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Research Article

A simple and efficient synthesis of [3α-^{3}H]5α-androst-16-en-3β-ol

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Summary
An efficient one step synthesis of [3α-^{3}H]5α-androst-16-en-3β-ol by $NaBT_4$ reduction of a ketone precursor is described. The specific activity of the product was 21.6 Ci/mmol with a radiochemical purity >99%. Synthesis of the precursor, 5α-androst-16-en-3-one, from commercially available 5α-androst-16-en-3α-ol is also presented.

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Key Words: tritium labeled steroids; $NaBT_4$

Introduction
We required [^{3}H]5α-androst-16-en-3β-ol with a specific activity >10 Ci/mmol. Römer and Wagner [1] reported a synthesis of [5α, 6α-$^3H_2$]5α-androst-16-en-3β-ol, **3**, at 50 Ci/mmol, as an intermediate in the synthesis of steroid **4** (Scheme 1). This synthesis had two disadvantages for our purposes: (1) forcing conditions were needed to reduce the 5, 6 double bond and (2) products **2** and **3** were difficult to separate from unsaturated compounds in the reaction mixtures. Our attempt to prepare compound **2** by reduction of compound **1** was not satisfactory, so we abandoned this route.

We subsequently developed an efficient, one step synthesis of [3α-^{3}H]5α-androst-16-en-3β-ol that gave an acceptable specific activity.

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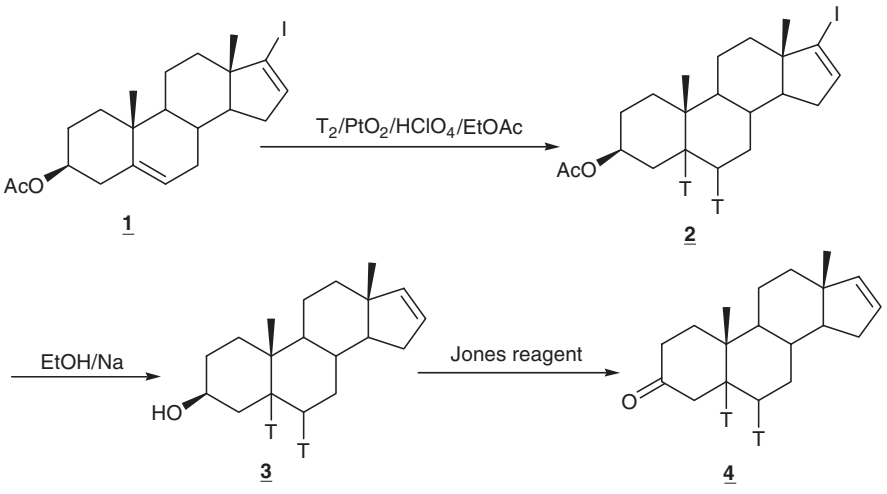

Scheme 1. Synthesis of [5α, 6α-$^3H_2$]5α-androst-16-en-3-one, 4

Results and discussion

Our initial attempt to synthesize [5α, 6α-$^3H_2$]5α-androst-16-en-3β-ol, **3**, is shown in Scheme 2.

Compound **1** was prepared in moderate yield from commercially available dehydroisoandrosterone-3-acetate, **5**, based on literature procedures.[2,3] Compound **1** (2.5 mg, 5.7 μmmol) was reduced with 800 mCi (13.9 μmmol) of tritium gas, using Römer and Wagner's conditions,[1] to give 4.8 mCi of crude product **2** at only 33% radiochemical purity. This route was not pursued further.

Tritium labeled alcohols are often prepared by reduction of an appropriate ketone with a complex tritide. $NaBT_4$ is commercially available at high specific activity, relatively stable and, therefore, the tritide of choice for this transformation. Ketone **8**, 5α-androst-16-en-3-one, was prepared by Ley-Griffith oxidation[4] of the inexpensive, commercially available alcohol 5α-androst-16-en-3α-ol, **7**.

