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Derivatives of 4-styrylpyridines: Synthesis, estrogen receptor binding affinity, and photophysical properties

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*In order to develop novel ligands for the estrogen receptor (ER) that might have high binding affinity and fluorescence properties suitable for assaying ER levels in cells, we have prepared a series of substituted 4'-hydroxyl-styrylpyridines and phenylethylpyridines and studied their optical spectroscopy and receptor binding properties. Several derivatives that contain alkyl substituents on the internal ethene or ethane carbons were prepared. While most of these compounds have only modest affinity for ER, one fluorescent analog, (E)-1-(4-hydroxyphenyl)-1-phenyl-2-(4-pyridinyl)ethene (**13**), has reasonably good binding affinity for ER and shows long wavelength fluorescence emission that is sensitive to solvent polarity and pH. This compound may prove to be a useful probe for detecting ER in cells.* (*Steroids* **60**:636–645, 1995)

Keywords: styrylpyridines; fluorescent estrogens; estrogen receptor binding; non-steroidal estrogens

Introduction

Fluorescent probes have been widely used for the characterization of cellular binding sites, since they enable quantitation and provide spatial resolution.[1] In this regard, there is a considerable interest in the development of fluorescent-based methods for detecting receptors for steroidal hormones, especially the estrogen receptor (ER). Such agents would permit a cell-by-cell assay of the quantity and distribution of ER in breast cancer cells,[2–6] thereby providing useful information for the prediction of response to hormonal therapy.[7]

The literature abounds with reports describing the preparation of fluorescent estrogens and their use in receptor assays; however, most of the agents described do not have optimal photophysical properties or binding affinity. Thus, reagents of the conjugate design, with a ligand linked to a fluorophore, generally have low affinity for the receptor and high nonspecific binding,[2,3] while inherently fluorescent ligands, in which the fluorochrome is encompassed within the structure of the ligand, have usually suffered from suboptimal fluorescence and binding characteristics.[4,5]

Stilbene structures which contain electron donor-acceptor systems with long-wavelength emission bands have been incorporated in ER ligands such as diethylstilbestrol and nafoxidine, and they present an intriguing case.[8,9] The bulky substituents on the stilbene skeleton, which are required for high receptor binding affinity in this series of compounds, cause a twisting about the aryl-ethene bond; this interrupts the conjugation, reducing the absorbance and compromising the fluorescence emission characteristics, since optimal fluorescence requires a planar stilbene chromophore.[6] Earlier studies have established that the introduction of a nitrogen atom into the phenyl ring of stilbenes (to give a styrylpyridine system) significantly affects their photophysical and photochemical behavior, because of the involvement of the (n,π*) state.[10,11] Furthermore, the fluorescence of these compounds shows high environmental sensitivity, with solvents of different polarity or alteration in pH causing substantial changes in emission intensity and/or wavelength.[12] This characteristic is often crucial for the development of fluorescent probes for biological systems, since the observation of spectral changes upon the interaction of the fluorophore with a biological molecule provides a new dimension of sensitivity and selectivity.[13] Thus, we were intrigued by the possibility of incorporating nitrogen heteroatoms into several non-steroidal estrogen and antiestrogen structures, and the consequences that this would have on their photophysical properties and binding affinity to ER.

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In this report we describe the synthesis of a series of stilbene and deoxyhexestrol analogues which contain in their structural framework the 4-pyridinyl moiety instead of the phenyl group (**7, 8, 10ab, 11ab, 13, 14;** see Figure 1). We have also measured the binding affinity of these novel compounds with the ER and studied the photophysical properties of the most potent ones. Among these compounds is one **(13)** whose ER binding affinity and fluorescence behavior make it a likely candidate for the detection of ER in cells.

Experimental

General Methods

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Reaction progress was followed with analytical thin-layer chromatography (TLC) performed on 0.25 mm silica gel precoated plastic plates with fluorescent indicator UV_{254} (Merck). All column chromatography was done by the flash chromatography technique using 32-63 μm silica gel packing (Merck).[14] Proton magnetic resonance (^1H NMR) spectra were recorded on either a General Electric QE 300 (300 MHz) or a Varian Unity 400 (400 MHz) spectrometer in the indicated solvents. Chemical shifts are reported in parts per million downfield from tetramethylsilane as an internal standard (δ scale). The number that specifies the positions of protons on aromatic rings is given relative to the site of attachment of the ring to the ethane or ethylene core of the molecule, rather than to the functionality (phenolic hydroxyl or pyridinyl nitrogen) on the ring (see structure **7**). Electron impact spectral data were obtained either on a Varian MAT CH-5 mass spectrometer at 70 eV (low resolution) or on a Varian 731 high-resolution mass spectrometer. Both low and high resolution chemical ionization mass spectra were recorded on a VG Analytical 70-VSE mass spectrometer using methane as the reagent gas. Data are presented in the form m/z (intensity relative to base peak 100). Microanalytical data were provided by the Microanalytical Service Laboratory of the University of Illinois.

All starting materials and solvents were purchased from Aldrich. The γ-picoline condensation products (*R,S*)-1-(4-methoxyphenyl)-2-(4-pyridinyl)ethanol **(2a)** and (*R,S*)-1-(4-benzyloxyphenyl)-2-(4-pyridinyl)ethanol **(2b)** were prepared according to a previously reported method.[12] Tetrahydrofuran (THF) was distilled from sodium-benzophenone immediately prior to use. Methylene chloride was distilled over CaH_2 prior to use. Dimethylformamide (DMF) was dried over 4-Å molecular sieves.

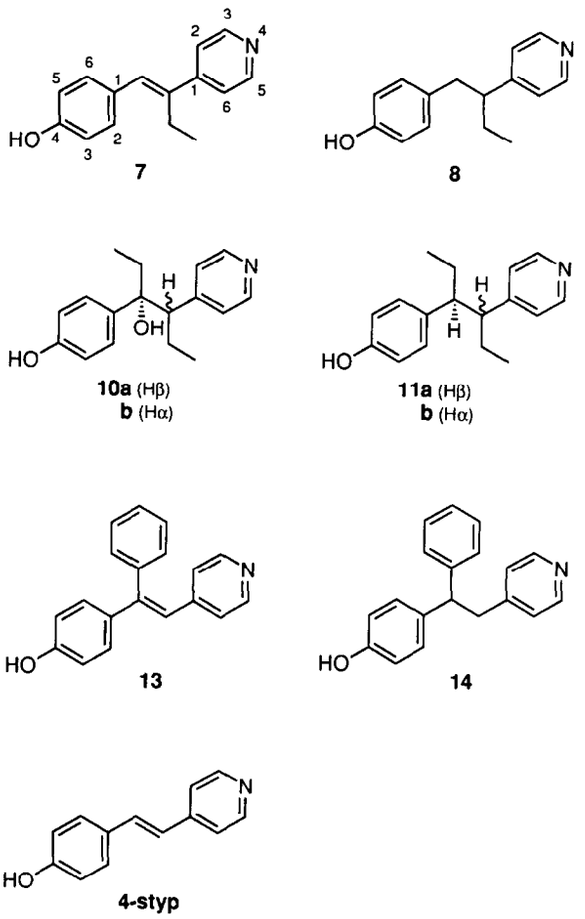


Figure 1

Chemical synthesis

4'-Methoxy-2-(4-pyridinyl)acetophenone (3a). To a stirred solution of compound **2a** (3.2 g, 14 mmol) in 250 mL acetone/water (9:1, vol/vol) at −15°C, 4 mL of Jones reagent[15] was added dropwise. After stirring for 1.5 h at that temperature, the solution was neutralized with a saturated solution of Na_2CO_3, and the green solid inorganic byproduct [$Cr_2(SO_4)_3$] was separated by filtration. The filtrate was evaporated under reduced pressure, and the resulting slurry was partitioned between ethyl acetate and water. The organic layer was separated, washed with brine, dried over Na_2SO_4, and evaporated to dryness to give a yellowish solid, which was decolorized by washing with cold diethyl ether. Recrystallization from ethyl acetate furnished 2.95 g (93% yield) of the analytically pure product as white needles; mp 100–101°C; ^1H NMR ($CDCl_3$) δ, *pyridinyl:* 8.55 (dd, J = 5.7 and 1.2 Hz, 2 H, H_3, H_5), 7.20 (dd, J = 5.7 and 1.2 Hz, 2 H, H_2, H_6); *phenyl:* 7.97 (d, J = 8.9 Hz, 2 H, H_2, H_6), 6.95 (d, J = 8.9 Hz, 2 H, H_3, H_5); 4.24 (s, 2 H, CH_2), 3.88 (s, 3 H, CH_3O); MS (EI) m/z 227 (M^+, 3.1), 136 (8.7), 135 (100), 107 (7.9), 92 (9.9), 77 (12.8); Analysis Calculated for $C_{14}H_{13}NO_2$ (227.25): C, 73.99; H, 5.77; N, 6.16. Found: C, 73.97; H, 5.76; N, 6.15.

