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Kinetico-Mechanistic Studies of Nucleoside and Nucleotide Substitution Reactions of Co^{III} Complexes of Fully Alkylated Cyclen

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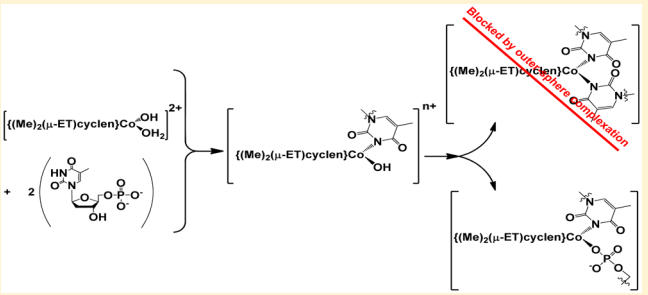
Supporting Information

ABSTRACT: The solution chemistry of complex $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(H_2O)_2]^{3+}$ containing a fully substituted tetraammine ligand designed for the avoidance of base-conjugated substitution mechanisms in the 6−8 pH range has been studied. The study should shed some light on the possible involvement of such Co^{III} skeleton in inert interactions with biomolecules. The reactivity and speciation of the complex has been found similar to that of the parent cyclen derivative with the presence of *mono-* and *bis*-hydroxo-bridged species; at pH < 7.1, all reactivity has been found to be related to the aqua/hydroxo monomeric complexes. Under these pH conditions, the substitution reactions of the aqua/hydroxo ligands by chloride, inorganic phosphate, thymidine, cytidine 5′-monophosphate (5′-CMP), and thymidine-5'-monophosphate (5′-TMP) have been studied at varying conditions; ionic strength has been kept at 1.0 $NaClO_4$ due to the high concentration of 2-(*N*-morpholino)ethanesulfonic acid (MES) or *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) used to ensure buffering. Except for chloride, the process occurs neatly in a one or two step process, showing dissociatively activated substitution mechanisms, having in general large $\Delta H^{\ddagger}$, positive $\Delta S^{\ddagger}$, and values of $\Delta V^{\ddagger}$ close to those corresponding to the liberation of an aqua ligand to the reaction medium. The actuation of noticeable encounter-complex formation equilibrium constants has been found to be the determinant for the reactions with nucleosides and nucleotides, a clear indication of the relevance of hydrogen-bonding interactions in the reactivity of these molecules, even in this highly ionic strength medium. For the substitution of the active aqua/hydroxo ligands with 5′-TMP, the first substitution reaction produces an $\boldsymbol{N}_{thymine}$-bound 5′-TMP complex that evolves to a *bis*-5′-TMP with an $\boldsymbol{N}_{thymine},\boldsymbol{O}_{phosphate}$-bonding structure. The formation of outer-sphere complexes between the dangling phosphate group of the $\boldsymbol{N}_{thymine}$-bound 5′-TMP and the thymine moiety of another entering 5′-TMP has been found to be responsible for this fact, which leaves only the phosphate group for coordination available.



INTRODUCTION

The use of coordination compounds to study possible modification in biologically relevant molecules is not new; nevertheless, any new information that can be extracted from their simple reactivity should not be underestimated.[1,2] Besides obvious thermodynamic requirements and no leaching of the metal center, the need for the processes to occur in a rate that allows a controlled reaction also has to be taken into consideration when designing processes able to act in biological systems. That is, the solvolysis or substitution processes that involve the metal centers have to be relatively slow in order to ascertain the maintenance of the active molecule or the interaction of the complex with the expected target.[3] Lately, an important amount of literature has appeared dealing with the speciation, hydrolysis, complexation, and polymerization of a lot of "biologically" active centers, underlining the key role on simple substitution processes actuating on biologically relevant coordination complexes.[4−7] Clearly, a rationalization of the solution behavior of metal complexes under conditions similar to those in biological conditions is fully desirable, including stability in plasma studies.[8] Mimicking *in vitro* the conditions of biological systems is extremely difficult, if possible at all, so that some simplifications have to be made. In this respect, the nontrivial aspect of so-called "innocent buffers" at pH values close to 7 has already been carefully studied by us,[9] and possible hydrogen bonding and stabilization of supramolecular interactions have appeared to have dramatic consequences. Another important aspect relates to the effect of the temperature on the activity of these coordination compounds,[10] which underlines the importance of determining activation parameters for these simple substitution reactivities, a point normally not considered.

Although the anticancer activity of *cis*-platinum still remains as the most important landmark in medicinal inorganic chemistry, the importance of other metal complexes should not be underestimated.[11] Antiartritic gold compounds, Gd compounds for MRI or Cu compounds for PET imaging could be cited as relevant examples.[12−15] Actually, the last decades have seen an increasing number of reports showing metal

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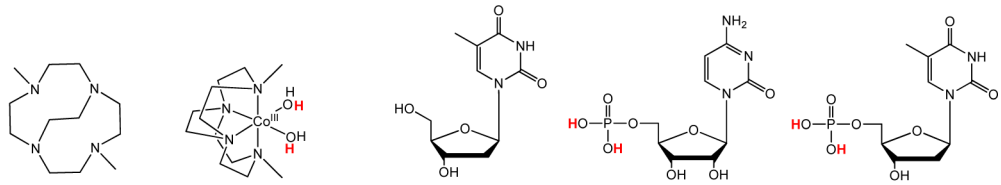
Scheme 1



complexes with promising medical applications and, for example, ruthenium complexes are now one of the most important groups of compounds with antitumor properties.[16−21] From this point of view, Co complexes with an inert tetradentate skeleton and two reactive positions in *cis* are obviously interesting as they could represent a cheaper and less toxic alternative to currently used compounds.[22] Even some Co alkyne complexes have shown promising activity associated with its capability to target specific enzymes.[23,24]

The possible use of complexes of type *cis*-$[Co(N)_4(H_2O)_2]^{3+}$, with $(N)_4$ being cyclen or tren, has already been explored by us[9,25] by studying its substitution processes with some nucleotides at physiological pH levels and taking into account the existence of rather fast processes due to the actuation of base-conjugate processes for nonfully substituted *N*-donors.[26−29] In the reactivity involved, the formation and cleavage of hydroxo-bridged dimeric units, kinetico-mechanistically detected some time ago,[30,31] has been found to be a keystone. As a whole, the solution chemistry of complex $[Co(cyclen)(H_2O)_2]^{3+}$ at pH values within the physiological range has been found to be of an extremely complex nature. It has been found dependent on concentration, pH levels and buffer used;[9] furthermore, it has been associated both with the base-conjugate substitution lability, induced on the Co^{III} center by the ligand,[27,29,32] and with the presence of good bridging hydroxo ligands.[31]

In view of these facts, the study of the complex with the alternative, fully substituted, and conformation rigid, $\{(Me)_2(\mu$-ET)cyclen$\}$ (Scheme 1, left), cyclen-based ligand has been carried out. The use of such ligand should prevent both the actuation of base-conjugate accelerated substitution processes and any isomerization reactions,[33] which could hamper the interpretation of data.[34] Furthermore, the absence of acidic hydrogens attached to the nitrogen donors should also prevent some of the interactions observed for the $[Co(cyclen)(H_2O)_2]^{3+}$ complex[9] with Good's buffer media.[35]

In this report, we present the study of the spontaneous solution chemistry of the $[Co\{(Me)_2(\mu$-ET)cyclen$\}(H_2O)_2]^{3+}$ in the 6−8 pH range and its reactivity with a series of biologically relevant potential ligands such as chloride, inorganic phosphate, and the nucleosides and nucleotides indicated in Scheme 1. The results collected agree with the expected slow dissociatively activated reactivity of a Co^{III} t_{2g}^6 metal center. The processes completely stop at pH > 7.1, where an equilibrium displacement to the *bis*-hydroxo dimeric form of the ion occurs. The data collected are also indicative of important outer-sphere interactions between the donors on the nucleobase moieties and the aqua ligands on the Co^{III} coordination center.

