95571-06-5; **6**, 95672-05-2; **7a**, 116561-60-5; **7b**, 116561-63-8; **7c**, 116561-62-7; **7d**, 116561-66-1; **7e**, 116561-61-6; **7f**, 116536-09-5; **7g**, 116536-10-8; **7h**, 95672-06-3; **7i**, 116561-67-2; **7j**, 95672-07-4; **8a**, 127792-62-5; **8a** (sulfide), 127792-51-2; **8b**, 127792-52-3; **8c**, 95672-02-9; **9a**, 127792-53-4; **9b**, 127792-54-5; **9c**, 127792-55-6; **9c** (sulfide; (*p*-methoxyphenyl)methyl ester), 127792-56-7; **9c** ((*p*-methoxyphenyl)methyl ester), 127792-57-8; **10** (sulfide), 95570-51-7; **10**, 95671-88-8; **11** (sulfide), 127792-58-9; **11**, 127792-59-0; **12** (sulfide), 95570-84-6; **12**, 95671-89-9; **13a**, 127792-60-3; **13b**, 127792-61-4; HS(C=S)OEt, 151-01-9; HSPh, 57658-36-3; L-$H_2NCH(CH_2Ph)C(O)OC_6H_4$-*p*-OMe, 47171-46-0; elastase, 9004-06-2; 5-mercapto-1-methyltetrazole, 13183-79-4; 1,2,5,6-tetrahydro-5,6-dioxo-3-mercapto-2-methyl-*as*-triazine, 58909-39-0; 5-mercapto-1,3,4-triazole, 3179-31-5; 2-methyl-5-mercapto-1,3,4-thiadiazole, 29490-19-5; 2-mercapto-1-methylimidazole, 60-56-0; 5-mercapto-1-tetrazolylacetic acid, 57658-36-3.

Aqua[1-(2,6-dichloro-4-hydroxyphenyl)-2-phenylethylenediamine](sulfato)platinum-(II) Complexes with Variable Substituents in the 2-Phenyl Ring. 1. Synthesis and Antitumor and Estrogenic Properties

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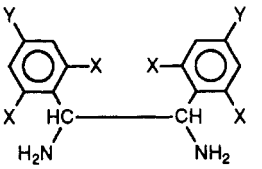
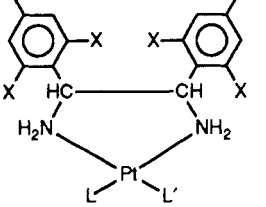
Erythro- and threo-configurated aqua[1-(2,6-dichloro-4-hydroxyphenyl)-2-phenylethylenediamine](sulfato)platinum(II) complexes with variable substituents in the 2-phenyl ring (**2**-$PtSO_4$ to **9**-$PtSO_4$: H, 4-F, 3-OH, 4-OH, 2,6-F_2, 2,6-Cl_2, 2-F/4-OH, 2-Cl/4-OH) were synthesized and tested for estrogenic and antitumor activities. The ligands were obtained by a three-step reaction. The stilbenes were reacted with a mixture of IN_3 and NaN_3 to yield the respective 1,2-diazido-1,2-diphenylethanes. The subsequent reduction with $LiAlH_4$ led to the corresponding 1,2-diphenylethylenediamines. The (sulfato)platinum(II) complexes were synthesized by reaction of Ag_2SO_4 with the diiodo complexes, which had been obtained by coordination of the diamines with K_2PtI_4. Two complexes, *erythro*-**8**-$PtSO_4$ and *erythro*-**9**-$PtSO_4$, possess antitumor and estrogenic effects and are therefore of interest for the therapy of breast cancer.

[*meso*-1,2-Bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) (*meso*-**1**-$PtCl_2$) shows a strong and specific effect on estrogen receptor positive (ER^+) mammary carcinoma (MC) models, e.g. on the MXT, ER^+-MC of the mouse.[1,13] This effect is determined by the ligand *meso*-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine (*meso*-**1**) which possesses a marked estrogenic activity. The exchange of *meso*-**1** by its diastereomeric diamine *d,l*-**1** leads to a complex (*d,l*-**1**-$PtCl_2$) which, in contrast to *meso*-**1**-$PtCl_2$, shows neither MC-inhibiting nor estrogenic properties.[1] At equimolar dosage *meso*-**1**-$PtSO_4$, a water soluble derivative of *meso*-**1**-$PtCl_2$, is significantly more active on the 9,10-dimethyl-1,2-benzanthracene (DMBA) induced, hormone-dependent MC of the Sprague-Dawley (SD) rat than its ligand *meso*-**1** (0.5×10^{-5} M/kg, sc, three times per week, duration of therapy 4 weeks; % change of tumor area: *meso*-**1**-$PtSO_4$, −84%; *meso*-**1**, +129%; cisplatin, −38%; control, +420%). The complex *meso*-**1**-$PtSO_4$ is also superior to cisplatin,[1] which in the therapy of metastatic breast cancer does not lead to convincing results.[2] In the DMBA–MC experiment we found a 22-fold enrichment of *meso*-**1**-$PtSO_4$ in the tumor compared with the skeletal muscle.[1] These results support a mode of action which needs an intact ER system (i.e. an intact cytoplasm nucleus translocation process). According to this concept platinum complexes that contain an ER-affinic ligand are supposed to be accumulated in the nuclei of hormone-dependent breast cancer cells by the receptor system, thereby causing a stronger cytotoxic effect (due to the PtLL'–residue) than non-estrogenic platinum complexes. In contrast to the very promising results on the MXT, ER^+-MC *meso*-**1**-$PtCl_2$ does not cause an inhibition of the hormone-independent MXT-MC.[1] It cannot be excluded that the estrogenic properties of *meso*-**1**-$PtCl_2$ are partially responsible for its effect on the hormone-dependent MC, since high-dosed estrogens also evoke MC-inhibiting properties.[3]



1: X = Y = H
meso-**1**: X = Cl; Y = OH
d,l-1: X = Cl; Y = OH



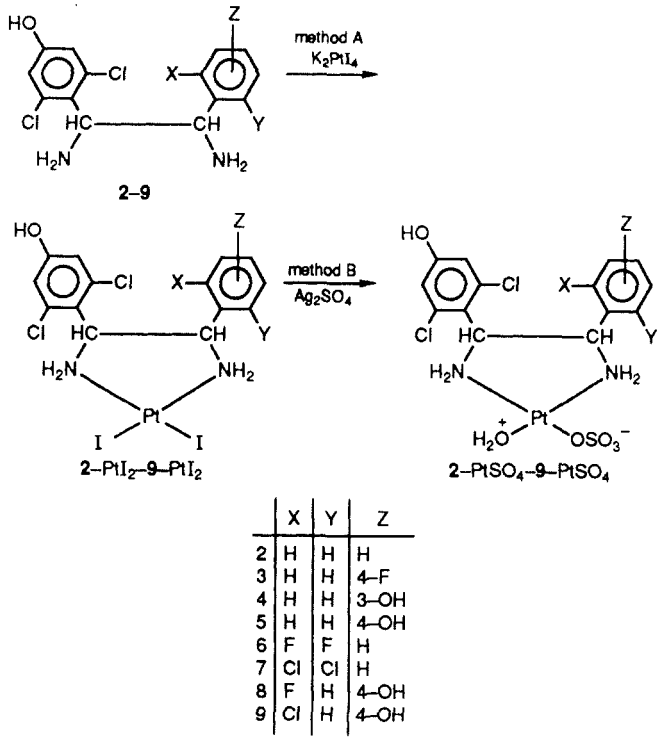
1-PtLL': X,Y = H; L,L' = variable leaving groups
meso-**1**-$PtCl_2$: X = Cl; Y = OH; L,L' = Cl
d,l-1-$PtCl_2$: X = Cl; Y = OH; L,L' = Cl
meso-**1**-$PtSO_4$: X = Cl; Y = OH; L = OH_2^+; L' = $O\text{–}SO_3^-$

Platinum(II) complexes, which show marked activities not only on the ER positive MC but also on the ER negative MC, should cause a delay of the development of resistance, a well-known process in the therapy of breast cancer (e.g. with antiestrogens).[4,5]

(1) Karl, J.; Gust, R.; Spruss, T.; Schneider, M.; Schönenberger, H.; Engel, J.; Wrobel, K.-H.; Lux, F.; Trebert-Haeberlin, S. *J. Med. Chem.* **1988**, *31*, 72.
(2) Prestayko, A. W. In *Cancer and Chemotherapy*; Crooke, S. T., Prestayko, A. W., Eds.; Academic Press: New York, 1981; Vol. III (Antineoplastic Agents), p 145.
(3) Hartmann, R. W. *Eur. J. Cancer Clin. Oncol.* **1983**, *19*, 959.
(4) Selby, P.; Bizzari, J.-P.; Buick, R. N. *Cancer Treat. Rep.* **1983**, *67*, 659.
(5) 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.