4'-Benzyloxy-2-(4-pyridinyl)acetophenone (3b). Compound **2b** (3 g, 10 mmol) was treated with Jones reagent by the procedure described for compound **3a** to give 2.83 g (95% yield) of **3b;** m.p. 165–166°C; ^1H NMR (CD_3COCD_3) δ, *pyridinyl:* 8.50 (d, J = 5.6 Hz, 2 H, H_3, H_5), 7.29 (d, J = 5.6 Hz, 2 H, H_2, H_6); *phenyl:* 8.06 (d, J = 8.8 Hz, 2 H, H_2, H_6), 7.44–7.34 (m, 5 H), 7.14 (d, J = 8.8 Hz, 2 H, H_3, H_5); 5.25 (s, 2 H, CH_2O), 4.39 (s, 2 H, CH_2); MS (EI) m/z 303 (M^+, 3.2), 211 (20), 183 (8.3), 92 (10.7), 91 (100), 65 (11.5); Analysis Calculated for $C_{20}H_{17}NO_2$ (303.35): C, 79.18; H, 5.65; N, 4.62. Found: C, 79.15; H, 5.62; N, 4.58.

4'-Methoxy-2-(4-pyridinyl)butyrophenone (4a). To a stirred solution of sodium hydride (0.4 g of 60% dispersion in oil, 11 mmol) in 15 mL dry DMF under an N_2 atmosphere, a solution of compound **3a** (2.27 g, 10 mmol) in 15 mL dry THF was added dropwise. After being stirred for 4h, ethyl bromide (0.85 mL, 11 mmol) was added and the mixture was stirred for an additional 20 h. A saturated solution of NH_4Cl was added to quench the reaction mixture, and the product was extracted twice with ethyl acetate. The combined organic layers were evaporated under vacuum, and the resulting red oil was chromatographed (ethyl acetate/hexane 3:1, v/v) to give the desired product as a colorless oil, which solidified in the freezer. Recrystallization from ether/petroleum

ether furnished 1.96 g of the analytically pure product as off white needles (yield 77%); m.p. 73–74°C; ^{1}H NMR ($CDCl_3$) δ, *pyridinyl:* 8.51 (dd, J = 5.8 and 1.4 Hz, 2 H, H_3, H_5); 7.25 (dd, J = 5.8 and 1.4 Hz, 2 H, H_2, H_6); *phenyl:* 7.94 (dd, J = 8.9 and 1.8 Hz, 2 H, H_2, H_6), 6.90 (dd, J = 8.9 and 1.8 Hz, 2 H, H_3, H_5); 4.42 (t, J = 7.1 Hz, 1 H, H_2), 3.83 (s, 3 H, CH_3O), 2.21 (ddq, J = 13.9, 7.3, and 7.1 Hz, 1 H, H_3), 1.85 (ddq, = 13.9, 7.3 and 7.1 Hz, 1 H, H_3, 0.90 (t, J = 7.3 Hz, 3 H, H_4); MS (EI) m/z 256 (M^+ + 1, 1.4), 255 (M^+, 7.5), 136 (8.7), 135 (100), 107 (6.9), 92 (9.1), 77 (13.1); Analysis Calculated for $C_{16}H_{17}NO_2$ (255.31): C, 75.27; H, 6.71; N, 5.49. Found: C, 75.29; H, 6.72; N, 5.50.

4'-Benzyloxy-2-(4-pyridinyl)butyrophenone (4b). Prepared on a 10 mmol scale from compound **3b** as white needles by the procedure described for compound **4a** (yield 80%); m.p. 109–110°C; ^{1}H NMR ($CDCl_3$) δ, *pyridinyl:* 8.51 (dd, J = 5.7 and 1.3 Hz, 2 H, H_3, H_5), 7.24 (dd, J = 5.7 and 1.3 Hz, 2 H, H_2, H_6); *phenyl:* 7.94 (d, J = 8.8 Hz, 2 H, H_2, H_6), 7.36 (m, 5 H), 6.96 (d, J = 8.8 Hz, 2 H, H_3, H_5); 5.09 (s, 2 H, CH_2O), 4.40 (t, J = 7.3 Hz, 1 H, H_2), 2.17 (ddq, J = 13.9, 7.3, and 7.2 Hz, 1 H, H_3), 1.86 (ddq, J = 13.9, 7.3 and 7.2 Hz, 1 H, H_3), 0.90 (t, J = 7.2 Hz, 3 H, H_4); MS (EI) m/z 331 (M^+, 3.9), 211 (38.4), 92 (11.8), 91 (100), 65 (10); Analysis Calculated for $C_{22}H_{21}NO_2$ (331.34): C, 79.73; H, 6.39; N, 4.23. Found: C, 79.74; H, 6.38; N, 4.26.

1-(4-Methoxyphenyl)-2-(4-pyridinyl)butanols (5 and 6). To a stirred solution of compound **4a** (0.51 g, 2 mmol) in 30 mL dry THF, under an N_2 atmosphere, $LiAlH_4$ (135 mg, 3.5 mmol) was added in small portions. After being stirred for 10 h the reaction mixture was quenched with a saturated solution of NH_4Cl and filtered through Celite. The filtrate was evaporated to dryness, and the resulting solid was chromatographed (ethyl acetate/hexane, 9:1, v/v) to give 0.39 g (75% yield) of **5** as the major product (more polar diaseteromer, R_f = 0.13 in ethyl acetate); m.p. 108–109°C; ^{1}H NMR ($CDCl_3$) δ, *pyridinyl:* 8.42 (d, J = 5.5 Hz, 2 H, H_3, H_5), 7.12 (d, J = 5.5 Hz, H_2, H_6); *phenyl:* 7.17 (d, J = 8.6 Hz, 2 H, H_2, H_6), 6.85 (d, J = 8.6 Hz, 2 H, H_3, H_5); 4.75 (d, J = 7.7 Hz, 1 H, H_1), 3.78 (s, 3 H, CH_3O), 2.85 (br, 1 H, OH), 2.72 (m, 1 H, H_2), 1.54 (m, 2 H, H_3), 0.66 (t, J = 7.4 Hz, 3 H, H_4); MS (CI) m/z 258 (MH^+, 35.1), 240 (68.9), 150 (38.8), 137 (37.8), 122 (84.3), 57 (100); HRMS for $C_{16}H_{20}NO_2$ (MH^+) calculated 258.1494; found 258.1493.

The minor (less polar) diastereomer (**6**); R_f = 0.22 in ethyl acetate (yield 17%); m.p. 100–102°C; ^{1}H NMR ($CDCl_3$) δ, *pyridinyl:* 8.21 (dd, J = 5.6 and 1.1 Hz, 2 H, H_3, H_5), 6.90 (dd, J = 5.6 and 1.1 Hz, H_2, H_6); *phenyl:* 6.98 (dd, J = 8.6 and 1.8 Hz, 2 H, H_2, H_6), 6.70 (dd, J = 8.6 and 1.8 Hz, H_3, H_5); 4.68 (d, J = 7 Hz, 1 H, H_1), 4.15 (br, 1 H, OH), 3.72 (s, 3 H, CH_3O), 2.78 (m, 1 H, H_2), 2.08 (m, 1 H, H_3), 1.68 (m, 1 H, H_3), 0.72 (t, J = 7.3 Hz, 3 H, H_4); MS (CI) m/z 258 (MH^+, 18.6), 242 (11.3), 240 (36.3), 150 (35.3), 122 (65.1), 109 (10.5), 57 (100); HRMS for $C_{16}H_{20}NO_2$ (MH^+) calculated 258.1494; found 258.1489.

