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DERIVATIVES OF ETHYNYLESTRADIOL WITH OXYGENATED 17α-ALKYL SIDE CHAIN: SYNTHESIS AND BIOLOGICAL ACTIVITY

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Summary—The coupling reaction of an acetylide ion with alkyl bromide or δ-valerolactone was used to synthesize twelve 17α-derivatives of ethynylestradiol having various 17α-side chain lengths and, except one, having mono- or di-oxygenated function on the side chain. All compounds had low (0.01–1.79%) relative binding affinity (RBA) for the rat uterine estrogen receptor. The highest RBA were obtained for compounds **26** (1.79%), **30** (1.55%) and **19** (0.42%). The length and polarity of the side chain decreases the affinity for the estrogen receptor. The *in vivo* estrogenic activity was measured on mouse uterine weight and was found to range from 0 to 35%, except for compound **30** (100%). The antiuterotrophic activity was measured by inhibition of estradiol-induced stimulation of uterine weight and was found to be 39% for compound **19**, 25% for compound **26** and 0% for all other compounds. These two compounds (**19** and **26**) possess mixed agonist and antagonist activity.

INTRODUCTION

Antiestrogens represent a logical approach to the treatment of estrogen-sensitive cancers, especially breast cancer [1]. Tamoxifen is the most widely used compound for the treatment of breast cancer [2, 3]. A limitation common to all known antiestrogens, including tamoxifen, is their intrinsic estrogenic activity which should limit their therapeutic efficacy [4, 6]. Following the discovery that 7α- and 17α-derivatives of 17β-estradiol maintain affinity for the estrogen receptor [7], some 17β-estradiol derivatives possessing an alkylamide side chain in the 7α-position have been found to be pure antiestrogen, i.e. estrogen antagonists with no detectable agonistic activity [4–6]. Bucourt *et al.* [7], have in fact measured the estrogen receptor affinity of a series of estradiol derivatives substituted at positions C2, 3, 4, 7α, 17α and 17β and concluded that positions 7α and 17α were the most suitable for maintaining affinity for the estrogen receptor. In order to assess the capacity of the 17α-position of 17β-estradiol to accept a side chain (alkylamide or others), we have synthesized several 17α-ethynylestradiol derivatives, evaluated their binding affinity for the rat uterine estrogen receptor, and measured their uterotrophic and antiuterotrophic activities in adult female ovariectomized Balb/c mice.

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EXPERIMENTAL

Most chemical reagents were purchased from Aldrich Chemical Company (Milwaukee, Wis.) or Sigma Chemical Company (St Louis, Mo.), while solvents were obtained from BDH Chemicals (Montréal). Thin-layer chromatography (TLC) was performed on 0.25 mm Kieselgel 60F254 plates (E. Merck, Darmstadt, F.R.G.), while 70–230-mesh Kieselgel 60F254 (E. Merck, Darmstadt, F.R.G.) was used for column chromatography. When the products were submitted for biological assays, the last chromatographic step was performed with freshly-distilled solvents.

Infrared spectra (i.r.) were obtained on a Perkin-Elmer 1310 spectrophotometer. Nuclear magnetic resonance spectra (NMR) were recorded with a Varian EM-360A (60 MHz) or a Varian XL-200 (200 MHz) spectrometer using tetramethylsilane (TMS) as internal standard. Ultraviolet spectra (u.v.) were obtained on a Beckman DU-6 spectrophotometer with appropriate solvents. Mass spectra (MS) were recorded with a V.G. Micromass 16F and exact mass spectra (EMS) were provided by Le Centre Régional de Spectrométrie de Masse, Université de Montréal, Montréal, Canada.

Chemical synthesis

Tetrahydropyranyl derivative of various bromo alcohols (1–7) (n = 2, 4, 6, 8, 11, 17, 22). When the bromo alcohol was commercially available ($Br(CH_2)_nOH$, $n = 2, 4, 6, 8, 11$), tetrahydropyranyl derivatives were obtained as described by Bucourt *et*

al. [7]. The products obtained were in agreement with i.r. and NMR analysis. Other tetrahydropyranyl derivatives **(6,7)** were prepared using the Wittig reaction and catalytic hydrogenation of resulting double bond (Fig. 1).

General procedure for Wittig reaction

Under anhydrous conditions, triphenylphosphine (47.8 mmol) was dissolved in dry benzene (25 ml) and 11-bromo undecanol tetrahydropyranyl ether (47.8 mmol) dissolved in benzene (20 ml) was added. The reaction mixture was refluxed with stirring. After 2 days, the solvent was evaporated, the viscous oil was washed with petroleum ether (35–60°C), and drying under vacuum provided an amorphous white solid. The crude product (36.5 mmol), dry THF (40 ml) and *n*-BuLi (40.2 mmol) were stirred at room temperature for 3 h (ylide formation). To this mixture, bromo aldehyde (36.5 mmol) dissolved in dry THF (16 ml) was added dropwise and the reaction was allowed to proceed at room temperature for 2 h. The reaction mixture was then diluted with water, extracted with diethyl ether and dried over $MgSO_4$. After removal of the solvent, the residue was chromatographed on a silica gel dry column (hexane–ethyl acetate, 99:1, v/v) in order to obtain the bromo alkene.

17-bromo[(tetrahydro-2'H-pyran-2'-yl)oxy]-11-heptadecene ***(8)***. Colorless oil (29% yield); i.r. ν (neat) 2905, 2835, 1640 vw, 1440, 1335 cm^{-1}; NMR-60 δ ($CDCl_3$) 1.2–2.2 (m, 32H), 3.2–3.9 (m, 6H, 1- and 17-CH_2 and OCH_2 of THP group), 4.55 (s, 1H, 2'-CH of THP group), 5.3 (t_{app}, J = 4 Hz, 2H, 6- and 7-CH); MS *m/e* (rel. intensity) 418 (M^+, 0.3), 416 (M^+, 0.3), 85 (100).

22-bromo[(tetrahydro-2'H-pyran-2'-yl)oxy]-11-docosene ***(9)***. Colorless oil; i.r. ν (neat) 2910, 2840, 1640 vw, 1450, 1340 cm^{-1}; NMR-60 δ ($CDCl_3$) 1.2–2.2 (m, 42H), 3.2–3.9 (m, 6H, 1- and 22-CH_2 and OCH_2 of THP group), 4.54 (s, 1H, 2'-CH of THP group), 5.3 (t, J = 4 Hz, 2H, 11- and 12-CH); MS *m/e* (rel. intensity) 488 (M^+, 0.1), 486 (M^+, 0.1), 277 (11), 85 (69), 55 (100).

General procedure for catalytic hydrogenation

The alkene (0.47 mmol) was dissolved in ethyl acetate (40 ml) containing 40 mg of 10% Pd/C. The reaction mixture was then shaken under atmospheric pressure of hydrogen for 90 min, filtered on celite and evaporated under vacuum. The crude product was purified by silica gel dry column chromatography (hexane–ethyl acetate, 97:3, v/v) to give the bromo alkane with a small quantity of hydrolysed product.

17-bromo[(tetrahydro-2'H-pyran-2'-yl)oxy] heptadecane ***(6)***. Amorphous white solid (50% yield); i.r. ν (KBr) 2905, 2840, 1450, 1340 cm^{-1}; NMR-60 δ ($CDCl_3$) 1.2–2.2 (m, 36H), 3.2–4.1 (m, 6H, 1- and 17-CH_2 and OCH_2 of THP group), 4.56 (s, 1H, 2'-CH of THP group); MS *m/e* (rel. intensity) 419 (M^+-1, 0.9), 417 (M^+-1, 1.0), 85 (100). 17-bromo heptadecanol (37% yield) is also obtained as a side product.

