

Carbonic anhydrase activity of dinuclear Cu^{II} complexes with patellamide model ligands†

Cite this: *Dalton Trans.*, 2014, **43**, 3144

Peter Comba,^{*a} Lawrence R. Gahan,^b Graeme R. Hanson,^c Marcel Maeder^d and Michael Westphal^a

The dicopper(II) complexes of six pseudo-octapeptides, synthetic analogues of ascidiacyclamide and the patellamides, found in ascidians of the Pacific and Indian Oceans, are shown to be efficient carbonic anhydrase model complexes with k_{cat} up to $7.3 \times 10^3 \text{ s}^{-1}$ (uncatalyzed: $3.7 \times 10^{-2} \text{ s}^{-1}$; enzyme-catalyzed: 2×10^5 – $1.4 \times 10^6 \text{ s}^{-1}$) and a turnover number (TON) of at least 1700, limited only by the experimental conditions used. So far, no copper-based natural carbonic anhydrases are known, no faster model systems have been described and the biological role of the patellamide macrocycles is so far unknown. The observed CO₂ hydration rates depend on the configuration of the isopropyl side chains of the pseudo-octapeptide scaffold, and the naturally observed *R*,S*,R*,S** geometry is shown to lead to more efficient catalysts than the *S*,S*,S*,S** isomers. The catalytic efficiency also depends on the heterocyclic donor groups of the pseudo-octapeptides. Interestingly, the dicopper(II) complex of the ligand with four imidazole groups is a more efficient catalyst than that of the close analogue of ascidiacyclamide with two thiazole and two oxazoline rings. The experimental observations indicate that the nucleophilic attack of a Cu^{II}-coordinated hydroxide at the CO₂ carbon center is rate determining, *i.e.* formation of the catalyst-CO₂ adduct and release of carbonate/bicarbonate are relatively fast processes.

Received 6th November 2013,
Accepted 27th November 2013

DOI: 10.1039/c3dt53135j

www.rsc.org/dalton

Introduction

Ascidiacyclamide and the patellamides are pseudo-octapeptides containing four N-based heterocycles and four amide nitrogen groups as possible donors for metal ion coordination (see Scheme 1). These and other cyclic peptides present in the ascidians of the Pacific and Indian Oceans have attracted scientific interest for many years and for various reasons. Their biosynthesis has been reported,^{1–4} there is a considerable interest in their pharmaceutical activity,^{1,5} and their rich

transition metal coordination chemistry, in particular with Cu^{II}, has been studied in some detail.^{6–16} However, the biological function of these cyclic pseudo-peptides is still largely unclear. An interesting observation in this context is that many of the ascidians are host to symbionts, and for *Lissoclinum patella*, from which ascidiacyclamide and the patellamides are isolated, the symbiont is photosynthetic, *i.e.* the prokaryote *Prochloron*.^{17,18} The natural role of the cyclic pseudo-octapeptides possibly involves Cu^{II} coordination, and among the suggested biological functions are Cu^{II} transport and storage,^{17,19} copper detoxification,¹⁷ CO₂ hydration (carbonic anhydrase reactivity),^{12–14,17,20,21} oxygen activation,^{17,19,22} and phosphoester hydrolysis (phosphatase reactivity),¹⁵ and the Cu^{II} cyclic peptide complexes might also be cofactors in enzymes.¹⁷ Carbonic anhydrase activity has been suggested on the basis of the structural and spectroscopic characterization of carbonato-bridged dicopper(II) complexes.^{12–14} Studies involving EPR spectroscopy in combination with molecular mechanics (MM) and density functional theory (DFT) based molecular modeling as a structural probe,^{14,23–25} together with ¹⁸OH[−] and ¹³CO₂ labeling and electrospray mass spectrometry (ESI-MS),¹⁴ have suggested that the carbonate emerges from atmospheric CO₂, and catalytic activity was assumed on the basis of NMR experiments and the gravimetric quantification of CO₃^{2−}. However, so far there has been no clear proof for catalytic turnover and the kinetics and catalytic efficiency have not

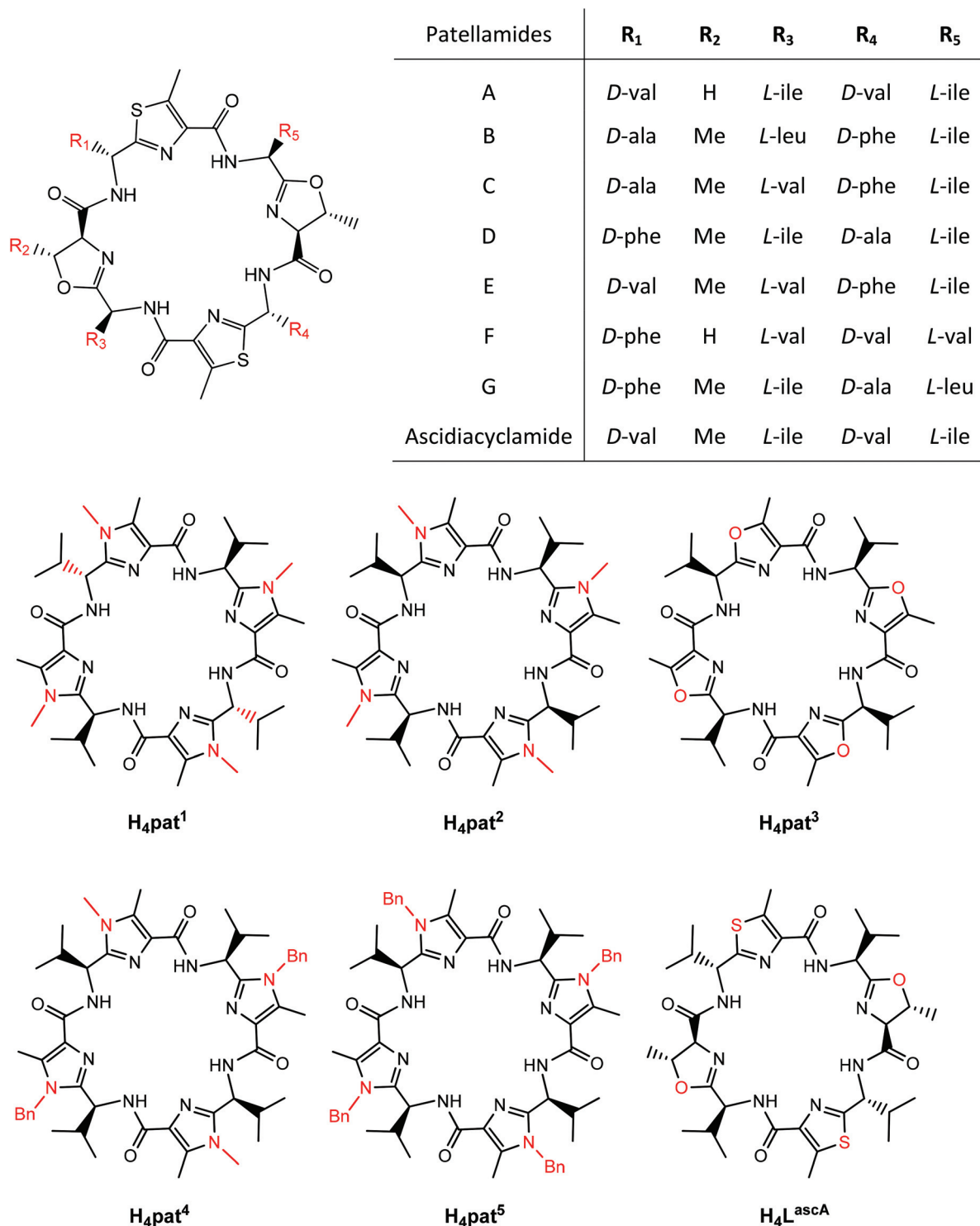
^aUniversität Heidelberg, Anorganisch-Chemisches Institut, INF 270, D-69120 Heidelberg, Germany. E-mail: peter.comba@aci.uni-heidelberg.de; Fax: +49-6226-546617

