Stereochemistry of $NaBH_4$ Reduction of a 19-Carbonyl Group of 3-Deoxy Androgens. Synthesis of [19*S*-^{3}H]- and [19*R*-^{3}H]-Labeled Aromatase Inhibitors Having a 19-Hydroxy Group

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To study the stereochemical aspects of the aromatase reaction of androst-4-en-17-one (1) and its 5-ene isomer 4, competitive inhibitors of aromatase, the [19*S*-^{3}H]- and [19*R*-^{3}H]-labeled 19-hydroxy derivatives 2 and 5, were synthesized through NaB^3H_4 reduction of the corresponding 19-aldehydes 3 and 6 as a key reaction. The hitherto unknown stereochemistry of the NaB^3H_4 reduction was established based on the deuterium-labeling experiments with NaB^2H_4. A comparison of ^{1}H-NMR spectra of the NaB^2H_4 reduction products of 19-als 3 and 6 with those of the respective authentic steroids revealed that the ratios of 19*S*-^{2}H to 19*R*-^{2}H were 90 : 10 for the 4-ene steroid 2 and 70 : 30 for the 5-ene isomer 5, respectively. Jones oxidation of the [19*S*-^{2}H]19-ols, followed by the non-labeled $NaBH_4$ reduction, gave the corresponding [19*R*-^{2}H]19-ols 2 and 5 (*R*-^{2}H : *S*-^{2}H = 90 : 10 for steroid 2 and 70 : 30 for steroid 5). The stereoselectively ^{3}H-labeled compounds 2 and 5 were similarly obtained in these sequences.

Key words aromatase inhibitor; isotope labeling; stereochemistry; [19-^{3}H]19-hydroxy androgen; [19-^{2}H]19-hydroxy androgen; NaB^3H_4 reduction

Human placental aromatase is a cytochrome P-450 enzyme complex that catalyzes the conversion of androgens into estrogens.[1—3] This process is illustrated by the conversion of androst-4-ene-3,17-dione (androstenedione, AD) into estrone through three steps, each of which required 1 mol of O_2 and 1 mol of NADPH (Fig. 1).[4] The first two steps occurred at the C-19 position to produce 19-hydroxy and 19-oxo intermediates, respectively.[5—9] The stereospecific 19-*pro* R hydrogen loss of the 19-hydroxy intermediate in the second oxygenation has been established using the [19*S*-^{3}H]-labeled 19-hydroxyAD as well as its [19*R*-^{3}H] stereoisomer.[10] The [19*S*-^{3}H]19-hydroxy steroid is stereoselectively obtained by the reduction of 19-oxoAD with NaB^3H_4, and the oxidation of the 19 S steroid followed by the reduction with $NaBH_4$ stereoselectively yields the [19*R*-^{3}H] stereoisomer. In the last step, C-19 and 1β,2β-hydrogens were eliminated as formic acid and water, respectively.[11—13]

Our laboratory has previously reported that 3-deoxy androgens, androst-4-en-17-one (**1**)[14] and its 5-ene isomer **4**,[15] are powerful and good competitive inhibitors of aromatase (Fig. 2). The structure–activity relationships of 3-deoxy androgen analogs have indicated that the binding geometries of the two 3-deoxy steroids **1** and **4** in the active site of aromatase are different principally in the region of an A–B ring system of the steroid molecule.[14—16] Gas chromatography-mass spectrometric analysis of the aromatase reaction of the inhibitors **1** and **4** has revealed that they are converted into the 19-oxo derivatives **3** and **6**, through their 19-hydroxy intermediates **2** and **5**, by aromatase, respectively, where the more potent inhibitor **1** is the less effective substrate. In contrast, the less potent inhibitor **4** is the more effective substrate.[17] On the basis of these previous findings, it is predicted that there will be a difference in the stereochemistry of the 19-hydrogen loss between 19-ols **2** and **5** in the conversion into the corresponding 19-als **3** and **6**. To determine this, we needed the stereoselectively 19-^{3}H-labeled steroids **2** and **5**. Thus, in this study, we initially explored the stereochemistry of the $NaBH_4$ reduction of 3-deoxy 19-als **3** and **6**, then synthesized [19*S*-^{3}H]19-ols **2** and **5** and their [19*R*-^{3}H] isomers.

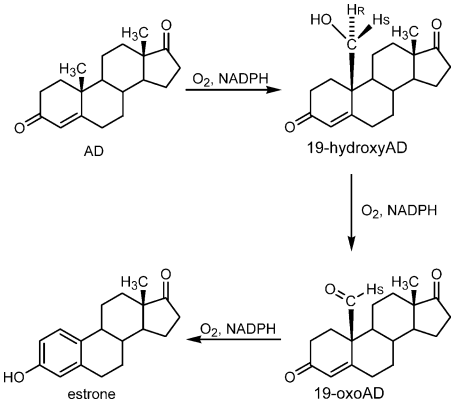

Fig. 1. Mechanism of Androstenedione (AD) Aromatization

Results and Discussion

A strategy of the stereoselective ^{3}H-labeling at C-19 of 19-hydroxy-4-en-17-oxo steroid **2** and its 5-ene isomer **5** employed a reduction of the corresponding 19-aldehydes **3** and **6** with NaB^3H_4 as a key reaction. The hitherto unknown stereochemistry of the reduction was initially determined using the deuterium-labeling experiments and ^{1}H-NMR spectroscopies; thus, the authentic [19*S*-^{2}H]-labeled 19-hydroxy

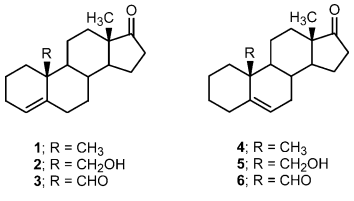

Fig. 2. Structures of 3-Deoxy Androgens

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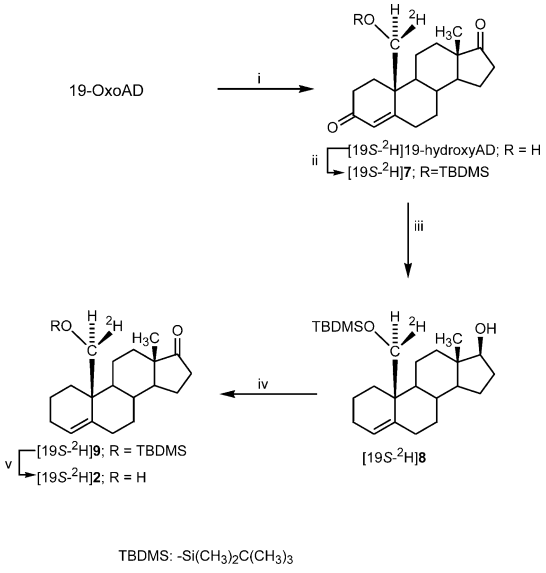

