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Possible Biotransformation Reactions of Polynuclear Pt(II) Complexes

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The reactions of the two complexes **BBR3464** [{*trans*-PtCl(NH$_3$)$_2$}$_2${μ-*trans*-Pt(NH$_3$)$_2$(NH$_2$(CH$_2$)$_6$NH$_2$)$_2$}]$^{4+}$ and **BBR3610** [{*trans*-PtCl(NH$_3$)$_2$}$_2${μ-C$_2$H$_4$(NH$_2$(CH$_2$)$_6$NH$_2$)$_2$}]$^{4+}$ and the corresponding diaqua complexes with the nucleophiles thiourea (tu) and L-methionine (L-Met), were investigated under pseudo-first-order conditions as a function of concentration and temperature, using UV−vis spectrophotometric and stopped-flow techniques. ^{1}H NMR spectroscopy was used to follow the stepwise substitution of the chloro ligands by guanosine-5′-monophosphate under second-order conditions. For the sulfur donor containing nucleophiles (tu and L-Met), a second reaction step, the displacement of the labilized amine chain linker, as a result of the strong trans-effect of tu and L-Met, was found. The activation parameters for all reactions studied suggest an associative substitution mechanism. The displacement of the chain linker by S-donor nucleophiles illustrates the limit of application of polynuclear complexes with monodentate aliphatic amine bridges and primary ammines, in agreement with previous studies reported in the literature.

Introduction

Attempts to overcome the drawbacks (severe side effects, development of resistance) of *cis*-[Pt(NH$_3$)$_2$Cl$_2$], cisplatin, in cancer therapy have focused on two different approaches: (1) the modification of the chloride leaving groups to alter the pharmacodynamic and -kinetic properties and thereby reduce toxicity and (2) fundamental changes in the structure of the complex or the substitution of primary ammine spectator ligands by chelating amines to influence the repair processes that are involved in the development of resistance, i.e., the cell response. Besides new mononuclear complexes with improved properties in terms of toxicity (Carboplatin) or cross-resistance to cisplatin (Oxaliplatin), the exclusively new class of multinuclear Pt(II) complexes has been developed by Farrell's group.[1−3] From a series of cis and trans complexes, comprising two or more platinum coordination units linked via aliphatic diamine bridges, the complex **BBR3464** has entered phase II clinical trials[4] and three other complexes, among **BBR3610**, have shown strong clinical potential. Therefore, these two complexes were chosen for a detailed kinetic investigation.

The apparent advantage of these type of complexes is the high charge (+4), compared to the neutral mononuclear complexes, resulting in good solubility, efficient electrostatic interaction with the polyanionic DNA (the major pharmacological target of platinating agents), and a fast uptake.[5] Furthermore, they are capable to form structurally different Pt−DNA adducts that may lead to a unique pattern of anticancer activity.[6] The length of the linker and the overall size of these complexes result in an increased flexibility as well in the DNA adduct. The structure lacks severe DNA distortion due to bending and twisting as reported for mononuclear complexes.[7,8] The cross-links being formed by polynuclear complexes span up to six base pairs, involving

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mainly both strands (interstrand cross-link) of the DNA,[9] compared to the formation of intrastrand cross-links by mononuclear complexes that only span a maximum of three base pairs.[7] Since interstrand cross-links persist longer than intrastrand cross-links, they are considered to be less susceptible to repair as both strands are affected by the damage.[8]

Unfortunately, the improved properties are accompanied by progressive biotransformation and drug degradation: **BBR3464** has encountered metabolism-related difficulties in clinical trials. Therefore, it is important to gain further insight into the mechanism of such transformation reactions. Especially S-containing molecules are involved in the thermodynamic and kinetic competition with nucleobase binding and the mechanism of metabolism of Pt(II) drugs.[10,11] Biotransformation products (e.g., [Pt(L-Met-S,N)$_2$]) found in the urine of patients undergoing cisplatin chemotherapy are reported in the literature and demonstrate that both the leaving group and the carrier ligand can be substituted by sulfur-containing nucleophiles.[12,13] These reaction products are inert to further substitution and therefore limit the active concentration of the drug. Although many of the reactions with S- and N-donor nucleophiles are well investigated and understood,[7] it is still controversially discussed which donor type wins the competition for the soft Pt(II) center. Studies indicate a kinetic preference for, e.g., thioethers over N-donors[10,14,15] and confirm what is expected from a theoretical point of view. However, still a sufficient amount of the Pt(II) drug reaches and reacts with the DNA target. Thus, it seems that the conditions under which the reactions proceed are of significant importance for the preferred pathway. In a previous study[16] on mononuclear complexes of the type [PtA$_2$(OH$_2$)$_2$] (A = diaminocyclohexane, ethylenediamine, aminomethylpyridine, *N*,*N*′-bipyridine), we demonstrated that the reaction of [Pt(aminomethylpyridine)(H$_2$O)$_2$]$^{2+}$ with guanosine-5′-monophosphate (5′GMP^{2-}) is faster than to the reaction with L-methionine (L-Met): k_1(5′GMP^{2-}, pH 2, 40 °C) = 12.5 ± 0.5 M^{-1} s^{-1}, k_1(L-Met, pH 2, 40 °C) = 4.1 ± 0.1 M^{-1} s^{-1}. Thus, the N-donor exhibits under the selected conditions a high affinity for this particular Pt(II) complex.

The understanding of the chemical transformations of Pt(II) complexes with biologically relevant nucleophiles under physiological conditions is of special concern for the pharmaceutical and biomedical research. 5′GMP^{2-} was used as a model for binding to a nucleobase. The well-investigated[17] nucleophile thiourea (tu), with well-known Pt(II) complex chemistry,[18] was selected because of its good solubility, neutral character, high nucleophilicity, and the fact that it combines ligand properties of thiolates[19] (π-donor) and thioethers[20] (σ-donor, π-acceptor). Furthermore, it was explored for its applicability as a chemoprotective agent, alleviating the side effect in normal tissue.[10,21] L-Methionine, a protein amino acid, is classified as an essential amino acid for humans, present in blood and available to react with the Pt(II) complex. Therefore, we studied the reactions of the polynuclear complexes containing two Pt−Cl or Pt−OH$_2$ bonds with tu, L-Met, and 5′GMP^{2-} using UV−vis spectrophotometric, stopped-flow, and NMR techniques in order to gain more insight into the mechanism of decomposition of reactions of potential biological importance.

Experimental Section

Chemicals. The nucleophiles L-Met, tu, and 5′-GMP^{2-} sodium salt used in the kinetic measurements were obtained from Acros Organics and Fluka. Nucleophile stock solutions were prepared shortly before use by dissolving the chemicals. The complexes **BBR3464** and **BBR3610** were kindly donated by Cell Therapeutics. K_2PtCl_4 was purchased from Strem Chemicals. 98% CF_3SO_3D (Aldrich), CF_3SO_3H (HOTf, Aldrich), and 99.9% D_2O (Deutero GmbH) are commercially available and used as received. All other chemicals were of the highest purity commercially available and used without further purification. Ultrapure water was used in all experiments involving aqueous solutions.

Preparation of Complex Solutions. Solutions of the aqua complexes of **BBR3464** and **BBR3610** for UV−vis experiments were prepared by dissolving a known amount of the dichloro complex in 0.01 M HOTf and adding slightly less than the stoichiometric excess (with respect to chloride) of silver triflate. The mixture was then stirred overnight at 50 °C. The precipitated silver chloride was removed with a Millipore filter, and the remaining solution was diluted with 0.01 M HOTf to give the desired complex concentration. The pH of the aqueous solutions was controlled using a Thermo Orion Ross Ultra Combination pH glass electrode, linked to a computer, or a WTW Inolab level 1 pH meter with a combined glass electrode. The electrodes were calibrated using standard buffer solutions of pH 4, 7, and 10 obtained from Sigma and WTW.

