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Synthesis and Evaluation of Estradiol Derivatives With 16α-(Bromoalkylamide), 16α-(Bromoalkyl) or 16α-(Bromoalkynyl) Side Chain as Inhibitors of 17β-Hydroxysteroid Dehydrogenase Type 1 Without Estrogenic Activity

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Abstract—To develop inhibitors of 17β-hydroxysteroid dehydrogenase (17β-HSD) without residual estrogenic activity, the synthesis of 16α-(bromoalkylamide) derivatives of estradiol was performed starting from a key intermediate aldehyde obtained from commercially available estrone. In addition, series of 16α-(bromoalkyl) and 16α-(bromoalkynyl) derivatives of estradiol were also prepared as model compounds. All new compounds inhibited human placental cytosolic 17β-HSD (type 1) with IC_{50} values ranging from 1.7 to 10.6 μM. From these results, we observed that a primary bromide produces a greater inhibition of 17β-HSD activity than secondary bromide, and that a shorter 16α-side chain increases the inhibiting activity. In the estrogen-sensitive ZR-75–1 human breast cancer cell line, the 16α-(bromoalkylamide)-estradiol series had no estrogenic activity at 30 nM, and only the compound with a shorter side chain length showed an estrogenic activity at 1000 nM. Interestingly, at this concentration, the compound with an intermediate side chain length showed an antiestrogenic activity of 74%, whereas the compound with the longer side chain length showed 34% of antiestrogenic activity. In this test, other 17β-HSD inhibitors (without bromoalkylamide side chain) were fully estrogenic. Among synthesized compounds, the estradiol derivative **4** (*N*-butyl, *N*-methyl, 9-[3′,17′β-(dihydroxy)-1′,3′,5′(10′)-estratrien-16′α-yl]-7-bromononamide) was the best compromise for a dual-action inhibitor. This compound inhibited moderately and reversibly the 17β-HSD type 1 activity, but possessed no estrogenic activity and exhibited antiestrogenic activity in the ZR-75–1 cell line. Copyright © 1996 Elsevier Science Ltd

Introduction

Estrogens are known to be involved in the growth of estrogen-dependent breast cancer. The action of estrogens in target tissues requires binding to the estrogen receptor; so a logical, now classic, approach to treating estrogen-dependent breast cancer is to use an antiestrogen to block the interaction of estrogens with their specific receptors.[1,2] Recently, a new generation of steroidal compounds was described as pure antiestrogens. These compounds, represented by ICI 164384,[3,4] EM-139,[5] RU 51625,[6] or ICI 182780,[7] contain a long side chain in the 7α- or 11β-position of an estradiol nucleus. The first two compounds have a long methyl butyl alkylamide side chain on the 7α-position of the estradiol nucleus. The difference between EM-139 and ICI 164384 is the chloride on the 16α-position of EM-139. The RU 51625 has a propyl methyl alkylamide side chain similar to that of ICI 164384, but at the 11β-position of the estradiol. For the last compound, ICI 182780, a new pentafluoropentylsulfinyl side chain is located in the 7α-position of the estradiol. In all cases reported above, the long alkylamide or fluorosulphinyl side chain is essential to obtain a good antiestrogenic activity.[8]

An alternate approach to the use of antiestrogens is to inhibit the enzymes involved in the synthesis of active steroidal hormones. Among these enzymes, 17β-hydroxysteroid dehydrogenase (17β-HSD) is responsible for the reversible transformation of estrogen, as well as androgens. In humans, three cDNAs encoding 17β-HSD have been cloned and characterized. Type 1 (interconverting estrone and estradiol)[9–12] and type 2 (interconverting estrone and estradiol as well as 4-androstenedione and testosterone)[13,14] were purified, respectively, from the cytosolic and microsomal fractions of human placenta, whereas type 3 (interconverting 4-androstenedione and testosterone) was found in human testis[15] and human hyperplastic prostate.[16] The 17β-HSD is expressed not only in classic steroidogenic tissues, such as the ovary, testis, and placenta, but also in a large series of peripheral tissues,[17–19] including normal and cancerous breast tissues.[20–23] Despite the fact that the formation of estrone from estradiol (oxidative activity) is predominant in most tissues, there is a higher level of 17β-HSD type 1 reductive activity in breast cancer cells,[24,25] which is responsible for the formation of mitogenic estradiol. Moreover, 17β-HSD activity has been reported to be higher in cancerous tissues

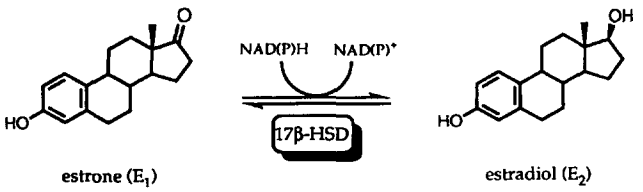
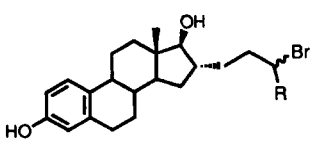


Figure 1. Interconversion of estrone (E_1) and estradiol (E_2) by 17β-hydroxysteroid dehydrogenase (17β-HSD) in the presence of cofactors (NAD(P)H or NAD(P)$^+$).

compared with normal breast tissues.[26,27] It is therefore attractive to lower the level of the most active estrogen, estradiol, by blocking the 17β-HSD type 1 activity (Fig. 1). Thus, inhibition of 17β-HSD type 1 would be a useful complementary strategy to the use of antiestrogen in the estrogen-dependent diseases.

Some work has been reported on the synthesis of 17β-HSD type 1 inhibitors. Chin et al.[28,29] synthesized the 16α-bromoacetoxy-estradiol 3-methyl ether and the 12β-bromoacetoxy-4-estrene-3,17-dione as irreversible inhibitors, while Thomas et al.[30] developed 16-methylene-estradiol as a suicide inhibitor. Recently, our group synthesized a series of 16α- and 16β-(halogenoalkyl)-estradiol.[31,32] Iodo and bromo compounds were revealed to be more potent irreversible inhibitors than chloro or fluoro analogues,[31] and the optimal side chain lengths were fixed to 3- and 4-carbons. Thus, 16α-(bromopropyl)-estradiol (**1**, Fig. 2), 16α-(bromobutyl)-estradiol, and 16β-(bromobutyl)-estradiol were the most potent inhibitors. Although the compounds reported above were good inhibitors of cytosolic 17β-HSD type 1, they were not useful for the eventual treatment of estrogeno-dependent diseases because of their residual estrogenic activity. So we decided to synthesize new compounds combining the two functional groups on the 16α-side chain: the alkylamide, which is responsible for the antiestrogenic activity, and the bromoalkyl, which causes irreversible inhibition of 17β-HSD type 1.

As we noted in our preliminary work,[33] the use of a 16α-(bromoalkylamide) side chain on an estradiol nucleus is responsible for the total inhibition of cytosolic 17β-HSD transformation of estrone to estradiol at 100 μM with an IC_{50} value of 10.6 μM. Consequently, we considered this bromoundecanamide



1 (R = H)
2 (R = $(CH_2)_7CONBuMe$)
3, 4 (R = $(CH_2)_nCONBuMe$; n = 3, 5)
5-7 (R = $(CH_2)_nCH_3$; n = 0, 2, 4)
8 (R = $C{\equiv}C(CH_2)_2CH_3$)

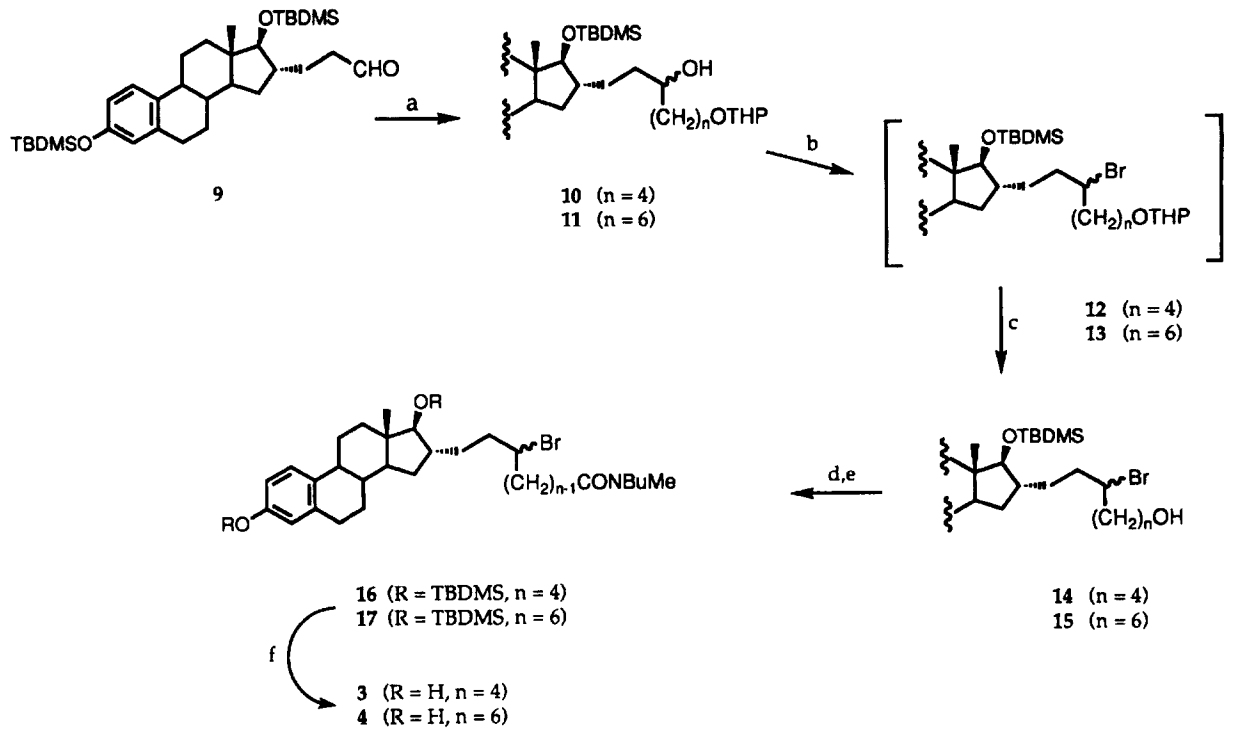
Figure 2. Chemical structures of 16α-estradiol derivatives **1–8** used in this study.

