Journal of Medicinal Chemistry

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[*N*-Ethyl- and [*N*,*N*‘-Diethyl-1,2-bis(2,6-difluoro-3-hydroxyphenyl)-ethylenediamine]dichloroplatinum(II): Structure and Cytotoxic/Estrogenic Activity in Breast Cancer Cells

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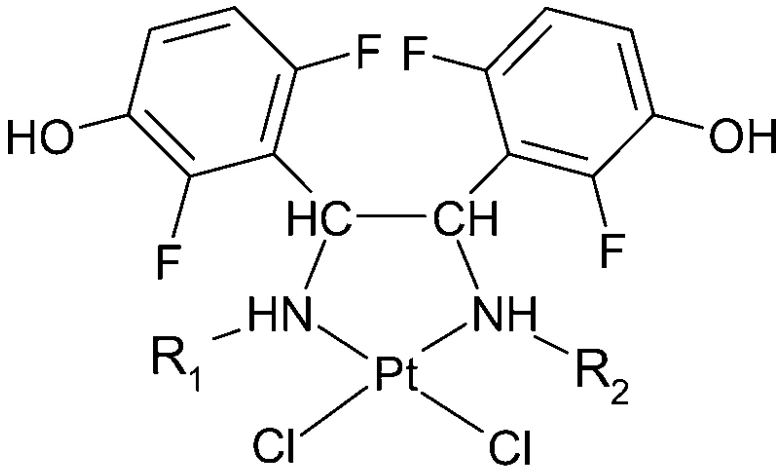
J. Med. Chem., **2005**, 48 (23), 7132-7144• DOI: 10.1021/jm050186i • Publication Date (Web): 14 October 2005



R_1, R_2 = H or ethyl

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[*N*-Ethyl- and [*N*,*N*′-Diethyl-1,2-bis(2,6-difluoro-3-hydroxyphenyl)-ethylenediamine]dichloroplatinum(II): Structure and Cytotoxic/Estrogenic Activity in Breast Cancer Cells

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Received February 28, 2005

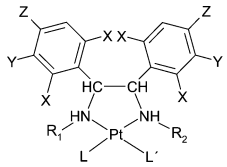
N-Ethyl and *N*,*N*′-diethyl derivatives (***erythro*-** and ***threo*-2-PtCl$_2$**; ***meso*-** and **D,L-3-PtCl$_2$**) of [*meso*- and [D,L-1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) (***meso*-** and **D,L-1-PtCl$_2$**) were synthesized and tested for cytotoxicity on the estrogen receptor-positive (ER$^+$) human MCF-7 breast cancer cell line. In this test, only **D,L-1-PtCl$_2$** and ***threo*-2-PtCl$_2$** showed strong cytotoxic properties. This revealed the existence of at least one NH_2 fragment as a prerequisite for antitumor activity. Furthermore, studies on the three-dimensional structure of the new compounds demonstrated that the aryl and alkyl residues at the five-membered chelate ring have to be arranged in equatorial positions for the triggering of cytotoxic effects, very likely due to the reaction with d(GpG) sequences in DNA resulting in GG−N7,N7 chelates. A contribution of the ER-mediated processes—(a) hindrance of the cellular processing of Pt-modified DNA by overexpression of high mobility group domain proteins and (b) interruption of the vicious circle of mutual growth stimulation of breast cancer cells and granulocytes/macrophages by reduction of the formation of key cytokines—to the anti-breast cancer activity of ***threo*-2-PtCl$_2$** is unlikely, since we did not observe transcription activation in the test on ER$^+$ MCF-7 breast cancer cells stably transfected with luciferase reporter plasmid ERE$_{wtc}$luc.

Introduction

[*meso*-1,2-Bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine]platinum(II) complexes (***meso*-1-PtLL′**, L, L′ = Cl_2 or L = OH_2 and L′ = OSO_3; for formula see Chart 1) are highly active in the test on the hormone-sensitive MXT-M-3,2 breast cancer of the mouse.[1] Their activity is mainly due to a mechanism based on the reduction of the physiological estrogen level that is necessary for the maintenance of breast cancer growth.[1,2] Two facts support the validity of this mechanism: (1) the neutralization of anti-breast cancer activity of ***meso*-1-PtLL′** by simultaneous administration of estrone[1] and (2) the capability of ***meso*-1-PtLL′** to interfere with the biosynthesis of testosterone (educt of estrogens).[3] Therefore, the compounds should be active in breast cancer patients with functioning ovarian steroid synthesis, but because of their low cytotoxicity they would be less efficient in postmenopausal patients, in whom anti-estrogens and aromatase inhibitors are effective drugs.[4]

Recently, Lippard and co-workers[5,6] reported on the increased cytotoxicity of cisplatin (cDDP) against estrogen receptor-positive (ER$^+$) breast cancer cells by simultaneous administration of estrogens. Estrogens caused an ER-mediated overexpression of high mobility group (HMG) domain proteins such as HMG1, which sensitize these tumor cells to cDDP by shielding its major DNA adducts from nucleotide excision repair.[5]

Chart 1. Structures of New Platinum Complexes and of Comparison Compounds



Compound	X	Y	Z	R_1	R2
1-PtLL'	F	OH	H	H	H
2-PtLL'	F	OH	H	H	Et
3-PtLL'	F	OH	H	Et	Et
4-PtLL'	H	OH	H	H	Et
5-PtLL'	Cl	OH	H	H	H
6-PtLL'	Cl	H	OH	H	H
7-PtLL'	Cl	H	OH	H	Et
8-PtLL'	H	H	F	H	H

Leaving groups: L,L' = Cl_2 or L = OH_2^+ and L' = OSO_3.

This mechanism may also contribute to the anti-breast cancer activity of ***meso*-1-PtCl$_2$**, since it causes significant cytotoxic and estrogenic effects [ER processing and progesterone receptor (PgR) synthesis] on the ER$^+$ human MCF-7 breast cancer cell line, however, only in concentrations higher than 10 μM (maximal effects at a 20 μM concentration; unpublished results).

To get more efficient [1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) complexes, which also impair the widespread postmenopausal mammary carcinoma (MC), we tried to increase the hormonal profile of ***meso*-** and **D,L-1-PtCl$_2$** by N-monoethylation (***erythro*-2-PtCl$_2$** and ***threo*-2-PtCl$_2$**) and N,N′-diethylation (***meso*-3-PtCl$_2$** and **D,L-3-PtCl$_2$**). This

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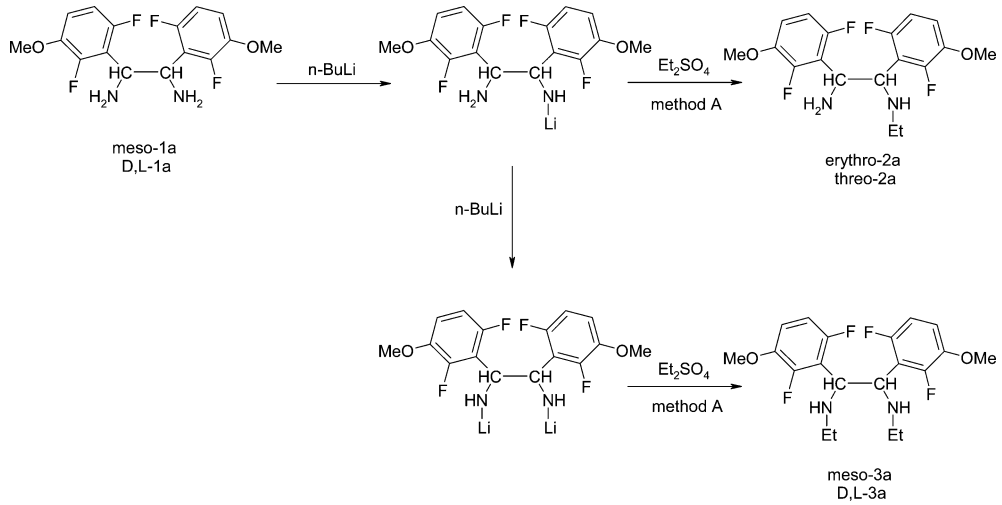
10.1021/jm050186i CCC: $30.25
Published on Web 10/14/2005

Scheme 1



structural modification was chosen because we already observed a marked increase of the estrogenic potency in the structurally related compound [*meso*-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) (***meso*-6-$PtCl_2$**; Chart 1) after its ethylation.[7]

***meso*-6-$PtCl_2$** strongly inhibited the premenopausal as well as the postmenopausal ER^+ MXT-M-3,2 breast cancer of the mouse (i.e. ovariectomized mouse bearing ER^+ MXT-M-3,2 breast cancer) mainly by its estrogenic potency; its low cytotoxicity played a subordinate role. Studies on the mode of action showed that ***meso*-6-$PtCl_2$** triggered an ER-mediated signal in breast cancer cells, which interrupted the tumor growth stimulation by cells of the phagocytic system and restored the natural immune defense, leading to tumor regression.[8−11]

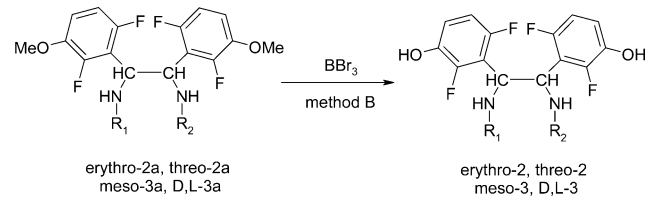
Estrogens generally inhibit the growth of the ER^+ murine breast cancer in supraphysiological concentrations irrespective of their chemical structure. In studies on a couple of estrogens, we found a correlation between anti-breast cancer activity and estrogenic potency.[12,13] Therefore, we supposed that platinum complexes possessing both marked estrogenic and cytotoxic properties would gain importance for breast cancer therapy.

In this paper we report the synthesis and ^1H NMR spectroscopic studies on diastereomeric [*N*-ethyl- and [*N*,*N*′-diethyl-1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) complexes and discuss the influence of their spatial structure on the cytotoxic and estrogenic activity against ER^+ MCF-7 breast cancer cells.

