

The Elusive Chemistry of Pyrroloquinoline Quinone Dimethyl Ester Lanthanide Complexes in Biomimetic Alcohol Oxidation

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Since its discovery, pyrroloquinoline quinone (PQQ) has been under constant investigation regarding its efficiency in biomimetic complexes for alcohol dehydrogenation. The discovery of lanthanide (Ln) dependent methanol dehydrogenases has led to the use of lanthanide complexes bearing PQQ derivatives to oxidize alcohols. However, the mechanism of these oxidations is still a subject of debate. Herein, La^{3+} and Lu^{3+} complexes of PQQ dimethyl ester (PQQDME), that are able to stoichiometrically oxidize an alcohol substrate, are reported. In the presence of air,

some catalytic turnover is observed, but less than with other, more heavily modified PQQ biomimetics known in the literature. To investigate the reason for this low turnover, the reduced counterpart, PQQDMEH₂, is synthesized. It is shown that in the presence of atmospheric oxygen, the complexes of the reduced form undergo oxidation and can then also convert alcohol to aldehyde. Additionally, the involvement of radicals in the alcohol oxidation reaction and the origin and nature of these radicals is investigated.

1. Introduction

In methylotrophic bacteria, methanol dehydrogenases (MDHs) are enzymes that catalyze the conversion of methanol to formaldehyde. One class of MDH, which is not dependent on NAD or NADP as cofactors, was discovered in 1964 by Anthony and Zatman^[1] and in the years following, efforts were made to identify the nature of the enzyme, more specifically, what prosthetic group is found in its active site.^[2,3] In 1979, Salisbury and coworkers isolated the cofactor as orange needles and upon single crystal analysis, the new molecule was finally described

as 4,5-dioxo-4,5-dihydro-1 H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylic acid, better known as pyrroloquinoline quinone or PQQ in short.^[4] In its early stages, this molecule was a source of controversy. For a period of time, it was thought that PQQ also represented the redox cofactor in copper-dependent amine oxidases,^[5,6] however this has since been rebutted^[7] and several other quinone cofactors were discovered (topaquinone TPQ, tryptophan tryptophylquinone TTQ, and lysine tyrosylquinone LTQ, which are covalently bound to the enzyme).^[8]

The metal cofactor also identified in PQQ-dependent MDHs is Ca^{2+} , and a refined crystal structure of Ca-MDH from *Methylobacterium extorquens* AM1 was published in 1995.^[9] Until 2011, Ca-MDH, expressed by a *mxoF*-gene, was thought to be the only type of PQQ-dependent MDH in nature, but the identification of a lanthanide-containing MDH encoded by a *xoxF*-gene in *Methylobacterium radiotolerans* led to the discovery of the field of lanthanide biochemistry.^[10] Since then, bacteria which can only grow in the presence of lanthanides and do not possess the gene in order to express the Ca-MDH have also been discovered.^[11]

A comparison of the active sites of Ca-MDH and La-MDH from the respective organisms is depicted in **Figure 1**. In both cases, the metal ion is coordinated by the redox cofactor PQQ in its ONO (see green/blue sphere in Figure 1) binding pocket with amino acid residues of the MDH saturating the coordination sphere of the metal ion. A crucial difference between the La and Ca-centered active sites lies in the additional aspartate residue required in the La-MDH to satisfy the higher coordination number of the lanthanide.

The two commonly proposed mechanisms for alcohol oxidation by Ca-MDH and Ln-MDH are the addition–elimination (AE) pathway and the hydride transfer (HT) pathway. Since the

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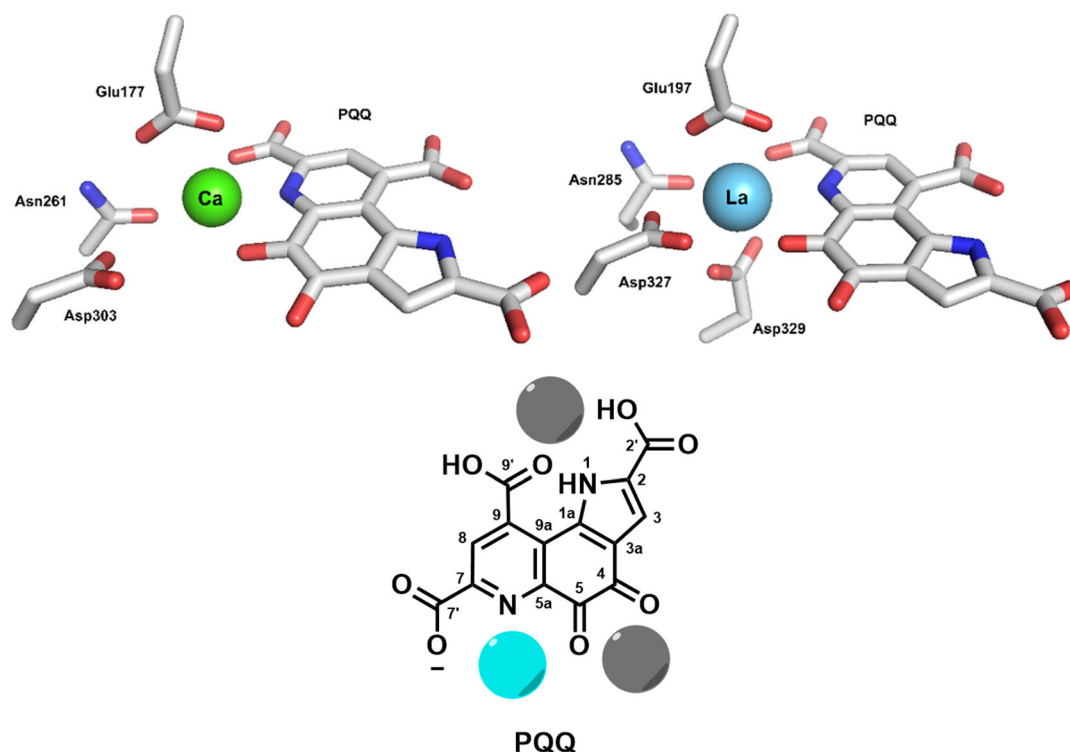


Figure 1. Comparison of the active sites of Ca-MDH (*M. extorquens*, PDB 1W6S)^[63] and La-MDH (*Mm. buryatense*, PDB 6DAM).^[64] Molecular structure of PQQ with possible binding sites (biologically relevant site depicted in turquoise). Atom numbering according to Unkefer et al.^[65]

discovery of these enzymes, extensive research has been conducted to elucidate the precise reaction mechanism. The isolation of Ca-MDH containing a PQQ-CD₃OH hemiketal adduct and later of a cyclopropanol adduct led to the initial conclusion that the AE is preferred as the hemiketal represents the first intermediate of this path.^[12–15] However, structural and kinetic studies on PQQ-dependent glucose dehydrogenase (GDH) provide strong evidence supporting a hydride transfer mechanism.^[16,17] Oubrie et al. proposed that, due to kinetic similarities between GDH and MDH, the catalytic mechanism of Ca-MDH may also involve hydride transfer; this hypothesis was subsequently supported by *in silico* studies conducted by Bruce in the following years.^[18–21] In light of the discovery of lanthanide-dependent MDH, recent computational studies have been undertaken, which tend to support an AE pathway for methanol oxidation.^[22,23] Accordingly, the exact catalytic mechanism within the enzymatic environment remains under investigation, as does the question of whether mechanistic differences exist between the calcium- and lanthanide-dependent enzymes.

The discoveries of PQQ-dependent Ca-MDHs and Ln-MDHs have led to the emergence of PQQ biomimetics, a field in which the aim is to better understand the alcohol oxidation mechanism and to develop bioinspired alcohol oxidation catalysts. Since the discovery of PQQ-dependent MDH, numerous biomimetic systems have been explored with ligands similar to the natural cofactor (see Figure 2). Table 1 provides a comprehensive overview of the complexes that have been successfully utilized in the biomimetic oxidation of alcohols to aldehydes. The first calcium-based model was reported by Itoh et al. in 1995.^[24] PQQDME was

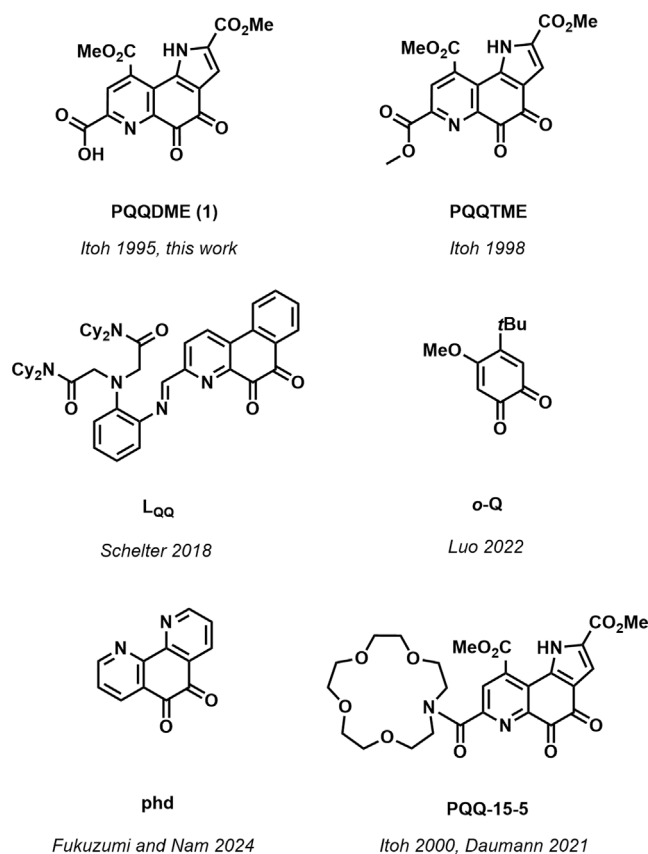


Figure 2. Overview of the ligands previously used for PQQ-dependent Ca and Ln-MDH biomimetic complexes.^[24–28,30,31]

Table 1. Overview of existing functional biomimetic models reported since the discovery of PQQ-dependent Ca and Ln-MDH with properties and catalytic performance.

