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SYNTHESIS AND ESTROGEN RECEPTOR BINDING OF FLUORINATED DIETHYLSTILBESTROL DERIVATIVES

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Abstract: Synthesis of fluorinated DES and hexestrol derivatives starting from 4-methoxybenzaldehyde is described. The structures of the fluorinated compounds were characterized by NMR. The fluorinated DES derivatives bind to the mouse uterine cytosol estrogen receptor with high affinity.

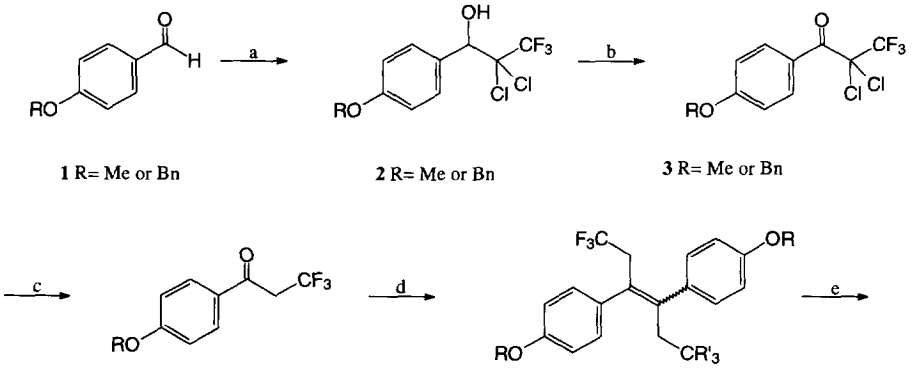
Diethylstilbestrol (DES) was first synthesized in 1938 by Dodds,[1] and had been widely used as a synthetic estrogen in humans and farm animals. However, it exhibits many toxic side effects in humans and laboratory animals.[2,3] DES posesses potent estrogenic biological activity and binds as well as estradiol, the natural estrogen, to the estrogen receptor (ER). This receptor plays an important role in the mechanism of action of estrogen,[4] and we have been investigating the binding and biological activity of a number of DES-related compounds,[5] some of which have even greater ER-binding affinity than estradiol.[6] We have reported the synthesis of 3,5,3',5'-tetrafluoro-DES[7] which had biological activity comparable to estradiol but with weaker binding affinity to the estrogen receptor. To further study the characteristics of the estrogen receptor and the requirements necessary for ligand binding, we have synthesized and evaluated several fluorinated DES derivatives for possible use as NMR active probes of estrogen receptor structure.
In this report, we describe the synthesis and ER binding properties of the fluorinated DES compounds.

As outlined in the Scheme **1**, treatment of 4-methoxybenzaldehyde or 4-benzyloxybenzaldehyde **1** and CCl_3CF_3 with Zn in DMF provided alcohol **2** in 70% yield.[8a] Alcohol **2** was transformed to ketone **3**[8b] in 90% yield (based on recovered **2**) with PCC by Corey's procedure.[9] Dechlorination of ketone **3** with Zn/HOAc proceeded smoothly to yield ketone **4** as a colorless oil in 93% yield.[10] Titanium mediated coupling via the McMurray procedure of ketone **4** gave a mixture of *Z*- and *E*-olefins **5** (4:1) in 75% yield.[11] Demethylation of isomers **5** with $BF_3{\cdot}S(Me)_2$ provided *Z* and *E* mixtures of the hexafluoro DES

derivatives, F6DES **6A** and **6B** in 90% yield. Each of these isomers was obtained pure by repeated recrystallization and preparative thin-layer chromatography. Each isomeric olefin (**6A** and **6B**) was hydrogenated to the hexafluorohexestrol derivatives F6HDES (**7A** and **7B**).

In a similar manner, the trifluoro DES derivatives (**8A** and **8B**) were prepared by a mixed coupling of **4** with 4-benzyloxypropiophenone. The desired **8A** and **8B** could be easily separated from DES and the hexafluoro DES derivatives by flash chromatography. Hydrogenation of the individual isomers **9A** and **9B** yielded the trifluorohexestrol derivatives F3HDES (**10A** and **10B**).

Scheme 1.



