Synthesis and Antiestrogenic Activity of the Compounds Related to the Metabolites of (*Z*)-4-[1-[4-[2-(Dimethylamino)ethoxy]phenyl]-2-(4-isopropylphenyl)-1-butenyl]phenyl Monophosphate (TAT-59)[1)]

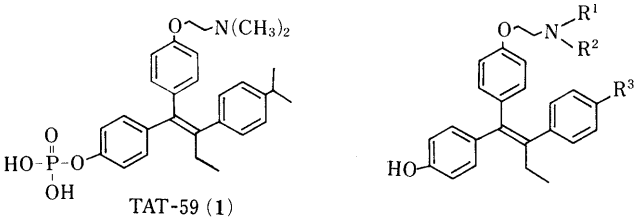
Kazuo OGAWA,* Yoh-ichi MATSUSHITA, Ichiro YAMAWAKI, Manabu KANEDA, Jiro SHIBATA, Toshiyuki TOKO, and Tetsuzi ASAO

Research Laboratory, Taiho Pharmaceutical Co., Ltd., Kawauchi-cho, Tokushima 771-01, Japan. *Received August 20, 1990*

The metabolites of (*Z*)-4-[1-[4-[2-(dimethylamino)ethoxy]phenyl]-2-(4-isopropylphenyl)-1-butenyl]phenyl monophosphate, TAT-59, (1), a potent antitumor agent for hormone-dependent tumors, and derivatives of TAT-59 were synthesized to confirm its proposed structure. The structure and the *Z*-configuration of the metabolites (2a—8a) were confirmed by comparison with synthesized authentic compounds. All of the metabolites and the derivatives of TAT-59 were tested for a binding affinity toward estrogenic receptors *in vitro* and antiuterotrophic activity *in vivo*. Most of the metabolites possessed remarkable binding affinity toward estrogenic receptors as well as fairly good antiuterotrophic activity.

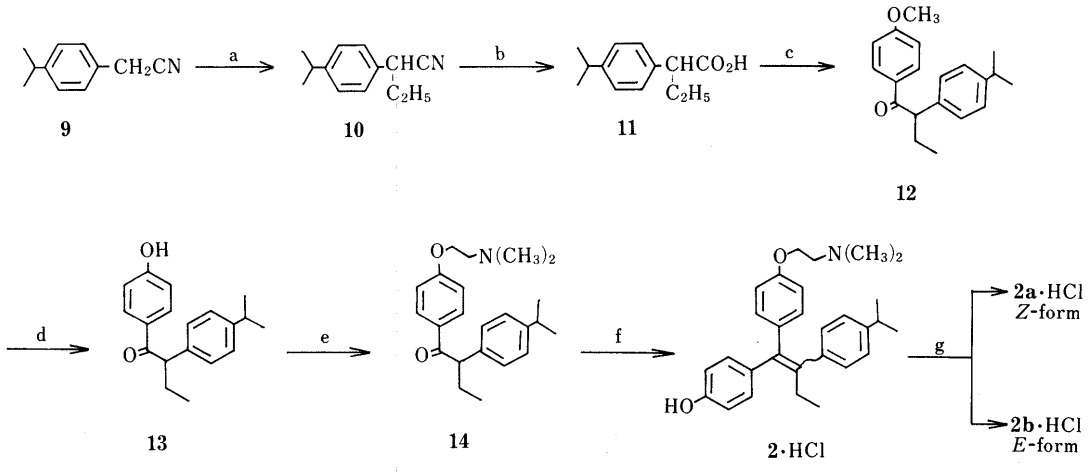
Keywords metabolite; synthesis; hormone-dependent tumor; antitumor agent; binding affinity; estrogen receptor; antiuterotrophic activity

Tamoxifen (TAM),[2)] a nonsteroidal antiestrogenic agent, is currently used as adjuvant in the surgical therapy of breast cancer. Moreover, several derivatives[3)] of TAM have been extensively studied in this field. In the course of a search for a non-steroidal antiestrogenic agent, we identified[4)] a novel non-steroidal antitumor agent, (*Z*)-4-[1-[4-[2-(dimethylamino)ethoxy]phenyl]-2-(4-isopropylphenyl)-1-butenyl]phenyl monophosphate (TAT-59) (**1**), which possesses much stronger antiestrogenic activity than TAM. Furthermore, TAT-59 is currently under study as a promising drug for the treatment of breast cancer. Recently, seven metabolites (**2a—8a**) have been identified in the study of TAT-59 biotransformation in the plasma of rat and dog. Specifically, a dephosphorylated derivative (**2**), three hydroxylated derivatives (**3**, **5** and **6**), an olefinic derivative (**4**), a demethylated derivative (**7**) and a hydroxylated derivative (**8**) with demethylation were proposed. The present study is undertaken to confirm their structure and



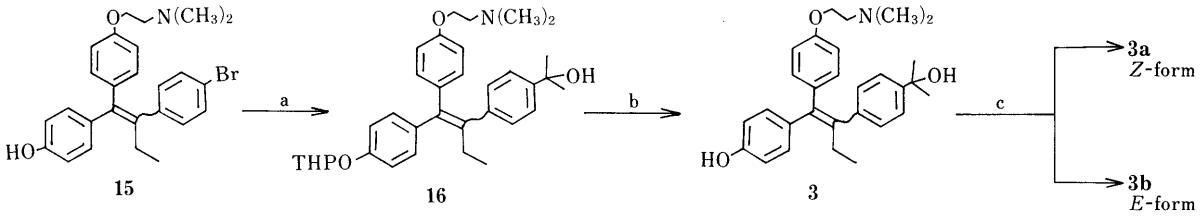
	R^1	R^2	R^3
2	CH_3	CH_3	
3	CH_3	CH_3	OH
4	CH_3	CH_3	
5	CH_3	CH_3	OH, OH
6	CH_3	CH_3	OH
7	H	CH_3	
8	H	CH_3	OH

Chart 1



a) C_2H_5Br, aqueous NaOH. b) H_2SO_4, 150°C. c) i) $SOCl_2$, ii) anisole, $AlCl_3$, CH_2Cl_2. d) pyridine hydrochloride, 230°C. e) $ClCH_2CH_2N(CH_3)_2$, NaOH, DMF. f) i) 4-(tetrahydropyran-2-yloxy)phenyl magnesium bromide, THF, ii) HCl-EtOH, reflux. g) recrystallization.

