Steroids

(17α,20*E/Z*)-Iodovinyl- and 16α-iodo-18-homoestradiol derivatives: synthesis and evaluation for estrogen receptor imaging

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Abstract

Three new ^{125}I-radioiodinated estrogens featuring a 13β-ethyl instead of the natural 13-methyl group, i.e. 18-homoestradiols, were synthesized and evaluated as potential estrogen receptor imaging agents. The 16α-iodo-18-methylestradiol and the ^{125}I-labeled analog were synthesized from the corresponding 16β-bromo analog by the halogen-exchange method. The cis-bromohydrin precursor was obtained by bromination of an estrone enolacetate, followed by epimerization and reduction. The isomeric (17α,20*E/Z*)-iodovinyl-18-methylestradiols were prepared via the vinyltin intermediates. Treatment of 18-methyl-17α-ethynylestradiol with tri-*n*-butyltin hydride, in the presence of azobis*iso*butyronitrile as catalyst and heating at 90–100°C afforded the (17α,20*E*)-tri-*n*-butylstannyl isomer as the major product. Changing the catalyst for triethyl borane, at room temperature, mainly gave the 20*Z*-isomer. The nca ^{125}I-labeled analogs were obtained from their corresponding tin intermediates upon treatment with [^{125}I]NaI in the presence of H_2O_2. The 16α-[^{125}I]iodo- and isomeric (17α,20*E/Z*)-[^{125}I]iodovinyl-18-methylestradiols were evaluated for estrogen receptor-mediated uterine uptake in immature female rats. Homologation of the C13-methyl group did improve the uterine uptake of the iodovinyl derivatives, but also increased blood retention, resulting in lower target uptake ratios. In the case of the 16α-iodo analog uterine retention decreased upon C13-homologation. © 2000 Elsevier Science Inc. All rights reserved.

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1. Introduction

The growth and proliferation of many estrogen-responsive cells is stimulated by estrogens, and tumors derived from these cells often retain this dependency [1,2]. Thus, for the proper management of breast cancer patients, early diagnosis of possible metastases and characterization of the estrogen receptor (ER) status, are of critical importance [3–6]. Analysis of receptor concentrations in biopsy material is routinely used to estimate tumor response to hormonal therapy [7]. Biochemical analyses have however limitations in that the receptor status may have been altered during tissue manipulation. Furthermore, inhomogeneity of the tumor may lead to samples with ER levels that do not represent the overall receptor status of the primary tumor or its metastases [8–10]. In addition to biochemical markers,

current diagnostic procedures include palpation, radiologic, or ultrasonic imaging and surgery [11]. Non-invasive imaging of breast tumors based on their content of hormonal receptors would be a useful adjunct to these methods [12]. Such an approach would require a receptor-ligand, labeled with a radionuclide suitable for imaging, with high affinity for the estrogen receptor, low affinity for non-specific binding sites and good in vivo metabolic stability.

Halogenation of the D-ring of estradiol (E$_2$), especially at the 16α-position, results in compounds with good affinity for the ER. Thus a number of estradiol derivatives were labeled at the 16α-position with radiohalogens [13–21]. The 16α-[^{125}I]iodoestradiol (IE$_2$) is an excellent ligand for the ER and routinely used for the in vitro quantification of ER in biopsy material. Although a few studies with [^{123}I]IE$_2$ have been reported [22,23], rapid metabolism limits the in vivo use of these agents. The ^{18}F-labeled analog, [^{18}F]FE$_2$ is stable and good images of primary and metastatic human breast cancer have been reported [24,25]. However the short half-life of ^{18}F (110 min) limits the availability of this agent

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to dedicated PET facilities. The 17α-iodovinylestradiols (IVE$_2$) were developed as a second generation of radiolabeled steroids with improved in vivo stability [13,26–29]. As expected, iodine attachment via the vinyl carbon renders the IVE$_2$ far less prone to in vivo deiodination as compared to the IE$_2$. Substitution at the 11β-position further increases the stability of the ER-steroid complex and reduces nonspecific binding. Likewise, addition of a 7α-methyl group enhances interaction of estradiol with ER [30–32], and several derivatives with various substituents at these positions have been put forth. The preparation of a series of IVE$_2$ derivatives, their receptor affinities and biodistribution pattern have been reported [33–37]. Only the ^{123}I-labeled 11β-methoxy-IVE$_2$ (MIVE$_2$) has been studied in a clinical setting [38–40].

Substitution of the 13β-methyl group in selected progestin receptor-ligands, for a 13β-ethyl group, increases their relative binding affinity for the receptor [41,42]. Furthermore, 11β-substitution of 13β-ethylgonane derivatives induces reversal of antiprogestational activity of the parent molecule [42]. A number of 13β-ethylsteroid analogs exhibit high affinities for androgen receptors and a 17α-iodovinyl derivative of ORG 33236 has been suggested as a potential receptor-binding radioligand [42]. However, although homologation of the C-13 methyl group in progestins leads to enhanced receptor-binding, for estrogen the opposite is the case [43–45]. Receptor binding is however only one of several parameters that control target tissue uptake and in vivo data on the effect of homologation of the C-13 group in estrogens have not been reported. Therefore, in this study we evaluated the effect of 13β-ethyl substitution of several radioiodinated estradiol derivatives on ER-mediated target tissue localization.

2. Experimental

2.1. General

Melting points (m.p.) were determined on a Fischer–Johns apparatus and are uncorrected. The ^{1}H NMR spectra were recorded on a Brücker AC 300 (300 MHz) in CDCl$_3$ or DMSO-d$_6$. Chemical shifts are referenced to the residual solvent signal (CDCl$_3$ δ = 7.26). Low and high resolution mass spectra (MS, HRMS) were carried out with a VG micromass model ZAB-1F apparatus at 70 eV ionization voltage. Analytical thin-layer chromatography (TLC) was performed with polygram silica gel plates coated with fluorescent indicator (UV$_{254}$) and the compounds were visualized by their UV absorbance and/or color response upon spraying with H$_2$SO$_4$/EtOH and heating at 120°C. Column chromatography was performed on silica gel (60–80 mesh). High-performance liquid chromatography (HPLC) was performed on a reversed-phase column (C-18, ODS-2 spherosorb, 5 μM, 25 × 0.94 cm, CSC, Montreal, Canada). The compounds were detected at 280 nm and where appro-

priate, by their γ-radiation that was registered via a sodium iodide detector.

All chemicals used are commercially available and were of the highest chemical grade. Carrier-free [^{125}I]NaI was purchased from Amersham Canada Ltd. Steroids were purchased from Sigma (St. Louis, MO, USA). All other chemicals were obtained from Aldrich (St. Louis, MO, USA).

2.2. D-(+)-13β-Ethylgon-4-ene-3,17-dione (2)

D(−)norgestrel (1) (1 g, 3.2 mmol) and silver carbonate on celite (5 g, 9.1 mmol) were heated in toluene (60 ml) to reflux for 1.5 h. The mixture was filtered and washed with ethyl acetate. The solvent was evaporated under reduced pressure to furnish compound D-(+)-13β-ethylgon-4-ene-3,17-dione (2) in a quantitative yield: m.p. 175–177°C (lit [46]. 174.5–175.5°C); ^{1}H NMR (CDCl$_3$) δ 0.80 (t, J = 7.5 Hz, 3 H, 13β-CH$_2$CH_3), 5.85 (s, 1 H, 4-CH); MS m/z (rel int) 286 (M$^+$, 62), 258 (45), 91 (100). HRMS calculated for C$_{19}$H$_{26}$O$_2$ 286.1933. Found: 286.1927.

2.3. 3-Hydroxy-18-methyl-estra-1,3,5(10)-trien-17-one (3)

D-(+)-13β-Ethylgon-4-ene-3,17-dione (2) (916 mg, 3.2 mmol) was dissolved in acetonitrile (400 ml) and CuBr$_2$ (1.46 g) and LiBr (287 mg) were added. The dark green mixture was stirred under nitrogen at room temperature for 2 h. Water was added until disappearance of the green color and acetonitrile was evaporated under reduced pressure. The product was extracted with ethyl acetate, dried over sodium sulfate (anhydrous) and concentrated to dryness. The residue was purified by column chromatography over silica gel. Elution with 8% EtOAc in hexane furnished 3-hydroxy 18-methyl-estra-1,3,5(10)-trien-17-one (3) (687 mg, 2.4 mmol; 75%): m.p. 242–245°C (lit [44]. 245–251°C).

2.4. 3,17-Diacetoxy-18-methyl-estra-1,3,5(10),16-tetraene (5)

3-Hydroxy-18-methyl-estra-1,3,5(10)-trien-17-one (3) (775 mg, 2.7 mmol) was dissolved in isopropenyl acetate (40 ml) and p-toluenesulfonic acid (195 mg) was added. The mixture was heated to reflux and stirred overnight. Isopropenyl acetate was evaporated under reduced pressure. The residue was poured into water and products were extracted with ethyl acetate and purified by column chromatography over silica gel with 5% EtOAc in hexane to yield 4 and 5.

