Synthesis and Receptor Binding Affinity of 7α- and 17α-Substituted 2- and 4-Chloroestradiol Derivatives

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Methods for the selective syntheses of 2- and 4-chloroestradiol derivatives substituted at the 17α- and 7α-positions are described. The relative binding affinities for estrogen receptors of these estrogens are also reported, and potential structure–activity relationships are put forth.

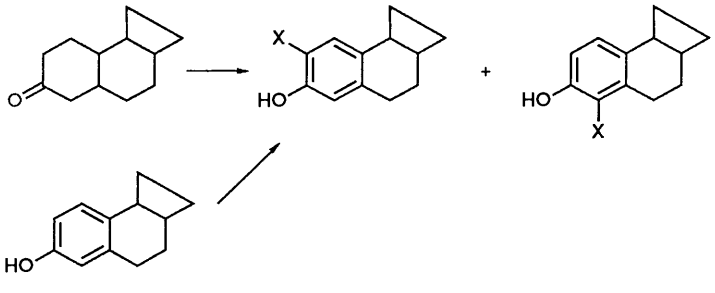
A number of radiohalogenated estrogens[1] have been prepared over the past years as potential estrogen-receptor based diagnostic probes to be used in conjunction with the therapy of hormone dependent tumors. Halogenated estradiol derivatives of high stability are required for this purpose. The synthesis of different analogues and the possibility of using radioactive isotopes, permits the evaluation of possible structure–activity relationships, both under *in vitro* and *in vivo* conditions. Substitution at the 2- and 4-positions of the aromatic A-ring of estrogens is known to yield products with enhanced *in vivo* stability due to their decreased rate of metabolism.[2] Several new methods to introduce various groups at the 2- and 4-positions have recently been reported. In general, these methods involve two main synthetic routes (Scheme 1). The first route uses 19-norsteroids with a suitable located double bond, followed by selective introduction of the functional group at position C-2 or C-4 and subsequent aromatization of the A-ring by established methods.[3] The other possible route is based on classical electrophilic substitution. However, the latter procedure usually yields a mixture of the two possible *ortho*-isomers.[4] Some reports for the selective monofunctionalization of estrone or estradiol and their derivatives at position C-2 have appeared,[5] whereas halogenation at position C-4 has received little attention.[6]

The development of positron emission tomography (PET) has resulted in an increased demand for radiopharmaceuticals labelled with positron-emitting radionuclides, such as ^{18}F, ^{11}C and ^{75}Br in order to study metabolic activities and receptor distribution pattern. Introduction of fluorine at selected positions of steroid molecules often leads to increased receptor binding affinities, suppression of undesired metabolic reactions and reduced non-specific binding to serum proteins.[2] A number of reagents have been developed for the preparation of organofluoro compounds, and their adaption for the introduction of ^{18}F, *via* substitution of Cl, has been reported.[7] Displacement of chloro- for fluoro-substituents generally improves receptor binding properties of steroid hormones and accordingly binding properties of selected chloroestrogens can serve as an indirect screening for potentially strong binding fluoro analogues.

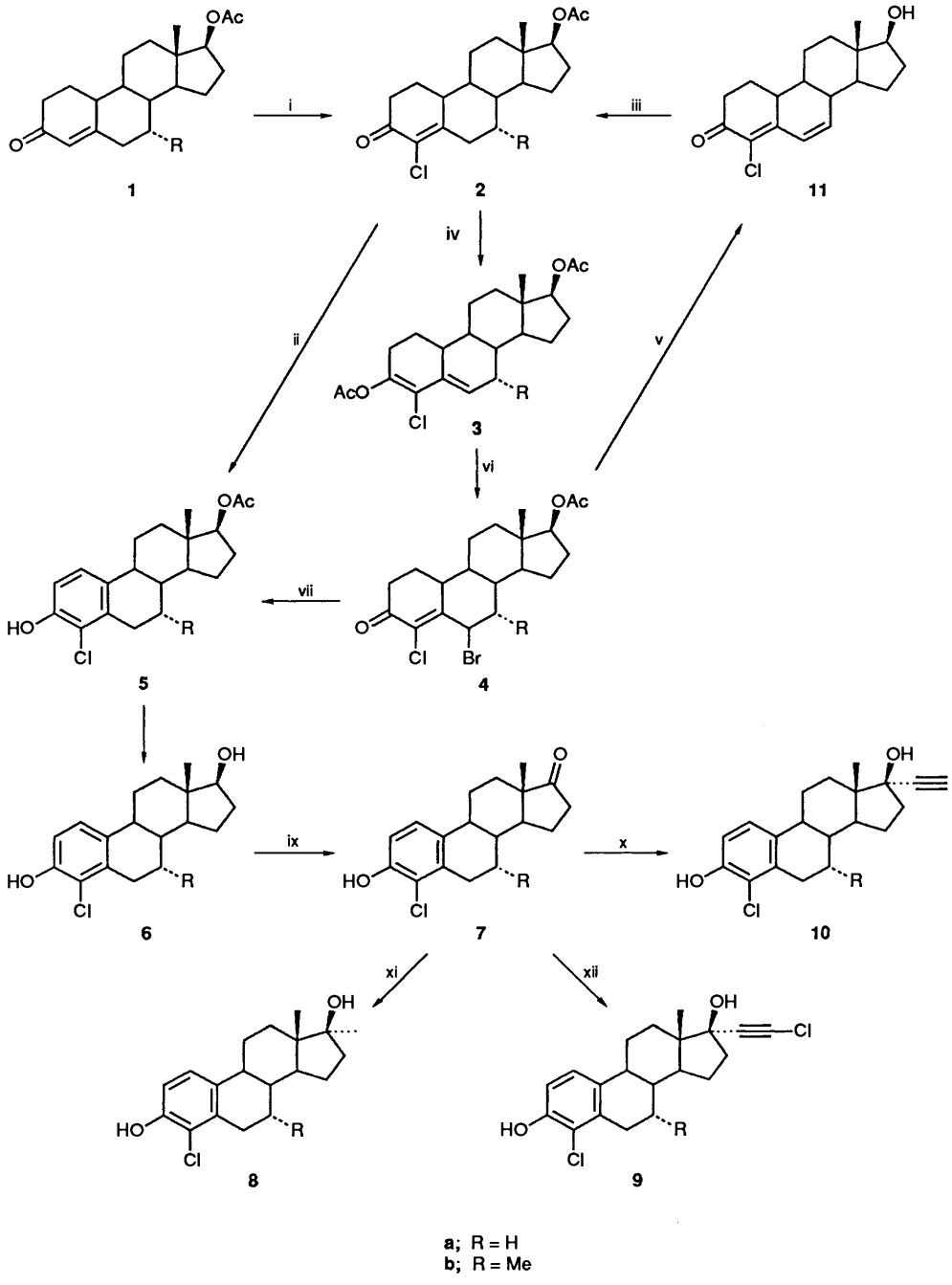
Previously reported methods for the synthesis of chloro derivatives of estradiol, using chlorine,[8] sulphuryl chloride,[8] *N*-chlorosuccinimide,[8–10] trichloroisocyanuric acid,[10] *tert*-butyl hypochlorite[11] or isocyanuric acid chloride[12] as the chlorinating agent, gave complex reaction mixtures. In general, these methods afford the 10β-chloroestra-1,4-diene-3-one as a major product together with the 2,10- or 4,10-dichloro analogues as minor products. The 10β-chloro group of the dichloro products can be removed selectively *via* $NaBH_4$ reduction, to yield the 2- and 4-chloro analogues.[10] In this paper we describe methods for the selective synthesis of 2- and 4-chloroestrogens substituted at the 7α- and/or 17α-positions. Their binding affinity for estrogen receptors and possible structure–activity relationships are also reported.

