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Rate and Equilibrium Data for Substitution Reactions of [Pd(*dien*)Cl]$^+$ with *L*-Cysteine and Glutathione in Aqueous Solution

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Summary. The kinetics of the complex formation reactions between monofunctional palladium(II) complex, [Pd(*dien*)Cl]$^+$, where *dien* is diethylene triamine or 1,5-diamino-3-azapentane, with *L*-cysteine and glutathione were studied in an aqueous 0.10 *M* perchloric acid medium by using variable stopped-flow spectrophotometry. Second-order rate constants, k_2^{298}, were $(3.89 \pm 0.02)\ 10^2\ M^{-1}s^{-1}$ for *L*-cysteine and $(1.44 \pm 0.01)\ 10^3\ M^{-1}s^{-1}$ for glutathione. The negative entropies of activation support a strong contribution from bond formation in the transition state of the process. The hydrolysis of Pd^{II} complex gave the monohydroxo species, [Pd(*dien*)(OH)]$^+$ and the dimer with a single hydroxo-bridge species, $[Pd_2(dien)_2OH]^{3+}$. *L*-Cysteine and glutathione ligands form complexes of 1:1 stoichiometry and a dimer with a single ligand bridge. The formation constants of the complexes were determined, and their concentration distribution as a function of *pH* was evaluated.

Keywords. Palladium; Complexes; Thiols; Equilibrium.

Introduction

For kinetics and mechanistic investigations of the mechanism of action of platinum(II) anticancer drugs their palladium(II) analogues are suitable model compounds since they exhibit *ca.* 10^4–10^5 times higher reactivities, whereas their structural and equilibrium behaviour is similar [1]. In recent years, our work has concentrated on reactions of Pt(II) and Pd(II) complexes with sulphur containing molecules [2–12] which could be of fundamental importance in understanding the nephrotoxicity of related platinum complexes.

To study this process, a very suitable compound appeared to be monofunctional [Pd(*dien*)Cl]$^+$ (*dien* = 1,5-diamino-3-azapentane). This complex, shown below, has

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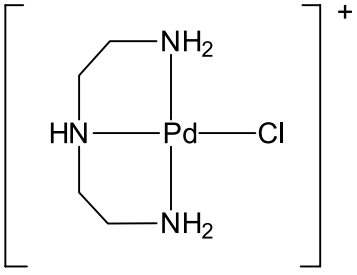
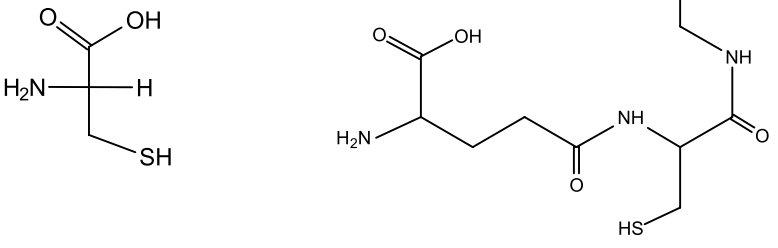


Formula 1



L-cysteine glutathione

Formula 2

only one exchangeable ligand and forms relatively stable complexes with S-donor ligands.

In continuation of our previous work [4–12], we here report detailed kinetic and equilibrium studies for the reactions of $[Pd(dien)Cl]^+$ complex with *L*-cysteine and glutathione in an aqueous solution (Eq. (1)). Also, the effect of temperature and *pH* on the reaction rate will be investigated.

$$[Pd(dien)Cl]^+ + L \rightleftharpoons [Pd(dien)L]^+ + Cl^- \qquad L = L\text{-cysteine and glutathione} \tag{1}$$

Results and Discussion

Equilibrium Studies

The acid dissociation constants of *L*-cysteine and glutathione were determined under the experimental conditions of $t = 25°C$ and a constant ionic strength of $0.10\,mol\,dm^{-3}$, which were used to determine the stability constants of the Pd^{II} complexes. The values obtained are consistent with those reported in the literature [13, 14]. The complex, $[Pd(dien)(H_2O)]^{2+}$, may undergo hydrolysis [15]. Its acid-base chemistry was characterized by fitting the potentiometric data to various acid-base models. The best fit model was found to be consistent with the species of the

stoichiometric coefficients 10-1 and 20-1. The dimer (20-1) with a single hydroxo-bridge may be formed by the reaction formulated in Eq. (2).