(*E*)-1-(4-Hydroxyphenyl)-2-(4-pyridinyl)but-1-ene (7). To a (4.5:1) mixture of diastereomers **5** and **6** (0.31 g, 1.2 mmol) dissolved in 25 mL dry methylene chloride and cooled at 0°C, under an N_2 atmosphere, 3 mL of boron trifluoride-dimethylsulfide complex was added dropwise with stirring. The reaction was allowed to reach room temperature and was stirred for 36 h. The resulting precipitate was separated by decantation and dissolved in 10 mL water. A saturated solution of $NaHCO_3$ was added (pH ~7.5), and the product was extracted twice with ethyl acetate. The organic layers were combined and the solvent was evaporated under reduced pressure. The resulting solid was dissolved in 15 mL MeOH and 3 mL HCl and refluxed for 8 h. Then the solvent was removed in vacuo, and the remaining slurry was dissolved in water and neutralized with a saturated solution of $NaHCO_3$. The product was extracted twice with ethyl acetate, and the combined organic layers were dried over Na_2SO_4 and evaporated to dryness to give a solid which was chromatographed (ethyl acetate/hexane 4:1, v/v), yielding 247 mg (91% total yield) of the desired product as an off-white solid; m.p. 178–179°C; ^{1}H NMR (DMSO-d_6) δ, *pyridinyl:* 8.52 (dd, J = 5.9 and 1.2 Hz, 2 H, H_3, H_5), 7.47 (dd, J = 5.9 and 1.2 Hz, 2 H, H_2, H_6); *phenyl:* 7.23 (d, J = 8.6 Hz, 2 H, H_2, H_6), 6.80 (d, J = 8.6 Hz, 2 H, H_3, H_5); 6.89 (s, 1 H, H_1), 2.72 (q, J = 7.4 Hz, 2 H, H_3), 1.04 (t, J = 7.4 Hz, 3 H, H_4); MS (EI) m/z 227 (M^+ + 2, 1.7), 226 (M^+ + 1, 15.8), 225 (M^+, 100), 210 (73.7), 196 (9.1), 182 (7.1), 119 (10.8), 117 (15), 107 (17.9), 77 (11.9); Analysis Calculated for $C_{15}H_{15}NO$ (225.28): C, 79.97; H, 6.71; N, 6.22. Found: C, 79.93; H, 6.72, N, 6.21.

(*R*,S)-1-(4-Hydroxyphenyl)-2-(4-pyridinyl)butane (8).** A solution of compound **7** (0.2 g, 0.9 mmol) in 30 mL methanol/acetic acid (1:1, vol/vol) was hydrogenated over 30 mg Pd/C (5%) at room temperature and 1 bar pressure for 5 h. Then the catalyst was removed by filtration, and the filtrate was evaporated producing a slurry, which was dissolved in water and neutralized with a saturated solution of $NaHCO_3$. The product was extracted twice with ethyl acetate, and the combined organic layers were dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The resulting colorless oil solidified upon standing in high vacuum. Recrystallization from methanol/petroleum ether gave 196 mg (97% yield) of the analytically pure material as white crystals; m.p. 114–115°C; ^{1}H NMR ($CDCl_3$) δ, *pyridinyl:* 8.40 (d, J = 5.9 Hz, 2 H, H_3, H_5), 7.05 (d, J = 5.9 Hz, 2 H, H_2, H_6); *phenyl:* 6.78 (d, J = 8.7 Hz, 2 H, H_2, H_6), 6.72 (d, J = 8.7 Hz, 2 H, H_3, H_5); 2.85 (m, 1 H, H_2), 2.70 (m, 2 H, H_1), 1.76 (m, 1 H, H_3), 1.63 (m, 1 H, H_3), 0.76 (t, J = 7.3 Hz, 3 H, H_4); MS (EI) m/z 228 (M^+ + 1, 2.2), 227 (M^+, 10.2), 121 (73.8), 107 (100), 106 (81.4), 78 (10.3), 77 (18.7), 57 (30.4), 43 (26), 41 (12.9); Analysis Calculated for $C_{15}H_{17}NO$ (227.30): C, 79.26; H, 7.54; N, 6.16. Found: C, 79.20; H, 7.62; N, 6.13.

(*3R*,4S)-3-(4-Benzyloxyphenyl)-4-(4-pyridinyl)hexan-3-ol (9a) and (*3R*,4R**)-3-(4-Benzyloxyphenyl)-4-(4-pyridinyl)hexan-3-ol (9b).** To a stirred solution of compound **4b** (1.22 g, 4 mmol) in 50 mL dry THF, under an N_2 atmosphere, 10 mL (20 mmol) of EtMgCl (2 M solution in diethylether) were added. The mixture was stirred for 2 h, then quenched with a saturated solution of NH_4Cl, and the product was extracted twice with ethyl acetate. The combined organic layers were evaporated under vacuum to produce a white solid, which was chromatographed (ethyl acetate/hexane 3:1, v/v) to give 0.72 g (54% yield) of a white crystalline product, identified as the 3*R**,4*R**) diastereomer (less polar, R_f = 0.32 with ethyl acetate) and 0.32 g (24% yield) of another white crystalline product identified as the (3*R**,4*S**) diastereomer (**9b,** more polar, R_f = 0.25 with ethyl acetate).

Physical and spectral data for the major (less polar) (3*R**,4*R**) diastereomer (**9b**) are as follows; m.p. 150–151°C; ^{1}H NMR ($CDCl_3$) δ, *pyridinyl:* 8.30 (dd, J = 5.8 and 1.5 Hz, 2 H, H_3, H_5), 6.88 (dd, J = 5.8 and 1.5 Hz, 2 H, H_2, H_6); *phenyl:* 7.38 (m, 5 H, *phenyl*), 7.04 (dd, J = 8.8 and 1.8 Hz, 2 H, H_2, H_6), 6.86 (dd, J = 8.8 and 1.8 Hz, 2 H, H_3, H_5); 5.01 (s, 2 H, CH_2O), 2.75 (dd, J = 11.9 and 3.1 Hz, H_4), 2.26 (s, 1 H, OH), 2.08 (m, 1 H, H_5), 1.92 (q, J = 7.3 Hz, 2 H, H_2), 1.65 (m, 1 H, H_5), 0.73 (t, J = 7.3 Hz, 3 H, H_1), 0.65 (t, J = 7.3 Hz, 3 H, H_6); MS (CI) m/z 362 (MH^+, 7.1), 344 ($MH^+ - H_2O$, 18.7), 241 (38.4), 178 (17.2), 150 (19.4), 12 (100), 91 (28.9); Analysis Calculated for $C_{24}H_{27}NO_2$ (361.47): C, 79.74; H, 7.53; N, 3.88. Found: C, 79.59; H, 7.56; N, 3.89.

The minor (more polar; 3*R**,4*S**) diastereomer (**9a**) has: m.p. 162–163°C; ^{1}H NMR ($CDCl_3$) δ, *pyridinyl:* 8.49 (d, J = 5.7 Hz, 2 H, H_3, H_5), 7.19 (d, J = 5.7 Hz, H_2, H_6); *phenyl:* 7.41 (m, 5H,

phenyl), 7.28 (d, J = 8.8 Hz, 2 H, H_2, H_6), 6.97 (d, J = 8.8 Hz, 2 H, H_3, H_5); 5.07 (s, 2 H, CH_2O), 2.75 (dd, J = 11.8 and 2 Hz, 1 H, H_4), 1.73 (m, 2 H, H_2) 1.45 (m, 2 H, H_5), 0.56 (t, J = 7.3 Hz, 3 H, H_1), 0.54 (t, J = 7.3 Hz, 3 H, H_6); MS (CI) m/z 362 (MH^+, 6.6), 344 ($MH^+ - H_2O$, 5.8), 316 (6), 241 (37.4), 178 (22.2), 150 (24.1), 122 (100), 91 (33); Analysis Calculated for $C_{24}H_{27}NO_2$ (361.47): C, 79.74; H, 7.53; N, 3.88. Found: C, 79.76; H, 7.54; N, 3.89.

