

The role of hydroxo-bridged dinuclear species and the influence of “innocent” buffers in the reactivity of cis -[Co^{III}(cyclen)(H₂O)₂]³⁺ and [Co^{III}(tren)(H₂O)₂]³⁺ complexes with biologically relevant ligands at physiological pH[†]

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In view of the relevance of the reactivity of inert tetraamine Co^{III} complexes having two substitutionally active *cis* positions capable of interact with biologically relevant ligands, the study of the reaction of cis -[Co(cyclen)(H₂O)₂]³⁺ and [Co(tren)(H₂O)₂]³⁺ with chlorides, inorganic phosphate and 5'-CMP (5'-cytidinemonophosphate) has been pursued at physiological pH. The results indicate that, in addition to the actuation of the expected labilising conjugate-base mechanism, the formation of mono and inert bis hydroxo-bridged species is relevant for understanding their speciation and reactivity. The reactivity pattern observed also indicates the key role played by the “innocent” buffers frequently used in most *in vitro* studies, which can make the results unreliable in many cases. The differences between the reactivity of inorganic and biologically relevant phosphates has also been found to be remarkable, with outer-sphere hydrogen bonding interactions being a dominant factor for the process. While for the inorganic phosphate substitution process the formation of μ - η^2 -OPO₂O represents the termination of the reactivity monitored, for 5'-CMP only the formation of η^1 -OPO₃ species is observed, which evolve with time to the final dead-end bis hydroxo-bridged complexes. The promoted hydrolysis of the 5'-CMP phosphate has not been observed in any of the processes studied.

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Introduction

The use of coordination compounds to study possible modifications 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, the need for the processes to occur at a rate that allows a controlled reaction has also to be taken into consideration when designing systems able to act in biological systems. That is, the solvolysis or substitution processes that involve the metal centres 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 In this respect, the metal centre in the desired coordination compound has to survive in the

biologically relevant environment in such a way that it is not leached to the medium, potentially producing unexpected or undesired reactivity. Recently a large amount of literature has appeared concerning the speciation, hydrolysis, complexation and polymerization of a lot of “biologically” active centres, underlining the key role of simple substitution processes actuating on biologically relevant coordination complexes.^{4–6} In this respect, the use of cis -[Pt(NH₃)₂(H₂O)₂]²⁺, derived from the aquation of the dichlorido precursor in biologically relevant media, fulfils most of the premises expected.^{6–8} Its substitution reactivity is relatively slow and polymerization is not expected to be relevant due to the relatively low acidity of M^{II}-bound water molecules.^{9,10} Furthermore, the cis -[Pt(NH₃)₂(H₂O)₂]²⁺/ cis -[Pt(NH₃)₂(H₂O)Cl]⁺/ cis -[Pt(NH₃)₂Cl₂] speciation equilibria even allow for a change in charge and polarity for its intake/permeability through cell membranes.^{11,12} Another important aspect, also being dealt with in the literature, refers to the effect of temperature on the activity of these coordination compounds,¹³ a fact that underlines the importance of determining activation parameters for this simple substitution reactivity. Although the anticancer activity of cisplatin still remains the most important landmark in

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medicinal inorganic chemistry, the importance of other metal complexes should not be underestimated.¹⁴ Antiarthritic gold compounds, Gd compounds for MRI or Cu compounds for PET imaging could be cited as relevant examples.^{15–18} Actually, the last few decades have seen an increasing number of reports showing metal complexes with promising medical applications and, for example, ruthenium complexes are now one of the most important groups of compounds with anti-tumor properties, their mode of action being different from that of cisplatin.^{19–24} With regard to cobalt, some Co alkyne complexes also show promising activity associated with their capability to target specific enzymes.^{25,26} Given the variety of interactions of metal complexes with biomolecules possible for the different complexes mentioned above, a rationalization of the solution behavior of metal complexes under conditions similar to those in biological conditions is desirable. From this point of view, cobalt complexes with two reactive positions in *cis* are obviously interesting as they could represent a cheaper and less toxic alternative to currently used compounds. On the other hand, it is evident that mimicking *in vitro* the conditions of biological systems is extremely difficult, if not impossible at all, so that some simplifications have to be made. In this sense, a non-trivial aspect that should also be clarified is the role of so-called “innocent buffers” due to its potential influence on the reactivity of these complexes in *in vitro* studies at pH close to 7, especially when considering possible hydrogen bonding and stabilization of supramolecular interactions. The results of the present work are also in line with the so-called “innocent ionic” medium used in other biologically relevant reactions, which has been proved to be not so innocent.^{27,28}

Co^{III} coordination compounds containing a set of two substitutionally active positions, plus a tetradentate very inert skeleton surviving any processes occurring on these sites, represent an obvious potential alternative.²⁹ Nevertheless, the use of complexes of the type *cis*-[Co(N)₄(H₂O)₂]³⁺, with (N)₄ being a ligand such as cyclen or tren, is hampered by the existence of rather fast processes occurring at physiological pH due to the actuation of the conjugate-base process for non-fully substituted N-donors.^{30–32} The metal centre being small (the first transition series) and highly charged (Co^{III}) are the key points for the prevalence of such reactivity. The high acidity of coordinated (N)₄ ligands is also relevant, and derives from possible internal hydrogen-bonding.³³ Nevertheless, the presence of a *cis*-dihydroxo complex as one of the dominant species at physiological pH can also be considered very important for the reactivity of such complexes in biological media, given the good ligand character of the OH⁻ group and the dissociatively activated mechanism involved in Co^{III} substitution processes.

