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Synthesis and Antitumor Activity of Enantiomerically Pure [1,2-Diamino-1-(4-fluorophenyl)propane]dichloroplatinum(II) Complexes

Enantiomerically pure 1,2-diamino-1-(4-fluorophenyl)propanes were synthesized by stereospecific and stereoselective procedures by use of the (1*R*,2*S*)- and (1*S*,2*R*)-2-amino-1-(4-fluorophenyl)propanols (**12 a**) as intermediates. The enantiomeric purity was determined by ^{1}H NMR spectroscopy after conversion of the propanolamines and the diamines with (1*R*)-myrtenal into mono- and diimines. For the coordination to platinum the diamines were reacted with K_2PtCl_4. The resulting dichloroplatinum(II) complexes **4F-Ph/Me-PtCl$_2$** were tested for antiproliferative activity on the MCF-7 breast cancer cell line. (*SS*)- and (*RR*)-**4F-Ph/Me-PtCl$_2$** produced the strongest inhibitory effect. Both complexes showed cytocidal effects, (*SS*)-**4F-Ph/Me-PtCl$_2$** even in a concentration of 1 µM. The (1*S*,2*R*)- and (1*R*,2*S*)-configurated complexes were far less active (*SS* > *RR* > *RS* = *SR*) and comparable in this respect with the standard cisplatin.

Keywords: Platinum complexes; MCF-7 cell line; Antitumor activity; Enantiomers

Received: November 26, 2001 [FP650]

Introduction

Since the first report of an inhibition of cell division by platinum complexes [1], a large number of platinum(II) compounds have been synthesized and tested for antitumor activity [2–4]. The first derivative approved for cancer treatment was cisplatin (**1**) which was later followed by carboplatin (**2**), oxaliplatin (**3**), and nedaplatin (**4**) (formulae see Figure 1) [3–5].

Although platinum complexes show high antitumor activity, their therapeutic effectiveness is often limited by toxic side effects and the occurrence of drug resistance [6]. This was also confirmed for **1**, **2**, and **4** and, to a lesser extent, for the chiral [(1*R*,2*R*)-1,2-diaminocyclohexane]dichloroplatinum(II) complex (**3**) [5]. Therefore, the use of optically pure chiral ligands was proposed as an alternative means in order to get better characteristics for platinum compounds [7–10].

In previous studies we synthesized enantiomerically pure [1,2-diamino-1-phenyl-propane]dichloroplatinum(II) and [1,2-diamino-1,2-diarylethane]dichloroplatinum(II) complexes and demonstrated the effectiveness of the separation of racemic compounds on the antitumor effects *in vitro* and *in vivo* [8, 11, 12]. The most potent inhibitory activity *in vitro* on the MCF-7 breast cancer cell line was caused by (*RR*)-**Ph/Me-PtCl$_2$** and the enantiomers of **4F-Ph/4F-Ph-PtCl$_2$** (see Figure 1 and Table 1). These results encouraged us to synthesize the 4 isomers of [1,2-diamino-1-(4-fluorophenyl)propane]dichloroplatinum(II) in order to get platinum complexes with outstanding antitumor activity.

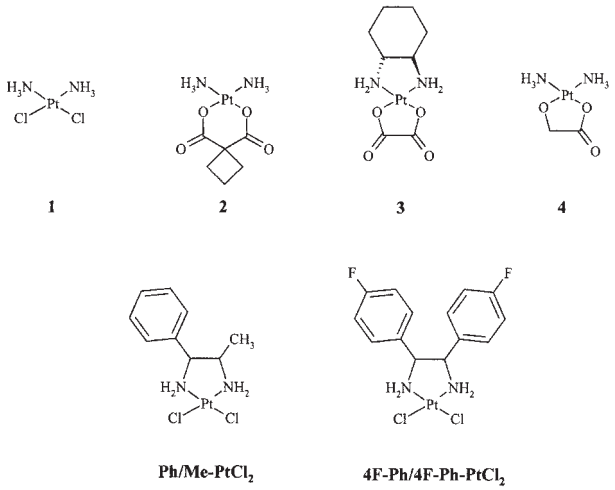

Figure 1. Structure of platinum complexes referred to in the paper.

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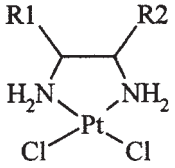
Table 1. Growth inhibiting effects of [1,2-diamino-1-(4-fluorophenyl)propane]dichloroplatinum(II) and [1,2-diamino-1,2-bis(4-fluorophenyl)ethane]dichloroplatinum(II) complexes on the MCF-7 breast cancer cell line (T/C_{corr} (%)[a] and time for best value).

Compounds			Concentration		
	R1	R2	0.5 µM	1 µM	5 µM
Test substances					
(*RS/SR*)-**4F-Ph/Me-PtCl$_2$**	4F-phenyl	–CH$_3$	92 (117 h)[b]	34 (117 h)[b]	2 (237 h)
(*RR/SS*)-**4F-Ph/Me-PtCl$_2$**	4F-phenyl	–CH$_3$	63 (120 h)[b]	32 (120 h)[b]	–20 (221 h)
(*RS*)-**4F-Ph/Me-PtCl$_2$**	4F-phenyl	–CH$_3$	71 (166 h)	44 (166 h)	6 (237 h)
(*SR*)-**4F-Ph/Me-PtCl$_2$**	4F-phenyl	–CH$_3$	116 (142 h)[b]	93 (142 h)[b]	6 (237 h)
(*SS*)-**4F-Ph/Me-PtCl$_2$**	4F-phenyl	–CH$_3$	11 (169 h)	–8 (169 h)	–52 (221 h)
(*RR*)-**4F-Ph/Me-PtCl$_2$**	4F-phenyl	–CH$_3$	36 (147 h)[b]	4 (147 h)	–41 (221 h)
Control substances					
Cisplatin			82 (166 h)	54 (166 h)	4 (237 h)
(*R/S*)-**4F-Ph/H-PtCl$_2$**	4F-phenyl	–H	88 (75 h)	64 (75 h)	4 (225 h)
(*RS*)-**Ph/Me-PtCl$_2$**[c]	phenyl	–CH$_3$	92 (72 h)	93 (72 h)	33 (117 h)
(*SR*)-**Ph/Me-PtCl$_2$**[c]	phenyl	–CH$_3$	98 (214 h)	95 (214 h)	29 (117 h)
(*SS*)-**Ph/Me-PtCl$_2$**[c]	phenyl	–CH$_3$	92 (72 h)	70 (72 h)	10 (143 h)
(*RR*)-**Ph/Me-PtCl$_2$**[c]	phenyl	–CH$_3$	76 (72 h)	44 (72 h)	–2 (214 h)
(*RR/SS*)-**4F-Ph/4F-Ph-PtCl$_2$**[d]	4F-phenyl	4F-phenyl	20 (170 h)	10 (170 h)	–5 (210 h)
(*RR*)-**4F-Ph/4F-Ph-PtCl$_2$**[d]	4F-phenyl	4F-phenyl	30 (170 h)	12 (170 h)	0 (210 h)
(*SS*)-**4F-Ph/4F-Ph-PtCl$_2$**[d]	4F-phenyl	4F-phenyl	43 (170 h)	13 (170 h)	–5 (210 h)
(*RS/SR*)-**4F-Ph/4F-Ph-PtCl$_2$**[d]	4F-phenyl	4F-phenyl	59 (90 h)[b]	15 (210 h)	0 (210 h)

[a] T/C_{corr} (%) = $(T - C_0)/(C - C_0)$ (see also "Experimental Part").
[b] Appearing of resistance.
[c] Previously published results [8].
[d] Previously published results [23].

