

Epoxidation of Diosgenin, 25(R)-1,4,6-Spirostatrien-3-one and 25(R)-4,6-Spirostadien-3 β -ol

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Abstract: Upon treatment of 25(R)-spirost-5-en-3 β -ol (diosgenin), 25(R)-1,4,6-spirostatrien-3-one and 25(R)-4,6-spirostadien-3 β -ol with 30% H₂O₂ and 5% NaOH in methanol, diosgenin did not react, 25(R)-1,4,6-spirostatrien-3-one was converted to 25(R)-1 α ,2 α -epoxy-4,6-spirostadien-3-one and 25(R)-4,6-spirostadien-3 β -ol was oxidized to give a small amount of 25(R)-4,6-spirostadien-3-one, while most of the original starting material remained unchanged. On the other hand, reactions of diosgenin, 25(R)-1,4,6-spirostatrien-3-one and 25(R)-4,6-spirostadien-3 β -ol with *m*-chloroperoxybenzoic acid in chloroform yielded 25(R)-5 α ,6 α -epoxyspirostan-3 β -ol, 25(R)-6 α ,7 α -epoxy-1,4-spirostadien-3-one and 25(R)-4 β ,5 β -epoxy-6-spirosten-3 β -ol, respectively.

Keywords: Diosgenin, Stereoselective epoxidation, H₂O₂, *m*-Chloroperoxybenzoic acid

Introduction

Diosgenin (25(R)-spirost-5-en-3 β -ol) is the steroidal saponin which is isolated from the Mexican yam (*Dioscorea*). Estrogenic, progestogenic and anti-inflammatory effects have been hypothesized for diosgenin due to its structural similarity to estrogen and progesterone precursors [1]. Diosgenin has been reported to lower serum cholesterol in chicken and rabbits fed cholesterol and to decrease liver cholesterol in cholesterol-fed rats, an action associated with increased rates of cholesterol synthesis, increased secretion of cholesterol into bile acid, and increased fecal excretion of neutral sterols [2-6].

Diosgenin was used commercially to produce steroid hormones such as cortisone, estrogen, and progesterone by *in vitro* chemical modification [7]. 1 α -Hydroxydiosgenin and 1 β -hydroxydiosgenin (ruscogenin) were used as starting materials in the synthesis of 1 α -hydroxycholesterol and 1 β -hydroxycholesterol by chemical removal of its side chain [8,9]. The study showed that 1 β -hydroxydiosgenin could be used as a potential starting material for 1 α - or 1 β -hydroxy vitamin D analogs [10,11]. Diosgenin was also used as the natural source to synthesize dehydroepiandrosterone (DHEA), a steroid that declines with age, but has significant activities as an antioxidant to modify serum lipid levels and which inhibits proliferation of cancer and noncancer cells in both *in vitro* and *in vivo* studies [12-14].

