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Supplementary Information for:  

Synthesis of a Silk-inspired Peptide-Oligothiophene Conjugate

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Abbreviations. ACN, Acetonitrile; ATR-FTIR spectroscopy, Attenuated total reflection Fourier-transform infrared spectroscopy; DCM, dichloromethane; DIPEA, N,N-diisopropyl-ethylamine; DMF, N,N-dimethylformamide; Et2O, diethyl ether; Fmoc, fluorenylmethoxycarbonyl; 1H-NMR, 1H nuclear magnetic resonance; PyBOP, benzotriazole-1-yl-oxy-tris-pyrroli-dino-phosphonium hexafluorophosphate; RP-HPLC, reversed-phase high pressure liquid chromatography; THF, tetrahydrofurane; TFA, trifluoroacetic acid.

Experimental Part

Materials. Unless stated otherwise, solvents and reagents were purified and dried by usual methods prior to use. Preparative column chromatography was performed on glass columns of different sizes packed with Kieselgel 60 (Merck, 0.06-0,2 mm). Amino acids and the Wang resin were purchased from Calbiochem-Novabiochem GmbH (Schwalbach, Germany). DMF was dried over molecular sieves (4 Å) prior to use. Benzylbromide (Merck), 1,8-diazabicyclo[5.4.0]undec-7-en (DBU) (Merck), iodine (Merck), tetrabutylammonium hydroxide (30hydrate) (Bu₄NOH) (Fluka) were purchased. 3',4-Dihexyl-2,2'-bithiophene-5-carboxylic acid 1, 3',4-dihexyl-2,2'-bithiophene-5-boronic acid propane-1,3-diy ester 4 and tetrakis(triphenyl-phosphino)palladium(0)² were prepared following literature procedures.

General methods. Melting points were determined with a Büchi B-545 melting point apparatus and are uncorrected. 1H NMR spectra were recorded on a Bruker WS 250 and a Bruker Avance 400 (400 MHz) spectrometer (with deuterated solvent as lock-in and tetramethylsilane as internal reference). 13C-NMR spectra were recorded on a Bruker WS 700 spectrometer at 176 MHz. Substance purity was assayed by RP-HPLC using a set-up consis-
ting of a HP 1100 quaternary gradient pump, a HP 1050 diode array detector (270 - 600 nm), a HP Chemstation and a Merck Purostar RP18e 55x4/3µm analytical column. Samples were eluted with a THF/ACN/DMF mixture employing a linear gradient starting with THF/ACN/DMF 10/80/10 (v/v/v) going to THF/DMF 90/10 (v/v) over a period of 10 minutes with a flow rate of 0.5 mL/min. ATR FT-IR spectroscopy was performed on a Bruker Vector 22 spectrometer with a Golden Gate A531 ATR unit. Samples were slightly pressed with a sapphire against the diamond of the ATR unit to obtain a thin film. The spectra were recorded at room temperature in the range of 200-8000 cm\(^{-1}\) with a resolution of 1 cm\(^{-1}\). Typically, 16 scans were averaged for a single spectrum. MALDI-TOF mass spectra have been measured on a Bruker Reflex III equipment. STM experiments were performed with a low-current microscope from RHK (Rochester Hills, MI) equipped with a RHK STM 1000 control system. Mechanically cut Pt/Ir tips were used. The STM images were acquired in the constant current mode under ambient conditions at solid-liquid as well as solid-air interfaces. The substrate employed was highly oriented pyrolytic graphite (HOPG). In the STM images, white or bright contrast corresponds to the highest tunnelling currents whereas black or dark contrast relates to the lowest values measured. Different settings for the tunnelling current (I) as well as the bias voltage (V) were used, as indicated in the figure captions.

**Synthetic procedures.**

**Mercuric hexanoate.** A mixture of 50.0 g (231 mmol) mercury(II)oxide (yellow) and 72,94 g (628 mmol) hexanoic acid in 500 mL chloroform is heated under reflux at a water separator till a clear yellow solution has formed and no water is separated further. Then, ~ 140 to 160 mL solvent is distilled off from the solution. After cooling to room temperature, the mercury hexanoate crystallizes as a colourless precipitate. The product is filtered with suction, washed with several portions of petroleum ether and dried in vacuum (yield: 98,2 g, 98 %). The mercuric hexanoate can be used without further purification. A small crop of product is isolated from the filtrate after evaporation of the solvent.

