Electronic Supplementary Material (ESI) for Chemical Communications
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Supplementary Information

New catalytic model systems of tyrosinase: Fine tuning of the reactivity with pyrazole-based *N*-donor ligands

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Experimental Section

General: The applied reagents were used as bought from Aldrich Chemical Co and ABCR GmbH & Co. KG. Solvents used were all reagent grade and were purified by refluxing over drying agents and distilling under argon atmosphere: Acetonitrile and dichloromethane were distilled from CaH_2; toluol from natrium. The NMR spectra were recorded at 300 K on a Bruker Avance 400 Pulse Fourier Transform spectrometer operating at a ^1H frequency of 400.13 MHz and a ^{13}C frequency of 100.62 MHz. Referencing was carried out using TMS as the substitutive standard. Optical absorption spectra were recorded in solution on an Agilent 8435 spectral photometer using a quartz cell with l = 1 mm. Furthermore optical absorption spectra at low temperatures were recorded in solution on an Agilent Cary 4000 spectral photometer using a CryoVAC KONTI cryostat with a quartz cell length 1 cm. Elemental analysis was performed using a Euro Vector CHNS-O-element analyzer (Euro EA 3000). Samples were burned in sealed tin containers in a stream of oxygen. The content of fluoride was determined with a potentiograph E536 from Metrohm, Herisau, using ion selective electrodes after decomposition of the compound according to Schöniger.[1] Complex syntheses were performed under N_2-atmosphere using Schlenk techniques. Preparations for oxygenation reactions were carried out in a MBRAUN Glovebox.

Synthesis of the ligands. The first step of synthesis was prepared according to instructions 1-(2-aminoethyl)pyrazol by Kenji *et al.* with variation of the pyrazole residue for the $\mathbf{L_{hpz}2}$.[2]

Synthesis of acetonitrile fluid of 2-chloroethyl amine.[2] 20.0 g (175 mmol) of 2-chloroethylamine hydrochloride were dissolved in 30 mL of dried acetonitrile under a nitrogen atmosphere. The suspension was cooled to 5 °C and then 21.9 mL (175 mmol) triethylamine was dropped over 30 min and it was made to react at 5 °C for 1 h. A white precipitate was formed during the reaction and was removed reaction mixture by filtration after ending reaction and the acetonitrile fluid of 2-chloroethyl amine was acquired. Yield was 13.83 g (100%).

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Synthesis of 1-(2-aminoethyl) pyrazole.[2] 2.00 g (30.0 mol) of 1H-pyrazole were dissolved in 40 mL of dried acetonitrile and 3.60 g (90.0 mmol) of sodium hydroxide was added into this solution and the solution was stirred for 30 min at ambient temperature. The reaction mixture was heated up to 75 °C and 4.78 g (60.0 mmol) of the acetonitrile fluid of 2-chloroethyl amine was dropped into the solution over 30 min. Afterwards, the solution was stirred for 5 h at 75 °C. After completion of reaction the solution was cooled to ambient temperature and the formed precipitate was removed by filtration. The solvent of the filtrate was removed in vacuo to gain 2.98 g of pale yellow oil. The yield was 89%. Elemental analysis calculated (%) for $C_5H_9N_3$: C, 54.0; H, 8.2; N, 37.8; found: C, 53.9; H, 8.1; N, 37.8; **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 7.69 (d, 1H, 5-H), 7.43 (d, 1H, 3-H), 6.22 (d, 1H, 4-H), 4.06 (t, 2H, -NCH_2), 2.88 (t, 2H, -CH_2NH$_2$), 1.37 (br s, 2H, -NH$_2$) ppm. **^{13}C NMR** (100.6 MHz, DMSO-d_6): δ = 138.96 (*C*5), 129.12 (*C*3), 104.82 (*C*4), 54.45 (-N*C*H$_2$), 41.54 (-*C*H$_2$NH$_2$) ppm.

Synthesis of 2-(3,5-dimethyl-1H-pyrazol-1-yl)ethanamine. The synthesis was prepared with 3,5-dimethyl-1H-pyrazole as previously described. The yield was 2.35 g (81 %) of colorless oil. Elemental analysis calculated (%) for $C_7H_{13}N_3$: C, 60.4; H, 9.4; N, 30.2; found: C, 60.2; H, 9.2; N, 29.8; **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 5.76 (s, 1H, 4-H), 3.87 (t, 2H, -NCH_2), 2.82 (t, 2H, -CH_2NH$_2$), 2.18 (s, 3H,-CH_3), 2.08 (s, 3H,-CH_3), 1.76 (br s, 2H, -NH$_2$) ppm. **^{13}C NMR** (100.6 MHz, DMSO-d_6): δ = 145.67 (*C*5), 138.74 (*C*3), 104.32 (*C*4), 50.83 (-N*C*H$_2$), 41.97 (-*C*H$_2$NH$_2$), 13.44 (-*C*H$_3$), 10.71 (-*C*H$_3$) ppm.

