J Fluoresc (2013) 23:1113–1120
DOI 10.1007/s10895-013-1240-8

ORIGINAL PAPER

Design and Synthesis of a Ruthenium(II) Complex-Based Luminescent Probe for Highly Selective and Sensitive Luminescence Detection of Nitric Oxide

Xiaojing Yu · Run Zhang · Zhiqiang Ye · Bo Song · Jingli Yuan

Received: 25 April 2013 / Accepted: 22 May 2013 / Published online: 2 June 2013

Abstract Nitric oxide (NO) is one of the most important intercellular signaling molecules, and plays important roles in various biological systems. In this work, a unique Ru^{II} complex, tris[(5-(4-methylamino-3-aminobenzylamino)-1,10-phenanthroline)] ruthenium(II) hexafluorophosphate $[Ru(MAA\text{-}phen)_3][PF_6]_2$, has been designed and synthesized as a luminescent probe for the detection of NO in aqueous media. The complex itself is almost non-luminescent, but can specifically react with NO under the aerobic conditions to afford its highly luminescent triazole derivative in aqueous media, $[Ru(MTA\text{-}phen)_3]^{2+}$ (MTA-phen: methyl-trazolebenzylamino-1,10-phenanthroline), accompanied by a 302-fold increase in luminescence intensity at 598 nm with a 130 nm Stokes shift. The luminescence response of $[Ru(MAA\text{-}phen)_3]^{2+}$ to NO is rapid, highly specific without interferences of other reactive oxygen/nitrogen species, and highly stable against the pH changes in the range of pH 4.5–9.5. These features enable $[Ru(MAA\text{-}phen)_3]^{2+}$ to be used as a probe for the highly selective and sensitive luminescence detection of NO in weakly acidic, neutral, and weakly basic media.

Keywords Ruthenium complex · Luminescent probe · Nitric oxide · Luminescence detection

X. Yu · R. Zhang · Z. Ye (✉) · B. Song · J. Yuan (✉)
State Key Laboratory of Fine Chemicals, School of Chemistry, Dalian University of Technology, Dalian 116024, People's Republic of China
e-mail: zhiqiangye2001@yahoo.com.cn
e-mail: jingliyuan@yahoo.com.cn

Introduction

In biosystems, nitric oxide (NO) is a ubiquitous intra- and extracellular signaling molecule produced by nitric oxide synthases. Since NO has only a shorter lifetime and is produced at lower concentrations, a variety of sensitive quantification methods for the detection of NO have been developed based on techniques of chemiluminescence [1], electron paramagnetic resonance [2, 3], electrochemistry [4, 5], colorimetry [6, 7], and fluorimetry [8, 9]. Of which, the fluorescence method using various organic dye-based NO-responsive fluorescent probes, such as the derivatives of fluorescein [10], rhodamine [11], BODIPY [12], and cyanine [13], offers distinct advantages including high selectivity, sensitivity, experimental feasibility, and availability for real-time detection. However, the organic dye-based probes have some disadvantages such as lower water-solubility and photostability, small Stokes shifts, and pH-dependent fluorescence behaviors.

In recent years, luminescent transition metal complexes were also found to be useful luminophores for the developments of various luminescent probes [14–17]. Compared to fluorescent organic dyes, transition metal complexes, especially Ru^{II} complexes with diimine ligands (2,2′-bipyridine, 1,10-phenanthroline, and their derivatives), have some outstanding photochemical and photophysical properties, such as intense visible excitation and emission, high photo-, thermal and chemical stabilities, low cytotoxicity, and large Stokes shifts. On the basis of these properties, some Ru^{II} complex-based luminescent probes have been developed for the detections of anions [18–20], metal cations [21–23], and bioactive molecules [24–28]. In a previous work, we found that a Ru^{II} complex bearing two 2,2′-bipyridine ligands and

one *o*-diamino-substituted 2,2′-bipyridine ligand, $[(Ru(bpy)_2(dabpy)]^{2+}$, could be used as a highly specific luminescent probe for NO [29]. The main drawback of this probe is its lower signal turn-on ratio. After reacting with NO under aerobic conditions, the luminescence of $[(Ru(bpy)_2(dabpy)]^{2+}$ can only be 16-fold enhanced.

