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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

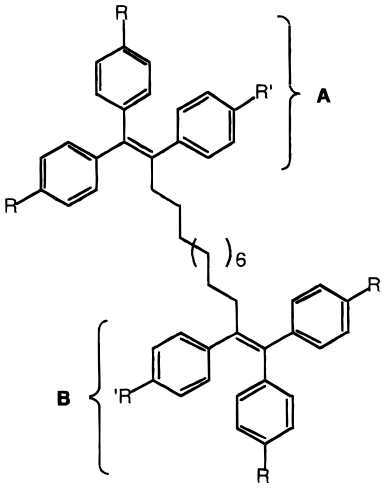
The synthesis of an estrogenic bivalent ligand could, theoretically, interfere with the step of receptor dimerization. This concept was first studied by the group of Professor Katzenellenbogen (*12*). They made several dimeric molecules that were analogs of hexestrol (see **2**, Scheme 1). Some symmetrical 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^-).

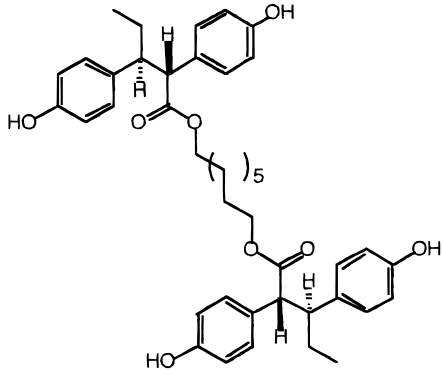
EXPERIMENTAL

Materials and Methods

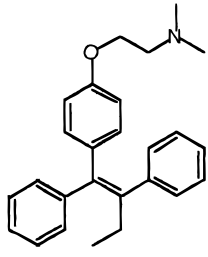
Anhydrous reactions were performed under an inert atmosphere, the setup assembled and cooled under dry nitrogen. Unless otherwise noted, starting material, reagents, and solvents were obtained commercially and were used as such or purified and dried by standard means (*14*). Organic solutions were dried 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**).

MP 117°C; MS (*m/z*) 814 (M^+), 359 ($M^+-C_{32}H_{39}O_2$). Exact mass calcd for $C_{56}H_{62}O_5$: 814.4597. Found: 814.4610.

1,1,2,14,15,15-Hexakis(4′-methoxyphenyl)-1,14-pentadecadiene (**1**, **3-3**). mp 101–102°C; IR (KBr, υ_{max}, cm^{-1}) 1600 (C=C), 1240 (C−O); ^1H NMR (δ ppm) 7.13 and 6.85, 7.01 and 6.69, 6.77 and 6.55 (24H, 3× 2d, J = 7.6, 8.1, and 8.2 Hz, 6× 4′-methoxyphenyl), 3.80, 3.74, and 3.68 (18H, 6× s, 6× OCH_3), 2.38 (4H, m, 2× C=C−CH_2), 1.3–1.0 (18H, m, $(CH_2)_9$); ^{13}C NMR (δ ppm) 158.17 (2), 157.70 (2), 157.33 (2), 139.47 (2), 137.52 (2), 136.59 (2), 136.18 (2), 135.19 (2), 131.94 (4), 130.66 (8), 113.43 (4), 113.29 (4), 112.79 (4), 55.22 (2), 55.10 (2), 55.03 (2), 35.94 (2), 35.79 (2), 29.58 (3), 29.37 (2), 29.04 (2); MS (*m/z*) 844 (M^+), 359 ($M^+-C_{33}H_{41}O_3$). Exact mass calcd for $C_{57}H_{64}O_6$: 844.4703. Found: 844.4697.

Synthesis of Hydroxylated Dimers **1** *(R or R′ = H or OH)*

The 1,14-pentadecadiene **1** (R or R′ = H or OCH_3) (0.54 mmol) was dissolved in dichloromethane (15 ml) and the resulting solution was cooled down to −60°C, under a nitrogen atmosphere. Then, a solution of boron tribromide (1.0 M in CH_2Cl_2, 1.89 mmol) was added and the mixture was stirred 15 min at −60°C. The mixture was allowed to warm to room temperature (22°C) and stirred for 18 h. Afterward, the mixture was refluxed for 2 h. The reaction was cooled down to 0°C, before 15 ml methanol was added. The resulting solution was concentrated to 1–2 ml, treated with saturated $NaHCO_3$ (30 ml), and extracted with ethyl acetate (5 × 5 ml). The organic phase was dried, filtered, and evaporated to give a residue which was purified by flash column choromatography (hexane:acetone, 9:1 to 7:3) to give the hydroxylated dimer **1** (0.50 mmol) (R or R′ = H or OH) as a yellow or orange solid. The yield of this reaction was 90%.

