Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 335–345

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis, Characterization and Biological Evaluation of 7α-Perfluoroalkylestradiol Derivatives

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Received 10 July 2002; accepted 19 September 2002

Abstract—Linkage of a long CH_2 side chain ('spacer') onto C-7α of estradio1-17β (E_2) does not abrogate the binding affinity of this hormone for its receptor. Our purpose was to assess whether the linkage of a CF_2 side chain, which is more bulky and rigid, could also be accommodated by the estrogen receptor (ER). We describe here the synthesis of 7α-perfluorohexylestradiol **7** by perfluoroalkylation of a key silylenolether **2** with FITS-6 (perfluorohexyl-phenyliodoniurn trifluoromethanesulfonate). 7α-Trifluoromethylestradiol **10a** was prepared as a fluorinated control compound by UV-light induced trifluoromethylation of **2** with Umemoto reagent (*S*-trifluoromethyldibenzo[*b*,*d*]thiophenium trifluoromethanesulfonate). Endocrine properties of these two E_2 derivatives were tested on the MCF-7 breast cancer cell line. Our data reveal that rigidity of the side chain of **7** affected the association of its hormone moiety with the ER to the same extent as a long alkyl side chain. Rigidity also failed to abrogate estrogenicity, as demonstrated by the ability of **7** to enhance ERE-dependent transcription and cell growth. Compound **7** retained the capacity of inducing down regulation of the receptor. Interestingly, no evidence of antiestrogenicity was recorded since this compound behaved like a weak estrogen, exerting a mitogenic effect at high concentration. Of note, control **10a** displayed a higher binding affinity than **7** for ER and consequently acted like the latter, albeit with a higher efficiency. Selection of appropriate residues to be linked at the end of a long 7α alkyl side chain is known to be essential for generating strong antiestrogenicity. One may hope that such a property may also hold for perfluorinated chains to produce antiestrogens with strong metabolic stability.

Introduction

Working on estrogen-bearing resins for the purification of the estrogen receptor (ER), Bucourt et al.[1] identified the C-7 position of estradiol (E_2) as an appropriate attachment point for long alkyl side chains. Once linked to matrices, such molecules retained a sufficiently high binding affinity for the receptor to be used in chromatographic extraction procedures. Subsequent studies conducted at the ICI company (now Astra Zeneca) revealed that linkage of selected residues at the end of side chains may generate compounds with very strong antiestrogenicity.[2,3] Some of these antagonists (i.e., ICI 164,384[2,4] and ICI 182,780[3]) appeared totally devoid of estrogenicity leading to their classification as 'pure antiestrogens'. This property was also found for similarly substituted 11β derivatives[5,6] and related triphenylethylenic analogues.[7] Hence, linkage of long CH_2 side chains in C-7α or C-11β of E_2 ('spacer') appeared appropriate as an approach for the design of compounds with substantial binding affinity for E_2 and potential therapeutic interest. The orientation of the aminoalkyl side chain of triphenylethylenic antiestrogens into a channel on the border of the hormone-binding domain of the receptor explains their antagonistic effect.[8,9] Steric hindrance provoked by the length of the side chain borne by these compounds does not seem to play a major role in antiestrogenic potency, as demonstrated by comparison of the endocrine profile of

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PII: S0968-0896(02)00457-1

short [$(CH_2)_2$] and long [$(CH_2)_8$] derivatives of the antiestrogen idoxifene which display similar antagonistic properties.[10] Interestingly, this channel in the vicinity of the binding pocket also accepts the 7-α side chain of pure antiestrogen ICI 164,384,[11] giving thereby an explanation for its antagonistic activity. Thus, it would be of interest to examine the impact of the potential steric hindrance associated with 7-α rigid side chains on binding and endocrine properties of 7-α derivatives.

Our purpose was to assess whether the linkage in C7-α of E_2 of a $(CF_2)_nCF_3$ side chain which is more bulky and rigid than its hydrocarbon counterpart[12] could be accommodated by the receptor channel. The potential benefits resulting from the introduction of highly fluorinated chains would stem from their resistance to metabolism in vivo,[13] as well as their intrinsic hydrophobicity and thermal stability.[12] Yet, the introduction of perfluoroalkyl substituents to any compound represents a significant challenge, and the necessity for stereo- and regiospecific functionalization in this case adds considerably to the complexity of the synthesis.[14] We describe here the strategy that we designed for the preparation of 7α-perfluoroalkylestradiol (compound **7**) as well as 7α-trifluoromethylestradiol (compound **10a**) that was used as a reference for the assessment of the impact of side-chain fluorination on biological properties.

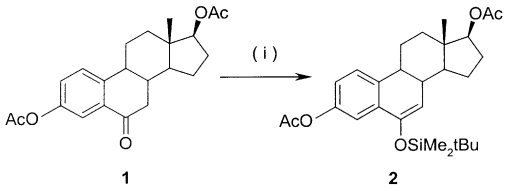
Results and Discussion

Chemical syntheses

Perfluorohexyl series. The synthesis of 7α substituted estradiol derivatives has classically been achieved via copper promoted nucleophilic conjugate addition to dienones such as hydroxyestra-4,6-dien-3-one.[2,4] Such additions usually afford a mixture of epimers, with the 7-α isomer formed in a large excess and subsequently require rearomatization to generate the appropriate estradiol skeleton. More recently, Kunzer et al. have shown that alkylation at the 7 position may be performed with the estradiol skeleton already in place.[15] The same strategy was used by Napolitano and Katzenellenbogen.[16,17]

Our own approach to a flexible synthesis of 7-perfluoroalkyl estradiol analogues is based upon a related perfluoroalkylation of a suitable nucleophilic intermediate **2**. Access to the required nucleophile from E_2 requires selective oxidation at the 6 position to afford the known ketone 1. Amongst the wealth of oxidants investigated for this process,[18] chromium reagents have proved most successful, but we have found, like many groups before us, that the selective oxidation of estradiol is not an easy task.

The key silyl enol ether **2** was subsequently readily produced from ketone 1 by reaction over several days with *t*-butyldimethylsilyl triflate in the presence of 2,6-lutidine (Scheme 1).



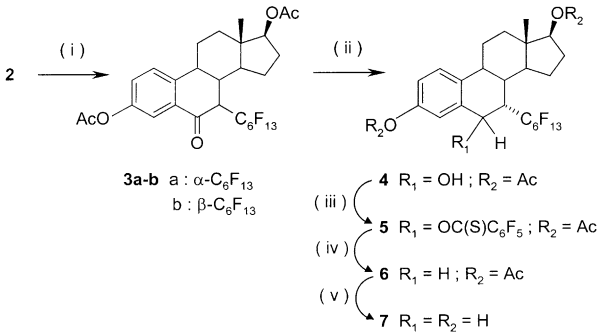
Scheme 1. Reagents and conditions: (i) CH_2C1_2, *t*-butyldimethylsilyl triflate, 2,6-lutidine.

Reaction of FITS-6 (perfluorohexyl-phenyliodonium trifluoromethanesulfonate)[19a,b] with silyl enol ether 2 provided the steroids **3a** and **3b** in 80% yield with high diastereoselectivity (**3a**:**3b** = 10:1) (Scheme 2). Definitive stereochemical identification proved impossible at that stage, due to the complexity of the proton NMR spectrum. However, correlation with its trifluoromethyl analogue showed the stereochemistry of the major stereoisomer **3a** to be α (vide infra).

In the original pioneering work of Napolitano[20] removal of the benzylic ketone was possible under classical conditions employing Et_3SiH and BF_3-diethyl etherate. Apparently in the case of ketone **3a**, the presence of the perfluoroalkyl substituent rendered this methodology unsuitable. It eventually proved possible to reduce the C_6F_{13} substituted ketone **3a** via prior transformation to the alcohol **4**, and esterification with pentafluorophenylthiocarbonate to afford the thioester **5**. Subsequent radical reduction of thiocarbonate **5** with Bu_3SnH under Barton conditions[21] followed by saponification of the acetate protecting groups generated the estradiol derivative **7**.

The perfluoroalkylated steroid **7** is thus available in eight steps from estradiol, in a global yield of 9%.

