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Synthesis of Pyridine Derivative-based Chemosensor for Formaldehyde Detection

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Abstract: *Compound of 3,3'-(4-(2-amino-4,5-dimethoxyphenyl)pyridine-2,6-diyl)dianiline (**CHP**) has been synthesized via three-step synthetic procedure from veratraldehyde as starting material and 4-(4,5-dimethoxy-2-nitrophenyl)-2,6-bis(3-nitrophenyl)pyridine (**CHP-1**) as an intermediate compound. The **CHP-1** was reduced using hydrazine hydrate catalyzed by 10% Pd/C to the final target of **CHP**. The spectroscopic study revealed that **CHP** in acetonitrile could detect formaldehyde through fluorescence enhancement and showed color change from yellow to blue under the 365 nm portable ultraviolet lamp as a response. Based on the fluorescence spectra, the emission wavelength of **CHP** in acetonitrile was shifted from 526 to 480 nm after addition of formaldehyde. Limit detection (LOD), selectivity, sensitivity, and computational study geometry of **CHP** as a chemosensor for formaldehyde has also been investigated. **CHP** could also be applied as a test paper for the detection of formaldehyde qualitatively.*

Keywords: *pyridine; chemosensor; formaldehyde; fluorescence*

■ INTRODUCTION

Formaldehyde is usually found in resins that used in manufactures of wood product, building materials, household products, fertilizers, and pesticides. In addition, formaldehyde is also used as a food preservative illegally, likely on fish and meat products, wet noodles, and soybean curds [1]. On the other hand, formaldehyde has serious effects on human health such as irritation of skin, eyes, nose, throat, and causes DNA damage by reacting with nucleophilic material actively [2]. Moreover, a high concentration of formaldehyde exposure has been reported to cause cancer [3]. For a reason, it is necessary to develop a formaldehyde chemosensor in a simple and cost-effective way.

Research regarding the formaldehyde detection method has been carried out, developed, and described, such as using chromatography, spectrophotometry, and enzymatic methods. Li et al. [4] and Yeh et al. [5] determined the formaldehyde level in a sample using HPLC and GC-MS. This method has a disadvantage due to longer analysis time, and complicated tools. Korpan et al. [6] and Nikitina et al. [7] have designed biosensor compounds to detect formaldehyde enzymatically, but it has a low potential because of a lot of interference and depending on the environmental conditions (pH and temperature). Dar et al. [8] used the Thin Layer Chromatography method for formaldehyde determination, but this method was unable to measure formaldehyde in high concentration. Analysis of spectrophotometric method has been used in this matter. Mohr [9] and Wei et al. [10] have developed a colorimetric method to detect formaldehyde, but this method has a lack of sensitivity. Meanwhile, fluorometric [11-17] has advantages over other methods, namely simpler, faster, cheaper, a great sensitivity, and it can be used for routine analysis [18-20].

Previously, pyridine derivative 4-phenyl-2,6-bis(4-aminophenyl)pyridine (**CHP-0**) has been reported [21] by our group as fluorescence chemosensor of formal dehyde. The sensor shows emission peak shifts from 489 to 442 nm after addition of formaldehyde. Inspired by their works, we developed a pyridine derivative compound 3,3'-(4-(2-amino-4,5-dimethoxyphenyl) pyridine-2,6-diyl)dianiline (**CHP**) from veratraldehyde modified with amine group for formaldehyde sensing.

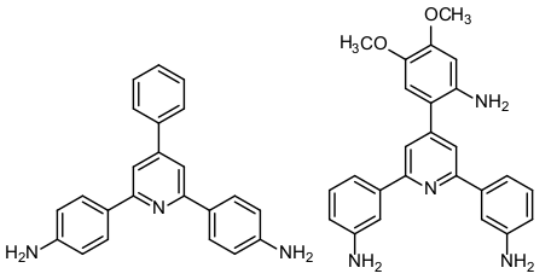


Fig 1. Chemical structure of **CHP-0** and **CHP**

The **CHP** has a different substituent and position in aryl group compared to **CHP-0**. **CHP** could detect formaldehyde and enhanced fluorescence intensity in acetonitrile. This study also discovered that **CHP** shows a clearer distinct color change, better selectivity, and sensitivity compared to the previously reported compounds (**CHP-0**).

