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Platinum(II) complexes with hybrid amine-imidazolin-2-imine ligands and their reactivity toward bio-molecules

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Two new Pt(II) complexes with imidazolin-2-imines as ancillary ligands, [Pt(DMEAIm^{iPr})Cl$_2$] and [Pt(DPENIm^{iPr})Cl$_2$], were synthesized and characterized. Substitution reactions of these complexes with nucleophiles – thiourea (TU), L-methionine (L-Met), L-histidine (L-His) and guanosine-5'-monophosphate (5'-GMP) were carried out in 25 mM Hepes buffer in the presence of 30 mM NaCl. The reactions were monitored using variable-temperature UV-Vis spectrophotometry and were followed under *pseudo*-first-order conditions with a large excess of nucleophiles. Slightly higher reactivity was found for [Pt(DMEAIm^{iPr})Cl$_2$], while the reactivity of the nucleophiles decreased in the order TU > L-Met > L-His > 5'-GMP. The negative values reported for the entropy of activation confirmed an associative substitution mode. Spectrophotometric acid-base titrations were performed to determine the pK_a values of the coordinated water molecules in the diaqua complexes [Pt(DMEAIm^{iPr})(H$_2$O)$_2$]$^{2+}$ and [Pt(DPENIm^{iPr})(H$_2$O)$_2$]$^{2+}$. Solubility measurements revealed good solubility of the studied imidazolin-2-imine complexes in water. The crystal structure of [Pt(DMEAIm^{iPr})Cl$_2$] was determined by X-ray diffraction analysis. The coordination geometries around the platinum atoms are distorted square-planar; the [Pt(DMEAIm^{iPr})Cl$_2$] complex displays Pt-N distances of 2.0162(19) and 2.0663(19) Å. Attempts to coordinate Au(III) ions to different imidazolin-2-imine ligands did not result in the formation of coordination complexes, but rather in the reduction of the Au(III) precursor. This was evidenced by the X-ray crystal structure of [(DACH(Im^{iPr}H)$_2$)(AuCl$_2$)$_2$], which formed during the reaction of KAuCl$_4$ with the ligand DACH(Im^{iPr})$_2$.

1. Introduction

The platinum group metals, Pt(II), Pd(II) and Au(III), are valence isoelectronic and often form characteristic isostructural square-planar complexes.[1] Accordingly, the chemical behavior in solution of structurally analogous Pt(II) and Pd(II) complexes is very similar.[2,3] Since the late 1970s, platinum(II) complexes are well-known for their anti-tumor activity.[4,5] In the past 50 years vast variety of structurally different platinum complexes were synthesized and tested, but platinum drug resistance and toxic side effects represent a limiting factor and continuing challenge.[4-8] The research field of platinum complexes as anti-tumor drugs is still growing and going in direction to design novel platinum drug with distinctly different structural and mechanistic profiles in comparison with cisplatin. In addition, research has been focused on the synthesis and investigation of complexes of different metal ions with the goal to establish compounds with good anti-tumor properties but with no side-effects and resistant such have shown platinum anti-tumor drugs.[6-8] In recent years, the anti-tumor activity of some Pd(II) complexes has been confirmed,[9-11] and also square-planar Au(III) compounds have shown to be excellent candidates for anticancer evaluation.[12,13]

Factors that influence the properties of the complexes such as geometry, steric hindrance, flexibility, electronic effects, and lipophilicity are important to be considered during the drug design. In an earlier publication,[14] we were able to show that the use of hybrid amine-imidazolin-2-imine as strong N-donor ligands[15-17] afforded Pd(II) complexes with improved properties such as water solubility and lower reactivity toward small bio-molecules, which should lead to a more selective distribution of these complexes in the human body.[14] Therefore, it was of interest to introduce imidazolin-2-imines in the coordination sphere of Pt(II) and Au(III) complexes and follow the way how this will affect the characteristics of such complex under physiological conditions as well as their reactivity towards small bio-molecules.

In this paper, we present the synthesis of imidazolin-2-imine Pt(II) complexes and their interactions with small bio-molecules, in an attempt to define preliminary structure-function relationships within

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this new class of imidazolin-2-imine complexes. In addition, we report attempts to prepare imidazolin-2-imine Au(III) complexes.

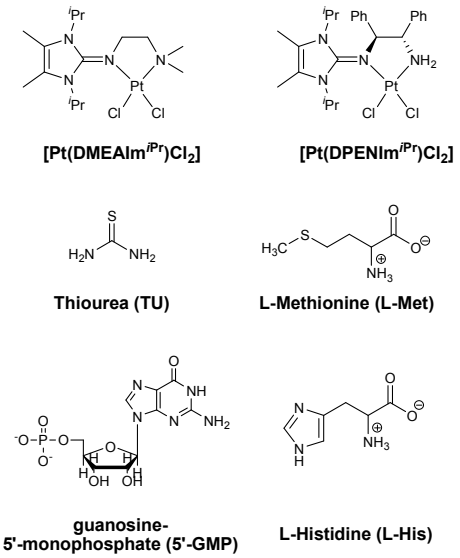


Figure 1. Structures of the investigated Pt(II) complexes and nucleophiles, along with their abbreviations.