^bSchool of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Queensland 4072, Australia

^cCentre for Advanced Imaging, The University of Queensland, Brisbane, Queensland 4072, Australia

^dDepartment of Chemistry, The University of Newcastle, Callaghan, NSW 2308, Australia

† Electronic supplementary information (ESI) available: The ESI includes a figure which illustrates the dependence of the k_{cat} values from the ligand system and $\text{p}K_{\text{a}}$ value of the sacrificial base, a plot of the pH as a function of time for the H₄pat¹-based system with the four base/indicator systems used, and the entire set of relevant kinetic traces, plots of experimental vs. fitted electronic spectra and concentration profiles not shown in the manuscript. See DOI: 10.1039/c3dt53135j



Scheme 1 Relevant cyclic pseudo-octapeptides; H₄Asc and H₄Pat^{A–G} are natural products, H₄pat^{1–5} and H₄L^{ascA} are the synthetic analogues used in the present study.

been studied; these topics are therefore the focus of the present report.

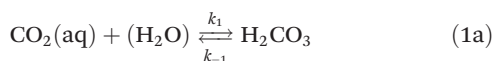
Carbonic anhydrases are ubiquitous and responsible for the efficient hydration of CO₂ at pH > 7 and dehydration of HCO₃[–] at pH < 7, allowing the fixation and transport of CO₂ in

biological systems (photosynthesis, respiration, bone metabolism) as well as the regulation of pH, *e.g.* in the blood and stomach. Carbonic anhydrases are among the most efficient enzymes and accelerate the hydration of CO₂ around 10⁸-fold. They generally have mononuclear Zn^{II} sites and their

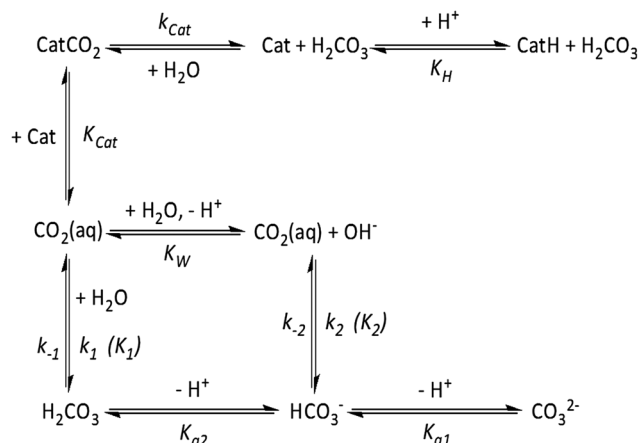
mechanism has been of interest to biochemists, biologically oriented coordination and computational chemists.^{26–35} One of the exciting features of the dicopper(II)-patellamide chemistry discussed here is that these complexes might be the first structural, spectroscopic and very efficient functional models for a copper-based carbonic anhydrase. Note however that the *in vitro* results presented only show that CO₂ hydration/HCO₃[−] dehydration *might be* a biological function, and confirmation of this suggestion obviously will need biological studies. Most of the small molecule model complexes reported so far are mononuclear Zn^{II} based but a few other mimics have been reported, in particular dicopper(II) complexes.^{36–39} The fixation of CO₂ also has potential for environmental and technological applications^{40,41} and, importantly, the dicopper(II) complexes of the patellamide-based cyclic peptides discussed here are the most efficient model systems for carbonic anhydrase known so far.

Results and discussion

The kinetic analysis of the uncatalyzed and catalyzed hydration of CO₂ in general is not trivial because the gas phase and ionized species have very different solubilities in H₂O with strong dependencies on temperature, pressure and pH. In addition, all relevant species are colorless and there are a number of protonation equilibria, also including those of the catalyst involved, which are difficult to assess quantitatively (Scheme 2, eqn (1)–(3); note that the kinetics related to eqn (1a) and (3c) are generally analyzed as pseudo-first-order processes; Ind: indicator, Cat: catalyst).



The analysis of the CO₂ hydration activity of carbonic anhydrase and model systems is usually based on stopped-flow kinetics, involving the spectrophotometric measurement of the time dependence of pH *via* the color change of a pH indicator (eqn (2)),^{26,30} using matched pairs of bases and pH indicators. The four pairs used in our studies are 3,5-lutidine and



Scheme 2 Equilibria involved in the hydration of CO₂.⁴³

chlorophenol red (pK_a 6.21, 6.25), imidazole and bromothymol blue (pK_a 7.14, 7.10), Tris [(tris-hydroxymethyl)-aminomethane] and metacresol purple (pK_a 8.07, 8.30), 1,2-dimethylimidazole and metacresol purple (pK_a 8.22, 8.30).^{26,42} The various equilibria shown in Scheme 2 (eqn (1) and (3)) have been analyzed in detail previously,^{43–45} and for the analysis of the Cu^{II}-patellamide systems presented here, we have adopted the same experimental setup and largely the same parameters. Note, however, that, due to solubility problems, we had to revert to methanol–water mixtures (10% MeOH).[‡] For the evaluation of the kinetic parameters (see below) we have therefore carefully tested the fundamental parameters involved in Scheme 2 and found that a small variation of these parameters did not lead to significant changes in the *k*_{Cat} values (Table 1 shows the parameters used for fitting the stopped-flow traces; see ESI[†] for the effect of variation of the least well-determined parameters *K*_{Cat} and *K*_H). The time-dependent spectra of a solution containing H₄pat¹:Cu:base (1:2:4) with CO₂ in the optimum pH range (Tris, pK_a = 8.07; note that the pH value varies during the reaction and, therefore, pK_a rather than pH values are discussed in the text and given in Table 1) are shown in Fig. 1 (similar Figures for the other dicopper(II) complexes of the synthetic analogues (H₄pat²–H₄pat⁵, H₄L^{ascA}) and at different pH values are given as ESI[†]).

