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Synthesis of the catechols of natural and synthetic estrogens by using 2-iodoxybenzoic acid (IBX) as the oxidizing agent

Muhammad Saeed, Muhammad Zahid, Eleanor Rogan, Ercole Cavalieri *

Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, 986805 Nebraska Medical Center, Omaha, NE 68198-6805, USA

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Abstract

A method for the synthesis of 2-hydroxyestrone/estradiol, 4-hydroxyestrone/estradiol, 3′-hydroxydiethylstilbestrol, 3′-hydroxyhexestrol, and 3′-hydroxydienestrol is reported, in which 2-iodoxybenzoic acid (IBX) and the corresponding phenolic estrogen are reacted. Treatment of the natural estrogens, estrone/estradiol, with stoichiometric amounts of IBX in dimethylformamide initially yielded a mixture of estrone/estradiol-2,3- and -3,4-quinones, which were reduced in situ to the corresponding catechols by treatment with a 1 M aqueous solution of ascorbic acid. Chromatographic separation of the reaction products afforded 2- and 4-hydroxyestrone/estradiol in good overall yields (79%). In the case of the synthetic estrogens containing two identical phenolic rings, protection of one ring is a prerequisite for the synthesis of the monocatechol. Thus, diethylstilbestrol and dienestrol were protected at one phenol ring as their methyl ethers. The resulting monophenols were treated with stoichiometric amounts of IBX for 1 h, followed by treatment with 1 M aqueous ascorbic acid to obtain the corresponding catechols in more than 70% yield. Furthermore, the catechol of diethylstilbestrol, protected at one ring, was reduced by catalytic hydrogenation at the C3–C4 double bond to obtain 3′-hydroxyhexestrol in 90% yield. Removal of the protected methoxy groups of the synthetic estrogen catechols was carried out by treatment with a 1 M solution of boron tribromide in dichloromethane. This method is highly efficient for the preparative scale synthesis of catechols of both natural and synthetic estrogens.

Keywords: Estradiol; Estrone; Diethylstilbestrol; Hexestrol; Dienestrol; Catechol synthesis; 2-Iodoxybenzoic acid (IBX)

1. Introduction

Several of the most common cancers in western societies occur in hormonally responsive tissues, including breast, endometrium, and ovary in women and prostate in men. These cancers have been causally linked to exposure to synthetic and endogenous steroidal hormones or their metabolites [1–3]. Endogenous estrogens, estradiol (E_2) and estrone (E_1), and synthetic estrogens, diethylstilbestrol (DES) and hexestrol (HES), have been found to be associated with cancer in animals and humans [4–10]. There is mounting experimental evidence suggesting that tumor initiation arises from specific metabolites of endogenous estrogens [11–13].

Both endogenous and synthetic estrogens are mainly metabolized to their respective catechols [14–19]. Catechol estrogens are metabolically oxidized to their electrophilic quinones, which react with DNA to form specific depurinating adducts that can lead to mutations and, eventually, to the initiation of cancer [11–13]. To study the mechanism of tumor initiation by endogenous and synthetic estrogens, it was necessary to synthesize the catechol metabolites of these estrogens on a preparative scale.

The standard chemical methods for preparing 2- and 4-hydroxyestrogens are laborious multistep chemical reactions [20,21], giving low overall yields and practically

Abbreviations: $CDCl_3$, deuterated chloroform; DES, diethylstilbestrol; DIES, dienestrol; DMF, *N*,*N*-dimethylformamide; DMSO-d_6, deuterated dimethylsulphoxide; E_1, estrone; E_2, estradiol; ESI-MS, electron spray ionization-mass spectrometry; HES, hexestrol; HMPA, hexamethylphosphoramide; HPLC, high performance liquid chromatography; IBX, 2-iodoxybenzoic acid; NMR, nuclear magnetic resonance; TBDMS, *tert*-butyldimethylsilyl; THF, tetrahydrofuran

* Corresponding author. Tel.: +1 402 559 7237; fax: +1 402 559 8068.
E-mail address: ecavalie@unmc.edu (E. Cavalieri).