Synthesis of L_{hpz}1. 1.00 g (8.99 mmol) 1-(2-aminoethyl) pyrazole was dissolved in 30 mL dried toluol and 0.78 g (9.00 mmol) trimethylacetaldehyde was added slowly. A small amount of *p*-toluene sulfonic acid was added and the reaction mixture was heated up to 120 °C for 3 d under nitrogen atmosphere. After completion of reaction the solvent was evaporated in vacuo to obtain 1.45 g (90 %) of pale yellow oil. Elemental analysis calculated (%) for $C_{10}H_{17}N_3$: C, 67.0; H, 9.6; N, 23.4; found: C, 66.5; H, 9.3; N, 23.0; **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 7.35 (s, 1H, -NC*H*), 7.34 (d, 1H, 5-H), 7.24 (d, 1H, 3-H), 5.99 (s, 1H, 4-H), 4.17 (t, 2H,-NCH_2), 3.57 (t, 2H,-CH_2NH$_2$), 0.81 (s, 9H, -C(CH_3)$_3$) ppm. **^{13}C NMR** (100.6 MHz, $CDCl_3$): δ = 173.89 (-N*C*H), 139.40 (*C*5), 130.71 (*C*3), 105.05 (*C*4), 61.06 (-N*C*H$_2$), 52.91 (-*C*H$_2$NH$_2$), 36.39 (-*C*(CH$_3$)$_3$), 26.92 (-C(*C*H$_3$)$_3$) ppm.

Synthesis of L_{hpz}2. The synthesis was prepared with 2-(3,5-dimethyl-1H-pyrazol-1-yl)ethanamine as previously described. The product was obtained as dark yellow oil (1.31 g, 88 %). Elemental analysis calculated (%) for $C_{12}H_{21}N_3$: C, 69.5; H, 10.2; N, 20.3; found: C, 69.1; H, 10.1; N, 20.0; **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 7.09 (s, 1H, -NC*H*), 5.62 (s, 1H, 4-H), 3.97 (t, 2H,-NCH_2), 3.54 (t, 2H,-CH_2NH$_2$), 2.06 (s, 3H, -CH_3), 1.95 (s, 3H, -CH_3), 0.79 (s, 9H, -C(CH_3)$_3$) ppm. **^{13}C NMR** (100.6 MHz, $CDCl_3$): δ = 173.72 (-N*C*H), 147.09 (*C*5), 140.07 (*C*3), 104.04 (*C*4), 61.60 (-N*C*H$_2$), 49.12 (-*C*H$_2$NH$_2$), 36.37 (-*C*(CH$_3$)$_3$), 26.83 (-C(*C*H$_3$)$_3$), 13.62 (-*C*H$_3$), 11.18 (-*C*H$_3$) ppm.

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Synthesis of [Cu(I)L_{hpz}1$(CH_3CN)_2$]PF_6. The synthesis was performed under argon atmosphere. A solution of 372 mg (1.00 mmol) tetrakis(acetonitrile)copper(I)hexafluoro phosphate dissolved in 10 mL acetonitrile was dropped into a solution of 179 mg (1.00 mmol) **L_{hpz}1** in 8 mL acetonitrile. The yellow solution was stirred for 30 min at ambient temperature followed by evaporated to dryness. A yellow solid was obtained with a yield of 368 mg (78 %). Elemental analysis calculated (%) for $C_{14}H_{23}N_5CuPF_6$: C, 35.8; H, 4.9; N, 14.9; F^-, 24.3; found: C, 34.7; H, 4.5; N, 13.1; F^-, 23.6; **^{1}H NMR** (400 MHz, acetone-d_6): δ = 7.97 (d, 1H, 5-H), 7.76 (d, 1H, 3-H), 7.35 (s, 1H, -NC*H*), 6.51 (t, 1H, 4-H), 4.67 (t, 2H,-NCH_2), 4.19 (t, 2H,-C$H_2$$NH_2$), 2.29 (s, 6H, -NCC$H_3$), 1.29 (s, 9H, -C(C$H_3$)$_3$) ppm. **^{13}C NMR** (100.6 MHz, acetone-d_6): δ = 183.28 (-N*C*H), 140.82 (*C*5), 132.55 (*C*3), 117.45 (-N*C*CH3), 106.53 (*C*4), 67.18 (-N*C*H_2), 52.39 (-*C*$H_2$$NH_2$), 36.00 (-*C*$(CH_3)_3$), 26.37 (-C(*C*$H_3$)$_3$) ppm.