^{1}H NMR kinetic experiments of the dichloro complexes with 5′GMP^{2-} were studied on a freshly prepared sample of the reactants. A 10 mM solution of the complex was prepared in 300 μL of 0.1 M NaCl/D_2O ~30 min prior to the start of the kinetic experiment and put in an ultrasonic bath until complete dissolution (2 min). A solution (300 μL) of 10 mM 5′GMP^{2-} in 0.1 M NaCl/D_2O, whose pH* (pD = pH + 0.4[22]) was adjusted with DOTf/0.1 M NaCl to 7.4, was added to initiate the reaction. NMR spectra were recorded at 310.7 K over a period of 60 h. After completion of the first substitution step, an equimolar amount of 5′GMP^{2-} in 0.1 M NaCl/D_2O/DOTf (at pH* 7.4) was added to the NMR tube to give a final concentration of 4.5 mM for both reactants in the second substitution step. No buffer was used to prevent increased activa-

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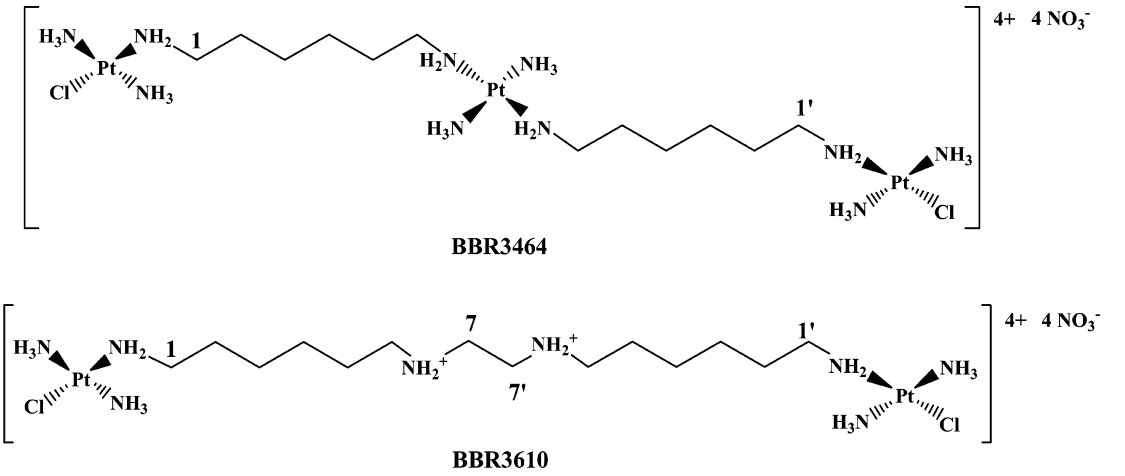

Figure 1. Structures of the multinuclear Pt(II) complexes.

Table 1. Summary of the Rate Constants for the Displacement of X (=H_2O, Cl^-) by a Range of Nucleophiles in the Multinuclear Pt(II) Complexes

				nucleophile			
				tu	L-Met	tu	L-Met
complex	*I* [M]	*T* [°C]	pH	$10^3\ k_1$, [$M^{-1}\ s^{-1}$]		$10^3\ k_2$ [$M^{-1}\ s^{-1}$] $10^5\ k_{-2}$ [s^{-1}]	
BBR3610−$(OH_2)_2$	0.01 OTf	25	2	4900 ± 100	171 ± 4	32.7 ± 0.6	2.84 ± 0.05
BBR3464−$(OH_2)_2$[a]	0.01 OTf	25	2	5200 ± 200			
BBR3610	0.1 NaCl	25	2	91 ± 3	20.8 ± 0.9	32 ± 1	5.3 ± 0.2 *1.4 ± 0.5*
BBR3610	0.1 NaCl	37.5	7.4		45 ± 1		11.5 ± 0.5 *1.1 ± 0.3*
BBR3464[b]	0.1 NaCl	37.5	7.4	200 ± 11	41 ± 1	94.6 ± 0.7	13.0 ± 0.5 *1.0 ± 0.2*
BBR3464[c]	0.1 NaCl	25	7.4	95 ± 9		33 ± 3	

[a] For concentration dependence see Figure S1 (Supporting Information) [b] For concentration dependence see Figures S6, S7, S11, and S12 (Supporting Information). [c] For concentration dependence see Figure S9 (Supporting Information)

tion of the chloro complexes due to coordination of phosphate[23] or interfering signals in the observed peak area. The pH* changed from 7.4 to 6.3 upon completion of both reaction steps.

Instrumentation and Measurements. NMR Spectroscopy and Elemental Analysis. The NMR spectra were acquired on a BRUKER AVANCE DRX 400WB or DPX 300 spectrometer. The measurements were performed with a commercial 5 mm Bruker broadband probe thermostated with a Bruker B-VT 3300 (DPX)/3000 (DRX) variable-temperature unit. All chemical shifts were referenced to TSP (trimethylsilylpropionic acid) in D_2O or TMS (tetramethylsilane) in DMSO, and downfield shifts recorded as positive numbers. In D_2O solutions, the pH* was measured with an inoLab SenTix Mic pH Microelectrode. Elemental analyses were performed on a Euro Vector, type EA 3000 (C, H, N, S) analyzer.

UV−Vis Kinetic Experiments. UV−vis spectra were recorded on Varian Cary 5G or Cary 1 spectrophotometers equipped with a thermostated cell holder or on a Shimadzu UV-2101-PC spectrophotometer equipped with a thermo-electrically controlled cell holder for the study of slow reactions. Kinetic measurements on fast reactions were performed on Applied Photophysics SX 18MV or Dionex Durrum stopped-flow instruments coupled to online data acquisition systems. The temperature of the instruments was controlled throughout all kinetic experiments to an accuracy of ±0.1 °C. All reactions of the diaqua complexes were studied at pH 2.0−2.2 to guarantee the presence of the diaqua form of the complexes, with respect to their pK_a values.[23] Thus, hydroxo and dihydroxo species, which play an important role under physiological conditions as they are practically inert to substitution, can be excluded. The ionic strength of solutions at pH 2 was maintained at 0.01 M using HOTf. The reactions of the dichloro complexes at pH 2 and 7.4 were performed at an ionic strength of 0.1 M using NaCl. *N*-2-Hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) (0.25 mM) and NaOH was used as a buffer for reactions studied at pH 7.4.

Spectral changes that accompany the reactions were first recorded over the wavelength range 190−500 nm to establish a suitable wavelength at which the kinetic trace could be followed. The wavelengths used for each reaction are listed in Table S1 (Supporting Information). The slow reactions were initiated by mixing 900 μL of complex with 900 μL of nucleophile solution in a tandem-cuvette, thermostated in a spectrophotometer cell compartment.

The ligand substitution reactions were studied under pseudo-first-order conditions. This was achieved by using at least a 20-fold (1:2 complex formation) or 40-fold (1:4 complex formation) excess of the nucleophile over the complex. All reported rate constants represent an average value of at least three to five independent kinetic runs for each experimental condition. The temperature dependence was studied in the range 15−40 or 22.5−42.5 °C.

Results and Discussion

To study the impact of the nature of the chain and the spectator ligands on the kinetic and thermodynamic proper-

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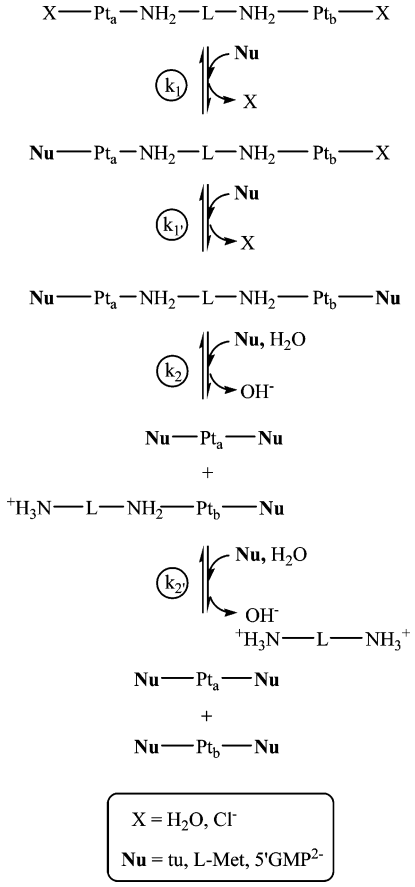
Scheme 1. Proposed Reaction Pathways for the Reaction of Multinuclear Pt(II) Complexes with a Series of Nucleophiles.[a]



[a] The Pt$_a$ center does not affect any changes on the Pt$_b$ center because of the length of the linker L, which results in the same rate constants for $k_1/k_{1'}$ and $k_2/k_{2'}$. Only k_1 and $k_{1'}$ for Nu = 5′GMP^{2-} could be obtained separately in the ^{1}H NMR kinetic experiment. No release of the bridging ligand was observed for 5′GMP^{2-}. Charges are omitted for clarity.