0022-2623/90/1833-2535$02.50/0

Scheme I



	X	Y	Z
2	H	H	H
3	H	H	4-F
4	H	H	3-OH
5	H	H	4-OH
6	F	F	H
7	Cl	Cl	H
8	F	H	4-OH
9	Cl	H	4-OH

The hormone-dependent MC often shows a transition to a gradually decreasing cellular differentiation and to autonomous growth. This process is accompanied by a reduction of the ER concentration. The phenomenon of tumor progression is thought to be a consequence of either clonal selection or epigenetic changes.[6] If a long-term survival of patients with hormone-dependent MC shall be achieved, the formation and spreading of autonomous clones must be prevented. This is only feasible with compounds which act against hormone-dependent and -independent MC cells as well.

Compounds with such properties were obtained by us by introduction of suitable substituents into the 2-phenyl ring of aqua[1-(2,6-dichloro-4-hydroxyphenyl)-2-phenylethylenediamine](sulfato)platinum(II) (2-$PtSO_4$). In this publication we describe the synthesis and the antitumor and estrogenic properties of the new compounds.

Chemistry

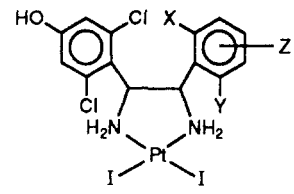
The diastereomeric diiodoplatinum(II) complexes 2-PtI_2–9-PtI_2 were synthesized by reacting K_2PtI_4 with the ligands **2–9** in water at room temperature (Scheme I, method A). The chemical data are listed in Table I (for elemental analyses and ^1H NMR data, see Table X and XIV in supplementary material). The analogous diastereomeric (sulfato)platinum(II) complexes 2-$PtSO_4$–9-$PtSO_4$ were obtained by addition of Ag_2SO_4 powder to a suspension of the respective diiodoplatinum(II) complex in water (Scheme I, method B). The analytical data are given in Table II (for elemental analyses, see Table XI in supplementary material).

Corresponding to the elemental analyses the (sulfato)platinum(II) complexes *erythro*- and *threo*-2-$PtSO_4$–9-$PtSO_4$ contain one or more H_2O molecules.

The question whether water-containing (ethylenediamine)(sulfato)platinum(II) complexes exist as unidentate aqua(ethylenediamine)(sulfato)platinum(II) complexes

(6) Rennie, P. S. In *Drug and Hormone Resistance in Neoplasia*; Bruchovsky, N., Goldie, J. H., Eds.; CRC-Press, Inc.: Boca Raton, FL, 1982; Vol. I (Basic Concepts), p 95.

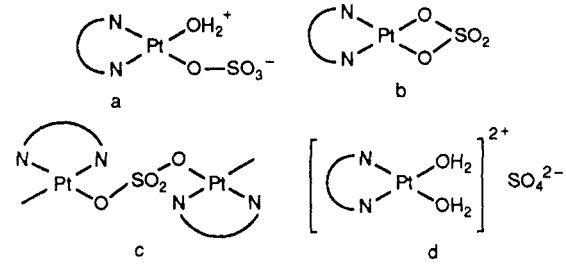
Table I. [1-(2,6-Dichloro-4-hydroxyphenyl)-2-phenylethylenediamine]diiodoplatinum(II) Complexes with Variable Substituents in the 2-Phenyl Ring[a]



compd	X	Y	Z	yield,[b] %	formula[c]
erythro-2-PtI_2	H	H	H	99	$C_{14}H_{14}Cl_2I_2N_2OPt$
threo-2-PtI_2				91	
erythro-3-PtI_2	H	H	4-F	78	$C_{14}H_{13}Cl_2FI_2N_2OPt$[d]
threo-3-PtI_2				91	
erythro-4-PtI_2	H	H	3-OH	73	$C_{14}H_{14}Cl_2I_2N_2O_2Pt$[e]
threo-4-PtI_2				93	
erythro-5-PtI_2	H	H	4-OH	64	$C_{14}H_{14}Cl_2I_2N_2O_2Pt$
threo-5-PtI_2				83	
erythro-6-PtI_2	F	F	H	68	$C_{14}H_{12}Cl_2F_2I_2N_2OPt$[f]
threo-6-PtI_2				83	
erythro-7-PtI_2	Cl	Cl	H	77	$C_{14}H_{12}Cl_4I_2N_2OPt$
threo-7-PtI_2				92	
erythro-8-PtI_2	F	H	4-OH	66	$C_{14}H_{13}Cl_2FI_2N_2O_2Pt$
threo-8-PtI_2				87	
erythro-9-PtI_2	Cl	H	4-OH	88	$C_{14}H_{13}Cl_3I_2N_2O_2Pt$
threo-9-PtI_2				98	

[a] Synthetic method A see under 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, except where noted. [d] *erythro*-3-PtI_2 H: calcd, 1.70; found, 2.35. [e] *erythro*-4-PtI_2 C: calcd, 22.06; found, 22.68. H: calcd, 1.84; found, 2.66. [f] *erythro*-6-PtI_2 C: calcd, 21.49; found, 22.52. *threo*-6-PtI_2 C: calcd, 21.49; found, 22.90. H: calcd, 1.54; found, 2.04.

Scheme II



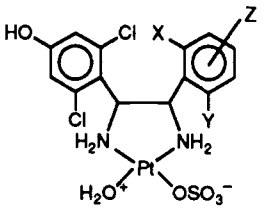
(Scheme II, a) or as diaqua(ethylenediamine)platinum(II) sulfates (Scheme II, d) was decided in favor of structure a by Rochon and Melanson.[7] They could show by X-ray analysis, that in a (*N*,*N*'-dimethylethylenediamine)(sulfato)platinum(II) complex containing two H_2O molecules, the sulfate residue is present as unidentate ligand and the second coordination site of the platinum atom is occupied by H_2O. This complex structure is further stabilized by two strong hydrogen bridges from the H_2O ligand to the sulfate ligand and to the lattice water.

For the amount of antitumor activity of the (ethylenediamine)(sulfato)platinum(II) complexes it is of no importance whether structure a or d is present, since in water solution the sulfato residue of unidentate (a) as well of bidentate (b,c) (ethylenediamine)(sulfato)platinum(II) complexes is quickly replaced by H_2O molecules, forming the active diaqua(ethylenediamine)platinum(II) ion (d).[1]

The IR spectra (KBr) show characteristics of ionic (1130 cm^{-1}, vs, ν_3, 620 cm^{-1}, s, ν_4) as well as of coordinated SO_4 residues (1040 cm^{-1}, s, ν_3, 950 cm^{-1}, m, ν_1) allowing no

(7) Rochow, F. D.; Melanson, R. *Inorg. Chem.* **1987**, *26*, 989.

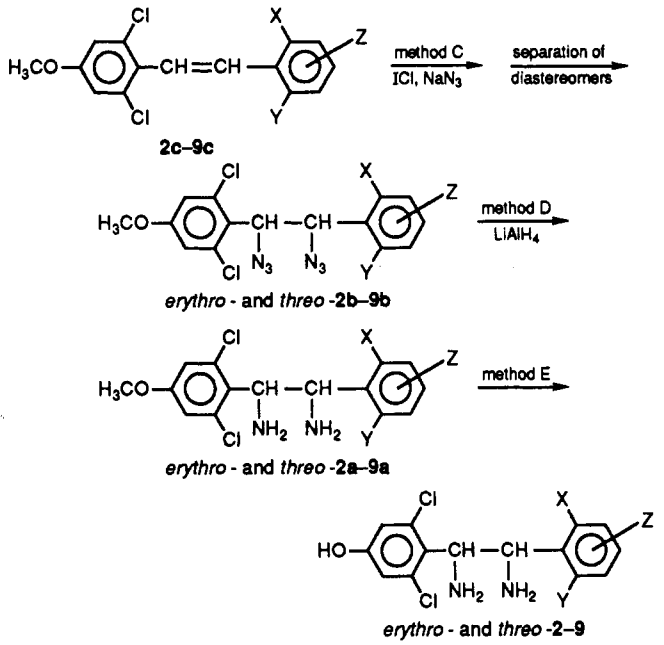
Table II. Aqua[1-(2,6-dichloro-4-hydroxyphenyl)-2-phenylethylenediamine](sulfato)platinum(II) Complexes with Variable Substituents in the 2-Phenyl Ring[a]



