A novel route for the preparation of betamethasone from 9α-hydroxyandrost-4-ene-3,17-dione (9αOH-AD) by chemical synthesis and fermentation

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A novel and efficient synthesis of betamethasone has been developed from the readily available 9α-hydroxyandrost-4-ene-3,17-dione (9αOH-AD). The 16α-methyl was introduced stereoselectively with CH_3Br and converted to the 16β-methyl, the 17-side chain was installed with 2-chlorovinyl ethyl ether in the place of the toxic KCN/HOAc, and a mild fermentation was employed for the 1,2-dehydrogenation, replacing the DDQ oxidation. By adjustments and improvements of the steps, this route produced betamethasone in 11 steps with a 22.9% overall yield, showing its potential for industrial application with relatively low toxicity and cost.

Keywords: betamethasone, phytosterol, 9αOH-AD, fermentation

Betamethasone is widely used in the clinic to treat many diseases, such as rheumatic disorders, skin diseases, allergic conditions, Crohn's disease and leukemia.[1–3] It is also a key intermediate for some important drugs, such as clobetasol and diflorasone.[4] Since it was first reported by Oliveto *et al*. in 1966, the synthesis of betamethasone has been studied in great detail.[5–9] Diosgenin, ticogenin and hecogenin (Fig. 1), which are extracted from specific plants in low relative amounts, are the main raw materials for its commercial production.[10] Thus, even though methods to produce betamethasone exist, the issues of environmental pollution and the rising price of raw materials urge chemists to develop new processes for its preparation from a cheaper starting material.[11]

Phytosterols are abundant natural resources and industrial waste. In recent years, the development of efficient fermentation processes using phytosterols has made 9α-hydroxyandrost-4-ene-3,17-one (9αOH-AD) and 4-ene-3,17-dione (4-AD)[12] readily available for the synthesis of glucocorticoids. It would be highly desirable if 9αOH-AD could be utilised for the preparation of betamethasone. However, two major challenges of the synthesis remain. They are the stereoselective introduction of the 16β-methyl group and the 17-side chain. Carruthers was able to introduce the 16β-methyl group stereoselectively *via* the oxalate activation/blocking method.[13,14] As for the synthesis of the side chain, the classical cyanohydrin method (KCN/HOAc) has been utilised. Even though this route provides a viable synthesis of betamethasone from 9αOH-AD, the need to use KCN and MeI makes the process less practical. Moreover, only a low overall yield was obtained. Herein we report an improved synthesis of betamethasone from 9αOH-AD. Notable features of this route involve direct 16-methylation with CH_3Br and a 1,2-dehydrogenation *via* a fermentation processes.[15,16]

Results and discussion

The 9α,11β-halohydrin functionality in steroids, which is essential for the biological activity of these compounds, is typically obtained from the corresponding $\Delta^{9,11}$ double bond. Normally dehydration of 11α-hydroxy steroid has been the common method to introduce the $\Delta^{9,11}$ double bond. However, a $\Delta^{11,12}$ side product is inevitable in this process.[17] Consequently, we decided to use 9αOH-AD as the starting material as it can be easily produced by fermentation. By treating 9α-hydroxy (**1**) with a mixture of AcOH, DCM and 70% H_2SO_4, the $\Delta^{9,11}$-ethene (**2**) was obtained in 95% yield, and more importantly, no $\Delta^{11,12}$ impurity was formed (Scheme 1).

The 3-keto group of **2** was easily protected as a vinyl ether with triethyl orthoformate to form intermediate **3** in excellent yield. Next, deprotonation of **3** with base and subsequent alkylation with CH_3I give **4** as the major product (Scheme 2). Large amounts of the double alkylation product **14** were formed when CH_3ONa and *t*-BuOK were used as base (Table