(*3R*,4S)-3-(4-Hydroxyphenyl)-4-(4-pyridinyl)hexan-3-ol (10a).** Compound **9a** (0.18 g, 0.5 mmol) was hydrogenated over 20 mg Pd/C (5%) as described for compound **7** to give 0.13 g (95% yield) of white crystalline product; m.p. 183–185°C; ^{1}H NMR (CD_3COCD_3) δ, *pyridinyl:* 8.41 (d, J = 5.1 Hz, 2 H, H_3, H_5), 7.39 (d, J = 5.1 Hz, 2 H, H_2, H_6); *phenyl:* 7.21 (d, J = 8.5 Hz, H_2, H_6), 6.76 (d, J = 8.5 Hz, H_3, H_5); 8.04 (br, 1 H, OH), 2.88 (dd, J = 11.8 and 2.7 Hz, 1 H, H_4), 2.30 (br, 1 H, OH), 1.73 (m, 2 H, H_2), 1.54 (m, 1 H, H_5), 1.34 (m, 1 H, H_5), 0.55 (t, J = 7.3 Hz, 6 H, H_1, H_6); MS (CI) m/z 273 (MH^+ + 1, 3.8), 272 (MH^+, 16.6), 254 ($MH^+ - H_2O$, 15.1), 178 (15.9), 151 (71.5), 150 (23.5), 122 (100), 57 (12.4); Analysis Calculated for $C_{17}H_{21}NO_2$ (271.35): C, 75.24; H, 7.80; N, 5.16. Found: C, 75.09; H, 7.96; N, 5.08.

(*3R*,4R)-3-(4-Hydroxyphenyl)-4-(4-pyridinyl)hexan-3-ol (10b).** Compound **9b** (0.36 g, 1 mmol) was hydrogenated over 40 mg Pd/C (5%) as described for compound **7** to give 0.25 g (93% yield) of white crystalline product; m.p. 154–156°C; ^{1}H NMR ($CDCl_3$) δ, *pyridinyl:* 8.25 (d, J = 5.7 Hz, 2 H, H_3, H_5), 6.92 (m, 2 H, H_2, H_6); *phenyl:* 6.92 (m, 2 H, H_2, H_6), 6.69 (d, J = 8.7 Hz, 2 H, H_3, H_5); 10.40 (br, 1 H, OH), 2.77 (dd, J = 11.6 and 2.6 Hz, 1 H, H_4), 2.13 (m, 1 H, H_5), 2.07 (br, 1 H, OH), 1.93 (m, 2 H, H_2), 1.63 (m, 1 H, H_5), 0.73 (t, J = 7.3 Hz, 3 H, H_1), 0.64 (t, J = 7.3 Hz, 3 H, H_6); MS (CI) m/z 273 (MH^+ + 1, 10.6), 272 (MH^+, 42), 254 (16.9), 178 (18.4), 151 (28.8), 150 (17.7), 122 (69.2), 59 (100); Analysis Calculated for $C_{17}H_{21}NO_2$ (271.35): C, 75.24; H, 7.80; N, 5.16. Found: C, 74.91; H, 7.67; N, 5.04.

(*3R*,4S)-3-(4-Hydroxyphenyl)-4-(4-pyridinyl)hexane (11a) and (*3R*,4R**)-3-(4-hydroxyphenyl)-4-(4-pyridinyl)hexane (11b).** A (1:2) mixture of diastereomers **9a** and **9b** (1.45 g, 4 mmol) was dissolved in 25 mL methanol and 5 mL HCl and refluxed for 7 h. The solvent was evaporated under vacuum, and the remaining slurry was dissolved in water and neutralized with a saturated solution of $NaHCO_3$. The product was extracted twice with ethyl acetate, and the combined organic layers were evaporated to dryness, resulting in a slurry which proved to be composed of several dehydration products. The two major products were identified as an equimolar mixture of *E/Z* 3,2-dehydration products, while the minor product was identified as an *E/Z* (8:1 by ^{1}H NMR) mixture produced from 3,4-dehydration. Subsequent hydrogenation of the crude mixture containing all the above products by the procedure described for compound **7** furnished a white solid, which was chromatographed (ethyl acetate/hexane 3:1, v/v) to give 0.65 g (yield 64%) of a white crystalline product identified as the (3*R**,4*S**) diastereomer **11a** (less polar, R_f = 0.33 in ethyl acetate) and 0.29 g (28% yield) of another white crystalline product identified as the (3*R**,4*R**) diastereomer **11b** (more polar, R_f = 0.30 in ethyl acetate).

Physical and spectral data for the major (less polar) product (**11a** (3*R**,4*S**)) are as follows: m.p. 177–178°C; ^{1}H NMR (DMSO-d_6) δ, *pyridinyl:* 8.46 (d, J = 4.9 Hz, 2 H, H_3, H_5), 7.23 (d, J = 4.9 Hz, 2 H, H_2, H_6); *phenyl:* 6.99 (d, J = 8.2 Hz, 2 H, H_2, H_6), 6.70 (d, J = 8.2 Hz, 2 H, H_3, H_5); 9.19 (s, 1 H, OH), 2.51 (m, 2 H, H_3, H_4), 1.25 (m, 2 H, H_5), 1.14 (m, 2 H, H_2), 0.44 (t, J = 7 Hz, 3 H, H_6), 0.42 (t, J = 7 Hz, 3 H, H_1); MS (EI) m/z 255 (M^+, 3.2), 135 (56.5), 121 (100), 120 (13.4), 107 (73), 106 (67.6), 93 (21.9), 92 (12.5), 77 (14), 43 (18.2), 41 (23.6); Analysis Calculated for $C_{17}H_{21}NO$ (255.35): C, 79.96; H, 8.29; N, 5.49. Found: C, 79.90; H, 8.39; N, 5.42.

The minor (more polar) diastereomer (**11b** (3*R**,4*R**)) has: m.p. 173–174°C; ^{1}H NMR ($CDCl_3$) δ, *pyridinyl:* 8.29 (d, J = 5.9 Hz, 2 H, H_3, H_5), 6.81 (d, J = 5.9 Hz, 2 H, H_2, H_6); *phenyl:* 6.60 (s, 4 H, H_2, H_3, H_5, H_6); 10.19 (br_s, 1 H, OH), 2.65 (m, 2 H, H_3, H_4), 1.90 (m, 2 H, H_5), 1.57 (m, 2 H, H_2), 0.76 (t, J = 7.2 Hz, 3 H, H_6), 0.74 (t, J = 7.2 Hz, 3 H, H_1); MS (EI) m/z 255 (M^+, 3.1), 135 (46.7), 121 (100), 107 (60.5), 106 (71.2), 91 (7.1), 77 (10.7), 41 (14.4); Analysis Calculated for $C_{17}H_{21}NO$ (255.35): C, 79.96; H, 8.29; N, 5.49. Found: C, 79.92; H, 8.30; N, 5.48.

(*R*,S)-1-(4-Methoxyphenyl)-1-phenyl-2-(4-pyridinyl)ethanol (12).** To a stirred solution of compound **3a** (0.61 g, 2.7 mmol) in 30 mL dry THF, under an N_2 atmosphere, 1.9 mL (3 mmol) of phenyllithium (1.6 M solution in cyclohexane-ether) was added dropwise. After being stirred for 15 h, the reaction mixture was quenched by addition of 30 mL of a saturated solution of NH_4Cl, and the product was extracted twice with ethyl acetate. The combined organic layers were evaporated to dryness and chromatographed (ethyl acetate/hexane 4:1, v/v) to give 0.25 g (30% yield) of the desired product (R_f = 0.23 with ethyl acetate), along with unreacted starting material (45% recovered) and an unidentified polyphenyl byproduct. Recrystallization from methylene chloride/petroleum ether afforded the title product as white crystals; m.p. 156–157°C; ^{1}H NMR ($CDCl_3$) δ, *pyridinyl:* 8.32 (d, J = 5.5 Hz, 2 H, H_3, H_5, *phenyl:* 7.43–7.24 (m, 7 H, H_2, H_6), *phenyl:* 6.82 (m, 4 H, H_3, H_5), 3.79 (s, 3 H, CH_3O), 3.57 (s, 2 H, H_2), 3.42 (br, 1 H, OH); MS (CI) m/z 306 (MH^+, 100), 288 ($MH^+ - H_2O$, 68), 228 (25), 213 (67), 198 (36), 122 (33), 94 (84); Analysis Calculated for $C_{20}H_{19}NO_2$ (305.36): C, 78.66; H, 6.27; N, 4.59. Found: C, 78.67; H, 6.26; N, 4.57.

