

Functionalized regioregular polythiophenes: towards the development of biochromic sensors

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Biochromic polythiophenes have been synthesized and the interaction of avidin with the biotinylated water-soluble polymers results in stunning chromic effects, opening the way to the detection of numerous biological components.

Thermally induced chromic effects (thermochromism) have been observed in polythiophene,¹ polysilane² and polydiacetylene³ derivatives. These optical effects have been described in terms of a reversible transition between a coplanar (highly conjugated) and a nonplanar (less conjugated) conformation of the main chain.⁴ Recently, a new chromic phenomena (ionochromism) has been reported in which a regioregular polythiophene derivative bearing long oligo(oxyethylene) side-chains shows stunning chromic effects upon the addition of alkali metal ions,⁵ in agreement with studies by other groups that non-covalent interactions between alkali ions and certain ether⁶ or crown-ether⁷ functionalized polythiophenes induce a slight shift of their absorption maximum in solution. Very recently, this approach has been extended further and photo-induced chromic effects have been produced in a polythiophene bearing an azobenzene moiety,⁸ where irradiation of this polymer induced the transformation of the azobenzene conformation from *trans* to *cis*, resulting in side-chain disorder that induces the rotation of the main chain.

On the other hand, it has been reported recently by Charych *et al.*⁹ that direct colorimetric detection of a receptor–ligand interaction can be obtained through the use of a polydiacetylene film bearing a sialic acid side-chain, where a colour transition from blue to red is obtained upon the complexation of the sialic acid and the influenza virus. More recently, these authors have extended this methodology by the use of UV-polymerized liposomes composed of polydiacetylenes incorporating different head groups that have affinities for various pathogens and important biological receptors.¹⁰ Recently, the incorporation of biotin in a random copolymer of 3-alkylthiophene and 3-(hydroxymethyl)thiophene obtained by the iron trichloride oxidation of mixtures of the two monomers has been reported by Kamath *et al.*¹¹ These authors have also reported the use of such biotinylated copolymers as fluorescence¹² and chemiluminescence-based biosensors.¹³

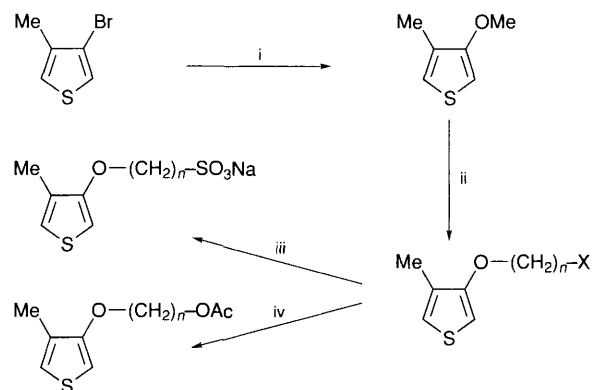
Here we report some new extensions to the use of chromic regioregular polythiophene derivatives by presenting novel synthetic methods which allow the obtention of thiophene monomers incorporating a large number of desirable functions, allowing the tuning of the polymer's properties and characteristics. Biotinylated water-soluble and regioregular polythiophene derivatives have been synthesized and, by taking advantage of the specific complexation between biotin and avidin,¹⁴ interesting chromic effects have been observed, opening the way to the design of simple biochromic sensors.

3-Methoxy-4-methylthiophene was synthesized from 3-bromo-4-methylthiophene in a solution of 1-methylpyrrolidin-2-one (NMP) and sodium methoxide in the presence of copper bromide. The methoxy substituent was subsequently replaced by a longer chain alcohol bearing different functionalities, such as hydroxy groups, halogens, carboxylic acids, amine, *etc.* by the reaction of the appropriate functionalized alcohol with the monomer in toluene with sodium hydrogen

sulfate, following a modified method reported by Zotti *et al.*¹⁵ For instance, 3-(2-bromoethoxy)-4-methylthiophene was obtained by the reaction of 2-bromoethanol with 3-methoxy-4-methylthiophene which can be further reacted with sodium hydrogen sulfite in H₂O–Me₂CO to yield sodium 2-(4-methyl-3-thienyloxy)ethanesulfonate. 3-(6-Chlorohexyloxy)-4-methylthiophene was reacted in DMF with sodium acetate to yield the 3-(6-acetoxyhexyloxy)-4-methylthiophene monomer (Scheme 1).