Synthesis of [Cu(I)L_{hpz}2$(CH_3CN)_2$]PF_6. The synthesis was prepared with **L_{hpz}2** as previously described. The product was obtained as pale yellow solid (399 mg, 80 %). Elemental analysis calculated (%) for $C_{16}H_{27}N_5CuPF_6$: C, 38.6; H, 5.5; N, 14.1; F^-, 22.9; found: C, 37.1; H, 5.2; N, 13.0; F^-, 21.8; **^{1}H NMR** (400 MHz, acetone-d_6): δ = 7.41 (s, 1H, -NC*H*), 5.82 (s, 1H, 4-H), 4.97 (t, 2H,-NCH_2), 4.54 (t, 2H,-C$H_2$$NH_2$), 2.27 (s, 2H,-NCC$H_3$), 2.16 (s, 3H, -C$H_3$), 2.01 (s, 3H, -C$H_3$), 1.25 (s, 9H, -C(C$H_3$)$_3$) ppm. **^{13}C NMR** (100.6 MHz, acetone-d_6): δ = 184.21 (-N*C*H), 149.32 (*C*5), 145.14 (*C*3), 118.68 (-N*C*CH_3), 106.04 (*C*4), 67.13 (-N*C*H_2), 59.62 (-*C*$H_2$$NH_2$), 37.70 (-*C*$(CH_3)_3$), 29.47 (-C(*C*$H_3$)$_3$), 14.60 (-*C*$H_3$), 11.98 (-*C*$H_3$) ppm.

Oxygenation reaction of [Cu(I)L_{hpz}1$(CH_3CN)_2$]PF_6 with DTBP-H and NEt_3. A 500 μM solution of the copper(I) complex [Cu(I)**L_{hpz}1**$(CH_3CN)_2$]PF_6 in 25 mL dried dichloromethane was prepared and 50 eq. of DTBP-H and 100 eq. of triethylamine were added under nitrogen atmosphere. The solution was oxygenated by bubbling molecular oxygen into the solution over 6 hours. During this time of oxygenation reaction UV/Vis spectra were measured in a 1 mm quartz cuvette. Measured spectra are shown in Figure 1.

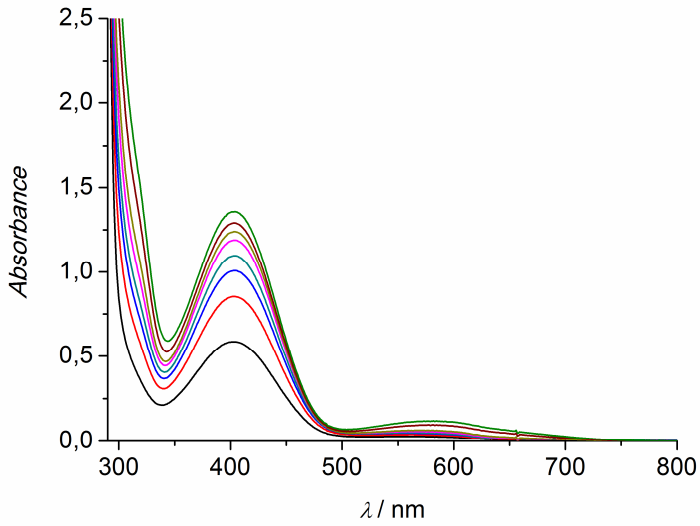

Figure 1. Measured UV/Vis spectra for a 500 μM solution of [Cu(I)Lhpz1$(CH_3CN)_2$]PF_6 in dichloromethane during the reaction with O_2 for six hours at ambient temperature; *l* = 1 mm.

Oxygenation reaction of $[Cu(I)L_{hpz}2(CH_3CN)_2]PF_6$ with DTBP-H and NEt_3. The oxygenation reaction of the $[Cu(I)L_{hpz}2(CH_3CN)_2]PF_6$ was performed as previously desribed. Measured spectra are shown in Figure 2.

