

# **DESIGN AND SYNTHESIS OF TURN-ON FLUORESCENT SENSORS FOR THE DETECTION OF ZINC IONS**

BY

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## **Dedications**

Dedicated to my Parents, who loved me unconditionally and teach me to work hard for the goals that I aspire to achieve. This work is also dedicated to my Wife, Brothers, Sisters, Teachers and Friends who have been a source of encouragement and continued support during the course of my studies and life endeavors. Surely, I am thankful to have you all in my life.

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## ABSTRACT

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The design and synthesis of seven chemosensors for  $\text{Zn}^{2+}$  detection in aqueous solutions are described. Chemosensors **1** and **2** contain dinitrophenol and 7-nitro-2,1,3-benzoxadiazole as a fluorophore, respectively. The fluorophores of both these chemosensors are attached to the 2,2'-dipicolylamine (DPA) receptor through 4,4'-oxydianiline. These sensors exhibited 3-fold and 2.4-fold enhancement in fluorescence intensity, respectively, upon the addition of 1 equiv. of  $\text{Zn}^{2+}$ . Moreover, these sensors displayed higher selectivities for  $\text{Zn}^{2+}$  in the presence of other competing ions, displaying detection limits of 124 nM and 1.06  $\mu\text{M}$ , respectively.

Likewise, three new 1,8-naphthalimide-based fluorescent sensors (**3-5**) for  $\text{Zn}^{2+}$  detection in an aqueous solution were synthesized. The structural architect of these sensors contains a 1,8-naphthalimide scaffold as a fluorophore attached to 2,2'-dipicolylamine (DPA) and bis(2-quinolinylmethyl)amine (DQA) receptors through an amide linkage. The addition of  $\text{Zn}^{2+}$  to the solutions of sensors (**3-5**) led to enhanced fluorescence intensity, ranging between 2.5 to 14 folds. At physiological pH (pH = 7.4), these sensors exhibited high selectivities for  $\text{Zn}^{2+}$  in the presence of similar competing ions, displaying detection limits of 0.12  $\mu\text{M}$ , 81.7 nM and 79.2 nM, respectively. This suggests their ability to detect the

chronic concentration of  $\text{Zn}^{2+}$  for freshwater ( $>1.84 \mu\text{M}$ ), designed by EPA (US). DFT simulations were performed on the more stable stacked conformations of unbound and  $\text{Zn}^{2+}$  bounded states of these sensors, which revealed that the latter display a higher density of excited states than the unbound sensors. Moreover, the stacked conformer of sensor **5** was significantly more stable as compared to sensors **3** and **4**, which was attributed to a stronger Van Der Waals (VDW) interaction between DQA and 1,8-naphthalimide. The  $\text{Zn}^{2+}$  binding leads to enhanced electronic coupling between the HOMOs and LUMOs, making excited states more populated which then undergo geometric relaxation before emitting light and relaxing back to the ground states.

In addition, this study describes the design and synthesis of two new fluorescent sensors **6** & **7** for the highly sensitive and selective detection of  $\text{Zn}^{2+}$ . The synthesis of chemosensors **6** and **7** was accomplished through easy synthetic procedures in a high-yielding reaction sequence. Titration experiments were performed to measure the responses of sensors **6** and **7** to the varying concentrations of neat  $\text{Zn}^{2+}$  and  $\text{Zn}^{2+}$  in the presence of other bio-relevant metal ions ( $\text{Cu}^{2+}$ ,  $\text{Cr}^{2+}$ ,  $\text{K}^{1+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$  and  $\text{Cd}^{2+}$ ), using UV and fluorescence spectroscopy. Extensive DFT simulations were performed to examine the impact of  $\text{Zn}^{2+}$  binding on the absorption spectra of **6** and **7**. The synergistic coordination of the N-3 atom of 1,2,3-triazolyl function and 2,2-dipicolylamine (DPA) receptor unit with  $\text{Zn}^{2+}$  enables highly sensitive and selective detection of  $\text{Zn}^{2+}$ , with a limit of detections (LOD) of 65.6 nM and 107 nM, respectively. At a physiological pH (pH = 7.4), the addition of  $\text{Zn}^{2+}$  to the solutions of **6** and **7** led to 4.7-fold and 6-fold enhancements in the fluorescence intensities, respectively. However, both sensors remain almost insensitive toward the competing ions. Job's plot analysis suggests the

formation of sensor/ $\text{Zn}^{2+}$  in 1:1. DFT simulations studies revealed that both sensors possess excellent binding with  $\text{Zn}^{2+}$ . The total change in energy after the complexation was -86.6 for **6** and -89.3 for **7** hinting their high thermodynamic stabilities. In the unbound states, the highest occupied molecular orbital (HOMO) electronic density was localized onto the DPA receptor, used for the coordination with  $\text{Zn}^{2+}$  whereas the lowest unoccupied molecular orbitals (LUMOs) density was on the fluorophores. Upon coordination with  $\text{Zn}^{2+}$ , however, the HOMOs and LUMOs were found to be localized on the fluorophores of **6** and **7**. The insertion of dinitrobenzene in **6** exhibited higher fluorescence enhancement as compared to **7** upon binding to  $\text{Zn}^{2+}$ .

|

## ملخص الرسالة

الاسم الكامل: توفير احمد

عنوان الرسالة: تصميم وتوليف أجهزة استشعار الفلورسنت الدوارة للكشف عن أيونات الزنك

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تم وصف تصميم وتركيب سبعة مستشعرات كيميائية للكشف عن أيون الزنك  $Zn^{2+}$  في المحاليل المائية. تحتوي المستشعرات الكيميائية 1 و 2 على ثنائي نتروفينول و 7-نيرو-2،1،3-بنزوكساديازول على شكل فلوروفورات على التوالي. الفلوروفورات لكل من هذه المستشعرات الكيميائية أرفقت بمستقبل 2،2'-ثنائي بيكوليل أمين (DPA - 2،2') من خلال 4،4'-اوكسي ثنائي أنيلين 4،4'-oxidianiline. أظهرت هذه المستشعرات تعزيزًا بمقدار 3 أضعاف و 2.4 ضعفًا في كثافة الاستشعار على التوالي، عند إضافة 1 مكافئ لأيون الزنك. بالإضافة إلى ذلك، أظهرت هذه المستشعرات انتقائية عالية لأيون الزنك  $Zn^{2+}$  في وجود أيونات منافسة مماثلة، حيث أظهرت حساسيات عالية بحد اكتشاف يبلغ 124 نانومتر و 1.06 ميكرومتر، على التوالي. بالمثل، تم أيضاً إصطناع ثلاثة مستشعرات استشعارية جديدة تعتمد على 1،8-نافثاليميد (naphthalimide - 1،8) للكشف عن أيون الزنك  $Zn^{2+}$  في محلول مائي. الهندسة التركيبية لهذه المستشعرات تحتوي على سقالة 1،8-، نفثاليميد (3-5) مرتبط بمستقبلات 2،2'-ثنائي بيكوليل أمين (DPA - 2،2') و بس (2-كوينولينيل ميثيل) أمين كفلوروفور مرتبط بمستقبلات 2،2'-ثنائي بيكوليل أمين (DPA - 2،2') و بس (2-كوينولينيل ميثيل) أمين (DQA (bis(2-quinolinylmethyl) amine من خلال رابط أميد. إضافة الزنك ( $Zn^{2+}$ ) إلى محاليل المستشعرات (3-5) أدت إلى تعزيز كثافة الاستشعار، والتي تتراوح بين 2.5 إلى 14 ضعفًا. في درجة الحموضة الفسيولوجية  $P^H$  (الرقم الهيدروجيني = 7.4)، هذه المستشعرات أظهرت انتقائية عالية لأيون الزنك ( $Zn^{2+}$ ) في وجود أيونات منافسة مماثلة، عارضاً الحساسيات العالية بحد الاكتشافات عن 0.12 ميكرون، 81.7 نانومتر، و 79.2 نانومتر على التوالي. يشير هذا إلى أن هذه المستشعرات يمكنها الكشف عن تركيز الزنك  $Zn^{2+}$  المزمّن للمياه العذبة (< 1.84 ميكرومتر)، المحدد من قبل وكالة حماية البيئة الأمريكية. عمليات محاكاة نظرية دالة الكثافة DFT التي تم إجراؤها على المطابقات المكسدة الأكثر استقرارًا للحالات غير المقيدة و المقيدة بالزنك  $Zn^{2+}$  اقترحت بأن الأخيرة تعرض كثافة أعلى للحالات المثارة من المستشعرات غير المقيدة. علاوة على ذلك، كان المطابق المكسّد للمستشعر 5 أكثر استقرارًا بشكل ملحوظ مقارنة بالمستشعرات 3 و 4، والذي يُعزى إلى تفاعل

فاندر فالز (Van Der Waals (VDW) الأقوى بين DQA و naphthalimide-1,8. ارتباط الزنك  $Zn^{2+}$  يؤدي إلى إقتران إلكتروني مُعزز بين أعلى المدارات الجزيئية المشغولة HOMOs و أدنى المدارات الجزيئية غير المشغولة LUMOs، مما يجعل الحالات المثارة أكثر اكتظاظًا بالسكان والتي تخضع بعد ذلك للاسترخاء الهندسي قبل انبعاث الضوء والاسترخاء مرة أخرى إلى الحالة الأرضية. الفصل المنخفض للطاقة (5.0 فولت) بين أعلى المدار الجزيئي المشغول HOMO وأول مدار  $Zn^{2+}$  d في المستشعر 5 مقارنة بالمستشعرات 3 و 4 ينتج عنه كثافة معززة للحالات المتولدة وبالتالي كثافة أعلى عند الارتباط بالزنك  $Zn^{2+}$ .

تصميم وتوليف اثنين من أجهزة الاستشعار الفلورسنت الجديدة 6 و 7 تم وصفهما. الهندسة التركيبية لـ 6 و 7 يحتوي على وحدة مستقبيلات 2،2'-ثنائي بيكوليل أمين (DPA) (2,2'-dipicolylamine) مرتبطة من خلال وظيفة 1،2،3-تريازوليل إلى الفلوروفورات (سفالة 4،2-نيتروفينيل أو 8،1-نافثاليميد). التنسيق التآزري لـ 3-N لوظيفة 1،2،3-triazolyl وشق DPA مع الزنك  $Zn^{2+}$  يتيح 6 و 7 للكشف عن الزنك  $Zn^{2+}$  بطريقة انتقائية وحساسة للغاية، مع حد من الاكتشافات (LOD) تبلغ 65.6 نانومتر و 107 نانومتر، على التوالي. عند درجة الحموضة الفسيولوجية (الرقم الهيدروجيني = 7.4)، أدت إضافة  $Zn^{2+}$  إلى المحلول 6 و 7 إلى تحسينات 4.7-fold و 6.0-fold في شدة التألق، على التوالي. علاوة على ذلك، أظهرت هذه المستشعرات انتقائية عالية للزنك  $Zn^{2+}$  على أيونات المعادن الأخرى المتنافسة. قدرات التتبع لهذه المستشعرات تشير إلى أنها يمكن أن تكون مفيدة للكشف عن تركيز الزنك  $Zn^{2+}$  المزمّن للمياه العذبة (< 1.84 ميكرومتر)، الذي حددته وكالة حماية البيئة الأمريكية. محاكاة نظرية دالة الكثافة DFT أجريت لفحص تأثير ارتباط الزنك  $Zn^{2+}$  على أطياف الامتصاص 6 و 7. أظهر كلا المستشعرين ارتباطاً ممتازاً مع الزنك  $Zn^{2+}$  مع ثبات ديناميكي حراري عالي. علاوة على ذلك، فإن الاقتران الإلكتروني المُحسّن لربط الزنك  $Zn^{2+}$  بين HOMOs و LUMOs، والذي بدوره يسكن حالات أكثر إثارة مع احتمال أكبر للتحويلات الإلكترونية. علاوة على ذلك، في المستشعرات المرتبطة بالزنك  $Zn^{2+}$ ، متضمن HOMOs الأعمق و LUMOs الأعلى في التحويلات الإلكترونية. مدارات  $Zn^{2+}$  لا تساهم في الانتقال الإلكتروني ولكن تم تحديد موقعها بعمق في مستويات الطاقة و فصل الطاقة بين HOMO وأول مدار  $Zn^{2+}$  d في 6 و 7 وُجد علي أنها 7.46 و 6.94 فولت، على التوالي. إدخال ثنائي نيترو بنزين في 6 أظهر تعزيز إستشعاع أعلى مقارنة بـ 7 عند الارتباط بـ  $Zn^{2+}$ .



# CHAPTER 1

## INTRODUCTION

Zinc ion ( $\text{Zn}^{2+}$ ) is one of the essential metals in the human body that is implicated in various biological processes such as pathology, brain function [1], gene transcription [2], immune function [3], DNA binding, apoptosis [4,5], mammalian reproduction [6] and muscle contraction [7]. As second most abundant metal ion after iron, zinc mainly exists inside cells and involves in the activation of approximately over three hundred enzymes [8,9]. In biological systems it is exclusively found in divalent form. Further to its role as a Lewis acid or structural cofactor in enzymes and other proteins, it is accumulated in various cell types as labial  $\text{Zn}^{2+}$  pools for proper functioning. For example, various evidence signals that synaptic plasticity is mediated by ionic burst of  $\text{Zn}^{2+}$  released during neuronal activity [10]. Approximately 10% of human protein requires it for their structure and/or normal functions. Normal metabolic process needs  $\text{Zn}^{2+}$  in their normal range (2-3 g). Zinc homeostasis is primarily maintained by change in the zinc absorption and excretions in the gastrointestinal tract. Adjustment in renal excretions also help in maintaining homeostasis during extremely low or high zinc intake. Redistribution of zinc in tissue and cell also contributes to maintaining zinc homeostasis [11]. Disruption in  $\text{Zn}^{2+}$  homeostasis results in several disorders like epileptic seizures, Alzheimer's disease, Parkinson's disease [8,9], infantile diarrhea, ischemic stroke [12], amyotrophic lateral sclerosis [13], breast cancer and types 1 and 2 diabetes [14]. Likewise, an acute brain injury such as seizures, ischemia

and trauma can result in the release of higher concentration of zinc, which leads to zinc-induced neurotoxicity. Thus, free  $\text{Zn}^{2+}$  can serve as a biochemical indicator to determine the degree of brain injury [6]. Moreover, the higher concentration of zinc can also disrupt the immune system, leading to gastrointestinal disorders and eye lesions [15,16]. Excess of  $\text{Zn}^{2+}$  in the body inhibits phagocytes activity, which in turn leads to weakness of the immune system. Industrial process like refining, mining and steel processing increases  $\text{Zn}^{2+}$  in the environment [17]. Likewise, higher levels of zinc in the environment are detrimental to the microbial activity, which results in decreased soil fertility [14].

Recently, a great deal of attention has been devoted for the selective tracking of  $\text{Zn}^{2+}$  in biological and environmental samples [8,9]. However, detection of  $\text{Zn}^{2+}$  is perplexing since it lacks optical signature due to its filled d-orbital ( $3d^{10}$ ) configuration. Moreover, the conventional analytical methods not only require high cost instruments and time-consuming procedures but are also unable to discriminate between the bounded zinc (less toxic) from bioavailable (unbounded) zinc [8]. In recent times, organic dye-based fluorescent sensors for the detection of  $\text{Zn}^{2+}$  have received a widespread interest due to their operational simplicity, quick-response, high sensitivity, and reproducibility [18]. Consequently, a diverse range of fluorescent chemosensors based on fluorescein [19], BODIPY [20], quinolone [21], anthracene [22], naphthalimide [23,24] and coumarin [25,26] have been reported. Although the detection abilities of these sensors are appealing, they still suffer from liabilities such as complicated synthesis, poor selectivity for  $\text{Zn}^{2+}$  in the presence of  $\text{Cd}^{2+}$  and reduced solubilities in polar solvents. In this context, it is a challenging task to design and synthesize chemosensors for  $\text{Zn}^{2+}$  with high sensitivity and selectivity in a cost-effective fashion. Consequently, search for fluorescent probes having

high sensitivity and selectivity for the detection of  $\text{Zn}^{2+}$  is a continued process. Until now, a wide range of  $\text{Zn}^{2+}$  fluorescent sensors have been reported.

The working mechanism of most fluorescent sensors relies upon photoinduced electron transfer (PET) wherein enhancement in the emission intensity is normally observed but without any or little change in the emission wavelength [27]. The basic principle involved in molecular sensing is based on Pearson's HSAB concept. According to this concept,  $\text{Zn}^{2+}$  is considered the borderline Lewis acid. Thus, the deployment of selecting Lewis bases (pyridine, aniline, etc) as receptor sites, the selectivity of the fluorescent probes towards  $\text{Zn}^{2+}$  could be achieved as compared to other interfering soft ( $\text{Cd}^{2+}$ ,  $\text{Cu}^+$ ,  $\text{Hg}^{2+}$  and  $\text{Ag}^+$ ), hard ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Fe}^{3+}$  and  $\text{Cr}^{3+}$ ) Lewis acids and some of the bio-relevant borderline Lewis acids ( $\text{Cu}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Co}^{2+}$ ) [28]. Therefore, 2,2-dipicolylamine (DPA) is retained as a receptor unit for  $\text{Zn}^{2+}$  over the years. It is known to hold a higher affinity for  $\text{Zn}^{2+}$  over alkali and alkaline metal ions which are present in a much higher concentration in biological samples [29]. Thus, DPA was employed as a receptor in chemosensor **1** and **2**.

Nevertheless, it is well documented that transition metal ions can decrease the fluorescence of the fluorophore by either redox activity, magnetic perturbations, or single electron transfer (SET) from the singlet excited state of the fluorophore to metal ion [30]. To overcome this problem, an amide linkage was installed between the DPA and naphthalimide fluorophore in chemosensor **3-5** in order to increase the inflexibility or impose conformational restrain of the receptor or rigidity of the ligand scaffold which may develop a specific topology or right coordination geometry for the receptor for enhanced

$\text{Zn}^{2+}$  selectivity. Moreover, after the anticipated coordination of the carbonyl oxygen of the amide function, in conjunction of DPA, with  $\text{Zn}^{2+}$  may block  $\text{Zn}^{2+}$  from sterically interacting with the naphthalimide. This in turn will help prevent  $\text{Zn}^{2+}$  from the close proximity of the fluorophore. While in chemosensor **6** and **7** a triazole function was installed in between the fluorophore and DPA receptor in order to impose conformational restrain or inflexibility, which in turn may create the right coordination geometry/specific topology that may enhance  $\text{Zn}^{2+}$  selectivity. Furthermore, the anticipated cooperative coordination of the N-3 atom of 1,2,3-triazolyl moiety and DPA with  $\text{Zn}^{2+}$  may restrict  $\text{Zn}^{2+}$  from sterically interacting with the fluorophore. Finally, the triazole function may serve as a sacrificial donor by participating in the SET process with the  $\text{Zn}^{2+}$  and thus would hinder SET from the singlet excited state of the fluorophore to  $\text{Zn}^{2+}$  [31]. Moreover, the N-3 atom of the 1,2,3-triazolyl function is expected to create a tetradentate coordination motif, which in turn can chelate  $\text{Zn}^{2+}$  [12].

## CHAPTER 2

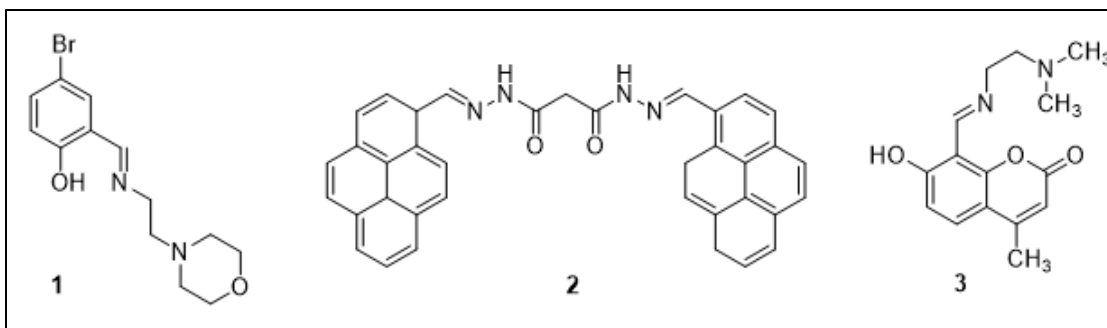
### LITERATURE REVIEW

#### 2.1 Fluorescence sensor for $\text{Zn}^{2+}$ detection

Several methods including atomic absorption spectroscopy, UV-vis spectroscopy and potentiometry are being used for the detection of  $\text{Zn}^{2+}$  [32]. However, these techniques require expensive and heavy instrumentation, coupled with their complicated operation makes them unfit for onsite application. Further, they are considered time-consuming as it requires laborious sample preparation. As an alternative to these techniques, fluorescent-based chemosensors are developed to selectively track  $\text{Zn}^{2+}$ . Fluorescent chemosensors enjoy the advantage of fast response time and simple operational procedures with high sensitivity. Until now, a wide range of fluorescent chemosensors based on variety of fluorophores such as anthracene [33], coumarin [34,35], pyrrins [36], quinolone [37–39], BODIPY [40,41], rhodamine [42–44] and naphthalimide [45] have been developed. Chelation-enhanced fluorescence [CHEF], photoinduced electron transfer [PET],  $\text{Zn}^{2+}$  and metal-ligand charge transfer [MLCT] are the common sensing mechanisms that the sensing of these fluorescent chemosensors relies upon [17]. The 2,2'-dipicolylamine (DPA) is the most widely used receptor for  $\text{Zn}^{2+}$  ion sensing. Besides this quinoline, triazole, polymer and Schiff base are used as receptors. However, due to spectral properties,  $\text{Zn}^{2+}$  and  $\text{Cd}^{2+}$  exhibit identical spectral responses[12]. Thus, the development of fluorescence

chemosensors which could effectively discriminate between  $\text{Zn}^{2+}$  and  $\text{Cd}^{2+}$  remains a formidable task.

In 2021, D. Maity and co-workers. reported 2-((2-morpholinoethylimino)methyl)-4-bromophenol based fluorescent sensor (**1**) [46] [**Fig. 2.1**]. The sensor **1** was synthesized from the condensation reaction of 5-bromo-2-hydroxybenzaldehyde and 2-morpholinoethaneamine. The chemosensor was found to enhance the fluorescent at 458 nm by 32-fold with 1 equiv. of  $\text{Zn}^{2+}$  when excited at 378 nm. The working mechanism of the sensor was based on PET and CHEF mechanism. The sensor **1** could selectivity detect  $\text{Zn}^{2+}$  in the presence of various competing cations, with LOD of  $4.41 \times 10^{-7}$  M. The sensor was successfully applied in cell imaging in C6 rat brain glioma. In 2020, Rani et al. reported the synthesis of pyrene and malonohydrazide-based chemosensor (**2**) for  $\text{Zn}^{2+}$  sensing [36] [**Fig. 2.1**]. The initial reaction of diethylmalonate with hydrazine hydrate leads to the synthesis of malonohydrazide which upon reaction with pyrene carboxaldehyde leads to the synthesis of the final product of **2**. Higher selectivity towards the  $\text{Zn}^{2+}$  in the presence of competing ions was displayed by **2**. DFT calculations revealed that the sensing mechanism of **2** was based on PET process. The LOD calculated for **2** was 5.1 nM. In addition, it was able for detecting intracellular  $\text{Zn}^{2+}$  distribution in the Hela cells. In another study, Bhasin and co-workers designed a coumarin-based chemosensor (**3**) for the detection of  $\text{Zn}^{2+}$  [34] [**Fig. 2.1**]. An enhancement of 2.5-fold in the fluorescence of the native **3** was reported with the addition of 1 Equiv. of  $\text{Zn}^{2+}$  ions in the presence of competing cations. The limits of detection were calculated to be 0.072 nM.



**Figure 2.1:** Structure of Chemosensor 1-3.

## 2.2 Mechanism of PET fluorescence sensing

The general architecture of a PET sensor consists of an analyte binding site (known as a receptor, coordination site, or chelating group) and a fluorophore [47]. The receptor is attached to the fluorophore through a spacer [Fig. 2.2]. The binding of the receptor to the analyte is translated to a fluorescence output signal by a fluorophore molecule. Common signal output is the change in absorption or fluorescence emission spectra. The signal in the PET sensors, depending on the special circumstances is either turn-on or turn-off. However, in case of  $\text{Zn}^{2+}$  mainly fluorescence turn-on sensors are employed.

To better understand the fluorescence mechanism, it is good to understand relative energies and resultant electronic transition in the sensor before and after binding with the analyte [48]. Such a system is characterized by relatively high-energy bonding nonbonding electrons pairs of the receptor in the unbound state. After excitation, an electron from the HOMO of the fluorophore is promoted to the LUMO. This allows a fast-intramolecular electron transfer from the HOMO of the receptor to the HOMO of the excited fluorophore. This results in quenching of fluorescence of the fluorophore (turn-off) due to the nonradiative

deactivation of the excited state. However, upon binding of the receptor with the analyte, the HOMO of the receptor is lowered from that of the fluorophore which results in censorship of the PET. This results in the resumption of the fluorescence (turn-on) [48].

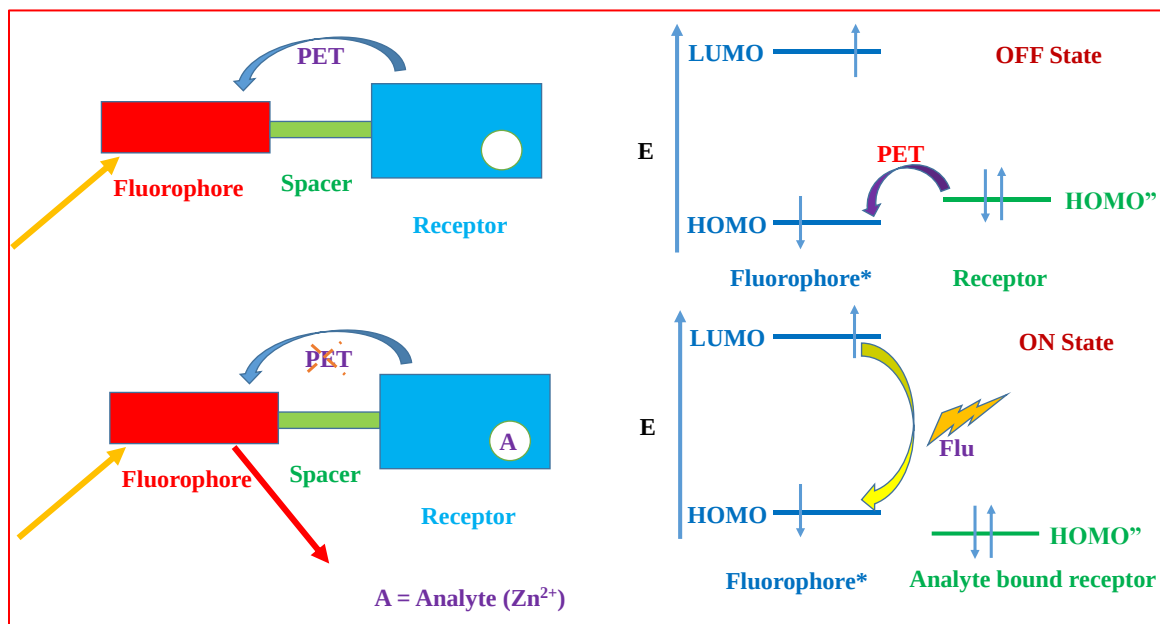
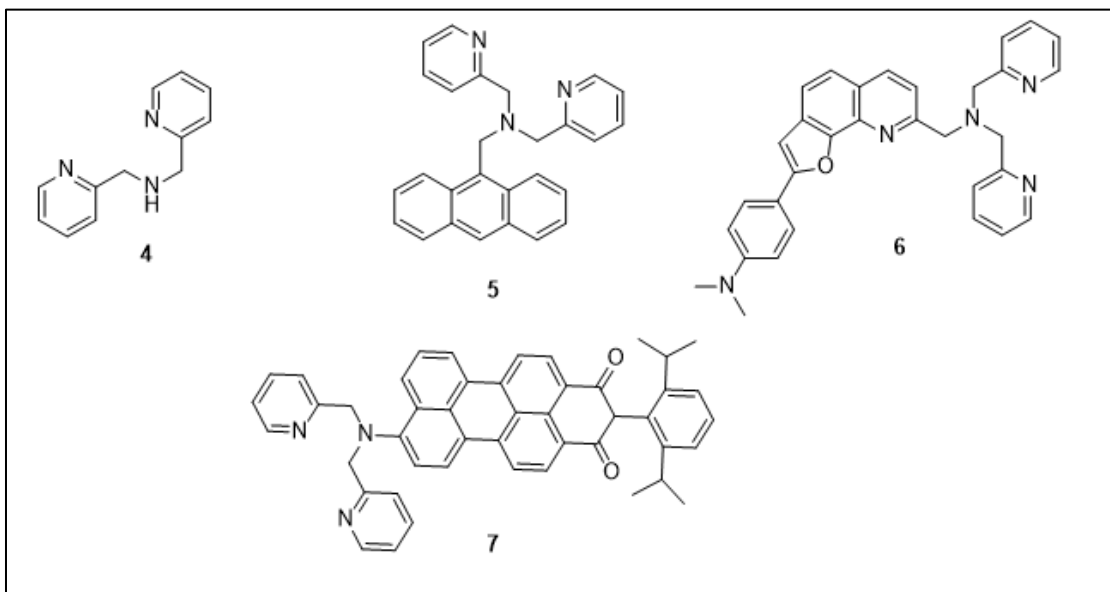


Figure 2.2: Working principle of turn-on fluorescence sensors.

### 2.3 DPA based fluorescent sensors

Bis(2-pyridylmethyl)amine (DPA) **4** moiety is widely used in the fluorescence sensing of  $\text{Zn}^{2+}$  due to its excellent selectivity for  $\text{Zn}^{2+}$  over other competing ions [12]. Furthermore, the DPA nitrogens are good electron donors during the photoinduced electron transfer. An easy way to develop DPA based chemosensor for  $\text{Zn}^{2+}$  detection is to directly connect DPA to a fluorophore. For example, in **5** the DPA is connected to anthracene fluorophore through a  $-\text{CH}_2-$  group. In the unbound state the PET towards the anthracene via methylene group results in fluorescence off. In the bound state, the fluorescence is turn-on once the

PET stops due to the binding of the analyte to the receptor [33]. Synthesis of probe **6** was reported for  $\text{Zn}^{2+}$  imaging in the living cell. The addition of  $\text{Zn}^{2+}$  to the solution of **6** in the  $\text{C}_2\text{H}_5\text{OH}/\text{H}_2\text{O}$  mixture results in fluorescence enhancement with a 75 nm red shift when excited at 405 nm. Further, a large Stokes effect of 199 nm was also displayed. However, the response of **6** towards  $\text{Cd}^{2+}$  was similar to that of  $\text{Zn}^{2+}$  [49].



**Figure 2.3:** Structure of DPA and DPA based Chemosensors **4-7**.

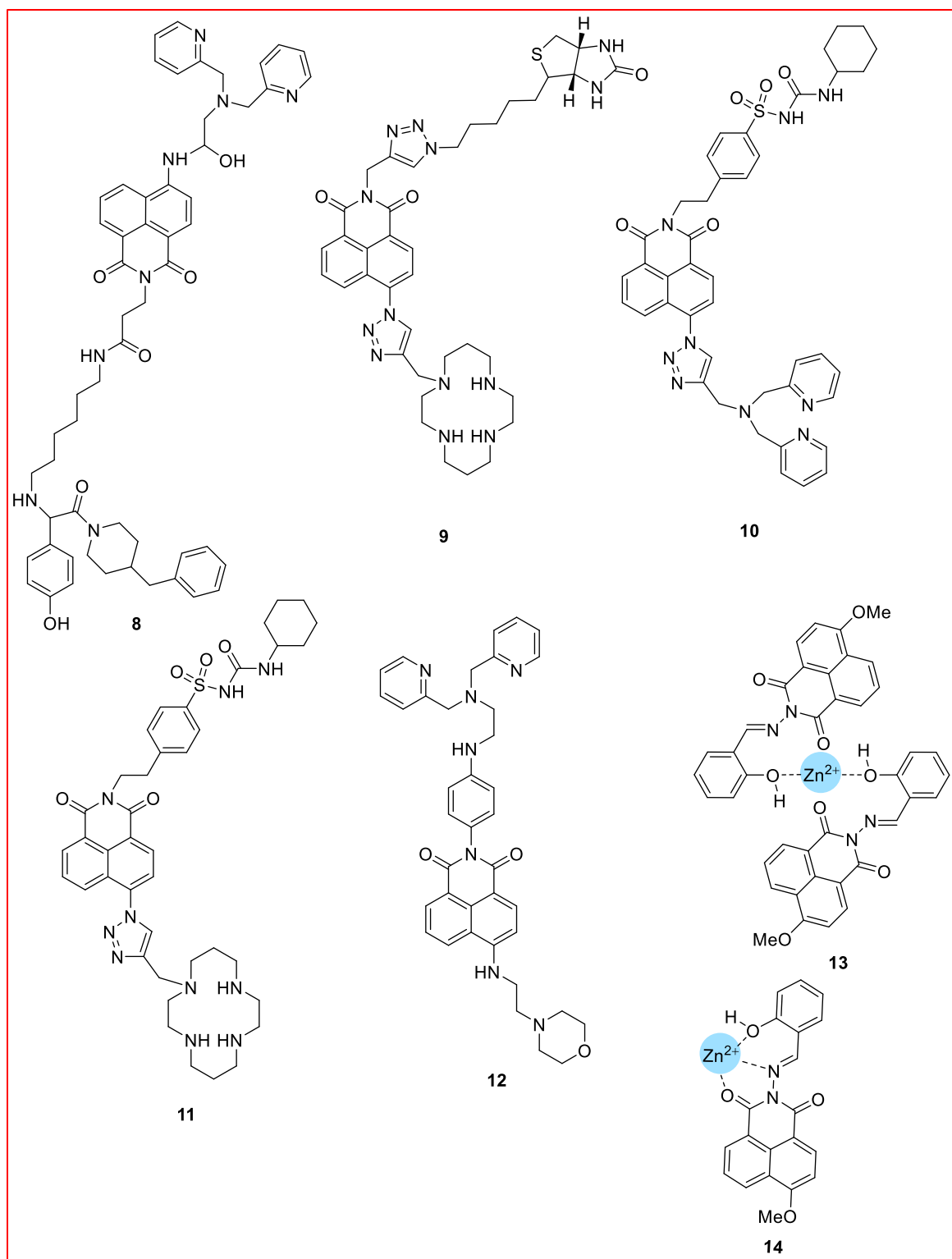
The *N, N*-bis(2-picolyl)-9-aminoperylene monoimide **7** was synthesized (**Fig. 2.3**) for the highly specific and responsive sensing of  $\text{Zn}^{2+}$ . Complex formation of  $\text{Zn}^{2+}$  ion and **7** was responsible for the sensing of  $\text{Zn}^{2+}$  which was characterized with different spectroscopic techniques like UV-vis spectroscopy,  $^1\text{H}$  NMR and FD-MS. Complex formation was confirmed by a shift from an absorption maximum of 534 nm to 501 nm when chemosensor **7** was reacted with  $\text{ZnCl}_2$ . Further confirmation comes from fluorescence maximum shift from 702 nm to 670 nm upon reaction with 1 equiv. of  $\text{Zn}^{2+}$ .  $^1\text{H}$  NMR also confirms complex formation due to variations in the chemical shift of proton peaks. Other similar

ions like  $\text{Ni}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Hg}^{2+}$ , etc. do not show any such changes under the specified conditions which suggested high selectivity of sensor **7** for  $\text{Zn}^{2+}$ . Sensor **7** was deposited in the sol-gel silica layer coated on the optical fiber where the fiber transmission technology was used to detect the zinc ions. It was observed that the transmission signal was considerably enhanced in the zinc solution as compared to pure water. Hence, synthesized chemosensor **7** worked well both in solution and when deposited on solid fiber [50].

## 2.4 Fluorescence sensors using naphthalimide fluorophores

Naphthalimide is a promising fluorophore for  $\text{Zn}^{2+}$  sensing due to its photophysical stability, commercial availability and low toxicity. In 2017, X. Chen et al. developed di-2-picolylamine containing chemosensor **8** for the sensing of zinc ions [51]. The detection limit of **8** was calculated to be in the sub-nanomolar range, enabling the detection of  $\text{Zn}^{2+}$  in live neurons. In 2018, the Watkinson group reported the synthesis of a naphthalimide based fluorescent sensor **9**, bearing DOTA and triazole rings, for imaging of free  $\text{Zn}^{2+}$  in cancer cells [52]. Upon excitation at 346 nm, the fluorescent emission intensity of **9** at 412 nm was enhanced when the cyclam-based receptor binds to the  $\text{Zn}^{2+}$  in the HEPES buffer solution. The developed fluorescent sensor was used for the  $\text{Zn}^{2+}$  imaging in breast cancer cells and the result suggests that it can permeate through the entire cell and can locate cancer selectively. In next year, the same research group have developed **10** and **11** for the imaging of  $\text{Zn}^{2+}$  in the endoplasmic reticulum. The deficiency or depletion of  $\text{Zn}^{2+}$  can lead to ER stress and upregulate the unfolded proteins response [53]. Probes **10** and **11** contain the sulfonyl urea as a targeting group for the endoplasmic reticulum. DFT and TDDFT study suggests the involvement of DPA and triazole ring of probe **10** with the attachment

of  $\text{Zn}^{2+}$ . Similarly, in probe **11**, the attachment of cyclen with the  $\text{Zn}^{2+}$  was supported by the triazole ring. The emission intensity at 414 nm of both probes when excited at 346 nm enhances the  $\text{Zn}^{2+}$  binding. Targeting behavior for both probes was also studied via co-staining assays results suggested good ER-targeting abilities of **10** and **11**. Huang et al. designed the synthesis of **12**, containing naphthalimide fluorophore and BPEN ligand as a receptor [54]. Upon binding with the  $\text{Zn}^{2+}$ , fluorescent emission enhancement at 535 nm was observed when excited at 450 nm. Panchenko et al. reported interesting chemosensors **13** and **14** for the detection of  $\text{Zn}^{2+}$  using salicylidene amino group as a binding site while naphthalimide as a fluorophore [55]. The intramolecular PET process quenches the fluorescence which was recovered when the receptor attaches to the  $\text{Zn}^{2+}$  resulting in fluorescent emission enhancement at 455 nm when excited at 375 nm. This literature indicates that naphthalimide works as an effective fluorophore. However, it is well understood that fluorescence properties are greatly dependent on the substituents attached to the naphthalimide ring system. Almost all the work is reported using naphthalimide substituted with electron-donating groups [56]. However, naphthalimide-based fluorophores bearing electron-withdrawing groups are far less explored.



**Figure 2.4:** Structure of Chemosensor **8-14**.

## 2.5 Research Objectives

1. Synthesis of new fluorescent chemosensors **1–7**.
2. Absorption and fluorescence spectroscopic studies of chemosensors **1–7** at known and varied concentration of  $\text{Zn}^{2+}$
3. Investigation of binding affinity of  $\text{Zn}^{2+}$  to chemosensor **1–7**
4. Competing experiments with cations ( $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Fe}^{2+}/\text{Fe}^{3+}$ ,  $\text{K}^{1+}$ ,  $\text{Mn}^{2+}$  etc.) to assess selectivity for  $\text{Zn}^{2+}$ .

## CHAPTER 3

### EXPERIMENTAL

#### 3.1 Materials and instrumentation

Commercial grade reagents and solvents were used, as received. Di-*tert*-butyl-dicarbonate (Sigma Aldrich, purity >99%), 2-(bromomethyl)pyridine hydrobromide (Sigma Aldrich, purity 98%), Trifluoroacetic acid (Sigma Aldrich, purity 99.7%), 1-Fluoro-2,4-dinitrobenzene (Sigma Aldrich, purity 99%), 4-chloro-7-nitrobenzofurazan (Sigma Aldrich, purity 98%), *N,N*-dimethyl formamide (Sigma Aldrich, purity 99.8%), Acetonitrile (Sigma Aldrich, anhydrous, purity 99.8%), 2-bromoethylamine (Sigma Aldrich, purity 99%), Sodium azide (Sigma Aldrich, purity 99.5%), Propargylamine (Sigma Aldrich, purity 98%), Trifluoroacetic acid (Sigma Aldrich, purity 99.7%), 4-nitro-1,8-naphthalic anhydride (Sigma Aldrich, purity 95%), *N,N*-dimethyl formamide (Sigma Aldrich, purity 99.8%),

GENESYS 10S UV-vis spectrophotometer were used for UV-Visible absorption study. Fluorescence spectra were recorded with the help of Edinburgh FL spectrophotometer while JEOL spectrometer (JNM-LA 500 MHz, JEOL USA inc.) were used for NMR analysis. TLC analysis were done with the help of 60 F254 plates while column chromatography was carried done with Merck silica gel (200-400 mesh, E. Merck). Stock solutions (1mM) of probe **1** to **7** and  $\text{Zn}^{2+}$  (Zinc trifluoromethanesulfonate,  $(\text{CF}_3\text{SO}_3)_2\text{Zn}$ ) were prepared in  $\text{CH}_3\text{CN}$  and (50  $\mu\text{M}$  in HEPES buffer, pH = 7.4) respectively. Similarly,

1 mM stock solutions of other ions were prepared in HEPES buffer (pH = 7.4). Stock solutions were diluted to get required concentration of solutions whenever needed.

## **3.2 Synthesis of intermediates and chemosensors 1 and 2**

### **3.2.1 Synthesis of tert-butyl (4-(4-aminophenoxy)phenyl)carbamate (9)**

4,4'-oxidianiline **8** (1 g, 5.0 mmol) was dissolved in a mixture of THF, H<sub>2</sub>O and DMF (15 mL) (2:2:1, v/v) and Na<sub>2</sub>CO<sub>3</sub> (0.265 g, 2.5 mmol) was added. This was followed by the addition of di-tert-butyl dicarbonate (0.55 g, 2.5 mmol) and the reaction mixture was stirred for overnight at room temperature. Upon completion of the reaction (TLC analysis), the volatiles were evaporated and ethyl acetate (10 mL) was added and then washed with brine (3 x 10 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness under vacuum. The crude residues were resolved over a silica column eluting with EtOAc–Hexane (80:20) to afford intermediate **9** as a brown solid (0.63 g, 84%). <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>): δ (ppm) 9.21 (s, 1H), 7.37 (d, 2H, *J* = 8.4), 6.8 (t, 2H, *J* = 2.0), 6.72 (q, 2H, *J* = 2.0), 6.57 (q, 2H, *J* = 2.0), 4.91 (s, 2H), 1.46 (s, 9H); <sup>13</sup>C-NMR (125.7 MHz, DMSO-d<sub>6</sub>): δ (ppm) 153.94, 153.36, 147.04, 145.45, 134.41, 120.59, 120.14, 117.68, 115.29, 79.24, 28.60.

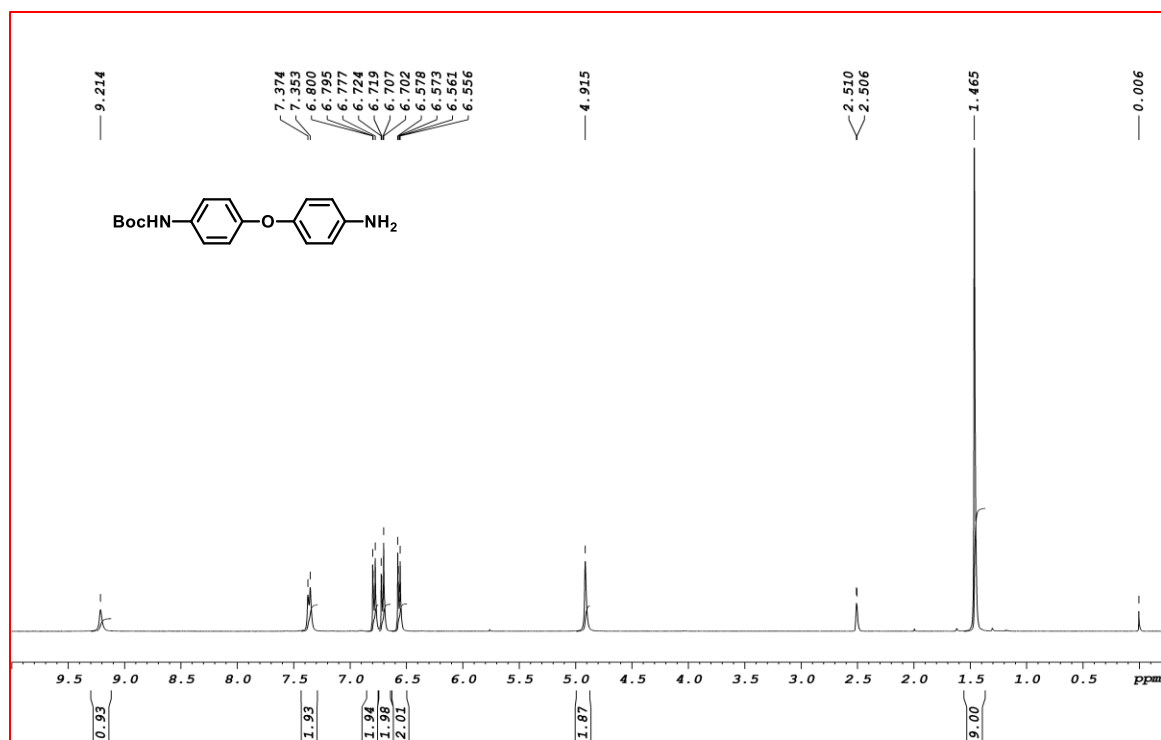


Figure 3.1:  $^1\text{H}$ -NMR of 9.

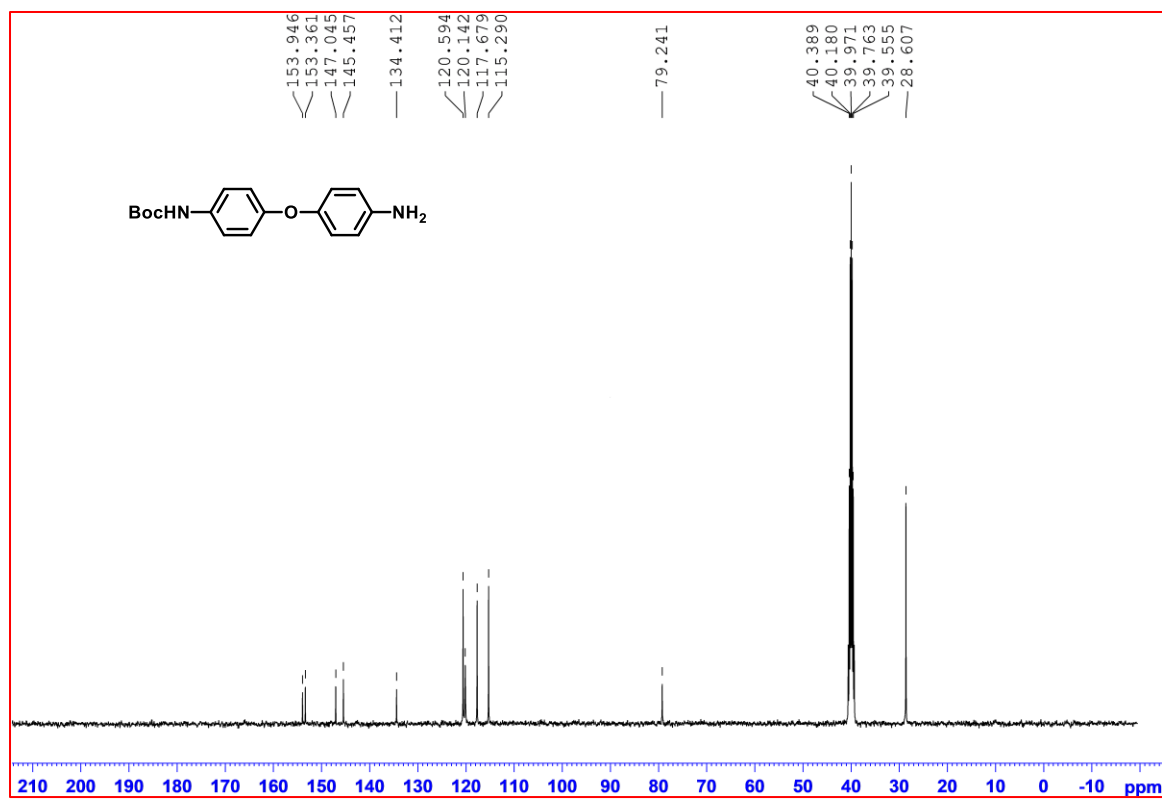


Figure 3.2: <sup>13</sup>C-NMR of 9.

### 3.2.2 Synthesis of tert-butyl (4-(4-(bis(pyridin-2-ylmethyl)amino)phenoxy)phenyl)carbamate (**11**)

To a mixture of intermediate **9** (0.8 g, 2.7 mmol) in acetonitrile was added  $K_2CO_3$  (2.2 g, 16.0 mmol) at room temperature and after being stirred for 30 minutes, bromoethylpyridine **10** (1.68 g, 6.7 mmol) was added and the reaction mixture was heated to reflux for 8 h under inert condition. Upon completion of the reaction (TLC analysis), the reaction was cooled to room temperature, filtered and the filtrate was concentrated by rotary evaporator. The crude residue was purified over a silica column, eluting with 98-02 ( $CH_3Cl:CH_3OH$ ) to afford the desired **11** as a black gummy thick liquid (1.17 g, 91%).  $^1H$ -NMR (400 MHz,  $CDCl_3$ ):  $\delta$  (ppm) 8.72 (d, 2H,  $J = 4.4$ ), 7.87 (t, 2H,  $J = 7.6$ ), 7.50 (d, 2H,  $J = 8.0$ ), 7.39 (t, 3H,  $J = 5.6$ ), 7.26 (s, 1H), 6.88 (dd, 4H,  $J = 8.8$ ), 6.67 (d, 2H,  $J = 9.2$ ), 4.96 (s, 4H), 1.51 (s, 9H);  $^{13}C$ -NMR (125.7 MHz,  $CDCl_3$ ):  $\delta$  (ppm) 171.18, 157.74, 153.70, 145.45, 153.01, 149.48, 147.57, 143.53, 139.26, 133.24, 123.09, 122.38, 120.27, 120.13, 118.62, 114.29, 80.41, 60.41, 28.34.

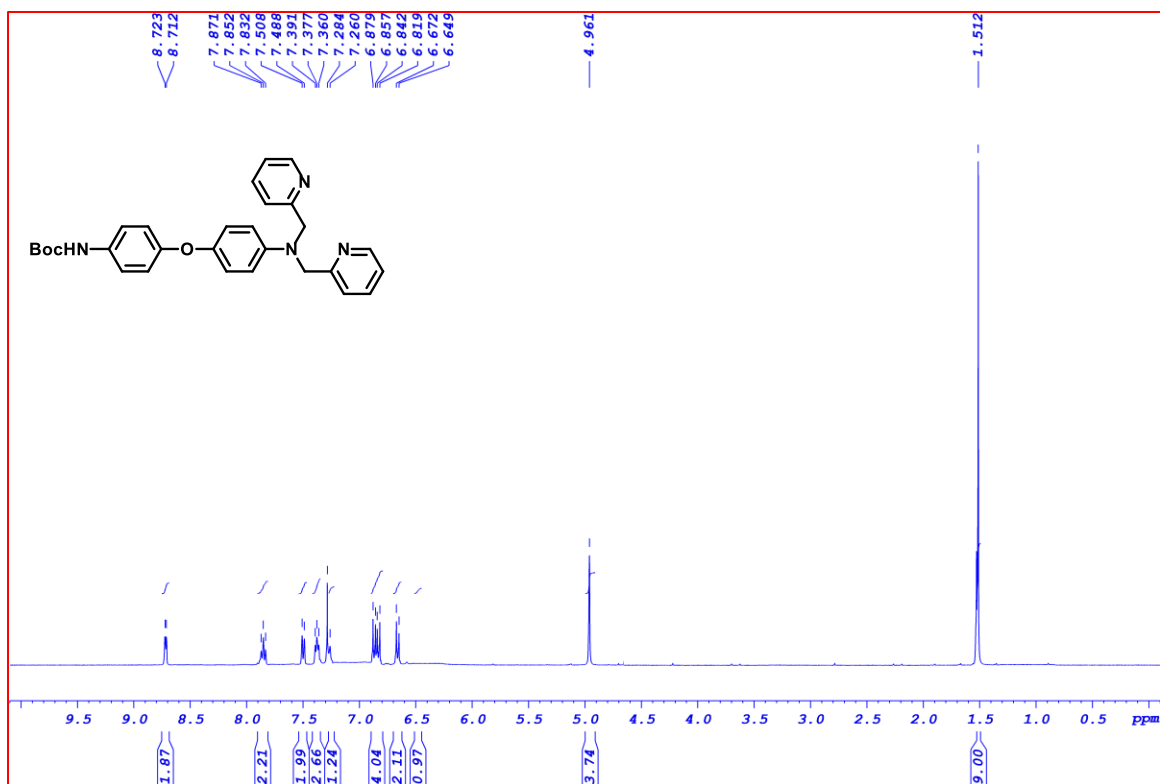


Figure 3.3: <sup>1</sup>H-NMR of 11.

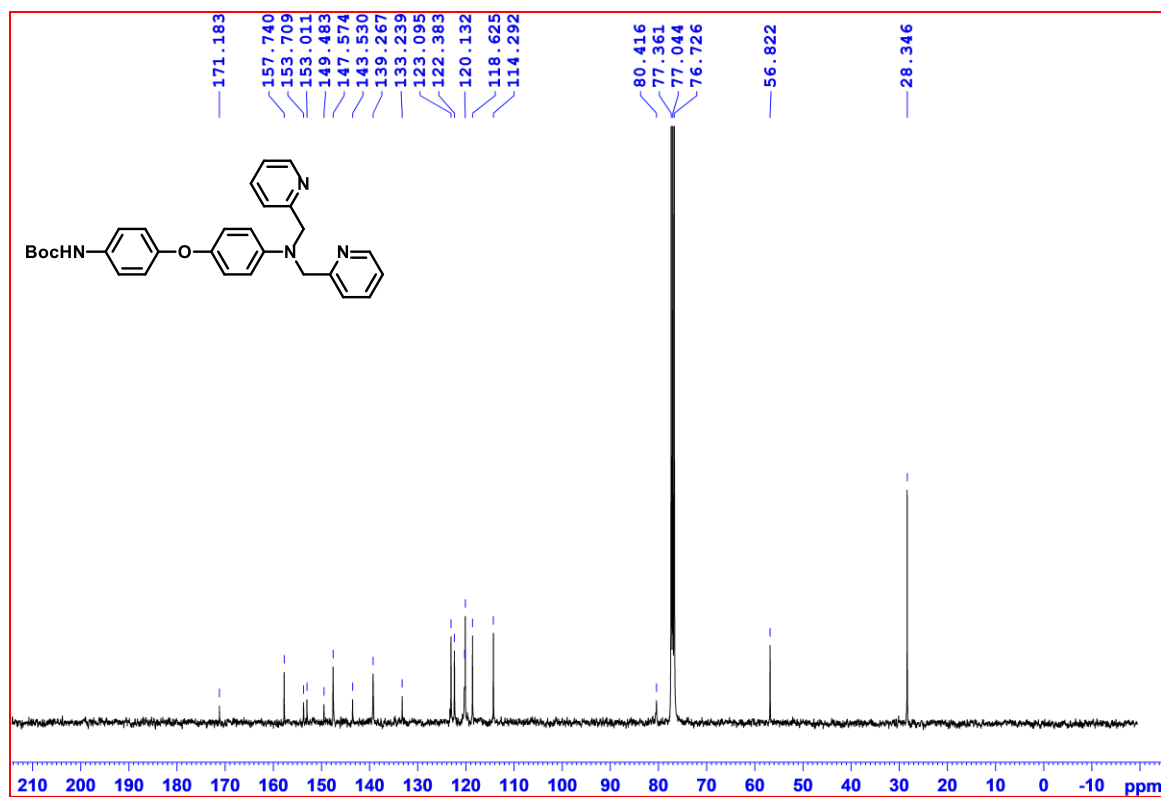


Figure 3.4: <sup>13</sup>C-NMR of 11.

