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Original article

Synthesis of 11β-ether-17α-ethinyl-3,17β-estradiols with strong ER antagonist activities

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ABSTRACT

We have previously found that several families of nonpolar short chain 11β-ethers and esters of estradiol are selective estrogen receptor modulators (SERMs). Surprisingly, the transformation from potent estrogen to anti-estrogen occurs when the 11β-side chain is increased slightly in length from four to five non-hydrogen atoms. To generate strong antagonists for preclinical development, we have synthesized other similar ER ligands with 11β-ethers and with an additional ethinyl group at the 17α-position in order to slow metabolism of the steroidal moiety. Here we report the synthesis and biological activity of two such compounds (11β-*i*-PrO-propyl and 11β-*t*-BuO-propyl ethers) with extremely strong antagonist activities.

1. Introduction

In recent years estrogen receptor antagonists have become very important therapeutic agents for the treatment of estrogen sensitive cancers, such as breast cancer [1]. Many anti-estrogenic compounds are typified by analogs of estradiol, substituted at C-7α (ICI 164,384) and C-11β (RU39411) or aromatic compounds, such as Tamoxifen. They share some similar structural features, mainly polar long chains containing tertiary amines or carboxylic groups (Fig. 1).

We have identified a series of unusual estradiol analogs with short and non-polar substituent groups at the 11β position [2,3]. One of the most remarkable examples is the simple and short chain ester, E11-2,2 (Fig. 1). The highly unusual aspect of these results is that a complete reversal of function occurs with a single methylene group, lengthening the side-chain from 4 atoms of a methyl ester in E11-2,1 to 5 atoms of an ethyl ester in E11-2,2 (Fig. 1). E11-2,1 is an estrogen receptor agonist, while E11-2,2 an antagonist. This same principle was found to apply to other substitutions at the 11β position, which include ketones, ethers (Fig. 1, E11-2,2$_{ether}$) and thiono esters [3].

The ethers were especially interesting as they were highly active anti-estrogens and were more stable than other substituents [3]. Among these ethers, two of them, 11β-(3-isopropoxypropyl)-estra-1,3,5(10)-trien-3,17β-diol and 11β-(3-*t*-butoxypropyl)estra-1,3,5(10)-trien-3,17β-diol (E11-3,*i*-Pr$_{ether}$ and E11-3,*t*-Bu$_{ether}$) were especially potent anti-estrogens that were totally without agonist activity. Consequently, in order to produce an ER antagonist that would be longer lived and could be administered orally, we introduced an ethinyl group at 17α position of estradiol to render the D-ring of the steroid resistant to oxidation [4]. These compounds are analogous to moxestrol, 11β-methoxy-17α-ethinyl-estradiol, an extremely potent estrogen that is highly resistant to metabolism [5]. The synthesis and biological activity of these two compounds 11β-(3-isopropoxypropyl)estra-17α-ethinyl-1,3,5(10)-trien-3,17β-diol and 11β-(3-*t*-butoxypropyl)estra-17α-ethinyl-1,3,5(10)-trien-3,17β-diol (17α-ethinyl-E11-3,*i*-Pr$_{ether}$ and 17α-ethinyl-E11-3,*t*-Bu$_{ether}$, Fig. 2) are reported here.

2. Experimental

2.1. Chemistry

General: ^{1}H NMR spectra were recorded with a Bruker Avance 400 spectrometer, and chemical shifts are reported relative to residual $CHCl_3$ (7.27 ppm). Purification by flash chromatography was performed according to the procedure of Still [6], using 230–400 mesh silica gel (EM Science, Darmstadt Germany). Unless otherwise noted, solvents (analytical or HPLC) and reagents were used as supplied, and all reactions were carried out under nitrogen. Thin-layer chromatography (TLC) was performed using Merck silica gel plates (F_{254}) (EM Science) and visualized using phosphomolybdic acid or UV illumination. TLC