22-bromo[(tetrahydro-2'H-pyran-2'yl)oxy] docosane ***(7)***. Amorphous white solid (83% yield); i.r. ν (KBr) 2900, 2835, 1455, 1350 cm^{-1}; NMR-60 δ ($CDCl_3$) 1.1–2.0 (m, 46H), 3.2–4.0 (m, 6H, 1- and

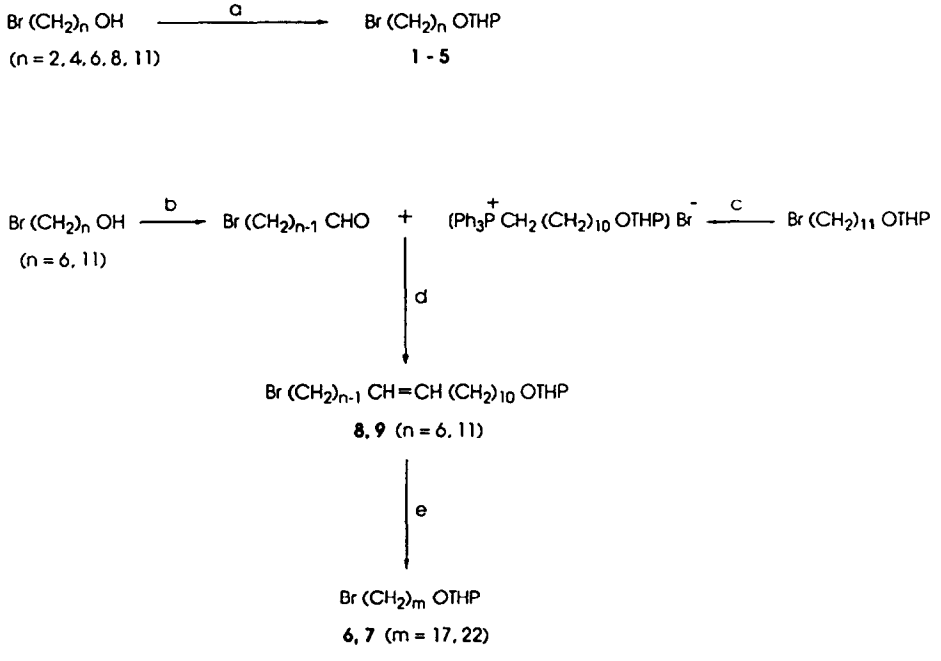


Fig. 1. Preparation of various side chains used in the coupling reaction with di-tetrahydropyranyl derivatives of ethynylestradiol. Reagents are: (a) DHP, C_6H_6, pTSA; (b) 1. DMSO, P_2O_5; 2. Et_3N; (c) Ph_3P; (d) n-BuLi, THF; (e) H_2 Pd/C (10%), AcOEt.

22-CH_2 and OCH_2 of THP group), 4.53 (s, 1H, 2'-CH of THP group); MS *m/e* (rel. intensity) 489 (M^+-1, 1.0), 487 (M^+-1, 1.2), 85 (100).

General procedure for coupling reaction of estradiol acetylide with tetrahydropyranyl derivative of various bromo alcohols

In a flame-dried flask under an argon atmosphere, 3,17β-bis tetrahydropyranyl ethynylestradiol (1.5 mmol) was dissolved in dry THF (40 ml) and HMPA (6.0 mmol). The solution was cooled at −78°C and *n*-BuLi (3.0 mmol) was added. After 2 or 3 h, tetrahydropyranyl bromo alcohol (6.0 mmol except for **6** and **7** where 3.0 mmol were used) in dry THF (10 ml) was added at −78°C. The mixture was allowed to return slowly to room temperature and was kept at this temperature overnight. Brine was then added and the reaction mixture was extracted with diethyl ether (1 ×) and dichloromethane (3 ×). The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (hexane–ethyl acetate, 9.6:0.4 to 7:3, v/v) to give the coupling product **11–17**, unreacted steroid **10** and by-product **25** (see Table 1).

3,17β-bis[(tetrahydro-2'H-pyran-2'-yl)oxy]-17α-{4'-[(tetrahydro-2"H-pyran-2"-yl)oxy]-1'-butyn-1'-yl} estra-1,3,5(10)-triene ***(11)***. Colorless oil; i.r. *ν* (neat) 2920, 2850, 2230 vw, 1605, 1570, 1490 cm^{-1}; NMR-60 *δ* ($CDCl_3$) 0.9 (s, 3H, 18-CH_3), 2.5 (t, J = 7 Hz, 2H, 3'-CH_2), 2.78 (m, 2H), 3.7 (m, 8H, 4'-CH_2 and OCH_2 of THP groups), 4.6 (s, 1H, 2'-CH of THP at 4'), 4.95 and 5.15 (2s, 1H, 2'-CH of THP at 17 (two isomers)), 5.3 (s, 1H, 2'-CH of THP at 3), 6.8 (m, 2H, 2- and 4-CH), 7.14 (d, J = 8 Hz, 1H, 1-CH); MS *m/e* (rel. intensity) 424 (M^+-2DHP, 1.1), 408 (2.6), 340 (11), 322 (5.1), 307 (2.7), 270 (4.2), 85 (100).

3,17β-bis[(tetrahydro-2'H-pyran-2'-yl)oxy]-17α-{6'-[(tetrahydro-2"H-pyran-2"-yl)oxy]-1'-hexyn-1'-yl} estra-1,3,5(10)-triene ***(12)***. Colorless oil; i.r. *ν* (neat) 2910, 2850, 1600, 1490 cm^{-1}; NMR-60 *δ* ($CDCl_3$) 0.9 (s, 3H, 18-CH_3), 2.8 (m, 2H), 3.7 (m, 8H, 6'-CH_2 and OCH_2 of THP groups), 4.53 (s, 1H, 2'-CH of THP at 6'), 4.98 and 5.18 (2s, 1H, 2'-CH of THP at 17 (two isomers)), 5.35 (s, 1H, 2'-CH of THP at 3), 6.8 (m, 2H, 2- and 4-CH), 7.17 (d, J = 8 Hz, 1H, 1-CH); MS *m/e* (rel. intensity) 536 (M^+-DHP, 0.3), 452 (5.0), 434 (5.7), 367 (10), 350 (11), 334 (10), 270 (4.8), 85 (100).

3,17β-bis[(tetrahydro-2'H-pyran-2'-yl)oxy]-17α-{8'-[(tetrahydro-2"H-pyran-2"-yl)oxy]-1'-octyn-1'-yl} estra-1,3,5(10)-triene ***(13)***. Colorless oil; i.r. *ν* (neat) 2920, 2850, 1595, 1485 cm^{-1}; NMR-60 *δ* ($CDCl_3$) 0.9 (s, 3H, 18-CH_3), 2.8 (m, 2H), 3.65 (m, 8H, 8'-CH_2 and OCH_2 of THP groups), 4.5 (s, 1H, 2'-CH of THP at 8'), 4.98 and 5.17 (2s, 1H, 2'-CH of THP at 17 (two isomers)), 5.3 (s, 1H, 2'-CH of THP at 3), 6.75 (m, 2H, 2- and 4-CH), 7.15 (d, J = 8 Hz, 1H, 1-CH); MS *m/e* (rel. intensity) 480 (M^+-2DHP, 1.3), 464 (1.3), 396 (7.5), 380 (9.4), 363 (4.0), 270 (9.1), 85 (100).