Results and Discussion

Synthesis of 4-Chloroestradiol Derivatives.—The starting material for the synthesis of the 4-chloroestradiol derivatives, the 17-acetoxy-4-chloroestr-4-en-3-one **2a**, was prepared from 17-acetoxyestr-4-en-3-one **1a**. Treatment of **1a** with 2 mol. equiv. of SO_2Cl_2 in pyridine gave the corresponding 4-chloro compound **2a** exclusively with no trace of the 2-chloro derivative.[6a,13] The presence of the chlorine atom at C-4 in **2a** was confirmed by a characteristic shift of the UV absorption band from λ 237 to 255 nm and the absence of the C-4 vinylic proton signals in the ^1H NMR spectrum. The 4-chloroestradiol **6a** was obtained by two different methods. In the first approach, compound **2a** was converted into the enol acetate **3a** in Ac_2O–Py–AcOCl and subsequently to the 6-bromo compound **4a** following treatment of **3a** with NBS (*N*-bromosuccinimide) in DMF (dimethylformamide). Compound **4a**, upon treatment with HCl gave 17β-acetoxy-4-chloroestradiol **5a** which upon hydrolysis under basic conditions gave **6a** with an overall yield of 50%. A higher yield of up to 80% was obtained by the second approach, which involves treatment of **2a** with SeO_2 in



Scheme 1



Scheme 2 *Reagents and conditions:* i, SO_2Cl_2; ii, SeO_2; iii, MeMgBr; iv, Py–CH_3COCl; v, $LiCO_3$–LiBr; vi, NBS–DMF; vii, Acetone–HCl; viii, MeOH–K_2CO_3; ix, Jones' reagent; x, LiC≡CEDA; xi, MeLi or MeMgBr; xii, LiC≡CCl

Bu^{t}OH. The assigned structure of **6a** was supported by the UV absorption maximum at 282 nm, which is characteristic for aromatic compounds, and the two doublets of the C-1 and C-2 protons at δ 6.8–7.0 in the ^{1}H NMR spectrum, indicative of chloro-substitution at C-4. Jones' oxidation of the alcohol **6a** gave the ketone **7a**. Reaction of **7a** with methyllithium gave the 17α-methyl analogue **8a**. Lithium chloroacetylide, generated *in situ* with butyllithium and 1,2-*cis*-dichloroethylene,[14] was treated with **7a** resulting in the formation of the 17α-chloroethynyl derivative **9a**, whereas treatment of **7a** with lithium acetylide ethylenediamine[15] gave the 17α-ethynyl derivative **10a**. All three 17α-substituted estradiol derivatives, *i.e.* **8a**, **9a** and **10a**, were obtained as single isomeric products, with the 17β-OH configuration assignment based on steric considerations.

The 4-chloro-7α-methylestradiol **6b** was prepared from **2b** in a similar manner to that described for **6a**, using **1b** or **11** as a starting material. Treatment of 17β-acetoxy-7α-methylestr-4-en-3-one **1b** with SO_2Cl_2 gave the 4-chloro derivative **2b**. Alternatively, the 4-chloro compound **2b** was prepared from the 6-bromo compound **4a**. Treatment of **4a** with lithium carbonate and lithium bromide gave the dienone **11** which upon treatment with Grignard reagent (MeMgBr) in the presence of cuprous chloride gave a mixture of isomeric products from which **2b** was isolated. The configuration of the 7α-methyl group was assigned from the *J* value in the ^{1}H NMR spectrum (doublet at δ 0.82, *J* 7 Hz). The aromatization of **2b** into **6b** can be achieved by two different procedures similar to those used for the preparation of **6a**. Compound **2b**, upon enolization and bromination gave **3b** and **4b**, respectively. Compound **4b** under acidic condition furnished the aromatized product **5b**. The latter was also obtained directly by treatment of 17β-acetoxy-4-chloro-7α-methylestr-4-en-3-one **2b** with SeO_2 in Bu^{t}OH.

