Synthesis of 2,16α- and 4,16α-difluoroestradiols and their 11β-methoxy derivatives as potential estrogen receptor-binding radiopharmaceuticals

Yann Seimbille, Hasrat Ali and Johan E. van Lier *

Department of nuclear medicine and radiobiology, Faculty of Medicine, Université de Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4. E-mail: jvanlier@courrier.usherb.ca; Fax: +1 819 564 5442; Tel: +1 819 564 5409

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We prepared the 2,16α- and 4,16α-difluoroestradiols and their 11β-methoxy derivatives *via* two different pathways. The first route permits large scale synthesis and characterization of the final products while the second route was selected to allow for fluorination as a final step to facilitate labeling with the short-lived [^{18}F]fluorine. The former route involves successive electrophilic fluorinations of intermediate bistrimethylsilyl enol ethers and 16α-fluoroestrones followed by reduction of the 17-ketone and chromatographic separation of the isomeric products. The second route proceeds *via* electrophilic substitution of estrone or 11β-methoxyestrone with *N*-fluoropyridinium salt to give the 2- and 4-fluoro derivatives followed by conversion to the reactive 16β,17β-cyclic sulfates. Stereoselective opening of the cyclic sulfates *via* nucleophilic fluorination with Me_4NF and subsequent removal of the protecting ether and sulfate groups *via* rapid hydrolysis in acidic ethanol, gave the desired 16α-fluoro derivatives. The latter procedure is readily adapted for radiolabeling with ^{18}F by substituting Me_4NF for $^{18}F^-$ in acetonitrile. Preliminary biological data suggest that the addition of both a 4-fluoro and 11β-methoxy group onto 16α-[^{18}F]fluoroestradiol (FES) may provide an improved radiopharmaceutical for positron emission tomography (PET) imaging of estrogen receptor densities in breast cancer patients.

I Introduction

The determination of estrogen receptor (ER) levels in breast tumors plays a crucial role in the choice of an appropriate therapy, and provides important prognostic information.[1] ER concentrations in breast tumors are routinely determined *via* a biochemical assay of biopsy samples. This procedure is invasive and may introduce sampling heterogeneity problems.[2] Estrogens bind with high affinity and selectivity to the ER, providing a mechanism for the selective accumulation of radiolabeled analogs within ER-positive tumors. It has been recognized for over two decades that radiolabeled estrogens could be used for the non-invasive imaging and quantification of ER, providing a powerful tool to confirm or complement the information obtained from conventional diagnosis.[3]

A substantial number of radiolabeled estrogens have been evaluated in animal models, but only few derivatives have been advanced to a clinical setting. Among the latter 11β-methoxy-(17α,20*Z*)-[^{123}I]iodovinylestradiol (MIVE)[4] and 16α-[^{18}F]-fluoroestradiol (FES)[5] have been used successfully in breast cancer patients, using single photon emission computed tomography (SPECT) and positron emission tomography (PET) imaging, respectively. However both radiopharmaceuticals have their inconveniences, MIVE is prepared with the rather expensive ^{123}I isotope ($t_{1/2}$ = 13.2 h) using a relatively unstable stannyl vinyl precursor while FES requires the short-lived ^{18}F ($t_{1/2}$ = 110 min) and consequently the proximity of a cyclotron facility. Also, FES is readily converted to circulating radiometabolites preventing optimal localization at the ER-binding sites.[6] Several attempts have been performed to improve the tissue localization properties of FES *via* the addition of various substituents at different positions on the steroid skeleton.[7,8] A particularly promising analog appeared to be the 16β-[^{18}F]fluoromoxestrol (FMOX), a derivative of the very potent estrogen moxestrol (11β-methoxy-17α-ethynylestradiol).[9] However, in spite of high binding affinity for the ER, metabolic stability and very high *in vivo* uptake in ER-rich rat uteri, FMOX failed to detect ER-positive tumors in human patients.[10] This lack of localization was attributed to the loss of affinity of FMOX for the sex hormone binding globulin (SHBG), which is absent in the rat but abundantly present in humans. Binding to SHBG protects the steroid ligand from attack by metabolizing enzymes and may also facilitate its delivery to ER-rich target cells.

The above findings suggest that further modifications of FES to improve *in vivo* selectivity should aim at maintaining good binding to both the ER and SHBG, while reducing the rate of metabolism of the tracer. We showed previously that the addition of a 2- or 4-fluoro substituent onto radioiodinated estrogens slows down their metabolism and that particularly the 4-fluoro, either alone or in combination with an 11β-methoxy group, enhances ER-mediated uterine uptake in the rat.[11] Also, the 2-fluoro derivative of estradiol shows strong binding to SHBG and its 2-[^{18}F]fluoro analog has been proposed as an ER scanning agent.[12] However, [^{18}F]F^- incorporation into the electron-rich phenolic A-ring of estradiol to give the desired radiopharmaceutical proceeds in low radiochemical yield. On such accounts we have prepared the 2- and 4-fluoro derivatives of FES and 11β-OMe-FES and conducted preliminary studies on their interaction with ER and SHBG.

II Results and discussion

We describe two distinct approaches to synthesize the 2,16α- and 4,16α-difluoroestradiols and their 11β-methoxy derivatives. The more elaborate second pathway was developed to accommodate the preparation of the ^{18}F-labeled analogs.

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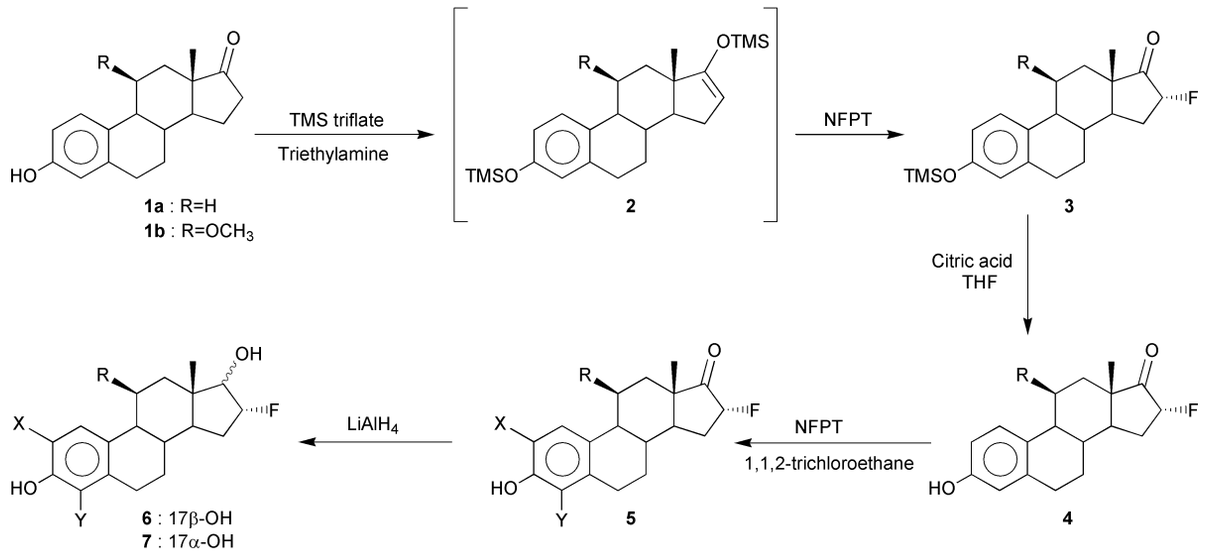


5 - 7	R	X	Y
a	H	F	H
b	H	H	F
c	OCH_3	F	H
d	OCH_3	H	F

Scheme 1

Method I. Synthesis of 2,16α-difluoroestradiol (6a), 4,16α-difluoroestradiol (6b), 2,16α-difluoro-11β-methoxyestradiol (6c) and 4,16α-difluoro-11β-methoxyestradiol (6d)

The 2- and 4-fluoro substituted 16α-fluoroestradiols (**6a** and **6b**) and their 11β-methoxy analogues (**6c** and **6d**) were synthesized from estrone (**1a**) and 11β-methoxyestrone (**1b**), respectively (Scheme 1). The 11β-methoxyestrone was prepared according to a route reported by Baran,[13] starting from Δ^1-adrenosterone. This synthetic pathway afforded the A-ring fluorinated 16α-fluoroestradiol derivatives **6a**–**d** in only five steps, representing a shorter and more convenient procedure as compared to the conventional method *via* F-substitution of the 16β-triflate† intermediates.[14,15]

