Preparation of 17α-iodoethynylandrosta- and 17α-(2-iodoethenyl)androsta-4,6-dien-17β-ol-3-ones as active site–directed photoaffinity ligands for androgen-binding proteins

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Unsaturated analogues of androst-4-en-17β-ol-3-one, each with a 17α-iodoethynyl or 17α-(2-iodoethenyl) substituent, were prepared, and their relative binding affinities (RBAs) for androgen-binding protein (ABP) were compared with those of 5α-androstan-17β-ol-3-one, androst-4-en-17β-ol-3-one, androsta-4,6-dien-17β-ol-3-one, and androsta-1,4,6-trien-17β-ol-3-one. These binding studies indicate that the iodine[^{125}I] analogues of 17α-iodoethynyl and 17α-[(E)-2-iodoethenyl] derivatives of androsta-4,6-dien-17β-ol-3-one and androsta-1,4,6-trien-17β-ol-3-one will have RBAs at least twice as great as that of 5α-androstan-17β-ol-3-one. They can be prepared from 17α-ethynylandrosta-4-en-17β-ol-3-one, the final synthetic step using N-[^{125}I]iodosuccinimide, and are potential radioiodinated, active site–directed photoaffinity ligands for ABP and testosterone-binding globulin. (*Steroids* **57**:569–576, 1992)

Keywords: steroids; photoaffinity ligands; androgen-binding proteins; 17α-(2-iodoethynyl)androsta-4,6-dien-17β-ol-3-one; 17α-(2-iodoethynyl)androsta-1,4,6-trien-17β-ol-3-one; 17α-(2-iodoethenyl)androsta-4,6-dien-17β-ol-3-one; 17α-(2-iodoethenyl)androsta-1,4,6-trien-17β-ol-3-one; relative binding affinity

Introduction

Androgen-binding protein (ABP)[1–4] and testosterone-binding globulin (TeBG),[5,6] also referred to as sex hormone-binding globulin (SHBG), are extracellular proteins present in several species, including humans, and are responsible for the transport of the androgens 5α-androstan-17β-ol-3-one (**1**, 5α-dihydrotestosterone, 5α-DHT) and androst-4-en-17β-ol-3-one (**2a**, testosterone) (Figure 1).[7–10] ABP is produced by the Sertoli cells of the testis,[1] whereas TeBG is produced by the liver.[11] Both proteins are considered to be involved in the regulation of male reproductive function. Some time ago we reported the synthesis of [1ξ,2ξ-$^3H_2$]androsta-4,6-dien-17β-ol-3-one ([^{3}H]**3a**, Δ^6-[^{3}H]testosterone), prepared by catalytic reduction of androsta-1,4,6-trien-17β-ol-3-one (**4a**, Δ^{1,6}-testosterone) with tritium, as an active site–directed photoaffinity radiolabel for the study of the physical and biological properties of androgen-binding proteins (Figure 2).[12] Subsequently, we reported the use of [^{3}H]**3a** in elucidating the physicochemical properties of ABP[13–15] and TeBG[16] and have recently used [^{3}H]**3a** as a probe for determining the amino acid sequence of the steroid-binding domain of ABP (Figure 2).[17]

Although 5α-DHT (**1**) and testosterone (**2a**) are the endogenous ligands for androgen-binding proteins, these androgens cannot be used as photoaffinity ligands for these proteins in cytosol. Cytosol has an intense

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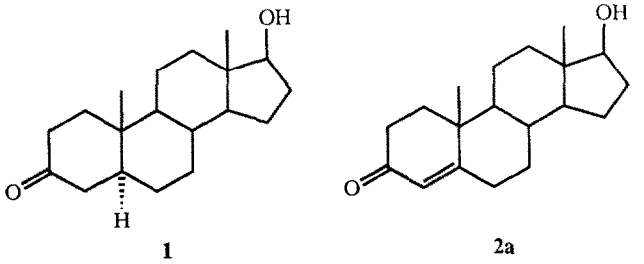


Figure 1 Structure of compounds **1** and **2a**.

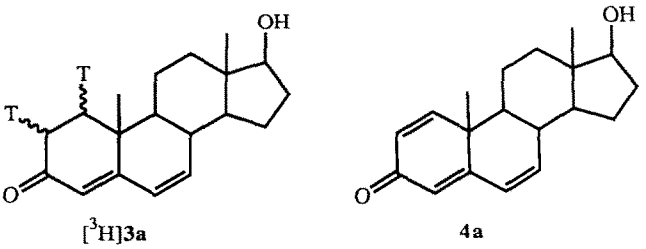


Figure 2 Structure of compounds [^{3}H]**3a** and **4a**.

absorption band centered at 270 nm with a long tail extending beyond 300 nm. Thus, photoexcitation of 5α-dihydrotestosterone and testosterone, with their n $\rightarrow$ π^* carbonyl group transitions centered at 280 (ε_{max} 25) and 305 nm (ε_{max} 100), respectively, cannot occur. In addition, a Pyrex filter with wavelength cut-off at about 300 nm is necessary to protect the proteins from photodegradation. The extended conjugation of Δ^6-testosterone **(3a)**, which has a binding affinity for ABP approximately half that of testosterone,[13] results in a carbonyl absorption band centered at 345 nm (ε_{max} 300). This absorption is sufficiently beyond the absorption band of cytosol and the cut-off of the Pyrex filter to permit photoexcitation of the unsaturated carbonyl group in [^{3}H]**3a**, which results in covalent bond formation between [^{3}H]**3a** and ABP and TeBG.[13,16] The covalently bonded, radiolabeled steroid-protein complexes can remain intact during electrophoresis under denaturing conditions and during other manipulations.

