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# Chemical synthesis of 7α-hydroxypregnenolone, a neuroactive steroid that stimulates locomotor activity

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ABSTRACT

7α-Hydroxypregnenolone is an endogenous neuroactive steroid that stimulates locomotor activity. A synthesis of 7α-hydroxypregnenolone from pregnenolone, which takes advantage of an orthogonal protecting group strategy, is described. In detail, the C7-position was oxidized with $CrO_3$ and 3,5-dimethylpyrazole to yield a 7-keto steroid intermediate. The resulting 7-ketone was stereoselectively reduced to the 7α-hydroxy group with lithium tri-*sec*-butylborohydride. In contrast, reduction of the same 7-ketone intermediate with $NaBH_4$ resulted in primarily the 7β-hydroxy epimer. Furthermore, in an alternative route to the target compound, the 7α-hydroxy group was successfully incorporated by direct C–H allylic benzoyloxylation of pregnenolone-3-acetate with CuBr and *tert*-butyl peroxybenzoate followed by saponification. The disclosed syntheses to 7-oxygenated steroids are amenable to potentially obtain other biologically active sterols and steroids.

## 1. Introduction

7α-Hydroxypregnenolone (Fig. 1, **4**) is a neurosteroid that has been shown to stimulate locomotor activity in newts [1], juvenile birds (biosynthetically produced in the pineal gland) [2], and salmon (stimulating upstream migration) [3]. The biological target that stimulates locomotor activity is unknown, however, it has been suggested that the $GABA_A$ and N-methyl-d-aspartate receptor may be the targets of this neurosteroid since pregnenolone stimulates these receptors [4]. Injection of 7α-hydroxypregnenolone at 6.25 mg/kg body weight has been shown to increase the immune response in mice [5]. Additionally, administration of 7α-hydroxypregnenolone had enhanced spatial memory retention in cognitively impaired aged rats [6]. Moreover, 7α-hydroxypregnenolone has been shown to promote microtubule polymerization [7].

### 1.1. Biosynthesis of 7α-hydroxypregnenolone

Cytochrome P450 7B1 is the enzyme responsible for directly converting pregnenolone to 7α-hydroxypregnenolone (Fig. 1, **3** to **4**) [8,9]. P450 7B1 has a broad substrate scope and has been shown to 7α-hydroxylate 27-hydroxycholesterol and dehydroepiandrosterone [10]. Mutations in *CYP7B1*, the gene that encodes the P450 7B1 protein, results in motor neuron degeneration and hereditary spastic paraplegia type 5 [11–13].

The 7-oxygenated C19-androgens (i.e. 7α-hydroxy-, 7β-hydroxy-, and 7-oxo- dehydroepiandrosterone) have been shown to be interconverted by 11β-hydroxysteroid dehydrogenase type 1 [14].

Other possible biosynthetic pathways to 7α-hydroxypregnenolone may arise from the activity of the cholesterol side chain cleavage enzyme, P450 11A1 (Fig. 1), onto 7α-hydroxycholesterol, which is the enzymatic product of P450 7A1 acting on cholesterol [15].

Despite its physiological importance, a report on the synthesis of 7α-hydroxypregnenolone with spectral characterization has yet to be disclosed. A previous synthesis without NMR data was reported by treating 7α-bromopregnenolone-3-acetate with acetic acid/sodium acetate – resulting in mixtures of 7α- and 7β- acetoxy epimers, which were saponified in $KOH/CH_3OH$ and purified by preparative silica gel thin-layer chromatography with ethyl acetate [16]. A more recent synthesis of 7α-hydroxypregnenolone has been reported through displacement of an allylic bromide intermediate with $CaCO_3$ in $H_2O$ [7]. A convenient chemical synthesis of this neuroactive steroid may open the possibility of the synthesis of other neuroactive steroid analogs. Here, two complementary syntheses of 7α-hydroxypregnenolone (**4**) from pregnenolone (**3**) are reported.

### 1.2. Retrosynthetic analysis

The ideal synthesis of 7α-hydroxypregnenolone would come from a one-step biomimetic oxidation (Fig. 1, **3** to **4**), where a diastereo- and

*Abbreviations:* GABA, gamma-aminobutyric acid; NMR, nuclear magnetic resonance; CYP, cytochrome P450; TLC, thin-layer chromatography

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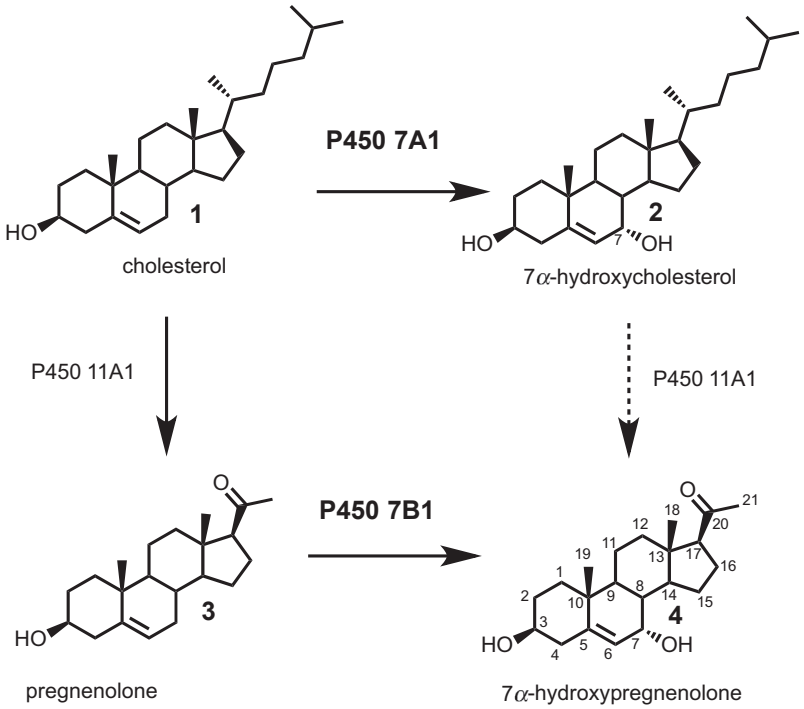



**Fig. 1.** Biosynthesis of 7α-hydroxypregnenolone (**4**).

regio- selective monohydroxylation would occur at the C7-position of pregnenolone to directly furnish 7α-hydroxypregnenolone. In this situation, there are two allylic positions (C4- and C7-) and during the allylic oxidation conditions, the 3β-hydroxy group will not be oxidized. Realistically, the 3-hydroxy group should be protected during the C7-H oxidation process to avoid oxidation to the 3-keto moiety.

Because of the potential oxidation of any free hydroxy during the proposed allylic oxidation process (*vide supra*, **3** to **4**), the chemical synthesis required the protection of the 3-hydroxy group of pregnenolone as the acetate (Fig. 2, Schemes 1 and 3, **9**). The available method to introduce a 7α-hydroxy substituent in the steroid backbone involved the regioselective oxidation of the C7-position to the 7-keto group using $CrO_3$ and 3,5-dimethylpyrazole, followed by diastereoselective reduction with L-selectride. This strategy was previously employed in the synthesis of 7α-hydroxycholestenone from cholesterol [17]. Other methods of C7-oxidation to the ketone exist, which involves a dirhodium-caprolactamate complex in the presence of *tert*-butyl hydroperoxide [18]. In order to follow the oxidation and subsequent diastereoselective reduction strategy, the C20-keto group of pregnenolone would have to be masked. The C20-ketone was therefore reduced to the alcohol, which was orthogonally protected as the silyl ether (Fig. 2, **8**), allowing the 3- and 7- hydroxy groups to be protected as acetates (**6**).

