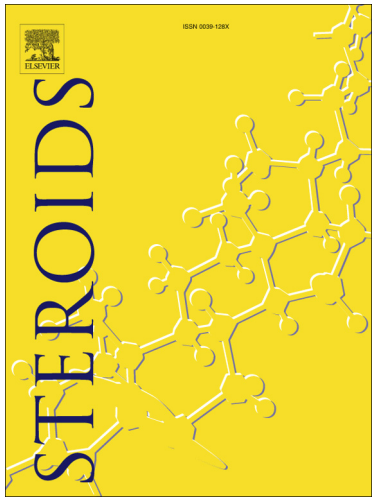

Chemical Synthesis of 7-Oxygenated 12α-Hydroxy Steroid Derivatives to Enable the Biochemical Characterization of Cytochrome P450 8B1, the Oxysterol 12α-Hydroxylase Enzyme Implicated in Cardiovascular Health and Obesity

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Chemical Synthesis of 7-Oxygenated 12α-Hydroxy Steroid Derivatives to Enable the Biochemical Characterization of Cytochrome P450 8B1, the Oxysterol 12α-Hydroxylase Enzyme Implicated in Cardiovascular Health and Obesity

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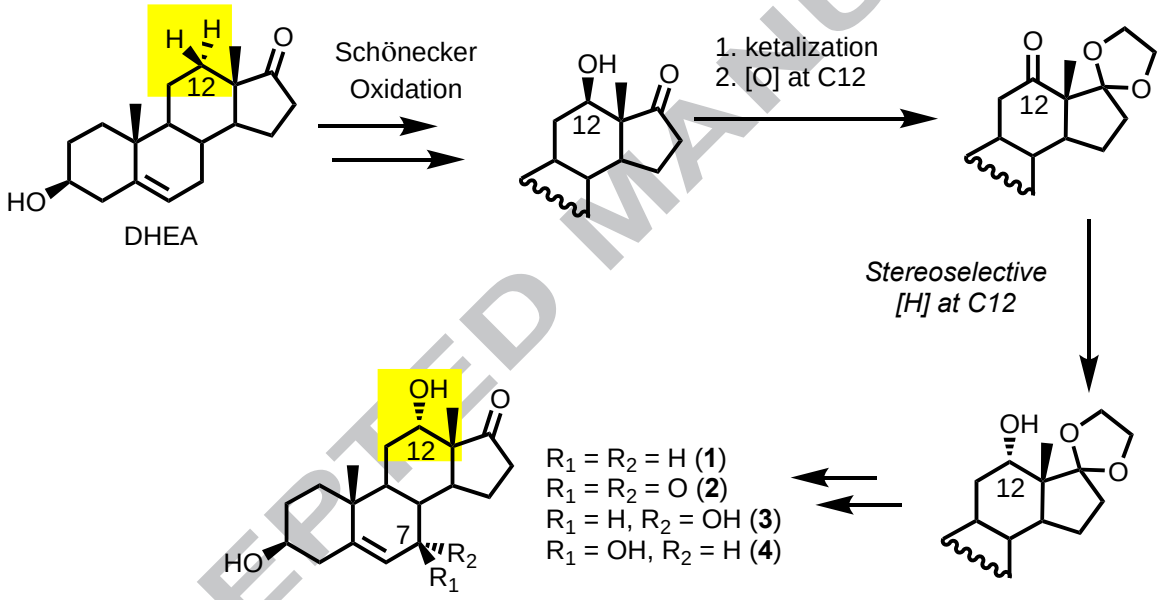
ABSTRACT

Cholic acid is the endogenous 12α-hydroxylated bile acid, which possesses enhanced cholesterol absorption properties compared to its 12-desoxy counterpart, chenodeoxycholic acid. The oxysterol 12α-hydroxylase enzyme is cytochrome P450 8B1 (P450 8B1), which regioselectively and stereoselectively incorporates the 12α-hydroxy group in 7α-hydroxycholest-4-en-3-one, the biosynthetic precursor of cholic acid. Despite the vital role of P450 8B1 activity in cardiovascular health, research studies of other 12α-hydroxy steroid derivatives are rare. A synthetic route to incorporate a C12α-hydroxy group into the C12-methylene (-CH_2-) in dehydroepiandrosterone derivatives is disclosed. The incorporation of the C12-oxygen was accomplished through a copper mediated Schönecker oxidation of an imino-pyridine intermediate, introducing the 12β-hydroxy group. The resulting 12β-hydroxy steroid derivative was oxidized to the C12-ketone, which was stereoselectively reduced with lithium tri-*sec*-

butylborohydride to afford the 12α-hydroxy stereochemistry. The C7-position was oxidized to yield the various 7-keto, 7β-hydroxy, and 7α-hydroxy derivatives. Furthermore, 7-ketodehydroepiandrosterone and 12α-hydroxy-7-ketodehydroepiandrosterone both displayed NMDA receptor antagonistic activities at 10 μM concentrations. These C12α-hydroxy steroids will be used as tools to identify new biochemical properties of the enzymatic products of P450 8B1, the oxysterol 12α-hydroxylase.

Abstract Figure:



Introduction

Cytochrome P450 8B1 is the oxysterol 12α-hydroxylase enzyme, which oxidizes 7α-hydroxy-cholest-4-en-3-one to form 7α-,12α-dihydroxycholest-4-en-3-one [1, 2] (Figure 1A), the biosynthetic precursor of cholic acid, the 12α-hydroxylated bile acid (Figure 1B). Bile acids form micelles, which regulate the intestinal absorption of lipids [3]. In particular, cholic acid supplementation has been shown to enhance the absorption of cholesterol [4]. Moreover, mice

lacking the *CYP8B1* gene encoding for P450 8B1, showed a resistance to weight gain when fed a high-cholesterol diet [5].

Furthermore, the isolation of other steroids with a 12α-hydroxy group has been reported. For instance, a 12α-hydroxypregnenolone derivative, named menarandroside A (Figure 1C), has been recently isolated from plants (*Cynanchum marnierianum*) [6]. This novel 12α-hydroxylated steroid was identified from a bio-guided fractionation assay that detected the stimulation of glucagon-like peptide 1 (GLP-1) secretion, suggesting anti-diabetic activity. Other 12α-hydroxylated sterol plant natural products have been previously isolated in plants [7].

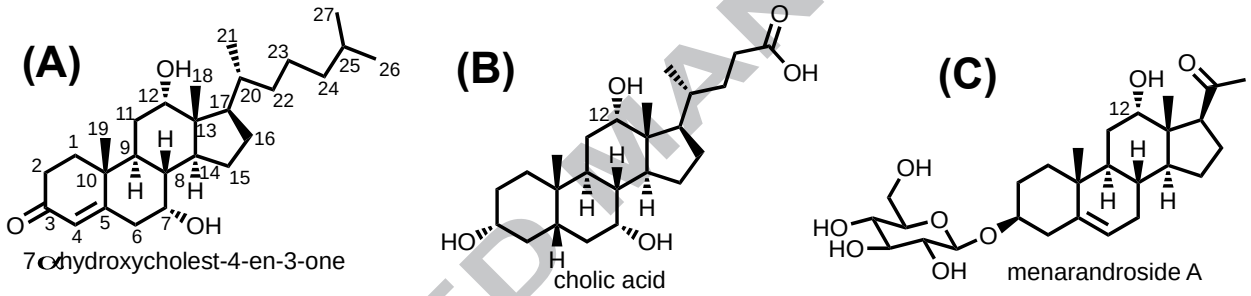


Figure 1. Examples of naturally occurring 12α-hydroxylated steroids: (A) 7α-hydroxycholest-4-en-3-one, (B) cholic acid, and (C) menarandroside A.

Moreover, although it is an endogenous steroid hormone, 7-ketodehydroepiandrosterone and its 3-acetate form have been used to treat certain health conditions. For example, 7-keto dehydroepiandrosterone-3-acetate (7-keto DHEA-3OAc) has been shown to reduce post-traumatic stress disorder (PTSD) symptoms [8] and also to reverse the resting metabolic rate associated with dieting [9]. 7-Keto DHEA has also decreased voluntary intake of ethanol in male rats [10]. The various 7-oxygenated DHEA forms (7β-hydroxy-, 7α-hydroxy, and 7-keto DHEA) are interconverted by liver 11β-hydroxysteroid dehydrogenase 1 [11]. In addition, 7-keto DHEA

has been shown to be effective at enhancing weight loss [12]. Due to the steroid backbone of 7-keto DHEA, it is reasonable to hypothesize that P450 8B1 would recognize 7-keto DHEA as a substrate and incorporate a 12α-hydroxy group into 7-keto DHEA.

Access to 12α-hydroxylated steroid structures would be necessary to explore their biochemical properties and facilitate the identification of new bioactive 12α-hydroxy steroid products arising from P450 8B1 activity. However, there is no known report to introduce a 12α-hydroxy group from a C12-desoxy steroid. *Therefore, a synthetic route to introduce a C12α-hydroxy group from the methylene (-CH_2-) in the steroid backbone was developed.* This report focuses on the syntheses of 12α-hydroxy dehydroepiandrosterone (Figure 2, **1**) and its 7-oxygenated derivatives (i.e. 7-keto-, 7α-hydroxy-, and 7β-hydroxy) (Figure 2, **2**, **3**, and **4**) beginning with dehydroepiandrosterone (DHEA). We further tested the hypothesis that 7-ketodehydroepiandrosterone is an *N*-methyl-D-asparatate (NMDA) receptor antagonist and found that both 7-ketodehydroepiandrosterone and 12α-hydroxy-7-ketodehydroepiandrosterone (**2**) had antagonistic effects towards the NMDA receptor.

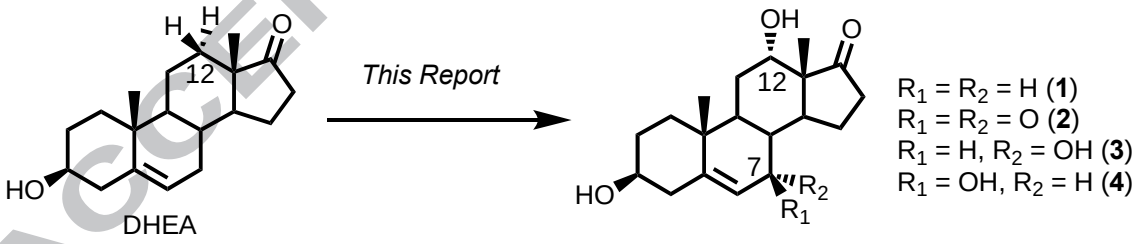
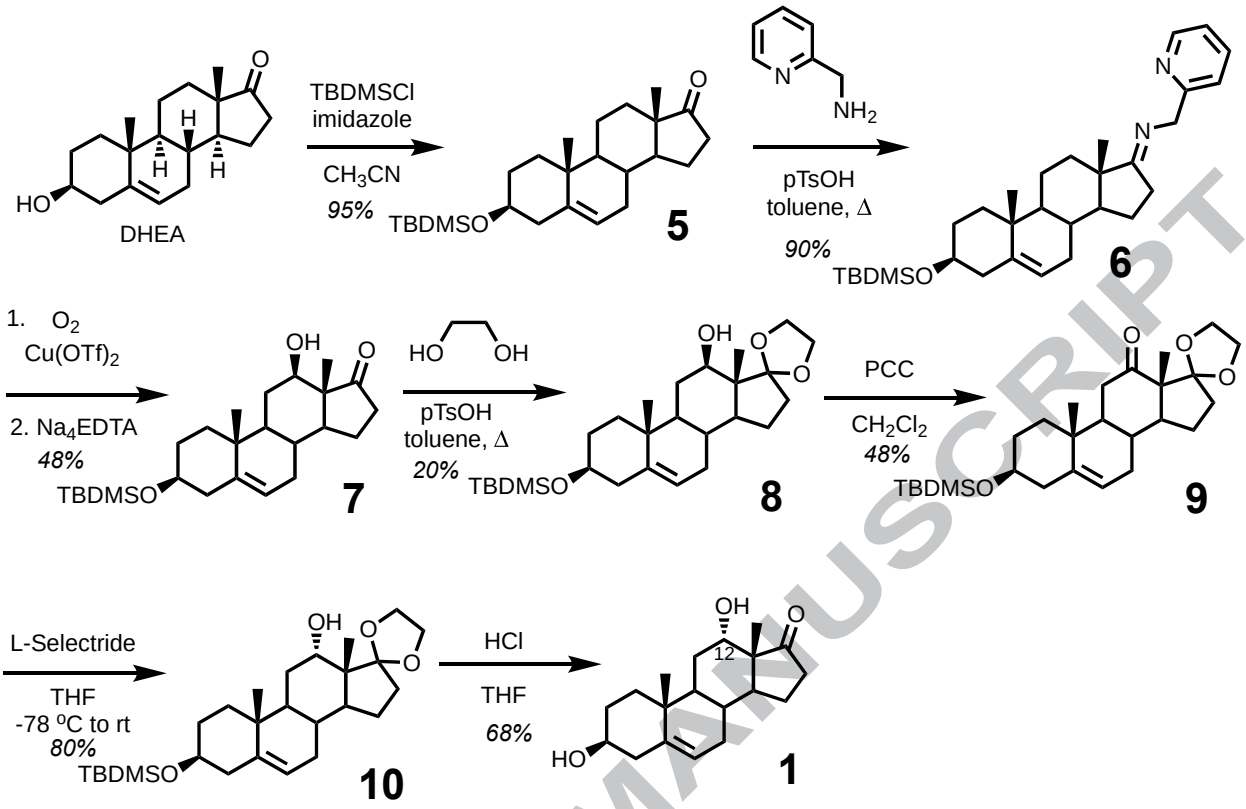


Figure 2. 12α-Hydroxy-dehydroepiandrosterone (**1**), 12α-hydroxy-7-keto-dehydroepiandrosterone (**2**), 7α-,12α-dihydroxy-dehydroepiandrosterone (**3**), and 7β-,12α-dihydroxy-dehydroepiandrosterone (**4**) were synthesized from dehydroepiandrosterone (DHEA).

