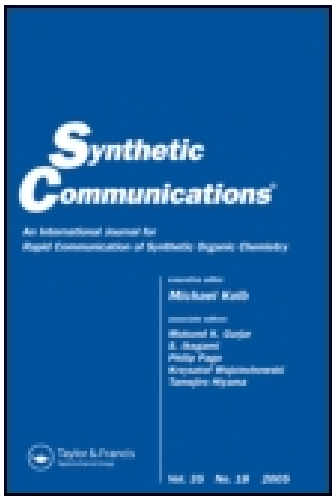

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A Novel Synthetic Approach from Diosgenin to a 17α-Hydroxy Orthoester via a Regio- and Stereo-Specific Rearrangement of an Epoxy Ester

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ABSTRACT

A cholesterol model compound, containing 16β-acetoxy, 17α-hydroxy, and (20*S*, 22*R*)-epoxy groups was synthesized from diosgenin in 13 steps and was rearranged regio- and stereo-specifically to an orthoester with $BF_3{\cdot}Et_2O$.

Key Words: Orthoester; Epoxy ester; Regio-specific and stereo-specific rearrangement; Diosgenin; Cholesterol.

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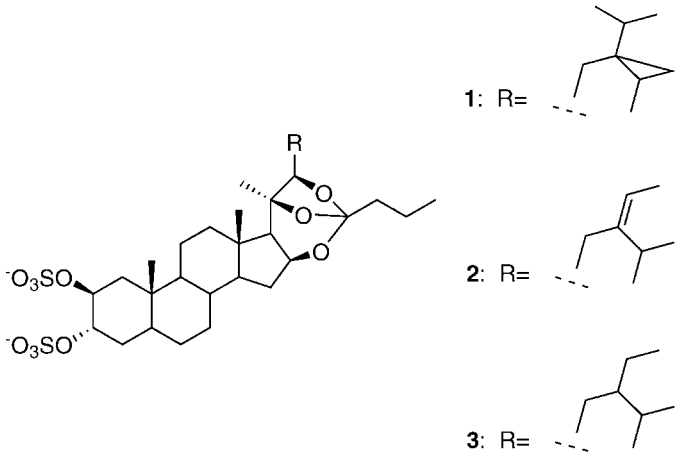

Figure 1. Structure of orthoesterol disulfate.

Biologically active compounds, isolated from marine sponges, include a group of orthoesters that have interesting structures and activities, such as the ergostanoids from *Petunia inflata*[1] and the antiviral orthoesterol disulfate A (**1**), B (**2**) and C (**3**) (Fig. 1) from *Petrosia weinbergi*.[2,3] Compounds **1–3** showed *in vitro* activity against feline leukemia virus (FELV), influenza PR8 virus and murine coronavirus (A59).[2,3] The natural occurrence of epoxy esters together with orthoesters suggested the possibility that the latter arise biosynthetically via rearrangement of the former either under enzymatic or mildly acidic conditions.[1] Recently, Giner et al.[4] reported a biomimetic approach to the synthesis of a steroidal orthoester via an epoxy ester–orthoester rearrangement using trifluoroacetic acid. This reaction also was used to synthesize 2-methyl-D-erythritol via the formation of a [2.2.1] bicyclic orthoester intermediate.[5]

The saponin OSW-1 has antitumor activity and has been the object of many synthetic efforts.[6] During our synthetic approach to OSW-1 aglycone, we noted that the two epoxy alcohols **17** and **19** obtained in 45% and 15% yield, respectively, from the oxidation of allyl alcohol **16** that had been synthesized from diosgenin (**4**) (Sch. 1), could serve as suitable compounds for rearrangement to orthoesters.

Our synthetic approach was aimed at creating the cholesterol side chain from the spiro-ring of the commercially inexpensive diosgenin (**4**) noting that the number of carbon atoms in the side chains are the same. We adapted the procedure reported by Basler et al.[7] for this purpose. As shown in Sch. 1, after acetylation, the spiroketal ring-opening of diosgenin acetate

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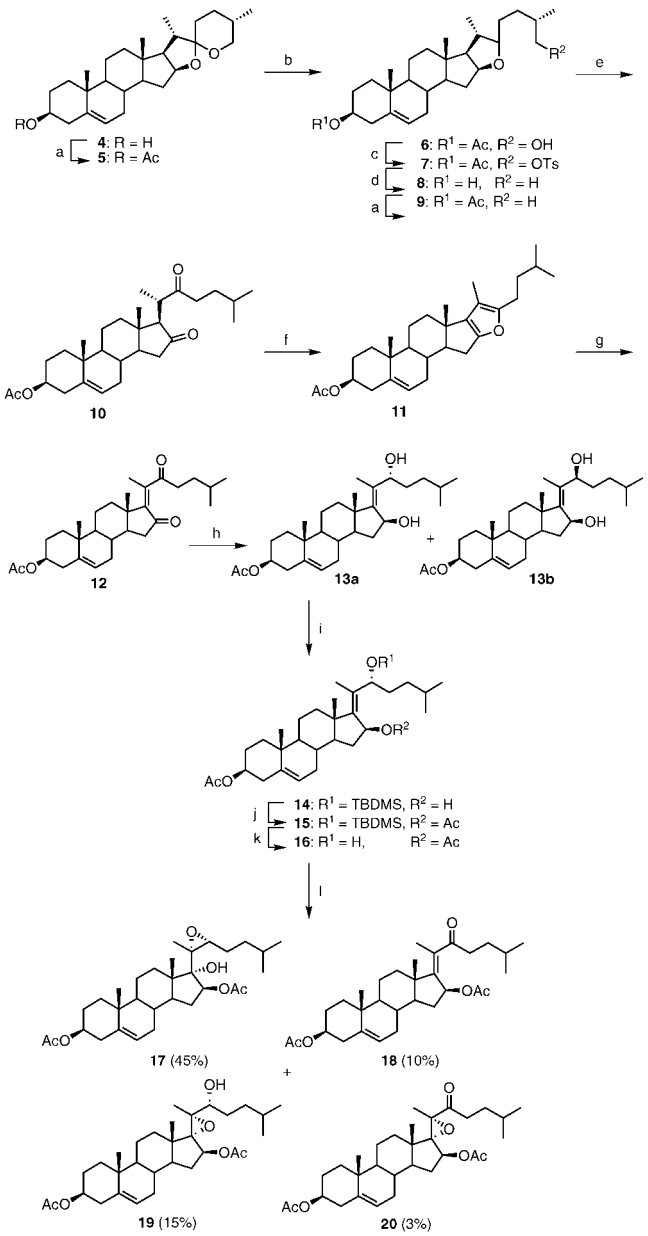

Scheme 1. Reagents and conditions: (a) Ac_2O, Py, RT, 99%; (b) $NaCNBH_3$, AcOH, CH_2Cl_2, RT, 94%; (c) TsCl, Py, DMAP, CH_2Cl_2, RT, 86%; (d) $LiAlH_4$, THF, RT, 78%; (e) $K_2Cr_2O_7$, AcOH, 70°C, 35%; (f) $HClO_4$, Ac_2O, benzene, RT, 76%; (g) *m*CPBA, CH_2Cl_2, RT, 86%; (h) $NaBH_4$, CH_3OH, RT, 82% (13a : 13b = 10 : 1); (i) TBDMSCl, imidazole, DMF, CH_2Cl_2, RT, 92%; (j) Ac_2O, Py, RT, 98%; (k) PPTS, CH_3OH, CH_2Cl_2, RT, 50%; (l) PDC, CH_2Cl_2, RT.