**3',4-Dihexyl-2,2'-bithiophene-5-carboxylic acid benzyl ester (2).** To a solution of 2.52 g (6.7 mmol) 3',4-dihexyl-2,2'-bithiophene-5-carboxylic acid in 25 mL toluene 1.07 g (7 mmol) DBU is added. The mixture is stirred for 0.5 h at room temperature prior to addition of a solution of 1.2 g (6.8 mmol) benzyl bromide dissolved in 15 mL toluene. A solid precipitate forms. The mixture is stirred over night at room temperature and then poured on water. After phase separation, the aqueous phase is extracted with ether twice. The combined organic
solutions are washed with 1 M HCl and water and dried over Na₂SO₄. Evaporation of the solvent leaves behind a dark brown oil which was purified on a column of silica with petroleum ether/dichloromethane mixture (8:2). Yield: 2.73 g (88%) of a slight yellow oil which solidifies on cooling; m.p. 31 °C; ¹H NMR (CDCl₃, 400 MHz) [ppm]: δ= 7.48–7.32 (m, 5H, Phenyl-H), 7.22 (d, 1H, 3J(5',4') = 5.2 Hz, H5'), 7.00 (s, 1H, H3), 6.93 (d, 1H, 3J(4',5') = 5.2 Hz, H4'), 5.35 (s, 2H, -CH₂O), 3.00 (t, 2H, 3J(H,H) = 7.6 Hz, α-CH₂), 2.81 (t, 2H, 3J(H,H) = 7.8 Hz, α'-CH₂), 1.71-1.64 (m, 4H, β,β'-CH₂), 1.41-1.21 (m, 12H, -CH₂-), 0.87 (t, 6H, -CH₃); IR (KBr) [cm⁻¹]: ν= 2950, 2840 (s, CH), 1650 (s, CO), 1450, 1250, 1110 (m, CH).

3',4-Dihexyl-5'-iodo-2,2'-bithiophene-5-carboxylic acid benzyl ester (3). To a solution of 2.73 g (5.8 mmol) bithiophene 2 in 120 mL of a mixture of chloroform and acetic acid (95:5) solid mercuric hexanoate is added. After 0.5 h stirring at room temperature the solution is cooled in an ice bath. A solution of 1.55 g (6.1 mmol) iodine in 60 mL chloroform/acetic acid is added within 2 h. A colourless precipitate is formed and the mixture is stirred over night warming up to room temperature. The mixture is washed with sat. NaHCO₃, with Na₂S₂O₃ solution and finally with water. The organic layer is dried over Na₂SO₄, the solvent distilled off and the crude product purified on a short column of silica with petroleum ether/dichloromethane (3:1). A bright yellow oil is isolated which solidifies on cooling. Yield: 3.15 g (92%); m.p. 42-43 °C; ¹H NMR (CDCl₃, 400 MHz) [ppm]: δ= 7.43–7.34 (m, 5H, Phenyl-H), 7.07 (s, 1H, H4'), 6.89 (s, 1H, H3), 5.35 (s, 2H, -CH₂O), 2.96 (t, 2H, 3J(H,H) = 7.8 Hz, α-CH₂), 2.77 (t, 2H, 3J(H,H) = 7.8 Hz, α'-CH₂), 2.77 (t, 2H, 3J(H,H) = 7.8 Hz, α'-CH₂), 1.68-1.60 (m, 4H, β,β'-CH₂), 1.39-1.21 (m, 12H, -CH₂-), 0.87 (t, 6H, -CH₃).

3",3',4"-Tetrahexyl-2,2':5',2":5",2"'-quaterthiophene-5-carboxylic acid benzyl ester (5). A mixture of 3.15 g (5.3 mmol) bithiophene 3 in 120 mL THF, 0.28 g (0.27 mmol) [Pd(PPh₃)₄] and 4.0 g (2.6 mmol) CsF is heated under reflux in an inert gas atmosphere. A solution of 4.26 g (10.2 mmol) boronic ester 4 in 80 mL THF is added slowly within 2.5 h. The pale yellow solution turns to a bright yellow mixture, which was refluxed for 8 h. After cooling down the precipitated solid is filtered off and rinsed with THF. Evaporation of the solvent from the filtrate leaves behind an oil, which was purified by chromatography on a column of silica with petroleum ether/dichloromethane (9:1). Besides a first fraction of 3,4'-dihexyl-2,2'-bithiophene (RF= 0.95) a main fraction was isolated which consisted of unreacted 3 and the quaterthiophene benzyl ester 5. From this mixture the product was isolated by recrystallization from ethanol/dichloromethane (4:1) in 1.85 g (44%) yield; m.p. 48-49 °C; ¹H
NMR (CDCl₃, 400 MHz) [ppm]: δH = 7.45 – 7.35 (m, 5H, Phenyl-H), 7.16 (d, 1H, 3J(5''',4'''')=5.2 Hz, H5'''), 6.99 (s, 1H, H3), 6.96 (s, 1H, H4'), 6.93 (s, 1H, H4''), 6.92 (d, 1H, 3J(4''',5''')=5.2 Hz, H4'''), 5.33 (s, 2H, -CH₂O), 3.00 (t, 2H, 3J(H,H)=7.8 Hz, α-CH₂), 2.78 (t, 6H, 3J(H,H)=7.8 Hz, α',α'',α'''-CH₂), 1.65-1.55 (m, 8H, β,β',β'',β'''-CH₂), 1.41-1.21 (m, 24H, -CH₂-), 0.87 (t, 12H, -CH₃).