### 3.2.3 Synthesis of ammonium salt of 4-(4-aminophenoxy)-N,N-bis(pyridin-2-ylmethyl)aniline (**12**)

To a solution of **11** (0.9 g, 1.86 mmol) in DCM (4 mL) was added trifluoroacetic acid (2 mL) at room temperature and the mixture was then stirred overnight. Upon completion of the reaction, the volatiles were evaporated under vacuum. The residues were triturated with diethyl ether and then filtered to obtain the entitled compound **12** as a dark brown gummy liquid (0.68 g, 95%).  $^1\text{H-NMR}$  (400 MHz, DMSO- $\text{d}_6$ ):  $\delta$  (ppm) 8.82 (d, 2H,  $J = 4.8$ ), 8.09 (t, 2H,  $J = 8.0$ ), 7.67 (d, 2H,  $J = 8.0$ ), 7.57 (t, 3H,  $J = 5.2$ ), 7.19 (d, 2H,  $J = 8.8$ ), 6.91 (d, 2H,  $J = 2.4$ ), 6.85 (d, 2H,  $J = 8.8$ ), 6.64 (d, 2H,  $J = 9.2$ ), 5.0 (s, 4H);  $^{13}\text{C-NMR}$  (125.7 MHz, DMSO- $\text{d}_6$ ):  $\delta$  (ppm) 158.77, 158.38, 156.23, 148.29, 147.32, 144.50, 139.48, 123.50, 123.09, 122.74, 120.85, 118.53, 113.77, 56.88.

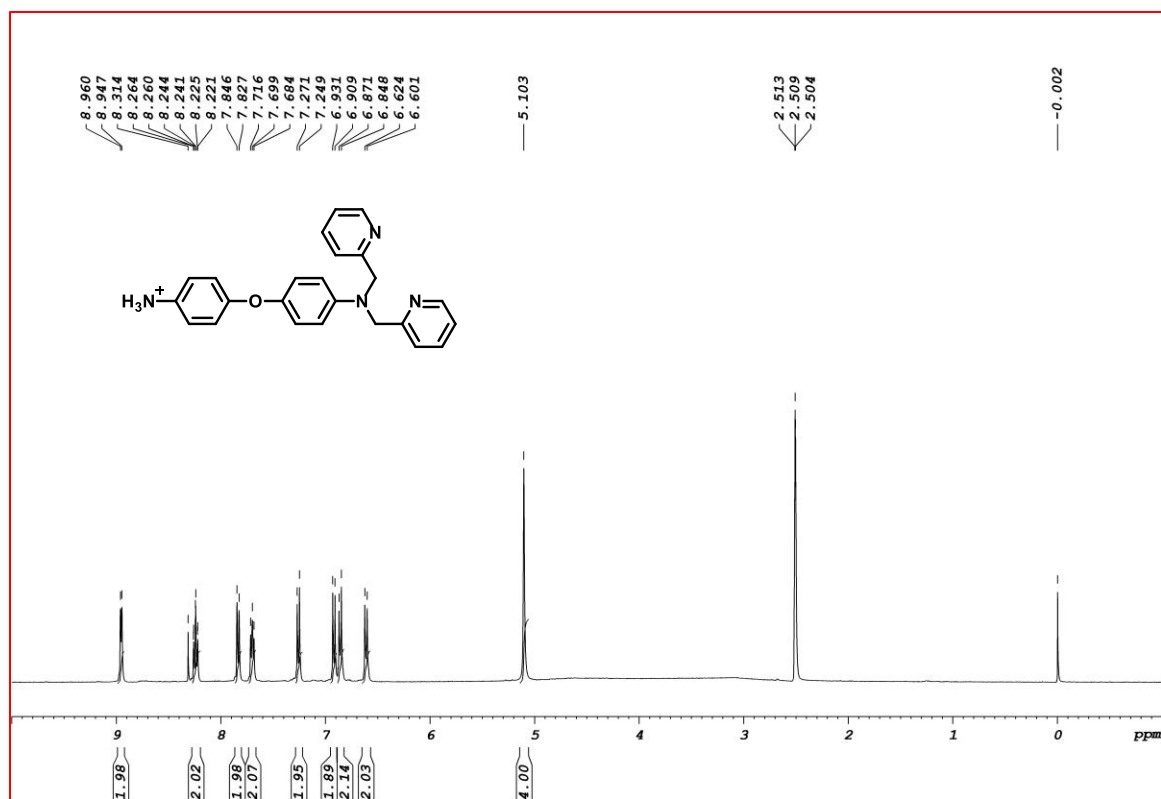


Figure 3.5: <sup>1</sup>H-NMR of 12.

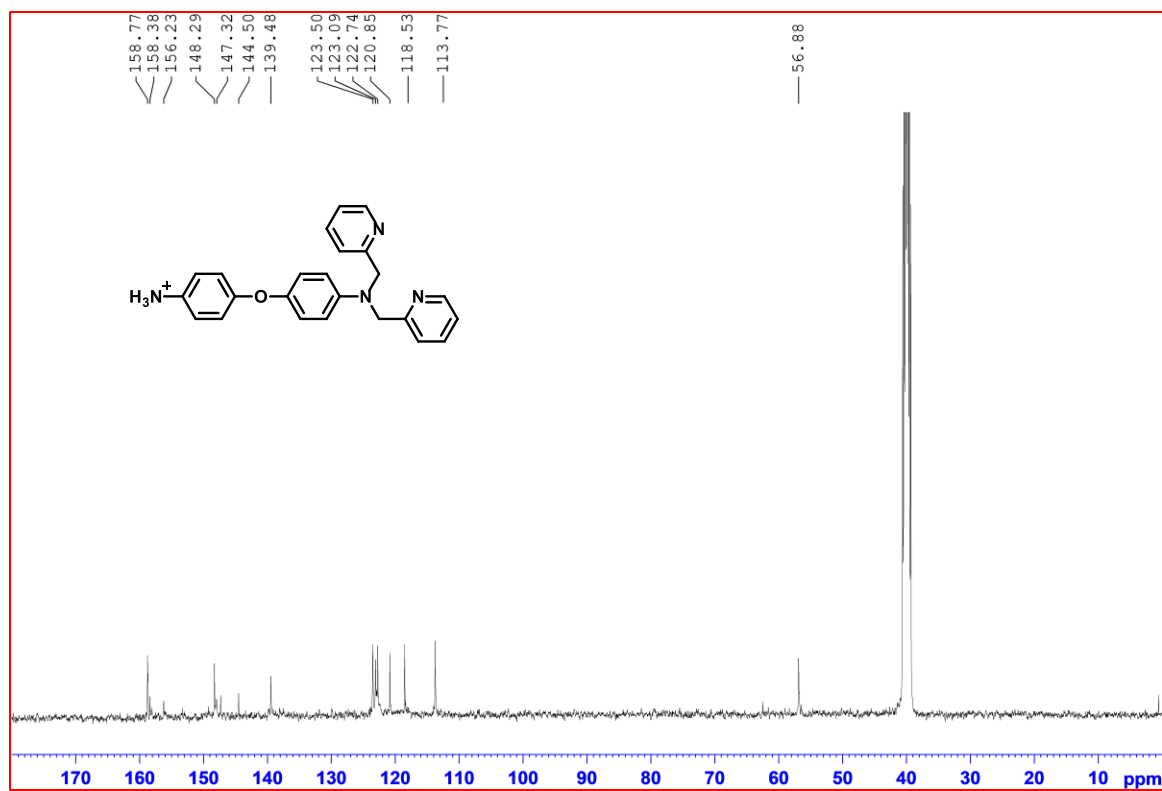


Figure 3.6: <sup>13</sup>C-NMR of 12.

### 3.2.4 Synthesis of chemosensor (**1**)

To a solution of compound **12** (0.5 g, 1.3 mmol) in DMF at 0 °C was added diisopropylethylamine (DIPEA) (0.84 g, 6.5 mmol) dropwise. After being stirred for 0.5 h, the mixture was brought to room temperature and 1-fluoro-2,4-dinitrobenzene **12** (0.29 g, 1.56 mmol) was added and the reaction mixture was stirred for 12 h at 40 °C. Upon completion of the reaction (TLC analysis), the mixture was diluted with dichloromethane (12 mL) and washed successively with brine (3 x 8 mL) and dis.H<sub>2</sub>O (8 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness. The crude residue was resolved over a silica column, eluting with EtOAc-n-hexane (1:1) and gradually changing to CH<sub>3</sub>OH-CH<sub>3</sub>Cl (2:98) to afford **1** as a light-yellow gummy solid (0.54 g, 75.3%). <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>CN): δ (ppm) 9.75 (s, 1H), 8.97 (d, 1H, *J* = 2.4), 8.56 (q, 2H, *J* = 4.8), 8.10 (q, 1H, *J* = 2.8), 7.74 (m, 2H, *J* = 1.6), 7.58 (s, 1H), 7.36 (d, 2H, *J* = 8.0), 7.26 (q, 4H, *J* = 7.2), 7.02 (d, 1H, *J* = 9.6), 6.95 (d, 2H, *J* = 8.8), 6.86 (d, 2H, *J* = 8.8), 6.71 (d, 2H, *J* = 9.2), 4.83 (s, 4H), 1.46 (s, 9H); <sup>13</sup>C-NMR (125.7 MHz, CD<sub>3</sub>CN): δ (ppm) 153.94, 153.36, 147.04, 145.45, 134.41, 120.59, 120.14, 117.68, 115.29, 79.24, 28.60.

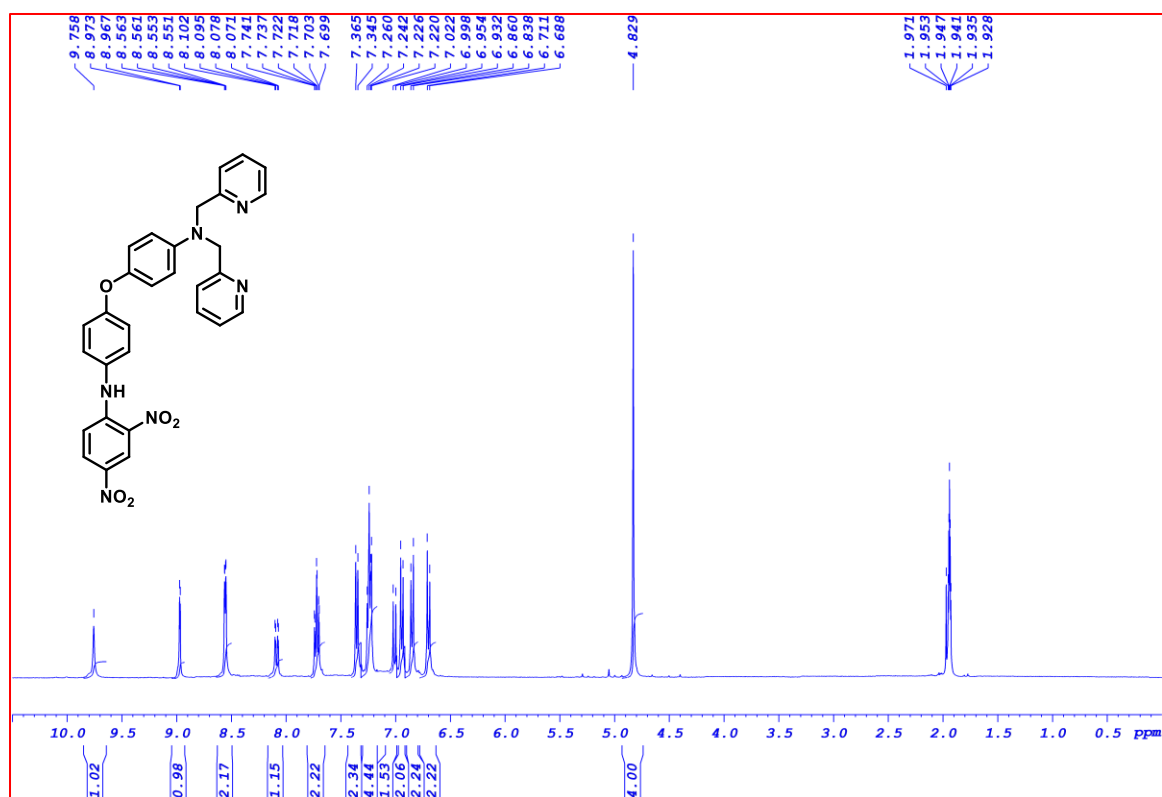


Figure 3.7: <sup>1</sup>H-NMR of **1**.

### 3.2.5 Synthesis of chemosensor (2)

To the solution of compound **12** (0.5 g, 1.3 mmol) in methanol was added potassium carbonate  $K_2CO_3$  (0.90 g, 6.5 mmol) at room temperature. After being stirring for 30 minutes, 4-chloro-7-nitrobenzofurazan **14** (0.31 g, 1.56 mmol) was added and the reaction mixture was stirred at 40 °C for 12 h. Upon completion of the reaction (TLC analysis), the mixture was diluted with dichloromethane and washed successively with brine and  $H_2O$ . The organic layer was dried over  $Na_2SO_4$  and evaporated to dryness. The crude was resolved over a silica column eluting with EtOAc–n-hexane (1:1) and gradually changing to  $CH_3OH-CH_3Cl$  (2:98) to afford chemosensor **2** as a light-pink gummy solid (0.58 g, 81.2%).  $^1H$ -NMR (400 MHz,  $CDCl_3$ ):  $\delta$  (ppm) 8.65 (d, 2H,  $J = 2.4$ ), 8.43 (d, 1H,  $J = 2.4$ ), 7.74 (m, 2H,  $J = 2.0$ ), 7.37 (d, 2H,  $J = 2.0$ ), 7.31 (m, 4H,  $J = 5.6$ ), 7.03 (d, 2H,  $J = 2.4$ ), 6.92 (d, 2H,  $J = 6.8$ ), 6.72 (d, 2H,  $J = 9.2$ ), 6.56 (d, 1H,  $J = 8.4$ ), 4.87 (s, 4H).  $^{13}C$ -NMR (125.7 MHz,  $CDCl_3$ ):  $\delta$  (ppm) 158.38, 158.13, 149.22, 146.99, 145.24, 144.64, 143.99, 142.05, 137.58, 136.12, 130.40, 125.76, 125.11, 122.49, 121.28, 119.50, 118.32, 117.85, 113.847, 100.55, 57.48.

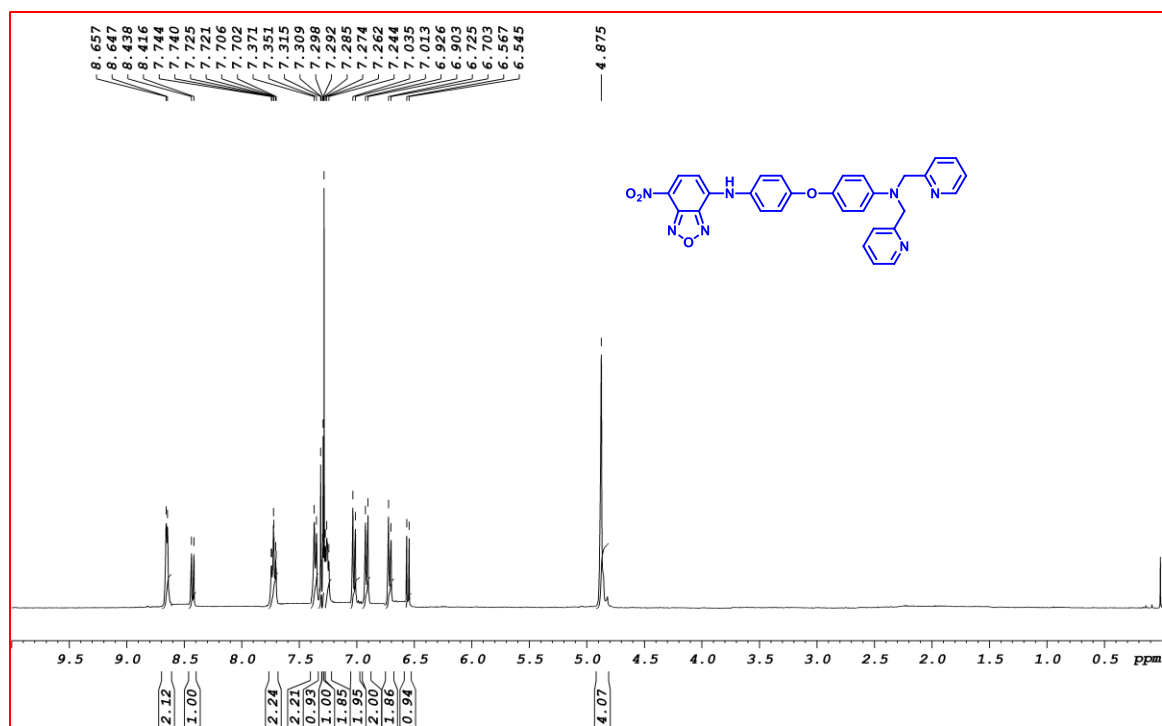


Figure 3.8:  $^1\text{H}$ -NMR of 2.

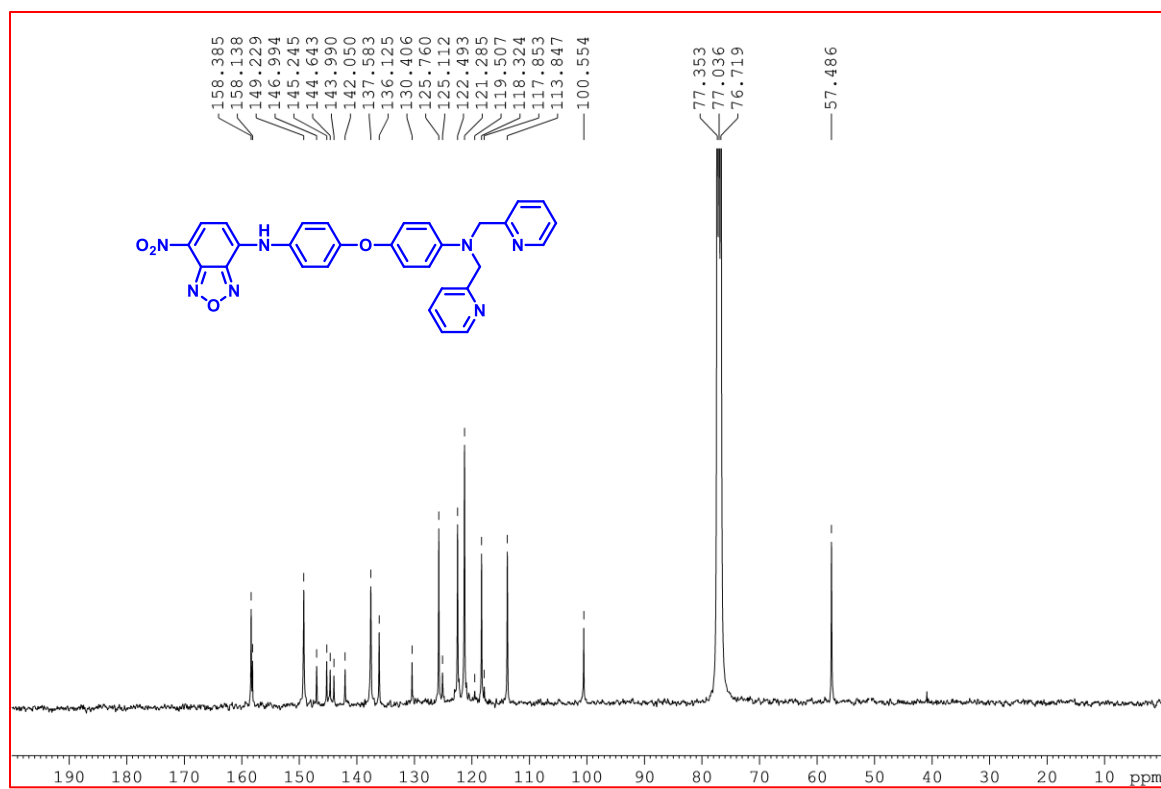


Figure 3.9: <sup>13</sup>C-NMR of 2.

### 3.3 Synthesis of intermediates and chemosensor 3-5

#### 3.3.1 Synthesis of tert-butyl (2-aminoethyl)carbamate (**16**)

To a cold solution of ethylene diamine **15** (0.3 g, 4.99 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) at 0 °C was added a solution of Boc<sub>2</sub>O ((1.1 g, 4.99 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added dropwise in 2 h, which led to the formation of white precipitates. Upon completion of the reaction, the mixture was dissolved in water and extracted with ethyl acetate (3 x 10 mL) and the organic layer was dried with Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness under reduced pressure. The yellowish oily residues were passed through a plug of silica gel, eluting with CHCl<sub>3</sub> (100%) and then changing to MeOH:CHCl<sub>3</sub> (1:99) to afford **16** as a colorless viscous oil. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>): δ (ppm) = 3.30-3.26 (m; 4H), 2.91 (s, 2H) and 1.43 (s, 9H); <sup>13</sup>C-NMR (125.7 MHz, CDCl<sub>3</sub>): δ (ppm) = 156.3, 79.12, 42.93, 41.61 and 28.4.

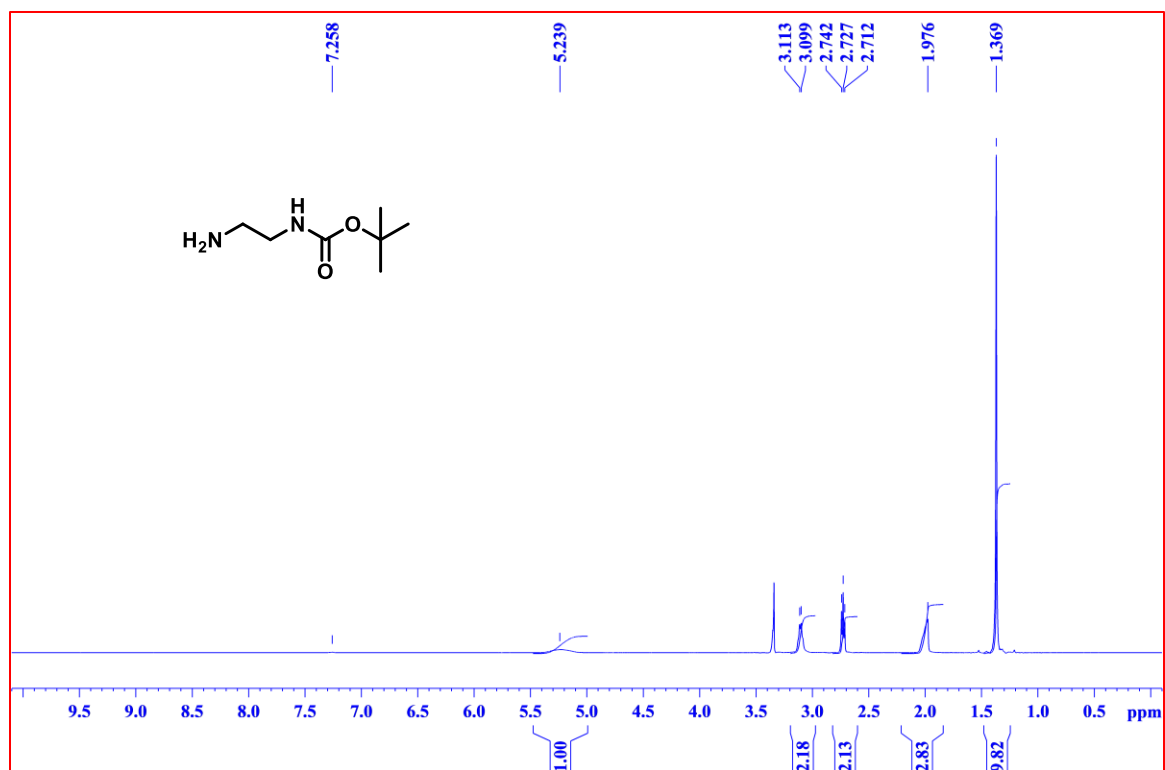


Figure 3.10: <sup>1</sup>H-NMR of **16**.

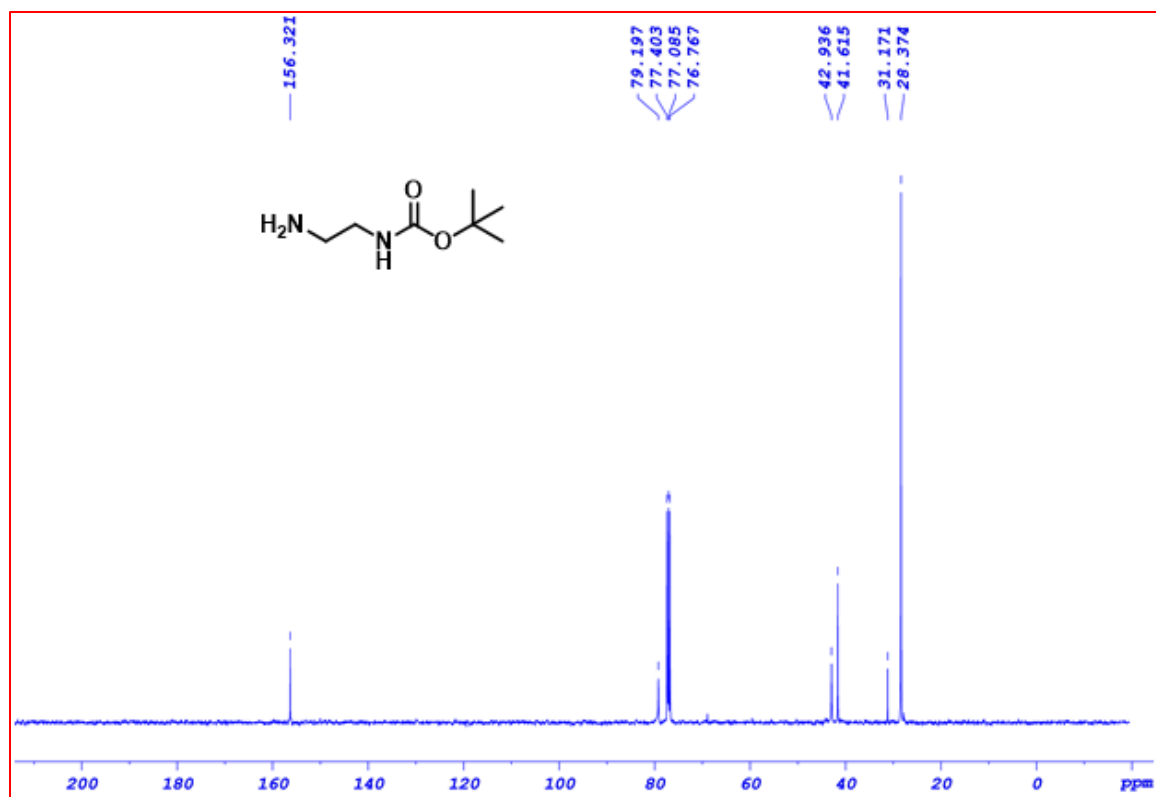


Figure 3.11: <sup>13</sup>C-NMR of 16.

### 3.3.2 Synthesis of tert-butyl (2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)ethyl)carbamate (**19**)

A mixture of solution of compounds **16** (0.90 g, 5.5 mmol) and **17** (1.0 g, 5.0 mmol) in EtOH (20 mL) was refluxed for 8 h. Upon completion of the reaction (TLC analysis), the reaction was concentrated under reduced pressure and the obtained solid was washed with diethyl ether (3 x 5 mL) to render the desired **19** as a light brown solid (1.57 g, 92%), m.p. 187–189 °C. IR (KBr): 3504, 3390, 2981, 1710, 1692, 1657, 1623, 1590, 1501, 1492, 1346, 1241.8, 1164, 1054, 1026, 785  $\text{cm}^{-1}$ .  $^1\text{H}$ -NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.59 (d, 2H,  $J = 7.2$  Hz), 8.20 (d, 2H,  $J = 8.0$  Hz), 7.36 (t, 2H,  $J = 7.6$  Hz), 5.00 (br. s, 1H), 4.35 (t, 2H,  $J = 5.6$ , Hz), 3.52 (br. s, 2H) and 1.28 (s, 9H);  $^{13}\text{C}$ -NMR (125.7 MHz,  $\text{CDCl}_3$ ):  $\delta$  164.8, 162.0, 134.0, 131.3, 127.0, 122.5, 39.8, 34.4, 28.4, 28.1. Anal. Calcd for  $\text{C}_{19}\text{H}_{20}\text{N}_2\text{O}_4$ : C 67.05, H 5.92, N 8.23. Found: C 67.00, H 5.96, N 8.17.

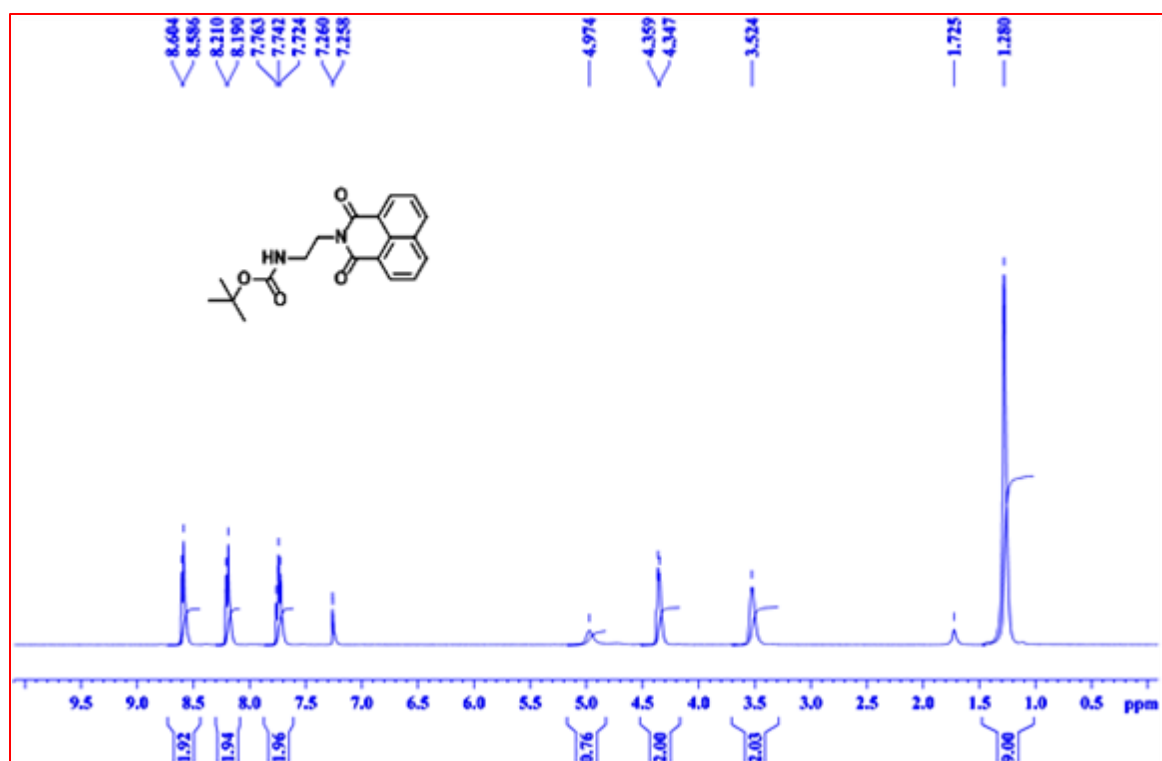


Figure 3.12: <sup>1</sup>H-NMR of 19.

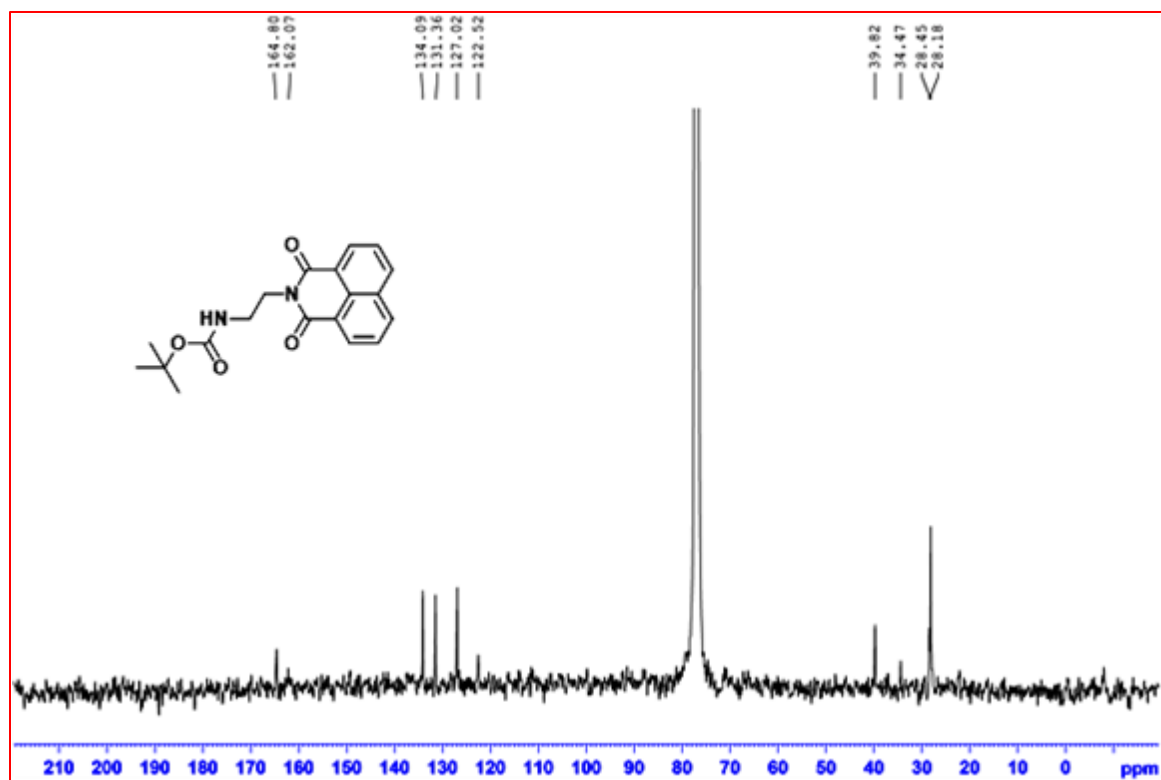


Figure 3.13: <sup>13</sup>C-NMR of 19.

### 3.3.3 Synthesis of tert-butyl (2-(6-nitro-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)ethyl)carbamate (**20**)

Following the same procedure adopted for the synthesis of compound **19**, the titled compound **20** was obtained from the reaction of compound **16** (1.0 g, 6.2 mmol) and **18** (1.37 g, 5.6 mmol) as a brown solid (2.03 g, 90%), m.p. 184–185 °C [57].

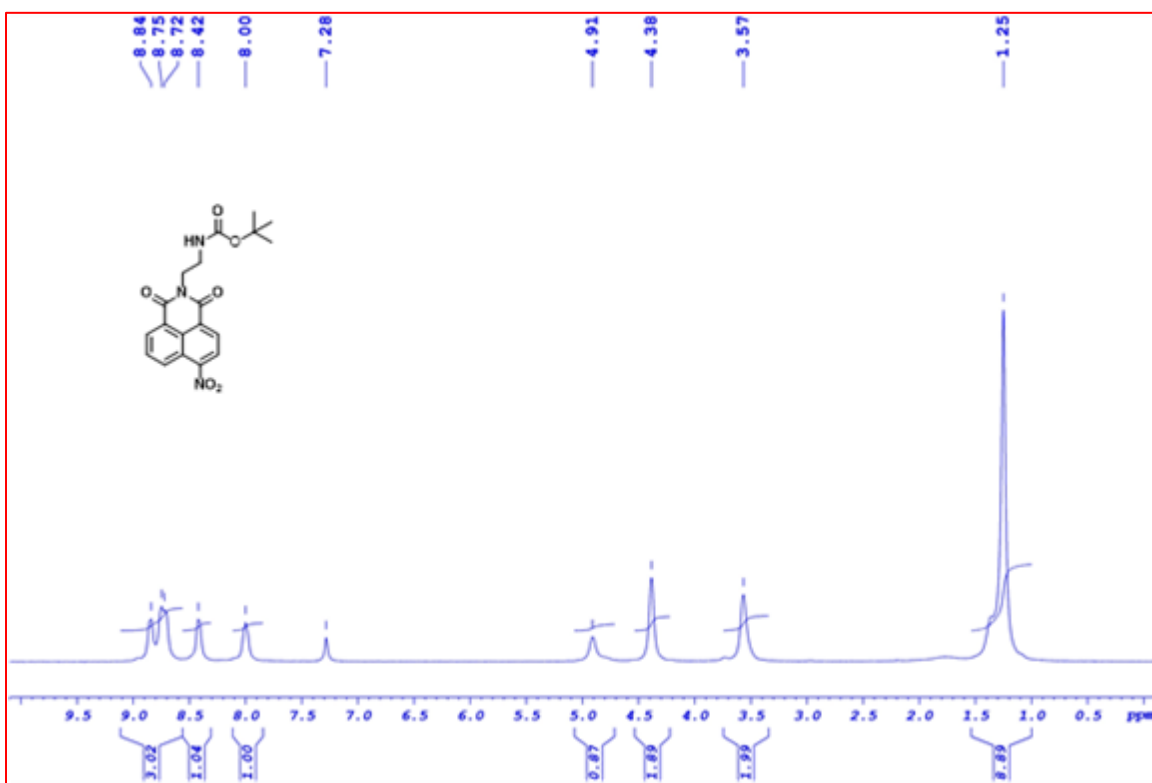


Figure 3.14: <sup>1</sup>H-NMR of **20**.

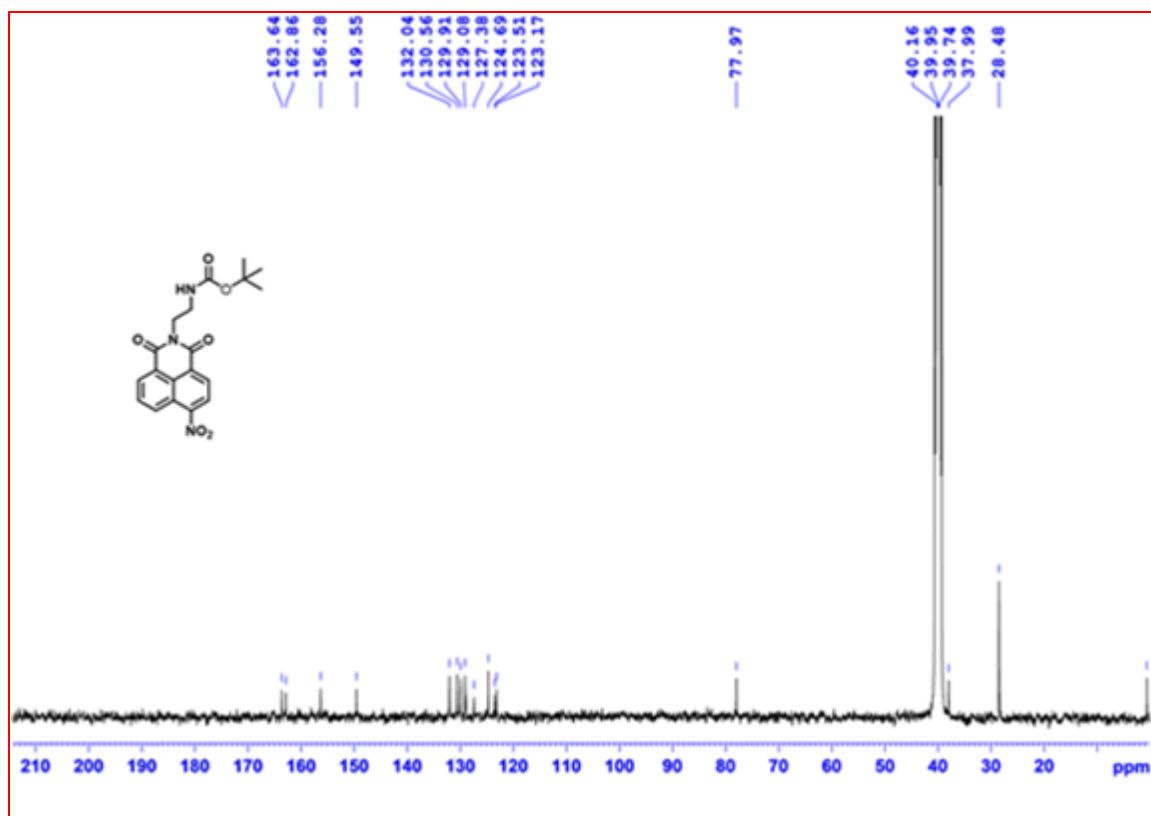


Figure 3.15:  $^{13}\text{C}$ -NMR of 20.

### 3.3.4 Synthesis of 2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)ethan-1-aminium TFA salt (**21**)

To a solution of compound **19** (0.50 g, 1.4 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (8 mL) was added trifluoroacetic acid (8.0 mL) dropwise at room temperature. The mixture was stirred overnight and volatiles were then evaporated under reduced pressure to obtain a brown thick oil, which was crystallized from diethyl ether to produce the desired **21** as a white solid (0.46 g, 92%), m.p. 212–213 °C. IR (KBr): 3439, 3064, 2923, 1685, 1659, 1594, 1514, 1438, 1387, 1350, 1249, 1204, 1174, 1141, 1092, 1027, 894, 841, 782, 722 cm<sup>-1</sup>. <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>): δ 8.48-8.44 (m, 4H), 7.96 (s, 2H), 7.86 (t, 2H, J = 8.0 Hz), 4.31 (s, 2H), 3.39 (br, s, 1H), 3.16 (s, 2H); <sup>13</sup>C-NMR (125.7 MHz, DMSO-d<sub>6</sub>): δ 167.5, 163.1, 139.3, 134.9, 132.2, 128.3, 122.2, 73.8 and 71.6. Anal. Calcd for C<sub>16</sub>H<sub>13</sub>F<sub>3</sub>N<sub>2</sub>O<sub>4</sub>: C 54.24, H 3.70, N 7.91. Found: C 54.17, H 3.77, N 7.80.

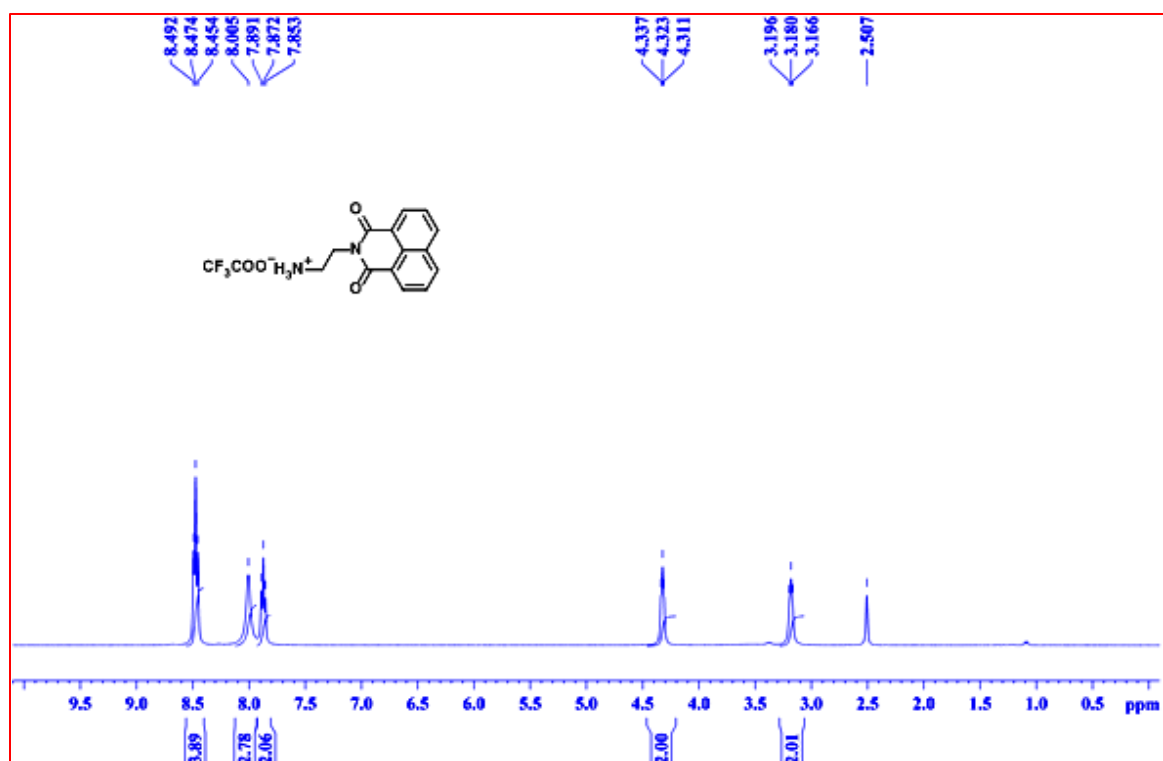


Figure 3.16: <sup>1</sup>H-NMR of 21.

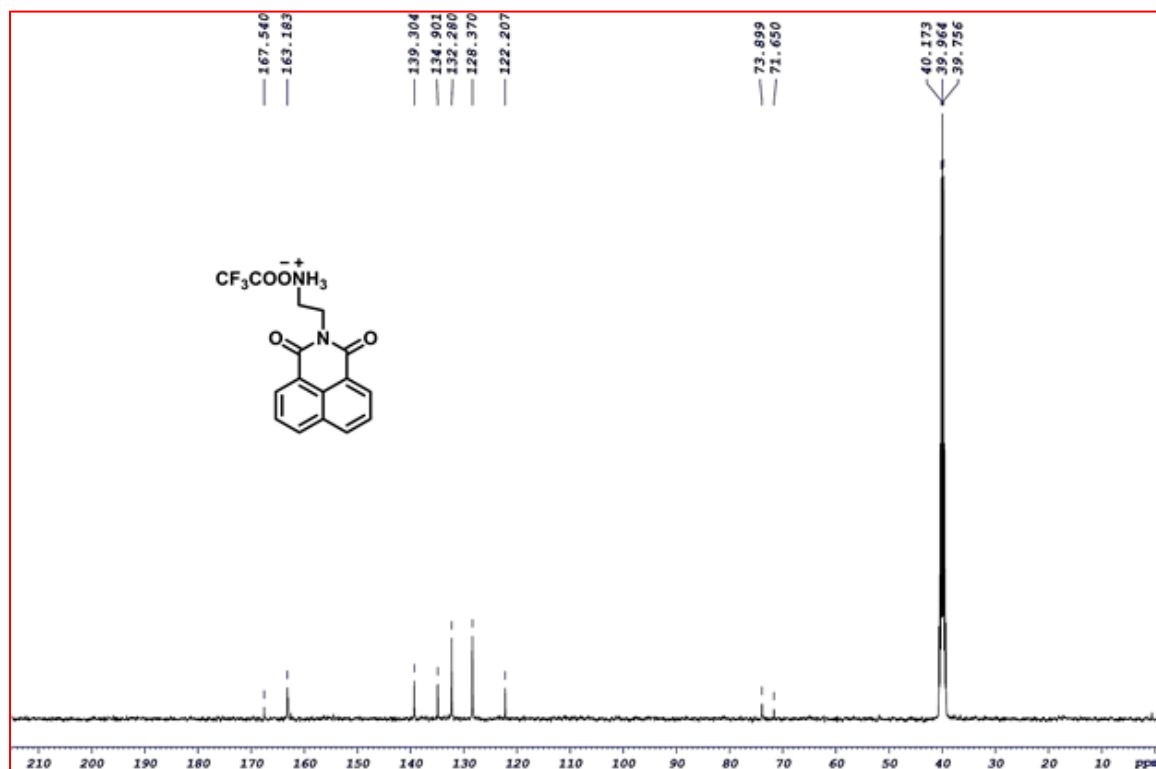


Figure 3.17: <sup>13</sup>C-NMR of 21.

### 3.3.5 2-(2-aminoethyl)-6-nitro-1H-benzo[de]isoquinoline-1,3(2H)-dione TFA salt (22)

The title compound was synthesized according to the synthesis procedure adopted for the synthesis of compound **20**. Brown solid (0.55 g, 91%), m.p. 211–212 °C [58].

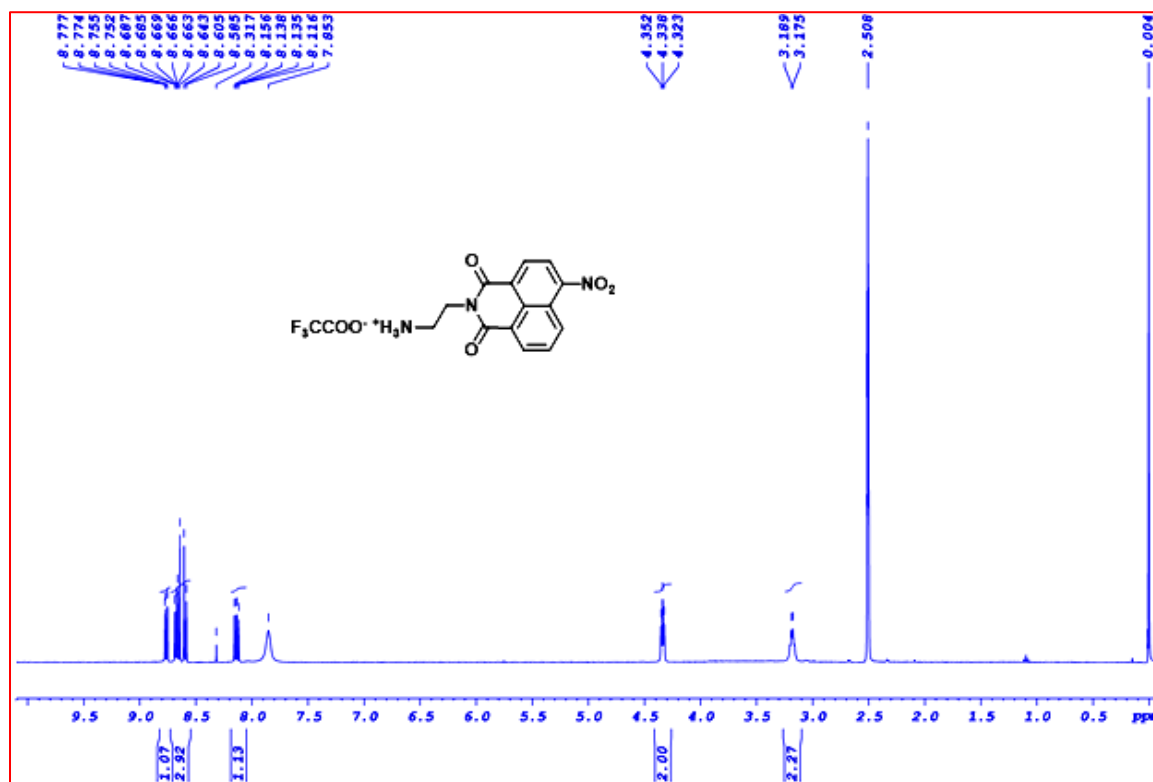


Figure 3.18: <sup>1</sup>H-NMR of 22.

### 3.3.6 Synthesis of (24)

To a solution of ester **23** (0.75 g, 2.52 mmol) in a mixture of methanol and water (1:2 v/v, 8 mL) was added NaOH (0.41 g, 10.22 mmol) at room temperature and the reaction was then stirred overnight, and volatiles were evaporated. The reaction mixture was neutralized by HCl (1.00 M) and then lyophilized to obtain a brownish solid. Dissolution of obtained solid in acetone and filtration led to afford salt free acid **24**. Solvent evaporated to get the desired acid as dark brown solid (yield 81.0 %). <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD): δ 8.56 (2H, d, J = 4.58), 7.70 (2H, d, J=7.62), 7.32 (4H, t, J = 7.63 Hz), 4.15 (4H, s), 3.63 (2H, s). <sup>13</sup>C-NMR (125.7 MHz, CD<sub>3</sub>OD): 149.0, 137.92, 123.26, 122.92, 77.00.

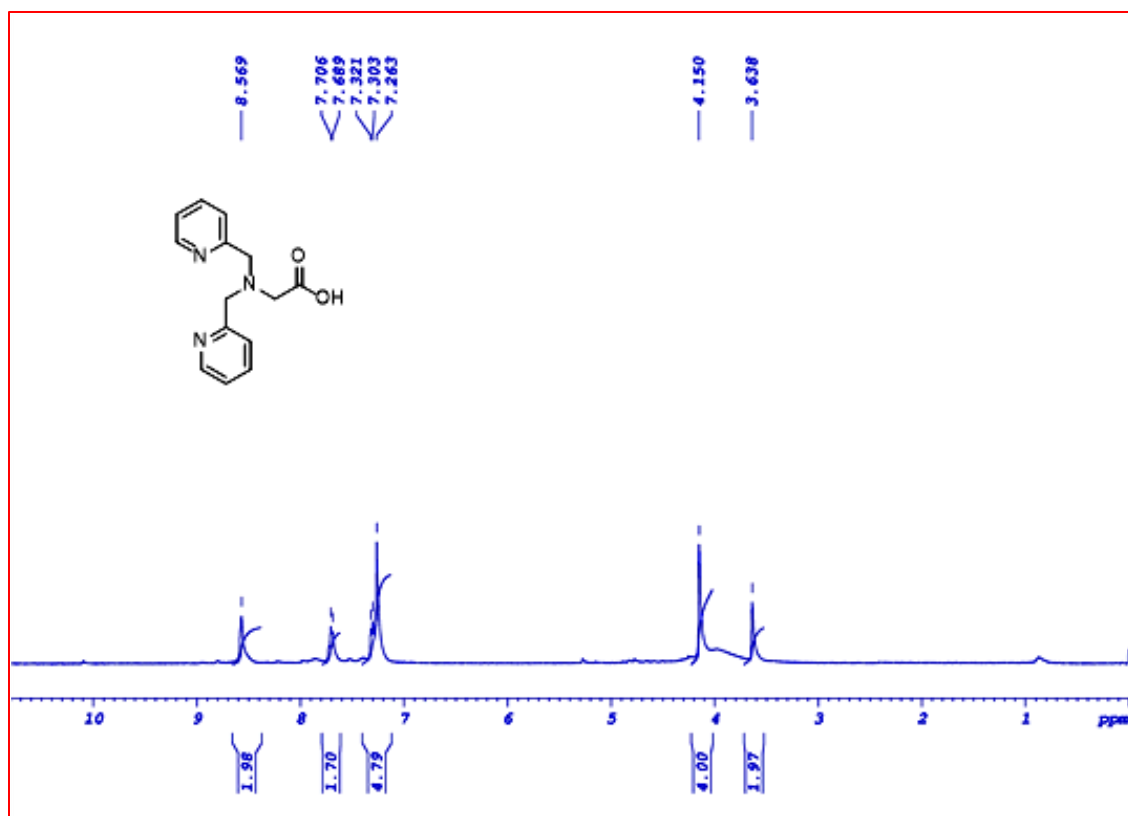


Figure 3.19: <sup>1</sup>H-NMR of 24.

