[1-(β-Mercapto-β,β-diethylpropionic acid),2-O-ethyltyrosine,4-valine,8-D-arginine]vasopressin (dEt$_2$Tyr(Et)-VDAVP, 18). A solution of the protected nonapeptide amide XVIII (0.135 g, 0.092 mmol) in sodium-dried and redistilled ammonia (400 mL) was treated at the boiling point and with stirring with sodium[19] from a stick of metal contained in a small-bore glass tube until a light blue color persisted in the solution for 30 s. Dry glacial acetic acid (0.4 mL) was added to discharge the color. The solution was evaporated, the residue was dissolved in aqueous acetic acid (0.2%, 800 mL), and this solution was treated with 2 M ammonium hydroxide solution to give a solution of pH 6.5. An excess of a solution of potassium ferricyanide (0.01 M, 16 mL)[20] was added gradually with stirring. The yellow solution was stirred for a further 10 min and for 10 min with anion-exchange resin (Bio Rad AG-3, Cl^- form, 10-g damp weight). The suspension was slowly filtered through a bed of resin (50-g damp weight). The bed was washed with aqueous acetic acid (0.2%, 200 mL), and the combined filtrate and washings were lyophylized. The resulting powder (1.45 g) was desalted on a Sephadex G-15 column (110 × 2.7 cm), eluting with aqueous acetic acid (50%)[22] with a flow rate 5 mL/h. The eluate was fractioned and monitored for absorbance of 280 nm. The fractions comprising the major peak were pooled and lyophylized, and the residue (100 mg) was further subjected to gel filtration on a Sephadex G-15 column (100 × 1.5 cm), eluting with aqueous acetic acid (0.2 M) with a flow rate of 4 mL/h.[22] The peptide was eluted in a single peak (absorbance 280 nm). Lyophylization of the pertinent fractions yielded the vasopressin analogue (18) [31 mg, 29.8% (based on the amount of protected peptide used in the reduction–reoxidation procedure)]. The physiochemical properties of this and of the remaining free peptides 1–17, which were prepared in the same way as for 18, are given in Table IV. Their pharmacological properties are presented in Tables I and II.

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Hydroxy Derivatives of Tamoxifen

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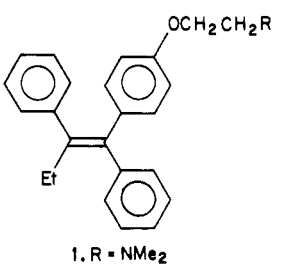
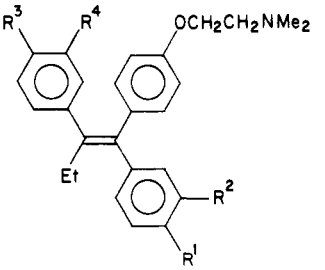
In the exploration of the structural features that affect the RBA (binding affinity for the estrogen receptor of rat uterus relative to that of estradiol) in the tamoxifen [*trans*-(*Z*)-1-[4-[2-(dimethylamino)ethoxy]phenyl]-1,2-diphenyl-1-butene] series, several derivatives variously substituted in the 1-phenyl group have been synthesized. [In the tamoxifen series, the descriptors *E* and *Z*, which define the configuration of the geometrical isomers and depend on the location and nature of substituents in the aromatic moieties and the ethyl group, may vary, although the relative configuration (cis or trans) does not. In order to avoid confusion the terms cis and trans will be used in this paper to refer to the relative positions of the 4-[2-(dimethylamino)ethoxy]phenyl and ethyl (or hydroxyethyl, hydroxypropyl, or bromo) substituents attached to the ethene moiety.] The final stage of each synthesis involved acid-catalyzed dehydration of a tertiary alcohol, and, in contrast to the known 3- and 4-hydroxy derivatives which were obtained as near-equimolar cis,trans mixtures, only the trans forms of the 2-hydroxy, 2-methyl, 2,4-dihydroxy, and 4-hydroxy-2-methyl derivatives were obtained. Also, in contrast to the trans forms of the 3- and 4-hydroxy derivatives, which are readily equilibrated to cis,trans mixtures, the trans 2-hydroxy derivative could not be isomerized. Tamoxifen and 2-methyltamoxifen had similar RBA's (~1% of that of E_2), but that of 2-hydroxytamoxifen was much lower (0.1%). Introduction of a second hydroxyl group (2,4-dihydroxy derivative) enhanced the RBA, and for the 4-hydroxy-2-methyl derivative, the RBA and growth inhibitory activity against the MCF-7 mammary tumor cell line in vitro were high and comparable to those of 4-hydroxytamoxifen, a metabolite of the parent drug. Tamoxifen derivatives hydroxylated at positions 3 or 4 of the 1-butene moiety and the 5-hydroxy-1-pentene analogue were also synthesized, but they had very low RBA values.

Tamoxifen [**1**, *trans*-(*Z*)-1-[4-[2-(dimethylamino)ethoxy]phenyl]-1,2-diphenyl-1-butene] is a triphenylethylene derivative presently in clinical use[1] for the treatment of hormone-dependent disseminated breast cancer and is thought to act[2] mainly by competing with estradiol for its protein receptor (ER). The relative binding affinity (RBA) of **1** for the ER of rat uterus is ~1% of that of estradiol. Of the human plasma metabolites of **1** so far identified, the *N*-desmethyl[3] (**2**, major metabolite), *N*-oxide[4] (**3**), and hydroxyethyl[5] (**4**) derivatives have RBA values comparable to that of the parent drug, whereas 4-hydroxytamoxifen [**5**, *trans*-(*Z*)-1-[4-[2-(dimethylamino)ethoxy]phenyl]-1-(4-hydroxyphenyl)-2-phenyl-1-butene, minor metabolite[3,6]] has an RBA value that is comparable to, and possibly greater than, that of estradiol. The high RBA of 4-hydroxytamoxifen (**5**) has raised the



1. R = NMe_2
2. R = NHMe
3. R = N(→O)Me_2
4. R = H



5. R^1 = OH, R^2 = R^3 = R^4 = H
6. R^1 = R^3 = R^4 = H, R^2 = OH
7. R^1 = R^2 = R^3 = H, R^4 = OH
8. R^1 = R^2 = R^4 = H, R^3 = OH

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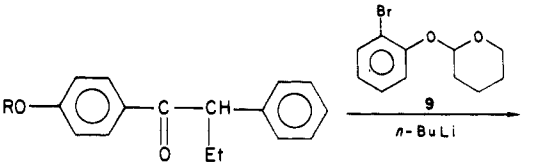
question[7] of the role of this metabolite in the expression of the biological activity of tamoxifen (1). As expected from the RBA values, 4-hydroxytamoxifen (5) is more effective than tamoxifen (1) in inhibiting the growth of the ER-positive MCF-7 breast cancer cell line.[4,8] However, the in vivo antitumor activity of 5 does not reflect the high RBA since it was less active[9] than tamoxifen (1) against a DMBA-induced ER-positive tumour in rats and only slightly more active against a hormone-dependent mammary tumour in mice.[10] These findings would be explained, at least in part, if the excretion of 5 was more rapid than that of 1, as would be expected since 5, is more polar and since it contains a hydroxy group, also amenable to glucuronidation.

