Synthesis and characterization of novel conducting homopolymers based on amino β-styryl terthiophene

Hakim Mehenni and Lê H. Dao

Abstract: Novel ECPs (electronic conducting polymers) based on amino β-styryl-substituted terthiophene (AST) were synthetized by direct electropolymerization. The ECPs were characterized by cyclic voltammetry, square-wave voltammetry, Fourier transform infrared (FTIR) spectroscopy, and X-ray photoelectron spectroscopy (XPS). The poly(amino β-styryl terthiophene) displayed cyclic and square-wave voltammograms with redox peaks that can be assigned to the aminophenyl moiety and the polyterthiophene backbone. The presence of free primary amine groups on the ECP film permitted further biological functionalization (i.e., covalent bonding of various bioreceptors on its surface). The electrochemical performance of Biotin grafted at the AST modified glassy carbon electrode was investigated to detect the Avidin protein in solution by cyclic voltammetry and square-wave voltammetry.

Key words: electronic conducting polymer, electrode surface modification, biosensor, β-styryl-substitued terthiophene, functionalization, cyclic, square-wave voltammetry.

Résumé : Faisant appel à une électropolymérisation directe, on a réalisé la synthèse de nouveaux polymères conducteurs électroniques (PCE) à base de terthiophènes portant des substituants β-styryles aminés (TSA). On a caractérisé les PCE par voltampérométrie cyclique, par voltampérométrie à onde carrée, par spectroscopie infrarouge à transformée de Fourier (IR-TF) et par spectroscopie photoélectronique de rayons X (SPX). Les poly(amino β-styryl terthiophènes) présentent des voltampérogrammes cyclique et à onde carrée comportant des pics redox qui peuvent être attribués à la portion aminophényle ainsi qu'au squelette polyterthiophène. La présence de groupes amines primaires libres sur le film de PCE a permis de réaliser une autre fonctionnalisation biologique, c'est-à-dire la formation de liaisons covalentes de divers biorécepteurs sur sa surface. On a évalué la performance électrochimique de la biotine fixée sur une électrode de carbone vitreuse modifiée par des TSA à détecter la protéine avidine en solution par les techniques de voltampérométrie cyclique et à onde carrée.

Mots-clés : polymère conducteur électronique, modification de la surface d'une électrode, biosenseur, terthiophène portant une β-styryle substitué, fonctionnalisation, voltampérométrie cyclique et à onde carrée.

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Introduction

In recent years, conducting polymers have been the subject of considerable academic and industrial research because of their potential uses in areas such as storage and energy conversion, electrocatalysis, corrosion-resistant materials, and sensors (1–3). Moreover, the use of electronic conducting polymers (ECP) as an immobilization matrix of biomolecules on the electrode surface and as an electronic transducer has taken a considerably important place in the development of biosensors (4, 5). Generally, a biosensor, consisting of a transduction system coupled to a receptor, is prepared by immobilizing the molecular recognition bioreceptor onto the substrate materials (e.g., ECP), and this bioreceptor (e.g., antigen, biotin) recognizes a biomolecule compound (e.g., antibody, avidin) according to its specificity. Therefore, bioreceptors have to be properly immobilized on the sensor surface, and a transducer signal has to be generated, which is related to the concentration of the biomolecule in the sample (6). This kind of device is called reagentless "third-generation biosensor" in which a redox probe is not required (7).

Oligo and polythiophenes have also been functionalized as advanced materials for electrode surface modifications allowing the development of chemosensor (8–10) and biosensor devices (11–13). Effectively, the advantage of making polythiophene films from functionalized terthiophene is to avoid reaching a highly positive potential, which may cause the degradation of delicate functional groups because terthiophene has a lower oxidation potential than thiophene or bithiophene; thus, electrochemical polymerization is easier (14). Moreover, conducting polymers from terthiophene

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are known for their simple functionalization and relatively good stability in air for the neutral and oxidized states (15).

Reeman et al. (16) developed an avidin biosensor based on a direct functionalization of terthiophene by biotin. Biotin was directly bonded to the central thiophene ring in the 3′ position by chemistry synthesis. A redox-inactive polythiophene film was obtained from an electrochemical polymerization of the biotin-functionalized terthiophene alone, because the bonded biotin has an insulating effect on the polythiophene backbone. Consequently, a polythiophene copolymer was realized from the biotin-functionalized terthiophene and 2,2′:5′,2″-terthiophene (1:5 molar ratio, respectively) to obtain a redox-active copolymer film. Another inconvenience was that the polythiohene redox signal was situated at a high potential, approximately 1 V vs. Ag/AgCl, leading usually to the degradation of the bioreceptor, i.e., of biotin. Also, no redox signal was clearly visible to allow the measurement of avidin by using a simple electrochemical method as cyclic voltammetry or square-wave voltammetry; for this reason, impedance was used without forgetting the inconvenience of using chemical synthesis to make biological functionalization of polythiophene film, because this type of biosensor system will be limited only for a single target biomolecule.

A new approach to the functionalization of the surface of polythiophene films appeared with the work of Shim et al. The terthiophene was functionalized by a carboxylic acid group (–COOH) in the 3′ position of the central thiophene ring to prepare the terthiophene 3′-carboxylic acid (TCA). Biosensors of vitellogenin (17), glutamatate (18), and H_2O_2 (19) have been developed after the biological surface functionalization of the poly(TCA) film electrodeposited on an electrode surface. The advantage of biosensor systems based on the poly(TCA) films is to be not restricted to a single target biomolecule. Indeed, various bioreceptors can be immobilized through a covalent bond by interaction with free carboxylic acid groups present on the poly(TCA) film surface. The inconvenience of this biosensor system is that, here also, the polythiophene backbone redox signal is situated at a high potential, which may cause the degradation of the immobilized bioreceptor on its surface.

So, another new approach is to functionalize the terthiophene by a redox group to use another redox signal rather than the polythiophene backbone redox signal to detect the target biomolecule. In this last approach, the terthiophene was used only to bind the redox group on the electrode surface by electropolymerization of the terthiophene moiety. This is the case of the terthiophene functionalization by a ferrocene (20) used to develop a cytochrome C biosensor. Wallace et al. have covalently bonded a ferrocene ring to the central thiophene ring in the 3′ position by a π-conjugated linker, allowing to make a redox-active polythiophene film from the ferrocene-functionalized terthiophene alone. Indeed, the π-conjuguated linker allows an electronic communication between the polythiophene backbone and the ferrocene group, so that the insulation of the polythiophene backone is avoided. The advantage of this last approach is the use of a redox signal from another redox group as electronic transductor (i.e., ferrocene ring) rather than from the polythiophene moiety of the poly(terthiophene 3′-ferrocene) film. Effectively, the ferrocene redox potentials were clearly visible to low potential (towards 0.4 V vs. Ag/AgCl), and were able to be exploited by a simple electrochemical technique such as cyclic voltametry to detect and to measure cytochrome C. On the other hand, the disadvantage of this biosensor system based on the poly(terthiophene 3′-ferrocene) film is that its use is restricted to a single biological target, here Cytochrome C. Also, the ferrocene ring is known for its toxicity in situ to the point that some of these by-products are the object of interest as anticancerous agents (21–23). This constitutes a major disadvantage in the development of biosensors for an in situ application in a medical field.