## 2. Results and discussion

To begin, pregnenolone was protected as the acetate to furnish pregnenolone-acetate (Scheme 1, **3** to **9**). The resulting C20-keto group was reduced with $NaBH_4$ to afford the C20-alcohol (**10**). The *R*-stereochemistry at the C20 position of alcohol **10** was determined by the chemical shift of the C18-methyl ($\delta$ 0.75 ppm) in comparison to the known literature value ($\delta$ 0.78 ppm for the C20-*R* stereochemistry compared with $\delta$ 0.68 ppm for the C20-*S* stereochemistry) [19]. In addition, the reduction of pregnenolone-3-acetate (**9**) has been performed with $NaBH_4$ [20] or L-selectride [21] to afford the alcohol (**10**) in 99% or 96% reported yields prior to purification. Furthermore, the

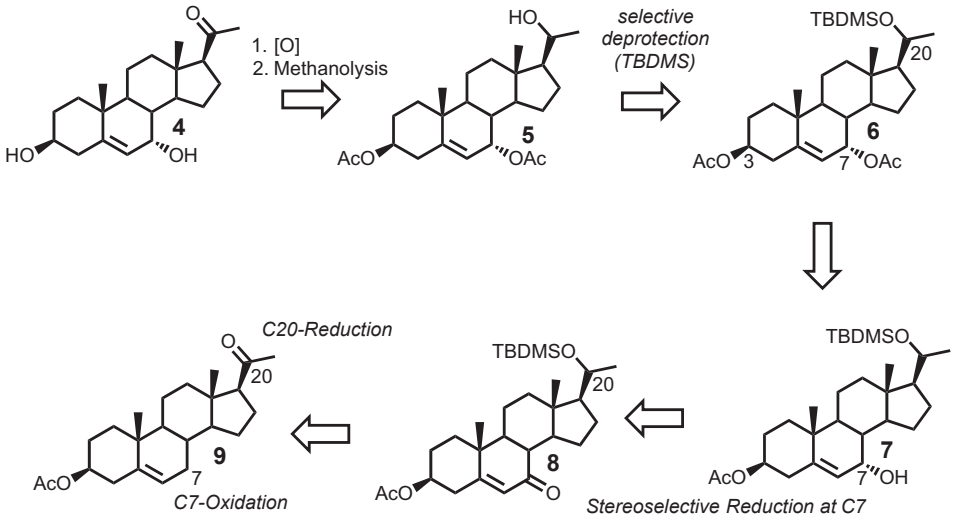
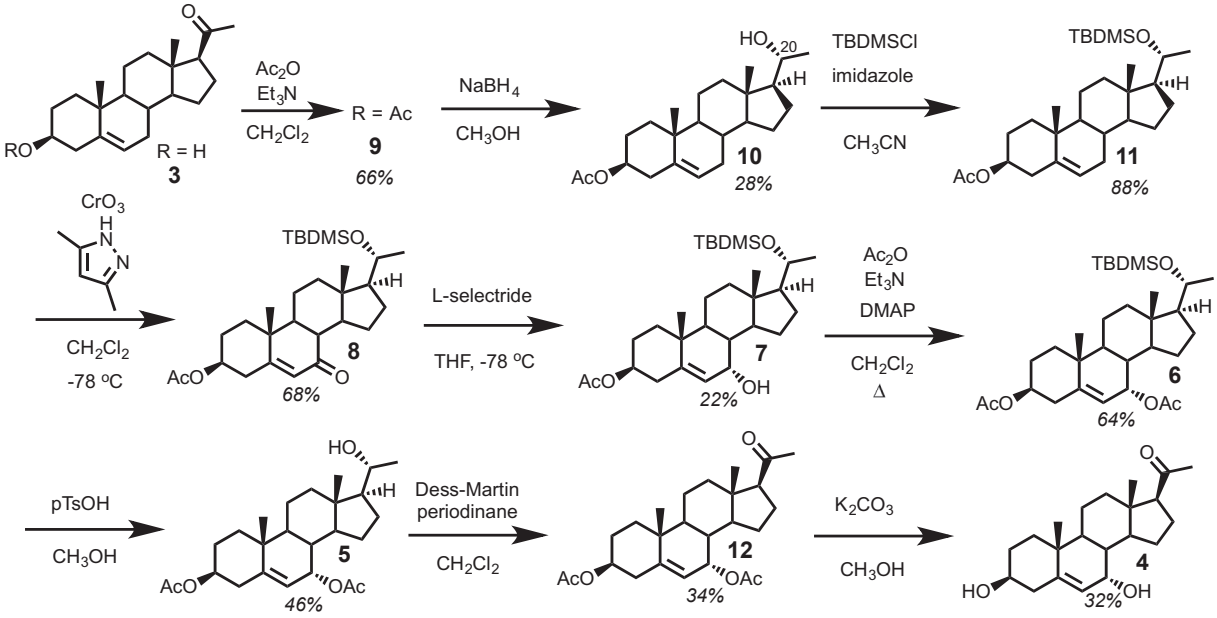



**Fig. 2.** Retrosynthetic analysis of 7α-hydroxypregnenolone using an orthogonal protecting group strategy at C3 and C7 vs. C20 (**6**).




**Scheme 1.** Synthesis of 7α-hydroxypregnenolone (**4**) from pregnenolone (**3**). The stereochemistry at C20 of compound **10** was assigned as the *R*-configuration based on the crystal structure (Fig. 3).

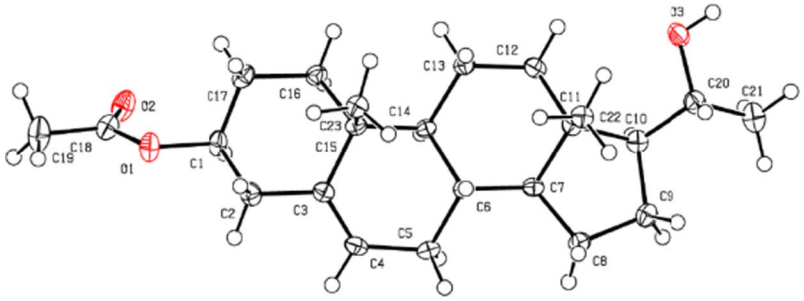



**Fig. 3.** Thermal ellipsoid plot of compound **10**. Ellipsoids are shown at 50% probability.

stereochemistry at C20 was also confirmed with the crystal structure of alcohol **10** (Fig. 3). The C20-hydroxy group of alcohol **10** was protected as the silyl ether (**11**). The C7-position was regioselectively oxidized with $CrO_3$ and 3,5-dimethylpyrazole to afford the ketone (**8**). The use of excess L-selectride afforded the 7α-hydroxy intermediate (**7**). Although the yield for this reduction was 22%, the stereoselectivity for the 1,2-reduction of the C7-ketone, yielding the 7α-hydroxy epimer over the 7β-hydroxy epimer, is evident from the analysis of the $^1$H NMR spectrum of the crude reaction mixture (*vide infra*, Fig. 4A). The loss of 78% of the starting material is probably due to other unwanted products that were not identified. Interestingly, the counterion does not play a role in the stereoselectivity as K-selectride has been reported to afford 7α-hydroxy epimers from 3,7-diketo bile acid precursors [22]. The resulting 7α-hydroxy epimer was protected as the bis acetate **6**. When the silyl ether **6** was treated with stoichiometric amounts of tetrabutylammonium fluoride, no reaction was observed, suggesting that the steric hindrance associated with the d-ring (C13-quaternary carbon center in **6**) prevented access to the silyl group. However, treatment of the silyl ether (**6**) with catalytic *p*-toluenesulfonic acid in methanol and dichloromethane (1:1, v/v) afforded the C20-alcohol (**5**). The C20-alcohol (**5**) was oxidized to the ketone (**12**) with Dess-Martin periodinane with at least half of the starting material recovered. The Dess-Martin periodinane mediated oxidation yielded the C20-ketone **12** at relatively low yield (34%) probably due to the sterically hindered environment of the C20-hydroxy group, which is near the C13-quaternary carbon center. 7α-Hydroxypregnenolone (**4**) was obtained through methanolysis of the bis-acetate (**12**) with $K_2CO_3$ in methanol.