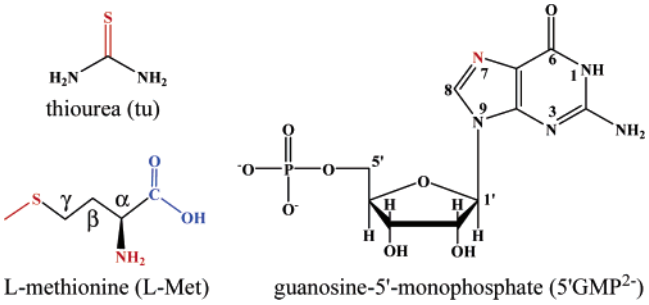


Figure 2. Structures of the investigated nucleophiles.

ties of each of the platinum centers, ligand substitution reactions with the three selected nucleophiles (tu, L-Met, 5′GMP^{2-}) were investigated. The structures of the complexes are summarized along with the used abbreviations in Figure 1.

The structural difference between **BBR3610** and **BBR3464** is located in the center part of the bridge. In **BBR3610**, ethylene links the two Pt(II) centers [{*trans*-PtCl(NH$_3$)$_2$}$_2$-{(NH$_2$(CH$_2$)$_6$NH$_2$)$_2$}]$^{2+}$, whereas in **BBR3464** a *trans*-[Pt-(NH$_3$)$_2$]$^{2+}$ moiety forms the center piece. As the two amines in **BBR3610** are quarternary amines and therefore positively charged, the overall charge of the chloride form of **BBR3610** is +4. The additional Pt(II) center in **BBR3464** leads to the same overall charge of the chloro complex of +4 and causes a kink in the linear structure (see Figure 1). It is known from literature that if the two Pt halves are separated by six or more atoms, an independent reaction of the two positively charged [Pt(NH$_3$)$_2$(NH$_2$R)OH$_2$]$^{2+}$ groups can be expected.[23,25] This would mean that an additional Pt(II) center in **BBR3464** compared to **BBR3610** should not increase the overall electrophilicity of the complex and therefore affect the substitution rates, although it can play an important role in cell uptake and DNA preassociation, especially due to 'noncovalent' interactions.[24]

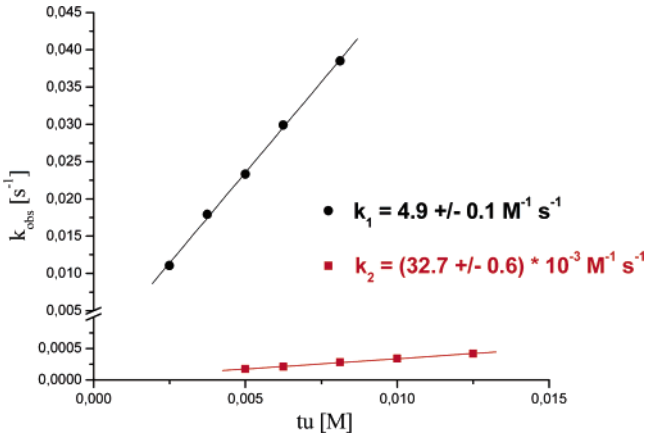


Figure 3. Plots of k_{obs} vs tu concentration for the reaction with **BBR3610-(OH$_2$)$_2$** (0.125 mM) at 25 °C, pH = 2 and I = 0.01 M.

Table 2. Summary of the Rate Constants for the Displacement of Chloride in the Multinuclear Pt(II) Complexes by 5′GMP^{2-} Followed by ^{1}H NMR

				nucleophile 5′GMP^{2-}	
complex	I [M]	T [°C]	pH	$10^3\,k_1$,[a] M^{-1} s^{-1}	$10^3\,k_1'$,[a] M^{-1} s^{-1}
BBR3610	0.1 NaCl	37.5	7.4	19.5 ± 0.3	21 ± 1
BBR3464	0.1 NaCl	37.5	7.4	19.3 ± 0.3	21.8 ± 0.3

[a] k_1 and $k_{1'}$ are defined in Scheme 1.

The substitution reactions of the diaqua complexes were studied at pH 2.0−2.2 to ascertain the complexes predominantly in their diaqua form.[23] Protonation of tu (pK_a = −1.3) under the selected conditions can be neglected as recently reported.[17] The chloro complexes were investigated at pH 7.4 in 0.1 M NaCl to suppress the formation of aqua and hydroxo species in solution. The kinetics of the substitution of coordinated water and chloride (see Scheme 1) was investigated spectrophotometrically by following the change in absorbance at suitable wavelengths (Table S1, Supporting Information) as a function of time using UV−vis and stopped-flow techniques. tu, L-Met, and 5′GMP^{2-} were investigated as nucleophiles because of their different nucleophilicity, steric hindrance, binding properties, and biological relevance (structures shown in Figure 2). Not all reactions were studied for both complexes at pH 2 in order to save some of the compounds for measurements at pH 7.4.

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Table 3. Activation Parameters for the Reactions of Different Nucleophiles with the Multinuclear Pt(II) Complexes

complex	nucleophile	pH	$\Delta H_1^{\#}$ [kJ mol^{-1}]	$\Delta S_1^{\#}$ [J K^{-1} mol^{-1}]	$\Delta H_2^{\#}$ [kJ mol^{-1}]	$\Delta S_2^{\#}$ [J K^{-1} mol^{-1}]
BBR3610-(OH$_2$)$_2$	tu	2	46.4 ± 0.5	−76 ± 2	54 ± 2	−90 ± 8
	L-Met	2	59 ± 1	−59 ± 3	62 ± 3	−85 ± 11
BBR3464-(OH$_2$)$_2$	tu	2	46 ± 1	−77 ± 4		

The dependence of the observed rate constant on the nucleophile concentration and temperature resulted in rate constants and activation parameters ($\Delta H^{\#}$ and $\Delta S^{\#}$) for the displacement of coordinated water and chloride, which are summarized in Tables 1 and 3. The resulting rate constants from the ^{1}H NMR kinetic experiments for the reaction with 5′GMP^{2-} are presented in Table 2.