Fig. 3. Synthesis of Authentic [19*S*-^{2}H]4-en-19-ol **2**

Reagents: (i) NaB^2H_4, MeOH; (ii) TBDMS chloride, imidazole, DMF; (iii) $AlCl_3$, $LiAlH_4$, THF; (iv) pyridinium dichromate, CH_2Cl_2; (v) 3 M HCl, THF, propan-2-ol.

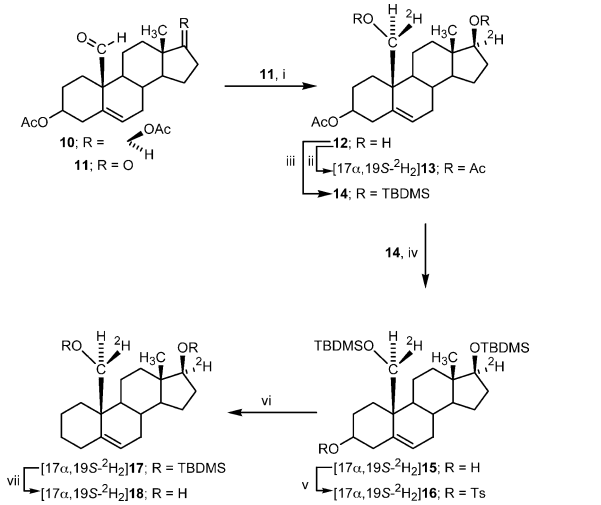

Fig. 4. Synthesis of Authentic [17α,19*S*-$^2H_2$]5-en-19-ol **18**

Reagents: (i) NaB^2H_4, MeOH; (ii) acetic anhydride, pyridine; (iii) TBDMS chloride, imidazole, DMF; (iv) 1 M NaOH, MeOH, acetone; (v) Ts chloride, pyridine; (vi) NaI, Zn, H_2O, 1,2-dimethoxyethane; (vii) 3 M HCl, THF, propan-2-ol.

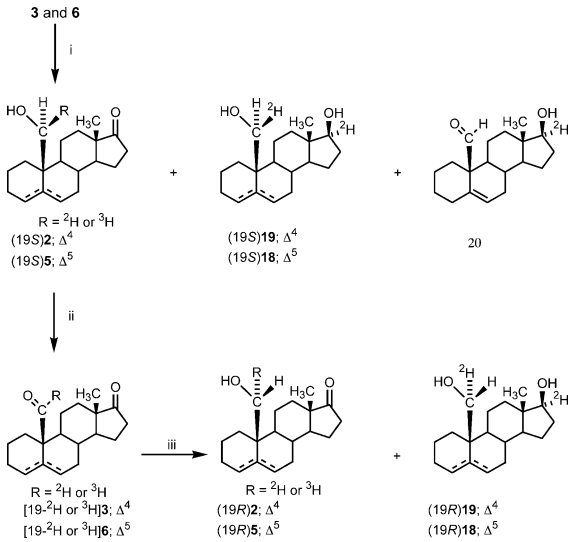

Fig. 5. Synthesis of the Stereoselectively Deuterium- or Tritium-Labeled 19-ols **2** and **5**

Reagents: (i) NaB^2H_4 or NaB^3H_4, MeOH; (ii) Jones reagent; (iii) $NaBH_4$, MeOH.

compounds **2** and **18** were synthesized, respectively, starting from [19*S*-^{2}H]19-hydroxyAD and [19*S*-^{2}H]3β-acetoxyandrost-5-ene-17β,19-diol (**12**) of which the stereochemistry at C-19 has previously been determined.[10]

The former starting material [a ratio of *S* to *R*=90 : 10; ^{1}H-NMR δ: 3.92 ppm (0.90H, H_R) and 4.06 ppm (0.10H, H_S)] was obtained by the reaction of 19-oxoAD with NaB^2H_4.[10] Treatment of this with *tert*-butyldimethylsilyl (TBDMS) chloride in the presence of imidazole in DMF followed by a reductive deoxygenation with $LiAlH_4$ and $AlCl_3$ in THF[18] yielded [19*S*-^{2}H]3-deoxy-17β-ol **8** (Fig. 3). This compound was oxidized with pyridinium dichromate in CH_2Cl_2 to give the [19-^{2}H]17-keto derivative **9** that was converted into the authentic [19*S*-^{2}H]19-hydroxy-4-en-17-one **2** by treatment with diluted HCl.

Another starting material [17α,19*S*-$^2H_2$]17β,19-diol **12** [a 70 : 30 ratio of *S* to *R*; ^{1}H-NMR δ: 4.47 ppm (0.70H, H_R) and 3.94 ppm (0.30H, H_S) as the triacetate derivative **13**], that was obtained by treatment of 17,19-dione **11** with NaB^2H_4, was converted into [$^2H_2$]17β,19-bis-TBDMS ether **14** by treatment with TBDMS chloride, as described above (Fig. 4). Hydrolysis of [$^2H_2$]compound **14** with NaOH, followed by treatment with *p*-toluenesulfonyl (Ts) chloride in pyridine, gave [$^2H_2$]3β-tosylate **16**. Reductive deoxygenation of compound **16** with zinc powder and NaI,[19] followed by hydrolysis of the two TBDMS groups with diluted HCl, produced the authentic [17α,19*S*-$^2H_2$]17β,19-diol **18**.