Trifluoromethyl series. For the synthesis of the lower trifluoromethyl analogue, we planned to introduce the trifluoromethyl group by reaction of silyl enol ether **2** with Umemoto reagent (*S*-trifluoromethyldibenzo[*b*,*d*]thiophenium trifluoromethanesulfonate). Under thermal conditions similar to those employed by Umemoto,[22] the yield was low (15%). However, UV irradiation of the reaction mixture led to excellent yield (90%) of the ketones **8** but with a poorer selectivity than



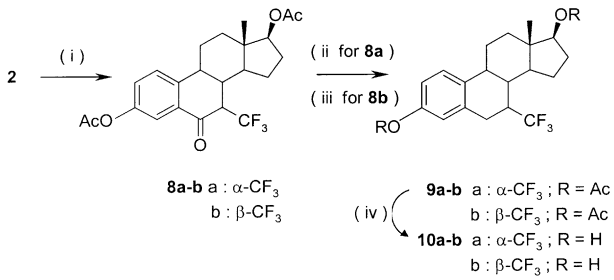
Scheme 2. Reagents and conditions: (i) CH_2Cl_2, FITS-6, 2,6-lutidine; (ii) (isomer **3a** only) THF/ethanol (10:1), $NaBH_4$; (iii) CH_2C1_2, pyridine, DMAP, $C_6F_5C(S)C1$; (iv) benzene, AIBN, Bu_3SnH, reflux; (v) MeOH, H_2O, NaOH.

the one observed in the C_6F_{13} series (**8a**:**8b** = 5:4) (Scheme 3).

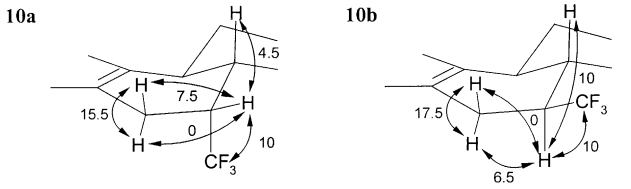
Attempts to use similar conditions employed for the CF_{13} compound in the reduction of the two epimers of the trifluoromethyl analogues failed. However, the C1emmensen reduction has been shown to be highly effective in the direct reduction of some benzylic ketones.[23] Indeed, slow addition of the ketone **8b** to a mixture of zinc amalgam and concentrated hydrochloric acid led to the saturated product **9b** in up to 40% yield from the minor epimer. Subsequent saponification of the acetate protecting groups in **9b** was consequently performed, thus finalizing the synthesis of the trifluoromethyl estrogen **10b.** However, C1emmensen reduction of the other, major epimer **8a** was much less successful (12% yield). Surprisingly, a reassessment of the classical conditions employing BF_3-diethyl etherate and Et_3SiH led to good results in this case, the reduced trifluoromethyl steroid **9a** being isolated in satisfactory yield (60%). Again, saponification of the acetate groups afforded smoothly steroid **10b.**

Thus, a suitable reduction route is available to each of the trifluoromethyl steroids **10**. It is noteworthy that the electronic nature and hence reactivity of the ketone appears to vary considerably with the stereochemistry at C-7. This could be an indication of the varying degree of conjugation between the ketone and the aromatic ring, this in turn being governed by the distortion caused by the perfluoroalkyl substituent.

In the absence of suitable crystalline intermediates for X-ray analysis, we undertook a careful study of the ^1H NMR spectra of the major and minor products in the trifluoromethyl case. Irradiation of the AB multiplet of the CH_2 at C-6 in the ^1H NMR spectra of each isomer of 7-trifluoromethyl estradiol **10a** and **10b** has revealed the other coupling constants of the proton multiplet at C-7 (Scheme 4). This set of coupling constants strongly suggests that for the CF_3 compound the major isomer **10a** produced was the desired α-CF_3 epimer. By analogy, when considering the NMR similarity between compounds **7** and **10a** (see Experimental), the major C_6F_{13} epimer may also be assumed to possess α-stereochemistry at the 7 position.



Scheme 3. Reagents and conditions: (i) DMF, Umemoto reagent, hv; (ii) CH_2Cl_2, Et_3SiH, $BF_3{\cdot}Et_2O$, reflux; (iii) Et_2O, Zn/Hg, HCl; (iv) MeOH, H_2O, NaOH.



Scheme 4. Selected NMR data for compounds **10a** and **10b** (coupling constants in Hz).

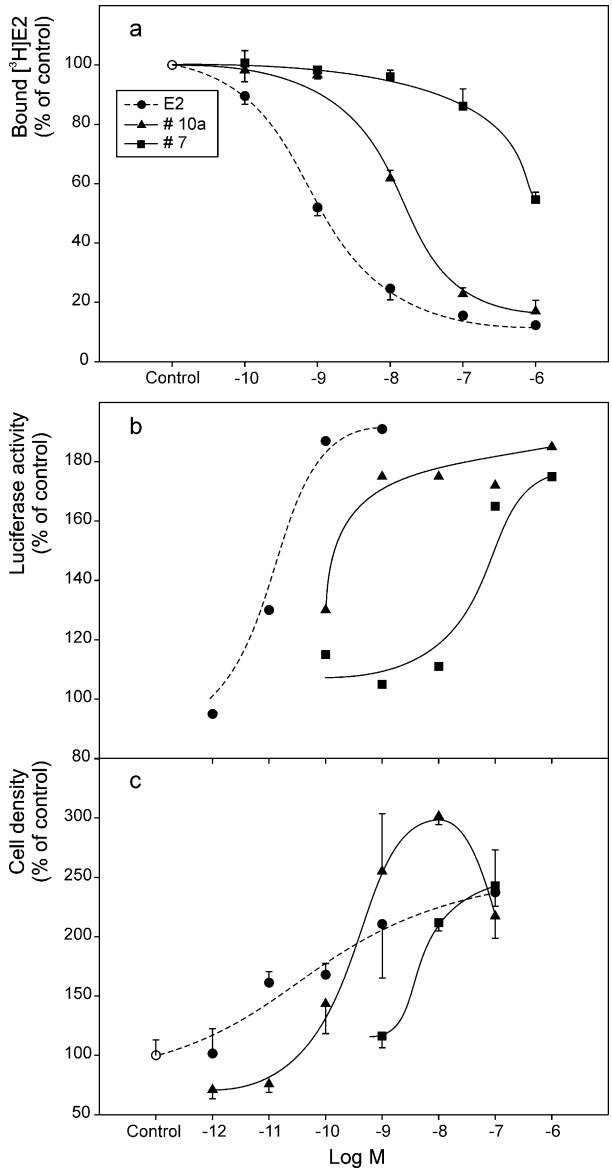


Figure 1. Binding to ER and related biological activity of compounds **7** (7α-perfluorohexylestradiol) and **10a** (7α-trifluoromethylestradiol) in MCF-7 cells: (a) competition with $\{^3H\}E_2$ for binding to ER assessed by whole cell assay (mean of two independent experiments performed in triplicate, control refers to the binding value in absence of competitor); (b) effect of E_2 and fluorinated derivatives on ER-mediated transcription in MVLN cells, representative result of an experiment repeated three times; (c) proliferative response of MCF-7 cells to E_2 and fluorinated derivatives (composite graph combining data from separate experiments, $n = 4$–8), cell densities are expressed relative to the mean value in absence of agonist. Bars: SD.

Biological evaluation of compounds 7 and 10a

Fig. 1a shows that compound **7** (IC_{50}, 1 μM) impeded the incorporation of [^{3}H]E_2 into MCF-7 cells with an efficiency ~1000 less than that of unlabeled E_2 (IC_{50}, 1 nM) (Relative Binding Affinity, RBA ~0.1). Although this value is relatively low, it does not markedly differ from that established in the past for ICI 134,384 under identical experimental conditions (RBA ~0.3).[24] Hence, a perfluorination-induced enhancement of rigidity of a C-7α side chain does not drastically affect binding affinity. As expected, compound **10a** displayed a largely higher binding affinity toward cognate receptors (IC_{50}, 20 nM) (RBA ~5). These results showing the capacity of compound **7** and **10a** to bind ERα (ERβ, if expressed at all, is extremely low in MCF-7 cells) were obtained with living cells, that is in conditions where various physiological/pharmacological factors may influence ligand association with the receptor (cell membrane permeability, interaction with intra- and extracellular lipophilic molecules, metabolism, …). None the less, competition DCC assays performed in parallel with partially purified recombinant human ERα gave values similar to those established by whole cell assay (data not shown), thus excluding major interference of the factors cited above.

The difference in binding affinity between these two C-7α E_2 derivatives reflected in their relative ability to induce luciferase in MVLN cells (ERE-dependent transcription) (Fig. 1b). Compound **7** enhanced luciferase expression to the same extent as E_2 at a ~1000-fold higher concentration (0.1 μM vs 0.1 nM) while compound **10a** already operated at a 10-fold higher concentration (Fig. 1b shows representative data out of three independent experiments). In similar experimental conditions, RU 58,668[6,25,26] (used at 0.1 μM as a reference antiestrogen) produced a decrease of approx. 50% in basal luciferase activity.

