Synthesis, Crystal Structure, Antitumor Activity, and DNA-Binding Properties of the New Active Platinum Compound (Bis(*N*-methylimidazol-2-yl)carbinol)dichloroplatinum(II), Lacking a NH Moiety, and of the Inactive Analog Dichloro(N^1,$N^{1'}$-dimethyl-2,2′-biimidazole)platinum(II)

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To obtain insight into the structure−activity relationships of new antitumor active platinum compounds the X-ray structure of the antitumor active Pt compound [Pt(bmic)Cl_2] (bmic = bis-(*N*-methylimidazol-2-yl)carbinol) (**1**) and its interaction with short DNA fragments has been investigated using NMR spectroscopy. For comparison also the structurally related compound [Pt(bmi)Cl_2] (bmi = N^1,$N^{1'}$-dimethyl-2,2′-biimidazole) (**2**), which is not antitumor active, has been studied. The structure of the compound [Pt(bmic)Cl_2] (**1**) was characterized by single-crystal X-ray structure determination. Compound **1** crystallizes in the monoclinic space group $P2_1/n$, with a = 10.055(3) Å, b = 11.802(3) Å, c = 10.620(3) Å, β = 103.78(2)°, V = 1224.0(6) Å^3 and Z = 4. Convergence was reached at wR2 = 0.1148 (all data) and R1 = 0.0476 ($I > 2$ (I)) for 2433 independent reflections and 156 adjustable parameters. The platinum atom is coordinated by two nitrogen and two chlorine atoms, resulting in a square planar PtN_2Cl_2 coordination sphere. The two best least-squares planes through the two imidazole rings of the bmic ligand show a dihedral angle of 30.6°. The *in vitro* and *in vivo* antitumor activity of **1** is significant whereas for compound **2** no antitumor activity could be detected. In P388 mice leukemia an increase of lifespan of 56% was found for complex **1**. The antitumor active Pt compound [Pt(bmic)Cl_2] binds to G bases in a similar fashion as cisplatin with a clear preference for N7. In reaction with d(GpG) two stereoisomers are formed, due to the unsymmetric bmic complex and the chiral d(GpG) molecule. Stereoisomer A, *i.e.* the isomer with the OH group of the bmic and the O6 of the G bases oriented on the same side of the Pt−N_4 plane, is preferentially formed. Modeling studies suggest that this preference is due to the presence of H bonds from the OH of the bmic moiety toward the O6 of the G bases. The presence of many conformers, present in solution, could also be due to these H bonds. For the inactive complex [Pt(bmi)Cl_2] only one GG-*N7*,*N7* chelate is observed. Differences in reactivity toward G bases were also detected for the two platinum complexes. The inactive bmi complex proves to be the most reactive one, whereas the antitumor active bmic compound is less reactive. Thus both structural and kinetic properties may explain the different biological properties of these new platinum compounds.

Introduction

Rosenberg's discovery of the cell-division inhibiting activity exerted by cisplatin (*cis*-diamminedichloroplatinum(II)) toward *E. coli* bacteria has initiated numerous fundamental studies of the coordination chemistry of platinum compounds towards nucleobases.[1] The interest in nucleobases arises from the widespread acceptance that DNA is the molecular target of this antitumor drug; in particular the d(GpG) intrastrand cross-link is known to be preferentially formed after reaction of cisplatin with DNA.[2] Structure-activity relationships (SAR) have been defined for chemotherapeutic agents based on cisplatin. They claim the necessity of the [*cis*-PtX_2(amine)$_2$] structure where X should be an anion with intermediate binding strength (such as Cl^-, SO_4^{2-} or carboxylic acid residues). The amine ligand (the nonleaving group) should possess at least one NH moiety which is thought to be necessary for H-bonding interactions toward DNA.[3] The role such NH groups play is not completely understood: both thermodynamic stability[4] and kinetics of complexation[5] have been employed to explain the strong preference of Pt(II) for adjacent G-N7 sites.

The requirement of a NH moiety for antitumor activity has become a subject of debate since, recently, an increasing number of platinum compounds have appeared in literature which violate this rule. Examples of platinum complexes containing no NH moiety but displaying significant antitumor activity are complexes with bipyridyl crown ethers,[6] platinum organoamides containing pyridine ligands,[7] and complexes containing bis-(imidazole) ligands.[8]

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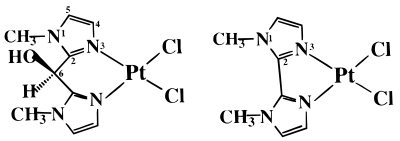

(1) (2)

Figure 1. Schematic representation of [Pt(bmic)Cl_2] (**1**) and [Pt(bmi)Cl_2] (**2**).

Previous work on the complexes containing bisimidazole ligands has shown significant cytostatic activity and reduced toxicity for the compound (bis(*N*-methylimidazol-2-yl)carbinol)-dichloroplatinum(II) ([Pt(bmic)Cl_2], see Figure 1). An analog of this complex is [Pt(bmik)Cl_2] (bmik = bis(*N*-methylimidazol-2-yl) ketone) which has been reacted with model nucleobases like 9-methylguanine (9MeGH).[9] The X-ray structures show that this complex can bind to nucleobases in a similar fashion as cisplatin, *i.e.* coordination of platinum at the N7 site of 9-MeGH is observed. The G base has no contact with the bmik ligand, contrary to cisplatin which does show H-bonding interactions from the ammine ligand toward O6.[4a]

To understand the mechanism of action of this new group of Pt complexes the interaction of two representative complexes, [Pt(bmic)Cl_2] and [Pt(bmi)Cl_2] (bmi = N^1,$N^{1'}$-dimethyl-2,2′-biimidazole) with (oligo)nucleotides containing a G base (such as 5′-GMP and d(GpG)) has been studied using NMR spectroscopy. As [Pt(bmic)Cl_2] is antitumor active and [Pt(bmi)Cl_2] is not, it is interesting to compare the DNA-binding properties of these compounds. Both structural and kinetical aspects will be compared in order to establish SAR's for this new series of antitumor active compounds. In addition, the X-ray structure of [Pt(bmic)Cl_2] is described in detail.

Experimental Section

Starting Materials. 5′-GMP was obtained from Sigma (Na-salt). d(GpG) was synthesized via an improved phosphotriester method and used as its sodium salt.[10]

Preparation of Compounds. The starting materials [Pt(bmic)Cl_2] (**1**) and [Pt(bmi)Cl_2] (**2**), were prepared from K_2[$PtCl_4$] by the following method: A solution of 0.415 g (1 mmol) of K_2[$PtCl_4$] in 25 mL of water was heated to 40 °C under constant stirring and dropwise addition of either 0.192 g of bis(1-methylimidazole-2-yl)carbinol (prepared by the method of Tang *et al.*[11]) or 0.162 g of 1,1′-dimethyl-2,2′-biimidazole (prepared by the method of Winder *et al.*[12]) in 25 mL of water. The mixtures were stirred for two additional hours and then cooled to 0 °C. The resulting [Pt(bmic)Cl_2] and [Pt(bmi)Cl_2] were removed by filtration and washed three times with 20 mL of water. The yield was 0.300 g for compound **1** (70%) and 0.321 g for compound **2** (75%). The compounds [Pt(bmi)$(NO_3)_2$] and [Pt(bmic)$(NO_3)_2$] were prepared by the following method: To a suspension of either 0.229 g of **1** (0.5 mmol) or 0.214 g of **2** (0.5 mmol) in 10 mL water was added 0.167 g of $AgNO_3$ (0.98 mmol). After the suspension had been stirred for 2 h at 50 °C, the solution was cooled to roomtemperature. The resulting AgCl precipitate was removed by centrifugation and the filtrate was lyophilized overnight.

