Cellular Uptake of Rhodamine 123 Derivatives. Chinese hamster V79 lung fibroblasts and human prostate PC3 adenocarcinoma cells were grown as monolayers at 37 °C in minimum essential medium (MEM) supplemented with L-glutamine (2 mM), penicillin (50 units/mL), streptomycin (50 μg/mL), 1% nonessential amino acids, and 15% fetal bovine calf serum (plating efficiency 60–80%).

Cell size determinations were carried out by using a Coulter Counter (Model Z_F) in conjunction with a Coulter Channelyzer (Model 256).

For the fluorescence microscopy studies, cells were grown overnight as monolayers on glass cover slips. The rhodamine 123 derivatives were dissolved in methanol (10 mM) and diluted (1:100) in 0.1 M phosphate buffered saline, pH 7.2 (PBS). The cells were rinsed gently with PBS, incubated for 1 h at 37 °C in the presence of the rhodamine 123 derivatives, washed with PBS, observed under the fluorescence microscope (Zeiss), and photographed.

Cellular Uptake of [^{125}I]Rhodamine 123 Derivatives. Logarithmically growing cells were trypsinized and suspended in calcium-free MEM (supplemented as above), the cell number was determined and adjusted to 2×10^5/mL, and 1-mL portions were dispensed into sterile tubes. Following a 4-h incubation at 37 °C on a roller shaker (Fisher Scientific), the radioiodinated dihydrorhodamine derivatives, dissolved in the same medium, were added. The cells were reincubated at 37 °C for 2 h, and their radioactive content was determined by the microfuge method.[26]

Note Added in Proof: Since the submission of this paper, we have used the radioiodination procedure described herein with carrier-free Na^{123}I (Atomic Energy Commission of Canada) and have obtained comparable results.

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Registry No. 1, 62669-70-9; 2, 1239-45-8; 3, 38483-26-0; 4, 109244-58-8; 5, 109244-59-9; 5 (I-125 labeled), 109244-63-5; 6, 109282-63-5; 6 (I-125 labeled), 109244-64-6; 7, 109244-60-2; 7 (I-125 labeled, 109244-65-7; 8, 109244-61-3; 8 (I-125 labeled), 109244-66-8; 9, 109244-62-4; 9 (I-125 labeled), 109244-67-9.

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Synthesis, Conformational Considerations, and Estrogen Receptor Binding of Diastereoisomers and Enantiomers of 1-[4-[2-(Dimethylamino)ethoxy]phenyl]-1,2-diphenylbutane (Dihydrotamoxifen)

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As part of a study into nonisomerizable antiestrogens, the diastereoisomeric dihydrotamoxifens **7** and **8** were prepared by catalytic transfer hydrogenation of (*Z*)- and (*E*)-tamoxifen and were shown by NMR spectrometry to exist in preferred conformations with hydrogen atoms in an antiperiplanar relationship. The corresponding 4-hydroxy derivatives **9** and **10** were prepared from hydrogenated precursors of (*Z*)- and (*E*)-4-hydroxytamoxifen. The relative binding affinities (RBA) of the compounds to estrogen receptors are consistent with the assigned conformations and parallel reported data on derivatives of the nonsteroidal estrogen hexestrol. The growth-inhibitory activity against the MCF-7 human breast cancer cell line in vitro was for **10** comparable to that of 4-hydroxytamoxifen, although increasing the concentration from 10^{-8} to 10^{-6} M did not significantly improve the growth inhibition. The derivative **9** analogous to (*E*)-4-hydroxytamoxifen antagonized the growth-stimulating effect of added estradiol and is therefore also an antiestrogen but at low concentration (10^{-8} M) in the absence of estradiol, MCF-7 cell growth was stimulated, indicating an estrogenic influence. The enantiomers of the dihydrotamoxifen **8** were individually prepared from the resolved enantiomers of 2-phenylbutanoic acid, the key reaction step being a lithium–ammonia reduction of the 1-(4-methoxyphenyl)-1,2-diphenyl-1-butanol to generate the triphenylbutane. The enantiomers of **8** gave identical RBA values in cytosol.

Appropriately substituted triarylethylene derivatives have antiestrogenic activity.[1] Of these, tamoxifen (**1**) is currently in clinical use for the treatment of estrogen-dependent breast cancer.[2] Its principal action is its competition with the natural hormone estradiol for binding to the estrogen receptor protein (ER), thereby reducing the ability of estradiol to stimulate nuclear transcription and consequent cell growth. On the other hand, the *E* isomer **2** of tamoxifen is an estrogen agonist.[3,4] Among the metabolites of tamoxifen detected in patients, the 4-hydroxylated derivative **3**[5,6] has a much higher potency in vitro than tamoxifen.[7,8] This derivative can undergo a facile isomerization as a consequence of conjugation between the central double bond and the hydroxyl group to give a mixture containing the *E* isomer **4**,[9-11] a process that complicates the metabolism picture. Moreover, whether the *E* isomer **4** is an estrogen agonist or antagonist is uncertain since it partially reverts to the more active *Z* isomer **3** during cell-culture experiments.[12,13]

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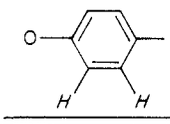
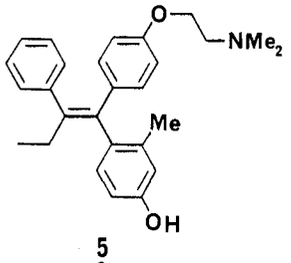
Table I. Selected Proton NMR Chemical Shift Data for Dihydrotamoxifens Compared with Tamoxifen Derivatives

		δ_H ($CDCl_3$)				
compound		Me_2N	CH_2	CH_2		
(*Z*)-tamoxifen	**1**	2.26	2.59	3.89	6.62	6.77
	8	2.26	2.62	3.88	6.60	7.00
(*E*)-tamoxifen	**2**	2.31	2.70	4.05	6.88	7.13
	7	2.32	2.70	4.03	6.87	7.29
(*Z*)-4-hydroxytamoxifen	**3**	2.35	2.73	3.91	6.29	6.69
	10	2.30	2.67	3.89	6.51	6.92
(*E*)-4-hydroxytamoxifen	**4**	2.36	2.78	4.03	6.63	6.68
	9	2.33	2.72	4.02	6.83	7.23

We sought analogues that do not isomerize in order to enable more reliable structure–activity relationships to be established and to simplify the metabolism profile. Since the clinical success of tamoxifen has been attributed largely to its low incidence of side effects, a property not shared by the related dihydronaphthalene derivative nafoxidine,[1] we aimed to prepare analogues bearing stereochemical features as close as possible to those of tamoxifen. Our finding that 2-methyl-4-hydroxytamoxifen (**5**) was resistant to isomerization yet retained the antitumor potency of 4-hydroxytamoxifen[14] initially prompted this work. Subsequently we were able to prepare the seven-membered ring analogue **6**, which was shown by X-ray crystallography



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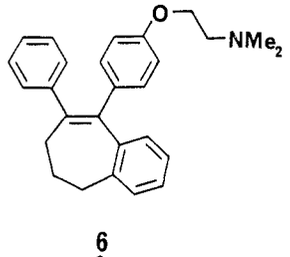


6

to have its phenyl rings in orientations closer to those of tamoxifen than do other known fused ring analogues.[15] We now report that the easily prepared dihydrotamoxifens derived by saturation of the ethylene linkage of tamoxifen represent alternative nonisomerizable tamoxifen analogues.

