SUPPORTING INFORMATION FOR

Self-assembling Nanofibers from Thiophene-Peptide Diblock Oligomers: a Combined Experimental and Computer Simulations Study

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1. Experimental details.

1.1. General procedures:

Copper powder (Merck), trifluoroacetic acid (Merck), tetrakis(acetonitrile)copper(I) hexafluorophosphate (Sigma-Aldrich), phosphoryl chloride (Merck), potassium carbonate (Merck), dimethyl(2-oxopropyl)-phosphonate (Merck), sodium hydride (Merck, 60% suspension in paraffin oil) and methanol (Merck, HPLC gradient grade) were used without further purification. Size exclusion chromatography (SEC) was performed by using Bio-Beads® S-X-1 and THF as eluent. 3,3'''-Didodecyl-[2,2';5',2";5",2'"]quaterthiophene **2**, tosylazide and PEO-β-sheet-peptide **5** were synthesized according to literature procedures.[1-3] Nuclear magnetic resonance spectra were recorded on a *Bruker AMX 500* (^{1}H NMR: 500 MHz, ^{13}C NMR: 125 MHz) or an *Avance 400* spectrometer (^{1}H NMR: 400 MHz, ^{13}C NMR: 100 MHz) at room temperature unless otherwise noted. Chemical shift values (δ) are given in parts per million using residual solvent protons (^{1}H NMR: δ_H = 6.00 for $C_2D_2Cl_4$ (tetrachloroethane-d_2), δH = 7.26 for $CDCl_3$, ^{13}C NMR: δC = 77.0 for $CDCl_3$) as internal standard. Spectra splitting patterns are assigned as follows: s (singulet), d (dublet), t (triplet) and m (multiplet). Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) measurements were carried out on a *Bruker Daltonik Reflex III* mass spectrometer with the following matrices: 1,2,3-trihydroxyanthracene (dithranol), 2,5-dihydroxybenzoic acid (DHB), α-cyano-4-hydroxy-cinamic acid (HCCA) and T-2-(3-(4-*t*-butyl-phenyl)-2-methyl-2-propenylidene) malononitrile (DCTB).[4] Compounds were mixed with a 100-1000 fold excess of matrix in THF and deposited on the stainless steel target as small spots (2 mm diameter). The matrix crystallized on evaporation of the solvent on air. Elemental analyses were performed on a *Elementar Vario EL* and a *Carlo Erba 1104*. Melting points are uncorrected and were determined by using a *Büchi B-545* apparatus.

1.2. Synthesis:

3,3'''-Didodecyl-2,2';5',2";5",2'"-quaterthiophene-5-carbaldehyde **3**: For the generation of the Vilsmeier reagent 70 µL dry *N*,*N*-dimethylformamide (0.9 mmol, 1.5 eq.) were dissolved in 1.3 mL of dry

dichloroethane and 90 µL (0.9 mmol, 1.5 eq.) of $POCl_3$ were added dropwise. The resulting slightly yellow mixture was stirred for two hours at room temperature. In a two-neck flask equipped with a reflux condenser, 400 mg 3,3’’’-didodecyl-2,2';5',2";5",2"'-quaterthiophene **2** (0.6 mmol, 1 eq.) were dissolved in 2 mL of dry dichloroethane. The solution was warmed to reflux and the Vilsmeier reagent was added dropwise. After 3h under reflux, the reaction mixture was quenched with 20 ml of saturated $NaHCO_3$-solution and stirred over night. The layers were separated, the aqueous layer was extracted three times with dichloromethane. The combined organic layers were washed with water and dried over Na_2SO_4. The solvent was removed in vacuo. The crude product was purified by column chromatography (silica, dichloromethane as eluent) and gave 261 mg (3.8 mmol, 63%) of **3** as a bright red solid. M.p.: 63-64 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.83 (s, 1H, carbonyl-H), 7.59 (s, 1H, H-4), 7.17 (m, 4H, H-4’,3’,4’’, 5’’’), 7.03 (d, $^3J_{(H\text{-}3'',H\text{-}4'')}$ = 3.78 Hz, 1H, H-3’’), 6.94 (d, $^3J_{(H\text{-}5''',H\text{-}4''')}$ = 5.20 Hz, 1H,H- 4’’’), 2.80 (m, 4H, H_a), 1.67 (m, 4H, H_b), 1.25 (m, 36H, $H_{c\text{-}k}$), 0.87 (m, 6H, H_l); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 182.4, 140.9, 140.4, 140.2, 140.1, 139.1, 138.9, 136.2, 135.9, 133.6, 130.1, 130.0, 128.2, 126.5, 124.5, 124.0, 124.0, 31.9, 30.6, 30.2, 29.6, 29.6, 29.5, 29.5, 29.5, 29.4, 29.4, 29.3, 29.2, 22.6, 14.0; MS (MALDI-TOF, dithranol): m/z $[M+H]^+$ = 695.0 (calc. for $C_{41}H_{58}OS_4$: 694.3); Elemental analysis: calc. (%) for $C_{41}H_{58}OS_4$: C 70.84, H 8.41, S 18.45; found: C 70.67, H 8.44, S 18.19; FT-IR (KBr), ν [cm^{-1}]: 3084, 2923, 2847, 2361, 1648, 1426, 1390, 1252, 1162, 801, 725.

3,3’’’-Didodecyl-5-ethynyl-2,2';5',2";5",2"'-quaterthiophene **4**: A suspension of 115 mg sodium hydride (2.9 mmol, 10 eq.) in 30 mL of dry tetrahydrofuran was cooled to 0 °C. 382 µl of dimethyl(2-oxopropyl)phosphonate (2.8 mmol, 9.6 eq.) were added dropwise to the solution. The resulting mixture was stirred at 0 °C for one hour. Then 567 mg (2.8 mmol, 10 eq.) of tosylazide were added and the reaction mixture was stirred for another 10 min at 0 °C. The reaction mixture then was quickly passed through a short filtration column (silica, eluent: ethyl acetate) and the solvent was removed in vacuo. The resulting colourless oil was dissolved in 14 mL of dry tetrahydrofuran and was added to a previously prepared suspension of 716 mg of potassium carbonate (5.2 mmol, 18 eq.) and 200 mg of **3** (287.7 µmol, 1 eq.) in 8 mL of dry methanol. The resulting intensely yellow mixture was stirred under