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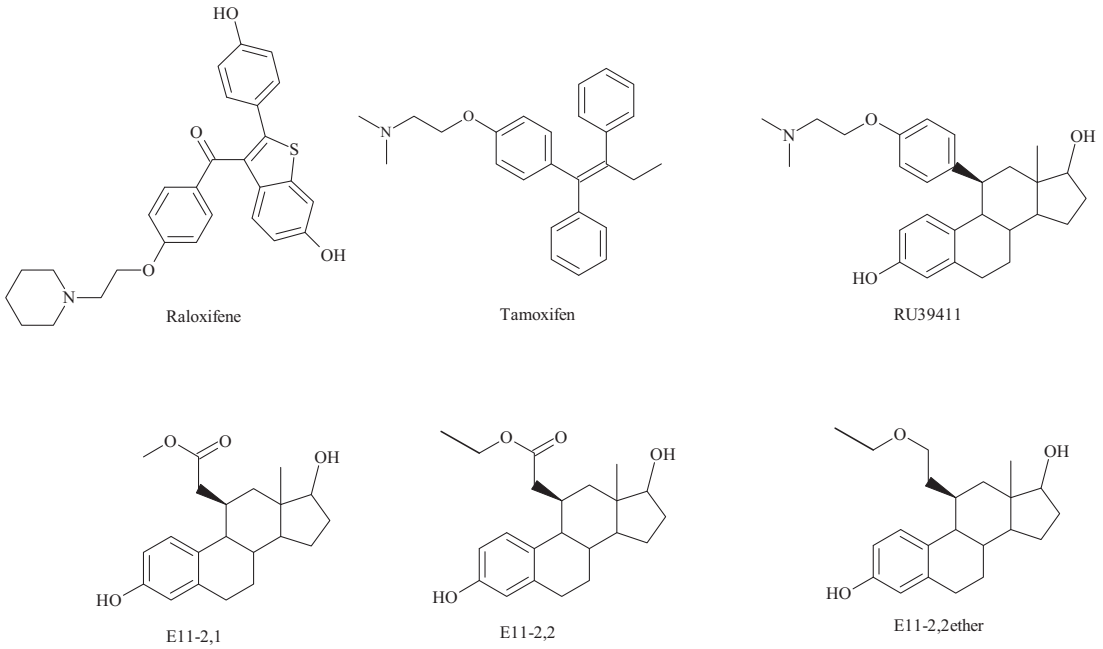


Fig. 1. The structures of the classical antiestrogens, raloxifene, tamoxifen, RU 39411; the estrogen E11-2,1 and the antiestrogens E11-2,2 and E11-2,2$_{ether}$.

system: *T*-1, hexanes/EtOAc (2:1); *T*-2, hexanes/EtOAc (1:1). Analytical high performance liquid chromatography (HPLC) was performed on a Beckman System Gold HPLC system (Beckman Coulter, Inc., Fullerton, CA) consisting of a model 126 solvent module and a model 168 diode array detector at 280 nm using the following columns and systems: with an RP-18 column (LiChrosorb RP-18, 5 μm, 4.6 mm × 25 cm, EM Science), with H-1, CH_3CN/H_2O (50/50) at 3 mL/min, 280 nm; with a Diol column (LiChrosphor Diol, 5 μm, 4.6 mm × 25 cm, EM Science) with, H-2, CH_2Cl_2 at 1 mL/min, 280 nm.

Synthesis of 3-hydroxy, 17β-hydroxy, 17α-ethinyl, 11β-substituted estradiols is shown in Scheme 1.

The synthesis of 3,17β-dibenzyloxyestra-1,3,5(10)-trien-11-one (**1**) was prepared as previously described in the literature [7]. Compound **1** was first converted to 11α-allyl-3,17β-dibenzyloxyestra-1,3,5(10)-triene-11β-ol by addition of allylmagnesium bromide. Ally group was introduced from the less sterically hindered alpha side. The hydroxyl group was then reduced by triethylsilane and $BF_3{\cdot}Et_2O$ at 0 °C to yield 11β-allyl-3,17β-dibenzyloxy estra-1,3,5(10)-triene (**2**), in which the configuration of 11-ally was inversed from 11α to 11β. Hydroboration of the terminal olefin with $LiBH_4$ and catecholborane followed by oxidation with H_2O_2 resulted in propanol compound **3** [8]. Tosylation of the hydroxyl group by p-toluenesulfonyl chloride in pyridine gave compound **4**, which was converted to the desired ethers in the next step.

To generate the 3,17-bis-protected E11-3,*i*-Pr$_{ether}$ (**5a**), isopropanol was first activated by reacting with a suspension of 35% dispersion of KH in the presence of 18-crown-6. Reacting with this anion of isopropanol in toluene at 80 °C converted tosylate (**4**) to the protected ether (**5a**), which was then deprotected by hydrogenolysis of the benzyl groups using 5% palladium on carbon under an atmosphere of H_2 to give **6a**.