The reaction is limited by the amount of base used (8.5 mmol); CO₂ was used in excess (17 mmol) and the catalyst concentration was 5 μmol (0.06 mol%), that of the indicator 12.5 μmol; depending on the base used, the pH variation is around 2 pH units (see ESI[†]). The complex formation and the various Cu^{II} complexes present in equilibrium in solution have

[‡] Note that our earlier work focusing on solution structures and equilibria of the mono- and dinuclear copper(II) complexes of cyclic pseudo-peptide ligands was performed in pure but not dry methanol, *i.e.* the only difference in terms of solvent to the experiments reported here is the amount of water present. Since in the various structural analyses reported before, and to which we refer in the present kinetic study,^{13,14} OH₂ and OH[−] are terminal and bridging ligands to the copper(II) centers, we assume that the moderate change in terms of conditions for our kinetic analysis does not lead to significant differences in terms of the reported structural models.

Table 1 Fixed general parameters for the hydration of CO₂ at 25 °C (see Scheme 2; the hydration of CO₂ (eqn (1a), k_1^*) is treated as a pseudo-first-order reaction, [H₂O] = 55.6 M)^{43,57–59 a}

k_1^* (s ⁻¹)	$3.7(2) \times 10^{-2}$	K_{a1} (M ⁻¹)	$6.3(4) \times 10^{-11}$	K_H^a	$18.0(2) \times 10^{-9}$
k_{-1} (s ⁻¹)	24.8(4)	K_{a2} (M ⁻¹)	$2.0(1) \times 10^{-5}$	K_{cat}^a	10^4
k_2 (M ⁻¹ s ⁻¹)	$12.1(4) \times 10^3$	K_1^*	$1.5(1) \times 10^{-3}$		
k_{-2} (s ⁻¹)	$40(1) \times 10^{-5}$	K_2 (M ⁻¹)	$3.02(1) \times 10^7$		

^a In addition to the usual fixed parameters for fitting kinetic data of CO₂ hydration, the pK_a value of Cu^{II}-coordinated water (see Schemes 2 and 3, K_H ; pK_a [Cu^{II}(H₂O)₆]²⁺ = 7.50)⁴⁸ and the association constant of CO₂ with the catalyst (see Scheme 2, log K_{cat} = 4)^{36,47,60} were estimated from published data (standard deviations given in brackets).

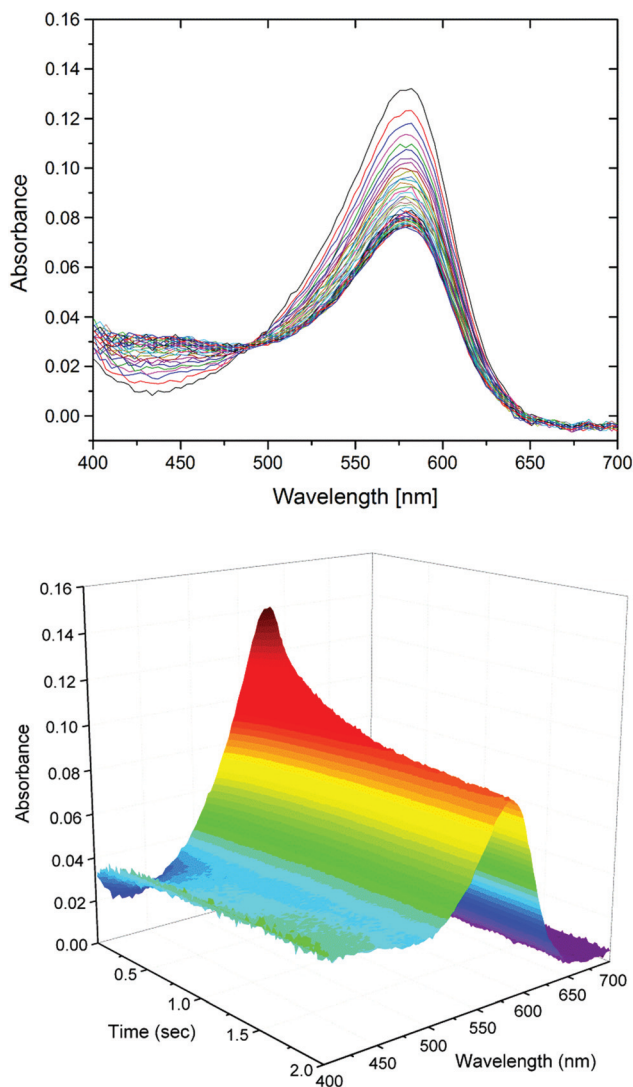


Fig. 1 Two-dimensional (absorbance vs. wavelength, recorded in the first 2 s, top) and three-dimensional (absorbance vs. wavelength vs. time, bottom) representation of absorbance data during the hydration of CO₂ by the [Cu₂^{II}(H₂pat¹)(OH)]⁺ complex. Reaction with 8.5 mM Tris [(tris-hydroxymethyl)-aminomethane] and 17 mM CO₂ in a water–methanol 9:1 mixture at 25 °C; corresponding figures for the other dicopper(II) cyclic peptide complexes are given as ESI.†

been studied in detail for the cyclic pseudo-octapeptides used here (see Scheme 3).[†]^{13,14}