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no selectivity for the synthesis of 4-hydroxyestrogens. Yoshizawa et al. [22] reported the chemical synthesis of 2-hydroxyestrogens using benzoyl peroxide; however, the synthesis of 4-hydroxyestrogens was not achievable by this method. A multistep method reported by Stubenrauch and Knuppen [23] was not successful for the direct conversion of estradiol to the 2- and 4-hydroxyestradiols, and the authors needed to reduce the corresponding 17-oxo compound with sodium borohydride for the preparation of estradiol catechols [23]. Use of potassium nitrosodisulfonate (FREMY's salt) for the one-step chemical conversion of estrone/estradiol to a mixture of the corresponding 2,3- and 3,4-quinones had been reported earlier by Gelbke et al. [24]. The method involved a laborious work-up and ended up with very low overall yields of catechol estrogens. Regarding the catechols of synthetic estrogens, DES and HES, our laboratory previously reported the chemical synthesis of 3′-hydroxydiethylstilbestrol (3′-OH-DES) and 3′-hydroxyhexestrol (3′-OH-HES) [25]; however, overall yields were low due to multi-step reactions, and the formation of a mixture of isomers was observed.

We report here a new method for the synthesis of the catechols of both natural and synthetic estrogens in good to excellent yields. After oxidation of E_1 (**1a**) or E_2 (**1b**) with 2-iodoxybenzoic acid (IBX) to the 2,3- and 3,4-quinones, subsequent reduction with either KI or a 1 M aqueous solution of ascorbic acid gives the corresponding catechol estrogens in good overall yield. This method was also used for the synthesis of the catechols of synthetic estrogens, demonstrating the general applicability of this method.

2. Experimental

2.1. Chemicals and instrumentation

DES, E_2, E_1, DIES and ascorbic acid were purchased from Sigma (St. Louis, MO) and used as such without further purification. 2-Iodobenzoic acid, oxone and dimethylformamide (DMF) were purchased from Aldrich Chemical Co. (Milwaukee, WI). IBX was synthesized from 2-iodobenzoic acid and oxone as previously described [26]. Preparative HPLC was performed on a Waters (Milford, MA) 600E solvent delivery system equipped with a 996 photodiode array detector and a Phenomenex (Torrance, CA) Luna (2) C-18 column (10 μm, 100 Å, 21.2 mm × 250 mm). ^{1}H NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ on a Varian-Inova 500 instrument at 499.835 MHz at 25 °C. Chemical shifts are reported relative to DMSO-d_6 (2.49 ppm) or $CDCl_3$ (7.26 ppm).

2.2. General procedure for the synthesis of catechols

IBX (1 mmol) was added to a stirred solution of phenol (1 mmol) in DMF (50 mL) in the dark. After 1 h, thin-layer chromatography indicated the complete conversion of the phenol to a polar product. The red-colored solution was treated with a 1 M solution of ascorbic acid in water until the color of the reaction mixture turned light yellow. The reaction product was extracted with ethyl acetate (3× 50 mL), and the combined organic phases were evaporated after drying over anhydrous sodium sulfate (Na_2SO_4). The residue was chromatographed over a column of silica gel impregnated with ascorbic acid as described by Gelbke and Knuppen [28]. The product was then purified by preparative HPLC using 10% acetonitrile and 90% H_2O (with 0.4% acetic acid) as a mobile phase for the initial 5 min and then a linear gradient to 100% acetonitrile in 25 min, to afford the catechol. The spectroscopic data of 2- and 4-OHE_1/E_2, 3′-OH-DES and 3′-OH-HES synthesized by this method agree with those reported earlier [24,25].

2.2.1. 3′-OH-4″-OCH_3-DES (**8**)

