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Synthesis and characterization of nucleobase functionalized monothiophenes

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ABSTRACT

The synthesis of a series of nucleobase functionalized thiophene monomers has been accomplished through the reaction of 2-bromo-1-thiophen-3-yl-ethanone with the corresponding DNA base anion. The distinctive pK_a values for the various amine groups in the nucleobases provided a pathway for the creation of specific anions through selective deprotonation of these groups. Using the appropriate anion it is possible to create an amine linkage between the thiophene and nucleobase that is, analogous to that found between the deoxyribose sugar and nucleobase, in the biologically occurring nucleoside.

There is an ever increasing need to develop new technologies for the sensing of important biomolecules such as metabolites, proteins, and DNA because of their important role in disease diagnosis. In the pursuit of this objective various materials, which exhibit ideal properties for sensing will need to be developed. Recently, interesting electrochemical and optical properties of conjugated polymers such as polythiophenes have been exploited for use in the development of such novel detection techniques.[1] It has been reported that the optical properties of a cationic conjugated polymer (CCP) can be used to probe the hybridization event between two complementary strands of DNA.[2] The positively charged backbone of the CCP was able to interact with anionic single stranded DNA to form a duplex that promotes complementary hybridization by diminishing electrostatic repulsion between the two DNA complementary anionic strands.[3] The transition from duplex to triplex conformation has been probed coulometrically because of changes in color associated with conversion of the polymer from its planar, highly conjugated conformation (duplex) to its slightly conjugated, non-planar conformation (triplex).[3]

The electrochemical and electrochromic properties of conjugated polymers functionalized with a biomolecule of interest can be modified by the formation of hydrogen bonds between the tethered biomolecule and a hydrogen bond donor or acceptor that is free in solution.[4] An example of this behavior is reported by Bäuerle and Emge [5] in which bi-thiophenes were functionalized with pyrimidine or purine analogs and then polymerized electrochemically onto the surface of platinum electrodes. The resulting films were characterized in the presence or absence of its complementary base. They found that the electrochemical properties of the films were indeed influenced by the presence of complementary base pairing and these effects were reversible.

Modified DNA bases are used to facilitate the synthesis of nucleobase functionalized materials because the naturally occurring ones are plagued by solubility and reactivity problems.[6] Herein we present the (i) synthesis of adenine and thymine functionalized monothiophenes produced through the reaction of thiophene-3-acetyl bromide and the corresponding purine or pyrimidine anion (Chart 1) and (ii) discussion regarding the reactivity of the nucleophilic sites of thymine. These nucleobase thiophene monomers may afford the corresponding polymers after oxidation (Chart 1).

There was precedent for using an acetyl bromide functionality to immobilize DNA nucleobases to an organic molecule.[7] Huang and co-workers incorporate an adenine nucleobase to a calyx[4]arene using an acetyl bromide functionality.[7a] They reported a base-mediated deprotonation of the nucleobases in DMF allows the solubilization of the adenine and other nucleobases. By adapting this approach we were able to introduce nucleobases on thiophene moieties. The 3-acetylbromothiophene required for this synthesis was prepared following a procedure described in the literature.[8] It reacts with the adenine anion to afford the corresponding functionalized thiophene as described in Scheme 1.[9]

^{1}H and ^{13}C NMR confirm that the amine linkage between the thiophene and adenine molecule was in a position that was analogous to the linkage between the nucleobase and sugar in the adenine nucleoside.

Thymine, being the complementary base to adenine was the next nucleobase chosen for linkage to the thiophene monomer. The structure and composition of thymine are much different than that of adenine. Unlike adenine, which has only the one secondary amine, thymine has two. This may affect the selectivity of the nucleophilic attack affording two isomers. In attempting to link the nucleobase thymine to the thiophene monomer, we found that

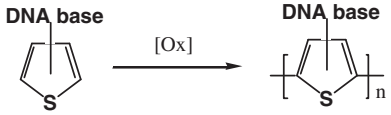

Chart 1. DNA base functionalized oligo/polythiophenes.