MCF-7 behaves as a bona fide estrogen-dependent cell line and exhibits a proliferative response upon exposure to estrogen agonists. Dose–response curves for E_2, compounds **7** and **10a** are illustrated in Figure 1c. The maximal responses of MCF-7 cells to E_2 and compound **7** observed in this study were quite modest (approx. 140% above baseline), due to the fact that agonists were added after a short period of estrogen deprivation (1 day, see Biological procedures). Indeed, cell reactivity to estrogenic stimulation increases with the duration of culture in an estrogen-free environment prior to drug addition (manuscript in preparation). Anyway, the potency of compound **7** as a mitogen (EC_{50}, *5* nM) was substantially lower (approx. 100×) than that of E_2 (EC_{50}, 60 pM), whereas compound **10a** was only four times less potent than E_2 (EC_{50}, 0.2 nM). On the other hand, compound **10a** seemed to elicit a maximal response higher than that induced by E_2, this being possibly due to an increased resistance of this fluorinated compound to metabolic inactivation.

In this context, it is noteworthy that the EC_{50}'s characterizing the potencies of E_2 and its fluorinated derivatives as agonists of ER (activation of ERE-dependent transcription, cell mitotic response) are systematically shifted to lower concentration values as compared to the

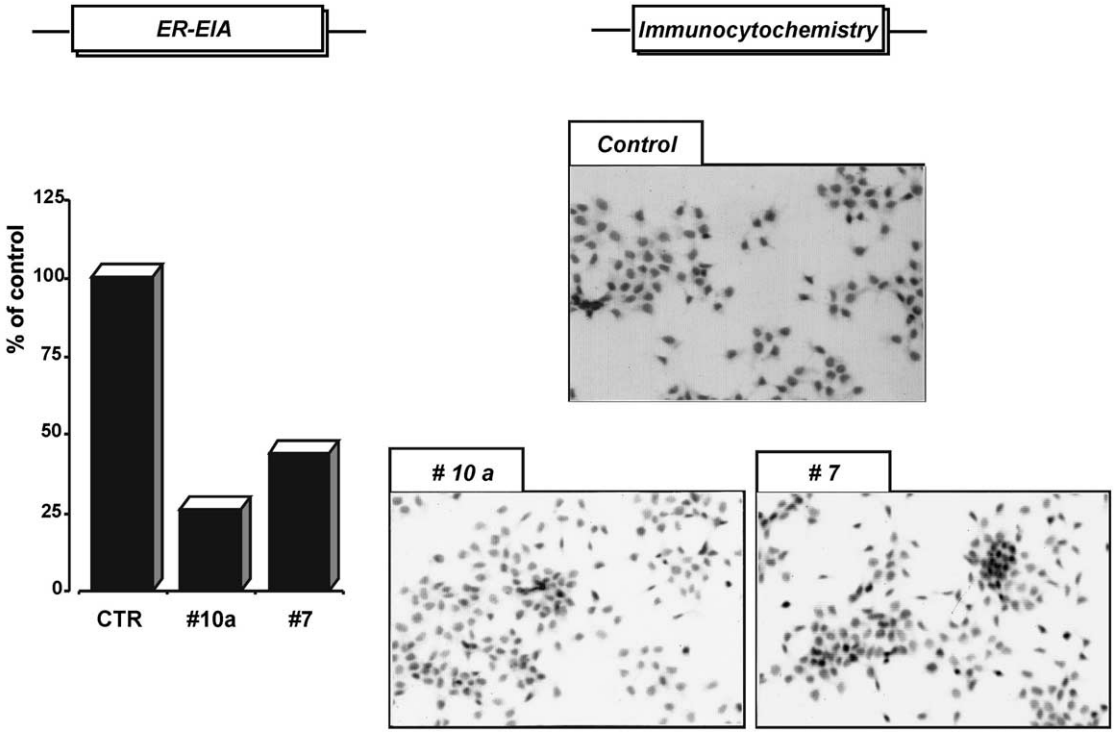


Figure 2. Down regulation of ER in MCF-7 cells exposed to compounds **7** and **10a** (overnight incubation at 0.1 μM). ER level was assessed on KC1 extract by enzyme immunoassay (left; 100% correspond to 728 fmol/mg prot.) or peroxidase-based procedure, bright-field microscopy (magnification: 250×).

IC_{50}'s for competitive binding, illustrating once again the fact that the agonists exert their optimal effect at a concentration largely below total receptor occupancy.[27]

Ligand-binding to ER induces the progressive degradation of the latter leading usually to a decrease of steady-state level (down regulation) when this elimination process exceeds synthesis.[24,28] Such a down regulation was documented by measuring the intracellular concentration of ER protein (ITA) and by the immunocytochemical (bright field microscopy and immunofluorescence) demonstration of ER in MCF-7 cells exposed to E_2 and fluorinated derivatives (Figs 2 and 3). As revealed by ITA, an overnight exposure of MCF-7 cells to compounds **10a** and **7** (0.1 μM) induced a disappearance of approx. 75 and 50%, respectively, of immunoreactive ER (Fig. 2). In similar experimental conditions, E_2 at 0.1 nM would invariably produce a nearly total elimination of the receptor (data not shown). Decrease of ER was confirmed by light microscopy examination after immunocytochemical staining of the receptor. Thus, as shown in Figure 2, exposure to compounds **7** and **10a** resulted in a diminished intensity of DAB staining in bright field microscopy. Observation at higher magnification of cells processed for ER immunofluorescence (Fig. 3) similarly showed a substantial reduction of the signal, which already occurred after 5 h of cell exposure to E_2 and fluorinated derivatives. Of note and as already reported for E_2,[28,29] the extent of this agonist-induced ER loss varied among individual cells, most probably due to the fact that they were in different phases of the cell division cycle (absence of synchronization). Indeed, while ER concentration in asynchronous MCF-7 cells peaks at G0/G1 and late S phases, it has been suggested that the receptor is more prone to down regulation during G0/G1 phase than during the other phases of the cell cycle.[29]

Conclusions

Our data indicate that linkage of a perfluorohexyl side chain in 7α of E_2 does not totally abrogate the ability of the hormone to bind to ER. Of note, the binding affinity for ER of this compound is quite similar to that measured for ICI 164,184 under similar experimental conditions (whole cell assay).[24] Thus, an increase in the rigidity of a 7α long side chain in E_2 analogues does not affect the association of the estrogen moiety with specific residues of the hormone binding pocket. Note also that compound **10a** activates ER with only a slightly lower efficiency than E_2 (~1:10) indicating that fluorinated substituents are relatively well tolerated within this pocket. Consistent with the fact that compounds **7** and **10a** both behave as ER ligands, they induce a down regulation of the receptor, albeit less efficiently than E_2.

Concerning the effect of compound **10a** on MCF-7 cell growth, no cytostatic activity was seen, even at high concentrations. Conversely, the compound stimulated cell proliferation with less potency than E_2, suggesting a weak estrogenicity. Hence, rigidity of the side chain of this compound failed to generate any antagonistic activity. Such a property was also recorded for ERE-dependent transcription (luciferase induction).

The size of 7α alkyl side chains is known to influence the estrogenic/antiestrogenic profile of substituted E_2

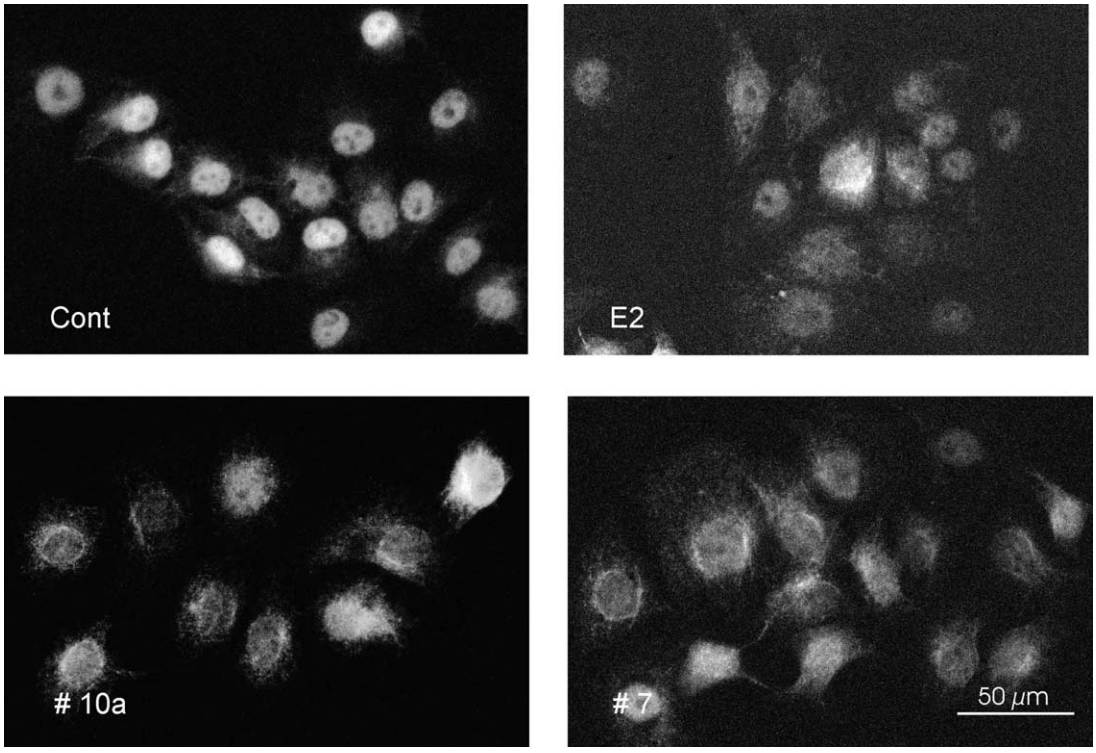


Figure 3. Down regulation of ER in MCF-7 cells, as revealed by immunofluorescence (Texas Red labeling). Cells were exposed for 5 h to 1 nM E_2, 10 nM compound **10a** or 0.1 μM compound **7**.