argon over night. The solvent was removed in vacuo and the residue was redissolved in dichloromethane and was washed with a satured solution of ammonium chloride. The layers were separated and the aqueous layer was repeatedly extracted with dichloromethane. The combined organic layers were dried over Na_2SO_4 which was filtered off and the solvent was removed in vacuo. The crude product was put onto silica gel and was purified by column chromatography (eluent: hexane) to yield 178 mg (257.5 µmol, 90%) of the desired product **4** as a bright yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.18 (d, $^3J_{(H\text{-}5''',H\text{-}4''')}$ = 5.14 Hz, 1H,H- 5'''), 7.12 (m, 3H, H-3'', H-4'', H-3'), 7.02 (m, 2H, H-4', H-4), 6.94 (d, $^3J_{(H\text{-}5''',H\text{-}4''')}$ = 5.24, 1H, H-4'''), 3.38 (s, 1H, acetylene-H), 2.74 (m, 4H, H_a), 1.62 (m, 4H, H_b), 1.25 (m, 36H, $H_{c\text{-}k}$), 0.88 ("t", $^3J_{(H\text{-}l,H\text{-}k)}$ = 6.82 Hz, 6H, H_l); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 139.8, 139.3, 137.3, 136.4, 136.0, 135.5, 133.9, 132.4, 130.1, 130.0, 126.9, 126.4, 124.0, 123.8, 123.7, 119.6, 82.0 (C-B), 76.9 (C-A), 31.9, 30.6, 30.3, 29.6, 29.6, 29.6, 29.5, 29.5, 29.4, 29.4, 29.3, 29.2, 29.1, 22.6, 14.1; MS (MALDI-TOF, dithranol): m/z $[M+H]^+$ = 690.3 (calc. for $C_{42}H_{58}S_4$: 690.3), m/z $[M\text{-}16]^+$ = 674.2, m/z $[M+dithranol+Na]^+$ = 940.4; FT-IR (KBr), ν [cm^{-1}]: 3309, 2923, 2852, 2101, 1645, 1465, 834, 791, 656, 587; Analytical HPLC**:** analytical nitrophenyl-column, eluent: 100 % hexane, 99% purity; UV-Vis (CH_2Cl_2): λ_{max} (ε) = 390 (29700).

PEO-peptide-quaterthiophene hybrid precursor **6**: To a solution of 25 mg (36 µmol, 1 eq.) of **4** in 1.5 mL of dichloromethane were added 77 mg (40 µmol, 1.1 eq.) of PEO-peptide **5**, 5.4 mg (14.5 µmol, 0.4 eq.) of tetrakis(acetonitrile)copper(I) hexafluorophosphate ($[Cu(CH_3CN)_4]PF_6$) and 0.9 mg (14.5 µmol, 0.4 eq.) of copper powder. The reaction was stirred at room temperature for 24h. The reaction mixture was washed with semi-concentrated ammonia and the aqueous layer was repeatedly reextracted with dichloromethane. The combined organic layers were washed with Brine, then the solvent was removed from the organic layer. The crude product was taken up in dioxane and was freeze-dried. For further purification, an SEC column was run (eluent: THF). The product containing fractions were combined and the THF was removed. The residue was taken up in dioxane and the desired product **6** was isolated by precipitation with diethyl ether under ice cooling and subsequent collection by centrifugation (washing with diethyl ether) as 68 mg (26 µmol, 71%) of an orange solid.^{1}H NMR (500

MHz, $C_2D_2Cl_2$/MeOH-d_4 3:1, 350K): δ = 8.20 (s, triazole-H, 1H), 7.64-7.62 (d, 2H, C_{ar}H aPhe), 7.36-7.34 (d, 2H, C_{ar}H aPhe), 7.26 (s, 1H, H-4), 7.13 (d, $^3J_{(H\text{-}5''',H\text{-}4''')}$ = 4.05 Hz 1H, H- 5'''), 7.09 (bs, 2H, H-3', H-4''), 7.03 (bs, 1H, H-4'), 6.96 (bs, 1H, H-3''), 6.87 (d, $^3J_{(H\text{-}5''',H\text{-}4''')}$ = 4.95, 1H, H-4'''), 5.30-5.21 (m, 1H, C<u>H</u>(CH_3)-O-CO Thr), 4.74-3.64 (m, 14H, 24 H α-C<u>H</u> + 1 C<u>H</u>-O-C(CH_3)$_2$ + 1 C<u>H</u>-O-C(CH_3)$_3$ Thr), 3.56 (m, 54H, O-C<u>H_2</u>-C<u>H_2</u>-O), 3.46 (m, 3H, O-C<u>H_3</u> PEG), 2.73 (m, 4H, H_a), 2.46 (m, 4H, CO-C<u>H_2</u>-C<u>H_2</u>-CO), 2.14-1.94 (m, 3H, C<u>H</u>(CH_3)$_2$ Val), 1.69-0.73 (m, 97H, 18H, C(C<u>H_3</u>)$_2$ Val + 9H CH(C<u>H_3</u>)-O Thr + 9H O-C(C<u>H_3</u>)$_3$ Boc + 9H O-C(C<u>H_3</u>)$_3$ ^{t}Bu + 6H C(C<u>H_3</u>)$_2$ ^{Me,Me}pro + 4H alkyl-H_b + 36H alkyl-$H_{c\text{-}k}$ + 6H, alkyl-H_l); MS (MALDI-TOF, DCTB): calculated: *m/z* = 2639.42, found: two homologous series with 44 Da mass difference of the PEO repeat units, each ($m/z_{(max\ intensity)}$ = 2661.9 and 2678.1, which can be assigned to $[M + Na]^+$ and $[M + K]^+$, respectively with 15 ethylene glycol repeat units and an end group mass of 1260.75 Da); GPC (THF, pS-standards) M_n = 1590, M_w/M_n = 1.38; UV-Vis (CH_2Cl_2/MeOH 1:1): λ_{max} (ε) = 394 (20800).