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(**5**) was achieved by reduction with sodium cyanoborohydride in acetic acid and dichloromethane to provide good yield of tetrahydrofuran **6**. Tosylation of **6** with *p*-toluenesulfonyl chloride in the presence of 4-(dimethylamino) pyridine (DMAP) in pyridine and dichloromethane yielded tosylate **7**. It was found that the ratio of pyridine and dichloromethane was crucial for this reaction. A ratio 3 : 5 of pyridine to dichloromethane was found to complete the reaction in 12 hr and also to reduce the formation of an ether by-product. Reduction of **7** with lithium aluminum hydride gave the desired 5-furosten-3β-ol (**8**). After reacetylation, oxidative opening of the tetrahydrofuran moiety of **8** to a diketone was accomplished by treatment with potassium dichromate in acetic acid at 70°C to give the 16,22-diketone **10** in moderate yield. Allylic oxidation at C-7 was found to be a significant side-reaction, but could be minimized by limiting the temperature to 70°C and controlling the volume of acetic acid. Under acidic conditions, the diketone **10** could be converted to the furan **11**, which then underwent autooxidation to provide the ene-dione **12**. The ene-dione **12** also could be produced from **11** with *m*-chloroperbenzoic acid (*m*CPBA) in dichloromethane. Sodium borohydride reduction of the ene-dione **12** in methanol gave two epimeric alcohols **13a** and **13b** in the ratio of about 10 : 1 (Sch. 1), which were assigned as the 22*R*- and 22*S*-epimer, respectively. The absolute configuration of the 22*S*-epimer was confirmed by x-ray crystallography. Treatment of the major 22*R*-epimer **13a** with *t*-butyldimethylchlorosilane (TBDMSCl) provided a selective protection of the hydroxyl group at C-22 in **14** in high yield. Protection of the hydroxyl group at C-16 of **14** as the acetate, followed by desilylation of the hydroxy at C-22 using pyridinium *p*-toluenesulfonate (PPTS) in MeOH and CH_2Cl_2, provided the allyl alcohol **16**. Oxidation of **16** was expected to provide the corresponding enone that could serve as an intermediate for the synthesis of the OSW-1 aglycone. Neutral oxidation condition with pyridinium dichromate (PDC) was chosen for this purpose. After treatment of **16** with PDC in CH_2Cl_2 for 10 hr, the products included the tertiary epoxy alcohol **17** (45%), the α,β-unsaturated ketone **18** (10%) and the epoxy alcohol **19** (15%), as well as the epoxy ketone **20** (3%), arising from **19**. It appeared that oxidation of the allylic hydroxyl group of **16** occurred at a slower rate than did epoxidation of the double bond. Thus, the enone **18** was formed in only a small amount (10%). The transformation of **16** into **17** involved rearrangement of $\Delta^{17(20)}$-22*R*-ol to the isomeric $\Delta^{20(22)}$-17α-ol system followed by epoxidation of the double bond; the tertiary hydroxyl group was left untouched. The epoxidation of the double bond presumably proceeded through formation of a chromate ester, followed by the transfer of an oxygen atom from this ester to the double bond. Thus, the epoxide moiety in **17** was formed on the same side of the molecule as the 17α-hydroxyl group. The epoxidation of double bond in the allylic

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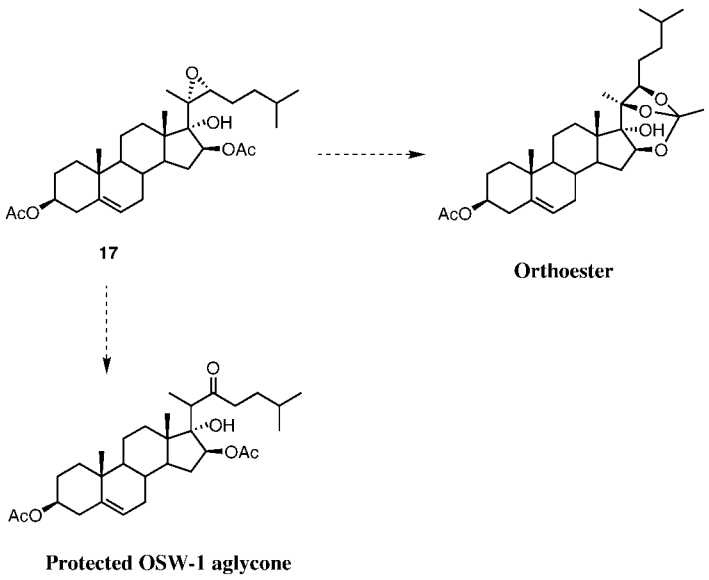
alcohol **16** to **19** could be explained in the same manner. Further oxidation of the secondary hydroxyl group of **19** provided the epoxy ketone **20**. To verify that the epoxy ketone **20** was formed by way of an epoxidation of the double bond in the allylic alcohol **16**, prior to the oxidation of the hydroxyl group, both the isolated epoxy alcohol **19** and the enone **18** were treated with PDC under the same condition as before. After 6 hr, only the epoxy alcohol **19** was converted to the epoxy ketone **20** in ~50% conversion as sole product, whereas no reaction occurred for the enone **18**. Similar epoxidations of other steroidal allylic alcohols with chromium trioxide have been reported by Glotter et al.[8] However, oxidation of **16** with *o*-iodoxybenzoic acid in 1,2-dichloroethane provided a high yield of the α,β-unsaturated ketone **18**.

The tertiary epoxy alcohol **17** could serve as a very useful intermediate for rearrangement to two classes of natural products: the protected OSW-1 aglycone and a hydroxy orthoester (Sch. 2). Treatment of **17** with boron trifluoride etherate ($BF_3{\cdot}OEt_2$) in dichloromethane at 0°C for a few minutes led to rearrangement to an [3.2.1] orthoester **22** in 80% yield with the same configuration as that reported in nature[4] (Sch. 3). A [2.2.1] orthoester **23**, which could be formed from the same intermediate, involving the tertiary 17α-hydroxy group, was not detected. Thus, the cyclization occurred



Scheme 2.

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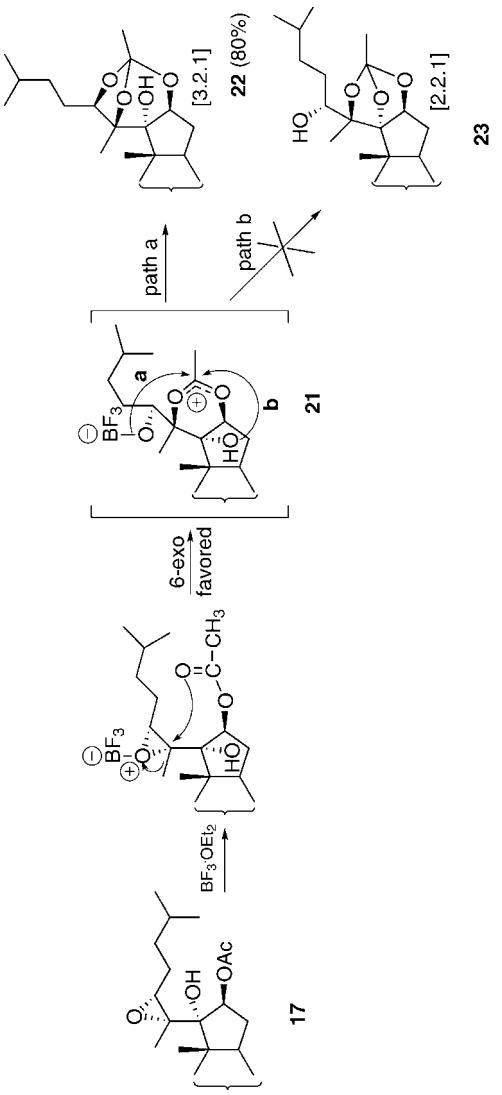

Scheme 3.

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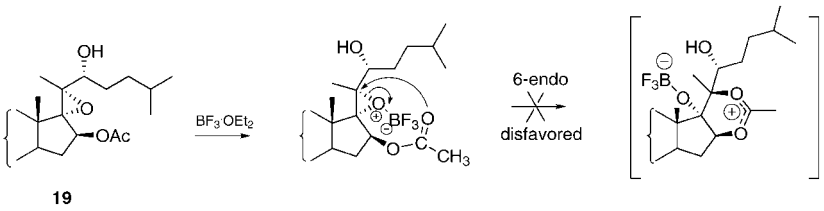

Scheme 4.

regio- and stereo-specifically. The epoxy alcohol **17** probably cyclized through energetically favored 6-exo route to form the dioxycarbocation **21**, thereby producing the more stable [3.2.1] bicyclic orthoester **22** as the only product (Sch. 3). The epoxy ester **19**, which could undergo a similar rearrangement, did not react and remained unchanged upon treatment either with $BF_3{\cdot}OEt_2$ or trifluoroacetic acid under the same conditions. When the reaction conditions were raised to room temperature, only complex mixtures were obtained, indicating that a 6-endo cyclization is a disfavored process (Sch. 4). The configurations at C-16 and C-22 of the orthoester **22** were established based on a nuclear overhauser effect between H-16 and H-22 and were found to have the reported natural configuration of such orthoesters. ^{1}H and ^{13}C NMR data of the relevant portions of **22** were in good agreement with those reported for orthoesterol B (**2**).[2]

Similar Lewis-acid-promoted rearrangement of α,β-epoxy acylates to the corresponding ketones and orthoesters have been intensively studied by Kita et al.[9] They have found that the dioxycarbocation intermediate was formed by neighboring group participation of acyloxy groups and that formation of such intermediates could be suppressed by a very strong electron-withdrawing group, such as 4-nitrobenzoyl group. Therefore, in order to make the desired epoxide-ketone rearrangement reaction applicable to OSW-1 system, the acetoxy group at C-16 in our β,γ-epoxy ester was replaced by a 4-nitrobenzoyl group in an attempt to minimize orthoester formation (Sch. 5). Surprisingly, even though containing a strong electron-withdrawing group, the epoxy 4-nitrobenzoate **26** rearranged into the orthobenzoate **27** with $BF_3{\cdot}OEt_2$ in a few minutes in high yield (95%). The epoxy 2,4-dinitrobenzoate **30** was also investigated and after 1 hr, the orthobenzoate **31** was obtained (25%) along with an unidentified product (20%), without any of the desired ketone product.

