SYNTHESIS, RECEPTOR BINDING AND
BIODISTRIBUTION OF THE
GEM-21-CHLORO-21-IODOVINYLESTRADIOL
DERivATIVES

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Summary—Radioiodinated 11β-methoxy-(17α,20E)iodovinylestradiol (11β-OMe-IVE₂) shows high estrogen receptor (ER)-mediated uterus uptake and good potential as an ER-imaging agent. In order to examine the tolerance of the ER for modification about the iodovinyl substituent, we prepared the (17α,20Z-chloro)21-chloro-21-iodovinylestradiol (4a) and several derivatives featuring 11β-methoxy (4b), 11β-ethoxy (4c) or 7α-methyl (4d) substituents. All gem-dihalogen derivatives 4a-d were prepared from the 17α-chloroethynyl precursors. The intermediate chlorostannylvinyl derivatives were obtained using tri-n-butyltin hydride and palladium acetate catalyst. Compounds 4a and 4b were labeled with ¹²⁵I via their corresponding tin intermediates and their tissue distribution was studied in immature female rats. Addition of a 21-CI to the 17α-ethynylestradiols reduced ER binding affinity, except for the 11β-substituted analogs which showed a pronounced increase. Surprisingly, addition of a 21-CI to the (17α,20E)IVE₂ resulted in increased ER binding affinities and augmented ER-mediated uterus uptake, which may result from the pronounced increase in the dipole moment of the molecule. Thus, further modifications at the C-21 position of IVE₂ are well tolerated by the ER. However, addition of the 21-CI also resulted in increased radioiodine uptake by the thyroid, much slower blood clearance and lower uterus to blood/nontarget ratios, suggesting increased in vivo instability of the C--I bond of the gem-chlorine-iodine atoms which may reflect the increase in steric and electronic interference.

INTRODUCTION

Radiolabeled estrogens have received a great deal of interest over the past decades as possible diagnostic agents for estrogen receptor (ER)-imaging in relation to breast cancer detection and treatment, using nuclear medicine procedures [1–3]. A number of different short-lived isotopes have been evaluated as radiolabels for this procedure, including ¹¹C [4, 5], ¹⁸F [6–8], ⁷⁷Br [9–12] and various radioisotopes of iodine [13–21]. Whereas ¹⁸F-estrogens have shown the best in vivo images to date [22], the availability of ¹⁸F limits the use of such derivatives. Promising results were also obtained with 16α-iodo [23] and 17α-iodovinyl [24, 25] derivatives of estradiol.

We previously reported the synthesis and biological properties of both isomeric 17α-(20E/Z)-iodovinylestradiols and showed that particularly the 20 Z-configuration provides good target selectivity [26]. The preparation of a number of 17α-iodovinylestradiols in which the vinyl iodide is systematically removed from the 17-position of the steroid nucleus, through the addition of methylene groups (n = 0–7) has also been reported [27]. Further improvements in the biodistribution pattern were sought through the addition of 11β-methoxy/ethoxy, 7α-methyl [28] as well as 2- or 4-fluoro substituents [29]. Such substitutions are known to reduce nonspecific binding, to increase stability of the ER-ligand complex and to reduce metabolism for the A- and D-rings [30, 31]. Thus, most structure–activity studies reported to date involved modification about the steroid skeleton. In this study we evaluated the effect of additional substitution on the 17α-iodovinyl side-chain. The synthesis of a series of C-21 gem-dihalo derivatives is reported. The steric influence of varying the C-21 substituents (hydrogen for chlorine) on receptor binding affinity, in vivo stability and receptor-mediated uptake by target tissues, was evaluated.
EXPERIMENTAL