We then explored the stereochemistry of the NaB^2H_4 reduction of 3-deoxy-19-oxo steroids **3** and **6** (Fig. 5). The two 19-oxo compounds were separately treated with NaB^2H_4 to give [17α,19-$^2H_2$] diols **19** and **18** as well as the [19-^{2}H]19-hydroxy-17-oxo derivatives **2** and **5**, respectively. In the experiment with the 5-ene steroid **6**, [17α-^{2}H]17β-hydroxy-19-one **20** was also isolated, in addition to the two products, suggesting that a 17-carbonyl function of compound **5** would be more reactive toward $NaBH_4$ than the 19-carbonyl function under the conditions used. A comparison of ^{1}H-NMR spectra of the ^{2}H-labeled compounds **2** and **18** with those of the respective authentic samples obtained above revealed that the major product was [19*S*-^{2}H]-labeled product in each case, where the stereoselectivity of the reactions was *S* : *R*=90 : 10 for 4-ene **3** and 70 : 30 for 5-ene **6** (Fig. 6). This indicates that the stereoselective *si*-face attack of the borohydride reagent gives rise principally to the (19*S*) introduction of the labeled group. The stereoselectivities obtained were almost the same as those previously reported in the borohydride reductions of the corresponding 3-deoxygenated compounds, 19-oxoAD and 3β-acetoxy-5-ene steroid **10**.[10] A 19-carbonyl function of 19-oxoAD and steroid **10** is thought to be oriented in the out-of-ring position, then the borohydride reagent approaches the carbonyl carbon from the less hindered over-A-ring side rather than the crowded over-C-ring side, thereby stereoselectively giving the [19*S*-^{2}H]-labeled 19-hydroxyAD and compound **12**.[10,20] We have previously reported the conformational analysis of 4-en-19-oxo steroid

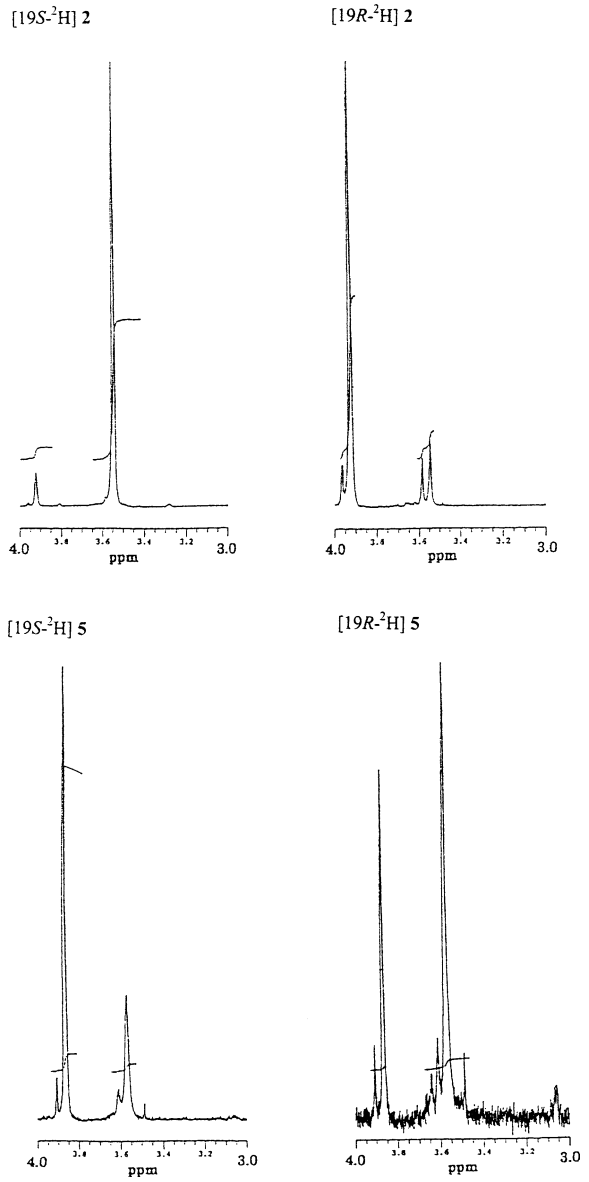

Fig. 6. ^{1}H-NMR Spectroscopic Analysis of 19-Protons of [19*S*-^{2}H]19-ols **2** and **5** and their [19*R*-^{2}H] Isomers

3 with semiempirical molecular orbital PM3 calculations, indicating that the 19-oxo function favors the over-A-ring conformation with the torsional angle C(5)–C(10)–C(19)–O(19) of $-31°$.[21] This conformation would also allow the attack of the reagent from the less hindered over-A-ring side, presumably from the C(4)–C(5) edge, resulting in the *si*-face attack. Although there is currently no information regarding the conformation of a C-19-carbonyl group of another 19-al **6**, the present ^{2}H-labeling result suggests that the borohydride reaction of the 19-carbonyl group would proceed in a steric course similar to that involved in the reduction of the 3β-acetoxy derivative **10**.

Oxidation of [19*S*-^{2}H]4-ene-17β,19-ols **19** and its 5-ene isomer **18** with Jones reagent yielded the corresponding [19-^{2}H] 19-als **3** and **6** (^{1}H-NMR δ: 9.79 ppm (19-H) 0.16H for **3**, and 9.71 ppm 0.13H for **6**). Treatment of the labeled 19-als with non-labeled reagent $NaBH_4$ produced [19*R*-^{2}H]19-hydroxy-17-ones **2** and **5**, the *si*-face attack products, as well as their [$^2H_2$]17β-hydroxy derivatives **19** and **18**, respectively. [^{1}H-NMR δ: 19-H; 3.52 ppm (0.10H, H_R) and 3.95 ppm (0.90H, H_S) for **2** and 3.89 ppm (0.30H, H_R) and 3.59 ppm (0.70H, H_S) for **5**].

Finally, we stereoselectively synthesized tritium-labeled 19-hydroxy-17-ones **2** and **5** with a high specific activity of the isotope in the same sequences employed for the deuterium-labeling experiments. To minimize the production of 17β,19-diols **19** and **18**, the corresponding 19-als **3** and **6** were treated with a limited amount of NaB^3H_4 and a brief reaction period. The products were purified by reverse-phase HPLC (C_{18} column, CH_3CN–H_2O) in the former experiment and by normal phase HPLC (silica, hexane–THF) in the later, giving the corresponding [19*S*-^{3}H]19-ols **2** and **5** (specific activity: 0.61 and 0.39 mCi/mmol, respectively). The [19*S*-^{3}H] compounds were converted into the corresponding [19*R*-^{3}H] isomers (specific activity: 0.55 and 0.21 mCi/mmol for **2** and **5**, respectively) through Jones oxidation, the non-labeled $NaBH_4$ reduction, and the HPLC purification. Their chemical and radiochemical purities were determined to be more than 98%, based on the HPLC analysis.

