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Synthesis of 19-trideuterated *ent*-testosterone and the $GABA_A$ receptor potentiators *ent*-androsterone and *ent*-etiocholanolone

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19-Trideuteromethyl enantiomers of androgens namely *ent*-testosterone, *ent*-androsterone and *ent*-etiocholanolone were prepared by total synthesis. The isotope labeling at the C-19 angular methyl group was achieved by using deuterated methyl iodide (99.5% d_3) for introduction of C-19 before closure of the steroid A-ring. This method yields 19,19,19-trideuterated steroids without increasing the number of steps involved in the total synthesis of *ent*-androgens. Analysis by mass spectrometry (MS) showed no loss of deuterium during incorporation of C-19 into *ent*-testosterone. The availability of the compounds will enable these *ent*-androgens to be distinguished by MS from their natural enantiomers in future pharmacokinetic and metabolic studies.

Keywords: *ent*-testosterone-19,19,19-d_3; trideuterium label; *ent*-androsterone-19,19,19-d_3; *ent*-etiocholanolone-19,19,19-d_3; $GABA_A$ receptor modulators

Introduction

Anxiolytic, anticonvulsant, sedative and anesthetic effects of neuroactive steroids are known to occur due to binding of neuroactive steroids to $GABA_A$ receptors.[1] As part of our structure–activity studies of neuroactive steroid modulation of $GABA_A$ receptors, we have investigated the enantioselectivity of neuroactive steroid action at these receptors.[2] Although we initially found that the naturally occurring enantiomers of several potent neuroactive steroids were more effective modulators of $GABA_A$ receptors than their synthetic non-naturally occurring enantiomers (*ent*-steroids), we recently found that *ent*-androsterone and *ent*-etiocholanolone enhanced GABA-mediated chloride currents at these receptors more effectively than their natural counterparts.[3] These previous results suggest that *ent*-androsterone and *ent*-etiocholanolone may have useful *in vivo* activity as GABAergic drugs of the future. In preparation for future pharmacokinetic and metabolic studies, we modified the total synthesis of *ent*-testosterone to obtain *ent*-testosterone-19,19,19-d_3 (Scheme 1). Modification was easily achieved by using trideuterated methyl iodide in place of methyl iodide during the introduction of the C-19 angular methyl group and subsequent closure of the *ent*-steroid A-ring. This trideuterated *ent*-testosterone was then converted into *ent*-androsterone-19,19,19-d_3 and *ent*-etiocholanolone-19,19,19-d_3 by established literature procedures (Figure 1).[3,6]

Results and discussion

Although deuterium labeling of the C-19 position of natural testosterone has been achieved previously by several different methods,[7,8] we wanted to incorporate the trideuterated C-19 methyl group into *ent*-testosterone without increasing the number of synthetic steps required to prepare this *ent*-steroid. Accordingly, tricyclic intermediate **1** was first prepared according to the literature.[4,5]

Compound **1** was then subjected to Li/liquid NH_3 reduction and the radical anion intermediate produced in the reaction was reacted with methyl iodide-d_3 (99.5% d_3) to obtain the desired C-19 position deuterium labeling in 35% yield. Compound **2** thus obtained was then treated with MeOH under acidic conditions to obtain *ent*-testosterone-19,19,19-d_3 (**3**) in 67% yield. The ^{1}H-NMR of product **3** lacked the resonance at δ 1.27 ppm for the C-19 methyl group in the natural abundance ^{1}H-NMR of this compound. Mass spectroscopic analysis of compound **3** showed complete retention (99.5% d_3) of the three deuterium atoms derived from the methyl iodide-d_3 in the product.