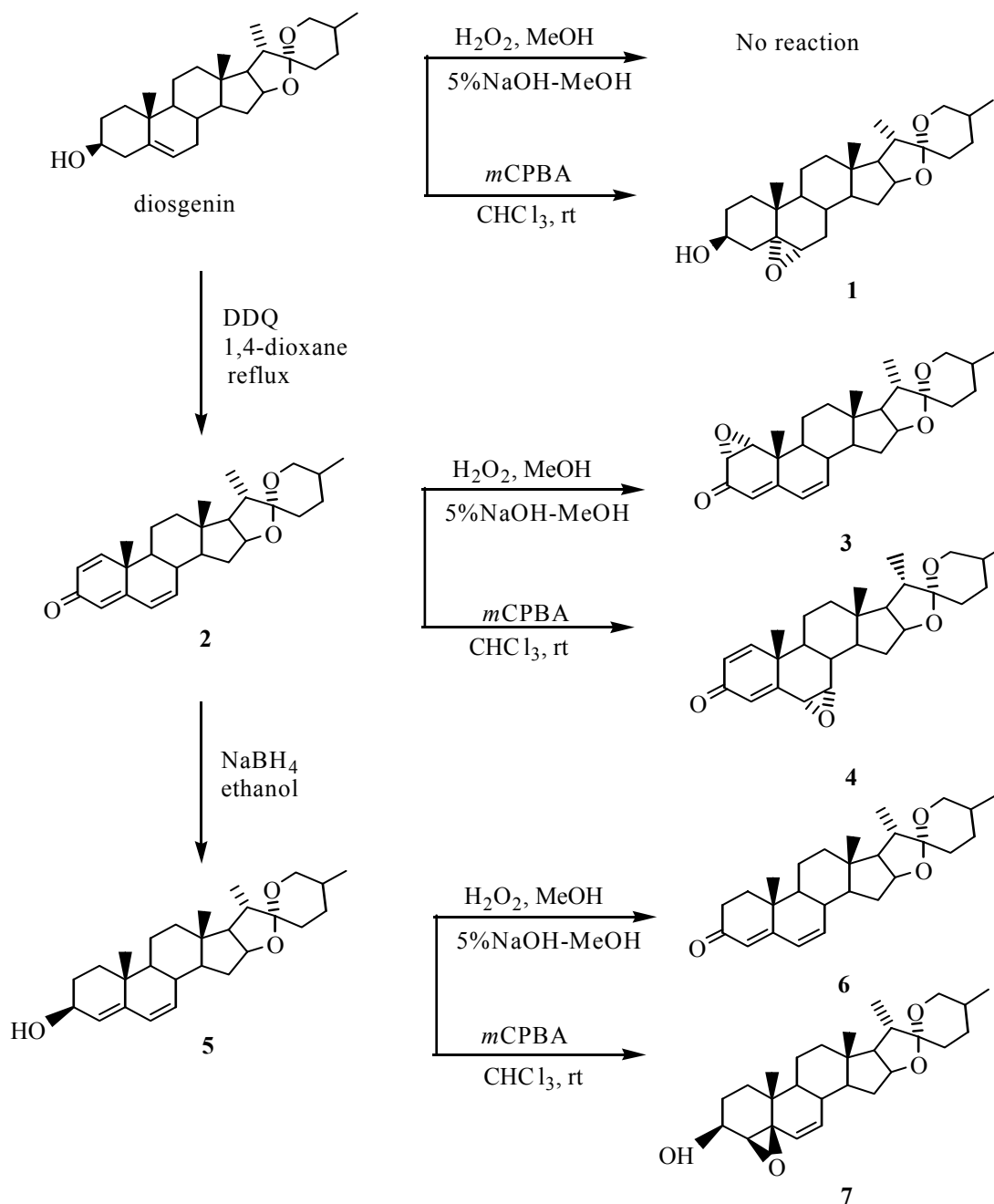
Since 1 α - or 1 β -hydroxydiosgenin is of great interest as a starting material in the synthesis of various active compounds, it is desirable to make hydroxydiosgenin derivatives more available for biological experimentation. The efficient stereoselective synthesis of hydroxydiosgenin derivatives requires a stereoselective epoxidation of double bond as an important intermediate step. Consequently we have examined position selective and reagent selective epoxidations of the olefinic position of diosgenin, 25(R)-1,4,6-spirostatrien-3-one [9] and 25(R)-4,6-spirostadien-3 β -ol using 30 % H₂O₂ and *m*-chloroperoxybenzoic acid as epoxidizing agents, respectively.