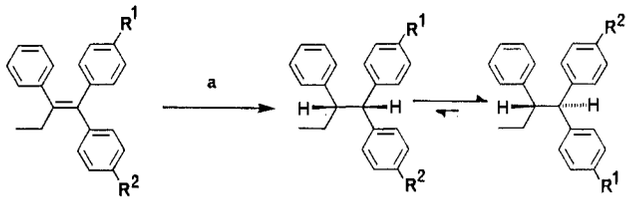
We have studied the racemic diastereoisomers of dihydrotamoxifen and their 4-hydroxy derivatives. In addition, in order to determine in detail the importance of the configuration of the triarylbutane, we have also studied the individual enantiomers of that diastereoisomer of dihydrotamoxifen which is analogous to (*Z*)-tamoxifen.

Although the preparation of racemic dihydrotamoxifens has been reported in a patent by Imperial Chemical Industries,[16] no biological properties or conformational studies of these compounds have been reported.

Results and Discussion

Synthesis of Diastereoisomeric Dihydrotamoxifens. Catalytic reduction of tamoxifen (**1**) and its *E* isomer **2** was conveniently carried out (Scheme I) under atmospheric pressure using ammonium formate as a hydrogen transfer agent.[17] The products **7** and **8**, respectively, were crystalline. The corresponding 4-hydroxy derivatives **9** and **10** were not prepared from (*Z*)- and (*E*)-4-hydroxytamoxifen because of the likelihood of isomerization of the starting material during the reduction. The preferred procedure was to reduce the (chloroethoxy)perfluorotolyl ethers **11** and **12**, which are easily prepared as a mixture of *Z* and *E* isomers that are separable by column chromatography and which have been used as intermediates in a synthesis of pure *Z* and *E* isomers of 4-hydroxytamoxifen.[18] The reduction products **13** and **14** were converted into **9** and **10** by treatment first with dimethylamine, which replaces chlorine by dimethylamino, and then with sodium methoxide in dimethylformamide, which cleaves the (perfluorotolyl)oxy function to generate the hydroxy group.

Scheme I. Synthesis of Racemic Dihydrotamoxifens[a]



1–**4**, **11**–**12** **7**–**10**, **13**–**15**

Triarylbutenes	R^1	R^2	Triarylbutanes
1	$-OCH_2CH_2NMe_2$	-H	**7**
2	-H	$-OCH_2CH_2NMe_2$	**8**
3	$-OCH_2CH_2NMe_2$	-OH	**9**
4	-OH	$-OCH_2CH_2NMe_2$	**10**
11	$-OCH_2CH_2Cl$	$-OC_6F_4CF_3$	**13**
12	$-OC_6F_4CF_3$	$-OCH_2CH_2Cl$	**14**

[a] Reagents: a, $NH_4^+HCOO^-$, Pd/C, EtOH, 80 °C.

Conformational Analysis Using NMR Spectrometry. The proton NMR spectrum of the dihydrotamoxifen **7** prepared from (*Z*)-tamoxifen had several features in common with that of (*E*)-tamoxifen and the spectrum of **8** resembled that of (*Z*)-tamoxifen (see Table I). Thus, diastereoisomer **8** had the OCH_2 resonance and the signals for the AB quartet of the disubstituted ring protons at the higher field. These features that are a consequence of the shielding influence of two adjacent phenyl rings in the

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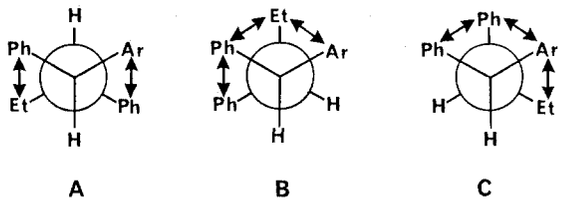
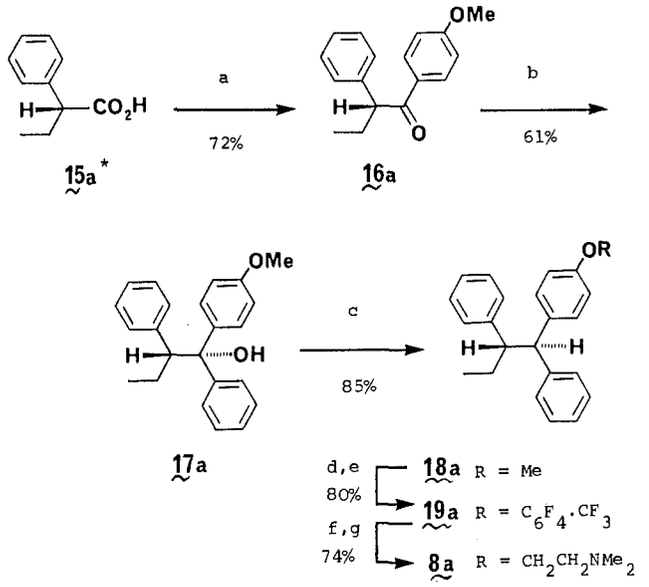


Figure 1. Conformations of the dihydrotamoxifen 8. Ar = $C_6H_4OCH_2CH_2NMe_2$. Interactions between non-hydrogen atoms are indicated by double headed arrows.

Scheme II. Synthesis of Enantiomers of Dihydrotamoxifen[a]



[a] Reagents: (a) C_6H_5OMe, $(CF_3CO)_2O$, 22 °C; (b) PhMgBr, Et_2O, 22 °C; (c) Na, NH_3(l), *t*-BuOH, THF, −33 °C; (d) Py·HCl, 220 °C; (e) C_7F_8, $Bu_4N^+HSO_4^-$, NaOH (aq), CH_2Cl_2, 22 °C; (f) NaOMe, DMF, 35 °C; (g) $Me_2NCH_2CH_2Cl$·HCl, NaH, DMF, 60 °C. Compounds **15b–19b** are enantiomers of the above (*).

isomer 8 but not in 7 have been amply demonstrated in the triphenylbutenes.[19,20] Bearing in mind that hydrogenation must have occurred by addition of the hydrogen atoms onto the same face of the double bond, the favored conformations must be as indicated in Scheme I. Also the high value of ~11.5 Hz in either isomer for the coupling constant between the benzylic hydrogen atoms indicates, by reference to the Karplus equation,[21] that these atoms are in an antiperiplanar orientation. The spectra of the 4-hydroxy derivatives **9** and **10** showed similar features (Table I).

