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A comparative kinetic study of modified Pt(dppf)Cl_2 complexes and their interactions with L-cys and L-met†

Graeme Puxty,[a] Haris Bjelosevic,[b] Tina Persson[b] and Sofi K. C. Elmroth*[a]

[a] *Inorganic Chemistry, Chemical Center, Lund University, P.O. Box 124, SE-221 00, Lund, Sweden. E-mail: sofi.elmroth@inorg.lu.se; Fax: + 46 (0)46 222 4439; Tel: + 46 (0)46 222 8106*

[b] *Organic Chemistry 1, Chemical Center, Lund University, P.O. Box 124, SE-221 00, Lund, Sweden*

Received 22nd March 2005, Accepted 4th April 2005
First published as an Advance Article on the web 4th August 2005

With the success of cisplatin (*cis*-diamminedichloroplatinum(II)), strong interest has developed in the application of inorganic metal complexes to the treatment of cancer. Research has focused on platinum(II) complexes with a variety of spectator ligands that provide novel physicochemical properties. In this paper we report a kinetic study of 1′,1′-bis(diphenylphosphino)ferrocenedichloroplatinum(II) and two related compounds with either an acetate or amide ester substituent attached to the cyclopentadienyl ring. For all compounds the reactivity towards L-cysteine and L-methionine in aqueous solution has been investigated (25 °C, $I = 0.010$ M and pseudo-first-order conditions). For the reactions with L-cysteine and L-methionine the reactions proceeded *via* a steady-state aquated intermediate to form mono (0.92(2)–3.25(4)) × 10^{-3} s^{-1}) and bis adducts (0.97(2)–3.67(4)) × 10^{-4} s^{-1}). For reactions with L-cysteine, direct reactions with the starting complex also contributed (mono adduct: 0.36(2)–1.41(4) M^{-1} s^{-1}, bis adduct: 0.080(1)–0.96(1) M^{-1} s^{-1}). The attached substituents were found to have a significant effect upon the reaction kinetics, with the substituted complexes found to have increased reactivity. It is proposed that the increased reactivity stems from hydrogen bonding between the substituent and the entering ligand and subsequent outer-sphere complex stabilisation. Evidence in support of this theory was obtained form measurements in dichloromethane with 1-propanethiol as the entering ligand. The reactivity of the dppf containing complexes was also compared to that of cisplatin (mono adduct: (0.170(1)–0.175(1)) × 10^{-3} s^{-1}, bis adduct: (0.183(1)–0.397(1)) × 10^{-4} s^{-1}) and found to be significantly enhanced.

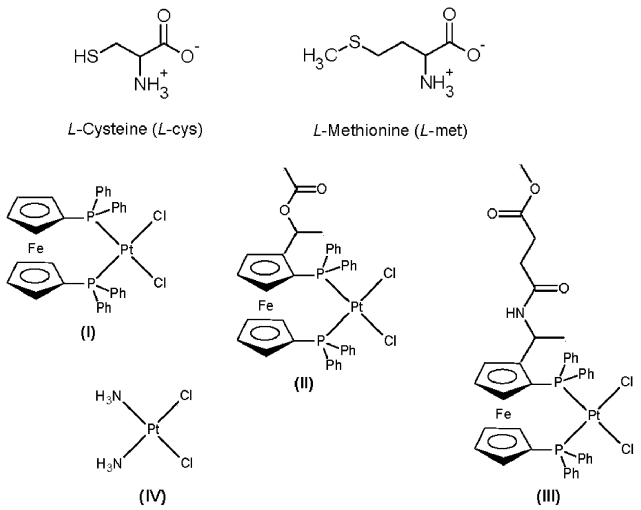
Introduction

Platinum based complexes have found extensive application in the treatment of a variety of cancers.[1,2] The first synthesised and most commonly used compound in this family is cisplatin, *cis*-$Pt(NH_3)_2Cl_2$ (*cis*-diamminechloroplatinum(II)), a square-planar Pt(II) complex whose antitumor properties were first discovered in the 1960s.[3,4] Unfortunatly, factors such as toxic side effects and spontaneous development of drug resistance in tumors limits the use of the drug.[5] There is thus great interest in development of new Pt-based drug candidates that circumvent these limitations, preferentially combined with an improved target selectivity and solubility. Intense research during the last couple of decades has resulted in the discovery of a handful of promising, alternative platinum-containing anticancer drug candidates including both structurally related ones, *e.g.* carbo-, oxali- and neda-platin,[6–9] and those with novel types of coordination environment.[10,11] Of particular interest in the latter category are the recently developed Pt(IV)-containing prodrugs, *e.g.* JM216 and multinuclear, polyamine-containing *trans*-Pt(II) complexes, *e.g.* BBR3464, both currently undergoing clinical trials.[12–16] The promising results obtained for BBR3464 clearly show that, in comparison with the interaction patterns established for cisplatin, disruption of alternative intracellular pathways could successfully be explored in the search for novel Pt-based antineoplastic reagents.

