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Polymerization of cysteine functionalized thiophenes

Rita Cagnoli[a], Massimiliano Lanzi[b], Adele Mucci[a], Francesca Parenti[a], Luisa Schenetti[a,*]

[a]Dipartimento di Chimica, Centro SCS, Università di Modena e Reggio Emilia, via G. Campi 183, 41100 Modena, Italy
[b]Dipartimento di Chimica Industriale e dei Materiali, Università di Bologna, viale Risorgimento 4, 40136 Bologna, Italy

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Abstract

Different synthetic pathways leading to polythiophenes (PTs) containing units derived from methyl *N*-(*tert*-butoxycarbonyl)-*S*-3-thienyl-L-cysteinate (**1**) and methyl *N*-(*tert*-butoxycarbonyl)-*S*-(2-thien-3-ylethyl)-L-cysteinate (**2**) were investigated. The oxidative coupling with $FeCl_3$ applied to *N*-deprotected monomer **1** generates a chemically fleeting PT, whereas when applied to *N*-deprotected monomer **2** generates a mixture of oligomers. Two co-polymers bearing cysteine moieties, poly{[methyl *N*-(*tert*-butoxycarbonyl)-*S*-3-thienyl-L-cysteinate]-*co*-thiophene} (*co*-**PT1**) and poly{[methyl *N*-(*tert*-butoxycarbonyl)-*S*-(2-thien-3-ylethyl)-L-cysteinate]-*co*-thiophene} (*co*-**PT2**), were eventually synthesized through Stille coupling of 2,5-bis(trimethylstannyl)thiophene and 2,5-dibromo derivative of compound **1** and through the post-functionalization with protected cysteine of a tosylate co-polymer, poly{[2-(3-thienyl)ethyl 4-methylbenzenesulfonate]-*co*-thiophene} (*co*-**PTTs**). UV–vis, CD, NMR and GPC analyses evidenced that these polymers are able to form chiral self-assembling structures, due to the formation of a hydrogen bond network and to π-stacks, not only in the solid state but also in solution.

Keywords: Polythiophenes; Cysteine; Stille coupling

1. Introduction

One of the most interesting fields of research is represented, at the present time, by the design, synthesis and characterization of organic semiconducting materials able to detect and transduce physical or chemical information into electrical or optical signals [1]. Among organic semiconductors, substituted polythiophenes (PTs) play a significant role since, they possess good stability and permit the insertion of functionalities which allow processability in different environments [2]. These materials combine the electroactive properties of PT with those due to the side chain groups, and PT based devices, as modified electrodes or sensors, have been developed for applications both in gas phase and in solution in order to identify organic compounds, ions and biomolecules [2,3]. The insertion of an amino acid on a synthetic polymer backbone is very intriguing since, it can lead to the obtainment of macromolecules useful for ion and molecular recognition and possessing biomimetic characteristics [4]. Nevertheless, only the Inganäs group has reported on the synthesis and properties of a PT functionalized with a serine moiety, linked through an ethylenic spacer to a thiophene ring [5].

We recently reported the preliminary results on a co-polymer of methyl *N*-(*tert*-butoxycarbonyl)-*S*-thien-3-yl-cysteinate and thiophene [6]. Cysteine directly bonded to thiophene ring (through the sulfur atom) was chosen on the basis of our previous studies on oligo- and poly(alkylsulfanyl)thiophenes, which have shown that the sulfur atom positively influences the characteristics of these PTs in comparison to polyalkylthiophenes, especially when filmability and p- and n-doping are concerned [7]. Polymers bearing a cysteinic side chain seem to us very attractive as they possess three potential sites (the thioether, the carboxy and the amino group) for molecular interaction, metal-ion detection and self-assembling through hydrogen bond formation. Furthermore, the chirality of the amino acid should make these polymers suitable for chiral recognition and induce chain helicity in aggregated phases [4].

In the present work we report on the generation of PTs bearing a cysteinic side group directly bonded, through the sulfur atom, to the thiophene ring or two-bond remote from it. Methyl *N*-(*tert*-butoxycarbonyl)-*S*-3-thienyl-L-cysteinate

* Corresponding author. Tel.: +39 59 2055076; fax: +39 59 373543.
E-mail address: schene@unimo.it (L. Schenetti).

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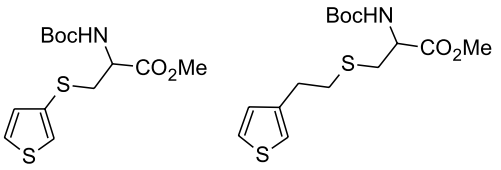


1 2

Scheme 1.

(**1**) and methyl *N*-(*tert*-butoxycarbonyl)-*S*-(2-thien-3-ylethyl)-L-cysteinate (**2**) [8] (Scheme 1) are the base units of these PTs.

Firstly, the oxidative polymerization with $FeCl_3$ was applied to **1** and **2**. Then, Stille coupling was utilized to co-polymerize the 2,5-dibromoderivatives of **1** and **2** with 2,5-bis(trimethylstannyl)thiophene. Finally, the post-functionalization of a tosylate PT was used to introduce the cysteine group on the ethylenic side chain. The PTs obtained were characterized through gel permeation chromatography (GPC), NMR, circular dichroism (CD), and UV–vis measurements.

2. Experimental section

2.1. General methods and materials

All air- or moisture-sensitive reactions were performed under pre-purified nitrogen with dry glassware. All solvents were dried by standard procedures. All reagents were purchased by Aldrich, Acros and Fluka used as received unless otherwise indicated. Iron(III) chloride was dried under vacuum.