*Synthesis of 12α-Hydroxy Dehydroepiandrosterone (**1**)*

The incorporation of a 12β-hydroxy group from a DHEA-imine derivative through a copper mediated oxygenation has been reported [13-15]. For the synthesis of 12α-hydroxy DHEA (compound **1**), dehydroepiandrosterone (DHEA, Scheme 1) was protected as the DHEA-3-O-*tert*-butyldimethylsilyl ether **5**, which was converted to the pyridine-imine derivative at C17 (Scheme 1, **6**). The Schönecker protocol through a copper mediated oxidation resulted in the introduction of the 12β-hydroxy group to yield 12β-hydroxy DHEA-3-OTBDMS ether **7** [13-15]. The resulting C17-ketone would be protected as ketal **8**. The C12β-hydroxy group of ketal **8** was oxidized to the C12-ketone **9**. Subsequent stereoselective reduction at the C12-position gave the 12α-hydroxy compound **10**. Various reducing conditions were explored to find the optimal hydride reagent as summarized in Table 1. In general, a sterically hindered hydride (i.e. lithium triethylborohydride and lithium tri-*sec*-butylborohydride) source gave the 12α-hydroxy epimer as the major diastereomer. Deprotection of the TBDMS group and the C17-ketal of intermediate **10** with dilute HCl (aqueous) in THF yielded 12α-hydroxy DHEA (**1**).



Scheme 1. Synthesis of 12α-hydroxy DHEA (**1**) from DHEA.

Stereoselective Reduction of the C12-Ketone (Compound **9**) with Various Reducing Agents

Entry	Hydride Source	12β-Hydroxy (**8**)[a]	12α-Hydroxy (**10**)[a]
1[b]	DIBAL	2.9	1.0
2[c]	$NaBH_4$	9.8	1.0
3[c]	$NaBH_4/CeCl_3$	12	1.0
4[b]	$LiAlH_4$	14	1.0
5[b]	Lithium triethylborohydride	1.0	10
6[b]	Lithium tri-*sec*-butylborohydride	1.0	9.2

Table 1. Summary of stereoselectivity of the different reduction conditions tested to yield the C12α-hydroxy epimer **10** or the C12β-hydroxy epimer **8** from C12-ketone **9**. Excess hydride source (5 mol equivalents) was used and the reaction was run for 3 hours in test tubes.

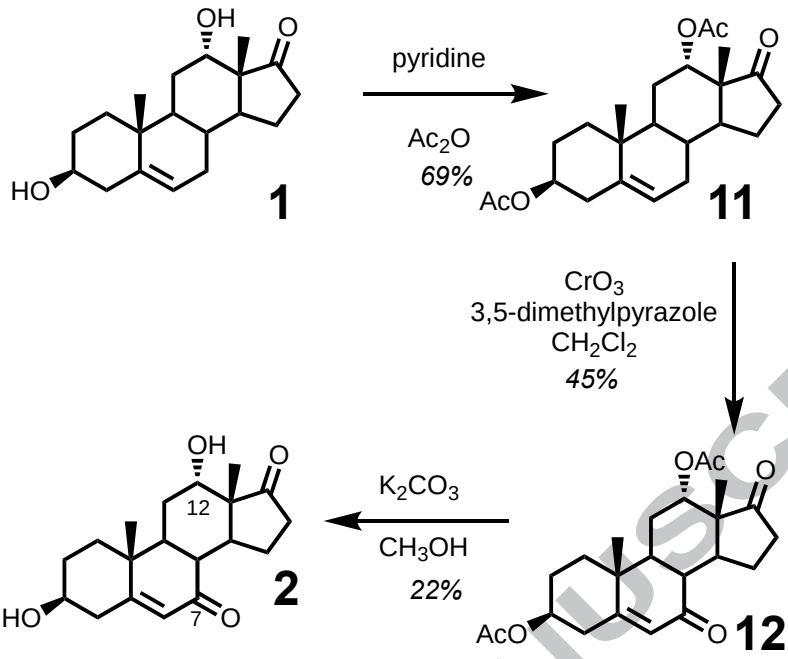
a. The ratios of the 12β-hydroxy and 12α-hydroxy products were determined by integration of the C18-methyl peak of the ^{1}H NMR spectra of the crude reaction material (δ 0.86 ppm for the 12α-hydroxy epimer, and δ 0.92 ppm for the 12β-hydroxy epimer).

b. THF (5 ml) was the solvent, the reaction was run at -78 °C with 30 mg of starting material (Compound **9**).

c. CH_3OH (5 ml) was the solvent, the reaction was run at 0 °C with 30 mg of starting material (Compound **9**).

*Synthesis of 12α-Hydroxy-7-Keto-Dehydroepiandrosterone (**2**)*

The synthesis of 12α-hydroxy-7-keto dehydroepiandrosterone (**2**) commenced with 12α-hydroxy DHEA (Scheme 2). The dihydroxy compound (**1**) was diacetylated with pyridine and acetic anhydride as the solvent (1:10, v/v) using experimental conditions previously employed [15]. The C7-position was oxidized to the C7-keto group using CrO_3 and 3,5-dimethylpyrazole in CH_2Cl_2 (Scheme 2). The resulting diacetate (**12**) was deprotected with K_2CO_3 in CH_3OH to yield 12α-hydroxy-7-keto DHEA (**2**) (Figure 3: crystal structure of compound **2**).



Scheme 2. Synthesis of 12α-hydroxy-7-keto-dehydroepiandrosterone (**2**) from 12α-hydroxy DHEA (**1**).

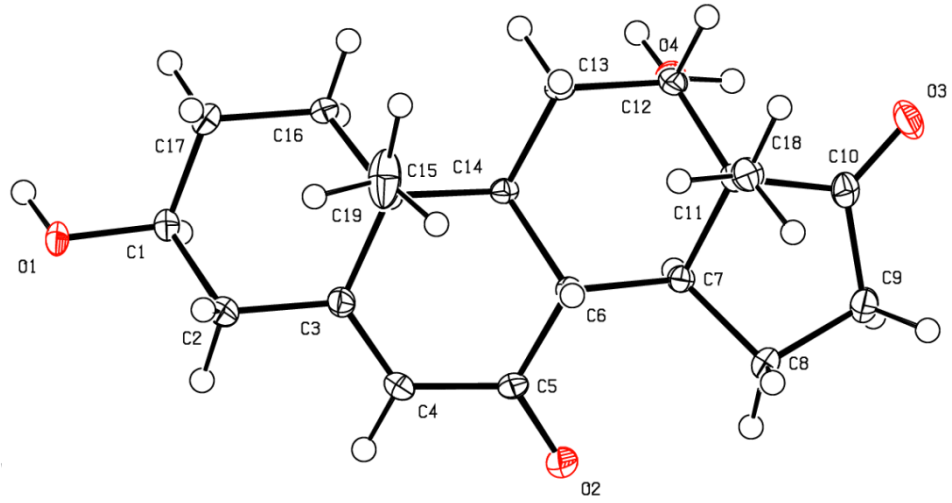
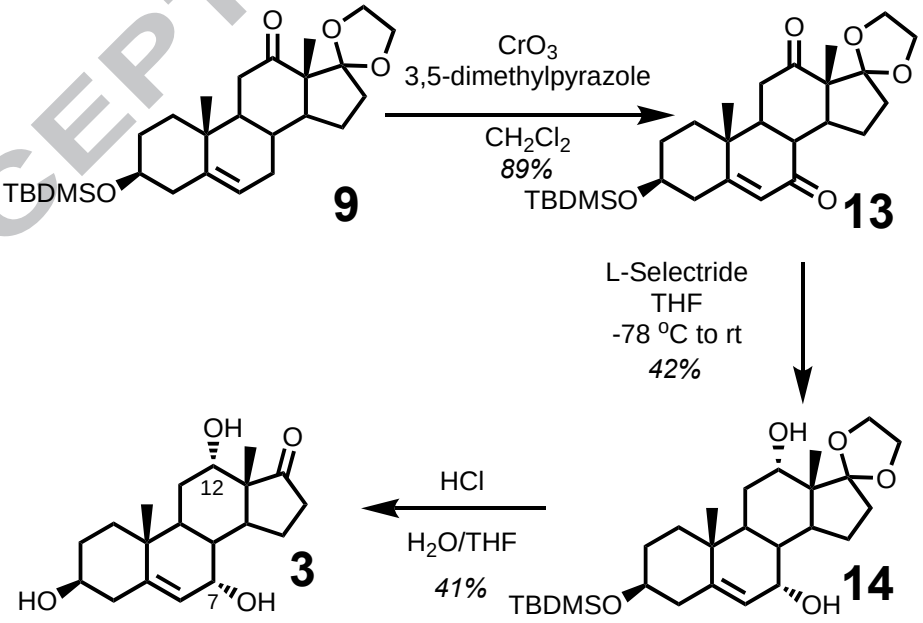


Figure 3. Crystal structure of 12α-hydroxy-7-keto DHEA (compound **2**).

Synthesis of 7α-,12α-Dihydroxy-Dehydroepiandrosterone (**3**)

In order to efficiently access 7α-,12α-dihydroxy DHEA (**3**), we hypothesized that a 7-,12-diketo steroid intermediate (e.g. Scheme 3, **13**) could be stereoselectively reduced by lithium tri-sec-butylborohydride (L-Selectride) to furnish the α-hydroxy stereochemistry at both the C7- and C12- positions. This stereoselective reduction of the diketone was based on the observation that L-Selectride results in the delivery of the hydride onto the β-face of C7-keto steroid backbones [16] and C12-keto backbones (*vide supra*, Scheme 1, **9** to **10**).

The synthesis of the 7α-,12α-dihydroxy DHEA (**3**) commenced with the oxidation of the C7-allylic position of 12-keto C17-ketal **9** with CrO_3 to furnish diketone **13**. Gratifyingly, diketone **13** was reduced with L-Selectride to afford the 7α-,12α-dihydroxy ketal **14**. Treating ketal **14** with dilute HCl in THF resulted in the deprotection of both the C17-ketal and the C3-*tert*-butyldimethylsilyl ether to afford 7α-,12α-dihydroxy DHEA (**3**) (Scheme 3) (Figure 4: crystal structure of compound **3**).



Scheme 3. Synthesis of 7α-,12α-dihydroxy DHEA (**3**).

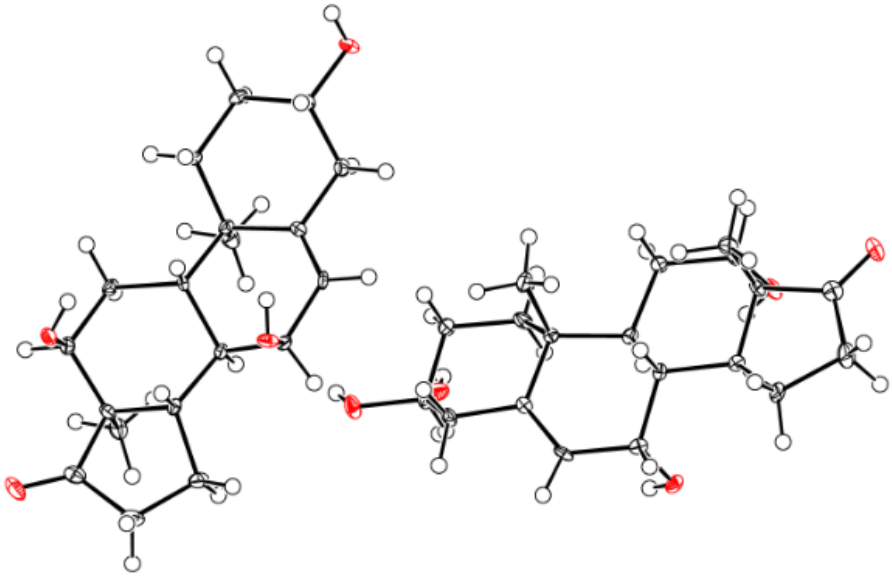


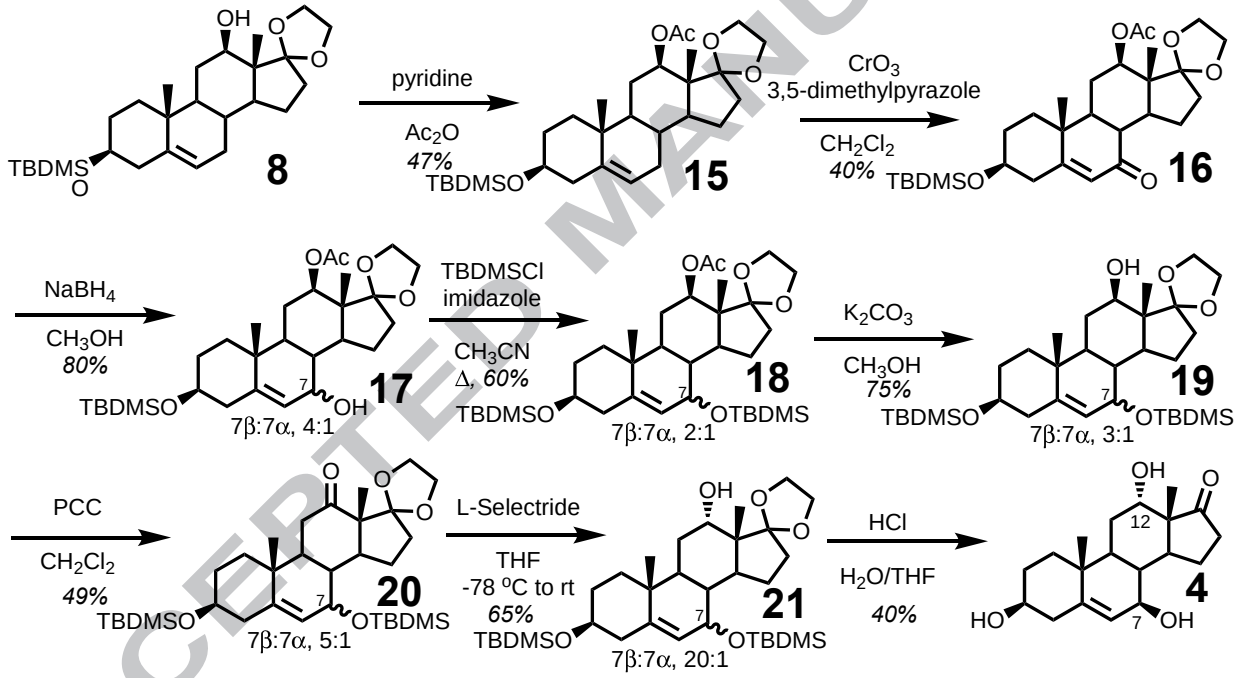
Figure 4. Crystal structure of 7α-,12α-dihydroxy DHEA (compound **3**).