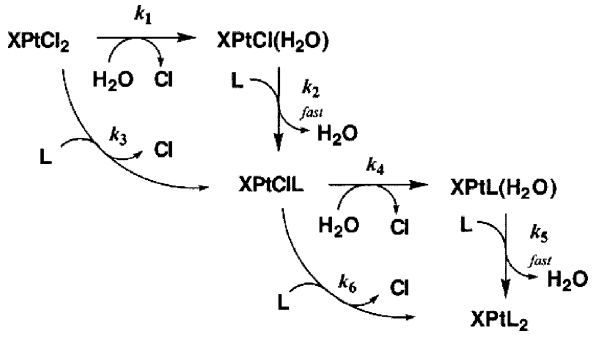
We have recently initiated research aimed at combining the well documented anticancer activity of the Pt(II) metal center with carefully designed organic moieties. The long term goal is to obtain compounds which allow for more specific interaction patterns towards biological targets compared to compounds such as *cis*-, carbo- and oxali-platin. As a first effort towards these goals, here we present a reactivity study of a series of *cis*-Pt(II) compounds coordinated to the 1′,1′-bis(diphenyldiphosphino)ferrocene (dppf) moiety. Compounds of this type have already been shown to exhibit both antineoplastic and antimicrobial activity.[17,18] An initial synthetic goal has been to improve the overall solubility of these compounds since the presence of the dppf-moiety is known to limit the solubility of these compounds in protic solvents. This has been done by the asymmetric attachment of organic fragments to the dppf-moiety that contain atoms which could easily interact with water through H-bonding interactions, see Scheme 1.[19] Such modulation of the ligand environment is likely to influence the kinetic properties of these compounds, however. First of all, the increased steric bulk of the metal complex is likely to limit the access of ligands to the metal center and thus may contribute towards a decrease in the reactivity. On the other hand, if favourable H-bonding patterns can be established between the entering group and the pendant organic fragment this may act to increase the overall reactivity. In order to test which of these two alternatives dominates the reactivity here, we present a comparative kinetic study in which either an acetate group (complex **II**) or an amide ester (complex **III**) has been attached to the dppf-moiety. The sulfur-containing amino acids L-cysteine (L-cys) and L-methionine (L-met) were chosen as reactants to allow for evaluation of both direct replacement reactions and hydrolysis pathways, compare Scheme 2. Reactivity studies of

† Electronic supplementary information (ESI) available: Pseudo-first-order rate constants for complexes **I–III** with L-met and of cisplatin with L-cys and L-met. Measured and calculated absorbance data for complexes **I**, **II** and cisplatin with L-cys and of complexes **I–III** and cisplatin with L-met. See http://dx.doi.org/10.1039/b504129e

DOI: 10.1039/b504129e



Scheme 1 Structures of the amino acids L-cysteine (L-cys) and L-methionine (L-met) used as ligands, the Pt(II)–dppf complexes **I–III** and cisplatin (**IV**). Ph denotes a phenyl group.



Scheme 2 Complete mechanism for the replacement of Cl^- by L-cys or L-met for all complexes. Direct replacement of Cl^- by L-met was not observed. All charges have been omitted for clarity and X represents the spectator ligand.

the corresponding Pt(II) complex with the unsubstituted dppf-ligand (complex **I**) and cisplatin (complex **IV**) were included as reference reactions. The results give rise to a uniform picture for both direct replacement reactions and hydrolysis pathways, in which an increasing access to potential H-bond acceptors contribute to increase the reactivity.

Experimental

Materials and equipment

The Pt(II)–dppf complexes **I–III** (see Scheme 1) were synthesised according to the procedures reported elsewhere,[19] resulting in racemic mixtures of (*R*),(*S*) and (*S*),(*R*) enantiomers. All other chemicals were used as purchased without further purification. Cisplatin was purchased from Sigma and L-cysteine and L-methionine were purchased from Aldrich. The non-ionic surfactant, Triton X-100 (octylphenol ethylene oxide condensate), was purchased from Sigma. The ionic strength of all aqueous solutions was adjusted to 0.010 M with sodium perchlorate (Merck), and the pH was adjusted by addition of perchloric acid (Merck) or sodium hydroxide (Aldrich). All aqueous solutions were prepared using deionized 18.2 megaohm water (ELGA PURELAB Ultra-genetic) and spectroscopy grade DMSO (Merck). Dichloromethane was purchased from Fluka, 1-propanethiol from Aldrich and triethylamine from Merck.

Kinetic measurements in aqueous solution were followed using the time-dependent absorbance changes at 239 nm on a Varian Cary 300 UV-visible spectrophotometer. Measurements were made in a batch mode using five cuvettes (and one additional reference cuvette) by use of a series II peltier thermostated 6 × 6 multicuvette holder coupled to a water-bath (Varian). The temperature was maintained at 25.0 ± 0.1 °C. Multiwavelength kinetic measurements made in aqueous solution for illustrative purposes and measurements for the determination of rate constants in dichloromethane were made using a Milton Roy Spectronic 3000 ARRAY spectrophotometer (diode array) with a thermoelectric cuvette holder coupled to a water bath (Lauda). The measurements were performed at 25.0 ± 0.1 °C. All pH measurements were made using a Metrohm 744 pH meter with a Ag/AgCl combination glass electrode at room temperature. The pH meter was regularly calibrated using standard pH 7.0 and pH 4.0 buffers (Merck).‡§

Kinetic measurements in aqueous solution

The experiments were carried out after thermostating of the ligand solutions at the required concentrations in the spectrophotometer for at least fifteen minutes. Fresh solutions of the required Pt(II) complexes were prepared directly before use by dissolution of a weighed amount of the complex in DMSO, followed by immediate addition of appropriate aliquots to the cuvettes to initiate the reaction. The resulting solutions contained 1.0% DMSO by volume. All measurements were repeated at least in duplicate. It should be noted that it has been assumed that the presence of DMSO in the reaction solutions had negligible effect on the observed kinetics. In regard to the dppf containing complexes this assumption is based on a study by Longato *et al.*,[20] in which it was found that for Pt(dppf)Cl_2 (complex **I**) dissolved in neat DMSO the mono-substituted DMSO product formed only on addition of $AgBF_4$ and the bis product did not form at all. In the case of cisplatin (complex **IV**) the first-order rate constant of solvolysis in neat DMSO has been determined as 6.24×10^{-5} s^{-1} at 26 °C.[21] Since the solutions of all complexes were freshly prepared for each measurement, the time spent in neat DMSO prior to addition to aqueous solution was less then five minutes (corresponding to less than 3% conversion of cisplatin to the solvolysis product).

