

Research Article

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

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Summary

An efficient one step synthesis of [3α - ^3H]5 α -androst-16-en-3 β -ol by NaBT₄ 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, 5 α -androst-16-en-3-one, from commercially available 5 α -androst-16-en-3 α -ol is also presented. Copyright © 2006 John Wiley & Sons, Ltd.

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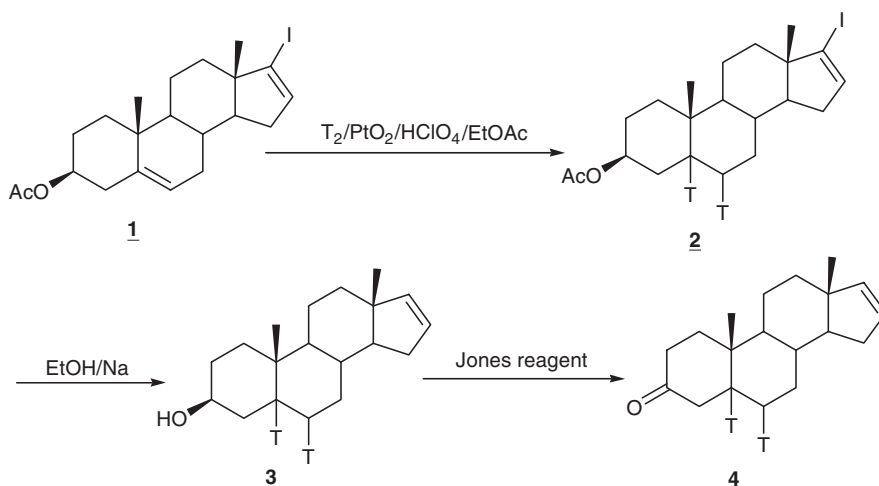
Key Words: tritium labeled steroids; NaBT₄

Introduction

We required [^3H]5 α -androst-16-en-3 β -ol with a specific activity > 10 Ci/mmol. Römer and Wagner¹ reported a synthesis of [5 α , 6 α - $^3\text{H}_2$]5 α -androst-16-en-3 β -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 - ^3H]5 α -androst-16-en-3 β -ol that gave an acceptable specific activity.

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Scheme 1. Synthesis of $[5\alpha, 6\alpha\text{-}^3H_2]5\alpha\text{-androst-16-en-3-one}$, **4**

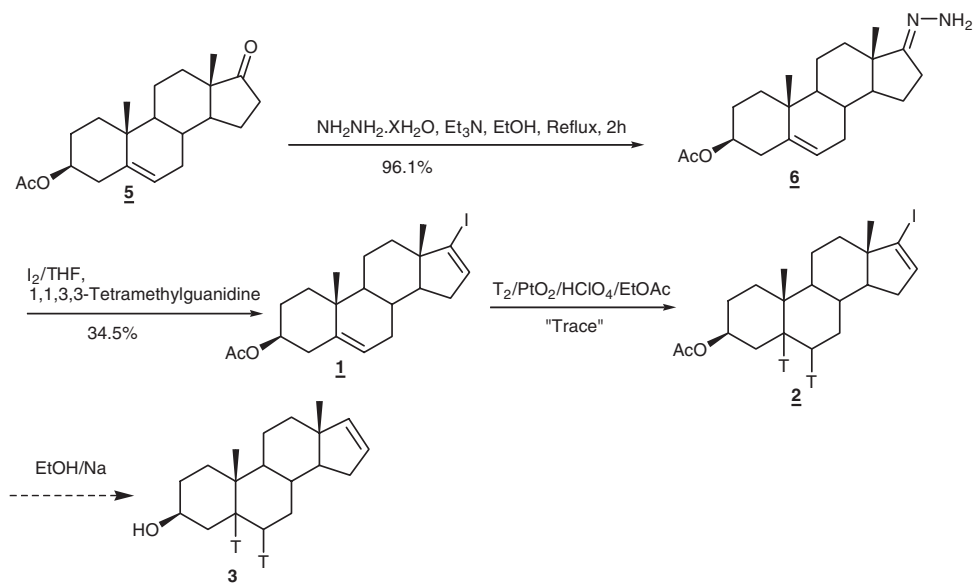
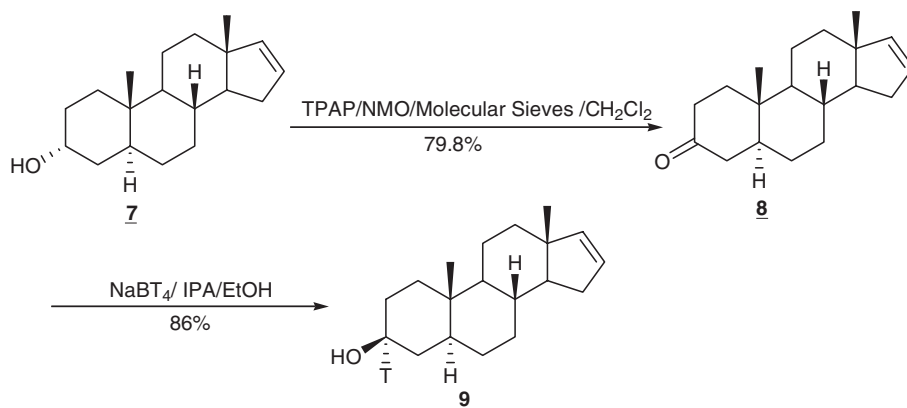
Results and discussion

