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Synthesis and biological evaluation of two new radiolabelled estrogens: [^{125}I](*E*)-3-methoxy-17α-iodovinylestra-1,3,5(10),6-tetraen-17β-ol and [^{125}I](*Z*)-3-methoxy-17α-iodovinylestra-1,3,5(10),6-tetraen-17β-ol

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Abstract

The synthesis of two novel radiolabelled estrogen derivatives, [^{125}I](*E*)-3-methoxy-17α-iodovinylestra-1,3,5(10),6-tetraen-17β-ol (***E***[^{125}I]IVDE) and [^{125}I](*Z*)-3-methoxy-17α-iodovinylestra-1,3,5(10),6-tetraen-17β-ol (***Z***[^{125}I]IVDE), was carried out aiming to study the influence of the introduction of a C6–C7 double bond on the biological properties of the estradiol molecule. 3-Methoxyestra-1,3,5(10),6-tetraen-17-one was synthesised starting from a suitably protected estrone and subsequently converted into the 17α-ethynyl derivative. The radioiodinated derivatives were stereoselectively formed by radioiododestannylation of the corresponding tributylstannyl precursors. The biodistribution of the novel [^{125}I]iodovinylestradiol derivatives was evaluated in immature female mice. Biological data indicated that the *Z*-isomer, owing to its higher in vivo uptake by the target tissue, has the preferable configuration for further development of similar compounds for estrogen receptor detection.

1. Introduction

Imaging of breast cancer tumours based on their content of estradiol receptor, ERα, poses a major challenge in the design of radiopharmaceuticals. Among the radioiodinated estrogens that have been advanced over past years as possible imaging agents for estrogen-positive human breast tumours, the 17α-iodovinylestradiol derivatives showed some promising properties (Hanson et al., 1989, 1993; Ali et al., 1988, 1993; Foulon et al., 1992; Ribeiro-Barras et al., 1992; Zeicher et al., 1996; Rijks et al., 1997a,b). Many of these compounds with high affinity for the estrogen receptor are still unsuitable for in vivo imaging mainly due to their high non-specific binding, rapid metabolism and instability. The few clinical studies reported to date for the 17α-iodovinylestradiol derivatives were carried out with the *E*- and *Z*-isomers of [^{123}I]11β-methoxy-17α-iodovinylestradiol (Ribeiro-Barras et al.,

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1992; Rijks et al., 1997a,b) but only the *Z*-isomer could fulfil the requisites to be considered an effective breast cancer radiotracer (Rijks et al., 1997a,b, 1998). Nevertheless, radiopharmaceuticals with improved biological profile are still required for estrogen receptor tumour imaging.

In connection with our work on the development of new radiopharmaceuticals (Inohae et al., 1999) based on estradiol derivatives, we have synthesised two new radiolabelled estrogen derivatives, [^{125}I](*E*)-3-methoxy-17α-iodovinylestra-1,3,5(10),6-tetraen-17β-ol (***E***[^{125}I]IVDE) and [^{125}I](*Z*)-3-methoxy-17α-iodovinylestra-1,3,5(10),6-tetraen-17β-ol (***Z***[^{125}I]IVDE). The reason for this work is that the most relevant research for the development of imaging agents for estrogen receptor-positive breast tumours has been based on subtle modifications of the natural estrogen structure through the placement of different chemical groups around the ring system. The introduction of a C6–C7 double bond has an effect on the conformation of the estradiol molecule due to the conformational change in ring B and gives a different topology to the steroid compared to that of the saturated analogue, which should be reflected in its biological characteristics. Indeed, preliminary in vitro receptor binding assays suggest that 3-methoxy-17α-ethynylestra-1,3,5(10),6-tetraen-17β-ol shows an enhanced binding to the estrogen receptor as compared to the saturated analogue (Marques et al., 1999 Unpublished results). Furthermore, steroidal estrogens with unsaturation at the C6–C7 positions can be useful intermediates in the synthesis of 7α-substituted estradiol derivatives, which are described as interesting compounds with estrogenic or antiestrogenic properties (Da Silva and van Lier, 1990) depending on the nature of the 7α-chain. Thus far, the effect of a double bond at C6–C7 on the metabolism and biological efficacy of estrogens has not been described.

The introduction of a 3-methoxy group in the estradiol molecule preserves estrogen receptor affinity (Franke and Hanson, 1984). In vivo, the 3-methoxy group is metabolically labile and will generate the parent estradiol by *O*-demethylation in the liver. Moreover, it can be expected that radiopharmaceuticals based on 3-methoxy-estradiol derivatives show higher in vivo stability. It is for this reason that the authors have chosen to maintain the methyl ether moiety at C-3.

Studies on the effect of changes within the steroid framework on the biodistribution of the compounds often involve an approach using the preparation of radioiodinated 17α-iodovinyl derivatives. The established synthetic procedures yield mainly the 20*E*-isomers. Accordingly, estrogen-receptor binding and in vivo distribution studies have been essentially performed with these isomers. Nevertheless, a superiority of the 20*Z*-isomers over 20*E*-isomers of some estradiol derivatives has been reported (Napolitano et al., 1991; Hughes et al., 1997; Rijks et al., 1997a,b). Thus, the authors deemed a study on the effect of the stereochemistry of the iodovinyl moiety on the biodistribution of these radiolabelled estrogens necessary. The target tissue uptake and uptake selectivity of both the *E*- and the *Z*- isomer were studied in immature female mice and are described in this paper.

2. Experimental

All reagents used were commercially available and were of analytical grade. 3-Methoxyestra-1,3,5(10)-trien-17-one (purity >98%) was purchased from Sigma. Carrier-free [^{125}I]sodium iodide (625 MBq/μg of iodide) was purchased from Amersham, UK. Melting points were determined on a Mitamuriken MELT THERMO and are uncorrected. IR spectra were recorded on a JASCO-102 spectrometer. NMR spectra were recorded at 270 and 300 MHz (proton) and at 67.9 and 75.4 MHz (carbon-13) with JEOL GSX-270 and Bruker WP-300 instruments, respectively, with tetramethylsilane as internal standard. *J*-values are given in Hz. Mass spectra were obtained on a JEOL JMS-O1SG-2 mass spectrometer at 70 eV using a direct inlet system. Column chromatography was carried out on silica gel (Waco gel, C-300, 70–230 mesh). High performance liquid chromatography was performed on a reverse-phase column (C_{18}, 250/8/4, Macherey-Nagel, Germany) eluted at 1 mL min^{-1} with 80% aqueous methanol. The eluted compounds were detected by their absorbance at 254 nm with a variable wavelength absorbance detector (LKB, Bromma) and by their gamma radiation with a scintillation counter equipped with a J100 cell (Berthold LB 505). Determination of the tissue radioactivity was carried out with a gamma counter (Hydragamma, Innotron Ltd, Oxford, England).

*2.1. 3-Methoxy-17,17-(2′,2′-dimethyltrimethylenedioxy)-estra-1,3,5(10)-triene (**2**)*

To 3-methoxyestra-1,3,5(10)-trien-17-one (**1**) (5.0 g, 17.6 mmol) in benzene (60 mL) was added 2,2-dimethyl-propan-1,3-diol (6.8 g, 65.3 mmol) and *p*-toluenesulfonic acid monohydrate (0.55 g, 2.9 mmol) and the mixture was heated at reflux for 4 h with continuous azeotropic removal of water (Dean–Stark condenser). The reaction was cooled to room temperature, poured into a saturated aqueous sodium bicarbonate solution and the organic layer separated. The organic layer was washed with brine, dried over sodium sulphate, filtered and evaporated to dryness to afford an oil. Chromatography of the residue by flash column

chromatography (petroleum ether: ethyl acetate; 4:1) gave 3-methoxy-17,17-(2′,2′-dimethyltrimethylenedioxy)-estra-1,3,5(10)-triene (**2**) (3.2 g), which was recrystallised from methanol to afford a colourless solid (2.6 g, 7.1 mmol, 40% yield). IR (KBr) 2940, 2850, 1600, 1570, 1500, 1460, 1270, 1250, 1100, 1030, 960, 900 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.22 (d, 1-H, 1H, J = 8.5 Hz), 6.78 (dd, 2-H, 1H, J = 2.7 Hz, J = 8.5 Hz), 6.71 (dd, 4-H, 1H, J = 2.7 Hz), 3.81 (s, -OCH_3, 3H), 3.38–3.50 (m, -CH_2- acetal, 4H), 1.16 (s,-CH_3 acetal, 3H), 0.83 (s,-CH_3 -C18, 3H), 0.73 (s,-CH_3 acetal, 3H).

