Electronic Supplementary Information

Hierarchical Self-Assembly of Azobenzene Dyad with Inverted Amide Connection into Toroidal and Tubular Nanostructures

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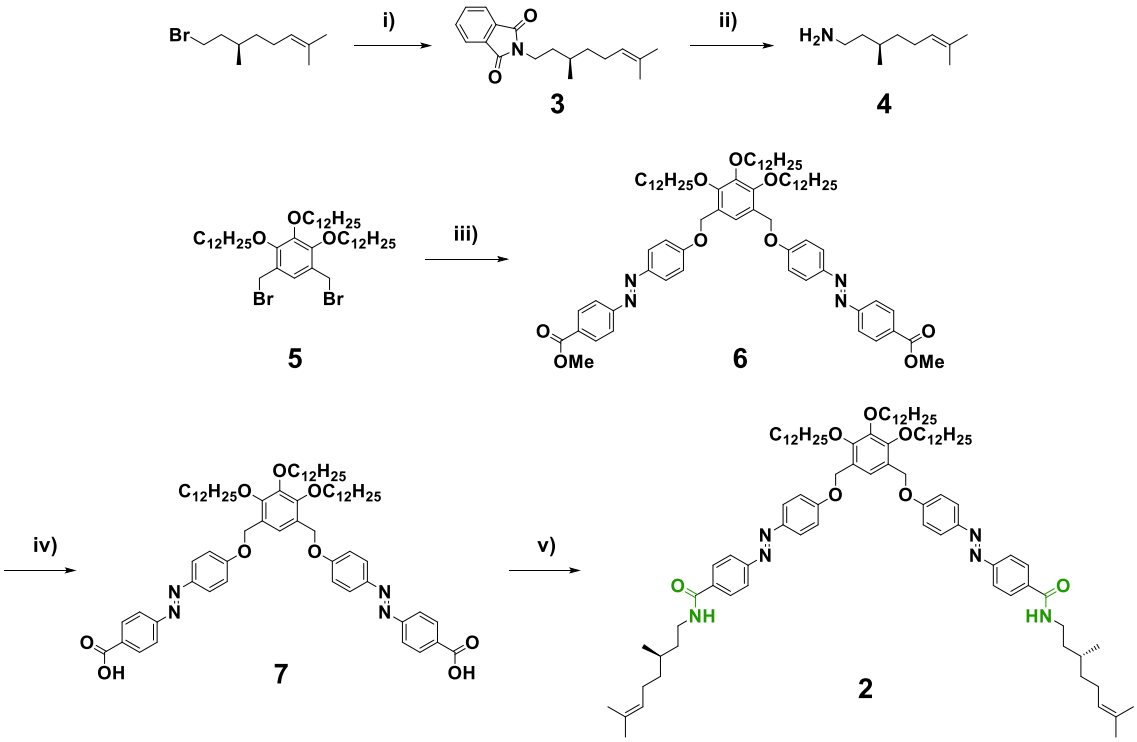
1. Materials and Methods

General. All commercially available reagents and solvents were reagent grade and used without further purification. Column chromatography was performed using 63−210 μm silica gel. All the solvents for the preparation of the assemblies were spectral grade and used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on Bruker-AVANCE Ⅲ-400M and JEOL JNM-ECS 500 NMR spectrometers and chemical shifts are reported in parts per million (ppm, δ) with the signal of tetramethylsilane (TMS) as an internal standard. ES and CI-MS spectra were measured on a JEOL JMS-700. ESI-MS spectra were measured on an Exactive (Thermo Scientific). MALDI Spiral-TOF-MS spectra were measured on a JMS-S3000. UV/Vis absorption spectra were recorded on JASCO V-760 spectrophotometer with a peltier device temperature-control unit using a screw-capped quartz cuvette of 1.0-mm path length. Circular dichroism (CD) spectra were recorded on JASCO J840 spectropolarimeter at ambient conditions. Fourier transform infrared (FT-IR) spectra were recorded on JASCO FT/IR-4600 spectrometer. Dynamic light scattering (DLS) measurements were conducted on Zetasizer Nano (Malvern Instruments) using noninvasive backscattering (NIBS) technology under 4.0-mW He-Ne laser (633 nm). The scattering angle was set at 173°. Atomic force microscopy (AFM) images were acquired under ambient conditions using a Multimode 8 Nanoscope V (Bruker AXS) in Scanasyst mode (cantilever: SCANASYST-AIR; spring constant = 0.4 N/m; frequency = 70 kHz). The samples were prepared by spin coating aliquots of the assembling solution onto freshly cleaved highly-oriented pyrolytic graphite (HOPG, 5 × 5 mm^2). Quantum chemical calculation was conducted by following density functional theory (DFT) with Gaussian 16W.[S1]

2. Synthesis and Characterization of Compounds

Compounds **2** was synthesized according to Scheme S1. Compound **1**[S2] and **5**[S3] were prepared according to reported procedures.



