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Synthesis of the aglycone of 26-*O*-deacetyl pavoninin-5

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Abstract—The aglycone of 26-*O*-deacetyl pavoninin-5, (25*R*)-cholest-5-en-3β,15α,26-triol, **5a**, was synthesized in 10 steps in 17% overall yield from diosgenin, **3**. Removing mercury from the Clemmensen reduction of diosgenin **3**, gave a higher yield of (25*R*)-cholest-5-en-3β,16β,26-triol, **4**, by a method, that is also more environmentally friendly. Attempted methods for the transposition of the C-16β hydroxyl to the 15α position are described. A successful method for this transposition via the 15α-hydroxy-16-ketone, **13**, using the Barton deoxygenation reaction on the 16-alcohol, **15**, is reported. © 2003 Published by Elsevier Science Ltd.

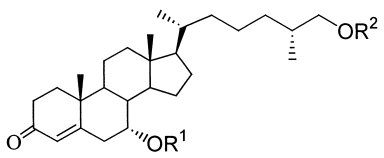
1. Introduction

Some species of fish are known to emit toxic substances that kill or repel their predators. These ichthyotoxic substances have a variety of structures and have been the topic of a number of reviews.[1–3] The sole, *Pardachirus pavoninus*, lives in the tropical region of the western Pacific and eastern Indian Ocean, is an ichthyotoxic fish. Six ichthyotoxic, shark-repelling steroidal *N*-acetylglucosaminides have been isolated and characterized as pavoninins **1**–**6**.[4] It is believed that the pavoninins are potent cell disrupters, which should have important pharmacological properties. The toxicity of these steroid glycosides is believed to relate to their surfactant properties. The synthesis of pavoninin-1, (**1**),[5] and the aglycones of pavoninin-1 (**1a**) and -2 (**2a**) have been reported.[6] In this article, we describe the first synthesis of the aglycone of 26-*O*-deacetyl pavoninin-5, **5a**.

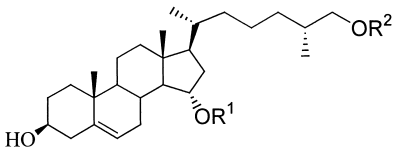
2. Results and discussion

The structure of 26-*O*-deacetyl pavoninin-5 aglycone, (**5a**), may be described as cholesterol with two additional hydroxyl groups at C-15α and C-26. A logical starting material for the synthesis of **5a** is the commercially available diosgenin, **3**, which has functionality in positions suitable for conversion to **5a**. Zinc and hydrochloric acid reduction of diosgenin **3**, afforded the 3β,16β,26-triol, **4**, in 85% yield. This is a significant improvement over the original Clemmensen reduction since the yield of **4** is higher, 85% verses ~60% previously.[7] Also not using mercury in the reduction, is more environmentally friendly.

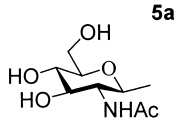
Initially we planned to dehydrate the C-16β alcohol via a *syn* elimination to afford the C-15 olefin. Regioselective hydroxylation from the less hindered α face should yield the



Pavoninin-1: **1** R^1 = β–glcNAc, R^2 = Ac
1a R^I = H, R^2 = Ac
Pavoninin-2: **2** R^1 = β–glcNAc, R^2 = H
2a R^I = H, R^2 = H



Pavoninin-5: **5** R^1 = β–glcNAc, R^2 = Ac
5a R^I = H, R^2 = H

β-glcNAc = 

Keywords: hydroxy steroids; *Pardachirus pavoninus*; shark repellent.

