Study on the colorimetric properties of 2,4,6-triarylpyridine derivative compound for imaging Formaldehyde

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Abstract. The derivative compound of 2,4,6-triarylpyridine was synthesized in two steps. The target compound was tested as colorimetric chemosensor against formaldehyde and the limit of detection was also determined using spectrophotometer UV-vis. The first step of the synthesis was the formation of 4-phenyl-2,6-bis(4-nitrophenyl)pyridine compound (1) from 4-nitroacetophenon and benzaldehyde. The second step of the synthesis was the formation of 4-phenyl-2,6-bis(4-aminophenyl)pyridine (2) from reduction of the nitro group in compound 1 by HCl 37% solution and Sn metal. Compound 1 and 2 were characterized by spectrometers FTIR, ¹H NMR and direct inlet-mass spectrometry. The results showed that compound 1 was synthesized with a yield of 78.3% and compound 2 with a yield of 68.9%. Compound 2 as chemosensor showed color transition from colorless to yellow in chemosensor test against formaldehyde in ethanol solvent. The limit of detection of formaldehyde was measured as 4.7×10⁻³ M using spectrophotometer UV-vis.

1. Introduction
Formaldehyde (FA) is classified as a known human carcinogen by IARC [1,2,3]. Formaldehyde is commonly found in the environment, since it is formed primarily from natural sources and human activities. Naturally formed by biomass burning process or decomposition and volcanic activity. Meanwhile, direct human activities such as emissions from industrial and fuel combustion vehicles¹⁶. Formaldehyde is one of the chemicals that in practice is used as a food preservative [4,5]. Early detection is important to ensure that formaldehyde residues have no harmful effects on the health of the body by controlling levels of formaldehyde. Many methods based on spectrophotometric, fluorometric, piezoresistive, amperometric or conductive measurements have been proposed for detecting the concentration of formaldehyde [6]. Other techniques such as liquid and gas chromatography have been shown to be more selective and accurate for determining formaldehyde [7]. However, sensor technology is required that can be used in real time and in-situ detection simultaneously and reusable [8,9].

Pyridine derivative compounds exhibit various activities such as antimalarial, antioxidant, anticonvulsant, anesthetic, antibacterial and antiparasitic [10,11]. While as a heterocyclic compound, pyridine derivative compounds have conjugated orbitals that can be added chromophore or fluorophore groups lead to optical signal changes in case of interaction with the substrate. Therefore pyridine derivative compounds can be developed as colorimetric sensor compounds [12]. The structure of 2,4,6-triaryl pyridine is a fluorescence material that is compatible with hole-transport capability and high fluorescence quantum yield for analysis [13]. Wang et al. (2014) succeeded in
synthesizing 2,4,6-triaryl pyridine by reacting benzaldehyde, acetophenone and sodium hydroxide powder into mortar, yielding

1,3,5-triarylpentane-1,5-dione in the form of white needle solids with 85% yield. The solids are reacted with ammonium acetate in ethanol, yielding a white solid with a yield of 75% [14]. Type of introduction of formaldehyde substrate which can be utilized as chemosensor that is imine formation. The formation of the covalent bond has the advantage of being strong, reversible and can be used in water or slightly protic solvent [15].

Herein, our research group has designed 4-phenyl-2,6-bis(4-aminophenyl)pyridine that possess an amine group that it can form imine bond with formaldehyde. The pyridine ring was introduced as chromophore and the amine group as recognition moiety. The synthesis of 2,4,6-triaryl pyridine generally resulting high yield and selectivity, while derivative substances have not been widely used as colorimetric chemosensors.

2. Experiment

2.1. Material and reagents
All commercial grade chemicals and solvents were purchased and were used without further purification. The 4-phenyl-2,6-bis(4-nitrophenyl)pyridine (1) was synthesized according to Scheme 1 and was modified from Tamami and Yeganeh (2001) procedure [16].