Scheme S1. Reagents and conditions: i) Phthalimide potassium salt, DMF, 65 °C; ii) Hydrazine monohydrate, EtOH, 70 °C; iii) Methyl (*E*)-4-((4-hydroxyphenyl)diazenyl)benzoate, K_2CO_3, acetone, 65 °C; iv) KOH, THF/MeOH/H_2O, 70 °C; v) *N,N′*-dicyclohexylcarbodiimide (DCC), *N,N′*-dimethyl-4-aminopyridine (DMAP), **4**, DMF, 85 °C.

Synthesis of 3: Phthalimide potassium salt (1.18 g, 6.39 mmol) was suspended in 30 mL of dry DMF at 65 °C. To this suspension, (*R*)-8-bromo-2,6-dimethyloct-2-ene (1.26 mL, 6.39 mmol) was added and the mixture was stirred vigorously for 24 h at 65 °C under N_2 atmosphere. The mixture was diluted with *n*-hexane/EtOAc mixture and washed with water and brine. The organic layer was dried over Na_2SO_4, filtrated and evaporated to dryness. The residue was purified by column chromatography over silica gel (eluent: $CHCl_3$) to give **3** as a colorless oil (1.74 g, 95% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 20 °C): δ = 7.86–7.81 (m, 2H), 7.72–7.68 (m, 2H), 5.09–5.05 (m, 1H), 3.74–3.67 (m, 2H), 2.04–1.91 (m, 2H), 1.74–1.68 (m, 1H), 1.65 (s, 3H), 1.59 (s, 3H), 1.52–1.16 (m, 4H), 0.98 (d, J = 6.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 20 °C): δ = 168.3, 133.8, 132.2, 131.3,

124.6, 123.1, 36.75, 36.23, 35.35, 30.23, 25.65, 25.30, 19.31, 17.60. HRMS (EI+): *m/z* calcd for $C_{18}H_{23}O_2N$ 285.1729 $[M]^+$, found 285.1730.

Synthesis of 4: To a solution of **3** (784 mg, 2.75 mmol) in 15 mL of EtOH was added hydrazine monohydrate (1.0 mL, 21 mmol), and the mixture was stirred for 24 h at 70 °C under N_2 atmosphere. The solvent was evaporated and the residue was dissolved in $CHCl_3$ and washed with cold aqueous NaOH (2 M) solution. The organic layer was dried over Na_2SO_4, filtrated and evaporated to dryness to give **4** as a thin yellow oil (389 mg, 91% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 20 °C): δ = 5.04–5.00 (m, 1H), 2.67–2.62 (m, 2H), 1.96–1.83 (m, 2H) 1.60 (s, 3H), 1.53 (s, 3H), 1.47–1.35 (m, 4H), 1.26–1.19 (m, 2H), 1.13–1.05 (m, 1H), 0.81 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 20 °C): δ = 131.0, 124.7, 40.98, 39.94, 37.13, 30.04, 25.61, 25.40, 19.45, 17.53. HRMS (CI+): *m/z* calcd for $C_{10}H_{22}N$ 156.1752 $[M+H]^+$, found 156.1748.