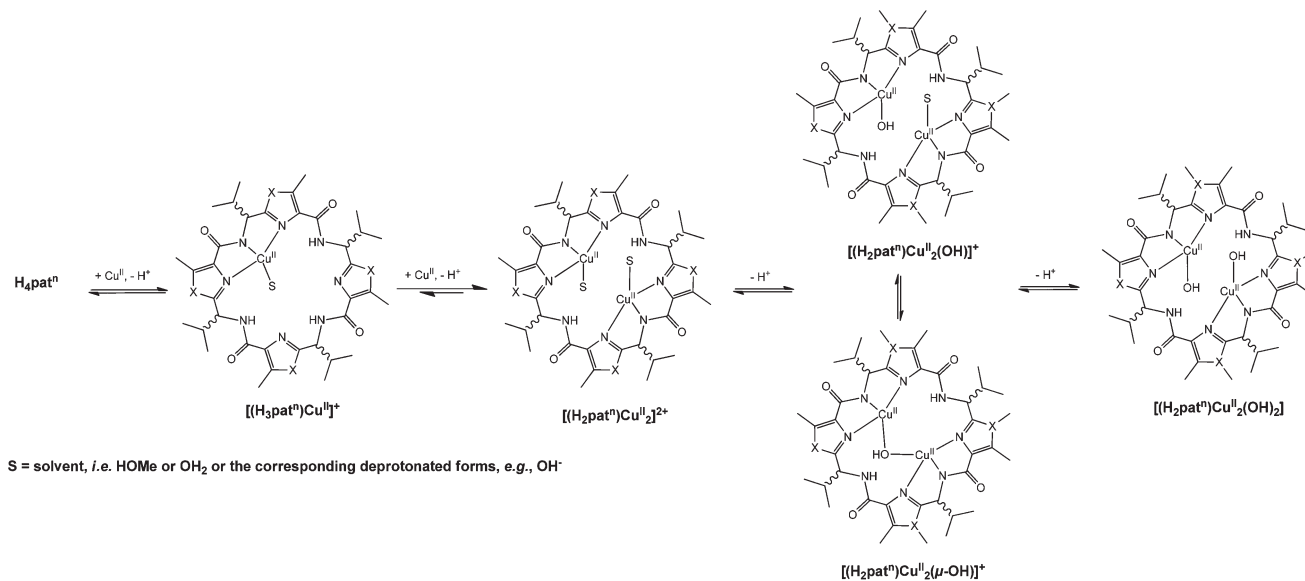
From these studies^{13,14} and the data presented below, it emerges that the hydroxido-dicopper(II) complexes of the cyclic

pseudo-octapeptides are the catalytically active species (see Scheme 3). The fitted rate constants of the six copper-patellamide catalysts used are given in Table 2. Fig. 2 shows a comparison of the experimental spectra at the beginning and end of the reaction with those simulated with the fitted kinetic parameters, using the dicopper(II) complex of H₄pat¹ as catalyst and Tris/metacresol purple as base/indicator couple for detection (similar plots of the other catalysts are given as ESI†). The good agreement between theory and experiment supports the quality of the fitting procedure and parameters given in Table 2. From the time-dependence of the concentration of the relevant species (CO₂, HCO₃⁻, pH, base, baseH⁺) given in Fig. 3 (similar plots of the other catalysts are given as ESI†) it follows that under the optimized conditions used there is a nearly quantitative yield of the hydration reaction (>8 mmol), leading to the maximum TON (turnover number) of close to 1700 under these conditions.

The kinetic parameters in Table 2 indicate that all six catalysts are of similar efficiency with a maximum catalytic rate between 1.7×10^3 and 7.3×10^3 s⁻¹ with Tris as base (pK_a = 8.07). The uncatalyzed hydration of CO₂ at 25 °C is 3.7×10^{-2} s⁻¹,^{26,43} *i.e.*, there is an approx. 10⁵-fold acceleration by the dicopper(II)-patellamide catalysts, compared to the enzymatic rates which are in the region of 2×10^5 – 1.4×10^6 s⁻¹,^{26,46} *i.e.*, leading to an up to approx. 10⁸-fold acceleration. Rates of other model systems generally are smaller (1×10^2 – 5×10^3 s⁻¹, typically in the pH range of 7–9),^{29–32,37,39,47} and the systems discussed here are the most efficient CO₂ hydration catalysts known so far.

The proposed mechanism (see Fig. 4) involves CO₂ coordination to one of the Cu^{II} centers, nucleophilic attack of a hydroxido group, coordinated to the other Cu^{II} center, at the CO₂ carbon atom, leading to a bridging HCO₃⁻ anion, and release of HCO₃⁻ to reform the catalytically active hydroxido-dicopper(II)-patellamide complex [the important forms of the complexes (unbridged, hydroxido-bridged, carbonato-bridged) have been thoroughly characterized by electrospray mass-spectrometry (ESI-MS), UV-vis-NIR, CD, EPR spectroscopy, X-ray crystallography, ¹³C and ¹⁸O labeling studies and computational methods)[†] and preliminary conformational analyses (Monte Carlo and molecular dynamics simulations) support the release of the bridging carbonate].^{13,14}

The maximum rate with Tris as the base (pK_a = 8.07, 10⁵-fold acceleration) and relatively small rates at lower pH (3,5-lutidine pK_a = 6.21, uncatalyzed; imidazole pK_a = 7.14, 10³-fold acceleration) indicate that the pK_a value of the



Scheme 3 Complexation and deprotonation reactions of the six patellamide ligands (for detailed structures see Scheme 1). The assumed catalytically active species is the monohydroxo dicopper(II) complex (water exchange rates of Cu^{II}_{aq} are in the range of 7×10^8 – 5×10^9 s⁻¹; the pK_a value [Cu^{II}(H₂O)₆]²⁺ is 7.50).⁴⁸

Table 2 Hydration rates of CO₂, catalyzed by the dicopper(II) complexes of the six analogues of the patellamides given in Scheme 1 (*k*_{cat}: reactions with 8.5 mmol base, 17 mmol CO₂, 5 μmol catalyst, 12.5 μmol indicator, in a water–methanol 9 : 1 at 25 °C; analysis based on parameters given in Table 1; average and standard deviation of 5 independent measurements)

Ligand	<i>k</i> _{cat} [s ⁻¹]			
	pK _a 6.21	pK _a 7.14	pK _a 8.07	pK _a 8.22
H ₄ pat ¹	$1.71 \times 10^{-3} \pm 9.1 \times 10^{-4}$	$7.08 \times 10^1 \pm 2.3 \times 10^0$	$7.32 \times 10^3 \pm 3.7 \times 10^2$	$6.00 \times 10^2 \pm 1.8 \times 10^2$
H ₄ pat ²	$1.54 \times 10^{-3} \pm 1.3 \times 10^{-3}$	$6.96 \times 10^1 \pm 1.4 \times 10^0$	$1.72 \times 10^3 \pm 1.1 \times 10^2$	$1.15 \times 10^2 \pm 1.3 \times 10^1$
H ₄ pat ³	$1.10 \times 10^{-3} \pm 9.4 \times 10^{-4}$	$7.32 \times 10^1 \pm 6.0 \times 10^0$	$1.86 \times 10^3 \pm 8.0 \times 10^1$	$1.18 \times 10^2 \pm 3.8 \times 10^1$
H ₄ pat ⁴	$2.27 \times 10^{-3} \pm 1.3 \times 10^{-3}$	$7.74 \times 10^1 \pm 1.6 \times 10^1$	$1.92 \times 10^3 \pm 1.1 \times 10^2$	$1.12 \times 10^2 \pm 2.2 \times 10^1$
H ₄ pat ⁵	$1.94 \times 10^{-3} \pm 1.3 \times 10^{-3}$	$7.25 \times 10^1 \pm 2.6 \times 10^0$	$2.05 \times 10^3 \pm 2.9 \times 10^1$	$1.11 \times 10^2 \pm 1.6 \times 10^1$
H ₄ L ^{ascA}	$1.41 \times 10^{-3} \pm 1.1 \times 10^{-3}$	$7.49 \times 10^1 \pm 4.6 \times 10^0$	$4.68 \times 10^3 \pm 2.0 \times 10^2$	$2.59 \times 10^2 \pm 2.3 \times 10^1$