To further improve the signal turn-on ratio of the Ru^{II} complex-based luminescent probe for NO, in this work, a Ru^{II} complex bearing three 1,10-phenanthroline derivative ligands, $[Ru(MAA\text{-}phen)_3][PF_6]_2$ [MAA-phen: 5-(4-methylamino-3-aminobenzylamino)-1,10- phenanthroline], was newly designed and synthesized as a luminescent probe for the detection of NO in aqueous media. In the complex, the 4-methylamino-3-aminophenyl group is a specific recognition moiety for reacting with NO, and the Ru^{II}-phen complex core acts as a signaling moiety for responding to the reaction. This complex is almost non-luminescent due to the strong luminescence quenching of the 4-methylamino-3-aminophenyl moiety to the excited-state of the Ru^{II}-phen complex based on a photoinduced electron transfer (PET) mechanism. After the 4-methylamino-3-aminophenyl moiety in the complex is reacted with NO under aerobic conditions to afford its benzotrazole derivative, the complex becomes strongly luminescent with a 302-fold increase in luminescence intensity. This remarkable improvement of the signal turn-on ratio allows $[Ru(MAA\text{-}phen)_3]^{2+}$ to be more useful than $[Ru(bpy)_2(dabpy)]^{2+}$ for the luminescence detection of NO at a lower concentration level. In addition, the luminescence response of $[Ru(MAA\text{-}phen)_3]^{2+}$ to NO is highly specific without interferences of other reactive oxygen/nitrogen species (ROS/RNS), and highly stable against the pH changes in the range of pH 4.5–9.5. These features enable $[Ru(MAA\text{-}phen)_3]^{2+}$ to be used for the detection of NO in weakly acidic, neutral, and weakly basic media. Scheme 1 shows the structure of $[Ru(MAA\text{-}phen)_3]^{2+}$ and its luminescence response reaction to NO under aerobic conditions.

Experimental

Materials and Physical Measurements

5-Amino-1,10-phenanthroline [30], 4-methylamino-3-nitrobenzaldehyde [31] and 1-hydroxy-2-oxo-3-(3-aminopropyl)-3-methyl-1-triazene (NOC-13, a NO donor with a half-life of 13.7 min) [32] were synthesized by using the literature methods. The saturated NO aqueous solution (2.2 mM) was prepared by bubbling NO gas (passing through a degassed KOH solution to remove trace impurities) for 3 h into an argon-deoxidized 0.1 M borate buffer of pH 7.4. The NO concentration was measured by using the Griess method [33]. Unless otherwise stated, all chemical materials were purchased from commercial sources and used without further purification.

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance spectrometer (400 MHz for ^1H, and 100 MHz for ^{13}C). ESI-mass spectra were measured on a HP1100LC/MSD MS spectrometer. Absorption spectra were measured on a Perkin- Elmer Lambda 35 UV–vis spectrometer. Elemental analysis was carried out on a Vario-EL CHN analyser. Luminescence spectra were measured on a Perkin-Elmer LS 50B luminescence spectrometer with the conditions of excitation wavelength, 468 nm; emission wavelength, 598 nm; excitation slit, 10 nm; and emission slit, 10 nm.

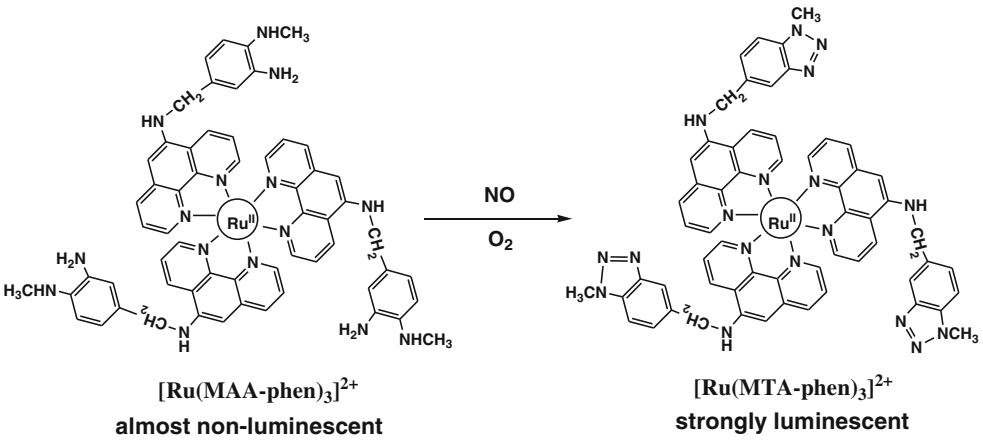
Synthesis of 5-(4-Methylamino-3-Nitrobenzylamino)-1,10-Phenanthroline (1)

A mixture of 5-amino-1,10-phenanthroline (780 mg, 4 mmol) and 4-methylamino-3-nitrobenzaldehyde (720 mg, 4 mmol) in 0.5 mL glacial acetic acid-80 mL anhydrous methanol was refluxed for 24 h with stirring. The red precipitate was filtered, washed with methanol, and dried in vacuum. After the product was dissolved in a mixture of 60 mL ethanol and 30 mL chloroform, $NaBH_4$ (1.52 g, 40 mmol) was added, and the mixture was stirred in a ice-water bath for 1 h and further refluxed for 3 h. The solvent was evaporated, and the residue was dissolved in 150 mL chloroform. The solution was washed once with saturated Na_2CO_3 solution (100 mL) and 3 times with distilled water (100 mL). After organic phase was dried with Na_2SO_4 and evaporated, Compound **1** was obtained as a red solid (1.29 g, 90 % yield). ^1H NMR ($CDCl_3$): δ=3.01 (d, *J*=4 Hz, 3H), 4.44 (s, 2H), 4.96 (s, 1H), 6.66 (s, 1H), 6.81 (d, *J*=12 Hz, 1H), 7.45~7.48 (m, 1H), 7.54~7.60 (m, 2H), 7.95~7.98 (m, 1H), 8.04 (d, *J*= 4 Hz, 1H), 8.22 (d, *J*=4 Hz, 1H), 8.35~8.38 (m, 1H), 8.87~ 8.89 (m, 1H), 8.91 (d, *J*=4 Hz, 1H), 9.15 (d, *J*=4 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ=29.83, 47.31, 100.86, 114.06, 122.14, 122.28, 123.34, 125.11, 125.74, 128.91, 130.19, 131.47, 133.92, 136.10, 140.71, 141.68, 145.84, 146.44, 146.55, 149.92. ESI-MS (m/z): 360.1 $[M+H]^+$.