To understand the steric course of the aromatase reaction of 3-deoxy-19-ols **2** and **5**, incubation studies of the stereoselectively [^{3}H]-labeled substrates **2** and **5** with human placental aromatase are now underway.

Experimental

Melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR 1725 spectrometer in KBr pellets, and ^{1}H-NMR spectra were obtained in $CDCl_3$ solution with a JEOL EX270 (270 MHz) spectrometer using tetramethylsilane as an internal standard. Mass spectra (MS) was obtained with a JEOL JMS-DX303 spectrometer. Column chromatography was conducted with silica gel (E. Merck, Darmstadt, Germany, 70—230 mesh) (solvent: hexane–EtOAc). TLC was performed on E. Merck precoated TLC silica gel plates (silica gel 60F-254, layer thickness 0.25 and 0.5 mm for analytical and preparation use, respectively; solvent: hexane–EtOAc). The HPLC system consisted of a Waters model 510 pump (Milford, MA, U.S.A.) and a Soma UV detector (Tokyo, Japan).

Synthesis of the Authentic [19*S*-^{2}H]19-Hydroxyandrost-4-en-17-one (2) [19*S*-^{2}H]19-HydroxyAD (150 mg, 0.50 mmol), which was synthesized by treatment of 19-oxo AD with NaB^2H_4 according to the method of Osawa's group,[10] was initially treated with TBDMS chloride (112 mg, 0.74 mmmol) and imidazole (50 mg, 0.74 mmol) in DMF (3 ml), according to the method previously reported,[22] to give [19*S*-^{2}H]19-(*tert*-butyldimethylsiloxy)androst-4-ene-3,17-dione (**7**) (179 mg, 87%) (Fig. 3): mp 156—157 °C (from acetone) (lit.[22] mp 156—157 °C for non-labeled **7**). ^{1}H-NMR δ: 0.04 (6H, s, $OSiMe_2$), 0.86 (9H, s, $SiCMe_3$), 0.92 (3H, s, 18-Me), 3.86 (0.90H, s, 19-H_R), 3.89 (0.10H, s, 19-H_S), 5.88 (1H, m, 4-H).

A solution of the [^{2}H]19-silyloxy steroid **7** (180 mg, 0.43 mmol) in THF (4 ml) was treated with a mixture of $LiAlH_4$ (44 mg, 1.15 mmol) and $AlCl_3$ (515 mg, 3.86 mmol) in ether (10 ml) for 1 h under reflux, according to the method previously reported,[19] and then the product was purified by column chromatography, yielding [19*S*-^{2}H]19-(*tert*-butyldimethylsiloxy)androst-4-en-17β-ol (**8**) (54 mg, 32%): mp 106 °C (from acetone) (lit.[14] mp 105.5—106 °C for non-labeled **8**). ^{1}H-NMR δ: 0.03 and 0.04 (3H, each, s, OSiMe), 0.87 (9H, s, $SiCMe_3$), 0.88 (3H, s, 18-Me), 3.61 (1H, dd, J=8.1, 8.7 Hz, 17α-H), 3.77 (0.90H, s, 19-H_R), 3.81 (0.10H, s, 19-H_S), 5.40 (1H, m, 4-H).

The [^{2}H]17β-ol **8** (49 mg, 0.12 mmol) was treated with pyridinium dichromate (53 mg, 0.14 mmol) in CH_2Cl_2 (2 ml) at room temperature for 10 h, diluted with EtOAc (50 ml), washed with 5% HCl, 5% $NaHCO_3$ solution, and H_2O, sequentially, and dried with Na_2SO_4. Evaporation of the solvent gave a solid that was recrystallized from MeOH, yielding [19*S*-^{2}H]19-(*tert*-butyldimethylsiloxy)androst-4-en-17one (**9**) (28 mg, 57%): mp 66—66.5 °C (lit.[14] mp 67—68 °C for non-labeled **9**). ^{1}H-NMR δ: 0.03 and 0.04 (3H, each, s, OSiMe), 0.88 (9H, s, $SiCMe_3$), 0.89 (3H, s, 18-Me), 3.79 (0.90H, s, 19-H_R), 3.83 (0.10H, s, 19-H_S), 5.42 (1H, m, 4-H).

The [^{2}H]19-siloxy compound **9** (28 mg, 0.07 mmol) was hydrolyzed with 3 M HCl (0.3 ml) in THF (0.5 ml) and propan-2-ol (0.8 ml) at room temperature, according to the previous method,[14] affording [19*S*-^{2}H]19-hydroxyan-

drost-4-en-17one (**2**) (13 mg, 66%): mp 154—155 °C (lit.[23] mp 154—156 °C for non-labeled **2**). ^{1}H-NMR δ: 0.88 (3H, s, 18-Me), 3.55 (0.90H, s, 19-H$_R$), 3.92 (0.10H, s, 19-H$_S$), 5.71 (1H, m, 4-H).

Synthesis of the Authentic [19*S*-^{2}H]Androst-5-ene-17β,19-diol (18) A mixture of 3β-acetoxyandrost-5-ene-17,19-dione (**11**) (368 mg, 1.07 mmol), NaB^{2}H$_4$ (55 mg, 1.3 mmol), and MeOH (12 ml) was stirred at 0 °C for 3 h. After this time, the mixture was diluted with EtOAc (200 ml), washed with H_2O, and dried with Na_2SO_4. Evaporation of the solvent gave a solid that was purified by column chromatography followed by recrystallization from acetone to yield [17α,19*S*-^{2}H$_2$]3β-acetoxyandrost-5-ene-17β,19-diol (**12**) (252 mg, 69%): mp 165—166 °C. ^{1}H-NMR δ: 0.82 (3H, s, 18-Me), 2.03 (3H, s, 3β-OCOMe), 3.63 (0.30H, s, 19-H$_S$), 3.84 (0.70H, s, 19-H$_R$), 4.64 (1H, m, 3α-H), 5.77 (1H, m, 6-H). MS *m*/*z*: (relat. int.): 290 (M-60, 18), 258 (100), 240 (21). The 17β,19-diol **12** is a new compound. Next, authentic non-labeled diol **12** was prepared, as described above, using $NaBH_4$ as the reducing reagent. Compound **12**: mp 165—166 °C (from acetone). ^{1}H-NMR δ: 0.82 (3H, s, 18-Me), 2.03 (3H, s, 3β-OCOMe), 3.59 (1H, m, 17α-H), 3.63 and 3.84 (1H each, d, J=11.4 Hz, 19-CH$_2$), 4.64 (1H, m, 3α-H), 5.77 (1H, m, 6-H). FT-IR cm^{-1}: 3573 and 3507 (OH), 1723 (C=O). *Anal.* Calcd for $C_{21}H_{32}O_4$: C, 72.26; H, 9.27. Found: C, 72.44; H, 9.36.