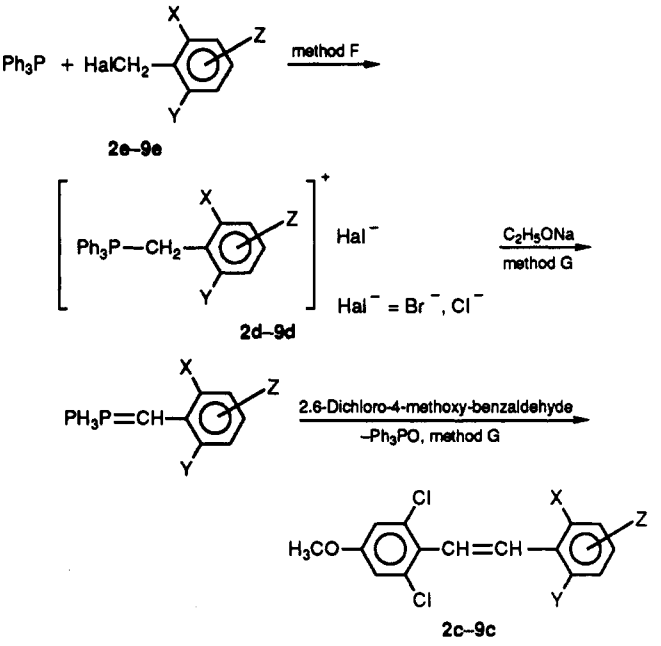
compd	X	Y	Z	yield,[b] %	formula[c]
erythro-**2**-$PtSO_4$	H	H	H	65	$C_{14}H_{16}Cl_2N_2O_6PtS\cdot H_2O$
threo-**2**-$PtSO_4$				46	$C_{14}H_{16}Cl_2N_2O_6PtS\cdot 2H_2O$
erythro-**3**-$PtSO_4$	H	H	4-F	45	$C_{14}H_{15}Cl_2FN_2O_6PtS\cdot H_2O$
threo-**3**-$PtSO_4$				37	$C_{14}H_{15}Cl_2FN_2O_6PtS\cdot 2H_2O$
erythro-**4**-$PtSO_4$	H	H	3-OH	47	$C_{14}H_{16}Cl_2N_2O_7PtS\cdot 5H_2O$[d]
threo-**4**-$PtSO_4$				39	$C_{14}H_{16}Cl_2N_2O_7PtS\cdot 2H_2O$
erythro-**5**-$PtSO_4$	H	H	4-OH	39	$C_{14}H_{16}Cl_2N_2O_7PtS\cdot 4H_2O$
threo-**5**-$PtSO_4$				54	$C_{14}H_{16}Cl_2N_2O_7PtS$
erythro-**6**-$PtSO_4$	F	F	H	72	$C_{14}H_{14}Cl_2F_2N_2O_6PtS$
threo-**6**-$PtSO_4$				55	$C_{14}H_{14}Cl_2F_2N_2O_6PtS$
erythro-**7**-$PtSO_4$	Cl	Cl	H	56	$C_{14}H_{14}Cl_4N_2O_6PtS\cdot 3H_2O$
threo-**7**-$PtSO_4$				24	$C_{14}H_{14}Cl_4N_2O_6PtS\cdot 6H_2O$
erythro-**8**-$PtSO_4$	F	H	4-OH	81	$C_{14}H_{15}Cl_2FN_2O_7PtS\cdot H_2O$
threo-**8**-$PtSO_4$				71	$C_{14}H_{15}Cl_2FN_2O_7PtS$[e]
erythro-**9**-$PtSO_4$	Cl	H	4-OH	60	$C_{14}H_{15}Cl_3N_2O_7PtS\cdot H_2O$
threo-**9**-$PtSO_4$				61	$C_{14}H_{15}Cl_3N_2O_7PtS\cdot H_2O$

[a] Synthetic method B see under Experimental Section. [b] Calculated with regard to the corresponding diiodoplatinum(II) complex. [c] All compounds were analyzed for C, H, and N within ±0.40% of the calculated values, except where noted. [d] H: calcd, 3.65; found, 3.05. [e] H: calcd, 2.34; found, 2.95.

Scheme III



Scheme IV



unambiguous assignment (see Table IX in supplementary material). Presumably the compounds are present as unidentate complexes which are contaminated with complex type d. The use of formula a (Scheme II) in this publication is without commitment to an unidentate complex structure.

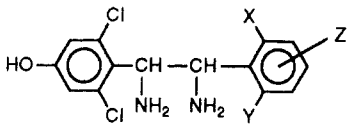
The 1-(2,6-dichloro-4-hydroxyphenyl)-2-phenylethylenediamines **2–9** were obtained in a four-step reaction (Scheme III, methods C–E). Their analytical data are listed in Table III (for elemental analyses and ^1H NMR data, see Table XII and XV in supplementary material).

In the first step the 1-(2,6-dichloro-4-methoxyphenyl)-2-phenylethenes **2c–9c** were added at −60 °C to a solution of IN_3 in acetonitrile to give the respective 1-azido-2-iodo-1,2-diphenylethanes which were not isolated (Fowler et al.[8]). The educt IN_3 was produced in situ by reaction of NaN_3 with ICl (Scheme III, method C). The addition of IN_3 to the C=C double bond and the subsequent exchange of I^- by N_3^- took place with high stereoselectivity at low temperatures.

The *E*-stilbenes **2c–9c**, which were predominantly formed by the reaction of 2,6-dichloro-4-methoxybenzaldehyde with the triphenylphosphonium halides (compare Scheme IV) yielded the *threo*-1,2-diazido-1-(2,6-dichloro-4-methoxyphenyl)-2-phenylethanes, while the *Z*-stilbenes **2c–9c** mainly afforded the erythro isomers. The purification of the erythro and threo isomers (**2b–9b**) was per-

(8) Fowler, F. W.; Hassner, A.; Levy, L. *J. Am. Chem. Soc.* **1967**, *89*, 2077.

Table III. 1-(2,6-Dichloro-4-hydroxyphenyl)-2-phenylethylenediamines with Variable Substituents in the 2-Phenyl Ring[a]



compd	X	Y	Z	yield,[b] %	mp, °C	formula[c]
erythro-**2**	H	H	H	49	90–95	$C_{14}H_{14}Cl_2N_2O$
threo-**2**				78	170–171	
erythro-**3**	H	H	4-F	60	145–146	$C_{14}H_{13}Cl_2FN_2O\cdot H_2O$
threo-**3**				50	180	
erythro-**4**	H	H	3-OH	42	194–196	$C_{14}H_{14}Cl_2N_2O_2$
threo-**4**				88	192–194	
erythro-**5**	H	H	4-OH	43	180–185	$C_{14}H_{14}Cl_2N_2O_2\cdot{}^1/_2H_2O$
threo-**5**				40	167–169	$C_{14}H_{14}Cl_2N_2O_2$
erythro-**6**	F	F	H	70	160–162	$C_{14}H_{12}Cl_2F_2N_2O$[d]
threo-**6**				41	148–152	$C_{14}H_{12}Cl_2F_2N_2O\cdot H_2O$
erythro-**7**	Cl	Cl	H	49	173–175	$C_{14}H_{12}Cl_4N_2O$
threo-**7**				53	156–158	$C_{14}H_{12}Cl_4N_2O\cdot H_2O$
erythro-**8**	F	H	4-OH	44	165–166	$C_{14}H_{13}Cl_2FN_2O_2$[e]
threo-**8**				63	163–164	
erythro-**9**	Cl	H	4-OH	27	163–165	$C_{14}H_{13}Cl_3N_2O_2$
threo-**9**				40	200	$C_{14}H_{13}Cl_3N_2O_2\cdot H_2O$

[a] Synthetic method E see under Experimental Section. [b] Calculated with regard to the methoxy substituted diamines. [c] All compounds were analyzed for C, H, and N within ±0.40% of the calculated values, except where noted. [d] *erythro*-**6** N: calcd, 8.42; found, 7.52. [e] *erythro*-**8** N: calcd, 8.47; found, 7.72. *threo*-**8** N: calcd, 8.47; found, 7.82.

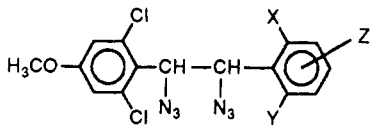
formed by column chromatography. Chemical data are listed in Table V (^{1}H-NMR data see Table XVII in supplementary material). The diastereomeric 1-(2,6-dichloro-4-methoxyphenyl)-2-phenylethylenediamines **2a–9a** were obtained by reduction of the related 1,2-diazido-1,2-diphenylethanes **2b–9b** with $LiAlH_4$ (Scheme III, method D; for chemical and ^{1}H NMR data, see Table IV and XVI in supplementary material). The ether cleavage of *erythro*- and *threo*-**2a–9a** to give the diastereomeric 1-(2,6-dichloro-4-hydroxyphenyl)-2-phenylethylenediamines **2–9** was performed with BBr_3 in CH_2Cl_2 (Scheme III, method E).

