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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 17α-ALKYNYLAMIDE DERIVATIVES OF ESTRADIOL

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Summary—Seven estradiol (E_2) derivatives with an alkynylamide side chain at the 17α position were synthesized starting from ethynylestradiol (EE_2). The main chemical step was the coupling reaction of the acetylide ion of EE_2 with carbon dioxide, glutaric anhydride or bromoalkyl *ortho* ester. The synthesis of these compounds is fast (3–6 steps according to the compound) and is easily achieved with good yield. Five compounds with different side chain lengths were evaluated for uterotrophic and antiuterotrophic activity in the CD-1 mouse. None of the tested compounds shows estrogenic activity in this sensitive *in vivo* system. At low doses (1 and 3 μg), a 14–57% inhibition of E_2-induced uterine growth was observed while no additional inhibition was observed at the 10, 20 and 30 μg doses. In human breast carcinoma cells in culture, all compounds show estrogenic activity at high concentrations while only compound **39** (*N*-butyl,*N*-methyl-8-[3′,17′β-dihydroxy estra-1′,3′,5′(10′)-trien-17′α-yl]-7-octynamide) possesses antiproliferative or antiestrogenic effects. No significant correlation could be demonstrated between alkynylamide side chain length and estrogenic or antiestrogenic activity. Among the compounds tested, the derivative of EE_2 possessing a five-methylene (CH_2) side chain (compound **39**) possesses the best antiestrogenic activity (44 ± 7% in the CD-1 mouse uterus assay at the 3 μg dose and 57 ± 4% at 0.1 nM in human ZR-75-1 cancer cells in culture).

INTRODUCTION

Since discovery of the first antiestrogen, namely ethamoxytriphetol (MER25) [1], several other non-steroidal compounds, especially nafoxidine, enclomifene, tamoxifen, LY117018, LY156758, toremifene, droloxifene and zindoxifen have been described as having antiestrogenic activity [2, 3]. Among these compounds, tamoxifen (Fig. 1A) [4, 5] is the best known and is the only compound currently used in the antihormonal therapy of breast cancer in women. Unfortunately, tamoxifen possesses intrinsic estrogenic activity under both *in vitro* and *in vivo* conditions which could well be responsible for its limited therapeutical efficacy [6, 7]. The search for pure antiestrogens or compounds devoid of agonistic activity is thus of major interest for potential improvement of the therapy of estrogen-sensitive cancer.

Recently, steroidal compounds were reported to possess antiestrogenic activity with lower or no agonistic estrogenic action. One of them, RU39411 (compound **2**, Fig. 1B) has a side chain at position 11β of the estradiol (E_2) nucleus [8]. The phenoxyalkylamine side chain is identical to that of tamoxifen and is positioned to be analogous to its position in tamoxifen. The other group of steroidal antiestrogens are 7α-alkylamide derivatives of E_2. The discovery of these compounds was based on the finding that 7α-substituted derivatives of E_2 linked to an affinity column retain their affinity for the estrogen receptor during affinity chromatography [9]. Compound ICI 164384 (**3b**) (Fig. 1B) has been found to possess pure antiestrogenic activity in the rat uterus assay and in human breast cancer cells [10, 11]. Additional modifications of the 7α-alkylamide E_2 skeleton, in particular the D-cycle (compound **3a**, Fig. 1B), have led to highly potent and specific antiestrogens in the mouse uterine assay [12, 13], human breast cancer cells in culture as well as dimethylbenz(*a*)anthracene(DMBA)-induced mammary carcinoma in the rat [F. Labrie *et al.*, unpublished data]. Since the 17α position of E_2 is also suitable for receiving a substituent which permits efficient binding of the estrogen receptor to an affinity colum [9], and we have found that a side chain can be introduced relatively easily at this position [14], we have substituted the 17α-ethynyl group of ethynylestradiol (EE_2)

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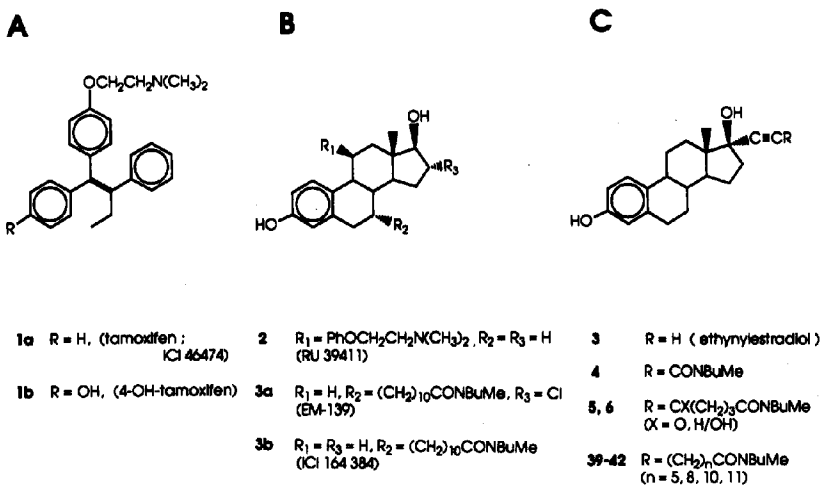

Fig. 1. Molecular structure of the non-steroidal antiestrogen tamoxifen **1a** and 4-hydroxytamoxifen **1b** (A), the new steroidal antiestrogens **2**, **3a**, and **3b** (B) and the new synthesized compounds **4–6** and **39–42** (C).

with various alkylamide side chains and have synthesized a series of 17α-alkynylamide E_2 derivatives (Fig. 1C). We have measured the biological activity of these new compounds in the following tests: (1) relative binding affinity (RBA) to the estrogen receptor [15]; (2) uterotrophic and antiuterotrophic activity in ovariectomized CD-1 mice [13, 14]; and (3) proliferative and antiproliferative activity in the estrogen-sensitive human breast cancer cell line (ZR-75-1) [16, 17].

EXPERIMENTAL

Chemical reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) or Sigma Chemical Co. (St Louis, MO), while most solvents were obtained from BDH Chemicals (Montréal, Canada). The compounds EM-139 and ICI 164384 were synthesized in our laboratory as described previously [7, 10, 11, 13]. Thin-layer chromatography (TLC) was performed on 0.25 mm Kieselgel $60F_{254}$ plates (E. Merck, Darmstadt, Germany), while 70–230 mesh Kieselgel $60F_{254}$ (E. Merck, Darmstadt, Germany) was used for column chromatography. When synthesized compounds were submitted to biological tests, last step chromatography was performed with freshly distilled solvents. Moreover, compounds tested for uterotrophic and antiuterotrophic activities were analyzed by high performance liquid chromatography (HPLC) (Waters Associates, Milford, MA) on C-18 μ-Bondapak reverse-phase columns (Waters Associates) using $CH_3CN:H_2O:MeOH$, 65:30:5, (v:v:v) or 40:38:22 (v:v:v) as eluant. The purity of the tested compounds was found to be >99%. Melting points were determined on a Gallenkamp apparatus and are uncorrected. Infrared spectra (i.r.) were obtained on a Perkin–Elmer 1310 spectrophotometer while nuclear magnetic resonance spectra (NMR) were recorded with a Varian EM-360A (60 MHz) or 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 solvent. Mass spectra (MS) were recorded with a V.G. Micromass 16F while high resolution mass spectra (HRMS) were provided by Le Centre Régional de Spectrométrie de Masse, Université de Montréal, Montréal, Canada.

Chemical Synthesis

Preparation of E_2 derivative with 17α-short amide side chain (compound 4, Fig. 2)

Synthesis of N-butyl,N-methyl-3-[3'-(i-butyl-oxy carbonyloxy)-17'β-hydroxy estra-1',3',5'(10')-trien-17'-yl]-2-propynamide (11). To carboxylic acid **10** [10] (200 mg, 0.588 mmol), resulting from addition of carbon dioxide to EE_2, was added dry CH_2Cl_2 (30 ml) and tributylamine (425 mg, 2.29 mmol) under anhydrous conditions. This mixture was cooled to −10°C and isobutyl chloroformate (337 mg,

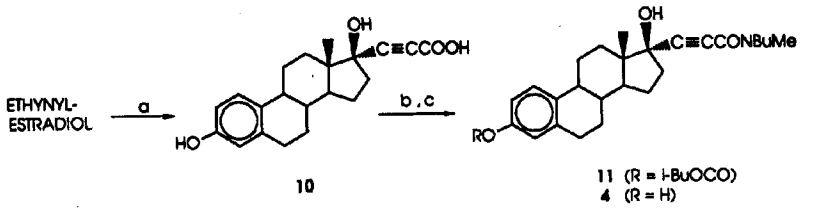

Fig. 2. Preparation of E_2 derivatives with a short amide side chain at the 17α position. Reagents are: (a) MeLi, CO_2; (b) (1) ClCOO*i*-Bu, $N(Bu)_3$, (2) NHBuMe; (c) K_2CO_3, H_2O, MeOH.

2.47 mmol) was then allowed to react for 30 min. *N*-methylbutylamine (1.47 g, 17 mmol) was next added in excess and the cooling bath was removed. After 3 h, CH_2Cl_2 was added and the organic phase was washed with HCl (1 N) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude product was purified by dry column chromatography (hexane–ethyl acetate, 5:5, v:v) to yield 206 mg (0.405 mmol, 69% yield) of amide product with the 3-isobutyl carbonate group **11**. Viscous colorless oil: i.r. ν (neat) 3340, 2940, 2910, 2860, 2210, 1750, 1610, 1485 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.91 (s, 3H, 18′–CH_3), 0.94 (m, 3H, $CH_2\underline{CH_3}$), 1.00 (d, $J = 7.0$ Hz, 6H, $(\underline{CH_3})_2CH$), 2.88 (m, 2H, 6′–CH_2), 2.96 and 3.18 (2s, 3H, CH_3NCO), 3.40 and 3.55 (2t, $J = 7.5$ Hz, 2H,$CH_2\underline{CH_2}NCO$), 4.03 (d, $J = 6.6$ Hz, 2H, *i*-Pr$\underline{CH_2}O$), 6.89 (d, $J = 2.6$ Hz, 1H, 4′–CH), 6.94 (dd, $J_1 = 2.6$ Hz and $J_2 = 8.1$ Hz, 1H, 2′–CH), 7.28 (d, $J = 8.1$ Hz, 1H, 1′–CH); MS *m/e* (rel. intensity) 509 (M^+, 14), 438 (2.9), 370 (5.9), 326 (2.3), 270 (100); HRMS M^+ calculated for $C_{31}H_{43}O_5N$—509.3141, found—509.3093.