Results

Synthesis of 1,2-Diarylethylenediamines and of Their Platinum(II) Complexes. The synthesis of the diastereomeric *N*-ethyl- and *N*,*N*′-diethyl-1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamines (***erythro*-** and ***threo*-2**; ***meso*-** and **D,L-3**) was performed according to an already established synthetic route (see Scheme 1).[7] We used as educts the diastereomeric 1,2-bis(2,6-difluoro-3-methoxyphenyl)ethylenediamines (***meso*-** and **D,L-1a**) obtained by stereoselective *meso*−*meso*- and D,L−D,L-diaza-Cope rearrangement reaction of *meso*- and D,L−*N*,*N*′-bis(2,6-difluoro-3-methoxybenzylidene)-1,2-bis(2-hydroxyphenyl)ethylenediamine.[14,15] In a first reaction step, it was necessary to increase the nucleophilicity of the amino groups of ***meso*-1a** and **D,L-1a** by NH/NLi exchange with *n*-butyllithium. The activated compounds subsequently treated with diethyl sulfate gave a mixture of *N*-ethyl and *N*,*N*′-diethyl derivatives (***erythro*-2a** and ***meso*-3a**; ***threo*-2a** and **D,L-3a**), which were separated by column chromatography on SiO_2. In the last step, the ether cleavage with BBr_3 yielded the 1,2-diarylethylenediamines ***erythro*-2**, ***threo*-2**, ***meso*-3**, and **D,L-3** (see Scheme 2). The coordination to platinum was achieved by reaction of the respective ethylenediamine ligand with $K[Pt(Cl)_3(DMSO)]$, freshly prepared by treatment of K_2PtCl_4 with an equimolar amount of DMSO.[16] The [chloro(DMSO)enPt]Cl complexes were isolated as intermediates and transformed into the dichloroplatinum(II) complexes (***erythro*-** and ***threo*-2-$PtCl_2$**; ***meso*-** and **D,L-3-$PtCl_2$**) by thermal decomposition or in a simpler, faster, and more specific preparation route by direct substitution of the coordinated DMSO with Cl^-. For this purpose, HCl was added to the reaction mixture. After heating for 1 h to 50−60 °C, the dichloroplatinum(II) complexes precipitated and were separated by filtration.

Scheme 2



compound	R1	R2
erythro-2a	H	Et
threo-2a	H	Et
erythro-2	H	Et
threo-2	H	Et
erythro-2-$PtCl_2$	H	Et
threo-2-$PtCl_2$	H	Et
meso-3a	Et	Et
D,L-3a	Et	Et
meso-3	Et	Et
D,L-3	Et	Et
meso-3-$PtCl_2$	Et	Et
D,L-3-$PtCl_2$	Et	Et

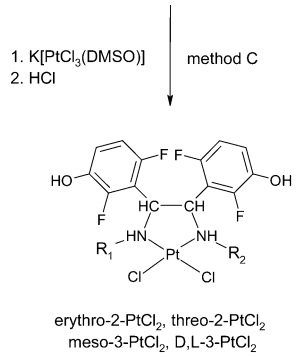
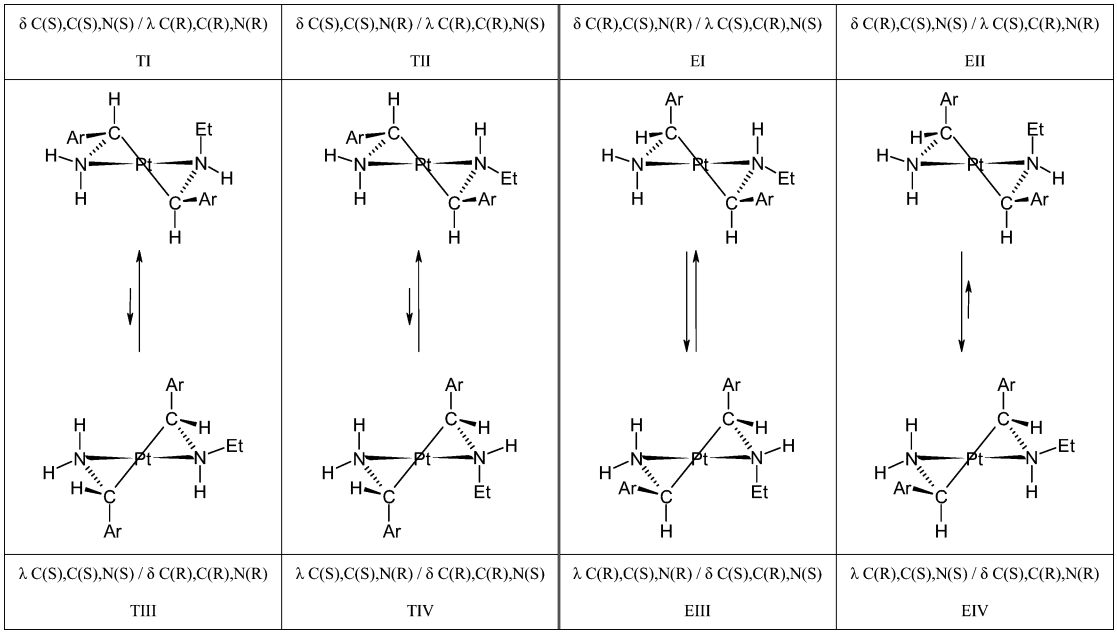


Chart 2. Theoretically Possible Conformers of ***erythro***- and ***threo*-2-PtCl$_2$**



Spectroscopic Studies on *N*-Ethyl and *N*,*N*′-Diethyl-Substituted [1,2-Diarylethylenediamine]-dichloroplatinum(II) Complexes: IR and ^{1}H NMR Spectroscopic Characterization. The dichloroplatinum(II) complexes ***erythro***- and ***threo*-2-PtCl$_2$** as well as ***meso***- and **D,L-3-PtCl$_2$** showed in their IR spectra characteristic Pt−Cl stretching vibrations in the region between 310 and 330 cm^{-1}.[17] Contamination with PtCl-(DMSO) compounds, intermediates of the coordination reaction, could be excluded, since the IR spectra did not exhibit ν S−O bands between 1110 and 1140 cm^{-1} and the ^{1}H NMR spectra did not show a DMSO signal at δ = 3.54 typical for coordinated DMSO.[16,18]

Conformational Studies. The coordination to platinum caused typical changes in the ^{1}H NMR spectra of the four diamines ***erythro*-2**, ***threo*-2**, ***meso*-3**, and **D,L-3**, giving a detailed insight into the spatial structures of the new dichloroplatinum(II) complexes.

Diastereomeric 2-PtCl$_2$ Complexes. In the spectra of the diastereomeric ligands, coupling constants of 10.9 Hz (***erythro*-2**) and 10.3 Hz (***threo*-2**) were observed for the doublets of the nonequivalent benzylic protons, pointing to a dihedral angle between the vicinal protons of about 180°. Thus the arrangement of the aromatic rings is predominantly antiperiplanar in ***erythro*-2** and synclinal in ***threo*-2**. This means that, during the coordination to platinum, the orientation of the phenyl residues in the erythro-configured ligand is changed from antiperiplanar to synclinal, while the synclinal orientation is conserved in the threo isomer.

The binding to platinum blocks rotation around the C−N axis, whereby the protons of the NH$_2$ group become diastereotopic due to the neighborhood of a chiral benzylic C atom with different signals for the axially and equatorially arranged NH. In accordance with this, the ^{1}H NMR spectra of ***erythro***- and ***threo*-2-PtCl$_2$** contained three NH resonances and two resonances for the nonequivalent benzylic protons in the range between 4 and 7 ppm. Upon coordination to platinum, the prochiral NHEt nitrogen in ***erythro*-2** as well as in ***threo*-2** can give rise to two pairs of enantiomers. If it is supposed that the five-membered chelate ring does not undergo $\delta \leftrightarrow \lambda$ interconversion, the existence of four racemic isomers is expected (for ***threo*-2-PtCl$_2$**, isomers TI−TIV, and for ***erythro*-2-PtCl$_2$**, isomers EI − EIV; see Chart 2).

***threo*-2-PtCl$_2$.** The ^{1}H NMR spectra of ***threo*-2-PtCl$_2$** (data see Table 1 and Figure 7 in Supporting Information) showed only one set of resonances, indicating the existence of only one of the isomers TI−TIV, which could be assigned by a coupling constant analysis. For this purpose, a complete NH/ND exchange was performed by addition of D$_2$O to the complex solution. In the resulting spectrum, two AB systems were present for the benzylic protons (Figure 1). The restricted rotation of the aryl residue neighboring the ethylamino group and the unsymmetric substituent pattern of this aryl ring induced at room temperature (297 K) two rotamers (see Figure 2) in which the corresponding benzylic protons (H$_A$ and H$_{A'}$, and H$_B$ and H$_{B'}$) were chemically and magnetically not equivalent (two AB systems). Furthermore, the large difference in the chemical shift between the two diastereotopic protons of the CH$_2$ group in the spectra of the complex (in ***threo*-2-PtCl$_2$**, $\Delta\delta$ = 1.1) compared to that in its free ligand (in ***threo*-2**, $\Delta\delta$ < 0.2) hints at a restricted rotation of the ethyl group around the C−N axis in the complex. The splitting of the CH$_2$ proton signal was the consequence of a stable chiral center at the NHEt nitrogen.

Upon increasing the temperature to 353 K (see Figure 1), the signals of the two AB patterns coalesced, owing to an unrestricted rotation of the aromatic rings. At this temperature, only one AB system was detected with a coupling constant of 12.5 Hz, indicating the existence of only one conformer with a dihedral angle of about 180° between the benzylic protons.

