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Synthesis and Preliminary *in Vitro* Cytotoxic Activity of New Triphenylethylene Dimers

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We have synthesized a series of six nonsteroidal homo- and heterobifunctional estrogenic dimers designed for the treatment of breast cancer. They are made of two triphenylethylene moieties linked by an aliphatic chain. The synthesis used six steps, from the known alcohol **5**, with an overall yield of more than 60%. This article describes the synthesis of these products and their *in vitro* biological activity on two human breast cancer cell lines: MCF-7 and MDA-MB-231. The dimers are generally less active than tamoxifen, which presents an $IC_{50} = 16$ and 40 μM on MCF-7 and MDA-MB-231 cell lines, respectively. However, the symmetrical dimer bearing six hydroxy functions possesses the best *in vitro* cytotoxic activity of the series, showing an $IC_{50} = 38$ μM on both types of cells. It was observed that the cytotoxicity of the dimers increases with the number of hydroxy groups present on the aromatic rings.

INTRODUCTION

The synthesis of new antiestrogenic molecules has attracted considerable interest because of their great therapeutic value in treating a number of hormone-dependent human cancers. For example, several research groups have reported various methodologies for the synthesis of steroidal and non-steroidal antiestrogens. The bulk of the work led to the study of the estradiol pharmacophore and a recent proposal of a model for the receptor binding site (*1*). Some new molecules have allowed a detailed mechanistic study of antiestrogen action on human breast cancer cells (*2–11*).

The known antiestrogens are competitive inhibitors of estrogen binding to the estrogen receptor (ER). Simply, they reduce the ability of estradiol to stimulate nuclear transcription and ultimately cell growth. The exact mechanism(s) by which pure antiestrogens achieve a complete ER blockade is still a matter of debate. Several mechanisms of action were observed: pure antiestrogens (**1**) reduce DNA binding by

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interfering with receptor dimerization (*7*); (**2**) induce conformational changes of the receptor that allow binding to DNA but do not promote events needed for gene transcription (inactivation of the two transcription activation functions AF1 and AF2) (*8*); (**3**) cause a rapid disappearance of the ER from the target tissue (degradation of the ER), resulting in an insufficient amount of ER to bind the native ligand (estradiol) and elicit agonist responses (*9,10*); and (**4**) inhibit nucleocytoplasmic shuttling of the ER (diffusion out of the nucleus and degradation) by blocking its nuclear uptake (*11*). It should be pointed out that pure antiestrogens could block completely the ER function by a combination of several of these processes.

DIMERS AS ANTIESTROGENS

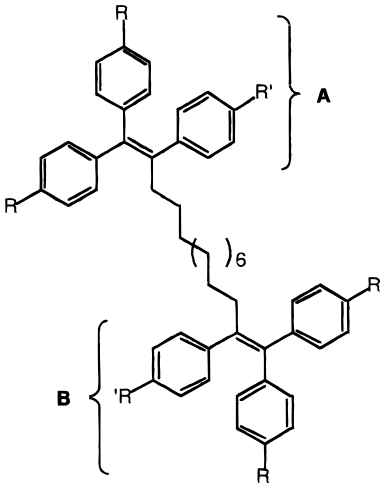
The synthesis of an estrogenic bivalent ligand could, theoretically, interfere with the step of receptor dimerization. This concept was first studied by the group of Professor Katzenellenbogen (*12*). They made several dimeric molecules that were analogs of hexestrol (see **2**, Scheme 1). Some symmetrical dimers showed interesting antiestrogenic activity (*12*). A bivalent ligand could act as an antiestrogen either by preventing the ER dimerization or, if there is impaired dimerization, by preventing its association to the estrogen response element located on DNA. These two modes of action would inhibit cell growth.

In order to further investigate the effect of a bivalent ligand on the ER, we have synthesized a series of nonsteroidal homo- and heterobifunctional dimers **1**. The chemical structure of these new dimers is based on a nonsteroidal triphenylethylene system. It was previously demonstrated that this type of system bearing two or three hydroxy groups can interact with the ER (*13*). The triphenylethylene portions are joined together with an 11-carbon-atom aliphatic chain. The choice of the length of the linking chain is important as it was reported that such length gives molecules a good estrogen receptor binding affinity (RBA, relative binding affinity (estradiol, RBA = 100%)) (*12*). Hexestrol dimer **2** with a similar linking chain length possesses a RBA value of 6.9% at 25°C. Therefore, the dimers are designed to interact with the ER, particularly when the triphenylethylene portion is hydroxylated. The nonhydroxylated systems were made as reference compounds. The new dimers are analogs of tamoxifen (**3**; TAM). Tamoxifen, a nonsteroidal antiestrogen, has been used for the treatment and more recently for the prevention of breast cancer. This article describes the synthesis of these new homo- and heterobifunctional dimers and reports on their *in vitro* cytotoxic activity on two neoplastic human breast cancer cells: MCF-7(ER^+) and MDA-MB-231 (ER^-).