All experiments were performed under pseudo-first-order conditions of either L-cys or L-met using at least a 20-fold excess of ligand. For reactions between the Pt(II)–dppf complexes and L-cys the following concentrations were used: [L-cys] = 4.0×10^{-4}–2.0×10^{-3} M and [Pt(II)] = 2.0×10^{-5} M. For the Pt(II)–dppf complexes and L-met, lower concentrations of L-met were used to avoid the formation of a precipitate that occurred at higher concentration: [L-met] = 2.0×10^{-4}–1.0×10^{-3} M and [Pt(II)] = 1×10^{-5} M. For reactions with cisplatin the following concentrations were used: [L-cys] = [L-met] = (1.2–6.0) $\times 10^{-3}$ M and [Pt(II)] = 6×10^{-5} M. A higher Pt(II) concentration was used for cisplatin as it has a smaller extinction coefficient than the Pt(II)–dppf complexes at the wavelength used for monitoring. The kinetics were followed for a minimum of 1.5 h up to a maximum of 10 hours, depending upon the reaction velocity. In all experiments, the non-ionic surfactant Triton X-100 was added to the solutions (0.020% by volume) to maintain adequate solubility of the Pt(II)–dppf complexes in aqueous solution. Without the addition of acid or base, the pH of the prepared solutions was in the range 5.5–7.0 (depending upon the concentration of amino acid). Some measurements were also completed in the presence of 2.0×10^{-4} M perchloric acid yielding a pH of approximately 3.5, with some variation depending upon the amino acid concentration.

‡ Attempts were also made to follow the kinetics in aqueous solution using NMR, however due to the insolubility of the dppf containing complexes, it was not possible to achieve the mM concentrations required for such measurements.

§ To allow study of the kinetics of water replacement, attempts were made to synthesise the diaqua analogs of the Pt(II)–dppf complexes according to procedures reported elsewhere.[20] The synthesis was unsuccessful as both the kinetics and mass spectrometry results indicated a hydroxide-bridged dimer was produced, the formation of which has also been reported elsewhere.[21]

pH Titration

Potentiometric titrations were carried out for L-cys and L-met to verify that the pK_a's were unaffected by the presence of 0.020% by volume Triton X-100. 50 mL solutions of 0.0050 M L-cys or L-met in 0.020% by volume Triton X-100 and 0.010 M $NaClO_4$ were prepared. While stirring, these solutions were titrated by use of a manual burette with 0.10 M NaOH. The pH was recorded after each addition. No attempt was made to eliminate carbon dioxide from the solution.

Kinetic measurements in dichloromethane

The possible influence of hydrogen bonding on the reaction kinetics was investigated by allowing 1-propanethiol to react with complexes **I–III**. The reactions were studied in dichloromethane and triethylamine was used as a base to deprotonate the 1-propanethiol. Multiwavelength measurements were made using the Milton Roy Spectronic 3000 spectrophotometer as the stability of the Pt(II)–dppf complexes in dichloromethane allowed reuse of stock solutions. In total, 42 wavelengths in the range 255–270 nm were used for data analysis. Shorter wavelengths were not used due to interference from the absorbance of triethylamine and the solvent. The measurements were performed under pseudo-first-order conditions with a 52-fold excess of 1-propanethiol. Following thermostating of ligand solutions containing 7.8×10^{-4} M 1-propanethiol and 8.0×10^{-4} M triethylamine, the reactions were initiated by addition of a small aliquot of the required Pt(II)–dppf complex dissolved in dichloromethane, yielding a Pt(II)–dppf concentration of 1.5×10^{-5} M. All experiments were repeated in duplicate.

Results and discussion

Spectral properties

Kinetic measurements in aqueous solution were followed by absorbance changes at 239 nm. For the dppf-containing complexes an absorbance decrease occurred, as shown in Fig. 1. In contrast, the reaction with cisplatin resulted in an absorbance increase. This absorbance maximum is due to a charge transfer band of the Pt(II) complexes,[22] and has a large extinction coefficient for the dppf containing complexes ($\sim 5 \times 10^4$ M^{-1} cm^{-1}). The extinction coefficient for cisplatin at this wavelength is an order of magnitude lower ($\sim 5 \times 10^3$ M^{-1} cm^{-1}). The larger extinction coefficient of the Pt(II)–dppf complexes is consistent with square-planar PtL_2Cl_2 moieties (L = substituted phosphine).[22] It is assumed that bond formation between the Pt(II) complexes and L-cys or L-met takes place *via* the S group of the amino acids, as would be expected from previous studies of Pt(II) complexes.[23–25]

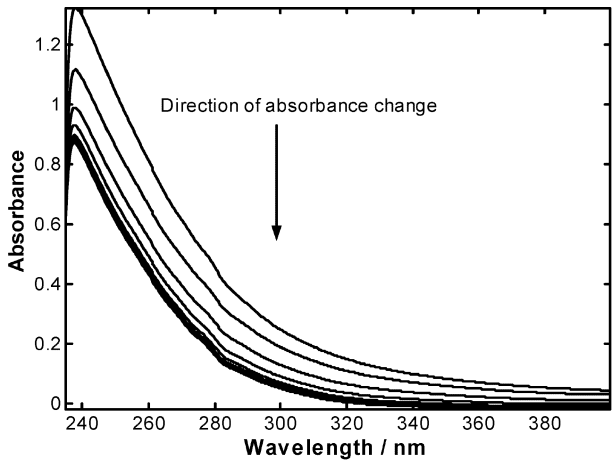


Fig. 1 Absorbance spectra between 235 and 400 nm for the reaction between complex **III** (2.5×10^{-5} M) and L-cys (5.6×10^{-5} M) over 2 h. The displayed spectra were collected every 3 min at 25 °C.