Materials and Methods

Melting points (m.p.) were determined on a Fisher-Johns apparatus and are uncorrected. Proton magnetic resonance (\(^1\)H NMR) spectra were obtained with a Bruker WM 25 spectrometer in CDCl\(_3\) + DMSO-d\(_6\) and chemical shifts are reported as parts per million (ppm, \(\delta\)) downfield from tetramethylsilane as an internal standard. Low resolution electron impact mass spectra (EI-MS) were obtained with a Hewlett-Packard model 5988A quadrupole instrument at 70 eV. Microanalysis data were obtained by Guelph Laboratories Ltd., Canada. Silica gel (60-200 mesh) was used for column chromatography. Silica gel plates coated with fluorescent indicator (u.v. 254) were used for analytical thin-layer chromatography (TLC) and the compounds were located by their u.v. absorbance or color response upon spraying with H\(_2\)SO\(_4\)/EtOH and heating at 120°C. High performance liquid chromatography (HPLC) was performed on a reverse-phase column (C-18, ODS-2 spherisorb, 5 \(\mu\)m, 25 x 0.94 cm, CSC, Montreal, Canada) with a flow rate of 2 ml/min, and the compounds were detected at 280 nm and where appropriate, by their \(^{14}C\) radiation which was registered via a sodium iodide detector. All chemicals used are commercially available and were of the highest chemical grade available. Carrier-free [\(^{125}\)I]NaI was purchased from Amersham Canada Ltd. Steroids were purchased from Steraloids Inc. or Sigma. All compounds used for the ER binding assay were shown to be >99% pure by HPLC.

Synthesis of 17\(\alpha\)-chloroethynylestradiol (2a--d)

A 0.5 mM solution of estrone (1a), 11\(\beta\)-methoxyestrone (1b), 11\(\beta\)-ethoxyestrone (1c) or 7\(\alpha\)-methylestrone (1d) in dry THF (5 ml) was added to a cold solution of lithium chloroacetylide in 5 ml of absolute THF to an ice cold solution of 0.3 ml of 1.4 M methyllithium in diethyl ether) in THF under N\(_2\). The mixture was stirred for 30 min after which it was diluted with water, extracted with ethyl acetate, dried over Na\(_2\)SO\(_4\) (anhy), filtered and evaporated to dryness under reduced pressure. Column chromatography on silica gel with ethyl acetate (10-20%) in hexane gave the title compounds (70-80% yield). The residue was also purified on a C-18 reverse phase semipreparative HPLC column in methanol–water (75:25, v/v).

17\(\alpha\)-Chloroethynylestradiol (2a). m.p. 110–113°C; HPLC, \(t\_R = 21\) min; \(^1\)H NMR (\(\delta\)) 0.72 (S, 3H, 18-CH\(_3\)), 6.44 (d, J = 2.5 Hz, 1H, 4-CH), 6.50 (dd, J = 2.5 and 8 Hz, 2-CH), 6.99 (d, J = 8 Hz, 1-CH). MS, \(m/z\) (relative intensity) 332 (M\(^+\), 24), 330 (M\(^+\), 83), 277 (10), 253 (20), 213 (100). Anal. calculated for C\(_{20}\)H\(_{23}\)C\(_{10}\): C, 72.3; H, 7.02; C1, 12.85.

11\(\beta\)-Methoxy-17\(\alpha\)-chloroethynylestradiol (2b). m.p. 255°C; HPLC, \(t\_R = 15\) min; \(^1\)H NMR (\(\delta\)) 0.82 (s, 3H, 18-CH\(_3\)), 3.05 (s, 3H, 11\(\beta\)-OCH\(_3\)), 3.97 (m, 1H, 11\(\beta\)-H), 6.32 (d, J = 2.5 Hz, 4-CH), 6.42 (dd, J = 2.5 and 8 Hz, 2-CH), 6.77 (d, J = 8 Hz, 1H, 1-CH), 8.19 (brs, 1H, -OH). MS, \(m/z\) (relative intensity) 362 (M\(^+\), 17), 360 (M\(^+\), 48), 327 (14), 293 (22), 285 (30), 283 (44), 275 (20), 211 (100). Anal. calculated for C\(_{21}\)H\(_{25}\)C\(_{12}\): C, 69.89; H, 6.98; C1, 9.82.

11\(\beta\)-Ethoxy-17\(\alpha\)-chloroethynylestradiol (2c). m.p. 225-255°C; HPLC, \(t\_R = 20\) min; \(^1\)H NMR (\(\delta\)) 0.89 (t, J=7Hz, 3H, 118-CH\(_2\)CH\(_3\)), 0.90 (s, 3H, 18-CH\(_3\)), 3.46 (q, J = 2 and 7Hz, 2H, 11\(\beta\)-OCH\(_2\)CH\(_3\)), 4.12 (m, 1H, 11\(\beta\)-H), 6.39 (d, J = 2.5 Hz, 1H, 4-CH), 6.47 (dd, J = 2.5 and 8 Hz, 1H, 1-CH), 6.81 (d, J = 8 Hz, 1H, 1-CH). MS, \(m/z\) (relative intensity) 376 (M\(^+\), 16), 374 (M\(^+\), 40), 313 (11), 292 (11), 285 (26), 283 (45), 211 (73), 169 (34), 156 (54), 145 (100). Anal. calculated for C\(_{22}\)H\(_{27}\)C\(_{12}\): C, 70.47; H, 7.27; Cl, 9.46. Found: C, 70.55; H, 6.9; Cl, 12.33.