In the case of the [D,L-1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine]platinum(II) complex (**D,L-1-PtLL′**), no stable rotamers could be observed at room temperature due to the absence of N-standing ethyl residues. In accordance with this, the spectrum of **D,L-1-PtI$_2$** showed a singlet resonance for the benzylic

Table 1. ^{1}H NMR Data[a] of [*N*-Ethyl- and [*N,N'*-Diethyl-1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine]dichloroplatinum(II)

compd	CH_3	CH_2	CH	NH	ArH	OH
erythro-2-PtCl$_2$/I	0.96 (t, ^3J = 7.2 Hz, 3H)	2.47 (m, 1H)	4.72, 4.96[b] ($^3J_{CH-CH}$ = 4.9 Hz)	4.50 (br s, 1H)	8.50−7.23 (m, 8H, isomers I and III)	10.0−10.6 (br s, 4H, isomers I and II)
		3.95 (m, 1H)		5.61 (br s, 1H)		
erythro-2-PtCl$_2$/II	1.44 (t, ^3J = 7.0 Hz, 3H)	3.49 (m, 1H)	4.72, 4.90[b] ($^3J_{CH-CH}$ = 7.2 Hz)	5.29 (br s, 1H)	8.50−7.23 (m, 8H, isomers I and III)	10.0−10.6 (br s, 4H, isomers I and II)
		d		6.22 (br s, 1H)		
threo-2-PtCl$_2$	1.57 (t, ^3J = 7.1 Hz, 3H)	2.26 (m, 1H)	4.88 (m, 1H)[b]	5.48 (br s, 1H)	6.78−7.06 (m, 4H)	10.0−10.5 (br, 2H)
		3.39 (m, 1H)	*e*	6.53 (br s, 1H)		
			5.06 (m, 1H)[b] ($^3J_{CH-CH}$ = 12.5 Hz)	6.70 (br s, 1H)		
meso-3-PtCl$_2$/I	1.04 (t, ^3J = 7.2 Hz, 3H)[f]	2.48 (m, 1H)[f]	4.10 (m, $^3J_{CH-Pt}$ = 80 Hz, 1H)[b,f]	5.43 (br s, 1H)[f]	6.44−7.30 (m, 4H)[f]	8.35 (s, 1H)[f]
	1.35 (t, ^3J = 7.1 Hz, 3H)[f]	3.45 (m, 1H)[f]	4.45 (dd, $^3J_{CH-CH}$ = 4.9 Hz, $^3J_{CH-NH}$ = 12.5 Hz, 1H)[b,f]	6.12 (br s, 1H)[f]		8.91 (s, 1H)[f]
meso-3-PtCl$_2$/II	1.48 (t, ^3J = 7.0 Hz, 6H)	3.56 (m, 2H)	4.73 (br s, 2H, $^3J_{CH-Pt}$ = 40 Hz)[b,f]	6.30 (br s, 2H)	6.83−7.16 (m, 4H)	10.34 (br s, 2H)
D,L-3-PtCl$_2$	1.50 (t, ^3J = 7.0 Hz, 6H)	2.18 (m, 2H)	4.95 (m, 2H)[b,e]	6.57 (br s, 2H)	6.80−7.06 (m, 4H)	10.38 (br s, 2H)
		3.43 (m, 2H)				

[a] The spectra were taken at 250 MHz in DMF-d_7 with TMS as internal standard; the chemical shifts are given as δ values. [b] Detected after addition of D_2O. [c] Unresolved in the ArH peak. [d] Covered by solvent peak. [e] Detailed information in the text and in Figures 1−4. [f] In acetone-d_6 solvent.

Figure 1. Signals of the benzylic protons of ***threo*-2-PtCl$_2$** in the 250 MHz ^{1}H NMR spectrum, in DMF-d_7/D_2O solvent, at variable temperatures (297, 313, 333, and 353 K).

Figure 2. Supposed rotamers of ***threo*-2-PtCl$_2$** at 297 K.

protons. Exchanging the I^- leaving groups for SO_4^{2-} and measuring the resulting sulfatoplatinum(II) complex **D,L-1-PtSO$_4$** in DMSO-d_6 split the $CH_{benzylic}$ resonance with ^3J = 12.2 Hz, due to the formation of an asymmetric Pt(DMSO-d_6)(SO_4) fragment.[14,15]

This means that in **D,L-1-PtLL'** (L, L' = Hal_2 or L = OH_2 and L' = OSO_3) and ***threo*-2-PtCl$_2$** the aromatic rings were equatorially arranged, and a conversion into a conformation with axially oriented rings was not favored.

The orientation of the *N*-ethyl group at the five-membered chelate ring was deduced from the chemical shift of the CH_3 signal, which depends on an axial or an equatorial position. It appeared in the spectrum of ***threo*-2-PtCl$_2$** at δ = 1.57 (t, ^3J = 7.1 Hz), very similar to that of the used reference substance [*threo*-*N*-ethyl-1,2-bis(3-hydroxyphenyl)ethylenediamine]dichloroplatinum(II), ***threo*-4-PtCl$_2$** (δ = 1.40, t, ^3J = 7.0 Hz), whose absolute configuration (TII) was determined with H,H-NOESY experiments (manuscript in preparation) and the structurally related compound [*threo*-*N*-ethyl-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]-dichloroplatinum(II) (***threo*-7-PtCl$_2$**). In the spectrum of the latter, the signal of the benzylic proton of the CH−NHEt fragment was split by two couplings, to the vicinal benzylic proton and to the proton of the NHEt group. The coupling constants point to dihedral angles of about 180° between these protons (see structure of TII in

Chart 2 and ref 7). Consequently, the ethyl group is equatorially oriented with a CH_3 resonance at $\delta = 1.61$.

The ^{1}H NMR studies revealed that only one of the four possible racemic isomers of ***threo*-2-PtCl$_2$** was formed in the course of the binding of the diamine ***threo*-2** to platinum (i.e., TII in Chart 2). In this compound, the three bulky aryl and ethyl residues were equatorially arranged. Moreover, two rotamers of ***threo*-2-PtCl$_2$** existed at 297 K.

***erythro*-2-PtCl$_2$/I and II.** The coordination of ***erythro*-2** to platinum resulted in two diastereomers, ***erythro*-2-PtCl$_2$/I** and ***erythro*-2-PtCl$_2$/II**, that could not be separated from each other. In the ^{1}H NMR spectrum, two sets of proton signals appeared (see Table 1), indicating a quantitative ratio of isomer I to isomer II of 1:7.

Assignment of the spatial structures was possible by comparison of the chemical shifts of their CH_3 protons (for isomer I, $\delta = 0.96$, t, $^3J = 7.2$ Hz; for isomer II, $\delta = 1.44$, t, $^3J = 7.0$ Hz) with those of the CH_3 protons in the [*erythro*-*N*-ethyl-1,2-bis(3-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) isomers ***erythro*-4-PtCl$_2$/I** ($\delta = 0.98$, t, $^3J = 7.2$ Hz) and ***erythro*-4-PtCl$_2$/II** ($\delta = 1.37$, t, $^3J = 7.0$ Hz).

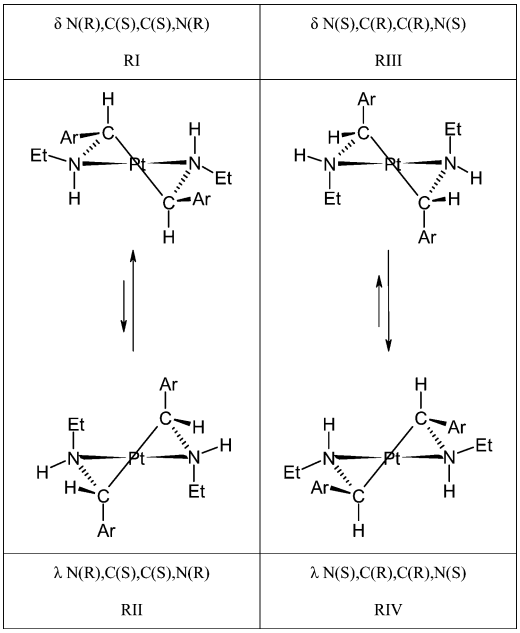
In a recent unpublished study, ***erythro*-4-PtCl$_2$/I** and ***erythro*-4-PtCl$_2$/II** were separated by virtue of their different solubilities in water and submitted to an investigation of their configuration and conformation by H,H-NOESY experiments. It revealed spatial structures for ***erythro*-4-PtCl$_2$/I** and ***erythro*-4-PtCl$_2$/II** that correspond to EIV and EI, respectively (see Chart 2). Contrary to diastereomer I possessing the conformation (configuration) λ C(*R*),C(*S*),N(*S*)/δ C(*S*),C(*R*),N(*R*) (compare EIV in Chart 2), diastereomer II exists in a $\delta \leftrightarrow \lambda$ equilibrium. Its conformers have the conformations (configurations) δ C(*R*),C(*S*),N(*R*)/λ C(*S*),C(*R*),N(*S*) and λ C(*R*),C(*S*),N(*R*)/δ C(*S*),C(*R*),N(*S*) with the following orientation of the aryl and ethyl residues: [Ar(ax),Ar(eq),Et(eq)] and [Ar(eq),Ar(ax),Et(ax)] (compare EI $\leftrightarrow$ EIII in Chart 2).

erythro*-2-PtCl$_2$** showed in its spectra after N-deuteration an AB system for the benzylic protons at $\delta = 4.72$ and 4.96 (isomer I) and $\delta = 4.72$ and 4.90 (isomer II). The coupling constant of isomer II amounted to $^3J_{CH-CH} = 7.2$ Hz, indicating a fast $\delta \leftrightarrow \lambda$ interconversion of the five-membered chelate ring. The same dynamic effects were determined for [*meso*-1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine]platinum(II) complexes (meso*-1-PtLL′**) on the example of the (DMSO)-sulfatoplatinum(II) derivative [***meso*-1-Pt(DMSO-*d*$_6$)-(SO$_4$)**].[14,15]

In contrast, the $^3J_{CH-CH} = 4.9$ Hz of the AB spectrum of ***erythro*-2-PtCl$_2$/I** correlates with a hampered $\delta \leftrightarrow \lambda$ interconversion and an arrangement of the vicinal CH protons in a dihedral angle of about 60°. Consequently, the orientation of the two phenyl rings and of the ethyl residue is mainly [Ar(eq),Ar(ax),Et(eq)] (Chart 2, EIV).

A restricted interconversion of the five-membered chelate ring was already demonstrated in earlier studies. We showed that [*erythro*-1-(2,6-dichloro-4-hydroxyphenyl)-2-phenylethylenediamine]diiodoplatinum(II) complexes followed the conformational behavior of either the type I or the type II isomer dependent on the substituent pattern in the 2-phenyl ring.[19] The spatial

Chart 3. Conformations of **D,L-3-PtCl$_2$**



structure of the type I isomers was confirmed on the example of two [*erythro*-1-(2,6-dichloro-4-hydroxyphenyl)-2-(2-halo-4-hydroxyphenyl)ethylenediamine]diiodoplatinum(II) complexes (Hal = F or Cl) by X-ray analyses.[20]

On the other hand, the ^{1}H NMR spectroscopic investigation on [*erythro*-*N*-ethyl-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) (***erythro*-7-PtCl$_2$**) revealed the existence of only one isomer.[7] The spectrum of this compound showed an AB pattern for the benzylic protons with a coupling constant ($^3J_{CH-CH} = 7.9$ Hz) favoring isomer II (***erythro*-7-PtCl$_2$/II**), in which the conformation equilibrium EI $\leftrightarrow$ EIII exists.

