A Potential Radioiodinated Ligand for Androgen Receptor: 7α-Methyl-17α-(2'-(*E*)-iodovinyl)-19-nortestosterone

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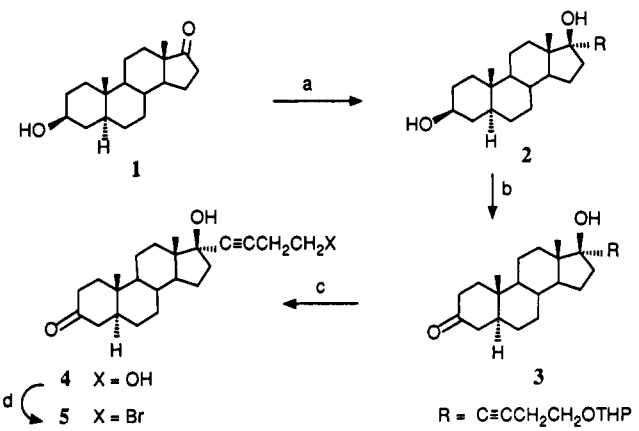
The presence of androgen receptor (AR) in prostate cancer has been linked to the androgen-dependent nature of the tumor and has also been shown to have prognostic significance; it also appears to be a positive prognostic indicator in breast cancer. However, due to the relatively low AR concentrations in most tumors and the inherently low specific activity of tritium, the assay of AR based on available ^{3}H-ligands is not sensitive enough to measure accurately the amount of receptor in small specimens. A ^{125}I-ligand like those available for the estrogen and progesterone receptors would be helpful, but development of such a ligand for AR has not been very successful. Although several androgen analogues containing iodine, bromine, or selenium have been synthesized specifically as potential probes for AR, none have shown any significant affinity or specificity for the receptor. We therefore undertook the synthesis of new potential AR ligands which could be radioiodinated, and determined their affinities for AR (from rat uterus and MCF-7 human breast cancer cells) by using a competition assay. We have examined both 5α-dihydrotestosterone (5α-DHT) and 19-nortestosterone analogues and have identified two such compounds which showed high AR affinity: (17α,20*E*)-17β-hydroxy-21-iodo-5α-pregn-20-en-3-one (17α-((*E*)-iodovinyl)-5α-DHT, **9**) and 17β-hydroxy-7α-methyl-(17α,20*E*)-21-iodo-19-norpregna-4,20-dien-3-one (7α-methyl-17α-((*E*)-iodovinyl)-19-nortestosterone, **11**). In fact, the affinity of the latter for human AR was found to be superior to that of 5α-DHT itself. These iodovinyl analogues could be easily prepared in the radioiodinated form, and should prove to be extremely useful in assaying low levels of AR in small specimens.

Introduction

The androgen receptor (AR) plays a critical role in male sexual differentiation and also in regulating a wide range of physiological processes during postnatal life. The androgen-dependent nature of prostate cancer has long been known, and the presence of AR is believed to have prognostic significance.[1] More recently, the significance of AR levels in breast cancer has also been investigated[2,3] with the finding that AR-positive patients have a longer survival and better response rates toward hormonal therapy than AR-negative patients. The detailed study of AR has lagged behind that of other sex steroid receptors, e.g. estrogen and progesterone receptors (ER and PgR), due in part to the relatively low levels of AR present in most target tissues, and also to the relatively low stability of this receptor. The AR assay based on available ^{3}H ligands such as 5α-dihydrotestosterone (5α-DHT), methyltrienolone (R1881), and mibolerone is not sensitive enough to measure accurately the amount of receptor in small specimens due to the relatively low specific activity of tritium. Moreover, all these available ligands have one or more disadvantages associated: 5α-DHT has substantial affinity for plasma proteins,[4] R1881 has affinity for the glucocorticoid and progesterone receptors, decomposes upon exposure to light, adsorbs to glass, and has high nonspecific binding,[4] while mibolerone has substantial affinity for PgR.[4] A specific high-affinity AR ligand labeled with high specific activity radioiodine or some other radionuclide would circumvent these problems. However, although several androgen analogues containing iodine, bromine, fluorine, or selenium have been synthesized specifically as probes for the AR,[5-10] none have shown any significant affinity or specificity for the receptor. We therefore undertook synthesis of several halogen-containing androgen analogues which could be prepared in radioiodinated form and determined their affinity and specificity for AR using a competition assay.

Scheme I[a]



[a] (a) LiC≡CCH$_2$CH$_2$OTHP; (b) CrO_3, pyridine; (c) *p*-toluenesulfonic acid, H_2O, EtOH; (d) Ph_3P, CBr_4.

Results and Discussion

Chemistry. Substitution at the 16α or 17α position in the D ring of the steroid nucleus has been a practical and effective approach for the development of high-affinity radiolabeled probes for estrogen and progesterone receptors (ER and PgR). Thus, 16α-[^{125}I]iodoestradiol[11,12] has

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(10) Brandes, S. J.; Katzenellenbogen, J. A. *Mol. Pharmacol.* **1987**, *32*, 391–403.
(11) For the sake of simplicity, the following general nomenclature has been used in the present discussion: estr-1,3,5(10)-trien-3,17β-diol is referred to as estradiol; 17β-hydroxy-4-estren-3-one is referred to as 19-nortestosterone; and 17β-hydroxy-5α-androstan-3-one is referred to as 5α-dihydrotestosterone (5α-DHT).