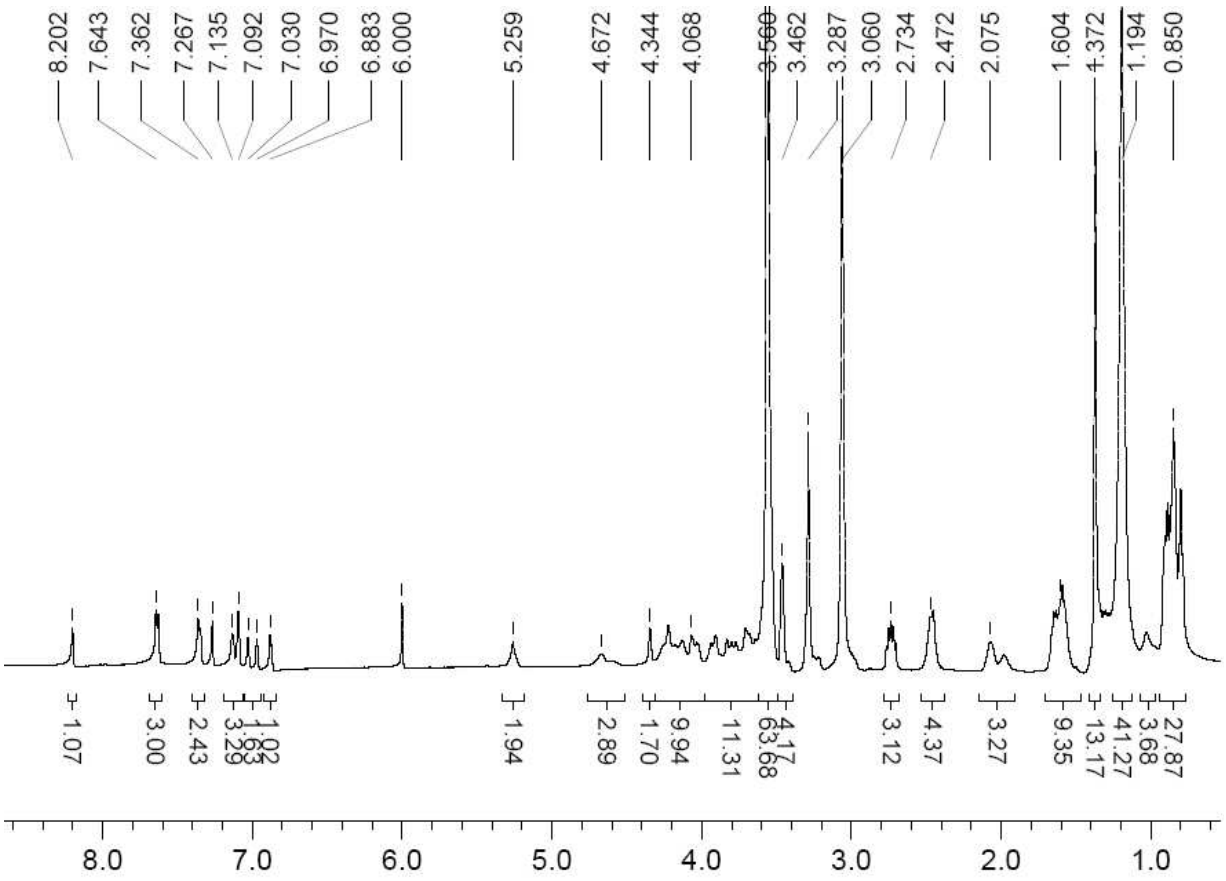


Figure S1. ^{1}H-NMR spectrum of PEO-peptide-quaterthiophene hybrid **6** ($C_2D_2Cl_4$ / MeOH-d_4 3:1, 77°C); δ = 6.00 ppm: tetrachloroethane, δ = 3.28 and 3.06 ppm: methanol

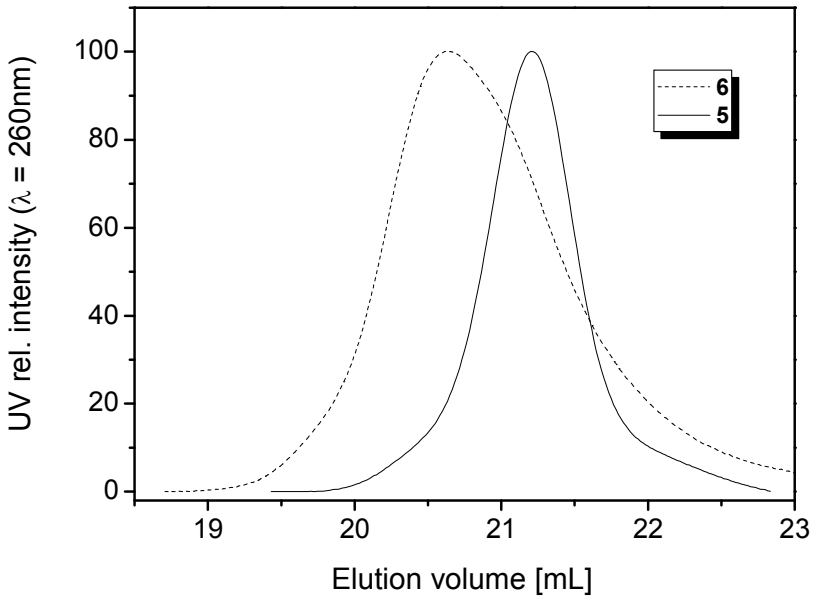


Figure S2. Comparison analytical GPC traces of PEO-peptide quaterthiophene hybrid precursor **6** and PEO-peptide **5** in THF.

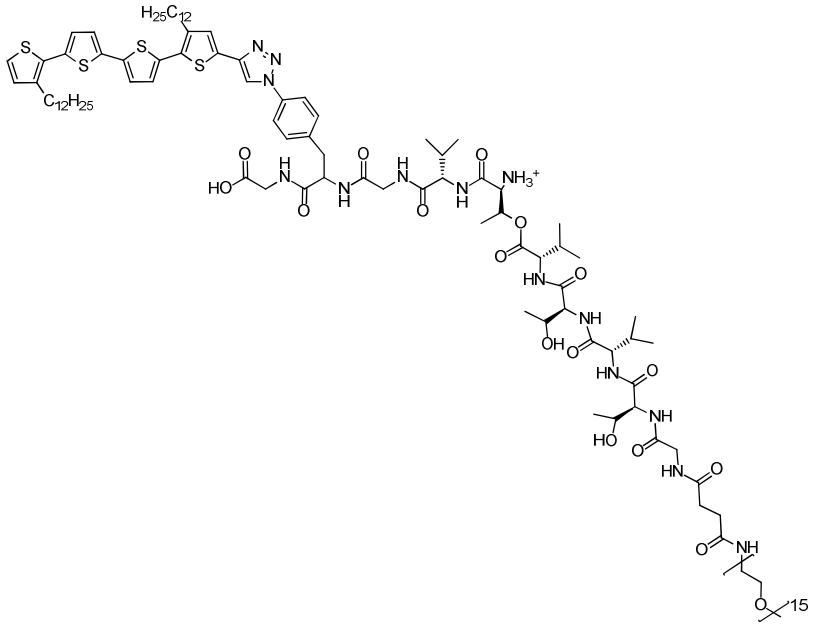


Figure S3. Structure of "kinked" PEO-peptide quaterthiophene hybrid precursor **7**.