Synthesis of Tris[5-(4-Methylamino-3-Nitrobenzylamino)-1,10-Phenanthroline]Ru^{II} Hexafluorophosphate (2)

To a solution of $RuCl_3$ (45 mg, 0.22 mmol) in ethanol-H_2O (1:1, v/v, 20 mL), compound **1** (390 mg, 1.09 mmol) in 30 mL ethanol was added. The mixture was refluxed with stirring for 24 h under an argon atmosphere. After filtering, the solvent was evaporated and the residue was purified by silica gel column chromatography using CH_3CN-H_2O-KNO_3 (sat.) (100:10:0.5, v/v/v) as the eluent. The fractions containing the target product were collected, and the solvent was evaporated. The solid was dissolved in 20 mL anhydrous CH_3CN to remove the excess KNO_3 by filtration.

Scheme 1 Structure of $[Ru(MAA\text{-}phen)_3]^{2+}$ and its luminescence response reaction to NO under aerobic conditions



After evaporation, the product was dissolved in a small amount of CH_3CN-H_2O (1:1), and then a saturated solution of NH_4PF_6 was added to give red precipitate. The product was filtered and washed with small amount of water. Compound **2** was obtained (239 mg, 75 % yield). ^1H NMR (CD_3CN): δ=2.98~3.00 (m, 9H), 4.58 (s, 6H), 6.41 (s, 3H), 6.89~6.91 (m, 3H), 6.97~7.00 (m, 3H), 7.32~7.34 (m, 3H), 7.55~7.61 (m, 6H), 7.64~7.67 (m, 3H), 7.94~7.97 (m, 6H), 8.09 (t, *J*=6 Hz, 3H), 8.24 (d, *J*=4 Hz, 3H), 8.62 (t, *J*=6 Hz, 3H). ^{13}C NMR (CD_3CN): δ=29.14, 45.76, 99.55, 114.45, 124.21, 124.28, 124.62, 124.70, 124.75, 124.89, 124.94, 125.67, 125.72, 125.79, 130.58, 131.28, 132.74, 132.77, 132.81, 133.53, 133.57, 136.10, 142.17, 143.34, 145.79, 147.42, 147.53, 148.66, 152.26. Elemental analysis calcd. (%) for $C_{60}H_{51}F_{12}N_{15}O_6P_2 \cdot 2.5H_2O$: C 47.59, H 3.73, N 13.38; found (%): C 47.73, H 3.62, N 13.47. ESI-MS (m/z): 1324.2 $[M\text{-}PF_6]^+$, 589.7 $[M\text{-}2PF_6]^{2+}$.

Synthesis of $[Ru(MAA\text{-}Phen)_3][PF_6]_2$

After a mixture of compound **2** (70 mg, 0.06 mmol), 10 % Pd/C (30 mg) and ethanol (80 mL) was stirred at room temperature for 30 min, 80 % hydrazine hydrate (30 μL) was added. The mixture was refluxed with stirring for 2 h, and then the Pd/C catalyst was removed by filtration. After evaporation, the product was dissolved in a small amount of CH_3CN-H_2O (1:1), and then a saturated solution of NH_4PF_6 was added to give a red precipitate. The product was filtered and washed with small amount of water. $[Ru(MAA\text{-}phen)_3][PF_6]_2$ was obtained (55.9 mg, 85 % yield). ^1H NMR (CD_3CN): δ=2.77 (s, 9H), 3.67 (s, 9H), 4.47 (s, 6H), 6.29 (d, *J*=4 Hz, 3H), 6.51 (d, *J*=8 Hz, 3H), 6.77 (s, 3H), 6.82 (d, *J*= 8 Hz, 3H), 6.92 (s, 3H), 7.29~7.35 (m, 3H), 7.51~7.59 (m, 6H), 7.92~8.00 (m, 3H), 8.08 (t, *J*=6 Hz, 3H), 8.60 (t, *J*= 6 Hz, 3H). ^{13}C NMR (CD_3CN): δ=30.65, 47.63, 99.81, 110.80, 115.11, 119.37, 124.82, 124.88, 125.09, 125.13, 125.17, 125.21, 126.24, 126.29, 136.36, 127.64, 131.21, 133.56, 133.59, 134.01, 135.70, 138.31, 142.60, 144.32, 147.70, 147.80, 149.27, 152.77, 152.88. Elemental analysis calcd. (%) for $C_{60}H_{57}F_{12}N_{15}P_2Ru \cdot 2H_2O$: C 50.92, H 4.34, N 14.85; found (%): C 50.81, H 4.39, N 14.50. ESI-MS (m/z): 1234.3 $[M\text{-}PF_6]^+$, 544.7 $[M\text{-}2PF_6]^{2+}$.