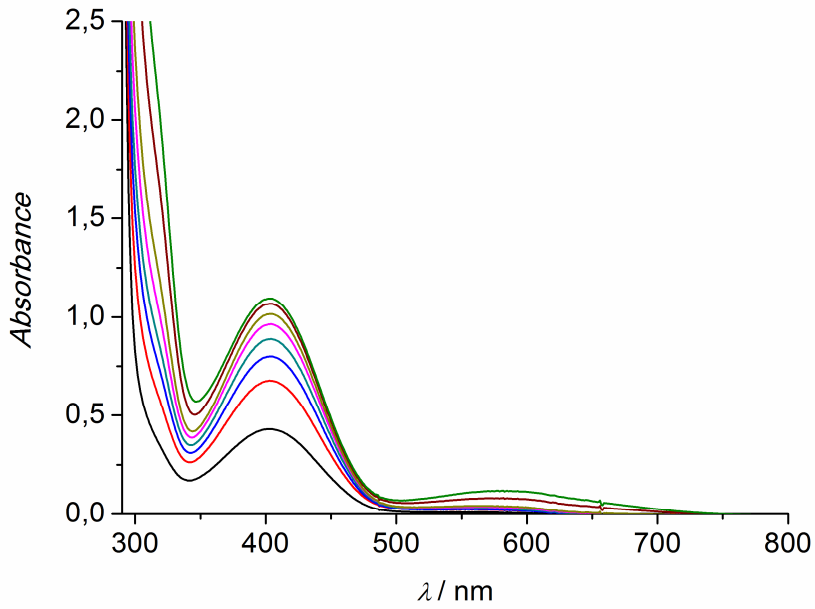

Figure 2. Measured UV/Vis spectra for a 500 µM solution of $[Cu(I)Lhpz1(CH_3CN)_2]PF_6$ in dichloromethane during the reaction with O_2 for six hours at ambient temperature; *l* = 1 mm.

Quenching of oxygenation solutions with HCl. After 30 minutes of oxygenation reaction a small amount of solutions were diluted to a 25 µM solution and were quenched with 6 M hydrochloric acid extracted with dichloromethane to eliminate the copper ions and were evaporated in vacou. Measured ^{1}H NMR spectra of $\mathbf{L_{hpz}1}$ and $\mathbf{L_{hpz}2}$ systems are shown (Figure 3-8).

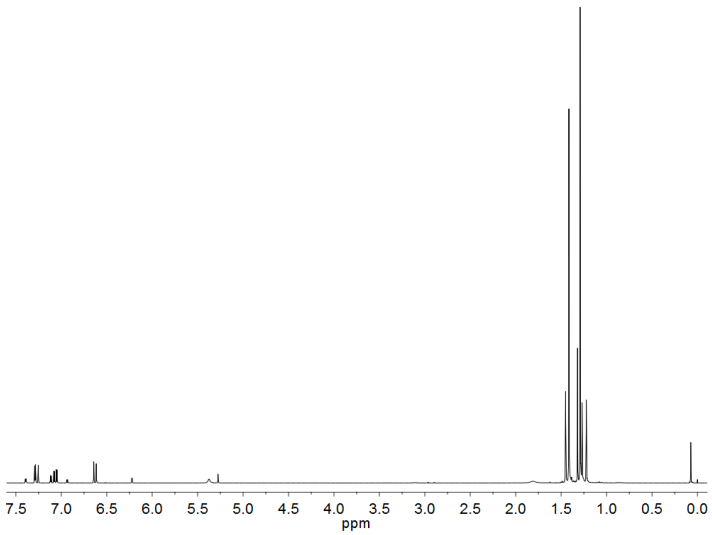

Figure 3. ^{1}H NMR overview spectrum of the organic phase of $L_{hpz}1$ system after quenching with HCl; measured in $CDCl_3$.

