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Synthesis of a silk-inspired peptide–oligothiophene conjugate†

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The first example of an oligothiophene–peptide conjugate, which was obtained by solid-phase acylation of a resin-bound silk-inspired oligopeptide sequence with a carboxylic acid functionalized regioregular tetra(3-hexylthiophene) derivative, is reported.

Introduction

Oligo- and polythiophenes are an attractive class of organic (semi)conducting materials which have received considerable attention for the development of organic electronic devices, such as light emitting diodes (LEDs),[1] field-effect transistors (FETs)[2] or solar cells.[3] Functionalization of oligo- and polythiophenes with receptor moieties that can selectively recognize and bind specific guests offers the possibility to transduce these events into an electronic signal and makes these materials attractive candidates for the development of novel sensor devices.[4] Of particular interest are polythiophenes containing side chains functionalized with specific nucleotide sequences,[5] saccharides,[6] or amino acids.[7] Since the performance of organic semiconductors, in particular regioregular head-to-tail coupled poly(3-alkylthiophenes) (HT-P3HT), has been found to be strongly influenced by their supramolecular organization and packing on a molecular level,[8] such motifs may not only be able to recognize and sense a variety of biologically relevant target molecules, but may also be powerful auxiliaries to control intermolecular forces and to consequently manipulate the organization of the hybrid materials. In particular, the conjugation of well-defined α-helical and β-strand peptide sequences with synthetic polymers is an attractive strategy that allows enhanced structural control at the nanometer level and to synergistically combine the properties of the two constituent components.[9]

In this communication, we describe the synthesis of a diblock-oligomer, combining a regioregular head-to-tail coupled oligo(3-alkylthiophene) (HT-O3AT) and an oligopeptide sequence. We chose as the conjugated oligomer part the corresponding tetramer, head-to-tail coupled tetra(3-hexylthiophene) (**4T**) which is functionalized by the pentapeptide sequence glycine-(L-alanine)-glycine-(L-alanine)-glycine (GlyAlaGlyAlaGly, **GAGAG**) inspired by *Bombyx mori* (silkworm) silk.[10] AlaGly has been identified as the most important repeat sequence in the crystalline β-sheet domains that is dominated by multiple hydrogen bonding and provides the strength of the silk fiber. On the other hand, on substrates such as graphite, β-alkylated oligothiophenes typically form highly organized, lamellar-type 2D-assemblies. This process is driven by relatively weak and unspecific van der Waals interactions of the alkyl side chains.[11] In contrast, the peptide–oligothiophene conjugate **GAGAG-4T** described here can also undergo directed hydrogen bonding interactions and represents a first model compound whose self-organization is driven by different competitive intermolecular interactions, which may lead to unexpected and novel 2D- and 3D-nanoscale structures and offers the possibility to fine-tune materials properties.

Results and discussion

The synthesis of the peptide–thiophene conjugate **GAGAG-4T** was realized by the activated amide coupling of two individually prepared building blocks, *i.e.*, the amino-terminated pentapeptide sequence **GAGAG** and the head-to-tail coupled tetra(3-hexylthiophene) carboxylic acid (**4T-COOH**). The synthesis of the semiconducting tetrameric building block was performed according to a recently developed strategy with which series of HT-O3ATs up to dodecamers were prepared serving as structurally defined model compounds for the above mentioned corresponding polydisperse HT-P3AT.[11b] First, 3,4′-dihexyl-2,2′-bithiophene-5-carboxylic acid **1**[12] is protected and transformed in 88% yield to benzyl ester **2**, which is subsequently selectively iodinated at the free α-position using a mercuration/iodination protocol to give α-iodobithienyl carboxylic acid benzyl ester **3** in 92% yield. Suzuki-type C–C cross coupling of **3** and 3,4′-dihexyl-2,2′-bithiophene-5-boronic acid propane-1,3-diyl ester **4**[12] gave the benzyl ester-capped quaterthiophene **5** in 44%, which was hydrolyzed to target molecule **4T-COOH** in 73% yield after recrystallization (Scheme 1).