15,15-Bis(4′-hydroxyphenyl)-1,1,2,14-tetraphenyl-1,14-pentadecadiene (**1**, **0-2**). MP 97°C; IR (KBr, υ_{max}, cm^{-1}) 3370 (OH), 1600 (C=C); ^1H NMR (δ ppm) 7.35–6.75 (24H, m, Ar-H and 4′-hydroxyphenyl), 6.72 and 6.46 (4H, 2d, J = 8.1 Hz, 4′-hydroxyphenyl), 4.63 and 4.44 (2H, 2× s, 2× Ar-OH), 2.41 (4H, 2× m, 2× C=C−CH_2), 1.40–1.00 (18H, m, $(CH_2)_9$); ^{13}C NMR (δ ppm) 154.12, 153.31, 143.52, 143.06, 142.91, 142.52, 141.15, 140.21, 139.02, 137.80, 136.45, 136.09, 132.09 (2), 130.83 (2), 130.72 (2), 129.58 (4), 129.51 (2), 128.09 (2), 127.81 (2), 127.77 (2), 127.33 (2), 126.54, 126.08, 125.89, 125.67, 114.91 (2), 114.24 (2), 35.93, 35.84, 29.70, 29.64, 29.46 (3), 29.25 (2), 28.89, 28.80; MS (*m/z*) 696 (M^+), 301 ($M^+-C_{30}H_{35}$). Exact mass calcd for $C_{51}H_{52}O_2$: 696.3967. Found: 696.3961.

14,15,15-Tris(4′-hydroxyphenyl)-1,1,2-triphenyl-1,14-pentadecadiene (**1**, **0-3**). mp 55–60°C; IR (KBr, υ_{max}, cm^{-1}) 3389 (OH), 1608 (C=C), 1254 (C−O); ^1H NMR (DMSO-d_6, δ ppm) 9.32, 9.16, 9.10 (3H, 3× s, 3× Ar-OH), 7.40–6.96 (15H, m, Ar-H), 6.94 and 6.73, 6.86 and 6.58, 6.62 and 6.43 (12H, 3× 2d, J = 8.0, 8.1, and 8.2 Hz, 3× 4′-hydroxyphenyl) 2.36 and 2.31 (4H, m, 2× C=C−CH_2), 1.3–1.0 (18H, m, $(CH_2)_9$); ^{13}C NMR (DMSO-d_6, δ ppm) 153.75, 155.20, 154.87, 142.86, 142.59, 141.75, 140.43, 138.63, 138.11, 137.37, 134.44, 134.29, 132.93, 131.28 (2), 130.15 (2), 130.04 (2), 129.99 (2), 129.12 (2), 128.87 (2), 128.12 (2), 127.70 (2), 127.38 (2), 126.55, 126.09, 125.67, 114.72 (2), 114.62 (2), 114.14 (2), 35.20, 35.06, 29.00, 28.75, 28.73 (2), 28.62, 28.58, 28.40, 28.37, 27.91; MS (*m/z*) 712 (M^+), 317 ($M^+-C_{30}H_{35}$). Exact mass calcd for $C_{51}H_{52}O_3$: 712.3916. Found: 712.3907.

