The MeOH solution was filtered and concentrated to yield the product as a white foam (2 g, 77% yield).

N^{α}-(*tert*-Butoxycarbonyl)-N^{G},$N^{G'}$-dimethyl-D-arginine (9a). N^{G},$N^{G'}$-Dimethyl-D-arginine (**7a**) was synthesized as described.[28] The crude product was not purified, but was converted directly to the N-protected compound **9a** as described for **10c**.

Registry No. 1, 82778-58-3; 2, 81608-49-3; 4, 89662-37-3; 6b, 98500-63-1; 6c, 98500-64-2; 6d, 89662-39-5; 6e, 98500-66-4; 6f, 98500-70-0; 6g, 98500-61-9; 7a, 110797-83-6; 8b, 110798-01-1; 8c, 110798-02-2; 8d, 110798-03-3; 8e, 110798-04-4; 8f, 110798-05-5; 8g, 110798-06-6; 9a, 110797-84-7; 10a, 98500-76-6; 10b, 110798-07-7; 10c, 110798-08-8; 10d, 110798-09-9; 10e, 110798-10-2; 10f, 110798-11-3; 10g, 110798-12-4; 11, 86855-16-5; 12, 90684-94-9; 13, 89662-32-8; 14, 89662-33-9; 15, 89662-29-3; 16, 89662-27-1; 17, 89662-28-2; 18, 89680-24-0; 19, 89662-30-6; 20, 110797-85-8; 21, 110797-86-9; 22, 110797-87-0; 23, 110850-65-2; 24, 110797-88-1; 25, 110797-89-2; 26, 93128-18-8; 27, 110797-90-5; 28, 110825-66-6; 29, 110797-91-6; 30, 89662-20-4; 31, 89662-21-5; 32, 89662-22-6; 33, 89662-18-0; 34, 89662-13-5; 35, 110797-92-7; 36, 89662-25-9; 37, 110797-93-8; 38, 89680-25-1; 39, 89662-31-7; 40, 106916-57-8; 41, 106881-68-9; 42, 110797-94-9; 43, 110797-95-0; 44, 110797-96-1; 45, 110797-97-2; 46, 110797-98-3; 47, 110797-99-4; 48, 110798-00-0; DCC, 538-75-0; EtN=C=NEt, 693-29-8; PrN=C=NPr, 821-79-4; *i*-PrN=C=NPr-*i*, 693-13-0; BuN=C=NBu, 693-64-1; $H_3C(CH_2)_5N{=}C{=}N(CH_2)_5CH_3$, 13296-55-4.

Ring-Substituted [1,2-Bis(4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) Complexes: Compounds with a Selective Effect on the Hormone-Dependent Mammary Carcinoma

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[1,2-Bis(4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) complexes with one substituent in the 2-position (CH_3, CF_3, F, Cl, Br, I: *meso*- and *d,l*-1-$PtCl_2$, *meso*-(3–5)-$PtCl_2$, *meso*-(7 and 8)-$PtCl_2$) or two substituents in the 2,6-positions (CH_3, Cl: *meso*-2-$PtCl_2$, *meso*- and *d,l*-6-$PtCl_2$) in both benzene rings were synthesized and tested for estrogenic and cytotoxic activities. Two complexes (*meso*-6-$PtCl_2$ and *meso*-7-$PtCl_2$) possess both effects. In comparative tests on estrogen receptor positive and negative mammary tumors in cell culture (MCF 7, ER^+ and MDA-MB 231, ER^-) and in animals (MXT, ER^+ and MXT, ER^-, mouse), *meso*-6-$PtCl_2$ shows a selective effect on the estrogen receptor positive mammary carcinoma. A further increase of efficacy was achieved with the water-soluble (sulfato)platinum(II) derivative (*meso*-6-$PtSO_4$). On the DMBA-induced hormone dependent mammary carcinoma of the SD rat, *meso*-6-$PtSO_4$ is significantly more active than its ligand (*meso*-6) and cisplatin.

Platinum complexes that contain an estrogen receptor (ER) affinic ligand should be enriched in the nuclei of hormone-dependent breast cancer cells by the receptor system, thereby causing a selective effect on this tumor (Figure 1). The first compounds of this type, the stereoisomeric [1,2-bis(4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) complexes, possess a marked activity on several ER-negative tumor models, e.g., ADJ/PC 6 plasmacytoma/mouse and P 388 leukemia/mouse,[1] but only a low activity on the 9,10-dimethyl-1,2-benzanthracene (DMBA) induced, hormone-dependent mammary carcinoma of the Sprague–Dawley (SD) rat ((±) compound: 6 × 10 mg/kg per day, ip, duration of therapy 4 weeks, increase of tumor area 238%, control 686%).[2] To achieve a stronger mammary tumor inhibiting activity, we introduced CH_3, CF_3, or Hal residues into positions 2 or 2,6 of both benzene rings of [1,2-bis(4-hydroxyphenyl)-ethylenediamine]dichloroplatinum(II), since by this kind of substitution in the class of 1,2-bis(4-hydroxyphenyl)-ethanes an increase of ER affinity was accomplished.[3] In addition we have shown that the introduction of two Cl atoms into 2,6-positions of *N,N'*-dialkyl-1,2-bis(4-hydroxyphenyl)ethylenediamines leads to compounds that have a high binding affinity to ER and a strong inhibitory effect on the DMBA-induced, hormone-dependent mammary carcinoma of the SD rat.[4]

Chemistry. The diastereomeric dichloroplatinum(II) complexes 1-$PtCl_2$ to 8-$PtCl_2$ were synthesized by reacting K_2PtCl_4 with the 2- and 2,6-substituted 1,2-bis(4-hydroxyphenyl)ethylenediamines **1–8** in *t*-BuOH/H_2O or DMF/H_2O solution at pH 5–6 and a temperature below 40 °C (Scheme I, methods A and B).

Owing to steric facts, the formation of meso-configurated complexes proceeds very slowly and requires a reaction time up to 3 days. The analytical data are listed in Table I. The new compounds 1-$PtCl_2$ to 8-$PtCl_2$ show IR spectra typical for [diamine]dichloroplatinum(II) complexes: (1) the N–H stretching vibration is decreased due to the formation of the metal–nitrogen bond (free ligand ν NH = 3400–3300 cm^{-1}; Pt bond ligand ν NH = 3300–3100 cm^{-1}); (2) two absorption bands appear in the far infrared region, one between 650 and 450 cm^{-1}, indicating a Pt–N stretching vibration, and another between 345 and 320 cm^{-1}, indicating a Pt–Cl stretching vibration.[5] The ^1H

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[∥] Technische Universität München.

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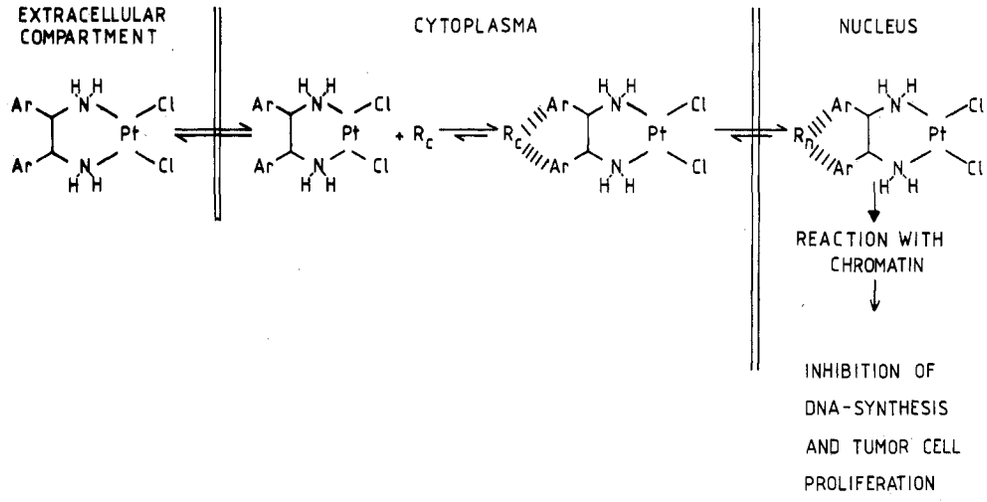
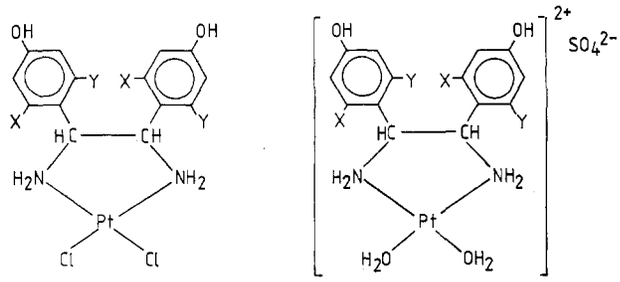


Figure 1. Proposed mechanism of action of platinum complexes with estrogen-receptor affinity: selective mammary tumor inhibiting activity by estrogen receptor mediated enrichment in the nucleus.

Table I. Ring-Substituted [1,2-Bis(4-hydroxyphenyl)ethylenediamine]platinum(II) Complexes

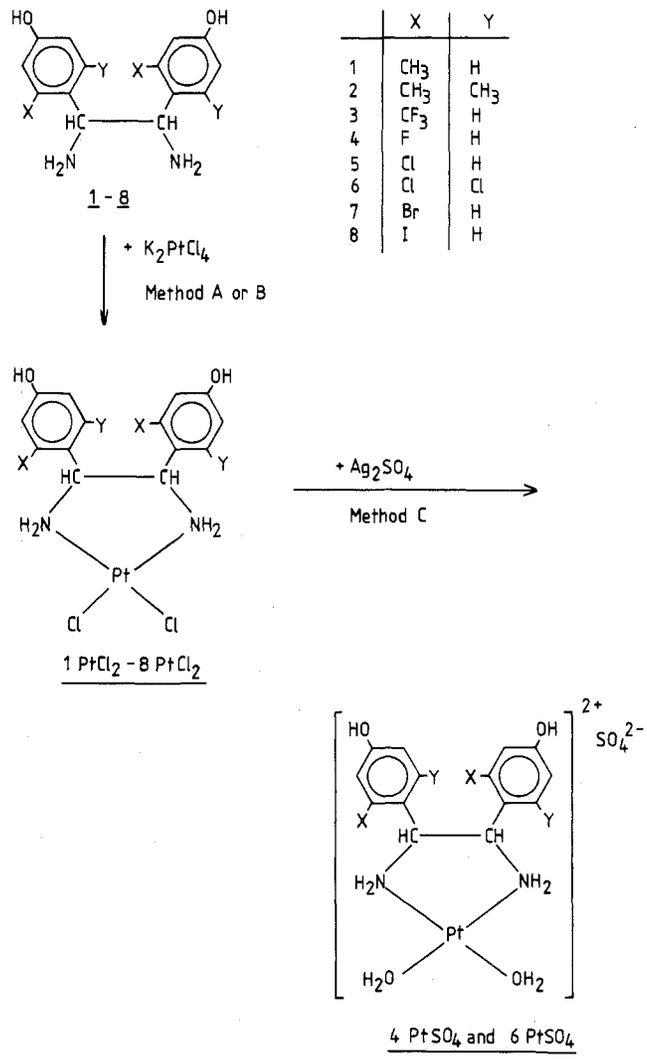


compd	X	Y	synth meth[a]	yield,[b] %	formula[c]
meso-1-$PtCl_2$	CH_3	H	A	80	$C_{16}H_{20}Cl_2N_2O_2Pt$
d,l-1-$PtCl_2$	CH_3	H	A	90	$C_{16}H_{20}Cl_2N_2O_2Pt$
meso-2-$PtCl_2$	CH_3	CH_3	B	90	$C_{18}H_{24}Cl_2N_2O_2Pt$
meso-3-$PtCl_2$	CF_3	H	A	60	$C_{16}H_{14}Cl_2F_6N_2O_2Pt$
meso-4-$PtCl_2$	F	H	A	73	$C_{14}H_{14}Cl_2F_2N_2O_2Pt$
meso-4-$PtSO_4$	F	H	C	98	$C_{14}H_{18}F_2N_2O_8SPt$
meso-5-$PtCl_2$	Cl	H	A	90	$C_{14}H_{14}Cl_4N_2O_2Pt$
meso-6-$PtCl_2$	Cl	Cl	A	64	$C_{14}H_{12}Cl_6N_2O_2Pt$
meso-6-$PtSO_4$	Cl	Cl	C	38	$C_{14}H_{16}Cl_4N_2O_8SPt$
d,l-6-$PtCl_2$	Cl	Cl	A	83	$C_{14}H_{12}Cl_6N_2O_2Pt$
meso-7-$PtCl_2$	Br	H	A	84	$C_{14}H_{14}Br_2Cl_2N_2O_2Pt$
meso-8-$PtCl_2$	I	H	A	90	$C_{14}H_{14}Cl_2I_2N_2O_2Pt$

