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Snap Together Bonds for Amine Capturing – New Spectroscopic and Amperometric Sensors

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Summary: A conjugated polymer (**P1**) containing a pendant aldehyde was anodically prepared on ITO electrodes. Aminothiophenes could be captured by reacting with an aldehyde group located along the polymer backbone resulting in a robust azomethine bond. This was confirmed both spectroscopically and electrochromically. **P1** undergoes absorbance and fluorescence bathochromic shifts when an aminothiophene is covalently linked to the aldehyde resulting from azomethine formation. Both the absorbance and fluorescence shifts are dependent on the aminothiophene used to form the azomethine bond.

Keywords: amine sensors; conjugated polymers; electropolymerization; light-emitting diodes; polyimines

Introduction

Conjugated materials have attracted much attention because of their electrochemical and photophysical properties that are ideally suited for photovoltaics,[1,2] emitting devices,[3] and conducting materials,[4] to name but a few. Thiophenes have been widely used in these electronic applications owing to their low oxidation potential and their possibility of undergoing electropolymerization resulting in conjugated polymers possessing extremely low oxidation potentials.[5] Polythiophenes also have good electrical properties and they are conductive when doped. They can therefore carry charges similar to an electrical wire while also being intensely colored and fluorescent.[5,6] Both these amperometric and spectroscopic properties are highly influenced by substituents along the conjugated backbone including molecular binding. Polythiophenes are extremely sensitive to these environmental changes as a result of their inherent high degree of conjugation. The combined effects of high sensitivity towards binding events arising from their high degree conjugation concomitant with two separate physical properties changes, signalling a binding event along their backbone, make polythiophenes suitable as sensors.

Azomethines are interesting connections involving the condensation of an aldehyde and an amine because these can be reversibly cleaved. The bonds can be hydrolyzed to their original constituents making them suitable protecting groups in synthetic organic chemistry.[7,8] Azomethines are also dynamic in that their constituents can be exchanged when exposed to different aldehyde or amines.[9–11] By judiciously choosing the aldehydes and amines, the imine bond can be made robust such that it is hydrolytically, reductively, and oxidatively resistant.[12–16]

Amines **2** and **3** are interesting precursors for azomethines because the resulting azomethines are robust. The resulting azomethines are also highly colored as a result of the inherently degree of conjugation. This is contrast to thiophenes such as **1** and **P1** that require substitution in the 3-position in order to achieve high coplanarity of the thiophenes and hence high degrees of conjugation. Given our on-going azomethine research, we decided to merge

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thiophenoazomethine with conventional polythiophenes in order to test the capacity of polymer **P1** to signal the covalent binding of aminothiophenes **3** and **4**. Discrete visible color changes of **P1** as a function of the amine are expected as a result of variations in degrees of conjugation. Given that most biologically biogens contain amines, their binding to **P1** would be signalled by discrete color changes dependent on the biogen linked to the polymer. Binding of a given biogen could therefore be detected by a simple color change. Herein, we report the preparation of **P1** and its color change as a function of binding aminothiophenes **3** and **4**. The objective is to report the proof-of-concept demonstrating discrete color and amperometric changes dependent on the type of analyte covalently bond, illustrating the usefulness of **P1** as a potential biosensor.

Experimental Part

Analyses

Spectroscopic Measurements

Absorbance measurements were made on a Cary-500 spectrometer while fluorescence measurements were done with an Edinburgh Instruments FLS-920 fluorimeter after deaerating the samples for 20 minutes with nitrogen. Fluorescence quantum yields were measured at low sample concentration in dichloromethane by exciting the compounds close to their maximum absorption wavelength and by comparing the emission to bithiophene (Φ_{fl}= 0.013) at the same wavelength.[17] Absolute measurements were done using the integrating sphere method.[18–20]