To bring a solution to the problematic context above, we chose to combine the strategies of Shim and Wallace. Herein, we report the synthesis and the characterization of novel conducting homopolymers based on amino β-styryl terthiophene, which have never been reported. The amino β-styryl terthiophene monomer corresponds to the functionalization of the terthiophene by an amino β-styryl group, covalently bonded to the central thiophene ring in the 3′ position. We have synthetized the 3′-(3-methyl-4-amino-β-styryl)-(2,2′:5′,2″-terthiophene) (monomer **4a**), the 3′-(3-chloro-4-amino-β-styryl)-(2,2′:5′,2″-terthiophene) (monomer **4b**), the 3′-(3-methoxy-4-amino-β-styryl)-(2,2′:5′,2″-terthiophene) (monomer **4c**), and the 3′-(2-methoxy-5-amino-β-styryl)-(2,2′:5′,2″-terthiophene) (monomer **4d**). This new strategy to functionalize the terthiophene by an amino β-styryl group gives a new ECP having the following properties:

(*i*) the ECP from amino β-styryl terthiophene monomers can be electrodeposited on an electrode surface by electropolymerization of the terthiophene moiety in which the redox signal of the aminophenyl moiety is used as an electronic transducer signal to detect and to measure target biomolecules. Also, we have introduced Y substitutes having different inductive, steric, and mesomeric effects on the aminophenyl ring to study their influences on its redox-activity to lower the aminophenyl anodic potential as low as possible towards 0 V vs. Ag/AgCl.

(*ii*) the π-conjuguated linker allows an electronic communication between the aminophenyl moiety and the polythiophene backbone to avoid an insulating effect on the polythiophene backbone. Consequently, copolymerization to obtain a redox-active polymer film is avoided.

(*iii*) the presence of free primary amine groups on the ECP film surface permits further biological functionalization of its surface by covalently bonding various bioreceptors. This biosensor system can be used for various targets biomolecules.

Experimental

Materials and instrumentation

All reagents were purchased from the Sigma-Aldrich Chemical Company (Canada) and used as received: 3-methyl-4-nitrobenzyl bromide, triphenylphosphine (PPh_3), 3-chlorobenzyl chloride, benzene, 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU), iron powder (Fe), ammonium chloride (NH_4Cl), acetic acid glacial (AcOH), sulfuric acid fuming (H_2SO_4), nitric acid (HNO_3), 2-methoxy-5-nitrobenzyl bromide, tin(II) chloride-dihydrate ($SnCl_2{\cdot}2H_2O$), 5-methyl-2-nitrophenol,

dimethyl sulfate ($(CH_3)_2SO_4$), dibenzoyl peroxide, *N*-hydroxysuccinimide (NHS), *N*-bromosuccinimide (NBS), magnesium sulfate ($MgSO_4$), sodium sulfate anhydrate (Na_2SO_4), ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDC), Biotin, and tetrabutylammonium perchlorate (TBAP, electrochemical grade). Dichloromethane (DCM, HPLC grade), avidin from egg white, and the phosphate buffer saline (PBS 0.1 mol/L) of pH 7.4 at 25 °C were purchased from Fischer Scientific (Canada). All experimental solutions were prepared using double-distilled water obtained from a Milli-Q water system.

Nuclear magnetic resonance (NMR) spectra were measured at 400.13 MHz (^{1}H) and 100.6 MHz (^{13}C) on a Bruker ARX 400. All NMR spectra were recorded in deuteroacetone solutions with tetramethylsilane as reference. Signals are described in terms of chemical shifts, multiplicity, and assignment. The following abbreviations were used: s (singlet), d (doublet), dd (doublet of doublet), m (multiplet), and b (broad). In the NMR spectrum interpretation, the phenyl protons will have a letter p accompagnying the position number to avoid the confusion with the protons of the first thiophene ring (e.g., H_{5p} corresponds to the proton of the phenyl in 5 position). Mass measurements were performed on a LC–MSD–TOF instrument from Agilent Technologies in positive electrospray mode. Either protonated molecular ions $(M + H)^+$ or sodium adducts $(M + Na)^+$ were used for peak assignment. The IR spectra were recorded on a NEXUS 670 FTIR apparatus over the 600–4000 cm^{-1} range. The melting points were measured using a Fisher–Johns melting point apparatus. The measuring set-up for the all electrochemical experiments consisted of a three-electrode system in which a glassy carbon electrode or a platinum electrode, Ag/AgCl (saturated KCl) electrode, and a platinum wire (0.5 mm of diameter) or platinum foil (dimension 1 cm × 1 cm, 0.025 mm^2 of thickness) were used as the working, the reference, and the auxiliary electrode, respectively. The cyclic voltammograms (CVs) and the square-wave voltammogram (SWVs) were recorded using a Solartron-SI 1287 potentiostat/galvanostat and a EG & G Princeton Applied Research 273 A one, respectively. The measurements were carried out in a 0.1 mol/L TBAP–DCM solution at room temperature.

General procedure for the synthesis of nitro β-styryl terthiophene (3a, 3b, 3c, and 3d)

A mixture of a terthiophene aldehyde (**1**) prepared according to the literature method (24) (1 mmol), the triphenylphosphonium bromide compounds (**2a**, **2c**, and **2d**) or triphenylphosphonium chloride compound (**2b**) (1.2 mmol), and DBU (1.2 mmol) in dichloromethane (15 ml) was heated under reflux. After 8 h, the reaction mixture was diluted with dichloromethane (100 ml) and washed with a 1 mol/L solution of HCl (2 × 40 ml), 10 % sodium bicarbonate solution (40 ml), and water (60 ml). The organic layer was dried and concentrated to give a crude organic solid. The pure product was obtained by chromatography on a silica-gel column using a dichloromethane/hexane solution (1:1) as eluent.

