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Novel electro-oxidizable chiral *N*-substituted dicarbazoles and resulting electroactive films for covalent attachment of proteins

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Abstract

Novel chiral electro-oxidizable dicarbazoles functionalized by *N*-succinimidyl/phthalimidyl or pentafluorophenyl carboxylates **6a–c** were synthesized from L-lysine methyl ester **1** and characterized electrochemically. The corresponding chiral polycarbazole films **8a–c** constitute polymers suitable for covalent grafting of proteins, as demonstrated for bovine serum albumin as a model compound.

Keywords: biosensors; polycarbazoles; chiral polymers; bovine serum albumin; protein attachment.

Recently developed microfabrication techniques, like microphotolithography, have facilitated the specific design and preparation of microdevices that integrate the recognition properties of biological macromolecules with the exquisite sensitivity of electrochemical transducers.[1] These transducers comprise of conductive microsurfaces, which can be modified by appropriate ligands, enabling them to detect and measure biological interactions occurring on them. Particularly attractive as microdevice transducers are microelectrodes covered by electropolymerized conductive polymers (ECPs) capable of immobilizing biomolecules.[2,3] The main advantages of ECPs are: (1) their capacity to be mildly electrogenerated onto microelectrodes of defined geometries; (2) their electroconductivity, allowing strictly controlled growth of the polymeric layers; and (3) the relative ease of chemically modifying the electro-polymerizable precursor monomers.[3,4]

Basically, two main strategies can be envisaged for stable immobilization of a biomolecule onto a microelectrode via ECPs: entrapment of the biomolecule within a polymer as it grows during the electrochemical process or covalent attachment of the biomolecule onto a preformed functionalized polymer.[3–5] Interestingly, the former procedure has been plagued by a number of problems: difficult access of analytes to the immobilized proteins and reduced activities of entrapped enzymes.[4b] Additionally, it should be noted that the direct grafting of precursor monomer-linked biological macromolecules has also been

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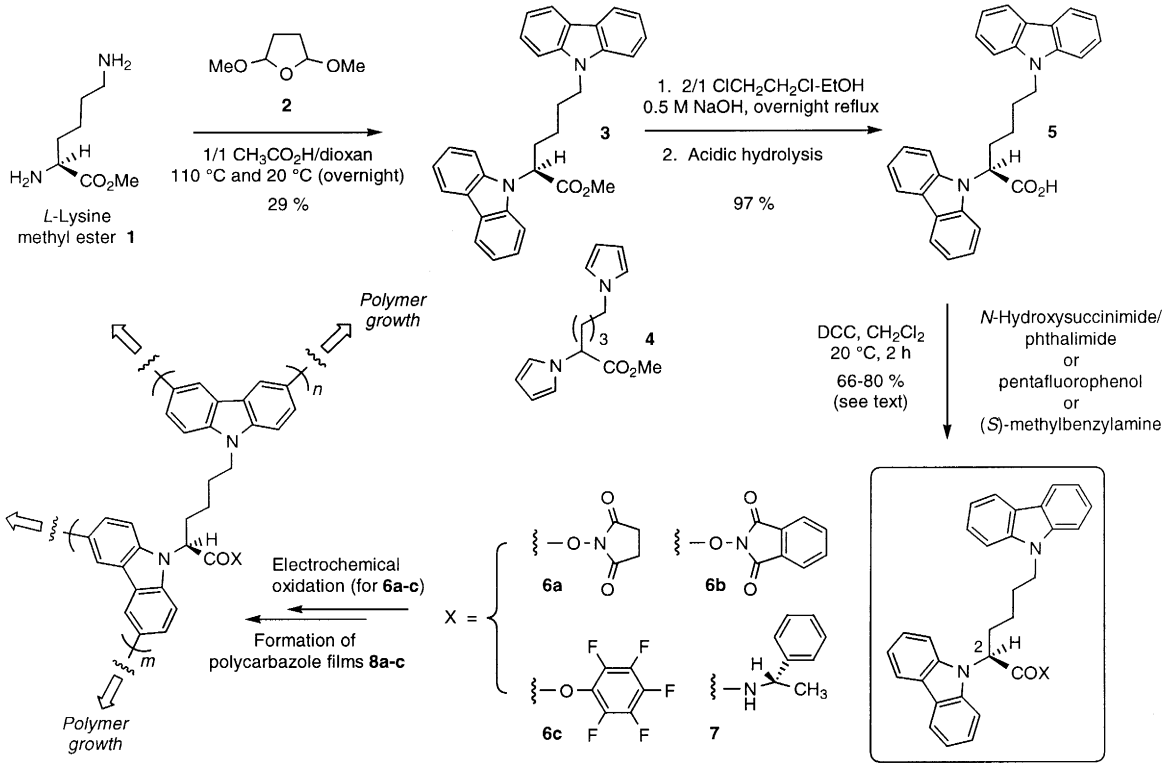
used but does not provide homopolymerization. Rather, an electrochemical copolymerization process with unsubstituted monomers was required for biopolymer formation.[5,6] Consequently, much of the effort to develop ECPs as matrices for the grafting of biomolecules has been directed towards the search for improved polymers in terms of performances of their chemically activated surfaces. For example, attractive precursor polymers have been produced by functionalizing some racemic thiophene and pyrrole monomers with easy leaving groups.[7,8]