[a] Synthetic methods A, B, and C: see Experimental Section. [b] Calculated with regard to the corresponding diamine. [c] All compounds were analyzed for C, H, and N within ±0.40% of the calculated values.

NMR spectra are also characteristic for [diamine]dichloroplatinum(II) complexes: all absorption bands of the ethylenediamines are shifted to lower fields by formation of the platinum complexes. Particularly the amine, benzylic, and ortho-localized aromatic protons are shifted to greatest extent. Since complexation blocks rotation around the C–N axis, both N-bound protons become diastereotopic owing to the neighborhood of asymmetric C atoms. This leads to the appearance of separate signals for the axially and equatorially arranged NH atoms. Due to a coupling between NH_2, benzylic CH, and ^{195}Pt, the NH and CH signals are broadened. With the *R,S*-configurated 2,6-disubstituted [1,2-bis(4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) complexes, *meso*-2-$PtCl_2$ and *meso*-6-$PtCl_2$, only one broad signal for NH and one for CH are observed (Figure 2, see supplementary material).

Scheme I

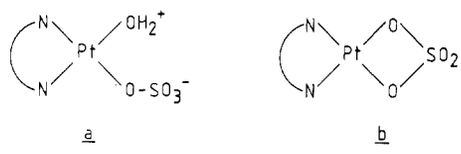


	X	Y
1	CH_3	H
2	CH_3	CH_3
3	CF_3	H
4	F	H
5	Cl	H
6	Cl	Cl
7	Br	H
8	I	H

This phenomenon can be explained by a rapid interchange between the δ and λ conformers relative to the NMR relaxation time (Figure 4).

On the other hand, two signals for the NH_2 group and one for the benzylic CH group in the spectrum of *d,l*-6-$PtCl_2$ (Figure 3, see supplementary material) suggest the presence of only one conformer, presumably that with equatorially arranged phenyl rings. Our conception of the conformation of *meso*-6-$PtCl_2$ and *d,l*-6-$PtCl_2$ corresponds

Scheme II



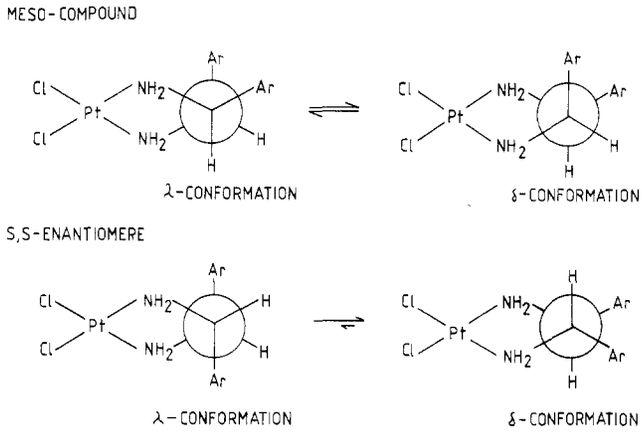


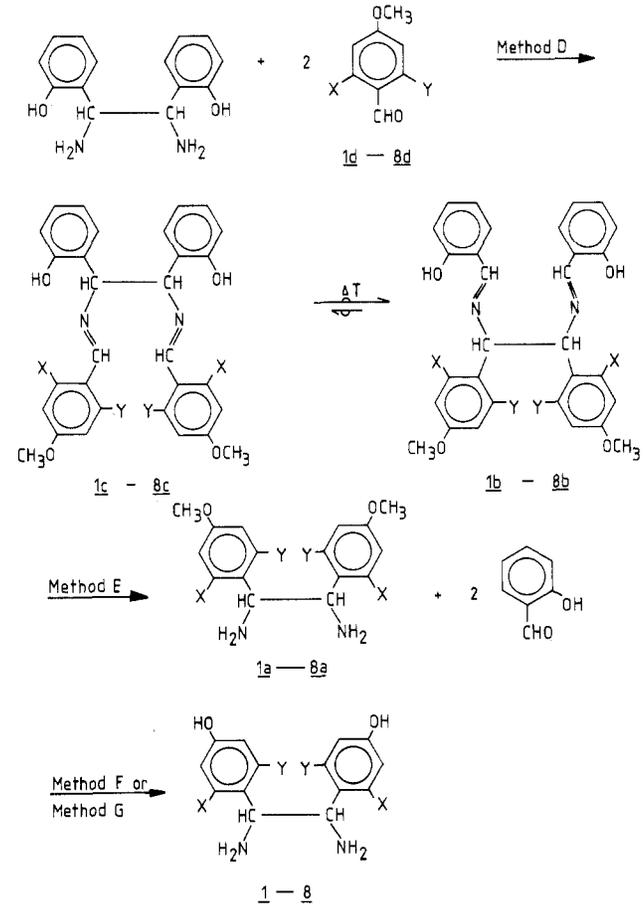
Figure 4. Conformation equilibria of stereoisomeric [diphenylethylenediamine]dichloroplatinum(II) complexes.

with that of Yano et al.[6] obtained with $[(en)Pt(stien)]^{2+}$. They could show by means of coupling constants between ^{195}Pt, ^{1}H, and ^{13}C that *S,S*- and *R,R*-configurated five-membered chelates of $[(en)Pt(stien)]^{2+}$ exist exclusively in the energetically favored δ and λ conformations, respectively (equatorially arranged phenyl rings). In contrast, the related *R,S*-configurated diastereomer interconverts rapidly between energetically equivalent conformers. Studies on the conformation of stereoisomeric (1,2-diphenylethylenediamine)(sulfato)platinum(II) complexes by means of circular dichroism spectrometry lead to the same result.[7]

The (sulfato)platinum(II) complexes *meso*-**4**-$PtSO_4$ and *meso*-**6**-$PtSO_4$ were synthesized by addition of Ag_2SO_4 to a suspension of *meso*-**4**-$PtCl_2$ or *meso*-**6**-$PtCl_2$, respectively, in H_2O at 40 °C (Scheme I, method C).[8] Different formulations for (sulfato)platinum(II) complexes have been reported in the literature: unidentate complex (a),[9] bidentate complex (b),[10] bridged bidentate complex (c),[11] and complex with free sulfate ion (d)[12] (see Scheme II).

On the basis of elemental analyses, we suppose structure d for *meso*-**4**-$PtSO_4$ and *meso*-**6**-$PtSO_4$. Also the IR spectra of both complexes are in accordance with structure d. They show the typical bands for the free sulfate ion (*meso*-**4**-$PtSO_4$, 1170–1120 cm^{-1} (vs, ν_3), 640 cm^{-1} (s, ν_4); *meso*-**6**-$PtSO_4$, 1150–1125 cm^{-1} (vs, ν_3), 620 cm^{-1} (s, ν_4)). However, a contamination by other coordination types cannot be excluded, as in the spectra of *meso*-**4**-$PtSO_4$ and *meso*-**6**-$PtSO_4$ further bands appear between 1060 and 970 cm^{-1} (*meso*-**4**-$PtSO_4$, 1170–1120 cm^{-1} (vs, ν_3), 1060 cm^{-1} (w, ν_3), 980 cm^{-1} (m, ν_1), 640 cm^{-1} (s, ν_4), 465 cm^{-1} (w, ν_2); *meso*-**6**-$PtSO_4$, 1150–1125 cm^{-1} (vs, ν_3), 1050 cm^{-1} (w, ν_3), 970 cm^{-1} (w, ν_1), 620 cm^{-1} (s, ν_4), 460 cm^{-1} (w, ν_2)). These bands are typical for the coordination of the sulfate ion through one oxygen atom (unidentate complex type a).[11,13] *meso*-**4**-$PtSO_4$ and *meso*-**6**-$PtSO_4$ are fairly soluble in water (approximately 52 and 4 mg/mL, respectively). The solubility in water can be increased by addition of polyethylene glycol 400, which is important for therapeutic use. In water solution the sulfato residue of unidentate and bidentate (sulfato)platinum(II) complexes (complex types a, b, and c) is quickly replaced by H_2O molecules, forming the diaquaplatinum(II) ion (complex type d). In accordance with this, an increase of conductance to a constant level can be observed within a few minutes.[14] Therefore, conductance measurements are not suitable for the distinction of complex types a, b, and c from type d. A constant conductance value was also rapidly attained with *meso*-**4**-$PtSO_4$ and *meso*-**6**-$PtSO_4$ (Figure 5, see supplementary material; Λ values in Ω^{-1} cm^2 mol^{-1}; for *meso*-**4**-

Scheme III[a]



[a] Substituents: see Scheme I.

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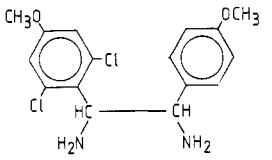


9a

Figure 6. Structural formula of **9a**.

$PtSO_4$, 360.5, 5.0×10^{-5} M water solution; for *meso*-**6**-$PtSO_4$, 253.8, 5.0×10^{-5} M water solution).