3′-(3-Methyl-4-nitro-β-styryl)-(2,2′:5′,2″-terthiophene) (3a)

Colour: orange. Solid, yield: 71%, mp 148 °C. IR (KBr): 3104, 3073, 2965, 2906, 1595, 1511 and 1333 (NO_2), 1078, 1039, 959, 844, 827, 753, 721, 706 cm^{-1}. ^{1}H NMR (acetone-d^6, 400 MHz) δ: 8.05 (dd, 1H, H_{5p}), 7.75 (s, 1H, $H_{4'}$), 7.68–7.63 (m, H_{6p}, H_b, H_5), 7.62–7.58 (2s, 1H, H_{2p}), 7.53 (dd, 1H, $H_{5''}$), 7.43–7.35 (m, H_2, H_3, $H_{3''}$), 7.25 (dd, 1H, H_4), 7.15 (dd, 1H, $H_{4''}$), 2.6 (s, 3H, $-CH_3$). ^{13}C NMR (acetone-d^6, 100.6 MHz) δ: 149, 143, 137, 136.8, 136.7, 135, 134.9, 133.7, 131.5, 129.5, 129.1, 128.9, 128.3, 128.1, 126.5, 126, 125.7, 125.5, 125, 123.3, 20 ($-CH_3$). MS *m*/*z*: 410 (MH^+).

3′-(3-Chloro-4-nitro-β-styryl)-(2,2′:5′,2″-terthiophene) (3b)

Colour: orange. Solid, yield: 20%, mp 115 °C. IR (KBr): 3100, 3063, 2960, 2854, 1588, 1515 and 1337 (NO_2), 1042, 953, 844, 831, 815, 746, 683 cm^{-1}. ^{1}H NMR (acetone-d^6, 400 MHz) δ: 8.15 (d, 1H, H_{5p}), 7.85 (d, 1H, H_{2p}), 7.8 (dd, 1H, H_{6p}), 7.75 (s, 1H, $H_{4'}$), 7.68 (d, 1H, H_b), 7.65–7.5 (m, 2H, H_5, $H_{5''}$), 7.45–7.38 (m, 3H, H_a, H_3, $H_{3''}$), 7.25 (dd, 1H, H_4), 7.15 (dd, 1H, $H_{4''}$). MS *m*/*z*: 430 (MH^+).

3′-(3-Methoxy-4-nitro-β-styryl)-(2,2′:5′,2″-terthiophene) (3c)

Colour: orange. Solid, yield: 15%, mp 148 °C. IR (KBr): 3100, 3082, 2965, 2930, 2843, 1595, 1504 and 1343 (NO_2), 1086, 1021, 830, 704 cm^{-1}. ^{1}H NMR (acetone-d^6, 400 MHz) δ: 7.89 (d, 1H, H_{5p}), 7.79 (d, 1H, H_b), 7.72 (s, 1H, $H_{4'}$), 7.6 (d, 1H, H_{2p}), 7.55–7.5 (m, 3H, H_a, H_5, $H_{5''}$), 7.44 (d, 1H, H_{6p}), 7.43–7.34 (m,2H, H_3, $H_{3''}$), 7.29 (dd, 1H, H_4), 7.15 (dd, 1H, $H_{4''}$), 4.04 (s, 3H, OCH_3). ^{13}C NMR (acetone-d^6, 100.6 MHz) δ: 149, 143, 137, 136.8, 136.7, 135, 134.9, 133.7, 131.5, 129.5, 129.1, 128.9, 128.3, 128.1, 126.5, 126, 125.7, 125.5, 125, 123.3, 20 ($-CH_3$). MS *m*/*z*: 426 (MH^+).

3′-(2-Methoxy-5-nitro-β-styryl)-(2,2′:5′,2″-terthiophene) (3d)

Colour: yellow. Solid, yield: 61%, mp 115 °C. IR (KBr): 3108, 3082, 2969, 2939, 2843, 1582, 1513 and 1339 (NO_2), 1095, 1026, 826, 743, 704 cm^{-1}. ^{1}H NMR (acetone-d^6, 400 MHz) δ: 8.38(d, 1H, H_{6p}), 8.19 (dd, 1H, H_{4p}), 8.01 (d, 1H, H_b), 7.8 (s, 1H, $H_{4'}$),7.65 (m, 2H, H_5, $H_{5''}$), 7.35 (d, 1H, H_a), 7.25 (m, 3H, H_{3p}, H_3, $H_{3''}$), 7.05 (m, 2H, H_4, $H_{4''}$), 4.04 (s, 3H, OMe). MS *m*/*z*: 426 (MH^+).

General procedure for the synthesis of amino β-styryl terthiophene monomers (4a, 4b, 4c, and 4d)

Amino-β-styryl terthiophene compounds **4a** and **4b** were obtained by the reduction of the nitro group of the corresponding compounds **3a** and **3b** using iron powder (Fe) in presence of NH_4Cl.

To nitrophenyl compounds (1 mmol) in THF/EtOH/H_2O with the ratio of 3:3:2 were added iron powder (3 mmol) and NH_4Cl (0.6 mmol). The reaction was stirred at 85 °C for 1 h, cooled to room temperature, and filtered through a Celite column. The filter cake was washed with $CHCl_3$, and the filtrate was concentrated to a low volume, diluted with CH_3Cl, and washed with H_2O. The organic extracts were dried over $MgSO_4$ and filtered. After removing the solvent, the compounds were separated by a silica column with EA:CH_2Cl_2 ratio of 2:1, then pure EA. After purification, the compounds were obtained with good yields. In this reduction process, to improve the solubility of the materials, the THF was used as co-solvent

3′-(3-Methyl-4-amino-β-styryl)-(2,2′:5′,2″-terthiophene) (4a)

Colour: brown. Liquid, yield: 100%. IR (NaCl): 3473 and 3373 (NH_2), 3104, 3073, 2965, 2926, 2873, 1604, 1260, 1082, 1039, 960, 821, 760, 695 cm^{-1}. ^1H NMR (acetone-d^6, 400 MHz) δ: 7.66 (dd, 1H, $H_{5''}$), 7.5 (dd, 1H, H_5), 7.43–7.36 (m, 2H, $H_{3''}$, H_3), 7.34 (d, 1H, H_a), 7.31 (d, 1H, H_b), 7.25–7.18 (m, 2H, $H_{4''}$, H_4), 7.16 (s, 1H, $H_{4'}$), 7.14 (m, 2H, H_{5p}, H_{6p}), 6.7 (d, 1H, H_{2p}), 3.8 (sl, 2H, $–NH_2$), 2.8 (s, 3H, $–CH_3$). MS *m*/*z*: 380 (MH^+, 100%).