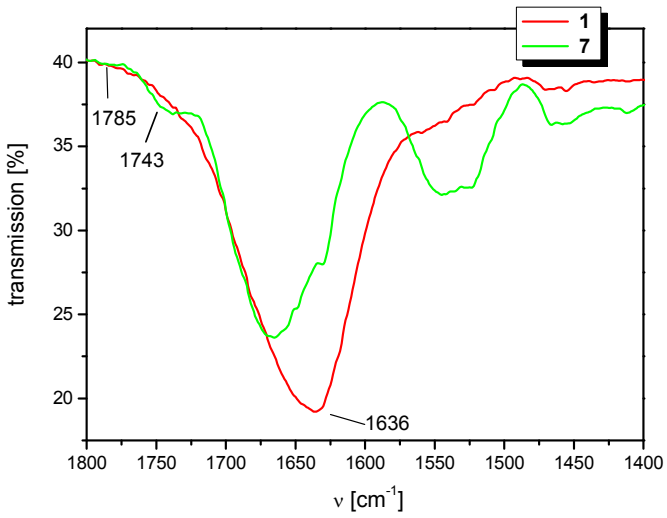


Figure S4. Carbonyl region of the FT-IR spectra of **7** (kinked state, green curve) and **1** (native state, red curve).

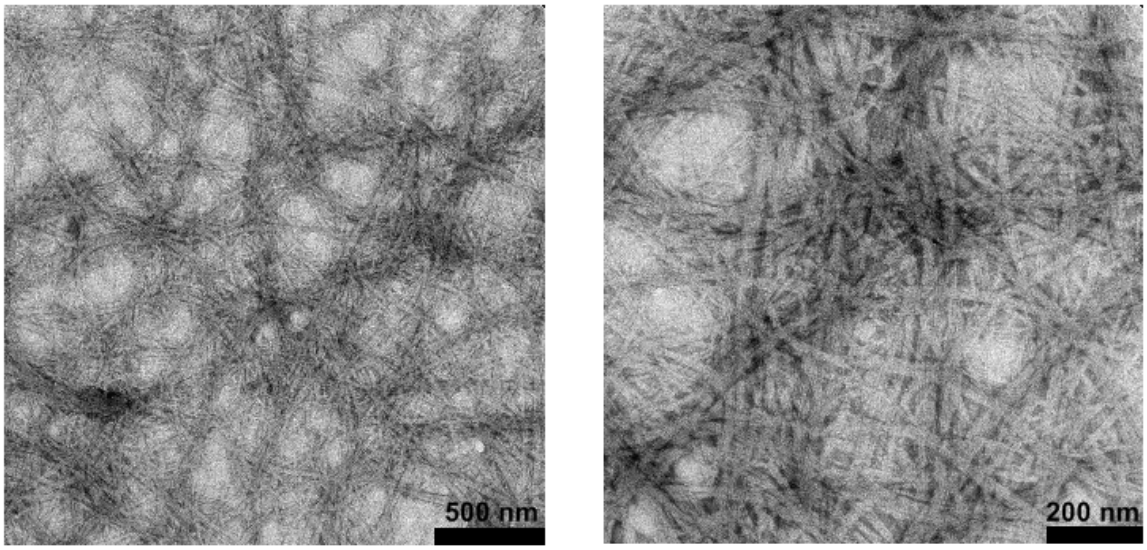


Figure S5. TEM images (unstained) of self-assembled fibers of **1**.

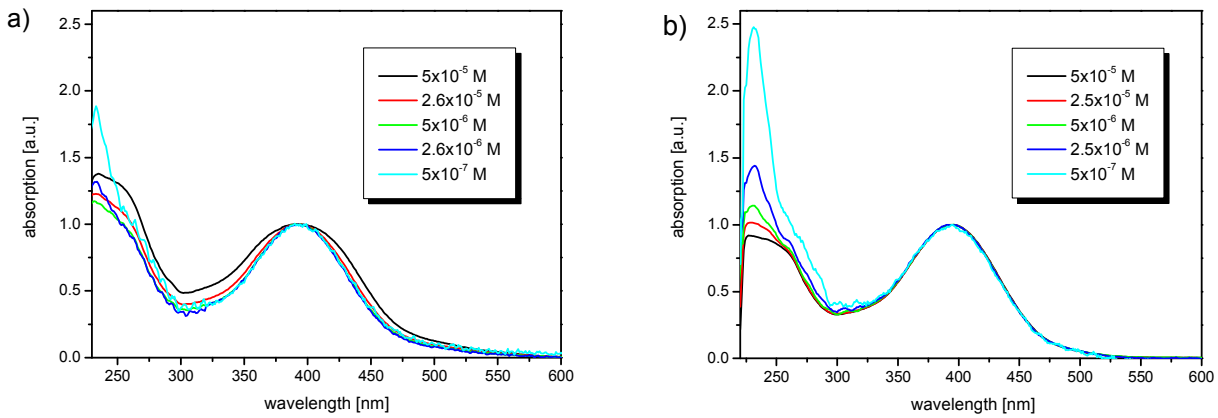


Figure S6. Normalized absorption spectra of PEO-peptide-oligothiophene hybrids: a) **1** and b) **7**. Samples were prepared in two different ways; case A (a): solution was prepared by slow addition of 0.001 M NaOH in methanol to a DCM solution by syringe pump (1:1); case B (b): solution was prepared by directly dissolving compound **7** in a 1:1 mixture of methanol and DCM.