3,17β-bis[(tetrahydro-2'-pyran-2'-yl)oxy]-17α-{10'-[(tetrahydro-2"H-pyran-2"yl)oxy]-1'-decyn-1'-yl} estra-1,3,5(10)-triene ***(14)***. Colorless oil; i.r. *ν* (neat) 2920, 2840, 2215 vw, 1600, 1485 cm^{-1}; NMR-60 *δ* ($CDCl_3$) 0.9 (s, 3H, 18-CH_3), 2.8 (m, 2H), 3.6 (m, 8H, 10'-CH_2 and OCH_2 of THP groups), 4.5 (s, 1H, 2'-CH of THP at 10'), 4.95 and 5.15 (2s, 1H, 2'-CH of THP at 17 (two isomers)), 5.3 (s, 1H, 2'-CH of THP at 3), 6.75 (m, 2H, 2- and 4-CH), 7.15 (d, J = 8 Hz, 1H, 1-CH); MS *m/e* (rel. intensity) 508 (M^+-2DHP, 1.2), 493 (1.2), 424 (9.5), 409 (9.1), 406 (5.4), 391 (3.0), 270 (20), 85 (100).

3,17β-bis[(tetrahydro-2'H-pyran-2'-yl)oxy]-17α-{13'-[(tetrahydro-2"H-pyran-2"-yl)oxy]-1'-tridecyn-1'-yl} estra-1,3,5(10)-triene ***(15)***. Colorless oil; i.r. *ν* (neat) 2920, 2845, 2220 vw, 1600, 1490 cm^{-1}; NMR-60 *δ* ($CDCl_3$) 0.88 (s, 3H, 18-CH_3), 2.8 (m, 2H), 3.6 (m, 8H, 13'-CH_2 and OCH_2 of THP groups), 4.5 (s, 1H, 2'-CH of THP at 13'), 4.95 and 5.15 (2s, 1H, 2'-CH of THP at 17 (two isomers)), 5.32 (s, 1H, 2'-CH of THP at 3), 6.77 (m, 2H, 2- and 4-CH), 7.17 (d, J = 8 Hz, 1H, 1-CH); MS *m/e* (rel. intensity) 550 (M^+-2DHP, 1.4), 535 (0.8), 466 (8.0), 451 (9.0), 448 (8.4), 433 (2.2), 270 (22), 85 (88), 55 (100).

3,17β-bis[(tetrahydro-2'H-pyran-2'-yl)oxy]-17α-{19'-[(tetrahydro-2"H-pyran-2"-yl)oxy]-1'-nonadecyn-1'-yl} estra-1,3,5(10)-triene ***(16)***. Colorless oil; i.r. *ν* (neat) 2910, 2840, 2220 vw, 1600, 1490 cm^{-1}; NMR-60 *δ* ($CDCl_3$) 0.9 (s, 3H, 18-CH_3), 2.8 (m, 2H), 3.6 (m, 8H, 19'-CH_2 and OCH_2 of THP groups), 4.57 (s, 1H, 2'-CH of THP at 19'), 5.0 and 5.2 (2s, 1H, 2'-CH of THP at 17 (two isomers)), 5.35 (s, 1H, 2'-CH of THP at 3), 6.8 (m, 2H, 2- and 4-CH), 7.2 (d, J = 8 Hz, 1H, 1-CH); MS *m/e* (rel. intensity) 634 (M^+-2DHP, 4.1), 617 (1.5), 550 (15), 533 (9.3), 270 (6.0), 85 (100).

3,17β-bis[(tetrahydro-2'H-pyran-2'-yl)oxy]-17α-{24'-[(tetrahydro-2"H-pyran-2"-yl)oxy]-1'-tetracosyn-1-yl} estra-1,3,5(10)-triene ***(17)***. Colorless oil; i.r. *ν* (neat) 2900, 2830, 2215 vw, 1600, 1485 cm^{-1}; NMR-60 *δ* ($CDCl_3$) 0.9 (s, 3H, 18-CH_3), 2.8 (m, 2H), 3.7 (m, 8H, 24'-CH_2 and OCH_2 of THP groups), 4.55 (s, 1H, 2'-CH of THP at 24'), 5.0 and 5.18 (2s, 1H, 2'-CH of THP at 17 (two isomers)), 5.35 (s, 1H, 2'-CH of THP at 3), 6.8 (m, 2H, 2- and 4-CH), 7.17 (d,

Table 1. Coupling reaction yield of ethynylestradiol with various tetrahydropyranyl derivatives of bromo alcohol

$R(CH_2)_nOTHP$	Yield (%)		
n	Coupling product (11–17)	Unreacted product (10)	By-product (25)
2	68	11	17
4	12	47	22
6	74	6	18
8	68	21	6
11	44	34	*
17	33	30	26
22	25	25	27

*Not recovered.

J = 8 Hz, 1H, 1-CH); MS *m/e* (rel. intensity) 706 (M^+-2DHP, 3.0), 620 (15), 605 (3.6), 602 (6.8), 85 (100).

3,17β-bis[(tetrahydro-2'H-pyran-2'-yl)oxy]-17α-(3'-hydroxy-1'-heptyn-1'-yl) estra-1,3,5(10)-triene ***(25)***. Colorless oil; i.r. *v* (neat) 3420, 2920, 2850, 2210 vw, 1600, 1485 cm^{-1}; NMR-60 δ ($CDCl_3$) 0.9 (s broad, 6H, 7'- and 18-CH_3), 2.83 (m, 2H), 3.7 (m, 4H, OCH_2 of THP groups), 4.45 (m, 1H, 3'-CH), 4.97 and 5.17 (2s, 1H, 2'-CH of THP at 17 (two isomers)), 5.34 (s, 1H, 2'-CH of THP at 3), 6.8 (m, 2H, 2- and 4-CH), 7.15 (d, J = 8 Hz, 1H, 1-CH); MS *m/e* (rel. intensity) 466 (M^+-DHP, 0.4), 382 (2.6), 364 (5.6), 348 (5.0), 296 (9.0), 270 (4.5), 85 (100).

General procedure for hydrolysis of the tetrahydropyranyl group

The tetrahydropyranyl derivative (0.1–1.1 mmol) was dissolved in MeOH (60–100 ml) and *p*-toluenesulfonic acid (0.09–0.29 mmol) was added. The resulting solution was stirred at room temperature and the reaction was followed by TLC. When the reaction was completed, water was added, MeOH was evaporated under reduced pressure and the residue was extracted with dichloromethane. After evaporation of the organic layer, the crude product was purified by column chromatography (hexane–ethyl acetate, appropriate solvent polarity ranging from 7:3 to 4:6, v/v) to yield the alcohol.

17α-(4'-hydroxy-1'-butyn-1'-yl) estra-1,3,5(10)-trien-3,17β-diol ***(18)***. Amorphous white solid (85% yield); i.r. *v* (KBr) 3340, 2910, 2850, 1600, 1490 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.87 (s, 3H, 18-CH_3), 2.54 (t, J = 6.4 Hz, 2H, 3'-CH_2), 2.81 (m, 2H), 3.74 (t, J = 7.0 Hz, 2H, 4'-CH_2), 4.60 (s, 1H, OH phenol), 6.56 (d, J = 2.9 Hz, 1H, 4-CH), 6.63 (dd, J_1 = 2.9 Hz and J_2 = 8.4 Hz, 1H, 2-CH), 7.16 (d, J = 8.4 Hz, 1H, 1-CH); MS *m/e* (rel. intensity) 340 (M^+, 10), 322 (7.2), 307 (7.7), 270 (100); EMS M^+ calculated for $C_{22}H_{28}O_3$: 340.2038, found: 340.2026.

