Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 557–560

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A Facile Synthesis of C_2-Symmetric 17β-Estradiol Dimers

Daniel Rabouin,[a] Valérie Perron,[a] Blaise N'Zemba,[a] René C.-Gaudreault[b] and Gervais Bérubé[a,*]

[a]*Département de Chimie-Biologie, Université du Québec à Trois-Rivières, C.P. 500, Trois-Rivières, Québec, Canada G9A 5H7*
[b]*Unité de Biotechnologie, Institut des Biomatériaux de Québec, C.H.U.Q., Hôpital Saint-François d'Assise, 10 Rue de l'Espinay, Québec, Canada G1L 3L5*

Received 29 May 2002; accepted 1 October 2002

Abstract—A rapid and efficient synthesis of a series of C_2-symmetric 17β-estradiol dimers is described. The new molecules are linked at position 17α of the steroid nucleus with either an alkyl chain or a polyethylene glycol chain. They are made from estrone in five chemical steps with an overall yield exceeding 30%. The biological activity of these compounds was evaluated in vitro on estrogen dependent and independent (ER^+ and ER^-) human breast tumor cell lines: MCF-7 and MDA-MB-231. Some of the dimers present selective cytotoxic activity against the ER^+ cell line.

Introduction

The design of C_2-symmetric ligands (bivalent ligands) as bioactive molecules have attracted considerable attention over the years because of their promising therapeutic value in treating a number of diseases. For example, bivalent ligands were designed as antagonist of the muscarinic receptor and of the *k* opiod receptor.[1,2] A variety of dimeric enzyme inhibitors were also studied such as HIV-1 protease inhibitors as well as the glycosidases inhibitors.[3,4] Some bivalent ligands were used as prodrugs with androgenic and myotrophic activities and a dimer of a naturally occurring monomeric naphthylisoquinoline alkaloid was prepared yielding an analogue with high antimalarial activity.[5,6] Recently, we have reported the synthesis of spermidine and norspermidine dimers as high affinity polyamine transport inhibitors.[7] It is generally believed that a bivalent ligand would be expect to show enhanced receptor affinity (or biological activity) relative to the monovalent ligand.[8] Hence, we can understand the great interest in making such dimeric compounds.

Furthermore, some attempts have been made to design estrogenic bivalent ligands in order to interfere with the process of estrogen receptor dimerization.[9,10] These dimeric molecules were constructed from non-steroidal estrogenic moieties analogues of hexestrol or triphenylethylene. It was demonstrated that some of the hexestrol bivalent ligands possess antiestrogenic activity.[9] In some cases, a biphasic interaction with the estrogen receptor was observed.[9] On the other hand, it was demonstrated that a symmetrical triphenylethylene dimer bearing six hydroxy functions, possesses a cytotoxic activity similar to that of tamoxifen. However, this type of molecule did not present selectivity towards ER^+ breast cancer cells.[10]

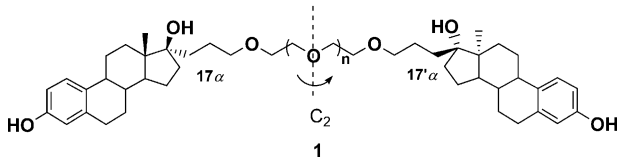
Recently, we have reported the synthesis of estrone dimers linked at position 16 of the steroid nucleus.[11] These dimers were linked via two ester groups with an alkyl chain or a polyethylene glycol (PEG) chain. It was shown that the estrone dimers were non-toxic towards ER^+ (MCF-7) and ER^- (MDA-MB-231) human breast cancer cell lines. In order to obtain dimers with a stronger linkage than the ester bonds found in the previously described molecules, we planned the synthesis of estradiol dimers which are linked together via ether bonds. The ether connection should increase the stability of the dimeric molecules while improving its solubility.

