# Theoretical and Experimental Approach Towards P-Cyano Stilbene Schiff Base as a Potential Linker in E-DNA Sensor

**H M Yusoff[1,2], N Hassan[1,2] and K H Ku Bulat[2]**

[1]Advanced Nanomaterials Research Group, School of Fundamental Science, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia
[2]School of Fundamental Science, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

E-mail: hanismy@umt.edu.my, norhafiefahassan@gmail.com, keriang@umt.edu.my

**Abstract.** This research aim to synthesis and characterize several p-cyano stilbene Schiff base molecules as a potential linker in electrochemical DNA sensor. Schiff base reaction is form from condensation reaction between primary amine and aldehyde at 79 °C using ethanol as solvent. All compounds are investigated and discussed by Fourier transform-infrared spectrometer (FTIR) and Uv-vis spectrophotometer. For computational study, structure drawings were constructed using GaussView 5.0. Geometry optimization has been carried out using density functional theory (DFT) at bB3LYP/6 31G and performed using Gaussian09 software. FTIR showed formation of C≡N stretching vibrations at 2224 cm$^{-1}$ also another important stretching at 1604 cm$^{-1}$ and 1249 cm$^{-1}$ which represent C=N and C-O mode respectively. In UV-vis, absorbance of C=N (imine) group can be observed at peak range 363 nm and transition of aromatic C=C can be seen at 238 nm. Theoretical and experimental results obtained are comparable and p-cyano stilbene Schiff base compounds were successfully synthesised and can be further applied as a linker in E-DNA sensor.

## 1. Introduction

Schiff base compounds has been applied in pharmaceutical and biological studies widely [1]. These compounds are also known for its low cost preparation and normally formed by the condensation of aldehydes with primary amines [2-4]. It is reported that Schiff base formed from aromatic aldehyde or aromatic ketones are stable with diverse structural aspects [5]. Application of Schiff base compounds in electrochemical DNA sensor seems as a promising approach. Development of biological sensor have started since 1960s [6]. Fabrication of biosensors include its materials and immobilization methods which required multidisciplinary research. To date there are many methods to detect enzymes, tissue and even DNA. One of the most sensitive and easiest method is electrochemical DNA sensor or known as E-DNA sensor [7].

This type of biosensor required a linker or recognition layer in the sensor system. Obtaining a suitable linker is very important in E-DNA sensor. The linker will be attached onto a suitable substrate. Typical substrates in E-DNA sensor are gold, platinum, silver or indium tin oxide (ITO). All molecular based biosensor reply on specific recognition and DNA probe must be sufficiently immobilised [8]. Furthermore, the length of linker may also affected DNA immobilisation pattern. Typical linker used in several studies normally involve molecules such as 3-mercaptopropionic acid (MPA) and 6-mercapto-1-hexanol (MCH) [9.10].

This paper focuses on synthesizing three different length of stilbene Schiff base having cyano at para position (p-stilbene) at one end of the synthesized molecule. Cyano group will bind with gold surface which act as metal substrate in E-DNA sensor application. Length were varied with 3, 6 and 9 carbon chain. The obtained compounds were also compared with theoretical study using Gaussian 09 software package program [11].

## 2. Experiment

### 2.1. Materials

All chemicals and solvents were purchased from Merck, Sigma Aldrich, Acros Organic, Fisher and HmbG chemicals and were used without further purification. To synthesis p-cyano stilbene Schiff base compounds, 4-(4-aminostyryl) benzonitrile was dissolved in 65 ml of ethanol and was treated with equivalent mole of 4-alkoxylbenzaldehyde.