The conformational preferences of the dihydrotamoxifens can be explained by consideration of Newman projections (Figure 1) which are illustrated for 8. In the two conformers in which the hydrogen atoms are in a synclinal arrangement (conformers B and C in Figure 1), the bulky groups are compacted together with consequent unfavorable steric repulsions. These interactions are less in conformer A, which is consequently favored. Similar arguments have been put forward to explain the favored conformations of hexestrol.[22]

Enantiomers of Dihydrotamoxifen. It was anticipated that attempts to find procedures for the enantios-

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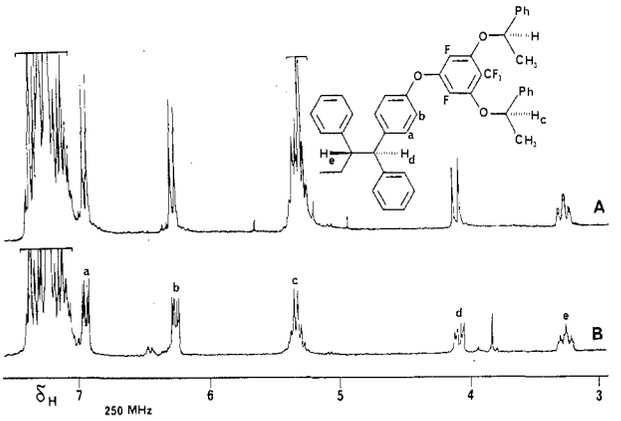

Figure 2. Part of the proton NMR spectra of **20** (spectrum A) and of the product from reaction of a 1:1 mixture of **19a** and **19b** with (+)-PhCH(OH)Me (spectrum B).

Table II. Relative Binding Affinities to Estrogen Receptor

compd[a]	config	calf uterine cytosol[b]	MCF-7 whole cells[b]
7	*rel*-(1*R*,2*R*)	<0.01	
8	*rel*-(1*R*,2*S*)	1.0	
8a	(1*R*,2*S*)	1.0	0.02
8b	(1*S*,2*R*)	1.0	0.04
9	*rel*-(1*R*,2*R*)	4.0	0.02
10	*rel*-(1*R*,2*S*)	100	0.6

[a] Compounds other than **8a** and **8b** are racemic. RBA values for triarylbutenes, cytosol/whole cell: (*Z*)-tamoxifen (**1**) = 1.0/0.06, (*E*)-tamoxifen (**2**) = ~0.1/~0.01, (*Z*)-4-hydroxytamoxifen (**3**) = 100/2.9, 2-methyl-4-hydroxytamoxifen (**5**) = 100/0.4. [b] Estradiol = 100.

elective hydrogenation of a triarylbutene would prove difficult. It was also considered difficult to resolve the enantiomers of the final product since there is no functional group close to the chiral centers. For these reasons, a new synthesis of dihydrotamoxifen was devised. 2-Phenylbutanoic acid was resolved by the method of Vigneron and Bloy[23] except that an enantiomerically purer product was obtained following recrystallization from ethanol of the intermediate salt that was formed with chiral α-methylbenzylamine. Scheme II shows the reaction sequence from the (−)-acid **15a**. Friedel–Crafts acylation of anisole by this acid gave the (−)-ketone **16a**. That racemization had not occurred was verified by examination of the NMR spectrum of **16a** in the presence of a chiral europium shift reagent. Reaction of **16a** with phenylmagnesium bromide gave the expected tertiary alcohol **17a**, which was reduced with lithium in liquid ammonia. The required diastereoisomer **18a** was the major product formed with a surprising degree of stereoselectivity (up to 13:1). The methyl ether group in **18a** was cleaved by pyridine hydrochloride. Conversion of the resulting phenol into its perfluorotolyl ether **19a** enabled removal of the minor proportion of the unwanted diastereoisomer by column chromatography. Also, reaction of a portion of the perfluorotolyl ether with (+)-1-phenylethanol in the presence of sodium hydride gave a product **20a**, the NMR spectrum of which (Figure 2), by comparison with that of a corresponding product obtained from a mixture of enantiomers, confirmed that **19a** was a pure enantiomer. Cleavage of **19a** with sodium methoxide, followed by dimethylaminoethylation, gave the chiral dihydrotamoxifen (**8a**). Its enantiomer (**8b**) was prepared similarly from (+)-2-phenylbutanoic acid (**15b**). Since the absolute configurations of the starting α-phenylbutanoic acids are

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Table III. Effect of Hydroxydihydrotamoxifens 9 and 10 on MCF-7 Cell Growth

		opt density (mean ± SD)[a]	
	concn, M	compd alone[c]	compd + 10^{-8} M estradiol
9	control	0.376 ± 0.072 (100)[b]	0.697 ± 0.122 (185)
	10^{-8}	0.557 ± 0.036 (148)	0.696 ± 0.061 (185)
	10^{-7}	0.486 ± 0.022 (129)	0.607 ± 0.044 (161)
	10^{-6}	0.428 ± 0.034 (114)	0.410 ± 0.111 (109)
10	control	0.328 ± 0.069 (100)	0.620 ± 0.115 (189)
	10^{-8}	0.121 ± 0.017 (37)	0.229 ± 0.011 (70)
	10^{-7}	0.120 ± 0.029 (37)	0.138 ± 0.026 (42)
	10^{-6}	0.131 ± 0.025 (40)	0.108 ± 0.055 (33)

[a] Each value corresponds to the mean of optical density measurements from four separate samples of culture. Values of optical density thus obtained are approximately proportional to cell numbers.[32] [b] Percentage of control value. [c] Effect of 4-hydroxytamoxifen using an identical experimental protocol: control, 0.453 ± 0.014 (100); 10^{-8} M, 0.218 ± 0.061 (48); 10^{-7} M, 0.171 ± 0.032 (38); 10^{-6} M; 0.067 ± 0.020 (15).

known,[24] there is no doubt as to the absolute configuration of the products.

Receptor Binding Affinity (RBA) Determinations and Effect on MCF-7 Cell Growth. Table II gives values for relative binding affinity to estrogen receptors in calf uterine cytosol and in MCF-7 whole cells. The cytosol RBA figures of the dihydrotamoxifens reflect the assigned conformations with the result that the RBA values of the *rel*-(1*R*,2*R*) and *rel*-(1*R*,2*S*) diastereoisomers were respectively virtually identical with those of the corresponding (*E*)- and (*Z*)-triarylbutenes. As with the triarylbutenes, para-hydroxylation of the 1-phenyl ring of the dihydrotamoxifens gave a 100-fold increase in the binding affinity. The enantiomers of the dihydrotamoxifen 8 both gave the same RBA as tamoxifen in the cytosol test, indicating that the central part of the molecule is not of importance in the interaction of the compound with the receptor. This conclusion is in agreement with the observation that epoxidation of the ethylene bond of tamoxifen does not modify the binding affinity (unpublished data).