■ EXPERIMENTAL SECTION

Materials and Instrumentation

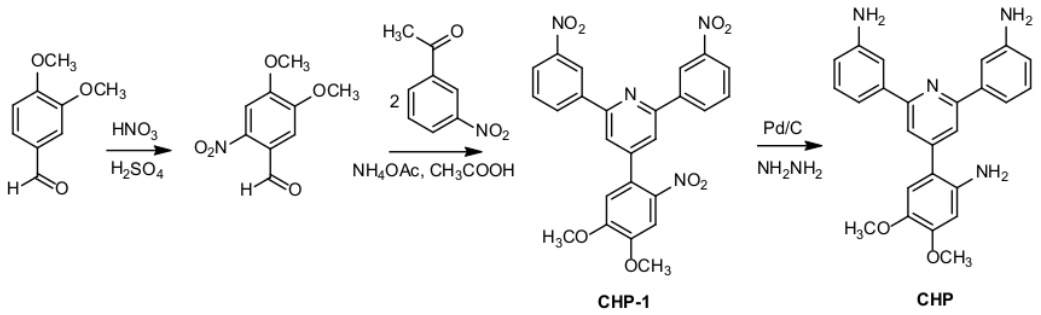
Materials used for the synthesis were veratraldehyde ($C_9H_{10}O_3$), 3-nitroacetophenone ($C_8H_7NO_3$), nitric acid 65%, sulfuric acid 95–98%, ammonium acetate, acetic acid glacial, Pd/C 10%, hydrazine hydrate 80%, ethanol 98%, and distilled water. The solvent used for fluorescence spectra measurement was acetonitrile. These materials and solvent were pro analytic reagents from Merck, except for distilled water.

Equipment used for the synthesis was a set of reflux, electric heater, magnetic stirrer, and laboratory glassware. Instruments employed for characterization of synthesis product were melting point (Electrothermal 9100), FT-IR spectrometer (Shimadzu Prestige 21), Mass Spectrometry (Shimadzu QP-2010S), NMR spectrometer (JEOL JNM-ECZ500R), and spectrofluorophotometer with a 1 cm standard quartz cell (Shimadzu RF-6000) for fluorescence measurements.

Procedure

Synthesis of 3,3'-(4-(2-amino-4,5-dimethoxyphenyl) pyridine-2,6-diyl)dianiline (CHP)

The synthesis of 3,3'-(4-(2-amino-4,5-dimethoxyphenyl)pyridine-2,6-diyl)dianiline (**CHP**) was modified from Tamami and Yeganeh [22] as shown in Scheme 1. A solution of 4-(4,5-dimethoxy-2-nitrophenyl)-2,6-bis(3-nitrophenyl)pyridine (**CHP-1**) in ethanol (0.6 mmol) was added 10% Pd/C (0.1 g). The mixture solution was stirred and heated about 50 °C, and 80% hydrazine hydrate 0.8 mL in 1.5 mL of ethanol was added slowly using dropping funnel over 1.5 h of the period. After the addition was completed, the mixture was refluxed for 2–3 h at 78 °C (the reaction was monitored by TLC) and filtered while it was still hot. The crude product as solid was obtained from the filtrate. The solid was recrystallized with absolute ethanol as yellowish solid (55%, 0.19 g, mp 218–219 °C). FT-IR (KBr) υ (cm^{-1}): 1041 and 1149 (C-O-C), 1080 and 1257 (C-N), 1589 (C=N), 3356 and 3433 (NH_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 3.23 (s, 6H), 3.84 (s, 3H), 3.87 (s, 3H), 6.38 (s, 1H), 6.75 (dd, 2H, J = 8.0, 2.0 Hz, 2H), 6.77 (s, 1H), 7.25 (t, 2H), 7.47 (d, 2H), 7.55 (t, 2H), 7.72 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 56.11,



Scheme 1. Synthesis route of compound **CHP**

56.90, 101.05, 113.89, 114.02, 116.10, 116.70, 117.63, 119.32, 129.78, 137.80, 140.72, 142.69, 147.00, 148.99, 150.53, 157.65; MS (EI) for $C_{25}H_{24}N_4O_2$ m/z: 412.0 (M^+).

General procedure for fluorescence spectra measurement

The stock solutions of **CHP** (2.5×10^{-4} M) were prepared in acetonitrile. The 37% formaldehyde in aqueous solutions was used to make the final concentration of 1×10^{-2} M. Formaldehyde concentration of 0, 1, 3, 5, 7, 9, 10, 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200×10^{-2} M (0.1 mL) was added into 4 mL of **CHP** in acetonitrile solution (2.5×10^{-4} M) and then the fluorescence spectra were recorded.

