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Synthesis of 7α-substituted derivatives of 17β-estradiol

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Abbreviations:
COSY, correlated spectroscopy;
LDA, lithium diisopropylamide;
E_2, 17β-estradiol;
MOM, methoxymethyl;
NMR, nuclear magnetic resonance;
PCC, pyridinium chlorochromate;
t-BuOK, potassium *t*-butoxide;
TEA, triethylamine;
TEMPO, tetramethylpyrrolidin-1-oxyl;
THF, tetrahydrofuran;
TLC, thin-layer chromatography;
TMS, tetramethylsilane

ABSTRACT

Estrogen receptor (ER) pure antagonists such as ICI-182,780 (fulvestrant) are effective alternatives to tamoxifen (an ER antagonist/weak partial agonist) in the treatment of post-menopausal, receptor-positive human breast cancers. Structurally, these pure antagonists contain the basic core structure of 17β-estradiol (E_2) with a long side chain attached to its C-7α position. We explored and compared in this study various synthetic routes for preparing a number of C-7α-substituted derivatives of E_2, which are highly useful for the design and synthesis of high-affinity ER antagonists, ER-based imaging ligands, and other ER-based multi-functional agents. Using E_2 as the starting material and 1-iodo-6-benzyloxyhexane as a precursor for the C-7α side chain, a seven-step synthetic procedure afforded 3,17β-bis(acetoxy)-7α-(6-hydroxyhexanyl)-estra-1,3,5(10)-triene (one of the derivatives prepared) in an overall yield of ~45% as compared to other known procedures that afforded substantially lower overall yield (8–27%). The synthetic steps for this representative compound include: (1) protection of the C-3 and C-17β hydroxyls of E_2 using methoxymethyl groups; (2) hydroxylation of the C-6 position of the bismethoxymethyl ether of E_2; (3) Swern oxidation of the C-6 hydroxy to the ketone group; (4) C-7α alkylation of the C-6 ketone derivative of E_2; (5) deprotection of the two methoxymethyl groups; (6) reprotection of the C-3 and C-6 free hydroxyls with acetyl groups; (7) removal of the C-6 ketone and the benzyl group on the side chain by catalytic hydrogenation in acetic acid. As predicted, two of the representative C-7α-substituted derivatives of E_2 synthesized in the present study retained strong binding affinities (close to those of E_2 and ICI-182,780) for the human ERα and ERβ subtypes as determined using the radioligand–receptor binding assays.

1. Introduction

Breast cancer is a serious health concern worldwide and is one of the leading causes of cancer-related mortality in women living in the United States [1]. The use of estrogen receptor (ER) antagonists has become a common strategy in the treatment of ER-positive human breast cancer [2,3]. Tamoxifen, a well-known antagonist of the human ER which also retains weak agonistic activity for the same receptor, is commonly prescribed nowadays for this purpose [2,3]. Partly because of its inherent partial agonistic activity (albeit very weak), breast cancer patients chronically treated with tamoxifen often experience relapse of the cancer. The newer ER antagonists such as ICI-182,780 (fulvestrant) and ICI-164,384 that are devoid of any ER agonistic activity have already been developed as effective alternatives to tamoxifen [4–6]. Studies have

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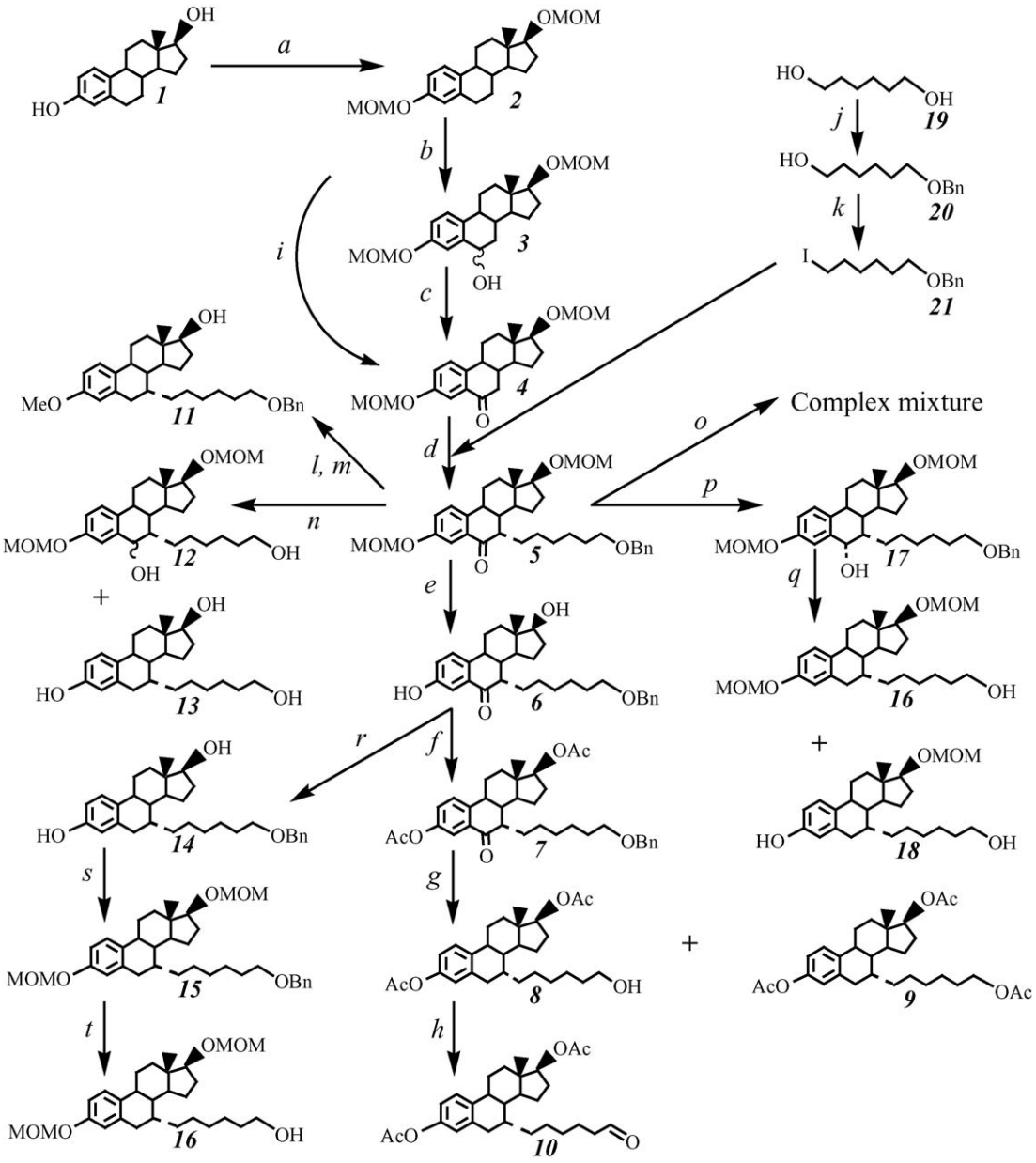

