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Monooxygenation of an appended phenol in a model system of tyrosinase: implications on the enzymatic reaction mechanism†

Jessica Nadine Hamann, Malte Rolff and Felix Tuczek*

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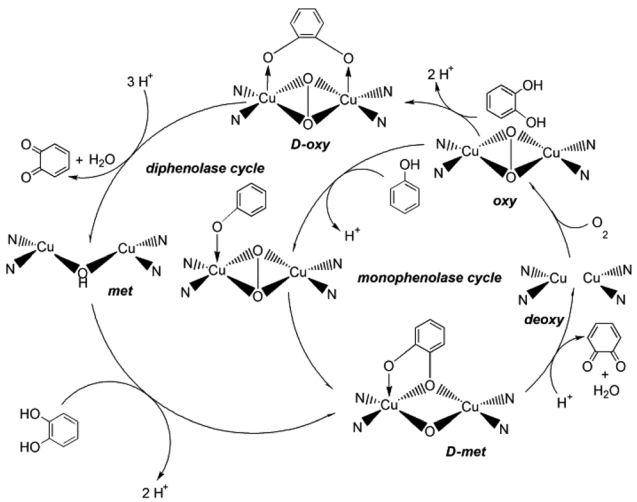
A new tridentate N-donor ligand and its corresponding copper(I) complex have been synthesized to investigate the tyrosinase-like aromatic hydroxylation of an attached phenol. The results of the oxygenation reactions are compared to related systems having attached phenyl and catechol groups, respectively. The title complex is the first system mediating the monooxygenation of a phenol in the absence of an external base.

Introduction

The ubiquitous enzyme tyrosinase (Ty) catalyses the conversion of monophenols to the corresponding *ortho*-quinones in a hydroxylation reaction followed by a two-electron oxidation.[1,2] This way tyrosine is converted to dopaquinone which is a precursor of melanin, an important pigment in living organisms.[3,4] Based on spectroscopic data and various crystal structures determined in the last few years, the active site of tyrosinase contains a binuclear type 3 copper centre, each copper ion being surrounded by three histidines.[5–9] The two other proteins in this class of metalloproteins are hemocyanin (Hc), which mediates oxygen transport in arthropods and molluscs, and catechol oxidase (CO), which is responsible for the oxidation of catechols to the corresponding *ortho*-quinones.[9] All of these proteins bind dioxygen as peroxide in a typical side-on bridging geometry (μ–η^2:η^2), whereby both copper ions are oxidized from Cu^I to Cu^{II}.[1,2] Through an aromatic hydroxylation mediated by the copper-peroxo core monophenolic substrates are converted to catechols; then the substrate is released as *ortho*-quinone, and the *deoxy*-state is reformed (monophenolase reactivity). The oxidation of catechols to *ortho*-quinones corresponds to the diphenolase activity of tyrosinase, which is also exhibited by catechol oxidase.[1,2,9–11] Both, monophenolase and diphenolase activity, are shown in Scheme 1.[3]

In the last years, many copper complexes have been synthesized and investigated as small-molecule models of tyrosinase. These systems either hydroxylate external monophenolic substrates[12–17] or are able to hydroxylate an aromatic part of the ligand framework.[18–23] One of the first model systems of the latter type has been published by Karlin and coworkers in 1984.[23] Their Cu_2**XYL** system is based on a binuclear copper(I) complex with a *m*-xylene bridged ligand providing six nitrogen donor atoms. Upon electrophilic attack of the side-on peroxo dicopper unit on the arene, the *m*-xylene bridged ligand is hydroxylated in *ortho*-position, leading to a μ-phenoxo-μ-hydroxo dicopper core (Scheme 2). After this discovery, a large number of other tyrosinase models were synthesized which exhibit this reactivity.

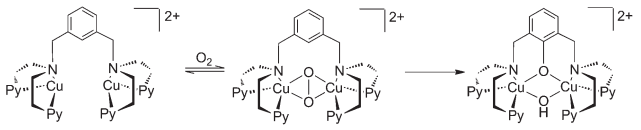
In the last years model systems for copper enzymes were established that contain *phenol* moieties in the ligand frame-



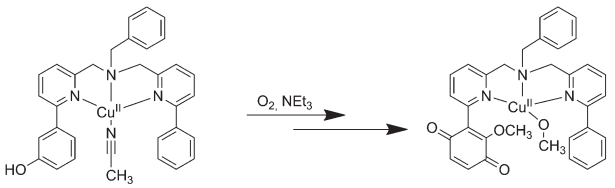
Scheme 1 Mono- and diphenolase cycle of tyrosinase (after Sánchez-Ferrer *et al.*).[3]

Christian-Albrechts-University Kiel, Max-Eyth-Straße 2, 24118 Kiel, Germany. E-mail: ftuczek@ac.uni-kiel.de; Fax: +49 (0)431 1520; Tel: +49 (0)431 1410

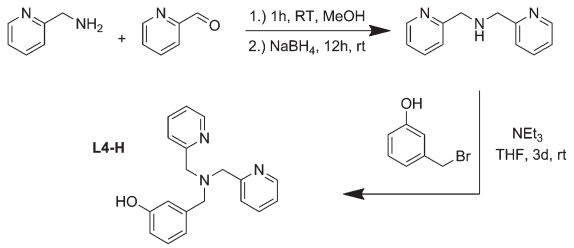
† Electronic supplementary information (ESI) available: Details of ligand syntheses with NMR spectra; fluorescence and UV/Vis spectra. See DOI: 10.1039/c4dt03010a



Scheme 2 Hydroxylation mechanism of Cu_2XYL by Karlin *et al.*[23]



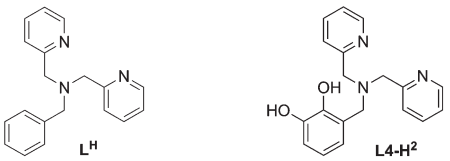
Scheme 3 The $Cu^{II}L^{m}H$ system by Itoh *et al.* that performs a hydroxylation of the appended phenol through a radical mechnanism.[25]



Scheme 4 Synthesis of the new ligand **L4-H**.