The few synthetic routes to vicinal chiral diamines with two chiral centers already described in the literature, starting from alkenes [13, 14], cyclic sulfates [15], imines [16], amino alcohols [8, 17], and nitrones [18] could not be applied for building unsymmetrical diamines containing an aromatic ring at the α-carbon of the aliphatic chain. Therefore, we adopted the same procedure as described for the synthesis of enantiomerically pure 1,2-diamino-1-phenylpropanes starting from (1*R*,2*S*)- or (1*S*,2*R*)-norephedrine [8].

(1*R*,2*S*)- or (1*S*,2*R*)-configurated 1,2-diamino-1-phenylpropanes were finally obtained stereospecifically by forming and subsequently opening a *trans*-oxazoline. The diasteriomeric 1,2-diamino-1-phenylpropanes ((1*R*,2*R*) or (1*S*,2*S*)) were synthesized via a *cis*-aziridine followed by its regio- and stereoselective opening. As the starting 4-fluoronorephedrine derivatives were not commercially available, their synthesis had to be first considered. Numerous methods described in the literature for obtaining this kind of optically pure amino alcohols [19] lead to final products with (1*S*,2*R*)- or (1*R*,2*S*)-configuration. In the present paper, we used a stereoselective procedure starting from (*R*)- or (*S*)-alanine (**7**) [20] and obtained for the amino alcohols **12 a**/**12 b** a ratio of about 80/20. The optical purity of amino alcohols and of their diamines was checked using the chiral aldehyde (1*R*)-myrtenal as a derivatization agent.

The coordination to platinum(II) of the 4 original enantiomers was achieved with K_2PtCl_4 and the resulting dichloroplatinum(II) complexes were tested on the

MCF-7 breast cancer cell line with cisplatin as a reference.

Results and discussion

Synthesis

Pure optical isomers of the amino alcohol **12 a** were prepared starting from commercially available (*R*)- or (*S*)-alanine (**7**). In Figure 2 the synthetic route is described for the example of (*R*)-**7**.

The procedure was accompanied by a complete retention of configuration for the carbon bearing the nitrogen atom. After protection of the amino group with ethyl chloroformate, the amino acid was converted into its acyl chloride (*R*)-**9** with oxalyl chloride, but the resulting

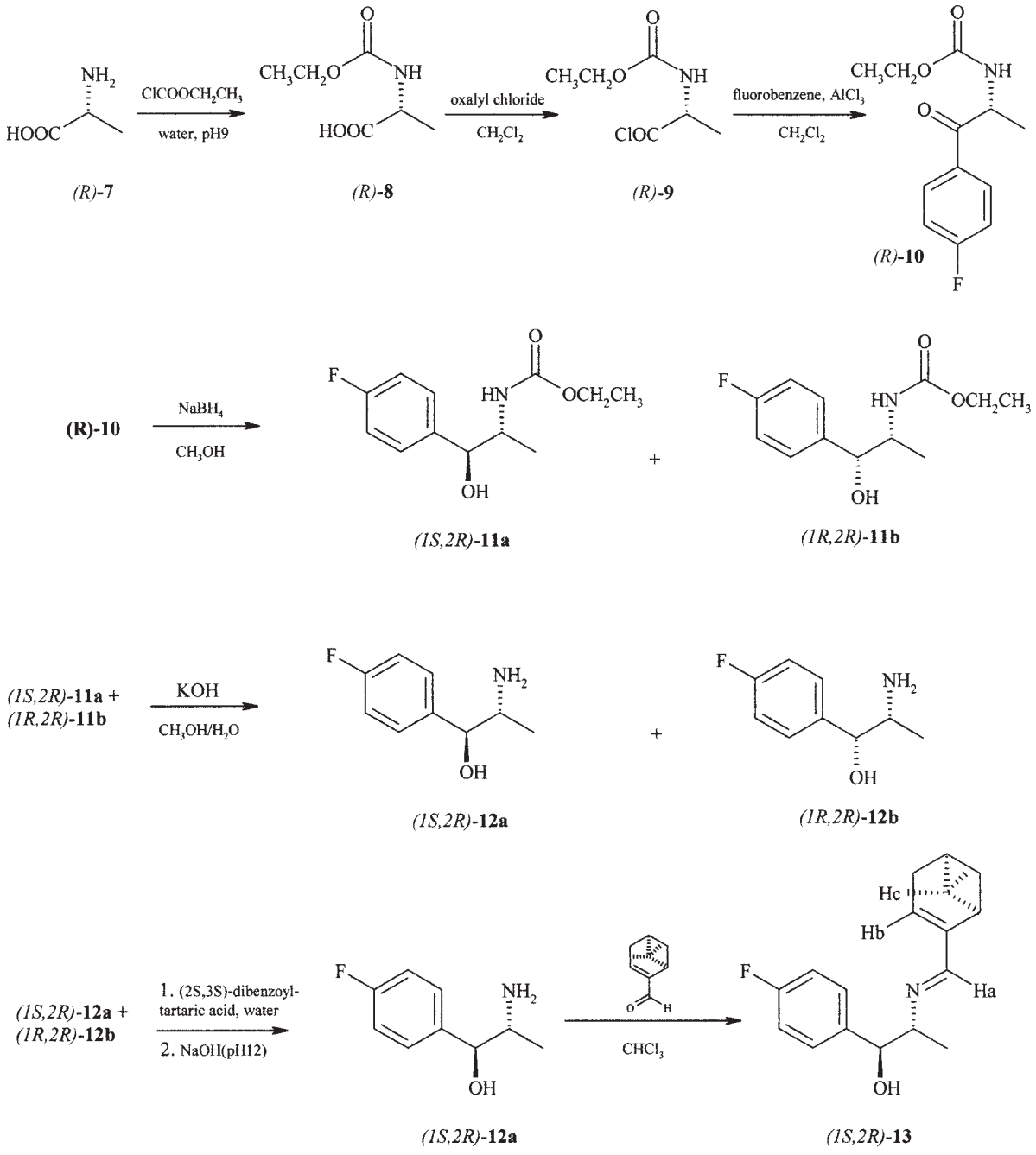


Fig. 2. Synthesis of enantiomeric (1*R*,2*S*)- and (1*S*,2*R*)-2-amino-1-(4-fluorophenyl)propanols (**12 a**).