The 3,17-bis-protected E11-3,*t*-Bu$_{ether}$ (**5b**) was prepared from the protected tosylate (**4**) using solid potassium *t*-butoxide instead of KH and isopropanol. Deprotection and purification as above gave **6b**.

To prepare 17α-ethinyl E11-3,*i*-Pr$_{ether}$, another strategy of protection/deprotection was employed. The 3-hydroxy group was selectively protected by *t*-butyldiphenylsilyl chloride in CH_2Cl_2 in the presence of 4-(dimethylamino)pyridine and triethylamine to give the 3-protected ether (**7a**). The 17-hydroxy group was then oxidized by pyridinium chlorochromate and sodium acetate in CH_2Cl_2 to give the 3-protected-17-keton (**8a**). Deprotection of 3-hyroxygroup by tetrabutylammonium fluroride in THF gave the 3-hydroxy-17-keton (**9a**).

Finally, to obtain 17α-ethinyl E11-3,*i*-Pr$_{ether}$ (**10a**), a solution of 18% sodium acetylide in xylenes was first added to the above deprotected ketone (**9a**) in DMSO. After reacting at r.t. for 3.5 h, the reaction was poured into saturated aqueous NH_4Cl and extracted with EtOAc. Flash chromatography gave the 17α-ethinyl E11-3,

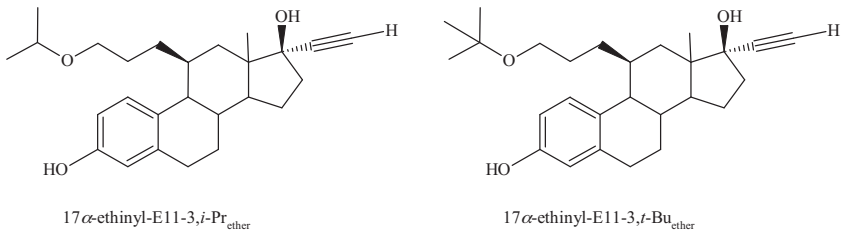
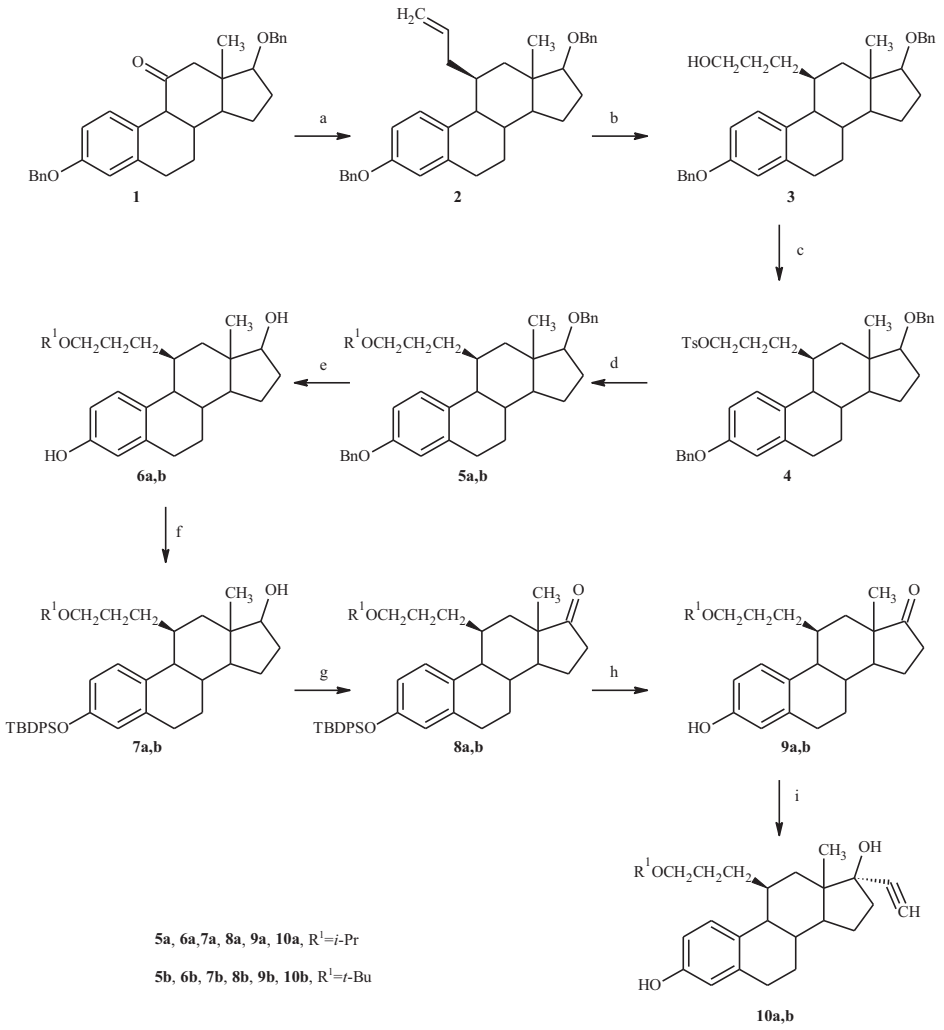