work to mimic tyrosine residues in the respective natural system.[24,25] The $\mathbf{Cu^{II}L^{m}H}$ complex was prepared by Itoh *et al.* as a model for copper amine oxidases.[25] This system is able to perform a *para*-hydroxylation of an appended phenol residue through a radical mechanism in the presence of triethylamine. A subsequent multistep reaction leads to the Topaquinone cofactor-like structure shown in Scheme 3, right.[25]

Nevertheless, no model system of tyrosinase has shown the formation of an *ortho*-quinone after *ortho*-hydroxylation of an appended phenol to date. However, only such a system would exactly mimic the conversion of tyrosine to dopaquinone in the enzyme. In this context it should be mentioned that tyrosinase-like conversion of *external* phenols to quinones, even catalytic, has been achieved with many copper-containing complexes,[12–17] but in all of these systems Brønsted bases were employed to first deprotonate the phenolic substrate.[1] In the absence of base, non-physiological C–C coupling products were always obtained exclusively.[1,26] Monophenolase activity of tyrosinase, on the other hand, does not require an external base. We therefore wanted to know whether a phenol residue, if properly positioned in vicinity of the active site, would be hydroxylated by an artificial copper–O_2 system *also in the absence of base*. To this end we synthesized the new tridentate N-donor ligand *N*-(3-hydroxyphenyl)methyl-bis-(2-picolyl)-amine (**L4-H**) and its corresponding copper(I) complex [Cu(I)-**L4-H**]PF_6 (Scheme 4). Herein the reactivity of [Cu(I)**L4-H**]PF_6 upon oxygenation is investigated. The results are compared with the $\mathbf{L^H}$ complex having the same ligand but *without* hydroxy group[27] and the $\mathbf{L4\text{-}H^2}$ complex having *two* hydroxy groups in the appended phenyl ring (Scheme 5). To interpret and support the experimental results DFT calculations are employed. The implications of the results on the reaction mechanism of tyrosinase are discussed.



Scheme 5 Further synthesized ligands $\mathbf{L^H}$ (left)[27] and $\mathbf{L4\text{-}H^2}$ (right) to investigate the reactivity of their corresponding copper(I) complexes with dioxygen at low and ambient temperature.

Results and discussion

The synthesis of **L4-H** proceeds in several steps. Ultimately, it was found most convenient to couple *m*-(bromomethyl)-phenol, which was prepared following Przybilla *et al.*,[28] with the literature-known secondary amine bis(2-pyridylmethyl)-amine.[29] After a reaction time of three days at ambient temperature **L4-H** was formed in good yield and converted to the mononuclear copper(I) complex [Cu(I)**L4-H**]PF_6. Oxygenation of [Cu(I)**L4-H**]PF_6 was performed in acetone at low and at ambient temperature and monitored by UV/Vis spectroscopy (Fig. 1).

ortho-Quinones show characteristic absorption bands between 400 and 450 nm, having ε values of ~1800 m^{-1} cm^{-1}.[30] In fact, upon oxygenation of [Cu(I)**L4-H**]PF_6 at −78 °C an intense band at 435 nm was observed, indicating hydroxylation and two-electron oxidation of the phenol residue in the ligand framework (Fig. 1). Based on the given ε value, the yield

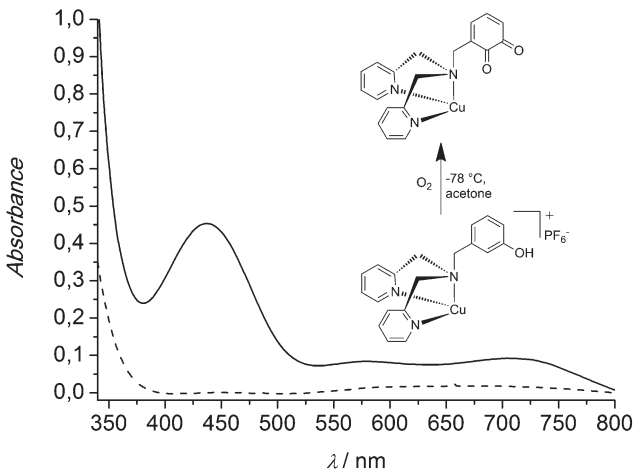
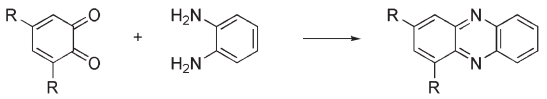


Fig. 1 UV/Vis spectra of a 1 mM solution of the complex [Cu(I)**L4-H**]PF_6 in acetone before (dashed) and after reaction with O_2 for 12 h at −78 °C, followed by warming up to rt (solid line); l = 1 cm.



Scheme 6 Synthesis of phenazines from *ortho*-quinones according to Zhu *et al.*[31]

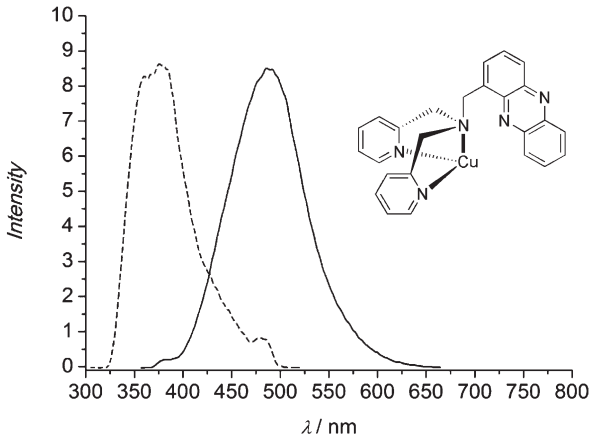


Fig. 2 Measured fluorescence spectra of a 25 μM solution of the generated phenazine derivative of Cu**L4**quinone in acetone; emission spectrum (solid line) and excitation spectrum (dashed), emission peak at 503 nm.

