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Neighboring group participation Part 16. Stereoselective synthesis and receptor-binding examination of the four stereoisomers of 16-bromomethyl-3,17-estradiols☆

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ABSTRACT

The four possible isomers of 3-benzyloxy-16-hydroxymethylestra-1,3,5(10)-trien-17-ol (**1a–4a**) with proven configurations were converted into the corresponding 3-benzyloxy-16-bromomethylestra-1,3,5(10)-triene-3,17-diols (**5e–8e**). Depending on the reaction conditions the *cis* isomers of 3-benzyloxy-16-hydroxymethylestra-1,3,5(10)-trien-17-ol (**1a** and **2a**) were transformed into 3-benzyloxy-16-bromomethylestra-1,3,5(10)-trien-17-yl acetate (**5b** and **6b**) or 16-bromomethyl-3-hydroxyestra-1,3,5(10)-trien-17-yl acetate (**5c** and **6c**) on treatment with HBr and acetic acid. The mechanism of the process can be interpreted as involving front-side neighboring group participation. Under similar experimental conditions, the *trans* isomers (**3a** and **4a**) yielded only 3-benzyloxy-16-acetoxymethylestra-1,3,5(10)-trien-17-yl acetates (**3b** and **4b**) or 16-acetoxymethylestra-1,3,5(10)-triene-3,17-diyl diacetates (**3d** and **4d**). Both the *cis* (**1a** and **2a**) and the *trans* (**3a**, and **4a**) isomers were transformed into 16-bromomethylestra-1,3,5(10)-trien-17-ol (**5a–8a**) by the Appel reaction on treatment with CBr_4/Ph_3P. Debenzylation of **5a–8a** was carried out with HBr and acetic acid to yield **5e–8e**. The debenzylation process in the presence of acetic anhydride produces the diacetates **5d–8d**. The structures of the compounds were determined by means of MS, ^1H NMR and ^{13}C NMR spectroscopic methods. Compounds **5c–8c** and **5e–8e** were tested in a radioligand-binding assay. Except for the affinity of **7e** for the estrogen receptor ($K_i = 2.55$ nM), the affinities of the eight compounds (**5c–8c** and **5e–8e**) for the estrogen, androgen and progesterone receptors are low ($K_i > 0.55$, 0.52 and 0.21 μM, respectively).

1. Introduction

On the basis of the observations that more than two-thirds of breast cancers occur in post-menopausal women, and that

☆ For a preceding paper in this series, see ref. [1].

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their proliferation and survival depend on the estrogens, there are now two major options for the treatment of estrogen-dependent breast cancers [2]. One is the use of aromatase inhibitors, which reduces the local in situ formation of estrogens. The other is the use of anti-estrogens, which block the interaction of estrogens with their specific receptor (estrogen receptor, ER) [3]. Synthetic non-steroidal compounds are significant anti-estrogens which are routinely used in the endocrine therapy of breast cancer [2]. Tamoxifen behaves as a mixed agonist/antagonist of estrogen action, thus limiting its therapeutic potential [4]. Attempts to improve the therapeutic efficacy have been focused on creating estradiol analogs with anti-estrogenic activity. It is known from the literature that halogen-containing estrogens are of great importance in the diagnosis [5,6] and chemotherapy [4] of estrogen receptor-positive human breast cancers [7]: the strongly electronegative halogens onto a sterane skeleton can enhance the receptor affinity, while at the same time it may reduce the tumorigenicity, too. Earlier examinations suggested that the ER tolerates reasonably large substituents at positions C-7α, C-11β, C-16α and C-17α in estradiol [8,9]. Moreover, Poirier and co-workers reported that 16α-(3-halopropyl)estradiol and 16α-bromoalkylamidoestradiol derivatives inhibit 17β-hydroxysteroid dehydrogenase, which is responsible for the interconversion of the less potent estrone into the most potent estrogen, estradiol, in the target tissues [10,11]. On the other hand, Heiman et al. [12] and Katzenellenbogen and co-workers [13] reported that halogenation of ring D of the steroid nucleus, especially at position C-16α, results in compounds with higher affinities for lamb and rat uterine ERs than that of 17β-estradiol itself. In consequence of the above observations, efforts have been directed toward the synthesis of estradiol derivatives with a halomethyl group at C-16, which may be hormonally inactive or at least may have a favorable ratio of tumor inhibitory to hormonal activity.

Herein, we report the synthesis of the four possible 16-bromomethyl-3-hydroxyestra-1,3,5(10)-trien-17-yl acetates (**5c–8c**) and the four possible 16-bromomethylestradiol derivatives (**5e–8e**) from the corresponding 3-benzyloxy-16-hydroxymethylestra-1,3,5(10)-triene-3,17-diols (**1a–4a**). The starting materials (**1a–4a**) were synthesized from estrone according to a recently reported method [14]. The receptor-binding examinations on the synthesized new compounds were carried out on the ERs, androgen receptors (ARs) and progesterone receptors (PRs). We wished to obtain answers to the following questions: (1) How do the receptor binding properties/processes of these eight derivatives (**5c–8c** and **5e–8e**) differ? (2) How is the receptor-binding influenced by the bromomethyl group at position C-16 and the acetoxy group at position C-17 in ring D of the estrane skeleton?

2. Experimental

2.1. General

Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. The reactions were monitored by TLC on Kieselgel-G (Merck Si 254 F) layers (0.25 mm thick); solvent system (ss): (A) ethyl acetate/$CHCl_3$ (2.5:97.5, v/v); (B) ethyl acetate/$CHCl_3$ (5:95, v/v); (C) ethyl acetate/$CHCl_3$ (10:90, v/v). The spots were detected by spraying with 5% phosphomolybdic acid in 50% aqueous phosphoric acid. The R_f values were determined for the spots observed by illumination at 254 and 365 nm. Flash chromatography: silica gel 60, 40–63 μm (Merck). All solvents were distilled prior to use. Specific rotations were measured in $CHCl_3$ (c 1.00) or ethyl acetate (c 1.00) at 25 °C with a Polamat-A (Zeiss-Jena) polarimeter and are given in units of 10^{-1}° $cm^2\ g^{-1}$. Elemental analyses were performed with a Perkin-Elmer CHN analyzer model 2400. ^1H NMR spectra were obtained in $CDCl_3$ or DMSO-d_6 solution at 500 MHz (Bruker DRX 500), and the ^{13}C NMR spectra were recorded at 125 MHz on the same instrument under the same conditions. Chemical shifts (δ) are reported relative to TMS, and are given in ppm; the coupling constants (J) are given in Hz. ^{13}C NMR spectra are ^1H-decoupled. Mass spectra were measured on a Varian MAT 311A spectrometer.

2.2. *Conversion of 3-benzyloxy-16-hydroxymethylestra-1,3,5(10)-trien-17-ol (1a–4a) and 3-benzyloxy-16-bromomethylestra-1,3,5(10)-trien-17-ol isomers (7a and 8a) with HBr in acetic acid*

2.2.1. *General procedures*

2.2.1.1. *Method A.* A solution of 1.18 g (3 mmol) of 3-benzyloxy-16-hydroxymethylestra-1,3,5(10)-trien-17-ol (**1a–4a**) [14] in 24 ml of anhydrous acetic acid was cooled to 10 °C and 2.4 ml of 33% HBr (in acetic acid) was added. The reaction mixture was diluted with water after stirring for 1 h. The precipitate was collected by filtration and dissolved in CH_2Cl_2, and the solution was washed with water and dried over Na_2SO_4. After evaporation in vacuo, the crude product was purified by column chromatography (silica gel, $CHCl_3$ or ethyl acetate/$CHCl_3$: 2.5:97.5).

2.2.1.2. *Method B.* According to procedure A, 1.18 g (3 mmol) of compound **1a–4a**, or 1.78 g (3 mmol) of compounds **7a–8a** and 2.4 ml of 33% HBr (in acetic acid) were allowed to react for 12 h. The reaction mixture was diluted with water, the precipitate was collected by filtration and dissolved in CH_2Cl_2, and the solution was washed with water and dried over Na_2SO_4. After evaporation in vacuo, the crude product was purified by column chromatography (silica gel, ethyl acetate/$CHCl_3$: 2.5:97.5).

2.2.1.3. *Method C.* According to procedure A, 1.18 g (3 mmol) of compounds **1a–4a**, or 1.78 g (3 mmol) **7a–8a** and 2.4 ml of 33% HBr (in acetic acid) were allowed to react for 12 h. Next, 1 ml of acetic anhydride was added, and the reaction mixture was diluted with water after stirring for 6 h. The precipitate was collected by filtration and dissolved in CH_2Cl_2, and the solution was washed with water and dried over Na_2SO_4. After evaporation in vacuo, the crude product was purified by column chromatography (silica gel, ethyl acetate/$CHCl_3$: 2.5:97.5).

2.2.2. *Reaction of 3-benzyloxy-16β-hydroxymethylestra-1,3,5(10)-trien-17β-ol (1a) with HBr in acetic acid*

2.2.2.1. *Method A.*

2.2.2.1.1. 3-Benzyloxy-16β-bromomethylestra-1,3,5(10)-trien-17β-yl acetate (5b) and 3-benzyloxy-16β-acetoxymethylestra-1,3,5(10)-trien-17β-yl acetate (1b). Compound

5b (1.24 g, 83%). Mp 144–146 °C; R_f = 0.65 (ss A); $[\alpha]_D^{20}$ + 54 (*c* 1.00 in $CHCl_3$). (Found: C, 67.55; H, 6.75. $C_{28}H_{33}O_3Br$ requires: C, 67.60; H, 6.69%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.84 (s, 3H, 18-H_3), 2.10 (s, 3H, OAc-CH_3), 2.84 (m, 2H, 6-H_2), 3.27 (t, 1H, *J* = 10.0 Hz) and 3.46 (dd, 1H, *J* = 10.0 Hz, *J* = 5.8 Hz): 16a-H_2, 4.86 (d, 1H, *J* = 10.0 Hz, 17-H), 5.02 (s, 2H, 3-benzyl-CH_2), 6.71 (d, 1H, *J* = 2.5 Hz, 4-H), 6.76 (dd, 1H, *J* = 8.6 Hz, *J* = 2.5 Hz, 2-H), 7.17 (d, 1H, *J* = 8.6 Hz, 1-H), 7.31 (t, 1H, *J* = 7.3 Hz, 4′-H), 7.36 (t, 2H, *J* = 7.3 Hz, 3′,5′-H), 7.41 (d, 2H, *J* = 7.3 Hz, 2′,6′-H); ^{13}C NMR ($CDCl_3$) δ, ppm: 13.2 (C-18), 20.9 (OAc-CH_3), 26.1, 27.3, 29.7, 32.6, 35.2, 37.5, 38.0, 41.5, 43.7, 44.0 (C-13), 48.2, 70.0 (3-benzyl-CH_2), 82.3 (C-17), 112.4 (C-2), 114.9 (C-4), 126.3 (C-1); 127.4 (2C, C-2′,6′), 127.8 (C-4′), 128.5 (2C, C-3′,5′), 132.6 (C-10), 137.4 (C-1′), 137.8 (C-5), 156.9 (C-3), 170.6 (OAc-CO). EI-MS (70 eV) *m*/*z* (%): 498 (24) [M^+ + 2], 496 (23) [M^+], 91 (100), 43 (8). **1b** (157 mg 11%). Mp 100–102 °C ([14], Mp 100–101 °C); R_f = 0.40 (ss A); $[\alpha]_D^{20}$ + 37 (*c* 1.00 in $CHCl_3$). (Found: C, 75.48; H, 7.52. $C_{30}H_{36}O_5$ requires: C, 75.60; H, 7.61%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.85 (s, 3H, 18-H_3), 2.02 and 2.06 (2 × s, 2 × 3H, 2 × OAc-CH_3), 2.84 (m, 2H, 6-H_2), 4.02 (dd, 1H, *J* = 11.0 Hz, *J* = 7.5 Hz) and 4.12 (dd, 1H, *J* = 11.0 Hz, *J* = 6.9 Hz): 16a-H_2, 4.89 (d, 1H, *J* = 10.1 Hz, 17-H), 5.01 (s, 2H, 3-benzyl-CH_2), 6.70 (d, 1H, *J* = 2.3 Hz, 4-H), 6.76 (dd, 1H, *J* = 8.5 Hz, *J* = 2.3 Hz, 2-H), 7.17 (d, 1H, *J* = 8.5 Hz, 1-H), 7.29 (t, 1H, *J* = 7.3 Hz, 4′-H), 7.35 (t, 2H, *J* = 7.3 Hz, 3′,5′-H), 7.41 (d, 2H, *J* = 7.3 Hz, 2′,6′-H); ^{13}C NMR ($CDCl_3$) δ, ppm: 12.9 (C-18), 20.8 (2C, 2 × OAc-CH_3), 26.1, 27.3, 29.3, 29.6, 37.4, 37.7, 38.0, 43.6 (C-13), 43.7, 48.8, 65.2 (C-16a), 70.0 (3-benzyl-CH_2), 81.6 (C-17), 112.4 (C-2), 114.9 (C-4), 126.2 (C-1); 127.4 (2C, C-2′,6′), 127.8 (C-4′), 128.5 (2C, C-3′,5′), 132.6 (C-10), 137.3 (C-1′), 137.8 (C-5), 156.8 (C-3), 170.7 (2C, 2 × OAc-CO); EI-MS (70 eV) *m*/*z* (%): 476 (60) [M^+], 91 (100), 43 (8).