Scheme 1 – The flow chart for the synthesis of 7α-substituted derivatives of E_2 (compounds 8, 10, 13 and 16) using E_2 (compound 1) as the starting material. The reagents and the reaction conditions used are summarized below: (a) MOM chloride, diisopropylethylamine, THF, reflux, overnight; (b) (1) LDA, t-BuOK, THF, −78 °C, (2) $B(OMe)_3$, 0 °C and (3) H_2O_2, H_2O, 25 °C; (c) Swern oxidation; (d) t-BuOK, THF, 0 °C; (e) 6 M HCl in THF, room temperatures, overnight; (f) Ac_2O, pyridine; (g) 10% Pd–C, H_2, AcOH, reflux 3 h; (h) Swern oxidation, 98%; (i) CrO_3, 3,5-dimethylpyrazole, CH_2Cl_2 or PCC, CH_2Cl_2, molecular sieves, reflux; (j) benzyl bromide, NaOH, 15-crown-5 ether, THF, 10 °C; (k) triphenylphosphine, imidazole, I_2, CH_2Cl_2; (l) $BF_3{\cdot}Et_2O$, Et_3SiH, CH_2Cl_2; (m) $NaBH_3CN$, $BF_3{\cdot}Et_2O$, THF, reflux; (n) 10% Pd–C, H_2, ethanol, 60 °C, 2 h; (o) H_2NNH_2, KOH, $(CH_2CH_2OH)_2$, 200 °C, 3 h; (p) $NaBH_4$, MeOH, 4 h, 98%; (q) 10% $Pd(OH)_2/C$, H_2, 40 psi, ethanol, 60 °C, 3 h; (r) $BF_3{\cdot}Et_2O$, Et_3SiH, CH_2Cl_2; (s) MOMCl, diisopropylethylamine, THF, reflux overnight, 93%; (t) 10% Pd–C, H_2, ethanol, 40 psi, 2 h.

shown that human breast cancer cells that became resistant to tamoxifen were still sensitive to the anticancer effect of fulvestrant, which was recently approved for use in the United States for treatment of postmenopausal, ER-positive progressive breast cancers [4,5]. Structurally, these ER pure antagonists all contain the E_2 core structure with a long side chain attached to the C-7α position. Several of the 7α-substituted derivatives of E_2 (such as compounds **8**, **10**, **13** and **16** in Scheme 1) are highly useful intermediates for the rational design and synthesis of high-affinity ER antagonistic analogs as well as ER-based imaging ligands for human breast cancers or other ER-based multi-functional agents [7–12]. While there were several published methods describing the syntheses of this type of intermediates, usually there were also limitations when they were used for large-scale production of the desired intermediates, such as the paucity of the expensive starting

materials required in some of the synthetic procedures, the highly stringent and difficult conditions for some of the reaction steps involved, and/or the relatively low overall yield for the final product (ranging from 8 to 27%) [8–10]. In the present study, we explored and also compared a number of synthetic procedures for preparing several 7α-substituted derivatives of E_2 by modifying the reaction steps and also by significantly improving the overall yield for the desired products.

2. Experimental

2.1. Chemicals and reagents

Most of the commercially available reagents were obtained from the Sigma–Aldrich Chemical Co. (Milwaukee, WI, USA) or ACROS (through Fisher Scientific, Atlanta, GA, USA). 17β-Estradiol (E_2) was purchased from Steraloids Inc. (Newport, RI, USA). Tetrahydrofuran (THF) was distilled over sodium/benzophenone ketyl and dichloromethane distilled over calcium hydride in our laboratory prior to use. Most of the chemicals and solvents used in this study were of ACS grade and used directly without additional steps of purification.

[2,4,6,7,16,17-^{3}H]E_2 (specific activity of 123 Ci/mmol) was obtained from NEN Life Sciences (Boston, MA, USA), and it was re-purified using an HPLC method before use [13]. The recombinant human ERα and ERβ proteins and bovine serum albumin (BSA) were obtained from PanVera Corporation (Madison, WI, USA). Hydroxylapatite (HAP) and bovine serum albumin were obtained from Calbiochem (San Diego, CA, USA).

2.2. Spectrometric analyses

Mass spectra were recorded with a VG70S analytical mass spectrometer. An aliquot of the ethanol solution of the test compound was used for the direct-probe mass analysis. The nuclear magnetic resonance (NMR) spectra of all test compounds were recorded in deuterochloroform solution using a Varian Mercury/VX 300 spectrometer with tetramethylsilane (TMS) as an internal standard ($\delta = 0$), unless noted otherwise.

2.3. Synthesis of 3,17β-bis(methoxymethoxy)estra-1,3,5(10)-trien-6-ol (compound 3)

Using E_2 (compound **1** in Scheme 1) as the starting material, the 3,17β-bisMOM ether of E_2 (compound **2**) was prepared according to a previously published method [14] by refluxing overnight with MOM chloride and diisopropylethylamine in THF. The yield of this reaction was ~96%.

After a solution of *t*-BuOK (9.95 g, 88.9 mmol) in dry THF (100 ml) was cooled to −78 °C, LDA (49.4 ml, 1.8 M, 88.9 mmol) was added and the mixture was stirred for 30 min at −78 °C. Compound **2** (8.0 g, 22.2 mmol) in THF (30 ml) was then added to the reaction vessel, and the mixture (in a dark red color) was stirred for 3 h at −78 °C. Trimethyl borate (30 ml) was added, and the reaction was warmed to 0 °C, yielding a milky yellow suspension. After stirring for 2 h, H_2O_2 (34 ml, 30% in water) was added, and the mixture was stirred for 1 h at room temperatures. The reaction mixture was cooled to 0 °C and 10% $Na_2S_2O_3$ (100 ml) was added. After extraction with ethyl acetate and drying over Na_2SO_4/Na_2CO_3, flash column chromatography (hexane:ethyl acetate = 3:1) yielded compound **3** (7.70 g, 93%). Data from spectrometric analyses of the afforded compound are summarized below. ^{1}H NMR (300 Hz, $CDCl_3$): 7.18 (1H, d, $J = 2.7$ Hz), 7.12 (1H, d, $J = 8.4$ Hz), 6.83 (1H, dd, $J = 8.4$, 2.4 Hz), 5.09 (1H, s), 5.08 (1H, s), 4.73 (1H, m), 4.57 (1H, s), 4.56 (1H, s), 3.53 (1H, t, $J = 8.7$ Hz), 3.39 (3H, s), 3.29 (3H, s), 2.23–1.89 (6H, m), 1.64–1.16 (8H, m) and 0.72 (3H, s). MS (*m*/z, $C_{22}H_{32}O_5$, M^+ 376): 358 (base peak), 376. HRMS: 376.2250 (calculated) and 376.2255 (observed).