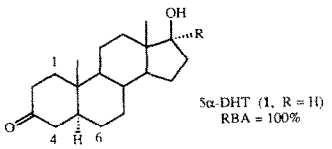
Although Δ^6-[^{3}H]testosterone-labeled androgen-binding proteins have had substantial use for the study of the physical properties and physiological role of androgen-binding proteins, an important extension of these studies would be the incorporation of iodine[^{125}I] into a Δ^6-testosterone or a $\Delta^{1,6}$-testosterone analogue. Androgen-binding proteins covalently radiolabeled with these ligands would have much greater specific radioactivity than can be obtained with tritium-labeled compounds. Thus, an iodine[^{125}I]-labeled probe would be of great utility in studies geared to examination of the in vivo tissue uptake of androgen-binding proteins and in the determination of the presence of receptors for them in tissues.[18]

Because an extensive study of the structural and configurational requirements for high binding affinity to androgen-binding proteins[19] showed that substitution at the 17α position has little effect on the relative binding affinity of otherwise structurally and configurationally similar androgens, we have prepared a number of androgens (Table 1), each with an ethynyl or an ethenyl substituent in the 17α position **(2b-f, 3b-f,** and **4b-f)**, some of the latter incorporating an iodine atom. The binding activity of these androgens was compared with those of 5α-DHT **(1)**, testosterone **(2a)**, Δ^6-testosterone **(3a)**, and $\Delta^{1,6}$-testosterone **(4a)** in order to evaluate the effect on binding affinity to ABP of unsaturation in the A/B-ring system, a two carbon group at the 17α position, and substitution of an iodine atom in the side chain. Those analogues with a 4,6-dien-3-one **(3b-f)** and 1,4,6-trien-3-one systems **(4b-f)** are potential photoaffinity ligands for androgen-binding proteins, whereas those incorporating an iodine atom **(3c,e,f** and **4c,e,f)** are also potential-radiolabeled photoaffinity ligands. Similar steroidal compounds containing an [^{125}I]iodovinyl group in the 17α position have been prepared for the estrogen,[20,21] progesterone,[22] and androgen[23] receptors. None of these iodinated steroids can function as a photoaffinity ligand in that none contains a conjugated dienone moiety.

Experimental

Melting points (MP) were determined in open capillary tubes and are corrected. Optical rotatory powers were obtained with a Rudolph Autopol III automatic polarimeter with a 1-dm sample tube. Ultraviolet (UV) absorption spectra were measured in methanol using matched 1-cm cells with a Cary 2390 spectrometer in auto gain mode or as indicated in ethanol with a Cary 14 spectrometer operating with the normal variable slit mode. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained

Table 1 RBAs of photoaffinity labels for rat androgen-binding protein



Compound	R	RBA (%) 2 (Δ^4)	3 ($\Delta^{4,6}$)	4 ($\Delta^{1,4,6}$)
a	H	49[a], 82[b]	33[a]	39[a]
b	C≡C—H	57, 92[b]	91	230
c	C≡C—I	200	200	820
d	H H | | C=C—H	28, 36[b]	35	140
e	H H | | C=C—I	41	33	100
f	H I | | C=C—H	60	390	230

[a] Previously reported.[1]
[b] Previously reported.[19]

in chloroform-*d* using a JEOL FX 90Q spectrometer operating at 90 MHz with tetramethylsilane as the internal standard. Chemical shifts are reported in ppm downfield from the standard. Radioactivity was determined using a Packard 1900CA scintillation counter. Microanalyses were conducted by Galbraith Laboratories, Inc., Knoxville, TN, USA.

17α-Ethynylandrost-4-en-17β-ol-3-one (2b, ethisterone)

Compound **2b** was purified by sublimation at 190–195 C (0.5 mm Hg): mp 270–273 C; $[\alpha]^{25}_D$ +31° (*c* 1.01, pyridine) [lit[24] mp 264–266 C; $[\alpha]_D$ +21.5° (dioxane)]; infrared (KBr) 3,275, 1,640 cm^{-1}; Proton NMR δ 0.88 (s, 3H, C-18), 1.18 (s, 3H, C-19), 2.55 (s, 1H, C-21), 5.71 (s, 1H, C-4).

17α-Iodoethynylandrost-4-en-17β-ol-3-one (2c)

17α-Ethynylandrost-4-en-17β-ol-3-one (**2b;** 0.94 g, 3.0 mmol) was suspended in acetone (25 ml), and *N*-iodosuccinimide (0.79 g, 3.5 mmol) and silver nitrate (0.050 g, 0.29 mmol) were added to the stirred suspension. After stirring for 1 hour at room temperature, the reaction mixture was poured onto an ice-water mixture (200 ml). The precipitate was removed by filtration and was dissolved in ethyl acetate (150 ml). This latter solution was washed with water (3 × 30 ml), and dried ($MgSO_4$). The solution was filtered, and the solvent was removed at reduced pressure. Recrystallization of the solid residue from acetone/hexane gave pure **2c** (0.97 g, 74%): mp 162–163 C dec (lit[25], mp 164 C); $[\alpha]^{22}_D$ −3° (*c* 0.481, $CHCl_3$) [lit[25] $[\alpha]^{22}_D$ -2.4 (*c* 0.5, $CHCl_3$)]; ^1H NMR δ 0.86 (s, 3H, C-18), 1.18 (s, 3H, C-19), 5.71 (br s, 1H, C-4). Analysis calculated for $C_{21}H_{27}IO_2$: C, 57.53; H, 6.22; I, 28.95. Found: C, 57.70; H, 6.31; I, 28.82.

17α-Ethenylandrost-4-en-17β-ol-3-one (2d)

17α-Ethynylandrost-4-en-17β-ol-3-one (**2b;** 0.310 g, 0.992 mmol) was dissolved in pyridine, and Lindlar's catalyst (70 mg; 5% palladium on calcium carbonate poisoned with lead acetate) was added. The mixture was stirred under an atmosphere of hydrogen (716 mm Hg, 25 C) until 1 mol equivalent of hydrogen (26 ml, 0.99 mmol) was absorbed (1 h). The catalyst was removed by filtration, and the solvent was evaporated at reduced pressure. The residue was dissolved in ether (100 ml), washed with water (2 × 20 ml), and dried ($MgSO_4$). The ether was removed at reduced pressure, and recrystallization of the solid residue from ethyl acetate gave pure **2d** (0.290 g, 93%): mp 141–143 C; $[\alpha]^{21}_D$ +96° (*c* 1.00, $CHCl_3$) [lit[26], mp 140 C; ^1H NMR δ 0.96 (s, 3H, C-18), 1.19 (s, 3H, C-19), 5.11 (dd, 1H, $J_{21,21}$ = 1 Hz and $J_{20,21}$ = 10 Hz, C-21), 5.14 (dd, 1H, $J_{21,21}$ = 1 Hz and $J_{20,21}$ = 18 Hz, C-21), 5.72 (br s, 1H, C-4), 6.03 (dd, 1H, $J_{20,21}$ = 11 Hz and $J_{20,21}$ = 18 Hz, C-20).