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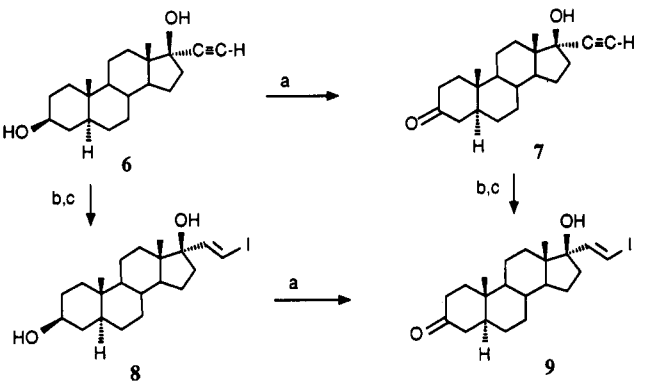
Scheme II[a]



[a] (a) *N*-Methylmorpholine *N*-oxide, $(n\text{-}C_3H_4)_4RuO_4$, molecular sieves; (b) nBu$_3$SnH, AIBN, benzene (dry); (c) iodine.

been a widely used radioiodinated probe for ER,[13] while 17α-((E)-[^{125}I]iodovinyl)-19-nortestosterone and 17α-(6′-[^{125}I]iodohex-1′-ynyl)-19-nortestosterone have been successfully used for assaying PgR.[14,15] Unfortunately, however, development of a radioiodinated probe for AR has not been so successful; 17β-hydroxy-16α-iodo-5α-androstanol-3-one, prepared by analogy to the corresponding estrogenic ligand, did not show any significant activity toward AR.[5] We therefore undertook synthesis of potential halogenated androgen ligands which could be radioiodinated, including both 5α-DHT and 19-nortestosterone analogues.

5α-DHT Analogues. Earlier,[16] we synthesized 17α-(2′,3′-epoxypropyl)-5α-DHT as a precursor for the development of reagents for affinity purification of AR and showed that these compounds, although they have resonable affinity for the AR, do not bind significantly to PgR as 17α-substituted-4-androstene analogues tend to do. On this basis of this and by analogy to our work on radioiodinated ligands for PgR,[15] we first undertook synthesis of 17α-(ω-halo-substituted) analogues of 5α-DHT as possible radioiodinated ligands for AR. We chose long (four carbon) bromoalkynyl and short (two carbon) iodovinyl 17α side chains as precursors for radioiodinated AR ligands. 17α-(4′-bromobut-1′-ynyl)-5α-DHT (**5**) and 17α-((*E*)-iodovinyl)-5α-DHT (**9**) were thus synthesized (Schemes I and II) as described below.

For the short two-carbon iodovinyl 5α-DHT, we chose to synthesize only the *E* isomer because it could be easily prepared stereospecifically while the preparation of *Z* iodovinyl could produce a mixture of *E* and *Z* isomer, as reported for analogous 17α-(iodovinyl)estradiol.[17] Furthermore, it has been shown with 17α-(iodovinyl)-19-nortestosterone[14] and also with 17α-(iodovinyl)estradiol[17] that while it is the *Z* isomer in both series of compounds which showed better affinity for the respective receptor, it is also less stable than the corresponding *E* isomer.[17] The *E* isomer of 17α-(iodovinyl)estradiol has been shown to be stable in vitro as well as in vivo.[18,19]

5α,17α-Pregn-20-yne-3β,17β-diol (**6**, Scheme II), available readily by reacting 3β-hydroxy-5α-androstan-17-one with excess lithium (trimethylsilyl)acetylide and then desilylating, could be envisaged as a common starting material for the preparation of **5** and **9**. Thus, conceivably, appropriate protection of the 3,17-diol, alkynyllithium formation with methyllithium, alkylation with 1,2-dibromoethane, and oxidation of the 3β-ol could afford **5**. Such an approach (alkynyl alkylation using four-carbon and six-carbon α,ω-diiodoalkanes) did afford facile preparation of 17α-(6′-iodohex-1′-ynyl) and 17α-(8′-iodooct-1′-ynyl) analogues of estradiol and 19-nortestosterone.[15,20] However, as we have reported,[15] attempted alkylation of alkynyllithium species using 1,2-diiodoethane led only to the iodoalkyne product. Apparently in this reaction C-iodination (via reductive elimination to produce ethylene) is preferred instead of C-alkylation. Furthermore, as also reported earlier,[15] α,ω-dibromoalkane did not react readily with steroidal 17α-alkynyllithium species. In view of these experiences, we assumed that reaction of appropriately protected **6** (bis(tetrahydropyranyl)ether) with 1,2-dibromoethane would not produce the desired four-carbon 17α side chain analogue **5**.

Alternatively, as reported for the synthesis of analogous 17α-(4′-bromobut-1′-ynyl)-19-nortestosterone,[15] a properly functionalized and suitably protected four-carbon side chain could be directly linked to the 17α position of 3β-hydroxy-5α-androstan-17-one (**1**). Thus, treatment of **1** with excess alkynyllithium derived from but-3-yn-1-yl tetrahydropyranyl ether and aqueous workup afforded 17α-[4-(tetrahydropyranyl)oxy]but-1′-ynyl]-5α-androstan-3β,17β-diol (**2**, Scheme I) in good yield. Sarret oxidation of **2** followed by aqueous acidic hydrolysis gave 17β-hydroxy-17α-(4′-hydroxybut-1′-ynyl)-5α-androstan-3-one (**4**). Treatment of **4** with carbon tetrabromide/triphenylphosphine afforded the desired ω-bromo compound **5**.