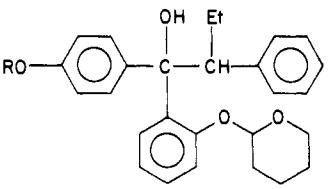
The recognition of the high RBA and in vitro antiestrogenic activity of 4-hydroxytamoxifen (5) has stimulated interest in other hydroxy derivatives accessible by synthesis but not formed by metabolism. Thus, Ruenitz et al.[11] described the trans 1-(3-hydroxyphenyl) (6) and the 2-(3- and 4-hydroxyphenyl) derivatives (7 and 8). The RBA values, expressed as the concentration (M) required to effect 50% inhibition of the binding at 2–4 °C of 5×10^{-9} M [^{3}H]estradiol to the estrogen receptor in rat uterine cytosol, were in the following sequence: 5 $\sim 0.4 \times 10^{-9}$; estradiol, $\sim 0.7 \times 10^{-9}$; 6 (3-hydroxytamoxifen), $\sim 10^{-8}$; 7, $\sim 0.3 \times 10^{-7}$; 8 $\sim 0.3 \times 10^{-7}$; tamoxifen (1), $\sim 10^{-6}$. Thus, the RBA is modestly enhanced on 3- and 4-hydroxylation of the 2-phenyl group of tamoxifen and markedly enhanced on 3- and 4-hydroxylation of the 1-phenyl group. However, Roos et al.[12] found that the enhancement of the RBA for 3-hydroxytamoxifen (6) was less marked in assays involving the cytosolic estrogen receptors of MCF-7 cells and a human mammary carcinoma. The hydroxy derivatives 5 and 6, like tamoxifen, showed strong antiestrogenic and weak estrogenic activity.[11]

We now report on the syntheses of several other hydroxy derivatives of tamoxifen, their antiestrogenic properties, and alternative syntheses of the 3- (6) and 4-hydroxy (5) derivatives.

Chemistry. Recent syntheses[11,13] of 4-hydroxytamoxifen (5) have involved the reaction of [4-(tetrahydropyran-2-yloxy)phenyl]magnesium bromide with an appropriate ketone. Erratic results with this Grignard reagent prompted the development of an alternative and improved procedure. Reaction of 4-(tetrahydropyran-2-yloxy)phenyl bromide with *n*-butyllithium under nitrogen followed by addition of the resulting lithium derivative to 1-[4-[2-(dimethylamino)ethoxy]phenyl]-2-phenylbutan-1-one (10) and acid-catalyzed dehydration of the resulting

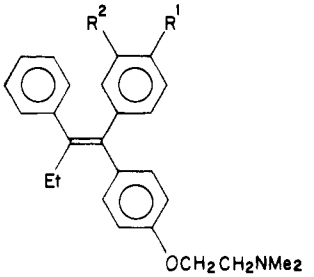


10, R = $CH_2CH_2NMe_2$
18, R = Me

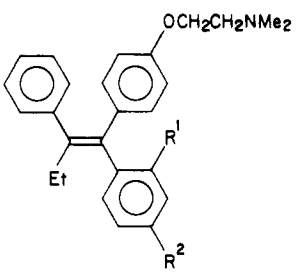


11, R = $CH_2CH_2NMe_2$
19, R = Me

tertiary alcohol gave a mixture of *trans*-4-hydroxytamoxifen (5) and its cis isomer (12). The isolation of these cis and trans isomers and their susceptibility to isomerization has been described in detail.[13]



12, R^1 = OH, R^2 = H
13, R^1 = H, R^2 = OH



14, R^1 = OH, R^2 = H
15, R^1 = Me, R^2 = H
20, R^1 = R^2 = OH
21, R^1 = Me, R^2 = OH

When the foregoing procedure was repeated with 3-(tetrahydropyran-2-yloxy)phenyl bromide, a cis,trans mixture (6, 13) of 3-hydroxytamoxifen was obtained from which the pure trans isomer (6) could be crystallized. Like Ruenitz et al.[11] we were not able to isolate the pure cis isomer 13. When the procedure was repeated with 2-(tetrahydropyran-2-yloxy)phenyl bromide (9), crystallization of the crude product obtained on dehydration of the intermediate tertiary alcohol 11 gave a 2-hydroxy derivative that was assigned the trans configuration (14) (see below). TLC (benzene-piperidine, 10:1) of the mother liquors from the crystallization of 14 revealed a second product (R_f 0.48; cf. 0.45 for 14) that, unlike 14 and the other hydroxy derivatives of tamoxifen noted above, was fluorescent under UV light and whose EI mass spectrum contained a peak (m/z 385) that was 2 mass units lower than that for the molecular ion of 14, suggesting[14] that it was a phenanthrene derivative. No evidence was obtained for the formation of the cis isomer of 14.

Attempts were therefore made to isomerize 14 on the basis of the observation that *trans*-(*Z*)-1-(4-hydroxyphenyl)-1,2-diphenyl-1-butene[15] (16) was converted into

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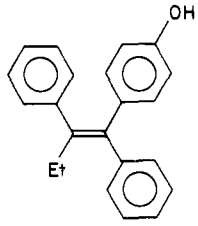

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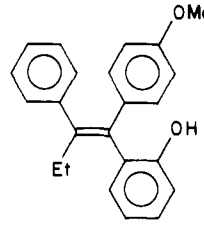


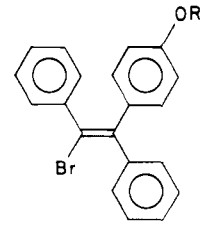
17

a 1:1 equilibrium cis,trans mixture by storage of a solution in 6 M HCl in ether at room temperature for 4 h. Unfortunately, this procedure could not be applied to 14 since the hydrochloride precipitated immediately. Attention was therefore turned to the model compound 1-(2-hydroxyphenyl)-1-(4-methoxyphenyl)-2-phenyl-1-butene (17), which lacks the basic substituent present in 14 and was prepared by the sequence 18 → 19 → 17. The intermediate tertiary alcohol 19 was obtained crystalline, but on dehydration only the trans isomer (17) appeared to be formed together with a fluorescent compound of slightly higher R_f value that, presumably, was a phenanthrene derivative analogous to that associated with 14 and described above. The crystalline trans isomer 17 dissolved readily in 6 M HCl in ether but was unchanged after storage of the solution for 4 h at room temperature.

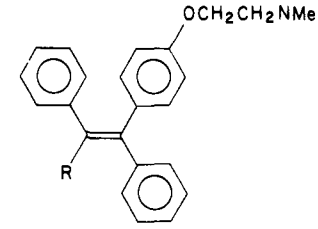
4-Hydroxytamoxifen (5) has a high RBA, but it readily equilibrates into a cis,trans mixture and the cis isomer (12) has[16,17] a low RBA. This unwanted isomerization can occur even on storage of the trans isomer (5) in the solid state and exceedingly easily under certain conditions,[13] particularly during chromatography of small amounts. Although there is no evidence for trans →cis,trans equilibration in vivo, it can occur readily under cell culture condtions.[17] Thus, prior to the use of the trans isomer (5) in in vitro or in vivo experiments, it is advisable to verify its stereochemical purity by TLC and, wherever possible, to monitor for trans → cis,trans equilibration. The formation of only the trans isomer of 2-hydroxytamoxifen (14), its resistance to isomerization, and the high RBA of 4-hydroxytamoxifen prompted syntheses of the 2-methyl (15), 2,4-dihydroxy (20), and 4-hydroxy-2-methyl (21) derivatives of tamoxifen.