Table 1. Crystal Data and Structure Refinement for **1**

formula	$PtC_9H_{12}N_4OCl_2$
fw	458.22
system	monoclinic
space group	$P2_1/n$
a, Å	10.055(3)
b, Å	11.802(3)
c, Å	10.620(3)
β, deg	103.78(2)
V, Å^3	1224.0(6)
Z	4
T, K	150
d_{calc}, g cm^{-3}	2.487
cryst size, mm	0.22 × 0.18 × 0.08
μ, mm^{-1}	11.888
abs cor	ψ-scan
transm factor	0.07−0.39
Index ranges	$0 \le h \le 17$, $0 \le k \le 16$, $-21 \le l \le 21$
θ range, deg	2.51−27.06
radiation (λ, Å)	Mo Kα (0.710 73)
no. of reflns collcd	2555
no. of indep reflns	2433
R_{int}[a]	0.0605
no. of obs reflns [I>2σ(I)]	1753
GOOF	1.009
R[b]	0.0476
R_w[c]	0.1148
a[d]	0.0539
b[d]	5.0449

[a] $R_{int} = \sum|F_o^2 - F_o^2(\text{mean})|/\sum[F_o^2]$. [b] $R = \sum||F_o| - |F_c||/\sum|F_o|$. [c] $R_w = [\sum w(F_o^2 - F_c^2)^2/\sum w(F_o^2)^2]^{1/2}$. [d] $w = 1/[\sigma^2(F_o^2) + (aP)^2 + bP]$ where $P = (\max(F_o^2 \text{ or } 0) + 2F_c^2)/3$.

Crystallization. Crystallization of [Pt(bmic)Cl_2] was achived by means of liquid diffusion. Aqueous solutions of the ligand bmic (0.02 M) and of K_2[$PtCl_4$] (0.02 M) were filled into 2 communicating tubes which were separated by a dialysis membrane. After 3 days yellow crystals appear, which were suitable for X-ray structure analysis.

Collection and Reduction of X-ray Data. Single-crystal X-ray diffraction data were measured on a Syntex $P2_1$ four-circle diffractometer using graphite-monochromated Mo Kα radiation. Unit cell parameters were obtained from a least-squares fit of 22 reflections of high 2θ angle. The details of the data collection are given in Table 1. Two standard reflections were measured after every 98 measurements during the data collection. Lorentz and polarization corrections were applied to the data. Empirical absorption corrections based on ψ-scan data were also applied. Intensity data for several reflections were recorded during a full turn around of the diffraction vectors, and relative transmissions were calculated.

Structure Solution and Refinement. The structure was solved by Patterson and Fourier methods[13] and refined on F^2 with anisotropic thermal parameters for all non-hydrogen atoms.[14] The complex crystallizes in the monoclinic crystal system and the structure was solved in space group $P2_1/n$. Further details of the structure solution and refinement are given in Table 1 (see Supporting Information for F_o and F_c). Neutral-atom scattering factors, anomalous dispersion corrections for all non-hydrogen atoms and hydrogen atom scattering factors were obtained as described previously.[15] The positions of all hydrogen atoms were placed at calculated positions (d(C−H_{arom}) = 0.95 Å, D(O−H) = 0.84 Å, d(C−H_{methyl}) = 0.98 Å, d(C−H_{alif}) = 0.95 Å) and constrained to "ride" on the carbon atoms to which they are attached. The isotropic thermal parameters for the aromatic hydrogen atoms and for the methyl protons were refined with 1.2 and 1.5 times the U_{eq} value of the corresponding carbon, respectively.

NMR Spectroscopy. Spectra were recorded on a Bruker WM 300 spectrometer equipped with a variable-temperature unit. The lyophi-

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lized products were dissolved in D_2O (99.95%, Merck). ^1H NMR data were collected at various temperature with TMA (tetramethyl ammonium nitrate, 3.18 ppm downfield from TMS) used as an internal reference.[16] ^{31}P NMR spectra were obtained at 297 K and referenced to H_3PO_4 (85 %). The pH dependence of the chemical shift of several nuclei was monitored by adding trace amounts of DCl and NaOD (0.1 and 1.0 M). The pH has not been corrected for deuterium isotope effects.[17] COSY spectra were measured according to standard procedures.[18]

Reactions. The (oligo)nucleotide (*i.e.* 1 or 2 equiv of 5′-GMP or 1 equiv of d(GpG)) was dissolved in deionized water at a concentration of 5×10^{-5} M. One equivalent of the platinum complex (*i.e.* [Pt(bmi)Cl_2], [Pt(bmi)$(NO_3)_2$], [Pt(bmic)Cl_2], or [Pt(bmic)$(NO_3)_2$]) was readily dissolved and added to the nucleotide solution. The pH was adjusted to 5.1 by adding a small amount of NaOH (0.1 M) and the solution was kept in the dark at 310 K for 7 days. After removal of the solvent using rotary evaporation, the reaction products were dissolved in 1.0 mL of D_2O and lyophilized. The reactions were also carried out in D_2O at 310 K and followed by ^1H NMR as a function of time (5 mM). For these reactions the chloride derivatives could not be used because of their insolubility at 5 mM conditions. The amounts of product (%) were measured by integration of the H1′ proton signals of the G base and the H4 protons of the imidazole moiety (experimental error 5%).

Computational Methodology. All of the modeling studies were conducted with the Quanta/CHARMm software (Molecular Simulations, version 3.3/22.0). Force field parameters for the metal-centered interactions were based on the work of Kozelka[19] and Hambley[20] since these parameters are not included in CHARMm 22.0. The starting structures were derived from the X-ray coordinates of [Pt(bmic)Cl_2] and the molecular editor routine of Quanta. Initially, solvent molecules were not explicitly included in the calculations, but the effect of the solvent was approximated by multiplying the electrostatic energy term by a $1/r$ screening function. The nonbonded interactions were switched off between 9.5 and 10.5 Å, with a cutoff distance of 11.5 Å using a switching function for the van der Waals interactions, a shift function for the electrostatic interactions, and a switching function applied between 4.0 and 7.5 Å for hydrogen bonds (with a cutoff distance of 7.0 Å).[21] All starting structures were fully minimized. Next, the structure was solvated with water molecules (TIP3P water model, 10 Å shell)[22] using the SOLVATE10.STR routine and further minimized using a dielectric constant of unity.

***In Vitro* and *in Vivo* Antitumor Activities of [Pt(bmic)Cl_2] and [Pt(bmi)Cl_2].** The pharmacological studies were carried out in the laboratories of ASTA Medica (Frankfurt am Main, Germany). For the *in vivo* studies six mice were used for each Pt compound (six animals constituted also the control group). The mice were inoculated intraperitoneally (ip) with a defined number of tumor cells. The mice perished after 30 days. To a similar group of mice (group 2) the Pt compounds were also injected ip. In the first group series one dosis of 316 mg/kg bodyweight was injected. Group 2 received four injections (each 100 mg/kg bodyweight) within 4 days. A detailed description of the tumor lines and the treatment is described elsewhere.[23] Cytolytic and cytostatic effects of [Pt(bmic)Cl_2] were determined *in vitro* in L1210 leukemia of mice. The IC_{90} value, *i.e.* the drug concentration (in μg/μL) at which 90 % of cell growth is inhibited, was obtained. No *in vitro* studies were carried out with [Pt(bmi)Cl_2], due to its very poor water solubility.