In this context, this letter describes our recent results in the synthesis of new chiral electro-oxidizable *N*-substituted dicarbazole monomers **6a–c** activated by *N*-succinimidyl/phthalimidyl or pentafluorophenyl carboxylate groups (Scheme 1). This is the first report of electropolymerization of these monomers to afford unique chiral polydicarbazole films that are chemically activated via lysine NH_2 functions for protein/antibody attachment onto microelectrodes.



Scheme 1.

It should be noted that the monomers **6a–c** contain two carbazole moieties able to generate, after oxidation, long polymeric chains **8a–c** (Scheme 1, indicated polymer growth directions). In addition, the resulting polymer films should exhibit a cross-linked skeleton and, hence, a better mechanical stability. Furthermore, the close vicinity of an electron-attracting ester group to one of the two carbazoles could differentiate them oxidatively, thus modulating the structural properties of related films.

Although usually applied for pyrrole synthesis, the Clauson–Kaas reaction, i.e. the condensation of an amine derivative with 2,5-dimethoxy-tetrahydrofuran **2**,[9] was modified to afford solely the dicarbazole methyl ester **3** from the L-lysine methyl ester **1** (dihydrochloride) under acid catalysis (**1**: 3.0 mmol, **2**: 6.8 mmol, 14 ml of 1:1 CH_3CO_2H:dioxan, 1 h at 110°C and stirring overnight at 20°C, **3**: 29%). Transient formation of the dipyrrole methyl ester **4** was observed (TLC check with a pure standard) accompanied by uncharacterized polymers produced from the acid-sensitive **2**. After saponification (**3**: 0.22 mmol,

6.0 ml of 2:1 $ClCH_2CH_2Cl$:EtOH, 0.5 M NaOH: 2.6 mmol, overnight reflux) and acidic hydrolysis (2 M HCl, **5**: 97%), the free acid **5** (0.56 mmol) was activated (DCC: 0.67 mmol, CH_2Cl_2: 5.0 ml) to couple with the nucleophiles *N*-hydroxysuccinimide/phthalimide and pentafluorophenol (0.56 mmol, 2 h, 20°C). This afforded purified activated oxidizable dicarbazole monomers **6a–c** (**6a**: 70%, **6b**: 66%, **6c**: 80%) ready for the preparation of the polycarbazole films. Application of these same coupling conditions to (*S*)-methylbenzylamine (99% optical purity, Aldrich), confirmed that this three-step sequence does not racemize the chiral center of **6a–c**, since the only diastereoisomer detected was dicarbazole amide **7** [85%; ^{1}H NMR check (500 MHz, $CDCl_3$); analytical HPLC: LichroCART® 125-4, LichroSpher® Si 60 (5 μm), petroleum ether PE:AcOEt 90:10, t_R=11.20 min, PE:CH_2Cl_2 50:50, t_R=11.18 min]. Furthermore, the (*S*)-absolute configuration depicted for C(2) of the dicarbazoles **6a–c** and **7** (retention of configuration) was based on an acid-catalyzed cationic mechanism analogous to the mechanism of the Clauson–Kaas pyrrole reaction but involving transient nucleophilic indole intermediates.[10]

The electrochemical behavior of the dicarbazole derivatives **6a–c** (1 mM) was examined by cyclic voltammetry in CH_3CN (Fig. 1A/B). In the positive region (0–1.2 V versus Ag/Ag^+ 10 mM in CH_3CN), the three monomers presented identical behavior, namely, two irreversible anodic peaks at 0.88 and 1.11 V. The later correspond to a one-electron oxidation of each carbazole group. The difference in oxidation potential is most probably due to the close vicinity of the electron-withdrawing ester group to one of them.