Synthesis of N-butyl,N-methyl-3-(3′,17′β-dihydroxy estra-1′,3′,5′(10′)-triene-17′α-yl)-2-propynamide (4). To the carbonate derivative **11** (188 mg, 0.369 mmol) dissolved in methanol (10 ml), K_2CO_3 (1%, w/v) in aqueous methanol (25:75, v:v) (10 ml) was added and the resulting solution was stirred at room temperature for 3 h. The reaction mixture was acidified with HCl (1 N) and MeOH was evaporated under vacuum. The residue was extracted with ethyl acetate and the organic phase was dried and evaporated. Purification by column chromatography (hexane–ethyl acetate, 6.5:3.5, v:v) gave 137 mg (0.335 mmol, 91%) of phenol **4**. Amorphous white solid: i.r. ν (film) 3320, 2950, 2910, 2850, 2205, 1600, 1480 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.85 (s, 3H, 18′–CH_3), 0.97 (t, $J =$ 7.0 Hz, 3H, $CH_2\underline{CH_3}$), 2.74 (m, 2H, 6′–CH_2), 2.99 and 3.22 (2s, 3H, CH_3NCO), 3.44 and 3.59 (2t, $J = 6.8$ Hz, 2H, $CH_2\underline{CH_2}NCO$), 6.56 (s_{app}, 1H, 4′–CH), 6.65 (dd, $J_1 = 2.2$ Hz and $J_2 = 8.4$ Hz, 1H, 2′–CH), 7.00 (d, $J = 8.4$ Hz, 1H, 1′–CH), 7.4 (s broad, 1H, OH phenol); u.v. λ_{max} (MeOH) 280 ($\epsilon = 2100$) nm; MS *m/e* (rel. intensity) 409 (M^+, 46), 394 (3.0), 391 (3.3), 376 (4.3), 338 (15), 270 (100); HRMS M^+ calculated for $C_{26}H_{35}O_3N$—409.2617, found—409.2608.

*Preparation of estradiol derivatives with 17α-oxygenated alkynylamide side chains **5** and **6** (Fig. 3)*

Synthesis of 7-{3′,17′β-bis[(tetrahydro-2″H-pyran-2″-yl)oxy] estra-1′,3′,5′(10′)-trien-17′α-yl}-5-oxo-6-heptynoic acid (13). In a flame-dried flask under argon atmosphere, 3,17β-bis tetrahydropyranyl EE_2 **12** [11] (704 mg, 1.52 mmol) was dissolved in dry THF (40 ml) and hexamethylphosphoramide (HMPA) (1.09 g, 6.07 mmol). The solution was cooled at −78°C and *n*-BuLi (3.03 mmol) was added. After 2 h, glutaric anhydride (242 mg, 2.12 mmol) in dry THF (6 ml) was added at −78°C and the mixture was allowed to return slowly to room temperature and kept at this temperature overnight. Then, brine was added and the reaction mixture was extracted with ethyl acetate (5 ×). The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The amide product was filtered on silica gel with hexane–ethyl acetate, 9:1, v:v in order to remove unreacted material. The silica gel was then washed with ethyl acetate to obtain the desired product. This product was washed with water in order to remove residual HMPA and provided 64 mg (0.110 mmol, 7.3% yield) of carboxylic acid **13**. Colorless oil: i.r. ν (neat) 3600–2300, 2910, 2860, 2780, 2190, 1705, 1660, 1600, 1480, 1450 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.95 (s, 3H, 18′–CH_3), 2.68 (t, $J = 7.0$ Hz, 2H, $CO\underline{CH_2}CH_2$), 2.83 (m, 2H, 6′–CH_2), 3.56 (m, 2H, OCH_2 of THP at 17′), 3.92 (m, 2H, OCH_2 of THP at 3′), 4.95 and 5.13 (2s, 1H, 2″–CH of THP at 17′ (two isomers)),

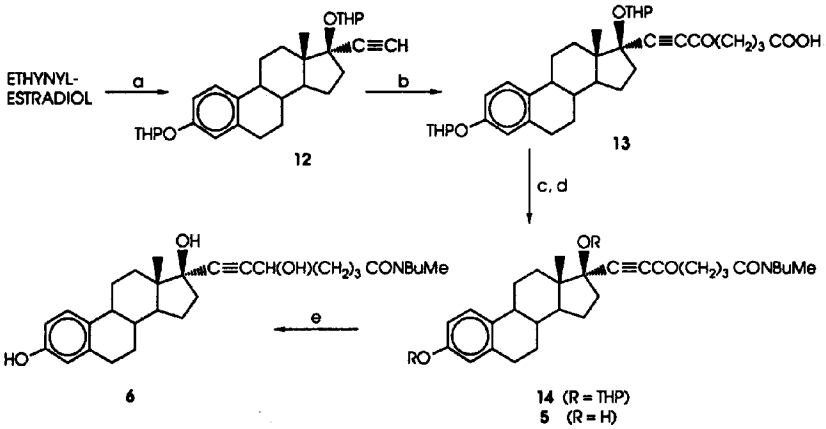

Fig. 3. Preparation of E_2 derivatives with an oxygenate alkynylamide side chain at the 17α position. Reagents are: (a) DHP, C_6H_6, *p*TSA; (b) (1) *n*-BuLi, HMPA, THF, (2) glutaric anhydride; (c) (1) ClCOO*i*-Bu, $N(Bu)_3$, (2) NHBuMe; (d) *p*TSA, MeOH; (e) $NaBH_4$.

5.39 (s, 1H, 2″–CH of THP at 3′), 6.78 (d, $J = 2.6$ Hz, 1H, 4′–CH), 6.84 (dd, $J_1 = 2.6$ Hz and $J_2 = 8.4$ Hz, 1H, 2′–CH), 7.19 (d, $J = 8.4$ Hz, 1H, 1′–CH); MS *m/e* (rel. intensity) 410 (M^+-2 DHP, 8.5), 395 (7.5), 392 (6.1), 377 (3.3), 364 (1.8), 349 (1.8), 338 (2.8), 324 (6.1), 295 (4.8), 270 (59), 85 (100).

Synthesis of N-butyl,N-methyl-7-{3′,17′β-bis[(tetrahydro-2″H-pyran-2″-yl)oxy] estra-1′,3′,5′(10′)-trien-17′α-yl}-5-oxo-6-heptynamide (14). Under anhydrous conditions, carboxylic acid **13** (63 mg, 0.109 mmol) was dissolved in dry CH_2Cl_2 (20 ml) and tributylamine (26 mg, 0.140 mmol). After cooling the mixture at −10°C, isobutyl chloroformate (21 mg, 0.154 mmol) was added and allowed to react for 30 min. *N*-methylbutylamine (0.10 ml) was then added in excess and the cooling bath was removed. After 2 h, CH_2Cl_2 was added and the organic phase was washed with HCl (1N) and dried over $MgSO_4$. The solvent was then removed under reduced pressure and the crude product was purified by flash chromatography (hexane–ethyl acetate, 5:5, v:v) to give 44 mg (0.068 mmol, 62% yield) of amide **14**. Colorless oil: i.r. *v* (neat) 2920, 2850, 2190, 1660, 1630, 1485 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.93 (m, 3H, $CH_2\underline{CH_3}$), 0.95 (s, 3H, 18′–CH_3), 2.72 (t, $J = 6.8$ Hz, 2H, $CO\underline{CH_2}CH_2$), 2.85 (m, 2H, 6′–CH_2), 2.89 and 2.94 (2s, 3H, CH_3NCO), 3.24 and 3.33 (2t, $J = 7.5$ Hz, 2H, $CH_2\underline{CH_2}NCO$), 3.55 (m, 2H, OCH_2 of THP at 17′), 3.93 (m, 2H, OCH_2 of THP at 3′), 4.96 and 5.12 (2s, 1H, 2″–CH of THP at 17′ (two isomers)), 5.39 (s, 1H, 2″–CH of THP at 3′), 6.79 (s_{app}, 1H, 4′–CH), 6.82 (dd, $J = 2.4$ Hz and $J_2 = 8.6$ Hz, 1H, 2′–CH), 7.19 (d, $J = 8.1$ Hz, 1H, 1′–CH); u.v. λ_{max} (hexane) 277 ($\epsilon = 1800$) nm; MS *m/e* (rel. intensity) 563 (M^+–DHP, 0.7), 546 (1.1), 479 (7.2), 461 (24), 444 (3.1), 238 (42), 184 (92), 85 (100).

Synthesis of N-butyl,N-methyl-7-(3′,17′β-dihydroxy estra-1′,3′,5′(10′)-trien-17′α-yl)-5-oxo-6-heptynamide (5). The tetrahydropyranyl derivative **14** (27.0 mg, 0.042 mmol) was dissolved in MeOH (20 ml) and *p*-toluenesulfonic acid monohydrate (5 mg, 0.026 mmol) was added. The resulting solution was stirred at room temperature for 2 h. Thereafter, water was added, MeOH was evaporated under reduced pressure and the residue was extracted with ethyl acetate. After evaporation of the organic phase, the crude product was purified by flash chromatography (hexane–ethyl acetate, 4:6, v:v) to give 13.0 mg (0.027 mmol, 65% yield) of hydrolyzed product **5**. Colorless oil: i.r. *v* (neat) 3300, 2910, 2850, 2180, 1650, 1600 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.91 (s, 3H, 18′–CH_3), 0.95 (m, 3H, $CH_2\underline{CH_3}$), 2.67 (t, $J = 7.2$ Hz, 2H, $CO\underline{CH_2}CH_2$), 2.81 (m, 2H, 6′–CH_2), 2.91 and 2.96 (2s, 3H, CH_3NCO), 3.25 and 3.36 (2t, $J = 7.0$ Hz, 2H, $CH_2\underline{CH_2}NCO$), 4.76 (s, 1H, OH phenol), 6.57 (s_{app}, 1H, 4′–CH, 6.63 (dd, $J_1 = 2.2$ Hz and $J_2 = 8.8$ Hz, 1H, 2′–CH), 7.14 (d, $J = 8.1$ Hz, 1H, 1′–CH); u.v. λ_{max} (MeOH) 281 ($\epsilon = 2400$) nm; MS *m/e* (rel. intensity) 479 (M^+, 2.8), 461 (7.5), 446 (1.7), 374 (3.5), 270 (38), 44 (100); HRMS M^+ calculated for $C_{36}H_{41}O_4N$—479.3035, found—479.3041.