The route to 15, 20, and 21 (see Experimental Section) was similar to that described above for 2-hydroxytamoxifen, and in each sequence, only the product with the trans configuration appeared to be formed (the assignments of configuration are discussed below). The tertiary alcohol precursor to the 2-methyl derivative 15 was obtained in crystalline form and in high yield (92%).

To further complete the series of hydroxylated derivatives of tamoxifen, the two hydroxyethyl compounds 24 and 25 were synthesized. Hydroxylation of the ethyl group of tamoxifen was proposed by Fromson et al.[18] as a minor metabolism pathway in the rat, although the monohydroxy derivatives 24 and 25 per se were not detected. The synthesis route to the *trans*-3- (24) and *trans*-4-hydroxy-1-butene (25) derivatives involved (dimethylamino)-ethylation of the known[19] triphenylethylene derivative (22) to give 23 followed by conversion of the product into the lithio derivative and reaction with acetaldehyde (24) or ethylene oxide (25). The *trans*-5-hydroxy-1-pentene derivative (26) was obtained by reaction of the lithio derivative with oxetan. Since a cis,trans mixture of products was formed when the trans bromide (23) was lithiated, there was no advantage in resolving the cis,trans mixture of the bromide 23 prior to lithiation. However, the cis,trans mixture of products was readily resolved by column chromatography.



22, R = H
23, R = $CH_2CH_2NMe_2$



24, R = $CH_3CH(OH)$-
25, R = $HOCH_2CH_2$-
26, R = $HOCH_2CH_2CH_2$-

Assignment of Cis and Trans Configurations. The isomers of tamoxifen derivatives in which the 1- and 2-phenyl groups are unsubstituted can be readily identified on the basis of the chemical shifts of the AB quartet for the protons in the remaining aromatic moiety. Thus, only in the trans isomer is this AB quartet upfield of the signals for the protons of the phenyl groups,[20] reflecting the combined shielding effect of these groups. On this basis 2-hydroxytamoxifen (14: AB q, δ 6.54–6.80; other aromatic protons, δ 6.84–7.22), 2-methyltamoxifen (15: AB q, δ 6.51–6.80; other aromatic protons, δ 7.12–7.30), and the model compound 17 (AB q, δ 6.42–6.76; other aromatic protons, δ 6.90–7.55) can be assigned the trans configuration. Although this relationship holds for the 3- (24) and 4-hydroxy-1-butene (25) derivatives and the 5-hydroxy-1-pentene derivative (26) (see Experimental Section), it does not hold for 2,4-dihydroxy (20) and 4-hydroxy-2-methyl (21) derivatives of tamoxifen (20: AB q, δ 6.51–6.76; other aromatic protons, δ 6.20–6.82. 21: AB q, δ 6.54–6.73; other aromatic protons, δ 6.62–7.24) but the chemical shifts of the AB quartet of 15, 20, and 22 (and also 14) are in the same range (δ 6.5–6.8) as the known trans isomers 1 and 5 and the trans configurations of 14 and 21 were proved by X-ray crystallography.[21] Also, the chemical shifts of the triplets for the OCH_2 protons in the compounds (20 and 21) assigned the trans configuration are below δ 4.0 (δ 3.80 and 3.86, respectively), consistent with the findings of Collins et al.[22] for triarylethylene derivatives.

Relative Binding Affinity and in Vitro Antimammary Tumor Activity. The relative binding affinities (RBA) of the tamoxifen derivatives for the uterine estrogen receptor[23] were as follows: 4-hydroxytamoxifen (5) = 4-hydroxy-2-methyltamoxifen (21) ≥ E_2, 100; 2,4-dihydroxytamoxifen (20), 25; 3-hydroxytamoxifen (6), 2.5; 2-methyltamoxifen (15) ≥ tamoxifen (1), 0.9; 2-hydroxytamoxifen (14), 0.1. A similar sequence was reported by Ruenitz et al.[13] for 5, 6, and 1, although the RBA values estimated from the inhibition curves reported by these authors suggest slightly higher values for 5 and 6. Our RBA values were estimated at 18 °C after incubation

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Table I. Effect of Tamoxifen and Some Hydroxy Derivatives on the Growth of MCF-7 Cells[a]

		DNA (μg ±SD)[b]	
	molarity	compd alone	compd + 10^{-8} M estradiol
control		16.7 ± 3.0 (100)	14.7 ± 1.0 (88)
tamoxifen (1)	10^{-7}	14.0 ± 2.2 (84)	15.2 ± 0.9 (91)
	10^{-6}	10.6 ± 1.0 (63)	15.1 ± 1.3 (90)
4-hydroxytamoxifen (5)	10^{-7}	6.0 ± 0.8 (36)	14.2 ± 0.6 (85)
	10^{-6}	3.8 ± 1.2 (23)	5.4 ± 0.8 (32)
3-hydroxytamoxifen (6)	10^{-7}	11.2 ± 0.9 (67)	15.0 ± 1.2 (90)
	10^{-6}	5.9 ± 1.5 (35)	11.3 ± 1.1 (68)
2-hydroxytamoxifen (14)	10^{-7}	14.6 ± 1.4 (87)	12.8 ± 1.8 (77)
	10^{-6}	14.2 ± 1.2 (85)	12.4 ± 1.8 (74)

[a] One-way variance analysis showed that tamoxifen (1) and the 4- (5) and 3-hydroxy (6) derivatives significantly inhibited the growth of MCF-7 cells: (1, $P < 0.05$; 5, $P < 0.001$; 6, $P < 0.001$). Newman–Keuls test showed that 5 was effective at both 10^{-7} and 10^{-6} M ($P < 0.001$), whereas 1 and 6 were effective only at 10^{-6} M ($P < 0.01$). [b] The figures in brackets are the percentages of the control value.

Table II. Effect of 4-Hydroxytamoxifen and Some Derivatives on the Growth of MCF-7 Cells[a]

	molarity	DNA (μg ± SD)[b]
control		8.2 ± 1.1 (100)
4-hydroxytamoxifen (5)	10^{-7}	1.2 ± 0.2 (15)
	10^{-6}	1.1 ± 0.3 (13)
2,4-dihydroxytamoxifen (20)	10^{-7}	6.6 ± 2.3 (80)
	10^{-6}	1.1 ± 0.5 (13)
4-hydroxy-2-methyltamoxifen (21)	10^{-7}	1.6 ± 0.4 (20)
	10^{-6}	1.0 ± 0.3 (12)

[a] One-way variance analysis showed that all compounds significantly inhibited the growth of MCF-7 cells ($P < 0.001$). Newman–Keuls test showed that 5 and 21 were effective at both 10^{-7} and 10^{-6} M ($P < 0.001$), whereas 20 was effective only at 10^{-6} M ($P < 0.01$). [b] The figures in brackets are the percentages of the control value.

for 30 min, whereas those of Ruenitz et al.[11] were determined at 4 °C after 4 h. These differences in the binding assays are probably responsible for the differences in RBA values, since the latter are markedly time and temperature dependent.[24] The RBA values of the 3- (24) and 4-hydroxy-1-butene (25) and the 5-hydroxy-1-pentene (26) derivatives were each ~0.05.