derivative (**2**, Fig. 2) to be a weak inhibitor of 17β-HSD type 1. Interestingly, this compound showed no estrogenic activity at a concentration of 30 nM when tested in vitro in a ZR-75–1 cells proliferative assay. Thus, it was possible to block the estrogenic activity with a bromo alkylamide side chain on the 16α-position of estradiol. To optimize these results, we considered the synthesis of two 16α-(bromoalkylamide) derivatives of estradiol with shorter side chain lengths (**3** and **4**, Fig. 2). We also synthesized a series of 16α-(bromoalkyl) and 16α-(bromoalkynyl) derivatives of estradiol (**5–8**, Fig. 2) to show the influence of a bromoalkyl side chain length and the effect of a secondary bromide on the inhibition of 17β-HSD type 1.

Results and Discussion

Chemical synthesis

Synthesis of 16α-(bromoalkylamide) derivatives of estradiol (3 and 4, Scheme 1). The synthesis of the key aldehyde **9** was performed in six steps from commercially available estrone following a procedure already described.[33] For the synthesis of compounds **3** and **4**, which have two different chain lengths, the same reaction sequence previously reported for the preparation of compound **2**[33] was used with few modifications. The first step was the addition of a Grignard reagent to the aldehyde **9** giving the secondary alcohols **10** and **11**. The next sequence of the reaction was the bromination of alcohols (**10** and **11**) using the triphenylphosphine–bromine complex, followed by deprotection of the THP-protective group to afford the primary alcohols **14** and **15**. Jones' oxidation of the primary alcohol gave the corresponding carboxylic acid. Without purification, this acid was transformed to alkylamides **16** and **17** by tributylamine and *iso*-butylchloroformate (activation) and *N*-butylmethylamine (amidation). The alkylamide side chain was easily observed by a duplication of characteristic ^1H and ^{13}C NMR signals ($CONCH_3$, $CONCH_2$, and CH_3 of the butyl group). The duplication of NMR signals for the protons and carbons (Table 1) surrounding the amide group was explained by the two preferred conformations of the amide bond, and has already been observed for compounds with a similar side chain.[4,5,33] The last step was the deprotection of di-TBDMS protective groups by HCl in methanol, leading to the final products **3** and **4**. No important differentiation of characteristic spectral signals was observed between both chain-length derivatives, except for the methyl-18 and CHBr. Indeed for compound **3**, the ^1H NMR signal of 18-CH_3 and CHBr was duplicated because of the rigidity resulting from the shortest alkylamide side chain. This fixed arrangement, combined with the presence of a chiral center on the side chain (CHX), explains the duplication of both signals. On the other hand, all intermediate (**10–17**) and final (**3** and **4**) compounds described above were obtained as a mixture of two isomers, at carbon bearing the atom of bromine. Although both isomers were not observed by ^1H NMR data (except **3**), their



Scheme 1. Synthesis of 16α-(bromoalkylamide)-estradiols **3** and **4**. The reagents are: (a) $BrMg(CH_2)_nOTHP$ ($n=4$ or 6); (b) (i) Ph_3P, Br_2, imidazole, (ii) **10** or **11**; (c) amberlyst resin; (d) Jones' reagent; (e) (i) tributylamine, *iso*-butylchloroformate, (ii) *N*-butylmethylamine; (f) HCl/MeOH (2:98).

presence was clearly shown by the NMR signals of carbon surrounding the chiral center and particularly the CHX signal (Table 1). Based on the ^{13}C NMR signals of CHX, the proportions of both isomers were estimated to ~50:50.

Synthesis of 16α-(bromoalkyl)-estradiol derivatives (5–7, Scheme 2). From di-TBDMS aldehyde **9**, appropriate Grignard reagents were used to lead to the formation of compounds **5–7**, with three side chain lengths (4, 6, and 8 carbons). Methylmagnesium iodide was used for the formation of alcohol **18**, propylmagnesium chloride for **19**, and pentylmagnesium bromide for **20**. Bromination of the secondary alcohol using a triphenylphosphine-bromine complex followed by the cleavage of di-TBDMS-groups with 2% HCl in methanol, provided the 16α-(bromoalkyl)-estradiol derivatives **5–7** in moderate yields. The formation of compounds **5–7** was confirmed by IR, ^1H NMR, ^{13}C NMR (Table 1), and MS data.

Synthesis of 16α-(bromoalkynyl)-estradiol derivative (8, Scheme 3). The lithium reagent generated from 1-pentyne and MeLi was added dropwise to the aldehyde **9**, providing the alkynyl alcohol **24**. The next step was bromination of the secondary alcohol into bromide **25**. For this transformation, a recently described bromination agent (BBr_3) was used successfully.[34] Without purification, the di-TBDMS protective groups of the crude bromide **25** were cleaved by 2% HCl in methanol. The formation of 16α-(bromoalkynyl)-estradiol **8** was confirmed by IR, ^1H NMR, ^{13}C NMR (Table 1), and MS data. By heteronuclear correlation experiment (HETCOR), the CHBr-proton signal at 4.61 ppm was correlated with the signal of carbon 38.94 ppm (Table 1). This low ^{13}C NMR chemical shift can be explained by the combined shielding effect of the bromine atom and triple bond and confirms the substitution of hydroxyl group by the bromine atom.

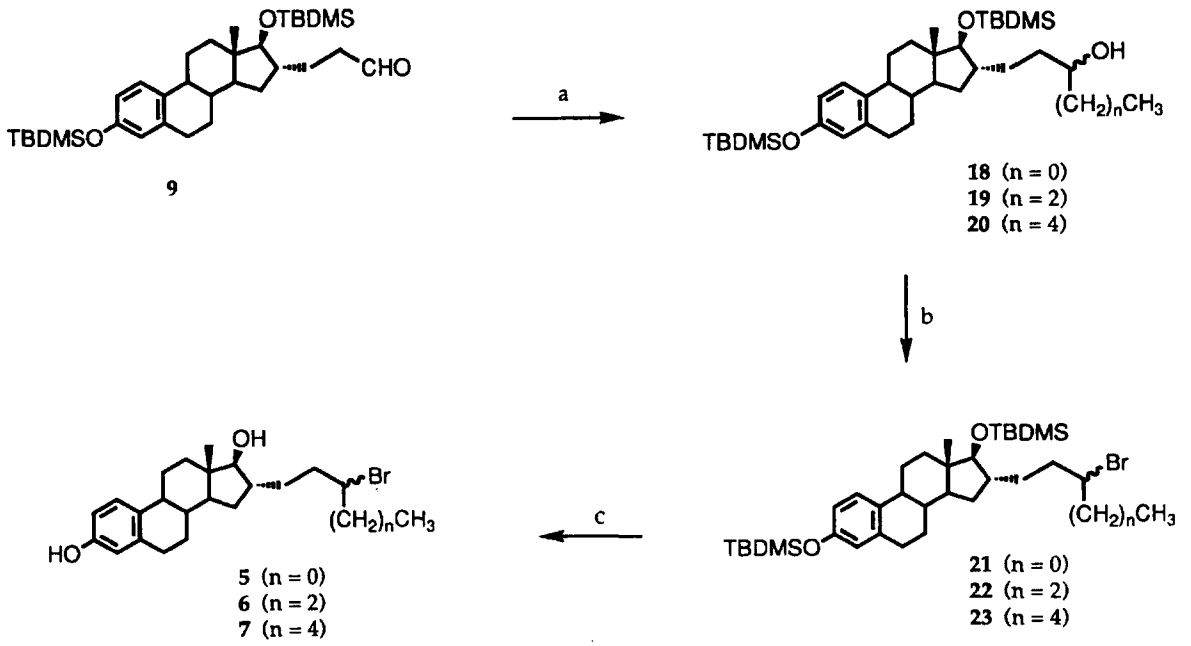
Inhibition of 17β-hydroxysteroid dehydrogenase (17β-HSD) type 1

The 17β-HSD activity of human placental cytosol (type 1) was used to evaluate the ability of compounds **1–8** to inhibit the transformation of estrone to estradiol. Since it is important to control the reduction of the less potent hormone estrone to the more potent hormone estradiol, we chose to observe the reductive sense of the 17β-HSD transformation with NADH as cofactor. In our enzymatic assay, increasing concentrations of tested compounds were used to inhibit the transformation of [^3H]-estrone to [^3H]-estradiol and obtain the inhibition curves. As can be seen for compounds **2–4** of the bromoalkylamide series (Fig. 3), a total inhibition of 17β-HSD type 1 was observed at a concentration of 100 μM. The IC_{50} values of 7.3, 10.4, and 10.6 μM for compounds **3**, **4**, and **2**, respectively, suggested that compound **3**, with a shorter side chain, was the most potent inhibitor of this series of the 16α-(bromoalkylamide) derivatives of estradiol (Table 2). The inhibiting activity of compounds **2–4** were, however, lower than that already reported for a good inhibitor of 17β-HSD type 1, the 16α-(bromopropyl)-estradiol (**1**, $IC_{50}=0.5$ μM).[31]