Methyl *N*-(*tert*-butoxycarbonyl)-*S*-3-thienyl-L-cysteinate **1** and methyl *N*-(*tert*-butoxycarbonyl)-*S*-(2-thien-3-ylethyl)-L-cysteinate **2** were synthesized as already described [8]. Methyl *N*-(*tert*-butoxycarbonyl)-L-cystine, obtained from L-cystine using literature procedures [9], was reduced to protected cysteine immediately prior to use with zinc in acetic acid [10]. 2,5-Bis(trimethylstannyl)thiophene **5** was obtained according to Ref. [11].

2.1.1. Methyl N-(tert-butoxycarbonyl)-S-(2,5-dibromothien-3-yl)-L-cysteinate *4*

N-Bromosuccinimide (NBS) (0.641 g, 3.6 mmol) was added in little portions to a solution of methyl *N*-(*tert*-butoxycarbonyl)-*S*-3-thienyl-L-cysteinate (0.571 g, 1.8 mmol) in 20 mL of CH_2Cl_2 placed in a three-necked flask equipped with magnetic stirring, condenser and thermometer. The mixture was warmed to 40 °C and stirring continued for 16 h. After cooling to room temperature, the organic solution was concentrated to little volume and diethyl ether (20 mL) was added. The organic phase was washed with water (2×10 mL), dried ($MgSO_4$) and evaporated. The crude product was purified by preparative HPLC (petroleum ether/diethyl ether 50:50) obtaining pure **4** as a pale yellow solid (0.274 g, 32%). mp 70–72 °C. ^{1}H NMR (400.13 MHz, $CDCl_3$, Me_4Si) δ: 6.95 (s, 1H, H-4), 5.35(broad *d*, 1H, J= 6.5 Hz, NH), 4.53 (broad m, 1H, CH), 3.31 (d, 2H, J= 4.5 Hz, CH_2), 3.68 (s, 3H, OCH_3), 1.42 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100.61 MHz, $CDCl_3$, Me_4Si) δ: 170.7 (C=O), 155.0 (C=ONH), 133.2 (C-4), 131.6 (C-3), 115.5 (C-2), 114.7 (C-5), 80.2 ($C(CH_3)_3$), 53.4 (CH), 52.6 (OCH_3), 37.6 (SCH_2), 28.3 ($C(CH_3)_3$).

2.1.2. Poly{[methyl N-(tert-butoxycarbonyl)-S-3-thienyl-L-cysteinate]-co-thiophene} co-*PT1*

A solution of **5** (0.233 g, 0.57 mmol) and **4** (0.271 g, 0.57 mmol) in 6 mL of anhydrous THF/DMF 1:1 was added dropwise to 7.0 mg (6.1×10^{-3} mmol) of $Pd(PPh_3)_4$ in 2 mL of anhydrous THF/DMF 1:1 under a flow of dry nitrogen. The mixture was stirred for 6 h at 45 °C then poured in $CHCl_3$ (15 mL) and washed with water (2× 10 mL). The organic layers were evaporated and a dark film formed on the flask. CH_3OH was added to detach the polymer and the free standing film thus obtained was redissolved in $CHCl_3$ and reprecipitated with CH_3OH. After filtration a very fine dark precipitate was obtained (0.134 g, 59%).

2.1.3. 2-(2,5-Dibromo-3-thienyl)ethyl 4-methylbenzenesulfonate *6*

Following a procedure analogue to that described by Ref. [12], to a stirred solution of 2-(3-thienyl)ethanol (1.00 g, 7.80 mmol) in acetic acid (1.60 mL) cooled to 5–10 °C a Br_2 solution (0.10 ml, 1.91 mmol) in acetic acid (1.60 ml) was added dropwise, keeping the temperature below 10 °C. The mixture was stirred for 2 h at room temperature, then poured into brine and extracted with diethyl ether (2×5 mL). The dried organic layers ($MgSO_4$) were evaporated and 2-(2,5-dibromo-3-thienyl)ethyl acetate was obtained (2.53 g, 7.71 mmol) as a pale orange syrup. ^{1}H NMR (400.13 MHz, $CDCl_3$, Me_4Si) δ: 6.85 (s, 1H, H-4), 4.20 (t, 2H, J=6.5 Hz, CH_2O), 2.86 (t, 2H, J=6.5 Hz, CH_2CH_2O), 2.05 (s, 3H, CH_3). ^{13}C NMR (100.61 MHz, $CDCl_3$, Me_4Si) δ: 170.8 (C=O), 138.3 (C-3), 131.0 (C-4), 110.9 (C-5), 109.7 (C-2), 62.9 (CH_2O), 28.9 (CH_2CH_2O), 20.9 (CH_3).

The acetate was hydrolyzed to the corresponding alcohol by adding water (35 ml), CH_3OH (35 ml) and K_2CO_3 (6.41 g, 46.0 mmol) and stirring for 2 h at room temperature. The aqueous layers were extracted with diethyl ether (2×30 mL) and the combined organic layers washed with water, dried over $MgSO_4$ and evaporated under vacuum, affording 2.20 g (7.69 mmol, 99%) of 2-(2,5-dibromo-3-thienyl)ethanol as a colorless viscous oil. ^{1}H NMR (400.13 MHz, $CDCl_3$, Me_4Si) δ: 6.88 (s, 1H, H-4), 3.83 (t, 2H, J=6.5 Hz, CH_2O), 2.80 (t, 2H, J=6.5 Hz, CH_2CH_2O). ^{13}C NMR (100.61 MHz, $CDCl_3$, Me_4Si) δ: 139.1 (C-3), 131.3 (C-4), 110.9 (C-5), 109.5 (C-2).