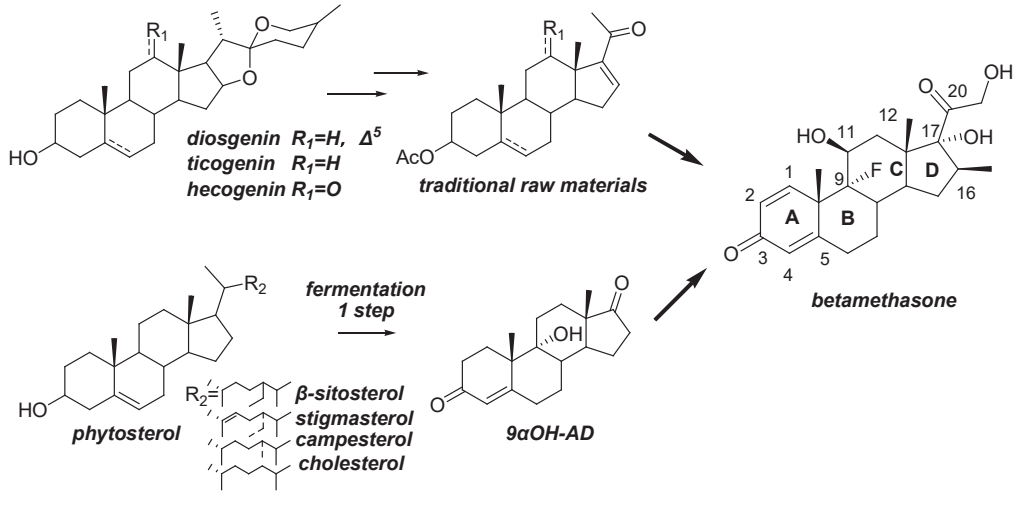

Fig. 1 Raw materials of betamethasone.

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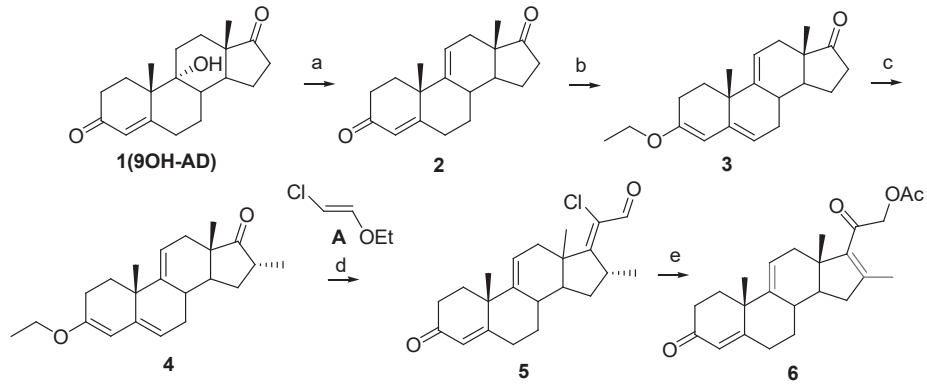
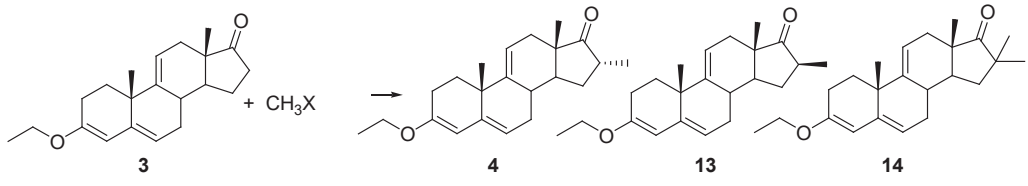


(a) AcOH/DCM, 70% H_2SO_4, r.t., 95%; (b) triethyl orthoformate/EtOH/*p*TSA, 40 °C, 96%; (c) LDA/HMPA/CH_3Br, THF, −40 °C–r.t., 92%; (d) *n*-BuLi/2-chlorovinyl ethyl ether(*trans*)/THF, −40 °C, HCl, 92%; (e) KOAc/DMF/Ac_2O, 115 °C, 88%

Scheme 1 Synthesis of critical intermediate **6** for betamethasone.



Scheme 2 Methylation of steroid **3**.

Table 1 Optimisation of 16-methylation[a]

Entry	Base	Temperature (°C)	Additive	CH_3X	Yield (%)	**3**/**4**/**13**/**14** (%)[b]
1	CH_3ONa (1.5 equiv.)	−20	–	CH_3I	86	14/**56**/2/20
2	*t*-BuOK (1.5 equiv.)	−20	–	CH_3I	72	9/**61**/2/21
3	LDA (1.5 equiv.)	−20	–	CH_3I	92	5/**89**/2/4
4	LDA (1.5 equiv.)	−40	–	CH_3I	92	5/**92**/1/2
5	LDA (1.2 equiv.)	−40	–	CH_3I	93	6/**91**/1/2
6	LDA (1.2 equiv.)	−40	–	CH_3Br	91	17/**80**/2/1
7	LDA (1.2 equiv.)	−40–r.t.	–	CH_3Br	91	11/**86**/1/1
8	LDA (1.2 equiv.)	−40–r.t.	–	CH_3Cl	–	No product
9	LDA (1.2 equiv.)	−40–r.t.	TMEDA	CH_3Br	88	3/**94**/2/1
10	LDA (1.2 equiv.)	−40–r.t.	HMPA	CH_3Br	92	1/**97**/1/1
11	LDA (1.2 equiv.)	−40–r.t.	HMPA	CH_3I	93	0/**98**/1/1
12	LDA (1.2 equiv.)	−40–r.t.	DMPU	CH_3Br	87	9/**88**/2/1
13	LDA (1.2 equiv.)	−40–r.t.	12-crown-4	CH_3Br	90	3/**94**/2/1

[a]All the reactions were carried out in the solvent of THF.