2. Results and Discussion

2.1. Pt(II) imidazolin-2-imine complexes

2.1.1. Complex synthesis

The Pt(II) complexes [Pt(DMEAIm^{iPr})Cl$_2$] and [Pt(DPENIm^{iPr})Cl$_2$] were synthesized by stirring equimolar amounts of K_2PtCl_4 and the respective imidazolin-2-imine ligand in THF. The complexes were characterized by ^{1}H and ^{13}C NMR spectroscopy, elemental analysis and ESI-MS mass spectrometry. The mass spectrum of [Pt(DPENIm^{iPr})Cl$_2$] in the *m*/*z* range of 200−600 includes main peaks at *m*/*z* = 293(2+) and 621(1+) which correspond to [Pt(DPENIm^{iPr})]$^{2+}$, [Pt(DPENIm^{iPr})Cl]$^+$ and represent characteristic fragments of the [Pt(DPENIm^{iPr})Cl$_2$] complex, (see Figure S1, ESI Supporting Information). The ^{1}H and ^{13}C NMR spectra indicate hindered rotation along the imine C-N bond on the NMR timescale at room temperature. Therefore, the C_s-symmetric [Pt(DMEAIm^{iPr})Cl$_2$] complex exhibits two doublets in the ^{1}H NMR spectrum, which can be assigned to the diasterotopic methyl groups of the isopropyl substituents. In contrast, C_1-symmetry of the chiral complex [Pt(DPENIm^{iPr})Cl$_2$] affords diastereotopic isopropyl groups, which gives rise to four doublets in the ^{1}H NMR spectrum. Such hindered rotation around the imine C-N bond was previously observed for related complexes.[15-18]

Orange single-crystals of [Pt(DMEAIm^{iPr})Cl$_2$] suitable for X-ray diffraction analysis were obtained from chloroform/diethyl ether solution, and the molecular structure is shown in Figure 2. The molecule crystallizes in the monoclinic space group $P2_1/n$, with two co-crystallized molecules of $CHCl_3$ per unit. The diimine ligand is coordinated to the platinum(II) ion in a chelating, bidentate fashion with an N1-Pt-N2 bite angle of 83.04(8)°. The Pt-N bond lengths are 2.0162(19) Å and 2.0663(19)Å for Pt-N1 and Pt-N2, respectively, indicating stronger coordination of the more basic imine nitrogen to the Pt atom. The electron-donating capacity of the imidazolin-2-imine is also reflected in the different Pt-Cl bond lengths, with the Pt-Cl1 (2.3368(6)Å) *trans* to the imine nitrogen being notably longer than the Pt-Cl2 (2.3076(5)Å) *trans* to the tertiary amine. These structural parameters are comparable to those observed for [Pd(DMEAIm^{iPr})Cl$_2$], [Pd(DPENIm^{iPr})Cl$_2$] and [Pd(BL^{iPr})Cl$_2$].[14,19]

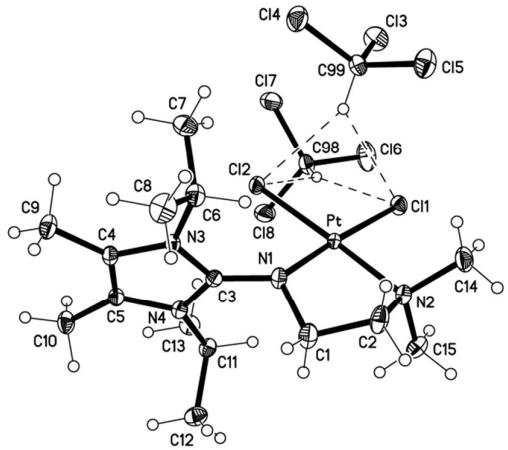


Figure 2. ORTEP drawing of [Pt(DMEAIm^{iPr})Cl$_2$]·2CHCl$_3$ with thermal displacement parameters drawn at 50% probability. Hydrogen atoms were omitted for clarity. Selected bond distances [Å] and angles [°] and contacts: Pt-N1 2.0162(19), Pt-N2 2.0663(19), Pt-Cl1 2.3076(5), Pt-Cl2 2.3368(6), C3-N1 1.365(3), C3-N3 1.351(3), C3-N4 1.350(3); N1-Pt-N2 83.04(8), Cl1-Pt-Cl2 90.68(2); H99···Cl1 2.79, C99-H99···Cl1 129.9, H99···Cl2 2.62, C99-H99···Cl2 150.3, H98···Cl1 2.64, C98-H98···Cl1 149.1, H98···Cl2 2.79, C98-H98···Cl2 135.1.

2.1.2. Solubility of the Pt(II) imidazolin-2-imine complexes and pK_a determination of the aqua Pt(II) complexes

Like the previously reported imidazolin-2-imine Pd(II) complexes,[14] the Pt(II) complexes reported herein show good solubility in water, as evidenced by UV-Vis spectrophotometric measurements (Table 1). The solubility of the Pt(II) complexes is greater than observed for cisplatin and oxaliplatin (see Table 1). Thus, the introduction of different imidazolin-2-imines affords Pt(II) complexes with satisfactory solubility in water. Good solubility in water is very important in designing metallo-drugs.

Table 1. Water solubility of Pt(II) and Pd(II) imidazolin-2-imine complexes at 298 K.