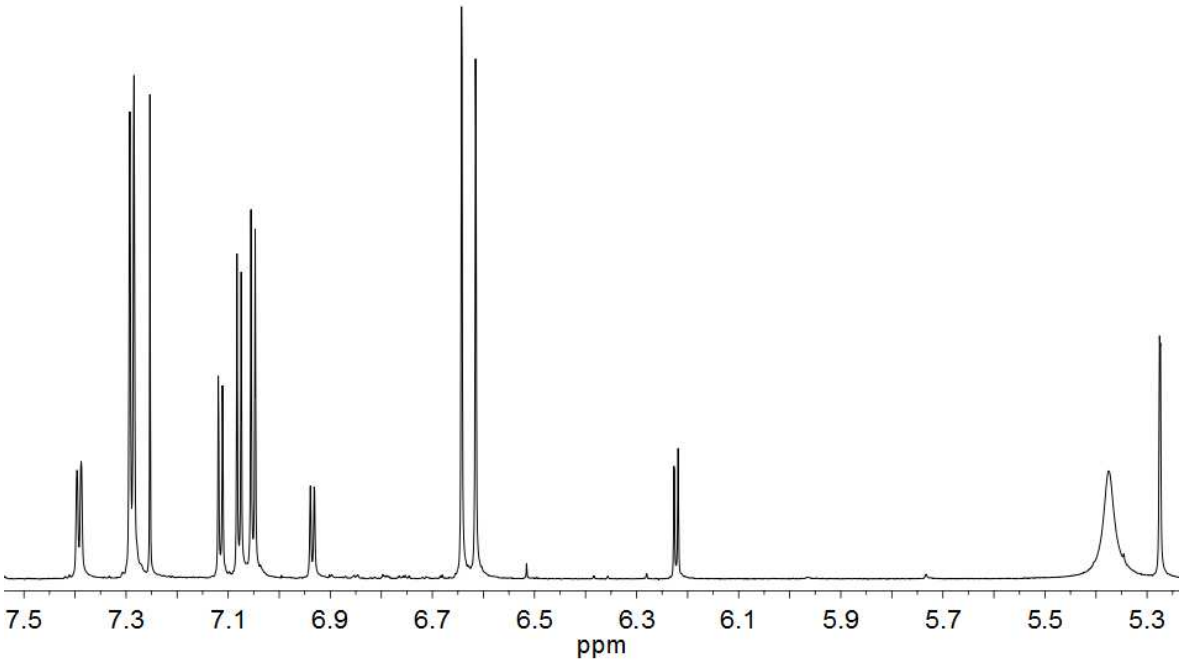

Figure 4. Aromatic area of ^{1}H NMR spectrum of organic phase after quenching with HCl (Figure 3).

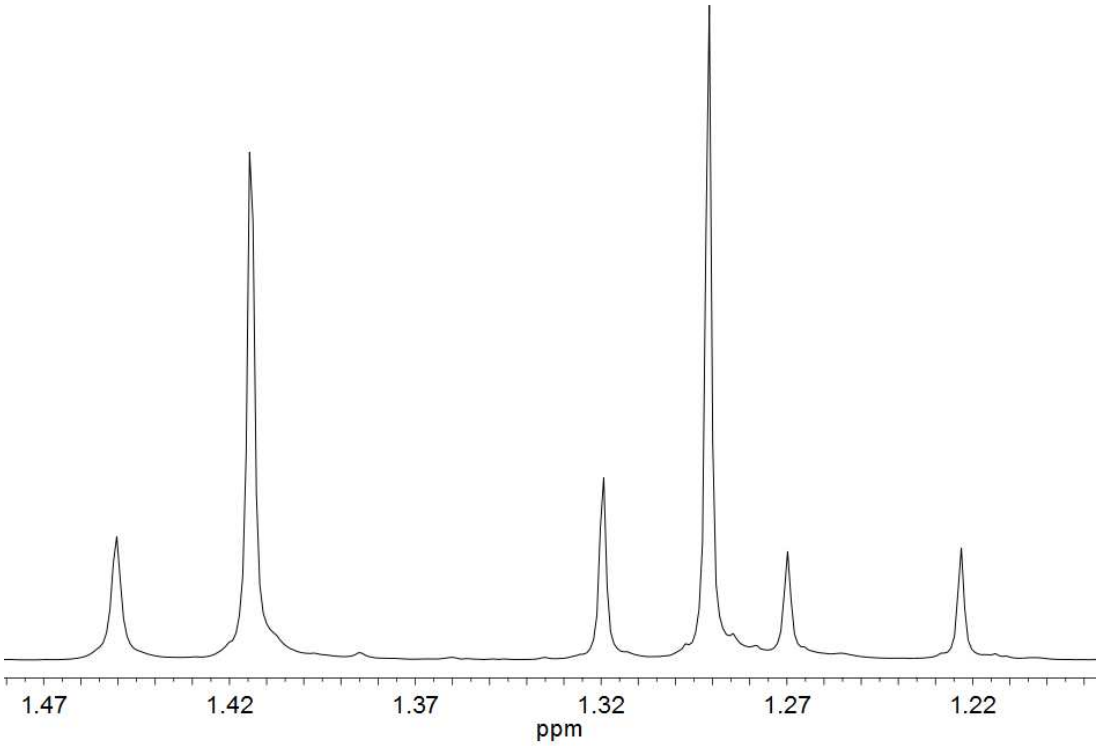

Figure 5. Aliphatic area of ^{1}H NMR spectrum of organic phase after quenching with HCl (Figure 3).

