Supporting information

Chiromers: Conformation-driven mirror-image supramolecular chirality isomerism identified in a new class of helical rosette nanotubes

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General Methods.

All reactions were performed under an atmosphere of N_2, unless stated otherwise. Reagent grade CH_2Cl_2 and MeOH were purified on an MBraun solvent purification system. All other solvents and reagents were used without further purification. Reactions were monitored by TLC analysis using UV254 pre-coated TLC plates and visualized under UV light. ^{1}H and ^{13}C NMR spectra were in the specified deuterated solvents. The NMR data is presented as follows: chemical shift δ (ppm), multiplicity, coupling constant and integration. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, bs = broad singlet. The ^{1}H NMR and ^{13}C NMR spectra were calibrated using 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (TMS = 0.0) as the internal reference.

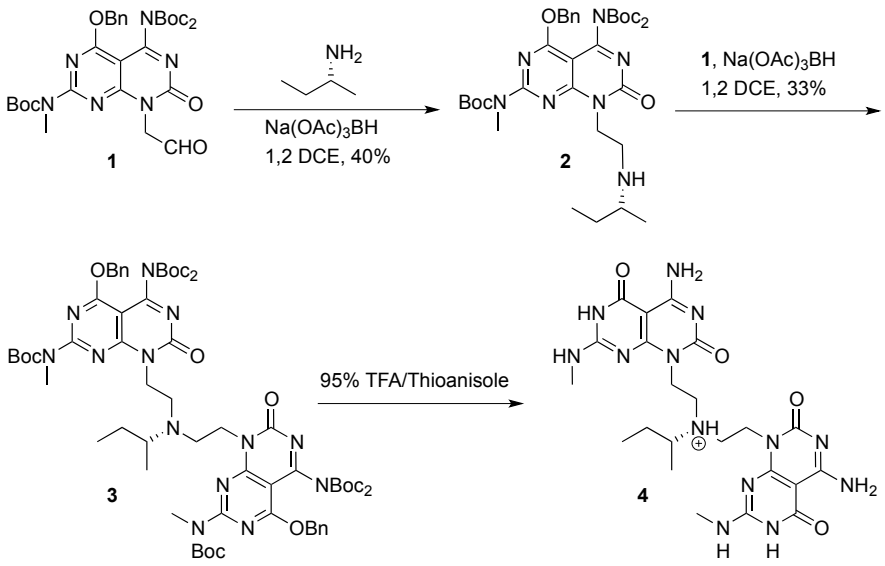


Fig. S1. Synthetic scheme for synthesis of R-TBL (**4**)

Synthesis of 2: (R)-butan-2-amine (26 μL, 0.352 mmol) was added to a solution of G∧C aldehyde **1**[1] (0.150 g, 0.234 mmol) in 1,2 DCE (20 mL) at room temperature under N_2 and stirred for 30 min. Sodium triacetoxy borohydride (0.078 g, 0.371 mmol) was added and the resulting mixture was stirred for an additional 24 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with water (20 mL), brine (20 mL), dried over Na_2SO_4 and concentrated. Purification by flash chromatography over silica gel (0-5% MeOH in CH_2Cl_2) gave **2** (0.066 g, 40%) as a white foam. R_f = 0.39 (5% MeOH in CH_2Cl_2); $[\alpha]_D^{25}$ = -2.4° (*c* = 1.5 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.45-7.33 (m, 5H), 5.56 (s, 2H), 4.47 (t, *J* = 6.6 Hz, 2H), 3.46 (s, 3H), 3.07 (m, 2H), 2.70 (m, 2H), 1.58 (s, 9H), 1.48-1.39 (m, 2H), 1.34 (s, 18H), 1.05 (d, *J* = 6.3 Hz, 3H), 0.85 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.7, 161.1, 160.9, 160.4, 155.8, 152.6, 149.2, 134.9, 128.6, 128.5, 128.3, 93.1, 83.8, 83.2, 70.1, 54.5, 44.2, 43.1, 34.9, 28.2, 27.8, 19.0, 10.2 ppm; HRMS: calcd for $C_{35}H_{52}N_7O_8$ $[M+H]^+$: 698.3872; found: 698.3873.