Then, the mixture was stirred under reflux for 2 hours and the k solvent was reduced to half by using dean stark apparatus. The reaction was monitored by thin layer chromatography (TLC). Precipitate formed was recrystallized using acetonitrile. Figure 1 shows the experimental scheme to synthesis the respective compounds. Figure 2 shows all three different lengths of synthesized p-cyano stilbene Schiff base compounds.
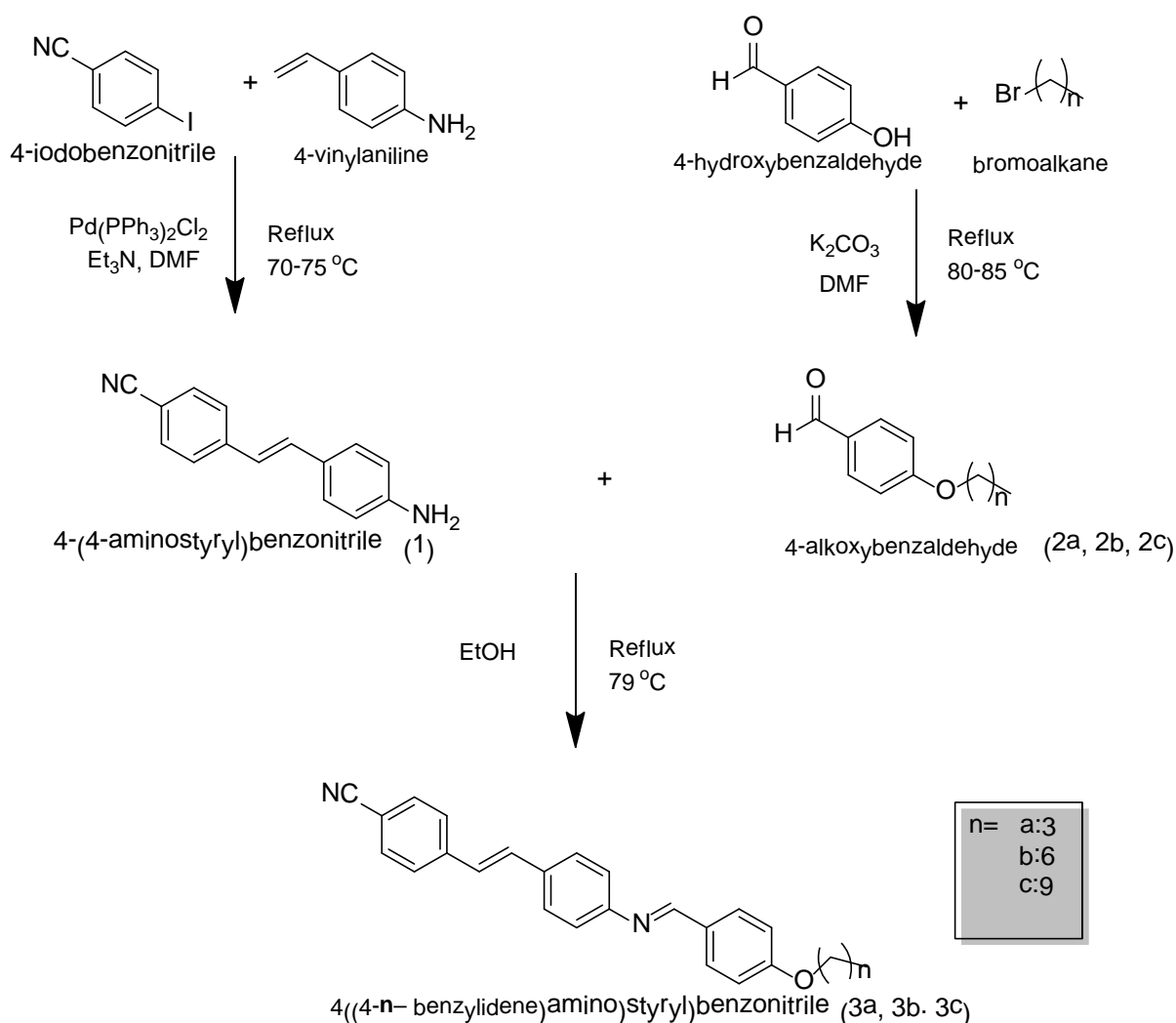


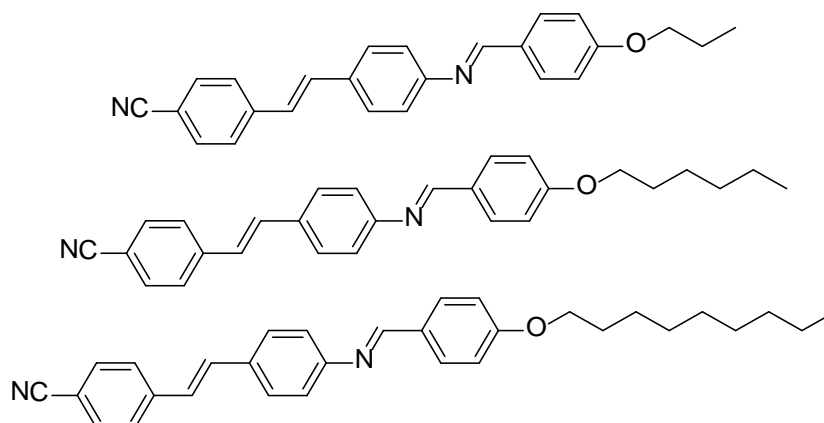**Figure 1.** Experimental procedures to synthesis p-cyano stilbene Schiff base.