Study	Ligand	Metal ion/other additives	Aldehyde yield	Proposed mechanism
Itoh 1995 ^[24a]	PQQDME	Ca ²⁺ /NH ₃	80% ^{b)}	AE
Itoh 1998 ^[25]	PQQTME	Ca ²⁺ /DBU	quantitative ^{c)}	AE
		Ca ²⁺ /DBU/O ₂	1450% ^{c)}	
Itoh 2000 ^[26]	PQQ-1-aza-15-crown-5	Ca ²⁺ /DBU/	quantitative ^{c)}	AE
Schelter 2018 ^[27]	L _{QQ}	La ³⁺ /DBU	63% ^{d)}	HT
		La ³⁺ /DBU/[Fc][PF ₆]	1920% ^{d)}	
Daumann 2021 ^[28]	PQQ-1-aza-15-crown-5	La ³⁺ /DBU	37% ^{d)}	Involvement of radicals observed
Luo 2022 ^[30]	o-Q	La ³⁺ /I ₃ ⁻ /O ₂	4750% ^{b)}	Proton-coupled HT, involvement of radicals observed
Fukuzumi and Nam 2024 ^[31]	phd	Ce ³⁺ /O ₂	1238% ^{b)}	HT

^{a)}Based on quinone. ^{b)}Benzyl alcohol to benzaldehyde. ^{c)}Ethanol to acetaldehyde. ^{d)}4-Methylbenzyl alcohol to 4-methylbenzaldehyde.

obtained through partial hydrolysis of PQQ trimethyl ester (PQQTME), and the subsequent addition of calcium nitrate resulted in the precipitation of a complex. Upon treatment with NH₃, this complex was capable of stoichiometrically oxidizing benzyl alcohol to the corresponding aldehyde. In 1998, Itoh further demonstrated that PQQTME in combination with calcium also constitutes a viable biomimetic model.^[25] The resulting complex quantitatively oxidized ethanol to acetaldehyde under stoichiometric conditions upon addition of DBU. When the reaction was conducted under aerobic conditions, catalytic turnover was achieved, with a turnover number (TON) of ≈ 15 . A subsequent model was reported by the same group in 2000, employing a 1-aza-15-crown-5 derivative of PQQDME with Ca²⁺ and DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) for the quantitative stoichiometric oxidation of ethanol.^[26] For these calcium-based PQQ biomimetic models, UV-Vis kinetic analyses were conducted to monitor the formation of hemiketal intermediates with alcohols. Based on these data, the authors concluded that alcohol oxidation proceeds *via* an AE mechanism.

Following the discovery of Ln-MDH, biomimetic complexes incorporating PQQ-like ligands and utilizing lanthanide ions have also been developed. The first functional model of Ln-MDH was reported by Schelter in 2018.^[27] They synthesized a quinoline quinone ligand (L_{QQ}) featuring a bulky chelating group to direct coordination to the desired site and successfully isolated the corresponding lanthanum complex. They demonstrated that the complex oxidizes 4-methylbenzyl alcohol, with the reaction rate enhanced by the addition of a base. Under stoichiometric conditions, an aldehyde yield of 63% was achieved and the introduction of ferrocenium hexafluorophosphate as a terminal oxidant enabled catalytic turnover, resulting in a TON of ≈ 19 . In 2021, Daumann employed the PQQ-1-aza-15-crown-5 ligand, previously used by Itoh for calcium-based biomimetics, to stoichiometrically oxidize 4-methylbenzyl alcohol to the corresponding aldehyde using its La³⁺ and Lu³⁺ complexes.^[28] They demonstrated that the La³⁺ complex facilitated the reaction more effectively than the Lu³⁺ complex, consistent with the enzymatic trend wherein early lanthanides exhibit higher activity than later ones. However, even higher yields were obtained using Ca²⁺ and Ba²⁺,

showing that not only ionic radius but also oxidation state of the metal ion play a crucial role. Furthermore, the same group showed that PQQ complexes precipitate in aqueous solution preferably with early lanthanides when a mixture is present, yet again highlighting the preference for these metal ions in the coordination environment of the cofactor PQQ.^[29] In 2022, Luo developed a particularly effective catalyst based on the La³⁺ complex of an ortho-quinone (o-Q) ligand, capable of oxidizing a broad range of primary and secondary alcohols to their corresponding aldehydes and ketones.^[30] The reaction proceeded using molecular oxygen as the terminal oxidant and did not require the addition of a base. The authors propose that the oxidation involves a radical species which is formed by the one electron reduction of o-Q by iodide ions. In small-scale reactions, yields corresponding to 4750% based on the quinone were achieved, and under optimized conditions, turnover numbers (TONs) of up to 250 were reported for the oxidation of benzylic alcohols. Both Schelter and Luo proposed that the oxidation of the alcohol involves a hydride transfer step. The most recent model of Ln-MDH was reported by Fukuzumi and Nam in 2024, utilizing the commercially available ligand 1,10-phenanthroline-5,6-dione to form a Ce³⁺ complex for the oxidation of alcohols.^[31] Unlike previous biomimetic systems, this ligand does not coordinate the metal ion through both the nitrogen and quinone oxygen atoms, as these donor groups create distinct coordination sites. In catalytic oxidation experiments using molecular oxygen as the terminal oxidant, TON of ≈ 12 was achieved. Based on the observation that the aldehyde product is formed even under anaerobic conditions, the authors concluded that the oxidation step likely proceeds *via* a hydride transfer mechanism.

Since the emergence of Ln-MDH biomimetics, several studies have undertaken more detailed mechanistic investigations, revealing that, beyond the AE versus hydride transfer debate, other factors also play significant roles. PQQ is known to access a one-electron reduced redox state as a semiquinone, which has been described as the resting state within the native enzyme.^[3,32–34] Furthermore, a 2021 spectroscopic and crystallographic study revealed a disulfide bridge between vicinal cysteine residues near the active site of MDH from *methylococcus*

capsulatus.^[35] The disulfide bridge was proposed to accept one electron after the hydride transfer to PQQ, leading to a PQQH• radical. The biradical formed is then oxidized by NAD⁺ to form PQQ and the disulfide bridge. These studies demonstrate not only that a PQQ-based radical is an easily accessible redox state inside the enzyme but also that PQQH• actively participates in the methanol oxidation mechanism. The aforementioned study by Luo and colleagues also proposes the involvement of a radical species in the oxidation of alcohols by the biomimetic o-Q Ln complex.^[30] The proposed mechanism begins with the one-electron reduction of o-Q by iodide ions, forming I₃[−]. The resulting semiquinone anion is responsible for alcohol oxidation and is subsequently reduced to the hydroquinone. A similar mechanism involving a semiquinone anion as the active species is proposed by the same group for the oxidation of thiols to disulfides using a La-o-Q complex, further highlighting the ease with which such quinones access radical states and their reactivity with the thiol/disulfide system.^[36]

Although recent studies on Ln-MDH biomimetics offer well-supported mechanistic proposals based on experimental and computational evidence, the mechanism remains incompletely understood for moieties more closely resembling PQQ. An alternative approach to understanding the reactivity of PQQ and related biomimetic compounds is to examine the redox properties of the isolated species involved in the reaction cycle. Although potentiometric and cyclic voltammetry studies of PQQ in aqueous media are available, investigations become significantly more challenging when examining PQQ or its derivatives in aprotic environments.^[37–40] For this reason, studies have aimed to synthesize the reduced counterpart of PQQ, the catechol PQQH₂, and look at its reactivity with oxidants such as O₂. PQQH₂ can be synthesized using several different routes by reducing PQQ, for example, using 1-benzyl-1,4-dihydronicotinamide (BNAH),^[41] NAD(P)H,^[42] or vitamin C.^[43] Several UV-Vis studies have examined the oxidation rate of PQQH₂ to PQQ, demonstrating that molecular oxygen can serve as the oxidant and that the reaction rate is pH-dependent, with higher rates observed under basic conditions.^[41–46]

This study initially aimed to elucidate the influence of Lewis acids (lanthanides) on alcohol oxidation by biomimetic PQQDME complexes and to develop bioinspired catalysts. However, PQQ exhibits relatively poor catalytic performance compared to other quinones, motivating a closer examination of the factors responsible. We thus closely examined the different steps of the reaction cycle and investigated the impact of the metal center on the oxidation of alcohols in the presence or absence of atmospheric O₂. Furthermore, we set out to investigate the impact of atmospheric O₂ on the re-oxidation of reduced PQQDME in dependence of the coordinated Lewis acid and last but not least, track the formation of radicals under these conditions.