2.2. 3-Methoxy-17,17-(2′,2′-dimethyltrimethylenedioxy)-estra-1,3,5(10)trien-6-ol (*3*)

3 was prepared analogously to a described procedure (Tedesco et al., 1995), as follows. To LIDAKOR reagent [prepared in situ by adding potassium *t*-butoxide (4.3 g, 38.3 mmol) and diisopropylamine (6 mL, 3.9 g, 38.3 mmol) to a cooled solution (−78°C) of *n*-butyllithium (17 mL, 15% in hexane, 38.3 mmol) in THF (100 mL)] was added 3-methoxy-17,17-(2′,2′-dimethyltrimethylenedioxy)-estra-1,3,5(10)-triene (**2**) (3.5 g, 9.6 mmol). A dark-red solution resulted, which was stirred for 3 h at −78°C. Thereafter, trimethyl borate (15 mL, 127 mmol) was added and the dry ice bath replaced by an ice-water bath. The mixture was stirred for 1–2 h (during which time the dark red solution turned milky) and then 30% aqueous hydrogen peroxide (40 mL) was added. After stirring for 1 h at room temperature the reaction mixture was partitioned between ethyl acetate and 10% aqueous sodium thiosulfate. The organic phase was washed with water, dried over sodium sulphate and evaporated to afford a residue from 3-methoxy-17,17-(2′,2′-dimethyltrimethylenedioxy)-estra-1,3,5(10)trien-6-ol (**3**) was obtained by chromatography (petroleum ether:ethyl acetate; 5:2) as a colourless solid (2.5 g, 6.5 mmol, 68% yield). IR (film) 3592 (OH), 1608, 1572, 1496 cm^{-1}. ^{1}H-NMR (270 MHz, $CDCl_3$) δ7.21 (d, 1H, J = 8.6 Hz, 1-H), 7.14 (d, 1H, J = 2.6 Hz, 4-H), 6.79 (dd, 1H, J = 2.6 Hz, J = 8.6 Hz, 2-H), 4.81–4.84 (m, 1H, 6-H), 3.80 (s, 3H, -OCH_3), 3.69–3.37 (m, 4H, -CH_2- acetal), 1.16 (s, 3H, -CH_3 acetal), 0.83 (s, 3H, 18-CH_3), 0.73 (s, 3H, -CH_3 acetal). ^{13}C-NMR (67.9 MHz, $CDCl_3$) δ158.02 (C3), 140.81 (C5), 132.65 (C10), 126.45 (C1), 113.71 (C2), 111.61 (C4), 108.57 (C17), 72.60 (C7), 70.67, 70.15 (2C, -CH_2-, acetal), 55.33 (O-CH_3), 47.25 (C13), 47.02, 44.18, 38.35, 32.22 (C6, C14, C9, C8), 30.39 (C, acetal), 29.43, 27.03, 26.11, 23.02 (C16, C12, C11, C15), 22.53, 21.03 (2x -CH_3, acetal), 13.98 (C18). MS (EI^+) m/z(%) = 386 (M^+, 7), 299 (11), 282 (35), 171 (13), 141 (100), 69 (21). HRMS: Found: 386.2460. Calcd. for $C_{24}H_{34}O_4$: 386.2457.

2.3. 3-Methoxy-17-(and 2′2′-dimethyltrimethylenedioxy)-estra-1,3,5(10),6-tetraene (*4*)

To a solution of 3-methoxy-17,17-(2′,2′-dimethyltrimethylenedioxy)-estra-1,3,5(10)trien-6-ol (**3**) (1.7 g, 4.4 mmol) in benzene (50 mL) was added *p*-toluenesulfonic acid monohydrate (112 mg, 0.65 mmol) and 2,2-dimethyl-propane-1,3-diol (2.0 g, 19.2 mmol). The mixture was heated under reflux for 4 h with continuous azeotropic removal of water (Dean–Stark condenser). The pale yellow solution was cooled to room temperature, poured into a 5% aqueous sodium carbonate solution and the organic layer separated. The organic layer was washed with water and brine, dried over sodium sulphate, filtered and evaporated to dryness to afford a residue from which 3-methoxy-17-(and 2′,2′-dimethyltrimethylenedioxy)-estra-1,3,5(10),6-tetraene (**4**) was obtained after flash column chromatography with petroleum ether: ethyl acetate (5:2) as a colourless solid (1.3 g, 3.5 mmol, 80% yield). IR (film) 2952, 1600, 1568, 1492 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$) δ7.19 (d, 1H, J = 8.2 Hz, 1-H), 6.75 (dd, 1H, J = 2.9 Hz, J = 8.2 Hz, 2-H), 6.66 (d, 1H, J = 2.9 Hz, 4-H), 6.44 (dd, 1H, J = 2.9 Hz, J = 9.6 Hz, 6-H), 6.00 (dd, 1H, J = 1.6 Hz, J = 9.6 Hz, 7-H), 3.81 (s, 3H -OCH_3), 3.68-3.41 (m, 4H, -CH_2- acetal), 1.20 (s, 3H, -CH_3 acetal), 0.86 (s, 3H, 18-CH_3), 0.76 (s, 3H, -CH_3 acetal). ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ158.70 (C3), 136.16 (C5), 133.91 (C7), 132.52 (C10), 128.30 (C1), 124.94 (C6), 112.41, 112.26 (C2, C4), 109.13 (C17), 73.29, 71.29 (-CH_2-, acetal), 55.92 (-OCH_3), 48.52 (C13), 46.50; 42.35, 39.48 (C14, C9, C8), 31.02 (C, acetal), 29.67, 27.60, 24.62, 23.62 (C11, C12, C15, C16), 23.21, 22.69 (-CH_3, acetal), 14.4 (C18). MS (EI^+) m/z(%) = 386 (M^+, 29), 282 (21), 238 (22), 225 (19), 184 (11), 167 (34), 149 (100), 141 (27), 128 (169), 69 (24). HRMS: Found: 368.2353. Calcd. for $C_{24}H_{32}O_3$: 368.2351.

2.4. 3-Methoxyestra-1,3,5(10),6-tetraen-17-one (*5*)

3-Methoxy-17-(and 2′,2′-dimethyltrimethylenedioxy)-estra-1,3,5(10),6-tetraene (288 mg, 0.78 mmol) in 70% acetic acid (6 mL) was heated at 60°C for 1.5 h. The reaction was cooled to room temperature, poured into saturated aqueous sodium bicarbonate (10 mL) and extracted with ethyl ether. The organic layer was washed with brine, dried over sodium sulphate, filtered and evaporated to dryness to afford a colourless residue from which 3-methoxyestra-1,3,5(10),6-tetraen-17-one was obtained after recrystallisation from methanol (198 mg, 0.7 mmol, 90% yield). IR (film) 1730, 1600, 1568, 1492, 1260, 1048, 1008 cm^{-1}. ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.16 (d, 1H, J = 8.4 Hz, 1-H), 6.75 (dd, 1H, J = 2.7 Hz, J = 8.4 Hz, 2-H), 6.66 (d,

1H, J = 2.7 Hz, 4-H), 6.51 (dd, 1H, J = 2.7 Hz, J = 9.6 Hz, 6-H), 6.06 (dd, 1H, J = 1.3 Hz, J = 9.6 Hz, 7-H), 3.80 (s, 3H, -OCH_3), 0.91 (s, 3H, 18-CH_3). ^{13}C-NMR (67.9 MHz, $CDCl_3$) δ220.35 (C17), 159.55 (C3), 136.53 (C5), 132.31 (C7), 132.20 (C10), 129.85 (C1), 125.55 (C6), 113.35, 113.19 (C2, C4), 56.59 (-OCH_3), 50.05 (C14), 49.74 (C13), 43.36 (C8), 39.50 (C9), 37.00 (C16), 32.17 (C12), 25.01 (C11), 22.79 (C15), 14.88 (C18). MS (EI^+) m/z(%) = 282 (M^+, 71), 225 (8), 197 (16), 184 (18), 171 (22), 167 (36), 158 (23), 149 (100). HRMS: Found: 282.1619. Calcd. for $C_{19}H_{22}O_2$: 282.1620.