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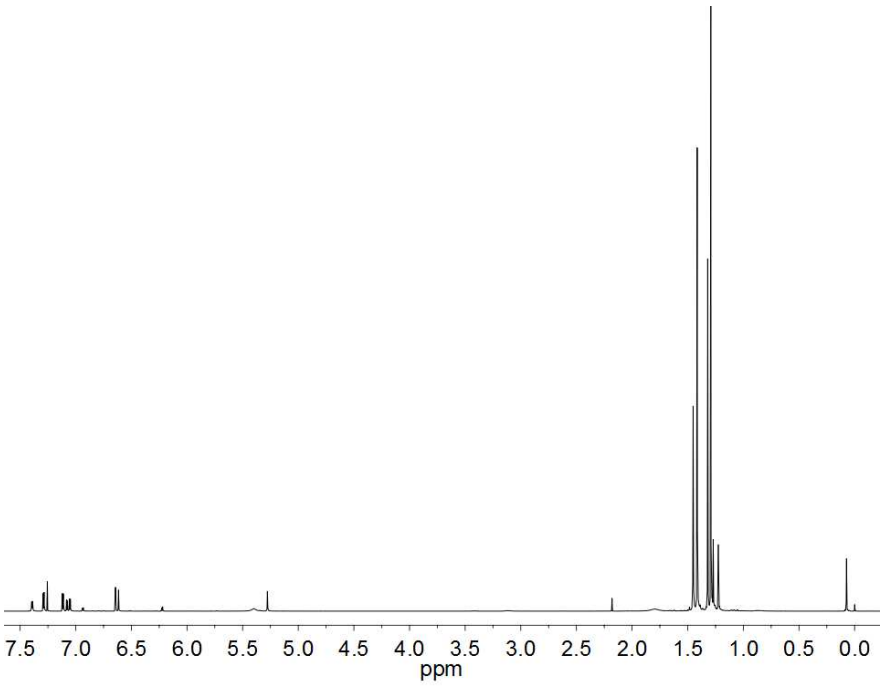

Figure 6. ^{1}H NMR overview spectrum of the organic phase of $L_{hpz}2$ system after quenching with HCl; measured in $CDCl_3$.

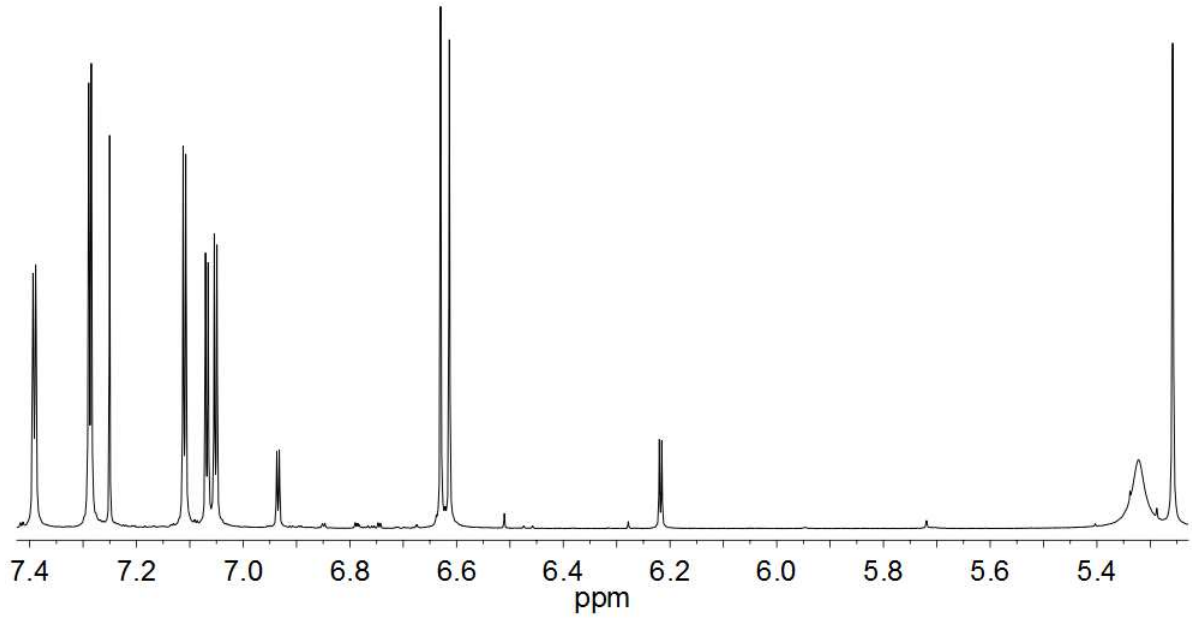

Figure 7. Aromatic area of ^{1}H NMR spectrum of organic phase after quenching with HCl (Figure 6).