In conclusion, we report herein the facile synthesis of a tertiary epoxy alcohol from diosgenin, that provided a key intermediate which readily rearranged regio- and stereo-specifically to yield a steroidal orthoester.

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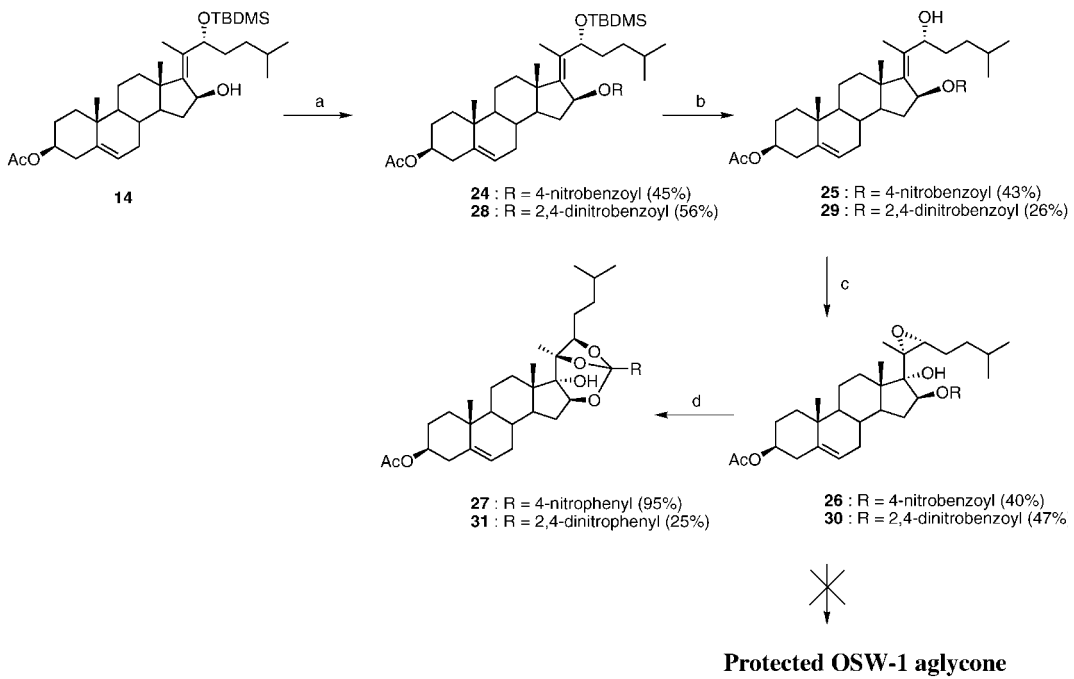

Scheme 5. Reagents and conditions: (a) 4-nitrobenzoic acid (or 2,4-dinitrobenzoic acid), DMAP, DCC, CH_2Cl_2, RT; (b) PPTS, MeOH, CH_2Cl_2, RT; (c) PDC, CH_2Cl_2, RT; (d) $BF_3{\cdot}OEt_2$, CH_2, Cl_2, 0°C.

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EXPERIMENTAL

The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrophotometer and on 400 MHz Brucker Advance DPX-400. Chemical shifts were recorded as δ values in ppm. Spectra were acquired in $CDCl_3$ unless otherwise stated. The peak due to residual $CHCl_3$ (7.26 ppm for ^{1}H and 77.23 ppm for ^{13}C) was used as the internal reference. Coupling constants (J) are given in Hz. Infrared (IR) spectra were recorded in cm^{-1} on a Perkin–Elmer 2000 Fourier transform infrared spectrophotometer. Mass spectra (MS) and accurate masses (HRMS) were obtained on a JEOL JMS-SX102 mass spectrometer and on a GC-MS-QP-5050A spectrometer in electron impact mode at 70 eV. Melting points (m.p.) were determined on a Mel-Temp electrothermal apparatus and are reported uncorrected in °C. Flash column chromatography was conducted using Merck silica gel 60 (mesh size 0.040–0.063 mm). Column chromatography was performed on Merck silica gel 60 (70–230 mesh), packed by the slurry method.

Diosgenin Acetate (5)

A solution of **4** (10 g, 0.024 mol), pyridine (110 mL), and acetic anhydride (10 mL, 0.105 mol) was stirred overnight (14 hr) at room temperature. The reaction was quenched by addition of ice and stirred for another 1 hr. The white precipitate was filtered to afford crude product **5** (10.95 g, 99.4%), which was used in the next step without further purification. Recrystallization of the crude product from ethyl acetate gave needles; m.p. 188.5–189°C. FTIR (KBr), ν_{max} 2940, 1720, 1235, 1045 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 5.36 (d, $J = 4.5$ Hz, 1H, C-6), 4.65–4.55 (m, 1H, C-3), 4.44–4.36 (m, 1H, C-16), 3.49–3.33 (m, 2H, C-26), 2.02 (s, 3H, AcO), 1.03 (s, 3H, H-18), 0.96 (d, $J = 6.9$ Hz, 3H, H-21), 0.78 (d, $J = 6.0$ Hz, 3H, C-27), 0.78 (s, 3H, C-19). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 170.7, 139.9, 122.6, 109.5, 81.0, 74.1, 67.0, 62.3, 56.6, 50.1, 41.8, 40.4, 39.9, 38.3, 37.2, 36.9, 32.2, 32.0, 31.6 (2C), 30.5, 29.0, 27.9, 21.6, 21.0, 19.5, 17.3, 16.5, 14.7 ppm. MS (FAB), m/z (relative intensity): 457 ($M^+ + 1$, 100), 397 (70), 313 (10), 282 (20), 253 (28), 154 (32), 91 (30), 55 (33). HRMS (FAB) calcd. for $C_{29}H_{45}O_4$ [$M^+ + 1$] 457.3318; found 457.3315.

(22β,25*R*)-3β-Acetoxyfurost-5-ene-26-ol (6)

To a stirred solution of **5** (10 g, 0.022 mol) in glacial acetic acid (100 mL) and dichloromethane (30 mL) was added sodium cyanoborohydride (4.2 g,

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0.066 mol) in portions at room temperature. After stirring for 8 hr, the reaction was quenched by addition of water and extracted with dichloromethane. The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution, then water, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (20% ethyl acetate/hexane) on silica gel to afford **6** (8.8 g, 88%); m.p. 105–106°C. FTIR (KBr), ν_{max} 3418, 2970, 1731, 1246, 1037 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.34 (d, $J = 4.2$ Hz, 1H, C-6), 4.58–4.55 (m, 1H, C-3), 4.31–4.24 (m, 1H, C-16), 3.49–3.36 (m, 2H, C-26), 3.33–3.27 (m, 1H, C-22), 2.00 (s, 3H, AcO), 1.00 (s, 3H, C-18), 0.97 (d, $J = 6.6$ Hz, 3H, C-21), 0.88 (d, $J = 6.6$ Hz, 3H, C-27), 0.78 (s, 3H, C-19). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 170.7, 139.8, 122.5, 90.5, 83.3, 74.0, 68.1, 65.2, 57.0, 50.1, 40.8, 39.5, 38.2, 38.0, 37.1, 36.8, 35.9, 32.4, 32.1, 31.7, 30.6, 30.3, 27.9, 21.6, 20.8, 19.5, 19.1, 16.8, 16.6 ppm. MS (FAB), m/z (relative intensity): 481 ($M^+ + Na$, 13), 459 ($M^+ + 1$, 55), 399 (25), 307 (25), 154 (100), 136 (75), 91 (25). HRMS (FAB) calcd. for $C_{29}H_{47}O_4$ [$M^+ + 1$] 459.3474; found 459.3481.