Interestingly, when $NaBH_4$ was used to reduce ketone **8** (Scheme 2, **8** to **13**), the resulting 7β-hydroxy epimer was obtained as the major product, which was the opposite stereochemistry obtained when L-selectride was used (i.e. Scheme 1, **8** to **7**). Similarly, $NaBH_4$ has been shown to reduce 7-ketocholesterol-3-benzoate to afford the 7β-hydroxy epimer as well [23].

### 2.1. Rationalization of the Stereoselectivity of the Reducing Agent at the C7-Ketone

The stereoselectivity of the C7-ketone reduction (Scheme 2) can be explained from the analysis of the Newman projection at the C7-C8 bond axis of ketone **8** (Fig. 4C). The difference in the chemical shifts of the C5-proton of the 7α-hydroxy epimer ($\delta$ 5.62 ppm, doublet, J = 5.15 Hz) vs. the 7β-hydroxy epimer ($\delta$ 5.31 ppm, singlet) allowed for quantification of the stereoselectivity of the reducing agent by $^1$H NMR spectroscopy. These chemical shifts agreed with previously reported chemical shifts of the C5-protons of 7α-hydroxycholesterol and 7β-hydroxycholesterol ($\delta$ 5.60–5.71 ppm (multiplet) and $\delta$ 5.27 ppm (singlet), respectively) [24].

A bulky reducing agent such as lithium tri-*sec*-butylborohydride (i.e. L-selectride) favored the addition of the hydride on the β-face, affording the 7α-hydroxy product (Fig. 4Ca, **8** to **7**). In this scenario, the addition of lithium tri-*sec*-butylborohydride from the Burgi-Dunitz angle shows that the hydride is more sterically accessible through pathway *a* in Fig. 4Ca (i.e. pathway *b* is unfavored with L-selectride due to the steric clash of the bulky hydride with C9). However, when a small hydride

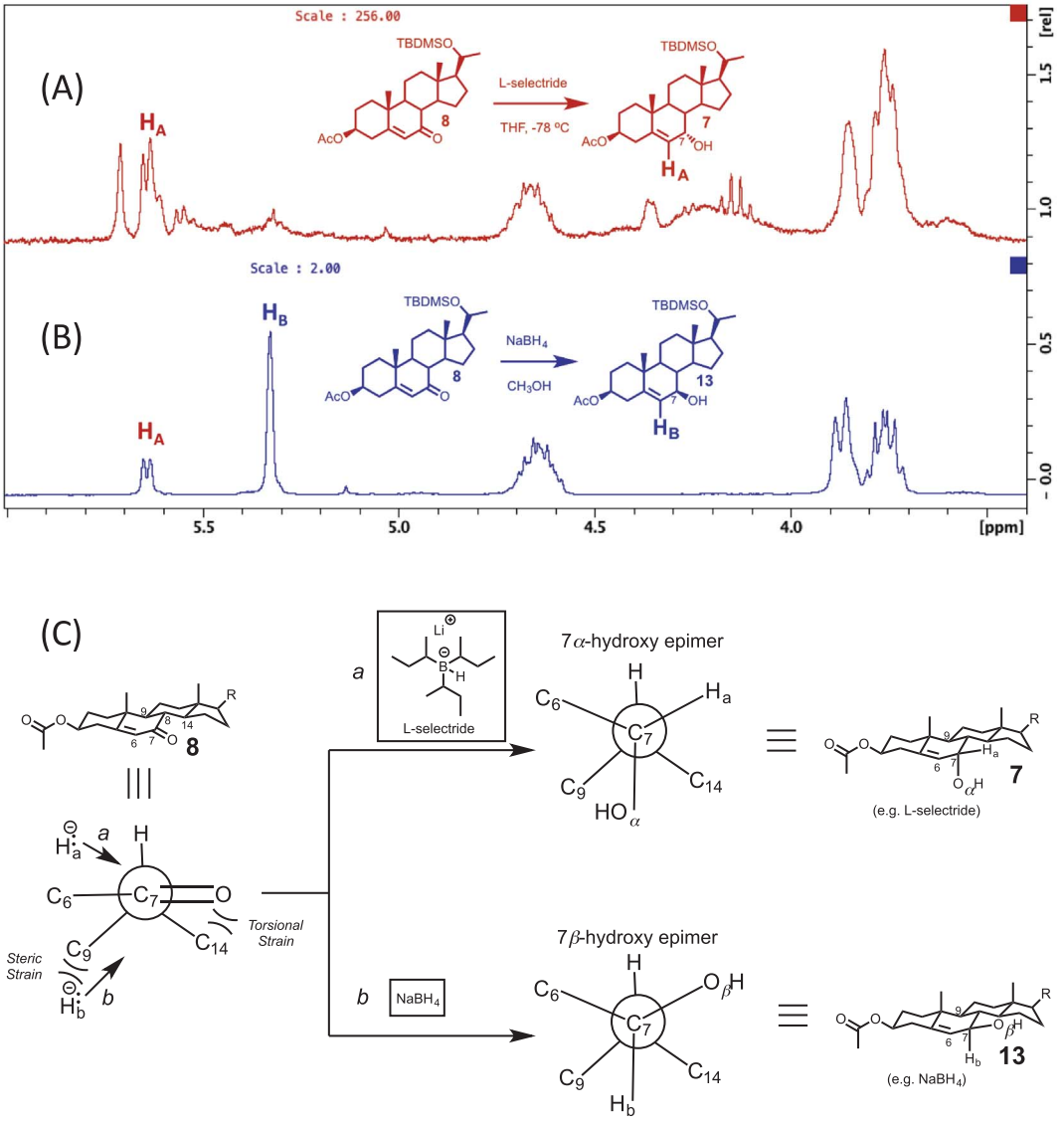
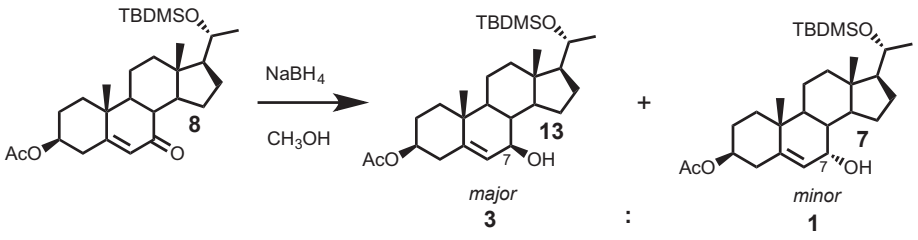



**Fig. 4.** Stereoselective reduction at the C7-ketone of compound **8**. $^1$H NMR overlay to show stereoselectivity of (A) lithium tri-*sec*-butylborohydride (red) vs. (B) $NaBH_4$ (blue). (C) Newman projection analysis to explain the stereoselectivities of (*a*) a bulky reducing hydride reagent and (*b*) a small reducing hydride reagent. Pathway *a*, which has torsional strain between C14 and the oxygen, is favored with a sterically hindered hydride source (e.g. lithium tri-*sec*-butylborohydride), yielding the 7α-hydroxy epimer, **7**. Pathway *b*, which has steric strain between the hydride reagent and C9, leads to the 7β-hydroxy epimer, **13** (e.g. $NaBH_4$). (A) and (B) are the $^1$H NMR spectra (3.4–6.0 ppm range) of the crude reaction mixtures of treatment of ketone **8** with lithium tri-*sec*-butylborohydride and $NaBH_4$, respectively. The C5-protons for the 7α-hydroxy and the 7β-hydroxy epimers are labeled at $\delta$ 5.62 and 5.31 ppm, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

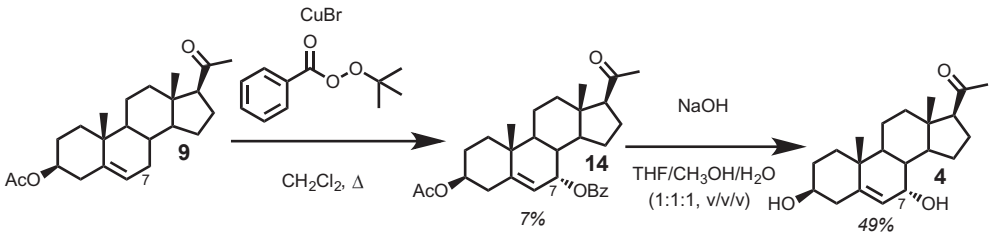



**Scheme 2.** Reduction of ketone **8** with $NaBH_4$ to afford the 7β-hydroxy and 7α-hydroxy stereochemistry (alcohols **13** and **7**) in a 3:1 ratio determined by $^1$H NMR spectroscopy of the crude reaction mixture (*vide infra*).

source such as $NaBH_4$ was used (Fig. 4, **8** to **13**), the 7β-hydroxy stereochemistry was favored (75%:25%, 7β-hydroxy (**13**):7α-hydroxy (**7**), determined by the $^1$H NMR spectrum of the crude reaction mixture, Fig. 4B). In the case of $NaBH_4$, to avoid the torsional strain between the oxygen and C14 in pathway *a*, the hydride is delivered primarily on the α-face, yielding the 7β-hydroxy epimer as the major product (i.e. pathway 4C*b* is favored with $NaBH_4$ to prevent torsional strain).