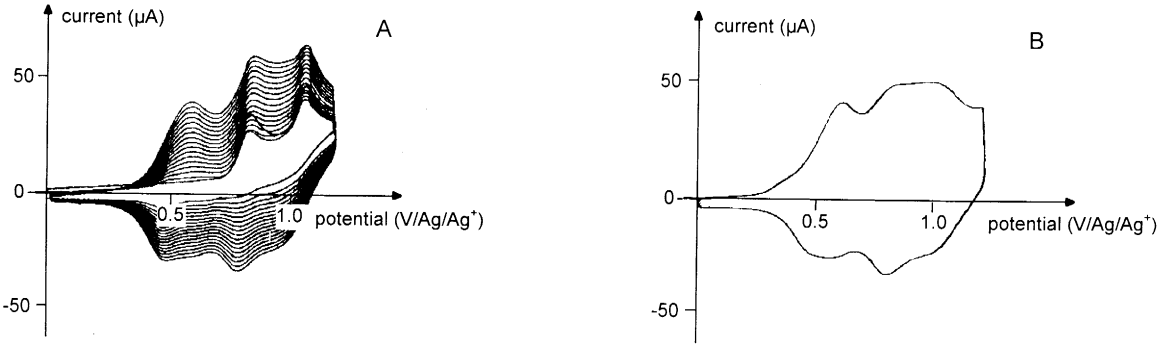


Fig. 1.

As previously reported for *N*-alkylcarbazoles, repeatedly scanning the potential over the range from 0 to 1.2 V results in the formation of polymeric films for all the monomers.[11] For instance, Fig. 1A [oxidative polymerization of **6a** (1 mM) by repeated potential scans between 0 and +1.2 V at a platinum electrode (Ø 5 mm) in CH_3CN+0.1 M TBAP, scan rate=0.1 $V.s^{-1}$] shows the appearance and growth of reversible peak systems, illustrating the electrogeneration of a poly-**6a** film **8a**. After 10 successive cycles and transfer to an CH_3CN solution free of monomer, the cyclic voltammogram of the modified electrode ($\Gamma_{\mathbf{6a}}$=1.15 $nmol.cm^{-2}$) exhibited an electroactivity similar to the regular electroactivity of classical poly-(*N*-alkylcarbazole) films [Fig. 1B, cyclic voltammogram of the poly-**6a** electrode, $\Gamma_{\mathbf{6a}}$=1.15 $nmol.cm^{-2}$, in CH_3CN+0.1 M TBAP, scan rate=0.1 $V.s^{-1}$].[12] If the potential range for the 10 cycles was restricted to the oxidation of only one carbazole group, the growth of the electrodeposited coating decreased markedly. Surprisingly, permeability of the resulting coating was lower than that of the former one. This may be due to the electrodeposition of short oligomers[13] (lack of polymer cross-linking). In contrast, the oxidation of the two carbazole groups should provide not only longer but also cross-linked polymeric chains and, hence, a polymer able to swell in organic media.

To illustrate the potential of such polymers for the easy covalent grafting of biological macromolecules, the immobilization of bovine serum albumin (BSA) as a model protein was investigated. The poly-**6a**

electrode was modified by soaking it for 2 h in an aqueous solution of BSA (0.5 mg.ml^{-1}), followed by 1 h in a stirred neutral phosphate buffer. Before and after the incubation with BSA, the permeability of the poly-**6a** film was tested by recording the cyclic voltammogram of ferrocene as a permanent electroactive probe in CH_3CN [Fig. 2, cyclic voltammograms of 1 mM ferrocene in CH_3CN+0.1 M TBAP at a poly-**6a** electrode (a) before, and (b) after, incubation with an aqueous BSA solution, scan rate 0.1 V.s^{-1}]. The ferrocene signal was drastically reduced after the incubation, indicating a decrease in permeability of the poly-**6a** film due to the insulating binding of BSA. A similar phenomenon was observed with the poly-**6b** and poly-**6c** films (similar curves not shown), illustrating the efficient grafting of BSA on **8b–c**.