Although the formation and cleavage of hydroxo-bridged tetraammine complexes of Co^{III} has already been studied kinetically-mechanistically some time ago,^{34,35} no comprehensive study of the substitution reactivity of this type of *cis*-[Co(N)₄(H₂O)₂]³⁺ complexes under biologically relevant conditions has been conducted so far. The role of these binuclear species in the chemistry of the tren and cyclen systems is

usually neglected. In this respect, it is important to note that the size and shape of the (N)₄ ligand determines the relative position of the two substitution-active coordination positions in the *cis* form in all cases.^{36,37} The knowledge of such reactivity should be relevant for its extrapolation to other inert metal centres that do not react by a dissociatively activated, hydrogen bonding assisted process.^{32,38}

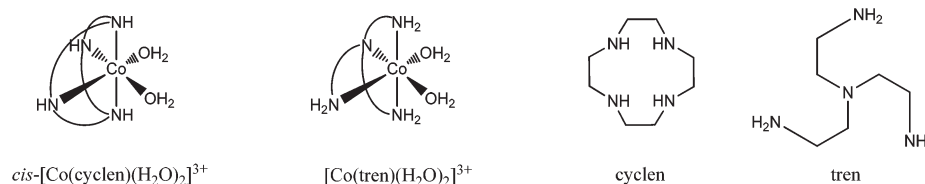
Results and discussion

Solution behaviour of *cis*-[Co(N)₄(H₂O)₂]³⁺ in non-buffered medium

The solution behaviour of the triflates (CF₃SO₃⁻, known to have no effects on the substitution chemistry of this type of complexes)^{39,40} of both *cis*-[Co(cyclen)(H₂O)₂]³⁺ and [Co(tren)(H₂O)₂]³⁺ complexes is usually considered to be dominated by the existence of two consecutive acid–base equilibria leading to the formation of *cis*-[Co(N)₄(H₂O)(OH)]²⁺ and *cis*-[Co(N)₄(OH)₂]⁺, the reported pK_a values being 5.4–5.9 and 7.6–8.2 for the tren complex and 5.6–5.8 and 8.0–8.2 for the cyclen complex.^{36,41,42} From these values it is evident that solutions prepared under physiologically relevant conditions will contain both diaquo complexes and significant amounts of species with one or two coordinated OH⁻. However, according to the literature about related complexes with NH₃, en or NTA,^{35,43–45} formation of simply- or double-OH⁻ bridged dinuclear species must be also considered. Even so, although formation of a dinuclear complex has been found to be relevant in the Co^{III}-cyclen catalysed hydrolysis of ethyl *p*-nitrophenyl phosphate,⁴⁶ the role of those dinuclear species remains essentially unexplored.

The reactivities of both *cis*-[Co(cyclen)(H₂O)₂](CF₃SO₃)₃ and [Co(tren)(H₂O)₂](CF₃SO₃)₃ complexes show similar behaviour when dissolved in a slightly alkaline medium; nevertheless, the time-scales of the changes observed differ noticeably. The conjugate-base activated substitution mechanism³¹ represents the key point for understanding the rate, reactivity and differences observed.^{33,47}

The results obtained for the *cis*-[Co(cyclen)(H₂O)₂]³⁺ system, featuring a simplified formal geometrical equivalence of the two water ligands (Scheme 1), will be discussed first. When a solution of the complex (*ca.* 1 × 10⁻³ M) is mixed with non-buffered aqueous slightly alkaline solutions at 25 °C (initial pH = 8.1–9.7), a fast (10–15 minutes) change in the electronic spectrum is observed (Fig. S1a†). The spectral characteristics of a *cis*-N₄O₂ chromophore attached to the Co^{III} centre are maintained during the reaction that is not a simple deprotonation process, which would be instantaneous. Monitoring of the pH during this time indicates that its value decreases, from the initial establishment of the *cis*-[Co(cyclen)(H₂O)₂]³⁺/*cis*-[Co(cyclen)(H₂O)(OH)]²⁺/*cis*-[Co(cyclen)(OH)₂]⁺ equilibrium to a value that involves the expected formation of [*cis*-Co(cyclen)(H₂O)₂(μ-OH)]⁵⁺.^{47,48} The examination of the process *via* ¹³C NMR spectroscopy indicates that the species in solution, once this fast process has finished, corresponds to the



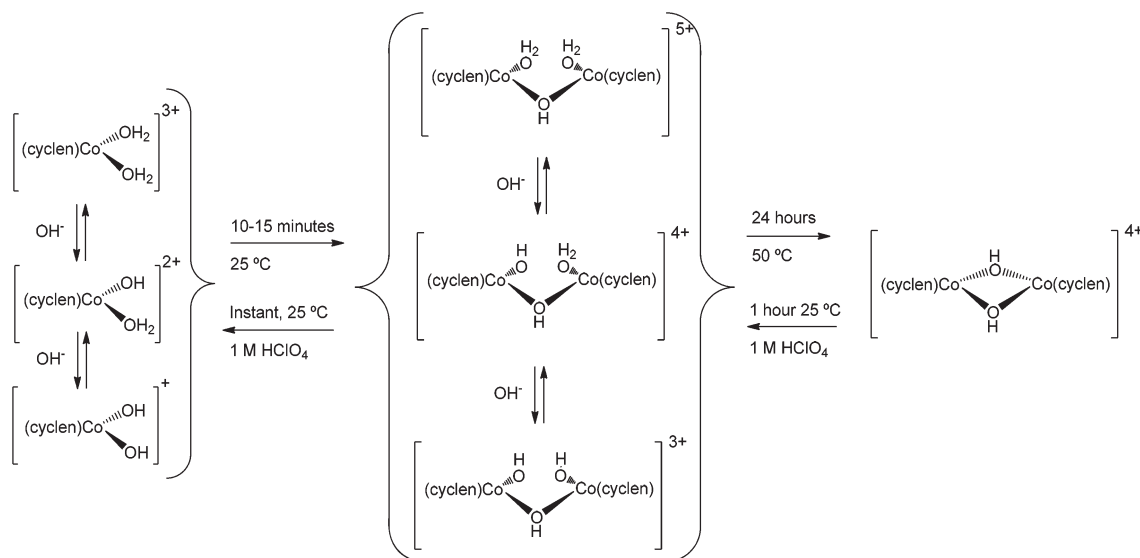
Scheme 1

initial *syn/anti* arrangement of the *cis*- equatorial NH groups of the macrocycle, as found for other systems (four equally intense signals at 49.8, 51.6, 56.5 and 59.0 ppm); no *syn/syn* or *anti/anti* forms are detected.^{49–51} Once this reaction is complete, continuous UV-Vis spectral monitoring of the same solutions at 50 °C for hours showed rather important changes (Fig. S1b†), which are indicative that the structure of the chromophore in solution is severely modified still maintaining the *cis*-{Co(cyclen){O}₂} structural unit. Monitoring of the pH during this slow process shows an increase in acidity of the medium during the reaction.