Amino-terminated **GAGAG** was synthesized using 9-fluorenylmethoxycarbonyl (Fmoc) solid-phase peptide synthesis (SPPS) on a *p*-benzyloxybenzyl alcohol (Wang) resin.[13] Peptide synthesis was performed *via in situ* activation of the appropriate N^{α}-Fmoc protected α-amino acids as 1-hydroxybenzotriazole (HOBt) esters using *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethaminium hexafluorophosphate *N*-oxide (HBTU)/HOBt in the presence of 2 eq. diisopropylethylamine (DIPEA). Then, in a second step, the resin-bound peptide was swollen in a 9 : 1 (v/v) mixture of dichloromethane (DCM) and *N*,*N*′-dimethylformamide (DMF) and treated with 4 eq. **4T-COOH**, 4 eq. benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) and 8 eq. DIPEA to couple the quaterthiophene carboxylic acid to the *N*-terminal amine group of the peptide. The crude product was released from the support using 90% (v/v) trifluoroacetic acid (TFA, aq.) (Scheme 2). After purification, the hybrid molecule **GAGAG-4T** was obtained in 24% yield with a purity >95%, as estimated from reversed-phase (RP) HPLC.

The structure of **GAGAG-4T** was confirmed by ^{1}H- and ^{13}C-NMR spectroscopy, MALDI-TOF mass spectrometry and FTIR spectroscopy. Fig. 1 shows the ^{1}H-NMR spectrum of **GAGAG-4T**. Comparison of the integrals of the α-CH protons of the L-alanyl residues of the pentapeptide (labelled "A" in Fig. 1) with the aromatic CH protons of the quaterthiophene

† Electronic supplementary information (ESI) available: Synthetic procedures for all compounds, as well as ^{13}C-NMR, RP-HPLC and MALDI-TOF mass spectrometry characterization of **GAGAG-4T**. See http://www.rsc.org/suppdata/ob/b4/b415454a/

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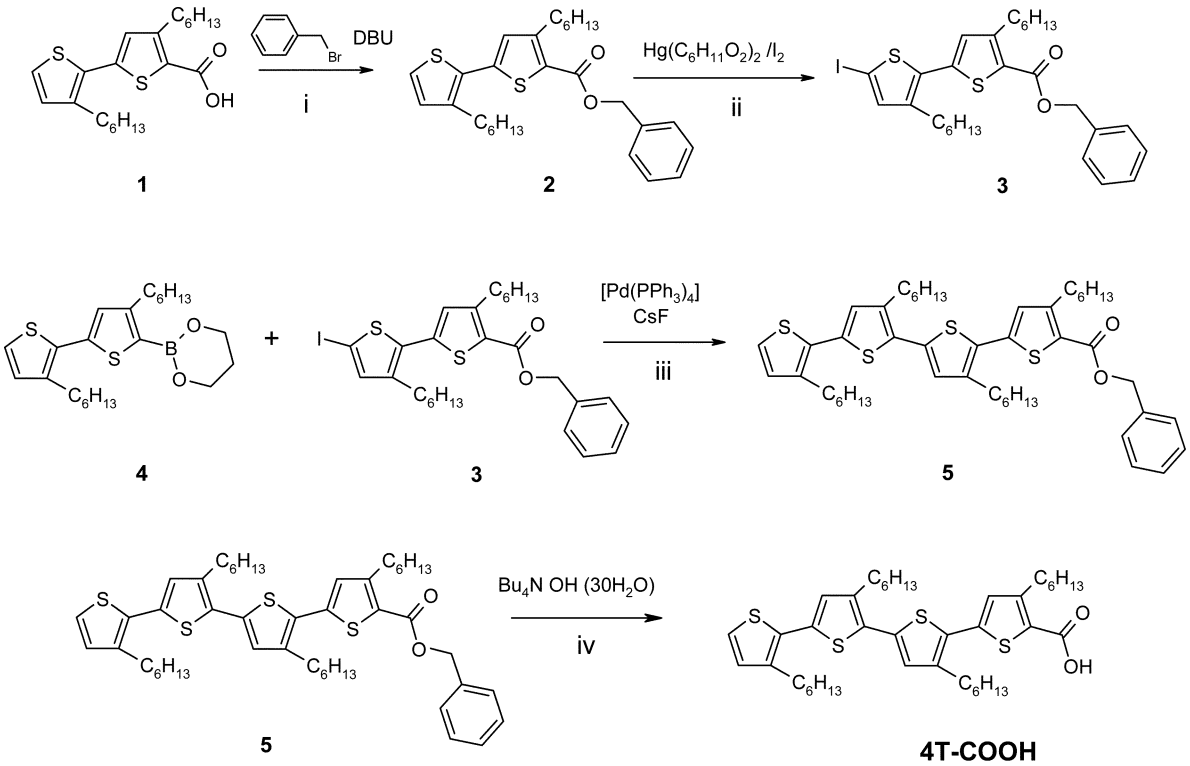
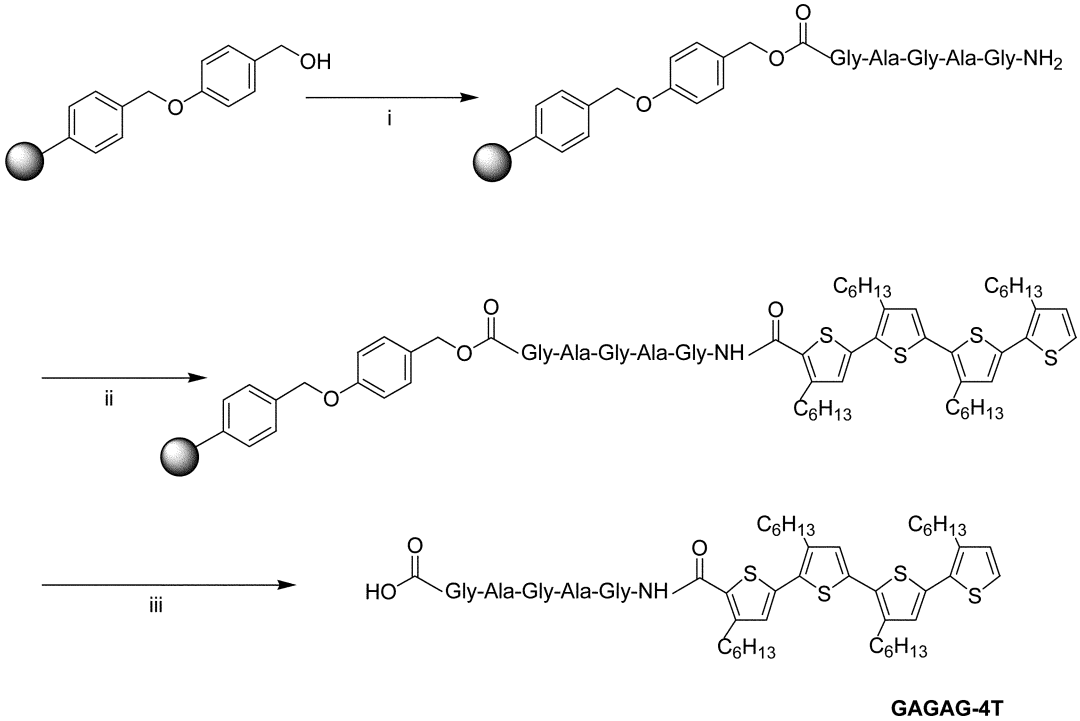