These investigations clearly demonstrated that the ortho substituents in the aromatic rings determined not only the conformational behavior but also the sterochemistry at the NH−Et group. ***erythro*-4-PtCl$_2$** without substituents in the 2,6-position existed in a proportion of about 18% as isomer I, while *o*-F (***erythro*-2-PtCl$_2$**) and *o*-Cl atoms (***erythro*-7-PtCl$_2$**) decreased this proportion to about 8% and 0%, respectively.

Diastereomeric 3-PtCl$_2$ Complexes. The spectra of the diastereomeric *N*,*N*′-diethyl-substituted complexes were highly analogous to those of their monoalkylated derivatives (see Figure 7 in Supporting Information). For **D,L-3-PtCl$_2$**, only one set of resonances was found, while two sets (***meso*-3-PtCl$_2$/I** and ***meso*-3-PtCl$_2$/II**; see Table 1) existed in the case of ***meso*-3-PtCl$_2$**.

D,L-3-PtCl$_2$. Comparison with the data of ***threo*-2-PtCl$_2$** shows great similarities in the resonances of the CH_3 as well as CH_2 fragments. This suggests the same environment at the ethylated nitrogens in ***threo*-2-PtCl$_2$** and **D,L-3-PtCl$_2$**. Therefore, the conformation (configuration) δ N(*R*),C(*S*),C(*S*),N(*R*)/λ N(*S*),C(*R*),C(*R*),N(*S*) can be assumed for **D,L-3-PtCl$_2$**, in which all bulky aryl and ethyl residues are equatorially arranged (compare Chart 3).

Furthermore, after exchange of the two N,N′-standing protons by deuterium [in deuterated *N*,*N*′-dimethyl-

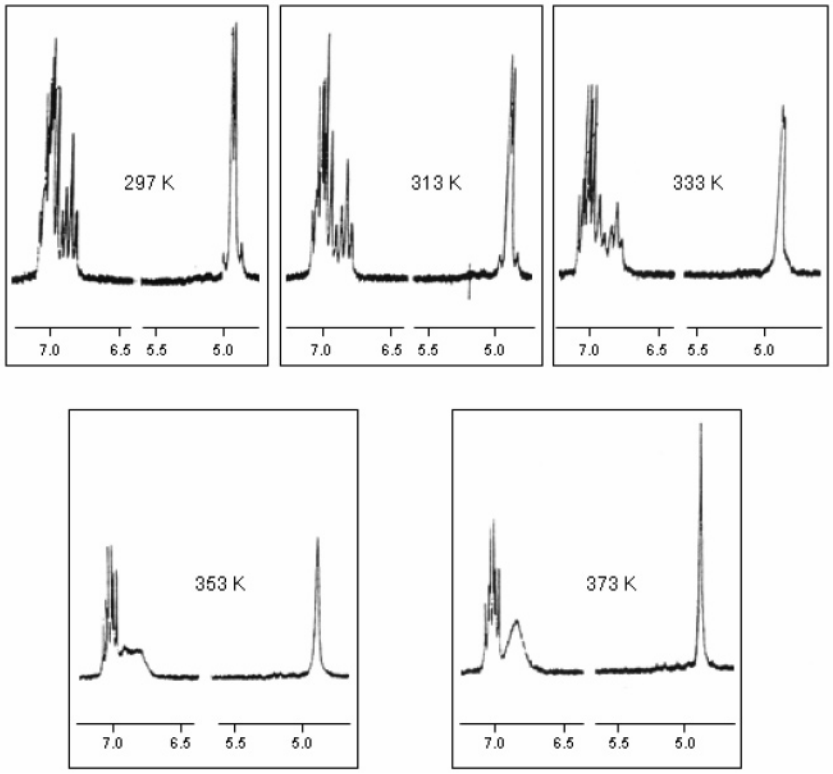


Figure 3. Signals of the benzylic protons of **D,L-3-PtCl$_2$** in the 250 MHz ^{1}H NMR spectrum, in DMF-d_7/D_2O solvent, at variable temperatures (297, 313, 333, 353, and 373 K).

formamide (DMF-d_7)/D_2O solvent], the spectrum of **D,L-3-PtCl$_2$** showed for the benzylic protons an AB pattern at $\delta = 4.89$ and 4.97 with a coupling constant of $^3J_{CH-CH} = 11$ Hz, as well as two singlets at $\delta = 4.92$ and 4.94 (Figure 3). A comparable splitting of the benzylic protons existed for [1,2-bis(2,6-dichloro-3-hydroxyphenyl)ethylenediamine]diiodoplatinum(II) complexes (**5-PtI$_2$**; Chart 1) due to the hindered rotation of the aromatic rings caused by the bulky *o*-chloro substituents.[21]

Upon increasing the temperature to 330 K, the signals of the AB pattern in the spectra of **D,L-3-PtCl$_2$** coalesced, while the two singlets remained unchanged. The coalescence of the latter began at 353 K, and after further elevation of the temperature by 20 K, only one singlet could be observed. At the same time, a coalescence of signals of the aryl protons took place (see Figure 3).

From this experiment, it can be concluded that three rotamers I−III (see Figure 4) exist at 297 K due to a restricted rotation of the aryl residues by neighboring ethylamino groups and the unsymmetric substituent pattern in these aryl rings. Both benzylic protons are chemically and magnetically equivalent in each of the rotamers I and II, yielding two singlets in the spectrum of the *N*,*N*′-deuterated **D,L-3-PtCl$_2$**, while in the rotamer III the different environment of the two benzylic protons gives rise to an AB pattern. The coupling constant of $^3J_{CH-CH} = 11$ Hz correlates with a dihedral angle of 170−180° and documents a bisequatorial arrangement of the aromatic rings.

meso*-3-PtCl$_2$/I and II.** The ^{1}H NMR spectrum confirmed the formation of two diastereomeric [*meso*-*N*,*N*′-diethyl-1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) complexes (meso*-3-PtCl$_2$/I** and **II**) during the synthesis of ***meso*-3-PtCl$_2$**.

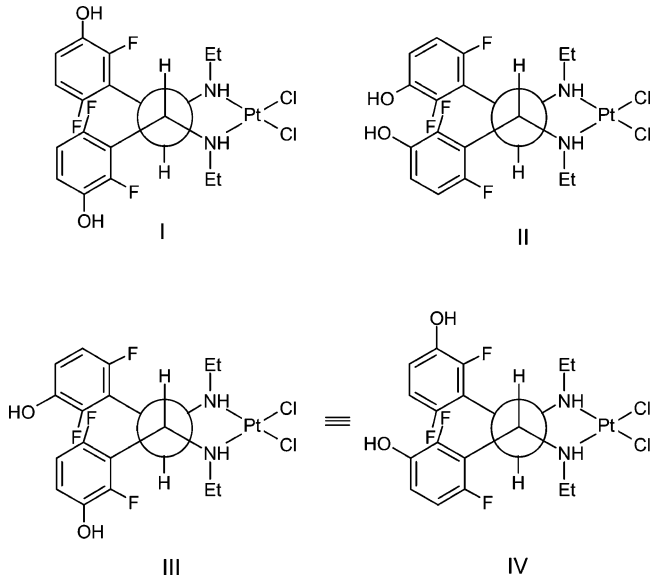
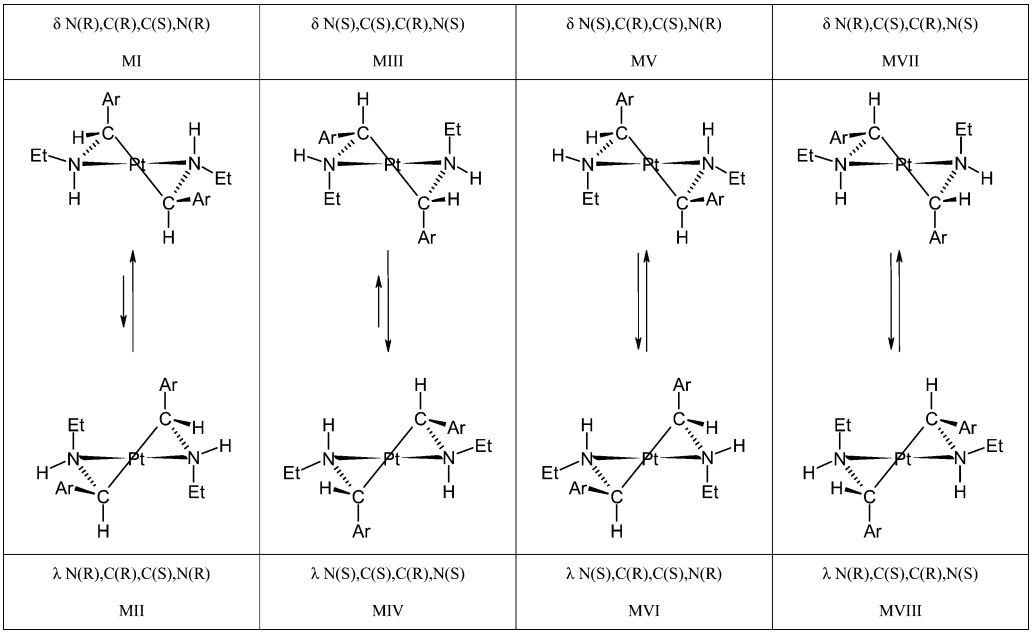


Figure 4. Supposed rotamers of **D,L-3-PtCl$_2$** at 297 K.

In the ^{1}H NMR spectrum measured in acetone-d_6, the unequal chemical shifts of the two CH_3 proton signals ($\delta = 1.04$, t, $^3J = 7.2$ Hz and $\delta = 1.35$, t, $^3J = 7.1$ Hz) point to different environments of the two chiral nitrogens of ***meso*-3-PtCl$_2$/I**. The value $\delta = 1.04$ corresponds with that of ***erythro*-2-PtCl$_2$/I** ($\delta = 0.96$, t, $^3J = 7.2$ Hz), a clue to the synclinal arrangement of the two bulky residues Ar(ax) and Et(eq). The benzylic protons show two signals split by CH−NH and CH−^{195}Pt couplings. The signal at $\delta = 4.10$, which is accompanied by platinum satellites with $^3J_{Pt-H} \approx 80$ Hz, indicates a

Chart 4. Conformations of ***meso*-3-PtCl$_2$/I** and ***meso*-3-PtCl$_2$/II**



predominantly equatorially oriented benzylic proton. The second signal of the benzylic protons at $\delta = 4.45$ couples to the amino group ($^3J_{CH-NH} = 12.5$ Hz) and the adjacent methine proton ($^3J_{CH-CH} = 4.9$ Hz). According to these coupling constants, both protons are axially oriented and the two bulky aryl and ethyl residues are equatorially oriented. Therefore, the conformation (configuration) δ N(*R*),C(*R*),C(*S*),N(*R*)/λ N(*S*),C(*S*),C(*R*),N(*S*) can be assumed for ***meso*-3-PtCl$_2$/I**. From steric reasons, a conversion into a conformer with three bulky residues in axial position is not favored (see Chart 4).