2.4. Synthesis of 3,17β-bis(methoxymethoxy)estra-1,3,5(10)-trien-6-one (compound 4)

After a solution of CH_2Cl_2 (70 ml) and oxalyl chloride (3.6 ml, 41.9 mmol) in a 250-ml three-neck round-bottom flask was cooled to −78 °C, Me_2SO (5.9 ml, 83 mmol, in 15 ml CH_2Cl_2) was added slowly to the stirred oxalyl chloride solution and the reaction mixture was stirred further for 5 min. Note that the addition of the Me_2SO solution needs to be relatively slow so that the temperatures of the reaction mixture would not rise above −50 °C (usually controlled between −50 and −60 °C), which is critical for this reaction. After that, compound **3** (13 g, 34.5 mmol, in 30 ml CH_2Cl_2) was slowly added over 15 min, and the mixture was stirred for an additional 25 min. Then TEA (24 ml) was added and the reaction mixture was stirred for 5 min and allowed to warm to room temperatures. Water (100 ml) was added and the aqueous phase was extracted with CH_2Cl_2 (3 × 50 ml). The organic phase was combined, washed with a saturated NaCl solution (100 ml) and dried over anhydrous Na_2SO_4. Flash chromatography afforded compound **4** (12.6 g, 98% yield). Data from spectrometric analyses of the title compound are summarized below. ^{1}H NMR (300 Hz, $CDCl_3$): 7.69 (1H, d, $J = 2.7$ Hz), 7.35 (1H, d, $J = 8.4$ Hz), 7.20 (1H, dd, $J = 8.4$, 2.7 Hz), 5.19 (2H, s), 4.65 (2H, brs), 3.62 (1H, t, $J = 8.4$ Hz), 3.46 (3H, s), 3.37 (3H, s), 2.73 (1H, dd, $J = 16.8$, 3.3 Hz), 2.60–1.86 (6H, m), 1.72–1.52 (3H, m), 1.46–1.30 (3H, m) and 0.80 (3H, s). MS (*m*/z, $C_{22}H_{30}O_5$, M^+ 374): 374 (base peak). HRMS: 374.2093 (calculated) and 374.2086 (observed).

2.5. Synthesis of 3,17β-bis(methoxymethoxy)-7α-(6-benzyloxyhexanyl)-estra-1,3,5(10)-trien-6-one (compound 5)

A 1.0 M solution of *t*-BuOK in THF (20 ml, 5.35 mmol) was added to a cooled solution (at 0 °C) of compound **4** (1.0 g, 2.67 mmol) in THF (20 ml). The reaction mixture was stirred at 0 °C for 40 min and then cooled to −78 °C. 6-Benzyl hexane iodide (compound **21**, 2.55 g, 8.02 mmol) was added dropwise to the solution. The reaction mixture was allowed to warm to 0 °C and stirred for 2 h, and then to room temperatures and stirred overnight. The reaction was quenched with water and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4 and solvent was evaporated in vacuo. Flash chromatography (hexane:ethyl acetate = 4:1) afforded compound **5** in 65% yield (0.98 g). Data from spectrometric analyses of the afforded compound are summarized below. ^{1}H NMR (300 Hz, $CDCl_3$): 7.68 (1H, d, $J = 3.0$ Hz), 7.29 (6H, m), 7.18 (1H, dd, $J = 8.7$, 3.0 Hz), 5.18

(2H, s), 4.64(2H, brs), 4.46 (2H, s), 3.63 (1H, t, J = 8.4 Hz), 3.45 (3H, s), 3.42 (2H, t, J = 6.6 Hz), 3.36 (3H, s), 2.69 (1H, t-d, J = 10.8, 4.2 Hz), 2.47–2.33 (2H, m), 2.16–1.98 (3H, m), 1.64–1.50 (8H, m), 1.41–1.28 (8H, m) and 0.79 (3H, s). MS (*m*/z, $C_{35}H_{48}O_6$, M^+ 564): 91 (base peak), 473, 564. HRMS: 564.3451 (calculated) and 564.3465 (observed).

2.6. Synthesis of 3,17β-bis(hydroxy)-7α-(6-benzyloxyhexanyl)-estra-1,3,5(10)-trien-6-one (compound 6)

A solution containing compound **5** (2.1 g, 3.7 mmol), HCl (25 ml, 6 M) and THF (25 ml) was stirred at room temperatures for 18 h. The reaction mixture was poured into water and then extracted with ethyl acetate (4 × 50 ml). The combined organics were dried over Na_2SO_4, concentrated and then chromatographed (hexane:ethyl acetate = 2:1) to afford compound **6** (1.67 g, 94% yield). Data from spectrometric analyses of the afforded compound are summarized below. ^{1}H NMR (300 Hz, $CDCl_3$): 7.57 (1H, d, J = 3.0 Hz), 7.33–7.26 (6H, m), 7.05 (1H, dd, J = 8.4, 2.7 Hz), 6.28 (1H, s), 4.48 (2H, s), 3.77 (1H, brs), 3.43 (2H, t, J = 6.6 Hz), 2.68 (1H, t-d, J = 11.1, 4.2 Hz), 2.43 (2H, m), 2.11 (3H, m), 1.77 (1H, s), 1.61–1.18 (16H, m) and 0.78 (3H, s). MS (*m*/z, $C_{31}H_{40}O_4$, M^+ 476): 91 (base peak), 385, 476. HRMS: 476.2927 (calculated) and 476.2934 (observed).

2.7. Synthesis of 3,17β-bis(acetoxy)-7α-(6-benzyloxyhexanyl)-estra-1,3,5(10)-trien-6-one (compound 7)

Under nitrogen, acetic anhydride (8 ml, 85 mmol) was added to a solution of compound **6** (2.5 g, 5.25 mmol) in dry pyridine (50 ml). The reaction mixture was stirred at room temperatures overnight. The solvents were removed in vacuo and the residue was purified by column chromatography (hexane:ethyl acetate = 4:1) to yield compound **7** (2.85 g, 97%). Data from spectrometric analyses of the afforded compound are summarized below. ^{1}H NMR (300 Hz, $CDCl_3$): 7.73 (1H, d, J = 2.7 Hz), 7.40 (1H, d, J = 8.7 Hz), 7.31–7.19 (6H, m), 4.71 (1H, t, J = 8.7 Hz), 4.46 (2H, s), 4.42 (2H, t, J = 6.6 Hz), 2.72 (1H, m), 2.47–1.91 (5H, m), 2.27 (3H, s), 2.04 (3H, s) and 1.87–1.20 (16H, m), 0.80 (3H, s). MS (*m*/z, $C_{35}H_{44}O_6$, M^+ 560): 91 (base peak), 469, 560. HRMS: 560.3138 (calculated) and 560.3149 (observed).

2.8. Synthesis of 3,17β-bis(acetoxy)-7α-(6-hydroxyhexanyl)-estra-1,3,5(10)-triene (compound 8) and 3,17β-bis(acetoxy)-7α-(6-acetoxyhexanyl)-estra-1,3,5(10)-triene (compound 9)

Compound **7** (2.5 g, 4.46 mmol) and 10% palladium on carbon (400 mg) were added to acetic acid (50 ml). The reaction vessel was purged with nitrogen, and hydrogen was added by continuous bubbling for 3 h at 70 °C. The mixture was filtered through celite and the solvent was removed in vacuo. Flash chromatography (hexane:ethyl acetate = 3:1) afforded compound **8** (1.83 g, 90% yield) and compound **9** (67 mg, 3% yield). Data from spectrometric analyses of the afforded compounds are summarized below. Compound **8**—^{1}H NMR (300 Hz, $CDCl_3$): 7.24 (1H, d, J = 8.4 Hz), 6.80 (1H, dd, J = 8.4, 2.4 Hz), 6.75 (1H, d, J = 2.4 Hz), 4.67 (1H, t, J = 7.5 Hz), 3.55 (2H, t, J = 6.6 Hz), 2.87 (1H, dd, J = 16.8, 5.1 Hz), 2.72 (1H, d, J = 16.8 Hz), 2.50 (1H, brs), 2.40–2.10 (3H, m), 2.24 (3H, s), 2.03 (3H, s), 1.86–1.0 (19H, m) and 0.79 (3H, s). MS (*m*/z, $C_{28}H_{40}O_5$, M^+ 456): 414 (base peak), 456. HRMS: 456.2875 (calculated) and 456.2862 (observed). Compound **9**—^{1}H NMR (300 Hz, $CDCl_3$): 7.24 (1H, d, J = 8.4 Hz), 6.80 (1H, dd, J = 8.4, 2.4 Hz), 6.75 (1H, d, J = 2.4 Hz), 4.68 (1H, t, J = 7.8 Hz), 4.0 (2H, t, J = 6.7 Hz), 2.88 (1H, dd, J = 16.8, 5.1 Hz), 2.72 (1H, d, J = 16.8 Hz), 2.38–2.16 (3H, m), 2.24 (3H, s), 2.02 (3H, s), 2.0 (3H, s), 1.86–0.94 (19H, m) and 0.79 (3H, s). MS (*m*/z, $C_{30}H_{42}O_6$, M^+ 498): 456 (base peak), 498. HRMS: 498.2981 (calculated) and 498.2983 (observed).

