were carried out in heavy water (D_2O) and are reported without correction for the deuterium isotope effect. An Orion digital pH meter was used for the pH measurements.

Synthesis of the Ligands. [(Thioethyl)amino]acetates (1-4). The derivatives were prepared according to literature procedures. Thus, 2-[(thioethyl)amino]acetic acid hydrochloride was prepared[20] from ethyl ethyleniminoacetate by the action of H_2S to -75 °C. The reaction product was subjected to acid hydrolysis to form 1. 2-[(Thioethyl)imino]diacetic acid **2** was isolated from [[(benzylthio)ethyl]imino]diacetic acid by the reaction of liquid ammonia and sodium metal.[21] Similarly, *N,N'*-bis(2-mercaptoethyl)ethylenediamine-*N,N'*-diacetic acid (**3**) was prepared from *N,N'*-bis[2-(benzylthio)ethyl]ethylenediamine-*N,N'*-diacetic acid.[22]

Bis[(thioethyl)amino]-*N,N,N',N'*-tetraacetic acid **4** was isolated by the action of chloroacetic acid to cystamine dihydrochloride.[22]

S-Substituted [(Thioethyl)imino]diacetates (5-8). The various thiols were converted to the corresponding S-substituted thioethylamines via ethylenimine reaction in -15 °C in methanolic solution.[23] The crude thioethylamines were treated with chloroacetic acid in aqueous media and alkaline pH to form the iminodiacetic acid derivatives as described for [[(benzylthio)-ethyl]imino]diacetic acid.[21] The derivatives were isolated from the alkaline solution by adjusting the pH to acidic.

Labeling Procedure. ^{99m}Tc Chelates 1-4. To 20 mg of the carrier in water solution was added, with stirring, 0.1 mL of 5 N HCl solution containing $SnCl_2$ (0.2 mg). The pH was then adjusted to 7 using 1 N NaOH. The mixture was filtered with a Millipore filter (0.22 μm), in an evacuated penicillin vial. $^{99m}TcO_4^-$ (30-40 μCi) was then added to the vial to give a final solution volume of 4 mL. The vial was agitated and left for 15 min at room temperature. The solution was then ready for further use.

N,N'-Bis(2-thioethyl)ethylenediamine-*N,N'*-diacetic acid ^{99m}Tc chelate **3** was prepared using 1 mg of the ligand, 0.4 mg of $SnCl_2$, and 0.7 mg of ascorbic acid as antioxidant. The radiolabeling of the derivatives **1-3** was carried out in a similar manner in acidic pH 3-4.

^{99m}Tc Chelates 5-8. Forty milligrams of the ligands was first dissolved at pH 7-7.5 with 1 N NaOH. $SnCl_2$ solution (0.4 mg in 0.1 mL of 5 N HCl) was added, and the pH of the mixture was adjusted again to 7. Radiolabeling with technetium-99m was then carried out as described above.

Radiochemical Analysis. ^{99m}Tc-[(thioethyl)amino] carboxylates were analyzed for complexed, reduced, or unbound technetium on silicic acid thin-layer strips (ITLC-s.a. Gelman Co.). The solvent systems used were acetonitrile-water (1:1) and 2-propanol for ^{99m}Tc complexes **1-4**, while acetonitrile-water (3:1) and NaCl (0.9%) were used for complexes **5-8**. The chromatograms were cut into sections of 0.5 cm and counted in a well-type γ-counter. The percent activity was determined, and the R_f values recovered were compared to those found for pertechnetate anion or reduced technetium.

Tissue Distribution Studies. Each radioactive solution (0.2 mL; 2 μCi) was administered intravenously into the tail vein of male swiss albino mice (20-25 g). The animals were put in metabolic cages in order to collect urine. The mice were killed 60 min postinjection with ether vapors, and the liver, kidneys, blood, intestines, stomach, muscles, and urine were dissected out, and the activity was measured. Urination of the animals during death was avoided by ligation of the penis. The organs or tissue samples collected were measured in a γ-counter (ICN-gamma set 500), and the percentage of the injected activity in each organ was calculated. Counts of the tail were subtracted from the total dose to obtain the total injected dose, to correct for any injected dose that infiltrated the tail and did not enter the circulation. Biodistribution studies in mice of ^{99m}Tc-mercaptosuccinate (^{99m}Tc-DMSA, Sorin Biomedica) and ^{99m}Tc-[(2,6-diethylacetanilido)imino]diacetate (^{99m}Tc-EHIDA, Solco Co.) were performed for comparison.