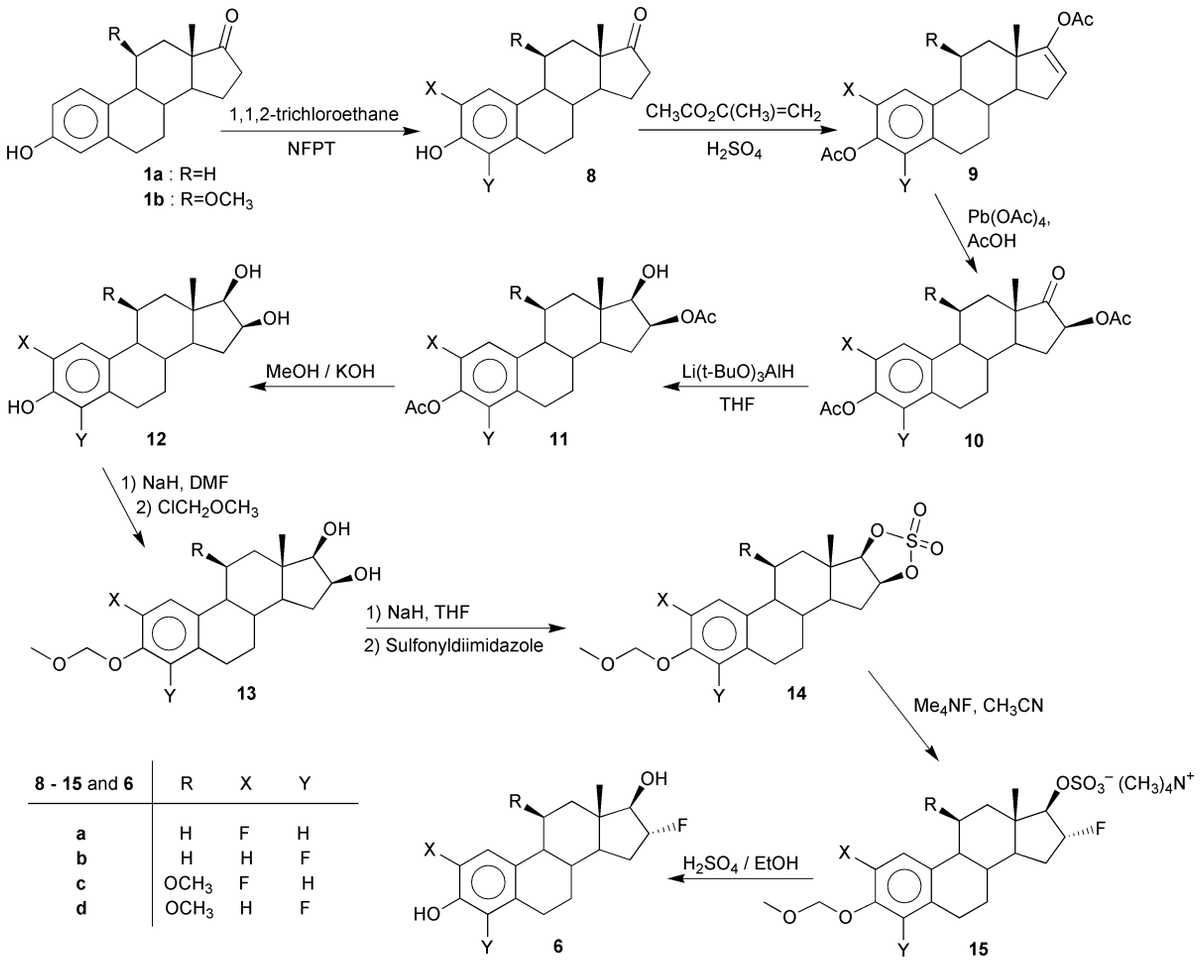
Estrone (**1a**) or 11β-methoxyestrone (**1b**) were converted to the 3,17-bis(trimethylsilyl) enol ether derivatives (**2a** or **2b**) by treatment with triethylamine and trimethylsilyl trifluoromethanesulfonate.[16,17] Electrophilic substitution of **2a** or **2b** with *N*-fluoropyridinium salt (NFPT) gave exclusively the 16α-fluoro derivatives (**3a** or **3b**). ^{1}H NMR spectra of **3a** and **3b** confirmed the stereospecificity of C-16 fluorination, *i.e.* a characteristic double doublet for 16β-H was observed at about 5 ppm, while the shielded double triplet for the 16α-H was missing in both spectra. The high stereoselectivity of the reaction reflects steric hindrance of fluorination from the β-side by the angular 18β-CH_3 group. Treatment of **3a** or **3b**, under mild acidic conditions, resulted in hydrolysis of the 3-OTMS ethers to yield **4a** or **4b**. The latter were converted to the 2- and 4-fluoro analogues **5a**–**b** or **5c**–**d**, *via* an electrophilic fluorination with NFPT conducted at a higher temperature than required for 16α-fluorination.[18,19] The mixed 17-keto compounds **5a**–**b** or **5c**–**d** were reduced with $LiAlH_4$ in THF at 0 °C to give a 17α/β epimeric mixture of 2,16α- or 4,16α-difluoroestra-3,17-diols (**6a**–**b** and **7a**–**b**), or their 11β-methoxy analogs (**6c**–**d** and **7c**–**d**).[20] The more polar 17β-OH compounds **6a**–**b** or **6c**–**d** were separated from the corresponding 17α-OH isomers **7a**–**b** or **7c**–**d** by flash chromatography. The stereochemistry of each mixture of separated products was confirmed by their characteristic signals in the ^{1}H NMR spectra. The 17β-OH configuration was assigned from the double doublet at 3.8 ppm (17α-H), while the 17α-OH isomers gave a doublet at 3.8 ppm (17β-H). Finally, the 2-fluoro compounds were separated from the corresponding 4-fluoro analogues by normal-phase HPLC to give, after recrystallisation, the single isomeric analytical samples.

Method II. Synthesis of the 2,16α- and 4,16α-difluoroestradiols (6a and 6b), and their 11β-methoxy derivatives 6c and 6d *via* the reactive 16β,17β-cyclic sulfate intermediates 14a–d

The above method I is not suitable for the preparation of the analogous 16α-[^{18}F]fluoro derivatives since the time required to complete the various reaction steps is incompatible with the short half-life of ^{18}F (110 min) (Scheme 2). Therefore we developed a second synthetic pathway *via* the key intermediate 16β,17β-cyclic sulfates, allowing for rapid and stereoselective incorporation of the 16α-fluoro substituent at the end of synthesis, using the method of Berridge.[21] This procedure was subsequently adapted for the preparation of the analogous [16α-^{18}F]**6a**–**d**.[22]

Electrophilic substitution of estrone (**1a**) or 11β-methoxyestrone (**1b**) with *N*-fluoropyridinium salt gave the 2- and 4-fluoro derivatives (**8a**–**d**). The latter were converted to the 3,17-enoldiacetates (**9a**–**d**) with isopropenyl acetate in the presence of acid catalyst.[11a] Treatment of **9a**–**d** with lead tetraacetate in acetic acid resulted in a rearrangement of the 17-enolacetate, to give exclusively the 3,16β-diacetate estrone derivatives (**10a**–**d**).[13] ^{1}H NMR spectra revealed the stereochemistry of the products, *i.e.* a characteristic triplet for the 16α-H at about 5 ppm *versus* a deshielded broad doublet for the 16β-H.[8] The 17-keto compounds **10a**–**d** were reduced with lithium tri-*tert*-butoxy aluminium hydride to yield the 17β-OH derivatives **11a**–**d**, followed by a base mediated hydrolysis to provide compounds **12a**–**d**. After protecting the 3-OH group as methoxymethyl (MOM) ethers,[23] *i.e.* compounds **13a**–**d**, the *cis*-configuration of the 16- and 17-OH groups was established by the formation of the 16β,17β-*O*-cyclic sulfate to give the key intermediates **14a**–**d**. These reactive intermediates were stereoselectively opened *via* a nucleophilic fluorination, under

† The IUPAC name for triflate is trifluoromethanesulfonate.