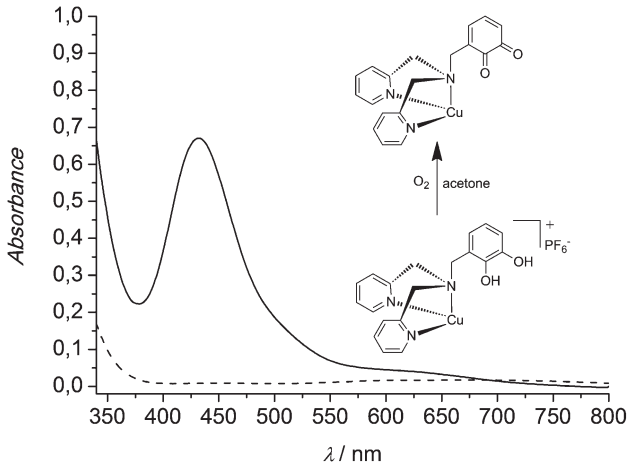


Fig. 3 UV/Vis spectrum of a 1 mM solution of [Cu(I)**L4-H²**]PF_6 in acetone before (dashed) and after (solid line) reaction with O_2 for 12 h at ambient temperature; l = 1 cm.

of the hydroxylation reaction is estimated to 25% per mononuclear copper precursor; *i.e.*, 50% per dicopper unit (see above). Oxygenation of the same Cu(I) complex in acetone at ambient temperature did not lead to a quinone band; only a broad ligand-field absorption at 680 nm was observed, indicating the formation of a Cu(II) complex (*cf.* ESI†). To gain insight into the reaction rate the formation of quinone was monitored during the first hour of oxygenation (Fig. S19†). During the first 15 min, most of quinone was formed, and after 60 min saturation was achieved.

In order to determine the identity of the quinone product, a derivatisation reaction was performed. To this end, fluorescence detection of phenazine derivatives was employed. Phenazines are stable compounds that can be synthesized by reaction of *ortho*-quinones with *ortho*-phenylenediamine (Scheme 6).[31]

In analogy, we prepared a phenazine derivative of the oxygenation product Cu**L4**quinone and measured its fluorescence spectrum. For comparison the phenazine derivative of 3,5-di-*tert*-butyl-*ortho*-quinone was synthesized independently (*cf.* ESI†). According to the literature, phenazines show characteristic emission bands around 500 nm.[32] Moreover, they exhibit intense optical absorption bands in the range of 350 to 450 nm.[33] Correspondingly, for the phenazine derivative of Cu**L4**quinone we observed an intense emission band in the range at 500 nm (Fig. 2); for the phenazine derivative of the 3,5-di-*tert*-butyl-*ortho*-quinone a similar fluorescence spectrum was obtained (*cf.* ESI†). Absorption measurements of these compounds also showed similar spectra, exhibiting an absorption band at 380 nm (*cf.* ESI†). We thus conclude that the phenazine derivative Cu**L4**quinone has been formed (Fig. 2).

Further chemical evidence that the spectroscopic features of the oxygenated [Cu(I)**L4-H**]PF_6 system are due to a hydroxylation of the appended phenol is provided by the investigation of a copper complex which is closely related to the **L4-H** system; *i.e.*, a complex supported by a ligand with *two* hydroxy groups (**L4-H²**).

The **L4-H²** system was oxygenated under the same conditions as the [Cu(I)**L4-H**]PF_6 complex, monitoring the reaction course by UV/Vis spectroscopy. Again, the characteristic absorption band of an *ortho*-quinone with a maximum at ~430 nm (Fig. 3) was observed after oxygenation at low temperature. In contrast to the **L4-H** complex, this band also appeared when the reaction was performed at room temperature.

As the measured UV/Vis spectrum (*cf.* Fig. 3) is similar to that of oxygenated [Cu(I)**L4-H**]PF_6 (*cf.* Fig. 1) we conclude that the same product is formed upon reaction with molecular oxygen. However, the yield is significantly higher (39% per mononuclear copper precursor; *i.e.*, 78% per dicopper unit based on the ε value given above).

While the presented results clearly indicate the formation of an *ortho*-quinone (as opposed to a *para*-quinone) after oxygenation of the **L4-H** system, the regioselectivity could in principle also be different; *i.e.*, hydroxylation could also occur in 4-position, leading to an *ortho*-quinone with oxygen atoms at 3- and 4-position. To actually prove that hydroxylation occurs at 2-position; *i.e.*, that an *ortho*-quinone with oxygen atoms at 2- and 3-position is formed, the oxygenation product of the [Cu(I)**L4-H**]PF_6 system was reduced with NH_2OH and the resulting product was investigated by NMR spectroscopy (*cf.* Fig. S10–S12†). Evidently the hydroxylamine reduction both reduces the Cu(II) to Cu(I) and the quinone to catechol. Importantly, the NMR spectra of the reduction product of Cu**L4**quinone (i) show that the signal of the hydrogen atom in

2-position of the phenol present in the **L4-H** starting complex is missing and (ii) are identical to the spectra of the complex [Cu(I)**L4-H**^{2}]PF_6 which exhibit the same splitting pattern (Fig. S13–S15†). Based on these results we conclude that during the oxygenation of the copper(I) complex [Cu(I)**L4-H**]PF_6 at −78 °C the hydroxylation of the appended phenol occurs at C_2; *i.e.*, in the *ortho*-position.

To prove that the O-atom incorporated into the appended phenol derives from molecular oxygen, oxygenation experiments were also performed with $^{18}O_2$. DFT calculations show that upon oxygenation of [Cu(I)**L4-H**]PF_6 a new, prominent vibration appears between 1600 and 1700 cm^{-1} which originates from the formed quinone. While the ^{16}O–^{16}O quinone just exhibits one single, intense band (corresponding to the antisymmetric combination of CO stretches), the calculation predicts a splitting of this band into two bands for a ^{16}O–^{18}O quinone moiety. Based on these findings the reaction product was investigated by infrared spectroscopy. In fact, oxygenation with $^{16}O_2$ leads just to one vibrational band for the carbonyl groups at 1699 cm^{-1} (*cf.* Fig. S17†) whereas for the ^{16}O–^{18}O mixed-isotope product the carbonyl band was found to split in two bands at 1697 cm^{-1} and 1663 cm^{-1} (*cf.* S17†). Accordingly, the source of the oxygen atom that is incorporated into the phenolic residue during the oxygenation of [Cu(I)**L4-H**]PF_6 is molecular oxygen.