Results and discussion

In this paper, we report the results of a study of the reagent selective and regioselective epoxidation reactions of diosgenin derivatives. The syntheses were accomplished by the procedures described in Scheme 1. 25(R)-Spirost-5-en-3 β -ol (diosgenin) was epoxidized with 30% H₂O₂ and 5% NaOH in methanol, but it did not react, whereas when diosgenin was treated with *m*-chloroperoxybenzoic acid (*m*CPBA) in CHCl₃ it gave 25(R)-5 α ,6 α -epoxy-spirostan-3 β -ol (**1**). In this case, the equatorial attack of the *m*CPBA to the double bond of diosgenin is destabilized by the steric interaction between the reagent and the axial CH₃ group (number 19), therefore the downside axial attack is preferred for reagent approach in the transition state. The oxidative dehydrogenation of diosgenin with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in refluxing 1,4-dioxane gave 25(R)-

1,4,6-spirostatrien-3-one (**2**). Only the 1,2-double bond of compound **2** was selectively epoxidized with 30% H_2O_2 and 5% NaOH in methanol to yield 25(R)-1 α ,2 α -epoxy-4,6-spirostadien-3-one (**3**) [9,15]. In the absence of directing effects, electrophilic epoxidation of the double bond takes place predominantly from the α -face of the molecule.

Scheme 1



On the other hand, when compound **2** was epoxidized with *m*CPBA, 25(R)-6 α ,7 α -epoxy-1,4-spirostadien-3-one (**4**) was obtained. This result is basically caused by the attack of the *m*CPBA directed on the less hindered α -face of 6,7-double bond. 25(R)-1,4,6-Spirostatien-3-one (**2**) was reduced with NaBH₄ in absolute ethanol to give 25(R)-4,6-spirosta-dien-3 β -ol (**5**) [11]. The $p\pi$ -electronic system of compound **5** is more stable than 25(R)-1,4- spirostadien-3 β -ol [16]. When compound **5** was epoxidized with 30% H₂O₂ and 5% NaOH in methanol, the 3-hydroxy group was dehydrogenated to yield only 25% of 25(R)-4,6-spirostdien-3-one (**6**) while most of the original starting material was recovered. In the case of H₂O₂, epoxidation seems to only occur in the presence of a carbonyl group conjugated with the double bond. Compound **5** was epoxidized with *m*CPBA to form 25(R)-4 β ,5 β -epoxy-6-spirosten-3 β -ol (**7**). In this case the presence of the 3 β -hydroxy group of the allylic alcohol directed the epoxidation exclusively to the β -face of the double bond of the molecule [16,17]. All synthesized compounds were identified by IR, ¹H-NMR, ¹³C-NMR and mass spectra, respectively.

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Experimental

General

Reactions were performed under nitrogen. Solvents were purified and dried prior to use. Melting points were measured on Thomas-Hoover melting point apparatus in open capillary tubes and are not corrected. ¹H- and ¹³C-NMR spectra were obtained on Gemini 200 MHz or 50MHz spectrometers in CDCl₃, chemical shifts δ are in ppm are relative to tetramethylsilane, coupling constants *J* in Hz. IR spectra were determined for KBr pellets on a Jasco FT-IR 300E spectrometer. FAB mass spectra were recorded on a Tandem mass spectrometer. Analytical TLC was performed on Merck precoated silica gel 60 F₂₅₄ plates. Column chromatography was carried out on Merck silica gel 9385 (230-400 mesh).

25(R)-5 α ,6 α -Epoxy-spirostan-3 β -ol (1). *m*-Chloroperoxybenzoic acid (480 mg, 2.78 mmol) was added at room temperature to a solution of 25(R)-spirost-5-en-3 β -ol (diosgenin, 1 g, 2.4 mmol) in CHCl₃ (100 mL). The mixture was stirred under nitrogen at room temperature for 18 hours. Water was added and the aqueous layer was extracted with several portions of CHCl₃ and the organic extracts were combined, dried with MgSO₄, filtered and evaporated to leave a crude solid that was recrystallized from ethyl acetate and *n*-hexane (1:5) to afford **1** as white crystals. Yield: 960 mg (93 %); mp : 202.3-204.5°C; IR (cm⁻¹): 3513, 3401; ¹H-NMR δ : 0.71 (3H, s, H-27), 0.78 (3H, s, H-18), 0.94 (3H, d,

