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PAPER

Reduction of copper(II) complexes of tridentate ligands by nitric oxide and fluorescent detection of NO in methanol and water media†

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Two copper complexes, **1** and **2**, with tridentate N-donor ligands, $\mathbf{L_1}$ and $\mathbf{L_2}$ [$\mathbf{L_1}$= (1-methyl-1*H*-imidazol-2-ylmethyl)-(2-pyridin-2-yl-ethyl)amine, $\mathbf{L_2}$ = (2-pyridin-2-yl-ethyl)-pyridin-2 yl-methylamine] respectively, have been synthesized and characterized. On exposure to nitric oxide, the copper(II) centers in complexes **1** and **2** were found to undergo reduction in various solvents. In acetonitrile solvent the reduction was accompanied by a simultaneous N-nitrosation on the secondary amine center on the ligand frameworks. Complexes **3** and **4** were prepared with ligands $\mathbf{L_3}$ and $\mathbf{L_4}$, respectively. $\mathbf{L_3}$ and $\mathbf{L_4}$ [$\mathbf{L_3}$ = 5-dimethylamino-naphthalene-1-sulfonic acid (1-methyl-1*H*-imidazol-2-ylmethyl)-(2-pyridin-2-yl-ethyl)-amide; $\mathbf{L_4}$ = 5-dimethylamino-naphthalene-1-sulfonic acid(2-pyridin-2-yl-ethyl)-pyridin-2-ylmethyl-amide] are the dansyl derivatives of $\mathbf{L_1}$ and $\mathbf{L_2}$, respectively. Complex **4**, due to paramagnetic quenching, does not display any fluorescence; however, on addition of nitric oxide to a methanol or water solution of complex **4**, the fluorescence intensity of the fluorophore has been found to be restored. This is attributed to the reduction of the Cu(II) center by nitric oxide to diamagnetic Cu(I). The turn-on of quenched fluorescence intensity has been observed both in methanol and water media.

Introduction

Nitric oxide (NO) has attracted enormous interest from chemists and biochemists since it has been discovered as a signalling agent in humans.[1–13] It is also known to play roles in various physiological processes like vascular regulation, neurotransmission and cytotoxicity.[14–18] These essentially inspired a wide range of research to identify the precise roles of nitric oxide in biology. To study the nitric oxide induced reactions in cellular systems, the most challenging aim is to detect the location of the nitric oxide formation. Thus, a selective probe to detect the formation and migration of nitric oxide with spatiotemporal resolution directly from living cells is highly desirable. In this aspect, the fluorescence-based detection technique is found to satisfy almost all the requirements.[19,20]

Starting from the early examples of fluorescence-based sensors such as *o*-diaminonaphthalene (DAN) and *o*-diaminofluoresceins (DAFs), a number of fluorescent probes have been reported to date.[21–36] However, they are unable to detect or monitor NO itself as their fluorescent response depends on the formation of a triazole species by oxidized NO products such as N_2O_3. Thus, the NO-related bio-events would not be detected in real time. Recently, a highly selective fluorescent imaging agent, NO_{550}, for nitric oxide has been reported which displays a rapid and linear response with a red-shifted turn-on signal.[37] A number of metal complex-based fluorescence sensors for nitric oxide have been reported recently based on a fluorophore displacement strategy.[38–43] However, the low sensitivity and water insolubility of most of the cobalt, ruthenium and di-rhodium complexes, precluded their further application in biological systems.[21–36] Though, a number of iron complexes were reported to sense nitric oxide in aqueous medium, some of them either display diminished fluorescence or are air sensitive and exhibit only modest turn-on emission with nitric oxide, which again precluded their biological applications.[44–46] On the other hand, it has been observed that the fluorophore displacement technique to sense nitric oxide works mostly in organic solvents.[38–43] In aqueous medium, since the replacement of the fluorophore ligand from the metal can also be achieved by water, the turn-on fluorescence may also be possible in the absence of nitric oxide. Hence, the reduction of the metal center by nitric oxide has been found to be a more effective strategy. The fluorescence intensity of a ligand comprising a fluorophore is expected to be quenched on its complexation with a paramagnetic Cu(II) center and the reduction of copper(II) center to diamagnetic copper(I) by nitric oxide will restore the quenched fluorescence intensity of the fluorescent ligand.[47–53]

In the continuation of our study of the reduction of copper(II) complexes by nitric oxide and ligand nitrosation,[54–57] we report here the nitric oxide reactivity of copper(II) complexes of two tridentate ligands; and the use of the copper(II) complexes of those

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† Electronic supplementary information (ESI) available. Characterization data of $\mathbf{L_1}$, $\mathbf{L_2}$, $\mathbf{L_4}$, **1**, **2**, **4**, $\mathbf{L_1}''$ and $\mathbf{L_2}''$ and cif files for complexes **1** and **2** are included. The UV-vis, EPR and fluorescence spectral studies for the reactions of complexes **1**, **2**, **3** and **4** with nitric oxide are also incorporated. CCDC reference numbers 816461–816462. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c1dt10773a

ligands with dansyl fluorophore to sense nitric oxide in aqueous and methanol media (Fig. 1).

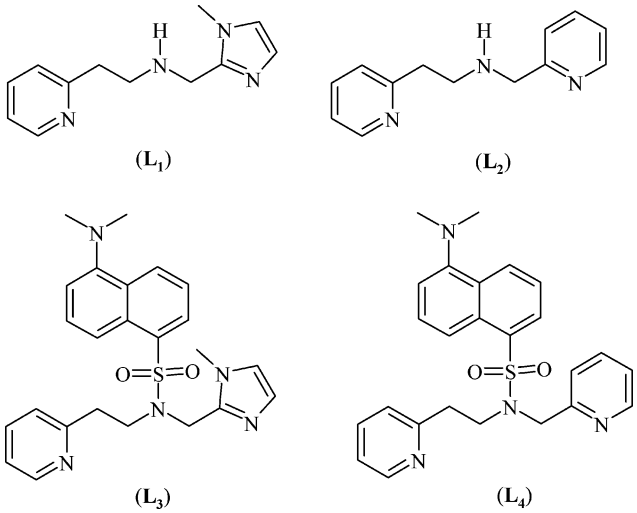


Fig. 1 Ligands used for the present study.