Acidification of the solutions obtained after the reaction occurring at room temperature to 1 M HClO₄ produced an instantaneous reversion to the initial *cis*-[Co(cyclen)(H₂O)₂]³⁺ UV-Vis spectrum. The reactivity observed at this time-scale (pH = 8.1–9.7, 10–15 minutes, 25 °C) clearly corresponds, from spectral and kinetic perspectives, to the simple formation of mono-hydroxo-bridged dimeric species, [*cis*-Co(cyclen)-(H₂O)₂(μ-OH)]⁵⁺ from the *cis*-[Co(cyclen)(H₂O)(OH)]²⁺ complex, as found for similar Co^{III} systems.⁴⁴ Unlike for some other model complexes,⁵² this species fully reverts to the initial diaqua monomer in acidic medium, preserving the *cis*-{Co(cyclen){O}₂} structural unit in a very fast process. Similarly, when the solution obtained after 24 h at 50 °C is acidified to 1 M HClO₄, the UV-Vis and ¹³C NMR spectra of the *cis*-[Co(cyclen)(H₂O)₂]³⁺ species are obtained after 1 hour at room

temperature. The formation of [*cis*-Co(cyclen)₂(μ-OH)₂]⁴⁺ dimeric units from the previous [*cis*-Co(cyclen)(H₂O)₂(μ-OH)]⁵⁺ species^{35,43,53} (see before) fully agrees both with the spectroscopic, pH and kinetic profiles associated with this slower process (24 h, 50 °C). It is also very relevant to indicate that leaching of Co^{III} or cyclen to the medium is prevented during the entire process (Fig. S2†). Scheme 2 presents a summary of the reactivity indicated above.

For the [Co(tren)(H₂O)₂](CF₃SO₃)₃ system, the situation is somewhat more complex because the two aquo ligands are non-equivalent, one of them is *trans* to one terminal -NH₂ group whereas the other is *trans* to the tertiary amino group (Scheme 1). The spectral changes observed, upon addition of a base to neutralise the initial acidic medium, show the occurrence of two consecutive steps that take *ca.* 5 and 30 minutes at pH = 6.5 to complete (Fig. S1c†). Again, during this time a definite decrease of the pH occurs, as for the cyclen analogue; nevertheless, the spectral characteristics of the {Co(tren){O}₂} structural unit are maintained. These changes can be considered to involve the bis-μ-OH formation between two cobalt units by comparison with similar systems.⁴³ Monitoring of the solution for extended periods at 50 °C does not produce any further changes in the UV-Vis spectrum, thus corroborating the inert bis-μ-OH nature complex formed after the two step process. As expected, the full process is accelerated on increasing the pH. Upon acidification of the solution with 1 M HClO₄



Scheme 2

at 25 °C, the spectrum of the solution reverts to the initial characteristics in a few minutes. As a whole the process is equivalent to that indicated in Scheme 2, although the time-scale of the reactions is much faster.

Solution behaviour of $cis\text{-[Co(N)}_4\text{(H}_2\text{O)}_2\text{]}^{3+}$ in buffered medium

In view of the complex nature of these Co^{III} complexes at physiological pH, the reactivity has been further pursued in HEPES buffered solutions. The reaction sequence observed under these pH-stabilised conditions is the same as that observed before, for both $cis\text{-[Co(cyclen)(H}_2\text{O)}_2\text{]}^{3+}$ and $[\text{Co(tren)(H}_2\text{O)}_2\text{]}^{3+}$, in non-buffered medium (Scheme 2); Fig. 1 shows the observed spectral changes for the cyclen derivative. For both complexes the faster process shows a decrease of the overall $t_{1/2}$ with the cobalt concentration, as expected for the formation of polymeric species, and important differences in the time-span of the full process are also obtained on modulating the pH of the medium.

For the $[\text{Co(tren)(H}_2\text{O)}_2\text{]}^{3+}$ species at pH = 6.0, 6.5, 7.0, 7.5 and 8.0, the reactions observed in MES, HEPES or Tris buffers (Scheme 3) are independent of both the nature of the buffer and its concentration, although its overall rate increases with pH. Given the difficulties in resolving the two-step sequence and the dead-end nature of the final complex obtained

(see the next section) no further studies were carried out. With respect to the time-resolved two-step reactivity of the $cis\text{-[Co(cyclen)(H}_2\text{O)}_2\text{]}^{3+}$ complex, apart from obvious differences in the time-scale and spectral changes of the processes (Fig. S1†), some other dramatic differences with the tren species have been observed. These can be related with the distinct structure of the two species shown in Scheme 1, and are dealt with in the next paragraphs.

For the $cis\text{-[Co(cyclen)(H}_2\text{O)}_2\text{]}^{3+}$ /HEPES system in the pH 6.0–8.0 range, no dependence on the pH is observed for the $t_{1/2}$ of the first process monitored (Scheme 2, left). Nevertheless, fitting of the UV-Vis spectral changes to a $2 \times A \rightarrow B$ sequence at pH = 7.0 showed a definite decrease of $t_{1/2}$ on increasing the complex concentration (from 170 s at 2.5×10^{-4} M to 70 s at 1.0×10^{-3} M, Table S1†). Thus, the assignment of this first fast reaction to the formation of $[\{cis\text{-Co(cyclen)(H}_2\text{O)}\}_2(\mu\text{-OH})]^{5+}$ from $cis\text{-[Co(cyclen)(H}_2\text{O)(OH)]}^{2+}$ is confirmed. As for the changes corresponding to the second slower process (15–20 hours at 50 °C, Scheme 2, right), they show a clear dependence on the alkalinity. Thus, while at pH = 8.0 the reaction is not observed, a definite acceleration occurs on increasing the pH from 6.0 to 6.5 to 7.0 and to 7.5 (from 1300 minutes to 750 minutes for the full process). Given the fact that $\text{p}K_{\text{a}2} = 8.1$ for the diaquo $cis\text{-[Co(cyclen)(H}_2\text{O)}_2\text{]}^{3+}$ complex,^{47,54} it is clear that the absence of the slower reaction