We believed that the used of the natural steroid nucleus in the design of such dimeric molecule could increase the chance of interactions with the estrogen receptor. Therefore, by increasing its interaction with the ER, one would theoretically enhance its biological activity as compared to the non-steroidal dimers. It is also believed that this type of molecules synthesized from the natural

*Corresponding author. Fax: +1-819-376-5084; e-mail: gervais_berube@uqtr.ca

0960-894X/03/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(02)00987-3

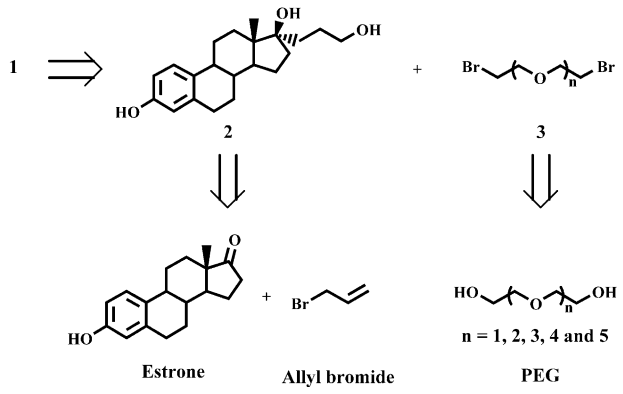
hormone would present essentially no toxicity towards healthy or cancerous cells but could possess interesting antiestrogenic activity. This manuscript describes the synthesis of several members of this new family of C_2-symmetric 17β-estradiol dimers (see general structure **1**). It also reports the in vitro cytotoxic activity of the dimers on two neoplastic human breast cancer cell lines: MCF-7 and MDA-MB-231 (ER^+ and ER^-).



1

Chemistry

A retrosynthetic analysis of the dimers is presented in Scheme 1. As one can observe, the 17α,17′α-estradiol dimer **1** possess a C_2 symmetry. The dimers **1** can easily be made from a suitable dihalogenated PEG **3** (or an α,ω-dibromoalkane) component and an adequately functionalised estradiol derivative **2** by selective alkylation on the primary alcohol. The latter can be prepared from estrone **4** itself and allyl bromide via a Grignard reaction. This analysis provides a simple path for the synthesis of a large variety of new estradiol dimers. Moreover, the dihalogenated PEG are ideal linkers because they are inexpensive, water soluble, and available in a variety of lengths. In addition, the calculated LogP of the dimers shows that their solubility remain almost constant whatever the length the linking arms (see Table 1).



Scheme 1. Retrosynthetic analysis for the 17-β-estradiol dimers.

As shown in Scheme 2, the di-, tri- tetra-, penta- and hexa-ethylene glycols were readily transformed into the dibrominated PEGs in two chemical steps with high yields. Initially, the PEGs were treated with methanesulfonyl chloride and triethylamine in ether to give the dimesylate intermediate which was not isolated. The precipitated triethylamine hydrochloride salt was filtered off and the residue evaporated to an oil. Afterwards, the dimesylate was treated with lithium bromide in acetone at reflux for 20 h to give the desired dibromides **3** with an average overall yield of 70%.

As shown in Scheme 3, six estradiol dimers were obtained using a straightforward reaction sequence. Initially, estrone (**4**) was protected as a benzyl ether using phase transfer catalysis (PTC) methodology. Thus, estrone was treated with benzyl bromide and tetrabutylammonium hydrogen sulfate in dichloromethane in the presence of a 10% aqueous sodium hydroxide solution.[12] The yield of the protection reaction is 98%. The derivative **5** was transformed into the 17α-(prop-2′-enyl) estradiol **6** upon treatment with freshly prepared allylmagnesium bromide in dry diethylether.[13] Derivative **6** was obtained with 95% yield. The hydroboration-oxydation sequence performed on compound **6** gave the diol **2** in 72% yield.[13] Hydrogenolysis of this intermediate with 10% Pd/C in tetrahydrofuran gave quantitatively the triol **7**.[12] Selective *O*-alkylation of diol **2** was performed upon treatment with sodium hydride for 30 min in a mixture of dry tetrahydrofuran and dimethylformamide to which was added the appropriate dihalogenated PEG chain **3** (or α,ω-dibromoalkane) at room temperature for 20 h. The dimers were obtained with an average yield of 50%. The final dimeric molecules were obtained quantitatively by hydrogenolysis of the benzyl ether with 10% Pd/C in tetrahydrofuran. The dimers were obtained with an average overall yield varying from 27% to 36%. All new compounds synthesized were characterized by their respective IR, ^1H NMR, ^{13}C NMR and mass spectra.[14]