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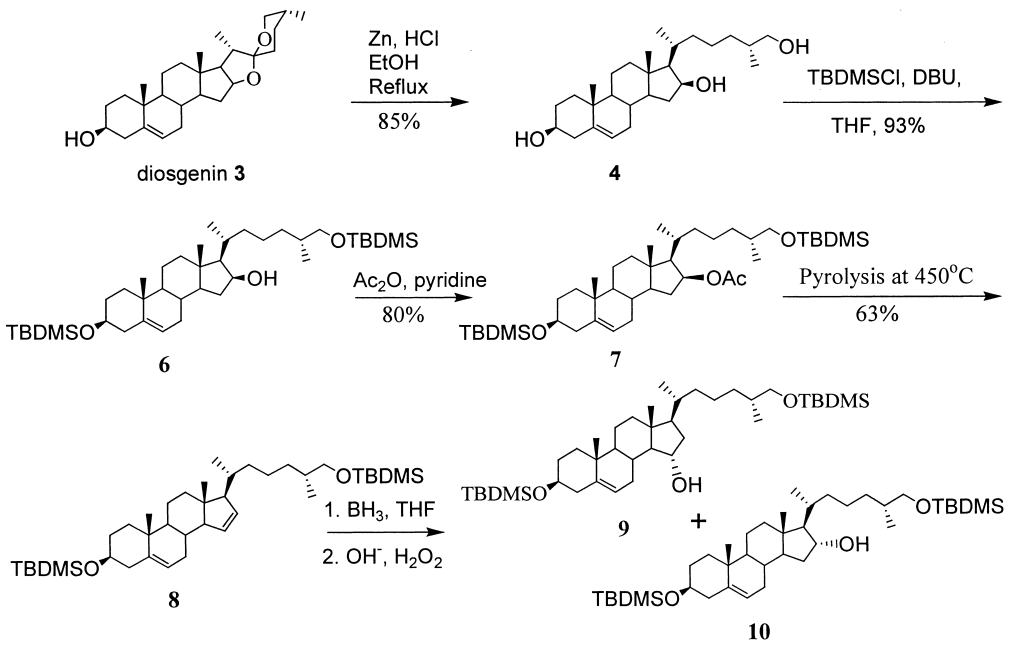
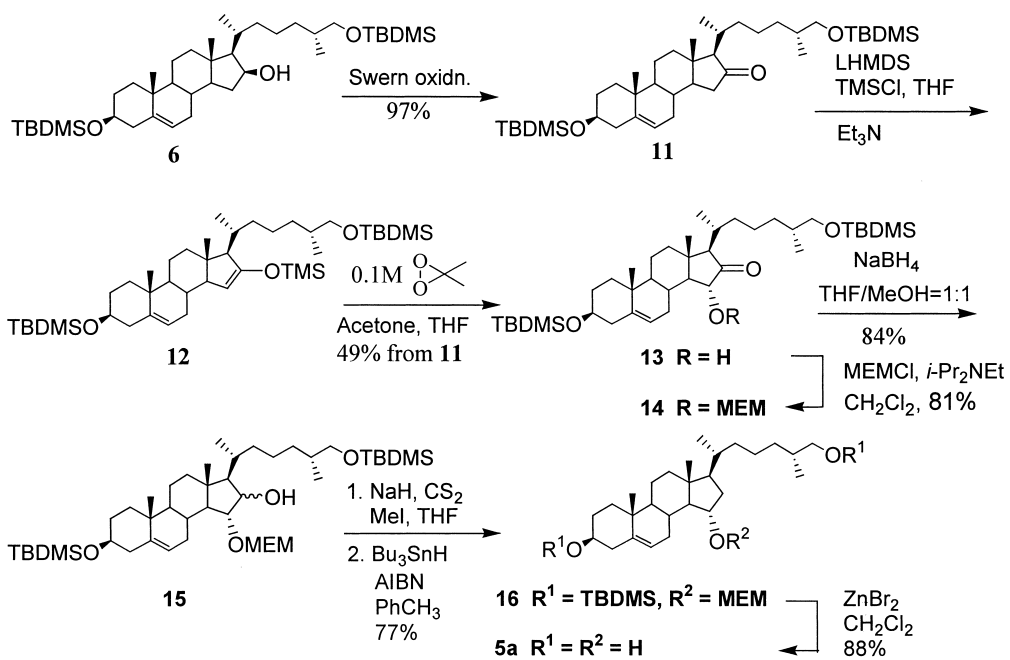


Scheme 1. Selective hydroboration–oxidation of the 3,26-bis-silyloxy-cholesta-5,15-diene **8**.

target aglycone **5a**, (Scheme 1). Djerassi had shown that one could selectively hydroborate and oxidize ring D in a 7,14-diene system to get a 15α-hydoxysteroid as the only product.[8] Since the C-16β alcohol is the most hindered of the 3β,16β,26-triol, **4**, reaction with *tert*-butyldimethylsilyl chloride (TBDMS) gave chemoselectively the 3β,26-bissilyl ether **6**, in 93% yield.[9] It was found that the use of THF and DBU gave the same yield 93%, but an easier workup than DMF and imidazole. Acetylation with acetic anhydride and pyridine afforded the C-16β acetate **7**. Pyrolysis of **7** at 450°C gave the desired C-15 alkene **8** in 63% yield. Attempts to regioselectively hydroxylate **8** using substituted alkyl boranes were unsuccessful as there was no reaction. When diborane followed by basic hydrogen peroxide oxidation was used, a mixture of C-15α and C-16α hydroxy steroids **9** and **10** was formed with the undesired C-16α hydroxy steroid **10** as the major component. In the ^{1}H NMR spectrum of the difficult to separate mixture, the 15β hydrogen in **9** occurs as a doublet of triplets with J=9.1 and 3.0 Hz at δ 3.96 ppm. where as the 16βH in **10** gives rise to a triplet with J=6.2 Hz at δ 4.03 ppm. Thus it appears that the C-15 olefin is very hindered and that the neighboring trisubstituted carbons C-14 and C-17 provide similar steric hindrance to diborane.