2.5. 3,17-Diacetoxy-18-methyl-estra-1,3,5(10),16-tetraene (5)

(124 mg, 0.34 mmol; 12%): m.p. 112–115°C (lit [44]. 114–116°C); ^{1}H NMR (CDCl$_3$) δ 0.79 (t, J = 7.5 Hz, 3 H, 13β-CH$_2$CH$_3$), 2.16 (s, 3 H, 17-OCOCH_3), 2.28 (s, 3 H,

3-OCOC*H₃*), 5.59 (brs, 1 H, 16-*H*), 6.79, 6.84 (2 H, 2 and 4-C*H*), 7.23 (d, 7.8 Hz, 1 H, 1-C*H*); MS *m/z* (rel int) 368 (8, M$^+$), 339 (30, M$^+$ − CHO), 326 (25, M$^+$ − CH$_2$CO), 297 (100, M$^+$ − C$_3$H$_2$O$_2$), 255 (70). HRMS calculated for C$_{23}$H$_{28}$O$_4$ 368.1987. Found: 368.1980.

2.6. 3-Acetoxy-18-methyl-estra-1,3,5(10)-trien-17-one (4)

(283 mg, 0.87 mmol; 32%): m.p. 155–157°C (lit [44]. 156–158°C); ^{1}H NMR (CDCl$_3$) δ 0.79 (t, *J* = 7.5 Hz, 3 H, 13β-CH$_2$C*H₃*), 2.28 (s, 3 H, 3-OCOC*H₃*), 6.80 (d, *J* = 2.2 Hz, 1 H, 4-C*H*), 6.85 (dd, *J* = 2.2 and 9.2 Hz, 1 H, 2-C*H*), 7.29 (d, *J* = 9.2 Hz, 1-C*H*); MS *m/z* (rel int) 326 (18, M$^+$), 284 (100, M$^+$ − CH$_2$CO). HRMS calculated for C$_{21}$H$_{26}$O$_3$ 326.1882. Found: 326.1872.

2.7. 16α-Bromo-3-acetoxy-18-methyl-estra-1,3,5(10)-trien-17-one (6)

3,17-Diacetoxy-18-methyl-estra-1,3,5(10),16-tetraene (5) (550 mg, 1.5 mmol) was dissolved in ether (10 ml) and acetate buffer (200 mg of potassium acetate in 4 ml of 85% acetic acid) was added. The mixture was cooled to 0°C and 1.6 ml of bromine solution was added (0.2 ml of Br$_2$ in 6.5 ml of acetic acid glacial) until the orange color persisted, whereafter the solution was stirred for an additional 20 min. The reaction was terminated by the addition of water and the organic phase was washed with water, 5% sodium thio-sulfate and 5% aqueous sodium bicarbonate, dried over sodium sulfate (anhydrous), filtered and evaporated to dryness to afford 16α-bromo-3-acetoxy-18-methyl-estra-1,3,5(10)-trien-17-one (6) in a quantitative yield: m.p. 133°C; ^{1}H NMR (CDCl$_3$) δ 0.79 (t, *J* = 7 Hz, 3 H, 13β-CH$_2$C*H₃*), 2.28 (s, 3 H, 3-OCOC*H₃*), 4.60 (dd, *J* = 7.5 Hz, 1 H, 16β-*H*), 6.81 (d, *J* = 2 Hz, 1 H, 4-C*H*), 6.85 (dd, *J* = 2.5 and 8 Hz, 1 H, 2-C*H*), 7.29 (1 H, 1-C*H*); MS *m/z* (rel int) 404, 406 (15, M$^+$), 364, 366 (100, M$^+$ − CH$_2$CO). HRMS calculated for C$_{21}$H$_{25}$BrO$_3$ 404.0987. Found: 404.0980.

2.8. 16α-Bromo-18-methyl-estra-1,3,5(10)-trien-17-one (7)

16α-Bromo-3-acetoxy-18-methyl-estra-1,3,5(10)-trien-17-one (6) (603 mg, 1.5 mmol) was hydrolyzed by treatment with concentrated sulfuric acid (0.3 ml) in ethanol (30 ml) at room temperature. Water was added and the compound was extracted with CHCl$_3$ and the organic phase was dried over sodium sulfate (anhydrous), filtered, and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel. Elution with 8% EtOAc in hexane furnished 16α-bromo-18-methyl-estra-1,3,5(10)-trien-17-one (7) (460 mg, 1.3 mmol; 85%): m.p. 97–98°C; HPLC (70:30 MeOH/H$_2$O) t$_r$ = 25 min; ^{1}H NMR (CDCl$_3$) δ 0.79 (t, *J* = 7 Hz, 3 H, 13β-CH$_2$C*H₃*), 4.61 (dd, *J* = 7.5 Hz, 1 H, 16β-*H*), 6.57 (d, *J* = 3 Hz, 1 H, 4-C*H*), 6.63 (dd, *J* = 3 and 8 Hz, 1 H, 2-C*H*), 7.13 (d, *J* = 8 Hz,

1 H, 1-C*H*); MS *m/z* (rel int) 362, 364 (100, M$^+$). HRMS calculated for C$_{19}$H$_{23}$BrO$_2$ 362.0881. Found: 362.0877.

## 2.9. 16β-Bromo-18-methyl-estra-1,3,5(10)-trien-17-one (9)

A mixture of the 16α-bromoestrogen (7) (140 mg, 0.38 mmol) and LiBr (140 mg) in 1 ml of 2-butanone was heated for 0.5 h at reflux. The reaction mixture was poured into 20 ml of water and extracted with EtOAc, washed with water, dried over sodium sulfate (anhydrous), and evaporated to dryness. The purple residue was filtered over a silica gel column to furnish a yellow residue that was purified by HPLC to gave 16β-bromo-18-methyl-estra-1,3,5(10)-trien-17-one (9) (37%): m.p. 187–190°C; HPLC (70:30 MeOH/H$_2$O) t$_r$ = 27 min; ^{1}H NMR (CDCl$_3$) δ 0.85 (t, *J* = 7 Hz, 3 H, 13β-CH$_2$C*H₃*), 4.12 (t, *J* = 8.5 Hz, 1 H, 16α-*H*), 6.57 (d, *J* = 3 Hz, 1 H, 4-C*H*), 6.63 (dd, *J* = 2 and 8.5 Hz, 1 H, 2-C*H*), 7.13 (d, *J* = 8.5 Hz, 1 H, 1-C*H*); MS *m/z* (rel int) 362, 364 (100, M$^+$), 228 (100). HRMS calculated for C$_{19}$H$_{23}$BrO$_2$ 362.0881. Found: 362.0877.

2.10. 16β-Bromo-18-methyl-estra-1,3,5(10)-trien-3,17β-diol (10)

A solution of NaBH$_4$ (105 mg, 2.8 mmol) in ethanol (6.6 ml) was added to 16β-bromo-18-methyl-estra-1,3,5(10)-trien-17-one (9) (122 mg, 0.33 mmol) in ethanol (6.6 ml). The mixture was stirred at room temperature for one hour. Excess NaBH$_4$ was destroyed with acetone and the reaction mixture was poured into water, extracted with ethyl acetate, washed with water, dried over sodium sulfate (anhydrous), and evaporated to dryness to furnished the crude product (119 mg). The residue was purified by column chromatography over silica gel. Elution with 5% CH$_3$CN in CHCl$_3$ furnished 16β-bromo-18-methyl-estra-1,3,5(10)-trien-3,17β-diol (10) (36 mg, 0.1 mmol, 34%): m.p. 195–199°C; HPLC (70: 30 MeOH/H$_2$O) t$_r$ = 29 min; ^{1}H NMR (CDCl$_3$) δ 1.01 (t, *J* = 7.5 Hz, 3 H, 13β-CH$_2$C*H₃*), 3.50 (t, *J* = 8.5 Hz, 1 H, 17α-*H*), 4.68 (td, *J* = 8 Hz, 1 H, 16α-*H*), 6.56 (d, *J* = 3 Hz, 1 H, 4-C*H*), 6.63 (dd, *J* = 3 and 8.5 Hz, 1 H, 2-C*H*), 7.14 (d, *J* = 8 Hz, 1 H, 1-C*H*); MS *m/z* (rel int) 364, 366 (30, M$^+$), 284 (90, M$^+$-Br), 159 (100). HRMS calculated for C$_{19}$H$_{25}$BrO$_2$ 364.1038. Found: 364.1033.