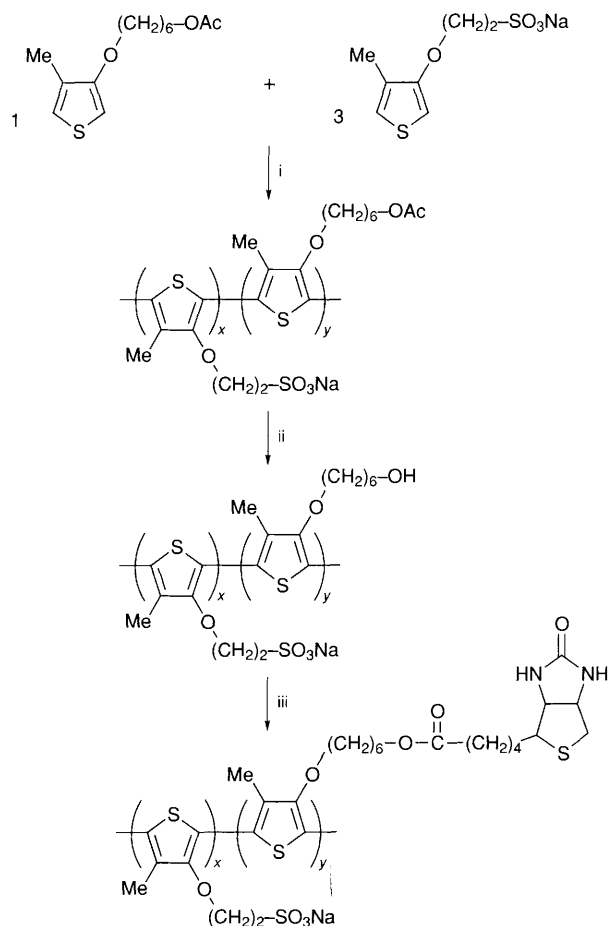
Polymerization of sodium 2-(4-methyl-3-thienyloxy)ethanesulfonate and other similar monomers by iron trichloride in CHCl₃¹⁶ results in self-doped, highly conducting and nearly transparent water-soluble regioregular polythiophene derivatives.¹⁷ Water-soluble functionalizable copolymers have been obtained by oxidation of a mixture of sodium 2-(4-methyl-3-thienyloxy)ethanesulfonate (3 equiv.) and 3-(6-acetoxyhexyloxy)-4-methylthiophene (1 equiv.) monomers with iron trichloride. This copolymer can be further hydrolysed in aqueous sodium hydroxide to yield the hydroxy/sodium sulfonate copolymer. This latter is reacted with D-biotin in CH₂Cl₂¹¹ in the presence of *N,N*-dicyclohexylcarbodiimide (DCC) and 4-pyrrolidinopyridine to yield the biotinylated water-soluble copolymer (Scheme 2). This latter copolymer was fully soluble in water, with a deep violet colour at room temperature which turns yellow upon heating to 95 °C, behaviour very similar to that of the parent homopolymers.

The UV–VIS spectra of a highly diluted aqueous solution of the biotinylated copolymer is shown in Fig. 1, with the absorption maximum around 550 nm. Upon the addition of aqueous avidin, a glycoprotein found in egg white which contains four identical units having a combined molecular mass of 68 000 Daltons with each subunit consisting of 128 amino acids sequences,¹⁸ the solution, initially violet in colour, becomes yellow with the disappearance of the absorption at 550 nm and the appearance of an absorption around 400 nm. The long biotinylated side-chains bind with high affinity to the avidin, which is so huge that it induces rotation of the polythiophene backbone. This behaviour is very similar to the chromic effects observed previously in this family of polymer



Scheme 1 Reagents and conditions: i, CuBr, NaOMe, NMP, 90%; ii, NaHSO₄, HO(CH₂)_nX (X = Br, Cl, OH, SH, NH₂ *etc.*, n = 2, 3, 6), PhMe, 65%; iii, Na₂SO₃, H₂O, Me₂CO, 60%; iv, AcONa, DMF, 85%

derivatives when subjected to external stimuli such as heat, light or ion incorporation. It must be noted that, in some cases and at very low concentration of avidin, a partial precipitation is observed due to the fact that avidin, which possesses four binding sites, can cause non-covalent crosslinking of the polymer. To verify that the complexation of the avidin with the biotin is the driving force behind this new chromic effect, a



Scheme 2 Reagents and conditions: i, FeCl_3 , CHCl_3 , 65%; ii, NaOH , H_2O , 95%; iii, D-Biotin, 4-pyrrolidinopyridine, DCC, CH_2Cl_2 , 85%

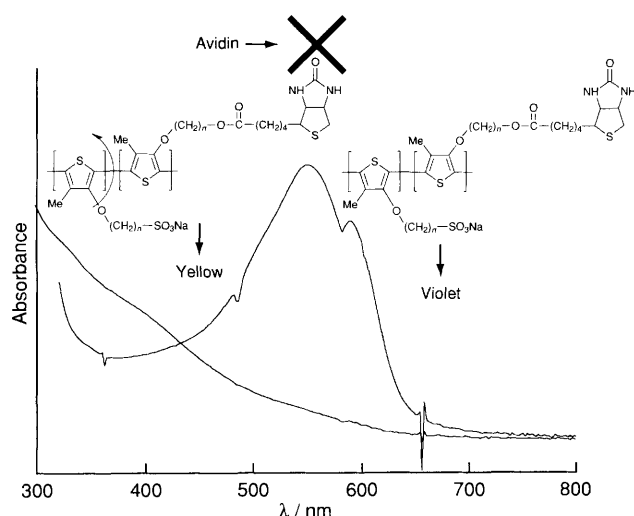


Fig. 1 UV-VIS absorption spectra of (a) biotinylated polythiophene derivative without avidin and (b) after the addition of avidin in water at room temp.

control experiment was carried out, in which a water-soluble copolymer containing no biotin is used. In the absence of biotin, no effect was detected and the absorption spectra remain unchanged, indicating that the introduction of the large avidin macromolecule is responsible for the planar to nonplanar twisting of the main chain and the accompanying chromic effects.

By combining the well known interactions between avidin and biotin and by using the unique chromic characteristics of novel highly regioregular polythiophene derivatives, it is possible to design new sensory devices capable of directly measuring the presence of the widely used and versatile glycoprotein avidin as well as other important biological functions. The precise molecular design of the starting monomers coupled to their asymmetric nature can lead to the design of new biochromic sensors incorporating both the biological molecular recognition component and the optical probe in the same macromolecular assembly by a simple polymerization method.

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