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Notes

Effect of *N,N'*-Diethyl-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamines on the 7,12-Dimethylbenz[*a*]anthracene-Induced Mammary Carcinoma of the Rat

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The syntheses and estrogen receptor affinities of *meso*- and (±)-*N,N'*-dialkyl-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamines (**2**) are described. They show high binding affinities in both diastereomeric forms but with a preference for the meso isomer, reaching a RBA value of 8.6 (*meso*-**2b**; 17β-estradiol = 100). Both stereoisomers of **2b** exhibit a strong inhibitory effect on the 7,12-dimethylbenz[*a*]anthracene (DMBA) induced hormone-dependent mammary carcinoma of the Sprague-Dawley rat, reducing the tumor area by 74 (*meso*-**2b**) and 24% [(±)-**2b**], respectively, after 4 weeks administration of 6 × 6 (mg/kg)/week. The high uterotrophic potency makes a mode of action likely which corresponds to the effect of high doses of estrogens.

In two previous papers we have reported the affinity of 1,2-bis(2,6-dichlorophenyl)ethylenediamines for the estradiol (E2) receptor[1] and the inhibitory activity of estrophilic 1,2-bis(4-hydroxyphenyl)ethylenediamines against the 7,12-dimethylbenz[*a*]anthracene (DMBA) induced hormone-dependent mammary carcinoma of the Sprague-Dawley rat.[2] It has been shown that the effect of mammary tumor inhibiting compounds correlates with their binding affinity for the estrogen receptor.[3] One way to increase the receptor affinity of the latter ethylenediamines can be the introduction of chlorine atoms into the

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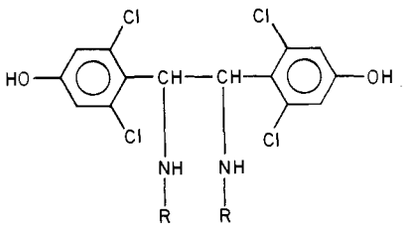
Table I. *N,N'*-Dialkyl-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamines (2)



compd	R	mp, °C	formula[a]	^{1}H NMR[b] chem shift, δ (*J* in hertz) aromatic H	benzylic H	alkyl H	RBA[c]
meso-2a	CH_3	198-200[d]	$C_{16}H_{16}Cl_4N_2O_2$	6.63 (s)	5.17 (s)	2.02 (s)	2.3
(±)-2a	CH_3	160-162	$C_{16}H_{16}Cl_4N_2O_2$	6.55 (d, *J* = 2) 6.35 (d, *J* = 2)	5.00 (s)	2.20 (s)	1.3
meso-2b	C_2H_5	147-150	$C_{18}H_{20}Cl_4N_2O_2$	6.60 (s)	5.17 (s)	2.30, 2.27 (q, *J* = 7) 0.88 (t, *J* = 7)	8.6
(±)-2b	C_2H_5	152-153	$C_{18}H_{20}Cl_4N_2O_2$	6.55 (d, *J* = 2) 6.33 (d, *J* = 2)	5.05 (s)	2.38 (q, *J* = 7) 1.10 (t, *J* = 7)	2.1
meso-2c	C_3H_7	167-169	$C_{20}H_{24}Cl_4N_2O_2$	6.63 (s)	5.17 (s)	2.20 (t, *J* = 7) 1.27 (sext, *J* = 7) 0.68 (t, *J* = 7)	8.2
(±)-2c	C_3H_7	155-157	$C_{20}H_{24}Cl_4N_2O_2$	6.52 (d, *J* = 2.5) 6.30 (d, *J* = 2.5)	5.03 (s)	2.35 (t, *J* = 7) 1.52 (sext, *J* = 7) 0.87 (t, *J* = 7)	1.0