17α-(6'-hydroxy-1'-hexyn-1'-yl) estra-1,3,5(10)-trien-3,17β-diol ***(19)***. Amorphous white solid (84% yield); i.r. *v* (film) 3370, 2920, 2860, 1605, 1490 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.87 (s, 3H, 18-CH_3), 2.30 (t, J = 6.6 Hz, 2H, 3'-CH_2), 2.81 (m, 2H), 3.69 (t, J = 5.9 Hz, 2H, 4'-CH_2), 4.86 (s, 1H, OH phenol), 6.56 (d, J = 2.6 Hz, 1H, 4-CH), 6.64 (dd, J_1 = 2.6 Hz and J_2 = 8.8 Hz, 1H, 2-CH), 7.16 (d, J = 8.8 Hz, 1H, 1-CH); MS *m/e* (rel. intensity) 368 (M^+, 9.5), 352 (52), 335 (61), 270 (50), 159 (100); EMS M^+ calculated for $C_{24}H_{32}O_3$: 368.2351, found: 368.2333.

17α-(8'-hydroxy-1'-octyn-1-yl) estra-1,3,5(10)-trien-3,17β-diol ***(20)***. Amorphous white solid (79% yield); i.r. *v* (film) 3340, 2920, 2845, 2220 vw, 1600, 1490 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.87 (s, 3H, 18-CH_3), 2.26 (t, J = 6.6 Hz, 2H, 3'-CH_2), 2.80 (m, 2H), 3.64 (t, J = 6.4 Hz, 2H, 4'-CH_2), 5.02 (s, 1H, OH phenol), 6.56 (d, J = 2.6 Hz, 1H, 4-CH), 6.63 (dd, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, 2-CH), 7.16 (d, J = 8.4 Hz, 1H, 1-CH); MS *m/e* (rel. intensity) 396 (M^+, 4.3), 380 (19), 362 (7.4), 270 (100); EMS M^+ calculated for $C_{26}H_{36}O_3$: 396.2664, found: 396.2608.

17α-(10'-hydroxy-1'-decyn-1'-yl)estra-1,3,5(10)-trien-3,17β-diol ***(21)***. Amorphous white solid (98% yield); i.r. *v* (film) 3360, 2920, 2840, 2220 vw, 1600, 1490 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.87 (s, 3H, 18-CH_3), 2.25 (t, J = 6.6 Hz, 2H, 3'-CH_2), 2.81 (m, 2H), 3.60 (t, J = 6.4 Hz, 2H, 4'-CH_2), 4.82 (s, 1H, OH phenol), 6.56 (d, J = 2.9 Hz, 1H, 4-CH), 6.63 (dd, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, 2-CH), 7.17 (d, J = 8.4 Hz, 1H, 1-CH); MS *m/e* (rel. intensity) 424 (M^+, 4.8), 409 (14), 391 (6.3), 270 (100); EMS M^+ calculated for $C_{28}H_{40}O_3$: 424.2977, found: 424.2964.

17α-(13'-hydroxy-1'-tridecyn-1'-yl) estra-1,3,5(10)-trien-3,17β-diol ***(22)***. Amorphous white solid (85% yield); i.r. *v* (film) 3340, 2910, 2840, 2220 vw, 1600, 1485 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.87 (s, 3H, 18-CH_3), 2.25 (t, J = 6.4 Hz, 2H, 3'-CH_2), 2.80 (m, 2H), 3.63 (t, J = 67.4 Hz, 2H, 4'-CH_2), 5.32 (s, 1H, OH phenol), 6.57 (d, J = 2.6 Hz, 1H, 4-CH), 6.64 (dd, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, 2-CH), 7.16 (d, J = 8.4 Hz, 1H, 1-CH); MS *m/e* (rel. intensity) 466 (M^+, 2.1), 451 (8.0), 448 (3.6), 433 (2.6), 270 (89), 55 (100); EMS M^+ calculated for $C_{31}H_{46}O_3$: 466.3447, found: 466.3390.

17α-(19'-hydroxy-1'-nonadecyn-1'-yl) estra-1,3,5(10)-trien-3,17β-diol ***(23)***. Amorphous white solid (89% yield); i.r. *v* (film) 3320, 2910, 2840, 2220 vw, 1605, 1490 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.87 (s, 3H, 18-CH_3), 2.25 (t, J = 6.9 Hz, 2H, 3'-CH_2), 2.82 (m, 2H), 3.67 (t, J = 6.6 Hz, 2H, 4'-CH_2), 5.16 (s, 1H, OH phenol), 6.57 (d, J = 2.7 Hz, 1H, 4-CH), 6.63 (dd, J_1 = 2.7 Hz and J_2 = 8.4 Hz, 1H, 2-CH), 7.17 (d, J = 8.4 Hz, 1H, 1-CH); MS *m/e* (rel. intensity) 550 (M^+, 3.7), 535 (6.7), 533 (4.8), 517 (1.7), 270 (84) 55 (100); EMS M^+ calculated for $C_{37}H_{58}O_3$: 550.4386, found: 550.4426.

17α-(24'-hydroxy-1'-tetracosyn-1'-yl) estra-1,3,5(10)-trien-3,17β-diol ***(24)***. Amorphous white solid (88% yield); i.r. *v* (film) 3280, 2900, 2835, 1600, 1490 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.86 (s, 3H, 18-CH_3), 2.24 (t, J = 6.8 Hz, 2H, 3'-CH_2), 2.81 (m, 2H), 3.65 (t, J = 6.4 Hz, 2H, 4'-CH_2), 4.83 (s, 1H, OH phenol), 6.56 (d, J = 2.6 Hz, 1H, 4-CH), 6.63 (dd, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, 2-CH), 7.16 (d, J = 8.4 Hz, 1H, 1-CH); MS *m/e* (rel. intensity) 620 (M^+, 3.1), 606 (4.8), 603 (2.8), 270 (72), 55 (100); EMS M^+ calculated for $C_{42}H_{68}O_3$: 620.5168, found: 620.5165.

17α-(3'-hydroxy-1'-heptyn-1'-yl) estra-1,3,5(10)-trien-3,17β-diol ***(26)***. Amorphous white solid (75% yield); i.r. *v* (film) 3320, 2920, 2860, 1605, 1490 cm^{-1}; ^{1}H-NMR-200 δ ($CDCl_3$) 0.88 (s, 3H, 18-CH_3), 0.91 (t, J = 6.8 Hz, 3H, 7'-CH_3), 2.80 (m, 2H), 4.46 (q_{app}, 1H, 3'-CH), 4.93 (s, 1H, OH phenol), 6.56 (d, J = 2.7 Hz, 1H, 4-CH), 6.63 (dd, J_1 = 2.7 Hz and J_2 = 8.4 Hz, 1H, 2-CH), 7.15 (d, J = 8.4 Hz, 1H, 1-CH); ^{13}C-NMR-200 δ ($CDCl_3$) 12.9, 14.1, 22.4, 22.9, 26.5, 27.3, 27.5, 29.7, 33.0, 37.6, 39.0, 39.4, 43.6, 47.3, 49.6, 62.6, 79.9, 87.0, 88.3, 112.7, 115.2, 126.4,

132.2, 138.0, 153.4; MS *m/e* (rel. intensity) 382 (M^+, 9.1), 364 (4.6), 349 (21), 296 (18), 270 (41), 213 (100); EMS M^+ calculated for $C_{25}H_{34}O_3$: 382.2508, found: 382.2523.