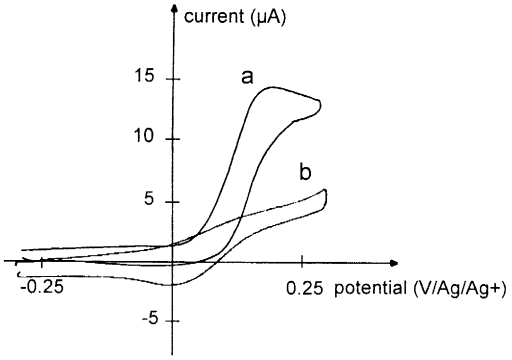


Fig. 2.

In summary, chiral *N*-substituted dicarbazoles bearing activated esters **6a–c** were synthesized and, for the first time, successfully electropolymerized to the corresponding polydicarbazole films **8a–c**. Our preliminary results emphasize the potential of these chemically activated chiral ECPs for microelectrode functionalization by covalent attachment of proteins. Additional applications of these novel films to immobilize redox mediators as well as antibodies/proteins suitable for the fabrication of protein biochips[14] are presently under investigation.[15]

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15. The new compounds were fully characterized spectroscopically (FT-IR, $^{1}H/^{13}C$ NMR, CI-MS) and their homogeneities checked by TLC and analytical HPLC for chromatographically stable compounds. *N*-Succinimidyl 2,6-di(carbazol-9-yl) hexanoate **6a** (yellow solid): ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.15 (d, *J*=8.5 Hz, 2H), 8.05 (d, *J*=8.6 Hz, 2H), 7.45–7.11 (m, 12H), 5.50 (dd, *J*=4.9, 10.6 Hz, 1H), 4.04 (m, 2H), 2.72 (s, 4H), 2.64–1.14 (m, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 168.4, 166.1, 140.1, 139.5, 126.0, 125.6, 123.6, 122.7, 120.4, 120.3, 120.1, 118.8, 109.7, 108.4, 54.9, 42.4, 29.5, 28.2, 25.5, 23.8; MS (CI, CH_4^+): 543 (51%) $[M]^{+\cdot}$, 167 (100%) $[carbazole]^{+\cdot}$; FT-IR (KBr, ν cm^{-1}): 1740 (s, C=O); $[\alpha]_D^{20}$=+9 (*c* 10 $g.l^{-1}$, acetonitrile). *N*-Phthalimidyl 2,6-di(carbazol-9-yl) hexanoate **6b** (yellow solid): ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.12 (d, *J*=7.7 Hz, 2H), 8.05 (d, *J*=7.7 Hz, 2H), 7.85–7.71 (m, 4H), 7.52–7.09 (m, 12H), 5.58 (dd, *J*=4.9, 10.4 Hz, 1H), 4.10 (m, 2H), 2.78–1.21 (m, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 167.1, 140.2, 139.6, 134.8, 128.8, 126.1, 125.7, 124.0, 123.7, 122.8, 120.5, 120.3, 120.1, 118.8, 109.7, 108.5, 55.0, 42.4, 29.6, 28.3, 23.9; MS (CI, CH_4^+): 591 (13%) $[M]^{+\cdot}$, 148 (100%) $[phthalimide+H]^+$; FT-IR (KBr, ν cm^{-1}): 1745 (s, C=O); $[\alpha]_D^{20}$=–1.3 (*c* 10 $g.l^{-1}$, acetonitrile). Pentafluorophenyl 2,6-di(carbazol-9-yl) hexanoate **6c** (yellow solid): ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.13 (d, *J*=7.7 Hz, 2H), 8.06 (d, *J*=7.7 Hz, 2H), 7.50–7.15 (m, 12H), 5.46 (dd, *J*=5.0, 10.6 Hz, 1H), 4.10 (m, 2H), 2.71–1.25 (m, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 166.9, 143.1, 140.2, 126.2, 125.7, 123.6, 122.8, 120.6, 120.4, 120.1, 118.9, 109.2, 108.5, 56.2, 42.4, 29.3, 28.3, 24.0; MS (CI, CH_4^+): 612 (100%) $[M]^{+\cdot}$; FT-IR (KBr, ν cm^{-1}): 1784 (s, C=O); $[\alpha]_D^{20}$=−9 (*c* 10 $g.l^{-1}$, acetonitrile).