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Amine functionalized polythiophenes: synthesis and formation of chiral, ordered structures on DNA substrates

Paul C. Ewbank,[a,c] Guido Nuding,[a] Hikaru Suenaga,[b] Richard D. McCullough[c,*] and Seiji Shinkai[a,*]

[a]*Chemotransfiguration Project, Japan Science and Technology Corporation, 2432 Aikawa-cho, Kurume, Fukuoka 839, Japan*
[b]*Biotechnology and Food Research Institute, Fukuoka Industrial Technology Center, 1465-5, Aikawa-cho, Kurume, Fukuoka 839, Japan*
[c]*Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA 15213, USA*

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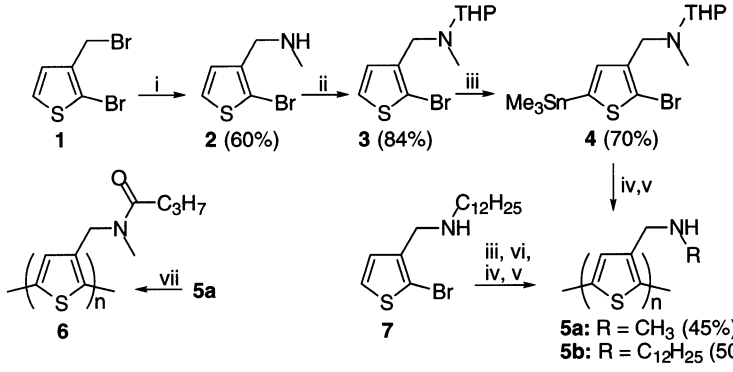
Abstract—Highly regioregular, head-to-tail coupled, 3-(amino functionalized)-polythiophenes can be synthesized by CuO co-catalyzed Stille coupling. Salts of one example are water soluble, and become helically ordered upon addition of DNA. © 2000 Elsevier Science Ltd. All rights reserved.

Polythiophenes (PT) have interesting optical and electronic properties that are sensitive to perturbations in the electronic structure, most notably deconjugative twisting arising from steric interactions.[1–3] Head-to-tail (HT) regioregular PT prepared via cross-coupling have few coupling defects, a common cause of twisting.[2] HTPT can be combined with environmentally-responsive substituents so that exposure to various analytes causes twisting of the polymer backbone, generating a colorimetric response.[1,3,4] We report the synthesis of two highly regioregular, amine functionalized polythiophenes, one of which (**5a**, Scheme 1) was water-soluble and could be induced to chirally order upon binding to DNA.

Compound **1** was dripped into 8 equivalents of CH_3NH_2 in EtOH and stirred for 1 h. The product **2**[5] was extracted into aqueous acid, made basic, re-extracted into Et_2O, then distilled (63°C, 0.34 T) in 60% yield. Compound **2** was dissolved in dry Et_2O, and 2-chlorotetrahydropyran (THP-Cl)[6] was added. The solution was stirred for 2 h, extracted with satd aq. Na_2CO_3, then compound **3**[7] was distilled (0.005 T, 105–115°C) in 84% yield. LDA was added to a cooled (−70°C) solution of **3** in THF, stirred for 1 h, then reacted with Me_3SnCl for 1 h. This was partitioned between Et_2O and satd aq. Na_2CO_3 before compound **4**[8] was distilled (0.005 T, 120°C) in 70% yield.



Scheme 1. *Reagents and conditions*: (i) CH_3NH_2, EtOH, 1 h; (ii) THP-Cl, Et_2O; (iii) (a) LDA, −70°C, 1 h, (b) Me_3SnCl, −70°C, 1 h; (iv) $Pd_2(dba)_3$, PPh_3, CuO, DMF, 100°C; (v) 3 M HCl; (vi) BOC_2O, DMAP; (vii) butyryl chloride.

* Corresponding authors.

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Polymerization[1] of **4** gave an insoluble solid that was hydrolyzed to **5a**. Polymer **5a** was soluble in water solutions (HCl) from pH 1 to 7, while pH 8 or greater gave a red solid. The UV–vis spectrum (sodium phosphate buffer, pH 6.8) exhibited a λ_{max} of 383 nm and a conjugation length of about four thiophene rings.[9] Polymer **5a** was not highly conjugated and adopted a twisted conformation. The product was indirectly characterized as the amide **6**.[10] Integration of the ^{1}H NMR α-methylene region of amide **6a** indicated at least a 90% HT coupling ratio. Since the polymer was not easily fractionated, the isolated polymer shows a broad polydispersity (GPC, $M_n = 1537$, PDI 3.0, DP = 12 rings). This general approach has produced the first amine derivative of a regioregular polythiophene.