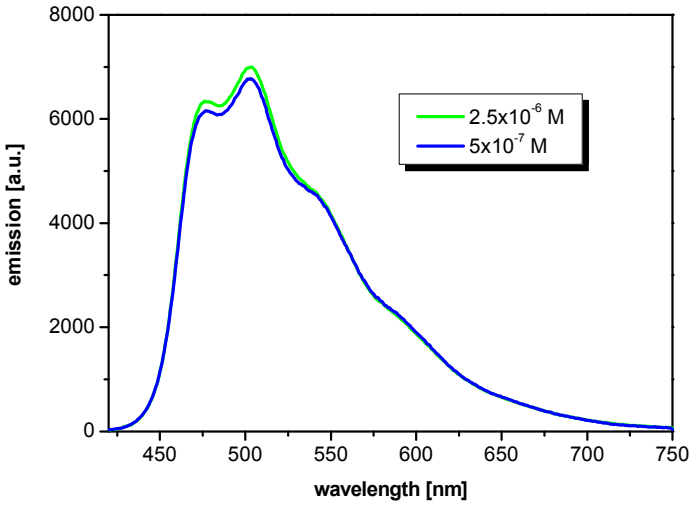


Figure S7. Emission spectra (normalized to the absorption at the excitation wavelength at $\lambda = 410$ nm) for quaterthiophene-peptide-PEO-hybrid **1** (case A).

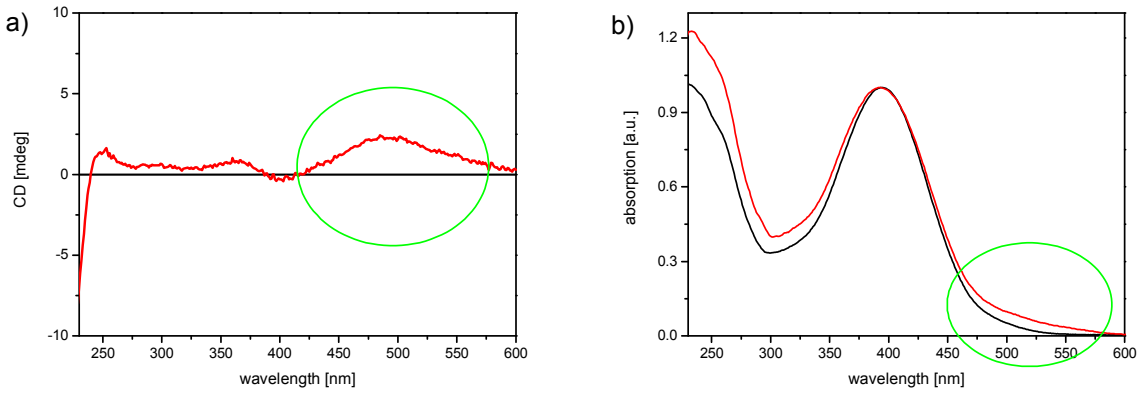
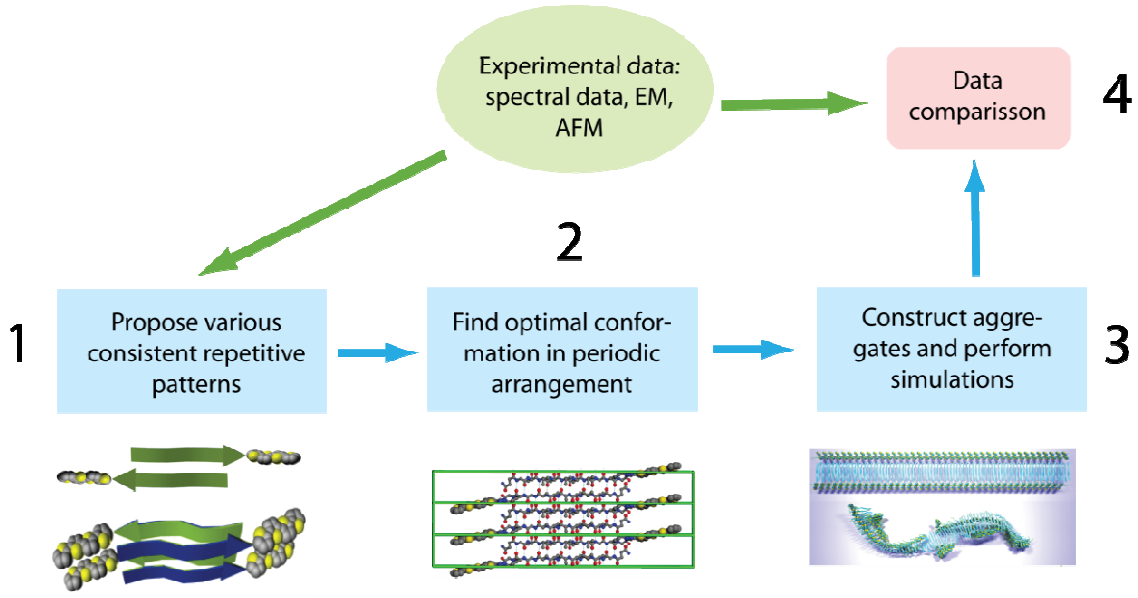


Figure S8: a) CD spectrum for hybrid **1** ($c \approx 4x10^{-4}$ M in DCM/MeOH 1:1) and b) normalized absorption spectra of **7** (black line) and **1** (red line) for band-shape comparison ($c \approx 2.5x10^{-5}$ M in DCM/MeOH 1:1).

2. Theoretical analysis workflow.

Our theoretical research methodology consisted of 4 main steps (see Scheme S1). During the first step (1) the available experimental data, including available X-ray structural data for the arrangement of peptide moieties in biological amyloid-like fibrils, was analyzed and possible periodic arrangement patterns of molecules consistent with experimental results were proposed. In the second step (2), a methodology relying on MD simulations was applied to obtain periodic arrangements of molecules that correspond to the local free energy minimum when all local degrees of freedom (torsion angles, side chain conformations, hydrogen bonds) would achieve their optimal positions. During step 3, these optimal arrangements were used to construct long fibrillar aggregates, the dynamic and statistical behavior of which was investigated by using MD simulations. At step 4, the obtained analysis results of the computer simulations are compared with experimental data, which enables us to suggest the most likely molecular arrangement pattern observed in the experiment.