2.11. 16α-Bromo-18-methyl-estra-1,3,5(10)-trien-3,17β-diol (8)

A solution of NaBH$_4$ (18 mg, 0.5 mmol) in ethanol (1 ml) was added to 16β-bromo-18-methyl-estra-1,3,5(10)-trien-17-one (7) (20 mg, 0.05 mmol) in ethanol (2 ml). The mixture was stirred at room temperature for 2 h. Excess NaBH$_4$ was destroyed with acetone and the reaction mixture was poured into water, extracted with ethyl acetate, washed with water, dried over sodium sulfate (anhydrous), and evaporated to dryness to furnish a crude product (19 mg). The residue was purified by HPLC to furnish 16α-bromo-
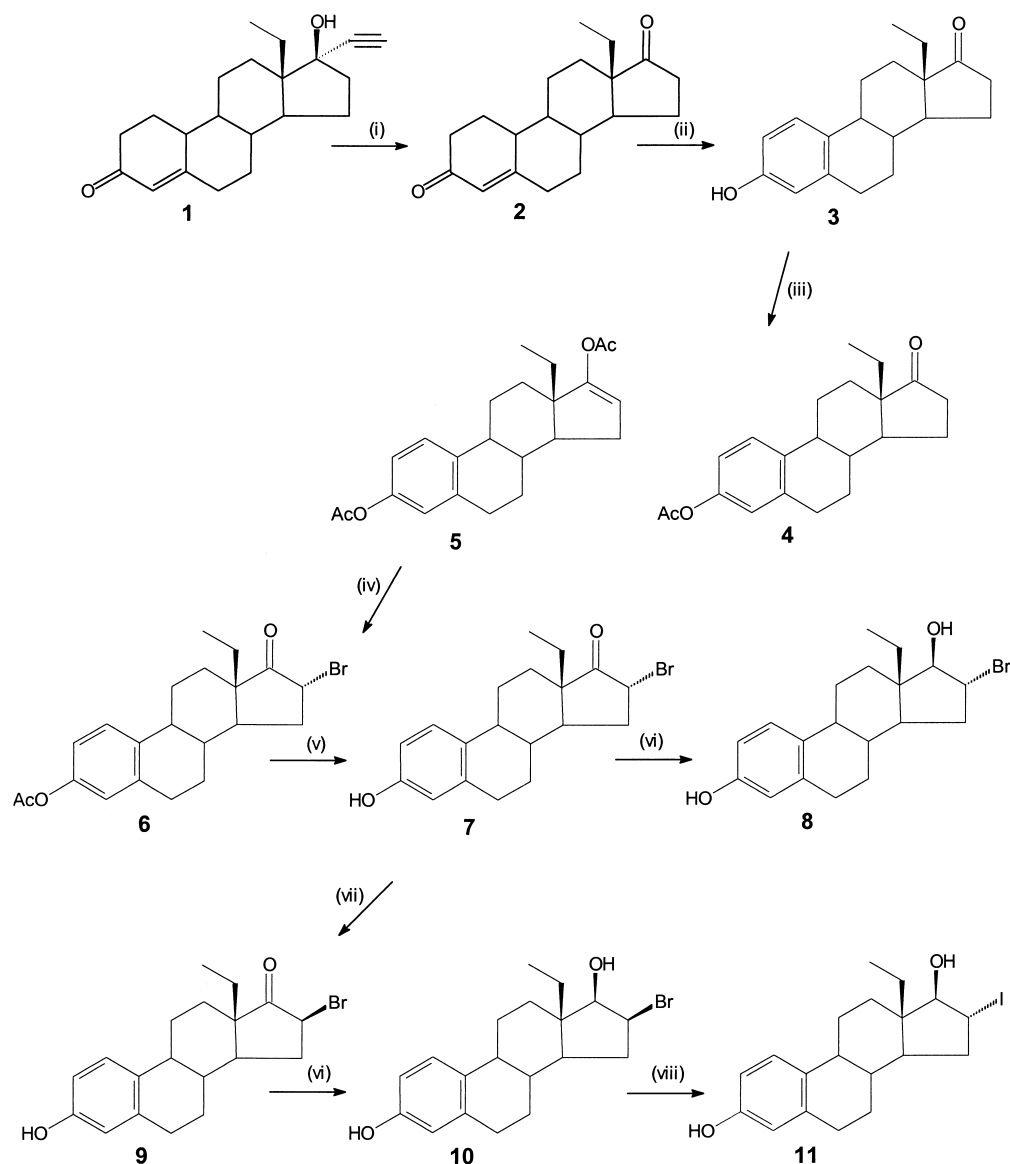
Scheme 1. Reagents: (i) Ag$_2$CO$_3$/Celite, toluene, (ii) CuBr$_2$, LiBr, CH$_3$CN, (iii) isopropenyl acetate, p-TsOH, (iv) ether, Br$_2$, acetate buffer, (v) EtOH, H$_2$SO$_4$, (vi) EtOH, NaBH$_4$, (vii) 2-butanone, LiBr, (viii) 2-butanone, NaI.

18-methyl-estra-1,3,5(10)-trien-3,17β-diol (**8**) (11 mg, 0.03 mmol, 60%): m.p. 98–101°C; HPLC (70:30 MeOH/H$_2$O) t$_r$ = 29 min; ^{1}H NMR (CDCl$_3$) δ 1.01 (t, J = 7 Hz, 13β-CH$_2$CH$_3$), 4.05 (d, J = 6.6 Hz, 1 H, 17α-H), 4.27 (1 H, 16β-H), 6.56 (d, J = 2.5 Hz, 1 H, 4-CH), 6.62 (dd, J = 3 and 8 Hz, 1 H, 2-CH), 7.14 (d, J = 8 Hz, 1 H, 1-CH); MS m/z (rel int) 364, 366 (38, M$^+$), 284 (75, M$^+$-Br), 159 (100). HRMS calculated for C$_{19}$H$_{25}$BrO$_2$ 364.1038. Found: 364.1033.

2.12. 16α-Iodo-18-methyl-estra-1,3,5(10)-trien-3,17β-diol (*11*)

A mixture of 16β-bromo-18-methyl-estra-1,3,5(10)-trien-3,17β-diol (**10**) (20 mg, 0.05 mmol) and NaI (50 mg) in 2-butanone (1 ml) was heated for 1 h at 120°C. After cooling, the mixture was poured into water and extracted with ethyl acetate. The organic phase was washed with water, dried over magnesium sulfate (anhydrous), and evaporated under reduced pressure. The residue was purified by HPLC to give 16α-iodo-18-methyl-estra-1,3,5(10)-trien-3,17β-diol (**11**) (16 mg, 78%): m.p. 160–163°C; HPLC (70:30 MeOH/H$_2$O) t$_r$ = 38 min; ^{1}H NMR (CDCl$_3$) δ 0.99 (t, J = 7.5 Hz, 3 H, 13β-CH$_2$CH$_3$), 2.82 (dd, J = 4 and 8.5 Hz, 1 H, 17α-H), 4.15 (d, J = 7 Hz, 1 H, 17β-OH), 4.26 (td, J = 5 Hz, 1 H, 16β-H), 6.56 (d, J = 3 Hz, 1 H, 4-CH), 6.63 (dd, J = 3 and 8 Hz, 1 H, 2-CH), 7.13 (d, J = 8 Hz, 1 H, 1-CH); MS m/z (rel int) 412 (70, M$^+$), 229 (85), 159 (90), 133 (100). HRMS calculated for C$_{19}$H$_{25}$IO$_2$ 412.0899. Found: 412.0894.
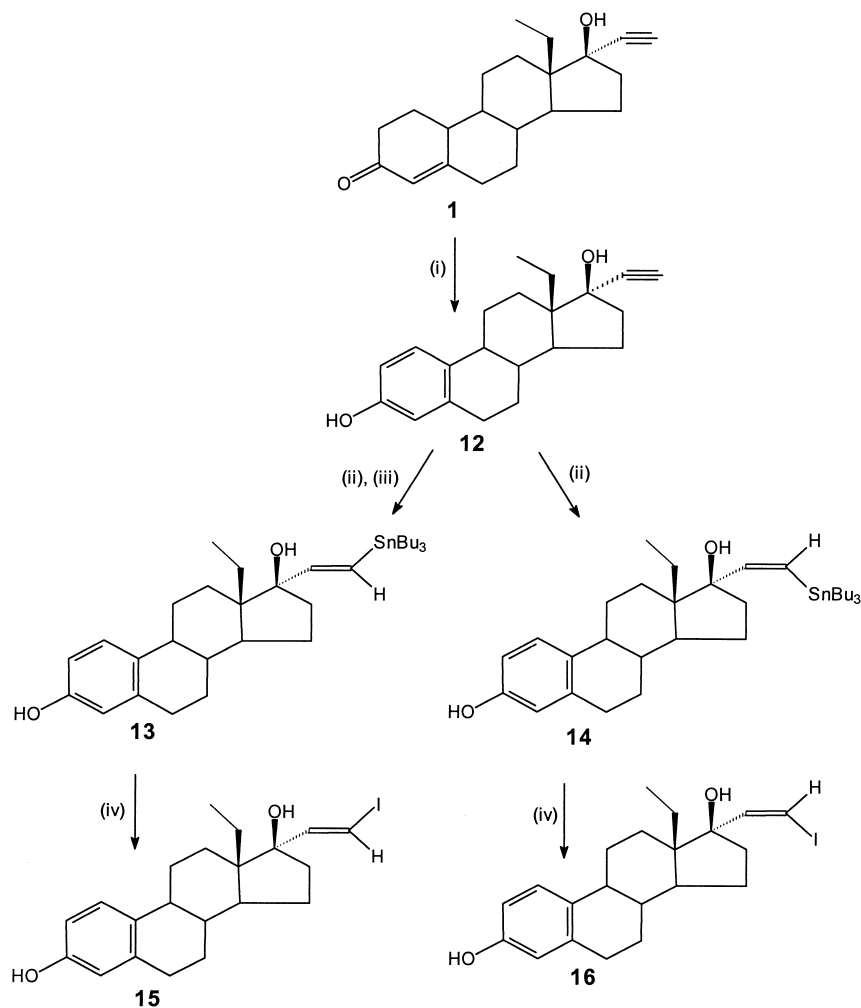
Scheme 2. Reagents: (i) CuBr$_2$, LiBr, CH$_3$CN, (ii) Bu$_3$SnH, AIBN, toluene, (iii) Bu$_3$SnH, Et$_3$B, THF, (iv) I$_2$, CHCl$_3$.