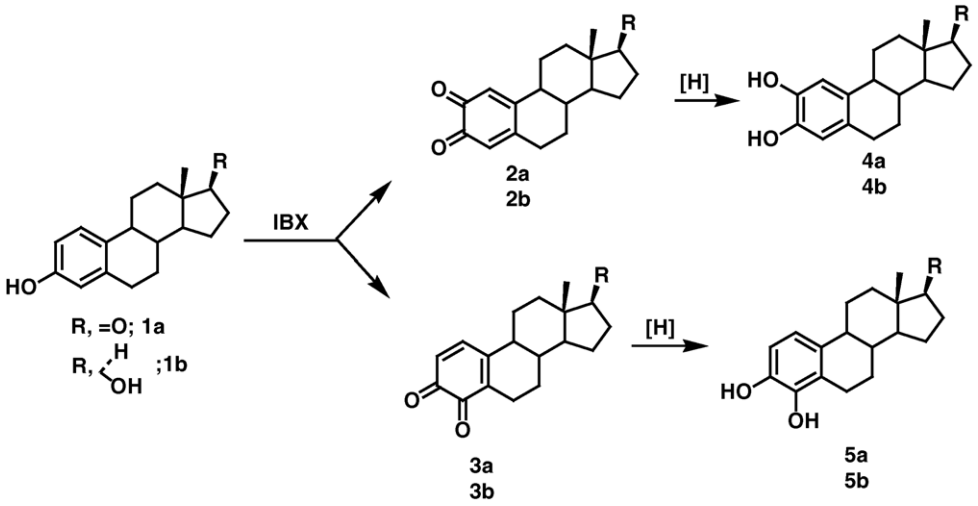
Compound **8** was synthesized from 4′-OCH_3-DES (**7**) according to the general method described above. Yield 68%; ^{1}H NMR (ppm, DMSO-d_6): δ 8.80 (s, 1H, Ar-OH, exchangeable with D_2O), 8.75 (s, 1H, Ar-OH, exchangeable with D_2O), 7.08 (d, J = 8.3 Hz, 2H, H-2″, H-6″), 6.92 (d, J = 8.8 Hz, 2H, H-3″, H-5″), 6.70 (d, J = 7.8 Hz, 1H, H-5′), 6.56 (d, J = 1.9 Hz, 1H, H-2′), 6.42 (dd, J = 7.8, 1.9 Hz, 1H, H-6′), 3.75 (s, 3H, OCH_3), 2.09 (q, J = 7.8 Hz, CH_2), 2.01 (q, J = 7.8 Hz, CH_2), 0.70 (t, J = 7.3 Hz, 2 × CH_3).

2.2.2. 3′-OH-4″-OCH_3-DIES (**14**)

Compound **14** was synthesized from 4′-OCH_3-DIES (**13**) according to the general method described above. Yield 75%; ^{1}H NMR (ppm, $CDCl_3$): δ 8.79 (bs, 2H, Ar-OH, exchangeable with D_2O), 7.01 (d, J = 8.3 Hz, 2H, H-2″, H-6″), 6.91 (d, J = 8.3 Hz, 2H, H-3″, H-5″), 6.70 (d, J = 7.8 Hz, 1H, H-5′), 6.50 (d, J = 1.9 Hz, 1H, H-2′), 6.36 (dd, J = 7.8, 1.9 Hz, 1H, H-6′), 5.29 (q, J = 6.8 Hz, CH), 5.15 (q, J = 6.8 Hz, CH), 3.75 (s, 3H, OCH_3), 1.43 (dd, J = 6.8, 6.8 Hz, 2 × CH_3).

2.3. General method for the synthesis of monomethylated synthetic estrogens

Sodium hydride (NaH) (358 mg, 60% suspension) was suspended in DMF (25 mL) at 0 °C in a 50-mL three-neck round-bottomed flask and mechanically stirred under argon for 5 min. DES or DIES (2 g, 7.46 mmol) was added slowly, and the ice bath was removed. A thick slurry of the salt of DES/DIES was formed after 1 h, which was thinned with a small amount of DMF to facilitate stirring. A solution of iodomethane (CH_3I, 566 μL) in DMF (20 mL) was added slowly into the mixture from a dropping funnel. After the formation of a clear solution, thin-layer chromatography of the reaction mixture was conducted, which indicated almost complete consumption of the starting material. The reaction mixture was plunged into crushed ice containing 5% HCl, and the reaction product was extracted with ethyl acetate (3× 50 mL). The combined organic layers were washed with brine and water, dried over sodium sulfate (Na_2SO_4), and the



Scheme 1. Synthesis of 2-OHE$_1$/E$_2$ (**4a** and **4b**) and 4-OHE$_1$/E$_2$ (**5a** and **5b**).

solvent was removed under low pressure. The residue was purified by flash column chromatography with 5% ethyl acetate in hexane to yield monomethylated estrogens as a white solid.