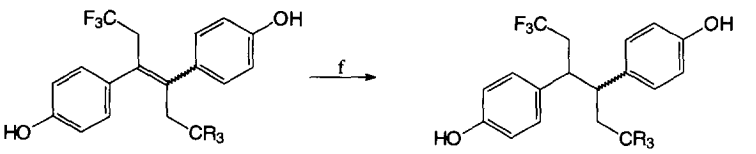
1 R= Me or Bn

2 R= Me or Bn

3 R= Me or Bn

4 R= Me or Bn

5A: *Z*-, **5B**: *E*-isomer (R= Me, R'= F)
8A: *Z*-, **8B**: *E*-isomer (R= Bn, R'= H)



6A: *Z*-, **6B**: *E*-isomer (R= F)
9A: *Z*-, **9B**: *E*-isomer (R= H)

7A: *meso*-, **7B**: *dl*-isomer (R=F)
10A: *syn*-, **10B**: *anti*-isomer (R=H)

Reagents: a) CCl_3CF_3, Zn, DMF (70%). b) PCC, Molecular Sieve 4Å, CH_2Cl_2 (90%). c) Zn/HOAc (93%). d) $TiCl_3$, Zn/Cu, THF for **5** (75%): 4-benzyloxypropiophenone, $TiCl_3$, Zn/Cu, THF for **8** (40-60%). e) BF_3SMe_2, CH_2Cl_2 (93%). f) H_2, Pd/C, EtOAc (94%).

The structures of *Z*- and *E*-F6DES (**5A** and **5B**) were assigned by comparing the signals of the CH_2 group of the *Z* isomer of dimethoxy DES which is shifted downfield (0.2 ppm) from that of the *E* isomer while the chemical shift of the methoxy group is moved upfield (0.3 ppm) compared with the corresponding signals of the *E* isomer.[12] The shift differences of the *E* and *Z* isomers of F6DES were found to be almost identical with those of the *E* and *Z* isomers of DES and the previously reported and related hexafluoro-dimethylstilbestrol. The ^{1}H signals of *E*- and *Z*-F3DES are similar to those of F6DES. The individual *E* and *Z* isomers of both the tri- and hexafluoro-DES derivatives were separated using a C-18 HPLC column with a linear acetonitrile-water gradient solvent system. As reported for the separation of *E*- and *Z*-DES by reversed phase HPLC,[13] *Z* isomers eluted after *E* isomers.

The binding affinities of the fluorinated compounds for the estrogen receptor were determined by a competitive cytosolic binding assay.[14] The binding pattern of the curves for the fluorinated compounds showed similar shapes to that of DES but have lower binding affinities than DES (Fig 1). The similar shape of the competition curves indicates competitive binding interactions with a single binding site. Relative binding affinities (RBA) were determined from the competitive binding curves (Table 1). The RBA values indicated that the *E* isomers (*E*-F3DES and *E*-F6DES) have higher binding affinities than the *Z* isomers (*Z*-F3DES and *Z*-F6DES) in both tri- and hexa-fluoro compounds. However, of the four fluorinated hexestrol derivatives, only the one derived from *Z*-F6DES is not a mixture of enantiomers as it is a *meso* compound. The three other hydrogenated derivatives exist as racemic mixtures. Since we have shown[5] that the estrogen receptor shows specificity in the binding of enantiomers, the activity of the individual fluorinated hexestrol isomers may be understandably different from the racemic mixture. When the double bond was saturated to hexestrol derivatives, the ER binding affinities slightly decreased. Interestingly with the unfluorinated DES isomers, *Z*-DES has approximately 1% the binding affinity of *E*-DES isomer[15] but upon fluorination of the side chain the difference is enhanced to 10% with the F3DES and 5% with F6DES isomers.