Synthesis of 7β-,12α-Dihydroxy-Dehydroepiandrosterone (**4**)

Among the four 12α-hydroxylated DHEA derivatives (**1-4**), 7β-,12α-dihydroxy-dehydroepiandrosterone (**4**) was the most challenging due to the opposing stereochemistry of the hydroxy groups at the C7- and C12- positions (i.e. C7β-hydroxy and C12α-hydroxy, cf. Figure 1, **4**).

The 12β-hydroxy group of ketal **8** was protected as the acetate with pyridine and acetic anhydride to yield the 12β-acetate **15** (Scheme 4). The C7-allylic position was oxidized with CrO_3 and 3,5-dimethylpyrazole to furnish enone **16**. The C7-ketone was stereoselectively reduced with $NaBH_4$ to yield a crude mixture of 7β-hydroxy and 7α-hydroxy epimers in a 4:1 ratio (determined by ^{1}H NMR, Supporting Information). This mixture was purified by silica gel

column chromatography, giving the 7β- to 7α- epimeric mixture, which would be resolved over the subsequent five steps towards the final product, **4**. The 7-hydroxy group of alcohol **17** was protected as the *tert*-butyldimethylsilyl diether **18** by refluxing with TBDMSCl and imidazole in CH_3CN. The acetate was methanolyzed with K_2CO_3 in CH_3OH to yield alcohol **19**. Alcohol **19** was oxidized with pyridinium chlorochromate (PCC) to yield C12-ketone **20**, which was stereoselectively reduced with L-Selectride to give the 12α-hydroxy epimer **21**. Deprotection of the two *tert*-butyldimethylsilyl groups and the C17-ketal with HCl in water and THF to furnish 7β-,12α-dihydroxy DHEA **4**.



Scheme 4. Synthesis of 7β-,12α-dihydroxy DHEA (**4**). For compounds **17**-**21**, the ratio of 7β- to 7α- epimers were determined from ^{1}H NMR spectrum by integration of the Δ^5 proton signals at δ 5.3 ppm and δ 5.5 ppm, respectively (See Supporting Information).

Synthesis of 12β-Hydroxy-Dehydroepiandrosterone (**22**)

12β-Hydroxydehydroepiandrosterone (**22**) was accessed through deprotection of the TBDMS group of Schoenecker product (compound **7**) with HCl and CH_3OH/CH_2Cl_2 (Figure 5: crystal structure of compound **22**).

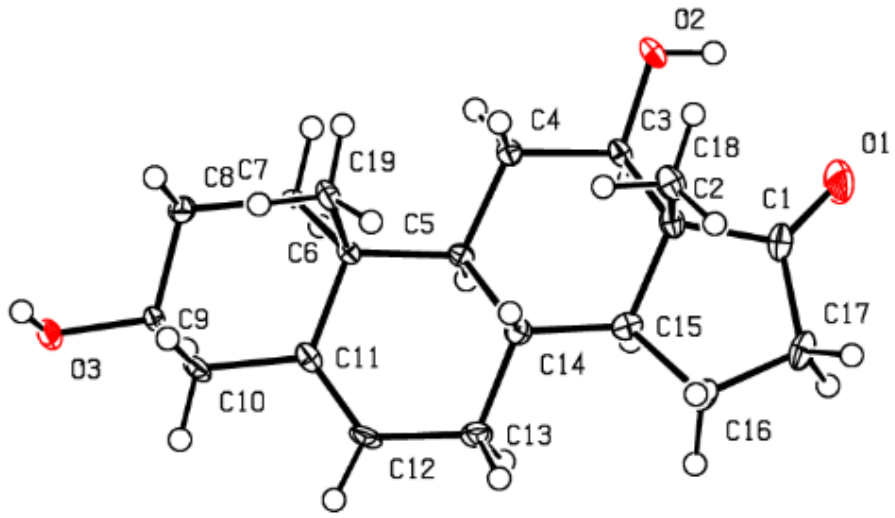


Figure 5. Crystal structure of 12β-hydroxy DHEA (compound **22**).

^{1}H NMR Analysis of C7- and C12- Oxygenated Steroid Derivatives – Diagnostic Signals

Intriguingly, when the C7-methylene position is converted to the C7-keto-$\Delta^{5,6}$-steroid scaffold, the Δ^5-proton shifts downfield from δ5.39 ppm (apparent doublet (J = 6 Hz) of compound **1**, Figure 6A) to δ5.75 ppm as a broad singlet (Figure 6B, compound **2**). On the other hand, when the C7-position bears a C7α-hydroxy intermediate (compound **3**) the Δ^5-proton appears as a

doublet (J = 6 Hz) at δ5.67 ppm (Figure 6C). However, when the C7-hydroxy group is in the β-orientation (compound **4**), the Δ^5-proton has an upfield shift and appears as a singlet at δ5.33 ppm (Figure 6D).

Furthermore, the C12-proton of the C12-oxygenated steroids had a diagnostic peak depending on whether the hydroxy group was in the 12β- or the 12α-orientation. For example, the C12α-proton (axial) of 12β-hydroxy DHEA (compound **22**) had a chemical shift of δ3.80 ppm, which appeared as a doublet of doublets (J = 11 and 4.6 Hz) (Figure 6E). In contrast, the C12β-proton (equatorial) of 12α-hydroxy DHEA (compound **1**) appeared as a broad singlet at δ4.14 ppm.

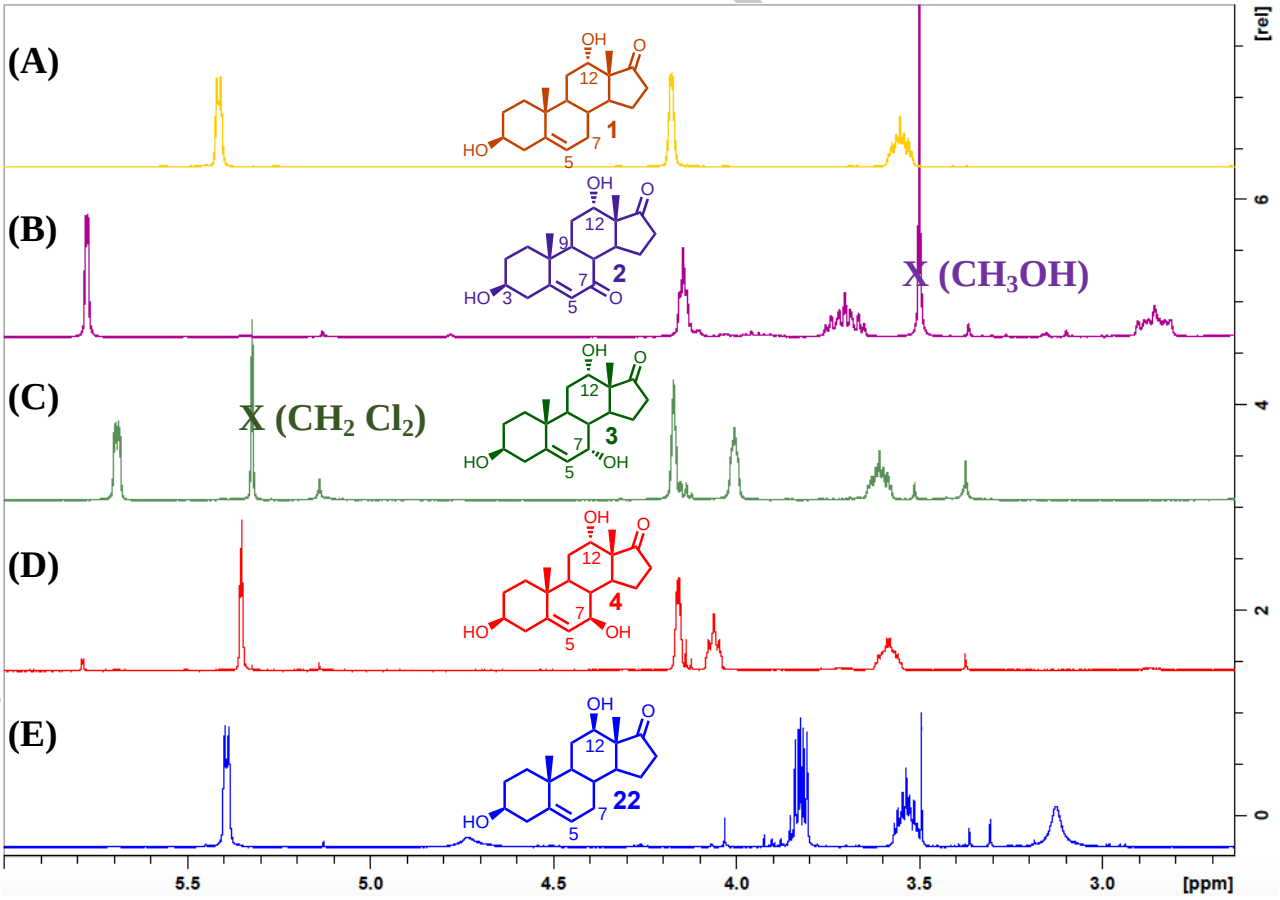


Figure 6. ^{1}H NMR spectral overlay of Compounds **1**, **2**, **3**, **4**, and **22** (500 MHz, δ 2.65-6.00 ppm range). (A) 12α-hydroxy DHEA (compound **1**), (B) 12α-hydroxy-7-keto DHEA (compound **2**), (C) 12α-,7α-dihydroxy DHEA (compound **3**), (D) 12α-,7β-dihydroxy DHEA (compound **4**), and (E) 12β-hydroxy DHEA (compound **22**).

Effect of 7-Ketodehydroepiandrosterone and 12α-Hydroxy-7-ketodehydroepiandrosterone on the NMDA Receptor

7-Ketodehydroepiandrosterone has been shown to reduce symptoms of post-traumatic stress disorder (PTSD) [8]. Therefore, we hypothesized that this compound can act as an *N*-methyl-D-aspartate (NMDA) receptor antagonist, which has been the activity associated with ketamine, another compound used to treat PTSD [17]. Furthermore, with access to the 12α-hydroxylated derivative (12α-hydroxy-7-ketodehydroepiandrosterone, compound **3**), the effect of the 12α-hydroxy group in the steroid scaffold was tested. Interestingly, both compounds were found to have antagonistic activity towards the NMDA receptor at 10 μM concentration (Figure 7). Other steroid derivatives have shown biological activity towards the NMDA receptor [18, 19], confirming steroids as potential ligands to modulate NMDA receptor activity.

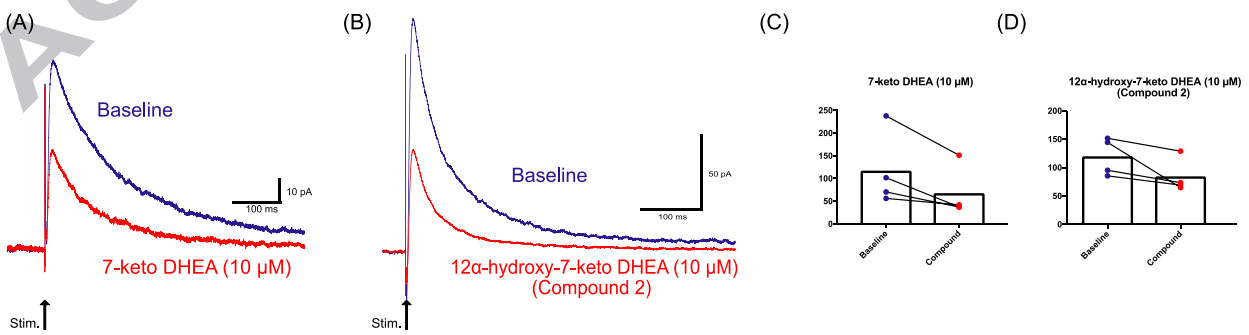


Figure 7. 7-Ketodehydroepiandrosterone and 12α-hydroxy-7-ketodehydroepiandrosterone (**2**) reduce the NMDA receptor-mediated current amplitude recorded from midbrain dopamine neurons. (A, B). Single pulse electrical stimulation of the slice elicits an outward current under baseline conditions (blue trace). When either (A) 7-ketodehydroepiandrosterone or (B) 12α-hydroxy-7-ketodehydroepiandrosterone (compound **2**) are bath applied to the horizontal brain slice, the peak current amplitude in response to the same electrical stimulation is decreased, indicating the effect of these compounds on NMDA receptors in the brain. (C, D). Summarized data of all recordings for each compound tested (C) 7-keto DHEA, and (D) 12α-hydroxy-7-ketodehydroepiandrosterone (compound **2**).