7\(\alpha\)-Methyl-17\(\alpha\)-chloroethynylestradiol (2d). m.p. 125–129°C; HPLC, \(t\_R = 19\) min; \(^1\)H NMR (\(\delta\)) 0.85 (d, J = 6 Hz, 7\(\alpha\)-CH\(_3\)), 0.87 (s, 3H, 18-CH\(_3\)), 6.43 (dd, 1H, J = 2.5 and 8 Hz, 2-CH), 6.55 (d, J = 2.5 Hz, 1H, 4-CH), 7.16 (d, 1H, J = 8 Hz, 1-CH). MS, \(m/z\) (relative intensity) 346 (M\(^+\), 29), 344 (M\(^+\), 96), 302 (15), 266 (30), 253 (12), 227 (100). Anal. calculated for C\(_{21}\)H\(_{23}\)C\(_{12}\): C, 73.14; H, 7.31; Cl, 10.28. Found: C, 73.49; H, 7.06; Cl, 10.08.

Synthesis of (17\(\alpha\),20\(\beta\)-chloro)21-chloro-21O-ri-n-butylstannyl)vinylestradiol (3a–d)

A solution of 2a–d (30 mg) and n-tributylstannyl hydride (80 \(\mu\)l) in CHCl\(_3\) (5 ml) at 0°C under nitrogen atmosphere was treated with palladium acetate (10–15 mg). After stirring for 30–60 min at room temperature, the solvent was removed under reduced pressure. The residue
was directly applied to a very small column of silica gel (10 g) and the products were eluted with 3–5% ethyl acetate in hexane. The eluting solvent was removed under reduced pressure and the residue was purified on a semipreparative reverse phase HPLC column using a 95–100% gradient of methanol in water over a 20 min period.

(17α,20Z-Chloro)21-chloro(tri-n-butylstannyl)vinylestradiol (3a). HPLC, tR = 14 min. MS, m/z (relative intensity) 565 (M⁺-C₄H₅, 11), 563 (M⁺-C₄H₉, 1), 529 (M⁺-C₄H₅Cl, 1), 296 (17), 291 (5), 277 (5), 269 (43), 213 (100).

(17α,20Z-Chloro)21-chloro-21(tri-n-butylstannyl)-7β-methoxyvinylestradiol (3d). HPLC, tR = 20 min. MS, m/z (relative intensity) 601 (M⁺-Cl, 4), 600 (M⁺-Cl, 5), 599 (M⁺-Cl, 5), 581 (M⁺-C₄H₅, 61), 579 (M⁺-C₄H₉, 100), 577 (77), 543 (31).

Conditions for the preparation of (17α,20Z-chloro)21-chloro-21-iodovinylestradiol derivatives (4a-d) from their corresponding tin intermediates (3a-d)

To a mixture of 3a–d (20–25 mg) in chloroform (5 ml) was gradually added at room temperature a 0.1 M solution of iodine in chloroform until the color of iodine persisted. This was followed sequentially by the addition of 0.2 ml of 1 M KF in methanol and 0.2 ml of 5% aqueous sodium bisulfite. The mixture was then extracted with chloroform (2 × 15 ml), dried over Na₂SO₄ (anh), filtered and evaporated to dryness. The residue were purified on a C-18 reverse phase semipreparative HPLC column in methanol-water (75:25, v/v).

(17α,20Z-Chloro)21-chloro-21-iodovinylestradiol (4a). m.p. 101–105°C; HPLC, tR = 30 min; 1H NMR (δ) 0.80 (s, 3H, 18-CH₃), 6.48 (d, J = 2.5 Hz, 4-CH), 6.54 (dd, J = 2.5 and 8 Hz, 2-CH), 6.64 (s, 1H, CH=CICl), 6.99 (d, J = 1H, CH=CICl). MS m/z (relative intensity) 460 (M⁺, 2), 458 (M⁺, 5), 313 (3), 277 (2), 243 (2), 239 (27), 226 (14), 213 (100). Anal. calculated for C₁₂H₁₄Cl₂I: C, 42.16; H, 3.23; CI, 54.61. Found: C, 42.37; H, 3.27; CI, 54.48.