For the synthesis of **9**, a reported general procedure for the preparation of analogous 17α-((*E*)-iodovinyl)estradiol[17] was used. Thus, treatment of **6** with tributyltin hydride and catalytic AIBN in refluxing benzene followed by stereospecific displacement of the tributylstannyl group with iodine gave (17α,20*E*)-21-iodo-5α-pregn-20-ene-3β,17β-diol (**8**, Scheme II). Oxidation of **8** with *N*-methylmorpholine *N*-oxide in the presence of a catalytic amount of *n*-tetrapropylammonium perruthenate and molecular sieves afforded the desired (*E*)-iodovinyl compound **9**. Alternatively, oxidation of **6**, as above, gave 17α-ethynyl-5α-DHT (**7**). Treatment of **7** with tributyltin hydride followed by displacement with iodine, as above, also gave **9**. For the synthesis of iodovinyl compounds **8** and **9**, we closely followed the Ali et al.[17] procedure. Under these experimental conditions (refluxing the appropriate alkynyl steroid and tributyltin hydride in benzene for 3 h in the presence of catalytic AIBN) no *E* isomer was detected. From the literature it is apparent that longer refluxing time (e.g. 24 h by Hoyte et al.[14]) or absence of AIBN and replacement of benzene by a polar solvent[17] tend to produce a mixture of *E* and *Z* isomers. As reported[17] for the analogous compounds, the stereochemistry of the iodovinyl

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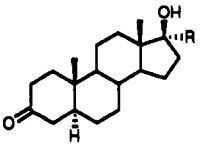
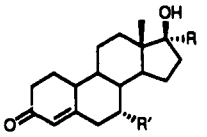
Table I. Relative Binding Affinities of 5α-DHT and 19-Nortestosterone Analogues for AR[a] and PgR[a]

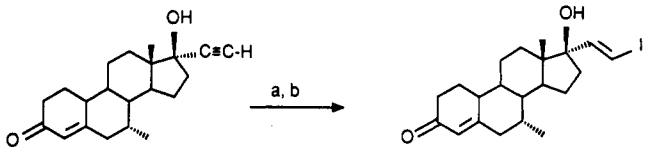
no.	source	17α-R	7α-R′	RBA[b] AR (rat)[c]	RBA[b] AR (human)[d]	RBA[b] PgR (human)[e]
						
D-1	(ref 16)	CH_2—CH=CH_2		3.0	5.8	0.3
D-2	5	C≡C—CH_2—CH_2Br		0.1	0.3	0.2
D-3	7	C≡CH		11.5	6.8	–
D-4	9	(*E*)-CH=CHI		4.7	11.5	0.1
						
N-1	com[f]	H	H	32	41	0.7
N-2	com	CH_3	H	26	25	4.5
N-3	com	C≡CH	H	1.8	2.0	12
N-4	(ref 27)	CH_2—CH=CH_2	H	3.3	3.8	–
N-5	(ref 15)	C≡C—CH_2—CH_2Br	H	0.4	0.6	23
N-6	com	H	CH_3	86	97	1.5
N-7	10	C≡CH	CH_3	28	31	8.3
N-8	11	(*E*)-CH=CHI	CH_3	34	120	8.5

[a] AR, androgen receptor, PgR, progesterone receptor. [b] Relative binding affinity: 5α-DHT = 100 for AR; R5020 = 100 for PgR. [c] Rat uterus. [d] MCF-7 cells. [e] T47D cells. [f] Commercially available from Steraloids.

group was established on the basis of a large coupling constant value for vinylic protons and also on the basis of carbon-13 NMR data. The ^1H NMR spectra of **8** and **9** gave two doublets at δ 6.2 and 6.7 with coupling constants of 14.4 Hz. The ^{13}C NMR spectrum of **9** showed characteristic vinyl carbon signals at δ 74.4 (C-21) and 150.6 (C-20). These ^1H and ^{13}C NMR data agree precisely with the reported chemical shifts of *E* isomer in analogous 17α-(iodovinyl)estradiol.[17]

19-Nortestosterone Analogues. Mibolerone (7α,17α-dimethyl-19-nortestosterone) binds to the AR (and PgR), while 17α-methyl-19-nortestosterone shows low affinities for AR as well as PgR.[21] It thus seems that introduction of a 7α methyl in this series enhances AR binding. 7α-Methyl-19-nortestosterone indeed binds strongly to AR and has reduced affinity for the PgR. On the basis of these observations we decided to synthesize the 17α-((*E*)-iodovinyl) analogue of 7α-methyl-19-nortestosterone and determine its binding affinities for AR and PgR. 7α-Methyl-17α-((*E*)-iodovinyl)-19-nortestosterone (**11**) was thus synthesized (Scheme III) in two steps starting from 7α-methyl-17α-ethynyl-19-nortestosterone (**10**) as described for the preparation of the analogous iodovinyl compound **9**. The tributyltin hydride reduction of **10**, unlike that of **7**, gave a mixture of products along with unreacted starting material. Presumably this difference was due to the presence of the 4-ene-3-one moiety in this case. Adding excess hydride or increasing the refluxing time did not help.[22] Purification of this mixture by preparative TLC afforded the tributyltin intermediate

Scheme III[a]



10 **11**

[a] (a) nBu_3SnH, AIBN, benzene (dry); (b) iodine.

which was then reacted with iodine, as described for the preparation of **8**, to afford the desired (*E*)-iodovinyl compound **11**. The stereochemistry of the iodovinyl group was established as described for **9**.

Receptor Binding. The relative binding affinities (RBAs) of both the 5α-DHT and the 19-nortestosterone series of newly synthesized compounds were determined versus [^3H]-5α-DHT for AR (rat and human) as well as versus [^3H]-R5020 for PgR (human). The results are listed in Table I.