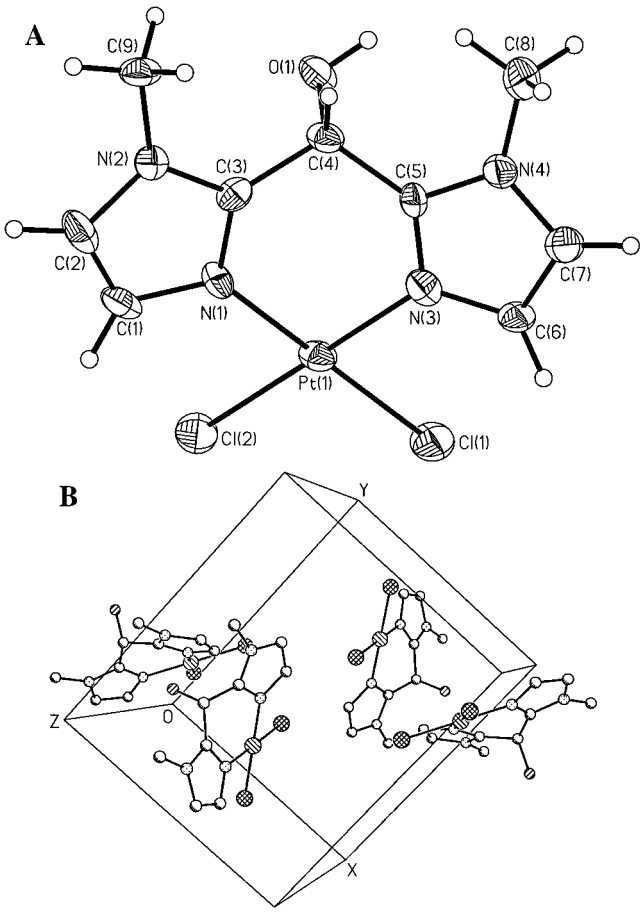

Figure 2. (A) Molecular structure and labeling of **1**. Ellipsoids are drawn at the 50% probability level. (B) Unit cell of **1**.

Results

X-ray Structure of [Pt(bmic)Cl_2]. The unit cell of **1** consists of four neutral complex molecules. The asymmetric unit contains one molecule. The platinum is coordinated besides the two nitrogen atoms of the chelating bidentate ligand (bmic) by two chlorine atoms. The coordination sphere of the platinum is square-planar. The deviation of the platinum from the best PtN_2Cl_2 plane is 0.003 Å. The average Pt−Cl distance is 2.302 Å (Pt(1)−Cl(1) 2.293(3) Å; Pt(1)−Cl(2) 2.309(3) Å). In the coordination sphere all angles are near the idealized value of 90°. The largest deviation appears in the angle N(1)−Pt−N(3) with 89.4(4)°. In the imidazole groups no unusual bond lengths or angles appear. The angles at the sp^3-hybridized bridging C(4) atom should be about 109°, but, due to coordination to platinum, the angle C(3)−C(4)−C(5) has widened to 113(1)°. The dihedral angle between the two best least-squares planes through the two imidazole rings amounts to 30.6°. In Figure 2A an ellipsoid plot of the complex is shown. The unit cell is illustrated in Figure 2B. The corresponding bond lengths and angles are listed in Table 2. The final atomic positional parameters, together with their estimated standard deviations, are given in Table 3.

Biological Studies. The *in vivo* activity of [Pt(bmic)Cl_2] was checked first at mice-bearing L1210 leukemia. An increase of lifespan of 10% was determined. This lies within the accuracy of the experiment. A significant antitumor activity was detected for P388 mice leukemia. After application of 316 mg/kg body

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Table 2. Bond Lengths (Å) and Angles (deg) for **1**

Pt(1)−N(1)	2.011(11)	Pt(1)−N(3)	2.029(9)
Pt(1)−Cl(1)	2.293(3)	Pt(1)−Cl(2)	2.309(3)
N(1)−C(3)	1.35(2)	N(1)−C(1)	1.38(2)
N(2)−C(3)	1.36(2)	N(2)−C(2)	1.39(2)
N(2)−C(9)	1.47(2)	N(3)−C(5)	1.32(2)
N(3)−C(6)	1.36(2)	N(4)−C(7)	1.36(2)
N(4)−C(5)	1.37(2)	N(4)−C(8)	1.48(2)
O(1)−C(4)	1.43(1)	C(1)−C(2)	1.35(2)
C(3)−C(4)	1.48(2)	C(4)−C(5)	1.49(2)
C(6)−C(7)	1.36(2)		
N(1)−Pt(1)−N(3)	89.4(4)	N(1)−Pt(1)−Cl(1)	179.7(3)
N(3)−Pt(1)−Cl(1)	90.3(3)	N(1)−Pt(1)−Cl(2)	90.5(3)
N(3)−Pt(1)−Cl(2)	179.8(3)	Cl(1)−Pt(1)−Cl(2)	89.8(1)
C(3)−N(1)−C(1)	107(1)	C(3)−N(1)−Pt(1)	124.6(8)
C(1)−N(1)−Pt(1)	128.4(9)	C(3)−N(2)−C(2)	107(1)
C(3)−N(2)−C(9)	126(1)	C(2)−N(2)−C(9)	127(1)
C(5)−N(3)−C(6)	108(1)	C(5)−N(3)−Pt(1)	123.2(8)
C(6)−N(3)−Pt(1)	128.8(8)	C(7)−N(4)−C(5)	108(1)
C(7)−N(4)−C(8)	125(1)	C(5)−N(4)−C(8)	128(1)
C(2)−C(1)−N(1)	109(1)	C(1)−C(2)−N(2)	108(1)
N(1)−C(3)−N(2)	109(1)	N(1)−C(3)−C(4)	126(1)
N(2)−C(3)−C(4)	124(1)	O(1)−C(4)−C(3)	106.6(9)
O(1)−C(4)−C(5)	113(1)	C(3)−C(4)−C(5)	113(1)
N(3)−C(5)−N(4)	109(1)	N(3)−C(5)−C(4)	129(1)
N(4)−C(5)−C(4)	122(1)	C(7)−C(6)−N(3)	109(1)
N(4)−C(7)−C(6)	107(1)		

Table 3. Positional Parameters ($\times 10^4$) of Non-Hydrogen Atoms and Thermal Parameters ($Å^2 \times 10^3$) for **1**

atom	*x*	*y*	*z*	*U*(eq)[a]
Pt(1)	69(1)	2243(1)	2653(1)	25(1)
Cl(1)	1095(3)	3855(3)	2130(3)	36(1)
Cl(2)	−78(3)	1549(3)	591(3)	38(1)
N(1)	−833(10)	835(9)	3118(8)	24(2)
N(2)	−2015(9)	−294(9)	4113(9)	25(2)
N(3)	190(9)	2855(10)	4462(9)	27(2)
N(4)	−394(9)	3198(9)	6285(9)	26(2)
O(1)	−3090(8)	2204(8)	4197(7)	29(2)
C(1)	−908(12)	−221(12)	2549(12)	32(3)
C(2)	−1626(13)	−916(12)	3146(12)	34(3)
C(3)	−1522(11)	773(10)	4066(10)	22(2)
C(4)	−1809(11)	1724(11)	4872(11)	24(2)
C(5)	−677(11)	2573(10)	5167(10)	22(3)
C(6)	1058(12)	3645(11)	5131(11)	25(3)
C(7)	699(12)	3867(11)	6266(11)	29(3)
C(8)	−1114(14)	3169(13)	7343(12)	41(4)
C(9)	−2812(12)	−707(12)	5016(11)	29(3)

[a] *U*(eq) is defined to be one third of the orthogonalized $\mathbf{U}_{ij}$ tensor.