Chart 2



a) i) DHP, *p*-TsOH, DMF, ii) *n*-BuLi, THF, iii) acetone, THF.
b) conc. HCl, THF, room temperature. c) ODS-chromatography.

Chart 3

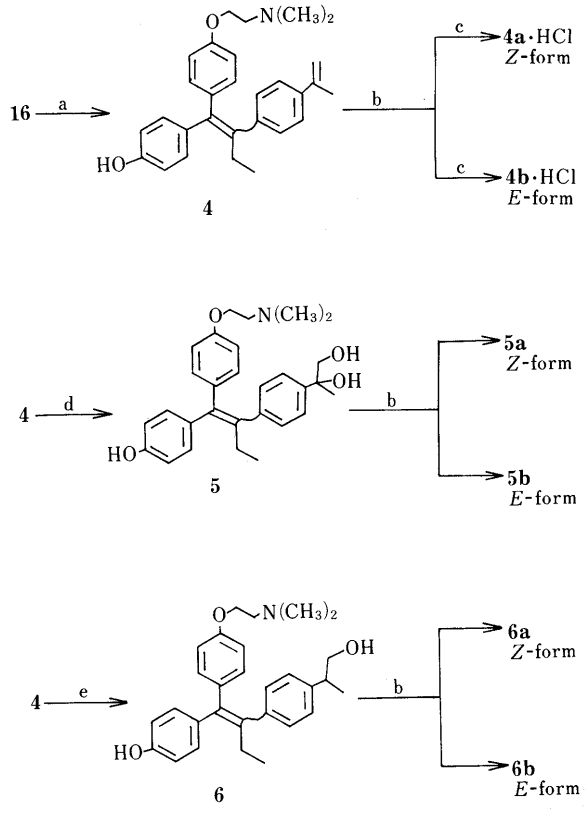
configuration (*E* or *Z*-form), and to clarify their potency in comparison with TAM and TAT-59 for the binding affinity *in vitro* toward estrogenic receptors and for the antiuterotrophic activity *in vivo*. In this paper, we wish to report on the synthesis of the metabolites (**2a**—**8a**) and related compounds (**2b**—**8b**), and their biological activity.

Synthesis The dephosphorylated compound (**2**) was prepared according to the method shown in Chart 2. *C*-Alkylation of 4-isopropylphenylacetonitrile (**9**)[5] with ethyl bromide resulted in compound (**10**). Compound (**11**) was subjected to chlorination with $SOCl_2$ followed by Friedel–Crafts reaction with anisole to give compounds (**12**) in a fair yield. Compound (**13**), obtained by *O*-demethylation of **12** with pyridinie hydrochloride, was again subjected to *O*-alkylation with 2-(dimethylamino)ethyl chloride to afford compound (**14**).

In accordance with a published method[6] on the synthesis of TAM derivatives, ketone (**14**) was allowed to react with [4-(tetrahydropyran-2-yloxy)phenyl]magnesium bromide,[7] followed by acid-catalytic deprotection of a tetrahydropyranyl moiety and dehydration to afford compound (**2**). The isomeric mixture (**2**) was combined in a ratio of about 1 : 1 (*E*:*Z*) on the basis of proton nuclear magnetic resonance (^{1}H-NMR) spectra. Each isomer could be separated by recrystallization to one isomer (**2a**) and another (**2b**), respectively.

The hydroxylated compound (**3**) was prepared by the method shown in Chart 3. Protection of the hydroxy group on the 4-bromophenyl-4′-hydroxyphenyl derivative (**15**)[6b] with 2,3-dihydropyrane (DHP) in the presence of a catalytic *p*-toluenesulfonic acid successfully resulted in a good yield. The phenyllitium derivative, prepared by treatment of the 4-bromophenyl-4′-(tetrahydropyran-2-yloxy)phenyl derivative with *n*-butyl lithium (*n*-BuLi), was allowed to react with acetone followed by acid-catalytic deprotection of the tetrahydropyranyl moiety to afford the tertiary alcohol derivative (**3**) as an isomeric mixture (*E*:*Z*=1 : 1). The mixture could not be separated by recrystallization but could be separated into the two regio-isomers (**3a** and **3b**) by means of chromatography on an octadecyl-bonded silica gel (ODS) column.

The olefinic (**4**), 1,2-dihydroxylated (**5**) and mono-hydroxylated (**6**) compounds were prepared by the method shown in Chart 4. Deprotection and dehydration of the 4-[(2-hydroxy-2-methyl)ethyl]phenyl-4′-(tetrahydropyran-2-yloxy)phenyl derivative (**16**) proceeded easily on heating in acetic acid to give an olefinic compound (**4**). Oxidation of **4** with osmium tetraoxide (OsO_4)[8] resulted in compound **5** by introducing diol moieties to the 1 and 2-positions on the alkyl chain. On the other hand, oxidation of **4** using



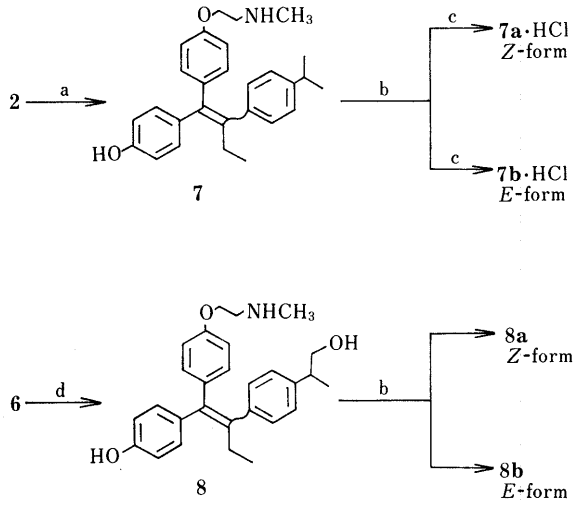
a) AcOH, reflux. b) ODS-chromatography. c) dioxane-HCl
d) OsO_4, pyridine, room temperature. e) i) 9-BBN, benzene, reflux, ii) H_2O_2-$NaOCH_3$, MeOH, 50°C.

Chart 4

9-borabicyclo[3.3.1]nonane (9-BBN)[9] gave compound (**6**), which involved selective introduction of a hydroxyl group to the terminal end of the alkyl chain. These compounds (**4**, **5** and **6**) were independently separated into two regio-isomers (**4a** and **4b**), (**5a** and **5b**), and (**6a** and **6b**), respectively, in the same manner as described for **3**.