2.2.2.2. Method B.

2.2.2.2.1. 16β-Bromomethyl-3-hydroxyestra-1,3,5(10)-trien-17β-yl acetate (5c) and 16β-acetoxymethyl-3-hydroxyestra-1,3,5(10)-trien-17β-yl acetate (1c). Compound **5c** (1.16 g, 75%). Mp 194–198 °C; R_f = 0.45 (ss C); $[\alpha]_D^{20}$ + 63 (*c* 1.00 in $CHCl_3$). (Found: C, 62.07; H, 6.54. $C_{21}H_{27}O_3Br$ requires: C 61.92; H 6.68%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.84 (s, 3H, 18-H_3), 2.11 (s, 3H, OAc-CH_3), 2.81 (m, 2H, 6-H_2), 3.27 (t, 1H, *J* = 10.0 Hz) and 3.46 (dd, 1H, *J* = 10.0 Hz, *J* = 5.9 Hz): 16a-H_2, 4.86 (d, 1H, *J* = 10.0 Hz, 17-H), 6.56 (d, 1H, *J* = 2.4 Hz, 4-H), 6.62 (dd, 1H, *J* = 8.4 Hz, *J* = 2.4 Hz, 2-H), 7.12 (d, 1H, *J* = 8.4 Hz, 1-H); ^{13}C NMR ($CDCl_3$) δ, ppm: 13.2 (C-18), 20.9 (OAc-CH_3), 26.1, 27.2, 29.5, 32.6, 35.2, 37.4, 38.0, 41.4, 43.7, 44.0 (C-13), 48.2, 82.3 (C-17), 112.8 (C-2), 115.3 (C-4), 126.4 (C-1); 132.3 (C-10), 138.0 (C-5), 153.5 (C-3), 170.8 (OAc-CO); EI-MS (70 eV) *m*/*z* (%): 408 (99) [M^+ + 2], 406 (100) [M^+], 267 (18), 213 (14), 172 (16), 159 (28), 133 (28), 43 (52). **1c** (157 mg, 12%). Mp 184 °C; R_f = 0.40 (ss C); $[\alpha]_D^{20}$ + 46 (*c* 1.00 in $CHCl_3$). (Found: C, 71.54; H, 7.65. $C_{23}H_{30}O_5$ requires: C 71.48; H 7.82%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.84 (s, 3H, 18-H_3), 2.04 and 2.08 (2 × s, 2 × 3H, 2 × OAc-CH_3), 2.81 (m, 2H, 6-H_2), 4.02 (dd, 1H, *J* = 11.0 Hz, *J* = 7.6 Hz) and 4.13 (dd, 1H, *J* = 11.0 Hz, *J* = 6.9 Hz): 16a-H_2, 4.89 (d, 1H, *J* = 10.0 Hz, 17-H), 6.57 (d, 1H, *J* = 2.2 Hz, 4-H), 6.64 (dd, 1H, *J* = 8.4 Hz, *J* = 2.2 Hz, 2-H), 7.12 (d, 1H, *J* = 8.4 Hz, 1-H); ^{13}C NMR ($CDCl_3$) δ, ppm: 12.9 (C-18), 21.0 (2C, 2 × OAc-CH_3), 26.1, 27.3, 29.5 (2C), 37.4, 37.6, 38.0, 43.6 (C-13), 43.7, 48.7, 65.4 (C-16a), 81.8 (C-17), 112.8 (C-2), 115.3 (C-4), 126.4 (C-1); 132.0 (C-10), 138.0 (C-5), 153.8 (C-3), 171.3 (2C, 2 × OAc-CO); EI-MS (70 eV) *m*/*z* (%): 386 (100) [M^+], 266 (4), 213 (12), 172 (12), 159 (16), 133 (12), 106 (13), 43 (48).

2.2.2.3. Method C.

2.2.2.3.1. 16β-Bromomethylestra-1,3,5(10)-triene-3,17β-diyl diacetate (5d) and 16β-acetoxymethylestra-1,3,5(10)-triene-3,17β-diyl diacetate (1d). Compound **5d** (1.02 g, 76%). Mp 122–123.5 °C; R_f = 0.45 (ss A); $[\alpha]_D^{20}$ + 58 (*c* 1.00 in $CHCl_3$). (Found: C, 61.58; H, 6.67. $C_{23}H_{29}O_4Br$ requires: C, 61.47; H, 6.50%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.84 (s, 3H, 18-H_3), 2.10 (s, 3H, 17-OAc-CH_3), 2.27 (s, 3H, 3-OAc-CH_3), 2.86 (m, 2H, 6-H_2), 3.26 (t, 1H, *J* = 10.0 Hz) and 3.46 (dd, 1H, *J* = 10.0 Hz, *J* = 5.8 Hz): 16a-H_2, 4.86 (d, 1H, *J* = 10.1 Hz, 17-H), 6.79 (d, 1H, *J* = 2.2 Hz, 4-H), 6.84 (dd, 1H, *J* = 8.5 Hz, *J* = 2.2 Hz, 2-H), 7.25 (t, 1H, *J* = 8.5 Hz, 1-H); ^{13}C NMR ($CDCl_3$) δ, ppm: 13.2 (C-18), 20.8 (17-OAc-CH_3), 21.0 (3-OAc-CH_3), 25.9, 27.1, 29.4, 32.6, 35.1, 37.4, 37.6, 41.5, 43.9, 44.0 (C-13), 48.2, 82.2 (C-17), 118.6 (C-2), 121.5 (C-4), 126.3 (C-1); 137.6 (C-10), 138.0 (C-5), 148.6 (C-3), 169.6 (3-OAc-CO), 170.6 (17-OAc-CO); EI-MS (70 eV) *m*/*z* (%): 450 (8) [M^+ + 2], 448 (8) [M^+], 408 (100), 406 (100), 267 (12), 172 (9), 159 (14), 133 (14), 43 (41). **1d** (103 mg, 8%). Mp 150–152 °C; R_f = 0.50 (ss B); $[\alpha]_D^{20}$ + 44 (*c* 1.00 in $CHCl_3$). (Found: C, 69.95; H, 7.45. $C_{25}H_{32}O_6$ requires: C, 70.07; H, 7.53%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.86 (s, 3H, 18-H_3), 2.02 and 2.07 (2 × s, 2 × 3H, 16a- and 17-OAc-CH_3), 2.27 (s, 3H, 3-OAc-CH_3), 2.86 (m, 2H, 6-H_2), 4.02 (dd, 1H, *J* = 11.0 Hz, *J* = 7.5 Hz) and 4.12 (dd, 1H, *J* = 11.0 Hz, *J* = 6.9 Hz): 16a-H_2, 4.89 (d, 1H, *J* = 10.1 Hz, 17-H), 6.79 (d, 1H, *J* = 1.8 Hz, 4-H), 6.83 (dd, 1H, *J* = 8.3 Hz, *J* = 1.8 Hz, 2-H), 7.26 (d, 1H, *J* = 8.3 Hz, 1-H); ^{13}C NMR ($CDCl_3$) δ, ppm: 12.8 (C-18), 20.8 (2C, 16a- and 17-OAc-CH_3), 21.0 (3-OAc-CH_3), 25.9, 27.1, 29.3, 29.4, 37.4, 37.6, 37.7, 43.5 (C-13), 43.8, 48.8, 65.1 (C-16a), 81.5 (C-17), 118.6 (C-2), 121.4 (C-4), 126.3 (C-1); 137.6 (C-10), 137.9 (C-5), 148.5 (C-3), 169.6 (3-OAc-CO), 170.7 (2C, 16a- and 17-OAc-CO); EI-MS (70 eV) *m*/*z* (%): 428 (11) [M^+], 386 (100), 172 (4), 159 (6), 133 (5), 43 (16).

2.2.3. Conversion of 3-benzyloxy-16α-hydroxymethylestra-1,3,5(10)-trien-17α-ol (2a) with HBr in acetic acid

2.2.3.1. Method A.

2.2.3.1.1. 3-Benzyloxy-16α-bromomethylestra-1,3,5(10)-trien-17α-yl acetate (6b) and 3-benzyloxy-16α-acetoxymethylestra-1,3,5(10)-trien-17α-yl acetate (2b). Compound **6b** was eluted with $CHCl_3$ (1.27 g, 85%). Mp 107–108 °C; R_f = 0.65 (ss A); $[\alpha]_D^{20}$ + 75 (*c* 1.00 in $CHCl_3$). (Found: C, 67.52; H, 6.75. $C_{28}H_{33}O_3Br$ requires: C, 67.60; H, 6.69%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.86 (s, 3H, 18-H_3), 2.11 (s, 3H, 17-OAc-H_3), 2.85 (m, 2H, 6-H_2), 3.36 (dd, 1H, *J* = 9.3 Hz, *J* = 7.6 Hz) and 3.44 (t, 1H, *J* = 9.3 Hz): 16a-H_2, 5.02 (s, 2H, 3-benzyl-CH_2), 5.08 (d, 1H, *J* = 5,5 Hz, 17-H), 6.70 (d, 1H, *J* = 2.4 Hz, 4-H), 6.77 (dd, 1H, *J* = 8.5 Hz, *J* = 2.4 Hz, 2-H), 7.17 (d, 1H, *J* = 8.5 Hz, 1-H), 7.30 (t, 1H, *J* = 7.4 Hz, 4′-H), 7.37 (t, 2H, *J* = 7.4 Hz, 3′,5′-H), 7.41 (d, 2H, *J* = 7.4 Hz, 2′,6′-H); ^{13}C NMR ($CDCl_3$) δ, ppm: 16.7 (C-18), 20.8 (17-OAc-CH_3), 25.9, 27.9, 29.8, 31.2, 31.9, 33.4, 38.9, 42.7, 43.5, 46.3 (C-13), 48.3, 70.0 (3-benzyl-CH_2), 81.5 (C-17), 112.4 (C-2), 114.9 (C-4), 126.3 (C-1); 127.4 (2C, C-2′,6′), 127.8 (C-4′), 128.5 (2C, C-3′,5′), 132.6 (C-10), 137.3 (C-1′), 137.8 (C-5), 156.8 (C-3), 170.3 (OAc-CO). EI-MS (70 eV) *m*/*z* (%): 498 (28) [M^+ + 2], 496 (28) [M^+], 91 (100), 43 (7). Continued elution with ethyl acetate/$CHCl_3$ (2.5:97.5) yielded **2b** (170 mg, 12%). Mp 106–108 °C ([14], Mp 106–108); R_f = 0.30 (ss A); $[\alpha]_D^{20}$ + 67 (*c* 1.00 in $CHCl_3$). (Found: C, 75.42; H, 7.56. $C_{30}H_{36}O_5$ requires: C 75.60%, H 7.61%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.85 (s,

3H, 18-H_3), 2.01 and 2.07 (2 × s, 2 × 3H, 2 × OAc-H_3), 2.84 (m, 2H, 6-H_2), 4.03–4.12 (overlapping multiplets, 2H, 16a-H_2), 5.02 (d, 2H, 3-benzyl-CH_2), 5.09 (d, 1H, J = 5.7 Hz, 17-H), 6.71 (d, 1H, J = 2.5 Hz, 4-H), 6.77 (dd, 1H, J = 8.6 Hz, J = 2.5 Hz, 2-H), 7.18 (d, 1H, J = 8.6 Hz, 1-H), 7.30 (t, 1H, J = 7.3 Hz, 4′-H), 7.36 (t, 2H, J = 7.3 Hz, 3′,5′-H), 7.41 (d, 2H, J = 7.3 Hz, 2′,6′-H); ^{13}C NMR ($CDCl_3$) δ, ppm: 16.8 (C-18), 20.8 (2C, 2 × OAc-CH_3), 25.9, 27.9, 28.1, 29.7, 31.6, 38.6, 38.9, 43.5, 46.0 (C-13), 48.1, 64.0 (C-16a), 70.0 (3-benzyl-CH_2), 80.7 (C-17), 112.3 (C-2), 114.9 (C-4), 126.2 (C-1); 127.4 (2C, C-2′,6′), 127.8 (C-4′), 128.5 (2C, C-3′,5′), 132.6 (C-10), 137.3 (C-1′), 137.8 (C-5), 156.8 (C-3), 170.2 and 170.9 (2C, 2 × OAc-CO); EI-MS (70 eV) m/z (%): 476 (16) [M^+], 91 (100), 43 (74).