2.9. Synthesis of 3,17(-bis(acetoxy)-7(-(6-hexanyladhyde)-estra-1,3,5(10)-triene (compound 10)

A solution of CH_2Cl_2 (10 ml) and oxalyl chloride (0.095 ml, 1.1 mmol) were placed in a 50-ml three-neck round-botton flask equipped with a thermometer. Me_2SO (0.16 ml, 2.2 mmol) dissolved in CH_2Cl_2 (5 ml) was added to the stirred oxalyl chloride solution at −50 to −60 °C. The reaction mixture was stirred for 5 min and compound **8** (0.5 g, 0.91 mmol, in 30 ml CH_2Cl_2) was added over 5 min, and stirring was continued for additional 25 min. TEA (2 ml) was added, and the reaction mixture was stirred for 5 min and then allowed to warm to room temperatures. Water (30 ml) was added and then the aqueous layer was re-extracted with CH_2Cl_2 (3 × 30 ml). The organic layer was combined, washed with saturated NaCl solution (50 ml), and dried over anhydrous Na_2SO_4. Flash chromatography afforded compound **10** (0.4 g, 98%). Data from spectrometric analysis of the afforded compound are summarized below. ^{1}H NMR (300 Hz, $CDCl_3$): 9.66 (1H, t, J = 1.8Hz), 7.21 (1H, d, J = 8.7 Hz), 6.78 (1H, dd, J = 8.7, 2.7 Hz), 6.72 (1H, d, J = 2.7 Hz), 4.65 (1H, t, J = 7.5 Hz), 2.85 (1H, dd, J = 16.8, 4.8 Hz), 2.69 (1H, d, J = 16.8 Hz), 2.33 (2H, t-d, J = 7.5, 1.8Hz), 2.24–2.07 (3H, m), 2.21 (3H, s), 1.99 (3H, s), 1.82–0.95 (17H, m) and 0.77 (3H, s). MS (*m*/z, $C_{28}H_{38}O_5$, M^+ 454): 412 (base peak), 454. HRMS: 454.2719 (calculated) and 454.2725 (observed).

2.10. Synthesis of 3-methoxy-7α-(6-benzyloxyhexanyl)-estra-1,3,5(10)-trien-17β-ol (compound 11)

Triethylsilane (30 ml) was added to a solution of compound **5** (1.46 g, 2.6 mmol) in CH_2Cl_2 (100 ml). The mixture was cooled to 0 °C and boron trifluoride etherate (100 ml) was added dropwise. The yellow-green solution was warmed to room temperatures and stirred overnight. The reaction was hydrolyzed with the slow addition of 10% K_2CO_3 (500 ml) and then passed through a plug of silica. The aqueous layer was extracted with CH_2Cl_2, and the organic fractions were combined and dried over Na_2SO_4. Following evaporation of the CH_2Cl_2, the residue was purified by flash chromatography (hexane:ethyl acetate = 5:2) to yield compound **11** (0.49 g, 40%) as the major compound. Data from spectrometric analyses of the afforded compound are summarized below. ^{1}H NMR (300 Hz, $CDCl_3$): 7.34 (5H, m), 7.14 (1H, d, J = 8.4 Hz), 6.62 (1H, dd, J = 8.4, 2.4 Hz), 6.53 (1H, d, J = 2.7 Hz), 4.51 (2H, s), 3.46 (2H, t, J = 6.6 Hz), 3.40 (3H, s), 3.36 (1H, t, J = 8.4 Hz), 2.85 (1H, dd, J = 16.8, 5.1 Hz), 2.68 (1H, d, J = 16.8 Hz), 2.30 (2H, m), 2.05 (2H, m), 1.71–1.08 (19H, m) and 0.81 (3H, s). MS (*m*/z, $C_{32}H_{44}O_3$, M^+ 476): 91 (base peak), 385, 476. HRMS: 476.3290 (calculated) and 476.3285 (observed).

2.11. Synthesis of 3,17β-bis(methoxymethoxy)-7α-(6-hydroxyhexanyl)-estra-1,3,5(10)-trien-6-ol (compound 12) and 7α-(6-hydroxyhexanyl)-17β-estradiol (compound 13)

Compound **5** (0.73 g, 1.3 mmol) and 10% palladium on carbon (100 mg) were added to ethanol (25 ml). The reaction vessel was purged with nitrogen, and hydrogen was added by continuous bubbling for 2 h at 60 °C. The mixture was filtered through celite and the solvent was removed in vacuo. Flash chromatography (hexane:ethyl acetate = 3:1) afforded compound **12** (0.12 g, 20% yield) and compound **13** (0.20 g, 42%). Data from spectrometric analyses of the afforded compounds are summarized below. Compound **12**—^{1}H NMR (300 Hz, $CDCl_3$): 7.30 (1H, d, J = 2.4 Hz), 7.16 (1H, d, J = 8.4 Hz), 6.88 (1H, dd, J = 8.4, 2.4 Hz), 5.16 (2H, s), 4.87(1H, d, J = 5.1 Hz), 4.64 (2H, s), 3.59 (3H, m), 3.46 (3H, s), 3.36 (3H, s), 2.22–1.95 (8H, m), 1.63–1.22 (16H, m) and 0.79 (3H, s). MS (*m*/z, $C_{28}H_{44}O_6$, M^+ 476): 444 (base peak), 458, 476. Compound **13**—^{1}H NMR (300 Hz, $CDCl_3$): 7.15 (1H, d, J = 8.4 Hz), 6.62 (1H, dd, J = 8.4, 2.4 Hz), 6.54 (1H, d, J = 2.7 Hz), 3.74 (1H, t, J = 8.7 Hz), 3.62 (2H, t, J = 6.6 Hz), 2.86 (1H, dd, J = 16.8, 5.1 Hz), 2.69 (1H, d, J = 16.8 Hz), 2.31–1.22 (25H, m) and 0.78 (3H, s). MS (*m*/z, $C_{24}H_{36}O_3$, M^+ 372): 372 (base peak).