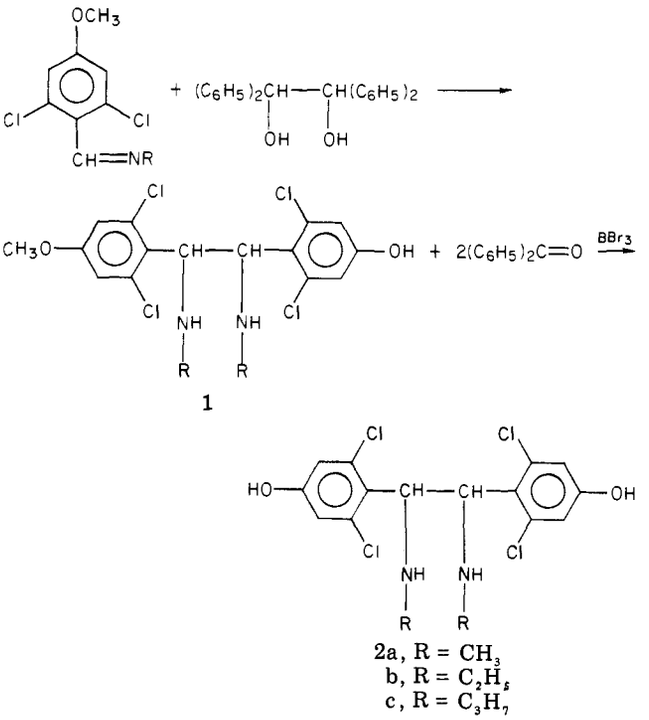
[a] All compounds were analyzed for C and H within ±0.40% of the calculated values, except *meso*-2c; C: calcd, 51.52; found, 50.81; H: calcd, 5.19; found, 5.63. [b] Dissolved in CD_3OD + NaOD, Me_4Si as internal standard. [c] Relative binding affinities for the estrogen receptor = ratio of molar concentrations of E2 and inhibitor required to decrease the amount of bound [^{3}H]E2 by 50% × 100. [d] Dihydrochloride.

ortho positions of the phenyl rings, leading to a higher lipophilicity in the center of the molecule. The contribution of chlorine atoms to the receptor binding has been demonstrated before.[1]

Chemistry. The *N,N'*-dialkyl-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamines (**2**) were synthesized as shown in Scheme I. Reductive dimerization of the corresponding 2,6-dichloro-4-methoxybenzaldehyde imines by 1,1,2,2-tetraphenylethanediol afforded a mixture of the diastereomeric ethylenediamines, which were separated by crystallization. The structures were assigned by converting them into imidazolidines by formaldehyde and studying the ^{1}H NMR spectra of the latter[1,4] (data not shown). The phenolic compounds were obtained by ether cleavage with BBr_3. A characteristic difference between the meso and the (±) form was observed in the ^{1}H NMR spectrum: The aromatic protons of the racemates appeared as two doublets [δ 6.33, 6.55 for (±)-**2b** in CD_3OD/NaOD) with a meta coupling constant of 2 Hz; the meso-isomers showed the expected singlet at δ 6.60 (*meso*-**2b**). The doublets merge in a singlet above 130 °C in C_4Cl_6, indicating a restricted rotation around the phenyl C-1 axis at room temperature.

Biological Properties. The binding affinity for the calf uterine estrogen receptor was measured by a competitive binding assay with [^{3}H]estradiol and the dextran-charcoal method.[5] We found a strong increase of receptor affinity in comparison to diarylethylenediamines lacking either the hydroxy groups (RBA values ≤ 0.03)[1] or the chlorine atoms (RBA values ≤ 0.3)[2] (Table I). Although the relative binding affinities of **2** were not determined concurrently with other ethylenediamines mentioned, the differences of more than one order of magnitude exceed experimental deviations considerably. The *N,N'*-diethyl compound (*meso*-**2b**) showed the highest binding affinity, reaching

Scheme I



a RBA value of 8.6 (17β-estradiol = 100). Surprisingly, there was no great difference between the RBA values of meso and racemic forms, which we usually observe in synthetic estrogens like hexestrol and its derivatives.[3]

The diastereomeric ethyl compounds *meso*- and (±)-**2b** were tested for their inhibitory activity against the established DMBA-induced mammary carcinoma of the rat, which is believed to have many similarities with the hormone-dependent human breast cancer.[6] For this exper-