Reduction of 3-keto steroids with hydrides, such as $NaBH_4$ and $LiAlH_4$, generally favor equatorial over axial alcohols in approximately a 10:1 ratio.[5] Reduction of ketone **8** with $NaBT_4$ in IPA:EtOH (1 · 2) gave the expected equatorial alcohol as the major product. The equatorial: axial (3β:3α) ratio was 85:15, comparable with the result from a trial reaction with $NaBH_4$. After purification by chromatography on silica gel, [3α-^{3}H]5α-androst-16-en-3β-ol, **9**, was obtained in 86% yield. The specific activity was 21.6 Ci/mmol. The synthesis is shown in Scheme 3.

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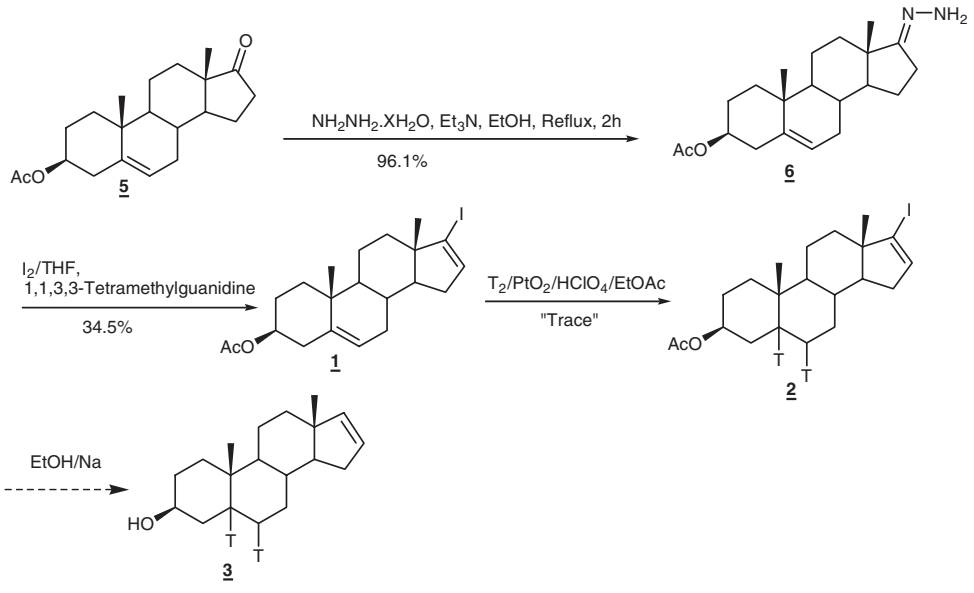
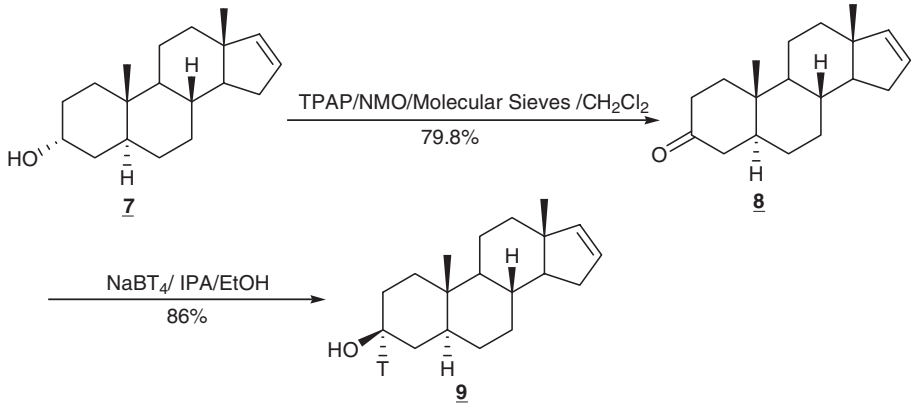


Scheme 2. Synthesis of [5α, 6α-$^3H_2$]5α-Androst-16-en-3β-ol 3



Scheme 3. Synthesis of [3α-^{3}H]5α-androst-16-en-3β-ol

Experimental

General

$NaBT_4$ (80 Ci/mmol) was purchased from American Radiolabeled Chemicals, Inc. All remaining reagents, authentic standards and solvents were purchased from Aldrich and used as received. Radioactivity was measured on a Packard 2200CA liquid scintillation analyzer using Scintiverse BD liquid scintillation cocktail. TLC plates were scanned on a Bioscan 1000 linear analyzer.

