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Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Unique norbornene based triazole molecule for selective Fe(II) sensing

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Triazole functionlized norbonene monomer (NFTZ) and it's corresponding homopolymer (NFTZH) are synthesized. Unique fluorescence property of NFTZ molecule is due to the conjugation of two aromatic ring through amide bond. Chelating behaviour of NFTZ monomer is explored through selective binding of Fe (II) in the presence of other metals. MALDI-TOF analysis and Job's plot clearly confirm the 1:2 mode of binding of NFTZ with Fe (II). ^{1}H NMR and IR spectroscopy clearly confirm the unique amide-iminol tautomerisation.

Fluorimetric quenching technique has been widely applied as the sensing response in metal ions detection. Mainly, the quenching process can be divided into two kinds of mechanism namely static and dynamic. For example, the static quenching mechanism is observed during estimation of Fe (III) and inorganic phosphate in blood serum by polyfluorene derivative.[1,2] On the contrary, dynamic quenching mechanism is observed while quantifying the molecular oxygen in aqueous and non-aqueous solutions.[3] In general static quenching is very common with respect to dynamic quenching. In this report, a sensor for Fe (II) with static quenching mechanism is demonstrated. As the life-time values are not changed while titrating the probe with the Fe (II) ion, it is believed that a static quenching mechanism is followed while sensing.

Iron is one of the most common elements among the all metals. Specially, Fe (II) is very essential element to all living organisms owing to its potent redox chemistry and possibility to engage in catalytic activity. If the Fe (II) concentration is not measured properly, it can lead to unrestrained oxidative chemistry causing tissue damage and fibrosis to various organs.[4] Although biological iron is observed frequently as Fe (II) in enzymes and storage proteins, the existence of labile iron within cells is controversial, resulting in high demand for selective sensors for iron.[5-8]

Compared to the number of fluorescent sensors for other transition metal ions, Fe (II)-specific fluorescent sensors are quite rare except very few examples.[9] Recently, "Calcein" with an EDTA-derived receptor has been reported to detect Fe (II) ions with ten-fold higher selectivity for Fe (II) over Fe (III).[10]

Herein we describe the synthesis and characterization of triazole functionlized norbornene monomer **(NFTZ)** and it's homopolymer **(NFTZH)**. Metal specific terminal chelating property of triazole molecule is studied with Fe (II) in presence of other metals. Quenching of fluorescence emission is observed as response. IR, Mass and NMR spectroscopic techniques clearly confirm the binding of Fe (II) to **NFTZ** monomer. To best of our knowledge this is the efficient method that clearly sense the Fe (II) over other metals particularly, Fe (III). Exo-oxabicylo[2.2.1]hept-5-ene-2,3-dicarboxylic anhydride was prepared following the reported procedure.[11] 3-(4-Carboxyphenylcarbamoyl)-7-oxabicyclo[2.2.1]hept-5-ene-2-carboxylic acid was prepared by using 4-aminobenzoic acid and of acetic anhydride in the presence of sodium acetate in dimethyl formamide solvent. Product formation was confirmed by ^{1}H NMR, FT-IR and mass spectroscopy techniques. Norbornene functionlized triazole (**NFTZ**) molecule was prepared by coupling of nadic acid and 3- amino-1,2,4-triazole-5-thiol using N,N'-dicyclohexylcarbodiimide as coupling reagent in dimethylformamide solvent. Formation of the product was confirmed by ^{1}H NMR, ^{13}C NMR and IR spectroscopy. Appearance of new proton signal at δ 7.9 (s,1H) was responsible for amide hydrogen which supported that triazole ring attached with -COOH group of nadic acid. The signals at δ 7.5 (s, 2H) & δ 7.2 (s, 2H) were responsible for the aromatic protons while signals at δ 6.5 (s, 2H) δ 5.3 (s, 2H) & δ 3.2 (s, 2H) were responsible for oxo-norbornene protons (Figure S1). In ^{13}C NMR spectrum, signal at δ 165 ppm was responsible for the attachment of triazole amino group with nadic acid. Further the presence of signals at δ 151 & δ 148 clearly indicated the formation of the product (Figure S2) The stretching frequency at 3244 cm^{-1} for free carboxylic acid was shifted to 3268 cm^{-1}, which indicated the formation of amide group as shown in the supporting information (FigureS5). All the monomers were characterized by micro-mass spectrometer (Q-TOF), using acetonitrile as solvent. Observed mass (m/z) and calculated mass for all the monomers were in good agreement, which confirmed the formation of product (Figure 2).

