SYNTHESIS OF 17α-[(E)-2-[125I]IODOETHENYL]ANDROSTA-4,6-DIEN-17β-0L-3-ONE,

AN ACTIVE-SITE-DIRECTED PHOTOAFFINITY RADIOLABEL FOR ANDROGEN-

BINDING PROTEINS

Pablo J. Diaz Cruz and Howard E. Smith*,
Department of Chemistry and
Center for Reproductive Biology Research,
Vanderbilt University,
Nashville, Tennessee 37235, U.S.A.,

Benjamin J. Danzo,
Departments of Obstetrics & Gynecology and Biochemistry and
Center for Reproductive Biology Research,
Vanderbilt University School of Medicine,
Nashville, Tennessee 37232, U.S.A.

and

Jeffrey A. Clanton and N. Scott Mason,
Department of Radiology and Radiological Sciences,
Vanderbilt University School of Medicine,
Nashville, Tennessee 37232, U.S.A.

SUMMARY

The active-site-directed photoaffinity radiolabel for androgen-binding proteins, 17α-[(E)-2-[125I]iodoethenyl]androsta-4,6-dien-17β-ol-3-one, was prepared by reaction of 17α-[(E)-2-tributyltin(IV)ethenyl]androsta-4,6-dien-17β-ol-3-one with carrier added sodium iodide-125 in the presence of hydrogen peroxide and acetic acid. Purification by HPLC gave the radiolabeled steroid in 52% radiochemical yield with a specific activity of 27 Ci/mmol and 100% radiochemical purity.

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*To whom communications concerning this paper should be sent.

INTRODUCTION

Androgen-binding protein (ABP) (1-4) and sex hormone-binding globulin (SHBG) (5,6) are extracellular glycoproteins present in several species, including humans, and function as transport proteins for the androgens, 5α-androstan-17β-ol-3-one (**1**, 5α-dihydrotestosterone, 5α-DHT) and androst-4-en-17β-ol-3-one (**2**, testosterone) (7-10), and are also considered to be involved in receptor mediated processes. ABP is a product of the Sertoli cells of the testis and is primarily confined to the testis and epididymis (1,4), whereas SHBG is produced by the liver (11) and is found solely in the blood (6,12,13). Both proteins bind their natural ligands, 5α-DHT and testosterone, with high affinity and specificity, but their interaction is reversible with a dissociation half-time of approximately five minutes (4,6).
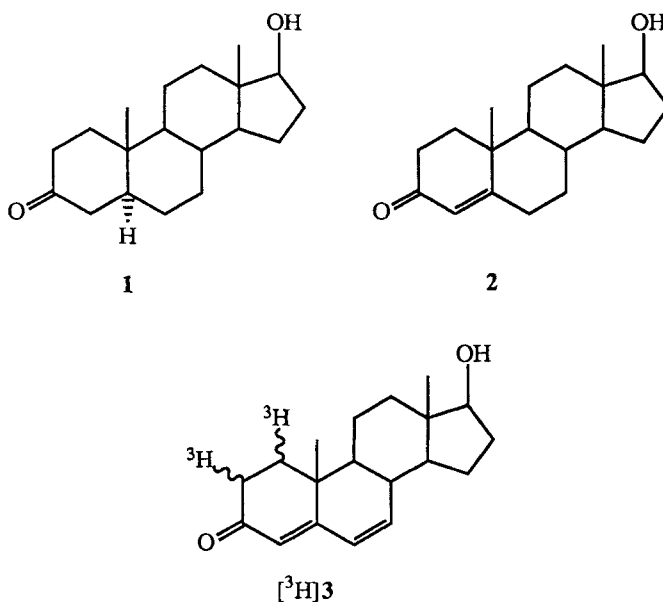

As an alternate to the reversible radiolabeling of ABP with [^{3}H]5α-dihydrotestosterone ([^{3}H]**1**), [1ξ, 2ξ-^{3}H$_2$]androsta-4,6-dien-17β-ol-3-one ([^{3}H]**3**, [^{3}H]Δ^6-testosterone) was prepared (14) as an irreversible, active-site-directed photoaffinity ligand for ABP. Subsequently, [^{3}H]**3** was used for the study of the physicochemical properties of ABP (15-17) and SHBG (18), and more recently, it functioned as a probe for the determination of the amino acid sequence of the steroid binding domain of ABP (19).