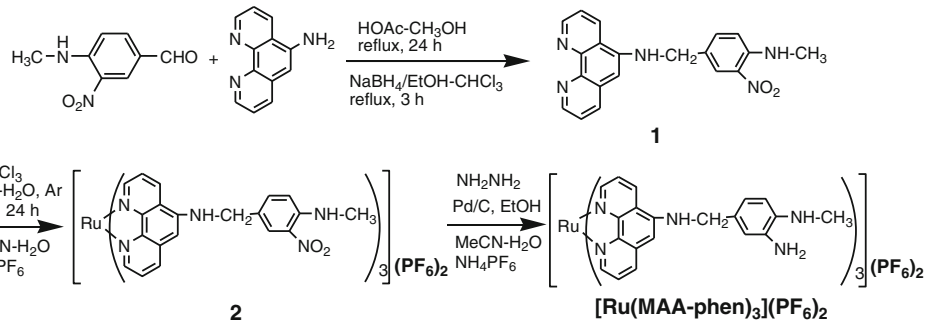
Reactions of $[Ru(MAA\text{-}Phen)_3]^{2+}$ with Different ROS and RNS

All the reactions were carried out in 0.1 M borate buffer of pH 7.4 with the same $[Ru(MAA\text{-}phen)_3]^{2+}$ concentration (10 μM) for 0.5 h at room temperature. Hydrogen peroxide (H_2O_2) was diluted immediately from a stabilized 30 % solution and was assayed by using its molar absorption coefficient of 43.6 m^{-1} cm^{-1} at 240 nm [34]. Hydroxyl radicals (·OH) were generated in the Fenton system from ferrous ammonium sulfate and hydrogen peroxide [35]. Peroxynitrite was synthesized from sodium nitrite (0.6 M) and H_2O_2 (0.65 M) in a quenched-flow reactor (excess H_2O_2 was used to minimize nitrite contamination). After the reaction, the solution was treated with MnO_2 to eliminate the excess H_2O_2. The concentration of the $ONOO^-$ stock solution was determined by measuring the absorbance at 302 nm with a molar extinction coefficient of 1670 M^{-1} cm^{-1} [36]. Singlet oxygen was chemically generated from the ClO^--H_2O_2 reaction [37]. Superoxide solution (O_2^-) was prepared by dissolving solid KO_2 in dry dimethyl sulfoxide (DMSO) and the mixture was stirred vigorously for 10 min before use. Freshly prepared aqueous solutions of NaOCl, $NaNO_2$ and $NaNO_3$ were used as hypochlorite anion (ClO^-), nitrite (NO_2^-), and nitrate (NO_3^-) sources, respectively.

Synthesis of the $[Ru(MTA\text{-}Phen)_3]^{2+}$ Solution

To a solution of 10 μM $[Ru(MAA\text{-}phen)_3]^{2+}$ in 2.9 mL of 0.1 M borate buffer of pH 7.4 was added 105 μL aqueous solution of 100 μM NOC-13 with stirring. After the solution was incubated for 3 h at 37 °C, the reaction was monitored by fluorometry to check the complete conversion of $[Ru(MAA\text{-}phen)_3]^{2+}$ to $[Ru(MTA\text{-}phen)_3]^{2+}$. The reaction

Scheme 2 Reaction pathway for the synthesis of $[Ru(MAA\text{-}phen)_3](PF_6)_2$



product was confirmed by ESI-MS. ESI-MS: m/z=633.5 (corresponding to the molecular ion peak of $[Ru(MTA\text{-}phen)_3]^{2+}$). The above solution was used for the luminescence property characterizations of $[Ru(MTA\text{-}phen)_3]^{2+}$.

Results and Discussion

Design and Synthesis of the Ru^{II} Complex-Based Luminescent Probe for NO

To the development of fluorescent probes for NO, one of the most useful strategies is to couple an electron-rich *o*-diaminophenyl group to various organic fluorophores [10, 12, 13]. In these probes, the *o*-diaminophenyl group plays two roles. One is to quench the fluorescence of fluorophores via a PET mechanism, and the other is to specifically react with NO derived by-products such as N_2O_3 formed by auto-oxidation of NO to form the triazole derivative, which makes the PET process be inhibited, thus to switch-on the fluorescence of fluorophores. Besides *o*-diaminophenyl group, *o*-methylamino-amino-phenyl group can be also used as a dual-functional moiety for the design of NO fluorescent probes to reduce the effect of pH on the fluorescence properties of the probes and their NO-reaction products [11].