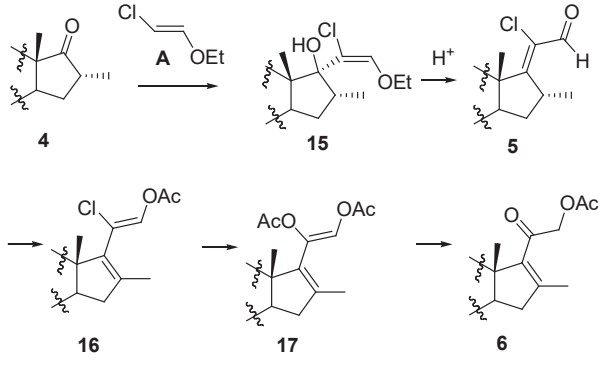
[b]Selectivity of products, the bold numbers indicate that the ratio of **4** was the main product of this reaction.

1, entries 1 and 2). When the base was switched to lithium diisopropylamide (LDA) and the reaction was run at a lower temperature, the formation of product **14** was largely suppressed and a yield as high as 92% was obtained (Table 1, entry 4). It is worth mentioning that the stereoselectivity of the methylation process was very high, possibly due to the steric hindrance of the 18β-methyl group; however, the reaction conversion was not satisfactory, about 5–6% of **3** remained. We also examined the possibility of replacing CH_3I with CH_3Br as it is considerably cheaper (Table 1, entries 5 and 6). However, a much lower conversion was obtained. This was to be expected as the reactivity of CH_3Br is much lower than the corresponding CH_3I. With CH_3Cl, we did not observe the formation of **4** at all (Table 1, entry 8). To speed up the alkylation process, some additives such as hexamethylphosphoric triamide (HMPA), *N*,*N*,*N*,*N*-tetramethylethylenediamine (TMEDA), *N*,*N*-dimethylpropyl-eneurea (DMPU) and 12-crown-4 were used. Among the additives tested, HMPA gave the best result and a 97% selectivity was obtained with CH_3Br (Table 1, entry 10). Based on the above results, we decided to affect the 16-methylation step with the LDA, HMPA and CH_3Br system.

For the introduction of the 17-side chain, 2-chlorovinyl ethyl ether (*trans*) was converted to the corresponding lithium olefin when mixed with *n*-butyl lithium at −45 °C under nitrogen. Subsequent addition of the 17-keto steroid **4** at approximately −45–−25 °C gave the 21-aldehyde **5** (mixture of *trans* and *cis*) in 92% yield after the reaction mixture was hydrolysed with hydrochloric acid. Compound **5** was smoothly transformed into **6** in 85% yield by a rearrangement process with NaOAc and acetic anhydride at high temperature (115 °C) (Scheme 3). The olefinic intermediates **16** and **17** were detected in the process. A catalytic amount of acetic anhydride was added to increase the reaction rate and yield. In summary, the critical intermediate 16-methly-4,9(11),16-triene-3,20-dione-21-acetate (**6**) was

efficiently synthesised from **1** in just five steps with an overall 67.9% yield.

Next we were faced with the challenge of installing the 17α-OH group. Epoxidation of the double bond at C16 of **6** with *m*-CPBA (1.2 equiv.) afforded the epoxy derivative **7**; however, some epoxidation of Δ^4 was also inevitable. So we attempted to carry out dehydrogenation of C1(2) first, thinking that subsequent epoxidation of C16 would be site specific. However, the low conversion in the dehydrogenation step led us to give up this strategy. Finally, the problem of epoxidation at Δ^4 was overcome by running the epoxidation at low temperature and the content of the side product was reduced to less than 11% (Scheme 4). After treating **7** with HBr and pyridine directly, **8** was obtained in 58% yield (two steps) after recrystallisation. Subsequent transformations of intermediates **9** and **10** were carried out by conventional chemistry without any difficulties. Hydrogenation with Wilkinson's catalyst gave the 16β-methyl **9** stereoselectively with 82% yield. Compound **9** was treated with 1,3-dibromo-5, 5-dimethyl hydantoin (DBH) in acetone followed by treatment with NaOH. The bromo-alcohol generated *in situ* was cyclised to produce the desired 9,11β-epoxide in 96% yield. 1,2-Dehydrogenation of **10** by microorganism fermentation gave **11** with a better yield and purity than DDQ. Crude **11** was recrystallised three times to remove all the impurities. Finally, betamethasone **12** was obtained by ring opening the 9,11β-epoxide with 70% HF.