Scheme 2

anhydrous conditions, with Me_4NF to yield the 16α-fluoro derivatives **15a**–**d**.[24] Rapid hydrolysis of the protecting ether and sulfate groups was accomplished in ethanolic acid solution to give the difluoroestradiols **6a**–**d**. The stereochemistry of products **6a**–**d** was confirmed by their characteristic signals in the ^1H NMR spectra. The 16α-F,17β-OH configuration was assigned from the double doublet at 3.8 ppm (17α-H) and a double multiplet at 4.9 ppm (16β-H). This pattern is readily distinguishable from that of the 16α-F,17α-OH isomer, which gives a doublet at 3.8 ppm (17β-H) and a double multiplet at 5.2 ppm (16β-H). On the other hand, the 16β-F,17β-OH isomer is characterized by a double doublet at 3.4 ppm (17α-H) and a double multiplet at 5.0 ppm (16α-H).[17]

III Conclusion

Two strategies were investigated to synthesize A-ring fluorinated 16α-fluoroestradiol derivatives with potential application for breast cancer imaging. Only the second synthetic pathway, *via* the formation of the reactive intermediate 16β,17β-*O*-cyclic sulfates, allows for the synthesis of the radioactive 16α-^{18}F analogs of **6a**–**d**.[14] Opening of the *O*-cyclic sulfate is accomplished with Me_4NF to yield the non radioactive **6a**–**d**, while [^{18}F]fluoride in the presence of Kryptofix[2,2,2] is used to obtain the radioactive analogues. Both ring opening reactions proceed in a stereoselective manner such as to yield the desired 16α-F,17β-OH configuration only. This procedure is more convenient for radiochemical synthesis than the conventional route, where a nucleophilic fluorination by $nBu_4N[^{18}F]F$ is conducted on a 16α-OTf,17-keto intermediate. The latter requires the conversion of [^{18}F]fluoride to $nBu_4N[^{18}F]F$ and the separation of the epimeric 17-OH products resulting from the final reduction with $LiAlH_4$.[20,21] Typical radiochemical synthesis times for the preparation of [16α-^{18}F]-**6a**–**d** were about 2 h from the end of bombardment with decay corrected yields varying between 30–50% and specific activities >3 000 Ci mmol^{-1}.

Preliminary receptor binding studies with the non-radioactive **6a**–**f** showed that addition of 16α-F to estradiol to yield FES has little effect on the binding affinity to the ER but strongly reduces affinity for the SHBG receptor. Addition of a 4-F to FES to yield **6b** slightly diminishes binding to the ER without affecting the affinity for the SHBG receptor while further addition of the 11β-OMe to yield **6d** lowers the binding affinity for the ER almost 4-fold and reduces the affinity for the SHBG receptor to negligible. Surprisingly, under *in vivo* conditions, the radioactive 16α-^{18}F-analog of **6d** showed the highest ER-mediated uterus uptake in immature female rats among the series suggesting that this analog is a good candidate radiopharmaceutical for clinical PET imaging of ER-rich target tissues. Detailed biological studies on these compounds are in progress and will be reported separately.

Experimental

Analytical thin layer chromatography (TLC) was performed on Aldrich aluminium oxide on polyester plates or Macherey-Nagel silica gel pre-coated plastic sheets, both with fluorescent indicator (UV 254). Visualization was achieved with short-wave ultraviolet light and/or color response upon spraying with H_2SO_4–EtOH and heating at 120 °C. Column chromatography was performed using silica gel (60–200 mesh) or florisil (60–100 mesh). HPLC was performed with a Waters 600 system, using a 4 μm silica gel column (8 mm × 200 mm, Waters, 8NVSi 4 μm) or a 6 μm preparative silica gel column (7.8 mm × 300 mm, Waters, Nova-Pak HR Silica 6 μm). HPLC eluents were monitored for UV absorbance at 280 nm.

^{1}H NMR spectra were taken in chloroform-d or dimethyl sulfoxide-d_6, on a Bruker AC-300 spectrometer (at 300.13 MHz) using the residual chloroform proton as an internal standard. Chemical shifts are expressed in ppm (δ) relative to the standard and coupling constants (J) in Hz. Mass spectra were obtained on a Micromass Model ZAB-1F high-resolution mass spectrometer (HRMS). The relative intensity of the salient fragment ions to the base peak (100) is given in parentheses. Chemicals were obtained from the following sources and were used as received, unless otherwise noted: Aldrich, Sigma or Fisher. Melting points were measured on a Fisher-Johns apparatus and are not corrected.

3,17-Bis(trimethylsilyloxy)estra-1,3,5(10),16-tetraene (2a) or 11β-methoxy-3,17-bis(trimethylsilyloxy)estra-1,3,5(10),16-tetraene (2b)

Estrone (**1a**) (1 g, 3.7 mmol) or 11β-methoxyestrone (**1b**) (1.1 g, 3.7 mmol) and triethylamine (1.13 mL, 8.1 mmol) in dry toluene (10 mL) were stirred under nitrogen at room temperature with exclusion of light. Trimethylsilyl (TMS) triflate (1.57 mL, 8.14 mmol) was added, and the mixture was heated at reflux for 3 h. After cooling to room temperature the mixture separated into two layers, dry hexane (10 mL) was added, the upper hexane–toluene layer was collected and evaporated to dryness to provide the crude silyl enol ether **2** as a pale yellow solid.

16α-Fluoro-3-trimethylsilyloxyestra-1,3,5(10)-trien-17-one (3a) or 16α-fluoro-11β-methoxy-3-trimethylsilyloxyestra-1,3,5(10)-trien-17-one (3b)

NFPT (0.94 g, 3.8 mmol) and dry dichloromethane (10 mL) were added to the unpurified silyl enol ether **2**. The mixture was stirred at room temperature for 20 h under nitrogen, with exclusion of light, than poured into water and extracted with dichloromethane (3 × 30 mL). The pooled extracts were dried (Na_2SO_4), filtered and evaporated to dryness to give a pale yellow residue (1.25 g, 94% from **1a** or 1.15g, 80% from **1b**) consisting of **3** (90% by integral of **3a** (16β-H); 85% from **1a** or 73% by integral of **3b** (16β-H); 58% from **1b**) and the 3-TMS ether of **1**.

3a: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.25 (s, 9H, $(CH_3)_3Si$), 0.96 (s, 3H, 18-CH_3), 5.15 (dd, 1H, J = 50.6, 7.2 Hz, 16β-H), 6.58 (br s, 1H, 4-H), 6.63 (dd, 1H, J = 8.6, 1.8 Hz, 2-H), 7.12 (d, 1H, J = 8.2 Hz, 1-H); MS m/z (relative intensity) 360 (M^+, 100%), 288 (34), 218 (24), 146 (10); HRMS calcd for $C_{21}H_{29}O_2SiF$, 360.1921, found 360.1914.

3b: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (s, 9H, $(CH_3)_3Si$), 1.03 (s, 3H, 18-CH_3), 3.16 (s, 3H, 11β-OCH_3), 4.13 (m, 1H, 11α-H), 5.38 (dd, 1H, J = 50.5, 7.3 Hz, 16β-H), 6.42 (br s, 1H, 4-H), 6.50 (dd, 1H, J = 8.6, 2.3 Hz, 2-H), 6.94 (d, 1H, J = 8.6 Hz, 1-H); MS m/z (relative intensity) 390 (M^+, 2%), 318 (94), 286 (64), 146 (100); HRMS calcd for $C_{22}H_{31}O_3SiF$, 390.2026, found 390.2018.

16α-Fluoro-3-hydroxyestra-1,3,5(10)-trien-17-one (4a) or 16α-fluoro-3-hydroxy-11β-methoxyestra-1,3,5(10)-trien-17-one (4b)

The crude product **3a** or **3b** was dissolved in tetrahydrofuran (10 mL), and aqueous citric acid (2.5 mL, 100 mg mL^{-1}) was added. The mixture was stirred at room temperature for 2 h. The THF was evaporated, the residue was poured into water, and the product was extracted with dichloromethane (3 × 30 mL). The extract was dried (Na_2SO_4), filtered and evaporated to provide a pale yellow solid, which was subjected to chromatography (dichloromethane–EtOAc 10 : 0 to 9 : 1, silica gel) to give **4a** (0.83 g, 78% from **1a**) or **4b** (0.61 g, 52% from **1b**) as white solids.

4a: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (s, 3H, 18-CH_3), 5.34 (dd, 1H, J = 50.7, 6.6 Hz, 16β-H), 6.44 (d, 1H, J = 2.3 Hz, 4-H), 6.50 (dd, 1H, J = 8.3, 2.4 Hz, 2-H), 7.03 (d, 1H, J = 8.4 Hz, 1-H); MS m/z (relative intensity) 288 (M^+, 100%), 240 (15), 214 (32), 172 (30), 146 (27); HRMS calcd for $C_{18}H_{21}O_2F_1$, 288.1525, found 288.1531.