Conclusion

In summary, a synthetic route to introduce a 12α-hydroxy group from the C12-methylene in steroid derivatives was established. The key feature of this route involved three transformations: (1) the copper-mediated Schönecker oxidation to introduce the 12β-hydroxy group, (2) oxidation of the 12β-hydroxy to the C12-keto moiety, and (3) stereoselective reduction to the 12α-hydroxy group using lithium tri-*sec*-butylborohydride (i.e. L-Selectride). Both 7-ketodehydroepiandrosterone and 12α-hydroxy-7-ketodehydroepiandrosterone (**3**) were found to have NMDA receptor antagonist properties at 10 μM concentrations. These novel 12α-hydroxy steroids and sterols will be used as chemical tools to explore the biochemistry of cytochrome P450 8B1, the oxysterol 12α-hydroxylase enzyme implicated in cardiovascular health.

Experimental Section

Materials. Reagents and solvents were purchased from commercial sources. TLC plates (silica gel) with 254 nm fluorescent indicator (Sigma, St. Louis, MO) were used to monitor reactions. Both a UV lamp and ceric ammonium molybdate staining were used to visualize compounds on TLC. Silica gel (Silicycle (Quebec, Canada), 40-63 μm, 60 Å) was used for flash column separations. A WXG-4 optical polarimeter (Bante Instruments, Ltd, Shanghai, China) was used to measure optical rotations with a 10 cm long cell. A melting point apparatus (Global Medical and Lab Solutions, India) was used to measure the melting points of each compound. A Bruker 500 Advance III High Definition (HD) NMR spectrometer (Billerica, MA) was used to record the spectra of synthesized intermediates at 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR. $CDCl_3$ (Cambridge Isotope Laboratories, Tewksbury, MA) was used to take NMR spectra. The chloroform peaks were referenced to δ 7.26 ppm and δ 77.16 ppm for the ^{1}H NMR and ^{13}C NMR, respectively. Infrared (IR) spectra were recorded using a Nicolet iS50 FT-IR Spectrometer (Thermo Fisher Scientific, Waltham, MA) equipped with the Attenuated Total Reflectance (ATR) module. OMNIC software (version 9.3.32) was used to analyze the IR data (Thermo Fisher Scientific, Waltham, MA).

X-Ray Crystallaography.

Single crystals of $C_{38}H_{58}O_9$ (7α-,12α-dihydroxy DHEA, compound **3**) and $C_{19}H_{30}O_6$ (12α-hydroxy-7-keto DHEA, compound **2**) were prepared by slow evaporation of 1:1 mixture of methanol and dichloromethane. Suitable colorless plate-like crystals for compounds **3** and **2**, with dimensions of 0.33 mm × 0.30 mm × 0.03 mm and 0.33 mm x 0.27 mm x 0.13 mm, were mounted in paratone oil onto a nylon loop. Single crystals of $C_{19}H_{28}O_3$ (12β-hydroxy DHEA, compound **22**) were prepared by slow evaporation of a 1:1 mixture of diethyl ether and acetone.

A suitable colorless plank-like crystal for compound **22** with dimensions of 0.50 mm × 0.20 mm × 0.10 mm, was mounted in paratone oil onto 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* software [20]. The total number of data were measured in the range $4.7° < 2\theta < 52.0°$, $4.7° < 2\theta < 52.0°$ and $5.0° < 2\theta < 51.0°$ for compounds **3**, **2**, and **22**, respectively, using ω scans. Data processing and absorption correction, giving minimum and maximum transmission factors (0.9144, 1.000 for compound **3**, 0.8617, 1.000 for compound **2**, 0.8958, 1.000 for compound **22**) were accomplished with *CrysAlisPro* [20] and *SCALE3 ABSPACK* [21], respectively. The structure, using Olex2 [22], was solved with the ShelXT [23] structure solution program using direct methods and refined (on F^2) with the ShelXL [22] refinement package using full-matrix, least-squares techniques. All non-hydrogen atoms were refined with anisotropic displacement parameters. All carbon bound hydrogen atom positions were determined by geometry and refined by a riding model. All oxygen bound hydrogen atom positions, on compound **3**, were determined by electron density plots, except for H6 hydrogen atom which was placed on a calculated position. All solvent bound hydrogen atom positions, on compound **2**, were determined by electron density plots. Non-solvent oxygen bound hydrogen atom positions were determined by geometry and refined by a riding model, except for hydrogen atom, H4A, which was determined by electron density plot. All oxygen bound hydrogen atom positions, on compound **22**, were determined by electron density plots. Crystal structures were submitted to the Cambridge Structural Database (CSD) with CSD numbers: 1870940, 1870939, and 1870941 for compounds **2**, **3**, and **22**.

Brain Slice Preparation and Electrophysiology

Male and female C57BL/6J mice were used. All procedures were approved by the University of Texas at San Antonio Institutional Animal care and Use Committees in accordance with the National Institutes of Health guidelines. Mice were deeply anesthetized with isoflurane followed by rapid decapitation. The brain was quickly removed and chilled in ice cold cutting solution containing (in mM): 110 cholineCl, 2.5 KCl, 1.25 NaH_2PO_4, 7 $MgCl_2$, 0.5 $CaCl_2$, 10 dextrose, 25 $NaHCO_3$, and oxygenated with 95% O_2 -5%CO_2. Horizontal brain slices (250 μm) containing VTA were incubated for 30 minutes at 35 ºC in a chamber filled with artificial cerebrospinal fluid (aCSF) and stored at room temperature for the remainder of the day. Slices were transferred to a recording chamber where they continuously received aCSF (33-35º C) at a rate of 2 ml per minute by a gravity-feed system. aCSF was composed of (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2 $MgCl_2$, 2 $CaCl_2$, 10 dextrose, 25 $NaHCO_3$, was equilibrated using 95% O_2 - 5%CO_2 and had a pH of 7.2.

Midbrain neurons were visualized using gradient contrast illumination through a 40X water-immersion lens attached to an Olympus BX51 upright microscope. Neurons were considered dopaminergic based on the following electrophysiological criteria: have a hyperpolarization-activated inward current, slow spontaneous firing frequency $\leq$ 5 Hz and an action potential half-width $\geq$ 1.5 ms. Dopamine (TH-positive) neurons filled with biocytin were further identified immunocytochemically.

Whole cell recording pipettes with a tip resistance between 4-6 MOhm were pulled from borosilicate glass using a P-97 Flaming/Brown electrode puller (Sutter Instruments) and filled with internal solution containing (in mm): 110 d-gluconic acid, 110 CsOH, 10 $CsCl_2$, 1 EGTA,

10 HEPES, 1 ATP, 10 mm phosphocreatine.

Postsynaptic currents (PSC) were evoked by placing a 250 µm bipolar stimulating electrode within 500 µm of the neuron being recorded and applying a single pulse (0.05 ms). Whole cell recordings were made using a Multiclamp 700B amplifier (Molecular Devices). Experiments were conducted in the presence of the AMPA receptor antagonist, NBQX (25 µM), to isolate NMDA receptor-mediated currents. Signals were digitized at 50 kHz and saved to a hard drive for analysis using Axograph X (Axograph). Access resistance was monitored and measured post hoc to ensure access resistance had not changed by less than 20%.

Statistics: A student's two-tailed t-test was used when comparing between two groups. A P-value < 0.05 was used as our significance level for all tests.

Chemical Synthesis.

3*β*-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-androst-5-en-17-one (Compound **5**)**:** *tert*-Butyldimethylsilyl chloride (9.05 g, 60 mmol, 3 eq) and Imidazole (6.81 g, 100 mmol, 5 eq) were added to a solution of Dehydroepiandrosterone (DHEA) (6.00 g, 20 mmol, 1 eq) in acetonitrile (100 ml). The reaction was stirred at room temperature for 6 h. The reaction mixture was diluted with water (50 ml) and extracted three times with ethyl acetate (3 x 50 ml). The organic layer was concentrated under reduced pressure to afford DHEA-3β-tert-butyldimethylsilyl ether (**5**) (7.70 g, 19 mmol, 95%) as a white solid. No further purification was done; R_f: 0.86 (hexanes:ethyl acetate, 4:1, v/v); IR (neat) 2926.42, 2889.06, 2856.05, 1738.49,

1462.61, 1379.73, 1250.67, 1076.66, 1059.33 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.34 (d, J = 6 Hz, 1H), 3.48 (m, 1H), 2.45 (dd, J = 19, 8 Hz, 1H), 2.27 (m, 1H), 2.19 (m, 1H), 2.08 (m, 2H), 1.94, (m, 1H), 1.82 (m, 2H), 1.74 – 1.42 (m, 7H), 1.29 - 1.27 (m, 2H), 1.01 (s, 3H), 1.09 -0.96 (m, 2H), 0.88 (s, 12H), 0.05 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 141.94, 120.54, 72.59, 51.95, 50.47, 47.70, 42.93, 37.45, 36.87, 35.99, 32.16, 31.67, 31.61, 30.97, 26.06, 22.03, 20.50, 19.60, 18.39, 13.68, -4.42; HRMS (*m/z*) calculated for $C_{25}H_{42}O_2SiNa$ $[M + Na]^+$, 425.2846; found, 425.2839 (Δ 1.64 ppm).

3*β*-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-17-[(2-pyridinylmethyl)imino]-androst-5-ene

(Compound 6): To a solution of DHEA-3*β*-*tert*-butyldimethylsilyl ether **5** (3.00 g, 7.5 mmol, 1 eq) in toluene (100 ml) was added 2-aminomethyl pyridine (2.20 g, 23 mmol, 3 eq) and (50 mg, 0.2 mmol, 0.03 eq) of *para*-toluenesulfonic acid. The solution was refluxed for 4 h with a Dean Stark apparatus. The progress of the reaction was monitored by ^{1}H NMR. The reaction was cooled to room temperature. The reaction mixture was diluted with ethyl acetate and subsequently washed three times with saturated $NaHCO_3$ (200 ml), dried with $MgSO_4$ and concentrated under reduced pressure to afford the imine **6** as a white solid (3.1904 g, 6.8 mmol, 90%). No further purification was done. IR (neat) 2940.26, 2927.23, 2890.02, 2856.88, 1745.74, 1678.36, 1590.54, 1567.92, 1471.49, 1435.35, 1382.00, 1254.46, 1083.24 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52 (d, J = 5 Hz, 1H), 7.65 (td, J = 8, 2 Hz, 1H), 7.42 (dt, J = 8 Hz, 1H) , 7.14 – 7.09 (m, 1H), 5.33 (d, J = 6 Hz, 1H), 4.58 (ABq, J_{AB} = 16.7 Hz, 2H), 3.48 (M, 1H), 2.45 (dd, J = 17, 9 Hz, 1H), 2.34 – 2.13 (m, 3H), 2.25 – 1.98 (m, 2H), 1.92 – 1.79 (m, 2H), 1.75 – 1.58 (m, 4H), 1.55 – 1.30 (m, 4H), 1.20 – 1.12 (m, 1H), 1.11 – 0.96 (m, 2H), 1.06 (s, 3H), 0.91 (s, 3H), 0.88 (s, 9H), 0.05 (s, 6H) ; ^{13}C NMR (125 MHz, $CDCl_3$) δ 186.19, 160.62, 149.07, 141.92,

136.65, 128.87, 126.07, 122.35, 121.66, 120.66, 120.73, 120.51, 72.63, 58.10, 53.43, 50.71, 42.93, 37.48, 36.48, 34.19, 32.17, 31.45, 28.16, 26.05, 23.47, 20.85, 19.67, 18.37, 16.34, -4.46; HRMS (*m/z*) calculated for $C_{31}H_{49}N_2OSi$ $[M + H]^+$, 493.3609; found, 493.3611 (Δ -0.48 ppm).