Scheme 3 Process for intermediate **6**.

Conclusion

A novel and improved synthesis of betamethasone has been developed using readily available 9αOH-AD as the starting material. The success of the synthesis critically hinges on the indirect 16β-methylation with CH_3Br, introduction of the 17-side chain with 2-chlorovinyl ethyl ether as well as using microorganism fermentation for the 1,2-dehydrogenation. This new synthesis has significant advantages, in terms of cost, safety and environmental friendliness, and gives betamethasone in 11 steps with a 22.9% overall yield.

Experimental

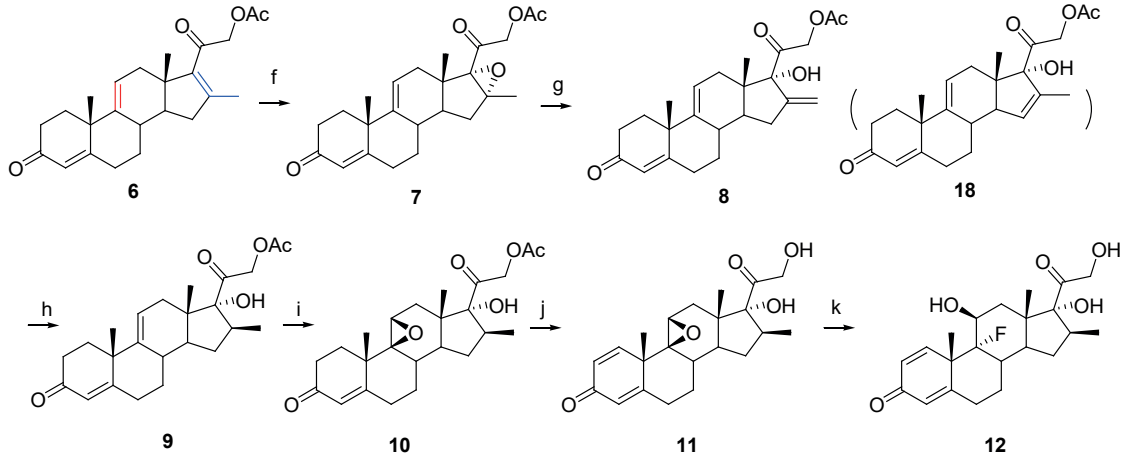
The raw material (9αOH-AD) was provided by Hunan Norchem Pharmaceutical Co. Ltd. All other chemicals were obtained commercially and used directly without further purification. The progress of the reactions was monitored by thin-layer chromatography (TLC) on silica gel. Melting points were determined in open capillary tubes with a MFB 595010 M Gallenkamp melting point apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz Advance (400 MHz for ^{1}H, 100.6 MHz for ^{13}C) spectrometer. Chemical shifts (Δ) are reported in ppm relative to the internal standard TMS. MS data were recorded on an Agilent Technologies 6120 mass spectrometer.

Synthesis of 4,9(11)-androstadiene-3,17-dione (**2**)

Steroid **1** (500 g, 1.65 mol) was added to a solution of AcOH (250 mL) and H_2SO_4 (70%, 150 mL) in CH_2Cl_2 (2000 mL). The solution was refluxed until **1** disappeared (measured by TLC, benzene:acetone = 6:1) and then H_2O (1000 mL) was added at r.t. The mixture was neutralised with NaOH (5 N). The organic phase was separated, washed with H_2O (3 × 1000 mL) and evaporated to give compound **2** as: Yellow solid; m.p. 200–203 °C (lit.[18] 202–204 °C); yield 446.7 g (95.0%); ^{1}H NMR (400 MHz, $CDCl_3$): Δ 5.72 (s, 1H), 5.530 (t, *J* = 4.8 Hz, 1H), 2.45–2.57 (m 1H), 2.34–2.45 (m, 5H), 2.08–2,14 (m, 7H), 1.59–1.62 (m, 1H), 1.32 (s, 3H), 1.11–1.16 (m, 1H), 0.85 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$): Δ 199.1, 169.0, 145.2, 124.3, 118.2, 48.1, 45.9, 41.2, 36.9, 36.3, 34.3, 33.9, 33.5, 32.7, 31.2, 31.1, 26.3, 22.7, 14.0; MS-ESI (*m*/*z*): 285.2 $[M + H]^+$.