The assignment of the ligands **2–9** to the erythro or threo series is possible by characteristic differences in the ^{1}H NMR spectra of their diiodoplatinum(II) complexes, especially of the absorption bands of the vicinal benzylic protons. However, the identification of the bands in the interesting range between 4 and 7 ppm is difficult. This interval contains, besides two signals of benzylic protons, two signals each for axially and equatorially arranged NH atoms caused by complexation, which blocks rotation around the C–N axis. Due to a coupling between NH_2, benzylic CH, and ^{195}Pt, the NH and CH signals are additionally broadened.

An exchange of N standing protons by deuterium, by which the assignment of signals is facilitated, does not take place after addition of D_2O to the complex. This observation is in accordance with results of Fanizzi et al.,[9] who found no H–D exchange and a stable chirality at the coordinated nitrogens of stereoisomeric (*N,N'*-dialkylethylenediamine)dichloroplatinum(II) complexes in a chloroform solution saturated with D_2O at room temperature. Therefore, the tetradeuterated complexes were synthesized by reaction of the related ligand with K_2PtI_4 in D_2O solution (see Table XIII).

As an example for the typical change in the ^{1}H NMR spectrum after transformation of the ligands **2–9** into their diiodoplatinum(II) complexes the diastereomeric ligands **9** and complexes **9**-PtI_2 are presented in the Figures 1 and

Table V. 1,2-Diazido-1-(2,6-dichloro-4-methoxyphenyl)-2-phenylethanes with Variable Substituents in the 2-Phenyl Ring[a]



compd[b]	X	Y	Z	yield,[c] %	mp, °C	formula[d]
erythro-**2b**	H	H	H	69	oil	$C_{15}H_{12}Cl_2N_6O$
threo-**2b**					oil	
erythro-**3b**	H	H	4-F	59	oil	$C_{15}H_{11}Cl_2FN_6O$
threo-**3b**					oil	
erythro-**4b**	H	H	3-OCH_3	91	oil	$C_{16}H_{14}Cl_2N_6O_2$
threo-**4b**					oil	
erythro-**5b**	H	H	4-OCH_3	93	oil	$C_{16}H_{14}Cl_2N_6O_2$
threo-**5b**					oil	
erythro-**6b**	F	F	H	99	96–100	$C_{15}H_{10}Cl_2F_2N_6O$
threo-**6b**					oil	
erythro-**7b**	Cl	Cl	H	84	95–98	$C_{15}H_{10}Cl_4N_6O$
threo-**7b**					oil	
erythro-**8b**	F	H	4-OCH_3	95	oil	$C_{16}H_{13}Cl_2FN_6O_2$
threo-**8b**					oil	
erythro-**9b**	Cl	H	4-OCH_3	94	oil	$C_{16}H_{13}Cl_3N_6O_2$
threo-**9b**					oil	

[a] Synthetic method C see under Experimental Section. [b] Separation by column chromatography on SiO_2: **2b**, CH_2Cl_2/petroleum ether 1:1; **3b**, CH_2Cl_2/cyclohexane 1:1; **4b**, 1,2 dichloroethane/hexane 1:1; **5b**, petroleum, ether/ether 1:1; **6b** and **7b**, petroleum ether/ether 5:1; **8b** and **9b**, petroleum ether/ether 4:1. [c] Calculated with regard to the stilbenes. [d] Analyzed by ^{1}H NMR, spectroscopy (see Table XVII).

2. *erythro*-**9** as well as *threo*-**9**, shows a doublet with a coupling constant of about 10 Hz for the nonequivalent benzylic protons. Therefore, *erythro*-**9** and *threo*-**9** exist in conformations in which the dihedral angle between the two vicinal protons amounts to about 180°. This means that one of the two synclinal positions of phenyl residues is preferred in the case of *threo*-**9** and the antiperiplanar one in *erythro*-**9** (see also Figures 3 and 4). After transformation of the diastereomeric ligands **9** into their N-deuterated diiodoplatinum(II) complexes a shift of both doublets to lower field takes place.

In the threo form the coupling constant (of about 10 Hz) remains essentially unchanged (Figures 1 and 3). This

(9) Fanizzi, F. P.; Maressa, L.; Natile, G.; Lanfranchi, M.; Manotti-Laufredi, A. M.; Tiripicchio, A. *Inorg. Chem.* **1988**, *27*, 2422.

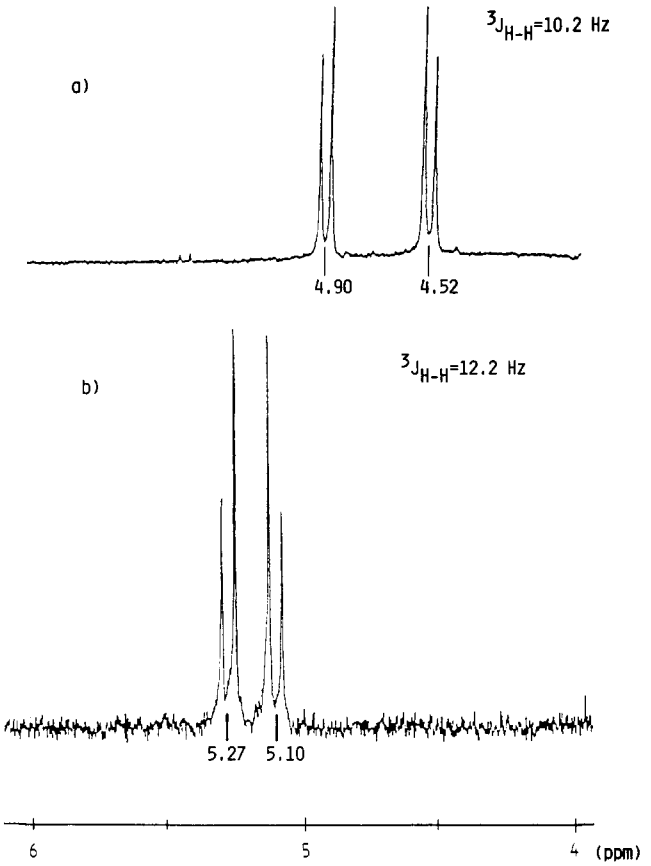


Figure 1. 250-MHz ^{1}H NMR spectrum of (a) *threo*-**9** in DMF-d_7 and (b) *threo*-**9**-PtI_2 in DMF-d_7. NH was replaced by deuterium.

finding is in accordance with a preferred arrangement of the two phenyl rings in equatorial positions of the five-membered chelate. Due to steric reasons a conformation with axially oriented phenyl rings is not favored. This means also that the transformation of the ligand *threo*-**9** into the diiodoplatinum(II) complex *threo*-**9**-PtI_2 essentially conserves the ligand conformation.

In contrast to *threo*-**9** the ligand *erythro*-**9** changes its conformation when transformed into the diiodoplatinum-(II) complex (compare Figures 2 and 4). The two vicinal protons of *erythro*-**9**-PtI_2 show a dihedral angle of about 60° as well in the δ-conformation as in the λ-conformation corresponding with a coupling constant of about 5 Hz. Therefore, the two phenyl rings are also synclinally arranged in both conformations (i.e. the axial and equatorial positions in the five-membered chelate).

From the coupling constant of the Pt satellites of the 2-chloro-4-hydroxyphenyl-substituted –CH– (δ = 4.78 ppm, ^{1}H, J_{H-H} = 5.2 Hz, J_{H-Pt} = 79 Hz) in *erythro*-**9**-PtI_2 (Figure 2) it can be concluded that on this C atom the hydrogen is equatorially and the phenyl ring axially arranged.[10] Therefore this complex exists in the δ-conformation (see Figure 4). In case of the simultaneous presence of a λ-conformer a reduction of J_{H-Pt} or a second –CH– doublet which possesses Pt satellites with $J_{H-Pt} \approx 40$ Hz should be expected. In accordance with the presence of the δ-conformation the –CH– doublet at lower field (2,6-dichloro-4-hydroxyphenyl-substituted –CH–: δ = 5.26 ppm, ^{1}H, J_{H-H} = 5.2 Hz) shows merely a broadening of its basis, which is caused by the two Pt satellites ($J_{H-Pt} \approx 30$ Hz).[10] The 2,6-dichloro-4-hydroxyphenyl ring is in the δ-conformer equatorially and the respective hydrogen is axially arranged. If a λ-conformer existed in the equilibrium, a $J_{H-Pt} > 30$ Hz or a second –CH– doublet which possesses Pt satellites with $J_{H-Pt} \approx 80$ Hz would be expected.