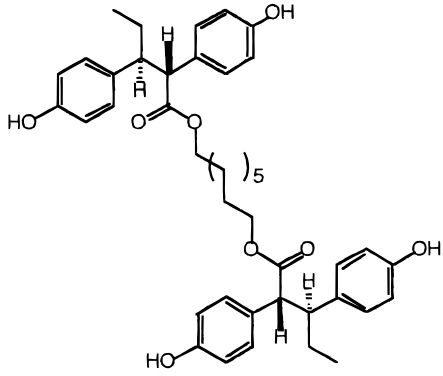
EXPERIMENTAL

Materials and Methods

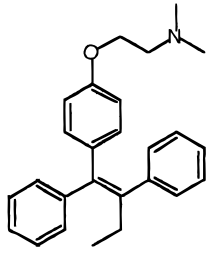
Anhydrous reactions were performed under an inert atmosphere, the setup assembled and cooled under dry nitrogen. Unless otherwise noted, starting material, reagents, and solvents were obtained commercially and were used as such or purified and dried by standard means (*14*). Organic solutions were dried over anhydrous magnesium sulfate and evaporated on a rotary evaporator and under reduced pressure. All reactions were monitored by thin-layer chromatography (TLC). The plates were visualized by



1 Dimer, R and R' = H or OH
Homobifunctional (A = B)
Heterobifunctional (A ≠ B)



2 Dimer analog of hexestrol



3 Tamoxifen

SCHEME 1

UV fluorescence at 254 nm. Commercial TLC plates were Sigma T 6145 (polyester silica gel 60 Å, 0.25 mm). Flash chromatography was performed according to the method of Still and co-workers on Merck Grade 60 silica gel, 230–400 mesh (*15*). All solvents used in chromatography were distilled. Melting points (mp) were recorded on an Electrothermal 9100 apparatus and are uncorrected. The infrared spectra (IR) were taken on a Perkin–Elmer 1430 IR or on a Nicolet Impact 420 FT–IR spectrophotometer. Mass spectral assays (MS, *m*/*z*) were obtained using a VG Micromass 7070 HS instrument (Université de Sherbrooke) using an ionization energy of 70 eV. Nuclear magnetic resonance (NMR) spectra were obtained in $CDCl_3$ solution on a Bruker AMX2 (500 MHz) instrument. Chemical shifts were measured relative to internal standards: tetramethylsilane (δ 0.0 ppm) for ^1H and $CDCl_3$ (δ 77.0 ppm) for ^{13}C NMR. The number of carbons assigned to a peak, on ^{13}C NMR spectra, is indicated in brackets when it is more than one carbon. Multiplicities are described by the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), 2d

(two doublets), dd (double doublet), dt (double triplet), m (multiplet), and so on. The NMR assignments were assisted by heteronuclear multiple-quantum correlation and by correlated spectroscopy 2-D spectra (*16*). Spectral data are presented completely for only one member of every family of molecules with the exception of the final dimers **1**, for which spectral data are completely described.

Synthesis of Tosylates **6**

Tosyl chloride (3.37 g, 17.68 mmol) dissolved in methylene chloride (15 ml) was added to a mixture of alcohol **5** (1.83 g, 3.54 mmol) and triethylamine (1.79 g, 17.67 mmol) in methylene chloride (35 ml). The reaction mixture was stirred at room temperature (22°C) for 18 h under a nitrogen atmosphere. Then, most of the solvent was evaporated and the residue was transferred to an extraction flask with ether (20 ml) and water (5 ml). The organic phase was washed with water (5 × 5 ml), dried, filtered, and concentrated to a viscous oil. The crude tosylate **6** was purified by flash column chromatography (hexane:acetone, 9:1 to 85:15). The yield was 95%.

12,13,13-Triphenyl-12-tridecenyl tosylate (**6a**). MS (*m*/*z*) 580 (M^+), 408 ($M^+-C_7H_8O_3S$), 269 ($M^+-C_{17}H_{27}O_3S$): Exact mass calcd for $C_{38}H_{44}O_3S$: 580.3011. Found: 580.3016.