The demethylated compounds (**7** and **8**) were prepared by the method shown in Chart 5. In accordance with the known method,[10] compounds (**2** and **6**) were allowed to react with vinyloxycarbonyl chloride followed by acidcatalytic removal of the vinyloxycarbonyl moiety to give the *N*-demethylated compounds (**7** and **8**), respectively. These compounds (**7** and **8**) were separated into the two regio-isomers (**7a** and **7b**) and (**8a** and **8b**), respectively, in the same manner as described for **3**.

Assignment of Configuration (*Z* and *E*) and Determination of Chemical Purity The configurational assignment of the



a) i) vinyloxycarbonyl chloride, dioxane, 170°C, ii) HCl-EtOH, 80°C. b) ODS-chromatography. c) dioxane-HCl. d) i) vinyloxycarbonyl chloride, dioxane, ii) 4 N-NaOH, reflux.

Chart 5

TABLE I. Binding Affinity toward Estrogenic Receptor and Antiuterotrophic Activity of Metabolites (**2a**—**8a**) and Their Isomers (**2b**—**8b**)

Compd. No.	Configuration[a]	Chemical purity[b] (%)	Binding affinity[c] RBA	Binding affinity[c] IC_{50} ($\times 10^{-8}$ M)	Antiuterotrophic activity[c] Inhibition[d] (%)
2a	*Z*	97.0	152.5	0.181	36.2
2b	*E*	97.2	1.7	16.7	10.1
3a	*Z*	99.8	48.5	0.569	11.4
3b	*E*	98.0	0.8	36.4	—[e]
4a	*Z*	95.4	53.8	0.513	38.4
4b	*E*	96.2	0.7	39.0	—[e]
5a	*Z*	99.5	31.5	0.876	29.3
5b	*E*	96.1	0.7	40.1	—[e]
6a	*Z*	99.2	28.9	0.959	36.2
6b	*E*	98.3	3.6	7.72	—[e]
7a	*Z*	95.8	66.2	0.417	37.5
7b	*E*	95.6	1.6	16.8	—[e]
8a	*Z*	98.5	58.5	0.472	15.4
8b	*E*	98.6	2.4	11.4	—[e]
TAT-59 (**1**)			5.1	5.37	42.5
TAM			0.6	45.3	17.8
Estradiol			100.0	0.276	0.0[f]

a) Configurational isomer. *b*) Determined by HPLC-Develosil ODS-5. *c*) See the experimental section. *d*) Dose of 40 μg/kg, i.p. *e*) Not tested. *f*) Dose of 0.5 μg/kg, i.p.

isomers (**2a**—**8a** and **2b**—**8b**) was confirmed on the basis of ^{1}H-NMR chemical shifts in accordance with the literature.[11] Namely, the chemical shift of triplets for the OCH_2 proton in the compounds (**2a** and **2b**) appeared at δ 4.21 and 4.37 ppm, respectively. Shani *et al.*[11] report that the substituents situated *trans* (*E*-form) to a phenyl ring are shifted to lower fields than those in a *Z*-form. On the basis of this observation, the resonance appearing at lower fields was assigned to be *E*-isomer in the compound (**2b**). On the other hand, for **2a**, resonance was considered to be *Z*-isomer. The other isomers (**3a**—**8a**) and (**3b**—**8b**) were confirmed in the same manner as described for **2a** and **2b**. The chemical purity of the regio-isomers was respectively determined by high performance liquid chromatography (HPLC) on Develosil ODS-5 with methanol–phosphate buffer (75 : 25) as an eluant. The data are shown in Table I.

Results and Discussion

All of the metabolites (**2a**—**8a**) were identified to be *Z*-isomers on the bases of ^{1}H-NMR chemical shifts and HPLC properties of the synthesized authentic compounds. During transformation of TAT-59, it was clarified that the double bond configuration of the metabolites did not change. All metabolites (**2a**—**8a**) and the corresponding regio-isomers (**2b**—**8b**) were examined for competitive activity *in vitro* toward estrogenic receptors and for antiuterotrophic activity *in vivo*. The data are shown in Table I. Binding affinity of the *Z*-isomers (**2a**—**8a**) *in vitro* toward estrogenic receptors was remarkably higher than that of the *E*-isomers (**2b**—**8b**) and TAM. In particular, among all of the metabolites (*Z*-isomers), **2a** showed the most potent activity ($IC_{50} = 1.8 \times 10^{-9}$ M). Almost all of the *Z*-isomers showed more potent antiuterotrophic activity *in vivo* in comparison with the *E*-isomers and TAM. On the other hand, the activity of **2a**, **4a**, **6a** and **7a** was as potent as TAT-59 (**1**). Based on pharmacological data, it was suggested that the potent antitumor activity of TAT-59 (parent compound) is mediated by the combination of its metabolites.

Experimental

All melting points were recorded with a Yanagimoto micromelting apparatus and are uncorrected. Spectral data were obtained as follows: mass spectra (MS) with a JEOL LMS-O1G-2 spectrometer; ^{1}H-NMR spectra with a JEOL JMN-FX 100 spectrometer (using tetramethylsilan as an internal standard). Chemical shifts of ^{1}H-NMR signals are given in δ values (ppm).

2-(4-Isopropylphenyl)butyronitrile (10) The title compound (**10**) was prepared from ethyl bromide (49.1 g), 4-isopropylphenylacetonitrile (**9**)[5] (70.0 g), triethylbenzylammonium chloride (TEBAC) (2.0 g) and 50% NaOH solution (60 ml) in accordance with the known method.[12] Yield 63.2 g (78.7%). bp 119—123 °C (5 mmHg). ^{1}H-NMR ($CDCl_3$) δ: 1.06 (3H, t, J=7.3 Hz), 1.24 (6H, d, J=7.1 Hz), 1.70—2.10 (2H, m), 2.70—3.15 (1H, m), 3.70 (1H, t, J=7.1 Hz), 7.23, 7.25 (4H, each s). MS *m/z*: 187 (M^+).

