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Synthesis of 16-(Bromoalkyl)-Estradiols having Inhibitory Effect on Human Placental Estradiol 17β-Hydroxysteroid Dehydrogenase (17β-HSD Type 1)

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Abstract—The activity of 17β-HSD type 1, the enzyme that converts estrone into its more potent metabolite estradiol, has been demonstrated in all classical steroidogenic tissues and almost all peripheral tissues from both rat and human. Since 17β-HSD is one of the most important enzymes involved in active steroid hormone formation, its inactivation could be a clinical approach to the treatment of hormono-dependent diseases like breast cancer. Herein we report the synthesis of 16-(bromoalkyl)-estradiols and their potency to inhibit the human placenta cytosolic estradiol 17β-HSD (type 1). Synthetic analogues possess various side chain lengths and orientation (α or β) at position 16 of the steroidal D ring. The most potent inhibitory effect was observed when the length of the side chain was 3 or 4 carbons. However, the 16β-(bromopropyl)-estradiol easily undergoes cyclization and its effect on 17β-HSD is lost. Consequently, 16α-(bromopropyl)-E_2, 16α-(bromobutyl)-E_2, and 16β-(bromobutyl)-E_2 were the best inhibitors discussed in this paper.

Introduction

Estrogens play a predominant role in human breast cancer.[1–4] It is well known that estrogens, like androgens, are more potent in their 17β-hydroxy than in their 17-keto configuration. The reversible interconversion of these two forms is catalyzed by 17β-hydroxysteroid dehydrogenase (17β-HSD). Since this enzyme is widely distributed,[5] it has been recognized to have a role in the transformation and regulation of intracellular steroid hormones from circulating precursors.[6,7] 17β-HSD, like aromatase and sulfatase, is the last enzyme involved in estrogen biosynthesis in peripheral tissues like breast tumor cells.[8] Consequently, inhibition of these enzymes could be a logical complementary approach to the treatment of breast cancer in women. Aromatase is a target that has been extensively exploited for the blockade of endogenous estrogen synthesis. However, aminoglutethimide, the best-known aromatase inhibitor, has a wide spectrum of inhibitory effects on other P_{450}-dependent steroid hydroxylases such as 11β-, 18- and 21-hydroxylase as well as 20-hydroxylase activity involved in cholesterol side-chain cleavage.[9] Although estrogen deprivation is known to be possible by inhibition of steroidogenesis enzymes, 17β-HSD inactivation remains to be investigated more fully. In this view, 16-bromoacetoxy-estradiol,[10] 16-oxo-estrone[11] and acetylenic 16-secoestradiol[12] have been shown to be good inactivators because of their strong alkylating activities on purified enzyme.

Recent developments on the enzymology of 17β-HSD warrant an update at this point. To date, three cDNAs encoding 17β-HSD have been cloned and characterized from humans. The first, type 1, encodes for a 34.9 kDa protein containing 327 amino acids. This soluble enzyme, often referred to as 17β-estradiol dehydrogenase because of its high substrate specificity, has been purified from the cytosol of human placenta.[13,14] The human 17β-HSD gene was localized to the q11–q12 region of chromosome 17,[15,16] and two distinct mRNA species that differ in their 5' noncoding regions were characterized.[17] Further studies on its expression in mammalian cells have demonstrated that this human 17β-HSD catalyzes the interconversion of estrone and estradiol; dehydroepiandrosterone (DHEA) and 5-androsten-3β,17β-diol (Δ^5-diol) are interconverted at a lower rate.[18] The 17β-HSD type 2, a microsomal 387 amino acid protein with a calculated molecular weight of 47.8 kDa, is capable of catalyzing the interconversion of testosterone and androstenedione as well as estrone and estradiol. This enzyme also possesses 20α-HSD activity.[19] Recently, the same group cloned a cDNA encoding for human testicular 17β-HSD (type 3), which favors the reduction of androstenedione to testosterone. This gene has been localized on chromosome 9q22 and encodes for a 34.5 kDa microsomal protein.[20] Earlier, another group solubilized and characterized a testosterone 17β-HSD in human hyperplastic prostate.[21]

More recently, our group demonstrated that the presence of a side chain located at 16-position of estradiol containing a good leaving group is favorable for inhibition of 17β-HSD type 1.[22] Moreover, a leaving group on a secondary carbon leads to a lower 17β-HSD inhibition than one on a primary carbon.[23] This was in agreement with Murdock's hypothesis suggesting that a

histidyl residue in the 17β-HSD active site acts like a nucleophilic agent.[24] To obtain further information about the effect of the side chain length and the 16-position stereochemistry on 17β-HSD inhibition, we synthesized nine novel 16-(bromoalkyl)-estradiols. Two different synthetic routes were achieved to induce the correct stereochemistry (α and β) at the 16-position (Figure 1). First, these compounds were submitted to activity screening on partially purified cytosolic 17β-HSD type 1 from human placenta. Then complete inhibition curves were determined for the three most potent analogues to establish the IC_{50} values. This study is the starting point of a fundamental research to develop molecules having double action: inhibition of 17β-HSD without estrogenic activity.

Chemistry

The approach to synthesizing target compounds depends on the expected stereochemistry at position 16 of the steroidal nucleus. The two different synthetic routes that lead mainly to 16α or 16β-derivatives are shown in Figure 1. To introduce different side chains onto position 16, the key step involves a C–C bond formation. Alpha-alkylation of steroidal 17-ketone, using LDA as base, could be done with activated electrophiles.[25] Since the 18-CH_3 group on the β-face of steroid is known to direct the attack on the electrophile at the less hindered α face, this approach produces a major 16α-alkylated product.[22,25] Several attempts by our group and others[25] to alkylate directly the 16-

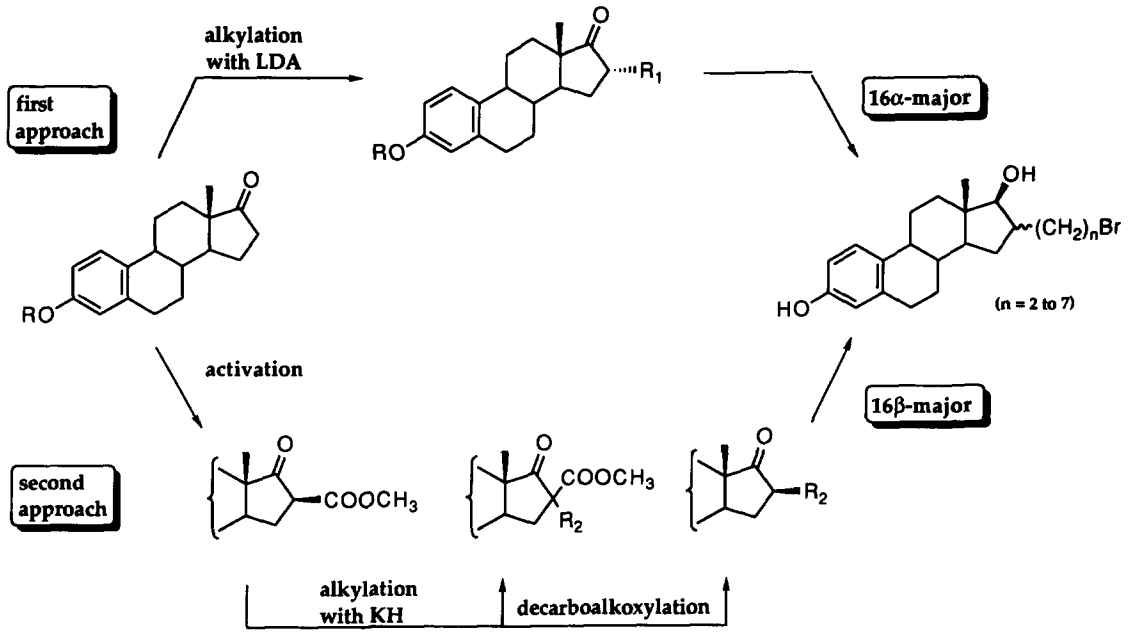
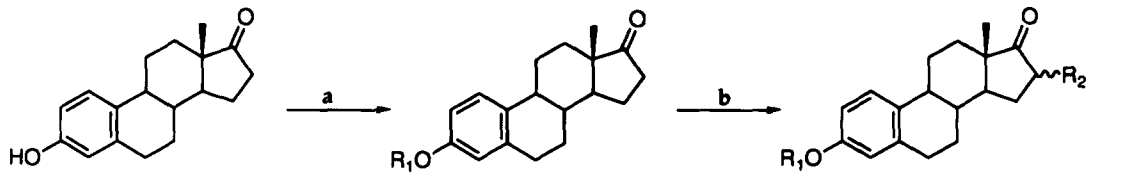


Figure 1. Synthetic approaches used to obtain mainly 16α- and 16β-diastereoisomers (R = protective group, R_1 = activated electrophiles and R_2 = activated or unactivated electrophiles).



1

2a (R_1 = TBDMS)
2b (R_1 = Me)

3 (R_1 = TBDMS, R_2 = CH_2CO_2Me) (16α:16β/85:15)
4 (R_1 = TBDMS, R_2 = $CH_2CH{=}CH_2$) (16α:16β/95:5)
5 (R_1 = Me, R_2 = $CH_2CH{=}CHCO_2Me$) (16α:16β/73:27)
6 (R_1 = Me, R_2 = COOMe) (see Ref. 27)

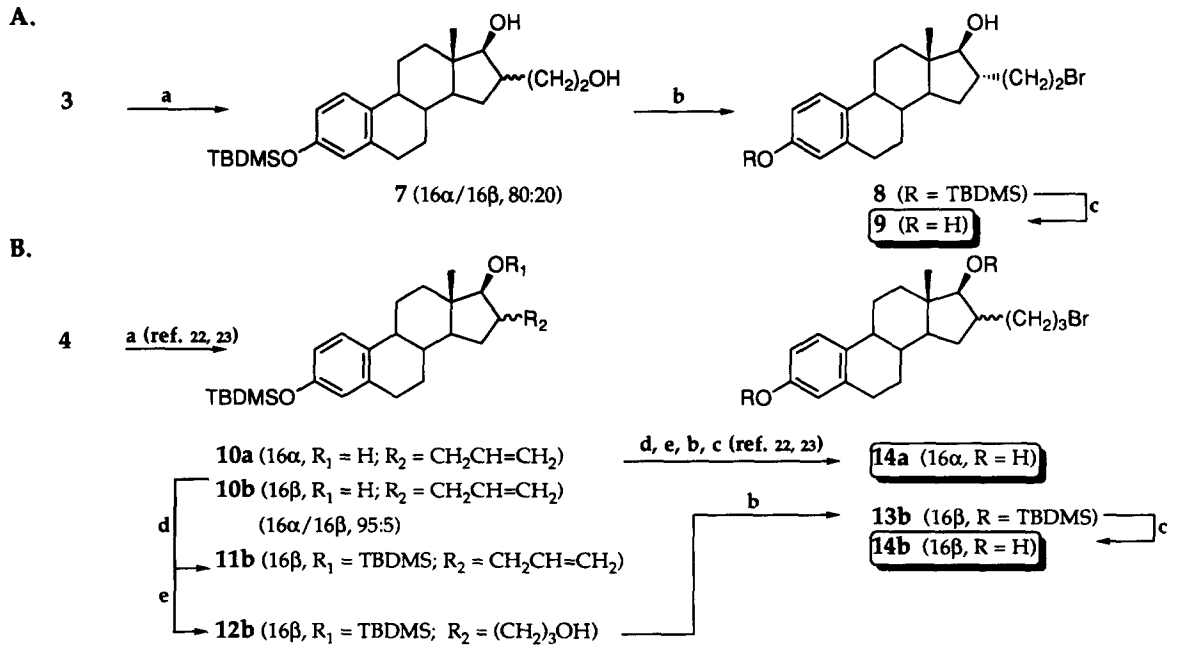
Scheme 1. Synthesis of key intermediates **3–6**. Reagents: (a) TBDMS-Cl, imidazole, DMF for **2a**; CH_3I, K_2CO_3, DMF, Δ for **2b**; (b) 1. LDA 2. $BrCH_2COOMe$ for **3**; $BrCH_2CH{=}CH_2$ for **4**; $BrCH_2CH{=}CHCOOMe$ for **5**; CNCOOMe for **6**.

position with unactivated electrophiles using strong bases have been unsuccessful. Although specific cases of direct alpha-alkylation on steroids with unactivated electrophiles have been reported,[26] alpha-alkylation on steroidal 17-ketones using unactivated electrophiles usually requires an activated carbonyl group. The addition of a 16-methoxycarbonyl group to promote enolization and alkylation of less reactive 17-ketone is a logical choice, as this group could be easily removed. Moreover, this approach led to a major 16β-epimer.[27] Thus, the 16α-configuration could be obtained mainly by direct alkylation on protected estrone, whereas previous activation of the latter was necessary to induce mainly the 16β-configuration, (Figure 1).

The two approaches reported above (Figure 1) need the key intermediates **3–6**, which were synthesized from estrone (**1**) according to the procedures described in Scheme 1. Protection of phenol with *tert*-butyldimethylsilyl (TBDMS), benzoate, and acetate groups have been shown to be unstable under KH deprotonation conditions (second approach), but the methoxy group (**2b**) was resistant. However, TBDMS (**2a**) was a good protective group for the synthesis of 16α-configured products (first approach), which need LDA as a base. Four different groups were introduced at position 16 to obtain, in excellent yields, ketone intermediates **3–6**. For this type of alkylation using a slight excess of LDA as base, the major diastereoisomer observed was 16α-configured for compounds **3**, **4** and **5** as predicted by the stereoselectivity rule. An unresolved mixture of 16α/16β-isomers in proportions of 95:5 to 73:27 was revealed by ^{1}H NMR spectroscopy. For the 16β-isomer, the 18-CH_3 signal is generally shielded compared with the 16α-isomer. These data indicate a 'pseudo' 1,3 diaxial interaction between the 18-CH_3 and the first methylene group of the 16β-isomer. This interaction between 18-CH_3 and the side chain was also observed for 15-alkylated estradiol derivatives.[28] These results will be confirmed when each isomer is fully characterized under its reduced form. On the other hand, methoxycarbonylation resulted in a 16β-isomer using these conditions (LDA, 1.2 eq.). We have established that the acidic proton at position 16 could be responsible for isomerization during both the work-up and the purification conditions[27] leading to the thermodynamically stable 16β-isomer.[29] The 16β-configuration of **6** has been proved chemically and by ^{1}H as well as ^{13}C NMR spectroscopy.[27]

*Synthesis of 16α-(bromoethyl)-estradiol (**9**), 16-(bromopropyl)-estradiols (**14a** and **14b**) and 16α-(bromobutyl)-estradiol (**17a**) by the first approach*

The sequence of reactions used for the synthesis of compounds **9**, **14a** and **14b** is illustrated in Scheme 2. First, γ-ketoester **3** was selectively reduced with $LiAlH_4$ to give the corresponding alcohol **7** as an 80:20 mixture of 16α/16β-isomers as evaluated by ^{1}H NMR analysis (Scheme 2A). The reaction must be performed at –78 °C to fix the 17β-hydroxy configuration[25] whereas reduction of ester could be completed at room temperature. Low temperature leads to high stereoselectivity of hydride attack on the electrophilic 17-ketone by the less hindered α face due to the 18-CH_3 group.[22,25] The 17β-OH configuration was confirmed by ^{1}H and ^{13}C NMR analysis.[27,30] The Table 1 shows our ^{1}H and ^{13}C NMR data for both 17-CH and 18-CH_3 signals of all 16-alkyl, 17β-OH estradiols synthesized in this study. In ^{1}H NMR, these results correlate exactly with data reported for 16-alkylated estradiol derivatives.[29,31–33] The C16 stereochemistry can also be discriminated by the C17 chemical shift in ^{13}C NMR spectroscopy. In fact, for 16α-diastereoisomers, the C17 signal is unshielded at 88 ppm but is shielded at 82 ppm for 16β-diastereoisomers (Table 1).