Following a procedure analogue to that described in Ref. [13], *p*-toluensulfonyl chloride (1.63 g, 8.53 mmol) was

added in 30 min to a stirred solution of 2-(2,5-dibromo-3-thienyl)ethanol (2.20 g, 7.69 mmol) in pyridine (2.51 mL, 31.2 mmol) cooled to 10 °C, maintaining the temperature below 20 °C. The reaction mixture was stirred for 3 h at a temperature below 20 °C, then diluted with a solution of hydrochloric acid (15%, 25 mL). The solid tosylate was crystallized from isopropanol affording 2.91 g (86%) of a yellow solid. ^{1}H NMR (400.13 MHz, $CDCl_3$, Me_4Si) δ: 7.72 (m, 2H, H-2′,6′), 7.31 (m, 2H, H-3′,5′), 6.68 (s, 1H, H-4), 4.13 (t, 2H, J=6.6 Hz, CH_2O), 2.87 (t, 2H, J=6.6 Hz, CH_2CH_2O), 2.45 (s, 3H, CH_3). ^{13}C NMR (100.61 MHz, $CDCl_3$, Me_4Si) δ: 144.9 (C-1′), 136.8 (C-3), 132.6 (C-4′), 130.9 (C-4), 129.8 (C-3′,5′), 127.8 (C-2′,6′), 111.0 (C-5), 110.0 (C-2).

2.1.4. Poly{[2-(3-thienyl)ethyl 4-methylbenzenesulfonate]-co-thiophene} co-**PTTs**

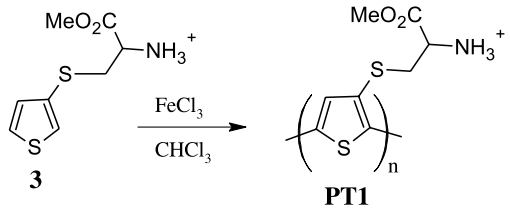
A solution of **5** (0.283 g, 0.69 mmol) and **6** (0.304 g, 0.69 mmol) in 6 mL of anhydrous THF/DMF 1:1 was added dropwise to 7.0 mg (6.1×10^{-3} mmol) of $Pd(PPh_3)_4$ in 2 mL of anhydrous THF/DMF 1:1 under a flow of dry nitrogen. The mixture was stirred at 80 °C for 45′ until a dark violet color appeared, then poured in CH_3OH. The brown precipitate was extracted in Soxhlet with CH_3OH (14 h), *n*-pentane (10 h) and $CHCl_3$ (14 h). $CHCl_3$ was removed under vacuum giving 0.241 g (96% yield) of *co*-**PTTs** as a green scaly solid.

2.1.5. Poly{[methyl N-(tert-butoxycarbonyl)-S-(2-thien-3-ylethyl)-L-cysteinate]-co-thiophene} co-***PT2***

K_2CO_3 (0.038 g, 0.27 mmol) and methyl *N*-(*tert*-butoxycarbonyl)cysteine (0.065 g, 0.27 mmol) were added, under a flow of dry nitrogen, to 0.020 g of *co*-**PTTs** in anhydrous DMF (25 mL). The reaction vessel was kept in an ultrasonic bath at 30 °C for 3 h, then this temperature was maintained for another 14 h. The reaction mixture was extracted with $CHCl_3$ (2×10 mL) and washed with water (10 mL), 5% $NaHCO_3$ (10 mL), and water (10 mL). The dried organic layers ($MgSO_4$) were reduced to small volume and poured into CH_3OH (20 mL). After some hours a precipitate formed, which was filtered, washed with CH_3OH and dried to give 0.019 g of *co*-**PT2** as a greenish-golden film (85%).

2.2. Measurements

GPC was carried out on a Hewlett–Packard system equipped with a Hewlett–Packard 5μ mixed PLgel column and a diode-array UV detector, using THF as the eluant, with a flow rate of 1.0 mL min^{-1}, at room temperature. The GPC system was calibrated using a series of monodisperse polystyrene standards. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AMX400 and Avance400 spectrometers operating at 400.13 and 100.61 MHz, respectively. UV–vis spectra were recorded on 2×10^{-5} g/mL solutions using a Perkin–Elmer Lambda Bio 20 spectrophotometer with 1 cm path length. CD spectra were acquired with a JASCO J-710 spectropolarimeter.



Scheme 2.

3. Results

3.1. Polymerization with $FeCl_3$

According to the fact that oxidative coupling with $FeCl_3$ is the most simple and common method utilized for the synthesis of functionalized PTs [14] and that one example is reported in the literature in which a β-functionalized thiophene with an aminoacidic moiety is polymerized with $FeCl_3$ [5a], we firstly followed this route for the polymerization of monomer **1**, of its *N*-deprotected trifluoroacetic salt (**3**), and of the completely deprotected *S*-3-thienyl-L-cysteine. A polymeric material was obtained only in the second case. The dropwise addition of a solution of **3** to a suspension of $FeCl_3$ in $CHCl_3$ (Scheme 2) generated (3 h at room temperature) a dark-brown precipitate, from which a dark-golden material was isolated after filtration and repeated washings with CH_3OH. The isolated polymer **PT1** is highly soluble in polar solvents, affording solutions of different colors: for example, in water it gives a dark-blue, whereas in DMSO gives a raspberry-red color.

The ^{1}H NMR spectrum, recorded in DMSO-d6 solution, supports the presence of a polymer: the singlet at 7.80 ppm and three signals of lower intensity in the aromatic region are attributable to H-4 protons of the main head-to-tail/head-to-tail (HT–HT) triad, and of the other three minor configuration triads, respectively [15]. The broad signal at 9.0 ppm is due to NH_3^+ protons, whereas those at 4.2 and 3.65 ppm are due to the CH and OCH_3 protons of the cysteine residue, respectively. Unfortunately, the initially formed polymer undergoes a degradation process with the

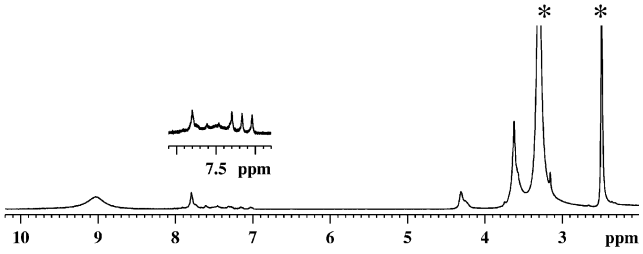


Fig. 1. ^{1}H NMR spectrum (DMSO-d_6, 400 MHz) of **PT1**. In the box, the aromatic region after two days, showing the increase of the triplet from NH_4^+ at 7.15 ppm. Starred peaks comes from H_2O and DMSO.

formation of free ammonium ion, as indicated by the progressive increase with time of its characteristic signal in the ^{1}H NMR spectrum, a triplet at 7.15 ppm [$^1J(^{14}N, H) = 50$ Hz] (Fig. 1).

