.2 (C-19), 21.6 (C-3), 22.4 (C-11), 22.5 (C-26), 22.6 (C-27), 24.9 (C-2), 27.0 (3C, Piv), 28.1 (C-25), 29.8 (C-8), 32.2 (C-23), 33.2 (C-1), 34.5 (C-7), 34.6 (C-15), 35.4 (C-5), 35.6 (C-24), 38.5 (Piv), 38.9 (C-20), 40.3 (C-12), 43.2 (C-10), 43.6 (C-13), 47.7 (C-9), 53.7 (C-14), 56.5 (OMe), 58.1 (C-17), 72.9 (C-22), 80.1 (C-16), 82.1 (C-6), 178.1 (Piv). HRMS-FAB-NBA (*m*/*z*): $[M+Na]^+$ calcd for $C_{33}H_{56}O_4Na$, 539.4076; found, 539.4080. 16α,22*S*-**12**. Colorless oil. $[\alpha]_D^{27}$ –31.7 (*c* 0.290 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.43 (1H, dd, *J* = 7.9, 5.2 Hz), 0.64 (1H, dd, *J* = 5.2, 3.8 Hz), 0.83 (3H, s), 0.86 (3H, d, *J* = 6.5 Hz), 0.87 (3H, d, *J* = 6.5 Hz), 1.02 (3H, s), 1.04 (3H, d, *J* = 6.5 Hz), 1.18 (9H, s), 1.19 (9H, s), 2.75 (1H, dd, *J* = 2.7, 2.7 Hz), 3.30 (3H, s), 4.60 (1H, ddd, *J* = 8.0, 5.5, 1.6 Hz), 4.85 (1H, dd, *J* = 6.7, 6.7 Hz). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 12.9, 13.0, 13.5, 19.3, 21.7, 22.3, 22.4, 22.8, 24.9, 27.0 (3C), 27.2 (3C), 28.0, 29.1, 29.6, 33.3, 34.5, 34.6, 35.4, 35.5, 37.4, 38.5, 38.9, 40.4, 43.3, 43.9, 47.7, 53.7, 56.6, 57.7, 76.1, 80.4, 82.2, 177.3, 178.7. HRMS-FAB-NBA (*m*/*z*): $[M+Na]^+$ calcd for $C_{38}H_{64}O_5Na$, 623.4651; found, 623.4653.

4.12. (22*R*)-16α-(2,2-Dimethylpropanoyloxy)-6β-methoxy-3α,5-cyclocholestan-22-ol (16α,22*R*-**11**), and (22*R*)-16α,22-bis(2,2-dimethylpropanoyloxy)-6β-methoxy-3α,5-cyclocholestane (16α,22*R*-**12**).



16α,22*R*-**11**

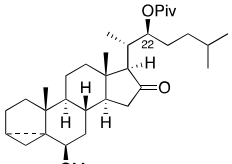


16α,22*R*-**12**

An ice-cold solution of 16α,22*R*-**9** (163 mg, 0.377 mmol) and DMAP (75.2 mg, 0.616 mmol) in anhydrous CH_2Cl_2 (9.0 mL) was treated with a solution of PivCl (49.9 mg, 0.414 mmol) dissolved in anhydrous CH_2Cl_2 (1.0 mL). After 16 h of stirring at room temperature, PivCl (0.07 mL, 0.6

mmol) and DMAP (73.2 mg, 0.599 mmol) were added to the mixture and stirring was continued for a further 8 h. The reaction was quenched by addition of a saturated aqueous solution of $NaHCO_3$ (10 mL). After 30 min of vigorous stirring at room temperature, the mixture was diluted with water (30 mL) and extracted with $CHCl_3$ (1 × 30 mL, 2 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 85/15–60/40, v/v). 16α,22*R*-**12** (86.4 mg, 38%) eluted first, followed by 16α,22*R*-**10** (87.2 mg, 45%) and then 16α,22*R*-**11** (10.4 mg, 5.3%). 16α,22*R*-**11**. Colorless oil. $[\alpha]_D^{27}$ –8.3 (*c* 0.165 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.43 (1H, dd, *J* = 8.0, 5.2 Hz, H-4α), 0.65 (1H, dd, *J* = 5.2, 3.7 Hz, H-4β), 0.82 (3H, s, H_3-18), 0.87 (1H, m, H-1β), 0.88 (3H, d, *J* = 6.2 Hz, H_3-27), 0.88 (1H, m, H-9α), 0.89 (3H, d, *J* = 6.2 Hz, H_3-26), 0.90 (1H, m, H-3), 0.97 (3H, d, *J* = 6.8 Hz, H_3-21), 1.02 (3H, s, H_3-19), 1.06 (1H, ddd, *J* = 13.3, 12.1, 2.8 Hz, H-7α), 1.09 (1H, m, H_a-24), 1.18 (9H, s, Piv), 1.29 (1H, m, H_a-23), 1.31 (2H, m, H-12α and H-14α), 1.37 (1H, m, H-15α), 1.38 (1H, m, H_b-24), 1.39 (1H, m, H_b-23), 1.40 (1H, m, H-11β), 1.44 (1H, m, H-11α), 1.47 (1H, dd, *J* = 11.4, 6.4 Hz, H-17α), 1.51 (2H, m, H-1α and H-25), 1.54 (1H, m, H-2β), 1.75 (1H, m, H-2α), 1.76 (1H, m, H-8β), 1.80 (1H, m, H-15β), 1.84 (1H, m, H-20), 1.85 (1H, ddd, *J* = 13.3, 5.8, 2.8 Hz, H-7β), 2.02 (1H, ddd, *J* = 12.3, 3.2, 3.2 Hz, H-12β), 2.75 (1H, dd, *J* = 2.8, 2.8 Hz, H-6α), 3.30 (3H, s, OMe), 3.50 (1H, br d, *J* = 10.4 Hz, H-22), 4.96 (1H, dd, *J* = 6.4, 6.4 Hz, H-16β). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 12.9 (C-4), 13.1 (C-21), 13.4 (C-18), 19.3 (C-19), 21.7 (C-3), 22.38 (C-26), 22.43 (C-11), 23.0 (C-27), 24.9 (C-2), 27.1 (3C, Piv), 28.2 (C-23), 28.8 (C-25), 29.7 (C-8), 33.3 (C-1), 34.6 (C-7), 35.2 (C-15), 35.5 (C-5), 35.7 (C-24), 38.5 (Piv), 40.2 (C-12), 41.7 (C-20), 43.3 (C-10), 44.2 (C-13), 47.8 (C-9), 53.5 (C-14), 56.6 (OMe), 57.9 (C-17), 73.8 (C-22), 80.0 (C-16), 82.2 (C-6), 178.3 (Piv). HRMS-FAB-NBA (*m*/*z*): $[M+Na]^+$ calcd for $C_{33}H_{56}O_4Na$, 539.4076; found, 539.4079. 16α,22*R*-**12**. Colorless oil. $[\alpha]_D^{27}$ –13.4 (*c* 0.864 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.43 (1H, dd, *J* = 7.9, 5.2 Hz), 0.64 (1H, dd, *J* = 5.2, 3.8 Hz), 0.79 (3H, s), 0.86 (3H, d, *J* = 6.5 Hz), 0.87 (3H, d, *J* = 6.5 Hz), 0.97 (3H, d, *J* = 6.9 Hz), 1.01 (3H, s), 1.19 (18H, s), 2.74 (1H, dd, *J* = 2.6, 2.6 Hz), 3.29 (3H, s), 4.73 (1H, ddd, *J* = 10.7, 2.4, 2.4 Hz), 5.07 (1H, dd, *J* = 6.9, 6.9 Hz). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 12.9, 13.2, 13.3, 19.2, 21.7, 22.3, 22.4, 22.9, 24.9, 25.3, 27.09 (3C), 27.13 (3C), 28.6, 29.7, 33.2, 34.5, 35.1, 35.2, 35.5, 38.3, 38.4, 38.8, 40.2, 43.3, 44.0, 47.8, 53.6, 56.5, 57.8, 75.9, 80.0, 82.2, 177.7, 177.8. HRMS-FAB-NBA (*m*/*z*): $[M+Na]^+$ calcd for $C_{38}H_{64}O_5Na$, 623.4651; found, 623.4651.

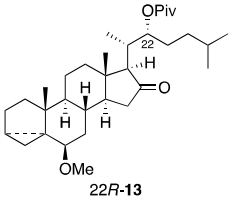
4.13. (22*S*)-22-(2,2-Dimethylpropanoyloxy)-6β-methoxy-3α,5-cyclocholestan-16-one (22*S*-**13**).



22*S*-**13**

A solution of 16α,22*S*-**10** (101 mg, 0.195 mmol) in anhydrous CH_2Cl_2 (10 mL) was treated with DMP (91.7 mg, 0.216 mmol). After 6.5 h of stirring at room temperature, the reaction mixture was diluted with $CHCl_3$ (40 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (20 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 90/10–70/30, v/v) to give 22*S*-**13** (94.6 mg, 94%) as a colorless oil, which solidified during storage at room temperature. Mp 118–120 °C. $[\alpha]_D^{30}$ –91.0 (*c* 0.946 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.47 (1H, dd, *J* = 7.9, 5.2 Hz), 0.68 (1H, dd, *J* = 5.2, 3.8 Hz), 0.84 (3H, s), 0.88 (3H, d, *J* = 6.5 Hz), 0.89 (3H, d, *J* = 6.5 Hz), 0.99 (3H, d, *J* = 7.2 Hz), 1.04 (3H, s), 1.21 (9H, s), 1.98–2.03 (1H, m), 2.09 (1H, ddd, *J* = 12.5, 3.2, 3.2 Hz), 2.27 (1H, dd, *J* = 18.4, 8.1 Hz), 2.80 (1H, dd, *J* = 2.7, 2.7 Hz), 3.33 (3H, s), 5.43 (1H, ddd, *J* = 7.9, 5.8, 1.7 Hz). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 11.3, 13.07, 13.10, 19.2, 21.3, 22.1, 22.49, 22.55, 24.9, 27.3 (3C), 27.9, 29.6, 30.2, 33.1, 34.6, 34.99, 35.03, 35.2, 38.3, 39.0, 39.5, 43.0, 43.4, 47.7, 50.7, 56.6, 64.2, 75.4, 81.9, 178.3, 218.2. HRMS-FAB-NBA (*m*/*z*): $[M+Na]^+$ calcd for $C_{33}H_{54}O_4Na$, 537.3920; found, 537.3919.

4.14. (22*R*)-22-(2,2-Dimethylpropanoyloxy)-6β-methoxy-3α,5-cyclocholestan-16-one (22*R*-**13**).