2.2.3.2. Method B.

2.2.3.2.1. 16α-Bromomethyl-3-hydroxyestra-1,3,5(10)-trien-17α-yl acetate (6c) and 16α-acetoxymethyl-3-hydroxyestra-1,3,5(10)-trien-17α-yl acetate (2c). Compound **6c** (953 mg, 78%). Mp 201–203 °C; R_f = 0.30 (ss A) $[\alpha]_D^{20}$ + 85 (*c* 1.00 in $CHCl_3$). (Found: C, 62.05; H, 6.72. $C_{21}H_{27}O_3Br$ requires: C, 61.92; H, 6.68%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.86 (s, 3H, 18-H_3), 2.12 (s, 3H, OAc-CH_3), 2.81 (m, 2H, 6-H_2), 3.36 (dd, 1H, J = 9.4 Hz, J = 7.5 Hz) and 3.44 (t, 1H, J = 9.4 Hz): 16a-H_2, 4.76 (s, 1H, 3-OH), 5.08 (d, 1H, J = 5.6 Hz, 17-H), 6.56 (d, 1H, J = 2.5 Hz, 4-H), 6.62 (dd, 1H, J = 8.4 Hz, J = 2.5 Hz, 2-H), 7.12 (d, 1H, J = 8.4 Hz, 1-H); ^{13}C NMR ($CDCl_3$) δ, ppm: 16.8 (C-18), 20.9 (OAc-CH_3), 25.9, 27.8, 29.6, 31.2, 31.9, 33.4, 38.9, 42.8, 43.5, 46.3 (C-13), 48.3, 81.6 (C-17), 112.8 (C-2), 115.3 (C-4), 126.5 (C-1); 132.4 (C-10), 138.1 (C-5), 153.5 (C-3), 170.5 (OAc-CO); EI-MS (70 eV) m/z (%): 408.1 (69) [M^+ + 2], 406 (67) [M^+], 267 (16), 172 (26), 160 (100), 146 (47), 133 (50), 107 (22), 43 (87). **2c** (115 mg, 10%) as a colorless oil. R_f = 0.45 (ss C); $[\alpha]_D^{20}$ + 57 (*c* 1.00 in $CHCl_3$). (Found: C, 71.36; H, 7.95. $C_{23}H_{30}O_5$ requires: C, 71.48; H, 7.82%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.85 (s, 3H, 18-H_3), 2.01 and 2.08 (2 × s, 2 × 3H, 2 × OAc-CH_3), 2.81 (m, 2H, 6-H_2), 4.04–4.13 (overlapping multiplets, 2H, 16a-H_2), 5.10 (d, 1H, J = 5.7 Hz, 17-H), 6.56 (d, 1H, J = 2.4 Hz, 4-H), 6.63 (dd, 1H, J = 8.5 Hz, J = 2.4 Hz, 2-H), 7.13 (d, 1H, J = 8.5 Hz, 1-H); ^{13}C NMR ($CDCl_3$) δ, ppm: 16.8 (C-18), 20.8 (2C, 2 × OAc-CH_3), 25.9, 27.9, 28.2, 29.6, 31.7, 38.6, 38.9, 43.5, 46.0 (C-13), 48.2,64.1 (C-16a), 80.8 (C-17), 112.8 (C-2), 115.3 (C-4), 126.5 (C-1); 132.4 (C-10), 138.1 (C-5), 153.6 (C-3), 170.4 and 171.2 (2C, 2 × OAc-CO); EI-MS (70 eV) m/z (%): 386 (100) [M^+], 266 (12), 213 (7), 172 (12), 159 (26), 133 (16), 106 (16), 43 (74).

2.2.3.3. Method C.

2.2.3.3.1. 16α-Bromomethylestra-1,3,5(10)-triene-3,17α-diyl diacetate (6d) and 16α-acetoxymethylestra-1,3,5(10)-triene-3,17α-diyl diacetate (2d). Compound **6d** (990 mg, 74%). Mp 120–122 °C; R_f = 0.50 (ss A); $[\alpha]_D^{20}$ + 72 (*c* 1.00 in $CHCl_3$). (Found: C, 61.66; H, 6.35. $C_{23}H_{29}O_4Br$ requires: C 61.47; H 6.50%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.86 (s, 3H, 18-H_3), 2.12 (s, 3H, 17-OAc-CH_3), 2.27 (s, 3H, 3-OAc-CH_3), 2.87 (m, 2H, 6-H_2), 3.36 (dd, 1H, J = 9.2 Hz, J = 7.5 Hz) and 3.44 (t, 1H, J = 9.2 Hz): 16a-H_2, 5.08 (d, 1H, J = 5.6 Hz, 17-H), 6.79 (d, 1H, J = 2.1 Hz, 4-H), 6.84 (dd, 1H, J = 8.4 Hz, J = 2.1 Hz, 2-H), 7.26 (d, 1H, J = 8.4 Hz, 1-H); ^{13}C NMR ($CDCl_3$) δ, ppm: 16.7 (C-18), 20.8 (17-OAc-CH_3), 21.1 (3-OAc-CH_3), 25.7, 27.7, 29.5, 31.2, 31.8, 33.3, 38.5, 42.7, 43.6, 46.2 (C-13), 48.4, 81.4 (C-17), 118.6 (C-2), 121.5 (C-4), 126.3 (C-1), 137.6 (C-10), 138.0 (C-5), 148.5 (C-3), 169.7 (3-OAc-CO) and 170.3 (17-OAc-CO); EI-MS (70 eV) m/z (%): 450 (10) [M^+ + 2], 448 (10) [M^+], 408 (96), 406 (100), 267 (87), 172 (7), 160 (26), 133 (12), 83 (14), 43 (39). **2d** (102 mg, 8%). Mp 67–68 °C; R_f = 0.45 (ss B); $[\alpha]_D^{20}$ + 64 (*c* 1.00 in $CHCl_3$). (Found: C, 69.92; H, 7.61. $C_{25}H_{32}O_6$ requires: C 70.07; H 7.53%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.85 (s, 3H, 18-H_3), 2.01 and 2.07 (2 × s, 2 × 3H, 16a- and 17-OAc-CH_3), 2.27 (s, 3H, 3-OAc-CH_3), 2.86 (m, 2H, 6-H_2), 4.03–4.13 (m, 2H, 16a-H_2), 5.10 (d, 1H, J = 5.2 Hz, 17-H), 6.79 (d, 1H, J = 2.3 Hz, 4-H), 6.83 (dd, 1H, J = 8.1 Hz, J = 2.3 Hz, 2-H), 7.26 (d, 1H, J = 8.1 Hz, 1-H); ^{13}C NMR ($CDCl_3$) δ, ppm: 16.7 (C-18), 20.8 (2C, 16a- and 17-OAc-CH_3), 21.0 (3-OAc-CH_3), 25.7, 27.7, 28.1, 29.4, 31.6, 38.5, 38.6, 43.6, 45.9 (C-13), 48.1, 64.0 (C-16a), 80.6 (C-17), 118.6 (C-2), 121.4 (C-4), 126.2 (C-1); 137.6 (C-10), 138.0 (C-5), 148.5 (C-3), 169.6 (3-OAc-CO), 170.2 and 170.9 (2C, 16a- and 17-OAc-CO); EI-MS (70 eV) m/z (%): 428 (14) [M^+], 386 (100), 172 (5), 159 (12), 133 (8), 43 (41).

2.2.4. Reaction of 3-benzyloxy-16α-hydroxymethylestra-1,3,5(10)-trien-17β-ol (3a) with HBr in acetic acid

2.2.4.1. Method A.

2.2.4.1.1.3-Benzyloxy-16α-acetoxymethylestra-1,3,5(10)-trien-17β-yl acetate (3b). Compound **3b** (1.36 mg, 95%). Mp 132–134 °C ([14] Mp 131–132 C); R_f = 0.30 (ss A); $[\alpha]_D^{20}$ + 7 (*c* 1.00 in $CHCl_3$). (Found: C, 75.58; H, 7.78. $C_{30}H_{36}O_5$ requires: C, 75.60; H, 7.61%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.85 (s, 3H, 18-H_3), 2.03 and 2.06 (2 × s, 2 × 3H, 2 × OAc-CH_3), 2.83 (m, 2H, 6-H_2), 4.06 (dd, 1H, J = 10.9 Hz, J = 6.9 Hz) and 4.12 (dd, 1H, J = 10.9 Hz, J = 6.4 Hz): 16a-H_2, 4.72 (d, 1H, J = 7.7 Hz, 17-H), 5.01 (s, 2H, 3-benzyl-H_2), 6.70 (d, 1H, J = 2.4 Hz, 4-H), 6.76 (dd, 1H, J = 8.6 Hz, J = 2.4 Hz, 2-H), 7.17 (d, 1H, J = 8.6 Hz, 1-H), 7.29 (t, 1H, J = 7.3 Hz, 4′-H), 7.35 (t, 2H, J = 7.3 Hz, 3′,5′-H), 7.41 (d, 2H, J = 7.3 Hz, 2′,6′-H); ^{13}C NMR ($CDCl_3$) δ, ppm: 12.7 (C-18), 20.8 and 21.0 (2C, 2 × OAc-CH_3), 26.1, 27.1, 27.4, 29.6, 36.9, 38.4, 40.3, 43.7, 44.3 (C-13), 48.7, 66.7 (C-16a), 70.0 (3-benzyl-CH_2), 83.8 (C-17), 112.4 (C-2), 114.9 (C-4), 126.3 (C-1); 127.4 (2C, C-2′,6′), 127.8 (C-4′), 128.5 (2C, C-3′,5′), 132.6 (C-10), 137.4 (C-1′), 137.8 (C-5), 156.8 (C-3), 170.7 and 170.9 (2C, 2 × OAc-CO); EI-MS (70 eV) m/z (%): 476 (48), 91 (100), 84 (20), 49 (52), 43 (11).

2.2.4.2. Method B.

2.2.4.2.1. 16α-Acetoxymethyl-3-hydroxyestra-1,3,5(10)-trien-17β-yl acetate (3c). Compound **3c** (1.00 g, 87%). Mp 145–147 °C; R_f = 0.40 (ss C); $[\alpha]_D^{20}$ − 3 (*c* 1.00 in $CHCl_3$). (Found: C, 71.57; H, 7.88. $C_{23}H_{30}O_5$ requires: C, 71.48; H, 7.82%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.85 (s, 3H, 18-H_3), 2.05 and 2.08 (2 × s, 2 × 3H, 2 × OAc-CH_3), 2.80 (m, 2H, 6-H_2), 4.08 (dd, 1H, J = 10.9 Hz, J = 6.8 Hz) and 4.12 (dd, 1H, J = 10.9 Hz, J = 6.5 Hz): 16a-H_2, 4.74 (d, 1H, J = 7.7 Hz, 17-H), 5.74 (s, 1H, 3-OH), 6.56 (d, 1H, J = 2.4 Hz, 4-H), 6.63 (dd, 1H, J = 8.4 Hz, J = 2.4 Hz, 2-H), 7.11 (d, 1H, J = 8.4 Hz, 1-H); ^{13}C NMR ($CDCl_3$) δ, ppm: 12.6 (C-18), 20.8 and 21.0 (2C, 2 × OAc-CH_3), 26.0, 27.1, 27.4, 29.4, 36.8, 38.4, 40.2, 43.6, 44.3 (C-13), 48.7, 66.9 (C-16a), 84.0 (C-17), 112.8 (C-2), 115.3 (C-4), 126.4 (C-1); 132.0 (C-10), 137.9 (C-5), 153.7 (C-3), 171.0 and 171.3 (2C, 2 × OAc-CO); EI-MS (70 eV) m/z (%): 386 (95) [M^+], 266 (12), 213 (12), 172 (19), 159 (34), 159 (34), 133 (27), 10 (8), 43 (100).

2.2.4.3. Method C.

2.2.4.3.1. 16α-Acetoxymethylestra-1,3,5(10)-triene-3,17β-diyl diacetate (3d). Compound **3d** (1.25 g, 98%). Mp 85–88 °C; R_f = 0.40 (ss B); $[\alpha]_D^{20}$ − 6.0 (*c* 1.00 in $CHCl_3$). (Found: C, 69.95; H, 7.65. $C_{25}H_{32}O_6$ requires: C, 70.07; H, 7.53%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.85 (s, 3H, 18-H_3), 2.04 and 2.07 (2 × s,

2 × 3H, 16a- and 17-OAc-CH_3), 2.27 (s, 3H, 3-OAc-CH_3), 2.85 (m, 2H, 6-H_2), 4.06 (dd, 1H, J = 10.8 Hz, J = 6.8 Hz) and 4.12 (dd, 1H, J = 10.8 Hz, J = 6.4 Hz): 16a-H_2, 4.74 (d, 1H, J = 7.7 Hz, 17-H), 6.79 (d, 1H, J = 2.2 Hz, 4-H), 6.83 (dd, 1H, J = 8.3 Hz, J = 2.2 Hz, 2-H), 7.25 (d, 1H, J = 8.3 Hz, 1-H); ^{13}C NMR ($CDCl_3$) δ, ppm: 12.6 (C-18), 20.8, 20.9 and 21.0 (3C, 3 × OAc-CH_3), 25.9, 26.9, 27.3, 29.3, 36.8, 38.0, 40.2, 43.8, 44.2 (C-13), 48.7, 66.7 (C-16a), 83.7 (C-17), 118.6 (C-2), 121.4 (C-4), 126.3 (C-1); 137.6 (C-10), 137.9 (C-5), 148.5 (C-3), 169.6 (3-OAc-CO), 170.6 and 170.9 (2C, 16a- and 17-OAc-CO); EI-MS (70 eV) *m/z* (%): 428 (11) [M^+], 386 (100), 172 (8), 159 (14), 133 (10), 43 (54).