The desired *ent*-androsterone-19,19,19-d_3 (**6**) was obtained from *ent*-steroid **3** using procedures reported previously for this transformation on natural abundance *ent*-testosterone (Scheme 2).[6] Reduction of compound **3** using Li/liquid NH_3 gave *ent*-steroid **4** in 65% yield. Jones oxidation converted ketoalcohol **4** into dione **5** (82% yield) and regio- and stereoselective reduction of dione **5** with K-selectride® gave *ent*-androsterone-19,19,19-d_3 (**6**) in 47% yield. Because the

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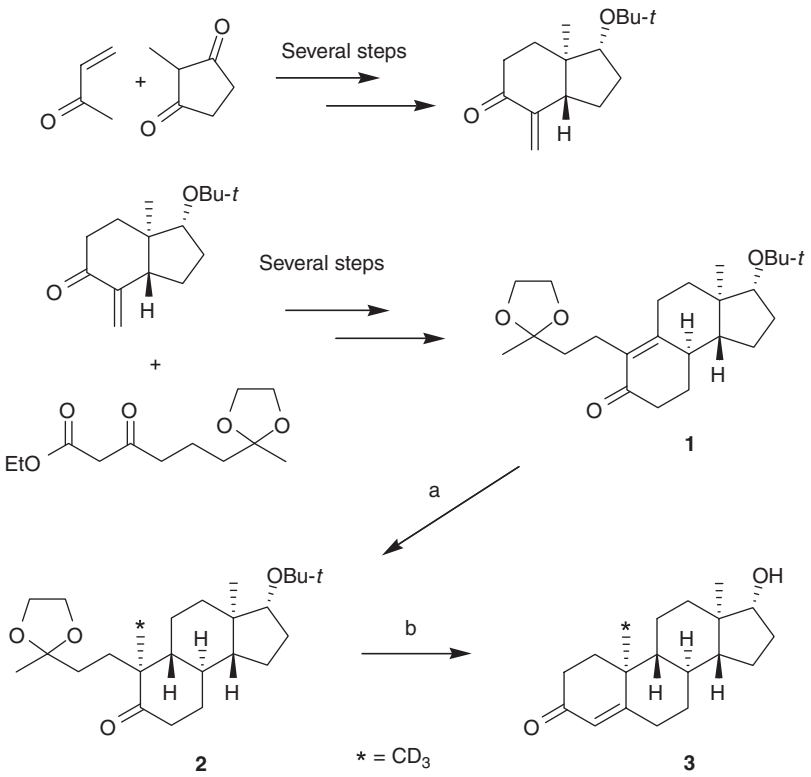


Scheme 1. Synthesis of *ent*-testosterone-19,19,19-d_3 (**3**). Reagents: (a) (i) Li/liquid NH_3, THF; (ii) CD_3I; and (b) 3 N HCl, MeOH. For complete description of the preparation of compound **1**, see References 4,5.

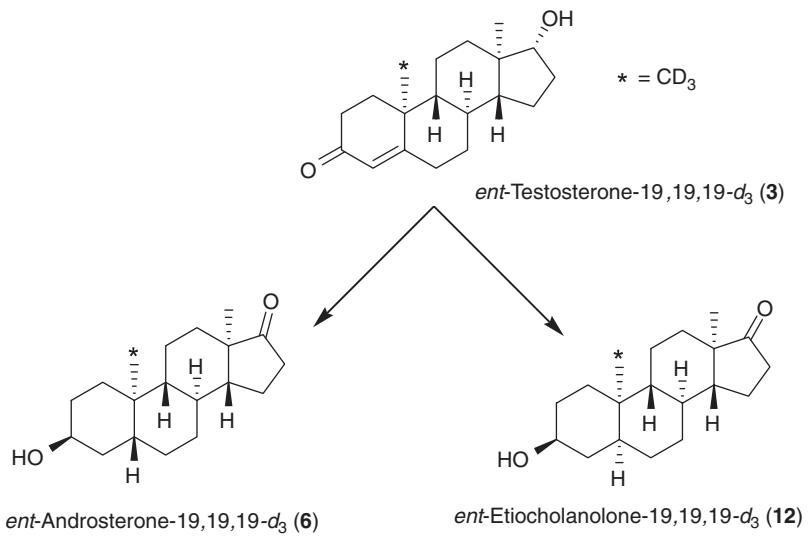
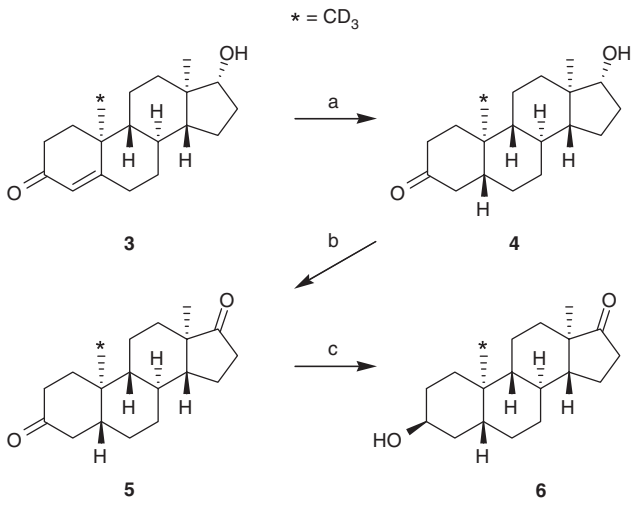