(17α,20Z-Chloro)21-chloro-21-iodo-7β-methoxyvinylestradiol (4b). m.p. 101–105°C; HPLC, tR = 30 min; 1H NMR (δ) 0.80 (s, 3H, 18-CH₃), 6.48 (d, J = 2.5 Hz, 4-CH), 6.54 (dd, J = 2.5 and 8 Hz, 2-CH), 6.64 (s, 1H, CH=CICl), 6.99 (d, J = 1H, CH=CICl). MS m/z (relative intensity) 460 (M⁺, 2), 458 (M⁺, 5), 313 (3), 277 (2), 243 (2), 239 (27), 226 (14), 213 (100). Anal. calculated for C₁₂H₁₄Cl₂I: C, 42.16; H, 3.23; CI, 54.61. Found: C, 42.37; H, 3.27; CI, 54.48.

(17α,20Z-Chloro)21-chloro-21-iodo-7β-methoxyvinylestradiol (4c). m.p. 101–105°C; HPLC, tR = 30 min; 1H NMR (δ) 0.80 (s, 3H, 18-CH₃), 6.48 (d, J = 2.5 Hz, 4-CH), 6.54 (dd, J = 2.5 and 8 Hz, 2-CH), 6.64 (s, 1H, CH=CICl), 6.99 (d, J = 1H, CH=CICl). MS m/z (relative intensity) 460 (M⁺, 2), 458 (M⁺, 5), 313 (3), 277 (2), 243 (2), 239 (27), 226 (14), 213 (100). Anal. calculated for C₁₂H₁₄Cl₂I: C, 42.16; H, 3.23; CI, 54.61. Found: C, 42.37; H, 3.27; CI, 54.48.

Conditions for the preparation of (17α,20Z-chloro)21-chloro-21-iodovinylestradiol derivatives (4a-d) from their corresponding tin intermediates (3a-d)

To a mixture of 3a–d (20–25 mg) in chloroform (5 ml) was gradually added at room temperature a 0.1 M solution of iodine in chloroform until the color of iodine persisted. This was followed sequentially by the addition of 0.2 ml of 1 M KF in methanol and 0.2 ml of 5% aqueous sodium bisulfite. The mixture was then extracted with chloroform (2 × 15 ml), dried over Na₂SO₄ (anh), filtered and evaporated to dryness. The residue were purified on a C-18 reverse phase semipreparative HPLC column in methanol-water (75:25, v/v).

(17α,20Z-Chloro)21-chloro-21-iodo-7β-methoxyvinylestradiol (4d). m.p. 101–105°C; HPLC, tR = 30 min; 1H NMR (δ) 0.80 (s, 3H, 18-CH₃), 6.48 (d, J = 2.5 Hz, 4-CH), 6.54 (dd, J = 2.5 and 8 Hz, 2-CH), 6.64 (s, 1H, CH=CICl), 6.99 (d, J = 1H, CH=CICl). MS m/z (relative intensity) 460 (M⁺, 2), 458 (M⁺, 5), 313 (3), 277 (2), 243 (2), 239 (27), 226 (14), 213 (100). Anal. calculated for C₁₂H₁₄Cl₂I: C, 42.16; H, 3.23; CI, 54.61. Found: C, 42.37; H, 3.27; CI, 54.48.

To a mixture of 3a or 3b (100 μg), and 50 μl of a 5% (w/v) solution of NaOAc in glacial AcOH was added [¹²⁵I]NaI (500 μCi), followed by 50 μl of an oxidant solution consisting of a 2:1 mixture (v/v) of H₂O₂ (30%) and AcOH. After stirring at room temperature for 10 min the reaction was terminated by the addition of 25 μl of an aqueous 5% NaHSO₃ solution (w/v). The mixture was extracted with CH₂Cl₂ and dried under a stream of nitrogen. The residue (450 μCi, 90%) was dissolved in MeOH and purified on an analytical C-18 reverse phase HPLC column operated at a flow rate of 1 ml/min. Elution with MeOH–H₂O (70:30, v/v) gave [¹²⁵I]4a (350 μCi, 70%, tR = 27 min), and MeOH–H₂O (75:25, v/v) gave [¹²⁵I]4b (350 μCi, 70%, tR = 11 min). The retention time of free iodine was 4 min.
Synthesis of (17α,20E)21-chloroestradiol (6α)