derivatives.[2–4] One may anticipate that such a property also holds for corresponding perfluorinated chains. Nevertheless, the rigidity of such chains may strongly modify their positioning into the channel bordering the receptor binding site,[11] thereby decreasing or enhancing receptor mediated transactivation. On the other hand, we know that the selection of appropriate residues to be linked at the end of a long 7α alkyl side chain is essential for the agonist–antagonist conversion of steroid estrogen. For example, the 7α side chain of the pure antiestrogen ICI 164,384 bears an amido group, whereas that of ICI 182,780 contains a sulfinyl residue. As shown by recent studies,[30] modifications of the sulfinyl group in ICI 182,780 (generating the sulfide or the sulfone analogue) modulate the antiestrogenic potency, thus stressing the importance of this residue in the 7α side chain. One may hope that such a property may also hold for perfluorinated chains to produce new antiestrogens with strong metabolic stability. Such a work will need further synthetic efforts, because bifunctional perfluoroalkyl chains are far less readily available than the monofunctional ones used in this study.[31]

Experimental

General analytical procedures

NMR spectra were recorded on a Bruker AC-300 spectrometer. Reported coupling constants and chemicals shifts were based on a first order analysis. Internal reference was the residual peak of $CHCl_3$ (7.27 ppm) for ^1H (300 MHz), central peak of $CDCl_3$ (77 ppm) for ^{13}C (75 MHz) spectra and internal $CFCl_3$ (0 ppm) for ^{19}F (282 MHz) NMR spectra. IR spectra were recorded as CCl_4 solutions on an Impact 400D Nicolet spectrophotometer. High resolution mass spectra were performed with a Finnigan MAT 95S spectrometer. Melting points were determined on a Mettler FP61 melting point apparatus.

FITS-6[19c] and Umemoto[22c] reagents were prepared according to published procedures.

Chemical synthesis

6-(*t*-Butyldimethylsilyloxy)-1,3,5 [10],6-estratetraene-3,17β-diol diacetate (2). To a stirred mixture of ketone **1** (276 mg, 0.746 mmol) and 2,6-lutidine (0.7 mL, 0.644 mg, 6.0 mmol) in CH_2Cl_2 (20 mL) at 0 °C was added dropwise *tert*-butyldimethylsilyl triflate (0.70 mL, 0.806 mg, 3.05 mmol). The solution was allowed to come to room temperature and stirred for 66 h, when another portion (2 mL) of each reagent was added. 24 h later, the mixture was evaporated onto florisil (60 g). Column chromatography (florisil/0–30% ether in hexane) gave the *silyl enol ether* **2** (333 mg, 0.687 mmol, 92%) as a colorless oil which crystallized on cooling.

Mp 143–144 °C. ^1H NMR ($CDCl_3$) δ 7.23 (d, 1H, $J=8.2$ Hz, H1); 7.18 (d, 1H, $J=2.5$ Hz, H4); 6.94 (dd, 1H, $J=8.2$, 2.5 Hz, H2); 5.3 (t, lH, $J=0.3$ Hz); 5.13 (d, 1H, $J=1.5$ Hz); 4.73 (dd, lH, $J=9.0$, 8.0 Hz, H17); 2.50–0.70 (m, 28H, including 2.31 (s, 3-OAc), 2.07 (s, 17-OAc), 1.00 (s, Si*t*Bu), 0.84 (s, 13-Me); 0.21 (s, 3H, SiMe); 0.18 (s, 3H, SiMe). ^{13}C NMR ($CDCl_3$, ^1H coupled) δ 171.1 (qd, $J=7.0$, 3.0 Hz); 169.6 (q, $J=6.9$ Hz, MeCO); 149.2, 147.8, 137.4, 135.4 (C3, 5, 6 and 10); 124.0 (dt, $J=158$, 2.3 Hz); 120.0 (dddd, $J=164$, 5.0, 3.8, 0.5 Hz); 115.3 (dd, $J=163$, 4.0 Hz); 109.1 (d, $J=157$ Hz, C7); 82.3 (d, $J=148$ Hz, C17); 48.7 (d, $J=126$ Hz, C14); 43.8 (C13); 42.7 (d, $J=142$ Hz); 37.6 (d, $J=125$ Hz); 36.3 (t, $J=125$ Hz, C12); 27.4 (t, $J=134$ Hz, C16); 25.8 (qquin, $J=134$, 5.5 Hz, CMe_3); 23.7, 23.2 (C11, C15); 21.1 (q, $J=130$ Hz); 18.2 (Me_3C); 11.8 (q, $J=125$ Hz, C18); −4.5, −4.5 (m, $SiMe_2$). I.R. (CCl_4, cm^{-1}) ν 1775 and 1743 (ester C=O), 1646 (aryl C=C), 1175.

6-Oxo-7-perfluorohexylestradiol diacetate (3a and 3b). To a stirred suspension of FITS-6 (0.563 g, 0.84 mmol) and 2,6-lutidine (0.088 mL, 0.081 g, 0.755 mmol) in CH_2Cl_2 (4 mL) was added dropwise a solution of silyl enol ether **2** (371 mg, 0.765 mmol) in CH_2Cl_2 (4 mL). After stirring for 42 h, the mixture was evaporated onto silica and column chromatography (6:1:13 CH_2Cl_2/ether/hexane) gave two epimers of *perfluorohexylestradiol derivative* **3** as colorless oils, the smallest fraction being eluted first. Yield of minor epimer **3b** 50 mg (0.073 mmol, 9.4%): yield of major epimer **3a** 351 mg (0.5 10 mmol, 67%).

Major epimer 3a. ^1H NMR (C_6D_6) δ 7.49–7.43 (m, 1H, H4); 7.11 (dd, 1H, $J=8.5$, 2.5 Hz, H2); 6.83 (d, 1H, $J=8.0$ Hz, H1); 4.64–4.53 (m, 1H, H17); 3.24 (dm, 1H,$J=28.0$ Hz, H7); 2.15–1.90 (m, 2H); 1.85–0.73 (m, 14H), (including 1.75 (s, OAc), 1.74 (s, OAc); 0.68 (s, 3H, 13-Me); 0.65–0.5 (m, 1H). ^1H NMR ($CDCl_3$) δ 7.38–7.24 (m, 3H, H1, 2 and 4); 4.79–4.68 (m, 1H, H17); 3.45 (dm, 1H, $J=27.0$ Hz, H7); 2.50–0.70 (m, 20H), (including 2.31 (s, 3-OAc), 2.08 (s, 17OAc), 0.97 (s, 13-Me)). ^{13}C NMR ($CDCl_3$) δ 193.6 (dd, $J=8.0$, 2.5 Hz, C6); 171.0, 169.0 (MeCO); 149.6, 140.1 (C3, C10); 137.4 (d $J=2.0$ Hz, C5); 126.4, 124.8, 118.3 (C1, 2, 4); 81.9 (C17); 53.7 (t $J=20.0$ Hz, C7); 51.9 (C14); 44.1 (C13); 41.0 (C9); 36.4 (C12); 36.2 (d, $J=3.5$ Hz, C8); 27.1 (d, $J=2.0$ Hz, C16); 24.4 (C15); 23.8 (d, $J=10.0$ Hz, C11); 21.1, 21.0 (CH_3CO); 12.2 (C18). ^{19}F NMR ($CDCl_3$) δ −81.3 (t, 3F, $J=9.0$ Hz, CF_3); −107.0 (ddt (AB), 1F, $J=285$, 28.0, 12.5 Hz, one of α-CF_2); −111.8 (dm (AB), 1F $J=285$ Hz, 18.0, one of α-CF_2); −118.3 (dm (AB), 1F, $J=300$ Hz, one of β-CF_2); −120.7 (dm (AB), 1F, $J=300$ Hz, one of β-CF_2); −121.1 (dm(AB), 1F, $J=300$ Hz, one of γ,δ-CF_2); −122.4 (dm (AB), 1F, $J=290$ Hz, one of γ,δ-CF_2); −123.1 (dm (AB), 1F, $J=300$ Hz, one of γ,δ-CF_2); −124.0 (dm (AB), 1F, $J=300$ Hz, one of γ,δ-CF_2); −125.8 (dm (AB), 1F, $J=295$ Hz, one of ε-CF_2); −127.4 (ddt (AB), 1F, $J=295$, 19.0, 11.5 Hz, one of ε-CF_2). I.R. (CCl_4, cm^{-1}) ν 1780, 1759, 1740, 1732 (ester C=O); 1705 (aryl C=O); 1164 (R_F).