2.2.5. *Conversion of 3-benzyloxy-16β-hydroxymethylestra-1,3,5(10)-trien-17α-ol* ***(4a)*** *with HBr in acetic acid*

2.2.5.1. *Method A.*

2.2.5.1.1. 16β-Acetoxymethyl-3-benzyloxy-17-methyl-18-nor-estra-1,3,5(10),13(17)-tetraene (16b), 3-benzyloxy-16β-hydroxymethylestra-1,3,5(10),13(17)-tetraene (16a) and 16β-acetoxymethyl-3-benzyloxyestra-1,3,5(10)-trien-17α-yl acetate (4b). Compound **16b** (290 mg, 23%) as colorless oil; R_f = 0.80 (ss A); $[\alpha]_D^{20}$ + 85 (*c* 1.00 in $CHCl_3$). (Found: C, 80.56; H, 7.83. $C_{28}H_{32}O_3$ requires: C, 80.73; H, 7.74%). ^{1}H-NMR δ, ppm 1.61 (s, 3H, 18-H_3), 2.03 (s, 3H, Ac-Me), 2.81 (m, 2H, 6-H_2), 3.98 (dd, 1H, J = 10.8 Hz, J = 6.9 Hz) and 4.22 (dd, 1H, J = 10.8 Hz, J = 4.8 Hz): 16a-H_2, 5.02 (s, 2H, benzyl-H_2), 6.70 (d, 1H, J = 2.3 Hz, 4-H), 6.78 (dd, 1H, J = 8.5 Hz, J = 2.3 Hz, 2-H), 7.24 (d, 1H, J = 8.5 Hz, 1-H), 7.30 (t, 1H, J = 7.5 Hz, 4′-H), 7.37 (t, 2H, J = 7.5 Hz, 3′-, 5′-H), 7.42 (d, 2H, J = 7.5 Hz, 2′-, 6′-H). ^{13}C-NMR δ, ppm: 12.3 (C-18), 21.4 (Ac-Me), 26.4, 28.1, 30.8, 31.8, 32.8, 42.8, 48.1, 49.6, 51.3, 68.0, 70.4, 113.0, 115.1, 127.6, 127.9, 128.2, 128.9, 132.8, 137.8, 138.8, 139.3, 157.1 (C-3), 171.7 (Ac-CO).

The white oily **16b** (104 mg, 0.25 mmol) obtained in the chromatographic separation was dissolved in methanol (10 ml) containing $NaOCH_3$ (5 mg, 0.092 mmol) and the solution was allowed to stand for 24 h. It was diluted with water, and the white precipitate that separated out was filtered off and recrystallized from a mixture of acetone/hexane; **16a** (76 mg, 81%). Mp 128–129 °C; R_f = 0.65 (ss A); $[\alpha]_D^{20}$ + 52 (*c* 1.00 in $CHCl_3$). (Found: C, 83.57; H, 7.92. $C_{26}H_{30}O_2$ requires: C, 83.38; H, 8.07%). ^{1}H-NMR δ, ppm: 1.67 (s, 3H, 18-H_3), 2.84 (m, 2H, 6-H_2), 3.72 (m, 2H, 16a-H_2), 5.06 (s, 2H, benzyl-H_2), 6.74 (d, 1H, J = 2.6 Hz, 4-H), 6.82 (dd, 1H, J = 8.6 Hz, J = 2.6 Hz, 2-H), 7.27 (d, 1H, J = 8.6 Hz, 1-H), 7.35 (t, 1H, J = 7.0 Hz, 4′-H), 7.41 (t, 2H, J = 7.0 Hz, 3′-, 5′-H), 7.46 (d, 2H, J = 7.0 Hz, 2′-, 6′-H). ^{13}C-NMR δ, ppm: 12.14 (C-18), 26.5, 28.0,28.1,28.2, 30.8, 31.7, 31.9,42.8,49.5, 51.3, 51.4,65.2, 70.4,113.0,115.2, 127.6, 127.9, 128.3, 129.0, 132.9, 137.8, 138.9,140.4, 157.1.

4b (850 mg, 59%). Mp 80–82 °C ([14], Mp 79–82 °C); R_f = 0.35 (ss A); $[\alpha]_D^{20}$ + 51 (*c* 1.00 in $CHCl_3$). (Found: C, 75.82; H, 7.51. $C_{30}H_{36}O_5$ requires: C, 75.60; H, 7.61%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.85 (s, 3H, 18-H_3), 2.06 and 2.07 (2 × s, 2 × 3H, 2 × OAc-CH_3), 2.85 (m, 2H, 6-H_2), 4.17 (dd, 1H, J = 10.9 Hz, J = 7.1 Hz) and 4.22 (dd, 1H, J = 10.9 Hz, J = 7.4 Hz): 16a-H_2, 4.71 (d, 1H, J = 1.7 Hz, 17-H), 5.03 (s, 2H, 3-benzyl-H_2), 6.71 (d, 1H, J = 2.2 Hz, 4-H), 6.77 (dd, 1H, J = 8.5 Hz, J = 2.2 Hz, 2-H), 7.19 (d, 1H, J = 8.5 Hz, 1-H), 7.30 (t, 1H, J = 7.3 Hz, 4′-H), 7.37 (t, 2H, J = 7.3 Hz, 3′,5′-H), 7.42 (d, 2H, J = 7.3 Hz, 2′,6′-H); ^{13}C-NMR ($CDCl_3$) δ, ppm: 17.1 (C-18), 20.9 and 21.1 (2C, 2 × OAc-CH_3), 25.9, 27.9, 29.3, 29.6, 32.2, 38.5, 43.4, 44.6 (C-13), 45.8, 50.0, 66.6 (C-16a), 70.0 (3-benzyl-CH_2), 83.6 (C-17), 112.4 (C-2), 114.9 (C-4), 126.3 (C-1), 127.4 (2C, C-2′,6′), 127.8 (C-4′), 128.5 (2C, C-3′,5′), 132.7 (C-10), 137.4 (C-1′), 137.9 (C-5), 156.8 (C-3), 170.3 and 171.0 (2C, 2 × OAc-CO); EI-MS (70 eV) *m/z* (%): 476 (39) [M^+], 91 (100), 43 (9).

2.2.5.2. *Method B.*

2.2.5.2.1. 16β-Acetoxymethyl-3-hydroxyestra-1,3,5(10)-trien-17α-yl acetate (4c). Compound **4c** (560 mg, 48%). Mp 155–157 °C; R_f = 0.40 (ss C); $[\alpha]_D^{20}$ + 57 (*c* 1.00 in $CHCl_3$). (Found: C, 71.55; H, 7.96. $C_{23}H_{30}O_5$ requires: C, 71.48; H, 7.82%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.84 (s, 3H, 18-H_3), 2.07 and 2.08 (2 × s, 2 × 3H, 2 × OAc-CH_3), 2.81 (m, 2H, 6-H_2), 4.18 (dd, 1H, J = 11.0 Hz, J = 7.2 Hz) and 4.22 (dd, 1H, J = 11.0 Hz, J = 7.5 Hz): 16a-H_2, 4.72 (d, 1H, J = 1.9 Hz, 17-H), 5.70 (s, 1H, 3-OH), 6.57 (d, 1H, J = 2.4 Hz, 4-H), 6.64 (dd, 1H, J = 8.4 Hz, J = 2.4 Hz, 2-H), 7.13 (d, 1H, J = 8.5 Hz, 1-H); ^{13}C NMR ($CDCl_3$) δ, ppm: 17.0 (C-18), 20.8 and 21.0 (2C, 2 × OAc-CH_3), 25.9, 27.8, 29.2, 29.5, 32.1, 38.5, 43.3, 44.5 (C-13), 45.6, 49.9, 66.6 (C-16a), 83.7 (C-17), 112.8 (C-2), 115.2 (C-4), 126.3 (C-1); 132.0 (C-10), 137.9 (C-5), 153.7 (C-3), 170.6 and 171.3 (2C, 2 × OAc-CO); EI-MS (70 eV) *m/z* (%): 386 (100) [M^+], 266 (10), 213 (6), 172 (10), 159 (23), 133 (15), 106 (13), 43 (10).

2.2.5.3. *Method C.*

2.2.5.3.1. 16β-Acetoxymethylestra-1,3,5(10)-triene-3,17α-diyl diacetate (4d). Compound **4d** (530 mg, 41%) as a colorless oil; R_f = 0.30 (ss A); $[\alpha]_D^{20}$ + 45 (*c* 1.00 in $CHCl_3$). (Found: C, 69.91; H, 7.45. $C_{25}H_{32}O_6$ requires: C, 70.07; H, 7.53%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.85 (s, 3H, 18-H_3), 2.06 and 2.07 (2 × s, 2 × 3H, 16a- and 17-OAc-CH_3), 2.27 (s, 3H, 3-OAc-CH_3), 2.87 (m, 2H, 6-H_2), 4.17 (dd, 1H, J = 10.9 Hz, J = 7.1 Hz) and 4.23 (dd, 1H, J = 10.9 Hz, J = 7.4 Hz): 16a-H_2, 4.71 (d, 1H, J = 1.64 Hz, 17-H), 6.79 (d, 1H, J = 1.9 Hz, 4-H), 6.84 (dd, 1H, J = 8.4 Hz, J = 1.9 Hz, 2-H), 7.27 (d, 1H, J = 8.4 Hz, 1-H); ^{13}C NMR ($CDCl_3$) δ, ppm: 16.9 (C-18), 20.8 (3-OAc-CH_3) and 21.0 (2C, 16a- and 17-OAc-CH_3), 25.7, 27.7, 29.2, 29.4, 32.1, 38.1, 43.5, 44.5 (C-13), 45.7, 50.0, 66.4 (C-16a), 83.5 (C-17), 118.6 (C-2), 121.4 (C-4), 126.2 (C-1); 137.6 (C-10), 138.0 (C-5), 148.5 (C-3), 169.6 (3-OAc-CO), 170.3 and 171.0 (2C, 16a- and 17-OAc-CO); EI-MS (70 eV) *m/z* (%): 428 (12) [M^+], 386 (100), 172 (6), 159 (12), 133 (9), 43 (57).

2.3. *Conversion of 3-benzyloxy-16-hydroxymethylestra-1,3,5(10)-trien-17-ol isomers (1a–4a) to 3-benzyloxy-16-bromomethylestra-1,3,5(10)-trien-17-ol (5a–8a) by Appel bromination*

2.3.1. *Method D., General procedure*

Compounds **1a–3a** or **4a** (1.18 g, 3 mmol) was suspended in 40 ml of anhydrous CH_2Cl_2, and 4.97 g (15 mmol) of CBr_4 was added. After cooling the suspension to 0 °C, 3.94 g (15 mmol) of Ph_3P in 20 ml anhydrous CH_2Cl_2 was added dropwise and the reaction mixture was allowed to become warm and stirred until it became a yellow solution. After evaporation in vacuo the crude product was purified on a silica gel column with ethyl acetate/$CHCl_3$ 2.5:97.5.

5a (1.13 g, 83%). Mp 109–110 °C; R_f = 0.60 (ss A); $[\alpha]_D^{20}$ + 66 (*c* 1.00 in $CHCl_3$). (Found: C, 68.35; H, 6.92. $C_{26}H_{31}O_2Br$ requires: C, 68.57; H, 6.86%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.79 (s, 3H, 18-H_3), 2.84 (m, 2H, 6-H_2), 3.33 (t, 1H, J = 9.7 Hz) and 3.72 (dd, 1H, J = 9.7 Hz, J = 6.4 Hz): 16a-H_2, 3.82 (d, 1H, J = 9.8 Hz, 17-H), 5.01

(s, 2H, benzyl-H_2), 6.71 (d, 1H, J = 2.3 Hz, 4-H), 6.77 (dd, 1H, J = 8.6 Hz, J = 2.3 Hz, 2-H), 7.18 (d, 1H, J = 8.6 Hz, 1-H), 7.30 (t, 1H, J = 7.3 Hz, 4′-H), 7.36 (t, 2H, J = 7.3 Hz, 3′,5′-H), 7.41 (d, 2H, J = 7.3 Hz, 2′,6′-H). ^{13}C NMR ($CDCl_3$) δ, ppm: 12.3 (C-18), 26.2, 27.4, 29.7, 32.6, 36.4, 37.6, 38.1, 43.3, 43.9, 44.5 (C-13), 48.2, 70.0 (benzyl-CH_2), 81.9 (C-17), 112.4 (C-2), 114.9 (C-4), 126.2 (C-1); 127.4 (2C, C-2′,6′), 127.8 (C-4′), 128.5 (2C, C-3′,5′), 132.8 (C-10), 137.4 (C-1′), 137.9 (C-5), 156.8 (C-3); EI-MS (70 eV) m/z (%): 456 (42) [M^+ + 2], 454 (41) [M^+], 91 (100).

6a (1.16 g, 85%). Mp 119–120 °C; R_f = 0.80 (ss A); $[\alpha]_D^{20}$ + 89 (c 1.00 in $CHCl_3$). (Found: C, 68.67; H, 6.79. $C_{26}H_{31}O_2Br$ requires: C, 68.57; H, 6.86%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.78 (s, 3H, 18-H_3), 2.83 (m, 2H, 6-H_2), 3.48 (dd, 1H, J = 9.4 Hz, J = 6.5 Hz) and 3.61 (t, 1H, J = 9.4 Hz): 16a-H_2, 3.85 (t, 1H, J = 4.4 Hz, 17-H), 5.02 (s, 2H, benzyl-H_2), 6.70 (d, 1H, J = 2.5 Hz, 4-H), 6.77 (dd, 1H, J = 8.5 Hz, J = 2.5 Hz, 2-H), 7.19 (d, 1H, J = 8.5 Hz, 1-H), 7.30 (t, 1H, J = 7.3 Hz, 4′-H), 7.36 (t, 2H, J = 7.3 Hz, 3′,5′-H), 7.41 (d, 2H, J = 7.3 Hz, 2′,6′-H). ^{13}C NMR ($CDCl_3$) δ, ppm: 17.2 (C-18), 26.0, 27.9, 29.8, 31.1, 31.2, 34.9 (C-16a), 39.0, 43.5, 44.0, 46.3 (C-13), 47.3, 70.0 (benzyl-CH_2), 79.9 (C-17), 112.3 (C-2), 114.9 (C-4), 126.3 (C-1); 127.4 (2C, C-2′,6′), 127.8 (C-4′), 128.5 (2C, C-3′,5′), 132.9 (C-10), 137.4 (C-1′), 137.9 (C-5), 156.8 (C-3); EI-MS (70 eV) m/z (%): 456 (23) [M^+ + 2], 454 (23) [M^+], 91 (100).