17α-[(Z)-2-Iodoethenyl]androst-4-en-17β-ol-3-one (2e)

3,3-Ethylenedioxy-17α-[(*Z*)-2-iodoethenyl]androst-5-en-17β-ol-3-one (**5c;** 3.30 g, 6.81 mmol) was dissolved in methanol (20 ml). Oxalic acid dihydrate (1.40 g, 11.1 mmol) and water (1.0 ml) were added, and the mixture was boiled for 1 hour. The cooled solution was diluted with ether (150 ml), and the organic layer was washed with 5% aqueous sodium bicarbonate (2 × 100 ml) and brine (100 ml) and dried (Na_2SO_4). Evaporation of the ether at reduced pressure gave crude **2e** as a yellow solid. Recrystallization of the solid residue from acetone/water gave pure **2e** (2.36 g, 79%) as fine, light yellow needles: mp 127–128 C dec; $[\alpha]^{24}_D$ +114° (*c* 0.140, $CHCl_3$); ^1H NMR δ 0.98 (s, 3H, C-18), 1.20 (s, 3H, C-19), 5.73 (br s, 1H, C-4), 6.34 (d, 1H, $J_{20,21}$ = 8 Hz, C-20), 6.76 (d, 1H, $J_{20,21}$ = 8 Hz, C-21). Analysis calculated for $C_{21}H_{29}IO_2$: C, 57.27; H, 6.64; I, 28.82. Found: C, 57.62; H, 6.61; I, 28.91.

17α-[(E)-2-Iodoethenyl]androst-4-en-17β-ol-3-one (2f)

3,3-Ethylenedioxy-17α-[(*E*)-2-iodoethynyl]androst-5-en-17β-ol (**5e;** 5.10 g, 10.5 mmol) and oxalic acid dihydrate (2.00 g, 15.9 mmol) in methanol/water (10:1, 55 ml) were boiled for 1.5 hours. The solvent was removed at reduced pressure, and the residue was dissolved in ether (250 ml). The ethereal solution was washed with 1 N sodium hydroxide (75 ml, 2x), brine (75 ml), and water (75 ml) and then dried ($MgSO_4$). Evaporation of the ether at reduced pressure gave a white solid foam, and crystallization of this solid from acetone/hexane gave pure **2f** (3.50 g, 76%) as a white solid: mp 106–108 C dec; $[\alpha]^{23}_D$ +34° (*c* 1.00, $CHCl_3$); ^1H NMR δ 0.93 (s, 3H, C-18), 1.19 (s, 3H, C-19), 5.72 (br s, 1H, C-4), 6.24 (d, 1H, $J_{20,21}$ = 14 Hz, C-20), 6.71 (d, 1H, $J_{20,21}$ = 14 Hz, C-21). Analysis calculated for $C_{21}H_{29}IO_2$: C, 57.27; H, 6.64; I, 28.82. Found: C, 57.48; H, 6.68; I, 29.64.

17α-Ethynylandrosta-4,6-dien-17β-ol-3-one (3b)

Compound **3b** was prepared by (A) dehydrobromination of 6-bromo-17α-ethynylandrost-4-en-17β-ol-3-one with calcium carbonate in boiling dimethylformamide[27]; (B) dehydrogenation of 3-ethoxy-17α-ethynylandrosta-3,5-dien-17β-ol-3-one with DDQ in aqueous acetone[28]; and (C) by dehydrogenation of 17α-ethynylandrost-4-en-17β-ol-3-one (**2b**) with chloranil[29] as outlined below. From each preparation, **3b** had identical physical properties: mp 260–262 C; $[\alpha]^{24}_D$ −84° (*c* 1.00, $CHCl_3$) [lit[27] mp 262–265 C; $[\alpha]_D$ −85° ($CHCl_3$)]; ^1H NMR δ 0.92 (s, 3H, C-18), 1.11 (s, 3H, C-19), 2.54 (s, 1H, C-21), 5.66 (s, 1H, C-4), 6.08 (s, 2H, C-6 and C-7). For the preparation using method (C), **2b** (2.00 g, 6.40 mmol) was suspended in *tert*-butyl alcohol (30 ml). Chloranil (1.73 g, 7.04 mmol) was added, and the mixture was boiled for 18 hours. The solvent was removed at reduced pressure, and the residue was dissolved in sufficient acetone for solution. This solution was passed through a neutral alumina column (50 g, Brockman activity I), using sufficient acetone for complete elution of **3b** from the column. The acetone was evaporated at reduced pressure, and recrystallization of the light brown, solid residue from ethyl acetate gave pure **3b** (0.96 g, 48%).

17α-Iodoethynylandrosta-4,6-dien-17β-ol-3-one (3c)

17α-Ethynylandrosta-4,6-dien-17β-ol-3-one (**3b,** 0.100 g, 0.322 mmol) was suspended in acetone (5 ml), and *N*-iodosuccinimide (0.090 g, 0.40 mmol) and silver nitrate (0.010 g, 0.059 mmol) were added to the stirred suspension. After 1 hour, the reaction mixture was poured into an ice-water mixture (50 ml), and the precipitate was removed by filtration and dissolved in ethyl acetate (50 ml). The ethyl acetate solution was washed with water (3 × 20 ml), dried ($MgSO_4$), and filtered. The ethyl acetate was evaporated at reduced pressure, and recrystallization of the solid residue from acetone/hexane gave pure **3c** (70 mg, 50%): mp 184–186 C; $[\alpha]^{25}_D$ −6° (*c* 0.500, $CHCl_3$); UV (EtOH) max 283 nm (ε 24,000); IR 3,300, 1,640, 1,605 cm^{-1}; ^1H NMR δ 0.91 (s, 3H, C-18), 1.11 (s, 3H, C-19), 5.67 (s, 1H, C-4), 6.09 (s, 2H, C-6 and C-7). Analysis calculated for $C_{21}H_{25}IO_2$: C, 57.80; H, 5.78; I, 29.08. Found: C, 57.72; H, 5.99; I, 28.90. Compound **3c** was also prepared from 17α-iodoethynylandrost-4-en-17β-ol-3-one (**2c**) by dehydrogenation with chloranil in *tert*-butyl alcohol as described above for the preparation of **3b** from **2b**. Recrystallization from

ethyl acetate gave pure **3c** (38%) as pale yellow needles with physical properties identical with those described above for **3c**.