The ability of [^{3}H]**3** to serve as a photoaffinity radiolabel resides in the excitation of the dienone system to an excited singlet state which then undergoes intersystem crossing to a triplet

state. The triplet state is then reduced by a process which involves initial hydrogen abstraction from the protein by the steroid radical and combination of the resultant steroid-protein radical pair to give a covalent bond (20). Although, 5α-DHT and testosterone are the endogeneous ligands for the androgen-binding proteins, they can not function as photoaffinity reagents for these proteins in cytosol. The cytosol has an absorption band centered at 270 nm with a long tail extending beyond 300 nm, whereas 5α-DHT and testosterone have n$\rightarrow$π* carbonyl group transitions centered at 280 (ε_{max} 25) and 305 nm (ε_{max} 100), respectively. The C-6 double bond in 3 is necessary to shift the absorption maximum of the photoaffinity reagent outside the cytosol absorption envelope. Thus the extended conjugation of Δ^6-testosterone results in a carbonyl absorption band centered at 345 nm (ε_{max} 300). This absorption is beyond the absorption band of cytosol and the cut-off of the Pyrex filter to allow photoactivation of the unsaturated carbonyl group and subsequent covalent bond formation with the protein.

Although [³H]3 has had substantial use for the study of the physical and physiological properties of androgen binding proteins, the incorporation of iodine-125 into a Δ^6-testosterone analogue will impart a much greater specific radioactivity than can be obtained with tritium-labeled compounds. Iodine-125 is a gamma emitting radionuclide containing high specific activity and its gamma emission can be directly detected. Thus, an iodine-[¹²⁵I]-labeled probe would be of great utility in studies geared to examination of the in vivo tissue uptake of androgen-binding proteins.

Recently, the synthesis of 17α-[¹²⁵I]iodoethynylandrosta-4,6-dien-17β-ol-3-one ([¹²⁵I]4)

$[^{125}I]4$ $[^{125}I]5$
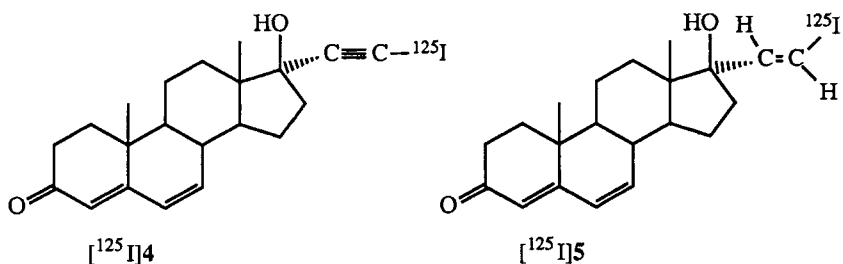
was reported (21) as an active-site-directed photoaffinity radiolabel for ABP, unlabeled 4 having a binding affinity for ABP twice as that of 5α-DHT, relative binding affinities of 200 and 100, respectively (22). Preliminary experiments indicated that [¹²⁵I]4 is able to bind covalently to ABP upon irradiation with UV light and to inactivate the protein binding sites, but [¹²⁵I]4 and the protein-[¹²⁵I]4 complex are unstable to electrophoresis conditions where the presence of reducing

agents such as β-mercaptoethanol and free thiol groups from reduced cystine residues are present (23). The decomposition of [125I]4 and its protein complex is attributed to the nucleophilic displacement of iodide by thiol groups, a similar mechanism attributed to the *in vivo* and *in vitro* deiodination of 17α-iodoethynylestradiol (24). The decomposition of [125I]4 and its protein complex in the presence of β-mercaptoethanol makes the utility of [125I]4 very limited.

We now report the synthesis of 17α-[(*E*)-2-[125I]iodoethenyl]androsta-4,6-dien-17β–ol-3-one ([125I]5) as an active-site-directed photoaffinity radiolabel for androgen-binding proteins. The iodovinyl group in the 17α position has been demonstrated to be chemically and metabolically stable under both *in vitro* and *in vivo* conditions (25,26), and relative binding affinity studies of unlabeled 5 with ABP indicated that 5 binds to ABP approximately 4 times better than does 5α-DHT, relative binding affinities of 390 and 100, respectively (22). Steroids containing an iodovinyl group in the 17α position have been reported for estrogen (27-30), progesterone (31), and androgen receptors (32), but none of these iodinated steroids can function as a photoaffinity ligand in that none contains a conjugated dienone moiety.