22*R*-**13**

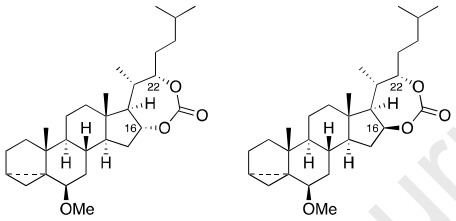
A solution of 16α,22*R*-**10** (167 mg, 0.323 mmol) in anhydrous CH_2Cl_2 (10 mL) was treated with DMP (155 mg, 0.365 mmol), before the mixture was stirred for 2 h at room temperature. The mixture was diluted with $CHCl_3$ (40 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (20 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 90/10–70/30, v/v) to give 22*R*-**13** (148 mg, 89%) as a colorless oil. $[\alpha]_D^{28}$ –71.7 (*c* 0.977 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.47 (1H, dd, *J* = 7.9, 5.2 Hz), 0.68 (1H, dd, *J* = 5.2, 3.8 Hz), 0.85 (3H, d, *J* = 6.5 Hz), 0.86 (3H, d, *J* = 6.5 Hz), 0.91 (3H, s), 0.95 (3H, d, *J* = 7.2 Hz), 1.05 (3H, s), 1.21 (9H, s), 2.10 (1H, ddd, *J* = 12.4, 3.1, 3.1 Hz), 2.24 (1H, dd, *J* = 18.2, 7.2 Hz), 2.30–2.36 (1H, m), 2.80 (1H, dd, *J* = 2.6, 2.6 Hz), 3.34 (3H, s), 5.46 (1H, ddd, *J* = 8.2, 6.2, 3.4 Hz). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 13.1, 13.4, 14.5, 19.2, 21.2, 22.1, 22.3, 22.8, 24.9, 26.8, 27.3 (3C), 27.9, 29.5,

33.1, 34.0, 34.4, 34.9, 35.4, 38.85, 38.93, 39.7, 43.4, 43.5, 47.8, 51.1, 56.6, 64.2, 75.0, 81.9, 177.6, 217.6. HRMS-FAB-NBA (*m*/*z*): $[M+Na]^+$ calcd for $C_{33}H_{54}O_4Na$, 537.3920; found, 537.3922.

4.15. Reduction of 22*S*-**13**.

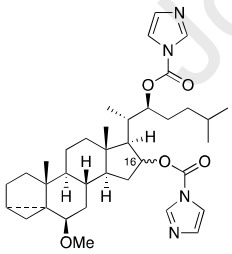
A solution of 22*S*-**13** (121 mg, 0.235 mmol) in anhydrous CH_2Cl_2 (4.0 mL) at –78 °C under an argon atmosphere was treated dropwise with a DIBAL solution (1.5 M in toluene, 0.80 mL, 1.2 mmol). After 3.5 h of stirring at the same temperature, additional DIBAL solution (1.5 M in toluene, 0.80 mL, 1.2 mmol) was added dropwise to the reaction mixture, before stirring was continued for 2 h at –78 °C. The mixture was then treated dropwise with water (2.0 mL), before the cooling bath was removed. The mixture was diluted with EtOAc (30 mL) and dried over anhydrous $MgSO_4$. Insoluble materials were filtered off, and the filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 75/25–60/40, v/v) to give 16αβ,22*S*-**9** (73.3 mg, 72%, α/β = 9/91) as a colorless solid.

4.16. (22*S*)-16α,22-Carbonyldioxy-6β-methoxy-3α,5-cyclocholestane (16α,22*S*-**14**) and (22*S*)-16β,22-carbonyldioxy-6β-methoxy-3α,5-cyclocholestane (16β,22*S*-**14**).



16α,22*S*-**14**

16β,22*S*-**14**

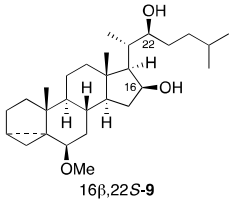


15
(16α/16β=33/67)

16αβ,22*S*-**9** (1.38 g, 3.19 mmol, α/β = 17/83) and CDI (779 mg, 4.80 mmol) were dissolved in toluene (220 mL), and the solution was stirred under reflux for 4 days before the mixture was cooled to room temperature and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 90/10–70/30, v/v) to give 16α,22*S*-**14** (188 mg, 13%) and 16β,22*S*-**14** (1.03 g, 70%) as colorless solids. 16α,22*S*-**14**: Mp 212 °C. $[\alpha]_D^{16}$ +57.0 (*c* 1.54 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.46 (1H, dd, *J* = 8.0, 5.2 Hz, H-4α), 0.66 (1H, dd, *J* = 5.2, 3.7 Hz, H-4β), 0.79 (3H, s, H_3-18), 0.87 (1H, ddd, *J* = 13.2, 11.8, 7.8 Hz, H-1β), 0.900 (3H, d, *J* = 6.6 Hz, H_3-26), 0.901 (3H, d, *J* = 6.6 Hz, H_3-27), 0.91 (1H, m, H-9α), 0.92 (1H, m, H-3),

1.00 (3H, d, *J* = 6.7 Hz, H_3-21), 1.02 (3H, s, H_3-19), 1.12 (1H, ddd, *J* = 13.3, 12.1, 2.8 Hz, H-7α), 1.16 (1H, m, H_a-24), 1.34 (1H, ddd, *J* = 13.1, 11.9, 3.7 Hz, H-12α), 1.40 (1H, m, H-11β), 1.46 (1H, m, H_b-24), 1.48 (1H, m, H_a-23), 1.49 (2H, m, H-1α and H-11α), 1.53 (1H, m, H-14α), 1.55 (1H, m, H-2β), 1.56 (1H, m, H-25), 1.62 (1H, dd, *J* = 10.3, 9.1 Hz, H-17α), 1.68 (1H, m, H_b-23), 1.75 (1H, m, H-8β), 1.76 (1H, m, H-2α), 1.79 (1H, m, H-15β), 1.81 (1H, ddd, *J* = 13.3, 2.8, 2.8 Hz, H-7β), 1.86 (1H, ddd, *J* = 11.9, 3.2, 2.6 Hz, H-12β), 1.96 (1H, ddd, *J* = 14.2, 8.5, 2.4 Hz, H-15α), 1.96 (1H, br s, H-20), 2.79 (1H, dd, *J* = 2.8, 2.8 Hz, H-6α), 3.33 (3H, s, OMe), 4.55 (1H, br s, H-22), 4.94 (1H, br s, H-16β). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 13.08 (C-4), 13.08 (br, C-21), 14.3 (br, C-18), 19.1 (C-19), 21.2 (C-3), 22.0 (C-11), 22.37 (C-26), 22.43 (C-27), 24.8 (C-2), 27.9 (C-25), 29.0 (br, C-23), 30.1 (C-8), 32.7 (br, C-15), 33.1 (C-1), 34.8 (br, C-20), 34.89 (C-7), 34.93 (C-5), 35.0 (C-24), 39.4 (C-12), 43.3 (C-10), 43.6 (C-13), 47.8 (C-9), 53.4 (C-14), 56.6 (OMe), 59.9 (br, C-17), 81.9 (C-6), 83.3 (br, C-22), 83.9 (br, C-16), 152.4 (br, C=O). HRMS-FAB-NBA (*m*/*z*): $[M+Na]^+$ calcd for $C_{29}H_{46}O_4Na$, 481.3294; found, 481.3299. 16β,22*S*-**14**: Mp 183 °C. $[\alpha]_D^{16}$ –46.0 (*c* 1.53 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.46 (1H, dd, *J* = 8.0, 5.2 Hz, H-4α), 0.66 (1H, dd, *J* = 5.2, 3.7 Hz, H-4β), 0.85 (1H, m, H-9α), 0.88 (1H, m, H-1β), 0.90 (3H, d, *J* = 6.8 Hz, H_3-26), 0.91 (1H, m, H-3), 0.91 (3H, d, *J* = 6.8 Hz, H_3-27), 0.92 (3H, s, H_3-18), 1.01 (3H, d, *J* = 6.9 Hz, H_3-21), 1.02 (1H, m, H-14α), 1.04 (3H, s, H_3-19), 1.09 (1H, ddd, *J* = 13.7, 12.6, 2.7 Hz, H-7α), 1.17 (1H, ddd, *J* = 17.6, 8.5, 3.9 Hz, H-12α), 1.27 (1H, m, H_a-24), 1.41 (1H, dd, *J* = 10.8, 7.4 Hz, H-17α), 1.46 (1H, m, H-11β), 1.47 (1H, m, H-11α), 1.50 (1H, m, H-1α), 1.54 (1H, m, H-2β), 1.56 (3H, m, H_2-23 and H_b-24), 1.58 (1H, m, H-25), 1.59 (1H, ddd, *J* = 13.8, 13.8, 4.8 Hz, H-15β), 1.73 (1H, dddd, *J* = 12.1, 12.1, 7.9, 4.2 Hz, H-2α), 1.88 (1H, m, H-8β), 1.89 (1H, m, H-12β), 1.90 (1H, m, H-7β), 2.26 (1H, ddd, *J* = 13.8, 8.3, 7.1 Hz, H-15α), 2.32 (1H, dqd, *J* = 10.8, 6.9, 3.9 Hz, H-20), 2.78 (1H, dd, *J* = 2.7, 2.7 Hz, H-6α), 3.33 (3H, s, OMe), 4.18 (1H, m, H-22), 4.85 (1H, ddd, *J* = 8.3, 7.4, 4.8 Hz, H-16α). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 13.0 (C-4), 13.3 (C-18), 15.8 (C-21), 19.2 (C-19), 21.4 (C-3), 22.2 (C-11), 22.3 (C-26), 22.6 (C-27), 24.9 (C-2), 27.0 (br, C-23), 27.8 (C-25), 29.8 (C-8), 32.2 (C-20), 32.5 (C-15), 33.2 (C-1), 34.9 (C-7), 35.1 (C-5), 35.2 (C-24), 39.7 (C-12), 42.3 (C-13), 43.3 (C-10), 48.0 (C-9), 53.8 (C-14), 54.5 (br, C-17), 56.6 (OMe), 80.5 (C-16), 81.9 (C-6), 85.7 (br, C-22), 153.5 (C=O). HRMS-FAB-NBA (*m*/*z*): $[M+Na]^+$ calcd for $C_{29}H_{46}O_4Na$, 481.3294; found, 481.3296. In this reaction, bis-imidazolylcarbonylated **15** (203 mg, 10%, 16α/16β = 33/67) was also obtained as a colorless solid. The representative ^{1}H NMR peaks of **15** are the following (600 MHz, $CDCl_3$, δ). For the 16β-isomer: 5.07 (1H, dd, *J* = 7.0, 7.0 Hz), 5.66 (1H, ddd, *J* = 7.6, 7.6, 4.1 Hz), 7.09 (1H, s), 7.37 (1H, s), 7.40 (1H, s), 8.09 (1H, s), 8.11 (1H, s). For the 16α-isomer: 4.95 (1H, dd, *J* = 7.0, 7.0 Hz), 5.26 (1H, dd, *J* = 6.7, 6.7 Hz), 6.99 (1H, s), 7.06 (1H, s), 7.16 (1H, s), 7.18 (1H, s), 7.92 (1H, s), 7.95 (1H, s).

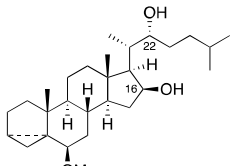
4.17. (22*S*)-6β-Methoxy-3α,5-cyclocholestane-16β,22-diol (16β,22*S*-**9**).