2. Results and Discussion

2.1. Ligand Synthesis

The ligand PQQDME (1) was synthesized according to Vetsova et al.^[28] The corresponding reduced PQQDMEH₂ (2) was

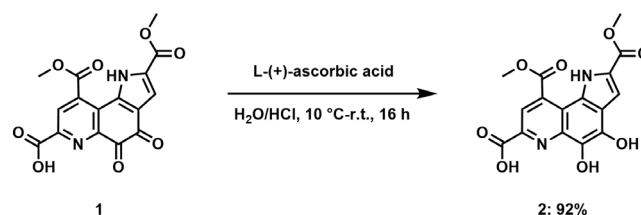
synthesized using an adapted version of the synthesis described by Mukai (see Scheme 1).^[43] To an acidic solution of L-(+)-ascorbic acid at 10 °C, a suspension of 1 was added dropwise over 1 h. After addition, the reaction mixture was left to warm to room temperature and stirred overnight. The product 2 was isolated as a brown precipitate in 92% yield.

2.2. Metal–Ligand Interaction

In 1995, Itoh and coworkers first synthesized 1 by selective hydrolysis of PQQ trimethyl ester with trifluoroacetic acid.^[24] They showed that the addition of calcium(II) nitrate to an acetonitrile solution of 1 leads to the precipitation of a one to one 1-Ca complex with the Ca²⁺ additionally coordinated by a nitrate anion. The formed complex could be employed in the oxidation of benzyl alcohol, showing that 1 can act as a biomimetic for Ca-MDH.

To assess the coordination behavior of 1 with lanthanide ions, we performed metal ion titrations. Increasing amounts of Ln(NO₃)₃·6H₂O (Ln = La, Lu) were added to solutions of 1 in acetonitrile and the change in absorbance was monitored by UV-Vis spectroscopy (see Figure 3). With both La³⁺ and Lu³⁺, the absorbances at ≈280 nm and 442 nm decrease, while the absorbances at 357 nm shift toward ≈375 nm, indicating coordination to 1. However, clear isosbestic points are absent, suggesting the presence of more than two species. The addition of small nucleophiles to the C-5 carbonyl of PQQ has long been well documented.^[47,48] Lumpe et al. demonstrated that PQQ forms a C-5 water adduct in an organic solvent upon addition of metal salts containing water of crystallization.^[49] The same group later investigated PQQ speciation in aqueous media using NMR, UV-Vis, and fluorescence spectroscopy and also observed water adducts.^[49,50] Thus, simultaneously to the complex formation, water addition to 1 or to 1-Ln³⁺ may also occur which can lead to the UV-Vis spectra not showing clear isosbestic points. Nevertheless, both titration curves show saturation in the 1–3 equiv. range of added metal salt. The 1-La³⁺ complex appears to fully form at slightly higher ratios, suggesting that Lu³⁺ may bind more tightly to 1 than La³⁺. However, due to the absence of clear isosbestic points, the calculation of binding constants was not pursued. Instead, a 1:2 ligand-to-metal ratio was selected for all subsequent experiments, as saturation is very likely guaranteed at this ratio in higher total concentrations.

The formation of 1-Ln³⁺ complexes was further confirmed by ESI mass spectrometry. In mixtures of 1 with two equivalents of La(OTf)₃ or Lu(OTf)₃ in methanol, the species [(1[−])Ln³⁺MeO[−]]⁺



Scheme 1. Synthesis of 2 by reduction of 1 using ascorbic acid. Synthesis modified from Ikemoto et al.^[43]

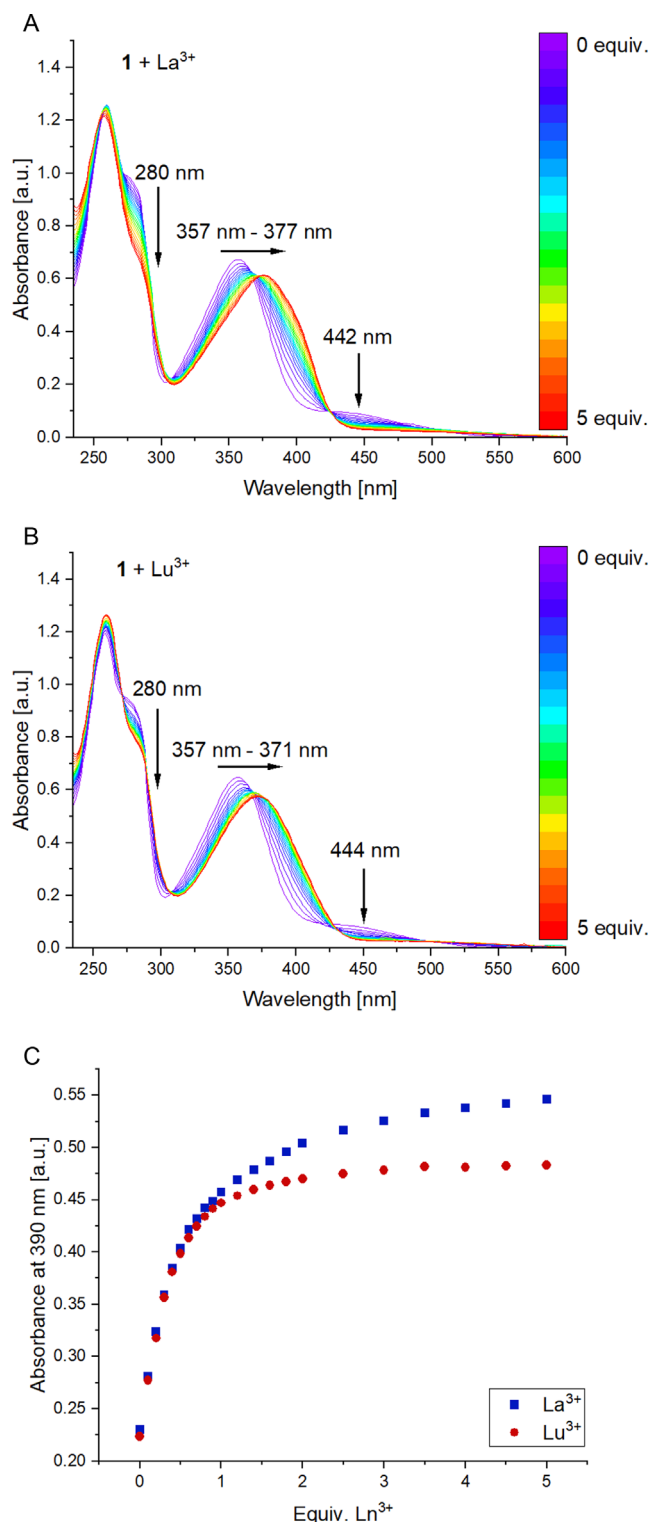


Figure 3. A) titration of **1** (50 μM in acetonitrile) with increasing amounts of $\text{La}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ at 25 $^\circ\text{C}$. B) titration of **1** (50 μM in acetonitrile) with increasing amounts of $\text{Lu}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ at 25 $^\circ\text{C}$. C) Absorbance change at 390 nm of the two titrations with increasing amounts of Ln^{3+} ions.

with m/z 526.957 (calc. 526.960) for La and m/z 562.990 (calc. 562.994) for Lu were identified among other species. Whether the MeOH is coordinating to the metal ion or is covalently bound

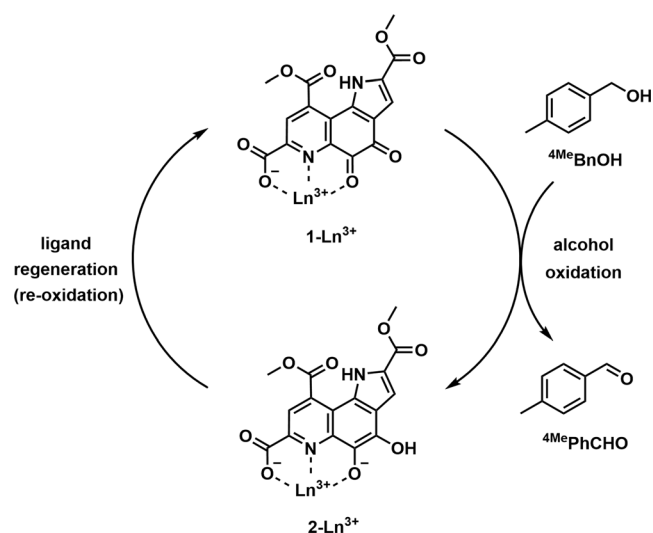
to C-5 (as hemiketal) cannot be deduced from the mass spectra. For isotopic patterns and relative abundance of these species, see supporting information, Figure S5 and S6, Supporting Information. The covalent addition hypothesis is supported by multiple literature reports demonstrating that nucleophiles including methanol readily add to the C-5 carbonyl of PQQ.^[26,47,48] The mass spectra also indicate the formation of 2:1 ligand-to-metal complexes in the gas phase. It is plausible that these species exhibit a greater tendency to ionize and enter the gas phase, especially considering their low relative abundance and the absence of any other 1-Ln^{3+} species in the spectra. Nonetheless, the results clearly demonstrate the ability of **1** to form complexes with both La^{3+} and Lu^{3+} .