Reactions of BBR3464-(OH$_2$)$_2$ and BBR3610-(OH$_2$)$_2$ at pH 2. Under the selected experimental conditions, the substitution of the aqua ligand in each $[Pt(NH_3)_2(NH_2R)OH_2]^{2+}$ moiety involves two reaction steps, viz. k_1 and $k_{1'}$ (see Scheme 1), respectively. pK_a measurements on H_2O/H_2O, Cl/H_2O, and OH/H_2O species of $[\{trans\text{-}PtCl(NH_3)_2\}_2(\mu\text{-}NH_2(CH_2)_6NH_2)]^{2+}$ and **BBR3464** performed by Farrell et al.[23,25] showed a pK_a value of 5.62 for all coordinated water molecules on the end groups, independent of inherent electrostatic repulsion of the end groups (a combination of charge and $\{Pt(NH_3)_2\}-\{Pt(NH_3)_2\}$ repulsive interactions). One can therefore assume that the two positively charged $[Pt(NH_3)_2(NH_2R)OH_2]^{2+}$ groups, separated by either a $[\mu\text{-}trans\text{-}Pt(NH_3)_2\{NH_2(CH_2)_6NH_2\}_2]^{2+}$ (**BBR3464**) or a $[\mu\text{-}C_2H_4\text{-}\{NH_2(CH_2)_6NH_2\}_2]^{2+}$ (**BBR3610**) chain linker (a total of 17 and 18 atoms, respectively) act independently of one another, such that $k_1 = k_{1'}$. Therefore, in the remainder of this report k_1 and k_2 will be used to indicate the different reaction steps and include both k_1 and $k_{1'}$, and k_2 and $k_{2'}$, respectively.

a. Reactions with tu. Kinetic traces for reactions with tu showed a fast step (k_1) for the substitution of both water molecules and a very slow second step (k_2), both depending on the tu concentration. The latter step can be ascribed to the substitution of the now labilized amine chain linker, as a result of the strong trans effect of tu. Thus, the linker is liberated and the multinuclear structure destroyed.

Stopped-flow techniques were used to study the first reaction and tandem cuvettes in combination with UV−vis spectrophotometry for the second reaction step. The so-obtained constants, k_{obs1} and k_{obs2}, were plotted against the concentration of the entering tu nucleophile. A linear dependence of the observed rate constants on the tu concentration was found, as shown by way of example for **BBR3610-(OH$_2$)$_2$** in Figure 3. k_{obs1} and k_{obs2} can be expressed by eqs 1 and 2. With the exception of the second reaction step of the chloro complexes with L-Met, the studied reactions do not show an intercept, and therefore, the reactions are irreversible and k_{-1} and k_{-2} are practically zero.

$$k_{obs1} = k_1[Nu] + k_{-1} \approx k_1[Nu] \quad Nu = \text{L-Met, tu, 5′GMP}^{2-} \quad (1)$$

$$k_{obs2} = k_2[Nu] + k_{-2} \approx k_2[Nu] \quad Nu = \text{L-Met, tu, 5′GMP}^{2-} \quad (2)$$

For the first substitution step no difference between the values of k_1 for **BBR3610-(OH$_2$)$_2$** (Figure 3) and **BBR3464-(OH$_2$)$_2$** (see Figure S1, Supporting Information) was found within the experimental error limits (see Table 1). Thus, the additional Pt(II) center in **BBR3464-(OH$_2$)$_2$** has no influence on the reaction rate when compared to **BBR3610-(OH$_2$)$_2$**. The trans labilization and substitution of the chain linker by another molecule of tu is significantly slower by a factor of 150.

Jaganyi et al.[26] recently investigated the reactions of a series of dinuclear Pt(II) complexes of the type $[\{trans\text{-}Pt(H_2O)(NH_3)_2\}_2(\mu\text{-}NH_2(CH_2)_nNH_2)]^{4+}$ (n = 2, 3, 4, 6) with tu and derivatives thereof at pH 2. On increasing the chain lengths from $n = 2$ to 6, they found a decrease in the rate of substitution of water by tu (at 25 °C) from 336×10^{-3} (n = 2) to 31×10^{-3} M^{-1} s^{-1} ($n = 6$). On considering the chain linker with $n = 6$, one can assume that the $[Pt(NH_3)_2(NH_2R)OH_2]^{2+}$ groups act independently of one another,[23,25] such that the kinetic data for $n = 6$ can be compared to our results. This value agrees exactly with the value we found in our systems, viz. for **BBR3610-(OH$_2$)$_2$** $k_2 = (32.7 \pm 0.6) \times 10^{-3}$ M^{-1} s^{-1}, although not for the substitution of the coordinated water molecule but for the substitution of the now-labilized amine chain linker. Jaganyi's group found an exceptionally high value for the complex with $n = 2$ (viz. 336×10^{-3} M^{-1} s^{-1}).[26] Only this complex was investigated by stopped-flow technique, and the resulting value is of the order of magnitude known from the literature for the substitution of a water molecule by tu in complexes of the type $[PtA_3(H_2O)]^{2+}$.[27] In contrast, the reactions of the complexes with $n = 3$, 4, 6 were followed using UV−vis spectrophotometry such that it was impossible to detect reactions being as fast as the value found for $n = 2$. Therefore, it seems that the first reaction step was apparently already over (for n = 3, 4, 6) such that the second step, the substitution of the linker, was in fact observed. Thus, the effect of increasing chain length of the linker on the reaction rate seems to have been misinterpreted in their work.[26]

b. Reactions with L-Met. At pH 2 the carboxylate group in L-Met is protonated to an extent of ca. 50% on the basis of the acid dissociation constants for L-Met (pK_{COOH} = 2.28,[28] 2.13,[29] pK_{NH3+} = 9.2),[28] resulting in an overall charge of +1. The rest are present as zwitterions. For electrostatic reasons it is reasonable to expect that the reaction will occur between the zwitterionic form and the positively charged complex. The nucleophilic attack occurs via the sulfur donor of the thioether group. Since it is known that the pK_a values

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of L-Met decrease significantly upon coordination to a metal ion, we can assume that even if the charged L-Met molecule reacts with the complex, it will deprotonate and the product complex will be of the same overall charge as the reactant complex.

Substitution of the aqua ligands of **BBR3610-(OH$_2$)$_2$** by L-Met at 25 °C involves two concentration-dependent steps, with rate constants differing by a factor of 60; viz. $k_1 = (171 \pm 4) \times 10^{-3}$ M^{-1} s^{-1}, $k_2 = (2.84 \pm 0.05) \times 10^{-3}$ M^{-1} s^{-1}. It follows that k_1 is 29 times and k_2 12 times slower than for the reactions with tu as nucleophile. Plots showing representative results for **BBR3610-(OH$_2$)$_2$** are presented in Figure S3 (for UV−vis spectra see Figure S2, Supporting Information).

For reactions of mononuclear complexes of the type [PtA$_2$X$_2$] (X = anionic leaving group; A = ammonia, amine, pyridine) with L-Met, it is known that following the nucleophilic attack via the sulfur donor of the thioether group, a six-membered chelate is formed via ring-closure by the nitrogen donor of the amine group.[12] Such a chelation process should be independent of the L-Met concentration (i.e., $k_{obs2} = k_2$).[16] Since the second step of the reaction of **BBR3610-(OH$_2$)$_2$** with L-Met clearly shows a concentration dependence, it follows that the trans-positioned linker must be substituted by another molecule of L-Met in this step.

The degradation of the polynuclear structure into mononuclear Pt(II) complexes and the diamine linker was already reported for the reaction with the tripeptide glutathione and *N*-acetyl-L-methionine[1,2] and is shown also in this work. The goal with this new class of complexes fails if the multinuclear structure that leads to different DNA adducts compared to mononuclear complexes, and therefore may exhibit a unique reactivity pattern, is destroyed. The subsequent reactions of the mononuclear complexes will resemble biotransformation reaction products of *cis*- and *trans*-Pt(NH$_3$)$_2$Cl$_2$, under loss of the two ammonia molecules.[12,13] This is very important since the resulting reaction products (e.g., [Pt(L-Met-S,N)$_2$]) are inert to further substitution reactions and will therefore limit the active concentration of the drug.