3′-(3-Chloro-4-amino-β-styryl)-(2,2′:5′,2″-terthiophene) (4b)

Colour: brown. Liquid, yield: 100%. IR (NaCl): 3465 and 3373 (NH_2), 3104, 3069, 2960, 2921, 2852, 1613, 1265, 1095, 1043, 956, 817, 760, 700 cm^{-1}. ^1H NMR (acetone-d^6, 400 MHz) δ: 7.62 (dd, 1H, $H_{5''}$), 7.5 (dd, 1H, H_5), 7.45 (d,1H, H_b), 7.37 (dd, 1H, $H_{3''}$), 7.33 (dd, 1H, H_3), 7.31 (d, 1H, H_a), 7.22 (m, 2H, $H_{4''}$, H_4), 7.19 (s, 1H, $H_{4'}$), 7.14 (m, 2H, H_{2p}, H_{6p}), 6.9 (d, 1H, H_{5p}), 3.85 (sl, 2H, $–NH_2$). MS *m*/*z*: 400 (MH^+, 100%).

For the amino-β-styryl terthiophene compounds 4c and 4d, the nitro groups of **3c** and **3d** compounds were reduced by $SnCl_2{\cdot}2H_2O$. To nitrophenyl compounds (1 mmol) in $EtOH/CH_2Cl_2$ with the ratio of 5:2 were added $SnCl_2{\cdot}2H_2O$ (12 mmol). The reaction was stirred at 90 °C for 17 h, cooled to room temperature, and then 1 g sodium bicarbonate was added. The reaction mixture was stirred during 30 min and then diluted with dichloromethane (100 ml) and filtered through Celite. The filtrate was washed with 5% sodium bicarbonate solution (2 × 100 mL), washed with H_2O (1 × 100 mL), and then dried over anhyd. Na_2SO_4. After removing the solvent, the product was obtained by column chromatography (silica gel) with ethyl acetate as eluent.

3′-(3-Methoxy-4-amino-β-styryl)-(2,2′:5′,2″-terthiophene) (4c)

Colour: brown. Liquid, yield: 74%. IR (NaCl): 3481 and 3373 (NH_2), 3108, 3073, 2973, 2930, 2873, 1617, 1252, 1126, 1056, 834, 695 cm^{-1}. ^1H NMR (acetone-d^6, 400 MHz) δ: 7.33 (s, 1H, $H_{4'}$), 7.6–7.55 (m, 2H, H_5, $H_{5''}$), 7.52 (d, 1H, H_b), 7.53–7.2 (m, 5H), 7.2–7.1 (m, 3H, phenyl), 3.95 (s, 3H, OCH_3), 3.6 (sl, 2H, NH_2). MS *m*/*z*: 396 (MH^+, 100%).

3′-(2-Methoxy-5-amino-β-styryl)-(2,2′:5′,2″-terthiophene) (4d)

Colour: brown. Liquid, yield: 54%. IR (NaCl): 3443 and 3360 (NH_2), 3104, 3069, 2960, 2926, 2856, 1617, 1265, 1100, 1030, 808, 756, 700 cm^{-1}. ^1H NMR (acetone-d^6, 400 MHz) δ: 7.78 (dd, 1H, $H_{5''}$), 7.68 (dd, 1H, H_5), 7.63 (dd, 1H, $H_{3''}$), 7.5 (dd, 1H, H_3), 7.42 (s, 1H, $H_{4'}$), 7.4 (d, 1H, H_a), 7.33 (d, 1H, H_b), 7.23–7.15 (m, 2H, H_4, $H_{4''}$), 6.95 (d, 1H, H_{6p}), 6.8 (dd, 1H, H_{3p}), 6.64 (dd, 1H, H_{4p}), 4.3 (sl, 2H, NH_2), 3.8 (s, 3H, OMe). MS *m*/*z*: 396 (MH^+, 100%).

General procedure for the synthesis of ylides compounds 2a, 2b, 2c, and 2d

A mixture of the 3-methyl-4-nitrobenzyl bromide (1 mmol) or 2-methoxy-5-nitrobenzyl bromide (1 mmol) and PPh_3 (1.2 mmol) in benzene was heated under reflux. After 3 h, the pure product **2a** or **2d** was recuperated as a white solid by removal of the solvent. The ylide **2b** was synthesized from the commercially available 3-chlorobenzyl chloride according to the literature method (25) to prepare the 3-chloro-4-nitrobenzyl chloride and then according the synthesis of **2a** and **2d**. In this reaction, the **2b** and **2b′** were given. Because of the difficulty of the separation, the mixture was not separated before used for the Wittig reaction. After the Wittig reaction, the pure desired isomer **3b** was isolated by TLC plates of silica gel (20 × 20 cm, thickness of 2000 μm), with cyclohexane/acetone (10:1) as eluent, from the reaction mixture following removal of the major isomer (**3b′**) by recrystallization from dichloromethane/ether. The ylide **2c** was synthesized according to the literature method (26) to prepare the 3-methoxy-4-nitrobenzyl bromide and then according the synthesis of **2a** and **2d**.

Electrochemistry

Electrochemical polymerization of monomers 4a, 4b, 4c, and 4d

The monomers **4a**, **4b**, **4c**, and **4d** were electrodeposited on a polished glassy carbon electrode by electropolymerization of 5 mmol/L of the monomer in a 0.1 mol/L TBAP–DCM solution by potential cycling between 0.0 and 1.1 V (vs. Ag/AgCl) at 10 mV s^{-1}. After the polymerization, the electrode surface was rinsed with DCM to remove the excess monomer. Post-polymerization cyclic voltammograms and square-wave voltammograms of homopolymers **4a**, **4b**, **4c**, and **4d** were investigated in 0.1 mol/L TBAP–DCM solution.

The neutral polymers were characterized by reflectance FTIR experiments and X-ray photoelectron spectroscopy (XPS). FTIR spectra of polymers films on gold-coated glass electrodes were recorded on NEXUS 670 FTIR over the range 600–4000 cm^{-1}. XPS examination were carried out on the polymers films grown on ITO-coated glass electrodes using a Escalab 220i XL spectrometer.