This behavior is probably analogue to that reported for glycine in the presence of $AuCl_4^-$ ions [16], where a O, N chelate become an imine intermediate that undergoes a facile hydrolysis to the corresponding aldehyde and NH_4^+. Similarly, $FeCl_4^-$ ion formed during the oxidative polymerization with $FeCl_3$, is probably at the origin of the deamination process observed for **PT1**. The degradation of **PT1** makes it insoluble in common organic solvents (DMF, DMSO, $CHCl_3$, CH_2Cl_2, pyridine, CS_2, benzene), preventing its processing and further characterization in solution. The IR spectrum of the insoluble polymer in KBr pellets shows the presence of a signal at 1625 cm^{-1} attributable to the stretching of the C=C double bond formed after deamination. Moreover, ring stretching vibrations in the range 1520–1440 cm^{-1} and typical methylester absorptions (2919–2849 ν_{as} and ν_{sim} of methyl group and 1742 stretching of C=O) are recognizable. Attempts to electrochemically polymerize **1**, performed in CH_3CN with tetrabutylammonium hexafluorophosphate as support electrolyte, were unsuccessful. No polymerization occurred both when platinum and glassy-carbon electrodes were used.

The oxidative polymerization with $FeCl_3$ of the totally protected **2** was a failure and no oligomer or polymer formation was observed. Moreover, the polymerization of the *N*-deprotected trifluoracetic salt of **2** gave, in low yield, a polymeric material that underwent a degradation during the separation process, becoming insoluble in all organic solvents. The lower reactivity of **2** and of its unprotected derivatives with respect to **1**, to the serine analogue [5a] and to 3-alkylthiophenes [17] is probably attributable to the formation of an iron complex promoted by the side chain sulfur atom, with a concomitant decrease of the concentration of the free oxidant. Nevertheless, the increase of $FeCl_3$/**2** molar ratio from 4/1 to 10/1 afforded no results.

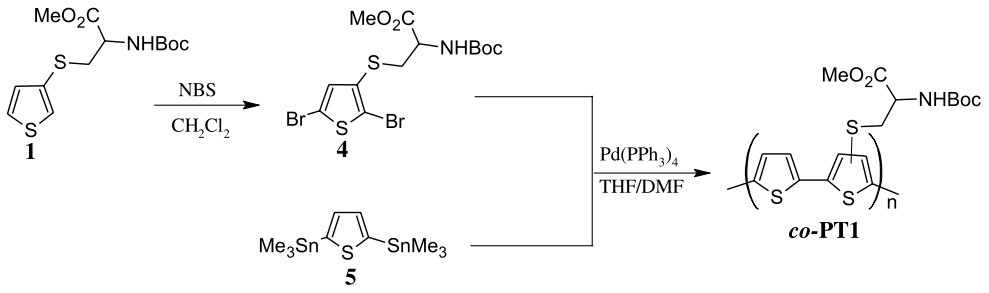
3.2. Polymerization through Stille coupling

The difficulties encountered in the polymerization with $FeCl_3$ of **1** and **2** moved us towards an alternative route, based on the Stille coupling [18] between a halo- and a stannyl derivative. This reaction would require, in order to generate a head-to-tail/head-to-tail polymer, the 2-halo-5-trimethylstannyl derivatives of **1** or **2** as reactants, but the hard experimental conditions necessary for the introduction of the trimethylstannyl group could induce racemization at the chiral centre, so we preferred to introduce the two complementary functionalities (halo and stannyl moieties) onto two different thiophene rings. The complete synthetic pathway for the generation of polymer *co*-**PT1**, based on the Stille coupling of methyl *N*-(*tert*-butoxycarbonyl)-*S*-(2,5-dibromothien-3-yl)-L-cysteinate **4** and 2,5-bis(trimethylstannyl)thiophene **5**, is reported in Scheme 3.

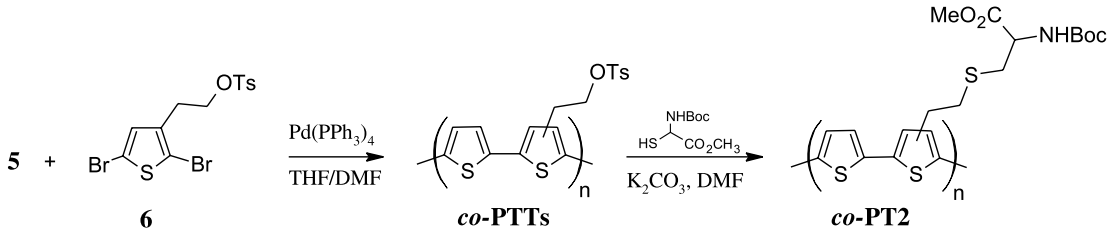
Bromination of **1** with NBS in CH_2Cl_2, afforded the dibromo derivative **4** in a 32% yield. The low yield is attributable to the concomitant formation of a 30% of a 2-bromo derivative and to the formation of *N*-deprotected species during the chromatographic purification process. The coupling of **4** and 2,5-bis(trimethylstannyl)thiophene **5** was done in anhydrous THF/DMF at 45 °C in the presence of $Pd(PPh_3)_4$ as catalyst and stopped after 6 h when a violet coloration indicative of a polymer formation was observed. The reaction mixture was poured in $CHCl_3$ and washed with water. The separated organic layers were evaporated giving the formation of a thin layer of *co*-**PT1** which was easily detached from the flask walls by adding CH_3OH. The free standing film is soluble in $CHCl_3$, CH_2Cl_2, THF, DMSO, partially soluble in acetone, insoluble in CH_3CN, H_2O and CH_3OH.