Synthesis of 6: Methyl (*E*)-4-((4-hydroxyphenyl)diazenyl)benzoate (105 mg, 0.410 mmol) was dissolved in 5 mL of dry acetone, and K_2CO_3 (185 mg, 1.34 mmol) was added to the solution. To this suspension heated at 65 °C, compound **5** (154 mg, 0.189 mmol) dispersed in 2 mL of dry acetone was added and the mixture was stirred for 24 h at 65 °C under N_2 atmosphere. The insoluble solid was removed by filtration, washed with $CHCl_3$ and evaporated to dryness. The residue was purified by column chromatography over silica gel (eluent: *n*-hexane: $CHCl_3$ = 1:1 → 0:1) to give **6** as an orange powder (191 mg, 87% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 20 °C): δ = 8.16 (d, *J* = 8.8 Hz, 4H), 7.94 (d, *J* = 9.0 Hz, 4H), 7.90 (d, *J* = 8.8 Hz, 4H), 7.29 (s, 1H), 7.09 (d, *J* = 9.1 Hz, 4H), 5.13 (s, 4H), 4.10 (t, *J* = 6.6 Hz, 4H), 4.03 (t, *J* = 6.7 Hz, 2H), 3.95 (s, 6H), 1.80–1.69 (m, 6H), 1.49–1.21 (m, 54H), 0.90–0.84 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 20 °C): δ = 166.6, 161.9, 155.4, 152.2, 147.1, 145.8, 131.2, 130.6, 125.3, 125.2, 124.6, 122.4, 115.1, 74.33, 73.76, 65.62, 52.25, 31.96, 31.94, 30.44, 30.41, 29.76, 29.74, 29.71, 29.68, 29.60, 29.56, 29.41, 29.39, 26.22, 26.19, 22.72, 22.70, 14.12. HRMS (Spiral MALDI): *m/z* calcd for $C_{72}H_{102}N_4O_9Na$ 1189.7546 $[M+Na]^+$, found 1189.7539.

Synthesis of 7 : Compound **6** (111 mg, 0.095 mmol) and KOH (32 mg, 0.57 mmol) were

dissolved in the mixture of THF (5 mL), MeOH (2 mL) and H_2O (5 mL), and the mixture was stirred for 4 h at 70 °C. The reaction mixture was acidified with aqueous HCl (2 M) solution and the resulting precipitates were collected by filtration, washed by cold water and dried *in vacuo* for 2 h at 70 °C to give compound **7** as a reddish orange powder (99 mg, 91% yield). ^{1}H NMR (500 MHz, DMSO-d_6, 100 °C): δ = 8.19 (s, 2H), 8.09 (d, J = 8.5 Hz, 4H), 7.89 (d, J = 8.9 Hz, 4H), 7.86 (d, J = 8.6 Hz, 4H), 7.31 (s, 1H), 7.18 (d, J = 9.0 Hz, 4H), 5.15 (s, 4H), 4.05 (t, J = 6.5 Hz, 4H), 3.99 (t, J = 6.4 Hz, 2H), 1.74–1.65 (m, 6H), 1.47–1.21 (m, 54H), 0.86–0.80 (m, 9H). HRMS (Spiral MALDI): *m/z* calcd for $C_{70}H_{97}N_4O_9$ 1137.7252 $[M-H]^-$, found 1137.7250. ^{13}C NMR spectrum of this compound could not be measured due to its very low solubility in common deuterated organic solvents, such as $CDCl_3$, DMSO-d_6, Toluene-d_8 and THF-d_8.

Synthesis of 2: Compound **7** (80 mg, 0.070 mmol), compound **4** (30 mg, 0.19 mmol) and DMAP (5 mg, 0.07 mmol) were dissolved in 5 mL of DMF at 85 °C under N_2 atmosphere. To this mixture, DCC (45 mg, 0.22 mmol) in 1 mL of DMF was added dropwise and the mixture was stirred for 28 h at 85 °C. The reaction mixture was added to 30 mL of cold MeOH. The resulting precipitates were collected by filtration and washed with cold MeOH. The residue was purified by column chromatography (eluent: $CHCl_3$) and further purified by preparative gel permeation chromatography (GPC) (eluent: $CHCl_3$) to give **2** as an orange flake (36 mg, 36% yield). ^{1}H NMR (500 MHz, $CDCl_3$, 20 °C): δ = 7.88 (d, J = 9.1 Hz, 4H), 7.84 (s, 8H), 7.24 (s, 1H), 7.03 (d, J = 9.1 Hz, 4H), 6.18 (d, J = 8.0 Hz, 2H), 5.14 (s, 4H), 4.03 (t, J = 6.6 Hz, 4H), 4.04–4.00 (m, 4H), 2.08–2.06 (m, 4H), 1.80–1.60 (m, 14H), 1.49–1.22 (m, 72H); 0.90–0.84 (m, 15H); ^{13}C NMR (125 MHz, $CDCl_3$, 20 °C): δ = 170.3, 166.1, 161.6, 154.2, 151.9, 147.0, 145.7, 136.2, 127.8, 125.2, 125.0, 122.6, 115.0, 74.25, 74.74, 65.53, 48.91, 33.25, 31.93, 30.43, 29.76, 29.72, 29.67, 29.59, 29.55, 29.41, 29.39, 26.20, 26.51, 25.00, 22.71, 14.16. HRMS (ESI): *m/z* calcd for $C_{90}H_{135}O_7N_6$ 1412.0387 $[M-H]^-$, found 1412.0387.