The next approach was to convert the C-16 alcohol in **6** into a ketone, introduce the C-15α hydroxyl and then remove the ketone, (Scheme 2). Swern oxidation of 3,26-bissilyl ether



Scheme 2. Synthesis of 26-*O*-deacetyl pavoninin-5 aglycone, **5a**.

6, gave the C-15 ketone **11** in 97% yield, an improvement over pyridinium chlorochromate oxidation.[9] A number of methods were attempted to hydroxylate **11** into **13** in one step but were unsuccessful. The Davis reagent,[10] was unsuccessful probably due to the large size of the reagent which prevented it from approaching the substrate.[11] In order to reduce the steric congestion in the hydroxylation reaction, a small reagent dimethyldioxirane was tried unsuccessfully.[12]

Due to the difficulty of direct hydroxylation of **11**, the ketone was hydroxylated through its silyl enol ether.[13] When trimethylsilyl chloride was added to the kinetic enolate of **11** in THF, formed by addition of lithium hexamethyldisilazane, (LHMDS) at −78°C, followed by quenching the reaction with water after 20 min, **12** was not obtained. We also tried more powerful silylating reagents, trimethylsilylmethyl trifluoromethanesulfonate[14] and *tert*-butyldimethylsilyl trifluoromethanesulfonate[15] and both methods of addition (either the triflate was added to the substrate or the other way), but **12** was not obtained. Even when we changed solvent to a more favorable solvent, such as 1,2 dichloromethane or triethyl amine,[16] it did not help. The silyl enol ether **12** was finally synthesized by adding LHMDS to the solution of **11** and trimethylsilyl chloride at −78°C, followed by addition of redistilled triethylamine, then quenching the reaction by saturated $NaHCO_3$.[17] The ^1H NMR showed the chemical shift of 15-H at 4.6 and indicated there were no side products. The silyl enol ether **12**, was not purified because it is sensitive to silica gel. Oxidation of **12** with dimethyldioxirane at −78°C afforded the C-15α-hydroxy ketone **13**. The coupling constant between 15-H and 14-H is 12.0 Hz, which shows the 15-H should be in β position. The formation of this structure maybe rationalized by the big groups at C-13β and C-17β blocking the oxidant attack from the β position. Attack by the oxidant on the α face, resulted in the unstable intermediate C-15α,16α-epoxide. Decomposition under mildly acidic conditions gave the desired 15α-hydroxy-16-ketone **13**.

Next step was the deoxygenation of the 16-ketone. However all methods to deoxygenate the α-hydroxy ketone using Raney Nickel on the dithioketal or Wolff–Kishner reduction failed. To remove the ketone we planned to reduce the carbonyl to a hydroxyl group followed by deoxygenation using Barton's reaction.[18] Scheme 2, Aglycone, was developed because the hydroxyl group in the Barton reaction functions as a nucleophile and attacks carbon disulfide, thereby avoiding the steric congestion of the C-16 position. Since both C-15 and C-16 would be hydroxyl groups, we first had to choose a different protecting group for the hydroxyl group in the 15α-position. Protection of the C-15α alcohol in **13** with methoxyethoxymethyl chloride (MEM),[19] gave the MEM ether **14**. Reduction of the C-16 ketone in **14** using sodium borohydride gave **15**, a mixture of epimeric alcohols at C-16. Deoxygenation of the alcohols **15**, via the xanthate, using the Barton reaction, yielded the MEM ether **16**. Treatment of the MEM ether, **16**, using dry zinc bromide in dry methylene chloride[19] cleaved the MEM group as well as the TBDMS groups to yield the target 26-*O*-deacetyl pavoninin-5 aglycone, **5a**. The ^1H NMR of **5a** showed the 15β hydrogen as a doublet of triplets with J=9.1 and 3.0 Hz at δ 3.83 ppm.

This route achieved the first synthesis of the aglycone of 26-deacetyl pavoninin-5, **5a**. However in order to synthesize pavoninin-5, better ways of introducing the C-15α hydroxyl group need to be developed.