Fig. 2. The structures of 17α-ethinyl-E11-3,*i*-Pr$_{ether}$ and 17α-ethinyl-E11-3,*t*-Bu$_{ether}$.



Scheme 1. Reagents and conditions: (a): (i) allylmagnesium bromide, THF, (ii) $HSiEt_3$, $BF_3.Et_2O$, 0 °C (**1**–**2**). (b): (i) catecholborane, $LiBH_4$, THF, (ii) NaOH, H_2O_2 (**2**–**3**). (c) TsCl, Pyr, 0 °C (**3**–**4**). (d): (i) KH, 18-crown-6, ROH, toluene, r.t., (ii) **4**, 80 °C (**4**–**5a**); (iii) *t*-BuOK (**4**–**5b**). (e) 5% Pd-C, H_2, EtOAc-EtOH, r.t. (**5**–**6**). (f) *t*-BuDPSO, Et_3N, 4-Me_2N-pyridine, CH_2Cl_2, r.t. (**6**–**7**). (g) pyridinium chlorochromate (PCC), NaOAc, CH_2Cl_2, r.t. (**7**–**8**). (h) tetrabutylammonium fluroride, THF, r.t. (**8**–**9**). (I) (i) 18% sodium acetylide, xylene, r.t., (ii) NH_4Cl (**9**–**10**).

i-Pr_{ether} (**10a**). 17α-ethinyl E11-3,*t*-Bu_{ether} (**10b**) was similarly prepared according to the above procedures.

Detailed experimental procedures and data for compounds **7a**, **8a**, **9a**, **10a** and **10b** are listed in ref. [12].

2.2. Biological studies

The binding of the two 11β-substituted ethers to rat uterine cytosol ER, human Estrogen Receptor α ligand Binding Domain (hERα-LBD) and human Estrogen Receptor β ligand Binding Domain (hERβ-LBD) [9] were determined by competition for the binding of 1 nmol/L [^{3}H] E_2 as previously described [3]. Relative binding affinity (RBA) was determined by analysis of the displacement curves by the curve-fitting program Prism. The results as RBAs compared to E_2 represent the ratio of the EC_{50} of E_2 to that of the steroid analogs × 100.

The anti-estrogenic potency of the two 11β-substituted ethers were determined by the inhibition of the effect of 1 nmol/L E_2 on the estrogen sensitive marker, alkaline Phosphatase, in Ishikawa cells [10] using the procedure previously described [3]. For antagonists, the effect (Ki) of each compound tested at a range from 10^{-6} to 10^{-12} mol/L was measured for the inhibition of the action of 10^{-9} mol/L E_2 (EC_{50} = 0.2 nmol/L). Each compound was analyzed in at least three separate experiments performed in duplicate. The Ki were determined using the curve fitting program Prism.

3. Results and discussion

The biological properties of the two 11β-ethers substituted with a 17α-ethinyl group were analyzed in several different assays: The affinity of the compounds for the estrogen receptor (ER) was determined by their competition for the binding of [^{3}H]E_2: rat uterine cytosol (native ER, predominantly ERα [11]); ligand binding domain (LBD) of human ERα and the LBD of human ERβ. Anti-estrogenic potency was measured by inhibition of the stimulation of alkaline phosphatase in Ishikawa cells by 1 nmol/L E_2. The results summarized in Table 1 are compared to the previously reported results [3] of the parent compounds (unsubstituted at 17α). As shown in Table 1, these two compounds, 17α-ethinyl-E11-3,*t*-Bu_{ether} and 17α-ethinyl-E11-3,*i*-Pr_{ether}, bind very strongly to both ERα and ERβ, with relative binding affinities approximately equal to that of E_2. In each case, the 17α-ethinyl substituted compounds bound equally, or slightly weaker, than the

Table 1
Biological activities.