(22β,25*R*)-3β-Acetoxyfurost-5-ene-26-(p-toluenesulfonate) (7)

A solution of **6** (16.68 g, 0.036 mol), pyridine (60 mL), *p*-toluenesulfonyl chloride (10.42 g, 0.054 mol) and 4-(dimethylamino) pyridine (2.28 g, 0.018 mol) in dichloromethane (100 mL) was stirred at room temperature for 12 hr. The reaction was quenched by addition of water and extracted with additional dichloromethane. The combined organic layers were washed with 10% HCl, saturated aqueous $NaHCO_3$ solution, brine and water and dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (10% ethyl acetate/hexane) to provide **7** (20 g, 90%); m.p. 120–121°C. FTIR (KBr), ν_{max} 2940, 1731, 1362, 1239, 1179 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 7.77 (d, $J = 8.4$ Hz, 2H, C-2′ and C-6′), 7.33 (d, $J = 8.1$ Hz, 2H, C-3′ and C-5′), 5.36 (d, $J = 4.5$ Hz, 1H, C-6), 4.63–4.55 (m, 1H, C-3), 4.30–4.23 (m, 1H, C-16), 3.90–3.78 (m, 2H, C-26), 3.26–3.20 (m, 1H, C-22), 2.44 (s, 3H, C-7′), 2.02 (s, 3H, AcO), 1.03 (s, 3H, C-18), 0.96 (d, $J = 6.6$ Hz, 3H, C-21), 0.88 (d, $J = 6.6$ Hz, 3H, C-27), 0.77 (s, 3H, C-19). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 170.7, 144.8, 139.9, 133.2, 130 (2C), 128.1 (2C), 122.6, 90.1, 83.4, 75.2, 74.1, 65.2, 57.1, 50.2, 40.8, 39.6, 38.3, 38.1, 37.2, 36.9, 33.2, 32.4, 32.2, 31.8, 30.8, 30.0, 27.9, 21.8, 21.6, 20.8, 19.5, 19.1, 16.6(2C) ppm. MS (FAB), m/z (relative intensity): 635 ($M^+ + Na$, 10), 613 ($M^+ + 1$, 45), 307 (25), 154 (100), 136 (72), 91 (20). HRMS (FAB) calcd. for $C_{36}H_{53}O_6S$ [$M^+ + 1$] 613.3563; found 613.3566.

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(22β)-3β-Hydroxyfurost-5-ene (8)

To a cooled suspension of $LiAlH_4$ (70 mg, 1.65 mmol) in dry tetrahydrofuran was added a solution of **7** (340 mg, 0.55 mmol) in dry tetrahydrofuran. The mixture was stirred under N_2 at room temperature for 7 hr. The reaction was quenched by careful addition of ethyl acetate and water and extracted with dichloromethane. The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (30% ethyl acetate/hexane) to provide **8** (192 mg, 78%); m.p. 139–140°C. FTIR (KBr), ν_{max} 3455, 2955, 1052 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.34 (d, $J = 5.1$ Hz, 1H, C-6), 4.33–4.26 (m, 1H, C-16), 3.55–3.45 (m, 1H, C-3), 3.33–3.27 (m, 1H, C-22), 1.02 (s, 3H, C-19), 0.99 (d, $J = 6.9$ Hz, 3H, C-21), 0.88 (d, $J = 6.3$ Hz, 6H, C-26 and C-27), 0.80 (s, 3H, C-19). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 141.0, 121.7, 90.7, 83.3, 71.9, 65.4, 57.2, 50.3, 42.5, 40.9, 39.7, 38.1, 37.5, 36.8, 36.1, 32.5, 32.2, 31.8 (2C), 31.6, 28.5, 22.7, 22.7, 20.9, 19.6, 19.3, 16.6 ppm. MS (FAB), m/z (relative intensity): 399 ($M^+ - 1$, 15), 369 (100), 355 (12). HRMS (FAB) calcd. for $C_{27}H_{43}O_2$ [$M^+ - 1$] 399.3263; found 399.3261.

(22β)-3β-Acetoxyfurost-5-ene (9)

A solution of **8** (2.0 g, 5.0 mmol), pyridine (20 mL) and acetic anhydride (2 mL, 0.02 mol) was stirred overnight (14 hr) at room temperature. The reaction was quenched by addition of ice and stirred for another 1 hr. The white precipitate was filtered to afford crude product **9** (2.2 g, 99%), which was used in the next step without further purification. Recrystallization of the crude product from methanol gave fine needles; m.p. 133–134°C. FTIR (KBr), ν_{max} 2955, 1720, 1239, 1033 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.36 (d, $J = 4.5$ Hz, 1H, C-6), 4.61–4.57 (m, 1H, C-3), 4.33–4.26 (m, 1H, C-16), 3.33–3.26 (m, 1H, C-22), 2.02 (s, 3H, AcO), 1.03 (s, 3H, C-18), 0.99 (d, $J = 6.9$ Hz, 3H, C-21), 0.88 (d, $J = 6.3$ Hz, 6H, C-26 and C-27), 0.80 (s, 3H, C-19). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 170.7, 139.9, 122.6, 90.7, 83.3, 74.1, 65.4, 57.1, 50.2, 40.9, 39.6, 38.3, 38.1, 37.2, 36.9, 36.1, 32.4, 32.2, 31.8, 31.6, 28.5, 27.9, 22.8, 22.7, 21.6, 20.8, 19.5, 19.3, 16.6 ppm. MS (FAB), m/z (relative intensity): 441 ($M^+ - 1$, 45), 415 (100). HRMS (FAB) calcd. for $C_{29}H_{45}O_3$ [$M^+ - 1$] 441.3369; found 441.3340.

3β-Acetoxycholest-5-ene-16,22-dione (10)

A solution of **9** (1.0 g, 2.26 mmol) and potassium dichromate (1.67 g, 5.68 mmol) in glacial acetic acid (10 mL) was stirred at 70°C for 10 hr. The

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reaction was quenched by addition of H_2O and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution, then water and dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (5% ethyl acetate/hexane) to afford recovered **9** (300 mg, 30%) and the product **10** (413 mg, 40%, 57% based on recovered **9**) as white solid; m.p. 151.5–152.5°C. FTIR (KBr), ν_{max} 2955, 1731, 1709, 1239, 1033 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.37 (d, $J = 4.8$ Hz, 1H, C-6), 4.66–4.55 (m, 1H, C-3), 2.61 (d, $J = 4$ Hz, 1H), 2.75–2.50 (m, 3H) 2.03, (s, 3H, AcO), 1.04 (s, 3H, C-21), 1.04 (d, $J = 6.9$ Hz, 3H, C-18), 0.90 (d, $J = 5.7$ Hz, 6H, C-26 and C-27), 0.79 (s, 3H, C-19). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 218.2, 214.2, 170.7, 140.1, 122.0, 73.9, 66.3, 51.3, 49.8, 43,5, 41.9, 40.6, 38.8, 38.2, 37.4, 36.9 (2C), 32.4, 31.9, 31.1, 27.8 (2C), 22.7 (2C), 21.6, 20.7, 19.5, 15.6, 13.2 ppm. MS (DCI), m/z (relative intensity): 456 (M^+, 2), 441 (10), 396 (35), 340 (100), 325 (30), 297 (35). HRMS (FAB) calcd. for $C_{29}H_{45}O_4$ [$M^+ + 1$] 457.3318; found 457.3300.

3β-Acetoxyfurost-5,16,20(22)-triene (11)

To a stirred solution of **10** (835 mg, 1.83 mmol) in benzene (6 mL) was added 1.5 mL of a solution of acetic anhydride (3 mL) and $HClO_4$ (2 drops). After 0.5 h, the mixture was quenched by addition of water and extracted with dichloromethane. The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution, then water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (10% ethyl acetate/hexane) to provide **11** (568 mg, 71%). FTIR (KBr), ν_{max} 2947, 1735, 1254, 1033 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.40 (d, $J = 4.8$ Hz, 1H, C-6), 4.61 (m, 1H, C-3), 2.04, (s, 3H, AcO), 1.90 (s, 3H, C-21), 1.08 (s, 3H, C-18), 0.91 (s, 3H, C-19), 0.91 (d, $J = 6.0$ Hz, 6H, C-26 and C-27). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 170.7, 155.0, 153.9, 140.2, 136.8, 122.4, 111.4, 74.0, 60.6, 50.8, 41.2, 38.3, 38.1, 37.2, 37.0, 35.6, 31.9, 30.4, 27.9, 27.9, 26.8, 24.6, 22.6, 22.6, 21.6, 20.6, 19.4, 18.1, 9.0 ppm. MS (DCI), m/z (relative intensity): 438 (M^+, 100), 423 (60), 381 (25), 363 (10), 91 (10), 43 (25). HRMS (FAB) calcd. for $C_{29}H_{42}O_3$ [M^+]438.3134; found: 438.3154.