Table 1. ^{13}C NMR data for several intermediates and target estradiol derivatives

CARBONS[a,b]	COMPOUNDS[a,c–e]											
	10	**11**	**14**	**15**	**16**	**17**	**3**	**4**	**5**	**6**	**7**	**8**
1	126.05	125.97	126.05	125.98	126.01	125.99	126.28	126.37	126.46	126.48	127.01	126.46
2	117.11	117.03	117.12	117.07	117.07	117.05	112.79	112.76	112.67	112.65	113.60	112.66
3	153.27	153.16	153.30	153.20	153.19	153.20	153.95	153.77	153.35	153.32	155.95	153.32
4	119.92	119.83	119.93	119.85	119.88	119.85	115.31	115.31	115.25	115.23	115.98	115.22
5	137.52	137.75	137.87	137.75	137.84	137.80	137.98	138.12	138.24	138.26	138.44	138.25
6	29.70	29.60	29.65	29.58	29.61	29.58	29.59	29.62	29.58	29.57	[f]	29.56
7	27.26	27.19	27.25	27.19	27.21	27.19	27.18	27.21	27.15	27.15	28.16	27.15
8	38.70	38.60	38.67	38.60	38.63	38.61	38.57	38.63	38.57	38.58	39.86	38.57
9	44.06	43.96	44.03	43.96	44.00	43.96	43.93	43.97	43.93	43.94	45.04	43.95
10	133.20	133.10	133.17	133.10	133.14	133.10	132.00	132.31	132.63	132.68	132.11	132.66
11	26.32	26.18	26.29	26.23	26.26	26.23	26.18	26.22	26.18	26.19	27.17	26.18
12	37.46	37.42	37.41	37.35	37.38	37.35	36.74	36.77	36.71	36.72	37.80	36.71
13	44.30	44.20	44.24	44.23	44.26	44.25	44.17	44.29	44.10	44.14	44.97	44.20
14	48.29	48.20	48.26	48.19	48.22	48.19	48.31	48.37	48.36	48.36	49.27	48.37
15	29.38	29.32	29.29	29.24	29.21	29.24	30.00	30.07	30.01	30.00	31.14	30.18
16	43.78	43.72	43.70	43.63	43.73	43.66	43.24	43.34	43.33	43.41	43.73	43.02
			(43.37)	(43.30)	(43.26)	(43.27)	(42.70)	(42.78)	(43.12)	(43.21)	(43.43)	
17	88.03	87.96	87.91	87.84	87.90	87.83	87.93	88.06	88.06	88.08	88.23	87.97
	(87.88)	(87.81)			(87.81)							
18	12.24	12.17	12.21	12.16	12.18	12.15	11.90	11.89	11.87	11.86	12.52	11.85
$Si(\underline{C}H_3)_2$-3	−4.38	−4.46	−4.38	−4.44	−4.34	−4.42	–	–	–	–	–	–
$Si(\underline{C}H_3)_2$-17	−4.00	−4.08	−4.00	−4.07	−4.03	−4.03	–	–	–	–	–	–
	(−3.93)	(−4.00)	(−3.90)	(−3.94)	(−3.89)	(−3.97)						
$SiC(CH_3)_3$-3	18.16	18.07	18.16	18.08	18.11	18.09	–	–	–	–	–	–
$SiC(CH_3)_3$-17	18.16	18.07	18.16	18.08	18.11	18.09	–	–	–	–	–	–
$SiC(\underline{C}H_3)_3$-3	25.97	25.90	25.97	25.93	25.95	25.93	–	–	–	–	–	–
$SiC(\underline{C}H_3)_3$-17	25.72	25.65	25.72	25.66	25.69	25.66	–	–	–	–	–	–
a	30.78	30.70	32.70	32.58	32.45	32.46	33.57[g]	33.62	33.84	33.66	34.65	33.27
									(33.72)	(33.60)	(34.49)	
b	37.34	37.42	38.33	38.24	38.33	38.37	37.76	38.02	40.01	38.03	39.01	38.82
	(36.94)	(37.02)	(38.12)	(38.03)	(38.12)	(37.40)	(37.40)	(37.65)	(39.77)	(37.79)	(37.79)	
c	72.10	72.00	58.58	58.70	58.56	58.81	58.36	58.92	52.06	58.69	60.51	38.94
	(71.81)	(71.79)	(58.39)	(58.49)	(58.29)	(58.58)	(58.04)	(58.65)			(60.44)	
d	36.39	36.31	38.97	39.05	38.88	38.97	38.57	38.90	26.55	41.33	39.86	79.52
	(36.29)	(36.23)	(38.67)	(38.83	(38.63)	(38.75)	(38.41)	(38.63)	(26.44)	(41.23)		
e	22.40	26.18	23.93	27.49	23.47	27.35	23.17	24.98	–	20.83	27.98	88.38
			(23.87)	(27.43)	(23.11)	(27.19)	(22.94)	(24.64)				
f	–	29.49	–	28.80	–	28.79	–	28.51	–	13.47	31.95	20.93
g	–	29.42	–	25.57	–	25.17	–	25.16	–	–	23.20	21.91
						(24.82)		(24.83)				
h	30.78	30.70	32.14	32.58	32.78	33.30	32.74	33.51	–	–	14.29	13.44
					(32.13)	(32.69)	(32.09)	(32.72)				
i	67.54	67.51	62.72	62.79	172.10	172.61	172.63	173.01	–	–	–	–
					(172.00)	(172.49)	(172.40)	(172.91				
j (c-2)	98.92	98.72	–	–	–	–	–	–	–	–	–	–
k (c-3)	30.43	30.31	–	–	–	–	–	–	–	–	–	–
l (c-4)	19.71	19.58	–	–	–	–	–	–	–	–	–	–
m (c-5)	25.49	25.45	–	–	–	–	–	–	–	–	–	–
n (c-6)	62.39	62.19	–	–	–	–	–	–	–	–	–	–
NCH_3	–	–	–	–	35.24	35.24	35.40	35.40	–	–	–	–
					(33.33)	(33.37)	(33.35)	(33.34)				
NCH_2	–	–	–	–	49.71	49.68	49.88	49.85	–	–	–	–
					(47.46)	(47.37)	(47.69)	(47.59)				
$NCH_2\underline{C}H_2$	–	–	–	–	30.61	30.60	30.56	30.64	–	–	–	–
					(29.40)	(29.37)	(29.43)	(29.41)				
$N(CH_2)_2\underline{C}H_2$	–	–	–	–	20.06	20.03	20.03	20.08	–	–	–	–
					(19.95)	(19.92)	(19.94)	(19.98)				
$N(CH_2)_3\underline{C}H_3$	–	–	–	–	13.83	13.83	13.82	13.85	–	–	–	–

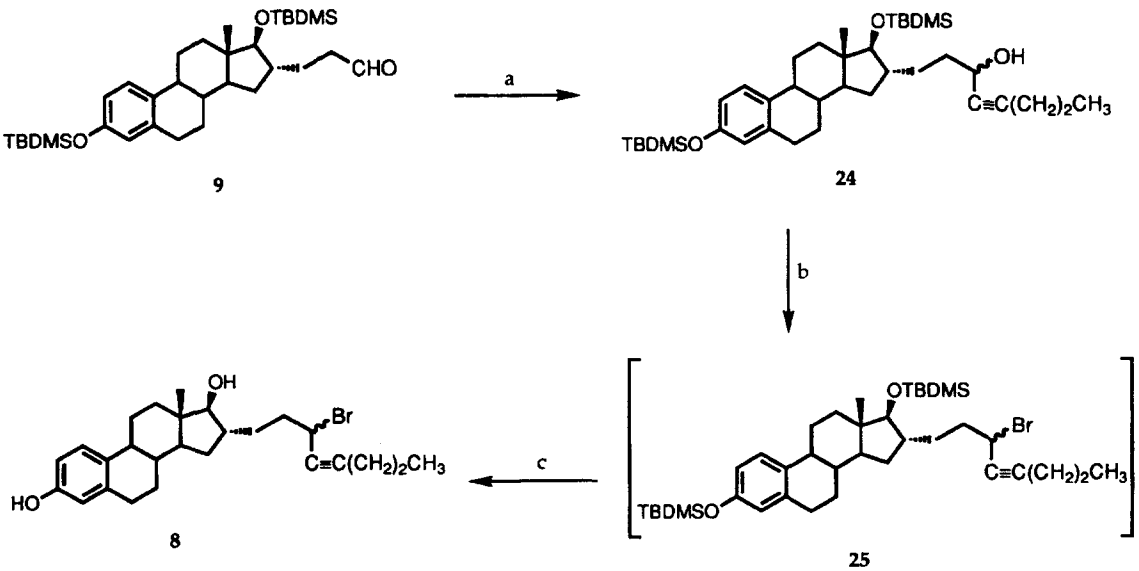
[a]For chemical structures, see Schemes 1–3.
[b]The numbers 1–18 correspond to steroid numerotation of carbons; the carbons of TBDMS group are directly represented; the letters a to i correspond to carbons of side chain moiety, starting from carbon connected to steroid and letters j to n correspond to THP group; the carbons of butyl methyl amide moiety are directly represented.
[c]$CDCl_3$ was used for all NMR spectra except for compound 7 which was taken in acetone-d_6.
[d]Assignments of carbons may be reversed when the chemical shifts are very similar.
[e]The duplication of a signal is represented by a second value indicated in parenthesis.
[f]The signal of C-6 is included in the solvent peaks.