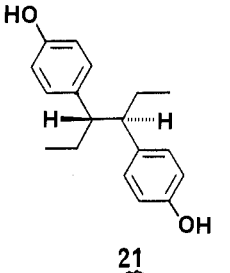
The recently developed[25] whole-cell RBA assay is complementary to the cytosol assay. Whereas the cytosol assay provides a measure of the binding of a compound to the receptor, the whole-cell assay gives a measure of its potency to activate the receptor.[25] While steroidal estrogens usually display similar values in both assays, the triarylbutanes described here give lower values in the whole-cell assay than they do in the cytosol assay, in common with values that have been reported for triarylbutene antiestrogens.[25] Interestingly, the whole-cell RBA of the 4-hydroxydihydrotamoxifen **10** (0.6) was significantly less than that of (*Z*)-4-hydroxytamoxifen **3** (2.9). A small difference was observed in the whole-cell RBA values of enantiomers of the dihydrotamoxifen 8 which may reflect a slight difference in activation potency not detectable in the cytosol assay.

Table III shows that the dihydrotamoxifen **10** analogous to (*Z*)-4-hydroxytamoxifen significantly inhibits the growth of MCF-7 cells in culture at 10^{-8} M and is able to antagonize the growth-stimulating effect of 10^{-8} M estradiol. However, in contrast to 4-hydroxytamoxifen,[14] increasing the concentration of **10** above 10^{-8} M in the absence of estradiol did not improve the growth inhibition, indicating that the ER–**10** complex still allows a certain level of growth. The dihydrotamoxifen **9** analogous to (*E*)-4-hydroxytamoxifen when added in 100-fold excess of estradiol suppressed the growth stimulation of the hormone, indicating a weak antiestrogenic activity. However, at low concentration (10^{-8} M), in the absence of added estradiol, the compound stimulated cell growth. At higher concentration its growth-promoting influence was reduced, presumably owing to the antiestrogenic component of its action.

Conclusions

The dihydrotamoxifens adopt favored conformations with the benzylic hydrogen atoms in an antiperiplanar orientation with the consequences that (i) the relative orientation of the substituents is determined by the relative configuration at the benzylic carbon atom and (ii) the biologically more potent diastereoisomer 8 is obtained by hydrogenation of the *E* isomer of tamoxifen. Since the relative binding affinities of the dihydrotamoxifens to estrogen receptors correlate with those of tamoxifen isomers of corresponding configuration and since the 4-hydroxy isomers **9** and **10** cannot interconvert, the dihydrotamoxifens **7**–**10** can be considered as nonisomerizable equivalents of (*E*)- and (*Z*)-tamoxifen and (*E*)- and (*Z*)-4-hydroxytamoxifen, respectively. Advantages of the nonisomerizability of **9** and **10** are that more reliable structure–activity relationships can be established and that the metabolism profile of their nonhydroxylated metabolic precursors should be simplified.

Since the RBA of the isomer **7** was much higher than that of (*E*)-tamoxifen, conformations analogous to B and C in Figure 1 cannot make any significant contribution to the biological activity which correlates with the favored conformation in solution. A similar situation has been reported in examination of the RBA of diastereoisomers of hexestrol where the meso form (**21**) has a much greater affinity to ER than its optically active diastereoisomers (*RR*)- and (*SS*)-hexestrol, explainable by the closer conformational similarity of *meso*-hexestrol to estradiol.[22] The result that the enantiomers of the dihydrotamoxifen 8 had the same relative binding affinity to cytosol ER and a similar affinity to ER in whole cells is also consistent with observations in the hexestrol series, although the derivative (**22**) had a 14 times greater affinity to ER than its enantiomer owing to an interaction between the receptor and the carboxylate function.[26,27] The conclusion can be made that the detailed stereochemistry of the central part of the molecule is not a parameter in determining binding affinity. The primary role of the molecular framework in the triarylbutanes is to hold the appropriate functionalities and phenyl rings at the correct spacing and in the correct relative orientation.



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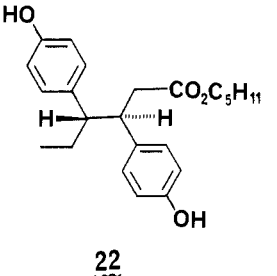

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The results of this study further show that the whole-cell RBA assay can give values that are more sensitive to

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structural variation than are cytosol RBA figures. Thus, although **10** and 4-hydroxytamoxifen give essentially the same cytosol RBA, there is an approximately fivefold lower RBA in the whole-cell assay of the 4-hydroxydihydrotamoxifen **10** (RBA = 0.6) compared with 4-hydroxytamoxifen (**3**) (RBA = 2.9). The difference seen can be accounted for by the absence of conjugation throughout the molecule in the dihydrotamoxifens. This reduced conjugation may influence the charge distribution of the molecule and/or alter the ability of the hydroxyl group to donate a hydrogen bond. A similarly reduced whole-cell relative binding affinity was recorded for 2-methyl-4-hydroxytamoxifen (**5**) (RBA = 0.4).[25] In this analogue, the 2-methyl group would be expected to inhibit conjugation between the hydroxyl group and the double bond because its steric bulk prevents the phenyl ring bearing the hydroxy group from approaching planarity with the ethylene linkage. However, there was no correlation between the whole-cell RBA data and the ability of the compounds to inhibit the growth of the MCF-7 cell line in culture. Therefore in the present study, as in a previous study,[28] the relevance of the whole-cell assay is unknown.

The diastereoisomer **9** analogous to (*E*)-4-hydroxytamoxifen displayed both definite antiestrogenic and estrogenic properties. The antiestrogenic effect is apparent at 10^{-6} M in the presence of estradiol and is presumably due to competitive displacement of estradiol from its binding site. Since the estrogenic effect occurs at low concentration (10^{-8} M) and since the compound has a low affinity for ER, it seems that this action does not take place by way of binding of **9** to the estradiol binding site but through an as yet unknown mechanism. One of a number of reasons may explain this phenomenon. It may reflect that the regulation of estrogen-stimulated growth is not simply confined to binding at a single site, rather that a complex regulatory mechanism is present, possibly related to observations that the estrogen receptor is an allosteric protein,[29] or may be due to easily saturable metabolism of **9** by the MCF-7 cells to give an estrogenic compound. Whether these properties are shown by (*E*)-4-hydroxytamoxifen cannot be determined because of isomerization to the more potent *Z* isomer during cell-culture experiments. In order to study processes such as those mentioned above through structure–activity studies, it is essential that the compounds are configurationally stable. For this purpose, triarylbutanes such as **9** and **10** are better suited than the configurationally labile hydroxylated triarylbutenes, such as 4-hydroxytamoxifen.