Reaction of BBR3610 at pH 2. Experiments with the chloro complex **BBR3610** at pH 2 (for UV−vis spectra see Figure S4, Supporting Information) were performed in 0.1 M NaCl to suppress the spontaneous aquation reactions. Farrell et al.[23] calculated on the basis of the equilibrium and dissociation constants that in blood plasma, where the concentration of chloride is 103 mM,[30] only 0.3% of the complex is aquated. This data will allow a comparison to be made between the substitution kinetics of the neutral leaving group (water) and a charged chloride ion at the same pH. Furthermore, the substitution of chloride could in addition be performed at the biologically relevant pH of 7.4.

a. Reactions with tu. The chloro complex **BBR3610** shows linear dependences of the observed rate constants k_{obs1} and k_{obs2} on the tu concentration (see Figure 4; for a typical kinetic trace see Figure S5, Supporting Information). The

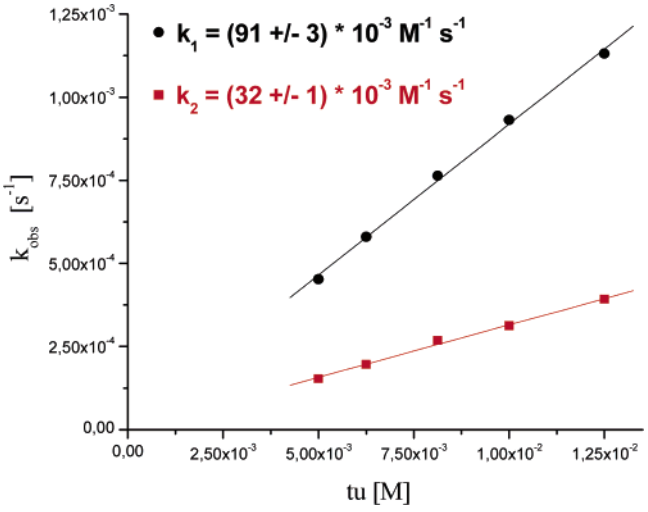

Figure 4. Plots of k_{obs} vs tu concentration for the reaction with **BBR3610** (0.125 mM) at 25 °C, $I = 0.1$ M (NaCl), pH = 2 and $\lambda = 310$ nm.

value of k_1 (at 25 °C) for **BBR3610-(OH$_2$)$_2$** decreases by a factor of ca. 50 on going to **BBR3610** (see Table 1).

This is consistent with the substitution of chloride in **BBR3610** in the first step, which is significantly slower than the substitution of a water molecule, whereas the value for k_2 was found to be the same as for **BBR3610-(OH$_2$)$_2$**, indicating a process that seems to be independent of the leaving group in the first step and involves displacement of the linker in the second step.

b. Reactions with L-Met. The substitution of chloride in **BBR3610** by L-Met is slower by a factor of 8 compared the substitution of a water molecule. The value for k_2 is expected to be the same as for the diaqua complex since it involves the displacement of the linker. k_2 was found to be $(5.3 \pm 0.2) \times 10^{-3}$ M^{-1} s^{-1} and is slightly higher than the value of k_2 for the diaqua complex, viz. $(2.84 \pm 0.05) \times 10^{-3}$ M^{-1} s^{-1}. The reason for this could be the difference in ionic strength, as it was $I = 0.01$ M (HOTf) for the aqua species and $I = 0.1$ M (NaCl) for the chloro complexes.

It can be seen from Figure S6, Supporting Information, that an intercept for both reaction steps was found. In the case of the first reaction step, $k_{-1} = (1.9 \pm 1.7) \times 10^{-5}$ s^{-1}, is negligible in terms of the large experimental error. For the second reaction k_{-2} is more significant (see Table 1). The intercept can be interpreted in terms of a small fraction of a parallel reaction independent of the L-Met concentration which leads to the release of the linker group. Since 0.1 M NaCl was used to suppress the aquation reaction of the chloro complex, the excess of Cl$^-$ over the nucleophile L-Met could lead to a small fraction of the linker being displaced by Cl$^-$. Due to the fact that the bond to the linker is labilized by the trans-located L-Met nucleophile after completion of the first reaction, it seems reasonable to assume that the linker can be substituted by Cl$^-$ and liberated under protonation.

Reactions of BBR3464 and BBR3610 at pH 7.4. In terms of the application of Pt(II) complexes in cancer therapy, investigations at the physiological pH of 7.4 are of importance. To work under biorelevant conditions, experiments with **BBR3610** and **BBR3464** were performed at 37.5 °C

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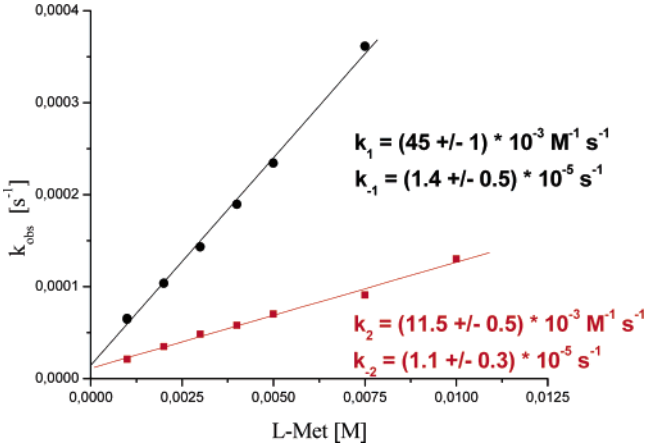

Figure 5. Plots of k_{obs} vs L-Met concentration for the reaction with **BBR3610** (0.125 mM) at 37.5 °C, pH = 7.4, I = 0. 1 M (NaCl), 2.5 mM HEPES and λ = 260 nm.

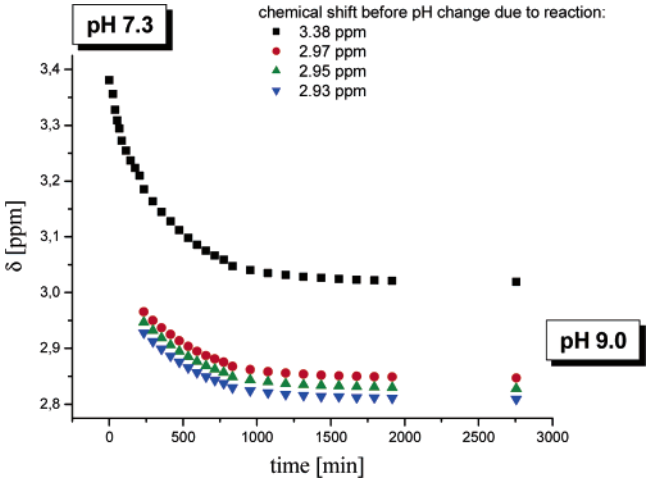

Figure 6. Time dependence of the chemical shift observed for two sets of resonances for the 1/1′ and 7/7′ protons (see Figure S13) of 5 mM **BBR3610** during the reaction with 20 mM L-Met at 37.5 °C in 0.1 M NaCl.

in 0.1 M NaCl and 2.5 mM HEPES buffer. This buffer was chosen as it is sterically more crowded than, for example, Tris buffer (tris-(hydroxymethyl)-aminomethane) and does, therefore, not coordinate to Pt(II) as efficiently as Tris.[31] Both buffers are used in cell tests and DNA binding studies of Pt(II) drugs.

a. Reactions with tu. Since tu is a neutral nucleophile, no differences compared to the measurements performed at pH 2 are to be expected. The measurements with **BBR3464** were done at two temperatures (25 and 37.5 °C) for a better comparison with the values at pH 2. The resulting values (see Table 1) clearly confirm the pH independence of the two step mechanism proposed on the basis of the experiments performed at pH 2 (for concentration dependences see Figures S7 and S8, Supporting Information).

b. Reactions with L-Met. At pH 7.4 the reaction will occur between the zwitterionic form (pK_{COOH} = 2.28,[28] 2.13,[29] pK_{NH3+} = 9.2[28]) and the positively charged complex. Since we already made the assumption at pH 2 that it will be the fraction of the zwitterion that reacts with the complex, we expect to find a similar behavior at pH 7.4. By way of example, kinetic data for **BBR3610** are shown in Figure 5, from which it follows that the substitution of the chloro ligands in both complexes by L-Met at pH 7.4 and 37.5 °C involves two reaction steps with rate constants that differ only by a factor of 4 (see Table 1). For further information on observed spectral changes, kinetic traces, and plots for the concentration dependence of k_{obs}, see Figures S9−S12.