17α-Ethenylandrosta-4,6-dien-17β-ol-3-one (3d)

Compound **3d** was prepared from 17α-ethenylandrost-4-en-17β-ol-3-one **(2d)** by dehydrogenation with chloranil in *tert*-butyl alcohol as outlined above for the preparation of **3b** from **2b**. Crystallization from acetone/hexane gave **3d** (42%) as a white solid: mp 135–138 C; $[\alpha]_D^{21}$ −3° (*c* 1.00, $CHCl_3$); UV max 284 nm (ε 24,000); ^{1}H NMR δ 1.01 (s, 3H, C-18), 1.12 (s, 3H, C-19), 5.12 (dd, 1H, $J_{21,21}$ = 1 Hz and $J_{20,21}$ = 11 Hz, C-21), 5.15 (dd, 1H, $J_{21,21}$ = 1 Hz and $J_{20,21}$ = 18 Hz, C-21), 5.67 (s, 1H, C-4), 6.04 (dd, 1H, $J_{20,21}$ = 11 Hz and $J_{20,21}$ = 18 Hz, C-20), 6.11(s, 2H, C-6 and C-7). Analysis calculated for $C_{21}H_{28}O_2$: C, 80.73; H, 9.03. Found: C, 80.77; H, 8.94.

17α-[(Z)-2-Iodoethenyl]androsta-4,6-dien-17β-ol-3-one (3e)

Compound **3e** was prepared by dehydrogenation of 17α-[(*Z*)-2-iodoethenyl]androst-4-en-17β-ol-3-one **(2e)** with chloranil in *tert*-butyl alcohol as outlined above for the preparation of **3b** from **2b**. Recrystallization from ethyl acetate gave pure **3e** (55%) as a yellow solid: mp 115–117 C dec; $[\alpha]_D^{24}$ −54° (*c* 1.0, C_2H_5OH); UV max 284 nm (ε 24,000), 212 (5800); ^{1}H NMR δ 1.01 (s, 3H, C-18), 1.12 (s, 3H, C-19), 5.66 (s, 1H, C-4), 6.09 (s, 2H, C-6 and C-7), 6.34 (d, 1H, $J_{20,21}$ = 8 Hz, C-20), 6.76 (d, 1H, $J_{20,21}$ = 8 Hz, C-21). Analysis calculated for $C_{21}H_{27}IO_2$: C, 57.54; H, 6.21; I, 28.95. Found: C, 57.27; H, 6.35; I, 28.60.

17α-[(E)-2-Iodoethenyl]androsta-4,6-dien-17β-ol-3-one (3f)

Compound **3f** was prepared by dehydrogenation of 17α-[(*E*)-2-iodoethenyl]androst-4-en-17β-ol-3-one **(2f)** with chloranil in *tert*-butyl alcohol as outlined for the preparation of **3b** from **2b**. Recrystallization from ethyl acetate/hexane gave pure **3f** (54%) as a colorless crystalline solid: mp 126–130 C dec; $[\alpha]_D^{21}$ −128° (*c* 1.00, $CHCl_3$); UV max 284 nm (ε 26,000), 219 (12,000); ^{1}H NMR δ 0.98 (s, 3H, C-18), 1.12 (s, 3H, C-19), 5.68 (s, 1H, C-4), 6.10 (s, 2H, C-6 and C-7), 6.26 (d, 1H, $J_{20,21}$ = 15 Hz, C-20), 6.72 (d, 1H, $J_{20,21}$ = 15 Hz, C-21). Analysis calculated for $C_{21}H_{27}IO_2$: C, 57.54; H, 6.21; I, 28.95. Found: C, 57.65; H, 6.24; I, 28.51, 28.53.

17α-Ethynylandrosta-1,4,6-trien-17β-ol-3-one (4b)

A mixture of 17α-ethynylandrosta-4,6-dien-17β-ol-3-one **(3b;** 0.45 g, 1.5 mmol) and DDQ (0.66 g, 2.9 mmol) in benzene (25 ml) was boiled for 16 hours. The solvent was removed at reduced pressure, and the dark brown solid was dissolved in a minimum of acetone. The acetone solution was passed through a column of neutral alumina (Brockman activity I), using additional acetone to remove **4b** completely from the column. Most of the acetone was removed at reduced pressure, and hexane was added to induce crystallization of the reaction product, which occurred on cooling. Recrystallization of the pale yellow solid from acetone/hexane gave pure **4b** (0.22 g, 48%) as pale yellow needles: mp 231–233 C; $[\alpha]_D^{21}$ −125° (*c* 1.00, $CHCl_3$) [lit[30] mp 233–235 C; $[\alpha]_D^{23}$ −104.2° (*c* 1.0, $CHCl_3$)]; UV max 300 nm (ε 12,000), 256 (8600); ^{1}H NMR δ 0.98 (s, 3H, C-18), 1.21 (s, 3H, C-19), 2.55 (s, 1H, C-21), 5.94-6.31 (4H, a series of doublets, C-2, C-4, C-6, and C-7), 7.08 (d, 1H, $J_{1,2}$ = 10 Hz, C-1).