Compound	Rat uterine ER RBA[a]	hERα-LBD RBA[a]	hERβ-LBD RBA[a]	Inhibition Ki (nmol/L)[b]
E11-3,*i*-Pr_{ether}[c]	114 ± 25	220 ± 62	93 ± 18	0.2 ± 0.2
17α-ethinyl-E11-3,*i*-Pr_{ether}	110 ± 14	97 ± 64	116	0.09 ± 0.07
E11-3,*t*-Bu_{ether}[c]	81 ± 18	128 ± 54	82 ± 16	0.09 ± 0.06
17α-ethinyl-E11-3,*t*-Bu_{ether}	56 ± 24	117 ± 46	124	0.06 ± 0.03

[a] RBA: relative binding activity where E_2 = 100.
[b] Inhibition: effect on the estrogenic stimulation of 1 nmol/L E_2 on alkaline phosphatase activity in Ishikawa cells. Experiments were performed in duplicate in at least 3 separate experiments (with the exception of hERβ-LBD which was done only once). The values were determined using the curve fitting using program, GraphPad Prism. Values are mean ± S.D.
[c] See ref. [3].

parents. More importantly, both compounds are extremely potent anti-estrogens with potencies equal to, or greater than, that of the unsubstituted parent compounds; most likely reflecting their resistance to metabolism in the cell. Thus, it is highly likely that both compounds are promising for antiestrogen drug development.

4. Conclusion

We have generated two estradiols with 11β-*i*-PrO-propyl and 11β-*t*-BuO-propyl ethers substitutions and demonstrated their extremely strong antagonist activities.