**Figure 2.** P-syano stilbene Schiff base molecule attached to 9C alkane chain.

### 2.2. Computational method

There are many computational chemistry methods and also theoretical studies about the determination of biological and chemical activity [12]. GaussView 5.0 has been used to do all the structure drawing. Calculations were performed with Gaussian 09 software package program [11]. In this study, quantum-chemical descriptors are suitable to determine the ranking of physical descriptors. The parameters involved are highest occupied orbitals (HOMOs), the lowest unoccupied molecular (LUMOs), the energy gap between LUMO and HOMO ($\Delta E_{GAP}$), dipole moment ($\mu$), hardness ($\eta$) softness ($\sigma$) and global electronegativity ($\chi$). Density functional theory (DFT) methods named Becke, 3-parameter, Lee-Yang-Parr (B3LYP/6 31G d,p) was selected to analyse and the rest of the atoms were selected as basis sets. Equations 1 to 4 were used to calculate the physical descriptors.

$$\Delta E_{GAP} = E_{LUMO} - E_{HOMO} \tag{1}$$
$$\text{Hardness, } \eta = (E_{LUMO} - E_{HOMO}) / 2 \tag{2}$$
$$\text{Softness, } \sigma = 1 / \eta \tag{3}$$
$$\text{Global electronegativity } \chi = - (E_{LUMO} + E_{HOMO}) / 2 \tag{4}$$

Figure 3 shows one of p-stilbene molecule that has been optimised using DFT with B3LYP 6 31G DP parameters.
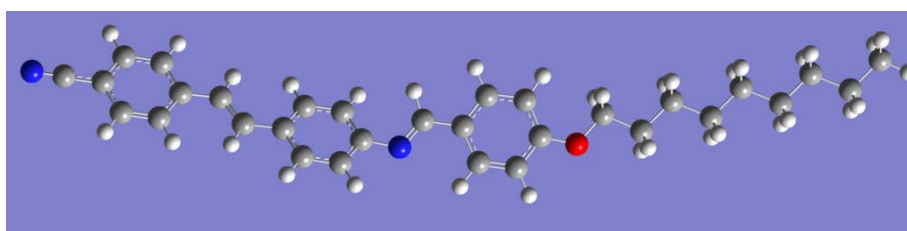
**Figure 3.** P-cyano stilbene Schiff base molecule attached to 9C alkane chain using GaussView 5.0.

## 3. Results and discussion

### 3.1. Infrared (IR) Spectroscopic Analysis

Figure 4 shows FTIR spectra for all different length of p-cyano stilbene Schiff base molecules. All the prominent peaks which represent the functional group present in the compounds can be seen. Peaks ranging at 2967 to 2853 cm$^{-1}$ show the presence of C-H stretching of alkyl chain in the compound. Peak that represents C≡N can be seen 2224 cm$^{-1}$. Peaks ranging at 1604 to 1609 cm$^{-1}$ are the

characteristic absorption bands of carbon-nitrogen double band of the imine group (-HC=N-) [13,14]. Furthermore, peaks at about 1249 cm$^{-1}$ corresponds to the C-O stretching [12].