Surface biofunctionalization

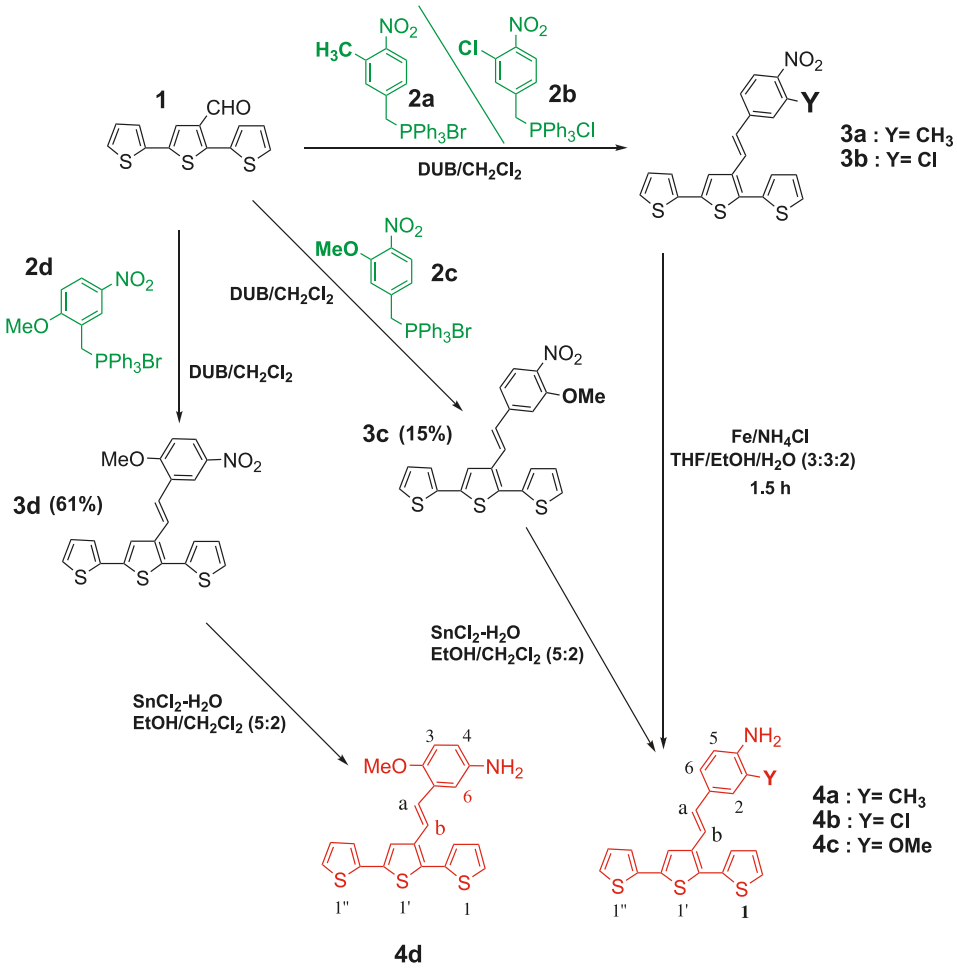
Poly(**4b**)- and poly(**4d**)-coated electrodes were immersed in a 0.1 mol/L phosphate buffer saline (PBS) solution (pH 8) containing 0.05 mol/L EDC and 0.05 mol/L biotin for 24 h at 40 °C. The biotin was immobilized through the formation of covalent bond with free primary amine groups on the polymer surface. These electrodes were then rinsed thoroughly with the same buffer to remove the non immobilized biotin, and their cyclic voltammograms (CVs) and square-wave voltammograms (SWVs) were recorded. Finally, this poly(**4c**)/ and poly(**4d**)/biotin-modified electrode were incubated in a 0.1 mol/L phosphate buffer saline (PBS) solution of 3.10^{-4} mol/L avidin for 15 min at room temperature to block active sites of avidin by a specific interaction with biotin. These electrodes were removed, rinsed with the same buffer to remove the non reacted avidin with biotin, and allowed to dry. Then, their CVs and SWVs were recorded.

Results and discussion

Synthesis of amino β-styryl terthiophene monomers (4a, 4b, 4c, and 4d)

Scheme 1 shows the synthetic routes for amino β-styryl terthiophene monomers (**4a**–**4d**). The terthiophene aldehyde precursor (**1**) was prepared according to ref. 24. Starting from compound **1**, two steps are necessary to prepare the monomers **4a**, **4b**, **4c**, and **4d**. The first step is a Wittig reac-

Scheme 1. The synthesis of the monomers.



tion to make the conjugated link between the aminophenyl moiety and the terthiophene. Compound **1** was treated with various ylide compounds (**2a**–**2d**) in DCM in presence of DBU to obtain the nitro β-styryl terthiophene compounds (**3a**–**3d**). The second step is a reduction of the nitro group of the compounds **3a**, **3b**, **3c**, and **3d** to provide the final amino β-styryl terthiophene monomers (**4a**–**4d**). Iron powder (Fe) was used as reducing agent for **3a** and **3b** and tin(II) chloride dihydrate ($SnCl_2{\cdot}2H_2O$) for **3c** and **3d**. The ylide compounds (**2a**–**2d**) were synthesized according to Scheme 2. The ylide compounds **2a** and **2b** were prepared from commercially available materials, the 3-methyl-4-nitrobenzyl bromide (**a**) and the 2-methoxy-5-nitrobenzyl bromide (**d**), which were treated by PPh_3 in benzene. The ylide **2b** was prepared according to ref. 27 to synthesize the 3-chloro-4-nitrobenzyl chloride, which was treated by PPh_3 in benzene. The ylide **2c** was synthesized according to ref. 28 to prepare the 3-methoxy-4-nitrobenzyl bromide (**c**), which was treated by PPh_3 in benzene.

Synthesis of poly(amino β-styryl terthiophene) (poly(4a), poly(4b), poly(4c), and poly(4d))

Monomers **4a**, **4b**, **4c**, and **4d** were electrodeposited on glassy carbon electrodes by cyclic voltammetry between 0 and 1.1 V (vs. Ag/AgCl). Figure 1 shows the CVs for the formation of poly(**4d**) film on a glassy carbon electrode. The cyclic voltammograms show the expected increase in current with increasing number of potential cycles, indicating the growth of electronic conducting polymers (ECP). The electrochemical polymerization of monomers **4a**, **4b**, **4c**, and **4d** results in the formation of a black uniform polymer film on the electrode surface.