Scheme 2. Reagents: (a) $LiAlH_4$, THF; (b) CBr_4, PPh_3, CH_2Cl_2; (c) MeOH/HCl (2% v/v); (d) TBDMS-Cl, imidazole, DMF; (e) 1. BH_3•THF; 2. NaOH, H_2O_2.

Table 1. ^1H and ^{13}C NMR data for 16-(bromoalkyl)-estradiols dissolved in $CDCl_3$

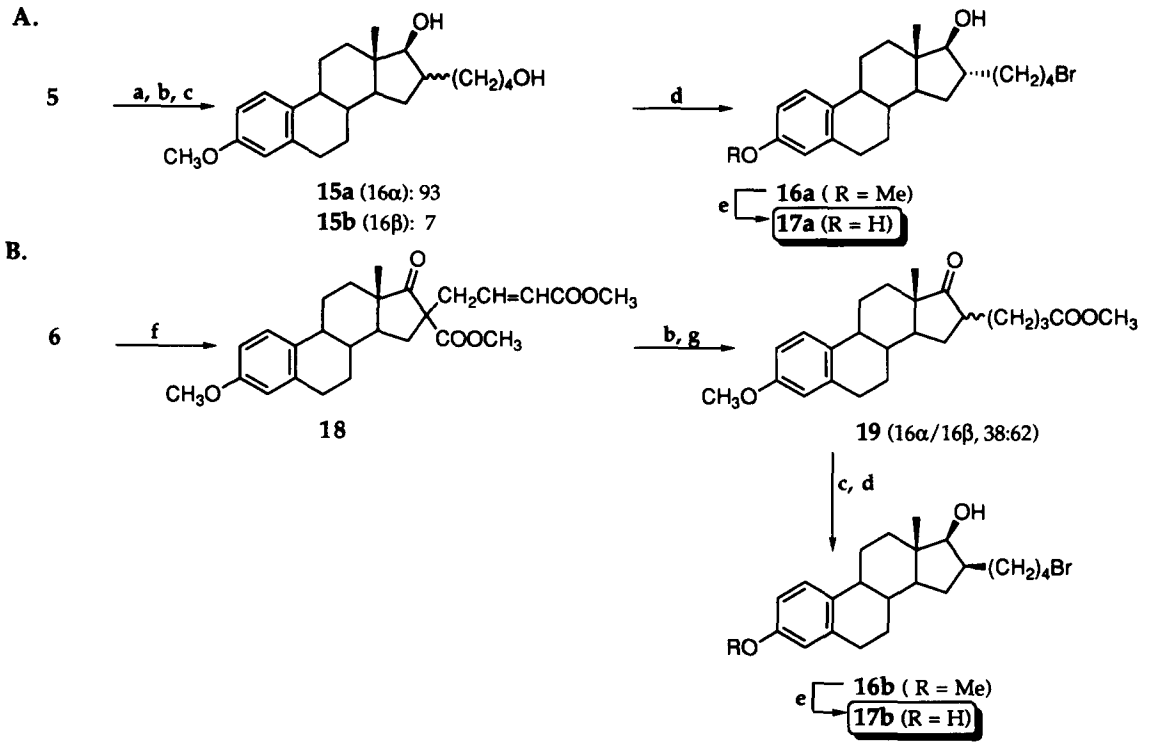
Compounds	Configuration 16α or 16β	Number of methylene (*n*)	17α-CH δ (ppm)		18-CH_3 δ (ppm)	
			^1H	^{13}C	^1H	^{13}C
7*	α	2	3.40	87.87	0.84	11.97
8	α	2	3.32	87.84	0.82	11.83
9**	α	2	3.32**	87.96**	0.82**	12.41**
14a***	α	3	3.30	88.04	0.80	11.87
15a	α	4	3.28	87.95	0.81	11.91
16a	α	4	3.27	88.06	0.81	11.86
17a**	α	4	3.27**	87.97**	0.81**	12.26**
29a	α	6	3.28	88.09	0.80	11.85
30a	α	7	3.26	88.12	0.80	11.86
31a	α	6	3.27	88.11	0.81	11.85
32a	α	7	3.26	88.21	0.80	11.88
33a	α	6	3.27	88.23	0.80	11.88
34a	α	7	3.27	88.26	0.80	11.92
12b	β	3	3.66	82.71	0.76	12.75
14b	β	3	3.78	82.25	0.78	12.38
16b	β	4	3.75	82.21	0.79	12.33
17b	β	4	3.75	82.36	0.77	12.40
23	β	5	3.74	82.39	0.77	12.37
24	β	5	3.76	82.48	0.77	12.39
29b	β	6	3.72	82.46	0.76	12.36
30b	β	7	3.72	82.34	0.77	12.31
31b	β	6	3.74	82.44	0.77	12.38
32b	β	7	3.74	82.42	0.78	12.36
33b	β	6	3.74	82.49	0.77	12.39
34b	β	7	3.75	82.54	0.77	12.39

*Only the major isomer is shown.
**Dissolved in acetone-d_6.
***Ref.[22]

Bromination with CBr_4 and PPh_3 is successful on primary alcohols, but only specific cases were observed on secondary alcohols under these conditions.[34] In this standard procedure, secondary hindered alcohol at position 17 of the steroidal nucleus is not substituted by bromine,[35] so compound **8** could be obtained without previous protection of 17β-OH. At this step, only the 16α-(bromoethyl)-E_2 derivative was observed. Cyclization of the 16β-diastereoisomer was thought to be responsible for this result. Our attempts to obtain the 16β-diastereoisomer by the second approach with activated ketone confirmed our hypothesis. In fact, the 16β-diastereoisomer underwent cyclization under bromination conditions. The presence of nucleophilic alcohol and electrophilic side chain with a *syn* configuration can explain the formation of a stable tetrahydrofuran ring in an intramolecular process. Finally, silyl ether of compound **8** was cleaved by a methanolic solution of HCl (2%) to afford the target bromide **9**.

The synthesis of 16α-(bromopropyl)-E_2 (**14a**) is described completely in our earlier report.[22] The same synthetic sequence was used to provide the other diastereoisomer **14b** (Scheme 2B). The stereoselective reduction of intermediate **4** under the conditions described above ($LiAlH_4$, –78 °C) gave only two diastereoisomers **10a** and **10b** in proportions of 95:5, which could be separated by silica gel flash chromatography. Even though 16β-configured product was the minor isomer, it was used for the synthesis of 16β-(bromopropyl)-E_2 (**14b**). The secondary alcohol at position 17 of minor 16β-diastereoisomer **10b** was protected with a TBDMS group to give the di-TBDMS olefin **11b**. Protection of 17β-alcohol was originally thought to prevent cyclization during functionalization of the side chain. The olefin **11b** was treated with borane followed by H_2O_2 and NaOH to produce primary alcohol **12b**. Subsequent bromination and hydrolysis of TBDMS groups led to 16β-(bromopropyl)-E_2 (**14b**). The spectral data of **14b** are indicated in Table 1, demonstrating that it belongs to the 16β-isomer family. Unfortunately, long-term storage of compound **14b** in ethanolic solution made cyclization occur, as predicted by previous observations, with the ethyl side-chain analogue. Consequently, this target bromide was tested immediately after its preparation.

The synthesis of the expected 16α-(bromobutyl)-E_2 (**17a**) is illustrated in Scheme 3A. The intermediate conjugated ester **5** was transformed to diols **15** by a sequence of three reductive steps: (1) $LiAlH_4$ reduction of 17-ketone exclusively; (2) catalytic hydrogenation (H_2, Pd/C) of double bond; (3) $LiAlH_4$ reduction of ester. The first reduction was originally thought to generate resolvable diastereoisomers. However, subsequent reduction steps have been shown to be necessary to resolve the mixture at the last step. The reduction sequence was then followed by chromatographic purification, which could yield separated



Scheme 3. Reagents: (a) $LiAlH_4$, THF, –78 °C; (b) H_2, Pd/C; (c) $LiAlH_4$, THF, –78 °C to rt; (d) CBr_4, PPh_3, CH_2Cl_2; (e) BBr_3, CH_2Cl_2, 0 °C; (f) KH, 18-crown-6, 4-methylbromocrotonate; (g) LiCl, H_2O, DMF, Δ.

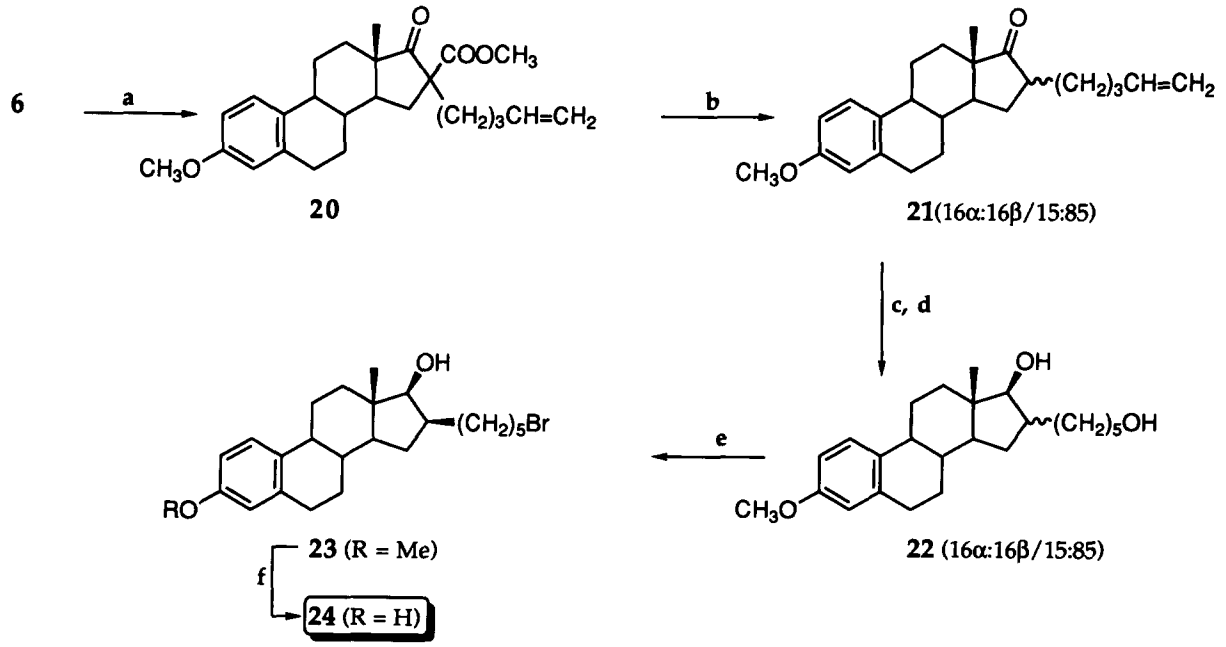
diastereoisomers. The major 16α-configured diol **15a** was submitted to bromination (PPh_3, CBr_4) and methoxy cleavage (BBr_3)[36] to give the target compound **17a** (Table 1).

Synthesis of 16β-(bromobutyl)-estradiol (17b), 16β-(bromopentyl)-estradiol (24), 16-(bromohexyl)-estradiols (33a and 33b) and 16-(bromoheptyl)-estradiols (34a and 34b) by the second approach

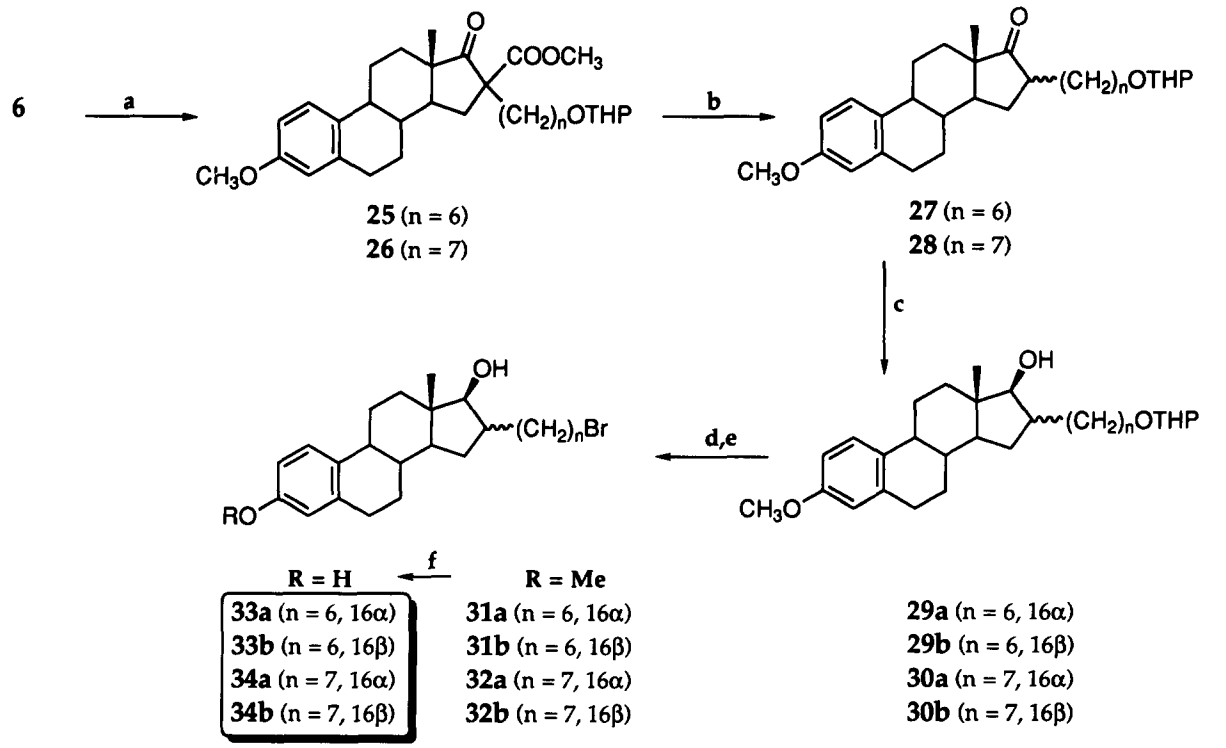
So far, alpha-alkylation of steroidal 17-ketone, using LDA as base, is generally limited to activated electrophiles, meaning that only short commercially available side chains can be introduced at position 16 of the steroidal nucleus. To introduce longer alkyl sidechains at position 16, a different synthetic approach was elaborated. As illustrated in Figure 1 and Schemes 3B, 4 and 5, the two key steps are a second alkylation on activated 16-position and a subsequent decarboalkoxylation. The starting compound **6**, obtained first by an acylation with LDA and methylcyanoformate, was revealed to be non-reactive for a second deprotonation with this moderately hindered base. Similar studies were recently done on cyclic β-ketoester with potassium carbonate in acetone as the enolate-generating agent.[37] However, the same approach was also unsuccessful in our case (steroidal 17-ketone). This observation, in combination with other groups' studies,[25] has shown that the steroidal 17-ketone does not react like similar cyclic ketones toward halogenoalkanes. Fortunately, potassium hydride has been shown to enolize the starting compound **6**. Alkylation with terminal dibromo side chain was next attempted and worked well, but the subsequent decarboalkoxylation was unsuccessful. In fact, primary bromide was unstable under conditions of decarboalkoxylation generating a polar compound. The best synthetic method was then alkylation with electrophiles, which allowed us to easily obtain a primary bromide such as methyl 4-bromocrotonate, 5-bromopentene, $I(CH_2)_6OTHP$ and $Br(CH_2)_7OTHP$.