Synthesis of N-butyl,N-methyl-7-(3',17β-dihydroxy estra-1',3',5'(10')-trien-17'α-yl)-5-hydroxy-6-heptynamide (6). A mixture of ketoamide **5** (7.5 mg, 0.0157 mmol), MeOH (10 ml) and $NaBH_4$ (in excess) was stirred at 0°C for 1 h. Then, the reaction mixture was acidified with diluted HCl and MeOH was evaporated under reduced pressure. After extraction with ethyl acetate, the organic phase was dried and the solvent was removed. The crude product was purified by TLC (20 × 20 cm, 0.25 mm, Kieselgel $60F_{254}$ plate) with hexane–ethyl acetate, 2:8, v:v (3 migrations) as eluent and 6.3 mg (0.0131 mmol, 83% yield) of alcohol **6** was obtained. Colorless viscous oil: i.r. ν (film) 3240, 2910, 2850, 2240 vw, 1605 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.87 (s, 3H, 18'–CH_3), 0.91 and 0.94 (2t, J = 7.0 Hz, 3H, $CH_2\underline{CH_3}$), 2.80 (m, 2H, 6'–CH_2), 2.90 and 2.96 (2s, 3H, CH_3NCO), 3.25 and 3.35 (2t, J = 7.0 Hz, 2H, $CH_2\underline{CH_2}NCO$), 4.47 (m, 1H, $\underline{CH}OH$), 4.87 (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 = 8.1 Hz, 1H, 2'–CH), 7.14 (d, J = 8.1 Hz, 1H, 1'–CH); MS *m/e* (rel. intensity) 481 (M^+, 1.7), 463 (1.9), 448 (1.7), 445 (5.3), 430 (5.1), 296 (7.4), 270 (25), 213 (29), 114 (44), 44 (100); HRMS M^+ calculated for $C_{30}H_{43}O_4N$—481.3192, found—481.3192.

*Preparation of bromo side chains **15–22** (Fig. 4)*

General procedure for ester formation. Under anhydrous conditions and an argon atmosphere, bromo acid (17 mmol) was dissolved in dry CH_2Cl_2 (30 ml) and oxalyl chloride (12 ml) was added. The reaction mixture was then stirred for 2 h at room temperature. Following the reaction, dry benzene was added to the mixture and solvent was evaporated under reduced pressure (2 ×) and dried under vacuum. This crude product was dissolved in dry CH_2Cl_2 (20 ml) and added at 0°C to a solution of 3-methyl 3-oxetanemethanol (17 mmol), CH_2Cl_2 (7 ml) and pyridine (1.4 ml). The reaction was kept at the same temperature for 4–8 h. Thereafter, the mixture was diluted with CH_2Cl_2, washed with $NaHCO_3$ (10%, w/v) and the organic phase was dried over $MgSO_4$. After removal of solvent, the residue was purified by chromatography (hexane–ethyl acetate–triethylamine, 80:20:1, v:v:v) to yield bromo ester.

*6-Bromo hexanoate ester of 3-methyl-3-hydroxymethyl oxetane (**15**).* Light yellow liquid (91% yield): i.r. ν (neat) 2930, 2860, 1725, 1450, 1370, 1160 cm^{-1}; NMR-60 δ ($CDCl_3$) 1.31 (s, 3H, CH_3), 1.1–2.1 (m, 6H, 3 × CH_2), 2.36 (t, J = 6.0 Hz, 2H, $CH_2\underline{CH_2}CO$), 3.36 (t, J = 6 Hz, 2H, $Br\underline{CH_2}CH_2$), 4.13 (s, 2H, $COOCH_2C$), 4.41 (AB systems $\Delta\nu$ = 8.3, J = 6 Hz, 4H, 2 × $\underline{CH_2}O$).

*9-Bromo nonanoate ester of 3-methyl-3-hydroxymethyl oxetane (**16**).* Colorless liquid (86% yield): i.r. ν (neat) 2920, 2840, 1725, 1450, 1370, 1150 cm^{-1}; NMR-60 δ ($CDCl_3$) 1.2–2.2 (s + m, 15H, CH_3 and 6 × CH_2), 2.40 (t, J = 6.0 Hz, 2H, $CH_2\underline{CH_2}CO$), 3.45 (t, J = 6.0 Hz, 2H, $Br\underline{CH_2}CH_2$), 4.20 (s, 2H, $COOCH_2C$), 4.48 (AB system $\Delta\nu$ = 8.2, J = 6.0 Hz, 4H, 2 × CH_2O).

*11-Bromo undecanoate ester of 3-methyl-3-hydroxymethyl oxetane (**17**).* Colorless liquid (85% yield): NMR-60 δ ($CDCl_3$) 1.0–2.0 (s + m, 19H, CH_3 and 8 × CH_2), 2.30 (t, J = 6.0 Hz, 2H, $CH_2\underline{CH_2}CO$), 3.35 (t, J = 6.0 Hz, 2H, $Br\underline{CH_2}CH_2$), 4.12 (s, 2H, $COOCH_2C$), 4.40 (AB system $\Delta\nu$ = 8.2, J = 6.0 Hz, 4H, 2 × CH_2O).

*12-Bromo dodecanoate ester of 3-methyl-3-hydroxymethyl oxetane (**18**).* Colorless liquid (86% yield): i.r. ν (neat) 2910, 2840, 1720, 1450, 1370, 1155 cm^{-1}; NMR-60 δ ($CDCl_3$) 1.1–2.0 (s + m, 21H, CH_3 and 9 × CH_2), 2.30 (t, J = 6.0 Hz, 2H, $CH_2\underline{CH_2}CO$), 3.33 (t, J = 6.0 Hz, 2H, $Br\underline{CH_2}CH_2$), 4.11 (s, 2H, $COOCH_2C$), 4.40 (AB system $\Delta\nu$ = 8.0, J = 6.0 Hz, 4H, 2 × CH_2O).

General procedure for ortho ester formation. To a solution of bromo ester (3.4–14.2 mmol) in dry CH_2Cl_2 (10–40 ml) at 0°C was added with stirring distilled boron trifluoride etherate (0.85–3.55 mmol). After 4 h at 0°C, the reaction mixture was quenched by the addition of triethylamine (3.4–14.2 mmol), diluted with diethyl ether and filtered to remove the amine–BF_3 complex. The filtrate was evaporated and the residue was purified by chromatography (hexane–ethyl acetate–triethylamine, 80:20:1, v:v:v) to give the bromo *ortho* ester.

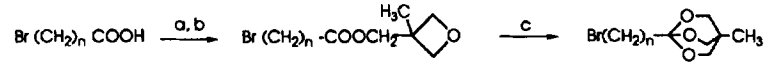

Fig. 4. Preparation of four bromo side chains. Reagents are: (a) oxalyl chloride; (b) 3-methyl-3-oxetanemethanol, pyridine; (c) $BF_3 \cdot O(Et)_2$.

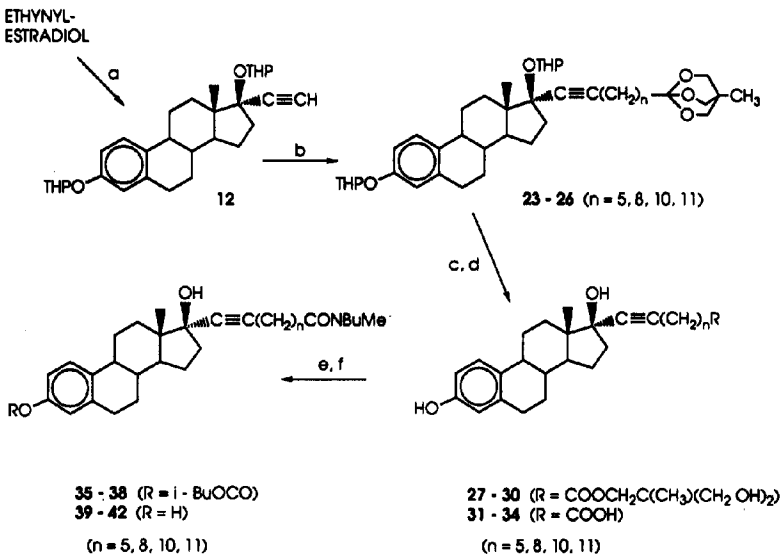

Fig. 5. Preparation of E_2 derivatives with an alkynylamide side chain at the 17α position. Reagents are: (a) DHP, C_6H_6, *p*TSA; (b) (1) *n*-BuLi, HMPA, THF, (2) $Br(CH_2)_nC(OCH_2)_3CCH_3$ ($n = 5, 8, 10, 11$); (c) *p*TSA, MeOH; (d) KOH, H_2O, MeOH; (e) (1) ClCOO*i*-Bu, $N(Bu)_3$, (2) NHBuMe; (f) K_2CO_3, H_2O, MeOH.

*1-(5′-Bromo pentanyl)-4-methyl-2,6,7-trioxabicyclo [2,2,2] octane (**19**).* Colorless oil (68% yield): i.r. ν (neat) 2940, 2915, 2855, 1450, 1390, 1050, 980 cm^{-1}; NMR-60 δ ($CDCl_3$) 0.79 (s, 3H, CH_3), 1.2–2.0 (m, 8H, 4 × CH_2), 3.35 (t, $J = 6.0$ Hz, 2H, $Br\underline{CH_2}CH_2$), 3.87 (s, 6H, 3 × OCH_2); MS *m/e* (rel. intensity) 280 (M^+, 0.2), 278 (M^+, 0.2), 250 (8.1), 248 (8.5), 197 (7.2), 195 (7.7), 179 (58), 177 (61), 72 (54), 69 (100).