Minor epimer 3b. ^1H NMR ($CDCl_3$) δ 7.66 (d, 1H, $J=2.5$ Hz, H4); 7.47 (d, 1H, $J=8.5$ Hz, H1); 7.32 (d, 1H, $J=8.5$, 2.5 Hz, H2); 4.75 (dd, 1H, $J=9.0$, 8.0 Hz, H17); 3.41 (tt, 1H, $J=16.5$, 2.5 Hz, H7); 3.26–3.12 (m, 1H); 1.85–0.73 (m, 14H, including 2.31 (s, AcO), 2.06 (s, AcO)); 0.82 (s, 3H, 13-Me). ^{13}C NMR ($CDCI_3$) δ 190.8

(t, $J=5.5$ Hz, C6); 171.1, 169.3 (MeCO); 149.4, 143.9 (C3, C10); 133.5 (d, $J=1.7$ Hz, C5); 128.0, 127.7, 120.5 (C1, 2, 4); 81.9 (C17); 45.9 (tm, $J=19.5$ Hz, C7); 43.7, 42.8 (two of C9, 13, 14); 38.9 (t, $J=1.7$ Hz, C8); 36.5, 29.7, 28.1, 27.2, 22.8 (C11, 12, 15, 16, and one of C9, 13, 14); 21.1, 21.0 ($\underline{C}H_3CO$); 11.2 (C18). ^{19}F NMR ($CDCl_3$) δ −81.4 (t, 3F, $J=10.0$ Hz, CF_3); −109.5 (d (AB), $J=15$ Hz, one of α-CF_2); −109.5 (d (AB), 1F, $J=15$ Hz, one of α-CF_2); −119.8 (dm (AB), 1F, $J=295$ Hz, one of β-CF_2); −121.3 (dm (AB), 1F, $J=290$ Hz, one of γ,δ-CF_2); −121.9 (dm (AB), 1F, $J=295$ Hz, one of β-CF_2); −122.7 (dm (AB), 1F, $J=295$ Hz, one of γ,δ-CF_2); −123.1 (dm (AB), 1F, $J=300$ Hz, one of γ,δ-CF_2); −123.9 (dm (AB), 1F, $J=300$ Hz, one of γ,δ-CF_2); −126.0 (dm (AB), 1F, $J=295$ Hz, one of ε-CF_2); −127.4 (dm (AB), 1F, $J=290$ Hz, one of ε-CF_2). I.R. (CCl_4, cm^{-1}) 1765 (ester C=O); 1754, 1153 (R_F).

7α - Perfluorohexyl - 1,3,5[10] - estratriene - 3,6,17β - triol 3,17-diacetate (4).

To a stirred solution of ketone **3a** (684 mg, 1.00 mmol) in THF/ethanol (10:1, 15 mL) at 0 °C was added sodium borohydride (128 mg, 3.38 mmol). After 30 min another portion of sodium borohydride (77.5 mg, 2.05 mmol) was added. The reaction was monitored closely by TLC, and 18 min after the second portion of hydride had been added, when a trace of starting material remained, the mixture was poured into ether (100 mL) and water (50 mL) and the aqueous layer separated. After further extraction with ether (2 × 50 mL), the combined organic phases were dried ($MgSO_4$) and evaporated under reduced pressure. Column chromatography (4–6% ether in CH_2C1_2) gave the *alcohol* **4** as a colorless oil. Yield 588 mg (0.85 mmol, 85%).

^{1}H NMR ($CDC1_3$) δ 7.24 (d, 1H, $J=2.5$ Hz, H4); 7.17 (d, 1H, $J=8.0$ Hz, H1); 6.99 (d, 1H, $J=2.5$ Hz, H2); 4.73 (dd, iH, $J=9.5$, 6.5 Hz, H17); 4.58 (br m, 1H, H6); 2.96 (br d, 1H, $J=20.0$ Hz, H7); 2.91 (d, 1H, $J=6.5$ Hz, OH); 2.40–1.20 (m, 17H, including 2.31 (s, 3-OAc), 2.08 (s, 17 OAc)); 0.88 (s, 3H 13-Me). ^{13}C NMR ($CDCl_3$) δ 171.4, 170.3 (MeCO); 149.5, 140.6, 136.2 (C3, 5, 10); 123.1, 119.5, 115.3 (Cl, 2, 4); 82.3 (C17); 66.7 (dd, $J=5.5$, 2.5 Hz, C6); 50.5 (C14); 45.7 (tm $J=19.0$ Hz, C7); 44.3 (C13); 41.1 (C9); 37.8 (dm $J=6.0$ Hz, C8); 36.6 (C12); 27.1 (C16); 24.5, C15); 23.5 (m, C11); 21.2, 21.1 ($\underline{C}H_3CO$); 12.2 (C18). ^{19}F NMR δ −81.3 (t, 3F $J=10.0$ Hz, CF_3); −104.0 (ddt (AB), 1F $J=290$, 10.0, 7.5 Hz, one of α-CF_2); −113.1 (dm (AB), 1F, $J=290$ Hz, one of α-CF_2); −118.6 (dm (AB), 1F, $J=290$ Hz, one of β-CF_2); −120.3 (dm (AB), 1F, $J=290$ Hz, one of β-CF_2); −121.6 (dm (AB), IF, $J=280$ Hz, one of γ,δ-CF_2); −122.5 (dm (AB), 1F, one of γ,δ-CF_2);−123.0 (dm (AB), 1F, one of γ,δ-CF_2); −123.8 (dm (AB), 1F, $J=305$ Hz, one of γ,δ-CF_2); −126.1 (dm (AB), 1F, $J=290$ Hz, one of ε-CF_2); −127.4 (dm (AB), 1F, $J=290$ Hz, one of ε-CF_2). MS (El) 690 (2%), 648 (100%). I.R. (CCl_4, cm^{-1}) ν 3529 (OH); 1743 (C=O).

6- [(Pentafluorophenoxythiocarbonyl)oxy]-7α-perfluorohexylestradiol diacetate (5). To a stirred solution of alcohol **4** (100 mg, 0.15 mmol) in CH_2C1_2 (2 mL) was added pyridine (36.4 μL, 35.5 mg, 0.45 mmol), 4-(*N*,*N*-dimethylamino)pyridine (55 mg, 0.45 mmol) and finally pentafluorophenoxy thiocarbonyl chloride (72 μL, 118 mg, 0.45 mmol). After 18 h, the mixture was poured into HC1 (2 M, 25 mL) and CH_2C1_2 (50 mL). The organic layer was washed with aqueous sodium hydrogencarbonate solution (satd, 20 mL), dried ($MgSO_4$) and evaporated under reduced pressure. Preparative scale tlc (20% ether in hexane, three elutions) gave the *thiocarbonate* **5** (59 mg, 0.06 mmol, 44%) as a colorless oil.