For ***meso*-3-PtCl$_2$/II**, there exists in the spectra only one CH_3 proton signal ($\delta = 1.48$, t, $^3J = 7.0$ Hz), which points to equal environments of the two chiral nitrogens. The value of $\delta = 1.48$ corresponds with that of ***erythro*-2-PtCl$_2$/II** ($\delta = 1.44$, t, $^3J = 7.0$ Hz), suggesting a $\delta \leftrightarrow \lambda$ equilibrium for ***meso*-3-PtCl$_2$/II**. The conformers have the conformations (configurations) δ N(*S*),C(*R*),C(*S*),N(*R*)/λ N(*R*),C(*S*),C(*R*),N(*S*) and δ N(*R*),C(*S*),C(*R*),N(*S*)/λ N(*S*),C(*R*),C(*S*),N(*R*) with the following orientation of the aryl and ethyl groups: [Et(ax)Ar(ax)Ar(eq)Et(eq)] and [Et(eq)Ar(eq)Ar(ax)Et(ax)] (compare Chart 4). In accordance with a fast $\delta \leftrightarrow \lambda$ interconversion of the five-membered chelate ring, we found only one broadened signal for the methine groups at $\delta = 4.73$, accompanied by platinum satellites with $^3J_{Pt-H} \approx 40$ Hz after exchange of the N-standing protons by deuterium.

Cytotoxic Properties. To find out, how the cytotoxic potency of diastereomeric [1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) complexes (***meso*-** and **D,L-1-PtCl$_2$**) is influenced by transformation into *N*-ethyl (***erythro*-** and ***threo*-2-PtCl$_2$**) and *N*,*N*′-diethyl derivatives (***meso*-** and **D,L-3-PtCl$_2$**), tests were performed on the human MCF-7 breast cancer cell line at concentrations of 0.5, 1.0, and 5.0 μM.

In the *R*/*S*-configured series the derivatization of the parent compound (***meso*-1-PtCl$_2$**), which itself is only marginally cytotoxic even at the highest concentration of 5.0 μM ($T/C_{corr} = 65\%$ at 5.0 μM, $t = 68$ h; Figure 5), led to inactive products (i.e., ***erythro*-2-PtCl$_2$** and ***meso*-3-PtCl$_2$**). In contrast to this, the ethylation of one NH_2 fragment in the *R*,*R*/*S*,*S*-configured parent compound **D,L-1-PtCl$_2$** [$T/C_{corr} = 57\%$ at 5.0 μM, drug−cell contact (dcc) = 68 h; Figure 5] caused a significant increase in cytotoxicity (for ***threo*-2-PtCl$_2$**, $T/C_{corr} = 24\%$ at 5.0 μM, dcc = 68 h; Figure 5). The time−activity curves of ***threo*-2-PtCl$_2$** indicated a concentration-dependent inhibition very similar to that of cDDP (Figure 5). Interestingly, the ethylation of the second NH_2 fragment in ***threo*-2-PtCl$_2$** led to a complete loss of activity (**D,L-3-PtCl$_2$**, Figure 5).

The comparison compounds [*erythro*- and [*threo*-*N*-ethyl-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) (***erythro*-** and ***threo*-7-PtCl$_2$**) proved to be inactive ($T/C_{corr} = 97\%$ and 93%) at the highest concentration of 5.0 μM. These results confirmed the supposed negative influence of 2,6-standing halogen atoms on the cytotoxicity of the compounds. The cytotoxic effect decreased after exchange of F for Cl due to the larger van der Waals radius of the latter.

Estrogenic Properties. The influence of *N*-ethyl- and *N*,*N*′-diethyl-substituted complexes on ER-mediated processes were evaluated in a transcriptional assay with hormone-dependent MCF-7 cells stably transfected with the luciferase reporter plasmid ERE_{wtc}luc (MCF-7-2a cells).[22]

Since it was already demonstrated that N-alkylation of [1,2-diarylethylenediamine]dichloroplatinum(II) complexes might increase their hormonal potency, the activation of the luciferase expression in MCF-7-2a cells was determined. As depicted in Figure 6, only ***meso*-3-PtCl$_2$** slightly activated the luciferase expression at the highest used concentration (10 μM) by 30%. All other compounds were inactive. Furthermore, the influence on estradiol (E2; 10^{-9} M) induced activation was very low. Only the simultaneous treatment with ***threo*-2-PtCl$_2$** (10 μM) reduced the E2 effect by 60%. However, this effect was mainly caused by a reduction of the cells' growth due to cytotoxicity and not by antiestrogenic properties.

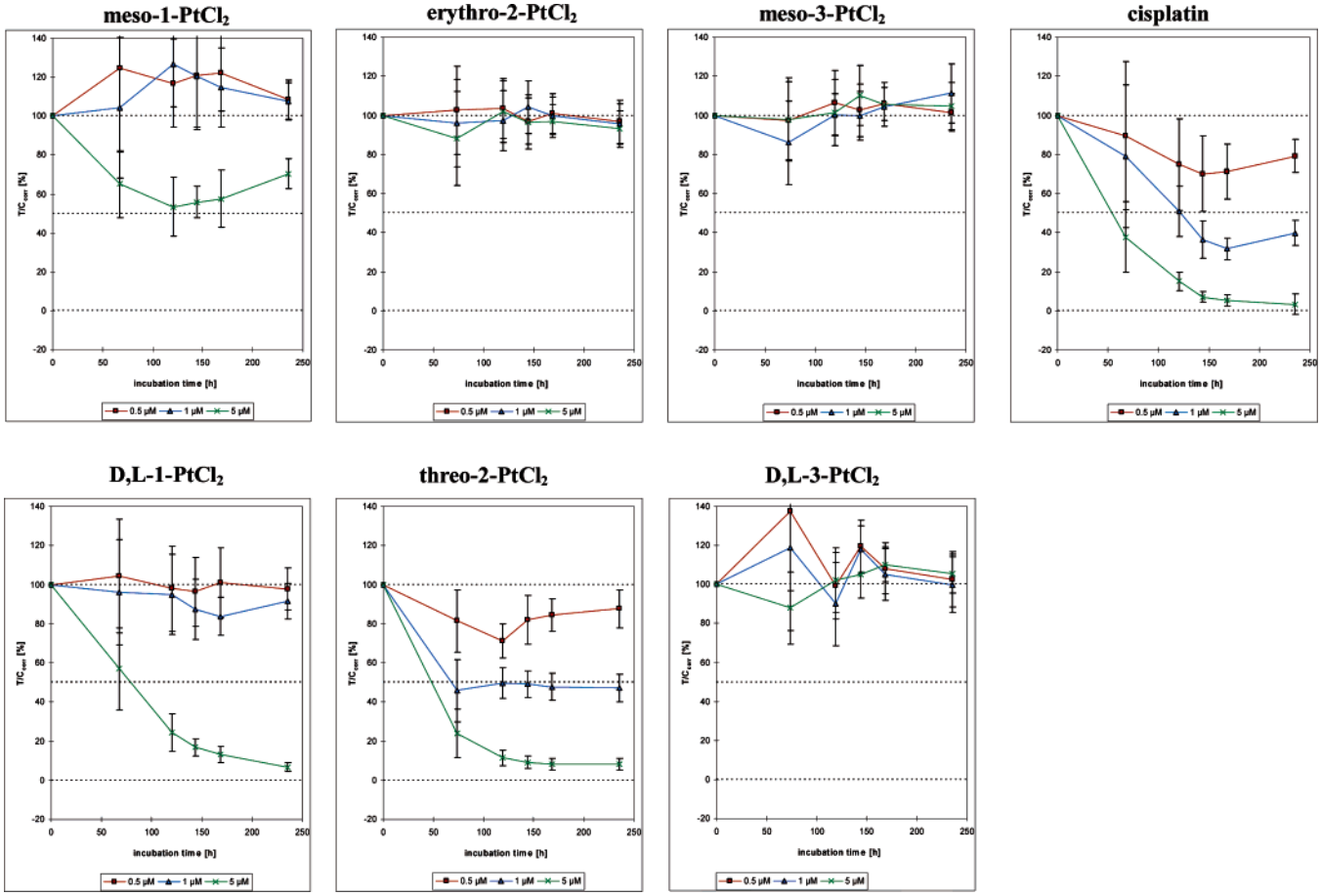


Figure 5. Growth-inhibiting effects of cisplatin, the parent compounds [*meso*- and [D,L-1,2-bis(2,6-difluoro-3-hydroxyphenyl)-ethylenediamine]dichloroplatinum(II) (***meso*-1-PtCl$_2$** and **D,L-1-PtCl$_2$**), and the *N*-ethyl- and *N*,*N*′-diethyl-substituted [1,2-bis-(2,6-difluoro-3-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) complexes ***erythro*-** and ***threo*-2-PtCl$_2$** and ***meso*-** and **D,L-3-PtCl$_2$** on the human MCF-7 breast cancer cell line.

Discussion

In this structure−activity study we tried to improve the anti-breast cancer activity of [*meso*- and [D,L-1,2-bis-(2,6-difluoro-3-hydroxyphenyl)ethylenediamine]dichloro-platinum(II) (***meso*-** and **D,L-1-PtCl$_2$**) by N-ethylation (***erythro*-** and ***threo*-2-PtCl$_2$**) and N,N′-diethylation (***meso*-** and **D,L-3-PtCl$_2$**). The compounds were tested for cytotoxic effects on hormone-dependent MCF-7 breast cancer cells and for (anti)estrogenic properties at the MCF-7-2a cell line stably transfected with the plasmid ERE_{wtc}luc.