Synthesis of 3-ethoxylandrosta-3,5,9(11)-diene-17-one (**3**)

4,9(11)-Androstadiene-3,17-dione (**2**, 500 g, 1.76 mol), absolute alcohol (1000 mL) and triethyl orthoformate (400 g, 2.70 mol) were slurried at room temperature (25 °C) and the atmosphere was replaced with nitrogen. *p*-Toluenesulfonic acid (9.0 g, 0.052 mol) was added and the mixture was heated with stirring at 40 °C for about 4 h until **2**



(f) *m*-CPBA/DCM, 0 °C; (g) HBr/dioxane/pyridine, 0 °C, 58% two steps; (h) $RhCl(PPh_3)_3/H_2$, 82%; (i) $HClO_4$/DBH, MeOH/NaOH, 96%; (j) *Nocardioides simplex*/glucose/phosphate buffer, 83%; (k) HF/DMF, 89%

Scheme 4 Synthesis of betamethasone from **6**.

disappeared (TLC, benzene:acetone = 12:1). The mixture was cooled to 20 °C and stirring was maintained for 1 h after water (100 mL) was added. The reaction mixture was poured into a solution of ice and sodium carbonate (0.1%, 5000 mL). The precipitate was collected by centrifugation, washed with absolute alcohol (100 mL) and dried at 30–35 °C to give compound **3** as: Yellow solid; m.p. 114–118 °C (decomposed); yield 527.4 g (96%); ^{1}H NMR (400 MHz, $CDCl_3$): Δ 5.49 (s, 1H), 5.24–5.25 (m, 1H), 5.15 (s, 1H), 3.73–3.81 (m, 2H), 2.41–2.59 (m, 4H), 1.87–2.39 (m, 7H), 1.54–1.64 (m, 3H), 1.22–1.35 (m, 3H), 1.14 (s, 3H), 0.86 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$): Δ 154.9, 144.9, 139.2, 124.3, 116.8, 115.0, 98.9, 62.4, 49.9, 46.4, 37.3, 36.3, 33.5, 32.3, 31.9, 27.4, 25.7, 22.9, 14.8, 14.0, 13.8; MS-ESI (*m*/*z*): 313.2 $[M + H]^+$.

Synthesis of 3-ethoxy-16α-methylandrosta-3,5,9(11)-diene-17-one (**4**)

A solution of diisopropylamine (148.6 mL, 1.06 mol) and HMPA (80 mL, 0.43 mol) in tetrahydrofuran (400 mL) was cooled to −40 °C under argon, and a solution of *n*-butyllithium in hexanes (1.6 M, 60 mL, 1.5 equiv.) was added slowly (<−20 °C) over 0.5–1.0 h. Then, the resulting LDA solution was cooled to −40 °C, **3** (200 g, 0.64 mol) was added in three portions and methyl bromide (100 g, 1.05 mol, dissolved in 100 mL THF) was added (<−40 °C). The mixture was stirred for 3 h from −40 °C to r.t. After reaction completion (TLC, benzene:acetone = 12:1), water (50 mL) was added to quench the reaction followed by acetic acid (30 mL). The organic solvent was evaporated under reduced pressure at 50 °C, more water (500 mL) was pumped in and the slurry was stirred for 0.5 h at 0–5 °C. The precipitate was collected and washed with water, added to methanol (200 mL) and the slurry was heated to 40–45 °C for 30 min, cooled to 0–5 °C for another 1 h, filtered and dried at 30–35 °C to give product **4** as: White solid; m.p. 123–125 °C (decomposed); yield 192.2 g (92%); ^{1}H NMR (400 MHz, $CDCl_3$): Δ 5.50–5.51 (m, 1H), 5.27 (s, 1H), 5.16 (s, 1H), 3.73–3.83 (m, 2H), 2.23–2.58 (m, 3H), 1.58–2.18 (m, 10H), 1.37–1.48 (m, 3H), 1.10–1.26 (m, 6H), 0.88 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$): Δ 155.0, 145.0, 139.4, 117,0, 115.3, 99.0, 62.5, 47.3, 47.1, 40.1, 37.4, 33.9, 33.3, 32.3, 32.0, 31.3, 31.1, 27.4, 25.8, 17.0, 14.8, 14.3; MS-ESI (*m*/*z*): 327.2 $[M + H]^+$.