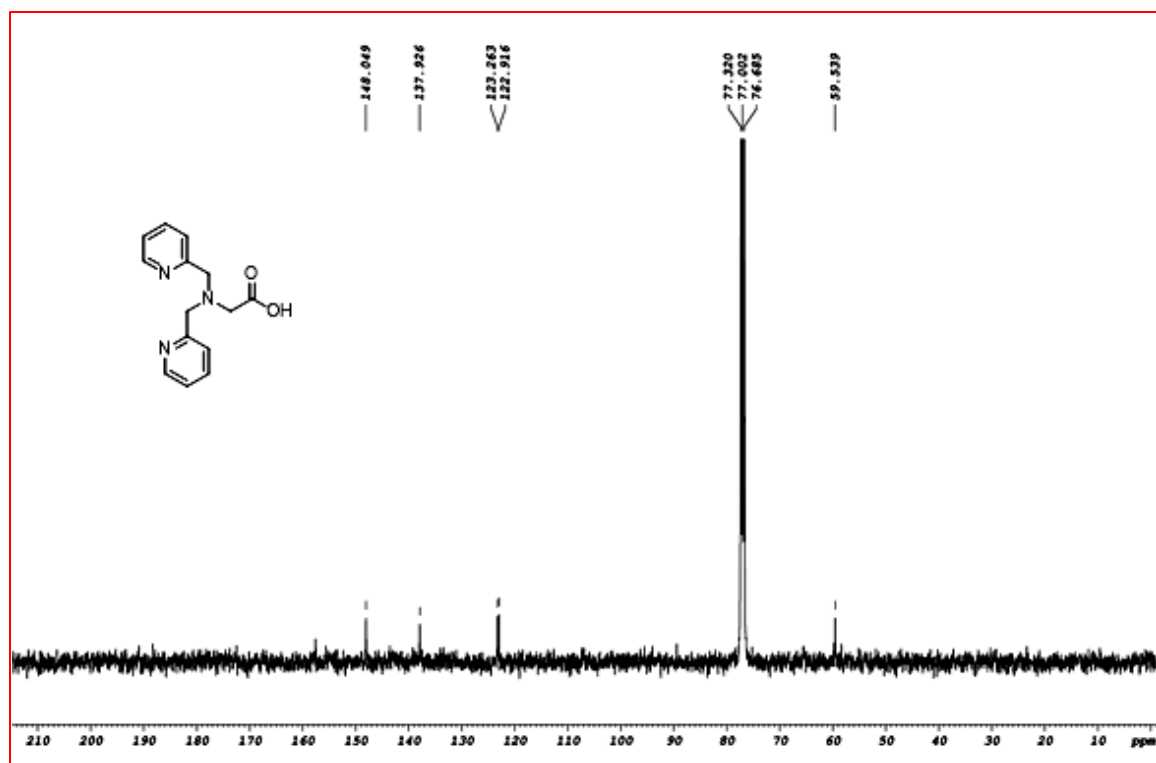


Figure 3.20:  $^{13}\text{C}$ -NMR of 24

### 3.3.7 Synthesis of bis(quinolin-2-ylmethyl)carbamic acid (26)

To a solution of ester **25** (0.6 g, 1.68 mmol) in a mixture of methanol and water (1:2 v/v, 12 mL) at room temperature was 1M sodium hydroxide (10 mL, 10.0 mmol) and mixture stirred for overnight. Upon completion of the reaction, the solvent was evaporated under vacuum and crude was purified by column chromatography, eluting with methanol-chloroform (5:95) to afford the title compound as a dark black oil (0.48 g, 84.0%), IR (KBr): 3429, 3064, 2372, 1961, 1729, 1647, 1597, 1505, 1466, 1505, 1466, 1427, 1376, 1211, 1144, 1114, 1045, 964, 876, 836, 755, 624 and 525  $\text{cm}^{-1}$ .  $^1\text{H}$ -NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.16 (d, 2H,  $J = 8.4$  Hz), 8.06 (d, 2H,  $J = 8.4$  Hz), 7.72 (q, 4H,  $J = 8.5$  Hz), 7.51 (t, 4H,  $J = 7.0$ ), 4.27 (s, 4H), 3.2 (s, 2H);  $^{13}\text{C}$ -NMR (125.7 MHz,  $\text{CDCl}_3$ ):  $\delta$  174.3, 157.4, 146.6, 137.6, 131.1, 128.1, 127.6, 126.8, 121.0, 59.7, and 50.6. Anal. Calcd for  $\text{C}_{22}\text{H}_{19}\text{N}_3\text{O}_2$ : C, 73.93; H, 5.36; N, 11.76; O, 8.95. Found: C 54.17, H 3.77, N 7.80.

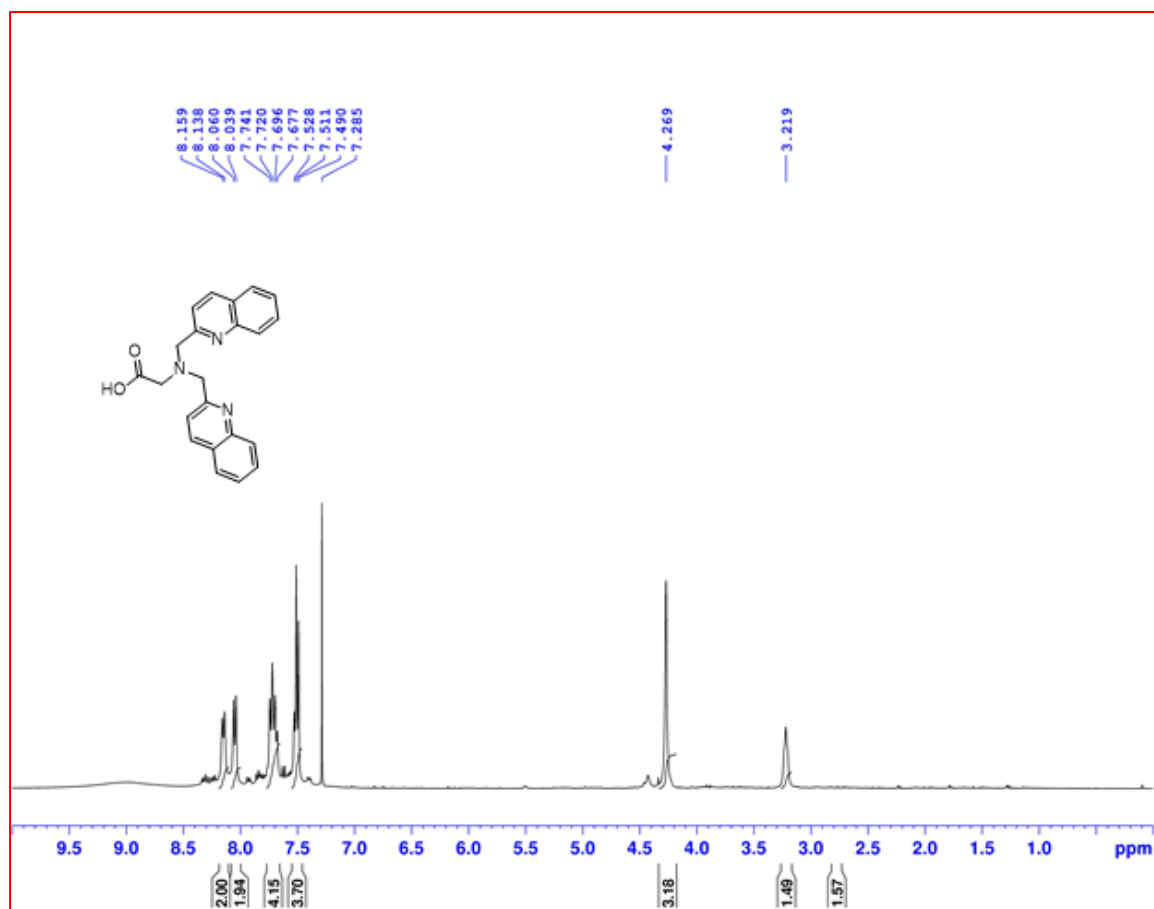


Figure 3.21: <sup>1</sup>H-NMR of 26.

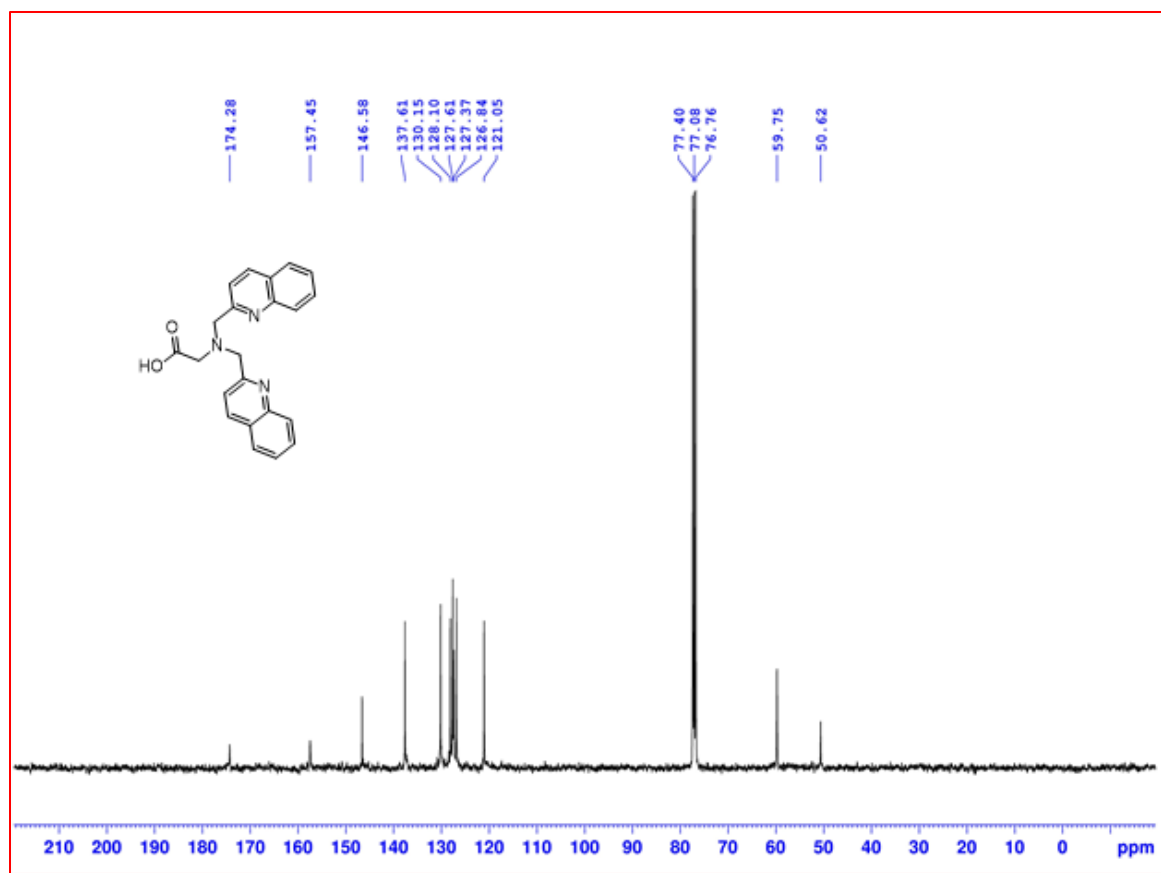


Figure 3.22: <sup>13</sup>C-NMR of 26.

### 3.3.8 Synthesis of chemosensor (3)

To a stirred solution of mixture of compound **21** (0.20 g, 0.83 mmol) and **24** (0.19 g, 0.73 mmol) in DMF (10 mL) was sequentially added hydroxybenzotriazole (0.17 g, 1.2 mmol), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (0.32 g, 1.6 mmol) and N,N-diisopropylethylamine (1.3 mL, 6.6 mmol) dropwise and the reaction was then stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate (10 mL) and washed with H<sub>2</sub>O (10 mL) and brine (3 x 10 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated under reduced pressure to obtain a light brown residue, which was resolved over silica column eluting with 2:98 (methanol:chloroform) to obtain the desired chemosensor **3** as a dark brown gummy oil (0.30, 86%). IR (KBr): 3428, 2924, 5823, 1696, 1658, 1590, 1438, 1383, 1346, 1237, 1184, 1093, 1051, 850, 780 cm<sup>-1</sup>. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>): δ 9.30 (d, 1H), 8.39 (d, 2H, J = 7.2 Hz), 8.19 (d, 2H, J = 4.4 Hz), 8.09 (d, 2H, J = 8 Hz), 7.61 (t, 3H, J = 8 Hz), 7.35 (t, 2H, J = 7.6 Hz), 7.12 (d, 2H, J = 7.6 Hz), 6.82 (t, 2H, J = 5.6 Hz), 4.41 (t, 2H, J = 4.8 Hz), 3.76 (br, s, 6H), 3.30 (s, 2H); <sup>13</sup>C-NMR (125.7 MHz, CDCl<sub>3</sub>): δ 171.7, 164.3, 157.7, 148.3, 136.8, 133.7, 131.4, 130.9, 127.9, 126.7, 123.2, 122.5, 122.0, 59.8, 58.1, 39.8, 37.7. Anal. Calcd for C<sub>28</sub>H<sub>25</sub>N<sub>5</sub>O<sub>3</sub>: C 70.13, H 5.26, N 14.60. Found: C 70.02, H 5.35, N 14.50.

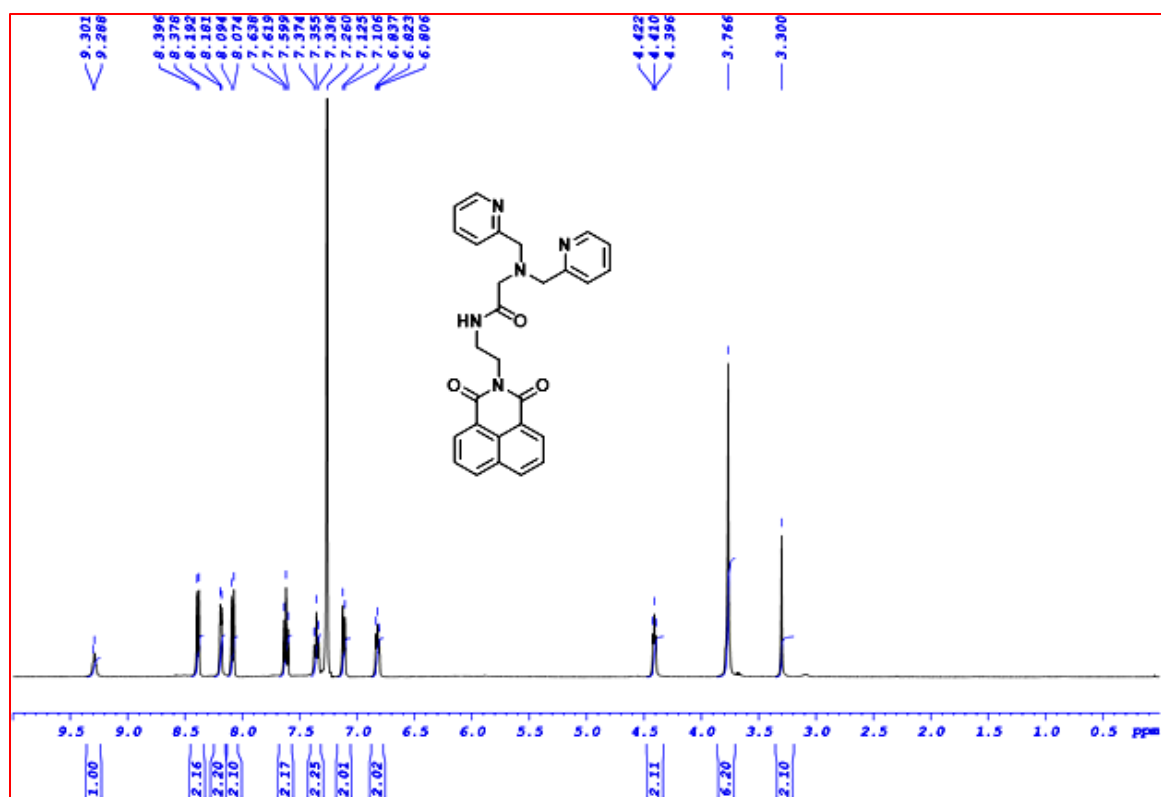


Figure 3.23: <sup>1</sup>H-NMR of 3.

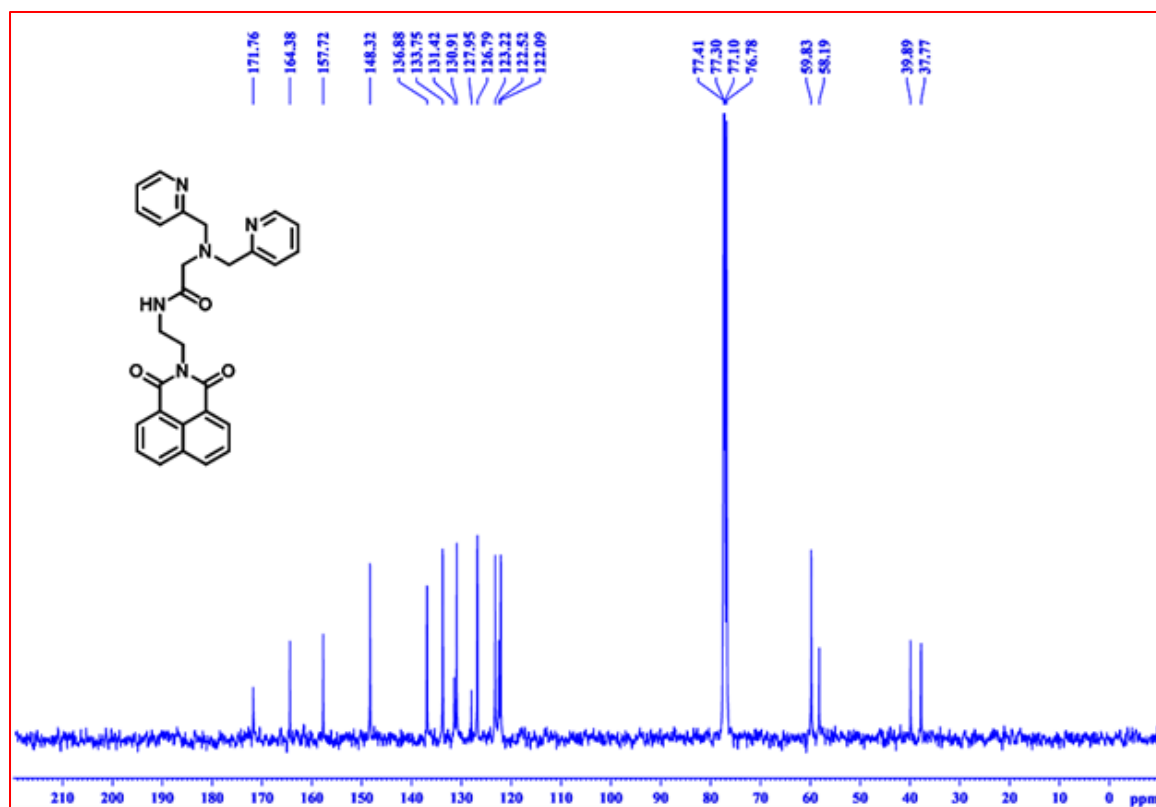


Figure 3.24:  $^{13}\text{C}$ -NMR of 3.

### 3.3.9 Synthesis of chemosensor (4)

Chemosensor **4** was synthesized from the reaction of compounds **22** and **24**, according to the procedure adopted for the synthesis of chemosensor **3**. Dark brown gum (0.29 g, 79%). IR (KBr): 3437, 2924, 2853, 1658, 1531, 1438, 1382, 1344, 1235, 1094, 728  $\text{cm}^{-1}$ .  $^1\text{H}$ -NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.30 (d, 1H), 8.39 (d, 2H,  $J = 7.2$  Hz), 8.19 (d, 2H,  $J = 4.4$  Hz), 8.09 (d, 2H,  $J = 8.0$  Hz), 7.61 (t, 3H,  $J = 8.0$  Hz), 7.35 (t, 2H,  $J = 7.6$  Hz), 7.12 (d, 2H,  $J = 7.6$  Hz), 6.82 (t, 2H,  $J = 5.6$  Hz), 4.41 (t, 2H,  $J = 4.8$  Hz), 3.76 (br, s, 6H), 3.30 (s, 2H);  $^{13}\text{C}$ -NMR (125.7 MHz,  $\text{CDCl}_3$ ):  $\delta$  172.0, 158.5, 148.8, 137.3, 132.3, 130.4, 129.9, 129.4, 127.5, 124.4, 123.6, 122.6, 59.8, 54.9, 54.3, 40.2, 37.5. Anal. Calcd for  $\text{C}_{28}\text{H}_{24}\text{N}_6\text{O}_5$ : C 64.12, H 4.61, N 16.02. Found: C 64.06, H 4.67, N 16.95.

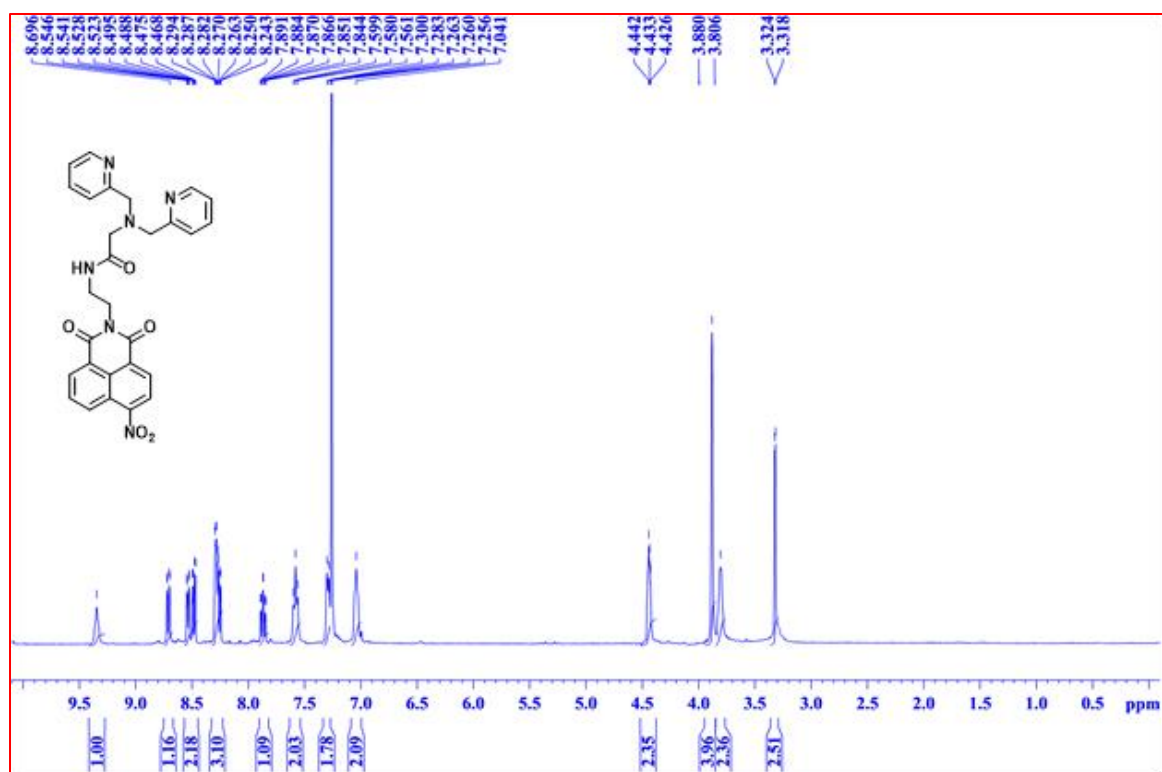


Figure 3.25: <sup>1</sup>H-NMR of 4.

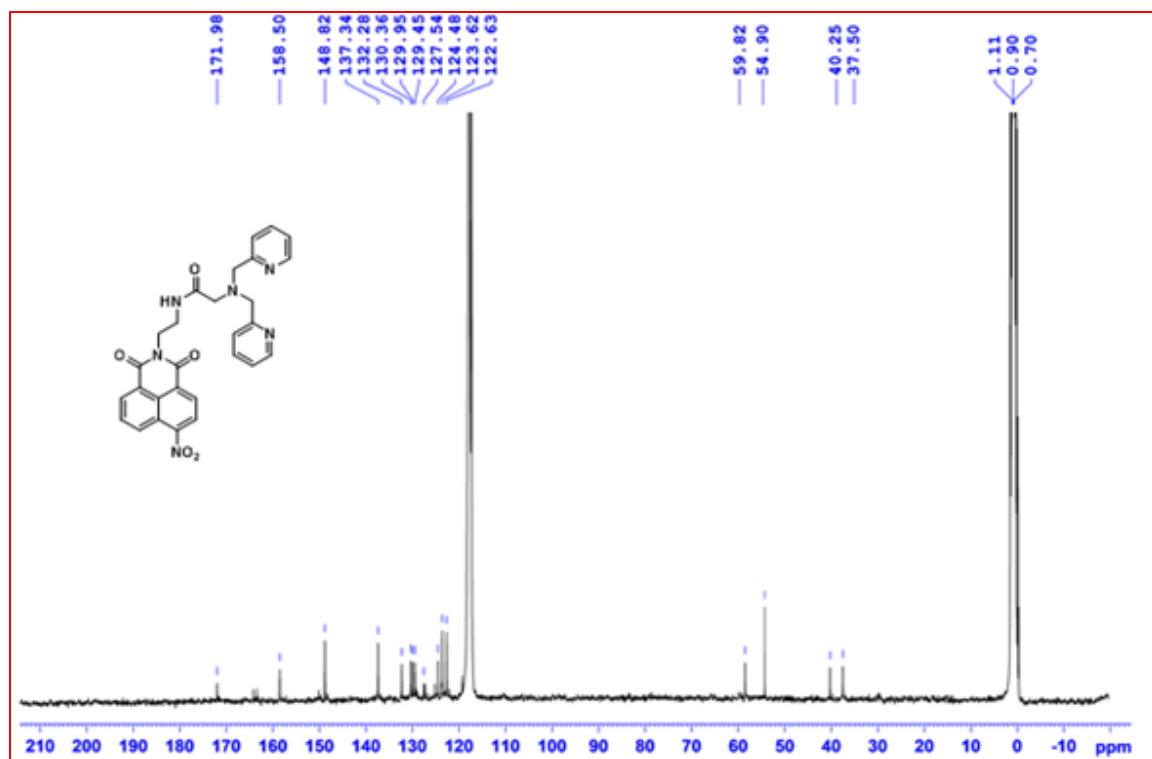


Figure 3.26:  $^{13}\text{C}$ -NMR of 4.

### 3.3.10 Synthesis of chemosensor (5)

Chemosensor **5** was synthesized from the reaction of compounds **21** and **26**, according to the procedure adopted for the synthesis of chemosensor **3**. Dark brown gum (0.35 g, 83%). IR (KBr): 3433, 2924, 2854, 1964, 1656, 1591, 1504, 1436, 1383, 1344, 1234, 1182, 1113, 1051, 842, 779, 726  $\text{cm}^{-1}$ .  $^1\text{H}$ -NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  10.08 (t, 1H,  $J = 5.6$  Hz), 8.09 (d, 2H,  $J = 7.2$  Hz), 7.84 (t, 4H,  $J = 8.8$  Hz), 7.60 (d, 2H,  $J = 8.4$  Hz), 7.45 (t, 2H,  $J = 2.4$  Hz), 7.41 (t, 4H,  $J = 8.8$  Hz), 7.285 (t, 4H, 6.4 Hz), 7.14 (d, 2H,  $J = 8.4$  Hz), 4.42 (d, 2H,  $J = 4.8$  Hz), 4.04 (s, 4H), 3.90 (d, 2H,  $J = 4$  Hz) and 3.53 (s, 2H);  $^{13}\text{C}$ -NMR (125.7 MHz,  $\text{CDCl}_3$ ):  $\delta$  171.4, 164.3, 157.6, 139.3, 138.7, 134.1, 133.4, 131.4, 131.2, 131.0, 130.6, 129.6, 129.0, 128.1, 127.8, 127.4, 127.0, 126.8, 12.5, 125.9, 122.0, 121.5, 119.4, 59.5, 59.1, 45.8, 40.0, 39.5, 38.1. Anal. Calcd for  $\text{C}_{36}\text{H}_{29}\text{N}_5\text{O}_3$ : C 74.59, H 5.04, N 12.08. Found: C 74.52, H 5.11, N 12.00.

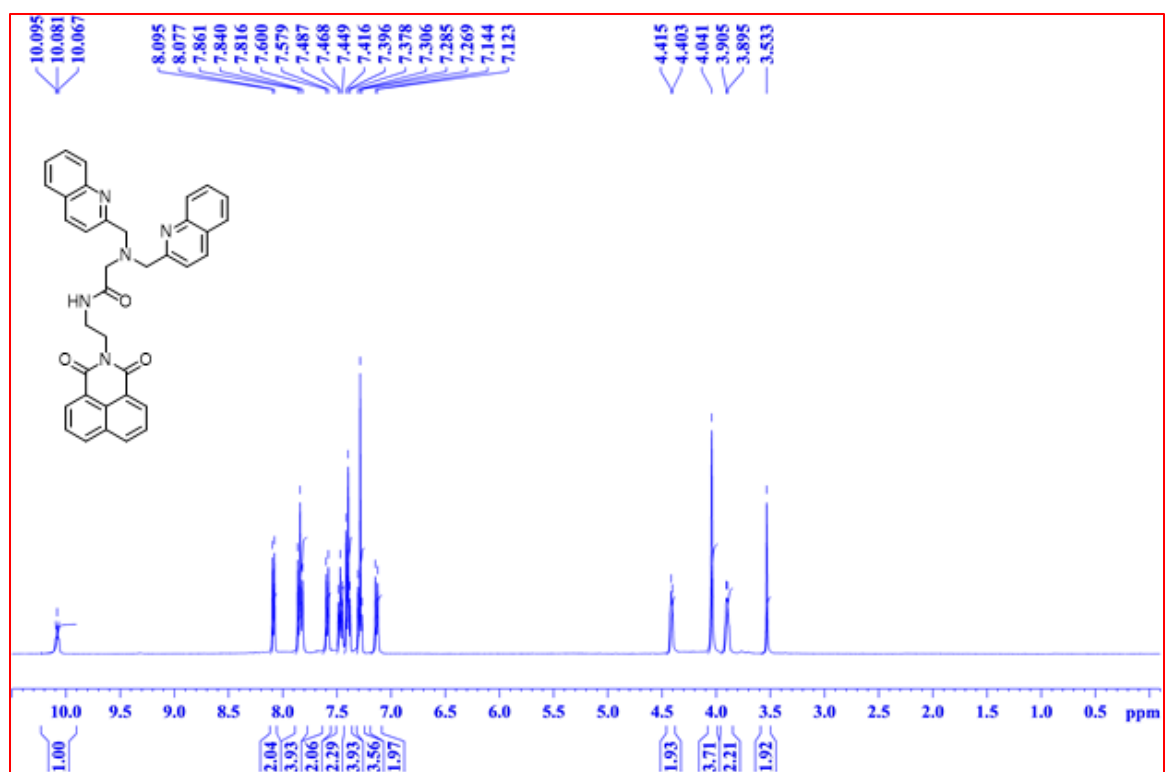


Figure 3.27:  $^1\text{H}$ -NMR of 5.

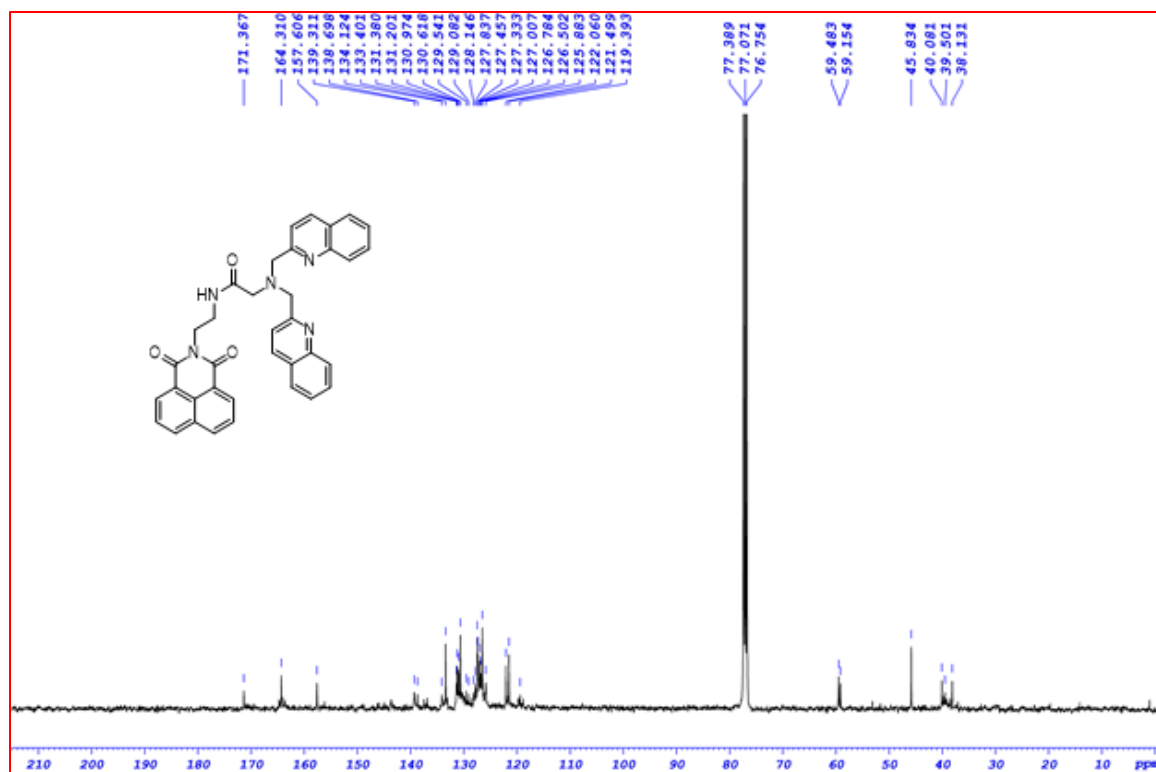


Figure 3.28: <sup>13</sup>C-NMR of 5.

### 3.4 Synthesis of intermediates and chemosensor 6 and 7

#### 3.4.1 Synthesis of tert-butyl (2-bromoethyl)carbamate (**28**)

Intermediate **28** was synthesized according to the reported procedure [30]. Di-tert-butyl dicarbonate (1.22 g, 5.6 mmol) was added to a suspension of **27** (1.0 g, 4.88 mmol) in THF (80 mL) and the reaction mixture was cooled to 0 °C before dropwise addition of diisopropylethylamine (1.6 mL, 9.19 mmol). The reaction was then stirred at room temperature for 10 hours until completion (TLC analysis). The solvent was evaporated under reduced pressure and crude residues were dissolved in ethyl acetate (8 mL) and washed with 10% aqueous K<sub>2</sub>CO<sub>3</sub> (8 mL) and brine (8 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness to afford **28** as a thick yellow oil. (1.02 g, 98%).  
<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>): δ (ppm) 5.20 (s, 1H), 3.46 (t, 2H, *J* = 5.2 Hz), 3.39–3.37 (t, 2H), 1.38 (s, 9H); <sup>13</sup>C-NMR (125.7 MHz, CDCl<sub>3</sub>): δ (ppm) 155.64, 79.59, 42.30, 32.48, 28.30.

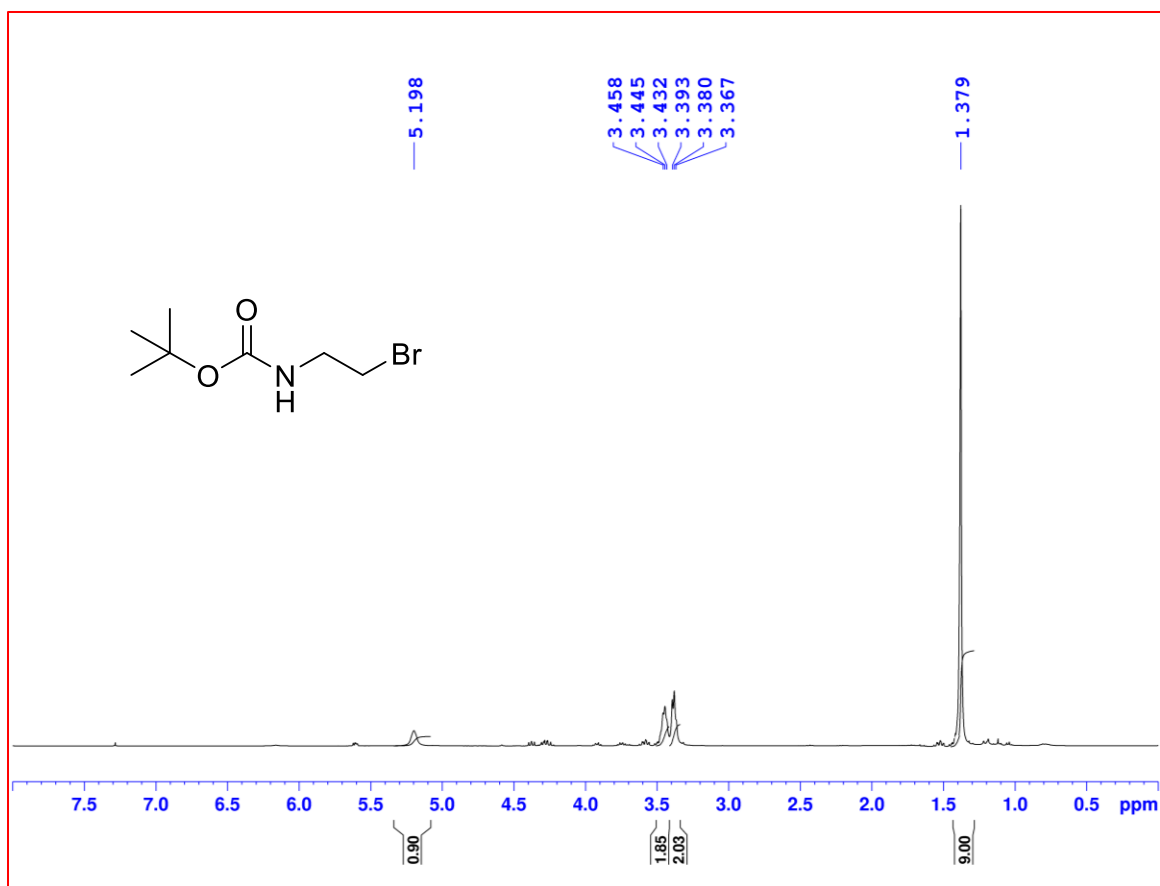


Figure 3.29:  $^1\text{H}$ -NMR of 28.

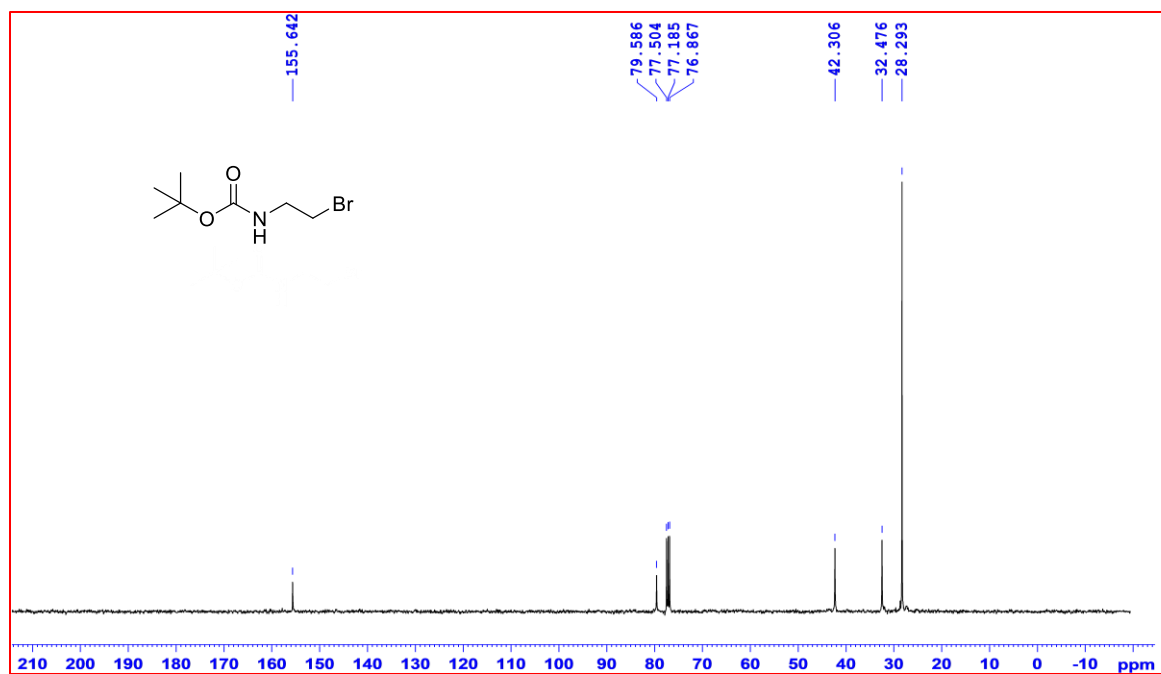


Figure 3.30:  $^{13}\text{C}$ -NMR of 28.

### 3.4.2 Synthesis of tert-butyl (2-azidoethyl)carbamate (**29**)

The title compound **29** was synthesized according to the reported procedure [31]. To a stirring solution of compound **28** (1.0 g, 4.46 mmol) in DMF (16 mL) was added NaN<sub>3</sub> (860 mg, 13.2 mmol) and the reaction mixture was stirred for 24 hours at room temperature. After completion of the reaction (TLC analysis), the mixture was diluted with ethyl acetate (100 mL) and washed with brine (40 mL x 3). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated under vacuum to afford the desired **29** as a colorless oil (0.79 g, 95%). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>): δ (ppm) 4.85 (s, 1H), 3.43 (t, 2H, *J* = 5.2 Hz), 3.33–3.30 (t, 2H), 1.47 (s, 9H); <sup>13</sup>C-NMR: (125.7 MHz, CDCl<sub>3</sub>): δ (ppm) 155.72, 79.87, 51.26, 40.11, 28.35.

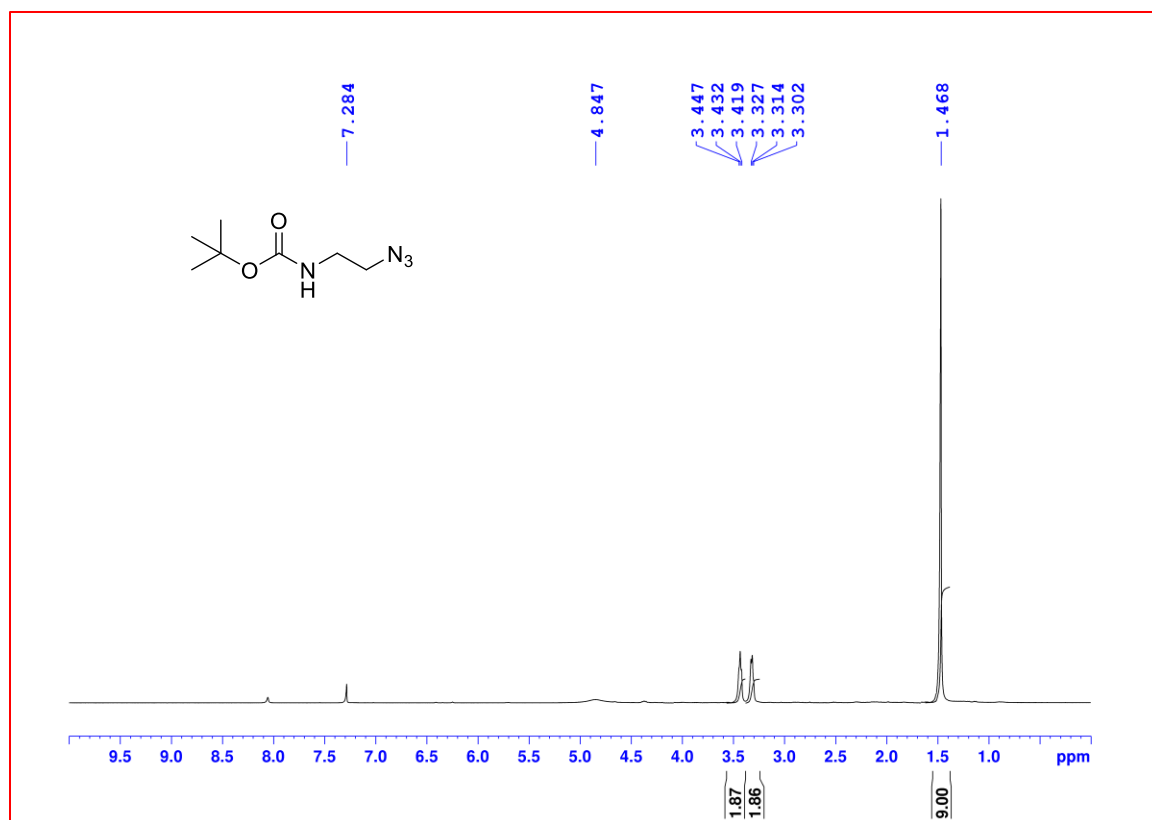


Figure 3.31: <sup>1</sup>H-NMR of 29.

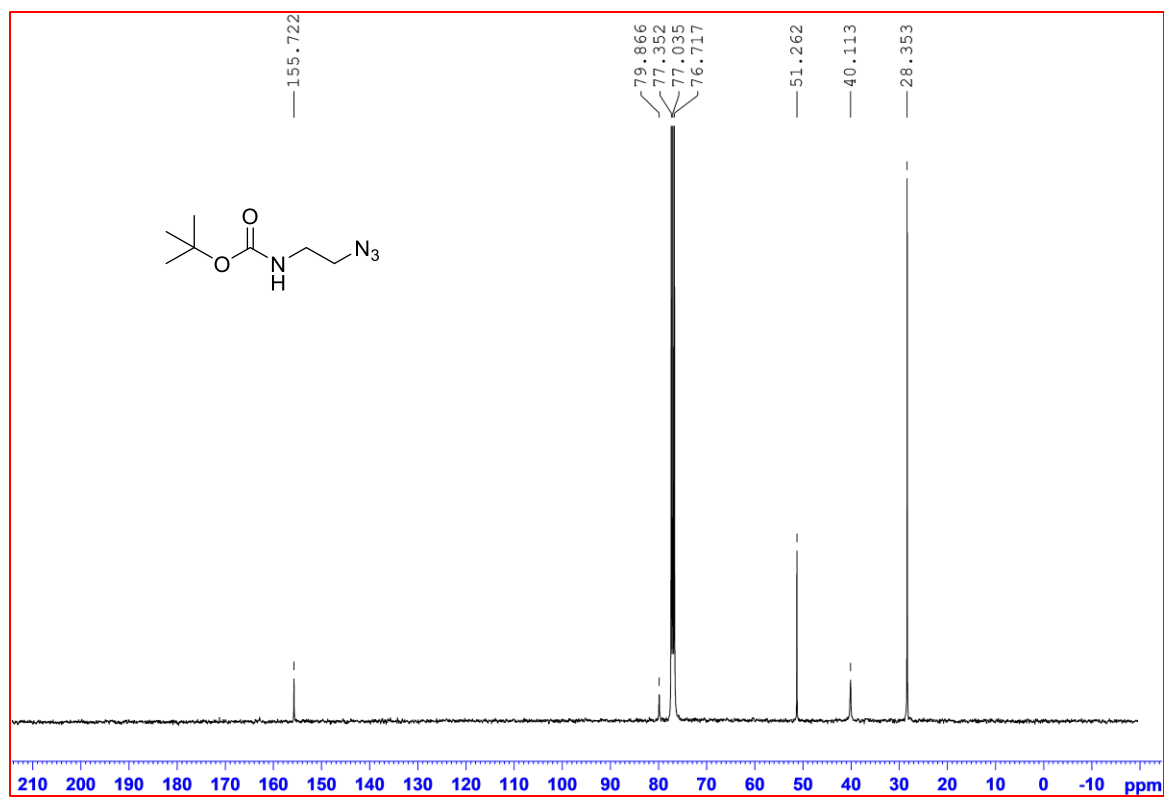


Figure 3.32:  $^{13}\text{C}$ -NMR of 29.

### 3.4.3 Synthesis of N-propargyl-di-2-picolyl)amine (32)

To a solution of propargylamine **30** (0.1 g, 1.8 mmol) in acetonitrile (10 mL) was added  $K_2CO_3$  (1.5 g, 11 mmol) at room temperature. After being stirred for 0.5 hour, bromoethylpyridine **31** (1.1 g, 4.5 mmol) was added to the mixture and the reaction was then heated to reflux for 8 h under inert atmosphere. Upon completion of the reaction (TLC analysis), the reaction was cooled to room temperature, filtered and the filtrate was concentrated under vacuum to obtain crude residues, which were resolved over silica column, eluting with 98-2.0 ( $CH_3Cl:CH_3OH$ ) to afford the desired **32** as a black gummy thick liquid (0.59 g, 98.7%).  $^1H$ -NMR (400 MHz,  $CDCl_3$ ):  $\delta$  (ppm) 8.60 (d, 2H,  $J = 5.2$  Hz), 7.74 (m, 2H), 7.60 (d, 2H,  $J = 5.6$  Hz), 7.23 (m, 2H), 4.00 (s, 4H), 3.48 (d, 2H,  $J = 2.3$  Hz), 2.30 (t, 1H,  $J = 2.3$  Hz);  $^{13}C$ -NMR: (125.7 MHz,  $CDCl_3$ ):  $\delta$  (ppm) 158.08, 148.74, 137.19, 123.61, 122.47, 74.04, 59.04, 42.73.

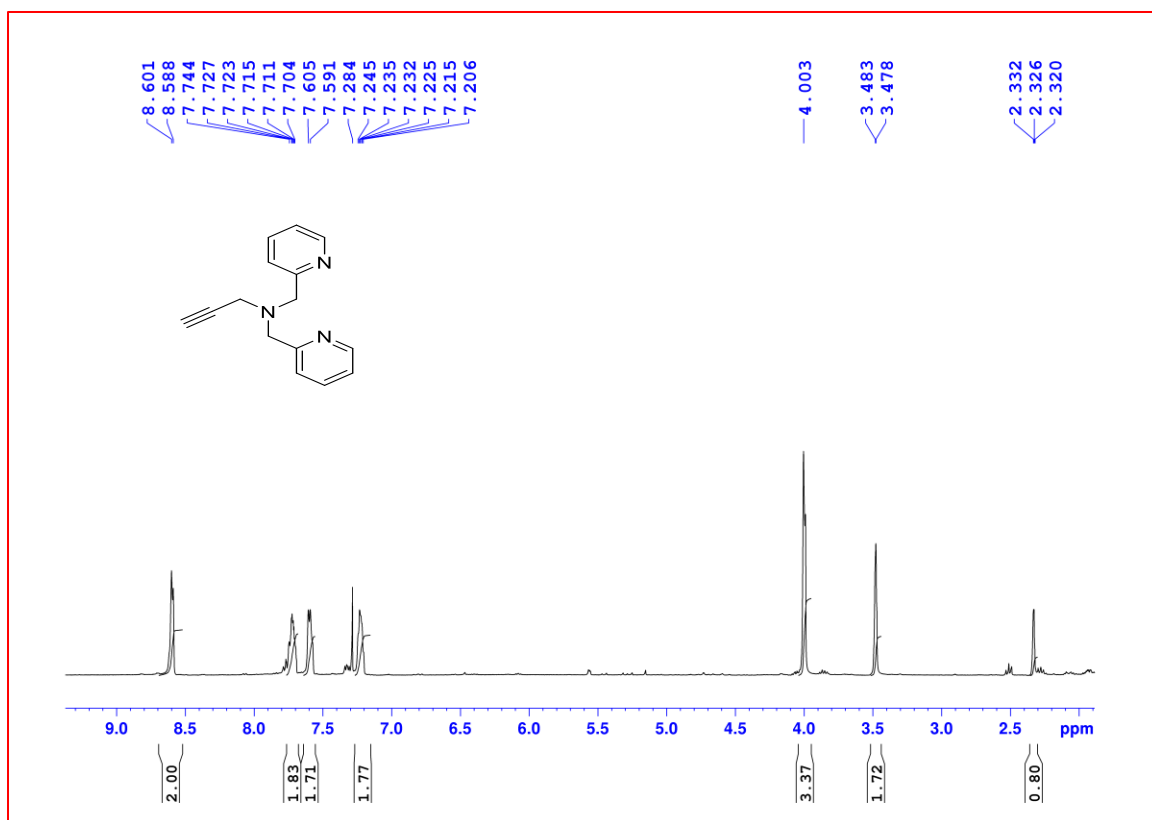


Figure 3.33: <sup>1</sup>H-NMR of 32.

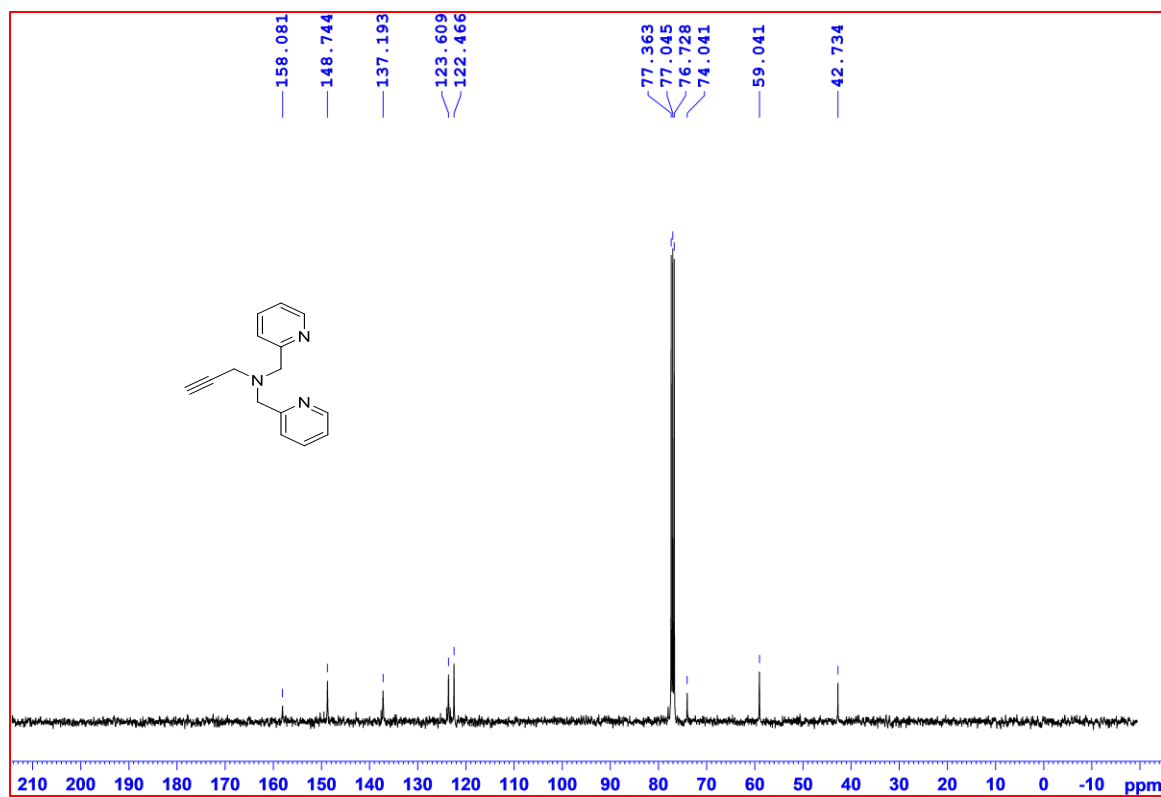


Figure 3.34:  $^{13}\text{C}$ -NMR of 32.