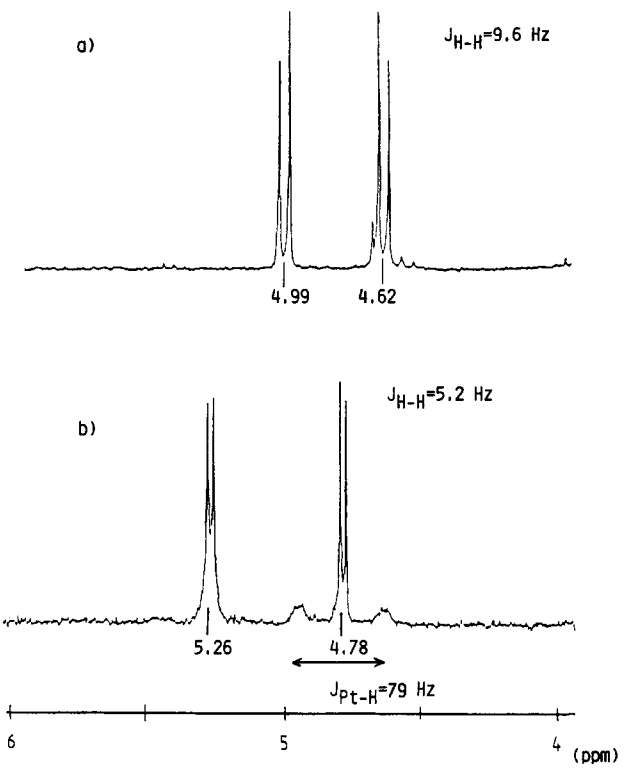


Figure 2. 250-MHz ^{1}H NMR spectrum of (a) *erythro*-**9** in DMF-d_7 and (b) *erythro*-**9**-PtI_2 in acetone-d_6. NH was replaced by deuterium.

The X-ray analysis of *erythro*-**9**-PtI_2 gave the same structure as ^{1}H NMR spectroscopy.

The complexes *threo*-**2**-PtI_2 to *threo*-**8**-PtI_2, *erythro*-**2**-PtI_2 to *erythro*-**5**-PtI_2, and *erythro*-**8**-PtI_2 show the same coupling constants as found for *threo*-**9**-PtI_2 and *erythro*-**9**-PtI_2 and can therefore be unequivocally assigned to either the threo or erythro form (Table XIII).

erythro-**6**-PtI_2 and *erythro*-**7**-PtI_2 are an exception to the above described examples. The coupling constants J_{H-H} of the benzylic protons are greater than 5 Hz, amounting to 7.5 Hz. Therefore, we suppose an equilibrium between the λ- and the δ-configurations. This is confirmed by the reduction of the J_{H-Pt} coupling constant of the 2,6-dichloro-4-hydroxyphenyl-substituted –CH– to 55 Hz for *erythro*-**6**-PtI_2. The difference of the chemical shifts of the benzylic protons of *erythro*-**7**-PtI_2 are small, so the Pt satellites cannot be detected. The ^{1}H NMR spectra of the 1,2-diphenylethylenediamines and the 1,2-diazido-1,2-diphenylethanes are insuitable for a configurational assignment because the substitution of the 2-phenyl ring induces various conformers in solution.

The synthesis of the 1-(2,6-dichloro-4-methoxyphenyl)-2-phenylethenes **2c–9c** was performed by Wittig reaction (Scheme IV; the analytical data are listed in Table VI and the ^{1}H NMR data in Table XVIII in supplementary material).

(10) Hawkins, C. J.; Palmer, J. A. *Coord. Chem. Rev.* **1982**, *44*, 1.
(11) Watson, C.; Medina, D.; Clark, J. H. *Endocrinology* **1980**, *107*, 1432.
(12) Leclercq, G.; Danguy, A.; Devleeschouwer, N.; Heuson, J. C.; *Cancer Chemother. Pharmacol. (Suppl)* **1982**, *9*, 30.
(13) The transplantable, hormone-dependent MXT-M 3.2 mammary carcinoma of the B6D2F1 mouse (MXT,ER$^+$) was described by Watson, Medina, and Clark.[11] This tumor contains a high estrogen receptor level (*ER* = about 59 fmol/mg protein) and is strongly inhibited by ovariectomy, tamoxifen, and high-dosed estrogens.[12] The MXT,ER$^+$ is a useful premenopausal MC model for the evaluation of new breast cancer drugs.

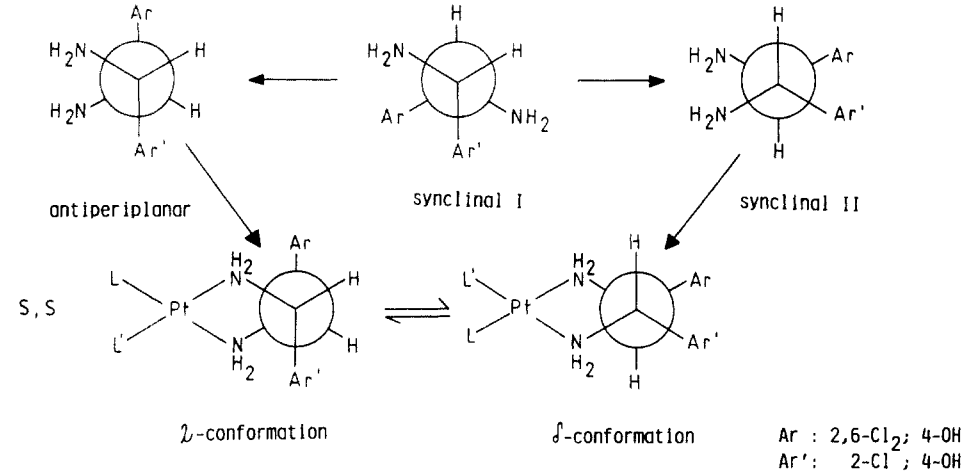


Figure 3. Transformation of the ligand *threo*-**9** into the complex *threo*-**9**-PtLL', *no* change of ligand conformation.

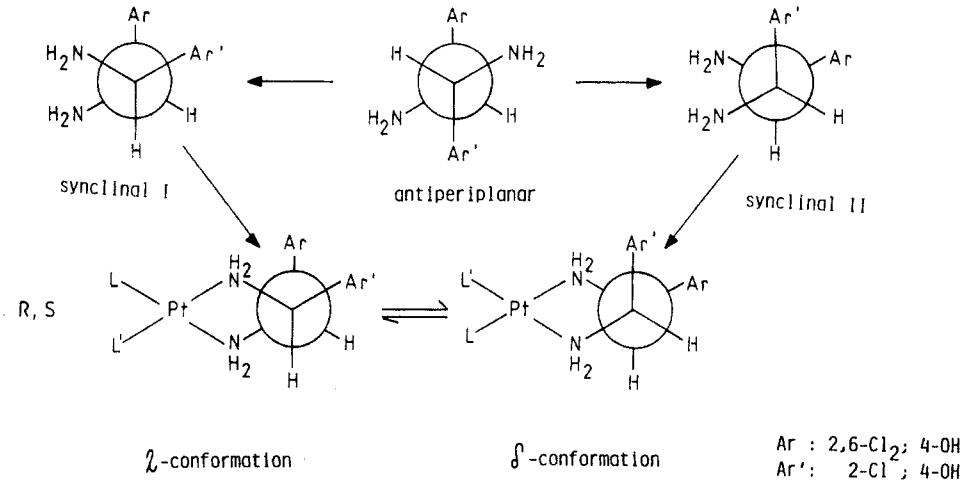


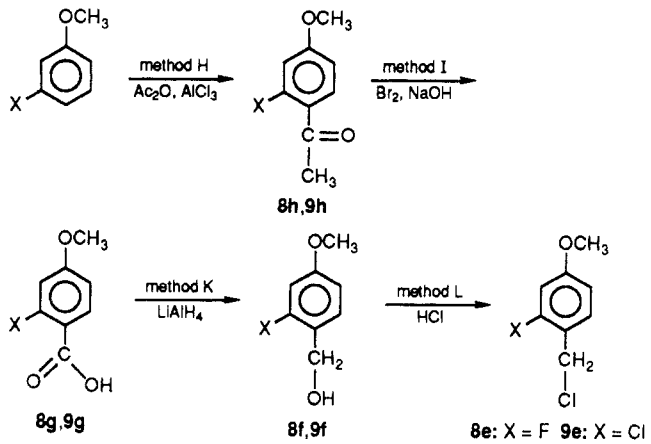
Figure 4. Transformation of the ligand *erythro*-**9** into the complex *erythro*-**9**-PtLL', change of ligand conformation.