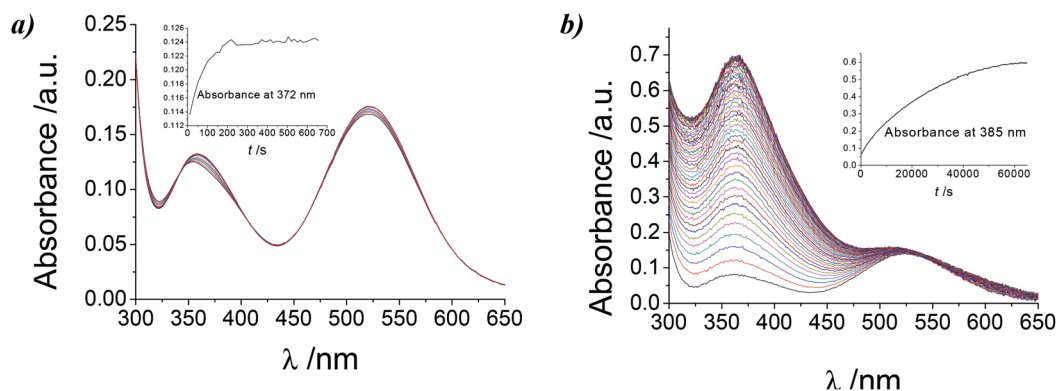
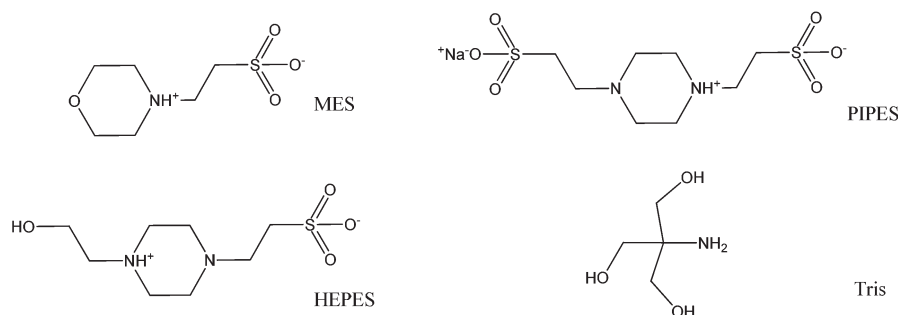


Fig. 1 (a) Initial spectral changes observed in the electronic spectrum at 25 °C of a solution of the $cis\text{-[Co(cyclen)(H}_2\text{O)}_2\text{]}(\text{CF}_3\text{SO}_3)_3$ complex (1×10^{-3} M) at pH = 7.0 in buffered aqueous solution (HEPES, 0.4 M) ($I = 1.0$ NaClO₄, 10 minutes); (b) consecutive slow changes for the same solution (50 °C, 19 hours).



Scheme 3

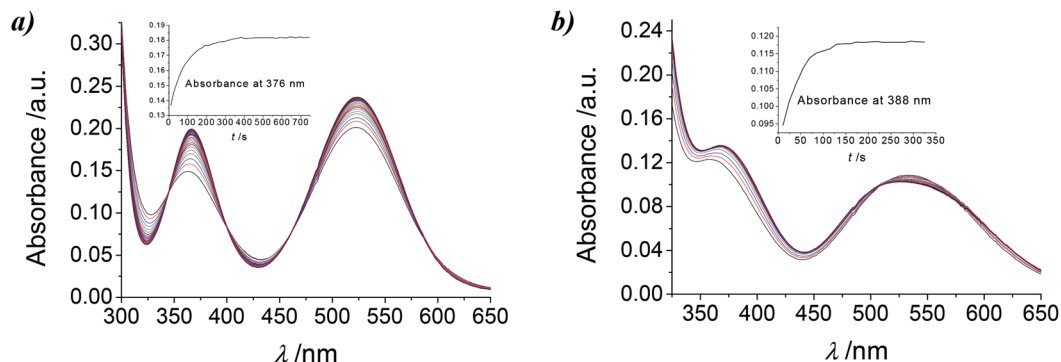


Fig. 2 Sole spectral changes observed in the electronic spectrum at 25 °C of a solution of the *cis*-[Co(cyclen)(H₂O)₂](CF₃SO₃)₃ complex (1 × 10⁻³ M, I = 1.0 NaClO₄ 10 minutes) at: (a) pH = 6.5 (MES 0.4 M, 13 minutes); (b) pH = 8.0 (Tris 0.4 M, 5 minutes).

at pH = 8.0 is associated with the increasing presence of *cis*-[Co(cyclen)(OH)₂]³⁺ in solution (Scheme 2, bottom left), which should be much more dissociatively inert than any of the aquo derivatives. As for the acceleration of the reaction (when observed) with increasing the pH, the $[\{cis\text{-Co(cyclen)-(H}_2\text{O)}_2(\mu\text{-OH)}\}^{5+} \rightleftharpoons [\{cis\text{-Co(cyclen)(OH)}\}(\mu\text{-OH)}\{cis\text{-Co(cyclen)-(H}_2\text{O)}_2\}^{4+} + \text{H}^+$ equilibrium shift, already described in similar systems (Scheme 2, centre),^{35,44} producing active bridging hydroxo/aquo ligand pairs, can be held responsible for the fact. This effect being a common feature of many anation reactions occurring on Co^{III} amino complexes.^{55,56}

In this respect, the higher acidity of the second aquo ligand in [Co(tren)(H₂O)₂]³⁺ (pK_{a1} = 5.5 and pK_{a2} = 7.6),^{47,54} in addition to the distinct nature of the cyclen and tren ligands, should be held responsible for the differences observed (Fig. S1†). The absence of mono-hydroxo-bridged species was already remarked for similar [Co(NTA)(H₂O)₂] derivatives.^{43,44}

Finally, it is extremely interesting to note that, contrary to [Co(tren)(H₂O)₂]³⁺, for *cis*-[Co(cyclen)(H₂O)₂]³⁺ the use of MES at pH = 6.5 and 7.0, PIPES at pH = 7.0 and 7.5, or Tris at pH = 7.5 and 8.0 (Scheme 3) produces dramatic differences in the reactivity of the system. For the first fast reaction step the use of MES or PIPES leads to definite differences in the amplitude of the spectral changes when compared to those observed when HEPES had been used (Fig. 1a and 2a). Even the use of Tris at pH = 8.0 produces such dramatic differences (Fig. 2b) that it has been completely avoided for the studies indicated in the next section. The effect of the concentration of the buffer has also been checked and no dependence of the *t*_{1/2} for the monitored reaction has been found. As for the second slower process, it is not observed at all when MES or PIPES is used instead of HEPES, thus indicating the formation of some aggregates that inhibit the follow up reaction, and changes the spectral characteristics of the B species in the 2 × A → B → C sequence determined in the previous section under non-buffered conditions.