$J=6.8\text{Hz}$, H-21), 1.07 (3H, s, H-19), 2.90 (1H, d, $J=14.0\text{Hz}$, H-6), 3.30-3.52 (2H, m, H-26), 3.87-3.96 (1H, m, H-3), 4.35-4.44 (1H, m, H-16); $^{13}\text{C-NMR}$ δ : 109.3, 80.7, 68.7, 66.9, 65.7, 61.9, 59.2, 56.7, 42.6, 41.7, 40.4, 39.9, 39.5, 35.1, 31.2, 30.4, 29.6, 29.1, 28.9, 20.6, 17.2, 16.4, 16.1, 14.6; FABMS $(\text{M}+\text{H})^+$: 431.2.

25(R)-1,4,6-Spirostatrien-3-one (2). A solution of diosgenin (5 g, 12 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (10 g, 88 mmol) in dioxane (125 mL) was refluxed for 24 hours under nitrogen. The reaction mixture was cooled down and the resulting precipitate was filtered off and washed with dichloromethane. The filtrate was rotary evaporated to dryness and the dark brown residue was purified by column chromatography on silica gel (ethyl acetate/*n*-hexane=1:7) to afford white crystals of **2**. Yield: 3.04 g (62 %); mp: 207.1-209.2°C; IR (cm^{-1}): 1661, 1452; $^1\text{H-NMR}$ δ : 0.80 (3H, d, $J=3.1\text{Hz}$, H-27), 0.90 (3H, s, H-18), 1.00 (3H, d, $J=3.4\text{Hz}$, H-21), 1.21 (3H, s, H-19), 3.36-3.51 (2H, m, H-26), 4.38-4.49 (1H, m, H-16), 6.00 (2H, d, $J=9.4\text{Hz}$, H-6, H-7), 6.23 (2H, dd, $J=2.0$, 12.0Hz, H-2, H-4), 7.05 (1H, d, $J=10.0\text{Hz}$, H-1); $^{13}\text{C-NMR}$ δ : 162.5, 152.9, 138.1, 128.3, 127.7, 123.9, 109.3, 80.5, 67.0, 62.0, 53.4, 48.5, 41.7, 41.1, 39.5, 37.8, 31.4, 31.3, 30.3, 29.8, 28.8, 21.8, 20.9, 17.2, 16.4, 14.5; FABMS $(\text{M}+\text{H})^+$: 409.2.

25(R)-1 α ,2 α -Epoxy-4,6-spirostadien-3-one (3). A mixture of 5 % NaOH-MeOH (0.6 mL) and 30 % H_2O_2 (6 mL) was added to a solution of the trienone **2** (3 g, 7.34 mmol) in methanol (60 mL). The mixture was stirred at room temperature for 22 hours, at which time TLC indicated reaction completion, then extracted with dichloromethane. The organic layers were combined and washed with brine and H_2O , dried over MgSO_4 , filtered, and concentrated to leave a crude solid. Column chromatography on silica gel, eluting with ethyl acetate/*n*-hexane (1:5), gave pure white crystals of epoxide **3**. Yield: 2.53 g (81 %); mp: 219.2-220.8°C; IR (cm^{-1}): 1669, 1459; $^1\text{H-NMR}$ δ : 0.73 (3H, d, $J=6.2\text{Hz}$, H-27), 0.88 (3H, s, H-18), 0.92 (3H, d, $J=6.6\text{Hz}$, H-21), 1.13 (3H, s, H-19), 3.27 (1H, br d, $J=10.8\text{Hz}$, H-26), 3.35-3.46 (2H, m, H-26, H-2), 3.51 (1H, d, $J=4.2\text{Hz}$, H-1), 4.43-4.46 (1H, m, H-16), 5.58 (1H, d, $J=1.4$, H-4), 6.01 (2H, m, H-6, H-7); $^{13}\text{C-NMR}$ δ : 193.6, 157.5, 138.8, 126.9, 118.6, 108.3, 79.3, 65.9, 58.4, 53.7, 52.0, 45.1, 40.7, 40.0, 38.2, 37.0, 36.1, 30.3, 30.2, 29.2, 27.8, 20.0, 17.5, 16.1, 15.1, 13.4; FABMS $(\text{M}+\text{H})^+$: 425.2.