### 2.2. A Second Approach to 7α-Hydroxypregnenolone (4) through C—H Benzoyloxylation with CuBr

Considering the nine steps required for the synthesis of 7α-hydroxypregnenolone described in Scheme 1, an alternative and more direct approach involving three steps from pregnenolone was explored. The stereoselective 7α-benzoyloxylation of both dehydroepiandrosterone-3-acetate and cholesterol-3-acetate using copper (I) bromide and *tert*-




**Scheme 3.** Synthesis of 7α-Hydroxypregnenolone (**4**) through stereoselective C7-benzoyloxylation of pregnenolone-3-acetate (**9**) with CuBr followed by saponification.

butylperoxybenzoate [25] has been recently reported. Therefore, employing this method to regioselectively and stereoselectively introduce the oxygen at the C7α-position of $\alpha^5$-bearing steroids, would allow access to 7α-hydroxypregnenolone in a two-step process from pregnenolone-3-acetate (**9**).

Gratifyingly, when pregnenolone-3-acetate was subjected to the copper allylic benzoyloxylation conditions, pregnenolone-3-acetate-7α-benzoate (Scheme 3, **14**) was obtained. Although the reaction yielded the desired product, the purification of the 7α-benzoate product was a significant challenge, due to the use of excess *tert*-butylperoxybenzoate (4 mol equivalents), which co-eluted with the desired product. To remove the excess benzoyl-containing impurity, the crude reaction mixture was purified a second time through column chromatography, followed by a final preparative TLC purification, and a NaOH (aqueous) wash to afford the 7α-benzoate product (**14**) (7% yield). The reaction was repeated at a larger scale with stoichiometric amounts of CuBr and *tert*-butylperoxybenzoate to furnish the similar yield of pregnenolone-3-acetate-7α-benzoate (**14**) (5.2% yield).

The acetate and benzoate groups were saponified with sodium hydroxide in tetrahydrofuran/methanol/water (1:1:1, v/v/v) to afford 7α-hydroxypregnenolone.

## 3. Conclusion

In conclusion, two convenient syntheses of 7α-hydroxypregnenolone were accomplished beginning with pregnenolone and pregnenolone-3-acetate (Schemes 1 and 3). In the first approach (Scheme 1), the 7α-hydroxy group was introduced by stereoselective reduction of a C7-ketone intermediate (**8**) using a sterically hindered hydride source, lithium tri-*sec*-butylborohydride (**8** to **7**). Alternatively, the 7α-hydroxy group was introduced using CuBr and *tert*-butylperoxy benzoate (Scheme 3). The insights gained from this report can be applicable to access other steroid analogs with intriguing biological properties. The syntheses shown in Schemes 1 and 3 describe our synthetic routes to 7α-hydroxypregnenolone from pregnenolone in nine steps and three steps (**4** from **3**) with overall yields of 0.08% and 2.3%.

## 4. Materials and methods

A Bruker (Billerica, MA) NMR spectrometer (300 MHz or 500 MHz) was used to record NMR spectra of synthesized intermediates. Deuterochloroform ($CDCl_3$, Cambridge Isotope Laboratories, Tewksbury, MA) was used as the solvent for NMR spectra and the chemical shift of the solvent was referenced to $\delta$ 7.26 ppm and $\delta$ 77.16 ppm for the $^1H$ and $^{13}C$ NMR spectra, respectively. Solvents used for reactions were obtained from Fisher Scientific (Hampton, NH). Topspin software was used to process NMR data. Thin-layer chromatography (TLC) plates (silica gel, Sigma, St. Louis, MO) with fluorescent indicator (254 nm) were used. Preparatory TLC plates (silica gel, 2 mm, Analtech, Newark, DE) with fluorescent indicator (254 nm) were used to purify compound **14**. Ceric ammonium molybdate stain (235 ml of $H_2O$, 15 ml of $H_2SO_4$, 12 g of ammonium molybdate, 0.5 g of ceric ammonium molybdate) was used to visualize compounds on TLC plates. Silica gel (40–63 μm, 60 Å) was purchased from SiliCycle (Quebec, Canada). Optical rotations were obtained using a WXG-4 manual polarimeter (Bante Instruments Ltd, Shanghai, China) with a 10 cm cell using a power transformer (220 V to 110 V). Infrared Spectra were recorded using a Nicolet iS50 FT-IR Spectrometer (Thermo Fisher Scientific, Waltham, MA). OMNIC 9.3.32 software (Thermo Fisher Scientific) was used to analyze IR data. Melting points were acquired using an Optimelt MPA100 automated melting point system from Stanford Research Systems; starting temperature of 95 °C to a final temperature of 200 °C at a rate of 10.0 °C/min.

### 4.1. Mass spectrometry

For ESI-MS analysis, all samples were diluted, without purification, into an aqueous solution containing 50% methanol and 0.1% acetic acid. Mass spectra were collected on a maXis plus quadrupole-time of flight (qTOF) mass spectrometer equipped with an electrospray ionization source (Bruker Daltonics, Billerica, MA) and operated in the positive ionization mode. Samples were introduced via syringe pump at a constant flow rate of 3 μl/min. Important source parameters are summarized as follows: capillary voltage, 3500 V with a set end plate offset of −500 V; nebulizer gas pressure, 0.4 bar; dry gas flow rate, 4.0 μl/min; source temperature, 200 °C. Mass spectra were averages of one minute of scans collected at a rate of 1 scan per second in the range $50 \leq m/z \leq 1500$. Compass Data Analysis software version 4.3 (Bruker Daltonics, Billerica, MA) was used to process all mass spectra.

### 4.2. Optical rotations

Solutions of synthesized intermediates (12 ml) for optical rotations were made in dichloromethane (0.5% to 1%, g/ml, w/v, as described). The polarimeter was calibrated with a blank solution of dichloromethane at ambient temperature (20 °C) to be 80.5. The samples were measured by arbitrarily turning the vernier (horizontal) knob until the black shade appeared on the right in the telescope. The measurement was made on the dial and recorded. The dial was moved the other direction until the black shade appeared on the left in the telescope and the number on the dial was recorded. The average number was calculated and subtracted from the measurement of the blank solution. The specific rotation was calculated: $[\alpha]_D^{20} = \alpha/(l*c)$. The α value is the difference between the calibrated angle from a blank solution and the measured angle with sample, $l$ is 1 dm, and c is the concentration of the sample.