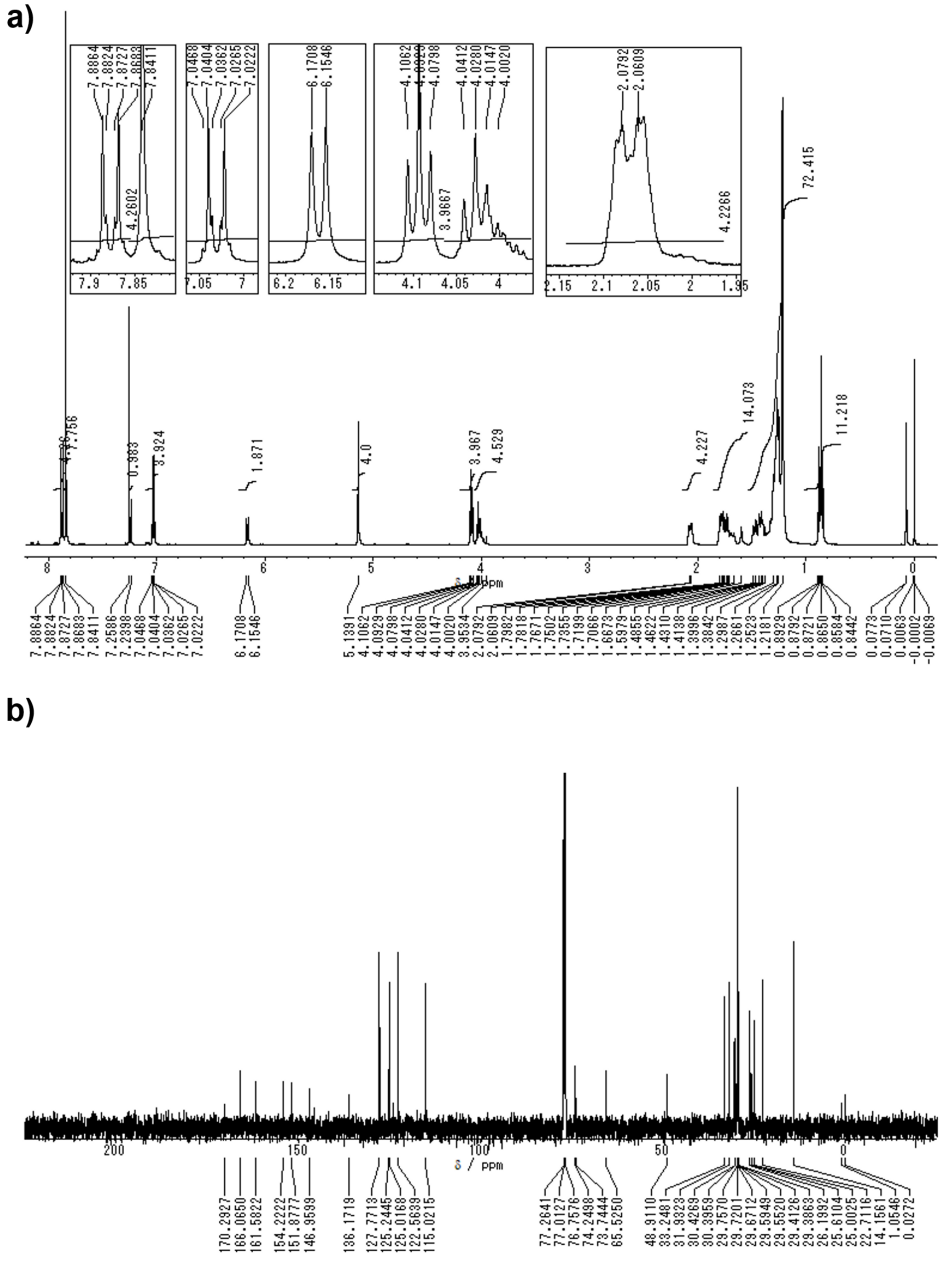


Chart S1. a) ^{1}H and b) ^{13}C NMR of **2** in $CDCl_3$ at 20 ºC.