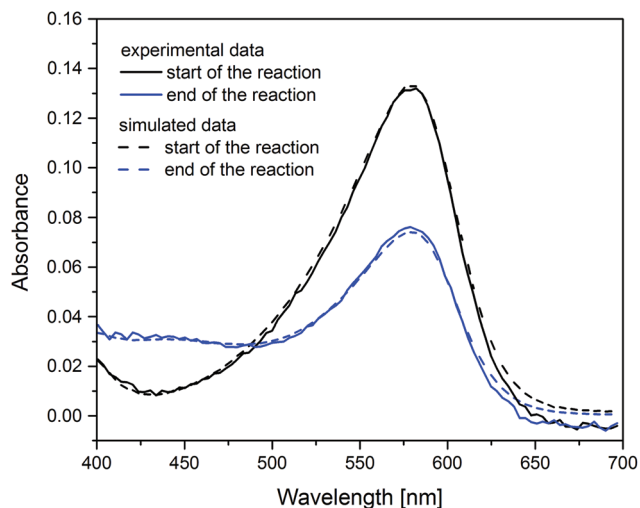


Fig. 2 Comparison of experimental (solid lines) and simulated (dotted lines); parameters listed in Tables 1 and 2 were used for the simulation) absorbance data of the CO₂ hydration reaction of the [Cu₂^{II}(H₂pat¹)(OH)]⁺ complex using the Tris/metacresol purple base/indicator system (pK_a 8.07/8.30; λ_{max} 578) at the beginning (black lines) and the end (blue lines) of the reaction; corresponding figures for the other dicopper(II) cyclic peptide complexes are given as ESI.†

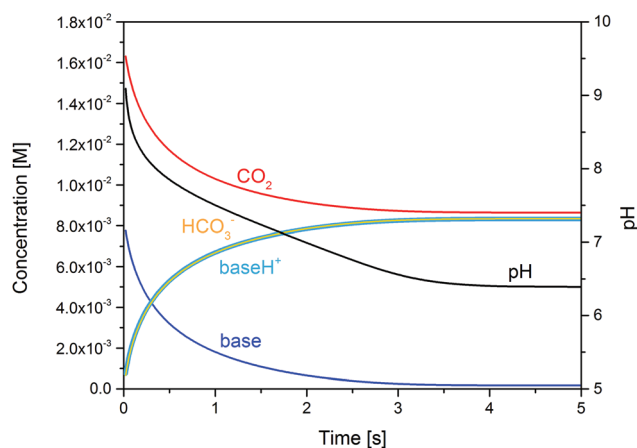


Fig. 3 pH and concentration profile of important species (CO₂, HCO₃⁻, base, baseH⁺) during the hydration of 17 mM CO₂ with 8.5 mM Tris by the [Cu₂^{II}(H₂pat¹)(OH)]⁺ complex at 25 °C; corresponding figures for the other cyclic peptide systems are given as ESI.†