Paramagnetic ^1H - and $^{13}\text{C}\{^1\text{H}\}$ -NMR spectroscopy was performed on the analogous 1-Eu^{3+} species in CD_3CN at ambient temperature to gain more insight into the complexes in solution. The most informative spectra were $^{13}\text{C}\{^1\text{H}\}$ NMR spectra (see Figure S8, Supporting Information). Due to the paramagnetic nature of the complex, only 11 relatively sharp resonances could be observed of the 16 carbon atoms in the ligand. The small number of resonances and the absence of visible exchange broadening indicates one single species in solution, an observation also supported by the corresponding ^1H NMR spectrum (see Figure S7, Supporting Information). The paramagnetism prevented meaningful 2D NMR spectra and consequently a rigorous assignment of all signals. Nevertheless, characteristic changes can be observed relative to the diamagnetic ligand signals.^[28] Both methyl ester carbons do not experience a relevant paramagnetic shift and remain at ≈ 54 ppm. In contrast, only two carbonyl carbons are visible in the usual range (≈ 161 ppm), indicating the shift/broadening of the two quinone and one carboxylic carbonyl carbon. Another very diagnostic feature is the absence of sharp ^{13}C signals in the region 140–150 ppm, which is characteristic for the 2- and 6-positions of the pyridine moiety in **1**. Taken together, the data shows that **1** forms a well-behaved complex with Eu^{3+} with metal coordination most likely taking place at the biologically relevant ONO binding pocket (compare to Figure 1).

2.3. Alcohol Oxidation

Next, we set out to investigate the alcohol oxidation properties of these complexes. Ideally, we aim to achieve a catalytic cycle as depicted in **Scheme 2**. We assume that the C-7' carboxylic acid is deprotonated by considering the pK_a value of native PQQ in aqueous media.^[38,51] The following results employ specific terms to describe individual steps; a brief overview is provided here for clarity. In the first step of the cycle, $^4\text{MeBnOH}$ (the alcohol substrate) undergoes two electron oxidation and yields the aldehyde product $^4\text{MePhCHO}$. The two electrons are transferred to PQQDME (**1**), which is reduced to PQQDMEH₂ (**2**). If a terminal oxidant such as O_2 is present, PQQDMEH₂ (**2**) can react back to PQQDME (**1**) in the ligand regeneration/re-oxidation step.

Generally, the 1-Ln complex was generated in situ by the addition of a lanthanide salt to a 3.25 mM solution of **1** in acetonitrile. The resulting complex solution was then added to pure



Scheme 2. Isolated and studied intermediates from the model oxidation reaction of 4MeBnOH by 1-Ln^{3+} complexes.

4MeBnOH , and the reaction was submitted to the suitable analysis method. For $^1\text{H-NMR}$ analysis of 4MePhCHO , the yield is expressed as percentage relating to the amount of quinone, **1**, used. In a stoichiometric reaction under inert conditions, 100% 4MePhCHO yield would mean that 100% of **1** has reacted to form one equivalent of 4MePhCHO . If a terminal oxidant is involved and the ligand regeneration/re-oxidation step of **2** can take place (see Scheme 2), the regenerated 1-Ln^{3+} complex can oxidize additional 4MeBnOH and yields of more than 100% can be achieved.

First, the ideal amount of metal ion needed for alcohol oxidation was determined. For this, the model reaction presented in Scheme 2. was performed under inert and ambient conditions with increasing amounts (1, 2, 5, and 10 equivalents) of $\text{La}(\text{OTf})_3$ and $\text{Lu}(\text{OTf})_3$, respectively. 4MeBnOH was added in 50-fold excess to 1-Ln^{3+} . The aldehyde yield was determined by $^1\text{H-NMR}$ spectroscopy (see experimental methods for details) and the results are presented in Figure 4. Control reactions with only **1** and 4MeBnOH gave only 5% aldehyde yield and the reaction with $\text{La}(\text{OTf})_3$ and no added **1** yielded only 2% aldehyde, demonstrating the necessity of both a lanthanide ion and **1** for substrate conversion. Under inert conditions, the 4MePhCHO yields with varying Ln^{3+} equivalents and **1** are in a similar range for both lanthanides. The minimum yield reached with one equivalent metal ion is $51\% \pm 6\%$ (La^{3+}) and $67\% \pm 3\%$ (Lu^{3+}), respectively. With the maximum amount tested here, 10 equivalents, $75\% \pm 14\%$ (La^{3+}) and $86\% \pm 5\%$ (Lu^{3+}) are reached. The same experiment was then performed in the presence of air. For both La^{3+} and Lu^{3+} , slightly higher yields are achieved, indicating that the presence of a terminal oxidant (oxygen from air in this case) leads to some regeneration of the complex and to some catalytic turnover. With Lu^{3+} , the yields show no large difference with increasing metal ion equivalents and range from $89\% \pm 6\%$ to $101\% \pm 22\%$. With La^{3+} , however, the yield increases from $108\% \pm 11\%$ with one equivalent to $182\% \pm 15\%$ with 10 equivalents, suggesting that La^{3+} ions may additionally support the

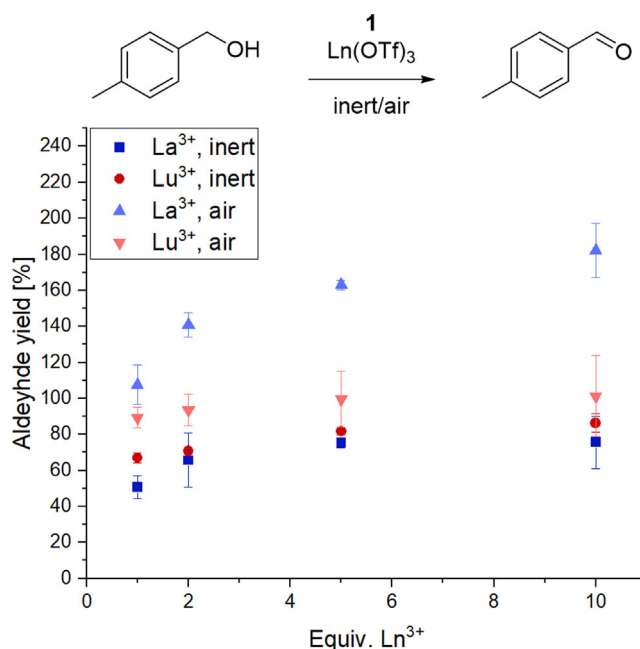


Figure 4. Oxidation of 4MeBnOH (162.50 mM) by **1** (3.25 mM) and increasing amounts $\text{Ln}(\text{OTf})_3$ ($\text{Ln} = \text{La}, \text{Lu}$) under inert or ambient conditions (acetone- d_6 , 293 K). 4MePhCHO yields determined by $^1\text{H-NMR}$ spectroscopy (400 MHz).

reoxidation/ligand regeneration step more efficiently than Lu^{3+} . Furthermore, Ln^{3+} ions may influence the reaction through other mechanisms; for instance, their Lewis acidity could activate not only the PQQ moiety but also the alcohol, potentially facilitating deprotonation. An increased concentration of Ln^{3+} ions may also promote the spatial proximity of the 1-Ln^{3+} complex and the substrate through coordination.

Subsequently, separate parts of the reaction sequence in Scheme 2. were investigated. To single out the alcohol oxidation reaction and prevent the ligand from re-oxidizing, the next experiment was performed under an inert atmosphere. The time course of 4MePhCHO formation from 4MeBnOH by the 1-La^{3+} and 1-Lu^{3+} complexes under inert conditions was followed by $^1\text{H-NMR}$ spectroscopy to assess the effectiveness of the stoichiometric alcohol oxidation (Figure 5). It was observed that with La^{3+} , $73\% \pm 6\%$ are reached, while with Lu^{3+} , $78\% \pm 7\%$ can be obtained. Although the yield after 24 h is the same within error, La^{3+} shows a slightly higher initial reaction rate than Lu^{3+} .