### 4.3. X-ray analysis

Single crystals of compound **10** were prepared by slow evaporation of a 50/50 mixture of ethyl acetate and hexanes (2 ml). A suitable colorless block-like crystal for compound **10**, with dimensions of 0.47 mm × 0.33 mm × 0.23 mm, was mounted in Partone oil on to a nylon loop. All data were collected at 98(2) K, using a Rigaku AFC12/Saturn 724 CCD fitted with MoKα radiation (λ = 0.71075 Å). Data collection and unit cell refinement were performed using *CrysAlisPro* [26]. The total number of data were measured in the range $5.1^\circ < 2\theta < 52.0^\circ$, using ω scans. Data processing and absorption correction, giving minimum and maximum transmission factors (0.8588, 1.000) were accomplished with *CrysAlisPro* [26] and *SCALE3*

*ABSPACK* [27], respectively. The structure, using Olex2 [28], was solved with the ShelXT [29] structure solution program using direct methods and refined (on $F^2$) with the ShelXL [30] refinement package using full-matrix, least-squares techniques. All non-hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atom positions were determined by geometry and refined by a riding model, except for the hydrogen atom on atom O3 which was determined by electron density map. The data was deposited in the Cambridge structural database with the deposition number CCDC 1559616.

### 4.4. Chemical syntheses

Pregnenolone-3-acetate (Compound **9**). Triethylamine (20 ml, 143 mmol, 2.3 mol eq) and acetic anhydride (10 ml, 106 mmol, 1.7 mol eq) were added to a solution of pregnenolone (20 g, 63.3 mmol, 1.0 mol eq) in $CH_2Cl_2$ (200 ml, 0.3 M). To the mixture was added 4-dimethylaminopyridine (0.4 g, 3.3 mmol, 0.05 mol eq). The reaction was stirred for 24 h. The resulting mixture was concentrated by reduced pressure and purified by flash column chromatography (100% hexanes to 30% ethyl acetate in hexanes) to afford pregnenolone-3-acetate as a white solid (15 g, 41.9 mmol, 66%); mp: 142.7–146.0 °C; IR (neat) 2942, 2914, 2891, 2873, 2847, 1727, 1701, 1467, 1454, 1434, 1387, 1372, 1357, 1290, 1268, 1246, 1232, 1193, 1170, 1152, 1135, 1114, 1085, 1028, 1019, 993, 979, 953, 940, 922, 912, 900, 883, 874, 854, 836 $cm^{-1}$; $R_f$: 0.74 (Hexanes:ethyl acetate, 4:1, v/v); $^1H$ NMR (500 MHz, $CDCl_3$)$\delta$ 5.38–5.35 (m, 1H), 4.63–4.55 (m, 1H), 2.53 (apparent t, J = 9.2 Hz, 1H), 2.35–2.25 (m, 2H), 2.21–2.13 (m, 1H), 2.12 (s, 3H), 2.03–2.01 (m, 1H), 2.03 (s, 3H), 2.02–1.95 (m, 1H), 1.90–1.82 (m, 2H), 1.72–1.52 (m, 5H), 1.52–1.40 (m, 3H), 1.27–1.18 (m, 1H), 1.18–1.10 (m, 2H), 1.01 (s, 3H, H-19), 1.04–0.96 (m, 1H, H-9), 0.62 (s, 3H, H-18); $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$ 209.8 (C-20), 170.7, 139.7 (C-4), 122.5 (C-5), 73.9, 63.8, 56.9, 50.0 (C-9), 44.1, 38.9, 38.2, 37.1, 36.7, 31.91, 31.86, 31.7, 27.8, 24.6, 22.9, 21.6, 21.1, 19.4 (C-19), 13.4 (C-18); HRMS (*m/z*) calculated for $C_{23}H_{34}O_3Na$ $[M + Na]^+$, 381.2400; found, 381.2399 ($\Delta$ −0.26 ppm).

Pregn-5-ene-3β-acetoxy-20β-ol (Compound **10**). $NaBH_4$ (0.38 g, 10.0 mmol, 0.3 mol eq) was added to a solution of pregnenolone-3-acetate (10.7 g, 29.9 mmol, 1 mol eq) in $CH_3OH$ (100 ml, 0.3 M). The reaction was stirred for 24 h at room temperature. The mixture was diluted with water (100 ml) and extracted with ethyl acetate (200 ml). The organic extract was concentrated under reduced pressure and purified by flash column chromatography (100% hexanes to 50% hexanes in ethyl acetate, v/v) to afford the alcohol **10** as a white solid (3.0 g, 8.27 mmol, 28%). The solid (~200 mg) was dissolved in ethyl acetate/hexanes (3 ml, 1:1, v/v), and left at rt to crystallize over a period of 5 days to form crystals to determine the stereochemistry at C20 as the *R*-configuration (Fig. 3); mp: 165.9–168.2 °C; IR (neat) 3555, 2969, 2948, 2939, 2909, 2889, 2866, 2854, 2823, 1720, 1681, 1464, 1450, 1425, 1399, 1367, 1334, 1277, 1255, 1197, 1147, 1129, 1108, 1079, 1048, 1029, 1012, 989, 968, 957, 943, 936, 917, 905, 896, 882, 871, 849, 839 $cm^{-1}$; $R_f$: 0.44 (Hexanes:ethyl acetate, 4:1, v/v); $^1H$ NMR (500 MHz, $CDCl_3$)$\delta$ 5.39–5.33 (m, 1H), 4.64–4.54 (m, 1H), 3.75–3.66 (m, 1H), 2.35–2.24 (m, 2H), 2.10–2.04 (m, 1H), 2.02 (s, 3H), 1.99–1.92 (m, 1H), 1.90–1.79 (m, 2H), 1.70–1.40 (m, 7H), 1.36–1.18 (m, 3H), 1.20–1.06 (m, 3H), 1.12 (d, J = 5.82 Hz, 1H), 1.06–0.99 (m, 1H), 1.01 (s, 3H), 0.99–0.92 (m, 1H), 0.75 (s, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$ 170.7, 139.8, 122.6, 74.0, 70.6, 58.5, 56.2, 50.1, 42.3, 39.9, 38.2, 37.1, 36.7, 32.0, 31.8, 27.8, 25.7, 24.6, 23.8, 21.6, 21.0, 19.4, 12.5; HRMS (*m/z*) calculated for $C_{23}H_{37}O_3$ $[M + H]^+$, 383.2557; found, 383.2557 ($\Delta$ 0.00 ppm).

Pregn-5-ene-3β-acetoxy-20-*tert*-butyldimethylsilyl ether (Compound **11**). *tert*-Butyldimethylsilyl chloride (8.0 g, 53 mmol, 3.3 mol eq) and imidazole (8.0 g, 118 mmol, 7.4 mol eq) were added to a solution of alcohol **10** (5.8 g, 16 mmol, 1.0 mol eq) in acetonitrile (200 ml, 0.08 M). The reaction flask was equipped with a Dean-Stark trap and heated under reflux for 20 h. The reaction mixture was cooled to room temperature, diluted with $H_2O$ (100 ml), and extracted with ethyl acetate (200 ml). The organic layer was concentrated by reduced pressure and purified by column chromatography (100% hexanes to 20% ethyl acetate in hexanes, v/v) to afford the silyl ether **11** as a clear oil (6.7 g, 14 mmol, 88%); IR (neat) 2933, 2854, 1732, 1471, 1462, 1438, 1371, 1239, 1119, 1092, 1048, 1029, 980, 956, 939, 918, 887, 834, 810, 773, 733 $cm^{-1}$; $R_f$: 0.90 (Hexanes:ethyl acetate, 4:1, v/v); $^1H$ NMR (500 MHz, $CDCl_3$)$\delta$ 5.39–5.33 (m, 1H), 4.64–4.54 (m, 1H), 3.77–3.68 (m, 1H), 2.33–2.27 (m, 2H), 2.18–2.12 (m, 1H), 2.02 (s, 3H), 1.99–1.91 (m, 1H), 1.89–1.81 (m, 2H), 1.78–1.74 (m, 1H), 1.66–1.52 (m, 4H), 1.52–1.34 (m, 5H), 1.19–1.08 (m, 3H), 1.07 (d, J = 5.84 Hz, 3H), 1.01 (s, 3H), 1.00–0.91 (m, 2H), 0.90 (s, 3H), 0.87 (s, 9H), 0.69 (s, 3H), 0.058 (s, 3H), 0.055 (s, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$ 170.7, 139.8, 122.7, 74.1, 71.2, 58.4, 56.5, 50.2, 42.2, 39.3, 38.2, 37.1, 36.7, 32.1, 31.9, 27.9, 26.2, 25.9, 25.8, 24.5, 23.9, 21.6, 20.9, 19.5, 18.2, 12.2, -3.3, -3.5, -3.9; HRMS (*m/z*) calculated for $C_{29}H_{51}O_3Si$ $[M + H]^+$, 497.3421; found, 497.3422 ($\Delta$ 0.20 ppm).