Both estrogens and antiestrogens bind to ER, but antiestrogenic activity can be assessed on the basis of inhibition of the growth in vitro of ER-positive and ER-negative cell lines derived from human breast cancers.[25,26] The results in Table I show that the growth of the ER-positive cell line MCF-7 was inhibited by tamoxifen (1) and its 4- (5) and 3-hydroxy (6) derivatives; the 2-hydroxy isomer (14) was ineffective. As expected[4,8,12] from the RBA values, 5 and 6 were more potent inhibitors than tamoxifen (1), with 5 being slightly the more potent; the inhibitions were suppressed by estradiol. Similarly, growth was inhibited by the 2,4-dihydroxy (20) and 4-hydroxy-2-methyl (21) analogues (Table II). Again, the inhibitory activity paralleled the RBA values: the 4-hydroxy-2-methyl derivative (21) was as strong an inhibitor as 4-hydroxytamoxifen (5) whereas the 2,4-dihydroxy derivative (20) was only a weak inhibitor. Like tamoxifen[4,25] (1) none of the derivatives inhibited the growth of the ER-negative cell line Evsa-T, indicating that they had no general cytotoxic properties.

The marked reduction in RBA on moving the 4-hydroxyl group in 5 to the 3-position (6) may reflect an increase in steric hindrance to hydrogen bonding of the hydroxyl group or an increased separation distance between the hydroxyl group and a hydrogen-bond acceptor at the binding site. That hydrogen bonding of the hydroxyl group in the 4-hydroxy derivative (5) is responsible for the enhancement of the RBA is suggested by the fact that replacement[27] of the hydroxyl group by, for example, Me or F results in a dramatic reduction in the RBA. The finding that 2-hydroxytamoxifen (14) has an RBA even lower than that of tamoxifen suggests the operation of an effect that does not occur in the 3- (6) and 4-isomer (5), possibly the intrusion of the hydroxyl group into a lipophilic zone of the molecule involved in the binding to ER. Consistent with this interpretation is the fact that introduction of a 2-methyl substituent (15) does not adversely affect the RBA.

As might be expected, the introduction of a second hydroxyl group into the 2-hydroxy derivative (14) to give the 2,4-dihydroxy compound 20 increased the RBA. The high RBA of the 4-hydroxy-2-methyl derivative (21), which is comparable to that of 4-hydroxytamoxifen (5), coupled with its resistance to trans → cis isomerization leads us to suggest that it would be a useful compound in ER-binding studies such as those recently described by Keene et al.[28] using 1-[4-[2-(diethylamino)ethoxy]phenyl]-1-(4-hydroxyphenyl)-2-(4-methoxyphenyl)-1-butene, a tamoxifen analogue that, like 4-hydroxytamoxifen (5), would be expected to readily undergo trans → cis,trans equilibration.

The biological activity of 4-hydroxy-2-methyltamoxifen (21) is being further explored.

Experimental Section

Routine 60-MHz ^{1}H NMR spectra (internal Me_4Si) were obtained on a Perkin-Elmer R12B spectrometer. The 250-MHz spectra (internal Me_4Si) were obtained by courtesy of the University of London Intercollegiate Research Service. Mass spectra (electron impact, 70 eV) were obtained with a VG 7070H spectrometer and VG 2235 data system, using the direct-insertion method. TLC was performed on Kieselgel 60 (Merck 7730) with benzene–piperidine[11] (10:1, v/v) unless stated otherwise, and reversed-phase (RP) TLC on Whatman $KC_{18}F$ using methanol–water–diethylamine (80:20:1, v/v). Developed plates were viewed under UV light (Hanovia Chromatolite). Column chromatography was performed on Kieselgel 60 (Merck 7734). Melting points were determined on a Kofler hot stage and are uncorrected.

The (tetrahydropyranyloxy)phenyl bromides were prepared routinely by stirring a mixture of the appropriate bromophenol, excess dihydropyran, and toluene-*p*-sulfonic acid (trace) at room temperature (with cooling if necessary) for 1 h. Each mixture was diluted with Et_2O, washed with dilute aqueous NaOH, dried ($MgSO_4$), and concentrated. The products had no IR absorption for OH, and those that were noncrystalline were used without further purification.

Derivatives of 1-[4-[2-(Dimethylamino)ethoxy]phenyl]-1,2-diphenyl-1-butene. (a) 4-Hydroxy Derivatives (12 and 5). *n*-Butyllithium (3.2 mmol, 2.4 mL of a 1.35 M solution in hexane) was added dropwise during 10 min to a solution of 4-(tetrahydropyran-2-yloxy)phenyl bromide[29] (0.84 g, 3.26 mmol; mp 50–52 °C) in tetrahydrofuran (THF, 5 mL) at −78 °C. The mixture was stirred for 30 min, and a solution of 1-[4-[2-(di-

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methylamino)ethoxy]phenyl]-2-phenylbutan-1-one (**10**) (1 g; mp 40–42 °C; previously reported[30] as a liquid bp 162 °C (0.75 mmHg)) in THF (5 mL) was added during 2–3 min from a syringe. The mixture was allowed to attain room temperature and then stirred for 10 h and partitioned between Et_2O (50 mL) and H_2O (25 mL). The aqueous layer was extracted with Et_2O (3 × 10 mL), and the combined Et_2O layer and extracts were washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated.

A solution of the resulting tertiary alcohol (1.86 g, no IR absorption for carbonyl) in MeOH (25 mL) and concentrated HCl (3 mL) was kept at 60–70 °C for 30 min, then cooled to room temperature, adjusted to pH 11 with concentrated aqueous NaOH, and partitioned between Et_2O (50 mL) and H_2O (20 mL). The aqueous layer was extracted with Et_2O (3 × 5 mL), and the combined Et_2O layer and extracts were washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated. The residue was crystallized from MeOH to give a ~1:1 (NMR) mixture (0.51 g, 44%) of the cis *E* (**12**) and trans *Z* isomer (**5**) as a white solid: mp 152–154 °C; ^1H NMR (60 MHz, $CDCl_3$) δ 2.28 (s, 6, NMe_2, *Z* isomer), 2.34 (s, 6, NMe_2, *E* isomer); TLC, R_f 0.29, 0.33; RP-TLC, R_f 0.24, 0.27.

The isolation of the *E* and *Z* isomers, and their susceptibility to isomerization, has been described in detail.[13]

(b) *trans*-(*E*)-3-Hydroxy Derivative (6). The procedure in (a) was followed but with 3-(tetrahydropyran-2-yloxy)phenyl bromide (0.84 g, 3.26 mmol), and the resulting *E,Z* mixture was eluted from a column (25 × 1 cm) of Kieselgel 60 with $CHCl_3$–MeOH (19:1, v/v) and then crystallized twice from benzene–*n*-hexane to give pure **6** (0.35 g, 30%) as a white solid: mp 152–156 °C [lit.[11] mp 162–164 °C]; ^1H NMR (60 MHz, $CDCl_3$) δ 2.30 [s, 6, NMe_2; cf. 2.37 for the cis *Z* isomer **13**]; RP-TLC, R_f 0.27 [cf. 0.24 for the cis *Z* isomer **13**]; the *E* and *Z* isomers could not be separated by TLC using benzene-piperidine (10:1, v/v) or benzene–triethylamine (10:1, v/v).