Table 1 Analytical data for chlorinated estrogens

Compound	Formula	Yield (%)	M.p. (°C) (decomp.)	Found (%) (Required) C	H
6a	$C_{18}H_{23}ClO_2$	80	202	70.37 (70.47)	7.62 (7.50)
7a	$C_{18}H_{21}ClO_2$	75	235	70.89 (70.9)	6.5 (6.89)
8a	$C_{19}H_{25}ClO_2$	70	214	70.94 (71.13)	8.19 (7.80)
9a	$C_{20}H_{22}ClO_2$	50	94	65.26 (65.75)	5.98 (6.02)
10a	$C_{20}H_{23}ClO_2$	80	190	72.61 (72.6)	6.99 (6.95)
6b	$C_{19}H_{25}ClO_2$	85	165	71.20 (71.13)	7.79 (7.80)
7b	$C_{19}H_{23}ClO_2$	80	192	71.30 (71.58)	7.39 (7.22)
10b	$C_{21}H_{25}ClO_2$	75	92–94	72.99 (73.14)	7.76 (7.25)
16a	$C_{20}H_{23}ClO_2$	78	205–225	72.58 (72.6)	6.93 (6.95)
16b	$C_{21}H_{25}ClO_2$	82	93–95	73.12 (72.95)	7.73 (7.27)

Table 2 Receptor binding affinity (RBA) of chloroestradiol derivatives for estrogen receptors[a]

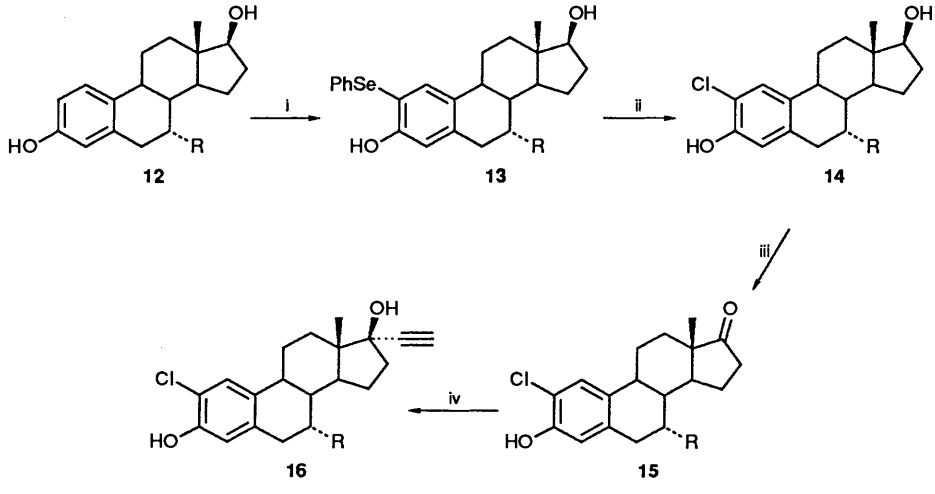
Compound		RBA
6a	4-Chloroestradiol	40
14a	2-Chloroestradiol	11
7a	4-Chloroestrone	1.0
8	4-Chloro-17α-methylestradiol	7.0
9	4-Chloro-17α-chloroethynylestradiol	8.0
10a	4-Chloro-17α-ethynylestradiol	48.7
16a	2-Chloro-17α-ethynylestradiol	17.3
6b	4-Chloro-7α-methylestradiol	56
7b	4-Chloro-7α-methylestrone	7
14b	2-Chloro-7α-methylestradiol	32
10b	4-Chloro-7α-methyl-17α-ethynylestradiol	22
16b	2-Chloro-7α-methyl-17α-ethynylestradiol	17.5
	Estrone	7
	17α-Ethynylestradiol	100
	7α-Methyl-17α-ethynylestradiol	73

[a] The binding affinity was determined relative to that of [^{3}H]estradiol by a competition binding assay. The relative binding affinity (RBA) is 100 times the ratio between the competitor and unlabelled estradiol concentration required for 50% competition. Accordingly, by definition, the RBA of estradiol equals 100.

Finally, oxidation of the alcohol **6b** with Jones' reagent gave the ketone **7b** which upon treatment with lithium acetylide–ethylenediamine gave the 17α-ethynyl derivative **10b**.

Synthesis of 2-Chloroestradiol Derivatives.—Recently we reported that A-ring chlorination of estradiol or estrone with benzeneselenenyl chloride (PhSeCl) exclusively yields the 2-chloro derivatives.[16] We have extended this method to the synthesis of the 2-chloro analogues of estradiol **12a** and 7α-methylestradiol **12b**. Using a 1:1.2 molar ratio of steroid/PhSeCl, the 2-phenylselenenyl estradiol derivatives **13a,b** were obtained as the major product. When the percentage of PhSeCl was increased, new products were obtained which were characterized as the 2-chloro derivatives **14a,b** by mass spectrometry and ^{1}H NMR spectroscopy. The yield of **14a,b** depends on the amount of PhSeCl in the reaction mixture. The pure 2-chloro derivatives were also obtained *via* the reaction of **13a,b** with ICI or PhSeCl. The alcohols **14a,b** were oxidized with Jones' reagents to the ketones **15a,b** which, by treatment with a lithium acetylide–ethylenediamine complex, were subsequently converted into the 17α-ethynyl derivatives **16a, b**.