Confiding on the success of the Stille reaction on **4** as substrate, we applied it on the 2,5-dibromo derivative of **2**, obtaining only an oligomeric mixture difficult to characterize. The increase of the reaction time from 6 h to 3 days had no influence in the obtainment of a polymer. These results prompted us to pursue an alternative synthetic way to generate *co*-**PT2**, based on the post-functionalization with protected cysteine of a tosylate polymeric precursor *co*-**PTTs**, obtained by coupling **5** and (2,5-dibromo-3-thienyl) ethyl 4-methylbenzenesulfonate **6** (Scheme 4).

co-**PTTs** was generated in high yield (96%) after 45 min reaction time and its post-functionalization was performed with methyl *N*-(*tert*-butoxycarbonyl)cysteinate in anhydrous DMF in the presence of K_2CO_3. *co*-**PT2** was obtained in good yield (85%) and with a high substitution percentage,



Scheme 3.



Scheme 4.

as can be inferred from the ^{1}H NMR spectrum reported in Fig. 2: the proton signals of the tosyl group of *co*-**PTTs**, Fig. 2(a) are absent, whereas those of the cysteine bonded to the thienylethyl units are clearly visible in the spectrum of *co*-**PT2** in Fig. 2(b).

co-**PT2** was obtained, after filtration, in the form of a film which is more fragile than that of *co*-**PT1** and only moderately soluble in $CHCl_3$, CH_2Cl_2, THF, DMSO, insoluble in acetone, CH_3CN, H_2O and CH_3OH.

3.3. Molecular weight analysis

GPC performed on *co*-**PT1** and *co*-**PT2** evidenced remarkable differences between the two polymers. The elugram of *co*-**PT1** shows the presence of two well resolved peaks ($M_w = 2.6\times10^5$ g mol^{-1}, $M_n = 9.3\times10^4$ g mol^{-1} and $M_w = 6.0\times10^3$ g mol^{-1}, $M_n = 4.3\times10^3$ g mol^{-1}), a major peak at lower retention times (higher masses) and a minor one at higher retention times (lower masses), which were attributed to an aggregated and to a free form, respectively [6]. Only one peak ($M_w = 5.7\times10^4$ g mol^{-1}, $M_n = 1.4\times10^4$ g mol^{-1}, polydispersivity index, M_w/M_n, of

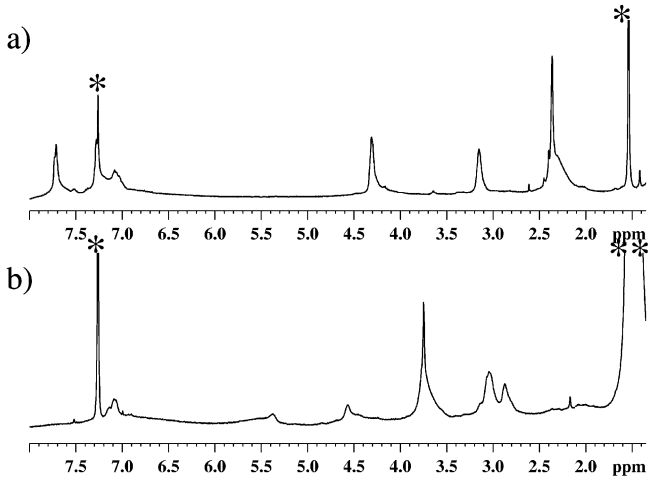


Fig. 2. ^{1}H NMR spectra in $CDCl_3$ of (a) *co*-**PTTs**: signals from tosyl residue at 7.72, 7.27 and 2.36 ppm; signals from the ethylenic spacer at 4.31 (CH_2OTs) and 3.15 ppm (CH_2 bonded to the thiophene ring); β-protons of the thiophene rings between 7.00 and 7.10 ppm; and (b) *co*-**PT2**: signals from cysteine residue at 5.38 ppm (NH), 4.57 ppm (CH), 3.57 ppm (OCH_3), 3.04 (SCH_2), 1.44 ppm ($C(CH_3)_3$); signals from the ethylenic spacer at 3.06 (CH_2S) and 2.87 ppm (CH_2 bonded to the thiophene ring), β-protons of the thiophene rings at 7.06, 7.09 and at 7.14 ppm; * denote residual water signals, ** denotes the out-of-scale signal from the Boc moiety.

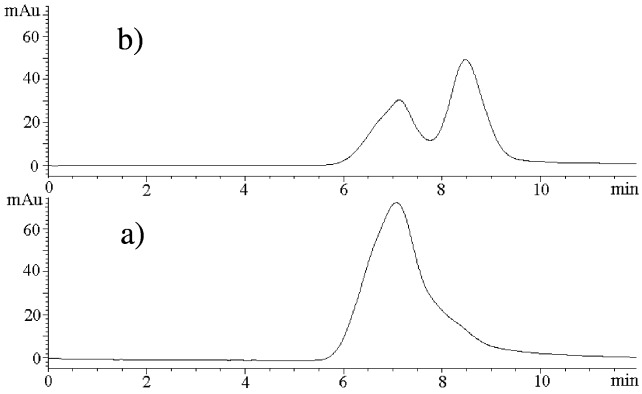


Fig. 3. GPC chromatogram of (a) *co*-**PT2** and (b) *co*-**PTTs**.