12*β*-Hydroxy-3*β*-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-androst-5-en-17-one (Compound **7**): Copper triflate, (2.11 g, 5.84 mmol, 1.3 eq) and sodium-(L)-ascorbate (1.78 g, 8.98 mmol, 2 eq) were added to the imine **6** (2.12 g, 4.4 mmol, 1 eq) in an oven baked round bottom flask backfilled with N_2. Acetone (18 ml) and methanol (18 ml) were added to the mixture and stirred for 10 minutes. The reaction solution turned brown after stirring for 5 minutes. The round bottom flask containing the reaction mixture was sealed with a rubber stopper and degassed. The reaction solution was bubbled with O_2 gas with the aid of a balloon and an exit needle until the color of the solution changed from brown to green. The reaction mixture was heated at 50 $^{\circ}$C under an atmosphere of O_2 for 2 h. The reaction was cooled to room temperature and then quenched by adding saturated Na_4EDTA (30 ml, pH = 5) and ethyl acetate (15 ml). The resulting solution was stirred for 1 h and then transferred to a separatory funnel and allowed to stand for 5 minutes to differentiate the organic layer from the aqueous layer. The organic layer was separated from the aqueous layer. The aqueous layer was extracted with ethyl acetate (150 ml). The combined organic layers were concentrated under reduced pressure to afford a crude brown oil. The crude brown oil was purified by flash column chromatography (100% hexanes to 50% ethyl acetate in hexanes) to afford the alcohol **7** as a white solid (0.9 g, 2.1 mmol, 48%). An analytical sample of compound 7 was recrystallized with hexane/ethyl acetate (1:1) to afford a white amorphous solid: mp: 175 – 177 $^{\circ}$C; R_f: 0.79 (hexanes:ethyl acetate, 1:1, v/v); $[\alpha]_D^{20}$ -25°

[0.02% in $CHCl_3$]; IR (neat) 3545.58, 2949.63, 2928.84, 2890.64, 1733.18, 1470.79, 1403.66, 1359.09, 1291.15, 1270.45, 1111.92, 1047.42, 1027.11, 1018.23 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.34 (d, J = 6 Hz, 1H), 3.8 (dd, J = 11.3, 4.9 Hz, 1H), 3.47 (m, 1H), 3.06 (s, 1H), 2.47 (dd, J = 19, 10 Hz, 1H), 2.31 – 2.16 (m, 2H), 2.17 – 2.04 (m, 2H), 2.02 – 1.95 (m, 1H), 1.86 – 1.77 (dt, J = 13, 5 Hz, 2H), 1.76 – 1.70 (m, 1H), 1.69 – 1.57 (m, 3H), 1.57 – 1.50 (m, 1H), 1.49 – 1.37 (m, 1H), 1.29 - 1.19 (m, 1H), 1.16 – 1.06 (m, 2H), 1.06 – 0.99 (m, 1H), 1.03 (s, 3H), 0.95 (s, 3H), 0.88 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 141.94, 120.35, 77.82, 77.51, 51.54, 49.72, 49.35, 42.83, 37.40, 37.00, 35.90, 32.10, 30.74, 30.55, 26.05, 21/84, 19.51, 18.38, 8.19, -4.45; HRMS (*m/z*) calculated for $C_{25}H_{42}O_3SiNa$ $[M + Na]^+$, 441.2795; found, 441.2795 (Δ -0.01 ppm).

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17-,17-Ethylenedioxy-3*β*-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-androst-5-en-12*β*-ol (Compound **8**): Ethylene glycol (2 ml, 35.9 mmol, 16 mol eq), *para*-toluenesulfonic acid (0.103 g, 0.59 mmol, 0.28 eq) were added to the ketone **7** (0.89 g, 2.13 mmol, 1 eq) in toluene (100 ml). The reaction was refluxed with a Dean Stark apparatus for 4 h. Upon completion of the reaction as judged by TLC, the reaction was cooled to room temperature. The reaction mixture was diluted with ethyl acetate (150 ml), washed three times with saturated aqueous $NaHCO_3$ (3 x 100 ml) and dried with magnesium sulfate. The resulting solution was concentrated under reduced pressure to form a crude yellowish-brown oil, which was the purified by silica gel column chromatography (100% hexanes to 50% ethyl acetate in hexanes) to afford the ketal **8** as a white solid (0.2064 g, 0.44 mmol, 20%); mp: 140 – 143 $^{\circ}C$; R_f: 0.15 (hexanes:ethyl acetate, 4:1, v/v);

$[\alpha]_D^{20}$ -12.5° [0.12% in $CHCl_3$]; IR (neat) 3574.56, 3545.91, 2929.47, 2891.15, 2854.47, 1733.91, 1470.18, 1434.08, 1381.34, 1371.54, 1248.77, 1217.62, 1080.94, 1048.63, 1033.95 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.31 (d, J = 5 Hz, 1H), 4.03 – 3.95 (m, 4H), 3.91 – 3.85 (m, 1H), 3.47 (m, 1H), 2.30 – 2.22 (m, 1H), 2.21 – 2.14 (m, 1H), 2.05 – 1.96 (m, 1H), 1.94 -1.86 (m, 1H), 1.86 – 1.77 (m, 2H), 1.76 – 1.68 (m, 2H), 1.67 – 1.59 (m, 2H), 1.57 – 1.45 (m, 2H), 1.50 – 1.41 (m, 2H), 1.42 – 1.36 (m, 3H), 1.10 - 0.99 (m, 2H), 1.02 (s, 3H), 0.93 (s, 3H), 0.88 (s, 9H), 0.05 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 141.65, 120.89, 119.25, 72.59, 71.51, 49.41, 49.25, 48.87, 42.84, 37.48, 36.84, 32.10, 35.95, 32.15, 31.38, 30.97, 29.53, 26.07, 22.47, 19.55, 18.37, 9.01, -4.41; HRMS (*m/z*) calculated for $C_{27}H_{46}O_4SiNa$ $[M + Na]^+$, 485.3058; found, 485.3051 (Δ 1.42 ppm).

17-,17-Ethylenedioxy-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-androst-5-en-12-one (Compound **9**): Pyridinium chlorochromate (0.90 g, 19 mmol, 1 eq) was added to a solution of the ketal-alcohol **8** (0.90 g, 19 mmol, 1 eq) in dichloromethane (50 ml). The solution was stirred at room temperature for 6 h. The reaction mixture was washed with 5% NaOH (3×50 ml) and then concentrated under reduced pressure to obtain a crude yellow solid residue. The crude residue was purified by silica gel column chromatography (100% hexanes to 10% ethyl acetate in hexanes) to afford ketone **9** as a white solid (0.419 g, 0.909 mmol, 48%). An analytical sample of compound **9** was recrystallized from 100% acetone to afford colorless needlelike crystals: mp 143 – 145 °C; R_f: 0.77 (hexanes:ethyl acetate, 4:1, v/v); $[\alpha]_D^{20}$ +33.3° [0.03% in $CHCl_3$]; IR (neat) 3545.09, 2934.26, 2893.37, 2854.03, 1714.58, 1460.25, 1434.19, 1381.52, 1359.66, 1246.17, 1178.01, 1074.15, 1050.79, 1025.94 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.34 (d, J = 6 Hz, 1H), 4.28 – 4.15 (m, 1H), 4.15 – 4.08 (m, 1H), 4.01 – 3.90 (m, 1H), 3.90 – 3.75 (m, 1H),

3.47 (m, 1H), 2.42 (dd, J = 15, 11 Hz, 1H), 2.31 – 2.19 (m, 2H), 2.19 – 2.12 (m, 1H), 2.05-1.91 (m, 1H), 1.84 – 1.75 (m, 2H), 1.68 – 1.60 (m, 1H), 1.65 – 1.55 (m, 1H), 1.54 – 1.37 (m, 2H), 1.33 – 1.16 (m, 5H), 1.11 (s, 3H), 1.07 - 0.98 (m, 1H), 1.05 (s, 3H), 0.85 (s, 9H), 0.04 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 210.90, 141.29, 120.81, 116.92, 72.28, 65.59, 65.02, 57.73, 50.83, 49.96, 42.77, 38.64, 37.09, 37.04, 34.58, 31.92, 31.36, 30.79, 26.06, 20.92, 19.21, 18.37, -4.45; HRMS (*m/z*) calculated for $C_{27}H_{44}O_4SiNa$ $[M + Na]^+$, 483.2901; found, 483.2903 (Δ -0.42 ppm).

17-,17-Ethylenedioxy-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-androst-5-en-12*α*-ol (Compound **10):** L- Selectride (6.0 ml of a 1.0 M solution in tetrahydrofuran, 6.0 mmol, 1.8 eq), was added to ketone **9** (1.5 g, 3.3 mmol, 1.0 eq) in THF (50 ml) at -78 °C under an N_2 atmosphere for 12 h. The reaction was quenched with the addition of water (20 ml) dropwise at -78 °C, allowed to warm to room temperature and then extracted with ethyl acetate (3×50 ml). The organic layer was concentrated under reduced pressure and purified by silica gel column chromatography to afford the 12α-hydroxylated compound (**10**) as a white waxy solid (1.20 g, 2.59 mmol, 80%). An analytical sample of compound **10** was recrystallized from 100% acetone to afford colorless needlelike crystals: mp 128 – 130°C; R_f: 0.29 (hexanes:ethyl acetate, 4:1, v/v); $[\alpha]_D^{20}$ +100° [0.01% in $CHCl_3$]; IR (neat) 3485.67, 3209.96, 2956.47, 2926.19, 2887.82, 2854.42, 1470.61, 1460.75, 1404.53, 1380.79, 1279.49, 1249.29, 1181.18, 1154.53, 1085.57, 1042.45, 1007.23, 1029.96 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) 5.32 (d, J = 6 Hz, 1H), 4.70 (s, 1H), 4.05 – 3.84 (m, 5H), 3.47 (m, 1H), 2.29 – 2.21 (m, 1H), 2.19 – 2.08 (m, 2H), 2.06 – 1.98 (m, 1H), 1.96 -1.87 (m, 1H), 1.86 – 1.63 (m, 6H), 1.62 – 1.46 (m, 4H), 1.38 – 1.23 (m, 2H), 0.99 (s, 3H), 0.88 (s, 9H), 0.85 (s, 3H), 0.05 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 141.88, 121.73, 120.94, 72.72,

71.79, 64.87, 63.53, 43.76, 43.18, 43.00, 42.61, 37.33, 36.52, 34.97, 32.76, 32.18, 39.49, 28.17, 26.08, 22.77, 19.41, 18.40, 15.71, 1.16, -4.43; HRMS (*m/z*) calculated for $C_{27}H_{46}O_4SiNa$ $[M + Na]^+$, 485.3058; found, 485.3051 (Δ 1.33 ppm).

3*β*-,12*α*-Dihydroxy-andros-5-en-17-one (Compound **1**)**:** Hydrochloric acid (4.0 ml of a 2.0 M solution, 8.0 mmol, 37 mol eq) was added to a solution of ketal **10** (100 mg, 0.22 mmol, 1.0 eq) in THF (10 ml). The reaction was stirred for 4 h and quenched with saturated $NaHCO_3$ (aqueous) and extracted with CH_2Cl_2 (50 ml). The CH_2Cl_2 extract was concentrated under reduced pressure and purified by silica gel column chromatography (70% hexanes to 50% hexanes in ethyl acetate) to afford the diol **1** as a white solid (45 mg, 0.15 mmol, 68%). An analytical sample of compound **1** was recrystallized from acetone/diethylether (1:1) to afford white crystals: mp 178 – 179°C; R_f: 0.17 (hexanes: ethyl acetate, 1:1, v/v); $[\alpha]_D^{20}$ -22.7° [0.02% in $CHCl_3$]; IR (neat) 3446.37, 3350.12, 2963.99, 2933.57, 2885.49, 1724.51, 1463.65, 1431.58, 1406.30, 1375.51, 1357.81, 1309.33, 1259.72, 1191.52, 1132.46, 1089.15, 1054.47, 1037.11, 1024.17, 1007.20 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 5.39 (d, J = 6 Hz, 1H), 4.14 (s, 1H), 3.52 (m, 1H), 2.45 (dd, J = 19, 9 Hz, 1H), 2.36 – 2.28 (m, 1H), 2.27 – 2.18 (m, 1H), 2.15 - 1.93 (m, 4H), 1.87 – 1.65 (m, 4H), 1.55-1.40 (m, 3H), 1.17 – 1.08 (m, 1H), 1.00 (s, 3H), 0.99 (s, 3H), 0.87 (s, 3H) ; ^{13}C NMR (125 MHz, $CDCl_3$) 141.03, 121.11, 71.60, 69.65, 53.04, 43.37, 42.33, 37.11, 36.40, 36.26, 31.64, 31.38, 30.63, 27.41, 25.77, 21.16 19.35, 14.31, 13.86; HRMS (*m/z*) calculated for $C_{19}H_{28}O_3Na$ $[M + Na]^+$, 327.1931; found, 327.1934 (Δ -0.94 ppm).