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Table II. Effect of 2b on the DMBA-Induced Mammary Carcinoma of the Sprague-Dawley Rat

compd	dose,[a] mg/kg	no. of animals	no. of tumors[b]	new tumors	complete remission,[c] %	partial remission,[d] %	static tumors,[e] %	progr tumors,[f] %	change of body wt,[g] %	change of tumor area,[h] %
control		10	25	41	3	18	38	41	+5	+235
(±)-2b	6	10	28	15	35	26	23	16	−2	−24[i]
meso-2b	6	10	26	6	28	53	16	3	−4	−74[i]
control		10	32	64	9	3	20	68	+4	+620
(±)-2b	2	10	34	18	14	11	21	54	+0	+282[j]
meso-2b	2	9	35	7	50	14	10	26	−6	+29[i]

[a] Dissolved in olive oil. The animals received a single dose daily from Monday to Thursday and a double dose on Friday. [b] At the beginning of the test. [c] Tumor not palpable. [d] Reduction of initial tumor size ≤ 50%. [e] Tumor size 51-150% of the initial size. [f] Tumor size > 150% of the initial size. [g] Average on the 7th day of therapy. [h] Average on the 28th day of therapy. The *U* test according to Wilcoxon, Mann, and Whitney was used to determine the significance. [i] Significant ($p < 0.01$). [j] Significant ($p < 0.05$).

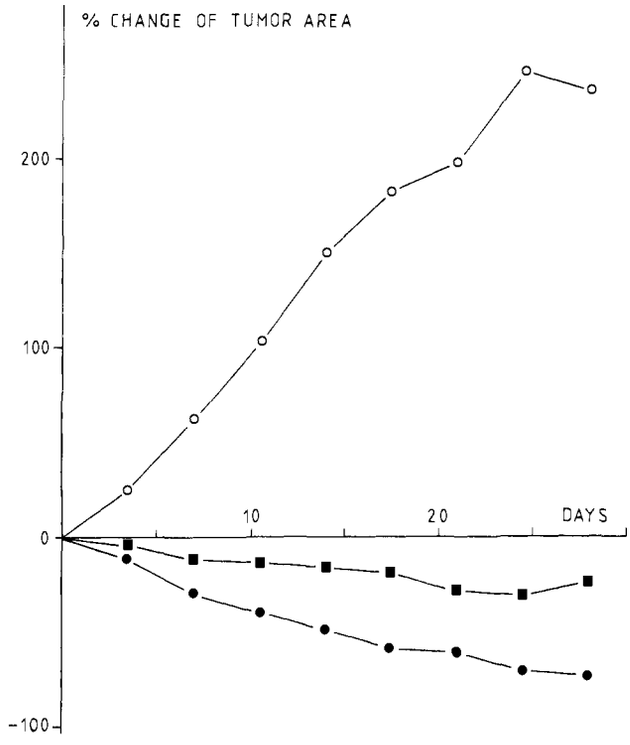

Figure 1. The effect of **2b** on the tumor area of SD rat bearing DMBA-induced mammary carcinoma. Animals were randomized in groups of 10 between the 35th and 70th day after tumor induction and treated simultanously: control (○); *meso*-**2b**, from Monday to Thursday, 6.0 (mg/kg)/day sc, on Friday, double dose (●); (±)-**2b**, from Monday to Thursday, 6.0 (mg/kg)/day sc, on Friday, double dose (■).