RESULTS AND DISCUSSION

Unlabeled 17α-[(*E*)-2-iodoethenyl]androsta-4,6-dien-17β-ol-3-one can be prepared as previously described (22), but for the radiosynthesis of [125I]4 a different approach is necessary for the introduction of iodine-125 during the last synthetic step. As shown in Scheme I, commercially available 17α-ethynylandrost-4-en-17β-ol-3-one (6, ethisterone) was dehydrogenated with chloranil to give 17α-ethynylandrosta-4,6-dien-17β-ol-3-one (7). Treatment of 7 with ethylene glycol in the presence of a catalytic amount of *p*-toluenesulfonic acid in benzene gave 3,3-ethylenedioxy-17α-ethynylandrosta-4,6-dien-17β-ol (8) (33). Compound 8 was unstable in protic polar solvents (ethanol, methanol) or when left exposed to air for a few days, it being hydrolized easily to the dienone 7. The presence of the ketal group of 8 is necessary for the stereoselective reaction of 8 with tri-*n*-butyltin(IV) hydride in the presence of α,α'-azobisisobutyronitrile (AIBN) in tetrahydrofuran giving 3,3-ethylenedioxy-17α–[(*E*)-2-tributyltin(IV)ethenyl]androsta-4,6-dien-17β-ol (9) without formation of the Z diastereomer (34). Similar reactions involving tri-*n*-butyltin(IV) hydride and other 17α-ethynylsteroids using other solvents gave a mixture of the *E* and Z stannylvinyl isomers (30,31,35). Hydrolysis of 9 in an ethanol-water mixture gave 17α-[(*E*)-2-tributyltin(IV)ethenyl]androsta-4,6-dien-17β-ol-3-one (10), the precursor for the synthesis of [125I]5 using sodium iodide-125.

SCHEME I
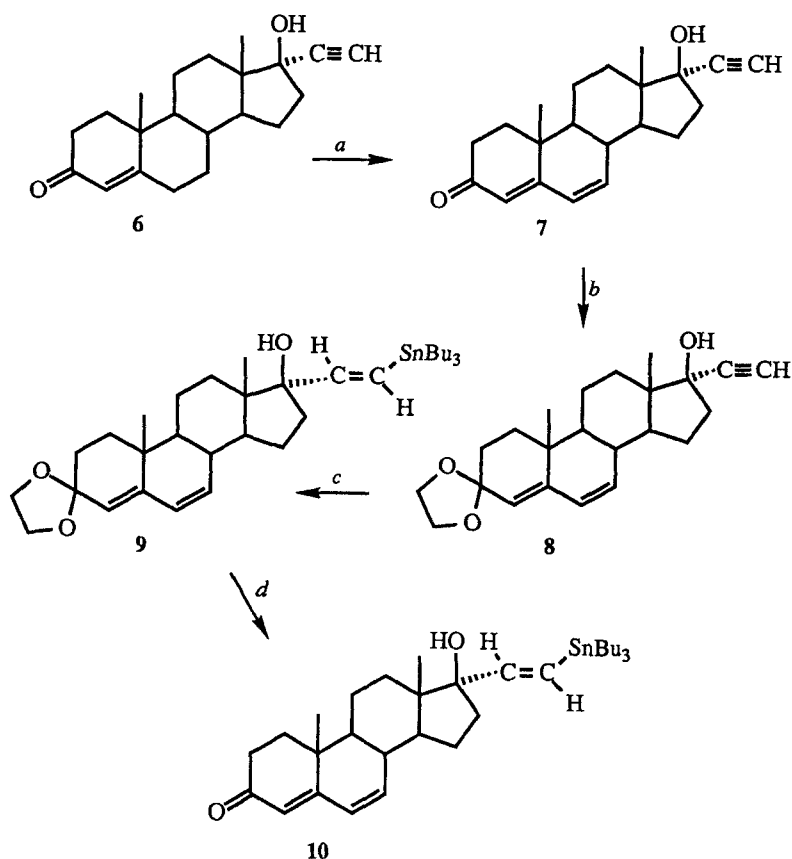
Reagent (yield): *a*, chloranil, *tert*-butyl alcohol (49%); *b*, ethylene glycol, benzene, *p*-toluenesulfonic acid (62%); *c*, tri-*n*-butyltin(IV) hydride, AIBN, THF (89%); *d*, ethanol, water (81%).