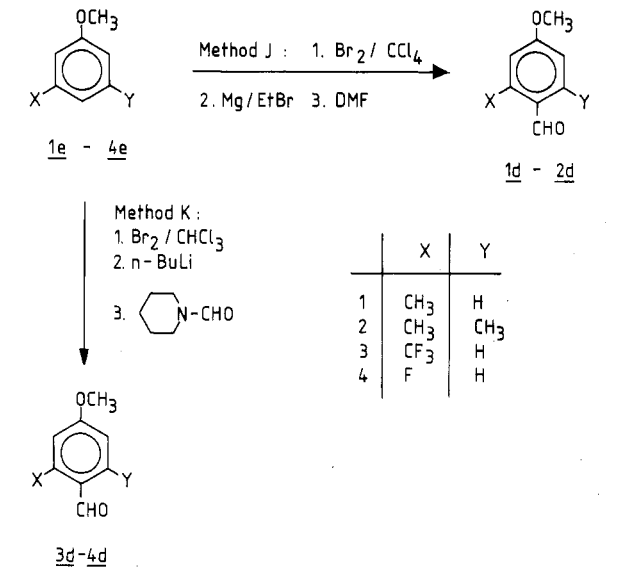
The 2- and 2,6-substituted 1,2-bis(4-hydroxyphenyl)-ethylenediamine ligands were synthesized by the [3,3] sigmatropic diaza-Cope rearrangement reaction according to the method of Vögtle and Goldschmitt.[15] At a temperature below 120 °C, *N,N'*-disalicylidene-*meso*-1,2-bis-(4-methoxyphenyl)ethylenediamines **1b** and **3b–8b** were formed quantitatively from *meso*-1,2-bis(2-hydroxyphenyl)ethylenediamine[7] and the anisic aldehydes **1d** and **3d–8d** via Schiff bases **c** in a stereospecific reaction (method D, Scheme III; chemical data, see Table II in supplementary material). However, in the case of diimine **2b**, an equilibrium between **2b** and **2c** turns up due to steric conditions. By hydrolysis (method E) and ether cleavage either with BBr_3 or 47% HBr (methods F and G) of **1a–8a**, the *meso*-1,2-bis(4-hydroxyphenyl)ethylenediamines **1–8** were generated. The *d,l*-configurated compound *d,l*-**1a** was achieved by the meso $\rightleftharpoons$ *d,l* stereoisomerization of *N,N'*-bis(4-methoxy-2-methylbenzylidene)-*meso*-1,2-bis(4-methoxy-2-methylphenyl)ethylenediamine, which takes place during the diaza-Cope rearrangement at high temperatures (>120 °C). After hydrolysis, the diastereomeric diamines *meso*-**1a** and *d,l*-**1a** were separated by fractional crystallization of their sulfates to yield the low water soluble *d,l*-**1a** (method H). Compound *d,l*-**1a** was demethylated to *d,l*-**1** with BBr_3.

The approach to synthesize the diamine *d,l*-**6a** by high-temperature stereoisomerization of *N,N'*-bis(2,6-dichloro-4-methoxybenzylidene)-*meso*-1,2-bis(2,6-dichloro-4-methoxyphenyl)ethylenediamine was unsuccessful. We assume that by thermolysis of this diimine an intramolecular nucleophilic substitution of an ortho chlorine atom takes place as described previously for *N,N'*-dialkyl-1,2-bis(2,6-dichloro-4-methoxyphenyl)ethylenediamines.[16] Therefore, *d,l*-**6a** was prepared by *d,l* $\rightleftharpoons$ *d,l* diaza-Cope rearrangement[15] of *N,N'*-bis(2,6-dichloro-4-methoxybenzylidene)-*d,l*-1,2-bis(4-methoxyphenyl)ethylenediamine in boiling MeOH. The byproduct **9a**, which was formed by transimination reaction, was separated from *d,l*-**6a** by column chromatography (method I).

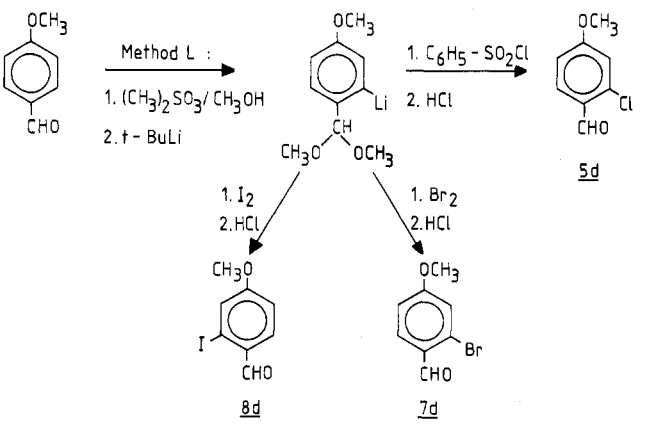
The structure of **9a** (Figure 6) was confirmed by ^1H and ^{13}C NMR spectroscopy. Because of the stereospecific *d,l* $\rightleftharpoons$ *d,l* rearrangement at the low temperature used, we assume a threo configuration of **9a**. Compound *d,l*-**6a** was transformed into the free phenol *d,l*-**6b** by ether cleavage with BBr_3. The chemical data of the ring-substituted 1,2-diphenylethylenediamines are listed in Table III.

The primary compounds for diamines **1** and **2**, 4-methoxy-2-methyl- and 2,6-dimethyl-4-methoxybenzaldehydes (**1d** and **2d**), were achieved by transformation of the anisoles **1e** and **2e** into the arylmagnesium bromides according to the method of Nelson and Uschak[17] and subsequent reaction with DMF (Scheme IV, method J). The bromination of 3-methyl- and 3,5-dimethylanisole (**1e** and **2e**) to the bromine derivatives occurs exclusively in the 4-position.

Scheme IV



Scheme V



The synthesis of 2-fluoro-4-methoxy- and 4-methoxy-2-(trifluoromethyl)benzaldehydes (**4d** and **3d**), starting compounds for the diamines **4** and **3**, was performed by reaction of 4-bromo-3-fluoro- and 4-bromo-3-(trifluoromethyl)anisole with *n*-BuLi at −78 °C and formylation with *N*-formylpiperidine according to the method of Olah and Arvanaghi[18] (Scheme IV, method K). Since the bromination of anisole derivatives **3e** and **4e** also yields a small quantity of 2-substituted products, a separation of isomeric aldehydes by column chromatography was necessary.

The 2-chloro-, 2-bromo-, and 2-iodo-4-methoxybenzaldehydes (**5d**, **7d**, and **8d**), which were required for the synthesis of diamines **5**, **7**, and **8**, were prepared by ortho metalation of 4-methoxybenzaldehyde dimethyl acetal[19] with *t*-BuLi at low temperatures according to the method of Plaumann et al.[20] and subsequent treatment with benzenesulfonyl chloride, bromine, or iodine (Scheme V, method L).

2,6-Dichloro-4-methoxybenzaldehyde (**6d**), primary compound for diamine **6**, was synthesized from 3,5-dichloroanisole (**6e**) by chloromethylation, followed by hydrolysis to the benzyl alcohol and oxidation to the aldehyde **6d** with MnO_2 (Scheme VI, method M). The stereoisom-

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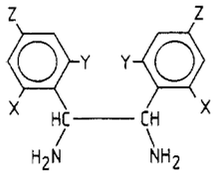
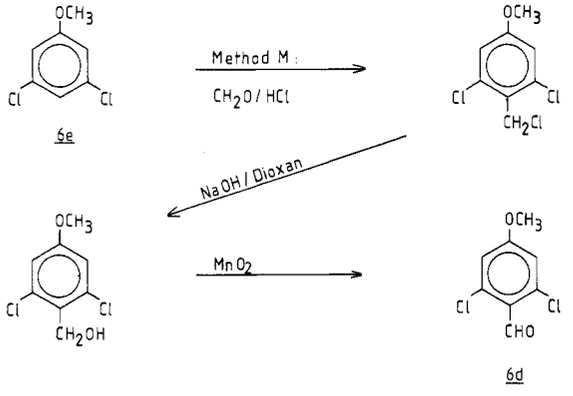
Table III. Ring-Substituted 1,2-Diphenylethylenediamines



compd	X	Y	Z	synth meth[a]	yield,[b] %	mp, °C	formula[c]
meso-**1a**	CH_3	H	OCH_3	D	62	156–157	$C_{18}H_{24}N_2O_2$
meso-**1**	CH_3	H	OH	E	72	175–177	$C_{16}H_{20}N_2O_2$[d]
d,l-**1a**	CH_3	H	OCH_3	G	30	218–220[e]	$C_{18}H_{24}N_2O_2 \cdot 2HCl \cdot H_2O$
d,l-**1**	CH_3	H	OH	E	82	162	$C_{16}H_{20}N_2O_2$[f]
meso-**2a**	CH_3	CH_3	OCH_3	D	30	165–166	$C_{20}H_{28}N_2O_2$
meso-**2**	CH_3	CH_3	OH	E	91	189–191	$C_{18}H_{24}N_2O_2$
meso-**3a**	CF_3	H	OCH_3	D	65	141–143	$C_{18}H_{18}F_6N_2O_2$
meso-**3**	CF_3	H	OH	F	85	185–187	$C_{16}H_{14}F_6N_2O_2$[g]
meso-**4a**	F	H	OCH_3	D	71	113–115	$C_{16}H_{18}F_2N_2O_2$
meso-**4**	F	H	OH	E	55	169–170	$C_{14}H_{14}F_2N_2O_2$
meso-**5a**	Cl	H	OCH_3	D	82	175	$C_{16}H_{18}Cl_2N_2O_2$
meso-**5**	Cl	H	OH	E	68	179–180	$C_{14}H_{14}Cl_2N_2O_2$
meso-**6a**	Cl	Cl	OCH_3	D	79	197	$C_{16}H_{16}Cl_4N_2O_2$
meso-**6**	Cl	Cl	OH	F	50	205–207	$C_{14}H_{12}Cl_4N_2O_2$
d,l-**6a**	Cl	Cl	OCH_3	H	29[h]	221[e]	$C_{16}H_{16}Cl_4N_2O_2 \cdot 2HCl \cdot 2H_2O$
d,l-**6**	Cl	Cl	OH	E	78	198–199	$C_{14}H_{12}Cl_4N_2O_2$[i]
meso-**7a**	Br	H	OCH_3	D	91	204–206	$C_{16}H_{18}Br_2N_2O_2$[j]
meso-**7**	Br	H	OH	F	81	184–185	$C_{14}H_{14}Br_2N_2O_2$
meso-**8a**	I	H	OCH_3	D	70	215–217	$C_{16}H_{18}I_2N_2O_2$[k]
meso-**8**	I	H	OH	F	87	187–189	$C_{14}H_{14}I_2N_2O_2$

[a] Synthetic methods E, F, G, H, and I: see Experimental Section. [b] Calculated with regard to the corresponding diimines (a) or to the methoxy-substituted diamines respectively. [c] All compounds were analyzed for C, H, and N within ±0.40% of the calculated values, except where noted. [d] C: calcd, 70.56; found, 70.03. [e] Dihydrochloride. [f] C: calcd, 70.56; found, 69.95. N: calcd, 10.29; found, 9.83. [g] C: calcd, 50.52; found, 51.19. [h] Calculated with regard to the *d,l*-1,2-bis(4-methoxyphenyl)ethylenediamine. [i] C: calcd, 44.02; found, 43.48. [j] C: calcd, 44.68; found, 43.53. [k] C: calcd, 36.68; found, 35.92.

Scheme VI



eric products were separated at the stage of the benzyl alcohols. The chemical data of the products are listed in Table IV in the supplementary material. ^1H NMR data are also available in the supplementary material.