25(R)-6 α ,7 α -epoxy-1,4-spirostadien-3-one (4). *m*CPBA (490 mg, 2.84 mmol) was added to a solution of trienone **2** (1 g, 2.45 mmol) in CHCl_3 (100 mL) at room temperature. The reaction mixture was stirred at the same temperature until no starting material could be detected by TLC. The mixture was stirred with H_2O and then extracted with dichloromethane. The extract was dried over MgSO_4 , filtered, and evaporated to give a pale yellow precipitate. Column chromatography on silica gel, eluting with ethyl acetate/*n*-hexane (1:5) gave the pure white crystals of epoxide **4**. Yield: 655 mg (63 %); mp: 264-265.5°C; IR (cm^{-1}): 1653, 1448; $^1\text{H-NMR}$ δ : 0.79 (3H, d, $J=6.2\text{Hz}$, H-27), 0.88 (3H, s, H-18),

0.98 (3H, d, $J=6.6\text{Hz}$, H-21), 1.21 (3H, s, H-19), 3.31-3.50 (2H, m, H-26, H-7), 3.64 (1H, d, $J=3.6\text{Hz}$, H-6), 4.50-4.67 (1H, m, H-16), 6.24 (1H, dd, $J=1.6, 10.2\text{Hz}$, H-2), 6.48 (1H, d, $J=1.8\text{Hz}$, H-4), 7.0 (1H, d, $J=16.4\text{Hz}$, H-1); $^{13}\text{C-NMR}$ δ : 185.2, 159.7, 153.2, 131.2, 127.8, 101.4, 80.7, 67.6, 61.9, 53.9, 51.8, 50.9, 41.8, 41.2, 41.1, 39.3, 38.4, 35.1, 31.5, 31.3, 30.3, 28.9, 22.0, 20.9, 17.2, 16.3, 14.6; FABMS (M+H) $^{+}$: 425.2.

25(R)-4,6-Spirostadien-3 β -ol (5). To a solution of compound **2** (1 g, 2.45 mmol) in absolute ethanol (100 mL), NaBH₄ (370 mg, 9.8 mmol) was added portionwise at room temperature. The resulting mixture was stirred overnight at this temperature, then concentrated by rotary evaporation to afford a white solid. This was stirred with water for 30 minutes, then extracted with dichloromethane. The organic layer was washed with water and dried with MgSO₄, filtered, and concentrated to give a white solid, which was recrystallized from ethyl acetate and *n*-hexane to afford white crystals. Yield: 900 mg (89 %); mp: 184-186°C; IR (cm⁻¹): 3418, 2929; $^1\text{H-NMR}$ δ : 0.77 (3H, d, $J=2.4\text{Hz}$, H-27), 0.81 (3H, s, H-18), 0.97 (3H, d, $J=6.8\text{Hz}$, H-21), 1.01 (3H, s, H-19), 3.34 (1H, br d, $J=9.0\text{Hz}$, H-26), 3.37-3.52 (1H, m, H-26), 4.23-4.31 (1H, m, H-3), 4.36-4.37 (1H, m, H-16), 5.37 (1H, br s, H-6), 5.62 (1H, br d, $J=10.0\text{Hz}$, H-4), 5.92 (1H, dd, $J=2.8, 9.8\text{Hz}$, H-7); $^{13}\text{C-NMR}$ δ : 144.7, 131.0, 128.2, 126.4, 109.3, 80.8, 68.1, 67.0, 62.2, 54.0, 51.6, 41.7, 41.5, 40.0, 37.1, 35.3, 33.7, 31.5, 30.4, 29.2, 28.9, 20.7, 18.3, 17.2, 16.5, 14.6; FABMS (M+H) $^{+}$: 413.2.