^{1}H NMR ($CDCl_3$) δ 7.30 (d, 1H, $J=8.5$ Hz, H1); 7.24 (d, 1H, $J=2.0$ Hz, H4); 7.12 (dd, 1H, $J=8.0$, 2.5 Hz, H2); 6.40–6.30 (dm, 1H, $J=5.5$ Hz, H6); 4.79 (dd, 1H, $J=9.5$, 7.0 Hz, H17); 3.62–3.45 (dm, lH, $J=25.0$ Hz, H7); 2.40–1.20 (m, 14H, including 2.34 (s, 3-OAc); 2.09 (s, 17-OAc)); 0.88 (s, 3H, 13-Me). ^{13}C NMR ($CDCl_3$) δ 191.1 (C=S); 171.1, 169.6 (MeCO); 149.6 (C3); 141.2 (dm, $J=249$ Hz, *ortho* CF); 140.8 (dm, $J=255$ Hz, *para* CF); 168.0 (dm, $J=255$ Hz, *meta* CF); 135.8, 134.4 (d, $J=1$ Hz (C5, 10); 127.3 (m, aromatic C–O–C=S); 123.8, 121.1, 115.5 (d, $J=1$ Hz, C1, 2, 4); 82.0 (C17); 79.0 (dd, $J=5.5$, 0.5 Hz, C6); 50.1 (C14); 44.5 (C13); 45.7 (dd, $J=22.0$, 19.0 Hz, C7); 41.5 (C9); 38.1 (d, $J=$ 5.5 *Hz,* C8); 36.4 (C12); 27.1 (C16); 24.3 (C15); 23.4 (d, $J=3$ Hz, C11); 21.1, 21.1 ($\underline{C}H_3CO$); 12.2 (C18). ^{19}F NMR ($CDCl_3$) δ −81.3 (t, 3F, $J=10.0$ Hz, CF_3); −104.7 (ddt (AB), 1F, $J=290$, 15.0 Hz, one of α-CF_2);−112.6 (dtd (AB), 1F, $J=290$, 23.0, 20.0 Hz, one of α-CF_2); −119.8 (dm (AB), 1F, $J=295$ Hz, one of β-CF_2); −121.5 (dm (AB), IF, $J=300$ Hz, one of γ-CF_2); −120.6–124.2 (m, 3F, one each of β,γ,δ-CF_2); −124.0 (dm (AB), 1F, $J=305$ Hz, one of δ-CF_2); −126.0 (dm (AB), 1F, $J=290$ Hz, one of ε-CF_2); −127.4 (dm (AB), 1F, $J=295$ Hz, one of ε-CF_2); −152.5 (d, 2F, $J=20.5$ Hz, *ortho*-F); −156.0 (t, 1F, $J=21.5$ Hz, *para*-F); −162.2 (d, 2F, $J=19.0$ Hz, *meta*-F). I.R. (CCl_4, cm^{-1}) ν 1785; 1765; 1729 (C=O); 1301 (aromatic C-F); 1164 (satd C-F).

Also, a faster running mixture, believed to consist of 7-perfluorohexyl-1,3,5[10],6-estratetraene-3,17β-diol diacetate and 7-perfluorohexyl-1,3,5[10],8[9]-estratetraene-3,17β-diol diacetate (40 mg, 0.06 mmol, 40%), as a colorless oil. MS (EI) 916 (2), 875 (80), 571 (100%). I.R. (CCl_4, cm^{-1}) ν 1785; 1765; 1729 (C=O); 1154 (satd C–F).

7α-Perfluorohexylestradiol diacetate (6). To a solution of thiocarbonate **5** (70 mg, 0.076 mmol) in benzene (6 mL) at reflux was added in l mL aliquots 9 min apart, 4 mL of a solution of AIBN (5.7 mg, 0.034 mmol) and tri-*n*-butyltin hydride (0.074 mL, 0.080 mg, 0.275 mmol) in benzene (12 mL). After the last addition, refluxing was continued for 30 min then four more aliquots added in the same fashion. After a total reaction time of 2 h the solution was cooled and evaporated. Preparative scale tlc (eluting with hexane then CH_2C1_2) gave the *diacetate* **6** as a colorless oil. Yield 40 mg (0.059 mmol, 85%).

^{1}H NMR ($CDC1_3$) δ 7.18 (d, 1H, $J=8.5$ Hz, H1); 6.96 (dd, 1H, $J=8.0$, 2.5 Hz, H2); 6.89 (d, 1H, $J=2.5$ Hz,

H4); 4.72 (dd, 1H, J= 9.5, 7.0 Hz, H17); 2.94 (d, 1H, J=14.0 Hz, one of H6); 2.84–2.64 (m, 2H, one of H6, H7); 2.30–1.30 (m, 14H, including 2.35 (s, 3-OAc); 2.04 (s, 17-OAc)); 0.88 (s, 3H, 13-Me). ^{13}C NMR ($CDCl_3$) δ: 171.1, 169.6 ($MeCO_2$); 148.9 (C3); 138.4, 137.3 (d, J=1.0 Hz (C5, 10); 123.5, 120.0 (d, J=1.2 Hz), 119.4 (C1, 2, 4); 82.3 (C17); 50.8 (C14); 44.0 (C13); 41.6 (C9); 39.3 (t, J=19.5 Hz, C7); 37.4 (d, J=2.8 Hz, C8); 36.5 (C12); 27.2 (m, C6); 27.2 (d, J=1.5 Hz, C16); 24.3 (C15); 23.7 (d, J=6.8 Hz, C11); 21.1, 21.0 (CH_3CO); 12.2 (C18). ^{19}F NMR ($CDCl_3$) δ 81.3 (t, 3F, J=10.0 Hz, CF_3); −106.7 (dm (AB), 1F, J=280 Hz, one of α-CF_2); −116.8 (dm (AB), 1F J=280 Hz, one of α-CF_2); −119.9 (dm (AB), 1F, J=295 Hz, one of β-CF_2); −121.1 (dm (AB), 1F, J=295 Hz, one of β-CF_2); −121.7 (dm (AB), 1F, J=305 Hz, one of γ,δ-CF_2); −122.4 (dm (AB), 1F, J=305 Hz, one of γ,δ-CF_2); −123.0 (dm (AB), 1F, J=305 Hz, one of γ,δ-CF_2); −123.9 (dm (AB), 1F, J=305 Hz, one of γ, δ-CF_2); −125.7 (dm (AB), 1F, J=295 Hz, one of ε-CF_2); −127.3 (dm (AB), 1F, J=295 Hz, one of ε-CF_2). I.R. (CCl_4, cm^{-1}) ν 1765; 1744 (C=O); 1729 (C=O); 1154 (sat. C-F).

7α-Perfluorohexylestradiol (7). To a stirred solution of the diacetate **6** (132 mg, 0.196 mmol) in methanol (6.6 mL) was added a solution of sodium hydroxide (0.66 g, 16.5 mmol) in water. The resulting suspension was stirred for 24 h, then poured into CH_2Cl_2 (200 mL) and HC1 (2 M, 70 mL). The layers were separated, and the aqueous phase extracted again with CH_2Cl_2 (2 × 100 mL). The combined organic layers were washed with brine (40 mL), dried ($MgSO_4$) and evaporated. Preparative scale tlc (eluting once with CH_2C1_2, then with 10% ether in CH_2C1_2) gave *perfluorohexylestradiol* **7** as a colorless oil. Yield 102 mg (0.172 mmol, 88%).

^{1}H NMR (acetone-d_6) δ 8.18 (br s, 1H, 3-OH); 7.02 (d, 1H, J=8.0 Hz, H1); 6.76–6.64 (m, 2H, H2, 4); 4.00–3.40 (m, 2H, H7, 17); 0.84 (s, 3H, 13-Me). ^{13}C NMR (acetone-d_6) δ 156.5 (C3); 138.3 (d, J=1 Hz), 135.2 (C5, C10); 124.1, 114.8 (br m), 113.7 (C1, 2, 4); 81.6 (C17); 51.4 (C14); 45.3 (C13); 42.2 (C9); 40.1 (t, J=19.5 Hz, C7); 39.2 (d, J=2.2 Hz, C8); 37.4, 30.4, 29.8, 27.7 (m), 24.4 (d, J=6.5 Hz) (C6, 11, 12, 15, 16); 11.7 (C18). ^{19}F NMR (acetone-d_6) δ −80.6 (t, 3F, J=10.0 Hz, CF_3); −104.9 (dm (AB), 1F, J=280 Hz, one of α-CF_2); −115.6 (dm (AB), 1F, J=275 Hz, one of α-CF_2); −118.8 (dm (AB), 1F, J=295 Hz, one of β-CF_2); −120.0 (dm (AB), 1F, J=295 Hz, one of β-CF_2); −120.6 (dm (AB), 1F, J=300 Hz, one of γ, δ-CF_2); −121.5 (dm (AB), 1F, J=295 Hz, one of γ, δ-CF_2); −122.2 (dm (AB), 1F, J=295 Hz, one of γ, δ-CF_2); −123.0 (dm (AB), 1F, J=300 Hz, one of γ, δ-CF_2); −125.0 (dm (AB), IF, J=300 Hz, one of ε-CF_2); −126.6 (dm (AB), IF, J=295 Hz, one of ε-CF_2). HRMS $C_{24}H_{23}O_2F_{13}$ (M^+) calculated 590.1490, observed 590.1497.