2.2. Synthesis of 4-phenyl-2,6-bis(4-aminophenyl)pyridine (2)
The synthesis route of chemosensor compound was demonstrated in Scheme 1. The ethanol solution (20 mL) of compound 1 (0.15 g, 0.38 mmol) and tin granular (0.27 g, 2.3 mmol) was stirred to a temperature of 60 °C. After that, the mixed solution was added HCl 37% (5 mL). Then, the reaction of mixture solution was stirred at 84 °C for 2h. The reaction result was kept at room temperature and then stored in a refrigerator for 24h. The reaction product was filtered and put in a solution of sodium hydroxide (6.0 g/30 mL). The solution was stirred until the yellow crystal precipitate was formed, then filtered and washed with distilled water three times. The crude were purified by recrystallization using ethanol solvent to get slightly yellow crystal of chemosensor in 68.9% yield (m.p. 199.1-199.4 °C, 208 °C) [17]. 1H NMR (CDCl3, 500 MHz) δ: 7.41-7.44 (t, 1H), 7.48-7.50 (t, 2H), 7.69-7.71 (d, 2H), 7.68 (s, 2H), 8.02-8.04 (d, 4H), 6.77-6.78 (d, 4H), 3.81 (s, 4H). FTIR (KBr) v: 1180 cm⁻¹ (C─N), 1612 cm⁻¹ (C═N), 3371 and 3425 cm⁻¹ (NH₂). GC-MS for C₂₃H₁₉N₃ m/z 337.0 (100%).

2.3. Instruments
FTIR spectra were recorded on a Shimadzu Prestige 21. Mass spectra were recorded on a Shimadzu QP-2010S. 1H NMR spectra were recorded on JEOL JNM-ECZ5000R/S1 spectrometer at 500 MHz. Chemical shifts are reported in ppm downfield from tetramethylsilane as internal standard and chloroform as a solvent. UV-vis spectra were recorded on a Shimadzu D-1800 spectrophotometer. Photoluminescence spectra were performed on a Shimadzu RF-6000 spectro fluorophotometer.
2.4. General procedure for colorimetric spectra experiments
A total of 2.5 mg of chemosensor compound was dissolved in 25 mL of solvents (acetonitrile, ethanol, DMSO, acetic acid, ethyl acetate). Then re-made the same solution for all solvents with the addition of 100 μL formaldehyde 37%. The two treatments before and after addition were subjected to spectral readings between 800-200 nm using a UV-vis spectrophotometer and observed. Both treatments before and after the addition of formaldehyde were done between 800 to 200 nm spectral scan using spectrophotometer UV-vis and observed.

2.5. Limit of detection calculations
About 3 mL of stock solution included in the 14 vial and then added a 37% formaldehyde (1.09 g / mL) with a volume of 0, 10, 20, 30, 40, 50, 60, 80, 100, 200, 400, 700, 1000 and 1500 μL. The absorbance readings were performed at a certain wavelength at maximum absorption wavelength using a UV-vis spectrophotometer.

3. Results and discussion

3.1. Synthesis of compound 1
In this work, compound 1 was prepared in a yield of 78.3% from benzaldehyde, 4-nitroacetophenone and ammonium acetate by a one-pot synthetic procedure. The chemical structure was confirmed by FTIR.

3.2. Synthesis of compound 2
The crude was purified by recrystallization using ethanol solvent, the chemosensor compound of solid with a yield was obtained by 68.9% and had melting point between 199.1-199.4 °C.

3.3. Structure determination of compound 2
The success of the reduction reaction of compound 1 was shown in the peak loss at 1350 cm$^{-1}$ wavenumber as the stretching asymmetric aryl-NO$_2$ vibration. This proves that in compound 2 does not have a nitro functional group. The molecular ion has M$^+$ value equal to the mass of the theoretical molecule of chemosensor molecule. Ingole et al. (2010) reported substituted 2,4,6-triaryl pyridine compounds were analyzed using GC-MS having M$^+$ molecular ions appearing as base peak [18]. $^1$H NMR showed the total hydrogen atoms in each molecule there are a number of 19 hydrogen atoms (6.7-8.0 ppm, C-H arom., 15H; 3.8 ppm, NH$_2$, 4H).