Biological Properties. The binding affinities for the ER of the ring-substituted 1,2-bis(4-hydroxyphenyl)-ethylenediamines and related platinum(II) complexes were measured by a competitive binding assay using the dextran coated charcoal technique (competitor, $[^3H]E_2$, E_2 = 17-β-estradiol; ER source, calf uterine cytosol).[24] The relative binding affinity (RBA) was evaluated as the ratio of the molar concentrations of E_2 and inhibitor required to decrease the receptor-bound $[^3H]E_2$ by 50%, multiplied by 100. Out of the 24 compounds tested, seven show a weak affinity to the ER (see Table V in supplementary material). Only substitution by CF_3 in the ortho position and by Cl in the ortho and ortho,ortho′ positions of the inactive diastereomeric parent compounds, *meso*- and *d,l*-1,2-bis-(4-hydroxyphenyl)ethylenediamine, yielded derivatives that bind weakly to the ER. The most active compounds are the diastereomeric 1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamines, but their RBA values are low compared to those of synthetic estrogens like hexestrol (HES, 27; *meso*-**6**, 0.45; *d,l*-**6**, 0.2). On the one hand, the aromatic rings in *meso*-**6** and *d,l*-**6** are forced into an antiperiplanar conformation by the two 2,6-standing bulky Cl atoms whereby both hydroxy groups are located in an optimal distance for the binding to the ER.[4] On the other hand, the two NH_2 groups markedly diminish the hydrophobic interaction with suitable receptor areas causing a low affinity to the ER. The unfavorable effect of the NH_2 groups can be compensated by transformation into the more lipophilic *N,N′*-dialkyl derivatives (RBA values of *N,N′*-diethyl derivatives of *meso*-**6** and *d,l*-**6**: 8.6 and 2.1).[4] In comparison with *meso*-**6** and *d,l*-**6**, the corresponding dichloroplatinum(II) complexes possess smaller RBA values (*meso*-**6**-$PtCl_2$, 0.3; *d,l*-**6**-$PtCl_2$, 0.1). A further decrease of the RBA values is observed by exchange of Cl by SO_4 (*meso*-**6**-$PtCl_2$, 0.3; *meso*-**6**-$PtSO_4$, 0.1). Due to their low ER affinity, the new complexes are not able to compete with endogenous estrogens for cytoplasmic ER binding. Therefore, a cytoplasmic receptor-mediated enrichment of these drugs seems unlikely (compare Figure 1). In spite of these results, we investigated the estrogenic effects of the new ligands and complexes.

Surprisingly, five out of 10 of the new ligands (required dose, nmol/animal per day, administered on days 1, 2, and 3: *meso*-**2**, 5000; *meso*-**3**, 1000; *meso*-**5**, 1000; *meso*-**6**, 2.5; and *meso*-**7**, 1000) reached the maximum effect of estrone (E_1: 1.5 nmol/animal per day) in the mouse uterine weight test (for detailed data, see Table VI in supplementary

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Table VIII. Antitumor Effect of Ring-Substituted [1,2-Bis(4-hydroxyphenyl)ethylenediamine]platinum(II) Complexes against Hormone-Independent MDA-MB 231 and Hormone-Dependent MCF 7 Breast Cancer Cells

	[^{3}H]thymidine incorporation				cell growth			
	MDA-MB 231		MCF 7		MDA-MB 231		MCF 7	
compd	T/C,[a] %	ED_{50},[b] M	T/C,[a] %	ED_{50},[b] M	T/C,[a] %	ED_{50},[b] M	T/C,[a] %	ED_{50},[b] M
meso-1-$PtCl_2$ (2-CH_3)	38	3.3×10^{-6}	25	2.4×10^{-6}	61	7.6×10^{-6}	57	6.8×10^{-6}
d,l-1-$PtCl_2$ (2-CH_3)	30	3.3×10^{-6}	17	2.3×10^{-6}	87	$>1 \times 10^{-5}$	32	2.5×10^{-6}
meso-2-$PtCl_2$ (2,6-$(CH_3)_2$)	28	2.7×10^{-6}	4	0.9×10^{-6}	34	2.7×10^{-6}	17	3.1×10^{-6}
meso-3-$PtCl_2$ (2-CF_3)	36	3.2×10^{-6}	27	2.2×10^{-6}	46	4.0×10^{-6}	61	5.1×10^{-6}
meso-4-$PtCl_2$ (2-F)	12	0.9×10^{-6}	16	1.5×10^{-6}	21	1.5×10^{-6}	49	4.8×10^{-6}
meso-4-$PtSO_4$	64	8.3×10^{-6}	44	3.0×10^{-6}	64	7.8×10^{-7}	27	3.0×10^{-6}
meso-5-$PtCl_2$ (2-Cl)	22	2.6×10^{-6}	14	0.9×10^{-6}	21	2.1×10^{-6}	47	4.7×10^{-6}
meso-6-$PtCl_2$ (2,6-Cl_2)	100	$>1 \times 10^{-5}$	67	4.2×10^{-6}	90	$>1 \times 10^{-5}$	89	$>1 \times 10^{-5}$
meso-6-$PtSO_4$	100	$>1 \times 10^{-5}$	62	8.8×10^{-6}	92	$>1 \times 10^{-5}$	55	7.2×10^{-6}
d,l-6-$PtCl_2$ (2,6-Cl_2)	95	$>1 \times 10^{-5}$	84	1.0×10^{-5}	94	$>1 \times 10^{-5}$	78	$>1 \times 10^{-5}$
meso-7-$PtCl_2$ (2-Br)	13	1.2×10^{-6}	15	1.4×10^{-6}	43	4.3×10^{-6}	30	3.1×10^{-6}
meso-8-$PtCl_2$ (2-I)	21	2.1×10^{-6}	3	0.7×10^{-6}	22	1.0×10^{-6}	11	0.8×10^{-6}
cis-Pt	17	3.2×10^{-7}	1	1.1×10^{-7}	26	2.4×10^{-7}	11	4.4×10^{-7}

[a] At 5×10^{-6} M. [b] ED_{50} = the effective dose, which decreases the tumor growth by 50%; mean of two or three tests.

material).[25] This result is contradictory to the known correlation of ER affinity and estrogenic activity. The dose–activity relationship of *meso*-**2** and *meso*-**7** resembled that of an "impeded estrogen" like estriol (E_3).[26] Up to doses of 100 nmol, the ligands *meso*-**3** and *meso*-**5** increased the uterine dry weight only slightly, but equaled the E_1 standard at a dose of 1000 nmol/animal per day. Compounds *meso*-**1**, *d,l*-**1**, *meso*-**4**, and *meso*-**8**, compounds with RBA < 0.01, exhibited no or just small estrogenic effects. On the other hand, *meso*-**6** was found to be a strong "true estrogen", which comes up to full uterotropic activity after administration of 2.5 nmol/animal per day. With the corresponding racemic compound *d,l*-**6**, only very small uterine growth stimulating effects were obtained, though its RBA value was greater than those of the estrogenic compounds *meso*-**2**, *meso*-**3**, *meso*-**5**, and *meso*-**7**. In the case of the *N,N'*-diethyl derivatives of **6**, both diastereoisomers show estrogenic effects comparable with those of E_1. However, the 10-fold dose is necessary for the *d,l* compound.[4] As expected, the uterotropic activities of the platinum complexes were decreased compared to those of the respective ligands. Only *meso*-**6**-$PtCl_2$, *meso*-**6**-$PtSO_4$, and *meso*-**7**-$PtCl_2$ show the desired estrogenic potencies. *meso*-**7**-$PtCl_2$, having an "impeded estrogen" as ligand, did not reach the E_1 level (55% of E_1 activity at 1000 nmol/animal per day). The only complexes matching the activity of E_1 were *meso*-**6**-$PtCl_2$ and *meso*-**6**-$PtSO_4$. Relative to their ligand (*meso*-**6**), the complexes need a higher dose (10 nmol/animal per day) to reach the maximum effect.

Since we know from previous studies that low estrogenicity is often associated with antiestrogenic activity (AA), we determined the antiuterotropic effect of the ligands by simultaneous administration of 0.4 μg of E_1. Except for *d,l*-**1** and *meso*-**8**, no significant antiestrogenic activity was found (*d,l*-**1**, 1000 nmol/animal per day, AA = 32%; *meso*-**8**, 2.5 nmol/animal per day, AA = 32%).

In a further study, we investigated whether the new platinum complexes also possess the desired cytotoxic effect. We chose the lymphocytic leukemia P 388 of the mouse[27] as test model, which is known to be very sensitive to platinum complexes. Except for the racemic compounds *d,l*-**1**-$PtCl_2$ and *d,l*-**6**-$PtCl_2$, all platinum complexes were active on this tumor model (see Table VII in supplementary material). Among these drugs, *meso*-**1**-$PtCl_2$, *meso*-**5**-$PtCl_2$, *meso*-**7**-$PtCl_2$, and *meso*-**8**-$PtCl_2$ exhibited similar antitumor activities (T/C, %: 164, 145, 155, and 164; 50 mg/kg on days 1, 5, and 9), but did not reach the % T/C value of cisplatin. *meso*-**6**-$PtCl_2$ and *meso*-**6**-$PtSO_4$ were marginally active. In a dose range between 12.5 and 50.0 mg/kg per day, no toxic side effects of any compound were observed. Since the platinum complexes, which were administered as suspensions in olive oil, ip, were presumably not optimally resorbed, the most interesting compound, *meso*-**4**-$PtCl_2$, was additionally tested as a solution of a 1:1 mixture of polyethylene glycol 400/H_2O. In this solvent, *meso*-**4**-$PtCl_2$ and cisplatin reached identical antitumor activities (cisplatin, 2 mg/kg on days 1, 5, 9, % T/C = 244; *meso*-**4**-$PtCl_2$, 50 mg/kg on days 1, 5, 9, % T/C = 239). The transformation of *meso*-**4**-$PtCl_2$ into the water-soluble *meso*-**4**-$PtSO_4$ led to a marked decrease of antitumor activity. Among the new platinum complexes, *meso*-**6**-$PtCl_2$, *meso*-**6**-$PtSO_4$, and *meso*-**7**-$PtCl_2$ are of special interest, since they show estrogenic as well as cytotoxic properties.

To determine a possible selective effect on the hormone-dependent breast cancer, the complexes were comparatively tested on both ER-positive and ER-negative human mammary carcinoma cell lines (MCF 7[29] and MDA-MB 231,[28] respectively). To evaluate the antitumor activity, we measured cell growth inhibition and [^{3}H]-thymidine incorporation. Except for *meso*-**6**-$PtCl_2$ and *meso*-**6**-$PtSO_4$, which inhibited exclusively the ER-positive MCF 7 breast cancer cell line, none of the platinum complexes showed a marked difference in its antitumor activity on both test models (Table VIII). For the majority of complexes, values for a 50% inhibition of [^{3}H]thymidine incorporation range about 2×10^{-6} M, equaling one-tenth of the activity of cisplatin.

On the grounds of these results, one could suppose that the action of *meso*-**6**-$PtCl_2$ and *meso*-**6**-$PtSO_4$ on the MCF 7 breast cancer cell line is mediated by the ER system; however, the ER affinity of these compounds would appear to be too weak for such an explanation.