Synthesis of 3: Compound **2** (0.150 g, 0.215 mmol) was added to a solution of G∧C aldehyde **1** (0.138 g, 0.216 mmol) in 1,2 DCE (20 mL) at room temperature under N_2 and stirred for 30 min. Sodium triacetoxy borohydride (0.051 g, 0.243 mmol) was added and

the resulting mixture was stirred for an additional 30 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and then washed with water (2 x 10 mL), brine (15 mL), dried over Na_2SO_4 and concentrated. Flash chromatography of the residue over silica gel (0-30% EtOAc in hexanes) gave **3** (0.103 g, 36%) as a white foam. R_f = 0.44 (30% EtOAc in hexanes); $[\alpha]_D^{25}$ = +10.6° (*c* = 0.7 in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.44-7.31 (m, 10H), 5.56 (s, 4H), 4.34 (m, 4H), 3.48 (s, 6H), 2.90 (m, 2H), 2.81 (q, *J* = 6.4 Hz, 1H), 2.73 (m, 2H), 1.55 (s, 18H), 1.48-1.39 (m, 2H), 1.30 (s, 36H), 0.90 (d, *J* = 6.0 Hz, 3H), 0.86 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 165.7, 161.3, 161.1, 160.3, 155.6, 152.6, 149.3, 149.2, 135.1, 128.7, 128.6, 92.9, 84.0, 82.8, 70.3, 54.0, 47.3, 43.0, 35.0, 29.5, 28.1, 27.9, 14.7, 11.7 ppm; HRMS: calcd for $C_{66}H_{92}N_{13}O_{16}$ $[M+H]^+$: 1322.6780; found: 1322.6783.

Synthesis of 4: Compound **3** (0.066 g, 0.050 mmol) was stirred in a 95% TFA in thioanisole (10 mL) for 72 h. Et_2O (60 mL) was then added to the reaction mixture and the precipitate formed, was centrifuged down. The residual solid was resuspended in Et_2O, sonicated and centrifuged down. This process was repeated until the spotting of the Et_2O produced no UV active spot. The solid was dried under vacuo for 72 h to give **4** (0.033 g, 89%) as an off-white powder in quantitative yield. ^{1}H NMR (600 MHz, d_6-DMSO) δ = 12.30 (s, 2H), 11.50 (bs, 1H) 9.17 (s, 2H), 8.84 (bs, 2H), 8.43 (bs, 2H), 4.53 (m, 4H), 3.42 (m, 5H, merged with H_2O peak), 2.95 (d, *J* = 4.8 Hz, 6H), 2.03 (m, 1H), 1.42 (m, 1H), 1.29 (s, 3H), 0.91 (s, 3H); ^{13}C NMR (150 MHz, [D_6]DMSO) δ = 161.3, 160.3, 156.6, 156.2, 148.3, 83.0, 60.6, 47.0, 46.8, 37.2, 28.4, 12.6, 10.8 ppm; HRMS: calcd for $C_{22}H_{33}N_{13}O_4$ $[M+H]^+$: 543.2768; found: 271.6381; elemental analysis calcd (%) for $C_{22}H_{31}N_{13}O_4(HCl)_4(H_2O)_2(Et_2O)_{0.25}$: C 37.23, H 5.64, N 24.54; found: C 37.74, H 5.22, N 24.13.