5α-DHT analogues having a larger substituent at 17α displayed weak AR binding affinities; 17α-(prop-2′-enyl)-5α-DHT[23] (D-1 in Table I) thus showed 3.0% and 5.8% RBA for rat and human AR, respectively, while 17α-(bromobutynyl)-5α-DHT (D-2) had almost negligible AR affinity. However, 5α-DHT analogues having shorter 17α substituents showed increased AR affinities. 17α-Ethynyl-5α-DHT (D-3) and 17α-((*E*)-iodovinyl)-5α-DHT (D-4) thus showed better affinities for the AR. Both of these compounds show species specificity; while 17α-ethynyl-5α-DHT has better affinity for rat AR, its affinity for human AR is only half of the affinity of 17α-((*E*)-iodovinyl)-5α-DHT.[24] None of these 5α-DHT analogues,

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(22) Hoyte et al. (ref 14) have reported the tributyltin hydride reduction of analogous 17α-ethynyl-19-nortestosterone and showed it to be incomplete even after 24 h of refluxing. However, no yields have been reported by these authors and therefore it is difficult to know if other products were also formed in their hands.

(23) The RBA of this compound was previously reported by us (ref 16) to be 50%. However, in repeated RBA experiments it consistently exhibited the lower RBA shown in Table I.

however, had any significant affinity for PgR, indicating once again that the presence of a 4-en-3-one substructure is critical for high PgR affinity.

The 19-nortestosterone analogues also quickly lost AR affinity with increasing size of the 17α substituent, while gaining PgR affinity (N-1 through N-5 in Table I). Introduction of a 7α-methyl in 19-nortestosterone has been shown to improve AR affinity;[21] indeed, 7α-methyl-19-nortestosterone (N-6) showed an AR affinity nearly comparable to 5α-DHT but also a somewhat increased PgR affinity as compared with the parent compound 19-nortestosterone (N-1). Introduction of a 7α-methyl in 17α-ethynyl-19-nortestosterone (N-3) also proved to be effective; 7α-methyl-17α-ethynyl-19-nortestosterone (N-7) thus had 15-fold higher affinity for human AR and slightly lower PgR affinity than N-3. Conversion of **10** (N-7) to the corresponding (*E*)-iodovinyl compound **11** (N-8) further markedly improved AR affinity. Indeed, in repeated RBA experiments, 7α-methyl-17α-((E)-iodovinyl)-19-nortestosterone (**11**, N-8) consistently showed better affinity for human AR than the reference 5α-DHT. Moreover, this iodovinyl compound N-8 showed considerable specificity for human AR, exhibiting no change in PgR affinity from the parent compound N-3. It is interesting that, like the (*E*)-iodovinyl-5α-DHT (D-4 above), but unlike other compounds of both the 5α-DHT and 19-nortestosterone series, N-8 has considerably higher affinity for human AR than for rat AR.

In summary, we have successfully synthesized 17α-((*E*)-iodovinyl) analogues of 5α-DHT and 7α-methyl-19-nortestosterone and have shown that both of these compounds bind to AR with high affinity; 17α-((*E*)-iodovinyl)-5α-DHT (**9**) had moderate AR affinity and almost negligible affinity for PgR, while 7α-methyl-17α-((*E*)-iodovinyl)-19-nortestosterone (**11**) had very high AR affinity and lower PgR affinity. Both of these iodovinyl analogues could be easily prepared in radioiodinated form. In view of their high affinity and relative specificity for AR, these ligands should be extremely useful for assaying AR in small specimens.

Experimental Section

Chemistry. Melting points were determined in open capillary tubes with an Electrothermal melting point apparatus and are uncorrected. IR spectra (as solutions in chloroform) were recorded on a Perkin-Elmer 283B spectrophotometer and values are expressed in cm^{-1}. ^1H NMR and ^{13}C NMR spectra were taken in deuterated chloroform on a JEOL FX 90Q or GN 300 spectrometer with TMS as internal standard; salient resonances are reported in subsequent individual experimental texts, with chemical shift values expressed in ppm (δ) relative to the standard and with coupling constants (*J*) in Hz.

Routine mass spectra were acquired on a Finnigan-MAT model 4615 mass spectrometer in conjunction with an INCOS data system. Samples were introduced by means of a direct insertion probe and were ballistically heated if necessary. The ion source temperature was 160 °C. Evaluation of spectra was made after background subtraction. Fast-atom bombardment (FAB) mass spectra were acquired on a Finnigan-MAT model 212 double focusing mass spectrometer in conjunction with an INCOS data system. The ion source temperature was 60 °C and the accelerating voltage was 3 kV. An Ion Tech saddle field atom gun was used with xenon at 8 kV. Samples were applied in solution to the copper probe tip and then approximately 2 μL of thioglycerol was added and mixed with the sample residue. Spectral evaluation was made after subtraction of the contribution from the matrix. Accurate mass FAB analyses were performed by the MSU-NIH Mass Spectrometry Facility and were obtained on a JEOL HX110 double focusing mass spectrometer (resolution ~10 000, acceleration potential 10 kV, ionization $^+$FAB 6 kV neutral xenon beam). The spectra were acquired by peak matching with nitrobenzyl alcohol cluster ions employed as the reference peaks.

Analytical and preparative TLC were performed on precoated silica gel glass plates (Type GF, Analtech, Newark, DE). The desired compounds were visualized by either UV, iodine, or potassium permanganate spray and were isolated from their silica gel bands by extraction with ethyl acetate. 3β-Hydroxy-5α-androstan-17-one, 7α-methyl-19-nortestosterone, 17α-methyl-19-nortestosterone, 17α-ethynyl-19-nortestosterone, and 19-nortestosterone were purchased from Steraloids, Wilton, NH. 7α-Methyl-17α-ethynyl-19-nortestosterone was a generous gift from Upjohn, Kalamazoo, MI. All other reagents and solvents were purchased from Aldrich, Milwaukee, WI.