2-(4-Isopropylphenyl)butyric Acid (11) Compound (**10**) (49.0 g) was added to 70% H_2SO_4 (50 ml) and then heated in an oil bath at 150 °C for 3 h. The reaction mixture was cooled to room temperature and then extracted with toluene (100 ml × 2). The organic layer was washed with water, dried over sodium sulfate and evaporated under reduced pressure to give **11** as a crude product, which was purified by distillation. Yield 46.2 g (85.6%). bp 156—160 °C (5 mmHg). (mp 95—96 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, t, J=7.3 Hz), 1.23 (6H, d, J=6.8 Hz), 1.60—2.30 (2H, m), 2.65—3.10 (1H, m), 3.42 (1H, t, J=7.5 Hz), 7.19, 7.20 (4H, each s). MS *m/z*: 206 (M^+). *Anal.* Calcd for $C_{13}H_{18}O_2$: C, 75.69; H, 8.80. Found: C, 75.35, H, 8.60.

2-(4-Isopropylphenyl)-1-(4-methoxyphenyl)-1-butanone (12) Anhydrous aluminium chloride (29.4 g) was gradually added to a solution of anisole (23.0 g) and acid chloride (47.0 g) (prepared by the treatment of **11** with thionyl chloride) in dichloromethane (250 ml) while maintaining the reaction temperature below 0 °C. The reaction mixture was stirred at room temperature for 3 h and then poured into 5% HCl (300 ml). The organic layer was washed with water, then dried over sodium sulfate and evaporated under reduced pressure to give **12**. Yield 60.0 g (82.2%). mp 47—48 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=7.4 Hz), 1.25 (6H, d, J=7.5 Hz), 1.50—2.50 (2H, m), 2.50—3.20 (1H, m), 3.66 (3H, s), 4.35 (1H, t, J=7.2 Hz), 6.77 (2H, d, J=8.8 Hz), 7.13, 7.16 (4H, each s), 7.92 (2H, d, J=8.8 Hz). MS *m/z*: 296 (M^+). *Anal.* Calcd for $C_{20}H_{24}O_2$: C, 81.04; H, 8.16. Found: C, 81.08; H, 7.92.

1-(4-Hydroxyphenyl)-2-(4-isopropylphenyl)-1-butanone (13) Compound (**12**) (17.3 g) was added to pyridine hydrochloride (20.0 g), and then heated in an oil bath at 230 °C for 2 h. The reaction mixture was poured into ice-water (30 ml) and extracted with dichloromethane (100 ml × 2). The organic layer was dried over sodium sulfate and evaporated under reduced pressure to give **13** as a crude product, which was recrystallized from toluene–hexane. Yield 12.4 g (75.2%). mp 132—133 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=7.3 Hz), 1.18 (6H, d, J=7.1 Hz), 1.60—2.40 (2H, m), 2.60—3.00 (1H, m), 4.40 (1H, t, J=7.3 Hz), 6.84 (2H, d,

J=8.8 Hz), 7.15, 7.18 (4H, each s), 7.92 (2H, d, J=8.8 Hz). MS m/z: 282 (M^+). *Anal.* Calcd for $C_{19}H_{22}O_2$: C, 80.81; H, 7.85. Found: C, 80.71; H, 7.59.

1-[4-[2-(Dimethylamino)ethoxy]phenyl]-2-(4-isopropylphenyl)-1-butanone (14) Compound (**13**) (12.4 g) was gradually added to a suspension of sodium hydride (about 60%, in oil) (1.8 g) in *N,N*-dimethylformamide (DMF) (50 ml) under stirring at room temperature. The reaction mixture was stirred for 1.5 h at the same temperature and then 2-(dimethylamino)ethyl chloride (5.5 g) [obtained by the treatment of 2-(dimethylamino)ethyl chloride hydrochloride with 10% NaOH solution] was added to the whole mixture. After stirring at 100 °C for 2 h, the reaction mixture was evaporated under reduced pressure, and the resulting residue was extracted with dichloromethane (200 ml), the obtained organic layer was extracted with 2 N HCl (50 ml × 2). The acid layer was adjusted to pH 10 with 10% NaOH solution. The resulting oily product was extracted with dichloromethane (100 ml × 2). The organic layer was dried over sodium sulfate and evaporated under reduced pressure to give **14**. Yield 12.6 g (80.1%). mp 55—56 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=7.3 Hz), 1.19 (6H, d, J=7.1 Hz), 1.60—2.30 (2H, m), 2.31 (6H, s), 2.71 (2H, t, J=5.6 Hz), 1.70—3.00 (1H, m), 4.07 (2H, t, J=5.6 Hz), 4.38 (1H, t, J=7.3 Hz), 6.88 (2H, d, J=9.0 Hz), 7.15, 7.19 (4H, each s), 7.96 (2H, d, J=9.0 Hz). MS m/z: 353 (M^+). *Anal.* Calcd for $C_{23}H_{31}NO_2$: C, 78.15; H, 8.84; N, 3.96. Found: C, 78.43; H, 8.85; N, 3.97.

(*Z*)- and (*E*)-1-[4-[2-(Dimethylamino)ethoxy]phenyl]-1-(4-hydroxyphenyl)-2-(4-isopropylphenyl)-1-butene Hydrochloride (2a and 2b) A solution of 4-(tetrahydropyran-2-yloxy)phenyl bromide[13] (51.4 g) in tetrahydrofurane (THF) (150 ml) was added dropwise in the presence of a catalytic amount of iodine to a suspension of magnesium (4.9 g) in THF (10 ml) under gentle reflux. The reaction was initiated by heating. After reflux for 2 h, the reaction mixture was cooled below 5 °C, then a solution of compound (**14**) (44.0 g) in THF (100 ml) was added to a solution of its Grignard reagent under ice-cooling. After stirring at room temperature for 1 h, the reaction mixture was refluxed for 2 h and then evaporated under reduced pressure. The resulting residue was poured into ice-water (300 ml) containing a saturated ammonium chloride solution and extracted with dichloromethane (300 ml × 2). The organic layer was dried over sodium sulfate and evaporated under reduced pressure. The obtained black oily product was dissolved in water (200 ml), then the whole was alkalized with ammonium hydroxide and extracted with ether (300 ml × 2). After removal of the solvent, the obtained residue was dissolved in a solution of conc. HCl (20 ml) in ethanol (200 ml). After refluxing for 2 h, the reaction mixture was evaporated under reduced pressure to give **2**·HCl as a crude product (isomeric mixture of E/Z=1/1), which was purified by recrystallization from a mixture of 2-butanone and ether. The yield was 35.2 g (65.8%). The isomeric mixture was separated to *E*- and *Z*-isomer as follows; From the recrystallization, *Z*-isomer (**2a**·HCl) was obtained. mp 200—202 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.84 (3H, t, J=7.1 Hz), 1.15 (6H, d, J=6.8 Hz), 2.20—2.60 (2H, m), 2.60—3.00 (1H, m), 2.77 (6H, s), 3.40 (2H, br t), 4.22 (2H, br t), 6.60—7.30 (12H, m), 9.52 (1H, s), 10.75 (1H, br s). MS m/z: 429 (M^+). *Anal.* Calcd for $C_{29}H_{35}NO_2$·HCl: C, 74.74; H, 7.78; N, 3.01. Found: C, 74.83; H, 7.71; N, 3.10. From the mother liquid, *E*-isomer (**2b**·HCl) was obtained. mp 171—173 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.84 (3H, t, J=6.8 Hz), 1.15 (6H, d, J=6.8 Hz), 2.20—2.60 (2H, m), 2.60—2.95 (1H, m), 2.84 (6H, s), 3.50 (2H, br t), 4.35 (2H, br t), 6.40 (2H, d, J=8.6 Hz), 6.60 (2H, d, J=8.6 Hz), 6.85—7.20 (8H, m), 9.22 (1H, s), 10.52 (1H, br s). MS m/z: 429 (M^+). *Anal.* Calcd for $C_{29}H_{35}NO_2$·HCl: C, 74.74; H, 7.78; N, 3.01. Found: C, 74.90; H, 7.85; N, 3.01.