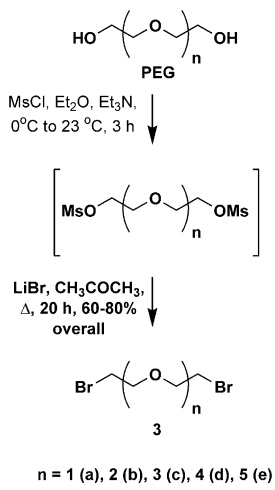
In Vitro Antitumor Activity

The toxicity of the dimers was evaluated on two human breast tumor cell lines using the Sulforhodamine B col-

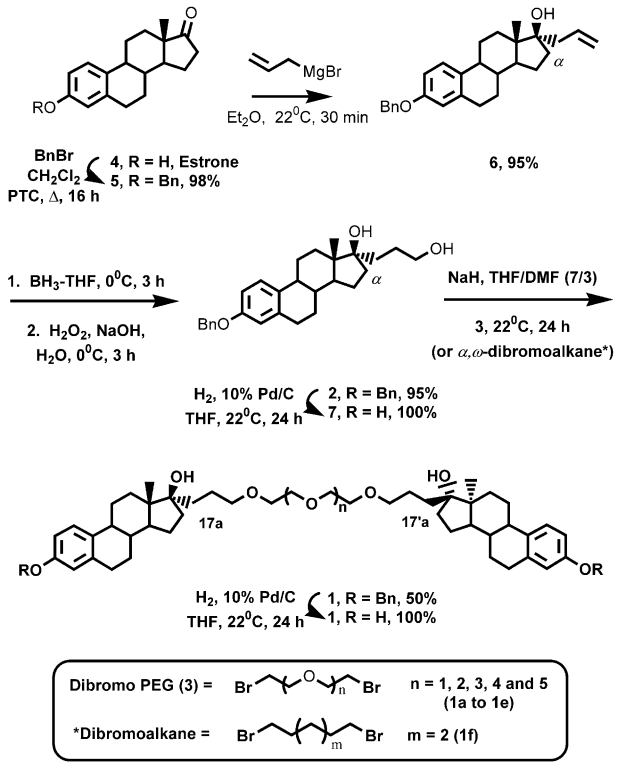
Table 1. Inhibitory concentration of drug on both ER^+ and ER^- breast cancer cell lines, chain length and CLogP

Compd	MCF-7 (ER^+) IC_{50} (μM)[a]	MDA-MB-231 (ER^-) IC_{50} (μM)[a]	Chain length *n* or *m*	CLogP[b]
Estradiol	32	>100	—	3.78
Tamoxifen	11	19	—	6.82
7	71	>100	—	3.37
1a	>200	>200	$n=1$	8.22
1b	62	>100	$n=2$	8.28
1c	63	>100	$n=3$	8.35
1d	>100	>100	$n=4$	8.41
1e	>100	>100	$n=5$	8.47
1f	>100	>100	$m=2$	9.32

[a]Inhibitory concentration as obtained by the SRB assay. Experiments were performed in octuplicate, errors are within ±10%.
[b]Calculated LogP as obtained with ChemDraw Ultra 6.0.



Scheme 2. Synthesis of dihalogenated PEG chains.