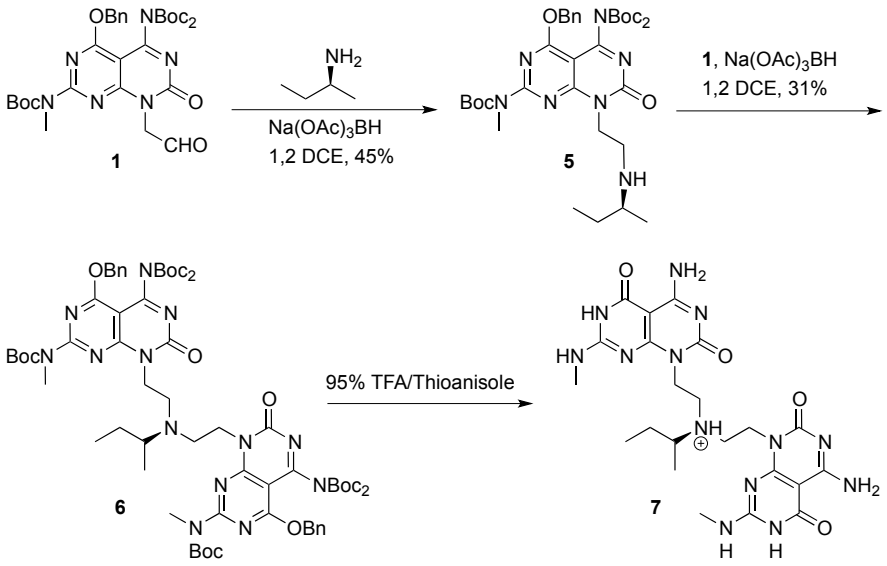


Fig. S2. Synthetic scheme for synthesis of S-TBL (**7**)

Synthesis of 5: (S)-butan-2-amine (26 μL, 0.352 mmol) was added to a solution of G∧C aldehyde **1** (0.150 g, 0.234 mmol) in 1,2 DCE (20 mL) at room temperature under N_2 and stirred for 30 min. Sodium triacetoxy borohydride (0.078 g, 0.371 mmol) was added and the resulting mixture was stirred for an additional 24 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with water (20 mL), brine (20 mL), dried over Na_2SO_4 and concentrated. Flash chromatography of the residue over silica gel (0-5% MeOH in CH_2Cl_2) gave **5** (0.074 g, 45%) as a white foam. R_f = 0.39 (5% MeOH in CH_2Cl_2); $[\alpha]_D^{25}$ = +2.0° (*c* = 2.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.44-7.32 (m, 5H), 5.56 (s, 2H), 4.47 (t, *J* = 6.7 Hz, 2H), 3.46 (s, 3H), 3.04 (m, 2H), 2.70 (m, 2H), 1.58 (s, 9H), 1.48-1.41 (m, 2H), 1.33 (s, 18H), 1.05 (d, *J* = 6.3 Hz, 3H), 0.85 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.7, 161.1, 160.9, 160.4, 155.8, 152.6, 149.2, 134.9, 128.6, 128.5, 128.3, 93.0, 83.8, 83.1, 70.1, 54.5, 44.2, 43.2, 34.9, 28.1, 27.8, 19.0, 10.1 ppm; HRMS: calcd for $C_{35}H_{52}N_7O_8$ $[M+H]^+$: 698.3872; found: 698.3876.

Synthesis of 6: Compound **5** (0.150 g, 0.215 mmol) was added to a solution of compound **1** (0.138 g, 0.216 mmol) in 1,2 DCE (20 mL) at room temperature under N_2 and stirred for 30 min. Sodium triacetoxy borohydride (0.051 g, 0.243 mmol) was added and the

resulting mixture was stirred for an additional 30 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and then washed with water (2 x 10 mL), brine (15 mL), dried over Na_2SO_4 and concentrated. Flash chromatography of the residue over silica gel (0-30% EtOAc in hexanes) gave **6** (0.096 g, 34%) as a white foam. R_f = 0.44 (30% EtOAc in hexanes); $[\alpha]_D^{25}$ = -11.1° (*c* = 0.7, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.45-7.31 (m, 10H), 5.56 (s, 4H), 4.36 (bs, 4H), 3.48 (s, 6H), 2.91 (bs, 2H), 2.83 (bs, 1H), 2.75 (bs, 2H), 1.55 (s, 18H), 1.47-1.38 (m, 2H), 1.30 (s, 36H), 0.92 (bs, 3H), 0.87 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ = 165.6, 161.2, 161.0, 160.2, 155.6, 152.5, 149.2, 149.1, 135.0, 128.5, 128.5, 92.8, 83.6, 82.8, 70.0, 53.4, 47.2, 35.0, 28.1, 27.8, 14.6, 11.6 ppm; HRMS: calcd for $C_{66}H_{92}N_{13}O_{16}$ $[M+H]^+$: 1322.6780; found: 1322.6779.