3β-Acetoxycholesta-5,17(20)-diene-16,22-dione (12)

To a stirred solution of **11** (447 mg, 1.02 mmol) in CH_2Cl_2 (10 mL) was added *m*CPBA (184 mg, 1.22 mmol). After 1 hr, the mixture was quenched by

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addition of aqueous 0.5 M sodium thiosulfate solution (5 mL) and extracted with dichloromethane. The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution, then water, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (10% ethyl acetate/hexane) to provide **12** (390 mg, 84%) as white solid with m.p. 136–137°C. FTIR (KBr), ν_{max} 2947, 1731, 1716, 1697, 1242, 1030 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.37 (d, $J = 4.5$ Hz, 1H, C-6), 4.60 (m, 1H, C-3), 2.03, (s, 3H, AcO), 1.93 (s, 3H, C-21), 1.10 (s, 3H, C-18), 1.06 (s, 3H, C-19), 0.89 (d, $J = 6.0$ Hz, 6H, C-26 and C-27). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 211.5, 205.7, 170.7, 145.6, 142.3, 140.1, 122.0, 73.9, 50.8, 49.8, 43.5, 39.2, 38.2, 37.9, 36.9, 36.8, 36.2, 32.5, 31.7, 30.9, 27.9, 27.8, 22.6, 22.6, 21.6, 20.9, 19.5, 17.2, 16.0 ppm. MS (CDI), m/z (relative intensity): 454 (M^+, 90), 439 (20), 394 (100), 383 (50), 323 (45), 183 (20), 43 (20). MS (FAB), m/z (relative intensity): 477 ($M^+ + Na$, 15), 455 ($M^+ + 1$, 100), 395 (20). HRMS (FAB) calcd. for $C_{29}H_{42}O_4$ [M^+] 454.3083; found: 454.3093.

(22*R*)-3β-Acetoxycholesta-5,17(20)-diene-16β,22-diol (13a) and (22*S*)-3β-Acetoxycholesta-5,17(20)-diene-16β,22-diol (13b)

To a solution of **12** (417 mg, 0.92 mmol) in methanol (8 mL) was added $NaBH_4$ (70 mg, 1.85 mmol) and the mixture was stirred at room temperature for 0.5 hr. The reaction was quenched by addition of water to destroy the excess $NaBH_4$. To the mixture was added saturated aqueous NaCl solution followed by extraction with dichloromethane. The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (15% ethyl acetate/hexane) to provide **13a** (317 mg, 75%) and **13b** (30 mg, 7%) and a mixture of **13a** and **13b** (25 mg, 6%); **13a** and **13b** were white solid with m.p. 137–138°C and 180.5–181.5°C, respectively.

13a: FTIR (KBr), ν_{max} 3402, 3305, 2947, 1723, 1261, 1205, 1037 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.40 (d, $J = 4.7$ Hz, 1H, C-6), 4.76 (t, $J = 7.2$ Hz, 1H, C-16), 4.67–4.59 (m, 1H, C-3), 4.55 (dd, $J = 4.1$, 8.8 Hz, 1H, C-22), 2.06 (s, 3H, AcO), 1.77 (s, 3H, C-21), 1.07 (s, 3H, C-18), 1.06 (s, 3H, C-19), 0.92 (d, $J = 6.6$ Hz, 6H, C-26 and C-27). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 170.8, 148.6, 139.9, 133.0, 122.4, 74.0, 73.9, 72.2, 52.1, 49.8, 44.7, 38.2, 37.7, 37.0, 36.8, 33.5, 31.8, 31.2, 29.9, 28.3, 27.9, 23.0, 22.7, 21.6, 21.2, 19.4, 17.2, 13.1 ppm. MS (EI), m/z (relative intensity): 440 ($M^+ - H_2O$, 1), 167 (22), 109 (26), 95 (23), 43 (100). HRMS (FAB) calcd. for $C_{29}H_{46}O_4$ $[m]^+$ 458.3396; found: 458.3372.

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13b: FTIR (KBr), ν_{max} 3298, 2940, 1727, 1466, 1436, 1365, 1242, 1048, 1026 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.40 (d, $J = 4.8$ Hz, 1H, C-6), 4.87 (t, $J = 7.4$ Hz, 1H, C-16), 4.67–4.59 (m, 1H, C-3), 4.50 (t, $J = 7.1$ Hz, 1H, C-22), 2.07 (s, 3H, AcO), 1.74 (s, 3H, C-21), 1.10 (s, 3H, C-19), 1.06 (s, 3H, C-18), 0.92 (d, $J = 6.6$ Hz, 6H, C-26 and C-27). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.3, 151.9, 140.4, 133.1, 122.8, 74.5, 73.1, 73.0, 52.8, 50.4, 44.6, 38.7, 37.8, 37.4, 37.2, 35.8, 35.7, 32.2, 32.0, 31.4, 28.8, 28.3, 23.4, 23.2, 22.1, 21.6, 19.9, 17.3, 12.7 ppm. MS (EI), m/z (relative intensity): 440 ($M^+ - H_2O$, 1), 167 (18), 109 (17), 95 (17), 43 (100).

(22*R*)-3β-Acetoxy-22-tert-butyldimethylsilyloxycholesta-5,17(20)-diene-16β-ol (14)

A solution of **13a** (420 mg, 0.92 mmol), *t*-butyldimethylchlorosilane (420 mg, 2.76 mmol) and imidazole (155 mg, 2.3 mmol) in *N*,*N*-dimethylformamide (4.2 mL) and dichloromethane (8 mL) was stirred at room temperature for 1 hr. The reaction was quenched by addition of water and extracted with dichloromethane. The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (2.5% ethyl acetate/hexane) to provide **14** (495 mg, 94.4%) as amorphous white solid; m.p. 63–64°C. FTIR (KBr), ν_{max} 3477, 2947, 1735, 1720, 1240, 1030, 832, 722 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.41 (d, $J = 4.7$ Hz, 1H, C-6), 4.69 (t, $J = 7.0$ Hz, 1H, C-16), 4.67–4.58 (m, 1H, C-3), 4.46 (dd, $J = 9.8$, 2.2 Hz, 1H, C-22), 2.06 (s, 3H, AcO), 1.73 (s, 3H, C-21), 1.06 (s, 3H, C-18), 1.05 (s, 3H, C-19), 0.92 (d, $J = 6.6$ Hz, 6H, C-26 and C-27), 0.90 (s, 9H, $-C(CH_3)_3$), 0.06 (s, 3H $-SiCH_3$), 0.00 (s, 3H, $-SiCH_3$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.2, 146.9, 140.4, 135.6, 122.8, 74.5, 74.0, 73.0, 52.5, 50.2, 44.9, 38.7, 37.9, 37.4, 37.2, 36.3 (2C), 35.0, 32.2, 31.5, 28.5, 28.3, 26.4 (3C), 23.5, 23.1, 22.0, 21.6, 19.9, 18.8, 17.7, 13.3, −3.8, −4.2 ppm. MS (EI), m/z (relative intensity): 572 (M^+, 1), 515 (2), 501 (28), 213 (7), 167 (20), 75 (100), 57 (24), 43 (78).

(22*R*)-22-tert-Butyldimethylsilyloxy-3β,16β-diacetoxycholesta-5,17(20)-diene (15)

A solution of **14** (100 mg, 0.17 mmol), acetic anhydride (0.1 mL, 0.10 mmol) in pyridine (1 mL) was stirred at room temperature for 12 hr. The reaction was poured into ice and stirred for the additional 1 hr. The precipitated white solid was filtered and washed with water for several times to

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afford the crude product **15** in quantitative yield, which could be used in the next step without purification. Recrystallization from hexane gave needles; m.p. 195–196°C. FTIR (KBr), ν_{max} 1731, 1250, 1235, 1033, 832, 769 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.65 (t, $J = 7.3$ Hz, 1H, C-16), 5.40 (d, $J = 4.4$ Hz, 1H, C-6), 4.66–4.59 (m, 1H, C-3), 4.03 (dd, $J = 9.9$, 2.0 Hz, 1H, C-22), 2.07 (s, 3H, AcO), 2.04 (s, 3H, AcO), 1.75 (s, 3H, C-21), 1.06 (s, 3H, C-18), 1.03 (s, 3H, C-19), 0.90 (d, $J = 6.6$ Hz, 6H, C-26 and C-27), 0.89 (s, 9H, $-C(CH_3)_3$), 0.04 (s, 3H, $-SiCH_3$), 0.00 (s, 3H, $-SiCH_3$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.2, 171.1, 141.3, 140.3, 136.6, 122.9, 74.9, 74.5, 74.0, 52.6, 50.2, 44.9, 38.6, 38.0, 37.4, 37.2, 36.7, 34.6, 33.6, 32.0, 31.6, 29.2, 28.3, 26.4 (3C), 23.5, 23.4, 22.1, 21.9, 21.6, 19.9, 18.8, 16.9, 12.8, −3.8, −4.3 ppm. MS (EI), m/z (relative intensity): 557 ($M^+ - C_4H_9$, 1), 117 (37), 73 (43), 55 (15), 43 (100).