2.13. Synthesis of 16α-[^{125}I]iodo-18-methyl-estra-1,3,5(10)-trien-3,17β-diol ([^{125}I]11)

The radiosynthesis of [^{125}I]11 was performed as described earlier [14]. A solution 16β-bromo-18-methyl-estra-1,3,5(10)-trien-3,17β-diol (10) (100 μg) in 20 μl of methanol was added to the reaction vial containing 1.5 mCi of Na[^{125}I] in 20 μl of water containing 20 μg of Na$_2$S$_2$O$_3$ and the solvent was evaporated (N$_2$) close to dryness at 60–65°C and 20 μl acetonitrile was added to the reaction mixture. The reaction vial was tightly closed and the reaction mixture was heated at 95–100°C for 2 h, whereafter acetonitrile was removed. The residue (1.3 mCi) was dissolved in methanol and purified on an analytical C-18 reversed-phase HPLC column operated at 1 ml/min with 70% methanol in 30% water. The compound [^{125}I]11 eluted at 35 min in 70% yield (based on radioactivity).

2.14. 18-Methyl-17α-ethynylestra-1,3,5(10)trien-3,17β-diol (12)

The D(-)norgestrel (1) (250 mg, 0.8 mmol) was dissolved in 115 ml of acetonitrile (conc. 0.007 M) and solid

CuBr$_2$ (367 mg) and LiBr (72 mg) were added. The dark green mixture was stirred under nitrogen at room temperature for 2–2.5 h. Water was added until disappearance of the green color and acetonitrile was removed under reduced pressure. The product was extracted with ethyl acetate, dried over sodium sulfate (anhydrous), filtered, and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel. Elution with 10% EtOAc in hexane furnished a white crystalline compound 18-methyl-17α-ethynylestra-1,3,5(10)trien-3,17β-diol (12) (150 mg, 0.48 mmol; 60%): m.p. 117–120°C (lit [44]. 134–136°C); ^{1}H NMR (CDCl$_3$) δ 0.98 (t, *J* = 7.5 Hz, 3 H, 13β-CH$_2$C*H*$_3$), 6.55 (d, *J* = 3 Hz, 1 H, 4-C*H*), 6.62 (dd, *J* = 3 and 8 Hz, 1 H, 2-C*H*), 7.16 (d, *J* = 8.5 Hz, 1 H, 1-C*H*); MS *m/z* (rel int) 310 (M$^+$, 32), 242 (12), 227 (100), 160 (40). HRMS calculated for C$_{21}$H$_{26}$O$_2$ 310.1933. Found: 310.1924.

2.15. (17α,20E)-21-(tributylstannyl)-18-methyl-1,3,5(10),20-tetraene-3,17β-diol (13)

18-Methyl-17α-ethynylestra-1,3,5(10)trien-3,17β-diol (12) (22 mg, 0.07 mmol) was dissolved in 10 ml of toluene

Table 1
Tissue uptake of 16α-[^{125}I]iodo-18-methylestradiol ([^{125}I]**11**) in immature female Fischer rats

%ID/g (SEM)[a]

Tissue	0.5 h	1 h	2 h	2 h (+E)	5 h	24 h
Blood	1.20 (0.06)	1.29 (0.09)	1.13 (0.08)	1.06 (0.18)	0.94 (0.10)	0.22 (0.19)
Plasma	1.45 (0.08)	1.50 (0.12)	1.35 (0.10)	1.33 (0.20)	1.07 (0.12)	0.19 (0.01)
Lungs	1.23 (0.13)	1.16 (0.03)	0.87 (0.09)	0.79 (0.14)	0.62 (0.03)	0.21 (0.04)
Spleen	0.92 (0.10)	1.32 (0.44)	0.92 (0.08)	0.97 (0.05)	0.63 (0.04)	0.17 (0.17)
Kidneys	1.43 (0.18)	1.33 (0.13)	1.25 (0.18)	1.08 (0.21)	0.77 (0.09)	0.14 (0.01)
Liver	2.88 (0.18)	2.82 (0.37)	3.10 (0.21)	2.71 (0.08)	1.83 (0.24)	0.68 (0.06)
Adrenals	3.75 (0.26)	3.09 (0.34)	1.81 (0.11)	1.96 (0.37)	1.27 (0.19)	0.33 (0.05)
Thyroid	388 (35)	710 (24)	732 (98)	1116 (48)	2277 (38)	3618 (258)
Muscle	0.75 (0.08)	0.61 (0.04)	0.39 (0.01)	0.36 (0.00)	0.42 (0.13)	0.09 (0.05)
Fat	2.85 (0.17)	3.86 (0.60)	1.88 (0.10)	1.98 (0.22)	0.79 (0.12)	0.20 (0.09)
Uterus	1.60 (0.04)	1.60 (0.16)	0.85 (0.08)	0.46 (0.14)	0.50 (0.07)	0.20 (0.09)
T/Blood	1.33 (0.04)	1.25 (0.10)	0.77 (0.11)	0.50 (0.19)	0.54 (0.02)	0.32 (0.01)
T/NT	1.67 (0.12)	1.44 (0.32)	1.05 (0.05)	0.71 (0.25)	0.72 (0.13)	0.47 (0.02)

[a] Mean organ uptake in percent injected dose per gram of tissue (%ID/g) and standard error of the mean (SEM) for 3 to 5 Fisher female immature (21 days) rats. Each animal received i.v. 10 μCi of [^{125}I]**11** in the presence (+E$_2$) or the absence of 60 μg of co-injected estradiol. T = target (Uterus); NT = Non-target (average value of Lung, Spleen, Muscle).

and tributyltin hydride (0.2 ml) and azobis*iso*butyronitrile (10 mg) were added. The mixture was heated at 90–100°C under nitrogen for 1 h. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography over silica gel. Elution with 8% EtOAc in hexane furnished (17α,20*E*)-21-(tributylstannyl)-18-methyl-1,3,5(10),20-tetraene-3,17β-diol (**13**).

2.16. (17α,20E)-21-(tributylstannyl)-18-methyl-1,3,5(10),20-tetraene-3,17β-diol (13) and (17α,20Z)-21-(tributylstannyl)-18-methyl-1,3,5(10),20-tetraene-3,17β-diol (14)

18-Methyl-17α-ethynylestra-1,3,5(10)trien-3,17β-diol (**12**) (25 mg, 0.08 mmol) was dissolved in THF (5 ml) and tributyltin hydride (0.1 ml) and triethylborane (0.1 ml) were added. The mixture was stirred under nitrogen at room temperature for 0.5 h. The solvent was evaporated under reduced pressure and the residue was purified by HPLC on a reversed-phase semipreparative column with a gradient of 5% H$_2$O in MeOH to 100% MeOH (20 min).

2.17. (17α,20E)-21-(tributylstannyl)-18-methyl-1,3,5(10),20-tetraene-3,17β-diol (13)

White solid, m.p. 121–122°C; HPLC t$_r$ = 12 min; MS *m/z* (rel int) 545 (100, M-C$_4$H$_9$)$^+$.

2.18. (17α,20Z)-21-(tributylstannyl)-18-methyl-1,3,5(10),20-tetraene-3,17β-diol (14)

Oil; HPLC t$_r$ = 17 min; MS m/z (rel int) 602 (5, M$^+$), 312 (45), 227 (100).

2.19. (17α,20E)-21-Iodo-18-methyl-estra-1,3,5(10),20-tetraene-3,17β-diol (15) and (17α,20Z)-21-Iodo-18-methyl-estra-1,3,5(10),20-tetraene-3,17β-diol (16)

Compound (**13**)/(**14**) was dissolved in chloroform and a solution of I$_2$ in chloroform was added until a pink color persisted. The color was removed with 5% sodium thiosulfate and the organic phase was dried over sodium sulfate (anhydrous), filtered and evaporated to dryness to afford (**15**)/(**16**).

2.20. (17α,20E)-21-Iodo-18-methyl-estra-1,3,5(10),20-tetraene-3,17β-diol (15)

M.p. 104–106°C; HPLC (80: 20 MeOH/H$_2$O) t$_r$ = 19 min; ^{1}H NMR (CDCl$_3$) δ 0.98 (t, *J* = 7.5 Hz, 3 H, 13β-CH$_2$C*H*$_3$), 6.27 (d, *J* = 14 Hz, 1 H, = C*H*I), 6.55 (d, *J* = 3 Hz, 1 H, 4-C*H*), 6.62 (dd, *J* = 3 and 8 Hz, 1 H, 2-C*H*), 6.80 (d, *J* = 14 Hz, 1 H, C*H* =), 7.13 (d, *J* = 8.5 Hz, 1 H, 1-C*H*); MS *m/z* (rel int) 438 (40, M$^+$), 311 (42, M$^+$-I), 242 (32), 227 (100). HRMS calculated for C$_{21}$H$_{27}$IO$_2$ 438.1056. Found: 438.1059.