Next, the same two experiments were also conducted under ambient conditions in order to further investigate whether atmospheric oxygen can lead to any catalytic turnover. Both the 1-La^{3+} and 1-Lu^{3+} complexes led to higher 4MePhCHO yields after 24 h, 1-La^{3+} reaching up to $141\% \pm 8\%$ and 1-Lu^{3+} up to $100\% \pm 5\%$ (Figure 6). In both cases, there is a difference to the reaction under inert atmosphere and the yields surpass 100%, indicating that ambient oxygen could be leading to some catalytic turnover. In terms of turnover numbers, the 1-La^{3+} complex barely reaches $\text{TON} = 1.4$.

Compared to previously reported biomimetic Ca- and Ln-MDH systems (Table 1), the complexes 1-La^{3+} and 1-Lu^{3+} exhibit

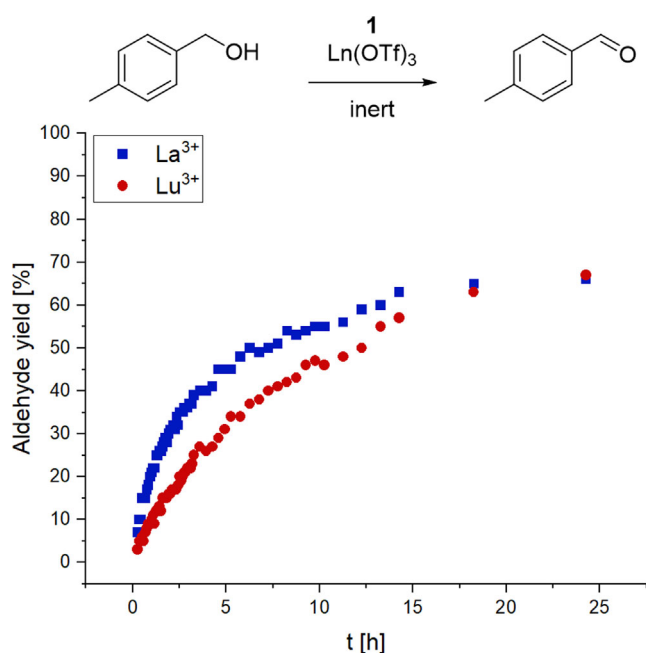


Figure 5. Time courses of the oxidation of $^4\text{MeBnOH}$ (162.50 mM) by **1** (3.25 mM) and $\text{Ln}(\text{OTf})_3$ (6.50 mM, $\text{Ln} = \text{La}$ or Lu) under inert conditions (acetonitrile- d_3 , 293 K). Aldehyde yields determined by ^1H -NMR spectroscopy (400 MHz).

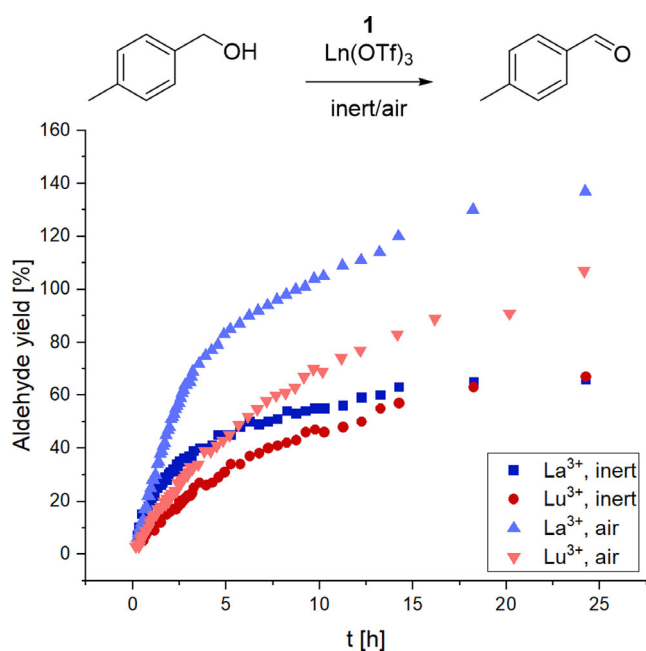


Figure 6. Time courses of the oxidation $^4\text{MeBnOH}$ (162.50 mM) by **1** (3.25 mM) $\text{Ln}(\text{OTf})_3$ (6.50 mM, $\text{Ln} = \text{La}$ or Lu) under inert or ambient conditions (acetonitrile- d_3 , 293 K). Aldehyde yields determined by ^1H -NMR spectroscopy (400 MHz).

significantly lower performance. For example, the most recent phd complex reported by Fukuzumi and Nam, employed under comparable conditions (triflate salt, acetonitrile as solvent, and molecular oxygen as terminal oxidant), achieves a TON of

≈ 12 .^[31] In a 1 mmol scale reaction involving *o*-Q, LaI_3 , and O_2 as the terminal oxidant, the oxidation of benzyl alcohol to benzaldehyde proceeds with a TON of around 48.^[30] Under further optimized conditions, TONs as high as 250–300 have been reported with this complex system. Interestingly, the authors also tested PQQTME as a quinone catalyst and with $\text{La}(\text{OTf})_3$ and tetrabutylammonium iodide as the redox mediator instead of LaI_3 , only traces of aldehyde were formed.^[30] The first reported Ln -MDH biomimetic by Schelter and coworkers operates in a concentration range comparable to that of this study; however, it employs DBU as a base, which clearly accelerates the reaction, and ferrocenium hexafluorophosphate as the terminal oxidant. Under optimized conditions and reaction times, a TON of ≈ 19 is achieved.^[27] The PQQTME-Ca complex reported by Itoh and co-workers in 1998 is the only Ca-MDH biomimetic tested under catalytic conditions. It oxidizes ethanol in acetonitrile using molecular oxygen as the terminal oxidant and DBU as a base, achieving a TON of ≈ 15 .^[25] A key observation is that catalysts with high TON values typically feature ligands that are extensively modified relative to PQQ, with the only exception being the Ca biomimetic by Itoh.^[25] Luo and coworkers observed that PQQTME does not produce aldehydes under their reaction conditions, consistent with our findings that PQQDME—also structurally similar to native PQQ—exhibits poor catalytic performance. It is possible that, outside the rigid and structured enzyme environment, the PQQ moiety displays overly complex reactivity that may lead to undesired side reactions.

The subsequent investigation focused on identifying the cause of the catalytic reaction's poor efficiency. Thus, our attention was directed toward the ligand regeneration/re-oxidation phase of the catalytic cycle. The previously synthesized **2** was stirred under ambient conditions with and without a metal triflate (La^{3+} , Lu^{3+} , and Ca^{2+}) present and the reaction was followed by UV-Vis spectroscopy. As shown in Figure 7, with La^{3+} , **2** is rapidly oxidized back to **1** by atmospheric oxygen in ≈ 50 min. The final spectra largely overlap with a reference spectrum of authentic **1** with La^{3+} (see Figure S27, Supporting Information); however, the presence of multiple species, such as the water adduct, results in slight variations in the spectra at the end of the reaction. With Lu^{3+} , the re-oxidation takes place at a much slower rate. Without the addition of lanthanide ions or with $\text{Ca}(\text{OTf})_2$, no oxidation of **2** was observed (see Figure S28, Supporting Information for the trace with Ca^{2+}). It thus appears that regeneration of **2** can take place using atmospheric oxygen, however, a Ln^{3+} ion is needed to promote this reaction. Ca^{2+} , which is also a natural cofactor of MDH, is not able to support the oxidation (in the absence of a base). This observation supports the conclusion by Itoh and coworkers that a base is required for the catalytic oxidation of alcohols with a PQQ biomimetic complex, likely due to the base facilitating the reoxidation of the ligand.^[25] The deprotonated form of catechols is more prone to oxidation, which leads to higher autooxidation rates under alkaline conditions, explaining the necessity of a base.^[52] In the presence of metal ions, the pK_a value of the quinol -OH groups coordinating the metal is decreased, so a more facile deprotonation in the presence of these ions also supports faster autooxidation.^[53,54]

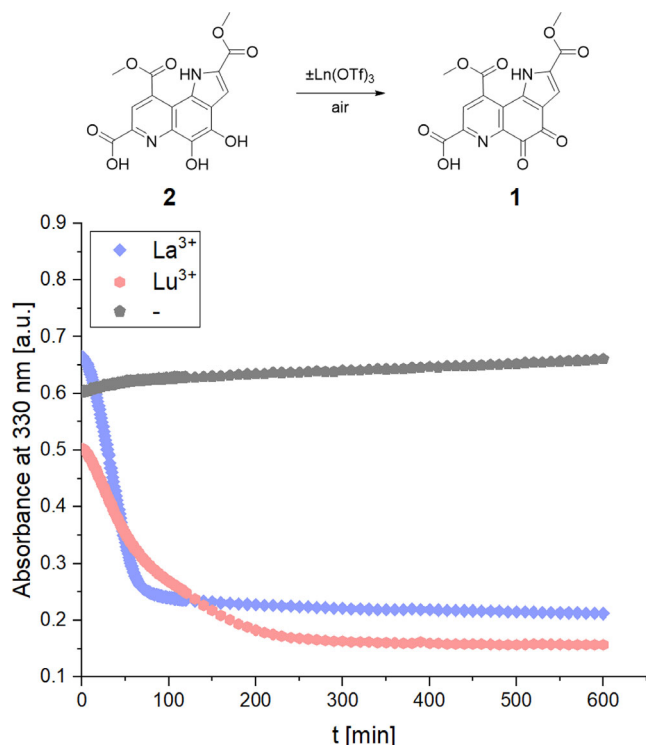


Figure 7. Time course of the oxidation of **2** (25 μM) in the presence or absence of $\text{Ln}(\text{OTf})_3$ (50 μM , $\text{Ln} = \text{La}, \text{Lu}$) under ambient conditions in acetonitrile at 298 K. Reaction followed using UV-Vis spectroscopy (for full spectra see Figure S9, Supporting Information).