Figure 1. Target compounds: 19-trideuterated androgens.

molecular ion from product **6** is weak, interference from background noise in the mass spectra of this product only permitted the d_3 content of the products to be accurately measured as $>95\%$.

The desired *ent*-etiocholanolone-19,19,19-d_3 (**12**) was also obtained from *ent*-steroid **3** using previously reported procedures described for this transformation on natural abundance *ent*-testosterone (Scheme 3).[3] Catalytic hydrogenation of *ent*-steroid **3** using Pd/C gave three isolated products (**4**, **7** and **8**). Compound **4** was easily separated from unseparated products **7** and **8** by column chromatography. Separation of products **7** and **8** required acetylation of the inseparable mixture and column chromatography was required to separate products **9** and **10**. Saponification of the ketoacetate **9** gave ketoalcohol **7** (37% overall yield). Jones oxidation converted ketoalcohol **7** into dione **11** (77% yield) and regio- and stereoselective reduction of

dione **11** with $LiAl[OC(CH_3)_3]_3H$ gave *ent*-etiocholanolone-19,19,19-d_3 (**12**) in 80% yield. As was the case for product **6**, mass spectrometry (MS) could only accurately determine the d_3 content of product **12** to be >95%.

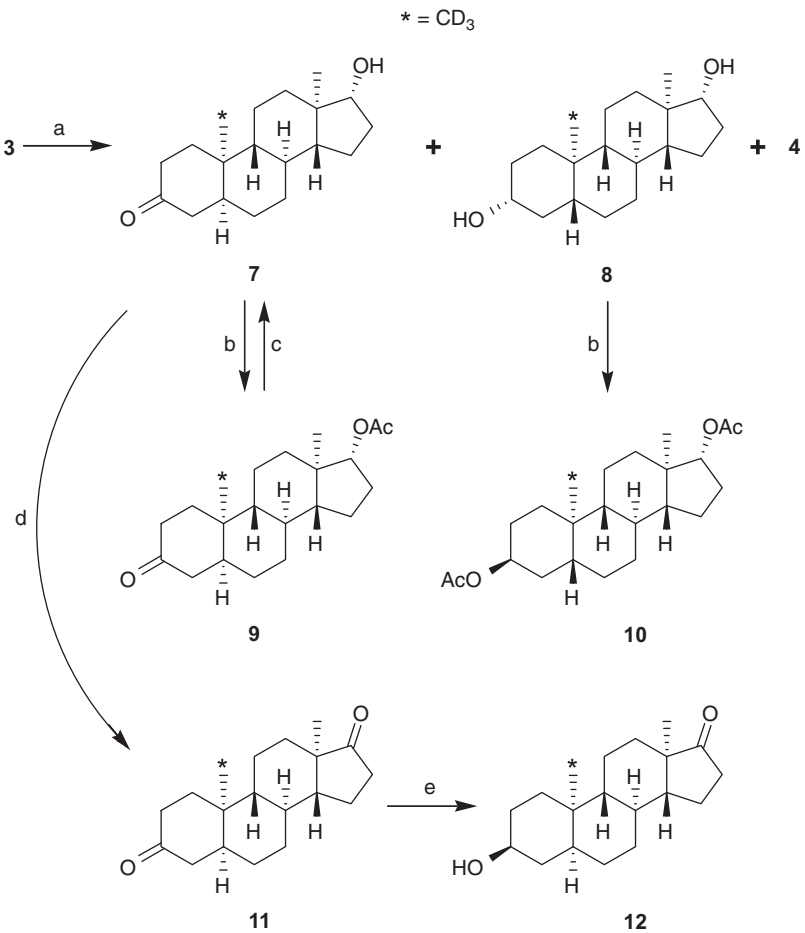


Scheme 2. Synthesis of *ent*-androsterone-19,19,19-d_3 (**6**). Reagents: (a) Li/liquid NH_3, THF, toluene, NH_4Cl, (b) Jones reagent, acetone, 0°C; and (c) (i) K-selectride®, THF; (ii) 10% NaOH, 30% aqueous H_2O_2.

Experimental

Materials and instruments

All solvents were purchased and dried and purified by standard methods. All air and/or moisture sensitive reactions were carried out under N_2 using oven-dried glassware and cooled under vacuum or N_2. All extraction solvents were dried with anhydrous Na_2SO_4 and the drying agent was removed by filtration before solvent removal. Extraction solvents were removed on a rotary evaporator. Flash chromatography was performed using silica gel (32–63 μm) purchased from Scientific Adsorbents (Atlanta, GA). Optical rotations were determined on a Perkin-Elmer Model 341 polarimeter. Melting points were determined on a Kofler micro hot stage and are uncorrected. IR spectra were recorded as films on an NaCl plate with a Perkin-Elmer Spectrum One FTIR spectrophotometer. NMR spectra were recorded at