2.12. Synthesis of 3,17β-bis(hydroxy)-7α-(6-benzyloxyhexanyl)-estra-1,3,5(10)-trien (compound 14)

Compound **6** (0.34 g, 0.71 mmol) was dissolved in methylene chloride (20 ml). To this solution was added triethylsilane (3.5 ml), followed by boron trifluoride etherate (12.5 ml) dropwise. The reaction was stirred for 2 days at room temperatures, cooled to 0 °C, and quenched with 10% potassium carbonate (20 ml). The heterogeneous solution was filtered through a short silica plug, and the organic layer separated. The aqueous layer was extracted twice with methylene chloride (20 ml). The organic fractions were combined, dried over sodium sulfate, and evaporated in vacuo to a yellow solid. Purification by flash chromatography (hexane:ethyl acetate = 2:5) provided compound **14** (0.23 g, 70%). Data from spectrometric analyses of the afforded compound are summarized below. ^{1}H NMR (300 Hz, $CDCl_3$): 7.23–7.15 (5H, m), 7.03 (1H, d, J = 8.4 Hz), 6.54 (1H, dd, J = 8.4, 2.8 Hz), 6.44 (1H, d, J = 2.8 Hz), 4.42 (2H, s), 3.66 (1H, t, J = 8.4 Hz), 3.36 (2H, t, J = 6.4 Hz), 2.75 (1H, dd, J = 16.8, 4.4 Hz), 2.58 (1H, d, J = 16.8 Hz), 2.20 (2H, m), 2.10–1.13 (22H, m) and 0.71 (3H, s). MS (*m*/z, $C_{31}H_{42}O_3$, M^+ 462): 91 (base peak), 371, 462. HRMS: 462.3134 (calculated) and 462.3141 (observed).

2.13. Synthesis of 3,17β-bis(methoxymethoxy)-7α-(6-benzyloxyhexanyl)-estra-1,3,5(10)-trien (compound 15)

MOMCl (0.19 ml, 2.5 mmol) was added dropwise to a cold (0 °C) solution of compound **14** (0.23 g, 0.5 mmol) and diisopropylethylamine (0.71 ml, 2.98 mmol) in THF (50 ml). Upon completion of the addition, the reaction mixture was allowed to warm up to room temperatures, stirred for 1 h at the same temperatures, and then heated at reflux overnight. The mixture was allowed to cool, and then saturated ammonium chloride solution (40 ml) was added. After extraction with ether (4 × 50 ml), the combined organics were washed with saturated brine (50 ml), dried over sodium sulfate, and concentrated. The crude product was purified by flash chromatography (hexane:ethyl acetate = 4:1) to afford compound **15** (0.26 g, 93%). Data from spectrometric analyses of the afforded compound are summarized below. ^{1}H NMR (300 Hz, $CDCl_3$): 7.27–7.17 (5H, m), 7.11 (1H, d, J = 8.4 Hz), 6.75 (1H, dd, J = 8.4, 2.4 Hz), 6.67 (1H, d, J = 2.4 Hz), 5.07 (2H, s), 4.58(2H, s), 4.42 (2H, s), 3.56 (1H, t, J = 8.1 Hz), 3.41 (3H, s), 3.37 (2H, t, J = 6.6 Hz), 3.29 (3H, s), 2.79 (1H, dd, J = 16.8, 4.8 Hz), 2.65 (1H, d, J = 16.8 Hz), 2.20 (2H, m), 2.24–1.12 (20H, m) and 0.73 (3H, s). MS (*m*/z, $C_{35}H_{50}O_5$, M^+ 550): 91 (base peak), 550. HRMS: 550.3658 (calculated) and 550.3651 (observed).

2.14. Synthesis of 3,17β-bis(methoxymethoxy)-7α-(6-hydroxyhexanyl)-estra-1,3,5(10)-trien (compound 16)

Compound **15** (98 mg, 0.18 mmol) and 10% palladium hydroxide on carbon (100 mg) were added to 20 ml of ethanol. The reaction vessel was evacuated of air and filled to a pressure of 40 psi with hydrogen, and then shaken for 2 h at room temperatures. The mixture was filtered through celite and the solvent was removed in vacuo. Flash chromatography (hexane:ethyl acetate = 3:1) afforded compound **16** (78 mg, 95% yield). Data from spectrometric analyses of the afforded compound are summarized below. ^{1}H NMR (300 Hz, $CDCl_3$): 7.12 (1H, d, J = 8.4 Hz), 6.75 (1H, dd, J = 8.4, 2.8 Hz), 6.67 (1H, d, J = 2.8 Hz), 5.06 (2H, s), 4.58 2H, s), 3.55 (1H, t, J = 8.7 Hz), 3.52 (2H, t, J = 6.8 Hz), 3.40 (3H, s), 3.29 (3H, s), 2.81 (1H, dd, J = 16.8, 5.2 Hz), 2.65 (1H, d, J = 16.8 Hz), 2.24 (2H, m), 2.01–1.13 (21H, m) and 0.73 (3H, s). MS (*m*/z, $C_{28}H_{44}O_5$, M^+ 460): 428 (base peak), 460. HRMS: 460.3189 (calculated) and 460.3184 (observed).

2.15. Synthesis of 3,17β-bis(methoxymethoxy)-7α-(6-benzyloxyhexanyl)-estra-1,3,5(10)-trien-6-ol (compound 17)

Compound **5** (0.5 g, 0.89 mmol) was dissolved in methanol (20 ml). To this solution was added sodium borohydride (0.68 g, 17.8 mmol). The reaction mixture was stirred at room temperatures for 4 h. The methanol was removed in vacuo, and the residue was then dissolved in water (20 ml). The aqueous solution was extracted with methylene chloride (3 × 20 ml). Organic fractions were combined, dried over sodium sulfate, and concentrated. The crude product was purified by flash chromatography (hexane:ethyl acetate = 3:1) to afford compound **17** (0.49 g, 98%). Data from spectrometric analyses of the afforded compound are summarized below. ^{1}H NMR (300 Hz, $CDCl_3$): 7.25–7.16 (6H, m), 7.11 (1H, d, J = 8.7 Hz), 6.82 (1H, dd, J = 8.7, 2.7 Hz), 5.08 (2H, d, J = 5.1 Hz), 4.80 (1H, d, J = 5.1 Hz), 4.58 (2H, s), 4.41 (2H, s), 3.56 (1H, t, J = 8.4 Hz), 3.39 (3H, s), 3.36 (2H, t, J = 6.6 Hz), 3.29 (3H, s), 2.31 (2H, m), 1.99 (4H, m), 1.59–1.05 (17H, m) and 0.73 (3H, s). MS (*m*/z, $C_{35}H_{50}O_6$, M^+ 566): 91 (base peak), 548, 566. HRMS: 566.3607 (calculated) and 566.3616 (observed).

2.16. Synthesis of 3-hydroxy-17β-methoxymethoxy-7α-(6-hydroxyhexanyl)-estra-1,3,5(10)-trien (compound 18)

Compound **17** (0.4 g, 0.7 mmol) and 10% palladium hydroxide on carbon (300 mg) were added to ethanol (50 ml). The reaction vessel was evacuated of air and filled to a pressure of 40 psi with hydrogen, and then shaken for 3 h at 60 °C. The mixture was filtered through celite and the solvent was removed

in vacuo. Flash chromatography (hexane:ethyl acetate = 3:1) afforded compound **16** (0.14 g, 43% yield) and compound **18** (0.11 g, 36% yield). Data from spectrometric analyses of the afforded compound are summarized below. (The data for compound **16** are listed under Section 2.14.) For compound **18**—^{1}H NMR (300 Hz, $CDCl_3$): 7.06 (1H, d, J = 8.4 Hz), 6.55 (1H, dd, J = 8.4, 2.7 Hz), 6.47 (1H, d, J = 2.7 Hz), 4.59 (2H, s), 3.56 (2H, t, J = 6.6 Hz), 3.31 (3H, s), 3.27 (1H, t, J = 8.7 Hz), 2.78 (1H, dd, J = 16.5, 4.8 Hz), 2.56 (1H, d, J = 16.5 Hz), 2.23 (2H, m), 2.02–1.12 (22H, m) and 0.72 (3H, s). MS (m/z, $C_{26}H_{40}O_4$, M^+ 416): 386 (base peak), 416. HRMS: 416.2927 (calculated) and 416.2933 (observed).