Reaction of I–III with L-cys

The kinetic behaviour of complexes **I–III** with L-cys was consistent with a mechanism where Cl^- undergoes replacement *via* two pathways, either by direct replacement by L-cys or through a steady-state aqua intermediate. The complete

$$\frac{d[Pt(L\text{-cys})]}{dt} = (k_1 + k_3[L\text{-cys}])[Pt] \quad (1)$$

$$\frac{d[Pt(L\text{-cys})_2]}{dt} = (k_4 + k_6[L\text{-cys}])[Pt(L\text{-cys})] \quad (2)$$

$$k_{obs1} = k_1 + k_3[L\text{-cys}] \quad (3)$$

$$k_{obs2} = k_4 + k_6[L\text{-cys}] \quad (4)$$

mechanism is shown in Scheme 2. If a steady-state assumption for the aquated intermediate is applied to the mechanism, it is described by the differential equations given in eqns. (1) and (2). For such mechanisms, a plot of first-order rate constants, k_{obs1} and k_{obs2}, fitted to data measured under pseudo-first-order conditions *vs.* ligand concentration should yield a straight line with a positive slope and non-zero intercept.[26] The intercept of the plot represents the rate constant of aquation and the slope represents the rate constant of direct Cl^- replacement. This relationship is given by eqns. (3) and (4). The absorbance data from each measurement was fitted to two psuedo-first-order rate constants using nonlinear regression[27] and the program Pro-KII.[28] Plots of k_{obs1} and k_{obs2} *versus* the concentration of L-cys for the complexes **I–III** are given in Fig. 2. The data treatment relies

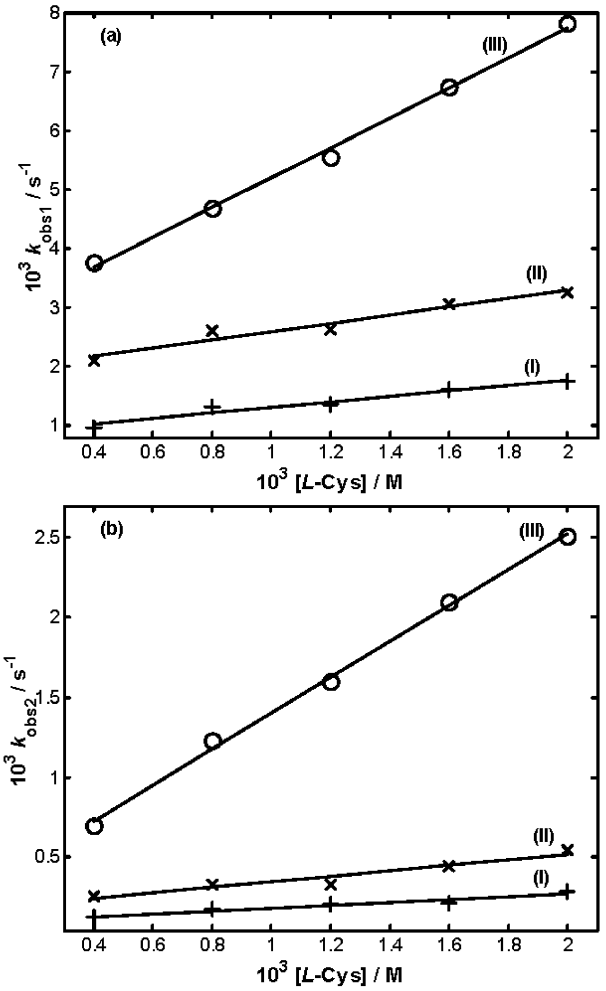


Fig. 2 Plots of pseudo-first-order rate constants *vs.* L-cys concentration for: (a) formation of the mono adduct and (b) formation of the bis adduct for complex **I** (+), **II** (×) and **III** (○).

on the assumption that the aquated intermediate is present under steady-state conditions, *i.e.* $k_2 \gg k_1$ and $k_5 \gg k_4$. This allows the determination of the rate constants for formation of the aqueous intermediate (k_1 and k_4) and direct Cl^- replacement (k_3 and k_6). The rate constants of water replacement (k_2 and k_5) cannot be determined. The linear relationship between the pseudo-first-order rate constants, k_{obs1} and k_{obs2} respectively, and the ligand concentrations is in agreement with this assumption. Thus, it is only possible to determine the rate constant of formation of the aqua species and the rate constant of direct Cl^- replacement.¶

Reaction of I–III with L-met

In contrast to the kinetic behaviour of compounds **I–III** with L-cys, their kinetic behaviour with L-met gave a linear relationship with zero slope (see ESI† for plots). This observation indicates that the direct replacement of Cl^- does not contribute significantly to the reaction mechanism when L-met is the entering ligand.

Reaction of cisplatin

For reactions between cisplatin and both L-cys and L-met again a linear relationship with zero slope was found (see ESI† for plots). Thus, for cisplatin direct replacement of Cl^- by neither L-cys nor L-met seems to contribute significantly to the reaction.