25(R)-4,6-Spirostadien-3-one (6). To a solution of compound **5** (500 mg, 1.2 mmol) in methanol (10 mL) was added a mixture of 5 % NaOH-MeOH (0.25 mL) and 30 % H₂O₂ (0.75 mL) and then it was treated as described for the synthesis of compound **3**. Pure compound **6** was obtained as white crystals by column chromatography (ethyl acetate-*n*-hexane=1:5). Yield: 123 mg (25 %); mp: 108-110°C; IR (cm⁻¹): 2940, 1664 ; $^1\text{H-NMR}$ δ : 0.80 (3H, d, $J=6.4\text{Hz}$, H-27), 0.87 (3H, s, H-18), 0.98 (3H, d, $J=6.4\text{Hz}$, H-21), 1.26 (3H, s, H-19), 3.37-3.50 (1H, m, H-26), 4.40-4.51 (1H, m, H-16), 5.67 (1H, s, H-4), 6.11 (2H, d, $J=2.4\text{Hz}$ H-6, H-7); $^{13}\text{C-NMR}$ δ : 163.4, 140.6, 127.8, 123.6, 109.2, 80.4, 66.8, 62.0, 53.1, 50.7, 41.6, 41.3, 39.5, 37.2, 36.1, 33.9, 33.8, 31.3, 31.2, 30.2, 29.6, 28.7, 20.4, 17.1, 16.3, 16.2, 14.4; FABMS (M+H) $^{+}$: 411.2.

25(R)-4 β ,5 β -Epoxy-6-spirosten-3 β -ol (7). *m*-Chloroperoxybenzoic acid (486 mg, 2.82 mmol) was added to a solution of dienol **5** (1 g, 2.43 mmol) in CHCl₃ (100 mL) at room temperature and then the reaction mixture was treated as described for synthesis of compound **1**. Pure compound **7** was obtained as white crystals by column chromatography (ethyl acetate-*n*-hexane=1:3). Yield: 603mg (58 %); mp : 114-116 °C; IR (cm⁻¹): 3500, 2950; $^1\text{H-NMR}$ δ : 0.74 (3H, d, $J=6.0\text{Hz}$, H-27), 0.84 (3H, s, H-18), 0.96 (3H, d, $J=6.8\text{Hz}$, H-21), 1.28(3H, s, H-19), 3.23-3.45 (2H, m, H-26), 3.55-3.65 (1H, m, H-3), 4.15 (1H, d, $J=3.4\text{Hz}$, H-4), 4.60-4.70 (1H, m, H-16), 5.60 (1H, dd, $J=2.2, 8.0\text{Hz}$, H-7), 5.66 (1H, d, $J=2.2\text{Hz}$, H-6); $^{13}\text{C-NMR}$ δ : 149.4, 134.6, 127.6, 125.0, 109.4, 80.6, 71.9, 69.3, 66.9, 61.9,

49.3, 44.0, 41.8, 40.3, 39.4, 37.2, 36.6, 35.9, 31.8, 31.4, 30.3, 28.8, 25.2, 20.2, 19.5, 17.2, 16.0, 14.6; FABMS (M+H)⁺: 429.2.