Synthesis of 3,17β-bis[(tetrahydro-2'H-pyran-2'-yl)oxy]-17α-(3'-oxo-1'-heptyn-1'-yl) estra-1,3,5(10)-trien-3,17β-diol ***(27)***

A solution of alcohol **25** (128 mg, 0.233 mmol) in benzene (40 ml) containing 5.5 g of polymer supported reagents (Poly. CrO_3, Polysciences, Warrington, PA) was heated with reflux for 4 days. The reaction mixture was filtered and benzene was evaporated. Dry column chromatography (hexane–ethyl acetate, 9:1, v/v) yielded 68 mg (0.124 mmol, 53% yield) of ketone **27**. Colorless oil; i.r. *v* (neat) 2920, 2850, 2190, 1660, 1600, 1485 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.92 (t, J = 7.7 Hz, 3H, 7'-CH_3), 0.95 (s, 3H, 18-CH_3), 2.57 (t, J = 7.3 Hz, 2H, 4'-CH_2), 2.83 (m, 2H), 3.56 (m, 2H, OCH_2 of THP at 17), 3.93 (m, 2H, OCH_2 of THP at 3), 4.97 and 5.15 (2m, 1H, 2'-CH of THP at 17 (two isomers)), 5.39 (m, 1H, 2'-CH of THP at 3), 6.78 (d, J = 2.9 Hz, 1H, 4-CH), 6.85 (dd, J_1 = 2.2 Hz and J_2 = 8.4 Hz, 1H, 2-CH), 7.20 (d, J = 9.1 Hz, 1H, 1-CH); u.v. λ_{max}(MeOH) 222 (ε = 16000), 277 (ε = 1700) nm; MS *m/e* (rel. intensity) 464 (M^+-DHP, 0.9), 380 (7.5), 365 (18), 85 (100).

Synthesis of 17α-(3'-oxo-1'-heptyn-1'-yl) estra-1,3,5(10)-trien-3,17β-diol ***(28)***

Following the general procedure for hydrolysis of the tetrahydropyranyl group, product **27** was hydrolysed and, following column chromatography (hexane–ethyl acetate, 9:1, v/v) we obtained the dihydroxyketone **28** (94% yield). Colorless oil; i.r. *v* (film) 3340, 2920, 2850, 2185, 1645, 1600, 1490 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.91 (s, 3H, 18-CH_3), 0.92 (t, J = 7.2 Hz, 3H, 7'-CH_3), 2.59 (t, J = 7.3 Hz, 2H, 4'-CH_2), 2.81 (m, 2H), 4.83 (s, 1H, OH phenol), 6.56 (d, J = 2.6 Hz, 1H, 4-CH), 6.63 (dd, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, 2-CH), 7.14 (d, J = 8.4 Hz, 1H, 1-CH); u.v. λ_{max}(MeOH) 222 (ε = 15700), 281 (ε = 2200) nm; MS *m/e* (rel. intensity) 380 (M^+, 50), 365 (22), 324 (18), 295 (22), 270 (31), 213 (100); EMS M^+ calculated for $C_{25}H_{32}O_3$: 380.2351, found 380.2353.

Synthesis of 3,17β-bis [(tetrahydro-2'H-pyran-2'-yl)oxy]-17α-(1'-heptyn-1'-yl) estra-1,3,5(10)-trien-3,17β-diol ***(29)***

Using the general procedure for the coupling reaction (see above), bromopentane was coupled with 3,17β-bis tetrahydropyranyl ethynylestradiol to give, after chromatography (hexane–ethyl acetate, 9.7:0.3–7:3, v/v), a by-product **25** (23%), the unreacted steroid **10** (50%) and the desired compound **29** (25%). Colorless oil; i.r. *v* (neat) 2920, 2840, 2220, vw, 1595, 1480 cm^{-1}; NMR-60 δ ($CDCl_3$) 0.9 (s broad, 6H, 7'- and 18-CH_3), 2.8 (m, 2H), 3.7 (m, 4H, OCH_2 of THP groups), 5.0 and 5.2 (2s, 1H, 2'-CH of THP at 17 (two isomers)), 5.35 (s, 1H, 2'-CH of THP at 3), 6.8 (m, 2H, 2- and 4-CH), 7.2 (d, J = 8 Hz, 1H, 1-CH); MS *m/e* (rel. intensity) 450 (M^+-DHP, 3.5), 435 (2.6), 366 (25), 349 (9.9), 85 (100).

Synthesis of 17α-(1'-heptyn-1'-yl) estra-1,3,5(10)-trien-3,17β-diol ***(30)***

The general procedure for hydrolysis of tetrahydroxypyranyl groups was used. Purification of crude product was achieved by column chromatography (hexane–ethyl acetate, 7:3, v/v) and yielded the compound **30** (92% yield). Amorphous white solid: i.r. *v* (film) 3360, 2910, 2845, 1605, 1490 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.87 (s, 3H, 18-CH_3), 0.89 (t, J = 6.6 Hz, 3H, 7'-CH_3), 2.24 (t, J = 6.8 Hz, 2H, 3'-CH_2), 2.81 (m, 2H), 4.69 (s, 1H, OH phenol), 6.56 (d, J = 2.6 Hz, 1H, 4-CH), 6.63 (dd, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, 2-CH), 7.17 (d, J = 8.4 Hz, 1H, 1-CH); MS *m/e* (rel. intensity) 366 (M^+, 43), 351 (68), 333 (12), 270 (23), 159 (100); EMS M^+ calculated for $C_{25}H_{34}O_2$: 366.2559, found: 366.2559.

Synthesis of 3,17β-bis[(tetrahydro-2'H-pyran-2'-yl)-oxy]-17α-(7'-hydroxy-3'-oxo-1'-heptyn-1'-yl) estra-1,3,5(10)-trien-3,17β-diol ***(31)***

The product was obtained by using the general procedure for the coupling reaction except that δ-valerolactone had replaced the bromo side chain and we used only 1.4 equivalent of δ-valerolactone rather than 4 equivalents of the bromo side chain. Purification was achieved by column chromatography (hexane–ethyl acetate, 7.5:2.5, v/v), thus giving the unreacted steroid **10** (60%) and the desired compound **31** (18%). Amorphous white solid; i.r. *v* (film) 3420, 2920, 2850, 2195, 1665, 1600, 1485 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.95 (s, 3H, 18-CH_3), 2.63 (t, J = 7.1 Hz, 2H, 4'-CH_2), 2.83 (m, 2H) 3.6 (m, 2H, OCH_2 of THP at 17), 3.65 (t, J = 6.2 Hz, 2H, 7'-CH_2), 3.93 (m, 2H, OCH_2 of THP at 3), 5.00 and 5.14 (2m, 1H, 2'-CH of THP at 17 (two isomers)), 5.38 (m, 1H, 2'-CH of THP at 3), 6.79 (s_{app}, 1H, 4-CH), 6.84 (dd, J_1 = 2.7 Hz and J_2 = 8.4 Hz, 1H, 2-CH), 7.19 (d, J = 8.4 Hz, 1H, 1-CH); u.v. λ_{max}(MeOH) 221 (ε = 22000), 276 (ε = 2700) nm; MS *m/e* (rel. intensity) 463 (M^+-(102: DHP + H_2O), 1.1), 447 (0.5), 396 (1.4), 379 (5.5), 363 (3.9), 296 (10), 270 (2.2), 85 (100).