(c) *trans*-(*E*)-1-[4-[2-(Dimethylamino)ethoxy]phenyl]-1-(2-hydroxyphenyl)-2-phenyl-1-butene (14). The procedure in (a) was followed (**10** → **11** → **14**) but with 2-(tetrahydropyran-2-yloxy)phenyl bromide (**9**) (0.84 g, 3.26 mmol). The crude product contained (NMR) only the trans *E* isomer (**14**), and crystallization from toluene gave a white solid (0.47 g, 40.3%): mp 136.5–138.5 °C; RP-TLC, R_f 0.27; ^1H NMR (250 MHz, Me_2SO-d_6) δ 0.78 (t, 1, *J* = 7 Hz, CH_2CH_3), 2.15 (s, 6, NMe_2), 2.27 (q, 2, *J* = 7 Hz, CH_2CH_3), 2.50 (t, 2, *J* = 5.7 Hz, NCH_2CH_2O), 3.87 (t, 2, *J* = 5.7 Hz, NCH_2CH_2O), 6.55–6.78 (AB q, 4, *J* = 8.7 Hz, CC_6H_4O), 6.84–6.86 (m, 2, ortho and para protons of OC_6H_4), 7.06–7.22 (m, 7, Ph and meta protons of OC_6H_4); mass spectrum (EI), *m/z* 387 (15%, $M^{+\cdot}$), 72 (23%, $[CH_2CH_2NMe_2]^{+\cdot}$), 58 (100%, $[CH_2{=}NMe_2]^+$). Anal. ($C_{26}H_{29}NO_2$) C, H, N.

The X-ray crystal structure of **14** is detailed in the following paper.[21]

(d) *trans*-(*E*)-1-[4-[2-(Dimethylamino)ethoxy]phenyl]-1-(2-methylphenyl)-2-phenyl-1-butene (15). The procedure in (a) was followed but with *o*-tolyl bromide (1.6 g, 6.4 mmol) to give 1-[4-[2-(dimethylamino)ethoxy]phenyl]-1-(2-methylphenyl)-2-phenyl-1-butanol (1.19 g, 92%): mp 133 °C (from cyclohexane); RP-TLC, R_f 0.19; ^1H NMR (250 MHz, $CDCl_3$) δ 0.84 (t, 3, *J* = 7.3 Hz, CH_2CH_3), 1.87–2.14 (m, 2, CH_2CH_3), 2.13 (s, 3, $C_6H_4CH_3$), 2.28 (s, 6, NMe_2), 2.39 (s, 1, OH), 2.62 (t, 2, *J* = 5.8 Hz, OCH_2CH_2N), 3.43 (dd, 1, *J* = 1.8 and 11.3 Hz, EtCH), 3.89 (t, 2, *J* = 5.8 Hz, OCH_2CH_2N), 6.50 and 6.72 (AB q, 4, *J* = 8.9 Hz, H-3,5 and H-2,6 of CC_6H_4O), 6.97–7.14 [m, 6, Ph and H-3 of CC_6H_4(*o*-Me)], 7.20 (dt, 1, *J* = 1.3 and 7.3 Hz, H-4 of CC_6H_4(*o*-Me)], 7.29 [dt, 1, *J* = 1.4 and 7.3 Hz, H-5 of CC_6H_4(*o*-Me)], 7.61 [d, 1, *J* = 7.3 Hz, H-6 of CC_6H_4(*o*-Me)]. Anal. ($C_{27}H_{33}NO_2$) C, H, N.

The foregoing tertiary alcohol (3 g) was dehydrated essentially as described in (e). The crude product was eluted from a column (4 × 45 cm) of Kieselgel 60 using a stepwise gradient of MeOH in $CHCl_3$ to give **15** (RP-TLC, R_f 0.08), which could not be obtained crystalline. HCl was bubbled through a solution of **15** (3 g) in Et_2O (10 mL). The precipitate was collected and recrystallized from EtOAc–EtOH to give the hydrochloride of **15** (1.6 g, 63%): mp 184–186 °C; ^1H NMR (250 MHz, $CDCl_3$) δ 0.83 (t, 3, *J* = 7.4 Hz, CH_2CH_3), 2.14 (s, 3, C_6H_4Me), 2.26 and 2.30 (2 dq, 2, *J* = 13.8 and 7.4 Hz, CH_2CH_3), 2.86 (s, 6, NMe_2), 3.36–3.41 (m, 2, OCH_2CH_2N), 4.31–4.34 (m, 2, OCH_2CH_2N), 6.51 and 6.80 (AB q, 4, *J* = 8.8 Hz, H-3,5 and H-2,6 of CC_6H_4O), 7.12–7.30 (m, 9, Ph and C_6H_4Me), 12.7 (bs, 1, $NHMe_2$). Anal. ($C_{27}H_{32}ClNO$) C, H, Cl, N.

(e) *trans*-(*E*)-1-(2,4-Dihydroxyphenyl)-1-[4-[2-(dimethylamino)ethoxy]phenyl]-2-phenyl-1-butene (20). *n*-Butyllithium (25 mmol, 14.8 mL of a 1.7 M solution in hexane) was added dropwise under N_2 to a solution of 2,4-bis(tetrahydropyranyloxy)phenyl bromide[31] (9.64 g, 27 mmol; mp 68–69 °C) in THF (20 mL) at −78 °C for 30 min, a solution of 1-[4-[2-(dimethylamino)ethoxy]phenyl]-2-phenylbutan-1-one (6 g, 19 mmol) in THF (10 mL) was then added during 2–3 min, and the mixture was stirred for 10 h at room temperature and worked up as in (a).

The resulting orange residue (14.8 g) was eluted from a column (5 × 70 cm) of Kieselgel 60 initially with CH_2Cl_2 and then with a stepwise CH_2Cl_2–MeOH gradient. At 1.75% MeOH the required tertiary alcohol was eluted and isolated as a viscous yellow oil (4.39 g): TLC, R_f 0.42 ($CHCl_3$–MeOH, 9:1, v/v); IR ν_{max} 3510 cm^{-1} (OH).

In order to minimize decomposition, the foregoing tertiary alcohol (1.5 g) was dehydrated under conditions milder than those described in (a), namely with concentrated HCl (1.5 mL) and MeOH (15 mL) at room temperature, for 30 min. Crystallization of the product from EtOAc at −25 °C gave **20** (0.17 g, 16.1%): mp 158–165 °C after turning yellow-orange at ~120 °C; TLC, R_f 0.16 ($CHCl_3$–MeOH, 9:1, v/v); ^1H NMR (250 MHz, Me_2SO-d_6) δ 0.77 (t, 3, *J* = 7.3 Hz, CH_2CH_3), 2.15 (s, 6, NMe_2), 2.29 (q, 2, *J* = 7.3 Hz, CH_2CH_3), 2.52 (t, 2, NCH_2CH_2O), 3.80 (t, 2, *J* = 5.8 Hz, NCH_2CH_2O), 6.20–6.24 [m, 1, H-5 of $C_6H_3(OH)_2$], 6.3 [d, 1, *J* = 2.2 Hz, H-3 of $C_6H_3(OH)_2$], 6.82 [d, 1, *J* = 8.1 Hz, H-6 of $C_6H_3(OH)_2$], 6.53, 6.75 (AB q, 4, *J* = 8.7 Hz, C_6H_4), 7.07–7.20 (m, 5, C_6H_5), 8.91 and 9.17 (2 s, 2, 2 OH). Anal. ($C_{26}H_{29}NO_3$) C, H, N.

