ORGANIC LETTERS

2008
Vol. 10, No. 17
3657−3660

A Conjoined Thienopyrrole Oligomer Formed by Using DNA as a Molecular Guide

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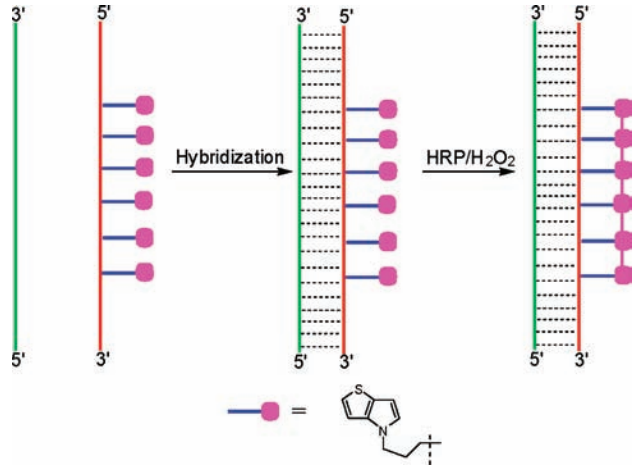
Received May 28, 2008

ABSTRACT



A thienopyrrole oligomer conjoined to DNA was prepared by means of a templated synthesis protocol. The oligomer was formed by reaction, initiated with HRP/H_2O_2, of thieno[3,2-*b*]pyrrole monomers attached to cytosine bases. The thienopyrrole oligomer was characterized spectroscopically.

The creation and manipulation of well-defined nanoscale structures with high precision depends ultimately on the implementation of synthetic methods that result in the "atom-level" control of their fabrication. In this context, DNA is a promising material for the construction of a variety of well-defined nanomaterials as a result of its highly sequence-specific self-assembling properties and its ability to form a flexible, well-ordered helical structure.[1−4] DNA-templated synthesis has proven to be a versatile tool useful for fabricating molecular nanostructures[5−8] including nanowires[9−11] and nanomachines.[12−14]

(1) Chen, J.; Seeman, N. C. *Nature* **1991**, *350*, 631–633.
(2) Lin, C.; Ke, Y.; Liu, Y.; Mertig, M.; Gu, J.; Yan, H. *Angew. Chem., Int. Ed.* **2007**, *46*, 6089–6092.
(3) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J. *Nature* **1996**, *382*, 607–609.
(4) Simmel, F. C.; Dittmer, W. U. *Small* **2005**, *1*, 284–299.
(5) Gartner, Z. J.; Tse, B. N.; Grubina, R.; Doyon, J. B.; Snyder, T. M.; Liu, D. R. *Science* **2004**, *305*, 1601–1605.
(6) Li, X.; Liu, D. R. *Angew. Chem., Int. Ed.* **2004**, *43*, 4848–4870.
(7) Park, S. H.; Pistol, C.; Ahn, S. J.; Reif, J. H.; Lebeck, A. R.; Dwyer, C.; LaBean, T. H. *Angew. Chem., Int. Ed.* **2006**, *45*, 735–739.
(8) Seeman, N. C. *Nature* **2003**, *421*, 427–431.
(9) Braun, E.; Eichen, Y.; Sivan, U.; Ben-Yoseph, G. *Nature* **1998**, *391*, 775–778.
(10) Burley, G. A.; Gierlich, J.; Mofid, M. R.; Nir, H.; Tal, S.; Eichen, Y.; Carell, T. *J. Am. Chem. Soc.* **2006**, *128*, 1398–1399.
(11) Nguyen, K.; Monteverde, M.; Filoramo, A.; Goux-Capes, L.; Lyonnais, S.; Jegou, P.; Viel, P.; Goffman, M.; Bourgoin, J.-P. *Adv. Mater.* **2008**, *20*, 1099–1104.
(12) Bath, J.; Turberfield, A. J. *Nat. Nanotechnol.* **2007**, *2*, 275–284.
(13) Liang, X.; Nishioka, H.; Takenaka, N.; Asanuma, H. *ChemBioChem* **2008**, *9*, 702–703.