3*β*-,12*α*-Diacetoxy-androst-5-en-17-one (Compound **11**)**:** Acetic anhydride (10.0 ml, 106 mmol, 17.9 eq) and pyridine (1.20 ml, 14.8 mmol, 2.5 eq) were added to diol **1** (1.8 g, 5.9 mmol, 1 eq). The reaction was stirred at room temperature for 12 h. The reaction mixture was diluted with 50 ml of water and extracted with ethyl acetate (150 ml). The organic layer was concentrated under reduced pressure and purified by silica gel chromatography (90% hexanes to 70% hexanes in ethyl acetate) to afford diacetate **11** as a white solid (1.69 g, 4.3 mmol, 72%); mp: 189 – 192 $^{\circ}$C; R_f: 0.29 (hexanes:ethyl acetate, 4:1, v/v); $[\alpha]_D^{20}$ -7.5° [0.2% in $CHCl_3$]; IR (neat) 3450.22, 2960.86, 2944.30, 2905.91, 2826.58, 2249.42, 1731.33, 1556.73, 1463.17, 1438.07, 1438.07, 1373.99, 1357.81, 1239.62, 1134.04, 1114.17, 1022.49, cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) 5.41 (d, J = 1 Hz, 1H), 5.19 (s, 1H), 4.57 (m, 1H), 2.45 – 2.25 (m, 3H), 2.17 – 2.05 (m, 2H), 2.01 (s, 3H), 2.00 – 1.96 (m, 2H), 1.98 – 1.94 (s, 3H), 1.90 – 1.80 (m, 2H), 1.79 – 1.50 (m, 6H), 1.33 – 1.18 (m, 1H), 1.12 – 1.05 (td, J = 14, 3 Hz, 1H), 0.98 (s, 3H), 0.90 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 216.69, 170.60, 170.20, 139.97, 121.94, 73.66, 71.77, 50.70, 45.48, 44.84, 38.17, 36.95, 36.44, 36.32, 30.99, 30.64, 27.68, 25.45, 21.50, 21.36, 21.27, 19.26, 13.94; HRMS (*m/z*) calculated for $C_{23}H_{32}O_5Na$ $[M + Na]^+$, 411.2142; found, 411.2144 (Δ -0.6 ppm)

3*β*-,12*α*-Diacetoxy-7-oxo-DHEA (Compound **12**)**:** Chromium trioxide (1.235 g, 12.35 mmol, 6 eq) was stirred in CH_2Cl_2 (50 ml) at -78 $^{\circ}$C for 15 minutes. 3,5-dimethylpyrazole (1.188 g, 12.35 mmol, 6 eq) was added to the reaction and stirred for another 20 minutes. Diacetate **11** (0.600 g, 2.059 mmol, 1 eq) in CH_2Cl_2 (10 ml) was added to the mixture and stirred for 24 h. The mixture was concentrated under reduced pressure to obtain a crude black mixture which was bounded to silica gel and introduced onto an already packed silica gel column and purified (80% hexanes to

50% hexanes in ethyl acetate) to afford diketone **12** as a white solid (0.370 g, 0.919 mmol, 45%); mp: 169 – 172 $^{\circ}$C; R_f: 0.48 (hexanes:ethyl acetate, 1:1, v/v); $[\alpha]_D^{20}$ +3.57° [0.28% in $CHCl_3$]; IR (neat) 3450.95, 3348.12, 2943.86, 2935.27, 1736.78, 1668.34, 1468.59, 1439.60, 1375.50, 1364.22, 1229.63, 1134.41, 1035.68, 1025.14, cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) 5.78 (s, 1H), 5.18 (s, 1H), 4.7 (m, 1H), 2.87 (m, 1H), 2.61 (m, 1H), 2.52 – 2.38 (s, 3H), 2.37 – 2.30 (td, J = 12, 7 Hz, 1H), 2.20 – 2.10 (m, 1H), 2.04 (s, 3H), 2.03 – 1.97 (m, 1H), 1.98 – 1.94 (m, 1H), 1.96 (s, 3H), 1.94 – 1.79 (m, 3H), 1.78 – 1.70 (m, 2H), 1.70 – 1.62 (m, 1H), 1.22 -1.17 (m, 4H), 0.91 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$), 215.98, 200.37, 170.39, 170.16, 164.99, 126.68, 71.94, 70.71, 51.08, 45.14, 43.86, 38.19, 38.01, 36.11, 36.07, 27.32, 25.60, 23.63, 21.35, 21.21, 17.33, 14.10; HRMS (*m/z*) calculated for $C_{23}H_{31}O_6$ $[M + H]^+$, 403.2115; found, 403.2115 (Δ 0.16 ppm).

3*β*-,12*α*-Dihydroxy-andros-5-en-7,17-dione (Compound **2**)**:** To a stirring solution of diketone **12** (0.35 g, 0.87 mmol, 1 eq) in methanol (30 ml) was added potassium carbonate (120 mg, 0.87 mmol, 1 eq). The reaction was stirred at room temperature for 24 h. The resulting reaction was diluted with water (30 ml) and extracted with ethyl acetate (100 ml). The ethyl acetate extract was concentrated under reduced pressure and then purified by silica gel column chromatography (50% hexanes to 100% ethyl acetate) to afford 12*α*-hydroxy-7-oxo DHEA (**2**) as a pale yellow solid (60 mg, 0.19 mmol, 22%); mp: 178 – 181 $^{\circ}$C; R_f: 0.78 (ethyl acetate:methanol, 4:1, v/v); $[\alpha]_D^{20}$ -8.33° [0.18% in $CHCl_3$]; IR (neat) 3619.00, 3357.93, 3296.12, 3296.12, 3131.07, 3109.09, 3035.64, 2979.73, 2938.52, 2875.31, 2846.05, 1738.61, 1722.26, 1670.31, 1654.82, 1627.01, 1442.62, 1434.02, 1382.14, 1328.99, 1297.17, 1258.99, 1186.50, 1164.83, 1050.61, 1037.49, 1028.92, cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) 5.75 (s, 1H), 4.12 (s, 1H), 3.68 (m, 1H), 2.81 (m, 1H), 2.58 – 2.50 (m, 1H), 2.48 – 2.34 (m, 4H), 2.17 – 2.06 (m, 2H), 2.06 – 1.96 (m, 2H), 1.96 –

1.87 (m, 2H), 1.85 – 1.76 (m, 3H), 1.76 – 1.68 (m, 2H), 1.66 – 1.58 (m, 2H), 1.32 – 1.22 (m, 1H), 1.18 (s, 3H), 0.88 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 200.83, 166.42, 126.15, 70.21, 68.70, 53.51, 44.46, 44.06, 42.00, 38.05, 37.82, 36.21, 36.04, 31.19, 27.50, 23.42. 17.41, 13.99; HRMS (*m/z*) calculated for $C_{19}H_{27}O_4$ $[M + H]^+$, 319.1904; found, 319.1904 (Δ -0.48 ppm). 12α-Hydroxy-7-oxo DHEA (40 mg) was dissolved in 10 ml of 1:1, methanol:dichloromethane (v/v) in a 40 ml screw cap vial. The solvent was slowly evaporated in the fume hood over 2 days to afford crystals, which were used to solve the crystal structure.

17-,17-Ethylenedioxy-3β-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-androst-5-en-7,12-dione (Compound **13):** Chromium trioxide (0.69 g, 6.51 mmol, 6 eq) was added to a stirring solution of CH_2Cl_2 (50 ml) in a round bottom flask at -78 $^{\circ}$C and left to stir for 15 minutes. 3,5-Dimethylpyrazole (0.63 g, 6.51 mmol, 6 eq) was added to the reaction and was stirred for 25 minutes. Ketone **9** (0.50 g, 1.09 mmol, 1 eq) was dissolved in CH_2Cl_2 (10 ml) and added to the reaction mixture and stirred for 12 h. In a separate round bottom flask A, a stirring solution of CH_2Cl_2 (50 ml) was cooled to -78 $^{\circ}$C and chromium trioxide (0.69 g, 6.51 mmol, 6 eq) and 3,5-dimethylpyrazole (0.63 g, 6.51 mmol, 6 eq) were added sequentially at a 15 minute-interval. After 1 h, the existing reaction was transferred to reaction flask A at -78 $^{\circ}$C and stirred for another 12 h. A crude black solution was obtained, bound to silica gel and loaded onto an already packed column and purified (hexane 100% to 80% hexanes in ethyl acetate) to afford diketone **13** as a pale-yellow solid (0.46 g, 0.97 mmol, 89%). An analytical sample of compound **13** was recrystallized from hexanes/ethyl acetate (1:1) to give yellow crystals: mp 268 – 270 $^{\circ}$C; R_f: 0.33 (hexanes:ethyl acetate, 4:1, v/v); $[\alpha]_D^{20}$ -12.19° [0.04% in $CHCl_3$] IR (neat) 2977.36, 2948.37,

2929.25, 2894.92, 1737.84, 1710.55, 1655.69, 1621.96, 1470.41, 1458.15, 1428.66, 1417.83, 1382.15, 1373.73, 1354.75, 1296.51, 1257.83, 1184.39, 1168.27, 1138.89, 1099.14, 1090.54, 1035.79, 1030.28, cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) 5.73 (s, 1H), 4.18 (m, 1H), 4.07 (m, 1H), 3.96 (m, 1H), 3.86 (m, 1H), 3.60 (m, 1H), 2.57 (m, 3H), 2.43 (d, J = 8, 2 Hz, 1H), 2.31 (m, 2H), 2.01 (m, 2H), 1.80 (m, 3H), 1.72 – 1.56 (m, 4H), 1.23 (s, 3H), 1.19 – 1.09 (m, 1H), 1.14 (s, 3H), 0.88 (s, 9H), 0.06 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) 208.80, 199.01, 166.09, 126.04, 116.20, 71.04, 65.99, 65.51, 57.91, 49.83, 44.58, 44.21, 42.62, 38.27, 36.10, 34.56, 31.58, 25.94, 23.28, 18.25, 17.07, 15.48, -4.49; HRMS (*m/z*) calculated for $C_{27}H_{43}O_5Si$ $[M + H]^+$, 475.2874; found, 475.2874 (Δ 0.07 ppm). *In subsequent procedures to oxidize the C7-allylic position using CrO_3 and 3,5-dimethylpyrazole, we found that increasing the mol equivalents of both CrO_3 and 3,5-dimethylpyrazole to 12 mol equivalents resulted in higher yields to afford the C7-keto steroid product.

17,17-Ethylenedioxy-3*β*-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-androst-5-en-7*α*,12*α*-diol (Compound **14**)**:** L-Selectride (15 ml of 1.0 M solution in THF, 15 mmol, 16 eq) was added to a solution of diketone **13** (0.4613 g, 0.9717 mmol, 1 eq) in THF (30 ml) dropwise at -78 °C under an atmosphere of N_2. The reaction was stirred for 12 h and then quenched by adding water (20 ml) to reaction solution at 78 °C. The mixture was warmed to room temperature and then extracted with ethyl acetate (3×50 ml). The ethyl acetate extract was concentrated under reduced pressure and purified by silica gel column chromatography (silica gel, 100% hexanes to 50 % hexanes in ethyl acetate) to afford diol **14** as a white solid (0.20 g, 0.42 mmol, 42%). An analytical sample of compound 14 was recrystallized with hexane/ethyl acetate (1:1) to afford white amorphous solid: mp: 120 – 122 °C; $[\alpha]_D^{20}$ + 7.4° [0.07% in $CHCl_3$]. R_f: 0.11 (hexanes:ethyl acetate, 4:1, v/v); IR (neat) 3578.41, 3416.56, 3357.55, 3207.67, 2953.08,

2953.08, 2930.93, 2883.81, 2854.33, 2260.15, 1670.03, 1629.45, 1470.72, 1416.44, 1307.65, 1282.04, 1229.08, 1193.51, 1166.41, 1118.48, 1101.42, 1042.51, 1007.59 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) 5.59 (dd, J = 5, 2 Hz, 1H), 4.77 (d, J = 2 Hz, 1H), 4.03 – 3.98 (m, 2H), 3.97 – 3.84 (m, 2H), 3.53 (m, 1H), 2.63 (td, J = 11, 7 Hz, 1H), 2.33 – 2.26 (m, 1H), 2.24 – 2.18 (m, 1H), 1.97 – 1.89 (m, 2H), 1.88 – 1.83 (m, 1H), 1.82 – 1.65 (m, 4H), 1.63 – 1.57 (m, 2H), 1.56 – 1.42 (m, 2H), 1.41 – 1.29 (m, 2H), 1.26 – 1.17 (m, 1H), 1.17 – 1.09 (td, J = 13, 4 Hz, 1H), 0.97 (s, 3H), 0.88 (s, 9H), 0.86 (s, 3H) 0.05 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) 147.14, 123.59, 121.56, 72.21, 71.46, 65.01, 64.87, 63.51, 47.39, 42.73, 38.48, 37.36, 37.05, 36.85, 36.33, 34.87 31.91, 27.80, 26.03, 22.71, 18.33, 18.18, 15.53, -4.44, -4.46; HRMS (*m/z*) calculated for $C_{27}H_{46}O_5SiNa$ $[M + Na]^+$, 501.3007; found, 501.2999 (Δ 1.64 ppm).