(f) *trans*-(*E*)-1-[4-[2-(Dimethylamino)ethoxy]phenyl]-1-(4-hydroxy-2-methylphenyl)-2-phenyl-1-butene (21). *n*-Butyllithium (6.4 mmol, 4 mL of a 1.6 M solution in hexane) was added dropwise during 15 min to a solution of 2-methyl-4-(tetrahydropyranyloxy)phenyl bromide [1.74 g, 6.4 mmol, prepared (see above) from 4-bromo-*m*-cresol[32] (mp 62–63 °C)] in THF (5 mL) at −78 °C. The mixture was stirred at −78 °C for 30 min and then treated with a solution of 1-[4-[2-(dimethylamino)ethoxy]phenyl]-2-phenylbutan-1-one (1 g) in dry THF (5 mL). The mixture was allowed to attain room temperature (~17 °C), stirred for 10 h there at, and then worked up as in (d) to give the crude tertiary alcohol (2.45 g): TLC, R_f 0.43 ($CHCl_3$–MeOH, 9:1, v/v); RP-TLC, R_f 0.16.

The foregoing tertiary alcohol (2.45 g) was dehydrated essentially as described in (e). Recrystallization of the product from MeOH gave **21** (0.5 g, 38.8%): mp 181–181.5 °C; TLC, R_f 0.24 ($CHCl_3$–MeOH, 9:1, v/v), RP-TLC, R_f 0.23; ^1H NMR (250 MHz, Me_2SO-d_6), δ 0.74 (t, *J* = 7.36 Hz, 3, CH_2CH_3), 2.01 [s, 3, Me of C_6H_3(OH)Me], 2.15 (s, 6, NMe_2), 2.19–2.27 (m, 2, CH_2CH_3), 2.5 (t, 2, *J* = 6 Hz, $OCH_2CH_2NMe_2$), 3.86 (t, 2, *J* = 5.8 Hz, $OCH_2CH_2NMe_2$), 6.56–6.71 (AB q, 4, *J* = 8.7 Hz, CC_6H_4O), 6.62 [s, 1, H-3 of C_6H_3(OH)Me], 6.62–6.66 [m, 1, H-5 of C_6H_3(OH)Me], 7.02–7.05 [d, 1, *J* = 7.8 Hz, H-6 of C_6H_3(OH)Me], 7.11–7.24 (m, 5, Ph), 9.26 (s, 1, OH). Anal. ($C_{27}H_{31}NO_2$) C, H, N.

In subsequent larger scale dehydrations coupled with column chromatography of the mother liquors, the total yield of **21** was increased to 60%.

TLC on Kieselgel 60 F_{254} (Merck, 5714) with benzene–piperidine (10:1, v/v) of the mother liquors remaining after the crystallization of **21** revealed two components [R_f 0.26 (**21**), 0.29]. The component with R_f 0.29 was not characterized, but it was not the *E* isomer of **21** (mass spectral data).

Under conditions (50 mg in 10 mL of 5% HCl in EtOH, reflux, 2 h) where 4-hydroxytamoxifen (**5**) was equilibrated to a cis,trans mixture, **21** was unaffected.

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1-(4-Methoxyphenyl)-1-[2-(tetrahydropyran-2-yloxy)-phenyl]-2-phenyl-1-butanol (19). The procedure was essentially that in (a) above except that 1-(4-methoxyphenyl)-2-phenylbutan-1-one[33] (18; 0.63 g, 2.48 mmol) was used together with 2 equiv of 2-(tetrahydropyran-2-yloxy)phenyl bromide (1.28 g, 4.96 mmol) and *n*-butyllithium (4.96 mmol, 2.9 mL of a 1.7 M solution in hexane) to promote complete reaction. Compound **19** crystallized from light petroleum (bp 60–80 °C) as a white solid (0.78 g, 73%): mp 178–180 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.81 (t, 3, J = 7.4 Hz, CH_2CH_3), 1.00–1.80 (m, 6, tetrahydropyran H-3,3,4,4,5,5), 2.16 (quint, 2, J = 7.5 Hz, CH_2CH_3), 3.64 (s and m, 3, MeO, EtC*H*), 3.92 (brt, 2, tetrahydropyran H-6,6), 4.96 (m, 1, tetrahydropyran H-2), 4.83 (s, 1, OH), 6.44, 6.73 (AB q, 4, J = 8.8 Hz, *p*-OC_6H_4C), 7.00–7.40 (m, 8, Ph and H-3,4,5 of *o*-OC_6H_4C), 7.58 (d, 1, J = 7.5 Hz, H-6 of *o*-OC_6H_4C). Anal. ($C_{28}H_{32}O_4$) C, H.

(*E*)-1-(2-Hydroxyphenyl)-1-(4-methoxyphenyl)-2-phenyl-1-butene (17). The procedure for deprotection and dehydration of **19** (0.3 g, 0.69 mmol) was essentially that in (a) above. The product **17** crystallized from light petroleum ether (bp 60–80 °C) as a white solid (0.11 g, 47%): mp 96–99 °C; ^{1}H NMR (60 MHz, $CDCl_3$) δ 0.88 (t, 3, J = 7 Hz, CH_2CH_3), 2.43 (q, 2, J = 7 Hz, CH_2CH_3), 3.61 (s, 3, MeO), 6.42, 6.76 (AB q, 4, J = 9 Hz, $MeOC_6H_4C$), 6.80–7.30 (m, 9, Ph and HOC_6H_4C). Anal. ($C_{23}H_{22}O_2$) C, H.

***trans*-(*E*)-1-Bromo-2-[4-[2-(dimethylamino)ethoxy]-phenyl]-1,2-diphenylethene (23).** To a stirred solution of (*E*,*Z*)-1-bromo-1,2-diphenyl-2-(4-hydroxyphenyl)ethene (**22**: 6.0 g, 17 mmol) in dry $HCONMe_2$ (75 mL) under N_2 at room temperature was added NaH (3.0 g, 125 mmol), and the mixture was heated to 60 °C under N_2. (Dimethylamino)ethyl chloride hydrochloride (6.0 g, 42 mmol) was then added portionwise during 20 min. After a further 15 min at 60 °C, the mixture was cooled, poured into ice water (200 mL), and extracted with Et_2O (2 × 150 mL). The combined extracts were washed with H_2O (200 mL), dried (Na_2SO_4), and concentrated, and the residue was crystallized from light petroleum (bp 40–60 °C) to give a 2:1 *E*,*Z* mixture (6.43 g, 89%). Further recrystallization from light petroleum (bp 80–100 °C) gave the pure trans *E* isomer (**23**): mp 117–118 °C; ^{1}H NMR (60 MHz, $CDCl_3$) δ 2.27 (s, 6, NMe_2), 2.61 (t, 2, J = 6 Hz, CH_2N), 3.91 (t, 2, J = 6 Hz, CH_2O), 6.59 (d, 2, J = 9 Hz, H-3,5 of CC_6H_4O), 6.83 (d, 2, J = 9 Hz, H-2,6 of CC_6H_4O), 7.0–7.4 (m, 10, 2 Ph). Anal. ($C_{24}H_{24}BrNO$) C, H, Br, N.

The cis *Z* isomer could not be obtained pure: ^{1}H NMR δ 2.35 (s, 6, NMe_2), 2.75 (t, 2, J = 6 Hz, CH_2N), 4.10 (t, 2, J = 6 Hz, CH_2O), 6.75–7.45 (m, 14, aromatic protons).