10.1021/ol801137t CCC: $40.75
Published on Web 08/08/2008

Extensive investigation of the properties of DNA has revealed that its electrical characteristics are not well-suited for functions that require the rapid and efficient transport of charge over long distances.[15−18] The application of DNA to molecular electronics as a "wire" therefore requires its major modification[9,19−22] or an appropriate restructuring of the DNA itself[23,24] while continuing to take advantage of its self-organizing and self-recognizing properties. An example of this approach is metallization procedures[9−11] that form conductive structures along a path originally defined by the DNA. Similarly, the fabrication of DNA attached to semiconductor nanoparticles[19,25] or to conducting polymers[26−28] offers considerable promise.

Conducting polymers such as polypyrrole and polyaniline[26−29] have been synthesized by taking advantage of electrostatic interactions to organize their positively charged monomers along the negatively charged phosphate backbone of DNA. This strategy is easy to execute, but it does not allow for specific attachment of the conducting polymer and does not take advantage of the sequence information inherent in DNA. We have been examining the possibility of forming DNA-linked conducting polymers that are attached covalently at specified bases, thus combining the templating and scaffolding roles of DNA. As an initial step toward this goal, we recently showed that either horseradish peroxidase (HRP) under mild oxidizing conditions or electrochemical methods can be employed to form polyaniline (PANI) from aniline derivatives linked to the bases of an oligonucleotide without destroying the duplex.[30,31] In this communication, we report the extension of this strategy to a heterocyclic monomer. Thieno[3,2-*b*]pyrrole monomers attached to cytosine bases through a flexible three-carbon chain were

DNA(1) : 5'-TAG CTA GCA CAX TXT XTX TXT XGT CGA ACC TGA-3'
DNA(2) : 5'-TAG CTA GCA CAX CCC CCG TCG AAC CTG A-3'
DNA(3) : 5'-TAG CTA GCA CAC TCT CTC TCT CGT CGA ACC TGA-3'
DNA(4) : 3'-ATC GAT CGT GTG AGA GAG AGA GCA GCT TGG ACT-5'
DNA(5) : 3'-ATC GAT CGT GTG GGG GGC AGC TTG GAC T-5'

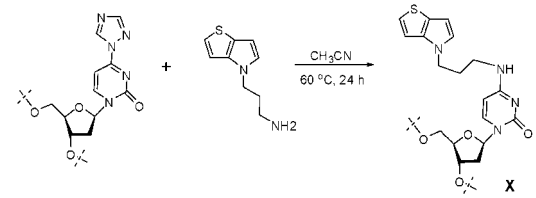


Figure 1. Schematic representation of the DNA oligomers used in this work. The symbol **X** stands for a cytosine nucleobase having a covalently attached thienopyrrole monomer prepared by postsynthetic modification of the oligonucleotide, as is shown.

oxidized, and optical absorption experiments indicated that this reaction results in the formation of a conducting polymer.

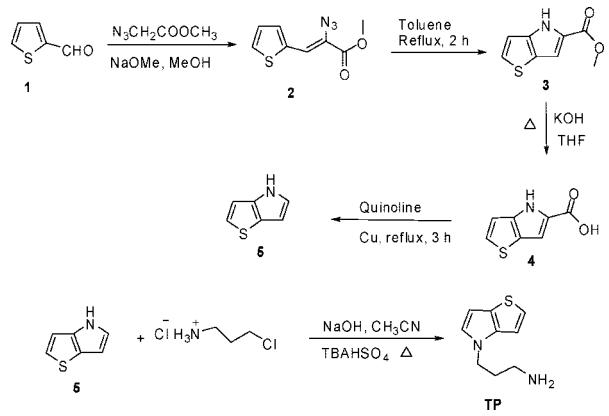
Thienopyrrole is an electron-rich heteroaromatic bicyclic compound containing pyrrole and thiophene units fused together. This compound is more easily oxidized ($E_{ox} = 0.6$ V vs Ag/Ag^+) than thiophene as a consequence of its greater π-electron delocalization. It has been predicted from ab initio theoretical calculations that poly(thienopyrrole) has a predominant α-α′ linkage between the monomer units. Similarly, poly(thienopyrrole) is expected to have good conductivity and be easily transformed between its oxidized and reduced states,[32] making it an ideal candidate for use as a nanowire conjoined to DNA. Moreover, preparation of oligonucleotides having thienopyrole groups on cytosines seemed straightforward and in fact was readily accomplished.