A portion of the nitric oxide sensor study has been reported as the preliminary findings of the present work.[56]

Experimental

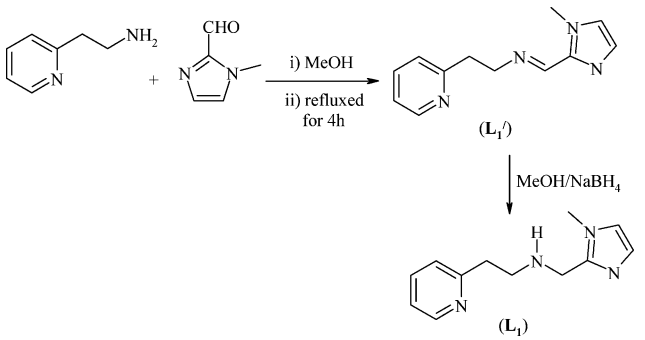
Materials and methods

All reagents and solvents were purchased from commercial sources and were of reagent grade. Acetonitrile was distilled from calcium hydride. Deoxygenation of the solvent and solutions were effected by repeated vacuum/purge cycles or bubbling with nitrogen for 30 min. NO gas was purified by passing through KOH and P_2O_5 column. UV-visible spectra were recorded on a Perkin Elmer Lambda 25 UV-visible spectrophotometer. FT-IR spectra of the solid samples were taken on a Perkin Elmer spectrophotometer with samples prepared as KBr pellets and for solutions, Varian 660-IR FT-IR spectrometer and NaCl cell of 2 mm path length were used and the spectra shown are the solvent subtracted ones. The fluorescence spectra were recorded in solution in VARIAN Cary Eclipse Fluorescence Spectrophotometer at room temperature. Quinine sulfate in acidic medium was used as the reference compound for the determination of fluorescence quantum yield. Solution electrical conductivity was checked using a Systronic 305 conductivity bridge. ^{1}H-NMR spectra were obtained with a 400 MHz Varian FT spectrometer. Chemical shifts (ppm) were referenced either with an internal standard (Me_4Si) or to the residual solvent peaks. The X-band Electron Paramagnetic Resonance (EPR) spectra were recorded on a JES-FA200 ESR spectrometer, at room temperature. Elemental analyses were obtained from a Perkin Elmer Series II Analyzer. The magnetic moment of complexes is measured on a Cambridge Magnetic Balance.

Single crystals were grown by a slow diffusion followed by slow evaporation technique. The intensity data were collected using a Bruker SMART APEX-II CCD diffractometer, equipped with a fine focus 1.75 kW sealed tube Mo-Kα radiation (λ = 0.71073 Å) at 273(3) K, with increasing ω (width of 0.3° per frame) at a scan speed of 3 s frame^{-1}. The SMART software was used for data acquisition. Data integration and reduction were undertaken with SAINT and XPREP software.[58] Multi-scan empirical absorption corrections were applied to the data using the program SADABS.[59] Structures were solved by direct methods using SHELXS-97 and refined with full-matrix least squares on F^2 using SHELXL-97.[60] All non-hydrogen atoms were refined anisotropically. Structural illustrations have been drawn with ORTEP-3 for Windows.[61]

Syntheses

Synthesis of the ligands L_1 and L_2. The ligands L_1 and L_2 were reported earlier[62–67] and have been prepared by the reaction of pyridine 2-ethylamine with the appropriate aldehyde followed by reduction of the imine with sodium borohydride (Scheme 1). The details are given in the supporting information†.



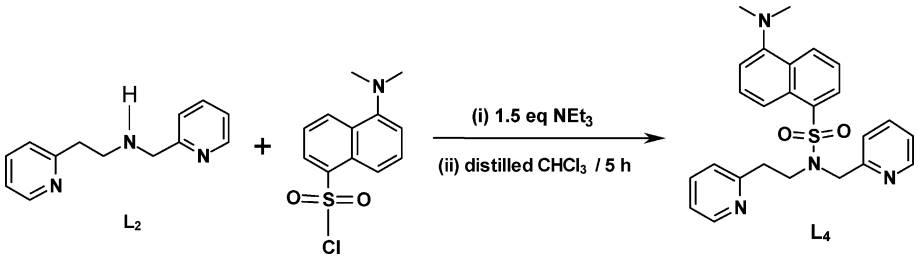
Scheme 1 Synthesis of ligand, L_1.

For L_1: Yield, 60%, 0.834 g. Elemental analyses: Calcd.(%) for $C_{12}H_{16}N_4$: C, 66.21; H, 7.22; N, 25.11. Found (%): C, 66.26; H, 7.23; N, 25.04. FT-IR in KBr: 3504, 2923, 1596, 1437 and 749 cm^{-1}. ^{1}H-NMR: (400 MHz, $CDCl_3$): δ_{ppm}: 2.86 (1H), 2.95 (2H), 3.01 (2H), 3.58 (3H), 3.83 (2H), 6.76 (1H), 6.86 (1H) 7.0–7.1 (2H), 7.52 (1H), 8.47 (1H). ^{13}C-NMR: (100 MHz, $CDCl_3$) δ_{ppm}: 32.47, 38.09, 45.47, 48.77, 121.06, 121.31, 126.64, 126.73, 136.66, 146.30, 149.04, 160.02. Mass, (M + H$^+$): calculated, 217.13; found: 217.48.