Scheme 3. Synthesis of 17β-estradiol dimers.

orimetric assay.[15,16] The cytotoxicity of the compounds was tested along with controls (estradiol and tamoxifen) on both estrogen-receptor positive (ER^+, MCF-7) and estrogen-receptor negative (ER^-, MDA-MB-231) human mammary carcinomas.[17]

As shown by the SRB assays on the two human breast cancer cell lines, the dimers are not very toxic towards breast cancer cells (Table 1). The reference products, estradiol and tamoxifen are more toxic than the dimers. Interestingly, there is a selective activity of dimers **1b** and **1c** against the ER^+ cell line. The lower homologue **1a** and the higher homologues **1d** and **1e** present no cytotoxicity showing an IC_{50} of more than 100 μM. The total length of the chain linking compounds **1b** and **1a** is 16 and 19 atoms long. The shorter linkage seen in derivative **1a** (13 atoms long) as well as the longer linkages seen in compounds **1d** and **1e** (22 and 25 atoms long) appear to be inadequate for selective biological activity on ER^+ cancer cells. The calculated LogP of the dimers is relatively constant throughout the sequence varying from 8.22 to 8.47. The dimer **1f** bearing a carbon atom linking chain is not toxic and possesses a CLogP of 9.32 and is therefore less soluble than the PEG dimeric analogues. On the other hand, the triol **7** show some toxicity against the ER^+ (MCF-7) breast cancer cells with a IC_{50} of 71 μM.

In summary, this manuscript presents a facile synthesis of C_2-symmetric 17β-estradiol dimers. They are made form estradiol in five chemical steps with an overall yield exceeding 30%. The key steps for the synthesis of these compounds is the selective *O*-alkylation of diol **2** with the dibrominated PEG chains **3**. The dimers are generally less toxic towards breast cancer cells as compare to the cognate hormone 17β-estradiol and the antiestrogen tamoxifen. Interestingly, dimers **1b** and **1c** show specific toxicity towards MCF-7, an hormone-dependent breast cancer cell line. Further investigation will be necessary to assess the complete biological potential of these new C_2-symmetric 17β-estradiol dimers.

Acknowledgements

This work was supported by the Université du Québec à Trois-Rivières. We are grateful to Mr. Jacques Lacroix for biological evaluation studies. We also thank Dr. Gilles Sauvé and Mr. Nicolas Le Berre, Pharmacor Inc. for the ^1H and ^{13}C NMR spectra and Mr. Gaston Boulay, Université de Sherbrooke, for the mass spectra.

References and Notes

1. Moser, U.; Gubitz, C.; Galvan, M.; Immel-Sehr, A.; Lambrecht, G.; Mutschler, E. *Drug Res.* **1995**, *45*, 449.
2. Bolognesi, M.-L.; Ojala, W. H.; Gleason, W. B.; Griffin, J. F.; Farouz-Grant, F.; Larson, D. L.; Takemori, A. E.; Portoghese, P. S. *J. Med. Chem.* **1996**, *39*, 1816.
3. Chakraborty, T. K.; Ghosh, S.; Ramana Rao, M. H. V.; Kunwar, A. C.; Cho, H.; Ghosh, A. K. *Tetrahedron Lett.* **2000**, *41*, 10121.
4. Merrer, Y. L.; Gauzy, L.; Gravier-Pelletier, C.; Depezay, J.-C. *Bioorg. Med. Chem. Lett.* **2000**, *8*, 307.
5. Millership, J. S.; Shanks, M. L. *J. Pharm. Sci.* **1988**, *77*, 116.
6. Bringmann, G.; Saeb, W.; Koppler, D. *Tetrahedron* **1996**, *52*, 13409.
7. Covassin, L.; Desjardins, M.; C.-Gaudreault, R.; Audette, M.; Bonneau, M.-H.; Poulin, R. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1709.
8. Portoghese, P. S. *J. Med. Chem.* **1992**, *35*, 1929.
9. Bergmann, K. E.; Wooge, C. H.; Carlson, K. E.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. *J. Steroid Biochem. Molec. Biol.* **1994**, *49*, 139.
10. Groleau, S.; Nault, J.; Lepage, M.; Couture, M.; Dallaire, N.; Bérubé, G.; C.-Gaudreault, R. *Bioorg. Chem.* **1999**, *27*, 383.