1,1,15,15-Tetrakis(4′-hydroxyphenyl)-2,14-diphenyl-1,14-pentadecadiene (**1**, **2-2**). MP 90–100°C; IR (KBr, υ_{max}, cm^{-1}) 3396 (OH), 1609 (C=C); ^{1}H NMR (acetone-d_6, δ ppm) 8.27 and 8.03 (4H, 2× s, 4× Ar-OH), 7.20–6.40 (26H, m, 4× 4′-hydroxyphenyl and 2× Ar-H), 2.45 (4H, m, 2× C=C−CH_2), 1.40–1.05 (18H, m, $(CH_2)_9$); ^{13}C NMR (acetone-d_6, δ ppm) 156.38 (2), 155.56 (2), 143.53 (2), 139.37 (2), 139.14 (2), 135.44 (2), 135.10 (2), 132.02 (4), 130.73 (2), 130.85 (4), 129.85 (4), 127.97 (4), 125.98 (2), 115.09 (4), 115.40 (4), 35.92 (2), 29.56, 29.51 (2), 29.31 (2), 28.91 (2) (The secondary carbons ($\underline{C}H_2$) were determined using a distortionless enhancement by polarization transfer experiment); MS (*m*/*z*) 728 (M^+), 301 (M^+–$C_{30}H_{35}O_2$). Exact mass calcd for $C_{51}H_{52}O_4$: 728.3865. Found: 728.3859.

1,1,14,15,15-Pentakis(4′-hydroxyphenyl)-2-phenyl-1,14-pentadecadiene (**1**, **2-3**). MP 98°C; IR (KBr, υ_{max}, cm^{-1}) 3345 (OH), 1600 (C=C); ^{1}H NMR (acetone-d_6, δ ppm) 8.32, 8.29, 8.14, 8.08, and 8.06 (5H, 5× s, 5× Ar-OH), 7.22–6.50 (25H, m, 5× 4′-hydroxyphenyl and Ar-H), 2.50 and 2.42 (4H, m, 2× C=C−CH_2), 1.40–1.00 (18H, m, $(CH_2)_9$); ^{13}C NMR (acetone-d_6, δ ppm) 156.36, 156.22, 155.73, 155.54, 155.38, 143.52, 139.37, 139.13 (2), 138.22, 135.79, 135.51, 135.43, 135.10, 134.31, 132.02 (4), 130.87 (2), 130.74 (4), 129.84 (2), 127.96 (2), 125.97, 115.09 (2), 115.05 (2), 114.92 (2), 114.39 (4), 35.88 (2), 29.79, 29.66, 29.58 (2), 29.51, 28.89, 28.74 (2 carbons were hidden by acetone-d_6); MS (*m*/*z*) 744 (M^+), 317 (M^+–$C_{30}H_{35}O_2$). Exact mass calcd for $C_{51}H_{52}O_5$: 744.3814. Found: 744.3809.

1,1,2,14,15,15-Hexakis(4′-hydroxyphenyl)-1,14-pentadecadiene (**1**, **3-3**). MP 90–100°C; IR (KBr, υ_{max}, cm^{-1}) 3340 (OH), 1616 (C=C), 1239 (C−O); ^{1}H NMR (acetone-d_6, δ ppm) 8.28, 8.13, and 8.05 (6H, 3× s, 6× Ar-OH), 7.04 and 6.82, 6.96 and 6.64, 6.71 and 6.50 (24H, 3× 2d, J = 8.8, 7.8, and 8.4 Hz, 6× 4′-hydroxyphenyl), 2.41 (4H, m, 2× C=C−CH_2), 1.4–1.1 (18H, m, $(CH_2)_9$); ^{13}C NMR (acetone-d_6, δ ppm) 156.81 (2), 156.32 (2), 155.98 (2), 139.74 (2), 138.83 (2), 136.38 (2), 136.11 (2), 134.91 (2), 132.62 (4), 131.48 (4), 131.35 (4), 115.66 (4), 115.53 (4), 114.99 (4), 35.88 (2) (9 carbons were hidden by acetone-d_6); MS (*m*/*z*) 760 (M^+), 317 (M^+–$C_{30}H_{35}O_3$). Exact mass calcd for $C_{51}H_{52}O_6$: 760.3764. Found: 760.3765.

Cytotoxicity Assay

Chemosensitivity of cell lines to TAM and the dimers were determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (*18*). This colorimetric assay uses the ability of viable cells to reduce a soluble tetrazolium salt, MTT, into an insoluble formazan precipitate.