Table 4. Results of the Pharmacological in Vivo Measurements of [Pt(bmic)Cl_2] and [Pt(bmi)Cl_2] Compared with Cisplatin at P388 (ip = Intraperitoneal)

		increase of life span (%)	
compound	toxicity (mg/kg)	LD_{50} 1 × ip	$^1/_3$ LD_{50} 4 × ip
cisplatin	13	55	
[Pt(bmic)Cl_2]	215−464	33	56
[Pt(bmi)Cl_2]	>1000	no activity	

weight a median survival time of 33% was reached and this was even increased to 56% by application of 100 mg/kg on 4 days. In contrast, no antitumor activity was detected for the bmi compound, although for both complexes the intraperitoneal treatment route was chosen.

Structural Aspects of Binding to the (Oligo)nucleotide GMP/GG. Reaction with 5′-GMP. After reaction of [Pt(bmi)Cl_2] or [Pt(bmi)$(NO_3)_2$] with 5′-GMP (one equivalent) the ^{1}H NMR spectrum of the reaction product shows a downfield shift of the H8 proton (δ(H8) = 5.71 ppm) compared to unreacted 5′-GMP (δ H8 = 4.91 ppm). The pH-dependent behavior of H8 shows no N7 protonation effect around pH 2−3, whereas the deprotonation of the 5′ phosphate group (pK_a 6.1) and the N1 (pK_a 8.5) can still be observed (see Figure 3A).[24] The coupling constant of H1′ ($^3J_{H1'-H2'}$) has change from 6.1 Hz (free GMP) to 4.1 Hz, characteristic for platinated nucleotides.[25] The H4 protons of the bmi ligand have become chemically inequivalent and have shifted upfield (δ(H4) = 3.35 and 4.14 ppm) compared to those of the starting complex (see Table 5). The H5 protons have also become inequivalent although less pronounced compared to the H4 protons. Relative integration of the H8/H1′ protons to the H4 protons of the product shows that one GMP has coordinated to the Pt complex. All these observations show that the major end product is [Pt(bmi)(GMP-*N7*)$(OH_2)]^+$. In addition a small amount of [Pt(bmi)(GMP-*N7*)$_2$] is observed (*vide infra*).

Reaction of [Pt(bmi)Cl_2] with 2 equiv of 5′-GMP results in the formation of [Pt(bmi)(GMP-*N7*)$_2$] (see Table 5). The pH-dependent behavior of H8 again confirms platination at N7 (see Figure 3A). The H4 and H5 protons of the bmi ligand have now become equivalent due to the coordination of two GMP ligands, which results in a symmetric compound. Both H4 protons are now shifted upfield, compared to those in the starting complex, indicating a shielding effect, induced by the two G bases (note that for the monosubstituted complex [Pt(bmi)(GMP-*N7*)$(OH_2)]^+$ one H4 shows a strong upfield shift, due to coordination of one 5′-GMP, whereas the shift of the other H4 proton is hardly influenced). Another indication for the close interaction between the bmi ligand and the guanine bases is the pH-dependent behavior of the two H4 protons (Figure 3A). The chemical shift of these protons shows a similar phosphate effect as found for the H8 protons. The two H5 protons are insensitive to the pH; as a result these protons must be oriented away from the phosphate moiety.

An interesting feature is the splitting of the two H8 protons at low pH whereas at high pH values (*i.e.* after deprotonation of the 5′ phosphate moiety) they cannot be observed separately. A temperature dependence study of [Pt(bmi)(GMP-N7)$_2$] at pH 4.1 shows coalescence of the two H8 signals at higher temperatures (see Figure 4). All other protons of [Pt(bmi)-(GMP-N7)$_2$] become broad upon increasing temperature. This effect proved to be reversible; *i.e.* after cooling to 293 K, two sharp H8 signals reappear.

A possible explanation for this phenomenon is restricted rotation around the Pt−N7 bond. This restricted rotation has been observed before for Pt compounds with 5′-GMP coordinated and can be due to steric hindrance[26] or the presence of H bonds.[27] Since, in our case, the effect is pH dependent, it is likely that H bonds, present at low pH, cause the inequivalence of the two GMP's by retarding the rotation around Pt−N7. These H bonds disappear after deprotonation of the 5′ phosphate groups or after an increase in the temperature, resulting in two equivalent 5′-GMP bases.

A molecular model of [Pt(bmi)(GMP-*N7*)$_2$] was constructed and compared to the NMR data. The energy-minimized structures of [Pt(bmi)(GMP-*N7*)$_2$] (either with protonated or deprotonated 5′ phosphate moieties) show the two coordinated G bases in a head-to-tail orientation (called HTT, as depicted in Figure 5 for the protonated structure). This HTT arrangement

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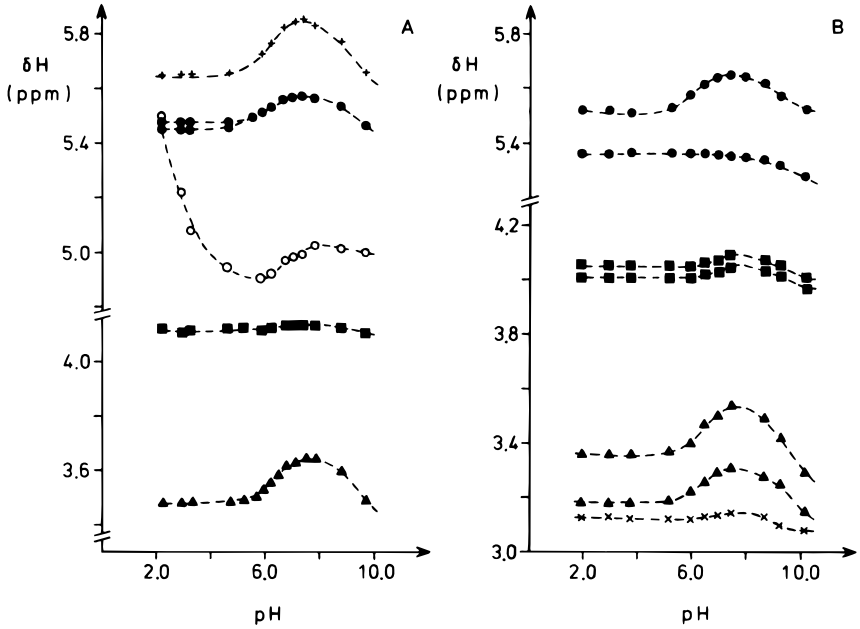

Figure 3. pH versus chemical shift profile of [Pt(bmi)(GMP-*N7*)$_2$] (A) and [Pt(bmic)(GMP-*N7*)$_2$] (B): (●) H8 signal; (▲) H4; (■) H5; (×) H6. Also 5′-GMP (H8 (○)) and [Pt(bmi)(GMP-*N7*)(aq)]$^+$ (H8 (+)) are depicted. All values are referenced to TMA.