4b: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04 (s, 3H, 18-CH_3), 3.17 (s, 3H, 11β-OCH_3), 4.14 (m, 1H, 11α-H), 5.38 (dd, 1H, J = 50.5, 7.1 Hz, 16β-H), 6.42 (d, 1H, J = 2.5 Hz, 4-H), 6.51 (dd, 1H, J = 8.4, 2.5 Hz, 2-H), 6.94 (d, 1H, J = 8.5 Hz, 1-H); MS m/z (relative intensity) 318 (M^+, 52%), 259 (8), 244 (10), 212 (15), 186 (8), 146 (100); HRMS calcd for $C_{19}H_{23}O_3F_1$, 318.1631, found 318.1628.

2,16α-Difluoro- (5a) and 4,16α-difluoro-3-hydroxyestra-1,3,5(10)-trien-17-one (5b) or 2,16α-difluoro- (5c) and 4,16α-difluoro-3-hydroxy-11β-methoxyestra-1,3,5(10)-trien-17-one (5d)

A mixture of **4a** or **4b** (1.92 mmol), NFPT (0.95 g, 3.84 mmol, 2 eq.) and 1,1,2-trichloroethane (8 mL) was refluxed under nitrogen for 24 h. The solvent was removed under reduced pressure, the residue poured into water and extracted with dichloromethane. Evaporation of the dried (Na_2SO_4) extract yielded a brown oil which was submitted to flash-chromatography (CH_2Cl_2–EtOAc 10 : 0 to 9 : 1, silica gel). The two isomers co-eluted to give a pale yellow solid (0.39 g, 66% from **4a** and 60% from **4b**).

5a and **5b** as a mixture: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (s, 6H, 18-CH_3), 5.34 (dd, 2H, J = 50.6, 6.8 Hz, 16β-H), 6.60 (**5a**) (d, 1H, J = 9.3 Hz, 4-H), 6.62 (**5b**) (t, 1H, J = 8.8 Hz, 2-H), 6.87 (**5b**) (d, 1H, J = 8.6 Hz, 1-H), 6.87 (**5a**) (d, 1H, J = 13.3 Hz, 1-H); MS m/z (relative intensity) 306 (M^+, 100%), 232 (67), 190 (31), 177 (19); HRMS calcd for $C_{18}H_{20}O_2F_2$, 306.1431, found 306.1438.

5c and **5d** as a mixture: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04 (s, 6H, 18-CH_3), 3.16 (s, 3H, 11β-OCH_3), 3.18 (s, 3H, 11β-OCH_3), 4.13 (m, 2H, 11α-H), 5.38 (dd, 2H, J = 49.6, 7.5 Hz, 16β-H), 6.58 (**5c**) (d, 1H, J = 9.4 Hz, C4-H), 6.71 (**5d**) (t, 1H, J = 8.8 Hz, C2-H), 6.78 (**5d**) (d, 1H, J = 8.7 Hz, C1-H), 6.87 (**5c**) (d, 1H, J = 13.5 Hz, C1-H); MS m/z (relative intensity) 336 (M^+, 74%), 316 (81), 276 (20), 242 (63), 164 (100); HRMS calcd for $C_{19}H_{22}O_3F_2$, 336.1537, found 336.1544.

2,16α-Difluoro- (6a) and 4,16α-difluoroestra-1,3,5(10)-triene-3,17β-diol (6b) or 2,16α-difluoro- (6c) and 4,16α-difluoro-11β-methoxyestra-1,3,5(10)-triene-3,17β-diol (6d)

Compound **5** (1 mmol) was dissolved in tetrahydrofuran (12 mL), and cooled to 0 °C in an ice bath. To the chilled solution was added dropwise a solution of $LiAlH_4$ in tetrahydrofuran (1.12 M, 1.8 mL, 2 mmol). The mixture was stirred at 0 °C for 40 min. An aqueous solution of hydrochloric acid (10%) was slowly added to dissolve all aluminium salts. Product isolation (EtOAc) gave a solid shown to be a two-component mixture (0.28 g, 85%, 2 : 1 of 17β- to 17α-epimer). The more polar 17β-OH compounds **6a**–**b** or **6c**–**d** were separated from the corresponding 17α-OH analogues **7a**–**b** or **7c**–**d** by flash chromatography (CH_2Cl_2–EtOAc 10 : 0 to 9 : 1, silica gel). Products were separated by HPLC (Waters Nova-Pak HR Silica 6-μm, 15% EtOAc in hexane; 2 mL min^{-1}) to yield **6a** (144 mg, t_R = 21 min) and **6b** (96 mg, t_R = 25 min), or using 35% EtOAc in hexane, to give **6c** (138 mg, t_R = 12 min) and **6d** (115 mg, t_R = 15 min). Recrystallization from EtOH provided the analytical samples as white powders.

6a: mp 185 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.80 (s, 3H, 18-CH_3), 3.86 (dd, 1H, J = 4.6, 28.5 Hz, 17α-H), 4.95 (dm, 1H, J = 51 Hz, 16β-H), 6.70 (d, 1H, J = 9.1 Hz, 4-H), 6.96 (d, 1H, J = 12.5 Hz, 1-H); MS m/z (relative intensity) 308 (M^+, 100%), 231 (25), 190 (14), 164 (14); HRMS calcd for $C_{18}H_{22}O_2F_2$, 308.1588, found 308.1591.

6b: mp 191 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.80 (s, 3H, 18-CH_3), 3.86 (dd, 1H, J = 4.6, 28.5 Hz, 17α-H), 4.98 (dm, 1H, J = 51 Hz, 16β-H), 6.81 (t, 1H, J = 8.8 Hz, 2-H), 6.94 (d, 1H, J = 8.6 Hz, 1-H); MS m/z (relative intensity) 308 (M^+, 100%), 231 (24), 190 (13), 164 (11); HRMS calcd for $C_{18}H_{22}O_2F_2$, 308.1588, found 308.1591.

6c: mp 224 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (s, 3H, 18-CH_3), 3.31 (s, 3H, 11β-OCH_3), 3.82 (dd, 1H, J = 4.5, 27.0 Hz, 17α-H), 4.06 (m, 1H, 11α-H), 4.96 (dm, 1H, J = 54 Hz, 16β-H), 6.66 (d, 1H, J = 9.2 Hz, 4-H), 6.80 (d, 1H, J = 12.5 Hz, 1-H); MS m/z (relative intensity) 338 (M^+, 100%), 261 (8), 190 (36), 164 (40); HRMS calcd for $C_{19}H_{24}O_3F_2$, 338.1693, found 338.1698.

6d: mp 246 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (s, 3H, 18-CH_3), 3.28 (s, 3H, 11β-OCH_3), 3.85 (dm, 1H, J = 30 Hz, 17α-H), 4.13 (m, 1H, 11α-H), 4.96 (dm, 1H, J = 55 Hz, 16β-H), 6.75–6.95 (m, 2H, aromatic-H); MS m/z (relative intensity) 338 (M^+, 100%), 261 (8), 190 (42), 164 (44); HRMS calcd for $C_{19}H_{24}O_3F_2$, 338.1693, found 338.1698.

2-Fluoro- (8a) and 4-fluoro-3-hydroxyestra-1,3,5(10)-trien-17-one (8b) or 2-fluoro- (8c) and 4-fluoro-3-hydroxy-11β-methoxyestra-1,3,5(10)-trien-17-one (8d)

Estrone (**1a**) (1 g, 3.7 mmol) or 11β-methoxyestrone (**1b**) (1.1 g, 3.7 mmol) and *N*-fluoropyridinium triflate (1.83 g, 7.4 mmol, 2 eq.) in 1,1,2-trichloroethane (16 mL) were refluxed under nitrogen for 24 hours. The solvent was removed under reduced pressure, and the residue was poured into water and extracted with CH_2Cl_2. Evaporation of the dried (Na_2SO_4) extract yielded a brown oil, which was purified by column chromatography using CH_2Cl_2–EtOAc (10 : 0 to 9 : 1) as eluent. The two isomers co-eluted and were recovered as a pale yellow solid (0.70 g, 66% from **1a** and 60% from **1b**).