*1-(8′-Bromo octanyl)-4-methyl-2,6,7-trioxabicyclo [2,2,2] octane (**20**).* Amorphous white solid (69% yield): i.r. ν (KBr) 2940, 2900, 2840, 1450, 1390, 1045, 985, 950 cm^{-1}; NMR-60 δ ($CDCl_3$) 0.80 (s, 3H, CH_3), 1.33 (s, 8H, 4 × CH_2), 1.0–2.1 (m, 6H, 3 × CH_2), 3.40 (t, $J = 6.0$ Hz, 2H, $Br\underline{CH_2}CH_2$), 3.93 (s, 6H, 3 × OCH_2); MS *m/e* (rel. intensity) 323 (M^+, 2.1), 321 (M^+, 2.0), 292 (4.4), 290 (5.1), 239 (8.6), 237 (7.1), 221 (34), 219 (33), 69 (71), 55 (100).

*1-(10′-Bromo decanyl)-4-methyl-2,6,7-trioxabicyclo [2,2,2] octane (**21**).* White solid (74% yield): m.p. 51–53°C; i.r. ν (KBr) 2940, 2900, 2850, 2830, 1455, 1390, 1055, 985, 955 cm^{-1}; NMR-60 δ ($CDCl_3$) 0.80 (s, 3H, CH_3), 1.27 (s, 12H, 6 × CH_2), 1.1–2.1 (m, 6H, 3 × CH_2), 3.39 (t, $J = 6.0$ Hz, 2H, $Br\underline{CH_2}CH_2$), 3.87 (s, 6H, 3 × OCH_2); MS *m/e* (rel. intensity) 350 (M^+, 1.2), 348 (M^+, 1.1), 321 (3.0), 319 (7.6), 269 (7.5), 248 (97), 144 (37), 55 (100).

*1-(11′-Bromo undecanyl)-4-methyl-2.6,7-trioxabicyclo [2,2,2] octane (**22**).* White solid (76%, yield): m.p. 47.5–48.5°C; i.r. ν (KBr) 2900, 2835, 1460, 1045, 975 cm^{-1}; NMR-60 δ ($CDCl_3$) 0.79 (s, 3H, CH_3), 1.25 (s, 14H, 7 × CH_2), 1.1–2.1 (m, 6H, 3 × CH_2), 3.37 (t, $J = 6.0$ Hz, 2H, $Br\underline{CH_2}CH_2$), 3.85 (s, 6H, 3 × OCH_2); MS *m/e* (rel. intensity) 364 (M^+, 3.5), 362 (M^+, 3.4), 334 (13), 332 (13), 283 (15), 263 (85), 261 (97), 144 (51), 55 (100).

*Preparation of E_2 derivatives with 17α-alkynylamide side chain **39–42** (Fig. 5)*

General procedure for coupling reaction. In a flame-dried flask under an argon atmosphere, 3,17β-bis tetrahydropyranyl EE_2 **12** (1.5 mmol) was dissolved in dry THF (40 ml) and hexamethylphosphoramide (HMPA) (6.0 mmol). The solution was cooled at −78°C and *n*-BuLi (3.0 mmol) was added. After 2 h, appropriate bromo *ortho* ester **19–22** (6.0 mmol) in dry THF (10 ml) was added at −78°C. The mixture was allowed to return slowly to room temperature and kept at the same temperature overnight. Then, brine was added and the reaction mixture was extracted with ethyl acetate. The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by chromatography (hexane–ethyl acetate–triethylamine, 96:4:1,

v:v:v to 50:50:1, v:v:v) to give coupling product **23–26** and unreacted steroid **12** (61, 22, 57 and 43%, respectively) and a small quantity of unknown product.

*1-{3′,17′β-Bis[(tetrahydro-2″H-pyran-2″-yl)-oxy] estra-1′,3′,5′(10′)-trien-17′α-yl}-7-(4′-methyl-2′,6′,7′-trioxabicyclo [2′,2′,2′] octan-1′-yl)-1-heptyne (**23**).* Colorless oil (15% yield): i.r. *ν* (neat) 2920, 2855, 2230 w, 1600, 1485 cm^{-1}; NMR-60 *δ* ($CDCl_3$) 0.75 (s, 3H, CH_3 of *ortho* ester group), 0.88 (s, 3H, 18′–CH_3), 2.80 (m, 2H, 6′–CH_2), 3.2–4.1 (m, 4H, OCH_2 of THP groups), 3.80 (s, 6H, 3 × OCH_2 of *ortho* ester group), 4.9–5.3 (m, 1H, 2″–CH of THP at 17′), 5.34 (s, 1H, 2″–CH of THP at 3′), 6.75 (m, 2H, 2′– and 4′–CH), 7.19 (d, $J = 8.0$ Hz, 1H, 1′–CH); MS *m/e* (rel. intensity) 579 (M^+–DHP, 4.0), 564 (1.1), 494 (12), 477 (12), 374 (13), 85 (100).

*1-{3′,17′β-Bis[(tetrahydro-2″H-pyran-2″-yl)oxy] estra-1′,3′,5′(10′)-trien-17′α-yl}-10-(4′-methyl-2′,6′,7′-trioxabicyclo [2′,2′,2′] octan-1′-yl)-1-decyne (**24**).* Colorless oil (15% yield): i.r. *ν* (neat) 2915, 2850, 2210 w, 1600, 1485 cm^{-1}; NMR-200 *δ* ($CDCl_3$) 0.79 (s, 3H, CH_3 of *ortho* ester group), 0.90 (s, 3H, 18′–CH_3), 2.24 (t, $J = 6.6$ Hz, 2H, C≡C$\underline{CH_2}CH_2$), 2.83 (m, 2H, 6′–CH_2), 3.55 (m, 2H, OCH_2 of THP at 17′), 3.89 (s, 6H, 3 × OCH_2 of *ortho* ester group), 3.95 (m, 2H, OCH_2 of THP at 3′), 4.98 and 5.19 (2s, 1H, 2″–CH of THP at 17′ (two isomers)), 5.39 (s, 1H, 2″–CH of THP at 3′), 6.78 (d, $J = 2.6$ Hz 1H, 4′–CH), 6.84 (dd, $J_1 = 2.6$ Hz and $J_2 = 8.4$ Hz, 1H, 2′–CH), 7.22 (d, $J = 8.4$ Hz, 1H, 1′–CH); MS *m/e* (rel. intensity) 620 (M^+–DHP, 4.8), 535 (13), 518 (8.9), 85 (100).

*1-{3′,17′β-Bis[(tetrahydro-2″H-pyran-2″-yl)-oxy] estra-1′,3′,5′,(10′)-trien-17′α-yl}-12-(4′-methyl-2′,6′,7′-trioxabicyclo [2′,2′,2′] octan-1′-yl)-1-dodecyne (**25**).* Colorless viscous oil (42% yield): i.r. *ν* (neat) 2920, 2850, 2210 vw, 1600, 1485 cm^{-1}; NMR-200 *δ* ($CDCl_3$) 0.79 (s, 3H, CH_3 of *ortho* ester group), 0.90 (s, 3H, 18′–CH_3), 2.25 (t, $J = 6.6$ Hz, 2H, C≡C$\underline{CH_2}CH_2$), 2.83 (m, 2H, 6′–CH_2), 3.55 (m, 2H, OCH_2 of THP at 17′), 3.89 (s, 6H, 3 × OCH_2 of *ortho* ester group), 3.95 (m, 2H, OCH_2 of THP at 3′), 5.0 and 5.2 (2s, 1H, 2″–CH of THP at 17′ (two isomers)), 5.39 (s, 1H, 2″–CH of THP at 3′), 6.78 (d, $J = 2.6$ Hz, 1H, 4′–CH), 6.84 (dd, $J_1 = 2.6$ Hz and $J_2 = 8.4$ Hz, 1H, 2′–CH), 7.21 (d, $J = 8.4$ Hz, 1H, 1′–CH); MS *m/e* (rel. intensity) 649 (M^+–DHP, 6.1), 634 (0.7), 564 (22), 547 (16), 85 (100).

*1-{3′,17′β-Bis[(tetrahydro-2″H-pyran-2″-yl)-oxy] estra-1′,3′,5′(10′)-trien-17′α-yl}-13-(4′-methyl-2′,6′,7′-trioxabicyclo[2′,2′,2′]octan-1′-yl)-1-tridecyne (**26**).* Colorless viscous oil (35% yield): i.r. *ν* (neat) 2915, 2850, 2220 vw, 1600, 1490 cm^{-1}; NMR-200 *δ* ($CDCl_3$) 0.80 (s, 3H, CH_3 of *ortho* ester group), 0.90 (s, 3H, 18′–CH_3), 2.25 (t, $J = 6.6$ Hz, 2H, C≡C$\underline{CH_2}CH_2$), 2.83 (m, 2H, 6′–CH_2), 3.53 (m, 2H, OCH_2 of THP at 17′), 3.89 (s, 6H, 3 × OCH_2 of *ortho* ester group), 3.95 (m, 2H, OCH_2 of THP at 3′), 5.0 and 5.2 (2s, 1H, 2″–CH of THP at 17′ (two isomers)), 5.39 (s, 1H, 2″–CH of THP at 3′), 6.78 (d, $J = 2.2$ Hz, 1H, 4′–CH), 6.84 (dd, $J_1 = 2.6$ and $J_2 = 8.4$ Hz, 1H, 2′–CH), 7.21 (d, $J = 8.4$ Hz, 1H, 1′–CH); MS *m/e* (rel. intensity) 662 (M^+–DHP, 8.3), 578 (19), 560 (31), 85 (75), 44 (100).

General procedure for ortho ester and di-THP hydrolysis. The product with *ortho* ester and di-THP group (0.22–0.63 mmol) was dissolved in MeOH (80–120 ml) and *p*-toluenesulfonic acid (0.17–0.23 mmol) was added. The solution was stirred at room temperature for 2–3 h. Then, water was added, MeOH was removed under reduced pressure and residue was extracted with ethyl acetate. After evaporation of solvent, the crude product was purified by column chromatography (hexane–ethyl acetate, 5:5, v:v) to give ester compound with free hydroxyl group.