(22*R*)-3β,16β-Diacetoxycholesta-5,17(20)-diene-22-ol (16)

A solution of **15** (100 mg, 0.16 mmol) and pyridinium *p*-toluenesulfonate (35 mg, 0.14 mmol) in methanol (6 mL) and dichloromethane (3 mL) was stirred at room temperature for 24 hr. The reaction was quenched by addition of saturated aqueous NaCl solution and extracted with dichloromethane. The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (5% and 10% ethyl acetate/hexane) to provide both recovered **15** (39 mg, 39%) and the product **16** (40 mg, 49%, 80% based on recovered **15**) as a white solid; m.p. 154.5–155.5°C. FTIR (KBr), ν_{max} 3498, 2944, 1730, 1712, 1465, 1373, 1251, 1240, 1030 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.63 (t, $J = 7.0$ Hz, 1H, C-16), 5.39 (d, $J = 5.0$ Hz, 1H, C-6), 4.66–4.57 (m, 1H, C-3), 4.18–4.15 (m, 1H, C-22), 2.05 (s, 3H, AcO), 2.02 (s, 3H, AcO), 1.78 (s, 3H, C-21), 1.04 (s, 3H, C-19), 1.03 (s, 3H, C-18), 0.91 (d, $J = 6.5$ Hz, 6H, C-26 and C-27). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 170.5, 170.4, 142.8, 139.7, 134.3, 122.2, 74.2, 73.8, 72.8, 52.0, 49.6, 44.4, 38.0, 37.4, 36.8, 36.5, 35.7, 33.0, 31.4 (2C), 31.1, 28.6, 27.7, 22.8, 22.7, 21.4, 21.3, 21.0, 19.2, 16.2, 11.5 ppm. MS (EI), m/z (relative intensity): 482 ($M^+ - H_2O$, 1), 440 (1), 380 (1), 298 (2), 95 (12), 81 (15), 43 (100).

Oxidation of (22*R*)-3β,16β-Diacetoxycholesta-5,17(20)-diene-22-ol (16)

A solution of **16** (117 mg, 0.23 mmol) and pyridinium dichromate (264 mg, 0.70 mmol) in CH_2Cl_2 (4 mL) was stirred at room temperature for

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10 hr. Then the reaction mixture was filtered through Celite and eluted with dichloromethane. The filtrate was washed with saturated aqueous $NaHCO_3$ solution, and then water, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (5% ethyl acetate/hexane) to give **17** (53 mg, 45%), **18** (12 mg, 10%), **19** (18 mg, 15%) and **20** (4 mg, 3%).

17: FTIR (KBr), ν_{max} 3500, 2947, 1735, 1256, 1033 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.40 (d, $J = 5.2$ Hz, 1H, C-6), 4.91 (dd, $J = 8.0$, 5.1 Hz, 1H, C-16), 4.63-4.61 (m, 1H, C-3), 3.14 (t, $J = 6.2$, 1H, C-22), 2.74 (s, 1H, OH), 2.07 (s, 3H, AcO), 2.05 (s, 3H, AcO), 1.45 (s, 3H, C-21), 1.09 (s, 3H, C-18), 1.06 (s, 3H, C-19), 0.92 (d, $J = 6.6$ Hz, 6H, C-26 and C-27). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 170.5, 169.6, 139.6, 122.3, 83.2, 82.2, 73.8, 62.3, 60.2, 49.5, 48.3, 47.1, 38.1, 36.9, 36.6, 35.4, 33.5, 31.6, 31.4, 31.1, 27.9, 27.7, 25.6, 22.6, 22.3, 21.4, 21.3, 20.1, 19.3, 17.3, 15.8 ppm. MS (EI), m/z (relative intensity): 456 (M^+ − HOAc, 1), 296 (2), 214 (4), 105 (7), 81 (12), 55 (15), 43 (100).

18: FTIR (KBr), ν_{max} 2940, 1735, 1727, 1690, 1231 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.66–5.61 (m, 1H, C-16), 5.40 (d, $J = 4.9$ Hz, 1H, C-6), 4.66–4.58 (m, 1H, C-3), 2.60–2.30 (m, 6H), 2.06 (s, 3H, AcO), 1.99 (s, 3H, AcO), 1.98 (d, $J = 1.6$ Hz, 3H, C-21), 1.09 (s, 3H, C-18), 1.05 (s, 3H, C-19), 0.92 (d, $J = 6.5$ Hz, 3H, C-26), 0.91 (d, $J = 6.5$ Hz, 3H, C-27). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 208.3, 170.5, 170.4, 148.7, 139.6, 133.3, 122.2, 74.4, 73.8, 51.3, 49.6, 44.7, 39.1, 38.0, 36.8, 36.6 (2C), 32.6, 32.2, 31.4, 30.7, 27.8, 27.7, 22.5, 22.4, 21.4, 20.9 (2C), 19.3, 16.1, 15.2 ppm. MS (EI), m/z (relative intensity): 498 (M^+, 1), 438 (1), 378 (2), 181 (3), 91 (7), 81 (11), 55 (11), 43 (100).

19: FTIR (KBr), ν_{max} 3462, 2947, 1735, 1246, 1231, 1037 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.39 (d, $J = 5.2$ Hz, 1H, C-6), 4.89 (dd, $J = 8.4$, 5.4 Hz, 1H, C-16), 4.66–4.68 (m, 1H, C-3), 3.16 (m, 1H, C-22), 2.05 (s, 6H, 2(AcO)), 1.44 (s, 3H, C-21), 1.07 (s, 3H, C-18), 1.04 (s, 3H, C-19), 0.93 (d, $J = 6.3$ Hz, 3H, C-26), 0.92 (d, $J = 6.3$ Hz, 3H, C-27). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 170.5, 169.4, 139.6, 122.0, 80.1, 79.2, 76.0, 73.7, 67.9, 50.9, 49.2, 42.3, 38.0, 36.8, 36.4, 35.9, 34.0, 31.4, 31.1 (2C), 30.9, 28.7, 27.6, 22.8, 22.5, 21.4, 21.1, 20.4, 19.3, 13.9, 12.4 ppm. MS (EI), m/z (relative intensity): 456 (M^+ − HOAc, 9), 296 (6), 270 (19), 105 (15), 81 (20), 55 (20), 43 (100).

20: FTIR (KBr), ν_{max} 2932, 1735, 1709, 1246, 1041 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.39 (d, $J = 4.7$ Hz, 1H, C-6), 4.93 (dd, $J = 8.0$, 6.6 Hz, 1H, C-16), 4.66–4.58 (m, 1H, C-3), 2.05 (s, 3H, AcO), 1.95 (s, 3H, AcO), 1.60 (s, 3H, C-21), 1.10 (s, 3H, C-18), 1.04 (s, 3H, C-19), 0.88 (d, $J = 5.7$ Hz, 6H, C-26 and C-27). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 210.4, 171.1, 170.2, 140.3, 122.5, 78.9, 78.4, 74.3, 67.9, 51.8, 49.9, 43.0, 38.6, 37.4, 37.3, 37.1, 34.6, 34.0, 32.7, 31.8, 31.7, 28.3, 28.2, 23.0, 22.9, 22.0, 21.0, 20.9, 19.9, 16.8, 14.5 ppm.

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MS (EI), m/z (relative intensity): 454 (M^+ − HOAc, 1), 295 (2), 295 (3), 157 (5), 91 (7), 81 (12), 55 (11), 43 (100).

3β,16β-Diacetoxycholesta-5,17(20)-diene-22-one (18)

A solution of **16** (30 mg, 0.06 mmol) and *o*-iodoxybenzoic acid (50 mg, 0.18 mmol) in 1,2-dichloroethane (2 mL) was refluxed for 1.5 hr. After cooling down, the reaction mixture was filtered through Celite and eluted with ethyl acetate. The filtrate was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (5% ethyl acetate/hexane) to give the desired product **18** (23.6 mg, 80%).

(20*S*,22*R*)-3β-Acetoxy-17α-hydroxycholesta-5-ene-16β,20, 22-orthoacetate (22)

To a solution of **17** (50 mg, 0.10 mmol) in CH_2Cl_2 (2 mL) at 0°C was added $BF_3{\cdot}OEt_2$ (2 drops). After stirring at 0°C for a few minutes, the reaction mixture was quenched by addition of water and extracted with dichloromethane. The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution, then water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (2.5% ethyl acetate/hexane) to give **22** (40 mg, 80%) as white solid with m.p. 232–232.5°C. FTIR (KBr), ν_{max} 3537, 2947, 1723, 1246, 1033 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.38 (d, $J = 4.5$ Hz, 1H, C-6), 4.65–4.55 (m, 1H, C-3), 4.16 (dd, $J = 7.9$, 3.7 Hz, 1H, C-16), 3.52 (dd, $J = 9.7$, 3.6 Hz, 1H, C-22), 2.03 (s, 3H, AcO), 1.38 (s, 3H, C-21), 1.13 (s, 3H, C-18), 1.06 (s, 3H, C-19), 0.90 (d, $J = 6.4$ Hz, 6H, C-26 and C-27). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 170.6, 139.6, 122.3, 116.7, 90.2, 83.7, 78.5, 77.8, 73.8, 49.8, 49.5, 47.4, 38.0, 37.4, 37.0, 36.6, 32.4, 31.8 (2C), 31.5, 28.3, 28.0, 27.7, 22.6, 22.5 (2C), 21.4, 20.3, 19.3, 17.2, 15.2 ppm. MS (EI), m/z (relative intensity): 456 (M^+ − HOAc, 1), 396 (1), 214 (45), 139 (53), 91 (22), 55 (100), 69 (72), 79 (20).