A regioregular polythiophene bearing a long alkylamine substituent, which should have improved solubility and processability, was also prepared. Thus monomer **7** was prepared analogously to **2**, and purified by chromatography. Polymerization yielded **5b**[11] which precipitated from solution as a brown tacky material ($M_n = 8769$; PDI = 1.83; DP = 31 rings). Polymer **5b** was soluble in many organic solvents. Solutions of this material were also yellow ($\lambda_{max} = 416$ nm) indicating a twisted polymer. Extractions and dialysis to remove any possible coordinated ions changed neither the solution or thin-film color nor the film consistency, suggesting that the twisted nature of the polymer is a consequence of the proximal amine substituent. Thin films were brown with a broad, optical absorption centered around 402 nm. The color and breadth of the absorption suggested a random mixture of ordered (red) and disordered (yellow) phases. Electrochemical studies show that an irreversible oxidation leads to dark films. Attempts to induce self-assembly in polymer films of **5b** by metal ion complexation have been unsuccessful. Protonated **5b** (w/CH_3COOH) formed tacky, yellow thin films, with a UV–vis spectrum identical to that of the solution.

Compound **5a** was designed to interact with DNA in order to probe the use of polythiophenes as biosensors. Aqueous solutions of polycationic polythiophenes could be ideally suited for binding to a polyanionic template such as DNA. Polymers designed for specific nucleobase recognition have been described;[12,13] however, unlike these models, **5a** could exhibit a non-specific response to all types of DNA. Non-specific binding is advantageous when **5a** is applied as a 'probe' to detect conformational changes in DNA, e.g. the resultant helical ordering in DNA should be spectroscopically detectable by a polythiophene sensor. To check this hypothesis, various DNA samples were added to the HCl salt of **5a** and polymer binding monitored by changes in CD activity (solutions of **5a** exhibit no CD optical activity). In a typical experiment, 0.2 mg polythiophene **5a** was dissolved in aqueous HCl, the solvent was removed, and the resultant brown solid was re-dissolved in 3 mL of 0.001 mol/L Na_2HPO_4/NaH_2PO_4 phosphate buffer (pH 6.8). The solution was titrated with DNA over a range of concentrations from 2×10^{-5} to 2×10^{-4} mol/L, and a distinct response associated with the polymer π–π* absorption was induced. Higher polymer concentrations could not be assayed because an aggregate precipitated upon addition of DNA.

Mixing calf thymus (CT) DNA with polymer **5a** did not perturb the CD spectral features of the DNA sample in the 260 nm region, suggesting that there was no conformational reorganization of DNA due to binding of polythiophene **5a** (Fig. 1). However, a new CD absorption centered at 480 nm was observed indicating that the highly-disordered polythiophene **5a** is chirally ordered by the DNA and was dependent upon DNA

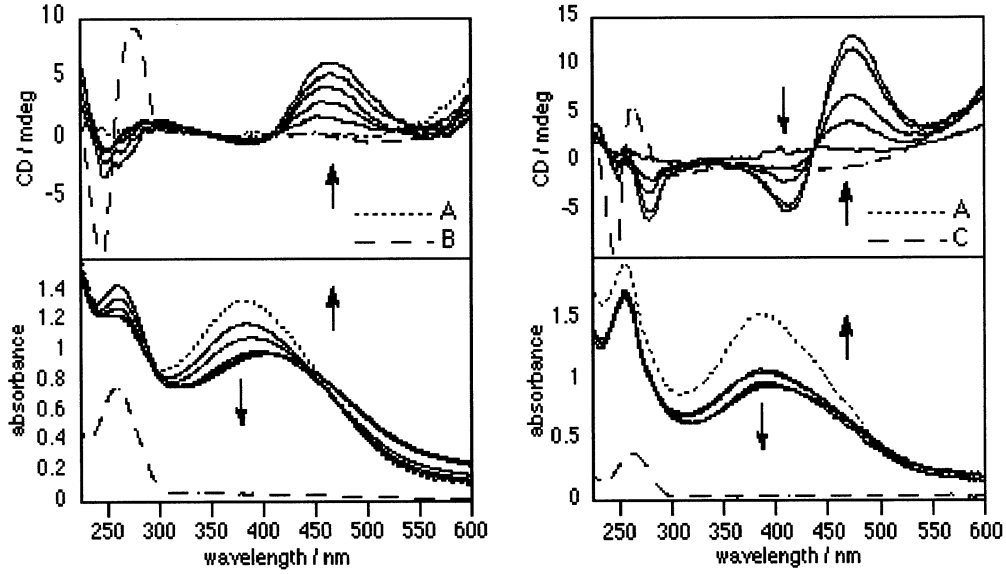

Figure 1. Electronic absorption (lower left) and CD (upper left) for CT DNA (B), **5a** (A), and CT DNA with **5a** (solid lines). Electronic absorption (lower right) and CD (upper right) for AT DNA (C), **5a** (A), and AT DNA with **5a** (solid lines).

concentration. The intensity increased with added DNA as indicated in Fig. 1. In the visible spectrum, growth of a corresponding shoulder in the 480 nm region was noted. This large 100 nm bathochromic shift of the lambda maximum indicated that association with DNA had induced a dramatic ordering of PT **5a**. Although distinct absorptions for DNA and DNA complexed PT were found, a clear isosbestic point could not be measured.