To determine the stereochemistry at C-19 of the [^{2}H$_2$]diol **12**, this (35 mg, 0.1 mmol) was converted into [17α,19*S*-^{2}H$_2$]androst-5-ene-3β,17β,19-yl triacetate (**13**) (32 mg, 74%) by acetylation with Ac_2O (0.15 ml) in pyridine (0.3 ml) (room temperature, overnight). The ^{1}H-NMR analysis of the product revealed that the 19-proton signals corresponded to those of the authentic [19*S*-^{2}H]triacetate **13**,[10] obtained by the NaB^{2}H$_4$ reduction of 3β,17β-diacetoxyandrost-5-en-19-one (**10**) followed by acetylation. [^{2}H$_2$]Compound **13** (oil): ^{1}H-NMR δ: 0.82 (3H, s, 18-Me), 2.02, 2.04, and 2.05 (3H each, s, OCOMe), 3.94 (0.30H, m, 19-H$_S$), 4.47 (0.70H, s, 19-H$_R$), 4.63 (1H, m, 3α-H), 5.64 (1H, m, 6-H). MS *m*/*z*: (relat. int.): 388 (M$^+$-44, 100), 328 (48).

A mixture of the [^{2}H$_2$]17β,19-diol **12** (550 mg, 1.58 mmol), TBDMS chloride (595 mg, 3.95 mmol), imidazole, and DMF (14 ml) was stirred at room temperature for 21 h, diluted with Et_2O (1000 ml), washed with saturated NH_4Cl solution and H_2O, and dried with Na_2SO_4. Evaporation of the solvent gave a solid product that was purified by column chromatography and recrystallized from acetone, yielding [17α,19*S*-^{2}H$_2$]17β,19-bis(*tert*-butyldimethylsiloxy)androst-5-en-3β-yl acetate (**14**) (822 mg, 91%). mp 148—149 °C. ^{1}H-NMR δ: 0.005, 0.010, 0.039, and 0.049 (3H each, s, OSiMe), 0.74 (3H, s, 18-Me), 0.88 [18H, s, SiCMe$_3$×2], 2.03 (3H, s, 3β-OCOMe), 3.56 (0.30H, s, 19-H$_S$), 3.86 (0.70H, s, 19-H$_R$), 4.62 (1H, m, 3α-H), 5.57 (1H, m, 6-H). MS *m*/*z*: (relat. int.): 578 (M$^+$, 1), 518 (18), 461 (100), 386 (83), 372 (43), 255 (26), and 73 (73). Bis-silyl ether **14** was a new compound. Next, authentic non-labeled steroid **14** was prepared from the non-labeled diol **13**, as described above. Compound **14**: mp 149 °C. ^{1}H-NMR δ: 0.005, 0.010, 0.039, and 0.049 (3H each, s, OSiMe), 0.74 (3H, s, 18-Me), 0.88 [18H, s, SiCMe$_3$×2], 2.03 (3H, s, 3β-OCOMe), 3.56 and 3.80 (1H each, d, J=10.5 Hz, 19-CH$_2$), 3.59 (1H, m, 17α-H), 4.62 (1H, m, 3α-H), 5.57 (1H, m, 6-H). FT-IR cm^{-1}: 1737 (C=O). *Anal.* Calcd for $C_{33}H_{60}O_4Si_2$: C, 68.76; H, 10.51. Found: C, 68.66; H, 10.33.

A mixture of the [17α,19-^{2}H$_2$]3β-acetate **14** (800 mg, 1.4 mmol), 1 M NaOH solution (4.2 ml), MeOH (17 ml), and acetone (35 ml) was stirred at room temperature for 30 min, then condensed to about 15 ml, diluted with EtOAc (450 ml), washed with 5% $NaHCO_3$ solution and H_2O, and dried with Na_2SO_4. After evaporation of the solvent, the solid product obtained was recrystallized from acetone to yield [17α,19*S*-^{2}H$_2$]17β,19-bis(*tert*-butyldimethylsiloxy)androst-5-en-3β-ol (**15**) (660 mg, 89%). mp 153—154 °C. ^{1}H-NMR δ: −0.0001, 0.0097, 0.030, and 0.038 (3H each, s, OSiMe), 0.74 (3H, s, 18-Me), 0.873 and 0.875 [9H each, s, SiCMe$_3$], 3.51 (0.30H, s, 19-H$_S$), 3.55 (1H, m, 3α-H), 3.71 (0.70H, s, 19-H$_R$), 5.54 (1H, m, 6-H). MS *m*/*z*: (relat. int.): 536 (M$^+$, 1), 518 (22), 479 (43), 461 (59), 386 (100), 372 (48), 255 (54), and 73 (64). Compound **15** was a new compound. Non-labeled compound **15** was also prepared from compound **14**, as described above. mp 154 °C. ^{1}H-NMR δ: −0.0001, 0.0097, 0.030, and 0.038 (3H each, s, OSiMe), 0.74 (3H, s, 18-Me), 0.873 and 0.875 [9H each, s, SiCMe$_3$], 3.51 and 3.71 (1H each, d, J=10.7 Hz, 19-CH$_2$), 3.55 (1H, m, 3α-H), 3.61 (1H, m, 17α-H), 5.54 (1H, m, 6-H). FT-IR (KBr) cm^{-1}: 3280 (OH). *Anal.* Calcd for $C_{31}H_{58}O_3Si_2$: C, 69.67; H, 10.96. Found: C, 69.50; H, 11.12.