Scheme 1 (i) Toluene, rt, 15 h; (ii) CH_2Cl_2–HOAc (95 : 5), 0 °C–rt, 18 h; (iii) THF, 5 mol% Pd-cat., reflux, 8 h; (iv) THF, reflux, 3 h.



Scheme 2 (i) Fmoc solid-phase peptide synthesis; (ii) **4T-COOH**, ByBOP, DIPEA (DCM–DMF 9 : 1 (v/v)); (iii) 90% TFA (aq.).

moiety ("D" and "E") indicates a successful coupling of the **4T-COOH** building block with the peptide sequence and the absence of residual free peptide or oligothiophene precursor. The ATR-FTIR spectrum of **GAGAG-4T** shown in Fig. 2 also contains features of both the pure pentapeptide and the quaterthiophene, thus providing additional support for the structure of the target compound. More interestingly, although there is some unfavourable overlap with bands of the **4T-COOH** precursor, the ATR-FTIR spectrum of **GAGAG-4T** reveals shoulders of ~1630 cm^{-1} and 1680 cm^{-1} at the major carbonyl band. These shoulders, which appear as signals in the spectrum of **GAGAG**, represent the amide I absorption band and suggest an antiparallel β-sheet organization of the peptide segments.[14]

First experiments concerning the electronic properties of the peptide–oligothiophene conjugate **GAGAG-4T** are under way, as well as detailed investigations of the self-organization properties on substrates. The optical and electrochemical properties of the hybrid molecule in dichloromethane solution (λ_{max} = 390 nm, E_p^a = 0.63 V *vs.* Fc/Fc^+) are quite comparable to those of the parent quaterthiophene carboxylic acid **4T-COOH** (λ_{max} = 393 nm, E°_1 = 0.45 V *vs.* Fc/Fc^+).[12c] The peptide residue obviously exerts a certain electron withdrawing effect onto the