$$[(dien)\mathrm{Pd(H_2O)}]^{2+} + [(dien)\mathrm{Pd(OH)}]^{+} \rightleftharpoons [(dien)\text{Pd-OH-Pd}(dien)]^{3+} + \mathrm{H_2O} \quad (2)$$

The equilibrium constant for the dimerization reaction (2) can be calculated with Eq. (3) from formation constants summarized in Table 1 and amounts to, $\log K_\mathrm{d} = 3.48$.

$$\log K_\mathrm{d} = \log \beta_{20\text{-}1} - \log \beta_{10\text{-}1} \quad (3)$$

The distribution diagram for $[(dien)\mathrm{Pd(H_2O)}]^{2+}$ and its hydrolysed species is shown in Fig. 1. The dimer with a single hydroxo-bridge reaches the maximum

Table 1. Formation constants of [Pd(*dien*)]$^{2+}$ with glutathione and *L*-cysteine

System	l[a]	p[a]	q[a]	log β
Pd(*dien*)-OH	1	0	−1	−4.79 (0.02)
	2	0	−1	−1.33 (0.04)
Pd(*dien*)-glutathione	0	1	1	9.63 (0.01)
	0	1	2	18.33 (0.01)
	0	1	3	21.84 (0.02)
	1	1	0	12.68 (0.04)
	2	1	0	15.87 (0.06)
	1	1	1	18.00 (0.03)
Pd(*dien*)-cysteine	0	1	1	10.34 (0.02)
	0	1	2	18.52 (0.03)
	1	1	0	9.28 (0.08)
	2	1	0	15.28 (0.10)

[a] l, p, and q are the stoichiometric coefficients corresponding to Pd(*dien*)$^{2+}$, glutathione or *L*-cysteine, and H^+ respectively

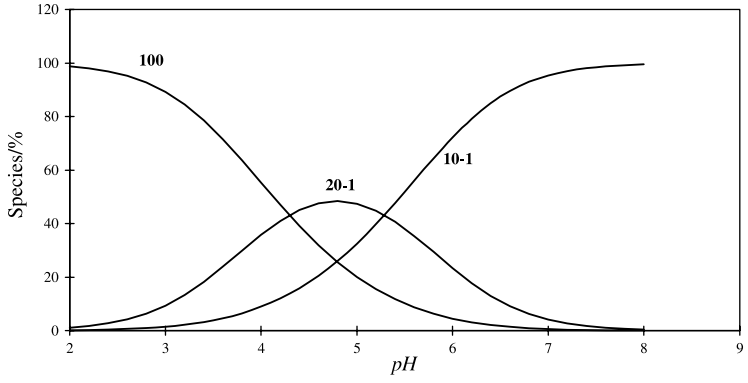


Fig. 1. Distribution of various species as a function of *pH* in the hydrolysis of [Pd(*dien*)]$^{2+}$ system (at a concentration of 1.25×10^{-3} mol dm^{-3})

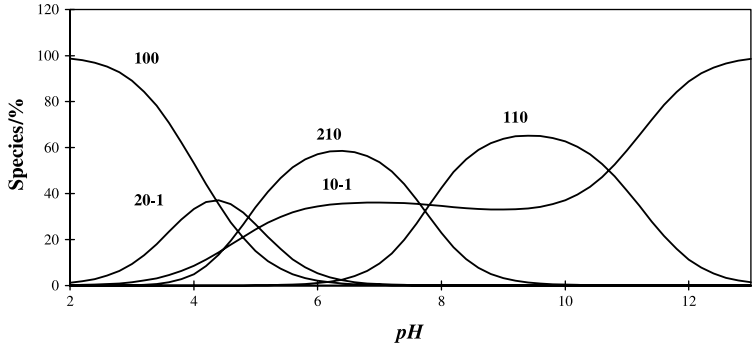


Fig. 2. Distribution of various species as a function of *pH* in the Pd(*dien*)-cysteine system (at concentrations of 1.25 and 1.25×10^{-3} mol dm^{-3}, respectively)

concentration of 47% at $pH = 4.8$, while the concentration of the mono-hydroxo species (10-1) increases with increase of *pH*.