*8-(3′,17′β-Dihydroxy estra-1′,3′,5′(10′)-trien-17′α-yl)-7-octynoate ester of 2′,2′-dihydroxymethyl propanol (**27**).* Colorless viscous oil (70% yield): i.r. *ν* (film) 3340, 2910, 2850, 1710, 1600, 1485 cm^{-1}; NMR-200 *δ* ($CDCl_3$) 0.83 and 0.86 (2s, 6H, 18′–CH_3 and CH_3 of ester group), 2.27 (t, $J = 6.4$ Hz, 2H, C≡C$\underline{CH_2}CH_2$), 2.38 (t, $J = 7.1$ Hz, 2H, CO$\underline{CH_2}CH_2$), 2.81 (m, 2H, 6′–CH_2), 3.54 (s broad, 4H, 2 × $\underline{CH_2}OH$), 4.17 (s, 2H, $COOCH_2C$), 4.87 (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) 512 (M^+, 14), 494 (97), 479 (17), 466 (11), 270 (48), 159 (100).

*11-(3′,17′β-Dihydroxy estra-1′,3′,5′(10′)-trien-17′α-yl)-10-undecynoate ester of 2′,2′-dihydroxymethyl propanol (**28**).* Colorless viscous oil (61% yield): i.r. *ν* (film) 3360, 2910, 2840, 2210 vw, 1710, 1600, 1485 cm^{-1}; NMR-200 *δ* ($CDCl_3$) 0.84 and 0.86 (2s, 6H, 18′–CH_3 and CH_3 of ester group), 2.24 (t, $J = 7.0$ Hz, 4H, C≡C$\underline{CH_2}CH_2$ and CO$\underline{CH_2}CH_2$), 2.79 (m, 2H,

6′–CH_2), 3.34 (s broad, 2H, 2 OH), 3.56 (s broad, 4H, 2 × $\underline{CH_2}OH$), 4.13 (s, 2H, $COOCH_2C$), 6.57 (s_{app}, 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) MS *m/e* (rel. intensity) 554 (M^+, 5.0), 536 (57), 520 (10), 507 (7.6), 435 (14), 419 (20), 270 (39), 160 (85), 133 (100).

13-(3′,17′β-Dihydroxy estra-1′,3′,5′(10′)-trien-17′α-yl)-12-tridecynoate ester of 2′,2′-dihydroxymethyl propanol (29). Colorless viscous oil (78% yield): i.r. *ν* (film) 3360, 2915, 2840, 1710, 1600, 1490 cm^{-1}; NMR-60 *δ* ($CDCl_3$) 0.83 (s, 6H, 18′–CH_3 and CH_3 of ester group), 2.25 (m, 4H, C≡C$\underline{CH_2}CH_2$ and CO$\underline{CH_2}CH_2$), 2.78 (m, 2H, 6′–CH_2), 3.53 (s broad, 4H, 2 × $\underline{CH_2}OH$), 4.09 (s, 2H, $COOCH_2C$), 6.6 (m, 2H, 2′– and 4′–CH), 7.10 (d, $J = 8.0$ Hz, 1H, 1′–CH); MS *m/e* (rel. intensity) 582 (M^+, 1.0), 563 (38), 548 (5.7), 535 (3.5), 463 (5.7), 446 (13), 270 (44), 160 (57), 133 (58), 55 (100).

14-(3′,17′β-Dihydroxy estra-1′,3′,5′(10′)-trien-17′α-yl)-13-tetradecynoate ester of 2′2′-dihydroxymethyl propanol (30). Colorless viscous oil (83%, yield): i.r. *ν* (film) 3360, 2910, 2840, 2220 vw, 1710, 1605, 1490 cm^{-1}; NMR-200 *δ* ($CDCl_3$) 0.85 0.87 (2s, 6H, 18′–CH_3 and CH_3 of ester group), 2.25 (t, $J = 6.6$ Hz, 2H, C≡C$\underline{CH_2}CH_2$), 2.33 (t, $J = 7.1$ Hz, 2H, CO$\underline{CH_2}CH_2$), 2.80 (m, 2H 6′–CH_2), 2.9 (m, 2H, 2 × OH), 3.58 (s broad, 4H, 2 × $\underline{CH_2}OH$), 4.20 (s, 2H, $COOCH_2C$), 5.72 (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), 7.15 (d, $J = 8.8$ Hz, 1H, 1′–CH); MS *m/e* (rel. intensity) 596 (M^+, 2.0), 578 (80), 563 (17), 550 (10), 477 (21), 461 (42), 270 (64), 160 (100).

General procedure for hydrolysis of ester followed by amide formation. At a solution of ester (0.14–0.49 mmol) in MeOH (12–50 ml) was added an aqueous solution of KOH 10%, w/v (6–25 ml) and the mixture was refluxed under an argon atmosphere for 24 h. Thereafter, water was added and MeOH was evaporated under reduced pressure. The resulting solution was acidified with HCl and extracted with ethyl acetate. The organic phase was washed with water, brine and dried over $MgSO_4$. Without purification, the crude carboxylic acid (i.r. acid band at 1700 and 2400–3600 cm^{-1}) was dissolved in dry CH_2Cl_2 (20–70 ml) and tributylamine (0.58–2.04 mmol). After cooling the mixture at −10°C, isobutyl chloroformate (0.68–2.41 mmol) was added and allowed to react for 30 min. At this time, *N*-methylbutylamine in excess (4.2–16.0 mmol) was added and the cooling bath was removed. After 2 h, CH_2Cl_2 was added and the organic phase was washed with HCl (1 N) and dried over $MgSO_4$. The solvent was removed and the crude amide was purified by column chromatography (hexane–ethyl acetate, 7:3, v:v).

N-Butyl,N-methyl-8-[3′-(i-butyloxy carbonyloxy)-17′β-hydroxy estra-1′,3′,5′(10′)-trien-17′α-yl]-7-octynamide (35). Colorless oil (79% yield): i.r. *ν* (neat) 3380, 2920, 2850, 1745, 1620 cm^{-1}; NMR-200 *δ* ($CDCl_3$) 0.87 (s, 3H, 18′–CH_3), 0.91 and 0.94 (2t, $J = 7.3$ Hz, 3H, $CH_2\underline{CH_3}$), 1.00 (d, $J = 6.6$ Hz, 6H, $(\underline{CH_3})_2CH$), 2.85 (m, 2H, 6′–CH_2), 2.89 and 2.91 (2s, 3H, CH_3NCO), 3.22 and 3.33 (2t, $J = 7.5$ Hz, 2H, $CH_2\underline{CH_2}NCO$), 4.02 (d, $J = 7.0$ Hz, 2H, *i*-Pr$\underline{CH_2}O$), 6.88 (d, $J = 2.6$ Hz, 1H, 4′–CH), 6.93 (dd, $J_1 = 2.6$ Hz and $J_2 = 8.4$ Hz, 1H, 2′–CH), 7.29 (d, $J = 8.4$ Hz, 1H, 1′–CH); MS *m/e* (rel. intensity) 579 (M^+, 12), 561 (26), 546 (11), 461 (6.7), 447 (3.7), 270 (84), 57 (100).

N-Butyl,N-methyl-11-[3′-(i-butyloxy carbonyloxy)-17′β-hydroxy estra-1′,3′,5′(10′)-trien-17′α-yl]-10-undecynamide (36). Colorless oil (67% yield): i.r. *ν* (neat) 3370, 2910, 2840, 1745, 1620 cm^{-1}; NMR-200 *δ* ($CDCl_3$) 0.87 (s, 3H, 18′–CH_3), 0.92 and 0.95 (2t, $J = 6.6$ Hz, 3H, $CH_2\underline{CH_3}$), 1.00 (d, $J = 7.0$ Hz, 6H, $(\underline{CH_3})_2CH$), 2.86 (m, 2H, 6′–CH_2), 2.90 and 2.94 (2s, 3H, CH_3NCO), 3.24 and 3.35 (2t, $J = 7.3$ Hz, 2H, $CH_2\underline{CH_2}NCO$), 4.03 (d, $J = 6.6$ Hz, 2H, *i*-Pr$\underline{CH_2}O$), 6.88 (d, $J = 2.6$ Hz, 1H, 4′–CH), 6.93 (dd, $J_1 = 2.6$ Hz and $J_2 = 8.8$ Hz, 1H, 2′–CH), 7.30 (d, $J = 8.1$ Hz, 1H, 1′–CH); MS *m/e* (rel. intensity) 621 (M^+, 2.1), 606 (2.4), 602 (6.2), 212 (43), 159 (69), 142 (68), 114 (100).

N-Butyl,N-methyl-13-[3′-(i-butyloxy carbonyloxy)-17′β-hydroxy estra-1′,3′,5′(10′)-trien-17′α-yl]-12-tridecynamide (37). Colorless oil (89% yield): i.r. *ν* (neat) 3370, 2920, 2840, 1745, 1620 cm^{-1}; NMR-200 *δ* ($CDCl_3$) 0.87 (s, 3H, 18′–CH_3), 0.92 and 0.95 (2t, $J = 7.0$ Hz, 3H, $CH_2\underline{CH_3}$), 1.00 (d, $J = 7.0$ Hz, 6H, $(\underline{CH_3})_2CH$), 2.86 (m, 2H, 6′–CH_2), 2.90 and 2.96 (2s, 3H, CH_3NCO), 3.25 and 3.35 (2t, $J = 7.4$ Hz, 2H, $CH_2\underline{CH_2}NCO$), 4.02 (d, $J = 6.6$ Hz, 2H, i-Pr$\underline{CH_2}O$), 6.88 (d, $J = 2.2$ Hz, 1H, 4′–CH), 6.93 (dd, $J_1 = 2.6$ Hz and $J_2 = 8.4$ Hz, 1H, 2′–CH), 7.30 (d, $J = 8.8$ Hz, 1H, 1′–CH); MS *m/e* (rel. intensity) 649 (M^+, 20), 633 (15), 631 (18), 616 (8.2), 531 (15), 516 (5.6), 270 (85), 57 (100).