To a solution of LiAlH₄ (1 ml, 1 M, 1 mmol) at −15°C in THF was added 17α-chloroethynylestradiol (2α, 33 mg, 1 mmol) under nitrogen while maintaining this temp. After the mixture was stirred for an additional 15 min, it was brought to 0°C and stirred for 30 min. Dry methanol (2 ml) was added to the reaction mixture while maintaining the temp below 10°C. The mixture was brought to room temp and then slowly poured into 10% HCl (50 ml). The compound was extracted with ethyl acetate, and worked up in the usual manner. The compound was purified on a reverse phase HPLC column in methanol-water (75 : 25, v/v) t_R = 22 min, m.p. 103–104°C (lit.[20], 100°C decomp.).

ER Binding Assay

Affinity of the estradiol derivatives for ER was determined by a competitive binding assay [32] and is expressed as the relative binding affinity (RBA). The RBA is defined as 100 times the ratio between competitor and unlabeled estradiol concentrations required for 50% competition for specific [³H]estradiol binding. Murine uterine cytoplasmic extracts were incubated at 0–4°C for 18 h with 20 nM of [³H]estradiol in the absence and presence of competitive steroids ranging from 2 nM to 20 μM. The bound steroid was separated from

<table>
<thead>
<tr>
<th>Substituents</th>
<th>R = H</th>
<th>R = Cl</th>
<th>R = H</th>
<th>R = Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>X = Y = H</td>
<td>100</td>
<td>53.4 (5.6)</td>
<td>39.7 (0.7)</td>
<td>49.4 (2.3)</td>
</tr>
<tr>
<td>X = H, Y = CH₃</td>
<td>73.1 (12.2)</td>
<td>37.6 (2.0)</td>
<td>39.3 (1.0)</td>
<td>36.8 (2.0)</td>
</tr>
<tr>
<td>X = OCH₃, Y = H</td>
<td>15.3 (0.5)</td>
<td>29.4 (4.8)</td>
<td>30.7 (3.3)</td>
<td>35.0 (1.0)</td>
</tr>
<tr>
<td>X = O₂C₂H₅, Y = H</td>
<td>21.0 (1.9)</td>
<td>33.1 (4.4)</td>
<td>17.1 (1.0)</td>
<td>31.7 (2.0)</td>
</tr>
</tbody>
</table>

*The RBA is defined as 100 times the ratio between competitor and unlabeled estradiol concentration required for 50% competition to specific [³H]estradiol binding. Competitive binding between 20 nM [³H]estradiol and 2 nM to 20 μM unlabeled ligand was plotted, and the concentration required for 50% competition was used to calculate the RBA values (mean of three experiments and standard deviation).
The gem-21-chloro-21-iodovinylestradiol derivatives

Table 2. Tissue distribution of (17a,20Z-chloro)21-chloro[125I]-iodovinylestradiol 4a, and its 11β-methoxy analog 4b, in immature female rats