Electrochemical Measurements

Cyclic voltammetry measurements were done with a Bio Analytical Systems Ec

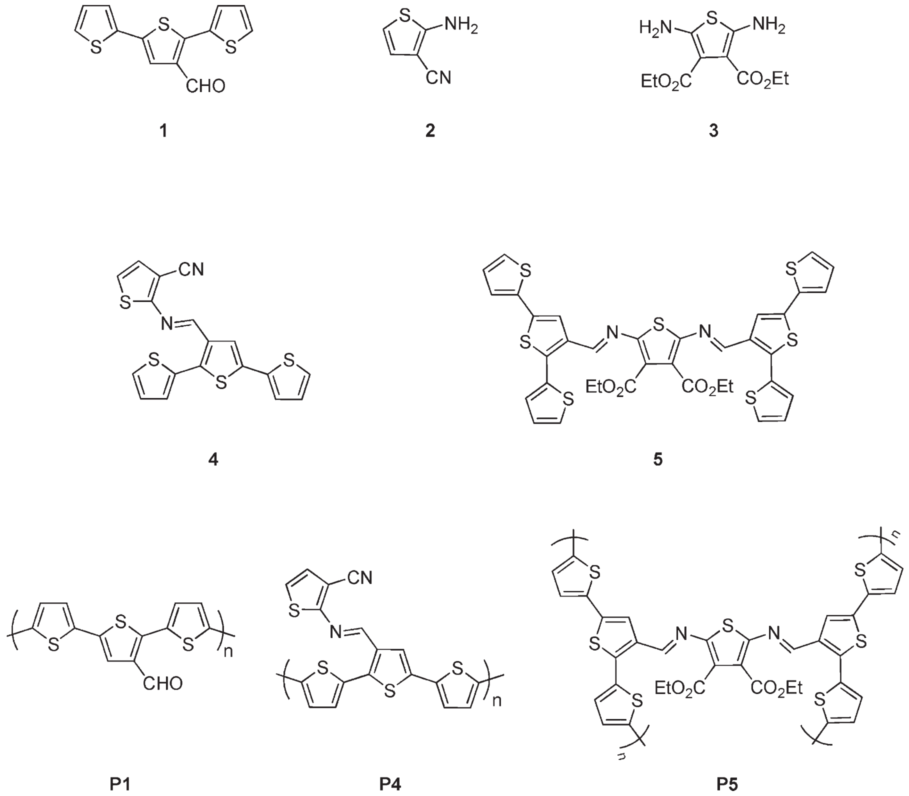


Chart 1.
Monomers and polymers prepared and investigated.

Epsilon potentiostat. The compounds of study were dissolved in anhydrous and deaerated dichloromethane with 0.1 M $TBAPF_6$. A platinum electrode was employed as the working electrode while a platinum wire was used as the auxiliary electrode. The reference electrode was a saturated Ag/AgCl electrode. The polymers were similarly characterized, but using ITO as the working electrode instead.

Electropolymerization

Polymers of the compounds were prepared chronoamperometrically similarly to the electrochromical measurements with the exception of using ITO glass electrodes as the working electrode. The polymers were deposited onto the ITO electrode as red films by sweeping the potential from 0 to 2 V at 100 mV/s for 16 cycles in the absence of oxygen. The resulting films were washed with dichloromethane and acetone and then spectroscopically and electrochemically measured. The glass slides were submerged into a solution of hydrazine-hydrate in ethanol in order to ensure that the reduced/neutral form of the polymer was characterized. The slides were then rinsed with ethanol and dichloromethane prior to analysis.

Syntheses

All chemicals were used as received from commercial suppliers and were used as received unless otherwise stated. Anhydrous solvents for synthetic and characterization purposes were obtained via a Glass Contour alumina solvent purification system.

The synthesis of 2-3 were done according to previously reported methods.[16,21,22]

2,5-Dibromothiophene-3-carbaldehyde

3-Formylthiophene (1.00 mL, 11.41 mmol) was added to a solution of aqueous hydrobromic acid 48% (3.4 mL) and diethyl ether (3.0 mL). Meanwhile, a solution of bromine (1.18 mL, 23.03 mmol) in aqueous hydrobromic acid 48% (3.4 mL) was prepared. This solution was added dropwise to the formylthiophene solution at 0 °C. The resulting mixture was heated at 50 °C for 3 hours. The reaction is then quenched by adding 30 mL of a solution of 10% aqueous sodium thiosulfate. The aqueous phase was then extracted three times with ether and the organic layers were combined and washed with water. The organic fraction was then dried with $MgSO_4$, filtered and concentrated. The crude product was loaded onto a silica column and eluted with 100% hexanes to give the title compound as a yellow solid (1.94 g, 63%). M.P. 44°C. ^{1}H-NMR (chloroform-d_6): δ= 9.68 (s, 1H), 7.22 (s, 1H). ^{13}C-NMR (chloroform-d_6): δ= 182.61, 138.96, 128.34, 123.91, 113.12.