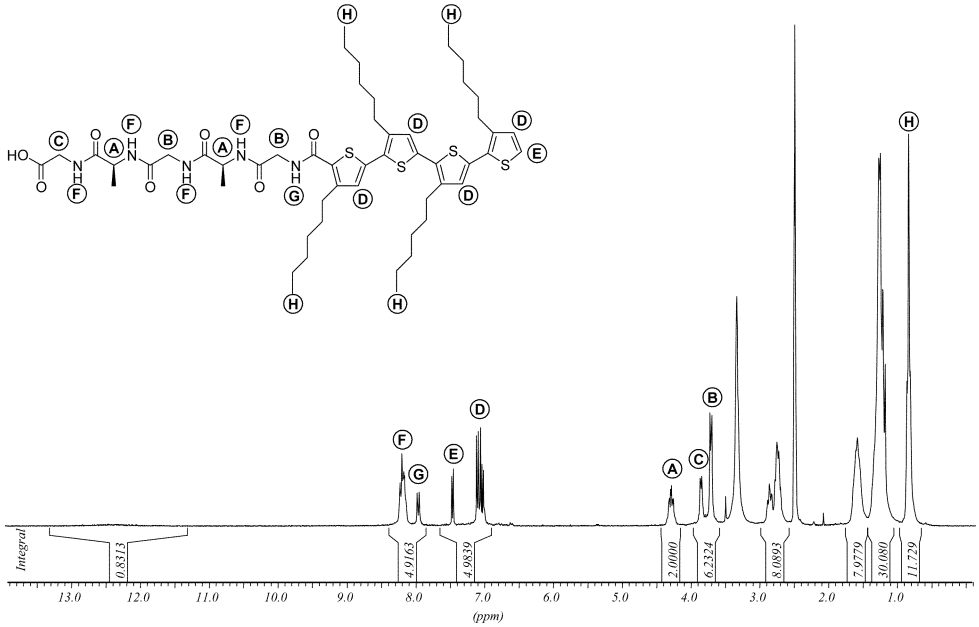


Fig. 1 ^{1}H-NMR spectrum (250 MHz, DMSO-d_6) of **GAGAG-4T**.

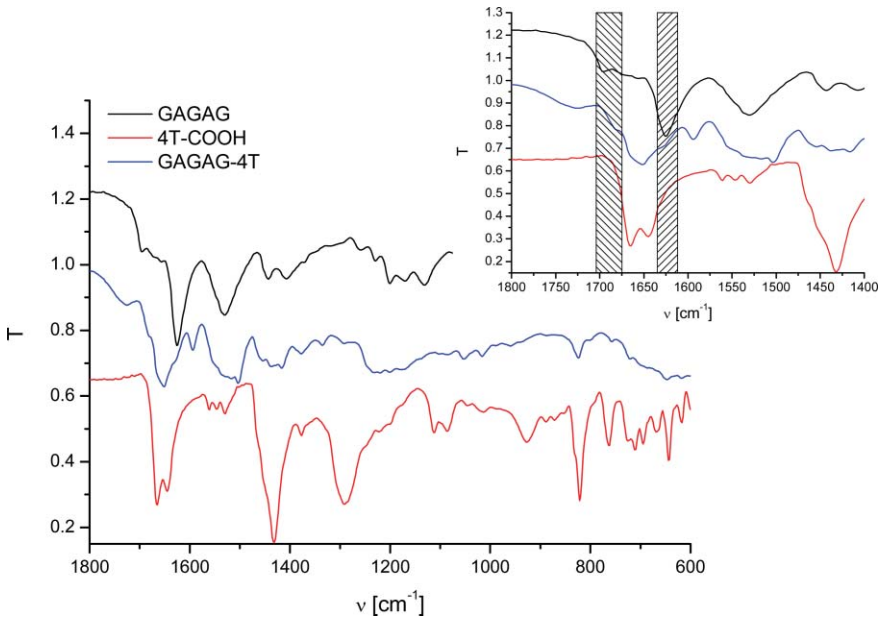


Fig. 2 ATR-FTIR spectra of **GAGAG** (black curve), **4T-COOH** (red curve), and **GAGAG-4T** (blue curve).

conjugated π-system, which can be seen in a positive shift of the first oxidation potential.