Determination of rate constants

For complexes **I–III** with L-cys the rate constants k_1, k_3, k_4 and k_6 were determined. The individual rate constants were obtained after a fit of the complete model given in Scheme 2 to the complete experimental dataset for a particular Pt(II) complex–ligand combination by use of second-order global analysis with local spectra.[29–31] This approach can be summarised as fitting of the model, consisting of a single set of rate constants, simultaneously to all the measurements (irrespective of the initial conditions) rather than treating each measurement individually. Again, the fitting of rate constants to the absorbance data was by nonlinear regression using Pro-KII.[28] The results of fitting using this method were in agreement with the results from the linear plots, but have the advantages of providing a more robust fit and model determination. The rate constants of water replacement (k_2 and k_5) were fixed to large enough values to maintain steady-state concentrations of the aquated intermediates. For the reactions of **I–III** with L-met and all reactions involving cisplatin (which all show a zero order dependence on ligand concentration) the steps in the mechanism involving direct replacement of Cl^- (k_3 and k_6) were removed from the model fitted to the data. The determined parameter values and associated errors of fit and literature values are listed in Table 1. It should be noted that the quoted errors of fit come solely from a statistical analysis of the data fitting, and as such are very likely an underestimate of the true errors. However, they do provide a useful point of comparison as to how well one parameter is defined relative to another. There was no indication that reverse reactions or dimerisation contributed to the kinetics. Following completion of some measurements the spectra were measured after 24 h and no additional change was apparent.

The quality of fit for all the measurements was excellent and the standard deviation of the residuals between the measured and calculated absorbance data was at the instrumental noise level ($\sim 5 \times 10^{-4}$ absorbance units). Plots of measured and calculated absorbance data for a series of measurements between complex **III** and L-cys are shown in Fig. 3. Plots for the other complexes are given in the ESI.†

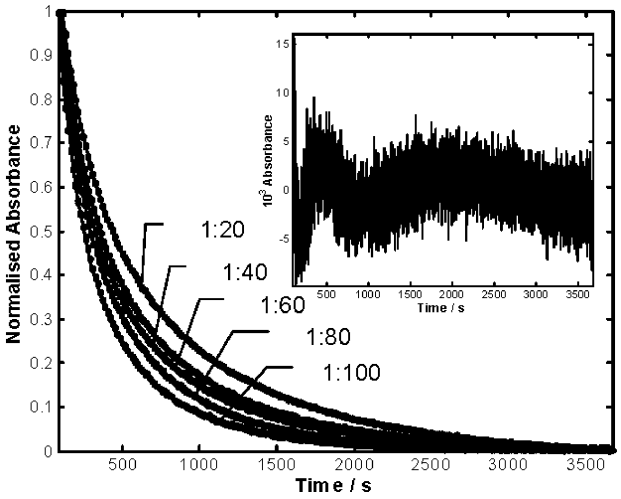


Fig. 3 Plots of measured (···) and calculated (—) absorbance at 239 nm for the reaction between complex **III** and L-cys for Pt : L-cys ratios of 1 : 20–100. The absorbance data has been normalised to lie between 0 and 1 for clarity. The inset shows the residuals from each kinetic trace overlaid.

pH-Dependence

The reaction of complex **III** with L-cys and L-met was studied in the interval pH 3.5–7.0. The kinetics exhibited no pH dependence in this pH range (see Table 2). This behaviour is consistent with the aquated intermediate being present under steady-state conditions. It should be noted that the presence of an equilibrium between the protonated and deprotonated form of the aqua complex should not be expected to influence the kinetics, as there is no evidence of accumulation of either species.

¶ Determination of the rate constants k_2 and k_5 was attempted by reducing the excess of amino acid and thus slowing the replacement of water. This was unsuccessful as at least a two-fold excess of amino acid was required to avoid formation of an insoluble white precipitate, which was most probably an insoluble hydroxide species. As mentioned, synthesis of the diaqua analogs also failed.

Table 1 Determined rate constants for the aquation and direct Cl^- replacement steps as defined by Scheme 2 at 25 °C, 0.010 M $NaClO_4$, 1.0% DMSO and 0.020% Triton X-100. Uncertainties are given as the error in the last significant digit to two standard deviations. Available literature data are given in brackets

	$10^3 k_1/s^{-1}$		$k_3/M^{-1}\ s^{-1}$	$10^4\ k_4/s^{-1}$		$k_6/M^{-1}\ s^{-1}$
Pt(II)–dppf complexes	L-cys	L-met	L-cys	L-cys	L-met	L-cys
I	0.92 ± 2	0.953 ± 5	0.36 ± 1	0.97 ± 2	0.98 ± 1	0.080 ± 1
II	1.48 ± 4	2.091 ± 8	0.94 ± 2	1.48 ± 2	2.74 ± 1	0.185 ± 2
III	3.25 ± 4	2.50 ± 1	1.41 ± 4	3.45 ± 6	3.67 ± 1	0.96 ± 1
IV	0.170 ± 1 (lit. 0.1512[a])	0.175 ± 1	—	0.183 ± 1 (lit. 0.240[b])	0.397 ± 1	—

[a] Overall rate constant at 25 °C and 0.10 M ionic strength ($HClO_4$) adjusted with respect to statistics to allow for direct comparison with those of complexes **I–III**.[32] [b] Rate constant at 25 °C and 1.0 M ionic strength ($NaClO_4$) calculated from activation parameters determined at 35–50 °C.[33]

Table 2 Comparison of rate constants determined for complex **III** at 25 °C, 0.010 M $NaClO_4$, 1.0% DMSO and 0.020% Triton X-100 with either no added acid or 2.0×10^{-4} M $HClO_4$. Uncertainties are given as the error in the last significant digit to two standard deviations