It is thus clear that the recurrent use of so-called innocent buffers for the *in vitro* study of the interaction of transition metal complexes with biologically relevant molecules has serious drawbacks. Taking into account that the substitution ability of such buffers is minimal by design,^{57,58} the

differences observed have to be related to outer-sphere complexation/aggregation with the metal complex. The presence of both hydrogen donor and acceptor centres in the same molecule (Scheme 3) has to be relevant for this sort of supra-molecular interaction, already found decisive in other circumstances.^{59,60} An examination of the structure of the buffers utilised⁵⁷ indicates that the most “interfering” Tris molecule might interact cooperatively *via* the three alcoholic protons with the deprotonated ligands of the complex in an outer-sphere fashion; thus the important changes seen in the electronic spectrum can be rationalised. As for the effects due to oxygen donors in the MES or PIPES molecules, they are a bit more uncertain. Its presence can block in an outer-sphere fashion (dead-end outer-sphere complex)^{61,62} the reactivity observed in HEPES or in non-buffered media. HEPES, being able to auto-aggregate, can be more innocent in the reaction medium. In this respect, the stabilization of the final deprotonated aggregates of the *cis*-[Co(cyclen)(H₂O)₂]³⁺ can also be held responsible for the differences observed both in the reaction rates and the spectra obtained.

Reaction of *cis*-[Co(cyclen)(H₂O)₂]³⁺ with chlorides, phosphates and 5'-CMP at physiological pH buffered medium

The study of the reaction of the complexes described above with chlorides and 5'-CMP (5'-cytidinemonophosphate) has obvious biological relevance. However, given the possible phosphate hydrolysis processes involving biologically relevant phosphates,⁶³⁻⁶⁷ the study of the reactivity with inorganic phosphate at pH 7.0 should also be pursued for comparison. Furthermore, the unexpected effect of innocent buffers in the solution chemistry of the cobalt complexes of this study indicated above, as well as the extended use of phosphate buffers in many biologically relevant substitution reactivity studies, also deserves a comprehensive study of the reactivity of inorganic phosphate on our systems. With these premises in mind, the study of the reaction of pre-incubated *cis*-[Co(N)₄(H₂O)₂]³⁺ species at physiological pH with Cl⁻, inorganic phosphate and 5'-CMP ligands has been pursued.

No reaction either with chlorides, phosphate or 5'-CMP has been observed for pre-equilibrated [Co(tren)(H₂O)₂]³⁺ solutions (15–30 minutes room temperature) at pH values between

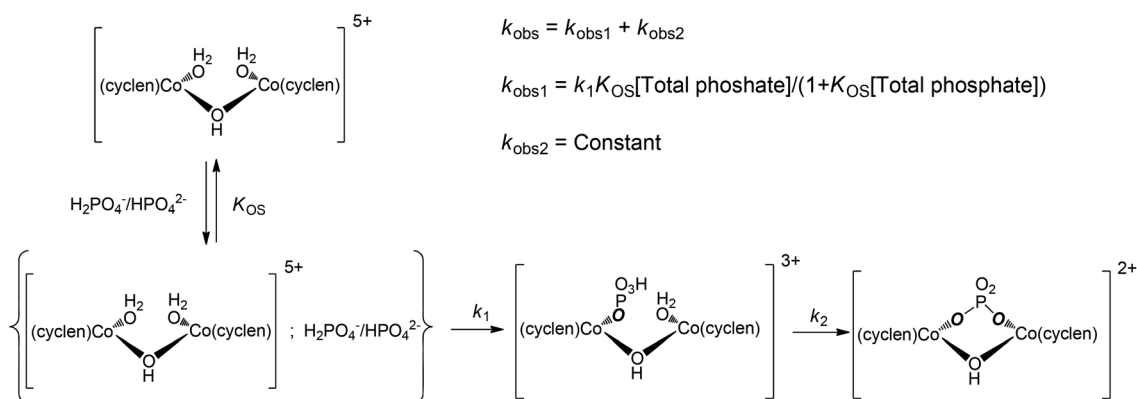
6.5 and 7.5, thus indicating the dead-end character of the species formed in this medium with respect to substitution with these ligands. For the *cis*-[Co(cyclen)(H₂O)₂]³⁺ complex, the equilibrium mixture obtained in HEPES buffer (pH = 6.5, 7.0 and 7.5) after the full equilibration process is completed (24 hours at 50 °C, Scheme 2, right) showed no reactivity with any of these three ligands at a reasonable time-scale either. It is clear that the final species formed is also too stable to interact with these ligands in this pH range. Both facts agree with formation of substitutionally non-active [*cis*-Co(cyclen)]₂(μ-OH)₂⁴⁺ units as dead-end complexes, as indicated in the previous sections; a similar reactivity has already been reported.⁴⁴ In contrast, formation of chlorocomplexes and other substituted species occurs readily when freshly prepared solutions are used, thus revealing the important differences in the reactivity between the mono and dinuclear species.

If the incubation of *cis*-[Co(cyclen)(H₂O)₂]³⁺ in HEPES buffer (pH = 6.5, 7.0 and 7.5) is limited to 10 minutes at 25 °C, thus only allowing for the first fast process to take place (Scheme 2, left), no reaction with chlorides is observed at the fast time scale expected for base-hydrolysis catalysed substitution processes. Longer reaction times produced the time-resolved spectral changes associated with the slow conversion to bis-μ-OH species, monitored in the absence of Cl⁻.

If the same procedure is carried out with at least 20-fold excess of inorganic phosphate anions at pH 7.0 (0.4 M HEPES, *I* = 1.0 NaClO₄) the expected UV-Vis spectral changes (Fig. S3†) for a substitution reaction are obtained. The process shows the actuation of two consecutive steps from which two pseudo-first order rate constants (*k*_{obs1} and *k*_{obs2}) can be calculated. While the value determined for *k*_{obs2} is found independent of the concentration of the entering ligand (*k*_{obs2} = *k*₂), for *k*_{obs1} a limiting [H₂PO₄⁻/HPO₄²⁻]_{TOTAL}-dependence is found (as for many other substitution reactions on cobalt-amino complexes involving the existence of outer-sphere precursor formation: *k*_{obs} = *k**K*_{OS}[ligand]/{1 + *K*_{OS}[ligand]}).⁶⁸ The values obtained for the kinetic parameters at pH = 7.0 (*k*₁ = 0.14 ± 0.1 s⁻¹; *K*_{OS} = 180 ± 60 M⁻¹; *k*₂ = 0.0010 ± 0.0001 s⁻¹) have to be related to a standard conjugate-base substitution reaction followed by a slower reorganization process, presumably chelate formation.