[2,2';5,2'']-Terthiophene-3'-carbaldehyde (1)

2,5-Dibromothiophene-3-carbaldehyde (250 mg, 0.93 mmol) and tetrakis(triphenylphosphine) palladium (171 mg, 0.15 mmol) were purged under nitrogen in a round bottom flask while 2-thiopheneboronic acid (326 mg, 2.55 mmol) was purged under nitrogen in another flask. The flask containing the palladium catalyst was protected from light to which was added 1,2-dimethoxyethane (6.0 mL) to dissolve the solids. The boronic acid was also dissolved in 1,2-dimethoxyethane (4.0 mL) and it was added to the other reaction mixture. Then, 8 mL of a 1 M aqueous sodium carbonate was added. The mixture was refluxed (100 °C) under nitrogen for 16 hours. The resulting solution was concentrated and then dissolved in dichloromethane. The organic layer was washed three times with water, dried with $MgSO_4$, filtered and concentrated. The product was then purified by flash chromatography using 100% hexanes and increasing the polarity to 92.5/7.5 hexanes/ethyl acetate to afford the product as a yellow solid (247 mg, 96%). M.P. 66-68°C. ^{1}H-NMR (acetone-d_6): δ= 10.02 (s, 1H), 7.68 (d, $^3J = 5$ Hz, 1H), 7.49 (s, 1H), 7.46-7.45 (m, 3H), 7.31 (d, $^3J = 3$ Hz, 1H), 7.19 (dd, $^3J = 4$ Hz, $^5J = 5$ Hz, 1H), 7.06 (dd, $^3J = 4$ Hz, $^5J = 5$ Hz, 1H). ^{13}C-NMR (acetone-d_6): δ= 184.5, 145.4, 138.0, 136.9, 135.5, 132.3, 130.1, 129.6, 128.9, 128.7, 126.6, 125.7, 122.7. HRMS (+) calculated for $[C_{13}H_8OS_3 + H]^+$: 276.981, found 276.981.

2-[([2,2'; 5', 2'']Terthiophen-3'-ylmethylene)-amino]-thiophene-3-carbonitrile (4).

1 (25 mg, 0.09 mmol) and 2-aminothiophene-3-carbonitrile (11 mg, 0.09 mmol) were mixed in a round bottom flask. A catalytic amount of trifluoroacetic acid was added to the mixture and it was heated at 60 °C for 10 minutes with a heat gun. The product was then dissolved in acetone, reprecipitated with hexanes and filtered. The product was obtained as a dark yellow solid (24.5 mg, 73%). M.P. 178-180 °C. ^{1}H-NMR (chloroform-d_6): δ= 8.71 (s, 1H), 7.84 (s, 1H), 7.50-7.49 (dd, ^3J= 5.2 Hz, ^5J = 1 Hz, 1H), 7.32 (dd, ^3J= 4.8 Hz, ^5J = 1 Hz, 1H), 7.28-7.26 (m, 2H), 7.19-7.14 (m, 2H), 7.08-7.06 (m, 2H). ^{13}C-NMR (chloroform-d_6): δ= 164.4, 154.9, 142.7, 137.5, 136.2, 135.6, 133.5, 129.0, 128.83, 128.8, 128.4, 126.2, 125.4, 123.3, 121.6, 115.2, 105.5, 100.0. HRMS (+) calculated for $[C_{18}H_{11}N_2S_4 + H]^+$: 382.97996, found 382.98138.

2-[([2,2'; 5', 2'']Terthiophen-3'-ylmethylene)-amino]-thiophene-3,4-dicarboxylic acid diethyl ester (5)