This may explain why oxidation of **2** only happens in the presence of La^{3+} and Lu^{3+} .

The superior promotion of alcohol oxidation by early lanthanides compared to later ones has been previously demonstrated in enzymatic systems and early Ln^{3+} have also been shown to support microbial growth more effectively than later ones.^[11,55,56] The earlier observation that **1**- La^{3+} promotes alcohol oxidation more effectively than **1**- Lu^{3+} under aerobic conditions, along with the faster reoxidation of **2** in the presence of La^{3+} compared to Lu^{3+} , suggests that La confers a distinct advantage in this biomimetic system. The nature of the metal appears to influence the reoxidation step, as the stoichiometric alcohol oxidation proceeds similarly with both La^{3+} and Lu^{3+} , whereas the aerobic reaction is more efficient with La^{3+} . The exact mechanistic basis for this trend remains unclear. Although Lu^{3+} , as a stronger Lewis acid, might be expected to perform better, studies suggest that additional factors also contribute to the observed differences. For example, Cotruvo and colleagues have proposed that the redox properties of the protein system are better tuned to accommodate the earlier lanthanides.^[57] Computational studies have also explored the basis for early lanthanide preference, suggesting that variations in binding modes and binding strengths may account for the observed preference.^[23,55,58,59] In biomimetic systems, a similar preference for La^{3+} over Lu^{3+} was observed by Vetsova et al. using the PQQ-1-aza-15-crown-5 ligand. Their findings indicated that both the size and the charge of the metal ion significantly influence the activity of the complexes, with the softer Lewis acid Ba^{2+} performing best.^[28]

With this information in hand, we investigated whether the **2**- La^{3+} complex could be reacted in the presence of air with the substrate 4^{Me}BnOH to yield $4^{\text{Me}}\text{PhCHO}$. This implies that, in the cycle shown in Scheme 2, the reaction initiates with ligand regeneration by air, producing the oxidized **1**- La^{3+} complex, which subsequently performs the alcohol oxidation. A control reaction with only **2** and 4^{Me}BnOH and no $\text{Ln}(\text{OTf})_3$ only yielded 7% $4^{\text{Me}}\text{PhCHO}$, yet again sowing the necessity of a Ln^{3+} ion for the reaction. The results in **Figure 8** demonstrate that the reaction proceeds even when initiated from the reduced **2**- La^{3+} PQQ derivative and $4^{\text{Me}}\text{PhCHO}$ is formed in $91\% \pm 7\%$ yield. With **2**- Lu^{3+} , $45\% \pm 22\%$ $4^{\text{Me}}\text{PhCHO}$ is formed. The initial delay observed in the otherwise similar time course may result from the prerequisite ligand regeneration step. Furthermore, the 24 h yields starting out with the **2**-complexes are generally lower than when starting with the **1**-complexes. It should be noted, that as the reactions were conducted in NMR tubes, the amount of available oxygen for the reaction is limited by the headspace in the tube.

Thus, the investigation into the limited catalytic turnover remains ongoing. It has been shown in previous biomimetic studies that PQQ inspired ligands readily form radicals during alcohol oxidation reactions.^[28,30] Using their previously published L_{QQ} model ligand, Schelter and coworkers had previously synthesized La^{3+} complexes and in later studies, they showed that by reducing the complex by one electron with one equivalent of Cp_2Co , the semiquinonate La^{3+} complex can be isolated.^[60] With a suitable oxidizing agent, this semiquinonate complex can also be employed in catalytic alcohol oxidation reactions. However, whether the semiquinonate form of the complex is part of the alcohol oxidation mechanism was not discussed. With this

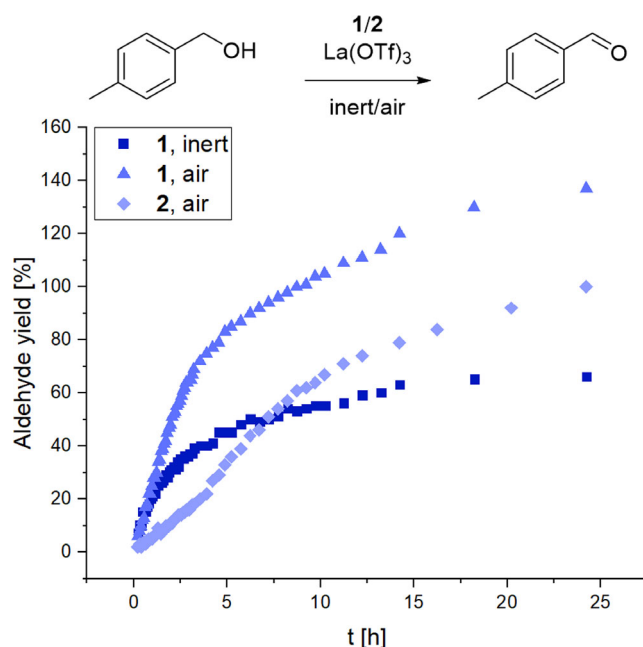


Figure 8. Time courses of the oxidation of 4^{Me}BnOH (162.50 mM) by **1** or **2** (3.25 mM) and $\text{La}(\text{OTf})_3$ (6.50 mM) under inert or ambient conditions (acetonitrile- d_3 , 293 K). Aldehyde yields determined by ^1H -NMR spectroscopy (400 MHz).

information in hand, we set out to investigate whether radicals are involved in the stoichiometric and catalytic oxidation of alcohols by the 1-La³⁺ complex.

Continuous wave (CW) X-band EPR spectroscopy was thus employed to follow the model oxidation reaction (see results in Figure 9). A radical is forming during the inert stoichiometric reaction of 1-La³⁺ with ⁴MeBnOH and its formation matches the time frame in which most of the ⁴MePhCHO is formed (¹H-NMR experiments). In the reaction under ambient conditions, only very little increase in intensity can be observed, followed by an immediate decrease. The radical forming under inert conditions was then investigated more closely. After leaving the 1-La³⁺ complex to react with ⁴MeBnOH for 24 h, the reaction was opened to air and the time course of the intensity of the radical signal was followed (Figure S32, Supporting Information). When exposed to atmospheric oxygen, the radical is quenched and disappears, explaining why under ambient conditions, there is barely any radical over the course of the reaction.

A possible explanation for the radical forming under inert conditions may be the comproportionation of formed 2 with remaining 1. In the literature, it has been proposed that PQQ and PQQH₂ can react to semiquinone PQQ• radicals.^[41] To assess whether this also applies to the 1-La³⁺ complex, equimolar amounts of 1 and 2 were mixed with La(OTf)₃ under inert conditions and analyzed using CW X-Band EPR spectroscopy. The radical that formed during these conditions disappears upon exposure to air (Figure 10, dark green), which would support the theory of comproportionation and subsequent quenching or continuing reaction. However, a radical can also be detected in a mixture of 2 with La(OTf)₃, which is also quenched when

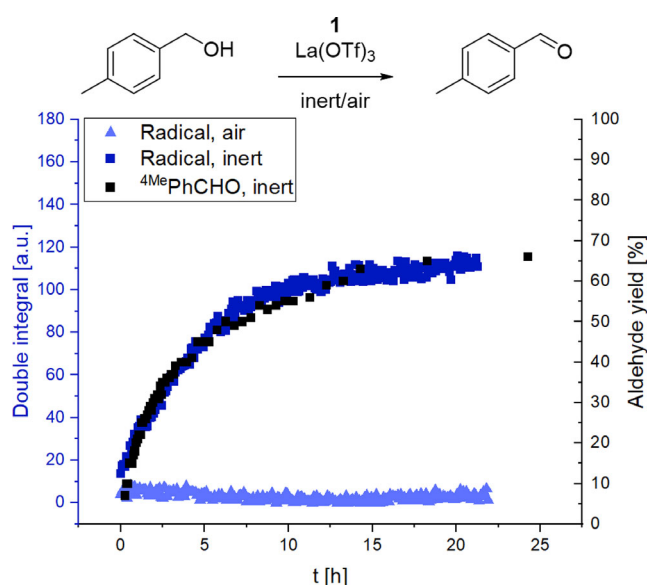


Figure 9. Time courses of the radical intensities of the oxidation of ⁴MeBnOH (162.50 mM) by 1 (3.25 mM) and La(OTf)₃ (6.50 mM) under inert or ambient conditions (acetonitrile, 293 K). Radicals detected using X-band EPR spectroscopy (for full spectra see Figure S29, Supporting Information). For comparison, the time course of ⁴MePhCHO yield (determined by NMR) under inert conditions is shown.