A comparative in vivo study of the platinum complexes was also performed on the ER-positive and ER-negative MXT mammary carcinoma of the mouse (MXT-MC, ER^+ and ER^-, respectively)[30] by using an equimolar dosage (2

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Table IX. Effect of Ring-Substituted [1,2-Bis(4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) Complexes on the Growth of Hormone-Dependent and Hormone-Independent MXT Mammary Carcinoma and of the Uterus of the BDF_1 Mouse

		MXT, ER^+				MXT, ER^-	
compd	dose,[a] mg/kg	med tumor weight,[b] mg	T/C, %	uterotropic effect[b,c] ± SD	T/C, %	med tumor area,[d] mm^2	T/C, %
meso-1-$PtCl_2$ (2-CH_3)	10.8	169	36[e]	80.4 ± 23.7	89	211	65[e]
d,l-1-$PtCl_2$ (2-CH_3)	10.8	74	16[f]	89.8 ± 30.4	100	228	70
meso-2-$PtCl_2$ (2,6-$(CH_3)_2$)	11.3	40	9[f]	86.6 ± 27.1	96	203	63[e]
meso-3-$PtCl_2$ (2-CF_3)	12.9	422[h]	78[g,h]	70.9 ± 18.2[h]	100[h]	234	72[g]
meso-4-$PtCl_2$ (2-F)	10.9	143	30[e]	89.9 ± 18.7	100	193	60
meso-5-$PtCl_2$ (2-Cl)	11.6	191	41	82.2 ± 27.9	91	265	82
meso-6-$PtCl_2$ (2,6-Cl_2)	13.0	3[h]	0.4[g,h]	149.1 ± 15.8[h]	219[h]	330	102
d,l-6-$PtCl_2$[i] (2,6-Cl_2)	13.0	–	–	–	–	241	74
meso-7-$PtCl_2$ (2-Br)	13.4	57	12[f]	89.3 ± 15.2	99	210	65[g]
meso-8-$PtCl_2$ (2-I)	15.2	341	72	80.5 ± 20.4	89	232	72
tamoxifen	8.0	16[h]	3[f,h]	72.3 ± 13.5[h]	80[h]	–	–
cis-Pt	2.0	15[h]	3[g,f]	95.0 ± 19.3[h]	114[h]	49	15[f]

[a] The compounds were administered in equimolar doses (2×10^{-5} M/kg) three times a week (Monday, Wednesday, and Friday), sc, as solution or suspension in polyethylene glycol 400/H_2O, 1:1. [b] Determined at the end of the 6-week therapy; the U test according to Wilcoxon, Mann, and Whitney was used. [c] Uterotropic effect = [uterus dry weight (mg)/body weight (g)] × 100. [d] Determined at the end of the 2-week therapy. [e] Significant ($p < 0.025$). [f] Significant ($p < 0.01$). [g] Significant ($p < 0.05$). [h] The compounds were administered analogously to administration described in *a* in olive oil as vehicle. [i] Not tested on MXT, ER^+.

Table X. Comparison of the Effects of [1,2-Bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) and Its Ligand on Growth of Hormone-Dependent MXT Mammary Carcinoma and Uterus of the BDF_1 Mouse

	dose[a]				uterotropic	
compd	mg/kg	M/kg	med tumor weight,[b] mg	T/C, %	effect[b,c] ± SD	T/C, %
meso-6	0.6	1.5×10^{-6}	91.5	18[d]	134 ± 15	158[d]
	1.8	4.6×10^{-6}	62.5	12[d]	201 ± 14	239[d]
	5.3	1.4×10^{-5}	61.0	12[d]	not tested	not tested
meso-6-$PtCl_2$	1.0	1.5×10^{-6}	23.5	5[d]	116 ± 16	138[d]
	3.0	4.6×10^{-6}	95.0	19[d]	135 ± 13	160[d]
	9.0	1.4×10^{-5}	39.0	8[d]	194 ± 31	229[d]
cis-Pt	1.4	4.6×10^{-6}	15.0	3[d,f]	96 ± 19	114
	4.2	1.4×10^{-5}	222.0	44[e,g]	100 ± 26	118

[a] The compounds were administered in olive oil as vehicle three times a week, sc. [b] Determined at the end of the 6-week therapy; the U test according to Wilcoxon, Mann, and Whitney was used. [c] Uterotropic effect = [uterus dry weight (mg)/body weight (g)] × 100. [d] Significant ($p < 0.01$). [e] Significant ($p < 0.05$). [f] Cisplatin was administered in water as vehicle three times a week, sc. [g] See *f*, application of one dose per week.

$\times 10^{-5}$ M/kg). Five out of seven of the ortho-substituted [1,2-bis(4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) complexes (*meso*-1-$PtCl_2$, *d,l*-1-$PtCl_2$, *meso*-3-$PtCl_2$, *meso*-4-$PtCl_2$, and *meso*-7-$PtCl_2$) caused a significant inhibition of MXT-MC, ER^+ (Table IX). Additionally, three of these active complexes (*meso*-1-$PtCl_2$, *meso*-3-$PtCl_2$, and *meso*-7-$PtCl_2$) were also significantly active on the hormone-independent MXT-MC. However, none of these compounds reached the activity of cisplatin on either tumor.

Among the ortho-substituted [1,2-bis(4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) complexes, *meso*-7-$PtCl_2$ was the most interesting substance. It proved to be markedly more active on the ER-positive than on the ER-negative MXT mammary carcinoma. These in vivo results are not consistent with those found in cell culture experiments, where *meso*-7-$PtCl_2$ was comparably active on ER-positive and ER-negative human breast cancer cell lines. Due to its estrogenic potency, in this case a more pronounced effect on the ER-positive cell line should be expected also. *meso*-7-$PtCl_2$ is a compound that possesses marked cytotoxic as well as estrogenic effects (Tables VI, supplementary material, and VIII), therefore being of interest for further evaluation. Compounds that show activities on the ER-positive and ER-negative MXT-MC possibly cause a delay of the development of resistance, a well-known process in the endocrine therapy of breast cancer.[31,32] By introduction of a second CH_3 or Cl residue, respectively into the ortho position of *meso*-1-$PtCl_2$ and *meso*-5-$PtCl_2$, we obtained two complexes (*meso*-2-$PtCl_2$ and *meso*-6-$PtCl_2$), which are more effective on the MXT-MC, ER^+ than their parent compounds (Table IX). Growth inhibitory activities of *meso*-2-$PtCl_2$ on hormone-dependent and -independent MXT-MC were comparable to those of *meso*-7-$PtCl_2$, making this substance also interesting for a closer study.

The most promising compound among the new [1,2-bis(4-hydroxyphenyl)ethylenediamine]dichloroplatinum-(II) complexes proved to be *meso*-6-$PtCl_2$, producing a % T/C value of 0.4 on the hormone-dependent MXT-MC of the mouse (Table IX). It was much more active on this tumor model than tamoxifen and cisplatin. In a further test series, *meso*-6-$PtCl_2$ and the related ligand *meso*-6 were compared (Table X). In three equimolar dosages they showed similar % T/C values. However, in both lower dosages the estrogenic side effects of *meso*-6-$PtCl_2$ were markedly weaker than those of *meso*-6. On the hormone-independent MXT-MC, *meso*-6-$PtCl_2$ and *meso*-6 caused no inhibition (Table XI in supplementary material). A selective effect of *meso*-6-$PtCl_2$ on the hormone-dependent MC was also observed on ER-positive and ER-negative human breast cancer cell lines (Table VIII). Presumably the low water solubility of *meso*-6-$PtCl_2$

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(32) The development of resistance in the endocrine therapy of the hormone-dependent breast cancer is often accompanied by a loss of ERs. In this process the resistant tumors possibly spring up from ER-negative subclones. They are only accessible to a cytotoxic chemotherapy.

Table XII. Effect of [1,2-Bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) and Its Ligand on Growth of DMBA-Induced, Hormone-Dependent Mammary Carcinoma of the SD Rat

compd	dose,[a] mg	no. of animals	no. of tumors		% of tumors with				% change of	
			B[b]	NT[c]	CR[d]	PR[e]	NC[f]	P[g]	body wt[h]	tumor area[i,j]
control		7	20	7	11	4	7	78	1.5	154
meso-6[l]	1.9	8	26	3	24	28	24	24	-1.8	-1[k]
meso-6-$PtCl_2$[m]	3.25	6	20	3	65	5	13	17	-6.3	-55[k]
	6.5	6	17	0	88	0	12	0	-5.4	-100[k]

[a] Dose per kilogram of body weight and day. The animals received this dose three times a week, sc, as suspension or solution in polyethylene glycol 400/H_2O, 1:1; duration of therapy 4 weeks. [b] At the beginning of the test. [c] Occurring during the test. [d] CR = complete remission, tumor not palpable. [e] PR = partial remission, reduction of initial tumor size ≥ 50%. [f] NC = no change; tumor size 51–150% of initial tumor size. [g] P = progression; tumor size > 150% of initial tumor size. [h] Average on the 7th day of therapy. [i] The U test according to Wilcoxon, Mann, and Whitney was used. [j] Average on the 28th day of therapy. [k] Significant ($p < 0.01$). [l] Dose received as suspension (see *a*). [m] Dose received as solution (see *a*).

Table XIII. Effect of *meso*-6, *meso*-6-$PtSO_4$, and Cisplatin on Growth of DMBA-Induced, Hormone-Dependent Mammary Carcinoma of the SD Rat

compd	dose,[a] mg	no. of animals	no. of tumors		% of tumors with				% change of		
			B[b]	NT[c]	CR[d]	PR[e]	NC[f]	P[g]	body wt, day 28	tumor area[h] day 14	tumor area[h] day 28
control		9	21	23	0	0	38	62	3.0	260	420
cis-Pt	1.5 (0.5 × 10^{-5} M/kg)	9	24	4	21	41	25	13	-0.9	51[j,l]	-38[i]
meso-6	1.9 (0.5 × 10^{-5} M/kg)	8	17	9	0	18	35	47	-4.6	62[i]	129[i]
meso-6-$PtSO_4$	3.5 (0.5 × 10^{-5} M/kg)	10	25	1	56	24	12	8	-5.4	-86[k]	-84[k]

[a–g] See Table XII; *cis*-Pt and *meso*-6-$PtSO_4$ were dissolved in H_2O. [h] The U test according to Wilcoxon, Mann, and Whitney was used. [i–k] Significant ($p < 0.01$, < 0.025, < 0.05) compared with the control. [l] Significant ($p < 0.01$) lower activity compared with *meso*-6-$PtSO_4$.

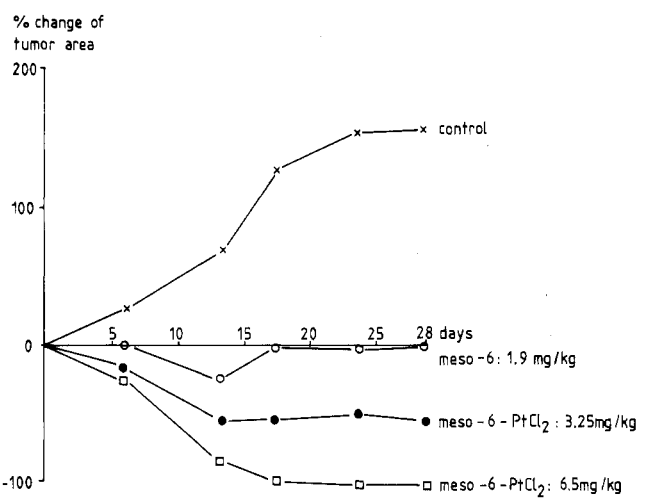

Figure 7. Effect of *meso*-6 and *meso*-6-$PtCl_2$ on growth of DMBA-induced, hormone-dependent mammary carcinoma of the SD rat. For experimental conditions, see Table XII.