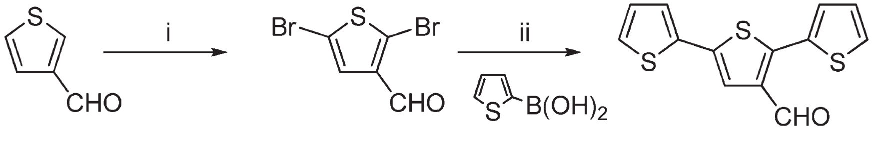
1 (50 mg, 0.18 mmol) and 2,5-diaminothiophene-3,4-dicarboxylate diethyl ester (23.2 mg, 0.09 mmol) were mixed in a round bottom flask. A catalytic amount of trifluoroacetic acid was added to the reagents and the mixture was heated at 60 °C with a heat gun for 15 minutes while under a nitrogen atmosphere. The resulting mixture was dissolved in acetone and the product was precipitated by adding a small amount of hexanes. The solution was then filtered to afford the product as a red solid (56.2 mg, 85%). M.P. 215-217°C. ^{1}H-NMR (chloroform-d_6): δ= 8.57 (s, 2H), 7.74 (s, 2H), 7.47 (dd, ^3J= 5.6 Hz, ^5J = 1 Hz, 2H), 7.29 (dd, ^3J= 5.2 Hz, ^5J = 1 Hz, 2H), 7.23 (m, 4H), 7.17-7.15 (m, 2H), 7.06-7.04 (m, 2H), 4.45-4.39 (q, ^1J = 7.2, 4H), 1.46-1.42 (t, ^1J = 7.2, 6H). ^{13}C-NMR (chloroform-d_6): δ= 163.4, 157.34, 152.9, 150.2, 140.9, 136.7, 135.9, 135.7, 133.4, 128.4, 128.1, 127.9, 127.2, 125.6, 124.7, 123.14, 61.4, 14.3.

Covalent linking of 2 to P1 was done by drop-casting a solution of 2 in ethanol onto the ITO electrode onto which P1 was electropolymerized. The glass electrode was then heated for 5 minutes with a heat gun. The plate was subsequently washed thoroughly with dichloromethane and acetone prior to absorbance measurements.

Results and Discussion

Synthesis

The synthesis of the required reagents for preparing the desired products were done according to previously reported protocols.[23] Both **2** and **3** were prepared by the Gewald method using activated methylenes and a dithialane along with elemental sulfur, respectively.[15,21,22] Meanwhile, **1** was prepared by Suzuki hetero-coupling between 2-thiophene boronic acid and 2,5-dibromo-3-thiophene carboxaldehyde as outlined in Scheme 1.[24] The latter was prepared from 3-thiophene carboxaldehyde with aqueous hydrobromic acid. The desired azomethine compounds were easily prepared by combining **1** with either **2** or **3** in the required number of equivalents in addition to a drop of catalytic TFA diluted in absolute ethanol. The bulk mixture without solvent was heated with a heat gun for ca. 15 minutes and the reaction progress was monitored by TLC. The desired product was obtained in high yield and was obtained by pouring the reaction



Scheme 1.
Synthetic route for the preparation of **1**. i) $HBr/Br_2/Et_2O$, ii) $Pd(PPh_3)_4$/DME/1M Na_2CO_3.

Table 1.
Spectroscopic and electrochemical data of various compounds measured in anhydrous and deaerated dichloromethane.

Compound	Absorbance (nm)	Fluorescence (nm)	Φ_{fl} (10^{-2})	E_{pa} (V)
1	260, 320,370	460	3.6	1.5
4	340, 410	510	0	1.4
5	355, 465	490	0	1.2
P1	485,860	–	–	1.2
P3	455, 810	–	–	1.2
P4	485, 955	–	–	1.2

mixture into acetone. The resulting precipitate was then filtered and washed with a cold acetone/hexanes mixture. Correct product formation was confirmed by ^{1}H and ^{13}C-NMR and MS. Each compound also exhibited a unique oxidation potential (E_{pa}) along with absorption and fluorescence spectra (Table 1).

Spectroscopy

Before testing the capturing and hence signaling behavior of the polymer **1** with mono-aminothiophene **2** and the diaminothiophene **3**, authentic compounds **1**, **4**, and **5** were prepared. This was done in order to see whether the compounds exhibited unique absorption and fluorescence spectra and E_{pa} allowing for spectroscopic and amperometric detection, respectively, when formed in thin films. As seen in Figures 1 and 2 and Table 1, both **4** and **5** possess absorption and fluorescence maxima that are unique and different from **1**. Although significant bathochromic absorbance shifts occur with 2, 5-thiophene azomethine analogues of **4** and **5**, the latter nonetheless exhibit absorbances that are different from **1**. The limited bathochromic shifts of **4** and **5** are owing to their limited degree of conjugation that is orthogonal to the main chain degree of conjugation of **P1**. Nonetheless, **1**, **4**, and **5** possess discrete absorption and fluorescence spectra providing the means to spectroscopically detect their formation.