In order to obtain further insight into the reactivity of the **L4-H** system we also investigated the copper complex [Cu(I)-$\mathbf{L^H}$(CH_3CN)]PF_6 that contains a benzyl group instead of the phenolic residue of **L4-H**. The $\mathbf{L^H}$ system has been studied before with respect to the structure of the copper-dioxygen adduct.[27,34,35] Low-temperature oxygenation of [Cu(I)-$\mathbf{L^H}$(CH_3CN)]PF_6 afforded a bright green solution, indicating the formation of a bis(μ-oxo) complex.[34] Even after prolonged reaction times no change of the spectrum was observed. Oxygenation at room temperature neither lead to a quinone product. To form an *ortho*-quinone through an aromatic hydroxylation of the ligand framework, the presence of a hydroxy group is therefore necessary in these systems.

To rationalize the experimental findings the following reactive scheme is proposed (Scheme 7).

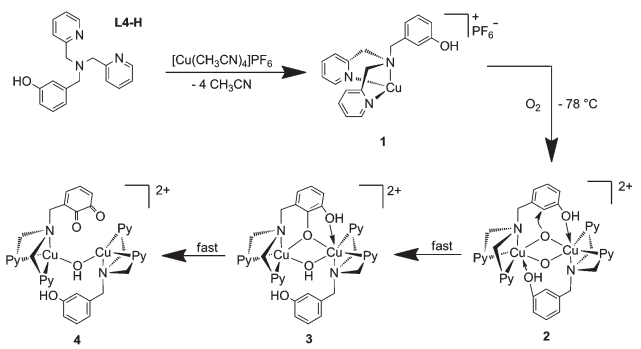
Upon oxygenation of the mononuclear copper(I) complex [Cu(I)**L4-H**]PF_6 (**1**) at low temperature dioxygen adduct **2** is formed. In the next step an *ortho*-hydroxylation of the appended phenol residue occurs, generating the μ-phenoxo-μ-hydroxo intermediate **3**. The reaction sequence is completed by the subsequent two-electron oxidation of **3**, leading to the *ortho*-quinone **4**, which is observable *via* UV/Vis spectroscopy (*cf.* Fig. 1).

The analogous complex [Cu(I)$\mathbf{L^H}$(CH_3CN)]PF_6 supported by the tridentate ligand $\mathbf{L^H}$ without a phenolic OH group forms a stable bis(μ-oxo) copper(III) adduct under the analogous experimental conditions.[34] We therefore anticipate that **2** having a similar ligand framework forms a bis(μ-oxo) core as well.[36] The fact that only at low temperature a hydroxylation of the appended phenol occurs whereas at ambient temperature formation of a Cu(II) complex is observed can be attributed to thermal instability of the dioxygen adduct and further decomposition of this intermediate in the presence of residual Cu(I) complex and traces of water.[22,37]

In order to obtain an impression of the geometry of intermediate **2**, DFT calculations were performed.[38]

The optimized structure shows weak interactions between the phenol residues and the copper centers, bringing the aromatic rings into a favorable position for the subsequent reaction with the bis(μ-oxo) moiety (Fig. 4). Just as a μ-η^2:η^2 peroxo dicopper unit, the Cu_2 bis(μ-oxo) core is capable to hydroxylate an arene in *ortho*-position.[1,39]

Due to a preorientation of the aromatic rings and the activating effect of the hydroxy group in *ortho*-position, the subsequent hydroxylation is rapid, even at −90 °C. The observation that the conversion to the *ortho*-quinone does not proceed with 100% yield can be attributed to an incomplete formation of the bis(μ-oxo) complex and/or a destabilisation of this intermediate by the presence of phenol groups. The [Cu(I)-$\mathbf{L^H}$(CH_3CN)]PF_6 complex, in contrast, forms a stable O_2 intermediate at low temperature, but does not mediate a hydroxylation reaction due to less favorable steric and electronic conditions.



Scheme 7 Proposed mechanism for the reaction of [Cu(I)**L4-H**]PF_6 with molecular oxygen at low temperature.

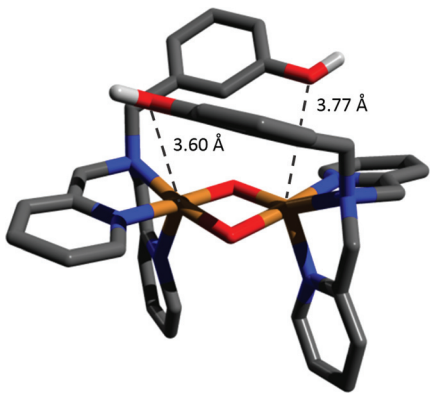


Fig. 4 Geometry-optimized structure of the intermediate **2**.

Summary and conclusions

The new N-donor ligand **L4-H** and its corresponding copper(I) complex have been synthesized to mimic a tyrosinase-like aromatic hydroxylation reaction. The model system was investigated under various conditions. Oxygenation of [Cu(I)**L4-H**]PF_6 in acetone at low temperature was found to convert the attached phenol to *ortho*-quinone. In order prove the identity of this product a derivatisation reaction was performed. Furthermore, two reference systems, $\mathbf{L^H}$ and $\mathbf{L4\text{-}H^2}$, were synthesized and investigated with regard to their reactivities in oxygenation reactions. The catechol analogue $\mathbf{L4\text{-}H^2}$ was found to generate *ortho*-quinone as well whereas oxygenation of the $\mathbf{L^H}$-system stops at the level of the dioxygen adduct.

In conclusion, the [Cu(I)**L4-H**]PF_6 complex shows for the first time that free (*i.e.*, undissociated) phenols can be converted to *ortho*-quinones if they are properly positioned in close vicinity to the copper dioxygen unit. Deprotonation by an external base (as applied in all synthetic model systems of tyrosinase to date) is therefore *not* a general precondition for the Cu-mediated conversion of phenols to *ortho*-quinones, in agreement with the reactivity of the enzyme.

Experimental section

General procedures

All starting materials were ordered by Sigma-Aldrich Co. LLC in reagent grade. Solvents used (acetone, acetonitrile, chloroform dichloromethane, diethyl ether, methanol, tetrahydrofuran) have had reagent grade and have been purified by refluxing over drying agents and distilled under nitrogen atmosphere. Oxygen sensitive syntheses were performed by using *Schlenk techniques* and stored under nitrogen atmosphere after preparation. NMR spectra were recorded at 300 K on a Bruker Avance 400 Pulse Fourier Transform spectrometer operating at a ^{1}H frequency of 400.13 MHz and a ^{13}C frequency of 100.62 MHz; TMS was used as substitutive standard. Elemental analyses were performed using a Euro Vector CHNS-O-element analyser (Euro EA 3000): In a stream of oxygen, prepared assays were burned in tin vessels. Optical absorption spectra were recorded in solution on an Agilent Cary 5000 spectrometer by using a quartz cell with length l = 1 cm. Fluorescence spectra were recorded in solution with a Perkin Elmer LS 55 Luminescence Spectrometer *precisely* (excitation wavelength 385 nm). Mass spectra (MALDI-TOF-MS) were recorded using a Bruker *Biflex III* spectrometer.