13,13-Bis(4′-methoxyphenyl)-12-phenyl-12-tridecenyl tosylate (**6b**). MS (*m*/*z*) 640 (M^+), 468 ($M^+-C_7H_8O_3S$), 329 ($M^+-C_{17}H_{27}O_3S$): Exact mass calcd for $C_{40}H_{48}O_5S$: 640.3222. Found: 640.3210.

*12,13,13-Tris(4′-methoxyphenyl)-12-tridecenyl tosylate (***6c**): IR (thin film, υ_{max}, cm^{-1}) 1600 (C=C), 1240 (C−O); ^1H NMR (δ ppm) 7.78 and 7.31 (4H, 2× d, J = 7.8 Hz, CH_3-*Ar*-SO_2), 7.13 and 6.86, 7.01 and 6.70, 6.78 and 6.55 (12H, 3× 2d, J = 8.1, 8.4, and 8.7 Hz, 3× methoxyphenyl), 4.01 (2H, t, J = 6.5 Hz, CH_2O), 3.80, 3.74, and 3.67 (9H, 3× s, 3× OCH_3), 2.42 (3H, s, Ar-CH_3), 2.40 (2H, m, C=C−CH_2), 1.65–1.58 (2H, m, C$H_2$$CH_2O$), 1.35–1.14 (16H, m, $(CH_2)_8$); ^{13}C NMR (δ ppm) 158.14, 157.67, 157.31, 144.58, 139.38, 137.52, 136.53, 136.11, 135.12, 133.32, 131.89 (2), 130.61 (4), 129.78 (2), 127.85 (2), 113.40 (2), 113.25 (2), 112.75 (2), 70.68, 55.17, 55.04, 54.97, 35.88, 29.71, 29.43, 29.40, 29.32, 29.26, 28.97, 28.89, 28.80, 25.31, 21.59; MS (*m*/*z*) 670 (M^+), 498 ($M^+-C_7H_8O_3S$), 359 ($M^+-C_{17}H_{27}O_3S$): Exact mass calcd for $C_{41}H_{50}O_6S$: 670.3328. Found: 670.3336.

Synthesis of Iodides **7**

A mixture of tosylate **6** (3.43 mmol) and sodium iodide (2.57 g, 17.15 mmol) in anhydrous acetone (25 ml) was heated to reflux for 8 h. After evaporation, the residue was diluted with ether (20 ml) and washed with water (5 × 5 ml). The ethereal phase was dried, filtered, and evaporated and the residue purified by flash column chromatography (hexane:acetone, 95:5) to produce iodide **7** (99% yield) as a viscous oil.

1-Iodo-12,13,13-triphenyl-12-tridecene (**7a**). MS (*m*/*z*) 536 (M^+), 269 ($M^+-C_{10}H_{20}I$). Exact mass calcd for $C_{31}H_{37}I$: 536.1940. Found: 536.1933.

1-Iodo-13,13-bis(4′-methoxyphenyl)-12-phenyl-12-tridecene (**7b**). MS (*m*/*z*) 596 (M^+), 468 (M^+–HI), 329 ($M^+-C_{10}H_{20}I$). Exact mass calcd for $C_{33}H_{41}O_2I$: 596.2151. Found: 596.2145.

1-Iodo-12,13,13-tris(4′-methoxyphenyl)-12-tridecene (**7c**). IR (thin film, υ_{max},

cm^{-1}) 1600 (C=C), 1240 (C−O); ^1H NMR (δ ppm) 7.13 and 6.86, 7.01 and 6.70, 6.78 and 6.55 (12H, 3× 2d, J = 8.8, 8.1, and 7.4 Hz, 3× methoxyphenyl), 3.81, 3.75, and 3.68 (9H, 3× s, 3× OCH_3), 3.17 (2H, t, J = 7.3 Hz, CH_2-I), 2.40 (2H, m, C=C−CH_2), 1.80 (2H, p, J = 7.3 Hz, C$H_2$$CH_2$I), 1.40–1.10 (16H, m, $(CH_2)_8$); ^{13}C NMR (δ ppm) 158.11, 157.64, 157.28, 139.38, 137.49, 136.54, 136.11, 135.12, 131.88 (2), 130.61 (4), 113.38 (2), 113.24 (2), 112.74 (2), 55.18, 55.06, 54.97, 35.87, 33.55, 30.48, 29.70, 29.45 (2), 29.36, 29.26, 28.97, 28.51, 7.25; MS (*m*/*z*) 626 (M^+), 498 (M^+–HI), 359 (M^+–$C_{10}H_{20}I$): Exact mass calcd for $C_{34}H_{43}O_3I$: 626.2257. Found: 626.2252.