The DNA oligomers used in the current study are shown in Figure 1 where **X** represents the modified cytosine bearing the thienopyrrole moiety. They were prepared by postsynthetic modification of the appropriate oligonucleotides. 1-(3-Aminopropyl)thieno[3,2-*b*]pyrrole (**TP**) was prepared from thieno[3,2-*b*]pyrrole (**5**), which in turn was efficiently obtained from thiophene-2-carboxaldehyde (**1**) by Hemetsberger−Knittel reaction methodology (see Scheme 1).[33] DNA(1) and DNA(2) are sequences with six and one **TP** units, respectively. The arrangement of alternating **X** and **T** nucleotides of DNA(1) was selected on the basis of molecular mechanics calculations so that the monomers were properly spaced for reaction. DNA(3) is a normal, unmodified sequence used in control experiments. DNA(4) and DNA(5) are the complementary sequences to the above oligonucleotides. All DNA oligomers were purified by reverse-phase HPLC, and the structures were confirmed by optical and ESI mass spectrometry.

Duplex DNA oligomers were prepared by hybridization of the appropriate single strands and probed by examination of their thermal melting behavior, first to confirm that duplexes are formed, but primarily to ensure that they are

(14) Niemeyer, C. M.; Adler, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 3779–3783.
(15) Cai, L. T.; Tabata, H.; Kawai, T. *Appl. Phys. Lett.* **2000**, *77*, 3105–3106.
(16) Cai, L. T.; Tabata, H.; Kawai, T. *Nanotechnology* **2001**, *12*, 211–216.
(17) Porath, D.; Bezryadin, A.; de Vries, S.; Dekker, C. *Nature* **2000**, *403*, 635–638.
(18) Storm, A. J.; van Noort, J.; de Vries, S.; Dekker, C. *Appl. Phys. Lett.* **2001**, *79*, 3881–3883.
(19) Coffer, J. L.; Bigham, S. R.; Li, X.; Pinizzotto, R. F.; Rho, Y. G.; Pirtle, R. M.; Pirtle, I. L. *Appl. Phys. Lett.* **1996**, *69*, 3851–3853.
(20) Keren, K.; Berman, R. S.; Buchstab, E.; Sivan, U.; Braun, E. *Science* **2003**, *302*, 1380–1382.
(21) Keren, K.; Krueger, M.; Gilad, R.; Ben-Yoseph, G.; Sivan, U.; Braun, E. *Science* **2002**, *297*, 72–75.
(22) Richter, J.; Seidel, R.; Kirsch, R.; Mertig, M.; Pompe, W.; Plaschke, J.; Schackert, H. K. *Adv. Mater.* **2000**, *12*, 507–510.
(23) Gu, J. H.; Tanaka, S.; Otsuka, Y.; Tabata, H.; Kawai, T. *Appl. Phys. Lett.* **2002**, *80*, 688–690.
(24) Lee, H. Y.; Tanaka, H.; Otsuka, Y.; Yoo, K. H.; Lee, J. O.; Kawai, T. *Appl. Phys. Lett.* **2002**, *80*, 1670–1672.
(25) Dittmer, W. U.; Simmel, F. C. *Appl. Phys. Lett.* **2004**, *85*, 633–635.
(26) Bae, A.-H.; Hatano, T.; Numata, M.; Takeuchi, M.; Shinkai, S. *Macromolecules* **2005**, *39*, 1609–1615.
(27) Ma, Y. F.; Zhang, J. M.; Zhang, G. J.; He, H. X. *J. Am. Chem. Soc.* **2004**, *126*, 7097–7101.
(28) Nagarajan, R.; Liu, W.; Kumar, J.; Tripathy, S. K. *Macromolecules* **2001**, *34*, 3921–3927.
(29) Nickels, P.; Dittmer, W. U.; Beyer, S.; Kotthaus, J. P.; Simmel, F. C. *Nanotechnology* **2004**, *15*, 1524–1529.
(30) Chen, W.; Josowicz, M.; Datta, B.; Schuster, G. B.; Janata, J. *Electrochem. Solid-State Lett.* **2008**, *11*, E11−E14.
(31) Datta, B.; Schuster, G. B. *J. Am. Chem. Soc.* **2008**, *130*, 2965–2973.
(32) Lassaroni, R.; De Pryck, A.; Debaisieux, C.; Riga, J.; Verbist, J. *Synth. Met.* **1987**, *21*, 189–195.
(33) Hemetsberger, H.; Knittel, D. *Monatsh. Chem.* **1972**, *103*, 194.