(*E,Z*)-1-[4-[2-(Dimethylamino)ethoxy]phenyl]-2-[4-(1-hydroxy-1-methylethyl)phenyl]-1-[4-(tetrahydropyran-2-yloxy)phenyl]-1-butene (16) DHP (61.5 g) was added to a solution consisting of DMF (100 ml), *p*-toluenesulfonic acid (6.6 g) and (*E,Z*)-**15** (15.9 g) (prepared starting from 4-bromophenylacetic acid according to the method of Robertson, *et al.*[6b]) After stirring overnight at room temperature, the reaction mixture was neutralized with triethylamine (70 g) under ice-cooling and evaporated under reduced pressure. The residue was dissolved in water (50 ml), adjusted to pH 12—13 with 2 N NaOH and extracted with ether (100 ml × 2). The organic layer was dried over sodium sulfate and evaporated under reduced pressure to give a crude (*E,Z*)-2-(4-bromophenyl)-1-[4-[2-(dimethylamino)ethoxy]phenyl]-1-[4-(tetrahydropyran-2-yloxy)phenyl]-1-butene, which was purified by chromatography on a silica gel column with chloroform–ethanol (10 : 1) as an eluent to give a light brown oil. Yield 13.2 g (70.2%). ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, t, J=7.3 Hz), 1.40—2.00 (6H, m), 2.29, 2.34 (6H, each s), 2.45 (2H, q, J=7.3 Hz), 2.65, 2.73 (2H, each t, J=5.9 Hz), 3.40—4.10 (2H, m), 3.94, 4.07 (2H, each t, J=5.9 Hz), 5.27, 5.41 (1H, each br s), 6.50—7.40 (12H, m). MS m/z: 549 (M^+).

A solution of *n*-BuLi in hexane (21.2 ml of 1.6 M) was added dropwise to a solution of the above 4-(tetrahydropyran-2-yloxy)phenyl compound (9.4 g) in THF (50 ml) at −50 °C in a nitrogen atmosphere. After stirring at the same temperature for 0.5 h, acetone (2.53 ml) was added dropwise to the reaction mixture while maintaining the temperature below −50 °C, and stirring was continued at the same temperature for 1 h. The reaction mixture was allowed to warm to room temperature and evaporated under reduced pressure. The residue was dissolved in water (40 ml), and then the solution was adjusted to pH 13 with 2 N NaOH and extracted with ether (100 ml × 2). The organic layer was dried over sodium sulfate and evaporated under reduced pressure to give **16** as a light brown oil (isomeric mixture of E/Z=1/1). Yield 8.2 g (90.9%). ^{1}H-NMR (DMSO-d_6) δ: 0.84 (3H, t, J=6.8 Hz), 1.37, 1.38 (6H, each s), 1.30—1.90 (6H, m), 2.15, 2.22 (6H, each s), 2.53, 2.63 (2H, each t, J=5.6 Hz), 3.30—4.00 (2H, m), 3.89, 4.05 (2H, each t, J=5.6 Hz), 4.90, 4.92 (1H, each s), 5.28, 5.45 (1H, each br s), 6.50—7.40 (12H, m). MS m/z: 529 (M^+). *Anal.* Calcd for $C_{34}H_{43}NO_4$: C, 77.09; H, 8.18; N, 2.64. Found: C, 77.30; H, 8.37; N, 2.81.

(*Z*)- and (*E*)-1-[4-[2-(Dimethylamino)ethoxy]phenyl]-2-[4-(1-hydroxy-1-methylethyl)phenyl]-1-(4-hydroxyphenyl)-1-butene (3a and 3b) Concentrated HCl (4 ml) was added to a solution of (*E,Z*)-**16** (4.46 g) in THF (40 ml). After stirring at room temperature for 15 min, the reaction mixture was neutralized with sodium bicarbonate and extracted with chloroform (50 ml × 2). The organic layer was dried over sodium sulfate and evaporated under reduced pressure to give **3** as a crude product (isomeric mixture of E/Z=1/1), which was purified by chromatography on a silica gel column with chloroform–methanol (5 : 1) as an eluent. The yield was 1.64 g (43.7%). MS m/z: 445 (M^+). The isomeric mixture was separated into *E*- and *Z*-isomers as follows: The compound (**3**) was chromatographed on an ODS column with methanol–phosphate buffer (75 : 25) as an eluent. The first eluate was collected and evaporated under reduced pressure at room temperature. 10% aqueous sodium bicarbonate was added to the residue and the whole was extracted with ether. The organic layer was dried over sodium sulfate and evaporated under reduced pressure to give **3a** (*Z*-isomer). mp 134—136 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.84 (3H, t, J=7.1 Hz), 1.37 (6H, s), 2.16 (6H, s), 3.89 (2H, t, J=6.7 Hz), 4.90 (1H, s), 6.50—7.40 (12H, m), 9.40 (1H, br s). MS m/z: 445 (M^+). *Anal.* Calcd for $C_{29}H_{35}NO_3 \cdot H_2O$: C, 75.13; H, 8.04; N, 3.02. Found: C, 75.27; H, 8.05; N, 3.05. The second eluate was collected, worked-up in the same manner as described for **3a** to give **3b** (*E*-isomer). mp 150—152 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.84 (3H, t, J=7.1 Hz), 1.38 (6H, s), 2.23 (6H, s), 2.63 (2H, t, J=6.7 Hz), 4.05 (2H, t, J=6.7 Hz), 4.93 (1H, s), 6.30—6.40 (12H, m), 9.16 (1H, br s). MS m/z: 445 (M^+). *Anal.* Calcd for $C_{29}H_{35}NO_3$: C, 78.17; H, 7.92; N, 3.14. Found: C, 77.92; H, 8.04; N, 3.12.