RESULTS AND DISCUSSION

Solution Behavior of $[Co\{(Me)_2(\mu$-ET)cyclen$\}(H_2O)_2]^{3+}$ in the 5.5−8.0 pH Range. The values of pK_a for the diaquo $[Co\{(Me)_2(\mu$-ET)cyclen$\}(H_2O)_2]^{3+}$ complex were determined to be 3.7 and 7.1 at 25 °C, by both alkaline potentiometric and spectrophotometric titrations at I = 1.0 ($NaClO_4$), as indicated in the Experimental Section. With these values, the prevalent species in the pH margin established for our next studies are $[Co\{(Me)_2(\mu$-ET)cyclen$\}(H_2O)(OH)]^{2+}$ and $[Co\{(Me)_2(\mu$-ET)cyclen$\}(OH)_2]^{+}$ in the 5.5−6.5 and 7.5−8.0 pH ranges, respectively. Determination of these pK_a values using different pH equilibration times was also carried out, and the same results were obtained, indicating that the processes being measured correspond effectively to the deprotonation equilibria. In this respect, the reverse spectrophotometric titration was also conducted on equilibrated solutions of the Co^{III} complex at pH ca. 7.0 for 2 h, followed by an increase in pH up to 0.1 M NaOH prior to acidic titration. No differences were observed in the pK_a values determined with respect to those obtained for alkaline titrations. The data indicate that there is no relevant contribution of any polymerization processes in the pK_a values determined.[28,36]

Once the acid/base prevalent species at these physiological pH levels were established, the possible slow polymerization processes occurring due to the generation of bridging hydroxo ligands with an increase in the pH was pursued.[28,36] Studies conducted on $(5−20) \times 10^{-4}$ M solutions of the $[Co\{(Me)_2(\mu$-ET)cyclen$\}(H_2O)_2]^{3+}$ complex at nonbuffered final pH = 6.8 do not show changes in the UV−vis spectrum for 24 h, even at 50 °C, which are expected to be specially relevant for the formation of the *bis*-hydroxobridged $[(Co\{(Me)_2(\mu$-ET)cyclen$\})_2(\mu$-$OH)_2]^{4+}$ complex.[9] Furthermore, the ^{13}C NMR spectrum of the above-mentioned samples, collected after equilibration, indicated a complete symmetrical arrangement of the $\{(Me)_2(\mu$-ET)cyclen$\}$ligand (52.4, 64.4, 65.8, and 70.7 ppm), with chemical shifts distinct from those of the diaqua species (54.0, 66.0, 67.2, and 71.1 ppm). It is thus clear that under these conditions relevant amounts of the *mono*-hydroxobridged, $[(Co\{(Me)_2(\mu$-ET)cyclen$\}(H_2O))_2(\mu$-$OH)]^{5+}$, species are not formed.

By the use of MES and HEPES buffers, a full pH screening of this solution reactivity has been conducted. In all cases, the above-mentioned irrelevant absorbance changes at pH values between 5.5 and 6.8 are observed after days at room temperature. As found for the cyclen parent system,[9] the use of MES significantly increases these UV−vis spectral changes. These become, nevertheless, fully relevant (Figure S1, Supporting Information) when monitored at pH > 7.1 (HEPES); under these conditions, they consist of a set of two processes with rather different time scales (2 *plus* 20 h). The fastest spectral changes (2 h) are found to increase its rate with the Co^{III} complex concentration, in good agreement with

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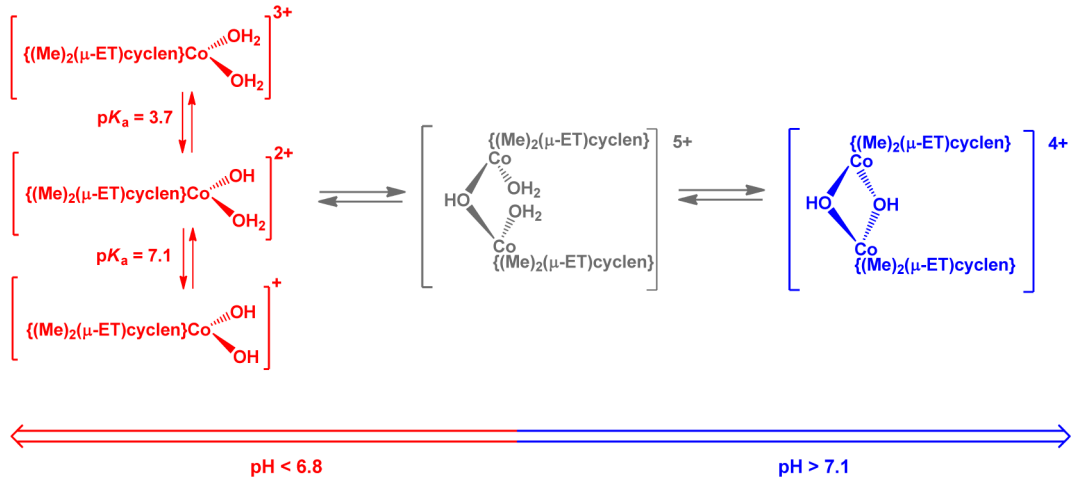
Scheme 2



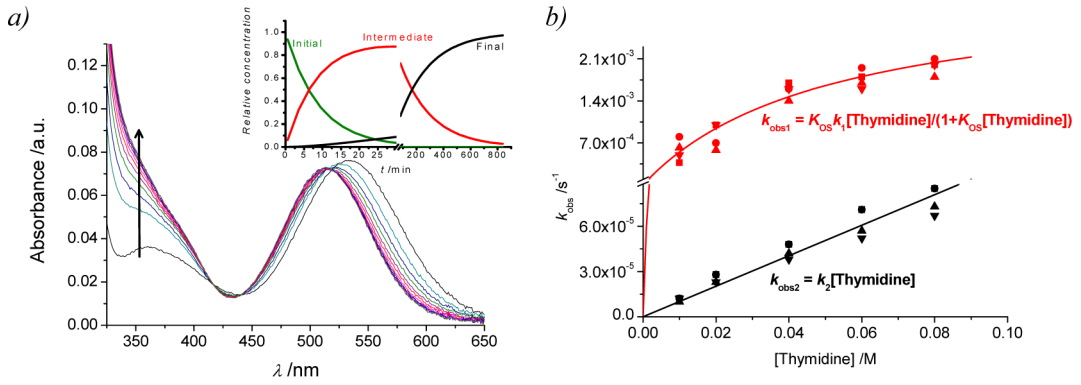


Figure 1. (a) Changes with time of the electronic spectrum of a 5×10^{-4} M solution of complex $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(H_2O)_2]^{3+}$ with 0.08 M thymidine (pH = 6.5, 0.4 M HEPES, I = 1.0 $NaClO_4$). (b) Plot of the values of the dependence of k_{obs} on [thymidine] at different pH values at 50 °C (▲, pH = 5.5; ▼, pH = 6.0; ■, pH = 6.5; ●, pH = 6.8; 0.4 M MES/HEPES, I = 1.0 $NaClO_4$).

the formation of a dimeric unit. Interestingly, increasing the pH has the opposite effect on the rate at which these spectral changes occur, as the amount of less reactive $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(OH)_2]^+$ complex increases. For the slowest spectral changes (20 h), their rate are found independent of the Co^{III} concentration, in full agreement with the formation of a *bis*-hydroxo $[(Co\{(Me)_2(\mu\text{-ET})cyclen\})_2(\mu\text{-OH})_2]^{4+}$ complex.