3. Conclusion

In summary, we report the first synthesis of 26-*O*-deacetyl pavoninin-5 aglycone, **5a**, from diosgenin **3**, via 10 steps in 17% overall yield. An improved method for the reductive cleavage of diosgenin, **3**, was exploited. Swern oxidation gave an improved yield for oxidation of the C-16 alcohol in **6**. It was found that the α-hydroxy ketone **13** was resistant to the usual ketone deoxygenation methods. A way around this problem using Barton's method of deoxygenation of alcohols was developed.

4. Experimental

4.1. (25*R*)-Cholest-5-en-3β,16β,26-triol (4)

To a magnetic mixer equipped 100 mL three neck flask was added sequentially diosgenin (0.22 g, 0.53 mmol), zinc dust (4.5 g, 68.8 mmol) and 50 mL of absolute alcohol. After the mixture was heated to reflux, 40 mL concentrated HCl was added drop wise during a 30 min period. The reaction was refluxed and stirred for an additional 30 min, then filtered to remove the zinc dust. Distilled water was added to the filtrate until a precipitate appeared. The solution was then heated until transparent, and then slowly cooled. The precipitate, which formed, was collected by suction filtration and washed three times with cold water. The crystals were then dried to yield the triol, **4**, as a white solid (0.18 g, 85% yield): mp 172–174°C; lit.[7] mp 176–178°C, IR (KBr) 3377 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3/CD_3OD$=2:1) δ 0.82 (s, 3H), 0.83 (d, J=7.0 Hz, 3H), 0.91 (d, J=6.5 Hz, 3H), 0.95 (s, 3H), 3.33–3.56 (m, 3H), 4.28 (m, 1H), 5.28 (m, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3/CD_3OD$=2:1) δ 13.40, 17.01, 18.63, 19.84, 21.25, 23.97, 30.29, 31.67, 32.04, 32.35, 35.89, 36.44, 37.06, 37.12, 37.75, 40.42, 42.38, 42.74, 50.66, 54.97, 61.99, 62.04, 68.23, 71.77, 72.50, 121.83, 141.55.

4.2. (25*R*)-3β,26-Bis[(*tert*-butyldimethylsilyl)oxy]-cholest-5-en-16β-ol (6)

To a solution of **4** (0.5 g, 1.2 mmol) in 20 mL THF were added TBDMSCl (0.9 g, 6 mmol), and then DBU (0.7 mL, 4.6 mmol) slowly. The mixture was stirred at rt for 16 h. Water was added and the mixture extracted with EtOAc. The organic layers were dried over anhydrous Na_2SO_4, filtered, and concd. under reduced pressure. Flash chromatography of the residue on silica gel (hexane/EtOAc, 20:1) afforded **6** as a white solid (0.72 g, 93% yield): mp 122–124°C; lit.[9] mp 123–124°C; ^1H NMR δ 0.04 (s, 6H), 0.06 (s, 6H), 0.86 (s, 3H), 0.88–0.89 (m, 18H), 0.99 (d, J=6.5 Hz, 3H), 1.01 (s, 3H), 3.33–3.56 (m, 3H), 4.33 (m, 1H), 5.34 (m, 1H); ^{13}C NMR (125.8 MHz) δ −5.40, −4.61, 13.00, 16.68, 18.18, 18.33, 19.39, 20.65, 22.64, 23.72, 25.64, 25.92, 25.94,

29.71, 31.45, 31.80, 32.02, 32.56, 35.75, 36.50, 36.54, 39.85, 42.15, 42.75, 50.13, 54.50, 61.36, 68.44, 72.39, 72.55, 120.89, 141.58.

4.3. (25*R*)-3β,26-Bis[(*tert*-butyldimethylsilyl)oxy]-cholest-5-en-16β-ol-16-acetate (7)