(*Z*)- and (*E*)-1-[4-[2-(Dimethylamino)ethoxy]phenyl]-1-(4-hydroxyphenyl)-2-(4-isopropenylphenyl)-1-butene Hydrochloride (4a and 4b) Compound (**16**) (5.76 g) was dissolved in acetic acid (25 ml). After stirring at 90 °C for 5 h, the reaction mixture was poured into saturated sodium carbonate (350 ml) and stirred at room temperature for 0.5 h. The resulting precipitate was collected to give **4** as a crude solid (isomeric mixture of E/Z=1/1), which was purified by chromatography on a silica gel with chloroform–methanol (6 : 1) as an eluent. The yield was 3.9 g (87.1%). The isomeric mixture was separated in the same manner as described for **3a** and **3b**. From the first eluate, the free base of **4a** was obtained and converted into the hydrochloride (**4a**·HCl) by the treatment with dioxane–hydrogen chloride. mp 213—215 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.85 (3H, t, J=6.8 Hz), 2.05 (3H, s), 2.42 (2H, q), 2.79 (6H, s), 4.20 (2H, br t), 5.05 (1H, s), 5.41 (1H, s), 6.60—6.85 (6H, m), 6.98 (2H, d, J=9.0 Hz), 7.08 (2H, d, J=8.4 Hz), 7.34 (2H, d, J=8.4 Hz), 9.47 (1H, s), 10.01 (1H, br s). MS m/z: 427 (M^+). *Anal.* Calcd for $C_{29}H_{33}NO_2$·HCl: C, 75.06; H, 7.38; N, 3.02. Found: C, 74.60; H, 7.34; N, 2.96. From the second eluate, the free base of **4b** was obtained and converted into the hydrochloride (**4b**·HCl) in the same manner as described for **4a**·HCl. mp 128—130 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.85 (3H, t, J=6.8 Hz), 2.06 (3H, s), 2.40 (2H, q), 2.86 (6H, s), 3.52 (2H, br t), 4.36 (2H, br t), 5.05 (1H, s), 5.41 (1H, s), 6.43 (2H, d, J=8.5 Hz), 6.63 (2H, d, J=8.5 Hz), 6.99 (2H, d, J=9.2 Hz), 7.07 (2H, d, J=8.2 Hz), 7.15 (2H, d, J=9.2 Hz), 7.34 (2H, d, J=8.2 Hz), 9.23 (1H, s), 10.25 (1H, br s). MS m/z: 427 (M^+). *Anal.* Calcd for $C_{29}H_{33}NO_2$·HCl: C, 75.06; H, 7.38; N, 3.02. Found: C, 74.61; H, 7.34; N, 2.96.

(*Z*)- and (*E*)-2-[4-(1,2-Dihydroxy-1-methylethyl)phenyl]-1-[4-[2-(dimethylamino)ethoxy]phenyl]-1-(4-hydroxyphenyl)-1-butene (5a and 5b) OsO_4 (1.0 g) was added to a solution of **4** (2.0 g) in pyridine (15 ml). After stirring at room temperature for 5 h, the reaction mixture was added to a solution consisting of sodium sulfite (1.8 g), pyridine (20 ml) and water (30 ml), then stirred at the same temperature for 1 h. The whole was

extracted with chloroform (100 ml × 2), dried over sodium sulfate and the organic layer was evaporated under reduced pressure to give **5** as a crude oil (isomeric mixture of $E/Z=1/1$), which was purified by chromatography on a silica gel column with chloroform–ethanol (5 : 1) as an eluent. The yield was 1.3 g (60.2%). The isomeric mixture was separated in the same manner as described for **3a** and **3b**. From the first eluate, *Z*-isomer (**5a**) was obtained. mp 147—149 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.84 (3H, t, $J=6.8$ Hz), 1.33 (3H, s), 2.15 (6H, s), 3.35 (2H, br s), 3.89 (2H, t, $J=5.8$ Hz), 4.60 (1H, t, $J=5.8$ Hz), 4.76 (1H, s), 6.56 (2H, d, $J=9.3$ Hz), 6.72 (2H, d, $J=9.3$ Hz), 6.74 (2H, d, $J=8.6$ Hz), 6.99 (2H, d, $J=8.6$ Hz), 7.01 (2H, d, $J=8.3$ Hz), 7.25 (2H, d, $J=8.3$ Hz), 9.39 (1H, s). MS *m/z*: 461 (M^+). *Anal.* Calcd for $C_{29}H_{35}NO_4 \cdot 1/2H_2O$: C, 74.01; H, 7.71; N, 2.98. Found: C, 73.69; H, 7.67; N, 3.01. From the second eluate, *E*-isomer (**5b**) was obtained. mp 165—167 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.86 (3H, t, $J=6.8$ Hz), 1.34 (3H, s), 2.23 (6H, s), 2.64 (2H, t, $J=5.7$ Hz), 3.35 (2H, br s), 4.05 (2H, t, $J=5.7$ Hz), 4.62 (1H, br t), 4.76 (1H, s), 6.39 (2H, d, $J=8.5$ Hz), 6.61 (2H, d, $J=8.5$ Hz), 6.91 (2H, d, $J=8.5$ Hz), 7.00 (2H, d, $J=8.3$ Hz), 7.08 (2H, d, $J=8.5$ Hz), 7.26 (2H, d, $J=8.3$ Hz), 9.15 (1H, s). MS *m/z*: 461 (M^+). *Anal.* Calcd for $C_{29}H_{35}NO_4 \cdot 1/2H_2O$: C, 74.01; H, 7.71; N, 2.98. Found: C, 74.13; H, 7.93; N, 2.90.