2.5. 3-Methoxy-17α-ethynylestra-1,3,5(10),6-tetraen-17β-ol (**6**)

A solution of 3-methoxyestra-1,3,5(10),6-tetraen-17-one (**5**) (300 mg, 1.06 mmol) in dry dimethyl sulphoxide (3 mL) was treated under nitrogen with lithium acetylide–ethylene diamine complex (147 mg, 1.6 mmol) and the mixture was stirred for 1.5 h at room temperature. The mixture was poured into ice-cold water, acidified with dilute acetic acid, extracted with ethyl acetate, washed with water and brine and then dried ($MgSO_4$). After evaporation of the solvent under reduced pressure, the residue was chromatographed on silica gel (ether:hexane; 1:1) to yield 3-methoxy-17α-ethynylestra-1,3,5(10),6-tetraen-17β-ol (**6**) (260 mg, 0.84 mmol, 80% yield). Mp 82–85°C (ether/hexane). IR (film) 3450, 3300, 1640, 1600, 1570, 1500, 1260, 1040, 1050, 1000 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$) δ7.17 (d, 1H, J = 8.3 Hz ,1-H), 6.74 (dd, 1H, J = 2.7 Hz, J = 8.3 Hz, 2-H), 6.65 (d, 1H, J = 2.7 Hz, 4-H), 6.45 (dd, 1H, J = 2.7 Hz, J = 9.6 Hz, 6-H), 5.97 (dd, 1H, J = 1.7 Hz, J = 9.6 Hz,7-H), 3.80 (s, 3H -OCH_3), 2.61 (s, 1H, C ≡ CH), 0.89 (s, 3H, 18-CH_3). ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 158.10 (C3); 135.38 (C5); 132.73 (C7); 131.38 (C10); 127.89 (C1); 124.28 (C6); 111.84 (C2); 111.73 (C4); 89.00 (C20) 79.68 (C17); 74.22 (C21); 55.31 (-OCH_3); 47.62 (C14); 45.11 (C13); 41.62 (C9); 39.24 (C8); 38.81 (C16); 32.24 (C12); 24.17 (C11); 22.65 (C15); 12.43 (C18). MS (EI^+) m/z (%) = 308 (M^+,100); 282 (13.5); 225 (27); 211 (18); 172 (39); 171 (46); 158 (16); HRMS (EI^+): Found: 308.1773. Calcd. for $C_{21}H_{24}O_2$: 308.1776.

2.6. (E)-3-Methoxy-17α-tributylstannylvinylestra-1,3,5(10),6-tetraen-17β-ol (**7-E**)

A mixture of 3-methoxy-17α-ethynylestra-1,3,5(10),6-tetraen-17β-ol (**6**) (100 mg, 0.32 mmol) and tributyltin hydride (0.4 mL, 0.15 mmol) in toluene (3 mL) were heated at 90°C in the presence of azobisisobutyronitrile (AIBN) (30 mg, 0.08 mmol) under nitrogen for 3 h. The mixture was allowed to cool, poured into ice water and then extracted with ethyl acetate (2 × 10 mL). The organic layer was separated and washed with water (2 × 10 mL) and brine (10 mL), dried (Na_2SO_4) and the solvent removed under reduced pressure and the residue was purified by flash column chromatography. Elution with ether:hexane (1:3) gave (*E*)-3-methoxy-17α-tributylstannylvinylestra-1,3,5(10),6-tetraen-17β-ol (**7-*E***) as the major product (oil, 114 mg, 0.19 mmol, 60% yield). IR (film) 3470, 2954, 2924, 1604, 1493, 1570, 1460, 1259, 1040 cm^{-1}. ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.15 (d, 1H, J = 8.3 Hz, 1-H), 6.73 (dd, 1H, J = 2.7 Hz, J = 8.3 Hz, 2-H), 6.65 (d, 1H, J = 2.7 Hz, 4-H), 6.45 (dd, 1H, J = 2.4 Hz, J = 9.0 Hz, 6-H), 6.21 (dd, 1H, J = 19 Hz, 20-H), 6.07 (d, 1H, J = 19 Hz, 21-H), 6.03 (d, 1H, J = 9.0 Hz, 7-H) 3.80 (s, 3H -OCH_3), 0.87 (s, 3H, 18-CH_3). ^{13}C-NMR (67.9 MHz, $CDCl_3$) δ 158.07 (C3), 152.21 (C20), 135.41 (C5), 133.20 (C7), 131.51 (C10), 127.75 (C1), 124.86 (C21), 124.26 (C6), 111.79 (C2), 111.66 (C4), 85.32 (C17), 55.29 (OCH_3), 47.19 (C14), 45.10 (C13), 41.92 (C9), 39.32 (C8), 35.75 (C16) 31.89 (C12), 29.71, 29.31, 29.18, 29.04, 27.99, 27.84, 27.62, 27.27, 26.86, 24.17, 23.28, 17.52 (-CH_2- n-butyl chain), 13.95, 13.76, 13.62 (-CH_3 *n*-butyl chain), 9.61 (C18). MS (FAB^+) m/z (%) = 599 (MH^+, 6), 543 (26), 291 (51), 171 (100). HRMS (FAB, MH^+): Found: 599.3071. Calcd. for $C_{33}H_{53}O_2^{118}Sn$: 599.3069. Found: 601.3080. Calcd. for $C_{33}H_{53}O_2^{120}Sn$: 601.3075.

2.7. (Z)-3-Methoxy-17α-tributylstannylvinylestra-1,3,5(10),6-tetraen-17β-ol (**7-Z**)

A mixture of 3-methoxy-17α-ethynylestra-1,3,5(10),6-tetraen-17β-ol (**6**) (70 mg, 0.23 mmol) and tributyltin hydride (1 mL, 3.7 mmol) was stirred in hexamethylphosphoramide (1.5 mL) at 70°C for 45 h under nitrogen. The mixture was allowed to cool, diluted with ethyl acetate (20 mL), washed with water (2 × 10 mL), dried (Na_2SO_4) and the solvent removed under reduced pressure. The residue was purified by flash column chromatography. Elution with 5% ethyl acetate in petroleum ether gave (*Z*)-3-methoxy-17α-tributylstannylvinylestra-1,3,5(10),6-tetraen-17β-ol (**7-*Z***) as the major product (oil, 34 mg, 0.06 mmol, 25% yield). IR (film) 3028, 2954, 2924, 1603, 1570, 1493, 1461, 1260, 1042 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.15 (d, 1H, J = 8.1 Hz, 1-H), 6.78 (d, 1H, J = 13.2 Hz, 20-H), 6.72, (dd, 1H, J = 3 Hz, J = 8.8 Hz, 2-H), 6.46 (dd, 1H, J = 9.6 Hz, J = 2.4 Hz, 6-H), 6.00 (dd, 1H, J = 9.6 Hz, J = 1.5 Hz, 7-H), 5.88 (d, 1H, J = 13.2 Hz, 21-H), 3.80 (s, 3H, -OCH_3), 0.89 (s, 3H, 18-CH_3). ^{13}C-NMR (75.9 MHz, $CDCl_3$) δ 158.11 (C3), 149.98 (C20), 135.39 (C5), 132.99 (C7), 131.50 (C10), 127.87 (C1), 125.72 (C21) 124.28 (C6), 111.83 (C2), 111.70 (C4), 85.03 (C17), 55.30 (OCH_3), 47.94 (C14), 47,47 (C13), 41.86 (C9), 39.47 (C8), 32.11, 29.33, 29.46, 24.17, 23.33, 14.01, 13.81, 12.02. MS (FAB^+, 3-nitrobenzyl

alcohol) m/z (%) = 599 (MH^+, 6), 543 (26), 291 (51), 171 (100); HRMS (FAB, MH^+): Found: 599.3049. Calcd. for $C_{33}H_{53}O_2^{118}Sn$: 599.3069. Found: 601.3053. Calcd. for $C_{33}H_{53}O_2^{120}Sn$: 601.3075.

2.8. (E)-3-Methoxy-17α-iodovinylestra-1,3,5(10),6-tetraen-17β-ol (**8-E**) and (Z)-3-methoxy-17α-iodovinylestra-1,3,5(10),6-tetraen-17β-ol (**92|||8-Z**)

The same procedure was used for the synthesis of both isomers. Thus, (*E*)-3-methoxy-17α-tributylstannylvinylestra-1,3,5(10),6-tetraen-17β-ol (**7-*E***) or (*Z*)-3-methoxy-17α-tributylstannylvinylestra-1,3,5(10),6-tetraen-17β-ol (**7-*Z***) (0.1 mmol) in chloroform (3 mL) were treated with a 0.1 M solution of iodine in chloroform until the colour of iodine persisted. This was followed by addition of potassium fluoride in methanol (0.2 mL 1 M KF solution) and of 5% aqueous sodium bisulfite (0.2 mL) and the mixture was extracted with ethyl acetate (3 × 10 mL). The organic phase was washed with water, dried (Na_2SO_4), and evaporated to dryness under reduced pressure and the residue purified by flash column chromatography. Elution with ether:hexane (1:1) gave (*E*)-3-methoxy-17α-iodovinylestra-1,3,5(10),6-tetraen-17β-ol (**8-*E***) as a colourless solid (25 mg, 0.06 mmol, 60% yield) or (*Z*)-3-methoxy-17α-iodovinylestra-1,3,5(10),6-tetraen-17β-ol (**8-*Z***) as a colourless solid (14 mg, 0.03 mmol, 32% yield).