3. Supporting Figures

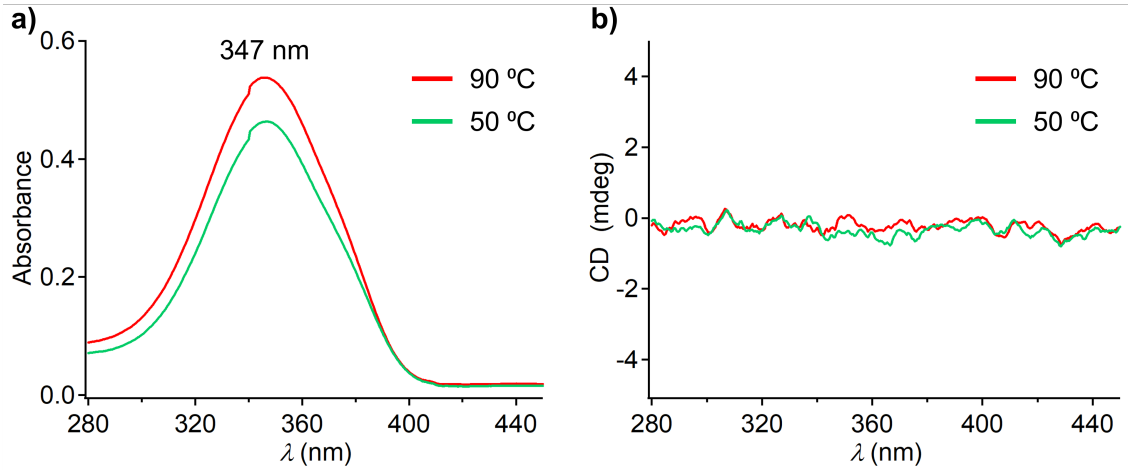


Figure S1. a) UV/Vis absorption and b) CD spectra of **2** in MCH (c = 100 μM) at 90 °C (red line) and 50 °C (green line).

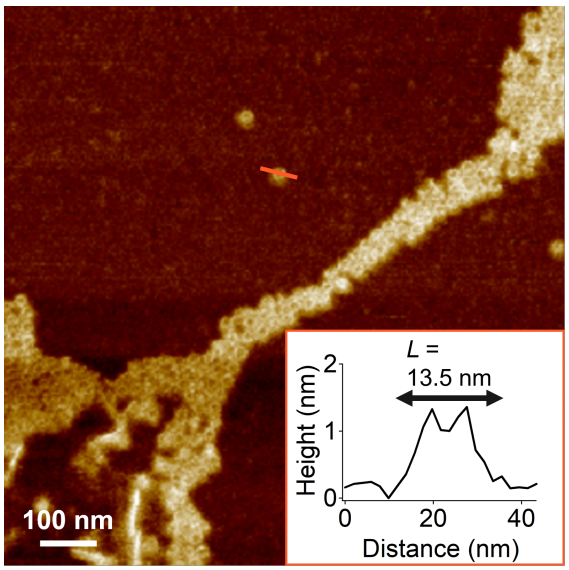


Figure S2. AFM image of toroids of **1** obtained by cooling a MCH solution of **1** (c = 100 μM) from 90 °C to 20 °C at a cooling rate of 1 °C/min. These samples were prepared by spin-coating the solution onto HOPG. Inset: AFM cross-sectional analysis along the reddish orange line in a).

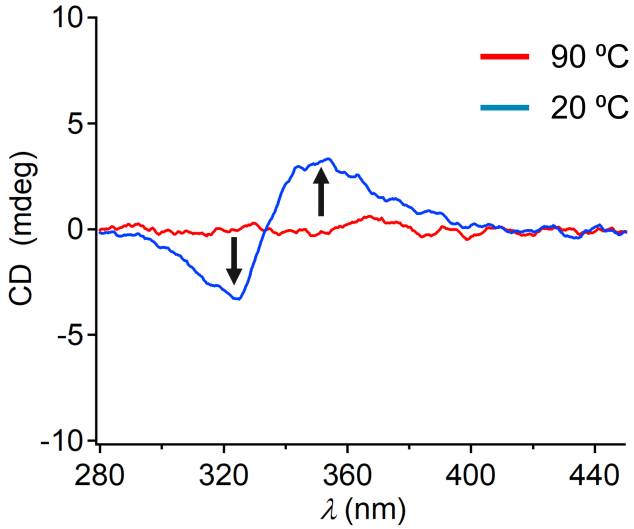


Figure S3. CD spectra of **1** in MCH (c = 100 μM) at 90 °C (red line) and 20 °C (blue line) obtained by cooling at a rate of 1 °C/min.