	L-cys		L-met	
Rate constant	No acid pH ~ 6	Acid pH ~ 3.5	No acid pH ~ 6	Acid pH ~ 3.5
$10^3 k_1/s^{-1}$	3.25 ± 4	3.41 ± 4	2.50 ± 1	3.10 ± 1
$k_3/M^{-1}\ s^{-1}$	1.41 ± 4	1.17 ± 4	—	—
$10^4 k_4/s^{-1}$	3.45 ± 6	3.51 ± 6	3.67 ± 1	3.21 ± 1
$k_6/M^{-1}\ s^{-1}$	0.96 ± 1	0.85 ± 1	—	—

Further, the reaction behaviour is also consistent with a single protolytic species dominating the speciation profile of the ligand replacing water in the coordination sphere of the Pt(II)-complexes, *i.e.* of L-cys and L-met. L-cys has pK_a's of 10.3, 8.18 and 1.90,[34] and L-met has pK_a's of 9.08 and 2.18[34] (values for 0.10 M ionic strength). According to species distribution calculations using the law of mass action and these pK_a-values, a single protolytic species represents 95–99% of the total concentration of L-cys or L-met in the pH range 3.5–7.0. The protolytic species distributions are shown in Fig. 4. pH titrations were made over the range pH 7.5–10.4 to verify the pK_a's were not significantly affected by the presence of the surfactant. Either L-cys or L-met was titrated with NaOH under the same conditions of surfactant and ionic strength as the kinetic experiments (0.010 M $NaClO_4$, 1.0% DMSO and 0.020% Triton X-100). Fitting of the data for L-cys and L-met was done by use of the standard equilibrium expression given by eqn. (5)[35] assuming a pK_w of 14. For L-met, the function F_a for a monoprotic acid was used (eqn. (6)), and for L-cys the function for a diprotic acid was used (eqn. (7)). The pK_a values were refined by nonlinear regression using Solver in Microsoft Excel® until a best fit was found between the measured values for the ratio of volume of added base (V_b) and volume of acid (V_a) and those calculated using eqn. (5). Plots of the titration curves and fits are given in the ESI.† The determined pK_a values were 10.27 ± 0.01 and 8.18 ± 0.01 for L-cys and 9.04 ± 0.01 for L-met, which are in good agreement with the respective literature values (10.30 ± 0.08, 8.18 ± 0.08 and 9.08 ± 0.04). The given errors are from the standard errors of fit calculated using the parameter

$$\frac{V_b}{V_a} = \frac{F_a C_a - \Delta}{C_b + \Delta}$$

where

V_b = volume of added base (NaOH)

V_a = initial volume of L-cys or L-met

C_a = initial concentration of L-cys or L-met

C_b = concentration of base

$$\Delta = [H^+] - \frac{10^{-pK_w}}{[H^+]} \quad (5)$$

$$F_a = \frac{K_{a1}}{[H^+] + K_{a1}} \quad (6)$$

$$F_a = \frac{[H^+]K_{a1} + 2K_{a1}K_{a2}}{[H^+]^2 + [H^+]K_{a1} + K_{a1}K_{a2}} \quad (7)$$

variance. Thus, the surfactant has no significant effect upon the pK_a's.

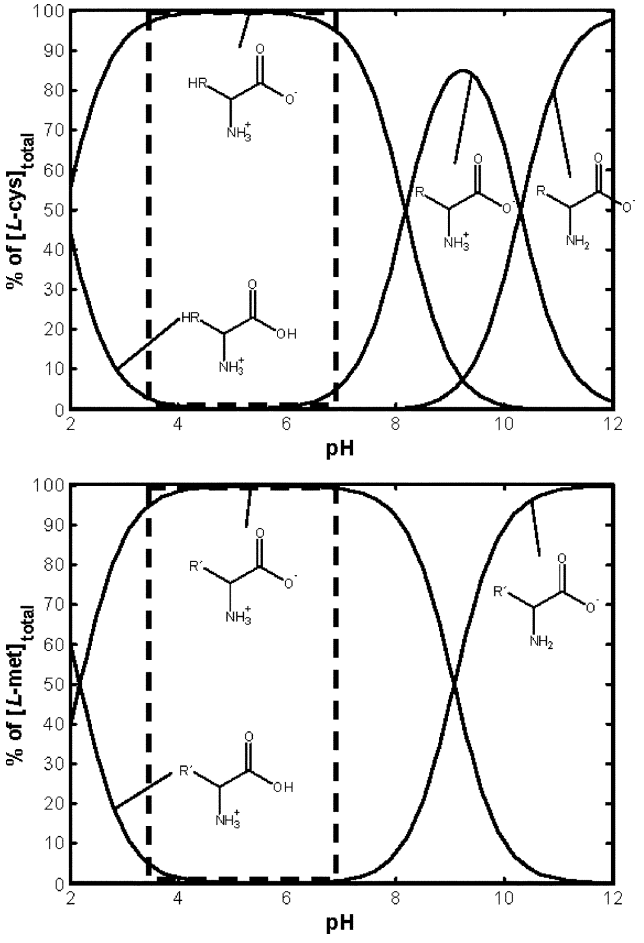


Fig. 4 pH dependent protolytic species distribution diagrams for L-cys (R = $-CH_2S^-$) and L-met (R′ = $-CH_2CH_2SCH_3$) calculated using literature pK_a values. The area within the dashed box indicates the pH range covered by the kinetic measurements.