Materials. (a) Drugs: The dimers (**1**) were synthesized as described previously. Tamoxifen was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI). (b) Cell lines and culture: Human breast cancer cell lines MCF-7 and MDA-MB-231 were obtained from the American Type Culture Collection (Rockville, MD). Both types of cells were propagated in RPMI 1640 supplemented with 2 mM glutamine, 10% bovine serum (Gibco, Burlington, Ontario, Canada), and 100 U gentamycin/ml (Sigma Chemical Company, St. Louis, MO). (c) Phosphate-buffered saline (PBS; pH 7.4) was prepared from PBS tablets (Oxford, Unipath Ltd., England) dissolved in water as per the manufacturer's instructions. (d) Microtiter plates (96 wells) were obtained from Flow Labs, Inc. (McClean, VA). (e) MTT and DMSO were obtained

from Sigma Chemical Company. (f) Plate reader was the Behring Elisa Processor II (Behring, Marburg, Germany).

Method. A stock solution of TAM or the dimers **1** (400 μM in fresh RPMI 1640, 1% DMSO) was prepared from an initial solution of 40 mM in DMSO. The cells (2000 cells/well) were grown in sterile 96-well culture plates at 37°C in a humidified incubator with 5% CO_2 for 24 h and then incubated for 72 h with TAM or the dimers **1** diluted in fresh culture medium (final concentration 0.1 to 400 μM). The test was performed in 8 wells for each dilution. After incubation, supernatant was discarded and 50 μl MTT (2.5 mg/ml into PBS:RMPI 1640, 1:4 v/v) was added and reincubated for 4 h at 37°C. The cells were washed with PBS, the formazan precipitate was solubilized in DMSO (100 μl, containing 1% glycine:NaOH 0.1 M; pH 11-12), and the plate was read spectrophotometrically at 570 nm. Absorbance was recorded and percentage cell survival was determined by comparing TAM- and dimer-treated cells with the untreated control.