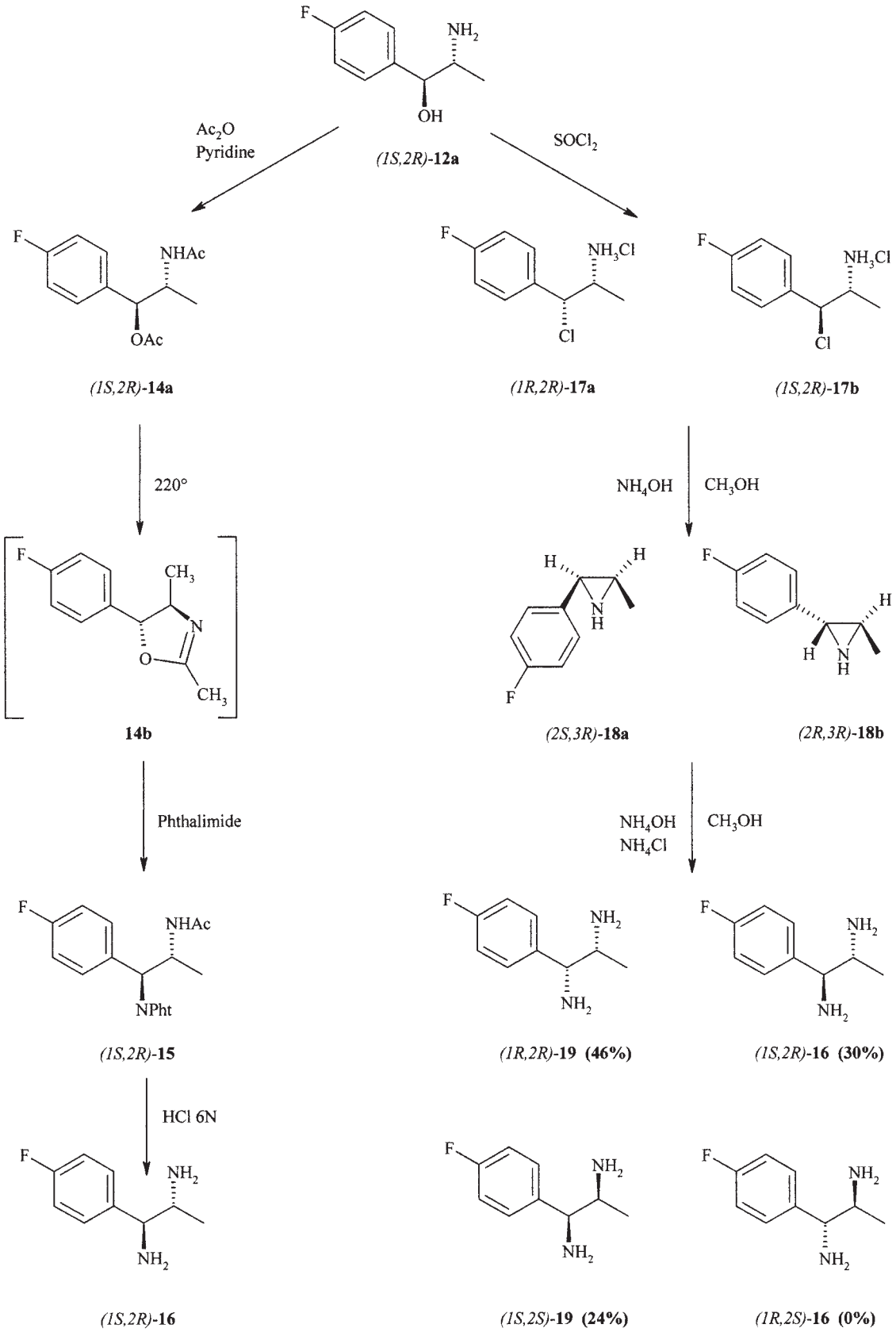


Fig. 3. Synthesis of enantiomeric 1,2-diamino-1-(4-fluorophenyl)propanes (**16** and **19**).

derivative was not isolated. A Friedel-Craft condensation of (*R*)-**9** with fluorobenzene was thereafter directly carried out at –15 °C, which resulted in the *N*-protected amino ketone (*R*)-**10** with the fluorine atom in the para position. This step has already been described in the literature regarding the use of benzene [20]. As expected, the yield was lower than that which was obtained with the non-substituted ring (90 % with benzene, 39 % with fluorobenzene). The last step consisted of a diastereoselective reduction of the ketone with sodium borohydride, which gave a mixture of (1*S*,2*R*)-**11 a**/(1*R*,2*R*)-**11 b** in a ratio of 77 : 23. After alkaline hydrolysis of the carbamates (1*S*,2*R*)-**11 a**/(1*R*,2*R*)-**11 b**, the resulting mixture of (1*S*,2*R*)-**12 a**/(1*R*,2*R*)-**12 b** was treated with (2*S*,3*S*)-dibenzoyltartaric acid to precipitate (1*S*,2*R*)-**12 a** (2*S*,3*S*)-dibenzoyltartrate [21].

Optically pure diamines **16** and **19** were synthesized by following a procedure previously described [8]. Figure 3 shows the stereospecific and stereoselective reaction of (1*S*,2*R*)-**12 a** to obtain (1*S*,2*R*)-**16**, and (1*R*,2*R*)-**19** and (1*S*,2*S*)-**19**. The use of (1*R*,2*S*)-**12 a** as educt yielded the 1,2-diaminoethane (1*R*,2*S*)-**16**.

(1*S*,2*R*)-**12 a** was first *O*- and *N*-acylated with acetic anhydride and then transformed into a *trans*-oxazoline **14 b** (not isolated) by cyclization and liberation of acetic acid [22]. The ring opening of **14 b** with phthalimide led to the exclusive formation of (1*S*,2*R*)-**15**, which was finally hydrolyzed in 6N HCl to give the enantiomerically pure compound (1*S*,2*R*)-**16** (enantiomeric excess, ee = 99 %). Starting from (1*R*,2*S*)-**12 a** the enantiomerically pure (1*R*,2*S*)-**16** was obtained.

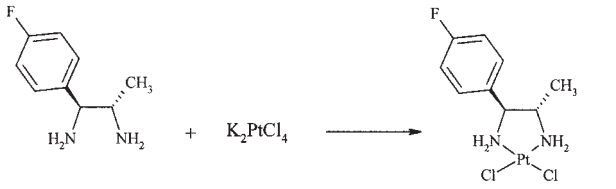
Enantiomeric derivatives **19** were obtained via a predominantly stereoselective two-steps procedure. As shown in Figure 3, the reaction of (1*S*,2*R*)-**12 a** with $SOCl_2$ gave a mixture of (1*R*,2*R*)-**17 a** and (1*S*,2*R*)-**17 b** (89 : 11), which was thereafter converted into the corresponding aziridines (2*S*,3*R*)-**18 a** and (2*R*,3*R*)-**18 b** (87 : 13). The ring opening of the aziridines with NH_3, which took place on C-2 as well as on C-3, yielded the diastereomeric diamines **16** and **19** only in the form of enriched products. In the (1*S*,2*S*/1*R*,2*R*)-series, ee was about 22 %. The pure enantiomers were obtained by serial fractional crystallizations of (1*R*,2*R*)-**19** (2*S*,3*S*)-dibenzoyltartrate or (1*S*,2*S*)-**19** (2*R*,3*R*)-dibenzoyltartrate (ee = 98 %).

The optical purity of **12 a**, **16** and **19** was confirmed by ^1H NMR (300 MHz, $CDCl_3$) analysis. Imines or diimines were formed directly in the NMR tube respectively by an *in situ* reaction of the amino alcohols or the diamines with (1*R*)-myrtenal. Reference compounds were (1*S*,2*R*)- and (1*R*,2*S*)-norephedrines for the amino alcohols **12 a**, and related unsubstituted diamines [8] for the diamines **16** and **19**. The protons Ha, Hb, and Hc of **13** were most typical for characterization of various isomers (Table 2).

Table 2. Chemical shifts of monoimines and diimines (δ in ppm).

Starting compounds	Ha[a]	Hb[a]	Hc[a]
(1*S*,2*R*)-norephedrine	7.57	5.94	0.68
(1*R*,2*S*)-norephedrine	7.79	5.99	0.77
(1*S*,2*R*)-**12 a**	7.63	5.93	0.71
(1*R*,2*S*)-**12 a**	7.72	5.97	0.78
(1*R*,2*S*)-1,2-diamino-1-phenylpropane[b]	7.33	5.73	0.74
	7.85	5.95	0.82
(1*S*,2*R*)-1,2-diamino-1-phenylpropane[b]	7.33	5.72	0.64
	7.85	5.96	0.71
(1*R*,2*R*)-1,2-diamino-1-phenylpropane[b]	7.63	5.83	0.76
	7.64	5.89	0.77
(1*S*,2*S*)-1,2-diamino-1-phenylpropane[b]	7.63	5.85	0.69
	7.63	5.90	0.79
(1*R*,2*S*)-**16**	7.34	5.65	0.79
	7.87	5.99	0.85
(1*S*,2*R*)-**16**	7.34	5.66	0.65
	7.85	6.00	0.72
(1*R*,2*R*)-**19**	7.63	5.85	0.76
	7.63	5.89	0.81
(1*S*,2*S*)-**19**	7.61	5.86	0.69
	7.61	5.91	0.78

[a] See **13**, Figure 2.
[b] Previously published results [8].