DFT-calculations

Geometric optimizations of structures were carried out using the LanL2DZ basis-set with the B3LYP functional. The calculations were performed with Gaussian09 on a NEC SX-9 array processor.[38]

Syntheses

***m*-(Bromomethyl)phenol.**[28] Into a suspension of 2.22 g (17.8 mmol) *m*-(hydroxybenzyl)alcohol in 10 mL chloroform was dropped a solution of 2.42 g (8.90 mmol) phosphorus tribromide in 10 mL chloroform over a period of 25 min at 0 °C. After complete addition, the reaction mixture was warmed up to ambient temperature and stirred for 2 h. The resulting yellow solution was poured over 25 g ice and the organic phase was separated. The aqueous was extracted with chloroform (2 × 25 mL) and the combined organic phases were dried over magnesium sulfate. The solution was dried under reduced pressure to yield 1.88 g (10.1 mmol, 56%) of yellow oil that was used without further purification. Anal. calc. for C_7H_7OBr: (m = 187.1 g mol^{-1}); C, 45.0; H, 3.8, found: C, 46.1; H, 4.3. ^{1}H-NMR (400 MHz, acetone-d_6/TMS): δ = 7.14 (t, 1H, H^3), 6.91–6.87 (m, 2H, H^2 + H^6), 6.77–6.74 (m, 1H, H^4), 4.52 (s, 2H, –CH_2Br) ppm. ^{13}C-NMR (100.6 MHz, acetone-d_6/TMS): δ = 157.4 (C^1), 139.6 (C^5), 129.7 (C^3), 120.2 (C^4), 115.9 (C^2), 115.3 (C^6), 33.5 (–CH_2Br) ppm.

Bis(2-pyridylmethyl)amine.[29] A modification of the literature procedure was used. To a solution of 3.00 g (27.7 mmol) 2-(aminomethyl)pyridine in 10 mL methanol were slowly added 3.00 g (28.0 mmol) pyridine-2-carboxaldehyde in 10 mL methanol at 0 °C. The dark yellow solution was stirred for 1 h at ambient temperature followed by reduction with 0.39 g (27.7 mmol) $NaBH_4$ at 0 °C. After the addition was completed, the reaction mixture was stirred for 12 h at ambient temperature and the colour of solution changed into light yellow. The solution was poured over ice and conc. HCl was added to adjust the pH to 4. The mixture was dried *in vacuo* and the yellow residue was dissolved in 15 mL H_2O. The clear yellow solution was washed with dichloromethane (6 × 20 mL) until the organic phase became colourless. To the aqueous layer was added saturated Na_2CO_3 to adjust the pH to 10. The solution was extracted with dichloromethane (3 × 25 mL) and dried over magnesium sulphate. The solvent was removed under vacuum to obtain 3.42 g (17.2 mmol, 62%) of a yellow oil. The product was used without further purification. Anal. calc. for $C_{12}H_{13}N_3$ (199.3 g mol^{-1}): C, 72.3; N, 21.1; H, 6.6, found: C, 72.5; H, 6.5; N, 21.0. ^{1}H-NMR (400 MHz, $CDCl_3$/TMS): δ = 8.56 (d, 2H, py H^6), 7.64 (dt, 2H, py H^4), 7.35 (dd, 2H, py H^3), 7.15 (dt, 2H, py H^5), 3.99 (s, 4H, –CH_2–py), 3.07 (bs, 1H, –NH) ppm. ^{13}C-NMR (100.6 MHz, $CDCl_3$/TMS): δ = 159.4 (py C^2), 149.3 (py C^6), 136.4 (py C^4), 122.3 (py C^3), 122.0 (py C^5), 54.7 (–CH_2–py) ppm.

***N*-(3-Hydroxyphenyl)methyl-bis-(2-picolyl)amine (L4-H).** A modification of the literature procedure for tertiary amines was used.[40] A solution of 1.97 g (9.84 mmol) bis(2-pyridylmethyl)amine and 0.81 g (9.84 mmol) triethylamine in 20 mL tetrahydrofuran was added dropwise to a solution of 1.85 g (9.84 mmol) *m*-(bromomethyl)phenol in 20 mL tetrahydrofuran. The dark yellow solution was stirred for 3 d at ambient temperature. After reaction was completed, the resulting precipitate was removed by filtration. The filtrate was dried under reduced pressure and the resulting yellow oil was purified by

column chromatography on silica gel with dichloromethane–methanol (8 : 1) as eluent to obtain 1.31 g (4.29 mmol, 44%) of pale yellow oil that crystallizes at 0 °C. Anal. calc. for $C_{19}H_{19}N_3O$ (m = 305.4 g mol^{-1}): C, 74.8; H, 6.3; N, 13.8, found: C, 74.7; H, 6.3; N, 13.5. ^{1}H-NMR (400 MHz, $CDCl_3$/TMS): δ = 8.45 (d, 2H, py H^6), 7.63–7.55 (m, 4H, py H^3 + py H^4), 7.13–7.08 (m, 3H, py H^5 + phenol H^5), 6.90–6.86 (m, 2H, phenol H^2 + phenol H^6), 6.70 (d, 1H, phenol H^4), 3.76 (s, 4H, –CH_2–py), 3.53 (s, 2H, –CH_2–phenol) ppm. ^{13}C-NMR (100.6 MHz, $CDCl_3$/TMS): δ = 159.4 (py C^2), 157.3 (phenol C^3), 148.5 (py C^6), 140.1 (phenol C^1), 137.0 (py C^4), 129.5 (phenol C^5), 123.2 (py C^3), 122.3 (py C^5), 120.0 (phenol C^6), 116.0 (phenol C^2), 114.7 (phenol C^4), 59.6 (–CH_2–py), 58.6 (–CH_2–phenol) ppm.