ambient temperature in $CDCl_3$ with a 5 mm probe on a Varian Gemini 2000 operating at 300 MHz (^1H) or 75 MHz (^{13}C). ^1H- and ^{13}C-NMR spectra were referenced to $CDCl_3$ (δ 7.27) and (δ 77.0), respectively. Mass spectroscopic analyses were performed by the UCR Mass Spectrometry Facility (Riverside, CA). Deuterated methyl iodide (99.5% d_3) was purchased from Cambridge Isotope Laboratories (Andover, MA).



Scheme 3. Synthesis of *ent*-etiocholanolone-19,19,19-d_3 (**12**): (a) Pd/C, KOH, ^iPrOH, H_2; (b) acetic anhydride, pyridine, DMAP; (c) K_2CO_3, MeOH; (d) Jones reagent, acetone; and (e) $LiAl[OC(CH_3)_3]_3H$, THF.

[3R-(3α,3aα,5aβ,6β,9aα,6β,9aα,9bβ)]-3-(1,1-dimethylethoxy)dodecahydro-3a,6-dimethyl-6-[2-(2-methyl-1,3-dioxolan-2-yl)ethyl]-7H-benz[e]inden-7-one-3a,3a,3a-d_3 (**2**)

A dry flask was equipped with a Dewar condenser mechanical stirrer, an addition funnel and a gas bubbler. After cooling the flask to −78°C in a dry ice–acetone bath, NH_3 (~1 L) was condensed in the flask. Small pieces of lithium (1.56 g, 0.225 mol) were added, and the blue solution was stirred for 20 min. Enone **1**[4,5] ($[\alpha]_D = -356.7$ ($c = 0.1$, toluene), 22 g, 0.056 mol) was dissolved in THF (100 mL) and added dropwise to the reaction mixture over 30 min. The reaction mixture was maintained at −78°C for 1 h with persistence of blue color. Then, deuterated methyl iodide (14.6 mL, 0.22 mmol) in THF (20 mL) was added dropwise to the solution. This led to a rapid change from blue to yellow color. The reaction was allowed to stir overnight to evaporate the ammonia. After addition of water (1 L) and NH_4Cl (40 g), the product was extracted with EtOAc (3 × 150 mL) and the combined EtOAc extractions were washed with brine and dried. After solvent evaporation, an orange–brown oil was obtained. Chromatography (silica gel, 15% EtOAc in hexanes) gave product **2** (8.41 g, 35%) as a white solid: m.p. 105–106°C; $[\alpha]_D = -34.2$ ($CHCl_3$, $c = 1.03$); IR 2966, 1696, 1358, 1201, 1078, 947 cm^{-1}; ^{13}C-NMR δ 214.83, 110.24, 80.50, 72.20, 64.47, 64.40, 50.39, 50.31, 47.53, 42.42, 38.09, 36.68, 34.71, 32.98, 30.97, 30.72, 28.88, 28.68, 23.73, 23.35, 20.85, 11.54; ^{1}H-NMR δ 3.93 (4H, m), 3.38 (1H, t, $J = 8.1$ Hz), 2.51(1H, m), 2.22–2.39 (1H, m), 1.35 (3H, s), 1.13 (9H, s), 0.78 (3H, s); HRMS (ESI) *m/z* calcd. for $C_{25}H_{39}D_3O_4$, $(M+Na)^+$ 432.3169; found 432.3178.