The substitution of chloride by the sulfur donor ligand L-Met occurs 5 times slower than with tu in the first step. The second step is even 8 times slower, indicating that tu is a stronger trans-labilizing nucleophile than L-Met. Again, for the second process a significant intercept was found, which can be ascribed to the contribution of a parallel reaction of the complex with Cl^- instead of a second L-Met molecule. The reaction of **BBR3610** with L-Met at pH 7.4 in 0.1 M NaCl was also investigated by NMR techniques to confirm the release of the linker during the substitution process. Intentionally no buffer was used in this experiment so that pH changes could be detected, and an increased activation rate reported for phosphate buffer[32] could be prevented.

The continuous shift of signals, as for the linker, with time (see Figures 6 and S13, Supporting Information, for peak assignment see Figures 1 and 2) can be ascribed to changes in pH. The pH of the solution was adjusted to 7.4 prior to the experiment and increased from 7.4 to 9 until completion of the reaction, which indicates the formation of OH^- during the reaction. Figure S13 shows ^{1}H NMR spectra over a time range of 380 h. The peak of the free S−CH_3 group at 2.143 ppm disappears with time and appears further downfield for the bound S−CH_3 group (2.595 ppm). The broad signals at 3.381 (BBR3610-linker 1,1′-CH_2) and 3.089 ppm (BBR3610-linker 7,7′-CH_2) shift upfield (see Figure 6) and result in a singlet at 3.022 ($linker_{free}$, 1,1′-CH_2) and a triplett at 2.832 ppm ($linker_{free}$, 7,7′-CH_2) with vicinal coupling constants ^3J(H7−H7′) = 7.6 ± 0.1 Hz. These signals correspond to the 1,1′- and 7,7′-CH_2 protons of the liberated aliphatic diamine linker, which undergo protonation (see pK_a values for aliphatic diamines[33]) under formation of OH^-. The retarded shift of α-CH of L-Met resembles deprotonation of the amine due to coordination to the Pt center.

c. NMR Kinetics of BBR3464 and BBR3610 with 5′GMP^{2-}. ^{1}H NMR spectroscopy was used to investigate the substitution reactions with 5′GMP^{2-} in aqueous solution at pH* 7.4 and 310 K. To confirm the assumption that the two positively charged $[Pt(NH_3)_2(NH_2R)Cl]^+$ halves, separated by a total of 17 (**BBR3464**) and 18 atoms (**BBR3610**), react independently of one another, the kinetics of the substitution of the first and the second chloride ligand (k_1 and $k_{1'}$) were studied separately, each in a 1:1 complex/nucleophile molar ratio. Although we formally have a 1:1 complex/nucleophile molar ratio in the first step (k_1), the relative amount of available sites for the attacking nucleophile is 2:1. Consider-

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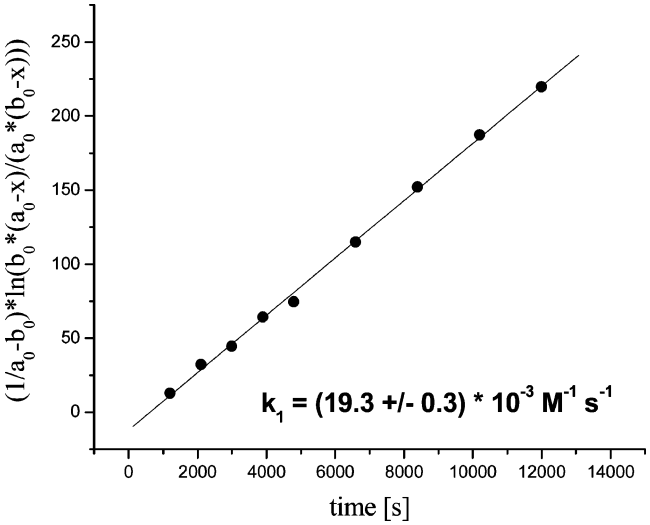

Figure 7. Second-order plot for the reaction of **BBR3464** (5 mM, available sites for substitution: 10 mM) with 5′GMP^{2-} (5 mM). The *y* axis presents the right-hand side term of eq 3. pH = 7.4, I = 0.1 M (NaCl), T = 37.5 °C.

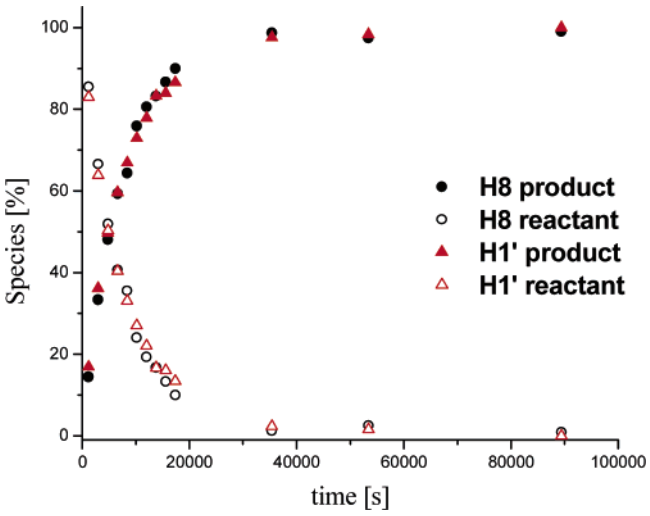

Figure 8. Time dependence of the concentrations (as a percentage of the total 5′GMP^{2-} concentration) of the reactant and product peaks (using the H8 and H1′ protons) during the reaction of 5 mM **BBR3610** with 5 mM 5′GMP^{2-}. pH = 7.4, I = 0.1 M (NaCl), T = 37.5 °C.

ing that $c_{a0} \neq c_{b0}$, eq 3 was applied to determine k_1. The amount of the 1:1 product, i.e., **BBR 3610**−5′GMP, is represented by x, and a_0 and b_0 are the initial concentrations of 5′GMP^{2-} and the complex, respectively.[34]

$$k_1 t = \frac{1}{c_{a0} - c_{b0}} \ln \frac{c_{b0}(c_{a0} - x)}{c_{a0}(c_{b0} - x)} \qquad (3)$$

A plot of the right side of eq 3 versus time results in a straight line passing through the origin, as shown by way of example for **BBR3464** in Figure 7 (for **BBR3610** see Figure S14, Supporting Information). The value of k_1 (M^{-1} s^{-1}) is obtained from the slope of this plot (see Table 2). A typical plot of the concentrations of reactant and product as a function of time, as obtained from the integration of the H8

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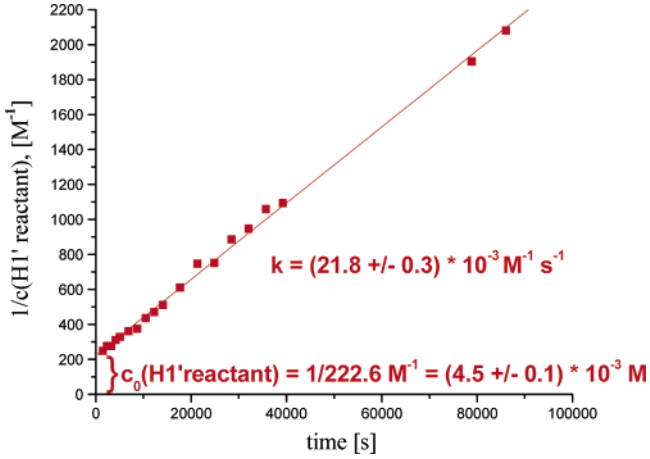

Figure 9. Second-order plot of $1/c_A$ vs time for the reaction of **BBR3464** (4.5 mM) with 5′ GMP^{2-} (4.5 mM). The slope equals $k_{1'}$ (M^{-1} s^{-1}) and the intercept is $1/c^0_A$. pH = 7.4, I = 0.1 M (NaCl), T = 37.5 °C.