2.3.1. 4″-OCH$_3$-DES (7)

Yield (1.47 g, 70%). ^{1}H NMR (ppm, DMSO-d_6): δ 9.30 (s, 1H, Ar-OH, exchangeable with D_2O), 7.09 (d, J – 8.3 Hz, 2H, H-1′,H-6′), 6.97 (d, J = 8.3 Hz, 2H, H-1″, H-6″), 6.93 (d, J = 8.3 Hz, 2 H, H-3′, H-5′), 6.75 (d, J = 8.3 Hz, 2 H, H-3″, H-5″), 3.75 (s, 3 H, OCH_3), 2.06 (m, 4 H, 2 × CH_2), 0.69 (t, J = 7.32 Hz, 6 H, 2 × CH_3). ^{13}C NMR 157.9, 156.0, 138.5, 137.9, 134.4, 132.5, 129.6, 129.5, 115.1, 113.7, 55.1, 28.2, 13.5, 13.4. Electron spray ionization-mass spectrometry (ESI-MS) m/z: 283.1 $(M + H)^+$.

2.3.2. 4″-OCH$_3$-D1ES (13)

Yield (1.50 g, 71%). ^{1}H NMR (ppm, $CDCl_3$): δ 7.10 (d, J = 8.3 Hz, 2H, H-1′,H-6′), 7.05 (d, J = 8.3 Hz, 2H, H-1″, H-6″), 6.93 (d, J = 8.3 Hz, 2 H, H-3′, H-5′), 6.86 (d, J = 8.3 Hz, 2 H, H-3″, H-5″), 5.30 (m, 2 H, CH), 3.85 (s, 3 H, OCH_3), 1.48 (d, J = 6.35 Hz, 6 H, 2 × CH_3). ^{13}C NMR 158.0, 154.0, 144.9, 144.8, 132.3, 132.2, 131.1, 130.9,125.1, 114.9, 113.4, 55.2, 15.2. ESI-MS m/z: 281.1 $(M + H)^+$.

2.4. Synthesis of 3′-OH-4″-OCH$_3$-HES (10)

A solution of **8** (50 mg, 0.17 mmol), 5% palladium on carbon (Pd/C, 3 mg) and ethyl acetate (3 mL) was stirred for 10 h under a hydrogen atmosphere (using a balloon filled with H_2 gas). The Pd/C was removed by filtration thorough a pad of Celite. The filtrate was dried to afford **10** as a diastereomeric mixture of DL- (**10a**) and meso-3′-OH-4-OCH$_3$-HES (**10b**) in 8:2 ratio, which were separated by preparative HPLC. The column [Phenomenex, Luna C-18(2), 250 mm × 21.2 mm i.d.] was eluted by using a linear gradient starting from 30% methanol in 70% water (0.4% TFA) to 100% methanol in 30 min at a flow rate of 7 mL/min.

2.4.1. DL-3′-OH-4″-OCH$_3$-HES (10a)

Yield (38.6 mg, 72 %). ^{1}H NMR (ppm, DMSO-d_6): δ 8.48 (bs, 2H, Ar-OH, exchangeable with D_2O), 6.82 (d, J = 8.8 Hz, 2H, H-2″, H-6″), 6.69 (d, J = 8.8 Hz, 2H, H-3″, H-5″), 6.47 (d, J = 7.8 Hz, 1H, H-5′), 6.32 (d, J = 1.5 Hz, 1H, H-2′), 6.17 (dd, J = 7.8, 1.5 Hz, 1H, H-6′), 3.66 (s, 3H, OCH_3), 2.59–2.47 (m, 2H, 2CH), 1.76 (m, 2H, CH_2), 1.48-1.36 (m, 2H, CH_2), 0.64 (t, J = 7.3 Hz, 3H, CH_3), 0.62 (t, J = 7.3 Hz, 3H, CH_3).