Complex	Solubility in water at 298 K mg/ml
$[Pt(DMEAIm^{iPr})(H_2O)_2]^{2+}$	9.8
$[Pt(DPENIm^{iPr})(H_2O)_2]^{2+}$	9.6
$[Pd(DMEAIm^{iPr})(H_2O)_2]^{2+}$,[14]	10.2
$[Pd(DPENIm^{iPr})(H_2O)_2]^{2+}$,[14]	10.1
Cisplatin[A]	2.5
Oxaliplatin[B]	5.0

[A] The Merck Index, 12th ed., Entry# 2378
[B] According to Sigma-Aldrich

The pK_a values of the complexes in aqueous solution were determined. This was performed *via* UV-Vis spectrophotometric pH titration with NaOH as a base in the pH range between 2 and 12. Each pK_a titration was performed twice and the average of both values was taken. The Figure 3 and S2 (ESI, Supportimg Information) show plots of absorbance versus pH at specific wavelengths, which was used to determine the pK_a values of the coordinated water molecules. The data were fitted using a nonlinear least-squares procedure, as shown in the insets in Figure 3 and S2. The overall process can be presented by Eqs (2) and (3). The titration data for the complexes were fitted to the following Eq. (1) for the determination of both pK_a values,[14,20-22] and the obtained data are presented in Table 2.

$$y = a + (b - a)/(1 + 2.718*((x - pK_{a1}/m) + (c - b)/(1 + 2.718*((x - pK_{a2})/n)) \quad (1)$$

The parameter a represents the value of the absorbance at the beginning of the titration, b represents the absorbance during the titration and c is the absorbance at the end of the titration. The parameters m and n are used to optimize the titration curve. In this equation y represents absorbance value and x refers to the pH. The data obtained for the pK_a values are summarized in Table 2.

$$[Pt(L)(H_2O)_2]^{2+} + H_2O \overset{K_{a1}}{\rightleftharpoons} [Pt(L)(OH)(H_2O)]^{+} + H_3O^{+} \quad (2)$$

$$[Pt(L)(OH)(H_2O)]^{+} + H_2O \overset{K_{a2}}{\rightleftharpoons} [Pt(L)(OH)_2] + H_3O^{+} \quad (3)$$

L = DMEAIm^{iPr}, DPEN(Im^{iPr})NH$_2$

Table 2. Summary of pK_a values obtained for the stepwise deprotonation of the coordinated water ligands in imidazolin-2-imine Pt(II) complexes.

	pK_{a1}	pK_{a2}
$[Pt(en)(H_2O)_2]^{2+}$,[22]	5.97	7.47
cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$,[22]	5.37	7.21
$[Pt(DMEAIm^{iPr})(H_2O)_2]^{2+}$	5.44 ± 0.15	7.73 ± 0.10
$[Pt(DPENIm^{iPr})(H_2O)_2]^{2+}$	6.11 ± 0.20	9.05 ± 0.20
$[Pd(DMEAIm^{iPr})(H_2O)_2]^{2+}$,[14]	5.75 ± 0.10	8.28 ± 0.10
$[Pd(DPENIm^{iPr})(H_2O)_2]^{2+}$,[14]	7.17 ± 0.20	11.21 ± 0.10

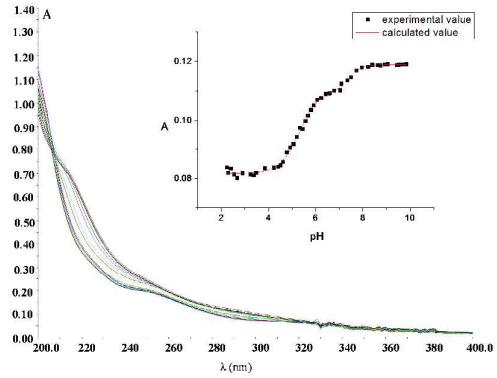
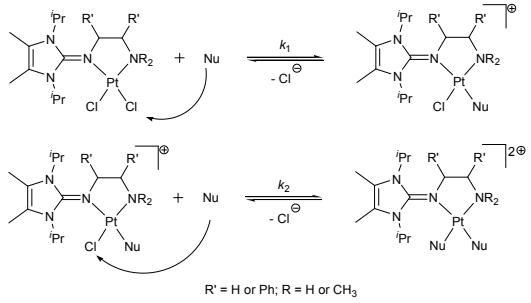


Figure 3. UV-vis spectra recorded for 0.1 mM $[Pt(DMEAIm^{iPr})(H_2O)_2]^{2+}$in the pH range 2 to 12 at 25 °C. Insert: Plot of absorbance *vs* pH at 230 nm.

For comparison, the data for $[Pt(en)(H_2O)_2]^{2+}$ and cisplatin have been included in Table 2 as they represent typical pK_a values for complexes coordinated by sp^3-hybridized amines.[23] It can be seen from Table 2 that both the Pt(II) as well as the Pd(II) complexes with imidazolin-2-imine ligands give higher pK_a values than cisplatin. This can be attributed to the electron-rich nature of the metal centers in these complexes induced by the electron-donating capacity of the imidazolin-2-imines. The studied complexes, $[Pt(DMEAIm^{iPr})(H_2O)_2]^{2+}$ and $[Pt(DPENIm^{iPr})(H_2O)_2]^{2+}$ have two different types of donors, *viz.* an imidazolin-2-imine moiety and an sp^3-hybridized primary amine unit. Therefore, it can be assumed that the first aqua ligand to be deprotonated would be that *trans* to the less electron donating amine donor. By comparing the pK_a values obtained for the Pt(II) and Pd(II) complexes bearing the same imidazolin-2-imines (Table 2), it can be noted that the Pd(II) imidazolin-2-imine complexes show higher pK_a values than the analogous Pt(II) complexes.

2.1.3. Kinetic studies

The substitution of two Pt(II) complexes with selected nucleophiles (Figure 1) was investigated and found to proceed in two successive reaction steps that are both dependent on the nucleophile concentration as presented in Scheme 1 (except for the substitution reaction with L-Met, see text below). The change in absorbance was followed, at suitable wavelengths, as a function of time at 310 K and pH ≈ 7.2. L-Methionine and L-His are essential amino acids, while 5'-GMP is the fragment of nucleic acid. Thiourea is used as a protective and rescue agent to prevent side effects which are caused by Pt(II) antitumor drugs.[24,25] Therefore, these compounds are useful to obtain more insights in the way how these Pt(II) complexes interact with bio-molecules, which can help to evaluate the possibility of applying them as anti-tumor agents.