Synthesis of 17α-(7'-hydroxy-3'-oxo-1'-heptyn-1'-yl) estra-1,3,5(10)-trien-3,17β-diol ***(32)***

Hydrolysis of the di-THP group was achieved using the general procedure described above, with slight modifications. At the end of hydrolysis, we added HCl (1N) and the reaction mixture was stirred for 4 min at room temperature. This modification was necessary to transform the acetal intermediate **35** to keto alcohol **32** (yield was not determined but reaction was quantitative according to TLC). Amorphous white solid; i.r. *v* (film) 3340, 2920, 2860, 2195, 1650, 1600, 1490 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.90 (s, 3H, 18-CH_3), 2.64 (t, J = 7.0 Hz, 2H, 4'-CH_2), 2.80

(m, 2H), 3.68 (t, J = 6.0 Hz, 2H, 7′-CH_2), 5.1 (s, 1H, OH phenol), 6.56 (d, J = 2.6 Hz, 1H, 4-CH), 6.62 (dd, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, 2-CH), 7.13 (d, J = 8.1 Hz, 1H, 1-CH); u.v. λ_{max}(MeOH) 222 (ϵ = 10000), 281 (ϵ = 1600) nm; MS *m/e* (rel. intensity) 396 (M^+, 5.2), 380 (9.2), 378 (12), 363 (3.5), 296 (29), 270 (20), 213 (100); EMS M^+ calculated for $C_{25}H_{32}O_4$: 396.2300, found: 396.2283. When treatment with HCl (1N) was not performed, we obtained the acetal compound: 17α-[2′-(2″-methoxy tetrahydro-2″H-pyran-2″-yl) ethyn-1′-yl] estra-1,3,5(10)-trien-3,17β-diol **(35)**. Amorphous light yellow solid; i.r. *v* (film) 3360, 2920, 2850, 1600, 1575, 1490 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.88 (s, 3H, 18-CH_3), 2.78 (m, 2H), 3.45 (s, 3H, 2″-OCH_3), 3.73 (m, 2H, OCH_2 of THP group), 5.3 (s, 1H, OH phenol), 6.55 (d, J = 2.6 Hz, 1H, 4-CH), 6.63 (dd, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, 2-CH), 7.12 (d, J = 8.4 Hz, 1H, 1-CH); MS *m/e* (rel. intensity) 410 (M^+, 0.6), 378 (97), 362 (42), 346 (17), 270 (37), 211 (41), 160 (100).

Synthesis of 17α-(3′,7′-dihydroxy-1′-heptyn-1′-yl) estra-1,3,5(10)-trien-3,17β-diol (33)

A mixture of trihydroxy ketone **32** (5.4 mg, 0.0136 mmol), MeOH (5 ml) and $NaBH_4$ (in excess) was stirred at room temperature. When the reaction was completed by TLC (30 min), the mixture was acidified with diluted HCl and MeOH was evaporated under reduced pressure. After extraction with ethyl acetate, the organic phase was dried and evaporated to give a crude product. Purifications was performed by thin-layer chromatography (20 × 20 cm, 0.25 mm Kieselgel 60F254 plate) with hexane–ethyl acetate, 2:8, v/v as eluant (several migrations) thus yielding 3.2 mg (0.0080 mmol, 59% yield) of alcohol **33**. Amorphous white solid; i.r. *v* (film) 3320, 2910, 2850, 1600, 1485 cm^{-1}; NMR-200 δ ($CDCl_3$ + trace of DMSO-d_6) 0.86 (s, 3H, 18-CH_3), 2.79 (m, 2H), 3.62 (m, 2H, 7′-CH_2), 4.45 (m, 1H, 3′-CH), 6.57 (d, J = 2.6 Hz, 1H, 4-CH), 6.64 (dd, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, 2-CH), 7.12 (d, J = 8.4 Hz, 1H, 1-CH), 8.10 (s, 1H, OH phenol); MS *m/e* (rel. intensity) 398 (M^+, 0.7), 380 (14), 364 (9.3), 346 (66), 321 (7.2), 296 (8.3), 270 (20), 39 (100).

Estrogen receptor binding assay

The affinity of the synthesized compounds for the estrogen receptor was measured in rat uterine cytosol receptor using [^{3}H]estradiol as ligand as described by Asselin and Labrie [8]. The incubations were performed at 25°C for 3 h and non-specific binding was determined from incubation medium containing the [^{3}H]estradiol accompanied by an excess (1000 nm) of radioinert estradiol.

Uterotrophic and antiuterotrophic assay

The estrogenic activity of the compounds was measured *in vivo* by stimulation of uterine weight while the antiestrogenic activity was also determined *in vivo* by inhibition of the estradiol-induced stimulation of uterine weight in adult female ovariectomized Balb/c mice (body wt = 19–20 g) sacrificed five days after ovariectomy. The compounds and/or estradiol dissolved in ethanol were injected subcutaneously in the appropriate groups in a solution of 0.9% (w/v) sodium chloride and 1% (w/v) gelatin at a dose of 20 μg in 0.2 ml for the test compounds, twice daily for 4.5 days, starting on the day of ovariectomy for a total of 9 injections. Estradiol was injected at the dose of 0.01 μg in 0.2 ml, twice daily, starting on the morning after ovariectomy for a total of 8 injections. After sacrifice, the uteri were rapidly removed, freed from fat and connective tissue and weighed. Results are the means ± SEM of 9–10 animals per group.

RESULTS AND DISCUSSION

Chemistry

Ethynylestradiol was used as the substrate of choice for the synthesis of 17α derivatives. The coupling reaction of an acetylide ion with alkyl halide [9] was used to synthesize a series of compounds with side chains **11–17** (Fig. 2) and **29** (Fig. 3). On the other hand, the same procedure was extended to permit the coupling of an acetylide ion with δ-valerolactone to obtain compound **31** (Fig. 3), which has two oxygenated functions on a 7-member chain.

The hydroxyl groups of bromo alcohols and ethynylestradiol used for the coupling reaction were protected as tetrahydropyranyl (THP) derivatives (**1–5** and **10**) according to the procedure described by Bucourt *et al.* [7]. Since bromo alcohols with 17 and 22 carbons were not commercially available, the corresponding THP derivatives **6** and **7** were synthesized using the Wittig reaction and catalytic hydrogenation (Fig. 1). Thus, the treatment of the triphenyl phosphonium salt of 11-bromo THP-undecanol with BuLi gave phosphorus ylide which reacted with 6-bromo hexanal or 11-bromo undecanal (freshly prepared by oxydation [10] of corresponding alcohol) to give the olefins **8** or **9** [11]. The yield was modest but comparable to similar olefin preparations reported by Igner *et al.* [12]. These olefins were transformed to saturated compounds by catalytic hydrogenation (H_2, Pd/C) with a 83% yield for 22-carbon chain **7** and a 50% yield for 17-carbon chain **6**. This later moderate yield was attributed to partial hydrolysis of THP group giving a 37% yield of 17-bromo heptadecanol.

The coupling reaction of acetylide ion with alkylhalide is generally easy for an acetylenic chain [9], but this reaction is much more difficult when 17α-ethynyl steroids, more sterically hindered, are used. As an example, Salman *et al.* [13] have reported a low yield (29 and 40% after THP hydrolysis) for the coupling of di-iodoalkane and 19-nortestosterone. Using a modification of Salman's procedure [13], we have obtained a yield ranging from 12 to 74% of coupling

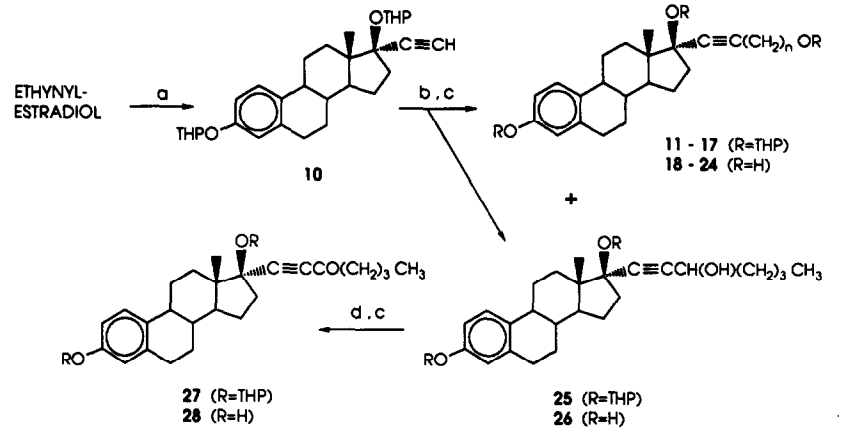


Fig. 2. Preparation of ethynylestradiol derivatives with various side chains ($n = 2,4,6,8,11,17,22$) at the 17α position. Reagents are: (a) DHP, C_6H_6, pTSA; (b) 1. n-BuLi, HMPA, THF; 2. $Br(CH_2)_nOTHP$; (c) pTSA, MeOH; (d) poly. CrO_3.

products **11** to **17** depending on the length of the side chain (Table 1). Except for the 4-carbon chain, the coupling yields were higher for small (68, 74 and 68%) than for large (44, 33 and 25%) chains. It should be mentioned that chains with 17 and 22 carbons were much less soluble than shorter ones and precipitated when the temperature of the reaction mixture decreased. Moreover, only 2 equivalents of these substrates were used comparatively to 4 equivalents for others.