Synthesis of the ligand L_2. Ligand L_2 was prepared following the same procedure as L_1 from pyridine-2- ethylamine (1.22 g, 10 mmol) and pyridine-2-carbaldehyde (1.07 g, 10 mmol) (yield, 70%, 1.11 g). Elemental analyses: Calcd.(%) for $C_{13}H_{14}N_3$: C, 73.55; H, 7.13; N, 19.82. Found (%): C, 73.49; H, 7.13; N, 19.74. FT-IR in KBr: 2928, 2852, 1594, 1476, 1435 and 765 cm^{-1}. ^{1}H-NMR: (400 MHz, $CDCl_3$): δ_{ppm}: 2.64–2.99 (4H), 3.86 (2H), 7.00–7.05 (2H), 7.08–7.10 (1H), 7.19–7.21 (1H), 7.47–7.54 (2H). ^{13}C-NMR: (100 MHz, $CDCl_3$) δ_{ppm}: 37.98, 48.58, 54.57, 120.82, 121.46, 121.77, 122.86, 135.93, 136.00, 148.72, 148.76, 159.23, 159.68. Mass, (M + H$^+$): calculated, 214.12; found: 214.13.

Synthesis of the ligands L_3 and L_4. Synthesis of L_3. The synthesis of L_3 has been reported earlier.[56] L_4 has been prepared following the same procedure (Scheme 2) and the details are given in the supporting information†.

Yield: ~70%, 0.625 g. Elemental analyses: Calcd.(%) for $C_{25}H_{26}N_4O_2S$: C, 67.24; H, 5.87; N, 12.55. Found (%): C, 67.25; H, 5.89; N, 12.44. FT-IR: 2921, 2850, 1590, 1436, 1323, 1142 and 791 cm^{-1}. ^{1}H-NMR: (400 MHz, $CDCl_3$) δ_{ppm}: 2.82–2.89 (8H, m), 3.70 (2H, t), 4.71 (2H, s), 6.81 (1H, d), 7.88 (1H, t), 7.12(2H,



Scheme 2 Synthesis of ligand $\mathbf{L_4}$.

m), 7.30–7.37 (2H, m), 7.43–7.47 (2H, m), 7.53 (1H, t) 8.19 (3H, m), 8.45 (2H, m). ^{13}C-NMR: (100 MHz, $CDCl_3$) δ_{ppm}: 36.29, 45.32, 47.47, 52.82, 115.08, 119.42, 121.24, 122.42, 122.47, 123.08, 123.13, 128.00, 129.74, 129.96, 130.32, 134.95, 136.03, 136.67, 148.88, 148.95, 151.62, 156.73, 158.03. Mass, ($M + H^+$): calculated, 447.17; found: 447.11.

Synthesis of complexes 1 and 2. Complexes **1** and **2** were synthesized following the same procedure. The details are given for complex **1**.

$[Cu^{II}(H_2O)_6](ClO_4)_2$ (0.370 g, 1.0 mmol) was dissolved in 10 ml distilled acetonitrile. To this solution, $\mathbf{L_1}$ (0.216 g, 1.0 mmol) was added slowly with constant stirring. The color of the solution turned into deep blue from light blue. The stirring was continued for 1 h at room temperature. The volume of the solution was then reduced to ~2 ml. To this, benzene (5 ml) was added to layer on it and kept it overnight on freezer. This resulted into blue color microcrystals of complex **1**. Yield: 0.423 g (83%). Elemental analyses: Calcd.(%) for $CuC_{12}H_{19}N_4O_{10}Cl_2$: C, 28.05; H, 3.70; N, 10.91. Found (%): C, 28.11; H, 3.69; N, 10.86. UV-vis. (acetonitrile): λ_{max}, 616 nm ($\varepsilon = 114\ M^{-1}\ cm^{-1}$). X-Band EPR: g_{av} = 2.060. FT-IR (KBr pellet): 2927, 1083, 1119 and 626 cm^{-1}. Molar conductivity in acetonitrile, Λ_M (S cm^{-1}), 244. μ_{obs}, 1.56 BM.

Complex **2** was synthesized from $[Cu^{II}(H_2O)_6](ClO_4)_2$ and $\mathbf{L_2}$. Yield: 78%, 0.405 g. Elemental analyses: Calcd.(%) for $CuC_{15}H_{17}N_4O_8Cl_2$: C, 34.90; H, 3.29; N, 10.86. Found (%): C, 34.95; H, 3.27; N, 10.81.UV-vis. (acetonitrile): λ_{max}, 606 nm (ε = 111 $M^{-1}\ cm^{-1}$). X-Band EPR: g_{av} = 2.042. FT-IR (KBr pellet): 2938, 1086, 1116, 1145 and 626 cm^{-1}. Molar conductivity in acetonitrile, Λ_M (S cm^{-1}), 236. μ_{obs}, 1.51 BM.

Synthesis of complex 3. The synthesis of complex **3** was reported elsewhere.[56]

Synthesis of complex 4. Copper(II)chloride dihydrate, $[Cu(H_2O)_2]Cl_2$ (0.170 g, 1.0 mmol) was dissolved in freshly distilled methanol (20 ml) and to this, $\mathbf{L_4}$ (0.449 g, 1.0 mmol) was added. The color of the solution changed from blue to light green. The resulting solution was stirred at room temperature for 3 h. Then the volume of the solution was reduced to 5 ml and diethyl ether (15 ml) was added. Storage in a freezer for overnight afforded a light green precipitate of complex **4**. Yield: ~85%, 0.522 g. Elemental analyses: Calcd.(%) for $CuC_{26}H_{30}N_4O_{11}SCl_2$: C, 42.14; H, 4.05; N, 7.56. Found (%): C, 42.10; H, 4.05; N, 7.52. UV-vis. (methanol): λ_{max}, 744 nm (ε, 70 $M^{-1}\ cm^{-1}$). FT-IR (KBr pellet): 2925, 1143 and 794 cm^{-1}. X-band EPR: g_{av} = 2.03. Molar conductivity: Λ_M (S cm^{-1}), 205. μ_{obs}, 1.52 BM.