Polymers of **1**, **4**, and **5** were anodically prepared on ITO glass electrodes as authentic samples in order to characterize their absorption spectra and oxidation potentials. As seen in Table 1, all the polymers immobilized on the ITO electrode show unique absorption and oxidation potentials. Of particular interest is the absorption at 928 nm for **P4** and 958 nm for **P5** arising from the azomethine bond as seen in Figure 3. Therefore, the formation

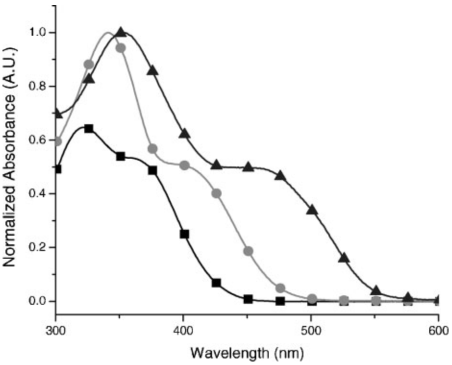


Figure 1.
Normalized absorbance spectra of **1** (■),**4** (•) and **5** (▲) in dichloromethane.

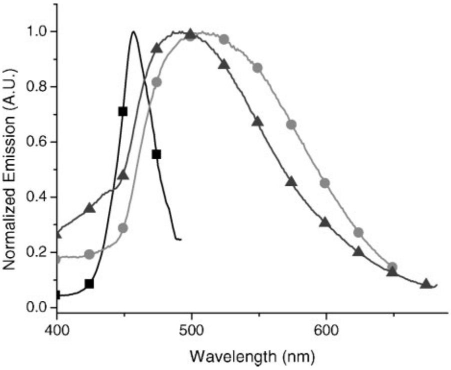


Figure 2.
Normalized fluorescence spectra of **1 (■)**, **4 (•)**, and **5 (▲)** in anhydrous and deaerated dichloromethane.

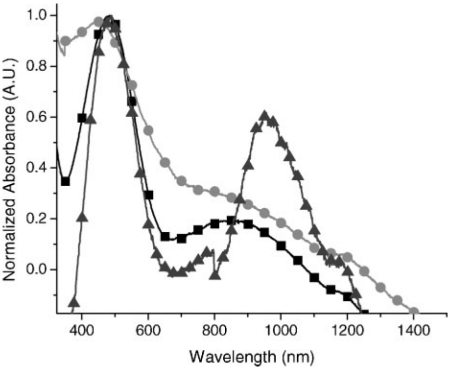


Figure 3.
Normalized absorbance of **P1** (■), **P4** (•) and **P5** (▲) electropolymerized on ITO electrodes.

of these characteristic absorbance signals the formation of the azomethine bond.

The capacity of **P1** to capture and hence detect amines was tested with **4**. Linking **4** to **P1** was done by drop-casting a solution of the corresponding amine in ethanol along with a catalytic amount of TFA. After evaporating the solvent, the ITO glass slide was heated to 80 °C for a couple of minutes. The unreacted amine was removed by rinsing the glass slide with ethanol followed by dichloromethane. The amine capture leading to the azomethine bond formation was confirmed by the absorption peak observed at 930 nm, characteristic of P4 (Figure 4).

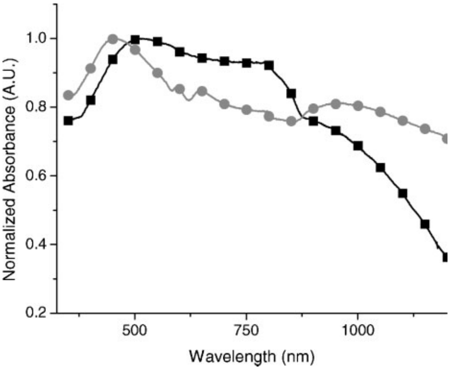


Figure 4.
Normalized absorbance of **P1** before (■) and after reaction with **3** (•) on ITO electrode.

Conclusion

It was confirmed that amines can be captured by aldehyde containing polymers. If aldehydes are used to capture the aldehydes, an azomethine bond results. The formation of the azomethines can be confirmed both spectroscopically and electrochromically. The discrete visible spectroscopic and electrochemical changes associated with the binding of amines along the conjugated backbone are useful signals for binding events. Polymers with azomethine linkages along the conjugated backbone are therefore interesting spectroscopic and amperometric sensors for amines and potentially are suitable as biosensors.

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