Anhydrous tetrahydrofuran containing less than 1% water was further purified by distillation from the blue sodium benzophenone ketyl. Pyridine was stored over sodium hydroxide and a decanted portion was distilled immediately prior to use. The titer of the alkyl lithium solutions was determined as reported.[25]

The reaction conditions described below are not necessarily optimized.

17α-[4′-[(Tetrahydropyranyl)oxy]but-1′-ynyl]-5α-androstane-3β,17β-diol (2). The title compound was prepared as described for the preparation of analogous diastereomeric 3,3-(ethylenedithio)-17α-[4′-[(tetrahydropyranyl)oxy]but-1′-ynyl]-estr-4-en-17β-ol.[5] Thus, reaction of 3β-hydroxy-5α-androstan-17-one (**1**) and a 5-fold excess of alkynyllithium (generated from but-3-yn-1-yl tetrahydropyranyl ether), purification by column chromatography (silica gel, 28–200 mesh), and recrystallization of the chromatographed solid from benzene/hexane afforded the title compound **2**: yield 75%; mp 146–156 °C (previous softening at 129 °C); IR no carbonyl; ^1H NMR δ 0.82 (s, 6 H, 18CH_3 and 19CH_3), 2.5 (t, 2 H, 3′-CH_2, *J* = 7), 3.33–4.0 (m, 5 H, 3α-*H*, CH_2OTHP and pyran OCH_2), and 4.6 (b s, 1 H, OC*H*O); MS *m*/*z* (relative intensity), 444 (M^+, 3), 429 (49), 411 (9), 360 (29), 342 (18), and 85 (100). Anal. ($C_{28}H_{44}O_4$)C, H.

17β-Hydroxy-17α-[4′-[(tetrahydropyranyl)oxy]but-1′-ynyl]-5α-androstan-3-one (3). A solution of **2** (133.2 mg, 0.3 mmol) in dry pyridine (9 mL) was added dropwise to Sarret's reagent [prepared by adding chromium trioxide (90 mg, 0.9 mmol) to dry pyridine (1 mL)] at 10 °C. After the addition was completed, the reaction mixture was stirred at room temperature for 24 h and diluted with ether (30 mL). The solid material was removed by filtration through Celite, and the filtrate was washed with dilute aqueous hydrochloric acid (3 × 15 mL). The combined aqueous phase was extracted with ether (3 × 20 mL), and the combined organic phase was washed with water to neutrality, dried over anhydrous sodium sulfate, and concentrated in vacuo. Purification by preparative TLC (45% ethyl acetate/benzene) followed by recrystallization of the chromatographed solid from benzene/hexane afforded the title compound **3**: yield 56 mg (42%); mp 117–118 °C; IR 1710; ^1H NMR δ 0.84 (s, 3 H, 18 CH_3), 1.01 (s, 3 H, 19 CH_3), 2.5 (t, 2 H, 3′-CH_2, *J* = 7), 3.33–4.0 (m, 4 H, CH_2OTHP and pyran OCH_2), and 4.58 (m, 1 H, OC*H*O); MS *m*/*z* (relative intensity), 442 (M^+, 1.5), 427 (22), 409 (4), 358 (20), 340 (11), and 85 (100). Anal. ($C_{28}H_{42}O_4$) C, H.

17β-Hydroxy-17α-(4′-hydroxybut-1′-ynyl)-5α-androstan-3-one (17α-(4′-Hydroxybut-1′-ynyl)-5α-DHT) (4). A mixture of **3** (442 mg, 1.0 mmol), *p*-toluenesulfonic acid monohydrate (38 mg, 0.2 mmol), water (3 mL), and ethanol (17 mL) was stirred at 75–80 °C for 2.5 h. The mixture was then cooled, poured over brine (100 mL), and stirred for 15 min. The solid thus separated

(24) After this manuscript was first submitted, one of our compounds was also reported in a paper by Hoyte et al.: Hoyte, R. M.; et al. The synthesis of *E*-17α-(2-iodovinyl)-5α-dihydrotestosterone and *Z*-17α-(2-iodovinyl)-5α-dihydrotestosterone as γ-emitting ligands for the androgen receptor. *J. Steroid Biochem.* **1990** (June), *36*, 125–132. These authors reported the relative binding affinity (RBA) for compound **9** (*E*-(iodovinyl)-5α-DHT) for androgen receptor (AR) from the rat prostate to be 5%, which agrees precisely with our own RBA data for rat uterine AR. However, these authors did not report affinities of compound **9** for human AR or for progesterone receptor.

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was collected through filtration, washed with water, and air dried. Crystallization and recrystallization from hot benzene afforded the title compound 4: yield 355 mg (99%); mg 170–171 °C; IR 1710; ^{1}H NMR δ 0.85 (s, 3 H, 18 CH_3), 1.02 (s, 3 H, 19 CH_3), 2.49 (t, 2H, 3'-CH_2, J = 7), and 3.68 (bt, 2 H, CH_2OH, J = 7); MS (relative intensity) 358 (M$^+$, 12), 343 (17), 340 (20), and 325 (100). Anal. ($C_{23}H_{34}O_3$) C, H.