2.17. *Synthesis of 1-iodo-6-benzyloxyhexane (compound 21)*

Using 1,6-hexanediol as starting material, its monobenzylation product (compound **20**) was first obtained by using a known procedure [25] with a yield of 86%. To the dry CH_2Cl_2 (40 ml) the following reactants were added in a sequential order: triphenylphosphine (3.14 g, 12 mmol), imidazole (0.82 g, 12 mmol) and iodine (3.05 g, 12 mmol). The mixture was stirred at room temperatures for 15 min. A solution of the compound **20** (2.1 g, 10 mmol) in dry dichloromethane (10 ml) was added and the mixture was stirred at room temperatures under N_2 for 5 h. The disappearance of compound **20** and the formation of an alkyl iodide were monitored using TLC. When the reaction was complete, the solvents were removed in vacuo and the product was purified by passing though a silica gel column with hexane as the mobile phase and the fractions containing the desired product were combined (3.1 g, 98% yield). Data from spectrometric analyses of the afforded compound are summarized below. ^{1}H NMR (300 Hz, $CDCl_3$): 7.36–7.24 (5H, m), 4.50 (2H, s), 3.62 (2H, t, J = 6.6 Hz), 3.47 (2H, t, J = 6.6 Hz), 1.65–1.54 (4H, m) and 1.42–1.36 (4H, m). MS (m/z, $C_{13}H_{19}IO$, M^+ 318): 91 (base peak), 227, 318. HRMS: 318.0481 (calculated) and 318.0488 (observed).

2.18. *ERα and ERβ binding assays*

The ERα and ERβ competitive binding assays were performed according to the method described in our recent study [15]. The ER binding buffer used for dilution of the receptor preparations consisted of 10% glycerol, 2 mM dithiothreitol, 1 mg/ml BSA and 10 mM Tris–HCl (pH 7.5). The ERα washing buffer contained 40 mM Tris–HCl and 100 mM KCl (pH 7.4), but the ERβ washing buffer contained only 40 mM Tris–HCl (pH 7.5). The 50% hydroxylapatite slurry was adjusted to a final concentration of 50% (v/v) by using the 50 mM Tris–HCl solution (pH 7.4). The reaction mixture contained 50 μl of varying concentrations of the test compound in the ER binding buffer, and 45 μl of [^{3}H]E_2 solution (at 22.22 nM). Then 5 μl of ERα or ERβ protein was added and mixed gently. Nonspecific binding by the [^{3}H]E_2 was determined by addition of a 400-fold concentration of the nonradioactive E_2. The binding mixture was incubated at room temperatures for 2 h. At the end of the incubation, 100 μl of the HAP slurry was added and the tubes were incubated on ice for 15 min with three times of brief vortexing. An aliquot (1 ml) of the washing buffer was added, mixed and centrifuged at 10,000 × g for 1 min, and the supernatants were discarded. This wash step was repeated twice. The HAP pellets were then resuspended in 200 μl ethanol, and the content was transferred to scintillation vials for measurement of the ^{3}H radioactivity in a liquid scintillation counter (Packard Tri-CARB 2900 TR; Downers Grove, IL, USA). The data obtained from duplicate measurements were expressed as the percent specific binding of [^{3}H]E_2 versus the log molar concentration of the competing compound. The IC_{50} values (calculated using the SigmaPlot software) represent the concentration of the test compound required to reduce the [^{3}H]E_2 binding by 50%.

3. Results and discussion

With E_2 (compound **1**) as the starting material, E_2 3,17β-bisMOM ether (compound **2**) was prepared according to a published method [14] by refluxing overnight with MOM chloride and diisopropylethylamine in THF. The reasons for using MOM chloride to protect the two free hydroxyls of E_2 are that the formation of E_2 3,17β-bisMOM ether gives a very high yield (almost quantitative) and that this reaction avoids the generation of diastereomer mixtures which would hamper facile assignment of the side chain stereochemistry by NMR.

There are two commonly used strategies to introduce a C-6 ketone group to compound **2**, one is the oxidation of the 6-benzyl position of the *O*-protected E_2 or estrone derivatives using chromium(VI) compounds [16,17], and the other is the oxidation of the C-6 organometallic derivatives of E_2 obtained through hydrogen–metal exchange [18,19]. For the first strategy, we tried with two methods, one that used CrO_3 and 3,5-dimethylpyrazole [20,21] and the other that used PCC [22], to oxidize E_2 bisMOM ether (compound **2**) to form a C-6 ketone derivative (compound **4**). However, these two methods only produced a rather low yield (20–25%), and there was a side product simultaneously formed which was difficult to separate from our desired product (compound **4**). After a number of trials with a few other approaches which also gave relatively low yields, the second strategy was then employed [19]. The bisMOM ether of E_2 (compound **2**) was first deprotonated under the "superbase" conditions employing a 1:1 ratio of *t*-BuOK and LDA. The anion was trapped with trimethyl borate to yield an intermediate borate ester that was subsequently oxidized with hydrogen peroxide to an epimeric mixture of alcohols (compound **3**). When we tried to obtain the C-6 ketone product (compound **4**) through oxidation of compound **3** (an epimeric mixture of alcohols) employing the reaction conditions described in the literature [8,9,18,19], such as PCC [9] or sodium hypochlorite in the presence of tetramethylpyrrolidin-1-oxyl (TEMPO) as catalyst [19], we noted that the overall yield was indeed substantially improved (65–75%). However, we encountered a similar situation which occurred in the first strategy, namely, the simultaneous formation of a side product (in considerable amounts) which was difficult to separate from the product of interest (compound **4**). This side product was not further identified. Finally, we found that compound **4** could be obtained quite readily and almost quantitatively from compound **3** by Swern oxidation [23]. This modified two-step synthesis procedure employed in this study afforded the C-6 ketone product with a very high overall yield (~91%).

Here it should be noted that a number of factors were considered when we selected the "synthon" as the side chain for

attachment to the C-7α position of the E_2 core structure: (i) the synthon should be a chain with an appropriate length and with any polar group(s) away from the C-7 carbon of the E_2 core structure after attachment, otherwise the ligand would not retain high ER binding affinity [24]. (ii) It should have a functional group at the end of the synthon that can be readily attached to (or transformed to) other functional groups for extension. Otherwise, after attachment of the side chain to the C-7α position, multi-step modifications of the functional group will be needed which will lose considerable amounts of the precious precursor (e.g., compound **5**). (iii) The side chain synthon should be easily synthesized with a high overall yield. There appeared to be no known procedures that met all of the aforementioned requirements.

With these considerations in mind, we had designed and tried a new approach. We used 1-iodo-6-benzyloxyhexane as a precursor for the side chain. Starting with 1,6-hexanediol, the monobenzylation product was obtained using a known procedure [25] which gave a yield of 86%. Then the 1-iodo-6-benzyloxyhexane was obtained almost quantitatively from the monobenzylation product under a reaction condition consisting of triphenylphosphine, imidazole and iodine in dry dichloromethane. The incipient hydroxyl group at the end of the side chain can be easily transformed into other functional groups that can be used for various purposes.