The parent compound ***meso*-1-PtCl$_2$** inhibited the growth of MCF-7 cells only at concentrations higher than 5.0 μM (Figure 5). It was postulated that the 2,6-standing F atoms in both phenyl rings as well as the axial orientation of one of the two phenyl rings caused this limited activity due to an impeded formation of DNA intrastrand cross-links.[1]

N-Monoethylation of ***meso*-1-PtCl$_2$** resulted in two isomers, ***erythro*-2-PtCl$_2$/I** and ***erythro*-2-PtCl$_2$/II**. In ***erythro*-2-PtCl$_2$/I** the aromatic ring of the ArCH−NH_2 fragment is predominantly equatorially oriented, while it is mainly axially standing at the ArCH−NHEt fragment. The neighboring ethyl group is located in an equatorial position ([Ar(eq),Ar(ax),Et(eq)]). ***erythro*-2-PtCl$_2$/II** exists in a $\delta \leftrightarrow \lambda$ equilibrium, whose two conformers possess [Ar(ax),Ar(eq),Et(eq)] and [Ar-(eq),Ar(ax),Et(ax)] orientations.

During the coordination of the *meso*-*N*,*N*′-diethyl-1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine to platinum(II), also two isomers (***meso*-3-PtCl$_2$/I** and **II**) were built having the following conformations: isomer I, [Et(eq),Ar(ax),Ar(eq),Et(eq)], and isomer II, [Et(ax),Ar-(ax),Ar(eq),Et(eq)] and [Et(eq),Ar(eq),Ar(ax),Et(ax)] in a $\delta \leftrightarrow \lambda$ equilibrium (Chart 4).

Both derivatives ***erythro*-2-PtCl$_2$** and ***meso*-3-PtCl$_2$** were inactive in the test on the human MCF-7 breast cancer cell line. Therefore, we supposed that the dynamic effects (e.g., the conversion of the five-membered chelate ring) and axially oriented residues at the chelate ring were responsible for the decline in cytotoxic potency by hindering the fit to target sequences in DNA. This hypothesis was confirmed by the results of **D,L-1-PtCl$_2$**. The aryl rings were equatorially oriented and a reorientation into an axial position due to an interconversion of the chelate ring did not take place. **D,L-1-PtCl$_2$** showed significant cytotoxic properties (Figure 5).

[*threo*-*N*-Ethyl-1,2-bis(2,6-difluoro-3-hydroxyphenyl)-ethylenediamine]dichloroplatinum(II) (***threo*-2-PtCl$_2$**) only exists in one isomer in which the ethyl group is also located in an equatorial position. This stable conformation leads to a reduction of the dynamic effects due to the interaction of the ethyl group with the neighboring aromatic ring. Consequently, two stable rotamers were detected for ***threo*-2-PtCl$_2$** at physiological temperature. The hindered rotation of the aromatic

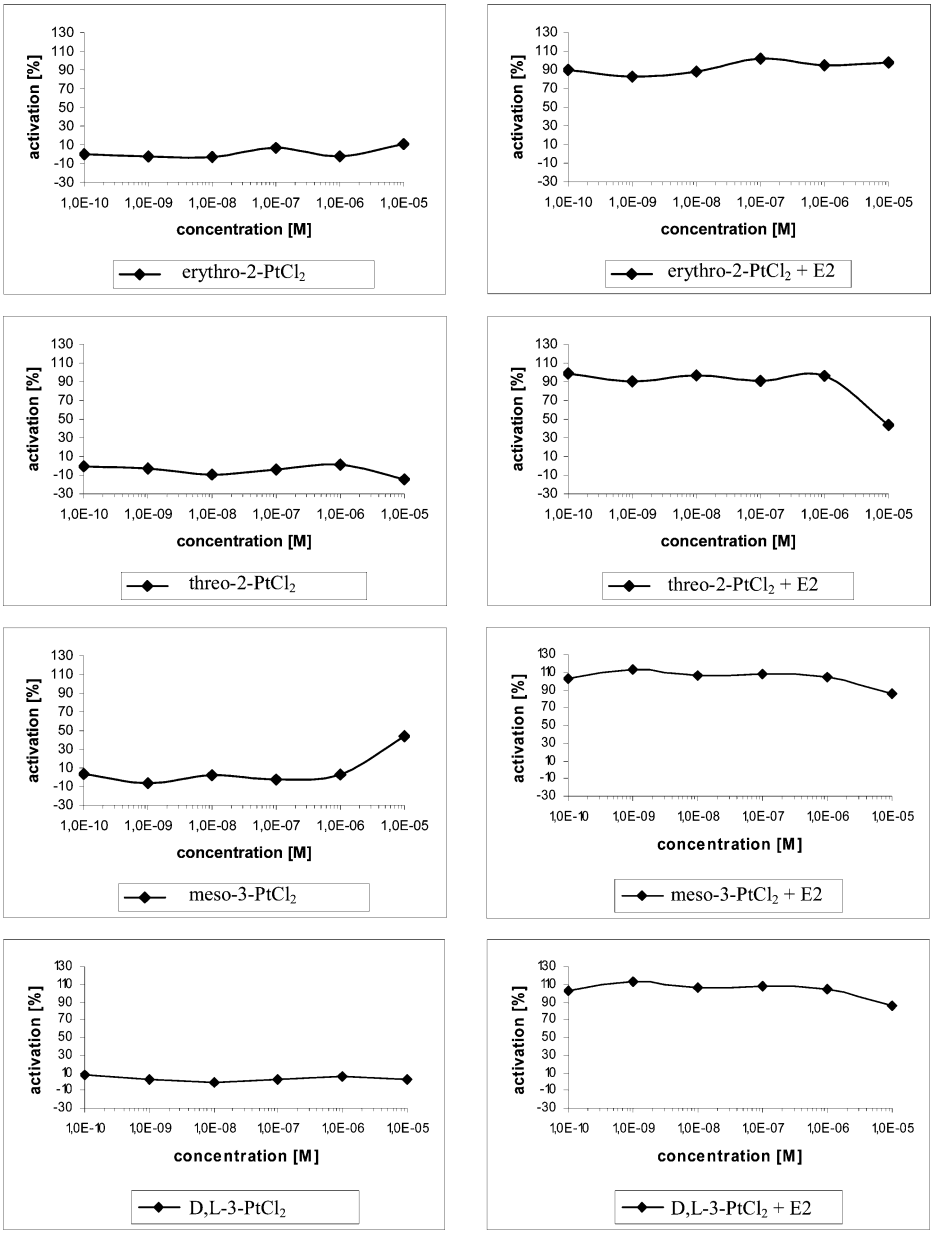


Figure 6. Evaluation of agonistic and antagonistic properties of ***erythro*-** and ***threo*-2-PtCl$_2$** and ***meso*-** and **D,L-3-PtCl$_2$** in ER$^+$ MCF-7-2a breast cancer cells stably transfected with the luciferase reporter plasmid ERE$_{wtc}$luc.

ring allowed a better approach to the target sequences in DNA, resulting in 5-fold increased cytotoxicity compared to the parent compound **D,L-1-PtCl$_2$** (Figure 5). A further ethyl group at the second NH_2 group diminished the cytotoxicity. [**D,L**-*N*,*N*′-Diethyl-1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) (**D,L-3-PtCl$_2$**) proved to be inactive despite the equatorial arrangement of all aryl and ethyl residues at the five-membered chelate ring and the existence of stable rotamers at physiological temperature.

These studies demonstrated that significant cytotoxic activity in this type of platinum(II) complexes requires both the existence of at least one NH_2 fragment and an equatorial arrangement of the bulky residues (Ar and Et).

An explanation can be given on the molecular level by means of a model in which the formation of an intramolecular NH_2···phosphate hydrogen bond essentially contributes to the reaction of Pt(II) complexes with DNA.[23,24]

Thorough studies in several laboratories revealed that the cytotoxic activity of Pt(II) complexes is caused by binding to biologically important sequences of DNA containing two or more adjacent guanosine nucleosides (for literature see the review articles[6,25−27]). The structure of the predominant platinum adduct [PtL$_2${d(GpG)-N7(1),N7(2)}] and its impact on the DNA conformation were studied by ^{1}H NMR spectroscopy and X-ray analysis mainly on the example of simple Pt(II) complexes such as cDDP.[6,28−32] The changes in the spatial structure of the affected DNA sequences were blamed for the inhibitory effects on gene expression[6,33,34] and DNA replication,[6,25−27] which in turn gave rise to tumor regression via triggering of apoptosis (see refs 1 and 6 and references therein).

In the reaction of Pt(II) complexes with d(GpG) units in DNA, the formation of the hydrogen bond takes place between the most easily available hydrogen in the neutral ligand of the Pt(II) complex and the 5′-terminal phosphate oxygen. This is suggested by a study of

Reedijk and co-workers[24] on the platination of oligodeoxynucleotides containing a GpG sequence with unsymmetrically alkyl-substituted cisplatin derivatives. In the ^{31}P NMR spectrum a significant downfield shift for the phosphorus resonance, characteristic for the presence of a NH···phosphate hydrogen bond, was observed (see ref 24 and references therein). Such hydrogen bonds appear to be important for the Pt−DNA interaction, both kinetically, that is, enhancing platinum coordination, and thermodynamically, that is, stabilization of the adduct.[24]

The fact that the *N*,*N*′-diethyl derivative **D,L-3-$PtCl_2$** was inactive in the test on the MCF-7 breast cancer cell line, while the *N*-ethyl derivative ***threo*-2-$PtCl_2$** was active, supports the importance of a hydrogen bond between the NH_2 and the adjacent 5′-terminal phosphate oxygen in the d(GpG) units of DNA for the cytotoxic potency. The exchange of one H atom in each of the two NH_2 groups of **D,L-1-$PtCl_2$** for an ethyl chain presumably impedes the formation of the essential hydrogen bond.