Scheme 1. Synthetic Route Used for the Preparation of 1-(3-Aminopropyl)thieno[3,2-*b*]pyrrole



stable at the temperatures where the oligomerization reactions are carried out. The duplex formed from DNA(3/4), which contains no modified cytosines, was observed to melt (T_m) at 72 °C, whereas DNA(1/4), which contains six modified cytosines, has T_m = 49 °C. Thus, modification of the DNA by incorporation of **TP** monomers results in a considerable stability decrease, but DNA(1/4) clearly exhibits the necessary stability for reaction with HRP at room temperature. We also determined the circular dichroism (CD) spectrum of DNA(1/4), which exhibits bands between 200 and 300 nm that resemble that of normal B-form DNA.[34] These experiments indicate that incorporation of the six **TP** units in DNA(1/4) does not destroy or overly distort the duplex structure.

It is well established that aniline-like monomers covalently attached to DNA can be oligomerized under oxidizing conditions.[30,31] The reaction of DNA(1/4) with H_2O_2 and HRP in citrate buffer at pH 4.5 was monitored by UV−vis spectroscopic measurements taken at 15-min intervals. As is shown in Figure 2, the reaction of DNA(1/4) under these conditions results in the growth of two new absorption bands, one at 547 nm and the other at 806 nm. These absorption bands are readily apparent within 15 min of the start of the reaction, reach a maximum intensity within 1 h, and then remain unaltered for several hours. No such absorption changes are observed when DNA(3/4), which does not contain a thieno[3,2-*b*]pyrrole unit, is subjected to these reaction conditions.

DNA(2/5) contains only one **TP**-containing modified nucleobase. It was examined in a control experiment intended to assess whether the spectral changes observed in the reaction of DNA(1/4) are the result of reactions between several monomer units or an oxidative process involving a single thieno[3,2-*b*]pyrrole group. Treatment of DNA(2/5) with H_2O_2 and HRP does not result in the appearance of significant absorption bands at 547 or 806 nm. Also, the reaction of the single strand DNA(1) with HRP/H_2O_2 results

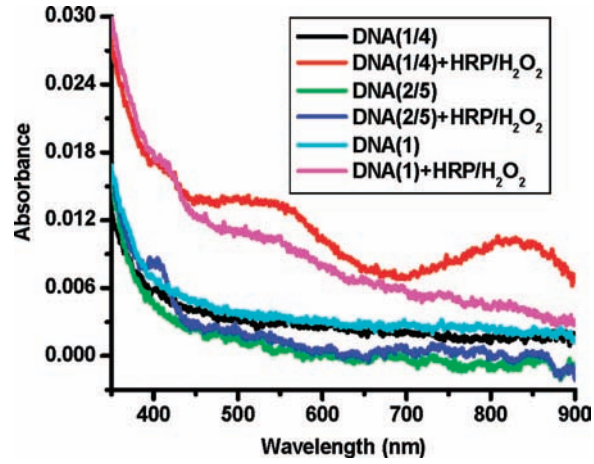


Figure 2. Absorption spectra of DNA(1/4), DNA(2/5), and DNA(1) (5 μM) before and after treatment with HRP/H_2O_2.

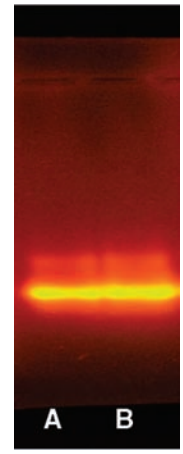


Figure 3. Agarose gel of duplex DNA(1/4) before (A) and after (B) treatment with HRP/H_2O_2.

in a product having an absorption band only at ca. 520 nm (see Figure 2), which is distinctly different from that formed in the reaction of DNA(1/4) duplex.

Melting temperature experiments show that after reaction with HRP/H_2O_2 the T_m of DNA(1/4) is broad with a maximum at 46 °C, which indicates that formation of the conjoined oligomer maintains a duplex structure but that it is distorted. However, attempted hybridization of the product formed from oxidation of single strand DNA(1) with its complement (DNA(4)) did not give a duplex. This finding supports the conclusion that the treatment of the single strand and the duplex with HRP and H_2O_2 give different products.