(*Z*)- and (*E*)-1-[4-[2-(Dimethylamino)ethoxy]phenyl]-2-[4-(2-hydroxy-1-methylethyl)phenyl]-1-(4-hydroxyphenyl)-1-butene (6a and 6b) Compound (**4**) (2.43 g) was added to a solution of 9-BBN (2.68 g) in benzene (80 ml) under a nitrogen atmosphere. After stirring under reflux for 10 h, the reaction mixture was evaporated under reduced pressure and the residue was dissolved in methanol (60 ml). 28% sodium methoxide–methanol (14.2 ml) followed by 30% hydrogen peroxide (7.9 ml) were carefully added to the above solution under ice-cooling. After stirring at 50 °C for 2.5 h, the reaction mixture was concentrated to 1/3 volume, then added to a 5% aqueous potassium carbonate (100 ml) and extracted with chloroform (100 ml × 2). The organic layer was dried over sodium sulfate and evaporated under reduced pressure to give **6** as a crude oil (isomeric mixture of $E/Z=1/1$), which was purified in the same manner as described for **5**. The yield was 1.12 g (44.3%). The isomeric mixture was separated in the same manner as described for **3a** and **3b**. From the first eluate, *Z*-isomer (**6a**) was obtained. mp 126—128 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.84 (3H, t, $J=7.0$ Hz), 1.14 (3H, t, $J=6.8$ Hz), 2.15 (6H, s), 2.74 (2H, t, $J=5.7$ Hz), 3.10—3.60 (2H, m), 3.89 (2H, t, $J=5.7$ Hz), 4.59 (1H, t, $J=5.6$ Hz), 6.56 (2H, d, $J=8.9$ Hz), 6.71 (2H, d, $J=8.9$ Hz), 6.73 (2H, d, $J=8.6$ Hz), 6.96 (2H, d, $J=8.6$ Hz), 7.01 (4H, s), 9.38 (1H, s). MS *m/z*: 445 (M^+). *Anal.* Calcd for $C_{29}H_{35}NO_3$: C, 78.17; H, 7.92; N, 3.14. Found: C, 78.11; H, 7.75; N, 3.09. From the second eluate, *E*-isomer (**6b**) was obtained. mp 155—157 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.83 (3H, t, $J=7.0$ Hz), 1.14 (3H, d, $J=6.8$ Hz), 2.22 (6H, s), 2.82 (2H, t, $J=5.6$ Hz), 3.10—3.60 (2H, m), 4.04 (2H, t, $J=5.6$ Hz), 4.60 (1H, br t), 6.39 (2H, d, $J=8.6$ Hz), 6.60 (2H, d, $J=8.6$ Hz), 6.90 (2H, d, $J=8.8$ Hz), 7.06 (2H, d, $J=8.8$ Hz), 7.01 (4H, s), 9.16 (1H, s). MS *m/z*: 445 (M^+). *Anal.* Calcd for $C_{29}H_{35}NO_3$: C, 78.17; H, 7.92; N, 3.14. Found: C, 78.09; H, 8.10; N, 3.06.

(*Z*)- and (*E*)-1-(4-Hydroxyphenyl)-2-(4-isopropylphenyl)-1-[4-[2-(methylamino)ethoxy]phenyl]-1-butene Hydrochloride (7a and 7b) Compound (**2**) (3.82 g) was added to a solution of vinyloxycarbonyl chloride (1.81 ml) in dioxane (90 ml) in a sealed tube and heated in an oil bath at 170 °C for 5 h. The reaction mixture was evaporated under reduced pressure and then the residue was dissolved in 5% hydrogen chloride–ethanol solution (40 ml). After stirring at 80 °C for 0.5 h, the whole was evaporated under reduced pressure to give **7** as a crude oil (isomeric mixture of $E/Z=1/1$), which was purified by chromatography on a silica gel column with chloroform–methanol (7 : 1) as an eluent. The yield was 1.96 g (53.1%). The isomeric mixture was separated in the same manner as described for **4a** and **4b**. From the first eluate, the free base of *Z*-isomer (**7a**) was obtained. **7a**·HCl was prepared by working up in the same manner as described for **4a**·HCl or **4b**·HCl. **7a**·HCl: mp 210—212 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.83 (3H, t, $J=6.8$ Hz), 1.15 (6H, d, $J=6.8$ Hz), 2.40 (2H, q, $J=6.8$ Hz), 2.56 (3H, s), 2.80 (1H, m), 3.22 (2H, br t), 4.11 (2H, br t), 6.50—7.00 (8H, m), 7.03 (4H, s), 8.93 (2H, br s), 9.46 (1H, s). MS *m/z*: 415 (M^+). *Anal.* Calcd for $C_{28}H_{33}NO_2 \cdot HCl$: C, 74.40; H, 7.58; N, 3.10. Found: C, 74.64; H, 7.48; N, 3.11. From the second eluate, the free base of *E*-isomer (**7b**) was obtained. **7b**·HCl: mp 195—197 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.84 (3H, t, $J=6.8$ Hz), 1.15 (6H, d, $J=6.8$ Hz), 2.38 (2H, q, $J=6.8$ Hz), 2.62 (3H, s), 3.30 (2H, br s), 4.27 (2H, t, $J=5.5$ Hz), 6.40 (2H, d, $J=8.8$ Hz), 6.60 (2H, d, $J=8.8$ Hz), 6.89 (2H, d, $J=8.6$ Hz), 7.03 (4H, s), 7.13 (2H, d, $J=8.6$ Hz), 9.10 (2H, br s), 9.22 (1H, s). MS *m/z*: 415 (M^+). *Anal.* Calcd for $C_{28}H_{33}NO_2 \cdot HCl$: C, 74.40; H, 7.58; N, 3.10. Found: C, 74.37; H, 7.28; N, 3.06.