Table 5. Summary of the ^{1}H NMR Chemical Shift (D_2O) of [Pt(bmi)Cl_2], [Pt(bmic)Cl_2], Their Aqua Derivatives, and Their 5′-GMP or GG Adducts at 297 K and pH 6.2[a]

	δ(H8)	δ(H4)	δ(H5)	δ(H6)	δ(CH_3)
[Pt(bmi)(NO_3)$_2$]		4.21	3.91		1.05
[Pt(bmi)Cl_2][b]		4.95	4.54		0.54
[Pt(bmi)(GMP-*N7*)(OH_2)]$^+$	5.74	4.14, 3.35	4.06, 4.00		1.04
[Pt(bmi)(GMP-*N7*)$_2$]	5.53	3.57	4.13		1.07
[Pt(bmi)(GG-*N7*,*N7*)]	5.71, 5.51	3.45, 3.54	4.12, 4.10		1.08
[Pt(bmic)Cl_2][b]		4.50	4.29	3.12	0.86
[Pt(bmic)(NO_3)$_2$]		4.10	3.93	3.01	0.73
[Pt(bmic)(GMP-*N7*)(OH_2)]$^+$	5.64, 5.33	4.07, 3.74, 3.54, 3.31	4.14, 4.12, 4.05, 3.72	3.18, 3.14	0.82, 0.80, 0.79
[Pt(bmic)(GMP-*N7*)$_2$]	5.63, 5.34	3.53, 3.28	4.06, 4.04	3.19	0.83, 0.79

[a] Values are referenced to TMA (3.18 ppm downfield of TMS). Data for [Pt(bmic)(GG-*N7*,*N7*)] have been omitted because of many conformers present in solution (see text). [b] In dmf-d_7.

is usually found for 2:1 Pt adducts.[28] Our NMR data are consistent with this HTT orientation of the nucleotides. If rotation around the Pt−N7 bond would be fast, only one H8 resonance is expected (as in the case at pH > 6). If, on the other hand, rotation around the Pt−N7 bond would be slow, two H8 signals are expected. Indeed two H8 signals are observed at pH < 6, indicating that restricted rotation around the Pt−N7 bond is present at acidic pH values. The origin of this restricted rotation at acidic pH, *i.e.* the presence of certain H bonds which retard the Pt−N7 rotation, might be explained by this model. H bonds between two oxygens of the 5′ phosphate of one GMP toward the NH protons of the other GMP (N(1)H and N(2)H) are found (at 1.96 and 1.99 Å, respectively) for the minimized structure of the protonated form. For the deprotonated [Pt(bmi)(GMP-*N7*)$_2$] structure, HTT orientation is also found; however, no H-bonding interactions between the two GMP's are observed.

After reaction of [Pt(bmic)Cl_2] with 1 equiv of 5′-GMP (10^{-5} M, 310 K) two reaction products are observed using NMR spectroscopy. Two H8 signals are present at 5.64 ppm and 5.35 ppm which is downfield of 5′-GMP (δ H8 = 4.92 ppm). In addition four H4 and four H5 resonances are present (see Table 5). A large upfield shift is found for the H4 protons which must be a result of coordination of 5′-GMP. The pH dependence of H8 confirms platination at N7 (data not shown). Raising the temperature does not result in coalescence of the two H8 protons; *i.e.*, the two H8 signals are not due to the presence of two conformers. Because of the unsymmetric bmic ligand, coordination of one chiral GMP molecule must result in the formation of two [Pt(bmic)(GMP-*N7*)(OH_2)]$^+$ stereoisomers.

Reaction of [Pt(bmic)Cl_2] (or [Pt(bmic)(NO_3)$_2$]) with 2 equiv of 5′-GMP results in the formation of [Pt(bmic)(GMP-*N7*)$_2$] as deduced from the ^{1}H NMR spectra. The pH-dependent behavior of the H8 proton chemical shift confirms platination at N7 and is depicted in Figure 3B. 1D NOE measurements show a small NOE contact between the two H8 protons at low temperature (283 K), suggesting a head-to-head bonding mode of the two GMP's. The difference in chemical shift of the two H8 signals is rather large ($\Delta\delta$(H8) = 0.27 ppm) just as usually found for HTH oriented bases.[29] It should be noted that for [Pt(bmi)-(GMP-*N7*)$_2$] (HTT), $\Delta\delta$(H8) is very small ($\Delta\delta$(H8) = 0 at pH > 5.3; $\Delta\delta$(H8) = 0.03 at pH 4.1), as listed in Table 5.

Another unusual observation is the absence of a phosphate deprotonation effect for the most upfield H8 signal as shown

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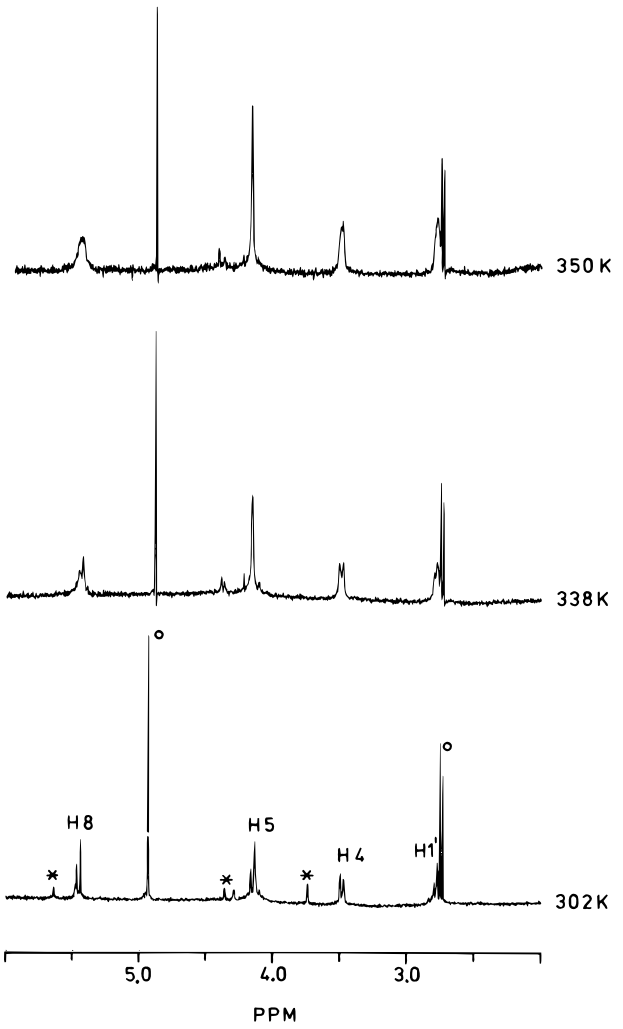

Figure 4. ^{1}H NMR spectra of [Pt(bmi)(GMP-*N7*)$_2$] at various temperatures and pH 4.1. An asterisk denotes [Pt(bmi)(GMP-*N7*)(OH$_2$)]$^+$; ○ is 5′-GMP.