Binding Affinity for the Calf Uterine Estrogen Receptor.—The affinity of the 2- and 4-chloroestradiol derivatives for the estrogen receptor was determined by competition studies with [^{3}H]estradiol using Sephadex LH-20 chromatography to separate bound from free steroid (Table 2).[17] Interaction with the receptor is expressed as the relative binding affinity (RBA), *e.g.* the ratio between unlabelled extradiol and competitor concentrations required for 50% displacement of [^{3}H]estradiol from the receptor. RBA values of reference estrogens lacking the 4-chloro substituents, *e.g.* estrone, 17α-ethynylestradiol and 17α-ethynyl-7α-methylestradiol, measured under the same experimental conditions, are also included in Table 2. Addition of the 4-chloro group into estradiol has been reported by others as having no effect on the binding affinity,[18] under our experimental conditions we observed a decrease in affinity for the estrogen receptor (RBA of **6a** = 40). A proportional decrease in receptor binding affinity is observed for the



Scheme 3 *Reagents:* i, PhSeCl; ii, PhSeCl or ICl; iii, Jones' reagent; iv, LiC≡CEDA

same substitution on estrone. Addition of substituents on the 17α-position of the 4-chloroestradiol **6a** further lowers affinity for the estrogen receptor, with the exception for the 17α-ethynyl group which appears to have little effect on the interaction of **6a** with the estrogen receptor (RBA of **10a** = 48). The lack of an effect of the 17α-ethynyl group on the binding affinities is also observed with the corresponding non-chlorinated derivatives since we find identical binding properties for estradiol and the 17α-ethynylestradiol under our experimental conditions (Table 2). In contrast, addition of a 7α-methyl group onto the 4-chloroestradiol **6a** augments interaction with the estrogen receptor (RBA of **6b** = 56). In the presence of 7% DMF, which suppresses non-specific binding, this value almost triples. Further substitution of **6a** at both the 7α- and 17α-positions, as in the 17α-ethynyl-4-chloro-7α-methylestradiol **10b**, results in diminished affinity for the estrogen receptor (RBA of **10b** = 22). Loss of estrogenic potency due to such simultaneous 7α,17α-substitutions is in agreement with earlier observations on related estrogens.[19]

Addition of a 2-chloro group onto estradiol, to yield the 2-chloroestradiol **14a**, results in a substantial loss (90%) of binding affinity for the estrogen receptor (Table 2). Further substitution of **14a** with either a 7α-methyl group, to yield **14b**, or a 17α-ethynyl group, to yield **16a**, increases the RBA substantially, particularly in the case of the 7α-methyl (RBA of **14b** = 32). Further substitution of **14b** with an additional 17α-ethynyl group to yield the disubstituted 2-chloroestradiol derivative **16b**, depresses the binding affinity to the level of the 17α-ethynyl derivative **16a** (RBA of **16b** = 17).

Overall the 4-chloro derivatives exhibit 1.5–4 times higher RBA values than the corresponding 2-chloroestradiol derivatives. This is in agreement with reported ratios of bonding data of other 4- *vs.* 2-substituted estradiols which range from 1.5 for the fluoro- to 8.5 for the bromo compounds.[20] Both the 7α-methyl- **6b** and 17α-ethynyl-4-chloroestradiols **10a** exhibited higher RBA values than the parent molecule, *i.e.* **6a**. Their binding affinities will likely further increase upon substitution of the chloro for a fluoro group. Accordingly, the fluoro analogues of **10a** and **6b** are good candidates for labelling with ^{18}F to yield potential imaging agents of estrogen receptor rich target tissues.

Experimental

General Procedures.—Reagents for the syntheses were obtained commercially and were of the highest chemical grade available. 19-Nortestosterone was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Thin-layer chromatography (TLC) was conducted on 0.25 mm thick Brinkmann silica gel plates coated with fluoroscent indicator (UV 254 nm) in 1:2 EtOAc–hexane. Chromatoplates were sequentially examined under 254 nm light and for colour response upon spraying with 50% H_2SO_4 in EtOH and heating at 120 °C. Crude reaction mixtures were purified by column chromatography on 60–200 mesh silica gel. High performance liquid chromatography (HPLC) was conducted on a 25 cm long × 0.94 cm i.d. column packed with C-18 ODS-2 on 5 μm Spherosorb (CSC, Montreal) operated at 2 cm^3 min^{-1} with mixtures of methanol in water. Steroids were detected by their absorption at λ 254 or 280 nm. ^1H NMR spectra were recorded on a Bruker WM 250 Spectrometer. Chemical shifts are reported in δ values from the internal standard Me_4Si deuteriated solvent(s). *J* Values are given in Hz. High- and low-resolution mass spectra (HRMS, MS) were determined with a V9 micromass model ZAB-1F apparatus at 70 eV ionization voltage. Combustion analyses were performed by Guelph Chemical Laboratories Ltd., Canada. M.p.s were determined on a Fisher-Johns apparatus and are not corrected.

Chlorination of **1a**.—17β-Acetoxyestr-4-en-3-one **1a** (1.2 g, 3.78 mmol) was dissolved in pyridine (5 cm^3) and SO_2Cl_2 (1.0 g, 7.56 mmol) was slowly added. The reaction mixture was stirred at room temp. for 1 h, poured into water (50 cm^3) and extracted with ethyl acetate (3 × 20 cm^3). Organic phases were combined, washed with water, 1 mol dm^{-3} HCl and water (20 cm^3 each) and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue (1.4 g) was purified. Chromatography on silica gel with ethyl acetate–hexane (1:9) as the eluent yielded 17β-acetoxy-4-cholestr-4-en-3-one **2a** (1.0 g, 75%), m.p. 164 °C; δ_H(250 MHz; $CDCl_3$) 0.83 (3 H, s, 18-Me), 2.02 (3 H, s, 17β-OCOMe) and 4.60 (1 H, m, 17α-H); *m/z* 352 (M^+, 11%), 350 (M^+, 33%), 308 (88, M − CH_2CO), 290 (68, M − CH_3CO_2H), 273 (28, M − CH_3CO_2 − Cl), 256 (28) and 144 (100).

Aromatization of **2a**.—*Method A.* A mixture of **2a** (300 mg, 0.85 mmol) and selenium dioxide (500 mg, 4.5 mmol) in Bu'OH (50 cm^3) was refluxed under N_2 for 24 h. The cooled mixture was filtered, the filtrate evaporated and the residue purified. Chromatography on silica gel with ethyl acetate–benzene (1:4) as the eluent yielded 17β-*acetoxy*-4-*chloroestradiol* **5a** (85%), m.p. 225 °C; δ_H(250 MHz; $CDCl_3$ + [$^2H_6$]-DMSO), 0.78 (3 H, s, 18-Me), 1.98 (3 H, s, 17β-OAc), 4.57 (1 H, m, 17α-H), 6.54 (1 H, d, *J* 8, 2-H) and 6.84 (1 H, d, *J* 8, 1-H); *m/z* 350 (M^+, 33%), 348 (M^+, 100), 259 (10), 247 (33), 253 (11), 193 (33) and 180 (22).