17β-Hydroxy-17α-(4'-bromobut-1'-ynyl)-5α-androstan-3-one (17α-(4'-Bromobut-1'-ynyl)-5α-DHT) (5). A solution of 4 (179 mg, 0.5 mmol) and triphenylphosphine (262 mg, 1.0 mmol) in dry methylene chloride (14 mL) was cooled at 0 °C, and carbon tetrabromide (249 mg, 0.75 mmol) was added at this temperature in one batch. The mixture was warmed to room temperature, stirred for 2 h, and concentrated in vacuo. Purification by preparative TLC (40% ethyl acetate/benzene) followed by recrystallization of the chromatographed solid from benzene/hexane afforded the title compound 5: yield 133 mg (63%); mp 108–109 °C; IR 1710; ^{1}H NMR δ 0.85 (s, 3 H, 18 CH_3), 1.02 (s, 3 H, 19 CH_3), 2.81 (t, 2H, 3'-CH_2, J = 7), and 3.45 (bt, 2 H, C*H2Br*, J = 7); MS (relative intensity), 422 (M$^+$, 5.6), 420 (7), 407 (7), 405 (10), 341 (100), and 323 (31); exact mass (MH$^+$) calcd for $C_{23}H_{33}BrO_2$ 421.1733, found 421.1735. Anal. ($C_{23}H_{33}BrO_2$) C, H.

5α,17α-Pregn-20-yne-3β,17β-diol (6). Under nitrogen, trimethylsilylacetylene (490 mg, 5.0 mmol) was dissolved in dry ether (30 mL), and the solution was cooled to 0 °C. At this temperature a solution of methyllithium in ether (1.4 M solution, 2.85 mL, 4.0 mmol) was added slowly; the mixture was warmed to room temperature and stirred for 1 h. The mixture was again cooled to 0 °C, and at this temperature a solution of 3β-hydroxy-5α-androstan-17-one (1, 290 mg, 1.0 mmol) in dry THF (50 mL) was added. The reaction mixture was warmed to room temperature, stirred for 4 h, quenched with saturated aqueous ammonium chloride (5 mL), and diluted with ethyl acetate (100 mL). The organic phase was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo.

The residue was dissolved in methanol (30 mL), and aqueous potassium hydroxide solution (5 N, 2.0 mL) was added to it. The mixture was stirred at 65–70 °C for 30 min, cooled, diluted with brine (100 mL), acidified with dilute HCl, and extracted with ethyl acetate (3 × 70 mL). The organic phase was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. Crystallization and recrystallization from hot ethyl acetate afforded the title compound 6: yield 225 mg (71%); mp 260–261 °C (lit.[26] mp 260–262 °C).

17β-Hydroxy-5α,17α-pregn-20-yn-3-one (17α-Ethynyl-5α-DHT) (7). Powdered 4A molecular sieves (3 g) were added to a solution of 6 (31.6 mg, 0.1 mmol) in dry THF (10 mL) followed by *N*-methylmorpholine *N*-oxide (17.55 mg, 0.15 mmol) and tetra-*n*-propylammonium perruthenate (1.75 mg, 0.005 mmol). The resulting dark green mixture was stirred at room temperature for 14 h, diluted with methylene chloride (50 mL), washed sequentially with aqueous sodium sulfite, brine, and aqueous copper sulfate, dried over anhydrous sodium sulfate, and concentrated in vacuo. Crystallization and recrystallization from THF/methylene chloride afforded the title compound 7: yield 18 mg (57%); mp 296–297 °C (lit.[26] mp 297–300 °C)

(5α,17α,20*E*)-21-Iodopregn-20-ene-3β,17β-diol (8). Under nitrogen, a mixture of 6 (316 mg, 1.0 mmol) and azobis(isobutyronitrile) (50 mg, 0.3 mmol) in dry benzene (60 mL) was heated at 70–80 °C, and cooled. Tri-*n*-butyltin hydride (960.3 mg, 3.3 mmol) was then added and the mixture was refluxed for 3 h, cooled, and concentrated in vacuo. Purification by column chromatography on silica gel (15 g, 28–200 mesh) eluting with 5% ethyl acetate in benzene afforded (5α,17α,20*E*)-21-(tributylstannyl)pregn-20-ene-3β,17β-diol: yield 460 mg (75%); MS (relative intensity), 608 (M$^+$, 0.5), 555 (16), 551 (100), 549 (81), 547 (47), and 533 (12).

The *E*-tributylstannyl intermediate thus prepared was dissolved in methylene chloride (15 mL) and to it a solution of iodine (0.1 M) in methylene chloride was gradually added until the color of iodine persisted. This was followed sequentially by the addition of potassium fluoride in methanol (1 M solution, 1.0 mL) and aqueous sodium sulfite (5% solution, 1.0 mL). The mixture was stirred for 10 min, diluted with methylene chloride (70 mL), washed with water, dried over anhydrous sodium sulfate, and concentrated in vacuo. Purification by column chromatography on silica gel (15 g, 28–200 mesh) with 5% ethyl acetate in benzene as eluant followed by recrystallization of the chromatographed solid from benzene/hexane afforded the title compound 8: yield 222 mg (71%); mp 128.5–129 °C dec; IR 1605; ^{1}H NMR δ 0.81 (s, 3 H, 18CH_3), 0.87 (s, 3 H, 19CH_3), 3.55–3.65 (m, 1 H, 3α-*H*), 6.23 (d, 1 H, =C*H*I, J = 14.4), and 6.72 (d, 1 H, *H*C=, J = 14.4); MS m/z (relative intensity) 444 (M$^+$, 5), 426 (12), 411 (14), 393 (8), 317 (48), 299 (67), 281 (48), and 107 (100); exact mass calcd for $C_{21}H_{33}IO_2$ 444.1516, found 444.1528.