Experimental Section

Chemical Methods. General Procedures. ^{1}H NMR 60-MHz spectra (internal Me_4Si) were obtained with a Perkin-Elmer R12B spectrometer and 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. Optical rotations were recorded with a Perkin-Elmer 141 polarimeter. Melting points were determined on a Kofler hot stage. Chromatography refers to column chromatography on silica gel (Merck 15111) with the eluant indicated applied at a positive pressure of 0.5 atm.

Hydrogenation of (*E*)- and (*Z*)-Tamoxifen. Preparation of Racemic Dihydrotamoxifens. (a) *rel*-(1*R*,2*S*)-1-[4-[2-(Dimethylamino)ethoxy]phenyl]-1,2-diphenylbutane (8). A solution of (*E*)-tamoxifen (1.526 g, 4.1 mmol) in EtOH (40 mL) containing 10% palladium on charcoal (300 mg) and ammonium formate (3.0 g, 48 mmol) was boiled under reflux for 2.5 h. Further ammonium formate (1.5 g, 24 mmol) was added and reflux continued for a further 2 h. The mixture was then cooled and filtered through Celite, and the filtrate was diluted with H_2O (100 mL) and extracted with Et_2O (100 mL). The Et_2O solution was washed with H_2O (100 mL), dried (Na_2SO_4), and concentrated. The residue was dissolved in petroleum ether (bp 40–60 °C). Cooling to −20 °C gave **8** (1.275 g, 83%): mp 96–99 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.63 (t, 3, J = 7.3 Hz, CH_3CH_2), 1.36–1.50 (m, 1, CH_3CH_2), 1.6–1.75 (m, 1, CH_3CH_2), 2.26 (s, 6, NMe_2), 2.62 (t, 2, J = 5.8 Hz, OCH_2CH_2N), 3.25 (dt, 2, J = 3.4, 11 Hz, CH_3CH_2CH), 3.88 (t, 2, J = 5.8 Hz, OCH_2CH_2N), 4.05 (d, 1, J = 11.6 Hz, $CHAr_2$), 6.60 (d, 2, J = 8.7 Hz, ArH ortho to OR), 7.00 (d, 2, J = 8.7 Hz, ArH meta to OR), 7.02–7.40 (m, 10, remaining ArH); mass spectrum, m/z 373 (M^+, 2), 254 (M^+ − PhCHEt, 22), 72 (100), 58 (85). Anal. ($C_{26}H_{31}NO$) C, H, N.

(b) *rel*-(1*R*,2*R*)-1-[4-[2-(Dimethylamino)ethoxy]phenyl]-1,2-diphenylbutane (7). Hydrogenation of (*Z*)-tamoxifen (502 mg) by the above method gave **7** (253 mg, 50%): mp 60–62 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.64 (t, 3, J = 7.3 Hz, CH_3CH_2), 1.35–1.55 (m, 1, CH_3CH_2), 1.6–1.8 (m, 1, CH_3CH_2), 2.32 (s, 6, NMe_2), 2.70 (t, 2, J = 5.8 Hz, OCH_2CH_2N), 3.25 (dt, 2, J = 3.3, 11.1 Hz, CH_3CH_2CH), 4.03 (t, 2, J = 5.8 Hz, OCH_2CH_2N), 4.06 (d, 1, J = 11 Hz, $CHAr_2$), 6.87 (m, 10, ArH), 7.29 (d, 2, J = 8.7 Hz, ArH meta to OR); mass spectrum, m/z 373 (M^+, 1), 254 (M^+ − PhCHEt, 21), 72 (100), and 58 (72). Anal. ($C_{26}H_{31}NO$) C, H, N.

Hydrogenation of (*Z*)- and (*E*)-1-[4-(2-Chloroethoxy)phenyl]-1-[4-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy]phenyl]-2-phenyl-1-butene (12) and (11). (a) *rel*-(1*R*,2*R*)-1-[4-(2-Chloroethoxy)phenyl]-1-[4-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy]phenyl]-2-phenylbutane (14). Hydrogenation of **12**[18] (1.248 g) by the method described above for the hydrogenation of (*E*)-tamoxifen gave **14** (1.033 g, 83%): mp 103 °C (from petroleum ether, bp 40–60 °C); ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.65 (t, 3, J = 7.3 Hz, CH_3CH_2), 1.35–1.5 (m, 1, CH_3CH_2), 1.55–1.75 (m, 1, CH_3CH_2), 3.21 (dt, 1, J = 3.3, 11 Hz, CH_3CH_2CH), 3.70 (t, 2, J = 5.9 Hz, OCH_2CH_2Cl), 4.06 (t, 2, J = 5.9 Hz, OCH_2CH_2Cl), 4.08 (d, 1, J = 11 Hz, $CHAr_2$), 6.60 (d, 2, J = 8.7 Hz, ArH ortho to OCH_2CH_2Cl), 6.9–7.2 (m, 9, ArH), 7.33 (d, 2, J = 8.7 Hz, ArH meta to OC_7F_7); mass spectrum, m/z 477 (M^+ − PhCHEt, 100), 415 (10), and 91 (10); M^+ not observed. Anal. ($C_{31}H_{25}ClF_7O_2$) C, H. Found: Cl, 5.99; F, 21.75. Calcd: Cl, 5.36; F, 22.27.

(b) *rel*-(1*R*,2*S*)-1-[4-(2-Chloroethoxy)phenyl]-1-[4-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy]phenyl]-2-phenylbutane (13). Hydrogenation of **11**[18] (730 mg) by the method described above for the hydrogenation of (*E*)-tamoxifen gave **13** (653 mg, 89%): mp 104 °C (from petroleum ether, bp 40–60 °C); ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.65 (t, 3, J = 7.3 Hz, CH_3CH_2), 1.35–1.55 (m, 1, CH_3CH_2), 1.6–1.8 (m, 1, CH_3CH_2), 3.18 (dt, 1, J = 3.3, 11 Hz, CH_3CH_2CH), 3.80 (t, 2, J = 5.9 Hz, OCH_2CH_2Cl), 4.04 (d, 1, J = 11.5 Hz, $CHAr_2$), 4.21 (t, 2, J = 5.9 Hz, OCH_2CH_2Cl), 6.66 (d, 2, J = 8.7 Hz, ArH ortho to OC_7F_7), 6.88 (d, 2, J = 8.7 Hz, ArH ortho to OCH_2CH_2Cl), 7.01 (d, 2, J = 8.7 Hz, ArH meta to OC_7F_7), 7.0–7.2 (m, 5, Ph), 7.28 (d, 2, J = 8.7 Hz, ArH meta to OCH_2CH_2Cl); mass spectrum, m/z 477 (M^+ − PhCHEt, 100), 415 (13), 91 (44), 63 (28). Anal. ($C_{31}H_{24}ClF_7O_2$) C, H, F; Cl: found, 5.83; required, 5.36.