4.1) is present in the elugram of *co*-**PT2** (Fig. 3(a)). The assignment of this peak to an aggregated form is made possible by comparison with the GPC of its parent polymer *co*-**PTTs** (Fig. 3(b)), which in turns displays a bimodal weight distribution ($M_w = 6.0\times10^4$ g mol^{-1}, $M_n = 4.3\times10^4$ g mol^{-1} and $M_w = 3.9\times10^3$ g mol^{-1}, $M_n = 3.0\times10^3$ g mol^{-1}), with the high mass peak of lower intensity than the low mass peak. The aggregation behavior of *co*-**PTTs** is very interesting because it enables the contribution to aggregation of π-stacking to be evidenced and distinguished from that of inter-residue hydrogen bond formation (which was the mechanism formerly hypothesized by us to explain the tendency of *co*-**PT1** to self-aggregation) [6]. The former mechanism is effective for all the three polymers, while the latter can contribute only for *co*-**PT1** and *co*-**PT2**, and, indeed, its contribution is higher for *co*-**PT2**. The stronger tendency of *co*-**PT2** to aggregate is probably also at the origin of its lower solubility with respect to *co*-**PT1**.

3.4. NMR characterization of co-*PT1* and co-*PT2*

The ^{1}H NMR spectra of *co*-**PT1** show that the polymer can be in a more or less aggregated form, depending on the solvent utilized: in $CDCl_3$ (Fig. 4(a)) a higher aggregation is evidenced by the presence of very broad components (see insert), whereas a reduction of aggregation is observed in THF-d_8 solution (Fig. 4(b)). This behavior closely parallels that deduced from UV–vis spectra in the same solvents [6]. Slight differences of chemical shifts are observed for all signals, except for NH (which passes from 5.4 to 6.6 ppm, on going from $CDCl_3$ to THF-d_8). The β-protons of the

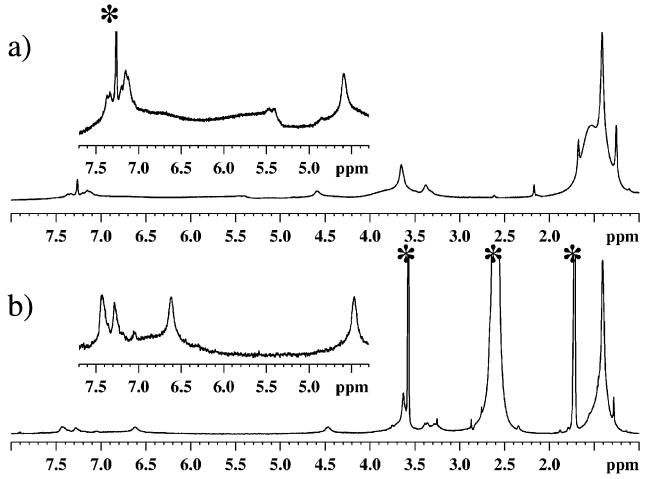


Fig. 4. ^{1}H NMR spectra of *co*-**PT1** (a) in $CDCl_3$; (b) in THF-d_8; asterisks denote signals from solvents and water.

thiophene rings are found in the region between 7.1 and 7.4 ppm, whereas the signals of the cysteine residue are found at around 4.5 ppm (CH), 3.6 ppm (OCH_3), 3.3–3.4 ppm (CH_2), 1.4 ppm ($C(CH_3)_3$). The two co-monomers, polymerized in a 1:1 ratio, maintain the same proportion in the product, as evidenced by the 1:3 integral ratio between the signal of CH at 4.5 ppm and the signals of aromatic protons.

The regiochemistry of *co*-**PT1** was deduced from the analysis of its one- and two-dimensional homo- and heteronuclear NMR spectra in THF-d_8.

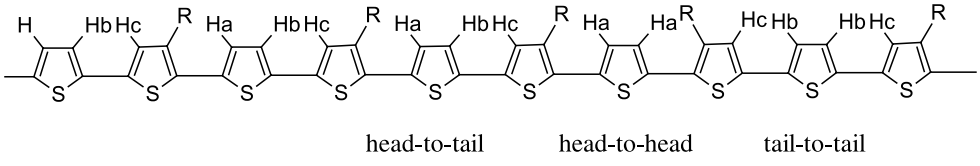
A total correlation spectroscopy (TOCSY) experiment [19] enables the presence of only one type of cysteinic side chain to be evidenced, confirming the absence of head-to-head junctions [20], and the couple of proton signals coming from the unsymmetrically 2,5-disubstituted units to be found at 7.40 and 7.27 ppm. Further information was obtained from a heteronuclear multiple quantum coherence (HMQC) experiment [21] through which five different correlations were observed in the aromatic region (attributable to β-thienyl H, C fragments), whereas only one type of cysteinic chain was detected in the aliphatic region (i.e. only one H, C correlation for each different aliphatic carbon). One carbon from trisubstituted thiophene units (129.7 ppm), whereas four carbons attributable to the 2,5-disubstituted thiophene units (127.8, 126.8, 125.8 and 124.4 ppm) were detected. These assignments were made on the basis of TOCSY results and by comparison with previously studied oligo- and polythiophenes [15], and support the presence of three types of 2,5-disubstituted thiophene units embedded in the polymer backbone, corresponding to head-to-tail (prevailing), head-to-head and tail-to-tail coupling with adjacent trisubstituted units (Scheme 5). NMR results are reported in Table 1.

The protons of the 2,5-disubstituted unit in the head-to-tail junctions show different chemical shifts, whereas only one chemical shift is found for those involved in head-to-head or tail-to-tail junctions.

A NOESY (nuclear overhauser enhancement spectroscopy) [22] experiment performed with a 100 ms mixing time enabled a cross-peak between the methyl protons of the Boc and the methoxy groups to be found. This can be related to the formation of inter-residue hydrogen bonds between two different polymeric chains (Scheme 6) that would explain the formation of aggregates, even though intra-chain bonds can not be excluded.