6-Oxo-7-trifluoromethylestradiol diacetate (8a and 8b). The silyl enol ether **2** (190 mg, 0.43 mmol) and pyridine (100 μL, 1.3 mmol) were dissolved in DMF (3.5 mL) in a quartz cuvette. Trifluoromethyl-benzothiophenium triflate (450 mg, 1.3 mmol) was added and the mixture subject to irradiation with a 245 nm high pressure Hg lamp for 3.5 h. The mixture was then allowed to cool, and was poured into EtOAc (50 mL). The EtOAc was extracted with sodium bicarbonate (5 × 10 mL), dried over $MgSO_4$ and the solvent removed in vacuo. Flash chromatography (SiO_2, 4:1 pentane/EtOAc) afforded *ketone* **8b** (73 mg, 0.17 mmol, 39%) then the *major isomer* **8a** (98 mg, 0.22 mmol, 52%).

6-Oxo-7β-trifluoromethylestradiol diacetate 8b. Mp: 146–148 °C, ^{1}H NMR ($CDCl_3$) δ 7.76 (d, 1H, J=2.6 Hz, H4), 7.48 (dd, 1H, J=7.9 Hz, H1), 7.30 (dd, 1H, J=8.5, 2.6 Hz, H2), 4.74 (dd, 1H, J=8.9, 8.2 Hz, H17), 3.27 (qd, 1H, J=9.8, 3.9 Hz, H7), 3.07 (br td, 1H, J=11.2, 5.6 Hz, H9), 2.46 (m, 1H, H11α), 2.31 (s, 3H, OAc), 3.38–2.20 (m, 3H, H8, 12, 16), 2.06 (s, 3H, OAc), 1.92 (dd,1H, J=9.2, 2.6 Hz, H11β), 1.96–1.78 (m, 2H, m, H14, 15), 1.68–1.32 (m, 3H, H12, 15, 16), 0.83, (s, 3H, 13-Me); ^{13}C NMR ($CDCl_3$) δ 190.0 (q, J=2.8 Hz, C6), 171.0 and 169.3 (2 × COMe), 149.3 (C), 144.1 (C), 132.4 (C), 127.9 and 127.9 (2 × CH_2), 125.1 (q, J=284 Hz, CF_3), 120.7 (CH), 81.8 (C17), 52.4 (q, J=24 Hz, C7), 45.5 (q, J=1.1 Hz, CH), 43.4 (C), 40.7 (CH), 37.9 (m, C9), 36.3 (CH), 27.2 (CH_2), 27.1 (CH_2), 22.8 (CH_2), 21.1 and 21.0 (2 × $COCH_3$), 11.4 (C18); ^{19}F NMR ($CDCl_3$) δ −60.4 (d, J=9.8 Hz, CF_3); IR (cm^{-1}, CCl_4): 2989 (m), 2941 (m), 1780 (s), 1748 (s), 1705 (s), 1497 (m), 1384 (s), 1283 (m), 1235 (s), 1165 (s), 1122 (s), 940 (m), 929 (m), 848 (m), 743 (m), 678 (w).

GCMS (El) 396 100%, (MH^+-CH_3CO), 438 8% (M^+). HRMS (DCI): $C_{23}H_{26}O_5F_3$ (MH^+) calculated 439.1732, observed 439.1728.

6-Oxo-7α-trifluoromethylestradiol diacetate 8a. Yellow oil, ^{1}H NMR ($CDCl_3$) δ 7.37–7.27 (m, 3H, H1, 2, 4), 4.70 (dd, 1H, J=9.2, 7.2 Hz, H17), 3.21 (qd, lH, J=9.5, 7.2 Hz, H7), 2.38 (td, 1H, J=11.5, 3.3 Hz), 2.32–2.10 (m, 3H), 2.38 (s, 3H, OAc), 2.05 (s, 3H, OAc), 1.97 (td, 1H, J=13.1, 3.0 Hz,), 1.81 (qd, 1H, J=12.8, 3.9 Hz,), 1.75–1.48 (m, 3H), 1.40 (td, 1H, J=11.8, 3.9 Hz), 0.92 (s, 3H, Me); ^{13}C NMR ($CDCl_3$) δ 192.4 (q, J=2.8 Hz, C6), 170.9 and 169.0 (2 × COMe), 149.5 (C), 140. (C), 135.6 (q, J=1.1 Hz, C), 127.0 (CH_2), 125.1 (CH_2), 118.9 (CH_2), 124.7 (q, J=280 Hz, CF_3), 81.7 (C17), 55.5 (q, J=24.0 Hz, C7), 53.4 (C13), 50.9 (CH), 43.8 (CH), 37.1 (CH), 36.4 (CH_2), 27.2 (CH_2), 24.1 (CH_2), 24.0 (q, J=3.5 Hz, CH_2), 21.0 and 20.9 (2 × $COCH_3$), 12.1 (C18); ^{19}F NMR ($CDC1_3$) δ −65.0 (d, J=9.5 Hz, CF_3); IR(CCl_4 cm^{-1}): 2925 (s), 2850 (s), 1780 (s), 1746 (s), 1700 (s), 1383 (s), 1256 (s), 1197 (s), 1122 (s), 1042 (s), 914 (s), 737 (s). GC–MS (EI) 396 100%, (MH^+-CH_3CO), 438 5% (M^+); HRMS (DCI): $C_{23}H_{26}O_5F_3$ (MH^+) calculated 439.1732, observed 439.1728.

7α-Trifluoromethylestradiol diacetate (9a). To a solution of the ketone **8a** (242 mg, 0.58 mmol) in CH_2C1_2 (15 mL), triethylsilane (1.20 mL, *7.5* mmol) and $BF_3{\cdot}OEt_2$ (4.0 mL, 31.0 mmol) were added in succession. The mixture was heated to reflux for 4 days. A further 0.50 mL of Et_3SiH and 3.8 mL of $BF_3{\cdot}OEt_2$ were added, and the mixture stirred for a further 3 days. The system was

quenched by pouring into a mixture of EtOAc (80 mL) and 5% sodium bicarbonate (30 mL). The layers were separated and the EtOAc layer was washed with water. The EtOAc was then dried over $MgSO_4$ and the solvent removed in vacuo to give an oil. The oil was purified by flash chromatography (SiO_2, 4:1 pentane/EtOAc) to afford the *diacetate* **9a** as an oil (118 mg, 0.34 mmol, 60%).

^{1}H NMR ($CDC1_3$) δ 7.20 (d, 1H, $J=7.9$ Hz), 6.94 (dd, 1H, $J=8.2$, 2.3 Hz), 6.90 (d, IH, $J=2.0$ Hz), 4.70 (dd, 1H, $J=5.5$, 7.2 Hz) 2.88 and 2.77, AB system (d, 1H, $J=14.8$ Hz) and (dd, 1H, $J=14.8$, 7.2 Hz), 2.60–2.45 (m, 1H) 2.41–1.20 (m, 10H), 1.82 (qd, 1H, $J=12.8$, 3.6 Hz), 2.27 (s, 3H, OAc), 2.05 (s, 3H, OAc), 0.85 (s, 3H, 13-Me);); ^{19}F ($CDCl_3$) δ −69.8 (d, $J=10.2$ Hz); IR (thin film, cm^{-1}): 2918 (s), 2851 (m), 1768 (m), 1740 (s), 1624 (w), 1605 (w), 1562 (w), 1499 (m), 1369 (m), 1153 (s), 1220 (s), 1249 (s), 1105 (s), 966 (w), 792 (m), 773 (m), 667 (w). GC–MS (El) 382 100%, (M^+-CH_3CO), 424 5% (M^+).

7α-Trifluoromethylestradiol (10a). To a solution of the diacetate **9a** (90 mg, 0.22 mmol) in a mixture of EtOH (3 mL) and water (2 mL), KOH (0.6 g, 11 mmol) was added. The mixture was stirred overnight, before pouring into a mixture of 2 M HC1 (10 mL) and EtOAc (50 mL). The layers were separated and the organic layer washed with water, dried over $MgSO_4$ and the solvent removed in vacuo. Preparative TLC (SiO_2, 2:1 pentane/EtOAc) afforded *trifluoromethylestradiol* **10a** as a foam (63 mg, 0.19 mmol, 86%). ^{1}H NMR ($CDCl_3$): 7.01 (d, 1H, $J=8.2$ Hz), 6.94 (dd, 1H, $J=8.2$, 2.3 Hz), 6.90 (d, 1H, $J=2.3$ Hz), 3.73 (dd, 1H, $J=8.5$, 7.9 Hz) 2.82 and 2.71 AB system (dd, 1H, $J=14.1$, 7.0 Hz) and (dd, 1H, $J=14.1$, 7.2 Hz), 2.20–1.95 (m, 7H), 1.81 (qd, 1H, $J=12.1$, 3.6 Hz), 1.70–1.41 (m, 3H), 0.81 (s, 3H, 13-Me); ^{13}C NMR ($CDC1_3$) δ 154.2 (C), 137.6 (C), 132.9 (C), 131.2 (q, $J=183$ Hz), 126.3 (CH), 114.3 (CH), 113.0 (CH), 81.7 (CH), 50.8 (CH), 44.2 (C), 41.6 (CH), 41.3 (q, $J=12.1$ Hz, CH), 38.4 (CH), 36.3 (CH_2), 30.0 (CH_2), 27.5 (CH_2), 24.4 (CH_2), 23.7 (CH_2), 11.2 (CH_3); ^{19}F ($CDCl_3$) δ −69.8 (d, $J=10.2$ Hz, CF_3); IR (thin film, cm^{-1}): 3389 (bs), 2922 (s), 2855 (m), 1567 (s), 1513 (m), 1451 (m), 1369 (w), 1263 (w), 1100 (s), 907 (w), 744 (s). HRMS $C_{19}H_{24}O_2F_3$ (MH^+) calculated 341.1728, observed 341.1733.