Preparation of Racemic 4-Hydroxydihydrotamoxifens. (a) *rel*-(1*R*,2*S*)-1-[4-[2-(Dimethylamino)ethoxy]phenyl]-1-(4-hydroxyphenyl)-2-phenylbutane (10). A solution of **14** (670 mg) in 33% w/v Me_2NH in EtOH (35 mL) was boiled under reflux for 72 h. The mixture was concentrated to dryness and the residue dissolved in Me_2NCHO (10 mL). NaOMe (2.0 g) was added and the mixture stirred at 40 °C for 2 h, then poured into saturated aqueous $NaHCO_3$ (60 mL), and extracted with Et_2O (2 × 40 mL). The Et_2O solutions were washed with H_2O (50 mL), dried (Na_2SO_4), and concentrated, and the residue was recrystallized from petroleum ether (bp 80–100 °C)–$CHCl_3$ (2:1) to give **10** (346 mg, 80%): mp 168 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.63 (t, 3, J = 7.3 Hz, CH_3CH_2), 1.3–1.5 (m, 1, CH_3CH_2), 1.6–1.8 (m, 1, CH_3CH_2), 2.30 (s, 6, NMe_2), 2.67 (t, 2, J = 5.8 Hz, OCH_2CH_2N), 3.17 (dt, 1, J = 3.3, 11 Hz, CH_3CH_2CH), 3.89 (t, 2, J = 5.8 Hz, OCH_2CH_2N), 3.97 (d, 1, J = 11.4 Hz, $CHAr_2$), 6.51 (d, 2, J = 8.7 Hz, ArH ortho to OR), 6.72 (d, 2, J = 8.5 Hz, ArH ortho to OH),

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6.92 (d, 2, J = 8.7 Hz, Ar*H* meta to OR), 7.0–7.25 (m, 5, Ph), 7.17 (d, 2, J = 8.5 Hz, Ar*H* meta to OH); mass spectrum, m/z 270 (M^+ – PhCHEt, 17), 72 (100), 58 (62); M^+ not observed. Anal. ($C_{26}H_{31}NO_2$) C, H, N.

(b) *rel*-(1*R*,2*R*)-1-[4-[2-(Dimethylamino)ethoxy]phenyl]-1-(4-hydroxyphenyl)-2-phenylbutane (9). Treatment of **13** (224 mg) with Me_2NH and then NaOMe as described above in (a) gave **9** (113 mg, 79%): mp 134–135 °C (from petroleum ether, bp 80–100 °C); ^1H NMR (250 MHz, $CDCl_3$) δ 0.63 (t, 3, J = 7.3 Hz, CH_3CH_2), 2.33 (s, 6, NMe_2), 2.72 (t, 2, J = 5.8 Hz, $OCH_2CH_2NMe_2$), 3.18 (dt, 1, J = 3.3, 11 Hz, CH_3CH_2CH), 3.98 (d, 1, J = 11.5 Hz, $CHAr_2$), 4.02 (t, 2, J = 5.8 Hz, $OCH_2CH_2NMe_2$), 6.48 (d, 2, J = 8.6 Hz, Ar*H* ortho to OH), 6.83 (d, 2, J = 8.7 Hz, Ar*H* ortho to OR), 6.91 (d, 2, J = 8.6 Hz, Ar*H* meta to OH), 7.0–7.2 (m, 5, Ph), 7.23 (d, 2, J = 8.7 Hz, Ar*H* (m, 5, Ph), 7.23 (d, 2, J = 8.7 Hz, Ar*H* meta to OR). Anal. ($C_{26}H_{31}NO_2$) C, H, N.

Resolution of 2-Phenylbutanoic Acid.[23] A solution of racemic 2-phenylbutanoic acid (162.6 g, 0.99 mmol) in Et_2O (2.5 L) was treated with a solution of (+)-α-methylbenzylamine (60 g, 0.49 mmol) in ether (0.4 L) and the mixture shaken vigorously for 5 min. The precipitated salt was collected by filtration and washed with ether (1 L). The filtrates were set aside. The salt (74.2 g) was partitioned between aqueous HCl (2 M; 200 mL, 0.4 mol) and ether (200 mL). The ether solution was concentrated and the residual oil distilled to give (*R*)-(–)-2-phenylbutanoic acid (**15a**, 35.1 g), bp 108 °C at 1 mmHg: $[\alpha]^{22}_D$ –91.1° (*c* 1, C_6H_6), indicating enantiomeric purity 94%. The filtrate of mainly (+)-acid from above was concentrated and the residue was dissolved in Et_2O (1.5 L). Treatment with (–)-α-methylbenzylamine (40 g) and collection of the salt, recrystallization, and cleavage with HCl as described above gave (*S*)-(+)-2-phenylbutanoic acid (**15b**, 43.0 g): $[\alpha]^{22}_D$ +93.4° (*c* 1, C_6H_6), indicating enantiometric purity (97%).

(*R*)-(–)-1-(4-Methoxyphenyl)-2-phenyl-1-butanone (16a). A mixture of (*R*)-(–)-2-phenylbutanoic acid (2.97 g, 18.1 mmol), anisole (3.0 g, 278 mmol), and trifluoroacetic anhydride (4.3 g, 205 mmol) was stirred at 22 °C. After 4 h, the mixture was poured into H_2O (50 mL) and extracted with Et_2O (2 × 50 mL). The Et_2O solutions were washed with saturated aqueous $NaHCO_3$ (50 mL), dried with Na_2SO_4, and concentrated. Crystallization of the residue from EtOH at –30 °C gave **16a** (3.32 g, 72%); mp 45–46 °C; $[\alpha]^{22}_D$ –79.8° (*c* 2, EtOH). Anal. ($C_{17}H_{18}O_2$) C, H.

The enantiomer **16b** was prepared similarly from (*S*)-(+)-2-phenylbutanoic acid: mp 44–46 °C; $[\alpha]^{22}_D$ +87.4° (*c* 2, EtOH). Anal. ($C_{17}H_{18}O_2$) C, H.

NMR in the presence of 0.1 mol % tris[3-[(trifluoromethyl)-hydroxymethylene]-*d*-camphorato]europium(III) [Eu(tfc)$_3$] (sample concentration = 100 mg/mL): δ_H ($CDCl_3$, 60 MHz) inter alia for **16a**, δ 8.37 (d, 2, J = 9 Hz, Ar*H* ortho to C=O). The (+)-enantiomer gave the corresponding signal at δ 8.42.