A six-step sequence led to the target 16β-(bromobutyl)-E_2 (**17b**) (Scheme 3B). The alkylation of starting ester **6** with methyl 4-bromocrotonate was performed to produce **18** with a good yield. Catalytic hydrogenation of the double bond and decarboalkoxylation[38] yielded a mixture of two unresolved ketoesters **19** (16α:16β, 38:62). As described above, the C16 stereochemistry was elucidated by observing the CH_3-18 signal in ^1H and ^{13}C NMR spectroscopy. Reduction using $LiAlH_4$ and selective bromination of primary alcohol were performed to yield **16b** as the major product, in contrast with the first synthetic approach, which gave the 16α-diastereoisomer **16a** from the starting compound **5** (Scheme 3A). All spectroscopic data are in agreement with the literature[29,31–33] (Table 1). Finally, methoxy ether cleavage of compound **16b** by BBr_3 afforded the 16β-(bromobutyl)-E_2 (**17b**).

As shown in Schemes 4 and 5, the synthetic route leading to analogues containing five, six and seven carbon bromoalkyl side chains is very similar to the previously described sequence in Scheme 3B. The synthesis of 16β-(bromopentyl)-E_2 (**24**) was started by introducing a side chain containing a terminal double bond to produce the intermediate **20**, which gives **21** after subsequent decarboalkoxylation (16α:16β, 15:85).



Scheme 4. Reagents: (a) KH, 18-crown-6, 5-bromopentene; (b) LiCl, H_2O, DMF, Δ; (c) $LiAlH_4$, THF; (d) 1. BH_3•THF; 2. NaOH, H_2O_2; (e) CBr_4, PPh_3, CH_2Cl_2; (f) BBr_3, CH_2Cl_2, 0 °C.



Scheme 5: Reagents: (a) KH, 18-crown-6, $X(CH_2)_nOTHP$ (X = Br or I, n = 6 or 7); (b) LiCl, H_2O, DMF, Δ; (c) $LiAlH_4$, THF; (d) MeOH/HCl (2%, v/v); (e) CBr_4, PPh_3, CH_2Cl_2; (f) BBr_3, CH_2Cl_2, 0 °C.

The stereoselective reduction by $LiAlH_4$ at –78 °C resulted in the corresponding mixture of diastereoisomers. In contrast to what was done previously, the mixture could not be separated at this step. Thus, the double bond was oxidatively hydroborated as described above to afford diols **22** as a mixture of diastereoisomers in 15:85 proportions respectively, for 16α and 16β. Starting with the mixture of diols **22**, functionalization of the side chain could be completed to give, after cleavage of methoxy group, the expected bromide **24**. Not enough 16α-diastereoisomer of diol was recuperated to follow up the sequence, and only the

major 16β-diastereoisomer was tested to evaluate its inhibition potency on 17β-HSD type 1.

Analogues containing six- and seven-carbon side chains (compounds **33a**, **33b**, **34a** and **34b**) were synthesized according to the second approach (Scheme 5).[27] First, the alkylation with halogeno side chains was achieved on activated 3-methoxy-estrone (**6**). The methoxycarbonyl group was removed under decarboalkoxylation conditions using LiCl and water in refluxing DMF to afford ketones **27** and **28**. Because the 17β-OH configuration is fixed by the reductive conditions, only two diastereoisomers of each THP-derivative **29a**, **29b** and **30a**, **30b** were obtained (Table 1), and the mixture could be resolved using classic silica gel chromatography purification. Hydrolysis of THP group and subsequent bromination of primary alcohol gave compounds **31a**, **31b**, **32a** and **32b**, which were deprotected using BBr_3 in CH_2Cl_2 to yield expected target bromides **33a**, **33b**, **34a** and **34b**.

Considerations focusing on the second approach

The target 16-(bromoalkyl)-estradiols were synthesized by two approaches (Figure 1) according to the expected C16 stereochemistry. The first approach, leading mainly to 16α-alkyl analogues, has been thoroughly discussed by our group[22,23] and by other authors.[25,29,32] However, the second approach involving three key steps (activation of 17-ketone, alkylation with KH and decarboalkoxylation) requires further explanation. Interestingly, this approach allowed us to obtain 16β-(bromoalkyl)-estradiols that could not be yielded easily by classic alkylation using LDA and activated electrophiles.

The three-step sequence could be achieved with yields varying from 17% to 39%. Synthesis of activated 3-methoxy-estrone (**6**), which is the first key intermediate, could be done with an excellent yield (83%). The alkylation with KH using unactivated electrophiles worked moderately well (42–68%), and activated bromide as methylbromocrotonate provided excellent yield (91%). Finally, decarboalkoxylation was achieved with yields generally varying from 41% to 71%. On the other hand, this synthetic method has been shown to generate an excess of 16β-isomer over 16α-isomer from 62:38 to 85:15. These experiments together with other studies[27] have demonstrated that the methoxycarbonyl group at position 16 could be a synthetic device to reverse the 16-position stereochemistry normally induced by 18-CH_3 during alkylation of estrone. In conclusion, combining two complementary approaches was used for the synthesis of nine novel 16-(bromoalkyl)-estradiols having 2 to 7 carbon side chains with 16α and 16β configurations.

Biological Results and Discussion

In order to observe the effect of the length and orientation of the bromoalkyl side chain on 17β-HSD type 1 activity, biological studies were performed on partially purified cytosolic enzyme from human placenta. Although tests using purified enzyme or transfected cells are actually available for 17β-HSD type 1,[14] a simple, fast test has been elaborated to assess, in the same protocol, the ability of several target compounds to inhibit the transformation of estrone to estradiol. The preparation of the enzymatic test begins with homogenization and biochemical fractionation of human placentas,[23] leading to a soluble cytosolic fraction, which contains 17β-HSD type 1 activity.[13] The reductive activity, as selected by NADH excess, was chosen because estradiol is more potent than estrone in regulating estrogeno-dependent tissues. Moreover, pH and temperature close to physiological conditions were used (pH = 7.4 and T = 37 °C).

As illustrated in Figure 2A and 2B, the various synthetic steroids were first tested by screening for the inhibitory effect. Two concentrations of inhibitor were incubated with cofactor NADH, tritiated estrone and partially purified enzyme in an appropriate phosphate buffer for 30 min at 37 °C. The enzymatic assay was performed at around 20% of transformation, respecting the steady-state condition. Some noteworthy relationships between structure and activity were found and deserve further mention. For 16α-configured compounds shown in Figure 2A, the shorter side chains were responsible for better inhibition than the longer ones. The 16α-(bromopropyl)-E_2 (**14a**) and 16α-(bromobutyl)-E_2 (**17a**) seemed the best inhibitors (Figure 2A) and did not appear significantly different from this screening. Despite the fact that 16α-(bromopentyl)-E_2 was not synthesized, tendencies for shorter side chains allowed us to exclude it as an interesting inhibitor. For 16β-configured compounds (Figure 2B), 16β-(bromopropyl)-E_2 (**14b**) was the most potent inhibitor, but only when tested immediately after its preparation. In fact, as reported above, the compound was not stable and underwent cyclization in solution with loss of inhibiting activity on 17β-HSD. Consequently, the compound **14b** was not retained for further studies. For the same reason, screening values are not shown for 16β-(bromoethyl)-E_2. Thus, 16β-(bromobutyl)-E_2 (**17b**) appeared to be a more potent inhibitor than compounds containing longer side chains and was the only compound retained from the 16β-configured series.

Recently, we have demonstrated this type of inhibitor as inactivators that covalently bond with the enzyme. In fact, 17β-HSD activity could not be recovered after preincubation of the enzymatic pool together with these compounds.[22] We also observed that unlabelled substrate estrone protects against the inactivation of enzyme, suggesting a competitive process (unpublished results). Therefore, two interactions can be suggested to rationalize our results. Firstly, the electrophile site of 16α-(bromoethyl)-E_2 must be too far from the opposite nucleophile site of the enzyme, often postulated to be a histidyl or another basic amino acid residue.[24] Secondly, the five-, six- and seven-carbon-long side-chain could turn in on itself to prevent the steroid taking place in the 17β-HSD active site with the correct orientation. Moreover, a long side chain can adopt far more

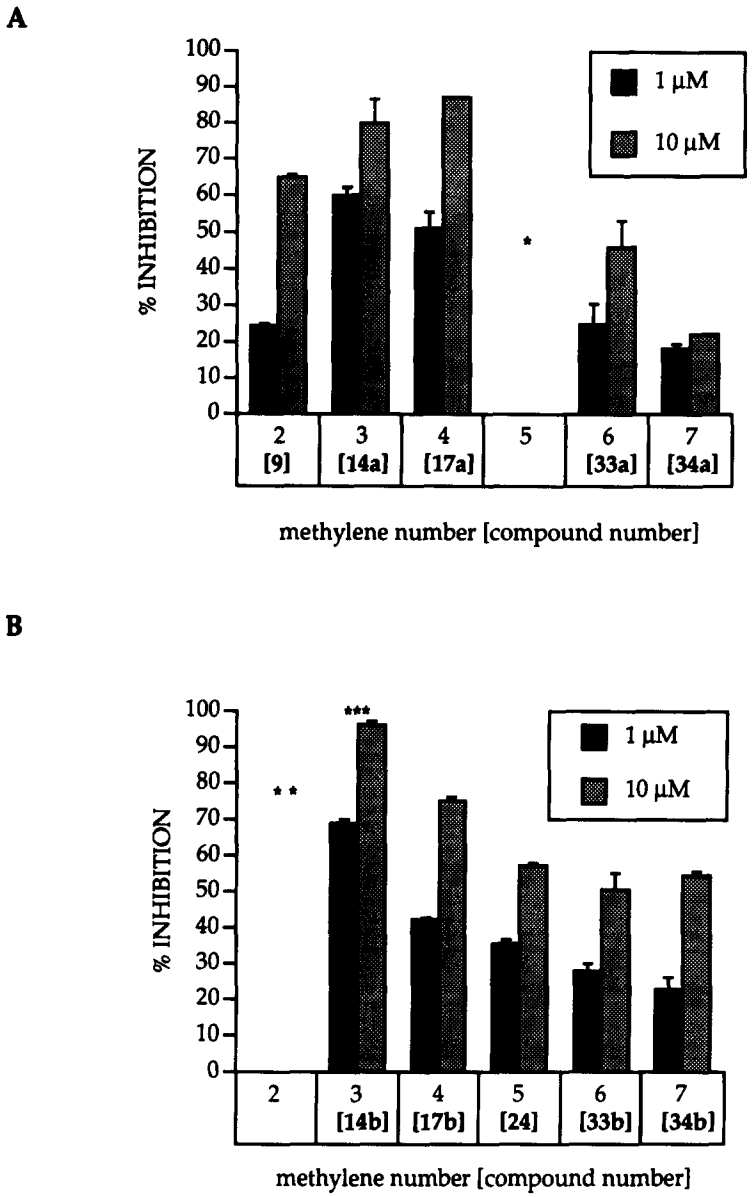


Figure 2. Inhibition of partially purified 17β-HSD type 1 by the synthetic 16-(bromoalkyl)-estradiols. **A**: 16α-configured analogues. **B**: 16β-configured analogues. Enzymatic tests were performed in a total volume of 1 mL containing 900 μL of phosphate buffer (50 mM KH_2PO_4, 1 mM EDTA, 0.25 M sucrose, pH 7.4, 1 mM NADH and 1.8 nM [^{3}H]-E_1), 10 μL of inhibitor at indicated concentration and 100 μL of crude enzyme as described in the Experimental section. Ao = 18 fmol E_2 min^{-1} mg^{-1} of proteins. Results are expressed as mean ± SEM of two experiments. *Compound not synthesized; **cyclized before characterization; ***tested immediately after preparation.

conformations than shorter ones, which could decrease the probability of obtaining an effective conformation favoring the nucleophilic and the electrophilic sites coming close to each other. In conclusion, the optimum length of the bromoalkyl side chain at the position 16 of estradiol for 17β-HSD inhibition was 3 or 4 carbons. At this step, we could not make any conclusions about the importance of the configuration at the position 16. Thus, complete inhibition curves should have been traced for the most potent inhibitors, which were **14a**, **17a** and **17b**.

Figure 3 shows the effect of increasing concentrations of inhibitors **14a**, **17a** and **17b** on the transformation of estrone to estradiol catalyzed by 17β-HSD type 1. These complete curves allowed us to calculate the concentration causing the half-inhibition (IC_{50}). As shown in Figure 3, the 16α-(bromopropyl)-E_2 (**14a**) is slightly better than 16α-(bromobutyl)-E_2 (**17a**), which is approximately 2-fold better than its 16β-configured isomer **17b**. In fact, IC_{50} values are, respectively, 870, 970 and 2140 nM for **14a**, **17a** and **17b**. In light of these results, 16α-(bromopropyl)-E_2 (**14a**), discussed in our earlier report,[22] is still the best inhibitor emerging from the 16-(bromoalkyl)-E_2 series.

Conclusions

A new series of 16-(bromoalkyl)-estradiols, showing an inhibitory effect on human 17β-HSD type 1, has been synthesized. Starting from commercially available

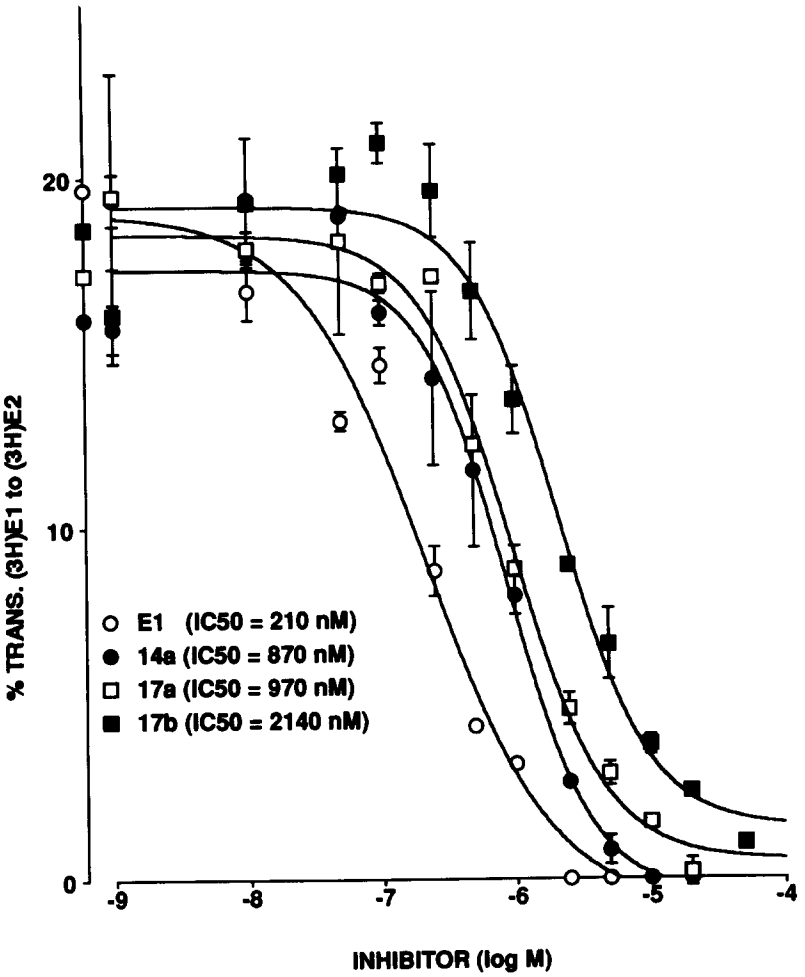


Figure 3. Effect of increasing concentrations of estrone (E_1) or inhibitors (**14a**, **17a** and **17b**) on partially purified 17β-HSD type 1 from human placenta. The enzyme preparation was incubated with 1.1 nM of [^{3}H]-estrone in the presence of indicated concentration of inhibitor for 30 min at 37 °C. Ao = 3 fmol E_2 min^{-1} mg^{-1} of proteins. Data points are expressed as mean ± SEM of two experiments.

estrone, two approaches have been described to obtain mainly the corresponding 16α or 16β-isomer (Figure 1). The direct alkylation at position 16 was performed using LDA as base and activated electrophiles (first approach). Under the same conditions, the direct alkylation of longer side chains was not successful. To overcome this chemical difficulty, the synthetic pathway using activated 17-ketone was elaborated. This second approach led to major 16β-isomers and allowed alkylation at a less accessible position 16 of the steroidal D-ring, using long unactivated side chains.