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[12] Detailed experimental procedures and data for compounds **7a**, **8a**, **9a**, **10a** and **10b**:3-*t*-Butyldiphenylsiloxy-11β-(3-isopropoxypropyl)estra-1,3,5(10)-trien-17β-ol (**7a**). A solution of 16 mg (0.0432 mmol) of E11-3,*i*-Pr_{ether} (**6a**), *t*-butyldiphenylsilyl chloride (124 μL, 0.475 mmol), dimethylaminopyridine (10 mg, 0.08 mmol) and triethylamine (200 μL, 1.434 mmol) in CH_2Cl_2 (1 mL) was allowed to stir at r.t. overnight. The reaction was poured into H_2O (50 mL) and extracted with EtOAc (3× 50 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo giving a clear colorless oil. Purification by flash chromatography on a 2 cm × 17 cm column of silica gel using 2:1 hexanes/EtOAc as eluent gave 14.4 mg (54%) of **7a**. Data for **7a**: TLC, *T*-1, Rf 0.35.3-*t*-Butyldiphenylsiloxy-11β-(3-isopropoxypropyl)estra-1,3,5(10)-trien-17-one (**8a**). A solution of **7a**, NaOAc (1 mg) in CH_2Cl_2 (2 mL) was stirred at r.t. as PCC (8 mg, 0.035 mmol) was added. The reaction was stirred at r.t. for 2 h, poured into H_2O (30 mL) and extracted with EtOAc (3× 20 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo giving a brown film. Purification by flash chromatography on a 2× 17 cm column of silica gel using 4:1 hexanes/EtOAc as eluent gave 10.3 mg (71%) of **8a**. Data for **8a**: TLC, *T*-1, Rf 0.55.3-Hydroxy-11β-(3-isopropoxypropyl)estra-1,3,5(10)-trien-17-one (**9a**). A solution of 1 mol/L tetra-n-butylammonium fluoride in THF (1 mL) was added to **8a** (10.3 mg, 0.0169 mmol) and stirred at r.t. for 1.5 h. The reaction was poured into H_2O (70 mL) and extracted with EtOAc (3× 20 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo giving a clear colorless oil. Purification by flash chromatography on a 2 cm × 17 cm column of silica gel using 2:1 hexanes/EtOAc as eluent gave 5 mg (79%) of **9a**. Data for **9a**: TLC, *T*-1, Rf 3.75; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.05 (s, 3H, H-18), 1.11 (d, 3H, *J* = 6.1 Hz, –CH_3), 1.12 (d, 3H, *J* = 6.1 Hz, –CH_3), 2.18 (dd, 1H, *J* = 13.9, 1.6 Hz, H-12β), 2.50–2.55 (m, 1H, H-11), 2.54 (dd, 1H, *J* = 18.9, 8.6 Hz, H-16β), 2.60 (dd, 1H, *J* = 10.9, 4.6 Hz, H-9), 2.72–2.87 (m, 2H, H-6), 3.28–3.37 (m, 2H, CH_2O), 3.51 (septet, 1H, *J* = 6.1 Hz, $CH(CH_3)_2$), 4.70 (br s, 1H, OH), 6.56 (d, 1H, *J* = 2.7 Hz, H-4), 6.65 (dd, 1H, *J* = 8.5, 2.7 Hz, H-2), 7.04 (d, 1H, *J* = 8.5 Hz, H-1).11β-(3-Isopropoxypropyl)estra-17α-ethinyl-1,3,5(10)-trien-3,17β-diol (**10a**). A solution of **9a** (5 mg) in DMSO (2 mL) was stirred at r.t. as an 18% solution of sodium acetylide in xylene/mineral oil (1 mL) was added over 5 min. The reaction was stirred at r.t. for 3.5 h, poured into saturated aqueous NH_4Cl (30 mL) and extracted with EtOAc (3× 30 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo giving a yellow oil (DMSO was azeotroped off with toluene). Purification by flash chromatography on a 1 cm × 17 cm column of silica gel using 1:1 hexanes/EtOAc as eluent gave product contaminated with a nonpolar impurity. Further purification in 6 portions by semiprep HPLC (RP-18) eluting at 3 mL/min with 50/50 CH_3CN/H_2O (tR, 15 min) followed by crystallization from acetone-petroleum ether gave 3.4 mg (63%) of **10a**. Data for **10a**: TLC, *T*-1, 0.35; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.04 (s, 3H, H-18), 1.12 (d, 3H, *J* = 6.1 Hz, –CH_3), 1.13 (d, 3H, *J* = 6.1 Hz, –CH_3), 2.51–2.56 (m, 1H, H-11), 2.60 (dd, 1H, *J* = 10.5, 4.3 Hz, H-9), 2.64 (s, 1H, ethinyl-H), 2.67–2.83 (m, 2H, H-6), 3.32 (t, 2H, *J* = 6.9 Hz, CH_2O), 3.52 (septet, 1H, *J* = 6.1 Hz, –$CH(CH_3)_2$), 6.54 (d, 1H, *J* = 2.7 Hz, H-4), 6.64 (dd, 1H, *J* = 8.5, 2.7 Hz, H-2), 7.05 (d, *J* = 8.5 Hz, H-1); HPLC system: H-2, tR = 11.16 min; system H-1, tR 15 min, >99% pure.11β-(3-*t*-Butoxypropyl)estra-17α-ethinyl-1,3,5(10)-trien-3,17β-diol (**10b**). A solution of **9b** (5.3 mg, 0.01378 mmol) in DMSO (2 mL) was stirred at r.t. as an 18% solution of sodium acetylide in xylene/mineral oil (2 mL) was added dropwise over 5 min. The reaction was stirred at r.t. for 3.5 h, poured into saturated aqueous NH_4Cl (60 mL) and extracted with EtOAc (2× 50 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo (DMSO was azeotroped off with toluene). Purification by flash chromatography on a 1× 17 cm column of silica gel using 2:1 hexanes/EtOAc as eluent gave 3.7 mg of product which was further purified by semiprep HPLC using an RP-18 column eluting with 50/50 CH_3CN/H_2O giving 2.7 mg **10b**. Crystallization from acetone-petroleum ether gave 1.9 mg (33%) of **10b** as a white solid. Data for **10b**: TLC, *T*-2, Rf 0.67; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.04 (s, 3H, CH_3), 1.15 (s, 9H, *t*Bu), 2.52 (m, 1H, H-11), 2.59 (dd, 1H, *J* = 10.2, 4.9 Hz, H-9), 2.63 (s, 1H, ethinyl-H), 2.67–2.83 (m, 2H, H-6), 3.20–3.29 (m, 2H, CH_2O), 6.54 (d, 1H, *J* = 2.7 Hz, H-4), 6.64 (dd, 1H, *J* = 8.6, 2.7 Hz, H-2), 7.05 (d, 1H, *J* = 8.6 Hz, H-1); HPLC system: H-2, tR = 10.6 min, >92% pure; system: H-1, tR 17.2 min, >99% pure.