7a (1.08 g, 79%). Mp 90–91 °C; R_f = 0.30 (ss A); $[\alpha]_D^{20}$ + 50 (c 1.00 in $CHCl_3$). (Found: C, 68.63; H, 6.95. $C_{26}H_{31}O_2Br$ requires: C, 68.57; H, 6.86%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.83 (s, 3H, 18-H_3), 2.84 (m, 2H, 6-H_2), 3.47 (d, 1H, J = 7.6 Hz, 17-H), 3.54–3.61 (overlapping multiplets, 2H, 16a-H_2, 5.02 (s, 2H, benzyl-H_2), 6.71 (d, 1H, J = 2.3 Hz, 4-H), 6.77 (dd, 1H, J = 8.6 Hz, J = 2.3 Hz, 2-H), 7.18 (d, 1H, J = 8.6 Hz, 1-H), 7.30 (t, 1H, J = 7.3 Hz, 4′-H), 7.36 (t, 2H, J = 7.3 Hz, 3′,5′-H), 7.41 (d, 2H, J = 7.3 Hz, 2′,6′-H). ^{13}C NMR ($CDCl_3$) δ, ppm: 12.0 (C-18), 26.1, 27.2, 29.7, 29.8, 36.6, 38.3, 38.9 (C-16a), 43.9, 44.6 (C-13), 45.8, 48.3, 69.9 (benzyl-CH_2), 85.8 (C-17), 112.3 (C-2), 114.8 (C-4), 126.2 (C-1); 127.4 (2C, C-2′,6′), 127.8 (C-4′), 128.5 (2C, C-3′,5′), 132.7 (C-10), 137.3 (C-1′), 137.9 (C-5), 156.7 (C-3); EI-MS (70 eV) m/z (%): 456 (24) [M^+ + 2], 454 (25) [M^+], 91 (100).

8a (1.09 g, 80%). Mp 51–53 °C; R_f = 0.35 (ss A); $[\alpha]_D^{20}$ + 35 (c 1.00 in $CHCl_3$). (Found: C, 68.62; H, 6.94. $C_{26}H_{31}O_2Br$ requires: C, 68.57; H, 6.86%. ^{1}H NMR ($CDCl_3$) δ, ppm: 0.76 (s, 3H, 18-H_3), 2.84 (m, 2H, 6-H_2), 3.51 (m, 2H, 16a-H_2),), 3.64 (s, 1H, 17-H), 5.02 (s, 2H, benzyl-H_2), 6.71 (d, 1H, J = 2.4 Hz, 4-H), 6.77 (dd, 1H, J = 8.6 Hz, J = 2.4 Hz, 2-H), 7.19 (d, 1H, J = 8.6 Hz, 1-H), 7.30 (t, 1H, J = 7.3 Hz, 4′-H), 7.36 (t, 2H, J = 7.3 Hz, 3′,5′-H), 7.41 (d, 2H, J = 7.3 Hz, 2′,6′-H). ^{13}C NMR ($CDCl_3$) δ, ppm: 17.9 (C-18), 25.9, 27.9, 29.7, 31.9, 32.1, 37.3 (C-16a), 38.6, 43.3, 45.1 (C-13), 49.3, 52.2, 70.0 (benzyl-CH_2), 84.1 (C-17), 112.3 (C-2), 114.9 (C-4), 126.2 (C-1); 127.4 (2C, C-2′,6′), 127.8 (C-4′), 128.5 (2C, C-3′,5′), 132.8 (C-10), 137.3 (C-1′), 137.9 (C-5), 156.8 (C-3); EI-MS (70 eV) m/z (%): 456 (6) [M^+ + 2], 454 (6) [M^+], 91 (100).

2.4. *Debenzylation of 3-benzyloxy-16-bromomethylestra-1,3,5(10)-trien-17-ol isomers (5a–8a) with Pd/C to 16-bromomethylestra-1,3,5(10)-triene-3,17-diols (5e–8e)*

2.4.1. *General procedure*

Compounds **5a–7a** or **8a** (700 mg, 1.54 mmol) was dissolved in 50 ml of ethyl acetate and 250 mg of Pd/C was added to the solution, which was stirred at room temperature for 6 h at 30 bar H_2 pressure. The reaction mixture was filtered over celite. After evaporation of the filtrate, the crude product was purified by column chromatography with ethyl acetate/$CHCl_3$ 10:90).

5e (542 mg, 96%). Mp 168–169 °C; R_f = 0.40 (ss B); $[\alpha]_D^{20}$ + 114 (c 1.00 in ethyl acetate). (Found: C, 62.55; H, 7.02. $C_{19}H_{25}O_2Br$ requires: C 62.47; H 6.90%). ^{1}H NMR (DMSO-d_6) δ, ppm: 0.68 (s, 3H, 18-H_3), 2.70 (m, 2H, 6-H_2), 3.28 (t, 1H, J = 10.9 Hz, 17-H), 3.63 (m, 1H) and 3.76 (dd, 1H, J = 9.6 Hz, J = 4.2 Hz): 16a-H_2, 4.84 (d, 1H, J = 5.5 Hz, 17-OH), 6.43 (s, 1H, 4-H), 6.50 (d, 1H, J = 7.9 Hz, 2-H), 7.02 (d, 1H, J = 8.5 Hz, 1-H), 8.96 (s, 1H, 3-OH). ^{13}C NMR (DMSO-d_6) δ, ppm: 12.4 (C-18), 25.9, 27.0, 29.1, 32.4, 37.1, 37.9, 39.3, 43.1, 43.4, 43.8, 47.4, 80.6 (C-17), 112.7 (C-2), 114.9 (C-4), 125.9 (C-1); 130.3 (C-10), 137.0 (C-5), 154.8 (C-3); EI-MS (70 eV) m/z (%): 366 (34) [M^+ + 2], 364 (36) [M^+], 267 (23), 213 (12), 185 (9), 159 (17), 145 (10), 133 (24), 83 (11), 43 (100).

6e (550 mg, 98%). Mp 181–183 °C; R_f = 0.50 (ss B); $[\alpha]_D^{20}$ + 70 (c 1.00 in ethyl acetate). (Found: C, 62.55; H, 7.02. C19$H_{25}O_2Br$ requires: C 62.47; H 6.90%). ^{1}H NMR (DMSO-d_6) δ, ppm: 0.68 (s, 3H, 18-H_3), 2.70 (m, 2H, 6-H_2), 3.41 (t, 1H, J = 8.6 Hz) and 3.63 (t, 1H, J = 8.6 Hz): 16a-H_2, 3.60 (d, 1H, J = 5.0 Hz, 17-H), 6.43 (d, 1H, J = 2.0 Hz, 4-H), 6.50 (dd, 1H, J = 8.4 Hz, J = 2.0 Hz, 2-H), 7.04 (d, 1H, J = 8.4 Hz, 1-H), 8.96 (s, 1H, 3-OH). ^{13}C NMR (DMSO-d_6) δ, ppm: 17.0 (C-18), 25.8, 27.6, 29.2, 30.6, 31.2, 36.7, 38.8, 43.2, 43.6, 46.1, 46.3, 78.5 (C-17), 112.7 (C-2), 114.8 (C-4), 125.9 (C-1); 130.4 (C-10), 137.1 (C-5), 154.8 (C-3); EI-MS (70 eV) m/z (%): 366 (99) [M^+ + 2], 364 (100) [M^+], 267 (15), 213 (21), 198 (15), 185 (11), 172 (22), 159 (35), 146 (20), 133 (37), 107 (13).

7e (547 mg, 97%). Mp 188–189 °C; R_f = 0.30 (ss B); $[\alpha]_D^{20}$ + 37 (c 1.00 in ethyl acetate). (Found: C, 62.36; H, 6.84. C19$H_{25}O_2Br$ requires: C 62.47; H 6.90%). ^{1}H NMR (DMSO-d_6) δ, ppm: 0.71 (s, 3H, 18-H_3), 2.70 (m, 2H, 6-H_2), 3.24 (m, 1H, 17-H), 3.55 (t, 1H, J = 9.6 Hz) and 3.73 (dd, 1H, J = 9.6 Hz, J = 3.4 Hz): 16a-H_2, 4.87 (d, 1H, J = 4.8 Hz, 17-OH), 6.43 (s, 1H, 4-H), 6.50 (d, 1H, J = 8.4 Hz, 2-H), 7.01 (d, 1H, J = 8.4 Hz, 1-H), 9.00 (s, 1H, 3-OH). ^{13}C NMR (DMSO-d_6) δ, ppm: 12.0 (C-18), 25.9, 26.8, 28.9, 29.1, 36.5, 38.3, 40.6, 43.4, 44.0, 45.6, 47.7, 83.4 (C-17), 112.7 (C-2), 114.9 (C-4), 125.9 (C-1); 130.3 (C-10), 137.1 (C-5), 154.8 (C-3); EI-MS (70 eV) m/z (%): 366 (87) [M^+ + 2], 364 (89) [M^+], 267 (31), 213 (34), 198 (43), 185 (32), 172 (72), 159 (89), 145 (44), 133 (100), 107 (27), 43 (19).

8e (540 mg, 96%). Mp 186–188 °C; R_f 0.30 (ss B); $[\alpha]_D^{20}$ + 61 (c 1.00 in ethyl acetate). (Found: C, 62.55; H, 7.08. $C_{19}H_{25}O_2Br$ requires: C 62.47, H 6.90%). ^{1}H NMR (DMSO-d_6) δ, ppm: 0.67 (s, 3H, 18-H_3), 2.69 (m, 2H, 6-H_2), 3.38 (d, 1H, J = 4.2 Hz, 17-H), 3.50 (t, 1H, J = 9.2 Hz) and 3.66 (t, 1H, J = 9.2 Hz): 16a-H_2, 4.67 (d, 1H, J = 4.6 Hz, 17-OH), 6.43 (s, 1H, 4-H), 6.50 (d, 1H, J = 8.4 Hz, 2-H), 7.03 (d, 1H, J = 8.4 Hz, 1-H), 8.95 (s, 1H, 3-OH). ^{13}C NMR (DMSO-d_6) δ, ppm: 17.8 (C-18), 25.8, 27.7, 29.2, 31.6, 32.0, 38.4, 38.9, 43.0, 44.6, 48.7, 51.7, 82.6 (C-17), 112.7 (C-2), 114.9 (C-4), 125.9 (C-1); 130.4 (C-10), 137.0 (C-5), 154.8 (C-3); EI-MS (70 eV) m/z (%): 366 (100) [M^+ + 2], 364 (98) [M^+], 267 (23), 213 (17), 198 (11), 172 (18), 159 (21), 146 (12), 133 (23).

2.5. *Reaction of 3-benzyloxy-16α-bromomethylestra-1,3,5(10)-trien-17β-ol (7a) with HBr in acetic acid*

2.5.1. *Method B*

2.5.1.1. 16α-Bromomethyl-3-hydroxyestra-1,3,5(10)-trien-17β-yl acetate (7c). Compound **7c** (950 mg, 78%). Mp 181–182 °C,

$[\alpha]_D^{20}$ − 3 (*c* 1.00 in $CHCl_3$); R_f = 0.60 (ss C). (Found: C, 62.05; H, 6.50. $C_{21}H_{27}O_3Br$ requires: C, 61.92; H, 6.68%). ^{1}H-NMR ($CDCl_3$) δ, ppm: 0.85 (s, 3H, 18-H_3), 2.09 (s, 3H, OAc-CH_3), 2.81 (m, 2H, 6-H_2), 3.43 (dd, 1H, *J* = 10.0 Hz, *J* = 7.6 Hz) and 3.57 (dd, 1H, *J* = 10.0 Hz, *J* = 5.0 Hz): 16a-H_2, 4.71 (d, 1H, *J* = 7.7 Hz, 17-H), 6.56 (d, 1H, *J* = 2.4 Hz, 4-H), 6.63 (dd, 1H, *J* = 8.4 Hz, *J* = 2.5 Hz, 2-H), 7.11 (d, 1H, *J* = 8.4 Hz, 1-H). ^{13}C-NMR ($CDCl_3$) δ, ppm: 12.9 (C-18), 21.1 (OAc-CH_3), 26.0, 27.0, 29.5, 29.8, 36.8, 37.6, 38.3, 43.6 (2C), 44.8 (C-13), 48.4, 85.3 (C-17), 112.8 (C-2), 115.3 (C-4), 126.4 (C-1); 132.2 (C-10), 138.0 (C-5), 153.5 (C-3), 171.4 (OAc-CO); EI-MS (7 eV) *m*/*z* (%): 408 (100) [M^+ + 2], 406 (99) [M^+], 267 (9), 172 (16), 159 (18), 146 (11), 133 (16), 107 (4), 43 (11).