Scheme 2. Synthesis of 16α-(bromoalkyl)-estradiols **5–7**. The reagents are: (a) MeMgBr, PrMgCl, or PnMgBr; (b) (i) Ph_3P, Br_2, imidazole, (ii) **18**, **19**, or **20**; (c) HCl/MeOH (2:98).

When compound **4**, at a concentration of 10, 50, or 100 μM, was incubated with 17β-HSD type 1 and cofactor, no inactivation of enzyme activity was observed (Fig. 4). Indeed, enzymatic activity is restored after the removal of inhibitor, suggesting that compound **4** (and probably compounds **2** and **3**) acts as a reversible inhibitor. In a similar experiment, we have previously observed that compound **1** acts as an irreversible inhibitor.[31] This result indicate that, contrary to primary bromine of compound **1**, the secondary bromine of compounds **2–4** does not inactivate 17β-HSD type 1. Another factor explaining the weak activity of this series of inhibitors is that a bulky alkylamide side chain decreases markedly the affinity of inhibitor for enzymatic active site.

To examine the influence of side chain, we synthesized four additional compounds: three 16α-(bromoalkyl)-estradiols **5–7** and the 16α-(bromoalkynyl)-estradiol **8**. The IC_{50} values of compounds **5–8** were determined as described above from the inhibition curves. As shown in Table 2, the compound with the shorter side chain (**5**, R = methyl) causes a greater inhibition of 17β-HSD type 1 than the other compounds with longer side chains (**6**, R = propyl and **7**, R = pentyl). For compound with a bromo alkynyl side chain (**8**, R = pentyn), the enzymatic assay gave an IC_{50} value of 2.9 μM. When comparing **8** with **7** with the same side chain length but saturated, we observed a slightly better inhibiting potency for the bromoalkynyl derivative (2.9 and 4.4 μM, respectively, for **8** and **7**; Table 2). The rigidifica-



Scheme 3. Synthesis of 16α-(bromoalkynyl)-estradiol **8**. The reagents are: (a) PrC≡CMgBr; (b) BBr_3; (c) HCl/MeOH (2:98).

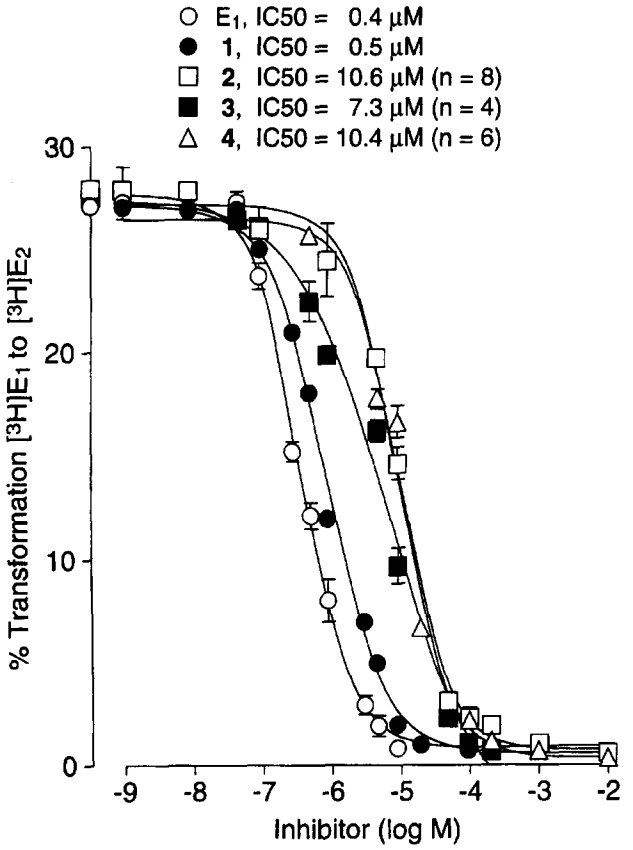


Figure 3. Inhibition of partially purified 17β-HSD type 1 by 16α-(bromoalkylamide)-estradiols **2–4**, 16α-(bromopropyl)-estradiol **1** and estrone (E_1). See Experimental for the conditions of enzymatic assay.

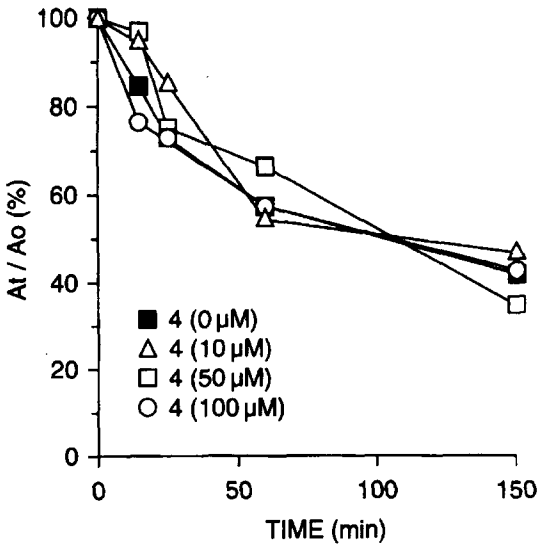
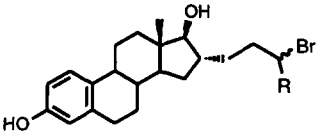


Figure 4. Inactivation of partially purified 17β-HSD type 1 by compound **4**. At, enzymatic activity at time t, and A_o, initial activity. See Experimental for the conditions of enzymatic assay.

Table 2. IC_{50} values obtained for compounds **1** to **8**



No	R	IC_{50} (µM)[a]
3	$(CH_2)_3CONBuMe$	7.3±2.1
4	$(CH_2)_5CONBuMe$	10.4±1.0
2	$(CH_2)_7CONBuMe$	10.6±1.4
1	H	0.49±0.05
5	CH_3	1.7±0.3
6	$(CH_2)_2CH_3$	5.4±0.8
7	$(CH_2)_4CH_3$	4.4±0.3
8	$C{\equiv}C(CH_2)_2CH_3$	2.9±0.8

[a]Transformation of estrone to estradiol by the cytosolic 17β-HSD of human placenta (17β-HSD type 1). See Experimental for the conditions used in this enzymatic assay and to calculate the IC_{50} values.

tion of side chain or activation of secondary bromine by a triple bond could explain the weak increase of 17β-HSD inhibition. However, these 16α-(bromoalkyl/alkynyl)estradiol derivatives **5–8**, including the primary bromide **1**, were weaker inhibitors than estrone (no 16α-side chain). Indeed, when estrone was used as a competitor of a tritiated substrate estrone), an IC_{50} value of 0.4 µM was obtained compared with 0.5 µM for **1** and 7.3 µM for **3** (Fig. 3). In view of these results, we concluded that 17β-HSD inhibition decreases with the side chain length of inhibitors.

Estrogenic and antiestrogenic activity

The ability of 16α-bromoalkylamide estradiol derivatives **2–4** to stimulate the proliferation (estrogenic effect) or to inhibit the estradiol (0.1 nM)-induced proliferation (antiestrogenic effect) was evaluated on the estrogen-sensitive human breast cancer ZR-75-1 cells. The bromoalkylamide series (compounds **2–4**) was especially synthesized to eliminate the antiestrogenic activity observed for the compounds (**1** and **5–8**) of the bromoalkyl series (results not shown). Compounds **2–4** were tested at two concentrations: 30 and 1000 nM. As shown in Figure 5, no proliferative effect was observed at a concentration of 30 nM for all tested compounds; a weak effect was observed only for compound **3** at 1000 nM (36%, related to estradiol stimulation). At this step, we could see that compound **3**, with a shorter bromoalkylamide side chain, was less interesting, taking into account this estrogenic activity. The antiestrogenic activity was also studied for those three compounds. At a lower concentration (30 nM) of compounds **2–4**, no antiestrogenic activity was observed. Interestingly, at a higher concentration of 1000 nM, compound **4**, with an intermediate side chain length, showed 74% antiestrogenic activity, whereas compound **2**, with the longest side chain, showed a moderate antiestrogenic activity of 34%. As expected, because of its estrogenic activity, compound **3** showed no antiestrogenic activity. Thus, the compound showing no estrogenic activity and the highest antiestrogenic

activity (74%) was compound **4**, with an optimal side chain length of five carbons between the carbonyl of the amide and the carbon-bearing an atom of bromine. The antiestrogenic potential of 16α-(bromoalkylamide) side chain of compound **4** is, however, lower than the potential of 7α-undecanamide side chain of EM-139 and ICI 164384 (data not shown). This result was in accordance with ER-binding affinity obtained for compound **4** (Fig. 6). Indeed, the binding affinity of compound **4**, with a 16α-bromoalkylamide side chain, was 430-fold lower than ICI 164384, with a 7α-undecanamide side chain, and 950-fold lower than estradiol (without side chain).