Synthesis of Ketones **8**

To a stirred suspension of sodium hydride (448 mg, 11.2 mmol, 60% dispersion in mineral oil) in a mixture of tetrahydrofuran and dimethyl sulfoxide (THF:DMSO, 9:1, 150 ml) was added the appropriate ketone **4a**, **4b**, or **4c** (10.2 mmol). The reaction mixture was heated in a water bath (45°C) for 1 h under a nitrogen atmosphere. After cooling, the required iodide **7a**, **7b**, or **7c** (5.1 mmol) dissolved in THF (10 ml) was added dropwise and the resulting mixture stirred overnight at room temperature (18 h, 22°C). Then, most of the solvent was evaporated and the residue was diluted with ether (200 ml) and treated with an aqueous solution of sodium thiosulfate (5%, 50 ml). The ethereal phase was washed thoroughly with water (6 × 50 ml), dried, filtered, and evaporated to give an oil that was purified by flash column chromatography (hexane:acetone, 98:2 to 9:1). A viscous oil was obtained. The yield was 85%.

NB: The following combinations 0-0, 0-1, 0-2, 0-3, 2-1, 2-2, 2-3, and 3-3, found next to the products' numbers, represent the number of methoxy or hydroxy groups on the dimeric molecule.

1,2,14,15,15-Pentaphenyl-14-pentadecen-1-one (**8**, **0-0**). MS (*m*/*z*) 604(M^+), 269 (M^+–$C_{24}H_{31}O$). Exact mass calcd for $C_{45}H_{48}O$: 604.3705. Found: 604.3701.

1-(4′-Methoxyphenyl)-2,14,15,15-tetraphenyl-14-pentadecen-1-one (**8**, **0-1**). MS (*m*/*z*) 634 (M^+). Exact mass calcd for $C_{46}H_{50}O_2$: 634.3811. Found: 634.3800.

1,2-Bis(4′-methoxyphenyl)-14,15,15-triphenyl-14-pentadecen-1-one (**8**, **0-2**). MS (*m*/*z*) 664 (M^+), 529 (M^+–$C_8H_7O_2$), 269 (M^+–$C_{26}H_{35}O_3$): Exact mass calcd for $C_{47}H_{52}O_3$: 664.3916. Found: 664.3909.

*1,15,15-Tris(4′-methoxyphenyl)-2,14-diphenyl-14-pentadecen-1-one (***8**, **2-1**). MS (*m*/*z*) 694 (M^+), 329 (M^+–$C_{25}H_{33}O_2$): Exact mass calcd for $C_{48}H_{54}O_4$: 694.4022. Found: 694.4011.

1,14,15,15-Tetrakis(4′-methoxyphenyl)-2-phenyl-14-pentadecen-1-one (**8**, **2-2**). MS (*m*/*z*) 724 (M^+), 359 (M^+–$C_{25}H_{33}O_2$): Exact mass calcd for $C_{49}H_{56}O_5$: 724.4128. Found: 724.4119.

1,2,14,15,15-Pentakis(4′-methoxyphenyl)-14-pentadecen-1-one (**8**, **3-2**). IR (thin film, υ_{max}, cm^{-1}) 1670 (C=O), 1600 (C=C), 1245 (C−O); ^1H NMR (δ ppm) 7.95 and 6.80, 7.22 and 6.84, 7.13 and 6.86, 7.02 and 6.69, 6.78 and 6.55 (20H, 5× 2d, J = 8.7, 6.3, 8.1, 8.7, and 8.0 Hz, 5× 4′-methoxyphenyl), 4.43 (1H, t, J = 7.0 Hz, CH−CO), 3.78, 3.77, 3.72, 3.71, and 3.65 (15H, 5× s, 5× OCH_3), 2.40 (2H, m, C=C−CH_2), 2.12 and 1.77 (2H, 2× m, CH_2CH−CO), 1.30–1.14 (18H, m, $(CH_2)_9$); ^{13}C NMR (δ ppm) 198.83, 163.15, 158.43, 158.12, 157.65, 157.29, 139.42, 137.48, 136.52, 136.13, 135.12, 132.31, 131.89 (2), 130.86 (2), 130.60 (4), 129.99, 129.10

(2), 114.18 (2), 113.63 (2), 113.39 (2), 113.24 (2), 112.74 (2), 55.33, 55.14, 55.12, 55.01, 54.94, 52.32, 35.88, 34.07, 29.72, 29.64, 29.55 (2), 29.49, 29.46, 29.29, 28.98, 27.71; MS (*m*/*z*) 754 (M^+), 359 ($M^+-C_{26}H_{35}O_3$): Exact mass calcd for $C_{50}H_{58}O_6$: 754.4233. Found: 754.4227.