Deprotonation of the C-7 position of compound **4** with *t*-BuOK generated the corresponding enolate, which was alkylated with 1-iodo-6-benzyloxyhexane (the reaction conditions were similar to those used in the literature) [8,9,26]. This reaction yielded the C-7α side chain compound (compound **5**) as a major product in ~65% yield. The 7α-configuration of compound **5** was confirmed based on the two-dimensional NMR analyses (the nuclear Overhauser effect and COSY).

To remove the C-6 ketone group from compound **5**, several different methods have been explored and compared. One of the ideal products is compound **16** that has two MOM-protecting groups on both 3 and 17β positions and a free hydroxyl group at the end of the side chain for extension of the side chain in future. A well-known reaction condition consisting of boron trifluoride etherate and triethylsilane was first employed [9,26]. However, no detectable amount of compound **16** (the desired product) was formed, but instead an unexpected product (compound **11**, proposed structure shown in Scheme 1 based on data from NMR, MS and HRMS) was separated as a major compound. The mechanism for the formation of this compound is not certain. Our subsequent attempts to remove the 6-keto group in compound **5** while keeping the 3- and 17β-protecting groups unchanged by several other methods, such as using a condition consisting of sodium cyanoborohydride, boron trifluoride etherate, THF and reflux [27], a condition consisting of Pd–C, H_2 and ethanol at 60 °C for 2 h [8], or Wolff–Kishner reduction condition [30,31], were also failed. Under the second experimental conditions, compounds **12** and **13** were separated as two major products (see Scheme 1).

The results from the above experiments suggested that the two protecting MOM groups in compound **5** might readily interact with the reducing agents or might be unstable under the employed reduction conditions. We then tried to remove the 3 and 17β MOM groups off compound **5** by treatment with 6 M HCl in THF, which afforded compound **6** almost quantitatively. From compound **6**, there are two main approaches that are of interest to us: One is to remove the 6-keto group while keeping the benzyl group at the end of the side chain unchanged, because this method would readily help distinguish the 3- and 17β-hydroxyls from the hydroxyl of the side chain. The other is to protect the 3- and 17β-hydroxyls with protecting groups that are sufficiently stable under the subsequent reduction conditions that were designed to remove the 6-keto group as well as the benzyl group on the side chain. For the first approach, we employed the boron trifluoride etherate and triethylsilane reduction system to remove the 6-keto group from compound **6**. We found that the benzyl group is stable enough under the conditions we devised. Notably, there was no published report on whether the benzyl group would be stable or not under our reaction conditions. Thus, compound **14** could be readily prepared from compound **6** in 70% yield under the boron trifluoride etherate and triethylsilane reduction conditions. Reprotection of compound **14** with MOM gave compound **15**. Then compound **16** was prepared by hydrogenolysis of compound **15** under the conditions of Pd–C and H_2 in EtOH. For the second approach, an excellent two-step reaction route was found: (i) re-protection of the two free hydroxyls of compound **6** by reaction with acetic anhydride in dry pyridine to yield the diacetate (compound **7**) almost quantitatively and (ii) hydrogenolysis of compound **7** under the reaction conditions of the Pd–C and H_2 in AcOH at 70 °C for 3 h to yield compound **8** in 90% yield [28]. It should be noted that we could almost selectively obtain compound **8** or **9** depending on the reaction conditions we would use. While a shorter reaction time (3 h) gave mostly compound **8**, compound **9** became the major product when the reaction time was prolonged to 2 days. Compound **8** was easily oxidized to compound **10**, which has a different functional group at the end of the side chain. Notably, this compound has a very similar structure as compounds that were used earlier for the synthesis of several pure ER antagonists [29].

Even though we found several excellent reaction conditions to remove the 6-keto group from compound **6**, it is still of considerable interest to have a useful method to remove the 6-keto group in compound **5** while keeping the 3- and 17β-protecting groups unchanged. Partly based our experience gained from the experiments described above, compound **5** was first reduced to compound **17** by sodium borohydride quantitatively, and then hydrogenolysis of compound **17** under the reaction conditions of the $Pd(OH)_2/C$ and H_2 at 45 psi in EtOH at 60 °C for 3 h yielded two major products, compound **16** (in 43% yield) and compound **18** (in 36% yield, proposed structure shown in Scheme 1 based on data from NMR, MS and HRMS). The amount of compound **18** increased as the reaction was prolonged.

In the present study, we also determined the relative binding affinities of compounds **13** and **14** for recombinant human ERα and ERβ proteins, and compared their binding affinities with those of ICI-182,780 and E_2 (data summarized in Fig. 1). The IC_{50} values of compound **13** for ERα and ERβ were 41.8 and 22.3 nM, respectively, and the IC_{50} values of compound **14** were 39.8 and 63.1 nM, respectively. The IC_{50} values of compounds **13** and **14** are very similar to those of ICI-182,780. The relative binding affinities of compound **13**, **14** or ICI-

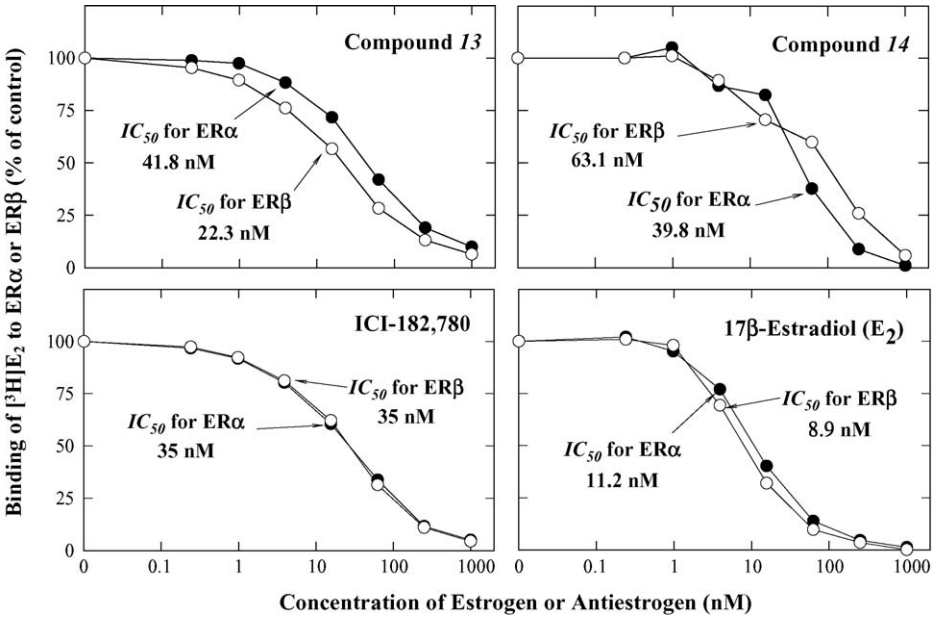

Fig. 1 – Comparison of the relative ER binding affinity of compounds 13 and 14 with those of E_2 and ICI-182,780. The relative binding affinity of each chemical was determined by measuring its inhibition of the binding of 10 nM [^{3}H]E_2 to the recombinant human ERα and ERβ. Eight concentrations (0.06, 0.24, 0.98, 3.9, 15.6, 62.5, 250 and 1000 nM) of each competing estrogen were tested. The IC_{50} values for each competing estrogen was calculated according to the sigmoid inhibition curves and the relative binding affinity (RBA) for each test compound was calculated against E_2 by using the following equation: RBA = IC_{50} for E_2/IC_{50} for the test compound. Each point was the mean of duplicate measurements, with average variations <5%. (●) ERα, (○) ERβ.