16β,22*S*-**9**

An ice-cold solution of 16β,22*S*-**14** (151 mg, 0.329 mmol) in anhydrous THF (5.0 mL) was slowly treated with $LiAlH_4$ (64.9 mg, 1.71 mmol), before the mixture was stirred for 1 h at 0 °C. The reaction mixture was successively treated dropwise with water (0.10 mL), a 10% aqueous solution of NaOH (0.10 mL), and water (0.30 mL), before stirring was continued for 1 h at room temperature. The mixture was diluted with EtOAc (30 mL) and dried over anhydrous $MgSO_4$. Insoluble materials were filtered off, and the filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 75/25–60/40, v/v) to give 16β,22*S*-**9** (129 mg, 91%) as a colorless solid. Mp 77 °C. $[\alpha]_D^{23}$ +23.1 (*c* 1.29 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.43 (1H, dd, *J* = 8.0, 5.1 Hz, H-4α), 0.64 (1H, dd, *J* = 5.1, 3.7 Hz, H-4β), 0.82 (1H, ddd, *J* = 10.9, 10.9, 5.1 Hz, H-9α), 0.86 (1H, ddd, *J* = 13.4, 12.0, 7.8 Hz, H-1β), 0.90 (1H, m, H-3), 0.90 (3H, d, *J* = 6.6 Hz, H_3-26), 0.91 (3H, d, *J* = 6.6 Hz, H_3-27), 0.96 (3H, s, H_3-18), 0.97 (3H, d, *J* = 7.1 Hz, H_3-21), 0.97 (1H, m, H-14α), 1.03 (3H, s, H_3-19), 1.06 (1H, ddd, *J* = 13.1, 11.9, 2.9 Hz, H-7α), 1.12 (1H, ddd, *J* = 12.3, 12.3, 4.9 Hz, H-12α), 1.17 (1H, m, H_a-24), 1.23 (1H, dd, *J* = 11.2, 7.0 Hz, H-17α), 1.26 (1H, ddd, *J* = 13.1, 13.1, 4.6 Hz, H-15β), 1.39 (1H, m, H_b-24), 1.40 (1H, m, H_a-23), 1.41 (1H, m, H-11β), 1.43 (1H, m, H-11α), 1.48 (1H, m, H_b-23), 1.50 (1H, m, H-1α), 1.52 (1H, m, H-2β), 1.57 (1H, m, H-25), 1.73 (1H, dddd, *J* = 12.0, 12.0, 7.9, 4.2 Hz, H-2α), 1.82 (1H, dddd, *J* = 11.9, 10.9, 10.9, 2.9 Hz, H-8β), 1.92 (1H, ddd, *J* = 13.1, 2.9, 2.9 Hz, H-7β), 1.94 (1H, ddd, *J* = 12.3, 3.4, 3.4 Hz, H-12β), 2.24 (1H, ddd, *J* = 13.1, 7.9, 7.5 Hz, H-15α), 2.27 (1H, dqd, *J* = 11.2, 7.1, 2.2 Hz, H-20), 2.77 (1H, dd, *J* = 2.9, 2.9 Hz, H-6α), 2.90 (1H, br s, OH), 3.32 (3H, s, OMe), 3.62 (1H, ddd, *J* = 9.4, 2.6, 2.2 Hz, H-22), 3.80 (1H, br s, OH), 4.35 (1H, ddd, *J* = 7.9, 7.0, 4.6 Hz, H-16α). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 13.0 (C-4), 13.4 (C-18), 16.2 (br, C-21), 19.3 (C-19), 21.6 (C-3), 22.4 (C-11), 22.5 (C-26), 22.7 (C-27), 24.9 (C-2), 28.1 (C-25), 30.0 (C-23), 30.1 (C-8), 33.3 (C-1), 34.7 (C-7), 34.9 (C-20), 35.4 (C-5), 35.8 (C-15), 36.1 (C-24), 40.5 (C-12), 42.9 (C-13), 43.3 (C-10), 48.0 (C-9), 54.4 (C-14), 56.4 (OMe), 57.4 (C-17), 71.9 (C-16), 77.6 (C-22), 82.3 (C-6). HRMS-FAB-NBA (*m*/*z*): $[M+Na]^+$ calcd for $C_{28}H_{48}O_3Na$, 455.3501; found, 455.3503. Using the same procedure, 16α,22*S*-**14** (154 mg, 0.336 mmol) was converted into 16α,22*S*-**9** (134 mg, 92%).

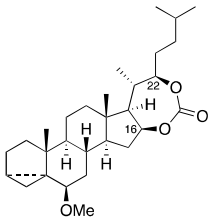
4.18. (22*R*)-6β-Methoxy-3α,5-cyclocholestane-16β,22-diol (16β,22*R*-**9**).

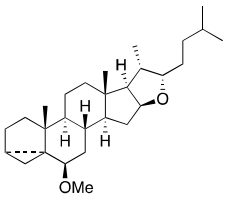


16β,22*R*-**9**

A boiling suspension of $LiAlH_4$ (56.0 mg, 1.48 mmol) in anhydrous THF (5.0 mL) was treated dropwise with a solution of 22*R*-**13** (148 mg, 0.287 mmol) in anhydrous THF (5.0 mL). The resulting mixture was stirred under reflux for 1 h and then cooled to 0 °C. This mixture was successively treated dropwise with water (0.10 mL), a 10% aqueous solution of NaOH (0.10 mL), and water (0.30 mL), before stirring was continued for 1 h at room temperature. The mixture was diluted with EtOAc (30 mL) and dried over anhydrous $MgSO_4$. Insoluble materials were filtered off, and the filtrate was concentrated *in vacuo*, before the residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 70/30–50/50, v/v) to give pure 16β,22*R*-**9** (106 mg, 85%) as a colorless solid. Mp 149–150 °C. $[\alpha]_D^{22}$ +55.0 (*c* 0.551 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.43 (1H, dd, *J* = 8.0, 5.1 Hz, H-4α), 0.65 (1H, dd, *J* = 5.1, 3.7 Hz, H-4β), 0.81 (1H, ddd, *J* = 10.7, 10.7, 5.5 Hz, H-9α), 0.86 (1H, ddd, *J* = 13.2, 11.3, 7.8 Hz, H-1β), 0.89 (1H, m, H-3), 0.90 (3H, d, *J* = 6.7 Hz, H_3-26), 0.91 (3H, d, *J* = 6.7 Hz, H_3-27), 0.93 (1H, m, H-14α), 0.95 (3H, s, H_3-18), 0.96 (3H, d, *J* = 6.7 Hz, H_3-21), 1.02 (3H, s, H_3-19), 1.06 (1H, dd, *J* = 11.0, 7.4 Hz, H-17α), 1.06 (1H, ddd, *J* = 13.4, 13.4, 2.9 Hz, H-7α), 1.11 (1H, ddd, *J* = 12.5, 11.7, 5.9 Hz, H-12α), 1.22 (1H, m, H_a-24), 1.25 (1H, ddd, *J* = 13.2, 13.2, 4.9 Hz, H-15β), 1.35 (1H, m, H_a-23), 1.40 (1H, m, H_b-24), 1.41 (1H, m, H-11β), 1.43 (1H, m, H-11α), 1.46 (1H, m, H_b-23), 1.50 (1H, m, H-1α), 1.52 (1H, m, H-2β), 1.55 (1H, m, H-25), 1.74 (1H, dddd, *J* = 12.0, 12.0, 7.8, 4.2 Hz, H-2α), 1.82 (1H, m, H-8β), 1.91 (1H, ddd, *J* = 13.4, 2.9, 2.9 Hz, H-7β), 2.00 (1H, ddd, *J* = 12.5, 3.3, 3.3 Hz, H-12β), 2.12 (1H, m, H-20), 2.25 (1H, ddd, *J* = 13.2, 7.8, 7.4 Hz, H-15α), 2.77 (1H, dd, *J* = 2.9, 2.9 Hz, H-6α), 3.32 (3H, s, OMe), 3.66 (1H, ddd, *J* = 8.9, 5.4, 2.1 Hz, H-22), 4.32 (1H, ddd, *J* = 7.8, 7.4, 4.9 Hz, H-16α). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 13.0 (C-4), 13.3 (C-18), 13.7 (C-21), 19.2 (C-19), 21.5 (C-3), 22.4 (2C, C-11 and C-26), 22.9 (C-27), 24.9 (C-2), 28.2 (C-25), 30.0 (C-8), 30.3 (C-23), 33.2 (C-1), 34.8 (C-7), 35.1 (C-24), 35.3 (C-5), 36.2 (C-15), 36.3 (C-20), 40.5 (C-12), 43.1 (C-13), 43.3 (C-10), 47.9 (C-9), 54.1 (C-14), 56.5 (OMe), 59.8 (C-17), 72.5 (C-16), 75.5 (C-22), 82.2 (C-6). HRMS-FAB-NBA (*m*/*z*): $[M+Na]^+$ calcd for $C_{28}H_{48}O_3Na$, 455.3501; found, 455.3507.

4.19. (22*R*)-16β,22-Carbonyldioxy-6β-methoxy-3α,5-cyclocholestane (16β,22*R*-**14**) and (22*S*)-6β-methoxy-3α,5-cyclofurostane (**16**).