(**8-*E***) Mp 133–135°C (from ether:hexane). IR (KBr) 3458, 2936, 2858, 1603, 1571, 1495, 1468, 1261, 1044, 761 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.15 (d, 1H, J = 8.4 Hz, 1-H), 6.79 (d, 1H, J = 14.4 Hz, 20-H), 6.73 (dd, 1H, J = 2.7 Hz, J = 8.4 Hz, 2-H), 6.65 (d, 1H, J = 2.7 Hz, 4-H), 6.46 (dd, 1-H, J = 9.6 Hz, J = 2.6 Hz, 6-H), 6.32, (d, 1H, J = 14.4 Hz, 21-H), 5.97 (dd, 1H, J = 9.6 Hz, J = 1.3 Hz, 7-H), 3.80 (s, 3H, -OCH_3), 0.93 (s, 3H, 18-CH_3). ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 158.12 (C3), 150.30 (C20), 135.32 (C5) 132.60 (C7), 131.28 (C10), 128.03 (C1), 124.26 (C1) 111.87 (C4) 111.75 (C2), 86.92 (C17), 74.87 (C21), 55.29 (-OCH_3), 47.44 (C14), 45.10 (C13), 41.71 (C9), 39.31 (C8), 36.44 (C12), 31.99 (C16), 24.03 (C11), 23.09 (C15), 13.67 (C18). MS (FAB^+, 3-nitrobenzyl alcohol) m/z (%) = 437 (18), 419 (19), 291 (76), 235 (56), 171 (100). HRMS: Found: 436.0907. Calcd. for $C_{21}H_{25}O_2I$: 436.0899.

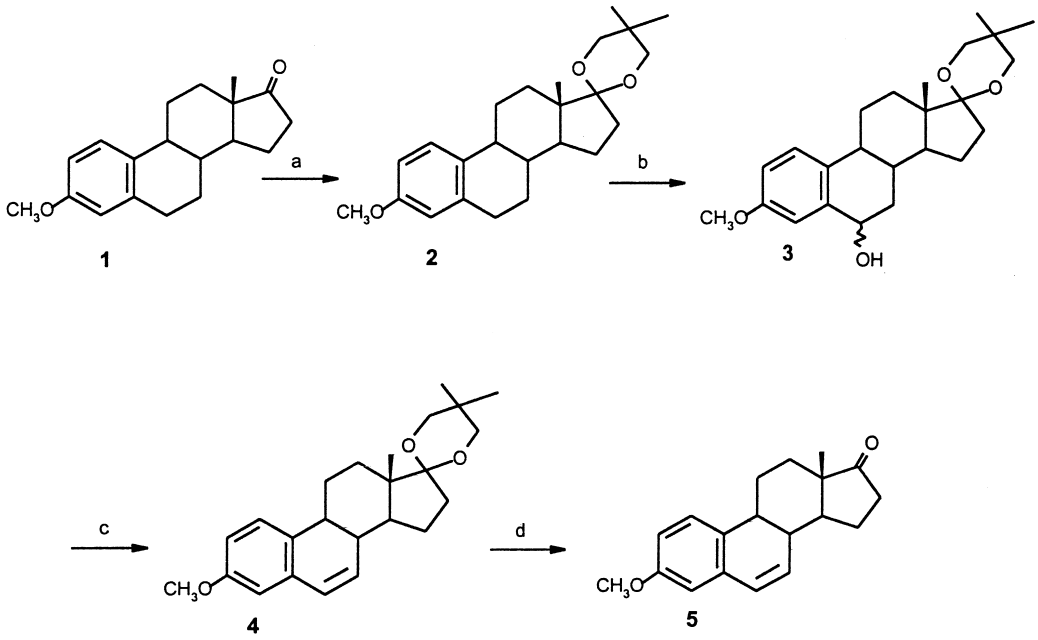
(**8-*Z***) IR (film) 3544, 2930, 2864, 1603, 1569, 1493, 1464, 1259, 1040, 756 cm^{-1}. ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.16 (d, 1H, J = 8.6 Hz, 1-H), 6.85 (d, 1H, J = 8.6 Hz, 20-H), 6.74, (dd, 1H, J = 3.0 Hz, J = 8.4 Hz, 2-H), 6.65 (d, 1H, J = 2.6 Hz, 4-H), 6.45 (dd, 1H, J = 9.6 Hz, J = 2.3 Hz, 6-H), 6.38 (dd, 1H, J = 8.6 Hz, 21-H), 5.97 (d, 1H, J = 1.6 Hz, J = 9.6 Hz, 7-H), 3.78 (s, 3H, -OCH_3), 0.96 (s, 3H, 18-CH_3). ^{13}C-NMR (67.9 MHz, $CDCl_3$) δ 158.15 (C3), 143.22 (C20), 135.35 (C5), 132.74 (C7), 131.30 (C10), 127.97 (C1), 124.27 (C6), 111.88 (C4), 111.77 (C2), 84.75 (C17), 76.53 (C21), 55.31 (OCH_3), 48.93 (C13), 47.82 (C14), 41.76 (C9), 39.39 (C8), 37.63 (C12), 31.57 (C16), 24.08 (C11), 23.09 (C15), 13.89 (C18). MS (FAB^+, 3-nitrobenzyl alcohol) m/z (%) = 436 (M^+, 29). HRMS: Found: 436.0907. Calcd. for $C_{21}H_{25}O_2I$: 436.0899.

2.9. [^{125}I](E)-3-Methoxy-17α-iodovinylestra-1,3,5(10),6-tetraen-17β-ol (E[^{125}I]IVDE, **8-E**-^{125}I) and [^{125}I](Z)-3-methoxy-17α-iodovinylestra-1,3,5(10),6-tetraen-17β-ol (Z[^{125}I]IVDE, **8-Z**-^{125}I)

The radioiodinated compounds **8-*E***-^{125}I and **8-*Z***-^{125}I were prepared by oxidative iodination of the corresponding tributylstannyl compounds **7-*E*** and **7-*Z***. They were purified by HPLC. To a mixture of **7-*E*** or **7-*Z*** (100 μg, 166 nmol) in ethanol (200 μL) was added [^{125}I]sodium iodide (18.5 MBq in 5 μL of a diluted sodium hydroxide solution, pH = 9), followed by a chloramine-T aqueous solution (50 μg, 1 μg/μL). The reaction was quenched after 4 min by addition of an aqueous sodium metabisulfite solution (50 μL, 1 μg/μL). The reaction mixture was purified on analytical C-18 reversed phase HPLC column operated at a rate of 1 mL/min. Elution with methanol–water (80:20) gave *E*[^{125}I]IVDE and *Z*[^{125}I]IVDE, respectively. The fractions containing the desired compound were combined and concentrated under a stream of nitrogen. The labelled compounds were taken up in ethanol and diluted with sterile saline to 111 kBq/100 μl for injection purposes.

2.10. Biodistribution studies in mice

Studies were carried out with immature (21–25 d) female mice CD-1 (randomly bred, Charles River, Wilmington, MA) in accordance with the Directives of EEC (Portuguese National Law 129/92). Animals were intravenously injected with 111 kBq (3 μCi) of the ^{125}I-labelled preparation via the lateral tail vein. The injection solutions were prepared by dilution of the ^{125}I-labelled estradiol derivative to give a final concentration of 10% (v/v) ethanol in water. A standard was prepared to determine the radioactive dosage administered. The mice were maintained on normal diet ad libitum. A separate group of animals was co-injected with unlabelled estradiol (50 μg) for the receptor saturation studies. Animals were sacrificed by cervical dislocation at 1, 4 and 24 h after injection with the radiotracer, and at 4 h in the case of the co-injected cold estradiol. The radioactivities in the various organs were determined in a gamma counter. The results were expressed as percent of injected dose per g organ (%ID/g organ). Mean values are given with the standard deviation. For blood and muscle the radioactivity was calculated assuming that these organs constitute 7



Scheme 1.