Isolation of $\mathbf{L_1}''$

Complex **1** (0.510 g, 1.0 mmol) was dissolved in 10 ml of distilled and degassed acetonitrile. To this solution an excess of NO gas was purged for 1 min. and the resulting colorless solution was stirred for 1 h at room temperature. The solvent was then removed under reduced pressure using a rotavapor. Water (5 ml) was added to the dried mass followed by the addition of 5 ml of saturated Na_2S solution. The black precipitate of CuS was filtered out. The crude organic part was then extracted from the aqueous layer using $CHCl_3$ (25 ml × 4 portions). The crude product, obtained after removal of solvent, was then purified by column chromatography using a neutral alumina column and a hexane/ethyl acetate solvent mixture to get the pure $\mathbf{L_1}''$. Yield: 0.137 g, 64%. Elemental analyses: Calcd.(%) for $C_{12}H_{15}N_5O$: C, 58.76; H, 6.16; N, 28.55. Found (%): C, 58.66; H, 6.16; N, 28.53. FT-IR (KBr pellet): 3399, 2926, 1437, 1116 and 752 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$) δ_{ppm}: 2.8 (2H, t), 3.22 (2H, t), 3.50 (3H, s), 3.95 (2H, s), 6.8–7.15 (4H, m), 7.55 (1H, s), 8.22 (1H, s). Mass, ($M + H^+$): calculated, 246.12; found: 246.11.

Isolation of $\mathbf{L_2}''$

$\mathbf{L_2}''$ was isolated after the reaction of complex **2** (0.513 g, 1.0 mmol) with nitric oxide in degassed acetonitrile following the same procedure for $\mathbf{L_1}''$. Yield: 62%, 0.134 g. Elemental analyses: Calcd.(%) for $C_{13}H_{14}N_4O$: C, 64.45; H, 5.82; N, 23.13. Found (%): C, 64.48; H, 5.83; N, 23.15. FT-IR (KBr pellet): 2924, 2853, 1593, 1436, 1454, 1436, 1128 and 760 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$) δ_{ppm}: 2.94 (2H, t), 3.32 (2H, t), 4.89 (2H, s), 6.91–7.03 (3H, m), 7.13 (1H, t), 7.42 (2H, s), 8.36 (2H, m). Mass, ($M + H^+$): calculated, 243.27; found: 243.29.

Results and discussion

Ligands $\mathbf{L_1}$ and $\mathbf{L_2}$ were prepared from the reaction of pyridine 2-ethylamine with the appropriate aldehyde followed by reduction of the imine (experimental section). The formation of the ligands were authenticated by elemental analysis and other spectroscopic studies (experimental section). The ligands $\mathbf{L_3}$ and $\mathbf{L_4}$ were synthesized by incorporating the dansyl group into the secondary amine site of $\mathbf{L_1}$ and $\mathbf{L_2}$, respectively, following the reported procedure (experimental section).[62–67] $\mathbf{L_4}$ was found to display satisfactory elemental analyses and was characterized further by various spectral analysis (experimental section). All of the complexes were prepared using a general method where hexa-aquacopper(II) perchlorate was made to react with equivalent

Table 1 Crystallographic data for complexes **1** and **2**

	(1)	(2)
Formulae	$C_{12}H_{15}Cl_2CuN_4O_{10}$	$C_{15}H_{17}Cl_2CuN_4O_8$
Mol. wt.	509.73	515.78
Crystal system	Triclinic	Monoclinic
Space group	$P\bar{1}$	$P21/c$
T /K	293(2)	296(2)
Wavelength /Å	0.71073	0.71073
a /Å	8.3087(2)	8.4963(9)
b /Å	10.3152(3)	9.0426(10)
c /Å	11.6673(3)	26.440(3)
α (°)	85.8490(10)	90.00
β (°)	83.5950(10)	90.769(7)
γ (°)	85.1690(10)	90.00
V /Å^3	988.18(5)	2031.1(4)
Z	2	4
Density /Mg m^{-3}	1.713	1.687
Abs. Coeff. /mm^{-1}	1.434	1.383
Abs. correction	None	None
F(000)	516.0	1048.0
Total no. of reflections	4211	2355
Reflections, $I > 2\sigma(I)$	3287	1670
Max. 2θ /°	27.50	21.70
Ranges (h, k, l)	$-10 \le h \le 10$ $-9 \le k \le 13$ $-15 \le l \le 15$	$-8 \le h \le 8$ $-9 \le k \le 9$ $-26 \le l \le 27$
Complete to 2θ (%)	92.8	98.3
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Goof (F^2)	1.277	1.056
R indices [$I > 2\sigma(I)$]	0.0486	0.0847
R indices (all data)	0.0623	0.1045

quantity of the respective ligands (experimental section). All the complexes showed satisfactory elemental analyses.

Crystal structure

Single crystal structures of complexes **1** and **2** have been determined. The perspective view of the ORTEP diagrams are shown in Fig. 2. In both of the complexes, copper(II) is found to be coordinated with the tridentate ligand and a solvent molecule in a distorted square planar geometry. Weak interactions between the perchlorate oxygens and copper(II) ion have been found in both the complexes. In complex **1**, the coordinated solvent is water and in complex **2**, it is acetonitrile. The crystallographic data, important bond angles and distances were listed in Tables 1, 2 and 3 respectively. From the spectral analysis, presumably, the complexes attain the distorted square planar geometry in solution also.