Analysis of the titration data for the Pd(*dien*)L system (L = cysteine or glutathione) showed the formation of the 1:1 complex species and of the dimeric form (21-0). The concentration of the dimer is especially high in presence of an excess of the $[Pd(dien)H_2O]^{2+}$ complex, but it is also an important species in equimolar solution as it is clearly indicated in Fig. 2. It is well known that both –SH and $–NH_2$ groups can coordinate to Pd^{II}. $[Pd(dien)H_2O]^{2+}$ complex has only one coordination site available, which is occupied by the thiolate group, based on the high preference of Pd^{II} ion toward sulphur. The other coordination site ($–NH_2$) group is coordinating to another $[Pd(dien)H_2O]^{2+}$ ion. Such reaction may be formulated as given in Eq. (4).

$$Pd(dien)\text{-}S\wedge\wedge\wedge\wedge NH_2 + Pd(dien)H_2O \rightleftharpoons Pd(dien)\text{-}S\wedge\wedge\wedge\wedge NH_2\text{-}Pd(dien) \quad (4)$$

The equilibrium constant for such a reaction (Eq. (4)) can be calculated using Eq. (5) and amounts to $\log K_c = 6.00$ and 3.19 for *L*-cysteine and glutathione respectively.

$$\log K_c = \log \beta_{210} - \log \beta_{110} \quad (5)$$

The equilibrium constant (K_c) corresponding to coordination of the amino group to $[Pd(dien)H_2O]^{2+}$ is lower than that corresponding to the coordination of the thiolate group, (β_{110}), which reflects the difference in ligation potential between sulphur and nitrogen atoms to $[Pd(dien)H_2O]^{2+}$.

Kinetic Studies

The spectrum of the reaction mixtures evolves in all cases in a first order fashion and with well mentioned isobestic points, from that of the chloro complex, $[Pd(dien)Cl]^+$, to that of an authentic sample of the substituted product, $[Pd(dien)(SR)]^+$, measured under the same experimental conditions [9, 10], clearly

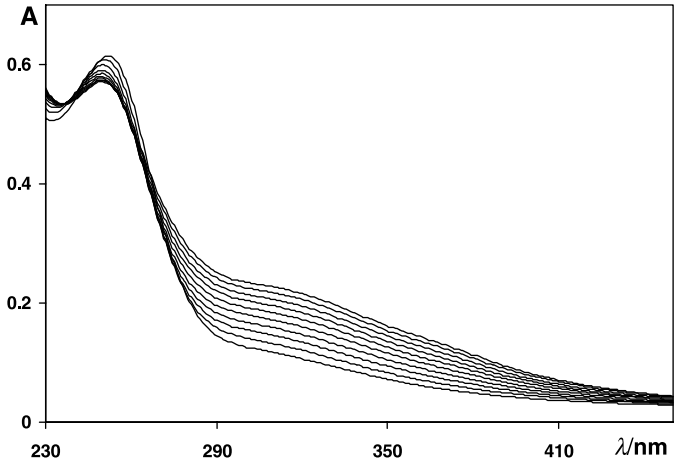


Fig. 3. UV-Vis spectroscopic changes during the reaction of the [Pd(*dien*)Cl]$^+$ with *L*-cysteine; [complex] $= 2\times10^{-4}$ mol dm^{-3}; [*L-cys*] $= 2\times10^{-3}$ mol dm^{-3}; $I = 0.1$ mol dm^{-3} $HClO_4$, $\Delta t = 30$ s

indicating that the process studied is the displacement of the coordinated chloro-ligand by the thiol (*e.g.* Fig. 3). The kinetic traces follow single exponentials, suggesting that only 1:1 complexes are formed according to Eq. (1). The observed