To a solution of **6** (1.0 g, 1.58 mmol) in 10 mL pyridine, were added Ac_2O (3.0 mL) and *N*,*N*-dimethylaminopyridine (0.1 g). The mixture was stirred at rt under argon for 18 h. Water (5 mL) was added, and the product was extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$, water and dried (Na_2SO_4). The solvent was evaporated and residual viscous oil dissolved in toluene. The solvent was evaporated to remove any remaining pyridine. The crude product was crystallized from acetone to yield **7**, (1.04 g, 93% yield): mp 58–60°C; IR (KBr) 1730, 1670 cm^{-1}; ^1H NMR δ 0.03 (s, 6H), 0.06 (s, 6H), 0.87 (s, 3H), 0.90 (s, 18H), 0.97 (d, *J*=6.5 Hz, 3H), 1.02 (s, 3H), 2.02 (s, 3H), 3.34 (dd, 1H *J*=9.6, 6.9 Hz), 3.44 (dd, 1H *J*=9.6, 5.7 Hz), 3.49 (m, 1H), 5.20 (m, 1H), 5.31 (m, 1H); ^{13}C NMR δ −5.4, −4.6, 12.5, 16.6, 18.2, 18.3, 19.4, 20.7, 21.2, 23.9, 25.6, 25.9, 29.9, 31.4, 31.6, 32.0, 33.4, 34.9, 35.8, 36.3, 36.5, 37.3, 39.7, 42.5, 42.8, 50.1, 54.6, 60.0, 68.4, 72.5, 75.3, 120.8, 141.5, 170.6; HRMS calcd for $C_{35}H_{61}0_3Si_2$ (M+ $-C_6H_{15}O$) 585.4161, found 585.4103.

4.4. (25*R*)-3β,26-Bis[(*tert*-butyldimethylsilyl)oxy]-cholest-5,15-diene (8)

Acetate **7** (0.25 g, 0.46 mmol) was heated at 400–440°C at less than 10 mm Hg for 30 min in a quartz tube. After cooling to rt, the quartz tube was rinsed and the quartz wool was extracted with ether. The ether washings were combined and dried ($MgSO_4$). The solvent was removed in vacuo and the crude product was purified by flash chromatography on silica gel (1% EtOAc–hexane) to afford **8** (0.137 g, 89% based on recovered starting material): mp 58–60°C; IR (neat) 3100 cm^{-1}; ^1H NMR δ 0.06 (s, 6H), 0.08 (s, 6H), 0.77 (s, 3H), 0.88 (d, *J*=6.6 Hz, 3H), 0.91 (s, 18H), 1.03 (s, 3H), 3.37 (dd, 1H, *J*=9.6, 6.6 Hz), 3.46 (dd, 1H, *J*=9.9, 6.0 Hz), 3.50 (m, 1H), 5.36 (m, 1H), 5.79 (m, 2H); ^{13}C NMR δ −5.3, −4.6, 12.5, 16.7, 18.3, 18.4, 18.5, 19.4, 20.8, 23.8, 26.0, 32.0, 32.1 (2), 33.5, 35.7, 36.5, 36.7, 37.2, 37.6, 42.8, 49.1, 50.5, 62.1, 62.2, 68.5, 72.6, 120.9, 131.0, 133.9, 141.8; MS *m*/*z* (relative intensity) M+ 628 (1), 513 (1), 497 (1), 481 (1), 454 (1), 453 (2), 439 (1), 425 (1), 411 (2), 227 (6), 75 (100); HRMS calcd for $C_{19}H_{23}$ (M+ $-C_{20}H_{46}O_2Si_2$) 251.1801, found 251.1798. Further elution (10% EtOAc–hexane) afforded recovered starting acetate **7** (0.079 g, 32%).

4.5. (25*R*)-3β,26-Bis[(*tert*-butyldimethylsilyl)oxy]-cholest-5-en-15α-ol (9) and (25*R*)-3β,26-bis[(*tert*-butyldimethylsilyl)oxy]cholest-5-en-16α-ol (10)

To a solution of **8** (100 mg, 0.159 mmol) at 0°C in dry THF (10 mL) was added BH_3 (0.2 mL of 1 M in THF). After stirring at 0°C for 0.5 h, the mixture was allowed to warm to room temperature and stirring was continued for 12 h. Aqueous NaOH (0.5 mL of 3 M) and 30% aqueous H_2O_2 (0.5 mL) were added, and the mixture was stirred for 2 h at rt. The mixture was diluted with brine (5 mL), and extracted with Et_2O. The combined organic extracts were dried (Na_2SO_4), the solvent removed in vacuo and the crude product was purified by flash chromatography on silica gel (20% ethyl acetate–hexane) to afford a 1:2 mixture of **9** and **10**. IR (KBr) 3349 cm^{-1}; ^1H NMR δ 0.05 (s, 6H), 0.06 (s, 6H), 0.70 (s, 3H), 0.85 (d, *J*=6.6 Hz, 3H), 0.89 (s, 18H), 1.03 (s, 3H), 3.33–54 (m, 4H), 3.96 (dt, 1H *J*=9.1, 3.0 Hz), 4.03 (t, 1H, *J*=6.2 Hz), 5.32 (m, 1H); ^{13}C NMR δ −4.91, −4.17, 13.52, 13.83, 17.05, 18.67, 18.78, 18.93, 19.31, 19.82, 21.16, 23.69, 24.46, 26.37, 30.11, 31.63, 31.99, 32.26, 32.47, 32.69, 33.86, 33.93, 34.39, 35.66, 36.17, 36.36, 36.50, 37.00, 37.42, 37.69, 37.78, 40.30, 41.12, 43.12, 43.21, 44.05, 44.56, 50.28, 50.45, 53.97, 54.09, 54.71, 63.74, 67.49, 68.97, 72.98 75.00, 77.10, 121.39, 141.67, 141.95.