### 3.4.4 Synthesis of tert-butyl (2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)ethyl)carbamate (**33**)

To a mixture of solution of **29** (1.03 g, 5.56 mmol) (11 mL) and **32** (1.32 g, 5.56 mmol) in CH<sub>3</sub>OH (11 mL) was sequentially added solutions of Cu(AcO)<sub>2</sub>·H<sub>2</sub>O (0.56 g, 3.1 mmol) in water (7 mL) and sodium ascorbate (0.856 g, 4.32 mmol) in water (27 mL). The reaction was allowed to stir at room temperature for 6 h until completion (TLC analysis, methanol-chloroform 2:98). The reaction was diluted with ethyl acetate (50 mL) and washed with sat. NaHCO<sub>3</sub> (25 mL) and brine (25 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated under vacuum to obtain a dark brown sticky gum, which was resolved on silica column eluting methanol-chloroform (2:98) to obtain the desired **33** as a dark brown thick oil (2.1 g, 86%). IR (KBr): cm<sup>-1</sup> 3436, 2995, 2907, 2085, 1683, 1563, 1519, 1434, 1367, 1252, 1169, 1055, 767. <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>): δ (ppm) 8.49 (d, 2H, *J* = 4.0 Hz), 8.01 (s, 1H), 7.79–7.75 (m, 2H), 7.59 (d, 2H, *J* = 7.6 Hz) 7.26 (m, 2H), 6.7 (s, 1H), 4.52 (t, 2H, *J* = 5.6 Hz), 4.27 (s, 4H), 4.24 (s, 2H), 3.65 (t, 2H, *J* = 5.2 Hz), 1.42 (s, 9H); <sup>13</sup>C-NMR (125.7 MHz, CDCl<sub>3</sub>): δ (ppm) 155.91, 154.21, 148.13, 140.52, 138.42, 126.16, 125.40, 123.59, 79.80, 57.50, 50.31, 48.64, 40.41, 28.34. Anal. Calcd for C<sub>22</sub>H<sub>29</sub>N<sub>7</sub>O<sub>2</sub>: C 62.39, H 6.90, N 23.15. Found: C 62.30, H 6.99, N 23.0.

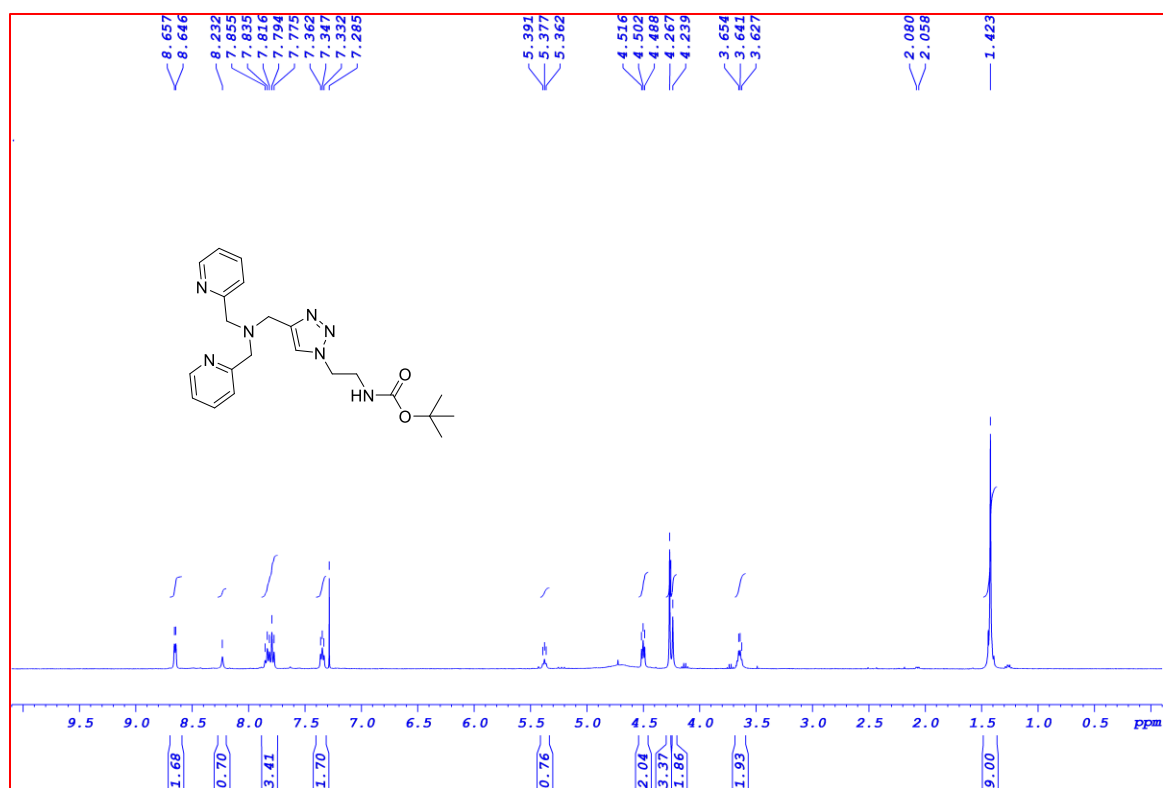


Figure 3.35: <sup>1</sup>H-NMR of 33.

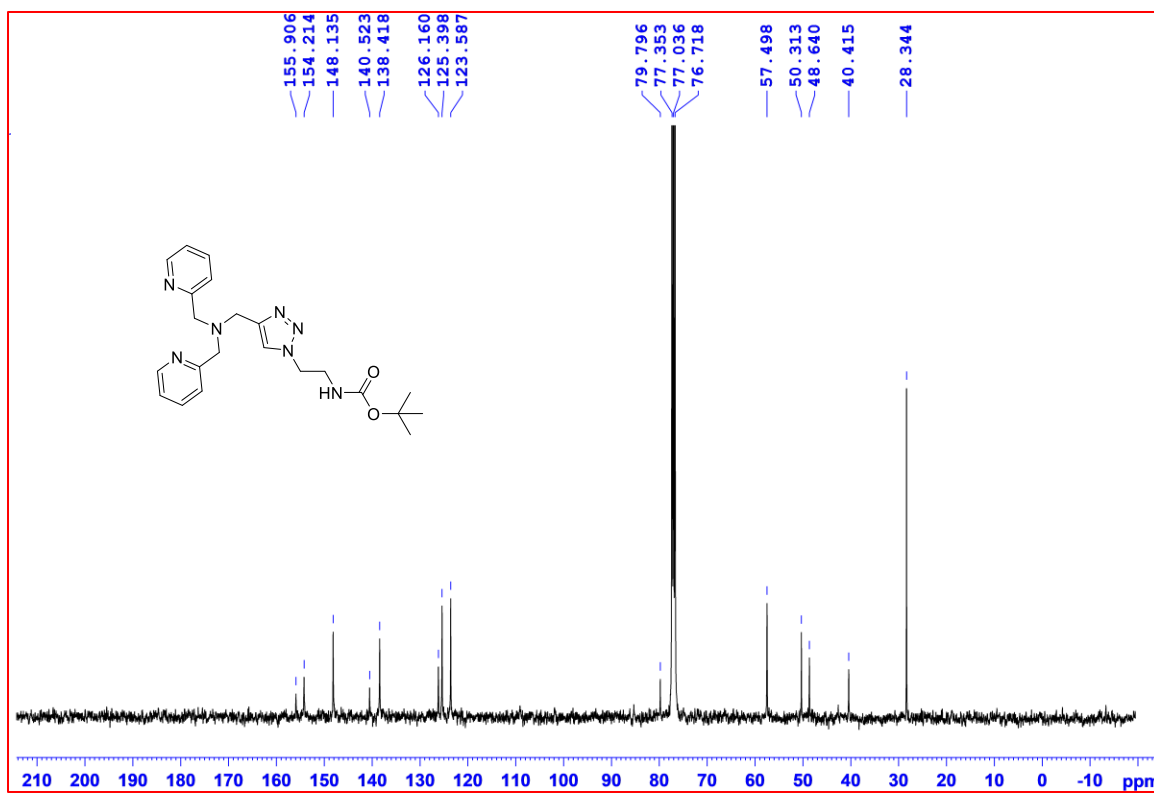


Figure 3.36:  $^{13}\text{C}$ -NMR of 33.

### 3.4.5 Synthesis of trifluoro ammonium salt of 2-(4-((bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)ethan-1-amine (34)

To a solution of **33** (1.0 g, 2.31 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) at room temperature was added trifluoroacetic acid (2 mL) and the mixture was stirred overnight at room temperature and volatiles were then evaporated under vacuum. The residues were triturated with ether thrice (3 x 5 mL) and filtered to afford the desired **34** as a dark brown gummy solid (0.74 g, 97%). IR (KBr): cm<sup>-1</sup> 3448, 2927, 2997, 2382, 1685, 1501, 1232, 1130, 835, 722. <sup>1</sup>H-NMR (400 MHz, DMSO): δ 8.69 (t, 2H, *J* = 0.8 Hz), 8.32 (s, 1H), 8.16 (bs, 3H), 8.02–7.98 (m, 2H), 7.63 (d, 2H, *J* = 8.0 Hz), 7.54–7.51 (m, 2H), 4.66 (t, 4H, *J* = 5.6 Hz), 4.35 (s, 4H), 4.34 (s, 2H); <sup>13</sup>C-NMR: (125.7 MHz, CDCl<sub>3</sub>): δ 158.74, 158.41, 153.81, 148.56, 139.01, 127.24, 124.94, 124.25, 118.34, 115.41, 57.12, 48.63, 47.51. Anal. Calcd for C<sub>19</sub>H<sub>22</sub>F<sub>3</sub>N<sub>7</sub>O<sub>2</sub>: C 52.17, H 5.07, N 22.41. Found: C 52.07, H 5.17, N 22.20.

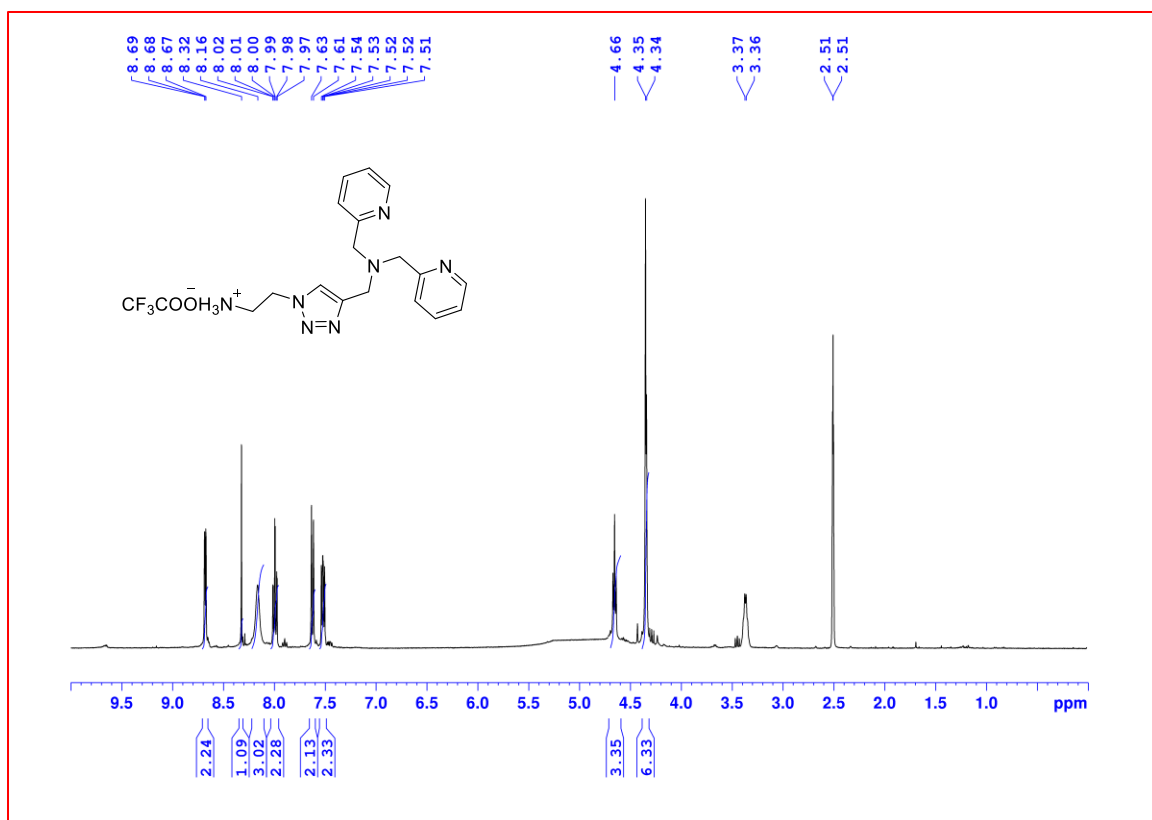


Figure 3.37: <sup>1</sup>H-NMR of 34.

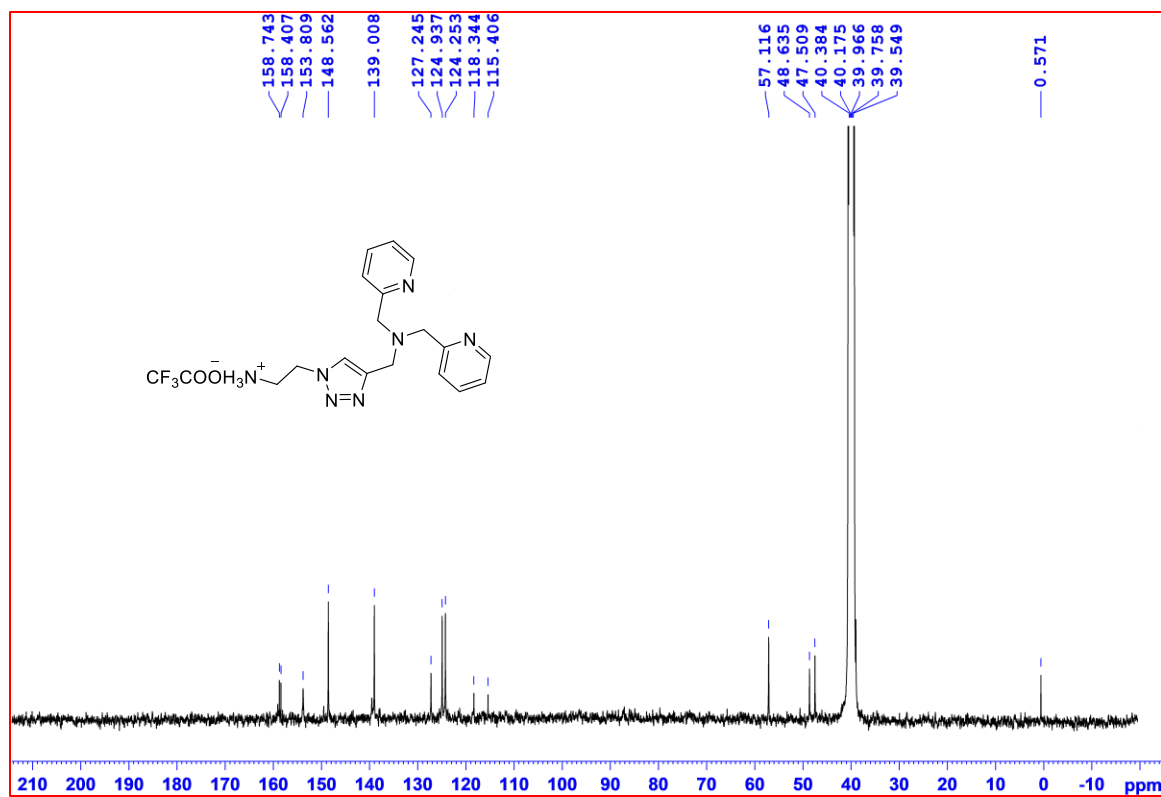


Figure 3.38:  $^{13}\text{C}$ -NMR of 34.

### 3.4.6 Synthesis of chemosensor (6)

To a solution of compound **34** (0.3 g, 0.93 mmol) in DMF at 0 °C was added diisopropylethylamine (DIPEA) (0.6 g, 4.63 mmol) dropwise. After being stirred for 30 minutes, 1-fluoro-2,4-dinitrobenzene **13** (0.19 g, 1.0 mmol) was added and reaction mixture was stirred at 40 °C for 12 h until completion (TLC analysis). The mixture was diluted with dichloromethane (15 mL) and washed successively with brine (6 mL x 3) and H<sub>2</sub>O (6 mL). The organic layer was separated, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated under vacuum. Column chromatography of the dark brown oily residues on silica column, eluting with ethyl acetate-hexane (1:1) and then changing to methanol-chloroform (5:95) afforded the desired chemosensor **6** as a light-yellow gummy solid (0.39 g, 86%). IR (KBr): cm<sup>-1</sup> 3478, 2877, 2101, 1747, 1620, 1483, 1427, 1366, 1254, 1078, 1026, 883, 786, 707, 573. <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>CN): δ 8.8 (d, 1H, *J* = 2.8 Hz), 8.48–8.46 (m, 2H), 8.16–8.13 (m, 1H), 7.84 (s, 1H), 7.74–7.62 (m, 2H), 7.52 (d, 2H, *J* = 7.6), 7.24–7.21 (m, 2H), 6.88 (d, 1H, *J* = 9.6 Hz), 4.70–4.67 (m, 2H), 4.01 (t, 2H, *J* = 6.0 Hz), 3.75 (s, 2H), 3.65 (s, 4H); <sup>13</sup>C-NMR (125.7 MHz, CD<sub>3</sub>CN): δ 158.88, 148.63, 148.39, 144.09, 136.80, 135.99, 130.55, 130.02, 124.67, 123.69, 123.02, 122.27, 114.14, 58.82, 48.87, 48.22, 42.95. Anal. Calcd for C<sub>23</sub>H<sub>23</sub>N<sub>9</sub>O<sub>4</sub>: C 56.44, H 4.74, N 25.75. Found: C 56.35, H 4.81, N 25.66.

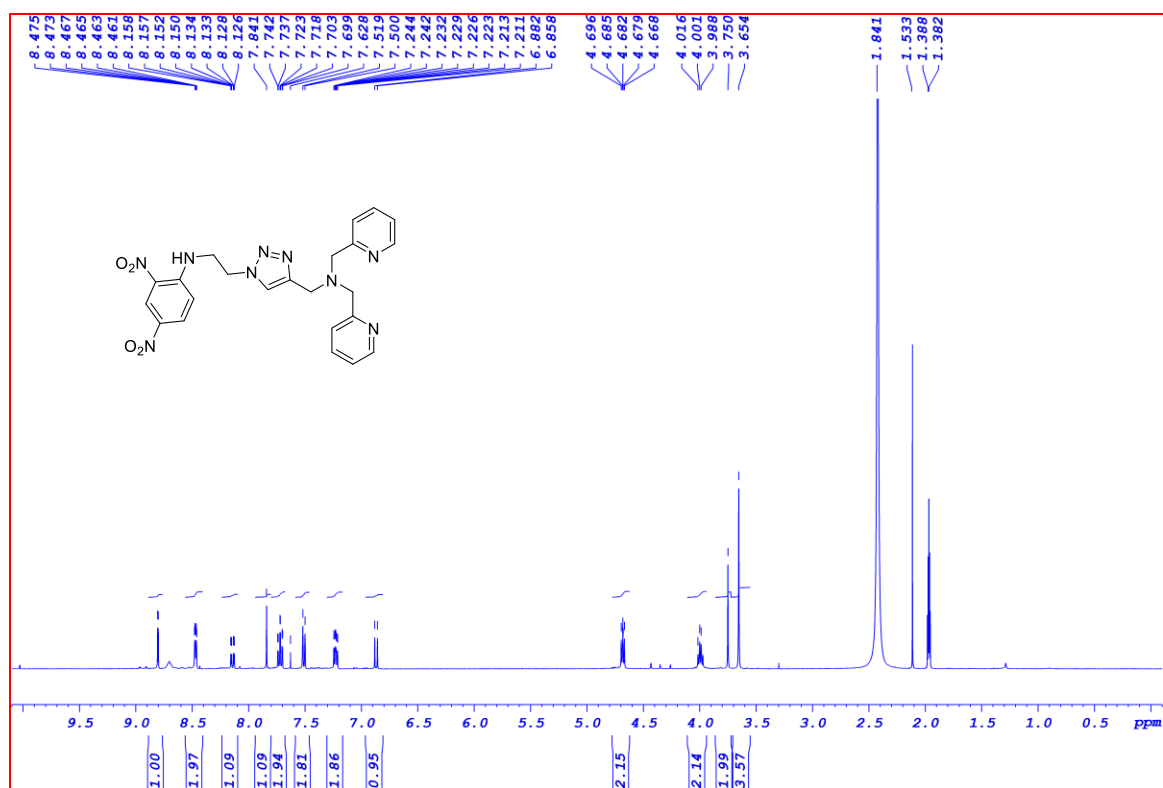


Figure 3.39: <sup>1</sup>H-NMR of 6.

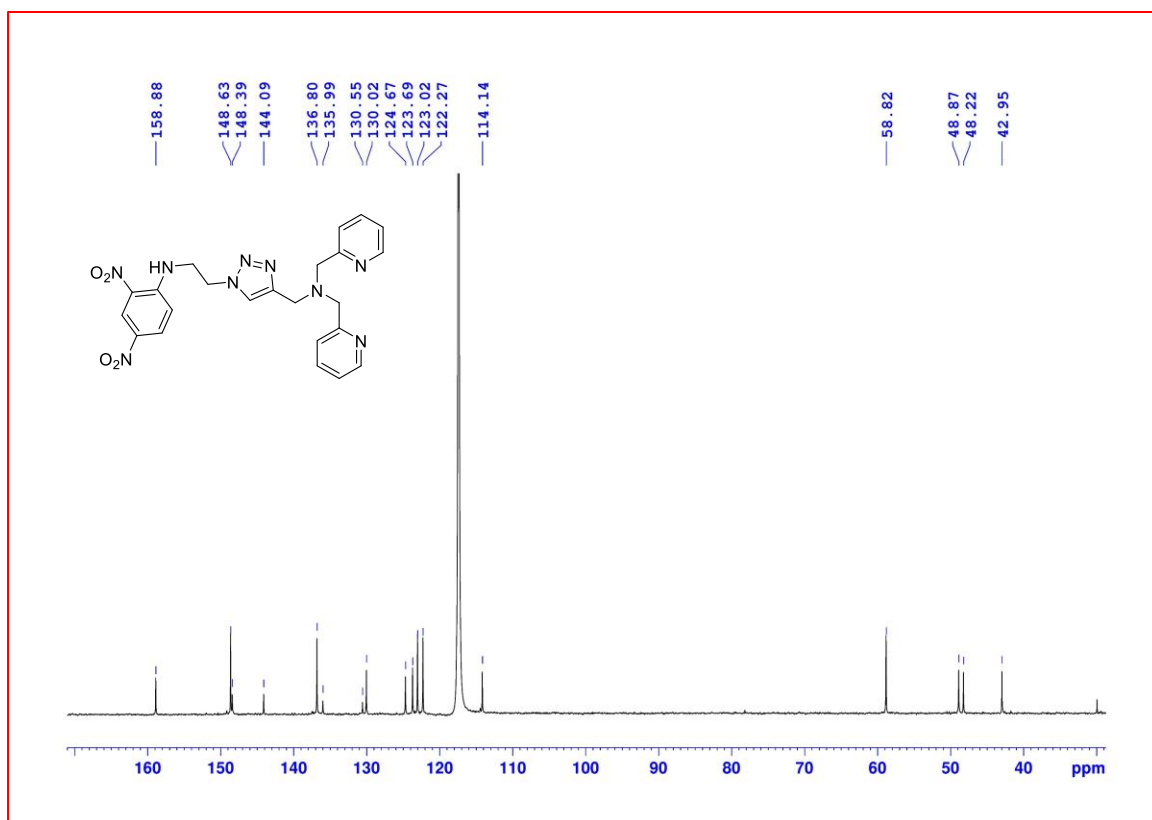


Figure 3.40: <sup>13</sup>C-NMR of 6.

### 3.4.7 Synthesis of chemosensor (7)

To a solution of compound **34** (0.3 g, 0.93 mmol) in ethanol at 0 °C was added K<sub>2</sub>CO<sub>3</sub> (0.64 g, 4.6 mmol) and after being stirred for 30 minutes at room temperature, compound **35** (0.243 g, 1.0 mmol) was added and the reaction mixture was refluxed for 12 h until completion (TLC analysis). The reaction was cooled to room temperature, filtered and residues were washed with chloroform (30 mL) and volatiles were then evaporated under vacuum. The dark brown residues were dissolved in chloroform (20 mL) and then washed with water (10 mL). The organic layer was separated, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated with rotary evaporator to afford a dark brown oil, which was resolved over silica column, eluting with ethyl acetate-hexane (1:1) and then changing to methanol-chloroform (1:9) to afford the desired chemosensor **7** as a brown gummy solid (0.43 g, 84%). IR (KBr): cm<sup>-1</sup> 3444, 3081, 2807, 1964, 1710, 1673, 1622, 1568, 1529, 1473, 1432, 1363, 1241, 1049, 833, 783, 759. <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>CN): δ 8.63 (dd, 1H, *J* = 7.6, 12.0 Hz), 8.47–8.42 (m, 4H), 8.26 (d, 1H, *J* = 8.0), 7.87–7.85 (m, 2H), 7.68–7.63 (m, 4H), 7.44 (d, 2H, *J* = 8.0), 7.21 (m, 2H), 4.78 (t, 2H, *J* = 5.2 Hz), 4.57 (t, 2H, *J* = 6.0 Hz), 3.69 (s, 2H), 3.65 (s, 2H); <sup>13</sup>C-NMR (125.7 MHz, CD<sub>3</sub>CN): δ 163.13, 162.37, 158.61, 149.59, 148.65, 143.44, 137.41, 136.80, 133.45, 132.08, 129.93, 129.22, 128.73, 126.26, 124.63, 124.07, 123.15, 122.38, 122.27, 78.21, 64.99, 58.64, 40.31. Anal. Calcd for C<sub>29</sub>H<sub>24</sub>N<sub>8</sub>O<sub>4</sub>: C 63.50, H 4.41, N 20.43. Found: C 63.42, H 4.47, N 20.32.

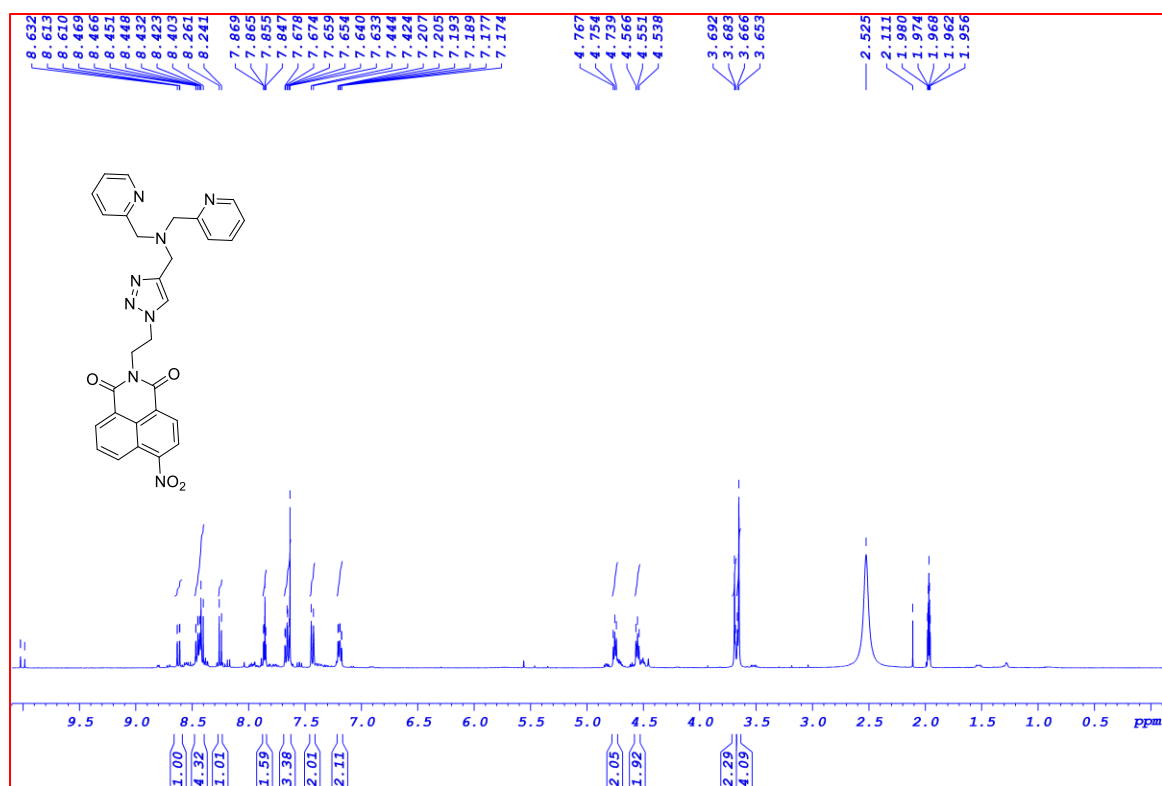


Figure 3.41: <sup>1</sup>H-NMR of 7.

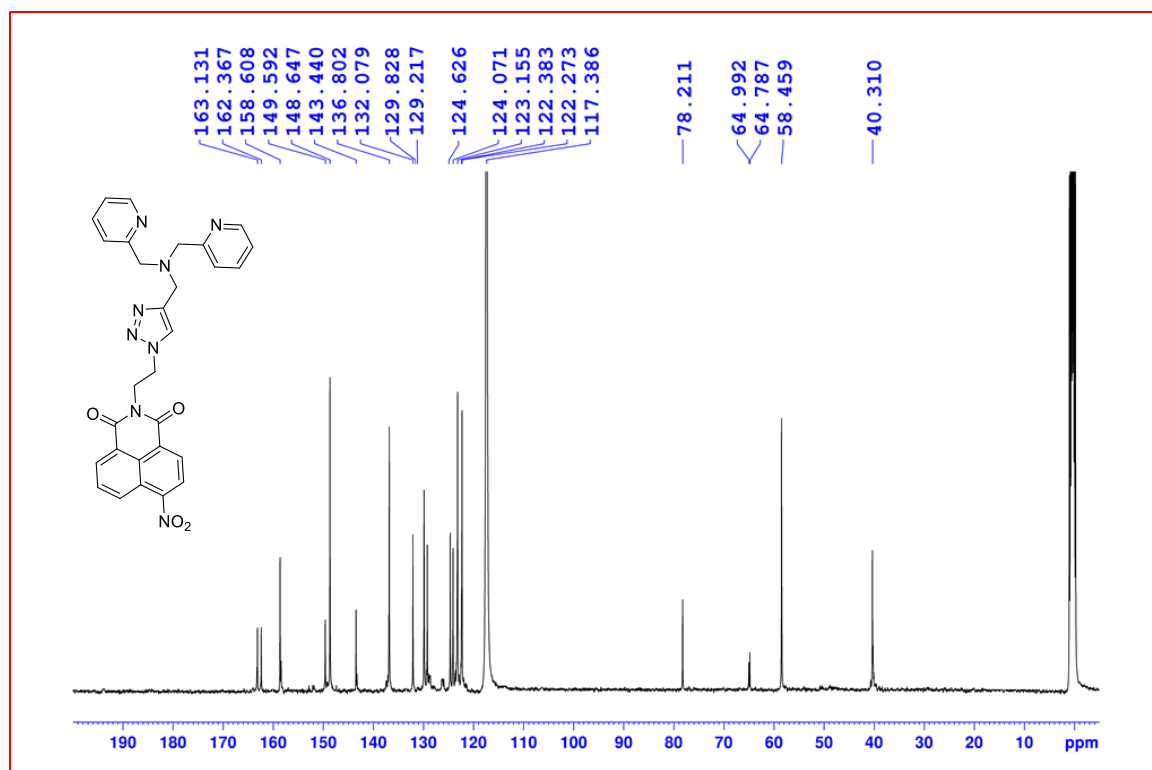


Figure 3.42: <sup>13</sup>C-NMR of 7.

## 3.5 Sensing methodology

### 3.5.1 Procedures for absorption and fluorescent studies

Stock solution of 1 mM of chemosensors were diluted to get 100 mL of 50  $\mu$ M sensor solutions for analysis. Required equivalence of  $\text{Zn}^{2+}$  (0.0 to 2.0 equiv.) were mixed with diluted solutions of sensors and then absorption and fluorescence measurements were recorded after 5 minutes (for **1** and **4**) and 1 hour (for **2**, **3** and **5-7**). Fluorescence studies and emission spectra were recorded at  $\lambda_{\text{ex}} = 350$  nm (for **1** and **3-5**) and  $\lambda_{\text{ex}} = 280$  nm (for **2**).

### 3.5.2 Determination of association constant ( $k_a$ )

Association constant was calculated with the help of Benesi Hildebrand equation .

$$1/(I - I_{\text{min}}) = 1/\{K(I_{\text{max}} - I_{\text{min}})[C]\} + 1/[I_{\text{max}} - I_{\text{min}}] \quad (1)$$

Where  $I$  and  $I_{\text{min}}$  are the emissions of sensors in the presence and absence of  $\text{Zn}^{2+}$  respectively.  $I_{\text{max}}$  is the absorbance of sensors in the presence of metal ( $M^{n+}$ )<sub>max</sub> while  $K$  is representing association constant.  $1/(I - I_{\text{min}})$  and  $1/[C]$  were plotted and the slop was used for the calculation of association constant which was found to  $1.5 \times 10^4 \text{ M}^{-1}$  for probe **3** and,  $1.6 \times 10^4 \text{ M}^{-1}$  for probe **4** and  $2.1 \times 10^4 \text{ M}^{-1}$  for probe **5**.

### 3.5.3 Determination of limit of detection

Limit of detection of sensors was determined by IUPAC method with the help of fluorescence spectroscopy (for **1** and **2**) and UV-vis spectroscopy (for **3-7**). Following equation was used to determine LOD of sensors.

$$\text{LOD} = 3\text{sB}/\text{sensitivity} \quad (2)$$

Here sB represents the standard deviation of blank while sensitivity is representing the slope of the calibration curve.

### 3.5.4 Determination of job's plot

Fluorescent method was applied for the determination of Job's plot. A series of sensors and  $\text{Zn}^{2+}$  solutions were prepared in such a way to maintain the total concentration at 50  $\mu\text{M}$ . The molar ratios of ( $\text{XS} = ([\text{Sen}]/[\text{Sen}] + [\text{Zn}^{2+}])$ ) was changed from 0.1 to 1.0. For all these solutions, the fluorescent spectra were recorded by exciting at 350 nm (for **1** and **3-5**) and  $\lambda_{\text{ex}} = 280$  nm (for **2**) and fluorescent intensities (at  $\lambda_{\text{em}} = 394$  nm for probe **1**,  $\lambda_{\text{em}} = 375$  nm for probe **2**,  $\lambda_{\text{em}} = 392$  nm for probe **3**,  $\lambda_{\text{em}} = 394$  nm for probe **4**,  $\lambda_{\text{em}} = 428$  nm for probe **5**,  $\lambda_{\text{em}} = 438$  nm for probe **6** and  $\lambda_{\text{em}} = 428$  nm for probe **7**) were plotted against the mole fractions.

## 3.6 Computational Details

Density functional theory (DFT) calculations were performed on chemosensors **3-7**, depicted in **Figures 3.43-4.45**, to underline the effect of  $\text{Zn}^{2+}$  ions on the spectra of sensors. The geometrical optimization and spectra calculations were performed by wB97xd [59–62] levels of theory. The solvent environment was taken into account using the PCM model

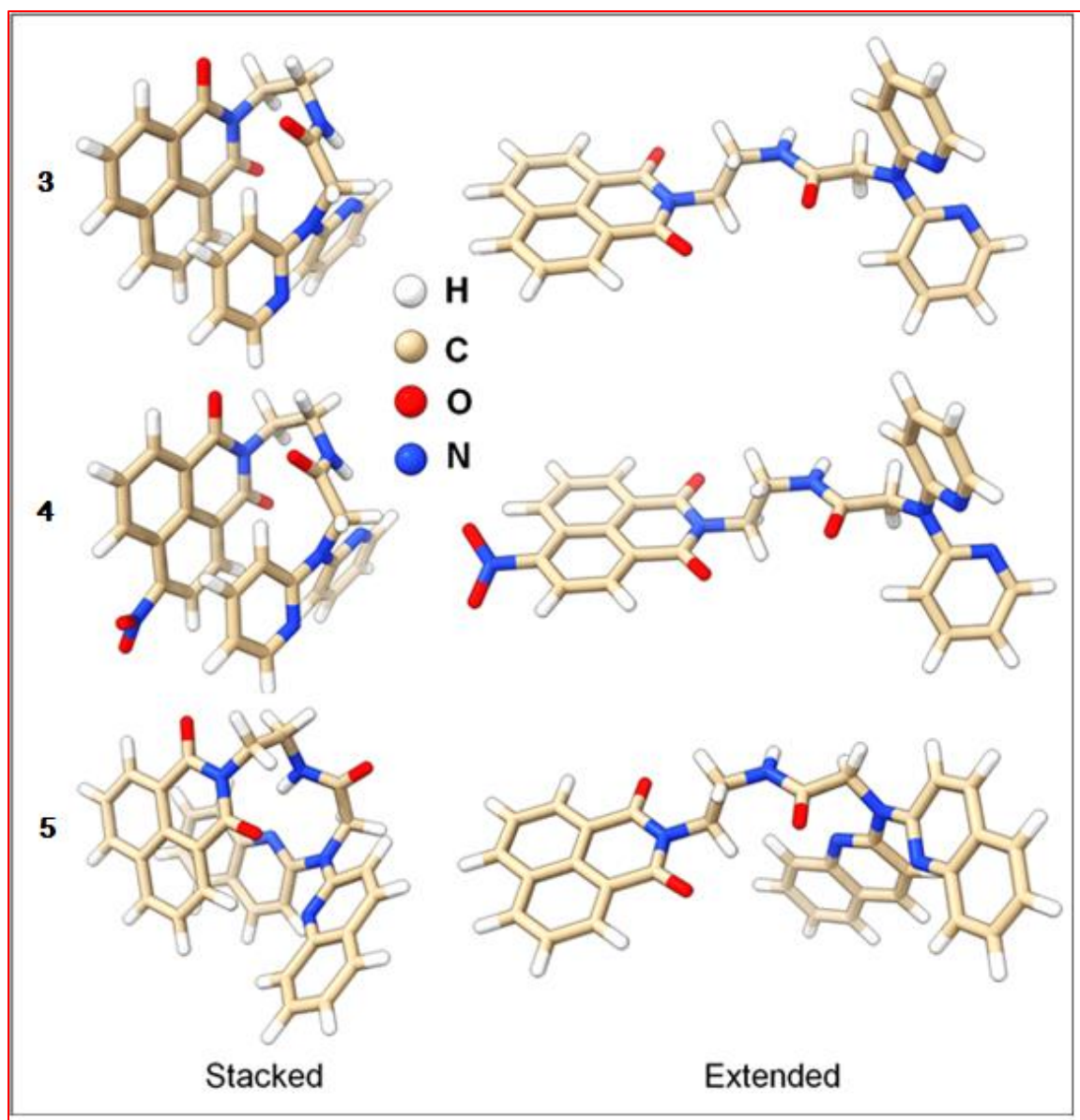
[63], as implemented in Gaussian 16 [64]. Two acetonitrile molecules were included to complete the  $\text{Zn}^{2+}$  coordination sphere. Prior DFT calculations, conformational sampling of chemosensors were performed and then selecting the most stable one for the binding and spectra calculations. These calculations were conducted by a cheaper and very efficient semi-empirical method GFN2-xTB, recently developed by Grimme [65]. The GFN2-xTB method along with the extended tight-binding Hamiltonian [66,67] was used, as implemented in XTB code. The iMTD-GC algorithm [68] was employed to generate the conformer/rotamer ensemble by an extensive metadynamics sampling (MTD) [69] and genetic z-matrix crossing (GC), as implemented in Conformer–Rotamer Ensemble Sampling Tool (CREST) code [70]. Such workflow is challenging for the DFT method [71]. The empirical parameters used in the GFN2-xTB method were fitted to reproduce the results of DFT (B97-3c) [72]. The metal binding free energies and spectra calculations were calculated on the most stable conformer generated by CREST, using equation 3.1 after further geometrical optimization by using wB97xd1,2/6-31+G(d,p) level of theory as mentioned above.

$$\Delta G_{\text{bind}} = G_{\text{complex}} - G [\text{Molecule} + \text{Metal}] \quad (3.1)$$

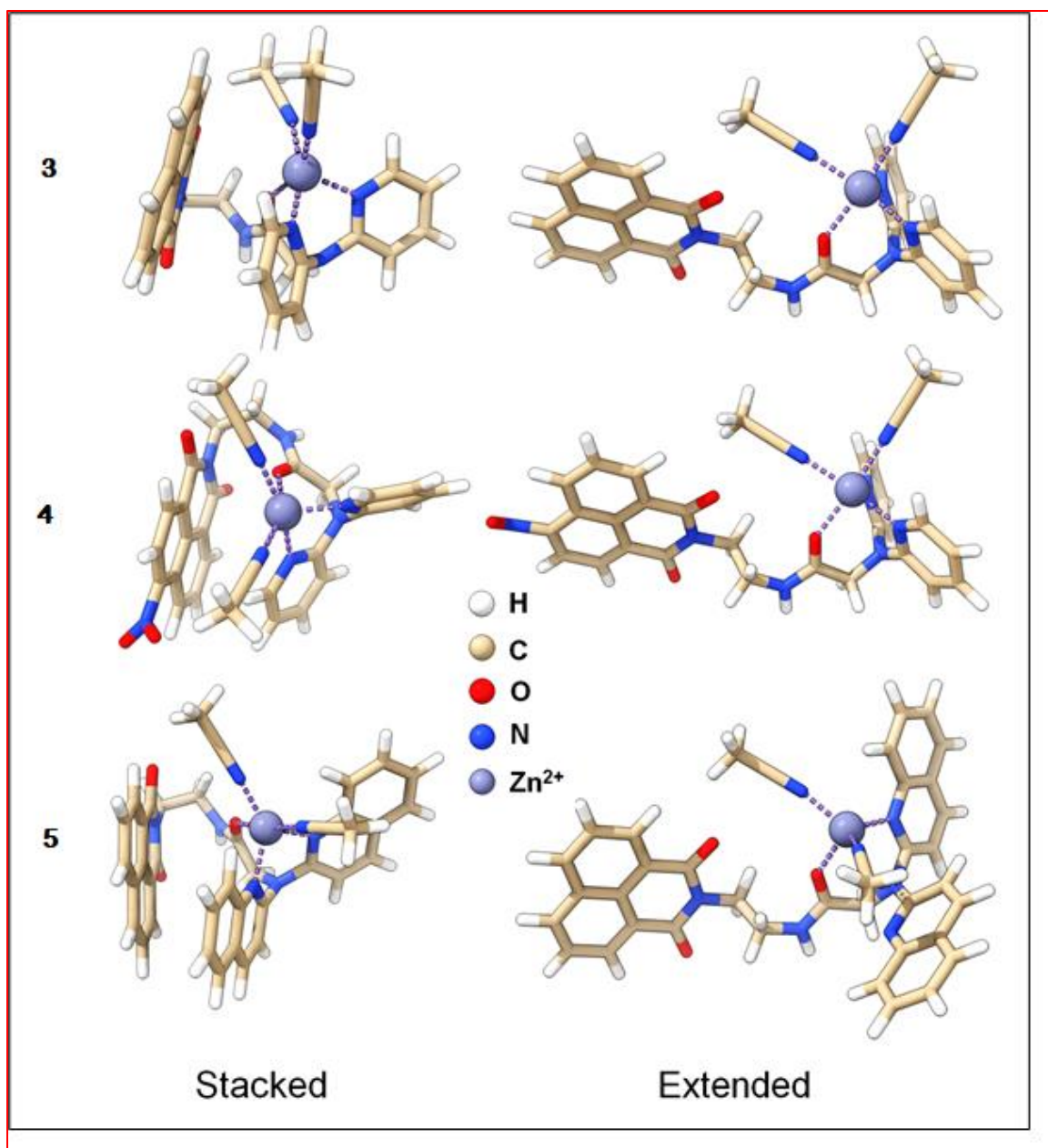
Equation 3.2 were applied to calculate the contribution of solvation free energy to the total metal-binding free energy.

$$\Delta G_{\text{solv}} = G_{\text{solv}}_{\text{complex}} - G_{\text{solv}} [\text{Molecule} + \text{Metal}] \quad (3.2)$$

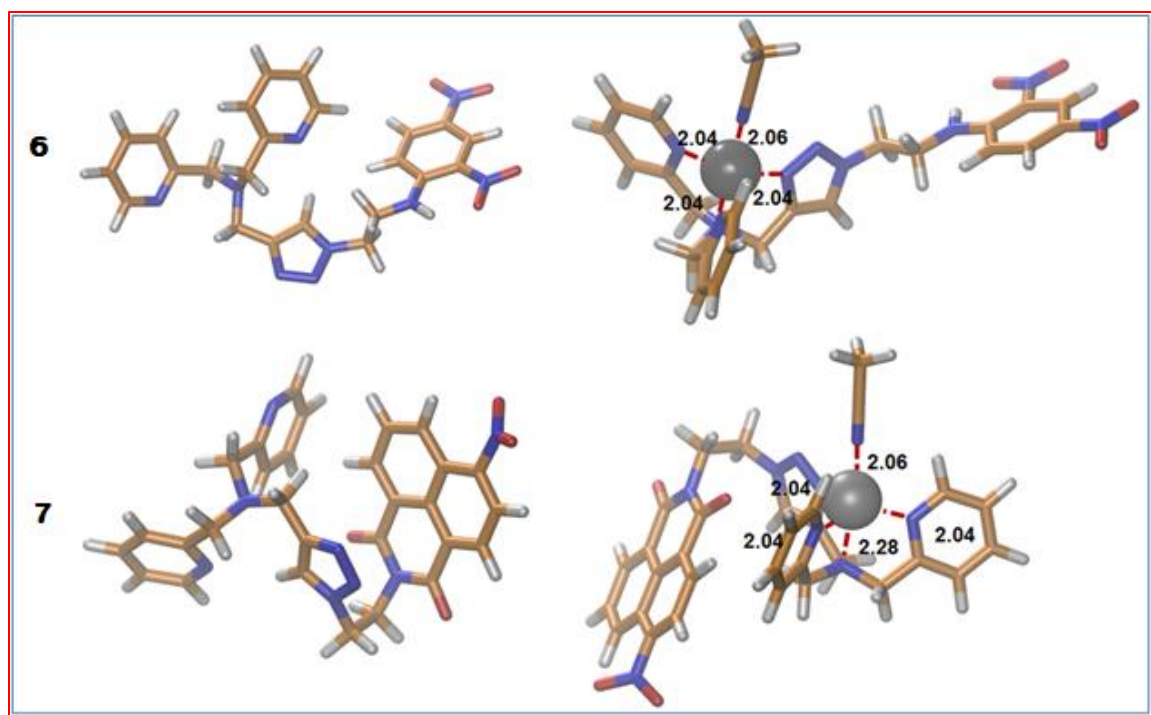
Using the counterpoise method, all binding free energies were corrected for the basis set superposition [73].



**Figure 3.43:** Optimized structures of the three chemosensors in the extended and stacked conformations. The stacked conformations are predicted as the most stable conformer generated by CREST code.



**Figure 3.44:** Optimized structures of the three chemosensors complexed with  $\text{Zn}^{2+}$  in the extended and stacked conformations. The stacked conformations are predicted as the most stable conformer generated by the CREST code.



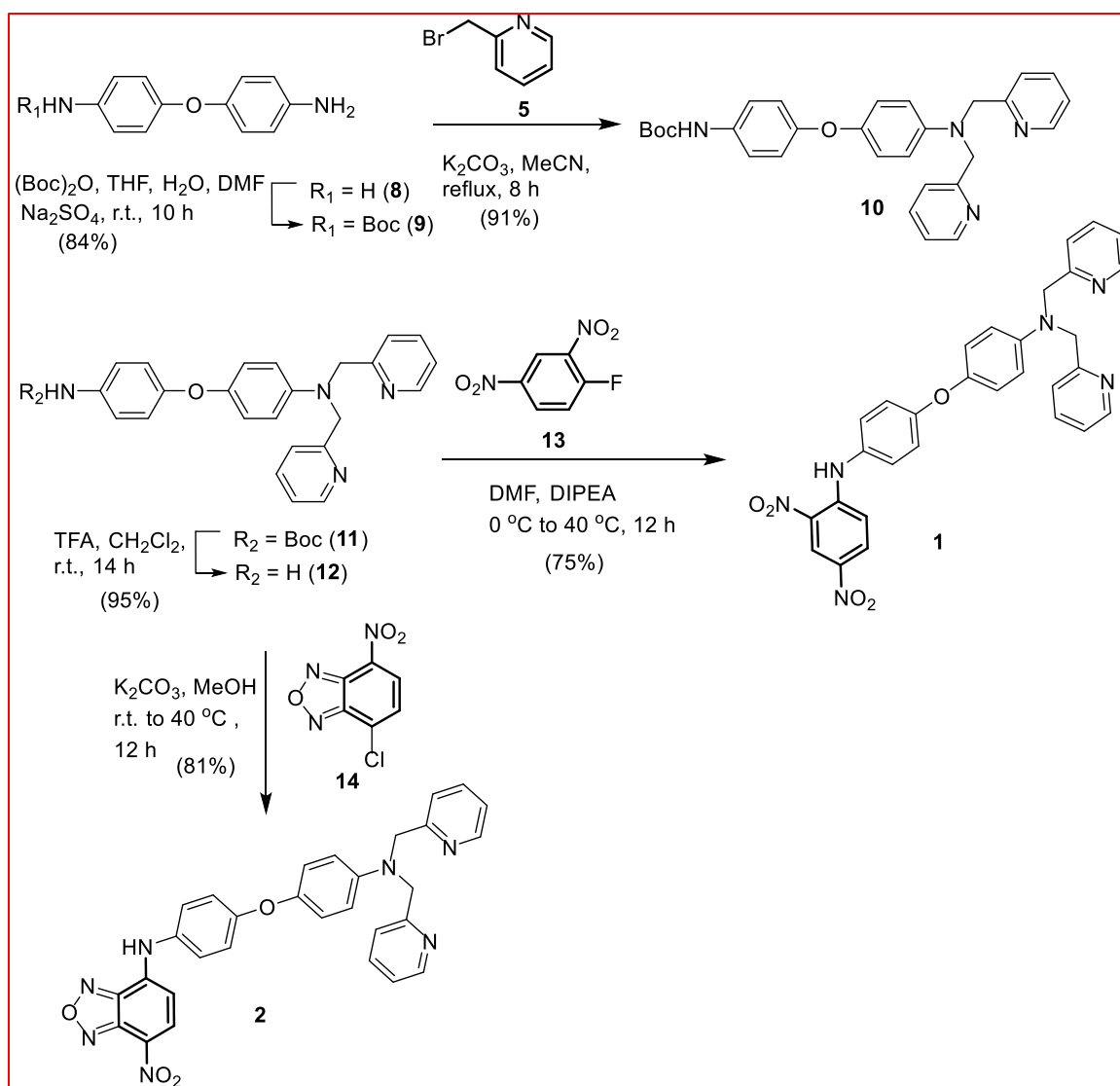
**Figure 3.45.** Optimized structures of sensors 6 and 7 in the unbound and  $\text{Zn}^{2+}$ -bound states.

## CHAPTER 4

### RESULTS AND DISCUSSION

#### 4.1 Synthesis of chemosensor 1 and 2

The synthetic route for chemosensors **1** and **2** is outlined in **figure 4.1**. The N-boc protection of 4,4'-oxidianiline **8** produced intermediate **9**, which was *N*-alkylated with bromomethylpyridine **10** to afford key intermediate **11** in 91% yield. Exposure of **11** to trifluoroacetic acid in CH<sub>2</sub>Cl<sub>2</sub> led to deprotection of N-boc function to produce intermediate **12**, which then reacted with fluoro-dinitrobenzene **13** to yield chemosensor **1**. Likewise, reaction of **12** with 4-chloro-7-nitrobenzofurazan **14** resulted in the synthesis of chemosensor **2** (**Figure 4.1**).



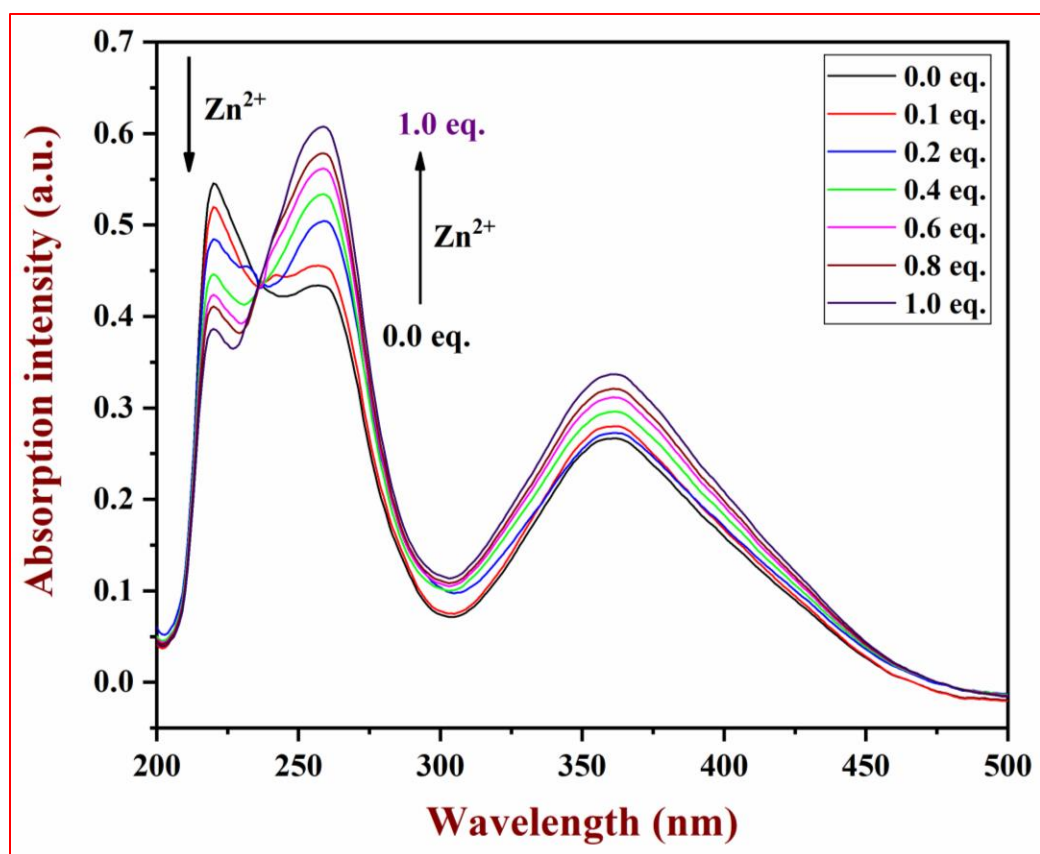
**Figure 4.1:** Synthesis of intermediates and chemosensors **1** and **2**.