Application of chemosensor as paper strip test

This procedure was modified from He et al. [23]. Filter papers were dipped into the **CHP** in acetonitrile solution (1×10^{-3} M) and flicked redundantly to make liquid away. The papers were then put over the formaldehyde solution (37%) and distilled water (as a negative control) respectively.

To confirm the detection ability of paper strip, meatball as food sample was tested by paper strip loaded with **CHP**. At first, the meatballs were immersed in 1 M formaldehyde solution for overnight. The meatballs were then weighed (1 g), crushed with a mortar, added with distilled water and filtered. The filtrate obtained was diluted to 25 mL. At last, meatball filtrate was dripped into the paper strip loaded with **CHP** compound.

Density Functional Theory (DFT) calculations

Computational studies using DFT based on the Becke three-parameter exchange/Lee-Yang-Parr correlation hybrid functional (B3LYP), PCM Solvent Model with acetonitrile as a solvent, and the 6-31G basis set as implemented in the Gaussian 09 programs were carried out for the geometry optimizations of **CHP** and **CHP-HCHO**.

■ RESULTS AND DISCUSSION

Synthesis of CHP

The chemosensor of **CHP** was produced via three-step synthetic procedures starting from veratraldehyde. The nitration reaction of veratraldehyde produced in 95% yield, followed condensation of 6-nitroveratraldehyde with 3-nitroacetophenone yielded a compound of **CHP-1** in 20% yield. The final step was the reduction of **CHP-1** with 80% hydrazine hydrate and 10% Palladium on carbon and resulted in the **CHP** in a 55% yield. The chemical structure of **CHP** was confirmed by FT-IR, MS, ^{1}H and ^{13}C NMR.

Fluorescence Response of CHP toward Formaldehyde

Compound **CHP** in acetonitrile (2.5×10^{-4} M) was added with 37% formaldehyde (HCHO) in aqueous solution to examine the response as a chemosensor toward formaldehyde. It has been revealed that **CHP** was showed an obvious color change from yellow to blue under the 365 nm portable ultraviolet lamp, implying that chemosensor **CHP** could be used for qualitative determination of formaldehyde. The interaction produced a blue-shift of emission wavelength from 526 nm to 480 nm with excitation at 363 nm (before and after the addition of formaldehyde) (see Fig. 2).

Upon the addition of equivalents of formaldehyde in acetonitrile, the emission intensity at 480 nm is greatly enhanced. In width concentration range of formaldehyde from 0 to 1.2 M, the fluorescence peak intensity of **CHP** showed a good linear relationship with

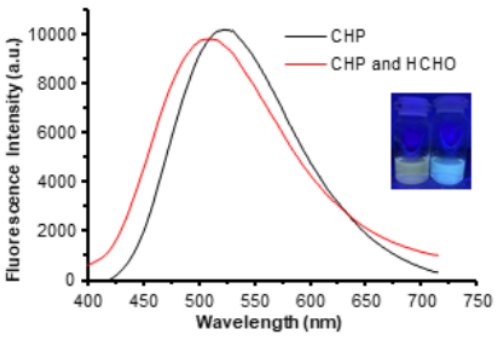


Fig 2. Fluorescence spectra of **CHP** (2.5×10^{-4} M in acetonitrile) before and after addition of 50 µL 37% formaldehyde-water solution with excitation at 363 nm. Inset: Photographs of compound **CHP** (2.5×10^{-4} M in acetonitrile) before (left) and after (right) addition of formaldehyde under 365 nm portable UV lamp