Scheme 1. Nu = TU, L-Met, L-His and 5'-GMP

The substitution reactions of square-planar metal complexes can proceed according to two parallel pathways.[26] One involves the formation of a solvent-coordinated complex, e.g. a diaqua complex, followed by rapid substitution of the coordinated solvent by the entering nucleophile (solvolytic pathway), whilst the other involves a direct nucleophilic attack by the entering nucleophile. To suppress the solvolytic pathway, a 30 mM NaCl solution was added (see ESI, Supporting Information, Figure S3). The rate constants for substitution could be determined, under *pseudo*-first-order conditions from a plot of the linear dependence of k_{obsd} *versus* the total nucleophile concentration, according to Eqs. (4) and (5). The slope of the line represents k_1 or k_2, whilst the intercept represents $k_{-1}[Cl^-]$ or $k_{-2}[Cl^-]$. The results are summarized in Table 3 and S10 (ESI, Supporting Information).

$$k_{obsd1} = k_1[Nu] + k_{-1}[Cl^-] \quad (4)$$
$$k_{obsd2} = k_2[Nu] + k_{-2}[Cl^-] \quad (5)$$

Nu = TU, L-Met, L-His and 5'-GMP

Figure 4 shows the dependence of k_{obsd} on the nucleophile concentration for the [Pt(DMEAIm^{iPr})Cl$_2$] complex (see also ESI, Supporting Information, Figures S4 and S5).

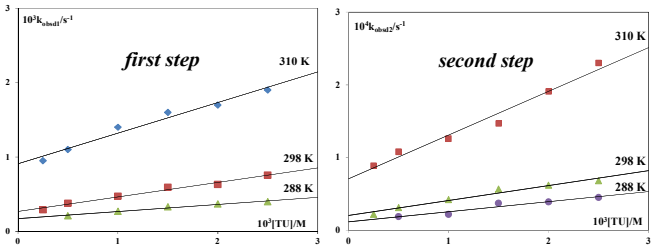
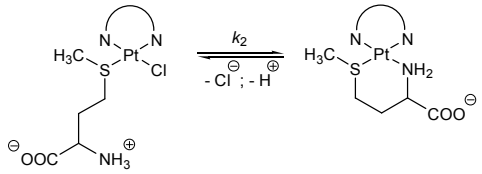


Figure 4. *Pseudo*-first-order rate constants plotted as a function of nucleophile concentration for the first step (left graph) and the second step (right graph) of the substitution reactions of the [Pt(DMEAIm^{iPr})Cl$_2$] complex with TU at pH = 7.2 and 310 K in 25 mM Hepes buffer and 30 mM NaCl.

[Pt(DMEAIm^{iPr})Cl$_2$] reacts faster than [Pt(DPENIm^{iPr})Cl$_2$] which is in accordance with the order of reactivity observed for analogous Pd(II) complexes.[14] The second substitution step is slower than the first substitution for both complexes. It can be assumed that the first substitution would take place next to the less sterically hindered side of the ligand *viz.* next to the amine donor, while, the second substitution would have to take place next to the bulky imidazolin-2-imine donor. In addition, the first substitution would result in a less electrophilic Pt(II) center, reducing the reaction rate for the second substitution.

The order of the reactivity of the investigated nucleophiles for the first reaction step is: TU > L-Met > L-His > 5'-GMP (Table 3). Thus, the sulfur-donor nucleophiles react faster with Pt(II) complexes than the nitrogen-donor nucleophiles.

The order of the reactivity for the second substitution step is: TU > L-His > 5'-GMP. Kinetic traces for reactions with L-Met gave fits with a double exponential function. However, when the constants, k_{obsd1} and k_{obsd2} were plotted against the concentration of the entering L-Met, it was observed that k_{obsd1} shows a linear dependence on the nucleophile concentration, while k_{obsd2} was found to be independent of the L-Met concentration, suggesting a chelate formation process as presented in Scheme 2 and Figure 5.



Scheme 2. The second step of the substitution reaction of investigated Pt(II) complexes with L-Met.

Table 3. The rate constants for the first and the second reaction step of the substitution reactions of the Pt(II) complexes with TU, L-Met, L-His and 5'-GMP at pH = 7.2 (25 mM Hepes buffer) in the presence of 30 mM NaCl at 310 K.

	[Pt(DMEAIm^{iPr})Cl$_2$]		[Pt(DPENIm^{iPr})Cl$_2$]	
	first step k_1[M^{-1} s^{-1}]	**second step** k_2[M^{-1} s^{-1}]	**first step** k_1[M^{-1} s^{-1}]	**second step** k_2[M^{-1} s^{-1}]
TU	$(41.10 \pm 0.10)\ 10^{-2}$	$(6.00 \pm 0.10)\ 10^{-2}$	$(20.90 \pm 0.20)\ 10^{-2}$	$(4.88 \pm 0.20)\ 10^{-2}$
L-Met	$(14.30 \pm 0.20)\ 10^{-2}$	/	$(11.90 \pm 0.10)\ 10^{-2}$	/
L-His	$(3.50 \pm 0.10)\ 10^{-3}$	$(3.00 \pm 0.10)\ 10^{-4}$	$(2.80 \pm 0.10)\ 10^{-3}$	$(2.16 \pm 0.20)\ 10^{-4}$
5'-GMP	$(3.30 \pm 0.10)\ 10^{-3}$	$(2.91 \pm 0.20)\ 10^{-4}$	$(2.59 \pm 0.20)\ 10^{-3}$	$(1.59 \pm 0.10)\ 10^{-4}$

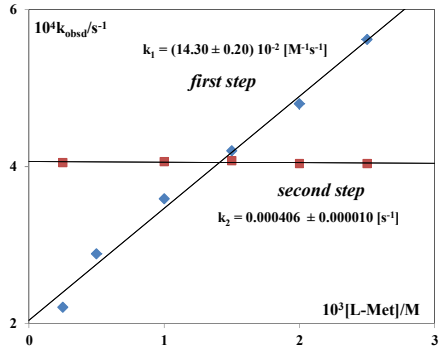


Figure 5. Plots of k_{obsd} versus L-Met concentration for the [Pt(DMEAIm^{iPr})Cl$_2$] complex (pH = 7.2, 310 K, 25 mM Hepes buffer, 30 mM NaCl).