(5α,17α,20*E*)-17β-Hydroxy-21-iodopregn-20-en-3-one (17α-((*E*)-Iodovinyl)-5α-DHT) (9). Method A. The title compound was prepared as described for the synthesis of 7. Thus, oxidation of 8 with *N*-methylmorpholine *N*-oxide in methylene chloride in the presence of 4A molecular sieves and a catalytic amount of tetra-*n*-propylammonium perruthenate, purification by preparative TLC (50% ethyl acetate/benzene), and recrystallization of the chromatographed solid from benzene/hexane afforded the title compound 9: yield 62%; mp 114–115 °C dec; IR 1710, 1610; ^{1}H NMR δ 0.91 (s, 3 H, 18 CH_3), 1.02 (s, 3 H, 19CH_3), 6.26 (d, 1 H, =C*H*I, J = 14.4), and 6.73 (d, 1 H, *H*C=, J = 14.4); ^{13}C NMR δ 11.47 (C-19), 14.01 (C-18), 21.05, 23.57, 28.82, 31.36, 32.49, 35.77, 36.17, 36.66, 38.08, 38.52, 44.65, 46.71, 50.24, 52.59, and 53.57 (steroid skeleton carbons), 74.40 (C-21), 87.01 (C-17), 150.57 (C-20), and 211.50 (C-3); MS m/z (relative intensity) 442 (M$^+$, 3), 424 (12), 409 (10), 315 (32), 297 (22), 282 (9), and 55 (100); FAB MH$^+$ 443 (100); exact mass (MH$^+$) calcd for $C_{21}H_{31}IO_2$ 443.1438, found 443.1434.

Method B. The title compound was prepared as described for the two-step synthesis of 8. Thus, reaction of 7 with tributyltin hydride and catalytic AIBN in refluxing benzene and subsequent reaction of the *E*-stannyl intermediate with iodine, purification by preparative TLC (50% ethyl acetate/benzene) followed by recrystallization of the chromatographed solid from benzene/hexane afforded the title compound 9: yield 54%. The physical and spectroscopic data were the same as described in method A.

(17α,20*E*)-17β-Hydroxy-21-iodo-7α-methyl-19-norpregna-4,20-dien-3-one (7α-Methyl-17α-((*E*)-iodovinyl)-19-nortestosterone) (11). The title compound was prepared as described for the two step synthesis of 8. Thus, a mixture of 7α-methyl-17α-ethynyl-19-nortestosterone (10, 312 mg, 1 mmol), tributyltin hydride (291 mg, 1 mmol), and AIBN (20 mg, 0.12 mmol) in dry benzene (15 mL) was refluxed for 3 h under nitrogen. The reaction mixture was then concentrated in vacuo and purified by preparative TLC (20% ethyl acetate/benzene). The band at *RF* 0.7 showed the desired vinylic protons in the ^{1}H NMR spectrum and was directly iodinated with use of iodine as described for the synthesis of 8. Purification by preparative TLC (20% ethyl acetate/benzene) and recrystallization of the chromatographed solid from benzene/hexane afforded the desired (*E*)-iodovinyl compound 11: yield 36 mg (8%); mg 179–180 °C (softens and starts decomposing at 97 °C); IR 1665 and 1610; ^{1}H NMR δ 0.78 (d, 3 H, 7αCH_3, J = 7), 0.96 (s, 3 H, 18CH_3), 5.84 (s, 1 H, C-4*H*), 6.29 (d, 1 H, =C*H*I, J = 14), and 6.72 (d, 1 H, C*H*=, J = 14); MS m/z(relative intensity) 440 (M$^+$, 8), 313 (83), 295 (16), and 55 (100); exact mass calcd for $C_{21}H_{29}IO_2$ 441.1282, found 441.1277.

Receptor Affinity Determinations. The relative binding affinities (RBAs) of the newly synthezied compounds were determined (vs [^{3}H]DHT) for androgen receptor from rat uterus, as described earlier.[16] In brief, androgen receptor cytosol was prepared from uteri of mature ovariectomized Harlan-Sprague-Dawley rats in TEDGM buffer (10 mM Tris-HCl, pH 7.4 at 0 °C, 1.5 mM EDTA, 0.5 mM dithiothreitol, 10% glycerol, 20 mM sodium molybdate). In each volume of cytosol, the pellet from a half volume of DCC suspension (10 mM Tris-HCl, pH 8.0 at 0 °C, 0.25% Norit A, 0.0025% dextran) was suspended for 10 min and then centrifuged 10 min at 2000*g* to remove endogenous steroids. For RBA determinations, triplicate sets of tubes received 200 μL of cytosol, 40 μL of 1.25 × 10^{-8} M [^{3}H]DHT (195.9 Ci/mmol, New England Nuclear), and 10 μL of unlabeled test compound at five or more concentrations in DMF, giving a final concentration of 4% DMF. After 18 h at 0–4 °C, all tubes received

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500 μL of DCC suspension and were mixed for 15 min and centrifuged. Supernatants (500 μL) were counted in a liquid scintillation counter.

Sets of tubes also containing 10^{-6} M DHT were used to determine nonspecific binding, which was subtracted from all values. Nonspecific binding was typically 20% of total [^{3}H]DHT bound. Results were plotted and RBAs determined as described earlier. The RBA values reported here are the average of three or more determinations.

The RBAs of these newly synthesized compounds were also determined for AR from MCF-7 human breast cancer cells. To prepare AR cytosol from this cell line, 0.05 mL packed cells per milliliter of TEDGM buffer were homogenized in a Dounce glass-teflon homogenizer with a motor driven pestle at 0-4 °C. The homogenate was centrifuged at 100000g for 30 min and the clear supernatant (cytosol) was collected. Endogenous steroids were removed with DCC and RBAs were determined as above.