The synthesis of [^{125}I]5 (Scheme II) was accomplished using the method reported by Hanson and Franke (29) with slight modifications. Thus, treatment of an excess of **10** with sodium iodide-125 of specific activity of 27 Ci/mmol in a sodium acetate-acetic acid buffered solution (5% sodium acetate (w/w)) and a solution of 30% hydrogen peroxide in glacial acetic acid (2:1, v/v) formed [^{125}I]5.

Purification by HPLC gave [^{125}I]5 in 52% radiochemical yield and 100% radiochemical purity. Only one radioactive peak was observed in the radiochromatogram (Figure 1) coinciding with the retention time (11.1 min) of an authentic sample of **5**. The addition of unlabeled sodium iodide to sodium iodide-125 to form carrier added 81:1.0 sodium iodide-125 was necessary for visualization of [^{125}I]5 on TLC plates and in HPLC systems. The same experimental procedure

SCHEME II

10           $[^{125}I]5$
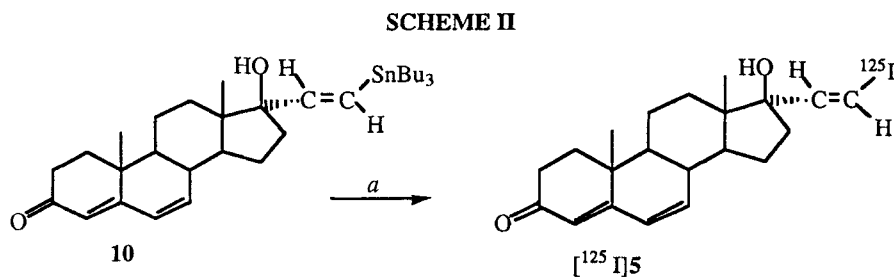
Reagent (yield): *a*, $Na^{125}I$, H_2O_2, NaOAc, AcOH, THF (52% radiochemical yield).
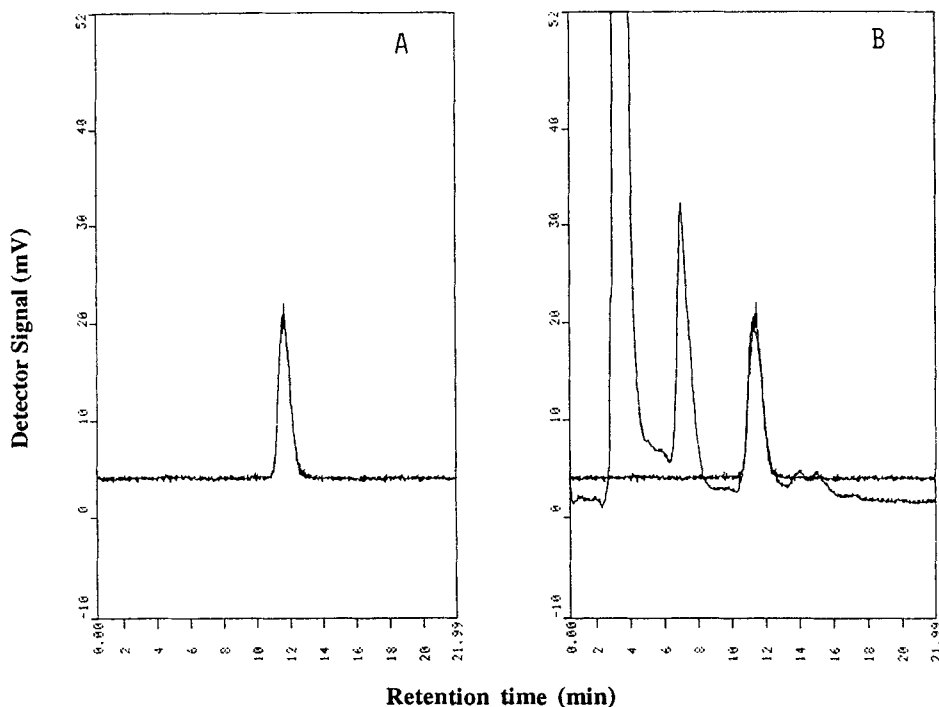