In this study, we demonstrated that analogues containing short bromoalkyl moieties at position 16 (n = 3 or 4) are more potent than those with longer side chains when tested on partially purified 17β-HSD type 1. Complete inhibition curves allowed us to conclude that the bromopropyl is the optimum side chain provoking inhibition on 17β-HSD type 1 (Fig. 3). Because 16β-configured compounds with short side chains (n = 2 and 3) are chemically unstable, comparison between the two possible configurations (16α and 16β) could have been made only for bromobutyl derivatives of estradiol. In this case, the 16α configuration improved by 2-fold the potency of the inhibitor. However, this tendency can not be generalized for other analogues with longer side chains (n = 5–7).

The 16-(bromoalkyl)-E_2 series were shown as growth stimulating agents when tested on human estrogeno-sensitive breast cancer cell line ZR-75-1 (unpublished results). Since this estrogenic effect[4] must be avoided for the endocrine therapy of breast cancer, we are now working on the synthesis of a compound that could inhibit the formation of estradiol from estrone by 17β-HSD, without providing estrogenic properties. Herein we have optimized the length and the orientation of the bromoalkyl side chain at the position 16 of estradiol. An additional step will introduce this optimized side chain on antiestrogenic steroidal nucleus.

Experimental

Chemical synthesis

General procedure. Chemical reagents and starting material (estrone) were purchased from Aldrich Chemical Company (Milwaukee, WI) or Steraloids

(Wilton, NH); solvents were obtained from BDH Chemicals (Montréal, Canada), Baker Chemicals (Montréal, Canada), or Fischer Chemical (Montréal, Canada). Thin-layer chromatography (TLC) was performed on 0.20-mm silica gel 60 F_{254} plates (E. Merck, Darmstadt, Germany), and 230–400 mesh ASTM silica gel 60 (E. Merck, Darmstadt, Germany) was used for flash column chromatography. Infrared spectra (IR) were reported in cm^{-1} and obtained on a Perkin–Elmer 1600 (FT-IR series) spectrophotometer. Nuclear magnetic resonance spectra (NMR) were obtained at 300 MHz for ^1H and 75.5 MHz for ^{13}C with a Bruker AC/F300 spectrometer. The chemical shifts (δ in ppm) were referenced to $CDCl_3$ (7.26 or 77.00) and acetone-d_6 (2.05 or 29.83), respectively, for ^1H and ^{13}C. In ^1H data, only specific peaks were reported from upfield to downfield. In ^{13}C data, only unambiguously assigned signals were reported and splitting signals were indicated between parentheses. Low-resolution mass spectra (MS) were recorded with a Hewlett Packard spectrometer by injecting directly on column the dissolved samples (30 eV or 60 eV, interface: 190 °C or 205 °C). Important peaks were reported with relative intensity between parentheses. High-resolution electron impact mass spectra (EIMS) were provided by the Centre Régional de Spectrométrie de Masse (Université de Montréal, Montréal, Canada).

*Synthesis of protected starting products **2a** and **2b** (Scheme 1)*

Protection of the phenolic group of estrone by *tert*-butyldimethylsilyloxy (TBDMS) or methoxy was achieved following the procedure described in our earlier reports,[22,23] and all data were in agreement with the literature.

*Synthesis of key intermediates **3**, **4**, **5** and **6** (Scheme 1)*

General procedure for alkylation using LDA. A solution of diisopropylamine (1.2 eq.) in dry THF was stirred under argon at –25 °C and butyllithium (1.2 eq.) was added dropwise. After 30 min, the solution was cooled at –78 °C and TBDMS-estrone (**2a**) or 3-methoxy-estrone (**2b**) dissolved in dry THF was added dropwise into the LDA solution. The mixture was allowed to stir for 1 h at 0 °C before another cooling at –78 °C. Then, dry HMPA (1.2 eq.) and 1 eq. of methyl bromoacetate (**3**), allyl bromide (**4**), methyl 4-bromocrotonate (**5**) or methyl cyanoformate (**6**) were slowly added to the solution, which was allowed to stir from –78 °C to room temperature for 1–12 h. The reaction was quenched by the addition of water, and the crude product was extracted with EtOAc. The organic phase was washed with a saturated NaCl solution, dried over $MgSO_4$ and evaporated under reduced pressure. Purification by flash chromatography [hexane/EtOAc, 80:20 (**3**), 97:3 (**4**), 99:1 (**5**) and 85:15 (**6**)] afforded the expected 16-alkylated-products **3**, **4**, **5** and **6**.

*Methyl 2-[3'-(*tert*-butyldimethylsilyloxy)-17'-oxo-1',3',5'-(10')-estratrien-16'α/β-yl]-acetate (**3**).* White solid (67% yield); IR ν (film): 1740 (C=O, ester and ketone); ^1H NMR δ ($CDCl_3$): 0.19 (*s*, 6H, $Si(CH_3)_2$), 0.88 and 0.99 (2*s*, 3H, CH_3-18', 16β:16α, 15:85), 0.98 (*s*, 9H, $SiC(CH_3)_3$), 2.80 (*m*, 2H, CH_2-6') 2.79 and 2.85 (2*m*, 2H, $\underline{CH_2}COOCH_3$), 2.98 (*m*, 1H, CH-16'), 3.71 (*s*, 3H, $COO\underline{CH_3}$), 6.57 (*d*, *J* = 2.2 Hz, 1H, CH-4'), 6.62 (*dd*, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, CH-2'), 7.12 (*d*, *J* = 8.4 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): only the major 16α-isomer is described: –4.52 ($Si(\underline{C}H_3)_2$), 14.37 (C-18'), 18.02 ($Si\underline{C}(CH_3)_3$), 25.57 ($SiC(\underline{C}H_3)_3$ and C-15'), 26.29, 27.74, 29.28, 31.46, 35.21, 38.09, 40.85, 43.84, 45.61, 48.13, 51.63 ($COO\underline{C}H_3$), 117.19 (C-2'), 119.83 (C-4'), 125.95 (C-1'), 132.23 (C-10'), 137.39 (C-5'), 153.35 (C-3'), 172.73 (C-1), 219.54 (C-17'); MS m/z (rel. intensity): 456 (M^+, 43), 399 (100), 271 (5), 215 (5), 163 (14).

*3-[3'-(*tert*-Butyldimethylsilyloxy)-17'-oxo-1',3',5' (10')-estratrien-16'α/β-yl]-propene (**4**).* White solid (69% yield); IR ν (film): 1740 (C=O, ketone); ^1H NMR δ ($CDCl_3$): 0.20 (*s*, 6H, $Si(CH_3)_2$), 0.87–0.99 (*m*, 12H, CH_3-18' of 16β:16α, 5:95 and $SiC(CH_3)_3$), 2.84 (*m*, 2H, CH_2-6'), 5.07 (*m*, 2H, $CH=C\underline{H_2}$), 5.79 (*m*, 1H, $C\underline{H}=CH_2$), 6.57 (*d*, *J* = 2.2 Hz, 1H, CH-4'), 6.62 (*dd*, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, CH-2'), 7.12 (*d*, *J* = 8.4 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): only the major 16α-isomer is described: –4.40 ($Si(\underline{C}H_3)_2$), 14.61 (C-18'), 18.15 ($Si\underline{C}(CH_3)_3$), 25.69 ($SiC(\underline{C}H_3)_3$ and C-15'), 26.45, 26.60, 29.43, 31.63, 35.03, 38.22, 44.03, 44.09, 48.01, 48.67, 116.37 ($CH=\underline{C}H_2$), 117.26 (C-2'), 119.96 (C-4'), 126.07 (C-1'), 132.48 (C-10'), 136.46 ($\underline{C}H=CH_2$), 137.60 (C-5'), 153.46 (C-3'), 221.24 (C-17'); MS m/z (rel. intensity): 424 (M^+, 76), 367 (100), 271 (6), 215 (6), 163 (18).

*Methyl 4-[3'-methoxy-17'-oxo-1',3',5'(10')-estratrien-16'α/β-yl]-2-butenoate (**5**).* White solid (50% yield); IR ν (KBr): 1730 and 1735 (C=O, conjugated ester and ketone); ^1H NMR δ ($CDCl_3$): 0.91 and 0.97 (2*s*, 3H, CH_3-18', 16β:16α, 27:73), 2.67 (*m*, 2H, $\underline{CH_2}CH=CH$), 2.89 (*m*, 2H, CH_2-6'), 3.74 (*s*, 3H, $COOCH_3$), 3.78 (*s*, 3H, $\underline{CH_3}OAr$), 5.88 (*d*, *J* = 15.6 Hz, 1H, $CH_2CH=\underline{CH}$), 6.65 (*d*, *J* = 2.3 Hz, 1H, CH-4'), 6.72 (*dd*, J_1 = 2.6 Hz and J_2 = 8.6 Hz, 1H, CH-2'), 6.94 (*m*, 1H, $CH_2\underline{CH}=CH$), 7.20 (*d*, *J* = 8.6 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): only the major 16α-isomer is described: 14.50 (C-18'), 25.79, 26.45, 26.83, 29.59, 31.66, 33.48, 38.28, 43.43, 43.97, 47.98, 48,58, 51.49 ($COO\underline{C}H_3$), 55.20 ($\underline{C}H_3OAr$), 111.61 (C-2'), 113.91 (C-4'), 122.52 (C-3), 126.28 (C-1'), 131.89 (C-10'), 137.68 (C-5'), 147.01 (C-2), 157.65 (C-3'), 166.69 (C-1), 219.98 (C-17'); MS m/z (rel. intensity): 382 (M^+, 100), 350 (13), 322 (15), 265 (39), 227 (25), 171 (49).

*Methyl 1-[3'-methoxy-17'-oxo-1',3',5'(10')-estratrien-16'β-yl]-formate (**6**).* Physical and spectroscopic data have been reported elsewhere.[27]

*Synthesis of 16α-(bromoethyl)-, 16α-(bromopropyl)- and 16β-(bromopropyl)-estradiols **9**, **14a** and **14b** (Scheme 2)*

Synthesis of 16α-(bromoethyl)-estradiol (9).
(a) *Procedure for reduction of ketone and/or ester using $LiAlH_4$.* To a solution of γ-ketoester **3** (160 mg, 0.35 mmol) in dry THF, lithium aluminum hydride (2 eq.) was slowly added. The mixture was stirred under argon at –78 °C and at room temperature (15 min) for the reduction of ester. Reaction time was monitored by TLC. The reaction was quenched by the addition of EtOAc at –78 °C and water at room temperature. Then the slurry was diluted with water and extraction was performed with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$ and solvent was removed under reduced pressure. The crude product was purified by flash chromatography (hexane:EtOAc, 7:3).

2-[3'-(tert-Butyldimethylsilyloxy)-17'β-hydroxy-1',3',5' (10')-estratrien-16'α/β-yl]-ethanol (7). White solid (68% yield); IR ν (film): 3300 (OH, alcohol); ^{1}H NMR δ ($CDCl_3$): 0.19 (*s*, 6H, $Si(CH_3)_2$), 0.80 and 0.84 (2*s*, 3H, CH_3-18', 16β:16α, 2:8), 0.98 (*s*, 9H, $SiC(CH_3)_3$), 2.78 (*m*, 2H, CH_2-6'), 3.40 and 3.75 (2*d*, 1H, CH-17', 16α:16β diastereoisomers), 3.80 (*m*, 2H, $\underline{CH_2}OH$), 6.57 (*d*, *J* = 2.2 Hz, 1H, CH-4'), 6.62 (*dd*, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, CH-2'), 7.12 (*d*, *J* = 8.4 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): only the major 16α-isomer is described: –4.39 ($Si(\underline{C}H_3)_2$), 11.97 (C-18'), 18.17 ($Si\underline{C}(CH_3)_3$), 25.69 ($SiC(\underline{C}H_3)_3$), 26.11, 27.24, 29.58, 31.37, 36.77, 38.51, 38.90, 41.85, 44.01, 44.30, 48.51, 62.53 (CH_2OH), 87.87 (C-17'), 117.15 (C-2'), 119.93 (C-4'), 126.06 (C-1'), 132.98 (C-10'), 137.78 (C-5'), 153.32 (C-3'); MS m/z (rel. intensity): 430 (M^+, 99), 373 (100), 163 (28).

(b) *Procedure for bromination of primary alcohol using CBr_4 and PPh_3.* A mixture of primary alcohol **7** (198 mg, 0.48 mmol), PPh_3 (2 eq.) and CBr_4 (2 eq.) in dry CH_2Cl_2 was stirred at 0 °C under argon. The reaction time was monitored by TLC. The crude mixture was preabsorbed on silica gel and chromatography performed with hexane/EtOAc, 95:5 as eluent.

2-[3'-(tert-Butyldimethylsilyloxy)-17'β-hydroxy-1',3',5'-(10')-estratrien-16'α-yl]-bromoethane (8). White solid (73% yield) and a small amount of cyclized product (not described); IR ν (film): 3410 (OH, alcohol); ^{1}H NMR δ ($CDCl_3$): 0.19 (*s*, 6H, $Si(CH_3)_2$), 0.82 (*s*, 3H, CH_3-18'), 0.99 (*s*, 9H, $SiC(CH_3)_3$), 2.80 (*m*, 2H, CH_2-6'), 3.32 (*d*, *J* = 6.9 Hz, 1H, CH-17'α), 3.51 (*m*, 2H, CH_2Br), 6.56 (*d*, *J* = 2.4 Hz, 1H, CH-4'), 6.62 (*dd*, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, CH-2'), 7.12 (*d*, *J* = 8.4 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): –4.35 ($Si(\underline{C}H_3)_2$), 11.83 (C-18'), 18.15 ($Si\underline{C}(CH_3)_3$), 25.70 ($SiC(\underline{C}H_3)_3$), 26.08, 27.23, 29.53, 29.82, 32.40 (CH_2Br), 36.70, 38.52, 39.21, 42.65, 44.00, 44.20, 48.52, 87.84 (C-17'), 117.17 (C-2'), 119.93 (C-4'), 126.02 (C-1'), 132.87 (C-10'), 137.70 (C-5'), 153.36 (C-3'); MS m/z (rel. intensity): 494 (M^+, 66), 492 (M^+, 62), 437 (100), 435 (94), 412 (8.2), 355 (13), 163 (32).