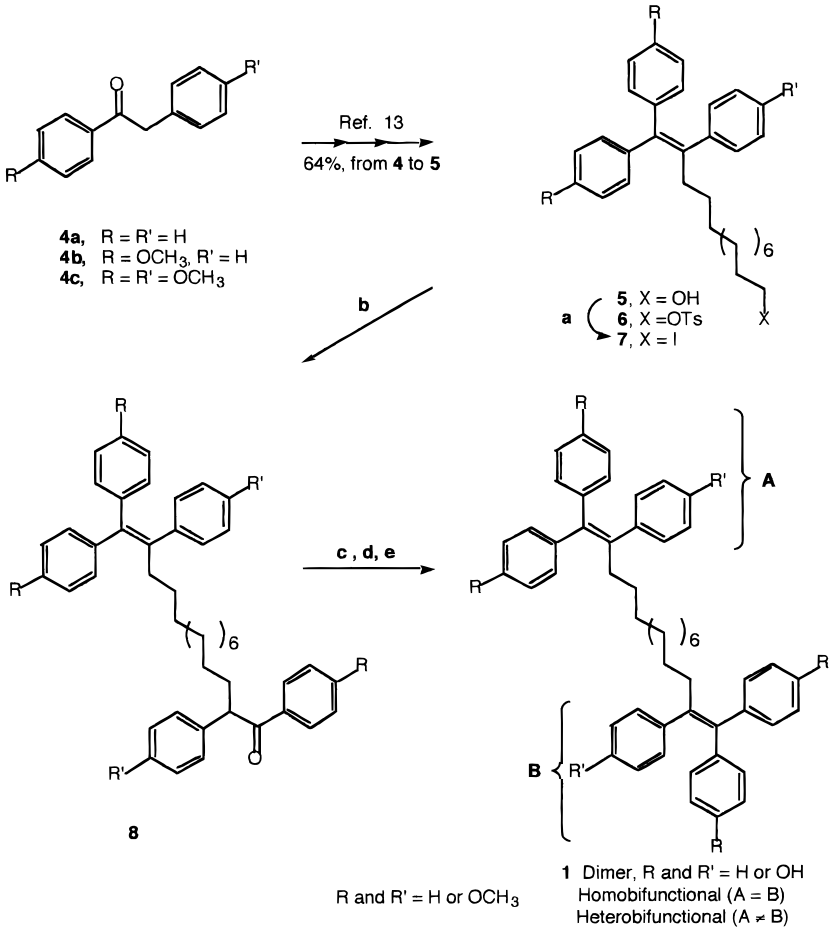
RESULTS AND DISCUSSION

Synthesis of Homo- and Heterobifunctional Dimers **1**

Scheme 2 presents the structure of three alcohols **5a** (R = R′ = H), **5b** (R = OCH_3, R′ = H), and **5c** (R = R′ = OCH_3), available in our laboratory, which were synthesized with the following starting materials: deoxybenzoin (**4a**), 1-(4′-methoxyphenyl)-2-phenyl-1-ethanone (**4b**), and desoxyanisoin (**4c**). These products were obtained with an overall yield of 65% (*13*). The alcohols were changed to the corresponding iodide in two chemical steps. First, the alcohols **5** were transformed into the corresponding tosylate intermediates **6** with a mixture of tosyl chloride and triethylamine in methylene chloride. The yield of this reaction was 95%. Treatment of the tosylate intermediates with sodium iodide in dry acetone gave the desired iodides **7** quantitatively.

The alkylation of ketones **4** (Scheme 2) was done in a mixture of tetrahydrofuran and dimethyl sulfoxide (9:1). Reaction of sodium hydride with the ketone **4** gave the sodium enolate which was immediately treated with the appropriate iodide **7** to give compound **8**. The yield of this reaction was 90%. Then, the addition of *n*-butyllithium to 4-bromoanisole in a mixture of dry tetrahydrofuran and ether (THF:Et_2O, 6:4) at −110°C gave the corresponding organolithium reagent (*17*). The organomagnesium reagent derived from 4-bromoanisole could also be used; however, being less reactive than the organolithium reagent it leads to lower yield of reaction. Phenyllithium is available commercially.

Addition of an excess of *p*-methoxyphenyllithium (or phenyllithium) to the ketone **8** and subsequent treatment of the crude tertiary alcohol intermediate with TsOH in ethanol heated to reflux gave the 1,14-pentadecadienes dimers **1** (R and R′ = H or OCH_3) with a yield of 90%. Finally, demethylation with an excess of boron tribromide in dichloromethane gave the hydroxylated dimers **1** (R and R′ = H or OH). The yield for this step was 93%. This highly efficient synthesis used six steps from the known alcohol **5**, with an overall yield of more than 60%. The global yield from ketone **4**, with nine chemical transformations, is 40%. All new compounds synthesized were homogeneous on TLC and were fully characterized by their IR, ^{1}H NMR, ^{13}C NMR, and mass spectra as well as high-resolution mass spectra.



Reagents: (a) TsCl, Et_3N, CH_2Cl_2, 22 °C, 15 h, (95%); NaI, $(CH_3)_2C{=}O$, 22 °C, 15 h, (99%); (b) NaH, **4a** **4b** or **4c**, 22 °C, 15 h, (85%); (c) R-C_6H_4Li, THF, 22 °C, 0.5 h, (90%); (d) Tertiary alcohol intermediate, PyH^+ TsO^- or TsOH, EtOH, reflux, 5h, (95%); (e) BBr_3, CH_2Cl_2, -60 °C to 22 °C, 15 h; Δ, 2 h (90%).

SCHEME 2

In vitro Cytotoxic Activity

The cytotoxic activity of our new dimers was evaluated *in vitro* on two human breast cancer tumor cell lines: MCF-7 and MDA-MB-231. The MCF-7 cell line is estrogen receptor positive (ER^+) while the MDA-MB-231 cell line is estrogen receptor negative (ER^-) (*19*). The cytotoxicity of dimers was tested along with tamoxifen **3**, as the control antiestrogen, on both human mammary carcinoma cell lines in order to assess the potential antineoplastic selectivity of these molecules. The cytotoxic activity was evaluated with the colorimetric MTT assay (*18*). The results are indicated in Table 1.

TABLE 1

Inhibitory Concentration of TAM and the Dimers on both ER^+ and ER^- Breast Cancer Cell Lines

Dimers	Number OH[a]	MCF-7 (ER^+) IC_{50} (μM)[b]	MDA-MB-231 (ER^-) IC_{50} (μM)[b]
TAM	0	16	40
1 (3-3)	6	38	38
1 (3-2)	5	74	50
1 (2-2)	4	N/A	N/A
1 (3-0)	3	90	>100
1 (2-0)	2	>100	>100
1 (0-0)	0	Insoluble	Insoluble

Note. N/A, data not available.

[a] Number of hydroxy functions on the molecule.

[b] Inhibitory concentration as obtained by the MTT assay.