Retention time (min)

Figure 1. HPLC of $[^{125}]5$: (panel A) radioactivity trace of the eluant and (panel B) UV absorption of the eluant at 235 nm overlayed on its radioactivity trace.

outline below, however, can be used also for the formation of carrier free $[^{125}I]5$ when high specific radioactivity is required for biological studies. Preliminary experiment using chloramine T as the oxidation agent gave a mixture of radioactive compounds.

EXPERIMENTAL SECTION

Melting points were determined in open capillary tubes and are corrected. Optical rotatory powers were obtained with a Rudolph Autopol III automatic polarimeter and a 1-dm sample tube.

Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained in chloroform-*d* using a Bruker spectrometer operating at 300 MHz with tetramethylsilane as the internal standard. Chemical shifts (δ) are reported in part per million (ppm) downfield from the standard. The ultraviolet (UV) absorption spectrum was measured in methanol using matched 1-cm cells with a Cary 2390 spectrometer operating in auto gain mode.

Reverse phase high performance liquid chromatography (HPLC) was done with a system consisting of a Kontron 420 pump and Kontron 432 scanning UV detector (wavelength 235 nm) in series with a NaI (Tl) gamma scintillation detector (Ortec 276) along with a Dynamax-60A C-18 reversed phase column. The eluting solvent was 60% aqueous ethanol with a flow rate of 1.0 mL/min. Thin layer chromatography (TLC) was done with Whatman K6F silica gel 60A plates (5 x 10 cm, 250 µm thick) using reagent grade solvents as eluants.

17α-[(E)-2-[^{125}I]iodoethenyl]androsta-4,6-dien-17β-ol-3-one ([^{125}I]5). To a magnetically stirred aqueous solution of sodium iodide (10 µL, 3.34 mM, 33 nmol) in a 500-µL Reacti-Vial were added sodium iodide-125 (20 µL, 900 µCi, 2200 Ci/mmol, 0.41 nmol, New England Nuclear, high concentration in 0.1 M NaOH), sodium acetate in glacial acetic acid (10 µL, 5% (w/w)), and 17α-[(E)-2-tributyltin(IV)ethenyl]androsta-4,6-dien-17β-ol-3-one (**10**) in THF (10 µL, 33.3 mM, 0.33 µmol). To this mixture, 30% H_2O_2 in glacial acetic acid (2:1 v/v) (10 µL), prepared no longer than 24 hours prior to use, was added, and the reaction vessel was sealed with a Teflon-lined cap. The mixture was stirred at room temperature for 1 h, and sodium metabisulfite (20 µL, 0.53 M, 11 µmol) was added. The total reaction mixture was injected into the HPLC column. Elution with ethanol-water (60:40, v/v) showed the radioactive compound eluting at 11.1 minutes. Collection of the radioactive peak between 10 and 12 min gave pure [^{125}I]5 (471 µCi, radiochemical yield 52%, specific radioactivity 27 Ci/mmol) in 2 ml of solvent. A small sample of this solution (2 µL) was subjected to TLC using hexane-ethyl acetate (7:3) as the eluant. The location of the radioactive spot which accounted for 100% of the radioactivity on the plate coincided with that occupied by an authentic sample of **5** (22) (R_f = 0.26) as visualized by ultraviolet light.

17α-Ethynylandrost-4-en-17β-ol-3-one (6) was purchased from Sigma and was used without further purification.

17α-Ethynylandrosta-4,6-dien-17β-ol-3-one (7) was prepared as reported earlier (22) and had mp 260-262 °C, $[\alpha]^{24}_D$ -84° (*c* 1.00, CHCl$_3$), and an ^{1}H NMR spectrum identical with that reported earlier (22).