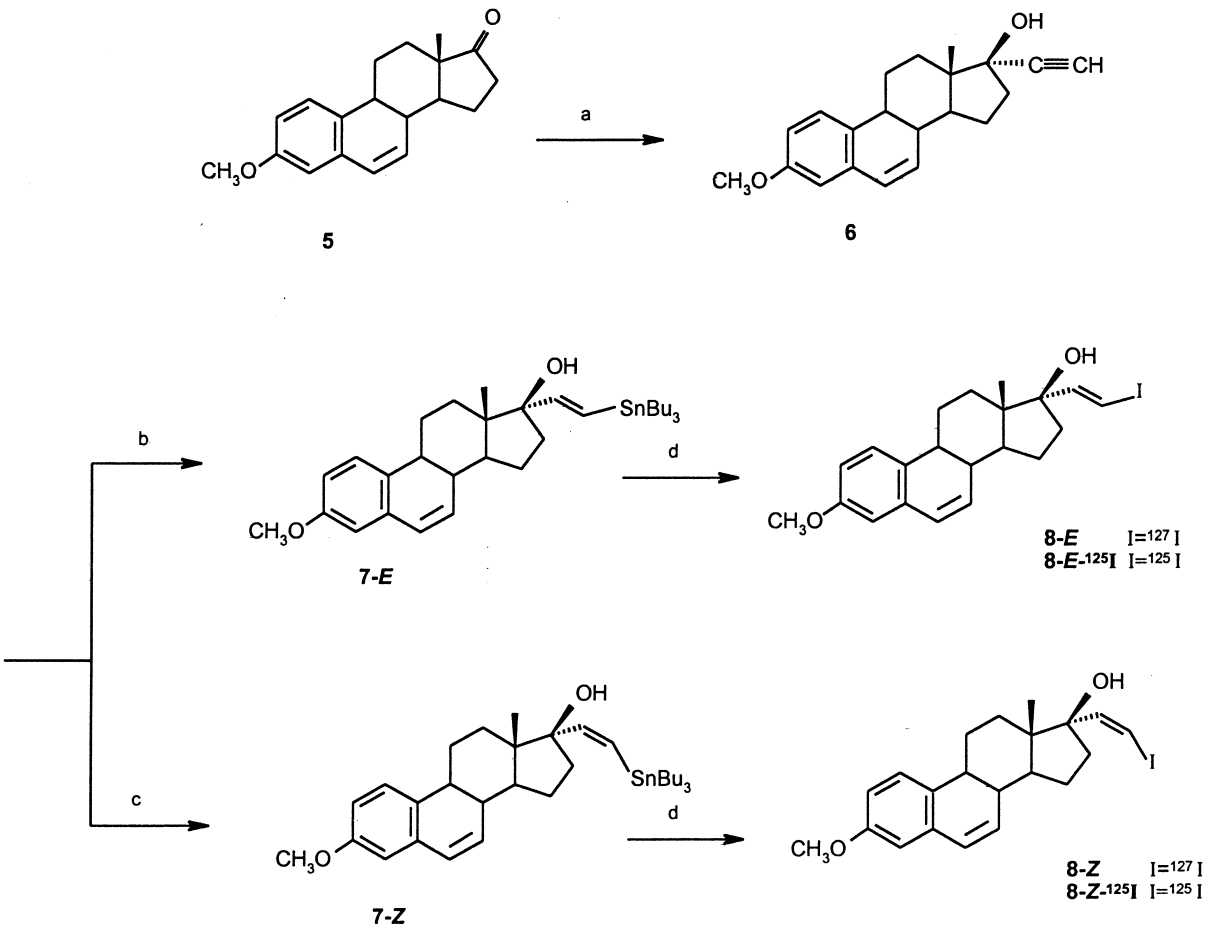
and 40% of the total weight body, respectively. The radioactivity ratios in target to blood and target to muscle were calculated. Results of the biodistribution were evaluated by an analysis of variance. The level of significance was set at 0.05.

3. Results and discussion

The synthetic route to prepare 3-methoxyestra-1,3,5(10),6-tetraen-17-one (**5**) is presented in Scheme 1. Acetalisation of 3-methoxyestra-1,3,5(10)-trien-17-one (**1**) with 2,2-dimethyl-propane-1,3-diol under standard conditions gave 3-methoxy-17,17-(2′,2′-dimethyltrimethylenedioxy)e stra-1,3,5(10)-triene (**2**) in 40% yield.

In principle, estradiol derivatives can undergo hydrogen-metal exchange with strong bases at the aromatic position C-2 and at the benzylic positions C-6 and C-9. Although benzylic protons are intrinsically more acidic than ring protons in benzene derivatives, aromatic rings bound to an electronegative atom such as oxygen can seriously compete with the benzylic position for deprotonation. The strong preference of LIDAKOR reagents (lithium diisopropylamide/potassium alkoxide) for benzylic deprotonation has been demonstrated by Takagishi and Schlosser (1991) in the metallation of some toluene derivatives bearing electronegative substituents on the ring. Tedesco et al. (1995) had already shown that careful equilibration leads to selective deprotonation at C-6 (vs C-9) in estrones. This finding prompted us to transform **2** oxidatively into **3** in a similar manner, via the anion of **2**, deprotonated at C-6. This can be achieved with a fourfold excess of LIDAKOR reagent (prepared by mixing equimolecular amounts of lithium diisopropylamide and potassium *tert*-butoxide in THF solution [−78°C, 3 h]). 3-Methoxy-17-(2′,2′-dimethyltrimethylenedioxy)estra-1,3,5(10)-triene-6-ol (**3**) was obtained in 68% yield after quenching the metallated species with trimethyl borate, followed by reaction with excess of hydrogen peroxide. This hydroxylation turned out to be remarkably stereoselective: a ratio of approximately 90:10 of 6α to 6β-epimer was obtained. The two epimeric alcohols were not separated but the composition of the mixture was established by inspection of its ^{1}H-NMR spectrum. The 6,7-olefinic bond was introduced efficiently by dehydration of the 6α-hydroxy derivative with *p*-toluenesulphonic acid giving 3-methoxy-17-(2′,2′-dimethyltrimethylenedioxy)estr a-1,3,5(10),6-tetraene (**4**) in 80% yield. After acidic cleavage of the 17,17-acetal group, 3-methoxyestra-1,3,5(10),6-tetraen-17-one (**5**) was obtained in 90% yield.

For radioiodination purposes, 3-methoxyestra-1,3,5(10),6-tetraen-17-one (**5**) was functionalised at the C-17α position with the ethynyl moiety followed by a hydrostannylation and iodination of the corresponding tributylstannyl precursors (Scheme 2). **5** was converted into the 17α-ethynyl analogue (**6**) by reaction with



Scheme 2.

lithium acetylide–ethylene diamine complex. The isomeric (*E*)-3-methoxy-17α-iodovinylestra-1,3,5(10),6-tetraen-17β-ol (8-*E*) and (*Z*)-3-methoxy-17α-iodovinylestra-1,3,5(10),6-tetraen-17β-ol (8-*Z*) were prepared via stannyl-iodo exchange (see also Ali et al., 1988) of the corresponding tributylstannyl precursors (7-*E* and 7-*Z*), obtained by hydrostannylation of the acetylene (6). The iodovinyl derivatives (8-*E*) and (8-*Z*) were obtained in 60% and 32% yield, respectively. This electrophilic substitution reaction has been shown to be stereospecific, ultimately resulting in good yields of radiopharmaceuticals with high specific radioactivities. In addition, the tributylstannyl group permits to iodinate selectively the vinyl substituent even in the presence of an unprotected phenolic hydroxy group. 3-Methoxy-17α-ethynylestra-1,3,5(10),6-tetraen-17-βol (**6**) was treated for 24 h with tributyltin hydride in toluene at 90–100°C in the presence of azobutyronitrile as a catalyst to yield (*E*)-3-methoxy-17α-tributylstannylvinylestra-1,3,5(10),6-tetraen-17β-ol (7-*E*). Replacing toluene by hexamethylphosphoramide (a more polar solvent) and lowering the reaction temperature to 60–70°C in the absence of a catalyst, leads to the selective formation of the *Z*-isomer. The tributylstannyl intermediates were converted with retention of configuration to the corresponding iodovinyl derivatives via reaction with iodine in chloroform. Stereochemical assignments of the isomeric (*E*)- and (*Z*)-3-methoxy-17α-iodovinylestra-1,3,5(10),6-tetraen-17β-ol (8-*E* and 8-*Z*) were based on high resolution ^{1}H- and ^{13}C-NMR spectra. The ^{1}H-NMR spectrum of the 20*E*-isomer gave two doublets at δ 6.32 and 6.79 with coupling constants of $J = 14$ Hz, characteristic of the 17α-vinyl protons with a *trans* (*E*) iodo-substituent. On the other hand, the 20*Z*-isomer showed two doublets at δ 6.38 and 6.85 with coupling constants of $J = 8$ Hz, characteristic of the 17α-vinyl protons with *cis* (*Z*)-iodo configuration (Hofmeister et al., 1986). The ^{13}C-