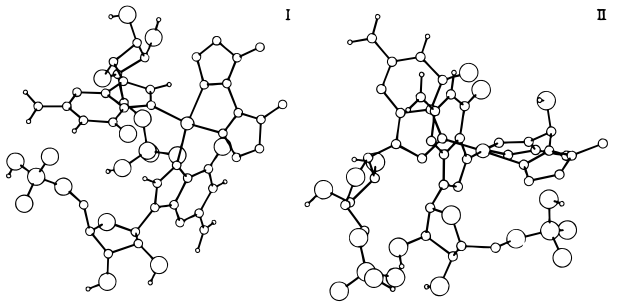

Figure 5. Energy-minimized structures of [Pt(bmi)(GMP-*N7*)$_2$] (I, HTT) and [Pt(bmic)(GMP-*N7*)$_2$] (II, HTH).

in Figure 3B. The ^{31}P NMR spectra show no signal for inorganic phosphate; *i.e.*, no dephosphorylation has occurred. In addition, the pH-dependent behavior of the phosphate groups shows protonation for both phosphates with reduced pK_a values compared to 5′-GMP (see Figure 6). Thus the observation that protonation of the 5′ phosphate moiety has no influence on the chemical shift of the H8 must be due to the orientation of this phosphate with respect to the position of H8. For the nucleotide 3′-GMP a very small influence of the phosphate protonation on the δ of H8 has been reported, due to the orientation of this phosphate group.[30] Therefore, in the case of [Pt(bmic)(GMP-*N7*)$_2$], one of the phosphate groups definitely must be oriented away from the H8 proton.

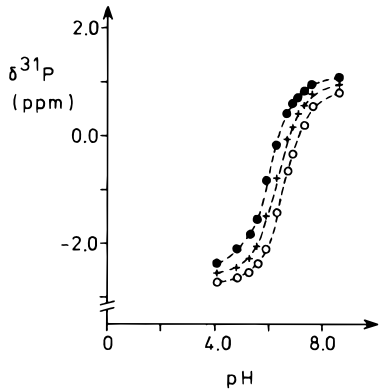

Figure 6. pH versus chemical shift of ^{31}P signals of [Pt(bmic)(GMP-*N7*)$_2$]: (○) 5′-GMP; (+ and ●) [Pt(bmic)(GMP-*N7*)$_2$].

The energy-minimized structure of [Pt(bmic)(GMP-*N7*)$_2$] shows a head-to-head (HTH) orientation of the two GMP moieties. This is surprising considering the generally observed HTT conformation of bis(oxopurine)platinum complexes,[28] which is also observed for the bmi complexes (*vide supra*). However, a close inspection of the minimized Pt(bmic) structure shows H bonds from the O6 atoms of both GMP's towards the OH of the bmic ligand (see Figure 5B). These H bonds apparently can stabilize the HTH orientation, generally thought to be energetically unfavorable compared to HTT (r(O6-(G)···*H*O(bmic)) = 3.16 Å and is 3.33 Å in the HTH model).[31] The observed NOE between the two H8 signals is in accordance with this HTH structure. One of the phosphate groups is involved in H-bonding interactions toward the other 5′-GMP and, as a result, is oriented away from the H8 making this proton insensitive to (de)protonation of the 5′ phosphate group (r(H8···*O*P) = 4.96 Å). Although this HTH model is in remarkably good agreement with the NMR data, one should note that this energy-minimized model represents only a local energy minimum and that a detailed conformational NMR study, together with a molecular dynamics simulation, would be required to obtain more evidence for this HTH model of [Pt(bmic)(GMP-*N7*)$_2$].

Reaction with d(GpG). Reaction of [Pt(bmi)Cl$_2$] or [Pt(bmi)(NO$_3$)$_2$] with d(GpG) at 310 K results in the formation of the chelate [Pt(bmi){d(GpG)-*N7(1),N7(2)*}] as deduced from the NMR data. The pH dependence of the H8 protons confirms platination at the N7 position (data not shown). The ^{31}P NMR shows a signal at 0.49 ppm, which is 0.9 ppm downfield of unplatinated GG and characteristic for platinated GG-*N7*,*N7* moieties.[29] Analysis of the HH-COSY spectrum of this product shows two spin systems for the deoxyribose sugars (not shown). One sugar lacks coupling of the H1′ toward the H2′, due to a conformational change which results in a N-type sugar, a common feature of platinated GG residues. In addition the two imidazole rings can be detected separately using 2D NMR techniques. In fact, these imidazole rings are almost chemically equivalent; only the H4 protons can be observed separately ($\Delta\delta$ = 0.08 ppm) (see Table 1). Therefore the H4 protons must be oriented close toward the chiral d(GpG) moiety, making them chemically and magnetically inequivalent, whereas the H5 and CH_3 protons, oriented away form the chiral center, show no resolved differences in their chemical shift values.

Reaction of [Pt(bmic)Cl$_2$] (or [Pt(bmic)(NO$_3$)$_2$]) with d(GpG) results in the formation of the chelate [Pt(bmic){d(GpG)-*N7(1),N7(2)*}] as deduced from the resulting NMR spectra. At

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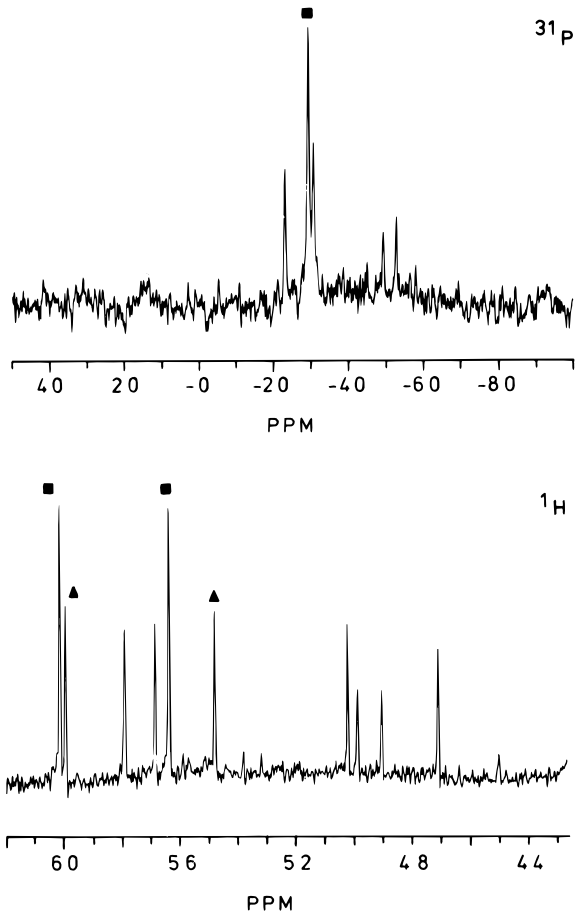

Figure 7. ^{1}H NMR (H8 region) and ^{31}P NMR spectra of the several conformers of [Pt(bmic){d(GpG)*N7,N7*}], present at room temperature: (■) stereoisomer A (major conformer); (▲) stereoisomer B (minor).

room temperature and 5 mM concentration, the ^{1}H NMR spectrum shows a very complicated pattern with 10 H8 proton signals present, whereas the ^{31}P NMR shows the presence of as much as five phosphodiester signals (see Figure 7). After the temperature is raised up to 353 K, only four H8 signals are left. This effect proved to be reversible, *i.e*. reducing the temperature to 297 K results in the reappearance of the other six H8 proton signals. Since the bmic ligand is unsymmetric, after it has coordinated to platinum, and because d(GpG) is a chiral ligand, the formation of two stereoisomers (*i.e*. four H8 signals) can be expected, just as reported by Hartwig and Lippard for a similar case.[32] Study of the NMR signals over a wide pH range has excluded possible Pt binding at N1 sites of G.