(1S,2S)-**16** *(SS)*-**4F-Ph/Me-PtCl$_2$**

Fig. 4. Coordination of the enantiomerically pure 1,2-diaminoethanes with K_2PtCl_4 on the example of (1*S*,2*S*)-**16**.

K_2PtCl_4 was used for the coordination of the pure 1,2-diaminoethanes **16** and **19** (Figure 4). The coordination to platinum was confirmed by NMR spectroscopy. The NH_3^+ resonances of the free 1,2-diaminoethanes **16** and

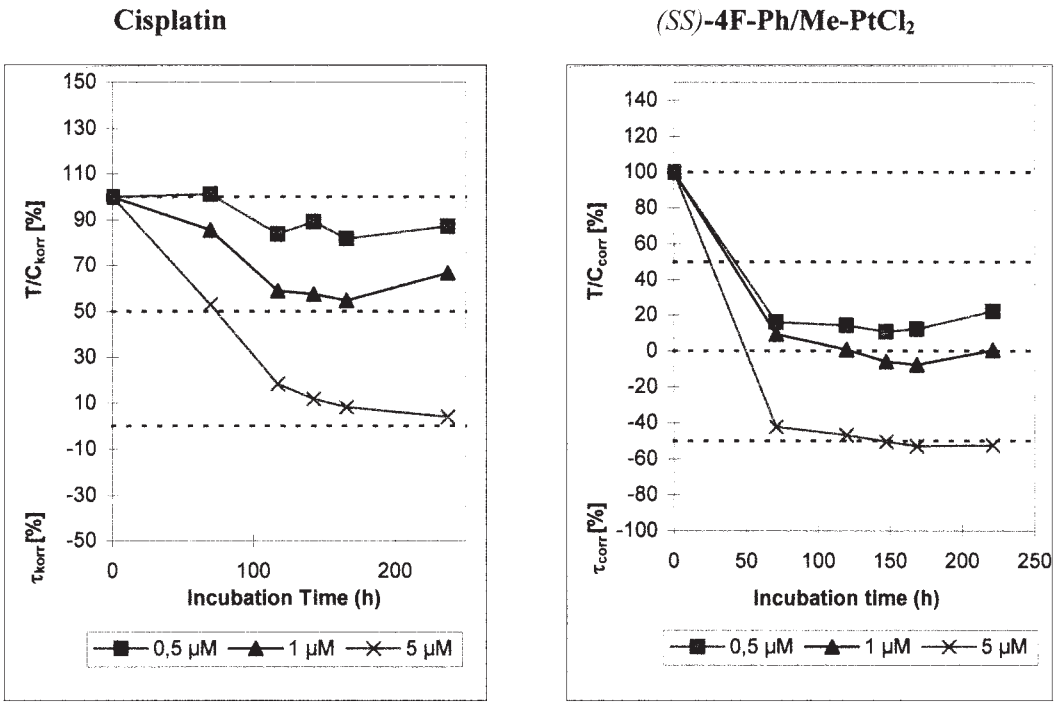


Figure 5. Effect of Cisplatin and (*SS*)-**4F-Ph/Me-PtCl$_2$** on the MCF-7 breast cancer cell line at concentrations of 0.5 µM, 1 µM, and 5 µM.

19 appear at δ = 8.93 and 9.12, respectively. Due to the coordination to platinum, the amine protons were diastereotopically split into four signals in the spectra of the [1,2-diamino-1-(4-fluorophenyl)propane]dichloroplatinum(II) complexes (see Experimental Part).

Pharmacological evaluation

Table 1 shows the results of the tests performed on the MCF-7 breast cancer cell line. One of the most striking observation was the diastereoselectivity of the activity between (1*S*,2*S*)/(1*R*,2*R*)- and (1*S*,2*R*)/(1*R*,2*S*)-isomers, with a more pronounced effect for unsymmetrical than for symmetrical compounds. The enantioselectivity is relatively low. (*SS*)-**4F-Ph/Me-PtCl$_2$** and (*RR*)-**Ph/Me-PtCl$_2$** were somewhat more active than their enantiomers. Interestingly, in the case of the symmetric complexes, the racemate and the enantiomers showed identical concentration time curves. In contrast, the separation of enantiomers is particularly profitable for threo-configurated compounds, (*RR*,*SS*)-**4F-Ph/Me-PtCl$_2$** was less active than the pure enantiomers.

The activity of **4F-Ph/Me-PtCl$_2$** complexes was higher or at least comparable to that of cisplatin. (*RR/SS*)-**4F-Ph/Me-PtCl$_2$** and its isomers were more active than cisplatin and cytocidal, while (*RS/SR*)-, (*RS*)- and (*SR*)-**4F-Ph/Me-PtCl$_2$** were equipotent to cisplatin and cytostatic. The most potent enantiomer (*SS*)-**4F-Ph/Me-PtCl$_2$** was also the only one which was cytocidal at a concentration of 1 µM (Figure 5).

After a prolonged exposure, the tumor cell proliferation recuperated, giving rise to a new ascent of the growth curve. This phenomenon can be explained either by the development of drug resistance or by a progressing drug inactivation due to an irreversible binding to plasma proteins. As far as resistance is concerned, it seems to depend on the optical purity of the complexes: both (*RR/SS*)-**4F-Ph/Me-PtCl$_2$** and (*RS/SR*)-**4F-Ph/Me-PtCl$_2$** showed resistance at concentrations of 0.5 and 1 µM. In contrast to their enantiomers resistance did not occur for (*RS*)-**4F-Ph/Me-PtCl$_2$** and (*SS*)-**4F-Ph/Me-PtCl$_2$**.

A comparison of the results obtained with test and control substances shows that the introduction of a fluorine atom in the *para* position of the aromatic ring in [1,2-diamino-1-phenylpropane]dichloroplatinum(II) and a methyl group instead of an aromatic ring at the C2-atom increase markedly the activity of enantiomeric pure complexes.

Discussion

The design of [1,2-diaminoethane]platinum(II) complexes with optimal anticancer activity and the possibility to overcome resistance by introduction of phenyl or alkyl substituents at the C1- and C2-atom shows the need for a critical examination of the position occupied by the aromatic moieties of the considered molecules. In a first series of platinum(II) complexes with ligands such as 1,2-diamino-1,2-diphenylethanes [11, 12, 23–26], 1,2-diamino-1-phenylpropane [8], 1,2-diamino-3-phenylpropanes [27], 2-picoline [28] or 1-(2-aminophenyl)isoquinolines [29], it was postulated that this kind of compounds should be more strongly bound to DNA, possibly by intercalating the aromatic rings between the DNA bases. This theory was later reconsidered [30] when it was proved that aromatic groups cause a steric hindrance during the attachment to the DNA bases. Although the precise mechanism of action of platinum(II) complexes still remains imperfectly known, two facts seem well established: (i) Platinum(II) complexes bind to DNA without dissociation of their neutral ligand [6], and (ii) fixation of the complexes induces changes in the conformation of DNA [31] and inhibits DNA polymerase which in turn inhibits DNA duplication and transcription, and finally cell replication [32, 33]. The presence of repair enzymes is held responsible for the apparition of resistance during the treatment with platinum complexes. The use of sterically demanding diamines as ligands for cisplatin become an interesting alternative since complexes can slow or block repair enzymes. This was already demonstrated for oxaliplatin (**3**) where both ammine groups of cisplatin are replaced by the bulky (1*R*,2*R*)-1,2-diaminocyclohexane [5].

To overcome resistance we synthesized [1,2-diamino-1,2-diarylethane]platinium(II) complexes. It could be shown, that the activity against cisplatin-resistance tumors depends on the nature and position of ring substituents and also on the ligand configuration [25]. Resuming this work, we developed 1,2-diamino-1-arylalkanes as neutral ligands for platinum(II) complexes [8]. These compounds are less voluminous than the 1,2-diamino-1,2-diarylethanes and should ensure a better accessibility of the platinum(II) atom to DNA. As a matter of fact, a higher antitumor activity could be demonstrated for [1,2-diamino-1-(4-fluorophenyl)propane]dichloroplatinum(II) complexes in comparison to [1,2-diamino-1,2-bis(4-fluorophenyl)ethane]dichloroplatinum(II) complexes.