Electrochemical characterization of poly(amino β-styryl terthiophene) films

Figure 2 shows cyclic voltammograms (CVs) of poly(**4a**), poly(**4b**), poly(**4c**), and poly(**4d**) films at scan rate 5 mV s^{-1} by potential cyclic between 0 and 1.0 V (vs. Ag/AgCl). The scanning from 0 to 1.0 V shows clearly two anodic peaks for poly(**4c**) and poly(**4d**) films, which we named a and c, and which we attributed to the oxidation of the aminophenyl moiety and to the polaronic oxidation of the polythiophene backbone, respectively. Indeed, it is reported that the aminophenyl moiety can be oxidized to give a radical cation (27). The oxidation removes an electron from the free electrons doublet of the nitrogen in its neutral state (NH_2), to give a radical cation nitrogen ($NH_2^{\bullet+}$). We were able to attribute the origin of the peaks a and c compared with the cyclic voltammogram of a polyterthiophene film prepared in the same conditions, and also by using literature results for polyterthiophene for which it is reported (28) that the polaronic oxidation peak is situated towards 1.05 V vs. SCE

Scheme 2. Synthesis of the ylide compounds.

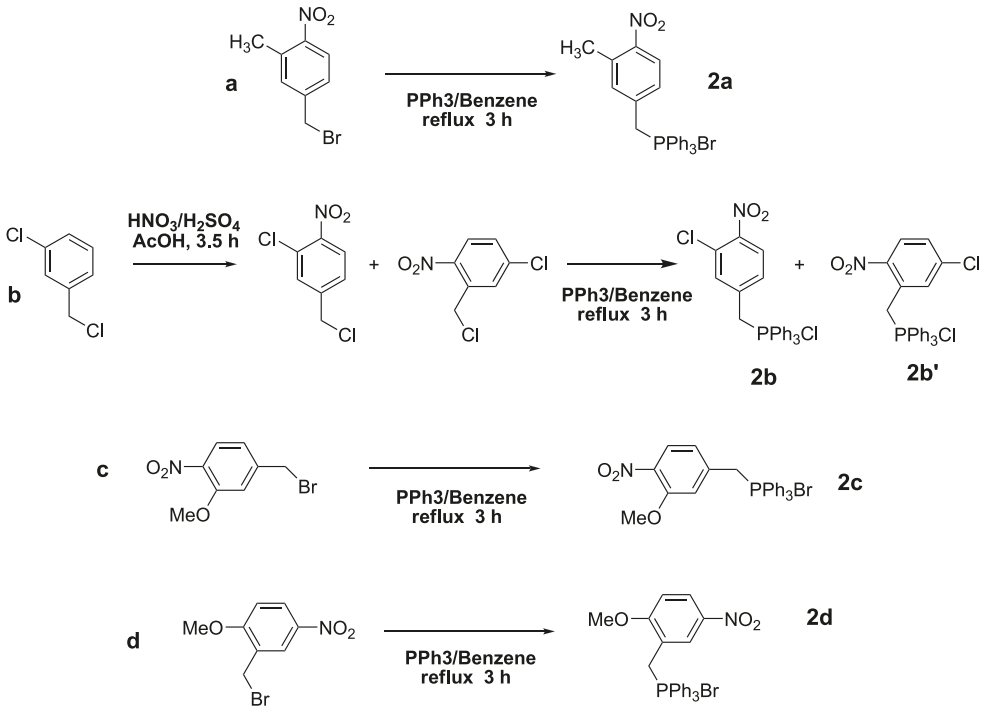


Fig. 1. Growth of poly(**4d**) by repetitive-scan cyclic voltammetry on a 7.068 mm^2 glassy carbon electrode between 0.0 and 1.1 V, auxiliary electrode: Pt foil, reference electrode: Ag/AgCl, scan rate: 10 mV s^{-1}m, 5 mmol/L monomer **4d**, 0.1 mol/L TBAP–DCM.

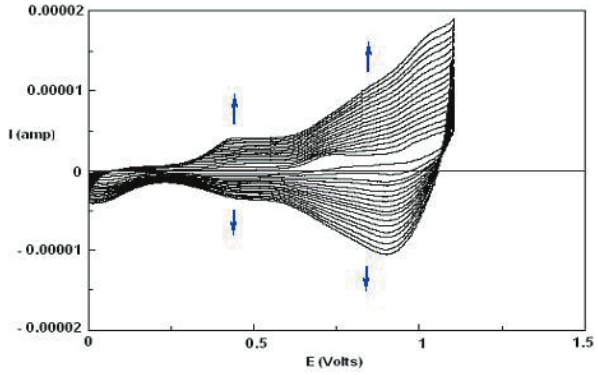
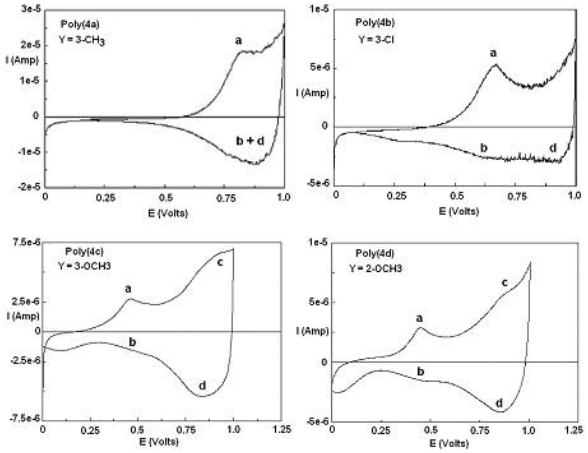


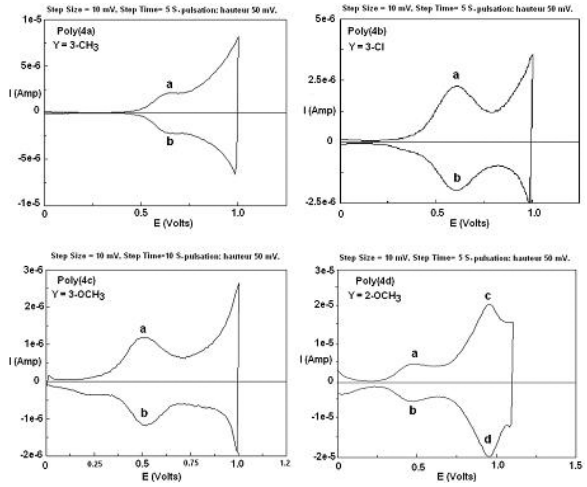
Fig. 2. Cyclic voltammogram of poly(**4a**), poly(**4b**), poly(**4c**), and poly(**4d**) films between 0.0 and 1.0 V, scan rate: 5 mV s^{-1} on a 7.068 mm^2 glassy carbon working electrode, auxiliary electrode: Pt wire, reference electrode: Ag/AgCl, 0.1 mol/L TBAP–DCM.