(c) *Procedure for hydrolysis of TBDMS group.* The TBDMS protected 16α-(bromoethyl)-E_2 (**8**) (166 mg, 0.33 mmol) was dissoved in a methanolic solution of HCl (2%, v/v) and the resulting solution was stirred at room temperature. After 3.5 h, water was added, MeOH was evaporated under vacuum and the resulting slurry was filtered. The white precipitate was recrystallized in MeOH:H_2O (99:1).

2-[3',17'β-Dihydroxy-1',3',5' (10')-estratrien-16'α-yl]-bromoethane (9). White needles (87%); IR ν (film): 3425 (OH, alcohol and phenol); ^{1}H NMR δ ($CDCl_3$): 0.82 (*s*, 3H, CH_3-18'), 2.80 (*m*, 2H, CH_2-6'), 3.32 (*d*, *J* = 7 Hz, 1H, CH-17'α), 3.50 (*m*, 2H, CH_2Br), 6.56 (*d*, *J* = 2.4 Hz, 1H, CH-4'), 6.62 (*dd*, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, CH-2'), 7.15 (*d*, *J* = 8.5 Hz, 1H, CH-1'); ^{13}C NMR δ (acetone-d_6): 12.41 (C-18'), 27.12, 28.09, 29.07, 29.33, 30.60, 33.47 (CH_2Br), 37.66, 39.78, 40.43, 43.16, 44.94, 49.24, 87.96 (C-17'), 113.59 (C-2'), 115.96 (C-4'), 126.99 (C-1'), 132.04 (C-10'), 138.41 (C-5'), 155.96 (C-3'); MS m/z (rel. intensity): 380 (M^+, 98), 378 (M^+, 100), 213 (48), 172 (52), 159 (49), 146 (52), 133 (51); EIMS: calcd for $C_{20}H_{27}O_2{}^{79}Br$ (M^+) 378.11945, found 378.11892.

Synthesis of 16α-(bromopropyl)-estradiol (14a). The synthesis of 3-[3',17'β-dihydroxy-1',3',5' (10')-estratrien-16'α-yl]-bromopropane (**14a**) (Scheme 2B) as well as intermediates **10a** and **10b** are entirely described in our recent report.[22]

Synthesis of 16β-(bromopropyl)-estradiol (14b).
(a) *TBDMS-protection.* The secondary alcohol of minor 16β-diastereoisomer **10b** was protected by TBDMS according to standard procedure (TBDMS-Cl, imidazole, DMF) to yield, after chromatography (hexane:EtOAc, 98:2), 54% of diTBDMS derivative **11b**. White solid; ^{1}H NMR δ ($CDCl_3$): 0.11 (*s*, 6H, 17'-$Si(CH_3)_2$), 0.23 (*s*, 6H, 3'-$Si(CH_3)_2$), 0.80 (*s*, 3H, CH_3-18'), 0.98 (*s*, 9H, 17'-$SiC(CH_3)_3$), 1.02 (*s*, 9H, 3'-$SiC(CH_3)_3$), 2.82 (*m*, 2H, CH_2-6'), 3.74 (*d*, *J* = 9.5 Hz, 1H, CH-17'α), 4.99 (*m*, 2H, $CH=\underline{CH_2}$), 5.82 (*m*, 1H, $\underline{CH}=CH_2$), 6.59 (*d*, *J* = 2.2 Hz, 1H, CH-4'), 6.62 (*dd*, J_1 = 2.2 Hz and J_2 = 8.3 Hz, 1H, CH-2'), 7.15 (*d*, *J* = 8.5 Hz, 1H, CH-1'). *Oxidative hydroboration.* To a solution of olefin **11b** (350 mg, 0.65 mmol) in dry THF (30 mL) was added 1.0 M borane–THF complex (1.6 mmol) at 0 °C under an atmosphere of argon. The mixture was stirred at 0 °C for 3 h. Then, 3 N NaOH (1.65 mmol) and 0.21 mL of hydrogen peroxide 30% (w/v) were added. After 1 h at room temperature, the reaction was quenched by the addition of water and extraction was performed with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$ and evaporated to dryness. Purification by chromatography (hexane: EtOAc, 9:1) gave 284 mg (79% yield) of alcohol **12b**.

3-[3',17'-di(tert-Butyldimethylsilyloxy)-1',3',5'(10')-estratrien-16'β-yl]-propanol (12b). White amorphous solid; IR ν (film): 3345 (OH, alcohol); ^{1}H NMR δ ($CDCl_3$): 0.06 and 0.07 (2*s*, 6H, 17'-$Si(CH_3)_2$), 0.20 (*s*, 6H, 3'-$Si(CH_3)_2$), 0.76 (*s*, 3H, CH_3-18'), 0.94 (*s*, 9H, 17'-$SiC(CH_3)_3$), 0.99 (*s*, 9H, 3'-$SiC(CH_3)_3$), 2.80 (*m*, 2H, CH_2-6'), 3.66 (*m*, 3H, $\underline{CH_2}OH$ and CH-17'α), 6.56 (*d*, *J* = 2.4 Hz, 1H, CH-4'), 6.62 (*dd*, J_1 = 2.4 Hz and J_2 = 8.4

Hz, 1H, CH-2'), 7.13 (*d*, *J* = 8.5 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): –4.58 (17-Si(CH_3)$_2$), –4.41 (3-Si(CH_3)$_2$), 12.75 (C-18'), 18.14 (17-Si<u>C</u>(CH_3)$_3$), 18.23 (3-Si<u>C</u>(CH_3)$_3$), 25.69 (17-SiC(<u>C</u>H_3)$_3$), 25.97 (3-SiC-(<u>C</u>H_3)$_3$), 26.33, 27.43, 28.24, 29.67, 31.91, 32.37, 38.14, 38.42, 40.60, 44.10, 44.35, 48.54, 63.34 (CH_2OH), 82.71 (C-17'), 117.11 (C-2'), 119.89 (C-4'), 126.05 (C-1'), 133.22 (C-10'), 137.81 (C-5'), 153.26 (C-3'); MS m/z (rel. intensity): 558 (M^+, 10), 501 (43), 409 (78), 273 (36), 119 (57), 73 (100).

(b) As described above for the synthesis of **8**, the bromination of alcohol **12b** by PPh_3 and CBr_4 afforded, after chromatography (hexane:EtOAc, 95:5), the primary bromide **13b** with 84% yield. ^{1}H NMR δ ($CDCl_3$): 0.08 (*s*, 6H, 17'-Si(CH_3)$_2$), 0.21 (*s*, 6H, 3'-Si(CH_3)$_2$), 0.77 (*s*, 3H, CH_3-18'), 0.96 (*s*, 9H, 17'-SiC(CH_3)$_3$), 1.00 (*s*, 9H, 3'-SiC(CH_3)$_3$), 2.83 (*m*, 2H, CH_2-6'), 3.42 (*t*, *J* = 6.6 Hz, 2H, CH_2Br), 3.70 (*d*, *J* = 9.3 Hz, 1H, CH-17'α), 6.57 (*d*, *J* = 2.5 Hz, 1H, CH-4'), 6.63 (*dd*, J_1 = 2.4 Hz and J_2 = 8.4 Hz, 1H, CH-2'), 7.14 (*d*, *J* = 8.5 Hz, 1H, CH-1'). Finally, TBDMS groups of compound **12b** were hydrolyzed for 10 h at room temperature by a methanolic solution of HCl (2%, v/v) to give, after flash chromatography (hexane:EtOAc, 8:2), 51 mg (69% yield) of expected 16β-(bromopropyl)-E_2 (**14b**).

3-[3',17'β-Dihydroxy-1',3',5' (10')-estratrien-16'β-yl]-bromopropane (14b). White solid; IR ν (film): 3360 (OH, alcohol and phenol); ^{1}H NMR δ ($CDCl_3$): 0.78 (*s*, 3H, CH_3-18'), 2.81 (*m*, 2H, CH_2-6'), 3.42 (*m*, 2H, CH_2Br), 3.78 (*d*, *J* = 9.9 Hz, 1H, CH-17'α), 6.57 (*d*, *J* = 2.6 Hz, 1H, CH-4'), 6.63 (*dd*, J_1 = 2.7 Hz and J_2 = 8.4 Hz, 1H, CH-2'), 7.14 (*d*, *J* = 8.5 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 12.38 (C-18'), 26.22, 27.37, 29.57, 30.30, 32.11, 32.34, 33.98 (CH_2Br), 37.60, 38.26, 39.37, 43.92, 44.10, 48.57, 82.25 (C-17'), 112.72 (C-2'), 115.25 (C-4'), 126.38 (C-1'), 132.37 (C-10'), 138.06 (C-5'), 153.59 (C-3'); MS m/z (rel. intensity): 394 (M^+, 15), 392 (M^+, 15), 312 (74), 213 (44), 97 (100); EIMS: calcd for $C_{21}H_{29}O_2{}^{79}Br$ (M^+) 392.13510, found 392.13394.

Synthesis of 16α- and 16β-(bromobutyl)-estradiols 17a and 17b (Scheme 3)

Synthesis of 16α-(bromobutyl)-estradiol (17a).
(a) *Reduction of 17-ketone.* The 17-ketone of conjugated ester **5** (1.0 g, 2.6 mmol) was submitted to $LiAlH_4$ reduction as described above. ^{1}H NMR analysis revealed a new characteristic signal at 3.32 ppm for CH-17α. *Reduction of double bond*: a suspension of the crude secondary alcohol (830 mg), MeOH (150 mL) and 10% Pd/C (150 mg) was stirred in hydrogen atmosphere at room temperature. After 18 h, the resulting suspension was filtered through Celite and MeOH was removed under reduced pressure. ^{1}H NMR analysis on the crude ester revealed the disappearance of vinylic protons at 5.88 and 6.94 ppm. *Reduction of ester*: the ester group was reduced following the procedure descibed above to provide, after a purification by flash chromatography (hexane:EtOAc, 5:5), 10 mg (1% yield for three steps) of minor 16β-diastereoisomer **15b** (not described) and 153 mg (16% yield for three steps) of major 16α-diastereoisomer of **15a**.

4-[3'-Methoxy-17'β-hydroxy-1',3',5' (10')-estratrien-16'α-yl]-butanol (15a). White amorphous solid; IR ν (film): 3400 (OH, alcohol); ^{1}H NMR δ ($CDCl_3$): 0.81 (*s*, 3H, CH_3-18'), 2.83 (*m*, 2H, CH_2-6'), 3.28 (*d*, *J* = 7.4 Hz, 1H, CH-17'α), 3.66 (*t*, *J* = 6.2 Hz, 2H, <u>C</u>H_2OH), 6.63 (*d*, *J* = 2.3 Hz, 1H, CH-4'), 6.71 (*dd*, J_1 = 2.6 Hz and J_2 = 8.6 Hz, 1H, CH-2'), 7.20 (*d*, *J* = 8.6 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 11.91 (C-18'), 24.36, 26.16, 27.18, 29.72, 30.20, 32.39, 35.48, 36.78, 38.56, 43.40, 43.91, 44.10, 48.33, 55.11 (<u>C</u>H_3OAr), 62.10 (CH_2OH), 87.95 (C-17'), 111.38 (C-2'), 113.74 (C-4'), 126.18 (C-1'), 132.62 (C-10'), 137.86 (C-5'), 157.33 (C-3'); MS m/z (rel. intensity): 358 (M^+, 100), 340 (2), 271 (6), 227 (19), 186 (20), 160 (15).

(b) The primary alcohol of **15a** (153 mg, 0.44 mmol) was brominated with PPh_3 and CBr_4 in dry CH_2Cl_2 as previously described. Purification by flash chromatography (hexane/EtOAc, 9:1) provided 141 mg (78% yield) of 16α-diastereoisomer **16a**.

4-[3'-Methoxy-17'β-hydroxy-1',3',5' (10')-estratrien-16'α-yl]-bromobutane (16a). Colorless oil; IR ν (film): 3440 (OH, alcohol); ^{1}H NMR δ ($CDCl_3$): 0.81 (*s*, 3H, CH_3-18'), 2.85 (*m*, 2H, CH_2-6'), 3.27 (*d*, *J* = 7.4 Hz, 1H, CH-17'α), 3.44 (*t*, *J* = 6.8 Hz, 2H, CH_2Br), 3.78 (*s*, 3H, <u>C</u>H_3OAr), 6.64 (*d*, *J* = 2.7 Hz, 1H, CH-4'), 6.72 (*dd*, J_1 = 2.8 Hz and J_2 = 8.6 Hz, 1H, CH-2'), 7.21 (*d*, *J* = 8.6 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 11.86 (C-18'), 26.16, 26.94, 27.21, 29.76, 30.06, 32.95, 33.88, 34.78 (CH_2Br), 36.76, 38.59, 43.65, 43.95, 44.10, 48.38, 55.17 (<u>C</u>H_3OAr), 88.06 (C-17'), 111.44 (C-2'), 113.80 (C-4'), 126.23 (C-1'), 132.59 (C-10'), 137.92 (C-5'), 157.43 (C-3'); MS m/z (rel. intensity): 422 (M^+, 100), 420 (M^+, 98), 335 (24), 333 (22), 227 (18), 186 (41).

(c) *Procedure for cleavage of methoxy group using BBr_3.*[36] To a solution of methoxy compound **16a** (141 mg, 0.34 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise 1.3 mL (1.3 mmol) of BBr_3 1.0 M in CH_2Cl_2 at 0°C. After 2 h at room temperature, the mixture was quenched by addition of water and acidified with 1 N HCl. The aqueous phase was extracted with CH_2Cl_2 and organic phase was dried with $MgSO_4$ and solvent was removed under reduced pressure. The crude compound was purified by flash chromatography (hexane:EtOAc, 8:2) to give 92 mg (68% yield) of 16α-(4'-bromobutyl)-E_2 (**17a**).

4-[3',17'β-Dihydroxy-1',3',5'(10')-estratrien-16'α-yl]-bromobutane (17a). White solid; IR ν (film): 3330 (OH, alcohol and phenol); ^{1}H NMR δ (acetone-d_6): 0.81 (*s*, 3H, CH_3-18'), 2.81 (*m*, 2H, CH_2-6'), 3.27 (*d*, *J* = 7.4 Hz, 1H, CH-17'α), 3.43 (*t*, *J* = 6.9 Hz, 2H, CH_2Br), 6.56 (*d*, *J* = 2.4 Hz, 1H, CH-4'), 6.62 (*dd*, J_1 = 2.7 Hz and J_2 = 8.5 Hz, 1H, CH-2'), 7.14 (*d*, *J* = 8.5 Hz, 1H, CH-1'); ^{13}C

NMR δ (acetone-d_6): 12.26 (C-18'), 26.89, 27.87 (2 x), 28.80, 30.54, 33.65, 34.57, 35.37 (CH_2Br), 37.55, 39.59, 43.69 (2 x), 44.68, 48.99, 87.97 (C-17'), 113.33 (C-2'), 115.70 (C-4'), 126.70 (C-1'), 131.84 (C-10'), 138.14 (C-5'), 155.66 (C-3'); MS m/z (rel. intensity): 408 (M^+, 100), 406 (M^+, 97), 326 (9), 213 (56), 172 (24); EIMS: calcd for $C_{22}H_{31}O_2{}^{79}Br$ (M^+) 406.15073, found 406.15136.