N-Butyl, N-methyl-14-[3′(i-butyloxy carbonyl-oxy)-17′β-hydroxy estra-1′,3′,5′(10′)-trien-17′α-yl]-13-tetradecynamide (38). Colorless oil (83%, yield): i.r. ν (neat) 3380, 2910, 2840, 1750, 1625 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.87 (s, 3H, 18′–CH_3), 0.92 and 0.95 (2t, J = 7.0 Hz, 3H, $CH_2\underline{CH_3}$), 1.00 (d, J = 6.6 Hz, 6H, $(\underline{CH_3})_2CH$), 2.85 (m, 2H, 6′–CH_2), 2.91 and 2.96 (2s, 3H, CH_3NCO), 3.25 and 3.36 (2t, J = 7.4 Hz, 2H, $CH_2\underline{CH_2}NCO$), 4.03 (d, J = 6.6 Hz, 2H, i-Pr$\underline{CH_2}O$), 6.88 (d, J = 2.6 Hz, 1H, 4′–CH), 6.93 (dd, J_1 = 2.9 Hz and J_2 = 8.4 Hz, 1H, 2′–CH), 7.30 (d, J = 8.8 Hz, 1H, 1′–CH); MS m/e (rel. intensity) 663 (M^+, 4.9), 647 (10), 645 (10), 545 (9), 88 (100).

Hydrolysis of carbonate. The hydrolysis of carbonates **35–38** was performed using the same procedure described previously for hydrolysis of compound **11**.

N-Butyl, N-methyl-8-[3′, 17′β-dihydroxy estra-1′, 3′, 5′(10′)-trien-17′α-yl]-7-octynamide (39). Purified by column chromatography (hexane–ethyl acetate, 4:6, v:v). Amorphous white solid (88% yield): i.r. ν (film) 3280, 2910, 2840, 1610 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.87 (s, 3H, 18′–CH_3), 0.91 and 0.94 (2t, J = 7.0 Hz, 3H, $CH_2\underline{CH_3}$), 2.80 (m, 2H, 6′–CH_2), 2.90 and 2.92 (2s, 3H, CH_3NCO), 3.22 and 3.34 (2t, J = 7.3 Hz, 2H, $CH_2\underline{CH_2}NCO$), 5.22 (s, 1H, OH phenol), 6.57 (d, J = 2.9 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) 479 (M^+, 11), 462 (18), 460 (38), 446 (18), 270 (30), 114 (56), 88 (67), 44 (100); HRMS M^+ calculated for $C_{31}H_{45}O_3N$—479.3399, found—479.3369.

N-Butyl, N-methyl-11-[3′, 17′β-dihydroxy-estra-1′,3′,5′,(10′)-trien-17′α-yl]-10-undecynamide (40). Purified by column chromatography (hexane-ethyl acetate, 4:6, v:v). Amorphous white solid (83% yield): i.r. ν (KBr) 3300, 2910, 2840, 1610 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.87 (s, 3H, 18′–CH_3), 0.93 and 0.95 (2t, J = 7.0 Hz, 3H, $CH_2\underline{CH_3}$), 2.80 (m, 2H, 6′–CH_2), 2.91 and 2.94 (2s, 3H, CH_3NCO), 3.23 and 3.35 (2t, J = 7.3 Hz, 2H, $CH_2\underline{CH_2}NCO$), 5.30 (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 = 8.1 Hz, 1H, 1′–CH); MS m/e (rel. intensity) 521 (M^+, 4.4), 505 (10), 502 (26), 489 (7.7), 487 (8.7), 270 (20), 114 (55), 88 (42), 44 (100); HRMS M^+ calculated for $C_{34}H_{51}O_3N$—521.3869, found—521.3897.

N-Butyl, N-methyl-13-[3′, 17′β-dihydroxy estra-1′, 3′, 5′(10′)-trien-17′α-yl]-12-tridecynamide (41). Purified by column chromatography (hexane–ethyl acetate, 7:3, v:v). Amorphous white solid (98% yield): i.r. ν (film) 3300, 2910, 2840, 1610 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.88 (s, 3H, 18′–CH_3), 0.93 and 0.95 (2t, J = 7.0 Hz, 3H, $CH_2\underline{CH_3}$), 2.80 (m, 2H, 6′–CH_2), 2.93 and 2.97 (2s, 3H, CH_3NCO), 3.25 and 3.38 (2t, J = 7.5 Hz, 2H, $CH_2\underline{CH_2}NCO$), 6.61 (d, J = 2.6 Hz, 1H, 4′–CH), 6.69 (dd, J_1 = 2.6 Hz and J_2 = 8.6 Hz, 1H, 2′–CH), 6.87 (s, 1H, OH phenol), 7.14 (d, J = 8.1 Hz, 1H, 1′–CH); MS m/e (rel. intensity) 549 (M^+, 8.7), 532 (17), 530 (23), 516 (12), 270 (30), 114 (35), 88 (45), 44 (100); HRMS M^+ calculated for $C_{36}H_{55}O_3N$—549.4182, found—549.4189.

N-Butyl, N-methyl-14-[3′, 17′β-dihydroxy-estra-1′,3′,5′(10′)-trien-17′α-yl]-13-tetradecynamide (42). Purified by column chromatography (hexane–ethyl acetate, 6:4, v:v). Amorphous white solid (93% yield): i.r. ν (film) 3280, 2915, 2840, 1615 cm^{-1}; NMR-200 δ ($CDCl_3$) 0.88 (s, 3H, 18′–CH_3), 0.94 and 0.95 (2t, J = 7.0 Hz, 3H, $CH_2\underline{CH_3}$), 2.80 (m, 2H, 6′–CH_2), 2.95 and 2.98 (2s, 3H, CH_3NCO), 3.26 and 3.39 (2t, J = 7.3 Hz, 2H, $CH_2\underline{CH_2}NCO$), 6.61 (d, J = 2.2 Hz, 1H, 4′–CH), 6.70 (dd, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, 2′–CH), 7.13 (m, 2H, 1′–CH and OH phenol); MS m/e (rel. intensity) 563 (M^+, 3.5), 545 (43), 530 (17), 254 (17), 114 (58), 88 (100); HRMS M^+ calculated for $C_{37}H_{57}O_3N$—563.4338, found—563.4305.

Estrogen Receptor Binding Assay

The relative binding affinities for the estrogen receptor of the synthesized and reference compounds as well as those E_2 and EE_2 were determined by competition of the binding of [^{3}H]E_2 to the rat uterine cytosol receptor as described by Asselin and Labrie [15]. The incubation was performed at 25°C for 3 h and non-specific binding was determined in the presence of an excess (1000 nM) of radioinert E_2. The affinities were expressed in relative binding affinity (RBA = 100 × ED_{50} E_2/ED_{50} tested compound, where ED_{50} is the concentration which inhibits [^{3}H]E_2 binding by 50%).

Uterotrophic and Antiuterotrophic Assay

The *in vivo* estrogenic activity of tested compounds was measured by stimulation of uterine weight in adult ovariectomized CD-1 mice (body wt = 19–20 g sacrificed 5 days after ovariectomy), while their antiestrogenic activity was

measured by inhibition of the E_2-induced stimulation of uterine weight in an assay performed under similar experimental conditions [13, 14]. The test compounds (at doses of 1, 3, 10, 20 or 30 μg) and/or E_2 dissolved in ethanol were injected s.c. in the appropriate groups in a solution of 0.9% (w/v) sodium chloride and 1% (w/v) gelatin in a total volume of 0.2 ml, twice daily for 4.5 days, starting on the day of ovariectomy for a total of 9 injections. E_2 was injected at the dose of 0.01 μg in 0.2 ml of the same vehicle, 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 weighted. Results are the means ± SEM of 9–10 mice per group.

Proliferative and Antiproliferative Cell Assay

Maintenance of stock cell cultures

The ZR-75-1 human breast cancer cells were obtained from the American type culture collection (Rockville, MD). The cells were grown in RPMI 1640 medium without phenol red, supplemented with 10% (v/v) fetal bovine serum (FBS), 1 nM estradiol, 2 nM L-glutamine, 1 mM sodium pyruvate, 15 mM HEPES, 100 IU penicillin per ml and 100 μg streptomycin sulfate per ml [16, 17]. All media and supplements were from Sigma (St Louis, MO) except for the fetal bovine serum which was obtained from Hyclone (Logan, UT). The ZR-75-1 cells were subcultured weekly and cells at passages 93 to 95 were used for this study.

Cell growth experiments

The ZR-75-1 cells in their exponential growth were harvested with 0.05% trypsin–0.02% EDTA (w/v) and resuspended in RPMI 1640 medium without phenol red supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 15 mM HEPES, 100 IU penicillin per ml, 100 μg streptomycin sulfate per ml and 5% (v/v) dextran-coated charcoal-treated FBS (SD medium). The cells were plated in Falcon 24-well tissue culture plates (2 cm^2/well) with 10,000 cells per dish and allowed to adhere to substrate for 3 days. Estrone (E_1) and tested compounds were added from concentrated stock solutions in 99% redistilled ethanol into fresh SD medium. Cells were then incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air for 10 days with a change of medium every second day. At the end of the incubation period, cell growth was assessed by measurement of DNA content by a modification of the Fiszer–Szafarz method [18] as previously described [19]. Medium was carefully removed from the dishes and 150 μl of methanol was added. Plates were then left to dry at room temperature and were either frozen until assayed or processed immediately. Salmon testis DNA (Pharmacia) was used as a standard. Standard solutions containing 0.5 to 20 μg DNA/tube in 1 M NH_4OH were used. Charcoal-treated 3,5-diaminobenzoic acid (DABA) reagent (200 mg/ml) was added (150 μl) to standards and dishes containing dried fixed cells. The reaction was carried out for 60 min at 60°C, before cooling on ice and dilution with 1.5 ml 1.0 N HCl. Fluorescence was measured with a LS2B Perkin–Elmer filter fluorimeter.