In a previous work, we have demonstrated that a Ru^{II} complex bearing two 2,2′-bipyridine ligands and one *o*-diaminophenyl-substituted 2,2′-bipyridine ligand, $[(Ru(bpy)_2(dabpy)]^{2+}$, can be used as a highly selective luminescent probe for the detection of NO [29]. To improve the signal turn-on ratio of the Ru^{II} complex probe, in this work, a new Ru^{II} complex, $[Ru(MAA\text{-}phen)_3]^{2+}$, was designed and synthesized as a luminescent probe for NO. The structure design of this probe is based on the consideration that the luminescence of $[Ru(MAA\text{-}phen)_3]^{2+}$ could be more effectively quenched by three *o*-methylamino-amino-phenyl groups, while the luminescence could be restored to the general level of the Ru^{II}-phen complex after $[Ru(MAA\text{-}phen)_3]^{2+}$ is reacted with NO under aerobic conditions to form the corresponding triazole derivative. Thus the signal turn-on ratio of the probe to respond to NO could be anticipated to be effectively increased.

Based on the above opinion, the complex $[Ru(MAA\text{-}phen)_3](PF_6)_2$ was synthesized according to the procedure as shown in Scheme 2. This complex was synthesized with three-step reactions by using 5-amino-1,10-phenanthroline and 4-methylamino-3-nitrobenzaldehyde as the starting materials. At first, 5-(4-methylamino-3-nitrobenzylamino)-1,10-phenanthroline (compound **1**) was synthesized by refluxing the two starting materials in a HOAc-MeOH mixture and then reducing by $NaBH_4$. After the Ru^{II} complex **2** was synthesized by reacting $RuCl_3$ with compound **1** in a 1:1 mixture of ethanol-H_2O, the target complex $[Ru(MAA\text{-}phen)_3][PF_6]_2$ was obtained by the reduction of the 4-methylamino-3-nitrobenzylamino group to the 4-

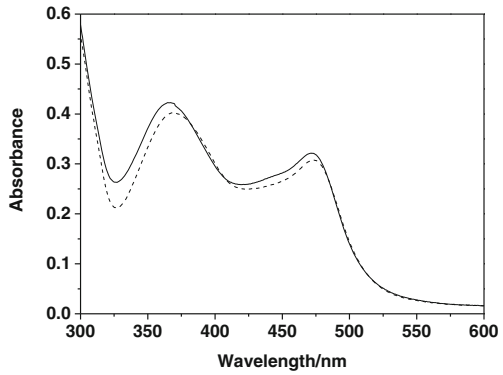


Fig. 1 UV–vis absorption spectra of $[Ru(MAA\text{-}phen)_3]^{2+}$ (10 μM, dash line) and $[Ru(MTA\text{-}phen)_3]^{2+}$ (10 μM, solid line) in 0.1 M borate buffer of pH 7.4

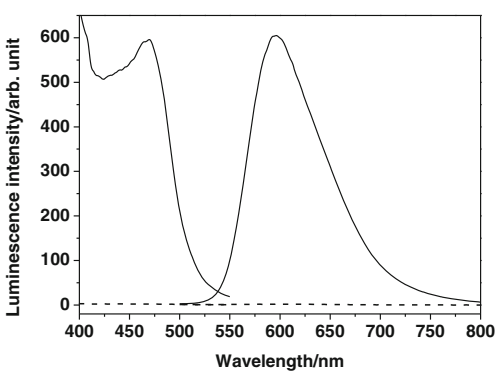


Fig. 2 Excitation (400–550 nm) and emission (500–800 nm) spectra of $[Ru(MAA\text{-}phen)_3]^{2+}$ (10 μM, dash lines) and $[Ru(MTA\text{-}phen)_3]^{2+}$ (10 μM, solid lines) in 0.1 M borate buffer of pH 7.4

Table 1 Luminescence properties of $[Ru(MAA\text{-}phen)_3]^{2+}$ and $[Ru(MTA\text{-}phen)_3]^{2+}$ in 0.1 M borate buffer of pH 7.4 at room temperature

complex	$\lambda_{ex,\ max}$ (nm)	$\varepsilon_{468\ nm}$ ($M^{-1}\cdot cm^{-1}$)	$\lambda_{em,\ max}$ (nm)	ϕ(%)[a]
$[Ru(MAA\text{-}phen)_3]^{2+}$	468	3.01×10^4	598	n.d.[b]
$[Ru(MTA\text{-}phen)_3]^{2+}$	468	3.20×10^4	598	2.3 %

[a] Luminescence quantum yield was measured by using $[Ru(bpy)_3]Cl_2$ (ϕ=2.8 %) [38] as a standard

[b] Luminescence was too weak to be determined

methylamino-3-aminobenzylamino group with hydrazine in the presence of Pd/C catalyst. The new complex was confirmed by NMR, ESI-MS, and elemental analyses.