To a stirred solution of **5a** (350 mg, 1 mmol) in 90% MeOH at room temp. was added anhydrous Na_2CO_3 (0.47 g, 3.4 mmol) after which the mixture was stirred under N_2 for an additional 24 h. Methanol was removed under reduced pressure and the residue was taken up in ice-cooled water, acidified with 10% acetic acid and extracted with ethyl acetate. The material was worked up and the residue (300 mg) was purified. Chromatography on silica gel with ethyl acetate–hexane (2:3) yielded 4-chloroestradiol **6a** (90%), m.p. 202 °C (lit.,[10] 255–261 °C). An analytical sample was obtained by HPLC (MeOH–H_2O, 7:3) t_R = 38 min; δ_H(250 MHz; $CDCl_3$) 0.76 (3 H, s, 18-Me), 6.72 (1 H, d, *J* 8, 2-H) and 6.98 (1 H, d, *J* 8, 1-H); *m/z* 308 (M^+, 10%), 306 (M^+, 32), 272 (100, M − HCl), 249 (6), 247 (18), 213 (39), 194 (17), 172 (32), 158 (21) and 145 (39).

Method B. A solution of **2a** (1.2 g, 3.78 mmol) in Ac_2O (1.5 cm^3), pyridine (0.8 cm^3) and AcOCl (6 cm^3) was heated on a reflux under N_2 for 3 h and concentrated under reduced pressure and the residue was purified. Chromatography on neutral alumina (activity I) with hexane as en eluent yielded a white crystalline compound: 4-*chloroestra*-3,5(6)-*diene*-3,17β-*diyl diacetate* **3a**, m.p. 154 °C; δ_H(250 MHz; $CDCl_3$) 0.82 (3 H, s, 18-Me), 1.98 (3 H, s, 17β-OAc), 2.08 (3 H, s, 3-OAc) 4.50 (1 H, t, 17α-H) and 5.98 (1 H, d, 6-H); *m/z* 392 (M^+, 1.3%), 350 (100, M − CH_2CO), 314 (49, M − 2 × CH_3CO), 255 (11), 156 (28), 147 (32) and 94 (43).

To a cooled suspension of the enol acetate **3a** (1.0 g, 2.55 mmol) in DMF (15 cm^3) and water (0.5 cm^3) was added over a period of 1 h *N*-bromosuccinimide (NBS) (1.0 cm^3) at 0 °C. The resulting solution was kept at 0 °C and stirred for an additional 30 min. The reaction mixture was poured into an excess of water, extracted with ethyl acetate and the extract washed with water, aqueous potassium iodide (5%), water, sodium thiosulphate (5%) and water, dried ($MgSO_4$) and evaporated. Chromatography of the residue on silica gel with ethyl acetate–hexane (1:9) as eluent afforded 17β-*acetoxy*-6-*bromo*-4-*chloroestr*-4-*en*-3-*one* **4a** (80%), m.p. 185 °C; δ_H(250 MHz; $CDCl_3$) 0.82 (3 H, s, 18-Me), 2.0 (3 H, s, 17β-OCOMe), 4.6 (1 H, m, 6-H) and 4.66 (1 H, t, 17α-H); *m/z* 428 (M^+, 27%), 430 (M^+, 21), 388 (15), 386 (20, M − CH_2CO), 356 (26), 349 (38, M − Br), 348 (97, M − Br), 347 (100), 315 (65), 314 (89), 227 (5), 213 (22) and 114 (23).

A solution of **4a** (250 mg, 0.58 mmol) in acetone (10 cm^3) and conc. HCl (0.3 cm^3) was refluxed for 2.5 h. Acetone was removed under reduced pressure and the residue poured into water and worked up. The recovered material showed two spots on TLC corresponding to **6a** and the 17β-acetate derivative **5a**. The mixture was hydrolysed as described in method A and purified to yield **6a** (overall yield 50%), which was in all aspects (TLC, m.p., ^1H NMR) identical with the product obtained by method A.

Oxidation of **6a**.—Jones' reagent (0.2 cm^3) was added to a stirred solution of **6a** (0.2 g, 0.65 mmol) in acetone (10 cm^3) at 0 °C. The reaction was kept at room temp. for about 20 min and excess of reagent was destroyed by the addition of methanol (5 cm^3). The solution was concentrated to half of its original volume and then diluted with water. Products were extracted with ethyl acetate, and worked up. After removal of the solvent the crude product was purified. Chromatography on silica gel with ethyl acetate–hexane (1:4) as eluent yielded 4-chloroestrone **7a** (75%): HPLC (70% MeOH in water) t_R = 38 min; δ_H(250 MHz; $CDCl_3$) 0.92 (3 H, s, 18-Me), 6.73 (1 H, d, *J* 8, 2-H) and 6.92 (1 H, d, *J* 8, 1-H); *m/z* 306 (M^+, 33%), 304 (M^+, 100), 268 (6, M − HCl), 261 (9), 259 (15), 248 (11), 246 (34), 219 (15), 216 (24), 194 (28) and 181 (26).