First hints to this mode of action of [1,2-diarylethylenediamine]platinum(II) complexes, with which we have thoroughly investigated the influence of substituents in the aromatic rings on their antitumor activity,[7,14,15,19−21,35−43] came from a study with [*meso*-1,2-bis(2,6-dichloro-4-hydroxyphenyl)ethylenediamine]-platinum(II) (***meso*-6-PtLL′**), [*meso*- and [D,L-1,2-bis(4-fluorophenyl)ethylendiamine]platinum(II) (***meso*-** and **D,L-8-PtLL′**) and cisplatin (cDDP).[44] In experiments on the human MCF-7 breast cancer cell line, the rate of complexation with DNA followed the order **D,L-8-PtLL′** > cDDP > ***meso*-8-PtLL′** > ***meso*-6-PtLL′**. This demonstrates that the predominant equatorially standing aryl residues in **D,L-8-PtLL′** do not cause steric hindrance in the reaction with DNA. In contrast to this, the five-membered chelate ring of ***meso*-8-PtLL′** interconverts between two conformers, each having one of the aryl residues in the axial position, and hampers the formation of DNA−Pt(II) adducts. This negative effect is further enhanced by the two 2,6-standing Cl atoms in the phenyl rings of ***meso*-6-PtLL′**.

We suppose that, in analogy to cDDP, [1,2-diarylethylenediamine]platinum(II) complexes react preferably with d(GpG) sequences in DNA to give GG-N7,N7 chelates, in which a hydrogen bond between the adjacent NH_2 and the 5′-terminal phosphate group contributes to the formation of the intrastrand cross-links essential for the triggering of antitumor effects.

With the exception of ***meso*-6-PtLL′**, which was inactive in the test on the human MCF-7 breast cancer cell line, cDDP and ***meso*-** and **D,L-8-PtLL′** caused complete inhibition of the cell growth at 5.0 μM concentration and drug−cell contact (dcc) of 240 h (T/C_{corr} = 0%). The differing extent of DNA platination by the equicytotoxic Pt(II) complexes cDDP and ***meso*-** and **D,L-8-PtLL′** points to an unequal perturbation in the spatial structure of DNA by the respective compound after formation of the intrastrand cross-link. We suppose that the strength of the hydrogen bond between the Pt(II) complex and adjacent 5′-phosphate oxygen is decisive for the assumed influence on the DNA conformation and therefore on the cytotoxic effect. However, for confirmation of this hypothesis, studies on the reaction of the diastereomeric **1-$PtCl_2$** to **3-$PtCl_2$** complexes with d(pGpG) and the structural clarification of the products, especially of their capability to form intramolecular NH···phosphate hydrogen bonds of differing strength, are necessary.

In the testing of the new [1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) derivatives on the hormone-sensitive, human MCF-7 breast cancer cell line, ***threo*-2-$PtCl_2$** proved to be a very interesting drug. It showed a strong, concentration-dependent cytotoxicity and might therefore be able to inhibit widespread postmenopausal breast cancer. In contrast, its parent compound ***meso*-1-$PtCl_2$** can only be applied in the therapy of premenopausal breast cancer (see Introduction). However, a complex mode of action including cytotoxic potency and estrogenlike effects, the latter probably giving rise to an improvement of anti-breast cancer activity, has been excluded. Estrogenlike effects would be (a) hindrance of the cellular processing of Pt-modified DNA by overexpression of high mobility group domain proteins[5] and (b) interruption of the vicious circle of mutual growth stimulation of breast cancer cells and granulocytes/macrophages by reducing the formation of key cytokines[8−11] (see Introduction). In the test on the MCF-7-2a cell line, the cytotoxic ***threo*-2-$PtCl_2$** did not stimulate luciferase expression as an indication for estrogenic properties. The weak reduction of luciferase activity in the competition experiment with estradiol might be an indication of antiestrogenicity. However, such a concentration−activity curve is always observed with drugs possessing cytotoxic properties causing reduction of cell proliferation. It should be mentioned that the only [1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) derivative endowed with weak estrogenicity, ***meso*-3-$PtCl_2$**, lacks cytotoxicity.

Conclusion

The assumed strong increase of estrogenic and cytotoxic activity of ***meso*-** and **D,L-1-$PtCl_2$** on ER^+ MCF-7 breast cancer cells could not be achieved by their transformation into the *N*-ethyl and *N*,*N*′-diethyl derivatives. Different prerequisites in the substituent pattern of the aromatic rings and in the spatial structure of the diamine region of the five-membered chelate ring for the appearance of maximum estrogenicity on one hand and for maximum cytotoxicity on the other hand are the cause of this result. [1,2-Diarylethylenediamine]platinum(II) complexes possessing both marked estrogenic and cytotoxic properties can be most probably obtained by variation of type and position of the ring substituents. In a further publication, we will report on current studies on this field.

Experimental Section

General Procedures. Melting points (uncorrected) were determined with a Büchi 510 instrument. ^1H NMR spectra were taken with a Bruker FT-NMR spectrometer WM 250 at 250 MHz with TMS as internal standard. Elemental analyses were performed by the "Mikroanalytisches Laboratorium der Universität Regensburg". IR spectra (KBr pellets) were recorded with a Perkin-Elmer 580 spectrophotometer.

Syntheses. ***meso*-** and **D,L-1**, ***meso*-** and **D,L-1a**, and ***meso*-** and **D,L-1-$PtCl_2$** have been synthesized according to methods reported in the literature.[14,15]

Method A: Synthesis of *N*-Ethyl- and *N,N′*-Diethyl-1,2-bis(2,6-difluoro-3-methoxyphenyl)ethylenediamines. *n*-BuLi (15 mmol in hexane) was added dropwise to a solution of the respective 1,2-bis(2,6-difluoro-3-methoxyphenyl)ethylenediamine (10 mmol) in 70 mL of dry THF at a temperature of −70 °C. After stirring for 15 min, the reaction mixture was supplemented dropwise with a solution of diethyl sulfate (10 mmol) in 10 mL of dry THF and was stirred for 5 h at room temperature. Subsequently, the organic layer was separated after addition of 40 mL of ether and 60 mL of 2 N NaOH, washed with water, and dried over $MgSO_4$. Removal of the solvent in vacuo left the *N*-ethyl and the *N,N′*-diethyl derivatives as a mixture, which was separated by column chromatography.

***erythro*-*N*-Ethyl-1,2-bis(2,6-difluoro-3-methoxyphenyl)ethylenediamine (erythro-2a) and *meso*-*N,N′*-Diethyl-1,2-bis(2,6-difluoro-3-methoxyphenyl)ethylenediamine (meso-3a). *meso*-1a** was used as educt. The products were separated by column chromatography (SiO_2, diethyl ether/petroleum ether 3:1).

***erythro*-2a**: yield 40%, colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, ^3J = 7 Hz, 3H, CH_3), 1.60 (br, 3H, NH), 2.10−2.52 (m, 2H, CH_2), 3.81 (s, 6H, OCH_3), 4.40 (d, ^3J = 10 Hz, 1H, $CH_{benzylic}$), 4.60 (d, ^3J = 10 Hz, 1H, $CH_{benzylic}$), 6.58−6.91 (m, 4H, Ar−H).

***meso*-3a**: yield 21%, colorless crystals, mp 118 °C. ^{1}H NMR ($CDCl_3$): δ = 0.89 (t, ^3J = 7 Hz, 6H, CH_3), 1.59 (br, 2H, NH), 2.18−2.64 (m, 4H, CH_2), 3.82 (s, 6H, OCH_3), 4.48 (s, 2H, $CH_{benzylic}$), 6.53−6.91 (m, 4H, Ar−H).

***threo*-*N*-Ethyl-1,2-bis(2,6-difluoro-3-methoxyphenyl)ethylenediamine (*threo*-2a) and D,L-*N,N′*-Diethyl-1,2-bis(2,6-difluoro-3-methoxyphenyl)ethylenediamine (D,L-3a). D,L-1a** was used as educt. The products were separated by column chromatography (SiO_2, diethyl ether/petroleum ether 8:1).

***threo*-2a**: yield 39%, colorless oil. ^{1}H NMR ($CDCl_3$): δ = 1.10 (t, ^3J = 7 Hz, 3H, CH_3), 2.10 (br, 3H, NH), 2.60 (q, ^3J = 7 Hz, 2H, CH_2), 3.75 (s, 6H, OCH_3), 4.38 (d, ^3J = 11 Hz, 1H, $CH_{benzylic}$), 4.64 (d, ^3J = 11 Hz, 1H, $CH_{benzylic}$), 6.57−6.86 (m, 4H, Ar−H).

D,L-3a: yield 23%, colorless oil. ^{1}H NMR ($CDCl_3$): δ = 1.08 (t, ^3J = 7 Hz, 6H, CH_3), 1.98 (br, 2H, NH), 2.48 (q, ^3J = 7 Hz, 4H, CH_2), 3.75 (s, 6H, OCH_3), 4.60 (s, 2H, $CH_{benzylic}$), 6.57−6.82 (m, 4H, Ar−H).

Method B: General Procedure for the Cleavage of the Methyl Ether. A solution of the methyl ether (3 mmol) in 60 mL of dry CH_2Cl_2 was cooled to −60 °C. At this temperature BBr_3 (13 mmol) was added. The reaction mixture was brought to room temperature and heated to reflux for 24 h. Subsequently, 20 mL of methanol was added under cooling, and the solvent was evaporated. The residue was dissolved in 5 mL of water, the resulting mixture was filtrated, and the eluent was made alkaline with 2 N NaOH. Unreacted methyl ether was filtered off, and the filtrate was brought to pH 8 with 2 N HCl. The precipitate was collected by suction filtration, washed with water, and dried over P_2O_5.

***erythro*-*N*-Ethyl-1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine (*erythro*-2). *erythro*-2** was obtained from ***erythro*-2a**: yield 82%, colorless powder, mp 187−188 °C. ^{1}H NMR (DMF-d_7): δ = 0.84 (t, ^3J = 7.1 Hz, 3H, CH_3), 2.22−2.40 (m, 1H, CH_2), 2.40−2.57 (m, 1H, CH_2), 4.44 (d, ^3J = 10.9 Hz, 1H, $CH_{benzylic}$), 4.55 (d, ^3J = 10.9 Hz, 1H, $CH_{benzylic}$), 6.78−7.00 (m, 4H, Ar−H).

***meso*-*N,N′*-Diethyl-1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine (*meso*-3). *meso*-3** was obtained from **meso-3a**: yield 83%, colorless powder, mp 227−228 °C. ^{1}H NMR (DMF-d_7): δ = 0.85 (t, ^3J = 7.1 Hz, 6H, CH_3), 2.29−2.39 (m, 2H, CH_2), 2.43−2.56 (m, 2H, CH_2), 4.51 (m, 2H, $CH_{benzylic}$), 6.82−6.99 (m, 4H, Ar−H).