ent-Testosterone-19,19,19-d_3 (**3**)

Benz[*e*]indenone **2** (6.12 g, 0.014 mol) in MeOH (160 mL) and 3 N HCl (40 mL) were heated to reflux for ~24 h. After the reaction mixture cooled to room temperature, the MeOH was removed on a rotary evaporator and water (135 mL) was added. The water was extracted with CH_2Cl_2 (3 × 150 mL) and the combined extracts were washed with $NaHCO_3$ (50 mL), brine (50 mL) and dried. Solvent evaporation gave a yellow oil. Column chromatography (silica gel, 20% EtOAc in hexanes) yielded product **3** (2.91 g, 67%) as a white solid: m.p. 151–153°C; $[\alpha]_D = -113$ ($CHCl_3$, $c = 1.1$); IR 2942, 1671, 1349, 1179, 940 cm^{-1}; ^{13}C-NMR δ 199.70, 171.50, 123.72, 81.43, 53.76, 50.36, 42.71, 38.37, 36.32, 35.54, 35.50, 33.83, 32.72, 31.43, 30.24, 23.33, 20.55, 10.97; ^{1}H-NMR δ 5.73 (1H, s), 3.64 (1H, m), 0.79 (3H, s); HRMS (ESI) *m/z* calcd. for $C_{19}H_{25}D_3O_2$, MH^+ 292.2356; found 292.2358. No natural abundance MH^+ peak for $C_{19}H_{28}O_2$ was detectable by MS analysis indicating that no deuterium loss accompanied the incorporation of the deuterated methyl group derived from deuterated methyl iodide (99.5% d_3) into product **3**.

(5β,8α,9β,10α,13α,14β,17α)-17-Hydroxyandrostan-3-one-19,19,19,-d_3 (**4**)

A solution of compound **3** (2.1 g, 7.2 mmol) in THF (20 mL) was added to a blue solution of liquid ammonia (500 mL) and dissolved lithium metal (0.16 g, 2.4 mol) in THF (6 mL) and toluene (60 mL) at −78°C. After 30 min, NH_4Cl (3 g) was added portionwise to discharge the blue color and the ammonia was allowed to evaporate. The reaction mixture was diluted with 10% aqueous HCl (50 mL) and extracted with EtOAc (3 × 25 mL). The combined organic extracts were washed with water (50 mL) and brine (50 mL) and dried. The solvent was removed to give a yellow oil that was purified by column chromatography (silica gel, 20% EtOAc in hexanes to 35% EtOAc in hexanes) to give compound **4** (1.35 g, 65% yield): m.p. 174–176°C; $[\alpha]_D = -32.6$ ($CHCl_3$, $c = 0.8$); IR 2925, 1713, 1358, 1201, 1076, 945 cm^{-1}; ^{13}C-NMR δ 212, 81.72, 53.82, 50.77, 46.65, 44.62, 42.93, 38.44, 38.08, 36.59, 35.44, 35.39, 31.19, 30.41, 28.74, 23.32, 20.97, 11.09; ^{1}H-NMR δ 3.63 (1H, t, $J = 8.3$ Hz), 0.75 (3H, s); HRMS (ESI) *m/z* calcd. for $C_{19}H_{27}D_3O_2$, MH^+ 294.2507; found 294.2512.