Reactivity trends: L-cys *vs* L-met

As would be expected for the mechanism of Scheme 2, the determined rate constants for formation of the aqua complexes (k_1 and k_4) are independent of whether L-cys or L-met was used as the entering group. On the other hand, significant differences were seen between L-cys and L-met in terms of the pathway where Cl^- undergoes direct replacement by an amino acid. While this pathway contributes significantly for the reactions between complexes **I–III** and L-cys, the kinetic data indicates there is little contribution from this pathway for L-met. The difference in reactivity between L-cys and L-met can be rationalised in terms of the steric bulk and reduced nucleophilicity of a thioether, L-met, *vs*. a thiolate, L-cys. In L-met the entering S group is attached to two C atoms. In contrast, the S group in L-cys is terminal and as such there would be less steric hindrance involved in complex formation. Thus, formation of a complex with L-met is favoured only when Cl^- has been replaced by a better leaving group, namely water. The trend seen in reactivity of the Pt(II) complexes is **III** > **II** > **I** > **IV** for all rate constants. This trend can be clearly seen in the bar graph of Fig. 5. Reactions with L-met were not included in the graph as the determined values for k_1 and k_4 are essentially the same as those determined for reactions with L-cys.

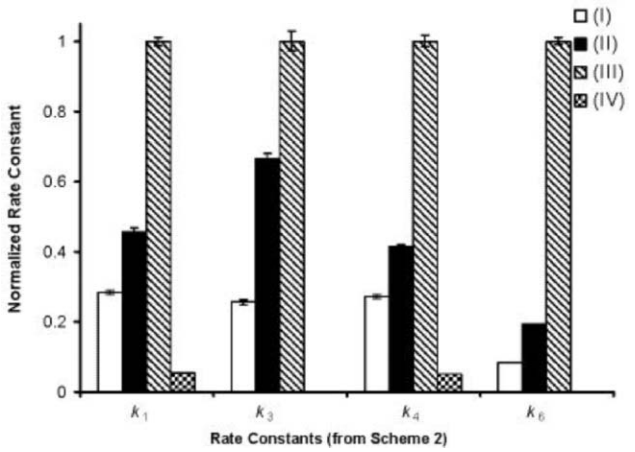


Fig. 5 Bar graph illustrating the variation of the rate constants k_1, k_3, k_4 and k_6 (see Scheme 2) for the complexes **I–IV** by normalization to 1 with respect to the rate constant obtained for **III**.

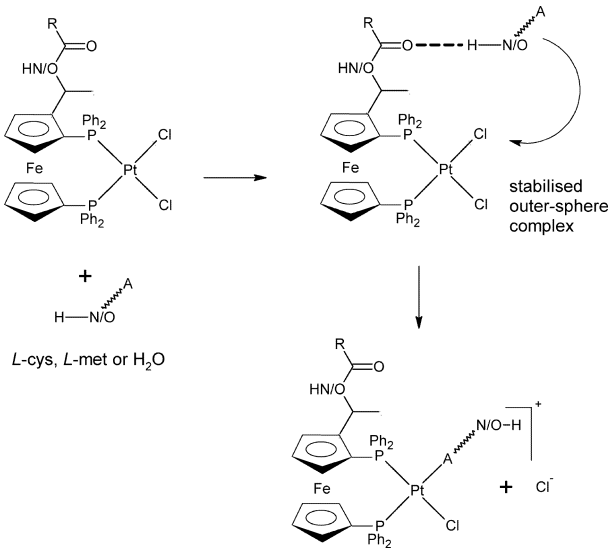


Fig. 6 Proposed mechanism for stabilisation of the outer-sphere complex for the Pt(II) complexes containing substituted dppf. The entering ligand represents either L-cys, L-met or water with hydrogen bonding *via* –OH or $–NH_3^+$; R = $–CH_3$ or $–CH_2CH_2CO_2CH_3$.

Reactivity comparison

The rate constants for complex **I** are approximately one order of magnitude larger than for cisplatin. It is not surprising that the dppf containing complexes show enhanced reactivity over cisplatin. This behaviour can be explained in terms of the enhanced *trans* effect of having substituted P groups in the *trans* position to a leaving group as opposed to the N groups present in cisplatin. The presence of a substituted P group *trans* to a leaving group enhances the rate of ligand exchange.[36,37] Also, a general trend for all the complexes was the rate constant for formation of the mono-substituted water products (k_1) was one order of magnitude faster then formation of the bis products (k_4). Such a change is not unexpected due to both statistical and steric considerations when going from the mono to the bis products. A reduction of smaller magnitude is also seen when moving from the mono- to the bis-substitued amino acid products for complexes **I–III** (k_3 and k_6 respectively).

As shown in Fig. 5, introduction of the substituted dppf-ligands in complexes **II** and **III** result in enhanced reactivity over complex **I**. It might be possible that the substituents on dppf allow the reaction to proceed *via* a hydrogen bonding interaction similar to an internal conjugate base type mechanism,[38,39] with the substituted dppf spectator ligand providing the basic donor atom and the entering ligand a proton. Complex substitution reactions of this type can generally be interpreted in terms of the Eigen–Wilkins mechanism[40] where the overall rate constant for a substitution step is the product of both the outer-sphere formation constant, K_{OS}, and the subsequent rate constant of coordinative bond formation. If either K_{OS} or the rate constant of coordinative bond formation is enhanced, then an increased reaction rate will be observed. The obvious dependence in reactivity on the nature of substituents on dppf for reactions with the potential H-bond donors L-cys and L-met indicates that outer-sphere complex formation may influence the reactivity (this is supported by reactions with 1-propanethiol, detailed shortly). The proposed mechanism is shown in Fig. 6, where the double bonded oxygens of complexes **II** and **III** act as basic donor atoms and form hydrogen bonds with protons of the entering ligand.[41] The difference in reactivity between the two substituted complexes can then be explained in terms of complex **III** providing access to three potential interaction sites: two double-bound oxygens and one amine, whereas complex **II** only allows for interaction with a single double-bound oxygen. Structural characteristics of the substituents may also play a role. If a substituent results in the hydrogen bonded entering ligand being oriented in a favourable manner, coordinative bond formation may also be enhanced.