3-((Bis(pyridin-2-ylmethyl)amino)methyl)benzene-1,2-diol (L4-H^2). A modification of a literature procedure was used.[41] A solution of 0.51 g (3.59 mmol) 2,3-dihydroxy-benzaldehyde in 30 mL dried dichloromethane was treated with 0.71 g (3.59 mmol) bis(2-pyridylmethyl)amine and a small amount acetic acid. The solution was stirred at ambient temperature under nitrogen atmosphere and 0.76 g (3.59 mmol) sodium triacetoxyborohydride was added in small portions. After stirring for 4 d at ambient temperature the dark yellow mixture was acidified with 6 M HCl and then evaporated to dryness. The residue was dissolved in saturated Na_2CO_3 and extracted with $CHCl_3$ (3 × 30 mL). The combined organic phases were dried over magnesium sulphate. The solution was dried under reduced pressure to yield dark yellow oil that was purified by column chromatography on silica gel with dichloromethane–methanol (8 : 1) as eluent to obtain 0.71 g (2.21 mmol, 62%) of a pale yellow solid. Anal. calc. for $C_{19}H_{19}N_3O_2$ (m = 321.4 g mol^{-1}): C, 71.0; H, 6.0; N, 13.1, found: C, 71.0; H, 6.0; N, 13.0. ^{1}H-NMR (400 MHz, acetone-d_6/TMS): δ = 8.41 (dq, 2H, py H^6), 7.60 (dt, 2H, py H^4), 7.26 (d, 2H, py H^3), 7.13 (dt, 2H, py H^5), 6.61 (dd, 1H, phenol H^4), 6.50–6.48 (m, 2H, phenol H^5 + phenol H^6), 3.74 (s, 4H, –CH_2–py), 3.64 (s, 2H, –CH_2–phenol) ppm. ^{13}C-NMR (100.6 MHz, acetone-d_6/TMS): δ = 158.6 (py C^2), 148.7 (py C^6), 145.8 (phenol C^2), 144.9 (phenol, C^3), 136.7 (py C^4), 123.3 (phenol C^1), 123.1 (py C^3), 122.2 (py C^5), 120.9 (phenol C^6), 118.6 (phenol C^5), 114.3 (phenol C^4), 58.4 (–CH_2–py), 56.0 (–CH_2–phenol) ppm.

Bis(2-pyridylmethyl)benzylamine (L^H). The known synthesis for L^H has been modified.[27] A solution of 2.50 g (12.5 mmol) bis(2-pyridylmethyl)amine and 1.27 g (12.5 mmol) triethylamine in 50 mL tetrahydrofuran was added dropwise to a solution of 2.14 g (12.5 mmol) benzyl bromide in 50 mL tetrahydrofuran. The reaction mixture was stirred for 4 d at ambient temperature. The resulting white precipitate was removed by filtration and the filtrate was dried *in vacuo*. The resulting yellow oil was purified by column chromatography on silica gel with dichloromethane–methanol (5 : 1) as eluent. The title compound was obtained as brown oil (2.50 g, 8.64 mmol, 69%). Anal. calc. for $C_{19}H_{19}N_3$ (m = 289.4 g mol^{-1}): C, 78.9; H, 6.6; N, 14.5, found: C, 78.8; H, 6.7; N, 14.4. ^{1}H-NMR (400 MHz, $CDCl_3$/TMS): δ = 8.44 (dq, 2H, py H^6), 7.58 (td, 2H, py H^4), 7.52 (dd, 2H, py H^3), 7.34 (dd, 2H, benzyl H^2, H^6), 7.24 (t, 2H, py H^5), 7.18–7.14 (m, 1H, benzyl H^4), 7.07–7.04 (m, 2H, benzyl H^3), 3.74 (s, 4H, –CH_2–py), 3.62 (s, 2H, –CH_2–benzyl) ppm. ^{13}C-NMR (100.6 MHz, $CDCl_3$/TMS): δ = 158.8 (py C^2), 147.9 (py C^6), 137.9 (benzyl C^5), 135.4 (py C^4), 127.8 and 127.2 (benzyl C^1, C^3, C^4, C^6), 126.0 (benzyl C^2), 121.7 (py C^3), 120.9 (py C^5), 59.0 (–CH_2–py), 57.4 (–CH_2–benzyl) ppm.

[Cu(I)L4-H]PF$_6$. Under argon atmosphere, a solution of 242 mg (655 μmol) tetrakis(acetonitrile)copper(I) hexa-fluorophosphat dissolved in 8 mL acetonitrile was dropped into a solution of 200 mg (655 μmol) **L4-H** in 3 mL acetonitrile. The resulting yellow solution was stirred for 30 min under nitrogen. The solution was evaporated to dryness *in vacuo* and 151 mg (0.294 mmol, 45%) of a yellow solid was obtained. Anal. calc. for $CuC_{19}H_{19}N_3OPF_6$ (m = 513.9 g mol^{-1}): C, 44.4; H, 3.7, N, 8.2, found: C; 44.2, H, 3.5; N, 8.1. MS (MALDI, m/z) 368 [M − PF_6]. ^{1}H-NMR (400 MHz, CD_3CN/TMS): δ = 8.56 (d, 2H, py H^6), 7.82 (dt, 2H, py H^4), 7.42–7.33 (m, 4H, py H^3 + py H^5), 7.13 (t, 1H, phenol H^5), 6.90 (d, 1H, phenol H^6), 6.85 (d, 1H, phenol H^2), 6.72 (d, 1H, phenol H^4), 3.81 (s, 4H, –CH_2–py), 3.77 (s, 2H, –CH_2–phenol) ppm. ^{13}C-NMR (100.6 MHz, CD_3CN/TMS): δ = 157.4 (py C^2), 157.3 (phenol C^3), 149.5 (py C^6), 138.6 (py C^4), 137.7 (phenol C^1), 129.8 (phenol C^5), 124.7 (py C^3), 124.6 (py C^5), 122.8 (phenol C^6), 118.1 (phenol C^2), 115.6 (phenol C^4), 60.4 (–CH_2–phenol), 59.2 (–CH_2–py) ppm.