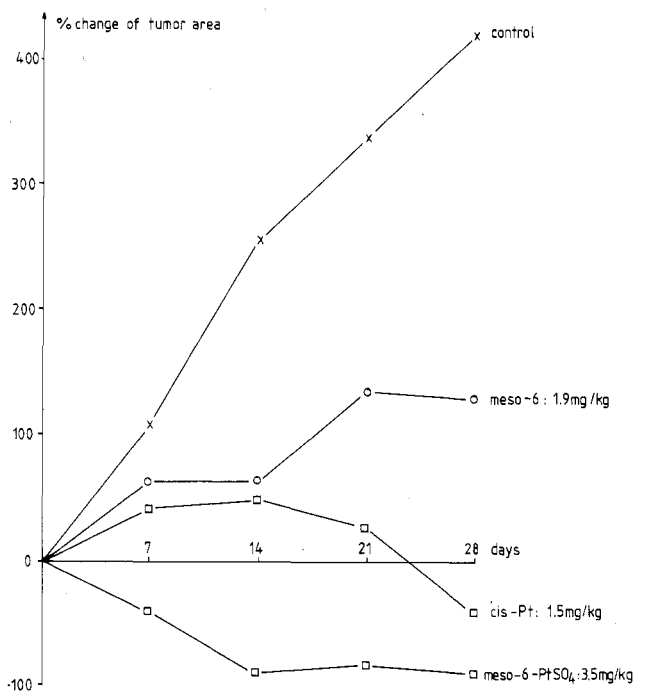

Figure 8. Comparison of the effects of *meso*-6-$PtSO_4$, *meso*-6, and *cis*-Pt (equimolar dosage: 0.5 × 10^{-5} M/kg) on the growth of DMBA-induced, hormone-dependent mammary carcinoma of the SD rat. For experimental conditions, see Table XIII.

is to blame for the lack of an exact dose–activity relationship. This is confirmed by experiments using the related water soluble (sulfato)platinum(II) complex *meso*-6-$PtSO_4$. *meso*-6-$PtSO_4$ is distinctly more active than *meso*-6-$PtCl_2$ when applied in an equimolar dosage (3.0 mg/kg three times a week, sc, duration of therapy 6 weeks; cf. Table X). Only one out of nine *meso*-6-$PtSO_4$-treated animals possessed a very small tumor (% T/C = 3) at the end of therapy. In the remaining animals, no tumor was detectable macroscopically. In this experiment, the estrogenic side effects of *meso*-6-$PtSO_4$ were comparable to those of *meso*-6-$PtCl_2$ (% T/C = 142 and 160 at a dose of 3 mg/kg). Body weights of control and therapy groups gave no hints to a general toxicity (average body weights after 6-week duration of therapy: control, 22.7 g/animal; therapy, 23.9 g/animal).

The interesting results that we achieved on the hormone-dependent MXT-MC of the mouse with *meso*-6-$PtCl_2$ and *meso*-6-$PtSO_4$ induced us to a detailed investigation of both compounds on the DMBA-induced, hormone-dependent mammary carcinoma of the SD rat[33] (DMBA-MC). In this lesser sensitive tumor model, *meso*-6-$PtCl_2$ and *meso*-6-$PtSO_4$ proved as well to be very promising compounds for the therapy of hormone-dependent breast cancer (Tables XII and XIII). For example, this follows from an experiment where *meso*-6-$PtCl_2$ was given for 4 weeks at a dose of 3 × 6.5 mg/kg per week (solvent: polyethylene glycol 400/H_2O, 1:1; Figure 8). At the end of therapy, a nearly complete remission of tumors

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Table XIV. Platinum Contents of Blood and of Different Tissues of SD Rats Bearing DMBA-Induced, Hormone-Dependent Mammary Carcinoma after a 4-Week Therapy with *meso*-6-$PtSO_4$[a]

blood $\overline{EC}$ ± SD, μg/g	spleen $\overline{EC}$ ± SD, μg/g	skeletal muscle $\overline{EC}$ ± SD, μg/g	uterus $\overline{EC}$ ± SD, μg/g	tumor		
				$\overline{EC}$ ± SD, μg/g	f_1	f_2
0.17 ± 0.071	5.6 ± 1.6	0.091 ± 0.040	0.51 ± 0.11	2.0 ± 0.91	22	3.9

[a] Dose: 3.5 mg (0.5×10^{-5} mol) per kg of body weight and day. The animals received this dose three times a week, sc, as aqueous solution. $\overline{EC}$ = mean values ($n = 5$) of the paltinum contents found by neutron activation analysis (see supplementary material), based on fresh weight. SD = standard deviation. The enrichment factor f_1 is the ratio $\overline{EC}_{tumor}/\overline{EC}_{skeletalmuscle}$, and f_2 is $\overline{EC}_{tumor}/\overline{EC}_{uterus}$.

(88%; compare Table XII and Figure 7) was found. It is of special interest that the new complex type is significantly more active than cisplatin (Table XIII, Figure 8). In this experiment, the highest tolerable dose of cisplatin ($3 \times 0.5 \times 10^{-5}$ M/kg per week; duration of therapy 4 weeks) was applied. *meso*-6-$PtSO_4$ was used at an equimolar dose. In addition, a stronger antitumor effect and a faster onset of tumor inhibition were also observed (Figure 8, Table XIII) even though the blood Pt level after treatment with *meso*-6-$PtSO_4$ reaches only about one-tenth of that after treatment with cisplatin (mean values: 0.17 μg/g, day 28, and 1.6 μg/g, day 28, respectively, as determined by neutron activation analysis). The ligand itself also produced a marked inhibition of tumor growth. This result is not surprising, since *meso*-6 is also active on MXT-MC, ER^+. Presumably, the estrogenic potency of *meso*-6 is responsible for this effect. It is known that high doses of estrogens inhibit the growth of hormone-dependent MC,[40] but the mechanism of action is not yet clear (see Discussion). However, in an equimolar dose, *meso*-6-$PtSO_4$ is significantly superior to *meso*-6. These differences speak for a contribution of cytotoxic effects to the antitumor activity of *meso*-6-$PtSO_4$.

With this in mind, it is of great interest that *meso*-6-$PtSO_4$ does not cause kidney damage. On day 28 after the beginning of therapy, the kidneys were histopathologically examined by using 5-μm paraffin sections. In cisplatin-treated animals all kidneys showed widely dilated S_3 segments of the proximal tubules while the kidneys of *meso*-6-$PtSO_4$-treated rats were not affected. Similar reductions of white blood cell counts were observed after administration of *meso*-6-$PtSO_4$ and cisplatin (day 28; control, 7270/μL; *meso*-6-$PtSO_4$, 3570/μL; cisplatin, 3750/μL).

Discussion

Our conception to develop platinum complexes with a selective effect on the hormone-dependent MC by exchanging the two NH_3 groups of cisplatin for a 1,2-bis(4-hydroxyphenyl)ethylenediamine derivative that possesses estrogenic activity was successful. The most interesting compound of this new platinum complex series, [*meso*-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]-dichloroplatinum(II) (*meso*-6-$PtCl_2$) and its water-soluble (sulfato)platinum(II) derivative (*meso*-6-$PtSO_4$), were significantly more active on the DMBA-MC than cisplatin and the related ligand 1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine (*meso*-6) (Figures 7 and 8, Tables XII and XIII). These results support the validity of the already-discussed mode of action (see Figure 1). According to this mechanism, *meso*-6-PtX (X = Cl_2 or SO_4) should be enriched in the nuclei of mammary tumor cells, which possess an intact ER system (i.e., an intact cytoplasma nucleus translocation process), causing thereby their very strong tumor growth inhibiting activity. Preliminary studies of *meso*-6-$PtSO_4$ on the DMBA-MC confirm this assumption. In the tumor tissue we found higher Pt levels than in uterine tissue, which also contains ERs (Table XIV). The Pt levels of the tumor tissue are much higher than those of skeletal muscle and of blood. A strong enrichment of Pt occurs in spleen. In biological experiments, both cytotoxic and estrogenic effects of *meso*-6-PtX can be proved. By UV difference spectroscopy an interaction between *meso*-6-PtX and DNA can be demonstrated.[34] An absorption maximum at 270 nm with a shoulder at 295 nm and a minimum at 248 nm is observed. An identical UV difference spectrum is obtained under the influence of stereoisomeric [1,2-bis(4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) complexes[1] and of cisplatin.[35,36] The increment ΔA_{270} is an indication for a substance-mediated loss in "base stacking", accompanied by a change in DNA secondary structure.[36] The ratio $\Delta A_{270}/\Delta A_{295}$ is used as a measure for this conformational disturbance,[36] having a value of approximately 2 in the case of cisplatin.[36] For *meso*-6-PtX the quotient is 1.85 ($r = 0.33$; r = [Pt]/[P]).[34] According to these findings, we assume a mechanism for the binding of *meso*-6-PtX to DNA corresponding to that of cisplatin. The antitumor effect of cisplatin is produced by a specific inhibition of DNA synthesis.[37,38] Considerable controversy exists on the question of whether the inter- or intrastrand cross-link or the DNA–protein cross-link is the "critical" lesion resulting in arrest of DNA synthesis.[39] Since the bifunctional coordination of Pt to the N(7) positions of adjacent guanosines of one DNA strand is the preferred reaction, the intrastrand DNA cross-link is thought to be the most important reason for antitumor activity.[39] On hormone-independent tumors, the cytotoxic effect of *meso*-6-PtX from DNA experiments could only partially be confirmed. Tests on the P 388 leukemia of the mouse yielded weak but significant activities (Table VII in supplementary material). On two hormone-independent mammary carcinoma models (MDA-MB 231 human breast cancer cell line and MXT-MC, ER^-, mouse), no significant inhibition of tumor growth could be detected (Tables VIII and IX). The selective activity of *meso*-6-PtX on the hormone-dependent MXT-MC shows the necessity of an intact ER system as a prerequisite for the incidence of antitumor activity. Presumably only an ER-mediated enrichment in the nuclei of MC cells brings about the essential level for cytotoxic activity. Though *meso*-6-PtX exerts a strong estrogenic activity, its low ER affinity is inconsistent with the enrichment theory (compare Tables V and VI in supplementary material). The latter requires the binding of the drug to cytoplasmic ERs, followed by the translocation into the nucleus. However, it is possible that *meso*-6-PtX binds to an estrogen-specific nuclear receptor, which also could cause an enrichment in the nucleus giving rise to an in-

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creased reaction with DNA. The significance of ERs for the antitumor effect is demonstrated by experiments using *d,l*-6-PtX. Though this complex shows a comparable reaction with DNA ($\Delta A_{270}/\Delta A_{295} = 1.90$, $r = 0.33$),[34] it has neither estrogenic nor mammary tumor inhibiting properties. The high stereospecific activity of *meso*-6-PtX could be explained by differences in the conformation equilibria of both diastereomeric complexes (*meso*-6-PtX and *d,l*-6-PtX). We suppose that a better fit to the ER is obtained by the high flexibility of the five-membered chelate ring of *meso*-6-PtX (i.e., by the rapid interconversion between δ and λ conformers; compare discussion of ^{1}H NMR spectroscopy data). Contrary to this idea is the observation that the related ligand (*d,l*-6), which is probably also flexible enough to interconvert between several conformations, shows no marked estrogenic effects. Further studies will be necessary before these relationships are understood.