Next, the homo polymerization of **NFTZ** was carried out by using second generation Grubbs' catalyst at room temperature in dry dichloromethane solvent and was monitored through NMR.[12] (SI Scheme 2) New signals were observed at δ 5.4 ppm and δ 4.9 ppm, suggested the formation of **NFTZH.** The molecular weight

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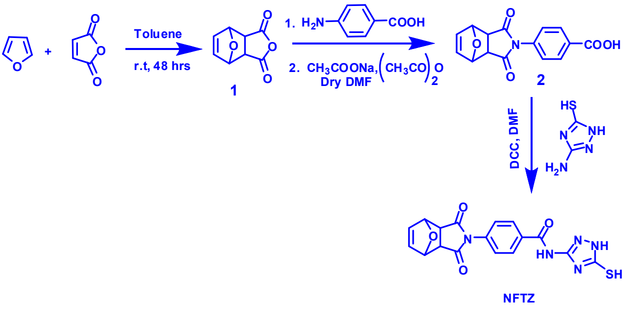
of **NFTZH** (**M_n** = 19000 with PDI 1.2) was determined by gel permeation chromatography (Figure S3).



Scheme 1. Synthesis of triazole functionlized **NFTZ** monomer

The unique bluish-white (Figure S8) emission from **NFTZ** was first observed while monitoring the product formation by thin layer chromatography (TLC) under the UV light. Both the compound, nadic acid and 3-amino-1,2,4-triazole-5-thiol were not showing any fluorescence property. But when both the compound attached through coupling reaction it showed the fluorescence. We hypothesized that it could be because of two aromatic group attached through amide bond and therefore the **NFTZ** created a long conjugative system where electron transfer was possible and hence the molecule was emissive in nature.[13] The absorbance maximum was found to be 350 nm for **NFTZ** in THF (Figure S6). Because of the transition of electron from nitrogen lone pair to the of norbornene double bond (n to π* transition) we observed an absorption at 350 nm. But as it was not in the visible region, we could not see any colour in **NFTZ** monomer. Quantum yield (φ) = 0.18 of compound **NFTZ** was calculated from its absorption and emission spectrum taking quine sulphate as standard. Optical events generated by inducing alternation to the optical properties of fluorophores through associating metal ions with their binding moieties could be witnessed either by the shift in wavelength (bathochromic/ hypsochromic) or quenching of the fluorescence intensity. Due to the presence of chelating functionality (terminal triazole ring), the molecule **NFTZ** was chosen to study the viability of metal binding which would facilitate its association with metal ions. Salts of Pb (II), Ba (II), Mn (II), Hg (II), Fe (II), Fe (III), Co (II), Ni (II), Cu (II), and Cd (II) (with concentrations of 0.015 M in water) were titrated with 1 mg/ml in THF and monitored through fluorescence. Addition of $FeSO_4$ in water to a solution of **NFTZ** in THF/water (1: 1) caused immediate quenching. On the contrary, addition of the other metals to solutions of **NFTZ** resulted in little or no effect on **NFTZ**. Titrating aqueous solutions of Fe (II) metal salts (sulfate) caused large quenching in the fluorescence of **NFTZ** about 90% reduction in fluorescence intensity occurred, which was clearly implying the preferential binding of **NFTZ** towards Fe (II) (Figure 1 a,b).[14] The instantaneous quenching of **NFTZ** by Fe (II) was first explained by ^{1}H NMR spectroscopy which clearly showed that aromatic region signals at 7-8 ppm as well as norbornene peak were shifted to upfield region along with that at **1.9** ppm region a new signal came because of unique amide-iminol tautomerisation.[15] As there was equlibrium between –CONH and -C(OH)=N, little amount of -NH functionality was still there in the reaction medium which was also responsible for binding to Fe (II) and therefore in ^{1}H NMR spectroscopy a upfield shift was observed. (Figure 3). This binding was further supported by I.R. spectra where about 30 cm^{-1} shifting in the position –NH stretching frequency which clearly proved that there was a strong binding interaction between **NFTZ** to Fe (II) (Figure S5).