The benzyltriphenylphosphonium halides **2d–9d** were prepared from triphenylphosphine and the benzyl halides **2e–9e** (Scheme IV, method F; for analytical data and ^1H NMR data, see Tables VII and XIX in supplementary material). The benzyltriphenylphosphonium halides **2d–9d** were converted into the ylides by treatment with sodium ethanolate in ethanol. To the colored reaction mixture the 2,6-dichloro-4-methoxybenzaldehyde[1] was added to give the stilbenes **2c–9c** (Scheme IV, method G).

Because of the bulky groups in the 2,6-positions of one phenyl ring, the thermodynamically more stable *E* isomer was predominant. A separation of the *E*/*Z* isomers was not carried out.

The preparation of the commercially not available benzyl halides is presented in Scheme V (methods H–L; for analytical and ^1H NMR data, see Tables VIII and XX in supplementary material). The syntheses of 2-chloro- and 2-fluorobenzoic acids **8g** and **9g** were performed by a Friedel–Crafts acylation (Scheme V, method H) and a subsequent bromoform reaction (Scheme V, method I). The isomeric acids were separated by fractional crystallization in ethanol/H_2O. Reduction of **8g** and **9g** with $LiAlH_4$ to the benzyl alcohols **8f** and **9f** (Scheme V, method K) and the following treatment with HCl gas led to the benzyl chlorides **8e** and **9e** (for analytical and ^1H NMR data, see Tables VIII and XX in supplementary material).

Scheme V



Biological Properties

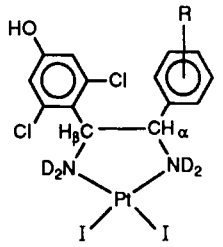
The cytotoxic activity of the new platinum complexes *erythro*- and *threo*-**2**-$PtSO_4$–**9**-$PtSO_4$ was evaluated in vivo experiments on the P388 leukemia of the CDF_1 mouse. The results are summarized in Table XXI.

All compounds showed a significant antitumor activity (% $T/C \geq 125$). However, none of these platinum complexes produced an effect which was comparable with that of cisplatin (0.5×10^{-5} M/kg ip, days 1, 5, 9; $T - C = -3$, 8; median day of survival 19 (17–21); % $T/C = 238$). The threo configurated compounds were often more toxic than their erythro diastereomers.

In the case of *threo*-**4**-$PtSO_4$ and *threo*-**5**-$PtSO_4$ the toxic side effects even led to a strong decrease of survival time at the highest used dose (40 μM/kg).

This study proved that a marked elevation of cytotoxic potency was already achieved by the replacement of one bulky chlorine atom with hydrogen in one of the two 2,6-dichloro-4-hydroxyphenyl rings of *meso*-**1**-$PtSO_4$ (see *er-*

Table XIII. ^{1}H NMR Data[a] of N-Deuterated [1-(2,6-Dichloro-4-hydroxyphenyl)-2-phenylethylenediamine]diiodoplatinum(II) Complexes



compd	R	H atoms	δ –CH–, ppm	$^3J_{H-H}$, Hz	$^3J_{Pt-H}$, Hz	$\delta H_\alpha - \delta H_\beta$, ppm
erythro-2-PtI_2	H	H_α	4.25	4.8	85	1.0
		H_β	5.25	4.8	nd[b]	
threo-2-PtI_2		H_α	5.10	12.3	nd	0.17
		H_β	5.27	12.3	nd	
erythro-3-PtI_2	4-F	H_α	4.28	5.0	80	0.98
		H_β	5.26	5.0	nd	
threo-3-PtI_2		H_α	5.13	12.3	nd	0.12
		H_β	5.25	12.3	nd	
erythro-4-PtI_2	3-OH	H_α	3.97	5.1	78	1.10
		H_β	5.07	5.1	nd	
threo-4-PtI_2		H_α	4.84	12.3	nd	0.24
		H_β	5.08	12.3	nd	
erythro-5-PtI_2	4-OH	H_α	4.17	5.0	80	1.03
		H_β	5.20	5.0	nd	
threo-5-PtI_2		H_α	5.02	12.3	nd	0.22
		H_β	5.22	12.3	nd	
erythro-6-PtI_2	2,6-F_2	H_α	4.99	7.5	55	0.44
		H_β	5.43	7.5	19	
threo-6-PtI_2		H_α	5.41	12.6	nd	0.07
		H_β	5.48	12.6	nd	
erythro-7-PtI_2	2,6-Cl_2	H_α	5.26	7.9	nd	0.07
		H_β	5.33	7.9	nd	
threo-7-PtI_2		H_α	5.63	12.6	nd	0.08
		H_β	5.71	12.6	nd	
erythro-8-PtI_2	2-F, 4-OH	H_α	4.59	5.1	82	0.66
		H_β	5.25	5.1	nd	
threo-8-PtI_2		H_α	5.06	12.3	nd	0.11
		H_β	5.17	12.3	nd	
erythro-9-PtI_2	2-Cl, 4-OH	H_α	4.78	5.2	79	0.48
		H_β	5.26	5.2	nd	
threo-9-PtI_2		H_α	5.10	12.3	nd	0.17
		H_β	5.27	12.3	nd	

[a] The spectra were taken at 250 MHz in acetone-d_6 with Me_4Si as internal standard. [b] nd = not detected.

ythro-9-$PtSO_4$ in Table XXI). Aqua[1-(2,6-dichloro-4-hydroxyphenyl)-2-phenylethylenediamine](sulfato)platinum(II) complexes which were unsubstituted or substituted with the smaller fluorine atoms in the 2,6-positions of the 2-phenyl ring showed a stronger cytotoxic activity in comparison with *erythro*-9-$PtSO_4$.

For assessment of estrogenic properties the relative binding affinity to the ER (RBA, E_2 (17β-estradiol) = 100, calf uterine cytosol) and the uterotrophic effect (mouse uterine weight test) were determined. The studies confirmed that estrogenic properties could only be found in (1,2-diphenylethylenediamine)platinum(II) complexes (1-PtLL') if both phenyl rings contained one para-standing OH group and at least one ortho-standing halogen atom and, in addition, if the ligand was *R,S* configurated.[1] Corresponding to this rule estrogenic properties were only expected with *erythro*-8-$PtSO_4$ and *erythro*-9-$PtSO_4$. Both complexes were active in the uterine weight test. They surpassed the maximum effect of estrone (E_1). Compared to the key substance *meso*-1-$PtSO_4$ they were, however, less active. For an optimal estrogenic activity *erythro*-8-$PtSO_4$ must be given at a 10-fold higher dose than *erythro*-9-$PtSO_4$. The reason for the inferior activity of both complexes (*erythro*-9-$PtSO_4$ > *erythro*-8-$PtSO_4$) may be the decrease of the hydrophobic character caused by the exchange of the 2-standing 2,6-dichloro-4-hydroxyphenyl residue in *meso*-1-$PtSO_4$ by 2-chloro-4-hydroxyphenyl or 2-fluoro-4-hydroxyphenyl. All other diastereomeric complexes obtained by a variation of the substitution pattern in the 2-phenyl ring (2-$PtSO_4$, H; 3-$PtSO_4$, 4-F; 4-$PtSO_4$, 3-OH; 5-$PtSO_4$, 4-OH; 6-$PtSO_4$, 2,6-F_2; 7-$PtSO_4$, 2,6-Cl_2) were not or only marginally active even at the highest dose.

In a further publication we will describe the mammary tumor inhibiting properties of the new complexes.

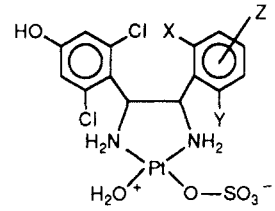
Experimental Section

General Procedures. Melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. The IR spectra were obtained with use of a Beckman Acculab 7 spectrophotometer. ^{1}H NMR were obtained with a Varian EM 360 L 60-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 carried out by the microlaboratory of the University of Regensburg.

Syntheses. Methods A–L are representative for the syntheses of the compounds reported in Tables I–VIII.