LC-MS: Waters Micromass with Waters 2695 Separation Module operating in the ES^+ ionization mode. Supelcosil LC-CN, 150 mm × 4.6 mm, 215 nm, isocratic, 0.1% HCO_2H in H_2O: 0.1% HCO_2H in CH_3CN (65:35), 1.0 ml/min.

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RadioHPLC: Waters 600 Multisolvent Delivery System with Waters 2487 Absorbance Detector and Radiomatic 525TR Radioflow Detector. YMC-Pack PVA-Sil, 150 mm × 4.6 mm, 215 nm. Hexanes:EtOH (98:2) for 15 min followed by a step gradient to EtOH, 0.5 ml/min., HPLC elute: Packard Flo-Scint III liquid scintillation cocktail (1:3).

^{1}H-NMR (400 MHz) spectra was obtained on a Varian spectrometer.

*Synthesis of 5α-androst-16-en-3-one (**8**)*

To a solution of 5α-androst-16-en-3α-ol (**7**, 95 mg, 0.346 mmol) in anhydrous CH_2Cl_2 (4.0 ml) at 0°C, 5Å molecular sieves (5 seeds), 4-methyl-morpholine N-oxide (NMO, 70 mg, 0.60 mmol), and tetrapropylammonium perruthenate (TPAP, 10 mg, 0.028 mmol) were added. The reaction was stirred for 1 h at room temperature and was complete by TLC (Hexane:EtOAc = 80 : 20, R_f: 3α-ol **7**: 0.53; 3β-ol: 0.40; ketone **8**: 0.76). The mixture was filtered through a celite pad and concentrated to dryness. The crude product was purified by flash chromatography (10g Sep-Pak, eluted with 1–5% EtOAc/Hexanes) to give 75 mg (79.8% yield) 5α-androst-16-en-3-one, **8**. ^{1}H NMR ($CDCl_3$), δ (ppm): 5.84 (m, 1H, C_{17} olefinic proton), 5.70 (m, 1H, H_{16} olefinic proton), 2.25–0.80 (m, 26H).

*Synthesis of [3α-^{3}H]5α-androst-16-en-3β-ol (**9**)*

$NaBT_4$ (300 mCi, 3.75 μmol, 80 Ci/mmol) was added to a suspension of 5α-androst-16-en-3-one, **8**, (4.0 mg, 14.7 μmol) in IPA (1 ml) and EtOH (2 ml) in a reaction vial. The reaction was stirred at room temperature for 2 h, and then solvent was removed under N_2. The residue was dissolved in CH_2Cl_2 (5 ml), washed with saturated NH_4Cl (5 ml), dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography on silica gel (elution with 1–10% EtOAc/Hexanes) to give 120 mCi of [3α-^{3}H] 5α-androst-16-en-3-beta-ol, **9**. Radiochemical purity (radioHPLC) was 99.5%. The [3α-^{3}H]5α-androst-16-en-3β-ol **9** co-eluted with an authentic standard by radioHPLC (t_R = 9.2 min). The specific activity was 21.6 Ci/mmol. LC-MS: t_R = 7.1 min, m/z: 259 [100, $(M\text{-}H_2O + H)^+$], unlabeled standard m/z: 257 [100, $(M\text{-}H_2O + H)^+$]. An additional 140 mCi of **9** (radiochemical purity = 97.5%) containing 2.2% [3β-^{3}H]5α-androst-16-en-3-alpha-ol (t_R = 7.5 min by radioHPLC) was also collected. In total, 260 mCi (86.7%) of **9** was obtained.

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