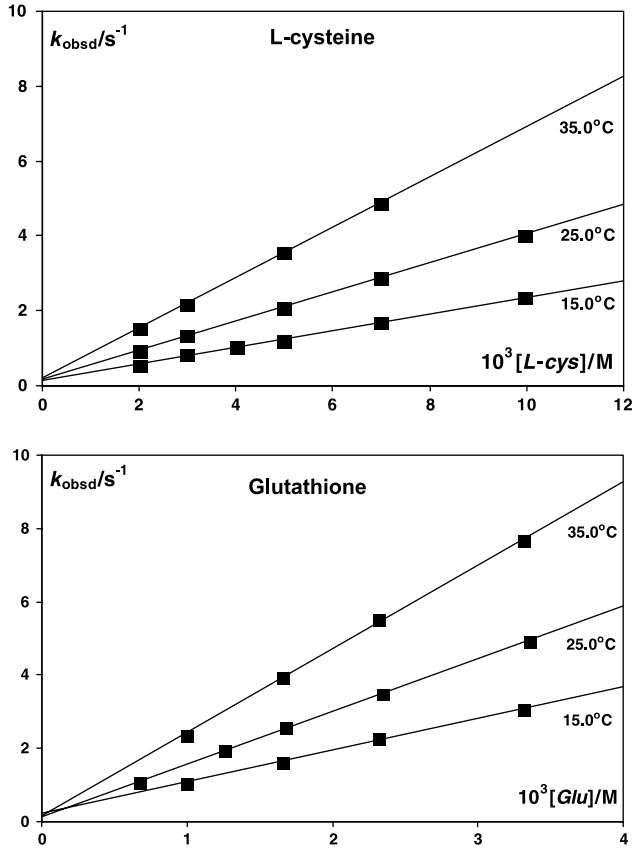


Fig. 4. Observed *pseudo*-first-order rate constants as a function of thiol concentration and temperature

rate constants, k_{obsd}, as a function of the total concentration of thiol, are described by Eq. (6).

$$k_{obsd} = k_1 + k_2\ [\text{thiol}] \tag{6}$$

When k_{obsd} is plotted against the concentration of the free ligand, [thiol], for the reaction of the chloro complex with thiol, the solvolysis rate constant (k_1) which is

Table 2. Observed *pseudo*-first-order rate constants as a function of *L*-cysteine concentration; complex, $[Pd(dien)Cl]^+$, concentration: 1×10^{-4} M

t/°C	10^3 $C_{L\text{-}cys}$/M	k_{obsd}/s^{-1} [a]
15.0	1.989	0.554 (6)
15.0	2.984	0.814 (4)
15.0	3.979	1.023 (5)
15.0	4.974	1.226 (5)
15.0	6.963	1.689 (6)
15.0	9.947	2.340 (6)
25.0	1.989	0.924 (5)
25.0	2.984	1.334 (5)
25.0	4.974	2.077 (4)
25.0	6.963	2.886 (6)
25.0	9.947	4.030 (7)
35.0	1.989	1.529 (7)
35.0	2.948	2.193 (5)
35.0	4.974	3.548 (7)
35.0	6.963	4.868 (7)

[a] Number of runs in parenthesis

Table 3. Observed *pseudo*-first-order rate constants as a function of glutathione concentration; complex, $[Pd(dien)Cl]^+$, concentration: 1×10^{-4} M

t/°C	10^3 C_{Glu}/M	k_{obsd}/s^{-1} [a]
15.1	0.994	1.069 (6)
15.1	1.658	1.624 (6)
15.1	2.321	2.280 (5)
15.1	3.316	3.060 (6)
25.0	0.670	1.085 (5)
25.0	1.257	1.942 (7)
25.0	1.676	2.552 (7)
25.0	2.346	3.501 (6)
25.0	3.352	4.941 (5)
34.9	0.994	2.378 (4)
34.9	1.659	3.931 (6)
34.9	2.321	5.535 (6)
34.9	3.316	7.660 (5)

[a] Number of runs in parenthesis

Table 4. Rate constants and activation parameters for the reactions between [Pd(*dien*)Cl]$^+$ and thiols; $I = 0.10$ mol dm^{-3} $HClO_4$

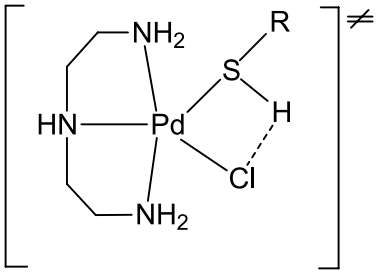
L	t/°C	k_2/M^{-1}s^{-1}	k_1/s^{-1}	$\Delta H_2^{298\neq}$/kJ mol^{-1}	$\Delta S_2^{298\neq}$/JK^{-1}mol^{-1}	Ref.
L-cysteine	25.0	$(3.89 \pm 0.02)10^2$	0.158	38 ± 1	-68 ± 2	This work
L-cysteine	15.0	$(2.22 \pm 0.02)10^2$	0.130			This work
L-cysteine	35.0	$(6.69 \pm 0.03)10^2$	0.214			This work
L-glutathione	25.0	$(1.44 \pm 0.01)10^3$	0.128	33 ± 1	-73 ± 2	This work
L-glutathione	15.1	$(8.63 \pm 0.01)10^2$	0.119			This work
L-glutathione	34.9	$(2.27 \pm 0.02)10^2$	0.163			This work
DL-penicillam	25.0	$(2.24 \pm 0.03)10^2$	0.117	47 ± 1	-43 ± 1	[10]