7-Oxo-pregn-5-ene-3β-acetoxy-20-*tert*-butyldimethylsilyl ether (Compound **8**). $CrO_3$ (9.7 g, 97 mmol, 13 mol eq) was stirred in $CH_2Cl_2$ (350 ml) at −78 °C for 10 min. 3,5-Dimethylpyrazole (9.9 g, 100 mmol, 14 mol eq) was added to the reaction as a solid and the reaction was stirred for 20 min at -78 °C. The TBDMS ether **11** (3.4 g, 7.2 mmol, 1.0 mol eq) in $CH_2Cl_2$ (20 ml) was added and the reaction was stirred for 20 h. The resulting mixture was directly loaded on a silica gel column and purified by column chromatography (100% hexanes to 20% ethyl acetate in hexanes, v/v, to 40% ethyl acetate in hexanes, v/v) to afford ketone **8** as a clear oil (2.3 g, 4.7 mmol, 68%); IR (neat) 2949, 2930, 2878, 2855, 2708, 1733, 1673, 1633, 1471, 1463, 1387, 1372, 1297, 1245, 1186, 1116, 1097, 1083, 1052, 1032, 980, 930, 906, 869, 834 $cm^{-1}$; $R_f$: 0.56 (Hexanes:ethyl acetate, 4:1, v/v); $^1H$ NMR (500 MHz, $CDCl_3$)$\delta$ 5.70–5.68 (m, 1H), 4.74–4.66 (m, 1H), 3.77–3.69 (m, 1H), 2.57–2.50 (m, 1H), 2.49–2.44 (m, 1H), 2.44–2.36 (m, 1H), 2.26–2.18 (m, 2H), 2.03 (s, 3H), 2.00–1.93 (m, 2H), 1.73–1.62 (m, 3H), 1.58–1.48 (m, 3H), 1.42–1.22 (m, 5H), 1.20 (s, 3H), 1.19–1.09 (m, 2H), 1.08 (d, J = 5.71 Hz, 3H), 0.90 (s, 3H), 0.87 (s, 9H), 0.70 (s, 3H), 0.06 (s, 3H), 0.05 (s, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$ 202.09, 170.4, 164.2, 126.7, 72.4, 71.4, 57.1, 50.1, 49.8, 45.5, 43.0, 38.5, 38.4, 37.9, 36.2, 27.5, 26.6, 26.3, 26.2, 25.8, 24.0, 21.4, 21.1, 18.2, 17.4, 12.2, -3.30, -3.45, -3.89; $[\alpha]_D^{20}$ + 948° [0.4% in $CH_2Cl_2$]; HRMS (*m/z*) calculated for $C_{29}H_{49}O_4Si$ $[M + H]^+$, 489.3395; found, 489.3398 ($\Delta$ 0.61 ppm).

7α-Hydroxy-pregn-5-ene-3β-acetoxy-20-*tert*-butyldimethylsilyl ether (Compound **7**). L-selectride (2.93 ml of a 1 M solution in THF, 2.9 mmol, 1.1 mol eq) was added to a solution of ketone **8** (1.3 g, 2.7 mmol, 1.0 mol eq) in tetrahydrofuran (50 ml) at −78 °C. The reaction was stirred for 30 min at −78 °C. The reaction was quenched with the addition of water (50 ml) and the resulting reaction mixture was extracted with ethyl acetate (100 ml). The organic layer was concentrated by reduced pressure and purified by column chromatography (100% hexanes to 50% hexanes in ethyl acetate, v/v) to afford 7α-hydroxy compound **7** as a transparent white solid (290 mg, 0.59 mmol, 22%); mp: 140.6–144.7 °C; IR (neat) 3495, 2962, 2935, 2905, 2891, 2870, 2855, 1712, 1668, 1470, 1442, 1414, 1373, 1366, 1333, 1314, 1257, 1200, 1155, 1135, 1082, 1041, 1019, 978, 957, 9445, 938, 920, 905, 893, 873 $cm^{-1}$; $R_f$: 0.31 (Hexanes:ethyl acetate, 4:1, v/v); $^1H$ NMR (500 MHz, $CDCl_3$)$\delta$ 5.62 (d, J = 5.2 Hz, 1H), 4.69–4.60 (m, 1H), 3.85–3.80 (m, 1H), 3.78–3.71 (m, 1H), 2.41–2.30 (m, 2H), 2.21–2.13 (m, 1H), 2.03 (s, 3H), 1.93–1.83 (m, 2H), 1.79–1.64 (m, 2H), 1.64–1.57 (m, 1H), 1.57–1.40 (m, 6H), 1.33–1.11 (m, 6H), 1.09 (d, J = 6.02 Hz, 3H), 1.01 (s, 3H), 0.88 (s, 9H), 0.71 (s, 3H), 0.07 (s, 6H); $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$ 170.5, 145.4, 124.9, 73.6, 73.1, 65.5, 58.2, 49.2, 42.5, 42.1, 38.8, 38.1, 37.7, 37.6, 36.9, 27.7, 26.3, 26.0, 24.5, 24.0, 21.5, 20.6, 18.4, 18.3, 12.0, 1.16, -3.27, -3.89; HRMS (*m/z*) calculated for $C_{29}H_{50}O_5SiNa$ $[M + Na]^+$, 513.3371; found, 513.3372 ($\Delta$ 0.19 ppm). ∗At a larger scale (~5 g scale of ketone **8**, it was necessary to add 2 mol equivalents of L-selectride for the reaction to proceed).

Pregn-5-ene-3β,7α-diacetoxy-20-*tert*-butyldimethylsilyl ether (Compound **6**). Triethylamine (1 ml, 14 mmol, 25 mol eq) and acetic anhydride (1 ml, 11 mmol, 20 mol eq) were added to a solution of the alcohol (274 mg, 0.56 mmol, 1.0 mol eq) in $CH_2Cl_2$ (100 ml). 4-Dimethylaminopyridine (100 mg, 0.82 mmol, 1.5 mol eq) was added and the reaction was heated at reflux 2 h. A second portion of acetic anhydride (1 ml, 11 mmol, 20 mol eq) was added and the reaction was stirred at reflux for 4 h. The reaction mixture was concentrated and purified by flash column chromatography (100% hexanes to 20% ethyl acetate in hexanes, v/v, to 50% ethyl acetate in hexanes, v/v) to afford diacetate **5** as a clear solid (191 mg, 0.36 mmol, 64%); mp: 125.5–131.1 °C; IR (neat) 2948, 2935, 2892, 2855, 1728, 1672, 1471, 1462, 1438, 1370, 1338, 1236, 1196, 1157, 1134, 1119, 1096, 1086, 1050, 1024, 982, 934, 910, 888, 830 $cm^{-1}$; $R_f$: 0.59 (Hexanes:ethyl acetate, 4:1, v/v); $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 5.55 (d, J = 5.07 Hz, 1H), 4.94 (apparent t, J = 4.70 Hz, 1H), 4.69–4.61 (m, 1H), 3.77–3.68 (m, 1H), 2.38–2.30 (m, 2H), 2.19–2.11 (m, 1H), 2.01 (s, 3H), 2.00 (s, 3H), 1.90–1.82 (m, 2H), 1.65–1.53 (m, 3H), 1.53–1.48 (m, 1H), 1.48–1.40 (m, 3H), 1.39–1.29 (m, 2H), 1.28–1.20 (m, 1H), 1.20–1.08 (m, 4H), 1.06 (d, J = 5.90 Hz, 3H), 1.00 (s, 3H), 0.87 (s, 9H), 0.68 (s, 3H), 0.053 (s, 3H), 0.047 (s, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$ 170.8, 170.5, 146.7, 120.9, 73.3, 71.1, 68.4, 58.2, 49.0, 43.3, 42.1, 38.7, 38.0, 37.4, 36.6, 35.8, 27.6, 26.2, 25.8, 24.3, 23.9, 21.43, 21.40, 20.6, 18.3, 18.2, 11.8, 1.11, -3.34, -3.90; $[\alpha]_D^{20}$ + 414° [0.4% in $CH_2Cl_2$]; HRMS (*m/z*) calculated for $C_{31}H_{52}O_5SiNa$ $[M + Na]^+$, 555.3476; found, 555.3478 (Δ 0.36 ppm).