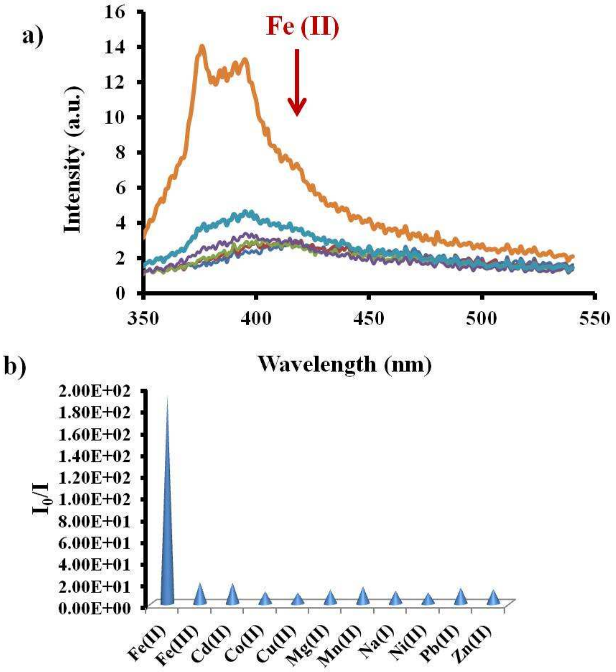


Figure 1. (a) Emission spectra of **NFTZ** as a function of increasing Fe (II) concentration. The spectra was collected in THF with an excitation wavelength (Ex) of 350 nm. (b) Selectively binding of **NFTZ** to Fe (II) over other metal ions**.**

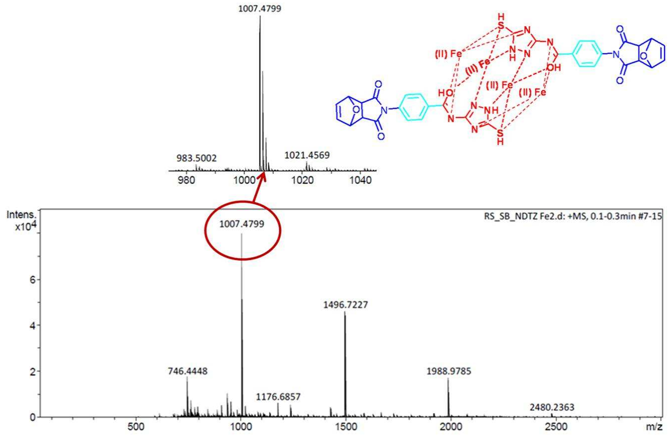


Figure 2. MALDI-TOF analysis of triazole functionlized **NFTZ** monomer coordinated with Fe (II) which is clearly showing Fe (II) is coordinated 1:2 fashion with triazole monomer.

The proposed complex formation was strongly supported by MALDI-TOF analysis which shows 1:2 binding mode (binding of triazole molecule to iron: **NFTZ: Fe (II) = 1:2**) (Figure 2). The calculated m/z value was (2 **NFTZ** + 4 Fe (II) + NH$_4^+$**)** 1007.47

(which is the characteristic peak of Fe (II) isotope) where as observed m/z value was 1007.36.[16] MALDI (Matrix-assisted laser desorption/ionization) is a soft ionization technique used in mass spectrometry. In the ionization mechanism, it is hypothesized that the matrix ions, such as, H^+, Na^+ and NH_4^+normally has been transferred to the analyte. As a result when we analysed the mass spectrum we observed the spectrum as a added value of NH_4^+. ESI-Mass spectra was further supported our MALDI-TOF analysis data (Figure S7). Job's plot (a continous variation method) was providing strong confirmation for the stoichiometry of **NFTZ**/Fe (II) was followed.[17] The results confirmed that 1:2 binding nature in the **NFTZ**-Fe(II) complex (Figure 4 b).

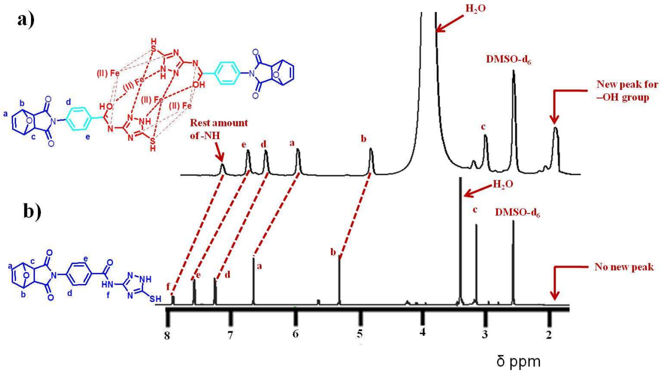
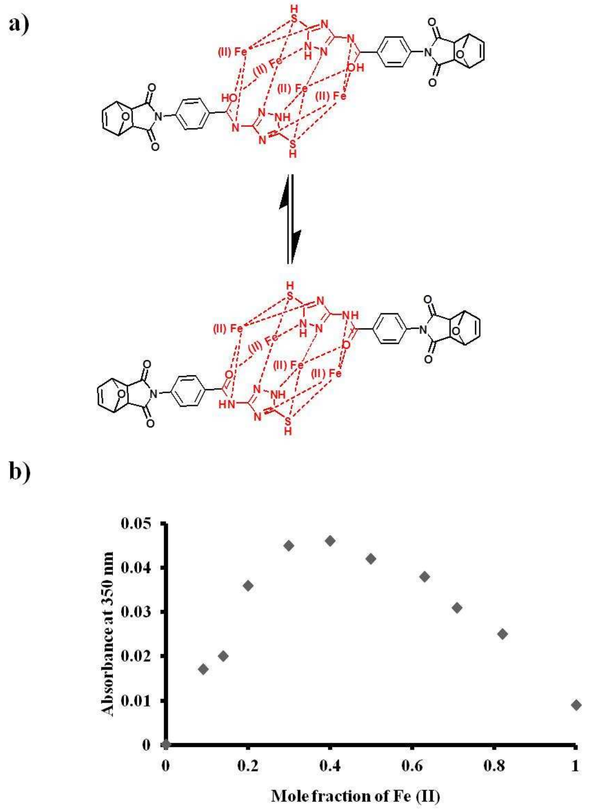