8a and **8b** as a mixture: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.80 (s, 6H, 18-CH_3), 6.60 (**8a**) (d, 1H, J = 9.3 Hz, C4-H), 6.70 (**8b**) (t, 1H, J = 8.9 Hz, C2-H), 6.88 (**8b**) (d, 1H, J = 8.6 Hz, C1-H), 6.96 (**8a**) (d, 1H, J = 13.2 Hz, C1-H); MS m/z (relative intensity) 288 (M^+, 100%), 244 (14), 231 (27), 203 (12), 190 (20); HRMS calcd for $C_{18}H_{21}O_2F$, 288.1525, found 288.1531.

8c and **8d** as a mixture: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (s, 6H, 18-CH_3), 3.16 (s, 3H, 11β-OCH_3), 3.18 (s, 3H, 11β-OCH_3), 4.12 (m, 2H, 11α-H), 6.58 (**8c**) (d, 1H, J = 9.3 Hz, C4-H), 6.72 (**8d**) (t, 1H, J = 8.8 Hz, C2-H), 6.78 (**8d**) (d, 1H, J = 8.7 Hz, C1-H), 6.87 (**8c**) (d, 1H, J = 13.5 Hz, C1-H); MS m/z (relative intensity) 318 (M^+, 100%), 286 (17), 259 (40), 188 (33), 164 (97); HRMS calcd for $C_{19}H_{23}O_3F$, 318.1631, found 318.1628.

3,17-Diacetoxy-2-fluoro- (9a) and 3,17-diacetoxy-4-fluoroestra-1,3,5(10),16-tetraene (9b) or 3,17-diacetoxy-2-fluoro- (9c) and 3,17-diacetoxy-4-fluoro-11β-methoxyestra-1,3,5(10),16-tetraene (9d)

A mixture of (0.7 g) **8a** and **8b** (or **8c** and **8d**) in isopropenyl acetate (7 mL) and catalyst solution (0.3 mL), prepared by mixing isopropenyl acetate (4 mL) and H_2SO_4 (0.1 mL), was refluxed for 2 h. Approximately 1 mL of the solvent was slowly distilled over a period of 1 h. An additional 2 mL of isopropenyl acetate and 0.1 mL of catalyst were added and the solution was concentrated to half the volume by slow distillation for 1 h. The solution was chilled and EtOAc was added. The EtOAc solution was washed with ice-chilled sodium bicarbonate (5%) in water and dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The residue was purified on a column of florisil (hexane–EtOAc, 10 : 0 to 9 : 1) to yield a mixture of **9a** and **9b** (or **9c** and **9d**) as white solids.

9a and **9b** as a mixture: ^{1}H NMR (300 MHz, d_6-DMSO) δ 0.85 (s, 6H, 18-CH_3), 2.14 (s, 6H, 3-$OCOCH_3$), 2.28 (s, 3H, 17-$OCOCH_3$), 2.29 (s, 3H, 17-$OCOCH_3$), 5.41 (m, 2H, 16-H), 6.95 (**9a**) (d, 1H, J = 8.2 Hz, C4-H), 7.0 (**9b**) (t, 1H, J = 8.2 Hz, C2-H), 7.10 (**9b**) (d, 1H, J = 8.2 Hz, C1-H), 7.18 (**9a**) (d, 1H, J = 12.4 Hz, C1-H); MS m/z (relative intensity) 372 (M^+, 4%), 330 ($M^+ - CH_2CO$, 32), 288 ($M^+ - 2\ CH_2CO$, 100); HRMS calcd for $C_{22}H_{25}O_4F$, 372.1737, found 372.1740.

9c and **9d** as a mixture: ^{1}H NMR (300 MHz, d_6-DMSO) δ 1.06 (s, 6H, 18-CH_3), 2.16 (s, 6H, 3-$OCOCH_3$), 2.28 (s, 6H, 17-$OCOCH_3$), 3.17 (s, 3H, 11β-OCH_3), 3.20 (s, 3H, 11β-OCH_3), 4.18 (m, 2H, 11α-H), 5.38 (br s, 2H, 16-H), 6.90–7.07 (m, 4H, aromatic-H); MS m/z (relative intensity) 402 (M^+, 9%), 360 ($M^+ - CH_2CO$, 52), 318 ($M^+ - 2\ CH_2CO$, 55), 300 (18), 164 (100); HRMS calcd for $C_{23}H_{27}O_5F$, 402.1842, found 402.1851.

3,16β-Diacetoxy-2-fluoro- (10a) and 3,16β-diacetoxy-4-fluoroestra-1,3,5(10)-trien-17-one (10b) or 3,16β-diacetoxy-2-fluoro- (10c) and 3,16β-diacetoxy-4-fluoro-11β-methoxyestra-1,3,5(10)-trien-17-one (10d)

A mixture of **9a** and **9b** (or **9c** and **9d**) (1.25 mmol), lead tetraacetate (0.75 g) and AcOH (6.5 mL) was stirred for 2.5 h. Then 0.10 g of $Pb(OAc)_4$ was added and the mixture was stirred for another 1 h. The reaction mixture was diluted with $CHCl_3$ (35 ml), washed (2 × 20 mL aqueous 5% sodium thiosulfate; 4 × 75 mL saturated aqueous $NaHCO_3$), then dried over anhydrous Na_2SO_4 and distilled to dryness. The crude product was subjected to chromatography (hexane–EtOAc 10 : 0 to 9 : 1, florisil) to give mixtures of **10a** and **10b** (0.36 g, 75%) or **10c** and **10d** (0.34 g, 65%) as white solids.

10a and **10b** as a mixture: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (s, 6H, 18-CH_3), 2.06 (s, 6H, 3-$OCOCH_3$), 2.28 (s, 3H, 16-$OCOCH_3$), 2.29 (s, 3H, 16-$OCOCH_3$), 5.09 (t, 1H, J = 8.8 Hz, 16-H), 5.10 (t, 1H, J = 8.8 Hz, 16-H), 6.95 (**10a**) (d, 1H, J = 8.1 Hz, C4-H), 7.02 (**10b**) (t, 1H, J = 8.1 Hz, C2-H), 7.14 (**10b**) (d, 1H, J = 8.6 Hz, C1-H), 7.22 (**10a**) (d, 1H, J = 12.4 Hz, C1-H); MS m/z (relative intensity) 388 (M^+, 3%), 346 (100), 232 (62); HRMS calcd for $C_{22}H_{25}O_5F$, 388.1686, found 388.1681.

10c and **10d** as a mixture: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04 (s, 6H, 18-CH_3), 2.06 (s, 6H, 3-$OCOCH_3$), 2.28 (s, 3H, 16-$OCOCH_3$), 2.29 (s, 3H, 16-$OCOCH_3$), 3.20 (s, 3H, 11β-OCH_3), 3.22 (s, 3H, 11β-OCH_3), 4.23 (m, 2H, 11α-H), 5.06 (t, 1H, J = 8.8 Hz, 16-H), 5.07 (t, 1H, J = 8.8 Hz, 16-H), 6.90–7.17 (m, 4H, aromatic-H); MS m/z (relative intensity) 418 (M^+, 22%), 376 (100), 304 (8), 262 (6); HRMS calcd for $C_{23}H_{27}O_6F$, 418.1792, found 418.1782.

3,16β-Diacetoxy-2-fluoro- (11a) and 3,16β-diacetoxy-4-fluoroestra-1,3,5(10)-trien-17β-ol (11b) or 3,16β-diacetoxy-2-fluoro- (11c) and 3,16β-diacetoxy-4-fluoro-11β-methoxyestra-1,3,5(10)-trien-17β-ol (11d)

A solution of the mixture of **10a** and **10b** (or **10c** and **10d**) (0.81 mmol), lithium tri-*tert*-butoxyaluminium hydride (0.7 g), and THF (20 mL) was stirred for 1 h and then poured with stirring into a mixture of ice (35 g), H_2O (35 mL), and AcOH (5 mL). The mixture was extracted with $CHCl_3$, washed (3 × 80 mL H_2O; saturated aqueous $NaHCO_3$), dried (Na_2SO_4), and distilled to dryness. Chromatography (CH_2Cl_2–EtOAc, 10 : 0 to 8 : 2, SiO_2) afforded mixtures of **11a** and **11b** (275 mg, 86%) or **11c** and **11d** (300 mg, 88%) as white solids.