The results were markedly different with weakly H-bonded poly AT DNA (Fig. 1). Polymer **5a** binds to DNA as evidenced by the absorption in the 480 nm region. The data show an exciton coupling band indicative of a very interesting bimolecular ordering of PT chromophores that is being induced by the DNA template. The intensity of the CD absorption varied as indicated with increasing DNA concentration. The shape and sign were characteristic of a right-handed helical form of polythiophene first observed by Meier,[14] and lead to the conclusion that helically ordered conducting polymer aggregates had formed. The normal bisignate CD spectrum of DNA near 260 nm disappeared. Reorganization of the nucleic acid as a consequence of PT binding had also occurred, and may reflect expansion of the major groove to accommodate stacked **5a**.

In summary, we have demonstrated a synthesis for amine functionalized polythiophenes. These polymers do not appear to self-organize, remaining highly twisted and disordered in solution. However, polythiophene **5a** can be induced to order differentially by different DNA structures in water, producing a low energy π–π^* absorption that is comparable to that seen in self-assembled PT thin films.[2] However, in the AT DNA case, the dramatic red shift of the absorption maximum, along with the observed exciton coupling, indicates that the π-systems on adjacent chains in PT are interacting in an organized (e.g. stacked) fashion. This is similar to an observation of DNA templating of dyes by Armitage and co-workers.[15] These preliminary observations demonstrate the remarkable application of highly regioregular polythiophene as a biological chemosensor. We expect that continued investigation of the PT/DNA interaction will allow us to further develop PT biosensors.

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5. Compound **2**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.46 (3H, s), 3.27 (2H, s), 6.91 (d, $J = 6.0$ Hz), 7.19 (d, $J = 6.0$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 35.8, 49.5, 110.3, 125.6, 128.2, 139.8.
6. Synthesis of *2-chlorotetrahydropyran (THP-Cl)*: A sample of 3,4-dihydro-2-*H*-pyran (10 mL, 190.6 mmol) was dissolved in anhydrous Et_2O and cooled in an ice bath. A 1.0 M solution of HCl in Et_2O (Aldrich; 130 mmol) was added dropwise, and the reaction was stirred for 3 h. Concentration and distillation (46°C, 0.16 T) afforded a colorless oil (6.8 g, 56 mmol, 51% yield). This is a simplification of the procedure first reported by: Eliel, E. L.; Daignault, R. A. *J. Org. Chem.* **1965**, *30*, 2450.
7. Compound **3** is a racemic mixture of chiral THP isomers. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38–1.53 (3H, m), 1.58–1.70 (2H, m), 1.82–1.91 (H, M), 2.33 (3H, s), 3.35–3.45 (H, m), 3.62–3.79 (2H, m), 3.86–4.02 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.9, 25.9, 29.9, 36.4, 51.0, 67.6, 92.9, 110.5, 125.2, 128.9, 139.5. Calculated: C, 45.52; H, 5.56; Br, 27.53; N, 4.83; Found: C, 45.3; H, 5.58; Br, 27.60; N, 4.86.
8. Compound **4** is a racemic mixture of chiral THP isomers. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.33 (9H, Sn t, $J = 28.5$ Hz), 1.37–1.54 (3H, m), 1.57–1.71 (2H, m), 1.81–1.92 (H, m), 2.34 (3H, s), 3.35–3.46 (H, m), 3.61 (3H, m), 3.85–4.04 (2H, m), 6.98 (H, Sn t, $J = 13.2$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ −8.3 (Sn t, $J = 707$ Hz), 23.8, 25.8, 29.7, 36.4, 50.7, 67.5, 92.8, 115.2, 136.9 (Sn t, $J = 48.6$ Hz), 138.0, 140.3. Calculated: C, 37.12; H, 5.34; Br, 17.64; N, 3.09. Found: C, 37.29; H, 5.15; Br, 17.87; N, 3.18.
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10. Compound **6**: ^{1}H NMR (300 MHz, 100°C, TCE-d_4) δ 0.94–0.99 (3H, m), 1.62–1.72 (2H, m), 2.30–2.37 (2H, m), 2.91–2.97 (3H, m), 4.50 (non-HT-α-methylene, 0.2H), 4.67 (HT-α-methylene, 1.8H), 7.02 (b, s).
11. Compound **5b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (3H, T, $J = 6.6$ Hz), 1.19–1.27 (18H, m), 1.44–1.51 (2H, m), 3.10–3.21 (2H, m), 4.40 (non-HT-α-methylene, 0.4H, b), 4.54 (HT-α-methylene, 1.6H), 6.98.
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