3*β*-,7*α*-,12*α*-Trihydroxy-andros-5-en-17-one (Compound **3**)**:** To diol **14** (0.20 g, 0.42 mmol, 1 eq) in THF (50 ml) was added dilute HCl (2.0 M, 10 ml in 40 ml of water, 20 mmol, 48 eq) and was stirred for 12 h. The solution was quenched with saturated $NaHCO_3$ and extracted with CH_2Cl_2 (50 ml). The CH_2Cl_2 layer was concentrated under reduced pressure and then purified by silica gel column chromatography (100% ethyl acetate to 20% methanol in ethyl acetate) to afford 7*α*-,12*α*-dihydroxy DHEA (**3**) as a white solid (55 mg, 0.17 mmol, 41%) and 7*β*-,12*α*-dihydroxy DHEA (**4**) as a white solid (5 mg, 0.015 mmol, 3.57%)*; mp of Compound **3**: 182 – 185 $^{\circ}C$; R_f of Compound **3**: 0.64 (ethyl acetate:methanol, 4:1, v/v) and 0.58 (ethyl acetate:methanol, 95:5, v/v); R_f of Compound **4**: 0.65 (ethyl acetate:methanol, 95:5, v/v); $[\alpha]_D^{20}$ of Compound **3**: -14.28° [0.07% in $CHCl_3$]; IR of **3**: (neat) 3529.25, 3400.97, 3327.91, 3247.75, 2956.64, 2930.31, 2916.06, 2901.19, 2856.06, 1727.94, 1682.69, 1660.17, 1646.95, 1563.35, 1442.02, 1400.44, 1376.85, 1338.38, 1272.60, 1225.54, 1190.00, 1170.52, 1054.59, 1038.17,

1022.50, 1000.76 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) 5.67 (d, J = 6 Hz, 1H), 4.14 (apparent s, 1H), 3.98 (apparent s, 1H), 3.65 – 3.58 (m, 1H), 2.58 – 2.51 (apparent td, J = 11, 5 Hz, 1H), 2.50 – 2.42 (m, 1H), 2.40 – 2.39 (m, 1H), 2.33 – 2.26 (m, 1H), 2.20 – 2.11 (m, 2H), 1.90 – 1.84 (m, 2H), 1.83 – 1.76 (m, 1H), 1.75 – 1.68 (m, 1H), 1.66 – 1.63 (m, 1H), 1.62 – 1.47 (m, 2H), 1.25 (s, 3H), 1.20 - 1.11 (td, J = 14, 3 Hz, 1H), 0.99 (s, 3H), 0.89 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 146.49, 123.92, 71.30, 69.45, 64.48, 52.71, 42.07, 37.93, 37.30, 37.16, 36.92, 36.90, 36.16 31.40, 27.11, 21.19, 18.24, 13.74; HRMS (*m/z*) calculated for $C_{19}H_{28}O_4Na$ $[M + Na]^+$, 343.1880; found, 343.1881 (Δ -0.31 ppm). *The minor 7*β*-hydroxy product (Compound **4**, 7*β*-,12*α*-dihydroxy DHEA) presumably arose from the L-Selectride step in the reduction of diketone **13** to diol **14**, (see above). 7*α*-,12*α*-Dihydroxy DHEA (40 mg) was dissolved in 2 ml of 1:1, methanol:dichloromethane (v/v) in a 40 ml screw cap vial. The solvent was slowly evaporated in the fume hood over 3 days to afford crystals, which were used to solve the crystal structure.

12*β*-Acetoxy-17,17-ethylenedioxy-3*β*-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-androst-5-ene (Compound **15):** To a solution alcohol **8** (1.25 g, 2.70 mmol, 1 eq) in acetic anhydride (15 ml, 157 mmol, 58 eq) was added pyridine (1.00 ml, 12.4 mmol, 4.59 eq). The reaction was stirred at room temperature for 24 h. The reaction mixture was diluted with water (50 ml) and extracted with ethyl acetate (3 × 50 ml). The ethyl acetate layer was concentrated under reduced pressure to produce a crude brown oil which was further purified by silica gel column chromatography (100% hexanes to 80% hexanes in ethyl acetate) to afford acetate **15** as a yellow oil (0.64 g, 1.26 mmol, 47%); R_f: 0.59 (hexanes:ethyl acetate, 4:1, v/v); $[\alpha]_D^{20}$ -12.6° [0.2% in $CHCl_3$]; IR (neat) 2951.91, 2928.59, 2892.49, 1732.37, 1669.40, 1471.11, 1462.39, 1371.33, 1361.30, 1306.50, 1277.95, 1244.36, 1184.86, 1163.59, 1088.61, 1035.07, 1021.88, 1004.07 cm^{-1}; ^{1}H NMR (500

MHz, $CDCl_3$) 5.29 (apparent d, J = 5 Hz, 1H), 5.17 (dd, J = 11, 5 Hz, 1H), 3.90 – 3.78 (m, 3H), 3.66 – 3.59 (m, 1H), 3.49 – 3.42 (m, 1H), 2.26 – 2.12 (m, 2H), 2.00 (s, 3H), 1.98 - 1.89 (m, 1H), 1.85 (dt, J = 12, 5 Hz, 1H), 1.80 – 1.73 (m, 2H), 1.73 – 1.64 (m, 3H), 1.55 – 1.22 (m, 6H), 1.14 – 1.00 (m, 2H), 0.99 (s, 3H), 0.97 (s, 3H) 0.86 (s, 9H), 0.03 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.51, 141.45, 120.85, 118.73, 73.88, 72.45, 64.92, 64.36, 49.80, 48.95, 48.30, 42.78, 37.38, 36.82, 34.75, 32.01, 31.36, 30.84, 26.74, 26.04, 22.13, 21.64, 19.44, 18.34, 9.90, -4.47; HRMS (*m/z*) calculated for $C_{29}H_{48}O_5SiNa$ $[M + Na]^+$, 527.3163; found, 527.3161 (Δ 0.48 ppm).

12*β*-Acetoxy-17,17-ethylenedioxy-3*β*-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-androst-5-en-7-one (Compound **16):** Chromium trioxide (1.9 g, 19 mmol, 15 eq) was stirred in CH_2Cl_2 (50 ml) at -78 °C for 15 minutes. 3,5-dimethylpyrazole (1.8 g, 18.7 mmol, 15 eq) was added to the reaction and stirred for another 20 min. The acetate **15** (0.64 g, 1.26 mmol, 1 eq) in CH_2Cl_2 (10 ml) was added to the reaction mixture and stirred for 24 h. The mixture was concentrated under reduced pressure to obtain a crude black mixture which was bound to silica gel and dry loaded onto an already packed silica gel column and purified (hexanes 100% to 80% hexanes in ethyl acetate) to afford ketone **16** as a yellow solid (0.2664 g, 0.5140 mmol, 40%); mp: 164 – 167 °C; R_f: 0.22 (hexanes:ethyl acetate, 4:1, v/v); $[\alpha]_D^{20}$ +7° [0.14% in $CHCl_3$]; IR (neat) 2952.03, 2929.47, 2856.95, 1731.88, 1666.73, 1634.24, 1471.28, 1384.89, 1373.47, 1245.27, 1137.82, 1093.40, 1032.28, 1022.03, 1004.85 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) 5.67 (s, 1H), 5.09 (dd, J = 11, 5 Hz, 1H), 3.90 – 3.78 (m, 3H), 3.65 – 3.55 (m, 2H), 2.53 – 2.34 (m, 3H), 2.22 (dd, J = 15, 12 Hz, 1H), 2.02 (s, 3H), 1.95 - 1.89 (m, 2H), 1.88 – 1.77 (m, 4H), 1.71 – 1.63 (dt, J = 13, 5 Hz, 1H), 1.60 (apparent s, 3H), 1.57 – 1.47 (m, 2H), 1.21 – 1.18 (m, 2H), 1.16 (s, 3H), 1.00 (s, 3H), 0.88 (s, 9H), 0.05 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 200.76, 170.49, 166.19, 125.91,

117.95, 72.86, 71.23, 64.90, 64.29, 48.56, 48.53, 44.53, 44.38, 44.14, 42.64, 38.51, 36.49, 34.76, 31.70, 26.70, 25.95, 24.46, 21.58, 18.26, 9.98, -4.50, -4.55; HRMS (*m/z*) calculated for $C_{29}H_{47}O_6Si$ $[M + H]^+$, 519.3136; found, 519.3136 (Δ 0.1 ppm). The reaction was scaled up to yield 1.59 g of compound **16**, which was used for the synthesis of compound **17**.

12*β*-Acetoxy-17,17-ethylenedioxy-3*β*-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-androst-5-en-7*β*-ol (Compound **17**): To a stirring solution of ketone **16** (1.5 g, 2.89 mmol, 1 eq) in methanol (50 ml) at 0 °C was added sodium borohydride powder (4.00 g, 105 mmol, 36 eq). The reaction was stirred from 0 °C to room temperature for 6 h. The solution was diluted with water (50 ml) and transferred to a separatory funnel, which was extracted with ethyl acetate (3 ×50 ml). The ethyl acetate layer was concentrated under reduced pressure to form crude white solid which was purified by silica gel column chromatography (100% hexanes to 70% ethyl acetate) to afford an epimeric mixture of 7*α*- and 7*β*- hydroxy products as a colorless solid (1.2 g, 2.3 mmol, 80%), which were unresolvable from TLC analysis. Based on the integration of the Δ^5-proton in the ^{1}H NMR spectrum, the mixture of the 7*α*-hydroxy and 7*β*-hydroxy products were in a 1:4 ratio. This ratio was determined by integrating the Δ^5-protons of the 7*α*-hydroxy epimer and the 7*β*-hydroxy epimer (**17**) in the ^{1}H NMR spectrum, which appeared at δ 5.56 ppm and 5.21 ppm, respectively. The upfield chemical shift (δ 5.21 ppm) was assigned as the 7*β*-hydroxy epimer as we have done in a previous study [16]; R_f: 0.45 (hexanes:ethyl acetate, 7:3, v/v); IR (neat) 3466.11, 2951.81, 2930.96, 2854.78, 1731.91, 1715.78, 1671.87, 1471.24, 1462.55, 1358.18, 1371.99, 1361.26, 1245.93, 1191.37, 1136.13, 1091.75, 1070.26, 1027.82, 1004.27 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.21 (s, 1H), 5.12 (dd, J = 12, 5 Hz, 1H), 3.89 – 3.78 (m, 3H), 3.65 – 3.60 (m, 1H), 3.52 – 3.44 (m, 1H), 2.30 – 2.15 (m, 2H), 2.00 (s, 3H), 1.98 - 1.90 (m, 2H), 1.89 – 1.78 (m, 2H),

1.77 – 1.57 (m, 3H), 1.55 – 1.31 (m, 5H), 1.25 – 1.17 (dt, J = 12, 6 Hz, 1H), 1.16 – 1.01 (m, 1H), 1.03 (s, 3H), 0.99 (s, 3H), 0.87 (s, 9H), 0.04 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 146.78, 144.07, 125.39, 123.37, 118.62, 118.25, 73.57, 73.35, 73.03, 72.13, 64.90, 64.45, 64.36, 64.32, 49.36, 48.54, 46.93, 43.33, 42.56, 42.26, 41.64, 40.12, 37.65, 37.10, 37.06, 36.81, 34.88, 34.72, 31.96, 31.77, 26.58. 26.01, 24.30, 21.61,19.15, 18.31, 9.88, -4.47, -4.49; HRMS (*m/z*) calculated for $C_{29}H_{48}O_6SiNa$ $[M + Na]^+$, 543.3112; found, 543.3108 (Δ 0.87 ppm)

12*β*-Acetoxy-17,17-ethylenedioxy-3*β*-,7*β*-di-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]]-androst-5-ene (Compound **18):** To the 7*α**/7*β*-epimeric mixture of alcohol **17** (*vide supra*) (1.2 g, 2.3 mmol, 1 eq) in acetonitrile (50 ml) was added *tert*-butyldimethylsilyl chloride (3.11 g, 20.6 mmol, 9 eq) and imidazole (2.5 g, 36 mmol, 15 eq). The reaction was refluxed at 90 °C for 12 h. The reaction was diluted with water (50 ml) and extracted with ethyl acetate (150 ml). The organic layer was concentrated under reduced pressure and purified by silica gel column chromatography (hexanes 90% to 80% hexanes in ethyl acetate) to afford the TBDMS ether **18** as a white solid (0.94 g, 1.42 mmol, 60%); R_f 0.63 (hexanes:ethyl acetate, 7:3, v/v); IR (neat) 2950.94, 2927.70, 2855.45, 1721.92, 1678.02, 1471.03, 1462.09, 1385.44, 1376.50, 1361.74, 1249.56, 1174.04, 1159.85, 1092.94, 1078.57, 1049.42, 1032.25, 1002.64 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.20 (s, 1H), 5.09 (dd, J = 12, 5 Hz, 1H), 3.95 (apparent d, J = 8 Hz, 1H), 3.89 – 3.75 (m, 3H), 3.65 – 3.59 (m, 1H), 3.50 – 3.41 (m, 1H), 2.34 – 2.08 (m, 2H), 2.00 (s, 3H), 1.97 - 1.81 (m, 3H), 1.79 – 1.41 (m, 7H), 1.40 – 1.23 (m, 2H), 1.20 – 1.13 (td, J = 11, 5 Hz, 1H), 0.97 (s, 3H), 0.90 (s, 3H), 0.87 (s, 9H), 0.86 (s, 9H), 0.08 – 0.01 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.55, 142.63, 126.10 (-CH-), 118.39, 74.41 (-CH-), 73.47, 72.11 (-CH-), 64.85, 64.29, 49.51 (-CH-), 48.61, 47.10, 43.05, 42.41, 39.87, 37.17, 36.63, 34.88, 32.01, 26.48, 26.35,

26.04, 21.62, 18.96, 18.29, 9.91, -2.46, -3.23, -4.45, -4.46 (the minor 7*α*-OTBDMS epimer product was observed through ^{13}C NMR spectroscopy, and the peaks corresponding to the major 7*β*-OTBDMS epimer were reported); HRMS (*m/z*) calculated for $C_{35}H_{62}O_6Si_2Na$ $[M + Na]^+$, 657.3977; found, 657.3985 (Δ -1.17 ppm). *The minor 7*α*-OTBDMS epimer product was observed through ^1H NMR spectroscopy (δ 5.45 (d, J = 6 Hz, 1H), which corresponds to the Δ^5 proton), however over the sequence of the next four synthetic steps toward 7*β*-,12*α*-dihydroxy-DHEA (Compound **4**), the amount of the 7*α*-hydroxy epimer decreases relative to the 7*β*-hydroxy epimer. Since the 7*α*/7*β* epimeric mixture was resolvable by TLC, an analytical sample of the TBDMS ether **18** was purified by column chromatography (isocratic 20% ethyl acetate in hexane). The isolated products were collected across 20 test-tubes (2 ml each) and then each tube was dried down using a stream of nitrogen. The ^1H NMR of each tube was taken and the integrations of the Δ^5-proton of the 7*α*- to 7*β*- epimers were calculated (doublet at δ 5.47 ppm and broad singlet at δ 5.21 ppm, respectively). Ratios of 7*α*- to 7*β*- epimer ranged from 2.5:1 in tube 1 to 0.005:1 in tube 20, respectively (See Supporting Information of ^1H NMR overlay and a table of 7*α*-:7*β*- epimeric ratios corresponding to the tube number).

