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Synthesis of (17α,20E/Z)Iodovinyl Testosterone and 19-Nortestosterone Derivatives as Potential Radioligands for Androgen and Progesterone Receptors

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To develop androgen and progesterone receptor-based radioligands for SPECT imaging we synthesized several radioiodinated 17α-iodovinyl testosterone and 19-nortestosterone analogs and evaluated their biological properties. The synthesis of these compounds proceeds via the (17α,20E/Z)stannyl intermediates and involves addition of tri-*n*-butyltin hydride to the 17α-ethynyl group of the steroid using either azo*bisiso*butyronitrile or triethylborane as a catalyst. The stannyl derivatives are stereospecifically converted to the corresponding (17α,20E/Z)iodovinyl derivatives using molecular iodine, or to the [^{125}I]iodovinyl analogs using [^{125}I]NaI and H_2O_2. Androgen and progesterone receptor (AR and PgR) binding affinities were measured via a competitive *in vitro* binding assay. In general 19-nortestosterone derivatives showed higher receptor affinities as compared to the testosterone derivatives. In the latter series the highest PgR binding affinities were observed with the (17α,20Z)iodovinyl-19-nortestosterone (IVNT) (92 vs 100 for R5020) followed by the 7α-methyl analog, whereas the highest AR binding affinity was observed with the 7α-Me-(17α,20Z)IVNT (54 vs 100 for 5α-dihydrotestosterone). These derivatives were also labeled with ^{125}I and evaluated for their *in vivo* target organ uptake (prostate and estrogen-primed uterus). The highest PgR-mediated target tissue uptake was observed with the (17α,20Z)-[^{125}I]IVNT and its 7α–methyl derivatives whereas only one derivative, the 7α-Me-(17α,20Z)-[^{125}I]IVNT, showed AR-mediated dorsal prostate retention. Although some of the IVNT derivatives have interesting binding properties, the lack of *in vivo* selectivity does suggest that the ^{123}I-labeled analogs are unlikely to be suitable for imaging of AR and PgR-rich tissues.

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INTRODUCTION

The design of steroid receptor-based radiopharmaceuticals for imaging and therapy of endocrine cancers has been pursued by several groups of investigators over the past decade. The emphasis of this research focused mainly on the estrogen receptors (ER) [1–3]. In addition to ER concentration, knowledge of the level of progesterone receptors (PgR) in breast tumors [4, 5] and androgen receptors (AR) in prostate tumors [6–10] also provides important prognostic information for the detection and treatment of hormone-responsive neoplasms. In the case of breast cancer, positive PgR levels have been shown more predictive of tumor response to hormonal therapy than positive ER levels [11–12]. Furthermore, a progestin based imaging agent for breast tumors might be preferred in patients on hormonal therapy (i.e. tamoxifen) since the circulating levels of tamoxifen and its metabolites will occupy the ER, rendering these sites inaccessible to ER-based radiopharmaceuticals [13]. In the case of prostate cancer, the correlation between tumor response to hormonal treatment and AR levels is less evident [8–10].

The synthesis of a number of radiolabeled progestins has been reported [14–18]. Most derivatives were obtained at low specific activity, exhibiting low affinity for the PgR, resulting in the absence of selective uptake by progestin target tissues under *in vivo* conditions. More recently ^{18}F-labeled analogs [19–22] and ^{99m}Tc-chelated

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derivatives [23] were prepared at higher specific activities but likewise lacking target tissue selectivity, due to high uptake in non-target tissues.

Several androgen analogs containing iodine (I), bromine (Br), fluorine (F), or selenium (Se) have been synthesized as specific probes for the AR, however none of them have shown significant affinity for the AR, or specificity for AR-rich target tissue [24]. The 2α-bromo [25, 26] and 2α-[^{125}]iodo [27] derivatives of 5α-dihydrotestosterone (5α-DHT) are rapidly dehalogenated *in vivo*, explaining their lack of selectivity. A, B-Ring substitution with I, Br, F as well as Se gave compounds capable of competing with 5α-DHT for AR, however, the radiolabeled analogs lacked *in vivo* specificity [25–28]. Recently Katzenellenbogen *et al.* [29–32] prepared a number of positron-emitting 16α-[^{18}F]fluoroandrogens and showed their uptake by AR-rich target tissue *in vivo*, confirming the potential of AR-based radiopharmaceuticals for prostate imaging. Introduction of a 16α-radioiodine onto 5α-DHT resulted in loss of tissue specificity [33], while the analogous 16α-[^{125}I]iodo-19-nortestosterone showed a high PgR binding affinity and good *in vivo* stability [34]. The use of 17α-iodovinyl radiolabeled androgens and progestins has not been fully explored. Only a limited number of possible analogs have been prepared in this series and insufficient *in vivo* biodistribution data are available [35–39]. On such accounts we prepared a number of (17α,20E/Z)iodovinyl derivatives of testosterone and 19-nortestosterone, as well as their 7α-methyl analogs, established their binding affinities for the AR and PgR and evaluated their *in vivo* distribution pattern.

EXPERIMENTAL

Materials and methods

7α-Methyl-17α-ethynyl-19-nortestosterone (**1b**) and 4-androsten-7α-methyl-17α-ethynyl-17-ol-3-one (**1d**) were obtained as gifts from The Upjohn Company (Kalamazoo, MI, U.S.A.). The 17α-ethynyl-19-nortestosterone (**1a**) and 4-androsten-17α–ethynyl-17-ol-3-one (**1c**) and all other steroids used in this work were purchased from Steraloids Inc. (Wilton, NH, U.S.A.). All chemicals were commercially available and were of the highest grade available. Carrier-free [^{125}I]NaI was purchased from Amersham Canada Ltd.

Melting points (m.p.) were determined on a Fisher-Johns apparatus and are uncorrected. Proton Magnetic Resonance (^{1}H NMR) spectra were obtained on a Bruker WM 25 Spectrometer in $CDCl_3$ + DMSO-d_6 and chemical shifts are reported as part per million (ppm) downfield from tetramethylsilane as an internal standard. Low resolution electron impact mass spectra (EIMS) were obtained on a Hewlett-Packard model 5988A quadrupole instrument at 70 eV. Microanalysis data were conducted by Guelph Laboratories Ltd (Canada). Silica gel (60–200 mesh) was used for column chromatography. Silica gel plates coated with fluorescent indicator (UV 254) were used for analytical thin-layer chromatography (TLC) and the compounds were located by their UV absorbance/or color response upon spraying with H_2SO_4/EtOH and heating at 120°C. High performance liquid chromatography (HPLC) was performed on a reverse-phase column (C-18, ODS-2 spherisorb, 5 μm, 25 × 0.94 cm, CSC, Montreal, Canada) at a flow rate of 2 ml/min, compounds were detected at 254 nm and were appropriate, by their γ-radiation which was registered via a sodium iodide detector.

*Synthesis of (17α,20E/Z)tri-n-butylstannyl derivatives (**2a–d** and **3a–d**)*

Method A: *with triethyl borane.* A hexane solution of triethyl borane (1 M, 0.1 ml, 0.1 mmol) was added to a solution of **1a** or **1c** (0.1 mmol) and *n*-tributylstannyl hydride (0.2 mmol) in THF (5 ml) at room temperature under nitrogen atmosphere. After stirring for 30–60 min, the THF was removed under reduced pressure.

Method B: *with azobisisobutyronitryle (AIBN).* A mixture of **1b** or **1d** (0.1 mmol) in 5 ml of toluene and 0.1 ml of tri-*n*-butyltin hydride (0.3 mmol) was heated at 90–95°C for 1–3 h in the presence of AIBN (9 mg, 0.5 mmol) under nitrogen. The solvent was removed under reduced pressure.

Purification. The residue obtained with either method A or B was chromatographed on silica gel (10 g). Elution with 3–5% ethyl acetate in hexane gave mixtures of products which were further purified by HPLC on a reverse phase, semipreparative column with a gradient of 5% water in methanol to 100% methanol, over a period of 20 min.

*(17α,20Z)-17β-Hydroxy-21-tri-n-butylstannyl-19-norpregna-4,20-dien-3-one (**2a**).* HPLC, t_R = 20 min. Yield: 20%; MS *m/z* (relative intensity) 577 (3), 533 (M^+-C_4H_9, 40), 515 (M^+-C_4H_9-H_2O, 100), 529 (78), 367 (50), 312 (65).

*(17α,20E)-17β-Hydroxy-21-tri-n-butylstannyl-19-norpregna-4,20-dien-3-one (**3a**).* HPLC, t_R = 14 min. Yield: 30%; MS *m/z* (relative intensity) 533 (M^+-C_4H_9, 100), 477 (10), 419 (20), 400 (12).

*(17α,20Z)-17β-Hydroxy-21-tri-n-butylstannyl-7α-methyl-19-norpregna-4,20-dien-3-one (**2b**).* HPLC, t_R = 24 min. Yield: 15%; MS *m/z* (relative intensity) 602 (M^+, 0.3), 601 (M^+, 0.2), 551 (5), 549 (9), 547 (M^+-C_4H_9, 28), 545 (22), 529 (M^+-C_4H_9-H_2O, 100), 527 (66), 415 (14), 411 (7), 314 (22).