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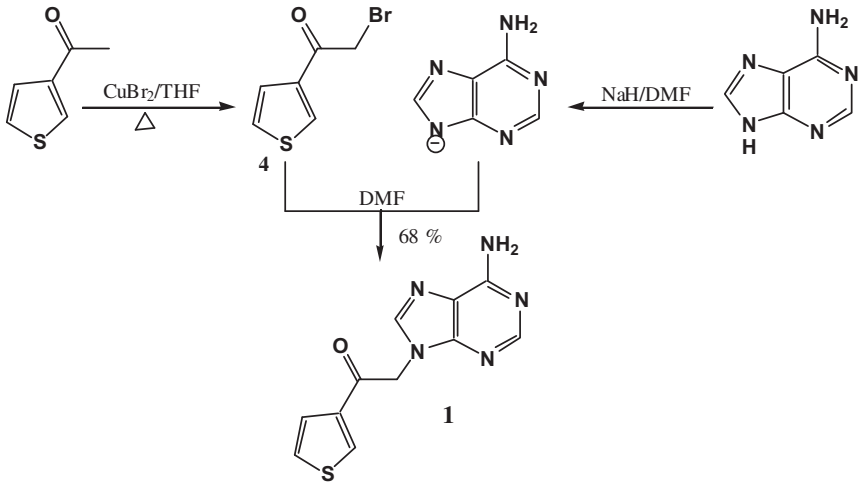


Scheme 1. Synthetic route for thiophene bearing adenine.

the base used to deprotonate the amine can have a large influence on the identity of the final product. This provided us with a way to selectively deprotonate the secondary amines of thymine and other nucleobases.

In performing the synthesis of the 3-acetylthyminethiophene using the same procedure that was used for the 3-acetyladenin-ethiophene, but using K_2CO_3 as the base for amine deprotonation, we found that thymine was attached to thiophene in the N1 position producing the product **2** after recrystallization.[10] However, using NaH as the base to deprotonate thymine gives the undesired N3-linked 3-acetylthyminethiophene (**3**).[11] Scheme 2 shows the synthetic route for the two thymine functionalized thiophene monomers.

The yields and optical properties of the newly synthesized nucleobase functionalized thiophene monomers can be found in Table 1.

The yield of monomer **2** (40%) is lower than the yield of **1** and **3**. The origin of this result is unclear. For deprotonation of thymine with K_2CO_3, the reaction did not afford any other isomer, only monomer **2**. The latter may degrade during the reaction. Moreover, it was reported that the yield of the reaction giving a thymin-1-yl acetic acid compound using a similar procedure is around 60%.[7b]

Using ^{1}H NMR of thymine N–H and ^{13}C NMR of thymine carbonyl, we were able to successfully characterize both isomers. Table 2 summarizes the spectroscopic data of the specific functional groups (NH and CO) present in the thymine moiety for both isomers **2** and **3**.

The selective substitution can be explained through an analysis of the pK_a's of thymine secondary amines as shown in Scheme 3.[13]

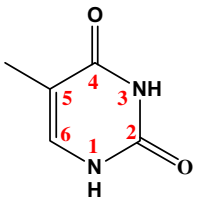
Table 1
Yields and optical properties of nucleobase functionalized thiophenes

Monomer	Yields (%)	λ (nm)	ε (cm^{-1} M^{-1})
1	68	256	4.96×10^4
2	40	260	1.46×10^4
3	70	261	3.54×10^4

Table 2
Spectroscopic data of **2**–**3**

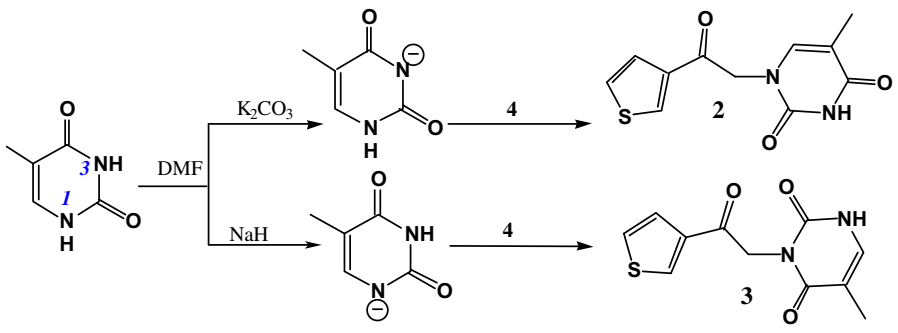
Spectroscopic data	**2**	**3**
^{1}H NMR of NH(1)	10.5 [10.6]	–[10.6]
^{1}H NMR of NH(3)	–[11.0]	12.1 [11.0]
^{13}C NMR of CO(2) (thymine)	150.3 [151.5]	156.6 [151.5]
^{13}C NMR of CO(4) (thymine)	169.6 [164.9]	163.9 [164.9]
^{13}C NMR of CO (acetyl)	192.9	187.3