In summary, the complete behavior of the $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(H_2O)_2]^{3+}$ system has distinct features, when compared with the already studied parent cyclen derivative.[9] In the present case, the equilibrium between the monomer and dimeric complex is only significantly displaced to the formation of the latter at pH values higher than 7.1. Under these conditions, the dead-end *bis*-hydroxo $[(Co\{(Me)_2(\mu\text{-ET})cyclen\})_2(\mu\text{-OH})_2]^{4+}$ complex is produced (see below). At lower pH, the amount of dinuclear species is minimal and only the reactivity of the $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(H_2O)(OH)]^{2+}$ complex is expected (Scheme 2).[9] Attempts to determine the value of the acidity constants of the *mono*-hydroxobridged dimeric species shown in Scheme 2 has been unsuccessful due to the fast cleavage of these type of μ-OH complexes on acidification.[9] In the same respect, it is clear that the values of the pK_a values determined for the starting $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(H_2O)_2]^{3+}$ complex could be affected by dimerization reactions; given the slowness of these processes (2−20 h) and the fact that the values were determined with different equilibration times (see above), the validity of the values determined seems to be reinforced.

Substitution Reactions on $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(H_2O)_2]^{3+}$ in the 5.5−7.5 pH Range by Chloride, Thymidine, Phosphate, 5′-Cytidinemonophosphate, and 5′-Thymidinemonophosphate. *Chloride Anion.* After the establishment of the equilibrated species of the $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(H_2O)_2]^{3+}$ in the 5.5−7.5 pH range and in view of some studies carried out about the involvement of complexes having the same Co^{III} core in biologically relevant processes,[22,37] the reactivity at physiological pH of the complex with this ligand was pursued. Keeping in mind the lack of reactivity of the parent cyclen complex with chloride[9] and the importance of such processes on the relevant chemistry of *cis*-platinum at different pCl,[38−41] the reactivity with this anion was studied at pH values between 5.5 and 7.0 and at $[Cl^-]$ = 0.050−0.075 M. No changes in the UV−vis spectrum are observed after 24 h at 50 °C, thus indicating that the behavior totally parallels that of *cis*-$[Co(cyclen)(H_2O)_2]^{3+}$, i.e., no reactivity.

Thymidine. The study of the substitution reactions of this Co^{III} complex with related biologically relevant ligands with nitrogen donors, i.e., nucleosides or nucleobases, was further intended. Given the limitations in solubility of the nucleobases in this pH range,[42,43] as well as the blocking of one of the nitrogen donors of the base by the sugar unit, nucleosides were the choice. In fact, inosine has already been studied as a

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Table 1. Summary of the Kinetic, Thermal, and Pressure Activation Parameters for the Reaction of $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(H_2O)_2]^{3+}$ with Chloride, Thymidine, Phosphate, 5′-CMP, and 5′-TMP at Different pH Values (0.4 M MES/HEPES, I = 1.0 ($NaClO_4$))

entering ligand	pH	$^{323}k/M^{-1}\ s^{-1}$	$\Delta H^{\ddagger}$/kJ mol^{-1}	$\Delta S^{\ddagger}$/J K^{-1} mol^{-1}	$\Delta V^{\ddagger}$/cm^3 mol^{-1}
Cl^-	5.5−7.0	no reaction			
thymidine	5.5, 6.0, 6.5, 6.8	$k_1 = 3.3 \times 10^{-3}$[a]	115 ± 5[b]	50 ± 15[b]	15 ± 2[c]
		$k_2 = 1.0 \times 10^{-3}$	120 ± 10[b]	55 ± 20[b]	18 ± 1[d]
	7.1	no reaction			
$H_2PO_4^-/HPO_4^{2-}$	5.5	9.3×10^{-3}			
	6.0	6.7×10^{-3}			
	6.5	4.6×10^{-3}	120 ± 5	80 ± 15	23 ± 1[e]
	6.8	3.6×10^{-3}			
	7.1	no reaction			
5′-CMP$^-$/5′-CMP^{2-}	5.5	3.9×10^{-3}			
	6.0	2.7×10^{-3}			
	6.5	0.94×10^{-3}	124 ± 1	77 ± 2	not determined
	6.8	0.77×10^{-3}			
	7.1	no reaction			
5′-TMP$^-$/5′-TMP^{2-}	6.2, 6.5. 6.8	$k_1 = 4.1 \times 10^{-3}$	66 ± 8	−88 ± 23	not determined
		$k_2 = 6.8 \times 10^{-5}$[f]	121 ± 7	45 ± 21	not determined
	7.1	no reaction			

[a]Limiting value, in s^{-1}; K_{OS} = 20 M^{-1}. [b]Determined at pH = 6.5. [c]Determined at pH = 6.5 and 30 °C using $k_1 \cdot k_{obs@[thymidine]=0.08\ M}$, according to Figure 1b. [d]Determined at pH = 6.5 and 55 °C using $k_2 = k_{obs@[\ thymidine]=0.08\ M}/0.08$, according to Figure 1b. [e]Determined at pH = 6.5 and 60 °C using $k = k_{obs@[phosphate]=0.015\ M}/0.015$, according to Figure 3b. [f]Limiting value, in s^{-1}; K_{OS} = 25 M^{-1}.

substituent species on *cis*-$[Co(cyclen)(H_2O)_2]^{3+}$ in a very wide pH range.[25] From the parent nucleosides available, thymidine (Scheme 1) was chosen due to the acid−base characteristics of its nitrogen donor (pK_a = 9.8),[44] which allows the simplification of the system; only the neutral species is prevalent under the conditions of the study. Monitoring the spectral changes on nonequilibrated freshly prepared solutions leads to a complex sequence where the first changes were equivalent to those observed for noncontaining thymidine solutions. Thus, by using the methodology indicated in the Experimental Section, as well as in previous studies,[9,25] the time-resolved UV−vis spectral changes were monitored at 50 °C on preequilibrated samples of $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(H_2O)_2]^{3+}$ at the relevant pH levels (according to the data collected in the previous paragraphs).

By using the standard software,[45,46] the spectral changes obtained were found to be indicative of the actuation of a two step sequential process (Figure 1a). These processes are not observed at pH > 7.1, in good agreement with the formation of the dead-end *bis*-hydroxobridged dimers (Scheme 2) and were only studied in the 5.5−6.8 pH range. That is, the substitution reactivity is only observed in the pH range where the $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(H_2O)(OH)]^{2+}$ is prevalent in the medium, and it is completely lost at pH values where $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(OH)_2]^{+}$ or $[(Co\{(Me)_2(\mu\text{-ET})cyclen\})_2(\mu\text{-OH})_2]^{4+}$ are dominant. Figure 1b collects the trends for the pseudo-first order rate constants obtained at different acidities. While the slow step shows the common linear dependence, i.e., $k_{obs2} = k_2$[thymidine] (eq 1 at low K_{OS}), on the concentration of the entering ligand, the fastest step clearly shows a limiting behavior on thymidine concentration,[36,47,48] i.e., $k_{obs1} = K_{OS} \times k_{lim1}$[thymidine]/(1 + K_{OS}[thymidine]) (eq 1 at intermediate K_{OS}). It is also interesting to note that no significant dependence of the data on the pH is observed and, both for the determination of the limiting value of fast step (k_1(s^{-1})) and for the slope of the linear dependence of the constants of the slow step (k_2(M^{-1} s^{-1})), a joint fitting for all the observed rate constants at different pH values has been used to evaluate the final data. The relevant values extracted from these plots are collected in Table 1.