17,17-Ethylenedioxy-3*β*-,7*β*-di-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]]-androst-5-en-12*β*-ol (Compound **19**)**:** Potassium carbonate (2.0 g, 14 mmol, 10 eq) was added to acetate **18** (0.90 g, 1.41 mmol, 1 eq) in methanol (50 ml). The reaction was refluxed at 60 °C for 6 h. The solution was cooled to room temperature and diluted with water (50 ml) and extracted with ethyl acetate (150 ml). The ethyl acetate extract was concentrated under reduced pressure and purified by silica gel column chromatography (hexanes 100% to 20% ethyl acetate in hexanes) to afford the 12*β* alcohol **19** as a white solid (0.643 g, 1.08 mmol, 75%); mp: 126 – 130 °C; R_f. 0.375

(hexanes:ethyl acetate, 4:1, v/v); $[\alpha]_D^{20}$ +7.5° [0.2% in $CHCl_3$] IR (neat), 3592.56, 3516.69, 2950.77, 2928.91, 2884.30, 2854.88, 1470.80, 1462.41, 1436.18, 1385.81, 1360.65, 1276.37, 1248.73, 1160.60, 1128.81, 1092.21, 1069.31, 1051.75, 1035.04, 1000.13 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.21 (s, 1H), 4.04 – 3.90 (m, 4H), 3.89 – 3.82 (m, 1H), 3.47 (m, 1H), 2.33 – 2.20 (m, 1H), 2.19 – 2.08 (m, 1H), 1.94 – 1.83 (m, 2H), 1.83 – 1.63 (m, 7H), 1.57 – 1.44 (m, 3H), 1.44 – 1.31 (m, 2H), 1.16 – 1.08 (m, 1H), 1.08 – 1.02 (s, 3H), 1.02 – 0.94 (m, 2H), 0.92 (s, 3H), 0.87 (s, 9H), 0.85 (s, 3H), 0.05 (m, 12H)); ^{13}C NMR (125 MHz, $CDCl_3$) δ 142.79, 126.10, 118.94, 74.70, 72.25, 70.07, 64.61, 64.06, 49.08, 48.91, 47.33, 42.46, 39.92, 37.26, 36.70, 33.85, 32.14, 29.22, 26.37, 26.03, 25.10, 19.10, 18.31, 9.03, -2.47, -3.22, -4.42, -4.43 (the minor 7*α*-OTBDMS epimer product was observed through ^{13}C NMR spectroscopy, and the peaks corresponding to the major 7*β*-OTBDMS epimer were reported); HRMS (*m/z*) calculated for $C_{33}H_{60}O_5Si_2Na$ $[M + Na]^+$, 615.3871; found, 615.3855 (Δ 2.75 ppm). The minor 7*α*-OTBDMS epimer was observed by ^1H NMR spectroscopy (δ 5.46 (d, J = 6 Hz, 1H), see experimental for Compound **18**).

17,17-Ethylenedioxy-3*β*-,7*β*-di-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]]-androst-5-en-12-one (Compound **20**)**:** Pyridinium chlorochromate (0.25 g, 1.16 mmol, 2.16 eq) was added to a solution of the alcohol **19** (0.319 g, 0.538 mmol, 1 eq) in CH_2Cl_2 (50 ml). The solution was stirred at room temperature for 8 h. The reaction mixture was washed with 5% NaOH (aqueous, 3 × 50ml, w/v) using a separatory funnel and then concentrated under reduced pressure to obtain a crude yellow solid residue. The crude residue was purified by silica gel column chromatography (100% hexanes to 90% hexanes in ethyl acetate) to afford ketone **20** as a white solid (0.157 g, 0.266 mmol, 49%); R_f. 0.83 (hexanes:ethyl acetate, 4:1, v/v); IR (neat) δ 2951.39,

2928.74, 2891.79, 2854.65, 1713.00, 1470.80, 1462.63, 1385.07, 1360.19, 1275.17, 1249.39, 1163.10, 1091.89, 1073.71, 1061.62, 1034.85, 1004.05 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.26 (s, 1H), 4.24 – 4.13 (m, 1H), 4.11 - 4.04 (m, 2H); 4.02 – 3.91 (m, 1H), 3.88 – 3.81 (m, 1H), 3.51 – 3.44 (m, 1H), 2.45 – 2.36 (m, 1H), 2.31 – 2.19 (m, 3H), 2.11 – 2.02 (m, 1H), 2.02 – 1.88 (m, 2H), 1.86 – 1.63 (m, 4H), 1.62 (s, 1H), 1.58 – 1.44 (m, 2H), 1.11 (s, 3H), 1.08 (s, 3H), 1.06 – 0.92 (m, 1H), 0.89 (s, 9H), 0.88 (s, 9H), 0.05 (d, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 210.60, 142.47, 126.07, 116.61, 74.10, 71.93, 65.98, 65.52, 57.88, 49.57, 48.73, 42.40, 39.82, 38.43, 36.88, 36.78, 34.69, 31.91, 26.39, 26.01, 23.29, 18.59, 18.33, 18.28, 15.34, -2.44, -3.21, -4.43, -4.45; HRMS (*m/z*) calculated for $C_{33}H_{58}O_5Si_2Na$ $[M + Na]^+$, 613.3715; found, 613.3721 (Δ -0.97 ppm). The minor 7*α*-OTBDMS epimer was observed by ^{1}H NMR spectroscopy (δ 5.51 (d, J = 6 Hz, 1H), see experimental for Compound **18**).

17,17-Ethylenedioxy-3*β*-,7*β*-di-[-[[(1,1-dimethylethyl)dimethylsilyl]oxy]]-androst-5-en-12*α*-ol (Compound **21):** To a stirring solution of ketone **20** (0.1573 g, 0.266 mmol, 1eq) in THF (50 ml) at -78 °C under an atmosphere of N_2, L-Selectride (8 ml of a 1.0 M solution in THF) was added via a syringe dropwise. The reaction was stirred for 12 h and then quenched with the slow addition of water (20 ml) at -78 °C. The resulting solution was extracted with ethyl acetate (150 ml) and the ethyl acetate extract was concentrated under reduced pressure, purified by silica gel column chromatography (hexanes 100% to 80% hexanes in ethyl acetate) to afford the 12*α* alcohol **21** as a white solid (0.103 g, 0.174 mmol, 65%); R_f 0.45 (hexanes:ethyl acetate, 4:1, v/v); IR (neat) δ 3501.97, 3459.13, 3214.33, 2949.55, 2927.31, 2895.49, 2854.47, 1672.44, 1470.20, 1461.30, 1385.02, 1360.37, 1280.85, 1249.53, 1180.10, 1074.60, 1053.57, 1024.36, 1004.78 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.22 (s, 1H), 4.72 (s, 1H), 4.08 (apparent d, J = 6 Hz, 1H); 4.02

– 3.97 (m, 1H), 3.97 – 3.86 (m, 4H), 3.51 – 3.42 (m, 1H), 2.29 – 2.16 (m, 2H), 2.15 – 2.09 (m, 1H), 2.06 – 1.97 (m, 1H), 1.93 – 1.85 (m, 1H), 1.80 – 1.69 (m, 3H), 1.68 – 1.46 (m, 7H), 1.35 – 1.19 (m, 1H), 1.10 – 0.99 (m, 4H), 0.88 (apparent d, 19H), 0.84 (s, 3H), 0.11 – 0.03 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.01, 126.27, 121.32, 75.33, 72.43, 71.60. 64.81, 63.50, 48.12, 42.60, 42.25, 41.66, 41.26, 37.12, 36.55, 35.05, 32.18, 27.98, 26.41, 26.05, 25.48, 18.94, 18.33, 18.28, 15.88, -2.63, -3.28, -4.40, -4.42; HRMS (*m/z*) calculated for $C_{33}H_{60}O_5Si_2Na$ $[M + Na]^+$, 615.3871; found, 615.3870 (Δ 0.24 ppm). The minor 7*α*-OTBDMS epimer was observed by ^{1}H NMR spectroscopy (δ 5.48 (d, J = 6 Hz, 1H), see experimental for Compound **18**).

3*β*-,7*β*-,12*α*-Trihydroxy-androst-5-en-17-one (Compound **4):** To the 12*α*-alcohol **21** (0.278 g, 0.468 mmol, 1 eq) in THF (50 ml) was added dilute HCl (2 M, 10 ml in 40 ml of water, 20 mmol, 43 eq). The reaction was stirred for 24 h and then quenched with saturated $NaHCO_3$ (aqueous). The resulting solution was extracted with CH_2Cl_2 (100 ml) and the organic layer was concentrated under reduced pressure to give a crude colorless oil. The crude oil was purified by silica gel column chromatography (ethyl acetate 100% to 20% methanol in ethyl acetate then to 100% methanol) to afford two triol products, 7*β*-hydroxy as a white solid (**4**, 60 mg, 0.187 mmol, 40%) as the major product R_f of compound **4**: 0.64 (ethyl acetate:methanol, 95:5, v/v) and the minor product, 7*α*-hydroxy (**3**, 15 mg, 0.046 mmol, 9%), which was more polar than the 7*β*-epimer. In fact, the 7*α*-hydroxy epimer was eluted from the column with 100% methanol. R_f of compound **3**: 0.58 (ethyl acetate: methanol, 95:5, v/v) as a white solid. Compound **4**: IR (neat) 3542.23, 3434.50, 3239.20, 2990.33, 2947.19, 2931.57, 2857.25, 1730.46, 1688.83, 1680.83, 1465.06, 1439.21, 1391.66, 1305.75, 1261.74, 1255.16, 1236.10, 1219.66, 1196.92, 1131.53,

1082.62, 1107.25, 1059.93, 1059.93, 1054.78, 1043.50, 1005.66 cm^{-1}; ^{1}H NMR of compound **4** (500 MHz, $CDCl_3$) 5.33 (s, 1H), 4.13 (s, 1H), 4.03 (apparent d, J = 7 Hz, 1H) 3.60 – 3.51 (m, 1H), 2.46 (dd, J = 19, 9 Hz, 1H), 2.37 – 2.32 (m, 1H), 2.30 – 2.22 (m, 1H), 2.22 – 2.16 (m, 1H), 2.11 (dt, J = 19, 9 Hz, 1H), 1.93 – 1.73 (m, 4H), 1.69 – 1.46 (m, 7H), 1.15 – 1.07 (td, J = 14, 5 Hz, 1H), 1.05 (s, 3H), 0.89 (s, 3H); ^{13}C NMR of compound **4** (125 MHz, $CDCl_3$) δ 143.79, 125.71, 72.79, 71.37. 69.26, 53.35, 42.84, 42.76, 41.81, 40.37, 36.84, 36.46, 36.43, 31.59, 27.39, 23.45, 19.14, 13.39; HRMS of compound **4** (*m/z*) calculated for $C_{19}H_{28}O_4Na$ $[M + Na]^+$, 343.1880; found, 343.1883 (Δ -0.93 ppm).

3*β*-,12*β*-dihydroxy-andros-5-en-17-one (Compound **22**). 12*β*-Hydroxy DHEA-3-OTBDMS ether (compound **7**) (200 mg, 478 mmol) was dissolved in CH_3OH (5 ml) and CH_2Cl_2 (10 ml). HCl (10% v/v in H_2O) was added and the reaction was monitored by TLC. The reaction was concentrated by reduced pressure and purified by flash column chromatography (100% hexanes to 50% ethyl acetate in hexanes to 80% ethyl acetate in hexanes) to afford 12*β*-hydroxy DHEA (100 mg, 329 mmol, 69%) as a white solid. For the crystallization conditions, 12*β*-hydroxy DHEA (70 mg) was dissolved in 2 ml of 1:1, acetone:diethyl ether (v/v) in a 40 ml screw cap vial. The solvent was slowly evaporated in the fume hood over 3 days to afford crystals, which were used to solve the crystal structure. ^{1}H NMR (500 MHz, $CDCl_3$) 5.37-5.36 (m, 1H), 3.80 (dd, , J = 11.3, 4.60 Hz, 1H), 3.54-3.48 (m, 1H), 2.47 (dd, , J = 18.9, 7.89 Hz, 1H), 2.35-2.29 (m, 1H), 2.26-2.19 (m, 1H), 2.16-2.07 (m, 2H), 2.02-1.95 (m, 1H), 1.88-1.81 (m, 3H), 1.70-1.57 (m, 4H), 1.54-1.40 (m, 2H), 1.28-1.21 (m, 1H), 1.14-1.07 (m, 2H), 1.04 (s, 3H), 0.95 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 141.2, 120.9, 72.8, 71.6, 51.5, 49.7, 49.2, 42.2, 37.3, 36.9, 35.9, 31.7, 30.7, 30.5, 28.3, 21.8, 19.5, 8.28.

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Highlight:

- Synthesis of 12α-hydroxy dehydroepiandrosterone derivatives
- Crystal structures for 12β-hydroxy DHEA, 7α-,12α-dihydroxy DHEA, 12α-hydroxy-7-keto DHEA
- Biological activity measured for 7-keto DHEA and 12α-hydroxy-7-keto DHEA