11a and **11b** as a mixture: ^{1}H NMR (300 MHz, d_6-DMSO) δ 0.76 (s, 6H, 18-CH_3), 1.97 (s, 6H, 3-$OCOCH_3$), 2.28 (s, 3H, 16-$OCOCH_3$), 2.29 (s, 3H, 16-$OCOCH_3$), 4.22 (t, 1H, J = 6.5 Hz, 17-H), 4.65 (t, 1H, J = 6.5 Hz, 17-H), 5.08 (m, 2H, 16-H), 6.93 (**11a**) (d, 1H, J = 8.1 Hz, C4-H), 7.00 (**11b**) (t, 1H, J = 8.1 Hz, C2-H), 7.11 (**11b**) (d, 1H, J = 8.6 Hz, C1-H), 7.19 (**11a**) (d, 1H, J = 12.5 Hz, C1-H); MS m/z (relative intensity) 390 (M^+, 5%), 348 (100), 288 (5), 273 (15), 231 (22); HRMS calcd for $C_{22}H_{27}O_5F$, 390.1842, found 350.1850.

11c and **11d** as a mixture: ^{1}H NMR (300 MHz, d_6-DMSO) δ 0.90 (s, 6H, 18-CH_3), 1.97 (s, 6H, 3-$OCOCH_3$), 2.28 (s, 6H, 16-$OCOCH_3$), 3.14 (s, 3H, 11β-OCH_3), 3.16 (s, 3H, 11β-OCH_3), 4.03 (m, 2H, 11α-H), 4.19 (t, 1H, J = 6.5 Hz, 17-H), 4.61 (t, 1H, J = 6.7 Hz, 17-H), 5.05 (m, 2H, 16-H), 6.50–6.90 (m, 4H, aromatic-H); MS m/z (relative intensity) 420 (M^+, 14%), 378 (100), 318 (16), 286 (23); HRMS calcd for $C_{23}H_{29}O_6F$, 420.1948, found 420.1960.

2-Fluoro- (12a) and 4-fluoroestra-1,3,5(10)-triene-3,16β,17β-triol (12b) or 2-fluoro- (12c) and 4-fluoro-11β-methoxyestra-1,3,5(10)-triene-3,16β,17β-triol (12d)

A mixture of compounds **11a** and **11b** (or **11c** and **11d**) (0.7 mmol) was dissolved in MeOH (8 mL), diluted with 15 ml of a solution of KOH (4 g) in MeOH (25 mL) and H_2O (20 mL), and kept on the steam bath for 2 h under N_2. The solution was concentrated to half the volume and then acidified to about pH 1 with 4 M HCl. The crude, crystalline product was collected by filtration and washed with H_2O. Then, the white solids were dried carefully to afford a mixture of **12a** and **12b** (200 mg, 93%) or **12c** and **12d** (211 mg, 90%).

12a and **12b** as a mixture: ^{1}H NMR (300 MHz, d_6-DMSO) δ 0.71 (s, 6H, 18-CH_3), 3.95 (m, 2H, 16-H), 4.12 (d, 1H, J = 7.1 Hz, 17-H), 4.52 (m, 1H, 17-H), 6.58 (**12a**) (d, 1H, J = 9.3 Hz, C4-H), 6.70 (**12b**) (t, 1H, J = 8.9 Hz, C2-H), 6.85 (**12b**) (d, 1H, J = 8.6 Hz, C1-H), 6.94 (**12a**) (d, 1H, J = 13.3 Hz, C1-H); MS *m*/*z* (relative intensity) 306 (M^+, 100%), 231 (32), 164 (10); HRMS calcd for $C_{18}H_{23}O_3F$, 306.1631, found 306.1636.

12c and **12d** as a mixture: ^{1}H NMR (300 MHz, d_6-DMSO) δ 0.87 (s, 6H, 18-CH_3), 3.13 (s, 3H, 11β-OCH_3), 3.15 (s, 3H, 11β-OCH_3), 3.94 (m, 2H, 16-H), 4.04 (m, 2H, 11α-H), 4.10 (d, 1H, J = 7.2 Hz, 17-H), 4.60 (m, 1H, 17-H), 6.50–6.90 (m, 4H, aromatic-H); MS *m*/*z* (relative intensity) 336 (M^+, 100%), 286 (5), 164 (55); HRMS calcd for $C_{19}H_{25}O_4F$, 336.1737, found 336.1743.

2-Fluoro- (13a) and 4-fluoro-3-*O*-methoxymethylestra-1,3,5(10)-triene-3,16β,17β-triol (13b) or 2-fluoro- (13c) and 4-fluoro-11β-methoxy-3-*O*-methoxymethylestra-1,3,5(10)-triene-3,16β,17β-triol (13d)

A mixture of **12a** and **12b** (or **12c** and **12d**) (0.6 mmol), DMF (anhydrous, 4 mL) and a magnetic stirrer were placed in a bulb. After adding NaH (60% suspension in mineral oil, 0.84 mmol, 34 mg), the suspension was stirred and a solution of methoxymethyl chloride (67 μL, 0.84 mmol) in DMF (0.2 mL) was added dropwise. After the suspension had been stirred for 1 h, EtOH (abs., 5 mL) was added. The solvent was removed *in vacuo*, and the residue extracted with ether. The ether extract was washed with water, dried (Na_2SO_4), and evaporated to yield **13a** and **13b** (95%, 200 mg) and **13c** and **13d** (216 mg).

13a and **13b** as a mixture: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (s, 6H, 18-CH_3), 3.42 (m, 2H, 17-H), 3.52 (s, 6H, 3-OCH_2–OCH_3), 4.23 (m, 2H, 16-H), 5.17 (s, 2H, 3-OCH_2–), 5.18 (s, 2H, 3-OCH_2–), 6.85–7.05 (m, 4H, aromatic-H); MS *m*/*z* (relative intensity) 350 (M^+, 100%), 320 (93), 245 (21), 192 (21); HRMS calcd for $C_{20}H_{27}O_4F$, 350.1893, found 350.1897.

13c and **13d** as a mixture: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.03 (s, 6H, 18-CH_3), 3.28 (s, 3H, 11β-OCH_3), 3.30 (s, 3H, 11β-OCH_3), 3.43 (d, 2H, 17-H), 3.50 (s, 6H, 3-OCH_2–OCH_3), 4.05 (m, 1H, 11α-H), 4.13 (m, 1H, 11α-H), 4.22 (m, 2H, 16-H), 5.16 (s, 2H, 3-OCH_2–), 5.17 (s, 2H, 3-OCH_2–), 6.8–7.1 (m, 4H, aromatic-H); MS *m*/*z* (relative intensity) 380 (M^+, 100%), 264 (11), 234 (12); HRMS calcd for $C_{21}H_{29}O_5F$, 380.1999, found 380.1996.

2-Fluoro- (14a) and 4-fluoro-3-*O*-methoxymethyl-16β,17β-*O*-sulfonylestra-1,3,5(10)-triene-3,16β,17β-triol (14b) or 2-fluoro- (14c) and 4-fluoro-11β-methoxy-3-*O*-methoxymethyl-16β,17β-*O*-sulfonylestra-1,3,5(10)-triene-3,16β,17β-triol (14d)

In a bulb fitted with a magnetic stirrer, **13a** and **13b** (or **13c** and **13d**) (0.57 mmol) was dissolved in anhydrous THF (8 mL) and NaH (60% suspension in mineral oil, 2.3 mmol, 91 mg) was added while stirring. After 10 min a solution of sulfonyldiimidazole (0.66 mmol, 130 mg) in anhydrous THF (2.5 mL) was added dropwise and stirring was continued. After 1 h the solution was filtered and evaporated. The residue was extracted with ether, washed with water and dried (Na_2SO_4). Upon evaporation of the ether, **14a** and **14b** (85%, 200 mg) (or **14c** and **14d**; 214 mg) were obtained as fine crystals. Products were separated by HPLC (Waters 8NVSi 4-μm, 15% EtOAc in hexane; 2 mL min^{-1}) to yield **14a** (108 mg, t_R = 20 min) and **14b** (72 mg, t_R = 15 min), or using 18% EtOAc in hexane, to give **14c** (105 mg, t_R = 20 min) and **14d** (88 mg, t_R = 23 min). Recrystallization from EtOH provided the analytical samples as white powders.

14a: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01 (s, 3H, 18-CH_3), 3.51 (s, 3H, 3-OCH_2–OCH_3), 4.59 (d, 1H, J = 7.5 Hz, 17-H), 5.16 (m, 1H, 16-H), 5.17 (s, 2H, 3-OCH_2–), 6.89 (d, 1H, J = 8.6 Hz, 4-H), 6.97 (d, 1H, J = 12.9 Hz, 1-H); MS *m*/*z* (relative intensity) 412 (M^+, 75%), 382 (100); HRMS calcd for $C_{20}H_{25}O_6SF$, 412.1356, found 412.1348.