20-Hydroxypregn-5-ene-3β,7α-diacetate (Compound **5**). *Para*-toluenesulfonic acid (6 mg, 0.03 mmol, 0.23 mol eq) was added to a solution of TBDMS ether (70 mg, 0.13 mmol, 1.0 mol eq) in $CH_3OH$:$CH_2Cl_2$ (4 ml, 1:1, v/v). The reaction was stirred for 1 h and loaded directly on a silica gel column for purification (100% hexanes to 50% hexanes in ethyl acetate, v/v) to yield the alcohol **5** as the major product, which was purified further with a second silica gel column (100% hexanes to 50% ethyl acetate in hexanes, v/v) to yield alcohol **5** as a white solid (25 mg, 0.06 mmol, 46%); mp: 178.6–183.6 °C; IR (neat) 3509, 2954, 2943, 2867, 1727, 1709, 1668, 1653, 1630, 1577, 1558, 1526, 1507, 1469, 1455, 1437, 1368, 1331, 1316, 1239, 1198, 1141, 1121, 1105, 1082, 1034, 1012, 992, 958, 934, 909, 895, 878, 835 $cm^{-1}$; $R_f$: 0.23 and 0.57 in (Hexanes:ethyl acetate, 4:1, v/v) and (Hexanes:ethyl acetate, 1:1, v/v), respectively; $^1H$ NMR (500 MHz, $CDCl_3$)$\delta$ 5.58 (d, J = 5.22 Hz, 1H), 4.97 (apparent t, J = 4.48 Hz, 1H), 4.72–4.63 (m, 1H), 3.78–3.68 (m, 1H), 2.38–2.32 (m, 2H), 2.12–2.07 (m, 1H), 2.05 (s, 3H), 2.03 (s, 3H), 2.03–2.01 (m, 1H), 1.92–1.85 (m, 2H), 1.75–1.58 (m, 4H), 1.56 (s, 3H), 1.55–1.45 (m, 3H), 1.44–1.33 (m, 3H), 1.33–1.16 (m, 5H), 1.15 (d, J = 6.13 Hz, 3H), 1.02 (s, 3H), 0.90–0.86 (m, 1H), 0.76 (s, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$ 170.9, 170.6, 146.8, 120.9, 73.3, 70.7, 68.4, 58.4, 48.9, 43.3, 42.4, 39.3, 38.0, 37.5, 36.7, 35.8, 27.6, 25.8, 24.5, 23.9, 21.5, 20.7, 18.3, 12.1; HRMS (*m/z*) calculated for $C_{25}H_{38}O_5Na$ $[M + Na]^+$, 441.2611; found, 441.2610 (Δ −0.23 ppm). ∗At a larger scale (~1 g starting material, with ~50 ml volume of solvent, the reaction mixture was washed with saturated sodium bicarbonate (aqueous) solution (100 ml) and extracted with dichloromethane (200 ml) before purification by flash column chromatography.

20-Oxo-preg-5-ene-3β,7α-diacetate (Compound **12**). Dess-Martin periodinane (30 mg, 0.071 mmol, 1.0 mol eq) was added to a solution of alcohol **5** (30 mg, 0.072 mmol, 1.0 mol eq) in $CH_2Cl_2$ (2 ml) in a screw cap vial and the reaction was stirred for 2 h at rt. The reaction mixture was directly loaded onto a silica gel column and purified (100% hexanes to 50% ethyl acetate in hexanes, v/v) to afford the C20-ketone **12** (10 mg, 0.024 mmol, 34%) as a white solid; IR (neat) 2941, 2872, 2854, 1730, 1703, 1667, 1467, 1452, 1438, 1372, 1241, 1196, 1139, 1035, 1015, 956, 933, 903, 886 $cm^{-1}$; $R_f$: 0.86 (Hexanes:ethyl acetate, 1:1, v/v). $^1H$ NMR (500 MHz, $CDCl_3$)$\delta$ 5.59 (d, J = 4.94 Hz, 1H), 4.99 (apparent t, J = 4.44 Hz, 1H), 4.72–4.63 (m, 1H), 2.57 (m, apparent t = 9.30 Hz, 1H), 2.22–2.14 (m, 1H), 2.13 (s, 3H), 2.045 (s, 3H), 2.037 (s, 3H), 1.93–1.86 (m, 1H), 1.74–1.54 (m, 4H), 1.53 (s, 3H), 1.52–1.39 (m, 3H), 1.31–1.17 (m, 2H), 1.02 (s, 3H), 0.64 (s, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$209.4, 170.6, 146.6, 120.7, 73.0, 68.0, 63.4, 55.5, 49.5, 43.8, 43.0, 38.4, 38.1, 37.8, 37.3, 36.5, 35.7, 31.6, 27.5, 24.3, 22.9, 21.3, 20.7, 18.2, 12.8; HRMS (*m/z*) calculated for $C_{25}H_{37}O_5$ $[M + H]^+$, 439.2455; found, 439.2456 (Δ 0.23 ppm).

7α-Hydroxypregnenolone (Compound **4**). $K_2CO_3$ (18 mg, 0.13 mmol, 6.8 mol eq) was added to a solution of diacetate **12** (8 mg, 0.019 mmol, 1.0 mol eq) in $CH_3OH$ (2 ml) in a vial. The reaction was stirred at 50 °C for 2 h. The resulting mixture was filtered through a short pad of silica gel (50% ethyl acetate/hexanes, v/v, to 100% ethyl acetate). The mono-acetate (presumably the 3-hydroxy-7-acetate) was observed as an intermediate from TLC analysis after 1 h before complete hydrolysis to the diol product **4** (2 mg, 0.0060 mmol, 32%); IR (neat) 3382, 2926, 2852, 1727, 1700, 1668, 1462, 1453, 1437, 1376, 1358, 1241, 1193, 1136, 1110, 1051, 1016, 955 $cm^{-1}$; $R_f$: 0.13 (Hexanes:ethyl acetate, 1:1, v/v); $^1H$ NMR (500 MHz, $CDCl_3$)$\delta$ 5.61 (dd, $J_1$ = 5.23, $J_2$ = 1.93 Hz, 1H), 3.87 (apparent t, J = 4.14 Hz, 1H), 3.63–3.55 (m, 1H), 2.58 (apparent t, J = 9.35 Hz, 1H), 2.38–2.15 (m, 4H), 2.13 (s, 3H), 2.09–2.01 (m, 2H), 1.91–1.76 (m, 3H), 1.78–1.70 (m, 2H), 1.69–1.58 (m, 3H), 1.58–1.38 (m, 8H), 1.34–1.18 (m, 7H), 1.26 (s, 3H), 1.19–1.10 (m, 3H), 0.65 (s, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$209.7, 146.4, 123.9, 71.4, 65.3, 63.6, 49.8, 43.9, 42.3, 42.1, 38.4, 37.6, 37.5, 37.2, 31.7, 31.4, 24.6, 23.1, 20.9, 18.4, 13.1; HRMS (*m/z*) calculated for $C_{21}H_{32}O_3Na$ $[M + Na]^+$, 355.2244; found, 355.2248 (Δ 1.13 ppm). $R_f$ of monoacetate intermediate: 0.48 (Hexanes:ethyl acetate, 1:1, v/v) (i.e. 3β-hydroxy-7α-acetate).