For both complexes a clear diastereoselectivity was determined, which is the consequence of different spatial structures for (1*R*,2*S*)/(1*S*,2*R*)- and (1*S*,2*S*)/(1*R*,2*R*)-configurated complexes. In the (1*R*,2*S*)/(1*S*,2*R*)-series the aromatic ring is equatorially and either the second phenyl ring or the methyl group is axially oriented. The presence of an axial standing group close to DNA is energetically unfavourable and in opposition to the formation of a stable complex between DNA and Pt(II)-containing drugs. This statement has already been made regarding several complexes and has been confirmed by molecular modelling [2], X-ray analysis [24], ^{1}H NMR [24] and NOESY experiments [28] on the adducts obtained between nucleosides sequence and Pt(II) complexes. In contrast, the substituents at (1*S*,2*S*)/(1*R*,2*R*)-configurated 1,2-diaminoethanes are located in a stable equatorial position and allow a much better attachment to the DNA [8].

Nevertheless, the observation that the less hindered complex [1,2-diamino-1-(4-fluorophenyl)ethane]dichloroplatinum(II) (*R/S*)-**4F-phenyl/H-PtCl$_2$** was less active than (*RS/SR*)- and (*RR/SS*)-**4F-Ph/Me-PtCl$_2$** on the MCF-7 cell line indicates that spatial considerations are not the only factors related to the activity. Differences in transport rates of the compounds through the cell wall may also be involved in the different behaviors of the diastereoisomers. This was suggested by the observation that the amount of (*RR/SS*)-**4F-Ph/4F-Ph-PtCl$_2$** accumulated in the cytoplasm was much higher than that of (*RS/SR*)-**4F-Ph/4F-Ph-PtCl$_2$**, which could be the consequence of a stereospecific transport by an active pumping system [34, 35].

The differences in the antitumor activity of enantiomeric complexes were explained by the chirality of the DNA [23, 36, 37]. In the first step the enantiomers hydrolyzed with the same kinetics followed by interaction with nucleobases. The resulting diastereomeric monoadducts are supposed to react at different rates in the second reaction step, which is the binding of Pt to a neighboring nucleobase with loop formation. This process finds its expression in different inhibition kinetics concerning DNA synthesis and therefore tumor cell proliferation. A comparison of the results listed in Table 1 shows that the preference of one enantiomers could not be predicted. On the MCF-7 cell line (*SS*)-**4F-Ph/Me-PtCl$_2$** is more active than (*RR*)-**4F-Ph/Me-PtCl$_2$** while in the case of **Ph/Me-PtCl$_2$** the (*RR*)-enantiomer is more active. In contrast to this, **4F-Ph/4F-Ph-PtCl$_2$** shows no enantioselectivity.

Besides the interaction with chiral targets like DNA, differences in pharmacokinetics could also be responsible for unequal antitumor activity of enantiomeric platinum(II) complexes. Therefore, investigations on the cellular uptake and accumulation of enantiomeric pure [1,2-diamino-1-(4-fluorophenyl)propane]dichloroplatinum(II) complexes are in progress and will be part of a forthcoming paper.

Experimental part

Determination of pH was performed using a Mettler Delta 340 pH meter. ^{1}H and ^{13}C NMR spectra were taken on a Bruker Avance 300 MHz spectrometer with TMS as internal standard. IR analysis was performed with a Shimadzu IR-470 spectrophotometer. Melting points (uncorrected) were measured with a Mettler FP1 apparatus. All CC purifications were done using silicagel Kieselgel® 100 (Merck). TLC were performed on Kieselgel® 60 F_{254} plates (Merck). Mass spectra were recorded on a Thermo-Fisons VG Auto Spec (70 eV). Specific rotations were measured with a Perkin-Elmer 141 polarimeter.

*(2R)- or (2S)-N-carbethoxy-2-aminopropanoic acid ((R)-***8** *and (S)-***8***)*

(2*R*)- or (2*S*)-2-aminopropanoic acid **7** (1 g, 11.2 mmol) was dissolved in 11.2 mL of 1 N NaOH. Ethyl chloroformate (1.2 mL, 12.2 mmol) was added to the solution (15 °C) maintained at pH 9 by addition of 1 N NaOH. After stirring for 1 hour, the mixture was cooled to 0 °C and washed three times with diethyl ether (3 × 50 mL). The aqueous solution, saturated with NaCl, was acidified to pH 1 with 3 N HCl and extracted with CH_2Cl_2 (3 × 50 mL). The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. Colorless oil: 1.81 g. Yield: quantitative. ^{1}H NMR ($CDCl_3$): δ = 1.26 (t, 3 H, *J* = 7.25 Hz, –CH_2CH_3), 1.46 (d, 3 H, *J* = 7.25 Hz, CH_3CHN), 4.14 (q, 2 H, *J* = 7.25 Hz, –OCH_2–), 4.40 (m, 1 H, CH), 6.75 (b, 1 H, NH), 9.89 (b, 1 H, COOH). IR (film, cm^{-1}): 3305 (NH, OH), 2970 (CH), 1705 (C=O), 1524. α^D_{20} (c = 2, CH_3OH) = +3.4 ((*R*)-**8**) or –3.3 ((*S*)-**8**)).

*(2R)- or (2S)-N-carbethoxy-2-aminopropanoyl chloride ((R)-***9** *or (S)-***9***, not isolated) and (2R)- or (2S)-N-carbethoxy-2-amino-1-(4-fluorophenyl)propanone ((R)-***10** *and (S)-***10***)*

To a cold solution (0 °C, ice-bath) of (2*R*)- or (2*S*)-**8** (1.46 g, 9.1 mmol) in a mixture of 25 mL of CH_2Cl_2 and 0.05 mL of anh. DMF, oxalyl chloride (0.85 mL, 9.7 mmol) was added in one portion. After stirring for 2 hours, the temperature of the bath was decreased to –15 °C and fluorobenzene (42 mL, 450 mmol) and $AlCl_3$ (2.54 g, 20 mmol) were successively added. After stirring vigorously for 20 hours, crushed ice (20 g) and 12 N HCl (5 mL) were added, and the product was extracted three times with CH_2Cl_2 (3 × 50 mL). The organic layer was washed with a saturated $NaHCO_3$ solution (50 mL), water (50 mL) and dried over $MgSO_4$. After evaporation of the solvent, the oily residue (1.86 g) was purified by CC (mobile phase: $CHCl_3$). Yellowish solid: 0.83 g, mp. 68 °C. Yield: 39 %. ^{1}H NMR ($CDCl_3$): δ = 1.26 (t, 3 H, *J* = 7.13 Hz, CH_2CH_3), 1.41 (d, 3 H, *J* = 6.94 Hz, $CHNCH_3$), 4.13 (q, 2 H, *J* = 7.31 Hz, CH_2), 5.29 (m, 1 H, CHN), 5.67 (br, 1 H, NH), 7.2 (m, 2 H, H3′ H5′), 8.01 (m, 2H, H2′H6′). IR (KBr, 1 %, cm^{-1}) 3355 (NH), 2910, 1709 (C=O), 1595, 1524, 1219 (C–F), 841. α^D_{20} (c = 1, CH_3OH) = +2.7 ((*R*)-**10**) or –2.7 ((*S*)-**10**).