In contrast, a great difference between the two compounds was noted with respect to their 2D-ordering which might be due to the tendency of the peptide sequence in **GAGAG-4T** to form β-sheets *via* multiple hydrogen bonding. As a preliminary result, we note that scanning tunnelling microscopy (STM) investigations on the hybrid molecule at the solid/liquid interface reveal completely novel features and superstructures, which explicitly differ from those of the parent compound **4T-COOH**. At first glance, long linear strands of 3.5 to 4.0 nm in width and 3 Å in height are formed on the surface (Fig. 3a). More experiments are necessary to deduce a structural model of how the molecules are arranged in the strands which explains the observed features. In contrast, in case of the quaterthiophene carboxylic acid **4T-COOH**, a lamellar long-range ordering in various domains due to interdigitation and van der Waals interactions of the hexyl side chains is observed, which is typical for head-to-tail coupled oligo-[8b] and polythiophenes[11b] (Fig. 3b). The lamellae consist of dimers, which are formed due to pairing of carboxylic acid groups *via* hydrogen bonding (Fig. 3c).[12c] X-Ray structure analyses of this compound reveal polymorphism and two different 3D arrangements in the crystal are found. Similar to the 2D-crystalline monolayer, both structures are dominated by a mutual interplay of different intermolecular interactions, which is the dimerization of the carboxylic acid groups *via* hydrogen bonding and the interdigitation of the alkyl side chains *via* van der Waals forces.[12c] The striking difference in STM structures observed for **4T-COOH** and **GAGAG-4T** clearly illustrates the effect of directed hydrogen bonding interactions on the two-dimensional supramolecular organization of these compounds.

Conclusions

In this communication we have described the first example of a peptide–oligothiophene conjugate composed of a head-to-tail coupled tetra(3-hexylthiophene) and a silk-inspired GAGAG pentapeptide sequence. The basic synthetic strategy involved acylation of the resin-bound peptide sequence with a carboxylic

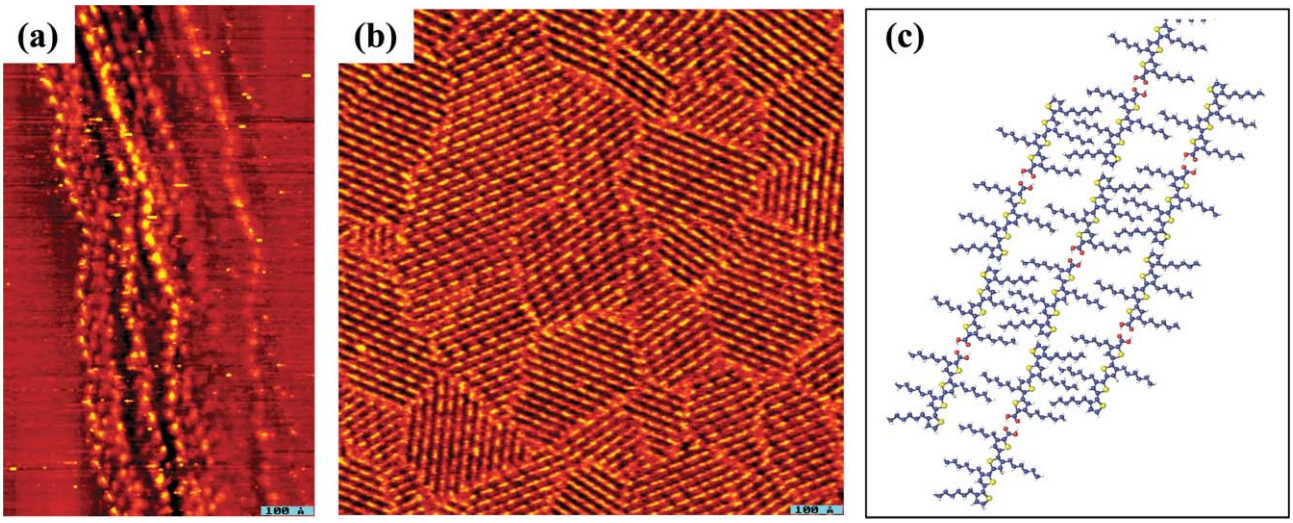


Fig. 3 (a) STM height image of **GAGAG-4T** on HOPG (62 × 100 nm^2, bias voltage = −600 mV, sample is negative, tunnel current = 68 pA); (b) STM height image of **4T-COOH** on HOPG (100 × 100 nm^2, bias voltage = −348 mV, sample is negative, tunnel current = 33 pA); (c) calculated model of the molecular arrangement of **4T-COOH**.

acid functionalized quaterthiophene derivative. This synthetic strategy is very flexible and may be easily adapted to other peptide sequences or other conjugated oligomers. FTIR experiments indicate that the peptide sequence retains its ability to form antiparallel β-sheet structures after conjugation to the oligothiophene. In conjunction with preliminary STM results, this suggests that short peptide sequences may indeed act as auxiliaries that can influence the nanoscale structure, and ultimately, properties of organic semiconducting materials.

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