***threo*-*N*-Ethyl-1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine (*threo*-2). *threo*-2** was obtained from *threo*-**2a**: yield 71%, colorless powder, mp 114−116 °C. ^{1}H NMR (DMF-d_7): δ = 1.07 (t, ^3J = 7.1 Hz, 3H, CH_3), 2.56 (q, ^3J = 7.1 Hz, 2H, CH_2), 4.39 (d, ^3J = 10.3 Hz, 1H, $CH_{benzylic}$), 4.61 (d, ^3J = 10.3 Hz, 1H, $CH_{benzylic}$), 6.62−6.81 (m, 4H, Ar−H).

D,L-*N,N′*-Diethyl-1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine (D,L-3). D,L-3 was obtained from D,L-**3a**: yield 73%, colorless powder, mp 108−110 °C. ^{1}H NMR (DMF-d_7): δ = 1.06 (t, ^3J = 7.1 Hz, 6H, CH_3), 2.47−2.57 (m, 4H, CH_2), 4.61 (s, 2H, $CH_{benzylic}$), 6.64−6.84 (m, 4H, Ar−H).

Method C: General Procedure for the Synthesis of Dichloroplatinum(II) Complexes. K_2PtCl_4 (0.5 mmol) and DMSO (0.5 mmol) were dissolved in 2.5 mL of H_2O and stirred for 24 h. To this reaction mixture were added 20 mL of water and a solution of the ligand (0.5 mmol) in 0.5 N HCl. Subsequently, the pH was adjusted to 4 with 0.5 N NaOH, and the mixture was heated to 50−60 °C for 8 h under stirring. During this time, the pH was maintained at 4−5. After this, 10 mL of 2 N HCl was added, and the mixture was stirred for an additional hour. The precipitate was separated from the hot solution by suction filtration, washed with hot water, and dried over P_2O_5.

[*erythro*-*N*-Ethyl-1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) (*erythro*-2-PtCl$_2$). *erythro*-2-PtCl$_2$ was obtained from ***erythro*-2**: yield 62%, beige powder. ^{1}H NMR: see Table 1. IR (KBr): ν = 320 cm^{-1} (Pt−Cl). Anal. ($C_{16}H_{16}Cl_2F_4N_2O_2Pt\cdot 0.5H_2O$) C H N.

[*meso*-*N,N′*-Diethyl-1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) (*meso*-3-PtCl$_2$). *meso*-3-PtCl$_2$ was obtained from **meso-3**: yield 59%, yellow powder. ^{1}H NMR: see Table 1. IR (KBr): ν = 325 cm^{-1} (Pt−Cl). Anal. ($C_{18}H_{20}Cl_2F_4N_2O_2Pt$) C H N.

[*threo*-*N*-Ethyl-1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) (*threo*-2-PtCl$_2$). *threo*-2-PtCl$_2$ was obtained from ***threo*-2**: yield 78%, yellow powder. ^{1}H NMR: see Table 1. IR (KBr): ν = 320, 330 cm^{-1} (Pt−Cl). Anal. ($C_{16}H_{16}Cl_2F_4N_2O_2Pt$) C H N.

[D,L-*N,N′*-Diethyl-1,2-bis(2,6-difluoro-3-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) (D,L-3-PtCl$_2$). D,L-3-PtCl$_2$ was obtained from **D,L-3**: yield 67%, yellow powder. ^{1}H NMR: see Table 1. IR (KBr): ν = 320 cm^{-1} (Pt−Cl). Anal. ($C_{18}H_{20}Cl_2F_4N_2O_2Pt\cdot 0.5H_2O$) C H N.

Biological Methods. A. Biochemicals, Chemicals, and Materials. Dextran, 17β-estradiol, L-glutamine (L-glutamine solution: 29.2 mg/mL of phosphate buffered saline (PBS)), and Minimum Essential Medium Eagle (EMEM) were from Sigma (Munich, Germany). Dulbecco's Modified Eagle Medium without phenol red (DMEM) was from Gibco (Eggenstein, Germany). Fetal calf serum (FCS) was from PAN (Aidenbach, Germany). *N*-Hexamethylpararosaniline (crystal violet) and gentamicin sulfate were from Fluka (Deisenhofen, Germany). Glutaric dialdehyde (25%) was from Merck (Darmstadt, Germany). Trypsin (0.05%) in ethylenediaminetetraacetic acid (0.02%) (trypsin/EDTA) was from Boehringer (Mannheim, Germany). Penicillin−streptomycin gold standard (10000 IE penicillin/mL, 10 mg of streptomycin/mL) and Geneticin disulfate (Geneticin solution: 35.71 mg/mL of PBS) were from ICN Biomedicals GmbH (Eschwege, Germany). Cell culture lysis reagent (5×) (diluted 1:5 with purified water before use) and the luciferase assay reagent were from Promega (Heidelberg, Germany). DMF was from Aldrich (Steinheim, Germany). PBS was prepared by dissolving 8.0 g of NaCl, 0.2 g of KCl, 1.44 g of $Na_2HPO_4\cdot 2H_2O$, and 0.2 g of KH_2PO_4 (all purchased from Merck or Fluka) in 1000 mL of purified water. TRIS-buffer (pH = 7.5) was prepared by dissolving 1.211 g of trishydroxymethylaminomethane, 0.37224 g of Titriplex III, and 0.19503 g of sodium azide (all from Merck or Fluka) in 1 L of purified water. Deionized water was produced by means of a Millipore Milli-Q Water System: resistivity > 18 MΩ. T-75 flasks, reaction tubes, 96-well plates, and 6-well plates were from Renner GmbH (Dannstadt, Germany).

B. In Vitro Chemosensitivity Assay at MCF-7 Cells. The in vitro testing of the complexes for antitumor activity was carried out by exponentially dividing human cancer cells according to a previously published microtiter assay.[45] Exponential cell growth is guaranteed during the whole time of incubation. Briefly, using 96-well microtiter plates, 100 μL of

a cell suspension was plated into each well at 7700 cells/mL of culture medium and incubated at 37 °C for 3 days in a humidified atmosphere (5% CO_2). By adding an adequate volume of a stock solution of the respective compound (solvent: DMF) to the medium, the desired test concentration was obtained. Sixteen wells were used for each test concentration and for the control, which contained the corresponding amount of DMF. The medium was removed after reaching the appropriate incubation time. Subsequently, the cells were fixed with a glutaric dialdehyde solution and stored under phosphate buffered saline (PBS) at 4 °C. Cell biomass was determined by means of a crystal violet staining technique as described earlier.[45] The effectiveness of the complexes is expressed as corrected % T/C_{corr} values according to the following equation:

$$\text{cytostatic effect: } T/C_{corr}\,[\%] = [(T - C_0)/(C - C_o)] \times 100$$

whereby T (test) and C (control) are the optical densities at 590 nm of the crystal violet extract of the cells in the wells (i.e. the chromatin-bound crystal violet extracted with ethanol 70%) with C_0 being the density of the cell extract immediately before treatment. For the automatic estimation of the optical density of the crystal violet extract in the wells, a Microplate Autoreader (Flashscan AnalytikJena, Germany) was used.

C. Luciferase Assay with ERα-Positive MCF-7-2a Cells Stably Transfected with the Reporter Plasmid ERE_{wtc}luc. MCF-7-2a cells were maintained as a monolayer culture at 37 °C in a humidified atmosphere (5% CO_2) in T-75 flasks using phenol red free DMEM supplemented with penicillin/streptomycin 1% (v/v), L-glutamine (2mM), FCS 5% (v/v), and Geneticin solution 0.5% (v/v) as growth media. Cell line banking and quality control were performed according to the seed stock concept reviewed by Hay.[46] One week before starting the experiment, MCF-7-2a cells were cultivated in DMEM supplemented with L-glutamine, antibiotics, and dextran/charcoal-treated FCS (ct-DMEM, 5% v/v). Cells from an almost confluent monolayer were removed by trypsinization and suspended in ct-DMEM to approximately 5×10^4 cells/mL. 100 μL of the cell suspension was seeded in the 60 inner wells of a white flat-bottomed 96-well plate (suitable for measuring luminescence). The border wells were filled with 200 μL of isoosmotic liquid (medium, PBS, e.g.) in order to avoid boundary problems. After 24 h, the media was replaced by 180 μL of ct-DMEM and 20 μL of medium containing either E2 or the test compounds in appropriate amounts to achieve final concentrations ranging from 10^{-7} to 10^{-12} M (E2) or 10^{-5} to 10^{-10} M (test compounds). The concentration of the solvent used to prepare stock solutions amounted to 0.1% (v/v). After 50 h of incubation under growth conditions, the medium was removed and 50 μL of cell culture lysis reagent was added into each well. The plate was incubated at room temperature under vigorous shaking (600 rpm, TiMix, Edmund Bühler, Germany). Luciferase was assayed using the Promega luciferase assay reagent. 50 μL of substrate reagent was added into each well, and luminescence (in relative light units, RLU) was measured for 10 s by use of a microlumat (Bethold). Measurements were corrected by correlating the RLU with the cell mass of each sample.

The cell mass was determined in a crystal violet assay[45] in crystal flat-bottomed 96-well plates analogously to the chemosensivity assay. After incubation for 50 h, the medium was removed and glutaric dialdehyde (1% in PBS; 100 μL/well) was added for fixation. After 15 min the solution of the aldehyde was replaced by 180 μL or PBS/well. The plates were stored at 4 °C until staining. Cells were stained by treating them for 30 min with 100 μL of an aqueous solution of crystal violet (0.02% (m/v)). After decanting, cells were washed several times with water to remove the adherent dye. After addition of 180 μL of ethanol (70%, (v/v)), plates were gently shaken for 4 h. The optical density of each well was measured in a microplate autoreader at 590 nm (Fashscan, AnalytikJena, Germany). The estrogenic activity was expressed as % activation of a 10^{-9} M E2 control (100%). The antiestrogenic activity was determined by incubation of the MCF-7-2a cells with the test compounds in concentrations from 10^{-5} to 10^{-10} M along with a constant concentration of E2 (10^{-9} M). IC_{50} is the concentration of the compound which is necessary to reduce the effect of E2 by 50%.

Acknowledgment. This work was supported by the Fonds der Chemischen Industrie. The technical assistance of S. Bergemann and I. Schnautz is gratefully acknowledged.

Supporting Information Available: Elemental analyses and ^1H NMR spectra of the target compounds ***erythro-*** and ***threo*-2-$PtCl_2$** and ***meso-*** and **D,L-3-$PtCl_2$**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM050186I