2.4.2. Meso-3′-OH-4″-OCH$_3$-HES (10b)

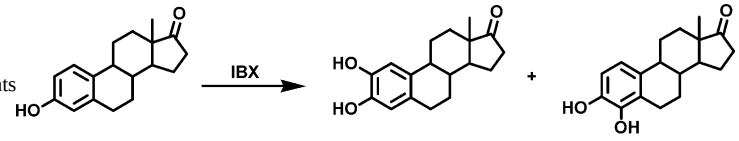
Yield (9.7 mg, 18%). ^{1}H NMR (ppm, DMSO-d_6): δ 8.63 (bs, 2H, Ar-OH, exchangeable with D_2O), 7.10 (d, J = 8.8 Hz, 2H, H-2″, H-6″), 6.87 (d, J = 8.8 Hz, 2H, H-3″, H-5″), 6.66 (d, J = 7.8 Hz, 1H, H-5′), 6.58 (d, J = 1.5 Hz, 1H, H-2′), 6.46 (dd, J = 7.8, 1.5 Hz, 1H, H-6′), 3.73 (s, 3H, OCH_3), 2.42-2.30 (m, 4H, 2CH, CH_2), 1.32-1.08 (m, 2H, CH_2), 0.45 (t, J = 7.3 Hz, 3H, CH_3), 0.44 (t, J = 7.3 Hz, 3H, CH_3).

2.5. General procedure for deprotection of methyl groups

Boron tribromide (BBr_3, 3 mL) was added over 15 min to a stirred solution of methylated catechol (1 mmol) in dichloromethane (50 mL) at −78 °C under argon. The reaction mixture was stirred at the same temperature for 1 h and then at room temperature for 2 h. The reaction mixture was plunged into a beaker containing crushed ice and sodium bicarbonate ($NaHCO_3$, 50 mg). The reaction products were extracted with ethyl acetate (3× 100 mL). The combined organic layers were washed with brine and water, dried over sodium sulfate (Na_2SO_4), and the solvent was removed under low pressure. The residue was purified by preparative HPLC as described above.

Table 1

Outcome of the reaction of E_1 with IBX in different solvents



1a **4a** **5a**

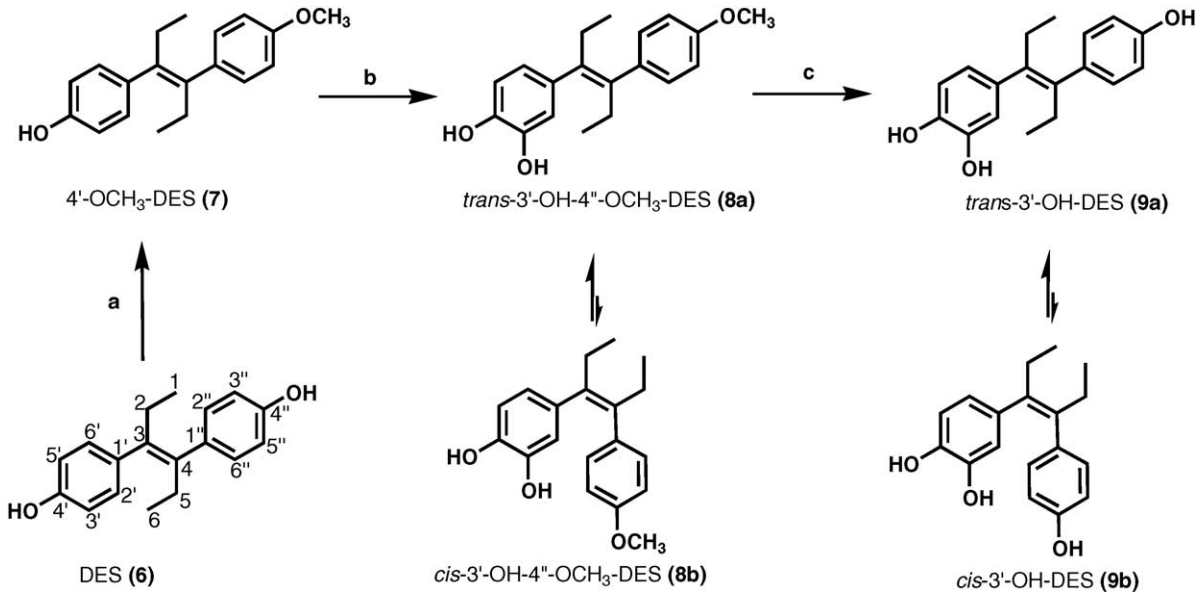
Solvent	Time (h)	Yield (%)	**4a**:**5a**	Recovered **1a** (%)
Acetone	10	50	2:1	39
Acetonitrile	10	55	1:1	33
DMF	3	79	1:1.2	0
Dioxane	3	65	1:1.1	5.1
DMF:HMPA	3	40	1:1	0
THF	3	76	1.2:1	0