Our initial attempt to synthesize $[5\alpha, 6\alpha\text{-}^3H_2]5\alpha\text{-androst-16-en-3}\beta\text{-ol}$, **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 μmol) was reduced with 800 mCi (13.9 μmol) 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**, $5\alpha\text{-androst-16-en-3-one}$, was prepared by Ley-Griffith oxidation⁴ of the inexpensive, commercially available alcohol $5\alpha\text{-androst-16-en-3}\alpha\text{-ol}$, **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 ($3\beta:3\alpha$) ratio was 85:15, comparable with the result from a trial reaction with $NaBH_4$. After purification by chromatography on silica gel, $[3\alpha\text{-}^3H]5\alpha\text{-androst-16-en-3}\beta\text{-ol}$, **9**, was obtained in 86% yield. The specific activity was 21.6 Ci/mmol. The synthesis is shown in Scheme 3.

Scheme 2. Synthesis of [5α , 6α - $^3\text{H}_2$]5 α -Androst-16-en-3 β -ol 3Scheme 3. Synthesis of [3α - ^3H]5 α -androst-16-en-3 β -ol

Experimental

General

NaBT₄ (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% HCO₂H in H₂O: 0.1% HCO₂H in CH₃CN (65:35), 1.0 ml/min.

RadioHPLC: Waters 600 Multisolvant Delivery System with Waters 2487 Absorbance Detector and Radiomatic 525TR Radioflow Detector. YMC-Pack PVA-Sil, 150 mm $\times$ 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).

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

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

To a solution of 5 α -androst-16-en-3 α -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 : 3 α -ol **7**: 0.53; 3 β -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) 5 α -androst-16-en-3-one, **8**. $^1\text{H NMR}$ (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 [3 α - ^3H]5 α -androst-16-en-3 β -ol (9)

NaBT_4 (300 mCi, 3.75 μmol , 80 Ci/mmol) was added to a suspension of 5 α -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 [3 α - ^3H] 5 α -androst-16-en-3-beta-ol, **9**. Radiochemical purity (radioHPLC) was 99.5%. The [3 α - ^3H]5 α -androst-16-en-3 β -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, $(\text{M}-\text{H}_2\text{O} + \text{H})^+$], unlabeled standard m/z : 257 [100, $(\text{M}-\text{H}_2\text{O} + \text{H})^+$]. An additional 140 mCi of **9** (radiochemical purity = 97.5%) containing 2.2% [3 β - ^3H]5 α -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.

Acknowledgements

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