17α-Iodoethynylandrosta-1,4,6-trien-17β-ol-3-one (4c)

Compound **4c** was prepared by (A) dehydrogenation of 17α-iodoethynylandrosta-4,6-dien-17β-ol-3-one **(3c)** with DDQ in benzene as outlined above for the preparation of **4b** from **3b**. Recrystallization from ethyl acetate gave **4c** (26%) as a white crystalline solid: mp 174–176 C dec; $[\alpha]_D^{21}$ −151° (*c* 1.00, $CHCl_3$); UV max 300 nm (ε 9,400), 254 (7,700); ^{1}H NMR δ 0.96 (s, 3H, C-18), 1.21 (s, 3H, C-19), 5.94–6.26 (series of doublets, 4H, C-2, C-4, C-6, and C-7), 7.08 (d, 1H, $J_{1,2}$ = 10 Hz, C-1). Analysis calculated for $C_{21}H_{23}IO_2$: C, 58.07; H, 5.34; I, 29.22. Found: C, 58.41; H, 5.24; I, 30.72. Compound **4c** was also prepared by (B) iodination of 17α-ethynylandrosta-1,4,6-trien-17β-ol-3-one **(4b)** with *N*-iodosuccinimide and silver nitrate in acetone as described above for the preparation of **3c** from **3b**. Recrystallization from ethyl acetate gave pure **4c** (71%) with physical properties identical to those described above for **4c**.

17α-Ethenylandrosta-1,4,6-trien-17β-ol-3-one (4d)

Compound **4d** was prepared by dehydrogenation of 17α-ethenylandrosta-4,6-dien-17β-ol-3-one **(3d)** with DDQ in benzene as described above for the preparation of **4b** from **3b**. Recrystallization from ethyl acetate/hexane gave pure **4d** (34%) as white needles: mp 161–163 C; $[\alpha]_D^{21}$ −49° (*c* 1.00, $CHCl_3$); UV max 300 nm (ε 12,000), 256 (9,100); ^{1}H NMR δ 1.03 (s, 3H, C-18), 1.20 (s, 3H, C-19), 5.10 (dd, 1H, $J_{21,21}$ = 1 Hz and $J_{20,21}$ = 11 Hz, C-21), 5.14 (dd, 1H, $J_{21,21}$ = 1 Hz and $J_{20,21}$ = 17 Hz, C-21), 6.00 (dd, 1H, $J_{20,21}$ = 10 Hz and $J_{20,21}$ = 17 Hz, C-20), 5.96–6.29 (a series of doublets, 4H, C-2, C-4, C-6, and C-7), 7.05 (d, 1H, $J_{1,2}$ = 10 Hz, C-1). Analysis calculated for $C_{21}H_{26}O_2$: C, 81.25; H, 8.44. Found: C, 80.76; H, 8.51.

17α-[(Z)-2-Iodoethenyl]androsta-1,4,6-trien-17β-ol-3-one (4e)

Compound **4e** was prepared by dehydrogenation of 17α-[(*Z*)-2-iodoethenyl]androsta-4,6-dien-17β-ol-3-one **(3e)** with DDQ in benzene as described above for the preparation of **4b** from **3b**. Recrystallization from ethyl acetate/hexane gave pure **4e** (50%) as yellow needles: mp 122–123 C; $[\alpha]_D^{24}$ −71° (*c* 1.00, C_2H_5OH); UV max 300 nm (ε 11,000), 256 (7,900), 219 (12,000); ^{1}H NMR δ 1.05 (s, 3H, C-18), 1.20 (s, 3H, C-19), 5.92–6.30 (4H, series of doublets, C-2, C-4, C-6, and C-7), 6.33 (d, 1H, $J_{20,21}$ = 9 Hz, C-20), 6.74 (d, 1H, $J_{20,21}$ = 8 Hz, C-21), 7.06 (d, 1H, $J_{1,2}$ = 11 Hz, C-1). Analysis calculated for $C_{21}H_{25}IO_2$: C, 57.81; H, 5.77; I, 29.08. Found: C, 57.73; H, 5.75; I, 29.44.

17α-[(E)-2-Iodoethenyl]androsta-1,4,6-trien-17β-ol-3-one (4f)

Compound **4f** was prepared by dehydrogenation of 17α-[(*E*)-2-iodoethenyl]androsta-4,6-dien-17β-ol-3-one **(3f)** with DDQ in benzene as described above for the preparation of **4b** from **3b**. Recrystallization from hexane/ethyl acetate gave pure **4f** (39%) as white needles: mp 134–164 C dec; $[\alpha]_D^{23}$ −120° (*c* 1.00, $CHCl_3$); UV max 300 nm (ε 14,000), 255 (11,000), 220 (22,000); ^{1}H NMR δ 1.01 (s, 3H, C-18), 1.20 (s, 3H, C-19), 5.93–6.29 (series of doublets and multiplets, 4H, C-2, C-4, C-6, and C-7), 6.26 (d, 1H, $J_{20,21}$ = 14 Hz, C-20), 6.69 (d, 1H, $J_{20,21}$ = 15 Hz, C-21), 7.04 ppm (d, 1H, $J_{1,2}$ = 11 Hz, C-1). Analysis calculated for $C_{21}H_{25}IO_2$: C, 57.81; H, 5.77; I, 29.08. Found: C, 57.85; H, 5.94; I, 29.23.

3,3-Ethylenedioxy-17α-ethynylandrost-5-en-17β-ol (5a)

Ethylene glycol (10 g, 0.16 mmol) and *p*-toluenesulfonic acid (1.2 g, 6.3 mmol) were added to benzene (150 ml), and the mixture boiled

under a Dean-Stark water trap for 2 hours. 17α-Ethynylandrost-4-en-17β-ol-3-one (**2b**; 10.0 g, 32.0 mmol) was added to the cooled solution, and the mixture was boiled again under the water trap for 20 hours. The cooled mixture was then added to 5% aqueous sodium bicarbonate (300 ml), and the benzene was removed by evaporation at reduced pressure. The precipitate was collected by filtration and washed with water. Recrystallization of the solid from 95% ethanol gave pure **5a** (9.10 g, 80%): mp 258–262 C (lit[31] mp 245–250 C); $[\alpha]_D^{21}$ −98° (*c* 1.00, $CHCl_3$); ^1H NMR δ 0.87 (s, 3H, C-18), 1.04 (s, 3H, C-19), 2.55 (s, 1H, C-21), 3.94 (s, 4H, OCH_2CH_2O), 5.34 (m, 1H, C-6).