Nitric oxide reactivity of complexes 1 and 2

Purging of excess nitric oxide to the degassed acetonitrile solution of complexes **1** and **2** resulted in the rapid reduction of the copper(II) center to copper(I). The reduction was monitored by UV-visible spectroscopy and Fig. 3(a) and (b) represent the observed spectral change during the reaction. The reduction of Cu(II) in the presence of NO was noticed in water, methanol and methanol/water mixture also. In a methanol and water medium, the reduction led to the formation of a methyl nitrite and a nitrite ion, respectively. The formation of methyl nitrite was confirmed quantitatively by GC-Mass spectral studies and nitrite ion in aqueous solution was authenticated by the Griess test.[68] Generation of H^+ (eqn (1)) was confirmed qualitatively in unbuffered aqueous solution by monitoring the decrease in pH as the reactions proceeded.

Table 2 Selected bond length (Å) for complexes **1**, **2**

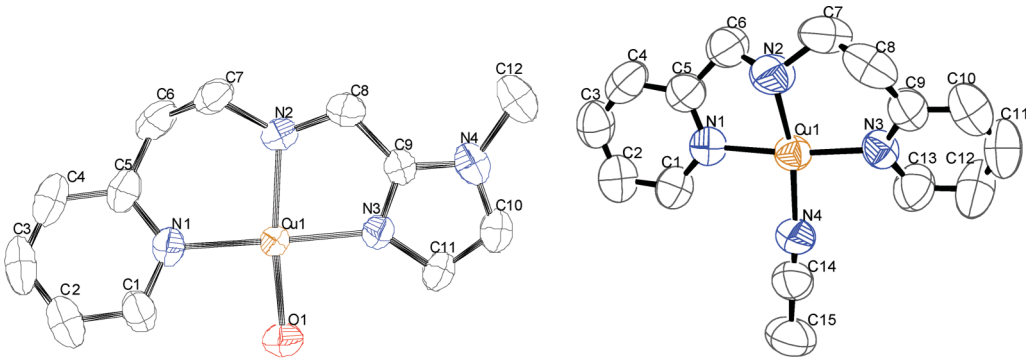
	(1)	(2)
Cu(1)–N(1)	2.000(3)	2.008(8)
Cu(1)–N(2)	2.024(4)	2.00(1)
Cu(1)–N(3)	1.955(3)	2.007(8)
Cu(1)–N(4)	—	1.97(1)
Cu(1)–O(1)	2.002(3)	—
C(2)–C(1)	1.369(6)	1.38(2)
C(2)–C(3)	1.362(8)	1.36(2)
C(1)–N(1)	1.354(6)	1.34(1)
C(5)–N(1)	1.356(4)	1.33(1)
C(10)–C(11)	1.334(6)	1.33(2)
N(2)–C(7)	1.485(5)	1.50(2)
C(7)–C(8)	—	1.48(2)
N(2)–C(8)	1.439(5)	—
C(9)–C(10)	—	1.40(2)
C(4)–C(5)	1.395(6)	1.43(2)
C(5)–C(6)	1.494(6)	1.49(2)
C(8)–C(9)	1.492(5)	1.54(2)
C(9)–N(3)	1.315(5)	1.32(1)

Table 3 Selected bond angles (°) for complexes **1**, **2**

	(1)	(2)
N(1)–Cu(1)–N(2)	95.3(1)	79.7(4)
N(2)–Cu(1)–N(3)	81.8(1)	96.9(4)
N(1)–Cu(1)–N(3)	170.1(1)	170.9(3)
C(1)–N(1)–C(5)	117.7(3)	117.7(8)
C(6)–C(5)–N(1)	118.3(3)	114.6(9)
C(4)–C(5)–C(6)	121.3(4)	126(1)
C(7)–N(2)–C(8)	114.8(3)	—
C(6)–N(2)–C(7)	—	114(1)
C(7)–C(6)–C(5)	115.0(3)	—
C(7)–C(8)–C(9)	—	111(1)
N(4)–C(14)–C(15)	—	178(1)
N(4)–C(10)–C(11)	106.9(4)	—
C(9)–N(3)–C(11)	106.3(3)	—
C(9)–N(3)–C(13)	—	117(1)
Cu(1)–N(1)–C(1)	117.4(3)	127.9(7)
Cu(1)–N(1)–C(5)	124.9(2)	114.4(6)
Cu(1)–N(2)–C(7)	116.7(3)	114.6(8)
Cu(1)–N(2)–C(8)	111.9(3)	—
Cu(1)–N(2)–C(6)	—	109.8(7)
Cu(1)–N(3)–C(9)	114.1(2)	124.0(7)
Cu(1)–N(3)–C(11)	139.5(2)	—
Cu(1)–N(3)–C(13)	—	119.0(7)
C(1)–C(2)–C(3)	119.4(5)	118(1)

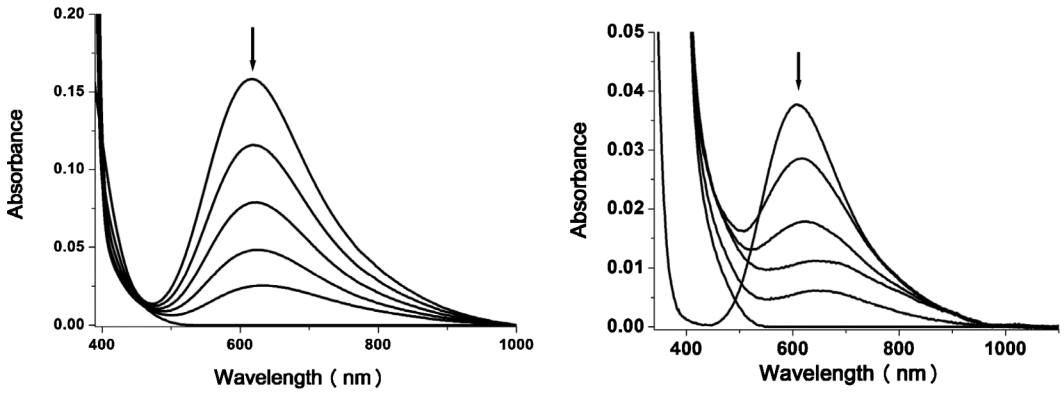
$$[Cu(L)(CH_3CN)]^{2+} + NO_{(g)} \xrightarrow{CH_3CN} [Cu(CH_3CN_4)]^{+} + L' + H^{+} \quad (1)$$