*(1S,2R)/(1R,2R)- or (1R,2S)/(1S,2S)-N-carbethoxy-2-amino-1-(4-fluorophenyl)propanol ((1S,2R)-***11 a***/(1R,2R)-***11 b***; (1R,2S)-***11 a***/(1S,2S)-***11 b***)*

To a solution of (*R*)- or (*S*)-**10** (20.17 g, 84 mmol) in CH_3OH (250 mL), $NaBH_4$ (4.89 g, 130 mmol) was added in small portions in order to maintain the temperature at 20 °C. Upon completion of the addition, the mixture was stirred at room temperature for 30 minutes. The solvent was evaporated under reduced pressure and 3 N HCl (100 mL) was added. The aqueous solution was extracted three times with $CHCl_3$ (3 × 50 mL). The organic layer, washed with brine, dried over $MgSO_4$ and filtered, was evaporated (20.3 g of yellowish oil that slowly crystallized). Yield: quantitative. Diastereoisomeric ratio (^{1}H NMR): *erythro : threo* 77 : 23. ^{1}H NMR ($CDCl_3$): *erythro* δ = 0.98 (d, 3 H, *J* = 6.94 Hz, CH_3CHN), 1.25 (t, 3 H, *J* = 6.94 Hz, CH_3CH_2), 3.99 (m, 1 H, CHN), 4.13 (q, *J* = 6.94 Hz, CH_2), 4.83 (2 H overlapped, NH and Ar-CH), 7.03 (m, 2 H, H3′H5′), 7.31 (m, 2 H, H2′H6′). *Threo* δ = 1.09 (d, 3 H, *J* = 6.94 Hz, CH_3CHN), 1.22 (t, 3 H, *J* = 6.94 Hz, CH_3CH_2), 3.91 (m, 1 H, CHN), 4.13 (q, *J* = 6.94 Hz, CH_2), 4.58 (d, 1 H, *J* = 5.48 Hz, Ar-CH), 4.83 (br, 1 H, NH), 7.03 (m, 2 H, H3′H5′), 7.31 (m, 2 H, H2′H6′). IR (KBr, 1 %, cm^{-1}) 3405 (NH + OH), 1683 (C=O), 1601, 1210 (C–F), 1059.

*(1S,2R)- or (1R,2S)-2-amino-1-(4-fluorophenyl)propanol ((1S,2R)-***12 a** *or (1R,2S)-***12 a***)*

(1*S*,2*R*)-**11 a**/(1*R*,2*R*)-**11 b** or (1*R*,2*S*)-**11 a**/(1*S*,2*S*)-**11 b** (14.92 g, 62 mmol) and KOH (14 g, 248 mmol) in 150 mL of CH_3OH/H_2O (3 : 1) were heated under reflux during 12 hours. After cooling and evaporation of CH_3OH, the suspension was extracted three times with $CHCl_3$ (3 × 100 mL). The organic phase was washed with brine, dried over $MgSO_4$ and evaporated. Yellowish oil: 10.46 g. Yield: quantiative. Diastereomeric ratio (^{1}H NMR): 77 : 23.

To a stirred solution of the diastereoisomers mixture **12 a**/**12 b** (10.46 g, 62 mmol) in acetone : water (200 : 200 mL), (2*S*,3*S*)- or (2*R*,3*R*)-dibenzoyltartaric acid (22.2 g, 62 mmol) was added. After elimination of the major portion of acetone with an air flow, the (2*S*,3*S*)-dibenzoyltartrate of (1*S*,2*R*)-**12 a** or the (2*R*,3*R*)-dibenzoyltartrate of (1*R*,2*S*)-**12 a** precipitated. After several recrystallisations in acetone : water (1 : 1), **12 a** isomers were obtained with a purity of 99 %. (checked by ^{1}H NMR in [D6]-DMSO). It was suspended in water (200 mL) and NaOH 30 % was added (pH 12). After extraction three times with $CHCl_3$ (3 × 100 mL), the organic layer was washed with brine, dried over $MgSO_4$ and evaporated (6.8 g of yellowish oil that slowly crystallized, mp.: 49 °C). Yield: 84 %. The optical purity was checked by ^{1}H NMR on the base using (1*R*)-myrtenal as derivatization agent. ee (%) = 98. ^{1}H NMR ([D6]-DMSO: *erythro*-**12 a** δ = 0.85 (d, 3 H, *J* = 6.62 Hz, CH_3), 2.89 (m, 1 H, CHN), 4.33 (d, 1 H, *J* = 5.15 Hz, Ar-CH), 7.12 (m, 2 H, H3′H5′), 7.33 (m, 2 H, H2′H6′). *Threo*-**12 b** δ = 0.77 (d, 3 H, *J* = 5.88 Hz, CH_3), 2.77 (m, 1 H, CHN), 4.13 (d, 1 H, *J* = 6.62 Hz, Ar-CH), 7.12 (m, 2 H, H3′H5′), 7.33 (m, 2 H, H2′H6′). IR (**12 a** base) (KBr, 1 %, cm^{-1}): 3330 (NH, OH), 2950 (CH), 1590, 1497, 1215 (C–F), 839. α^D_{20} (base, c = 1, CH_3OH) = +41.7 ((1*S*,2*R*)-**12 a**) or –41.2 (1*R*,2*S*)-**12 a**).

*(1S,2R)- or (1R,2S)-N,O-diacetyl-2-amino-1-(4-fluorophenyl)-propanol ((1S,2R)-***14 a** *or (1R,2S)-***14 a***)*

Acetic anhydride (7 mL, 75 mmol) was slowly added to (1*S*,2*R*)- or (1*R*,2*S*)-**12 a** (5.02 g, 30 mmol) dissolved in a mixture of anh. toluene and anh. pyridine (20 : 8 mL). The solution was stirred at room temperature overnight and then refluxed for 15 minutes. After cooling, water was added and $NaHCO_3$ in small portions until cessation of reaction. The organic phase washed with 3 N HCl (50 mL), was dried over $MgSO_4$ and evaporated to give 7.51 g of yellow viscous oil. Yield: quantitative. ^{1}H NMR ($CDCl_3$): δ = 1.07 (d, 3 H, *J* = 6.58 Hz, CH_3CHN), 1.94 (s, 3 H, CH_3 amide), 2.14 (s, 3 H, CH_3 ester), 4.42 (m, 1 H, CHN), 5.81 (d, 1 H, *J* = 4.02 Hz, Ar-CH), 7.04 (m, 2 H, H3′H5′), 7.29 (m, 2 H, H2′H6′). IR (film, cm^{-1}): 3275, 3050, 2920, 1740 (C=O ester), 1642 (C=O amide), 1533, 1502, 1441, 1362, 1224 (C–F), 1028, 827.

*(1S,2R)- or (1R,2S)-N-acetyl-2-amino-1-(4-fluorophenyl)-1-phthalimidopropane ((1S,2R)- or (1R,2S)-***15***)*

In a round-bottomed flask equipped with a condenser and a magnetic stirrer, a mixture of (1*S*,2*R*)- or (1*R*,2*S*)-**14 a** (4.1 g, 16 mmol) and phthalimide (2.38 g, 16 mmol) was heated at 220 °C. After 4 hours, the acetic acid formed was eliminated under vacuum (150 mm Hg). The brown residue was purified by

CC (mobile phase: $CHCl_3$: CH_3OH (9 : 1)). 2.62 g of brownish solid, mp. 195 °C. Yield: 81 %. ^1H NMR ($CDCl_3$): δ = 1.25 (d, 3 H, *J* = 6.21 Hz, C*H*$_3$CHN), 1.80 (s, 3 H, CH_3 amide), 5.17 (d, 1 H, *J* = 10.97 Hz, Ar-CH), 5.32 (bd, 1 H, *J* = 9.13 Hz, NH), 5.51 (m, 1 H, CHN), 6.99 (m, 2 H, H3′H5′), 7.59 (m, 2 H, H2′H6′), 7.70 (m, 2 H, H5′′H6′′ phthalimide), 7.82 (m, 2 H, H4′′H7′′ phthalimide). IR (KBr, 1 %, cm^{-1}): 3275, 3055, 2915, 1712 (C=O phthalimide), 1650 (C=O amide), 1539, 1439, 1419, 1375, 1226 (C–F), 837, 780, 717.