2.5.2. *Method C*

2.5.2.1. 16α-Bromomethylestra-1,3,5(10)-triene-3,17β-diyl diacetate (7d). Compound **7d** (1.01 g, 75%) as a colorless oil. $[\alpha]_D^{20}$ − 1 (*c* 1.00 in $CHCl_3$); R_f = 0.50 (ss A). (Found: C, 61.32; H, 6.68. $C_{23}H_{29}O_4Br$ requires: C 61.47, H 6.50%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.85 (s, 3H, 18-H_3), 2.09 (s, 3H, 17-OAc-CH_3), 2.27 (s, 3H, 3-OAc-CH_3), 2.86 (dd, 2H, *J* = 7.6 Hz, *J* = 3.2 Hz, 6-H_2), 3.43 (dd, 1H, *J* = 10.0 Hz, *J* = 7.7 Hz) and 3.58 (dd, 1H, *J* = 10.0 Hz, *J* = 4.8 Hz): 16a-H_2, 4.70 (d, 1H, *J* = 7.7 Hz, 17-H), 6.78 (d, 1H, *J* = 1.8 Hz, 4-H), 6.83 (dd, 1H, *J* = 8.4 Hz, *J* = 2.2 Hz, 2-H), 7.25 (d, 1H, *J* = 8.4 Hz, 1-H). ^{13}C NMR ($CDCl_3$) δ, ppm: 12.8 (C-18), 21.1 (2C, OAc-CH_3), 25.8, 26.8, 29.3, 29.7, 36.7, 37.7, 37.9, 43.6, 43.8, 44.7 (C-13), 48.4, 84.9 (C-17), 118.6 (C-2), 121.4 (C-4), 126.3 (C-1), 137.6 (C-10), 138.0 (C-5), 148.4 (C-3), 169.7 (3-OAc-CO), 171.0 (17-OAc-CO); EI-MS (7 eV) *m*/*z* (%): 450 (8) [M^+ + 2], 448 (8) [M^+], 408 (100), 406 (98), 267 (9), 172 (11), 159 (12), 133 (10), 83 (30), 57.1 (39), 43 (27).

2.5.3. *Reaction of 3-benzyloxy-16β-bromomethylestra-1,3,5(10)-trien-17α-ol (8a) with HBr in acetic acid*

2.5.3.1. Method B.

2.5.3.1.1. 16β-Bromomethyl-3-hydroxyestra-1,3,5(10)-trien-17α-yl acetate (8c). Compound **8c** (940 mg, 77%). Mp 210–212 °C; R_f = 0.60 (ss C); $[\alpha]_D^{20}$ + 38 (*c* 1.00 in $CHCl_3$). (Found: C, 61.25; H, 6.81. $C_{21}H_{27}O_3Br$ requires: C 61.92, H 6.68%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.86 (s, 3H, 18-H_3), 2.09 (s, 3H, OAc-CH_3), 2.83 (m, 2H, 6-H_2), 3.43 (t, 1H, *J* = 9.7 Hz) and 3.74 (dd, 1H, *J* = 9.7 Hz, *J* = 6.6 Hz): 16a-H_2, 4.63 (d, 1H, *J* = 2.0 Hz, 17-H), 4.75 (s, 1H, 3-OH), 6.57 (d, 1H, *J* = 2.5 Hz, 4-H), 6.63 (dd, 1H, *J* = 8.4 Hz, *J* = 2.5 Hz, 2-H), 7.14 (d, 1H, *J* = 8.4 Hz, 1-H). ^{13}C-NMR ($CDCl_3$) δ, ppm: 17.5 (C-18), 21.1 (OAc-CH_3), 25.9, 27.8, 29.6, 32.2 (2C), 36.7, 38.5, 43.3, 44.8 (C-13), 49.5, 50.1, 85.2 (C-17), 112.7 (C-2), 115.2 (C-4), 126.5 (C-1); 132.4 (C-10), 138.1 (C-5), 153.5 (C-3), 170.6 (OAc-CO); EI-MS (7 eV) *m*/*z* (%): 408 (96) [M^+ + 2], 406 (100) [M^+], 267 (17), 172 (21), 160 (78), 146 (39), 133 (50), 43 (100).

2.5.3.2. Method C.

2.5.3.2.1. 16β-Bromomethylestra-1,3,5(10)-triene-3,17α-diyl diacetate (8d). Compound **8d** (1.03 g, 76%) as a colorless oil; R_f = 0.50 (ss A); $[\alpha]_D^{20}$ + 36 (*c* 1.00 in $CHCl_3$). (Found: C, 61.58; H, 6.82.$C_{23}H_{29}O_4Br$ requires: C, 61.47; H, 6.50%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.86 (s, 3H, 18-H_3), 2.08 (s, 3H, 17-OAc-CH_3), 2.27 (s, 3H, 3-OAc-CH_3), 2.86 (m, 2H, 6-H_2), 3.43 (t, 1H, *J* = 9.6 Hz) and 3.74 (dd, 1H, *J* = 9.6 Hz, *J* = 6.6 Hz): 16a-H_2, 4.62 (d, 1H, *J* = 1.8 Hz, 17-H), 6.79 (d, 1H, *J* = 1.8 Hz, 4-H), 6.83 (dd, 1H, *J* = 8.4 Hz, *J* = 1.8 Hz, 2-H), 7.27 (d, 1H, *J* = 8.4 Hz, 1-H); ^{13}C NMR ($CDCl_3$) δ, ppm: 17.4 (C-18), 21.1 (2C, OAc-CH_3), 25.6, 27.6, 29.4, 32.2 (2C), 36.6, 38.1, 43.4, 44.7 (C-13), 49.5, 50.1, 85.1 (C-17), 118.6 (C-2), 121.5 (C-4), 126.3 (C-1), 137.6 (C-10), 138.0 (C-5), 148.4 (C-3), 169.8 (3-OAc-CO), 170.4 (17-OAc-CO); EI-MS (7 eV) *m*/*z* (%): 450 (16) [M^+ + 2], 448 (16) [M^+], 408 (17), 406 (19), 160 (6), 85 (15), 83 (26), 57 (10), 43 (100).

2.6. *3-Benzyloxy-16-bromomethylestra-1,3,5(10)-trien-17-yl acetate (7b and 8b)*

2.6.1. *General procedure*

Compound **7a** or **8a** (455 mg, 1 mmol) was dissolved in 2 ml of pyridine and 2 ml of acetic anhydride. The solution was allowed to stand at room temperature for 6 h. The mixture was then diluted with water. The precipitate was collected by filtration and dissolved in CH_2Cl_2, and the solution was washed with water and dried over Na_2SO_4. After evaporation in vacuo, the product was chromatographed on silica gel with ethyl acetate/$CHCl_3$ (2.5:97.5).

7b (483 mg, 97%). Mp 120–121 °C; R_f = 0.65 (ss A); $[\alpha]_D^{20}$ + 12 (*c* 1.00 in $CHCl_3$). (Found: C, 67.51; H, 6.75. $C_{28}H_{33}O_3Br$ requires: C, 67.60; H, 6.69%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.85 (s, 3H, 18-H_3), 2.08 (s, 3H, OAc-CH_3), 2.83 (m, 2H, 6-H_2), 3.42 (dd, 1H, *J* = 9.8 Hz, *J* = 7.7 Hz) and 3.57 (dd, 1H, *J* = 9.8 Hz, *J* = 5.1 Hz): 16a-H_2, 4.70 (d, 1H, *J* = 7.7 Hz, 17-H), 5.01 (s, 2H, Bzl-H_2), 6.70 (d, 1H, *J* = 2.2 Hz, 4-H), 6.76 (dd, 1H, *J* = 8.5 Hz, *J* = 2.5 Hz, 2-H), 7.17 (d, 1H, *J* = 8.6 Hz, 1-H), 7.31 (t, 1H, *J* = 7.3 Hz, 4′-H), 7.36 (t, 2H, *J* = 7.3 Hz, 3′,5′-H), 7.41 (d, 2H, *J* = 7.3 Hz, 2′,6′-H). ^{13}C NMR ($CDCl_3$) δ, ppm: 12.8 (C-18), 21.1 (Ac-CH_3), 26.0, 27.1, 29.6, 29.8, 36.8, 37.7, 38.3, 43.6 (2C), 44.7 (C-13), 48.4, 69.9 (C-Bzl-CH_2), 85.0 (C-17), 112.3 (C-2), 114.8 (C-4), 126.2 (C-1); 127.4 (2C, C-2′,6′), 127.8 (C-4′), 128.5 (2C, C-3′,5′), 132.5 (C-10), 137.3 (C-1′), 137.8 (C-5), 156.7 (C-3), 171.0 (OAc-CO); EI-MS (7 eV) *m*/*z* (%): 498 (25) [M^+ + 2], 496 (25) [M^+], 91 (100), 43 (11).

8b (478 mg, 96%) as a colorless oil; R_f = 0.70 (ss A); $[\alpha]_D^{20}$ + 37 (*c* 1.00 in $CHCl_3$). (Found: C, 67.48; H, 6.75. $C_{28}H_{33}O_3Br$ requires: C, 67.60; H, 6.69%). ^{1}H NMR ($CDCl_3$) δ, ppm: 0.86 (s, 3H, 18-H_3), 2.08 (s, 3H, OAc-CH_3), 2.85 (m, 2H, 6-H_2), 3.42 (t, 1H, *J* = 9.7 Hz) and 3.74 (dd, 1H, *J* = 9.7 Hz, *J* = 6.5 Hz): 16a-H_2, 4.62 (d, 1H, *J* = 2.1 Hz, 17-H), 5.02 (s, 2H, Bzl-H_2), 6.71 (d, 1H, *J* = 2.5 Hz, 4-H), 6.77 (dd, 1H, *J* = 8.6 Hz, *J* = 2.5 Hz, 2-H), 7.19 (d, 1H, *J* = 8.6 Hz, 1-H), 7.31 (t, 1H, *J* = 7.3 Hz, 4′-H), 7.37 (t, 2H, *J* = 7.3 Hz, 3′,5′-H), 7.42 (d, 2H, *J* = 7.3 Hz, 2′,6′-H). ^{13}C NMR ($CDCl_3$) δ, ppm: 17.44 (C-18), 21.1 (OAc-CH_3), 25.8, 27.8, 29.7, 32.2 (2C), 36.7, 38.5, 43.3, 44.8 (C-13), 49.5, 50.1, 69.9 (Bzl-CH_2), 85.1 (C-17), 112.3 (C-2), 114.8 (C-4), 126.3 (C-1); 127.4 (2C, C-2′,6′), 127.8 (C-4′), 128.5 (2C, C-3′,5′), 132.6 (C-10), 137.3 (C-1′), 137.8 (C-5), 156.8 (C-3), 170.5 (OAc-CO); EI-MS (7 eV) *m*/*z* (%): 498 (9) [M^+ + 2], 496 (13) [M^+], 91 (100), 43 (10).

2.7. *Receptor-binding assay*

Eight synthesized compounds (**5c–8c** and **5e–8e**) were evaluated for their ability to inhibit competitively the binding of [^{3}H]estra-1,3,5(10)-triene-3,17β-diol ([^{3}H]E2; 110 Ci mmol^{-1}) to ERs, of [^{3}H]ORG2058 (34 Ci mmol^{-1}) to PRs in cytosol prepared from ovariectomized rat uteri, and of [^{3}H]dihydrotestosterone ([^{3}H]DHT; 110 Ci mmol^{-1}) to ARs in castrated rat prostate cytosol [15].

Mature female (200–300 g) and male (300–400 g) rats were gonadectomized under general anesthesia (sodium

pentobarbital 1 g/kg i.p.) 48 or 24 h before the tissue preparation, respectively.