independent of [thiol], can be determined from the intercept, whereas the second order rate constant (k_2) for the direct nucleophilic attack, can be determined from the slope [16]. Figure 4 shows the concentration dependence of k_{obsd} (values entered are summarized in Tables 2 and 3).

The temperature dependencies of those rate constants allowed for the calculations of enthalpies and entropies of activation by use of *Eyring*'s equation [17]. Rate constants and activation parameters derived from these experiments are summarised in Table 4.

From Table 4 it could be seen that L-cysteine and glutathione are very good entering groups for [Pd(*dien*)Cl]$^+$ complex. Glutathione is a better nucleophile than *L*-cysteine although it is bigger. Moreover, glutathione is considerably more reactive than expected. This anomaly seems to suggest an appreciable anchimeric effect capable of reducing the activation energy of the substitution, arising from hydrogen bonding interactions between the acidic group located in a suitable position of the nucleophile. The anchimeric effect has been reported for other reactions at Pt(II) complexes and is well known for organic reactions [16]. A trigonal bipyramidal transition state of the reaction shown in Eq. (1) is probably stabilized by hydrogen bonding between the entering thiol and the leaving chloro ligand as already proposed for the reaction of $[Pd(H_2O)_4]^{2+}$ with monodentate acetate, propionate, glycolate, carboxylic acids [17, 18], and $[Pt(H_2O)_4]^{2+}$ with thioglycolic acid [5].

A large negative values of the entropy of activation is compatible with an associative mode of activation I_a or A mechanism. These findings indicate that bond-making with the entering ligand is important in the activation processes



Formula 3

and that the leaving group is still tightly bound to the metal centre in the transition state.

The general substitution behaviour of the $[Pd(dien)Cl]^+$ complex is very similar to that of $[Pt(dien)Cl]^+$, in the reactions with the same thiols [8], only the Pd(II) analogues react *ca.* 10^5 times faster.

Experimental

The complex [Pd(*dien*)Cl]Cl was prepared according to a standard procedure [20]. The chemical analysis and UV-Vis spectral data were in good agreement with those obtained for previous preparation. *L*-Cysteine (Fluka, 99.5%) and glutathione (Fluka, 99%) were used without further purification. For kinetic investigation ligand stock solutions were prepared shortly in 0.10 mol dm^{-3} $HClO_4$ (Merck, p.a.) as supporting electrolyte. Under these experimental conditions, $pH = 1.0$, the complex $[Pd(dien)Cl]^+$ was stable and the hydrolysis of the complex was negligible [15, 21, 22]. Water was doubly distilled from quartz. All solutions were flushed with nitrogen to remove dissolved oxygen.

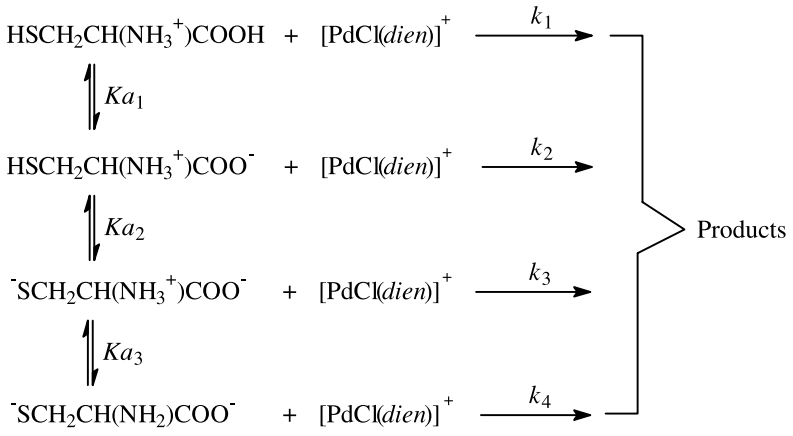
Potentiometric measurements were performed using a Metrohm 686 titroprocessor equipped with a 665 dosimat. The electrode and titraprocessor were calibrated with standard buffer solutions prepared according to NBS specifications [23]. The *pH* meter readings were converted into hydrogen ion concentration by titrating a standard acid solution (0.01 mol dm^{-3}), the ionic strength of which was adjusted to 0.1 mol dm^{-3} with $NaClO_4$, with standard base (0.10 mol dm^{-3}) at 25°C. The *pH* is plotted against *p*[H]. The relationship $pH - p[H] = 0.05$ was observed. [OH^-] value was calculated using a pK_w value of 13.997 [24].