*(17α,20E)-17β-Hydroxy-21-tri-n-butylstannyl-7α-methyl-19-norpregna-4,20-dien-3-one (**3b**).* HPLC, t_R = 17 min. Yield: 25%; MS *m/z* (relative intensity) 602 (M^+, 0.3), 601 (M^+, 0.5), 551 (5), 549 (69), 547 (M^+-C_4H_9, 100), 529 (22), 527 (15), 491 (20), 489 (17), 415 (12), 314 (10).

*(17α,20Z)-17β-Hydroxy-21-tri-n-butylstannyl-androsten-4,20-dien-3-one (**2c**).* HPLC, t_R = 22 min. Yield: 25%; MS *m/z* (relative intensity) 602 (M^+, 0.3)

551 (16), 549 (9), 547 (M^+-C_4H_9, 100), 546 (47), 544 (35), 491 (8), 489 (7), 487 (4), 433 (18), 431 (14).

(17α,20E)-17β-Hydroxy-21-tri-n-butylstannyl-androsten-4,20-dien-3-one (3c). HPLC, $t_R = 16$ min. Yield: 25%; MS *m/z* (relative intensity) 547 (M^+-C_4H_9, 33), 545 (27), 543 (1.5), 531 (71), 529 (M^+-C_4H_9-H_2O, 100), 433 (10), 431 (9), 415 (12), 314 (12).

(17α,20Z)-17β-Hydroxy-21-tri-n-butylstannyl-7α-methyl-androsten-4,20-dien-3-one (2d). HPLC, $t_R = 26$ min. Yield: 15%; MS *m/z* (relative intensity) 562 (11), 561 (M^+-C_4H_9, 31), 543 (M^+-C_4H_9-H_2O, 100), 314 (27).

(17α,20E)-17β-Hydroxy-21-tri-n-butylstannyl-7α-methyl-androsten-4,20-dien-3-one (3d). HPLC, $t_R = 18$ min. Yield: 20%; MS *m/z* (relative intensity) 562 (30), 561 (M^+-C_4H_9, 100), 558 (56), 505 (20), 503 (15), 447 (6), 429 (8).

Preparation of iodovinyl derivatives (4a–d and 5a–d) from their corresponding tin intermediates (2a–d and 3a–d)

To compounds **2a–d** and **3a–d** (0.1 mmol) in chloroform (5 ml) was gradually added at room temperature a 0.1 M solution of iodine in chloroform until the color of iodine persisted. This was followed sequentially by the addition of 0.2 ml of 1 M KF in methanol and 0.2 ml of 5% sodium bisulfite. The mixture was then extracted with chloroform (2 × 15 ml). The organic phase was dried over magnesium sulfate (anh), filtered and evaporated to dryness. The residues were purified over a C-18 reverse-phase semipreparative HPLC column. The compounds were obtained in 70–80% yield.

(17α,20Z)-17β-Hydroxy-21-iodo-19-norpregna-4,20-dien-3-one (4a). m.p. 141–144°C (lit[35] 125°C decom; lit[34] 117–118°C); HPLC: (methanol 75%, water 25%) $t_R = 20$ min.

(17α,20E)-17β-Hydroxy-21-iodo-19-norpregna-4,20-dien-3-one (5a). m.p. 136–139°C (lit[35] 130°C decom; lit[34] 111–114°C); HPLC: (methanol 75%, water 25%) $t_R = 23$ min.

(17α,20Z)-17β-Hydroxy-21-iodo-7α-methyl-19-norpregna-4,20-dien-3-one (4b). m.p. 65–70°C (lit[39] 60–65°C); HPLC: (methanol 80%, water 20%) $t_R = 17$ min; Anal. calculated for $C_{21}H_{29}IO_2$: C, 57.28; H, 6.63; I, 28.82. Found: C, 57.35; H, 6.57, I, 28.72.

(17α,20E)-17β-Hydroxy-21-iodo-7α-methyl-19-norpregna-4,20-dien-3-one (5b). m.p. 95–99°C (lit[38] 97°C decom; lit[39] 76–79°C); HPLC: (methanol 80%, water 20%) $t_R = 18$ min; Anal. calculated for $C_{21}H_{29}IO_2$: C, 57.28; H, 6.63; I, 28.82. Found: 57.21; H, 6.61; I, 28.95.

(17α,20Z)-17β-Hydroxy-21-iodo-androsten-4,20-dien-3-one (4c). m.p. 143–145°C; HPLC: (acetonitrile 55%, water 45%) $t_R = 31$ min; ^{1}H NMR δ 0.91 (s, 3H, 18-CH_3), 1.14 (s, 3H, 10-CH_3), 2.35 (s, 1H, OH), 5.66 (d, J = 0.9 Hz, 1H, 4-H), 6.29 (d, J = 8.5 Hz, 1H, =CHI), 6.69 (d, J = 8.5 Hz, 1H, CH=); MS *m/z* (relative intensity) 440 (M^+, 4), 407 (7), 398 (10), 313 (M-I, 100), 295 (44), 245 (68). Anal. calculated for $C_{21}H_{29}IO_2$: C, 57.28; H, 6.63; I, 28.82. Found: 57.38; H, 6.65; I, 28.80.

(17α,20Z)-17β-Hydroxy-21-iodo-androsten-4,20-dien-3-one (5c). m.p. 65–67°C; HPLC: (acetonitrile 55%, water 45%) $t_R = 28$ min; ^{1}H NMR δ 0.87 (s, 3H, 18-CH_3), 1.13 (s, 3H, 10-CH_3), 5.66 (d, J = 1.3 Hz, 1H, 4-H), 6.16 (d, J = 14.3 Hz, 1H, =CHI), 6.64 (d, J = 14.3 Hz, 1H, CH=); MS *m/z* (relative intensity) 440 (M^+, 5), 422 (54), 407 (78), 313 (M-I, 100), 295 (86), 245 (50). Anal. calculated for $C_{21}H_{29}IO_2$: C, 57.28; H, 6.63; I, 28.82. Found: C, 57.15; H, 6.51; I, 27.82.

(17α,20Z)-17β-Hydroxy-21-iodo-7α-methyl-androsten-4,20-dien-3-one (4d). m.p. 121–123°C; HPLC: (acetonitrile 60%, water 40%) $t_R = 26$ min; ^{1}H NMR δ 0.72 (d, J = 7 Hz, 7α-CH_3), 0.97 (s, 3H, 18-CH_3), 1.15 (s, 3H, 10-CH_3), 5.67 (d, J = 1.4 Hz, 1H, 4-H), 6.30 (d, J = 8.6 Hz, 1H, =CHI), 6.68 (d, J = 8.5 Hz, 1H, CH=); MS *m/z* (relative intensity) 454 (M^+, 5), 327 (M-I, 100), 309 (28), 259 (23), 180 (78). Anal. calculated for $C_{22}H_{31}IO_2$: C, 58.16; H, 6.87; I, 27.93. Found: C, 57.90; H, 6.79; I, 27.80.

(17α,20E)-17β-Hydroxy-21-iodo-7α-methyl-androsten-4,20-dien-3-one (5d). m.p. 123–128°C; HPLC: (acetonitrile 60%, water 40%) $t_R = 23$ min; ^{1}H NMR δ 0.70 (d, J = 7 HZ, 7α-CH_3), 0.86 (s, 3H, 18-CH_3), 1.12 (s, 3H, 10-CH_3) 5.64 (d, J = 1.5 Hz, 1H, 4-H), 6.18 (d, J = 14.3 Hz, 1H, =CHI), 6.65 (d, J = 14.3 Hz, 1H, CH=); MS *m/z* (relative intensity) 454 (M^+, 7), 327 (M-I, 100), 309 (28), 259 (18), 180 (71). Anal. calculated for $C_{22}H_{31}IO_2$: C, 58.16; H, 6.87; I, 27.93. Found: C, 57.80; H, 6.80; I, 28.04.

(17α,20E/Z)-21[^{125}I]iodovinyl derivatives

To a mixture of **2a–d** and **3a–d** (100 μg), and 50 μl of a 5% (w/v) solution of NaOAc in glacial AcOH was added [^{125}I]NaI (500 μCi), followed by 50 μl of an oxidant solution consisting of a 2:1 mixture (v/v) of H_2O_2 in (30%) AcOH. After stirring at room temperature for 10 min, the reaction was terminated by the addition of 25 μl of an aqueous 5% $NaHSO_3$ solution (w/v). The mixture was extracted with CH_2Cl_2 and dried under a stream of nitrogen. The residue (450 μCi, 90%) was dissolved in MeOH and purified on an analytical C-18 reverse phase HPLC Altech column operated at a flow rate of 1 ml/min. Elution with 75:25 MeOH–H_2O gave [^{125}I]**5a** ($t_R = 19$ min); [^{125}I]**4a** ($t_R = 22$ min); [^{125}I]**5b** ($t_R = 29$ min); [^{125}I]**4b** ($t_R = 29$ min). The retention time of free iodine was 4 min.

AR binding assay

Affinity of the androgen derivatives was determined by a competitive binding assay and is expressed as the relative binding affinity (RBA). The RBA is defined as 100 times the ratio between the competitor and the

unlabeled 5α-DHT concentrations required for 50% competition to specific [^{3}H]5α-DHT binding. Uterine cytoplasmic extracts were incubated at 0–4°C for 18 h with 20 nM of [^{3}H]5α-DHT in the absence and presence of competitive steroids ranging from 2.5 nM to 25 μM. The bound steroid was separated from free steroid by Sephadex LH-20 chromatography. The non-specific binding (equivalent to that observed in the presence of a 100-fold excess of unlabeled 5α-DHT) was 7% of the total binding which was subtracted from the total binding to estimate the specific binding.