Figure3: (a) ^1H NMR spectra of **NFTZ**+ Fe (II) complex. (b) ^1H NMR spectra of **NFTZ** monomer. Here marked Peak was shifted after the addition of Fe (II) to **NFTZ** monomer along with a new signal at 1.9 ppm responsible for hydroxyl group which is absent in **NFTZ** NMR spectra.



4 (a) Possible binding mode of Fe (II) with **NFTZ** monomer along with amide-iminol tautomerise structure. (b) Job's plot of **NFTZ** monomer and Fe (II) The absorption at 350 nm was plotted against the mole fraction of Fe (II) at a constant total concentration [Fe (II) + **NFTZ** monomer] of 80 M showing that 1:2 binding stoichiometry in 1:1 (MeOH:H_2O).

It is well-known in the literature that $FeSO_4$ salt is ESR active.[18] However, it is also well documented that low spin Fe (II) complex does not give ESR signal.[19] From the ESR experiment, we came to know that when **NFTZ** formed complex with Fe (II) the complex did not give signal in ESR spectrum. From this experiment it was clear that **NFTZ** monomer complexed with Fe (II) (low spin) in the specific structural orientation. To evaluate the sensitivity of this binding system, we determined the Stern-Volmer quenching constant K_{sv} which was given by the Stern-Volmer equation, [5] $I_0/I = 1 + K_{sv}[Q]$. At low concentration, I_0/I increased linearly as the concentration of quencher increase. On the basis of the correlation curve, a linear range of the plot was from 1.3 × 10^{-7} M to 1.1 ×10^{-6} M and the K_{sv} (quenching constant) was calculated to be 1.5 × 10^5 M^{-1} (Figure S4).[20] The analytical detection limit of chemosensor, **NFTZH,** for the detection of Fe (II), determined by standard methods was found to be 1.3 × 10^{-7} M (Figure S9) .

In case of homopolymer of **NFTZ** showed same kind of fluoremetric response in presence of Fe (II). Homopolymer **NFTZH** was more useful in the sensing application as without the need of special equipment we could sense Fe (II) just by polymer-coated paper strips, where as in the case of monomer it leeched out from the paper.[21] Therefore, towards the motivation of developing paper strips, homopolymer (**NFTZH**) was synthesized to demonstrate versatile utility of our unique sensing system. As the more number of terminal chelating functionality (triazole moiety) is present in case of polymer, Stern Volmer quenching constatnt (K_{sv}) of **NFTZH** is 2.3 × 10^5 M^{-1} , which is higher than it's monomer analogue (**NFTZ**).

In conclusion, norbornene functionlized triazole monomer (**NFTZ**) was synthesized and thoroughly characterized by ^1H, ^{13}C and mass spectroscopic analysis. Unusual fluorescence property of the monoer and polymer was explored through Fe (II) quenching process. The unique binding mode of **NFTZ** with Fe (II) was supported by mass and IR spectroscopic analysis. Job's plot and ESR analysis further confirmed the nature of binding and specific structural orientation of **NFTZ**-Fe (II) complex. In ^1H NMR spectra supported the amide-iminol tautomerised structure. The highly sensitive sensing nature of **NFTZ** and **NFTZH** suggested the uniqueness of the design.

ACKNOWLEDGMENT

SB thanks CSIR, New Delhi for the fellowship. RS thanks DST, New Delhi for Ramanujan Fellowship. RS thanks IISER-Kolkata for the infrastructure.

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