Ts chloride (720 mg, 3.78 mmol) was added to a chilled solution of the [17α,19-^{2}H$_2$]3β-ol **15** (410 mg, 0.39 mmol) in pyridine (4.4 ml) with stirring, then the mixture was allowed to stand at room temperature overnight; it was then diluted with EtOAc (150 ml), washed sequentially with 5% HCl, 5% $NaHCO_3$ solution, and H_2O, and dried with Na_2SO_4. Evaporation of the solvent gave a crude [17α,19*S*-^{2}H$_2$]17β,19-bis(*tert*-butyldimethylsiloxy)androst-5-en-3β-yl tosylate (**16**) (213 mg).

The crude [^{2}H$_2$]compound **16** (210 mg) was, without isolation, treated with Zn powder (110 mg, 0.8 mmol) and NaI (543 mg, 1.21 mmol) in ethyleneglycol dimethyl ether (6 ml) and H_2O (0.5 ml) under reflux for 2 h.[19] After this time, the mixture was diluted with EtOAc (150 ml), washed sequentially with 5% $Na_2S_2O_3$ solition, 5% $NaHCO_3$ solition, and H_2O, and dried with Na_2SO_4. Evaporation of the solvent gave an oil that was purified by column chromatography and recrystallized from acetone, giving [17α,19*S*-^{2}H$_2$]17β,19-bis(*tert*-butyldimethylsiloxy)androst-5-ene (**17**) (52 mg, 33%). mp 74—75 °C. ^{1}H-NMR δ: −0.002, 0.009, 0.020 and 0.031 (3H each, s, OSiMe), 0.74 (3H, s, 18-Me), 0.87 and 0.89 [9H each, s, SiCMe$_3$], 3.56 (0.30H, s, 19-H$_S$), 3.94 (0.70H, s, 19-H$_R$), 3.60 (1H, m, 17α-H), 5.46 (1H, m, 6-H). MS *m*/*z*: (relat. int.): 520 (M$^+$, 1), 463 (100), 388 (36), and 331 (20). Non-labeled compound **17** was also obtained as described above. Compound **17**: mp 74—75 °C. ^{1}H-NMR δ: −0.002, 0.009, 0.020 and 0.031 (3H each, s, OSiMe), 0.74 (3H, s, 18-Me), 0.87 and 0.89 [9H each, s, SiCMe$_3$], 3.56 and 3.94 (1H each, d, J=10.5 Hz, 19-CH$_2$), 3.60 (1H, m, 17α-H), 5.46 (1H, m, 6-H). *Anal.* Calcd for $C_{31}H_{58}O_2Si_2$: C, 71.74; H, 11.27. Found: C, 71.50; H, 11.00.

3 M HCl (0.15 ml) was added to a solution of [17α,19*S*-^{2}H$_2$] compound **17** (18 mg, 0.034 mmol) in THF (0.24 ml) and propan-2-ol (0.4 ml), and the mixture was allowed to stand at room temperature for 36 h; it was then diluted with EtOAc (100 ml), washed with 5% $NaHCO_3$ solution and H_2O, and dried with Na_2SO_4. Evaporation of the solvent gave a solid that was purified by preparative TLC (hexane–EtOAc, 3 : 1, v/v) followed by recrystallization from acetone to give [17α,19*S*-^{2}H$_2$]androst-5-ene-17β,19-diol **18** (7.1 mg, 70%). mp 126—127 °C (mp for non-labeled **18**[14] 137—138 °C). ^{1}H-NMR δ: 0.82 (3H, s, 18-Me), 3.57 (0.30H, s, 19-H$_S$), 3.84 (0.70H, s, 19-H$_R$), 5.68 (1H, m, 6-H). MS *m*/*z*: (relat. int.): 292 (M$^+$, 22), 260 (100), 242 (40) (d_1: 11. d_2: 89).

Reaction of 19-Oxo-4-ene Steroid 3 with NaB^{2}H$_4$ NaB^{2}H$_4$ (1.9 mg, 50 μmol) was added to a solution of 19-oxo-4-ene **3** (67 mg, 0.23 mmol) in dry MeOH (1 ml) under ice-cooling, and the mixture was stirred for 1 h. After this time, another NaB^{2}H$_4$ (1.2 mg, 31 μmol) was added to the mixture, and the mixture was further stirred at 0 °C for 2.7 h, diluted with EtOAc (50 ml), washed with H_2O, and dried with Na_2SO_4. After evaporation of the solvent, the residue obtained (71 mg) was purified by preparative TLC (hexane–EtOAc, 3 : 1, v/v, multiple developments), yielding two products (Fig. 5). The less polar product was recrystallized from acetone to give [19*S*-^{2}H]19-hydroxyandrost-4-en-17-one (**2**) (23 mg, 33%). mp 140—145 °C (lit.[20] mp 154—156 °C for non-labeled **2**). ^{1}H-NMR δ: 0.88 (3H, s, 18-Me), 3.55 (0.90H, s, 19-H$_R$), 3.92 (0.10H, s, 19-H$_S$), 5.71 (1H, m, 4-H). MS *m*/*z*: (relat. int.): 289 (M$^+$, 25), 257 (100), 239 (35) (d_0: 2, d_1: 98). The more polar compound was recrystallized from ether to afford [19*S*-^{2}H$_2$]androst-4-ene-17β,19-diol (**19**) (22 mg, 33%). mp 108—110 °C (lit.[14] mp 97—101 °C for non-labeled **19**). ^{1}H-NMR δ: 0.75 (3H, s, 18-Me), 3.52 (0.90H, s, 19-H$_R$), 3.93 (0.10H, s, 19-H$_S$), 5.71 (1H, m, 4-H). MS *m*/*z*: (relat. int.): 292 (M$^+$, 20), 260 (100), 240 (58) (d_1: 4, d_2: 96).