$NaBH_4$ reduction of ketone **8** to afford 7β-hydroxy epimer (compound **13**). $NaBH_4$ (90 mg, 2.38 mmol, 11 mol eq) was added to a mixture of ketone **8** (106 mg, 0.217 mmol, 1.0 mol eq) in $CH_3OH$ (3 ml) in a screw cap vial. The reaction mixture, open to air, was stirred for 30 min at rt. The reaction was poured into a separatory funnel containing $H_2O$ (50 ml). The reaction vessel was rinsed out with ethyl acetate (5 ml) and added to the water layer. The mixture was extracted with ethyl acetate (100 ml) and the organic layer was dried with reduced pressure to afford a yellow oil (61 mg, crude amount). The dried extract was diluted in $CDCl_3$ (0.7 ml) and an NMR spectrum was taken of the sample to show the signals obtained for the 7β-hydroxy epimer **13**. $R_f$: 0.33 (Hexanes:ethyl acetate, 4:1, v/v). ∗The $R_f$ values were indistinguishable between the 7β-hydroxy epimer **13** and the 7α-hydroxy epimer **7**. From $^1H$ NMR (300 MHz) analysis, there was a mixture of the 7α-hydroxy epimer (**7**) and the 7β-hydroxy epimer (**13**) in a 25:75 ratio (Fig. 4B). The crude mixture (**7** and **13**) was purified by flash column chromatography (100% hexanes to 10% ethyl acetate in hexanes, v/v, to 30% ethyl acetate in hexanes, v/v). The separated products were collected in ten test tubes (2 ml of eluent in each tube), which were identified by TLC analysis and not combined despite the identical $R_f$ values, from the column chromatography purification. The solvent (ethyl acetate/hexanes mixture) in each test tube was evaporated in a nitrogen evaporator and the NMR was taken of the dried down samples in each tube. Based on the integrations of the corresponding $\Delta^5$-protons, the ratios of the 7β-hydroxy epimer (**13**) to the 7α-hydroxy epimer (**13**) in tubes 2, 3, 4, 5, 6, and 7 were: 0.1:1.0, 0.2:1.0, 0.3:1.0, 0.5:1.0, 0.6:1.0, and 0.6:1.0 (See Supplementary Data for an overlay of the different fractions). Tube 2 (5 mg) contained mostly the 7β-hydroxy epimer (compound **13**). Tube 1 (1 mg) did not contain any detectable 7α-hydroxy epimer (**7**) and only contained the 7β-hydroxy epimer (**13**). IR (neat) 2931, 1733, 1248, 1031 $cm^{-1}$; $^1H$ NMR of compound **13** (300 MHz, $CDCl_3$)$\delta$ 5.31 (broad s, 1H), 4.67–4.55 (m, 1H), 3.85 (apparent t, J = 7.5 Hz, 1H), 3.78–3.69 (m, 1H), 2.38–2.32 (m, 2H), 2.19 (dt, $J_1$ = 13, $J_2$ = 3.5 Hz, 1H), 2.03 (s, 3H), 2.04–2.01 (m, 1H), 1.92–1.82 (m, 3H), 1.71–1.60 (m, 2H), 1.54–1.33 (m, 7H), 1.26–1.14 (m, 3H), 1.10 (s, 3H), 1.09 (d, J = 6.2 Hz, 3H), 1.07 (s, 3H), 0.88 (s, 9H), 0.72 (s, 3H), 0.072 (s, 3H), 0.065 (s, 3H); HRMS (*m/z*) calculated for $C_{29}H_{51}O_4Si$ $[M + H]^+$, 513.3371; found, 513.3369 (Δ −0.39 ppm).

7α-Benzoyloxy-pregnenolone-3-acetate (Compound **14**). Copper

bromide (370 mg, 2.6 mmol, 2.0 mol eq) was added to a stirring solution of compound **9** (500 mg, 1.3 mmol, 1.0 mol eq) in $CH_2Cl_2$ (25 ml). The reaction vessel was evacuated and backfilled with $N_2$ before being set to reflux at 40 °C. After 15 minutes, *tert*-butyl peroxybenzoate (0.98 ml, 5.2 mmol, 4.0 mol eq) was added dropwise. The reaction continued to stir at 40 °C for 2 h. The reaction mixture was concentrated under $N_2$ flow. The crude mixture was loaded onto a silica gel column (100% hexanes to 50% ethyl acetate in hexanes, v/v). The fractions containing a mixture of the product and some starting material were dissolved in $CH_2Cl_2$ (~5 ml) and loaded onto a preparatory TLC plate (4:1 hexanes:ethyl acetate, v/v) and the lowest band was isolated. The resulting solid was dissolved in $CH_2Cl_2$ (100 ml) and washed with NaOH (aqueous, 5% w/v, 3 × 30 ml) to remove benzoic acid. The organic extracts were concentrated under reduced pressure to give the product as an opaque solid (44.7 mg, 0.089 mmol, 6.8%); IR (neat) 2941, 1729, 1701, 1601, 1450, 1359, 1313, 1267, 1238, 1197, 1174, 1107, 1069, 1025, 992, 955, 935, 904, 887, 863, 733, 711 $cm^{-1}$; $R_f$: 0.41 (hexanes:ethyl acetate, 4:1, v/v); $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$8.01 (d, J = 7.9 Hz, 2H), 7.66 (apparent t, J = 7.1 Hz, 1H), 7.46 (t, J = 7.11 Hz, 2H), 5.73 (d, J = 5.5 Hz, 1H), 5.24 (t, J = 4.4 Hz, 1H), 4.68–4.60 (m, 1H), 2.54 (t, J = 8.3 Hz, 1H), 2.38 (d, J = 8.32 Hz, 2H), 2.12 (s, 3H), 2.02 (s, 3H), 1.97–1.88 (m, 3H), 1.78–1.69 (m, 2H), 1.65–1.58 (m, 6H), 1.31 (s, 2H), 1.25 (s, 2H), 1.06 (s, 3H), 0.66 (s, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$209.4, 170.5, 166.1, 147.2, 133.0, 131.0, 129.7, 128.5, 120.8, 73.2, 70.4, 68.8, 63.5, 53.9, 50.0, 44.0, 43.6, 38.4, 37.9, 37.6, 36.8, 36.3, 31.7, 27.6, 24.4, 23.1, 21.4, 20.9, 18.4, 13.0; HRMS (*m/z*) calculated for $C_{30}H_{38}O_5Na$ $[M + Na]^+$, 501.2611; found, 501.2603 ($\Delta$ −1.60 ppm).

Conversion of 7α-Benzoyloxy-pregnenolone-3-acetate (Compound **14**) to 7α-hydroxypregnenolone (Compound **4**). NaOH (220 mg, 5.4 mmol, 4.0 mol eq) was added to a stirring solution of 7α-benzoyloxy-pregnenolone-3-acetate (680 mg, 1.35 mmol) in 1:1 (v/v) THF:$H_2O$ (100 ml). The reaction was set to reflux at 70 °C for 2 h. The reaction was cooled to rt, and a second portion of NaOH (216 mg, 5.40 mmol, 4.0 mol eq) and $CH_3OH$ (50 ml) were added. The reaction was left to reflux for 16 h at 110 °C before diluting with $H_2O$ (200 ml) and extracting with ethyl acetate (3 × 100 ml). The organic extracts were dried with $MgSO_4$ and concentrated under reduced pressure. The crude material was dissolved in $CH_2Cl_2$ (~5 ml) and loaded onto a silica gel column (100% hexanes to 30% ethyl acetate in hexanes, v/v, to 10% $CH_3OH$ in $CH_2Cl_2$, v/v) to yield 7α-hydroxypregneolone (compound **4**, 220 mg, 0.663 mmol, 49%); $R_f$: 0.63 ($CH_3OH$ in $CH_2Cl_2$, 15%, v/v).

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2017.10.004.

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