[Cu(I)L4-H^2]PF$_6$. Under argon atmosphere, a solution of 174 mg (467 μmol) tetrakis(acetonitrile)copper(I) hexa-fluorophosphat dissolved in 10 mL acetonitrile was dropped into a solution of 150 mg (467 μmol) **L4-H^2** in 5 mL acetonitrile. The resulting light yellow solution was stirred for 30 min under nitrogen. The solution was evaporated to dryness *in vacuo* and 104 mg (0.197 mmol, 42%) of a yellow solid was obtained. Anal. calc. for $CuC_{19}H_{18}N_3O_2PF_6$ (m = 528.9 g mol^{-1}): C, 43.2; H, 3.4; N, 8.0, found: C, 43.5; H, 3.7; N, 8.0. MS (MALDI, m/z) 384 [M − PF_6]. ^{1}H-NMR (400 MHz, CD_3CN/TMS): δ = 8.54 (d, 2H, py H^6), 7.81 (t, 2H, py H^4), 7.40–7.35 (m, 4H, py H^3 + py^5), 6.80 (dd, 2H, py H^5), 6.61 (dd, 2H, phenol H^4 + phenol H^6), 6.66 (t, 1H, phenol H^5), 3.91 (s, 2H, –CH_2–phenol), 3.84 (s, 4H, –CH_2–py) ppm. ^{13}C-NMR (100.6 MHz, CD_3CN/TMS): δ = 157.7 (py C^2), 149.4 (py C^6), 144.9 (phenol C^2), 144.6 (phenol, C^3), 138.4 (py C^4), 124.6 (py C^3), 124.4 (py C^5), 122.7 (phenol C^1), 119.8 (phenol C^6), 118.9 (phenol C^5), 115.6 (phenol C^4), 59.1 (–CH_2–py), 53.9 (–CH_2–phenol) ppm.

[Cu(I)L^H(CH$_3$CN)]PF$_6$. Under argon atmosphere, a solution of 73.6 mg (225 μmol) tetrakis(acetonitrile)copper(I) hexa-fluorophosphat dissolved in 8 mL acetonitrile was dropped into a solution of 72.3 mg (250 μmol) L^H in 5 mL acetonitrile. The resulting yellow solution was stirred for 10 min under nitrogen and reduced to 3 mL. The solution was diluted with 80 mL diethyl ether to precipitate a yellow solid. The solid was removed by filtration and evaporated to dryness *in vacuo* to obtain 74.6 mg (0.138 mmol, 67%) of a pale yellow solid. Anal. calc. for $CuC_{21}H_{22}N_4PF_6$ (m = 538.9 g mol^{-1}): C, 46.8; H, 4.1; N, 10.4, found: C, 46.6; H, 4.3; N, 10.8. MS (MALDI, m/z) 352 [M − $NCCH_3PF_6$]. ^{1}H-NMR (400 MHz, CD_3CN/TMS): δ = 8.59 (d, 2H, py H^6), 7.84 (dt, 2H, py H^4), 7.45–7.41 (m, 4H, py H^3 + py H^5), 7.36–7.30 (m, 5H, benzyl H^2, H^3, H^4, H^5, H^6), 3.90 (s, 2H, –CH_2–benzyl), 3.85 (s, 2H, –CH_2–py), 2.19 (s, 3H, –NCCH_3)

ppm. ^{13}C-NMR (100.6 MHz, CD_3CN/TMS): δ = 157.4 (py C^2), 149.5 (py C^6), 138.6 (py C^4), 136.0 (benzyl C^1), 128.8 (benzyl C^2, C^3, C^4, C^5, C^6), 124.7 (py C^3), 124.6 (py C^5), 60.7 (–CH_2–benzyl), 59.2 (–CH_2–py), 30.5 (–NCCH_3) ppm.

3,5-Di-*tert*-butyl-*ortho*-quinone (DTBQ). To a solution of 111 mg (500 μmol) 3,5-di-*tert*-butylcatechol in 25 mL acetone was added 56 mg (500 μmol) triethylamine slowly. The yellow reaction mixture was stirred for 30 min at ambient temperature during dioxygen bubbled into solution. During the reaction with dioxygen, the solution became dark red. The solvent was removed *in vacuo* to provide a dark red solid (110 mg, 0.499 mmol, 100%). Anal. calc. for $C_{14}H_{20}O_2$ (m = 220.3 g mol^{-1}): C, 76.3; H, 9.2, found: C, 76.4; H, 9.4.

Derivatisation of DTBQ with *ortho*-phenylenediamine[31]

To a solution of 108 mg (1 mmol) *ortho*-phenylenediamine and 220 mg (1 mmol) DTBQ in 25 mL acetone was added 20 mL H_2O containing 1% trifluoroacetic acid. The green solution was stirred and heated to 75 °C for 10 min. The solvent was removed *in vacuo* to obtain 210 mg (0.718 mmol, 72%) of a green solid. Anal. calc. for $C_{20}H_{24}N_2$ (m = 292.4 g mol^{-1}): C, 82.2; H, 8.3; N, 9.6, found: C, 81.4; H, 8.8; N, 9.8.

Derivatisation of CuL4quinone with *ortho*-phenylenediamine. To a solution of 5.80 mg (54.2 μmol) *ortho*-phenylenediamine and 20.7 mg (54.2 μmol) Cu**L4**quinone in 25 mL acetone was added 20 mL H_2O containing 1% trifluoroacetic acid. The dark green solution was stirred and heated to 75 °C for 10 min. The solvent was removed *in vacuo* to obtain 16.3 mg (35.8 μmol, 65%) of a dark green solid. Anal. calc. for $CuC_{25}H_{21}N_5$ (m = 455.1 g mol^{-1}): C, 65.9; H, 4.7; N, 15.4, found: C, 66.1; H, 5.1; N, 14.9. MS (MALDI, m/z) 456 [M + 2H].