$$Co + L \overset{K_{OS}}{\rightleftarrows} \{Co;\ L\} \xrightarrow{k_{lim}} Co{-}L$$

$$\begin{aligned} &\text{at low } K_{OS} && k_{obs} = K_{OS} \times k_{lim} \times [L] = k \times [L] \\ &\text{at intermediate } K_{OS} && k_{obs} = K_{OS} \times k_{lim} \times [L]/(1 + K_{OS} \times [L]) \\ &\text{at high } K_{OS} && k_{obs} = k_{lim} \end{aligned} \quad (1)$$

By using the water Presat proton NMR experiment on the reacting solutions, the nature of the two species appearing during the full process (Figure 1a) in the reaction medium has been established. After 1 h at 50 °C at nonbuffered pH = 6.7, apart from the intense signal at 7.6 ppm corresponding to the proton of the ring of the free thymidine, a small signal at 8.5 ppm is also evident. This lower field shift is expected due to the coordination of a Co^{III} center to the nitrogen donor of the ring. After further 24 h under the same reaction conditions, a new signal at 8.1 ppm appears, and its relative intensity increases versus that of the signal at 8.5 ppm (Figure S2, Supporting Information). These data agree with the initial formation of a *mono*-thymidine complex (8.5 ppm) that evolves to a *bis*-thymidine species (8.1 ppm) with time. Neither attempts to isolate these *mono*- and *bis*-substituted derivatives, nor determination of the acidity constant of the *mono*-substituted derivative, have been conducted due to its irrelevance for the present study. The same reasoning applies for the rest of the substitution processes in this study.

In summary, the substitution reaction of complex $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(H_2O)_2]^{3+}$ with thymidine at pH values between 5.5 and 6.8 corresponds to a simple non-base-catalyzed substitution process. The reaction produces the *bis*-substituted species as the final complex via a clear dissociative activation mechanism for each step. This is evidenced by the thermal and pressure dependence of the data shown in Figure 2

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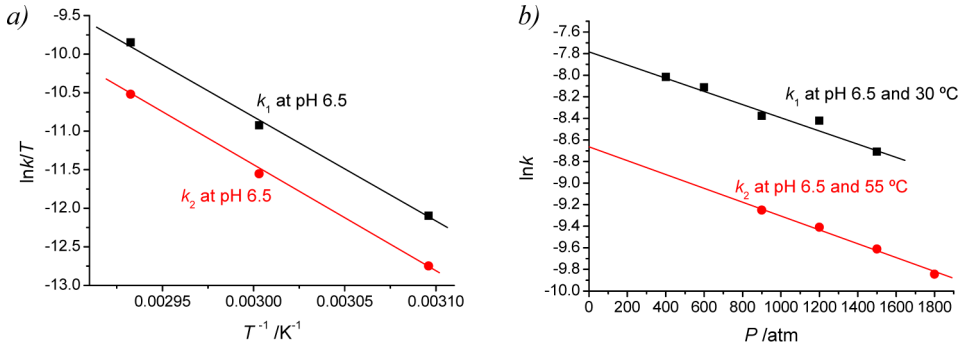


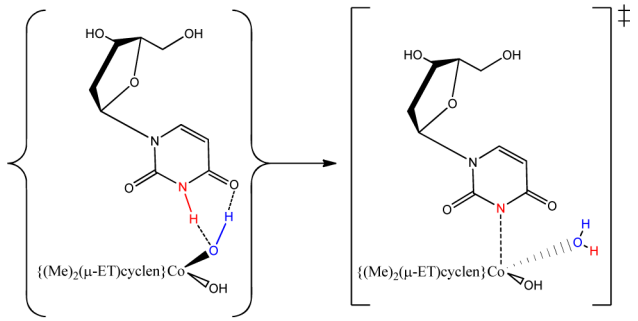
Figure 2. (a) Eyring plot for the dependence of the rate constants of the fast and slow step observed on the reaction of $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(H_2O)_2]^{3+}$ with thymidine. (b) Pressure dependence ln$k$ versus P for the same processes.

and collected in Table 1, which produces very large values of $\Delta H^{\ddagger}$ as well as positive values for both $\Delta S^{\ddagger}$ and the activation volumes; the latter is close to the expected +18 $cm^3\ mol^{-1}$ for the liberation of a water molecule. At higher pH levels, the reaction does not take place, due to the formation of the dead-end $[(Co\{(Me)_2(\mu\text{-ET})cyclen\})_2(\mu\text{-OH})_2]^{4+}$ species.

For the entry of the first thymidine nucleoside, the reaction involves the limiting formation of an outer-sphere complex (K_{OS} = 20 M^{-1}; eq 1, Figure 1b, and Table 1), as found for other substitution reactions on Co^{III} amine systems.[49,50] This encounter complex should originate from the interaction of the {ONO} unit of the nucleoside (see Scheme 1) with the protons of the remaining aqua ligand in the Co^{III} complex at this pH. A similar interaction has already been described for the equivalent substitution on Zn^{II} complexes,[51] despite the absence of the NH groups in the ligand in the present case. As for the entry of the second thymidine ligand, the value of the outer-sphere association equilibrium constant has definitively decreased in a way that a simple linear dependence between the value of k_{obs2} and [thymidine] is obtained (Figure 1b, eq 1 at low K_{OS}). Both the decrease of positive charge of the Co^{III} complex, produced by the first substitution of H_2O by thymidine (including its deprotonation to thymidine$_{\{-H\}}^{-}$ on coordination),[7,51] and the absence of acidic protons in the remaining $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(thymidine_{\{-H\}})(OH)]^{+}$ hydroxo complex can easily explain this fact.

The fact that the first substitution reaction of water by thymidine is not affected by the pH in the 5.5−6.8 range is surprising, given the increasing amounts of the more labile H_2O/OH^{-} Co^{III} species with a decrease in the pH. Only the protonation of putative and unreactive $\{[Co\{(Me)_2(\mu\text{-ET})cyclen\}(OH)_2]^{+};thymidine\}$ outer-sphere complexes, similar to those indicated in the previous paragraph, can explain this fact. In these species, the Brönsted basicity of the hydroxo ligand can be enhanced by the involvement of its remaining proton in the interaction with the nucleoside. As a consequence protonation of the hydroxo ligand of $\{[Co\{(Me)_2(\mu\text{-ET})cyclen\}(OH)_2]^{+};$ thymidine$\}$, the outer-sphere species, is achieved even at this pH; similar effects have already been described for other outer-sphere complexes in substitution reactions.[52] The effect can also be referred to, in this case, as a proton ambiguity (Scheme 3).[53] As for the absence of pH dependence on the rate constant for the entry of the second nucleoside, the effect can be related to an increased acidity of the remaining aqua ligand in $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(thymidine_{\{-H\}})(H_2O)]^{2+}$, probably remaining deprotonated at all the acidities studied. This can be associated with the attachment of a poorer Lewis base ($pK_{a(thymidine)}$ = 9.8)[44] (compared with the aqua ligand), thus

Scheme 3



leaving a higher positive charge density on the Co^{III} center, making the protonation of the remaining hydroxo ligand more difficult. Although computational methods should potentially improve the understanding of these types of interactions and diffusion-controlled proton transfers, inclusion of specific solvent interactions in the mechanisms of reactions is not a generally solved problem.[34,54−56]