Potentiometric Measurements

The acid-dissociation constants of the ligands were determined by titrating 1×10^{-4} mol dm^{-3} solutions of each with standard NaOH solution. The hydrolysis constants of $[Pd(dien)H_2O]^{2+}$ complex was determined by titrating 1×10^{-4} mol dm^{-3} of the solution complex with NaOH. The formation constants of the complexes were determined by titrating solution mixtures of $[Pd(dien)H_2O]^{2+}$ (1×10^{-4} mol dm^{-3}) and the ligand in concentration ratios of 1:1 and 2:1 (metal:ligand). The titration solution mixtures had a volume of 40 ml. The titrations were carried out at 25°C in a thermostated double-wall titration vessel and under a slow and constant stream of N_2 over the test solution. The ionic strength was adjusted to 0.10 mol dm^{-3} by addition of $NaClO_4$. A 0.10 mol dm^{-3} NaOH solution was used as titrant. The equilibrium constants for the species of the general formula $M_lL_pH_q$ ($M = [Pd(dien)H_2O]^{2+}$, $L =$ L-cysteine or glutathione) were calculated using the program [23] MINIQUAD-75. The stoichiometry and stability constants of the complexes formed were determined by testing various possible composition models. The selected model gave the best statistical fit and was chemically consistent with the titration data without giving any systematic drifts in the magnitude of various residuals, as described elsewhere [25]. The results are summarized in Table 1. The species distribution diagrams were obtained using the program SPECIES [26] under the experimental conditions employed.

Kinetic Measurements

Protolysis constants. Protolysis constants of the ligands are defined in Scheme 1 below. At 25°C and $\mu = 1.0$, their values are as follows: for cysteine [27] $pK_{a1} = 1.9$, $pK_{a2} = 8.10$ and $pK_{a3} = 10.1$. Protolysis constants for glutathione at 25°C and ionic strength of 0.2–0.55 M have been reported as $pK_{a1} = 2.05$, $pK_{a2} = 3.40$, $pK_{a3} = 8.72$ and $pK_{a4} = 9.49$ [28]. However, under the experimental conditions, where $[H^+] \gg K_a$, all ligands were protonated, so the reactions pathways described by k_2, k_3, and k_4 in Scheme 1 can be neglected at $pH = 1.0$, where all reported rate constants and activation parameters have been determined. At this *pH*, reactions with rate constants k_2, k_3, and k_4 contribute less than



Scheme 1

5% to the overall kinetics, which is within the error limits of the kinetics measurements and the determinations of the activation parameters.

The spectral changes resulting from mixing of complex and ligand solutions were recorded with a Hewlett-Packard 8452A diode-array spectrophotometer over the range $230 < \lambda > 450$ nm (Fig. 3) to establish a suitable wavelength at which kinetic measurements could be performed. These measurements were performed by use of a Hi-Tech stopped-flow spectrofotometer. Reactions were initiated by mixing equal volumes of the complex and thiol solutions directly in the stopped-flow instrument and were followed for at least 8 half-lives. Complex formation was monitored as an increase of absorbance at $\lambda = 320$ nm under *pseudo*-first-order conditions, with thiol in at least 10-fold excess. All kinetic runs could be described by single exponential, and no subsequent reactions were observed. The observed *pseudo*-first-order rate constants, k_{obsd}, were calculated as average values from five to eight independent runs. The variable temperature measurements were performed between 298 and 308 K. All kinetic runs were best described by single exponential. Kinetic data were collected and analyzed by use of the OLIS computer program [29].

Acknowledgments

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