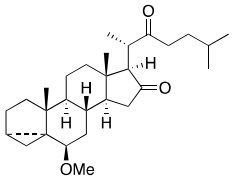

16β,22*R*-**14**


16

16αβ,22*R*-**9** (129 mg, 0.298 mmol, α/β = 10/90) and CDI (73.7 mg, 0.455 mmol) were dissolved in toluene (20 mL), before the resulting solution was stirred under reflux for 4 days. The mixture was then cooled to room temperature and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 90/10–80/20, v/v) to give 16β,22*R*-**14** (58.2 mg, 43%) as a colorless solid, and **16** (53.4 mg, 43%) as a colorless oil, respectively. 16β,22*R*-**14**: Mp 132–133 °C. $[\alpha]_D^{19}$ +33.1 (*c* 0.274 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.46 (1H, dd, *J* = 8.0, 5.2 Hz, H-4α), 0.66 (1H, dd, *J* = 5.2, 3.7 Hz, H-4β), 0.84 (1H, m, H-9α), 0.88 (1H, m, H-1β), 0.90 (3H, d, *J* = 6.5 Hz, H_3-26), 0.90 (1H, m, H-3), 0.92 (3H, d, *J* = 6.5 Hz, H_3-27), 0.93 (3H, s, H_3-18), 1.01 (1H, ddd, *J* = 14.1, 10.9, 6.8 Hz, H-14α), 1.03 (3H, d, *J* = 6.5 Hz, H_3-21), 1.04 (3H, s, H_3-19), 1.10 (1H, ddd, *J* = 13.7, 12.7, 2.8 Hz, H-7α), 1.21 (1H, m, H-12α), 1.39 (1H, dd, *J* = 10.6, 7.9 Hz, H-17α), 1.39 (1H, m, H_a-24), 1.45 (1H, m, H-11β), 1.46 (1H, m, H-11α), 1.49 (1H, m, H-1α), 1.50 (1H, m, H_b-24), 1.54 (1H, m, H-2β), 1.56 (1H, ddd, *J* = 13.5, 10.9, 5.5 Hz, H-15β), 1.57 (1H, m, H-25), 1.62 (1H, m, H_a-23), 1.74 (1H, dddd, *J* = 12.1, 12.1, 7.9, 4.2 Hz, H-2α), 1.80 (1H, dddd, *J* = 14.1, 11.2, 5.1, 2.7 Hz, H_b-23), 1.87 (1H, m, H-8β), 1.89 (1H, ddd, *J* = 13.7, 2.8, 2.8 Hz, H-7β), 1.99 (1H, ddd, *J* = 12.5, 3.3, 3.3 Hz, H-12β), 2.08 (1H, ddq, *J* = 10.6, 10.6, 6.5 Hz, H-20), 2.22 (1H, ddd, *J* = 13.5, 8.4, 6.8 Hz, H-15α), 2.79 (1H, dd, *J* = 2.8, 2.8 Hz, H-6α), 3.33 (3H, s, OMe), 4.04 (1H, ddd, *J* = 10.6, 8.1, 2.7 Hz, H-22), 4.92 (1H, ddd, *J* = 8.4, 7.9, 5.5 Hz, H-16α). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 13.1 (C-4), 13.4 (C-18), 15.9 (C-21), 19.2 (C-19), 21.3 (C-3), 22.2 (C-26), 22.4 (C-11), 22.8 (C-27), 24.9 (C-2), 27.8 (C-25), 29.8 (C-8), 31.3 (C-23), 32.2 (C-15), 33.2 (C-1), 33.4 (C-24), 33.7 (C-20), 35.0 (C-7), 35.1 (C-5), 40.5 (C-12), 42.7 (C-13), 43.3 (C-10), 47.8 (C-9), 53.6 (C-14), 56.6 (OMe), 59.6 (C-17), 80.7 (C-16), 81.9 (C-6), 87.9 (C-22), 152.9 (C=O). HRMS-FAB-NBA (*m*/*z*): $[M+Na]^+$ calcd for $C_{29}H_{46}O_4Na$, 481.3294; found, 481.3294. **16**: $[\alpha]_D^{19}$ +20.0 (*c* 0.534 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.44 (1H, dd, *J* = 8.0, 5.1 Hz, H-4α), 0.65 (1H, dd, *J* = 5.1, 3.7 Hz, H-4β), 0.82 (1H, ddd, *J* = 10.5, 10.5, 5.8 Hz, H-9α), 0.86 (1H, ddd, *J* = 13.4, 8.2, 3.7 Hz, H-1β), 0.88 (3H, s, H_3-18), 0.886 (3H, d, *J* = 6.7 Hz, H_3-26), 0.888 (3H, d, *J* = 6.7 Hz, H_3-27), 0.89 (1H, m, H-3), 0.91 (3H, d, *J* = 7.2 Hz, H_3-21), 1.03 (1H, ddd, *J* = 13.9, 11.0, 5.5 Hz, H-14α), 1.04 (3H, s, H_3-19), 1.07 (1H, ddd, *J* = 13.3, 12.1, 2.9 Hz, H-7α), 1.11 (1H, m, H-12α), 1.13 (1H, m, H_a-24), 1.28 (1H, ddd, *J* = 13.9, 12.2, 6.4 Hz, H-15β), 1.32 (1H, m, H_a-23), 1.33 (1H, m, H_b-24), 1.44 (1H, m, H-11β), 1.46 (1H, m, H-11α), 1.47 (1H, m, H_b-23), 1.51 (1H, dd, *J* = 13.4, 7.7 Hz, H-1α), 1.53 (1H, dd, *J* = 12.2, 7.7 Hz, H-2β), 1.54 (1H, m, H-25), 1.73 (1H, dd, *J*

= 8.6, 2.7 Hz, H-17α), 1.74 (1H, m, H-2α), 1.76 (1H, ddd, *J* = 12.5, 3.5, 3.5 Hz, H-12β), 1.88 (1H, m, H-8β), 1.90 (1H, ddd, *J* = 13.3, 2.9, 2.9 Hz, H-7β), 2.02 (1H, ddd, *J* = 12.2, 7.5, 5.5 Hz, H-15α), 2.19 (1H, m, H-20), 2.77 (1H, dd, *J* = 2.9, 2.9 Hz, H-6α), 3.32 (3H, s, OMe), 3.99 (1H, ddd, *J* = 8.6, 5.6, 4.6 Hz, H-22), 4.50 (1H, m, H-16α). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 13.0 (C-4), 15.1 (C-18), 16.2 (C-21), 19.3 (C-19), 21.4 (C-3), 22.4 (C-11), 22.56 (C-26), 22.61 (C-27), 24.9 (C-2), 28.1 (C-23), 28.2 (C-25), 29.5 (C-8), 33.2 (C-1), 33.3 (C-15), 35.2 (2C, C-5 and C-7), 35.3 (C-20), 35.8 (C-24), 40.2 (C-12), 41.3 (C-13), 43.5 (C-10), 48.2 (C-9), 55.3 (C-14), 56.5 (OMe), 64.5 (C-17), 81.5 (C-16), 82.2 (C-6), 84.4 (C-22). HRMS-FAB-NBA (*m*/*z*): $[M+Na]^+$ calcd for $C_{28}H_{46}O_2Na$, 437.3396; found, 437.3396.

4.20. 6β-Methoxy-3α,5-cyclocholesta-16,22-dione (**17**).



17

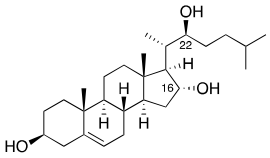
A solution of 16α,22*S*-**9** (502 mg, 1.16 mmol) in anhydrous CH_2Cl_2 (20 mL) was treated with DMP (1.25 g, 2.95 mmol), before the mixture was stirred for 21 h at room temperature under an argon atmosphere. The mixture was diluted with $CHCl_3$ (80 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (40 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 90/10–70/30, v/v) to give **17** (465 mg, 94%) as a colorless oil, which solidified during storage at room temperature. Mp 88–89 °C. $[\alpha]_D^{30}$ –106 (*c* 1.04 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.48 (1H, dd, *J* = 7.9, 5.2 Hz), 0.69 (1H, dd, *J* = 5.2, 3.8 Hz), 0.84 (3H, s), 0.92 (3H, d, *J* = 6.4 Hz), 0.92 (3H, d, *J* = 6.4 Hz), 1.05 (3H, d, *J* = 6.8 Hz), 1.05 (3H, s), 2.20 (1H, dd, *J* = 18.6, 7.9 Hz), 2.57 (1H, ddd, *J* = 17.5, 9.3, 5.5 Hz), 2.60–2.66 (2H, m), 2.71 (1H, ddd, *J* = 17.5, 9.8, 5.3 Hz), 2.81 (1H, dd, *J* = 2.7, 2.7 Hz), 3.34 (3H, s). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 13.2, 13.3, 15.4, 19.2, 21.2, 22.1, 22.4 (2C), 24.8, 27.6, 29.7, 32.2, 33.1, 34.9, 35.4, 37.3, 39.1, 40.4, 42.1, 43.3, 43.5, 47.7, 51.1, 56.6, 66.4, 81.9, 214.1, 218.1. HRMS-FAB-NBA (*m*/*z*): $[M+H]^+$ calcd for $C_{28}H_{45}O_3$, 429.3369; found, 429.3365.

4.21. Reduction of **17**.

An ice-cold solution of **17** (89.0 mg, 0.208 mmol) in anhydrous THF (10 mL) was slowly treated with $LiAlH_4$ (43.8 mg, 1.15 mmol), before stirring was continued for 1 h at 0 °C. The mixture was successively treated dropwise with water (0.10 mL), a 10% aqueous solution of NaOH (0.10 mL), and water (0.30 mL), before the resulting mixture was stirred for 1 h at room temperature. The

mixture was diluted with EtOAc (30 mL) and dried over anhydrous $MgSO_4$. Insoluble materials were filtered off, and the filtrate was concentrated *in vacuo*, before the residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 70/30–55/45, v/v) to give 16αβ,22*S*-**9** (50.5 mg, 56%, 16α/16β = 12/88) and pure 16β,22*R*-**9** (23.8 mg, 26%) as colorless oils.

4.22. (22*S*)-Cholest-5-ene-3β,16α,22-triol (16α,22*S*-**1**).

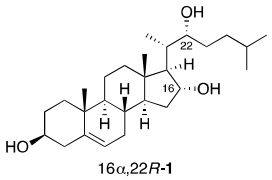


16α,22*S*-**1**

A mixture of 16α,22*S*-**9** (612 mg, 1.41 mmol), a catalytic amount of *p*-TsOH•H_2O, 1,4-dioxane (70 mL), and water (35 mL) was stirred under reflux for 2 h. Then, the mixture was cooled to room temperature, diluted with $CHCl_3$ (280 mL), and washed successively with a saturated aqueous solution of $NaHCO_3$ (110 mL) and brine (110 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*, before the residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 60/40–50/50, v/v) to give 16α,22*S*-**1** (588 mg, 99%) as a colorless solid. Mp 230 °C (MeOH). $[\alpha]_D^{19}$ –53.1 (*c* 0.202 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.70 (3H, s, H_3-18), 0.90 (6H, d, *J* = 6.6 Hz, H_3-26 and H_3-27), 0.93 (3H, d, *J* = 6.8 Hz, H_3-21), 1.00 (3H, s, H_3-19), 1.01 (1H, m, H-9α), 1.09 (1H, ddd, *J* = 13.6, 13.6, 3.8 Hz, H-1α), 1.16 (1H, m, H_a-24), 1.34 (1H, m, H-12α), 1.36 (1H, m, H_b-24), 1.39 (1H, m, H-17α), 1.41 (1H, m, H_a-23), 1.44 (1H, m, H-11β), 1.46 (2H, m, H-8β and H-14α), 1.50 (1H, m, H-2β), 1.54 (1H, m, H-11α), 1.55 (1H, m, H_b-23), 1.56 (1H, m, H-25), 1.58 (1H, br s, OH-3β), 1.59 (2H, m, H-15α and H-20), 1.60 (1H, m, H-7α), 1.64 (1H, ddd, *J* = 13.3, 13.3, 8.0 Hz, H-15β), 1.84 (2H, m, H-1β and H-2α), 1.96 (2H, m, H-7β and H-12β), 2.23 (1H, m, H-4β), 2.30 (1H, ddd, *J* = 13.0, 4.8, 2.0 Hz, H-4α), 2.59 (2H, br s, OH-16α and OH-22), 3.54 (1H, dddd, *J* = 11.3, 11.3, 4.8, 4.8 Hz, H-3α), 3.85 (1H, ddd, *J* = 8.2, 4.5, 2.4 Hz, H-22), 4.23 (1H, ddd, *J* = 8.0, 6.7, 1.3 Hz, H-16β), 5.35 (1H, m, H-6). ^{1}H NMR (600 MHz, C_5D_5N, δ): 0.79 (3H, s, H_3-18), 0.91 (3H, d, *J* = 6.5 Hz, H_3-27), 0.93 (3H, d, *J* = 6.5 Hz, H_3-26), 1.02 (1H, ddd, *J* = 12.3, 11.2, 4.8 Hz, H-9α), 1.07 (3H, s, H_3-19), 1.12 (1H, ddd, *J* = 14.7, 13.3, 5.2 Hz, H-1α), 1.19 (3H, d, *J* = 6.2 Hz, H_3-21), 1.33 (1H, ddd, *J* = 12.3, 11.8, 4.4 Hz, H-12α), 1.34 (1H, m, H_a-24), 1.45 (1H, dddd, *J* = 13.2, 12.3, 12.3, 3.5 Hz, H-11β), 1.49 (1H, m, H-8β), 1.52 (1H, m, H-11α), 1.56 (1H, m, H_a-23), 1.60 (1H, m, H-25), 1.60 (1H, ddd, *J* = 13.0, 13.0, 6.4 Hz, H-14α), 1.62 (1H, m, H-7α), 1.64 (1H, m, H_b-24), 1.74 (1H, ddd, *J* = 13.0, 13.0, 8.4 Hz, H-15β), 1.78 (1H, m, H-20), 1.80 (1H, dd, *J* = 13.0, 6.8 Hz, H-17α), 1.81 (1H, m, H-2β), 1.83 (1H, m, H-1β), 1.84 (1H, m, H_b-23), 1.92 (1H, ddd, *J* = 13.0, 6.4, 0.9 Hz, H-15α), 1.98 (1H, dddd, *J* = 17.3, 5.4, 4.9, 2.0 Hz, H-7β), 2.02 (1H, ddd, *J* = 11.8, 3.5, 2.6 Hz, H-12β), 2.10 (1H, m, H-2α), 2.62