(5β,8α,9β,10α,13α,14β)-Androstane-3,17-dione-19, 19, 19-d_3 (**5**)

Jones reagent (8 M) was titrated dropwise to a stirred solution of compound **4** (1.38 g, 4.6 mmol) in acetone (100 mL) at 0°C until an orange color persisted. After an additional 5 min, ^{i}PrOH was also titrated until the excess reagent was destroyed and water (100 mL) was added. The mixture was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (70 mL) and dried. The solvent was removed to give a solid that was recrystallized from hexanes to give product **5** (1.10 g, 82%) as a white solid: m.p. 124–125°C; $[\alpha]_D = -113$ ($CHCl_3$, $c = 1$); IR 2971, 1732, 1596, 1200, 1098, 940 cm^{-1}; ^{13}C-NMR δ 220.78, 211.46, 53.74, 51.13, 47.62, 46.49, 44.50, 38.27, 37.99, 35.72, 35.50, 34.87, 31.40, 30.43, 28.53, 21.68, 20.62, 13.72; ^{1}H-NMR δ 0.89 (3H, s); HRMS (ESI) *m/z* calcd. for $C_{19}H_{25}D_3O_2$, MH^+ 292.2350; found 292.2352.

ent-Androsterone-19,19,19-d_3 (**6**)

K-selectride® (1.0 M solution in THF, 5.2 mL, 5.2 mmol) was added to a stirred solution of compound **5** (1.10 g, 3.7 mmol) in dry THF (5.6 mL) at −78°C. After 1.5 h, 10% aqueous NaOH (20 mL) and then 30% aqueous H_2O_2 (7.6 mL) were added. The mixture was allowed to warm to room temperature, stirred for another 30 min and extracted with CH_2Cl_2. The combined organic layers were washed with brine and dried. Solvent removal gave a solid that was recrystallized from EtOAc to give compound **6** as a white solid (520 mg, 47%): m.p. 177–178°C; $[\alpha]_D = -92.1$ ($CHCl_3$, $c = 1.3$); IR 2923, 1738, 1371, 1200, 1072, 960 cm^{-1}; ^{13}C-NMR δ 221.44, 66.32, 54.33, 51.45, 47.76, 39.03, 35.98, 35.80, 35.74, 35.01, 32.02, 31.52, 30.79, 28.95, 28.21, 21.70, 20.0, 13.78; ^{1}H-NMR δ 4.05 (1H, m), 0.86 (3H, s); HRMS (ESI) *m/z* calcd. for $C_{19}H_{27}D_3O_2$, MH^+ 294.2507; found 294.2510; > 95% d_3.

(5α,8α,9β,10α,13α,14β,17α)-17-Hydroxyandrostan-3-one-19,19,19-d_3 (**7**)

KOH (0.48 g) and ^{i}PrOH (30 mL) were added to a hydrogenation bottle; the solution was stirred for 30 min to allow some of the KOH to dissolve. Pd/C (0.11 g, 5%) and *ent*-steroid **3** (2 g, 6.8 mmol) dissolved in ^{i}PrOH (30 mL) were then added and the total volume was increased to ~100 mL with ^{i}PrOH. The solution was hydrogenated using a Parr hydrogenator for 18 h at 45 psi and the catalyst removed by filtration through a pad of Celite, which was washed with MeOH. After removal of the solvent, a yellow oil remained. To this oil, brine (150 mL) and Et_2O (250 mL) were added, and the aqueous phase was neutralized with 6 N HCl. After thorough mixing, the aqueous layer was removed and the organic layer was washed with brine (3 × 200 mL) and dried. Solvent removal gave a white solid. Column chromatography (silica gel, 20% EtOAc/hexanes to 40% EtOAc/hexanes) separated ketoalcohol **4** from an inseparable mixture of ketoalcohol **7** and diol **8**. Acetylation of the mixture of products **7** and **8** in the standard manner[9] (pyridine 5.48 mL,