The ^{1}H NMR spectra of *co*-**PT2** in $CDCl_3$ (Fig. 5(a)) and in THF-d_8 solution (Fig. 5(b)) evidence that the polymer is in a more aggregated form with respect to *co*-**PT1** and confirm the GPC results.

This behavior closely parallels that deduced from UV–vis spectra in the same solvents (see below). Also for *co*-**PT2** slight differences of chemical shifts are observed for all signals, except for NH (which passes from 5.4 to 6.5 ppm, on going from $CDCl_3$ to THF-d_8). The β-protons of the thiophene rings are found between 7.1 and 7.3 ppm, in a narrower region with respect to those of *co*-**PT1**, whereas the signals of the ethylic and cysteine residues are found around 4.5 ppm (CH), 3.7 ppm (OCH_3), 3.1–2.8 ppm ($CH_2CH_2SCH_2$) and 1.4 ppm ($C(CH_3)_3$). A homonuclear double-quantum spectroscopy (DQS) [23] experiment allowed us to clearly distinguish signals from the cysteine residue and from the ethylenic spacer and to find the proton signals from the asymmetrically substituted 2,5-disubstituted thiophene units (7.19 and 7.24 ppm). The absence of head-to-head junctions between pairs of trisubstituted thiophenes may be ruled out by the absence of high field aliphatic signals deriving from the ethylenic group [20]. Due to the higher degree of aggregation in both solvents, only one set of aromatic carbon resonances (attributed to thienyl units in the main head-to-tail arrangement by comparison with previous results [24]) was detected through ^{1}H, ^{13}C inverse detection HMQC experiments (NMR data are reported in Table 1). The NOESY experiment on *co*-**PT2**, performed in the same conditions employed for *co*-**PT1**, gave very similar results: the cross-peak between the



Scheme 5.

Table 1
^{1}H and ^{13}C chemical shift (δ, THF, TMS) of *co*-**PT1** and *co*-**PT2**

	CH_2CH_2S	CH_2S	SCH_2	CH	C=O	OCH_3	NH(CO)	$C(CH_3)_3$	$C(CH_3)_3$
co-**PT1**									
^{1}H	–	–	3.37/3.27	4.46	–	3.63	6.6	–	1.40
^{13}C	–	–	38.6	54.5	171.2	52.3	nd	79.1	28.5
co-**PT2**									
^{1}H	3.05	2.92	3.00/2.86	4.57	–	3.66	6.5	–	1.40
^{13}C	30.6	33.0	34.7	54.4	nd	52.4	nd	nd	28.5

	C_a/H_a	C_b/H_b	C_c/H_c
co-**PT1**			
Head-to-tail	126.8/7.40	125.8/7.27	129.9/7.40
Head-to-head	127.8/7.39		129.9/7.40
Tail-to-tail		124.4/7.27	129.9/7.40
co-**PT2**			
Head-to-tail	127.5/7.19	125.3/7.24	127.5/7.26

methyl protons of the Boc and the methoxy groups is the most intense one confirming the strong association between cysteinic side chains.

3.5. UV–vis and CD spectra

The UV–vis spectra on $CHCl_3$ solutions of the two PTs (Fig. 6) display absorption maxima at 548 and 594 nm for *co*-**PT1**, and at 545 nm, with a shoulder at 587 nm, for *co*-**PT2**, and evidence the presence of strongly aggregated forms. Despite this similarity, a different behaviour is observed when attempts to disrupt the aggregation are made: *co*-**PT1** slowly disaggregates by changing the solvent to THF or after sonication, as is evident from the appearance of a blue shifted component at about 480 nm [6], whereas the shape of the UV–vis spectra of *co*-**PT2** does not change under the same conditions.

Notwithstanding the presence of aggregates in solution, only *co*-**PT1** displays a small but measurable cotton effect (CE) in $CHCl_3$ [6], whereas films of both PTs, cast from $CHCl_3$ solutions on quartz, display a CE (Fig. 7) with a maximum g factor ($\Delta A/A$) of -9×10^{-3} at 566 nm for *co*-**PT1**, and of -3×10^{-4} at 561 nm for *co*-**PT2**. *co*-**PT2** is not only characterized by a lower optical activity with respect to *co*-**PT1** but also generates much more heterogeneous films.

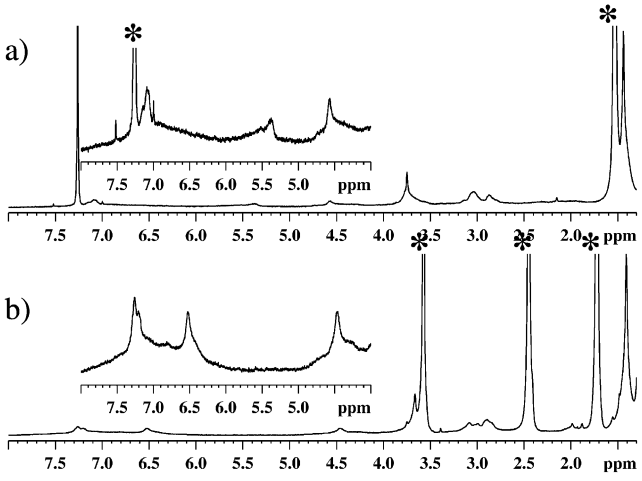
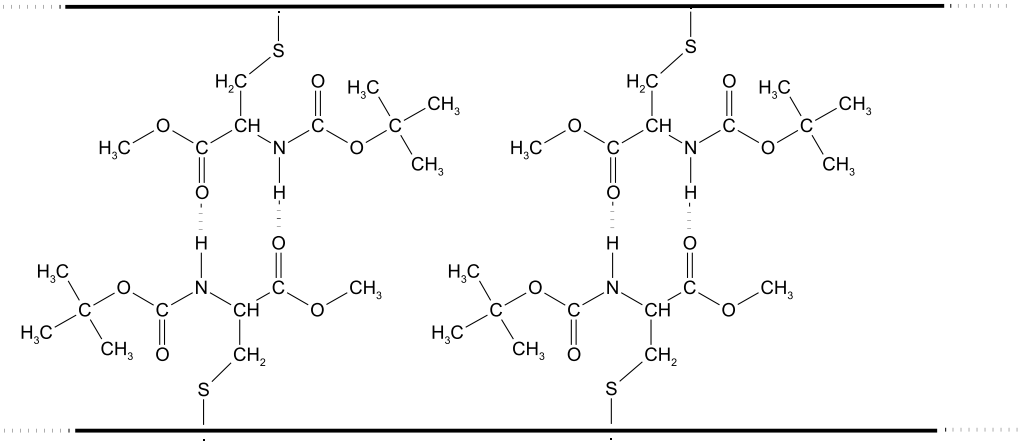