2.21. (17α,20Z)-21-Iodo-18-methyl-estra-1,3,5(10),20-tetraene-3,17β-diol (16)

M.p. 75–80°C; HPLC (80: 20 MeOH/H$_2$O) t$_r$ = 19 min; ^{1}H NMR (CDCl$_3$) δ 1.02 (t, *J* = 7.5 Hz, 3 H, 13β-CH$_2$C*H*$_3$), 6.34 (d, *J* = 8.5 Hz, 1 H, = C*H*I), 6.55 (d, *J* = 3 Hz, 1 H, 4-C*H*), 6.62 (dd, *J* = 3 and 8 Hz, 1 H, 2-C*H*), 6.85 (d, *J* = 8.5 Hz, 1 H, C*H* =), 7.14 (d, *J* = 8.5 Hz, 1 H, 1-C*H*); MS *m/z* (rel int) 438 (28, M$^+$), 311 (20, M$^+$-I), 242 (35), 227 (100). HRMS calculated for C$_{21}$H$_{27}$IO$_2$ 438.1056. Found: 438.1059.

Table 2
Tissue uptake of $(17\alpha,20E)$-[^{125}I]iodo-18-methylestradiol ([^{125}I]**15**) and $(17\alpha,20Z)$-[^{125}I]iodo-18-methylestradiol ([^{125}I]**16**) in immature female Fischer rats

%IDg (SEM)[a]

Tissue	0.5 h	1 h	2 h	2 h (+E)	5 h	24 h
Blood	0.79 (0.12)	0.59 (0.04)	0.56 (0.04)	0.52 (0.05)	0.42 (0.06)	0.12 (0.03)
Plasma	1.06 (0.13)	0.82 (0.04)	0.74 (0.06)	0.67 (0.05)	0.52 (0.05)	0.11 (0.01)
Lungs	2.63 (0.52)	1.36 (0.10)	1.23 (0.08)	1.18 (0.21)	0.76 (0.10)	0.12 (0.01)
Spleen	1.42 (0.25)	0.87 (0.08)	0.93 (0.07)	0.77 (0.14)	0.51 (0.07)	0.13 (0.00)
Kidneys	2.63 (0.60)	1.64 (0.17)	1.39 (0.12)	1.36 (0.31)	0.87 (0.13)	0.16 (0.00)
Liver	5.18 (0.63)	3.60 (0.24)	3.27 (0.20)	3.08 (0.40)	2.41 (0.24)	1.05 (0.00)
Adrenals	9.36 (1.43)	5.94 (0.56)	4.28 (0.22)	4.32 (0.75)	2.73 (0.35)	0.49 (0.09)
Thyroid	145 (2.81)	177 (19.17)	363 (22.68)	265 (17.84)	893 (18.7)	1295 (121.05)
Muscle	1.70 (0.22)	1.10 (0.13)	0.88 (0.14)	0.89 (0.10)	0.59 (0.09)	0.10 (0.01)
Fat	2.83 (0.53)	4.33 (0.78)	6.63 (0.73)	4.90 (0.76)	6.08 (0.79)	2.11 (0.18)
Uterus	3.09 (0.48)	2.27 (0.23)	1.84 (0.21)	0.91 (0.28)	1.12 (0.08)	0.30 (0.12)
T/Blood	3.90 (0.16)	3.83 (0.22)	3.29 (0.26)	1.93 (0.64)	2.56 (0.15)	2.52 (0.38)
T/NT	1.61 (0.04)	2.06 (0.15)	1.82 (0.14)	1.85 (0.15)	2.63 (1.02)	1.54 (0.49)
(20Z)-[^{125}I]**16**						
Blood	3.58 (0.22)	3.37 (0.12)	2.98 (0.41)	2.63 (0.03)	2.05 (0.20)	1.18 (0.30)
Plasma	2.89 (0.20)	2.44 (0.10)	1.96 (0.27)	1.79 (0.50)	1.20 (0.07)	0.28 (0.03)
Lungs	3.35 (0.33)	2.68 (0.10)	2.34 (0.25)	1.87 (0.01)	1.34 (0.04)	0.35 (0.09)
Spleen	2.60 (0.27)	2.29 (0.16)	1.89 (0.21)	2.46 (0.19)	1.24 (0.12)	0.45 (0.06)
Kidneys	4.09 (0.57)	2.94 (0.19)	2.13 (0.35)	1.74 (0.02)	1.25 (0.10)	0.27 (0.04)
Liver	6.71 (0.85)	4.92 (0.07)	4.24 (0.26)	3.76 (0.24)	2.40 (0.19)	0.97 (0.03)
Adrenals	14.96 (4.64)	8.60 (0.59)	6.15 (1.08)	3.38 (0.05)	3.36 (0.37)	0.41 (0.03)
Thyroid	145 (15.88)	217 (10.52)	375 (8.18)	333 (4.18)	729 (97.90)	1004 (61.29)
Muscle	2.58 (0.82)	1.58 (0.30)	1.25 (0.31)	0.88 (0.08)	0.56 (0.06)	0.10 (0.02)
Fat	4.10 (1.11)	6.23 (1.07)	7.04 (1.28)	4.60 (0.73)	6.48 (0.70)	0.92 (0.12)
Uterus	5.48 (0.23)	7.52 (0.68)	7.19 (1.12)	1.82 (0.04)	2.60 (0.14)	1.07 (0.19)
T/Blood	1.53 (0.03)	2.23 (0.18)	2.39 (0.07)	0.69 (0.01)	1.29 (0.13)	0.93 (0.08)
T/NT	1.97 (0.24)	3.15 (0.43)	3.29 (0.86)	2.52 (0.21)	3.59 (0.00)	1.05 (0.05)

[a] Mean organ uptake in percent injected dose per gram of tissue (%ID/g) and standard error of the mean (SEM) for 3 to 5 Fisher female immature (21 days) rats. Each animal received i.v. 10 μCi (20E)-[^{125}I]**15** or (20Z)-[^{125}I]**16** in the presence (+E$_2$) or the absence of 60 μg of co-injected estradiol.

2.22. Synthesis of $(17\alpha,20E)$-21-[^{125}I]Iodovinyl-18-methyl-estra-1,3,5(10),20-tetraene-3,17β-diol ([^{125}I]**15**) and $(17\alpha,20Z)$-21-[^{125}I]Iodovinyl-18-methylestra-1,3,5(10),20-tetraene-3,17β-diol ([^{125}I]**16**)

Compound (**13**)/(**14**) was dissolved in ethanol and 10 μl of Na^{125}I and 40 μl of solution H$_2$O$_2$/acetic acid (2:1) were added. After 5 min, 50 μl of 5% sodium thiosulfate was added and the mixture was dried. Water was added and product was extracted with chloroform. Organic phases were dried and the residue was dissolved in 500 μl of methanol and was purified by HPLC on a spherisorb S5 ODS2 column to furnish ([^{125}I]**15**)/([^{125}I]**16**): HPLC (75:25 MeOH/H$_2$O), flow 1 ml min, λ 280 nm, t_r = 19 and 18 min, respectively.

2.23. In vivo studies

The animal experiments were conducted in accordance with the recommendations of the Canadian Council of Animal Care and of the in-house Ethic Committee for Animal Experiments as previously described [26]. Briefly immature female Fischer rats (21 days old, 36 g SEM = 2.35 g, Charles River) were injected 200 μl of the ^{125}I-labeled preparation (10 μCi, 370 KBq) via the lateral tail vein. The radiopharmaceuticals were dissolved in ethanol and diluted with sterile physiological saline (0.9% NaCl in H$_2$O) containing 1% Tween-80, to give a final ethanol concentration of 9%. For the receptor saturation studies 60 μg of unlabeled estradiol was co-injected with the radiopharmaceutical. The animals were sacrificed under deep halothane anesthesia by severing the axillary artery to collect blood followed by chest opening [47]. Tissues of interest were removed washed in physiological saline, blotted dry and placed in pre-weighted tubes. Fats were removed from the flank of the animals. The radioactivity was counted in a Model 1282 Compugamma γ-counter (LKB Wallac, Finland) and specific activity was expressed as percent of injected dose per gram (%ID/g). Statistical variations are presented as the SEM [48].