Phosphate Anion. The reactivity of the complex with inorganic phosphate was also studied as a model for the substitution processes occurring with nucleotides. The changes observed in the UV−vis spectrum after pre-equilibration of the sample (Figure 3a) agree, using the standard software,[45,46] with a single step reaction in this case. The maxima in the electronic spectrum move to lower energies, which corresponds to the change to a lower field oxoanionic donor ligand. As in the previous system, the set of processes are not observed at pH > 7.1, in good agreement with the formation of the dead-end *bis*-hydroxobridged dimers, indicated in Scheme 2, at this acidity. The spectral changes were analyzed as described in the Experimental Section, and the values of the pseudo-first order observed rate constants obtained at different pH levels indicate a linear dependence on the total concentration of phosphate, Figure 3b (eq 1, low K_{OS}). ^{31}P NMR spectroscopy of final reaction ([Co]/[P] = 1:10) mixtures indicated the presence of a monodentate phosphato ligand (10.5 ppm downfield from the signal of the free anion)[57] at the same concentration as the cobalt center ($[P]_{10.5\ ppm}/[P]_{free}$ = 1:9). It is thus clear that a simple $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(HPO_4)(OH)]$ complex is formed after the reaction, with no *bis*-complexes of dinuclear units. Furthermore, as seen in Figure 3b, the reaction is faster at lower pH values (Table 1), despite the entering ligand decreasing its charge (from HPO_4^{2-} to $H_2PO_4^{-}$; pK_a = 7.2).[57] This fact agrees with the increasing amounts of more

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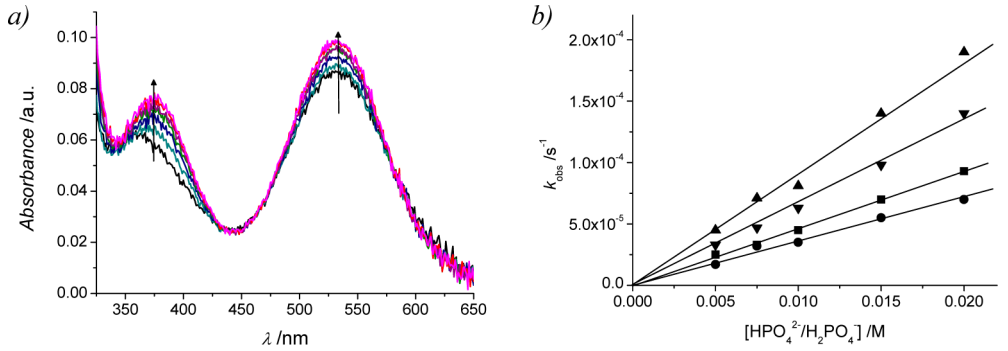

Figure 3. (a) Changes in the electronic spectrum of a solution of $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(H_2O)_2]^{3+}$ complex (5×10^{-4} M) in buffered (pH = 6.5, 0.4 M HEPES) aqueous 0.0075 M solution of inorganic phosphate (50 °C, I = 1.0 ($NaClO_4$)). (b) Plot of the values of the dependence of k_{obs} on [phosphate] at different pH values at 50 °C (▲, pH = 5.5; ▼, pH = 6.0; ■, pH = 6.5; ●, pH = 6.8; 0.4 M MES/HEPES, I = 1.0 ($NaClO_4$)).

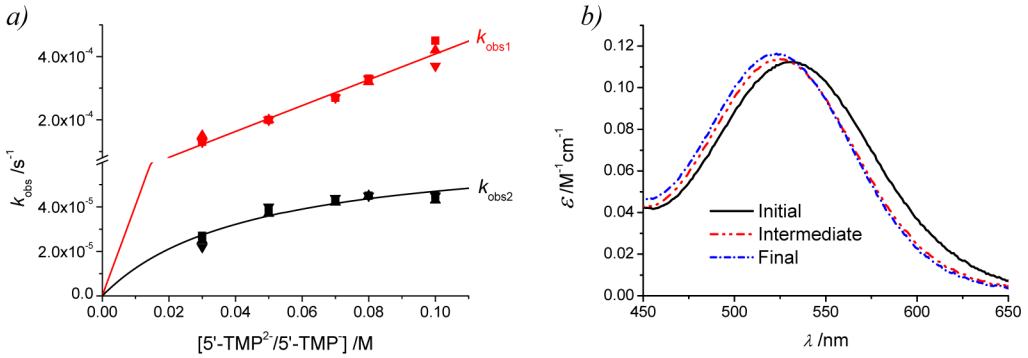

Figure 4. (a) Plot of the values of the dependence of k_{obs} on [5′-TMP] at different pH values at 50 °C (▲, pH = 6.2; ▼, pH = 6.5; ■, pH = 6.8; 0.4 M HEPES, I = (1.0 $NaClO_4$)). (b) SPECFIT-calculated spectra for the species derived from process k_{obs1} (2.3×10^{-4} s^{-1}) and k_{obs2} (3.3×10^{-5} s^{-1}) at [5′-TMP] = 0.08 M and pH =6.5 (0.4 M HEPES, I = 1.0 ($NaClO_4$), 50 °C).

dissociatively labile aqua ligands at lower pHs. For this system, no limiting kinetics is observed (eq 1 at high K_{OS}), similar to other phosphorus oxoanions substitution reactions on amine Co^{III} complexes.[49,58,59] The absence of the above-mentioned thymidine {ONO} unit can be held responsible for the differences observed with the system indicated above.[51] In this respect, the activation parameters (Table 1) determined for this system agree with a very dissociatively activated process, again dominated by bond breaking ($\Delta H^{\ddagger}$), and with a rather positive (+23 cm^3 mol^{-1}) volume of activation. These values are, in fact, in line the ones obtained for the substitution reactions with thymidine, agreeing with the expected independence on the entering ligand for a dissociative activation process.

As for the lack of a second substitution reaction, as that observed for the above-mentioned process with the thymidine nucleoside, it is somehow surprising. The fact that the second, negatively charged, phosphate entering ligand has to approach a position adjacent to another external negative charge (Co^{III}-***O***PO(OH)***O***$^-$) can explain this fact. In this respect, only η^2-phosphato (Co^{III}-***OO***PO_2) complexes have been described for simple *cis*-$[Co(en)_2(H_2O)_2]^{3+}$ complexes,[57] a coordination that seems highly improbable in the present system due to the pH range used, which does not allow for deprotonation of the $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(HPO_4)(OH)]$ species formed after the first substitution reaction.

Cytidine 5′-Monophosphate Anion. Once the reactivity with simple inorganic phosphate was established, the substitution processes of the complex by nucleotides were pursued. Given the fact that for the cyclen parent compound the studies had been carried out with cytidine 5′-monophosphate (5′-CMP),[9] this was our first choice. As for the $H_2PO_4^-/HPO_4^{2-}$ system, the $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(H_2O)_2]^{3+}$ complex shows some reactivity with 5′-CMP at 50 °C within the 5.5−6.8 pH range, where the ligand is in the phosphate-equivalent 5′-CMP$^-$/5′-CMP^{2-} forms.[60] The reaction features the same trends on the values of the observed rate constants for those observed for the substitution with inorganic phosphate (Figure S3, Supporting Information) and also stops completely at pH > 7.1. ^{31}P NMR spectroscopy indicated that a single species is formed after 36 h of reaction at 50 °C; the signal, being 9.7 ppm downfield from that of the free ligand, corroborates the formation of a single substituted species.