The substitution reactions of the investigated Pt(II) complexes with L-Met proceed as shown in Scheme 2 with the formation of the six-membered ring *via* the sulphur and nitrogen atoms of L-Met. Ring-closure and formation of a six-membered ring also occurs in the substitution reactions of the analogous Pd(II) complexes and with L-Met.[14] To confirm that the second step is chelation, the kinetics were studied with the Pt(II) complexes in excess rather than L-Met. This would mean that a two-step reaction can only occur if ring closure is involved. The obtained kinetic traces for such reactions gave fits to a double exponential function. Similar values for the rate constants were obtained, as were observed in the experiments in which L-Met was added in excess (see ESI, Supporting Information Figure S6).

The activation parameters $\Delta H^{\neq}$ and $\Delta S^{\neq}$ (Tables S1, ESI, Supporting Information) were calculated using the Eyring equation for the reactions with TU for the first and second reaction step. The activation parameters support an associative mechanism for each of these reactions which is supported by the significantly negative activation entropies.

The Pd(II) imidazolin-2-imine complexes shows a huge slowdown in the reactivity compared with others Pd(II) complexes. Thus, [Pd(DMEAIm^{iPr})Cl$_2$] and [Pd(DPENIm^{iPr})Cl$_2$] react 100 times slower than [Pd(en)Cl$_2$] (en = ethylenediamine) and their reactivity approaches the reactivity of Pt(II) aqua complexes.[14] However, the analogous Pt(II) complexes do not show such a pronounced decrease in reactivity as they react only 1.5 - 2.0 times slower compared to [Pt(en)Cl$_2$], Table 4.

Table 4. The rate constants for the first reaction step of the substitution reactions of the Pt(II) complexes with L-His and 5'-GMP.

	L-His $10^3 k_1$[M^{-1} s^{-1}]	5'-GMP $10^3 k_1$[M^{-1} s^{-1}]
[Pt(en)Cl$_2$][27]	7.90 ± 0.70	4.40 ± 0.30
[Pt(DMEAIm^{iPr})Cl$_2$]	3.50 ± 0.10	3.30 ± 0.10
[Pt(DPENIm^{iPr})Cl$_2$]	2.80 ± 0.10	2.59 ± 0.20

The observed intercepts in the Figure 4, S4 and S5 are ascribed to the back reaction with the excess of chloride present in solution. The obtained values of these rate constants are summarized in Table S10 (ESI, Supporting Information) and they are much smaller in comparison to the values of rate constants for the direct reactions.

2.2. Au(III) and imidazolin-2-imines

Gold(III) complexes can be significantly stabilized, even at neutral pH, by the appropriate choice of the ligand, preserving its interesting biological properties. It was shown that the presence of at least two

nitrogen donors directly coordinated with the Au(III) center leads to a significant decrease in the redox potential of such complexes.[12,13,28] Keeping this in mind, we attempted to use imidazolin-2-imines as spectator ligands in the synthesis of Au(III) complexes and to find out whether these ligands can stabilize Au(III) ion. However, the isolation of stable complexes have been failed.

The only stable product which we were able to isolate is the product of the reaction between the bis(imidazolin-2-imine) ligand DACH(Im^{iPr})$_2$[18] and $KAuCl_4$ in THF. The product of this reaction was obtained as a yellow solid, ^{1}H and ^{13}C NMR spectroscopy shows a characteristic signals of ligand[18] (see ESI, Supporting Information).

Yellow crystals suitable for X-ray diffraction analysis were isolated from a chloroform/diethyl ether solution, and the molecular structure of [(DACH($Im^{iPr}H$)$_2$)]($AuCl_2$)$_2$ is shown in Figure 6. The molecule crystallizes in the monoclinic space group $P2_1$ as a chloroform solvate. The protonated ligand shows H···Cl contacts with the $AuCl_2$ counterions of 2.54(7)Å (H01-Cl1) and 2.46(8)Å (H02-Cl4). In addition, the protonation of the imine nitrogen atoms causes clear delocalization of the imine double bond, which is indicated by the C-N bond distances of the imines of 1.381(6)Å (C7-N1) and 1.373(6)Å (C18-N2).

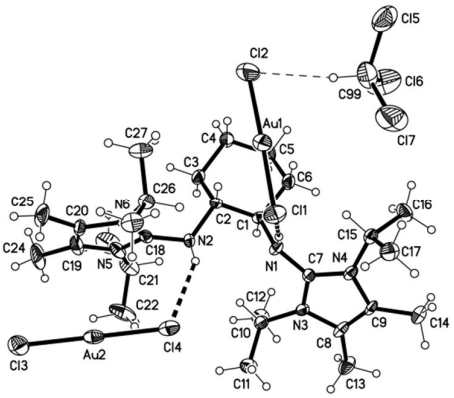


Figure 6. ORTEP drawing of [(DACH($Im^{iPr}H$)$_2$)]($AuCl_2$)$_2$·($CHCl_3$) with thermal displacement parameters drawn at 50% probability. Hydrogen atoms were omitted for clarity. Selected bond distances [Å] and angles [°] and contacts: C7-N1 1.381(6), C7-N3 1.340(7), C7-N4 1.340(7), C18-N2 1.373(6), C18-N5 1.343(7), C18-N6 1.342(7); N3-C7-N4 109.4(4), N5-C18-N6 108.1(4); H01···Cl1 2.54(7), N1-H01···Cl1 165(6), H02···Cl4 2.46(8), N2-H02···Cl4 146(7), H99···Cl2 2.54, C99-H99···Cl2 170.3.