<table>
<thead>
<tr>
<th>Tissue</th>
<th>1 h</th>
<th>1 h (+ E2)</th>
<th>2 h</th>
<th>5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcerus</td>
<td>8.20 (0.08)</td>
<td>2.61 (0.26)</td>
<td>8.38 (0.61)</td>
<td>4.27 (0.25)</td>
</tr>
<tr>
<td>Blood</td>
<td>2.46 (0.09)</td>
<td>2.28 (0.24)</td>
<td>2.19 (0.25)</td>
<td>1.33 (0.06)</td>
</tr>
<tr>
<td>Plasma</td>
<td>2.92 (0.07)</td>
<td>2.68 (0.23)</td>
<td>2.44 (0.30)</td>
<td>1.46 (0.09)</td>
</tr>
<tr>
<td>Liver</td>
<td>6.50 (0.44)</td>
<td>5.90 (0.37)</td>
<td>4.87 (0.37)</td>
<td>3.30 (0.20)</td>
</tr>
<tr>
<td>Lungs</td>
<td>2.69 (0.11)</td>
<td>2.81 (0.06)</td>
<td>1.99 (0.19)</td>
<td>1.21 (0.05)</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.16 (0.16)</td>
<td>1.74 (0.05)</td>
<td>1.73 (0.13)</td>
<td>1.37 (0.08)</td>
</tr>
<tr>
<td>Kidneys</td>
<td>2.56 (0.06)</td>
<td>2.25 (0.07)</td>
<td>2.01 (0.09)</td>
<td>1.24 (0.10)</td>
</tr>
<tr>
<td>Brain</td>
<td>1.19 (0.04)</td>
<td>1.34 (0.03)</td>
<td>0.67 (0.06)</td>
<td>0.28 (0.01)</td>
</tr>
<tr>
<td>Fat</td>
<td>8.52 (0.80)</td>
<td>6.28 (1.26)</td>
<td>8.61 (1.21)</td>
<td>5.35 (0.29)</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.51 (0.10)</td>
<td>1.72 (0.20)</td>
<td>1.67 (0.22)</td>
<td>0.85 (0.11)</td>
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<tr>
<td>Thyroid</td>
<td>285.05 (20.06)</td>
<td>203.61 (10.70)</td>
<td>616.53 (48.93)</td>
<td>1234.3 (36.17)</td>
</tr>
<tr>
<td>Ulcerus/blood</td>
<td>3.37 (0.22)</td>
<td>1.17 (0.35)</td>
<td>3.89 (0.43)</td>
<td>3.22 (0.24)</td>
</tr>
<tr>
<td>Ulcerus/nontarget</td>
<td>3.98 (0.41)</td>
<td>1.25 (0.22)</td>
<td>4.95 (0.34)</td>
<td>1.17 (0.21)</td>
</tr>
</tbody>
</table>

% ID/g (SE)°

In Vivo Studies

The animal experiments were conducted in accordance with the recommendations of the Canadian Council on Animal Care and of the in-house Ethics Committee for Animal Experiments. Immature female Fischer rats (21-24 days old, 38.4 ± 1.2 g) (Charles River) were injected with 200 μl of the [125I]-labeled steroid (3 μCi, 111 kBq) via the lateral tail vein. The animals were placed in retention cages and therefore not anaesthetized during the injection procedure. The radiopharmaceutical was dissolved in ethanol and diluted with sterile physiological saline (0.9% NaCl in H2O) containing 1% Tween-80, to give a final ethanol concentration of 9%. For the receptor saturation studies 60 μg of unlabeled estradiol was coinjected with the radiopharmaceutical. Animals were bled under deep ether anaesthesia by severing the axillary artery, followed by chest opening [33]. Blood was collected, tissues of interest were removed, washed with 0.154 M KCl, and blotted dry and samples were weighed. The radioactivity was counted in a model 1282 Compugamma gamma counter (LKB Wallac, Finland) and concentrations were expressed as percent of the injected dose per gram of tissue (% ID/g). Statistical variations are presented as the standard error [34].