Synthesis of 20-chloro-3-keto-16α-methylpregna-4,9(11),17(20)-triene-21-al (**5**)

Anhydrous THF (400 mL) and 2-chloro-1-ethoxyethylene (36 g, 0.338 mol) were cooled to −45 °C under nitrogen. *n*-BuLi in hexanes (400 mL) was added over 30 min (−30 °C). The mixture was stirred for about 15 min. Compound **4** (100 g, 0.306 mol) was added at once and the solution was stirred for 3 h at approximately −40−−45 °C The mixture was then poured into hydrochloric acid (6 N, 300 mL) and stirring was maintained at 15–20 °C for 2 h (TLC, benzene:acetone = 10:1). The THF was removed under reduced pressure and water (500 mL) was added. The slurry was filtered at 5–10 °C and recrystallisation from ethyl acetate gave compound **5** as: White solid; m.p. 145–147 °C; yield 101.1 g (92%); ^{1}H NMR (400 MHz, $CDCl_3$): Δ 9.76 (s, 1H), 5.75 (s, 1H), 5.55 (d, J = 4.0, 1H), 3.59 (m, 1H), 2.89–2.94 (dd, J_1 = 7.9, J_2 = 2.9, 1H), 2.11–2.59 (m, 10H), 1.73 (m, 4H), 1.74 (s, 3H), 1.25–1.27 (m, 4H), 1.02 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$): Δ 199.2, 184.6, 175.6, 169.2, 145.3, 126.5, 124.3, 118.9, 48.3, 47.6, 46.4, 41.2, 41.1, 38.4, 36.0, 35.9, 33.9, 32.7, 32.6, 32.0, 26.3, 14.4. MS-ESI (*m*/*z*): 359.1 $[M + H]^+$.

Synthesis of 21-acetoxy-16-methylpregna-4,9(11),16-triene-3,20-dione (**6**)

Anhydrous sodium acetate (60 g, 0.73 mol) and DMF (300 mL) containing acetic anhydride (12 mL, 0.13 mol) were stirred and heated to 115 °C under nitrogen. Compound **5** (100 g, 0.279 mol) dissolved in DMF (300 mL) was added dropwise over 0.5 h, and the mixture was stirred for 1.5 h at 115 °C (TLC, benzene:acetone = 10:1). Some of the DMF was removed under reduced pressure and the remaining solution was poured into ice water (1000 mL). The precipitate was collected by filtration to give compound **6** as: Brown solid; m.p. 166–169 °C; yield 93.8 g (88%); ^{1}H NMR (400 MHz, $CDCl_3$): Δ 7.73 (s, 1H), 5.51 (d, J = 4.0, 1H), 4.91 (d, J = 8.0, 1H), 4.77 (d, J = 8.0, 1H), 2.46–2.58 (m, 1H), 2.44–2.45 (m, 2H), 2.24–2,37 (m, 6H), 2.18 (s, 3H), 2.08–2.17 (m, 2H), 2.09 (s, 1H), 1.98–2.01 (m, 1H), 1.53–1.55 (m, 1H), 1.34 (s, 3H), 1.11–1.15 (m, 1H), 0.91 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$): Δ 199.3, 192.6, 170.5, 169.5, 154.2, 145.3, 143.7, 124.2, 119.3, 68.5, 51.1, 47.0, 41.2, 40.9, 38.2, 35.1, 34.4, 33.8, 32.8, 31.9, 26.3, 20.6, 18.5, 16.0. MS-ESI (*m*/*z*): 382.5 $[M + H]^+$.

Synthesis of 21-acetoxy-16 (17) α-epoxy-16-methylpregna-4,9(11)-diene-3,20-dione (**7**)

Compound **6** (100 g, 0.261 mol) was dissolved in CH_2Cl_2 (500 mL) and the solution was cooled to −5–0 °C. *m*-CBPA (54.1 g, 0.314 mol) was added in five portions every 20 min, then the mixture was stirred for 6 h at 0 °C (TLC, benzene:acetone = 6:1) and then filtered. The solution was washed with sodium carbonate solution (1%, 300 mL) and the CH_2Cl_2 was removed under reduced pressure. Dioxane (300 mL) was added and the solution of **7** was used directly for the next step.