(*Z*)- and (*E*)-2-[4-(2-Hydroxy-1-methylethyl)phenyl]-1-(4-hydroxyphenyl)-1-[4-[2-(methylamino)ethoxy]phenyl]-1-butene (8a and 8b) Compound (**6**) (1.78 g) was added to a solution of vinyloxycarbonyl chloride (1.7 ml) in dichloromethane (40 ml) in a sealed tube and heated in an oil bath at 170 °C for 6 h. The reaction mixture was evaporated under reduced pressure and then the residue was dissolved in ethanol (50 ml) and added to 4 N NaOH (10 ml). After stirring under reflux for 5 h, the whole was evaporated under reduced pressure to give **8** as a crude solid (isomeric mixture of $E/Z=1/1$), which was purified by chromatography on a silica gel with chloroform–methanol–triethylamine (10 : 1 : 1) as an eluent. The yield was 1.06 g (61.5%). The isomeric mixture was separated in the same manner as described for **3a** and **3b**. From the first eluate, *Z*-isomer (**8a**) was obtained. mp 155—157 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.83 (3H, t, $J=7.0$ Hz), 1.14 (2H, d, $J=6.8$ Hz), 2.28 (3H, s), 2.73 (2H, t, $J=5.6$ Hz), 3.10—3.50 (2H, m), 3.85 (2H, t, $J=5.6$ Hz), 4.55 (1H, br s), 6.56 (2H, d, $J=9.1$ Hz), 6.71 (2H, d, $J=9.1$ Hz), 6.73 (2H, d, $J=8.6$ Hz), 6.97 (2H, d, $J=8.6$ Hz), 7.00 (4H, s). MS *m/z*: 431 (M^+). *Anal.* Calcd for $C_{28}H_{33}NO_3$: C, 77.93; H, 7.71; N, 3.25. Found: C, 77.88; H, 7.68; N, 3.13. From the second eluate, *E*-isomer (**8b**) was obtained. mp 180—182 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.84 (3H, t, $J=6.8$ Hz), 1.14 (3H, d, $J=6.8$ Hz), 2.34 (3H, s), 2.82 (3H, t, $J=5.6$ Hz), 3.00—3.50 (2H, m), 4.01 (2H, t, $J=5.6$ Hz), 4.55 (1H, br s), 6.39 (2H, d, $J=8.5$ Hz), 6.61 (2H, d, $J=8.5$ Hz), 6.91 (2H, d, $J=8.7$ Hz), 7.01 (4H, s), 7.05 (2H, d, $J=8.7$ Hz). MS *m/z*: 431 (M^+). *Anal.* Calcd for $C_{28}H_{33}NO_3$: C, 77.93; H, 7.71; N, 3.25. Found: C, 77.97; H, 7.61; N, 3.18.

Binding Affinity toward Estrogenic Receptor (*in Vitro*)[14] Uterus and tumors from female rats were obgained as soon as possible after decapitation, and stored under −80 °C until use. Tissues were homogenized in the buffer (20 mM Tris, 1.5 mM ethylenediaminetetraacetic acid (EDTA), 5% glycerol, 12 μM monothioglycerol, pH 7.8) using a Bitron homogenizer. The homogenate was then centrifuged at 105000 × *g* for 1 h, and the supernatant was subjected to an estrogenic receptor (ER) binding assay. In order to distinguish between specific and non-specific binding capacity, the non-specific binding was measured by using a 100-fold excess of non-radioactive estradiol. Relative binding affinity toward ER of uterine cytosol was determined in the competition experiments (18 h at 4 °C) using 5 nM (^{3}H)-estradiol (105 Ci/mmol) and increasing concentrations of antiestrogens or unlabeled estradiol. Relative binding affinity (RBA) is expressed by the following equation.

$$\mathrm{RBA}=\frac{\mathrm{IC}_{50}\text{ of estradiol}}{\mathrm{IC}_{50}\text{ of test compound}}\times 100$$

Antiuterotrophic Activity (*in Vivo*)[15] Female Sprague-Dawley rats (4 weeks old, 7 rats/group) were used to determine antiuterotrophic activities of the compounds. In order to estimate the value of inhibition without endogenous estrogen, the animals were ovariectomized under ether anesthesia. The rats were injected intraperitoneally for 3 d at daily doses of 40 μg/kg of each metabolite and 0.5 μg/body of estradiol as a control. Animals were sacrificed on the fourth day, and the wet weight of the uterus was measured. Percent inhibition of uterine growth was calculated based on the wet weight of the uterus using the following equation.

$$\text{inhibition }\%=\frac{(\text{control}-\text{tested})}{(\text{control}-\text{ovariectomized})}\times 100$$

References

1) A part of the present work was presented at the 16th International Congress of Chemotherapy, Jerusalem, Israel, June 1989, Abstracts of Papers, p. 281.
2) S. S. Legha. H. L. Davis, and F. M. Muggia, *Ann. Intern. Med.*, **88**, 69 (1978).
3) *a*) L. Kangas, A. L. Nieminen, G. Blanco, M. Gronroos, S. Kallio, A. Karjalainen, M. Perila, M. Södervall, and R. Toivola, *Cancer Chemother. Pharmacol.*, **17**, 109 (1986); *b*) W. Roos, L. Oeze, R. Lösen, and V. Eppenberger, *J. Natl. Cancer. Inst.*, **71**, 55 (1983).
4) T. Asao, T. Toko, Y. Sugimoto, S. Takeda, Y. Yamada, K. Ogawa, and M. Yasumoto, The 16th International Congress of Chemotherapy, Jerusalem, Israel, June 1989, Abstracts of Papers, p. 248.
5) P. K. Yonan, R. L. Novotney, C. M. Woo, K. A. Prodan, and F. M. Hershenson, *J. Med. Chem.*, **23**, 1102 (1980).
6) *a*) P. C. Ruenitz, J. R. Bagley, and C. M. Mokler, *J. Med. Chem.*, **25**, 1056 (1982); *b*) D. W. Robertson and J. A. Katzenellenbogen, *J. Org. Chem.*, **47**, 2387 (1982).
7) A. B. Foster, M. Jarman, O. T. Leung, R. McCague, G. Leclercq, and N. Devleeschouwer, *J. Med. Chem.*, **28**, 1491 (1985).

8) J. S. Baran, *J. Org. Chem.*, **25**, 257 (1960).
9) C. G. Scouten and H. C. Brown, *J. Org. Chem.*, **38**, 4092 (1973).
10) R. A. Olofson, R. C. Schnur, L. Bunes, and J. P. Pepe, *Tetrahedron Lett.*, **1977**, 1567.
11) J. Shani, A. Gazit, T. Livshitz, and S. Biran, *J. Med. Chem.*, **28**, 1504 (1985).
12) M. Makosza and A. Jonczyk, "Organic Syntheses," Coll. Vol. VI, ed. by W. E. Noland, John Wiley and Sons, Inc., New York, 1988, p. 897.
13) W. E. Parham and E. L. Anderson, *J. Am. Chem. Soc.*, **70**, 4187 (1948).
14) A. E. Wakeling, "Steroid Hormones," ed. by B. Green and R. E. Leake, IRL Press Ltd., Washington D.C., 1987, pp. 219—236.
15) V. C. Jordan, M. M. Collins, L. Rowsby, and G. Prestwich, *J. Endocrinol.*, **75**, 305 (1977).