In order to ascertain such assumptions, the ³¹P NMR spectrum of a 1 : 5 (Co-phosphate) solution of the final reaction mixture was monitored. The spectrum showed a single signal (1 : 9 intensity) at 19 ppm low field from that of the free phosphate, thus indicating the stoichiometric formation of a μ-η²-OPO₂O bridge between the two cobalt centres, of the [*cis*-Co(cyclen)(H₂O)₂(μ-OH)]⁵⁺ units obtained on incubation at room temperature for 15 minutes. The absence of any signal at 23 ppm low field from that of free phosphate excludes the formation of a simple η²-OPO₂ chelate.^{47,69} In all cases the values of these shifts are indicative of the expected preferential formation of Co^{III}-OPO₃ bonds. Scheme 4 presents a summary of the reactivity monitored.

When the same procedure is carried out with at least 20-fold excess of 5'-CMP (in the dianionic form in this pH range),⁷⁰ a series of fast spectral changes are also obtained (Fig. S4†). These can be fitted to a set of two consecutive first-order reactions using the standard Specfit or ReactLab software.^{71,72} Clearly, the actuation of a catalytic process producing the hydrolysis of the phosphate derivative, as found for similar {Co^{III}(N)₄} units under harsher conditions, can be discarded in our system.^{63,64,66} The values of *k*_{obs1} and *k*_{obs2} obtained are collected in Table S1† as a function of the conditions used, and Fig. 3a shows their concentration-dependence. No dependence on the concentration of 5'-CMP²⁻ was observed for the amplitude of the spectral changes associated with *k*_{obs1}, while those associated with *k*_{obs2} do show an increase with increasing 5'-CMP²⁻ concentration (Fig. S4†), thus indicating the presence of equilibrium under the conditions used. In this respect, the ³¹P NMR spectral monitoring of the final reaction mixture is revealing. A reacted solution of 1 × 10⁻³ M in cobalt complex and 5 × 10⁻³ M in 5'-CMP at pH 7.0 shows two signals 8.1 and 8.9 ppm low field from that of free 5'-CMP²⁻, in agreement with the formation of two η¹-O(5'-CMP²⁻) different species, [*cis*-Co(cyclen)(H₂O)](μ-OH){*cis*-Co(cyclen)(5'-CMP)}³⁺ and [*cis*-Co(cyclen)(5'-CMP)]₂(μ-OH)⁴⁺.⁴⁷ The combined final intensity ratio is *ca.* 1 : 9, indicating that the dominant species in solution corresponds to the mono-substituted dimeric species. The assignment of the signal at lower field (8.9 ppm with respect to free 5'-CMP²⁻, 50% relative intensity) to the



Scheme 4

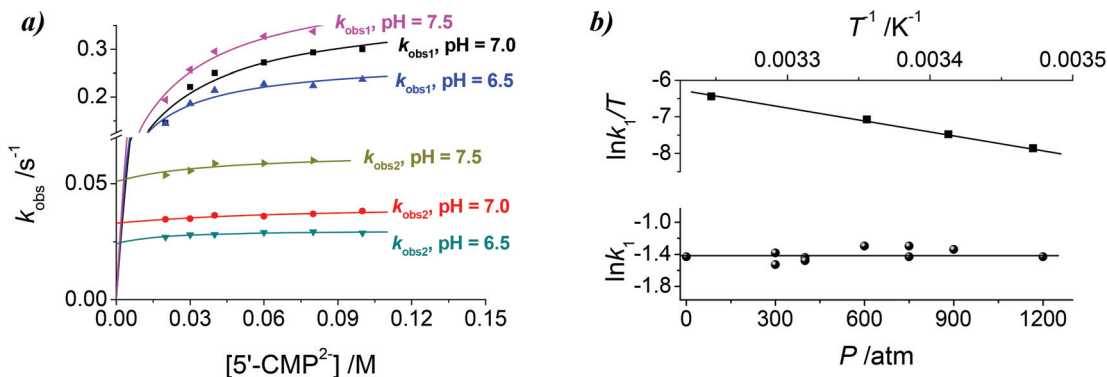
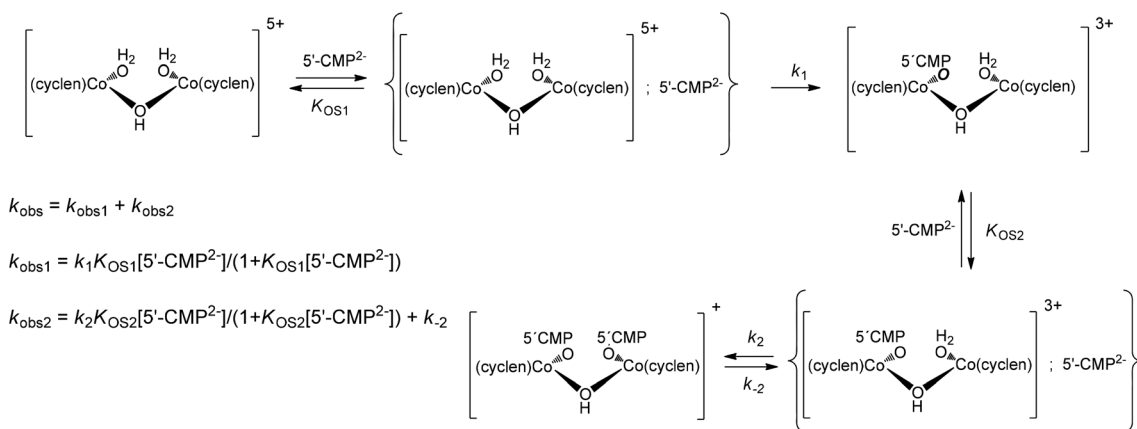


Fig. 3 (a) Plot of the values of the dependence of $k_{\text{obs}1}$ and $k_{\text{obs}2}$ as a function of $[5\text{-CMP}^{2-}]$ at different pH values at 25 °C (HEPES 0.4 M, $I = 1.0$ NaClO₄). (b) Top, Eyring plot for first order rate constants k_1 ; bottom, $\ln k_1$ versus P for the same system at pH = 7.0.