11. Rabouin D. M.Sc. Thesis, Université du Quebec à Trois-Rivières, Trois Rivières, August 2001.
12. Greene, T. W.; Wuts, P. G. M. In *Protective Groups in Organic Synthesis*, 3rd Ed., John Wiley & Sons: New York, 2000.
13. Dionne, P.; Tchédam Ngatcha, B.; Poirier, D. *Steroids* **1997**, *62*, 674.

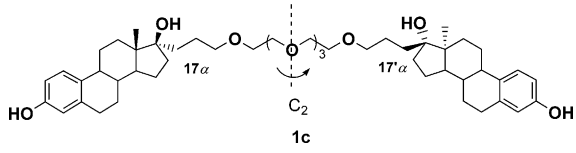
14. Spectral data for 3-*O*-benzyl-17α-(3′-hydroxypropyl)-1,3,5(10)-estratrien-17β-ol (**2**): **IR** (NaCl, ν_{max}, cm^{-1}): 3360 (O–H), 1600 (C=C), 1254 and 1017 (C–O). **^{1}H NMR** ($CDCl_3$, δ ppm): 7.41 (2H, d, $J=7.0$ Hz, a-CH), 7.36 (2H, t, $J=7.2$ Hz, b-CH), 7.30 (1H, d, $J=6.7$ Hz, c-CH), 7.18 (1H, d, $J=8.5$ Hz, 1-CH), 6.76 (1H, dd, $J=7.9$ Hz and $J=2.0$ Hz, 2-CH), 6.71 (1H, br s, 4-CH), 5.00 (2H, s, C**H**$_2$Ph), 3.67 (2H, m, 3′-C**H**$_2$OH), 2.86 (2H, m, 6-CH_2), 2.59 (2H, br s, 2× OH), 2.31–1.20 (17H, #m, 3× CH and 7× CH_2), 0.90 (3H, s, 18-CH_3). **^{13}C NMR** ($CDCl_3$, δ ppm): 156.7 (C-3), 138.0 (**C**CH$_2$O), 137.3 (C-5), 132.9 (C-10), 128.5 (C-b), 127.8 (C-c), 127.4 (C-a), 126.2 (C-1), 114.8 (C-4), 112.2 (C-2), 83.2 (C-17), 69.9 (**C**H$_2$Ph), 63.3 (C-3′), 49.5, 46.7, 43.8, 39.6, 34.4, 33.4, 31.5, 29.8, 27.5, 26.9 (C-2′), 26.3, 23.4, 14.3 (C-18). **MS** (*m*/*e*): 420 (M^+), 402 (M^+-H_2O), 362 ($M^+-C_3H_6O$). **Exact mass**: calculated for $C_{28}H_{36}O_3=420.2664$; found = 420.2656.

NB: a-CH, b-CH and c-CH are *ortho*, *meta* and *para* protons on the benzyl protecting group. Similarly, a-C, b-C and c-C are the corresponding carbons on the benzyl group.