Reaction of Methyllithium with **7a**.—A solution of **7a** (40 mg, 0.13 mmol) in dry THF (10 cm^3) was added to a stirred ice-cooled solution of methyllithium in diethyl ether (1.4 mol dm^{-3}; 2 cm^3) under N_2 over a period of 10 min. The mixture was stirred for an additional 45 min at 0–10 °C after which it was diluted with water, extracted with ethyl acetate and worked up. HPLC (80% MeOH in 20% water) t_R = 34 min, afforded 4-chloro-17α-methyl-17β-estradiol **8a**; δ_H(250 MHz; $CDCl_3$) 0.88 (3 H, s, 18-Me), 1.27 (3 H, s, 17-Me), 5.5 (1 H, br, OH), 6.85 (1 H, d, *J* 9, 2-H) and 7.15 (1 H, d, *J* 9, 1-H); *m/z* 322 (M^+, 11%), 320 (M^+, 35), 304 (6, M − H_2O), 302 (18, M − H_2O), 287 (30, M − Me), 261 (18), 247 (35), 220 (29) and 205 (100).

Reaction of Lithium Chloroacetylide with **7a**.—A solution of **7a** (62 mg, 0.2 mmol) in dry THF (5 cm^3) was added to a cold solution of lithium chloroacetylide (prepared by addition of 0.2 cm^3 of *cis*-1,2-dichloroethylene in 5 cm^3 of absolute THF to an ice cold solution of 0.3 cm^3 of methyllithium, 1.4 mol dm^{-3} 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 and worked up in the usual manner. Column chromatography on silica gel with ethyl acetate–hexane (1:9) afforded 4-chloro-17α-(2-chloroethynyl)-17β-estradiol **9a**; HPLC (80% MeOH in water) t_R = 31 min; δ_H(250 MHz; $CDCl_3$) 0.86 (3 H, s, 18-Me), 6.78 (1 H, d, *J* 8, 1-H) and 7.2 (1 H, d, *J* 8, 1-H); *m/z* 364 (M^+, 27%), 329 (6, M − Cl), 287 (16), 273 (7, 287 − CH_3), 262 (23), 247 (76) and 193 (100).

Reaction of Lithium Acetylide–Ethylene Diamine Complex with **7a**.—A solution of **7a** (100 mg, 0.32 mmol) in dry dimethyl sulphoxide (DMSO) (10 cm^3) under nitrogen was treated with lithium acetylide–ethylenediamine complex (300 mg) and the mixture was stirred for 20 h at room temp. The mixture was poured into ice-cold water, acidified with diluted 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 with ethyl acetate–hexane (1.5:8.5) to yield the desired compound 4-chloro-17α-ethynyl-17β-estradiol **10a**; HPLC (75% MeOH in water) t_R = 21 min; δ_H(250 MHz; $CDCl_3$) 0.87 (3 H, s, 18-Me), 2.60 (1 H, s, ≡CH), 5.52 (1 H, br s, OH), 6.85 (1 H, d, *J* 8, 2-H) and 7.15 (1 H, d, *J* 8, 1-H); *m/z* 332 (M^+, 11%), 330 (M^+, 32), 262 (22) and 247 (100).

Synthesis of **2b**.—*Method A. Chlorination of* **1b**. Sulphuryl chloride (300 mg, 2.2 mmol) was added dropwise to a stirred solution of 17β-acetoxy-7α-methylestr-4-en-3-one **1b** (360 mg, 1.1 mmol) in pyridine (4 cm^3) at room temp. After being stirred for 1 h, the mixture was poured into cold water, extracted with ethyl acetate and the extract worked up. The crude product (500 mg) was purified. Chromatography on silica gel with ethyl acetate–hexane (1:9) as an eluent yielded 17β-acetoxy-4-chloro-7α-methylestr-4-en-3-one **2b** (80%), m.p. 55 °C (from hexane); δ_H(250 MHz; $CDCl_3$) 0.82 (3 H, d, *J* 7, 7α-Me), 0.84 (3 H, s, 18-Me), 2.02 (3 H, s, 17β-OCOMe) and 4.61 (1 H, m, 17α-H); *m/z* 366 (M^+, 23%), 364 (M^+, 66), 324 (33, M − CH_2CO), 322 (100, M − CH_2CO), 306 (21, M − CH_3CO_2H), 304 (63, M − CH_3CO_2H), 287 (18), 269 (45), 162 (100) and 139 (78).

Method B. (*a*) *Dehydrohalogenation of* **4a**. To a solution of **4a** (429 mg, 1 mmol) in DMF (10 cm^3) was added lithium carbonate (300 mg) and lithium bromide (150 mg). The mixture was heated at 90 °C for 3 h, poured into ice cooled water (200 cm^3) containing acetic acid (25 cm^3) and worked up. Column chromatography on silica gel with ethyl acetate–hexane gave 4-chloro-17β-hydroxyestra-4,6-dien-3-one **11** (80%), m.p. 122 °C; δ_H(250 MHz; $CDCl_3$) 0.82 (3 H, s, 18-Me), 2.14 (3 H, s, 17β-OCOMe), 4.62 (1 H, t, 17α-H), 6.78 (1 H, dd, *J* 2 and 8, 7-H) and 7.08 (1 H, d, *J* 8, 6-H); *m/z* 350 (M^+, 14%), 348 (M^+, 13), 313 (100), 217 (7) and 172 (18).

(*b*) *Addition of methyl magnesium bromide to* **11**. A solution of compound **11** (250 mg, 0.7 mmol) in dry THF (10 cm^3) was added to a stirred mixture of MeMgBr (1 mmol) and cuprous chloride (100 mg) at −10 °C over a period of 30 min. The reaction mixture was stirred at the same temp. for an additional 30 min and poured into ice cold 1 mol dm^{-3} HCl. The reaction mixture was worked up to yield **2b** (70%), which was identical (m.p., HPLC, TLC, ^1H NMR, MS) with **2b** obtained by method A.