*(1S,2R)- or (1R,2S)-1,2-diamino-1-(4-fluorophenyl)propane ((1S,2R)- or (1R,2S)-***16***)*

(1*S*,2*R*)- or (1*R*,2*S*)-**15** (2.62 g, 11 mmol) was refluxed in 6 N HCl (200 mL) for 48 hours. After cooling, the suspension was filtered and washed with $CHCl_3$ (50 mL). The aqueous solution neutralized with Na_2CO_3 and alkalized with NaOH 30 % (pH 12) was extracted three times with $CHCl_3$ (3 × 100 mL). After washing with brine, the organic layer was dried over $MgSO_4$ and evaporated. Upon addition of ethereal HCl (20 mL), the dihydrochloride of **16** precipitated, was sucked off and air dried. Further purification was achieved by recrystallization from ethanol/water (10 : 1). 1.15 g of white solid, mp: 285 °C with some dec. Yield: 51 %. The optical purity was checked by ^1H-NMR on the base using (1*R*)-myrtenal as derivatization agent. ee (%) = 99. ^1H NMR (hydrochloride) ([D6]-DMSO): *erythro* δ = 1.36 (d, 3 H, *J* = 6.42 Hz, CH_3), 3.76 (m, 1 H, CHN), 4.68 (d, 1 H, *J* = 5.5 Hz, Ar-CH), 7.35 (m, 2 H, H3′H5′), 7.71 (m, 2 H, H2′H6′), 8.93 (bs, 6 H, NH_3^+). ^{13}C NMR (hydrochloride) ([D6]-DMSO): *erythro* δ = 14.44 (CH_3), 49.46 (CHN), 55.25 (Ar-CH), 115.51 (C3′C5′, J_{C-F} = 22 Hz), 129.74 (C1′, J_{C-F} = 4 Hz), 130.08 (C2′C6′, J_{C-F} = 9 Hz), 162.13 (C4′, J_{C-F} = 246 Hz). IR (base) (film): 3345 (NH), 3160 (NH),1600 ,1506, 1446, 1368, 1221 (C–F), 831. MS (base): *m/z* (%) = 168 (40) [M^+], 152 (91), 136 (7), 124 (100), 97 (26), 77 (14). α_{20}^D (hydrochloride, c = 1, CH_3OH) = –28.9 (1*S*,2*R*)-**16** or +28.7 (1*R*,2*S*)-**16**.

*(1R,2R)/(1S,2R)- or (1S,2S)/(1R,2S)-2-amino-1-chloro-1-(4-fluorophenyl)propane hydrochloride ((1R,2R)-***17 a***/(1S,2R)-***17 b*** *or (1S,2S)-***17 a***/(1R,2S)-***17 b***)*

To cold stirred $SOCl_2$ (ice-bath) (6.2 mL, 85 mmol), (1*S*,2*R*)- or (1*R*,2*S*)-**12 a** (2.06 g, 12 mmol) was added in small portions. The mixture was allowed to stand at room temperature for 1 hour and was then refluxed 30 minutes. The excess of $SOCl_2$ was evaporated under reduced pressure to give 7.96 g of a brown solid. Yield: quantitative. Diastereoisomeric ratio (^1H NMR): *erythro : threo* 11 : 89. ^1H NMR (hydrochloride) ([D6]-DMSO): *erythro* δ = 1.23 (d, 3 H, *J* = 6.88 Hz, CH_3), 3.76 (m, 1 H, CHN), 5.61 (d, 1 H, *J* = 4.59 Hz, Ar-CH), 7.27 (m, 2 H, H3′H5′), 7.56 (m, 2 H, H2′H6′), 8.49 (bs, 3 H, NH_3^+). *Threo* δ = 1.06 (d, 3 H, *J* = 6.88 Hz, CH_3), 3.88 (m, 1 H, CHN), 5.29 (d, 1 H, *J* = 9.18 Hz, Ar-CH), 7.27 (m, 2 H, H3′H5′), 7.56 (m, 2 H, H2′H6′), 8.61 (bs, 3 H, NH_3^+). IR (hydrochloride) (KBr, 1 %, cm^{-1}): 2980 (NH_3^+), 2850, 1600, 1513, 1455, 1383, 1230 (C–F), 834.

(2R,3S)/(2S,3S)- or (2S,3R)/(2R,3R)-2-(4-fluorophenyl)-3-methylaziridine (**18 a**–**b**)

To a solution of (1*S*,2*S*)-**17 a**/(1*R*,2*S*)-**17 b** or (1*R*,2*R*)-**17 a**/(1*S*,2*R*)-**17 b** (8.71 g, 39 mmol) in CH_3OH (30 mL), a solution of NH_3 (25 % (*m/V*) in H_2O) (45 mL) was added. After 20 hours at room temperature, the reaction was complete (control by TLC, mobile phase $CHCl_3/CH_3OH/NH_4OH$ (25 %) 9 : 1 : 0.1) and an aliquot was extracted with $CHCl_3$ for analysis. The aziridines **18 a**–**b** were not isolated and the following step was undertaken directly on the solution. Diastereoisomeric ratio (^1H NMR): *cis : trans* = 87 : 13. ^1H NMR ($CDCl_3$): *cis* δ = 0.89 (d, 3 H, *J* = 5.85 Hz), 1.26 (br, 1 H, NH), 2.40 (m, 1 H, CHN), 3.22 (d, 1 H, *J* = 6.58 Hz, Ar-CH), 7.00 (m, 2 H, H3′H5′), 7.30 (m, 2 H, H2′H6′). *Trans* δ = 1.26 (bs, 1 H, NH), 1.33 (d, 3 H, *J* = 5.48 Hz, CH_3), 2.05 (m, 1 H, CHN), 2.66 (d, 1 H, *J* = 2.93 Hz, Ar-CH), 7.00 (m, 2 H, H3′H5′), 7.16 (m, 2 H, H2′H6′). IR (film, cm^{-1}): 3235 (NH), 2975, 1600, 1507, 1442, 1217 (C-F), 1151, 841.

*(1S,2S)- or (1R,2R)-1,2-diamino-1-(4-fluorophenyl)propane ((1S,2S)-***19** *or (1R,2R)-***19***)*