Synthesis of [19*R*-^{2}H]19-Hydroxy-4-ene Steroid 2 Jones reagent was added dropwise to a solution of a 1 : 1 mixture of [19*S*-^{2}H]19-ol **2** and [17α,19*S*-^{2}H$_2$]diol **19** (111 mg, *ca.* 0.38 mmol) in acetone (1.9 ml) until the orange color of the reagent remained; it was then and stirred at 0 °C for 4 min. After the usual treatment, the crude isolated product [19-^{2}H]androst-4-ene-17,19-dione (**3**) (100 mg), without further purification, was dissolved in MeOH (2 ml). $NaBH_4$ (19 mg, 0.46 mmol) was added to this solution, and the mixture was stirred at 0 °C for 1.5 h. After this time, the solvent was evaporated under a stream of N_2 gas to give a residue that was dissolved in EtOAc (50 ml), washed with H_2O, and dried with Na_2SO_4. A solid product obtained after evaporation of the solvent was subjected to column chromatography followed by recrystallization from acetone, yielding [19*R*-^{2}H]19-hydroxyandrost-4-en-17-one (**2**) (37 mg, 34%) along with its 17β-reduced analog [19*R*-^{2}H$_2$]androst-4-ene-17β,19-diol (**19**) (21 mg, 21%). [19*R*-^{2}H]Compound **2**: mp 144—146 °C. ^{1}H-NMR δ: 0.89 (3H, s, 18-Me), 3.56 (0.90H, d, J=10.3 Hz, 19-H$_R$), 3.94 (0.10H, d, J=10.3 Hz, 19-H$_S$), 5.74 (1H, s, 4-H). MS *m*/*z*: (relat. int.): 289 (M$^+$, 21), 257 (100), 239 (28) (d_0: 12, d_1: 88). [^{2}H$_2$]Compound **19**: mp 107—110 °C (lit.[14] mp 97—101 °C for non-labeled **19**). ^{1}H-NMR δ: 0.75 (3H, s, 18-Me), 3.54 (0.10H, d, J=10.3 Hz, 19-H$_R$), 3.62 (1H, t, J=8.5 Hz, 17α-H), 3.95 (0.90H, d, J=10.3 Hz, 19-H$_S$), 5.72 (1H, s, 4-H). MS *m*/*z*: (relat. int.): 291 (M$^+$, 24), 259 (100), 241 (42) (d_0: 15, d_1: 85).

Reaction of 19-Oxo-5-ene Steroid 6 with NaB^{2}H$_4$ NaB^{2}H$_4$ (2.7 mg, 71 μmol) was added to a solution of 19-oxo-5-ene **6** (81 mg, 0.28 mmol) in MeOH (8 ml), and the mixture was stirred at 0 °C for 1 h. After this time, the reaction mixture was evaporated to a small volume by a stream of N_2 gas, then diluted with EtOAc (50 ml), washed with H_2O, and dried with Na_2SO_4.

After evaporation of the solvent, the oily product obtained was purified by preparative TLC (hexane–EtOAc, 2 : 1, v/v), affording two steroidal fractions. The less polar fraction (Rf=0.65) was further purified by normal phase HPLC using a silica gel column (R-SIL-5-06, 4.6×250 mm) (YMC, Kyoto, Japan) and hexane–THF (75 : 25, v/v, 1 ml/min) as a mobile phase, giving two compounds, [19S-^{2}H]19-hydroxyandrost-5-en-17-one (**5**) (6 mg, 8%) (t_R=8.1 min) and [17α-^{2}H]17β-hydroxyandrost-5-en-19-one (**20**) (23 mg, 28%) (t_R=9.6 min). [19-^{2}H]Compound **5**: mp 86—90 °C (lit.[15] mp 120—124 °C for non-labeled **5**). ^{1}H-NMR δ: 0.94 (3H, s, 18-Me), 3.59 (0.30H, d, J=13.5 Hz, 19-H$_S$), 3.89 (0.70H, d, J=13.5 Hz, 19-H$_R$), 5.72 (1H, s, 6-H). MS m/z: (relat. int.): 289 (M$^+$, 24), 257 (100), 239 (40) (d_0: 10, d_1: 90). [17-^{2}H]Compound **20**: mp 125—131 °C (lit.[17] mp 148—151 °C for non-labeled **20**). ^{1}H-NMR δ: 0.70 (3H, s, 18-Me), 5.75 (1H, m, 6-H), 9.69 (1H, d, J=1.5 Hz, 19-CHO). MS m/z: (relat. int.): 289 (M$^+$, 11), 257 (100), 242 (55) (d_0: 10, d_1: 90). The starting material **6** (41 mg, 50%) was recovered from the more polar fraction on TLC (Rf=0.40). Treatment of the recovered steroid **6** (41 mg, 0.143 mmol) with an excess of NaB^{2}H$_4$ (0.11 mmol) and a longer reaction time (2.5 h), followed by the preparative TLC and the subsequent HPLC, as described above, yielded [17α,19S-^{2}H$_2$]androst-5-ene-17β,19-diol (**18**) (9 mg, 22%) (Rf=0.40) as well as the [19S-^{2}H]19-ol **5** (1.7 mg, 4%) and [17α-^{2}H]17β-ol **20** (23 mg, 56%). [17α,19-^{2}H$_2$]Diol **18**: mp 79—85 °C (lit.[15] mp 137—138 °C for non-labeled **18**). ^{1}H-NMR δ: 0.82 (3H, s, 18-Me), 3.57 (0.30H, br s, 19-H$_S$), 3.83 (0.70H, br s, 19-H$_R$), 5.68 (1H, m, 6-H). MS m/z: (relat. int.): 292 (M$^+$, 18), 260 (100), 242 (54) (d_0: 2, d_1: 12, d_2: 86).

Synthesis of [19R-^{2}H]19-Hydroxy-5-ene Steroid 5 Jones reagent was added dropwise to a solution of [17α,19S-^{2}H]17β,19-diol **18** (60 mg, 0.21 mmol) in acetone (2 ml) until the orange color of the reagent remained, and the mixture was stirred at 0 °C for 5 min. After this time, the reaction mixture was poured into ice-water (10 ml), then extracted with EtOAc (100 ml). The organic layer was washed with saturated $NaHCO_3$ solution and H_2O, and dried with Na_2SO_4. Evaporation of the solvent gave a solid that was purified by preparative TLC (hexane–EtOAc=4 : 1, v/v) to give [19-^{2}H]androst-5-ene-17,19-dione (**6**) (36.5 mg, 56%). mp 106—112 °C (lit.[15] mp 110—112 °C for non-labeled **6**). ^{1}H-NMR δ: 0.83 (3H, s, 18-Me), 5.78 (1H, m, 6-H), 9.71(0.13H, d, J=1.2 Hz, 19-H). MS m/z: (relat. int.): 287 (M$^+$, 14), 257 (100), 239 (47) (d_0: 9, d_1: 91).