Apparently, the reaction between DACH(Im^{iPr})$_2$ and $KAuCl_4$ leads to reduction of Au(III) to Au(I), indicating that electron-rich imidazolin-2-imine ligands are not suited for the complexation of Au(III). This reactivity can be ascribed to the reducing nature of the imidazolin-2-imine ligands and the oxidizing nature of Au(III), this is also in agreement with the redox properties reported for guanidine-type ligands.[29-32]

3. Conclusion

In this study, two novel platinum(II) complexes with mono(imidazolin-2-imines) were synthesized and characterized; for [Pt(DMEAIm^{iPr})Cl_2], a crystal structure could be determined. Furthermore, the influence of the imidazolin-2-imine ligands on the solubility, acid-base characteristics and reactivity towards bio-molecules of these complexes were studied. We performed spectrophotometric acid-base titrations to determine the pK_a values for the coordinated water ligands in the respective diaqua complexes. In general, we found two pK_a values for both studied Pt(II) complexes. Solubility measurements have shown that Pt(II) complexes with imidazolin-2-imines revealed good solubility in water, which is higher than that of cisplatin and oxaliplatin. The performed kinetic measurements have shown that the complex [Pt(DMEAIm^{iPr})Cl_2] reacts faster than [Pt(DPENIm^{iPr})Cl_2]. The order of reactivity of studied bio-molecules decreases in the order TU > L-Met > L-His > 5'-GMP. The investigated nitrogen-donor bio-molecules react with the Pt(II) complexes in two successive reaction steps that are both dependent on the nucleophile concentration. However, L-methionine reacts by forming a six-membered ring *via* its sulphur and nitrogen atoms. The negative values reported for the entropy of activation confirmed an associative substitution mode. The Pd(II) imidazolin-2-imine complexes shows a large decrease of reactivity compared with others Pd(II) complexes, and their reactivity approaches to that of Pt(II) aqua complexes. However, the analogous Pt(II) complexes do not show such a pronounced decrease of reactivity as they react 1.5 – 2.0 times slower compared to [Pt(en)Cl_2]. Our attempts to coordinate Au(III) ion to imidazolin-2-imines were unsuccessful; instead, reduction to Au(I) was observed.

4. Experimental

4.1. Chemicals and solutions

Thiourea, L-methionine, L-histidine, guanosine-5'-monophosphate sodium salt, *N,N*-dimethylethylenediamine, (1*S*,2*S*)-(-)-1,2-diphenylethylenediamine, (1*R*,2*R*)-(-)-1,2-diaminocyclohexane, $NaBF_4$, KF, $NaNH_2$, KO^{t}Bu, K_2PtCl_4 and $KAuCl_4$ were obtained from Acros Organics or Sigma Aldrich, and were used without further purification. Hepes buffer (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) was obtained from Sigma Aldrich. All the other chemicals were of the highest purity commercially available and were used without further purification. Ultra-pure water was used in all experiments. Nucleophile stock solutions were prepared shortly before use by dissolving the chemicals.

4.2. Preparation of the complexes

All reactions were performed in a glove box, under dry argon atmosphere (MBraun 200B) or on a high-vacuum line using standard Schlenk techniques, unless noted otherwise. Commercial grade solvents were purified by use of a solvent purification system from MBraun GmbH and stored over molecular sieves (4 Å) under dry argon atmosphere. The ligands, DMEAIm^{iPr}, DPENIm^{iPr} and

DACH(Im^{iPr})$_2$ were prepared according to the literature procedure.[14,18]

4.2.1. Synthesis and characterization of [Pt(DMEAIm^{iPr})Cl$_2$] complex

To a suspension of 100 mg (0.24 mmol; 1 eq) of K_2PtCl_4 in 5 ml of THF was added 65 mg (0.24 mmol; 1 eq) of the DMEAIm^{iPr} ligand in 5 mL of THF. The reaction mixture was stirred overnight at the 50 °C affording a yellow solution. The complex was precipitated from solution by the addition of 100 mL of n-hexane. The precipitate was dissolved in chloroform to remove KCl, filtered and dried *in vacuo*. The product was obtained as a yellow-orange solid, the orange crystals were obtained from chloroform/diethyl ether (108 mg, 0.20 mmol, 84.2%).

^{1}H NMR (300 MHz; $CDCl_3$): δ = 5.41 (sept, 2H, J_{HH} 7.2 Hz, C*H*Me$_2$), 2.93 (s, 6H, N(C*H*$_3$)$_2$), 2.75 (t, 2H, J_{HH} 4.5 Hz, C=NC*H*$_2$CH$_2$), 2.45 (t, 2H, J_{HH} 4.5 Hz, CH$_2$C*H*$_2$NMe$_2$), 2.10 (s, 6H, CC*H*$_3$), 1.55 (d, 6H, J_{HH} 7.0 Hz, CH(C*H*$_3$)$_2$), 1.43 (d, 6H, J_{HH} 7.0 Hz, CH(C*H*$_3$)$_2$) ppm.