Luminescence Properties of the Ru^{II} Complexes

To investigate the luminescence properties of $[Ru(MAA\text{-}phen)_3]^{2+}$ and its reaction product with NO, $[Ru(MTA\text{-}phen)_3]^{2+}$, at first, the UV–vis absorption spectra of $[Ru(MAA\text{-}phen)_3]^{2+}$ and $[Ru(MTA\text{-}phen)_3]^{2+}$ in 0.1 M borate buffer of pH 7.4 were measured. As shown in Fig. 1, the two complexes showed almost the same UV–vis absorption spectra in the wavelength range of 300–600 nm with two absorption peaks at ~370 nm and ~468 nm. The absorption at ~370 nm can be belonged to the π–π* transition of the ligands, while the absorption at 468 nm is attributed to the metal-to-ligand charge transfer (MLCT) transition typically observed in the spectra of Ru(II)-diimine complexes.

Figure 2 shows the excitation and emission spectra of $[Ru(MAA\text{-}phen)_3]^{2+}$ (10 μM) and $[Ru(MTA\text{-}phen)_3]^{2+}$ (10 μM) in 0.1 M borate buffer of pH 7.4 at room temperature. The luminescence properties of the two complexes are summarized in Table 1. Although the UV–vis absorption spectra of $[Ru(MAA\text{-}phen)_3]^{2+}$ and $[Ru(MTA\text{-}phen)_3]^{2+}$ are similar, the luminescence spectra of the two complexes are remarkably different. Compared to almost non-luminescent $[Ru(MAA\text{-}phen)_3]^{2+}$, $[Ru(MTA\text{-}phen)_3]^{2+}$ emits strong luminescence with a 302-fold increase in emission intensity. This result indicates that the signal turn-on ratio can be remarkably improved by using $[Ru(MAA\text{-}phen)_3]^{2+}$ as a luminescent probe instead of $[(Ru(bpy)_2(dabpy)]^{2+}$ for the NO detection. Because the luminescence quantum yield of $[Ru(MTA\text{-}phen)_3]^{2+}$ (ϕ=2.3 %) is similar to that of the product of the $[(Ru(bpy)_2(dabpy)]^{2+}$-NO reaction (ϕ= 2.2 %) [29], the remarkable improvement of the signal turn-on ratio using $[Ru(MAA\text{-}phen)_3]^{2+}$ as a probe can be attributed to the big difference of the emission efficiency of $[Ru(MAA\text{-}phen)_3]^{2+}$ and $[(Ru(bpy)_2(dabpy)]^{2+}$, which is too low to be determined for $[Ru(MAA\text{-}phen)_3]^{2+}$ but relatively high (ϕ=0.13 %) for $[(Ru(bpy)_2(dabpy)]^{2+}$ [29]. Since there is no significant difference between the UV–vis spectra of $[Ru(MAA\text{-}phen)_3]^{2+}$ and $[Ru(MTA\text{-}phen)_3]^{2+}$, it can be concluded that the emission efficiency change from $[Ru(MAA\text{-}phen)_3]^{2+}$ to $[Ru(MTA\text{-}phen)_3]^{2+}$ is indeed modulated by the PET mechanism.

Luminescence Response of $[Ru(MAA\text{-}Phen)_3]^{2+}$ Towards NO

To quantitatively investigate the luminescence response of $[Ru(MAA\text{-}phen)_3]^{2+}$ to NO in aqueous media, the excitation and emission spectra of the products of $[Ru(MAA\text{-}phen)_3]^{2+}$ (10 μM) reacted with different concentrations of NOC-13 in the air-saturated 0.1 M borate buffer of pH 7.4 were recorded. As shown in Fig. 3a, upon reaction with NOC-13, the luminescence intensity of $[Ru(MAA\text{-}phen)_3]^{2+}$ was sensitively increased. Interestingly, the luminescence intensity response of $[Ru(MAA\text{-}phen)_3]^{2+}$ at 598 nm to NOC-13 displayed a complicated behavior with three linear dynamic ranges against the NOC-13 concentration increase (Fig. 3b). This result reveals that the reaction of three *o*-methylamino-

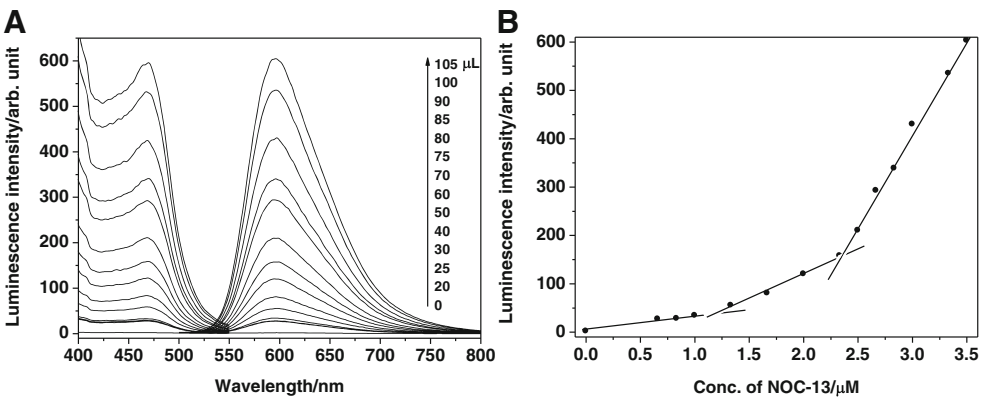


Fig. 3 **a** Excitation and emission spectra of the products of $[Ru(MAA\text{-}phen)_3]^{2+}$ (10 μM) reacted with different amounts of NOC-13 solution (100 μM, 0–105 μL) in 0.1 M borate buffer of pH 7.4 (the total volume of the solution was kept at 3.0 mL). **b** Luminescence intensity (598 nm) responses of $[Ru(MAA\text{-}phen)_3]^{2+}$ to different concentrations of NOC-13