Synthesis of 7: Compound **6** (0.063 g, 0.048 mmol) was stirred in a 95% TFA in thioanisole (10 mL) for 72 h. Et_2O (60 mL) was then added to the reaction mixture and the precipitate formed, was centrifuged down. The residual solid was resuspended in Et_2O, sonicated and centrifuged down. This process was repeated until the spotting of the Et_2O produced no UV active spot. The solid was dried under vacuo for 72 h to give **7** in quantitative yield. ^{1}H NMR (600 MHz, d_6-DMSO) δ = 12.30 (s, 2H), 11.42 (bs, 1H), 9.17 (s, 2H), 8.84 (bs, 2H), 8.41 (bs, 2H), 4.53 (m, 4H), 3.38 (m, 5H, merged with H_2O peak), 2.94 (d, *J* = 4.8 Hz, 6H), 2.00 (m, 1H), 1.45 (m, 1H), 1.29 (s, 3H), 0.91 (s, 3H); ^{13}C NMR (150 MHz, [D_6]DMSO) δ = 161.2, 160.3, 156.6, 156.3, 148.2, 83.0, 60.5, 47.0, 46.4, 37.2, 28.4, 12.6, 10.8 ppm; HRMS: calcd for $C_{22}H_{33}N_{13}O_4$ $[M+H]^+$: 543.2768; found: 271.6382; elemental analysis calcd (%) for $C_{22}H_{31}N_{13}O_4(HCl)_{3.5}(H_2O)_2(Et_2O)_{0.25}$: C 38.17, H 5.71, N 25.16, found C 38.01, H 5.39, N 25.24.