(22*R*)-3β-Acetoxy-22-tert-butyldimethylsilyloxy-16β-4-nitrobenzoylcholesta-5,17(20)-diene (24)

To a stirred solution of **14** (163 mg, 0.28 mmol), 4-nitrobenzoic acid (62 mg, 0.37 mmol) and 4-(dimethylamino) pyridine (10.4 mg, 0.09 mmol) in

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dichloromethane was added dicyclohexylcarbodiimide (76.5 mg, 0.37 mmol) at room temperature. After a few minutes, a white solid precipitated from the solution. The suspension was stirred at room temperature for 24 hr. The precipitated white solid was filtered and the filtrate was extracted with dichloromethane. The combined organic layers were washed with saturated aqueous NH_4Cl solution, then water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (hexane) to provide **24** (94 mg, 45%, 73% based on recovered **14**) as white solid; m.p. 128–129°C. FTIR (KBr), ν_{max} 2952, 1731, 1720, 1530, 1276, 1242, 1074, 1030, 832, 716 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.25 (d, $J = 8.8$ Hz, 2H, C-3′ and C-5′), 8.15 (d, $J = 8.8$ Hz, 2H, C-2′ and C-6′), 5.99 (t, $J = 7.1$ Hz, 1H, C-16), 5.36 (d, $J = 4.2$ Hz, 1H, C-6), 4.65–4.50 (m, 1H, C-3), 4.02 (d, $J = 8.8$ Hz, 1H, C-22), 2.50–2.57 (m, 1H, C-15), 2.01 (s, 3H, AcO), 1.74 (s, 3H, C-21), 1.10 (s, 3H, C-18), 1.02 (s, 3H, C-19), 0.84 (s, 9H, $-C(CH_3)_3$), 0.54 (d, $J = 6.5$ Hz, 3H, C-26), 0.32 (d, $J = 6.5$ Hz, 3H, C-27), 0.00 (s, 3H, $-SiCH_3$), -0.04 (s, 3H, $-SiCH_3$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.1, 164.4, 151.1, 141.0, 140.4, 137.7, 136.5, 131.3 (2C), 124.1 (2C), 122.7, 76.1, 74.4, 74.0, 52.8, 50.2, 45.1, 38.7, 38.0, 37.4, 37.2, 36.4, 34.8, 33.7, 32.0, 31.8, 28.7, 28.3, 26.4 (3C), 23.0, 22.9, 22.0, 21.6, 19.9, 18.7, 17.1, 12.8, -3.8, -4.3 ppm.

(22*R*)-3β-Acetoxy-16β-4-nitrobenzoylcholesta-5,17(20)-diene-22-ol (25)

A solution of 24 (87 mg, 0.12 mmol) and pyridinium *p*-toluenesulfonate (23 mg, 0.09 mmol) in methanol (6 mL) and dichloromethane (2 mL) was stirred at room temperature for 20 hr. The reaction was quenched by addition of saturated aqueous NaCl solution and extracted with dichloromethane. The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (hexane and 10% ethyl acetate/hexane) to provide both recovered **24** (35.8 mg, 41%) and the product **25** (31.4 mg, 43%, 73% based on recovered **24**) as white solid; m.p. 170–171°C. FTIR (KBr), ν_{max} 3564, 2952, 1731, 1716, 1528, 1277, 1244, 1030 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.26 (d, $J = 8.8$ Hz, 2H, C-3′ and C-5′), 8.14 (d, $J = 8.8$ Hz, 2H, C-2′ and C-6′), 5.99 (t, $J = 7.0$ Hz, 1H, C-16), 5.35 (d, $J = 4.8$ Hz, 1H, C-6), 4.65–4.50 (m, 1H, C-3), 4.16 (d, $J = 7.7$ Hz, 1H, C-22), 2.56–2.47 (m, 1H, C-15), 2.01 (s, 3H, AcO), 1.79 (s, 3H, C-21), 1.11 (s, 3H, C-18), 1.02 (s, 3H, C-19), 0.58 (d, $J = 6.5$ Hz, 3H, C-26), 0.40 (d, $J = 6.5$ Hz, 3H, C-27). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 170.7, 163.9, 150.6, 142.5, 139.8, 135.8, 135.3, 130.6 (2C), 123.6 (2C), 122.1, 75.6, 73.8, 72.8,

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52.1, 49.6, 44.6, 38.0, 37.2, 36.8, 36.6, 35.6, 33.3, 33.0, 31.5, 31.2, 28.2, 27.8, 22.3, 22.2, 21.4, 21.0, 19.3, 16.5, 11.6 ppm.

(20*R*)-3β-Acetoxy-16β-4-nitrobenzoyl-20,22-epoxycholesta-5-ene-17α-ol (26)

A solution of **25** (23 mg, 0.04 mmol) and pyridinium dichromate (43 mg, 0.12 mmol) in dichloromethane (2 mL) was stirred at room temperature for 3 hr. Then the reaction mixture was filtered through Celite and eluted with dichloromethane. The filtrate was washed with saturated aqueous $NaHCO_3$ solution, then water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (5% ethyl acetate/hexane) to give **26** (9 mg, 38%) as white solid; m.p. 174–175°C. FTIR (KBr), ν_{max} 3440, 3104, 2955, 1731, 1705, 1522, 1268, 1238, 1033 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.35 (d, $J = 9.0$ Hz, 2H, C-3′ and C-5′), 8.28 (d, $J = 9.0$ Hz, 2H, C-2′ and C-6′), 5.41 (d, $J = 4.8$ Hz, 1H, C-6), 5.26 (dd, $J = 8.1$, 4.9 Hz, 1H, C-16), 4.68–4.60 (m, 1H, C-3), 3.14 (t, $J = 6.1$ Hz, 1H, C-22), 2.57–2.50 (m, 2H, C-15), 2.06 (s, 3H, AcO), 1.43 (s, 3H, C-21), 1.18 (s, 3H, C-18), 1.07 (s, 3H, C-19), 0.82 (d, $J = 6.6$ Hz, 3H, C-26), 0.81 (d, $J = 6.6$ Hz, 3H, C-27). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.2, 164.3, 151.2, 140.3, 136.3, 131.4 (2C), 124.4 (2C), 122.8, 84.6, 83.8, 74.4, 63.0, 60.4, 50.1, 49.3, 47.5, 38.7, 37.6, 37.2, 36.0, 34.1, 32.2 (2C), 31.7, 28.4, 28.3, 26.3, 23.1, 22.8, 22.0, 20.8, 19.9, 17.8, 15.9 ppm.

(20*S*,22*R*)-3β-Acetoxy-17α-hydroxycholesta-5-ene-16β,20,22-ortho-4-nitrobenzoate (27)

To a solution of **26** (9 mg, 0.014 mmol) in CH_2Cl_2 (1 mL) at 0°C was added BF_3 OEt_2 (1 drop), followed by stirring at 0°C for a few minutes. The reaction mixture was quenched by addition of water and extracted with dichloromethane. The combined organic layer were washed with saturated aqueous $NaHCO_3$ solution, then water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (10% ethyl acetate/hexane) to give **27** (8.3 mg, 95%) as white solid; m.p. 234–235°C. FTIR (KBr), ν_{max} 3522, 2947, 1731, 1522, 1257, 1033 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.25 (d, $J = 9.0$ Hz, 2H, C-3′ and C-5′), 7.84 (d, $J = 9.0$ Hz, 2H, C-2′ and C-6′), 5.42 (d, $J = 4.5$ Hz, 1H, C-6), 4.68–4.60 (m, 1H, C-3), 4.42 (dd, $J = 8.0$, 3.4 Hz, 1H, C-16), 3.77 (dd, $J = 9.8$, 3.7 Hz, 1H, C-22), 2.07 (s, 3H, AcO), 1.50 (s, 3H, C-21), 1.25 (s, 3H, C-18), 1.10 (s, 3H, C-19), 0.96 (d, $J = 6.4$ Hz, 6H, C-26 and C-27). ^{13}C

NMR ($CDCl_3$, 100 MHz) δ: 171.2, 149.1, 144.2, 140.3, 127.9 (2C), 123.9 (2C), 122.8, 115.7, 91.8, 85.7, 79.4 (2C), 74.4, 50.0, 50.2, 48.2, 38.7, 38.0, 37.6, 37.2, 33.0, 32.4 (2C), 32.2, 28.9, 28.4, 28.3, 23.2, 23.1, 22.0, 20.9, 19.9, 17.8, 15.9 ppm.