Besides a carrier-mediated cytotoxic activity, we discuss a mode of action for *meso*-6-PtX, which is analogous to that of breast cancer therapy with high estrogen doses.[40] In this context it is important to note that, on the ER-positive mammary carcinoma of the ovariectomized rat, estrogens cause a growth stimulation in low dosage and a growth inhibition in high doses.[41] This process can be described by a model proposed by Bruchovsky et al.,[42-47] in which the hormonal control of growth in a target tissue is ascribed to the expression of intrinsic cellular mechanisms. The three phases, each of which is regulated by a separate homeostatic constraint mechanism, are termed initiation (I), negative feedback (N), and autophagia (A) (I, hormone-induced increase of DNA synthesis and cell proliferation; N, inhibition of process I; A, process that causes injury of cells, followed by their removal by phagocytic cells). Nonfunctional constraint mechanisms (reason for resistance in initially hormone dependent cancer cells) are always accompanied by depletion of specific receptors. According to this concept, the inhibiting effect of highly dosed estrogens on the hormone-dependent mammary carcinoma would be caused by triggering an autophagic mechanism. The same process can also be set off by withdrawal of endogenous estrogens (e.g., by ovariectomy).[41,48]

In breast cancers with partially depleted homeostatic constraint mechanisms, ERs are detectable, but the tumors respond only incompletely or not at all to endocrine therapy. If an autophagic mechanism is operative for *meso*-6-PtX, the related ligand *meso*-6, which shows a higher estrogenic potency, should possess a more pronounced mammary tumor inhibiting activity. The opposite is true (Figure 7, Table XII). Presumably the PtX residue increases the potency of *meso*-6 for cellular injury, which becomes apparent in an elevated cellular autolysis and involution of tumors. Therefore, *meso*-6-PtX should also be active on mammary carcinoma which possess a nonintact constraint mechanism for autophagia (e.g., $I^+N^-A^-$; minus symbols denote the absence of the corresponding constraint mechanism). In such a case the cellular autolysis would be started exclusively by the reactive PtX residue, but a selective activity should be caused by an ER-mediated enrichment. The inactivity of *meso*-6-PtX on the hormone-independent MXT-MC, in which all three constraint mechanisms are possibly deleted, can be explained by the low cytotoxic activity of the compounds as well as by a lack of an enrichment mechanism. The markedly higher activity of *meso*-6-PtX in vivo than in cell cultures points to the existence of an autophagic mechanism involved in the inhibition of the hormone-dependent mammary carcinoma.

There are similarities between the processes of estrogen-induced inhibition of hormone-dependent mammary carcinoma and of breast involution after suppression of lactation by high estrogen doses.[48,49] In both cases autophagic processes are set off by estrogens. These are followed by heterophagic involutional processes and invasion of connective tissue into the areas of involuted glandular structure.

These changes are histologically recognizable by (1) appearance of autophagic cytoplasmic vacuoles and of pyknoses and hyperchromatism of nuclei (signs for cellular injury); (2) invasion of areas of mammary tissues or mammary tumor, respectively (with lethal cellular damage), by macrophages and other phagocytic cells for removal of autolytic and necrotic cells; and (3) increase of activities of lysosomal enzymes as a result of autophagic and heterophagic processes.

However, it must be emphasized that the biochemical mechanism of estrogen-induced involution of parenchymal mammary tissue or mammary tumor, which require the ER system, is not well-understood. The histopathological examination of *meso*-6-PtX-treated DMBA-MC revealed signs of cellular injury and heterophagic processes, which are similar to those seen after estrogen treatment.

The diverging results obtained by *meso*-6-PtX in various biological experiments give rise to the hypothesis that both cytotoxic and estrogenic properties are responsible for the strong mammary tumor inhibiting activity observed. Future studies will investigate whether *meso*-6-PtX, as a result of its biological properties, also inhibits the development of resistance, which is often seen in the endocrine therapy of hormone-dependent breast cancer and which leads to a reduction of remission periods.

Experimental Section

General Procedures. Melting points, which are uncorrected, were taken on a Büchi 510 melting point apparatus or on a Petri melting point apparatus if higher than 220 °C. The IR data were obtained with a Perkin-Elmer Model 580 A spectrophotometer. ^{1}H NMR spectra were obtained with a Varian EM 390 A 90-MHz spectrometer. The ^{1}H NMR spectra of the platinum complexes were measured with a Bruker PFT-NMR spectrometer, WM 250, at 250 MHz. Elemental analyses were accomplished by the microlaboratory of the University of Regensburg.

Syntheses. Methods A–M are representative for the syntheses of the compounds reported in Tables I–IV. For analytical data, see the supplementary material.

Method A. [*meso*-1,2-Bis(4-hydroxy-2-methylphenyl)-ethylenediamine]dichloroplatinum(II) (*meso*-1-$PtCl_2$). Compound *meso*-1 (242 mg, 1 mmol) was suspended in 20 mL of H_2O and heated to 30–40 °C. The ligand was dissolved by addition of 2 mL of 2 N HCl and 20 mL of *t*-BuOH. Subsequently the pH of the clear solution was adjusted to 5.0–6.0 by treatment

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with 0.5 N NaOH, and then K_2PtCl_4 (415 mg, 1 mmol), dissolved in 5 mL of H_2O, was added. The mixture was kept in the dark, with stirring, and adjusted to pH 6 several times. After the pH value was constant (up to 3 days), the precipitate was collected, washed with 2 N HCl and H_2O, and dried over P_2O_5 to yield 431 mg of *meso*-1-$PtCl_2$ (80%, pale yellow powder).

Method B. [*meso*-1,2-Bis(2,6-dimethyl-4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) (*meso*-2-$PtCl_2$). K_2PtCl_4 (415 mg, 1 mmol) was dissolved in 5 mL of a 1:1 mixture of DMF/H_2O, and a suspension of *meso*-**2** (300 mg, 1 mmol) in 20 mL of DMF was added. The mixture was stirred in the dark at room temperature for 3 days, and the resulting yellow solution was evaporated to dryness. The residue was treated with 5% KCl solution and stirred for 5 h. The yellow precipitate was collected, washed with 2 N HCl and H_2O, and dried over P_2O_5 to yield 510 mg of *meso*-**2**-$PtCl_2$ (90% pale yellow powder).

Method C. [*meso*-1,2-Bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]diaquaplatinum(II) Sulfate (*meso*-6-$PtSO_4$). Solid Ag_2SO_4 (312 mg, 1 mmol) was added to 50 mL of an aqueous suspension of *meso*-**6**-$PtCl_2$ (648 mg, 1 mmol). The mixture was stirred for 3 days at 40–50 °C with protection from light. Silver chloride deposited was filtered off, and the clear filtrate was evaporated to dryness under reduced pressure. The residue was treated with a small amount of methanol, and the unreacted Ag_2SO_4 was filtered off. To this solution was added diisopropyl ether (100 mL), and the precipitated *meso*-**6**-$PtSO_4$ was collected and dried at 80 °C under reduced pressure (38%, white powder).

Method D. *N,N'*-Disalicylidene-*meso*-1,2-bis(4-methoxy-2-methylphenyl)ethylenediamine (1b). *meso*-1,2-Bis(2-hydroxyphenyl)ethylenediamine[15] (4.0 g, 17 mmol) and **1d** (4.9 g, 33 mmol) were suspended in 50 mL of acetonitrile. The mixture was refluxed with vigorous stirring until yellow cubic crystals were formed. After concentration of the volume to half of it, the yellow precipitate was collected on a Büchner funnel, washed with small amounts of acetonitrile, and dried over P_2O_5 to obtain 5.25 g of **1b** (61%, yellow powder, mp 207–209 °C).

In the case of compound **2b**, toluene was used as solvent. Only a mixture of the stereoisomeric diimines **2b** and **2c** could be isolated, which was used for further reactions without separation.

Method E. *meso*-1,2-Bis(4-methoxy-2-methylphenyl)ethylenediamine (*meso*-1a). Compound **1b** (5.08 g, 0.01 mol) was hydrolyzed with 200 mL of 3 N H_2SO_4 and the forming salicylic aldehyde removed by steam distillation. The solution was filtered and alkalized with 20% NaOH with cooling. The precipitated diamine was extracted with CH_2Cl_2, and the organic layer was washed with H_2O, dried over $MgSO_4$, and evaporated. The crude product was recrystallized in CH_2Cl_2/ether to give 1.86 g of *meso*-**1a** (62%, colorless crystals, mp 156–157 °C).

In the case of **6b**, **7b**, and **8b**, 40% H_2SO_4 was used for hydrolysis.

Method F. *meso*-1,2-Bis(2-methyl-4-hydroxyphenyl)ethylenediamine (*meso*-1). A solution of *meso*-**1a** (1.5 g, 5 mmol) in 100 mL of dry CH_2Cl_2 was cooled to −60 °C. BBr_3 (6.26 g, 25 mmol) was added in a N_2 atmosphere. After 1 h, the reaction mixture was brought to room temperature and stirred for 16 h. Subsequently, 50 mL of MeOH was added slowly with cooling and the solvents were removed under reduced pressure. The residue was dissolved in 50 mL of H_2O, filtered, and alkalized with 2 N NaOH. Undissolved *meso*-**1a** was filtered off, and the filtrate was neutralized with 2 N HCl. The precipitate was collected by suction filtration, washed with generous amounts of H_2O, and dried over P_2O_5 to yield 1 g of *meso*-**1** (72%, colorless powder, mp 175–177 °C).

Method G. *meso*-1,2-Bis[4-hydroxy-2-(trifluoromethyl)phenyl]ethylenediamine (*meso*-3). Compound *meso*-**3a** (4.08 g, 0.01 mol) was suspended in 60 mL of 47% HBr and refluxed for 48 h. After cooling, the dihydrobromide was filtered off and washed with ice-cold 47% HBr. The liberation of the diamine was performed according to method F to yield 3.23 g of *meso*-**3** (85%, colorless powder, mp 185–187 °C).

Method H. *d,l*-1,2-Bis(4-methoxy-2-methylphenyl)ethylenediamine (*d,l*-1a). Compound **1d** (3.0 g, 0.02 mol) was added to a solution of *meso*-**1a** (3.0 g, 0.01 mol) in 100 mL of acetonitrile and refluxed for 2 h. After reduction of the volume and cooling, 4.9 g of *N,N'*-bis(4-methoxy-2-methylbenzylidene)-*meso*-1,2-bis(4-methoxy-2-methylphenyl)ethylenediamine crystallized (87%, colorless crystals, mp 174–175 °C). The stereoisomerization of the latter (4.9 g, 8.7 mmol) was performed by heating to the melting temperature (180 °C) for 10 min. After cooling and addition of 250 mL of 3 N H_2SO_4, steam distillation was performed. After the removal of the aldehyde **1d**, the hot solution was filtered and the sulfate of *d,l*-**1a** crystallized at room temperature. From its suspension in 2 N NaOH, the free diamine was extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried over $MgSO_4$, and evaporated. The oily residue was dissolved in MeOH, and the dihydrochloride of the diamine was precipitated by treatment with ethereal HCl to give 1.1 g of *d,l*-**1a**·2HCl (30%, colorless powder, mp 218–220 °C).