Synthesis of 21-acetoxy-17α-hydroxy-16-methylenepregna-4,9(11)-diene-3,20-dione (**8**)

A solution of **7** in dioxane was stirred at r.t. (25–30 °C). Aqueous hydrobromic acid (40%, 100 mL) was added dropwise into P_2O_5 and anhydrous hydrobromic acid was bubbled into the solution over 1 h (<35 °C). The solution was stirred for another 4 h and then cooled to 0–5 °C. Pyridine (40 mL, 0.523 mol) was added dropwise at 0–5 °C to neutralise the reaction (TLC, benzene:acetone = 6:1), the resulting mixture (pH = 6–7) was poured into ice water (1000 mL) and the slurry was filtered at 0–5 °C. The wet product was added to methanol (100 mL) and the slurry was stirred at r.t. for 30 min, cooled to 0–5 °C for another 1 h, filtered and dried at 30–35 °C to yield compound **8** (with the isomer **18**) as: Yellow solid; yield 60.4 g (two steps, 58%).

Synthesis of 21-acetyloxy-17α-hydroxy-16β-methylpregna-4,9(11)-diene-3,20-dione (**9**)

Compound **8** (20 g, 0.05 mol) was dissolved in anhydrous ethyl acetate (400 mL). A rhodium catalyst (Wilkinson's catalyst $RhCl(PPh_3)_3$, 0.60 g) was added to the mixture under nitrogen. The reaction mixture was then stirred under hydrogen (1 atm, hydrogen balloon) at 60–65 °C for 6–8 h. The solution was washed with water (200 mL) and the ethyl acetate was removed under reduced pressure. Crystallisation at 0–5 °C gave compound **9** as: Yellow solid; m.p. 208–212 °C (lit.[19] 210–214 °C); yield 16.5 g (82%); ^{1}H NMR (400 MHz, $CDCl_3$): Δ 5.72 (s, 1H), 4.56 (d, J = 8.2, 1H), 4.32 (d, J = 8.2, 1H), 3.35 (m, 2H), 2.46–2.58 (m, 1H), 2.45–2.48 (m, 4H), 2.11–2.32 (m, 8H), 1.90 (m, 1H), 1.61–1.72 (m, 4H), 1.39 (s, 3H), 1.09 (d, J = 3.2, 3H), 1.08 (m, 1H), 0.96 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$): Δ 202.8, 199.5, 171.3, 124.1, 89.7, 65.3, 60.3, 50.4, 48.2, 48.1, 44.7, 44.6, 39.6, 35.9, 34.6, 34.0, 31.4, 31.2, 29.5, 26.0, 23.7, 23.6, 20.1, 17.7; MS-ESI (*m*/*z*): 401.1 $[M + H]^+$.

Synthesis of 21-acetoxy-17α-hydroxy-9β,11β-epoxy-16β-methylpregna-4-ene-3,20-dione (**10**)

Compound **9** (20 g, 0.05 mol) was suspended in acetone (300 mL) and H_2O (30 mL) at 0 °C, and $HClO_4$ (70%, 1.52 g) was added to the mixture. This was followed by the addition of DBH at 0–5 °C over about 30 min. The mixture was stirred for 2 h at 0–5 °C. Potassium carbonate solution (20%, 200 mL) was then added dropwise and the temperature of the slurry was allowed to rise to r.t. (25–30 °C) for 2 h (TLC, benzene:acetone = 4:1). Acetic acid was then added to modify the pH to 6–7. The solvents were removed by distillation under reduced pressure, and the resulting slurry was cooled to about 0 °C, filtered and dried at 50 °C to provide compound **10** as: White solid; m.p. 212–214 °C (lit.[8] 212–216 °C); yield 19.9 g (96%); ^{1}H NMR (400 MHz, $CDCl_3$): Δ 5.75 (s, 1H), 4.99 (d, J = 7.6, 1H), 4.80 (d, J = 7.6, 1H), 3.40 (s, 1H), 3.01 (s, 1H), 2.46–2.47 (m, 3H), 2.13–2.15 (m, 6H), 1.83–1.87 (m, 2H), 1.56–1.62 (m, 3H), 1.40 (s, 3H), 1.09–1.01 (m, 4H), 0.9 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$): Δ 204.7, 199.4, 170.0, 170.8, 124.2, 89.0, 69.4, 65.3, 65.2, 60.4, 48.6, 48.2, 44.9, 39.6, 35.9, 34.7, 31.4, 31.0, 29.5, 26.1, 23.7, 20.7, 20.0, 17.3; MS-ESI (*m*/*z*): 417.2 $[M + H]^+$.