acetic anhydride 5.35 mL, DMAP, catalytic amount) gave acetylation products **9** and **10** that were easily separated by column chromatography (silica gel, 180 g, 5–15% EtOAc in hexanes, 3 × 500 mL). Saponification of **9** using K_2CO_3 (200 mg and MeOH 20 mL, reflux for an hour and purification by column chromatography on silica gel eluted with 30% EtOAc in hexanes) gave pure product **7** as a white solid (730 mg, 37%): m.p. 140–141°C; $[\alpha]_D = -28.90$ (EtOH, $c = 0.6$); IR 2865, 1712, 1073, 855 cm^{-1}; ^{13}C-NMR δ 213.24, 81.78, 50.96, 44.21, 43.09, 42.27, 40.87, 37.14, 36.91, 36.79, 35.57, 34.72, 30.52, 26.43, 25.33, 23.32, 20.76, 11.09; ^{1}H-NMR δ 3.6 (1H, t, $J = 8.5$ Hz), 2.67 (1H, t, $J = 14.3$ Hz), 0.76 (3H, s); HRMS (ESI) *m/z* calcd. for $C_{19}H_{27}D_3O_2$, MH^+ 294.2507; found 294.2513.

*(5α,8α,9β,10α,13α,14β)-Androstane-3,17-dione-19,19,19-d_3 (**11**)*

Compound **7** (730 mg, 2.48 mmol) was dissolved in acetone (20 mL) and while stirring Jones reagent (8 M) was titrated dropwise to this solution until a yellow color persisted. The solution was stirred at room temperature for 30 min after which ^{i}PrOH was titrated dropwise to quench any remaining Jones reagent. The reaction mixture was poured into brine (150 mL) and the aqueous solution was extracted with EtOAc (3 × 200 mL). The organic extracts were combined and dried. After solvent removal, a blue solid remained. This solid was chromatographed (silica gel, 50% EtOAc in hexanes) to yield compound **11** (560 mg, 77%) as a white solid: m.p. 125–126°C; $[\alpha]_D = -104.66$ (EtOH, $c = 0.4$); IR 2931, 1738, 1712, 1373, 1202, 1093, 915 cm^{-1}; ^{13}C-NMR δ 220.66, 212.60, 51.30, 47.72, 44.03, 42.13, 40.90, 37.01, 36.76, 35.75, 35.04, 34.74, 31.57, 26.25, 24.61, 21.67, 20.39, 13.70; ^{1}H-NMR δ 2.68 (1H, t, $J = 14.2$ Hz), 0.89 (3H, s); HRMS (ESI) *m/z* calcd. for $C_{19}H_{25}D_3O_2$, MH^+ 292.2350; found 292.2350.

*ent-Etiocholanolone-19,19,19-d_3 (**12**)*

To a dry flask, compound **11** (560 mg, 1.92 mmol) dissolved in dry THF (30 mL) was added. While under N_2, the solution was cooled to −42°C in a bath of acetonitrile and dry ice. Lithium tri-*tert*-butoxyaluminohydride (1.9 mL, 2 mmol, 1 M in THF) was added dropwise and the solution was stirred for 2 h at −42°C under N_2. TLC after 2 h confirmed that the reaction was not complete; hence, it was warmed to −20°C for another 1 h. The reaction was quenched with 3 N HCl (20 mL). EtOAc (250 mL) was added and the organic solution was washed with aqueous $NaHCO_3$ (150 mL) and brine (150 mL). The organic layer was dried and the solvent was removed to give a white solid. Column chromatography (silica gel, 30% EtOAc/hexanes) yielded product **12** (480 mg, 80%) as a white solid: m.p. 149–150°C; $[\alpha]_D = -95.3$ (EtOH, $c = 0.7$); IR 2929, 1738, 1370, 1089, 947 cm^{-1}; ^{13}C-NMR δ 221.35, 71.59, 51.45, 47.82, 41.92, 40.69, 36.27, 35.89, 35.39, 35.19, 34.48, 31.69, 30.44, 26.86, 25.33, 21.78, 20.04, 13.75; ^{1}H-NMR δ 3.67–3.60 (1H, m), 2.44 (dd, $J = 18.9$, 8.7 Hz), 0.85 (3H, s); HRMS (ESI) *m/z* calcd. for $C_{19}H_{27}D_3O_2$, MH^+ 294.2507; found 294.2508; > 95% d_3.

Conclusion

An efficient synthesis of *ent*-testosterone, *ent*-androsterone and *ent*-etiocholanolone containing 19,19,19-d_3 deuterium labeling was developed.

Acknowledgement

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