In conclusion, the synthesis of 16α-(bromoalkylamide, 16α-(bromoalkyl), and 16α-(bromoalkynyl) derivatives of estradiol (**2–8**) were performed in 3–6 steps from the key intermediate aldehyde **9**. All new compounds inhibited human placental cytosolic 17β-HSD (type 1), with IC_{50} values ranging from 1.7 to 10.6 μM. In the ZR-75-1 cell assayed, the 16α-(bromoalkylamide) derivatives of estradiol **2–4** had no estrogenic activity

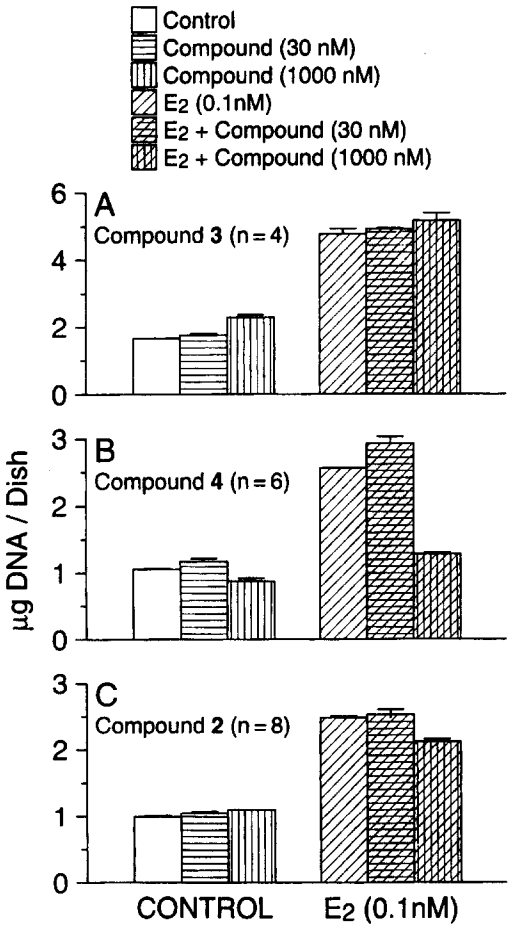


Figure 5. Effects of compounds **2–4** on the proliferation (estrogenic activity) and inhibition of estradiol (E_2) (0.1 nM)-induced proliferation (antiestrogenic activity) of estrogen-sensitive human breast cancer ZR-75-1 cells. Three days after plating, the cells were incubated for 9 days with the indicated concentrations of compounds **2–4** in the presence or absence of E_2 (0.1 nM). Media were changed every second day. Results are expressed as the mean ±SEM of triplicate.

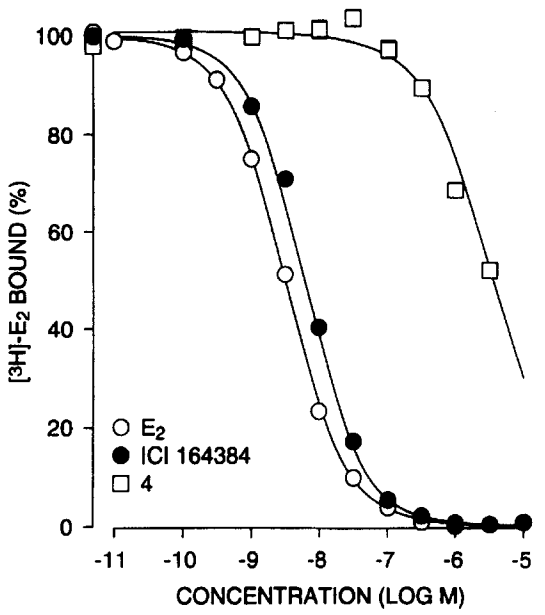


Figure 6. Competition for binding of estradiol to rat uterine estrogen receptor by compound **4** (□), pure antiestrogen ICI 164384 (●), and unlabeled estradiol (○). The IC_{50} values calculated by linear regression analysis were 2870, 6.7, and 3.0 nM, respectively, for compound **4**, ICI 164384, and estradiol (E_2).

at 30 nM, and only compound **3**, with its shorter side chain length, showed estrogenic activity at 1000 nM. Interestingly, at this concentration, compound **4**, with its side chain of intermediate length, showed antiestrogenic activity. This compound can bind to estrogen receptor, but its 16α-bromoalkylamide side chain decreased markedly its ER-binding. A bulky 16α-bromoalkylamide side chain abolished also the ability of bromine atom to form a covalent bond with 17β-HSD type 1 and, consequently, compound **4** was a reversible inhibitor. Thus, this later compound inhibited moderately 17β-HSD type 1, showed no estrogenic activity, and possessed antiestrogenic activity in the in vitro test used, and can be considered as a dual-action inhibitor. Since it was not possible to decrease the alkylamide side chain length, which was optimized regarding the estrogenic and antiestrogenic activities, two strategies could be considered to restore the alkylating potential of bromine atom and to improve the 17β-HSD type 1 inhibiting potency of compound **4**: (1) to develop a good activation of secondary bromine or (2) to include a primary bromine group in the alkylamide side chain. The later strategy is under progress.

Experimental

Chemical synthesis

General procedure. Chemical reagents and starting steroid estrone were purchased from Aldrich Chemical Company (Milwaukee, Wisconsin) or Sigma Chemical Company (St Louis, Missouri); solvents were obtained from BDH Chemicals (Montréal, Canada) or Baker Chemicals (Montréal, Canada). Flash-column chroma-

tography were performed on silica gel 60 (230–400 mesh ASTM), and TLC were performed on 0.20 mm silica gel F_{254} plates. IR spectra were recorded on a Perkin–Elmer 1600 (series FTIR) spectrophotometer and expressed in cm^{-1}. ^1H and ^{13}C NMR spectra were recorded, respectively, at 300 and 75.47 MHz on a Bruker AC/F 300 and are reported in ppm (δ) downfield from residual $CDCl_3$ as reference (7.26 and 77.00 ppm). Low-resolution mass spectra (LRMS) were obtained from electron impact (EI) and recorded with a V.G. Micromass 16F or Hewlett–Packard spectrometer. High-resolution mass spectra (HRMS) obtained from electron impact (EI) or fast-atom bombardment (FAB) were recorded at the Centre Régional de Spectrométrie de Masse (Université de Montréal, Montréal, Canada).

Synthesis of 16α-(bromoalkylamide)-estradiol derivatives 3 and 4 (Scheme 1)

The sequence of reactions used for the synthesis of 16α-(bromoalkylamide) estradiol derivatives **3** and **4** was previously reported by Pelletier et al.[33] for compound **2**. Herein we briefly described the experimental procedure, with an indication of modifications, and we fully characterized the new synthesized intermediates and final compounds.

Synthesis of alcohols 10 and 11 from aldehyde 9 (Grignard reaction)

To reduce the possibility of a secondary reaction (reduction of aldehyde to corresponding alcohol), the solution of Grignard reagent was added dropwise to a solution of aldehyde **9** in dry THF.[33] Purification by chromatography (hexane:EtOAc, 95:5) gave alcohols **10** (25%) and **11** (46%) with starting aldehyde **9** (35 and 10%, respectively).

7-[3′,17′β-(Di-*tert*-butyldimethylsilyloxy)-1′,3′,5′(10′)-estratrien-16′α-yl)]-5-hydroxy-1-[(tetrahydro-2′*H*-pyran-2′-yl)oxy]-heptane (10). Colorless oil; IR (film): 3450 (OH, alcohol); ^1H NMR ($CDCl_3$): 0.06 [s, 6H, 17′β-Si$(CH_3)_2$], 0.19 [s, 6H, 3′-Si$(CH_3)_2$], 0.78 (s, 3H, 18′-CH_3), 0.90 [s, 9H, 17′β-SiC$(CH_3)_3$], 0.98 [s, 9H, 3′-SiC$(CH_3)_3$], 2.78 (m, 2H, 6′-CH_2), 3.24 (d, $J=7.2$ Hz, 1H, 17′α-CH), 3.41, 3.51, 3.76, and 3.88 (4m, 4H, 2× CH_2O of THP and side chain), 3.61 (m, 1H, C<u>H</u>OH), 4.58 (t, $J=4.0$ Hz, 1H, CH of THP), 6.54 (d, $J=2.2$ Hz, 1H, 4′-CH), 6.60 (dd, $J_1=2.6$ Hz and $J_2=8.4$ Hz, 1H, 2′-CH), 7.11 (d, $J=8.5$ Hz, 1H, 1′-CH); LRMS (EI) *m/e* (relative intensity): 714 (M^+, 0.5), 630 (9.8), 574 (7.3), 555 (13), 481 (68), 273 (36), 85 (100).

9-[3′,17′β-(Di-*tert*-butyldimethylsilyloxy)-1′,3′,5′(10′)-estratrien-16′α-yl)]-7-hydroxy-1-[(tetrahydro-2′*H*-pyran-2′-yl)oxy]-nonane (11). Colorless oil; IR (film): 3450 (OH, alcohol); ^1H NMR ($CDCl_3$): 0.07 [s, 6H, 17′β-Si$(CH_3)_2$], 0.19 [s, 6H, 3′-Si$(CH_3)_2$], 0.79 (s, 3H, 18′-CH_3), 0.91 [s, 9H, 17′β-SiC$(CH_3)_3$], 0.98 [s, 9H, 3′-SiC$(CH_3)_3$], 2.79 (m, 2H, 6′-CH_2), 3.24 (d, $J=7.2$ Hz, 1H, 17′α-CH), 3.39, 3.50, 3.74, and 3.84 (4m, 4H, 2× CH_2O of THP and side chain), 3.59 (m, 1H, C<u>H</u>OH), 4.58 (t, $J=4.1$ Hz, 1H, CH of THP), 6.56 (d, $J=2.4$ Hz, 1H, 4′-CH), 6.61 (dd, $J_1=2.5$ Hz and $J_2=8.4$ Hz, 1H, 2′-CH), 7.11 (d, $J=8.5$ Hz, 1H, 1′-CH); LRMS (EI) *m/e* (relative intensity): 658 (M^+-THP, 4.2), 641 (4.8), 599 (6.2), 584 (43), 510 (72), 253 (61), 85 (72), 73 (100).

Synthesis of bromides 14 and 15 (bromination of alcohol following by THP deprotection)

The next step was the bromination of the secondary alcohol of compounds **10** and **11** by triphenyl phosphine–bromine complex to give bromides **12** and **13**.[33] Afterwards, the selective hydrolysis of the THP group (without hydrolysis of di-TBDMS groups) was performed with amberlyst-15 resin. After purification of the crude compounds by chromatography (hexane:EtOAc, 98:2), the corresponding primary alcohols **14** and **15** were obtained in the same yield (35%, two steps).