(22*R*)-3β-Acetoxy-22-tert-butyldimethylsilyloxy-16β-2,4-dinitrobenzoylcholesta-5,17(20)-diene (28)

To a stirred solution of **27** (280 mg, 0.49 mmol), 2,4-dinitrobenzoic acid (210 mg, 0.99 mmol) and 4-(dimethylamino) pyridine (30 mg, 0.25 mmol) in dichloromethane was added dicyclohexylcarbodiimide (200 mg, 0.98 mmol) at room temperature. After a few minutes, a white solid precipitated from the solution. The suspension was stirred at room temperature for 9 hr. The precipitated white solid was filtered and the filtrate was extracted with dichloromethane. The combined organic layers were washed with saturated aqueous NH_4Cl solution, then water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (10% ethyl acetate/hexane) to provide **28** (215 mg, 56%). FTIR (KBr), ν_{max} 3105, 2947, 1731, 1548, 1541, 1280, 1242, 1074, 1030, 832, 772 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.72 (d, $J = 2.2$ Hz, 1H, C-3′) 8.52 (dd, $J = 8.4$, 2.2 Hz, 1H, C-5′), 8.02 (d, $J = 8.4$ Hz, 1H, C-6′), 5.90 (t, $J = 7.2$ Hz, 1H, C-16), 5.42 (d, $J = 4.5$ Hz, 1H, C-6), 4.67–4.59 (m, 1H, C-3), 4.08 (dd, $J = 9.9$, 2.5 Hz, 1H, C-22), 2.62–2.55 (m, 1H, C-15), 2.07 (s, 3H, AcO), 1.74 (d, $J = 0.9$ Hz, 3H, C-21), 1.07 (s, 3H, C-18), 1.04 (s, 3H, C-19), 0.89 (s, 9H, $C(CH_3)_3$), 0.64 (d, $J = 6.6$ Hz, 3H, C-26), 0.52 (d, $J = 6.6$ Hz, 3H, C-27), 0.07 (s, 3H, -$SiCH_3$), 0.01 (s, 3H, -$SiCH_3$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.2, 163.3, 149.7, 149.5, 140.5, 140.2, 138.2, 132.9, 132.3, 127.5, 122.7, 120.0, 78.6, 74.4, 73.8, 52.6, 50.2, 45.0, 38.6, 37.9, 37.4, 37.2, 36.5, 34.6, 32.4, 32.0, 31.6, 28.9, 28.3, 26.4 (3C), 23.1, 23.0, 22.0, 21.6, 19.9, 18.8, 16.7, 12.8, −3.8, −4.3 ppm.

(22*R*)-3β-Acetoxy-16β-2,4-dinitrobenzoylcholesta-5,17(20)-diene-22-ol (29)

A solution of **28** (194 mg, 0.25 mmol) and pyridinium *p*-toluenesulfonate (49 mg, 0.20 mmol) in methanol (15 mL) and dichloromethane (5 mL) was stirred at room temperature for 36 hr. The reaction was quenched by addition of saturated aqueous NaCl solution and extracted with dichloromethane. The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (5% ethyl acetate/hexane and 10% ethyl

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acetate/hexane) to provide both recovered **28** (110 mg, 56.7%) and the product **29** (42 mg, 26%, 60% based on recovered **28**), the latter as a white solid; m.p. 134–135°C. FTIR (KBr), ν_{max} 3447, 3104, 2947, 1727, 1544, 1283, 1246, 1030 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.73 (d, $J = 2.1$ Hz, 1H, C-3′), 8.54 (dd, $J = 8.4$, 2.2 Hz, 1H, C-5′), 8.02 (d, $J = 8.4$ Hz, 1H, C-6′), 5.91 (t, $J = 7.2$ Hz, 1H, C-16), 5.42 (d, $J = 4.6$ Hz, 1H, C-6), 4.68-4.60 (m, 1H, C-3), 4.21 (dd, $J = 9.1$, 3.0 Hz, 1H, C-22), 2.63-2.57 (m, 1H, C-15), 2.07 (s, 3H, AcO), 1.78 (d, $J = 0.8$ Hz, 3H, C-21), 1.08 (s, 3H, C-18), 1.04 (s, 3H, C-19), 0.65 (d, $J = 6.4$ Hz, 3H, C-26), 0.55 (d, $J = 6.5$ Hz, 3H, C-27). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.2, 163.4, 149.7, 149.4, 142.5, 140.4, 136.2, 133.0, 132.2, 127.6, 122.7, 120.1, 78.6, 74.4, 73.3, 52.6, 50.2, 45.2, 38.6, 38.0, 37.4, 37.2, 36.2, 33.6, 32.3, 32.0, 31.7, 28.9, 28.3, 23.0, 22.9, 22.1, 21.6, 19.9, 16.7, 12.0 ppm.

(20*R*)-3β-Acetoxy-16β-2,4-dinitrobenzoyl-20,22-epoxycholesta-5-ene-17α-ol (30)

A solution of **29** (30 mg, 0.05 mmol) and pyridinium dichromate (52 mg, 0.14 mmol) in dichloromethane (2 mL) was stirred at room temperature for 3 hr. Then the reaction mixture was filtered through Celite and eluted with dichloromethane. The filtrate was washed with saturated aqueous $NaHCO_3$ solution, then water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (7.5% ethyl acetate/hexane) to give **30** (15 mg, 47%) as white solid; m.p. 165–166°C. FTIR (KBr), ν_{max} 3440, 3106, 2947, 1738, 1705, 1544, 1280, 1250, 1030 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.83 (d, $J = 2.1$ Hz, 1H, C-3′), 8.58 (dd, $J = 8.4$, 2.2 Hz, 1H, C-5′), 8.04 (d, $J = 8.4$ Hz, 1H, C-6′), 5.27 (dd, $J = 8.2$, 5.3 Hz, 1H, C-16), 5.42 (d, $J = 4.8$ Hz, 1H, C-6), 4.68–4.60 (m, 1H, C-3), 3.10 (dd, $J = 6.9$, 5.4 Hz, 1H, C-22), 2.54–2.48 (m, 1H, C-15), 2.07 (s, 3H, AcO), 1.37 (s, 3H, C-21), 1.07 (s, 3H, C-18), 0.98 (s, 3H, C-19), 0.88 (d, $J = 6.5$ Hz, 3H, C-26), 0.86 (d, $J = 6.5$ Hz, 3H, C-27). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.2, 163.2, 149.6, 148.6, 140.2, 133.5, 131.9, 128.8, 122.8, 120.2, 85.6, 84.6, 74.4, 63.5, 60.8, 50.1, 48.8, 47.5, 38.6, 37.5, 37.2, 36.2, 33.0, 32.3, 32.2, 31.7, 28.5, 28.3, 26.4, 23.2, 22.9, 22.0, 20.8, 19.9, 17.6, 15.6 ppm.

(20*S*,22*R*)-3β-Acetoxy-17α-hydroxycholesta-5-ene-16β,20,22-ortho-2,4-dinitrobenzoate (31)

To a solution of **30** (10 mg, 0.015 mmol) in CH_2Cl_2 (1 mL) at 0°C was added $BF_3{\cdot}OEt_2$ (1 drop), followed by stirring at 0°C for 1 hr. The reaction

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mixture was quenched by addition of water and extracted with dichloromethane. The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution, then water, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (5% ethyl acetate/hexane) to give **31** (2.5 mg, 25%) as white solid. FTIR (KBr), ν_{max} 3514, 3104, 2947, 1731, 1716, 1548, 1254, 1045 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.38 (dd, $J = 8.7$, 2.2 Hz, 1H, C-5′), 8.33 (d, $J = 2.2$ Hz, 1H, C-3′), 8.09 (d, $J = 8.7$ Hz, 1H, C-6′), 5.41 (d, $J = 4.8$ Hz, 1H, C-6), 4.68–4.60 (m, 1H, C-3), 4.39 (dd, $J = 8.0$, 3.4 Hz, 1H, C-16), 3.67 (dd, $J = 10.2$, 3.4 Hz, 1H, C-22), 2.25–2.29 (m, 1H, C-15), 2.07 (s, 3H, AcO), 1.50 (s, 3H, C-21), 1.18 (s, 3H, C-18), 1.09 (s, 3H, C-19), 0.94 (d, $J = 6.6$ Hz, 3H, C-26 and C-27).

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