## 4.2 Characterization of **1** and **2** as a sensor for $\text{Zn}^{2+}$

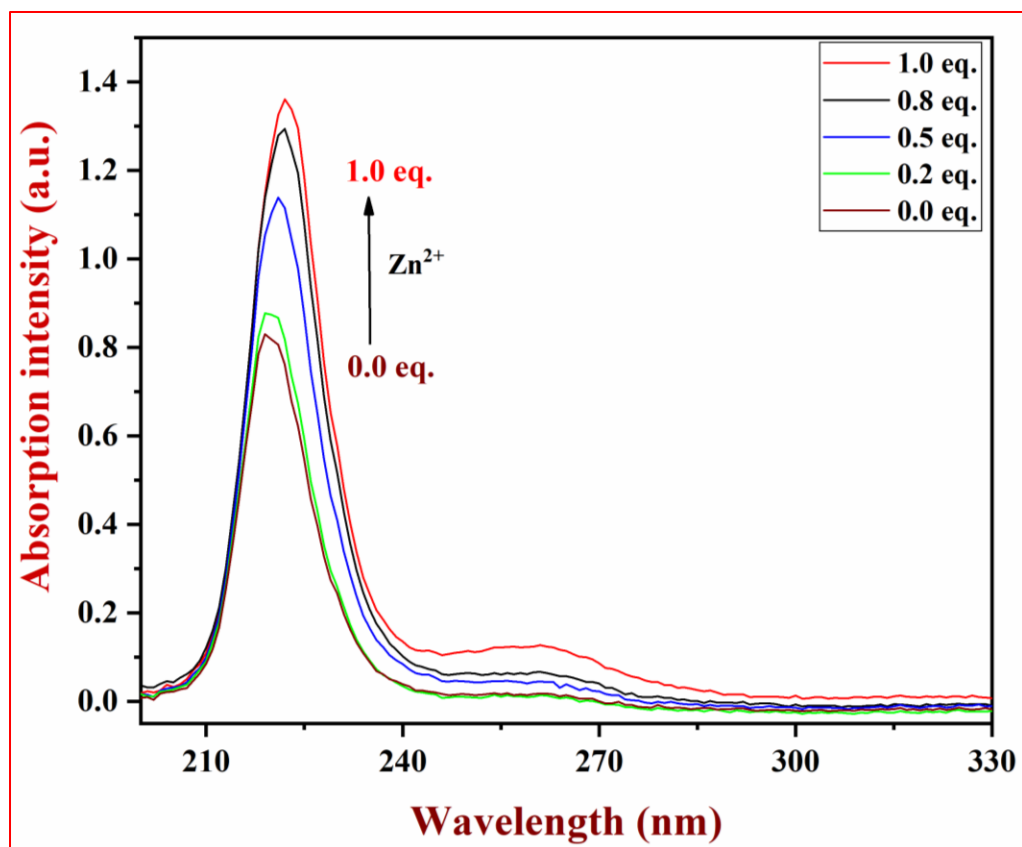
The sensing performance of chemosensors **1** and **2** towards  $\text{Zn}^{2+}$  was evaluated through an absorption study and fluorescence study.

#### 4.2.1 UV-Vis spectral response of **1** and **2** towards $\text{Zn}^{2+}$

The sensor response toward changing concentrations of  $\text{Zn}^{2+}$  was evaluated through a  $\text{Zn}^{2+}$  titration experiment in  $\text{CH}_3\text{CN}$  medium. The absorption spectra of neat **1** [50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ] is presented in **Figure 4.2**. It exhibited two prominent absorption peaks centered at 220 and 362 nm. However, the absorption spectra of **1** displayed marked changes with the addition of  $\text{Zn}^{2+}$  [50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ]. Peak at 220 nm starts to decreasing whereas a new peak at 259 nm starts appearing when  $\text{Zn}^{2+}$  was added. With the continuous addition of  $\text{Zn}^{2+}$  from 0.1 eq to 1.0 eq to the solution of **1**, the intensity of peak at 259 was significantly enhanced. This suggested the formation of new specie having altered electronic rearrangement, leading to an altered absorption spectrum. Similarly, the absorption spectra of **2** [50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ] displayed a sharp absorption peak at 225 nm. However, the absorption at 220 nm of pristine chemosensor was quenched upon the addition of  $\text{Zn}^{2+}$  [50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ] (**Fig. 4.3**), suggesting their complexation with the  $\text{Zn}^{2+}$  [74].



**Figure 4.2:** UV-vis absorption spectra of chemosensor **1** (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ) with the addition of  $\text{Zn}^{2+}$  (0.0-1.0 equiv.) in  $\text{CH}_3\text{CN}$ .

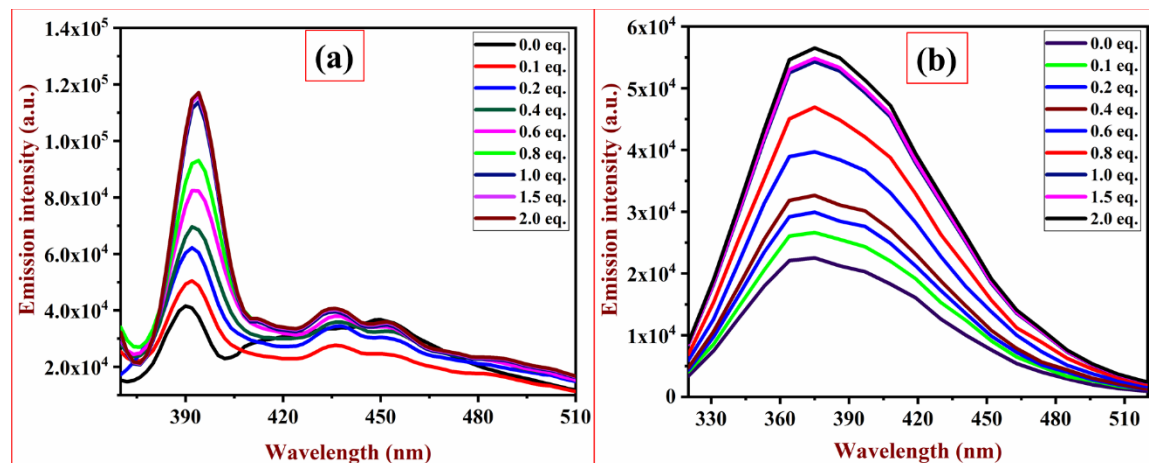


**Figure 4.3:** UV-vis absorption spectra of chemosensor **2** (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ) with the addition of  $\text{Zn}^{2+}$  (0.0-1.0 equiv.) in  $\text{CH}_3\text{CN}$ .

#### 4.2.2 Fluorescence response of **1** and **2** towards $\text{Zn}^{2+}$

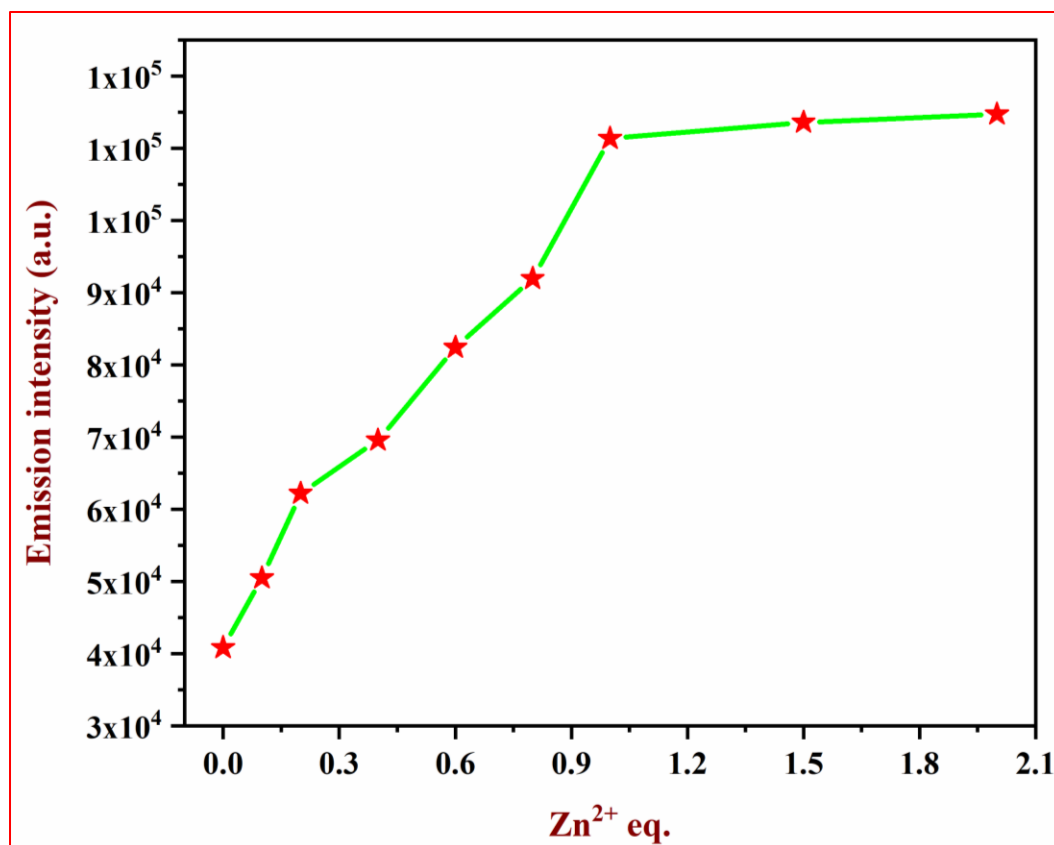
The fluorescence response of the **1** and **2** towards the varying concentration of  $\text{Zn}^{2+}$  was evaluated in the  $\text{CH}_3\text{CN}$ /HEPES mixture. Chemosensor **1** [50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ] in the free state, displayed very weak emission peaks at 390 nm (sharp) and 430 nm (broad) wavelength when excited at 350 nm. However, the fluorescence intensity of **1** at 390 nm was slightly red-shifted to 394 nm with the addition of the  $\text{Zn}^{2+}$  [50  $\mu\text{M}$  in HEPES buffer, pH = 7] (**Fig. 4.4a**). In addition, an enhancement in the fluorescent intensity was observed. However, the intensity of the broad peak centered at 430 nm was almost unchanged. The

intensity of emission wavelength at 394 nm linearly increased with the addition of  $\text{Zn}^{2+}$  until 1:1 addition of  $\text{Zn}^{2+}$  where it reached a stable value. Any further addition didn't bring any significant alteration in the emission intensity (**Fig. 4.5**). However, it enhances the fluorescence by 3-folds before it becomes a plateau.

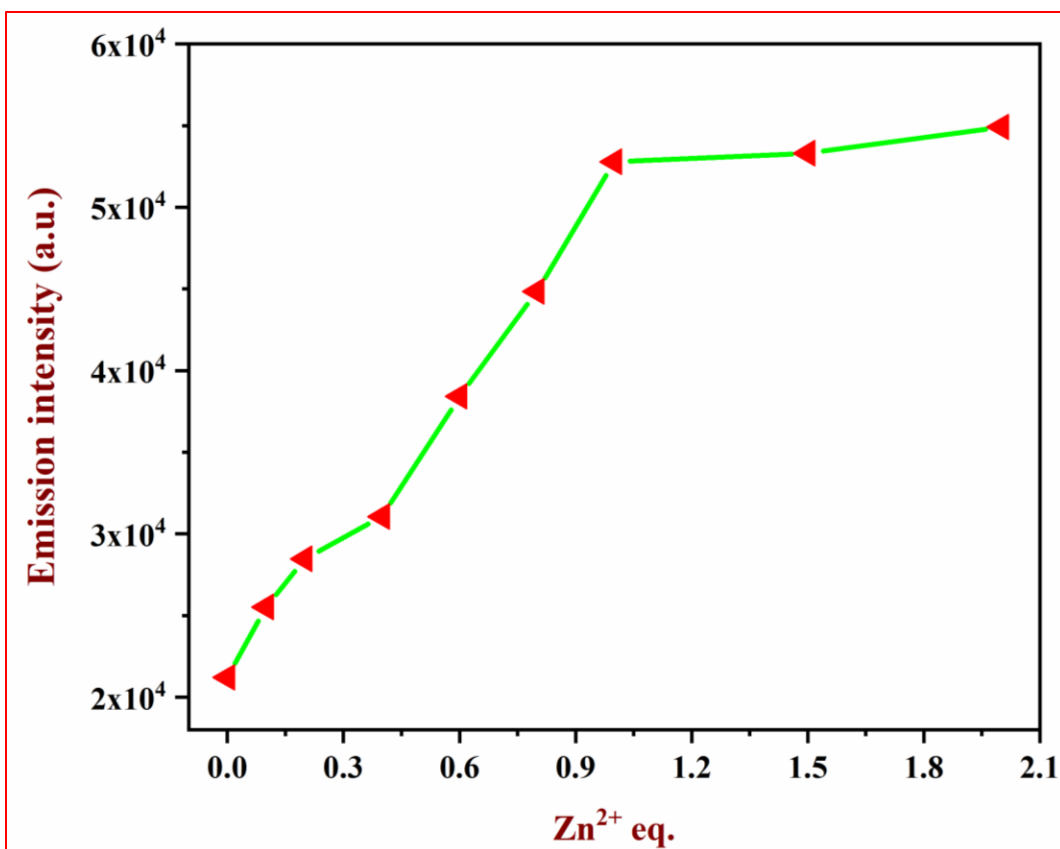


**Figure 4.4:** Emission spectra of chemosensor **1** ( $\lambda_{\text{ex}} = 350$  nm) (a) and **2** ( $\lambda_{\text{ex}} = 280$  nm) (b) upon the titration of  $\text{Zn}^{2+}$  [0-2.0 equiv.) in  $\text{CH}_3\text{CN-H}_2\text{O}$  at pH = 7.4.

The enhancement in the emission wavelength along with the red shift during the  $\text{Zn}^{2+}$  addition is most probably due to the intramolecular charge transfer and photoinduced electron transfer mechanism [75]. However, probe **2** in the free state displayed a broad peak centered at 375 nm when excited at 280 nm (**Fig. 4.4b**). Again, a slight red shift in the emission wavelength and enhancement in the emission intensity was observed with the addition of  $\text{Zn}^{2+}$  suggesting complexation of **2** with  $\text{Zn}^{2+}$  [76]. Enhancement in the fluorescent was significant till the addition of 1 equiv. of  $\text{Zn}^{2+}$ . No considerable fluorescence enhancement was observed after the addition of 1 equiv.  $\text{Zn}^{2+}$  (**Figure 4.6**). A 2.4-folds enhancement in the fluorescence was realized before reaching saturation.

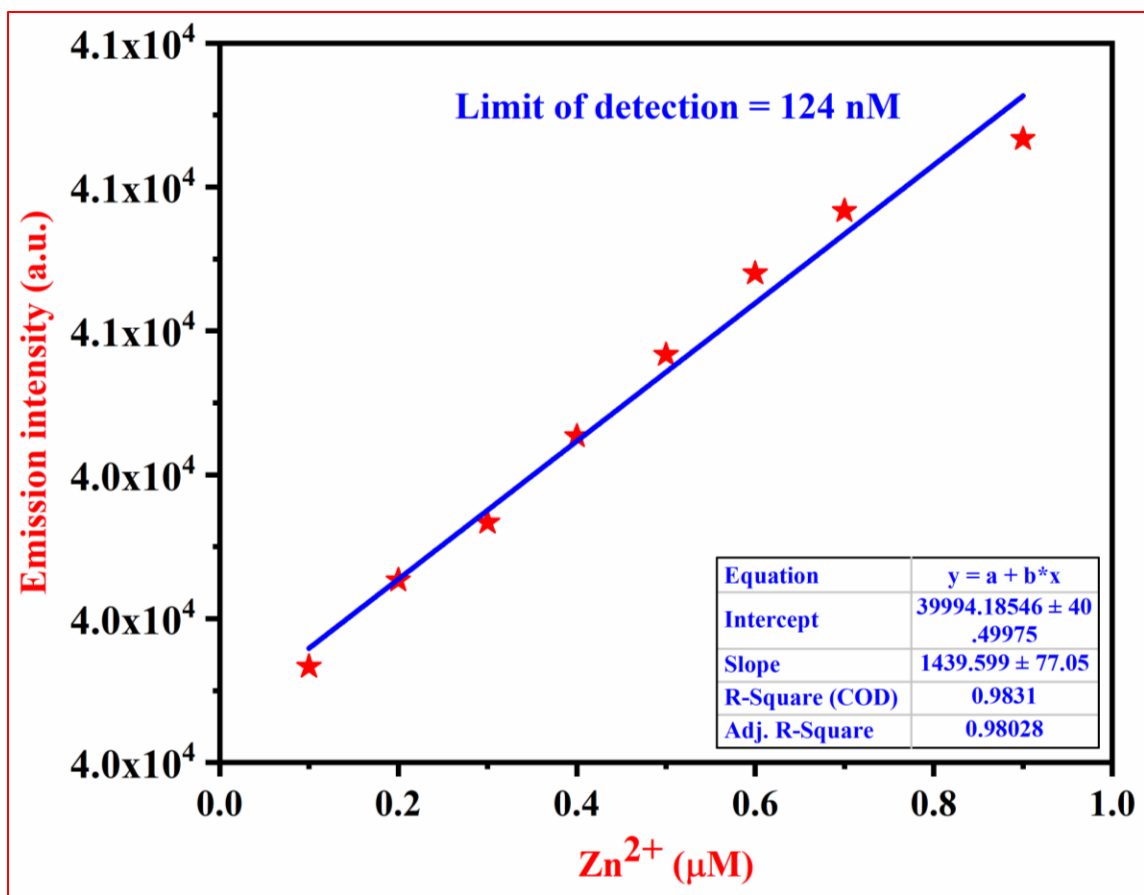


**Figure 4.5:** Change in the emission intensity of chemosensor **1** (50  $\mu$ M in CH<sub>3</sub>CN) with addition of Zn<sup>2+</sup> (0.0-2.0 equiv.) in HEPES buffer, pH = 7.4) at 394 nm.

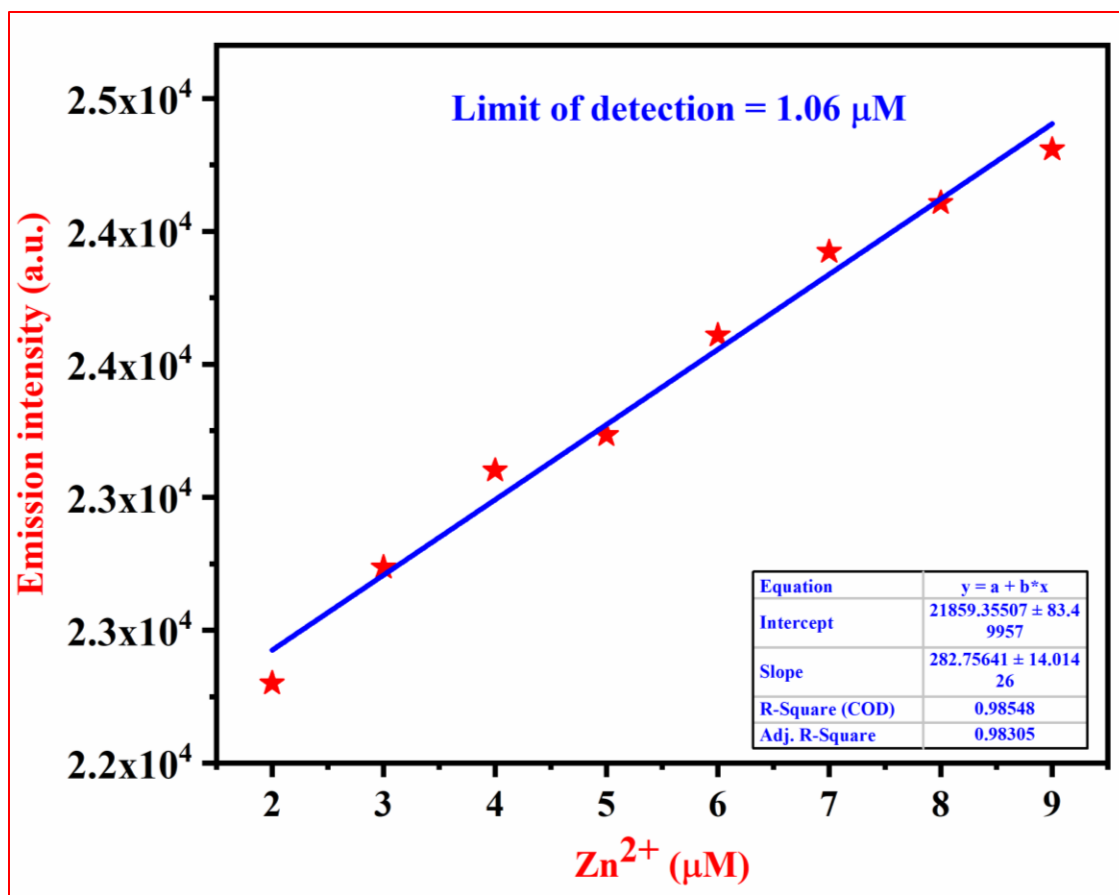


**Figure 4.6:** Change in the emission intensity of chemosensor **2** (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ) with addition of  $\text{Zn}^{2+}$  (0.0-2.0 equiv.) in HEPES buffer, pH = 7.4) at 375 nm.

The limit of detection (LOD) and limit of quantification (LOQ) of **1** was calculated through the IUPAC method and found to be 124 nM and 378 nM respectively (**Fig. 4.7**). Similarly limit of detection (LOD) and the limit of quantification (LOQ) of **2** was 1.06  $\mu\text{M}$  and 3.22  $\mu\text{M}$  respectively (**Fig. 4.8**).



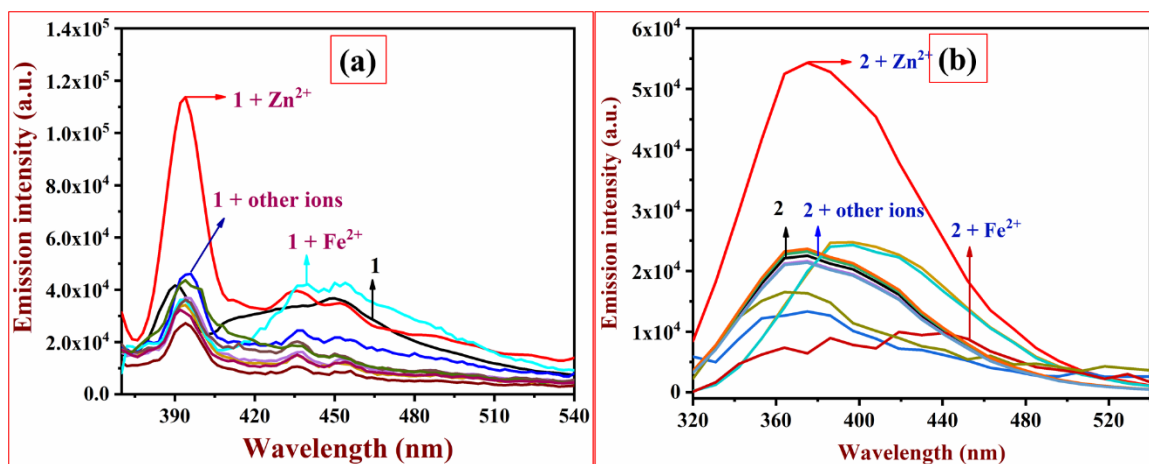
**Figure 4.7:** Standard calibration curve of **1** for the detection of  $\text{Zn}^{2+}$ .



**Figure 4.8:** Standard calibration curve of **2** for the detection of  $\text{Zn}^{2+}$ .

The fluorescence response of the chemosensor **1** and **2** towards metal ions [ $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{K}^+$ ,  $\text{Mn}^{2+}$  and  $\text{Pb}^{2+}$ ] [50  $\mu\text{M}$  in HEPES buffer at pH = 7.4]. When each common metal ion was added separately to the **1** solution (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ )  $\text{Zn}^{2+}$  could significantly enhance the fluorescence intensity of the **1** at 394 nm. Although all of the other tested ions also shifted the emission wavelength of the **1** from 390 nm to 394 nm after their addition, however, other metal ions had no significant effect on the emission intensity of the **1** (**Fig. 4.9a**). Instead, in most cases, fluorescence quenching was observed. Only  $\text{Cd}^{2+}$  and  $\text{Mn}^{2+}$  show very little enhancement.  $\text{Fe}^{2+}$  displayed a slight enhancement in the emission wavelength at 430 nm however quenching was observed at 394 nm. In the case

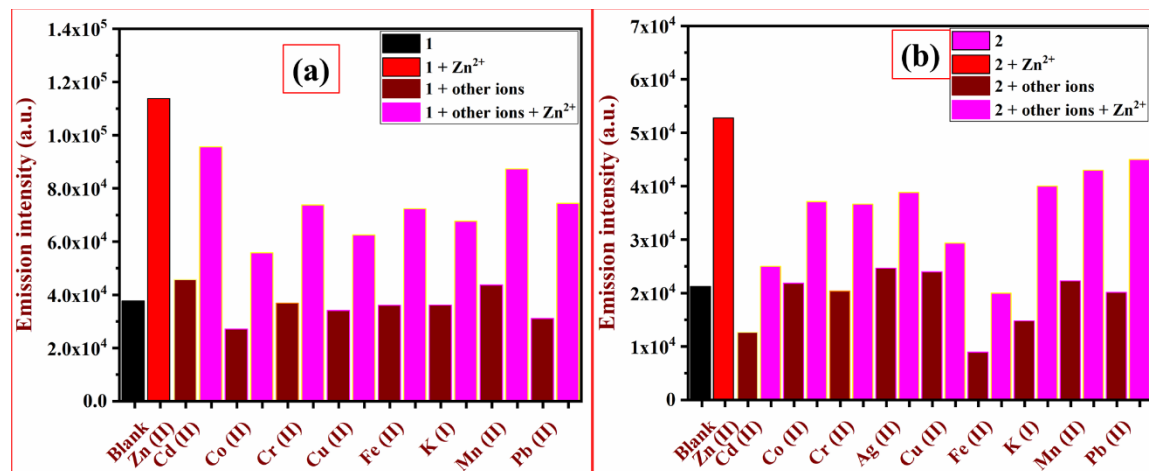
of chemosensor **2**, the addition of  $\text{Zn}^{2+}$  brings red-shift and emission enhancement (**Fig. 4.9b**). However, the addition of other metal ions, only  $\text{Ag}^{2+}$  and  $\text{Cu}^{2+}$  red shifted the emission wavelength but, no enhancement in the emission intensity was observed. Other metal ions have no significant effect neither on the emission wavelength or the emission intensity. But the  $\text{Fe}^{2+}$  displayed a very strong quenching of the **2** fluorescence.



**Figure 4.9:** The fluorescence response of chemosensors **1** (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ) and **2** (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ) ( $\lambda_{\text{ex}} = 350$  nm for **1**,  $\lambda_{\text{ex}} = 280$  nm for **2**) towards the addition of various individual ions including  $\text{Zn}^{2+}$  (concentration of all ions = 50  $\mu\text{M}$  in HEPES buffer, pH = 7.4).

To evaluate the selectivity of the chemosensors towards  $\text{Zn}^{2+}$ , the fluorescence response of both the chemosensors towards  $\text{Zn}^{2+}$  was evaluated when the other metal ions coexisted with the  $\text{Zn}^{2+}$ . First, the fluorescence response of chemosensors **1** and **2** (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ) with various individual ions (1 equiv. 50  $\mu\text{M}$  in HEPES buffer, pH = 7.4) was studied as indicated by the dark red color in **Figs. 4.10a** and **4.10b**. As evident there was almost no significant or minor fluorescence enhancement was observed. However, a good fluorescence enhancement of **1** was observed with the addition of  $\text{Zn}^{2+}$  in all cases [pink bars in (**Fig. 4.10a**)]. However somewhat less fluorescence recovery was observed with

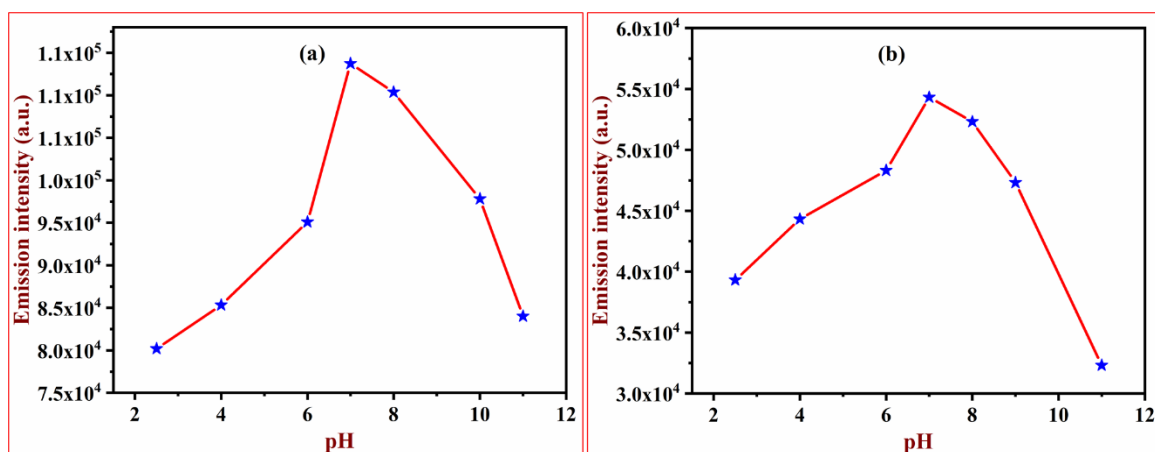
$\text{Co}^{2+}$ . Similarly, a fluorescence recovery for **2** with other metal ions was observed with the addition of  $\text{Zn}^{2+}$  except in  $\text{Fe}^{2+}$  and  $\text{K}^+$  which causes severe quenching of fluorescence intensity (**Fig. 4.10b**). This indicates excellent selectivity of chemosensors **1** and **2** for the detection of  $\text{Zn}^{2+}$ .



**Figure 4.10:** The fluorescence response of chemosensors **1** and **2** ( $\lambda_{\text{em}} = 394$  nm for **1**,  $\lambda_{\text{em}} = 375$  nm for **2**) towards the addition of various individual ions and  $\text{Zn}^{2+}$  (concentration of all ions =  $50 \mu\text{M}$ ) in  $\text{CH}_3\text{CN-H}_2\text{O}$  at pH = 7.4.

The effect of pH on the detection performance of the chemosensors **1** and **2** was also evaluated (**Figs. 4.11a & 4.11b**). The fluorescence emissions of the chemosensors- $\text{Zn}^{2+}$  complexes were recorded in  $\text{CH}_3\text{CN/HEPES}$  (1:1, v/v) in a pH changing from 2.5 to 11. The fluorescence intensity (at 394 nm) of **1** and **2** was comparatively low in the pH below 4 due to the protonation of the binding site of the chemosensors which blocks their complex formation with the  $\text{Zn}^{2+}$ . Although this led to exclusion of PET resulting in a false enhancement in fluorescence however not as effective as due to the chemosensors- $\text{Zn}^{2+}$  complex formation [9]. With an increase in the pH, the fluorescence enhancement was observed which was more intensified at neutral and weakly basic pHs. These results are

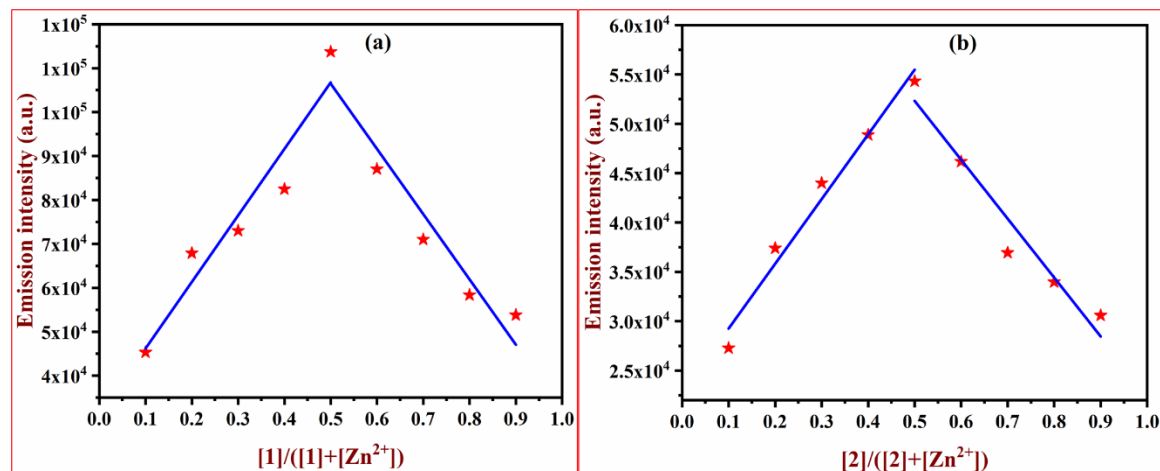
suggesting an effective binding of chemosensors **1** and **2** with the  $\text{Zn}^{2+}$  leading to an effective fluorescence enhancement due to the availability of the active sites of the receptor at neutral and weakly basic pHs. However, a drastic decrease in fluorescent emission intensity at a strong basic pH was observed due to the binding of  $\text{Zn}^{2+}$  with hydroxyl forming  $\text{Zn}(\text{OH})_2$  instead of complex formation with the chemosensors [8]. This indicates that the optimum pH condition of the pH for chemosensors **1** and **2** with  $\text{Zn}^{2+}$  is pH close to neutral.



**Figure 4.11:** The effect of pH on the fluorescence response of a) **1**/ $\text{Zn}^{2+}$  and b) **2**/ $\text{Zn}^{2+}$  ( $\lambda_{\text{em}} = 394 \text{ nm}$  for **1**/ $\text{Zn}^{2+}$ ,  $\lambda_{\text{em}} = 375 \text{ nm}$  for **2**/ $\text{Zn}^{2+}$ ) complexes in  $\text{CH}_3\text{CN}/\text{HEPES}$  buffer (1:1, v/v) solution.

To understand the binding proportion of chemosensors **1** and **2** with  $\text{Zn}^{2+}$  during complexation, the job's plot analysis was carried out. The molar ratios of the  $\text{Zn}^{2+}$  were changed from 0 to 1 during the complexation while maintaining the sum of the initial concentrations of the chemosensor and  $\text{Zn}^{2+}$  at  $50 \mu\text{M}$ . The emission intensities of all the different molar ratio complexes were determined and plotted against the molar ratios (**Figs.**

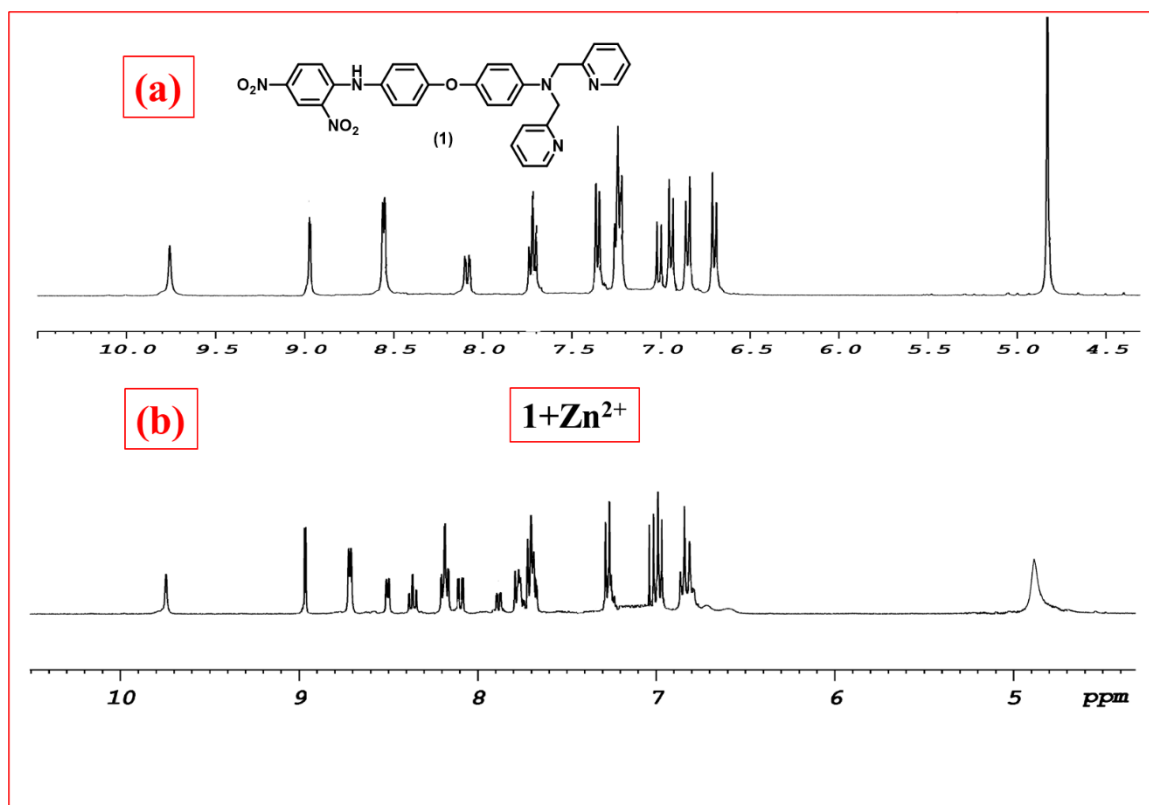
**4.12a & 4.12b).** The emission intensity was highest at 0.5 mol fraction of  $\text{Zn}^{2+}$  which suggested a 1:1 complexation between the chemosensor and  $\text{Zn}^{2+}$ .



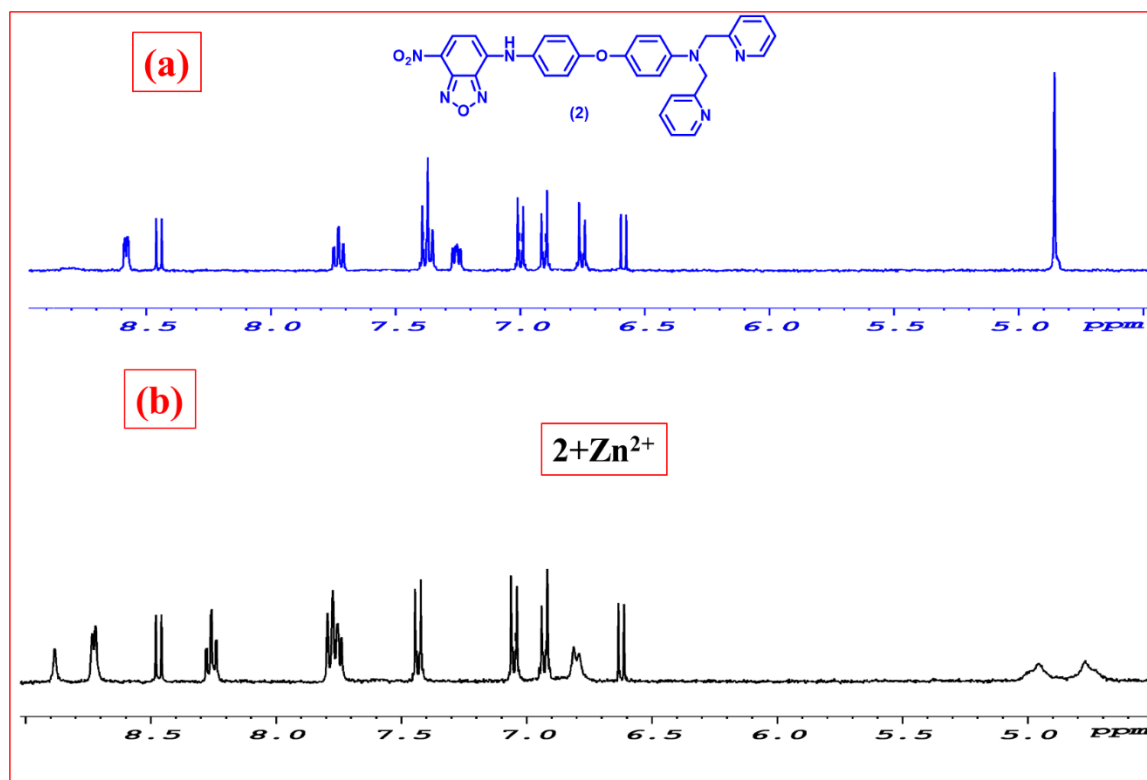
**Figure 4.12:** Job's plot for the chemosensors **1** and **2** (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ) in 50  $\mu\text{M}$  HEPES buffer solution (pH = 7.4): a) **1** ( $\lambda_{\text{em}} = 394 \text{ nm}$ ), b) ( $\lambda_{\text{em}} = 375 \text{ nm}$ ) the total concentration of the chemosensor and  $\text{Zn}^{2+}$  is 50  $\mu\text{M}$ .

### 4.2.3 $^1\text{H}$ -NMR and FT-IR analysis of **1** and **2** complexation with $\text{Zn}^{2+}$

To determine the binding behavior of the chemosensors, a  $^1\text{H}$ -NMR titration experiment was performed. It is clear from the  $^1\text{H}$ -NMR of both the sensors that after the completion with the  $\text{Zn}^{2+}$  (sensor- $\text{Zn}^{2+}$ ), a distinct downfield in the receptor protons (DPA's and methylene) occurred (**Figs. 4.13 & 4.14**). This observation is suggesting that the nitrogen atom of the DPA and tertiary nitrogen atom were involved in complexation with the  $\text{Zn}^{2+}$  resulting in the downfield of their signals due to the deshielding effect of the metal ions [18].

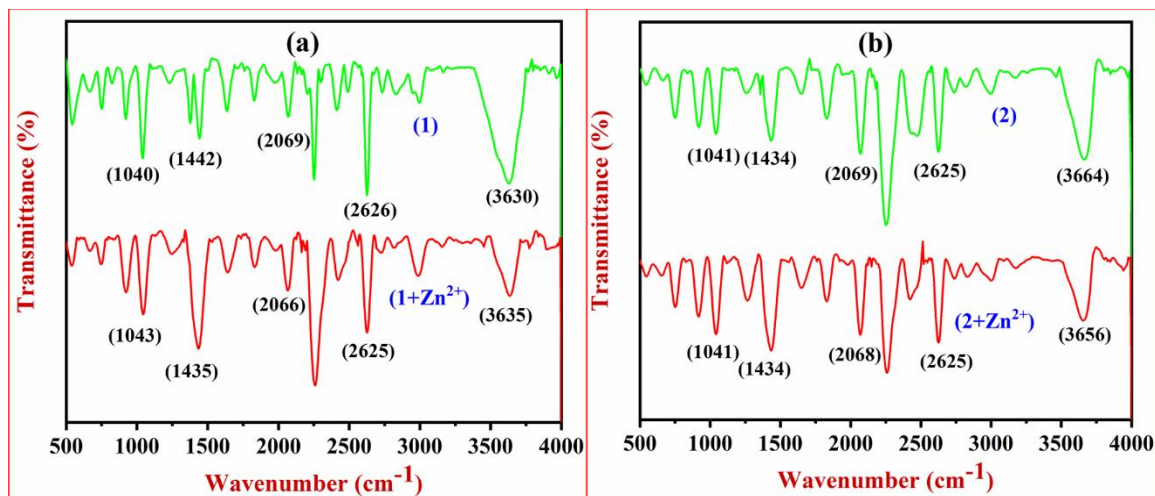


**Figure 4.13:** <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>CN) of a) **1** and b) **1**/Zn<sup>2+</sup> complex from 4.3 to 10.5 ppm.



**Figure 4.14:**  $^1\text{H}$ -NMR (400 MHz,  $\text{CD}_3\text{CN}$ ) of a) **2** and b)  $2/\text{Zn}^{2+}$  complex from 4.5 to 9 ppm.

Similarly, FT-IR spectra of the pristine sensors were recorded and compared with the sensor complex in acetonitrile solutions. Some changes in the absorption peaks were observed after the complexation hinting at the complex formation between the sensors and  $\text{Zn}^{2+}$  (Figs. 4.15a & 4.15b).



**Figure 4.15:** Comparative FT-IR of a) chemosensor **1** and **1**/ $\text{Zn}^{2+}$  complex, b) chemosensor **2** and **2**/ $\text{Zn}^{2+}$  complexes recorded in acetonitrile solution.

### 4.3 Synthesis of chemosensor 3-5

The synthesis of the designed chemosensors commenced with the N-Boc protection of hydrazine **15** to yield **16**, which was subsequently condensed with 1,8-naphthalic anhydride **17** and **18** under refluxing ethanol to produce intermediates **19** [77] and **20** [57], respectively, in high yields. Exposure of intermediates **19** and **20** to trifluoroacetic acid in dichloromethane then furnished the desired key intermediates **21** [77] and **22** [78], respectively. Next, amidation of intermediate **21** with acids **24** [79] and **26** rendered fluorescent probes **3** and **5**, respectively, in high yields. Likewise, amidation of intermediate **22** with acid **24** under standard conditions readily provided probe **2** in 79%. (Fig. 4.16).

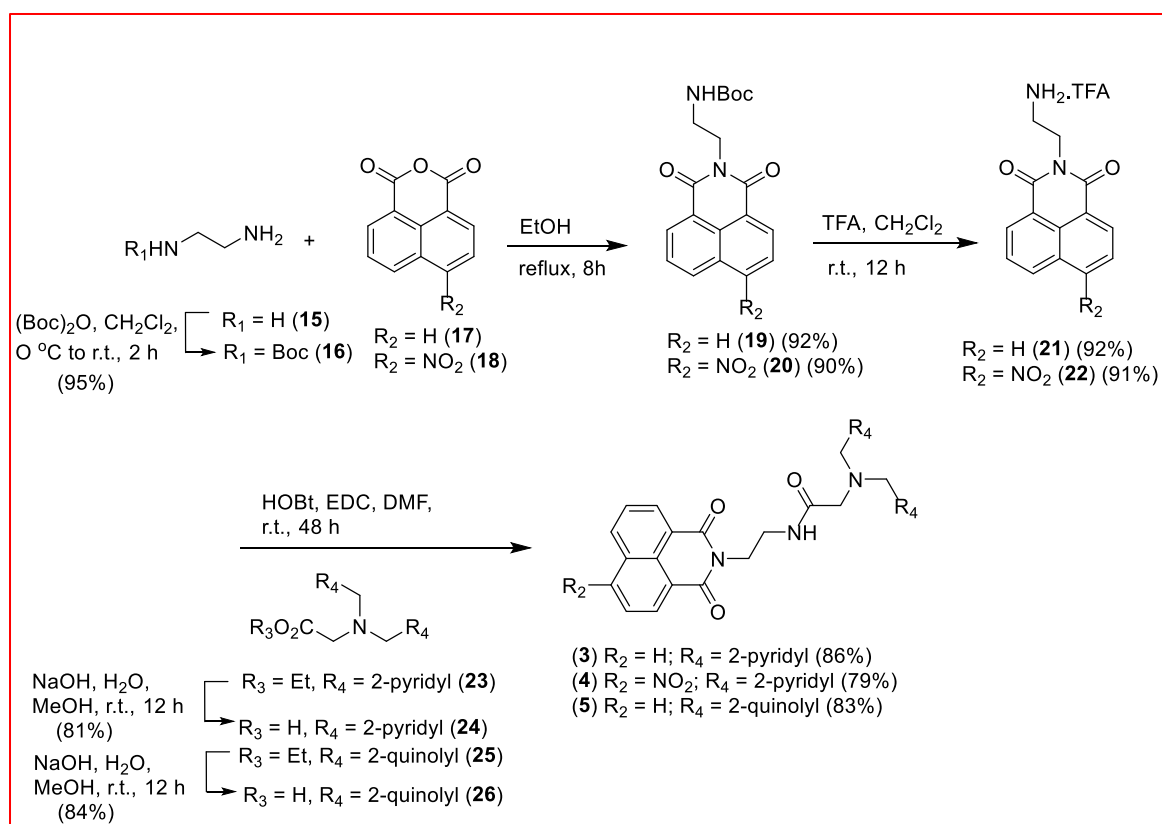
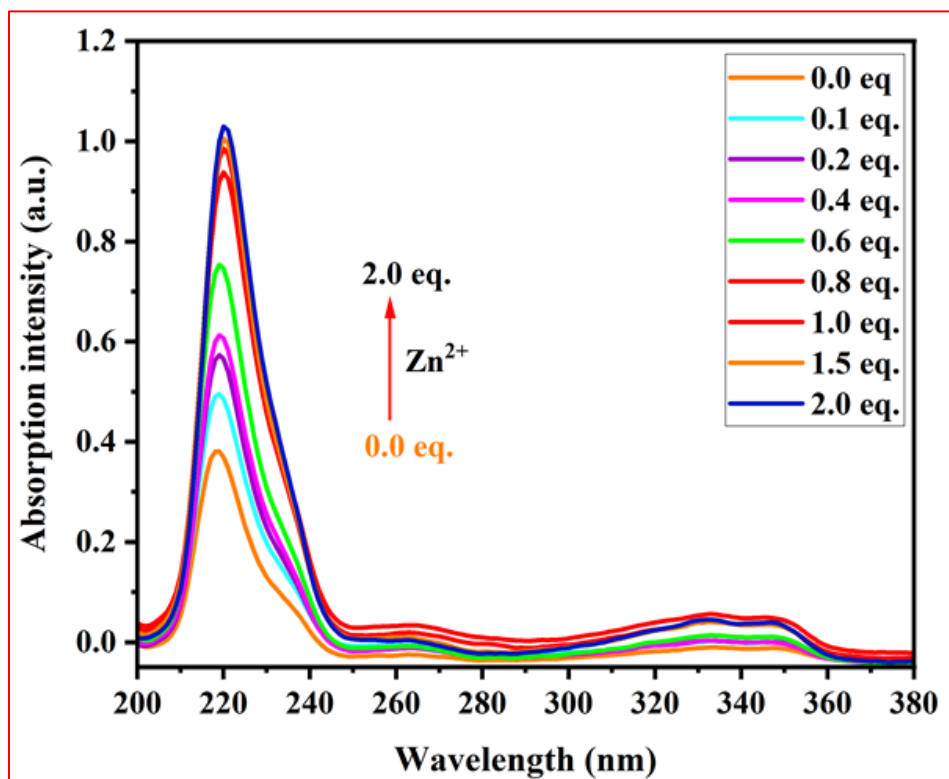


Figure 4.16: Synthesis of intermediates and chemosensors 3-5

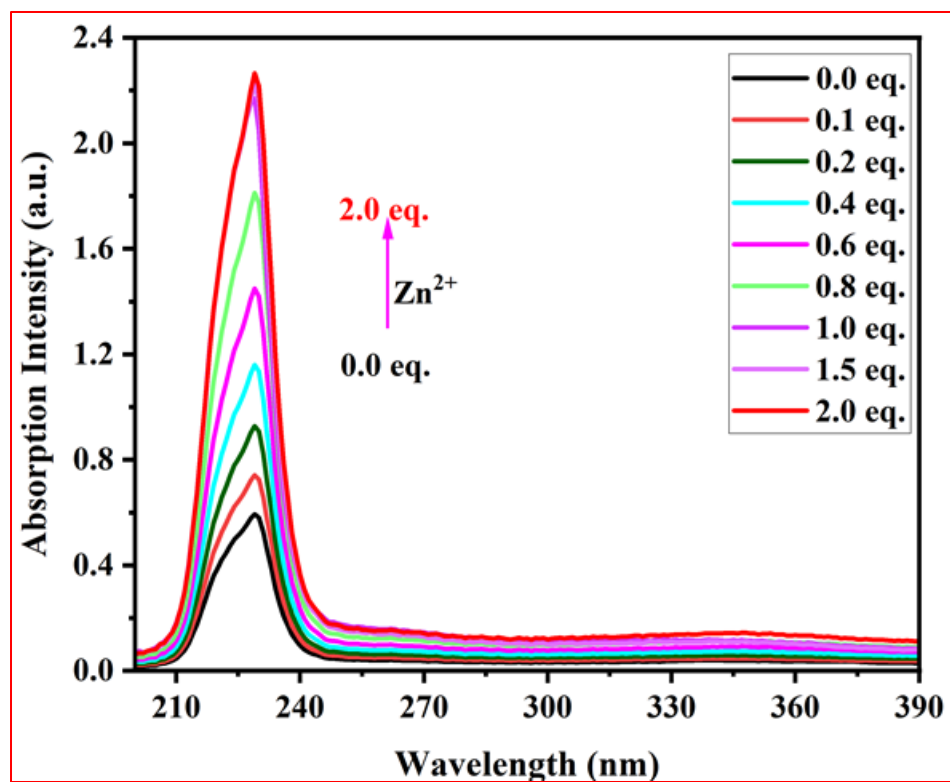
#### 4.4 Characterization of chemosensors (3-5) as $\text{Zn}^{2+}$ sensor

The sensors responses to different zinc ions concentrations were measured by zinc titration experiments in the  $\text{CH}_3\text{CN}$  medium. The absorption spectra of the neat sensors 3–5 exhibited intense and sharp bands at around 220, 230, and 219 nm, respectively. The absorption spectrum of 3 displayed very weak and broad absorption bands at 263 and 332 nm. Similarly, absorption spectra of 4 and 5 showed weak and broad bands at around 345 and 329 nm, respectively (Figs. 4.17-4.19). However, upon gradual addition of  $\text{Zn}^{2+}$  (0.0–2.0 equiv.) to the solutions of 3-5 led to increase in the absorption's maxima until the addition of 1.0 equiv. of  $\text{Zn}^{2+}$ . Afterwards, no appreciable alteration in the absorption intensity was realized up to the addition of 2.0 equiv. of  $\text{Zn}^{2+}$ . These observations suggested

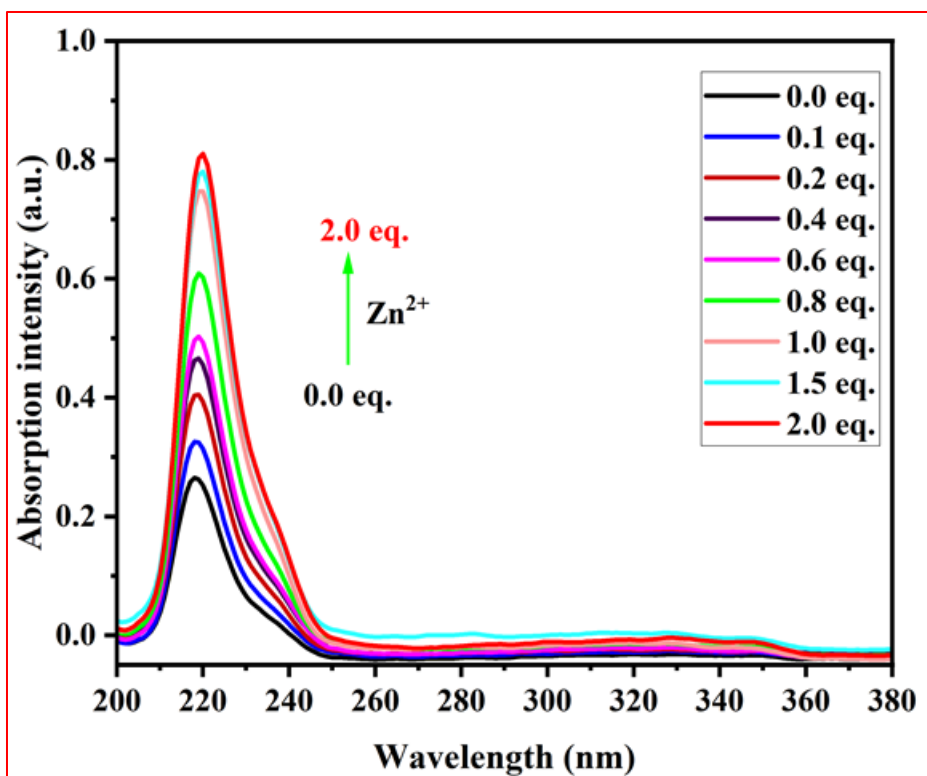
that addition of  $\text{Zn}^{2+}$  to the solutions of sensors **3-5** resulted in the formation of new species through the coordination of  $\text{Zn}^{2+}$  to the receptor, which led to the electron rearrangement in sensors **3-5** (Figs. 4.17-4.19) [9].