Finally, agarose gel electrophoresis analysis (see Figure 3 and Supporting Information) of DNA(1/4) after its reaction with HRP/H_2O_2 shows that there has been no cross-linking of the DNA strands or branching of **TP** monomers in the formation of oligomer. These results indicate that oxidation of duplex DNA(1/4) with HRP/H_2O_2 results in the formation

(34) Gray, D. M.; Hung, S.-H.; Johnson, K. H. *Methods Enzymol.* **1995**, *246*, 19–36.

of a short-chain, linear thienopyrrole oligomer that has the absorption spectrum expected for the conducting polymer.

The UV−vis absorption spectrum of poly(thienopyrrole) has not been previously reported. We prepared this compound by standard procedures[35,36] in order to confirm that the absorption changes observed upon oxidation of DNA(1/4) are indeed characteristic of formation of the expected conjoined oligomer. The oxidation of thieno[3,2-*b*]pyrrole (**5**) in acetonitrile with $FeCl_3$ results in the formation of a polymer having optical absorption bands at 546 and 797 nm (see Figure 4). These bands are similar to those that result from the reaction of DNA(1/4) with HRP/H_2O_2, which indicates that the latter process gives a poly(thienopyrrole).[37]

In this work we have successfully demonstrated the DNA-directed synthesis of thienopyrrole oligomers covalently tethered to DNA. This DNA conjoined oligomer system was formed by taking advantage of the highly specific self-assembly properties of DNA. It shows that the formation of DNA-conjoined polymers is a general phenomenon not limited to simple derivatives of aniline. This finding significantly expands the number of possible monomers that may be used to create conjoined oligomers along a pathway defined by DNA. Further studies are underway that include extending the chain length of the oligomer and evaluating the ability of a single polymer molecule to function as an electical conductor.

(35) Li, H.; Lambert, C.; Stahl, R. *Macromolecules* **2006**, *39*, 2049–2055.

(36) Machida, S.; Miyata, S.; Techagumpuch, A. *Synth. Met.* **1989**, *31*, 311–318.

(37) Differences in the absorption maxima of the oligomer bands shown in Figures 2 and 4 likely result because the former is recorded in citrate buffer solution, the oligomer is constrained by its covalent attatchment to DNA, and its chain length is a maximum of 6 monomer units. Whereas the polymer whose spectrum is shown in Figure 4 is dissolved in acetonitrile solution, its structure is unconstrained and it is composed of a variable number of monomer units. The changes in solvent, structure, and length may be responsible for the 10 nm shift in the observed absorption maxima.

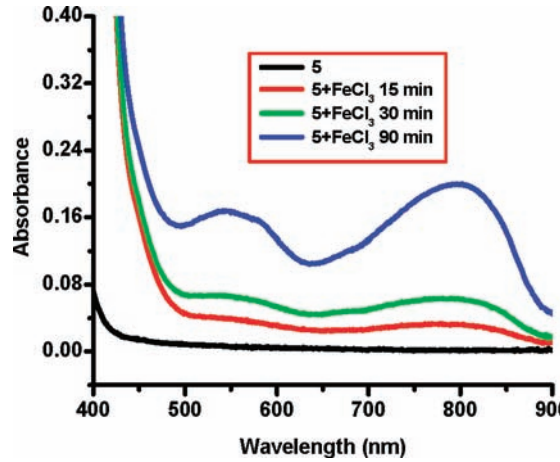


Figure 4. Absorption spectra representing polythienopyrrole formation in acetonitrile with $FeCl_3$ oxidant. The UV region is not shown because the $FeCl_3$ absorption obscures the region.

Acknowledgment. We thank the Vassar Woolley Foundation for financial support, and Dr. Bhaskar Datta and Dr. Joshy Joseph (School of Chemistry and Biochemistry, Georgia Institute of Technology) for assisting with DNA syntheses and for helpful discussions.

Supporting Information Available: Procedures for the synthesis of **TP** monomer, **TP** modified DNA oligomers and agarose gel electrophoresis, ESI mass spectrometric characterization of modified DNA sequences, and thermal melting curves and circular dichroic spectra of DNA(1/4). This material is available free of charge via the Internet at http://pubs.acs.org.

OL801137T