Synthesis of 16β-(bromobutyl)-estradiol (17b).
(a) *Procedure for alkylation using KH as base.* A mixture of activated 3-methoxy-estrone **6** (2.0 g, 5.8 mmol), potassium hydride (23 mmol) and 18-crown-6 (1.2 mmol) in dry THF (100 mL) was stirred under argon at refluxing temperature for 1 h. The resulting mixture was cooled at room temperature before addition of methyl 4-bromocrotonate (18 mmol). The reaction time was monitored by TLC. The reaction was quenched by addition of water and extraction was performed with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$ and evaporated to dryness. Purification by chromatography (hexane:EtOAc, 9:1) provided 2.34 g (91% yield) of diester **18**.

Methyl 4-[16'-(methoxycarbonyl)-3'-methoxy-17'-oxo-1',3',5'(10')-estratrien-16'-yl]but-2-enoate (18). Colorless oil; IR ν (film): 1764 (C=O, diester) and 1713 (C=O, ketone); ^{1}H NMR δ ($CDCl_3$): 0.98 (*s*, 3H, CH_3-18'), 2.88 (*m*, 2H, CH_2-6'), 3.71 and 3.73 (2*s*, 6H, CHCOOC$\underline{H}_3$ and COOC$\underline{H}_3$), 3.75 (*s*, 3H, C$\underline{H}_3$OAr), 5.87 (*d*, *J* = 15.5 Hz, 1H, CH=C$\underline{H}$COO), 6.63 (*d*, *J* = 2.6 Hz, 1H, CH-4'), 6.69 (*dd*, J_1 = 2.6 Hz and J_2 = 8.6 Hz, 1H, CH-2'), 6.81 (*m*, 1H, $CH_2\underline{C}H$=CH-COO), 7.17 (*d*, *J* = 8.6 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 13.93 (C-18'), 25.57, 26.41, 29.38, 30.33, 32.07, 37.16, 37.70, 43.93, 46.00, 49.44, 51.45 (COO$\underline{C}H_3$), 52.78 (COO$\underline{C}H_3$), 55.06 ($\underline{C}H_3$OAr), 59.33 (C-16'), 111.56 (C-2'), 113.78 (C-4'), 124.49 (C-3), 126.12 (C-1'), 131.52 (C-10'), 137.46 (C-5'), 143.46 (C-2), 157.60 (C-3'), 166.12 (C-1), 170.94 ($\underline{C}OOCH_3$), 212.78 (C-17'); MS m/z (rel. intensity): 440 (M^+, 82), 380 (27), 365 (13), 341 (22), 322 (17), 309 (100), 227 (89), 186 (45), 160 (51).

(b) *Reduction of double bond and decarboalkoxylation.* A suspension of diester **18** (2.0 g, 4.5 mmol), MeOH (200 mL) and 175 mg of 10% Pd/C was stirred under hydrogen atmosphere at room temperature. After 21 h, the resulting suspension was filtered through Celite and MeOH was evaporated to dryness. ^{1}H NMR analysis on the crude diester revealed the disappearance of previously described vinylic protons at 5.87 and 6.81 ppm. Decarboalkoxylation was performed according to the procedure described by Krapcho *et al.*[38] To a saturated solution of the crude diester in DMF (200 mL) was added LiCl (85.5 mmol) and H_2O (85.5 mmol). The resulting mixture was heated at refluxing temperature. The rate of the reaction was monitored by TLC. In order to quench the reaction, EtOAc was added in cooled mixture and the organic layer was washed with 1 N HCl, brine and dried over $MgSO_4$. Solvent was removed under reduced pressure before purification by flash chromatography (hexane:EtOAc, 7:3) to provide 85 mg of starting material (4%) and 840 mg (48% yield for 2 steps) of two unresolved 16α/16β diastereoisomers of ketoester **19**.

Methyl 4-[3'-methoxy-17'-oxo-1',3',5'(10')-estratrien-16'α/β-yl]-butanoate (19). Amorphous white solid; IR ν (film): 1730 (C=O, ester and ketone); ^{1}H NMR δ ($CDCl_3$): 0.85 and 0.94 (2*s*, 3H, CH_3-18', 16β:16α, 62:38), 2.89 (*m*, 2H, CH_2-6'), 3.67 (*s*, 3H, COOC$\underline{H}_3$), 3.77 (*s*, 3H, C$\underline{H}_3$OAr), 6.64 (*d*, *J* = 2.5 Hz, 1H, CH-4'), 6.71 (*dd*, J_1 = 2.6 Hz and J_2 = 8.6 Hz, 1H, CH-2'), 7.19 (*d*, *J* = 8.6 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 13.98 and 14.55 (C-18' of 16β-isomer and 16α-isomer, respectively), 23.46, 25.81 (26.44), 26.68 (27.29), 28.47, 29.60 (30.46), 31.65 (31.87), 33.79 (33.88), 37.92 (38.28), 43.94 (44.04), 44.57, 48.25 (48.34), 48.56, 48.91 (49.00), 51.45 (COO$\underline{C}H_3$), 55.14 ($\underline{C}H_3$OAr), 111.52 (C-2'), 113.84 (C-4'), 126.20 (C-1'), 131.01 (C-10'), 137.67 (C-5'), 157.56 (C-3'), 173.74 (C-1), 221.51 and 221.99 (C-17'); MS m/z (rel. intensity): 384 (M^+, 100), 353 (19), 337 (29), 284 (23), 227 (29), 186 (35), 160 (31).

(c) *Reduction of 17-ketone and ester.* The ketoester **19** (617 mg, 1.61 mmol) was reduced under the same procedure as that used for the synthesis of **7**. IR and ^{1}H NMR analysis on the crude product confirmed the reduction of ketone-17 and aliphatic ester (appearance of OH alcohol band at 3380 cm^{-1} and disappearance of C=O band at 1730 cm^{-1}; appearance of C$\underline{H}$-OH at 3.28 ppm [16α-diastereoisomer], 3.75 ppm [16β-diastereoisomer] and $C\underline{H}_2OH$ at 3.66 ppm and disappearance of COOC$\underline{H}_3$ at 3.67 ppm). The crude diol was used without purification for the bromination of the primary alcohol following the standard procedure to provide, after a purification by flash chromatography (hexane:EtOAc, 9:1), a small amount of 16α-diastereoisomer (not recovered) and 380 mg (56% yield for 2 steps) of the 16β diastereoisomer **16b**.

4-[3'-Methoxy-17'β-hydroxy-1',3',5'(10')-estratrien-16'β-yl]-bromobutane (16b). Colorless oil; IR ν (film): 3460 (OH, alcohol); ^{1}H NMR δ ($CDCl_3$): 0.79 (*s*, 3H, CH_3-18'), 2.87 (*m*, 2H, CH_2-6'), 3.45 (*t*, *J* = 6.8 Hz, 2H, CH_2Br), 3.75 (*d*, *J* = 10.0 Hz, 1H, CH-17'α of 16β-diastereoisomer), 3.79 (*s*, 3H, C$\underline{H}_3$OAr), 6.65 (*d*, *J* = 2.7 Hz, 1H, CH-4'), 6.72 (*dd*, J_1 = 2.7 Hz and J_2 = 8.6 Hz, 1H, CH-2'), 7.21 (*d*, *J* = 8.6 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 12.33 (C-18'), 26.20, 27.18, 27.39, 29.73, 30.60, 32.28, 32.88, 33.95 (CH_2Br), 37.61, 38.24, 39.86, 44.06, 44.90, 48.51, 55.10 ($C\underline{H}_3OAr$), 82.21 (C-17'), 111.38 (C-2'), 113.72 (C-4'), 126.19 (C-1'), 132.59 (C-10'), 137.81 (C-5'), 157.35 (C-3'); MS m/z (rel. intensity): 422 (M^+, 97), 420 (M^+, 100), 340 (27), 335 (19), 333 (17), 285 (4), 227 (32), 186 (30), 160 (29).
(d) According to procedure described for the synthesis of **17a**, the methoxy compound **16b** (170 mg) was demethylated by BBr_3 to yield, after purification by flash chromatography (hexane:EtOAc, 75:25), 91 mg (55% yield) of 16β-(4'-bromobutyl)-E_2 (**17b**).

4-[3',17'β-Dihydroxy-1',3',5' (10')-estratrien-16'β-yl]-bromobutane (**17b**). White solid; IR ν (film): 3360

(OH, alcohol and phenol); ^{1}H NMR δ ($CDCl_3$): 0.77 (*s*, 3H, CH_3-18'), 2.81 (*m*, 2H, CH_2-6'), 3.43 (*t*, *J* = 6.9 Hz, 2H, CH_2Br), 3.75 (*d*, *J* = 9.9 Hz, 1H, CH-17'α), 6.56 (*d*, *J* = 2.4 Hz, 1H, CH-4'), 6.62 (*dd*, J_1 = 2.7 Hz and J_2 = 8.5 Hz, 1H, CH-2'), 7.15 (*d*, *J* = 8.5 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 12.40 (C-18'), 26.29, 27.40, 27.65, 29.62, 30.68, 32.37, 32.94, 33.98 (CH_2Br), 37.68, 38.31, 39.92, 43.99, 44.17, 48.61, 82.36 (C-17'), 112.68 (C-2'), 115.23 (C-4'), 126.48 (C-1'), 132.72 (C-10'), 138.23 (C-5'), 153.38 (C-3'); MS m/z (rel. intensity): 408 (M^+, 100), 406 (M^+, 97), 326 (31), 213 (81), 172 (38), 160 (45); EIMS: calcd for $C_{22}H_{31}O_2{}^{79}Br$ (M^+) 406.15073, found 406.15190.

Synthesis of 16β-(bromopentyl)-estradiol 24 (Scheme 4)

(a) The activated 3-methoxy-estrone **6** (106 mg, 0.30 mmol) was submitted to alkylation with potassium hydride (1.5 mmol), 18-crown-6 (0.06 mmol) using 5-bromopentene as electrophile following the same procedure as that described for **18**. Purification by chromatography (hexane:EtOAc, 95:5) provided 14 mg of starting material (13%) and 53 mg (42% yield) of olefin **20**.

5-[17'-Oxo-16'β-(methoxycarbonyl)-3'-methoxy-1',3',5'-(10')-estratrien-16'α/β-yl]-pentene (20). Colorless oil; IR ν (film): 1750 (C=O, ester) and 1723 (C=O, ketone); ^{1}H NMR δ ($CDCl_3$): 0.94 (*s*, 3H, CH_3-18'), 2.91 (*m*, 2H, CH_2-6'), 3.73 (*s*, 3H, $COO\underline{CH_3}$), 3.78 (*s*, 3H, $\underline{CH_3}OAr$), 4.99 (*m*, 2H, $CH=\underline{CH_2}$), 5.78 (*m*, 1H, $\underline{CH}=CH_2$), 6.65 (*d*, *J* = 2.3 Hz, 1H, CH-4'), 6.72 (*dd*, J_1 = 2.6 Hz and J_2 = 8.6 Hz, 1H, CH-2'), 7.21 (*d*, *J* = 8.6 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 14.00 (C-18'), 24.66, 25.68, 26.46, 29.51, 30.58, 32.02, 33.68, 37.90, 43.94, 45.94 (2 x), 49.43, 52.52 ($COO\underline{C}H_3$), 55.12 ($\underline{C}H_3OAr$), 59.98 (C-16'), 111.55 (C-2'), 113.81 (C-4'), 114.97 (C-1), 126.18 (C-1'), 131.77 (C-10'), 137.58 (C-5'), 137.89 (C-2), 157.59 (C-3'), 171.76 ($\underline{C}OOCH_3$), 213.85 (C-17'); MS m/z (rel. intensity): 410 (M^+, 100), 310 (89), 227 (70), 173 (31).

(b) The olefin **20** (356 mg, 0.87 mmol) was decarboalkoxylated following the Krapcho's procedure as reported for the synthesis of compound **19**. Purification of the crude product by chromatography (hexane) gave 216 mg (71% yield) of the ketone **21**. The 16α:16β ratio was evaluated by ^{1}H NMR based on the shift of the CH_3-18'.

5-[3'-Methoxy-17'-oxo-1',3',5' (10')-estratrien-16'α/β-yl]-pentene (21). White solid; IR ν (film): 1734 (C=O, ketone); ^{1}H NMR δ ($CDCl_3$): 0.87 and 0.95 (2*s*, 3H, CH_3-18', 16β:16α, 85:15), 2.91 (*m*, 2H, CH_2-6'), 3.78 (*s*, 3H, $\underline{CH_3}OAr$), 4.99 (*m*, 2H, $CH=\underline{CH_2}$), 5.81 (*m*, 1H, $\underline{CH}=CH_2$), 6.65 (*d*, *J* = 2.3 Hz, 1H, CH-4'), 6.72 (*dd*, J_1 = 2.6 Hz and J_2 = 8.6 Hz, 1H, CH-2'), 7.21 (*d*, *J* = 8.6 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 13.94 and 14.57 (C-18' of 16β-isomer and 16α-isomer, respectively), 25.83 (2 x), 26.70, 27.40, 28.56, 29.62, 31.92, 33.57, 37.93, 44.05, 44.80, 48.35, 48.93 (49.23), 55.13 ($\underline{C}H_3OAr$), 111.50 (C-2'), 113.84 (C-4'), 114.62 (C-1), 126.21 (C-1'), 132.06 (C-10'), 137.67 (C-5'), 138.41 (C-2), 157.55 (C-3'), 222.42 (C-17'); MS m/z (rel. intensity): 352 (M^+, 100), 337 (5), 297 (11), 284 (35), 174 (17), 147 (11).

(c) *Reduction of 17-ketone.* The ketone of **21** (216 mg, 0.60 mmol) was reduced under the same conditions used for **4**, **5** and **19**. The crude alcohol (228 mg) was used without purification for the next step. *Oxidative hydroboration*: to a solution of the latter alcohol dissolved in dry THF (10 mL) was added borane–THF complex 1.0 M (1.6 mmol) at 0 °C under an atmosphere of argon. The mixture was stirred at 0 °C for 3 h. Then, 3 N NaOH (1.6 mmol) and 0.22 mL of hydrogen peroxide 30% (w/v) were added. After 2 h at room temperature, the reaction was quenched with the addition of water and extraction was performed with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$ and evaporated to dryness. Purification by chromatography (hexane:EtOAc, 8:2) gave 169 mg (71% yield for 2 steps) of two unresolved diastereoisomers **22**.

5-[3'-Methoxy-17'β-hydroxy-1',3',5'(10')-estratrien-16'α/β-yl]-pentanol (22). Amorphous white solid; IR ν (film): 3460 (OH, alcohol); ^{1}H NMR δ ($CDCl_3$): 0.78 and 0.82 (2*s*, 3H, CH_3-18', 16β:16α, 85:15), 2.85 (*m*, 2H, CH_2-6'), 3.65 (*t*, *J* = 6.5 Hz, 2H, $\underline{CH_2}OH$), 3.27 and 3.74 (2*d*, *J* = 7.4 Hz and *J* = 9.8 Hz respectively, 1H, CH-17'α, 16α:16β, 15:85), 3.78 (*s*, 3H, $\underline{CH_3}OAr$), 6.65 (*d*, *J* = 2.3 Hz, 1H, CH-4'), 6.72 (*dd*, J_1 = 2.6 Hz and J_2 = 8.6 Hz, 1H, CH-2'), 7.21 (*d*, *J* = 8.6 Hz, 1H, CH-1'). In this case, only specific ^{13}C NMR signals are shown due to the presence of two diastereoisomers that make accurate attribution of some carbons difficult. ^{13}C NMR δ ($CDCl_3$): 11.88 and 12.36 (C-18' of 16α-diastereoisomer and 16β-diastereoisomer, respectively), 55.16 ($\underline{C}H_3OAr$), 62.95 (C-1), 82.42 and 88.17 (C-17' of 16β-diastereoisomer and 16α-diastereoisomer, respectively), 111.44 (C-2'), 113.81 (C-4'), 126.24 (C-1'), 132.71 (C-10'), 137.91 (C-5'), 157.42 (C-3'); MS m/z (rel. intensity): 372 (M^+, 100), 285 (11), 227 (31), 174 (21).