Nevertheless, the kinetic data for this substitution, collected in Table 1, indicates a definitive decrease on the rate of the reaction with respect to that observed for the inorganic phosphate substitution. Given the fact that the thermal activation parameters measured indicate the actuation of a very similar dissociately activation process, differences have to be related to the formation of the kinetically undetected outer-sphere encounter complex (k(M^{-1} s^{-1}) = K_{OS}(M^{-1}) × k(s^{-1})) according to eq 1 at low K_{OS}.[53] The simple Fuoss approach involving charges[61] cannot be held responsible for the difference, these being equivalent (or even more favorable) for the nucleotide ligand ($pK_{a(5'\text{-CMP}-/5'\text{-CMP}^{2-})}$ = 6.1 versus $pK_{a(H_2PO_4^-/HPO_4^{2-})}$ = 7.2)[57,60] in this pH range. Similarly, a well-oriented outer-sphere complexation is not expected to have this effect, with phosphate anions being common to both substitution reactions. Thus, only the formation of dead-end (wrong-oriented) outer-sphere complexes with 5′-CMP can explain the trend observed. Similar effects have been reported

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in numerous occasions in a wide variety of reactions,[9,48,62] even such association with "innocent" buffers has been quantified by us.[9] Effectively, examination of the nucleotide structure (Scheme 1) shows that some potential outer-sphere hydrogen-bonding interaction points exist at the nucleobase side to the nucleotide. These would produce "ineffective" (in view of the final phosphate moiety coordination) outer-sphere complexes, thus slowing down the substitution reaction rate.

Thymidine-5′-Monophosphate Anion. Nucleotide 5′-thymidine-5′-monophosphate (5′-TMP) (Scheme 1) was also used for the studies in order to generalize the substitution trends observed for the different phosphates with this Co^{III} complex. Monitoring the UV−vis spectra at 50 °C, after incubation of the $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(H_2O)_2]^{3+}$ sample indicated in the previous sections, showed a series of changes that could be easily fitted to a set of two observed rate constants by following the same procedure indicated above and in the Experimental Section. The reaction totally stops at pH >7.1, as in the previous processes indicated, and the use of MES had to be avoided due to competitive decomposition of the 5′-TMP ligand at pH < 6.0 using this buffer. The trend on ligand concentration and pH of the values of k_{obs1} and k_{obs2} is shown in Figure 4a, where it is made clear that no pH dependence is observed in this range. For the faster reaction, a linear dependence is obtained, while for the slower step a limiting behavior is obtained (see Figure 1b, eq 1, and thymidine-related paragraphs); Table 1 collects the relevant data derived from the plots.

^{31}P NMR spectroscopy indicates that, after 3 h at 50 °C (while k_{obs1} is operating), only a very small signal of 9.3 ppm downfield from the free ligand is evident, which increases on further monitoring after 24 h to an intensity corresponding to a Co/P = 1:1 ratio. It seems thus clear that the formation of a Co-***O***-PO_3 unit only occurs as the second step of the process. By using a water Presat ^1H NMR experiments on the reacting solution at 50 °C and at nonbuffered pH = 6.2, further knowledge of the product of the first step is obtained. After 3 h at 50 °C, an intense signal at 8.5 ppm is evident, indicative of the ***N***-coordination of the thymine moiety of 5′-TMP. The full NMR data thus agrees with the initial formation of a *mono*-***N***-(5′-TMP) complex that evolves to a *bis*-***N***,***O***-$(5'\text{-TMP})_2$ species at longer times. In this respect, the SPECFIT-calculated spectra for the intermediate and final complex (Figure 4b) agree with these data; the first reaction produces a clear shift of λ_{max} to higher energies due to the change form an ***O***-donor (H_2O/OH^-) to a ***N***-donor (***N***-bound 5′-TMP), while the second step practically leads to no changes of λ_{max} due to the formation of a ***O***-bound 5′-TMP species from an aqua/hydroxo derivative.

In view of the NMR and UV−vis data indicated above, the values of k_{obs1} would have been expected to be very similar to those collected for the first substitution observed on the $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(H_2O)_2]^{3+}$ *plus* thymidine system. This is not so, but the reason can be associated with a definite lower value of K_{OS}, which produces a linear dependence (Figure 4a, eq 1 at low K_{OS}) of the k_{obs1} versus [5′-TMP] plots. As a result, the limiting value of k_1 indicated in Table 1 for the entry of a thymidine ligand is never reached (Figure S4a, Supporting Information, and eq 1). The bulkier and more complex 5′-TMP ligand can easily explain this fact, both from a size perspective and from the possible involvement of the {ONO} unit in hydrogen-bonding interactions with the phosphate group of another ligand unit, in a similar fashion than that depicted in the outer-sphere complex of Scheme 3. In this respect, the comparison of the data obtained for the entry of a ***O***-bound 5′-CMP or 5′-TMP is also revealing (see Table 1 and Figure S4b, Supporting Information); it is clear that for 5′-TMP a limiting plot (eq 1 at high K_{OS}) producing lower values of k_{obs2} is operative. The above-mentioned hydrogen-bonding interactions between the phosphate side of the ***N***-bound 5′-TMP and the amine side of the entering 5′-TMP produce a dead-end outer-sphere complex with an important K_{OS} value (25 M^{-1}, see Table 1). This effect limits the rate constant for the entry of the dangling phosphate moiety of the outer-sphere bonded 5′-TMP ligand to the value obtained. In this respect, the values for the thermal activation parameters for these two processes, indicated in Table 1, are rather surprising. While for the entry of the second 5′-TMP, in an $O_{phosphate}$-bound fashion, the values determined for $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are equivalent to those found for the entry of inorganic phosphates or 5′-CMP; as expected, this is not so for the coordination of the first 5′-TMP in an $N_{thymidine}$-bound manner. Clearly, the negative values of the activation entropy, together with the smaller values of $\Delta H^{\ddagger}$, indicate an important degree of association in the transition state as defined by k_{obs1}. Given the fact that the value determined for k_1 includes an outer-sphere complex equilibrium constant (eq 1 low K_{OS}),[36] the reason for this associativeness indicating parameter in an inherently[28,63] dissociative substitution process should then be related to this fact. If the degree of ordering involved in the necessary formation of the outer-sphere complex is very large, lower enthalpy requirements and an important degree of decrease in entropy should be observed.[53] Clearly, the known outer-sphere hydrogen-bonding involving nucleobases is rather relevant in this process.

CONCLUSIONS

The kinetico-mechanistic studies on the substitution of the aqua ligands on the $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(H_2O)_2]^{3+}$ complex by nucleosides and nucleotides at pH levels close to neutrality have been conducted. Although at pH higher than 7.1 the formation of *bis*-hydroxo (mono- or dimeric) represents no thoroughfare for the reactivity, at lower pH levels (5.5−6.8), only the aqua/hydroxo $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(OH)(H_2O)]^{2+}$ monomeric species is relevant. The substitution reactions studied on the latter have indicated a determinant influence of hydrogen-bonding and outer-sphere interactions in the processes. These include the formation of both active and dead-end species that limit the values of the rate constant for the aqua ligand substitution by the entering ligand. In this respect, the active outer-sphere complexation role of the {ONO} thymine unit, both in thymidine and in 5′-TMP, is specially noticeable, which favors the substitution of a water molecule by an ***O***-bound 5′-TMP ligand even when the reaction is favored to the formation of an ***N***-bound isomer.

As a whole, it is made clear that the influence of the medium in the speciation of transition metal complexes having a potential application in biological chemistry is crucial and cannot be underestimated. Classical ion-pair interaction seems to have a rather minute role in the reactivity; in the present study, this has been enhanced by the use of high ionic strength to avoid pH shifts at high nucleoside/nucleotide concentrations.

EXPERIMENTAL SECTION

Compounds and Procedures. The cobalt $[Co\{(Me)_2(\mu\text{-ET})cyclen\}(H_2O)_2](ClO_4)_3$ complex has been prepared directly by

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recrystallization in the minimum amount of 1 M $HClO_4$ of the already described [Co{(Me)$_2$(μ-ET)cyclen}(CO_3)]Cl complex.[22] The complex was characterized by its elemental analyses, ^{13}C NMR, and UV−vis spectra. MES and HEPES solutions were prepared at a 0.4 M concentration at I = 1.0 ($NaClO_4$) by weighing the desired amounts of the commercially available reactants. Final pH was adjusted with suitable $HClO_4$ or NaOH solutions.[35] In all cases, buffering was checked during and after the reactions studied. These stock solutions were used as the solvent for all the ligands solutions utilized in the study.