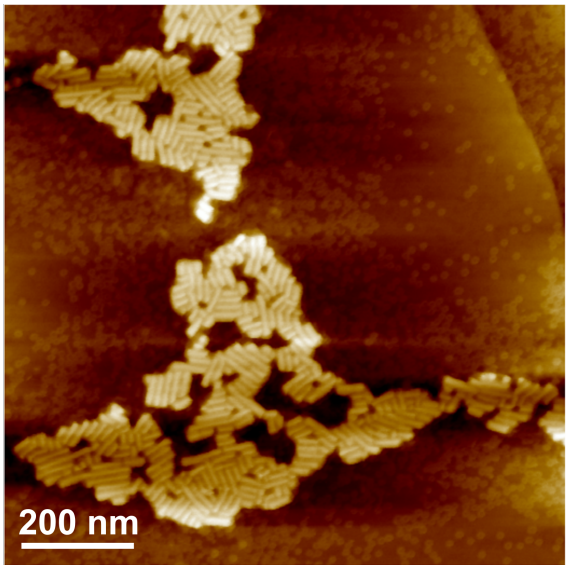


Figure S4. AFM image of nanotubes of **1** obtained by cooling a MCH solution of **1** (c = 100 μM) from 90 °C to 0 °C at a cooling rate of 1 °C/min. The sample was prepared by spin-coating the solution onto HOPG.

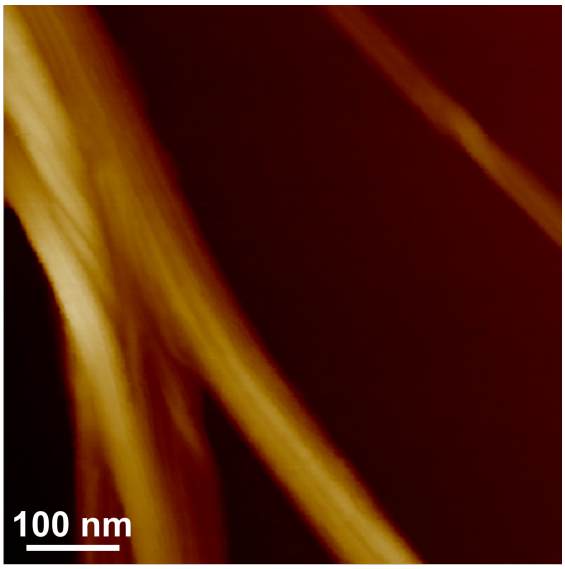


Figure S5. AFM image of left-handed helical fibrils of **1** obtained by aging a MCH solution of **1** (c = 100 μM) at 0 °C for 2 days. The sample was prepared by spin-coating the solution onto HOPG.

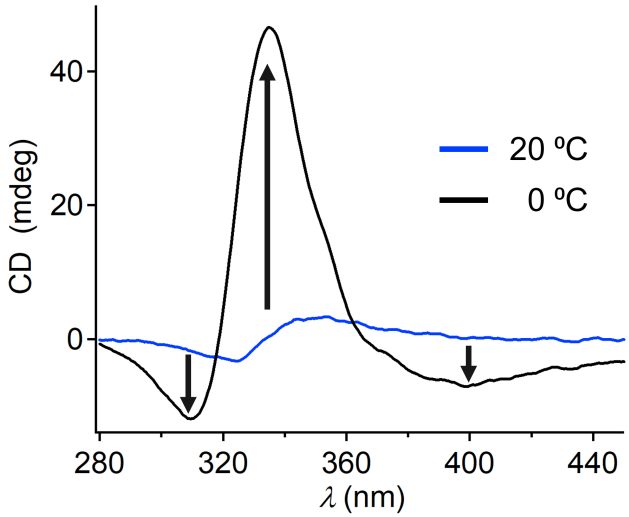


Figure S6. CD spectra of **1** in MCH (c = 100 μM) at 20 °C (blue line) and 0 °C (black line) obtained by cooling at a rate of 1 °C/min.

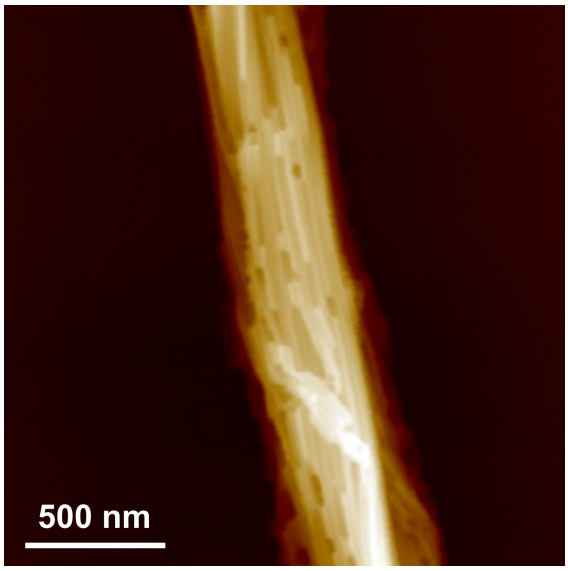


Figure S7. AFM image of non-helical nanotubes of **2** obtained by cooling a MCH solution of **2** (c = 100 μM) from 90 °C to 20 °C. The sample was prepared by spin-coating the solution onto HOPG.