Similar reduction was reported with $[Cu(dmp)_2(H_2O)]^{2+}$ and $[Cu(phen)_2(H_2O)]^{2+}$ (dmp = 2,9-dimethyl-1,10-phenanthroline; phen = 1,10-phenanthroline).[50,51] The striking difference between the these two cases and the present ones is, in the case of $[Cu(dmp)_2(H_2O)]^{2+}$ and $[Cu(phen)_2(H_2O)]^{2+}$, the reduction was observed only in presence of a protic solvent, but not in pure acetonitrile and dichloromethane.[50,51] On the other hand, the Cu(II) centers in $[Cu(tren)(CH_3CN)]^{2+}$, $[Cu(tiaea)(CH_3CN)]^{2+}$ and $[Cu(teaea)(CH_3CN)]^{2+}$ [tren = *tris*(2-aminoethyl)amine; tiaea = *tris*(2-isopropylaminoethyl)amine and teaea = *tris*(2-ethylaminoethyl)amine] were found to undergo reduction in the presence of nitric oxide in pure acetonitrile also.[54,55]



(a) (b)

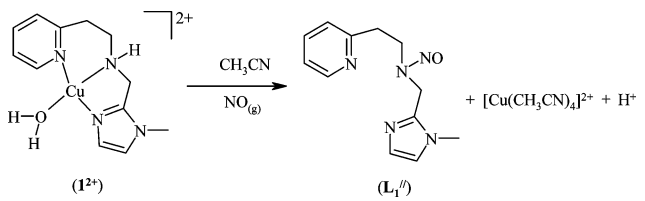
Fig. 2 ORTEP diagrams of complexes (a) **1** and (b) **2** (50% thermal ellipsoid plot). (Hydrogen atoms, perchlorate anions and solvent of crystallization were removed for clarity.)



(a) (b)

Fig. 3 UV-visible spectral changes of complexes (a) **1** and (b) **2** after their reactions with nitric oxide in acetonitrile.

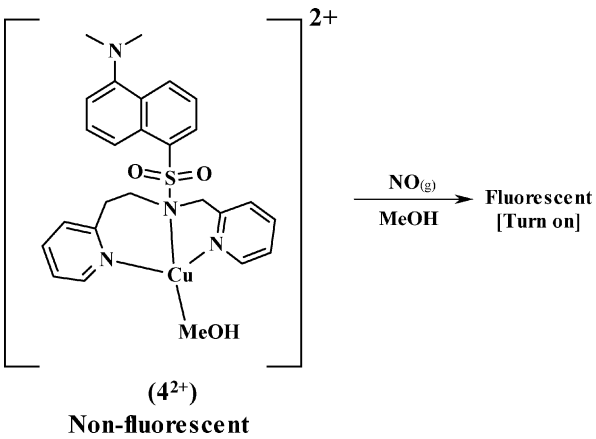
In these cases, the reduction was accompanied with either the N-nitrosation or the diazotization of the primary amine center of the ligand followed by ligand transformation.[54,55] In the present cases, the reduction in acetonitrile medium, led to the N-nitrosation of the ligand (Scheme 3). The N-nitrosation was reported in case of $[Cu(DAC)]^{2+}$ also (DAC = 1,8-bis(9-anthracylmethyl)-derivative of the macrocyclic tetraamine cyclam).[69,70]



Scheme 3

The *d-d* bands at 616 and 606 nm in the case of complexes **1** and **2**, respectively, were studied during the reduction of Cu(II) centers in the presence of nitric oxide (Fig. 3). In the presence of excess NO, the reduction of Cu(II) to Cu(I) was found to follow simple first-order kinetics in water, methanol and acetonitrile media and the rate behavior was observed to be independent of the initial concentration of the complexes. The observed rate constants at 298 K for complexes **1** and **2** are 15.55×10^{-5} s^{-1} and 7.35×10^{-5} s^{-1}, respectively. For $[Cu(tiaea)(CH_3CN)]^{2+}$ and $[Cu(teaea)(CH_3CN)]^{2+}$, the observed rates at 298 K were 5.64×10^{-2} and 6.55×10^{-3} s^{-1}, respectively.[55] The pseudo first order rate constants for $[Cu(pymea)_2]^{2+}$ and $[Cu(baea)(CH_3CN)]^{2+}$ [pymea = pyridine-2-methylamine and baea = bis(2-aminoethyl)amine] are also reported to be 3.10×10^{-3} and 2.20×10^{-3} s^{-1}, respectively, at 298 K.[57] Thus, the rates of reduction of the Cu(II) center to Cu(I) for the present set of complexes are much slower than the earlier reported ones. This is, presumably, because of the difference in ligand denticity and the nature of the N-donor atoms. From eqn (1) and Scheme 3, generation of H^+ is obvious during the reduction, it is expected to be catalyzed by the presence of base. However, no effect of pH was observed on the rates of the reactions indicating that the conjugate base has very little role in the reaction. It would be worth mentioning here that the rate of reductive nitrosylation of ferriheme proteins in aqueous medium is demonstrated to be strongly dependent on the hydroxide ion concentration in the pH range 6–9.[71] This was presumably because of the rate limiting attack of hydroxide ion on the Fe^{III}-coordinated NO.