**Figure 4.17:** UV-vis absorption spectra of probe **3** (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ) with addition of  $\text{Zn}^{2+}$  (0.0-2.0 equivalent) in acetonitrile.



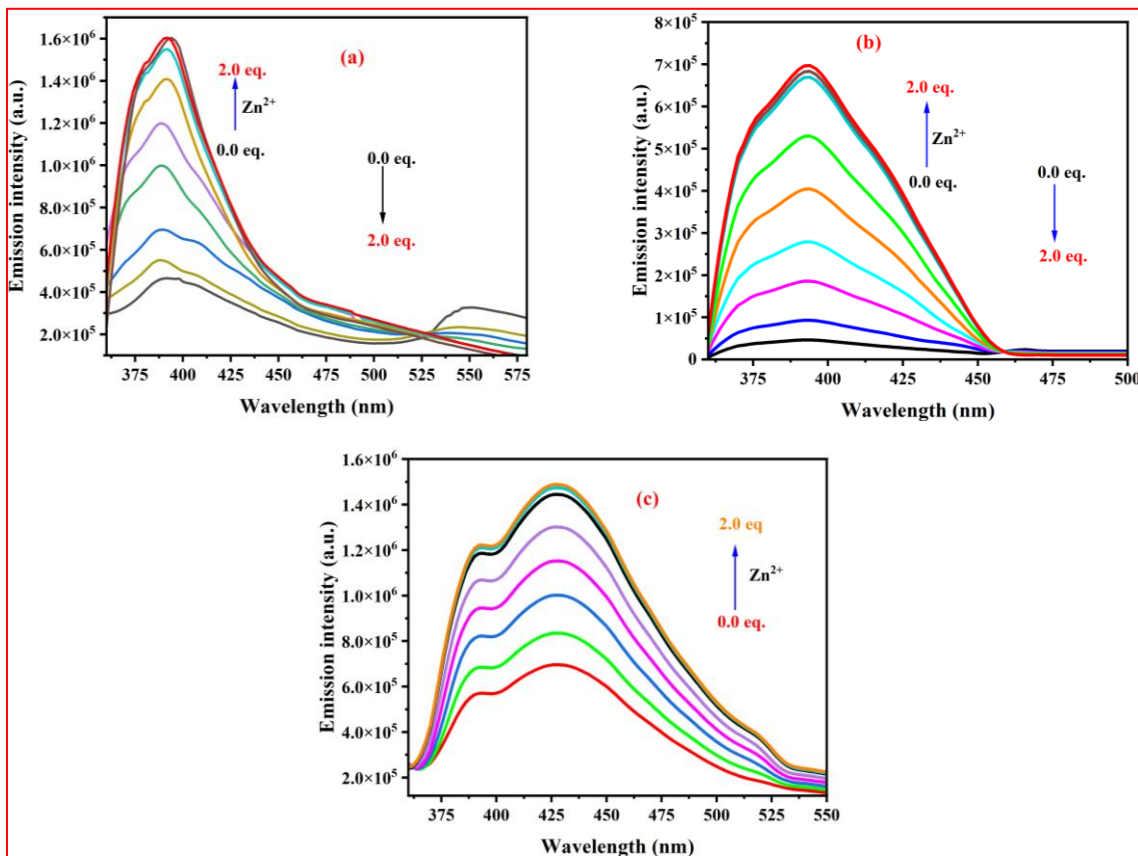
**Figure 4.18:** UV-vis absorption spectra of probe **4** (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ) with addition of  $\text{Zn}^{2+}$  (0.0-2.0 equivalent) in acetonitrile.



**Figure 4.19:** UV-vis absorption spectra of probe **5** (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ) with addition of  $\text{Zn}^{2+}$  (0.0-2.0 equivalent) in acetonitrile.

The sensing performance of these sensors was also investigated by studying their fluorescence properties in aqueous solution (50  $\mu\text{M}$  in HEPES buffer, pH = 7.4) (**Fig. 4.20**). The neat **3** exhibited two weak emission bands at around 392 nm and 550 nm when excited at 350 nm. However, with increasing equiv. of  $\text{Zn}^{2+}$  (0.0–2.0 equiv.), the band around at 550 nm was gradually disappeared whereas the intensity of emission bands at 392 nm was kept increasing with increase in  $\text{Zn}^{2+}$  concentration. Increase in fluorescence was realized until saturation i.e., addition of 1.0 equiv.  $\text{Zn}^{2+}$  (**Fig. 4.20a**). However, the fluorescence intensity was remained almost plateaued after the addition of more than 1.0 equiv. of  $\text{Zn}^{2+}$  (**Fig. 4.21**). Similarly,  $\text{Zn}^{2+}$  sensing performance of sensors **4** and **5** was also investigated. The emission spectrum of neat **4** displayed band at around 394 nm, whereas neat **5** showed

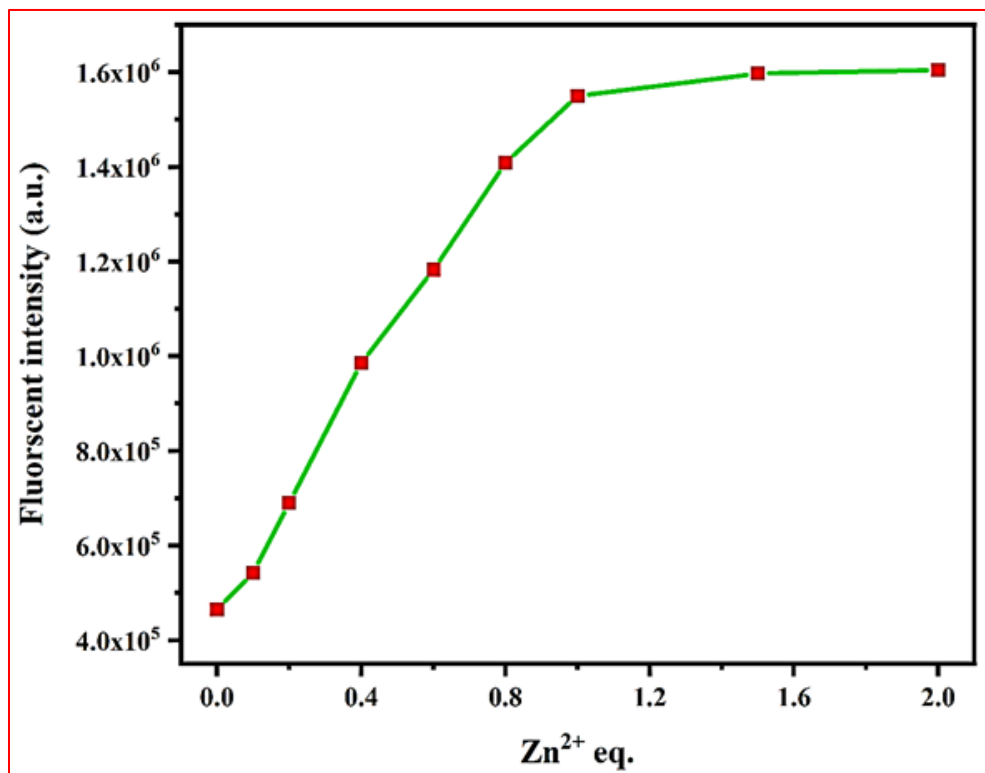
bands at around 392 nm and 428 nm. The fluorescence intensity of these bands gradually increased with increase in  $\text{Zn}^{2+}$  concentration (**Figs. 4.20b & 4.20c**).



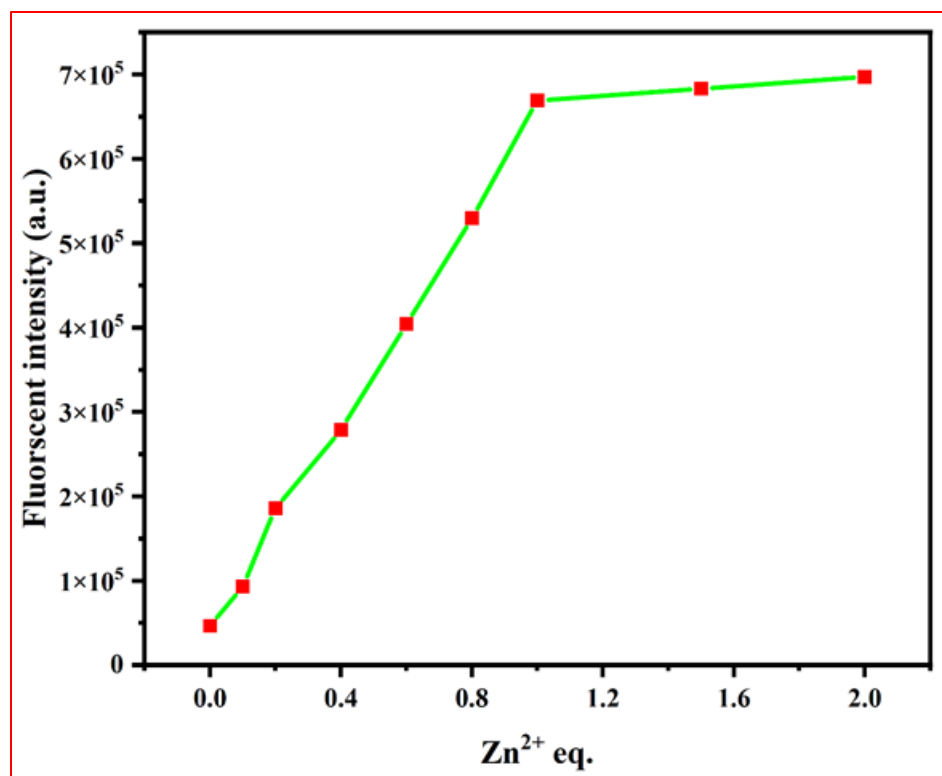
**Figure 4.20:** The emission spectra of sensors 3-5 (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ): a) **3** ( $\lambda_{\text{ex}} = 350$  nm), b) **4** ( $\lambda_{\text{ex}} = 350$  nm), and c) **5** ( $\lambda_{\text{ex}} = 350$  nm) upon addition of 0.0–2.0 equiv. of  $\text{Zn}^{2+}$  (50  $\mu\text{M}$  in HEPES buffer, pH = 7.4).

The titration experiment revealed a 14-folds and 2.5-folds enhancement in the fluorescence emission when 1.0 equiv. of  $\text{Zn}^{2+}$  was added to the solutions of **4** and **5**, respectively (**Figs. 4.22 & 4.23**). The above observations suggested that the fluorescence of sensors 3-5 quenched due to the PET. Nevertheless, addition of increasing  $\text{Zn}^{2+}$  concentration caused the formation of sensor/  $\text{Zn}^{2+}$  complex, which in turn prevented PET from occurring [9].

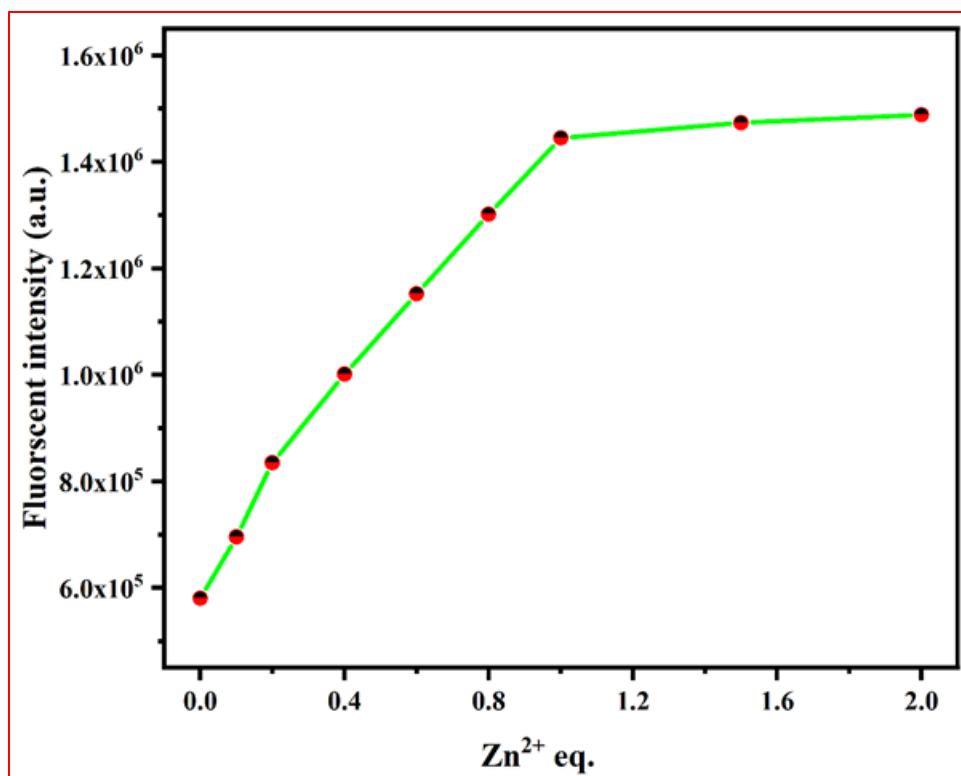
By employing IUPAC method [80], the limit of detection (LOD) of sensors **3-5** was calculated to be 0.12  $\mu\text{M}$ , 81.7 nM and 79.2 nM, respectively. Likewise, the limits of quantification (LOQ) for **3-5** was found to be 0.37  $\mu\text{M}$ , 247.5 nM and 239.9 nM, respectively (Figs. 4.24-4.26).



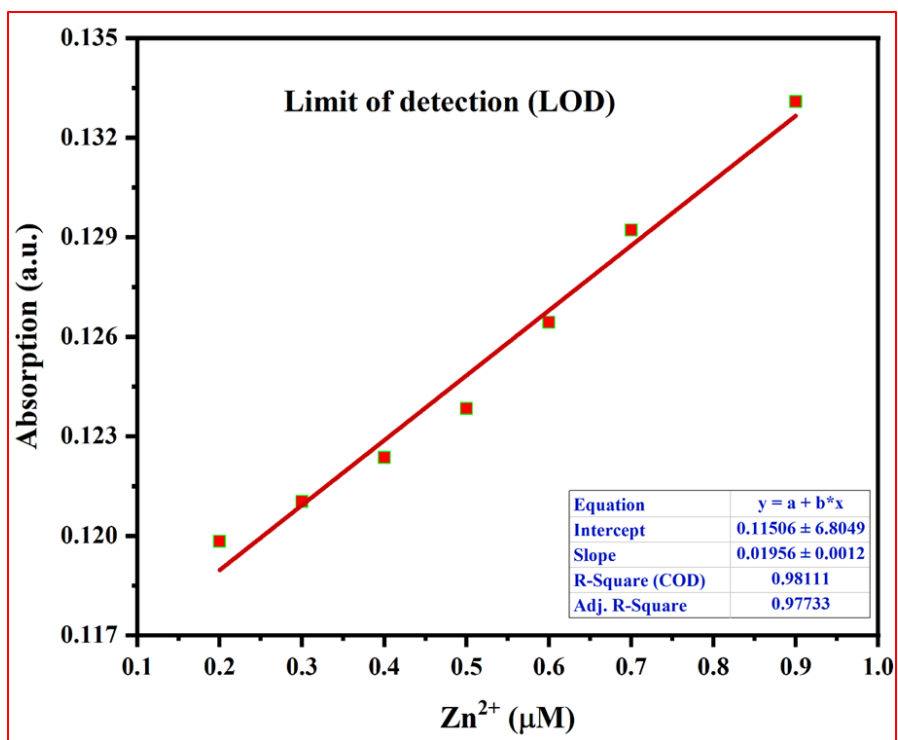
**Figure 4.21:** Change in emission intensity of probe **3** (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ) with increasing  $\text{Zn}^{2+}$  concentration (0.0-2.0 eq.) at 392 nm.



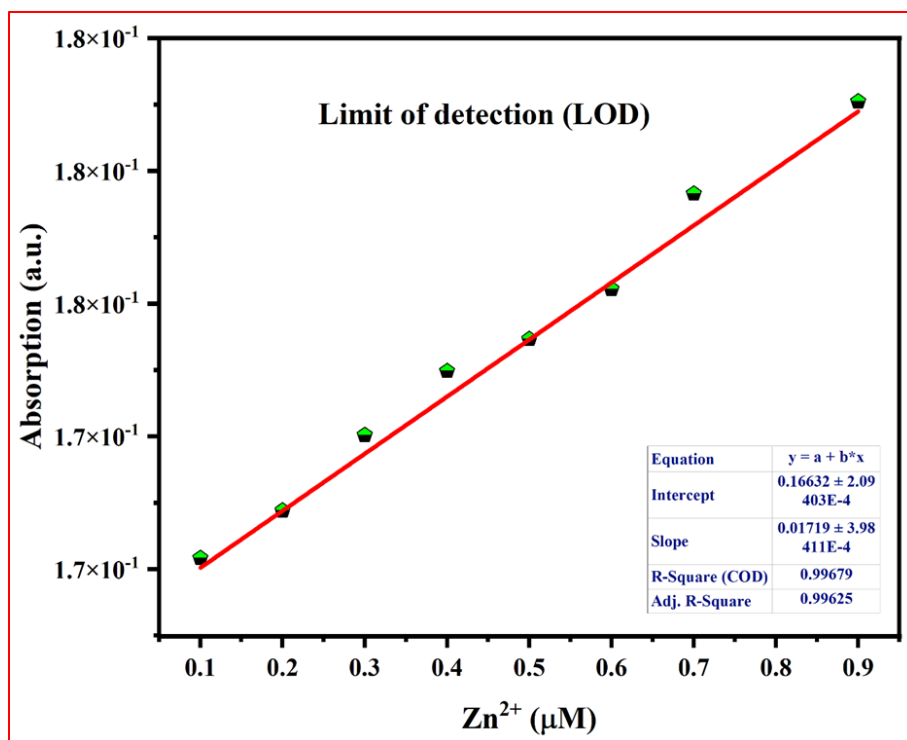
**Figure 4.22:** Change in emission intensity of probe **4** (50  $\mu$ M in CH<sub>3</sub>CN) with increasing Zn<sup>2+</sup> concentration (0.0-2.0 eq.) at 394 nm.



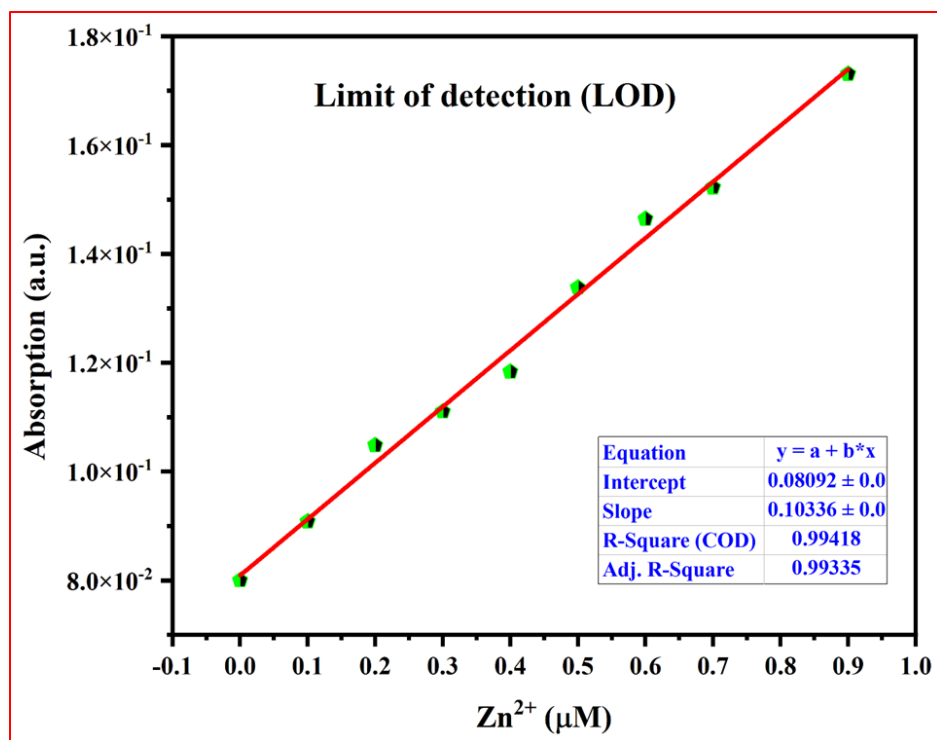
**Figure 4.23:** Change in emission intensity of probe 5 (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ) with increasing  $\text{Zn}^{2+}$  concentration (0.0-2.0 eq.) at 428 nm.



**Figure 4.24:** Standard calibration curve of probe **3** for the detection of  $\text{Zn}^{2+}$ .

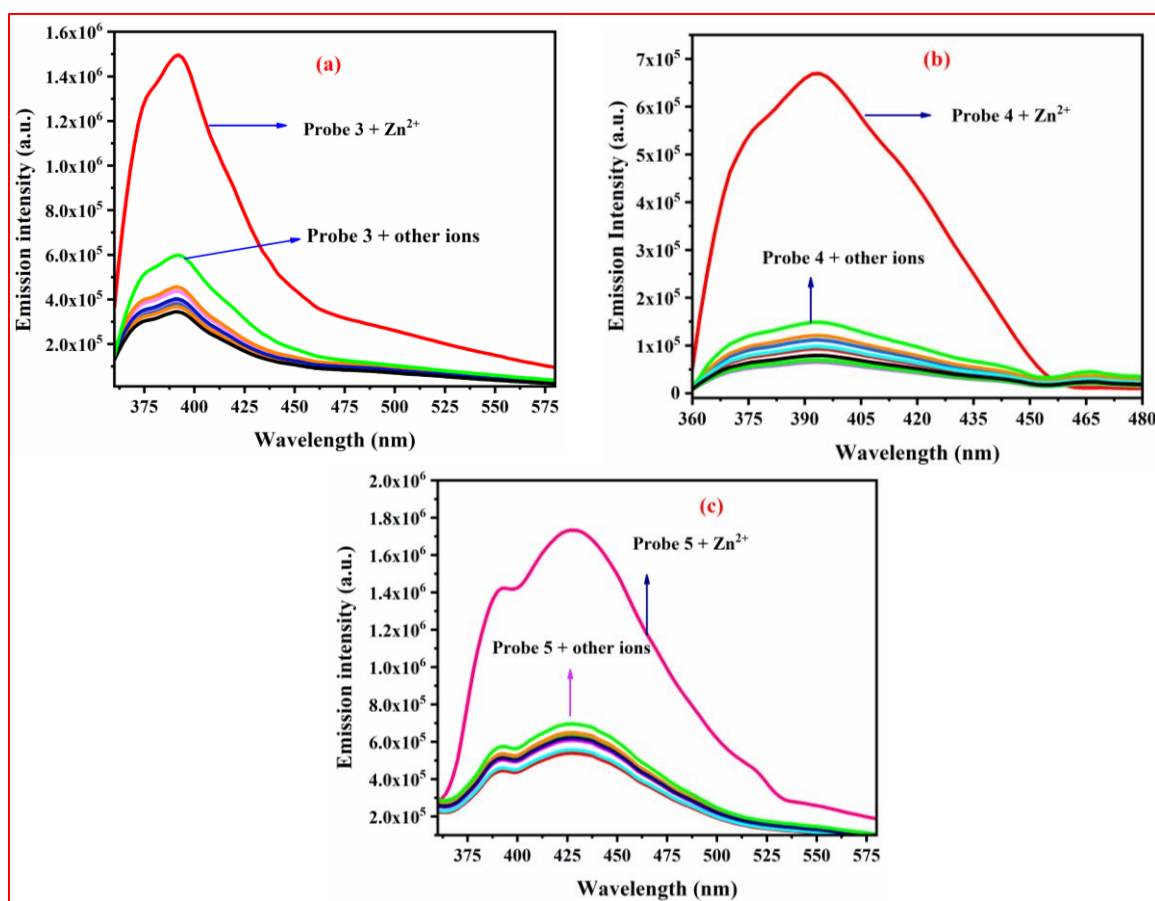


**Figure 4.25:** Standard calibration curve of probe **4** for the detection of  $\text{Zn}^{2+}$ .



**Figure 4.26:** Standard calibration curve of probe 5 for the detection of  $\text{Zn}^{2+}$ .

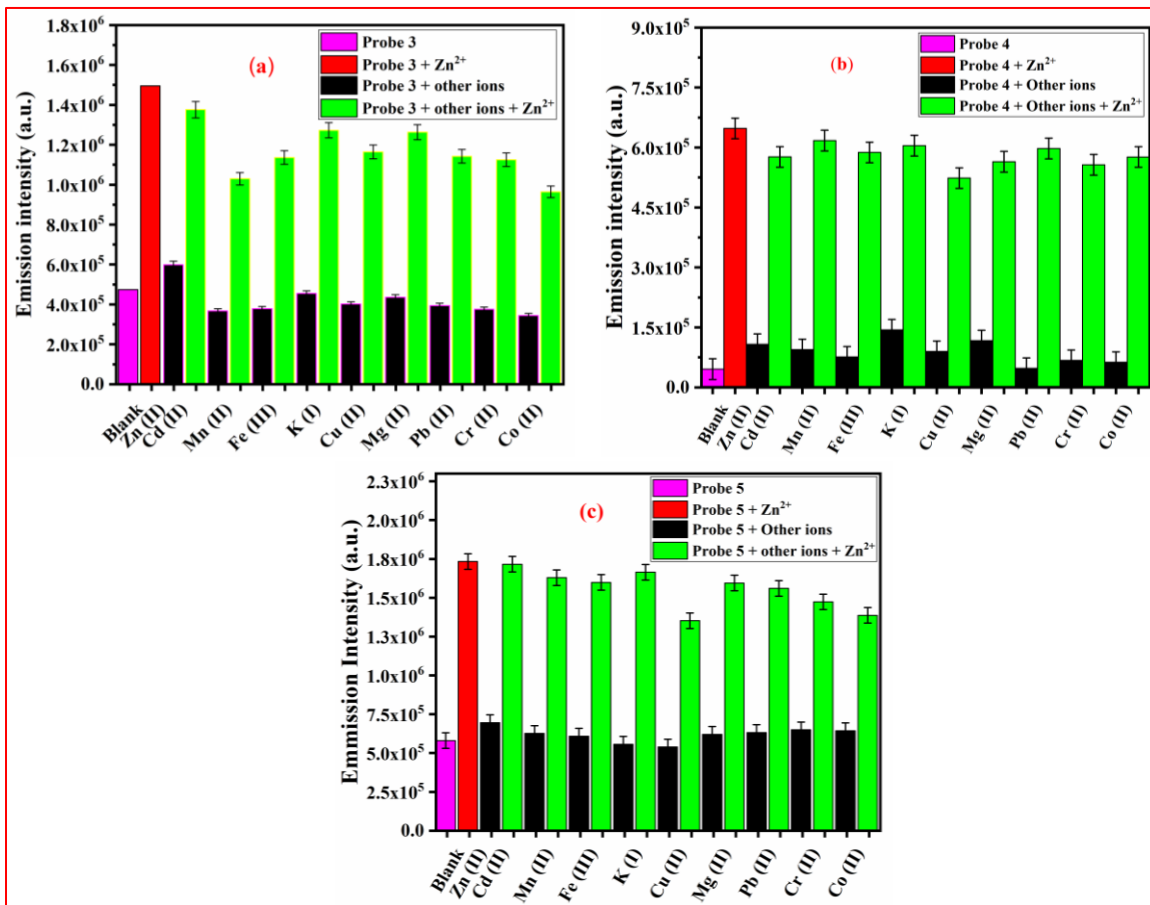
The fluorescence behavior of sensors **3-5** towards various metal ions ( $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{K}^{1+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Co}^{2+}$  and  $\text{Cr}^{2+}$ ) was monitored in HEPES buffer at pH 7.4. Apart from sensor **3** that displayed minor fluorescence enhancement upon addition of  $\text{Cd}^{2+}$ , fluorescent sensors **4** and **5** were found to be insensitive to the studied other metals ions (Fig. 4.27).



**Figure 4.27:** The fluorescence response of sensors **3-5** (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ): a) **3** ( $\lambda_{\text{ex}} = 350 \text{ nm}$ ), b) **4** ( $\lambda_{\text{ex}} = 350 \text{ nm}$ ), and c) **5** ( $\lambda_{\text{ex}} = 350 \text{ nm}$ ) upon addition of various individual metal ions (50  $\mu\text{M}$ ) and  $\text{Zn}^{2+}$  (50  $\mu\text{M}$ ) in HEPES buffer, pH = 7.4.

To determine the selectivity of fluorescent sensors **3-5** for  $\text{Zn}^{2+}$  over other coexisting competitive metal ions, competitive fluorescence experiments were performed. Initially, the fluorescence response of sensors **3-5** (50  $\mu\text{M}$ ) with various metal cations (1.0 equiv., 50  $\mu\text{M}$ ) was studied. The black bars in **Fig. 4.28** represent the relative fluorescence response of sensors **3-5** mixed with other metal cations. As evident, either no significant or minor fluorescence enhancements was observed. Thereafter, addition of  $\text{Zn}^{2+}$  (1.0 equiv., 50  $\mu\text{M}$ ) to the solutions of fluorescent sensors **3-5** in the presence of other ions

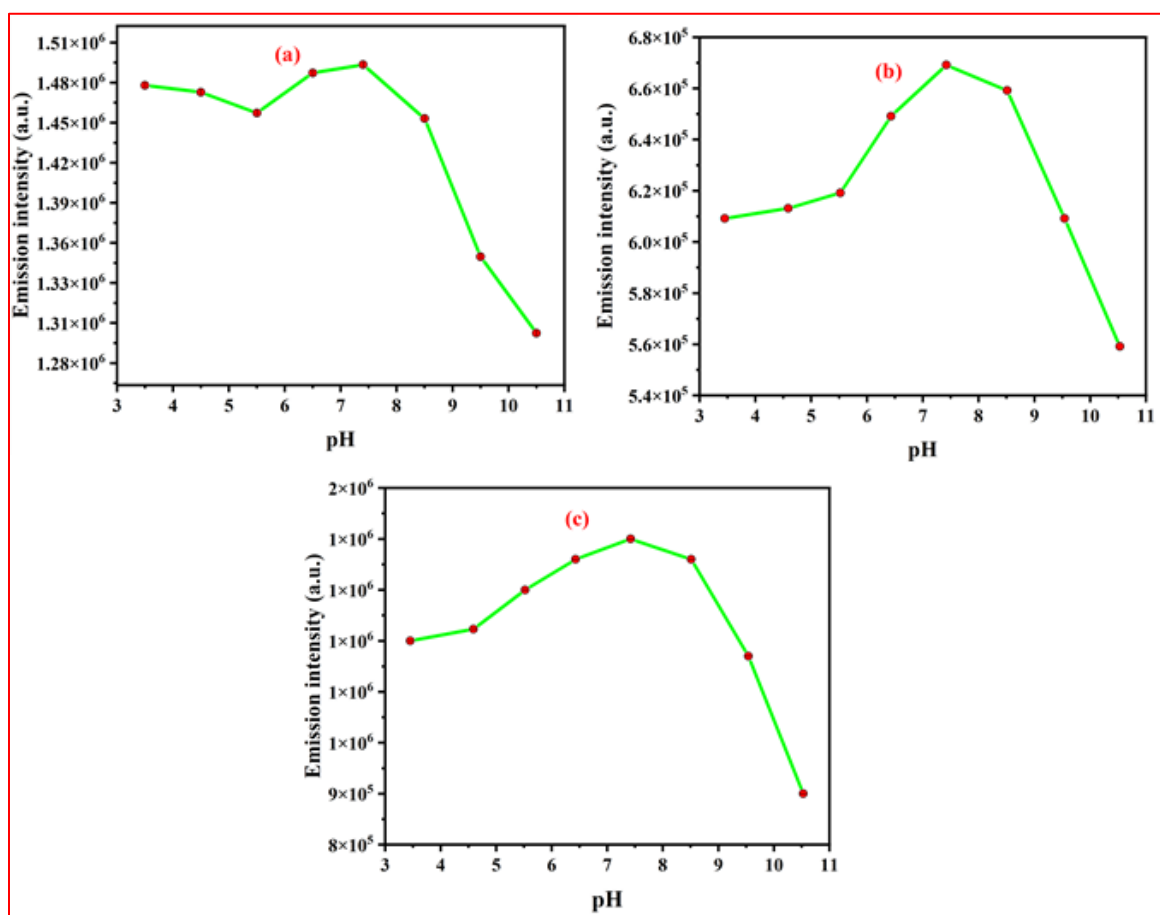
resulted in enhanced emission intensities, a similar pattern to that of with  $\text{Zn}^{2+}$  alone. These results suggested that amide-DPA and amide-DQA receptors of sensors **3-5** exhibited high selectivity for the detection of  $\text{Zn}^{2+}$  over other competitive metal ions in aqueous solution (Fig. 4.28).



**Figure 4.28:** The fluorescence response of sensors **3-5** (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ): a) **3** ( $\lambda_{\text{ex}} = 350 \text{ nm}$ ), b) **4** ( $\lambda_{\text{ex}} = 350 \text{ nm}$ ), and c) **5** ( $\lambda_{\text{ex}} = 350 \text{ nm}$ ) upon addition of various individual metal ions (50  $\mu\text{M}$ ) and  $\text{Zn}^{2+}$  (50  $\mu\text{M}$ ) in HEPES buffer, pH = 7.4

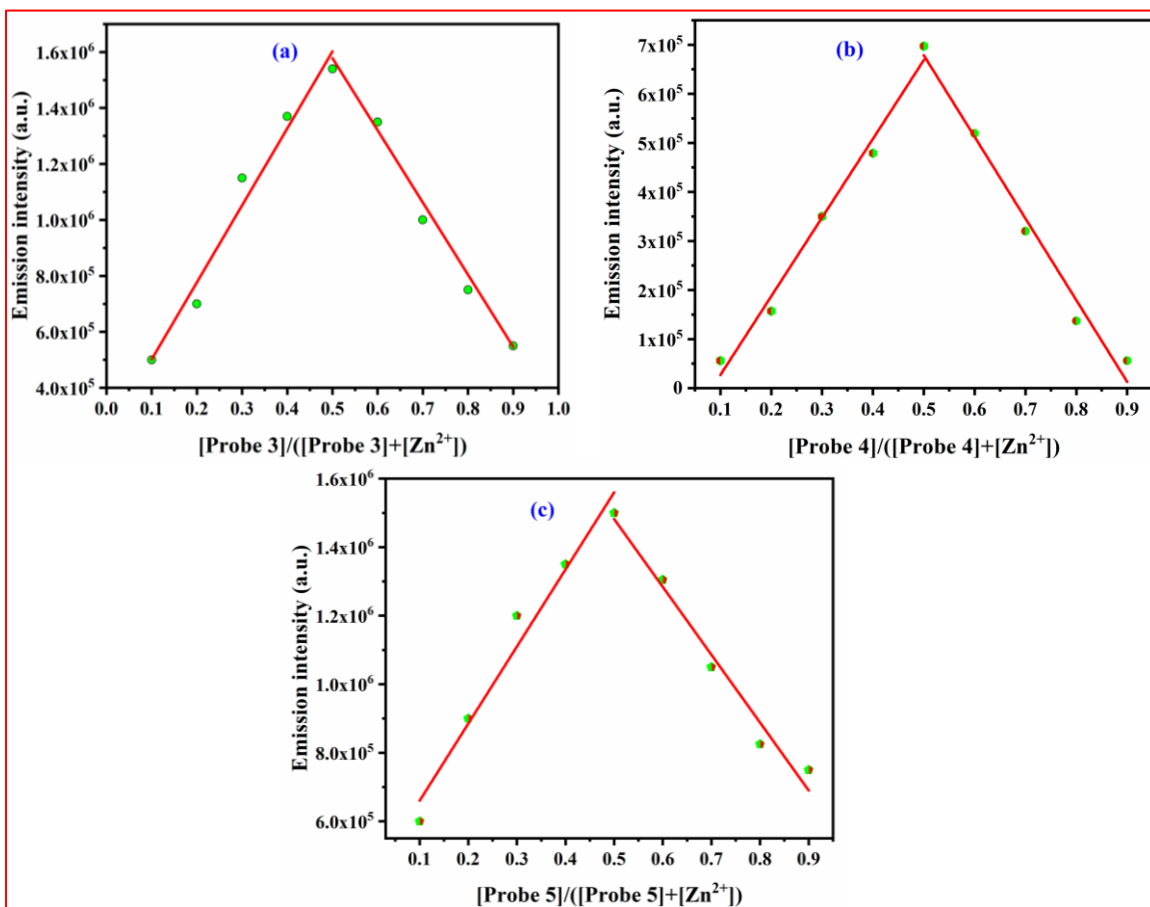
In addition, the effect of pH on the fluorescence emission of sensors- $\text{Zn}^{2+}$  complexes were studied in  $\text{CH}_3\text{CN}$ /HEPES (1:1 v/v). The pH of solutions was varied from 3.5-10.5, using

hydrochloric acid or sodium hydroxide to achieve the desired pH values. sensors- $\text{Zn}^{2+}$  complexes indicated strong pH dependence (**Fig. 4.29**). In the basic medium, the fluorescent intensity was substantially lowered, suggesting resumption of PET process from the electron donor (DPA and DQA) to the excited fluorophore (1,8-naphthalimide) owing to the conceivable generation of  $\text{Zn}^{2+}$  hydroxide [9]. Likewise, relatively reasonable enhancement in the fluorescence intensity in acid medium ( $\text{pH} < 5$ ) could be attributed to the protonation of DPA and DQA.



**Figure 4.29:** The effect of pH on the fluorescence response of a) 3/ $\text{Zn}^{2+}$  ( $\lambda_{\text{em}} = 392 \text{ nm}$ ), b) 4/ $\text{Zn}^{2+}$  ( $\lambda_{\text{em}} = 394 \text{ nm}$ ), and c) probe 5/ $\text{Zn}^{2+}$  ( $\lambda_{\text{em}} = 428 \text{ nm}$ ) complexes in  $\text{CH}_3\text{CN}/\text{HEPES}$  buffer (50:50, v/v) solution.

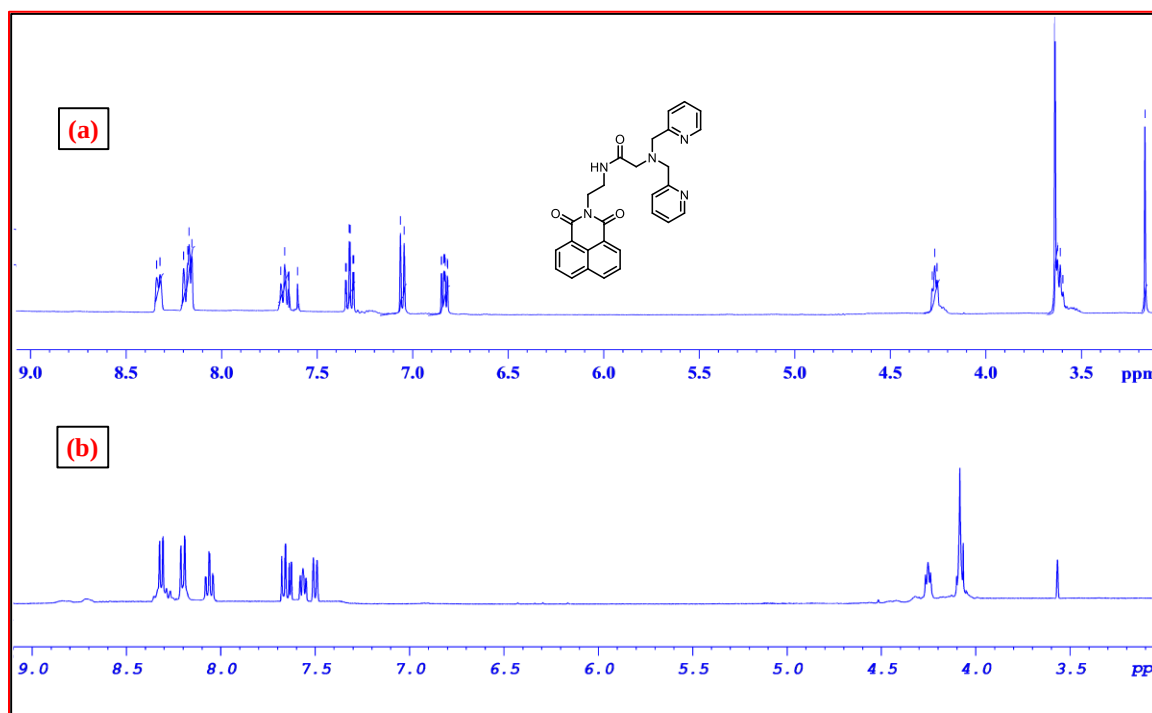
The stoichiometry and binding behavior sensors- $\text{Zn}^{2+}$  complexes were studied by fluorescence titration test of sensors **3-5** with different  $\text{Zn}^{2+}$  concentrations in  $\text{CH}_3\text{CN}$ /HEPES buffer (50:50, v/v) solution. The total concentration of sensors and  $\text{Zn}^{2+}$  at 50  $\mu\text{M}$ . The Job's plot analysis displayed a maximum at the 0.5 M fraction of  $\text{Zn}^{2+}$ , suggesting 1:1 binding ratio between  $\text{Zn}^{2+}$  and the sensors **3-5** (Fig. 4.30).



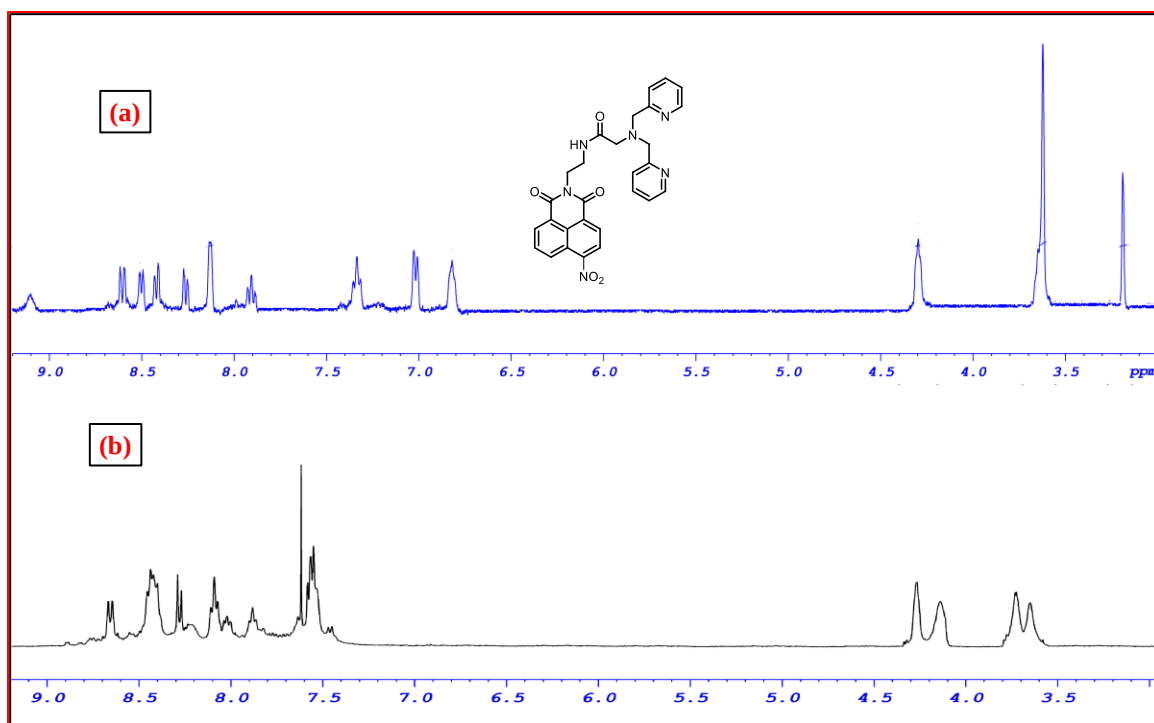
**Figure 4.30:** Job's plot for the sensors **3-5** (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ) in 50  $\mu\text{M}$  HEPES buffer solution (pH = 7.4): a) **3** ( $\lambda_{\text{em}} = 392$  nm), b) **4** ( $\lambda_{\text{em}} = 394$  nm), and c) **5** ( $\lambda_{\text{em}} = 428$  nm). The total concentration of the sensors **3-5** and  $\text{Zn}^{2+}$  is 50  $\mu\text{M}$ .

The binding behavior of sensors **3-5** with  $\text{Zn}^{2+}$  was also substantiated by monitoring through  $^1\text{H}$  NMR titration experiments. The  $^1\text{H}$  NMR spectra of neat **5** and sensor **5**- $\text{Zn}^{2+}$

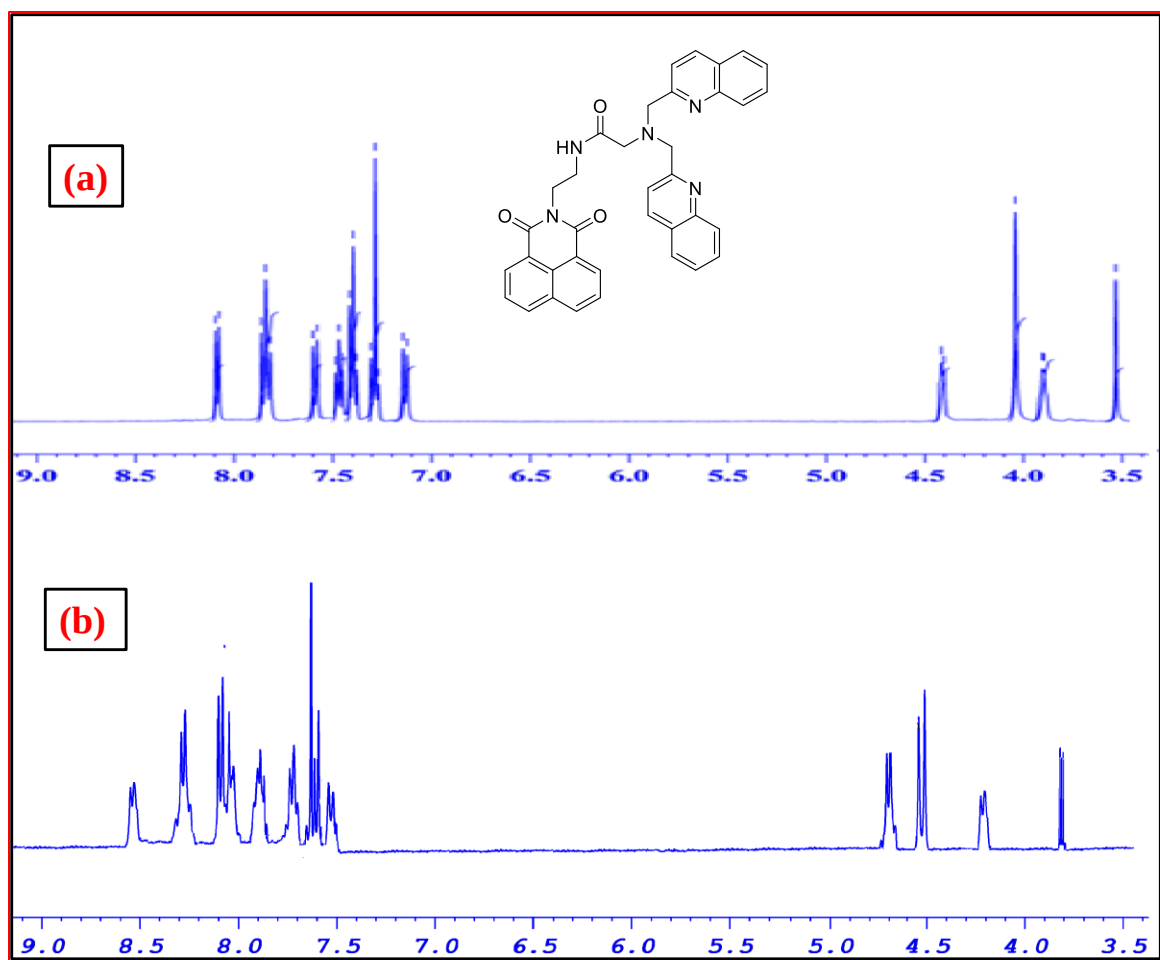
complex in CD<sub>3</sub>CN revealed marked downfield shift of pyridine protons of DPA along with methylene protons attached to tertiary N (**Fig. 4.33**). This observation suggested that the N atoms of DPA and tertiary N atom were involved in Zn<sup>2+</sup> coordination, which led to downfield shifts of the said signals owing to the deshielding effect of the metal ion. In a similar fashion, <sup>1</sup>H NMR spectra of sensors **3** and **4** complexed with Zn<sup>2+</sup> also revealed distinct variations in the resonance peaks for the pyridine protons of DPA as well as methylene protons attached to tertiary N (**Fig. 4.31 & 4.32**).



**Figure 4.31:** <sup>1</sup>H-NMR (400 MHz, acetonitrile-d<sub>3</sub> of **a**) sensor **3** and **b**) sensor **3**/Zn<sup>2+</sup> complex from 3.0 to 9.1 ppm.

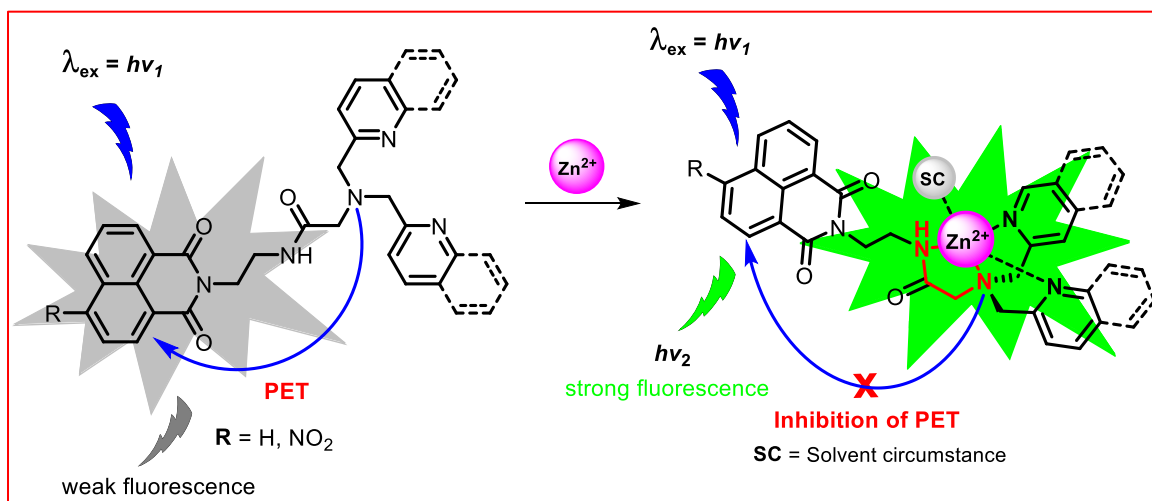


**Figure 4.32:**  $^1\text{H}$ -NMR (400 MHz, acetonitrile- $\text{d}_3$ ) of **a**) sensor **4** and **b**) sensor **4**/ $\text{Zn}^{2+}$  complex from 3.0 to 9.2 ppm.



**Figure 4.33:**  $^1\text{H}$  NMR (400 MHz, acetonitrile  $\text{d}_3$ ) of a) sensor 5 and b) sensor-5/ $\text{Zn}^{2+}$  complex from 3.4 to 9.1 ppm.

The PET mechanism of sensors **3-5** with  $\text{Zn}^{2+}$  can be explained as depicted in **Figure 4.31**. In the absence of  $\text{Zn}^{2+}$ , the HOMO of the unbound receptors (DPA and DQA) contains higher energy than the HOMO of the excited fluorophore. As a result, electron transfer from the receptor to the excited fluorophore readily occurs, which in turn quenches the fluorescence and thus resulting in weak fluorescence. Contrary, when DPA and DQA moieties bound to  $\text{Zn}^{2+}$ , the HOMO of receptors gets lower in energy than the HOMO of excited fluorophore, which makes electron transfer energetically unfavorable and hence leading to the fluorescence “switch on”.

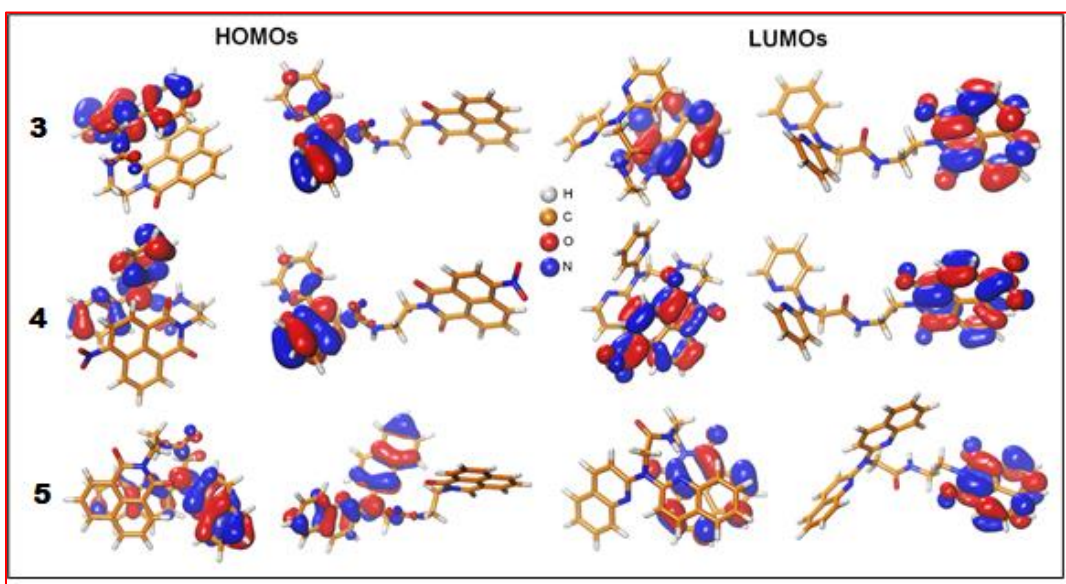


**Figure 4.34:** PET mechanism of sensors **3-5** with  $\text{Zn}^{2+}$ .

## 4.5 Computational studies

### 4.5.1 $\text{Zn}^{2+}$ Recognition and the Frontier Molecular Orbitals (FMO)

DFT simulations were employed to elucidate the impact of  $\text{Zn}^{2+}$  on the fluorescence of the three new synthesized sensors. Seeking the most stable structures of the three sensors, we explored the conformational space using the GFN2-xTB method and the iMTD-GC algorithm implemented in the CREST code. Further geometrical optimizations were carried out on the extended (optimization without the CREST) and the stacked structures (predicted as the most stable conformer by CREST), using wB97xd1,2/6-31+G(d,p) method. The stacked structures (**Figs. 3.43 & 4.35**) are predicted to be more stable than the extended ones.