(1H, m, H-4β), 2.64 (1H, m, H-4α), 3.85 (1H, m, H-3α), 4.26 (1H, m, H-22), 4.53 (1H, m, H-16β), 5.21 (1H, br s, OH-22), 5.41 (1H, br d, *J* = 4.9 Hz, H-6), 6.21 (1H, br s, OH-3β), 6.56 (1H, br d, *J* = 3.5 Hz, OH-16α). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 12.5 (br, C-21), 13.9 (C-18), 19.4 (C-19), 20.8 (C-11), 22.6 (C-26), 22.7 (C-27), 28.2 (C-25), 31.4 (C-8), 31.6 (C-2), 31.7 (C-7), 32.1 (br, C-23), 35.8 (C-24), 36.2 (C-15), 36.5 (C-10), 37.1 (C-1), 37.4 (br, C-20), 39.9 (C-12), 42.2 (C-4), 44.0 (C-13), 49.9 (C-9), 53.7 (C-14), 63.0 (br, C-17), 71.7 (C-3), 74.0 (br, C-22), 75.7 (C-16), 121.4 (C-6), 140.7 (C-5). ^{13}C NMR (151 MHz, C_5D_5N, δ): 12.7 (br, C-21), 13.4 (C-18), 19.0 (C-19), 20.5 (C-11), 22.2 (C-26), 22.3 (C-27), 27.9 (C-25), 31.2 (C-8), 31.5 (C-7), 31.9 (2C, C-2 and C-23), 35.9 (C-24), 36.17 (C-15), 36.23 (C-10), 37.0 (C-1), 38.1 (br, C-20), 39.8 (C-12), 42.8 (C-4), 43.3 (C-13), 49.8 (C-9), 53.6 (C-14), 62.9 (C-17), 70.5 (C-3), 72.8 (br, C-22), 74.4 (C-16), 120.4 (C-6), 141.3 (C-5). HRMS-FAB-NBA (*m*/*z*): $[M+Na]^+$ calcd for $C_{27}H_{46}O_3Na$, 441.3345; found, 441.3345.

4.23. (22*R*)-Cholest-5-ene-3β,16α,22-triol (16α,22*R*-**1**).

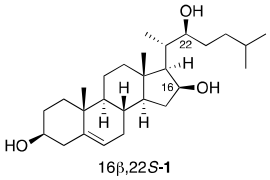


16α,22*R*-**1**

Using the same procedure as for the synthesis of 16α,22*S*-**1**, 16α,22*R*-**1** (913 mg, 95%) was obtained from 16α,22*R*-**9** (997 mg, 2.30 mmol) as a colorless solid. Mp 118–119 °C (MeOH). $[\alpha]_D^{17}$ –56.4 (*c* 0.208 g/dL, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.74 (3H, s, H_3-18), 0.89 (3H, d, *J* = 6.6 Hz, H_3-26), 0.90 (3H, d, *J* = 6.6 Hz, H_3-27), 0.97 (3H, d, *J* = 6.8 Hz, H_3-21), 0.99 (1H, m, H-9α), 1.00 (3H, s, H_3-19), 1.09 (1H, ddd, *J* = 14.0, 13.1, 3.3 Hz, H-1α), 1.20 (1H, dd, *J* = 10.9, 6.0 Hz, H-17α), 1.21 (1H, m, H_a-24), 1.31 (1H, ddd, *J* = 13.8, 12.5, 4.2 Hz, H-12α), 1.36 (1H, m, H_a-23), 1.38 (1H, m, H_b-24), 1.40 (1H, m, H-14α), 1.43 (1H, m, H-11β), 1.47 (1H, m, H-8β), 1.50 (1H, m, H-2β), 1.52 (1H, m, H-11α), 1.55 (1H, m, H-25), 1.57 (1H, m, H-15α), 1.60 (2H, m, H-7α and H_b-23), 1.67 (1H, ddd, *J* = 13.3, 13.3, 8.2 Hz, H-15β), 1.75 (1H, dqd, *J* = 10.9, 6.8, 4.5 Hz, H-20), 1.84 (2H, m, H-1β and H-2α), 1.96 (1H, dddd, *J* = 17.5, 5.2, 5.2, 2.8 Hz, H-7β), 2.01 (1H, ddd, *J* = 12.5, 3.4, 3.4 Hz, H-12β), 2.23 (1H, dddd, *J* = 13.1, 11.3, 5.1, 2.8 Hz, H-4β), 2.30 (1H, ddd, *J* = 13.1, 5.0, 1.9 Hz, H-4α), 3.54 (1H, dddd, *J* = 11.3, 11.3, 5.0, 4.2 Hz, H-3α), 3.68 (1H, ddd, *J* = 9.0, 4.5, 2.4 Hz, H-22), 4.16 (1H, m, H-16β), 5.35 (1H, m, H-6). ^{1}H NMR (600 MHz, C_5D_5N, δ): 0.79 (3H, s, H_3-18), 0.94 (3H, d, *J* = 6.5 Hz, H_3-27), 0.95 (3H, d, *J* = 6.5 Hz, H_3-26), 1.02 (1H, ddd, *J* = 11.6, 10.9, 4.8 Hz, H-9α), 1.07 (3H, s, H_3-19), 1.11 (1H, ddd, *J* = 13.1, 13.1, 3.5 Hz, H-1α), 1.30 (3H, d, *J* = 6.8 Hz, H_3-21), 1.34 (1H, ddd, *J* = 12.7, 12.7, 3.9 Hz, H-12α), 1.44 (1H, m, H-11β), 1.48 (1H, m, H-8β), 1.52 (1H, m, H-11α), 1.56 (1H, ddd, *J* = 13.0, 10.9, 7.0 Hz, H-14α), 1.61 (2H, m, H-7α and H-17α), 1.64 (1H, m, H_a-24), 1.67 (1H, m, H_a-23), 1.68 (1H, m, H-25), 1.73 (1H, ddd, *J* =

13.0, 12.4, 8.3 Hz, H-15β), 1.81 (1H, m, H-2β), 1.84 (1H, ddd, *J* = 13.1, 3.5, 3.5 Hz, H-1β), 1.89 (1H, dd, *J* = 12.4, 7.0 Hz, H-15α), 1.89 (1H, m, H_b-24), 1.98 (1H, m, H-7β), 2.05 (1H, m, H_b-23), 2.08 (1H, m, H-12β), 2.09 (1H, m, H-2α), 2.20 (1H, qdd, *J* = 6.8, 6.8, 3.7 Hz, H-20), 2.62 (1H, m, H-4β), 2.64 (1H, m, H-4α), 3.84 (1H, m, H-3α), 4.41 (1H, m, H-16β), 4.42 (1H, m, H-22), 5.41 (1H, br d, *J* = 4.0 Hz, H-6), 5.66 (1H, d, *J* = 5.1 Hz, OH-22), 6.02 (1H, d, *J* = 5.5 Hz, OH-16α), 6.21 (1H, br d, *J* = 3.8 Hz, OH-3β). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 13.3 (C-18), 15.4 (C-21), 19.4 (C-19), 20.7 (C-11), 22.5 (C-26), 22.8 (C-27), 28.2 (C-25), 30.9 (C-23), 31.3 (C-8), 31.6 (C-2), 31.7 (C-7), 35.5 (C-24), 36.4 (C-10), 36.8 (C-15), 37.1 (C-1), 39.9 (C-12), 40.8 (C-20), 42.2 (C-4), 44.6 (C-13), 49.8 (C-9), 53.4 (C-14), 63.6 (C-17), 71.7 (C-3), 76.63 (C-22), 76.64 (C-16), 121.4 (C-6), 140.7 (C-5). ^{13}C NMR (151 MHz, C_5D_5N, δ): 12.8 (C-18), 13.9 (C-21), 18.9 (C-19), 20.5 (C-11), 22.3 (C-26), 22.6 (C-27), 28.0 (C-25), 29.2 (C-23), 31.0 (C-8), 31.7 (C-7), 31.9 (C-2), 36.2 (C-24), 36.3 (C-10), 37.0 (C-1), 37.3 (C-15), 39.7 (C-12), 41.8 (C-20), 42.8 (C-4), 43.6 (C-13), 49.8 (C-9), 53.4 (C-14), 62.9 (C-17), 70.5 (C-3), 73.7 (C-22), 75.5 (C-16), 120.5 (C-6), 141.3 (C-5). HRMS-FAB-NBA (*m*/*z*): $[M+Na]^+$ calcd for $C_{27}H_{46}O_3Na$, 441.3345; found, 441.3346.

4.24. (22*S*)-Cholest-5-ene-3β,16β,22-triol (16β,22*S*-**1** = calibagenin).