PgR binding assay

RBA of the steroids for PgR were determined by a competitive binding assay. The RBA is defined as 100 times the ratio between competitor and the unlabeled R5020 concentrations required for 50% competition to specific [^{3}H]R5020 binding. Uterine cytoplasmic extracts were incubated at 0–4°C for 18 h with 50 nM of [^{3}H]R5020 in the absence and presence of competitive steroids ranging from 5 nM to 5 μM. The bound steroid was separated from free steroid by Sephadex LH-20 chromatography. The non-specific binding (equivalent to that observed in the presence of a 100-fold excess of unlabeled R5020) was 5% of the total binding which was subtracted from the total binding to estimate the specific binding. The specific binding (average of three experiments) in the receptor preparation was equivalent to 13.3 nM.

In vivo studies

The animal experiments were conducted in accordance with the recommendations of the Canadian Council on Animal Care and of the in-house Ethics Committee for Animal Experiments. The ventral and dorsal prostate of adult male Sprague–Dawley rats (150–200 g) were used as a model to measure the AR localization potential of the compounds under study. To suppress the endogenous production of testosterone the animals were conditioned by subcutaneous injection of 1 mg diethylstilbestrol (DES) in 200 μl of sun flower oil the day before and the morning of the experiment.

The PgR localization potential of these compounds was estimated in immature female Sprague–Dawley rats. To block ER receptors, animals were primed by i.p. administration of 5 μg of estradiol in 100 μl of 5% ethanol sun flower oil on 3 successive days and used on the fourth day. The ^{125}I labeled steroids were injected i.v. through the tail vein (0.2 ml, 111 kBq). The radiopharmaceuticals were dissolved in ethanol and diluted with physiological saline (0.9% NaCl in H_2O) containing 1% Tween-80 to give a final ethanol concentration of 9%. To evaluate receptor-mediate uptake, 60 μg of testosterone or 15.5 μg of progesterone were used in the appropriate animals, to block the adrenogenic and progesterone receptors, respectively. Animal were sacrificed under deep halothane anesthesia by severing the axillary artery, followed by chest opening [40]. Blood was collected, tissues of interest were removed, washed with physiological saline, and blotted dry, and samples were weighted. The radioactivity was counted in a Model 1282 Compugamma gamma-counter (LKB Wallac, Finland) and radioactivity concentration were expressed as percent of the injected dose per gram of tissue (%ID/g). Statistical variations are presented as the standard error of the mean (SEM) [41].

RESULTS

Chemistry

Synthesis of (17α,20E/Z)iodovinyl derivatives

The synthesis of IVNT has been reported independently by Hoyte *et al.* [34] and Hofmeister *et al.* [35]. In order to obtain the two different isomers the authors used the conventional method involving tri-*n*-butyl tin hydride, with or without catalyst, and different reaction conditions (temp, solvent polarity) to favor formation of either isomer. This method was found suitable for the synthesis of (17α,20E/Z)iodovinyl derivatives of 19-nortestosterone and testosterone. The synthesis of the 7α-methyl derivatives of the 20E isomer, i.e. 7α-Me-(17α,20E)IVNT, has been reported by Salman and Chamness [38]. However they were not able to isolate the corresponding 20Z isomer but subsequently Hoyte *et al.* [39] reported the synthesis of both 17α,20E/Z isomers. In our own hands, isolation of 7α-Me-(17α,20Z)IVNT was also unsuccessful using the conventional synthetic route [34,35–39,42,43]. As we previously observed with the analogous estrogen series, this problem can be overcome using alternative reaction conditions and by changing the catalyst. Napolitano *et al.* [44] reported the selective synthesis of either the 20E or 20Z isomer using UV light or thermal reaction conditions. Nozaki *et al.* [45] reported a more convenient method for the formation of the 20E/Z vinylstannes from the acetylenic compound using triethylborane as a catalyst. We previously adapted this method for the synthesis of 2F- and 4F-(17α,20E/Z)iodovinyl-estradiol derivatives [46]. Likewise we found that 7α-Me-17α-ethynyl-19-nortestosterone and 7α-Me-17α-ethynyltestosterone readily reacted with tri-*n*-butyl tin hydride in the presence of the triethyl borane (catalyst) to yield a mixture of isomeric (17α,20E/Z)vinylstannes in moderate to high yield (Scheme-I). The reaction also gave products resulting from the tin hydride addition to the double bond of the 4-en-3-one (10–15%). The latter adducts were readily distinguishable from the 17α-ethynyl addition products by the absence of the appropriate UV absorbance. The reaction was completed at room temp over a period of 15 min to 1 h, depending on the concentration and the type of substrate used and the isomeric ratio required. Prolonged reaction times or increased amounts of tributyl stannyl hydride in the

reaction mixture resulted in a decrease in the isomeric 20Z/E ratio. Similar effects were also observed upon varying the reaction temp. The 20Z isomers are less polar, with R_f values (silica gel TLC, hexane–ethylacetate) slightly higher than those observed for the corresponding 20E isomers and longer retention times on reverse-phase HPLC (water–methanol). The tin intermediates were characterized via mass spectroscopy by their weak molecular ion, or base peak corresponding to loss of C_4H_9 and by the presence of characteristic tin-isotope clusters. Addition of a 0.1 M solution of iodine in chloroform to the isomeric tin intermediates resulted in an immediate destannylation to give (17α,20E/Z)iodovinyl derivatives, with retention of configuration, in overall high yields. The iodovinyl derivatives gave the expected molecular ion peak in the mass spectrum and their assigned configuration was confirmed by the ^{1}H NMR coupling constants of the vinyl protons (20E isomer, J = 14Hz; 20Z isomer, J = 8Hz). The 20Z isomers are in general less polar than the 20E isomers and possess different solubility properties.

Biological Properties

Binding affinities for AR and PgR

RBA were determined by competitive binding assays in the case of AR vs [^{3}H]5α-DHT (RBA = 100) (murine uterine cytoplasmic extracts) and in the case of PgR vs [^{3}H]R5020 (RBA = 100) for PgR (murine uterine cytoplasmic extracts). The results are presented in Table 1. The AR and PgR binding affinities of the 19-nortestosterone derivatives are in general higher than those of the corresponding testosterone analogs. In general, the 20Z isomers showed higher PgR binding values than the corresponding 20E isomers. The AR binding values of the 20E isomers were either the same as those observed for the 20Z isomers or higher, except for the 7α-Me-(17α,20)IVNT where the 20Z gave a higher AR binding value than the 20E isomer.

In vivo tissue distribution studies in adult male rats. In order to suppress *in vivo* androgen biosynthesis and increase the concentration of the AR in target tissue, male Sprague–Dawley rats were treated with 1 mg of DES in 0.2 ml sunflower oil at 24 h and 3–5 h prior to injection of the radiolabeled androgens [47]. The purified ^{125}I labeled (17α,20E/Z)IVNT and their 7α-methyl analogs ([^{125}I]**4a**, [^{125}I]**5a**, [^{125}I]**4b** or [^{125}I]**5b**) were i.v. injected and the radioactivity concentration (%ID/g) in the various organs was measured at different intervals (0.5, 1, 2, 4 and 6 h) postinjection (Tables 2 and 3). To ascertain that the uptake was receptor mediated one group of rats was also given 60 μg of unlabeled testosterone to occupy the AR (Tables 2 and 3; 2 h, blocked). The target to blood and to non-target ratios are also presented in these tables. The prostate uptake measured at 2 h p.i. in DES-treated and untreated animals, as well as testosterone blocked animals does not show any significant variations. There is considerable activity in liver and kidney, known organs for metabolism and excretion of steroids. Thus the overall organ uptake does not appear to be very selective.

In vivo tissue distribution studies in estrogen-primed immature rats. A similar study as described above for the male rats was performed in immature, 21–25 days old female Sprague–Dawley rats, primed with estradiol to increase the PgR concentration of the uteri. One group of rats were coinjected with 15.5 μg of ORG2058 to block the PgR binding site. ORG2058 is a highly selective ligand for the PgR which does not affect other steroid receptors (glucocorticoids, mineralocorticoids) present in rat tissue [48]. The uterus to blood and non-target (muscle, spleen and lung) ratios are presented in Tables 4 and 5. High PgR-mediated uterus uptake values of 4–5 %ID/g (1 h p.i.) were observed with the (17α,20Z)IVNT [Fig. 1(a)]. The same derivative showed advantageous uterus to non-target ratios as well [Fig. 1(b)]. The persistent high concentration of ^{125}I in the blood results in relatively low uterus to blood ratios. Thyroid uptake of the different [^{125}I]IVNT

Table 1. RBA of testosterone and 19-nortestosterone analogs for AR and PgR

Compound	X	R	AR	PgR
1c	H	C≡CH	14.0 (1.1)	0.7 (0.2)
4c	H	(Z)CH=CHI	11.9 (2.5)	4.2 (0.2)
5c	H	(E)CH=CHI	30.5 (2.3)	0.7 (0.1)
1d	CH_3	C≡CH	3.3 (0.5)	1.2 (0.1)
4d	CH_3	(Z)CH=CHI	20.8 (4.4)	2.6 (0.1)
5d	CH_3	(E)CH=CHI	23.5 (5.4)	0.7 (0.1)
1a	H	C≡CH	16.2 (3.0)	10.4 (1.8)
4a	H	(Z)CH=CHI	41.0 (0.3)	92.7 (11)
5a	H	(E)CH=CHI	43.4 (13.5)	9.1 (1.8)
	CH_3	H	43.5 (4.1)	4.4 (0.6)
1b	CH_3	C≡CH	55.6 (3.8)	10.6 (1.8)
4b	CH_3	(Z)CH=CHI	54.0 (1.45)	64.5 (7.0)
5b	CH_3	(E)CH=CHI	26.1 (1.9)	3.3 (0.4)

RBA were determined relative to 5α-DHT (RBA = 100) for AR and relative to R5020 (RBA = 100) for PgR. Values are the average of at least three determinations and are reported together with the standard deviation (SD). Various concentrations of unlabeled steroid were incubated with [^{3}H]5α-DHT or [^{3}H]5020 in murine uterine cytoplasmic extracts and the concentration required for 50% competition were used to calculate RBA values.