RESULTS AND DISCUSSION

Chemistry

The compound with methylbutylamide added directly to the ethynyl group **4** was synthesized in 3 steps (Fig. 2). According to the procedure described by Bucourt *et al.* [9], carbon dioxide was added to the acetylide ion of EE_2, thus yielding carboxylic acid **10**. This compound was then transformed to amide **11** by the general procedure of amide formation. In this case, isobutyl chloroformate reacted with carboxylic acid to give a mixed anhydride which was transformed to the amide by the action of methyl butylamine. In this process, the phenolic group reacts also with isobutyl chloroformate to form a carbonate group at the 3-position. The formation of compound **11** was confirmed by i.r. bands at 1750 (C=O, carbonate group) and 1610 (C=O, amide group). By NMR, we observed two doublets at 1.00 and 4.03 ppm for the isobutyl carbonate group, while the amide group showed two singulets at 2.96 and 3.18 ppm (N—CH_3, two rotamers) and two triplets at 3.40 and 3.55 ppm (N—$\underline{CH_2}$—CH_2—, two rotamers). Hydrolysis of the carbonate group was easily achieved with K_2CO_3 in aqueous methanol [20] to give the phenol amide **4** in excellent yield.

Synthesis of the EE_2 derivatives with oxygenated alkylamide side chains (17α-oxygenated alkynylamide E_2-derivatives) **5** and **6** is shown in Fig. 3. In the first steps, the two hydroxyl groups of starting material (EE_2) were protected as tetrahydropyranyl ether **12** [10]. The intro-

duction of the oxygenated side chain was then achieved by adding glutaric anhydride to acetylide of EE_2 di-THP. As expected in a similar reaction [14], attack of the carbonyl group by the acetylide ion and particularly the acetylide of EE_2 di-THP, is not easy and the yield is very low. Nevertheless, separation of the minor acid derivative **13** and the major unreacted product **12** was easily achieved by simple filtration on silica gel. The formation of ketoacid **13** was confirmed by i.r. as a large hydroxyl band (2300–3600 cm^{-1}; —COOH), two carbonyl bands (1705 cm^{-1}; —COOH and 1660 cm^{-1}; C=O conjugated to alkyne) and a sharp alkyne band (2190 cm^{-1}; C≡C conjugated to ketone). We have observed that the stretching band of alkyne conjugated to ketone is more intense than that of the non-conjugated alkyne. By NMR, disappearance of the alkyne proton (C≡C—H) at 2.6 ppm is characteristic of side chain introduction.

The carboxylic acid **13** was transformed to amide **14** by the above-described amidation. The characteristic bands of the amide group observed by i.r. and NMR were similar to the amide band of compound **4**. To obtain the ketoamide **5** with free hydroxyl groups, the di-THP groups were hydrolyzed in good yield by *p*-toluenesulfonic acid (*p*-TSA) in methanol [21]. Reduction of ketoamide **5** by $NaBH_4$ permitted to obtain hydroxyamide **6**. This compound was confirmed under i.r. by disappearance of the carbonyl band at 1650 cm^{-1} (C≡C—C=O) and a decrease in the alkyne band intensity. By NMR, a new signal corresponding to one proton appeared at 4.47 ppm. This signal was attributed to the proton on the neighboring-carbon of the triple bond which is deshielded by an hydroxyl group. Moreover, the new compound **6** does not absorb u.v. light.

The four other synthesized compounds **39–42** possess a saturated alkylamide side chain added to the ethynyl group of EE_2. In a first approach, we used the coupling reaction of an acetylide ion with bromoalcohol di-THP ($Br(CH_2)_nOTHP$) to introduce the first part of the side chain [14]. After hydrolysis of three THP groups and protection of phenol, we have attempted to oxidize the primary alcohol to carboxylic acid in order to transform the later to a methyl butylamide derivative. Unfortunately, we were unable to obtain carboxylic acid starting from the primary alcohol. We rather observed the formation of E_1 resulting from the cleavage between carbon 17 and the side chain with oxidation of the 17-hydroxyl group. The second approach was more successful and consisted in coupling an acetylide ion with a bromoalkyle side chain [14, 22] which possesses a protecting group for carboxylic acid (Figs 4 and 5). In our search for this protecting group, we eliminated all groups having a labile proton able to react with the acetylide of EE_2. We have finally chosen for this purpose a bridged *ortho* ester group [23].

The bromo *ortho* esters **19–22** were easily synthesized starting from appropriate bromo acids (Fig. 4). Using oxalic acid chloride, the appropriate bromo acids were transformed to bromo acid chloride, before being esterified by 3-methyl 3-oxetanemethanol to give the corresponding bromo esters **15–18**. When these esters were submitted to Lewis acid treatment ($BF_3 \cdot O(Et)_2$) [23], the desired bromo *ortho* esters **19–22** were obtained. This was confirmed by NMR spectroscopy with signals at 0.79 (s, 3H) and 3.87 (s, 6H) which are characteristic of 4-methyl-2,6,7-trioxabicyclo [2,2,2] octanyl group (CH_3—$C(CH_2O)_3C$—) as well as by mass spectrometry. The global yields were very good, ranging from 59 to 63% (3 steps).

Following the synthesis of these bromo alkyls with appropriate acid protection, we carried out the coupling reaction with the acetylide of EE_2 [4] (Fig. 5). The coupling products **23–26** were obtained in moderate yields (14–42%), although these yields were not optimized. In all cases, the unreacted steroid was the major compound obtained, while a small quantity of unknown material was also present. The disappearance of the alkyne proton at 2.6 ppm in NMR is characteristic of coupling product formation. Moreover, the mass spectrum did not show the molecular peak (M^+) but rather a M^+-84 peak which corresponds to the loss of one tetrahydropyranyl group. The following step was an acidic treatment (*p*-TSA/MeOH) of compounds **23–26** to hydrolyze the di-THP group and thus transform the bridged *ortho* ester to ester groups **27–30**. The hydroxyl and carbonyl bands of resulting esters were observed by i.r. By NMR, signals at 0.83 ppm (s, 3H), 3.54 ppm (s broad, 4H) and 4.17 ppm (s, 2H) were attributed to the —$COOCH_2C(CH_3)(CH_2OH)_2$ group while the signal characteristic of the THP group disappeared. Molecular peak (M^+) was also observed by mass spectrometry for the three compounds.

Saponification of compounds **27–30** gave the corresponding carboxylic acids **31–34** which

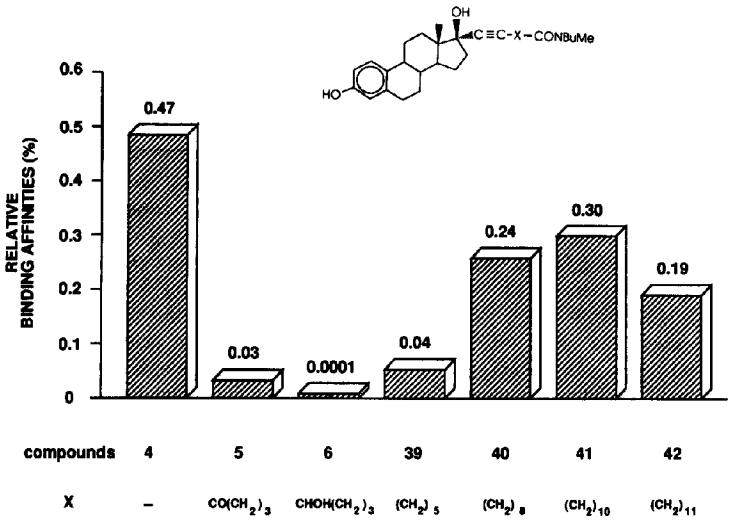

Fig. 6. RBA on estrogen receptor of 17α-alkynylamide derivatives of E_2 (compounds **4–6** and **39–42**). RBA = (ED_{50}) of $E_2/(ED_{50})$ of test compound × 100, where ED_{50} is the concentration of inhibitor required to reduce the binding of tritium-labelled E_2 by 50%. The RBA of E_2 is taken as 100%. Values are the mean of 2 ($n = 2$) independent determinations.

were used with no further purification for the next step. Using the previously described procedure for amide formation, we obtained the methyl butylamide derivatives **35–38** in good yields (79–89%). In this process, the phenol was also transformed to the isobutyl carbonate group. The spectroscopic data confirmed the formation of the desired compounds which have the general characteristics of other carbonate-amide derivatives already described. The final step was easily performed and consisted in the hydrolysis of the carbonate group [20] to obtain compounds with a free phenol group **39–42**.

Biological Activity

In order to assess the potential estrogenic and antiestrogenic activity of the new alkynylamide E_2-derivatives, we have performed the three following assays of biological activity, namely: (1) relative binding affinity to the estrogen receptor: (2) stimulation of uterine weight (estrogenic) or inhibition of estrogen-stimulated uterine weight (antiestrogenic); and (3) proliferation of the human breast cancer ZR-75-1 cells.

Although, the mechanism of action of antiestrogens is not fully understood, their first action is thought to be binding to the estrogen receptor, thus preventing access of endogenous E_2 to its specific binding site. Following binding to the estrogen receptor, the antiestrogen blocks the formation of an activated complex which is required for hormonal action. Using the ED_{50} values of displacement by E_2 as reference, we

Table 1. Comparison of the biological activity of the new synthesized compounds **4**, **39–42** and the pure antiestrogens EM-139 and ICI 164384 according to three biological tests

		AUA[b] (%)		APA[c] (%)	
		Dose		Conc.	
Compounds	RBA[a]	3 μg	30 μg	0.1 nM	1 μM
4	0.47	14 ± 3	34 ± 11	12 ± 11	0
39	0.04	44 ± 7	29 ± 6	57 ± 4	29 ± 1
40	0.24	21 ± 6	8 ± 9	0	0
41	0.30	27 ± 6	19 ± 6	0	0
42	0.19	57 ± 5	31 ± 6	0	0
EM-139	1.2	74 ± 7	99 ± 6[d]	−4 ± 17	101 ± 7
ICI 164384	1.2	40 ± 9	86 ± 10[d]	5 ± 4	90 ± 4

[a]RBA: relative binding affinity on estrogen receptor (see legend to Fig. 6), for EM-139 and ICI 164384 (see Ref. [13]).
[b]AUA: antiuterotrophic activity in female ovariectomized CD-1 mice (see legend to Fig. 7), for EM-139 and ICI 164384, Balb/c mice were used instead of CD-1 mice (see Ref. [13]).
[c]APA: antiproliferative activity in ZR-75-1 human breast cancer cells (see legend to Fig. 8).
[d]At a dose of 20 μg.