Aromatization of **2b**.—*Method A*. A mixture of **2b** (250 mg, 0.68 mmol) and selenium dioxide (400 mg, 3.63 mmol) in Bu^{t}OH (4 cm^3) was refluxed under N_2 for 15 h. The cold mixture was filtered, evaporated under reduced pressure to dryness and the residue was purified. Chromatography on silica gel with ethyl acetate–hexane (1:9) as an eluent gave 17β-acetoxy-4-chloro-7α-methyl-17β-estradiol **5b** as a crystalline compound (85%), m.p. 165 °C; δ_H(250 MHz; $CDCl_3$) 0.88 (3 H, s, 18-Me), 0.90 (3 H, d, *J* 7, 7α-Me), 2.06 (3 H, s, 17β-OCOMe), 4.62 (1 H, t, 17α-H), 5.98 (1 H, br s, OH), 6.70 (1 H, d, *J* 8, 2-H) and 7.0 (1 H, d, *J* 8, 1-H); *m/z* 364 (M^+, 33%), 362 (M^+, 100), 248 (4), 261 (15) and 208 (30).

A solution of **5b** (100 mg, 0.27 mmol) in 5% KOH in MeOH (25 cm^3) was refluxed for 1 h. Half of the methanol was evaporated off under reduced pressure and the remaining solution was diluted with water and acidified with 0.1 mol dm^{-3} HCl. The mixture was extracted with ethyl acetate and the extract worked up. After solvent removal under reduced pressure the residue was chromatographed on silica gel with ethyl acetate–hexane (1:4) as en eluent to yield 4-*chloro*-7α-*methyl*-17β-*estradiol* **6b**; HPLC (70% MeOH in water) t_R = 22 min; δ_H(250 MHz; $CDCl_3$) 0.80 (3 H, s, 18-Me), 0.82 (3 H, d, *J* 7, 7α-Me), 3.70 (1 H, m, 17α-H), 6.74 (1 H, d, *J* 8, 2-H) and 7.02 (1 H, d, *J* 8, 1-H); *m/z* 322 (M^+, 4%), 320 (M^+, 12), 287 (23), 286 (100), 279 (4), 227 (23) and 149 (50).

Method B. A solution of **2b** (400 mg, 1.1 mmol) in Ac_2O (1 cm^3), pyridine (0.5 cm^3) and AcOCl (3 cm^3) was refluxed for 3 h under N_2 and then evaporated to dryness under reduced pressure. The residue was dissolved in ethyl acetate and the solution washed with water, dried ($MgSO_4$), and concentrated. Chromatography of the residue on neutral alumina column (activity I) with hexane yielded 4-chloro-7α-methylestra-3,5(6)-diene-3,17β-diyl diacetate **3b** as an oil (80%); δ_H(250 MHz;

$CDCl_3$), 0.88 (3 H, s, 18-Me), 0.90 (3 H, d, 7α-Me), 2.02 (3 H, s, 6-OCOMe), 4.64 (1 H, m, 17α-H) and 6.8 (1 H, d, 6-H); *m/z* 364 (60%, $M^+ - CH_2CO$), 362 (70, $M^+ - CH_2CO$), 342 (24), 328 (100), 314 (65) and 134 (48).

To a cooled suspension of the enol acetate **3b** in DMF (10 cm^3) and water (0.5 cm^3) was added NBS (1.0 g) under N_2 over a period of 1 h. The solution was stirred for an additional 0.5 h at 0 °C, poured into an excess of water and extracted with ethyl acetate. Work-up of the extract and chromatography of the residue on silica gel with ethyl acetate–hexane (1:20) yielded 17β-acetoxy-6β-bromo-4-chloro-7α-methylestr-4-en-3-one **4b** (60%); δ_H(250 MHz; $CDCl_3$) 0.92 (3 H, d, *J* 7, 7α-Me), 0.94 (3 H, s, 18-Me), 2.0 (3 H, s, 17β-OCOMe), 4.0 (1 H, d, *J* 6, 6-H) and 4.5 (1 H, t, 17α-H); *m/z* 363 (18%, $M^+ - Br$), 342 (196), 329 (100), 299 (14), 286 (14), 185 (53) and 166 (93).

Compound **4b** was dissolved in acetone (10 cm^3) and 0.1 mol dm^{-3} HCl (0.3 cm^3) and refluxed for 2.5 h. After work-up as described for **5a**, the product was hydrolysed as in method A. The chloro compound **6b** was isolated with an overall yield of 30% and found to be identical (TLC, HPLC, ^1H NMR, MS) with **6b** obtained *via* method A.

Oxidation of **6b**.—Jones' reagent (0.2 cm^3) was added slowly to a stirred solution of **6b** (9.2 g, 0.62 mmol) in acetone (10 cm^3) at 0 °C. The reaction mixture was maintained at room temp. for *ca.* 20 min, after which excess of reagent was destroyed by the addition of MeOH (5 cm^3). The solution was then concentrated, diluted with an excess of water, extracted with ethyl acetate and the extract worked up. Chromatography of the residue on silica gel with ethyl acetate–hexane (1:5) as eluent yielded 4-chloro-7α-methylestrone **7b**; HPLC (75% methanol in water) t_R = 23 min; δ_H(250 MHz; $CDCl_3$), 0.86 (3 H, d, *J* 7, 7α-Me), 0.88 (3 H, s, 18-Me), 5.58 (1 H, br s, 17β-OH), 6.72 (1 H, d, *J* 8, 2-H) and 7.02 (1 H, d, *J* 8, 1-H); *m/z* 320 (M^+, 33%), 318 (M^+, 100), 263 (6), 261 (18), 247 (13), 232 (2) and 191 (15).

Reaction of Lithium Acetylide–Ethylenediamine Complex with **7b**.—A solution of **7b** (100 mg, 0.31 mmol) in dry DMSO (10 cm^3) under N_2 was treated with lithium acetylide–ethylenediamine complex (300 mg) and the mixture was stirred for 72 h at room temp.The mixture was poured into ice-cold water and acidified with diluted acetic acid. The organic compound was extracted with ethyl acetate, and the extract washed with brine and worked up. Chromatography of the residue on silica gel with ethyl acetate–hexane (1:4) as eluent afforded 4-chloro-17α-ethynyl-7α-methyl-17β-estradiol **10b**; HPLC (70% methanol in water) t_R = 26 min; δ_H(250 MHz; $CDCl_3$ + [$^2H_6$]DMSO) 0.80 (3 H, d, *J* 7, 7α-Me), 0.82 (3 H, s, 18-Me), 2.71 (1 H, s, ≡H), 4.43 (1 H, s, 17β-OH), 6.48 (1 H, d, *J* 9, 2-H) and 6.75 (1 H, d, *J* 9, 1-H); *m/z* 346 (M^+, 15%), 346 (M^+, 45), 311 (4), 300 (4), 288 (4), 277 (13), 263 (34), 261 (100), 208 (49) and 123 (21).