Scheme S1. Theoretical analysis workflow.

3. Simulation protocols.

All simulations were performed using the LAMMPS simulation package.[5]

3.1. Simulations of periodic arrangement

Periodic systems were at first subjected to energy minimization using Polak-Ribiere version of the conjugate gradient (CG) algorithm with the same potential parameters (described further) used during MD simulations. MD simulations for periodic systems employed cut-off radius for Van-der-Waals interactions of 1.0 nm, Coulomb interactions were accounted using particle-particle particle-mesh solver technique[6] with 1.0 nm cut-off in real space and precision parameter of 10^-4. Integration step was 1 fs. NVT ensemble with T=300K was maintained by adding friction and stochastic terms to the equations of motion according to Langevin equation, the inverse fiction constant of 1 ps was used.

3.2. Simulations of fibrillar aggregates

The production run was preceded by a relaxation run of 1 ps with the integration step of 0.1 fs. After that, a production run of 10 ns was employed with integration step of 1 fs. Cut-off radius for Coulomb and Van-der-Waals interactions was 1.5 nm. NVT ensemble was implemented using Langevin algorithm. During the relaxation run the temperature was maintained at 100 K with inverse friction constant of 1 ps, while during the production run, these parameters were 300 K and 2 ps respectively.

2.3. Simulations of aggregates on substrate

The substrate was modeled by 4 layers of highly oriented pyrolytic graphite. The system was assembled by placing the fibrillar aggregate in its conformation obtained after the bulk simulation studies near the surface of graphite. The simulations employed 1 fs integration step, Cut-off radius for Coulomb and Van-der-Waals interactions was 1.5 nm. To implement NVT ensemble, the Nose-Hoover algorithm[7] was used separately for two subsystems (the fibril and the substrate), because of the high difference in absolute heat capacities of two subsystems. The relaxation constant of 1 ps was used, the simulations were performed at 300 K.

3.4. Virtual AFM measurements

The system consisted of a graphite substrate (15x25 nm wide) and a double-layer fibril of type c, each sheet containing 40 β-strands. The fibril proved to be planar and stable during the 10 ns simulations. The procedure of virtual AFM measurements was accomplished by custom-made software. In our algorithm, a probe sphere 1 nm in diameter approached the surface of the substrate form above at each point of the horizontal grid with 0.1 nm spacing. Then the highest position of the probe sphere was determined when the sphere did not overlap with any of the van der Waals spheres of atoms in the system. The vertical position of the sphere was devised using bisection method with accuracy of 0.1 nm. The obtained data was further visualized using WSxM imaging software.