(*E*)-1-(4-Hydroxyphenyl)-1-phenyl-2-(4-pyridinyl)ethene (13). A solution of compound **12** (0.15 g, 0.5 mmol) in 12 mL dry methylene chloride was treated with 1.4 mL boron trifluorine-dimethylsulfide complex as described for compound **7.** Workup of the reaction mixture gave 0.12 g (92% yield) of the deprotected/dehydrated product as 2.5:1 mixture (by ^{1}H NMR) of the *E/Z* diastereomers. Chromatographic separation (3:1 ethyl acetate/hexane, v/v) and recrystallization from methylene chloride-petroleum ether resulted in the separation of the pure *E* isomer; m.p. 199–200°C; ^{1}H NMR (DMSO-d_6) δ, *pyridinyl:* 8.26 (d, J = 5.9 Hz, 2 H, H_3, H_5), 6.80 (d, J = 5.9 Hz, 2 H, H_2, H_6); *phenyl:* 7.10–7.44 (m, 5 H), 7.14 (d, J = 8.8 Hz, 2 H, H_2, H_6), 6.73 (d, J = 8.8 Hz, 2 H, H_3, H_5); 9.69 (br, 1 H, OH), 6.94 (s, 1 H, H_2); MS (CI) m/z 276 (MH^+ + 2, 3), 275 (MH^+ + 1, 23.2), 274 (MH^+, 100), 273 (28.2), 196 (12), 180 (10.7); Analysis Calculated for $C_{19}H_{15}NO$ (273.32): C, 83.49; H, 5.53; N, 5.12. Found: C, 83.62; H, 5.42; N, 5.01.

(*R,S*)-1-(4-Hydroxyphenyl)-1-phenyl-2-(4-pyridinyl)ethane (14). Compound **13** (0.14 g, 0.5 mmol) was hydrogenated over 20 mg Pd/C (5%) as described for compound **7,** affording 135 mg (96% yield) of a colorless oil, which solidified upon standing in the freezer; m.p. 147–149°C; ^{1}H NMR ($CDCl_3$) δ, *pyridinyl:* 8.35 (dd, J = 5.8 and 1.4 Hz, 2 H, H_3, H_5), 7.28–6.95 (m, 10 H, OH and aromatic), *phenyl:* 6.69 (d, J = 8.6 Hz, 2 H, H_3, H_5), 3.92 (m, 1 H, H_2), 3.07 (dd, J = 13.1 and 1.1 Hz, 2 H, H_2); MS (EI) m/z 275 (M^+, 2.8), 183 (100), 165 (16.2), 153 (8.1), 115 (5.3), 93 (58.8), 55 (8.3), 39 (7.8); Analysis Calculated for $C_{19}H_{17}NO$ (275.34): C, 82.88; H, 6.23; N, 5.09. Found: C, 82.85; H, 6.43; N, 4.74.

Biological methods

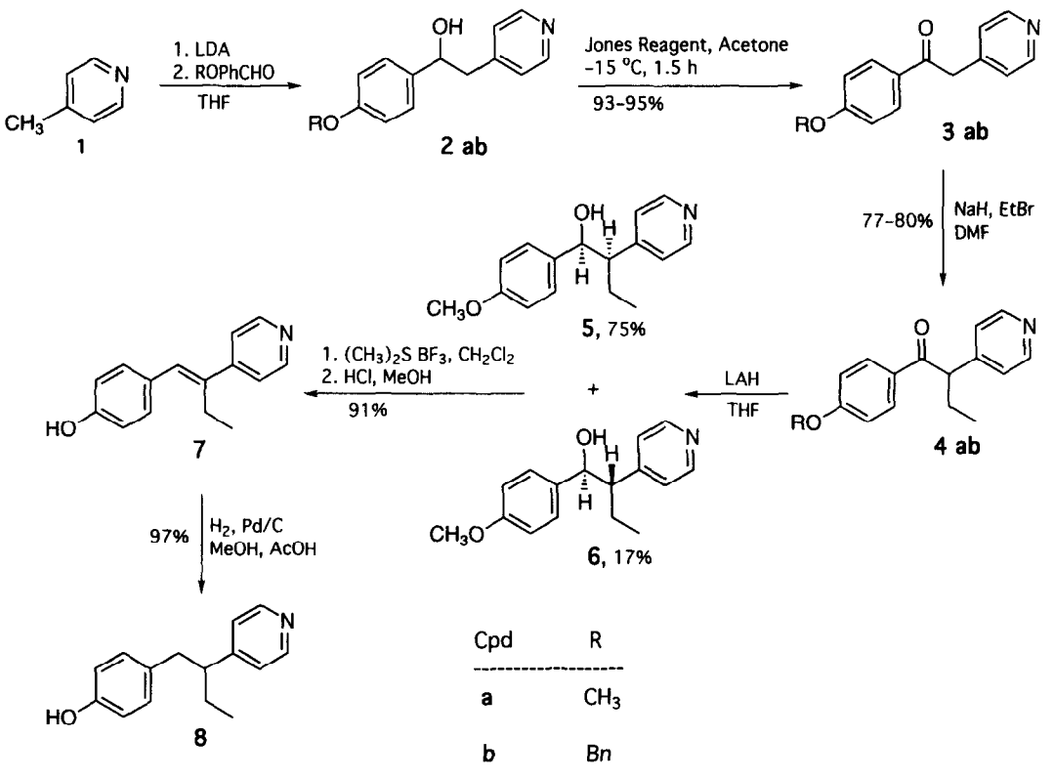
Determination of receptor binding affinity (RBA). Relative binding measurements were performed as previously reported,[16] using lamb uterine cytosol, diluted to ~1.5 nM receptor. The protein solution was incubated with buffer or several concentrations of unlabeled competitor together with 10 nM [^{3}H]estradiol at 0°C for 18–24 h. The unlabeled competitor was diluted in 1:1 (v/v) dimethylformamide/TEA buffer (10 mM Tris, 1.5 mM EDTA, 3 mM sodium azide, pH 7.4 at 25°C) to ensure solubility. All data are reported relative to estradiol = 100%.

UV-Vis and fluorescence spectra. Ultraviolet-visible (UV-vis) spectra were recorded on a Hewlett-Packard 8450A Diode Array spectrophotometer. Fluorescence spectra were acquired by photon counting on a Spex Fluorolog 2 Spectrophotometer (Model 111C) with a Datamate microprocessor using four 1.25 mm slits. All spectra were recorded at room temperature and are corrected for phototube sensitivity and by subtraction of the solvent background. Excitation was at the wavelength of maximum absorbance. Samples were prepared from stock solution (10^{-3} M) of the corresponding compounds in EtOH, giving final concentrations of 10^{-5} M and 5×10^{-6} M for UV-vis and fluorescence, respectively. Acidic or basic solutions were prepared by addition of concentrated HCl or a 6N KOH solution in water, to give a final concentration of 0.1N.