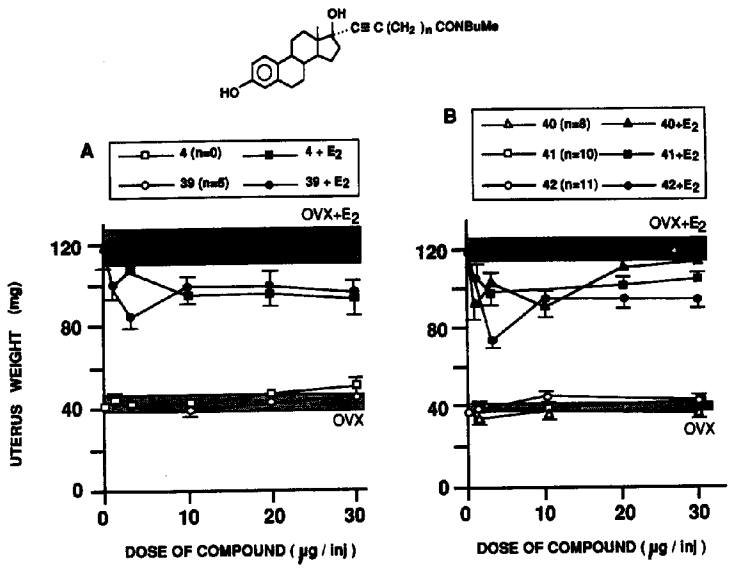

Fig. 7. Uterine weight assay in female ovariectomized CD-1 mice of compounds **4**, **39–42** at doses of 1, 3, 10, 20 and 30 μg/inj. (total of 9 inj.). OVX, uterine weight in ovariectomized animals and OVX + E_2, uterine weight in ovariectomized mice treated with E_2 (0.01 μg/inj.). Part A, compounds with short 17α-alkynylamide side chain (**4**, $n = 0$ and **39**, $n = 5$); part B, compounds with long 17α-alkynylamide side chain (**40–42**, $n = 8$, 10, 11).

have measured the relative binding affinity (RBA) of the new compounds to the rat uterine estrogen receptor, taking arbitrarily the binding of estradiol as 100. The RBA values of 17α-alkynylamide E_2-derivatives were low, ranging from 0.0001 to 0.47% that of E_2 (Fig. 6). Such low RBA values are likely to result, up to a large extent, from a high level of non-specific binding to cytosol components [24]. For this reason, the RBA-test is relatively insensitive and it was only used as one of the indicators of potential estrogenic or antiestrogenic action.

When examining the affinity for the estrogen receptor versus the alkynylamide chain length, it can be seen that compounds with longer side chains (**40–42**) have better RBA value than compounds with intermediate side chain length (**5, 6** and **39**) (0.19–0.30% and 0.0001–0.04%, respectively). It is also clear that introduction of an oxygenated group near the alkyne (compounds **5** and **6**) does not improve the RBA values. Interestingly, the shorter side chain compound (**4**) with no methylene group between the alkyne and amide groups (i.e. conjugated amide) has the best RBA value (0.47%). From this first assay, it appears that compounds with longest ($n = 8$, 10, 11) and shorter ($n = 0$) side chains have a better affinity than compounds with intermediate side chain length, the side chain being oxygenated ($n = 4$) or not ($n = 5$). With another type of 17α-side chain (alkylnyl-alcohol), we have already observed that the optimum side chain length for competition with estrogen binding is six carbons [14]. These findings probably reflect the major importance of the functionality on the side chain, namely the tertiary amide group and the primary alcohol. Introduction of functionality on the side chain can favor one conformational form which could well affect the actual length, thus changing the influence of the number of carbons. For comparative purposes, the antiestrogen ICI 164384 has a RBA of 1.2% while the present 17α-alkynylamide E_2-derivatives have RBA values ranging from 0.0001 to 0.47% (Fig. 6 and Table 1). In particular, compound **40** which has the same side chain length as ICI 164384, but at a different position (17α vs 7α), has a somewhat lower binding (0.24 vs 1.2%, respectively).

The compounds **4, 39–42** were then evaluated for their ability to stimulate mouse uterine weight (uterotrophic or estrogenic activity) and/or to inhibit the stimulation of uterine weight induced by E_2 (antiuterotrophic or antiestrogenic activity). None of the compounds tested had stimulatory effect on uterine weight at the doses used (1, 3, 10, 20 and 30 μg/injection) (Fig. 7, lower curves), thus

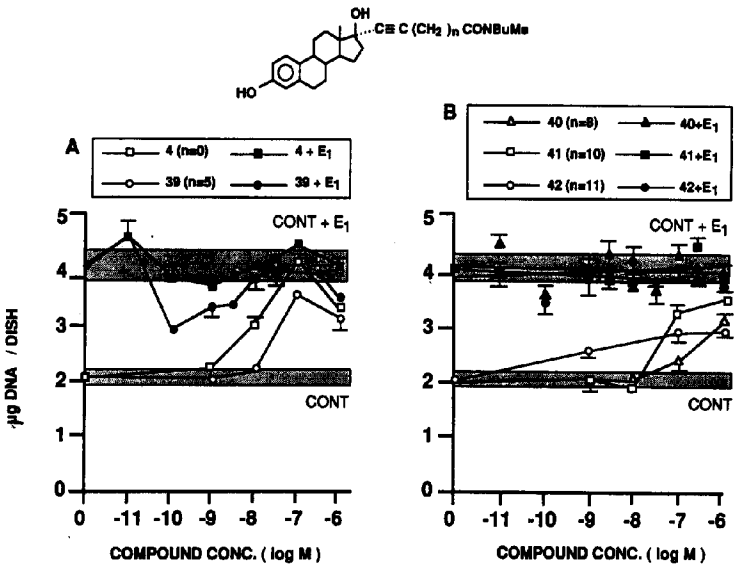

Fig. 8. Human breast cancer ZR-75-1 cell growth assay of compounds **4** and **39–42**. Control: basal level of cell proliferation and E_1: cells stimulated by E_1 (1 nM). Part A, compounds with short 17α-alkynylamide side chain (**4**, $n = 0$ and **39**, $n = 5$) and part B, compounds with long 17α-alkynylamide side chain (**40–42**, $n = 8, 10, 11$).

demonstrating the absence of intrinsic agonistic activity of the compounds in this sensitive *in vivo* system. As can also be seen in Fig. 7 (higher curves), the antiuterotrophic activity of these compounds is low at the doses used and only partial reversal of the stimulatory effect of E_2 on uterine weight can be observed. In fact, all compounds exert their maximal (14–57%) inhibition at low doses (3 or 10 μg), this inhibition remaining constant up to the highest dose used, namely 30 μg. For compound **40**, however, a lack of antiestrogenic activity was seen at the 20 and 30 μg doses. The best inhibition values were obtained at the 3 μg dose for compounds **42** and **39** (57 ± 5 and 44 ± 7%, respectively). In this test, the pure antiestrogens EM-139 and ICI 164384 give no stimulation of uterus weight at such doses [13]. At the 3 μg dose, compounds **39** and **42** inhibited the uterotrophic effect of E_2 at a level comparable to ICI 164384 (44 ± 7 and 57 ± 5% vs 40 ± 9%) while EM-139 inhibited by 74 ± 7%. At the 30 μg dose, however, while EM-139 and ICI 164384 inhibited by 99 ± 6 and 86 ± 10%, respectively, compounds **39** and **42** only inhibited the stimulatory effect of E_2 by 29 ± 6 and 31 ± 6%, respectively (Table 1).

The third test, namely the proliferation of ZR-75-1 cells permits us to assess the *in vitro* estrogenic or antiestrogenic activity of the new compounds in estrogen-sensitive human breast cancer cells. Thus, compounds **4, 39–42** were evaluated for their ability to stimulate the proliferation of ZR-75-1 cells and/or to inhibit E_1-stimulated proliferation of ZR-75-1 cells. As illustrated in Fig. 8, all new compounds stimulated ZR-75-1 cell proliferation at high concentrations. Half-maximal stimulatory effects were observed at 10, 30, 30 and 200 nM for compounds **4, 39, 41** and **40**, respectively, while compound **42** caused only a 30% increase at 1 μM followed by a near-plateau up to the last dose used, namely 1 μM. It can be seen in the same figure that only compound **39** exerted a significant (but partial) antiestrogenic activity in human breast carcinoma cells in culture. In fact, a 57 ± 4% reversal of the stimulatory effect of estrone was observed at the low concentration of 0.1 nM with a progressive loss of the antiestrogenic activity at higher concentrations. The progressive loss of antiestrogenic activity is probably due to the intrinsic estrogenic action of the compound at such concentrations. In this *in vitro* system, the compounds EM-139 and ICI 164384 give no stimulation of ZR-75-1 proliferation [6]. Their antiproliferative or antiestrogenic activity, although not detectable at 0.1 nM, causes a complete reversal of the stimu-

latory effect of E_1 while compounds **4**, **39–42** had no significant effect (Table 1).

In conclusion, these new 17α-alkynylamide derivatives of E_2 are easily synthesized (3–6 steps) starting from EE_2. Using rat uterine cytosol, all compounds have a low ability to compete for E_2 binding ranging from 0.0001 to 0.47% relative to E_2. These RBA-values are however of the same order as those of the pure antiestrogens EM-139 and ICI 164384 which possess an alkylamide side chain at the 7α position. Five compounds with highest RBA value were evaluated for uterotrophic and antiuterotrophic activity in the mouse. In this *in vivo* system, at the doses used, these 5 compounds have no estrogenic or uterotrophic activity while their antiestrogenic or antiuterotrophic activities are relatively low except at the 3 μg dose for compounds **39** and **42** (44–57%). In the ZR-75-1 cell culture assay, all compounds show an estrogenic effect at high concentrations while only compound **39** possesses a significant antiestrogenic activity at low concentrations (0.1–10 nM). There is no detectable correlation between 17α-side chain length and biological activity. In agreement with recent data [26], the present findings suggest that the 17α region is not important for antiestrogenic action. In fact, the present compounds are less antiestrogenic than the recently described pure antiestrogens EM-139 and ICI 164384 [6, 7, 10–13] (Table 1), but it should be mentioned that one of them (compound **39**) is more antiestrogenic in mice than tamoxifen, the compound currently used in the human for breast cancer therapy. In fact, tamoxifen in the *in vivo* uterine mouse assay is a full estrogen with no significant antiestrogenic activity [13, 25].

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