3,3-Ethylenedioxy-17α-ethynylandrosta-4,6-dien-17β-ol (8). 17α-Ethynylandrosta-4,6-dien-17β-ol-3-one (1.20 g, 3.87 mmol), ethylene glycol (25.0 g, 403 mmol), p-toluenesulfonic acid monohydrate (0.060 g, 0.32 mmol), and benzene (200 mL) were placed in a flamed dried, 500-mL round bottom flask equipped with a Dean-Stark trap and a reflux condenser. The magnetically stirred mixture was boiled for 18 h. After cooling, the purple mixture was poured into aqueous 5% Na$_2$CO$_3$ (300 mL). The aqueous layer was separated and extracted with ether (2 x 100 mL). The benzene layer and the ether extracts were combined, washed with water (1 x 150 mL) and dried (Na$_2$SO$_4$). The solvent was removed under reduced pressure, and the yellow residue was washed with a minimum amount of acetone to remove most of the yellow color. The light yellow solid was recrystallized from acetone (2 x) to give **8** (0.85 g, 62%) as white needles: mp 243-246 °C; $[\alpha]^{23}{}_D$ -9° (c 1.0, CHCl$_3$); ^{1}H NMR δ 0.89 (s, 3H, C-18), 0.97 (s, 3H, C-19), 2.52 (s, 1H, C-21), 3.85-4.00 (m, 4H, OCH$_2$CH$_2$O), 5.24 (s, 1H, C-4), 5.65 (d, 1H, J = 9.8 Hz, C-6), 5.91 ppm (dd, 1H, J = 2.2 and 9.8 Hz, C-7); too unstable for successful combustion analysis.

3,3-Ethylenedioxy-17α-[(E)-2-tributyltin(IV)ethenyl]androsta-4,6-dien-17β-ol (9). Tri-n-butyltin(IV) hydride (2.16 g, 7.42 mmol) and α,α'-azobisisobutyronitrile (AIBN) (0.080 g, 0.49 mmol) were added to a magnetically stirred solution of 3,3-ethylenedioxy-17α-ethenylandrosta-4,6-dien-17β-ol (0.830 g, 2.34 mmol) in dry tetrahydrofuran (15 mL) under nitrogen. The mixture was boiled for 16 h, and the solvent was removed at reduced pressure. The residual oil was dissolved in hexane and was subjected to chromatography on silica gel (100 g). Elution with hexane removed unreacted tri-n-butyltin(IV) hydride from the column while elution with hexane-ethyl acetate (9:1), followed by hexane-ethyl acetate (9:2) removed **9**. The fractions containing the steroid as detected by TLC were combined, and removal of the solvent gave **9** (1.35 g, 89%) as a colorless oil: UV (methanol) λ $_{max}$ 238 nm (ε 20,000); ^{1}H NMR δ 3.85-4.02 (m, 4H, OCH$_2$CH$_2$O), 5.23 (s, 1H, C-4), 5.68 (d, 1H, J = 9.7 Hz, C-6) 5.91 (dd, 1H, J = 2.4 and 9.8 Hz, C-7), 6.00 (d, 1H, J = 19.4 Hz, C-21), 6.09 ppm (d, 1H, J = 19.3 Hz, C-20).

17α-[(E)-2-Tributyltin(IV)ethenyl]androsta-4,6-dien-17β-ol-3-one (10) was prepared by boiling 3,3-ethylenedioxy-17α-[(E)-2-tributyltin(IV)ethenyl]androsta-4,6-dien-17β-ol (1.35 g, 2.09 mmol) in ethanol-water (5:1, 50 mL) for 2 h. The solvent was removed at reduced pressure, and the residue was dissolved in ethyl acetate (35 mL). The ethyl acetate solution was washed with 0.1 N NaOH (25 mL), water (25 mL), and then dried (NaSO$_4$). Evaporation of the solvent gave **10** (1.02 g, 81%) as a pale yellow oil: $[\alpha]^{22}{}_D$ -71° (c 1.00, CHCl$_3$); ^{1}H NMR δ 0.98 (s, 3H, C-18), 1.10 (s, 3H, C-19), 5.65 (s, 1H, C-4), 6.01 (d, 1H, J = 19.4 Hz, C-21), 6.10 (s, 2H, C-6 and C-7), 6.10 ppm (d, 1H, J = 19.4 Hz, C-20).

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