Method I. *d,l*-1,2-Bis(2,6-dichloro-4-methoxyphenyl)ethylenediamine (*d,l*-6a). *d,l*-1,2-Bis(4-methoxyphenyl)ethylenediamine[15] (6.8 g, 0.025 mol) and **6d** (10.25 g, 0.05 mol) were dissolved in MeOH and refluxed for 24 h. The solvent was removed, and the oily residue was chromatographed on a SiO_2 column (toluene/CH_2Cl_2, 1:1). The diimines were decomposed on SiO_2, and the forming aldehydes were eluted. Both diamines were separated by subsequent chromatography with MeOH. The first fraction was identified as *d,l*-**6a** and the second as *threo*-**9a**. Compound *d,l*-**6a** could be crystallized as the dihydrochloride by using method H to yield 3.5 g of *d,l*-**6a**·2HCl (29%, colorless powder, mp 221 °C).

Method J. 4-Methoxy-2-methoxybenzaldehyde (1d). To a solution of 3-methylanisole (**1e**) (97.6 g, 0.8 mol) in 500 mL of CCl_4 was added 2 g of Fe powder. Br_2 (128 g, 0.8 mol), dissolved in 200 mL of CCl_4, was given dropwise to the mixture. The reaction temperature was kept below 0 °C.. After being stirred for 2 h at this temperature, the whole was mixed with 1000 mL of H_2O, and the organic layer was extracted, washed with 10% NaOH and H_2O, and dried over $MgSO_4$. The solvent was removed, and the crude product was purified by fractional distillation to give 144 g of 4-bromo-3-methylanisole (90%, colorless liquid, bp 109–112 °C (14 mmHg). This product (40.2 g, 0.2 mol) and EtBr (43.6 g, 0.4 mol), dissolved in 500 mL of anhydrous ether, were added dropwise to a suspension of Mg turnings (15.8 g, 0.65 g-atom) in 1000 mL of anhydrous ether. The mixture was refluxed for 1 h. Subsequently a solution of DMF (43.9 g, 0.6 mol) in 150 mL of anhydrous ether was added slowly, while the reaction temperature was kept below 0 °C. The mixture was stirred for an additional hour at this temperature. The inorganic residue was dissolved in 10% NH_4Cl solution, and the ethereal layer was separated. The water layer was extracted with ether. The combined ethereal layers were washed successively with 2 N HCl, saturated $NaHCO_3$ solution, and H_2O and dried over $MgSO_4$. The solvent was removed, and the oily residue was purified by fractional distillation under reduced pressure to give 15.9 g of **1d** (53%, colorless liquid, bp 134–136 °C (14 mmHg)).

Method K. 4-Methoxy-2-(trifluoromethyl)benzaldehyde (3d). The reaction of 3-(trifluoromethyl)anisole (**3e**) (12.3 g, 0.07 mol), Fe powder (0.2 g), and Br_2 (11.2 g, 0.07 mol) in 150 mL of $CHCl_3$ was performed in analogy to method J at a temperature of −60 °C to yield 8.1 g of a mixture of stereoisomeric brominated products (45%, colorless liquid). The crude product (7.65 g, 0.03 mol), dissolved in 50 mL of anhydrous ether, was added dropwise to a solution of *n*-BuLi (1.92 g, 0.03 mol) in 150 mL of anhydrous ether at a temperature of −70 °C in a N_2 atmosphere. After the mixture was stirred for 15 min, an ethereal solution of *N*-formylpiperidine (3.4 g, 0.03 mol) was dropped slowly into the reaction mixture at a temperature below −55 °C. After a negative color test (with Michler's ketone) for organometallic compounds, the mixture was left at room temperature for 1 h and then acidified with 2 N HCl. The organic layer was separated, washed with saturated $NaHCO_3$ solution and H_2O, and dried over $MgSO_4$. The solvent was removed, and the isomers were separated by column chromatography (SiO_2, ether/petroleum ether, 1:4) to give 3.1 g of **3d** (51%, colorless needles, mp 38–39 °C).

Method L. 2-Chloro-4-methoxybenzaldehyde (5d). The reaction of 4-methoxybenzaldehyde (116 g, 0.85 mol) with dimethyl sulfite (101 g, 0.92 mol) and 5 mL of 11% methanolic HCl in 100 mL of anhydrous MeOH according to the procedure of Voss[19] afforded 139 g of 4-methoxybenzaldehyde dimethyl acetal (90%, colorless liquid, bp 120–121 °C (14 mmHg)). The acetal (18.2 g,

0.1 mol) was dissolved in 500 mL of anhydrous ether and cooled to −70 °C. To this solution was added *t*-BuLi (7.05 g, 0.11 mol) in hexane with stirring within 1 min in a N_2 atmosphere. A yellow color appeared upon addition of *t*-BuLi. After the mixture was stirred for 3 h at −25 °C, a yellow precipitate of ortho-metalated acetal was formed. To the mixture was added an ethereal solution of benzenesulfonyl chloride (17.7 g, 0.1 mol) within 1 min at −60 °C. After being stirred for 1 h at room temperature, the mixture was poured into ice/water and extracted with ether. The organic layer was successively washed with 2 N HCl, saturated $NaHCO_3$ solution, and H_2O. After drying with $MgSO_4$ and evaporation of the solvent, a crystalline solid was obtained. The crude product was purified by column chromatography (SiO_2, ether/petroleum ether, 1:2) to give 6.5 g of **5d** (38%, colorless needles, mp 60–61 °C).

In the case of compounds **7d** and **8d**, the ortho-metalated acetal was treated with Br_2 or I_2 in the same manner.

Method M. 2,6-Dichloro-4-methoxybenzaldehyde (6d). A mixture of 3,5-dichloroanisole (**6e**) (100 g, 0.56 mol) and paraformaldehyde (30.3 g) in 1.5 L of concentrated HCl and 15 mL of concentrated H_2SO_4 was stirred at 60 °C for 7 h. After cooling, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with H_2O, dried over $MgSO_4$, and evaporated. To the oily residue were added 1 L of 1 N NaOH and 500 mL of dioxane, and the mixture was refluxed for 3 h. The organic layer was separated, washed with H_2O, and dried over $MgSO_4$. The solvent was removed, and 100 mL of $CHCl_3$ was added to the oily residue. The precipitate was filtered off, the filtrate was evaporated, and the resulting isomeric benzyl alcohols were separated by column chromatography (SiO_2, ether/petroleum ether, 1:1) to obtain 46.4 g of 2,6-dichloro-4-methoxybenzyl alcohol (40%, colorless needles, mp 78–79 °C). A mixture of the latter (46.4 g, 0.22 mol) and freshly precipitated MnO_2 (102 g, 1.17 mol) in 800 mL of benzene was refluxed for 18 h on a water separator. The reaction mixture was filtered, the filter contents were washed with benzene, and the filtrate was evaporated. The crude product was recrystallized from MeOH to give 42.5 g of **6d** (94%, colorless needles, mp 109–111 °C).

Neutron Activation Analysis.[55-57] For experimental procedure, see the supplementary material.

Biochemical and biological methods: estradiol receptor binding assay;[50,51] immature mice uterine weight test;[24,25] P 388 leukemia;[27,53] hormone-independent MDA-MB 231 human breast cancer cell line;[28,52] hormone-dependent MCF 7 human breast cancer cell line;[29] hormone-dependent MXT mammary tumor;[30] hormone-independent MXT mammary tumor;[30] DMBA-induced, hormone-dependent mammary carcinoma of SD rat.[33,54] For experimental procedures, see the supplementary material.

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Registry No. *meso*-**1**, 111086-48-7; *d,l*-**1**, 111086-50-1; *meso*-**1**-$PtCl_2$, 105928-12-9; *d,l*-**1**-$PtCl_2$, 105855-88-7; *meso*-**1a**, 111086-47-6; *d,l*-**1a**, 111086-49-8; **1b**, 111086-71-6; **1c**, 111112-52-8; **1d**, 52289-54-0; **1e**, 100-84-5; *meso*-**2**, 111086-52-3; *meso*-**2**-$PtCl_2$, 105856-21-1; *meso*-**2a**, 111086-51-2; **2b**, 111086-72-7; **2c**, 111086-64-7; **2d**, 19447-00-8; **2e**, 874-63-5; *meso*-**3**, 111086-54-5; *meso*-**3**-$PtCl_2$, 105856-20-0; *meso*-**3a**, 111086-53-4; **3b**, 111086-73-8; **3c**, 111086-65-8; **3d**, 106312-36-1; **3e**, 454-90-0; *meso*-**4**, 111086-55-6; *meso*-**4**-$PtCl_2$, 105856-18-6; *meso*-**4**-$PtSO_4$, 111112-53-9; *meso*-**4a**, 111112-51-7; **4b**, 111086-74-9; **4c**, 111086-66-9; **4d**, 331-64-6; **4e**, 456-49-5; *meso*-**5**, 111086-57-8; *meso*-**5**-$PtCl_2$, 105856-16-4; *meso*-**5a**, 111086-56-7; **5b**, 111086-75-0; **5c**, 111086-67-0; **5d**, 54439-75-7; *meso*-**6**, 111086-58-9; *meso*-**6**-$PtCl_2$, 105856-23-3; *d,l*-**6**, 111112-21-1; *d,l*-**6**-$PtCl_2$, 105928-14-1; *meso*-**6**-$PtSO_4$, 111112-54-0; *meso*-**6a**, 83363-98-8; *d,l*-**6a**, 111086-59-0; **6b**, 111086-76-1; **6c**, 111086-68-1; **6d**, 82772-93-8; **6e**, 33719-74-3; *meso*-**7**, 111086-61-4; *meso*-**7**-$PtCl_2$, 105856-14-2; *meso*-**7a**, 111086-60-3; **7b**, 111086-77-2; **7c**, 111086-69-2; **7d**, 43192-31-0; *meso*-**8**, 111086-63-6; *meso*-**8**-$PtCl_2$, 105856-12-0; *meso*-**8a**, 111086-62-5; **8b**, 111086-78-3; **8c**, 111086-70-5; **8d**, 105469-13-4; Br_2, 7726-95-6; I_2, 7553-56-2; *meso*-1,2-bis(2-hydroxyphenyl)ethylenediamine, 58519-80-5; *N,N'*-bis(4-methoxy-2-methylbenzylidene)-*meso*-1,2-bis(4-methoxy-2-methylphenyl)ethylenediamine, 111086-79-4; 4-bromo-3-methylanisole, 27060-75-9; 4-bromo-3-(trifluoromethyl)anisole, 400-72-6; 4-methoxylbenzaldehyde, 123-11-5; 4-methoxybenzaldehyde dimethyl acetal, 2186-92-7; benzenesulfonyl chloride, 98-09-9; paraformaldehyde, 30525-89-4; 2,6-dichloro-4-methoxybenzyl alcohol, 86111-47-9.

Supplementary Material Available: Discussion of neutron activation analysis and biochemical and biological methods, Tables II, IV–VII, XI, and XV–XIX containing biological and chemical data, and Figures 2, 3, and 5 consisting of ^1H NMR spectra of *meso*-**6**-$PtCl_2$ and *d,l*-**6**-$PtCl_2$ and a conductance graph for *meso*-**6**-$PtSO_4$ (39 pages). Ordering information is given on any current masthead page.

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