The NO reduction of copper(II) centers in complexes **1** and **2** can be rationalized by an inner-sphere mechanism involving three steps: (i) reversible displacement of the solvent by NO from the coordination sphere of copper(II) leading to the formation of a inner-sphere $[Cu^{II}–NO]$ intermediate; (ii) nucleophilic attack of H_2O or CH_3OH (in the case of water and methanol



Scheme 4

media) or the generation of highly electrophilic NO^+ owing to [Cu^{II}–NO ↔ Cu^{I}–NO^+] charge distribution followed by the (iii) release of NO_2^- (or CH_3ONO) or N-nitrosated ligand. Step three, perhaps, became more facile owing to the geometrical preference of Cu^{I} complexes for tetrahedral coordination. In our earlier studies with $[Cu(tren)(CH_3CN)]^{2+}$, $[Cu(tiaea)(CH_3CN)]^{2+}$ and $[Cu(teaea)(CH_3CN)]^{2+}$ the formation of the transient [Cu^{II}–NO] intermediate complex was observed.[54,55] Similar instances of nucleophilic attack at the coordinated NO were reported in the reaction of hydroxide ion in [Ru^{II}–NO] to result in the corresponding nitro complexes and also the in the reaction of alcohols with Ir(III)–NO to yield alkyl nitrite complexes.[72,73]

In the present cases, we have not observed any indication of the formation of an [Cu^{II}–NO] inner-sphere complex (Fig. 3). Similarly, with $[Cu(dmp)_2(H_2O)]^{2+}$ and $[Cu(phen)_2(H_2O)]^{2+}$, even at the early stage of mixing no spectral change for the inner-sphere complex formation was reported.[50,51] This can be rationalized in two ways: (i) either the spectral patterns of the complexes **1** and **2** are very similar to their respective [Cu^{II}–NO] intermediates or (ii) the values of the equilibrium constants, K_{NO} are much lower. Since, in FT-IR studies also, no υ_{NO} frequency corresponding to the formation of [Cu^{II}–NO] was observed, the second option is more logical.

The complete reduction of the Cu^{II} centers in complexes **1** and **2** by nitric oxide was further confirmed by X-band EPR studies. Though both the complexes in acetonitrile solvent displayed characteristic EPR spectra, the colorless solutions are found to be silent (Fig. 4). This can be attributed to the reduction of paramagnetic Cu(II) to diamagnetic Cu(I).

The N-nitrosated ligands in both the cases were isolated and characterized completely using various spectroscopic techniques.

NO reactivity of complexes 3 and 4

Since the copper(II) centers in complexes **1** and **2** exhibit rapid reduction in presence of nitric oxide, these in combination with a pendant fluorophore might be good sensors for nitric oxide. Ligands $\mathbf{L_3}$ and $\mathbf{L_4}$ are prepared by incorporating the pendant dansyl group into $\mathbf{L_1}$ and $\mathbf{L_2}$. $\mathbf{L_3}$ and $\mathbf{L_4}$ display moderate fluorescence at room temperature in methanol, water and methanol/water solvent systems. The fluorescence quantum yields were calculated to be 0.198 and 0.177 for $\mathbf{L_3}$ and $\mathbf{L_4}$, respectively, in methanol at room temperature. Metallation with paramagnetic copper(II), resulted in the quenching of the fluorescence intensity of free $\mathbf{L_3}$ and $\mathbf{L_4}$ (Fig. 5, supporting information†). The quenched fluorescence intensity of the ligand fluorophore is expected to be restored on reduction of the copper(II) center by nitric oxide (Scheme 4).[29] The use of complex **3** as a fluorescent sensor of nitric oxide in water and methanol media has already been reported.[56] Complex **4** is also found to detect nitric oxide by fluorescence turn-on in both methanol and pH 7.2 buffered aqueous solutions. Except for a few, all earlier examples of copper(II) complexes as fluorescent sensor in aqueous methanol or in an aqueous pH 7.0 buffer and in cells, were found to detect nitric oxide by a different mechanism, and that involves the reduction of Cu^{II} by nitric oxide followed by dissociation of the N-nitrosated ligand.[56,73] [$Cu(Ds\text{-}en)_2$] and [$Cu(Ds\text{-}AMP)_2$], where Ds-en and Ds-AMP are the conjugate bases of dansylethylenediamine (Ds-Hen) and dansyl aminomethylpyridine (Ds-HAMP), respectively, were reported recently as a probe for fluorescence-based NO detection in aqueous solution.[48,49] However, these Cu(II) dansyl compounds were unable to sense NO at a physiologically more relevant pH.[73]

Addition of an equivalent amount of copper(II) in the methanol solution of $\mathbf{L_4}$, displayed a significant (>85%) quenching of the ligand fluorescence at 298 K (Fig. 5). This has been observed in aqueous medium buffered at pH 7.2 using TRIS-HCl buffer

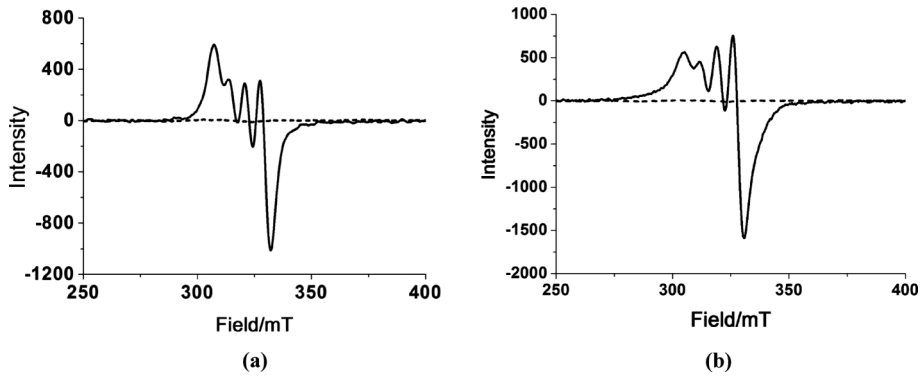


Fig. 4 X-band EPR spectra of the reaction of complexes **1** (a) and **2** (b) with nitric oxide in acetonitrile solvent at room temperature. (solid traces correspond to the respective complexes and dashed traces represent the spectra of the colorless solutions obtained after reaction of the respective complexes with nitric oxide).