Method A. [*erythro*-1-(2-Chloro-4-hydroxyphenyl)-2-(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]diiodoplatinum(II) (*erythro*-9-PtI_2). K_2PtCl_4 (415 mg, 1 mmol) and KI (1.49 g, 9 mmol) were dissolved in 40 mL of H_2O and stirred for 30 min. This solution was added to a stirred suspension of *erythro*-9 (347 mg; 1 mmol) in 30 mL of H_2O. The reaction mixture was heated to 40–50 °C and kept in the dark. While the mixture was stirred for 6 h, the pH was adjusted to 7.0 several

Table XXI. Antitumor Effect of Aqua[1-(2,6-dichloro-4-hydroxyphenyl)-2-phenylethylenediamine](sulfato)platinum(II) Complexes on the P388 Leukemia of the CD_2F_1 Mouse



compd	X	Y	Z	dose,[a] μM/kg	(mg/kg)	$T - C$, g[b]	median day of survival (range)[c]	% T/C[d]
cisplatin				5	1.50	-3.8	19 (17-21)	238
erythro-**2**-$PtSO_4$	H	H	H	40	24.96	-2.1	15 (14-17)	188
				20	12.48	-1.7	14 (14-15)	175
				10	6.24	-1.1	13.5 (12-15)	169
threo-**2**-$PtSO_4$	H	H	H	40	25.68	-3.8	15 (11-18)	166
				20	12.84	-2.0	13.5 (9-16)	150
				10	6.42	-1.0	13.5 (12-14)	150
erythro-**3**-$PtSO_4$	H	H	4-F	40	25.68	-2.2	15.5 (15-17)	194
				20	12.84	-1.3	14.5 (14-16)	181
				10	6.42	-1.0	14 (13-16)	175
threo-**3**-$PtSO_4$	H	H	4-F	40	26.40	-2.5	15 (14-16)	188
				20	13.20	-2.5	15 (14-16)	188
				10	6.60	-1.2	13.5 (11-14)	169
erythro-**4**-$PtSO_4$	H	H	3-OH	40	28.48	-2.6	15.5 (14-16)	194
				20	14.24	-1.9	13.5 (12-16)	169
				10	7.12	-1.5	13 (12-13)	163
threo-**4**-$PtSO_4$	H	H	3-OH	40	26.32	-4.6	8 (6-23)	90
				20	13.16	-1.7	14.5 (13-23)	161
				10	6.58	-0.9	14 (13-15)	156
erythro-**5**-$PtSO_4$	H	H	4-OH	40	27.76	-2.8	15 (14-16)	166
				20	13.88	-1.7	14 (13-14)	155
				10	6.94	-1.7	14 (14-15)	155
threo-**5**-$PtSO_4$	H	H	4-OH	40	27.76	-4.6	10 (8-18)	111
				20	13.88	-3.1	15.5 (15-17)	172
				10	6.94	-1.3	15 (14-19)	166
erythro-**6**-$PtSO_4$	F	F	H	40	25.68	-3.1	16.5 (16-17)	183
				20	12.84	-1.7	14 (11-17)	155
				10	6.42	-1.3	14 (13-15)	155
threo-**6**-$PtSO_4$	F	F	H	40	25.68	-2.0	15 (15-16)	166
				20	12.84	-1.4	14 (14-15)	155
				10	6.42	-1.4	14 (13-15)	155
erythro-**7**-$PtSO_4$	Cl	Cl	H	40	29.16	-1.8	12.5 (11-14)	139
				20	14.58	-1.5	12 (12-14)	133
				10	7.29	-1.2	11 (11-16)	122
threo-**7**-$PtSO_4$	Cl	Cl	H	40	31.32	-1.9	12.5 (12-14)	139
				20	15.66	-1.3	13 (12-14)	144
				10	7.83	-1.1	10.5 (10-12)	117
erythro-**8**-$PtSO_4$	F	H	4-OH	40	26.32	-2.4	14 (14-16)	155
				20	13.16	-1.2	14 (13-15)	155
				10	6.58	0.9	12 (12-14)	133
threo-**8**-$PtSO_4$	F	H	4-OH	40	25.60	-3.8	15.5 (11-17)	172
				20	12.80	-2.2	15 (14-15)	166
				10	6.40	-2.4	14 (14-16)	155
erythro-**9**-$PtSO_4$	Cl	H	4-OH	40	26.98	-1.9	14 (13-19)	155
				20	13.49	-1.2	13 (11-15)	144
				10	6.75	-2.4	11.5 (10-13)	128
threo-**9**-$PtSO_4$	Cl	H	4-OH	40	26.98	-2.9	14.5 (14-19)	161
				20	13.49	-1.8	14 (14-16)	155
				10	6.75	-1.8	14 (13-14)	155

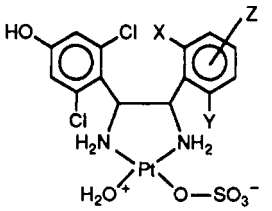
[a] The compounds were administered ip on day 1, 5, and 9 as a solution in water. [b] T = change of body weight between day 5 and day 1 of the treated group. C = change of body weight between day 5 and day 1 of the control group. [c] Median survival time = $X + Y/2$. X = first day for which the number of dead animals was $N/2$. Y = first day for which the number of dead animals was $N/2 + 1$. The median survival time of the control groups for *erythro*-**2**-$PtSO_4$, *erythro*-**3**-$PtSO_4$, *threo*-**3**-$PtSO_4$, and *erythro*-**4**-$PtSO_4$ was 8 days. For all other complexes the median survival time of the control groups was 9 days. [d] T = median survival time of the treated group; C = median survival time of the control group.

times. After the addition of 20 mL of 2 N HCl, the precipitate was collected, washed with H_2O, and dried over P_2O_5 to yield 700 mg of *erythro*-**9**-PtI_2 (88%, yellow powder).

Method B. Aqua[*erythro*-1-(2-chloro-4-hydroxyphenyl)-2-(2,6-dichloro-4-hydroxyphenyl)ethylenediamine](sulfato)platinum(II) (*erythro*-9-$PtSO_4$). Solid Ag_2SO_4 (281 mg, 0.9 mmol) was added to 50 mL of an aqueous suspension of *erythro*-**9**-PtI_2 (796 mg, 1 mmol). The mixture was stirred for 24 h at 40 °C with protection from light. Precipitated silver iodide was filtered off, and the clear, colorless filtrate was lyophilized (60%, colorless powder).

Method C. *erythro*-1,2-Diazido-1-(2-chloro-4-methoxyphenyl)-2-(2,6-dichloro-4-methoxyphenyl)ethane (*erythro*-9b). To a stirred slurry of NaN_3 (6.5 g, 0.1 mol) in 100 mL of dry acetonitrile was added slowly ICl (8.1 g, 0.05 mol). After the mixture was cooled in an 2-propanol–ice bath the stilbene **9c** (8.6 g, 0.025 mol) was added. The suspension was allowed to warm to room temperature, stirred for 20 h, and finally refluxed for 1

Table XXII. Estrogenic Properties of Aqua[1-(2,6-dichloro-4-hydroxyphenyl)-2-phenylethylenediamine](sulfato)platinum(II) Complexes and Related Ligands



					ligand		$PtSO_4$ complex	
compd	X	Y	Z	dose, μM animal^{-1} kg^{-1}	estrogenic effect[a]	RBA, %[b]	estrogenic effect[a]	RBA, %[b]
erythro-2	H	H	H	10	0		1	
				100	6	0.017	2	<0.001
				1000	0		5	
threo-2	H	H	H	10	0		0	
				100	0	0.190	0	<0.001
				1000	0		0	
erythro-3	H	H	4-F	10	0		0	
				100	0	0.026	0	<0.001
				1000	0		5	
threo-3	H	H	4-F	10	0		0	
				100	0	0.120	0	<0.001
				1000	0		16	
erythro-4	H	H	3-OH	10	7		0	
				100	23	0.090	8	<0.001
				1000	32		0	
threo-4	H	H	3-OH	10	0		0	
				100	0	0.110	0	<0.001
				1000	19		0	
erythro-5	H	H	4-OH	10	0		5	
				100	0	0.040	17	<0.001
				1000	9		20	
threo-5	H	H	4-OH	10	20		1	
				100	28	0.036	3	0.024
				1000	48		36	
erythro-6	F	F	H	10	0		7	
				100	0	0.045	0	<0.001
				1000	33		12	
threo-6	F	F	H	10	2		13	
				100	12	0.059	4	<0.001
				1000	26		12	
erythro-7	Cl	Cl	H	10	6		0	
				100	12	0.562	0	0.041
				1000	34		54	
threo-7	Cl	Cl	H	10	1		5	
				100	12	0.003	6	<0.001
				1000	42		3	
erythro-8	F	H	OH	10	11		0	
				100	13	0.174	44	<0.001
				1000	52		161	
threo-8	F	H	OH	10	9		1	
				100	11	0.047	1	<0.001
				1000	20		8	
erythro-9	Cl	H	OH	10	11		39	
				100	22	1.500	141	0.095
				1000	91		117	
threo-9	Cl	H	OH	10	9		10	
				100	21	0.500	14	0.030
				1000	86		35	

[a] Mouse uterine weight test: compounds were administered at three consecutive days sc. The uteri were removed 24 h after the last injection, estrogenic effect = $[(E_I - E_V)/(E_S - E_V)100$; effect = uterus weight (mg)/body weight (g)$]100$; E_T = effect of test compound; E_V = effect of vehicle; E_S = effect of estrone standard (0.4 μg). [b] Relative binding affinity (RBA), % = $[E_2]/[I]100$; $[E_e]$ and [I] are the molar concentrations of nonradioactive E_2 and inhibitor required to decrease the bound radioactivity by 50%; E_2 = 17β-estradiol.

h. The red-brown slurry was poured into 250 mL of water, and the mixture was extracted with 250 mL of ether in three portions. The combined organic layers were washed with 150 mL of 5% $Na_2S_2O_3$, leaving a colorless solution, which was washed with 1000 mL of water in four portions and dried over $MgSO_4$. Removal of the ether in vacuo left an oily mixture of *erythro*-**9b** and *threo*-**9b**. The diastereomers were separated by column chromatography (SiO_2, ligroin/ether 4:1) to give 4.6 g of *threo*-**9b** (43%, colorless oil) and 2.1 g of *erythro*-**9b** (20%, colorless powder, mp 73–76 °C).