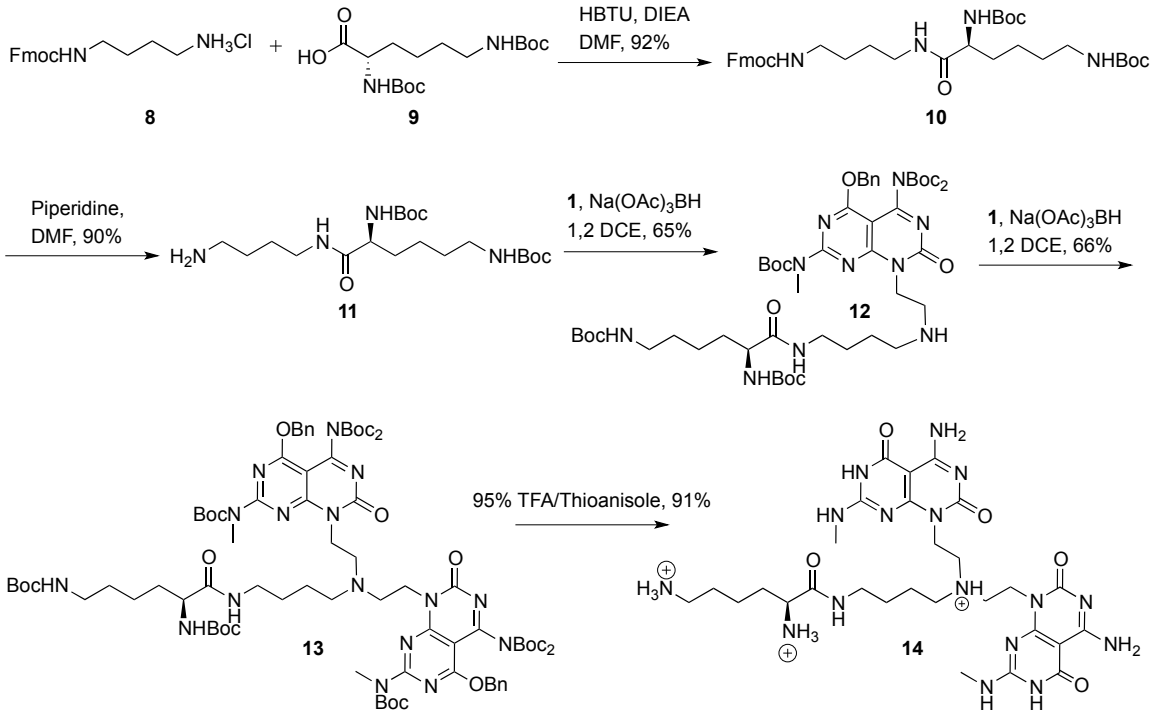


Fig. S3. Synthetic scheme for synthesis of K-C4-TBL (**14**)

Synthesis of 10: A stirred solution of (9*H*-fluoren-9-yl)methyl 4-aminobutylcarbamate hydrochloride **8** (1.00 g, 2.89 mmol) in DMF (10 mL) was treated with DIPEA (0.51 mL, 2.9 mmol) and the mixture was stirred for 20 min at room temperature under N_2. N-α,ε-di-Boc-L-lysine **9** (1.00 g, 2.89 mmol) and HBTU (1.64 g, 4.33 mmol) were then added and the resulting mixture was stirred for another 7 h. The reaction mixture was diluted with Et_2O (50 mL) and then washed with water (2 x 10 mL), citric acid (10 mL), $NaHCO_3$ (10 mL), brine (15 mL), dried over Na_2SO_4, filtered and concentrated. Purification by flash chromatography over silica gel (10-100% EtOAc in hexanes) gave **10** as an off-white foam in 92% yield. R_f = 0.33 (50% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.74 (d, J = 7.6 Hz, 2H), 7.58 (d, J = 7.6 Hz, 2H), 7.38 (t, J = 7.6 Hz, 2H), 7.29 (t, J = 7.0 Hz, 2H), 6.31 (bs, 1H), 5.12 (bs, 1H), 5.01 (bs, 1H), 4.61 (bs, 1H), 4.38 (d, J = 6.4 Hz, 2H), 4.19 (t, J = 6.8 Hz, 1H), 3.99 (m, 1H), 3.26 (m, 2H), 3.19 (m, 2H), 3.08 (t, J = 6.4 Hz, 2H), 1.80-1.46 (m, 8H), 1.42 (s, 18H), 1.38-1.33 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$)□δ□ = 158.8, 158.0, 144.0, 141.4, 127.6, 127.0, 125.1, 119.9,

80.1, 79.2, 66.6, 47.3, 40.6, 39.9, 38.6, 31.9, 29.7, 28.4, 28.3, 27.3, 26.6, 22.7 ppm; HRMS: calcd for $C_{35}H_{50}N_4O_7$ $[M+Na]^+$: 661.3572; found: 661.3571.

Synthesis of 11: Compound **10** (1.19 g, 1.87 mmol) was stirred in DMF (10 mL) and piperidine (1.6 mL) for 2 h at room temperature under N_2. The reaction mixture was concentrated. Purification of the residue by flash chromatography over silica gel (100-90% EtOAc in MeOH) gave **11** as a colorless oil in 90% yield. R_f = 0.21 (75% EtOAc in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 6.89 (bs, 1H), 5.21 (bs, 1H), 4.72 (bs, 1H), 4.01 (q, J = 6.3 Hz, 2H), 3.26 (q, J = 5.7 Hz, 2H), 3.10 (q, J = 6.6 Hz, 2H), 2.75 (t, J = 6.2 Hz, 2H), 1.86-1.75 (m, 2H), 1.67 (s, 9H), 1.63-1.48 (m, 6H), 1.44 (s, 9H), 1.39-1.23 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.0, 156.2, 155.2, 79.9, 79.0, 50.6, 41.2, 39.6, 39.1, 32.1, 29.9, 29.5, 28.3, 28.2, 26.7, 22.5 ppm; HRMS: calcd for $C_{20}H_{40}N_4O_5$ $[M+H]^+$: 417.3076; found: 417.3072.