Table 2. Tissue distribution of the isomeric (17α,20E/Z)-[125I]IVNT in adult male Sprague–Dawley rats

Tissue	0.5 h	1 h	2 h	2 h (+Test.)	2 h (−DES)	4 h	6 h
	%ID/g (SE)[a]						
	[125I](20E-isomer) (5a)						
Blood	0.06 (0.00)	0.07 (0.01)	0.03 (0.00)	0.05 (0.01)	0.04 (0.01)	0.03 (0.00)	0.03 (0.01)
Ventral prostate	0.21 (0.01)	0.16 (0.02)	0.09 (0.01)	0.10 (0.02)	0.09 (0.03)	0.07 (0.01)	0.04 (0.01)
Dorsal prostate	0.20 (0.01)	0.16 (0.04)	0.09 (0.00)	0.07 (0.01)	0.10 (0.01)	0.06 (0.01)	0.04 (0.01)
Thyroid	4.53 (0.39)	9.45 (1.89)	12.36 (1.31)	14.31 (1.89)	10.00 (1.46)	32.69 (5.07)	59.27 (13.74)
Fat	0.72 (0.07)	0.83 (0.13)	0.45 (0.04)	0.55 (0.07)	0.55 (0.11)	0.32 (0.02)	0.17 (0.02)
Muscle	0.17 (0.03)	0.14 (0.04)	0.03 (0.00)	0.05 (0.01)	0.04 (0.01)	0.03 (0.01)	0.03 (0.01)
Spleen	0.13 (0.02)	0.18 (0.04)	0.05 (0.01)	0.06 (0.02)	0.09 (0.02)	0.06 (0.02)	0.05 (0.02)
Kidneys	0.22 (0.01)	0.16 (0.02)	0.06 (0.00)	0.09 (0.01)	0.08 (0.01)	0.06 (0.01)	0.05 (0.01)
Lungs	0.16 (0.01)	0.10 (0.01)	0.05 (0.01)	0.05 (0.00)	0.05 (0.01)	0.03 (0.00)	0.04 (0.01)
Liver	0.49 (0.01)	0.27 (0.01)	0.21 (0.02)	0.20 (0.03)	0.21 (0.02)	0.14 (0.05)	0.08 (0.01)
VP/blood	3.39 (0.31)	2.38 (0.14)	2.86 (0.42)	2.10 (0.16)	2.38 (0.36)	2.06 (0.47)	1.42 (0.22)
DP/blood	3.11 (0.20)	2.24 (0.23)	2.64 (0.22)	1.59 (0.05)	2.69 (0.32)	1.94 (0.36)	1.58 (0.46)
VP/non-target[b]	1.43 (0.17)	1.18 (0.06)	2.09 (0.15)	1.89 (0.10)	1.50 (0.30)	1.84 (0.16)	0.96 (0.14)
DP/non-target[b]	1.31 (0.12)	1.15 (0.24)	1.95 (0.07)	1.44 (0.11)	1.66 (0.12)	1.78 (0.21)	1.01 (0.12)
	[125I](20Z-isomer) (4a)						
Blood	0.36 (0.02)	0.22 (0.01)	0.24 (0.02)	0.30 (0.02)	0.24 (0.02)	0.24 (0.03)	0.17 (0.02)
Ventral prostate	0.48 (0.01)	0.35 (0.05)	0.26 (0.01)	0.28 (0.01)	0.26 (0.03)	0.18 (0.02)	0.11 (0.01)
Dorsal prostate	0.46 (0.03)	0.33 (0.02)	0.21 (0.02)	0.22 (0.03)	0.28 (0.09)	0.15 (0.02)	0.17 (0.04)
Thyroid	28.91 (3.07)	42.71 (4.98)	88.28 (13.61)	96.03 (17.82)	77.15 (11.30)	146.35 (10.28)	171.15 (31.42)
Fat	2.21 (0.51)	0.97 (0.11)	0.93 (0.17)	0.64 (0.04)	1.55 (0.26)	0.85 (0.30)	0.41 (0.09)
Muscle	0.32 (0.04)	0.22 (0.05)	0.24 (0.05)	0.20 (0.04)	0.21 (0.06)	0.29 (0.01)	0.18 (0.05)
Spleen	0.24 (0.02)	0.18 (0.01)	0.25 (0.11)	0.23 (0.02)	0.17 (0.01)	0.17 (0.03)	0.10 (0.00)
Kidneys	0.57 (0.01)	0.36 (0.02)	0.36 (0.06)	0.42 (0.09)	0.35 (0.03)	0.30 (0.03)	0.22 (0.01)
Lungs	0.37 (0.01)	0.21 (0.01)	0.17 (0.02)	0.22 (0.02)	0.18 (0.03)	0.17 (0.01)	0.12 (0.01)
Liver	1.27 (0.11)	0.76 (0.05)	0.79 (0.09)	0.87 (0.09)	0.78 (0.07)	0.63 (0.08)	0.58 (0.03)
VP/blood	1.35 (0.09)	1.57 (0.17)	1.09 (0.10)	0.95 (0.10)	1.08 (0.14)	0.77 (0.06)	0.66 (0.03)
DP/blood	1.31 (0.10)	1.47 (0.13)	0.88 (0.06)	0.76 (0.08)	1.10 (0.28)	0.54 (0.26)	0.99 (0.21)
VP-non-target[b]	1.55 (0.04)	1.40 (0.14)	1.30 (0.27)	1.29 (0.03)	1.40 (0.20)	0.88 (0.10)	0.90 (0.15)
DP/non-target[b]	1.51 (0.13)	1.36 (0.26)	1.03 (0.16)	1.01 (0.07)	1.47 (0.48)	0.55 (0.25)	1.43 (0.50)

*Mean ^{125}I organ uptake in % injected dose per gram of tissue (%ID/g) and standard error (SE) for untreated (−DES) or DES primed adult Sprague–Dawley rats ($n = 3$), after injection of 3 μCi (111 kBq) of ^{125}I-labeled steroid in the presence (+Test) or absence of 60 μg of coinjected testosterone.

[b]Non-target organs include the muscle, spleen and lungs.

Table 3. Tissue distribution of the isomeric 7α-Me-(17α,20E/Z)[[^{125}I]IVNT in adult male Sprague–Dawley rats