14b: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01 (s, 3H, 18-CH_3), 3.51 (s, 3H, 3-OCH_2–OCH_3), 4.59 (d, 1H, J = 7.5 Hz, 17-H), 5.17 (m, 1H, 16-H), 5.18 (s, 2H, 3-OCH_2–), 6.90–7.05 (m, 2H, aromatic-H); MS *m*/*z* (relative intensity) 412 (M^+, 65%), 382 (100); HMRS calcd for $C_{20}H_{25}O_6SF$, 412.1356, found 412.1348.

14c: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (s, 3H, 18-CH_3), 3.31 (s, 3H, 11β-OCH_3), 3.50 (s, 3H, 3-OCH_2–OCH_3), 4.07 (m, 1H, 11α-H), 4.55 (d, 1H, J = 7.5 Hz, 17-H), 5.14 (m, 1H, 16-H), 5.17 (s, 2H, 3-OCH_2–), 6.84 (d, 1H, J = 13.0 Hz, 1-H), 6.89 (d, 1H, J = 8.6 Hz, 4-H); MS *m*/*z* (relative intensity) 442 (M^+, 100%), 380 (25); HRMS calcd for $C_{21}H_{27}O_7SF$, 442.1461, found 442.1454.

14d: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (s, 3H, 18-CH_3), 3.30 (s, 3H, 11β-OCH_3), 3.51 (s, 3H, 3-OCH_2–OCH_3), 4.16 (m, 1H, 11α-H), 4.55 (d, 1H, J = 7.6 Hz, 17-H), 5.14 (m, 1H, 16-H), 5.18 (s, 2H, 3-OCH_2–), 6.83 (d, 1H, J = 8.8 Hz, 1-H), 6.89 (t, 1H, J = 8.6 Hz, 2-H); MS *m*/*z* (relative intensity) 442 (M^+, 100%), 380 (25); HRMS calcd for $C_{21}H_{27}O_7SF$, 442.1461, found 442.1454.

Tetramethylammonium 2,16α-difluoro- (15a) or 4,16α-difluoro-3-*O*-methoxymethyl-3-hydroxyestra-1,3,5(10)-trien-17β-yl sulfate (15b)

Tetramethylammonium fluoride tetrahydrate (19 mg) was carefully dried by azeotropic distillation of acetonitrile (3 × 2.5 mL). A solution of compound **14a** or **14b** (35 mg) in absolute MeCN (3.5 mL) was added and refluxed under dry nitrogen for 15 min. The solvent was removed under reduced pressure to yield **15a** or **15b** (89%, 38 mg) as Me_4N^+ salts.

15a: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.68 (s, 3H, 18-CH_3), 3.09 (s, 12H, $(CH_3)_4$-N^+), 3.37 (s, 3H, 3-OCH_2–OCH_3), 4.17 (dd, 1H, J = 29.0, 3.9 Hz, 17α-H), 4.95 (dm, 1H, J = 54 Hz, 16β-H), 5.15 (q, 2H, 3-OCH_2–), 6.90 (d, 1H, J = 8.8 Hz, 4-H), 7.07 (d, 1H, J = 13.3 Hz, 1-H).

15b: ^{1}H NMR (300 MHz, d_6-DMSO) δ 0.68 (s, 3H, 18-CH_3), 3.09 (s, 12H, $(CH_3)_4$-N^+), 3.37 (s, 3H, 3-OCH_2–OCH_3), 4.18 (dd, 1H, J = 29.0, 3.9 Hz, 17α-H), 4.96 (dm, 1H, J = 54 Hz, 16β-H), 5.15 (br s, 2H, 3-OCH_2–), 6.90–7.10 (m, 2H, aromatic-H).

Tetramethylammonium 2,16α-difluoro- (15c) or 4,16α-difluoro-11β-methoxy-3-*O*-methoxymethyl-3-hydroxyestra-1,3,5(10)-trien-17β-yl sulfate (15d)

Tetramethylammonium fluoride tetrahydrate (17 mg) was dried *via* azeotropic distillation of acetonitrile (3 × 2.5 mL). A solution of compound **14c** or **14d** (35 mg) in absolute MeCN (3.5 mL) was added and the mixture was refluxed under dry nitrogen for 15 min. The solvent was evaporated to yield **15c** or **15d** (91%, 39 mg) as Me_4N^+ salts.

15c: ^{1}H NMR (300 MHz, d_6-DMSO) δ 0.84 (s, 3H, 18-CH_3), 3.09 (s, 12H, $(CH_3)_4$-N^+), 3.14 (s, 3H, 11β-OCH_3), 3.38 (s, 3H, 3-OCH_2–OCH_3), 4.06 (m, 1H, 11α-H), 4.15 (dd, 1H, J = 29.1, 3.8 Hz, 17α-H), 4.95 (dm, 1H, J = 54 Hz, 16β-H), 5.15 (q, 2H, 3-OCH_2–), 6.87 (d, 1H, J = 8.9 Hz, 4-H), 6.98 (d, 1H, J = 13.4 Hz, 1-H).

15d: ^{1}H NMR (300 MHz, d_6-DMSO) δ 0.84 (s, 3H, 18-CH_3), 3.09 (s, 12H, $(CH_3)_4$-N^+), 3.12 (s, 3H, 11β-OCH_3), 3.38 (s, 3H, 3-OCH_2–OCH_3), 4.10 (m, 1H, 11α-H), 4.15 (dd, 1H, J = 29.0,

3.9 Hz, 17α-H), 4.95 (dm, 1H, J = 54 Hz, 16β-H), 5.15 (q, 2H, 3-OCH_2–), 6.90–7.1 (m, 2H, aromatic-H).

2,16α-Difluoro- (6a) or 4,16α-difluoroestra-1,3,5(10)-triene-3,17β-diol (6b)

The crude product **15a** or **15b** (38 mg) was dissolved in a mixture of EtOH (20 mL) and concentrated sulfuric acid (100 μL). The solution was heated to 110 °C for 5 min, solvent was removed under reduced pressure, the residue extracted with ether, washed with water, dried (Na_2SO_4), and evaporated to yield **6a** or **6b** (90%, 21 mg). The analytical samples were obtained as white powders after recrystallization from ether–EtOH.

6a: mp 185 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.80 (s, 3H, 18-CH_3), 3.86 (dd, 1H, J = 4.6, 28.5 Hz, 17α-H), 4.95 (dm, 1H, J = 51 Hz, 16β-H), 6.70 (d, 1H, J = 9.1 Hz, 4-H), 6.96 (d, 1H, J = 12.5 Hz, 1-H); MS m/z (relative intensity) 308 (M^+, 100%), 231 (34), 190 (18); HRMS calcd for $C_{18}H_{22}O_2F_2$, 308.1588, found 308.1591.

6b: mp 191 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.80 (s, 3H, 18-CH_3), 3.86 (dd, 1H, J = 4.6, 28.5 Hz, 17α-H), 4.98 (dm, 1H, J = 51 Hz, 16β-H), 6.81 (t, 1H, J = 8.8 Hz, 2-H), 6.94 (d, 1H, J = 8.6 Hz, 1-H); MS m/z (relative intensity) 308 (M^+, 100%), 231 (46), 190 (27); HRMS calcd for $C_{18}H_{22}O_2F_2$, 308.1588, found 308.1591.

2,16α-Difluoro- (6c) or 4,16α-difluoro-11β-methoxyestra-1,3,5(10)-triene-3,17β-diol (6d)

A solution of **15c** or **15d** (39 mg) in EtOH (20 mL) and concentrated sulfuric acid (100 μL) was heated to 110 °C for 5 min. The EtOH was evaporated, water was added, and the product was extracted with ether. The extract was dried (Na_2SO_4), filtered and evaporated to yield **6c** or **6d** (90%, 22 mg). Crystallization of the crude products from ether and EtOH gave the analytical samples as white powders.

6c: mp 224 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (s, 3H, 18-CH_3), 3.31 (s, 3H, 11β-OCH_3), 3.82 (dd, 1H, J = 4.5, 27.0 Hz, 17α-H), 4.06 (m, 1H, 11α-H), 4.96 (dm, 1H, J = 54 Hz, 16β-H), 6.66 (d, 1H, J = 9.2 Hz, 4-H), 6.80 (d, 1H, J = 12.5 Hz, 1-H); MS m/z (relative intensity) 338 (M^+, 100%), 306 (38), 190 (50); HRMS calcd for $C_{19}H_{24}O_3F_2$, 338.1693, found 338.1698.