RESULTS AND DISCUSSION

Chemistry

The ketones (1a–d) were converted to the 17α-chloroethynyl derivatives 2a–d by treatment with lithium chloroacetylide generated in situ with butyllithium and 1,2-cis-dichloroethylene (Scheme 1) [35]. For the introduction of the radioiodine onto the vinyl substituent of the 17α-iodovinylestradiol we used the rapid destannylation method, which gives radiopharmaceuticals in high yield and of high specific activity [36]. The 20E and 20Z stannyl intermediates can be obtained using tri-n-butyl tinhydride, with or without catalyst,
under different reaction conditions. The reaction of 17α-chloroethynyl estradiol with these conventional free radical initiators gave mainly the nonchlorinated ethynyl analog and only a small amount of the desired product. Recently, a number of other catalysts have been proposed to improve stereo-selectivity and yield of the vinylstannanes. Among them, palladium and molybdenum were found to catalyze successfully the hydrostannation of hetero disubstituted alkynes [37]. In particular, palladium chloride and palladium(II) acetate gave satisfactory yields of chloro-vinylstannanes intermediates for the preparation of the (17α,20Z-chloro)21-chloro-21-iodovinyl (4a–d) derivatives of estradiol. Treatment of the 17α-chloroethynyl estradiol with tri-n-butyl tin hydride in chloroform at low temperature gave the chlorinated tin product 3 (75–90%, based on the starting material) and the corresponding 17α-ethynyl estradiol analog (10–15%). Longer reaction times gave dechlorinated products. Increasing the concentration of the tin reagent resulted in a decrease of the yield of chlorinated product. The highest yields were obtained when the reaction was monitored by TLC and stopped at about 75% conversion of the starting material. Separation of the 17α-(tri-n-butylstannyl)chlorovinylestradiols (3a–d) from the reaction mixture was achieved by chromatographic methods. The tin intermediates gave a weak ion with the loss of Cl or C₂H₅ from the molecular ion with the characteristic pattern of tin cluster in the mass spectrum. Addition of a 0.1 M molar solution of iodine in chloroform to the tin intermediates resulted in an immediate destannylation to give the chloroiodovinylestradiol derivatives 4a–d with retention of configuration, in 60–80% yield. The chloroiodovinyl derivatives gave an appropriate molecular ion in the mass spectrum and their assigned stereochemistry was deduced from the ¹H NMR and transformation of the tin intermediate to the known chlorovinyl compound 5. The ¹H NMR of 4 showed a singlet centered between δ 6.47–6.74 which was assigned to the 20-H vinylic proton. The stereochemistry of the chloro/iode substituent at C-21 was established by protodestannylation of the tin intermediate. Thus, treatment of 3a with TFA gave 5a, which was assigned a trans configuration (Z) based on the ¹H NMR data. Since it is well established that the destannylation reaction is stereospecific, the tin substituent can be considered in the cis configuration (E) and consequently the iodine should also be in the E configuration. For comparison, we also prepared the (17α,20E)chlorovinyllic derivative 6 from 2. Lithium-(17α,20E-chloro)21-chloro-21-alkenylestradiol alanate intermediate was prepared in a highly stereo- and regioselective trans addition of LiAlH₄ in THF to the triple bond of the readily available 21-chloro-alkyne [38]. These halovinylalanates, upon methanolysis, provide in excellent yields the corresponding (17α,20E)chloro derivative 6.

![Fig. 1. Uterus uptake in % ID/g of [²²]H4a,b, (17,20E)IVE₂ and 11β-OMe-(17,20E)IVE₂ in immature female Fischer rats. Values for the latter mono-halogenated steroid were taken from the literature [26, 28]. The error bar represents the standard error.](image-url)
The gem-21-chloro-21-iodovinylestradiol derivatives at the molecular level were evaluated via semi-empirical computer modelling of the structure. The minimized energies were calculated and corresponded to 33.66 kcal/mol for the 21-chloro derivative (4a), 31.708 kcal/mol for the (17a,20E)IVE₂ and 35.72 kcal/mol for the (17a,20Z)IVE₂ isomer. These values reveal a good stability for the gem-dihalo derivative. The van der Waals' space filling model shows little effect of the Cl atom on the overall configuration of the molecule. However, the high electron density over the C-17 OH due to the added C-21 Cl atom, strongly increases the dipole moment of the gem-dihalo molecule (1.527 vs 0.518).

**Biological properties**

**Binding affinities for the murine uterine ER.** The binding to the intracellular receptor constitutes the final process in the localization of the steroid hormone radioligand. Effectiveness as an imaging agent is related to the affinity of the radioligand for the receptor, the specific activity of the radioligand, retention by the receptor as well as interactions with transport proteins and metabolic enzymes.

The affinity of the compounds for ER were measured in murine uterine cytosol preparations using a competitive binding assay with [³H]estradiol. RBA represent the ratio between ligand and unlabeled estradiol concentrations required for 50% displacement of [³H]estradiol.
from the receptor (Table 1). In both the 17α-ethynyl- and (17α,20E)IVE₂ series, the highest RBA values were observed with the nonsubstituted analogs. Addition of the 21-chloro onto the ethynylestradiol resulted in a marked decrease in the RBA (100 vs 53.4). A similar decrease in RBA was observed for the analogous 7α-methyl derivatives (73.1 vs 37.6). However, in the case of the 11β-methoxy/ethoxy ethynylestradiols, addition of the 21-chloro resulted in an increase in binding affinity (Table 1). Surprisingly, addition of the 21-chloro onto the (17α,20E)IVE₂ increased binding affinity for the ER which may be due to a change in lipophilicity and/or the increase in dipole moment. The latter can be expected to facilitate hydrogen bonding between the 17β-OH of the ligand and the ER. Addition of the 21-chloro onto the 7α-Me or 11β-OMe derivatives of (17α,20E)IVE₂ had little effect on the RBA. A particular strong synergistic effect, resulting in increased RBA values, was observed.