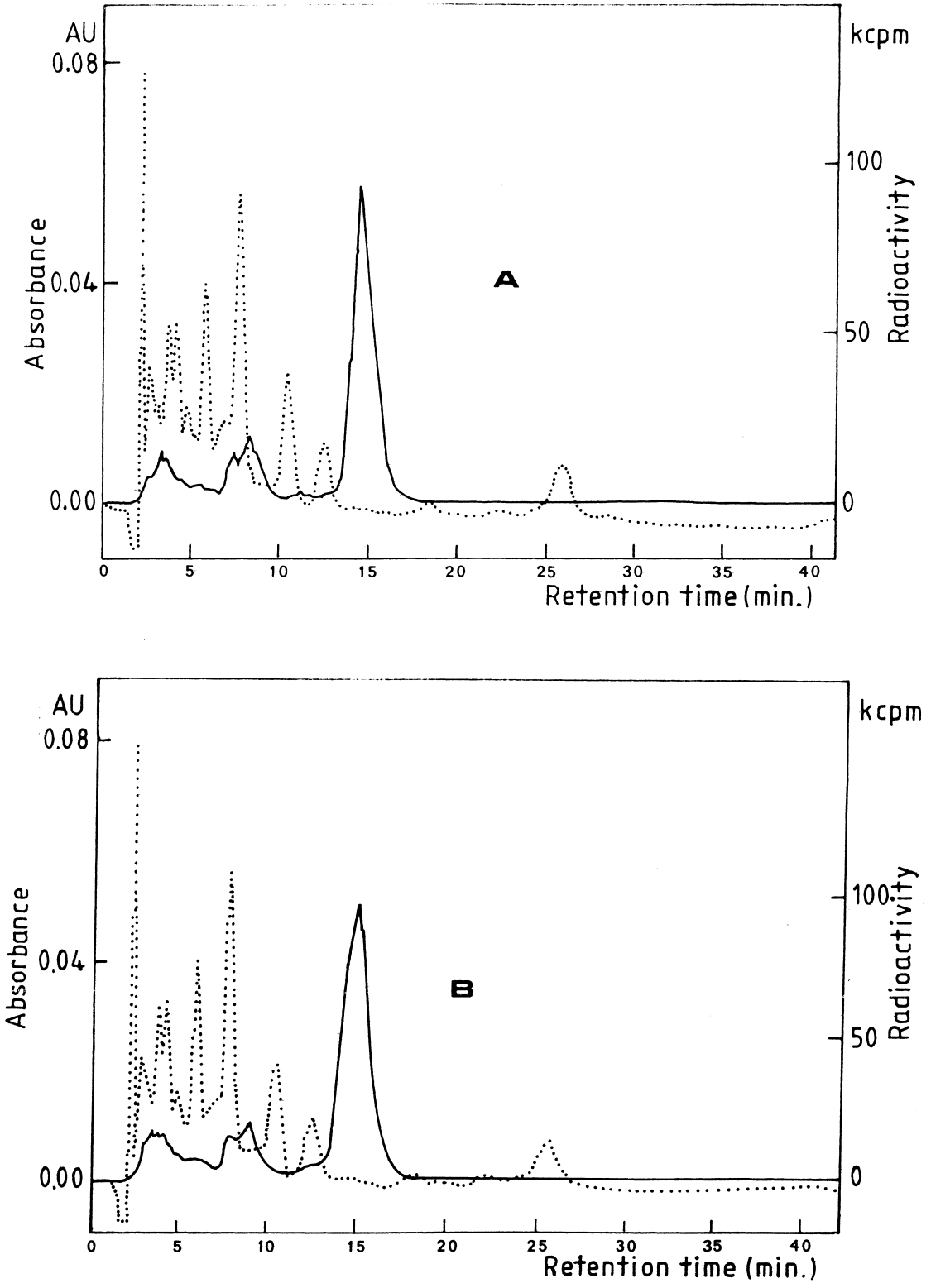


Fig. 1. UV absorbance (···) and radioactivity (—) profiles for the HPLC separation of E[^{125}I]IVDE (A) and Z[^{125}I]IVDE (B) from reaction mixtures. Experimental conditions: C18 reverse-phase column (Nucleosil 10 μm ET 250/8/4; Macherey–Nagel); flow rate: 1 mL/min; mobile phase: 80% methanol.

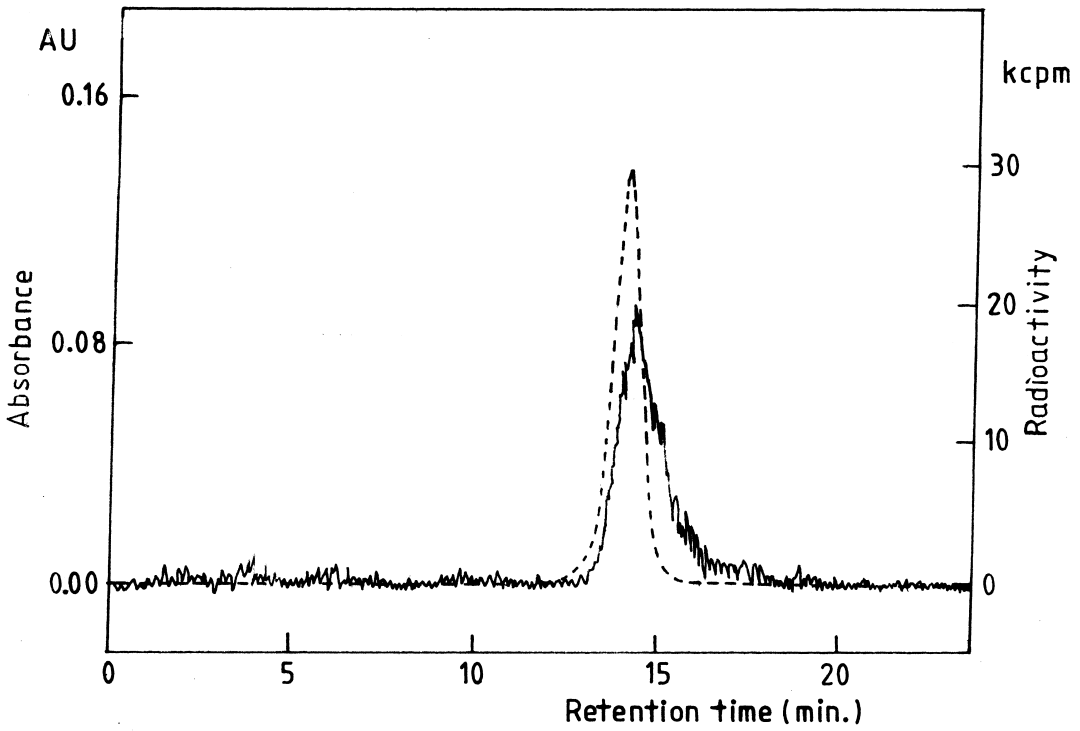


Fig. 2. HPLC analysis of radioiodinated E[^{125}I]IVDE and iodinated **8-*E*** with simultaneous radioactivity (—) and UV absorbance (···) detection. Experimental conditions: C18 reverse-phase column (Nucleosil 10 μm ET 250/8/4; Macherey-Nagel); flow rate: 1 mL/min; mobile phase: 80% methanol.

NMR spectrum of the 20E-isomer showed characteristic vinyl carbon signals at δ 150.30 (C-20) and 74.87 (C-21), while the 20Z-isomer gave corresponding signals at δ 143.22 (C-20) and 76.53 (C-21).

The corresponding [^{125}I](E)-3-methoxy-17α-iodovinylestra-1,3,5(10),6-tetraen-17β-ol (***E***[^{125}I]IVDE) and [^{125}I](Z)-3-methoxy-17α-iodovinylestra-1,3,5(10),6-tetraen-17β-ol (***Z***[^{125}I]IVDE) were readily obtained in

Table 1
Tissue distribution of E[^{125}I]IVDE and Z[^{125}]IVDE in immature female mice expressed in percentage of injected dose/g organ

Organ	1 h		4 h		4 h (co-injection of Estradiol)		24 h	
	E	Z	E	Z	E	Z	E	Z
Blood	0.4 ± 0.2	1.0 ± 0.3	0.4 ± 0.1	0.6 ± 0.1	0.6 ± 0.4	0.7 ± 0.0	0.2 ± 0.1	0.2 ± 0.0
Uterus	0.8 ± 0.4	1.6 ± 0.3	0.8 ± 0.3	1.6 ± 0.4	0.8 ± 0.4	0.9 ± 0.2	0.2 ± 0.1	0.2 ± 0.0
Ovaries	1.5 ± 0.6	3.6 ± 1.3	2.0 ± 0.5	1.9 ± 0.6	2.3 ± 0.2	1.3 ± 0.2	0.9 ± 0.5	0.2 ± 0.1
Liver	13.1 ± 3.5	8.6 ± 2.0	8.5 ± 1.6	4.3 ± 0.5	5.8 ± 1.2	3.2 ± 1.3	1.4 ± 0.4	1.2 ± 0.3
Intestines	9.5 ± 4.3	10.4 ± 0.8	16.7 ± 2.9	11.3 ± 4.8	16.8 ± 3.9	9.0 ± 4.3	1.2 ± 0.6	0.6 ± 0.4
Spleen	0.5 ± 0.2	2.0 ± 0.8	0.4 ± 0.1	1.3 ± 0.1	0.5 ± 0.1	0.6 ± 0.6	0.2 ± 0.1	0.4 ± 0.1
Lung	0.9 ± 0.6	1.0 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.5 ± 0.1	0.6 ± 0.2	0.1 ± 0.0	0.1 ± 0.0
Kidney	1.1 ± 0.3	1.3 ± 0.1	0.5 ± 0.2	0.8 ± 0.3	0.8 ± 0.4	0.7 ± 0.2	0.1 ± 0.0	0.2 ± 0.0
Brain	1.1 ± 0.3	0.7 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Muscle	0.8 ± 0.3	0.8 ± 0.2	0.4 ± 0.1	0.6 ± 0.1	0.5 ± 0.2	0.4 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
Stomach	0.9 ± 0.4	2.8 ± 0.8	2.9 ± 0.7	4.9 ± 0.9	1.6 ± 1.2	4.0 ± 0.3	0.4 ± 0.4	0.2 ± 0.1
Thyroid	2.9 ± 1.0	42.3 ± 8.5	21.1 ± 12.8	112.6 ± 33.0	16.9 ± 4.7	81.3 ± 2.7	48.8 ± 25.9	233 ± 181
Urinary excretion	3.1 ± 3.2	17.5 ± 5.0	5.3 ± 2.0	20.6 ± 9.0	8.0 ± 1.7	16.0 ± 2.8	83.0 ± 3.4	91.5 ± 1.5