7β-Trifluoromethylestradiol diacetate (9b). Zinc amalgam was prepared by the addition of $HgC1_2$ (120 mg, 0.25 mmol) in concentrated HC1 (0.50 mL) to a suspension of 2 M HC1 washed zinc (0.50 g, 7.9 mmol) in water (5 mL). After 5 min, the aqueous phase was decanted and the amalgam washed with water (2 × 5 mL). The amalgam was used immediately.

To a suspension of the amalgam in ether (6 mL) at reflux, a solution of the ketone **8b** (30 mg, 0.068 mmol) in ether (2.0 mL) was added via syringe pump over 5 h. Concentrated HC1 (3.0 mL) was added in 0.2 mL portions concurrently. A further 0.3 g of zinc was added after 3.5 h. After being allowed to cool, the reaction was quenched by filtering into a separating funnel containing ether (20 mL) and saturated sodium bicarbonate (10 mL). The layers were separated and the ether layer was washed with water, dried over $MgSO_4$ and the solvent removed in vacuo. Purification by preparative TLC (SiO_2, 2:1 pentane/EtOAc) afforded the *diacetate* **9b** as an oil (12 mg, 0.028 mmol, 41%).

^{1}H NMR ($CDC1_3$): 7.28 (d, 1H, $J=8.5$ Hz, H1), 6.85 (dd, 1H, $J=8.5$, 2.4 Hz, H2), 6.78 (d, 1H, $J=2.4$ Hz, H4), 4.70 (dd, 1H, $J=8.5$, 7.7 Hz, H17) 3.14 and 3.03 AB system (dd, 1H, $J=18.2$, 7.0 Hz, H6) and (d, 1H, $J=18.2$ Hz, H6), 2.70–2.50 (m, 1H, H7), 2.41–1.20 (m, 11H), 2.26 (s, 3H, OAc), 2.04 (s, 3H, OAc), 0.83 (s, 3H, 13-Me); ^{13}C NMR ($CDCl_3$) δ 171.1 (COMe), 169.7 (COMe), 148.3 (C), 134.5 (C), 132.0 (C), 127.3 (CH), 121.3 (CH), 119.2 (CH), 82.3 (C17), 46.5 (CH), 43.4 (C13), 39.2 (q, $J=1.1$ Hz, CH), 38.3 (m, CH), 37.7 (q, $J=24$ Hz, C7), 36.5 (CH_2), 30.3 (q, $J=3.2$ Hz, CH_2), 27.7 (CH_2), 27.3 (CH_2), 23.0 (CH_2), 21.2 and 21.1 (COCH3), 11.5 (C18); ^{19}F NMR ($CDC1_3$) δ −64.7 (d, $J=10.7$ Hz, CF_3); GC–MS (EI) 382 100%, (M^+-CH_3CO), 424 5% (M^+).

7β-Trifluoromethylestradiol (10b). To a solution of diacetate **9b** (15 mg, 0.035 mmol) in a mixture of MeOH (1 mL) and water (1 mL), NaOH (100 mg, 2.5 mmol) was added. The mixture was stirred for 4 h, before pouring into a mixture of 2 M HCl (20 mL) and CH_2C1_2 (50 mL). The layers were separated and the organic layer washed with water, dried over $MgSO_4$ and the solvent removed in vacuo. Preparative TLC (SiO_2, 2:1 pentane/EtOAc) afforded *trifluoromethylestradiol* **10b** as a foam (10 mg, 0.029 mmol, 84%). ^{1}H NMR (THF-d_6) δ 7.16 (d, 1H, $J=8.5$ Hz, H1), 6.57 (dd, 1H, $J=8.5$, Hz, H2), 6.60 (d, lH, $J=$ Hz, H4), 3.76 (m, 1H, H17), 3.19 (dd, 1H, $J=17.5$, 6.2 Hz, H6), 2.99 (d, 1H, $J=17.5$ Hz, H6), 2.74–2.54 (m, 2H), 2.44 (m, 1H), 2.10–1.70 (m, 3H), 1.60–1.30 (m, 7H), 0.83 (s, 3H, 13-Me); ^{13}C NMR (THF-d_6) δ 156.3 (C), 134.9 (C), 130.7 (C), 127.4 (CH), 115.4 (CH), 113.9 (CH), 81.4 (C17), 47.9 (CH), 44.6 (C13), 40.9 (CH), 39.5 (CH), 38.5 (q, $J=24.3$ Hz, C7), 37.5 (CH_2), 31.0 (q, $J=2.8$ Hz, CH_2), 30.8 (CH_2), 29.0 (CH_2), 23.5 (CH_2), 10.9 (C 18); ^{19}F NMR (THF-d_6) δ −64.7 (d, $J=10.2$ Hz, CF_3); HRMS $C_{19}H_{24}O_2F_3$ (MH^+) calculated 341.1728, observed 341.1728.

Biological procedures

Assessment of estrogenicity. Potential binding to ER of fluorinated estrogen derivatives (**7** and **10a**) as well as associated transcriptional activity were assessed in MCF-7 breast cancer cells, according to experimental protocols previously reported.[32,33] Binding to ER was evaluated on living MCF-7 cells by a competitive assay using E_2 as a reference compound (whole cell assay).[32] Thus, MCF-7 cells were incubated for 1 h with 1 nM [^{3}H]E_2 and increasing amounts (1 nM–1 μM) of unlabeled E_2 or investigated fluorinated estrogens. Incorporated [^{3}H]E_2 was subsequently extracted with ethanol and measured by scintillation counting. Analysis of ligand binding by DCC assay was performed as described previously,[32] using recombinant human ERα produced in yeast (a kind gift from M. Carlquist, Karo-Bio,

Huddinge, Sweden). ERE-dependent transcriptional activity was assessed on MVLN cells[33] (pVit-tk-Luc reporter piasmid stably transfected in MCF-7 cells).[34] Cells were incubated overnight with the investigated estrogens at concentrations ranging from 0.1 nM to 1 μM; RU 58 668 at 0.1 μM was used as reference compounds. After extraction, luciferase activity was measured by luminometry.

The proliferative response of MCF-7 cells to E_2 and fluorinated estrogen derivatives was estimated as described in a previous publication.[35] Briefly, cells were plated at a density of 10^4 cells/cm^2 in 12-well dishes containing phenol red-free medium supplemented with 10% charcoal-stripped serum. One day after seeding, cell cultures were fed fresh medium containing E_2 or investigated estrogens at concentrations ranging from 1 pM to 0.1 μM. After 3 days of culture, cells were harvested by trypsinization and suspended in medium. Concentrations of cells in suspension were measured in an electronic cell counter.

Demonstration of estrogen receptor down regulation. Potential ER down regulation was analyzed by peroxidase-based immunocytochemistry (observation of diaminobenzidine staining by bright field microscopy)[28] or immunofluorescence (using Texas Red as a fluorochrome),[35] after incubation of MCF-7 cells in the presence of E_2 (1 nM) or investigated fluorinated estrogens (0.01–0.1 μM). ER level was also measured in cell extracts (0.5 M KC1) by enzyme immunoassays (ER-EIA, Abbott).[28]

Acknowledgements

Work of the Versailles group enters in the frame of the European TMR programme ERB FMRXCT970 120, entitled 'Fluorine As a Unique Tool for Engineering Molecular Properties'. Experiments conducted at the Institute Jules Bordet were supported by a grant from the 'Fonds Medic'. Guy Laurent is Senior Research Associate of the National Fund for Scientific Research (Belgium) and recipient of a grant (no. 3.4512.03) from the Belgian Fund for Medical Scientific Research.

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