From table 1, the values obtained from theoretical and experimental are comparable except for C-O stretching however the value obtained from experimental is still acceptable since the range for C-O stretching are from 1000 to 1300 cm$^{-1}$.
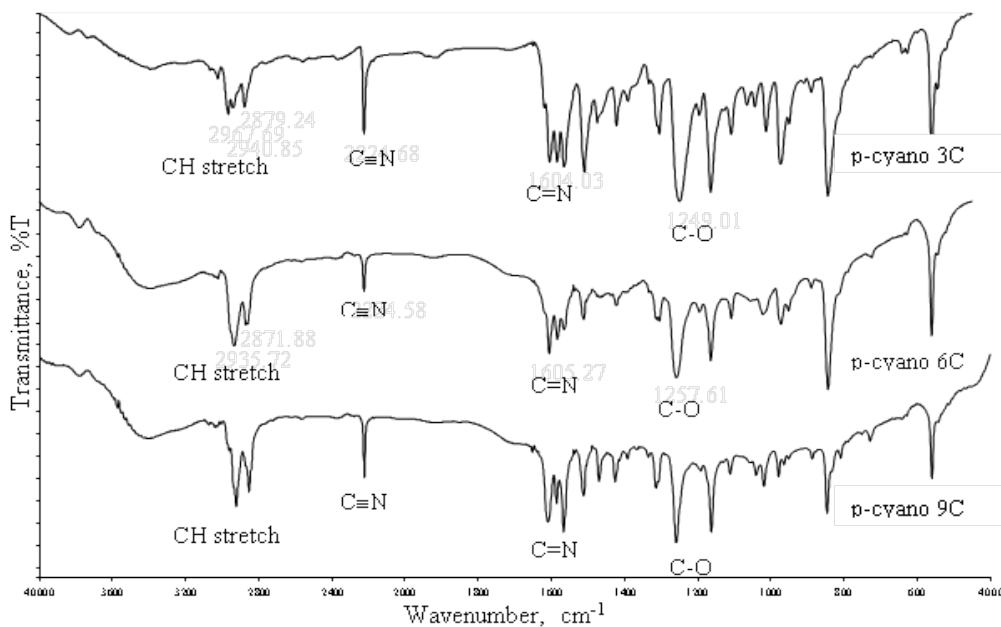


**Figure 4.** FTIR spectra of p-nitro stilbene Schiff base compounds.

**Table 1.** Comparison of frequency assignments between theoretical and experimental.

| Frequency Assignment | Theoretical (Wavenumber, cm-1) | Experimental (Wavenumber, cm-1) |
|---|---|---|
| C-H stretching (alkene/aromatic) | 3090, 3114 and 3124 | 2879, 2940 and 2967 |
| C-H stretching (alkane) | 3045, 3055 | 2940, 2968 |
| C-H stretching (alkane, CH$_2$-O, CH$_2$-N) | 3002 | 2879 |
| C≡N stretching | 2341 | 2224 |
| C=N stretching | 1614 | 1604 |
| C=C stretching (aromatic) | 1560, 1592 | 1565, 1584 |
| C-H deformation | 1421 | 1509 |
| CH$_3$ symmetry deformation | 1344 | 1391 |
| C-C asymmetry deformation (aromatic ring) | 1307 | 1304 |
| C-O (sp2) stretching | 1067 | 1249 |
| C-H deformation (out of phase) | 1005 | 1164 |
| C-O (sp3) stretching | 920 | 973 |
| C-H deformation (aromatic) [out of plane] | 859 | 843 |
| C-H asymmetry deformation (aromatic) [ring breathing] | 639, 650 | 626, 641 |
| C-H deformation [out of plane] (aromatic) | 577 | 560 |

*3.2. Ultraviolet-visible (UV-vis) spectroscopy*

The Ultraviolet-visible (UV-vis) spectroscopy analysis of compound 3a-3b was done by using acetonitrile (1 x 10$^{-5}$ M) as a solvent wih cut-off point at 190.0 nm (Figure 5). The ultraviolet-visible