(corresponding to 1.09 V vs. Ag/AgCl). For poly(**4a**) and poly(**4b**) films, we can only see the peak a and an oxidation slope attributed to the polythiophene backbone oxidation. When the scanning direction is inverted from 1.0 to 0.0 V vs. Ag/AgCl, we see two cathodic peaks, d and b, for poly(**4c**) and poly(**4d**) films, which we attributed to the reduction of the polythiophene backbone and to the aminophenyl moiety reduction, respectively. The top of the peak b will be more clearly visible by SWV for the poly(**4b**) film. For the poly(**4a**) film, the last peaks are not distinctly visible because they are overlapping. So, the introduction of Y substitutes in the aminophenyl ring has for consequence to make the aminophenyl redox couple (a/b) visible and different from the polythiophene backbone redox couple (c/d). It lowers its anodic potential, in the first place situated at 0.98 V vs. Ag/AgCl (corresponding to the aminophenyl anodic potential (29)) towards a lower potential, especially when Y = OCH_3, which is a strong electronic donor and for which the aminophenyl anodic potential is slightly below 0.5 V vs. Ag/AgCl.

The square-wave voltammetry allows to eliminate the current overload (capacitive current *Ic*) visible in cyclic voltammetry (30–32). In other words, we can subtract *Ic* from the total current measured. Consequently, we used it to see clearly the redox couple (a/b) attributed to the aminophenyl moiety of poly(**4a**), poly(**4b**), poly(**4c**), and poly(**4d**) films. The square-wave voltammograms of polymer films are reported in Fig. 3. During the oxidation scanning from 0.0 to 1.0 V vs. Ag/AgCl, we can observe two

Fig. 3. Square-wave voltammograms of poly(**4a**), poly(**4b**), poly(**4c**), and poly(**4d**) films between 0.0 and 1.0 V on a 7.068 mm^2 glassy carbon working electrode, auxiliary electrode: Pt foil, reference electrode: Ag/AgCl, 0.1 mol/L TBAP–DCM.



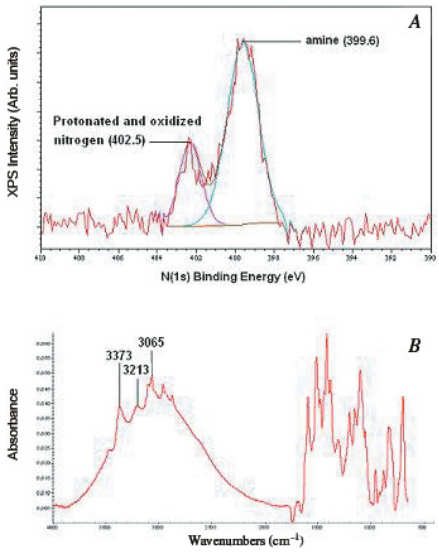
oxidation peaks, a and c, for the poly(**4d**) film. The anodic peak a situated towards 0.5 V vs. Ag/AgCl corresponds to the oxidation of the aminophenyl moiety, and the anodic peak c situated towards 0.9 V vs. Ag/AgCl corresponds to the polaronic oxidation of the polythiophene backbone. But for poly(**4a**), poly(**4b**), and poly(**4c**) films, we observe only the anodic peak a, situated towards 0.6 V vs. Ag/AgCl for poly(**4a**) and poly(**4b**), and towards 0.5 V vs. Ag/AgCl for poly(**4c**). In higher potentials, we observe an oxidation slope attributed to the oxidation of the polythiophene backbone. During the reduction scanning from 1.0 to 0.0 V vs. Ag/AgCl, we can observe two reduction peaks, d and b, for the poly(**4d**) film, corresponding to the reduction of the polythiophene backbone and to the aminophenyl moiety reduction, respectively. But for the poly(**4a**), poly(**4b**) and poly(**4c**), we observe a reduction slope corresponding to the reduction of the polythiophene backbone and the cathodic peak b.

Spectroscopic FTIR and XPS characterization

The studies of the poly(**4a**), poly(**4b**), poly(**4c**), and poly(**4d**) films by FTIR and XPS techniques, clearly show that there are free primary amine groups on the polymer film surface after electrochemical polymerization. This point proves that the amino β-styryl moiety is not destroyed during electropolymerization, and it does not bind anything by the amine groups. As an example, the XPS spectrum of the poly(**4d**) film and the FTIR spectrum of the poly(**4b**) film are shown in Fig. 4. FTIR spectrum of poly(**4b**) film grown on gold-coated glass shows two strong sharp peaks at 3373 and 3213 cm^{-1} (amine I band, N–H stretching vibration (33)) corresponding to the primary amine groups on the aminophenyl moiety, and a C–H stretching vibration of the aromatic ring (34) is also observed at 3065 cm^{-1}.

XPS spectrum shows the deconvoluted N1s spectrum of the poly(**4d**) film surface grown on ITO-coated glass electrode. The two features at 402.5 and 399.6 eV must be ascribed to a cationic nitrogen species (NH_3^+ and $NH_2^{\bullet+}$) and an amine I species (NH_2), respectively (35, 36).

Fig. 4. (*A*) XPS spectrum of the poly(**4d**) film and (*B*) FTIR spectrum of the poly(**4b**) film, grown on ITO-coated glass and gold-coated glass, respectively, at room temperature.



Biotin-modified electrodes

We have begun to investigate the possibility of using the poly(**4b**) and poly(**4d**) films in biosensor devices in which the redox signal of the aminophenyl moiety is used as an electronic transducer signal to detect a target biomolecule. We chose to begin with biotin–avidin interaction. Avidin is a highly stable glycoprotein and is able to be specifically bound by biotin to form an exceedingly strong complex (37). The steps used to link the biotin to the poly(amino β-styryl terthiophene)-coated electrode surface are summarized in Fig. 5. This procedure has the advantage of using simple conditions and of forming a covalent bond. The cyclic and the square-wave voltammograms of the poly(**4b**)- and poly(**4d**)-coated working electrode, before and after immobilization of biotin on their surfaces, are reported in Fig. 6*a* and Fig. 7*a*, respectively. We can observe for both polymer films that the biotin immobilization causes a shift towards a higher potential of the anodic peak initially attributed to the oxidation of the aminophenyl moiety. After biotin immobilization, the anodic peak corresponds to the oxidation of the amidophenyl resulting on the polymer film surface. Indeed, it is reported (29) that the amidophenyl anodic peak (Acetoamidophenyl *E*pA = + 1.24 V vs. Ag/AgCl) is higher than that of the aminophenyl (*E*pA = + 0.98 V vs. Ag/AgCl).