(d) The primary alcohol of **22** (168 mg, 0.45 mmol) was brominated using PPh_3 (0.90 mmol) and CBr_4 (0.90 mmol) in dry CH_2Cl_2 (10 mL) as described above to provide 151 mg (77% yield) of major 16β diastereoisomer **23**. Minor 16α-diastereoisomer was not recovered.

5-[3'-Methoxy-17'β-hydroxy-1',3',5' (10')-estratrien-16'β-yl]-bromopentane (23). Colorless oil; IR ν (film): 3460 (OH, alcohol); ^{1}H NMR δ ($CDCl_3$): 0.77 (*s*, 3H, CH_3-18'), 2.85 (*m*, 2H, CH_2-6'), 3.42 (*t*, *J* = 6.7 Hz, 2H, CH_2Br), 3,74 (*d*, *J* = 9.9 Hz, 1H, CH-17'α), 3.78 (*s*, 3H, $\underline{CH_3}OAr$), 6.65 (*d*, *J* = 2.3 Hz, 1H, CH-4'), 6.72 (*dd*, J_1 = 2.6 Hz and J_2 = 8.6 Hz, 1H, CH-2'), 7.21 (*d*, *J* = 8.6 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 12.37 (C-18'), 26.27, 27.45, 27.79, 28.35, 29.80, 31.29, 32.40, 32.79, 33.90 (CH_2Br), 37.70, 38.33, 39.92, 43.99, 44.14, 48.57, 55.17 ($\underline{C}H_3OAr$), 82.39 (C-17'), 111.45 (C-2'), 113.80 (C-4'),

126.27 (C-1'), 132.68 (C-10'), 137.90 (C-5'), 157.43 (C-3'); MS m/z (rel. intensity): 436 (M^+, 100), 434 (M^+, 94), 349 (17), 347 (15), 285 (5), 227 (40), 186 (32).

(e) According to the procedure described for the synthesis of **17a** as well as **17b**, the methoxy compound **23** was demethylated by BBr_3 to yield, after purification by flash chromatography (hexane:EtOAc, 8:2), 73 mg (50% yield) of 16β-(5'-bromopentyl)-E_2 (**24**).

5-[3',17'β-Dihydroxy-1',3',5'(10')-estratrien-16'β-yl]-bromopentane (24). White amorphous solid; IR ν (film): 3470 (OH, alcohol and phenol); ^{1}H NMR δ ($CDCl_3$): 0.77 (*s*, 3H, CH_3-18'), 2.81 (*m*, 2H, CH_2-6'), 3.41 (*t*, *J* = 6.9 Hz, 2H, CH_2Br), 3.76 (*d*, *J* = 9.9 Hz, 1H, CH-17'α), 6.56 (*d*, *J* = 2.7 Hz, 1H, CH-4'), 6.62 (*dd*, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, CH-2'), 7.15 (*d*, *J* = 8.4 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 12.39 (C-18'), 26.28, 27.39, 27.78, 28.34, 29.68, 31.28, 32.39, 33.78, 33.92 (CH_2Br), 37.66, 38.32, 39.89, 43.97, 44.13, 48.56, 82.48 (C-17'), 112.69 (C-2'), 115.24 (C-4'), 126.47 (C-1'), 132.67 (C-10'), 138.24 (C-5'), 153.40 (C-3'); MS m/z (rel. intensity): 422 (M^+, 92), 420 (M^+, 93), 335 (13), 333 (12), 228 (12), 213 (100); EIMS: calcd for $C_{23}H_{33}O_2{}^{79}Br$ (M^+) 420.16638, found 420.16242.

Synthesis of 16-(bromohexyl)-estradiols 33a, 33b and 16-(bromoheptyl)-estradiols 34a, 34b (Scheme 5)

(a) *Preparation of appropriate bromo and iodo side chains $X(CH_2)_nOTHP$ (n = 6,7).* Tetrahydropyranyl (THP) derivatives of 6-bromohexanol and 7-bromoheptanol were obtained according to a known procedure described by Poirier *et al.*[28] and Bucourt *et al.*[39] The products obtained were in agreement with IR, NMR and MS analysis. Before their use, these bromo side chains were filtered through neutral alumina (Merck type I, 70–230 mesh) with diethyl ether as solvent and dried over molecular sieves (4 Å). A subsequent step was necessary to obtain the 6-iodo-1-[(tetrahydro-2'*H*-pyran-2'-yl)oxy]-hexane. To a solution of the corresponding THP derivative of bromohexanol (6.43 g, 24.3 mmol) in acetone (100 mL) was added NaI (121 mmol) and the resulting suspension was heated at refluxing temperature overnight. The reaction was quenched by addition of water and acetone was evaporated under reduced pressure. Extraction with diethyl ether was performed and the organic layer was dried over $MgSO_4$. The resulting slurry was filtered through neutral alumina (Merck type I, 70–230 mesh) with hexane as solvent and dried over molecular sieves (4 Å). ^{1}H NMR analysis revealed the shift of CH_2X-signal from 3.35 ppm (X = Br) to 3.19 ppm (X = I).

(b) *General procedure for alkylation at 16-position with THP bromo or iodo side chains.* The THP derivatives of 6-bromohexanol and 7-bromoheptanol were introduced at the 16-position on 3-methoxy-16β-methoxycarbonyl-estrone (**6**) (2.2–5.1 mmol) following the procedure described earlier for **18**. Purification of the crude products by flash chromatography (hexane:EtOAc, 95:5 to 9:1) gave the expected alkylated-products **25** and **26**.

6-[17'-Oxo-16'α/β-(methoxycarbonyl)-3'-methoxy-1',3',5'-(10')-estratrien-16'β/α-yl]-1-[(tetrahydro-2'H-pyran-2'-yl)oxy]-hexane (25). White amorphous solid (50% corrected yield); IR ν (film): 1738 (C=O, ketone and ester); ^{1}H NMR δ ($CDCl_3$): 0.91 and 0.96 (2*s*, 3H, CH_3-18', 94:6), 2.89 (*m*, 2H, CH_2-6'), 3.36, 3.48, 3.71 and 3.83 (4m, 4H, 2 x OCH_2 of side chain and THP group), 3.71 (*s*, 3H, $COOCH_3$), 3.76 (*s*, 3H, $\underline{CH_3}OAr$), 4.55 (*t*, *J* ≈ 2 Hz, 1H, CH of THP group), 6.63 (*d*, *J* = 2.2 Hz, 1H, CH-4'), 6.69 (*dd*, J_1 = 2.6 Hz and J_2 = 8.6 Hz, 1H, CH-2'), 7.17 (*d*, *J* = 8.6 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 13.29 and 14.04 (C-18'), 19.70 (CH_2 of THP), 25.39, 25.50, 25.80, 25.99 (26.11), 26.40, 26.54, 29.58, 29.66, 30.55, 30.78, 31.95, 32.10 (35.52), 37.97, 44.01, 45.97, 49.50, 52.54 (54.06) ($COO\underline{C}H_3$), 55.19 ($\underline{C}H_3OAr$), 58.49 (60.16) (C-16'), 62.33 (OCH_2 of THP), 67.53 (CH_2O of THP), 98.87 (CH of THP), 111.63 (C-2'), 113.90 (C-4'), 126.28 (C-1'), 131.87 (C-10'), 137.66 (C-5'), 157.67 (C-3'), 169.86 ($\underline{C}OOCH_3$), 211.97 and 213.99 (C-17'); MS m/z (rel. intensity): 386 (M^+ – 140, 57), 371 (100), 293 (27), 84 (13).

7-[17'-Oxo-16'α/β-(methoxycarbonyl)-3'-methoxy-1',3',5'-(10')-estratrien-16'β/α-yl]-1-[(tetrahydro-2'H-pyran-2'-yl)oxy]-heptane (26). Physical and spectroscopic data have been reported elsewhere.[27]

(c) *General procedure for decarboalkoxylation.* The methoxycarbonyl group of the ketoesters **25** or **26** (1.11–1.29 mmol) was removed under the decarboalkoxylation conditions described by Krapcho[38] to provide, after purification by flash chromatography (hexane:EtOAc, 9:1), the expected ketones **27** and **28**.

6-[17'-Oxo-3'-methoxy-1',3',5'(10')-estratrien-16'α/β-yl]-1-[(tetrahydro-2'H-pyran-2'-yl)oxy]-hexane (27). Colorless oil (41% corrected yield); IR ν (film): 1740 (C=O, ketone); ^{1}H NMR δ ($CDCl_3$): 0.84 and 0.92 (2*s*, 3H, CH_3-18', 16β:16α, 84:16), 2.88 (*m*, 2H, CH_2-6'), 3.37, 3.50, 3.72 and 3.86 (4*m*, 4H, 2 x OCH_2 of side chain and THP group), 3.76 (*s*, 3H, $\underline{CH_3}OAr$), 4.56 (*t*, *J* ≈ 2 Hz, 1H, CH of THP group), 6.63 (*d*, *J* = 2.7 Hz, 1H, CH-4'), 6.70 (*dd*, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, CH-2'), 7.18 (*d*, *J* = 8.6 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 13.84 (C-18'), 19.60 (CH_2 of THP), 25.41, 25.45, 25.77, 25.98, 26.62, 27.93, 28.47, 29.19, 29.57, 30.68, 31.85, 32.30, 37.88, 43.98, 48.26, 48.87, 49.25, 55.03 ($\underline{C}H_3OAr$), 62.19 (OCH_2 of THP), 67.43 (67.79) (CH_2O of THP), 98.74 (CH of THP), 111.38 (C-2'), 113.73 (C-4'), 126.11 (C-1'), 132.04 (C-10'), 137.64 (C-5'), 157.47 (C-3'), 222.50 (C-17'); MS m/z (rel. intensity): 341 ((M^+ – 127, 25), 281 (23), 267 (46), 207 (100), 193 (36), 83 (90).

7-[17'-Oxo-3'-methoxy-1',3',5'(10')-estratrien-16'α/β-yl]-1-[(tetrahydro-2'H-pyran-2'-yl)oxy]-heptane (28). Physical and spectroscopic data have been reported elsewhere.[27]

(d) *General procedure for the reduction of 17-ketone.* The ketone of **27** or **28** was reduced using $LiAlH_4$ following the procedure described above. Purifications

were performed by flash chromatography (hexane: EtOAc, 95:5 or 8:2) and allowed us to resolve the diastereoisomeric mixture of respective alcohols **29a**, **29b** as well as **30a**, **30b**.

6-[3'-Methoxy-17'β-hydroxy-1',3',5'(10')-estratrien-16'α-yl]-1-[(tetrahydro-2'H-pyran-2'-yl)oxy]-hexane (29a). 16α-Diastereoisomer: white amorphous solid (13% yield); IR ν (film): 3455 (OH, alcohol); ^{1}H NMR δ ($CDCl_3$): 0.80 (*s*, 3H, CH_3-18'), 2.84 (*m*, 2H, CH_2-6'), 3.28 (*d*, 1H, CH-17'α), 3.37, 3.52, 3.71 and 3.86 (4*m*, 4H, 2 x OCH_2 of side chain and THP group), 3.77 (*s*, 3H, $\underline{C}H_3OAr$), 4.58 (*t*, $J \approx 2$ Hz, 1H, CH of THP group), 6.63 (*d*, J = 2.6 Hz, 1H, CH-4'), 6.71 (*dd*, J_1 = 2.7 Hz and J_2 = 8.6 Hz, 1H, CH-2'), 7.21 (*d*, J = 8.6 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 11.85 (C-18'), 19.64 (CH_2 of THP), 25.45, 26.19 (2 x), 27.19, 28.30, 29.69 (2 x), 29.76, 30.07, 30.73, 35.66, 36.77, 38.59, 43.87, 43.93, 44.05, 48.33, 55.13 ($\underline{C}H_3OAr$), 62.29 (OCH_2 of THP), 67.62 (CH_2O of THP), 88.09 (C-17'), 98.80 (CH of THP), 111.39 (C-2'), 113.76 (C-4'), 126.21 (C-1'), 132.65 (C-10'), 137.92 (C-5'), 157.37 (C-3'); MS m/z (rel. intensity): 386 (M^+ – DHP, 100), 299 (12), 227 (29), 186 (28).

6-[3'-Methoxy-17'β-hydroxy-1',3',5'(10')-estratrien-16'β-yl]-1-[(tetrahydro-2'H-pyran-2'-yl)oxy]-hexane (29b). 16β-Diastereoisomer: white amorphous solid (64% yield); IR ν (film): 3455 (OH, alcohol); ^{1}H NMR δ ($CDCl_3$): 0.76 (*s*, 3H, CH_3-18'), 2.85 (*m*, 2H, CH_2-6'), 3.39, 3.50, 3.72 and 3.87 (4*m*, 5H, OCH_2 of side chain, OCH_2 of THP group and CH-17'α), 3.76 (*s*, 3H, $\underline{C}H_3OAr$), 4.57 (*t*, $J \approx 2$ Hz, 1H, CH of THP group), 6.63 (*d*, J = 2.6 Hz, 1H, CH-4'), 6.71 (*dd*, J_1 = 2.7 Hz and J_2 = 8.6 Hz, 1H, CH-2'), 7.21 (*d*, J = 8.6 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 12.36 (C-18'), 19.68 (CH_2 of THP), 25.49, 26.24, 26.29, 27.46, 28.55, 29.70 (2 x), 29.82, 30.79, 31.39, 32.41, 37.73, 38.36, 40.01, 44.00, 44.12, 48.58, 55.17 ($\underline{C}H_3OAr$), 62.33 (OCH_2 of THP), 67.64 (CH_2O of THP), 82.46 (C-17'), 98.84 (CH of THP), 111.43 (C-2'), 113.80 (C-4'), 126.26 (C-1'), 132.74 (C-10'), 137.93 (C-5'), 157.43 (C-3'); MS m/z (rel. intensity): 386 (M^+ – DHP, 9), 227 (3), 85 (100).

7-[3'-Methoxy-17'β-hydroxy-1',3',5'(10')-estratrien-16'α-yl]-1-[(tetrahydro-2'H-pyran-2'-yl)oxy]-heptane (30a). Physical and spectroscopic data have been reported elsewhere.[27]

7-[3'-Methoxy-17'β-hydroxy-1',3',5'(10')-estratrien-16β-yl]-1-[(tetrahydro-2'H-pyran-2'-yl)oxy]-heptane (30b). Physical and spectroscopic data have been reported elsewhere.[27]

(e) *General procedure for cleavage of THP group and bromination of alcohol.* The tetrahydropyranyl derivatives **29a**, **29b**, **30a** and **30b** were dissolved in MeOH with a catalytic amount of *p*-toluenesulfonic acid and the resulting solution was stirred at room temperature. When the reaction was completed (0.5–3 h), water was added, MeOH was evaporated under reduced pressure and the residue was extracted with EtOAc. The crude diols (no THP group in agreement with ^{1}H NMR analysis) were submitted to bromination without purification according to the procedure described above. Purifications by flash chromatography (hexane:EtOAc, 95:5 or 9:1) gave the respective bromides **31a**, **31b**, **32a** and **32b**.