3. Results and discussion

3.1. Chemistry

The 3-hydroxy-18-methylestra-1,3,5(10)-trien-17-one (**3**) was prepared from commercially available D(-)norgestrel
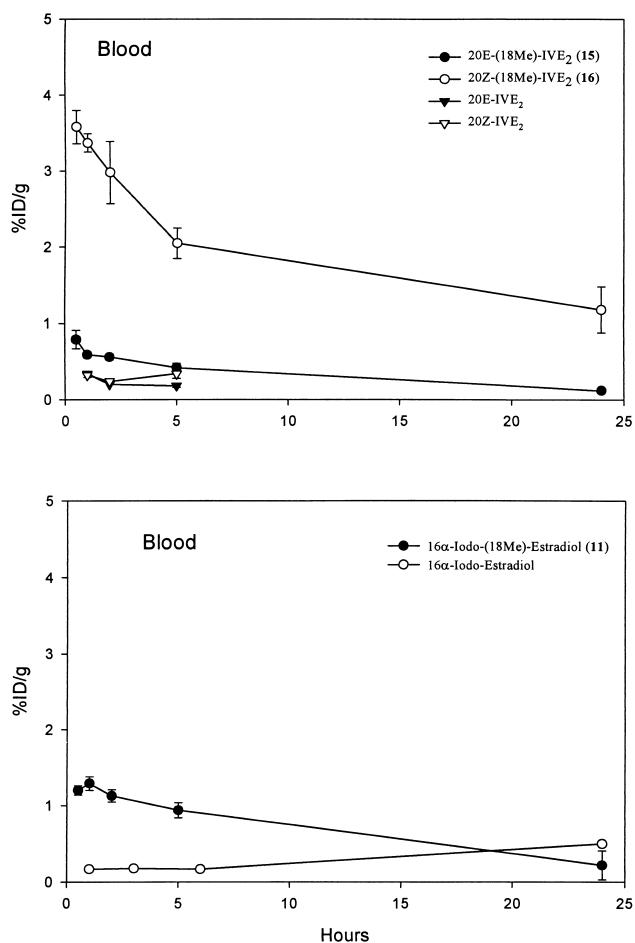
Fig. 1. Blood clearance in %ID/g of 20*E*-(18-methyl)-IVE$_2$ ([^{125}I]**15**), 20*Z*-(18-methyl)-IVE$_2$ ([^{125}I]**16**) and 16α-iodo-18-methylestradiol ([^{125}I]**11**) in immature female Fischer rats. Values for the ^{125}I-labeled isomeric 20*E/Z*-IVE$_2$ and 16α-iodoestradiol are taken from the literature [18,33]. The error bars represent the standard error (SE).
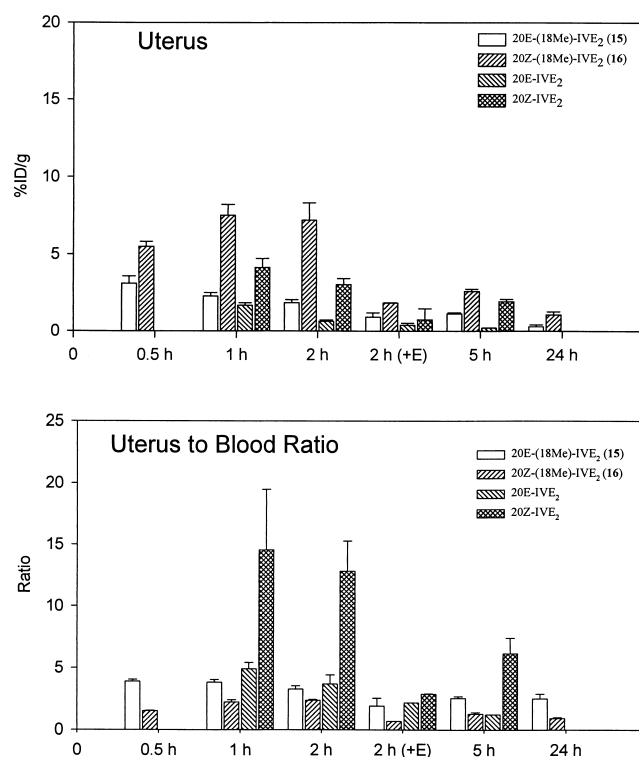

Fig. 2. Uterine uptake and uterus-to-blood ratios following i.v. administration of 20*E*-(18-methyl)-IVE$_2$ ([^{125}I]**15**) and 20*Z*-(18-methyl)-IVE$_2$ ([^{125}I]**16**) to immature female Fischer rats. Values for the ^{125}I-labeled isomeric 20*E/Z*-IVE$_2$ are taken from the literature [33]. The error bars represent the SE.

(**1**) accordingly to a published procedure. The initial step involves de-ethynylation of D(-)norgestrel (**1**) catalyzed by Fetizons reagent (silver carbonate on celite) according to the procedure of Lenz [49] that yielded D-(+)-13-ethylgon-4-ene-3,17-dione (**2**). Reaction of **2** with CuBr$_2$/CH$_3$CN in the presence of LiBr afforded 3-hydroxy-18-methylestra-1,3,5(10)-trien-17-one (**3**) in high yield [50]. The 16α-halosteroids were prepared by the adaptation of the Johnson and Johns procedure [51]. Reaction of 3-hydroxy-18-methylestra-1,3,5(10)-trien-17-one (**3**) with isopropenyl acetate in the presence of the acid catalyst gave 3,17-diacetoxy-18-methylestra-1,3,5(10)-16-tetraen (**5**) along with 3-acetoxy-18-methylestra-1,3,5(10)-trien-17-one (**4**). The enol diacetate (**5**) was selectively converted to the 16α-bromo-3-acetoxy-18-methylestra-1,3,5(10)-trien-17-one (**6**) by treatment with Br$_2$ in acetic acid. Acid hydrolysis of the 3-acetate group of **6** with sulfuric acid in alcohol at room temperature gave the 16α-bromo-18-methylestra-1,3,5(10)-trien-17-one (**7**) that was converted to the 16β-bromo-18-methylestra-1,3,5(10)-trien-17-one (**9**) via epimerization

with LiBr in 2-butanone. The 16β-bromo configuration of **9** was confirmed by the characteristic downfield shift of the C-18 proton in the ^{1}H NMR. Reduction of the 17-one group of 16β-bromo-18-methylestra-1,3,5(10)-trien-17-one (**9**) was achieved with NaBH$_4$ and gave 16β-bromo-18-methylestra-1,3,5(10)-trien-3,17β-diol (**10**) together with a small amount of the debrominated analog 18-methylestra-1,3,5(10)-trien-3,17β-diol. Similarly, the reduction of the 16α-bromo-18-methylestra-1,3,5(10)-trien-17-one (**7**) with NaBH$_4$ gave 16α-bromo-18-methylestra-1,3,5(10)-trien-3,17β-diol (**8**) (Scheme 1). The presence of a pair of molecular ions, of equal intensity, in the mass spectra of **6**–**10** confirmed the presence of bromine in the products. The 16α-iodo-18-methylestra-1,3,5(10)-trien-3,17β-diol (**11**) was obtained by halogen exchange of 16β-bromo-18-methylestra-1,3,5(10)-trien-3,17β-diol (**10**) with NaI and the radioiodinated analog was obtained with [^{125}I]NaI. The radiochemical yield of the product was 70%. The compounds were purified by reversed-phase HPLC.

18-Methyl-17α-ethynylestra-1,3,5(10)trien-3,17β-diol (**12**) was obtained by aromatization of the commercially available D(-)norgestrel (**1**) using CuBr$_2$/CH$_3$CN in the presence of LiBr, as reported by Rao et al. [50]. Treatment of 18-methyl-17α-ethynylestra-1,3,5 [10]trien-3,17β-diol (**12**) with tri-*n*-butyltin hydride using azo*iso*butyronitrile as catalyst in toluene at 90–95°C gave the 20*E*-isomer of the
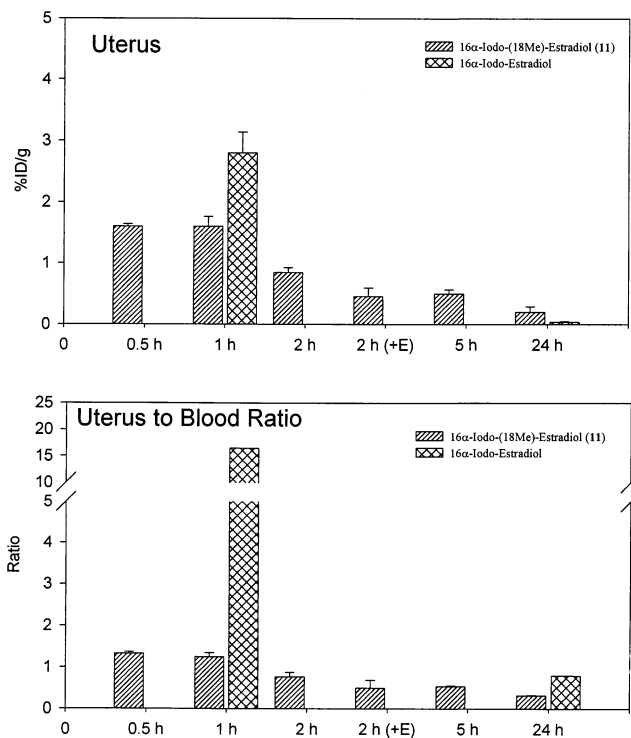
Fig. 3. Uterine uptake and uterus-to-blood ratios following i.v. administration of 16α-iodo-18-methylestradiol ([^{125}I]**11**) to immature female Fischer rats. Values for the ^{125}I-labeled 16α-iodoestradiol are taken from the literature [18]. The error bars represent the SE.

vinylstanne **13** as the major product. Nozaki reported [52] a convenient method for the formation of the 20*E/Z*-vinyl-stannes from the acetylenic compound using triethylborane as a catalyst. Likewise we found that compound **12** with tri-*n*-butyltin hydride in the presence of the triethylborane as catalyst yielded a mixture of the isomeric 20*E/Z*-vinylstanne derivatives **13** and **14** (Scheme 2). The isomeric tin intermediates failed to separate on TLC plates developed in ethylacetate/hexane or chloroform/acetonitrile. However using reversed-phase (C-18) HPLC in methanol/water the separation can be achieved. The isomeric ratio of the mixture can be altered on varying the reaction time and the amount of the reagent used. Longer reaction times and high amounts of the reagent resulted in the 20*E*-isomer as the major product [35,53]. The electrophilic destannylation of 18-homo-17α-(tri-*n*-butylstannyl)vinylestradiol proceeds in high yield with retention of configuration. Addition of a 0.1 M solution of iodine in chloroform to the isomeric intermediates **13** and **14** resulted in an immediate destannylation to give the iodovinyl derivatives **15** and **16**. The stereochemistry of the product was determined by ^{1}H NMR spectroscopic analysis. The coupling constant for the vinylic protons are greater for the 20*E*-isomer as compared to that of the 20*Z*-isomer, consistent with the reported value for analogous compounds [26]. The 20*Z*-isomer also is less polar than the 20*E*-isomer on normal phase silica gel TLC and the isomers separate well on reversed-phase HPLC in MeOH/

H_2O. The stannyl intermediates **13–14** were converted to the radioiodinated products [^{125}I]**15** and [^{125}I]**16** using nca Na[^{125}I] in the presence of H_2O_2 with a radiochemical yield of 60–70%. The labeled compounds were purified by reversed-phase HPLC in batches of about 0.2 mCi and their identity were confirmed by coinjection with the authentic iodinated products. During the purification, eluting ^{125}I-labeled products could not be detected by the UV monitor. Since the detection limit on our HPLC system is about 10^{-7} mmol, the minimum specific activity should be 2000 Ci/mmol. Thus the specific activities likely are in the same range as that of the starting [^{125}I]NaI, e.g. up to 2200 Ci/mmol.