Results and discussion

Chemical synthesis

The approach to the monoethyl functionalized 4-styrylpyridine derivatives **7** and **8** is shown in Scheme 1. The anion generated by treatment of γ-picoline (**1**) with lithium diisopropylamide (LDA) was allowed to react with 4-methoxy- (or 4-benzyloxy-) benzaldehyde, as previously described.[12] The resulting adduct (**2ab**) was oxidized in nearly quantitative yield (93–95%) to the ketone **3ab**, using Jones reagent[15]; mild conditions (−15°C for 1.5 h) were used to avoid oxidation of the pyridine ring. The ethyl group was introduced into ketone **3** in good yield (77–80%) by allowing it to react with equimolar amounts of sodium hydride and ethyl bromide; use of an excess of the reagent or the more reactive ethyl iodide gave large amounts of N-ethylation byproduct. Reduction of the substituted ketone **4a** with lithium aluminum hydride (LAH) produced a mixture of two diastereomeric alcohols (**5** and **6**). Although these diastereomers were separated and characterized individually, their stereochemical assignment could not be made with certainty. In practice they need not to be separated, however, since both of them were converted by sequential treatment with boron trifluoride-dimethyl sulfide complex and then refluxing with HCl to give (*E*)-1-(4-hydroxyphenyl)-2-(4-pyridinyl)but-1-ene (**7**). The *E* geometry of alkene **7** was assigned initially on the basis of its long wavelength ultraviolet absorbance and its fluorescence (see Tables 1 and 2, below), which are characteristic of *E*-4-hydroxystilbazoles.[12] This assignment was verified further with the aid of NOE studies. Irradiation of the vinylic proton (δ 6.89) yielded enhancements of the neighboring aromatic protons meta to the phenolic hydroxyl (δ 7.23, 15.0% enhancement) and meta to the pyridinyl nitrogen (δ 7.47, 18.4% enhancement), without exhibiting detectable enhancement of the methylene protons trans to it. This compound was hydrogenated catalytically to give the corresponding saturated analog **8**.

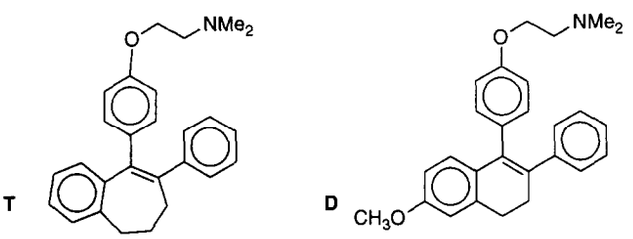


Scheme 1

Table 1 Estrogen binding affinity of 4-styrylpyridine derivatives

Compound	RBA%[a]
4-styp[b]	0.031 ± 0.010
7	0.0085 ± 0.0005
8	0.0065 ± 0.0015
10a	0.018[c]
10b	0.0015 ± 0.0002
11a	6.6 ± 1.0
11b	0.085 ± 0.006
13	5.4 ± 0.9
14	0.057 ± 0.012
T[d]	1[c]
D[d]	0.5 ± 0.02

[a]Values are the average ± range of duplicate determinations. The coefficient of variation in these assays are typically 0.3.[16]
[b]4'-Hydroxy-4-styrylpyridine, from reference 12.
[c]Single determination.
[d]T = 6,7-Dihydro-8-phenyl-9-[4-[2-(dimethylamino)ethoxy]phenyl]-5H-benzocycloheptene, D = 1-[4-[2-(dimethylamino)ethoxy]phenyl]-6-methoxy-2-phenyl-3,4-dihydronaphthalene, both are rigid triarylethylene tamoxifen analogues.[19]



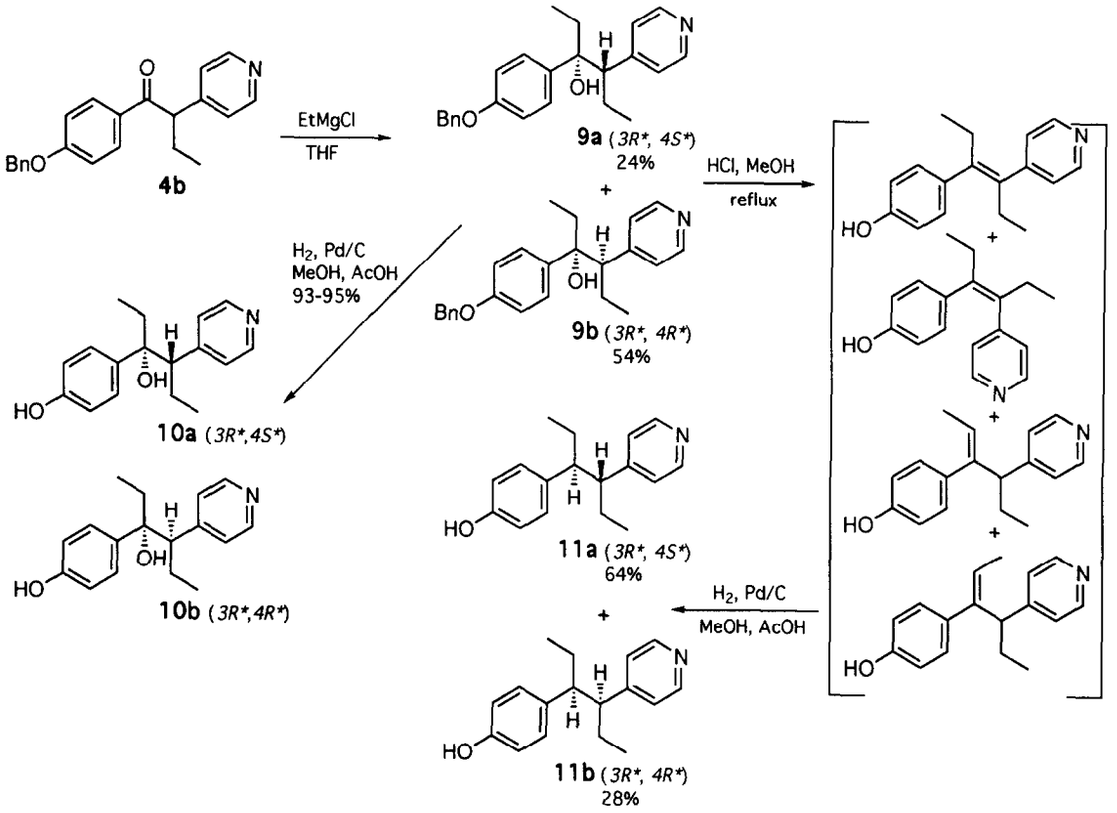
The route to the heterocyclic deoxyhexestrol analogs **10ab** and **11ab** is illustrated in Scheme 2. Ethyl magnesium chloride addition to the ketone **4b** gave a mixture (1:2) of diastereomeric benzylic alcohols **9ab**: each stereoisomer could be debenzylated by catalytic hydrogenolysis to give the diastereomeric alcohols **10ab.** Unlike the case with diastereomers **5** and **6,** the alcohols **9ab** and **10ab** are analogs of *meso*- and *dl*-hexestrol, and their relative stereochemistry can be assigned on the basis of the consistent upfield shift experienced by the chain-terminal methyl group in the ^{1}H NMR of the (*R**,*S**) isomers **9a** and **10a** (δ 0.54–0.56) compared to the (*R**,*R**) isomers **9b** and **10b** (δ 0.64–0.73); this shift is well precedented in the hexestrol series.[17] The *R**,*S** system is a convenient one to specify relative stereochemistry. (The *R**,*S** system of designating relative stereochemistry (IUPAC 1968 Tentative Rules, Section E) is used to define unambiguously the appropriate diastereomer of each hexestrol derivative. In this system, the *R**,*S** diastereomers correspond in each case to those referred to traditionally as the *meso* or *erythro* diastereomers. The other diastereomers (*R**,*R**,*dl*, or *threo*) typically have much lower binding affinity for the estrogen receptor.[18]) Although the alcohols **9ab** and **10ab** have a 3-hydroxyl group, not the pure hexane backbone of the hexestrols (and the hexane derivatives **11ab,** see below), a molecular mechanics evaluation of the steric energies of the three staggered conformers of **9ab** and **10ab** (Tripos forcefield implemented in the Sybyl modeling package, Tripos Associates, St. Louis, MO, USA) confirms that they have the same relative energies as the *meso*/*dl* hexestrols (data not shown). Therefore, we believe that these ^{1}H NMR chemical shift arguments can be applied to the **9ab** and **10ab** systems.