Tissue	%ID/g (SE)[a] 0.5 H	1 h	2 h (+Test.)	2 h (−DES)	2 h	4 h	6 h
[^{125}I](20E-isomer) (5b)							
Blood		0.17 (0.01)	0.16 (0.02)	0.19 (0.01)	0.15	0.12	0.11 (0.01)
Ventral prostate		0.24 (0.02)	0.17 (0.00)	0.20 (0.02)	0.19	0.12	0.09 (0.01)
Dorsal prostate		0.33 (0.00)	0.15 (0.01)	0.20 (0.00)	0.16	0.10	0.09 (0.01)
Thyroid		23.45 (6.01)	49.85 (12.98)	134.04 (23.10)	63.35	184.58	163.23 (40.27)
Fat		0.71 (0.03)	0.81 (0.07)	0.78 (0.01)	1.34	0.35	0.51 (0.13)
Muscle		0.15 (0.00)	0.19 (0.07)	0.29 (0.05)	0.11	0.05	0.23 (0.04)
Spleen		0.17 (0.00)	0.17 (0.05)	0.19 (0.03)	0.12	0.08	0.13 (0.02)
Kidneys		0.32 (0.00)	0.25 (0.03)	0.27 (0.03)	0.22	0.13	0.13 (0.00)
Lungs		0.22 (0.00)	0.17 (0.01)	0.17 (0.02)	0.35	0.10	0.07 (0.03)
Liver		0.80 (0.07)	0.56 (0.03)	0.58 (0.03)	0.70	0.37	0.27 (0.06)
VP/blood		1.39 (0.23)	1.13 (0.09)	1.09 (0.12)	1.27	1.00	0.87 (0.13)
DP/blood		1.93 (0.19)	0.97 (0.14)	1.07 (0.04)	1.07	0.83	0.88 (0.06)
VP/non-target[b]		1.31 (0.13)	0.98 (0.06)	0.95 (0.09)	0.98	1.57	0.64 (0.07)
DP/non-target[b]		1.83 (0.06)	0.84 (0.11)	0.94 (0.08)	0.83	1.30	0.68 (0.12)
[^{125}I](20Z-isomer) (4b)							
Blood	0.26 (0.01)	0.24 (0.01)	0.22 (0.02)	0.24 (0.02)	0.25 (0.03)	0.22 (0.03)	0.16 (0.01)
Ventral prostate	0.41 (0.04)	0.40 (0.05)	0.31 (0.03)	0.22 (0.02)	0.31 (0.09)	0.18 (0.01)	0.13 (0.00)
Dorsal prostate	0.52 (0.03)	0.51 (0.06)	0.56 (0.04)	0.25 (0.03)	0.44 (0.01)	0.38 (0.01)	0.37 (0.02)
Thyroid	86.93 (8.21)	105.76 (7.13)	219.02 (3.52)	220.27 (30.57)	234.33 (44.78)	426.76 (76.47)	863.46 (75.54)
Fat	0.61 (0.05)	0.93 (0.02)	0.47 (0.00)	0.46 (0.05)	0.54 (0.02)	0.34 (0.02)	0.28 (0.10)
Muscle	0.18 (0.01)	0.19 (0.05)	0.21 (0.07)	0.11 (0.03)	0.10 (0.02)	0.13 (0.01)	0.07 (0.01)
Spleen	0.18 (0.00)	0.19 (0.02)	0.17 (0.02)	0.18 (0.01)	0.23 (0.01)	0.21 (0.05)	0.10 (0.01)
Kidneys	0.39 (0.01)	0.31 (0.02)	0.31 (0.09)	0.17 (0.02)	0.28 (0.01)	0.22 (0.00)	0.13 (0.01)
Lungs	0.29 (0.00)	0.22 (0.01)	0.17 (0.01)	0.15 (0.01)	0.18 (0.02)	0.14 (0.01)	0.09 (0.03)
Liver	0.81 (0.11)	0.72 (0.13)	0.31 (0.04)	0.28 (0.02)	0.66 (0.15)	0.23 (0.03)	0.19 (0.03)
VP/blood	1.56 (0.10)	1.62 (0.14)	1.40 (0.04)	0.90 (0.08)	1.30 (0.16)	0.91 (0.07)	0.82 (0.05)
DP/blood	1.98 (0.08)	2.13 (0.32)	2.54 (0.35)	1.11 (0.11)	1.88 (0.62)	1.72 (0.92)	2.52 (1.53)
VP/non-target[b]	2.00 (0.08)	1.99 (0.22)	1.76 (0.30)	1.14 (0.30)	2.15 (0.08)	1.25 (0.17)	1.49 (0.33)
DP/non-target[b]	2.54 (0.02)	2.65 (0.51)	3.05 (0.24)	1.33 (0.21)	2.92 (0.61)	2.17 (1.00)	4.41 (2.40)

[a]Mean ^{125}I organ uptake in % injected dose per gram of tissue (%ID/g) and standard error (SE) for untreated (−DES) or DES primed adult Sprague–Dawley rats ($n = 3$), after injection of 3 μCi (111 kBq) of ^{125}I-labeled steroid in the presence (+Test) or absence of 60 μg of coinjected testosterone.

[b]Non-target organs include the muscle, spleen and lungs.

steroids varied substantially, suggesting *in vivo* instability of the C—I bond. It can be seen that the configuration about the 20-position affects *in vivo* instabilities of the radioligand. In general the blood clearance of the [125I]IVNT derivatives featuring the 20Z configuration is slower than that of the analogs with the 20E configuration. There is also high uptake by various non-target organs, suggesting metabolism and excretion of the steroids via the liver and the kidneys. Binding to corticoid receptors may also account for some of the uptake by organs rich in these receptors [49, 50]. The high fat uptake suggests that non-specific uptake may be due to the high lipophilicity of the IVNT derivatives.

Table 4. Tissue distribution of the isomeric (17α,20E/Z)-[125I]IVNT in immature female Sprague–Dawley rats

Tissue	%ID/g (SE)[a] Time in hours 1	2	2 block	4	6
	[125I](20E-isomer) (5a)				
Uterus	0.72	0.49	0.60	0.36	0.21
	(0.10)	(0.10)	(0.05)	(0.04)	(0.03)
Blood	1.50	1.19	1.41	0.94	0.56
	(0.14)	(0.18)	(0.14)	(0.17)	(0.10)
Thyroid	32.78	72.90	75.48	89.93	161.56
	(12.95)	(9.62)	(28.75)	(26.77)	(21.63)
Fat	1.49	0.98	1.66	1.22	0.72
	(0.18)	(0.48)	(0.10)	(0.02)	(0.04)
Muscle	0.59	0.51	0.47	0.46	0.23
	(0.03)	(0.11)	(0.02)	(0.11)	(0.03)
Kidneys	1.82	1.22	1.58	1.08	0.57
	(0.27)	(0.16)	(0.31)	(0.19)	(0.14)
Spleen	0.60	0.44	0.47	0.44	0.30
	(0.06)	(0.06)	(0.06)	(0.17)	(0.07)
Lungs	0.77	0.58	1.01	0.52	0.32
	(0.20)	(0.24)	(0.01)	(0.10)	(0.06)
Liver	2.59	2.14	2.38	1.54	1.08
	(0.30)	(0.37)	(0.41)	(0.27)	(0.22)
Uterus/blood	0.47	0.41	0.44	0.40	0.39
	(0.02)	(0.02)	(0.05)	(0.03)	(0.04)
Uterus/non-target[b]	1.13	1.06	0.93	0.82	0.77
	(0.19)	(0.23)	(0.10)	(0.11)	(0.05)
	[125I](20Z-isomer) (4a)				
Uterus	4.22	2.88	1.26	1.73	1.33
	(0.18)	(0.26)	(0.25)	(0.22)	(0.12)
Blood	2.45	1.94	2.04	1.64	1.61
	(0.17)	(0.16)	(0.42)	(0.28)	(0.18)
Thyroid	130.18	383.87	296.20	568.05	1192.75
	(36.14)	(129.34)	(88.94)	(95.41)	(375.37)
Fat	2.20	1.85	1.69	1.02	1.01
	(0.19)	(0.22)	(0.45)	(0.16)	(0.06)
Muscle	1.14	1.13	0.92	0.69	1.09
	(0.05)	(0.08)	(0.31)	(0.13)	(0.10)
Kidneys	2.84	2.26	2.42	1.65	2.35
	(0.12)	(0.17)	(0.58)	(0.56)	(0.89)
Spleen	1.91	2.64	2.07	1.76	2.19
	(0.21)	(0.49)	(0.93)	(0.87)	(0.55)
Lungs	1.71	1.48	1.15	0.96	0.79
	(0.12)	(0.21)	(0.35)	(0.17)	(0.04)
Liver	6.01	3.93	3.72	2.68	2.16
	(0.21)	(0.84)	(0.79)	(0.67)	(0.24)
Uterus/blood	1.73	1.48	0.62	1.07	0.83
	(0.05)	(0.03)	(0.04)	(0.09)	(0.04)
Uterus/non-target[b]	2.67	1.72	1.03	1.73	1.05
	(0.13)	(0.20)	(0.20)	(0.34)	(0.22)

[a]Mean organ uptake in % injected dose per gram of tissue (%ID/g) and standard error (SE) for estradiol-primed immature female Sprague–Dawley rats (n = 3–5), after injection of 3 μCi (111 kBq) of 125I-labeled steroid in the presence (block) or absence of 15.5 μg of coinjected progestin (ORG 2058).

[b]Non-target organs include the muscle, spleen and lungs.

Table 5. Tissue distribution of the isomeric 7α-Me-(17α,20E/Z)-[125I]IVNT in immature female Sprague–Dawley rats

Tissue	%ID/g (SE)[a] Time in hours 1	2	2 block	4	6
	[125I](20E-isomer) (5b)				
Uterus	1.03	0.81	0.80	0.65	0.59
	(0.01)	(0.06)	(0.03)	(0.05)	(0.10)
Blood	0.66	0.67	0.81	0.74	0.72
	(0.04)	(0.03)	(0.07)	(0.06)	(0.11)
Thyroid	72.01	147.29	207.28	408.85	481.79
	(11.99)	(19.41)	(41.75)	(56.48)	(112.89)
Fat	1.33	2.17	1.49	1.38	1.29
	(0.10)	(0.29)	(0.21)	(0.18)	(0.21)
Muscle	0.82	1.60	0.91	0.55	0.65
	(0.07)	(0.65)	(0.09)	(0.06)	(0.19)
Kidneys	1.50	1.37	1.91	0.92	0.84
	(0.01)	(0.05)	(0.73)	(0.14)	(0.15)
Spleen	0.92	1.31	1.08	1.12	0.98
	(0.09)	(0.19)	(0.21)	(0.27)	(0.12)
Lungs	0.98	0.82	0.92	0.65	0.60
	(0.01)	(0.06)	(0.11)	(0.05)	(0.11)
Liver	4.33	2.95	3.17	2.23	1.50
	(0.35)	(0.25)	(0.11)	(0.21)	(0.41)
Uterus/blood	1.57	1.20	0.99	0.88	0.81
	(0.10)	(0.03)	(0.06)	(0.06)	(0.03)
Uterus/non-target[b]	1.13	0.65	0.84	0.87	0.80
	(0.02)	(0.02)	(0.07)	(0.08)	(0.02)
	[125I](20Z-isomer) (4b)				
Uterus	2.99	2.70	1.18	1.75	0.93
	(0.19)	(0.10)	(0.05)	(0.24)	(0.12)
Blood	1.23	1.33	1.23	1.07	0.81
	(0.07)	(0.13)	(0.07)	(0.06)	(0.09)
Thyroid	63.90	283.63	376.48	723.53	1265.75
	(16.30)	(75.99)	(70.11)	(529.23)	(177.54)
Fat	1.55	1.37	1.13	0.98	0.64
	(0.27)	(0.15)	(0.06)	(0.01)	(0.08)
Muscle	1.35	0.87	1.00	0.71	0.87
	(0.16)	(0.07)	(0.11)	(0.14)	(0.14)
Kidneys	1.55	2.45	2.43	1.28	1.54
	(0.18)	(0.38)	(0.24)	(0.26)	(0.22)
Spleen	1.72	2.85	3.2	1.60	1.92
	(0.17)	(0.40)	(0.13)	(0.31)	(0.26)
Lungs	1.02	0.93	0.97	0.73	0.50
	(0.05)	(0.04)	(0.02)	(0.02)	(0.05)
Liver	3.26	2.44	2.35	2.61	1.25
	(0.32)	(0.20)	(0.22)	(0.93)	(0.04)
Uterus/blood	2.45	2.09	0.96	1.65	1.14
	(0.15)	(0.15)	(0.02)	(0.31)	(0.07)
Uterus/non-target[b]	2.20	1.74	0.71	1.73	0.85
	(0.16)	(0.17)	(0.01)	(0.01)	(0.07)