Values within square braces represents, spectroscopic data of thymine[12].



	pKa's
N1	9.86
N3	13.86

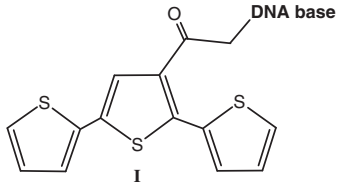
Scheme 3. Structure of thymine showing the pK_a values of its amine group at N1 and N3 positions.



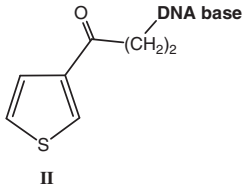
Scheme 2. Synthetic route for thymine functionalized thiophenes.

In the case of thymine both N1 and N3 can act as weak acids and can be deprotonated. Looking at the pK_a's of thymine, N1 will lose its proton at a lower whereas N3 will lose its proton when the media becomes more basic. It is through the N1 position that thymine associates with its sugar in DNA, therefore it is at this position we wish to substitute the thiophene monomer. The fact that we obtained the N3-linked thiophene when using NaH as the base for deprotonation and the N1-linked thiophene when using K_2CO_3 as the base for deprotonation relates back to the strength of these bases. Of the two bases used, NaH is the stronger one. Therefore by using the K_2CO_3 we were able to selectively deprotonate the thymine molecule at N1.

Attempts to polymerize the prepared monomers via electrochemical oxidation in ACN and DMF using cyclic voltammetry at the platinum electrode were unsuccessful. The electropolymerization process to form a carbon–carbon bond via radical cation–radical cation coupling was not able to occur due to the high oxidation peak potential of these monomers (the oxidation peak potential is outside the potential window of the solvent/supporting electrolyte). The alternative to the electrochemical polymerization is the chemical oxidation using either $FeCl_3$ (Ferric chloride) or $NOBF_4$ (Nitronium tetrafluoroborate) as oxidizing agent. Most of these chemical polymerizations are carried out in chloride solvents such as chloroform and dichloromethane. Due to the poor solubility of the prepared monomers **1–3** in these solvents, it is very hard to have a clear cut statement regarding their chemical polymerizations. Further investigations concerning the synthesis of (i) terthiophenes bearing nucleobases with low oxidation potential (**I**) and (ii) new monothiophenes (**II**) with enhanced solubility, are currently underway and will be published in due course.



I



II

In summary, we have explored the synthesis of a series of new thiophene monomers bearing the nucleobases adenine and thymine. These new compounds were fully characterized with different spectroscopic techniques and they are stable in air and in the presence of a variety of organic solvents. We have also shown by exploiting the pK_a's of the various amine groups of the nucleobases it is possible to selectively deprotonate and functionalize a specific amino group by using bases of varying strengths such as sodium hydride and potassium carbonate. The use of thiophenes substituted with an acetyl bromide functionality shows a great promise for nucleobase functionalization of a variety of thiophene monomers, which can be chemically/electrochemically oxidized to form the corresponding polymers.

The ability of the nucleobase functionalized thiophene to form hydrogen bonds with their complementary base could be examined, since this is the mechanism through which DNA hybridization takes place and is the key principle behind the use of these polymers in DNA biosensors.