Method D. *erythro*-1-(2-Chloro-4-methoxyphenyl)-2-(2,6-dichloro-4-methoxyphenyl)ethylenediamine (*erythro*-9a). A solution of *erythro*-**9b** (2.1 g, 5 mmol) in 20 mL of dry ether was added dropwise to a stirred suspension of $LiAlH_4$ (760 mg, 20 mmol) in dry ether at ice bath temperature. After being stirred for 30 min at room temperature the mixture was refluxed for 1 h. Subsequently 20 mL of water was added with cooling, and the precipitate was filtered off. The etheral layer was dried over $MgSO_4$ and evaporated to dryness (95%, yellow oil).

Method E. *erythro*-1-(2-Chloro-4-hydroxyphenyl)-2-(2,6-

dichloro-4-hydroxyphenyl)ethylenediamine (*erythro*-9). A solution of *erythro*-**9a** (1.13 g, 3 mmol) in 60 mL of dry CH_2Cl_2 was cooled to −60 °C. At this temperature BBr_3 (1.14 mL, 12 mmol) was added. The reaction mixture was allowed to warm to room temperature and stirred for 48 h. Subsequently 20 mL of methanol was added with cooling, and the solvent was evaporated. The residue was dissolved in a small amount of water, filtered, and alkalized with 2 N NaOH. Unreacted *erythro*-**9a** was filtered off, and the filtrate was brought to pH 8 with 0.5 N HCl. The precipitate was collected by suction filtration, washed with water, and dried over P_2O_5 to obtain 281 mg *erythro*-**9** (27%, colorless powder, mp 163–165 °C).

Method F. (2-Chloro-4-methoxybenzyl)triphenylphosphonium Chloride 9d). Compound **9e** (3.82 g, 20 mmol) and triphenylphosphine (5.25 g, 20 mmol) were mixed and melted for 15 min. The crude product which crystallized upon cooling was suspended in 100 mL of ether and collected by suction filtration. The residue was washed several times with ether and air dried (97%, colorless powder, mp 203–204 °C).

Method G. (*E*/*Z*)-1-(2-Chloro-4-methoxyphenyl)-2-(2,6-dichloro-4-methoxyphenyl)ethene (9c). Na (4.6 g, 0.2 mol) was dissolved in 100 mL of dry ethanol while cooling in an ice bath. To this compound was added **9d** (9.07 g, 20 mmol) in a N_2 atmosphere to give an orange suspension. After the mixture was stirred for 30 min, solid 2,6-dichloro-4-methoxybenzaldehyde (4.10 g, 20 mmol) was added and stirred for 1 h. Water (100 mL) was given to the reaction mixture, and the precipitate was collected on a Büchner funnel. The crude product was dissolved in CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$ and evaporated to dryness (99%, yellow oil).

Method H. 2-Chloro-4-methoxyacetophenone (9h). To a suspension of $AlCl_3$ (20.0 g, 0.15 mol) and 3-chloranisole (14.3 g, 0.1 mol) in 150 mL of 1,2-dichlorethane was dropped acetanhydride (10.2 g, 0.1 mol) at a temperature of 0–5 °C. After the mixture was stirred for 2 h at room temperature, the mixture was poured into ice and extracted with CH_2Cl_2. The organic layer was washed with 2 N NaOH, dried over $MgSO_4$, and evaporated. (81%, colorless oil).

Method I. 2-Chloro-4-methoxybenzoic Acid (9g). NaOH (32 g, 0.8 mol) was dissolved in 250 mL of water. After Br_2 (38.4 mL, 0.24 mol) was added at a temperature below 10 °C, the ketone **9h** (14.9 g, 0.08 mol), dissolved in 50 mL of dioxane, was given dropwise to the mixture while cooling in an ice bath. It was stirred for 1 h at room temperature and the $CHBr_3$ was separated. The aqueous solution was washed with 100 mL of CH_2Cl_2 and the benzoic acid **9g** precipitated upon the addition of concentrated HCl. The crude product was collected by suction filtration and recrystallized in ethanol (61%, colorless powder, mp 210 °C).

Method K. 2-Chloro-4-methoxybenzyl Alcohol (9f). To a stirred suspension of $LiAlH_4$ (3.79 g, 0.1 mol) in 100 mL of dry ether was given dropwise **9g** (9.33 g, 0.05 mol), dissolved in 100 mL of dry ether, while cooling in an ice bath. After the reaction mixture was heated to reflux for 1 h it was hydrolized with water. The precipitate was filtered off, and the etheral layer was evaporated to dryness after drying over $MgSO_4$ (96%, colorless oil).

Method L. 2-Chloro-4-methoxybenzyl Chloride (9e). The benzyl alcohol **9f** (8.63 g, 0.05 mol) was dissolved in 100 mL of dry benzene and treated with HCl gas while cooling in an ice bath. After 4 h the reaction mixture was washed with 100 mL of water, and the organic layer was dried over $MgSO_4$. The solvent was removed to give 9.36 g of **9e** (98%, colorless oil).

P388 Leukemia. The P388 Leukemia was maintained by routine passage in female DBA/2 mice. For determination of antitumor activity, female CDF_1 mice (18–22 g) were inoculated ip 1×10^6 leukemia cells in 0.1 mL PBS-buffer (day 0). The animals were assigned randomly to groups of six (10 animals to the solvent control), and the compounds were administrated ip as a solution in water on days 1, 5, and 9. Cisplatin served as positive control. The antitumor activity (% T/C) was evaluated as median day of survival time, compared to the untreated control multiplied by 100 (T = median survival time of the treated group; C = median survival time of the control group).

Immature Mice Uterine Weight Test. Immature female NMRI mice (19 days old) were randomly divided into groups of six animals. To determine estrogenic activity, compounds were dissolved in water and a 0.1-mL aliquot was injected sc on days 1, 2, and 3. Control animals received the vehicle alone. The positive control animals received estone (0.4 μg). Twenty four hours after the last injection, the animals were killed and weighed. The uteri were dissected free of fat and fixed in Bouin solution for 4 h. Uteri were freed from connective tissue, washed with ethanol, dried at 100 °C for 18 h, and weighed. The uterotrophic effect (E) was computed by the ratio of uterine dry weight (mg) and body weight (g). The estrogenic effect (EE) of a test compound in percent of the standard (estrone) was calculated by the following formula:

$$(E_T - E_V)/(E_S - E_V)100 = EE$$

where E_T = uterotrophic effect of test compounds, E_V = uterotrophic effect of vehicle, and E_S = uterotrophic effect of standard.

The uterotrophic effect was calculated by the formula:

$$[\text{uterine dry weight (mg)}/\text{body weight (g)}]100 = E$$

Estradiol Receptor Binding Assay. Test compounds were incubated with cytosol from calf uteri and [^{3}H]estradiol at 4 °C for 16 h. Incubation was stopped by adding dextran-coated charcoal. After centrifugation the radioactivity of a 100-μL supernatant aliquot was counted. The percentage of bound radioligand was plotted vs the concentration of unlabeled test compounds. Six concentrations of the competitors were tested. They were chosen to provide a linear portion of a semilog plot crossing the point of 50% competition. From this plot, the molar concentrations of unlabeled estradiol and test compounds reducing radioligand binding by 50% were determined. The effectiveness of an inhibitor was established by calculating the ratio of these concentrations. Multiplied by 100 gave the relative binding affinity (RBA).

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Supplementary Material Available: Tables IV, VI–XII, XIV–XX showing IR, ^{1}H NMR, and elemental analysis data and compound yields (19 pages). Ordering information is given on any current masthead page.