The cyclic and the square-wave voltammograms of the poly(**4b**)/ and poly(**4d**)/biotin-modified-coated working electrode, before and after exposure to a solution of 3.10^{-4} mol/L avidin in PBS 0.1 mol/L for 15 min are shown in Fig. 6*b* and Fig. 7*b*, respectively. We can observe in both cases, i.e., by CV or SWV, that the exposure of the poly(**4b**)/ or poly(**4d**)/biotin-modified-coated working electrode has a drastic effect upon its electrochemical response causing a significant diminution of the oxidation current measured on the anodic peak. This unusual voltammetry response after avidin exposure is explained by the presence of avidin covering the polymer film surface and thus causing the reduc-

Fig. 5. Schematic representation of the immobilization of receptive biomolecule (biotin) via the activation of carboxylic functional groups (actived by EDC) of the biotin.

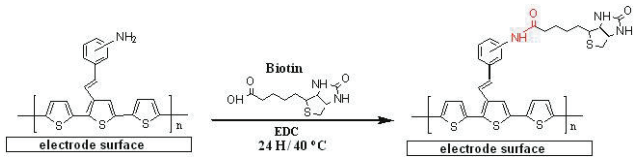
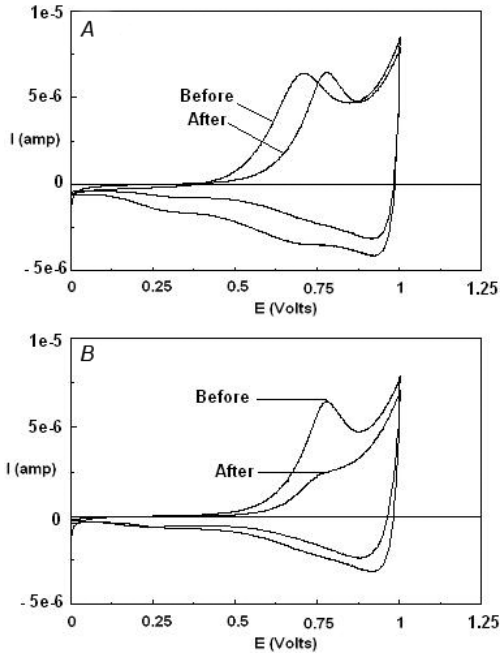


Fig. 6. (*a*) Cyclic voltammograms of poly(**4b**)-coated platinum working electrode before and after immobilization of biotin. (*b*) Cyclic voltammograms of poly(**4b**)/biotin-modified-coated platinum working electrode before and after exposure in a solution of avidin 3.10^{-4} mol/L in PBS 0.1 mol/L during 15 min, auxiliary electrode: Pt foil, reference electrode: Ag/AgCl, scan rate: 25 mV s^{-1}, 0.1 mol/L TBAP–CH_2Cl_2.

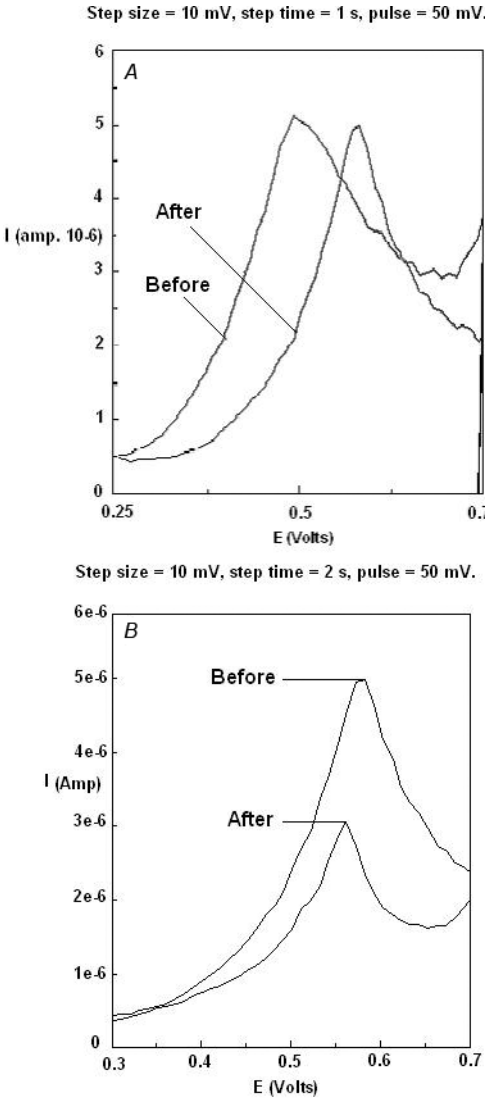


tion in the number of amidophenyl moieties able to be oxidized at the polymer-film surface.

Conclusions

The terthiophene was successfully functionalized by an amino β-styryl moiety. We synthesized four new monomers according to the Y substituent, the 3′-(3-methyl-4-amino-β-styryl)-(2,2′:5′,2″-terthiophene) (monomer **4a**), the 3′-(3-chloro-4-amino-β-styryl)-(2,2′:5′,2″-terthiophene) (monomer **4b**), the 3′-(3-methoxy-4-amino-β-styryl)-(2,2′:5′,2″-terthiophene) (monomer **4c**), and the 3′-(2-methoxy-5-amino-β-styryl)-(2,2′:5′,2″-terthiophene) (monomer **4d**). The homopolymers from monomers **4a**, **4b**, **4c**, and **4d** have been successfully electrosynthesized. The electrochemical studies of these polymer films display both the aminophenyl moiety redox couple as well as the polythiophene backbone. The cyclic and square-wave voltammogram responses of the poly(**4b**)/ and poly(**4d**)/biotin-modified film on working

Fig. 7. (*a*) Square-wave voltammograms of poly(**4d**)-coated glassy carbon working electrode before and after immobilization of biotin. (*b*) Square-wave voltammograms of poly(**4d**)/biotin-modified-coated glassy carbon working electrode before and after exposure in a solution of avidin 3.10^{-4} mol/L in PBS 0.1 mol/L during 15 min, auxiliary electrode: Pt foil, reference electrode: Ag/AgCl, 0.1 mol/L TBAP–CH_2Cl_2.



electrode, after exposure to an avidin solution, suggest that these polymer films have potential application as biosensors.

We project to develop an avidin biosensor by using this last system to measure an unknown avidin concentration. Some of our results will be submitted soon. To investigate the possibility of using the poly(amino β-styryl terthiophene) films for various target biomolecules, we project also to make futher functionalization on the last ECPs surfaces by other bioreceptors.

Acknowledgements

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