4.6. (25*R*)-3β,26-Bis[(*tert*-butyldimethylsilyl)oxy]-cholest-5-en-16-one (11)

DMSO (0.4 mL, 5.6 mmol) in dry CH_2Cl_2 was added dropwise to a stirred solution of oxalyl chloride 0.4 mL (2.0 mmol, 2 M solution) in CH_2Cl_2 at −70°C over 5 min. After 10 min, **6** (0.25 g, 0.4 mmol) in 2 mL of CH_2Cl_2, was added at −70°C over 5 min. The reaction mixture was stirred for 10 min, an excess of Et_3N (0.6 mL, 4.3 mmol) added and the reaction mixture allowed to slowly attain rt. After 4 h the cloudy mixture was concentrated at a reduced pressure, Et_2O added and the precipitated salts were filtered off. The solvent was removed in vacuum and the product was recrystallized from acetone to afford **11** as white solid (0.24 g, 97%): mp 78–80°C; lit.[9] mp 81.5–82.5°C. IR (KBr) 1736 cm^{-1}; ^1H NMR δ 0.06 (s, 6H), 0.08 (s, 6H), 0.85 (s, 3H), 0.87 (d, *J*=6.5 Hz, 3H), 0.88–0.89 (m, 18H), 0.99 (d, *J*=7 Hz, 3H), 1.05 (s, 3H), 3.33–3.56 (m, 3H), 5.34 (m, 1H); ^{13}C NMR δ −4.69, −3.87, 14.22, 17.45, 18.77, 18.94, 19.37, 20.08, 21.20, 25.25, 26.59, 26.65, 31.44, 31.88, 32.51, 32.70, 33.95, 36.34, 36.59, 37.30, 37.75, 39.43, 39.66, 43.46, 43.53, 50.61, 51.60, 68.51, 68.98, 73.01, 121.11, 142.20, 218.33.

4.7. (25*R*)-3β,26-Bis[(*tert*-butyldimethylsilyl)oxy]-cholest-5-en-15α-ol-16-one (13)

To a cooled (−78°C) solution of the ketone **11** (0.22 g, 0.34 mmol) in 1 mL dry THF under argon was added TMSCl (0.5 mL, 3.9 mmol). After 5 min, LHMDS (1 M solution in THF, 0.8 mL, 0.8 mmol) was added drop wise over a 6 min period. The solution was stirred for 5 min and Et_3N (1 mL, 7.2 mmol) was added. After 15 min, the mixture was warmed to rt, then quenched with saturated $NaHCO_3$ (5 mL) and diluted with 10 mL of Et_2O. The layers were separated, and the aqueous layer was further extracted with Et_2O. The combined organic layers were washed with water, brine, dried (Na_2SO_4), filtered, and concentrated in vacuo to provide a golden oil (0.23 g). The oil was dissolved in THF (1 mL) and cooled to −78°C, a dimethyldioxirane solution (0.099 M in acetone, 3 mL) was added, and the mixture was stirred at −78°C for one hour. The temperature of the mixture was slowly raised to rt, quenched with 3 mL saturated $NaHCO_3$ and diluted with 10 mL of THF. The layers were then separated and the aqueous layers were re-extracted with 5 mL of Et_2O. The combined organic layer was washed with water, brine, dried (Na_2SO_4), filtered and