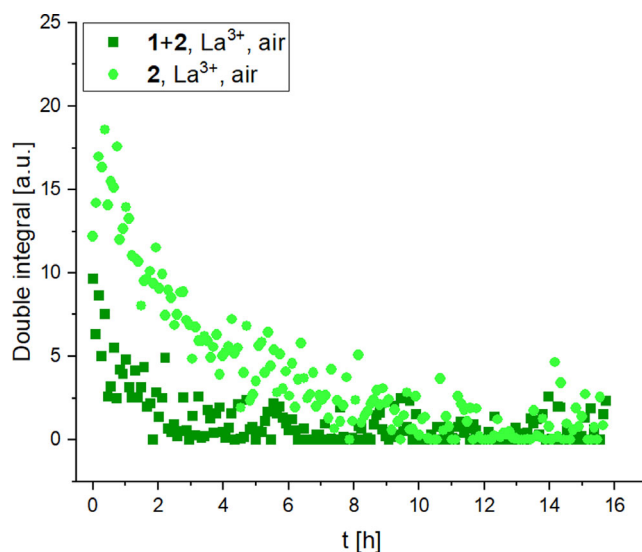


Figure 10. Change in radical intensity (double integral) over time when exposing an equimolar mixture of 1 and 2 (1.63 mM each) and La(OTf)₃ (6.50 mM) in acetonitrile to air versus when exposing 2 (3.25 mM) and La(OTf)₃ (6.50 mM) in acetonitrile to air. Radicals detected using CW X-band EPR spectroscopy.

exposed to air (Figure 10, light green). The observation of a radical even in the absence of 1 would therefore speak against the comproportionation theory, however, without advanced pulsed EPR methods the nature of this radical cannot be unambiguously determined.

Regardless of whether the radical is mechanistically relevant or merely a side product, its absence under ambient conditions indicates that it cannot account for the low turnover observed with the 1-Ln³⁺ complexes. In literature, it had been often theorized that the re-oxidation of PQQH₂ is accompanied by the formation of H₂O₂.^[42,61] Itoh and coworkers indeed showed in their study on PQQH₂ that during autoxidation, H₂O₂ is formed and this can be followed spectrophotometrically by the formation of I₃⁻ out of H₂O₂ and added I⁻ ions.^[41] Furthermore, Murphy and coworkers later showed that PQQ could induce cell death. The authors also traced this back to the formation of H₂O₂, as the effect could be eliminated by the addition of catalase.^[62] Whether H₂O₂ is present in solution after the re-oxidation step of 2 remains to be investigated, as well as whether its presence can hinder further oxidation of alcohol substrate.

The different functionalities of PQQ and derivatives such as PQQDME (1) appear to be leading to a much more complicated reactivity compared to other rationally designed PQQ biomimetic ligands that exist in literature.^[27,30,31] The still unclear involvement of radical pathways, pH influence due to the carboxylic acid moieties, increased number of possible coordination sites and possible formation of H₂O₂ may all be reasons why 1 is not able to perform in catalysis as good as its synthetic counterparts. Even after 60 years of its discovery and continuous investigation, PQQ still refuses to reveal all its intricacies and remains elusive regarding its exact mechanism in enzymatic and biomimetic alcohol oxidation.

3. Conclusion

We present investigations on PQQDME (1) and its interaction with La^{3+} and Lu^{3+} ions as complexes for methanol dehydrogenase biomimetics. We show that 1 forms a 1:1 complex with lanthanides with metal coordination most likely taking place at the biologically relevant binding pocket. We show that these complexes are able to oxidize 4-methylbenzyl alcohol stoichiometrically and that in the presence of atmospheric oxygen, the yield increases, indicating slight catalytic activity. Under ambient conditions, the La^{3+} complexes achieve higher aldehyde yields than the Lu^{3+} complexes, a trend that is also found in the enzyme. In our pursuit of a possible reason for the low catalytic turnover compared to other MDH biomimetics, we synthesized the reduced species PQQDMEH₂ (2), an intermediate in the proposed reaction sequence. We show that 2 can undergo autoxidation in the presence of air and that lanthanide addition is necessary for this process. 4-Methylbenzyl alcohol can also be oxidized when starting out with 2- Ln^{3+} complexes under ambient conditions. By continuing to explore the factors limiting turnover, we find that radical species are formed during the inert stoichiometric reactions of the complexes with alcohol substrate. When coming in contact with air, the radical is quenched. We set out to investigate whether the radical is formed because of the comproportionation of 1 and 2, however, the results were inconclusive. The nature and the origin of the radical are still unclear, as is the question of its involvement as an intermediate in the oxidation reaction mechanism. We hope that our studies shed some light on the intricate chemistry of biomimetic lanthanide complexes with ligand moieties that very closely resemble the natural cofactor, PQQ, and inspire further studies on this fascinatingly complicated molecule.

4. Experimental Section

ESI (electrospray ionization) mass spectra were measured on a LTQ XL Orbitrap mass spectrometer from Thermo Scientific or with a Thermo Finnigan LTQ FT Ultra Fourier Transform Ion Cyclotron Resonance mass spectrometer with acetonitrile or methanol as carrier solvent.

^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance III spectrometer at room temperature operating at 400 MHz for ^1H nuclei and 100 MHz for ^{13}C nuclei. ^1H -chemical shifts are reported in units of ppm relative to CD_3CN ($\delta_{\text{H}} = 1.94$ ppm) or $\text{DMSO}-d_6$ ($\delta_{\text{H}} = 2.50$ ppm). ^{13}C -chemical shifts are given in units of ppm relative to $\text{DMSO}-d_6$ ($\delta_{\text{C}} = 39.52$ ppm). The software used for data processing was MNova Version 14.1.1. Two-dimensional heterobinuclear multiple quantum correlation (HMQC) and heterobinuclear multiple bond connectivity (HMBC) experiments were used to assign resonances. The assignment of the ^1H and ^{13}C NMR shifts of PQQTME and PQQDME (1) was performed according to the reference of their synthesis.^[28]

The paramagnetic ^1H NMR spectra were recorded at room temperature with a Bruker AVII+500 spectrometer operating at 500.13 MHz with a 5 mm triple resonance broad band probe (TBO) probe head. The ^1H chemical shifts in ppm are relative to CD_3CN ($\delta_{\text{H}} = 1.94$). CD_3CN ($D \geq 99.8\%$) was purchased from Sigma Aldrich. For processing the data, Bruker Topspin 4.1.4 was used.

EPR measurements were performed using a BRUKER EMXNano X-band (9.5 GHz) spectrometer at room temperature. The spectra

for the kinetic measurements were recorded with a sweep width of 70.0 G and a sweep time of 10.50 s. The tuning process was automated and the attenuation used was 60.00 dB.

UV-Vis spectroscopy measurements were conducted with an Agilent Cary 60 UV-Vis with Peltier Element with stirred, thermostatted cuvette holder. 10 mm Quartz Suprasil cuvettes from Hellma were used.

Synthesis of PQQDME (1)

In a dry, nitrogen flushed Schlenk tube (100 mL), previously heat gun dried potassium carbonate (5.19 g, 37.6 mmol, 25 equiv.) and PQQ (533 mg, 1.53 mmol, 1.0 equiv.) were added to 20 mL dry DMF. Dimethyl sulfate (15.4 mL, 1.33 g mL⁻¹, 162 mmol, 106 equiv.) was added and the mixture was stirred overnight at room temperature (23 °C) to give an orange suspension. To quench remaining potassium carbonate and dimethyl sulfate, HCl (first 80 mL 2 M were slowly added followed by 10 mL conc. HCl) was added while stirring and the mixture was then stirred for another 5 h. The precipitate was separated by filtration (glass frit), washed with water and dried overnight at 100 °C to give PQQ trimethyl ester (PQQTME) in 84% yield (477 mg, 1.28 mmol). $^1\text{H-NMR}$ (400 MHz, $\text{DMSO}-d_6$): δ /ppm = 12.51 (s, 1H, H-1), 8.56 (s, 1H, H-8), 7.30 (d, $J = 2.2$ Hz, 1H, H-3), 4.05 (s, 3H, H-Me-9'), 3.96 (s, 3H, H-Me-7'), 3.89 (s, 3H, H-Me-2'). $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO}-d_6$): δ (ppm) = 177.0 (C-5), 173.3 (C-4), 166.6 (C-9'), 163.8 (C-7'), 159.9 (C-2'), 140.0 (C-5a), 145.7 (C-7), 134.2 (C-9), 133.6 (C-1a), 128.5 (C-8), 126.6 (C-9a), 126.5 (C-2/3a), 125.0 (C-2/3a), 113.9 (C-3), 54.2 (C-9'-Me), 53.0 (C-7'-Me), 52.4 (C-2'-Me). HRMS (ESI) m/z calcd for $\text{C}_{17}\text{H}_{13}\text{N}_2\text{O}_8^+$: 373.0666 $[M+H]^+$; found: 373.0660. Elemental microanalysis (CHN): % calc. for $[\text{C}_{17}\text{H}_{12}\text{N}_2\text{O}_8]$: C 54.85, H 3.25, N 7.52, found: C 54.66, H 2.97, N 7.49.