To the solution of (2*R*,3*S*)-**18 a**/(2*S*,3*S*)-**18 b** or (2*S*,2*R*)-**18 a**/(2*R*,3*R*)-**18 b** from the previous step, NH_4Cl (12.04 g, 225 mmol) and a solution of NH_3 (25 % *m/V* in H_2O) (30 mL) were added. After heating the reaction mixture to 60 °C for 48 hours, CH_3OH was evaporated. Water (50 mL) and a sufficient quantity of NaOH 30 % (pH 12) were added. The mixture was extracted three times with $CHCl_3$ (3 × 100 mL). The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. 5.17 g of brown oil. Yield for two steps from **17**: 79 %. Diastereoisomeric ratio (^1H NMR): *erythro : threo* 30 : 70. Enantiomeric ratio for *threo* isomers (^1H NMR with (1*R*)-myrtenal): 66 : 34. The crude mixture of (1*S*,2*S*)-**19**/(1*R*,2*R*)-**19**/(1*R*,2*S*)-**16** or (1*R*,2*R*)-**19**/(1*S*,2*S*)-**19**/(1*S*,2*R*)-**16** (46 : 24 : 30) (32 mmol) was dissolved in 60 mL of CH_3OH and (2*R*,3*R*)- or (2*S*,3*S*)-dibenzoyltartaric acid (12.81 g, 36 mmol) was added. Only the (1*S*,2*S*)/(1*R*,2*R*)- or (1*R*,2*R*)/(1*S*/2*S*)-isomer precipitated. After 3 recrystallizations of the mixture of salts (9.02 g) in ethanol, 4.79 g of the (2*R*,3*R*)-dibenzoyltartaric acid salt of (1*S*,2*S*)-**19** or (2*S*,3*S*)-dibenzoyltartaric acid salt of the (1*R*,2*R*)-**19** were obtained. The salts were suspended respectively in water (200 mL) and NaOH 30 % was added (pH 12). After extraction three times with $CHCl_3$ (3 × 100 mL), the organic layer was washed with brine, dried over $MgSO_4$ and evaporated to yield the free base. Upon addition of ethereal HCl (20 mL), the dihydrochloride could be obtained, which was sucked off and air dried. The optical purity was checked by ^1H NMR on the base using (1*R*)-myrtenal as derivatization agent. ee (%) for the two isomers = 98. ^1H NMR (hydrochloride) ([D6]-DMSO): δ = 1.10 (d, 3 H, *J* = 6.6 Hz, CH_3), 3.95 (m, 1 H, CHN), 4.8 (d, 1 H, *J* = 6.42 Hz, Ar-CH), 7.34 (m, 2 H, H3′H5′), 7.73 (m, 2 H, H2′H6′), 9.12 (bs, 6 H, NH_3^+). ^{13}C NMR (hydrochloride) ([D6]-DMSO): δ = 14.51 (CH_3), 48.4 (CHN), 55.18 (Ar-CH), 115.51 (C3′C5′, J_{C-F} = 22 Hz), 129.19 (C1′, J_{C-F} = 3 Hz), 130.59 (C2′C6′, J_{C-F} = 9 Hz), 162.17 (C4′, J_{C-F} = 246 Hz). IR (base) (film): 3350 (NH), 3305 (NH), 1600, 1505, 1221 (C–F), 829. MS (base): *m/z* (%) = 168 (40) [M^+], 152 (91), 136 (7), 124 (100), 97 (26), 77 (14). α_{20}^D (hydrochloride, c = 1, CH_3OH) = +18.9 ((1*S*,2*S*)-**19**) or –18.6 ((1*R*,2*R*)-**19**).

Optical purity determination of amino alcohols and diamines with (1R)-myrtenal: general procedure

To a solution of the free base (amino alcohol or diamine) in $CDCl_3$, a solution of (1*R*)-myrtenal (1 or 2 equiv.) in $CDCl_3$ was added. The mixture was left at room temperature for 2 days to give mono- and diimines. The sample was analyzed by ^1H NMR (Table 2).

Synthesis of [1,2-diamino-1-(4-fluorophenyl)propane]dichloroplatinum(II) complexes

To a solution of the respective 1,2-diamino-1-(4-fluorophenyl)-propane (1 mmol) in 15 mL of water, K_2PtCl_4 (1 mmol) dissolved in 8 mL water was added after the pH was adjusted with 0.5 N NaOH to 6.5 to 7.5. The reaction mixture was stirred in the dark for 24 hours while the pH was adjusted several times. Subsequently, it was acidified with 1N HCl and the yellow precipitate was sucked off and dried over P_2O_5 *in vacuo.*

[(1R,2R)- or (1S,2S)-1,2-diamino-1-(4-fluorophenyl)propane]-dichloroplatinum(II)

^{1}H NMR ([D7]-DMF): δ = 1.06 (d, 3 H, *J* = 6.5 Hz, CH_3), protected by solvent (2 H, CH-alkyl), 3.95 (m, 1 H, Ar-CH), 5.27 (br, 1H, NH), 5.52 (br, 1 H, NH), 5.90 (br, 1 H, NH), 6.09 (br, 1 H, NH), 7.20–7.29 (m, 2 H, H3′H5′), 7.65–7.72 (m, 2 H, H2′H6′).

(*RR*)-**4F-Ph/Me-PtCl$_2$**: yield 99%. **$C_9H_{13}FI_2N_2Pt$** (616.88): calc. C 24.97 H 3.03 N 6.47 found C 24.95 H 3.14 N 6.70.

(*SS*)-**4F-Ph/Me-PtCl$_2$:** yield 80 %, **$C_9H_{13}FI_2N_2Pt$** (616.88): calc. C 24.97 H 3.03 N 6.47 found C 25.10 H 3.17 N 6.53.

[(1R,2S)- or (1S,2R)-1,2-diamino-1-(4-fluorophenyl)propane]-dichloroplatinum(II)

^{1}H NMR ([D7]-DMF): δ = 1.18 (d, 3 H, *J* = 6.8 Hz, CH_3), 3.30 (2 H, CH-alkyl), 4.39 (m, 1 H, Ar-CH), 5.21 (br, 1 H, NH), 5.73 (br, 1 H, NH), 5.82 (br, 1 H, NH), 6.06 (br, 1 H, NH), 7.20–7.31 (m, 2 H, H3′H5′), 7.86–7.94 (m, 2 H, H2′H6′).

(RS)-**4F-Ph/Me-PtCl$_2$:** yield 61 %, **$C_9H_{13}FI_2N_2Pt$** (616.88): calc. C 24.97 H 3.03 N 6.47 found C 25.10 H 3.11 N 6.65.

(SR)-**4F-Ph/Me-PtCl$_2$:** yield 92 %, **$C_9H_{13}FI_2N_2Pt$** (616.88): calc. C 24.97 H 3.03 N 6.47 found C 24.90 H 3.26 N 6.31.

Biological methods

Cytotoxicity studies on the MCF-7 cell line

The human MCF-7 breast cancer cell line was obtained from the American Type Culture Collection (ATCC, Rockville, Md.; USA). Cell line banking and quality control were performed according to the seed stock concept reviewed by Hay [38]. The MCF-7 cells were maintained in L-glutamine containing Eagle's MEM (Sigma München, Germany), supplemented with $NaHCO_3$ (2.2 g/L) sodium pyruvate (110 mg/L), gentamycin (50 mg/L; Sebio Walchsing, Germany), and 10 % fetal calf serum (FCS; Gibco Eggenheim, Germany) using 75 cm^2 culture flasks (Falcon Plastics 3023) in a water-saturated atmosphere (95 % air/5 % CO_2) at 37 °C. The cell line was weekly passaged after treatment with trypsin (0.05 %)/ethylenediaminetetraacetic acid (0.02 %; EDTA; Boehringer, Mannheim, Germany). Mycoplasma contamination was routinely monitored, and only mycoplasma-free cultures were used.

In vitro chemosensitivity assay

In vitro testing of the platinum complexes for antitumor activity was carried out on exponentially dividing human breast cancer cells according to a previously published microtiter assay [39, 40]. Briefly, in 96-well microtiter plates (Costar), 100 µL of a cell suspension at 500 cells/mL culture medium were plated into each well and incubated at 37 °C for 2–3 days in a humidified atmosphere (5 % CO_2). By addition of an adequate volume of a stock solution of the respective compound (solvent: DMF) to the medium the desired test concentration was obtained (max. content of DMF in the medium: 1 ppm). For each test concentration and for the control, which contained the corresponding amount of DMF, 16 wells were used. After the proper incubation time the medium was removed, the cells were fixed with a glutardialdehyde solution and stored at 4 °C. Cell biomass was determined by a crystal violet staining technique as described in ref. [37, 38]. The effectiveness of the complexes is expressed as corrected *T/C* values according to the following equations: Cytostatic effect: T/C_{corr} [%] = $[(T - C_o)/(C - C_o)] \times 100$, where *T* (test) and *C* (control) are the optical densities at 578 nm of the crystal violet extract of the cell lawn in the wells (i.e. the chromatin-bound crystal violet extracted with ethanol 70 %), and C_o is the density of the cell extract immediately before treatment. Cytocidal effect: T [%] = $[(T - C_o)/C_o] \times 100$. For automatic estimation of the optical density of the crystal violet extract in the wells a Microplate EL 309 Autoreader was used.

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