concentrated in vacuo to give a colorless oil. The oil was dissolved in Et_2O and stirred with aqueous HCl (0.1 M, 2 mL) overnight. The layers were separated, and the aqueous layer was extracted with Et_2O, and the combined organic layers were washed with water, brine, dried (Na_2SO_4), filtered and concd in vacuo, followed by flash chromatography on silica gel (hexane/EtOAc, 10:1) to give **13** (110 mg, 49% yield): mp 75–77°C; IR (KBr) 3431, 2954, 1740 cm^{-1}; ^1H NMR δ 0.03 (s, 6H), 0.06 (s, 6H), 0.92 (s, 3H), 0.86 (d, J=7.0 Hz, 3H), 0.88–0.89 (m, 18H), 1.00 (d, J=7.0 Hz, 3H), 1.05 (s, 3H), 3.33–3.56 (m, 3H), 3.74 (dd, J=11, 1.5 Hz, 1H), 5.34 (m, 1H); ^{13}C NMR δ −5.39, −4.63, 15.71, 16.62, 18.20, 19.44, 19.55, 20.34, 24.53, 25.90, 30.84, 31.37, 31.93, 33.21, 34.91, 35.55, 36.55, 37.09, 38.71, 39.64, 42.61, 49.55, 56.38, 64.34, 68.39, 72.37, 76.53 121.06, 140.82, 219.35; HRMS calcd for $C_{39}H_{72}0_4Si_2$ (M+Na^+) 683.4840, found 683.4866.

4.8. (25*R*) 3β,26-Bis[(*tert*-butyldimethylsilyl)oxy]-cholest-5-en-15α-(2-methoxyethoxymethyl)oxy-16-one (14)

A solution of **13** (350 mg, 0.53 mmol), MEMCl (0.6 mL, 5.3 mmol) and *i*-Pr_2NH (1.2 mL, 6.7 mmol) in dry CH_2Cl_2 (5 mL) under argon was stirred overnight at rt, then quenched with saturated $NaHCO_3$ (2 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with water, brine, dried (Na_2SO_4), filtered and concd. in vacuo. Flash chromatography on silica gel (hexane/EtOAc, 10:1) gave **14** as a colorless oil (320 mg, 81% yield); IR (film) 1742 cm^{-1}; ^1H NMR δ 0.03 (s, 6H), 0.05 (s, 6H), 0.85 (s, 3H), 0.86 (d, J=6.5 Hz, 3H), 0.88–0.89 (m, 18H), 0.99 (d, J=7 Hz, 3H), 1.04 (s, 3H), 3.33–3.45 (m, 2H), 3.40 (s, 3H), 3.47–3.56 (m, 3H), 3.71 (d, J=11 Hz, 1H), 3.76–3.80 (m, 2H), 4.78–5.14 (m, 2H), 5.33 (m, 1H); ^{13}C NMR δ −4.70, −3.94, 16.12, 17.32, 18.89, 19.00, 19.68, 20.10, 21.02, 25.34, 26.57, 26.62, 31.41, 31.89, 32.41, 32.65, 33.95, 36.20, 36.41, 37.25, 37.80, 39.34, 39.97, 43.32, 50.32, 55.71, 59.71, 65.61, 68.82, 69.12, 72.46, 73.09, 80.61, 96.14, 121.58, 141.70, 217.63; HRMS calcd for $C_{43}H_{80}0_6Si_2$ (M+Na^+) 771.5386, found 771.5391.

4.9. (25*R*) 3β,26-Bis[(*tert*-butyldimethylsilyl)oxy]cholest-5-en-15α-(2-methoxyethoxymethyl)oxy-16-ol (15)

To a solution of **14** (310 mg, 0.41 mmol) in THF (1 mL) and MeOH (1 mL) under argon, $NaBH_4$ (280 mg, 7.4 mmol) was added. After stirring for two and half hours, the reaction was quenched with saturated $NaHCO_3$. The mixture was extracted with Et_2O, and the organic layer was washed with water, brine, dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was flash chromatographed on silica gel (hexane/EtOAc, 10:1) to give **15** (277 mg, 90% yield); IR (film) 3506 cm^{-1}; ^1H NMR δ 0.03 (s, 6H), 0.06 (s, 6H), 0.73 (s, 3H), 0.88–0.89 (m, 18H), 0.93 (d, J=6.6 Hz, 3H), 1.00 (s, 3H), 3.1 (d, J=3.3 Hz, 1H), 3.33–3.45 (m, 2H), 3.39 (s, 3H), 3.47–3.56 (m, 3H), 3.71 (d, J=11 Hz, 1H), 3.76–3.8 (m, 2H), 4.71–4.84 (m, 2H), 5.30 (m, 1H); ^{13}C NMR δ −5.35, −4.59, 15.02, 16.66, 18.25, 18.35, 19.13, 19.41, 20.47, 24.17, 25.96, 29.69, 31.39, 32.05, 32.10, 33.54, 34.07, 35.55, 35.84, 36.50, 37.29, 39.20, 40.14, 42.67, 49.61, 56.28, 59.02, 63.51, 68.26, 68.60, 71.76, 72.49, 74.08, 83.60, 97.38, 121.36, 140.90; HRMS calcd for $C_{43}H_{82}O_6Si_2$ (M+Na^+) 773.5543, found 773.5547.