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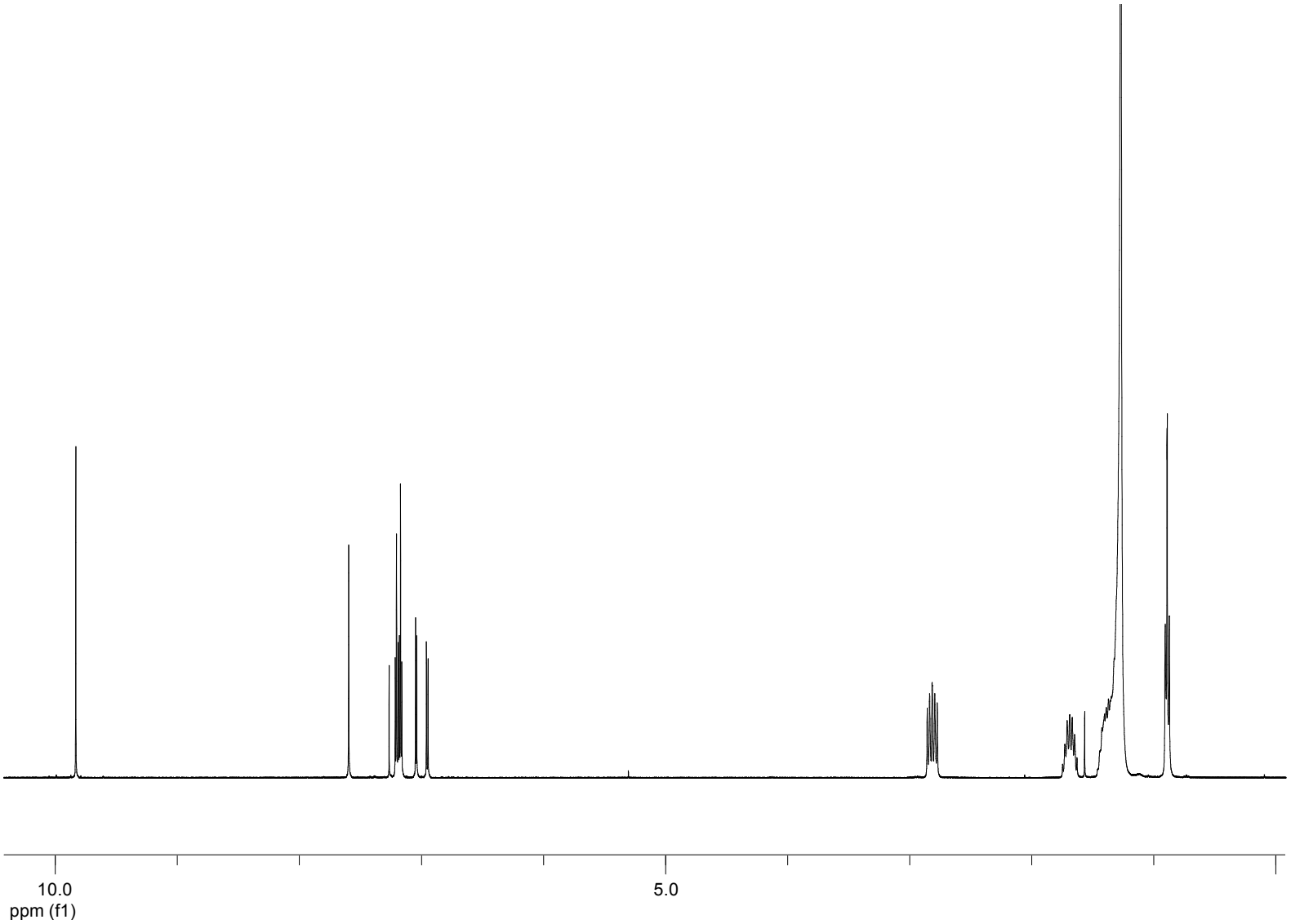
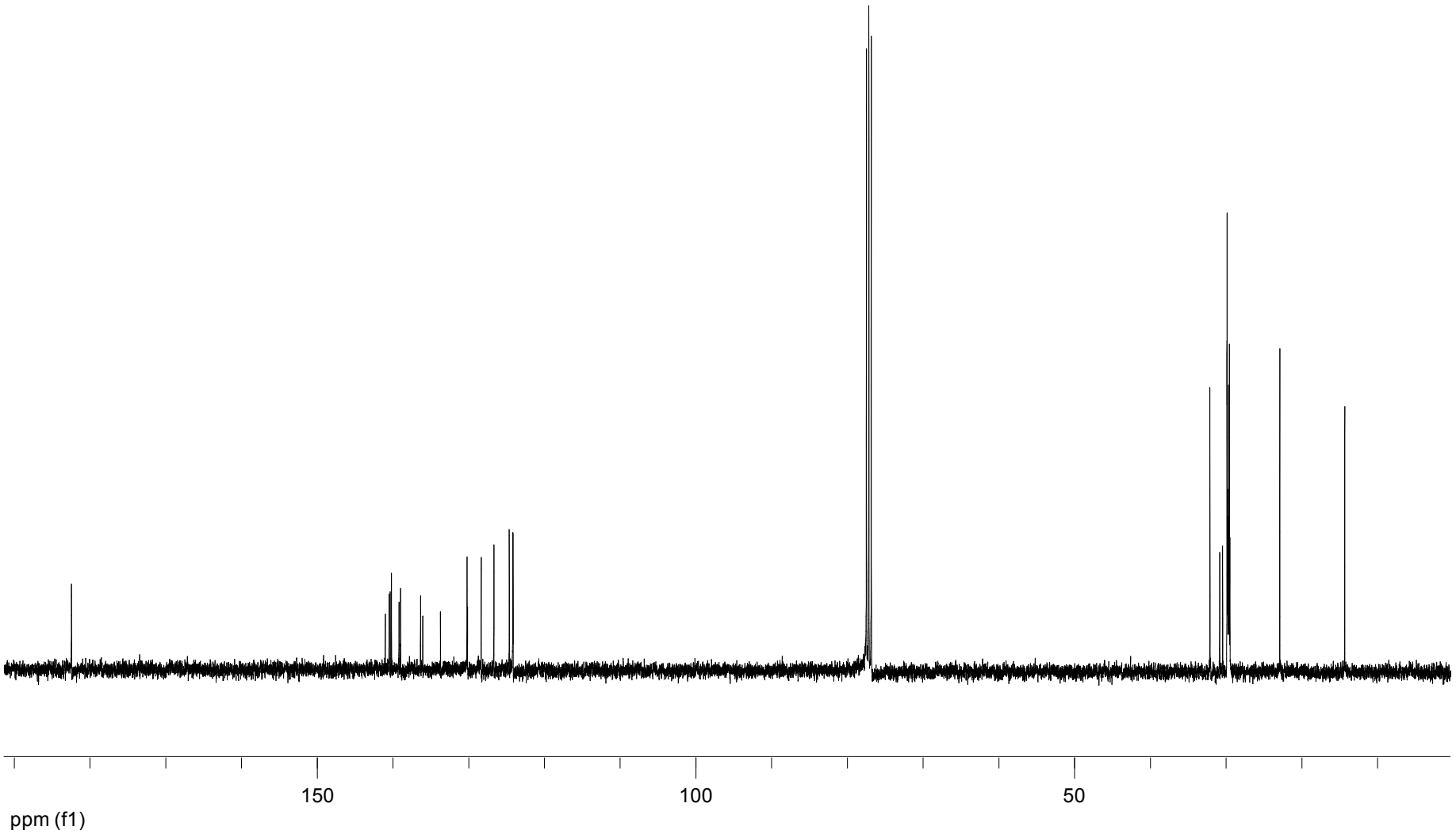
5. Plimpton, S., Fast Parallel Algorithms for Short-Range Molecular Dynamics. *J. Comput. Phys.* **1995,** *117*, 1-19.
6. Hockney, R. W.; Eastwood, J. W., *Computer simulation using particles*. Taylor and Fracis: New York, 1988.
7. Hoover, W., Canonical dynamics: Equilibrium phase-space distributions. *Phys. Rev. A* **1985,** *31*, 1695-1697.