Synthesis of 12: Compound **1** (0.443 g, 0.692 mmol) was added to a stirred solution of **11** (0.288 g, 0.692 mmol) in 1,2 DCE (20 mL) at room temperature under N_2. The mixture was stirred for 30 min before addition of sodium triacetoxyborohydride (0.176 g, 0.800 mmol) and the resulting mixture was stirred for an additional 15 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and then washed with water (2 x 10 mL), brine (15 mL), dried over Na_2SO_4 and concentrated. Purification by flash chromatography over silica gel (0-3% MeOH in CH_2Cl_2) gave **12** as a white foam in 65% yield. R_f = 0.37 (5% MeOH in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.43-7.32 (m, 5H), 6.75 (bs, 1H), 5.56 (s, 2H), 5.31 (d, J = 8.0 Hz, 2H) 4.75 (bs, 1H), 4.50 (m, 2H), 4.03 (bs, 1H), 3.46 (s, 3H), 3.22 (m, 2H), 3.12-3.03 (m, 4H), 2.74 (m, 2H), 1.85-1.59 (m, 4H), 1.57 (s, 9H), 1.54-1.46 (m, 2H), 1.41 (s, 18H), 1.39-1.33 (m, 2H), 1.32 (s, 18H), 1.28-1.19 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 172.5, 165.6, 161.0, 160.8, 156.1, 155.8, 155.7, 152.4, 149.3, 134.6, 128.5, 128.0, 127.6, 113.8, 93.3, 84.0, 83.5, 79.5, 78.8, 70.2, 54.3, 48.0, 46.3, 40.8, 40.0, 38.3, 35.0, 32.3, 29.3, 28.3, 28.2, 28.0, 27.7, 26.2, 23.8, 22.5 ppm; HRMS: calcd for $C_{51}H_{81}N_{10}O_{13}$ $[M+H]^+$: 1041.5979; found: 1041.5983.

Synthesis of 13: Compound **1** (0.192 g, 0.298 mmol) was added to a solution of **12** (0.311 g, 0.299 mmol) in 1,2 DCE (20 mL) at room temperature under N_2 and stirred for

30 min. Sodium triacetoxy borohydride (0.074 g, 0.352 mmol) was added and the resulting mixture was stirred for an additional 15 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and then washed with water (2 x 10 mL), brine (20 mL), dried over Na_2SO_4 and concentrated. Flash chromatography of the residue over silica gel (10-80% EtOAc in hexanes) gave **13** (0.326 g, 66%) as a white foam. R_f = 0.42 (70% EtOAc in hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.45-7.28 (m, 10H), 6.64 (bs, 1H), 5.57 (s, 4H), 4.77 (bs, 1H), 4.37 (m, 4H), 4.11 (m, 2H), 3.48 (s, 6H), 3.33 (m, 1H), 3.18 (m, 1H), 3.08 (m, 2H), 2.90 (t, J = 6.5 Hz, 4H), 2.67 (app. q, J = 7.5 Hz, 2H), 1.62-1.58 (m, 2H), 1.56 (s, 18H), 1.54-1.43 (m, 6H), 1.42 (s, 9H), 1.41 (s, 9H), 1.32 (s, 18H), 1.31 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 172.2, 171.0, 165.5, 161.2, 160.9, 160.2, 156.2, 156.0, 155.6, 152.4, 149.2, 149.1, 134.8, 128.4, 128.3, 127.7, 113.8, 92.8, 83.6, 82.8, 70.0, 60.3, 54.4, 50.8, 41.1, 40.1, 38.9, 34.9, 32.4, 29.4, 28.3, 28.2, 28.0, 27.7, 26.7, 24.6, 22.7 ppm; HRMS:calcd for $C_{82}H_{120}N_{16}O_{21}Na$ $[M+Na]^+$: 1687.8706; found: 1687.8700.