(1*S*,2*R*)-(+)-1-(4-Methoxyphenyl)-1,2-diphenyl-1-butanol (17a). To a solution of phenylmagnesium bromide [prepared in the usual manner from bromobenzene (2.61 g, 16.6 mmol) and Mg (400 mg, 16.6 mmol) in Et_2O (30 mL)] was added a solution of **16a** (2.11 g, 8.3 mmol) in Et_2O (10 mL). The mixture was stirred at 22 °C for 22 h, then poured into 0.5 N HCl (100 mL), and extracted with Et_2O (3 × 40 mL). The Et_2O solutions were washed with H_2O, dried with Na_2SO_4, and concentrated. On dissolution of the residual oil in petroleum ether (bp 80–100 °C; 50 mL) needles of **17a** crystallized (1.67 g, 61%): mp 109–110 °C; $[\alpha]^{22}_D$ +200° (*c* 2, EtOH). Anal. ($C_{23}H_{24}O_2$) C, H.

The enantiomer **17b** was prepared similarly from the (+)-ketone (70% yield): mp 109–110 °C; $[\alpha]^{22}_D$ –201° (*c* 2 EtOH). Anal. ($C_{23}H_{24}O_2$) C, H.

(1*R*,2*S*)-(–)-1-(4-Methoxyphenyl)-1,2-diphenylbutane (18a). A solution of **17a** (1.026 g, 3.1 mmol) was prepared in a mixture of liquid NH_3 (30 mL), tetrahydrofuran (10 mL), and *t*-BuOH (2 mL). The ammonia was allowed to reflux while lithium (50 mg, 7.2 mmol) cut into small pieces was added. After 15 min, the mixture was poured into H_2O (50 mL) and extracted with Et_2O (2 × 25 mL). The ether layers were concentrated, and the residue was chromatographed on silica gel (20 g). Elution with 1:5 CH_2Cl_2/petroleum ether (bp 60–80 °C) gave **18a** (830 mg, 85%) as an oil: $[\alpha]^{22}_D$ –2.1° (*c* 15, EtOH). NMR spectroscopy showed that this sample was a 13:1 mixture of **18a** [δ_H 3.40 (OMe)] and the 1*S*,2*S* diastereoisomer [δ_H 3.56 (OMe)].

The enantiomer **18b** was prepared similarly from the (–)-tertiary alcohol (86% yield) as a 7:1 mixture of the 1*S*,2*R* and 1*R*,2*R* diastereoisomers; $[\alpha]^{22}_D$ +4.3° (*c* 15, EtOH).

Preparations of Enantiomeric Dihydrotamoxifens (8a and 8b). (1*R*,2*S*)- and (1*S*,2*R*)-1-[4-[2-(Dimethylamino)ethoxy]phenyl]-1,2-diphenylbutane. A mixture of **18a** (mainly 1*R*,2*S* diastereoisomer, 324 mg, 1.02 mmol) and pyridine hydrochloride (500 mg, 4.4 mmol) was heated at 220 °C for 2 h, and then the mixture was partitioned between Et_2O (20 mL) and 5 N H_2SO_4 (20 mL). The Et_2O solution was concentrated and the residue dissolved in CH_2Cl_2 (10 mL) containing octafluorotoluene (315 mg, 1.33 mmol) and $Bu_4N^+HSO_4^-$ (30 mg, 0.1 mmol). This solution was stirred with 1.5 M NaOH (10 mL) for 2 h at 22 °C, and then the organic layer was concentrated and the residue chromatographed on silica gel. Elution with petroleum ether (bp 60–80 °C) gave the perfluorotolyl ether **19a** as an oil which was a pure isomer by TLC and was crystallized from petroleum ether (bp 60–80 °C) (423 mg, 80%): mp 73–74 °C; $[\alpha]^{22}_D$ +29° (*c* 5, EtOH). Anal. ($C_{29}H_{21}F_7O$) C, H, F. Similar treatment of **18b** gave the enantiomer **19b** (453 mg, 85%): mp 73–74 °C; $[\alpha]^{22}_D$ –29° (*c* 5, EtOH). Anal. ($C_{29}H_{21}F_7O$) C, H, F.

A solution of the perfluorotolyl ether **19a** (423 mg, 0.82 mmol) in Me_2NCHO (4 mL) was treated with NaOMe (500 mg, 9 mmol). The mixture was stirred at 35 °C for 2 h and then poured into 2 N HCl (20 mL) and extracted with Et_2O (2 × 15 mL). The combined Et_2O solutions were dried with Na_2SO_4 and concentrated, and the residual oil was dissolved in Me_2NCHO (10 mL). The solution was stirred under N_2 and NaH (200 mg, 8 mmol) added, and then the mixture was warmed to 60 °C and $Me_2NCH_2CH_2Cl$·HCl (300 mg, 2.1 mmol) added in portions over 10 min. The resulting mixture was maintained at 60 °C for 1 h and then cooled and excess NaH destroyed by addition of Me_2CHOH (1 mL). The mixture was poured into water (20 mL) and extracted with Et_2O (2 × 15 mL). The combined extracts were concentrated. Chromatography of the residue [1:10:10 NEt_3/Et_2O/petroleum ether (bp 60–80 °C)] gave **8a** (223 mg, 74%, 57% from **18a**): mp 78–80 °C; $[\alpha]^{22}_D$ +1.4° (*c* 10, EtOH). Anal. ($C_{26}H_{31}NO$) C, H, N.

Similar treatment of the perfluorotolyl ether **19b** gave **8b** (242 mg, 74%, 63% yield overall from **18b**): mp 79–81 °C; $[\alpha]^{22}_D$ –1.4° (*c* 10, EtOH). Anal. ($C_{26}H_{31}NO$) C, H, N.

In order to determine the enantiomeric purity, a solution of the perfluorotolyl ether (**19a** or a racemic mixture) (554 mg, 1.07 mmol) and (+)-PhCH(OH)Me (2.61 mg, 2.14 mmol) in dry Me_2NCHO (2.5 mL) was stirred at 5 °C. NaH (100 mg, 4.2 mmol) was added. Hydrogen was evolved immediately. After 30 min, usual aqueous workup and chromatography [10% CH_2Cl_2 in petroleum ether (bp 60–80 °C)] gave **20** (411 mg, 53%) as an oil having the NMR spectrum in Figure 2.

Binding Studies. Biochemical Assay.[29] Calf uterine cytosol was incubated at 18 °C for 30 min with 5 × 10^{-9} M [^{3}H]estradiol in the absence and presence of increasing amounts (10^{-9} to 10^{-5} M of the test compound or unlabeled estradiol (control). Unbound compounds were then removed with dextran-coated charcoal and the amounts of estrogen receptor bound [^{3}H]estradiol were measured. The relative concentrations of estradiol and test compound required to achieve 50% inhibition of [^{3}H]estradiol binding is the RBA; i.e., RBA = ([I_{50}] estradiol/[I_{50}] test compound) × 100. This procedure gives values of the same order of magnitude with cytosol from either rat immature uterus, human breast tumors or MCF-7 cells.