6d: mp 246 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (s, 3H, 18-CH_3), 3.28 (s, 3H, 11β-OCH_3), 3.85 (dm, 1H, J = 30 Hz, 17α-H), 4.13 (m, 1H, 11α-H), 4.96 (dm, 1H, J = 55 Hz, 16β-H), 6.75–6.95 (m, 2H, aromatic-H); MS m/z (relative intensity) 338 (M^+, 100%), 306 (41), 190 (50); HRMS calcd for $C_{19}H_{24}O_3F_2$, 338.1693, found 338.1698.

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References

1 (*a*) W. L. McGuire, K. B. Horwitz, O. H. Pearson and A. Segaloff, *Cancer*, 1977, **39**, 2934–2947; (*b*) C. A. Bertelsen, A. E. Guiliano, D. H. Kern, B. D. Mann, D. J. Roe and D. L. Morton, *J. Surgical Res.*, 1984, **37**, 257–263; (*c*) L. Vollenweider-Zeragui, G. Barrelet, Y. Wong, T. Lemarchand-Béraud and F. Gomez, *Cancer*, 1986, **57**, 1171–1180; (*d*) G. M. Clarck, G. W. Sledge, Jr., C. K. Osborne and W. L. McGuire, *J. Clin. Oncol.*, 1987, **5**, 55–61.

2 J. P. van Netten, J. B. Armstrong, S. S. Carlyle, N. L. Goodchild, I. G. Thornton, M. L. Brigden, P. Coy and C. Fletcher, *Eur. J. Cancer Clin. Oncol.*, 1988, **24**, 1885–1889.

3 (*a*) J. A. Katzenellenbogen, D. F. Heiman, K. E. Carlson, J. E. Lloyd, *In vivo* and *in vitro* steroid receptor assays in the design of estrogen pharmaceuticals. In: *Receptor binding radiotracers*, ed. W. C. Eckelman, CRC Press, Boca Raton, FL, 1982, 93–126; (*b*) C. H. Cummins, *Steroids*, 1993, **58**, 245–259.

4 (*a*) L. J. Rijks, P. J. Bakker, G. van Tienhoven, L. A. Noorduyn, G. J. Boer, R. C. Rietbroek, C. W. Taat, A. G. Janssen, C. H. Veenhof and E. A. van Royen, *J. Clin. Oncol.*, 1997, **15**, 2536–2545; (*b*) O. Nachar, J. Rousseau, B. Lefebvre, R. Ouellet and J. E. van Lier, *J. Nucl. Med.*, 1999, **40**, 1728–1736; (*c*) O. Nachar, J. Rousseau, R. Ouellet, A. Rioux, B. Lefebvre, H. Ali and J. E. van Lier, *J. Nucl. Med.*, 2000, **41**, 1324–1331.

5 (*a*) M. A. Mintun, M. J. Welch, B. A. Siegel, C. J. Mathias, J. W. Brodack, A. H. McGuire and J. A. Katzenellenbogen *Radiology*, 1988, **169**, 45–48; (*b*) A. H. McGuire, F. Dehdashti, B. A. Siegel, A. P. Lyss, J. W. Brodack, C. J. Mathias, M. A. Mintun, J. A. Katzenellenbogen and M. J. Welch, 1991, **32**, 1526–1531; (*c*) F. Dehdashti, J. E. Mortimer, B. A. Siegel, L. K. Griffeth, T. J. Bonasera, M. J. Fusselman, D. D. Detert, P. Duffy Cutler, J. A. Katzenellenbogen and M. J. Welch, *J. Nucl. Med.*, 1995, **36**, 1766–1774.

6 D. A. Mankoff, T. J. Tewson and J. F. Eary, *Nucl. Med. Biol.*, 1997, **24**, 341–348.

7 M. G. Pomper, H. VanBrocklin, A. M. Thieme, R. D. Thomas, D. O. Kiesewetter, K. E. Carlson, C. J. Mathias, M. J. Welch and J. A. Katzenellenbogen, *J. Med. Chem.*, 1990, **33**, 3143–3155.

8 H. F. VanBrocklin, A. Liu, M. J. Welch, J. P. O'Neil and J. A. Katzenellenbogen, *Steroids*, 1994, **59**, 34–45.

9 H. F. Vanbrocklin, P. A. Rocque, H. V. Lee, K. E. Carlson, J. A. Katzenellenbogen and M. J. Welch, *Life Sci.*, 1993, **53**, 811–819.

10 S. D. Jonson, T. A. Bonasera, F. Dehdashti, M. E. Cristal, J. A. Katzenellenbogen and M. J. Welch, *Nucl. Med. Biol.*, 1999, **26**, 123–130.

11 (*a*) H. Ali, J. Rousseau, T. G. Gantchev and J. E. van Lier, *J. Med. Chem.*, 1993, **36**, 4255–4263; (*b*) H. Ali, J. Rousseau and J. E. van Lier, *J. Med. Chem.*, 1993, **36**, 3061–3072; (*c*) H. Ali, J. Rousseau, M. A. Ghaffari and J. E. van Lier, *J. Med. Chem.*, 1991, **34**, 854–860; (*d*) J. E. Zielinski, H. Yabuki, S. L. Pahuja, J. M. Larner and R. B. Hochberg, *Endocrinology*, 1986, **119**, 130–139; (*e*) J. E. Zielinski, J. M. Larner, P. B. Hoffer and R. B. Hochberg, *J. Nucl. Med.*, 1989, **30**, 209–215; (*f*) R. N. Hanson, The influence of structure modification on the metabolic transformations of radiolabeled estrogen derivatives, in *Radiopharmaceuticals, Chemistry and Pharmacology*, ed. A. Nunn, Marcel Dekker, NY, 1992, pp. 333–364.

12 E. D. Hostetler, S. D. Jonson, M. J. Welch and J. A. Katzenellenbogen, *J. Org. Chem.*, 1999, **64**, 178–185.

13 J. S. Baran, *J. Med. Chem.*, 1967, **10**, 1188–1190.

14 D. O. Kiesewetter, J. A. Katzenellenbogen, M. R. Kilbourn and M. J. Welch, *J. Org. Chem.*, 1984, **49**, 4900–4905.

15 D. O. Kiesewetter, M. R. Kilbourn, S. W. Landvatter, D. F. Heiman, J. A. Katzenellenbogen and M. J. Welch, *J. Nucl. Med.*, 1984, **25**, 1212–1221.

16 T. B. Patrick and R. Mortezania, *J. Org. Chem.*, 1988, **53**, 5153–5155.

17 A. C. Stalford, J. L. Maggs, T. L. Gilchrist and B. K. Park, *Steroids.*, 1997, **62**, 750–761.

18 (*a*) T. Umemoto, K. Kawada and K. Tomita, *Tetrahedron Lett.*, 1986, **27**, 4465–4468; (*b*) T. Umemoto, S. Fukami, G. Tomizawa, K. Harasawa, K. Kawada and K. Tomita, *J. Am. Chem. Soc.*, 1990, **112**, 8563–8575.

19 P. C. Bulman Page, F. Hussain, J. L. Maggs, P. Morgan and B. K. Park, *Tetrahedron*, 1990, **46**, 2059–2068.

20 S. G. Senderoff, K. D. McElvany, K. E. Carlson, D. F. Heiman, J. A. Katzenellenbogen and M. J. Welch, *Int. J. Appl. Radiat. Isot.*, 1982, **33**, 545–551.

21 M. S. Berridge, M. P. Franceschini, E. Rosenfeld and T. J. Tewson, *J. Org. Chem.*, 1990, **55**, 1211–1217.

22 (*a*) J. W. Brodack, M. R. Kilbourn and M. J. Welch, *Appl. Radiat. Isot.*, 1988, **39**, 689–698; (*b*) J. W. Brodack, M. R. Kilbourn, M. J. Welch and J. A. Katzenellenbogen, *Appl. Radiat. Isot.*, 1986, **37**, 217–221.

23 J. Romer, J. Steinbach and H. Kasch, *Appl. Radiat. Isot.*, 1996, **47**, 395–399.

24 J. L. Lim, L. Zheng, M. S. Berridge and T. J. Tewson, *Nucl. Med. Biol.*, 1996, **23**, 911–915.