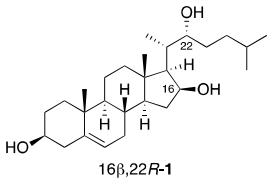


16β,22*S*-**1**

Using the same procedure as for the synthesis of 16α,22*S*-**1**, 16β,22*S*-**1** (756 mg, 95%) was obtained from 16β,22*S*-**9** (819 mg, 1.89 mmol) as a colorless solid. Mp 189 °C ($MeOH/H_2O$) (lit.[1] mp 195–196 ($MeOH/H_2O$)). $[\alpha]_D^{18}$ –55.8 (*c* 0.198 g/dL, $CHCl_3$) (lit.[1] $[\alpha]_D^{20}$ –56 ($CHCl_3$); lit.[3] $[\alpha]_D$ –62.0 ($CHCl_3$)). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.91 (3H, d, *J* = 6.6 Hz, H_3-26), 0.92 (3H, d, *J* = 6.6 Hz, H_3-27), 0.92 (1H, m, H-14α), 0.94 (3H, s, H_3-18), 0.94 (1H, m, H-9α), 0.98 (3H, d, *J* = 7.1 Hz, H_3-21), 1.02 (3H, s, H_3-19), 1.07 (1H, ddd, *J* = 14.0, 14.0, 4.1 Hz, H-1α), 1.13 (1H, m, H-12α), 1.19 (1H, m, H_a-24), 1.21 (1H, dd, *J* = 11.1, 6.9 Hz, H-17α), 1.22 (1H, ddd, *J* = 13.2, 13.2, 4.6 Hz, H-15β), 1.37 (1H, m, H_b-24), 1.42 (1H, m, H_a-23), 1.47 (1H, d, *J* = 4.4 Hz, OH-3), 1.50 (2H, m, H-2β and H-11β), 1.51 (1H, m, H_b-23), 1.52 (1H, m, H-7α), 1.53 (1H, m, H-11α), 1.55 (1H, m, H-8β), 1.58 (1H, m, H-25), 1.84 (2H, m, H-1β and H-2α), 1.97 (1H, ddd, *J* = 12.5, 3.5, 3.5 Hz, H-12β), 2.01 (1H, m, H-7β), 2.19 (1H, br s, OH-16/22), 2.23 (1H, ddd, *J* = 13.2, 8.0, 7.5 Hz, H-15α), 2.24 (1H, m, H-4β), 2.29 (1H, m, H-20), 2.30 (1H, m, H-4α), 3.53 (1H, m, H-3α), 3.54 (1H, br s, OH-16/22), 3.63 (1H, ddd, *J* = 9.7, 2.3, 2.3 Hz, H-22), 4.35 (1H, ddd, *J* = 8.0, 6.9, 4.6 Hz, H-16α), 5.34 (1H, m, H-6). The assignment of H-2, H-7, H-23, H-24, H-26, and H-27 by Christ and co-workers (400 MHz, $CDCl_3$)[7] was revised. ^{1}H NMR (600 MHz, C_5D_5N, δ): 0.899 (3H, d, *J* = 6.6

Hz, H_3-27), 0.902 (3H, d, *J* = 6.6 Hz, H_3-26), 0.97 (1H, ddd, *J* = 13.4, 10.5, 7.4 Hz, H-14α), 1.01 (1H, m, H-9α), 1.09 (3H, s, H_3-19), 1.15 (1H, ddd, *J* = 13.4, 13.4, 3.5 Hz, H-1α), 1.21 (3H, s, H_3-18), 1.22 (1H, m, H-12α), 1.24 (3H, d, *J* = 7.1 Hz, H_3-21), 1.37 (1H, m, H_a-24), 1.54 (2H, m, H-11α and H-11β), 1.55 (1H, ddd, *J* = 13.4, 12.8, 4.6 Hz, H-15β), 1.59 (1H, m, H-7α), 1.61 (2H, m, H-8β and H-25), 1.69 (1H, dd, *J* = 11.2, 7.1 Hz, H-17α), 1.72 (1H, m, H_a-23), 1.74 (1H, m, H_b-24), 1.81 (1H, m, H_b-23), 1.82 (1H, m, H-2β), 1.86 (1H, ddd, *J* = 13.4, 3.5, 3.5 Hz, H-1β), 2.00 (1H, m, H-7β), 2.10 (1H, m, H-12β), 2.11 (1H, m, H-2α), 2.33 (1H, ddd, *J* = 12.8, 7.9, 7.4 Hz, H-15α), 2.61 (1H, qdd, *J* = 7.1, 7.1, 1.8 Hz, H-20), 2.63 (1H, m, H-4β), 2.65 (1H, m, H-4α), 3.87 (1H, m, H-3α), 4.19 (1H, m, H-22), 4.79 (1H, dddd, *J* = 11.2, 7.9, 4.6, 3.6 Hz, H-16α), 5.43 (1H, br d, *J* = 5.0 Hz, H-6), 5.96 (1H, d, *J* = 3.6 Hz, OH-16β), 6.25 (1H, br d, *J* = 3.8 Hz, OH-3β), 6.41 (1H, br d, *J* = 5.2 Hz, OH-22). The ^{1}H NMR spectrum in C_5D_5N was not in accordance with that of Kuroda and co-workers (400 MHz, C_5D_5N).[6] ^{13}C NMR (151 MHz, $CDCl_3$, δ): 12.9 (C-18), 16.3 (C-21), 19.4 (C-19), 20.8 (C-11), 22.5 (C-26), 22.8 (C-27), 28.1 (C-25), 29.9 (C-23), 31.5 (C-8), 31.6 (C-2), 31.8 (C-7), 34.7 (C-20), 35.8 (C-15), 36.1 (C-24), 36.5 (C-10), 37.2 (C-1), 40.0 (C-12), 42.3 (C-4), 42.5 (C-13), 50.1 (C-9), 54.6 (C-14), 57.2 (C-17), 71.8 (C-3), 72.0 (C-16), 78.0 (C-22), 121.5 (C-6), 140.8 (C-5). The assignment of Christ and co-workers (100 MHz, $CDCl_3$)[7] was interchanged for the pairs C-2 and C-23; C-5 and C-6; and C-7 and C-8. ^{13}C NMR (151 MHz, C_5D_5N, δ): 12.9 (C-18), 14.6 (br, C-21), 19.0 (C-19), 20.5 (C-11), 22.1 (C-26), 22.4 (C-27), 27.8 (C-25), 31.4 (C-8), 31.5 (C-23), 31.6 (C-7), 32.0 (C-2), 35.4 (C-20), 36.1 (C-24), 36.3 (C-10), 36.6 (C-15), 37.2 (C-1), 39.8 (C-12), 42.0 (C-13), 42.8 (C-4), 50.0 (C-9), 54.4 (C-14), 57.4 (C-17), 70.6 (C-3), 70.8 (C-16), 74.5 (br, C-22), 120.6 (C-6), 141.4 (C-5). The ^{13}C NMR spectrum in C_5D_5N was not in accordance with that of Kuroda and co-workers (100 MHz, C_5D_5N).[6] HRMS-FAB-NBA (*m*/*z*): $[M+Na]^+$ calcd for $C_{27}H_{46}O_3Na$, 441.3345; found, 441.3344.

4.25. (22*R*)-Cholest-5-ene-3β,16β,22-triol (16β,22*R*-**1** = saxosterol).



16β,22*R*-**1**

Using the same procedure as for the synthesis of 16α,22*S*-**1**, 16β,22*R*-**1** (552 mg, quant) was obtained from 16β,22*R*-**9** (573 mg, 1.32 mmol) as a colorless solid. Mp 185–186 °C (MeOH) (lit.[2] mp 182–183 °C (MeOH)). $[\alpha]_D^{18}$ –32.6 (*c* 0.204 g/dL, $CHCl_3$) (lit.[2] $[\alpha]_D^{20}$ –27 (*c* 0.2 g/dL, $CHCl_3$)). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.89 (1H, ddd, *J* = 13.2, 10.4, 7.2 Hz, H-14α), 0.90 (3H, d, *J* = 6.7 Hz, H_3-26), 0.91 (3H, d, *J* = 6.7 Hz, H_3-27), 0.92 (3H, s, H_3-18), 0.93 (1H, m, H-9α), 0.97 (3H, d, *J* = 7.0 Hz, H_3-21), 1.02 (3H, s, H_3-19), 1.06 (1H, dd, *J* = 11.1, 7.2 Hz, H-17α), 1.07 (1H, m, H-1α),

1.14 (1H, ddd, *J* = 12.6, 11.8, 5.9 Hz, H-12α), 1.20 (1H, ddd, *J* = 13.2, 13.2, 5.0 Hz, H-15β), 1.22 (1H, m, H_a-24), 1.37 (1H, m, H_b-24), 1.38 (1H, m, H_a-23), 1.48 (1H, m, H-11β), 1.50 (2H, m, H-2β and H-11α), 1.51 (1H, m, H_b-23), 1.52 (1H, m, H-7α), 1.54 (1H, m, H-8β), 1.55 (1H, m, H-25), 1.84 (2H, m, H-1β and H-2α), 2.00 (1H, m, H-7β), 2.04 (1H, ddd, *J* = 12.6, 3.6, 3.6 Hz, H-12β), 2.12 (1H, dqd, *J* = 11.1, 7.0, 6.1 Hz, H-20), 2.23 (1H, ddd, *J* = 13.2, 8.0, 7.2 Hz, H-15α), 2.24 (1H, m, H-4β), 2.30 (1H, ddd, *J* = 13.1, 5.0, 2.1 Hz, H-4α), 3.52 (1H, m, H-3α), 3.65 (1H, ddd, *J* = 8.6, 6.1, 6.1 Hz, H-22), 4.35 (1H, ddd, *J* = 8.0, 7.2, 5.0 Hz, H-16α), 5.35 (1H, m, H-6). ^{1}H NMR (600 MHz, C_5D_5N, δ): 0.94 (6H, d, *J* = 6.5 Hz, H_3-26 and H_3-27), 0.95 (1H, ddd, *J* = 13.2, 10.4, 7.6 Hz, H-14α), 1.04 (1H, ddd, *J* = 10.6, 10.6, 6.4 Hz, H-9α), 1.09 (3H, s, H_3-19), 1.17 (1H, ddd, *J* = 13.4, 13.4, 3.8 Hz, H-1α), 1.20 (3H, s, H_3-18), 1.21 (1H, ddd, *J* = 12.4, 12.4, 5.6 Hz, H-12α), 1.29 (1H, dd, *J* = 11.5, 6.9 Hz, H-17α), 1.30 (3H, d, *J* = 6.9 Hz, H_3-21), 1.50 (1H, m, H_a-24), 1.52 (1H, m, H-11β), 1.55 (1H, m, H-11α), 1.57 (1H, ddd, *J* = 13.2, 12.8, 4.4 Hz, H-15β), 1.62 (2H, m, H-7α and H-8β), 1.66 (1H, m, H-25), 1.68 (1H, m, H_a-23), 1.75 (1H, m, H_b-23), 1.81 (1H, m, H_b-24), 1.83 (1H, m, H-2β), 1.88 (1H, ddd, *J* = 13.4, 3.3, 3.3 Hz, H-1β), 2.01 (1H, m, H-7β), 2.11 (1H, m, H-2α), 2.12 (1H, ddd, *J* = 12.4, 3.5, 3.5 Hz, H-12β), 2.36 (1H, ddd, *J* = 12.8, 7.6, 7.6 Hz, H-15α), 2.63 (1H, m, H-4β), 2.66 (1H, m, H-4α), 2.81 (1H, qdd, *J* = 6.9, 6.9, 4.3 Hz, H-20), 3.87 (1H, m, H-3α), 4.45 (1H, m, H-22), 4.66 (1H, dddd, *J* = 11.5, 7.6, 4.4, 4.4 Hz, H-16α), 5.44 (1H, br d, *J* = 5.0 Hz, H-6), 5.90 (1H, d, *J* = 4.4 Hz, OH-16β), 5.93 (1H, br d, *J* = 3.2 Hz, OH-22), 6.23 (1H, br d, *J* = 3.2 Hz, OH-3β). ^{13}C NMR (151 MHz, $CDCl_3$, δ): 13.0 (C-18), 14.2 (C-21), 19.4 (C-19), 20.8 (C-11), 22.4 (C-26), 22.9 (C-27), 28.2 (C-25), 31.0 (C-23), 31.5 (C-8), 31.6 (C-2), 31.8 (C-7), 34.9 (C-24), 36.1 (C-20), 36.2 (C-15), 36.5 (C-10), 37.2 (C-1), 40.1 (C-12), 42.2 (C-4), 42.8 (C-13), 50.0 (C-9), 54.4 (C-14), 60.1 (C-17), 71.7 (C-3), 72.6 (C-16), 76.3 (C-22), 121.5 (C-6), 140.8 (C-5). ^{13}C NMR (151 MHz, C_5D_5N, δ): 12.8 (C-18), 12.9 (C-21), 19.0 (C-19), 20.5 (C-11), 22.0 (C-26), 22.7 (C-27), 27.9 (C-25), 29.1 (C-23), 31.3 (C-8), 31.6 (C-7), 32.0 (C-2), 35.9 (C-24), 36.3 (C-10), 36.8 (C-20), 37.17 (C-1), 37.19 (C-15), 39.8 (C-12), 42.3 (C-13), 42.8 (C-4), 50.0 (C-9), 54.2 (C-14), 59.1 (C-17), 70.6 (C-3), 70.7 (C-16), 72.6 (C-22), 120.5 (C-6), 141.4 (C-5). HRMS-FAB-NBA (*m*/*z*): $[M+Na]^+$ calcd for $C_{27}H_{46}O_3Na$, 441.3345; found, 441.3344.