3.2. Biologic properties

The biodistribution and uterine uptake of the ^{125}I-labeled estradiol derivatives of **11**, **15,** and **16**, were studied in immature Fischer female rats. The animals were injected through the tail vein with 10 μCi (370 KBq) of the HPLC-purified ^{125}I-labeled steroid in 200 μl of a 9% ethanol-saline solution containing 1% Tween-80. The animals were sacrificed at 0.5, 1, 2, 5, and 24 h post injection. In order to establish that the uterine uptake was receptor-mediated, some rats of the 2 h group were co-injected with 60 μg of unlabeled estradiol. The radioactivity in the various organs is presented in Tables 1 and 2.

Blood and plasma radioactivity remained the highest with 20*Z*-(18-methyl)-IVE$_2$ ([^{125}I]**16**) followed by 16α-(18-methyl)-IE$_2$ ([^{125}I]**11**) and 20*E*-(18-methyl)-IVE$_2$ ([^{125}I]**15**) (Fig. 1). The plasma radioactivity concentration followed the same pattern as the total blood activity except that differences in plasma radioactivity levels between the 3 compounds were less pronounced. The non-target tissue distribution patterns were similar for all three compounds, with most of the radioactivity being retained in the adrenals, liver, and fat. The muscle, lungs and spleen contain the least activity. Tissue specific activities decreased in the following order, 20*Z*-(18-methyl)-IVE$_2$ > 20*E*-(18-methyl)-IVE$_2$ > 16α-(18-methyl)-IE$_2$. Adrenal uptake of 20*Z*-(18-methyl)-IVE$_2$ showed a 45% drop in specific activity in the presence of cold estradiol, no such effect was observed for the 20*E*-isomer or the 16α-(18-methyl)-IE$_2$.

The highest uterine uptake, uterus-to-non target ratio and specificity were observed with the 20*Z*-(18-methyl)-IVE$_2$ (Fig. 2). The 20*E*-iosomer however presented slightly higher uterus-to-blood ratios due to more rapid blood clearance. In the presence of cold estradiol the uterine specific activity dropped by 75%, 50%, and 46%, respectively, for 20*Z*-(18-methyl)-IVE$_2$, 20*E*-(18-methyl)-IVE$_2$ and 16α-(18-methyl)-IE$_2$. The plasma-to-blood ratio for 20*Z*-(18-methyl)-IVE$_2$ indicate that more than half of the blood radioactivity is associated with the blood cellular elements. Binding of 20*Z*-(18-methyl)-IVE$_2$ to blood cells seems to be strong, because plasma-to-blood ratios decrease with time. Thyroid uptake reveals that the 20*E*- and 20*Z*-isomers un-

dergo diiodination at similar rates. Thus the higher activity levels 20Z-isomer in blood cells likely concerns steroid-bound radioiodine.

Addition of the 18-methyl group to the 16α-IE_2 derivative results in a drop of uterine radioactivity uptake (Fig. 3). Combined with a pronounced increase in blood and non-target tissue specific activity thus results in poor uterus-to-blood and uterus-to-non target ratios. In the case of 20E- and 20Z-IVE_2 addition of a 18-methyl group results in an improved uterine uptake (Fig. 2). This effect is more pronounced with the 20Z-(18-methyl)-IVE_2 isomer, but higher blood activity results overall in a low uterus-to-blood ratio than observed with the parent steroid. Also, the addition of a 18-methyl group to 20E/Z-IVE_2 results in an increased uptake by most non-target tissues. Our data suggest that 18-homologation of 16α-IE_2 and IE_2 does not improve their potential as ER-based tumor imaging agents.

Acknowledgments

References

[1] Beatson GT. On the treatment of inoperable cases of carcinoma of the mamma. Suggestions for a new method of treatment with illustrative cases. Lancet 1896;104–7.

[2] Jensen EV, Jacobsen HI. Basic guide to mechanism of estrogen receptor action. Recent Prog Horm Res 1962;18:387–414.

[3] Bertelsen CA, Guiliano AE, Kern DH, Mann BD, Roe DJ, Morton DL. Breast cancer: estrogen and progesterone receptor status as a predictor of in vitro chemotherapeutic response. J Surgical Res 1984; 37:257–63.

[4] Jensen EV, Greene GL, Closs LE, DeSombre ER, Nadji M. Receptor reconsidered: a 20-year prospective. Recent Prog Horm Res 1982;38: 1–40.

[5] Clark GM, Sledge GW Jr, Osborne CK, McGuire WL. Survival from first recurrence: relative importance of prognostic factors in 1 015 breast cancer patients. J Clin Oncol 1987;5:55–61.

[6] Rose C, Thorpe SM, Andersen KW, Pedersen BV, Mouridsen HT, Blichert-Toft M, Rasmussen BB. Beneficial effect of adjuvant tamoxifen therapy in primary breast cancer patients with high oestrogen receptor value. Lancet 1985;1:16–9.

[7] Vollenweider–Zeragui L, Barrelet G, Wong Y, Lemarchand–Béraud T, Gomez F. The predictive value of estrogen and progesterone receptor concentration on the clinical behaviour of breast cancer in women. Cancer 1986;57:1171–80.

[8] van Netten JP, Armstrong JB, Carlyle SS, Goodchild NL, Thornton IG, Brigden ML, Coy P, Fletcher C. Estrogen receptor distribution in the peripheral, intermediate and central regions of breast cancers. Eur J Cancer Clin Oncol 1988;24:1885–9.

[9] Mobbs BG, Fisch EB, Pritchard KI, Oldfield G, Hanna WH. Estrogen and progesterone receptor content of primary and secondary breast carcinoma influence of time and treatment. Eur J Cancer Clin Oncol 1987;23:819–26.

[10] Holdaway IM, Bowditch JV. Variation in receptor status between primary and metastatic breast. Cancer 1983;52:479–85.

[11] Quinn Youngkin E, Szmania DM. Women's Health. A primary care clinical guide. Stamford, CT: Appleton Lange, 1998. p. 994.

[12] Schober O, Scheidhauer K, Jackich C, Schicha H, Smolarz K, Bolte A, Reiners C, Hoffken K, Biersack HJ, Briele B, et al. Breast cancer imaging with radioiodinated oestradiol [letter]. Lancet 1990;335: 1522.

[13] Cummins CH. Radiolabeled steroidal estrogens in cancer research. Steroids 1993;58:245–59.

[14] Ali H, Rousseau J, van Lier JE. 7α-Methyl- and 11β-ethoxy-substitution of [^{125}I]-16-iodoestradiol: effect on estrogen receptor-mediated target tissue uptake. J Med Chem 1993;36:264–71.

[15] Ali H, Rousseau J, Gantchev TG, van Lier JE. 2- and 4-Fluorinated 16α- [^{125}I]iodoestradiol derivatives: synthesis and effect on estrogen receptor binding and receptor-mediated target tissue uptake. J Med Chem 1993;36:4255–63.

[16] Hochberg RB. Iodine-125-labeled estradiol: a gamma emitting analog of estradiol that binds to the estrogen receptor. Science 1979;205: 1138–40.

[17] Katzenellenbogen JA, Senderoff SG, McElvany KD, O'Brien HA Jr, Welch MJ. 16α-[^{77}Br]bromoestradiol 17β: a high specific activity, γ-emitting tracer with uptake in rat uterus and induced mammary tumor. J Nucl Med 1981;22:42–7.

[18] Zielinski JE, Yabuki H, Pahuja SL, Larner JM, Hochberg RB. 16α-[^{125}I]iodo-11β-methoxy-17β-estradiol: a radiochemical probe for estrogen-sensitive tissue. Endocrinology 1986;119:130–9.

[19] Zielinski JE, Larner JM, Hoffer PB, Hochberg RB. The synthesis of 11β-methoxy-[16α-^{123}I]iodoestradiol and its interaction with the estrogen receptor in vivo and in vitro. J Nucl Med 1989;30:209–15.