7-[3′,17′β-(Di-*tert*-butyldimethylsilyloxy)-1′,3′,5′(10′)-estratrien-16′ α-yl)]- 5 -bromoheptanol (14). Colorless oil; IR (film): 3375 (OH, alcohol); ^1H NMR ($CDCl_3$): 0.08 and 0.09 [2s, 6H, 17′β-Si$(CH_3)_2$], 0.19 [s, 6H, 3′-Si$(CH_3)_2$], 0.79 (s, 3H, 18′-CH_3), 0.92 [s, 9H, 17′β-SiC$(CH_3)_3$], 0.98 [s, 9H, 3′-SiC$(CH_3)_3$], 2.79 (m, 2H, 6′-CH_2), 3.25 (d, $J=7.2$ Hz, 1H, 17′α-CH), 3.67 (t, $J=5.7$ Hz, 2H, C<u>H</u>$_2$OH), 4.04 (m, 1H, CHBr), 6.55 (d, $J=2.5$ Hz, 1H, 4′-CH), 6.62 (dd, $J_1=2.6$ Hz and $J_2=8.4$ Hz, 1H, 2′-CH), 7.12 (d, $J=8.4$ Hz, 1H, 1′-CH); LRMS (EI) *m/e* (relative intensity): 695 (M^+, 1.8), 693 (M^+, 2.0), 636 (5.0), 634 (4.3), 611 (11), 555 (100), 480 (45), 275 (59), 73 (93).

9-[3′,17′β-(Di-*tert*-butyldimethylsilyloxy)-1′,3′,5′(10′)-estratrien-16′ α-yl)]- 7 -bromononanol (15). Colorless oil; IR (film): 3400 (OH, alcohol); ^1H NMR ($CDCl_3$): 0.09 and 0.11 [2s, 6H, 17′β-Si$(CH_3)_2$], 0.20 [s, 6H, 3′-Si$(CH_3)_2$], 0.80 (s, 3H, 18′-CH_3), 0.93 [s, 9H, 17′β-SiC$(CH_3)_3$], 1.00 [s, 9H, 3′-SiC$(CH_3)_3$], 2.80 (m, 2H, 6′-CH_2), 3.26 (d, $J=7.2$ Hz, 1 H, 17′α-CH), 3.65 (t, $J=6.4$ Hz, 2H, C<u>H</u>$_2$OH), 4.04 (m, 1H, CHBr), 6.56 (d, $J=2.3$ Hz, 1H, 4′-CH), 6.63 (dd, $J_1=2.6$ Hz and $J_2=8.3$ Hz, 1H, 2′-CH_2), 7.12 (d, $J=8.5$ Hz, 1H, 1′-CH); LRMS (EI) *m/e* (relative intensity): 723 (M^+, 0.4), 721 (M^+, 0.6), 665 (3.7), 663 (3.3), 640 (7.1), 583 (55), 509 (16), 273 (50), 73 (100).

Synthesis of bromoalkylamides 16 and 17 (oxidation of alcohol to carboxylic acid followed by amide formation)

Two steps were used to transform the primary alcohol to the methylbutylamide group.[33] The first step was the oxidation of primary alcohol by Jones' reagent to give the corresponding acid. The last step was the amide formation using tributylamine, isobutylchloroformate, and *N*-methylbutylamine. The bromoalkylamides **16** and **17** (52 and 77% yield, two steps) were obtained

after final purification by chromatography (hexane: EtOAc, 9:1).

***N*-Butyl,*N*-methyl,7-[3′,17′β-(di-*tert*-butyldimethylsilyloxy)-1′,3′,5′(10′)-estratrien-16′α-yl]-5-bromoheptanamide (16).** Colorless oil; IR (film): 1650 (C=O, amide); ^{1}H NMR ($CDCl_3$): 0.07 and 0.08 [2s, 6H, 17′β-$Si(CH_3)_2$], 0.18 [s, 6H, 3′-$Si(CH_3)_2$], 0.77 (s, 3H, 18′-CH_3), 0.90 [s, 9H, 17′β-$SiC(CH_3)_3$], 0.93 (m, 3H, CH_3 of butyl group masked under two singlets of di-TBDMS groups), 0.97 (s, 9H, 3′-$SiC(CH_3)_3$), 2.78 (m, 2H, 6′-CH_2), 2.91 and 2.97 (2s, 3H, CH_3NCO), 3.24 (d, J=5.9 Hz, 1H, 17′α-CH), 3.28 and 3.36 (2t, J=7.4 Hz, 2H, CH_2NCO), 4.04 (m, 1H, CHBr), 6.54 (d, J=2.1 Hz, 1H, 4′-CH), 6.60 (dd, J_1=2.2 Hz and J_2=8.4 Hz, 1H, 2′-CH), 7.10 (d, J=8.4 Hz, 1H, 1′-CH); LRMS (EI) *m/e* (relative intensity): 720 [M^+-$C(CH_3)_3$, 2.7], 696 (9.4), 638 (100), 569 (81).

***N*-Butyl, *N*-methyl, 9-[3′,17′β-(di-*tert*-butyldimethylsilyloxy)-1′,3′,5′(10′)-estratrien-16′α-yl]-7-bromononamide (17).** Colorless oil; IR (film): 1645 (C=O, amide); ^{1}H NMR ($CDCl_3$): 0.07 and 0.08 [2s, 6H, 17′β-$Si(CH_3)_2$], 0.18 [s, 6H, 3′-$Si(CH_3)_2$], 0.78 (s, 3H, 18′-CH_3), 0.91 [s, 9H, 17′β-Si-$C(CH_3)_3$], 0.94 (m, 3H, CH_3 of butyl group masked under two singlets of di-TBDMS groups), 0.97 [s, 9H, 3′-$SiC(CH_3)_3$], 2.78 (m, 2H, 6′-CH_2), 2.91 and 2.96 (2s, 3H, CH_3NCO), 3.24 (d, J=7.4 Hz, 1H, 17′α-CH), 3.25 and 3.36 (2t, J=7.4 Hz, 2H, CH_2NCO), 4.02 (m, 1H, CHBr), 6.54 (d, J=2.5 Hz, 1H, 4′-CH), 6.60 (dd, J_1=2.4 Hz and J_2=8.4 Hz, 1H, 2′-CH), 7.10 (d, J=8.5 Hz, 1H, 1′-CH); LRMS (EI) *m/e* (relative intensity): 748 [M^+-$C(CH_3)_3$, 0.6], 746 [M^+-$C(CH_3)_3$, 1.6], 725 (8.7), 666 (100), 591 (23).

Synthesis of dihydroxy bromoalkylamides 3 and 4 (cleavage of di-TBDMS groups)

Di-TBDMS groups were cleaved under acidic conditions. Briefly, di-TBDMS amides **16** and **17** were dissolved in a methanolic solution of HCl (2%, v/v), and the resulting solution was stirred at room temperature. After standard work up (addition of water, partial evaporation of MeOH and extraction), the crude products were purified by chromatography (hexane: EtOAc, 5:5) to lead to dihydroxy compounds **3** and **4** (82 and 89% yield, respectively).

***N*-Butyl,*N*-methyl,7-[3′,17′β-(dihydroxy)-1′,3′,5′(10′)-estratrien-16′α-yl]-5-bromoheptanamide (3).** Colorless oil; IR (film): 3360 (OH, alcohol and phenol), 1620 (C=O, amide); ^{1}H NMR ($CDCl_3$): 0.78 and 0.79 (2s, 3H, 18′-CH_3, two isomers), 0.91 and 0.95 (2t, J=7.3 Hz, 2H, CH_3 of butyl group), 2.78 (m, 2H, 6′-CH_2), 2.92 and 2.98 (2s, 3H, CH_3NCO), 3.27 (m, 2H, 17′α-CH and 1H of CH_2NCO), 3.36 (t, J=7.4 Hz, 1H of CH_2NCO), 4.08 and 4.32 (2m, 1H, CHBr, two isomers), 6.58 (d, J=2.3 Hz, 1H, 4′-CH), 6.65 (dd, J_1=2.4 Hz and J_2=8.4 Hz, 1H, 2′-CH), 7.11 (d, J=8.5 Hz, 1H, 1′-CH); LRMS (EI) *m/e* (relative intensity): 467 (M^+-HBr, 36), 449 (6.7), 398 (15), 380 (35), 80 (100).

***N*-Butyl,*N*-methyl,9-[3′,17′β-(dihydroxy)-1′,3′,5′(10′)-estratrien-16′α-yl]-7-bromononamide (4).** Colorless oil; IR (film): 3340 (OH, alcohol), 1622 (C=O, amide); ^{1}H NMR ($CDCl_3$): 0.80 (s, 3H, 18′-CH_3), 0.92 and 0.95 (2t, J=7.2 Hz, 2H, CH_3 of butyl group), 2.80 (m, 2H, 6′-CH_2), 2.92 and 2.97 (2s, 3H, CH_3NCO), 3.28 (m, 2H, 17′α-CH and 1H of CH_2NCO), 3.36 (t, J=7.6 Hz, 1H of CH_2NCO), 4.11 (m, 1H, CHBr), 6.58 (d, J=2.5 Hz, 1H, 4′-CH), 6.64 (dd, J_1=2.6 Hz and J_2=8.4 Hz, 1H, 2′-CH), 7.13 (d, J=8.4 Hz, 1H, 1′-CH); LRMS (EI) *m/e* (relative intensity): 495 (M^+-HBr, 37), 477 (28), 88 (100); HRMS (FAB): calcd for $C_{32}H_{51}NO_3{}^{79}Br$ (M^++H) 576.3052, found 576.3035.