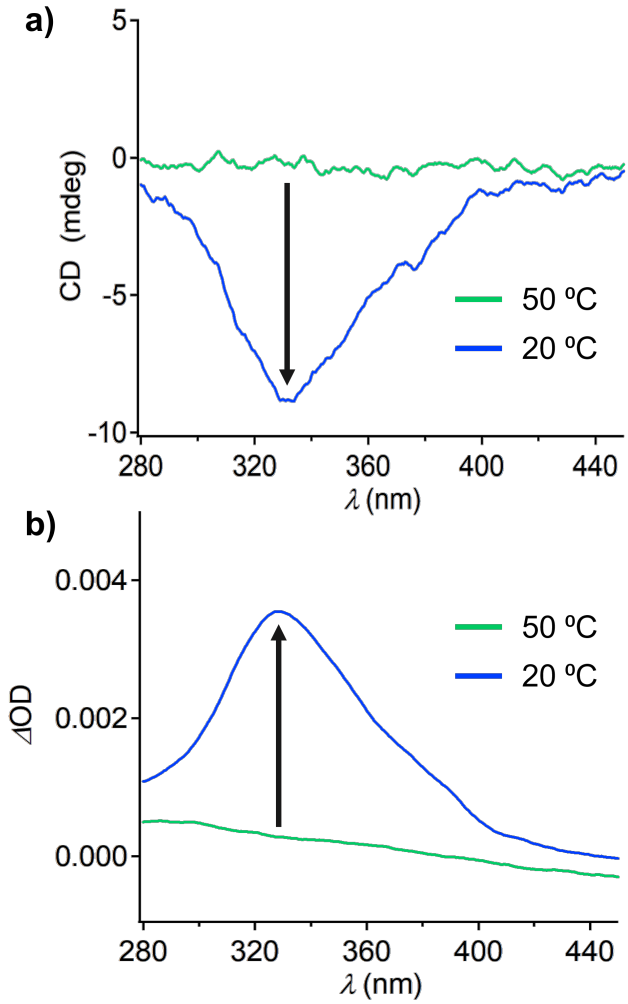


Figure S8. a) CD and b) LD spectra of **2** in MCH (c = 100 μM) at 50 °C (green line) and 20 °C (blue line).