Scheme 5

bis-substituted species results in a $[[\{cis\text{-Co}(\text{cycLen})\text{-}(5'\text{-CMP})\}_2(\mu\text{-OH})]^{3+}]/[[\{cis\text{-Co}(\text{cycLen})(\text{H}_2\text{O})\}_2(\mu\text{-OH})\{cis\text{-Co}(\text{cycLen})\text{-}(5'\text{-CMP})\}_2]^{3+}]$ concentration ratio of *ca.* 0.25, in agreement with the dominance of the mono-substituted species indicated above. Scheme 5 summarises the reactivity pattern for this system.

From the data shown in Fig. 3a it is clear that $k_{\text{obs}1}$ shows a limited dependence on $[5'\text{-CMP}^{2-}]$, in line with the outer-sphere formation of aggregates between the cobalt species and the substituting species.^{73,74} The treatment of the data, according to the equation indicated in Scheme 5, produces the kinetic parameters collected in Table 1. It is clear that a slight increase in the values of k_1 (operating from the encounter complex characterised by $K_{\text{OS}1} \approx 45 \text{ M}^{-1}$) is obtained on increasing pH in the range where only the dianionic form of the 5'-CMP ($\text{p}K_{\text{a}} = 6.0$)⁷⁰ is prevalent. Given the fact that dissociatively activated substitution of aquo ligands should be faster than that of hydroxo, the rate increase must be associated with the actuation of the base-assisted mechanism established for Co^{III}-amine complexes. Nevertheless, the increase obtained is rather small in comparison with that for similar systems,⁴⁷ which suggests that the deprotonation of the aquo to hydroxo ligands is also hampering its dissociation as leaving groups in $[\{cis\text{-Co}(\text{cycLen})(\text{H}_2\text{O})\}_2(\mu\text{-OH})]^{5+}$ (see Scheme 2).

Table 1 Summary of the kinetic (see text for the equation fitted) and thermal and pressure activation parameters^a for the reaction of *cis*-[Co(cycLen)(H₂O)₂]³⁺ with 5'-CMP at pH = 6.5, 7.0 and 7.5 (0.4 M HEPES, $I = 1.0$ M NaClO₄)

pH	²⁹⁸ k_1/s^{-1}	²⁹⁸ $K_{\text{OS}1}/\text{M}^{-1}$	$\Delta H_1^\ddagger/\text{kJ mol}^{-1}$	$\Delta S_1^\ddagger/\text{J K}^{-1} \text{mol}^{-1}$	$\Delta V_1^\ddagger/\text{cm}^3 \text{mol}^{-1}$
6.5	0.28 ± 0.01	65 ± 13	61 ± 1	-55 ± 2	Not determined
7.0	0.39 ± 0.03	40 ± 8	58 ± 2	-65 ± 6	~ 0
7.5	0.45 ± 0.03	45 ± 8	64 ± 1	-41 ± 2	Not determined
pH ^b	²⁹⁸ k_2/s^{-1}	²⁹⁸ $K_{\text{OS}2}/\text{M}^{-1}$			
6.5	0.0060 ± 0.001	54 ± 25			
7.0	0.0083 ± 0.004	15 ± 14			
7.5	0.013 ± 0.02	25 ± 60			

^a Determined at 0.08 M 5'-CMP, where the limiting value of $k_{\text{obs}1}$ ($\approx k_1$) applies, see Fig. 3a. ^b $k_{-2} \approx k_2/0.25$ according to ³¹P NMR measurements, see text.

As for the associated thermal activation parameters (Fig. 3b, top), the data collected in Table 1, derived from the first order rate constant k_1 , are rather surprising for a substitution process occurring on intimately dissociatively activated Co^{III} complexes,³² becoming even more dissociative under base-catalysed conditions.^{75,76} The values for ΔH^\ddagger and

ΔS^\ddagger were expected to be, respectively, large and positive,^{77,78} which is not the case (Table 1). Nevertheless, the values determined for ΔV^\ddagger are zero within error (Fig. 3b, bottom), thus showing the typical lack of $\Delta S^\ddagger/\Delta V^\ddagger$ correlation associated with the involvement of mechanistically determinant hydrogen bonding interactions in the transition state of the reaction.^{62,79–82} This fact fully agrees with the important outer-sphere interactions indicated in the previous paragraphs, not even hampered by the high ionic strength used in the study.⁸³ Hydrogen bonding seems to be crucial for this process, the fact being a keystone in biologically relevant reactivity; the inherent dissociatively activated Co^{III} substitution mechanisms are severely tuned by associative interactions in the outer-sphere encounter complex.

With reference to the slower reaction, the values of $k_{\text{obs}2}$ show a ligand-concentration dependence that does not agree with the formation of a chelate or bridging ligand structure (see Scheme 4),^{68,84} in full concordance with the ^{31}P NMR data. In Fig. 3a a limiting dependence of the data according to Scheme 5 is observed; the fitting has been carried out with a $k_2/k_{-2} = 0.25$ constraint, as determined by phosphorus NMR (see above). Given the approximate methodology used, the quality of the data fitted in this way is not good enough to determine thermal and pressure activation parameters (*i.e.* variation of $k_{\text{obs}2}$ with $[5'\text{-CMP}^{2-}]$ is very small, the reaction rate being dominated by the reverse process). The data, nevertheless, indicate that the values of $K_{\text{OS}2}$ are of the same magnitude as those determined for $K_{\text{OS}1}$, which is not in agreement with a merely electrostatic interaction between the entering ligand and the metal centre, a good indication of the actuation of specific, probably hydrogen bonding, outer-sphere interactions between these species.⁶²

The lack of formation of a $\mu\text{-}\eta^2\text{-OPO}_2\text{O}$ bridge in the $5'\text{-CMP}^{2-}$ substitution reactions studied merits some further comments. For the $5'\text{-CMP}^{2-}$ entering ligand system, the substitution process leading to mono $\eta^1\text{-OPO}_3\text{CMP}$ complexes is faster than for the inorganic phosphato derivatives, while the reaction producing the bis $\eta^1\text{-OPO}_3\text{CMP}$ compounds is faster than the chelate-bridge formation for the $\text{H}_2\text{PO}_4^-/\text{HPO}_4^{2-}$ system. This is rather remarkable, given the expected dissociative character of the substitution reactions studied; the presence of important amounts of the monoanionic H_2PO_4^- species at this pH ($\text{p}K_{\text{a}2}(\text{H}_3\text{PO}_4) = 7.21$)⁶⁹ should explain the differences. Both from electrostatic and hydrogen bonding perspectives, the formation of bis $\eta^1\text{-OPO}_3$ derivatives for the inorganic phosphate system may be hampered. In this respect, it is also interesting to note that when the final solutions containing a mixture of mono and bis $\eta^1\text{-OPO}_3\text{CMP}$ substituted derivatives are kept at 50 °C for *ca.* 24 h important changes in the UV-Vis spectrum are observed. The final spectrum resembles that of the extremely inert dead-end $[\{\text{cis-Co}(\text{cyclen})\}_2(\mu\text{-OH})_2]^{4+}$ indicated in previous sections (Scheme 2). Furthermore, ^{31}P NMR spectroscopy on these solutions shows the disappearance of the signals associated with the substituted species characterised above. Clearly the substitution of the coordinated $5'\text{-CMP}^{2-}$ by a bridging hydroxo ligand is favoured in these harsher conditions.