182,780 for the human ERα or ERβ are slightly lower than those of E_2.

In summary, we have explored and compared in the present study various synthetic methods for removal of the 6-keto group from C-6 position of 7α-substituted derivatives of E_2. We described here the improved methods for the chemical synthesis of several C-7α-substituted derivatives of E_2 by modifying the reaction steps and also by improving the overall yield for the desired products. For instance, using E_2 as the starting material and 1-iodo-6-benzyloxyhexane as a precursor for the C-7α side chain, we developed a seven-step synthetic procedure that produced 3,17β-bis(acetoxy)-7α-(6-hydroxyhexanyl)-estra-1,3,5(10)-triene (compound **8**) with an overall yield of ~45%. The two C-7α-substituted E_2 derivatives (compounds **13** and **14**) retained strong binding affinities (close to those of E_2 and ICI-182,780) for the human ERα and ERβ subtypes as determined using the radioligand–receptor binding assays.

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REFERENCES

[1] Parkin DM, Bray F, Ferlay J. Estimating the world cancer burden: Globocan. Int J Cancer 2001;94:153–6.

[2] Jordan VC. Third annual William L. McGuire Memorial Lecture. Studies on the estrogen receptor in breast cancer—20 years as a target for the treatment and prevention of cancer. Breast Cancer Res Treat 1995;36:267–85.

[3] MacGregor JI, Jordan VC. Basic guide to the mechanisms of antiestrogen action. Pharmacol Rev 1998;50:151–96.

[4] Osborne CK, Wakeling A, Nicholson RI. Fulvestrant: an estrogen receptor antagonist with a novel mechanism of action. Br J Cancer 2004;90(Suppl. 1):S2–6.

[5] Bowler J, Lilley TJ, Pittam JD, Wakeling AE. Novel steroidal pure antiestrogens. Steroids 1989;54:71–99.

[6] Jones SE. Fulvestrant: an estrogen receptor antagonist that downregulates the estrogen receptor. Semin Oncol 2003;30(5 Suppl. 16):14–20.

[7] Adamczyk M, Johnson D, Reddy E. A stereoselective synthesis of 7α-(3-carboxypropyl) estradiol from a noncontrolled substance. Steroids 1997;62:771–5.

[8] Hussey SL, He E, Peterson BR. Synthesis of chimeric 7α-substituted estradiol derivatives linked to cholesterol and cholesterylamine. Org Lett 2002;4:415–8.

[9] Skaddan MB, Wust FR, Katzenellenbogen JA. Synthesis and binding affinities of novel re-containing 7α-substituted estradiol complexes: models for breast cancer imaging agents. J Org Chem 1999;64:8108–21.

[10] Seimbille Y, Benard F, van Lier JE. Synthesis of 16α-fluoro ICI, 182,780 derivatives: powerful antiestrogens to image estrogen receptor densities in breast cancer by positron emission tomography. J Chem Soc, Perkin Trans 2002;1:2275–81.

[11] Mitra K, Marquis JC, Hillier SM, Rye PT, Zayas B, Lee AS, et al. A rationally designed genotoxin that selectively destroys estrogen receptor-positive breast cancer cells. J Am Chem Soc 2002;124:1862–3.

[12] Hussey SL, Muddana SS, Peterson BR. Synthesis of a β-estradiol-biotin chimera that potently heterodimerizes estrogen receptor and strepavidin proteins in a yeast three-hybrid system. J Am Chem Soc 2003;125:3692–3.
[13] Lee AJ, Kosh JW, Conney AH, Zhu BT. Characterization of the NADPH-dependent metabolism of 17β-estradiol to multiple metabolites by human liver microsomes and selectively expressed human cytochrome P450 3A4 and 3A5. J Pharmacol Exp Ther 2001;298:420–32.
[14] Lovely CJ, Gilbert NE, Liberto MM, Sharp DW, Lin YC, Brueggemeier RW. 2-(Hydroxyalkyl)estradiols: synthesis and biological evaluation. J Med Chem 1996;39:1917–23.
[15] Liu ZJ, Zhu BT. Concentration-dependent mitogenic and antiproliferative actions of 2-methoxyestradiol in estrogen receptor-positive human breast cancer cells. J Steroid Biochem Mol Biol 2004;88:265–75.
[16] Pearson JA, Han GR. Benzylic oxidation using *tert*-butyl hydroperoxide in the presence of chromium hexacarbonyl. J Org Chem 1985;50:2791.
[17] Rather R, Saxena N, Chandrasekaran S. A convenient method of benzylic oxidation with pyridinium chlorochromate. Synth Commun 1986;16:1493.
[18] Takagishi S, Schlosser M. Fluorine- and trifluoromethyl-substituted toluenes: site selective metalation of aromatic or benzylic positions. Synlett 1991:119–21.
[19] Tedesco R, Fiaschi R, Napolitano E. 6-Oxoestradiols from estradiols: exploiting site selective metalation of aralkyl systems with superbases. Synthesis 1995:1493–5.
[20] Akanni AO, Marples BA. Improved preparation of 3β,17β-diacetoxyoestra-1,3,5(10)-trien-6-one. Synth Commun 1984;14:713–5.
[21] Takagi H, Komatsu K, Yoshizawa I. Synthesis and mechanism of hydrolysis of estrogen 6-sulfates: model compounds for demonstrating the carcinogenesis of estrogen. Steroids 1991;56:173–9.
[22] Parish EJ, Chitrakorn S, Wei TY. Pyridinium chlorochromate-mediated allylic and benzylic oxidation. Synth Commun 1986;16:1371–5.
[23] Mancuso AJ, Huang SL, Swern D. Oxidation of long-chain and related alcohols to carbonyls by dimethyl sulfoxide "activated" by oxalyl chloride. J Org Chem 1978;43: 2480–2.
[24] Bucourt R, Vignau M, Torelli V, Richard-Foy H, Geynet C, Secco-Millet C, et al. New biospecific adsorbents for the purification of estradiol receptor. J Biol Chem 1978;253:8821–8.
[25] Bessodes M, Boukarim C. A new highly efficient procedure for the monobenzylation of symmetrical diols. Synlett 1996:1119–20.
[26] Tedesco R, Katzenellenbogen JA, Napolitano E. An expeditious route to 7α-substituted estradiol derivatives. Tetrahedron Lett 1997;38:7997–8000.
[27] Srikrishana A, Sattigeri JA, Viswajanani R, Yelamaggad CV. A simple and convenient one-step method for the reductive deoxygenation of aryl ketones to hydrocarbons. Synlett 1995:93–4.
[28] Burnham JW, Eisenbraun EJ. Hydrogenolysis of carbonyl derivatives as a route to pure aliphatic–aromatic hydrocarbons. J Org Chem 1971;36:737–8.
[29] Bowler J, Lilley TJ, Pittam JD, Wakeling AE. Novel steroidal pure antiestrogens. Steroids 1989;54:71–99.
[30] Huang M. Reducing steroid ketones to their corresponding methylene analogs. US Patent 2,648,686. Merck & Co. Inc.; 11 August 1953.
[31] Huang M. Reduction of steroid ketones and other carbonyl compounds by modified Wolff–Kishner method. J Am Chem Soc 1949;71:3301–3.