^{13}C NMR (100 MHz; $CDCl_3$): δ = 151.8 (N$_2$*C*=N), 119.2 (*C*Me), 67.5 (CH$_2$*C*H$_2$NMe$_2$), 51.5 (C=N*C*H$_2$CH$_2$), 50.8 (N(*C*H$_3$)$_2$), 48.1 (*C*HMe$_2$), 22.4 (CH(*C*H$_3$)$_2$), 21.9 (CH(*C*H$_3$)$_2$), 10.3 (C*C*H$_3$) ppm.

Anal. Calcd. for ($C_{15}H_{30}Cl_2N_4Pt$) C: 33.84; H: 5.68; N: 10.52. Found: C: 33.53; H: 5.49; N: 10.11.

4.2.2. Synthesis and characterization of [Pt(DPENIm^{iPr})Cl$_2$] complex

To 50 mg (0.12 mmol; 1 eq) of K_2PtCl_4 was added 35.2 mg (0.12 mmol; 1 eq) of DPENIm^{iPr} in10 mL of THF. The reaction mixture was stirred for 7 h at 50°C, and for two days at room temperature affording a yellow solution. The complex was precipitated from solution by the addition of 100 ml of n-hexane. The precipitate was dissolved in chloroform to remove KCl filtered and dried *in vacuo*. The product was obtained as a yellow solid (67.6 mg, 0.10 mmol, 85.4%).

^{1}H NMR (300 MHz; $CDCl_3$): δ 7.37 – 6.85 (m, 10H, H_{Ar}), 5.29 (sept, 2H, J_{HH} 7.0 Hz, C*H*Me$_2$), 4.96 (d, 1H, J_{HH} 9.0 Hz, NH$_2$HC*H*(Ph)CH), 4.32 (d, 1H, J_{HH} 9.0 Hz, CNHC*H*(Ph)CH), 2.14 (s, 6H, CC*H*$_3$), 2.10 (s, 2H, N*H*$_2$), 1.64 (d, 3H, J_{HH} 7.1 Hz, CH(C*H*$_3$)$_2$), 1.43(d, 3H, J_{HH} 7.1 Hz, CH(C*H*$_3$)$_2$), 1.03 (d, 3H, J_{HH} 7.1 Hz, CH(C*H*$_3$)$_2$), 0.84 (d, 3H, J_{HH} 7.1 Hz, CH(C*H*$_3$)$_2$), ppm.

^{13}C NMR (100 MHz; $CDCl_3$): δ 156.8 (N$_2$*C*=N), 140.1 (*ipso*-*C*$_{Ar}$(CHNH$_2$)), 133.2(*ipso*-*C*$_{Ar}$(CHN), 125.5 (*C*$_{Ar}$), 123.1 (*C*$_{Ar}$), 122.2 (*C*$_{Ar}$), 124.7 (*m*-*C*$_{Ar}$(CHNH$_2$)), 124.2 (*m*-*C*$_{Ar}$(CHNH), 123.9 (*C*$_{Ar}$), 119.8 (*C*Me) 73.5 (CNH*C*H(Ph)CH), 60.4 (NH$_2$H*C*H(Ph)CH), 49.1 (*C*HMe$_2$), 48.0 (*C*HMe$_2$), 22.8 (CH(*C*H$_3$)$_2$), 21.9 (CH(*C*H$_3$)$_2$), 21.3 (CH(*C*H$_3$)$_2$), 20.1 (CH(*C*H$_3$)$_2$), 11.2 (C*C*H$_3$), 10.8 (C*C*H$_3$) ppm.

Anal. Calcd. for ($C_{25}H_{34}Cl_2N_4Pt$) C: 45.73; H: 5.22; N: 8.53. Found: C: 45.96; H: 5.37; N: 8.71.

4.2.3. Preparation of aqua complexes

The aqua complexes of Pt(II) complexes were prepared starting from the corresponding chlorido complexes. The conversion was performed by addition of the corresponding amount of $AgClO_4$ to a water solution of the chloride complex and stirring for 5 h at 50 °C. The white precipitate that formed (AgCl) was filtered off using a Millipore filtration unit, and the solutions were diluted. Great care was taken to ensure that the resulting solution was free of Ag^+ ions and that the chlorido complexes had been completely converted into the aqua form. Since it is well known that perchlorate ions do not coordinate to Pd(II) and Pt(II) in aqueous solution,[33] pH titrations were studied in perchlorate medium.

4.3. Instrumentation and measurements

NMR spectra were recorded on Bruker DPX 200 and AV 300 devices. Chemicals shifts (δ) are reported in ppm and referenced to tetramethylsilane. Coupling constants (*J*) are reported in Hertz (Hz) and splitting patterns are indicated as s (singlet), d (doublet), t (triplet), sept (septet), bs (broad signal) and m (multiplet). Elemental analyses (C, H, N) were performed by combustion and gas chromatographic analysis with an Elementar Vario MICRO elemental analyzer. High resolution electron spray ionization (ESI) mass spectroscopy was performed on a Finnigan MAT 95 XL Trap device. pH measurements were carried out using a Mettler Delta 350 digital pH meter with a resolution ± 0.01 mV, with a combination glass electrode. This electrode was calibrated using standard buffer solutions of pH 4, 7 and 9 obtained from Sigma. Kinetic measurements of the Pt(II) complex were carried out on a PerkinElmer Lamda 25 and 35 double-beam spectrophotometer in thermostated 1.00 cm quartz Suprasil cells. The temperature was controlled to ± 0.1 ^{o}C. All kinetic measurements were performed under *pseudo*-first-order conditions, *i.e*., at least a 10-fold excess of the nucleophile was used.