Dehydration of either the pure (3*R**,4*S**) isomer (**9a**) or a mixture of both isomers (**9ab**) with refluxing HCl furnished several unsaturated products, which were separated chromatographically and identified as a major crop composed of an equimolar mixture of *E*/*Z* 2,3-dehydration products and a minor crop composed of an *E*/*Z* mixture (8:1 by ^{1}H NMR) produced by 3,4-dehydration. It is also noteworthy that in all cases the benzyl protecting group was removed. Alternative dehydration conditions (e.g., $SOCl_2$/pyridine) gave a similar product mixture, but with the 3,4-dehydration products being the predominant. Catalytic hydrogenation/hydrogenolysis of the mixture of these unsaturated products over palladium on charcoal gave a diastereomeric mixture of the desired diethyl functionalized derivatives (**11ab**). The configuration of these diasteroisomers was assigned on the basis of proton magnetic resonance chemical shifts as described above.[17] Thus, the meth-

Table 2 Long wavelength UV absorbance maxima for 4'-hydroxy-4-styrylpyridine derivatives

		λ_{abs}^{max} (ε)			
Compound	Condition[a]	THF	CH_3CN	EtOH	H_2O
7	neutral	302 (22,700)	298 (25,300)	308 (22,200)	300 (21,100)
	acid	364 (24,300)	366 (27,500)	376 (24,400)	354 (21,000)
	base	[b]	378 (34,900)	364 (34,200)	342 (24,700)
13	neutral	326 (23,100)	320 (20,900)	332 (22,300)	322 (11,800)
	acid	382 (25,700)	382 (24,500)	392 (25,800)	370 (17,800)
	base	[b]	400 (25,100)	386 (25,100)	358 (17,000)
4-styp[c]	neutral		322 (8,700)	332 (10,900)	320 (4,200)
	acid	[d]	378 (8,800)	390 (10,200)	366 (6,700)
	base		392 (10,800)	384 (11,400)	358 (6,700)

[a]Acid = 0.1N HCl, Base = 0.1N KOH.
[b]Not soluble.
[c]4'-Hydroxy-4-styrylpyridine, data from reference 12.
[d]Not available.



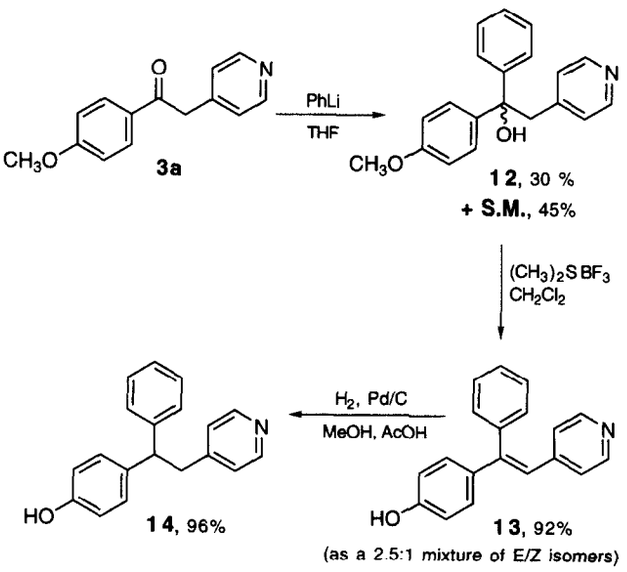
Scheme 2

yl protons of the major isomer (**11a**, 3*R**,4*S**) resonate upfield at δ 0.42–0.44, while in the other isomer (**11b**, 3*R**,4*R**), these signals appear at δ 0.74–0.76.

The synthesis of N-heterocyclic triarylethylene analogs **13** and **14** was performed by treatment of ketone **3a** with a 10% excess of phenyllithium (Scheme 3). The reaction furnished only a poor yield (30%) of the desired product (**12**) and never reached completion, since 45% of the unreacted starting material was recovered along with a polyphenylated byproduct. Attempts to force the reaction to completion by increasing the amount of phenyllithium gave a yet lower yield of the desired product and increased the amount of byproduct. This suggests that the competing enolization of ketone **3a** is limiting the yield of the addition product. Removal of the methoxy protecting group with boron trifluoride-dimethylsulfide complex caused the simultaneous dehydration of the tertiary benzylic alcohol and the formation of the desired conjugated product as a 2.5:1 mixture (by ^{1}H NMR) of the *E*/*Z* diastereoisomers. Chromatographic separation and recrystallization from methylene chloride and petroleum ether resulted in the separation of the desired pure *E* isomer (**13**). Catalytic hydrogenation of the latter compound gave the corresponding saturated compound (**14**). The assignment of stereochemistry of alkene **13** was initially based on the long wavelength absorbance and fluorescence expected for the E isomer (see Tables 1 and 2), due to its (*E*)-4-hydroxystylbazole substructure.[12] Once again, NOE studies confirmed this assignment, as irradiating the vinylic proton (δ 6.94) showed enhancements of the neighboring aromatic protons meta to the phenolic hydroxyl (δ 7.14, 17.5% enhancement) and meta to the pyridinyl nitrogen (δ 6.80, 13.1% enhancement), without exhibiting detectable enhancement of the aromatic protons trans to it.

Estrogen receptor binding affinity (RBA)

The estrogen RBAs of the new compounds were determined by a competitive binding assay and are shown in Table 1.



Scheme 3

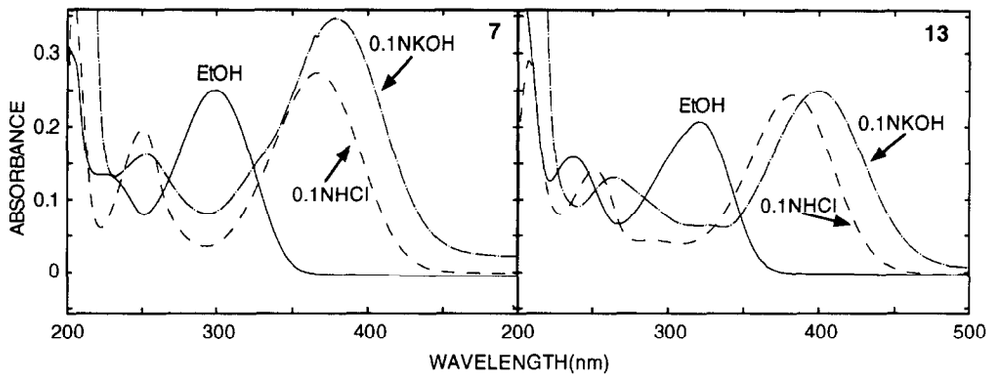


Figure 2 UV-Visible spectra of compounds **7** and **13**. Spectra were recorded in ethanol solution at 10^{-5} M, alone or with 0.1N HCl or 0.1N KOH.

The affinities are obtained by competition with the tracer compound [^{3}H]estradiol and are expressed on a percent scale, relative to estradiol, whose affinity is considered to be 100%.

Even though there are differences in their affinity, all of the new compounds, except **11a** and **13,** showed limited affinity for ER (i.e., less than 1% that of estradiol). As has been noted before,[18] the configuration of C-3 and C-4 of deoxyhexestrol analogues greatly influences their RBA values (compare **11a** versus **11b**); as expected, the *erythro* (*R*,S**) diastereomer **11a** has ~100-fold higher affinity.[18] The introduction of a second ethyl group in the alkane backbone has marked effect on the RBA value (compare **11a** versus **8,** even **11b** versus **8**). Although the compounds are not structurally homologous, when a phenyl group replaces a single ethyl substituent, the RBA is slightly affected in the alkane series (compare **14** versus **8**), but increases markedly in the alkene series (compare **13** and **7**). Reduction of the double bond has a negligible effect on the RBA of the monoethyl substituted derivative (compare **8** versus **7**), in contrast to the phenyl substituted derivative **13,** where the RBA decreases markedly (compare **13** versus **14**). The latter compound **13** exhibits an RBA value that is 5- or 11-fold greater than those of previously reported triarylethylene tamoxifen analogues T and D.[19] This is the only fluorescent compound in this series with substantial affinity for the estrogen receptor; the saturated system **11a** is not fluorescent.