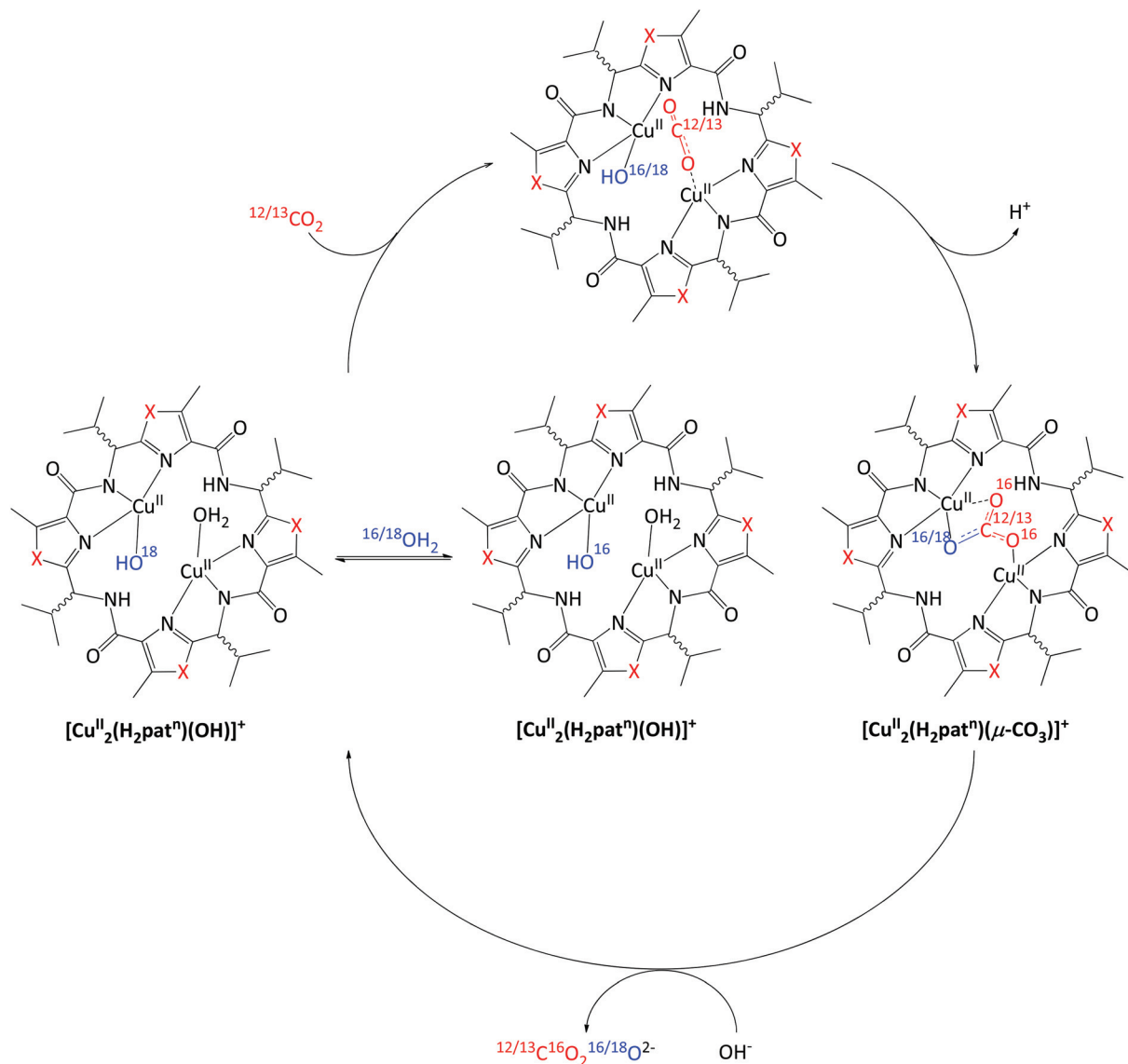


Fig. 4 Proposed mechanism for CO₂ hydration by the [Cu₂^{II}(H₂patⁿ)(OH)]⁺ complexes.¹⁴

Cu^{II}-coordinated OH₂ molecule, responsible for a maximum concentration of the catalytically active hydroxido complex, is $7.1 < \text{p}K_{\text{a}} < 8.1$, and this is as expected for Cu^{II} aqua ions⁴⁸ and the assumed $\text{p}K_{\text{a}}$ values of the two coordinated water molecules in our complexes in H₂O–MeCN as solvent ($\text{p}K_{\text{a}} = 6.8$ and 7.5), based on the analysis of the phosphoester hydrolysis kinetics.¹⁵ We assume that coordination of CO₂ (see Fig. 4) is a fast process since the water exchange rates of Cu^{II}_{aq} are in the range of 7×10^8 – 5×10^9 s⁻¹.⁴⁹ Moreover, the fact that the hydration rate decreases at higher pH (see Table 2 and ESI†) suggests that the dihydroxido complex is a much less efficient catalyst (see discussion of the $\text{p}K_{\text{a}}$ values above). This is in agreement with the expectation that ligand exchange (rate of CO₂ coordination) of the hydroxido complex is less efficient. The nucleophilic attack by the OH⁻ group is expected to be rate determining, and the subsequent release of the emerging

carbonate or bicarbonate to reform the resting state is assumed to be a facile process.¹⁴ This is supported by the fact that the observed optimum rates of all six catalysts are similar (1.5 – 7.5×10^3 s⁻¹), and this is due to the similarity of the geometric and electronic structures of the catalytically active hydroxido complexes.¹⁴ While we do not have quantitative information on the stability constants of the carbonato-bridged intermediate, it is interesting to note that for the most efficient of the catalysts studied here, the dicopper(II) complex of H₄pat¹, we have, in contrast to the other systems, not been able to trap and spectroscopically characterize any carbonato-bridged complexes. This might indicate that the configuration of the substituents, which for H₄pat¹ is identical to that of the natural systems, is related to the high efficiency of this system. The only other macrocycle with the same configuration, H₄L^{ascA}, which also has the same heterocycle combination as observed for

ascidiacyclamide and the patellamides, has a significantly lower efficiency but still a faster catalytic rate than the complexes of the four other ligand systems studied here. This suggests that both the orientation of the side chain and the type of heterocycle have an influence on the structure, stability and flexibility of the macrocycle and its complexes, and this is as expected on extensive computational work of the metal-free macrocycles (which also define the type of side-chain as decisive)^{13,50–52} and some initial studies of the dicopper(II) complexes.¹⁴

Conclusion

The structures and solution equilibria of the Cu^{II} complexes of six synthetic pseudo-octapeptides [Scheme 1, H₄patⁿ (*n* = 1–5) and H₄L^{ascA}] are known in much detail, and under the conditions of the experiments discussed here (neutral to slightly basic aqueous solution), the main species present are the cyclic-peptide-dicopper(II)-hydroxido complexes (Scheme 3). There are various possible forms of these compounds (*i.e.* mono- or dihydroxido, moreover, one of the hydroxides can bridge the two copper centers) and the corresponding structures have been analyzed in detail by X-ray crystallography and/or the combination of EPR spectroscopy with computational structure optimization.^{13,14} The hydroxido complexes are known to hydrate CO₂ in a reaction that involves a complex-CO₂ adduct and the nucleophilic attack of a coordinated hydroxide at the CO₂ carbon atom, leading to a bridging carbonate or bicarbonate.¹⁴ The results presented here clearly show that this reaction is catalytic and very efficient, *i.e.* the fastest of the systems (H₄pat¹) hydrates CO₂ with $k_{\text{cat}} = 7.3 \times 10^3 \text{ s}^{-1}$, and this is five orders of magnitude faster than the uncatalyzed reaction and only one to two orders of magnitude slower than carbonic anhydrases.

From a comparison of the rates for the dicopper complexes of cyclic pseudo-octapeptides with different heterocyclic donor groups (imidazole *vs.* thiazole *vs.* oxazoline) and from the configuration of the side chains (significant dependence on the configuration of the isopropyl side chain, small influence only on the heterocycle) it emerges that (i) the nucleophilic attack at the CO₂ carbon atom is rate determining, (ii) coordination of CO₂ to one of the Cu^{II} centers is in fast preequilibrium (*i.e.* the electronic perturbation by the heterocyclic N donor is relatively unimportant), and (iii) inhibition by the carbonate product is only of minor importance and consequently only slightly influences the catalytic efficiency at the concentrations formed in these catalytic reactions.

The important result is that our *in vitro* data suggest that a possible biological function of the patellamides is carbonic anhydrase activity of their copper(II) complexes. This is exciting for two reasons, *i.e.*, (i) there is no reliable information on the role of the patellamide macrocycles in the ascidians and (ii) so far no copper-based carbonic anhydrases have been reported. However, we point out that there is not even proof that Cu^{II} complexation of the patellamides is relevant for the ascidians

(however, the relative stabilities of the patellamide–Cu^{II} complexes and the relative metal ion concentrations in the ocean compared to that in various species support the assumption that these complexes play an important role).¹⁹ The fact that many ascidians live in symbiosis with photosynthetic prokaryotes shows the need for efficient carbonic anhydrases, and the function of the patellamide–Cu^{II} complexes might be to provide the *Prochloron* symbionts with CO₂. That the dehydration of HCO₃[–] which is abundant in sea water, rather than hydration of CO₂, might be of importance for the *L. patella* – *Prochloron* couple is supported by the fact that the optimum pH for the hydration reaction is relatively high (around pH 8), and the reverse reaction should then occur at around physiological pH.