3. Results and discussion

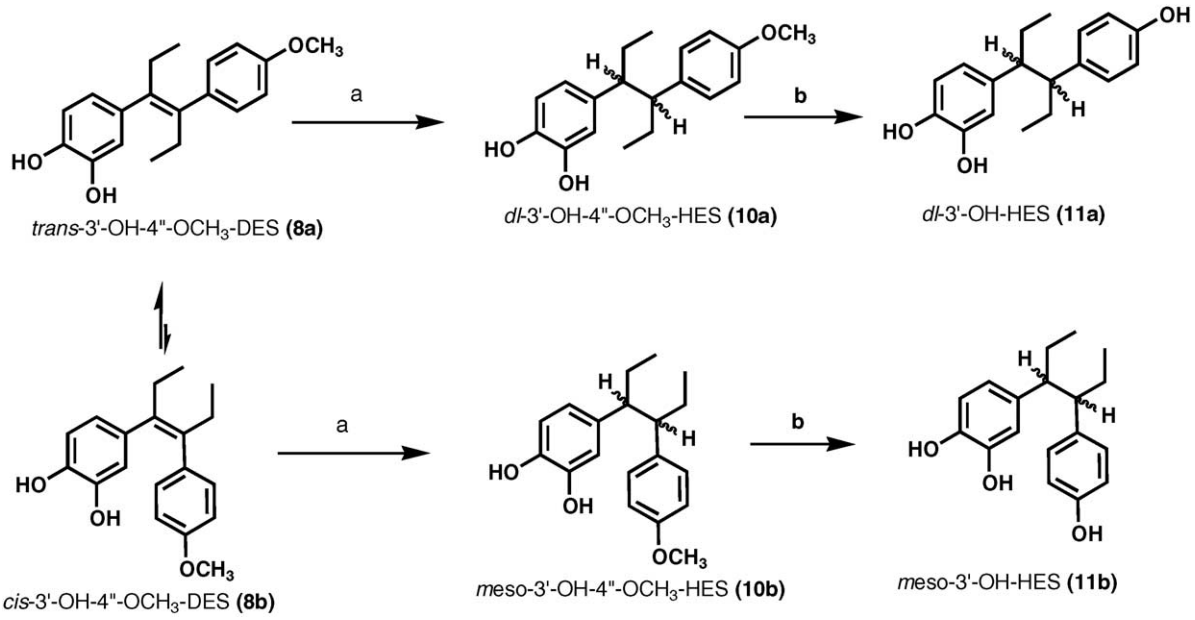
In light of a recently reported method for the preparation of o-quinones by the oxidation of the corresponding monophenolic compounds with IBX [27], the synthesis of catechol estrogens was carried out according to Scheme 1. Treatment of E_1 (**1a**) or E_2 (**1b**) with a stoichiometric amount of IBX in DMF at room temperature resulted in the formation of a mixture of the corresponding o-quinones (**2a/b** and **3a/b**). The quinones were easily reduced to the catechols 2-OHE_1/E_2 (**4a/b**) and 4-OHE_1/E_2 (**5a/b**) by treating with a 1 M solution of ascorbic acid or KI/acetic acid [24]. The separation of 2- and 4-OHE_1/E_2, thus formed, was achieved by column chromatography on silica gel impregnated with ascorbic acid as described by Gelbke and Knuppen [28].

Different solvents were tested to increase the overall yield of the reaction and to observe any selectivity in the formation of 4-OHE_1/E_2 over 2-OHE_1/E_2. Use of acetone or acetonitrile as solvent was not successful, and low reaction yields with the recovery of starting material were observed, even after the reaction mixture was stirred for a longer time. The low stability of the quinones rendered the reaction mixtures complex, thus making a longer reaction time practically nonfeasible. Use of dioxane as the solvent (Table 1) gave a 65% reaction yield as a 1:1 mixture of both catechols; however, some of the starting E_2 was also observed after 3 h. No starting E_2 was observed when DMF, DMF:hexamethylphosphoramide (HMPA) or tetrahydrofuran (THF) was used as solvent, and the overall reaction yields were found to be 79, 40, and 76%, respectively. Although the yield of reaction varied with different solvents, there was no practical selectivity in the synthesis of 4-OHE_1/E_2 over 2-OHE_1/E_2, and we always obtained a nearly 1:1 mixture of both catechols.