[a]Mean organ uptake in % injected dose per gram of tissue (%IS/g) and standard error (SE) for estradiol-primed immature female Sprague–Dawley rats (n = 3–5), after injection of 3 μCi (111 kBq) of 125I-labeled steroid in the presence (block) or absence of 15.5 μg of coinjected progestin (ORG 2058).

[b]Non-target organs include the muscle, spleen and lungs.

DISCUSSION

In the estrogen series, substitution at the 16α- or 17α-position of the D-ring produces radiolabeled ligands with high affinity for ER. Thus, 16α-iodoestradiol and its 11β-methoxy analog labeled with ^{125}I are excellent ligands for quantifying the ER in breast tumor biopsy specimens whereas the ^{123}I-analog has been proposed for the *in vivo* probing of the ER receptor [51–54]. The (17α,20)iodovinyl estrogens (IVE_2) exhibit good binding affinities for ER and high *in vivo* stability and furthermore their ^{123}I analogs have been tested in humans for ER imaging [55]. In this work, we present a simple method to obtain the (17α,20E/Z)iodovinyl derivatives of testosterone and 19-nortestosterone (Scheme I). The intermediate steroidal (17α,20E/Z)vinylstannes were obtained using azo*bisiso*butyronitrile or triethyl borane as catalysts, as described previously for the analogous estrogens [46]. The corresponding iodovinyl derivatives were synthesized by iodine-cleavage of the vinylstannyl intermediates and the radioiodinated steroids were obtained using radioiodine produced *in situ* by oxidation of ^{125}I with H_2O_2 [42]. The ^{125}I-labeled derivatives were purified by HPLC, and their identities were confirmed by their chromatographic mobilities which were identical to those of the corresponding unlabeled analogs.

The receptor binding data reveal a systematic pattern of changes in RBA values following structural modifications of the steroid skeleton (Table 1). The 7α-methyl-19-nortestosterone binds strongly to AR (RBA = 43.5) but exhibits relative low affinity for PgR (RBA = 4.4). The AR binding affinity of this compound increased slightly upon addition of the 17α-ethynyl group (43.5 to 55.6) while binding to the PgR augmented as well (4.4 to 10) (Table 1). Introduction of a 7α-methyl group to 19-nortestosterone also improved binding affinity to the AR and in the case of the 17α-ethynyl-19-nortestosterone the RBA increased over 3-fold (55 vs 16), although binding affinity for the