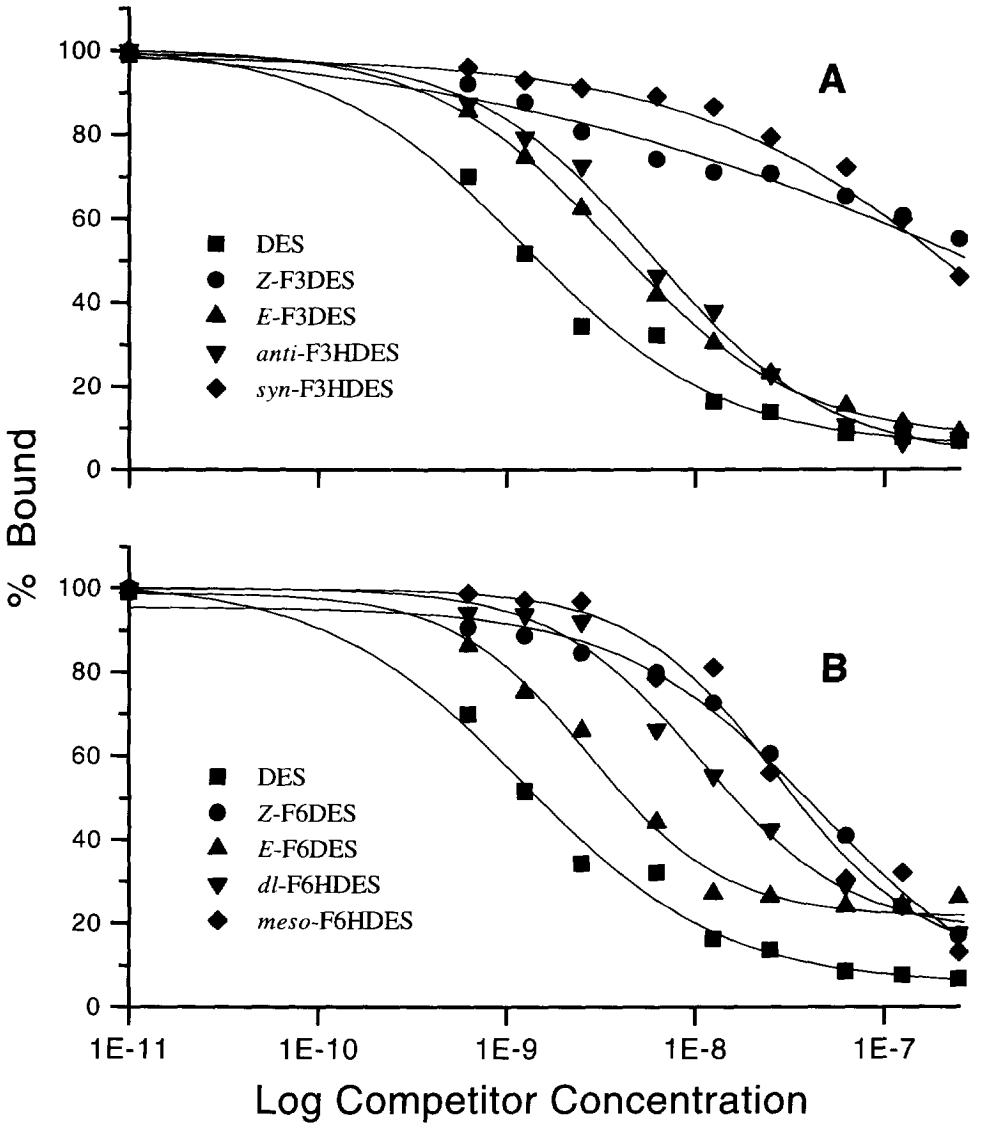


Figure 1. Competitive binding of DES, tri- (**A**) and hexa- (**B**) fluorinated DES derivatives to the estrogen receptor preparation from mouse uterine cytosol as described in Ref. 14.

TABLE 1

Relative binding affinities of DES and fluorinated DES derivatives

Compound	Relative Binding Affinity
E_2	100
DES	286
Z-F3DES (**9A**)	12.3
E-F3DES (**9B**)	117
anti-F3HDES (**10B**)	71
syn-F3HDES (**10A**)	2.6
Z-F6DES (**6A**)	9.6
E-F6DES (**6B**)	193
dl-F6HDES (**7B**)	48
meso-F6HDES (**7A**)	18

Relative binding affinity was determined by the competitive binding assay.[14] Binding affinity for E_2 was set to 100.

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14. The competitive binding assay was carried out as described previously.[16]
Aliquots of 100μL mouse uterine cytosol were incubated with 5 nM [^{3}H]E_2 and increasing concentrations (0.5 nM - 5 μM) of unlabeled competitor (fluorinated DES). After incubating the mixtures for 18 hours at 4°C, 250 μL of 60% HAP (hydroxyapatite) in TEGM buffer (10 mM Tris, 1.5 mM EDTA, 10% glycerol, 3 mM $MgCl_2$, pH 7.6) was added to each tube. Tubes were then centrifuged at 1,000 x g for 10 min, and the resulting HAP pellet was washed twice with 3 ml TEGM buffer then suspended in Ecoscint-O scintillation cocktail.
The radioactivity was measured on a Beckman CS 9800 scintillation counter. The Kd values were determined using Accufit Ligand Competition Analysis Software by Lundon Software. Specific binding was determined as the difference between total (radioactive ligand only) and non-specific binding (radioactive ligand with excess unlabeled ligand) in the preparation.

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