**Figure 4.35:** The frontier molecular orbitals (HOMOs & LUMOs) of chemosensors (3-5) considered in our molecular modeling study for the stacked and extended conformations. The isosurfaces of the FMO's electron density represented at 0.03  $\text{e}/\text{\AA}^3$  of resolution.

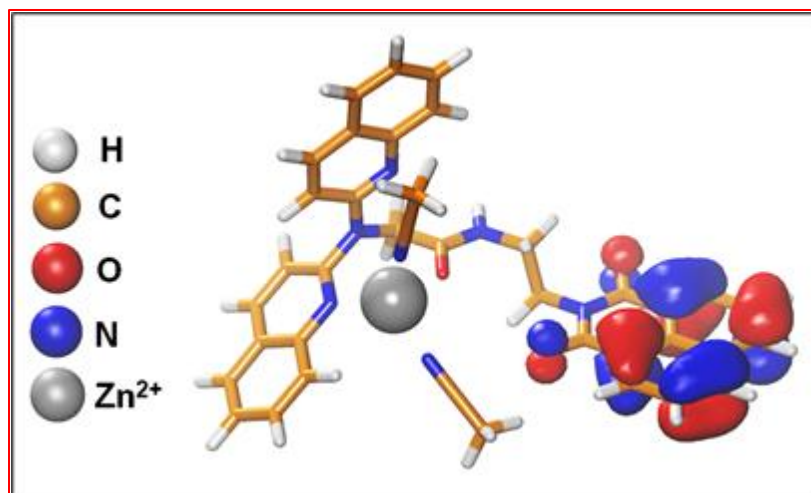
The relative free energies of the three sensors (**3-5**) were found to be -7.42, -7.62, and -11.10 kcal/mol, respectively. The stacked structure's stability is mainly due to the strong Van Der Waals (VDW) interactions between 1,8-naphthalimide and 4-nitro-1,8-naphthalimide with DPA and DQA moieties. Moreover, the presence of the NO<sub>2</sub> group does not affect the stability of the stacked structure of sensor **4**. However, 2-quinolyl groups in DQA further stabilize the stacked form in sensor **5**. The FMO of these structures were examined to investigate the impact of the conformational changes on the electronic structure of these sensors. Within the FMOs of the three sensors, the electronic density of the highest occupied molecular orbital (HOMO) was found to be localized on DPA and DQA moieties whereas the density of the lowest unoccupied molecular orbitals (LUMOs) was localized on the 1,8-naphthalimide and 4-nitro-1,8-naphthalimide (**Fig. 4.35**). In addition, the bandgap of the sensors **3-5** did not change significantly upon the conformational changes from the extended to the stacked (compact) structures or by the chemical modifications from **1** to **3**. Indeed, the bandgaps of sensors **3-5** for the extended form were determined to be 6.85, 6.20, 6.71 eV and for the stacked form were 6.82, 6.11, and 6.81 eV (**Fig. 4.35**).

Zn<sup>2+</sup> binding energies were studied on both the stacked and extended structures. Zn<sup>2+</sup> is pentacoordinate in the three complexes, four nitrogen atoms (two are solvent molecules) and one oxygen. The average bond distance was about 2.0 Å in both stacked and extended form structures. The metal binding energy calculations indicated significant preference to the favorable binding of Zn<sup>2+</sup> by stacked conformation, especially in case of sensors **3** and **5** (**Table 4.1**).

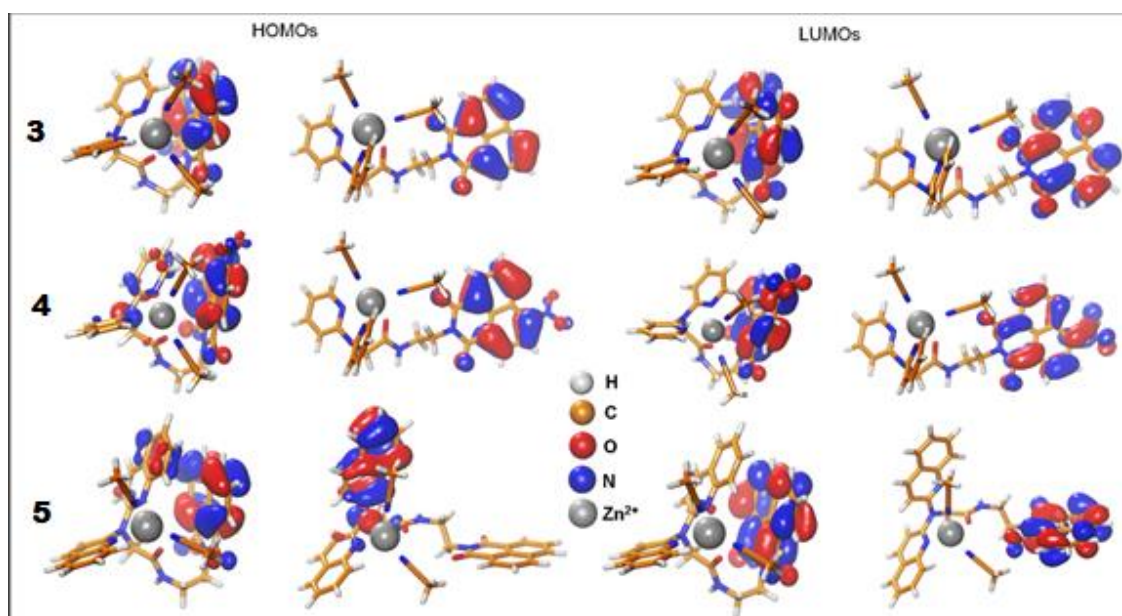
**Table 4.1:** Total  $\text{Zn}^{2+}$  binding energies ( $\Delta E_{\text{Total}}$ ) of the three sensors are reported in kcal/mol with the gas phase ( $\Delta E_{\text{gas}}$ ), and solvation contributions ( $\Delta E_{\text{Sol}}$ ). The binding energies for the extended conformation are reported in parenthesis

|          | $\Delta E_{\text{gas}}$ | $\Delta E_{\text{Sol}}$ | $\Delta E_{\text{Total}}$ |
|----------|-------------------------|-------------------------|---------------------------|
| <b>3</b> | -451.6 (-439.9)         | 357.0 (359.2)           | -94.6 (-80.7)             |
| <b>4</b> | -430.2 (-434.2)         | 348.0 (353.8)           | -82.2 (-80.4)             |
| <b>5</b> | -440.2 (-425.2)         | 358.8 (360.3)           | -81.4 (-64.9)             |

FMOs of the  $\text{Zn}^{2+}$ -sensor complexes were also examined to investigate the impact of  $\text{Zn}^{2+}$  on the FMOs of the three molecules. The electron density of HOMOs and LUMOs in the presence of  $\text{Zn}^{2+}$  was localized onto 1,8-naphthalimide and 4-nitro-1,8-naphthalimide and there was no contribution from DPA nor DQA except for the HOMO of complex 5 in the extended form. However, the HOMO-1 was completely localized on 1,8-naphthalimide and was found to be slightly more stable than the HOMO (the energy difference was only 0.19 eV, **Fig. 4.36**). Similarly, the bandgap does not change upon the conformational transition from the extended to the stacked form. Further,  $\text{Zn}^{+2}$  slightly increases the bandgap in sensor-  $\text{Zn}^{+2}$  complexes as compared to the free state due to stabilizing the HOMOs (**Fig. 4.37**). The bandgaps of the stacked complexes were found to be 7.50, 7.24, 7.40 eV and whereas for the extended they were determined to 7.51, 7.29, and 7.32 eV.



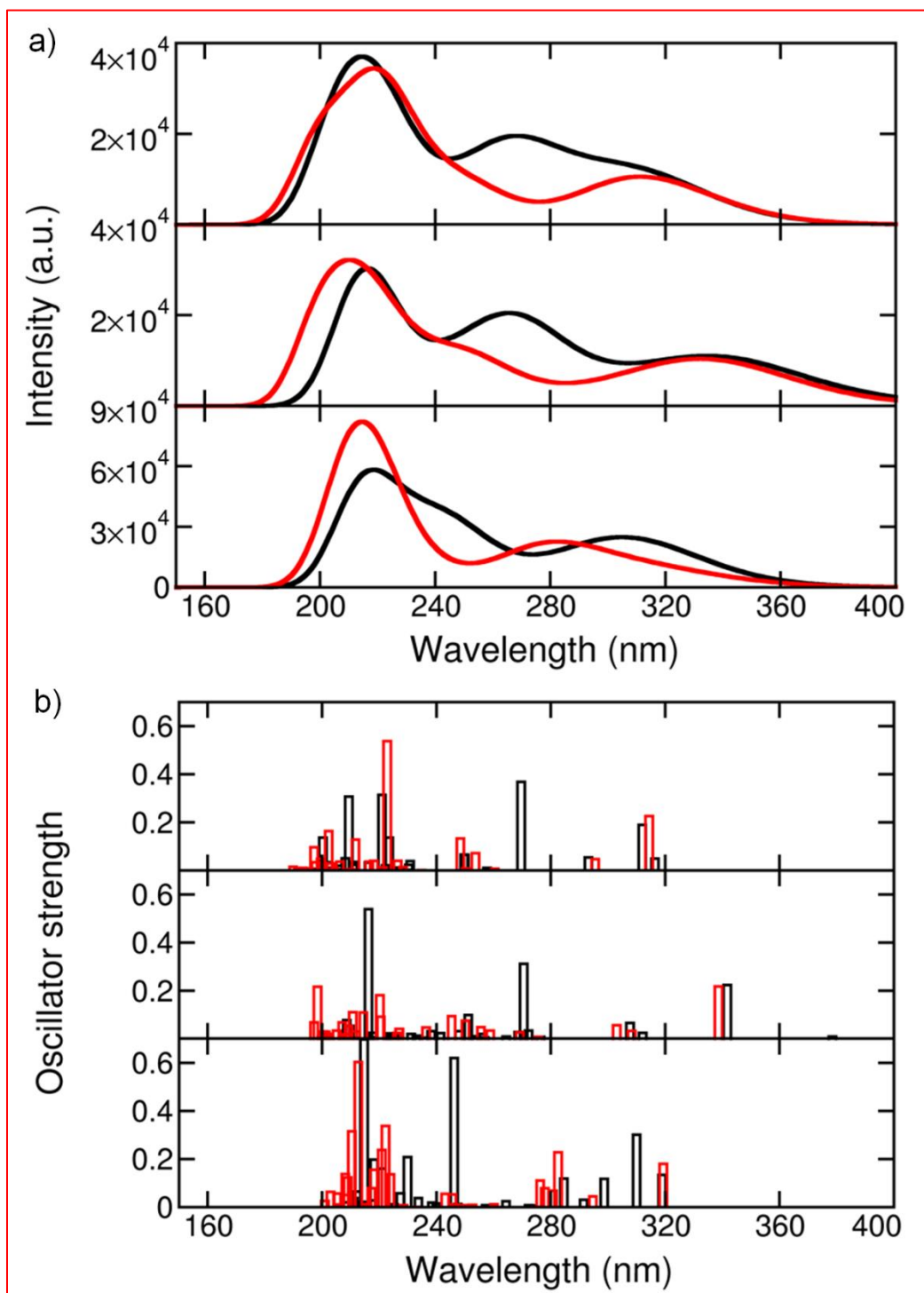
**Figure 4.36:** The HOMO-1 of the  $\text{Zn}^{2+}$  complex with sensor **5** in the extended form showing the electron density localized on 1,8-naphthalimide. The isosurfaces of the FMO's electron density is represented at  $0.03 \text{ e}/\text{\AA}^3$ .



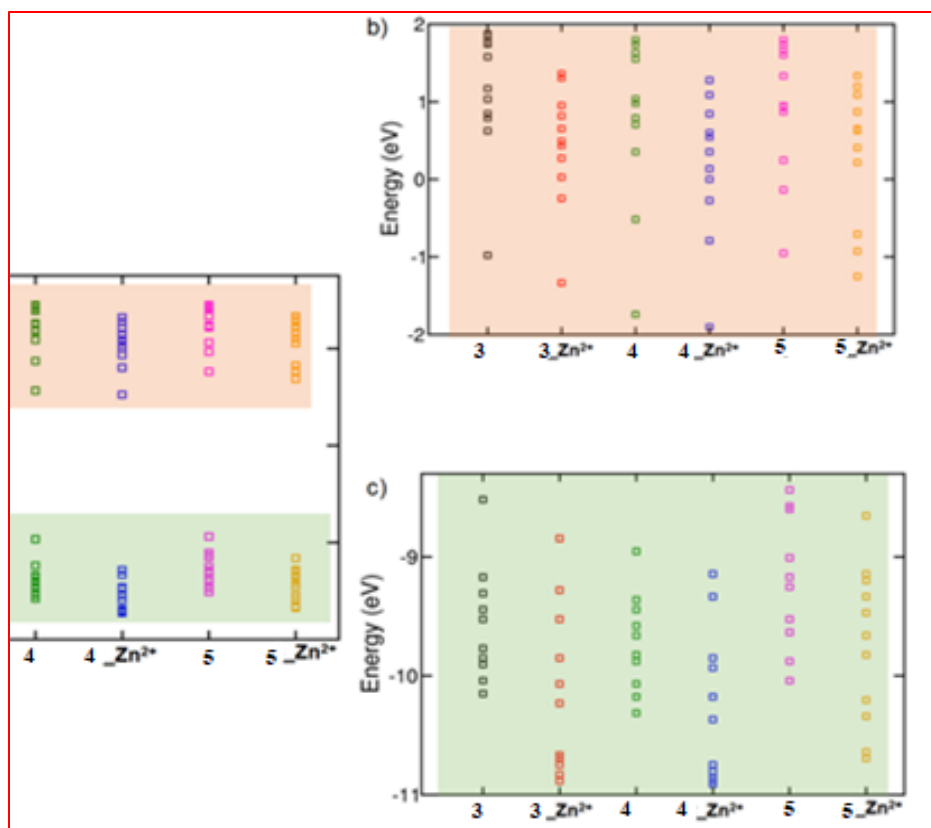
**Figure 4.37** The frontier molecular orbitals (HOMOs & LUMOs) of the three  $\text{Zn}^{2+}$ -chemosensors complexes in the two conformations (stacked and extended). The isosurfaces of the FMO's electron density are shown at  $0.03 \text{ e}/\text{\AA}^3$ .

#### 4.5.2 The Effect of $\text{Zn}^{2+}$ -Binding on the UV-Visible Spectra

DFCT study on the free sensors **3-5** and sensors- $\text{Zn}^{2+}$  complexes to investigate their UV-visible absorbance spectra. The stimulation of the UV-visible spectra was limited to the stacked conformations since they are more stable and exhibit higher binding preference for  $\text{Zn}^{2+}$ . Thirty excited states altogether were calculated in the TD-DFT simulations. Sensors **3-5** displayed a broadband absorption in the range of 200-350 nm; the sensors displayed  $\lambda_{\text{max}}$  at 214, 217, and 218 nm, respectively. The transitions originated from the intra-charge transfer of  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  types. Moreover, the electron withdrawing  $\text{NO}_2$  group does not affect the spectra of sensor **4**. On the other hand, the spectra of free sensors (unbound) did not show any appreciable changes except the intensity of peak at 270 nm in the spectrum of sensor **5** was decreased. Furthermore, a shoulder peak at 246 nm was also appeared. There are no changes in the spectra of the three sensors in the unbound state except at 270 nm of the peak intensity of sensor **5** is decreased. However, a shoulder peak at 246 nm was also appeared (**Fig. 4.38a**). The oscillator strength ( $f$ ) is a dimensionless factor that expresses the probability of the light absorbance upon electronic transitions. In the unbound states, sensor **3** has important absorbance probabilities at 269.6 nm (0.37), 223.5 nm (0.14), 220.9 nm (0.31), and 209.4 nm (0.31) (**Fig. 4.38b**).



**Figure 4.38:** The UV-visible absorbance spectra of the three sensors in the unbound- (black) and  $Zn^{2+}$ -bound states (red) and the stacked conformation a). The oscillator strength of the different excited states of the three sensors b).



**Figure 4.39:** The frontier molecular orbitals of the three sensors in the unbound and  $\text{Zn}^{2+}$ -bound states (a). b) Zoon of the orbitals from LUMO to LUMO+10, and c) is zoom in of the orbitals from HOMO to HOMO-10.

The peaks at 220.9 and 209.4 nm involved the electronic transitions from the top valence and deep valence orbitals (HOMO-1, HOMO-2, HOMO-9, HOMO-10, HOMO-11, and HOMO-13) to the LUMO, and LUMO+2. The densities of the LUMO and LUMO+2 was either localized on 1,8-naphthalimide or delocalized over the entire molecule. The densities of the involved HOMOs in these transitions were mainly localized on 1,8-naphthalimide. The highest  $f$  values for sensor **4** were 0.54, located at 216.2 nm, and 0.31 located at 270.5 nm. The absorbance at 216.2 nm was mainly due to the multiple transitions with major contributions from HOMO-1, HOMO-5, and HOMO-10 to LUMO+1, LUMO+2, and LUMO+4, respectively. The orbitals involved in these transitions were mainly localized

on the 1,8-naphthalimide group, the peptide linker with very limited contributions from DPA, which explains the similarity between sensors **3** and **4** spectra. Sensor **5** displayed a significant  $f$  factor (0.62,  $\lambda_{\text{max}}$ =246.2 nm), mainly due to the transition from HOMO-1, HOMO-2, HOMO-3, HOMO-4 to LUMO+1 LUMO+2, LUMO+5, respectively. The HOMOs of this transition were localized on 1,8-naphthalimide (HOMO-1 & HOMO-2) and DQA (HOMO-3 & HOMO-4), while LUMOs are all localized on DQA. The latter provided more degenerate states that enhanced the probability of the electronic transitions compared to sensors **3** and **4**. The energy separations from HOMO-1 to HOMO-4 was only 0.6 eV, and from LUMO+1 to LUMO+5 was 1.1 eV. Adding more pi-electron density in sensor **5** increased the energy of HOMOs and lowers the energy of the LUMOs. The highest  $f$  values of sensor **5** was found to be 0.70 at 214.7 nm, which involved mainly the electronic transitions from the HOMO to LUMO+6. The HOMO was localized on 1,8-naphthalimide, whereas the LUMO+6 was localized on DQA. The main DQA contributions in the spectra of sensor **5** was significant compared to DPA in sensors **3** and **4**.

Regarding the  $\text{Zn}^{2+}$ -bound states, a slight blue-shift in the absorbance spectra of sensors **3-5** was observed, with a maximum shift in sensor **4** (**Fig. 4.38a**). The intensity of the absorbance peak at 269 nm was decreased in sensors **3** and **4** whereas red-shifted in sensor **5** to 280 nm. The notable impact of  $\text{Zn}^{2+}$  on the sensors spectra was evidenced by the generation of more excited states (**Fig. 4.38b**). The presence of  $\text{Zn}^{2+}$  leads to stabilizing the HOMOs and LUMOs states compared to the unbound states; the effect was persisting in all sensors (**Fig. 4.39**). However, the effect was more pronounced in the LUMOs energy levels. The energy differences between the LUMOs were squeezed compared to the unbound states (**Fig. 4.39b**). Focusing on sensor **3**; the highest plausible excited state was

located at  $\lambda_{\text{max}}$  of 222.8 nm ( $f=0.54$ ), with contributions from HOMO, HOMO-2, and deep valence HOMO-7 to LUMO and LUMO+4. The densities of HOMO and HOMO-2 were localized on the 1,8-naphthalimide, while the HOMO-7 was delocalized on 1,8-naphthalimide and DPA. The LUMOs of this transition were also localized on 1,8-naphthalimide with minimum contributions from DPA. Upon  $\text{Zn}^{2+}$  binding, DPA started to contribute to the density of the HOMOs involved in the electronic transitions, which was presumably the reason for the improved intensities upon  $\text{Zn}^{2+}$ -binding. Another important note was that the binding of  $\text{Zn}^{2+}$  led to electronic transitions from higher valence HOMOs (HOMO-7) compared to from the highly deep orbitals in the unbound state (HOMO-13). Similarly, in  $\text{Zn}^{2+}$ -bound states, higher LUMOs (LUMO+4) were involved in the electronic transition than in the unbound state (LUMO+2).

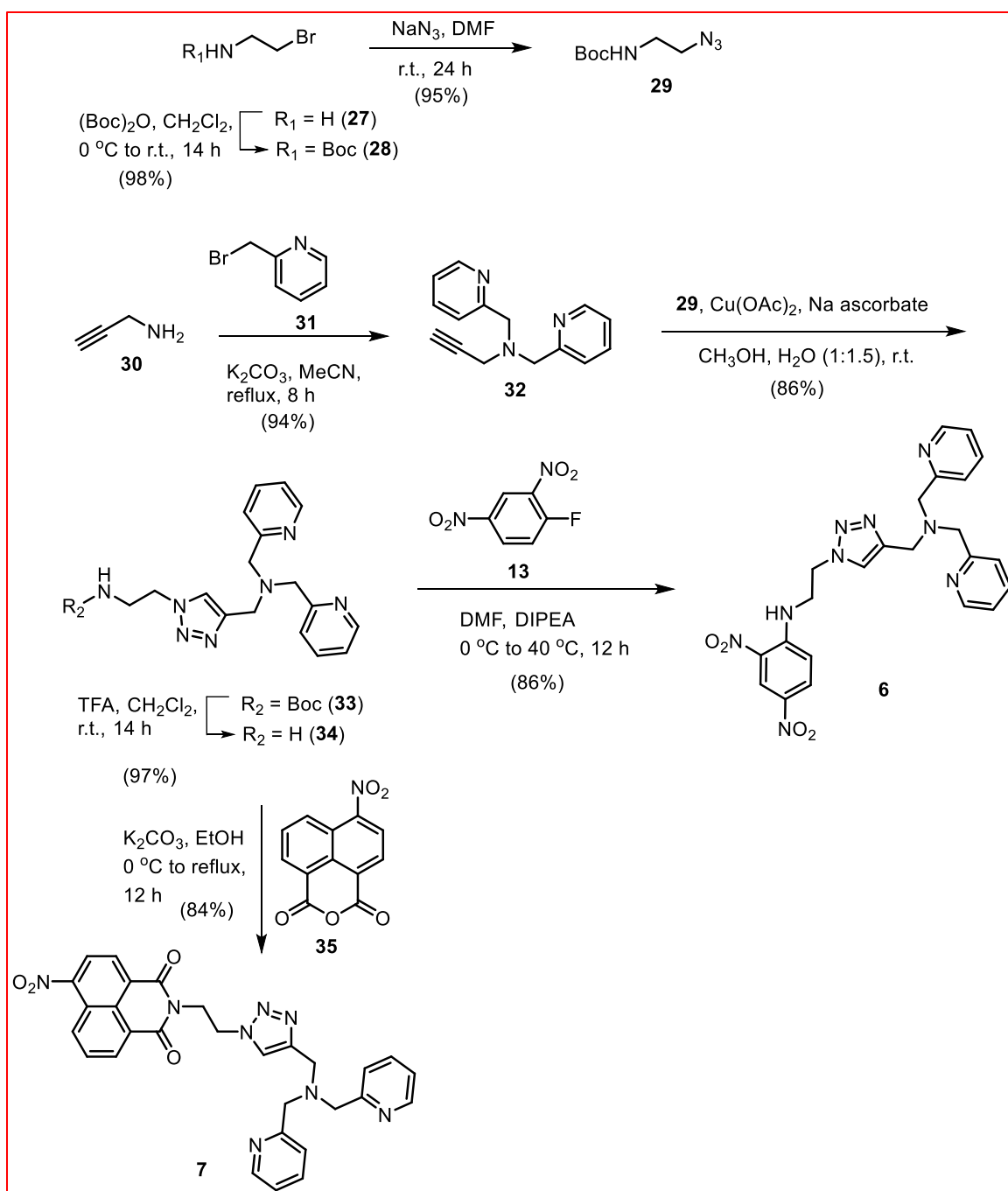
In sensor **4**, binding to  $\text{Zn}^{2+}$  increased the number of possible excited states with an average probability of  $f=0.2$ ; however, the probabilities were lower than the unbound state. Focusing on the excited state at  $\lambda_{\text{max}}$  of 220.2 nm, this transition involved HOMO, HOMO-1, and HOMO-4 to LUMO+4, LUMO+6, and LUMO+2, respectively. The transition from HOMO  $\rightarrow$  LUMO+4 mainly localized on 1,8-naphthalimide with a small contribution from one 2-pyridyl group. HOMO-1  $\rightarrow$  LUMO+6 transition in which the entire molecule is involved. The transition HOMO-4  $\rightarrow$  LUMO+6 was localized on DPA. A similar observation was applied in sensor **4**; upon  $\text{Zn}^{2+}$  binding, higher HOMOs and LUMOs contributed to the electronic transitions. Also, DPA contributed to the electronic transition, which was very limited in the unbound state.

In sensor **5**, the  $\text{Zn}^{2+}$ -bound state exhibited more excited states with significant probabilities as compared sensors **3** and **4**, which was mainly due to more  $\pi$ -electrons added to the

system via the 2-quinolyl compared as compared to the 2-pyridyl group. Focusing on the state with the highest probability ( $f=0.60$ ) at 212.9 nm, it involved the transitions HOMO  $\rightarrow$  LUMO+3/LUMO+5, HOMO-1  $\rightarrow$  LUMO+4, HOMO-2  $\rightarrow$  LUMO+4, and HOMO-3  $\rightarrow$  LUMO+3. The first transition was from PQA to 1,8-naphthalimide, and the remaining transitions were localized on the PQA. Inversely to the unbound state in which the LUMOs of the excited states were localized on DQA. Insertion of DQA with  $\text{Zn}^{2+}$  binding resulted in higher density of excited states with higher electronic coupling.

## 4.6 Synthesis of chemosensor 6 and 7

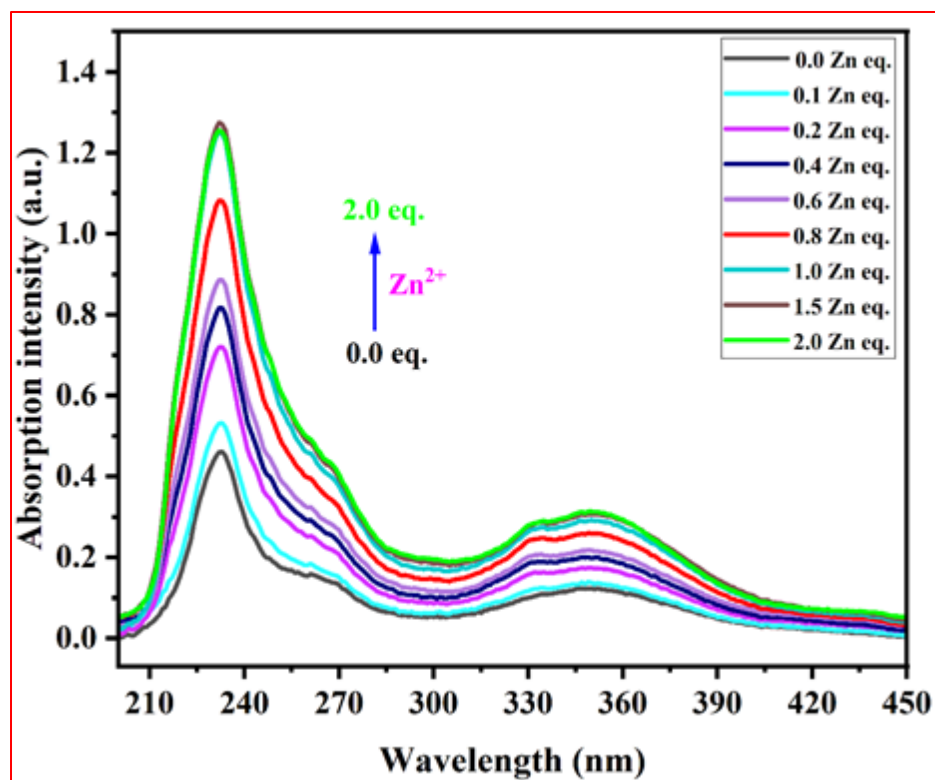
The synthesis of chemosensors **6** and **7** was accomplished as outlined in **Fig. 4.40**. The N-Boc protection of 2-bromoethylamine **27** afforded intermediate **28** [81], which was then reacted with NaN<sub>3</sub> in DMF to get azide **29** [81]. in 93% overall yield from **27**. Likewise, *N*-alkylation of propargylamine **30** with bromomethylpyridine **31** generated alkyne **32** [9]. Click reaction between alkyne **32** and azide **29**, using Cu(OAc)<sub>2</sub> and sodium ascorbate (reducing agent) then smoothly furnished the desired 1,2,3-triazole **33** in high yield (86%). Exposure of triazole **33** to trifluoroacetic acid in dichloromethane generated the key intermediates **34**, which was subsequently condensed with either fluoro-dinitrobenzene **13** or 1,8-naphthalic anhydride **35** to produce the desired chemosensors **6** and **7**, respectively (**Fig. 4.40**).



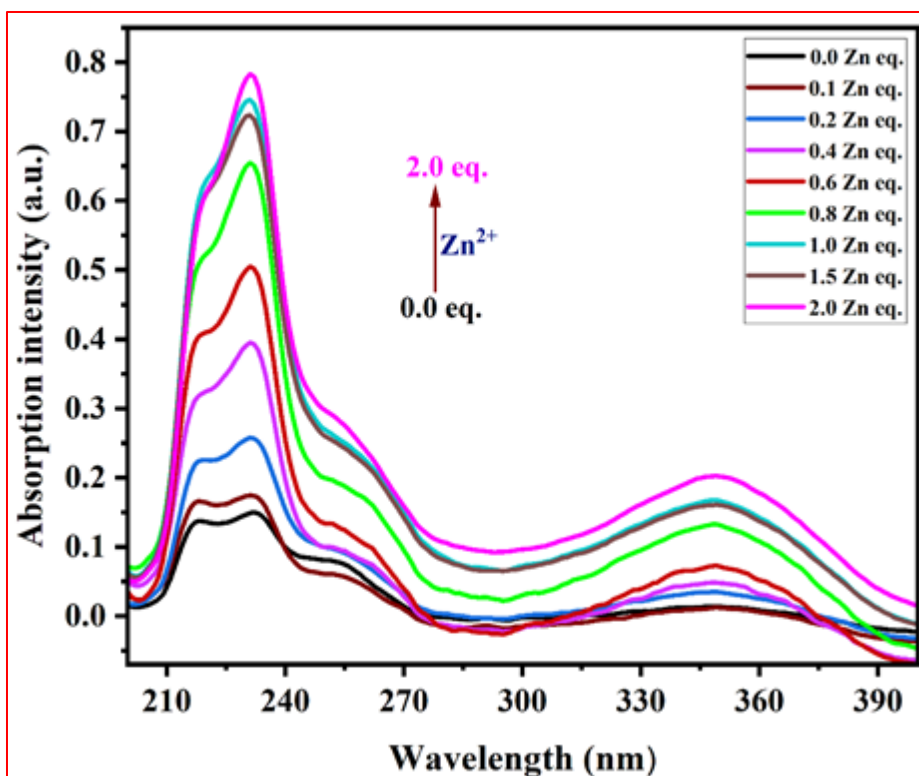
**Figure 4.40:** Synthesis of intermediates and chemosensors **6** and **7**

#### 4.7 Characterization of **6** and **7** as $\text{Zn}^{2+}$ sensors

Titration experiments were performed to measure the responses of sensors **6** and **7** to the varying concentrations of  $\text{Zn}^{2+}$ , using UV and fluorescence spectroscopy. Prior to the titration experiments, absorption spectra of neat **6** and **7** were recorded in  $\text{CH}_3\text{CN}$ . The absorption spectrum of **6** displayed a medium but sharp absorbance at 234 nm, a weak broader peak at 272 nm and a broad peak at 350 nm (**Fig. 4.41**). Likewise, sensor **7** exhibited a medium sharp peak at 231 nm, weak peaks at 219 nm and 255 nm and a broad peak at 349 nm (**Fig. 4.42**). Nevertheless, the absorptions maxima kept increasing with progressive addition of  $\text{Zn}^{2+}$  solution (0.0–2.0 equiv.; in 50  $\mu\text{M}$  in HEPES buffer, pH = 7.4) to the sensors **6** and **7** (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ) until 1.0 equiv. of  $\text{Zn}^{2+}$  was added. Thereafter, further addition of  $\text{Zn}^{2+}$  did not cause any significant increase in the absorptions maxima. These observations hinted that the receptors of sensors **6** and **7** must have coordinated with  $\text{Zn}^{2+}$ , producing new species in the solutions that altered electron rearrangement in sensors **6** and **7** (**Figs. 4.41 and 4.42**) [9].



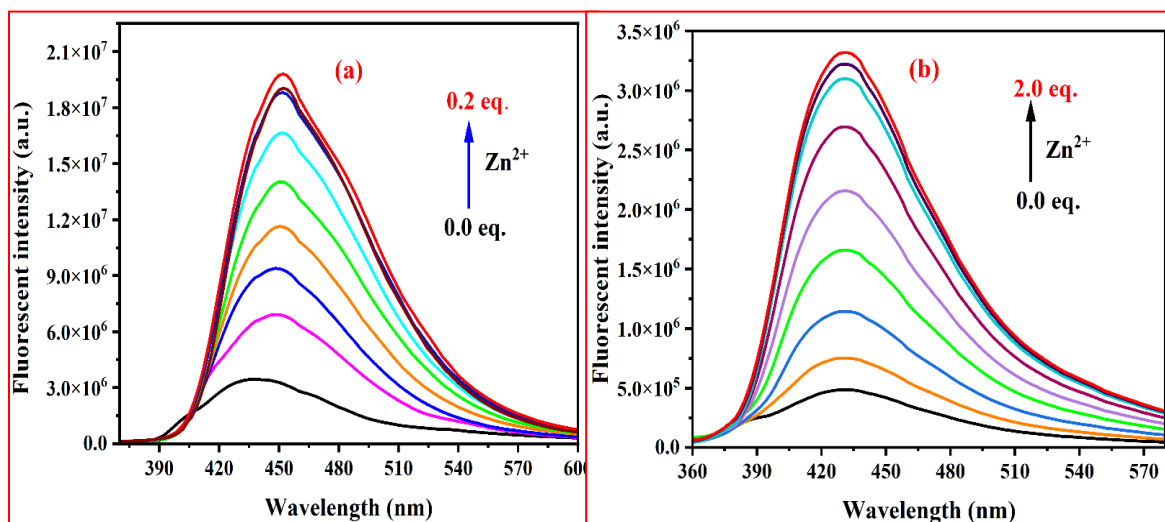
**Figure 4.41:** UV-vis absorption spectra of probe **6** (50 μM in CH<sub>3</sub>CN) with addition of Zn<sup>2+</sup> (0.0–2.0 equiv.) in CH<sub>3</sub>CN.



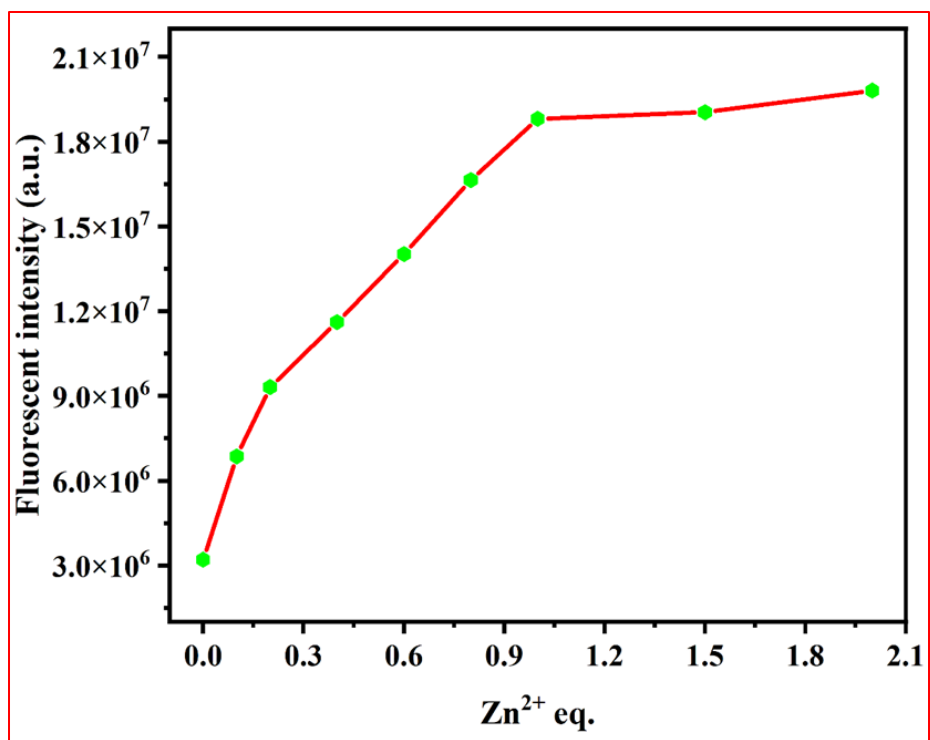
**Figure 4.42:** UV-vis absorption spectra of probe **7** (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ) with addition of  $\text{Zn}^{2+}$  (0.0–2.0 equiv.) in  $\text{CH}_3\text{CN}$ .

In order to study fluorescence responses, the fluorescence emission spectra of neat sensors **6** and **7** were recorded. Upon excitation at 350 nm ( $\lambda_{\text{ex}}$ ), neat sensors **6** and **7** displayed ( $\lambda_{\text{em}}$ ) at 438 nm and 430 nm, respectively. The fluorescence properties of sensors **6** and **7** were then studied by titrating  $\text{Zn}^{2+}$  (0.0–2.0 equiv.) in aqueous solution (50  $\mu\text{M}$  in HEPES buffer, pH = 7.4). **Figure 4.43a**, shows the intensity of emission band at 438 nm of sensor **6** kept increasing with progressive increase in  $\text{Zn}^{2+}$  concentration until the addition of 1.0 equiv. of  $\text{Zn}^{2+}$  when a 4.7-fold enhancement in the fluorescence intensity was observed. Likewise, the gradual addition of  $\text{Zn}^{2+}$  to the solution of sensor **7** also resulted in more than 6-folds enhancement in the intensity of emission band 430 nm, until 1.0 equiv. of  $\text{Zn}^{2+}$  was added (**Fig. 4.43b**). These observations hinted that **6** and **7** function as “turn-on”

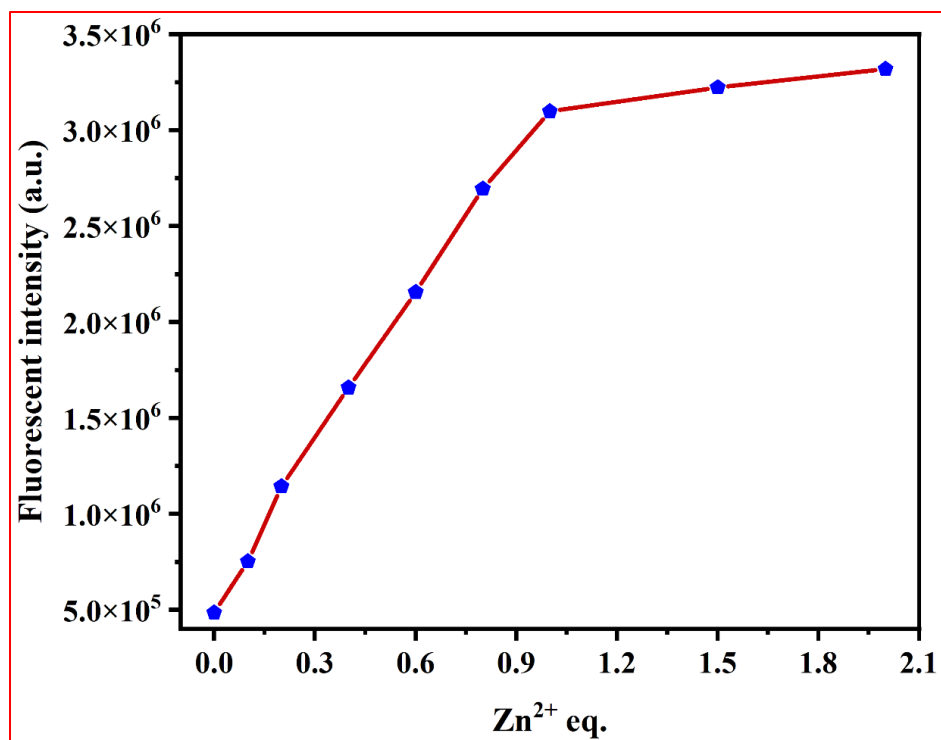
fluorescent sensors for  $\text{Zn}^{2+}$ . In the absence of  $\text{Zn}^{2+}$ , photoinduced electron transfer (PET) from the electron donor to the excited fluorophore results in fluorescence quenching. However, addition of  $\text{Zn}^{2+}$  with gradual increase in its concentration produced sensor/ $\text{Zn}^{2+}$  complex, which halted PET process from happening [9]. After saturation point (1.0 equiv. of  $\text{Zn}^{2+}$ ), further addition of  $\text{Zn}^{2+}$  did not bring significant spike in the intensity of emission peak of sensors **6** and **7** (Figs. 4.44 and 4.45). At saturation point, it is likely that almost all the sensor molecules have formed complex with  $\text{Zn}^{2+}$ .



**Figure 4.43:** The emission spectra of sensors **6** and **7** (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ): a) **6** ( $\lambda_{\text{ex}} = 350 \text{ nm}$ ), and b) **7** ( $\lambda_{\text{ex}} = 350 \text{ nm}$ ), upon addition of 0.0–2.0 equiv. of  $\text{Zn}^{2+}$  (50  $\mu\text{M}$  in HEPES buffer, pH = 7.4).

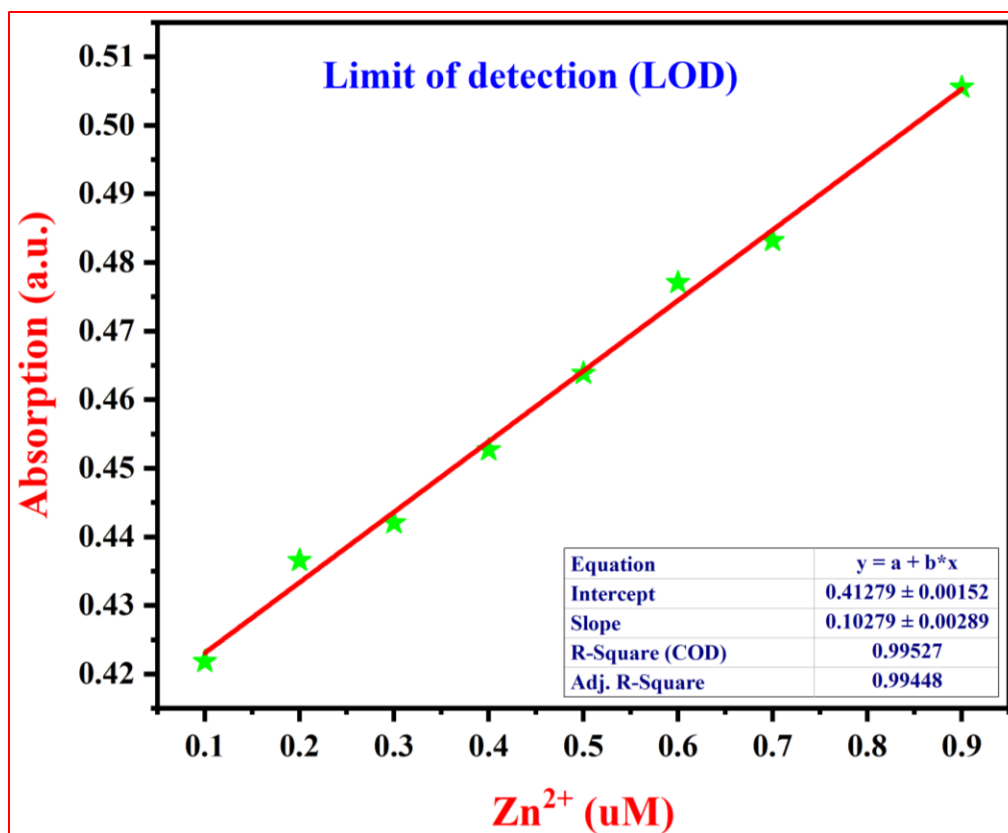


**Figure 4.44:** Change in emission intensity of probe **6** (50  $\mu$ M in CH<sub>3</sub>CN) with increasing Zn<sup>2+</sup> concentration (0.0–2.0 equiv.) at 440 nm.

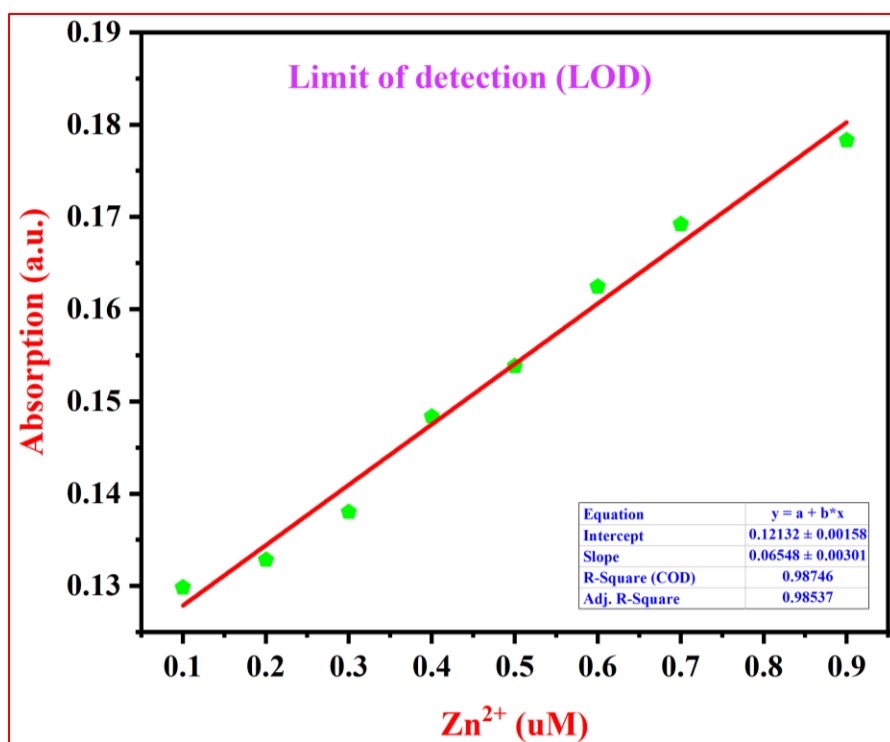


**Figure 4.45:** Change in fluorescent intensity of probe **7** (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ) with increasing  $\text{Zn}^{2+}$  concentration (0.0–2.0 equiv.) at 428 nm.

Moreover, by applying IUPAC method [80], limit of detection (LOD) and limit of quantification (LOQ) for sensor **6** were found to be 65.6 nM and 0.199  $\mu\text{M}$ , respectively. Likewise, LOD and LOQ for sensor **7** were turned out to be 107 nM and 0.325  $\mu\text{M}$ , respectively (**Figs. 4.46 & 4.47**).



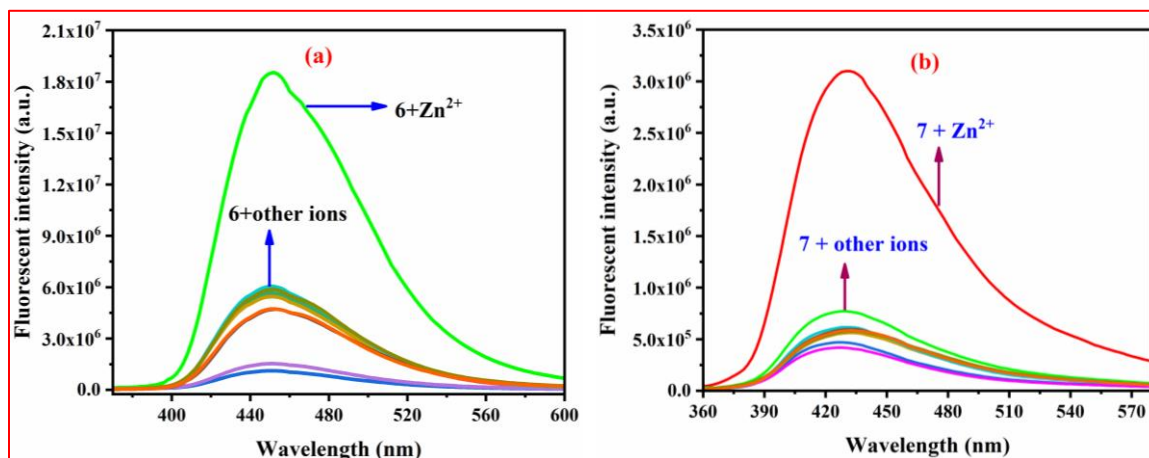
**Figure 4.46:** Standard calibration curve of probe 6 for the detection of  $\text{Zn}^{2+}$ .



**Figure 4.47:** Standard calibration curve of probe 7 for the detection of  $\text{Zn}^{2+}$ .

The fluorescence behavior of sensors **6** and **7** in the presence of other metal ions ( $\text{Cu}^{2+}$ ,  $\text{Cr}^{2+}$ ,  $\text{K}^{1+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$  and  $\text{Cd}^{2+}$ ) in HEPES buffer at pH 7.4 was also studied (**Fig. 4.48**). Significant fluorescence enhancements were observed when  $\text{Zn}^{2+}$  was added to solution of sensors **6** and **7**. Nevertheless, the addition of other metal ions under identical conditions led to either no fluorescence enhancements or minor/insignificant fluorescence spectral changes. Even common interfering metal ion such as  $\text{Cd}^{2+}$  did not interfere, albeit the addition of  $\text{Cu}^{2+}$  and  $\text{Co}^{2+}$  displayed quenching effect on the emission of sensor **7** (**Fig. 4.48**). It is well known that fluorescence quenching by the competing ions due to the electron energy transfer, redox activity or magnetic perturbations is a common problem associated with the fluorescence sensing of metal ions. Therefore, “turn-on”

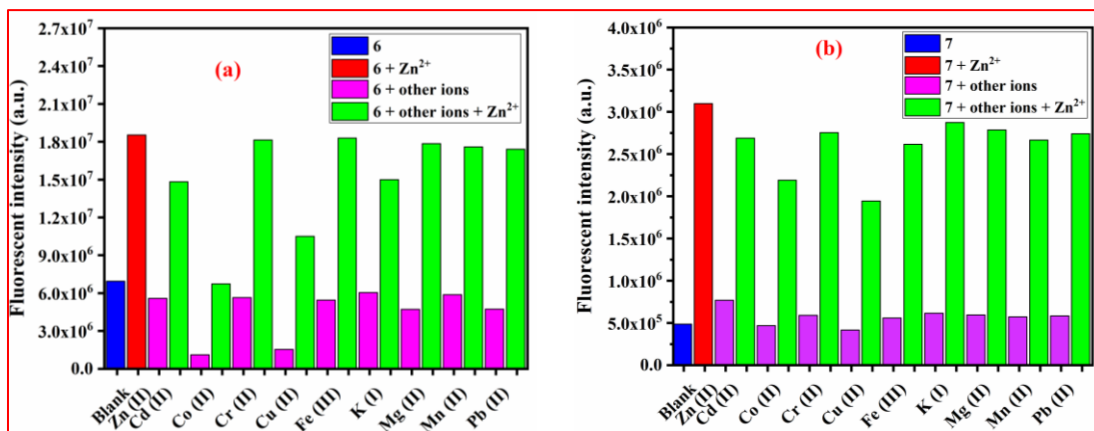
fluorescence sensing is believed to be more advantageous than compare to its “turn-off” counterpart [34].



**Figure 4.48:** Fluorescence response of sensors **6** and **7** (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ): a) **6** ( $\lambda_{\text{ex}} = 350 \text{ nm}$ ) and b) **7** ( $\lambda_{\text{ex}} = 350 \text{ nm}$ ) upon addition of various individual metal ions (50  $\mu\text{M}$ ) and  $\text{Zn}^{2+}$  (50  $\mu\text{M}$ ) in HEPES buffer, pH = 7.4.

Competitive fluorescence experiments were also performed in order to ascertain the selectivity of sensors **6** and **7** for  $\text{Zn}^{2+}$  over other coexisting competitive metal ions. Initially, the fluorescence responses of solutions of mixture of sensors **6** and **7** (50  $\mu\text{M}$ ) with various metal cations (1.0 equiv. 50  $\mu\text{M}$ ) was recorded. The corresponding fluorescence responses obtained are illustrated in pink bars in **Figure 4.49**. Thereafter, solution of  $\text{Zn}^{2+}$  (1.0 equiv. 50  $\mu\text{M}$ ) was added to the solutions of mixture of sensors **6** and **7** with various metal cations. The addition of  $\text{Zn}^{2+}$  resulted in the enhancement of emission intensities, in a similar fashion to that when  $\text{Zn}^{2+}$  was added alone to the solution of neat sensors **6** and **7** (**Fig. 4.49**). These observations suggested that sensors **6** and **7** are highly

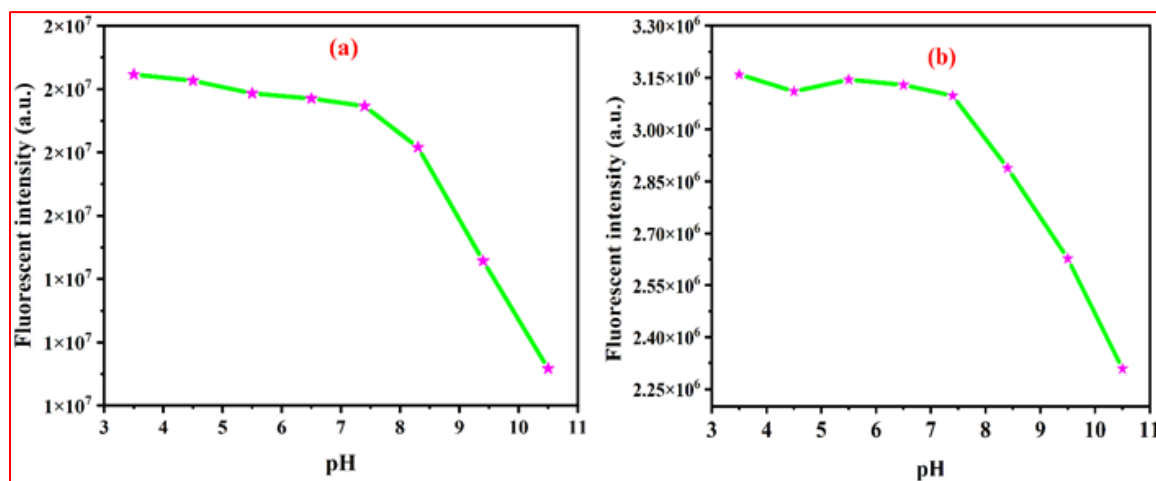
selective for the detection of  $\text{Zn}^{2+}$  and that other metal ions do not interfere with the binding affinity of these sensors with  $\text{Zn}^{2+}$ .