Experimental section

Materials and methods

All materials obtained commercially were of reagent grade and were used without further purification. Indicators, bases, Cu^{II} triflate (Cu(CF₃SO₃)₂) and methanol were purchased from Fluka and Aldrich, respectively. Solvents were used in pure analytical grade. All solvents were degassed and kept under argon to prevent any dissolution of CO₂ from the air. The Cu^{II} complexes were prepared according to published procedures,^{13,14,16} and were generated *in situ* by mixing the solution of the corresponding cyclic peptide (1 mM, MeOH) with the Cu^{II} solution (Cu(CF₃SO₃)₂, 25 mM, MeOH) and base ([N(C₄H₉)₄](OMe), 25 mM, MeOH).

Stopped-flow measurements were performed with a P.D.1 photodiode-array stopped-flow spectrometer from Applied Photophysics. Experiments were performed in a mixture of water–methanol ratio of 9 : 1. 30 min prior to the catalysis experiments a methanol solution of complex H₄patⁿ–Cu^{II}–MeO[–] 1 : 2 : 4 (*c*(H₄patⁿ) 1 mM) was prepared. In the catalytic experiments 220 μL of the complex (*c* = 10 μM), indicator (*c* = 25 μM) and base (*c* = 17 mM) containing water–methanol (80 : 20) solution were mixed with 220 μL of CO₂-saturated water (*c* = 34 mM) at room temperature. The CO₂ concentration was attained by relative gas-flow rates and published saturation concentrations.⁵³ After mixing, the rapid absorbance change was recorded and the absorption change correlated to the amount of hydrated CO₂. Each measurement was performed five times to ensure consistency of the measuring conditions and settings. The *ReactLab*TM software was used for the kinetic analysis (Jplus Consulting Ltd).⁵⁴ This software suite is designed to carry out the fitting of reaction models of either kinetic or equilibrium multiwavelength data sets. For fitting the kinetic parameters the software suite's "Kinetics" tool was used. All calculations are handled internally and the user provides the experimental measurements and a reaction scheme that is to be fitted to the data. A range of relevant supporting options is available as well as comprehensive graphics tools for visualization of the data and results. Data, models and results are provided in pre-formatted Excel^(R) spreadsheets.^{55,56}

Acknowledgements

Financial support by the German Science Foundation (DFG), the University of Heidelberg and the University of Queensland are gratefully acknowledged.

References

- B. F. Milne, P. F. Long, A. Starcevic, D. Hranueli and M. Jaspars, *Org. Biomol. Chem.*, 2006, **4**, 631.
- E. W. Schmidt, J. T. Nelson, D. A. Rasko, S. Sudek, J. A. Eisen, M. G. Haygood and J. Ravel, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 7315.
- W. E. Houssen, J. Koehnke, D. Zollman, J. Vendome, A. Raab, M. C. M. Smith, J. H. Naismith and M. Jaspars, *ChemBioChem*, 2012, **13**, 2683.
- J. Koehnke, A. Bent, W. E. Houssen, D. Zollman, F. Morawitz, S. Shirran, J. Vendome, A. F. Nneoyiege, L. Trembleau, C. H. Botting, M. C. M. Smith, M. Jaspars and J. H. Naismith, *Nat. Struct. Mol. Biol.*, 2012, **19**, 767.
- B. M. Degnan, C. J. Hawkins, M. F. Lavin, E. J. McCaffrey, D. L. Parry and D. J. Watters, *J. Med. Chem.*, 1989, **32**, 1354.
- A. L. van den Brenk, D. P. Fairlie, G. R. Hanson, L. R. Gahan, C. J. Hawkins and A. Jones, *Inorg. Chem.*, 1994, **33**, 2280.
- P. Comba, R. Cusack, D. P. Fairlie, L. R. Gahan, G. R. Hanson, U. Kazmaier and A. Ramlow, *Inorg. Chem.*, 1998, **37**, 6721.
- P. V. Bernhardt, P. Comba, D. P. Fairlie, L. A. Gahan, G. R. Hanson and L. Lötzbeyer, *Chem.–Eur. J.*, 2002, **8**, 1527.
- A. L. van den Brenk, J. D. A. Tyndall, R. M. Cusack, A. Jones, D. P. Fairlie, L. R. Gahan and G. R. Hanson, *J. Inorg. Biochem.*, 2004, **98**, 1857.
- L. Grondahl, N. Sokolenko, G. Abberate, D. P. Fairlie, G. R. Hanson and L. R. Gahan, *J. Chem. Soc., Dalton Trans.*, 1999, 1227.
- P. Wipf and C. Wang, *Org. Lett.*, 2006, **8**, 2381.
- A. L. van den Brenk, K. A. Byriel, D. P. Fairlie, L. R. Gahan, G. R. Hanson, C. J. Hawkins, A. Jones, C. H. L. Kennard, B. Moubaraki and K. S. Murray, *Inorg. Chem.*, 1994, **33**, 3549.
- P. Comba, N. Dovalil, G. R. Hanson and G. Linti, *Inorg. Chem.*, 2011, **50**, 5165.
- P. Comba, N. Dovalil, L. R. Gahan, G. Haberhauer, G. R. Hanson, C. J. Noble, B. Seibold and P. Vadivelu, *Chem.–Eur. J.*, 2012, **18**, 2578.
- P. Comba, L. R. Gahan, G. R. Hanson and M. Westphal, *Chem. Commun.*, 2012, 9364.
- P. Comba, N. Dovalil, G. Haberhauer, K. Kowski, N. Mehrkens and M. Westphal, *Z. Anorg. Allg. Chem. (Special Issue Bioinorg. Chem.)*, 2013, **639**, 1395.
- L. A. Morris and M. Jaspars, in *A Cu²⁺ selective marine metabolite*, RSC Special Publications, 2000, vol. 257, p. 140.
- C. E. Salomon and D. J. Faulkner, *J. Nat. Prod.*, 2002, **65**, 689.
- S. H. Wright, A. Raab, J. Feldmann, E. Krupp and M. Jaspars, in *Handbook of Marine Natural Products*, ed. E. Fattorusso, W. H. Gerwick and O. Tagliatalata-Scatati, Springer, Heidelberg, New York, 2012, p. 861.
- B. P. Kremer, R. Pardy and R. A. Lewin, *Phycologia*, 1982, **21**, 258.
- M. Köhl and A. W. D. Larkum, in *Cellular Origin and Life in Extreme Habitats*, ed. J. Seckbach, Kluwer Acad. Publ., Dordrecht, 2002, vol. 3: Symbiosis, Mechanisms and Model Systems, p. 273.
- R. Latifi, M. Bagherzadeh, B. F. Milne, M. Jaspars and S. P. De Visser, *J. Inorg. Biochem.*, 2008, **102**, 2171.
- P. V. Bernhardt, P. Comba, T. W. Hambley, S. S. Massoud and S. Stebler, *Inorg. Chem.*, 1992, **31**, 2644.
- P. Comba, Y. D. Lampeka, A. Prikhod'ko and G. Rajaraman, *Inorg. Chem.*, 2006, **45**, 3632.
- P. Comba, L. R. Gahan, G. Haberhauer, G. A. Hanson, C. J. Noble, B. Seibold and A. L. van den Brenk, *Chem.–Eur. J.*, 2008, **14**, 4393.
- R. G. Kalifah, *J. Biol. Chem.*, 1971, **246**, 2561.
- S. Lindskog and J. E. Coleman, *Proc. Natl. Acad. Sci. U. S. A.*, 1973, **70**, 2505.
- D. N. Silverman and S. Lindskog, *Acc. Chem. Res.*, 1988, **21**, 30.
- E. Kimura, T. Shiota, T. Koike, M. Shiro and M. Kodama, *J. Am. Chem. Soc.*, 1990, **112**, 5805.
- X. P. Zhang, R. van Eldik, T. Koike and E. Kimura, *Inorg. Chem.*, 1993, **32**, 5749.
- R. Alsfasser, S. Trofimenko, A. Looney, G. Pakin and H. Vahrenkamp, *Inorg. Chem.*, 1991, **30**, 4098.
- R. Alsfasser, M. Ruf, S. Trofimento and H. Vahrenkamp, *Chem. Ber.*, 1993, **126**, 703.
- E. Kimura, *Acc. Chem. Res.*, 2001, **34**, 171.
- J. H. Satcher Jr., S. E. Baker, H. J. Kulik, C. A. Valdez, R. L. Krueger, F. C. Lightstone and R. D. Aines, *Energy Procedia*, 2011, **4**, 2090.
- W. Sattler and G. Parkin, *Chem. Sci.*, 2012, **3**, 2015.
- C. Bazzicalupi, A. Bencini, A. Bianchi, V. Fedi, V. Fusi, C. Giorgi, P. Paoletti, L. Tei and B. Valtancoli, *J. Chem. Soc., Dalton Trans.*, 1999, 1101.
- C. Bazzicalupi, A. Bencini, A. Bencini, A. Bianchi, F. Corana, V. Fusi, C. Giorgi, P. Paoli, P. Paoletti, B. Valtancoli and C. Zanchini, *Inorg. Chem.*, 1996, **35**, 5540.
- A. Escuer, F. A. Mautner, E. Penalba and R. Vicente, *Inorg. Chem.*, 1998, **37**, 4190.
- A. Company, J.-E. Jee, X. Ribas, J. M. Lopez-Valbuena, L. Gomez, M. Corbella, A. Llobet, J. Mahia, J. Benet-Buchholz, M. Costas and R. v. Eldik, *Inorg. Chem.*, 2007, **46**, 9098.
- E. A. Parson and D. W. Keith, *Science*, 1998, **282**, 1053.
- H. Arakawa, M. Aresta, J. N. Armor, M. A. Barteau, E. J. Beckman, A. T. Bell, J. E. Bercaw, C. Creutz, E. Dinjus, D. A. Dixon, K. Domen, D. L. DuBois, J. Eckert, E. Fujita, D. H. Gibson, W. A. Goddard, D. W. Goodman, J. Keller, G. J. Kubas, H. H. Kung, J. E. Lyons, L. E. Manzer, T. J. Marks, K. Morokuma, K. M. Nicholas, R. Periana,