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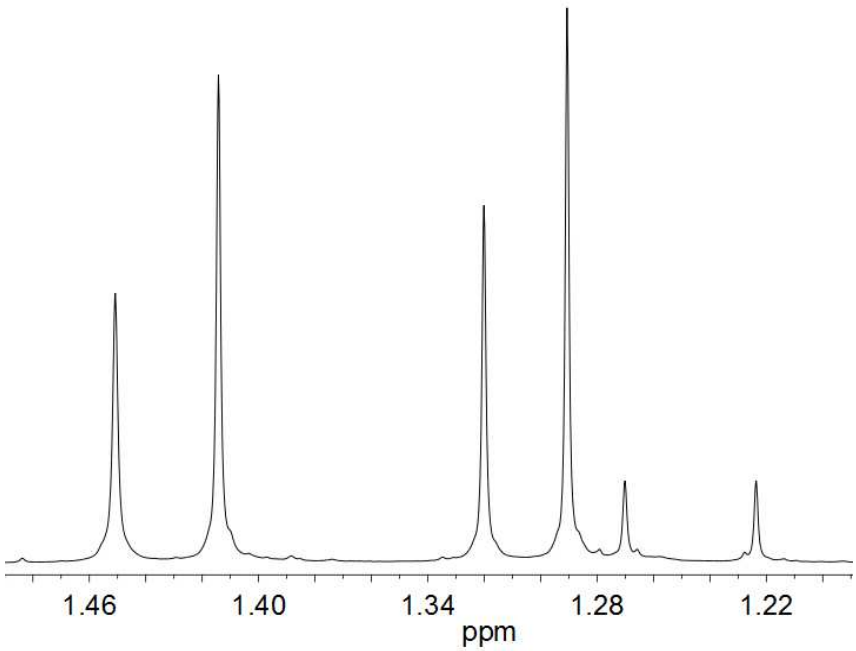

Figure 8. Aliphatic area of ^{1}H NMR spectrum of organic phase after quenching with HCl (Figure 6).

Oxygenation reaction of [Cu(I)L_{py}1$(CH_3CN)_2$]PF_6. A 3 mM solution of the copper(I) complex[3] [Cu(I)$\mathbf{L_{py}1}(CH_3CN)_2]PF_6$ in 40 mL dried acetone was prepared under nitrogen atmosphere. The pale yellow solution was oxygenated by bubbling molecular oxygen into the solution at 180 K. During the reaction with dioxygen the color of the solution changed from pale yellow to deep purple (Figure 9) and two absorption bands at ~ 350 nm and ~ 580 nm emerged, indicating the formation of the peroxo complex (Figure 10, red and black line). Upon bubbling N_2 into the solution and warming up the color of the solution changed from deep purple to intense blue and a broad band at 600 nm emerged, indicating the decay of the peroxo complex (Figure 10, green line).

Figure 9. 3 mM Solution of $[Cu(I)L_{py}1(CH_3CN)_2]PF_6$ in anhydrous acetone at 180 K at the beginning of the oxygenation (left) and after 5 minutes of oxygenation (right); the color of the solution changes from pale yellow to deep purple.