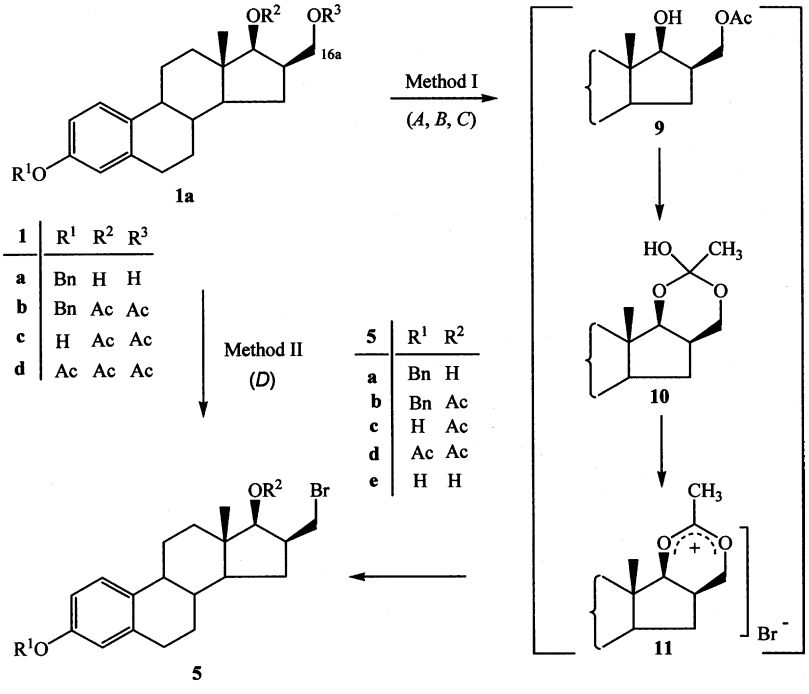
The tritiated ligands were purchased from Perkin-Elmer, USA, except for [^{3}H]ORG2058, which was obtained from Amersham, UK. All subsequent steps were carried out at 4 °C. The minced uterine tissues were homogenized in 10 volume (w/v) of buffer A (25 mM Tris–HCl, 1.5 mM EDTA disodium salt, 10 mM α-monothioglycerol, 10% glycerol and 10 mM sodium molybdate, pH 7.4) to measure the abilities of the compounds to bind to the ERs or PRs [16]. The ventral prostates tissues were homogenized in 3 volume (v/v) of buffer I (20 mM Tris–HCl, 1 mM EDTA disodium salt, 1 mM dithiothreitol and 10% glycerol, pH 7.5) to measure the abilities of the compounds to bind to the ARs [17]. The homogenates were centrifuged at 105,000 × *g* for 60 min and the supernatants (cytosol) were used for competitive binding. Aliquots of 100 μl of cytosol were incubated with 5 nM [^{3}H]E2, 4 nM [^{3}H]ORG2058 or 5 nM [^{3}H]DHT in the presence of synthesized molecules in 11 different concentrations (from 10^{-12} to 10^{-5} M) for 3 h at 25 °C for ER, for 24 h at 4 °C for PRs, and for 4 h at 4 °C for ARs. To determine the total bound radiolabeled ligand, we incubated 100 μl of cytosol with 100 μl of isotope and 100 μl of assay buffer. Unbound steroids were separated by incubation for 15 min at 4 °C with dextran-coated charcoal (0.5% Norit-A and 0.05% dextran T-70 in buffer B (1.5 mM EDTA disodium salt, 10 mM α-monothioglycerol and 10 mM Tris–HCl, pH 7.4) for ERs and PRs, and for 30 min at 4 °C with 1.25% Norit-A and 0.625% dextran T-70 in buffer II (10 mM Tris–HCl and 1 mM EDTA disodium salt, pH 7.5) for ARs. After incubation, the samples were centrifuged at 3000 rpm for 10 min, and the radioactivity of the supernatant was determined in a Wallac 1409 liquid scintillation counter. The percentage of the bound radioligand in the presence of competitor was plotted against the concentration of unlabeled steroid. The molar concentration of the steroid competitor that reduced the radioligand binding by 50% (IC_{50}) and the K_i value (inhibition constant) were calculated with GraphPad 2.0 software. To compare the ER-binding abilities of the investigated molecules with the K_i value of the reference compound, we calculated the relative binding affinity (RBA) according to the following equation: $RBA = (K_{i,E2}/K_{i,x}) \times 100$. All assays were carried out at least three times.

3. Results and discussion

3.1. Synthetic studies

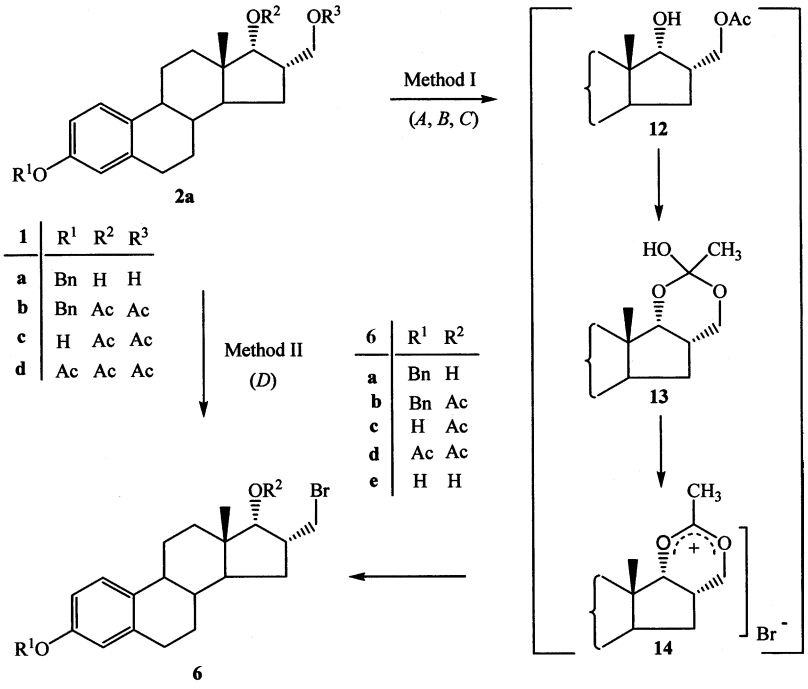
In preliminary experiments, the four possible isomers of 3-benzyloxy-16-hydroxymethylestra-1,3,5(10)-trien-17-ol isomers (**1a–4a**) were synthesized via a multistep pathway [14] in order to obtain the 16-bromomethyl-3-hydroxyestra-1,3,5(10)-trien-17-yl acetates (**5c–8c**) and the parent 16-bromomethyl-diols (**5e–8e**) derivatives.

First we studied the behavior of the four starting materials **1a–4a** in the presence of HBr and acetic acid. From the *cis* diols **1a**, and **2a**, we obtained different 16-bromomethyl-17-acetates (**5b–d** and **6b–d**) which, depending on the reaction time or conditions, differed from each other in the substituent at position C-3 (Schemes 1 and 2). Reactions for 1 h led to **5b** and **6b**, which have benzyl protecting groups at position C-3. After 12 h, the bromo substitution was at C-16a and debenzylation also occured. By this method, the *cis* diols furnished



A: HBr / acetic acid 1h
B: HBr / acetic acid 12h
C: HBr / acetic acid 12 h + acetic anhydride 6h
D: CBr_4 / $(Ph)_3P$

Scheme 1



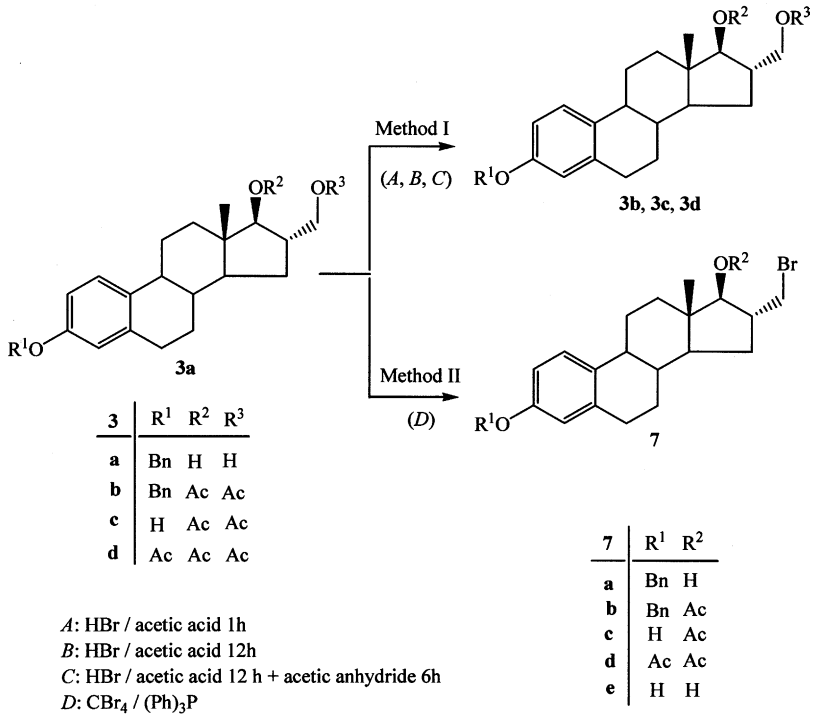
Scheme 2

3-deprotected 16-bromomethyl derivatives (**5c** and **6c**), which were subjected to receptor-binding examinations directly. In the following experiments, the 3-hydroxy function was transformed into an acetoxy group by adding acetic anhydride to the reaction mixture, yielding **5d** and **6d**. This procedure allowed us to replace the benzyl protecting group with the acetate group, which is easily removable and convenient. The 16-bromomethylestradiol derivatives were accompanied by 16-acetoxymethylestradiol derivatives (**1b–d** and **2b–d**) as by-products in low yields.

In contrast, from the *trans* diols (**3a** and **4a**), 16-bromomethyl products were not formed (Schemes 3 and 4). Instead of bromination, acetylation occurred, yielding different diacetoxyestradiol derivatives (**3b–d** and **4b–d**), depending on the reaction conditions. In the case of 3-benzyloxy-16β-hydroxymethylestra-1,3,5(10)-trien-17α-ol (**4a**), compound **16b** was formed as a by-product via Wagner–Meerwein rearrangement.

The experimental results reveal that the four diols (**1a–4a**) take part in stereoselective substitution in the presence of HBr and acetic acid. The formation of 16-bromomethyl-estradiol derivatives **5b** and **6b** can easily be explained in terms of front-side neighboring group participation [18]. According to the earlier observations the 16-hydroxymethyl group of **1a** and **2a** undergoes acetylation and generates **9** and **12**, which are formed under the appropriate reaction conditions. These monoacetates (**9** and **12**) cyclized into an *ortho* acid–*ortho* ester (**10** and **13**) in the acid medium, which are transformed via the ambidentate 2′-methyl-1′,3′-dioxenium ions **11** and **14** into **5b** and **6b**. During the formation of **5b** and **6b**, no change in configuration occurs, since ring cleavage in the ambidentate cations **11** and **14** takes place at the sterically favored site C-16a [19]. Furthermore, the absence of 16-bromomethylestradiol derivatives in the case of the *trans* diols **3a** and **4a** can be explained by the rigidity of the estrane skeleton and the unfavorable configuration of the substituents, which should take part in a formation of six-membered acyloxonium cation intermediates. The formation of **16b** from **4a** can be explained by a Wagner–Meerwein rearrangement: the secondary hydroxy function at C-17 is protonated and a carbocation **15** is generated after the elimination of water under the appropriate reaction conditions. This carbocation (**15**) furnishes **16b** through migration of the angular methyl group and subsequent deprotonation. The isolated double bond of the tetraene of type **16b** during a prolonged reaction time with HBr addition produced very sensitive undefined compounds.

The above experimental findings led us to develop a convenient method for the synthesis of *trans* 16-bromomethyl-17-acetate derivatives (**7** and **8**). First we brominated the *trans* diols **3a** and **4a** by the Appel reaction and obtained the *trans* 3-benzyloxy-16-bromomethylestra-1,3,5(10)-trien-17-ols (**7a** and **8a**). These compounds (**7a** and **8a**) permitted direct synthesis of the *trans* 16-bromomethyl-3,17-diacetates **7d** and **8d** by conversion of the substituent at C-3. The acetylation of **7a** and **8a** with acetic anhydride yielded 17-acetoxy compounds **7b** and **8b** in good yields. The transprotected derivatives **7d** and **8d** were synthesized from **7b** and **8b** by debenzylation with HBr and the subsequent addition of acetic anhydride to the reaction mixture in one step.



3	R^1	R^2	R^3
a	Bn	H	H
b	Bn	Ac	Ac
c	H	Ac	Ac
d	Ac	Ac	Ac

7	R^1	R^2
a	Bn	H
b	Bn	Ac
c	H	Ac
d	Ac	Ac
e	H	H

A: HBr / acetic acid 1h
B: HBr / acetic acid 12h
C: HBr / acetic acid 12 h + acetic anhydride 6h
D: CBr_4 / $(Ph)_3P$

Scheme 3

After the synthesis of 17-acetoxy-16-bromomethyl-3-hydroxy compounds (**5c–8c**) we set out to produce the appropriate 17-hydroxy derivatives, too. This was reasonable in view of their potential biological activities, while 17-hydroxyestradiol derivatives presumably have higher RBAs than their 17-acetoxy counterparts. The *cis* 16-bromomethylestradiol derivatives **5a** and **6a** could also be obtained by application of the Appel reaction. The 16-bromomethyl-17-hydroxy compounds **5a–8a** were deprotected by hydrogenolysis, which yielded the corresponding estradiols (**5e–8e**). The synthesized products could be subjected to receptor-binding examinations directly.

The NMR spectra demonstrated certain interesting features. In the ^{1}H NMR spectra of the 16-bromomethyl-17-acetates (**5b–8b, d**) and 16-bromomethylestradiol derivatives (**5e–8e**), the double doublet or triplet of the 16a-H_2 appears at $\delta = 3.26$–3.76 ppm. The analogous signals of the 16-acetoxymethyl-17-acetates (**1b–4b, d**) are found at higher chemical shifts, around 4.0–4.2 ppm). They also occur at higher ppm values than the similar signals of the *cis* isomers.

Further, the doublet assigned to 17-H in 16-bromomethyl,17-acetates (**5b–8b, d**) and 16-acetoxymethyl-17-acetates (**1b–4b, d**) appears at $\delta = 4.62$–5.10 ppm. For 16-bromomethylestradiol derivatives (**5e–8e**), the signal of 17-H is observed at higher frequencies, $\delta = 3.47$–3.82 ppm, as compared with the 17-acetates. As opposed to the 16a-H signals, the doublets of 17-H of the corresponding *cis* isomers lie at higher ppm values than in the spectra of the *trans* isomers. In agreement with the literature [20], the coupling constants $J_{16,17}$ display the following sequence: $J_{16\alpha H,17\beta H} < J_{16\beta H,17\beta H} < J_{16\beta H,17\alpha H} < J_{16\alpha H,17\alpha H}$. The ^{13}C NMR resonance of C-17 is usually observed at $\delta = 79.9$–85.8 ppm and this signal of the corresponding *trans* isomer is found at higher ppm values than for the analogous signals of the *cis* isomers.