C₄₂H₆₀O₂S₄ (725.2); calcd: C 69.58, H 8.36, S 17.82; found: C 69.56, H 8.34, S 17.69.

3″,3′,4-Tetrahexyl-2,2″:5″,2′′″-quaterthiophene-5-carboxylic acid (4T-COOH).

3″,3′,4-Tetrahexyl-2,2″:5″,2′′″-quaterthiophene-5-carboxylic acid [glycine-(L-alanyl)-glycyl-(L-alanyl)-glycyl]amide (GAGAG-4T). The peptide sequence, -Gly-Ala-Gly-Ala-Gly-, was prepared in a 0.25-mmol scale on an Applied Biosystems 433A automated peptide synthesizer using a Wang resin and standard Fmoc-chemistry. After the peptide portion of the target molecule was synthesized, the resin was removed from the synthesizer. Then, the resin was swollen in dry DCM/DMF (9/1 v/v) and two equivalents 4T-COOH, four equivalents PyBop and eight equivalents DIPEA were added. The mixture was allowed to stir under an argon atmosphere at ambient temperature for seven days. After that, the resin was filtered and washed intensively with DMF and DCM. Cleavage of the target peptide-oligothiophene was done by treating the resin-bound product with in 90 % aqueous TFA for two hours at 20 °C. The cleavage mixture and three subsequent DCM and TFA washings were filtered into a round bottom flask. The red solution was evaporated yielding a dark red film. Water soluble impurities were separated by extraction with water. The residue was dissolved in hot DMF and filtered. The solution was reduced to ~1 mL in vacuo. Addition of Et₂O led to a red precipitate, which was collected via filtration, washed with Et₂O and dried.
under vacuum. The resulting red gum was triturated with ACN, resulting in 56 mg (0.06 mmol, 24% related to the amount of the resin) of **GAGAG-4T** as a red powder. The purity of the substance as estimated from RP-HPLC was > 95%. ¹H-NMR (250 MHz, DMSO-d₆, 283K) [ppm]: δ_H = 12.3 (br, 1H, COOH), 8.25-7.92 (m, 5H, NH), 7.46 (d, 1H, H-5”), 7.11-6.96 (m, 4H, H-3,4’,4’’,4’’’), 4.36-4.20 (m, 2H, CH), 3.86 (d, 2H, CH₂), 3.75-3.65 (m, 4H, CH₂), 2.85 (t, 2H, α-CH₂), 2.78-2.62 (m, 6H, α-CH₂), 1.70–1.45 (m, 8H, γ-CH₂), 1.40–1.10 (m, 24H, γ-CH₂ + 6H, CH₃), 0.82 (t, 12H, CH₃). ¹³C-NMR (176 MHz, DMF-d₇, 333K) δ_C = 178.6, 178.3, 176.4, 175.1, 174.4, 168.3 (CO), 152.8, 146.9, 146.3, 145.7, 141.7, 140.1, 139.8, 136.1, 135.5, 135.4, 135.3, 134.9, 134.8, 134.5, 133.3, 130.3 (C-2,3,4,5,2’,3’,4’,5’,2’’,3’’,4’’,5’’), 55.1, 54.3 (CH), 48.9, 48.5, 46.5 (CH₂), 37.0, 35.9, 34.6, 34.5, 27.9 (CH₂), 23.3, 22.9 (CH₃), 19.1 (CH₃). MS (MALDI-TOF) m/z 1024 [M+] 

**References**

$^{13}$C-NMR spectrum (176 MHz, DMF-d7) of GAGAG-4T
3D HPLC Chromatogram of GAGAG-4T

HPLC trace of GAGAG-4T (UV-Vis detection at 380 nm)
MALDI-TOF mass spectrum of GAGAG-4T