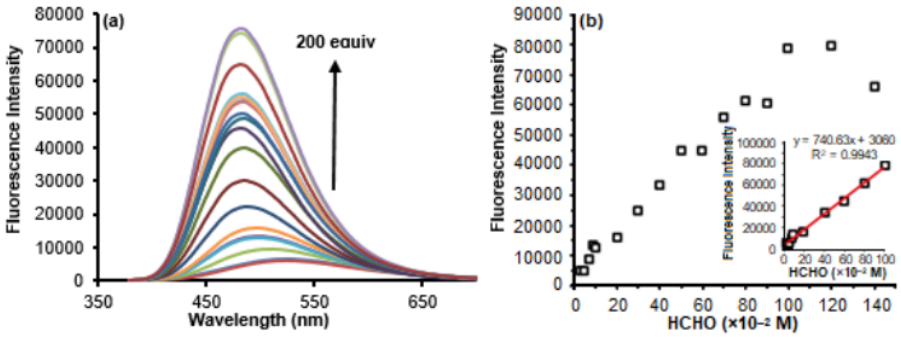
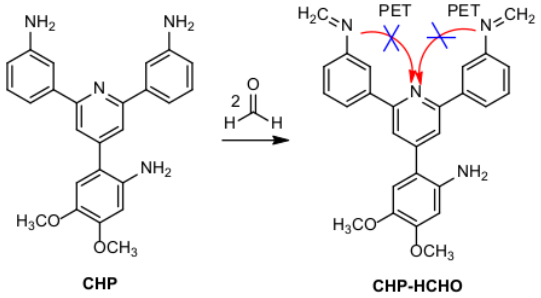


Fig 3. (**a**) Emission spectra of **CHP** (2.5×10^{-4} M in acetonitrile) upon addition of formaldehyde-water solution (0–200 equiv.) with excitation at 363 nm; (**b**) Emission intensity of **CHP** at 480 nm as a function of formaldehyde concentration (0–1.4 M). Inset: Linear relationship between fluorescence intensity and the concentration of formaldehyde

the formaldehyde concentration (R = 0.9943) (see Fig. 3). Thus, this result indicates that formaldehyde can be quantitatively detected in a wider concentration range. The detection limit (LOD) of **CHP** was calculated with the formula: LOD = 3σ/m, where σ represents the standard deviation of blank measurements and m is the slope between intensity versus sample concentration [24]. The calculated detection limit (LOD) of probe **CHP** for formaldehyde was found to be 0.58 mM. The results led us to conclude that **CHP** could be applied as an effective fluorescence chemosensor for formaldehyde.

Formaldehyde Sensing Mechanism of CHP

Based on the structure, two amines from **CHP** reacted with formaldehyde to form imine functional groups (Scheme 2) and showed fluorescence enhancement that could be described by the prohibition of PET process upon sensing. To verify the possible response mechanism, the molecular geometries of probe **CHP** and the product from the reaction of **CHP** with formaldehyde were optimized by Density Functional Theory (DFT). As shown in Fig. 4, the frontier orbital diagram indicates that the HOMO energy of the fluorophore (-5.249 eV) was lower than the HOMO energy of the aniline (-5.220 eV) before the reaction **CHP** with formaldehyde, which implied that PET process could happen from the aniline moieties to the pyridine fluorophore. However, after reaction of **CHP** with formaldehyde, electron transfer from



Scheme 2. Possible reaction mechanism of **CHP** with formaldehyde

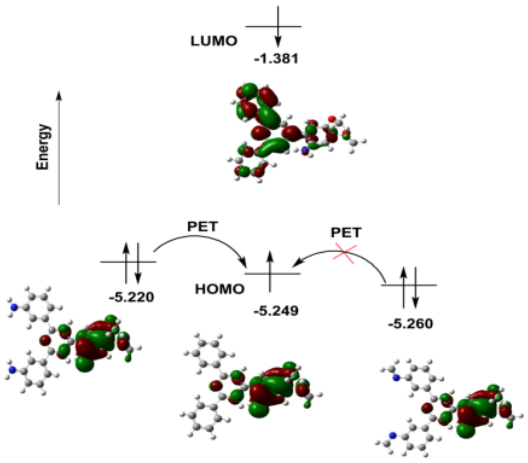


Fig 4. Frontier molecular orbital energies of **CHP** in different conditions that are relevant to PET process

the electron-deficient imine moieties to the electron-rich pyridine fluorophore became difficult to occur (blocked the PET process), because the HOMO energy of imine (-5.260 eV) was lower than the pyridine fluorophore.

Kinetic Study and Selectivity

The time course of the fluorescence spectra of **CHP** toward formaldehyde has also been studied. As shown in Fig. 5, emission intensity increased gradually and reached a maximum in 65 min after the addition of formaldehyde.

To examine the selectivity of **CHP** toward formaldehyde, various of aldehyde compounds were added into **CHP** in acetonitrile. As shown in Fig. 6, the addition of formaldehyde produced a significant enhancement in the fluorescence intensity at 480 nm, whereas the other aldehyde compounds did not generate significant fluorescence enhancement to the **CHP** solution.