electronic spectra of three compound showed the presence n-π* and π-π* electronic transition of C=C (aromatic) at the wavelength of 238 nm. While, band at wavelength 363 nm indicate to the π-π* electronic transition of the C=N group [13,14,15].  Table 2 shows the comparison of peak assignments of theoretical and experimental Uv-vis.
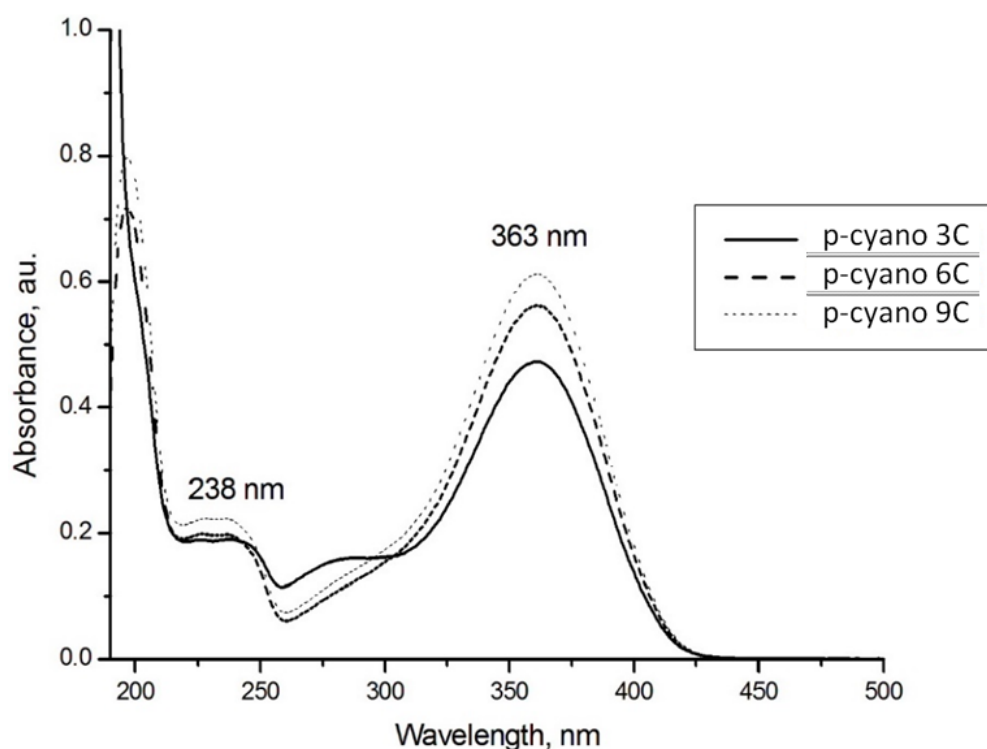


**Figure 5.** UV-Vis spectra of p-cyano stilbene Schiff base compounds.

**Table 2.** peak assignment of UV-Vis spectra from theoretical finding.

| Peak Assignment $\lambda$ | Oscillator strength f (f ≥ 0.01) | Transition |
|---|---|---|
| 413 | 1.9767 | HOMO → LUMO (π → π*) |
| 275 | 0.3003 | (HOMO-1) → (LUMO+1) (π → π*) |
| 254 | 0.0885 | (HOMO-2) → (LUMO+1) (π → π*) |
| 218 | 0.1187 | (HOMO-6) → (LUMO+1) (π → π*) (HOMO-1) → (LUMO+5) (π → π*) |

*3.3. Physical descriptors calculation*

HOMO is an important quantum chemical descriptor and mainly associated with electron donating ability of molecule. The higher the value of HOMO, the higher the tendency of electron transfer to acceptor molecule. LUMO value indicates that the electron-accepting ability of the molecules is higher. Lower value of $E_{gap}$ explains the eventual charge transfer interaction taking place within the molecules. The lower value of this descriptor shows that the molecule is more active [12].

**Table 3.** Calculated quantum physical descriptors at B3LYP method.

|  | p-cyano stilbene 3C | p-cyano stilbene 6C | p-cyano stilbene 9C |
|---|---|---|---|
| $E_{SCF}$ | -1150.6497 | -1268.5992 | -1386.5489 |
| Dipole moment, $\mu$ (Debye) | 9.6513 | 9.8435 | 9.8745 |
| $E_{HOMO}$ | -0.20153 | -0.20128 | -0.20128 |
| $E_{LUMO}$ | -0.07735 | -0.07721 | -0.07729 |
| $\Delta E_{gap}$ | 3.38 | 3.38 | 3.37 |
| Hardness, $\eta$ (eV) | 1.69 | 1.69 | 1.68 |
| Softness, $\sigma$ (eV$^{-1}$) | 0.5917 | 0.5917 | 0.5952 |
| Global Electronegativity, $\chi$ (eV) | 3.79 | 3.79 | 3.79 |

Generally there is no significant change in value obtained from the calculated physical descriptors especially for $\Delta E_{gap}$, hardness, $\eta$ and softenss, $\sigma$ since the attached functional group is the same which is cyano. Chemical hardness is associated with the stability and reactivity of a chemical system. Soft molecules are preferable because they can easily interact with biological molecules.

## 4. Conclusions
Three different length of p-cyano stilbene Schiff base compounds has been successfully synthesized and characterized. Experimental results obtained in FTIR analysis is comparable with theoretical study using Gaussian 09 at B3LYP 6 31G (d, p). These molecules will be further applied as a linker in E-DNA sensor and will be studied in both experimental and theoretical level. DFT is a promising tool to study theoretical aspect of recognition layer's length in E-DNA sensor.

## 5. References
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