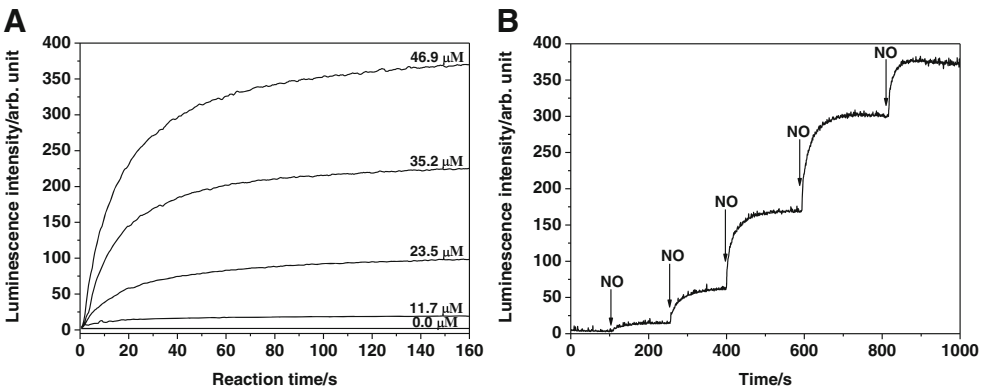


Fig. 4 **a** Luminescence response kinetic curves of $[Ru(MAA\text{-}phen)_3]^{2+}$ (10 μM) to different concentrations of NO in 0.1 M borate buffer of pH 7.4. **b** Real-time luminescence responses of $[Ru(MAA\text{-}phen)_3]^{2+}$ (10 μM) in 3.0 mL of 0.1 M borate buffer of pH 7.4 to the five-time repeat additions of 15 μL saturated NO aqueous solution (2.2 mM)

amino-phenyl groups in $[Ru(MAA\text{-}phen)_3]^{2+}$ with NO generated from NOC-13 is the step-by-step reaction, and during the reaction, three triazole derivatives of $[Ru(MAA\text{-}phen)_3]^{2+}$, $[Ru(MAA\text{-}phen)_2(MTA\text{-}phen)]^{2+}$, $[Ru(MAA\text{-}phen)(MTA\text{-}phen)_2]^{2+}$ and $[Ru(MTA\text{-}phen)_3]^{2+}$, were progressively formed with the increase of the NO concentration.

To investigate the luminescence response kinetics of $[Ru(MAA\text{-}phen)_3]^{2+}$ to NO in aqueous media, the temporal dynamics of the luminescence responses of $[Ru(MAA\text{-}phen)_3]^{2+}$ (10 μM) to the additions of different concentrations of NO in 0.1 M borate buffer of pH 7.4 were determined. As shown in Fig. 4a, the luminescence intensity of $[Ru(MAA\text{-}phen)_3]^{2+}$ itself was very weak and stable under the continuous excitation. Upon additions of different concentrations of NO, rapid increases in luminescence intensity were observed in a few seconds. After ~100 s reaction, the luminescence intensity reached a plateau and remained stable afterwards. Figure 4b shows the real-time luminescence responses of $[Ru(MAA\text{-}phen)_3]^{2+}$ (10 μM) in 3.0 mL of 0.1 M borate buffer of pH 7.4 to the five-time repeat additions of 15 μL saturated NO aqueous solution (2.2 mM). After each addition of NO, the luminescence intensity of $[Ru(MAA\text{-}phen)_3]^{2+}$ was immediately increased to reach the maximum value, and then kept at a steady level. All of above results demonstrate that the reaction of $[Ru(MAA\text{-}phen)_3]^{2+}$ with NO in air-saturated aqueous media is rapid, which is favorable for the detection of short-lived NO in complicated samples to avoid other undesirable reactions.