To further investigate the selectivity of **CHP** as a formaldehyde sensing, the fluorescence intensity of **CHP** upon addition of simultaneous formaldehyde and various aldehyde was recorded. Fig. 7 presented that all of the selected aldehyde compounds have no interference in the detection of formaldehyde. This result implies that **CHP** has good selectivity for formaldehyde.

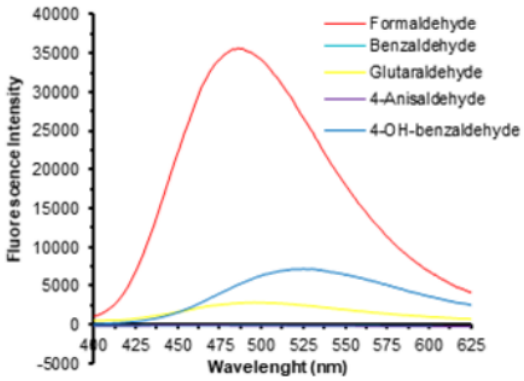


Fig 6. Emission spectra of **CHP** (1×10^{-3} M in acetonitrile) after addition of different aldehyde compounds with excitation at 363 nm

Application of CHP as Test Paper

A simple test paper was developed to demonstrate the practical application of chemosensor **CHP** and used to detect formaldehyde visually. As shown in Fig. 8, the presence of formaldehyde on the paper showed strong fluorescence relative to the paper over the water. The phenomenon was clearly observed by naked eyes under a portable UV lamp with excitation at 365 nm. Then, to demonstrate the applicability of paper strip for formaldehyde detection directly, meatball as a food sample was tested. As a result, paper strip added meatball

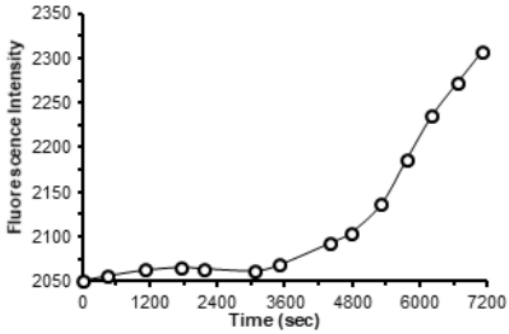


Fig 5. Time-course fluorescence response spectra of **CHP** (1.0×10^{-3} M in acetonitrile) toward formaldehyde (200 equiv.)

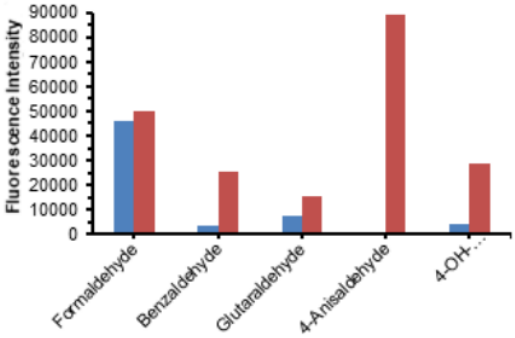


Fig 7. Fluorescence intensity of **CHP** (1×10^{-4} M in acetonitrile) at 480 nm after addition of different aldehyde compounds (200 equiv.) (**blue bars**); the mixture of **CHP** and formaldehyde after addition of other aldehyde compounds (**red bars**)

Fig 8. Photographs of probe **CHP** as test paper for the detection of formaldehyde under 365 nm portable UV lamp (filter paper loaded with **CHP** in acetonitrile over the water (**left**), formaldehyde (**center**), and application of meatball test (**right**)

filtrate containing formaldehyde showed bright appearance, implying that the test paper could be used to detect formaldehyde qualitatively.

■ CONCLUSION

In conclusion, chemosensor-based pyridine derivative of formaldehyde has been developed, which shows high sensitivity and selectivity fluorescence recognition for formaldehyde in acetonitrile. The chemosensor produces a blue-shift from 562 to 480 nm with excitation at 363 nm. Formaldehyde can be detected quantitatively in a concentration range from 0–1.2 M, and the detection limit was 0.58 mM. In addition, the test paper loaded **CHP** was successfully applied in the detection of formaldehyde qualitatively.

■ ACKNOWLEDGMENTS

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