3,3-Ethylenedioxy-17α-iodoethynylandrost-5-en-17β-ol (5b)

3,3-Ethylenedioxy-17α-ethynylandrost-5-en-17β-ol (**5a**) was converted to **5b** using *N*-iodosuccinimide and silver nitrate in acetone as described above for the preparation of **2c** from **2b**. Recrystallization from acetone/hexane gave pure **5b** (42%): mp 140–142 C; $[\alpha]_D^{24}$ −84° (*c* 0.450, $CHCl_3$) [lit[32] mp 140 C; $[\alpha]_D$ −80° (*c* 0.73, dioxane)]; ^1H NMR δ 0.86 (s, 3H, C-18), 1.04 (s, 3H, C-19), 3.95 (s, 4H, OCH_2CH_2O), 5.37 (m, 1H, C-6). Analysis calculated for $C_{23}H_{31}IO_3$: C, 57.26; H, 6.49; I, 26.31. Found: C, 57.39; H, 6.63; I, 26.09.

3,3-Ethylenedioxy-17α-[(Z)-2-iodoethenyl]androst-5-en-17β-ol (5c)

3,3-Ethylenedioxy-17α-iodoethynylandrost-5-en-17β-ol (**5b**; 4.10 g, 8.50 mmol) was mixed with methanol (100 ml) and stirred for 5 minutes. 2,4,6-Triisopropylbenzenesulfonic acid hydrazide (5.60 g, 18.8 mmol) and triethylamine (1.90 g, 18.8 mmol) were added, and the mixture was stirred for 4 hours at room temperature. Ether (200 ml) was added, and the solution was washed with 1 N sodium hydroxide (2 × 100 ml) and brine (100 ml) and then dried (Na_2SO_4). Evaporation of the solvent left a pale yellow, solid residue. Recrystallization of the latter from 95% ethanol gave pure **5c** (3.00 g, 73%) as white plates: mp 180–182 C; $[\alpha]_D^{23}$ −7° (*c* 1.00, $CHCl_3$); ^1H NMR δ 0.94 (s, 3H, C-18), 1.04 (s, 3H, C-19), 3.94 (s, 4H, OCH_2CH_2O), 5.34 (m, 1H, C-6), 6.32 (d, 1H, $J_{20,21}$ = 8 Hz, C-20), 6.76 (d, 1H, $J_{20,21}$ = 9 Hz, C-21).

3,3-Ethylenedioxy-17α-[(E)-2-(tributyltin(IV))ethenyl]androst-5-en-17β-ol (5d)

Tributyltin(IV)hydride (21.6 g, 74.2 mmol) and α,α′-azobisisobutyronitrile (AIBN, 0.85 g, 5.2 mmol) were added to a stirred solution of 3,3-ethylenedioxy-17α-ethynylandrost-5-en-17β-ol (**5a**; 8.40 g, 23.6 mmol) in dry tetrahydrofuran (150 ml) under nitrogen. The mixture was boiled for 18 hours, and the solvent was removed at reduced pressure. The residual oil was subjected to chromatography on silica gel. Elution with hexane removed unreacted tributyltin(IV)hydride from the silica gel column, whereas elution with hexane/ethyl acetate (8 : 2) removed **5d**. The fractions containing **5d** were combined, and removal of the solvent gave **5d** (14.8 g, 97%) as a pale yellow oil, which was used without further purification for the formation of 3,3-ethylenedioxy-17α-[(*E*)-2-iodoethenyl]androst-4-en-17β-ol (**5e**).

3,3-Ethylenedioxy-17α-[(E)-2-iodoethenyl]androst-4-en-17β-ol (5e)

N-Iodosuccinimide (1.20 g, 5.33 mmol) was added to a stirred solution of 3,3-ethylenedioxy-17α-[(*E*)-2-(tributyltin(IV)ethenyl]androst-5-en-17β-ol (**5d**; 2.60 g, 4.02 mmol) in tetrahydrofuran (50 ml), and the mixture stirred for an additional hour. The solution was diluted with ethyl acetate (200 ml), washed with water (2 × 75 ml), and dried ($MgSO_4$), and the solvent was removed at reduced pressure. Recrystallization of the pale yellow, solid residue from 95% ethanol gave pure **5e** (1.28 g, 66%) as white plates: mp 118–121 C dec; $[\alpha]_D^{23}$ −58° (*c* 1.00, $CHCl_3$); ^1H NMR δ 0.91 (s, 3H, C-18), 1.04 (s, 3H, C-19), 3.94 (s, 4H, OCH_2CH_2O), 5.34 (m, 1H, C-6), 6.23 (d, 1H, $J_{20,21}$ = 15 Hz, C-20), 6.7 (d, 1H, *J* = 15 Hz, C-21).

Biological testing

The relative binding affinities (RBAs) of the androgens shown in Table 1 were determined as previously described.[1] Briefly, competition studies were conducted in which fixed amounts of rat epididymal cytosol containing ABP were incubated with 4 nM [^{3}H]5α-DHT (40-60 Ci/mmol) and various concentrations of unlabeled 5α-DHT or test compound. After binding equilibrium had been achieved, the unbound [^{3}H]5α-DHT was separated from bound [^{3}H]5α-DHT using a charcoal absorption procedure.[1] The supernatant fluids containing [^{3}H]5α-DHT bound to ABP were counted in a liquid scintillation spectrometer. The RBAs were calculated by dividing the concentration of 5α-DHT that caused a 50% inhibition of binding of [^{3}H]5α-DHT to ABP by the concentration of the test androgen that also caused a 50% inhibition of the binding of [^{3}H]5α-DHT to ABP. The quotients were then multiplied by 100.