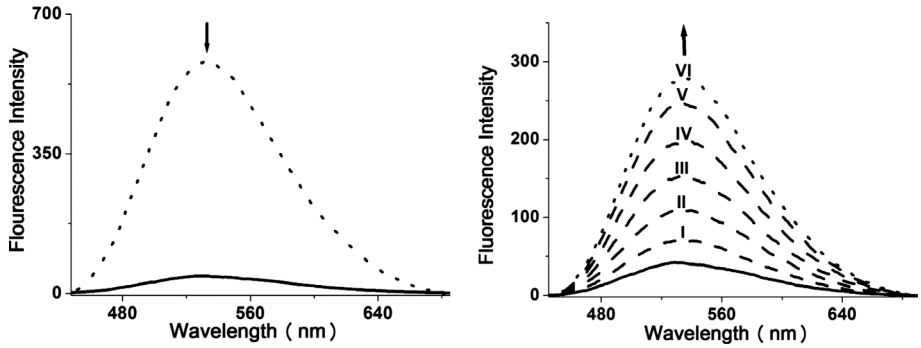


Fig. 5 Fluorescence responses (λ_{ex}, 350 nm) for (a) 25 μM solution of free ligand, $\mathbf{L_4}$ (dotted line) and after addition of one equivalent of $[Cu(H_2O)_6]^{2+}$ in methanol (solid line); (b) for a deoxygenated methanol solution of complex **4** before (solid line) and after (dashed lines) purging of 5 equivalent of NO at 10, 20, 30, 40, 50 and 60 min at 298 K (lines I–VI, respectively).

also (supporting information†). In the case of complex **3**, similar quenching was observed in methanol and water solutions.[56] It has been found that the addition of 2–5 equivalents of nitric oxide into a degassed methanol solution of complex **4** immediately restored the emission intensity significantly. However, the restored emission was observed to be less in aqueous medium buffered at pH 7.2, compared to that in methanol. Similar behavior was observed in the case of complex **3** also.[56] In the case of [Cu(Ds-en)$_2$] and [Cu(Ds-AMP)$_2$], the ligand fluorescence intensity was found to quench to 31(± 2)- and 23(± 0.5)-fold relative to free Ds-Hen and Ds-HAMP (40 μM), respectively, upon addition of Cu(II).[48,49] In buffered aqueous or methanol solution of the complexes [Cu(Ds-en)$_2$] and [Cu(Ds-AMP)$_2$], under anaerobic conditions, the emission intensity was found to be restored significantly upon addition of 100 equivalents of nitric oxide and the enhancements in integrated fluorescence were reported to be 6.1(±0.2)- and 8.8(±0.1)-fold, respectively.[48,49] In the case of complex **3**, the restored emission intensities are found to be 12.5 (±0.2)- and 8.3(±0.2)-fold in methanol and aqueous (at pH 7.2) medium, respectively.[56] Addition of 5 equivalents of nitric oxide to a degassed aqueous (buffered at pH 7.2) or methanol solution of complex **4** restored the emission intensity to 6.7 (±0.2)- and 4.6(±0.2)-fold, respectively. The fluorescence enhancement in the case of the Ds-HAMP ligand is attributed to both the formation of Cu(I) species and dissociation of the sulphonamide functionality following protonation by H^+ formed in the reaction.[48] The dissociation was confirmed by the presence of a sulphonamide group ($\nu_{N-H} \approx 3083$ cm^{-1} in KBr) in the FT-IR spectrum of the reaction product which indicates that ligand protonation also occurs. The protonated sulfonamide group is anticipated to contribute to the fluorescence intensity.[48] On the contrary, in the cases of complexes **3** and **4**, no indication of dissociation of the sulphonamide group was observed in the FT-IR spectra of the reaction products (supporting information†). Hence, the restored emission intensity, in these cases, is attributed to the reduction of Cu(II) center to the diamagnetic Cu(I) by nitric oxide. In the present case, no N-nitrosoamine formation was observed because of the reaction of NO^+, formed during the reaction of Cu(II) with nitric oxide, with methanol solvent to form CH_3ONO and H^+.[48,51] The formation of the NO_2^- in aqueous medium has been established by the Griess's test.[68]

Conclusion

In conclusion, two Cu(II) complexes have been prepared with two tridentate N-donor ligands and their nitric oxide reactivity were studied. The copper(II) centers in complexes **1** and **2** were found to undergo reduction in the presence of nitric oxide in acetonitrile, methanol and water media. No [Cu^{II}–NO] intermediate was observed in the present cases presumably because of very low value of K_{NO}. The reduction in acetonitrile solvent was found to be accompanied by N-nitrosation of the ligands. However, in water and methanol solvents, the formation of NO_2^- and CH_3ONO were observed. Complex **3** has already been reported to behave as nitric oxide sensor in methanol or water media. In the case of complex **4**, addition of nitric oxide to methanol or aqueous (at pH 7.2) solutions resulted in a significant increase in fluorescence intensity. Thus, complex **4** can function as a fluorescence-based nitric oxide sensor. The fluorescence enhancement is attributed mostly to the formation of diamagnetic Cu(I) species after reduction of paramagnetic Cu(II) center by nitric oxide.

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