Conclusions

The solution speciation of the triflate salts of complexes $[\text{Co}(\text{tren})(\text{H}_2\text{O})_2]^{3+}$ and $\text{cis-}[\text{Co}(\text{cyclen})(\text{H}_2\text{O})_2]^{3+}$ at pH close to the physiological values has been studied in non-buffered and classical Good's buffer media. In all cases the formation of mono and bis hydroxo-bridged derivatives is observed. While for the tren derivative the process is fully labilised by the actuation of the conjugate-base mechanism producing dead-end $(\mu\text{-OH})_2$ species, for the cyclen analogue system the build-up of relatively inert, but still reactive for hours, mono bridged species is effective. In all cases the effect of different buffering media has been studied with remarkable results that indicate the absolute necessity for a proper medium screening for *in vitro* studies of biologically relevant reactivity.

The reaction of the incubated solutions of $\text{cis-}[\text{Co}(\text{cyclen})(\text{H}_2\text{O})_2]^{3+}$ at pH 6.5, 7.0 and 7.5 HEPES buffered medium for 15 minutes allowed the study of the reactivity of the $[\{\text{cis-Co}(\text{cyclen})(\text{H}_2\text{O})\}_2(\mu\text{-OH})]^{5+}$ species with chlorides, H_2PO_4^- and $5'\text{-CMP}^{2-}$ at different temperatures and pressures. While no reactivity with the chloride anions has been observed, reaction with the two types of phosphate ligands takes place with rather different outcomes. While for the inorganic phosphate anions the sequential formation of mono $\eta^1\text{-OPO}_3$ and $\mu\text{-}\eta^2\text{-OPO}_2\text{O}$ is observed, for the reaction of $5'\text{-CMP}^{2-}$ the sequential process produces mixtures of mono and bis $\eta^1\text{-OPO}_3\text{CMP}$ derivatives, which evolve with time to the dead-end $(\mu\text{-OH})_2$ species with no hydrolysis of the phosphate-sugar bond or appearance of $\mu\text{-}\eta^2\text{-OPO}_2\text{CMPO}$ complexes. In all cases the substitution processes observed show pre-equilibrium outer-sphere interactions that do not follow simple electrostatic patterns; hydrogen-bonding interactions seem to be dominant. In this respect, the thermal and pressure activation parameters determined for some of the reactions have rather unexpected values for dissociatively activated reactions occurring on Co^{III} -amine complexes. A certain degree of hydrogen-bonded associativeness is evident, which is also supported by the lack of the expected $\Delta S^\ddagger/\Delta V^\ddagger$ correlation.

Experimental

Compounds and procedures

The dichlorido complexes, $\text{cis-}[\text{Co}(\text{tetraamine})\text{Cl}_2]\text{Cl}$, have been prepared according to the published procedures.^{85,86} The derived diaqua complexes, $\text{cis-}[\text{Co}(\text{tetraamine})(\text{H}_2\text{O})_2]^{3+}$, were prepared *via* triflate solvolysis^{87,88} from the corresponding dichlorido precursors. The resulting solid from the triflate solvolysis was dissolved in the minimum amount of water and after 1–2 h the solution was cooled at 0 °C and a few drops of triflic acid were added. On standing the corresponding triflates of the diaqua complexes crystallise. Their characterization was conducted on the basis of their quantitative UV-Vis spectrum, ^{13}C NMR spectroscopy and elemental analysis. The remaining chemicals are commercially available and were used as received.

Buffer solutions were prepared according to the desired final concentration of at least 10-fold the species to be buffered. The final pH was adjusted with suitable HClO₄ or NaOH solutions. According to their pK_a values⁵⁷ they were used within the expected pH ranges (6.5–8.5 HEPES, 5.2–7.2 MES, 5.8–7.8 PIPES, and 7.3–9.3 Tris). Nevertheless, in all cases buffering was checked during and after the reactions studied. All final solutions were set to a final ionic strength of 1.0 M with NaClO₄.

¹³C and ³¹P NMR spectroscopy was carried out on a Varian Mercury 400 MHz instrument in H₂O adjusted at the desired pH with a D₂O 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 NaTMPs (¹³C) and H₃PO₄ (³¹P).

Kinetics

Solutions of the different non-metal species involved in the kinetic runs were prepared in the corresponding buffer solutions at the desired ionic strength (1.0 M NaClO₄). Solutions of the metal complexes were prepared normally at much higher concentrations in water (producing an effective acidic pH) and aliquots were added to the desired medium to achieve the final conditions. All the kinetic runs with different time-scales (manual mixing or stopped flow) were conducted following a well described methodology. For all the substitution processes, pseudo-first order flooding conditions were used. Atmospheric pressure runs were carried out on HP8453 or J&M (connected to an Applied-Photophysics stopped-flow mixing unit) instruments depending on the *t*_{1/2} of the monitored reactions. For runs at elevated pressure a described high-pressure stopped-flow mixing unit connected to the above mentioned J&M spectrophotometer was used.⁸⁹ All post-run fittings were carried out using the standard commercially available software.

All data were collected as full (300–700 nm) spectra and treated with the standard Specfit or ReactLab Kinetics software;^{71,72} the 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 were applied; for consecutive reactions with the same characteristics, an A → B → C two exponential sequence was fitted. Alternatively spectral changes were fitted to the equation derived from a 2 × A → B process when dimerisation second order reactions were involved.⁸⁴ Table S1† collects all the values obtained for *k*_{obs} as a function of the different compounds and variables studied.

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