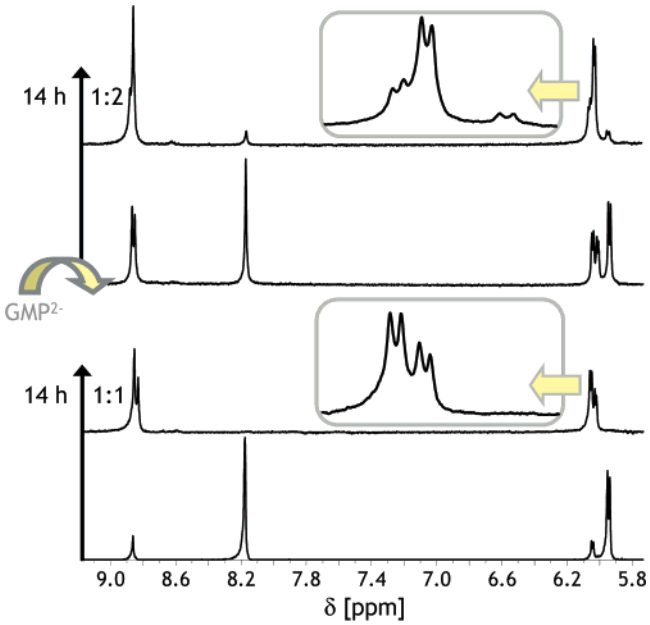

Figure 10. ^{1}H NMR spectra of the H8 (left) and H1′ (right) signals during the reaction of 5 mM 5′GMP^{2-} with 5 mM **BBR3610** at 37.5 °C in 0.1 M NaCl and pH = 7.4. After completion of the first (1:1) substitution step, an equimolar amount of 5′GMP^{2-} in 0.1 M NaCl/D_2O/DOTf was added to give a final concentration of 4.5 mM for both reactants in the second substitution step. The insets show two reaction product peaks for both steps, respectively.

proton and the doublet of the H1′ proton (for peak assignment see Figure 2), is presented in Figure 8.

For the substitution of the second chloro ligand, another molar ratio of 5′GMP^{2-} was added to the sample after completion of the first reaction step, which is now the monosubstituted **BBR**−5′GMP complex. This corresponds to a 1:1 ratio of available sites for the nucleophile. Under the special circumstance that $c_{a0} = c_{b0}$, eq 4 was applied to determine the rate constants of the second step (k_1′).

$$\frac{1}{c_a} = \frac{1}{c_{a0}} + kt \qquad (4)$$

The plot of $1/c_a$ vs t is linear with a slope equal to $k_{1'}$ (M^{-1} s^{-1}) and the intercept being $1/c_{a0}$,[34] as can be seen in

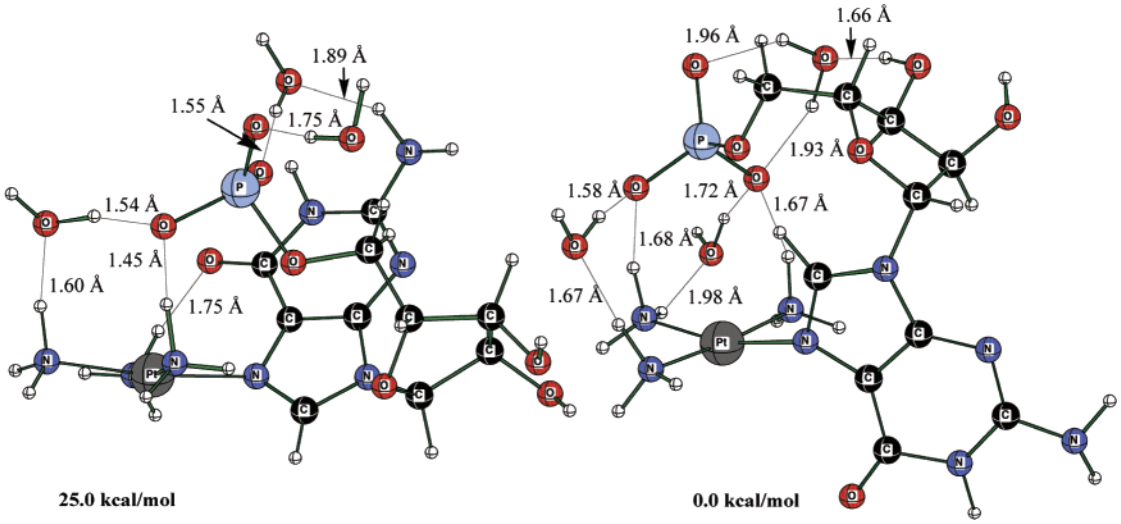

Figure 11. Calculated conformers (B3LYP/LANL2DZp) of a model compound for the reaction product of 5′GMP^{2-} with multinuclear complexes (1:1), namely [Pt(NH$_3$)$_3$-N75′GMP]·3H$_2$O.

Figure 9 (for **BBR3610** see Figure S15, Supporting Information).

The resulting values for the rate constants are summarized in Table 2. The rate constants for the first and second substitution reactions of chloride on each of the [Pt(NH$_3$)$_2$(NH$_2$R)Cl]$^+$ halves were found to be equal and independent of each other.

The N-donor nucleophile 5′GMP^{2-} exhibits an affinity for Pt(II) complexes which is of the same order of magnitude as the S-donor L-Met under these conditions (see rate constants in Tables 1 and 2). The reaction with L-Met is faster, but only by a factor of 2. This points to a direct attack of the nucleophile 5′GMP^{2-} on the Pt(II) center and may be of importance in the DNA binding mechanism of multinuclear Pt(II) drugs.

In comparison to the sulfur-containing nucleophiles tu and L-Met, 5′GMP^{2-} does not labilize the trans-positioned amine significantly, and therefore this step (k_2 and k_2') was not investigated. The kinetics of the reaction of **BBR3464**/**BBR3610** with 5′GMP^{2-} (k_1) and **BBR3464**−5′GMP/**BBR3610**−5′GMP with 5′GMP^{2-} ($k_{1'}$) to give the disubstituted complex, was followed by measuring the intensities of the singlet of the H8 proton and the doublet of the H1′ proton, respectively (see Figure 10), as this region is most suitable to reflect the spectral changes associated with coordination at N7 (see Figure 2).

The first spectrum after mixing **BBR3610** and 5′GMP^{2-} in the ratio 1:1 shows a downfield-shifted product peak for the H8 proton at 8.862 ppm and for the H1′ doublet at 6.043 ppm. During the reaction both signals split into two sets of signals at 8.833 and 8.856 and at 6.022 and 6.0582 ppm with vicinal coupling constants ^3J(H1′−H2′) = 4.6 ± 0.1 Hz. The more downfield-shifted signals reach 66% of the total product integral in comparison to 33% for the upfield located signals at the end of the first substitution step. In the time between completion (175 h) of the first step and the start of the second step, the ratio of the two signals changes toward 54% (downfield) and 46% (upfield), indicating a slow interconversion process. Addition of an equimolar concentration of 5′GMP^{2-} for the substitution of the second chloro ligand leads to an enhanced growth of the signals at 6.022 and at 8.833 ppm, respectively.

After completion of the overall reaction of **BBR3610** with 5′GMP^{2-}, a ^{1}H NMR spectrum was recorded at 80 °C (see Figure S16, Supporting Information). On the assumption that the two doublets for the H1′ protons result from two different isomers, one could expect the protons to exchange with each other at elevated temperatures, resulting in a joint signal. Indeed, at 80 °C both doublets shift closer together. This indicates that the magnetic field experienced by the protons becomes similar and it corroborates the assumption that the existence of two sets of signals for the reaction product can be attributed to different possible rotamers of 5′GMP^{2-} attached to the Pt(II) center.