As shown by the MTT assays, this type of molecule does not present selectivity toward the ER^+ breast cancer cells. The dimers **1** 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 **1** (**3-3**) bearing six hydroxy functions possesses the best *in vitro* cytotoxic activity of the series showing an $IC_{50} = 38$ μM on both type of cells. The cytotoxicity of the dimers increases with the number of hydroxy functions on the aromatic rings. The dimer with no hydroxy function (**1** (**0-0**)) was insoluble and could not be tested. The biological activity seems to be linked to the solubility of these molecules rather than the strategic location of the hydroxy functions on the dimers. Comparative biological assays with dimers bearing a polyethelene chain and those bearing aliphatic chain **1** could verify this observation. Unfortunately, the present dimeric molecules provide little improvement over the known hexestrol derivatives **2** (*12*). New dimers with enhanced cytotoxic activity must be designed, using the strategy described herein, before a complete biological evaluation of this type of dimeric molecule is done.

In conclusion, it was possible to synthesize and characterize six new nonsteroidal triphenylethylene dimers containing an aliphatic linking chain. A polyvalent strategy allowing the synthesis of symmetrical and asymmetrical molecules was used. This synthetic path is highly efficient and used six chemical steps from alcohol **5** with an overall yield of 60%. Also, it was found that cytotoxicity of the dimers **1** is correlated to the number of hydroxy groups on the molecule.

Work is in progress to synthesize symmetrical hexahydroxylated dimers with polyethylene side chains of varying length. This will allow us to perform comparative biological tests with dimers bearing alkyl (less soluble) and polyethylene linking chains (more soluble) in order to better understand the relation between the molecular structure of the dimers and their antitumoral potential.

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REFERENCES

1. Anstead, M. A., Carlson, K. E., and Katzenellenbogen, J. A. (1997) *Steroids* **62,** 268–303.
2. Jordan, V. G., and Murphy, C. S. (1990) *Endocr. Rev.* **11,** 578–610.
3. Rochefort, H. (1994) *Presse Méd.* **23,** 1211–1216.
4. Ethier, S. P. (1995) *J. Natl. Cancer Inst.* **87,** 964–973.
5. Goussard, J. (1996) *Ann. Pathol.* **16,** 91–97.
6. Schwabe, J. W. R., Chapman, L., and Finch, J. T. (1993) *Cell* **75,** 567–578.
7. Fawell, S. E., White, R., Hoare, S., Sydenham, M., Page, M., and Parker, M. G. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 6883–6887.
8. Sabbah, M., Gouilleux, F., Sola, B., Redeuilh, G., and Baulieu, E.-E. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 390–394.
9. Gibson, M. K., Nemmers, L. A., Beckman, W. C., Davis, V. L., Curtis, S. W., and Korach, K. S. (1991) *Endocrinology* **129,** 2000–2010.
10. Parczyk, K., and Schneider, J. (1996) *J. Cancer Res. Clin. Oncol.* **122,** 383–396.
11. Dauvois, S., White, R., and Parker, M. G. (1993) *J. Cell Sci.* **106,** 1377–1388.
12. Bergmann, K. E., Wooge, C. H., Carlson, K. E., Katzenellenbogen, B. S., and Katzenellenbogen, J. A. (1994) *J. Steroid Biochem. Mol. Biol.* **49,** 139–152.
13. Bérubé, G., He, Y., Groleau, S., Séné, A., Thérien, H.-M., and Caron, M. (1997) *Inorg. Chim. Acta* **262,** 139–145.
14. Perrin, D. D., and Armarego, W. L. F. (1988) Purification of Laboratory Chemicals, 3rd ed., Pergamon Press, Oxford.
15. Still, W. C., Kahn, M., and Mitra, A. (1978) *J. Org. Chem.* **43,** 2923–2925.
16. Sanders, J. K. M., and Hunter, B. K. (1993) Modern NMR Spectroscopy, 2nd ed., Oxford Univ. Press, New York.
17. Wakefield, B. J. (1988) Organolithium Methods, pp. 27–32, Academic Press, London.
18. Carmichael, J., DeGraff, W. G., Gazdar, A. F., Minna, J. D., and Mitchell, J. B. (1987) *Cancer Res.* **47,** 943–946.
19. Horwitz, K. B., Zava, D. T., Thilagar, A. K., Jensen, E. M., and McGuire, W. L. (1978) *Cancer Res.* **38,** 2434–2437.