***trans*-(*E*)-1-[4-[2-(Dimethylamino)ethoxy]phenyl]-1,2-diphenyl-1-buten-3-ol (24).** To a stirred solution of **23** (360 mg, 0.85 mmol) in dry THF (6 mL) at −78 °C under N_2 was added *n*-butyllithium (1.2 mmol, 0.8 mL of a 1.5 M solution in hexane) followed, after 5 min, by acetaldehyde (0.3 mL, 5 mmol). The mixture was allowed to attain 0 °C during 20 min, then quenched with H_2O (2 mL), and partitioned between Et_2O (20 mL) and H_2O (20 mL), the Et_2O layer was washed with H_2O (15 mL), dried (Na_2SO_4), and concentrated, and the the residue was subjected to chromatography on silica gel H (Merck, 8 g). Elution with an increasing proportion of Et_3N in Et_2O–light petroleum (bp 40–60 °C) (1:1, v/v) afforded the following: (1) with 1% Et_3N, (*E*,*Z*)-1-[4-[2-(dimethylamino)ethoxy]phenyl-1,2-diphenylethene (56 mg, 21%); (2) with 10% Et_3N, **24** (108 mg, 33%), mp 126–128 °C [from light petroleum (bp 80–100 °C)]. [Anal. ($C_{26}H_{29}NO_2$) C, H, N.] (3) with 20% Et_3N, the *Z* isomer of **24** (71 mg, 22%), mp 99–100 °C (from light petroleum). [Anal. ($C_{26}H_{29}NO_2$) C, H, N.]

^{1}H NMR (250 MHz, $CDCl_3$) for trans *E* isomer (**24**): δ 1.19 [d, 3, J = 6.5 Hz, $CH_3CH(OH)$], 1.7 (br s, 1, OH), 2.26 (s, 6, NMe_2), 2.62 (t, 2, J = 5.8 Hz, OCH_2CH_2N), 3.90 (t, 2, J = 5.8 Hz, OCH_2CH_2N), 4.83 [q, 1, J = 6.5 Hz, $CH_3CH(OH)$], 6.55 (d, 2, J = 8.7 Hz, H-3,5 of CC_6H_4O), 6.80 (d, 2, J = 8.7 Hz, H-2,6 of CC_6H_4O), 7.1–7.4 (m, 10, 2 Ph).

^{1}H NMR for cis *Z* isomer (60 MHz, $CDCl_3$): δ 1.19 [d, 3, J = 7 Hz, $CH_3CH(OH)$], 2.31 (s, 6, NMe_2), 2.73 (t, 2, J = 6 Hz, OCH_2CH_2N), 3.8 (br s, 1, OH), 4.07 (t, 2, J = 6 Hz, OCH_2CH_2N), 4.90 [q, 1, J = 7 Hz, $CH_3CH(OH)$], 6.75–7.30 (m, 14, aromatic protons).

***trans*-(*Z*)-1-[4-[2-(Dimethylamino)ethoxy]phenyl]-1,2-diphenyl-1-penten-5-ol (26).** To a stirred solution of **23** (360 mg, 0.85 mmol) in oxetan (1.5 mL) at −78 °C under N_2 was added *n*-butyl lithium (1.5 mmol, 1 mL of a 1.5M solution in hexane). The mixture was allowed to attain room temperature and, after 1 h, was poured into H_2O (20 mL). The product was extracted with Et_2O and subjected to chromatography as described above for **24**. A forerun containing *trans*-(*Z*)-1-[4-[2-(dimethylamino)ethoxy]phenyl]-1,2-diphenylethylene, the major product, was discarded. With 20% Et_3N, **26** (35 mg, 10%) was obtained; mp 96–98 °C [from light petroleum (bp 80–100 °C)]. Anal. ($C_{27}H_{31}NO_2$) C, H, N. With 40% Et_3N, the *E* isomer of **26** was obtained as an oil (23 mg, 7%).

^{1}H NMR (250 MHz, $CDCl_3$), for trans *Z* isomer (**26**): δ 1.59 (pent, J = 7 Hz, 2, $HOCH_2CH_2CH_2$), 1.63 (br s, 1, OH), 2.31 (s, 6, NMe_2), 2.49 (m, 2, $HOCH_2CH_2CH_2$), 2.67 (t, 2, J = 5.7 Hz, $OCH_2CH_2NMe_2$), 3.51 (t, 2, J = 6.6 Hz, $HOCH_2CH_2CH_2$), 3.94 (t, 2, J = 5.7 Hz, $OCH_2CH_2NMe_2$), 6.56 (d, 2, J = 8.8 Hz, H-3,5 of CC_6H_4O), 6.78 (d, 2, J = 8.8 Hz, H-2,6 of CC_6H_4O), 7.0–7.4 (m, 10, 2 Ph).

^{1}H NMR (60 MHz, $CDCl_3$) for cis *E* isomer: δ 2.33 (s, 6, Me_2N), 2.65 (t, 2, J = 7 Hz, $HOCH_2CH_2CH_2$), 2.74 (t, 2, J = 6 Hz, OCH_2CH_2N), 3.31 (t, 2, J = 8 Hz, $HOCH_2CH_2CH_2$), 4.09 (t, 2, J = 6 Hz, OCH_2CH_2N), ~4.5 (br s, 1, OH), 6.8–7.3 (m, 14, aromatic protons); the signal for $HOCH_2CH_2CH_2$ not detectable at 60 MHz.

***trans*-(*Z*)-1-[4-[2-(Dimethylamino)ethoxy]phenyl]-1,2-diphenyl-1-buten-4-ol (25).** To a stirred solution of **23** (341 mg, 0.8 mmol; 2:1 *E*,*Z* mixture) in dry THF (5 mL) at −78 °C under N_2 was added *n*-butyllithium (1.2 mmol, 0.8 mL of a 1.5 M solution in hexane). After 5 min, ethylene oxide (0.4 mL, 8 mmol) was added and the mixture was allowed to attain room temperature during 30 min and then poured into water (20 mL). The products were extracted with ether and subjected to chromatography as described above for **24**. With 1% Et_3N, (*E*,*Z*)-[4-[2-(dimethylamino)ethoxy]phenyl]-1,2-diphenylethene was obtained as an oil (50 mg, 18%). With 15% Et_3N, **25** (114 mg, 36%) was obtained; mp 114–115 °C [from light petroleum (bp 80–100 °C)]. Anal. ($C_{26}H_{29}NO_2$) C, H, N. With 25% Et_3N, the *Z* isomer of **25** (85 mg, 27%) was obtained; mp 112–114 °C (from light petroleum). Anal. ($C_{26}H_{29}NO_2$) C, H, N.

^{1}H NMR (60 MHz, $CDCl_3$): for the trans *Z* isomer (**25**), δ 1.75 (br s, 1, OH), 2.28 (s, 6 H, NMe_2), 2.63 (t, 2, J = 6 Hz, OCH_2CH_2N), 2.74 (t, 2, J = 7 Hz, $HOCH_2CH_2$), 3.59 (t, 2, J = 7 Hz, $HOCH_2CH_2$), 3.91 (t, 2, J = 6 Hz, OCH_2CH_2N), 6.55 (d, 2, J = 9 Hz, H-3,5 of CC_6H_4O), 6.79 (d, 2, J = 9 Hz, H-2,6 of CC_6H_4O), 7.1–7.35 (m, 10, 2 Ph); for the cis *E* isomer, δ 2.31 (s, 6, NMe_2), 2.6–2.9 (m, 5 H, OH, $HOCH_2CH_2$, OCH_2CH_2N), 3.59 (t, 2, J = 7 Hz, $HOCH_2CH_2$), 4.08 (t, 2, J = 6 Hz, OCH_2CH_2N), 6.7–7.3 (m, 14, aromatic protons.