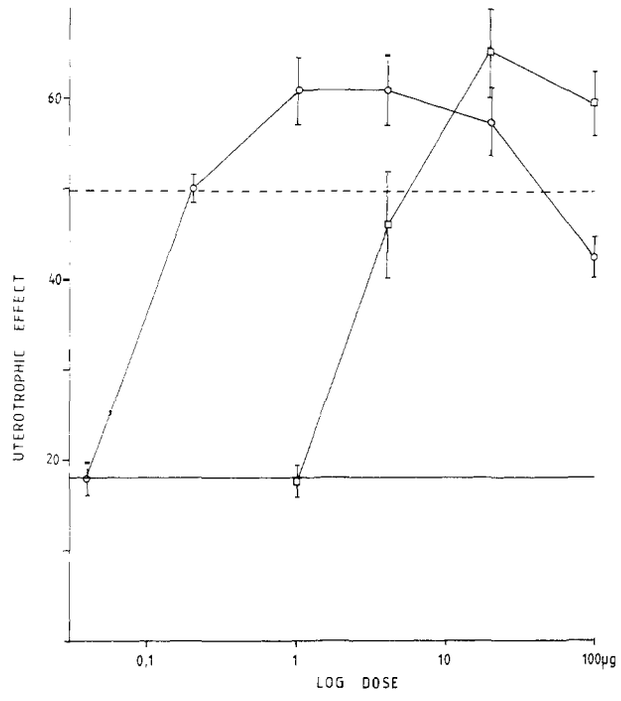

Figure 2. Uterotrophic activity of *meso*- and (±)-**2b** in the mouse uterine weight test. Compounds were administered at 3 consecutive days sc; the uteri were removed 24 h after the last injection. The uterotrophic effect is given as uterine dry weight (mg)/body weight (g) × 100; the values are means of 10 animals/group ± SD. The control (oil vehicle) uterine weights are shown as a solid horizontal line, the uterine weights after administration of 0.4 µg of estrone are shown as a dashed line: *meso*-**2b** (○); (±)-**2b** (□).

iment, animals that developed tumors between the 5th and 10th week after administration of DMBA were used. The estrogen dependence of these tumors can be demonstrated by ovariectomy, leading to a complete regression of most of the tumors.[3] A strong inhibition of the tumor growth was observed. In accordance with the higher receptor affinity, the meso form produced the better results. The administration of 6 × 6 (mg/kg)/week led to a strong reduction of the original tumor size (Figure 1); a high percentage of the tumors regressed after treatment with 6 × 2 and 6 × 6 (mg/kg)/week (Table II). The higher dose affected only the tumor size. A dose dependence can be assumed by these results but is not proved because of the great difference in the tumor growth of the control groups (235 vs. 620%). We can not offer an explanation for this difference, which is often observed.[3]

In order to find out by which mode of action the antitumor effect was accomplished, we determined the uterotrophic activity of **2b** as a measure of estrogenicity. The semilogarithmic plot of uterotrophic response vs. dose of **2b** showed two parallel curves with a slope typical of true estrogens (Figure 2). In the mouse, the full uterotrophic effect was reached after administration of 1 µg of *meso*-**2b** or 20 µg of (±)-**2b** respectively. In the rat, similar curves were obtained with a maximum stimulation by 25 µg of *meso*-**2b** or 100 µg of (±)-**2b**, respectively. No antiuterotrophic effect was observed in the mouse after simultaneous administration of 0.4 µg of estrone and various doses of **2b** (data not shown).

Discussion

In this and previous studies,[1,2] we have tried to develop antiestrogens by substitution of the methylene groups of hexestrol by imino groups. The N-isosteric hexestrol did not exhibit any estrogen receptor affinity, but this affinity

can be restored be elongation of the alkyl chains[2] and, in a better way, by introduction of two chlorine atoms in the 2,6-positions of the phenyl rings. The estrogenic potency that was lost also reappeared in the latter compounds to an extent which correlates to the binging affinities. Interestingly, the diastereomeric forms exhibited rather similar receptor affinities with a preference for the meso compounds. For comparison, the RBA value of *meso*-hexestrol is 100 times higher than the value for the racemate,[7] and only the (±)-1,2-bis(4-hydroxyphenyl)-ethylenediamines[2] showed receptor affinity but not the corresponding meso forms.

Two reasons for the binding affinities of both stereoisomers of **2** have to be considered: an increase of lipophilicity in the center of the molecule and a steric arrangement favoring a good binding interaction with the receptor site. The first effect explains the enhancement of affinity of the racemic ethylenediamines but can not be solely responsible for the binding of the meso form, which is inactive without chlorine substituents. From studies of Katzenellenbogen et al.,[7] we know, that only the antiperiplanar arrangement of the phenyl rings allows a strong binding interaction with the receptor site. Because of the restricted rotations in the molecule, it can be demonstrated by ^{1}H NMR spectroscopy that the aromatic rings are probably arranged in this way: Considering a plane going through C-1 and C-2 of the ethane skeleton, as well as through C-1 and C-4 of the phenyl rings, the aromatic protons of the racemate lie in a different surrounding, which explains the appearance of two doublets. In the meso compound, the amino groups are located on both sides of this plane, leading to a similar influence on all aromatic protons and, consequently, to a singlet. By these considerations, it seems likely that the four chlorine atoms in the ortho positions force these ethylenediamines into the antiperiplanar conformation.