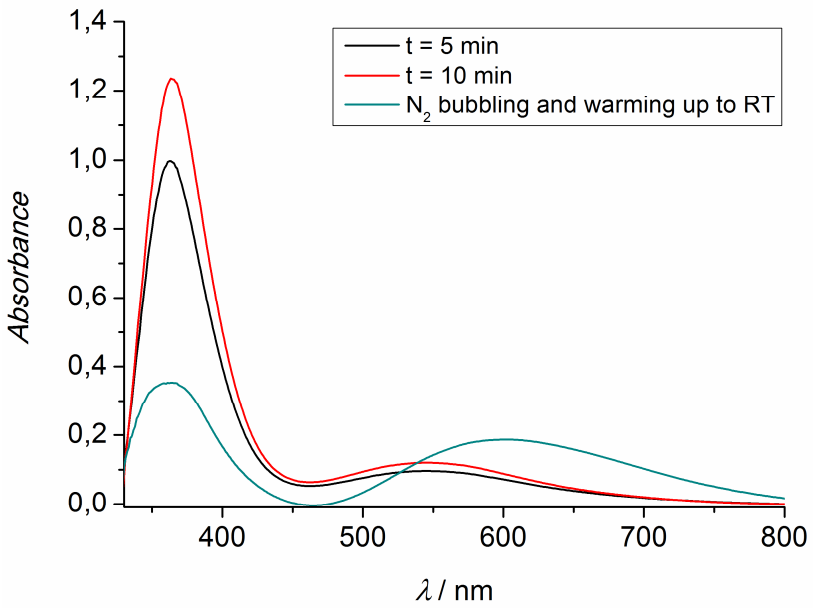

Figure 10. Difference spectra measured during the reaction of a 3 mM solution of $[Cu(I)L_{py}1(CH_3CN)_2]PF_6$ in anhydrous acetone at 180 K with molecular oxygen for 10 min and subsequent purging with nitrogen and warming up to room temperature; *l* = 1 cm.

Alternative Substrates.

In order to check the reactivity of our systems we oxygenated two other substrates, 3-*tert*-butylphenol and phenol, in the same procedure as previously described in the manuscript. A 500 µM solution of the copper(I) complex [Cu(I)$\mathbf{L_{hpz}1}$$(CH_3CN)_2$]$PF_6$ in dichloromethane was prepared and 50 eq. of 3-*tert*-butylphenol respectively phenol and 100 eq. triethylamine were added, followed by a subsequent oxygenation at ambient temperature. We found the characteristic absorption band at ~ 407 nm, too (Figure 11).

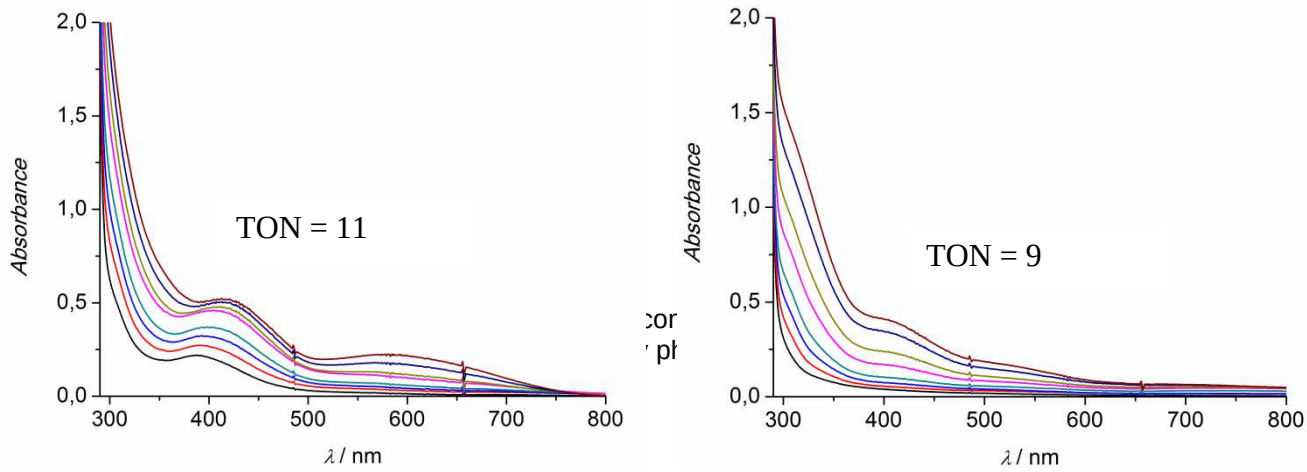

Figure 11. UV/Vis spectra of a 500 µM solution of the complex [Cu(I)$L_{hpz}1(CH_3CN)_2$]PF_6 in CH_2Cl_2 after addition of 50 eq. 3-*tert*-butylphenol (left) respectively phenol (right), 100 eq. NEt_3 and oxygenation between 15 min. and 4 h; *l* = 1 mm.

Regarding the formation of the corresponding *o*-quinones the TON were calculated to 11 for the substrate 3-*tert*-butylphenol and 9 for phenol after 4 hours of reaction with molecular oxygen. The investigated reactions happened slower with a lower yield of quinone as compared to our substrate DTBP-H.

References

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