Indeed at high temperature four H8 signals (*i.e*. two GG-*N7,N7* stereoisomers) can be detected. At low concentration (0.5 mM) also as many as 10 H8 signals are visible. Therefore this observation must be due to different conformations of [Pt-(bmic){d(GpG)-*N7(1),N7(2)*}], present at room temperature, which interconvert slowly on the NMR time scale. Raising the temperature results in rapid interconversion of the conformers and ends up with the two stereoisomers which cannot interconvert.

Interestingly, the formation of one stereoisomer is preferred (A/B ratio 2.44, as determined from relative integration of H8 protons). For [Pt(bmic){d(GpG)-*N7(1),N7(2)*}] two stereoisomers can be expected, named A and B. Isomer A has the O6 atoms of the guanine bases oriented at the same side of the Pt-N4 plane as the OH group of the bmic ligand, whereas isomer B has the O6 and the hydroxyl on opposite sides of the Pt-N4 coordination plane. In case of stereoisomer A a number of conformations apparently are present in solution at room temperature. One can expect a conformation with H bonds from both O6 atoms toward the OH group, two conformations with one H bond from, either O6(5′) or O6(3′) toward the OH group and one conformation with no H bonds toward the OH moiety. All these possible conformations already result in eight H8 signals. Stereoisomer B, which cannot form H bonds toward the OH group, contributes two extra H8 signals resulting in the observed 10 H8 signals. Raising the temperature results in rapid interconversion of all the conformations of isomer A, ending up in one average structure. Because of the extra H bonds present for isomer A, this isomer is likely to be energetically more favoured than isomer B; hence the A/B ratio is 2.44.

Kinetic Aspects. Preliminary results of the reaction kinetics of [Pt(bmi)$(NO_3)_2$] and [Pt(bmic)$(NO_3)_2$] with 5′-GMP are presented here. Because of the limited number of data points the results of the kinetics can only be interpreted in a qualitative way. More experiments with short time intervals during the measurements are needed in order to gain quantitative data.

[Pt(bmi)$(NO_3)_2$]. The reaction of [Pt(bmi)$(NO_3)_2$] with 5′-GMP has been studied in a 1:2 molar ratio. ^{1}H NMR spectroscopy was used to follow the time course of the reaction at 310 K (5 mM, pH 5.1). Since H8 protons of platinated G bases are susceptible to exchange with deuterium[33] and broad, due to unresolved coupling with the ^{195}Pt nucleus,[34] these signals are usually not suitable to obtain accurate integrals; therefore, the H1′ resonances had to be used. These can be observed separately at pH 5.1. Plots of the time dependence of the starting material, intermediates, and end products are depicted in Figure 8A. Initially, the monofunctional complex [Pt(bmi)-(GMP-*N7*)$(OH_2)]^+$ is formed, but soon the bifunctional end product [Pt(bmi)(GMP-*N7*)$_2$] becomes the dominant species ($t_{1/2}$ $\sim$ 26 min). Coordination of the second 5′-GMP must occur faster than coordination of the first 5′-GMP, since the amount of [Pt(bmi)(GMP-*N7*)$(OH_2)]^+$ remains relatively small (<25%) during the course of the reaction. Reaction of [Pt(bmi)$(NO_3)_2$] with 1 equiv of 5′-GMP also results in the formation of the bifunctional adduct [Pt(bmi)(GMP-*N7*)$_2$] (*vide supra*), in addition some unreacted Pt compound and the monosubstituted [Pt-(bmi)(GMP-*N7*)$(OH_2)]^+$ have been observed for this reaction (data not shown).

[Pt(bmic)$(NO_3)_2$]. The reaction of [Pt(bmic)$(NO_3)_2$] with 2 equiv of 5′-GMP was also followed by ^{1}H NMR spectroscopy and the results are depicted in Figure 8B. Initially, only the formation of the monofunctional product [Pt(bmic)(GMP-*N7*)-$(OH_2)]^+$ is observed. After ~20 min., however, the bifunctional end product [Pt(bmic)(GMP-N7)$_2$] has also been detected by ^{1}H NMR. Compared to the reaction profile of [Pt(bmi)$(NO_3)_2$] ($t_{1/2}$(5′-GMP) = 14 min), the overall reaction is much slower for [Pt(bmic)$(NO_3)_2$] ($t_{1/2}$(5′-GMP) = 45 min). Formation of the end product [Pt(bmic)(GMP-*N7*)$_2$] is relatively slow, compared to the formation of the intermediate product [Pt(bmic)-(GMP-*N7*)$(OH_2)]^+$ (see Figure 8B), since this monofunctional product is the predominant species during the first 24 h of the reaction. Coordination of the first 5′-GMP, therefore, must occur relatively faster than coordination of the second 5′ GMP. This is a remarkable difference compared to the reaction of 5′-

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(33) Girault, J.-P.; Chottard, J.-C.; Guittet, E. R.; Lallemand, J.-Y.; Huynh-Dinh, T.; Igolen, J. *Biochem. Biophys. Res. Commun.* **1982**, *109*, 1157.

(34) Lallemand, J.-Y.; Soulie, J.; Chottard, J.-C. *J. Chem. Soc. Chem. Commun.* **1980**, 436.

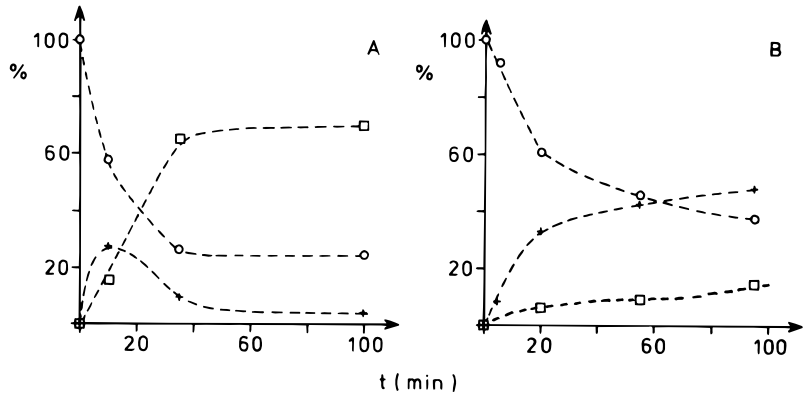

Figure 8. Formation of the products between 5′-GMP and [Pt(bmi)$(NO_3)_2$] (A) and between 5′-GMP and [Pt(bmic)$(NO_3)_2$] (B) (pH 5.1, 5 mM, T = 310 K). Indicated values denote relative amounts (%) determined by H1′ integrals. (○) 5′-GMP; (+) [Pt(bmi)(GMP-*N7*)(OH_2)] (A) or [Pt(bmic)(GMP-*N7*)(OH_2)] (B); (□) [Pt(bmi)(GMP-*N7*)$_2$] (A) or [Pt(bmic)(GMP-*N7*)$_2$] (B).

GMP with [Pt(bmi)$(NO_3)_2$] where coordination of the first G base accelerates the coordination of the second 5′-GMP.

Discussion

Structure−activity relationships (SAR's) defining the earlier-discovered antitumor active Pt compounds have indicated that least one NH moiety would be necessary for antitumor activity. The new complex [Pt(bmic)Cl_2], clearly violating this rule by showing antitumor activity and lacking a NH moiety, has therefore been investigated with respect to its interaction with short (oligo)nucleotides. [Pt(bmi)Cl_2], lacking a NH moiety, but also lacking antitumor activity, has been studied for comparison.