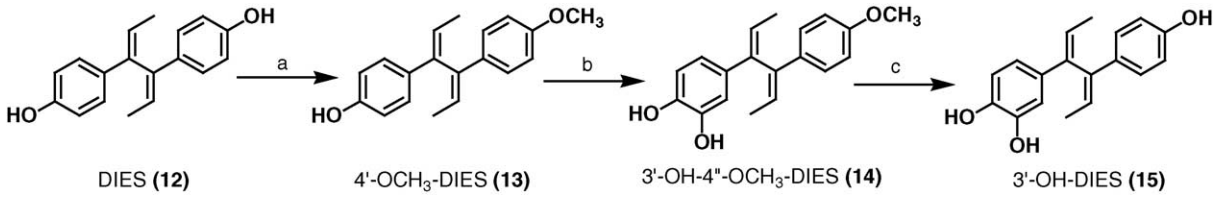
Synthesis of 3′-OH-DES (**9**) and 3′-OH-DIES (**15**) was initiated from the commercially available DES (**6**) and DIES (**12**), respectively (Schemes 2 and 4). Since both DES (**6**) and DIES (**12**) contain two phenolic rings, one of them must be protected with some easily removable protective group.



Scheme 2. Synthesis of *trans*- (**9a**) and *cis*-3′-OH-DES (**9b**). (a) NaH (1.2 eq), DMF, CH_3I (1.1 eq); (b) IBX, DMF, rt, in the dark for 1 h and then add 1 M ascorbic acid; (c) BBr_3, CH_2Cl_2, −78 °C.



Scheme 3. Synthesis of DL-3′-OH-HES (**11a**) and its meso-isomer (**11b**). (a) 5% Pd/C, H_2, ethyl acetate, rt; (b) BBr_3, CH_2Cl_2, −78 °C.



Scheme 4. Synthesis of 3′-OH-DIES (**15**). (a) NaH (1.2 eq), DMF, CH_3I (1.1 eq); (b) IBX, DMF, rt, in the dark for 1 h and then add 1 M ascorbic acid; (c) BBr_3, CH_2Cl_2, −78 °C.

We used both *tert*-butyldimethylsilyl (TBDMS) and methyl-ether as protective groups; however, we preferred methyl-ether groups over TBDMS ethers, because of the highly volatile nature of the latter. After careful monomethylation of DES (**6**) and DIES (**12**) with iodomethane (CH_3I) and sodium hydride (NaH) in DMF, methylated phenols **7** and **13** were achieved in 70 and 71% yields, respectively. Methylether-phenol **7** or **13** was then converted to the catechol **8** or **14**, respectively, by IBX in DMF, followed by treatment with ascorbic acid. Removal of the protective group in **8** or **14** was achieved by treating each compound with BBr_3 in dichloromethane to produce the target catechols 3′-OH-DES (**9**) or 3′-OH-DIES (**15**), respectively, in almost quantitative yields (Schemes 2 and 4).

Reduction of the C3-C4 double bond in **8** with 5% palladium on carbon (Pd/C) under a hydrogen atmosphere produced the HES derivative, **10**. We have observed that 3′-OH-4″-OCH_3-DES (**8**) was present as an equilibrium mixture of *trans*- (**8a**) and *cis*-isomers (**8b**) in an 8:2 ratio, respectively [25], if stored at room temperature for a longer time. Therefore, it may be desirable to separate both isomers before reduction, which can be achieved by using preparative HPLC. Alternatively, reduction of the mixture as such yielded both the DL-3′-OH-4″-OCH_3-HES (**10a**) and its meso-isomer (**10b**) in 8:2 ratio, respectively, as determined by HPLC and NMR analyses (Scheme 3). Separation of the diastereomers was achieved conveniently by preparative reverse phase HPLC. Deprotection of the methyl group by reacting **10a** or **10b** with BBr_3 in CH_2Cl_2 yielded the corresponding 3′-OH-HES (**11a** or **11b**) in good yield from **6**.

In conclusion, we report a one-step method for the synthesis of catechols of both natural and synthetic estrogens, starting from the corresponding monophenolic compounds.

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