3.4. Solvatochromic test of compound 2
Test of compound 2 was dissolved in acetonitrile, ethanol, DMSO, acetic acid, ethyl acetate solvent and showed a colorless appearance except in acetic acid showed yellow color. Then compared with compound 2 with the addition of saturated formaldehyde in various solvents, it was found that only ethanol solvents can give rise to peaks with observable differences before and after the addition of formaldehyde in the visible light region.

The UV-vis spectrophotometer spectra of Figure 2 showed that the spectra changes with the appearance of peaks in the region 400-600 nm after the addition of formaldehyde occur in acetonitrile, ethanol and acetic acid solvents. Between acetonitrile and ethanol showed the similarity of the new peak shape which indicates the color change of the solution and indicates a positive solvatochromic phenomenon, but the peak on ethanol has a shift approaching the visible light region. It showed that ethanol is more effective as a qualitative colorimetric chemosensor. Ethanol is a protic solvent, including acetic acid, while the other solvents is aprotic solvent. It can be concluded that compound 2
can be used as a chemosensor compound using a protic solvent as ethanol because it qualitatively and quantitatively can provide color change before and after addition of formaldehyde effectively.

**Figure 1.** Compound 2 (top) and after addition of formaldehyde (below) in the solvent: (a) acetonitrile, (b) ethanol, (c) DMSO, (d) acetic acid, (e) ethyl acetate.

**Figure 2.** UV-vis spectrophotometer spectra before and after addition of formaldehyde to the solvent: (a) acetonitrile, (b) ethanol, (c) DMSO, (d) acetic acid, (e) ethyl acetate.
3.5. **Test of chemosensor against formaldehyde**

The result of the chemosensor test of compound 2 on formaldehyde as the substrate dissolved in the ethanol solvent, gives positive response in the form of color change. Compound 2 in the ethanol solvent at a concentration of $2.97 \times 10^{-4}$ M (solution A) and the formaldehyde-added compound 2 (solution B) were read in the 340-600 nm wavelength range, and then obtained the maximum absorption wavelength at 430 nm. The wavelength region of light appears where the blue-purple color is absorbed, complementary to the yellow observation color. The yellow color corresponds to the color of the solution after it has been added formaldehyde (Figure 3).

![Figure 3. Spectra of spectrophotometer UV-vis of chemosensor compound 2.97×10^{-4} M in ethanol (blue) and after adding 50 µL HCHO 37% (yellow).](image)

3.6. **Limit of detection calculations**

The value of the detection limit is calculated based on the formula $(3 \times \text{SD}) / \text{slope}$ in which SD is the standard deviation of the blank solutions (Table 1).

| Blank   | Absorbance | Blank   | Absorbance |
|---------|------------|---------|------------|
| Blank 1 | 0.048      | Blank 5 | 0.048      |
| Blank 2 | 0.048      | Blank 7 | 0.050      |
| Blank 3 | 0.048      | Blank 8 | 0.049      |
| Blank 4 | 0.048      | Blank 9 | 0.049      |
| Blank 5 | 0.049      | Blank 10| 0.049      |

**Standard deviation = 0.000699**

![Graph showing the relationship between absorbance and equivalent volume of HCHO (µL) with the equation $y = 0.0015x + 0.049$ and $R^2 = 0.9973$.](image)
Figure 4. The standard curve of absorbance of compound 2 on formaldehyde using a UV-vis spectrophotometer at λ 430 nm.

Using the absorbance plot versus the equivalent volume of formaldehyde, Figure 4 showed the linearity relationship below the 60 μL equivalent volume. Based on these data, the standard deviation value of 0.000699 and slope value 0.0015 μL⁻¹, obtained detection limit value of 4.7×10⁻³ M.

4. Conclusions
In conclusion, we have presented a rapid and efficient chemosensor for formaldehyde, which showed colorimetric recognition for HCHO in ethanol solutions. The colorimetric spectra showed difference appearance between before and after addition of formaldehyde to chemosensor solution about visual color changes from colorless to yellow at 430 nm. The limit of detection of formaldehyde was measured as 4.7×10⁻³ M. We believe that these characteristics of chemosensor through formation of imine bond, make it attractive for molecular modifications and applications as colorimetric sensor for HCHO.

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