^{13}C and ^{31}P NMR spectroscopy was carried out on Bruker 400-Crio instrument in H_2O adjusted at the desired pH and with a D_2O inset containing the corresponding reference, at the *Unitat d'RMN* from the *Centres Científics i Tecnològics de la Universitat de Barcelona (CCiTUB)*. The spectra were referenced externally to DSS (^{13}C) and H_3PO_4 (^{31}P). ^1H NMR spectra from the same aqueous solutions were collected using a water Presat proton NMR experiment on the same instrument. UV−vis spectra were collected using either a Cary 50 or a HP8453 instrument equipped with thermostated multicell transports. pH measurements were conducted on a Crison instrument using either fast response or microsample glass combined electrodes. Time-resolved UV−vis spectra were collected with the same instruments and exported to the relevant software packages indicated below.

pK_a determination was carried out both by potentiometric and UV−vis spectroscopy titrations. A Titrando 888 Metrohm instrument was used for the alkaline (0.1 M NaOH) potentiometric titrations on 2 × 10^{-3} M solutions of the cobalt complex taken to 0.01 M $HClO_4$ and using the built-in Tiamo 2.3 software. pH equilibration waiting time was varied in order to establish the independence of the acidity constants determined from possible polymerization processes occurring at the pH on the Co^{III} complexes. UV−vis spectroscopy titrations were carried out on 2 × 10^{-3} M solutions of the Co^{III} complex, taken to 0.01 M $HClO_4$, by adding small aliquots of 0.1 M NaOH; electronic spectra (Figure S5, Supporting Information) were recorded by using a Helma 661.202-UV All Quartz Immersion Probe connected to a Cary 50 instrument with optical fibers. The determination of the pK_a values was carried out in this case using the standard Specfit or ReactLab Equilibrium software.[45,46] Reverse acidic titrations were conducted in the same way but on 1 × 10^{-3} M solutions of the Co^{III} complex, taken to 0.1 M NaOH.

Kinetics. Solutions of the different ligands involved in the kinetic runs were prepared in the corresponding 0.4 M buffer solutions at I = 1.0 described above. The solutions of the metal complex were prepared at much higher concentrations (20−30-fold) in water; thus, an effective acidic pH was achieved, which prevented its polymerization processes. Small aliquots of this stock solutions were added to achieve the final conditions of the runs ([Co^{III}]= (2−7) × 10^{-4} M, [ligand] = 0.01−0.1 M). For all the substitution processes, pseudo-first order flooding conditions were used.

All the time-resolved experiments (by pH and NMR or UV−vis spectral monitoring) were conducted following three types of setups. (i) For solution chemistry experiments in nonbuffered medium, the desired aliquot of the stock Co^{III} complex solution was added to a solution at a chosen acidity; pH was immediately registered and further UV−vis, NMR, and pH changes were monitored. (ii) For solution chemistry experiments in buffered media, the desired aliquot of the stock Co^{III} complex solution was added to the chosen 0.4 M buffer solution; pH was registered and further UV−vis, NMR, and pH changes were monitored. (iii) For substitution experiments, requiring a preequilibration process, the desired aliquot of the stock Co^{III} complex solution was added to the chosen 0.4 M buffer solution without reactants; pH was registered, and UV−vis monitoring was carried out. When the spectral changes associated with the equilibration process were completed, a solution of the desired ligands in the chosen 0.4 M buffer was added to the final desired concentration; pH was registered, and further UV−vis, NMR, and/or pH changes were monitored. Atmospheric pressure runs were carried out on HP8453 or Cary 50 instruments; for runs at elevated pressure, a described high-pressure unit, connected to an J&M spectrophotometer, was used.[64]

All data were collected as full (300−750 nm) spectra and treated with the standard Specfit or ReactLab Kinetics software;[45,46] observed rate constants were obtained from the full changes of the spectra or alternatively at the wavelength where a maximum change was observed. The changes were fitted to the relevant A → B single exponential equation when first or pseudo-first order conditions applied; for consecutive reactions with the same characteristics, an A → B → C two exponential sequence was fitted. Table S1, Supporting Information, shows all the values obtained for k_{obs} as a function of the different compound and variables studied.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The values of the observed rate constants for the experiments described, its concentration dependence, and figures showing the spectral changes associated. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.inorgchem.5b00581.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Gloe, K. *Macrocyclic chemistry: Current trends and future perspectives*; Springer: Dordrecht, 2005.
(2) Cowan, J. A. *Inorganic Biochemistry. An Introduction*, 2nd ed.; Wiley-VCH: New York, 1997.
(3) Albrecht, M.; Rodríguez, G.; Schoenmaker, J.; van Koten, G. *Org. Lett.* **2000**, *2* (22), 3461−3464.
(4) Wegner, S. V.; Spatz, J. P. *Angew. Chem., Int. Ed.* **2013**, *52* (29), 7593−7596.
(5) Wexselblatt, E.; Yavin, E.; Gibson, D. *Angew. Chem., Int. Ed.* **2013**, *52* (23), 6059−6062.
(6) Bugarcic, Z. D.; Bogojeski, J.; Petrovic, B.; Hochreuther, S.; van Eldik, R. *Dalton Trans.* **2012**, *41* (40), 12329−12345.
(7) Busto, N.; Martínez-Alonso, M.; Leal, J. M.; Rodríguez, A. M.; Domínguez, F.; Acuña, M. I.; Espino, G.; García, B. *Organometallics* **2014**, *34* (1), 319−327.
(8) Pierroz, V.; Joshi, T.; Leonidova, A.; Mari, C.; Schur, J.; Ott, I.; Spiccia, L.; Ferrari, S.; Gasser, G. *J. Am. Chem. Soc.* **2012**, *134* (50), 20376−20387.
(9) Basallote, M. G.; Martínez, M.; Vázquez, M. *Dalton Trans.* **2014**, *43*, 11048−11058.
(10) Clavel, C. M.; Paunescu, E.; Nowak-Sliwinska, P.; Dyson, P. J. *Chem. Sci.* **2014**, *5* (3), 1097−1101.
(11) Bruijnincx, P. C. A.; Sadler, P. J. *Curr. Opin. Chem. Biol.* **2008**, *12*, 197−206.
(12) Stigers, D. J.; Ferdani, R.; Weisman, G. R.; Wong, E. W.; Anderson, C. J.; Golen, J. A.; Mooree, C.; Rheingold, A. L. *Dalton Trans.* **2010**, *39*, 1699−1701.
(13) Yang, C. T.; Cguang, K. H. *Chem. Commun.* **2012**, 552−565.
(14) Sun, R. W. Y.; Ma, D. L.; Wong, E. L. M.; Che, C. M. *Dalton Trans.* **2007**, 4884−4892.
(15) Guo, Z.; Sadler, P. J. *Angew. Chem., Int. Ed.* **1998**, *38*, 1512−1531.
(16) Han Ang, W.; Dyson, P. J. *Eur. J. Inorg. Chem.* **2006**, *2006*, 4003−4018.