Whole-Cell Assay.[25,31] MCF-7 cells were incubated at 37 °C for 50 min with 10^{-9} M [^{3}H]estradiol in the absence or presence of increasing amounts (10^{-10} to 10^{-5} M) of the test compound or unlabeled estradiol (control). Bound compounds were then extracted with EtOH and the amounts of estrogen receptor bound [^{3}H]estradiol were measured. The RBA values were calculated as for the biochemical assay.

Effect of Compounds 9 and 10 on MCF-7 Cell Growth. MCF-7 cells were plated at a density of 5000 cells/mL in 96-

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multiwell dishes. After 24 h of culture, compounds **9** and **10** were added to the culture dishes according to the protocol described previously.[32] Estradiol was also added to evaluate its extent of antagonism of growth inhibition of compounds **9** and **10**. Final concentrations were as follows: estradiol, 10^{-8} M; compounds **9** and **10**, 10^{-8}, 10^{-7}, and 10^{-6} M. After 5 days of culture, the monolayer was fixed with 90% ethanol and colored with hematoxylin.[32] The intensity of the coloration giving a measure of the number of cells was determined with a multiscan spectrophotometer at 540 nm (Flow Laboratories Inc.).

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Registry No. (±)-**7**, 109640-20-2; (±)-**8**, 109640-21-3; (1*R*,2*S*)-**8a**, 109717-23-9; (1*S*,2*R*)-**8b**, 109717-24-0; (±)-**9**, 109669-15-0; (±)-**10**, 109669-16-1; (*E*)-**11**, 103628-15-5; (*Z*)-**12**, 103628-14-4; (±)-**13**, 109640-22-4; (±)-**14**, 109640-23-5; (*R*)-(−)-**15a**, 938-79-4; (*R*)-(−)-**15a**·(*R*)-(+)-PhCH(Me)NH_2, 109640-25-7; (±)-**15b**, 7782-29-8; (*S*)-(+)-**15b**, 4286-15-1; (*S*)-(+)-**15b**·(*S*)-(−)-PhCH(Me)NH_2, 13491-02-6; (*R*)-(−)-**16a**, 109640-26-8; (*S*)-(+)-**16b**, 109640-27-9; (1*S*,2*R*)-(+)-**17a**, 109640-28-0; (1*R*,2*S*)-(−)-**17b**, 109640-29-1; (1*R*,2*S*)-(−)-**18a**, 109640-30-4; (1*S*,2*S*)-**18a**, 109640-31-5; (1*S*,2*R*)-**18b**, 109640-32-6; (1*R*,2*R*)-**18b**, 109640-33-7; (1*R*,2*S*[)-(+)-**19a**, 109640-36-0; (1*S*,2*R*)-(−)-**19b**, 109640-37-1; **20a**, 109640-38-2; (1*R*,2*S*)-EtCH(Ph)CH(Ph)C_6H_4OH-*p*, 109640-34-8; (1*S*,2*S*)-EtCH(Pg)CH(Ph)C_6H_4OH-*p*, 109640-35-9; (*Z*)-tamoxifen, 10540-29-1; (*E*)-tanoxifen, 13002-65-8; (*R**,*R**)-(±)-1-(4-(2-dimethylaminoethoxy)phenyl)-1-(4-(2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy)phenyl)-2-phenylbutane, 109640-24-6.

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Synthesis and Antineoplastic Activity of 1a-Formyl and 1a-Thioformyl Derivatives of Mitomycin C and 2-Methylaziridine

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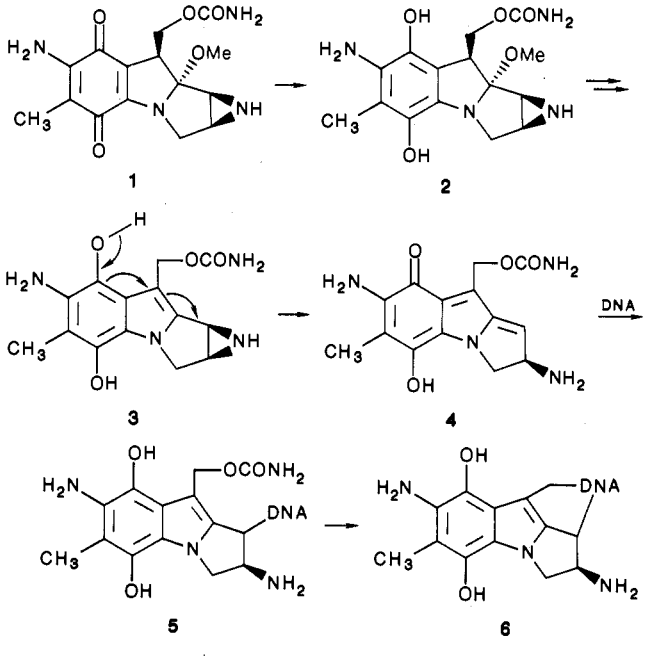
A select number of 1-formyl- and 1-thioformyl-2-methylaziridine derivatives and the corresponding 1a-substituted mitomycin C analogues were synthesized and tested for antineoplastic activity by using an in vivo test with murine P388 leukemia. Select compounds were also tested in vivo with murine melanoma B16. Several of the mitomycin C derivatives displayed activity and some of the mitomycin C analogues were comparable in activity to the parent compound.

Mitomycin C (**1**) is a clinically used anticancer drug.[1] It acts as a prodrug that requires initial reductive activation in a cancer growth.[2] The antitumor agent is believed to express its action by mono- and dialkylating (cross-linking) DNA.[2] It has been suggested that the reaction with the genetic material involves quinone methide **4**.[2] This species then undergoes attack by DNA at carbon 1 to give the monoalkylated intermediate **5** followed by an intramolecular attack at carbon 10 to give the bisalkylated product **6** as shown in Scheme I.[2]

The clinical use of mitomycin C is limited by its toxicity.[3] An explanation for the toxicity involves the aziridine ring.[4] Aziridines undergo acid-catalyzed ring opening.[5] Mitomycin C is known to undergo ring opening in acid media to generate a reactive carbon 1 cationic species.[6] A comparable intermediate might form in vivo before reaching a cancer cell and serve as a potent electrophile for other biomolecules, thus being toxic. 1a-Acyl- and 1a-sulfonylmitomycin C derivatives have been shown to be less toxic than the parent compound **1**.[7] The presence of electron-withdrawing groups at the 1a-position is expected to decrease the basicity of the aziridine nitrogen atom, and thereby diminish the possibility of an acid-catalyzed ring

Scheme I



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