4.10. (25*R*)3β,26-Bis[(*tert*-butyldimethylsilyl)oxy]-cholest-5-en-15α-(2-methoxyethoxymethyl)oxy ether (16)

A mixture of **15** (200 mg, 0.27 mmol), NaH dispersion (65% in mineral oil, 21 mg, 0.54 mmol), imidazole (2 mg, 0.029 mmol) and dry THF (3 mL) was stirred for a half an hour at rt. CS_2 (0.20 mL, 3.33 mmol) was added and the stirring continued for 1 h. Iodomethane (0.03 mL, 0.48 mmol) was added. After stirring for another hour, the mixture was poured into water (5 mL), and extracted with Et_2O. The organic layer was washed with water, brine, dried (Na_2SO_4), filtered and concd in vacuo to give a yellow oil. After adding a trace amount of AIBN, (ca. 10 mg), the oil was refluxed in toluene (10 mL) under argon while a solution of *n*-Bu_3SnH (0.3 mL, 1.12 mmol) in toluene (3 mL) was added over 30 min. The mixture was refluxed overnight. The reaction was quenched by aqueous saturated $NaHCO_3$, and the aqueous layer was extracted with Et_2O. The combined organic phases were dried (Na_2SO_4), the solvent evaporated, and the residue was purified by flash chromatography on silica gel (hexane/ethyl acetate, 10:1) to afford **16** as a colorless oil (169 mg, 77% yield); ^1H NMR δ 0.03 (s, 6H), 0.06 (s, 6H), 0.69 (s, 3H), 0.84 (d, J=7 Hz, 3H), 0.88–0.89 (m, 18H), 0.93 (d, J=7 Hz, 3H), 1.01 (s, 3H), 3.1 (d, J=3.3 Hz, 1H), 3.33–3.45 (m, 2H), 3.39 (s, 3H), 3.47–3.56 (m, 3H), 3.71 (d, J=11 Hz, 1H), 3.76–3.8 (m, 2H), 4.66–4.74 (m, 2H), 5.30 (m, 1H); ^{13}C NMR δ −4.67, −3.93, 13.74, 14.24, 17.29, 18.17, 19.18, 20.12, 21.41, 24.02, 26.62, 27.50, 28.50, 32.30, 32.74, 32.80, 34.21, 35.97, 36.41, 36.73, 37.20, 38.00, 38.06, 40.49, 43.30, 43.38, 50.55, 54.24, 59.71, 61.18, 68.25, 69.20, 72.50, 73.22, 80.76, 96.03, 122.14, 141.59; HRMS calcd for $C_{43}H_{82}0_5Si_2$ (M+Na^+) 757.5592 found 757.5598.

4.11. (25*R*)-Cholest-5-en-3β,15α,26-triol (5a)

To a solution of **16** (18 mg, 0.024 mmol) in 8 mL of dry CH_2Cl_2 was added zinc bromide (21 mg, 0.096 mmol). The mixture was kept stirring overnight under nitrogen, then run through a short silica gel column (CH_3Cl/MeOH=10:1). The solvent was evaporated, and the residue was purified by preparative TLC (CH_3Cl/MeOH, 20:1) to afford **5a** as the white solid (8.8 mg, 88% yield): mp 205–208°C; IR (KBr) 3253 cm^{-1}; ^1H NMR ($CDCl_3/CD_3OD$=2:1) δ 0.67 (s, 3H), 0.84 (d, J=6.5 Hz, 3H), 0.87 (d, J=5.5 Hz, 3H), 0.98 (s, 3H), 3.25–3.39 (m, 3H), 3.83 (dt, 1H, J=9.1, 3.0 Hz), 5.33 (m, 1H); ^{13}C NMR δ 13.29, 16.70, 18.76, 19.63, 21.28, 23.85, 30.10, 31.57, 32.20, 32.49, 34.08, 35.86, 36.17, 36.62, 36.94, 37.85, 40.50, 41.00, 42.22, 50.57, 54.11, 63.02, 68.12, 71.70, 74.27, 122.29, 140.96; HRMS calcd for $C_{27}H_{45}O_2$ (M+H−H_2O^+) 401.3419, found 401.3415.

4.12. Supporting information available

^1H, and ^{13}C NMR spectra for compounds **4**, **5a**, **6**–**8**, **9**+**10**, **11**–**16**.

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