4.26. Single-crystal X-ray structure determination.

For the X-ray experiments, 16α,22*S*-**1**, 16α,22*R*-**1**, 16β,22*S*-**1**, and 16β,22*R*-**1** were recrystallized from MeOH, $CHCl_3$, acetone/H_2O, and EtOAc/hexane, respectively. Single crystals of **1** suitable for X-ray diffraction studies were analyzed using a 724+ CCD detector with synchrotron radiation from beamlines BL40XU (λ = 0.78229 Å) and BL02B1 (λ = 0.70060 Å) of SPring-8 (Hyogo, Japan). The structures of **1** were solved by direct methods and refined using the full-matrix least squares method. The positions of all non-hydrogen atoms were found using difference Fourier electron density maps

and refined anisotropically. All calculations were performed using the Rigaku "Crystal Structure" crystallographic software package or Yadokari-XG,[18] and thermal-ellipsoid plots were drawn using ORTEP. The crystallographic data have been deposited at the Cambridge Crystallographic Data Centre under reference numbers CCDC-1978232, 1974315, 1974687, and 1974686 for compounds 16α,22*S*-**1**, 16α,22*R*-**1**, 16β,22*S*-**1**, and 16β,22*R*-**1**, respectively. Copies of the data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge, CB2 1EZ, UK; Fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

Acknowledgments

This work was supported by the 2nd Seiken Academic Incentive Fund provided by the Research Institute for Production Development, Kyoto, Japan (to B.W.) as well as the Collaborative Research Program of Institute for Chemical Research, Kyoto University (to M.M. and B.W.; 2017-83). H.T. thanks RIKEN for the provision of beam time at SPring-8 (BL40XU: 2018A1173, 2018B0948, and 2019A0948 as well as BL02B1: 2018B0946). Some of the experimental measurements were carried out using the Bruker and JEOL 600 MHz NMR spectrometers and the JEOL JMS-700 mass spectrometer at the Joint Usage/Research Center (JURC) at the Institute for Chemical Research, Kyoto University. The authors are greatly indebted to Ms. Kyoko Ohmine and Ms. Akiko Fujihashi for performing instrumental analyses.

Supplementary Information

Assignment and comparison of the ^{1}H and ^{13}C NMR spectra of **1**, crystallographic data for **1**, isolation of the minor product in the hydroboration of 22*S*-**2**, preparation of **4** and **5**, and ^{1}H and ^{13}C NMR spectra of all synthetic compounds.

References

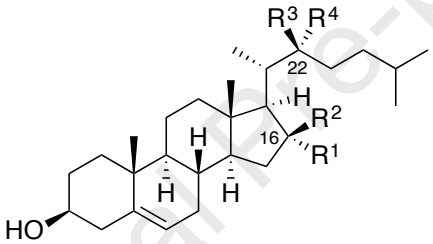
1. Giral, F.; Rivera, C. *Phytochemistry* **1975**, *14*, 793.
2. Stabursvik, A.; Holen, B. *Phytochemistry* **1988**, *27*, 1893.
3. Kawashima, K.; Mimaki, Y.; Sashida, Y. *Chem. Pharm. Bull.* **1991**, *39*, 2761.
4. Mimaki, Y.; Kuroda, M.; Yokosuka, A.; Sashida, Y. *J. Nat. Prod.* **2001**, *64*, 1069.
5. Yin, J.; Kouda, K.; Tezuka, Y.; Tran, Q. L.; Miyahara, T.; Chen, Y.; Kadota, S. *J. Nat. Prod.* **2003**, *66*, 646.

6. Kuroda, M.; Ori, K.; Takayama, H.; Sakagami, H.; Mimaki, Y. *Steroids* **2015**, *93*, 96.
7. Christ, B.; Xu, C.; Xu, M.; Li, F. S.; Wada, N.; Mitchell, A. J.; Han, X. L.; Wen, M. L.; Fujita, M.; Weng, J. K. *Nat. Commun.* **2019**, *10*, 3206.
8. Izzo, I.; Di Filippo, M.; Napolitano, R.; De Riccardis, F. *Eur. J. Org. Chem.* **1999**, *1999*, 3505.
9. Iida, T.; Hikosaka, M.; Kakiyama, G.; Shiraishi, K.; Schteingart, C. D.; Hagey, L. R.; Ton-Nu, H.-T.; Hofmann, A. F.; Mano, N.; Goto, J.; Nambara, T. *Chem. Pharm. Bull.* **2002**, *50*, 1327.
10. Williams, J. R.; Gong, H.; Hoff, N.; Olubodun, O. I.; Carroll, P. J. *Org. Lett.* **2004**, *6*, 269.
11. Nonappa; Maitra, U. *Eur. J. Org. Chem.* **2007**, *2007*, 3331.
12. Pellicciari, R.; Gioiello, A.; Sabbatini, P.; Venturoni, F.; Nuti, R.; Colliva, C.; Rizzo, G.; Adorini, L.; Pruzanski, M.; Roda, A.; Macchiarulo, A. *ACS Med. Chem. Lett.* **2012**, *3*, 273.
13. Houston, T. A.; Tanaka, Y.; Koreeda, M. *J. Org. Chem.* **1993**, *58*, 4287.
14. Deng, S.; Yu, B.; Lou, Y.; Hui, Y. *J. Org. Chem.* **1999**, *64*, 202.
15. Watanabe, B.; Nakagawa, Y.; Miyagawa, H. *J. Pestic. Sci.* **2003**, *28*, 188.
16. Steele, J. A.; Mosettig, E. *J. Org. Chem.* **1963**, *28*, 571.
17. Challinor, V. L.; Hayes, P. Y.; Bernhardt, P. V.; Kitching, W.; Lehmann, R. P.; De Voss, J. J. *J. Org. Chem.* **2011**, *76*, 7275.
18. Kabuto, C.; Akine, S.; Nemoto, T.; Kwon, E. *J. Cryst. Soc. Jpn.* **2009**, *51*, 218 (in Japanese). DOI: https://doi.org/10.5940/jcrsj.51.218.

Table 1. Comparison of the ^{1}H NMR, $[\alpha]_D$, and mp data of calibagenin, saxosterol, and **1**.

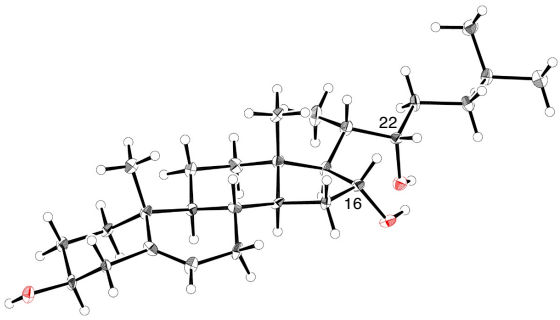
	^{1}H NMR[a]			$[\alpha]_D$[b]	Mp	
	H-18	H-16	H-22		(°C)	Solvent[c]
calibagenin	0.91 (s)	4.36 (m)[d]	3.56 (m)[e]	–56[f]	195–196	MeOH/H_2O
saxosterol	0.92 (s)	4.35 (m)	3.65 (m)	–27[f]	182–183	MeOH
16α,22*S*-**1**	0.70 (s)	4.23 (ddd, 8.0, 6.7, 1.3)	3.85 (ddd, 8.2, 4.5, 2.4)	–53.1[g]	230	MeOH
16α,22*R*-**1**	0.74 (s)	4.16 (m)	3.68 (ddd, 9.0, 4.5, 2.4)	–56.4[h]	118–119	MeOH
16β,22*S*-**1**	0.94 (s)	4.35 (ddd, 8.0, 6.9, 4.6)	3.63 (ddd, 9.7, 2.3, 2.3)	–55.8[i]	189	MeOH/H_2O
16β,22*R*-**1**	0.92 (s)	4.35 (ddd, 8.0, 7.2, 5.0)	3.65 (ddd, 8.6, 6.1, 6.1)	–32.6[i]	185–186	MeOH

[a] Recorded in $CDCl_3$ at 60 MHz (calibagenin), 270 MHz (saxosterol), or 600 MHz (**1**); [b] Measured in $CHCl_3$ (*c* 0.2 g/dL). [c] Solvent used for recrystallization. [d] Assigned as H-22 in the original literature. [e] Assigned as H-16 in the original literature. [f] Measured at 20 ºC. [g] Measured at 19 ºC. [h] Measured at 17 ºC. [i] Measured at 18 ºC.