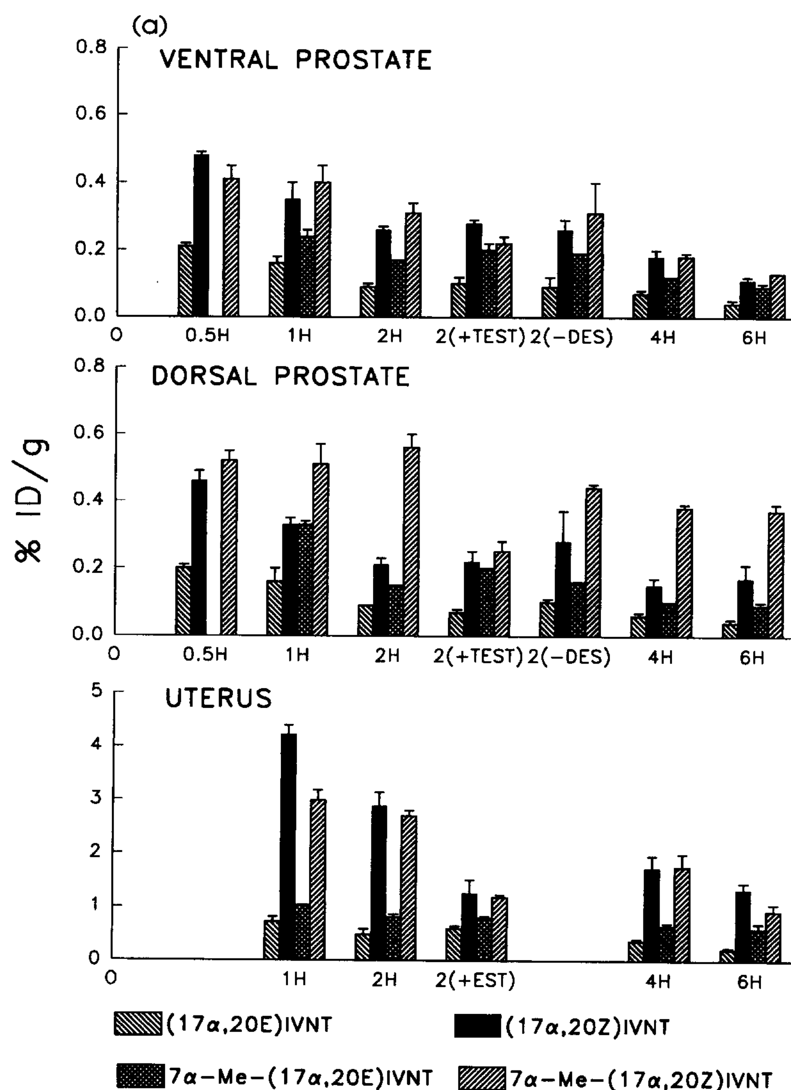


Fig. 1—*caption on p. 25.*

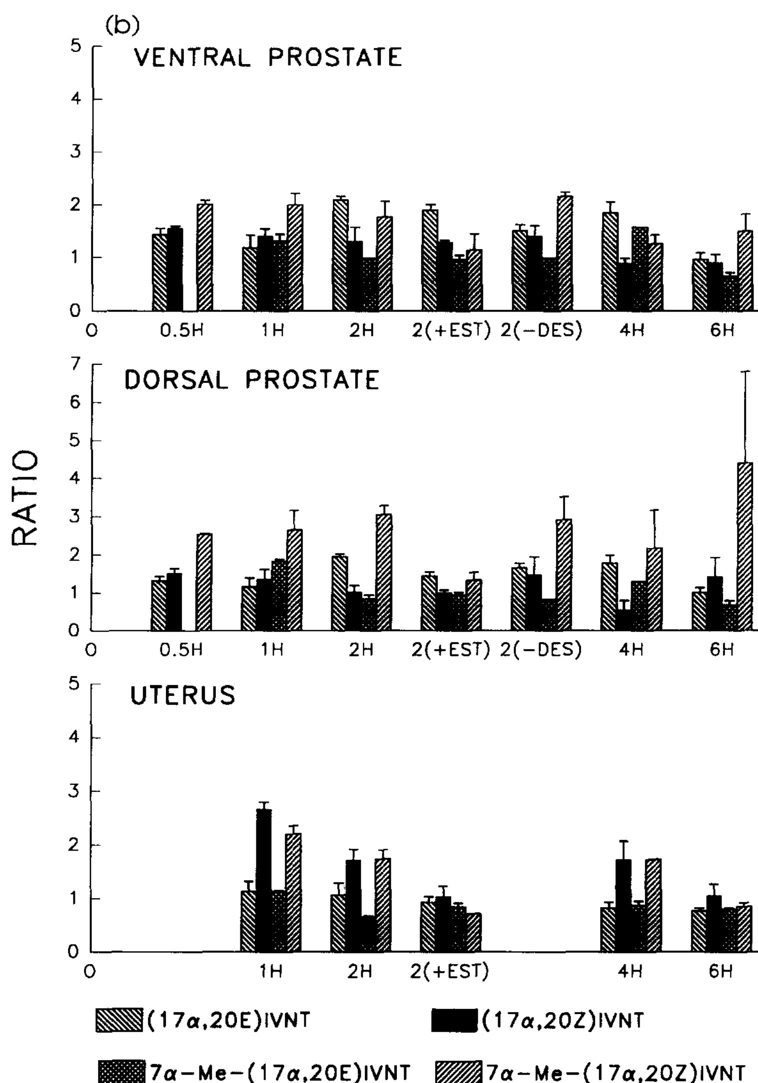


Fig. 1—*caption opposite.*

PgR remained unchanged (RBA ≃ 10). Similarly, it has been reported that mibolerone (7α,17α-dimethyl-19-nortestosterone) binds to AR and PgR, while 17α-methyl-19-nortestosterone shows low affinities for both receptors suggesting that introduction of a 7α-methyl onto androgens enhances AR binding [56, 57]. In the testosterone series, which in general exhibit lower binding affinities than the corresponding 19-nortestosterones, addition of a 7α-methyl to 17α-ethynyltestosterone further decreases the binding affinity for the AR (14 to 3.3) while the PgR binding values are unaffected and remain low (RBA ≃ 0.7). Conversion of the 17α-ethynyl group to an iodovinyl group strongly affects the receptor binding properties. Both isomeric (17α,20E/Z)IVNT exhibited good binding affinities for the AR (43 vs 41) and in accordance with literature data [36] large differences are observed in the PgR binding affinities (9 vs 92). Similar patterns have been reported for ER binding in the case of (17α,20)iodovinyl estrogens, with the 20Z isomer always exhibiting the higher binding affinity [46]. In contrast, in the case of the testosterone derivative, the 20E isomer shows a higher AR binding affinity than the 20Z isomer (30 vs 12) whereas PgR binding values are lower and exhibit a reversed pattern (0.7 vs 4.2). Addition of a 7α-methyl to IVNT increases AR binding affinity in the case of the 20Z isomer (54 vs 41) but lowered the AR binding of the 20E isomer (26 vs 43). PgR binding affinities of both 20E/Z isomers were depressed by 7α-methylation to yield the IVNT. The same pattern, albeit with overall lower RBA values, is observed in the case of the testosterone derivatives (Table 1).

Comparison of the biodistribution pattern of (17α,20E/Z)-[^{125}I]IVNT and 7α-Me-(17α,20E/Z)-[^{125}I]IVNT to evaluate PgR-mediated uptake (uterus) were made in estrogen-primed immature female rats whereas AR-mediated tissue localization (prostate) was evaluated in adult male rats treated with DES [Fig. 1(a)]. The overall tissue distribution pattern of the ^{125}I-labeled compounds were the same in male

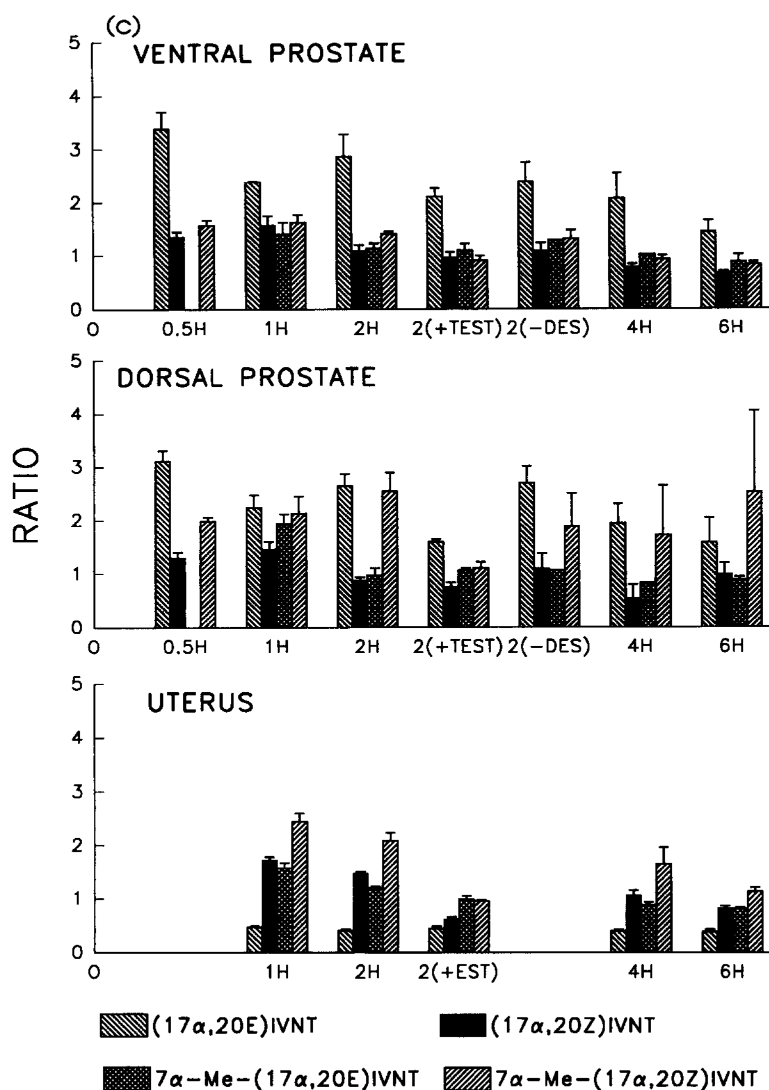
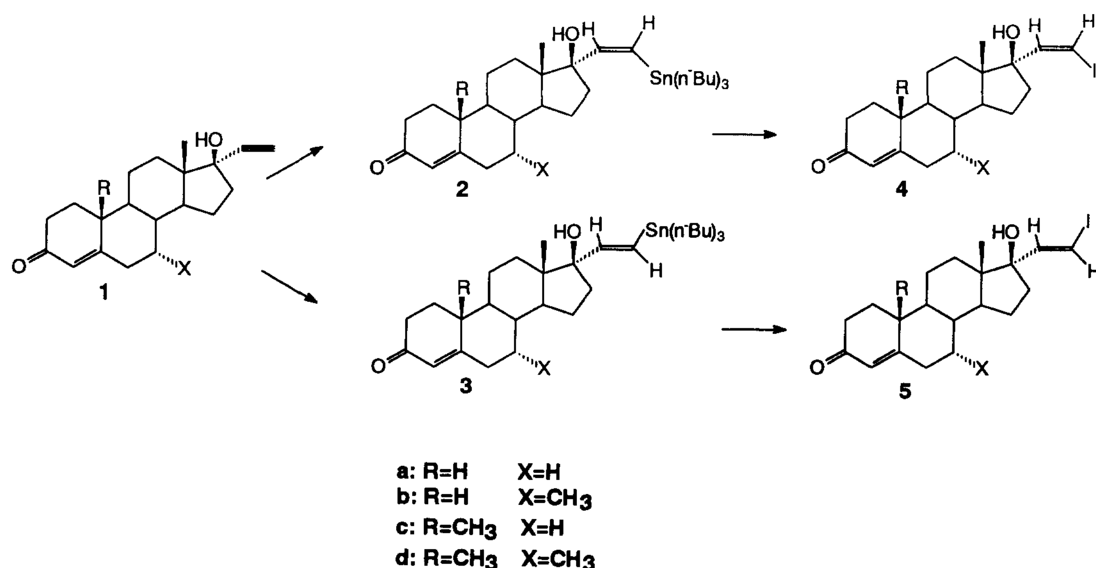


Fig. 1. Target tissue uptake and uptake ratios of (17α,20E/Z)-[^{125}I]IVNT and the corresponding 17α-Me derivative in the prostates of adult male rats and the uteri of immature female rats. (a) %ID/g, (b) target to non-target ratios, (c) target to blood ratios.

or female rats, however the blood radioactivity concentration was consistently higher in the female animals, reflecting their smaller body volume.

The highest uterus uptake was observed with the (17α,20Z)-[^{125}I]IVNT. The 20E isomer of this compound did not exhibit tissue selectivity which correlates well with its 10-fold lower PgR binding affinity as compared to the 20Z isomer (Table 1). Addition of the 7α-methyl group to (17α,20Z)-[^{125}I]IVNT initially reduces the uterus uptake by 29% (1 h p.i.), however 2–6 h p.i. both 20E/Z isomers show identical retention profiles. Addition of a 7α-methyl to (17α,20E)-[^{125}I]IVNT did not affect uterus uptake. The initial decrease in uterus uptake observed upon addition of 7α-methyl group to the 20Z isomer may reflect the decrease (30%) in binding affinity for the PgR (Table 1).

The (17α,20Z)-[^{125}I]IVNT also gave the highest uterus to non-target ratio among the 4 compounds tested, but the highest uterus to blood ratios were recorded with its 7α-methyl derivative [Fig. 1(b) and (c)]. These discrepancies in distribution ratios can be explained by examining the blood clearance (Fig. 2) as well as the fat uptake profiles (Fig. 3) which reveal that addition of the 7α-methyl to the 20Z isomer facilitate blood clearance and lowers fat uptake. The *in vivo* stability of our radioiodinated steroids is reflected in their thyroid uptake profile (Fig. 4) which shows that the 20E isomers are in general more stable than the corresponding 20Z isomers, and also that 7α-methyl substitution does not further affect *in vivo* stability of the parent compound. The higher stability of the 20E-iodovinyl as compared to 20Z-isomer has already been reported in the estrogen series [42, 43].