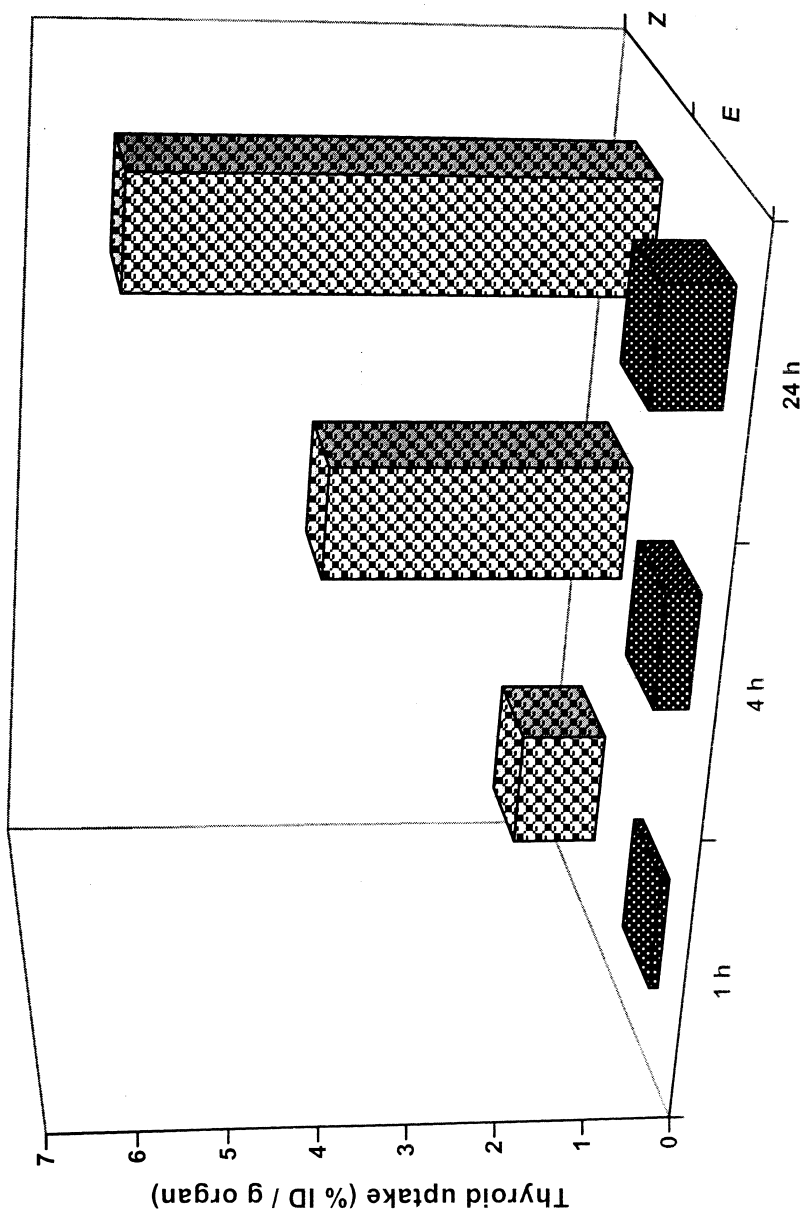


Fig. 3. Radioiodine accumulation in the thyroid of mice at 1, 4 and 24 h after administration of $E[^{125}I]IVDE$ and $Z[^{125}I]IVDE$ (mean ratio values ± standard deviation).

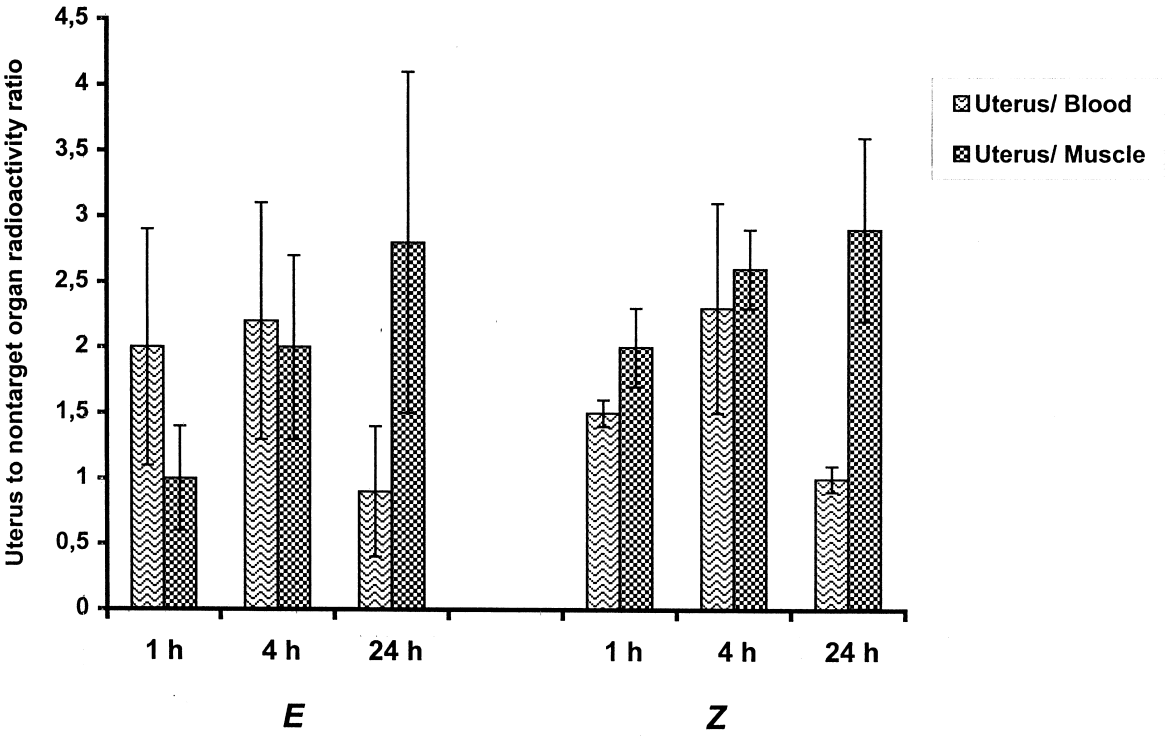


Fig. 4. Uterus to non-target (blood and muscle) radioactivity ratios of E[^{125}I]IVDE and Z[^{125}I]IVDE at 1, 4 and 24 h after administration.

good radiochemical yield, 80% and 70%, respectively, by treating the tributylstannyl derivatives with [^{125}I]sodium iodide in the presence of chloramine-T. The radioiodinated products (Fig. 1) were isolated by HPLC (radiochemical purity >95%) and compared to their corresponding non-radioactive iodinated analogues, **8-*E*** or **8-*Z***, by HPLC with simultaneous UV absorbance and radioactivity detection as shown in Fig. 2 for the *Z*-isomer. Similar profiles were also obtained for the *E*-isomer.

The biodistribution of the radiolabelled estrogens on the tissue can predict their usefulness as a breast cancer imaging agent better than the in vitro binding affinity, since factors such as protein binding, receptor dissociation, recirculation and metabolism are taken into account and must be considered in predicting the properties of the radioligands. The results of the biodistribution studies of the ^{125}I-labelled 3-methoxy-iodovinyl-6,7-dehydroestradiol derivatives in immature Charles River female mice are presented in Table 1. The results are expressed in percentage of injected dose per g organ (ID %/g organ) at 1, 4, and 24 h after injection with radiotracer, and at 4 h in the case of co-injected cold estradiol.