4.4. Determination of the pK_a value of the Pt(II) complexes

Spectrophotometric pH titrations of the solutions of the complexes were performed with NaOH as a base at 298 K. To avoid absorbance corrections due to dilution, a large volume (300 mL) of the complex solution was used in the titration. The change in pH from 2 to approximately 3 was achieved by addition of known amounts of crushed pellets of NaOH. The consecutive pH changes were obtained by adding drops of saturated solutions of NaOH, 1 or 0.1 M, using a micropipette. To avoid contamination released by the pH electrode, it was necessary to take 2-mL aliquots from the solution into narrow vials for the pH measurements. The aliquots were discarded after the measurements. The total reversibility of the titration could be achieved by subsequent addition of $HClO_4$.

4.5. Solubility measurements

The concentrations of saturated solutions of the studied Pt(II) complexes were determined by UV-Vis spectrophotometry. The specific absorptivity of the compounds in the water was determined first. This was measured using five dilution series (5, 10, 30, 40, 50 mM) of the studied complexes, then the calibration curve was calculated using Lambert-Beer law. The slope of the curve gave specific absorptivity.

The required quantity of water solution was added to the 5 ml volumetric flask. The solution was heated up to 298 K. Previously weighed quantity of Pt(II) complexes was added to the volumetric flask until the saturation point occurs. Stirring was continued up to 7 hours at 298 K. The sample was filtered through 0.20 μm membrane filter. A measured quantity of filtered sample was transferred into another volumetric flask and made further dilutions. The absorbance was measured using UV-Vis spectrophotometry. The same process was repeated two times.

4.6. Kinetic Measurements

The kinetics of the substitution of the coordinated chloride were followed spectrophotometrically by following the change in absorbance at suitable wavelengths as a function of time. The working wavelengths were determined by recording spectra of the reaction mixture over the wavelength range 220 to 450 nm. All kinetic experiments were performed under *pseudo*-first-order conditions, for which the concentration of the nucleophile was always in at least a 20-fold excess. The reactions were initiated by mixing 0.5 ml of the Pt(II) complex solution with 2.5 ml of thermally equilibrated nucleophile solution in the UV-Vis cuvette, and reactions were followed for at least 8 half-lives. The observed *pseudo*-first-order rate constants, k_{obsd}, represent an average value of two to four independent kinetic runs for each experimental condition. Some of the reactions were studied at three temperatures (288, 298 and 308K).

The experimental data are summarized in the ESI, Supporting Information, Tables S2 – S9. The values of the constants and other thermodynamic parameters were determined using the computer programs Microsoft Excel 2007 and OriginPro 8.

4.7. X-ray diffraction studies

Data were recorded at 100(2) K using an Oxford Diffraction Eos diffractometer with monochromated Mo *K*α radiation. The structures were refined anisotropically using the SHELXL-97 program.[34] Hydrogen atoms were either (i) located and refined isotropically (NH), (ii) included as idealized methyl groups allowed to rotate but not tip or (iii) placed geometrically and allowed to ride on their attached carbon atoms. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publications nos. CCDC 1431738 and 1431739. Copies of the data can be obtained free of charge from www.ccdc.cam.ac.uk/data_request/cif .

	$[Pt(DMEAIm^{iPr})Cl_2]\cdot 2(CHCl_3)$	$[(DACH(Im^{iPr}H)_2)](AuCl_2)_2\cdot(CHCl_3)$
Empirical Formular	$C_{17}H_{32}Cl_8N_4Pt$	$C_{29}H_{53}Au_2Cl_7N_6$
Formular Weight	771.16	1127.86
Crystal System	monoclinic	monoclinic
Space Group	$P2_1/n$	$P2_1$
a/Å	11.5923(3)	11.9748(3)
b/Å	16.9429(3)	10.2420(3)
c/Å	14.1236(3)	16.6859(4)
β/°	90.040(2)	97.307(3)
Volume [Å^3]	2773.98(10)	2029.84(9)
Z	4	2
Reflections Collected	105084	143811
Independent reflections	8427 [R_{int} = 0.0558]	12219 [R_{int} = 0.0786]
ρ_C/g cm^{-3}	1.846	1.845
μ/mm^{-1}	5.843	7.708
$R(F_O)$, [$I > 2\sigma(I)$]	0.0231	0.0365
Rw (F_O^2)	0.0419	0.0789
Goodness of fit on (F^2)	1.065	1.055
Flack parameter	–	0.009(5)
$\Delta\rho$/eÅ^{-3}	1.530/–1.080	1.630/–1.486

Abbreviations

en = ethylendiamine
DMEAIm^{iPr} = N^2-[1,3-diisopropyl-4,5-dimethylimidazolin-2-ylidene]-N^1,N^1-dimethyl-1,2-ethanediamine
DPENIm^{iPr} = N^1-[1,3-diisopropyl-4,5-dimethylimidazolin-2-ylidene]-1,2-diphenyl-(1*S*,2*S*)-1,2-ethanediamine
DACH(Im^{iPr})$_2$ = N^1,N^2-bis[1,3-diisopropyl-4,5-dimethylimidazolin-2-ylidene]-(1*R*,2*R*)-1,2-cyclohexanediamine
TU = thiourea
L-Met = L-methionine
L-His = L-histidine
5'-GMP = guanosine-5'-monophosphate

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Graphical Abstract

Platinum(II) complexes with hybrid amine-imidazolin-2-imine ligands and their reactivity toward bio-molecules

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Two new Pt(II) complexes with imidazolin-2-imines as ancillary ligands were synthesized and characterized and their reactivity toward bio-molecules were tested.