6-[3'-Methoxy-17'β-hydroxy-1',3',5'(10')-estratrien-16'α-yl]-bromohexane (31a). 16α-Diastereoisomer: white amorphous solid (62% yield for two steps); IR ν (film): 3398 (OH, alcohol); ^{1}H NMR δ ($CDCl_3$): 0.81 (*s*, 3H, CH_3-18'), 2.85 (*m*, 2H, CH_2-6'), 3.27 (*d*, J = 7.4 Hz, 1H, CH-17'α), 3.41 (*t*, J = 6.8 Hz, 2H, CH_2Br), 3.78 (*s*, 3H, $\underline{C}H_3OAr$), 6.64 (*d*, J = 2.7 Hz, 1H, CH-4'), 6.71 (*dd*, J_1 = 2.8 Hz and J_2 = 8.6 Hz, 1H, CH-2'), 7.21 (*d*, J = 8.6 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 11.85 (C-18'), 26.17, 27.21, 28.17, 28.98 (2 x), 29.76, 30.09, 32.76, 33.99 (CH_2Br), 35.60, 36.77, 38.58, 43.81, 43.94, 44.05, 48.34, 55.16 ($\underline{C}H_3OAr$), 88.11 (C-17'), 111.39 (C-2'), 113.78 (C-4'), 126.23 (C-1'), 132.62 (C-10'), 137.92 (C-5'), 157.38 (C-3'); MS m/z (rel. intensity): 450 (M^+, 98), 448 (M^+, 100), 363 (16), 361 (15), 227 (37), 186 (33), 160 (31).

6-[3'-Methoxy-17'β-hydroxy-1',3',5'(10')-estratrien-16'β-yl]-bromohexane (31b). 16β-Diastereoisomer: white amorphous solid (72% yield for two steps); IR ν (film): 3460 (OH, alcohol); ^{1}H NMR δ ($CDCl_3$): 0.77 (*s*, 3H, CH_3-18'), 2.85 (*m*, 2H, CH_2-6'), 3.41 (*t*, J = 6.8 Hz, 2H, CH_2Br), 3.74 (*d*, J = 9.9 Hz, 1H, CH-17'α), 3,78 (*s*, 3H, $\underline{C}H_3OAr$), 6.63 (*d*, J = 2.3 Hz, 1H, CH-4'), 6.72 (*dd*, J_1 = 2.6 Hz and J_2 = 8.5 Hz, 1H, CH-2'), 7.21 (*d*, J = 8.6 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 12.38 (C-18'), 26.29, 27.47, 28.19, 28.46, 28.99, 29.82, 31.37, 32.42, 32.81, 33.96 (CH_2Br), 37.72, 38.35, 39.99, 44.01, 44.15, 48.59, 55.18 ($\underline{C}H_3OAr$), 82.44 (C-17'), 111.46 (C-2'), 113.80 (C-4'), 126.29 (C-1'), 132.72 (C-10'), 137.94 (C-5'), 157.44 (C-3'); MS m/z (rel. intensity): 450 (M^+, 97), 448 (M^+, 100), 363 (21), 361 (20), 227 (39), 186 (58), 160 (44).

7-[3'-Methoxy-17'β-hydroxy-1',3',5'(10')-estratrien-16'α-yl]-bromoheptane (32a). 16α-Diastereoisomer: white amorphous solid (81% yield for two steps); IR ν (film): 3420 (OH, alcohol); ^{1}H NMR δ ($CDCl_3$): 0.80 (*s*, 3H, CH_3-18'), 2.84 (*m*, 2H, CH_2-6'), 3.26 (*d*, J = 7.3 Hz, 1H, CH-17'α), 3.42 (*t*, J = 6.8 Hz, 2H, CH_2Br), 3.78 (*s*, 3H, $\underline{C}H_3OAr$), 6.63 (*d*, J = 2.4 Hz, 1H, CH-4'), 6.71 (*dd*, J_1 = 2.7 Hz and J_2 = 8.6 Hz, 1H, CH-2'), 7.21 (*d*, J = 8.6 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 11.88 (C-18'), 26.22, 27.26, 28.14, 28.29, 28.74, 29.67, 29.81, 30.15, 32.81, 34.01 (CH_2Br), 35.72, 38.63, 38.82, 43.10, 43.98 (2 x), 48.39, 55.19 ($\underline{C}H_3OAr$), 88.21 (C-17'), 111.44 (C-2'), 113.82 (C-4'), 126.27 (C-1'), 132.68 (C-10'), 137.98 (C-5'), 157.45 (C-3'); MS m/z (rel. intensity): 464 (M^+, 95), 462 (M^+, 100), 447 (3.6), 445 (4.0), 378 (15), 376 (14), 227 (45), 186 (60), 173 (57), 160 (59).

7-[3'-Methoxy-17'β-hydroxy-1',3',5'(10')-estratrien-16'β-yl]-bromoheptane (32b). 16β-Diastereoisomer: white amorphous solid (92% yield for two steps); IR ν (film):

3465 (OH, alcohol); ^{1}H NMR δ ($CDCl_3$): 0.78 (*s*, 3H, CH_3-18'), 2.86 (*m*, 2H, CH_2-6'), 3.42 (*t*, *J* = 6.8 Hz, 2H, CH_2Br), 3.74 (*d*, *J* = 9.8 Hz, 1H, CH-17'α), 3.78 (*s*, 3H, $\underline{C}H_3OAr$), 6.64 (*d*, *J* = 2.3 Hz, 1H, CH-4'), 6.72 (*dd*, J_1 = 2.6 Hz and J_2 = 8.6 Hz, 1H, CH-2'), 7.22 (*d*, *J* = 8.6 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 12.36 (C-18'), 26.26, 27.33, 28.11, 28.50, 28.74, 29.61, 29.79, 31.38, 32.39, 32.78, 33.98 (CH_2Br), 37.68, 38.32, 39.97, 43.96, 44.10, 48.54, 55.15 ($\underline{C}H_3OAr$), 82.42 (C-17'), 111.41 (C-2'), 113.76 (C-4'), 126.25 (C-1'), 132.68 (C-10'), 137.89 (C-5'), 157.39 (C-3'); MS m/z (rel. intensity): 464 (M^+, 99), 462 (M^+, 100), 446 (11), 444 (12), 377 (10), 375 (8.8), 267 (13), 227 (55), 186 (43), 173 (60), 160 (55), 147 (47).

(f) *General procedure for cleavage of methoxy group.* Compounds **31a**, **31b**, **32a** and **32b** were demethylated by BBr_3 according to the procedure described above (see synthesis of **17a**). However, 4 equivalents of BBr_3 were used. A purification by flash chromatography (hexane:EtOAc, 9:1 or 8:2) yielded the respective phenols.

6-[3',17'β-Dihydroxy-1',3',5'(10')-estratrien-16'α-yl]-bromohexane (33a). Amorphous white solid (59% yield); IR ν (film): 3300 (OH, alcohol and phenol); ^{1}H NMR δ ($CDCl_3$): 0.80 (*s*, 3H, CH_3-18'), 2.81 (*m*, 2H, CH_2-6'), 3.27 (*d*, *J* = 7.0 Hz, 1H, CH-17'α), 3.41 (*t*, *J* = 6.9 Hz, 2H, CH_2Br), 6.56 (*d*, *J* = 2.6 Hz, 1H, CH-4'), 6.62 (*dd*, J_1 = 2.8 Hz and J_2 = 8.6 Hz, 1H, CH-2'), 7.15 (*d*, *J* = 8.3 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 11.88 (C-18'), 26.19, 27.16, 28.17 (2 x), 28.98, 29.58, 30.10, 32.77, 33.99, 35.58, 36.75, 38.57, 43.84, 43.93, 44.05, 48.34, 88.23, 112.65 (C-2'), 115.22 (C-4'), 126.42 (C-1'), 132.58 (C-10'), 138.20 (C-5'), 153.41 (C-3'); MS m/z (rel. intensity): 436 (M^+, 100), 434 (M^+, 97), 332 (26), 326 (35), 213 (71), 163 (80); EIMS: calcd for $C_{24}H_{35}O_2{}^{79}Br$ (M^+) 434.18204, found 434.18208.

6-[3',17'β-Dihydroxy-1',3',5'(10')-estratrien-16'β-yl]-bromohexane (33b). Amorphous white solid (58% yield); IR ν (film): 3380 (OH, alcohol and phenol); ^{1}H NMR δ ($CDCl_3$): 0.77 (*s*, 3H, CH_3-18'), 2.82 (*m*, 2H, CH_2-6'), 3.41 (*t*, *J* = 6.8 Hz, 2H, CH_2Br), 3.74 (*d*, *J* = 10.0 Hz, 1H, CH-17'α), 6.56 (*d*, *J* = 2.7 Hz, 1H, CH-4'), 6.62 (*dd*, J_1 = 2.6 Hz and J_2 = 8.4 Hz, 1H, CH-2'), 7.15 (*d*, *J* = 8.4 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 12.39 (C-18'), 26.30, 27.40, 28.19, 28.46, 28.99, 29.63, 31.38, 32.43, 32.83, 33.96 (CH_2Br), 37.71, 38.34, 39.99, 43.99, 44.15, 48.58, 82.49 (C-17'), 112.65 (C-2'), 115.22 (C-4'), 126.49 (C-1'), 132.81 (C-10'), 138.26 (C-5'), 153.32 (C-3'); MS m/z (rel. intensity): 436 (M^+, 55), 434 (M^+, 57), 418 (16), 416 (16), 354 (47), 213 (100); EIMS: calcd for $C_{24}H_{35}O_2{}^{79}Br$ (M^+) 434.18204, found 434.18259.

7-[3',17'β-Dihydroxy-1',3',5'(10')-estratrien-16'α-yl]-bromoheptane (34a). Amorphous white solid (48% corrected yield); IR ν (film): 3400 (OH, alcohol and phenol); ^{1}H NMR δ ($CDCl_3$): 0.80 (*s*, 3H, CH_3-18'), 2.81 (*m*, 2H, CH_2-6'), 3.27 (*d*, *J* = 7.4 Hz, 1H, CH-17'α), 3.41 (*t*, *J* = 6.8 Hz, 2H, CH_2Br), 6.56 (*d*, *J* = 2.5 Hz, 1H, CH-4'), 6.62 (*dd*, J_1 = 2.5 Hz and J_2 = 8.3 Hz, 1H, CH-2'), 7.15 (*d*, *J* = 8.4 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 11.92 (C-18'), 26.23, 27.19, 28.15, 28.75, 28.80, 29.62, 29.68, 30.16, 32.62, 34.02 (CH_2Br), 35.71, 36.81, 38.61, 43.95 (2 x), 44.10, 48.40, 88.26 (C-17'), 112.66 (C-2'), 115.23 (C-4'), 126.47 (C-1'), 132.72 (C-10'), 138.27 (C-5'), 153.35 (C-3'); MS m/z (rel. intensity): 450 (M^+, 97), 448 (M^+, 100), 363 (9), 361 (8), 213 (93), 172 (56), 146 (62), 133 (59); EIMS: calcd for $C_{25}H_{37}O_2{}^{79}Br$ (M^+) 448.19769, found 448.19564.

7-[3',17'β-Dihydroxy-1',3',5' (10')-estratrien-16'β-yl]-bromoheptane (34b). Amorphous white solid (41% corrected yield); IR ν (film): 3460 (OH, alcohol and phenol); ^{1}H NMR δ ($CDCl_3$): 0.77 (*s*, 3H, CH_3-18'), 2.81 (*m*, 2H, CH_2-6'), 3.41 (*t*, *J* = 6.8 Hz, 2H, CH_2Br), 3.75 (*d*, *J* = 10.0 Hz, 1H, CH-17'α), 6.57 (*d*, *J* = 2.4 Hz, 1H, CH-4'), 6.63 (*dd*, J_1 = 2.5 Hz and J_2 = 8.4 Hz, 1H, CH-2'), 7.15 (*d*, *J* = 8.4 Hz, 1H, CH-1'); ^{13}C NMR δ ($CDCl_3$): 12.39 (C-18'), 26.26, 27.39, 28,12, 28.47, 28.74, 29.18, 29.61, 31.38, 32.38, 32.79, 34.02 (CH_2Br), 37.67, 38.31, 39.92, 43.96, 44.11, 48.53, 82.54 (C-17'), 112.70 (C-2'), 115.25 (C-4'), 126.43 (C-1'), 132.56 (C-10'), 138.19 (C-5'), 153.50 (C-3'); MS m/z (rel. intensity): 450 (M^+, 100), 448 (M^+, 100), 363 (8), 361 (6), 213 (98), 172 (44), 160 (70), 146 (49), 133 (54); EIMS: calcd for $C_{25}H_{37}O_2{}^{79}Br$ (M^+) 448.19769, found 448.19648.

Biology

Inhibition of 17β-HSD type 1. The cytosolic fraction of human placenta containing 17β-HSD type 1 was used to assess the ability of the synthetic steroids to inhibit the transformation of estrone to estradiol. The procedure for partial purification of 17β-HSD type 1 has already been described in our earlier report.[23] For the screening of inhibition potency (Figure 2), 16-(bromoalkyl)-estradiols were carefully purified by silica gel chromatography using HPLC grade solvents. Briefly, the assay for 17β-HSD type 1 inhibition 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 ~1 pmol of [^{3}H]-estrone, 1 μmol NADH, 10 μL of ethanolic solution of inhibitor (final concentration of 1 and 10 μM) and 100 μL of partially purified 17β-HSD type 1 (3.2 mg of protein mL^{-1}). 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 immediately adding unlabelled estrone (E_1) and estradiol (E_2) as carriers. The tubes were then extracted twice with diethyl ether. After evaporation of the organic solvent, the extract was dissolved in 50 μL of CH_2Cl_2 and applied on TLC plates (Kieselgel 60 F 254). Plates were developed in a mixture of toluene:acetone, 4:1. Iodine vapors were used to locate E_1 and E_2. The corresponding areas were cut from the plates and radioactivity was counted in a liquid scintillation mixture after extraction of the labelled steroids with 1 mL of ethanol. The percentage

of transformation of [^{3}H]-E_1 into [^{3}H]-E_2 was calculated as follows: % trans. = 100 × ([^{3}H]-E_2 / ([^{3}H]-E_1 + [^{3}H]-E_2)), and subsequently, % inh. = [(% trans. of control − % trans. of compound)/(% trans. of control)] × 100. The concentration of proteins from the crude enzymatic pool was determined according to Bradford's method.[40]

Before submitting **14a**, **17a** and **17b** to evaluation of complete inhibition curves (Figure 3), their purities were checked by HPLC (Waters Associates, Milford, MA) with a reverse-phase column (C-18, Nova-pak, 4 μm, 0.5 cm × 10 cm) using a mixture of MeCN/MeOH/H_2O as eluent. All purities were greater than 99%. Then the enzymatic assay was performed as described above except that concentrations of inhibitor ranged from 1 nM to 75 μM. The IC_{50} values were calculated using an unweighted iterative least-squares method for 4-parameters logistic curve fitting.

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