Reaction of Lithium Acetylide–Ethylenediamine Complex with **15a**.—A stirred solution of **15a**[16] (100 mg, 0.32 mmol) in dry DMSO (8 cm^3) under nitrogen after treatment with lithium acetylide–ethylenediamine complex (280 mg), at room temp. was worked up in the manner described for **10a**. Chromatography of the residue on silica gel with ethyl acetate–hexane (1:5) as eluent yielded 2-chloro-17α-ethynyl-17β-estradiol **16a**; HPLC (75% MeOH in water) t_R = 21 min; δ_H(250 MHz; $CDCl_3$ + [$^2H_6$]DMSO), 0.78 (3 H, s, 18-Me), 6.61 (1 H, s, 1-H) and 7.09 (1 H, s, 4-H); *m/z* 332 (M^+, 10%), 330 (M^+, 23), 304 (11), 262 (18), 249 (34) and 247 (100).

Synthesis of **14b**.—To a stirred solution of 7α-methylestradiol **12b** (286 mg, 1 mmol) in dry chloroform (15 cm^3) was added benzeneselenenyl chloride (230 mg, 1.2 mmol). The reaction mixture was stirred at room temp. for about 6 h during which period the dark brown colour of the solution changed slowly to yellow. The reaction mixture was poured into water (30 cm^3) and extracted with chloroform (2 × 20 cm^3). The extract was washed with water (20 cm^3), dried (Na_2SO_4) and evaporated under reduced pressure. Chromatography of the residue on silica gel (40 g) with ethyl acetate–hexane (1:10) as eluent gave a mixture which was further purified by HPLC on a reverse-phase C-18 column to give 2-phenylselenenyl-7α-methyl-17β-estradiol **13b**; t_R = 27 min (gradient of 30% H_2O in MeOH to 100% MeOH over 40 min), m.p. 187–189 °C (from MeOH); *m/z* 442 (M^+, 100), 438 (16) and 361 (11). To a solution of **13b** (0.1 mmol) in chloroform (10 cm^3) was added either iodine monochloride or benzeneselenenyl chloride (0.2 mmol). The reaction mixture was stirred at room temp. for 3 h, poured into water and worked up. Chromatography of the residue on silica gel with ethyl acetate–hexane (1:5) as eluent afforded 2-chloro-7α-methyl-17β-estradiol **14b** (80%, from **13b**). An analytical sample was obtained after purification by HPLC; t_R = 20 min (MeOH–H_2O; 70:30), m.p. 187–189 °C; δ_H(250 MHz; $CDCl_3$), 0.69 (3 H, s, 18-Me), 0.72 (3 H, d, *J* 7, 7α-Me), 3.62 (1 H, m, 17α-H), 6.6 (1 H, s, 1-H) and 7.11 (1 H, s, 4-H); *m/z* 322 (M^+, 6%), 320 (M^+, 18), 261 (29), 208 (35) and 192 (27).

Synthesis of **16b**.—Jones' reagent (0.2 cm^3) was added slowly to a stirred solution of **14b** in acetone (10 cm^3) at 0 °C. The reaction mixture was maintained at room temp. for *ca.* 20 min after which it was worked up as described above for **7b**. Column chromatography of the residue on silica gel with ethyl acetate–hexane (1:5) as eluent yielded 2-chloro-7α-methylestrone **15b**, m.p. 198–202 °C (from MeOH); *m/z* 320 (M^+, 34%), 318 (M^+, 100), 284 (10), 276 (13), 261 (54), 246 (27) and 221 (10).

A solution of **15b** in dry DMSO (5 cm^3) under N_2 was treated with lithium acetylide–ethylenediamine complex (250 mg) and the reaction mixture was poured into ice cold water and worked up as described for **10b**. The compound 2-chloro-17α-ethynyl-7α-methyl-17β-estradiol **16b** was purified by HPLC; t_R = 21 min (MeOH–H_2O, 75:25); δ_H(250 MHz; $CDCl_3$ + [$^2H_6$]-DMSO) 0.80 (3 H, d, *J* 7, 7α-Me), 0.85 (3 H, s, 18-Me), 6.67 (1 H, s, 1-H) and 7.18 (1 H, s, 4-H); *m/z* 346 (M^+, 9%), 348 (M^+, 29), 287 (6), 263 (32), 261 (100), 233 (6), 210 (19), 208 (59), 193 (31), 181 (23), 157 (20), 144 (23), 124 (33) and 115 (32).

Estrogen Receptor Binding Assay.—The affinity of the 2- and 4-chloroestradiol derivatives for estrogen receptors was determined by a competitive binding assay[17] and is expressed as the relative binding affinity (RBA). The RBA is defined as 100 times the ratio between the competitor and unlabelled estradiol concentrations required for 50% competition to specific [^3H]estradiol binding. Murine uterine cyctoplasmic extracts were incubated at 0–4 °C for 18 h with 20 × 10^{-9} mol dm^{-3} of [^3H]estradiol in the absence and presence of competitive steroids at concentrations ranging from 2 × 10^{-9} to 20 × 10^{-6} mol dm^{-3}. The bound steroid was separated from free steroid by Sephadex LH-20 chromatography. The nonspecific binding (equivalent to that observed in the presence of a 100-fold excess of unlabelled estradiol) was 4% of the total binding, which was subtracted from the total binding to estimate the specific binding. The specific binding in the receptor preparation was equivalent to 4.77 × 10^{-9} mol dm^{-3}.

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