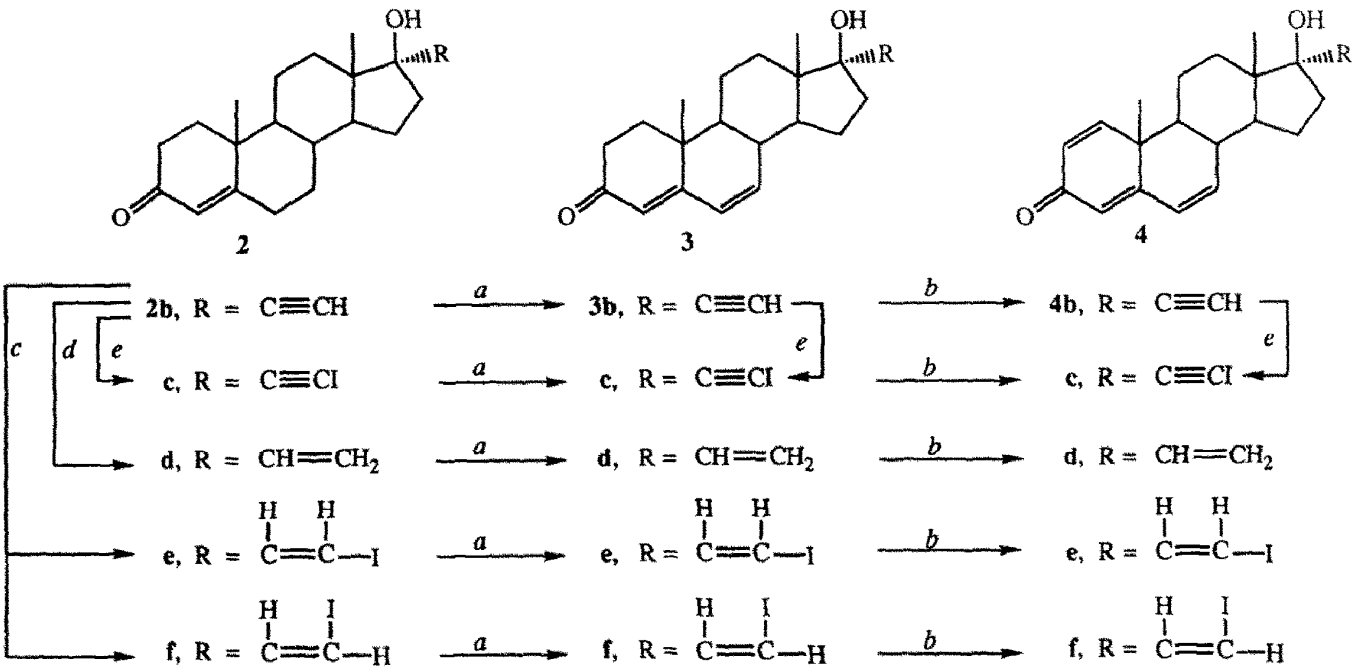
Results and discussion

Synthesis

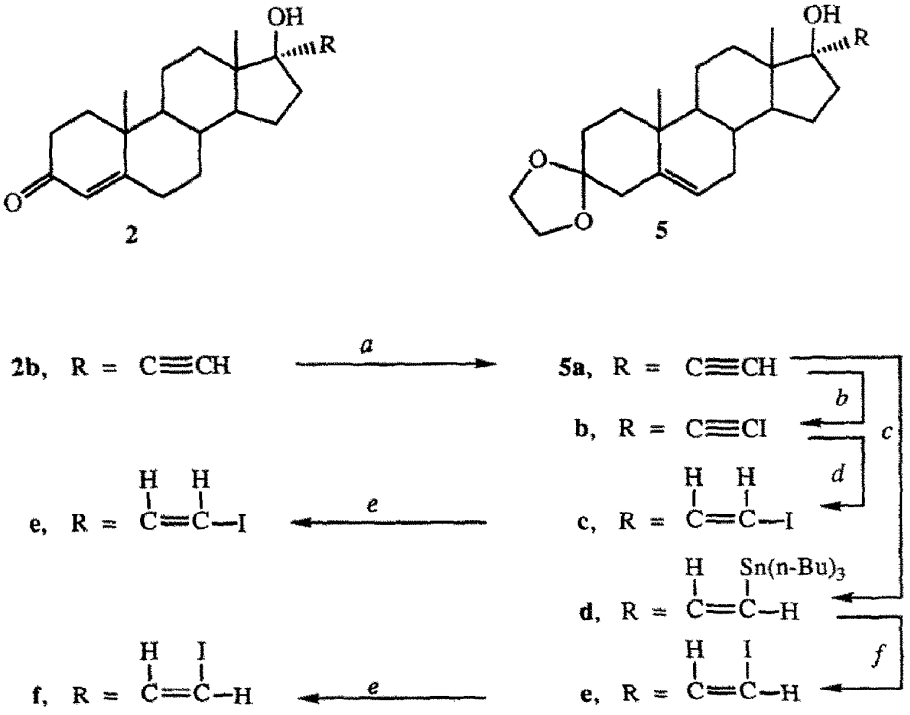
As shown in Scheme 1, the starting substance in all preparations was ethisterone (**2b**, 17α-ethynylandrost-4-en-17β-ol-3-one), which on oxidation (dehydrogenation) with 2,3,5,6-tetrachloro-1,4-benzoquinone (chloranil) gave 17α-ethynylandrosta-4,6-dien-17β-ol-3-one (**3b**). Treatment of the latter with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) resulted in further dehydrogenation and gave 17α-ethynylandrosta-1,4,6-trien-17β-ol-3-one (**4b**). Treatment of **2b**, **3b**, and **4b** in acetone with *N*-iodosuccinimide and silver nitrate as the catalyst gave the 17α-iodoethynyl analogues **2c**, **3c**, and **4c**. Compounds **3c** and **4c** were also prepared by dehydrogenation of **2c** to give **3c** and then dehydrogenation of **3c** to give **4c**.

Reaction of ethisterone (**2b**) with hydrogen using Lindlar's catalyst (platinum oxide poisoned with lead acetate) resulted in the reduction of the ethynyl moiety and formation of 17α-ethenylandrost-4-en-17β-ol-3-one (**2d**). The latter was also oxidized by dehydrogenation with chloranil to give 17α-ethenylandrosta-4,6-dien-17β-ol-3-one (**3d**), which was further dehydrogenated with DDQ to 17α-ethenylandrosta-1,4,6-trien-17β-ol-3-one (**4d**).