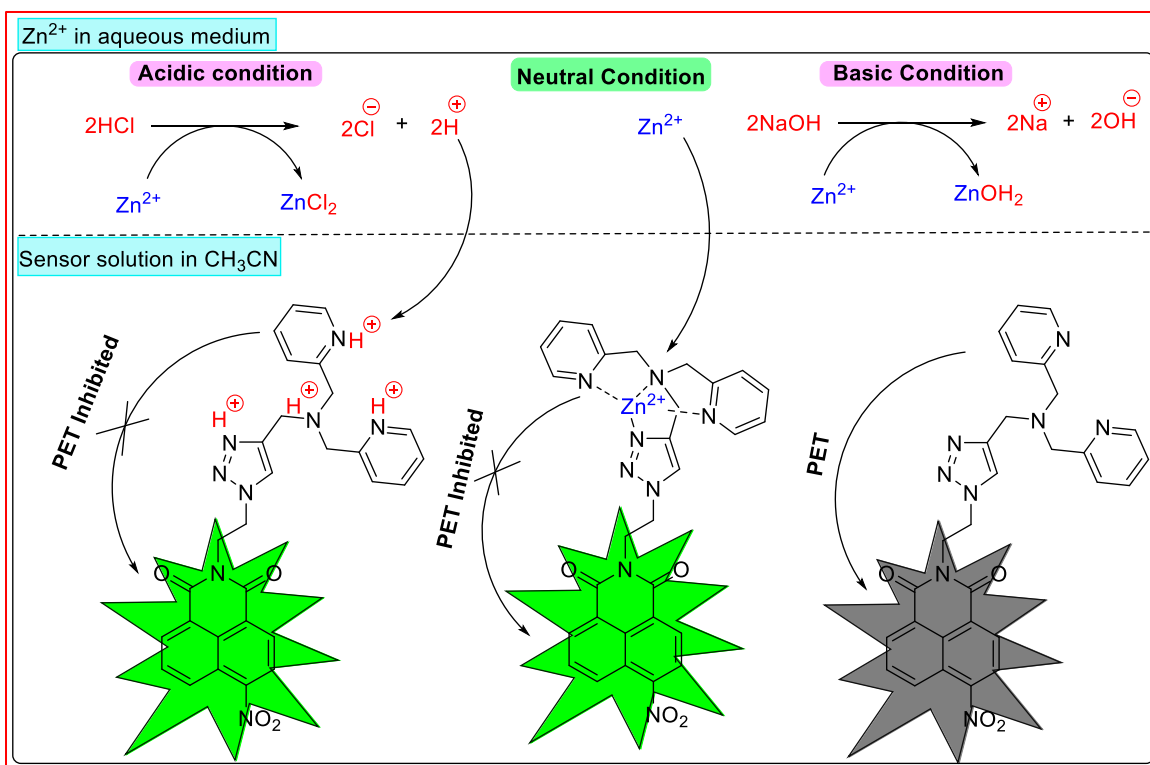
**Figure 4.49:** The fluorescence response of sensors **6** and **7** (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ): a) **6** ( $\lambda_{\text{ex}} = 350$  nm) and b) **7** ( $\lambda_{\text{ex}} = 350$  nm), upon addition of various individual metal ions (50  $\mu\text{M}$ ) and  $\text{Zn}^{2+}$  (50  $\mu\text{M}$ ) in HEPES buffer, pH = 7.4.

The fluorescence emission of sensors- $\text{Zn}^{2+}$  complexes was recorded in  $\text{CH}_3\text{CN}$ /HEPES (1:1 v/v) under varying pH, ranging from 3.5 to 10.0, in order to ascertain the effect of pH on the sensing performance of these sensors (**Fig. 4.50 & 4.51**). In the basic pH medium, the fluorescent intensity got substantially diminished, which suggested that the conceivable production of  $\text{Zn}^{2+}$  hydroxide led to the resumption of PET process from the electron donor (DPA) to the excited fluorophore of sensors [9]. Similarly in the acid medium ( $\text{pH} < 5$ ), protonation of DPA would stop PET process, and hence the recovery of fluorescence of fluorophore, that in turn results in the relatively reasonable enhancement in the fluorescence intensity. Nevertheless, within the pH range of 6.5 to 8.0, a relatively stable

enhanced fluorescence intensity for the sensors- $\text{Zn}^{2+}$  complexes were observed which hinted formation of sensor/ $\text{Zn}^{2+}$  complex [1] (**Fig. 4.50**).

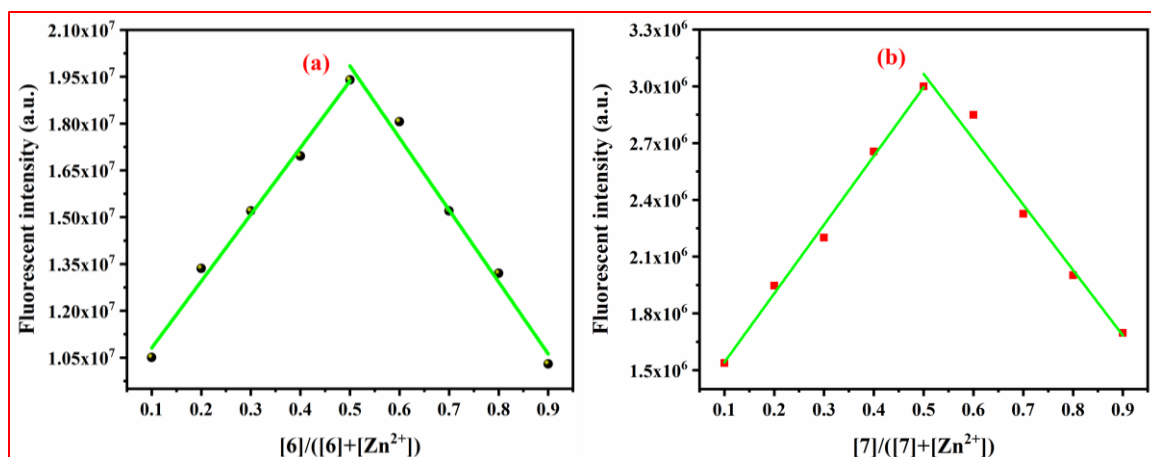


**Figure 4.50:** Effect of pH on fluorescence response of (a) 6/ $\text{Zn}^{2+}$  at 438 nm; (b) 7/ $\text{Zn}^{2+}$  at 430 nm complex in  $\text{CH}_3\text{CN}$ /HEPES buffer (50 : 50) solution at various pH.



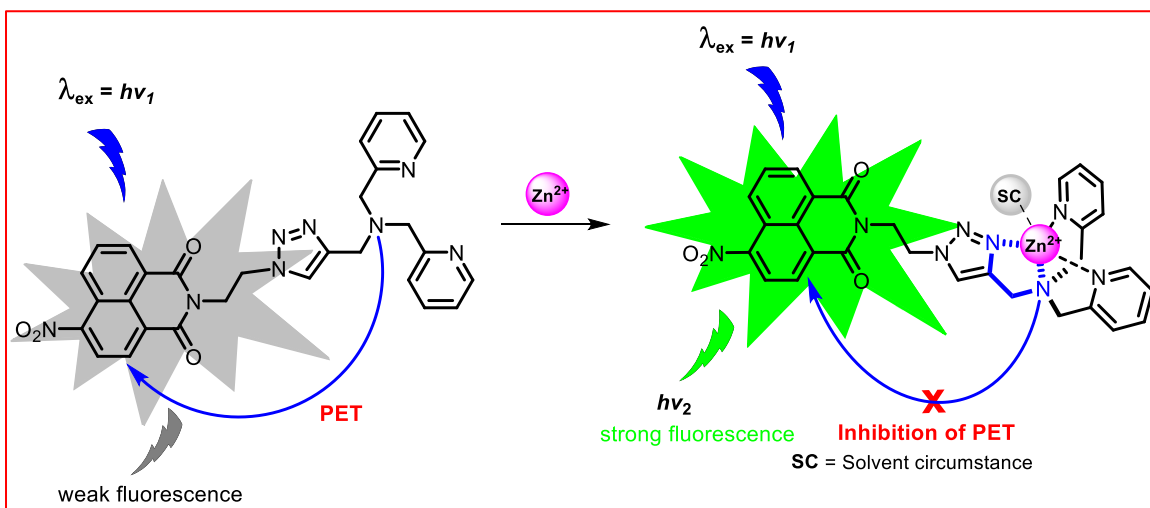
**Figure 4.51:** Mechanism for the responses of the probes towards Zn<sup>2+</sup> in different pH conditions.

The binding stoichiometry of sensors **6** and **7** was deduced from fluorescence titration test in CH<sub>3</sub>CN/HEPES buffer (50:50, v/v) solution. The Job's plot was generated by recording emission spectra of sensors **6** and **7** by continuously varying the addition of molar fraction of Zn<sup>2+</sup> while keeping the total concentration at 50 μM concentrations (**Fig. 4.52**). The Job's plot analysis for both sensors **6** and **7** revealed the maximum intensity of emissions a maximum at 0.5 M fraction, hinting the formation of 1:1 complex between the sensors and Zn<sup>2+</sup>.



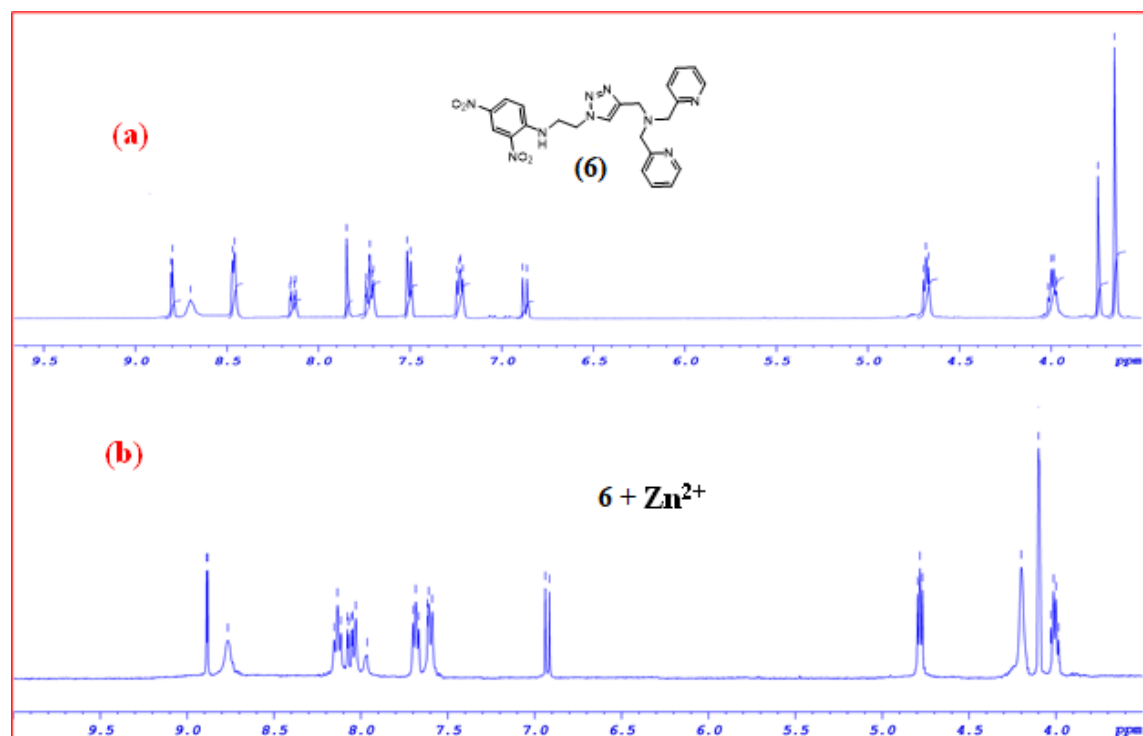
**Figure 4.52:** Job's plot of (a) **6** (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ), ( $\lambda_{\text{ex}} = 350 \text{ nm}$ ,  $\lambda_{\text{em}} = 438 \text{ nm}$ ) and (b) **7** (50  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ) ( $\lambda_{\text{ex}} = 350 \text{ nm}$ ,  $\lambda_{\text{em}} = 430 \text{ nm}$ ), keeping total concentration of sensor and  $\text{Zn}^{2+}$  (50  $\mu\text{M}$  in HEPES buffer, pH = 7.4) at 50  $\mu\text{M}$ .

The PET mechanism of sensors with  $\text{Zn}^{2+}$  illustrated in **Figure 4.53**. In the absence of  $\text{Zn}^{2+}$ , the higher energy of HOMO of the unbound receptors (DPA) readily allow its electron transfer to the LUMO of the excited fluorophore, which leads to nonradiative deactivation of the excited state and hence quenching the fluorescence. Nevertheless, in case when DPA bound to  $\text{Zn}^{2+}$ , the energy of HOMO gets lower than that of the fluorophore, making electron transfer energetically unfavorable that leads to fluorescence “switch on” [9].

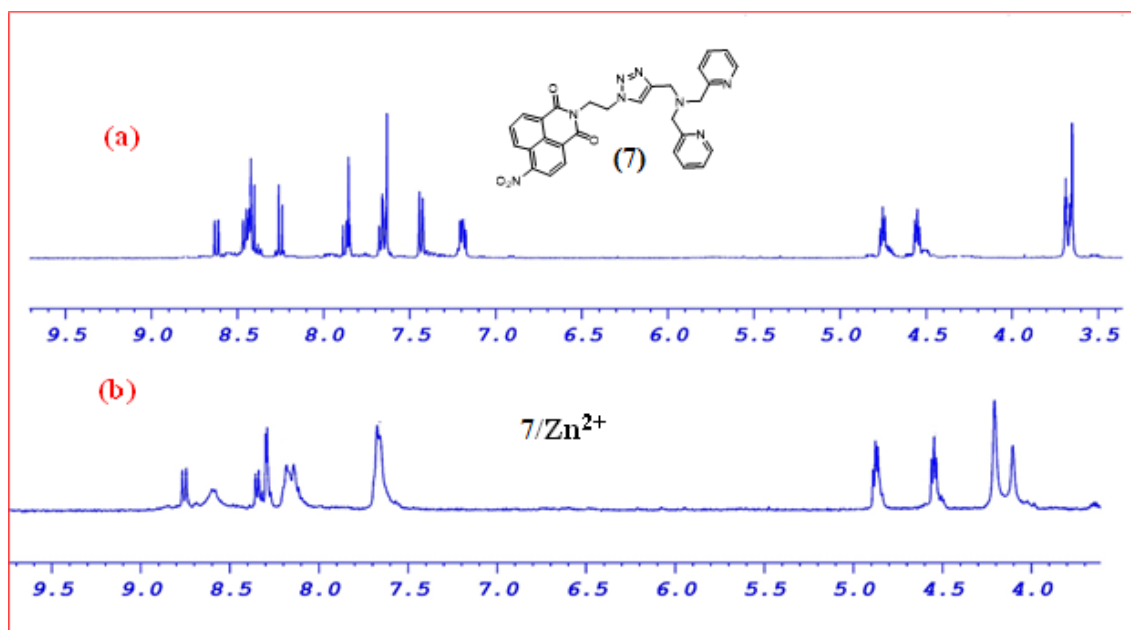


**Figure 4.53:** PET mechanism of sensors with  $\text{Zn}^{2+}$ .

The  $^1\text{H}$ -NMR titration experiments were also performed to ascertain binding behavior of sensors **6** and **7** with  $\text{Zn}^{2+}$ . The  $^1\text{H}$ -NMR spectra of neat sensors and sensors complexed with  $\text{Zn}^{2+}$  (sensor- $\text{Zn}^{2+}$ ) revealed distinct downfield shift of DPA's pyridine protons along with methylene protons attached to tertiary N-atom (**Figs. 4.53 & 4.55**). These observations hinted that N atoms of DPA and tertiary N atom were involved in  $\text{Zn}^{2+}$  coordination, resulting in downfield shifts of these signals owing to the deshielding effect of the metal ion.



**Figure 4.54:**  $^1\text{H}$  NMR (400 MHz, acetonitrile  $\text{d}_3$ ) of **a**) probe **6** and **b**) probe **6**/ $\text{Zn}^{2+}$  from 3.5 to 9.5 ppm.



**Figure 4.55:** <sup>1</sup>H NMR (400 MHz, acetonitrile d<sub>3</sub> of **a**) probe **7** and **b**) probe **7**/Zn<sup>2+</sup> from 3.5 to 9.5 ppm.

## 4.8 Computational studies

### 4.8.1 Zn<sup>2+</sup> Recognition and the Frontier Molecular Orbitals (FMO)

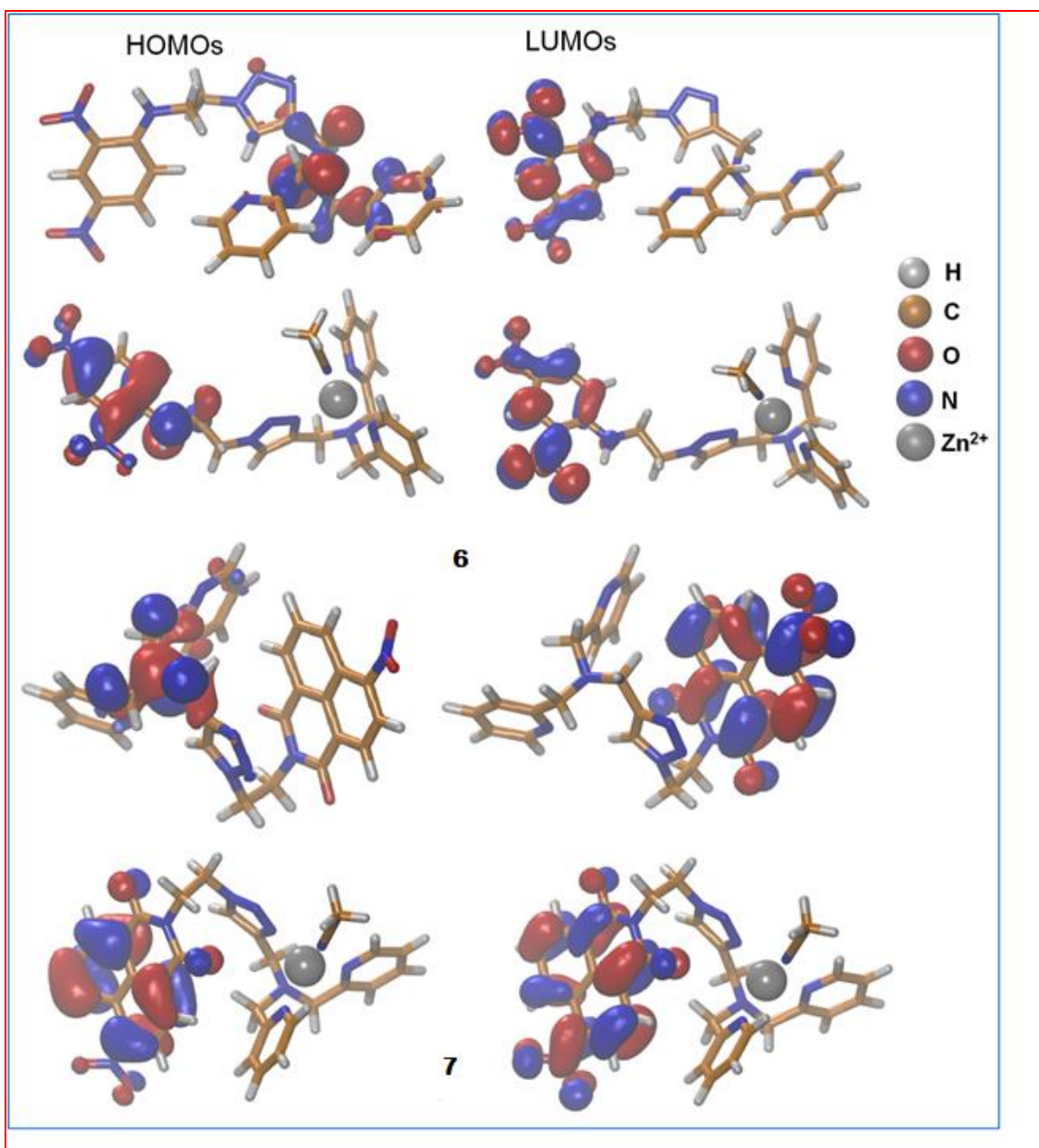
DFT simulations were performed to study the impact of Zn<sup>2+</sup> binding on the fluorescence of sensors **6** and **7**. To determine the most stable conformational structures of the sensors, the conformational space was explored with GFN2-xTB method and the iMTD-GC algorithm implemented in CREST code, as detailed in the methodology section. For further refinements of structures obtained by GFN2-xTB method, geometrical optimizations were performed with wB97XD/def2-TZVPP level of theory. Binding energies revealed that the

two sensors display favorable interactions energies (**Table 4.2**), indicating that the  $\text{Zn}^{2+}$ -sensor complexes are thermodynamically stable.

**Table 4.2:** Total  $\text{Zn}^{2+}$  binding energies ( $\Delta E_{\text{Total}}$ ) of the two sensors are reported in kcal/mol with the gas phase ( $\Delta E_{\text{gas}}$ ), and solvation contributions ( $\Delta E_{\text{Sol.}}$ ).

|          | $\Delta E_{\text{gas}}$ | $\Delta E_{\text{Sol.}}$ | $\Delta E_{\text{Total}}$ |
|----------|-------------------------|--------------------------|---------------------------|
| <b>6</b> | -424.2                  | 337.6                    | -86.5                     |
| <b>7</b> | -438.8                  | 349.5                    | -89.3                     |

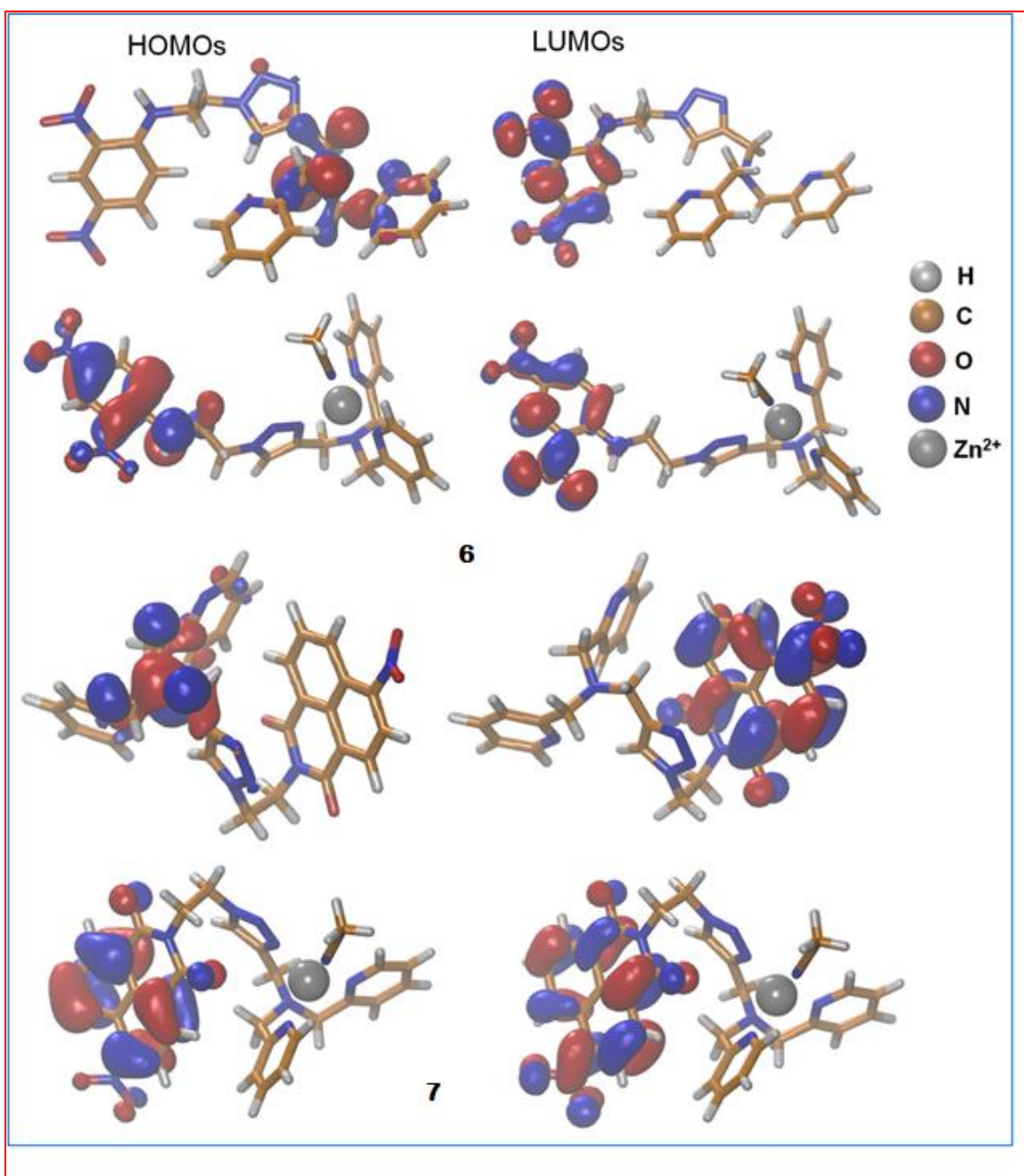
The energetic penalties owing to the high dehydration free energies of  $\text{Zn}^{2+}$  and the two sensors (in the unbound states) were surmounted easily by the strong enthalpic interaction energies (mainly electrostatic) between  $\text{Zn}^{2+}$  and the sensors. Concerning the geometry of the  $\text{Zn}^{2+}$  complexes, both sensors adopted trigonal bipyramidal geometry around  $\text{Zn}^{2+}$ , as expected owing to similar binding motif in both sensors. The average  $\text{Zn}^{2+}$ -N coordinating bond was found to be around 2.0 Å (**Fig. 4.56**).



**Figure 4.56:** The frontier molecular orbitals (HOMOs & LUMOs) of the two Zn<sup>2+</sup>-chemosensors complexes in the two conformations (stacked and extended). The isosurfaces of the FMO's electron density are shown at 0.03 e/Å<sup>3</sup>.

The bandgaps of sensors **6** and **7** in the unbound state was found to be 7.07 and 6.61 eV, respectively. Likewise, in the Zn<sup>2+</sup>-bound states, they were determined to be 7.46 and 7.29 eV, respectively. The presence of 1,8-naphthalimide function in sensor **7** resulted in 0.46

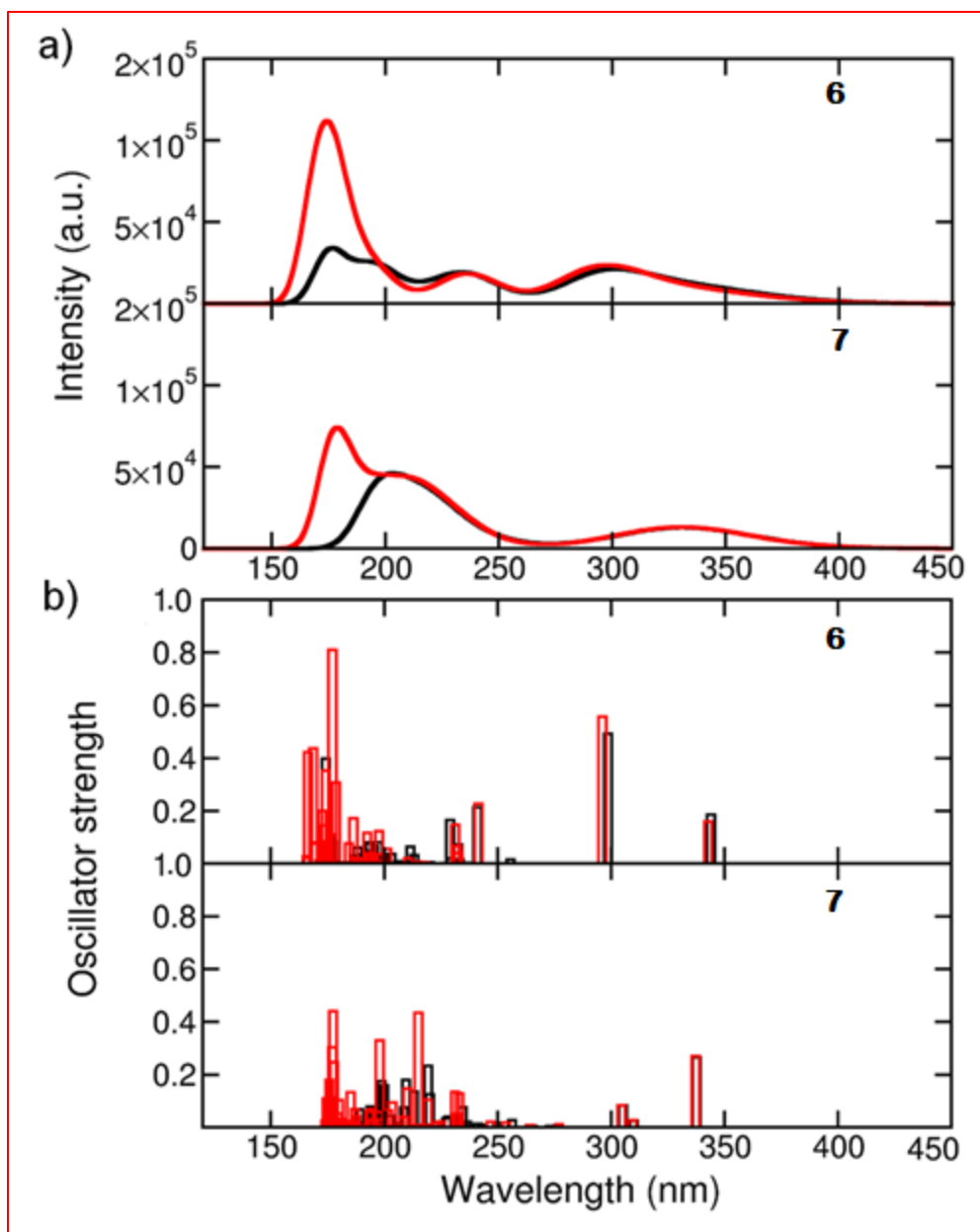
eV redshift in the electronic bandgap as compared to **6**, bearing dinitrobenzene function. Nonetheless, coordination of  $\text{Zn}^{2+}$  in the  $\text{Zn}^{2+}$ -bound states caused blue shift of 0.39 and 0.68 eV in the electronic bandgaps for **6** and **7**, respectively. The influence of the two fluorophores (dinitrobenzene and 1,8-naphthalimide) on the electronic structure of **6** and **7** was investigated frontier molecule orbitals (FMO). In the unbound states, the highest occupied molecular orbital (HOMO) electronic density was localized onto the DPA receptor whereas lowest unoccupied molecular orbitals (LUMOs) density was on the fluorophores. Upon coordination with  $\text{Zn}^{2+}$ , however, the HOMOs and LUMOs were found to be localized on the fluorophores of **6** and **7** (**Fig. 4.57**).



**Figure 4.57:** The frontier molecular orbitals (HOMOs & LUMOs) of the two Zn<sup>2+</sup>-chemosensors complexes in the two conformations (stacked and extended). The isosurfaces of the FMO's electron density are shown at 0.03 e/Å<sup>3</sup>.

#### 4.8.2 The Effect of $\text{Zn}^{2+}$ -Binding on the UV-Visible Spectra

In order to study the effect of  $\text{Zn}^{2+}$ -binding on the UV-visible spectra of **6** and **7**, time-dependent DFT (TD-DFT) simulations were performed on the unbound and  $\text{Zn}^{2+}$ -bound states of the two sensors. Fifty excited states in the TD-DFT simulations were calculated. As evident from **Figure 4.58**, both sensors displayed a broadband absorption spectrum in the range of 150-400 nm (**Fig. 4.58a**).



**Figure 4.58:** The UV-visible absorbance spectra of **6** and **7** a) in the unbound- (black) and  $\text{Zn}^{2+}$ -bound states (red), b) The corresponding oscillator strength factors of the different excited states of **6** and **7**

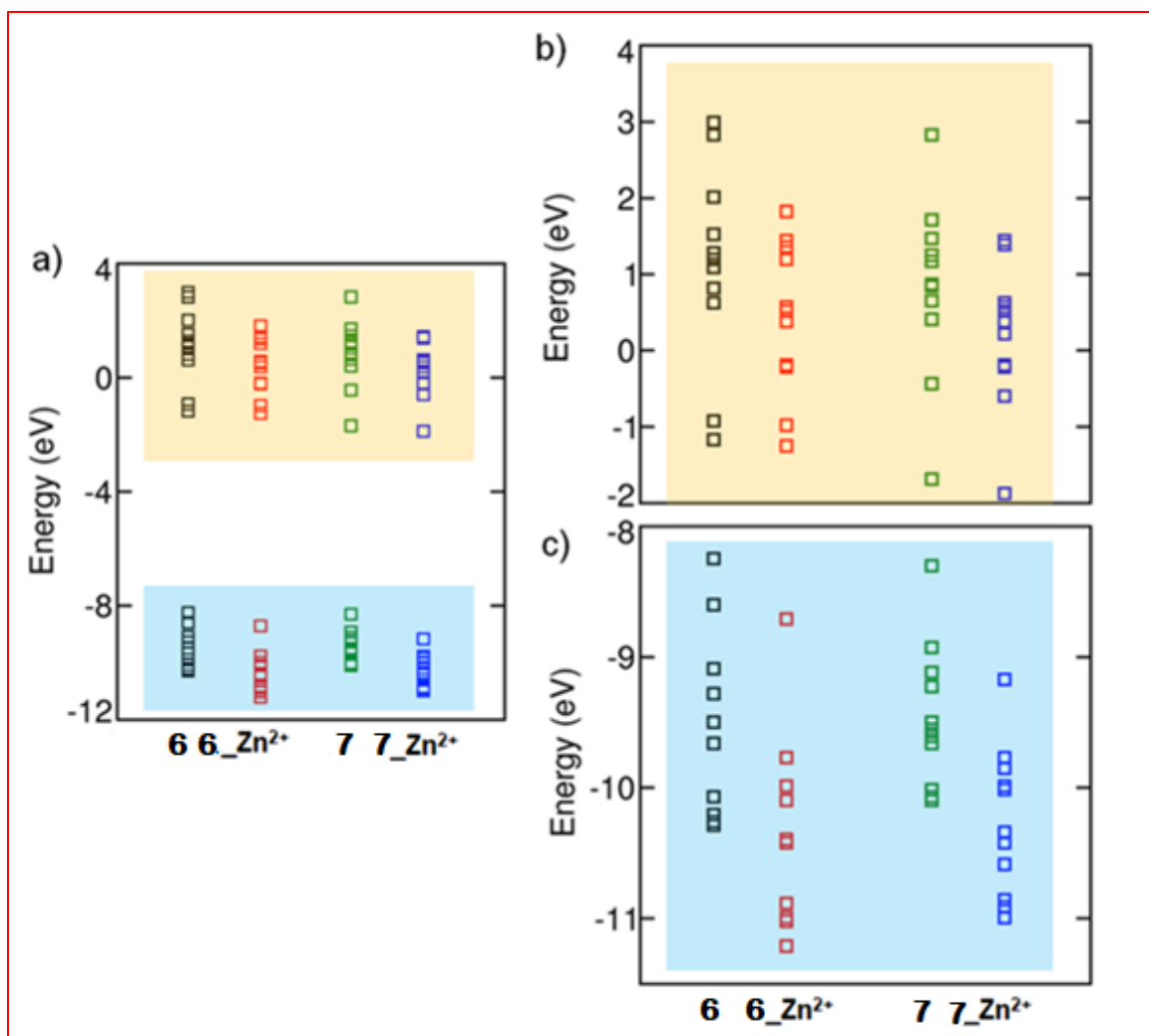
The oscillator strength ( $f$ ) is a dimensionless factor that expresses the probability of the light absorbance upon electronic transitions. In the unbound states, **6** showed important absorbance probabilities with  $\lambda_{\text{max}}$  at 174.3 (0.40), 176.6 (0.10), 229.1 (0.17), 241.1 (0.21),

298.6 (0.49), and 344.0 (0.19) nm (oscillator strength of each peak is shown in parenthesis, **Fig. 4.58a**). These transitions originated from the intra-charge transfer of  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  types. Likewise, **7** displayed a redshift as compared to **6**, bearing peaks at 198.8 (0.17), 200.0 (0.16), 209.6 (0.18) 213(0.14), 219.4 (0.23), 220.1 (0.13), and 337.7 (0.27) nm (**Fig. 4.58b**). The addition of the dinitrobenzene function has a significant effect on the molecular absorption spectra of **6**; it induces a blue shift as compared to 1,8-naphthalimide fluorophore of **5**, which significantly increases the electronic coupling between the ground and excited states, and hence enhancing the transition probability (**Fig. 4.58a**). The presence of dinitrobenzene fluorophore in **6** further stabilizes the molecular orbitals, especially the HOMOs, which in turn enhances the electronic coupling (**Fig. 4.59**). While Focusing on the highest probability peaks at 298.6 (0.49) and 174.3 (0.40), the first peak involves the transition from the HOMO-1 to LUMO+1. The electron densities of these two orbitals were localized on the dinitrobenzene fluorophore. Likewise peak at 174.3 (0.40) involves excitation from deep valence orbitals; HOMO-9  $\rightarrow$  LUMO+3, HOMO-8  $\rightarrow$  LUMO+2, and HOMO-2  $\rightarrow$  LUMO+7. The electron densities of these orbitals were localized on the triazole and DPA functions, which are spatially close; therefore, the electronic coupling between these orbitals was maximized. Likewise, the electronic transitions in **7** with the highest probabilities were located at 219.4 (0.23) and 337.7 (0.27) nm. The absorption peak at 337.7 nm involves significant excitation from HOMO-1 to LUMO. Moreover, the electron density of both orbitals was localized on the 1,8-naphthalimide moiety, which is a similar pattern of transition as in **6** (i.e., the orbitals involved in the electronic transition are localized on the fluorophore). Similarly, the second important absorption peak at 219.4 nm involves excitation from deep valence orbitals:

HOMO-6 $\rightarrow$  LUMO, and HOMO-1 $\rightarrow$  LUMO+2 excitations. The electron density of these orbitals was localized on the 1,8-naphthalimide function, except that of HOMO-6 was on the DPA group. Nevertheless, the Zn<sup>2+</sup>-binding led to absorption enhancement in both sensors, although more significantly in case of **6**. The number of absorption peaks was increased, and the transition probabilities were also significantly enhanced. The blue shift observed in the sensors upon Zn<sup>2+</sup> binding is more manifested in **7** spectrum than in the case of probe **6**. The absorption peaks of **6** were found to be 342.8 (0.16), 296.1 (0.56), 241.6 (0.23), 231.3 (0.15), 197.7 (0.12), 192.2 (0.12), 186.4 (0.17), 178.8 (0.31), 177.1 (0.81), 176.9 (0.11), 174.3 (0.35), 173.3 (0.15), 172.6 (0.20), 168.7 (0.44), and 166.5 (0.42). The major electronic transition at 296.1 nm was from HOMO to LUMO+1; the electron density of the HOMO was localized on the dinitrobenzene whereas LUMO+1 was on the DPA function. Similarly, absorption at 177.1 (0.81) nm involves multiple electronic transitions from deep valence orbitals, i.e., HOMO-7 $\rightarrow$  LUMO+3, HOMO-8 $\rightarrow$  LUMO+2, HOMO-2 $\rightarrow$  LUMO+5, and HOMO-10 $\rightarrow$  LUMO+4. The electron density of these orbitals was localized on the triazole and DPA groups, which enhanced their electronic coupling and maximizing transition probabilities. On the other hand, absorption peaks of **7** exhibited several peaks as well: 337.3 (0.27), 233.0 (0.13), 231.2 (0.13), 219.2 (0.11), 215.1 (0.43), 210.1 (0.15), 200.0 (0.33), 185.2 (0.13), 177.9 (0.25), 177.5 (0.44), 177.1 (0.30), 176.8 (0.11), (175.9) 0.18, and 174.6 (0.11) nm. The absorption peaks with the highest probabilities were found at 215.1 (0.43) and 177.5 (0.44). The electronic transitions at 215.1 nm were emerging mainly from the DPA to 1,8-naphthalimide function (HOMO-3 $\rightarrow$  LUMO, HOMO-1 $\rightarrow$  LUMO+1, and HOMO $\rightarrow$  LUMO+4). Likewise, absorption at 177.5 nm was due to multiple electronic transitions; HOMO-19 $\rightarrow$  LUMO,

HOMO→LUMO+4 which have electron density localized on 1,8-naphthalimide function. It also involves other transitions, e.g. HOMO-3→ LUMO+4, HOMO-7→ LUMO+5, and HOMO-7→ LUMO+6. The electron density of these orbitals were expanded over the entire sensor.

Altogether, DFT simulations are carried out on two newly synthesized sensors (**6** and **7**) to examine the impact of  $\text{Zn}^{2+}$  binding on their absorption spectrum. Both sensors display perfect binding with  $\text{Zn}^{2+}$  and show high thermodynamic stabilities. The binding of  $\text{Zn}^{2+}$  induced further stability of the frontier molecular orbitals, especially HOMOs. The electronic coupling between the HOMOs and LUMOs is enhanced, which in turn populates more excited states with a high probability of electronic transitions. Further, upon  $\text{Zn}^{2+}$  binding, deeper HOMOs and higher LUMOs are involved in the electronic transitions than unbound states. A similar effect is observed for the dinitrobenzene fluorophore with less extent than  $\text{Zn}^{2+}$  binding. The result of  $\text{Zn}^{2+}$  binding on the absorbance spectrum of **6** is more significant than on **7** spectrum. The  $\text{Zn}^{2+}$  orbitals do not contribute to the electronic transition and are localized deep in the energy levels; the energy separation between the HOMO and the first  $\text{Zn}^{2+}$  d-orbital is 7.46 and 6.94 eV in sensors **6** and **7** respectively. The insertion of dinitrobenzene enhances the luminescence of sensor **6** compared to **7**. It displays a similar effect as  $\text{Zn}^{2+}$  ions; therefore, upon  $\text{Zn}^{2+}$  binding, the intensity of luminescence of sensor **6** is significantly enhanced.



**Figure 4.59:** The frontier molecular orbitals of the two sensors (6 & 7) in the unbound and  $\text{Zn}^{2+}$ -bound states (a); b) Zoom-in of the orbitals from LUMO to LUMO+10, and c) zoom in from HOMO to HOMO-10.

## CHAPTER 5

### 5.1 Conclusion

In conclusion, we have designed and synthesized seven new turn-on fluorescence sensors for the detection of  $\text{Zn}^{2+}$  in aqueous solutions. The addition of 1 equiv.  $\text{Zn}^{2+}$  to the solutions of sensors led to enhancement in fluorescence intensities. Moreover, all these sensors showed high selectivities for  $\text{Zn}^{2+}$  in the presence of similar competing ions. In the case of sensors **1** and **2**, a 3-fold and 2.4-fold enhancement in fluorescence intensity, respectively, was observed. The LOD was found to be 124 nM and 1.06  $\mu\text{M}$ , respectively. Likewise, fluorescent sensors (**3-5**) displayed up to 14-fold enhancement in the fluorescence intensity when mixed with  $\text{Zn}^{2+}$ . These sensors also showed high selectivity for  $\text{Zn}^{2+}$  over other competing cations, with LODs of 0.12  $\mu\text{M}$ , 81.7 nM and 79.2 nM, respectively. DFT calculation performed on the stacked conformations of unbound and  $\text{Zn}^{2+}$  bounded sensors (**3-5**) revealed that the latter exhibited a better binding preference for  $\text{Zn}^{2+}$ . Moreover, it displayed a higher density of excited states than the unbound sensors. The  $\text{Zn}^{2+}$  binding leads to FMO stabilizations that result in enhancing the electronic coupling between the HOMOs and LUMOs, which makes excited states more populated. These excited states then undergo geometric relaxation before emitting light and relaxing back to the ground states. In addition, the  $\text{Zn}^{2+}$  orbitals are localized deep in the energy levels; the energy separation between the HOMO and the first  $\text{Zn}^{2+}$  d-orbital in sensors **3-5** was determined to be 5.0 eV, 6.6 eV, and 6.9 eV, respectively. It was observed that the closer the  $\text{Zn}^{2+}$  orbitals to the FMO, the more significant was its impact on the spectra. Indeed, in sensor

5, the density of the generated states and the intensity upon  $\text{Zn}^{2+}$  binding was higher than compared to sensors **3** and **4**, without almost notable changes in the bandgaps. Further, the insertion of DQA contributes significantly to the spectra upon binding with  $\text{Zn}^{2+}$ .

Finally, we synthesized two more fluorescent sensors **6** and **7** for highly sensitive and selective detection of  $\text{Zn}^{2+}$  in an aqueous solution. The synthesis of these sensors has been achieved from commercially available starting materials in straightforward high yielding reactions sequence. In the presence of  $\text{Zn}^{2+}$ , synergistic coordination of N-3 of 1,2,3-triazolyl function and DPA moiety with  $\text{Zn}^{2+}$  leads to inhibition of PET process, resulting in “turn on” fluorescence response by the sensors, which leads to 4.7-folds and 6-folds enhancements in the intensities of emission bands of **6** and **7**, respectively. These sensors displayed high sensitivity and selectivity for  $\text{Zn}^{2+}$  over other coexisting competing cations in a physiological pH (7.4), with LODs of 65.6 nM and 107 nM, respectively. The impact of  $\text{Zn}^{2+}$  binding on the absorption spectra of **6** and **7** was studied by DFT calculations. Both sensors demonstrated excellent binding with  $\text{Zn}^{2+}$  with high thermodynamic stabilities. The  $\text{Zn}^{2+}$  binding enhanced electronic coupling between the HOMOs and LUMOs, which in turn populated more excited states with a higher probability of electronic transitions. The insertion of dinitrobenzene in **6** exhibited higher fluorescence enhancements as compared to **7** upon binding to  $\text{Zn}^{2+}$ . The  $\text{Zn}^{2+}$  orbitals do not contribute to the electronic transition but rather localized deep in the energy levels. The energy separation between HOMO and the first  $\text{Zn}^{2+}$  d-orbital in **6** and **7** were found to be 7.46 and 6.94 eV, respectively. Considerable structural changes in sensor **6** in the  $\text{Zn}^{2+}$  bound state were observed as compared to **7** which had an almost unchanged structure. To get further insights,  $\text{Zn}^{2+}$  was removed from sensors and absorption spectra were recalculated for the same conformation.

As a result, almost identical absorption spectra were obtained, which suggested that fluorescence enhancements of sensors were due to  $\text{Zn}^{2+}$  binding, not to the conformational changes.

## 5.2 Future Work

For biological applications, it is good that the sensor could be excited in a visible region which is safe for the biological organism. Thus, there is a need to develop sensors that can be excited in the visible range.

Further, there is a need to come up with the synthesis and development of portable sensors. The cost of the product is always important from a commercial point of view. So, there is a need to develop better chromophores that are cheap.

In addition, there is a need for a design receptor that can enhance the selectivity towards  $\text{Zn}^{2+}$  as compared to  $\text{Cd}^{2+}$ ,  $\text{Fe}^{3+}$  and  $\text{Cu}^{2+}$ .

And finally, there is a need for a water-soluble sensor that is a safe solvent for biological applications.

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## Vitae

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1. **Lecturer B**, teaching general chemistry Chem-101, organic chemistry Chem-201 and Chem-202 at King Fahd University of Petroleum and Minerals, Dhahran, Saudi Arabia, January 2018 – Dec 2022.
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## Research Publications in peer-Reviewed Journals

### Book Chapter

**T. Ahmad**, C. Basheer and B. Saad, Derivatization Strategies for the Determination of Biogenic Amines with Chromatographic Techniques, B. Saad, R Tofalo (eds) Biogenic Amines in Food: Analysis, Occurrence and Toxicity, RSC, **2019**, doi.org/10.1039/9781788015813

| SN | Peer-Reviewed Journals Publications                                                                                                                                                                                                                                                                  | Latest IF | Quartile Ranking |
|----|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|------------------|
| J1 | <b>T. Ahmad</b> , S. Khan, Safwat Abdel-Azeim, N. Ullah. Turn-on fluorescent sensors for nanomolar detection of zinc ions: Synthesis, properties, and DFT studies. <i>Journal of the Taiwan Institute of Chemical Engineers</i> 139 (2022) 104507                                                    | 5.477     | Q1               |
| J2 | <b>T. Ahmad</b> , N. Ullah. The oxa-Michael reaction in the synthesis of 5-and 6-membered oxygen-containing heterocycles. <i>Org. Chem. Front.</i> , <b>2021</b> , 8, 1329-1344.                                                                                                                     | 5.456     | Q1               |
| J3 | <b>T. Ahmad</b> , A. Waheed, Safwat Abdel-Azeim, N. Ullah. Three new turn-on fluorescent sensors for the selective detection of Zn <sup>2+</sup> : Synthesis, properties, and DFT studies, <i>Arabian Journal of Chemistry</i> , <b>2022</b> , DOI: 10.1016/j.arabjc.2022.104002.                    | 6.212     | Q2               |
| J4 | S. Khan; <b>T. Ahmad</b> ; T. Rasheed; N. Ullah; Recent Applications of Vinylethylene Carbonates in Pd-Catalyzed Allylic Substitution and Annulation Reactions: Synthesis of Multifunctional Allylic and Cyclic Structural Motifs, <i>Coordination Chemistry Reviews</i> , <b>2022</b> , 462, 214526 | 24.833    | Q1               |
| J5 | <b>T. Ahmad</b> , M. Mansha, I.W. Kazi, A. Waheed, N. Ullah, Synthesis of 3,5-diaminobenzoic acid containing crosslinked porous polyamine resin as a new adsorbent for efficient removal                                                                                                             | 7.34      | Q1               |

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|     | of cationic and anionic dyes from aqueous solutions, <i>J. Water Process Eng.</i> 43, <b>2021</b> , 102304.                                                                                                                                                                                                                                              |       |    |
| J6  | <b>T. Ahmed</b> , M. S. Manzar, S. U. Khan, I. W. Kazi, N. Ullah and N.D. Mu'azu, Synthesis and ultrahigh adsorptive performance of a novel triazine-core containing resin for removal of malachite green from water. <i>Arab J Sci Eng</i> ( <b>2022</b> ).<br>doi.org/10.1007/s13369-022-07015-w                                                       | 2.807 | Q2 |
| J7  | <b>T. Ahmad</b> ; S. Khan; N. Ullah, Recent Advances in the Catalytic Asymmetric Friedel-Crafts Reactions of Indoles, <i>ACS Omega</i> <b>2022</b> ,7,35446–35485                                                                                                                                                                                        | 4.132 | Q2 |
| J8  | A. Waheed, <b>T. Ahmad</b> , M. Haroon, N. Ullah, A Highly Sensitive and Selective Fluorescent Sensor for Zinc(II) Ions Based on a 1,2,3-Triazolyl-Functionalized 2,2'-Dipicolylamine (DPA), <i>Chemistry Select.</i> 5 (2020) 5300–5305.                                                                                                                | 2.307 | Q3 |
| J9  | M. Mansha, A. Waheed, <b>T. Ahmad</b> , I.W. Kazi, N. Ullah, Synthesis of a novel polysuccinimide based resin for the ultrahigh removal of anionic azo dyes from aqueous solution, <i>Environ. Res.</i> 184 ( <b>2020</b> ).                                                                                                                             | 8.431 | Q1 |
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| J11 | A. Waheed, I.W. Kazi, M.S. Manzar, <b>T. Ahmad</b> , M. Mansha, N. Ullah, N.I. Ahmed Blaisi, Ultrahigh and efficient removal of Methyl orange, Eriochrom Black T and acid Blue 92 by triazine based cross-linked polyamine resin: Synthesis, isotherm and kinetic studies, <i>Colloids Surfaces A Physicochem. Eng. Asp.</i> 607 ( <b>2020</b> ) 125472. | 5.518 | Q2 |

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| J12 | M. Mansha, I.W. Kazi, M.S. Manzar, <b>T. Ahmed</b> , A. Waheed, N. Ullah, N.I. Blaisi, Ultrahigh removal of methyl orange, acid blue-92 and malachite green by a novel triazine-based polyamine resin: synthesis, isotherm and kinetic studies, <i>Int. J. Environ. Anal. Chem.</i> (2020). <a href="https://doi.org/10.1080/03067319.2020.1858072">https://doi.org/10.1080/03067319.2020.1858072</a>                | 2.731 | Q3 |
| J13 | M. S. Manzar, <b>T. Ahmad</b> , N. Ullah, P. V. Chellam, J. John, M. Zubair, R. J.Brandão, L. Meilif, O. Alagha, E. Çevik. Comparative adsorption of Eriochrome Black T and Tetracycline by NaOH-modified steel dust: Kinetic and process modeling. <i>Sep. Purif. Technol.</i> , 2022, 287, 120559                                                                                                                  | 9.136 | Q1 |
| J14 | M. Mansha, <b>T. Ahmad</b> , N. Ullah, S. Khan, M. Ashraf, S. Ali and I. Khan. Photocatalytic water-splitting by organic conjugated polymers: Opportunities and challenges, <i>Chem. Rec.</i> 2022, e202100336. <a href="https://doi.org/10.1002/TCR.202100336">https://doi.org/10.1002/TCR.202100336</a> .                                                                                                          | 6.935 | Q2 |
| J15 | T. Rasheed; S. Khan; <b>T. Ahmad</b> ; N. Ullah, Covalent Organic Frameworks-Based Membranes as Promising Modalities from Preparation to Separation Applications: An Overview <i>Chem. Rec.</i> 2022, <a href="https://doi.org/10.1002/tcr.202200062">doi.org/10.1002/tcr.202200062</a>                                                                                                                              | 6.935 | Q2 |
| J16 | M. Rauf, S. S. Shah, S. K. Shah, S. N. A. Shah, T. Ul Haq, J. Shah, A. Ullah, <b>T. Ahmad</b> , Y. Khan, Md. A. Aziz, K. Hayat. Facile hydrothermal synthesis of zinc sulfide nanowires for high-performance asymmetric supercapacitor, <i>Journal of Saudi Chemical Society</i> , 2022, 101514, <a href="https://doi.org/10.1016/j.jscs.2022.101514">https://doi.org/10.1016/j.jscs.2022.101514</a> .               | 4.712 | Q2 |
| J17 | M. Nawaz, M. Taha, F. Qureshi, N. Ullah, M. Selvaraj, S. Shahzad, S. Chigurupati, S. A. Abubshait, <b>T. Ahmad</b> , S. Chinnam & S. Hisaindee. Synthesis, $\alpha$ -amylase and $\alpha$ -glucosidase inhibition and molecular docking studies of indazole derivatives $\alpha$ -amylase and $\alpha$ -glucosidase inhibition and molecular docking studies of indazole derivatives, <i>Journal of Biomolecular</i> | 5.235 | Q1 |

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|     | <i>Structure and Dynamics</i> , <b>2021</b> , DOI:<br>10.1080/07391102.2021.194789                                                                                                                                                                                |      |    |
| J18 | G. Ashraf, <b>T. Ahmad</b> , M. Z. Ahmed, Murtaza, Y. Rasmi,<br>Advances in Metal-<br>Organic Framework (MOFs) based biosensors for diagnosis: An<br>update, Current Topics in Medicinal Chemistry <b>2022</b> ; 22.<br>doi.org/10.2174/1568026622666220829125548 | 3.57 | Q2 |