Annex – NMR spectra

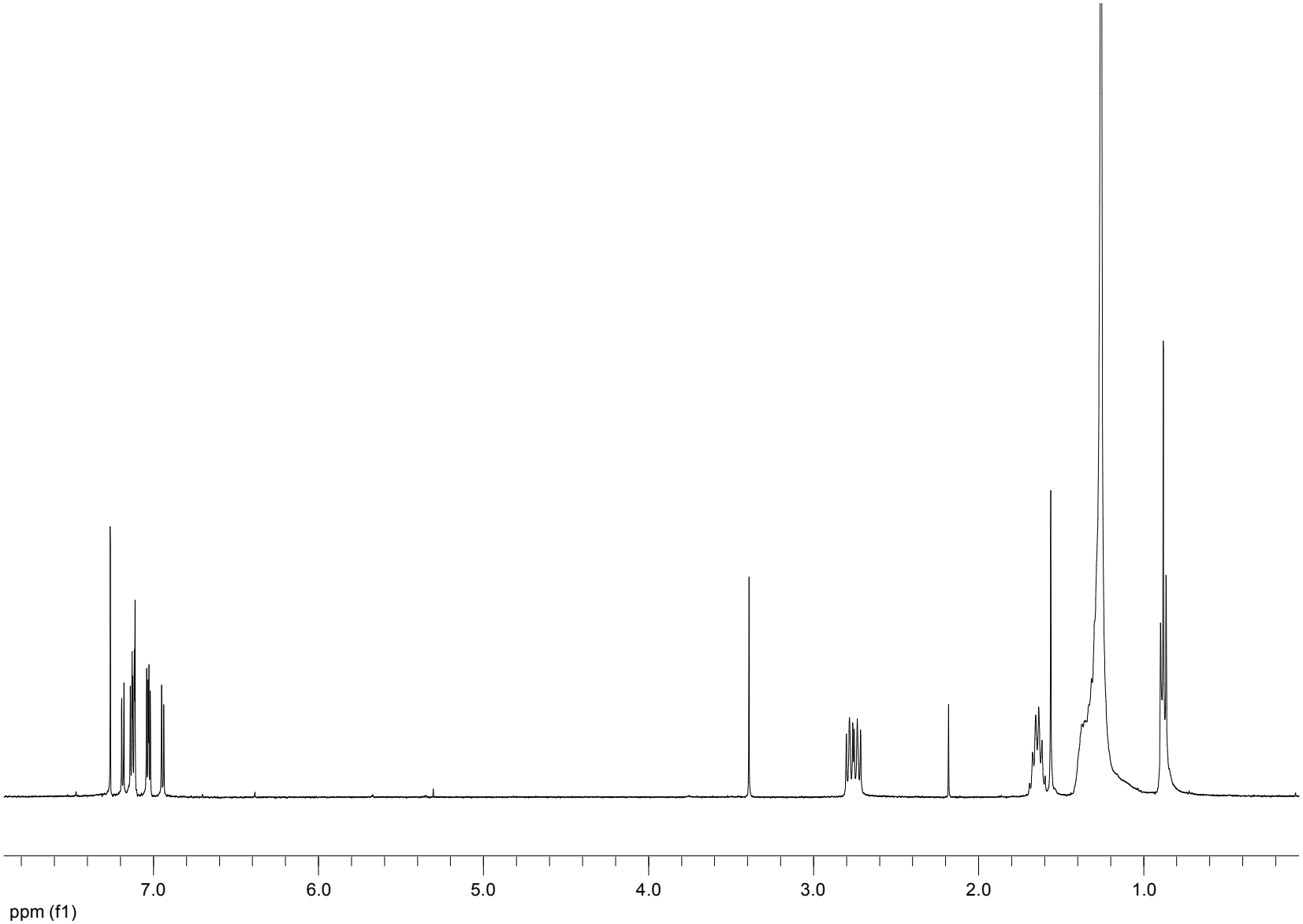
3,3’’’-Didodecyl-2,2';5',2";5",2"'-quaterthiophene-5-carbaldehyde **3**



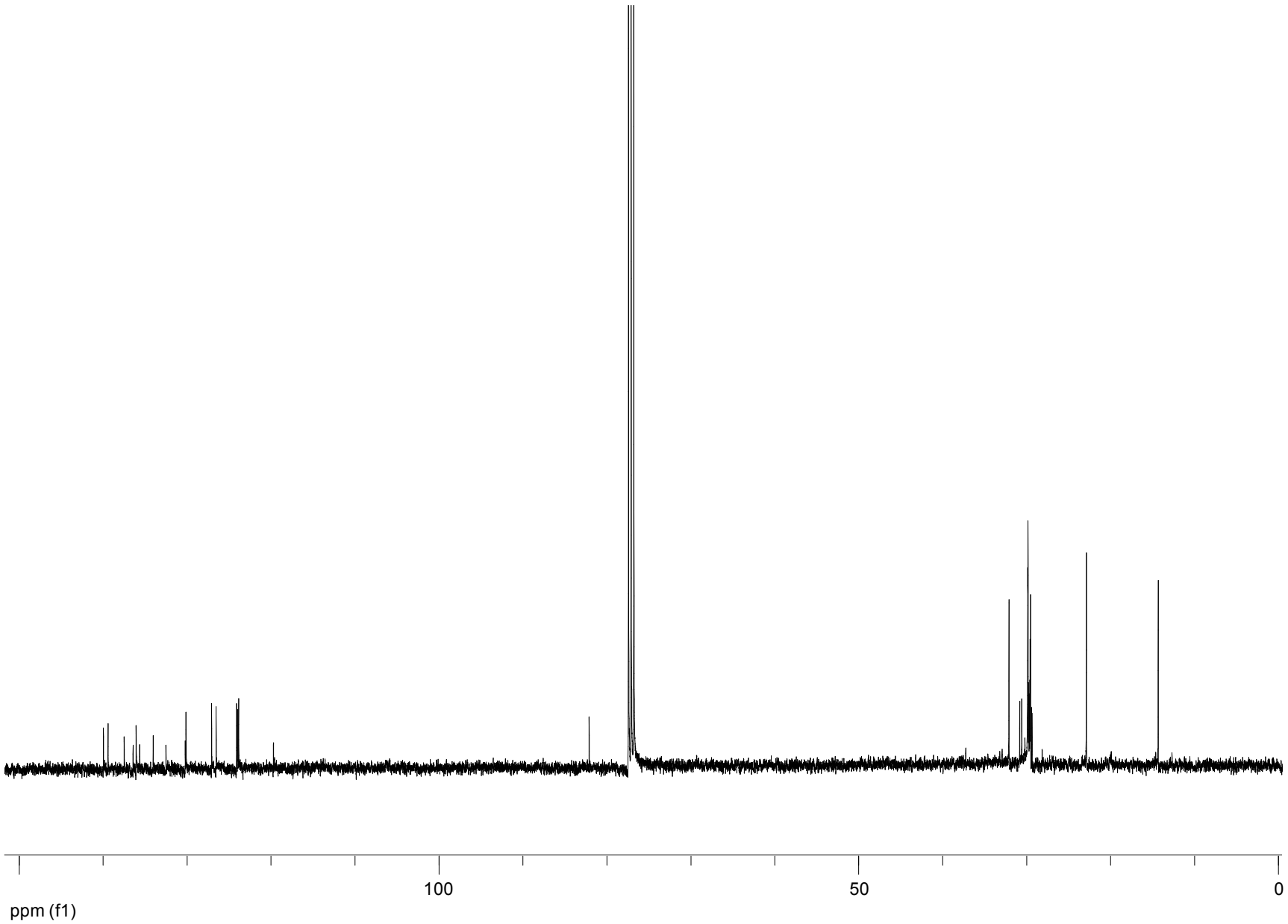
Note: Signal at δ = 1.55 ppm results from water.


150
100
50
ppm (f1)

3,3’’’-Didodecyl-5-ethynyl-2,2';5',2";5",2'"-quaterthiophene **4**



Note: Signals at δ = 1.54 ppm and δ = 2.17 ppm result from water and acetone, respectively.


100
50
0
ppm (f1)