Spectral data for 3-hydroxy-17α-(3′-hydroxypropyl)-1,3,5(10)-estratrien-17β-ol (**7**): **IR** (NaCl, ν_{max}, cm^{-1}): 3351 (O–H), 1156 and 1241 (C–O). **^{1}H NMR** (Acetone-d_6, δ ppm): 8.10–7.70 (1H, br s, 3-OH), 7.08 (1H, d, $J=8.5$ Hz, 1-CH), 6.76 (1H, dd, $J=1.9$ Hz and $J=8.4$ Hz, 2-CH), 6.59 (1H, dd, $J=2.4$ Hz and $J=8.7$ Hz, 2-CH), 6.52 (1H, d, $J=1.6$ Hz, 4-CH), 3.64– 3,50 (2H, m, C**H**$_2$OH), 3.30 - 3.05 (2H, br s, 17-OH and CH$_2$O**H**), 2.78 (2H, m, 6-CH_2), 2.40–1.10 (17H, m, 7× CH_2, 3× CH), 0.92 (3H, s, 18-CH_3). **^{13}C NMR** (Acetone-d_6, δ ppm): 155.9 (C-3), 138.5 (C-5), 132.2 (C-10), 127.0 (C-1), 115.9 (C-4), 113.6 (C-2), 83.1 (C-17), 63.5 (C**H**$_2$OH), 50.5, 47.7, 44.8, 40.9, 34.8, 34.3, 32.6, 30.6 (hidden by acetone), 28.4, 28.1, 27.3, 27.2, 15.1 (18-CH_3). **MS** (*m*/*e*): 330 (M^+), 312 (M^+-H_2O), 271 ($M^+-C_3H_7O$). **Exact mass:** calculated for $C_{21}H_{30}O_3=330.2195$; found = 330.2198.

Spectral data for 1,11-dibromo-3,6,9-trioxadecane (**3c**, $n=3$): **IR** (NaCl, ν_{max}, cm^{-1}): 1277 and 1116 (C–O). **^{1}H NMR** ($CDCl_3$, δ ppm): 3.80 (4H, t, $J=6.3$ Hz, 2× BrCH$_2$C**H**$_2$O), 3.66 (8H, s, 4× C**H**$_2$O), 3.46 (4H, t, $J=6.3$ Hz, 2× C**H**$_2$Br). **^{13}C NMR** ($CDCl_3$, δ ppm): 71.2 (BrCH$_2$**C**H$_2$O), 70.6 and 70.5 (**C**H$_2$O), 30.3 (**C**H$_2$Br)

Spectral data for dimer **1c** ($n=3$): **IR** (NaCl, ν_{max}, cm^{-1}): 3385 (O–H), 1610 (C=C), 1287 and 1036 (C–O). **^{1}H NMR** ($CDCl_3$, δ ppm): 7.05 (2H, d, $J=8.5$ Hz, 2× 1-CH), 6.59 (2H, d, $J=8.2$ Hz, 2× 2-CH), 6,51 (2H, d, $J=1,4$ Hz, 2× 4-CH), 3.65–3.42 (20H, s and m, 10× C**H**$_2$O), 3.06 (4H, br s, 3-OH and 17-OH), 2.76 (4H, m, 2× 6-CH_2), 2.30–1.15 (34H, m, 14× CH_2, 6× CH), 0.84 (6H, s, 2× 18-CH_3). **^{13}C NMR** ($CDCl_3$, δ ppm): 155.1 (C-3), 138.9 (C-5), 132.7 (C-10), 127.1 (C-1), 116.2 (C-4), 113.6 (C-2), 84.0 (C-17), 73.0, 71.3, 70.8, 50.4, 47.7, 44.6, 40.6, 34.8, 34.2, 32.5, 30.6, 28.4, 27.2, 24.8, 24.3, 15.4 (C-18). **MS** (*m*/*e*): 800 (M^+-H_2O), 782 (M^+-2H_2O). **Exact mass**: calculated for $C_{50}H_{72}O_8$ (M^+-H_2O) = 800.5227; found = 800.5231.



15. Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91.
16. Martin, A.; Clynes, M. *Cytotechnology* **1993**, *11*, 48.
17. Horwitz, K. B.; Zava, D. T.; Thilagar, A. K.; Jensen, E. M.; McGuire, W. L. *Cancer Res.* **1978**, *38*, 2434.