Procedure for the Preparation of p-Methoxyphenyllithium

4-Bromoanisole (145 μl, 1.16 mmol) was dissolved in a mixture of dry THF:Et_2O (6:4, 10 ml). The reaction mixture was cooled down to −110°C by means of a light petroleum ether/acetone/isopropanol/liquid nitrogen slush bath and kept under nitrogen (*17*). A solution of *n*-butyllithium in hexane (1.6 M, 725 μl, 1.16 mmol) was added to the solution and the mixture stirred for 30 min. Stirring was continued for a further 90 min, as the temperature was allowed to rise to *ca*. −60°C. The freshly prepared *p*-methoxyphenyllithium was used as such for the synthesis of the 1,14-pentadecadienes **1** (R and R′ = H or OCH_3). Phenyllithium is available commercially.

Synthesis of 1,14-Pentadecadienes 1 (R and R′ = H or OCH_3)

The freshly prepared organolithium derivative was added to a solution of ketone **8** (0.29 mmol) in a mixture of dry THF:Et_2O (6:4, 10 ml). The reaction mixture was stirred at −110°C for 30 min under a nitrogen atmosphere. Afterward, the reaction mixture was diluted with ether (40 ml) and a solution of ammonium chloride was added (20 ml, 10% aqueous). The phases were separated and the organic phase was washed with water (3 × 10 ml), dried, and evaporated to give the tertiary alcohol intermediate. This alcohol was dehydrated in 25 ml 95% ethanol in the presence of *p*-toluenesulfonic acid (TsOH) (10 mg, 0.04 mmol) heated to reflux for 3 h. After evaporation of the solvent, the residue was taken with ether (30 ml) and extracted with water (3 × 10 ml). The ethereal phase was dried, filtered, and evaporated to a viscous oil. Flash column chromatography (hexane:acetone, 100:0 to 85:15) gave compound **1** (0.26 mmol) (R and R′ = H or OCH_3) in 90% yield either as a viscous oil or as a solid.

1,1,2,14,15,15-Hexaphenyl-1,14-pentadecadiene (**1**, **0-0**). mp 113–114°C; IR (KBr, υ_{max}, cm^{-1}) 1590 (C=C); ^{1}H NMR (δ ppm) 7.35–6.80 (30H, m, Ar-H), 2.42, (4H, m, 2× C=C−CH_2), 1.4–1.1 (18H, m,$(CH_2)_9$); ^{13}C NMR (δ ppm) 143.64 (2), 143.17 (2), 142.63 (2), 141.25 (2), 139.19 (2), 130.85 (4), 129.70 (4), 129.63 (4), 128.22 (4), 127.91 (4), 127.47 (4), 126.68 (2), 126.23 (2), 125.82 (2), 35.98 (2), 29.77 (2), 29.63, 29.58 (2), 29.37 (2), 28.94 (2); MS (*m*/*z*) 664 (M^+), 269 ($M^+-C_{30}H_{35}$). Exact mass calcd for $C_{51}H_{52}$: 664.4069. Found: 664.4066.

15,15-Bis(4′-methoxyphenyl)-1,1,2,14-tetraphenyl-1,14-pentadecadiene (**1**, **0-2**). MS (*m*/*z*) 724 (M^+), 329 ($M^+-C_{30}H_{35}$). Exact mass calcd for $C_{53}H_{56}O_2$: 724.4280. Found: 724.4276.

14,15,15-Tris(4′-methoxyphenyl)-1,1,2-triphenyl-1,14-pentadecadiene (**1**, **0-3**). MS (*m*/*z*) 754 (M^+), 359 ($M^+-C_{30}H_{35}$). Exact mass calcd for $C_{54}H_{58}O_3$: 754.4386. Found: 754.4368.

1,1,15,15-Tetrakis(4′-methoxyphenyl)-2,14-diphenyl-1,14-pentadecadiene (**1**, **2-2**). MS (*m*/*z*) 784 (M^+), 329 ($M^+-C_{32}H_{39}O_2$). Exact mass calcd for $C_{55}H_{60}O_4$: 784.4491. Found: 784.4484.

1,1,14,15,15-Pentakis(4′-methoxyphenyl)-2-phenyl-1,14-pentadecadiene (**1**, **2-3**).