![Graphs showing blood clearance and thyroid uptake](image-url)

Fig. 3. Blood clearance in % ID/g of [³²P]4α,b, (17α,20E)IVE₂ and 11β-OMe-(17α,20E)IVE₂ in immature female Fischer rats. Values for the latter mono-halogenated steroid were taken from the literature [26, 28]. The error bar represents the standard error.

Fig. 4. Thyroid uptake in % ID/g of [³²P]4α,b, (17α,20E)IVE₂ and 11β-OMe-(17α,20E)IVE₂ in immature female Fischer rats. Values for the latter mono-halogenated steroid were taken from the literature [26, 28]. The error bar represents the standard error.
upon addition of the 21-Cl to the 11β-OEt (17α,20E)IVE₂ (Table 1).

**Biodistribution in immature female Fischer rats.** The biodistribution of the 131I-labeled 4a and its 11β-methoxy derivatives 4b was studied in immature Fischer female rats (Table 2). The animals were sacrificed at 1, 2 and 5 h post-injection, and their tissue radioactivity is expressed in terms of percent injected dose per gram of tissue (% ID/g). Uterus uptake and uterus to blood/nontarget ratios are also presented in Table 2. Both 21-chloro derivatives show substantial accumulation of 131I in the thyroid. Among the other organs, the uterus showed the highest radioactivity uptake for both compounds, followed by fat and the liver.

We have previously shown that among the analogs lacking the 21-Cl, the 11β-methoxy derivative with the (17α,20E)iodovinyl configuration showed the highest uterus uptake and uterus to blood/nontarget ratios [17]. Therefore, comparison of the retention data of the 21-chloro analogs 4a and 4b with the (17α,20E)IVE₂ and its 11β-methoxy analog were made. The following uterus uptake pattern can be observed (Fig. 1): (i) Addition of a 21-Cl to the (17α,20E)IVE₂ results in augmented uterus uptake which correlates with the 25% increase in ER affinity (Table 1); (ii) addition of a 21-Cl to the 11β-OMe-(17α,20E)IVE₂ has little effect on the uterus uptake levels, but provides for longer retention; and (iii) as expected, the 11β-methoxy derivative 4b showed higher uterus uptake as compared to the analog lacking the 11β-substituent 4a, reflecting low affinity for nonspecific protein binding sites. A strong drop in uterus uptake (70–80%) in the presence of unlabeled E₂, indicates that the augmented uterus uptake for both 4a and 4b involves an ER-mediated process.

Uterus to blood/nontarget ratios (Fig. 2) are similar for the (17α,20E)IVE₂ and its 21-Cl analog 4a whereas in the case of the 11β-OMe-(17α,20E)IVE₂, the 21-Cl analog 4b shows substantial lower uptake ratios. These observations can be explained from the blood clearance (Fig. 3) and thyroid uptake pattern (Fig. 4). For both 21-Cl derivatives 4a and 4b a much slower blood clearance is observed as compared to the analogs lacking the 21-Cl (Fig. 3). Furthermore, both 21-Cl derivatives 4a and 4b reveal substantially higher radiiodine accumulation by the thyroid as compared to the analogs lacking the 21-Cl (Fig. 4). This indicates release of radiiodine from the gem-dihalogen analogs 4a,b reflecting the increased steric interference of the two bulky halogens upon substitution of the 21-H for a 21-Cl. The higher blood radioactivity levels observed with the 21-Cl derivatives may also reflect formation of free radiiodine.

In summary, the addition of a 21-Cl to (17α,20E)IVE₂ derivatives increases the dipole moment of the molecule, coinciding with augmented binding affinity for the ER receptor as well as ER-mediated uterus uptake. These data reveal a high tolerance of the ER binding site for further substitution about the C-21 position of the vinylestradiol ligands. However, the steric and electronic interference between the gem-dihalogen at the 21-position increases the instability of the radiolabel resulting in high background radioactivity and low uterus to blood/nontarget ratios.

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