Preparation of the 17α-(2-iodoethenyl) analogues **2e,f**, **3e,f**, and **4e,f** also proceeded from ethisterone (**2b**) by way of its 3,3-ethylenedioxy derivative, 3,3 ethylenedioxy-17α-ethynylandrost-5-en-17β-ol (**5a**) (Scheme 2). Compound **5a** was converted to 3,3-ethylenedioxy-17α-iodoethynylandrost-5-en-17β-ol-3-one (**5b**) using *N*-iodosuccinimide and silver nitrate in acetone. Reduction of **5b** with 2,4,6-triisopropyl benzenesulfonic acid hydrazide and triethylamine gave 3,3-ethylenedioxy-17α-[(*Z*)-2-iodoethenyl]androst-5-en-17β-ol (**5c**). In this latter reaction, masking of the 3-oxo group as an ethylenedioxy moiety was necessary to prevent formation of the corresponding hydrazone. Hydrolysis of **5c** gave 17α-[(*Z*)-2-iodoethenyl]androst-4-en-17β-ol-3-one (**2e**), and dehydrogenation of **2e** with chloranil (Scheme 1) gave 17α-[(*Z*)-2-iodoethenyl]an-



Scheme 1 Reagent (yield): *a*, chloranil, *tert*-butyl alcohol (38–55%); *b*, DDQ, benzene (26–49%); *c*, see Scheme 2; *d*, H_2, Lindlar's catalyst, pyridine (93%); *e*, *N*-iodosuccinimide, $AgNO_3$, acetone (50–74%).



Scheme 2 Reagent (yield): *a*, ethylene glycol, *p*-toluenesulfonic acid, benzene (80%); *b*, *N*-iodosuccinimide, $AgNO_3$, acetone (42%); *c*, tributyltin(IV)hydride, AIBN, THF (98%); *d*, 2,4,6-triisopropylphenylsulfonylhydrazide, triethylamine, methanol (73%); *e*, oxalic acid, methanol (76–79%); *f*, *N*-iodosuccinimide, THF (66%).

drosta-4,6-dien-17β-ol-3-one **(3e)** and then with DDQ gave 17α-[(*Z*)-2-iodoethenyl]androsta-1,4,6-trien-17β-ol-3-one **(4e)**.

Treatment of 3,3-ethylenedioxy-17α-ethynylandrost-5-en-17β-ol **(5a)** with tributyltin(IV)hydride and α,α′-azobisisobutyronitrile (AIBN) (Scheme 2) gave 3,3-ethylenedioxy-17α-[(*E*)-2-(tributyltin(IV))ethenyl]-androst-5-en-17β-ol **(5d)**, which on reaction with *N*-iodosuccinimide in tetrahydrofuran gave 3,3-ethylenedioxy-17 α-[(*E*)-2-iodoethenyl]androst-5-en-17β-ol **(5e)**. Hydrolysis of **5e** gave 17α-[(*E*)-2-iodoethenyl]-androst-4-en-17β-ol-3-one **(2f)**.

Binding studies

The RBA to rat ABP from rat epididymides of the Δ^4-, $\Delta^{4,6}$-, and $\Delta^{1,4,6}$-androgens incorporating a 17α substituent as well as their analogues without this moiety are compared in Table 1 to the RBA of 5α-DHT **(1)**. As seen in Table 1, unsaturation of the A and B rings of 5α-DHT decreases the respective RBA as compared with 5α-DHT, but the 17α-iodoethynyl and 17α-[(*E*)-2-iodoethenyl] derivatives **3c, 3f, 4c,** and **4f** of Δ^6- and $\Delta^{1,6}$-testosterone **(3a** and **4a)** have RBAs at least twice as great as that of 5α-DHT and make these iodinated androgens potential radioiodinated active site–directed photoaffinity ligands for ABP and other androgen-binding proteins (ABPs).

Additional experiments indicate that the RBAs in Table 1 represent reversible binding to ABP rather than irreversible binding as a result of covalent bond formation due to displacement of the iodine atom by some nucleophilic moiety in the binding site of ABP. Excess of the iodinated steroids **3c, 3f,** and **4f** were incubated for about 3 hours with rat cytosol containing a known number of ABP binding sites. After the iodinated ligands were removed by a 1-hour charcoal extraction, the binding activity of the cytosol for [^{3}H]5α-DHT was not diminished.

Because **3c** and **4c** can be prepared in one synthetic step by the silver nitrate–catalyzed reaction of *N*-iodosuccinimide with **3b** and **4b,** it is anticipated that [^{125}I]**3c** and [^{125}I]**4c** can be prepared in high radiochemical yield from **3b** and **4b** and [^{125}I]*N*-iodosuccinimide, the latter prepared from sodium iodide[^{125}I].[33] Since the tributyltin(IV) cyclic ketal **5d** also gives on reaction with *N*-iodosuccinimide, the iodinated cyclic ketal **5e** that can be easily hydrolyzed in situ to **2f,** a similar procedure using the cyclic ketal of **3a** and [^{125}I]*N*-iodosuccinimide would afford a one-pot synthetic scheme for the formation of 17α-[(*E*)-2-[^{125}I]iodoethenyl]androsta-4,6-dien-17β-ol-3-one ([^{125}I]**3f**). Work is now in progress for the preparation of [^{125}I]**3c,** [^{125}I]**4c,** and [^{125}I]**3f** and for their evaluation as active site–directed radioiodinated photoaffinity ligands for ABP and other ABPs.

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Names and abbreviations

5α-DHT	5α-dihydrotestosterone
RBA	relative binding affinity (5α-DHT = 100%)
ABP	androgen-binding protein
TeBG	testosterone-binding globulin
Δ^6	double bond from C-6 to C-7
$\Delta^{1,6}$	double bond from C-1 to C-2 and from C-6 to C-7
$\Delta^{1,4,6}$	double bond from C-1 to C-2, from C-4 to C-5, and from C-6 to C-7
UV	ultraviolet
^{1}H NMR	proton nuclear magnetic resonance
IR	infrared
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
AIBN	α,α′-azobisisobutyronitrile
THF	tetrahydrofuran

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