The recovered unreacted product was important when low yield of the desired product was observed. However, in all cases, we also observed formation of a by-product (6–27% yield). i.r. analysis of this product indicated an hydroxyl group (3420 cm^{-1}) while NMR indicated a di-THP-steroidal part with a side chain coupled with the ethynyl group and an additional signal at 0.9 ppm (3H). In order to facilitate structure elucidation, the THP group was cleaved by pTSA/MeOH [14] and the corresponding alcohol was analysed by spectroscopy. i.r. gave no additional information, but ^{1}H-NMR indicated a triplet at 0.91 ppm corresponding to an ethyl group ($-CH_2\underline{CH_3}$) and an apparent quadruplet at 4.46 ppm (1H) attributed to $-\underline{CH}(OH)-$ moiety. ^{13}C-NMR data supported these findings by a signal at 62.6 ppm (characteristic of secondary alcohol) and signals at 14.1, 22.4, 33.0, 39.4 ppm (characteristic of aliphatic chain: $CH_3CH_2CH_2CH_2-$) [15]. These data and mass spectra analysis ($M^+ = 382$) permitted to attribute structure **25** to the by-product.

In order to obtain additional proof of the proposed structure, by-product **25** was submitted to mild oxidation (poly. CrO_3) [16]. As expected, ketone **27** was obtained. Evidence of the ynone group was observed in i.r. (absorption at 1660 cm^{-1}, conjugated carbonyl)

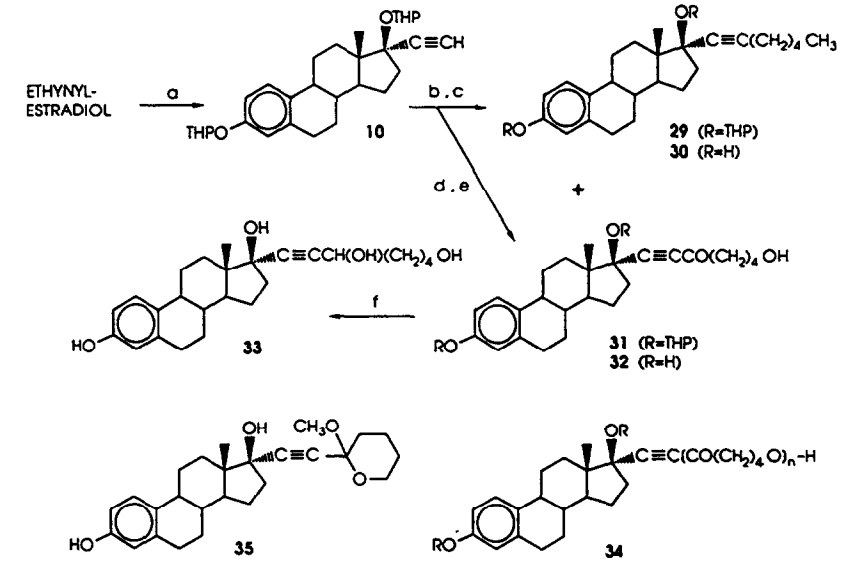


Fig. 3. Preparation of ethynylestradiol derivatives with 5-carbon side chain at the 17α position. Reagents are: (a) DHP, C_6H_6, pTSA; (b) 1. n-BuLi, HMPA, THF; 2. $Br(CH_2)_4CH_3$; (c) pTSA, MeOH; (d) 1. n-BuLi, HMPA, THF; 2- δ-valerolactone; (e) 1. pTSA, MeOH; 2. HCl (1N); (f) $NaBH_4$.

and u.v. (absorption at 222 and 277 nm, carbonyl conjugated to triple bond). Moreover, NMR indicated the disappearance of the signal at 4.46 ppm (CHOH) and the appearance of a triplet at 2.57 ppm ($O\overline{=}C-CH_2CH_2$) and mass spectra gave a peak at 464 (M^+-84, loss of dihydropyran group).

Bromopentane was also coupled with the THP derivative of ethynylestradiol using the general procedure, and the results were similar to those obtained for the THP derivative of bromo alcohol. The yield of the desired 17α-heptynyl derivative **29** was 25% while the yield of by-product **25** was 23%.

Prior to assaying the synthesized compounds for biological activity, THP groups were cleaved under mild conditions (pTSA/MeOH) [14]. Following this step, THP derivatives **11** to **17**, **25**, **27** and **29** were easily hydrolysed to compounds **18** to **24**, **26**, **28** and **30** with good yields ranging from 75 to 98%.

Ethynylestradiol was also used as starting material for the synthesis of 17α side chains with two oxygenated functions: **32** and **33** (Fig. 3). In order to obtain these derivatives, the corresponding acetylide of lithium was allowed to react with δ-valerolactone. Acetylide addition to the carbonyl group is well known in the case of small metallic acetylides (M–C≡C–H, Nef synthesis [17]), but not in the case of other metallic acetylides (M–C≡C–R where R = alkyl or steroid group). To the best of our knowledge, no acetylide ion was coupled with a carbonyl group when alpha protons were present. The general procedure for coupling the di-THP derivative of 17α-ethynylestradiol **10** and δ-valerolactone (4 equivalents) did not give the desired keto alcohol **31**. In fact, we observed products of di or several additions as **34** ($n > 1$) and the unreacted product (52%). Better results were obtained when only 1.4 equivalent of δ-valerolactone was used. In this case, we obtained the keto alcohol **31** with a 18% yield and the unreacted steroid (60%). i.r. and NMR analysis confirmed the formation of desired keto alcohol **31**. i.r. indicated an hydroxyl group (3240 cm^{-1}), conjugated alkyne (2195 cm^{-1}, more intense band than non-conjugated alkyne band) and conjugated carbonyl (1665 cm^{-1}), while NMR showed a triplet at 2.63 ppm for methylene group ($O\overline{=}CCH_2CH_2-$).

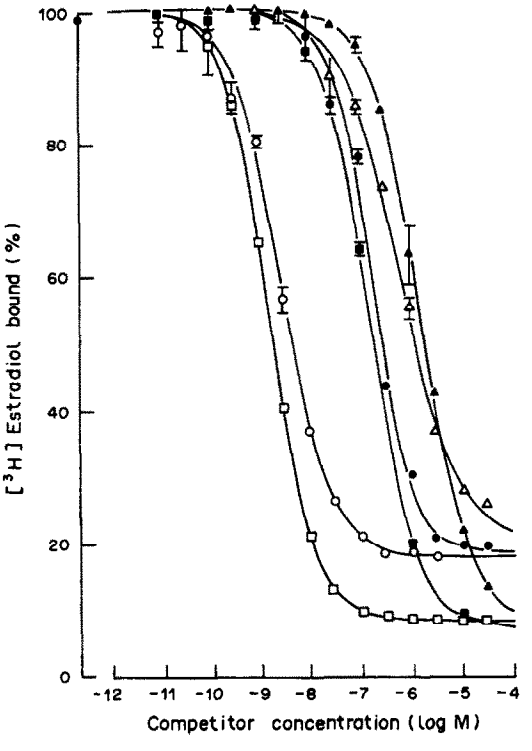


Fig. 4. Relative binding of ethynylestradiol (EE_2), E_2 and EE_2 17α-derivatives in the rat uterine estrogen receptor assay. □——□, ethynylestradiol ($ED_{50} = 1.61 \times 10^{-9}$); ○——○, estradiol ($ED_{50} = 2.75 \times 10^{-9}$); ■——■, **26** ($ED_{50} = 1.54 \times 10^{-7}$); ●——●, **30** ($ED_{50} = 1.77 \times 10^{-7}$); △——△, **19** ($ED_{50} = 6.54 \times 10^{-7}$); ▲——▲, **20** ($ED_{50} = 1.64 \times 10^{-6}$).