Relative Binding Affinities (RBA).[23] Rat uterine cytosol was incubated with 5 × 10^{-9} M [^{3}H]estradiol (E_2, saturating amount) in the presence of increasing amounts (10^{-9}–10^{-5} M) of tamoxifen or hydroxytamoxifen or E_2 (control). Unbound components were then removed with dextran-coated charcoal, and the amount of ER-bound [^{3}H]-E_2 was measured. The relative concentrations of estradiol and tamoxifen or its derivatives required to achieve 50% inhibition of [^{3}H]-E_2 binding is the RBA (=$[[I_{50}]_{E2}/[I_{50}]_{TAM}]$ × 100).

Cell Culture Assays.[26] The action of tamoxifen and its derivatives on breast cancer cell lines was determined by measuring the amounts of DNA after culture for 120 h. In practice MCF-7 (ER positive) and Evsa-T (ER negative) were grown in closed Falcon plastic flasks (75 cm^2) containing Earle's minimum essential medium supplemented with L-glutamine (0.6 mg/mL), gentamycin (40 μg/mL), penicillin (100 μg/mL), streptomycin (100 μg/mL), and 10% fetal calf serum. At confluency the cells were removed by trypsinization (0.05% trypsin, 0.025% EDTA) and suspended (50–200 × 10^3 cells/mL) in the growth medium supplemented with charcoal-stripped fetal calf serum (0.5% charcoal, 0.005% dextran in 1.5 mL of medium/mL serum, incubated overnight at 4 °C). Cells were then plated in 35-mm Petri dishes

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containing the same culture medium and cultured at 37 °C in humidified air–CO_2 (95:5). After 24 h, a solution of tamoxifen or a derivative in ethanol was added (0.1% final concentration of ethanol), and after 48 h, the medium was replaced by fresh medium containing tamoxifen or a derivative for an additional 72 h. Both at 24 h (drug addition) and at 144 h (end of experiment) the cells were washed twice with Earle's base (2 mL) and suspended in trypsin–EDTA (1.5 mL). The DNA of the collected cells was precipitated with 0.5 M perchloric acid and quantified by the diphenylamine method.[34] Quadruplicate cultures were used throughout.

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Registry No. **5**, 65213-48-1; **6**, 82413-20-5; **9**, 57999-46-9; **10**, 68047-07-4; 11, 97150-94-2; **12**, 68047-06-3; **13**, 83647-29-4; 14, 97150-95-3; **15**, 97150-96-4; **15**·HCl, 97150-97-5; **17**, 97150-98-6; **18**, 78423-10-6; **19**, 97135-10-9; **20**, 97150-99-7; **21**, 96474-35-0; *trans*-(*E*)-**22**, 97151-00-3; *cis*-(*Z*)-**22**, 61923-53-3; *trans*-(*E*)-**23**, 19118-19-5; *cis*-(*Z*)-**23**, 97151-01-4; *trans*-(*E*)-**24**, 97151-02-5; *cis*-(*Z*)-**24**, 97170-41-7; *trans*-(*Z*)-**25**, 97151-03-6; *cis*-(*E*)-**25**, 97151-04-7; *trans*-(*Z*)-**26**, 97151-05-8; *cis*-(*E*)-**26**, 97151-06-9; Cl-$(CH_2)_2NMe_2$, 4584-46-7; CH_3CHO, 75-07-0; 4-(tetrahydropyran-2-yloxy)phenyl bromide, 36603-49-3; 1-[4-[2-(dimethylamino)-ethoxy]phenyl]-2-phenyl-1-[4-(tetrahydropyran-2-yloxy)-phenyl]-1-butanol, 68047-08-5; 3-(tetrahydropyran-2-yloxy)phenyl bromide, 57999-49-2; *o*-tolyl bromide, 95-46-5; 1-[4-[2-(dimethylamino)ethoxy]phenyl]-1-(2-methylphenyl)-2-phenyl-1-butanol, 97151-07-0; 2,4-bis(tetrahydropyran-2-yloxy)phenyl bromide, 31963-61-8; 1-[2,4-bis(tetrahydropyran-2-yloxy)-phenyl]-1-[4-[2-(dimethylamino)ethoxy]phenyl]-2-phenyl-1-butanol, 97170-42-8; 2-methyl-4-(tetrahydropyran-2-yloxy)phenyl bromide, 97151-08-1; 1-[4-[2-(dimethylamino)ethoxy]phenyl]-1-[4-(tetrahydropyran-2-yloxy)-2-methylphenyl]-2-phenyl-1-butanol, 97151-09-2; (*E*)-1-[4-[2-(dimethylamino)ethoxy]phenyl]-1,2-diphenylethene, 97151-10-5; (*Z*)-1-[4-[2-(dimethylamino)ethoxy]phenyl]-1,2-diphenylethene, 97151-11-6; oxetane, 503-30-0; ethylene oxide, 75-21-8.

Structural Studies on Some Tamoxifen Derivatives

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The crystal structures of four derivatives of the antiestrogenic drug tamoxifen are described. These are of 2-hydroxy-, 3-hydroxy-, and 2-methyl-4-hydroxytamoxifen and of 1-(4-methoxyphenyl)-2-phenyl-1-[(tetrahydropyran-2-yloxy)phenyl]-1-butanol, the synthetic precursor to 2-hydroxytamoxifen. All compounds have trans stereochemistry about the ethylene double bond, as in tamoxifen itself. The orientations of the hydroxy substituents have been found to differ by 180°, depending on the nature of the compound. Empirical energy calculations have been used to show that the barrier to free rotation for the hydroxy-substituted phenyl rings is too high for interconversion to take place. These orientational differences are, it is suggested, related to the marked differences in estrogen receptor binding ability.

The accompanying paper reports synthesis, receptor-binding, and biological data for *trans*-tamoxifen derivatives with hydroxy and methyl substitution at several positions on the phenyl ring cis to the ethyl group on the ethene moiety. This study reports the molecular structures of several of these: 2- and 3-hydroxy- and 2-methyl-4-hydroxytamoxifen, as well as that of the synthetic precursor to the 2-hydroxy compound, 1-(4-methoxyphenyl)-1-[2-(tetrahydropyran-2-yloxy)phenyl]-2-phenyl-1-butanol. All have been determined by X-ray crystallography. The conformational dynamics of these molecules has been examined by semiempirical energy calculations in order to ascertain (i) whether the ground-state crystal structures correspond to unique energy minima and (ii) what the energy pathways between different conformers are. These studies have defined the stereochemistries of the derivatives and their relationships to both isomerization properties and estrogen receptor binding abilities.[1]

The molecular structures of a number of tamoxifen derivatives have now been determined. Both parents compounds *cis*-[2] and *trans*-tamoxifen[3] have been examined, as well as their 4-iodo derivatives,[4] and a synthetic precursor to *trans*-tamoxifen that contains only the methoxy part of the full side chain.[5] Both clomiphene[6] (with chlorine replacing the ethyl group) and the 1-(*p*-2-pyrrolidinineethoxyphenyl) derivative nafoxidine[7] have antiestrogenic activity and retain the trans arrangement.

These molecular structures have been examined in terms of their possible interrelationships[7] and also in relation to the structures of the estrogens estradiol and diethylstilbesterol.[8]

Experimental Section

Crystal Data. The 2-hydroxy and 2-methyl-4-hydroxy compounds were recrystallized from methanol; the 3-hydroxy com-

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