[20] Katzenellenbogen JA, McElvany KD, Senderoff SG, Carlson KE, Landvatter SW, Welch MJ. 16α[^{77}Br]bromo-11β-methoxyestradiol-17β: a γ-emitting estrogen imaging agent with high uptake and retention by target organs. J Nucl Med 1982;23:411–9.

[21] Pomper MG, van Brocklin H, Thieme AM, Thomas RD, Kiesewetter DO, Carlson KE, Mathias CJ, Welch MJ, Katzenellenbogen JA. 11β-Methoxy-, 11β-ethyl- and 17α-ethynyl-substituted 16α-fluoroestradiols receptor-based imaging agents with enhanced uptake efficiency and selectivity. J Med Chem 1990;33:3143–55.

[22] Preston DF, Spicer JA, Barankzuk RA, Fabian C, Baxter KG, Martin NL, Robinson RG. Clinical results of breast cancer detection by imageable estradiol (I-123 E_2). Eur J Nucl Med 1990;16:430.

[23] Scheidhauer K, Müller S, Smolarz K, Bräutigam P, Briele B. Tumorszintigraphie mit 123j-markiertem Östradiol beim Maamakarzinom-Rezeptorszintigraphie. Nucl Med 1991;30:84–99.

[24] McGuire AH, Dehdashti F, Siegel BA, Lyss AP, Brodack JW, Mathias CJ, Mintun MA, Katzenellenbogen JA, Welch MJ. Positron tomographic assessment of 16α-[^{18}F]fluoro-17β-estradiol uptake in metastatic breast carcinoma. J Nucl Med 1991;32:1526–31.

[25] Dehdashti F, Mortimer JE, Siegel BA, Griffeth LK, Bonasera TJ, Fusselman MJ, Detert DD, Cutler PD, Katzenellenbogen JA, Welch MJ. Positron tomographic assessment of estrogen receptors in breast cancer—comparison with FDG-PET and in vitro receptor assays. J Nucl Med 1995;36:1766–74.

[26] Ali H, Rousseau J, Ghaffari MA, van Lier JE. Synthesis, receptor binding and tissue distribution of ($17\alpha,20E$)- and ($17\alpha,20Z$)-21-[^{125}I]iodo19-norpregna-1,3,5(10),20-tetraene-3,17β-diol. J Med Chem 1988;31:1946–50.

[27] Hanson RN, Frank LA, Kaplan ML. Synthesis and evaluation of ($17\alpha,20E$)21[^{125}I]iodo-11β-substituted-19-norpregna 1,3,5(10),20-tetraene-3,17β-diols: the influence of 11β-stereochemistry on tissue distribution of radioiodinated estrogens. Nucl Med Biol 1989;16:2–9.

[28] Hanson RN, Seitz DE, Boltaro JC. E-17α-[^{125}I]iodovinyl estradiol: an estrogen-receptor seeking radiopharmaceuticals. J Nucl Med 1982; 23:431–6.

[29] Nakatsuka I, Ferreria NL, Eckelman WC, Francis BE, Rzeszotarski WJ, Gibson RE, Jagoda EM, Reba RC. Synthesis and evaluation of ($17\alpha,20E$)-21-[^{125}I]iodo-19-norpregna-1,3,5(10)-20-tetraene-3,17β-diol and ($17\alpha,20E$)-21-[^{125}I]-11β-methoxy-19-norpregna-1,3,5(10)-

20-tetraene-3,17β-diol (17α-(iodovinyl)estradiol derivatives) as high specific activity potential radiopharmaceuticals. J Med Chem 1984; 27:1287–91.

[30] Raynaud JP, Ojasoo T. Receptor binding as a tool in the development of selective new bioactive steroids and non steroids. In: Harms AF (editor), Innovative Approaches in Drug Research. Amsterdam: Elsevier Science 1986. pp. 47–72.

[31] Raynaud JP, Ojasoo T, Bouton MM, Bignon E, Pons M, Crastes de Poulet A. In: McLachlan JA (editor), Estrogen in the Environment 2. Amsterdam: Elsevier Science, 1985. pp. 22–42

[32] Kalvoda J, Krahnenbuhl C, Desaulles PA, Anner GA. 7α-Methyl oestrogens. Helv Chim Acta 1967;50:281–8.

[33] Ali H, Rousseau J, Ghaffari MA, van Lier JE. Synthesis, receptor binding and tissue distribution of 7α- and 11β-substituted (17α,20E)- and (17α,20Z)-21-[^{125}I]iodo19-norpregna-1,3,5(10),20-tetraene-3,17β-diol. J Med Chem 1991;34:854–60.

[34] Ali H, Rousseau J, van Lier JE. Synthesis, receptor binding and biodistribution of the gem-21-chloro-21-iodovinylestradiol derivatives J Steroid Biochem Mol Biol 1993;46:613–22.

[35] Ali H, Rousseau J, van Lier JE. Synthesis of A-ring fluorinated derivative of (17α,20E/Z)[^{125}I]iodovinylestradiols: effect on receptor binding and receptor-mediated target tissue uptake. J Med Chem 1993;36:3061–72.

[36] Foulon C, Guilloteau D, Baulieu JL, Ribeiro–Barras MJ, Desplanches G, Frangin Y, Besnard JC. Estrogen receptor imaging with 17α-[^{123}I]iodovinyl-11β-methoxyestradiol (MIVE2)—Part I. Radiotracer preparation and characterization. Nucl Med Biol 1992;19:257–61.

[37] Zeicher M, Delcorde A, Quivy J, Dupuis Y, Versist A, Frühling J. Radioimaging of human breast carcinoma xenografts in mice by [^{123}I]-labeled Z-17α-iodovinyl-11β-chloromethyl estradiol. Nucl Med Biol 1996;23:69–73.

[38] Rijks LJM, van den Bas Jan C, van Doremalen PAPM, Boer GJ, de Bruin K, Doornbos T, Vekemans JAJM, Posthumus MA, Jansen AGM, van Royen EA. Synthesis, estrogen receptor binding, and tissue distribution of a new iodovinylestradiol derivative (17α,20E)-21-[^{123}I]iodo-11β-nitrato-19-norpregna 1,3,5(10),20-tetraene-3,17β-diol (E-[^{123}I]NIVE). Nucl Med Biol 1998;25:411–21.

[39] Rijks LJM, Boer GJ, Endert E, de Bruin K, Jansen AGM, van Royen EA. The Z-isomer of 11β-methoxy 17α-[^{123}I]iodovinylestradiol is a promising radioligand for estrogen receptor imaging in human breast cancer. Nucl Med Biol 1997;24:65–75.

[40] Nachar O, Rousseau J, Lefebvre B, Ouellet R, Ali H, van Lier JE. Biodistribution, dosimetry, and metabolism of 11β-methoxy-(17α,20E/Z)-[^{123}I]iodovinylestradiol (MIVE) in healthy women and female breast cancer patients. J Nucl Med 1999;40:1728–36.

[41] Moguilewsky M, Deraedt R. Interaction between glucocorticoid and progestin receptors. J Steroid Biochem 1981;15:329–35.

[42] Rijks LJM. Imaging of steroid hormone receptors in human breast cancer. Ph.D. thesis, 1997.

[43] Raynaud J-P, Ojasoo T, Bouton MM, Philibert D. In: Ariëns EJ (editor), Drug Design, Vol. VIII. New York: Academic Press, 1979. pp. 169–214.

[44] Rufer C, Schröder E, Gibian H. Synthese und eigenschaften von modifizierten östratrien-derivaten. Liebigs Ann Chem 1971;752:1–13.

[45] Anstead GM, Carlson KE, Katzenellenbogen JA. The estradiol pharmacophore: ligand structure-estrogen receptor binding affinity relationships and a model for the receptor binding site. Steroids 1997; 62:268–303.

[46] Micheli RA, Hajos ZG, Cohen N, Parrish LA, Sciamanna W, Scott MA, Wehrli PA. Total synthesis of optically active 19-norsteroids (+)-estr-4-ene-3,7-dione and (+)-(13β-ethylgon-4-en-3,17-dione). J Org Chem 1975;40:675–81.

[47] Waynforth HB. Chapter 2. Methods of obtaining body fluids. In: Experimental and Surgical Technique in the Rat. London: Academic Press, 1980. pp. 67–8.

[48] Sherrer B. Chapter 4. Description de séries statistiques. In: Morin G, editor. Biostatistique. Quebec, Canada: Chicoutimi, 1984. pp. 161–62.

[49] Lenz GR. Fragmentation reactions catalysed by Fétizon's reagent (silver carbonate on celite). JCS Chem Comm 1972:468.

[50] Rao PN, Cessac JW, Kim HK. Preparative chemical methods for aromatization of 19-nor-Δ^4-3-oxosteroids. Steroids 1994;59:621–7.

[51] Johnson WS, Johns WS. 14-Isoestrone methylether and its identity with totally synthetic material. J Am Chem Soc 1957;79:2005–9.

[52] Nozaki K, Oshima K, Utimoto K. Et$_3$-Induced radical reaction to R$_3$SnH to acetylene and its application to cyclization reaction. J Am Chem Soc 1987;109:2457–549.

[53] Hanson RN, Napolitano E, Fiaschi R. Synthesis and evaluation of 11β-substituted 21-chloro/iodo-(17α,20E/Z)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diols: high-affinity ligands for the estrogen receptor. J Med Chem 1998;41:4686–92.