We therefore computed the energies of a model compound of the reaction product of 5′GMP^{2-} with the multinuclear complexes, namely [Pt(NH$_3$)$_3$-N7-5′GMP]·3H$_2$O (see Figure 11). Only the secondary chain amine was simplified with a primary amine. Three water molecules were included in the calculations to model the influence of H-bonded solvent molecules. We computed (RB3LYP/LAN2DZp)[35−42]

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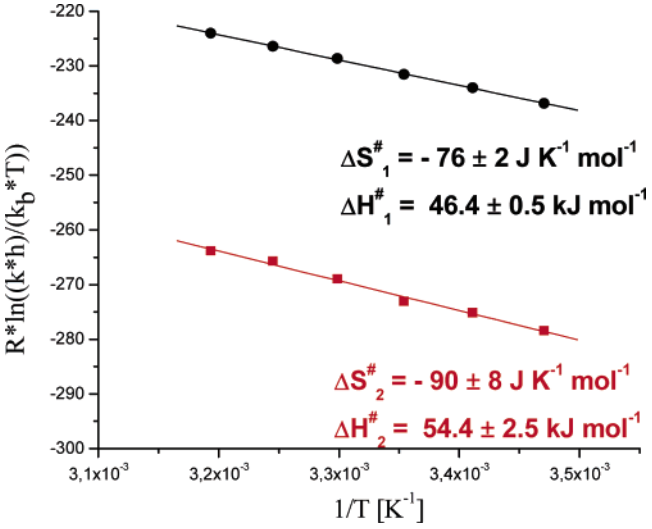

Figure 12. Eyring plots for the two reaction steps of **BBR3610-(OH$_2$)$_2$** with tu at pH = 2, I = 0.01 M (HOTf) and λ = 300 nm.

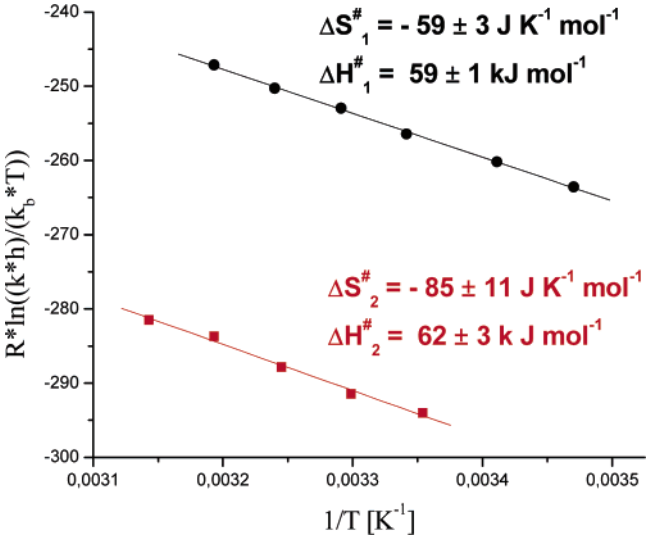

Figure 13. Eyring plots for the two reaction steps of **BBR3610** with L-Met at pH = 2, I = 0.01 M (HOTf) and λ = 250 nm.

two possible rotamers with a stability difference of 25 kcal/mol. As can be seen from Figure 11, H-bonds play a major role in the stability of the different rotamers. The more stable rotamer exhibits in total nine H-bonds: seven of them between the water molecules and the amines (2), the phosphate group (4) and the hydroxyl group of the sugar moiety (1); two intramolecular H-bonds between the phosphate group and the amine. The H-bonds have a big impact on the steric position of the 5′GMP^{2-} purine ring, relative to the plane of the complex, and therefore on the signals that were followed in the proton NMR. This indicates that H-bonds mediate the conformation of the metal drug and furthermore the interaction with the final target DNA.

Activation Parameters at pH 2. The thermal activation parameters were determined through a systematic variation of temperature. The values were calculated using the Eyring equation and are summarized in Table 3. Figures 12 and 13 show typical Eyring plots for the reaction of **BBR3610-(OH$_2$)$_2$** with tu and L-Met at pH 2 (for **BBR3464-(OH$_2$)$_2$** see Figure S17, Supporting Information). The negative $\Delta S^{\#}$ values are indicative of an associative substitution mechanism, which is well known from the literature[27,16] for square-planar Pt(II) systems.

The second substitution step in the reaction with tu and L-Met shows a more negative activation entropy compared to the first step. This can be ascribed to the cleavage of the linker, which results in formation of charge due to the protonation of the diamine linker and generation of OH$^-$. Furthermore, it can be interpreted in terms of a stronger associative character of the transition state due to the S-containing ligand, which is present after completion of the first step and enhances the stabilization of the incoming electron density of another S-containing nucleophile in the second step.

The apparent misinterpretation of the rate constants for the reactions of a series of dinuclear Pt(II) complexes of the type [{*trans*-Pt(H$_2$O)(NH$_3$)$_2$}$_2$(μ-NH$_2$(CH$_2$)$_n$NH$_2$)]$^{4+}$ (n = 2, 3, 4, 6) with tu and derivates thereof at pH 2 mentioned before[26] can also be seen in the interpretation of the activation parameters. The reported activation parameters for the reaction of the n = 6 complex with tu are $\Delta H^{\#}$ = 57 ± 1 kJ mol^{-1} and $\Delta S^{\#}$ = −85 ± 2 J K^{-1} mol^{-1}. These correspond within the experimental error limits much better to the values we found for the second reaction step in our system, viz. $\Delta H_2^{\#}$(**BBR3610-(OH$_2$)$_2$**) = 54 ± 3 kJ mol^{-1} and $\Delta S_2^{\#}$-(**BBR3610-(OH$_2$)$_2$**) = −90 ± 8 J K^{-1} mol^{-1}, than to those for the first reaction step, viz. $\Delta H_1^{\#}$(**BBR3610-(OH$_2$)$_2$**) = 46 ± 1 kJ mol^{-1} and $\Delta S_1^{\#}$(**BBR3610-(OH$_2$)$_2$**) = −76 ± 2 J K^{-1} mol^{-1}.

Conclusions

The results of this study clearly demonstrate that the substitution of the leaving group by the S-donor nucleophiles tu and L-Met in the first step, induces a second process: the labilization of the Pt−N bond in the trans position to the bound S-donor and thus, the displacement of the linker by a second S-donor during degradation. The degradation of **BBR3464** upon coordination of sulfur was already reported by Farrell's group.[1,2] It is well known from the literature that in mononuclear complexes, nonchelated ammines (carrier ligand) as in cisplatin, are more reactive toward substitution and hydrolysis compared to chelated amines (e.g., Oxaliplatin). Furthermore, the trans configuration of this type of polynuclear complexes facilitates the labilizing effect of strong donor nucleophiles.

In the present studies tu was found to be the strongest nucleophile, followed by L-Met and 5′GMP^{2-}, whereas the rate constants show that the N-donor 5′GMP^{2-} reacts in the same order of magnitude as the S-donor L-Met.

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In the investigated time frame, degradation upon binding of 5′GMP^{2-} was not observed, only the substitution of the leaving group Cl^-. 5′GMP^{2-} turned to be a very efficient nucleophile that indeed competes with L-Met under physiological conditions. In 0.1 M NaCl, no prior aquation step but a direct attack of the nucleophile on the Pt(II) center was found. The substitution of chloride on each $[Pt(NH_3)_2(NH_2R)Cl]^+$ half was found to be identical and independent of each other.

The mechanism of the substitution reactions is associative in nature as supported by the large and negative values of $\Delta S^{\#}$. The transition state of the linker cleavage seems to be more compact than that of the first step, indicated by significantly larger negative values of $\Delta S^{\#}$. Finally, no difference was found in the reaction rates and mechanistic behavior for **BBR3464** and **BBR3610**.

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Supporting Information Available: Table S1 summarizes the selected wavelengths for the kinetic measurements. Figures S1−S12 show different UV−vis spectral changes, kinetic traces, and concentration dependences. Figure S13−S15 report second-order plots obtained from ^{1}H NMR measurements for the reactions with 5′GMP^{2-}. Figure S16 compares the ^{1}H NMR spectrum of the product of the reaction between **BBR3610** and 5′GMP^{2-} at different temperatures. Figure S17 summarizes the temperature dependence of k_1 for the reaction of **BBR3464** with tu. This material is available free of charge via the Internet at http://pubs.acs.org.

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