Absorption and fluorescence properties

The ultraviolet spectra of compounds **7** and **13** were measured in tetrahydrofuran, acetonitrile, ethanol, and water, under neutral, acidic (0.1N HCl), and basic (0.1N KOH) conditions. These results, along with those of the parent 4′-hydroxy-4-styrylpyridine **(4-styp),** are presented in Table 2. In addition, the spectra of these two compounds in ethanol are shown in Figure 2. The absorption maxima of the substituted compounds **(7, 13)** in different solvents and at different pH are similar to those of the unsubstituted one **(4-styp),** but are 2–3 fold more intense (data not shown). It is also characteristic that the behavior of these compounds in different solvents does not show the conventional dependence upon solvent polarity; rather, they display the unusual property of reverse solvatochromism. This phenomenon is difficult to explain, but has been rationalized in the past by invoking solvent perturbation of the relative contribution of different tautomeric forms in the ground state.[20]

The suitability of fluorescent probes for biological systems, however, often depends upon the extent to which their fluorescent properties are sensitive to their environment (solvent and pH).[13] In a preliminary investigation, we have measured the fluorescence emission of novel compounds **7, 13,** and precursor **4-styp,** and the effect of pH (acidic, basic, and neutral) and solvent polarity (tetrahydrofuran, acetonitrile, ethanol, and water). The results are exemplified for ethanol solutions in Figure 3 and are summa-

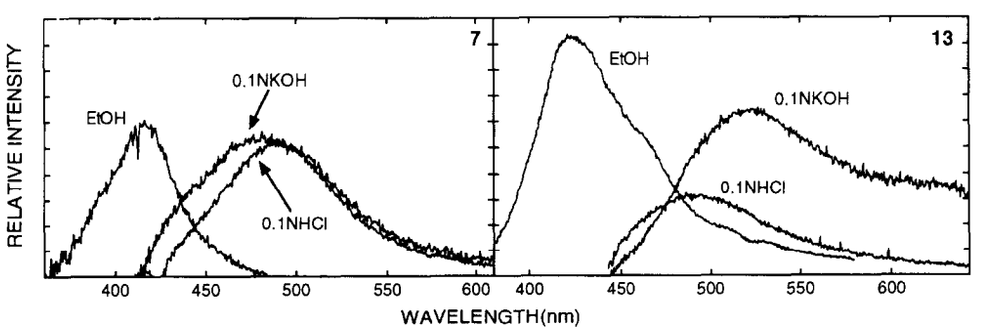


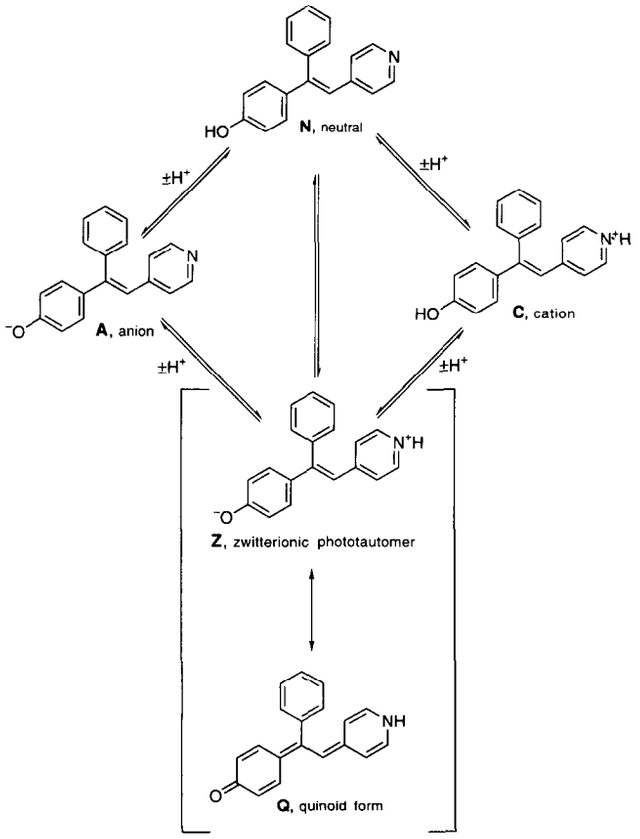
Figure 3 Fluorescence emission spectra of compounds **7** and **13**. Spectra were recorded in 5×10^{-6} M ethanolic solution, alone or with 0.1N HCl or 0.1N KOH. Excitation was at the major long wavelength band (302–400 nm): see Table 2 and Figure 2.

Table 3 Fluorescence solvatochromism of 4'-hydroxy-4-styrylpyridine derivatives

Compound	Condition[a]	λ_{em}^{max} (relative intensity)[b]			
		THF	CH_3CN	EtOH	H_2O
7	Neutral	360 (1)	377 (10.3)	416 (1.2)	430 (0.3)
	Acid	483 (1)	503 (1.2)	490 (1.1)	501 (0.6)
	Base	[c]	522 (2)	481 (1.1)	503 (0.6)
13	Neutral	392 (24)	415 (21)	420 (4.7)	489 (0.9)
	Acid	491 (2.4)	501 (3.2)	487 (0.8)	491 (0.8)
	Base	[c]	555 (8.9)	527 (1.7)	510 (0.2)
					630 (1.2)
4-styp[d]	Neutral	[e]	408 (0.1)	414 (0.2)	440 (0.2)
					505 (0.2)
	Acid	[e]	500 (1.6)	498 (1.7)	505 (1.1)
	Base	[e]	516 (5.2)	506 (2.6)	517 (1.5)

[a]Acid = 0.1N HCl, Base = 0.1N KOH. Excitation was at the major long wavelength absorption band in the UV under each condition (see Table 2 and Figure 1).
[b]Numbers in parentheses represent the relative intensity of emission ($\times 10^4$ cps) at λ_{em}^{max}.
[c]Not soluble.
[d]4'-Hydroxy-2-styrylpyridine, data from reference 12.
[e]Not available.

rized for all solvents in Table 3. It is evident that the fluorescence emission maxima of these compounds are extremely solvent dependent, indicating that there is a large dipole moment in the excited state.[1] Furthermore, the emission maximum under neutral conditions is shifted to the red as the solvent polarity increases, and in each solvent there is an additional red shift upon going from neutral to acidic or basic conditions.



Scheme 4

In a number of respects, the emission properties of the monoethyl substituted derivative (**7**) are similar to those of the unsubstituted compound **4-styp.** The phenyl substituted derivative **13,** however, possesses much better fluorescent properties, since its emission maxima in acid and base are considerably red-shifted (100–140 nm). Moreover, in aqueous acidic and basic solution, the long-wavelength emission maximum appears at ~630 nm, and it shows dual fluorescence (see Table 3, spectra not shown).

The unusual behavior of dual fluorescence can be rationalized[12] by considering that in the excited state, the acidity of the phenol and the basicity of the pyridine both increase[21,22]; so, the emission band at ~490 nm in acidic and neutral conditions arises from the cationic species (**C,** Scheme 4), as expected from the pKa values,[23] while the emission band at 510 nm is due to the anionic species (**A**). The extreme long wavelength emission at ~630 nm is due to the phototautomeric zwitterion **Z** (one resonance representation of which has a quinoid resonance structure **Q**); the emission band of this species is red shifted to such a great extent because of its capacity for resonance stabilization. This second long wavelength emission band is most evident in water, as this solvent most readily facilitates the proton transfer steps required for its formation. Moreover, it is worth noting that this emission band is at a longer wavelength than most cellular autofluorescence, making compound **13** suitable for use as fluorescent probe for biological molecules in a whole cell context.

Conclusion

The goal of preparing a practical fluorescent probe for the assay of estrogen receptor remains elusive. (*E*)-1-(4-Hydroxyphenyl)-1-phenyl-2-(4-pyridinyl)ethene (**13**), however, is a moderate affinity ER ligand, which does not require stereospecific synthesis or complicated isomer separations. In addition, it exhibits interesting photophysical properties, having emission maxima which are extremely solvent and pH dependent, and which occur under

certain conditions from a zwitterionic phototautomer at very long-wavelength (longer than most cellular autofluorescence). Thus, this compound is an attractive candidate for further development and use as a fluorescent probe for the assay of the estrogen receptor. Furthermore, studies involving the incorporation of this styrylpyridine system into a more rigid and higher affinity nafoxidine molecular framework (Scribner AW, Haroutounian SA, Katzenellenbogen JA, unpublished) and the design of derivatives containing an optimal hydroxyl substitution pattern and/or the inclusion a second side-chain are currently underway.

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