Acknowledgments

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9. *Synthesis of 2-(6-amino-purin-9-yl)-1-thiophen-3-yl-ethanone* (**1**). Adenine (0.132 g, 0.975 mmol) and sodium hydride (NaH) (0.045 g, 1.87 mmol) were added to 10 mL of dimethylformamide (DMF), which had been dried over 4 A° molecular sieves and degassed for 1 h with N2. The reaction mixture was allowed to stir for 2–3 h, at this point the adenine had been deprotonated and a white precipitate had formed. Compound **4** (0.209 g, 1.00 mmol) was then introduced to the flask. Immediately upon the addition of **4**, the precipitate disappeared and the reaction mixture became a light yellow. As the reaction progressed the reaction mixture transitioned from yellow to orange and then to red, once **4** had been consumed (ca. 45 min.). The DMF was then removed under vacuum, and the desired product was obtained as a white solid after recrystalization from a 50/50 mixture of water and ethanol. Yield = 68%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 4.85 (s, 2H), 6.36 (s, 2H), 6.71 (m, 1H), 6.85 (m, 1H) 7.21 (s, 2H) 7.90 (m, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 50.0, 118.5, 126.6, 128.3, 134.9, 138.9, 141.8, 150.1, 152.6, 156.2, 187.5. IR υ(cm^{-1}) = 1676 (CO). UV–vis (ACN), $\lambda_{max}(\varepsilon)$ = 256 nm (4.96×10^4 M^{-1} cm^{-1}). HRMS (EI) for $C_{11}H_9N_5OS$ [M$^+$]: calcd 259.0522; found 259.0524.
10. *Synthesis of 5-methyl-3-(2-oxo-2-thiophen-3-yl-ethyl)-1H-pyrimidine-2,4-dione* (**2**). Thymine (0.126 g, 1.00 mmol) and NaH (0.0245 g, 1.02 mmol) were added to 10 mL of dimethylformamide (DMF), which had been dried over 4 A° molecular sieves and degassed for 1 h with N2. The reaction mixture was allowed to stir for 2–3 h, at this point the thymine had been deprotonated and a white precipitate had formed. Compound **4** (0.209 g, 1.00 mmol) was then introduced to the flask. Immediately upon the addition of **4**, the precipitate disappeared and the reaction mixture became a light yellow. As the reaction progressed the reaction mixture transitioned from yellow to orange and then to red, once **4** had been consumed (ca. 1–2 h). The DMF was then removed under vacuum, and the desired product was obtained as a white solid after recrystallization from a 50/50 mixture of water and ethanol. Yield = 40%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.92 (s, 3H), 4.30 (s, 2H), 6.63 (d, J = 1.2 Hz, 1H), 6.72 (m, 1H), 6.86 (m, 1H), 7.84 (m, 1H), 10.52 (br s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 17.1, 59.0, 113.5, 131.5, 133.2, 139.8, 144.1, 147.26, 156.3, 169.6, 192.9. IR υ(cm^{-1}) = 1680 (CO). UV–vis (ACN), $\lambda_{max}(\varepsilon)$ = 260 nm (1.46×10^4 M^{-1} cm^{-1}). HRMS (EI) for $C_{11}H_{10}N_2OS$ [M$^+$]: calcd 250.0407; found 250.0407.
11. *Synthesis of 5-methyl-1-(2-oxo-2-thiophen-3-yl-ethyl)-1H-pyrimidine-2,4-dione* (**3**). Thymine (0.125 g, 0.992 mmol) and potassium carbonate (K_2CO_3) (0.150 g, 1.09 mmol) were added to 10 mL of dimethylformamide (DMF), which had been dried over 4 Å molecular sieves and degassed for 1 h with N2. The reaction mixture was allowed to stir for 2–3 h, at this point the thymine had been deprotonated and a white precipitate had formed. Compound **4** (0.208 g, 0.995 mmol) was then introduced to the flask. Immediately upon the addition of 4, the precipitate disappeared and the reaction mixture became a light yellow. As the reaction progressed the reaction mixture transitioned from yellow to orange and then to red, once **4** had been consumed (ca. 1–2 h). The DMF was then removed under vacuum, and the desired product was obtained as a white solid after recrystalization from a 50/50 mixture of water and ethanol. Yield = 70%. ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.43 (s, 3H), 5.85 (s, 2H), 8.20 (s, 1H), 8.28 (m, 1H), 8.42 (m, 1H), 9.41 (m, 1H), 12.1 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 11.5, 53.8, 107.6, 125.8, 127.6, 134.1, 138.4, 141.6, 150.6, 163.9, 187.3. IR υ(cm^{-1}) = 1680 (CO). UV–vis (CH_2Cl_2), $\lambda_{max}(\varepsilon)$ = 261 nm (3.54×10^4 M^{-1} cm^{-1}). HRMS (EI) for $C_{20}H_{21}NO_3S_3$ [M$^+$]: calcd 250.0407; found 250.0411.
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