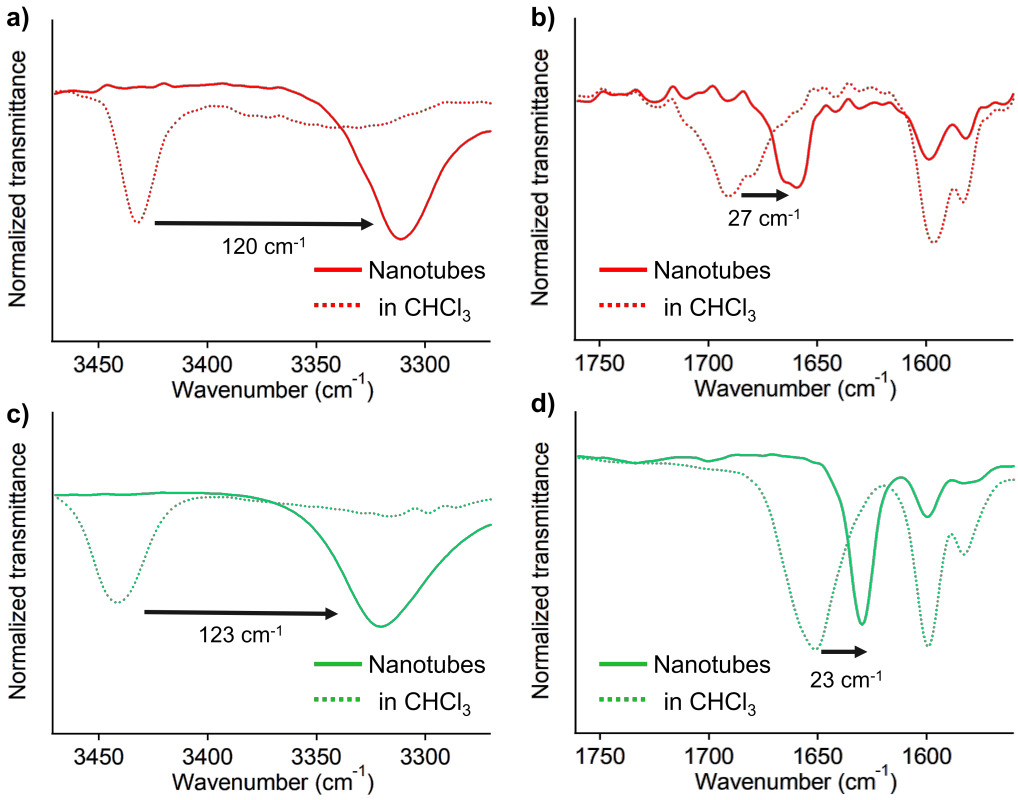


Figure S9. FT-IR spectra of a, b) **1** (red line) and c, d) **2** (green line) in $CHCl_3$ (c = 500 μM, dashed lines) and precipitates (solid lines) at 20 °C. The spectra display a, c) NH stretching bands and b, d) carbonyl stretching bands.

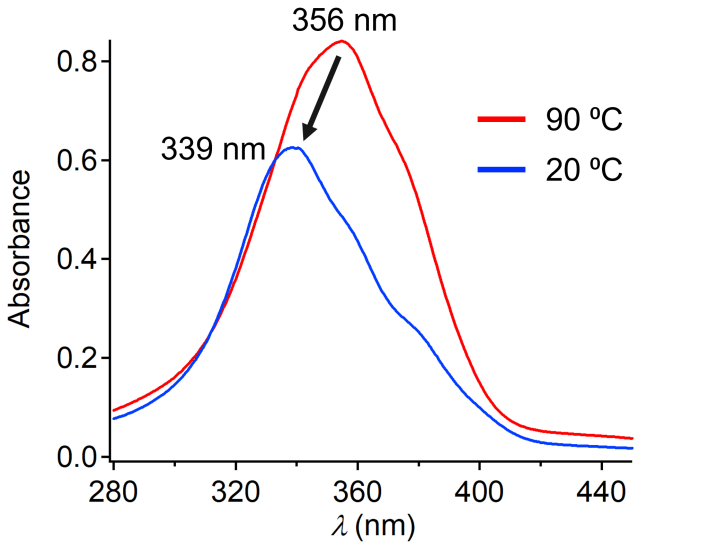


Figure S10. UV/Vis absorption spectra of **1** in MCH (c = 100 μM) at 90 °C (red line) and 20 °C (blue line) obtained by cooling at a rate of 1 °C/min.

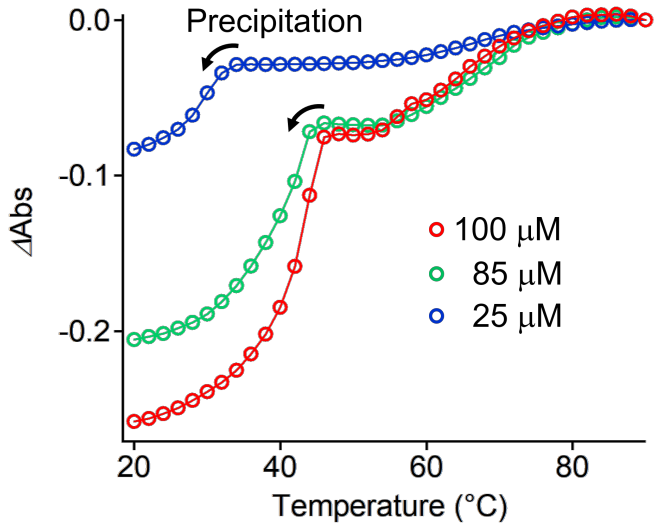


Figure S11. Temperature-dependent absorbance change (ΔAbs = Abs_T − $Abs_{90°C}$) at 347 nm as a function of the temperature at different concentrations of **2** (red plots: 100 μM, green plots: 85 μM, blue plots: 25 μM).

4. Supporting References

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S3. S. Yao, U. Beginn, T. Gress, M. Lysetskam and F. Würthner, *J. Am. Chem. Soc.,* 2004, **126**, 8336−8348.