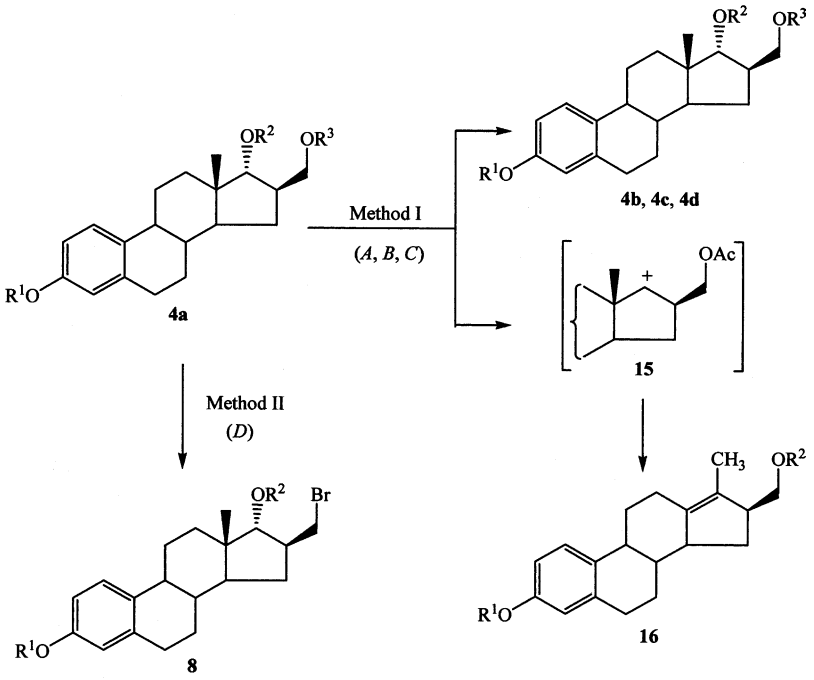
For the products containing a benzyl protecting group at position C-3 (**1a–8a, b, d**), the singlet of the benzyl-CH_2 appears in the ^{1}H NMR spectra at $\delta = 5.02$ ppm and in the ^{13}C NMR spectra at $\delta = 70.0$ ppm. The resonance of C-3 in the ^{13}C NMR spectra is observed at $\delta = 148.5$–156.8 ppm. The singlet at $\delta = 156.8$ ppm can be assigned to bearing the C-3 benzyloxy group, that at $\delta = 153.5$ ppm to the C-3 hydroxy function, and that at $\delta = 148.5$ ppm to the C-3 acetoxy group. Additionally, the OAc-CH_3 at position C-3 in **1d–8d** resonates at $\delta = 2.27$ ppm in ^{1}H NMR spectra and at 5 = 21.0 ppm in the ^{13}C NMR spectra. Finally, the signal from C=O (3-OAc) is found at $\delta = 169.9$ ppm.

3.2. *Radioligand-binding assay*

The RBAs of 16-bromomethyl-3-hydroxyestra-1,3,5(10)-trien-17-yl 17-acetates (**5c–8c**) and 16-bromomethylestra-1,3,5(10)-triene-3,17-diols (**5e–8e**) and their derivatives for the ERs, PRs and ARs were determined by radioligand-binding assay.

Our reference molecule for the ERs was estra-1,3,5(10)-triene-3,17β-diol (**E2**), the inhibition constant of which under our experimental conditions ($K_i = 2.31 \pm 0.76$ nM) was in good agreement with the literature K_i value (2.48 nM) [16].

Specifically bound [^{3}H]E2 was displaced from the ovariectomized rat uterine ERs by **5c** and its stereoisomers (**6c–8c**). 16α-Bromomethylestra-1,3,5(10)-triene-3,17-diyl 17β-acetate (**7c**) displays the highest binding affinity to the ERs in this series, though its K_i is two orders of magnitude lower than that of **E2**, the endogenous ligand of the ER. The other three molecules (**5c, 6c** and **8c**) have K_i values one order of magnitude higher than that for **7c**, and there is no major difference between the RBAs of the compounds. The K_i values are listed



4	R^1	R^2	R^3
a	Bn	H	H
b	Bn	Ac	Ac
c	H	Ac	Ac
d	Ac	Ac	Ac

8	R^1	R^2
a	Bn	H
b	Bn	Ac
c	H	Ac
d	Ac	Ac
e	H	H

16	R^1	R^2
a	Bn	OH
b	Bn	Ac

A: HBr / acetic acid 1h
B: HBr / acetic acid 12h
C: HBr / acetic acid 12 h + acetic anhydride 6h
D: CBr_4 / $(Ph)_3P$

Scheme 4

in Table 1, and representative curves from the ER displacement analysis are depicted in Fig. 1.

The reference molecule for the PR was **ORG2058**, with $K_i = 7.40 \pm 0.98$ nM under our experimental circumstances. All of the 17-acetate derivatives of estradiol (**5c–8c**) bind weakly to the PRs, with K_i values in excess of 3000 nM, and thus this receptor does not recognize them. Three compounds in this series exhibit a higher affinity to the ARs than to the ERs. The

Table 1 – Inhibition constants (K_i) of estra-1,3,5(10)-triene-3,17β-diol (E2), ORG2058 and dihydrotestosterone (DHT) as reference compounds and currently synthesized derivatives for the uterine ERs and PRs and for the prostate ARs

Investigated compounds	K_i (nM) ER	RBA (%)	K_i (nM) PR	K_i (nM) AR
E2	2.31 ± 0.76	100	–	–
ORG2058	–	–	7.40 ± 0.98	–
DHT	–	–	–	9.17 ± 0.71
5c	3855 ± 2350	0.06	≥3000	Not measurable[a]
7c	553.8 ± 198.3	0.42	≥3000	475.5 ± 7.28
8c	5254 ± 1040	0.04	≥3000	2963 ± 1528
6c	4143 ± 2269	0.09	≥3000	237.2 ± 23.76
5e	461.4 ± 145.6	0.50	≥3000	≥3000
7e	2.55 ± 0.64	90.59	525.2 ± 142.7	≥3000
8e	781.1 ± 149.1	0.29	≥3000	≥3000
6e	684.4 ± 154.0	0.34	≥3000	214.7 ± 10.96

The following radioligands were used: 5 nM [^{3}H]estra-1,3,5(10)-triene-3,17β-diol for the ERs, 4 nM [^{3}H]ORG2058 for the PRs and 5 nM [^{3}H]dihydrotestosterone for the ARs. The relative binding affinities (RBAs) on the ERs were calculated according to the following equation: $RBA = (K_{i;E2}/K_{i;x}) \times 100$.

[a] $K_i > 10{,}000$ nM; the ligand is not able to displace the radioligand from the receptor-binding site.

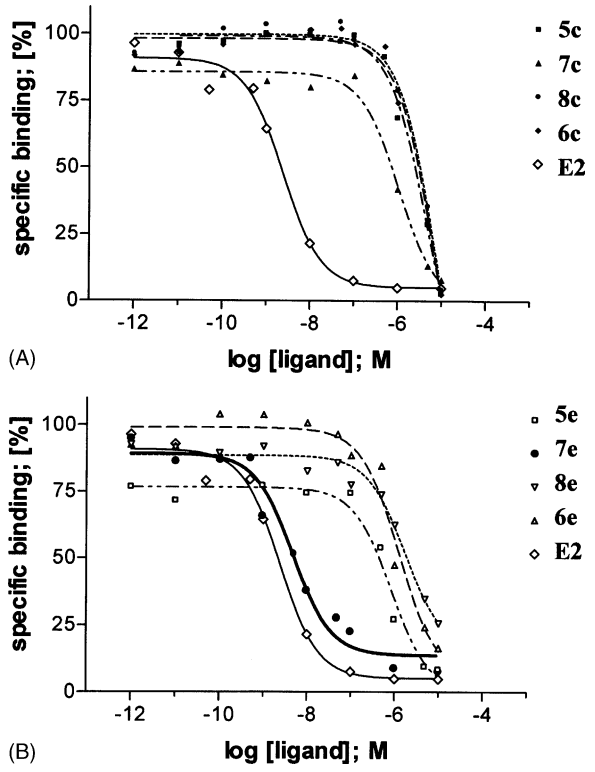


Fig. 1 – Competitive inhibition of [^{3}H]estra-1,3,5(10)-triene-3,17β-diol binding in rat uterine cytosol by 16-bromomethyl-3-hydroxyestra-1,3,5(10)-trien-17-yl 17-acetate (A) and 16-bromomethylestra-1,3,5(10)-triene-3,17-diol (B) and their stereoisomers. The reference molecule is estra-1,3,5(10)-triene-3,17b-diol (E2).

most marked difference is observed in the case of **6c**, which binds one order of magnitude more strongly to the ARs than to the ERs. Its K_i value for the ARs is the lowest in this group. The isomer (**5c**) could not displace specifically bound [^{3}H]DHT from the castrated rat prostate ARs.

The 16-bromomethylestrane derivatives of 3,17-estradiol (**5e–8e**) recognize the ERs well. Compound **7e** exhibits the highest affinity to this receptor ($K_i = 2.55 \pm 0.64$ nM), and this compound therefore binds to the ERs as strongly as **E2** does. The K_i values of the other molecules (**5e, 6e** and **8e**) are at least 200-fold higher than that for **7e**.

Compound **7e** displays the highest affinity for the PRs, but this interaction is 200-fold weaker than that with the ERs. The remaining three derivatives have K_i values higher than 3000 nM. The members of this series display weak affinity for the ARs ($K_i > 3000$ nM), except for the 16α-bromomethylestra-1,3,5(10)-triene-3,17α-diol substituent (**6e**). This latter exhibits the strongest RBA to the ARs. It binds 3-fold more strongly to the ARs than to the ERs, but its interactions with the ARs are 23-fold weaker than that of to the reference compound **DHT** ($K_i = 9.17 \pm 0.71$ nM).

3.3. *Discussion*

In the present study, the binding affinities of eight estradiol derivatives containing substituents at positions C-16 and C-17 to the ERs, PRs and ARs have been characterized. The molecules belong in two different substituted stereoisomer series: 16-bromomethylestra-1,3,5(10)-triene-3,17-diyl 17-acetates (**5c–8c**) and 16-bromomethylestra-1,3,5(10)-triene-3,17-diols (**5e–8e**).

Examination of the binding abilities of these compounds to the ERs proved that this receptor recognizes them. Considerable differences are observed between the K_i values, which can be explained by the different modes of receptor binding. Tanenbaum et al. [21] suggest that estrogens adopt a well-defined orientation in the ligand-binding domain (LBD) of the ERs: ring D is in contact with helix-11, the 17β-hydroxy group is H-bonded to His 524, ring A is projected toward helix-3, the 3-phenolic hydroxy forms a water-mediated H-bond with Glu 353, and the molecule is fixed by van der Waals contact around the binding pocket. The biggest divergence in the K_i values is exhibited between the members of the 17-acetate epimers and the 3,17-diols, suggesting that a hydroxy group at position 17β of the estradiol ring is necessary to form the H-bond with the His 524 residue. Highly polar and bulky groups, such as acetate, are poorly tolerated by ER LBD at positions 17α and β [22].

Several research groups have investigated the effects of C16 substitution on the affinity of estradiol derivatives for the ERs [13,23]. They concluded that ER tolerance for substituents at position 16α is mixed, although groups of moderate size and polarity, such as halogens or haloalkyl, are generally tolerated, but position 16β is sterically less permissive, even the smallest functional group (e.g. fluorine) decreasing the RBA substantially. Our experimental work with the four estradiol epimer pairs lend support to these findings, because the tested 17β derivatives with 16α substituents (**7c** and **7e**) possess K_i values at least one order of magnitude lower than those of 16β-substituted molecules. The RBA of **7e** is 90.95%, and this epimer has a two-orders of magnitude lower K_i than that of its *cis* counterpart **5e**. Ring D of estradiol derivatives makes non-polar contacts with the Ile 424, Gly 521 and Leu 525 residues, and the Ile 424 residue can be found close to C-16 of the ligand in the ERLBD [24]. Our results suggest that the position 16β is sterically unfavorable because of the side-chain of the Ile 424 residue, which is close to the ligand.

The investigated molecules are able to bind to the ARs and PRs, because the C-3 hydroxy group can act not only as an H-donor, but also as an H-acceptor [22], and in the AR and PR there is a good H-donor Glu residue instead of the Gln 353 in the ERs [21,25].

In summary, four substituted estradiol epimer pairs were tested in the radioligand-binding assay. The ER recognizes them, but only **7e** exhibits a really high binding affinity for the ERs. The selectivities of the investigated compounds are variable: only three derivatives (**5e**, **7e** and **8e**) are considered ER-selective. The others, (**7c**, **8c** and **6c** and **6e**) bind slightly more strongly to the ARs than to the ERs, and **5c** does not show any affinity to the ARs.

Acknowledgments

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