The rapid blood clearance observed indicates that the concentration rapidly drops to low values in the blood in the first hours after injection and continues to decline over the period of study. Since the primary route for excretion of estrogens is through the faeces, high levels of activity in the intestines were observed. Although similar biodistribution patterns for both isomeric iodovinyl estrogens were found, a significant difference in their biodistribution was observed. The clearance of radioactivity from both blood and muscle was faster for E[^{125}I]IVDE. The uterus uptake at 1 and 4 h after injection and the ovaries uptake at 1 h after injection were statistically higher ($p > 0.05$) for Z[^{125}I]IVDE. The higher specificity of the 20*Z*-isomers has already been reported (Napolitano et al., 1991; Hughes et al., 1997, Rijks et al., 1997b) for other 17α-iodovinyl estrogen derivatives. On the other hand, no evidence of receptor mediation in uterine uptake was observed from a 4 h blocked experiment, when an excess of inactive estradiol was co-injected with E[^{125}I]IVDE. However, the higher thyroid and stomach uptake as well as the higher urinary excretion found for Z[^{125}I]IVDE indicates its higher in vivo decomposition rate. The extent of deiodination of the two isomers, as evaluated from the thyroid uptake at 1, 4 and 24 h after injection is presented in Fig. 3. The urinary excretion was 3.1 ± 3.2, 5.3 ± 5.0, 83.0 ± 3.4 and 17.5 ± 5.0, 20.6 ± 9.0, 91.5 ± 1.5 at 1, 4 and 24 h for

E[^{125}I]IVDE and Z[^{125}I]IVDE, respectively. The radioactivity ratio (target to non-target organ) has been used as indicator for the uptake selectivity, since it is the difference between target and non-target organ uptake which is responsible for the contrast needed in imaging. The uterus to blood and uterus to muscle ratios are presented in Fig. 4. From the analysis of these values the higher specificity of Z[^{125}I]IVDE (the uterus uptake decreased to one half when a co-injection of inactive estradiol was administered) is not evident, since the E[^{125}I]IVDE isomer, although showing a non-specific uterus uptake, undergoes a faster clearance from blood and muscle. In conclusion, the biological data obtained for both E[^{125}I]IVDE and Z[^{125}I]IVDE isomers indicate clearly that the Z-configuration for these compounds is the preferable configuration for estrogen receptor detection, although the Z-configured compounds have a higher in vivo decomposition rate. Nevertheless, higher uterus to blood and uterus to non-target tissues ratios are required to obtain a promising estrogen receptor-imaging agent, in spite of the reasonable specificity observed for the Z-isomer.

4. Concluding remarks

We have described the synthesis and the biological distribution of two new iodovinyl estradiol derivatives, which possess an unsaturation at positions C6–C7 within the steroidal framework and which may be regarded as the parent compounds for analogous C-7 substituted estratetraenes. The reaction sequence of the 20E- and 20Z-isomers constitutes an appropriate route for an extension of the procedure to the short-lived ^{123}I-labelled compounds of interest for single photon emission computed tomography imaging (Foulon et al., 1992; Ribeiro-Barras et al., 1992, Rijks et al., 1997b). The radiochemical purity of the high specific radioactivity radioiodinated compounds was greater than 95% after HPLC purification. Biological data indicated that the Z-isomer owing to its higher in vivo uptake by the target tissue has the preferable configuration for estrogen receptor detection.

In spite of the reasonable specificity observed for the Z-isomer, the introduction of the C6/C7 double bond on the estradiol frame does not increase the uterus to blood and the uterus to non-target tissue ratios compared to [^{123}I](Z)-11β-methoxy-17α-iodovinylestra-1,3,5(10)-trien-17β-ol, the most suitable tracer at the present time (Rijks et al., 1997b).

The synthesis and evaluation of 7α-substituted estra-1,3,5(10),6-tetraen-17β-ols are in progress.

Acknowledgements

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References

Ali, H., Rousseau, J., Ghaffari, M.A., van Lier, J.E., 1988. Synthesis, receptor binding and tissue distribution of (17α,20E)- and (17 α,20Z)-21-[^{125}I]iodo-19-norpregna-1,3,5(10),20-tetr aene-3,17-diol. J. Med. Chem. 31, 1946–1950.

Ali, H., Rousseau, J., van Lier, J.E., 1993. Synthesis of A-ring fluorinated derivatives of (17α,20E/Z)-[^{125}I] iodovinylestradiols: effect on receptor binding and receptor-mediated target tissue uptake. J. Med. Chem. 36, 3061–3072.

Da Silva, J.N., van Lier, J.E., 1990. Synthesis and structure-affinity of a series of 7α-undecylestradiol derivatives: a potential vector for therapy and imaging of estrogen-receptor-positive cancers. J. Med. Chem. 33, 430–434.

Foulon, C., Guilloteau, D., Baulieu, J.L., Ribeiro-Barras, M.J., Desplanches, G., Frangis, Y., Besnard, J., 1992. Estrogen imaging with 17α-[^{123}I]Iodovinyl-11β-methoxyestradiol (MIVE2)- Part I. Radiotracer preparation and characterization. Nucl. Med. Biol. 19, 257–261.

Franke, L., Hanson, R., 1984. Radioiodinated ligands for the estrogen receptor: effect of 3-O-methylation on tissue distribution. J. Nucl. Med. 25, 1116–1121.

Hanson, R., Frank, L., Kaplan, M., 1989. Synthesis and evaluation of (17 α,20E)-21-[^{125}I]Iodo-11-substituted-19-norpregna-1, 3,5(10),20-tetraene-3,17β-diols: the influence of 11-stereochemistry on tissue distribution of radioiodinated estrogens. Nucl. Med. Biol. 16, 3–9.

Hanson, R., Ghoshal, M., Murphy, F., Rosenthal, C., Gibson, R., Ferreira, N., Sood, V., Ruch, J., 1993. Synthesis, receptor binding and tissue distribution of 17α-E[^{125}I]iodovinyl 11β-ethyl-estradiol. Nucl. Med. Biol. 20, 351–358.

Hofmeister, H., Laurent, H., Schulze, P.E., Wiechert, R., 1986. Synthesis of 17α-bromovinyl- and 17α-iodovinylnortestosterone derivatives. Tetrahedron 42, 3575–3578.

Hughes, A., Larson, S.M., Hanson, R.N., DeSombre, E.R., 1997. Uptake and interconversion of the Z and E isomers of 17α-iodovinyl-11β-methoxyestradiol in the immature female rat. Steroids 62, 244–252.

Inohae, E., Thiemann, T., Mataka, S., Melo e Silva, M.C., Patrício, L., 1999. Synthesis of 7-ω-amidoalkyl-substituted 6,7-dehydroestra-3,17β-diols. Kyushu Daigaku Kino-bushitsu Kenkyusho Hokoku 13 (1), 31–36.

Napolitano, E., Fiaschi, R., Hanson, R.N., 1991. Structure-activity relationships of estrogenic ligands: synthesis and evaluation of (17α,20E)-and (17α,20Z)-21-halo-19-norpregna-1,3,5(10),20-tetraene-3,17β-diols. J. Med. Chem. 34, 2754–2759.

Ribeiro-Barras, M.J., Foulon, C., Baulieu, J.L., Guilloteau,

D., Bougnous, P., Lansac, J., Besnard, J.C., 1992. Estrogen receptor imaging with 17α-[^{123}I]Iodovinyl-11β-methoxyestradiol (MIVE2)-Part II. Preliminary results in patients with breast carcinoma. Nucl. Med. Biol. 19, 263–267.

Rijks, L.J., Bakker, P.J., van Tienhoven, G., Noorduyn, L.A., Boer, G.J., Rietbroek, R.C., Taat, C.W., Janssen, A.G., Veenhof, C.H., van Royen, E.A., 1997a. Imaging of estrogen receptors in primary and metastatic breast cancer patients with iodine-123-labeled Z-MIVE. J. Clin. Oncol. 15, 2536–2545.

Rijks, L.J.M., Boer, G.J., Endert, E., de Bruin, K., Janssen, A.G., van Royen, E.A., 1997b. 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. 24, 65–75.

Rijks, L.J., Sokole, E.B., Stabin, M.G., de Bruin, K., Janssen, A.G., van Royen, E.A., 1998. Biodistribution and dosimetry of iodine-123-labelled *Z*-MIVE: an oestrogen receptor ligand for breast cancer imaging. Eur. J. Nucl. Med. 25, 40–47.

Takagishi, S., Schlosser, M., 1991 Fluorine- and trifluoromethyl-substituted toluenes: Site selective metalation of aromatic or benzylic positions, Synlett 119–121.

Tedesco, R., Fiaschi, R., Napolitano, , E, , 1995 6-Oxoestradiols from estradiols: Exploiting site selective metalation of aralkyl systems with superbases, Synthesis 1493–1495.

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