Synthesis of 16α-(bromoalkyl)-estradiol derivatives 5–7 (Scheme 2)

Synthesis of di-TBDMS alcohols 18–20.

Synthesis of 4-[3′,17′β-(di-*tert*-butyldimethylsilyloxy)-1′,3′,5′(10′)-estratrien-16′α-yl]-2-hydroxybutane (18). To a stirred solution of 255 mg (0.458 mmol) of aldehyde **9**[33] dissolved in 100 mL of dry THF, 0.458 mL (1.375 mmol) of methylmagnesium iodide (3.0 M) was added dropwise at 0 °C. The resulting mixture was stirred for 85 min under argon before the addition of a satd aq soln of NH_4Cl. The crude compound was extracted by EtOAc dried over $MgSO_4$ and purified by column chromatography with hexane:EtOAc (95:5) as eluent to give 207 mg (79% yield) of alcohol **18**. Light yellow oil; IR (film): 3340 (OH, alcohol); ^{1}H NMR ($CDCl_3$): 0.07 [s, 6H, 17′β-$Si(CH_3)_2$], 0.19 [s, 6H, 3′-$Si(CH_3)_2$], 0.78 (s, 3H, 18′-CH_3), 0.91 [s, 9H, 17′β-$SiC(CH_3)_3$], 0.98 [s, 9H, 3′-$SiC(CH_3)_3$], 1.21 [d, J=6.1 Hz, 3H, $CH(OH)C\underline{H}_3$], 2.79 (m, 2H, 6′-CH_2), 3.24 (d, J=7.3 Hz, 1H, 17′α-CH), 3.82 [m, 1H, $C\underline{H}(OH)$], 6.55 (d, J=2.1 Hz, 1H, 4′-CH), 6.61 (dd, J_1=2.5 Hz and J_2=8.4 Hz, 1H, 2′-CH), 7.10 (d, J=8.5 Hz, 1H, 1′-CH); LRMS (EI) *m/e* (relative intensity): 572 (M^+, 1.3), 554 (4.6), 515 (13), 497 (39), 423 (55), 273 (33), 73 (100).

Synthesis of 6-[3′,17′β-(di-*tert*-butylmethylsilyloxy-1′,3′,5′(10′)-estratrien-16′α-yl]-4-hydroxyhexane (19). The procedure described above for the synthesis of **18** was used with minor modifications. Thus, 0.607 mL (1.21 mmol) of propylmagnesium chloride (2.0 M) was added to a stirred solution of 225 mg (0.405 mmol) of aldehyde **9**.[33] After the usual work up, the crude compound was purified by column chromatography with hexane:EtOAc (96:4) as eluent to give 158 mg (65% yield) of alcohol **19**. Light yellow oil; IR (film): 3420 (OH, alcohol); ^{1}H NMR ($CDCl_3$): 0.09 [s, 6H, 17′β-$Si(CH_3)_2$], 0.21 [s, 9H, 17′β-$Si(CH_3)_2$], 0.80 (s, 3H, 18′-CH_3), 0.93 [s, 6H, 3′β-$SiC(CH_3)_3$], 0.97 (CH_3 signal of butyl masked under two singlets of di-TBDMS groups), 1.00 (s, 9H, 3′-$SiC(CH_3)_3$), 2.79 (m, 2H, 6′-CH_2), 3.26 (d, J=7.1 Hz, 1H, 17′α-CH), 3.62 (m, 1H, C$\underline{H}$OH), 6.56 (d, J=2.5 Hz, 1H, 4′-CH), 6.63 (dd,

J_1=2.6 Hz and J_2=8.3 Hz, 1H, 2'-CH), 7.12 (d, J=8.6 Hz, 1H, 1'-CH); LRMS (EI) *m/e* (relative intensity): 600 (M^+, 1.2), 582 (5.5), 543 (13), 525 (40), 451 (69), 273 (36), 73 (100).

Synthesis of 8-[3',17'β-(di-*tert*-butyldimethylsilyloxy)-1',3',5'(10')-estratrien-16'α-yl]-6-hydroxyoctane (20). (a) Preparation of Grignard reagent (solution stock: ~0.38 M): A solution stock of Grignard reagent (pentylmagnesium bromide) was prepared under an argon atmosphere by the dropwise addition of 8-bromopentane (1.1 g, 7.17 mmol) dissolved in 5 mL of dry THF on 183 mg of magnesium (7.55 mmol) and 15 mL of dry THF. The magnesium was firstly activated by iodine and heat. The solution was stirred at room temperature for 2 h before use.

(b) The addition of Grignard reagent to aldehyde: To a stirred solution of aldehyde **9**[33] (400 mg, 0.72 mmol) 4.0 mL of the solution stock (1.52 mmol) was added dropwise at room temperature. The resulting mixture was stirred for 25 min and quenched by the addition of a satd aq soln of NH_4Cl before extraction with EtOAc. The organic phase was washed with a satd aq soln of NaCl and dried over $MgSO_4$. After evaporation, the crude compound was purified by column chromatography with hexane:EtOAc (98:2) as eluent to give 334 mg (74% yield) of alcohol **20**. Light yellow oil; IR (film): 3420 (alcohol); ^{1}H NMR ($CDCl_3$): 0.07 [s, 6H, 17'β-Si(CH_3)$_2$], 0.19 [s, 6H, 3'-Si(CH_3)$_2$], 0.79 (s, 3H, 18'-CH_3), 0.89 (t, J=7.0 Hz, 3H, CH_3 of butyl), 0.91 [s, 9H, 17'β-SiC(CH_3)$_3$], 0.98 [s, 9H, 3'-SiC(CH_3)$_3$], 2.79 (m, 2H, 6'-CH_2), 3.24 (d, J=7.2 Hz, 1H, 17'α-CH), 3.65 (m, 1H, C<u>H</u>OH), 6.55 (d, J=2.5 Hz, 1H, 4'-CH), 6.61 (dd, J_1=2.4 Hz and J_2=8.4 Hz, 1H, 2'-CH), 7.11 (d, J=8.4 Hz, 1H, 1'-CH).

Synthesis of bromides 5–7 (bromination of alcohol following by hydrolysis of di-TBDMS groups)

The bromination of alcohols **18–20** was carried out following the procedure described by Pelletier et al.[33] After 45 min, the crude compound was purified by column chromatography (hexane:EtOAc, 99:1) to give a mixture of the corresponding bromides **21–23** containing triphenylphosphine oxide. Without additional purification, this mixture was used for the next step (hydrolysis of di-TBDMS groups). Di-TBDMS bromides **21–23** were dissolved in a methanolic solution of HCl (2%, v/v), and the resulting mixture was stirred at room temperature. After standard work up (addition of water, partial evaporation of MeOH and extraction), the crude bromides **5–7** were purified by chromatography (hexane:EtOAc, 8:2).

4-[3',17'β-(Dihydroxy)-1',3',5'(10')-estratrien-16'α-yl]-2-bromobutane (5). Colorless oil (31% yield, two steps); IR (film): 3300 (OH, alcohol and phenol); ^{1}H NMR ($CDCl_3$): 0.80 (s, 3H, 18'-CH_3), 1.73 (d, J=6.6 Hz, 3H, CHBrC<u>H</u>$_3$), 2.81 (m, 2H, 6'-CH_2), 3.30 (d, J=7.0, 1H, 17'α-CH), 4.14 (m, 1H, CHBr), 6.56 (d, J=2.7 Hz, 1H, 4'-CH), 6.63 (dd, J_1=2.7 Hz and J_2=8.3 Hz, 1H, 2'-CH), 7.15 (d, J=8.4 Hz, 1H, 1'-CH); LRMS (EI) *m/e* (relative intensity): 408 (M^+, 18), 406 (M^+, 18), 326 (60), 111 (100); HRMS (EI): calcd for $C_{22}H_{31}O_2{}^{79}Br$ (M^+) 406.1507, found 406.1514.

6-[3',17'β-(Dihydroxy)-1',3',5'(10')-estratrien-16'α-yl]-4-bromohexane (6). Colorless oil (30% yield, two steps); IR (film): 3400 (OH, alcohol and phenol); ^{1}H NMR ($CDCl_3$): 0.80 (s, 3H, 18'-CH_3), 0.93 (t, J=7.3 Hz, 3H, CH_2C<u>H</u>$_3$), 2.81 (m, 2H, 6'-CH_2), 3.30 (d, J=6.5 Hz, 1H, 17'α-CH), 4.07 (m, 1H, CHBr), 4.59 (s, 1H, OH phenol), 6.56 (d, J=2.6 Hz, 1H, 4'-CH), 6.62 (dd, J_1=2.8 Hz and J_2=8.4 Hz, 1H, 2'-CH), 7.15 (d, J=8.5 Hz, 1H, 1'-CH); LRMS (EI) *m/e* (relative intensity): 436 (M^+, 31), 434 (M^+, 31), 354 (55), 339 (35), 213 (76), 159 (100); HRMS (EI): calcd for $C_{24}H_{35}O_2{}^{79}Br$ (M^+) 434.1820, found 434.1813.