Fig. 5. ^{1}H NMR spectra of *co*-**PT2** (a) in $CDCl_3$; (b) in THF-d_8; asterisks denote signals from solvents and water.



Scheme 6.

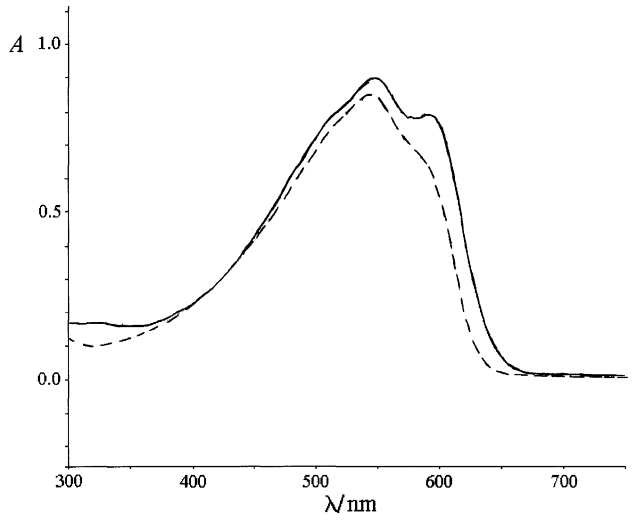


Fig. 6. UV–vis spectra of solutions of (a) *co*-**PT1** (solid line) (b) *co*-**PT2** (dashed line) in $CHCl_3$.

4. Conclusions

Two cysteine functionalized PTs, *co*-**PT1** and *co*-**PT2**, were prepared. The Stille coupling of the 2,5-dibromoderivatives of **1** and **2** with 2,5-bis(trimethylstannyl)thiophene **5** showed to be effective only for the former in generating a stable polymer, *co*-**PT1**. *co*-**PT2** was obtained through post-functionalization with protected cysteine of a tosylate PT (*co*-**PTTs**), derived from the co-polymerization of the 2,5-dibromoderivative **6** with **5**. The widely employed oxidative polymerization with $FeCl_3$, directly applied to the *N*-deprotected derivative of **1** afforded a HT–HT regioregular but chemically unstable polymer, **PT1**, whereas it was inefficient when applied directly to **2** and to its deprotected derivatives.

The GPC, NMR, and UV–vis characterization of the synthesized polymers evidences the presence in solution of both free and aggregated forms, the relative amounts of which depend on the different functionalities. In particular, the NOESY experiments on *co*-**PT1** and *co*-**PT2**, indicate a spatial proximity of the methoxy and the *t*-butoxy groups in the aggregates, that can be related to the formation of inter-residue hydrogen bonds between cysteine units belonging to different polymeric chains, even though intra-chain bonds can not be excluded. The self-assembling behavior is not only favored by the cysteinic side chains, but also by the alternation of β-substituted and β-unsubstituted thiophene rings, which favours π-stacking.

As a matter of fact, both *co*-**PT1** and *co*-**PT2** exhibit optical activity in the solid state, but their CE is low or absent in solution (despite the presence of aggregates), as already observed for another chiral PT synthesized in our laboratory [7a]. Even though aggregation can not be considered, in principle, a necessary condition for chirality [25], the main chain chirality induced by the cysteine residues is amplified in the solid state for the present PTs.

Eventually, *co*-**PT1** seems to be more promising than *co*-**PT2**, on the basis of its higher solubility, filmability and optical activity. These results are not surprising, because *co*-**PT1** belongs to a class of materials (oligo- and polythiophenes carrying the sulfur atom directly bonded to the thiophene backbone) which possess very interesting physical properties, as already reported by us in previous papers [7,26].

Further studies, concerning the synthetic pathways leading to differently protected and to deprotected forms of *co*-**PT1** and *co*-**PT2**, their stability in the presence of metal ions and their self-organization properties will be undertaken, in order to better understand the potential applications of these two PTs in the field of molecular recognition and biosensors.

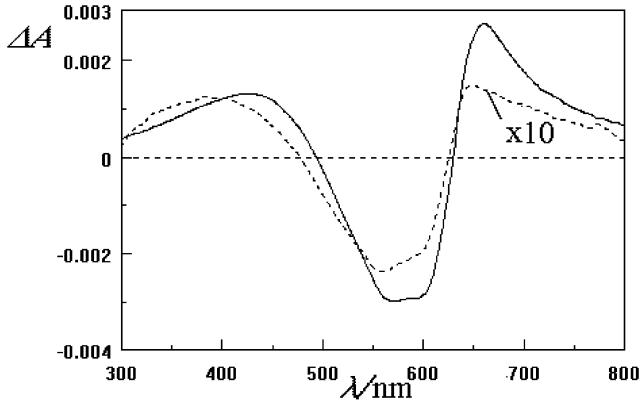


Fig. 7. CD spectra of films slowly cast from $CHCl_3$ on quartz of *co*-**PT1** (solid line) and *co*-**PT2** (dotted line, ten times enhanced).

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