To PQQTME (467 mg, 1.26 mmol, 1.0 equiv.) in a 25 mL round bottom flask, 18 mL of a 2:1 trifluoroacetic acid/water mixture were added at once and the red solution was stirred at 60 °C overnight. After cooling to room temperature (23 °C), 50 mL water were added and after 1 h of stirring, the red precipitate was filtered on a glass frit, washed with water and dried at 100 °C overnight to give 1 in 94% yield (422 mg, 1.18 mmol). $^1\text{H-NMR}$ (400 MHz, $\text{DMSO}-d_6$): δ /ppm = 12.52 (s, 1H, H-1), 8.57 (s, 1H, H-8), 7.29 (d, $J = 1.4$ Hz, 1H, H-3), 4.05 (s, 3H, H-Me-9'), 3.89 (s, 3H, H-Me-2'). $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO}-d_6$): δ (ppm) = 177.3 (C-5), 173.4 (C-4), 166.7 (C-9'), 164.8 (C-7'), 159.9 (C-2'), 148.9 (C-5a), 147.0 (C-7), 134.1 (C-9), 133.8 (C-1a), 128.5 (C-8), 126.4 (C-2/3a), 126.3 (C-9a), 124.8 (C-2/3a), 113.8 (C-3), 54.2 (C-9'-Me), 52.3 (C-2'-Me). Assignments of 5, 4, 5a and 7 based on literature-known PQQ shifts. HRMS (ESI) m/z calcd for $\text{C}_{16}\text{H}_{11}\text{N}_2\text{O}_8^+$: 359.0510 $[M+H]^+$; found: 359.0508. Elemental microanalysis (CHN): % calc. for $[\text{C}_{16}\text{H}_{10}\text{N}_2\text{O}_8 \cdot 0.2\text{H}_2\text{O}]$: C 53.11, H 2.90, N 7.74, found: C 53.03, H 2.80, N 7.67.

Synthesis of PQQDMEH₂ (2)

A 250 mL round bottom flask was charged with L-(+)-Ascorbic acid (1770 mg, 10.10 mmol, 18.0 equiv.) and then 15 mL H_2O were added. The pH was set to 3 using aqueous HCl (2 M). PQQDME (1) (200 mg, 0.56 mmol, 1.0 equiv.) was suspended in 80 mL H_2O and added dropwise over 1 h at 10 °C to the solution. The reaction was allowed to warm to room temperature (23 °C) and stirred overnight. The precipitate was centrifuged (4500 rpm, 5 min), washed two times with HCl (1 M, 20 mL each) and dried using lyophilization. The product 2 was obtained as a brown solid in 92% yield (205 mg, 0.52 mmol). $^1\text{H-NMR}$ (400 MHz, $\text{DMSO}-d_6$): δ /ppm = 12.11 (s, 1H, H-1), 10.06 (s, 1H, -OH), 9.17 (s, 1H, -OH), 8.53 (s, 1H, H-8), 7.41 (d, $J = 2.4$ Hz, 1H, H-3), 4.10 (s, 3H, H-Me-9'), 3.93 (s, 3H, H-Me-2'). $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO}-d_6$): δ

(ppm) = 168.0 (C-9'), 164.8, 160.8 (C-2'), 142.3, 140.6, 137.6, 132.6, 131.1, 126.5, 123.3, 122.8, 118.8 (C-8), 110.4 (C-7/C-9), 105.8 (C-3), 54.0 (C-Me-9'), 52.2 (C-Me-2'). **Elemental microanalysis (CHN):** % calc. for $[C_{16}H_{12}N_2O_8 \cdot 2H_2O]$: C 48.49, H 4.07, N 7.07, found: C 47.70, H 3.42, N 6.90.

Preparation of Samples for ESI-MS Analysis

Solutions of **1** (3.25 mM) and $Ln(OTf)_3 \cdot xH_2O$ (6.50 mM) were prepared in methanol. The solutions were then diluted to 0.33 mM **1** and submitted to ESI-MS analysis.

UV-Vis Titration of Metal Salts to PQQDME

100 μ l of a 1.50 mM PQQDME solution in acetonitrile were added to 2850 μ l acetonitrile and the first spectrum was recorded. Stock solutions (15 mM) of $Ln(NO_3)_3 \cdot 6H_2O$ ($Ln = La, Lu$) in acetonitrile were added were added step by step to the ligand solutions and spectra were recorded after each addition.

Paramagnetic 1H NMR Spectra of the $1-Eu^{3+}$ Complex

1 (2.32 mg, 6.5 μ mol, 1.0 equiv.) was dissolved in CD_3CN (2.0 mL) and was added to solid $Eu(OTf)_3 \cdot 4H_2O$ (8.1 mg, 12.1 μ mol, 1.9 equiv.). 1H -NMR (500 MHz, CD_3CN , 299 K): δ (ppm) = 12.50 (s, 1H), 8.25 (s, 1H), 4.06 (s, 3H), 3.66 (s, 3H). $^{13}C\{^1H\}$ -NMR (151 MHz, CD_3CN , 299 K): δ (ppm) = 163.3, 160.5, 130.7, 129.5, 114.7, 104.2, 102.2, 100.0, 97.9, 55.2, 55.4.

H-NMR Alcohol Oxidation Reactions

In an NMR tube, to 0.5 mL solution of PQQDME/PQQDMEH₂ (3.25 mM) and $Ln(OTf)_3 \cdot xH_2O$ (6.50 mM) in acetonitrile- d_3 , 4-methylbenzyl alcohol (162.50 mM) was added. For the control experiments, the mentioned component was excluded ($1/2/Ln(OTf)_3$). For quantification of the oxidation product, 4-methylbenzaldehyde, hexamethyldisiloxane (3.25 mM) was added. For inert atmosphere reactions, the procedure was carried out inside an oxygen-free nitrogen glovebox. Immediately after the addition of 4-methylbenzyl alcohol, the NMR tube was submitted to 1H -NMR analysis. Spectra were recorded every 3–4 min for the first 3 h, then in larger time intervals until 24 h reaction time. For the analysis, the baseline in the spectra was corrected with an ablative fit and the intensity of the signals was normalized on the largest peak, belonging to the standard TMS_2O . The aldehyde yield was determined by comparison of the aldehyde hydrogen integral (9.96–9.92 ppm) to the integral of the added hexamethyldisiloxane (0.13–0.00 ppm). The resulting yields referenced to the PQQDME/PQQDMEH₂ quantity were plotted against reaction time.

For the 1H NMR spectra, the following parameters were used: spectrometer frequency 400.18 MHz, number of scans 16, pulse angle 30°, acquisition time 6.55 s, relaxation delay 4.00 s, spectral width 10 000 Hz.

CW X-band EPR Alcohol Oxidation Reactions

To 0.5 mL solution of PQQDME/PQQDMEH₂ (3.25 mM) and $Ln(OTf)_3 \cdot xH_2O$ (6.50 mM) in acetonitrile- d_3 , 4-methylbenzyl alcohol (162.50 mM) was added. A small portion of the reaction solution was added *via* syringe to a glass capillary, which was then sealed with

wax. For inert atmosphere reactions, the procedure was carried out inside an oxygen-free nitrogen glovebox. Immediately after, the capillary was analyzed using CW X-Band EPR spectroscopy. Spectra were recorded ca. every 5 min with 5 scans per measurement. The signals in the resulting spectra were integrated twice and plotted against reaction time.

UV-Vis PQQDMEH₂ Oxidation under Ambient Conditions

In an oxygen-free nitrogen glovebox, a solution of **2** (25 mM) and $M(OTf)_x$ (50 μ M, $M = La/Lu$ with $x = 3$ and Ca with $x = 2$) was prepared and directly upon opening to air, UV-Vis spectra were recorded every minute for 2 h and then every 10 min until 10 h reaction time.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data (EPR, NMR, ESI-MS, UV-vis) that support the findings of this study are openly available at Radar4chem under the DOI <https://doi.org/10.22000/yt84fag5h2kgzadz> with the title "Data for Publication: The Elusive Chemistry of Pyrroloquinoline Quinone Dimethyl Ester Lanthanide Complexes in Biomimetic Alcohol Oxidation" and additional Figures are included in the supporting information.

Keywords: alcohol oxidation · bioinorganic chemistry · biomimetic complex · cofactors · lanthanides

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