H-Bond influence illustrated by the use of 1-propanethiol

To investigate the proposed role of hydrogen bonding on the reactivity of complexes **I–III**, reactions of these complexes with 1-propanethiol were measured in dichloromethane. 1-Propanethiol was chosen as a ligand and dichloromethane as a solvent as neither can hydrogen bond with the Pt(II)–dppf complexes. Also, the synthesis and structure of a range of Pt(dppf)–thiols have been described in the literature under similar conditions,[42] with 1-propanethiol able to replace both chlorides to form a bis adduct without the formation of any other species. The measurements were done under psuedo-first-order conditions of 1-propanethiol. A single set of Pt(II)–dppf and ligand concentrations were used. Two pseudo-first-order rate constants, k'_{obs1} and k'_{obs2} (see Table 3), were fitted by nonlinear regression to absorbance data measured over the range 255–270 nm using Pro-KII.[28] The absorbance in this wavelength range is mainly due to 1-propanethiol. Fits at three wavelengths for the reaction between complexes **I** and **III** and 1-propanethiol are shown in Fig. 7. The trend in reactivity seen is: for k'_{obs1}, **I** > **II** ≈ **III**; and for k'_{obs2}, **I** ≈ **II** > **III**. The trend is clear that complexes **II** and **III** containing the substituted dppf spectator ligand react overall more slowly than **I**. This is the reverse trend to that seen for L-cys and L-met in aqueous media. These results are consistent with the proposal that hydrogen bonding between the additional substituents on complexes **II** and **III** results in their enhanced reactivity in aqueous solution. If it is not possible for hydrogen bonding to take place, then the substituents seem to sterically and/or electrostatically hinder the approach of the entering ligand.

Table 3 Determined pseudo-first-order rate constants for 1.5×10^{-5} M Pt(II)–dppf and 7.8×10^{-4} M 1-propanethiol at 25 °C in dichloromethane. Uncertainties are given as the error in the last significant digit to two standard deviations

Complex	$10^2 k'_{obs1}/s^{-1}$	$10^4 k'_{obs2}/s^{-1}$
I	1.20 ± 3	6.3 ± 3
II	0.567 ± 8	6.27 ± 3
III	0.504 ± 8	0.47 ± 2

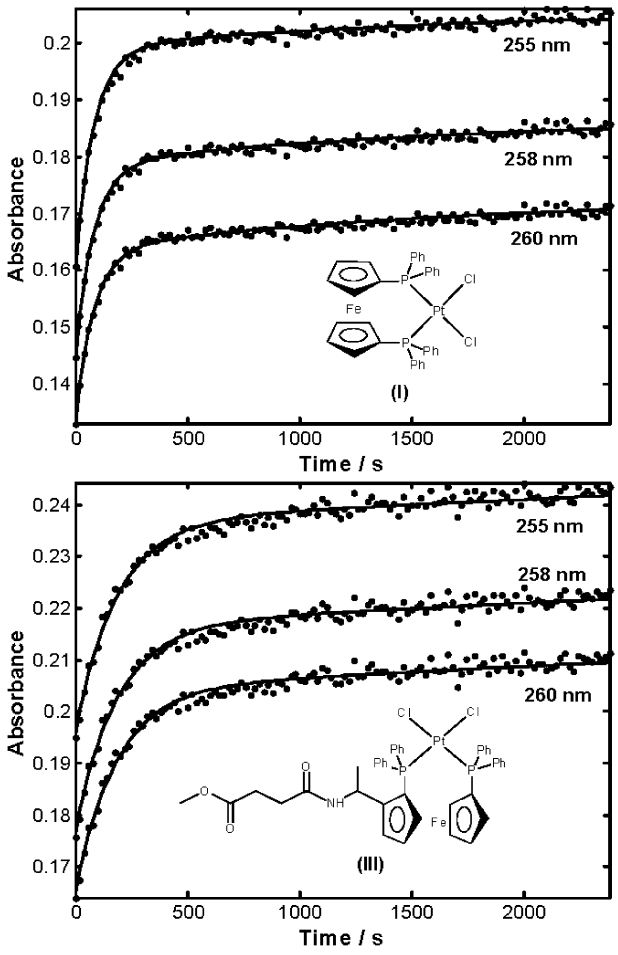


Fig. 7 Measured (···) and calculated (—) absorbance data at three wavelengths for the reaction between complex **I** and complex **III** and 1-propanethiol in dichloromethane.

Conclusions

The present study was designed with the aim of documenting the influence of non-reactive pendant H-bond acceptors on the reaction kinetics of a series of dppf-containing Pt(II) complexes, representing a novel series of tentative anticancer active drug candidates. The results indicate that the presence of the dppf moiety in Pt(II) complexes results in significantly enhanced reactivity over cisplatin, which is not surprising considering the expected more pronounced *trans*-effect of the coordinated phosphine ligands compared to ammonia. Furthermore, it was determined that the effect of the substituent attached to the dppf spectator ligand was to enhance the reactivity in aqueous solution. It is proposed that this enhanced reactivity could be a consequence of hydrogen bonding between the basic donor atoms of the dppf substituent and the entering ligand, resulting in a stabilised outer-sphere complex. Support for this proposal was found from the reversal of the reactivity trend seen for reactions between the dppf containing complexes and 1-propanethiol in dichloromethane (a non-hydrogen bonding ligand and solvent). These results indicate that H-bonding patterns between entering groups and the pendant arm is a parameter to consider for the future design of novel dppf-containing anticancer drugs. By modification of the substituent, it may be possible to direct it to or away from a well defined interaction site, which in conjunction with the more potent reactivity of the Pt(II)–dppf complexes, would result in a more active, selective and lower toxicity drug.

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