References

1. Dewick, P. M. *Medicinal Natural Products*, 2nd ed.; John Wiley & Sons: Chichester, U.K., **2002**; pp. 237-289.
2. Laguna, J.; Gomez-Puyoy, A.; Pena, A.; Guzman-Garcia, J. Effect of diosgenin on cholesterol metabolism. *J. Atheroscler. Res.* **1962**, 2, 459-470.
3. Peifer, J. J.; Guzman, J. Hypocholesterolemic effects of diosgenin in the rat. *Circulation* **1966**, 34, III-25.
4. Accatino, L.; Pizarro, M.; Solis, N.; Koenig, C. S. Effects of diosgenin a plant-derived steroid, on bile secretion and hepatocellular cholestasis induced by estrogens in the rat. *Hepatology* **1998**, 28, 129-140.
5. Cayen, M. N.; Dvornik, D. Effect of diosgenin on lipid metabolism in rats. *J. Lipid Res.* **1979**, 20, 162-174.
6. Uchida, K.; Takase, H.; Nomura, Y.; Takeda, K.; Takeuchi, N.; Ishikawa, Y. Changes in biliary and fecal bile acids in mice after treatments with diosgenin and β -sitosterol. *J. Lipid Res.* **1984**, 25, 236-245.
7. Datt, K.; Datta, S. K.; Datta, P. C. Pharmacognostic evaluation of potential yams *Dioscorea*. *J. Econ. Taxon. Bot.* **1984**, 5, 181-196.
8. Noam, M.; Tamir, I.; Breuer, E.; Mechoulam, R. Conversion of ruscogenin into 1 α - and 1 β -hydroxycholesterol. *Tetrahedron* **1981**, 37, 597-604.
9. Liu, M.; Yu, B.; Hui, Y. First total synthesis of 25(R)-ruscogenin-1-yl β -D-xylopyranosyl-(1 $\rightarrow$ 3)-(β -D-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-fucopyranoside, an Ophiopogonis saponin from the tuber of *Liliope muscari* (Decne.). *Tetrahedron Lett.* **1998**, 39, 415-418.
10. Furst, A.; Labler, L.; Meier, W. An effect procedure for preparing 1 α ,3 β -dihydroxy- Δ^5 -steroids by reduction of 1 α ,2 α -epoxy-4,6-dien-3-ones with lithium in ammonia. *Helv. Chim. Acta* **1981**, 64, 1870-1892.
11. Kobayashi, Y.; Taguchi, T.; Mitsuhashi, S.; Eguchi, T.; Ohshima, E.; Ikekawa, N. Studies on organic fluorine compounds. XXXIX. Studies on steroids. LXXIX. Synthesis of 1 α ,25-dihydroxy-26,26,26,27,27,27-hexafluorovitamin D. *Chem. Pharm. Bull.* **1982**, 30, 4297-4303.
12. Araghiniknam, M.; Chung, S.; Tresa, N.-W.; Eskelson, C.; Watson, R. R. Antioxidant activity of dioscorea and dehydroepiandrosterone (DHEA) in older humans. *Life Sci.* **1996**, 59, 147-157.
13. Schulz, S.; Klann, R. C.; Schonfeld, S.; Nyce, J. W. Mechanisms of cell growth inhibition and cell cycle arrest in human colonic adenocarcinoma cells by dehydroepiandrosterone: role of isoprenoid biosynthesis. *Cancer Res.* **1992**, 52, 1372-1376.

14. Boros, L. G.; Puigjaner, J.; Cascante, M.; Lee, W. N.; Brandes, J. L.; Brassilian, S. Oxythiamine and dehydroepiandrosterone inhibit the nonoxidative synthesis of ribose and tumor cell proliferation. *Cancer Res.* **1997**, *57*, 4242-4248.
15. Gao, H.; Dias J. R. Synthetic studies toward 1 α -hydroxytestosterone from testosterone. *Fourth International Electronic Conference on Synthetic Organic Chemistry (ECSOC-4)*, **2000**, September, 1-30.
16. Gamoh, K.; Hirayama, M.; Ikekawa, N. Stereocontrolled synthesis of Withanolide D and related compounds. *J. Chem. Soc., Perkin Trans. I* **1984**, 440-454.
17. Sharpless, K. B.; Verhoeven, T. R. Metal-catalysed, highly selective oxygenations of olefins and acetylenes with *tert*-butyl hydroperoxide. Practical considerations and mechanisms. *Aldrichim. Acta.* **1979**, *12*, 63-75.

Sample Availability: Samples of compounds **1**, **2**, **3**, **4**, **5** and **7** are available from the author.

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