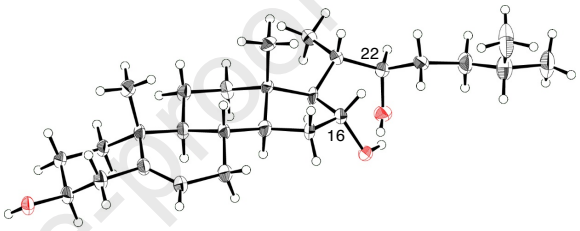


1

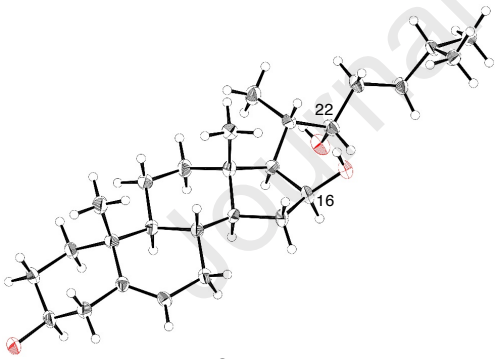
16α,22*S* (R^1 = OH, R^2 = H, R^3 = OH, R^4 = H)
16α,22*R* (R^1 = OH, R^2 = H, R^3 = H, R^4 = OH; calibagenin (previously proposed))
16β,22*S* (R^1 = H, R^2 = OH, R^3 = OH, R^4 = H)
16β,22*R* (R^1 = H, R^2 = OH, R^3 = H, R^4 = OH; saxosterol (previously proposed))



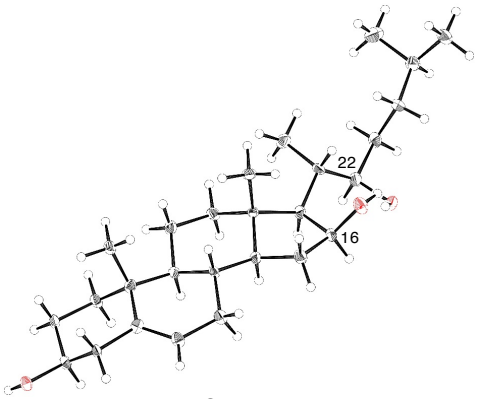
16α,22*S*-**1**



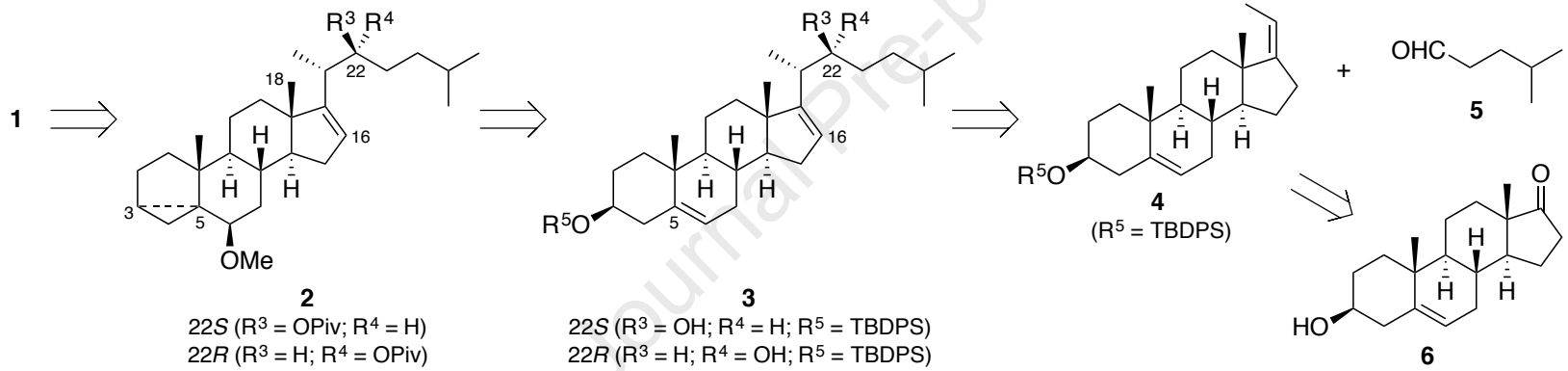
16α,22*R*-**1**

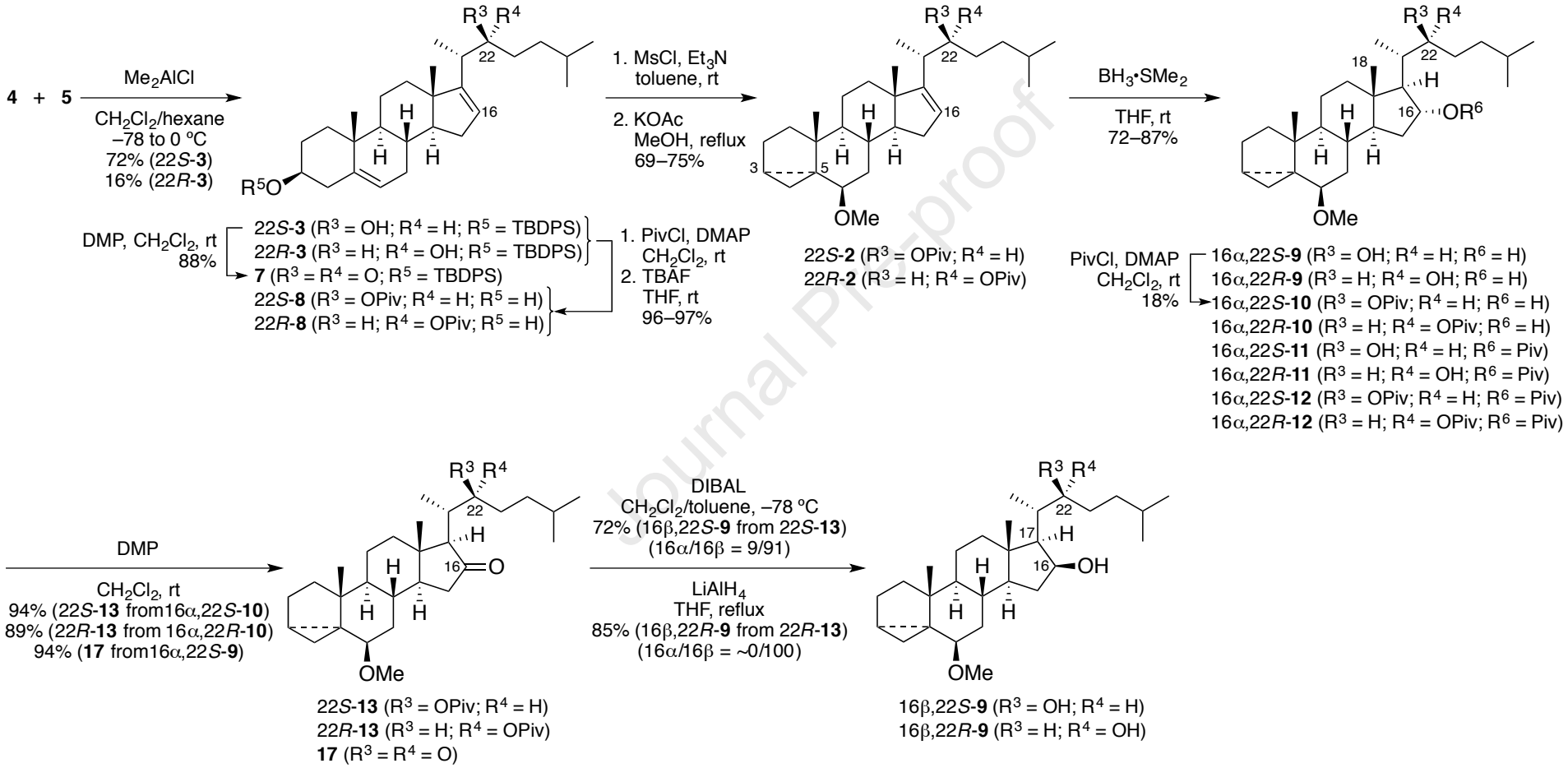


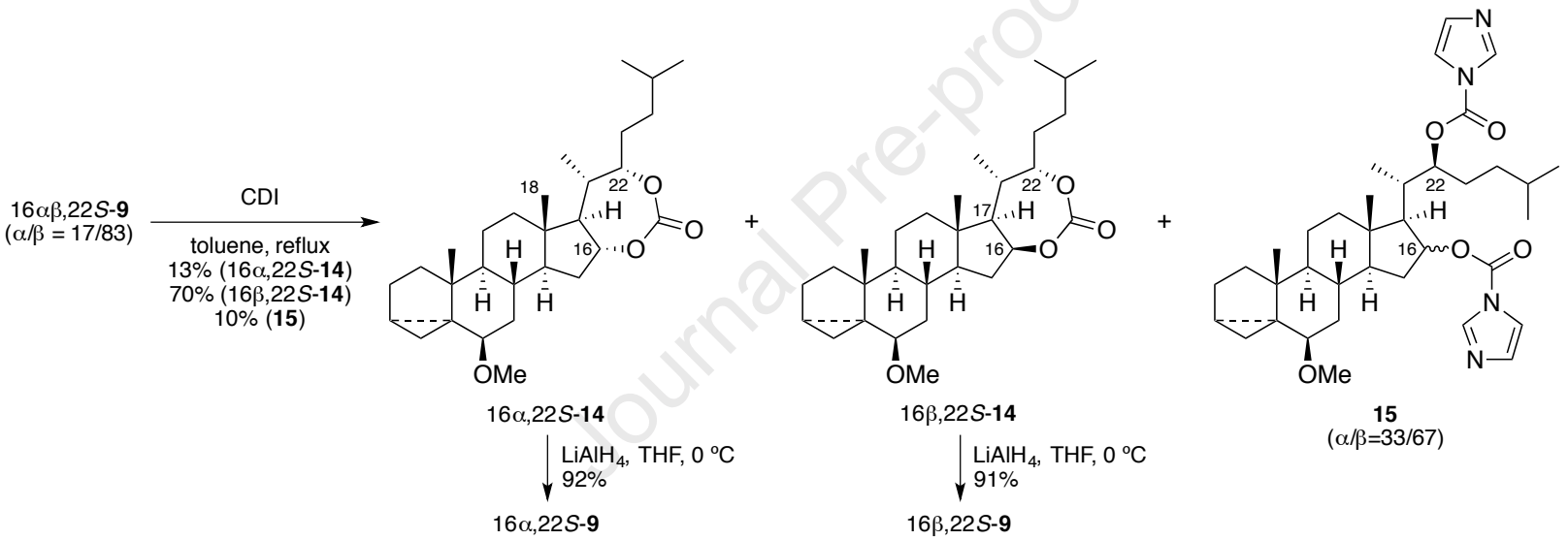
16β,22*S*-**1**

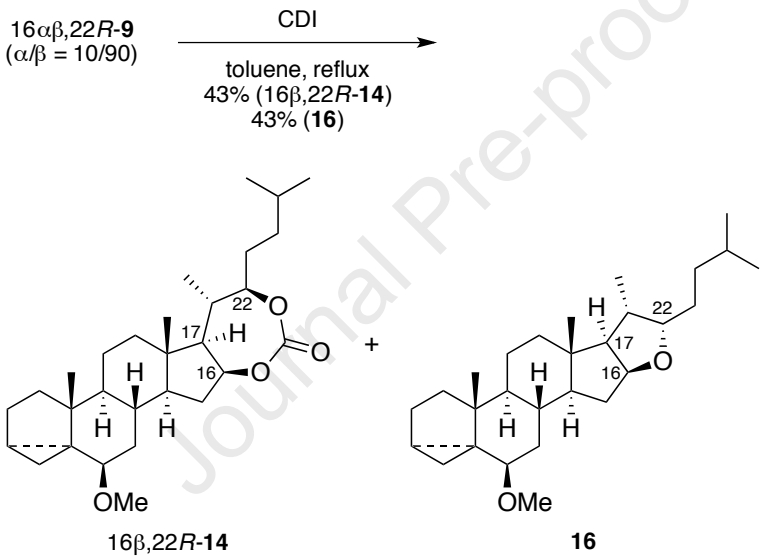


16β,22*R*-**1**









Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: