Self-assembly of chiral supramolecular ureido-pyrimidinone-based poly(ethylene glycol) polymers via multiple pathways

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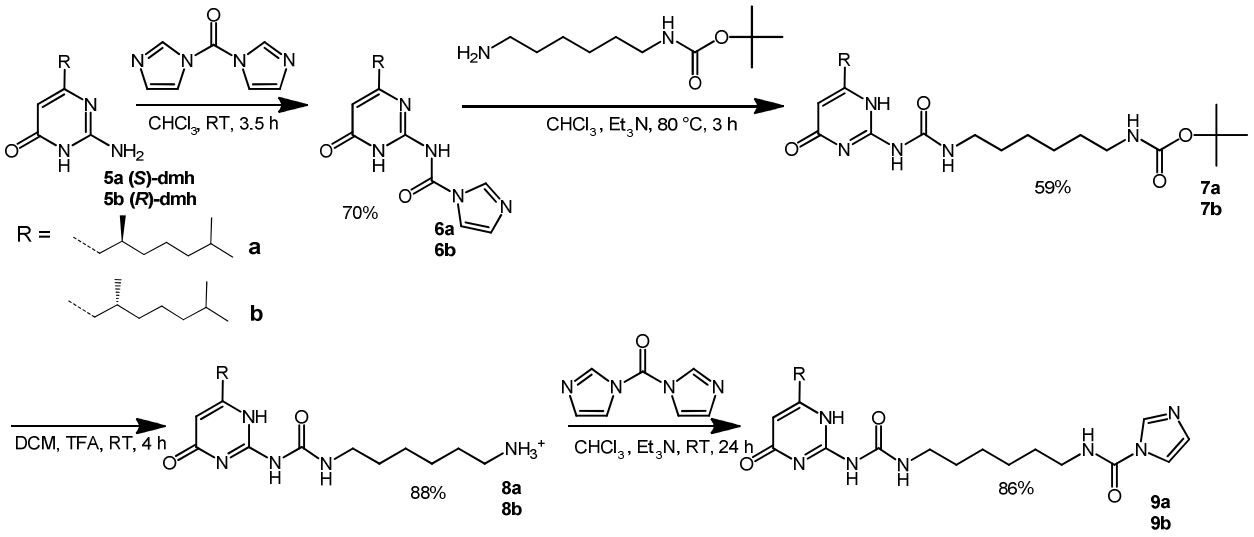
Supporting info

1. Materials and methods

Chemicals. All solvents and chemicals, were of reagent grade quality or better, and purchased from Biosolve, Sigma-Aldrich or Acros. $CHCl_3$ was dried over molsieves and triethylamine was stored on KOH pellets. Purified water was obtained using a MilliQ Advantage A-10 equipped with a Q-Guard T2 purification pack. Two PEG batches were used, one having impurities of higher PEG masses, as observed in MALDI-TOF MS. All other chemicals were used as received.

Synthesis



Scheme S1. Synthetic route to CDI activated UPy synthons.

Synthesis of (*S*)-*N*-(6-(3-(6-(2,6-dimethylheptyl)-4-oxo-1,4-dihydropyrimidin-2-yl)ureido) hexyl)-1H-imidazole-1-carboxamide (9a)

*Synthesis of (S)-N-(6-(2,6-dimethylheptyl)-4-oxo-1,4-dihydropyrimidin-2-yl)-1H-imidazole-1-carboxamide (**6a**)*

(*S*)-dimethylheptyl-isocytosine (**5a**) (0.46 g, 1.94 mmol) and 1,1'-carbonyldiimidazole (CDI) (0.48 g, 2.96 mmol) were dissolved in dry $CHCl_3$ (15 mL) under an argon atmosphere and stirred at room temperature for 3.5 h. Work up via extraction was performed to yield a slightly yellow solid (0.45 g, 1.36 mmol, 70%). ^{1}H-NMR ($CDCl_3$): δ = 8.8 (s, 1H, CDI), 7.6 (s, 1H, CDI), 7.0 (s, 1H, CDI), 5.8 (s, 1H, UPy-alkylidene), 2.6-2.4 (dq, 2H, CH_2), 1.95 (m, 1H, C*H chiral*), 1.6-1.1 (m, 7H, CH_2 C*H*), 1.0 (d, 3H, CH_3), 0.9 (d, 6H, CH_3) ppm, NH not visible.

*Synthesis of (S)-tert-butyl 6-(3-(6-(2,6-dimethylheptyl)-4-oxo-1,4-dihydropyrimidin-2-yl)ureido)hexylcarbamate (**7a**)*

Compound **6a** (0.45 g, 1.36 mmol) and *N*-boc-1,6-hexanediamine (0.36 g, 1.66 mmol) were dissolved in dry $CHCl_3$ (15 mL) under an argon atmosphere and refluxed for 3 h. Triethylamine (0.85 g, 8.32 mmol) was added because the hexanediamine salt was used. An acid-base extraction was followed by a recrystallization from MeOH (10 mL) to yield a white solid (0.39 g, 0.80 mmol, 59 %) ^{1}H-NMR ($CDCl_3$): δ = 13.2 (s, 1H, N*H*), 11.9 (s, 1H, N*H*), 10.2 (s, 1H, N*H*), 5.8 (s, 1H, UPy-alkylidene), 4.7 (s, 1H, N*H*), 3.3 (q, 2H, CH_2-NH), 3.1 (q, 2H, CH_2-NH), 2.6-2.2 (dq, 2H, CH_2), 1.8-1.1 (m, 16H, CH_2 C*H*), 1.4 (s, 9H, *t*-boc), 1.0 (d, 3H, CH_3), 0.9 (d, 6H, CH_3) ppm. ^{13}C-NMR ($CDCl_3$) δ = 173.1, 156.6, 155.9, 154.7, 151.5, 106.9, 78.9 (t-boc), 40.5, 39.7, 39.0, 36.7, 31.9, 29.9, 29.4, 28.4, 27.9, 26.4, 24.5, 22.6, 22.5, 19.3 ppm. IR (cm^{-1}): 3373, 3219, 2956, 2928, 2857, 1698, 1683 1645, 1577, 1519, 1464, 1445, 1384, 1364, 1250, 1171, 1140, 1126, 1099, 1040, 1017, 1001, 962, 941, 909, 887, 867, 804. MALDI-TOF MS: calculated MW = 479.66 g/mol, observed m/z = 518.31 $[M+K]^+$, 502.34 $[M+Na]^+$, 480.33 $[M+H]^+$.

*Synthesis of (S)-1-(6-(3-(6-(2,6-dimethylheptyl)-4-oxo-1,4-dihydropyrimidin-2-yl)ureido) hexyl)uronium (**8a**)*

Compound **7a** (0.23 g, 0.48 mmol) was dissolved in a DCM TFA-mixture (1:1, v:v, 10mL). This was stirred at room temperature for 4 h. The solvents were evaporated and the TFA was coevaporated with toluene. ^{19}F-NMR showed that no TFA traces were left (0.16 g, 0.42 mmol, 88%). ^{1}H-NMR ($CDCl_3$): δ = 8.1 (s, 1H, N*H*), 5.9 (s, 1H, UPy-alkylidene), 3.3 (q, 2H, CH_2-NH), 3.1 (q, 2H, CH_2-NH), 2.6-2.2 (dq, 2H, CH_2), 1.9 (s, 1H, C*H*) 1.8-1.1 (m, 15H, CH_2 C*H*), 1.0 (m, 3H, CH_3), 0.9 (m, 6H, CH_3) ppm, N*H* and NH_2 not visible.

*Synthesis of (S)-N-(6-(3-(6-(2,6-dimethylheptyl)-4-oxo-1,4-dihydropyrimidin-2-yl)ureido) hexyl)-1H-imidazole-1-carboxamide (**9a**)*

Compound **8a** (0.16 g, 0.42 mmol) and triethylamine (0.35 mL, 2.5 mmol) were mixed in dry $CHCl_3$ (4 mL) under an argon atmosphere. A precipitate was formed and the suspension was stirred for 5 min. CDI (0.13 g, 0.80 mmol) was added and the solution was stirred for 24 h. Work up via extraction was performed to yield a slightly yellow solid (0.17 g, 0.36 mmol, 86%). ^{1}H-NMR ($CDCl_3$): δ = 13.2 (s, 1H, N*H*), 11.9 (s, 1H, N*H*), 10.1 (s, 1H, N*H*), 8.3 (s, 1H, CDI), 7.6 (s, 1H, CDI), 7.0 (s, 1H, CDI), 5.7 (s, 1H, UPy-alkylidene), 3.4 (q, 2H, CH_2-NH), 3.3 (q, 2H, CH_2-NH), 2.6-2.2 (dq, 2H, CH_2), 1.8 (s, 1H, C*H*), 1.7-1.1 (m, 15H, CH_2 C*H*), 1.0 (m, 3H, CH_3), 0.9 (m, 6H, 2CH_3) ppm.

Synthesis of (*R*)-*N*-(6-(3-(6-(2,6-dimethylheptyl)-4-oxo-1,4-dihydropyrimidin-2-yl)ureido) hexyl)-1H-imidazole-1-carboxamide (9b)

*Synthesis of (R)-N-(6-(2,6-dimethylheptyl)-4-oxo-1,4-dihydropyrimidin-2-yl)-1H-imidazole-1-carboxamide (**6b**)*

(*R*)-dimethylheptyl-isocytosine (**5b**) (0.6 g, 2.53 mmol) and 1,1'-carbonyldiimidazole (CDI) (0.49 g, 3.04 mmol) were dissolved in dry $CHCl_3$ (15 mL) under an argon atmosphere and stirred

at room temperature for 3.5 h. Work up via extraction was performed to yield a slightly yellow solid (0.5 g, 1.51 mmol, 60%). ^{1}H-NMR ($CDCl_3$): δ = 13.4 (s, 1H, N*H*), 12.1 (s, 1H, N*H*), 8.8 (s, 1H, CDI), 7.6 (s, 1H, CDI), 7.0 (s, 1H, CDI), 5.8 (s, 1H, UPy-alkylidene), 2.6-2.4 (dq, 2H, CH_2), 1.95 (m, 1H, C*H chiral*), 1.6-1.1 (m, 7H, CH_2 C*H*), 1.0 (d, 3H, CH_3), 0.9 (d, 6H, CH_3) ppm.

*Synthesis of (R)-tert-butyl 6-(3-(6-(2,6-dimethylheptyl)-4-oxo-1,4-dihydropyrimidin-2-yl)ureido)hexylcarbamate (**7b**)*

Compound **6b** (0.5 g, 1.51 mmol) and *N*-boc-1,6-hexanediamine (0.46 g, 1.81 mmol) were dissolved in dry $CHCl_3$ (10 mL) under an argon atmosphere and refluxed for 3 h. Triethylamine (0.3 mL, 1.99 mmol) was added because the hexanediamine salt was used. An acid-base extraction was followed by a recrystallization from MeOH (10 mL) to yield a white solid (0.36 g, 0.75 mmol, 50 %) ^{1}H-NMR ($CDCl_3$): δ = 13.2 (s, 1H, N*H*), 11.9 (s, 1H, N*H*), 10.2 (s, 1H, N*H*), 5.8 (s, 1H, UPy-alkylidene), 4.6 (s, 1H, N*H*), 3.3 (q, 2H, CH_2-NH), 3.1 (q, 2H, CH_2-NH), 2.5-2.2 (dq, 2H, CH_2), 1.8-1.1 (m, 16H, CH_2 C*H*), 1.4 (s, 9H, *t*-boc), 1.0 (d, 3H, CH_3), 0.9 (d, 6H, CH_3) ppm.

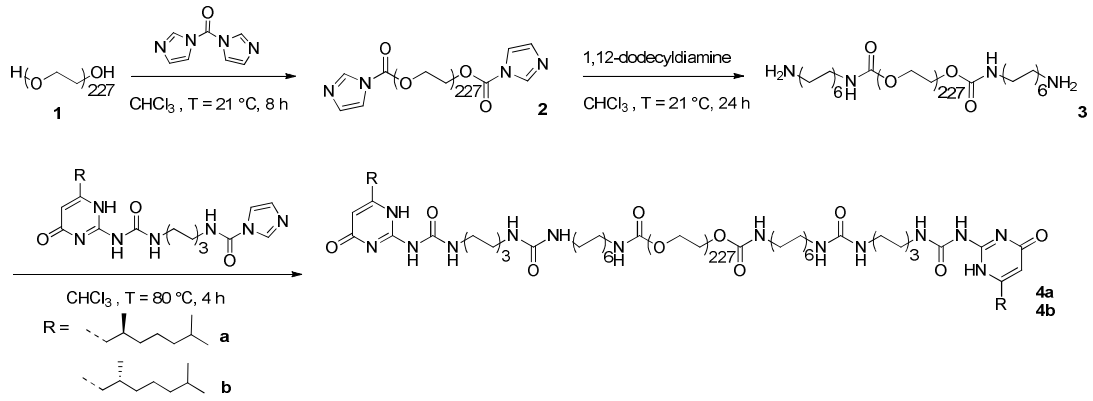
*Synthesis of (R)-1-(6-(3-(6-(2,6-dimethylheptyl)-4-oxo-1,4-dihydropyrimidin-2-yl)ureido) hexyl)uronium (**8b**)*

Compound **7b** (0.36 g, 0.75 mmol) was dissolved in a DCM TFA-mixture (1:1, v:v, 10 mL). This was stirred at room temperature for 4 h. The solvents were evaporated and the TFA was coevaporated with toluene. ^{19}F-NMR showed that no TFA traces were left (0.26 g, 0.68 mmol, 90%). ^{1}H-NMR ($CDCl_3$): δ = 8.1 (s, 1H, N*H*), 5.8 (s, 1H, UPy-alkylidene), 3.3 (q, 2H, CH_2-NH), 3.1 (q, 2H, CH_2-NH), 2.5-2.2 (dq, 2H, CH_2), 1.9-1.1 (m, 16H, CH_2 C*H*), 1.0 (m, 3H, CH_3), 0.9 (m, 6H, CH_3) ppm, N*H* and NH_2 not visible.

*Synthesis of (R)-N-(6-(3-(6-(2,6-dimethylheptyl)-4-oxo-1,4-dihydropyrimidin-2-yl)ureido) hexyl)-1H-imidazole-1-carboxamide (**9b**)*

Compound **8b** (0.26 g, 0.68 mmol) and triethylamine (0.52 mL, 3.75 mmol) were mixed in dry $CHCl_3$ (10 mL) under an argon atmosphere. A precipitate was formed and the suspension was stirred for 5 min. CDI (0.24 g, 1.5 mmol) was added and the solution was stirred for 24 h. Work up via extraction was performed to yield a slightly yellow solid (0.26 g, 0.55 mmol, 81%). ^{1}H-NMR ($CDCl_3$): δ = 13.3 (s, 1H, N*H*), 11.9 (s, 1H, N*H*), 10.0 (s, 1H, N*H*), 8.2 (s, 1H, CDI), 7.5 (s, 1H, CsDI), 7.1 (s, 1H, CDI), 5.7 (s, 1H, UPy-alkylidene), 3.4 (q, 2H, CH_2-NH), 3.3 (q, 2H, CH_2-NH), 2.5-2.2 (dq, 2H, CH_2), 1.8-1.1 (m, 16H, CH_2 C*H*), 1.0 (m, 3H, CH_3), 0.9 (m, 6H,2CH_3) ppm.

Synthesis of poly(ethylene glycol)-bis(12-(3-(6-(3-(6-((*S*)-2,6-dimethylheptyl)-4-oxo-1,4-dihydropyrimidin-2-yl)ureido)hexyl)ureido)dodecylcarbamate) (4a) and Synthesis of poly(ethylene glycol)-bis(12-(3-(6-(3-(6-((*R*)-2,6-dimethylheptyl)-4-oxo-1,4-dihydropyrimidin-2-yl)ureido)hexyl)ureido)dodecylcarbamate) (4b)



Scheme S2. Synthetic route to telechelic PEGs functionalized with alkyl spacers and chiral dimethylheptyl-UPy groups.

*Synthesis of poly(ethylene glycol)-bis(1H-imidazole-1-carboxylate) (**2**)*

CDI (0.8 g, 4.9 mmol) was dissolved in dry $CHCl_3$ (5 mL). The dried hydroxy-terminated poly(ethylene glycol) (Mn=10,000 g/mol, 10 g, 1 mmol) was dissolved in dry $CHCl_3$ (30 mL) and added slowly. The mixture was stirred at room temperature under an inert argon atmosphere for 8 h. The work up was done by precipitation in diethyl ether. The white solid was isolated by filtration and dried over P_2O_5 in a vacuum oven at 40 °C overnight (8.3 g, 0.81 mmol, 81%). ^{1}H-NMR ($CDCl_3$): δ = 8.2 (s, 2H, CDI), 7.4 (s, 2H, CDI), 7.1 (s, 2H, CDI), 4.6 (q, 4H, C*H*$_2$), 3.8-3.4 (m, 4nH, PEG) ppm. MALDI-TOF MS: calculated MW = 10190 g/mol, observed m/z = 11531.3 g/mol $[M+H]^+$, 22054.9 g/mol [PEG impurity], 33581.6 g/mol [PEG impurity].

*Synthesis of poly(ethylene glycol)-bis(12-aminododecylcarbamate) (**3**)*

In the next step 1,12-dodecyldiamine (0.92 g, 4.6 mmol) was dissolved in $CHCl_3$ (5 mL). This mixture was stirred under an argon atmosphere and reflux conditions. Compound **2** (4 g, 0.4 mmol) was dissolved in $CHCl_3$ (50 mL) and added slowly to the dodecyldiamine. The reaction mixture was stirred for 24 h. The work up was done by a precipitation in an excess of diethyl ether, dissolution in $CHCl_3$, filtration of the mixture over celite, and a second precipitation of the filtrate in an excess of diethylether. The white solid was isolated by filtration and dried over P_2O_5 in a vacuum oven at 40 °C overnight (3.1 g, 0.3 mmol, 76%). ^{1}H-NMR ($CDCl_3$): δ = 4.9 (s, 2H, N*H*), 4.2 (q, 4H, C*H*$_2$-NH), 3.9- 3.2 (m, 4nH, PEG), 3.1 (q, 4H, C*H*$_2$-NH), 2.7 (t, 4H, C*H*$_2$-NH$_2$), 1.6-1.2 (40H,C*H*$_2$) ppm, N*H*$_2$ not visible. MALDI-TOF MS: calculated MW = 10454.7 g/mol, observed m/z = 11364.8 g/mol $[M+H]^+$, 23090.8 g/mol [PEG impurity], 33788.8 g/mol [PEG impurity].

*Synthesis of poly(ethylene glycol)-bis(12-(3-(6-(3-(6-((S)-2,6-dimethylheptyl)-4-oxo-1,4-dihydropyrimidin-2-yl)ureido)hexyl)ureido)dodecylcarbamate) (**4a**)*

Compound **3** (1.5 g, 0.14 mmol) and UPy-synthon **9a** (0.14 g, 0.29 mmol) were dissolved in $CHCl_3$ (30 mL). The reaction mixture was stirred under an argon atmosphere and reflux

conditions for 4 h. The conversion was followed with ^{1}H-NMR. The work up was done by the use of a resin (poly(styrene-co-divinyl-benzene) amino methylated), to couple the excess of compound **9a**. The reaction mixture was then filtered over celite and a precipitation in diethyl ether was performed to yield a white solid. (1.07 g, 0.09 mmol, 65%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 13.2 (s, 2H, N*H*), 11.9 (s, 2H, N*H*), 10.1 (s, 2H, N*H*), 5.8 (s, 2H, UPy alkylidene), 4.9 (s, 2H, N*H*), 4.7 (s, 2H, N*H*), 4.5 (2H, N*H*), 4.2 (t, 4H, CH_2-OCO), 3.8-3.3 (m, 4nH, PEG), 3.3 (q, 4H, CH_2-NH), 3.1 (m, 12H, CH_2-NH), 2.5-2.2 (dq, 2H, CH_2), 1.8 (s, 2H, C*H*-CH_3), 1.7-1.1 (m, 70H, CH_2 C*H*), 1.0 (m, 3H, CH_3), 0.9 (m, 6H, CH_3) ppm. ^{13}C-NMR ($CDCl_3$) δ = 73.4 70.5 (PEG), 63.8, 41.0, 40.5, 40.2, 39.6, 39.0, 38.7, 36.7, 31.9, 30.3, 29.9, 29.8, 29.5, 29.3, 27.9, 26.9, 26.7, 26.2, 24.5, 22.7, 22.5, 19.2 ppm. IR (cm^{-1}): 3540, 3342, 2880, 2172, 1980, 1717, 1701 (C=O stretch), 1658, 1626, 1586 (C-N stretch, N-H deform), 1530 1467 (CH_2 bend), 1413, 1360 (CH_2 wag), 1342 (CH_2 wag), 1281, 1241, 1146, 1097, 1060, 962, 946, 841. MALDI-TOF MS: calculated MW = 11265.8 g/mol, observed m/z = 12118.8 g/mol $[M+H]^+$, 24341.1 g/mol [PEG impurity], 35510.8 g/mol [PEG impurity].

*Synthesis of poly(ethylene glycol)-bis(12-(3-(6-(3-(6-((R)-2,6-dimethylheptyl)-4-oxo-1,4-dihydropyrimidin-2-yl)ureido)hexyl)ureido)dodecylcarbamate) (**4b**)*

Compound **3** (0.5 g, 0.05 mmol) and UPy-synthon **9b** (0.046 g, 0.1 mmol) were dissolved in $CHCl_3$ (10 mL). The reaction mixture was stirred under an argon atmosphere and reflux conditions for 4h. The conversion was followed with ^{1}H-NMR. The reaction mixture was filtered over celite and a precipitation in diethyl ether was performed to yield a white solid. (0.48 g, 0.04 mmol, 88%). ^{1}H-NMR ($CDCl_3$): δ = 13.2 (s, 2H, N*H*), 11.9 (s, 2H, N*H*), 10.1 (s, 2H, N*H*), 5.8 (s, 2H, UPy alkylidene), 4.9 (s, 2H, N*H*), 4.7 (s, 2H, N*H*), 4.4 (2H, N*H*), 4.2 (t, 4H, CH_2-OCO), 3.8-3.3 (m, 4nH, PEG), 3.3 (q, 4H, CH_2-NH), 3.1 (m, 12H, CH_2-NH), 2.5-2.2 (dq, 2H, CH_2), 1.9-1.1 (m, 72H, CH_2 C*H*), 1.0 (m, 3H, CH_3), 0.9 (m, 6H, CH_3) ppm. MALDI-TOF MS: calculated MW = 11265.8 g/mol, observed m/z = 11516.4 g/mol $[M+H]^+$.

Characterization methods. ^{1}H-NMR measurements were conducted on a Varian 400 MHz. Proton chemical shifts were reported in ppm downfield from tetramethylsilane (TMS). The following splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; dq, double quartet; m, multiplet. ^{13}C-NMR measurements were recorded on a Varian 400 MHz. Frequency was set at 100 MHz. Carbon chemical shifts were expressed in ppm downfield from TMS using the resonance of the deuterated solvent as internal standard ($CHCl_3$, 77.2 ppm). Maldi-TOF-MS were acquired using a Perserptive Biosystem Voyager-DE PRO spectrometer. In all cases, α-cyano-4-hydroxycinnamic acid or t-2-[3-(4-t-butyl-phenyl)-2-methyl-2-propenylidene] malononitrile were employed as the matrix material. Fourier transform infrared (FT-IR) measurements were performed on a Perkin Elmer spectrum one.

Cryogenic transmission electron microscopy measurements were performed on the TU/e cryoTITAN (FEI, www.cryotem.nl) operated at 300 kV, equipped with a field emission gun (FEG), a post-column Gatan Energy Filter (GIF) and a post-GIF 2k x 2k Gatan CCD camera.

Sample solutions of 0.09 mM (*S*)-dimethylheptyl-UPy-PEG, **4a**, 0.44 mM **4a** and **4b**, used for cryoTEM analysis were prepared and cryoTEM sample preparation was performed using an automated vitrification robot (FEI Vitrobot Mark III) for plunging in liquid ethane.[1] For cryoTEM, 200 mesh Cu grids with Quantifoil R 2/2 holey carbon films (Quantifoil Micro Tools GmbH) were used. All TEM grids were surface plasma treated for 40 seconds using a Cressington 208 carbon coater prior to use. Gatan DigitalMicrograph and ImageJ were used for TEM image analysis. The fiber diameter and pitch length were determined by manually measuring independent areas in calibrated TEM images in MATLAB. The results are reported as mean ± standard deviation. For a sample of **4a** a second preparation method was investigated by dissolving in water, heating to 70 °C, quenching to 0 °C and vitrifying from 4 °C.

Cryogenic electron tomography. The samples as prepared in the above section were also used for lowdose cryo-TEM tomography; 3 µL of anionic gold tracer suspension (Ø 10 nm, Cell Microscopy Center) was added to 27 µL sample solution for feature tracking during the reconstruction procedure. The region of interest was selected and placed in optimal position for a maximal tilt angle to both sides. The tilt series was recorded from +64 to – 64 degrees with a 2° interval. Images were taken under low-dose conditions (total dose tilt series ~100 e-/Å2) with a 3.7 Å pixel size on the CCD camera using the FEI Xplore3D software.

Dynamic light scattering. DLS experiments were conducted on an ALV/CGS-3 MD-4 compact goniometer system equipped with a multiple tau digital real time correlator (ALV-7004) (solid state laser: λ = 532 nm; 40 mW). Typical experiments covered a scattering angle from 30 to 150 °, averaging over 3 x 30 s runs at a temperature of 20 °C. Data was processed using after ALV software, and a Contin analysis.

Small-angle X-ray scattering. Synchrotron radiation X-ray scattering data were collected at the highbrilliance beamline ID02 of the ESRF in Grenoble, France[2], operating at 12.46 keV. The scattering intensity was measured as a function of momentum transfer vector $q = 4\pi(\sin\theta)/\lambda$, where λ = 0.1 nm is the radiation wavelength and 2θ is the scattering angle. Three sample-to-detector distances of 1.5 and 3, and 10 m were used to cover an angular range of $0.061 < q < 3.17$ nm^{-1}. Samples were measured in a polycarbonate (ENKI, KIBeam) flow through capillary with a diameter of d = 1.9 mm kept in a temperature-controlled holder at T = 20 and 40 °C. SAXS patterns were azimuthally averaged to obtain the one dimensional SAXS profiles. For each sample, 10 frames of 0.3 s were collected and averaged after checking for radiation damage. This corresponds to a total data collection time of 3 s per sample with a reduced flux of about 1012 photons s^{-1}. To obtain the scattering curve, the normalized background scattering profile of the solvent and polycarbonate cell was subtracted from the sample scattering profiles. We have fitted the scattering profiles to a model for a core-shell cylinder over the entire recorded q-range using the software package SASfit.

Fluorescence microscopy. Fluorescence microscopy was conducted using a Varian Cary Eclipse fluorimeter at 20 °C. All measurements were performed in a 1 cm quartz cuvette. Solutions of

0.44 mM (*S*)-dimethylheptyl-UPy-PEG in water were kinetically followed to equilibrium. Subsequently, 2.5 mM Nile red in methanol was added to the sample (final Nile red concentration of 5 μM) and the spectrum was recorded (565-750 nm) after excitation of Nile red at 550 nm. Corresponding temperature-dependent measurements were performed with a temperature range of 20 - 70 °C.

Circular dichroism and UV-Vis. CD and UV-Vis measurements were performed on a Jasco J-815 CD-spectrometer at normal sensitivity, with a data pitch of 0.1 nm, a scanning speed of 20 nm/min, a band width of 1 nm and a response of 2 s. All measurements were performed in a 1 mm quartz cuvette. Corresponding temperature-dependent measurements were performed with a PFD-425S/15 Peltier-type temperature controller with a temperature range of 20 - 70 °C and adjustable temperature slopes. Further conditions or changes are noted in the text.

2. ^{1}H-NMR spectra

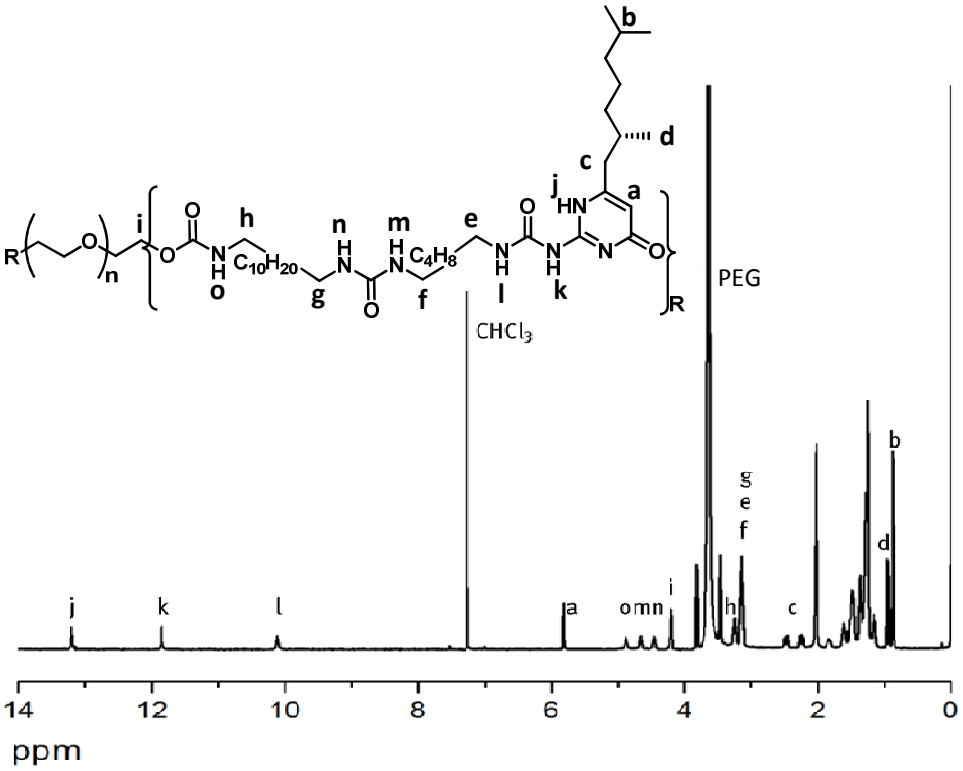


Figure S1. ^{1}H-NMR of compound **4a**, (*S*)-dimethylheptyl-UPy-PEG-10k.

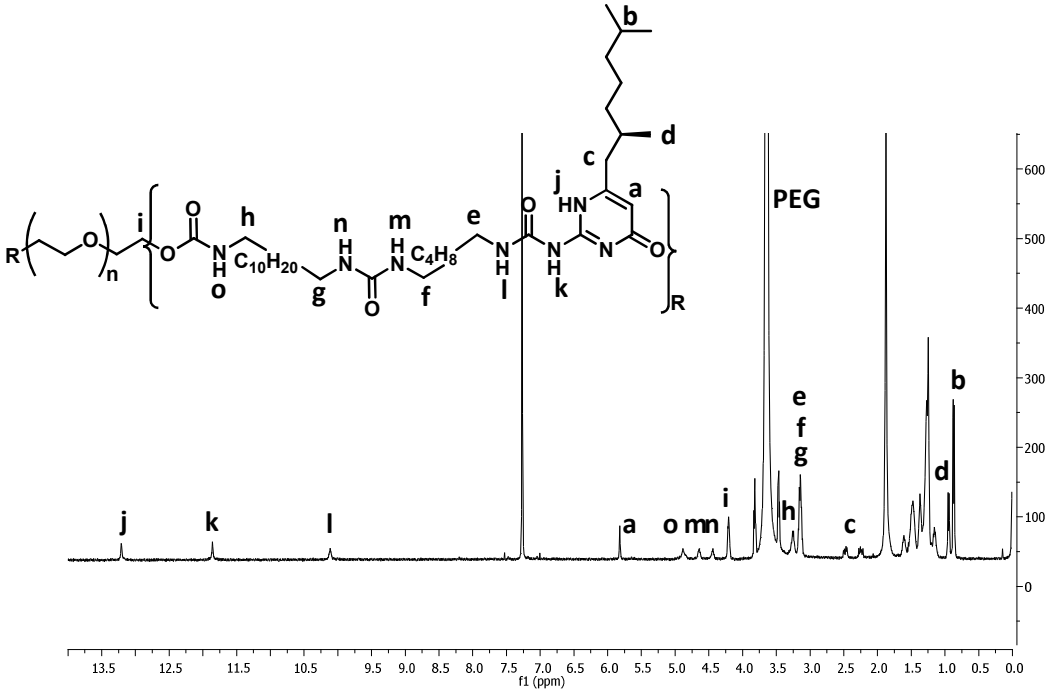


Figure S2. ^{1}H-NMR of compound **4b**, (*R*)-dimethylheptyl-UPy-PEG-10k.

3. CryoTEM micrographs

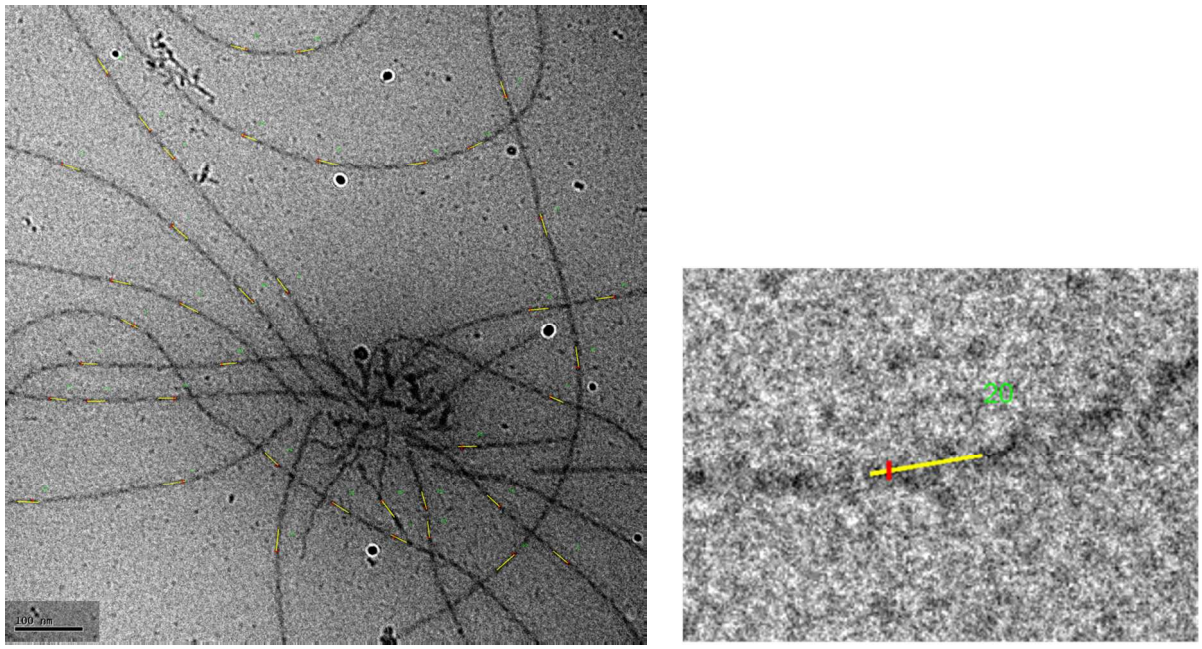
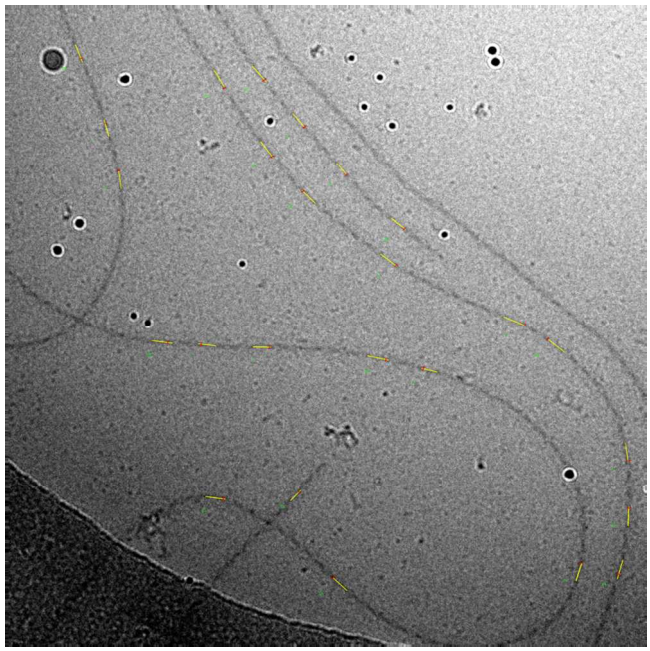


Figure S3. CryoTEM image of a 0.09 mM solution of compound **4a** in ultra pure water (gold particles added for tomography), with 40 measurements of pitch and diameter (see magnification of one measurement on the right). The picture is 20 µm under focus.



Average pitch: 28.1 ± 3.0 nm

Average diameter: 6.1 ± 1.0 nm

Figure S4. CryoTEM image of a 0.09 mM solution of compound **4a** in ultra pure water (gold particles added for tomography), with 25 measurements of pitch and diameter. The picture is 20 µm under focus.

The fiber diameter and pitch length were determined by manually measuring 65 independent areas in calibrated TEM images in MATLAB. The results are reported as mean ± standard deviation.

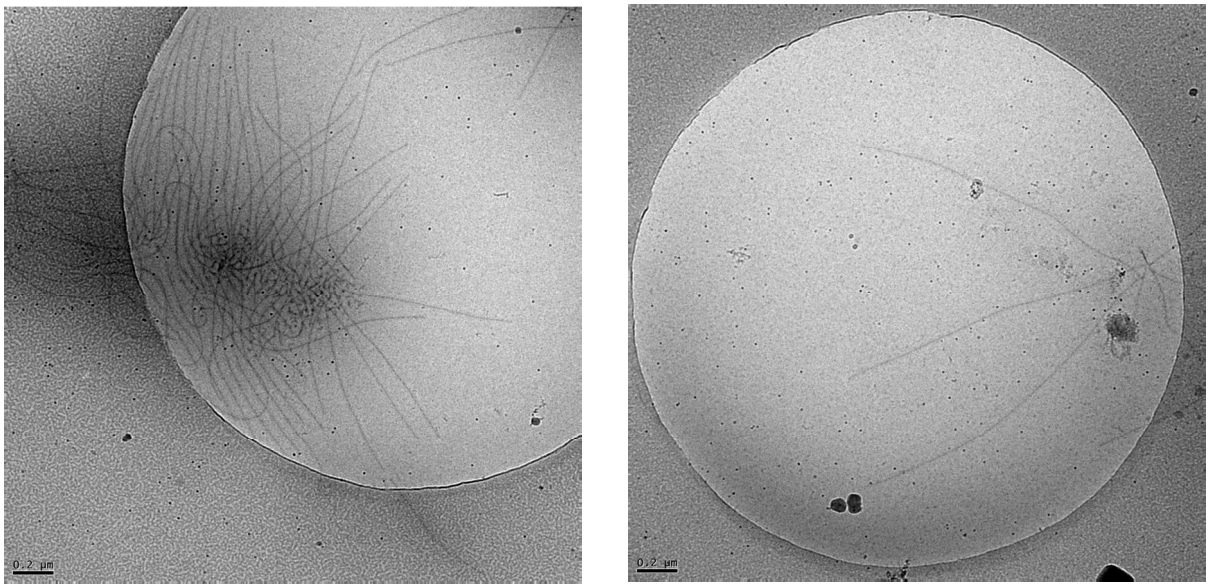
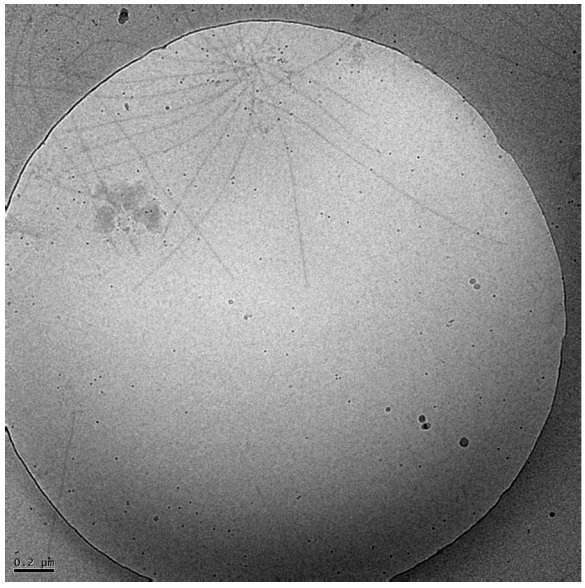


Figure S5. CryoTEM image of a 0.5 wt% (0.44 mM) solution of compound **4a** (left) and compound **4b** (right) in ultra pure water (gold particles added for tomography). The picture is 40 µm under focus.

4a. The fiber diameter was determined by manually measuring 148 independent areas in calibrated TEM images in MATLAB. Average diameter: 10.5 ± 1.4 nm

Due to the defocus effect in TEM imaging the diameter is larger than the ones measured for Figure S3 and S4, the previously mentioned diameter is however more accurate.

4b. The fiber diameter by manually measuring 100 independent areas in calibrated TEM images in MATLAB. Average diameter: 12.0 ± 1.7 nm



Average diameter: 12.0 ± 1.9 nm (100 measurements)

Figure S6. CryoTEM image of a quenched 0.5 wt% (0.44 mM) solution of compound **4a** in ultra pure water (gold particles added for tomography). The picture is 40 µm under focus.

4. Cryo electron tomography data

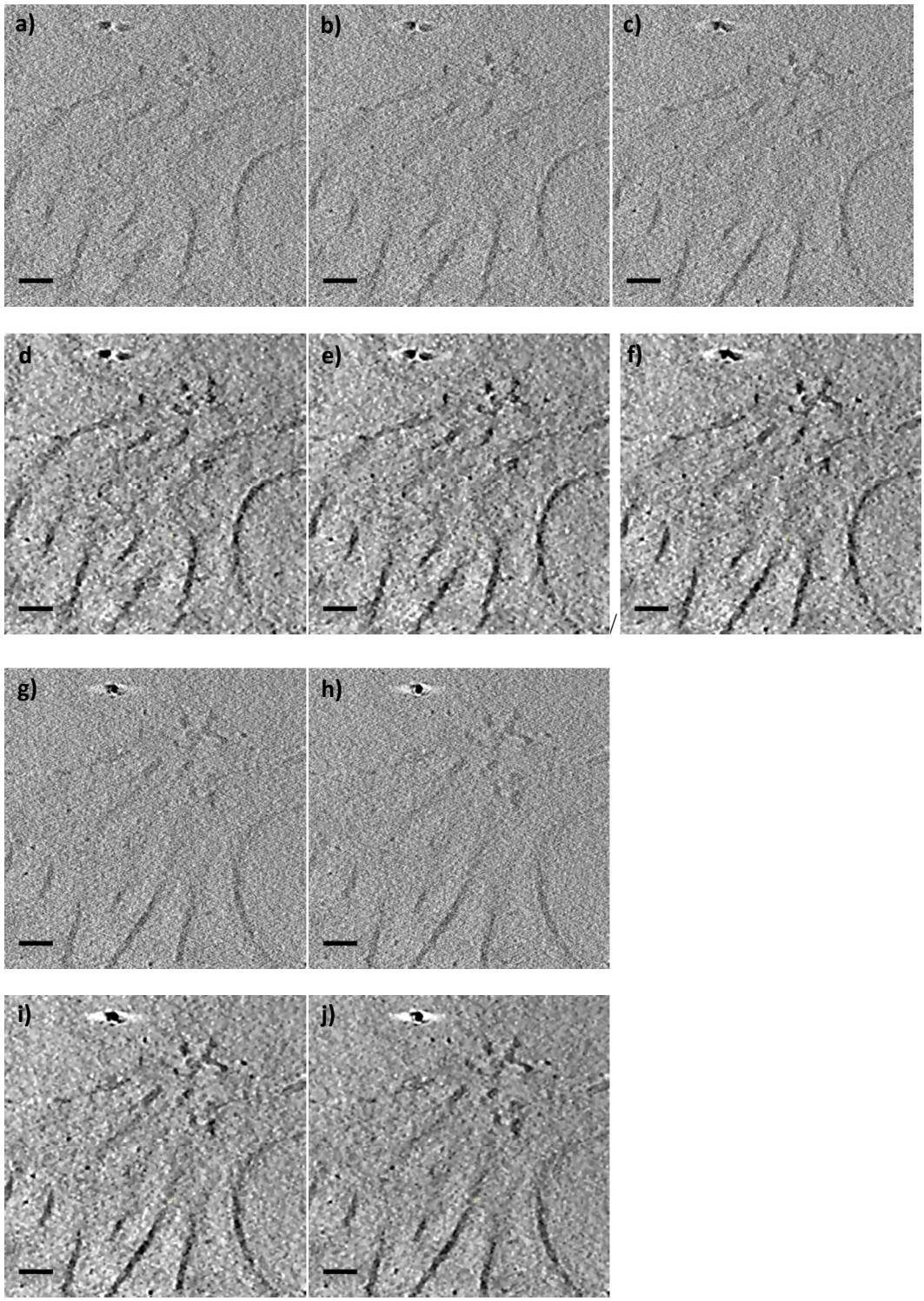


Figure S7. Cryo electron tomography images of a 0.09 mM solution of compound **4a** in ultra pure water with gold. Unfiltered (a-c, g, h) and filtered (d-f, i, j) data with 3.5 nm in depth divided in a series of 10 pictures, showing here pictures: 2, 4, 6, 8 and 10, scale bar represents 20 nm.

5. Fluorescence spectra of 4a with Nile red

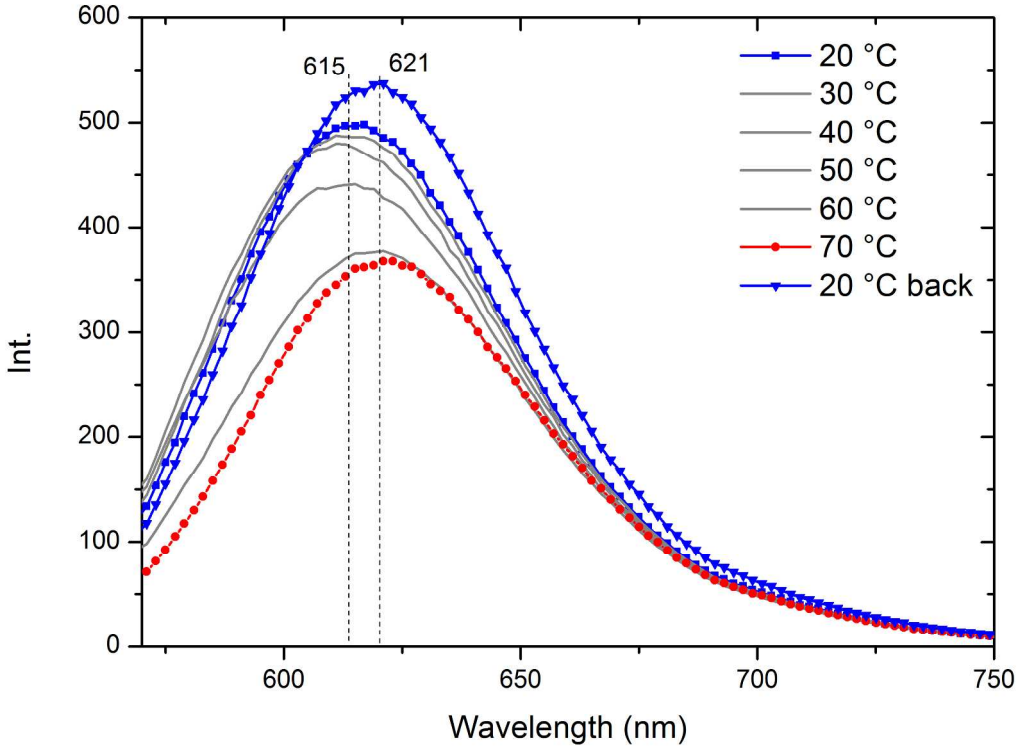


Figure S8. Fluorescence spectra of 0.44 mM **4a** in water, with 5 μM Nile red, during heating from 20 °C to 70 °C. Then the sample was cooled from 70 °C to 20 °C.

6. Circular Dichroism and UV-Vis spectra

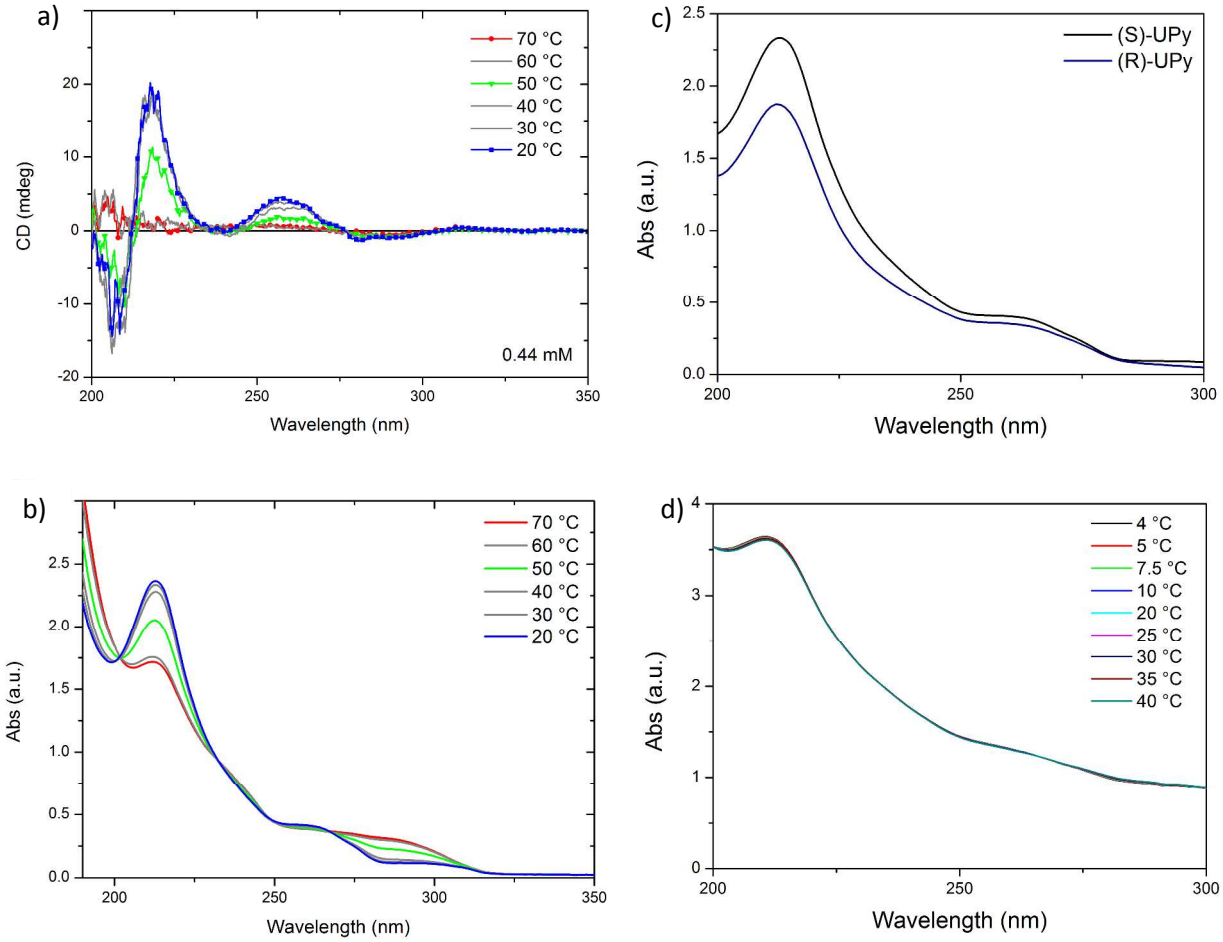


Figure S9. Circular dichroism spectroscopy: a) CD spectra of 0.44 mM **4a** in water during cooling from 70 ° C to 20 °C b) UV-Vis spectra of 0.44 mM **4a** in water during cooling from from 70 °C to 20 °C c) UV-Vis spectra of both enantiomers **4a** and **4b** at 0.44 mM in water d) UV spectra of **4a** of preparation method 2: quenching the sample from 70 °C in an ice bath and measuring at 4 °C, the sample was slowly heated up leading to a change in Cotton effect at 40 °C, but no change in UV-Vis.

7. References

1) M.R. Vos, P.H.H. Bomans, P.M. Frederik, N.A.J.M. Sommerdijk, *Ultramicroscopy* **2008**, *108*, 1478-1483.

2) M. Sztucki, E. Di Cola and T. Narayanan, *J. Appl. Crystallogr.* **2010**, *43*, 1479–1487.