The structural information, obtained from the NMR data, shows that both the bmic compound and the bmi complex can bind to small DNA fragments in a fashion similar to cisplatin. In case of d(GpG), N7 coordination of [Pt(bmic)Cl_2] or [Pt-(bmi)Cl_2] induces a downfield shift in the ^{31}P NMR spectrum, also reported for cisplatin, after binding to d(GpG). Coordination to the N7 position is accompanied by deformation of the sugar phosphate backbone, also a characteristic feature of cisplatin. The pH-dependent behavior of the imidazole protons shows a close interaction between the H4 protons and the coordinated nucleotide. The strong upfield shift of H4, after coordination of a G base, provides further evidence for this close interaction.

Some structural characteristics of the bmic or bmi adducts, however, are different compared to the cisplatin adducts. Slow rotation around the Pt−N7 bond, as in the case of [Pt(bmi)-(GMP-*N7*)$_2$], has not been reported for [*cis*-Pt$(NH_3)_2$(GMP-*N7*)$_2$]. In addition, the lack of a phosphate deprotonation effect on the H8 chemical shift of one of the two GMP's of [Pt(bmic)-(GMP-*N7*)$_2$] has, so far, not been observed for cisplatin analogs. For [Pt(bmic)(GMP-*N7*)$_2$], the HTH orientation of the two GMP's, stabilized by the presence of H bonds towards the OH group of the bmic ligand, is also a very unusual observation. The large number of conformers of [Pt(bmic){d(GpG)-*N7-(1),N7(2)*}], also due to the presence of these H bonds, deviates from [*cis*-Pt$(NH_3)_2${d(GpG)-*N7(1),N7(2)*}] and may induce significantly different distortions in the DNA structure *in vivo*. This may also be related to the fact that diastereoisomers can be formed.

Since it has been stated that platination of DNA is controlled kinetically, rather than thermodynamically,[35] it is of significant interest to compare the kinetic properties of [Pt(bmi)$(NO_3)_2$] and [Pt(bmic)$(NO_3)_2$]. In reaction with 5′-GMP a clear difference in reactivity is found between these two platinum compounds of which [Pt(bmi)$(NO_3)_2$] proves to be the most reactive complex. Both the initial reaction step, *i.e.* coordination of the first G base, and the second reaction step, resulting in the bifunctional adduct, are faster for [Pt(bmi)$(NO_3)_2$]. This can be understood considering the amount of steric hindrance around the Pt nucleus. Compared to the bmic ligand, the bmi ligand shows less steric hindrance as is obvious from the X-ray structures of [Pt(bmi)Cl)$_2$] and [Pt(bmic)Cl_2]. The dihedral angle between the two best least-squares planes through the two imidazole rings as shown in the structure of [Pt(bmi)Cl_2] and the related structure [Pt(mimim)Cl_2] (mimim = (1-methylimidazole-2-yl)(imidazol-2-yl) amounts to 3.1 and 2.6°, respectively.[36]

The bmi ligand is almost completely flat and lies in the plane of the Pt−N_2HCl_2 moiety. The bmic ligand is not flat, due to the carbinol group between the two imidazole rings, its imidazole rings are tilted out of the Pt−N_2Cl_2 plane, and the carbinol moiety is also out of the Pt−N_2Cl_2 plane (see Figure 2). This results in severe steric constraints around the Pt atom, making the bmic complex less reactive. This increased steric hindrance may also be responsible for the reported biological activity of [Pt(bmic)Cl_2]. The complex cannot be easily inactivated by other biomolecules (such as S-containing proteins) before binding to DNA and therefore may be antitumor active, whereas [Pt(bmi)Cl)$_2$] is inactive. One may expect that hydrolysis of [Pt(bmic)Cl_2] is relatively slow, compared to [Pt-(bmi)Cl_2], due to the steric effects of the bmic ligand. A detailed study determining the two Pt−Cl hydrolysis rate constants of [Pt(bmi)Cl_2] and [Pt(bmic)Cl_2], using reversed phase HPLC,[37] will be necessary to investigate this hypothesis.

The recently reported antitumor properties of *cis*-bis(pyridine)platinum(II) organoamides,[38] compounds also lacking an NH group, have been attributed to the large steric effects of the organoamide ligand, which makes the complex less reactive toward inactivating biomolecules. For these compounds the cytotoxic moiety {*cis*-Pt$(py)_2$}$^{2+}$ can bind to GG with the organoamide ligand acting as a leaving group.[39] If the orga-

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noamide ligand, which is rather bulky and not labile, is replaced by a relatively small and labile group (as in the case of [*cis*-$Pt(py)_2Cl_2$]) the compound is found to be inactive. The observed moderate activity of [*cis*-$Pt(4\text{-Pr-py})_2Cl_2$] (4-Pr-py = 4-isopropylpyridine) and [*cis*-$Pt(py)_2(C_2O_2)$] is supportive for these conclusions. These complexes are thought to be less reactive, compared to [*cis*-$Pt(py)_2Cl_2$], due to the increased steric hindrance ([*cis*-$Pt(4\text{-Pr-py})_2Cl_2$]) or the strongly coordinated leaving group ([*cis*-$Pt(py)_2(C_2O_2)$]). Therefore, they cannot be easily activated by other biomolecules before reaching DNA (as [*cis*-$Pt(py)_2Cl_2$]) and show moderate activity.

A possible explanation for the requirement of NH moieties in "classical" platinum complexes could be the increase of steric hindrance around the N atom after substitution of NH by an alkyl group. This steric hindrance on the N atom is known to retard the reaction with DNA. However, if this hindrance on the N atom is not present, as is the case with the "nonclassical" complexes containing aromatic ligands (such as bmic or pyridine), the close interaction with DNA is not hindered and Pt binding can occur relatively fast. The introduction of alkyl substituents on the pyridine ligands of the organoamide complexes results in reduced antitumor activity,[40] probably as a result of increased steric hindrance upon binding to DNA. So, for this new class of Pt compounds, the presence of an NH group is not necessary, because of the absence of steric hindrance around the nitrogen. It would be interesting to design other Pt complexes, lacking a NH moiety, but also lacking steric hindrance around the N atom, and determine their antitumor properties. From such compounds one can conclude if, for "classical" platinum complexes, the requirement of NH is due to the actual presence of a H bond from NH to DNA or due to the absence of steric hindrance on the N atom. Perhaps even N-donor ligands can be replaced by other donor ligands (such as certain P donor ligands) as long as they do not oppose the interaction with DNA or alter the properties of the leaving group due to the *trans* effect of these P ligands.

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Conclusions

The X-ray structure of $[Pt(bmic)Cl_2]$ is in accordance with the reduced reactivity of this complex towards G bases. Due to "tilting" of the coordinated imidazole rings of the bmic moiety, the complex is less reactive. Reaction of $[Pt(bmi)Cl_2]$ or $[Pt(bmic)Cl_2]$ (*inactive* and *active*, respectively) with 5′-GMP or d(GpG) results in the formation of *N7,N7* adducts for both complexes. Compared to cisplatin adducts, significant differences are found, in particular for the bmic adducts. A large variety of conformers is found in aqueous solution due to H bonds toward the OH group of the bmic ligands. Differences in the reactivity of the two complexes have also been detected. The most reactive complex proved to be the inactive Pt compound. Thus both the structural and the kinetic properties agree with the different biological behavior of these two Pt complexes. Finally, we cannot rule out that a different water solubility for the two compounds may also be related to a difference in activity.

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Supporting Information Available: Tables of isotropic and anisotropic displacement parameters and hydrogen coordinates of **1** (1 page). Ordering information is given on any current masthead page.

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