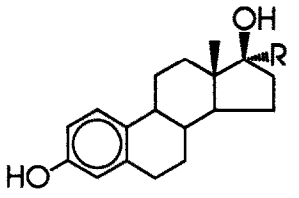
Hydrolysis of compound **31** by the general procedure (pTSA/MeOH) gave a product less polar than the starting material. This compound had an acetal structure **35** which resulted from cyclization of the side chain. The primary alcohol reacted with ketone and the oxygen anion was trapped by methanol (solvent). Acetal **35** was easily hydrolysed to trihydroxy compound **32** by more acidic conditions (HCl, 1N). Reduction of the carbonyl group of compound **32** was achieved by $NaBH_4$ in methanol, thus giving the desired alcohol **33**.

Biological activity

Figure 4 shows some examples of competition binding curves obtained for E_2, EE_2 and EE_2-17α-derivatives measured in the rat uterine estrogen receptor assay. The curves were used to calculate ED_{50} values which permitted to measure relative binding affinities (RBA). Estradiol was used as the reference compound with an RBA of 100. The RBA calculated for twelve synthesized compounds are shown in Table 2. It can be seen that the values obtained range from 0.01 to 1.79 while the steroidal antiestrogen compound (ICI 164384) has also similar low RBA (1.6 ± 0.5). In a first attempt, we have compared the effect of side chain length (compounds **18–24**) on RBA values. Generally, the RBA values decrease with the number of carbons added, except for the compound with 2 carbons added. In this case, the RBA value was similar to compounds with 11 or 17 carbons added, but much lower than the compound having a 4-carbon chain added. The compound having a 4-carbon side chain had the highest affinity for the estrogen receptor with an RBA of 0.42. Steric hindrance of hydroxyalkyl side chain was probably responsible for decreased affinity.

The best RBA value (1.79) was obtained for compound **26** which has a 5-carbon side chain added to the ethynyl group with an hydroxyl in the alpha position of the alkyne. When the hydroxy group was replaced by a proton (compound **30**), the RBA was slightly affected (1.55), but oxidation of the hydroxy

Table 2. Relative binding affinities of tested compounds for the rat uterine estrogen receptor



Compounds	R		RBA[a]
E_2[b]	H		100
EE_2[c]	C≡CH		171
18	$C{\equiv}C(CH_2)_nOH$,	$n=2$	0.07
19		$n=4$	0.42
20		$n=6$	0.17
21		$n=8$	0.11
22		$n=11$	0.05
23		$n=17$	0.06
24		$n=22$	0.02
26	$C{\equiv}CCX(CH_2)_3CH_3$,	X = H, OH	1.79
28		X = O	0.16
30		X = H, H	1.55
32	$C{\equiv}CCX(CH_2)_4OH$,	X = O	0.02
33		X = H, OH	0.01

[a]RBA (relative binding affinity) = (ED_{50}) estradiol/(ED_{50}) test compound × 100 where ED_{50} is the concentration of inhibitor required to reduce the binding of tritium-labeled estradiol by 50%. [b]Estradiol (E_2). [c]Ethynylestradiol (EE_2).

Table 3. Estrogenic and antiestrogenic activities of 17α-derivatives of ethynylestradiol in the *in vivo* mouse uterine assay

Compounds	Stimulation (%)[a]	Inhibition (%)[b]
E_2	100	0
18	12	0
19	10	39
20	0	0
21	0	0
22	14	0
24	15	0
26	35	25
30	100	0

[a]The stimulation of uterine weight (UW) by test compounds (20 μg/injection) is calculated according to the following equation. Stimulation of estradiol (0.01 μg/injection) is taken as 100%.

% of stimulation

$$= \frac{\text{UW of test compound} - \text{UW of control}}{\text{UW of } E_2 - \text{UW of control}} \times 100.$$

[b]The inhibition of E_2-induced uterine weight (estradiol, 0.01 μg) by test compound (20 μg/injection) is calculated according to the following equation.

% of inhibition

$$= \frac{\text{UW of } E_2 - \text{UW of test compound}}{\text{UW of } E_2 - \text{UW of control}} \times 100.$$

Uterine weight of adult female ovariectomized mice (UW of control): 20.61 ± 1.36 ($n = 12$).

Uterine weight of adult female ovariectomized mice stimulated by estradiol (UW of E_2): 65.37 ± 3.67 ($n = 13$).

group to give the ketone (compound **28**) dramatically decreased the RBA value (0.16). We also tested compounds with a terminal hydroxyl group and an oxygenated function in the alpha position of the alkyne (compounds **32** and **33**). These compounds have a very low RBA value: 0.02 and 0.01, respectively. In fact, for a similar side-chain length, RBA values decreased according to the number and the position of the hydroxyl group on the side chain. The best RBA value was obtained for compounds with a mono-hydroxyl group in the alpha position of the alkyne (such as **26**), intermediate RBA values for compounds with mono-hydroxyl at the end of the side chain (such as **19**) and lowest values for compounds with two hydroxyl groups (such as **32**). It should be mentioned that compound **30**, which has no hydroxyl group on the side chain, does not have higher RBA than mono-hydroxyl compound **26**.

Estrogenic activity of the compounds was measured by stimulation of uterine weight in adult female ovariectomized Balb/c mice (Table 3). The stimulation of estradiol at a dose of 0.01 μg/injection was taken as 100%. Among the compounds tested, the stimulation was low (0–15%) except for compounds **30** and **26**. Compound **30**, which has a n-pentyl added to the ethynyl group, possessed the highest estrogenic potenty (100%) while compound **26** (with an hydroxy n-pentyl group) had a stimulation of 35%. As can be seen in Table 2, these two compounds also had the best RBA in the estrogen receptor assay.

The antiuterotrophic activity of the test compounds was measured by inhibition of the estradiol-induced stimulation (0.01 μg/injection) of uterine weight in adult female ovariectomized Balb/c mice (Table 3). Only two compounds showed antiuterotrophic activity. These compounds, **19** and **26**, possessed respectively a 4-hydroxybutyl and a 1-hydroxy n-pentyl added to the ethynyl group of EE_2. Their activity was low as indicated by the inhibition values of 39% for **19** and 25% for **26**.

It can be concluded from the present data that addition of a side chain in the 17α position of EE_2 decreases the affinity for the estrogen receptor. RBA values generally decrease according to the length or polarity of the side chain. Among the 17α derivatives of EE_2 synthesized, compounds with a side chain of 4 or 5 carbons with or without hydroxyl, show the best affinity for the estrogen receptor. The estrogenic activity of all compounds is very low except for compound **30**. Despite the high concentration (2000-fold) of the test compounds used to inhibit E_2-induced uterine weight, 7 of 9 synthesized derivatives show no antiestrogenic activity. The two other compounds (**19** and **26**) have mixed agonist and antagonist activities, a characteristic shared by tamoxifen [5, 6]. All the synthesized compounds, except compound **30**, have a mono- or di-oxygenated function (hydroxyl or ketone group) on the 17α side chain. The conclusions obtained are thus limited to this category of EE_2 derivatives.

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