DOI: 10.1021/acs.inorgchem.5b00581
Inorg. Chem. 2015, 54, 4972−4980

(17) Liu, H. K.; Berners-Price, S. J.; Wang, F.; Parkinson, J. A.; Xu, J.; Bella, J.; Sadler, P. J. *Angew. Chem., Int. Ed.* **2006**, *45*, 8153−8156.
(18) Scolaro, C.; Bergamo, A.; Brescacin, L.; Delfino, R.; Cocchietto, M.; Laurenczy, G.; Geldbach, T. J.; Sava, G.; Dyson, P. J. *J. Med. Chem.* **2005**, *48*, 4161−4171.
(19) Habtemariam, A.; Melchart, M.; Fernández, R.; Parsons, S.; Oswald, I. D. H.; Parkin, A.; Fabbiani, F. P. A.; Davidson, J. E.; Dawson, A.; Aird, R. E.; Jodrell, D. I.; Sadler, P. J. *J. Med. Chem.* **2006**, *49*, 6858−6868.
(20) Yan, Y. K.; Melchart, M.; Habtemariam, A.; Sadler, P. J. *Chem. Commun.* **2005**, *38*, 4764−4776.
(21) Bergamo, A.; Sava, G. *Dalton Trans.* **2011**, *40*, 7817−7823.
(22) Chang, J. Y. C.; Lu, G. L.; Stevenson, R. J.; Brothers, P. J.; Clark, G. R.; Botting, K. J.; Ferry, D. M.; Tercel, M.; Wilson, W. R.; Denny, W. A.; Ware, D. C. *Inorg. Chem.* **2013**, *52* (13), 7688−7698.
(23) Failes, T. W.; Cullinane, C.; Diakos, C. I.; Yamamoto, N.; Lyons, J. G.; Hamblet, T. W. *Chem.—Eur. J.* **2007**, *13*, 2974−2982.
(24) Ott, I.; Schmidt, K.; Kircher, B.; Schumacher, P.; Wiglenda, T.; Gust, R. *J. Med. Chem.* **2005**, *48*, 622−629.
(25) Gómez, K.; González, G.; Martínez, M.; Mendoza, C.; Sienra, B. *Polyhedron* **2006**, *25*, 3509−3518.
(26) Curchod, B. F. E.; Rotzinger, F. P. *Inorg. Chem.* **2011**, *50*, 8728−8740.
(27) Tobe, M. L. In *Advances in Inorganic and Bioinorganic Mechanisms*; Academic Press: London, 1983; pp 1−94.
(28) Tobe, M. L.; Burgess, J. *Inorganic Reaction Mechanisms*; Longman: New York, 1999.
(29) Curtis, N. J.; Hendry, P.; Lawrance, G. A. *J. Chem. Soc., Dalton Trans.* **1988**, 47−51.
(30) Jackson, W. G. *Aust. J. Chem.* **2009**, *62*, 1308−1317.
(31) DeMaine, M. M.; Hunt, J. B. *Inorg. Chem.* **1971**, *10*, 2106−2113.
(32) Poon, C. K.; Tobe, M. L. *Inorg. Chem.* **1968**, *7*, 2398−2404.
(33) Castillo-Blum, S. E.; Sosa-Torres, M. E. *Polyhedron* **1995**, *14* (2), 223−229.
(34) Aullón, G.; Bernhardt, P. V.; Bozoglián, F.; Font-Bardía, M.; Macpherson, B. P.; Martínez, M.; Rodríguez, C.; Solans, X. *Inorg. Chem.* **2006**, *45*, 8551−8562.
(35) Good, N. E.; Winget, G. D.; Winter, W.; Connolly, T. N.; Izawa, S.; Singh, R. M. M. *Biochemistry* **1966**, *5* (2), 467−477.
(36) Wilkins, R. G. *Kinetics and Mechanisms of Reactions of Transition Metal Complexes*; VCH: New York, 1991.
(37) Zhang, T.; Zhu, X.; Prabhakar, R. *Organometallics* **2014**, *33* (8), 1925−1935.
(38) Kozelka, J.; Legendre, F.; Reeder, F.; Chottard, J. C. *Coord. Chem. Rev.* **1999**, *190−192*, 61−82.
(39) Hohmann, H.; Hellquist, B.; van Eldik, R. *Inorg. Chim. Acta* **1991**, *188*, 25−32.
(40) Hohmann, H.; van Eldik, R. *Inorg. Chim. Acta* **1990**, *174*, 87−92.
(41) de Barrios, N.; González, G.; Grandas, A.; Martínez, M.; Moreno, V. *Inorg. React. Mech.* **1999**, *1*, 205−218.
(42) Wada, T.; Moriguchi, T.; Sekine, M. *J. Am. Chem. Soc.* **1994**, *116*, 9901−9911.
(43) Rodrguez-Pérez, T.; Fernández, S.; Sanghvi, Y. S.; Detorio, M.; Schinazi, R. F.; Gotor, V.; Ferrero, M. *Bioconjugate Chem.* **2010**, *21*, 2239−2249.
(44) Thibaudeau, C.; Plavec, J.; Chattopadhyaya, J. *J. Org. Chem.* **1996**, *61* (1), 266−286.
(45) Binstead, R. A.; Zuberbuhler, A. D.; Jung, B. *SPECFIT32. [3.0.34]*; Spectrum Software Associates: Marlborough, MA, 2005.
(46) *ReactLab*; Jplus Consulting Pty Ltd: East Fremantle, WA. Australia, 2009.
(47) Espenson, J. H. *Chemical Kinetics and Reaction Mechanisms*; McGraw-Hill: New York, 1981.
(48) Lappin, A. G. *Redox Mechanisms in Inorganic Chemistry*; Ellis Horwood: New York, 1994.
(49) Martínez, M.; Ferrer, M. *Transition Met. Chem.* **1984**, *9*, 395−397.
(50) González, G.; Martínez, M.; Rodríguez, E. *Eur. J. Inorg. Chem.* **2000**, 1333−1338.
(51) Aoki, S.; Kimura, E. *J. Am. Chem. Soc.* **2000**, *122* (19), 4542−4548.
(52) Ferrer, M.; Martínez, M.; Pitarque, M. A. *J. Chem. Soc., Dalton Trans.* **1990**, 1629−1633.
(53) Jordan, R. B. *Reaction mechanisms of inorganic and organometallic systems*; Oxford University Press: New York, 2007.
(54) Algarra, A. G.; Aullón, G.; Bernhard, P. V.; Martínez, M. *Inorg. Chem.* **2014**, *53*, 512−521.
(55) Algarra, A. G.; Feliz, M.; Fernández-Trujillo, M. J.; Llusar, R.; Safont, V. S.; Vicent, C.; Basallote, M. G. *Chem.—Eur. J.* **2009**, *15* (18), 4582−4594.
(56) Aullón, G.; Chat, R.; Favier, I.; Font-Bardía, M.; Gómez, M.; Granell, J.; Martínez, M.; Solans, X. *Dalton Trans.* **2009**, 8292−8300.
(57) von Seel, F.; Bohnstedt, G. *Z. Anorg. Allg. Chem.* **1977**, *435*, 257−267.
(58) Coronas, J. M.; Vicente, R.; Ferrer, M. *Inorg. Chim. Acta* **1981**, *49*, 259.
(59) Vinaixa, J.; Ferrer, M. *J. Chem. Educ.* **1983**, *60*, 155−166.
(60) Levene, P. A.; Simms, H. S. *J. Biol. Chem.* **1925**, *65*, 519−534.
(61) Fuoss, R. M. *J. Am. Chem. Soc.* **1958**, *80*, 5059−5061.
(62) Bernhardt, P. V.; Martínez, M.; Rodríguez, C.; Vázquez, M. *Dalton Trans.* **2012**, *41*, 2122−2130.
(63) Albert, J.; Bosque, R.; Crespo, M.; Granell, J.; Rodríguez, J.; Zafrilla, J. *Organometallics* **2010**, *29* (20), 4619−4627.
(64) van Eldik, R. High Pressure Kinetics; Fundamental and Experimental Aspects. In *Inorganic High Pressure Chemistry*; Elsevier: New York, 1986; pp 1−68.

DOI: 10.1021/acs.inorgchem.5b00581
Inorg. Chem. 2015, 54, 4972−4980