To evaluate the effect of pH on the luminescence response of $[Ru(MAA\text{-}phen)_3]^{2+}$ to NO, the luminescence intensities of $[Ru(MAA\text{-}phen)_3]^{2+}$ (10 μM) and $[Ru(MTA\text{-}phen)_3]^{2+}$ (10 μM) in 0.1 M phosphate buffers with different pHs were determined. As shown in Fig. 5, when pH is less than 4.5, due to the decrease of the PET efficiency caused by the protonations of the *o*-methylamino-amino-phenyl groups in $[Ru(MAA\text{-}phen)_3]^{2+}$, the luminescence intensity of $[Ru(MAA\text{-}phen)_3]^{2+}$ is gradually increased with the decrease of pH value. However, the luminescence of $[Ru(MAA\text{-}phen)_3]^{2+}$ is weak and stable in the pH range of 4.5 to 9.5, and that of $Ru(MTA\text{-}phen)_3]^{2+}$ is strong and stable in the pH range of 3.0 to 9.5. These results indicate that $[Ru(MAA\text{-}phen)_3]^{2+}$ can be used as a luminescent probe for the detection of NO in weakly acidic, neutral, and weakly basic buffers.

The luminescence response specificity of $[Ru(MAA\text{-}phen)_3]^{2+}$ to NO was also investigated in air-saturated

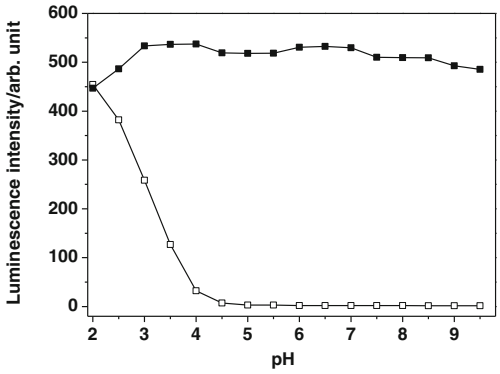


Fig. 5 Effects of pH on the luminescence intensities of $[Ru(MAA\text{-}phen)_3]^{2+}$ (10 μM, □) and $[Ru(MTA\text{-}phen)_3]^{2+}$ (10 μM, ■) in 0.1 M phosphate buffers with different pHs

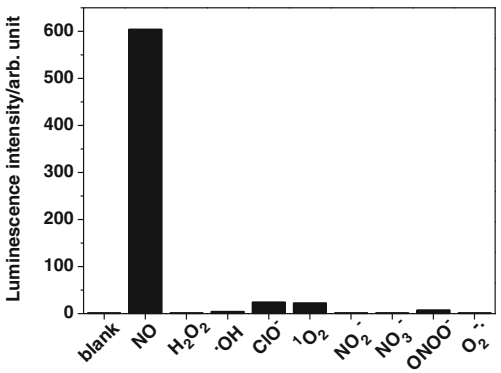


Fig. 6 Luminescence intensities of the products of $[Ru(MAA\text{-}phen)_3]^{2+}$ (10 μM) reacted with various ROS and RNS in 0.1 M borate buffer of pH 7.4. NO: 40 μM; H_2O_2 : 100 μM; $^{\cdot}OH$: 100 μM H_2O_2+ 100 μM $(NH_4)_2Fe(SO_4)_2$; ClO^-: 100 μM NaOCl; $^1O_2$: 100 μM H_2O_2+ 100 μM NaOCl; NO_2^-: 100 μM $NaNO_2$; NO_3^-: 100 μM $NaNO_3$; $ONOO^-$: 100 μM NaONOO; $O_2^{\cdot-}$: 100 μM KO_2

0.1 M borate buffer at pH 7.4. After $[Ru(MAA\text{-}phen)_3]^{2+}$ (10 μM) was reacted with different ROS and RNS, respectively, the luminescence intensity changes at 598 nm were recorded. As shown in Fig. 6, the luminescence intensity of $[Ru(MAA\text{-}phen)_3]^{2+}$ did not give any significant responses to the additions of H_2O_2, $^{\cdot}OH$, ClO^-, $^1O_2$, NO_2^-, NO_3^-, $ONOO^-$ and O_2^-, whereas it was remarkably increased after treated with NO. These results demonstrate that the luminescence response of $[Ru(MAA\text{-}phen)_3]^{2+}$ to NO is highly specific without interferences of other ROS/RNS.

Conclusions

By incorporating 4-methylamino-3-aminobenzyl group into 1,10-phenanthroline ligand, a Ru^{II} complex-based luminescent probe for NO, $[Ru(MAA\text{-}phen)_3]^{2+}$, was successfully developed for the highly selective and sensitive luminescence detection of NO in aqueous media. The almost non-luminescent probe can specifically react with NO in the air-saturated aqueous media to afford its highly luminescent derivative, $[Ru(MTA\text{-}phen)_3]^{2+}$, accompanied by a 302-fold luminescence enhancement. Compared to the previously reported NO probe $[Ru(bpy)_2(dabpy)]^{2+}$, the new probe shows remarkable improvement on the signal turn-on ratio, which enables it to be favorably useful for the detection of NO at a lower concentration level. The new probe has the advantages of visible-light excitation and emission with a large Stokes shift, good water-solubility, widely available pH range, and rapid luminescence response to NO with high specificity and sensitivity, which would be anticipated to be a useful tool for the detection of NO in various chemical and biological systems.

Acknowledgments Financial supports from the National Natural Science Foundation of China (Nos. 20975017, 21275025) and the Fundamental Research Funds for the Central Universities (No. DUT12LK41) are gratefully acknowledged.

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