8-[3',17'β-(dihydroxy)-1',3',5'(10')-estratrien-16'α-yl]-6-bromooctane (7). Colorless oil (51% yield, two steps); IR (film): 3420 (OH, alcohol and phenol); ^{1}H NMR (acetone-d_6): 0.82 (s, 3H, 18'-CH_3), 0.90 (t, J=6.5 Hz, 3H, CH_2C<u>H</u>$_3$), 2.76 (m, 2H, 6'-CH_2), 3.27 (d, J=6.8 Hz, 1H, 17'α-CH), 4.18 (m, 1H, CHBr), 6.52 (d, J=2.3 Hz, 1H, 4'-CH), 6.59 (dd, J_1=2.6 Hz and J_2=8.3 Hz, 1H, 2'-CH), 7.09 (d, J=8.4 Hz, 1H, 1'-CH); LRMS (EI) *m/e* (relative intensity): 464 (M^+, 11), 462 (M^+,13), 382 (67), 367 (43), 270 (36), 213 (59), 159 (100); HRMS (EI): calcd for $C_{26}H_{39}O_2{}^{79}Br$ (M^+) 462.2133, found 462.2163.

Synthesis of 16α-(bromoalkynyl)-estradiol derivative 8 (Scheme 3)

Synthesis of 8-[3',17'β-(di-*tert*-butyldimethylsilyloxy)-1',3',5'(10')-estratrien-16'α-yl]-6-hydroxy-4-octyne (24). To a solution of 0.72 mL (1.15 mmol) of BuLi (1.6 M) in 20 mL of dry THF, 145 μL (1.47 mmol) of 1-pentyne was added dropwise at −78 °C. The resulting mixture was stirred 1 h before the addition of 458 mg (0.824 mmol) of aldehyde **9**[33] dissolved in 12 mL of dry THF. After 3 h at −78 °C, the reaction mixture was quenched by the addition of a satd aq soln of NH_4Cl. The compound was extracted with EtOAc, dried over $MgSO_4$, and purified by column chromatography (hexane:EtOAc, 98:2) to give 397 mg (77% yield) of alcohol **24**. Colorless oil; IR (film): 3360 (OH, alcohol), 2250 (C≡C, alkyne); ^{1}H NMR ($CDCl_3$): 0.08 [s, 6H, 17'β-Si(CH_3)$_2$], 0.20 [s, 6H, 3'-Si(CH_3)$_2$], 0.80 (s, 3H, 18'-CH_3), 0.91 [s, 9H, 17'β-SiC(CH_3)$_3$], 0.99 [s, 9H, 3'-SiC(CH_3)$_3$], 1.00 (t, J=7.4 Hz, 3H, CH_2CH_3), 2.20 (t, J=5.5 Hz, 2H, C≡CCH_2), 2.80 (m, 2H, 6'-CH_2), 3.27 (d, J=7.1 Hz, 1H, 17'α-CH), 4.38 (m, 1H, CHOH), 6.56 (d, J=2.2 Hz, 1H, 4'-CH), 6.62 (dd, J_1=2.4 Hz and J_2=8.4 Hz, 1H, 2'-CH), 7.12 (d, J=8.5 Hz, 1H, 1'-CH); LRMS (FAB) *m/e* (relative intensity): 625 (M^++H, 27), 607 (7.8), 583 (16), 567 (9.0), 511 (11), 509 (10), 493 (19), 475 (32), 273 (100), 271 (66).

Synthesis of 8-[3',17'β-(dihydroxy-1',3',5'(10')-estratrien-16'α-yl]-6-bromo-4-octyne (8). Two steps were used to transform di-TBDMS-alcohol **24** to bromide **8**.

First was the bromination of the secondary propargylic alcohol; the second step was the cleavage of di-TBDMS groups. A solution of 73 mg (0.117 mmol) of alcohol **24** in 60 mL of dry CH_2Cl_2 was stirred at 0 °C under argon, and 276 μL (0.276 mmol) of BBr_3 (1 M) was added dropwise. After 45 min of stirring, a satd aq soln of $NaHCO_3$ was added and the crude compound was extracted with CH_2Cl_2, dried over $MgSO_4$, and filtered on silica gel with hexane as eluent. Without purification, the crude di-TBDMS bromide **25** (45 mg) was dissolved in 70 mL of a methanolic solution of HCl (2%, v/v) and stirred at room temperature. After 1.5 h, the reaction mixture was evaporated, and the residue was extracted with EtOAc and dried over $MgSO_4$. After evaporation of solvent, the crude compound was purified by column chromatography (hexane:EtOAc, 95:5) to give 17 mg (32% yield, two steps) of the corresponding dihydroxybromide **8**. White solid; IR (film): 3350 (OH, alcohol and phenol), 2250 (C≡C, alkyne); ^{1}H NMR ($CDCl_3$): 0.80 (s, 3H, 18′-CH_3), 0.99 (t, J=7.3 Hz, 3H, CH_2CH_3), 2.23 (t, J=4.9 Hz, 2H, C≡CCH_2CH_2), 2.81 (m, 2H, 6′-CH_2), 3.31 (d, J=7.0 Hz, 1H, 17′α-CH), 4.61 (m, 1H, CHBr), 6.56 (d, J=2.2 Hz, 1H, 4′-CH), 6.62 (dd, J_1=2.7 Hz and J_2=8.4 Hz, 1H, 2′-CH), 7.15 (d, J=8.4 Hz, 1H, 1′-CH); LRMS (FAB) *m/e* (relative intensity): 460 (M^+, 11), 458 (M^+, 10), 443 (5.5), 441 (6.0), 378 (25), 213 (28), 159 (100); HRMS (FAB): calcd for $C_{26}H_{35}O_2{}^{79}Br$ (M^+) 458.1820, found 458.1832.

Inhibition of human placental cytosolic 17β-hydroxysteroid dehydrogenase (type 1)

The partially purified 17β-HSD activity found in cytosolic fractions of human placenta (type 1) was used to evaluate the ability of compounds **1–8** to inhibit the transformation of [^{3}H]-estrone to [^{3}H]-estradiol.

Partial purification of 17β-HSD type 1. Human placentas were obtained immediately upon delivery from Department of Obstetrics, St-Sacrement Hospital (Québec, Canada) and stored on ice during transportation to the laboratory. All manipulations of placental tissues were performed at 4 °C. Placental tissue was homogenized in two volumes of buffer (40 mM Tris–HCl, pH 7.5, 0.25 M sucrose, 5 mM EDTA, 7 mM β-mercaptoethanol, 1 mM PMSF) before centrifugation at 1000 × g for 15 min. The supernatant from this centrifugation step was brought to 10,000 × g for 30 min, and then the new supernatant was centrifuged at 100,000 × g for 1 h. From this supernatant, a fraction precipitating between 30 and 50% saturation $(NH_4)_2SO_4$ was diluted with buffer as described above and used as a source of 17β-HSD type 1 activity in enzymatic assay.

Enzymatic assay (inhibition of 17β-HSD type 1). Assay for the determination of 17β-HSD inhibitors was performed in a final volume of 1 mL of buffer (20% glycerol, 1 mM EDTA, 50 mM KH_2PO_4, pH 7.4) containing between 2 and 6 nM of [^{3}H]-estrone, 1 μM NADH, 100 μL of partially purified 17β-HSD activity and 10 μL of ethanolic solution of tested inhibitor. Several concentrations ranging from 1 nM to 10 mM were used to obtain the inhibition curves from which the IC_{50} value was determined. Tubes were incubated for 30 min at 37 °C with shaking. After incubation, the reaction was stopped by cooling the tubes in a mixture of ice and water and by immediately adding unlabeled estrone and estradiol as carriers. The tubes were then extracted twice with diethyl ether. After the evaporation of organic solvent, the extract was dissolved in 50 μL of CH_2Cl_2 and applied on TLC plates (Kieselgel 60F 254) for chromatography. The plates were developed in a mixture of toluene:acetone (4:1). Iodine vapor or UV light were used to locate estrone and estradiol, which were cut from the plates, and the radioactivity of the labeled steroids was counted in liquid scintillation cocktail (9 mL) after the addition of ethanol (1 mL). The percent of transformation of estrone into estradiol was calculated for each inhibitor concentration as follows: % trans. = 100 × [^{3}H-E_2]/([^{3}H-E_1] + [^{3}H-E_2]), where [^{3}H-E_2] and [^{3}H-E_1] are the concentrations of labelled estradiol and labelled estrone expressed in cpm. The inhibition curves were plotted using the percent of transformation versus the concentration of inhibitor. From these curves, the IC_{50} values (the concentration that causes 50% of enzymatic inhibition) were calculated using an iterative least square regression method.[35,36]

Inactivation of 17β-HSD type 1 by compound 4. A solution containing the preparation of enzyme, NADH (100 μM), and ethanol (control) or ethanolic solution of compound **4** (test compound, final concentration of 10, 50, and 100 μM) was prepared and used for the inactivation test. Then the tubes containing 100 μM of this stock solution were incubated at 37 °C. At specific intervals, the sample tubes (duplicate) were diluted 20-fold with 1.9 mL of phosphate buffer solution (as above) containing [^{3}H] estrone (5 nM), and an enzymatic assay was performed as described in the previous section. The data were plotted according to Kitz and Wilson.[37]

Estrogenic and antiestrogenic activity

The proliferation of estrogen-sensitive human breast cancer cells ZR-75–1 permits assessment of the in vitro estrogenic/antiestrogenic activity of 16α-(bromoalkylamide)-estradiol derivatives **2–4**. The ability of tested compounds to stimulate the proliferation of ZR-75–1 cells shows estrogenic activity, and their ability to inhibit the 0.1 nM estradiol induced proliferation shows antiestrogenic activity. The compounds were tested at two concentrations: 30 and 1000 nM. The procedure of this assay has been described recently.[38,39]

Estrogen receptor binding affinity

As described by Asselin and Labrie,[40] the binding affinity for the rat uterine estrogen receptor of compound **4** and unlabeled estradiol (E_2) was determined by competition of the binding of labeled estra-

diol ([^{3}H]-E_2). The incubation was performed at 25 °C for 3 h.

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