- L. Que, J. Rostrup-Nielsen, W. M. H. Sachtler, L. D. Schmidt, A. Sen, G. A. Somorjai, P. C. Stair, B. R. Stults and W. Tumas, *Chem. Rev.*, 2001, **101**, 953.
- 42 DATABASE, *United States National Library of Medicine and Chem Dplus Advaced online database*, 2013.
- 43 X. Wang, W. Conway, R. Burns, N. McCann and M. Maeder, *J. Phys. Chem. A*, 2010, **114**, 1734.
- 44 X. Wang, W. Conway, D. Fernandes, G. A. Lawrance, R. Burns, G. Puxty and M. Maeder, *J. Phys. Chem. A*, 2011, **115**, 6405.
- 45 W. Conway, X. Wang, D. Fernandes, R. Burns, G. A. Lawrance, G. Puxty and M. Maeder, *J. Phys. Chem. A*, 2011, **115**, 14340.
- 46 J. C. Kernohan, *Biochim. Biophys. Acta*, 1965, **96**, 304.
- 47 D. A. Palmer and R. van Eldik, *Chem. Rev.*, 1983, **83**, 651.
- 48 G. Schwarzenbach, *Angew. Chem.*, 1958, **70**, 451.
- 49 D. H. Powell, L. Helm and A. E. Merbach, *J. Chem. Phys.*, 1991, **95**, 9258.
- 50 P. Wipf, *Alkaloids: Chemical and Biological Perspectives*, Pergamon, New York, 1998.
- 51 G. Haberhauer, E. Drosdow, T. Oeser and F. Rominger, *Tetrahedron*, 2008, **64**, 1853.
- 52 G. Haberhauer, A. Pinter, T. Oeser and F. Rominger, *Eur. J. Org. Chem.*, 2007, 1779.
- 53 D. Linde, *CRC Handbook of Chemistry and Physics*, CRC Press, Boca Raton FL, 2007.
- 54 Jplus, Jplus Consulting Pty Ltd, <http://www.jplusconsulting.com>, Palmyra, WA 6957, Australia.
- 55 M. Maeder, P. King and K. Varmuza, *Analysis of Chemical Processes, Determination of the Reaction Mechanism and Fitting of Equilibrium and Rate Constants, Chemometrics in Practical Applications*, InTechs, 2012.
- 56 M. Maeder and Y.-M. Neuhold, *Practical Data Analysis in Chemistry*, Elsevier, Amsterdam, 2007.
- 57 H. S. Harned and S. R. Scholes, *J. Am. Chem. Soc.*, 1941, **63**, 1706.
- 58 M. Maeda, O. Hisada, Y. Kinjo and K. ITO, *Bull. Chem. Soc. Jpn.*, 1987, **60**, 3233.
- 59 H. S. Weast and R. J. Davis, *Handbook of Chemistry and Physics*, Chemical Rubber Co., Cleveland, 1970–1971, p. D122.
- 60 G. M. Harris and T. P. Dasgupta, *J. Indian Chem. Soc.*, 1977, **54**, 62.