Scheme 1

Among the ^{125}I-labeled steroids tested for their uptake in the rat prostate, only the 7α-Me-(17α,20Z)IVNT showed AR-mediated retention and this selectivity was mainly associated with the dorsal prostate. At the 0.5 h time point both the 7α-Me-(17α,20Z)IVNT and the derivative lacking a 7α-methyl group show selective prostate retention. However, the ventral prostate rapidly released the ^{125}I in the case of both derivatives whereas the dorsal prostate only retained the ^{125}I associated with the 7α-methyl compound. The best prostate to non-target/blood ratios were however not recorded with these 20Z isomers, instead the (17α,20E)IVNT gave the highest ratios reflecting its rapid blood clearance (Fig. 2) and high *in vivo* stability (i.e. low thyroid uptake, Fig. 4). The liver and kidneys concentrate some of the radioactivity since these organs are actively involved in the metabolism and excretion of steroids. Fat uptake of these hormones plays an important role in their availability for target tissues. Sequestration in adipose tissues will

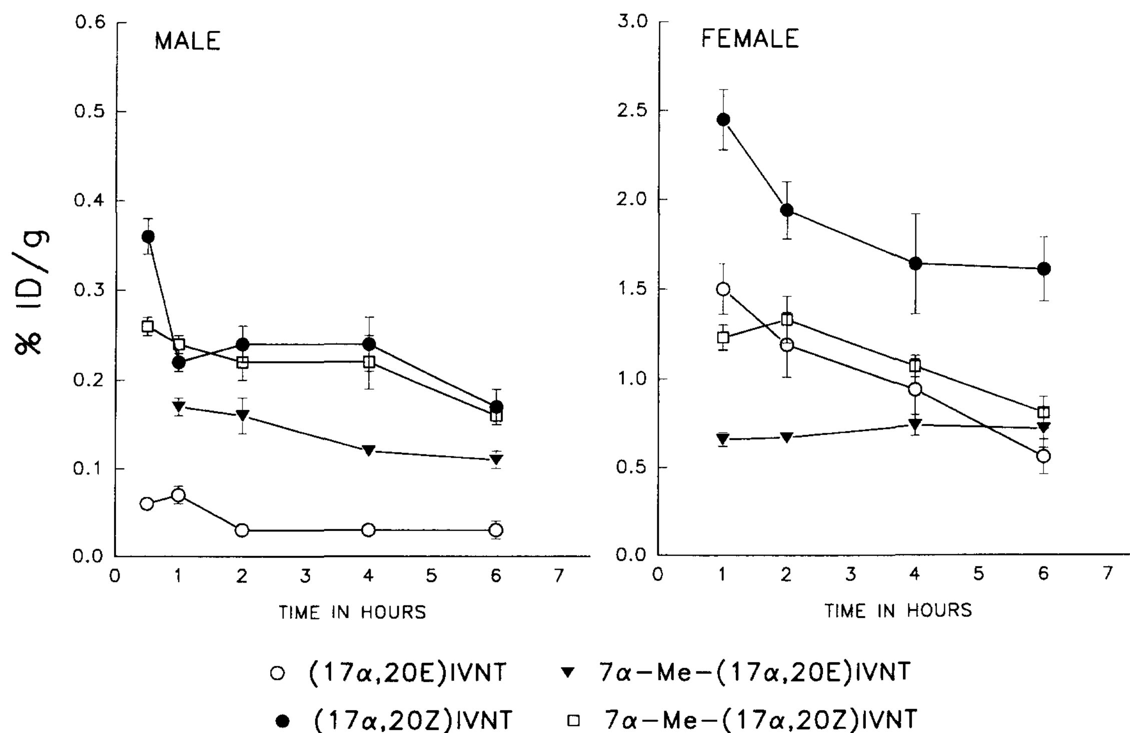


Fig. 2. Blood clearance in %ID/g of (17α,20E/Z)-[^{125}I]IVNT and the corresponding 17α-methyl derivative in adult male (prostate) and immature female estrogen-primed (uterus) rats.

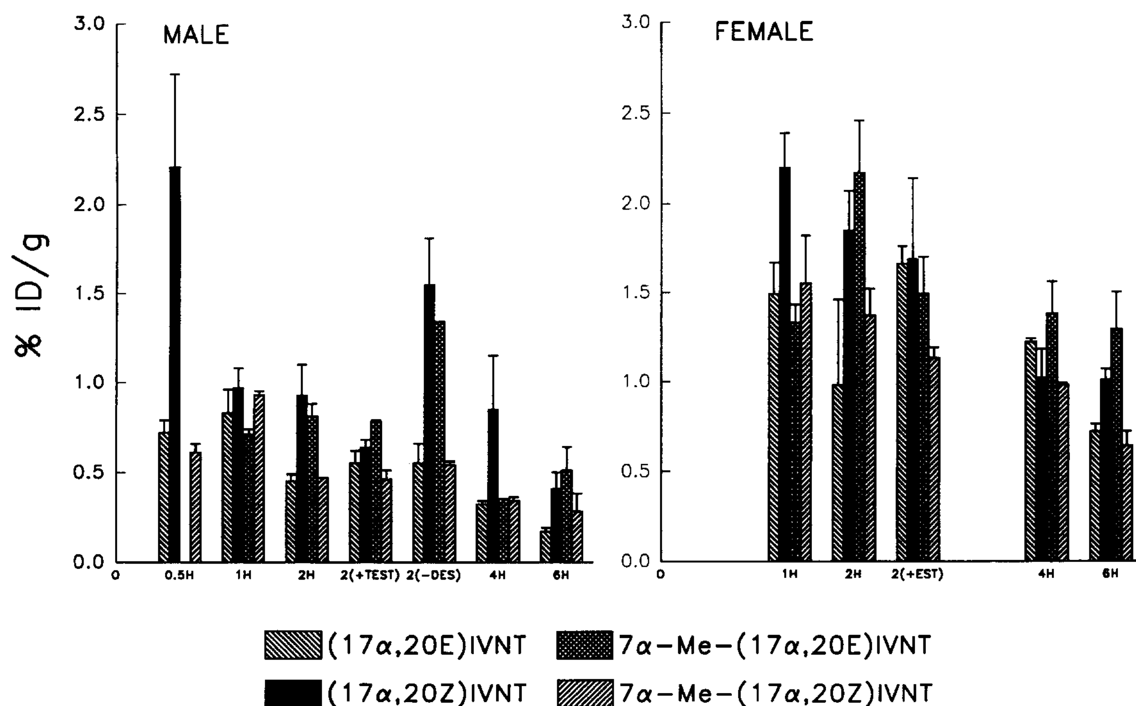


Fig. 3. Fat uptake in %ID/g of (17α,20E/Z)-[^{125}I]IVNT and the corresponding 17α-methyl derivative in adult male (prostate) and immature female estrogen-primed (uterus) rats.

lessen the amount of circulating steroids available for receptor binding, on the other hand it can prolong their availability by increasing their plasmal transition time upon slow release into circulation.

In conclusion, our data show that the (17α,20Z)-IVNT provides the highest *in vivo* PgR-mediated uterus uptake among the steroids tested. Interaction with the AR was less pronounced and only the 7α-Me-(17α,20Z)IVNT revealed AR-mediated dorsal prostate retention. However, their low target specificities make it unlikely that these IVNT derivatives will be useful as nuclear imaging agents.

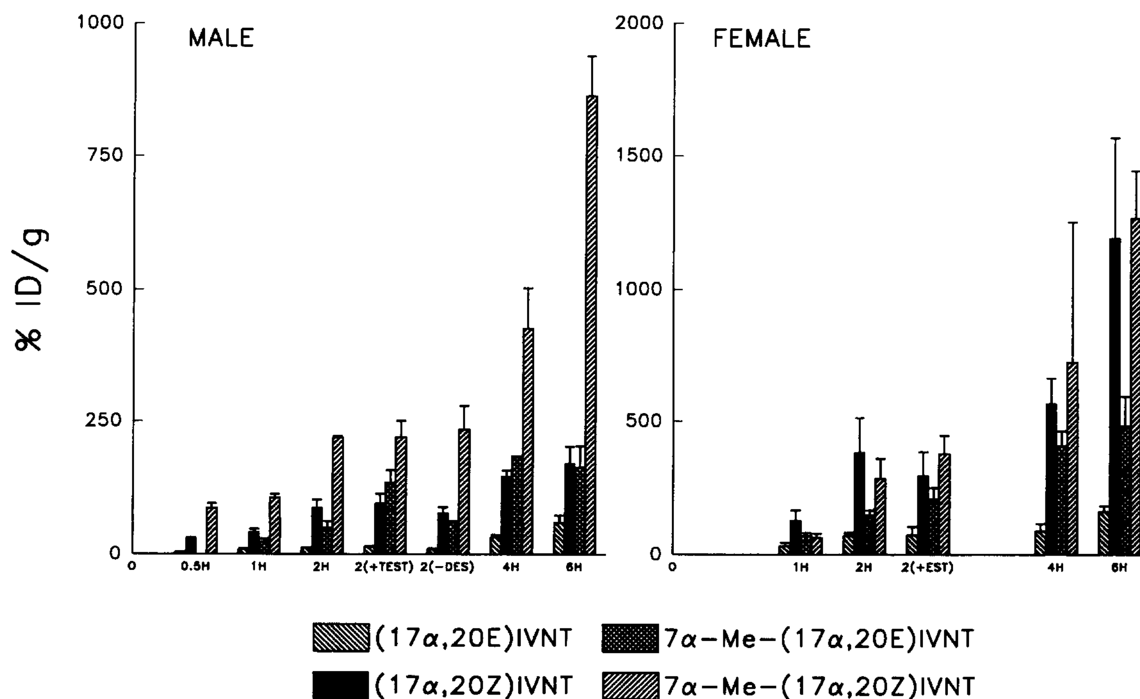


Fig. 4. Thyroid uptake in %ID/g of (17α,20E/Z)-[^{125}I]IVNT and the corresponding 17α-methyl derivatives in adult male (prostate) and immature female estrogen-primed (uterus) rats.

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