Synthesis of 17α,21-dihydroxy-9β,11β-epoxy-16β-methylpregna-1,4-diene-3,20-dione (**11**)

The steroid bioconversions used *Nocardioides simplex* VKM Ac-2033D cultures grown in fermentation medium (5.6 L) at 28 °C (200 rpm). Medium: glucose (15.0 g L^{-1}), corn steep liquor (20.0 g L^{-1}), yeast extract (2.0 g L^{-1}), K_2HPO_4 (2.5 g L^{-1}), antifoam (0.4 g L^{-1}), pH = ~7.5–7.6. After 18 h growth to the late-exponential phase (OD_{600}, ~1.5–2.0), the steroid substrate **10** (50 g, 0.12 mol) was added as a fine powder and bioconversion was monitored by TLC (benzene:acetone = 4:1) for 76 h. Then the mixture was filtered and the filter cake was dissolved in chloroform:methanol (2:1, 1500 mL), filtered again, and the methanol was replaced with chloroform under reduced pressure. The precipitate was crystallised from chloroform at 5–10 °C, filtered and dried to give the steroid **11** as: White solid; m.p. 215–218 °C (lit.[8] 215–220 °C); yield 37.1 g (83%); ^{1}H NMR (400 MHz, DMSO-d_6): Δ 6.60 (m, 1H), 6.09 (m, 2H), 5.22 (s, 1H), 4.35 (d, *J* = 10, 1H), 4.10 (d, *J* = 10, 1H), 3.18 (s, 1H), 2.67 (m, 1H), 2.50 (m, 1H), 2.34–2.36 (m, 1H), 2.18–2.21 (m, 2H), 2.04–2.05 (m, 2H), 1.60–1.61 (m, 1H), 1.50–1.54 (m, 1H), 1.37 (m, 4H), 1.00–1.01 (m, 3H), 0.9 (m, 1H), 0.86 (s, 3H); ^{13}C NMR (100.6 MHz, DMSO-d_6): Δ 211.7, 187.8, 165.6, 152.6, 127.2, 124.1, 87.2, 67.5, 65.7, 62.1, 47.1, 46.8, 45.9, 43.6, 35.6, 33.2, 30.9, 30.3, 28.7, 23.5, 20.0, 17.9. MS-ESI (*m*/*z*): 373.2 $[M + H]^+$.

Synthesis of betamethasone (**12**)

Aqueous hydrogen fluoride (70%, 100 mL) and DMF (3 mL) were cooled to −30 °C in a polyethylene flask. Then compound **11** (20 g, 0.054 mol) was added with stirring in five portions and the temperature was maintained below −15 °C. The mixture was stirred for 2–3 h (TLC, benzene:acetone = 2:1) and then poured into ice water (1600 mL). Ammonium hydroxide (10%, 300 mL) was used to adjust the pH to 6–7, and the precipitate was collected by filtration, washed with water and dried. The product **12** was dissolved in $CHCl_3$/MeOH (1:2, 240 mL), the chloroform was removed under reduced pressure and crystallisation from MeOH gave compound **12** as: White solid; m.p. 231–234 °C (lit.[20] 231–234 °C); yield 18.8 g (89%); ^{1}H NMR (400 MHz, DMSO-d_6): Δ 7.28 (d, *J* = 0.4, 1H), 6.20–6.22 (m, 1H), 6.00 (s, 1H), 5.20 (s, 1H), 5.10 (s, 1H), 4.35–4.44 (m, 2H), 4.11–4.15 (m, 2H), 2.62–2.63 (m, 1H), 2.30–2.35 (m, 2H), 1.89–2.08 (m, 5H), 1.50 (s, 3H), 1.30–1.40 (m, 2H), 1.02–1.04 (m, 5H), 0.97 (s, 3H); ^{13}C NMR (100.6 MHz, DMSO-d_6): Δ 212.2, 185.3, 167.0, 152.8, 129.0, 124.1, 102.1, 100.4, 87.8, 70.8 (d, *J* = 18.5), 67.8, 48.0, 46.8, 42.9, 39.5, 36.2, 34.6, 30.4, 27.6, 23.0, 19.8, 17.0. MS-ESI (*m*/*z*): 393.2 $[M + H]^+$.

Electronic Supplementary Information

The ESI is available through: stl.publisher.ingentaconnect.com/content/stl/jcr/supp-data

Received 19 March 2017; accepted 23 March 2017
Paper 1704660
https://doi.org/10.3184/174751917X14925986241025
Published online: 27 April 2017

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