Although we failed to synthesize an estrogen antagonist, we have obtained a potent new estrogen with a strong mammary tumor inhibiting effect. The possibility of a tumor growth stimulation at low doses was not checked, since we know from previous experiments[8,9] that only tumors from ovariectomized rats can be stimulated significantly. For the inhibition of the tumor growth, we assume a mode of action similar to the effect of high doses of estrogens[10] because we have only observed estrogenic properties in the uterine weight test.

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(9) Griswold, D. P., Jr.; Green, C. H. *Cancer Res.* **1970**, *30*, 819.
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Experimental Section

Melting points were determined on a Büchi 510 apparatus and are uncorrected. Elemental analyses were performed by the Mikroanalytisches Laboratorium, University of Regensburg, and were within ±0.4% of the calculated values. NMR spectra were obtained on a Varian EM 360A spectrometer and were consistent with the assigned structures.

General Preparation of 2,6-Dichloro-4-methoxybenzaldehyde Alkylimines. The alkylamine (0.33 mol) was added to a stirred solution of 2,6-dichloro-4-methoxybenzaldehyde (0.30 mol) in $CHCl_3$ (300 mL) and heated to 50 °C for 1 h. After the solution was cooled, the organic layer was separated and dried over $MgSO_4$. The solvent was removed in vacuo, and the residue used without further purification.

General Preparation of *N*,*N*′-Dialkyl-1,2-bis(2,6-dichloro-4-methoxyphenyl)ethylenediamines (1). A mixture of 2,6-dichloro-4-methoxybenzaldehyde alkylimine (0.04 mol) and 1,1,2,2-tetraphenyl-1,2-ethanediol (0.04 mol) in 2-propanol (200 mL) was refluxed for 24 h. After the mixture was cooled, the precipitate containing the meso isomer was separated by filtration. The solvent was removed from the mother liquor to afford a brown oil, from which the (±) isomer (±)-1 was obtained by column chromatography (SiO_2, elution with CH_2Cl_2, Et_2O and MeOH subsequently). The (±) isomers of **2b** and **2c** resisted crystallization and were used without further purification; the yields were 25–30%; (±)-**2a** was recrystallized from MeCN, mp 110 °C. The precipitate was dissolved in ether. After extraction with HCl (2 N) the aqueous solution was made alkaline with NaOH (2 N) and extracted several times with $CHCl_3$. The organic layer was washed with water and dried over $MgSO_4$ to afford a crystalline solid after evaporation of the solvent; the yields were between 30 and 35%. The product was recrystallized from EtOH: mp of *meso*-**1a**, 185–186 °C; mp of *meso*-**1b**, 157–159 °C; mp of *meso*-**1c**, 172–173 °C.

General Procedure of Ether Cleavage. *N*,*N*′-Dialkyl-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamines (2). A solution of 1 (0.01 mol) in dry CH_2Cl_2 (150 mL) was cooled to −60 °C under a nitrogen atmosphere, and BBr_3 (0.04 mol) was added with stirring. After 30 min, the cooling bath was removed, and the reaction mixture was kept at room temperature for 15 h. With cooling, the mixture was poured into an aqueous solution of $NaHCO_3$. The organic layer was separated, and the aqueous phase was extracted three times with EtOAc. The combined organic layers were dried ($MgSO_4$). After evaporation of the solvent, the residue was recrystallized from MeOH/Et_2O; the yields ranged from 55 to 72%. Melting points are reported in Table I.

Biological Methods. All procedures have been described previously in detail and were used without alterations. We applied the following methods: estrogen receptor binding assay with calf uterine cytosol as receptor source,[5] Dorfman uterine weight test with the dry weight of mouse uteri,[5] and growth inhibition of the established DMBA-induced mammary carcinoma of the SD rat.[5]

Acknowledgment. The author thanks Professor H. Schönenberger for helpful discussions, Mrs. M. Horn, Miss G. Dorn, and Mr. R. Ringshandl for their excellent technical assistance, and the Deutsche Forschungsgemeinschaft for financial support.