To check the receptor specificity, these compounds were also tested for their ability to bind PgR. RBAs for PgR (from T47D human breast cancer cells) were thus determined with respect to [^{3}H]R5020 as described earlier.[15] Nonspecific binding was typically 4% of total [^{3}H]R5020 binding.

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Registry No. 1, 481-29-8; 2, 131545-84-1; 3, 131545-85-2; 4, 131545-86-3; 5, 131545-87-4; 6, 13611-96-6; 7, 131611-97-7; 8, 131545-88-5; 9, 129163-98-0; 10, 1162-60-3; 11, 131545-89-6; but-3-yn-1-yltetrahydropyranyl ether, 40365-61-5; alkynyllithium, 67654-73-3; trimethylsilylacetylene, 1066-54-2; (5α,17α,20*E*)-21-(tributylstannyl)pregn-20-ene-3β,17β-diol, 131545-90-9.

Perfluorinated Analogues of Poison Ivy Allergens. Synthesis and Skin Tolerogenic Activity in Mice

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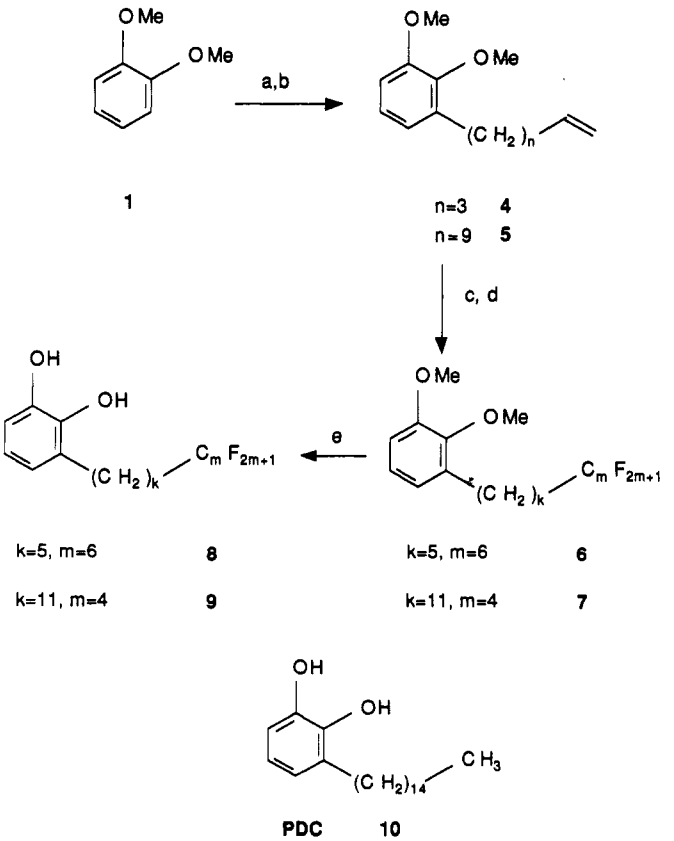
3-(Tridecafluoroundecyl)catechol (8) and 3-(nonafluoropentadecyl)catechol (9), perfluorinated analogues of pentadecylcatechol (PDC), a constituent of poison ivy, have been synthesized. These compounds were nonsensitizers in mice. Compounds 8 and 9, however, were elicitors of allergic contact dermatitis in PDC-sensitized animals. Moreover, compound 9 exhibited tolerogenic properties to sensitization by poison ivy allergens, i.e. mice pretreated with perfluorinated compounds could not be sensitized to PDC.

The allergenic principles contained in poison ivy (*Rhus toxicodendron* or *Toxicodendron radicans*), poison oak (*Rhus diversiloba* or *Toxicodendron diversilobum*), and poison sumac (*Toxicodendron vernix*) are the main causes of allergic contact dermatitis in the United States.[1,2] The allergens in these plants have been shown to be a catechols with linear 3-*n*-alkyl chains containing 15 (such as pentadecylcatechol, PDC) or 17 (such as heptadecylcatechol, HDC) carbon atoms, commonly referred to as urushiols.[2,3] Until now, there have been only a few studies directed at induction of immune tolerance to allergic contact dermatitis; tolerance can be induced by modifying the chemical structures of urushiol allergens.[3] The importance of the 3-alkyl carbon chain, number of atoms in the side chain,[4] and influence of branching and rigidity[5] has been evaluated. It was found that the sensitizing power (the contact allergy inducing capacity) was strongly dependent on the nature of the side chain.

In view of the expected biological effect of replacing hydrogen by fluorine, it seemed to us that, by introduction on the side chain of a perfluorinated segment, some interesting biological properties might result. For instance, it has been shown that perfluorinated alkyl chain of the fatty acids exhibited detergent-like activity in human and murine B cell, by physically altering cell membranes.[6] We have investigated the sensitizing power of PDC (10) and side-chain perfluorinated catechol analogues 3-(tridecafluoroundecyl)catechol (8) and 3-(nonafluoropentadecyl)catechol (9) in mice. Although guinea pigs are usually a better animal model of human skin than mice, evaluation of biological data is *qualitative* and based on

Scheme I[a]



[a] *n*-BuLi, THF; (b) bromo-1-undecene **2** or bromo-1-pentene **3**; (c) IC_mF_{2m+1} (m = 4 or m = 6), AIBN, reflux 3 h; (d) Bu_3SnH, AIBN, reflux 3 h; (e) BBr_3, CH_2Cl_2.

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