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References

1 M. Rolff, J. Schottenheim, H. Decker and F. Tuczek, *Chem. Soc. Rev.*, 2011, **40**, 4077.
2 E. I. Solomon, D. E. Heppner, E. M. Johnston, J. W. Ginsbach, J. Cirera, M. Qayyum, M. T. Kieber-Emmons, C. H. Kjaergaard, R. G. Hadt and L. Tian, *Chem. Rev.*, 2014, **114**, 3659–3853.
3 Á. Sánchez-Ferrer, J. N. Rodríguez-López, F. García-Cánovas and F. García-Carmona, *Biochim. Biophys. Acta, Prot. Struct. Mol. Enzym.*, 1995, **1247**, 1.
4 K. E. van Holde, K. I. Miller and H. Decker, *J. Biol. Chem.*, 2001, **276**, 15563.
5 Y. Matoba, T. Kumagai, A. Yamamoto, H. Yoshitsu and M. Sugiyama, *J. Biol. Chem.*, 2006, **281**, 8981.
6 Y. C. Li, Y. Wang, H. B. Jiang and J. P. Deng, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 17002.
7 M. Sendovski, M. Kanteev, V. S. Ben-Yosef, N. Adir and A. Fishman, *J. Mol. Biol.*, 2011, **405**, 227.
8 W. T. Ismaya, H. J. Rozeboom, A. Weijn, J. J. Mes, F. Fusetti, H. J. Wichers and B. W. Dijkstra, *Biochemistry*, 2011, **50**, 5477.
9 H. Decker, T. Schweikardt and F. Tuczek, *Angew. Chem., Int. Ed.*, 2006, **45**, 4546.
10 K. Selmeczi, M. Réglier, G. Michel and G. Speier, *Coord. Chem. Rev.*, 2003, **245**, 191.
11 I. A. Koval, P. Gamez, C. Belle, K. Selmeczi and J. Reedijk, *Chem. Soc. Rev.*, 2006, **35**, 814.
12 L. Casella, M. Gullotti, M. Bartosek, G. Pallanza and E. Laurenti, *J. Chem. Soc., Chem. Commun.*, 1991, 1235.
13 M. Réglier, C. Jorand and B. Waegell, *J. Chem. Soc., Chem. Commun.*, 1990, 1752.
14 M. Rolff, J. Schottenheim, G. Peters and F. Tuczek, *Angew. Chem., Int. Ed.*, 2010, **49**, 6438.
15 L. M. Mirica, M. Vance, D. J. Rudd, B. Hedman, K. O. Hodgson, E. I. Solomon and T. D. P. Stack, *Science*, 2005, **308**, 1890.
16 A. Hoffmann, C. Citek, S. Binder, A. Goos, M. Rübhausen, O. Troeppner, I. Ivanović-Burmazović, E. C. Wasinger, T. D. P. Stack and S. Herres-Pawlis, *Angew. Chem., Int. Ed.*, 2013, **52**, 5398.
17 J. N. Hamann and F. Tuczek, *Chem. Commun.*, 2014, **50**, 2298.
18 K. D. Karlin, S. Kaderli and A. D. Zuberbühler, *Acc. Chem. Res.*, 1997, **30**, 139.
19 M. Rolff, J. N. Hamann and F. Tuczek, *Angew. Chem., Int. Ed.*, 2011, **50**, 6924.
20 E. A. Lewis and W. B. Tolman, *Chem. Rev.*, 2004, **104**, 1047.
21 L. M. Mirica, X. Ottenwaelder and T. D. P. Stack, *Chem. Rev.*, 2004, **104**, 1013.
22 A. De, S. Mandal and R. Mukherjee, *J. Inorg. Biochem.*, 2008, **102**, 1170.
23 K. D. Karlin, J. C. Hayes, Y. Gultneh, R. W. Cruse, J. W. Mckown, J. P. Hutchinson and J. Zubieta, *J. Am. Chem. Soc.*, 1984, **106**, 2121.
24 K. D. Karlin, B. I. Cohen, A. Farooq, S. Liu and J. Zubieta, *Inorg. Chim. Acta*, 1988, **153**, 9–11.
25 K. Tabuchi, M. Z. Ertem, H. Sugimoto, A. Kunishita, T. Tano, N. Fujieda, C. J. Cramer and S. Itoh, *Inorg. Chem.*, 2011, **50**, 1633–1647.
26 P. P. Paul, Z. Tyeklar, R. R. Jacobson and K. D. Karlin, *J. Am. Chem. Soc.*, 1991, **113**, 5322.
27 A. Kunishita, J. D. Scanlon, H. Ishimaru, K. Honda, T. Ogura, M. Suzuki, C. J. Cramer and S. Itoh, *Inorg. Chem.*, 2008, **47**, 8222.
28 K. J. Przybilla and F. Vögtle, *Chem. Ber.*, 1989, **122**, 347.
29 A. Neves, M. Aires de Brito, V. Drago, K. Griesar and W. Haase, *Inorg. Chim. Acta*, 1995, **237**, 131–135.
30 W. Flaig, T. Ploetz and A. Küllmer, *Z. Naturforsch., B: Anorg. Chem. Org. Chem. Biochem. Biophys. Biol.*, 1955, **10**, 668.

31 J. H. Zhu, J. A. Olmstead and D. G. Gray, *J. Wood Chem. Tech.*, 1995, **15**, 43.
32 S. D. Choudhury and S. Basu, *Chem. Phys. Lett.*, 2003, **373**, 67.
33 G. M. Badger and I. S. Walker, *J. Chem. Soc.*, 1956, 122.
34 H. Lucas, L. Li, A. Sarjeant, M. Vance, E. I. Solomon and K. D. Karlin, *J. Am. Chem. Soc.*, 2009, **131**, 3230–3245.
35 The ligand $\mathbf{L^H}$ is named as $^{\mathbf{Bz}}\mathbf{L}$ in ref. 34.
36 For the **L4-H** system even at −90 °C no UV/Vis spectrum of a bis(μ-oxo) species was detectable.
37 O. Sander, C. Näther and F. Tuczek, *Z. Anorg. Allg. Chem.*, 2009, **635**, 1123.
38 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Son-nenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, *Gaussian 09, revision A.1*, Gaussian, Inc., Wallingford CT, 2009.
39 A. Company, S. Palavicini, I. Garcia-Bosch, R. Mas-Ballesté, L. Que Jr., E. V. Rybak-Akimova, L. Casella, X. Ribas and M. Costas, *Chem. – Eur. J.*, 2008, **14**, 3535.
40 Y. Gultneh, B. Ahvazi, A. R. Khan, R. J. Butcher and J. P. Tuchagues, *Inorg. Chem.*, 1995, **34**, 3633.
41 R. Mayilmurugan, K. Visvaganesan, E. Suresh and M. Palaniandavar, *Inorg. Chem.*, 2009, **48**, 8771–8783.