Non-labeled NaBH$_4$ (2.4 mg, 63 μmol) was added to a solution of [19-^{2}H]dione **6** (33 mg, 0.12 mmol) in dry MeOH (3 ml), and the mixture was stirred at 0 °C for 5 min. After the same work up as above, the oil product obtained was purified by preparative TLC (hexane : AcOEt=2 : 1, v/v) and normal phase HPLC (under the same conditions as above) to give the following three products. [19R-^{2}H]19-Hydroxy-17-one **5** (2.0 mg, 6%) (oil). ^{1}H-NMR δ: 0.94 (3H, s, 18-Me), 3.59 (0.63H, d, J=13.6 Hz, 19-H$_S$), 3.89 (0.37H, d, J=13.6 Hz, 19-H$_R$), 5.72 (1H, m, 6-H). MS m/z: (relat. int.): 291 (M$^+$, 23), 257 (100), 239 (33) (d_0: 12, d_1: 88). [19-^{2}H]17β-Hydroxy-19-one **20** (19 mg, 58%). mp 142—145 °C. ^{1}H-NMR δ: 0.70 (3H, s, 18-Me), 3.64 (1H, t, J=8.5 Hz, 17α-H), 5.75 (1H, m, 6-H), 9.69 (0.13H, d, J=1.2 Hz, 19-CHO). MS m/z: (relat. int.): 289 (M$^+$, 11), 259 (100), 241 (50) (d_0: 14, d_1: 86). [19R-^{2}H]17β,19-Diol **18** (9.2 mg, 27%) (oil). ^{1}H-NMR δ: 0.82 (3H, s, 18-Me), 3.59 (0.63H, d, J=13.1 Hz, 19-H$_S$), 3.64 (1H, t, J=8.5 Hz, 17α-H), 3.86 (0.37H, d, J=13.1 Hz, 19-H$_R$), 5.68 (1H, m, 6-H). MS m/z: (relat. int.): 291 (M$^+$, 18), 259 (100), 241 (48) (d_0: 14, d_1: 86).

Synthesis of [19S-^{3}H]19-Hydroxy-4-ene Steroid 2 and Its 5-ene Isomer 5 Non-labeled NaBH$_4$ (2.7 mg, 71 μmol) was added to a solution of NaB^{3}H$_4$ in 0.01 M NaOH solution (0.2 ml, 100 mCi, 0.3 μg, 6.52 nmol) obtained from NEN Life Science Products (Boston, MA, U.S.A.). Half of the NaB^{3}H$_4$ solution diluted with NaBH$_4$ was separately added to a solution of 19-oxo steroid **3** or **6** (21 mg, 73 μmol) in dry MeOH (1.5 ml) at 0 °C, and the mixture was stirred for 30 min. After this time, the solvent was evaporated under a stream of N_2 gas, then the residue was dissolved in EtOAc (5 ml), washed with H_2O (2 ml), and dried with Na_2SO_4. Evaporation of the solvent under reduced pressure gave a residue which was purified by HPLC under the conditions described below, yielding [19S-^{3}H]compound **2** or **5**. A) Purification of [^{3}H]steroid **2**: column, PureSil C$_{18}$ (4.6×150 mm); mobile phase, CH$_3$CN : H$_2$O=70 : 30, v/v, 1 ml/min; retention time, 4.9 min for [^{3}H]**2**. B) Purification of [^{3}H]steroid **5**: column, R-SIL-5-06 (4.6×250 mm); mobile phase, hexane : THF=75 : 25, v/v, 1 ml/min; retention time, 8.1 min for [^{3}H]**5**.

Radiochemical yields: 28% for [^{3}H]compound **2** and 5.7% for [^{3}H]compound **5**. Specific activity was obtained on the basis of a ratio of the radioactivity to the absorbance at 200 nm: 0.61 mCi/mmol for [^{3}H]compound **2** and 0.39 mCi/mmol for [^{3}H]compound **5**. Their chemical and radiochemical purities were more than 98% on the basis of HPLC analysis under the conditions described above, respectively.

Conversion of [19S-^{3}H]Compounds 2 and 5 into Their [19R-^{3}H] Isomers Jones reagent (20 μl) was added to a solution of [19S-^{3}H]19-ol **2** (5.8 mg, 20 μmol) in acetone (0.8 ml) at 0 °C, and the mixture was stirred for 3 min. After this time, an excess of the reagent was quenched by adding propan-2-ol (30 μl), then the product was extracted with EtOAc (2 ml). The organic layer was washed with H_2O and the solvent was evaporated under a stream of N_2 gas to give a solid that was, without purification, dissolved in MeOH (1 ml) and treated with 50 μl of non-labeled NaBH$_4$ solution [NaBH$_4$ (29 mg, 0.76 mmol) in 50 ml of 0.01 M NaOH] at 0 °C for 30 min. The product obtained was purified by reverse phase HPLC, as described above, and [19R-^{3}H] isomer **2** was obtained in 12% radiochemical yield (specific activity, 0.55 mCi/mmol).

[19S-^{3}H]Compound **5** (1.1 mg, 3.8 μmol) was similarly converted into the [19-^{3}H]19-oxo derivative **6** by treatment of Jones reagent (Jones reagent, 6 μl; acetone, 0.2 ml; reaction period 3 min), then this was treated with non-labeled NaBH$_4$ [NaBH$_4$ (28.8 mg, 0.76 mmol) in 1 ml of 0.01 M NaOH solution, 110 μl] and MeOH (1 ml) at 0 °C for 30 min, and the product was purified by the normal phase HPLC, as described above, producing [19R-^{3}H]compound **5** (radiochemical yield: 3.3%; specific activity 0.21 mCi/mmol).

The chemical and radiochemical purities of the [19R-^{3}H]compounds were determined by HPLC analysis under the conditions described above, respectively, to be more than 98%.

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