Synthesis of 14: Compound **13** (0.093 g, 0.0558 mmol) was stirred in 95% TFA in thioanisole (10 mL) for 72 h. Et_2O (60 mL) was then added to the reaction mixture and the precipitate formed was stirred for another 30 min, after which it was centrifuged down. The residual solid was resuspended in Et_2O, sonicated and centrifuged down. This process was repeated until the spotting of the Et_2O produced no UV active spot. The white solid obtained (0.069 g, 91%) was dried under vacuo. ^{1}H NMR (600 MHz, d_6-DMSO) δ = 12.32 (s, 2H), 11.50 (s, 1H), 9.20 (s, 2H), 9.83 (bs, 3H), 8.46 (bs, 2H), 8.31 (bs, 3H), 8.03 (bs, 3H), 4.50 (bs, 4H), 3.77 (m, 1H), 3.52-3.40 (m, 6H, merged with water peak), 3.19-3.09 (m, 2H), 2.92 (d, J = 4.8 Hz, 6H), 2.79-2.74 (m, 2H), 1.82-1.65 (m, 4H), 1.61-1.43 (m, 4H), 1.41-1.32 (m, 2H); ^{13}C NMR (125 MHz, [D_6]DMSO) δ = 168.2, 160.7, 159.7, 156.2, 155.7, 148.0, 82.6, 51.8, 48.5, 38.1, 37.8, 30.2, 28.9, 28.5, 27.8, 26.1, 25.8, 21.1 ppm; HRMS calcd for $C_{28}H_{45}N_{16}O_5$ $[M-2H]^+$: 685.3753; found: 685.3759; elemental analysis calcd (%) for $C_{28}H_{44}N_{16}O_5(TFA)_{5.5}(H_2O)_2$: C 34.75, H 4.00, N 16.63; found C 35.15, H 4.40, N 16.40; elemental analysis calcd (%) for $C_{28}H_{44}N_{16}O_5(HCl)_8(H_2O)_3(Et_2O)_{0.25}$: C 33.20, H 5.81, N 21.36; found C 33.12, H 5.93, N 21.42.

Sample preparation for characterization by microscopy and spectroscopy

Scanning electron microscopy (SEM)

The SEM samples were prepared by floating a carbon-coated 400-mesh copper grid (Electron Microscopy Sciences) on a droplet of the diluted RNT solution for 10 s. The grid was blotted using filter paper. The RNT-coated grid was then air-dried and heated on a hotplate (70 °C) for 15 min before imaging to remove any residual solvents. SEM images were obtained without negative staining (unless indicated otherwise), at 5 kV or at 30 kV accelerating voltage, 20 μA and a working distance of 5 to 8 mm on a high-resolution Hitachi S-4800 cold field emission SEM. For the STEM images, uranyl acetate (2% aqueous solution) was used for staining the samples.

Transmission electron microscopy (TEM)

Samples were prepared by depositing a drop of prepared solution on a carbon-coated 400-mesh copper grid and blotted after 10 s. The sample was stained using uranyl acetate (2% aqueous solution). The grid was then blotted, air-dried and heated on the hotplate prior to imaging. The samples were visualized on JEOL 2200 FS TEM – 200kV Schottky field emission instrument equipped with an in-column omega filter. Bright field TEM images are acquired using energy filtered zero loss beam (slit width 10 ev).

UV–Vis spectroscopy

All UV-Vis spectra were recorded on an Agilent 8453 UV/Vis spectrometer. The absorption spectra of the diluted samples were recorded by taking aliquots of the stock solutions and diluting them to 2.2 x 10^{-5} M at 20 °C in unbuffered water at different time intervals.

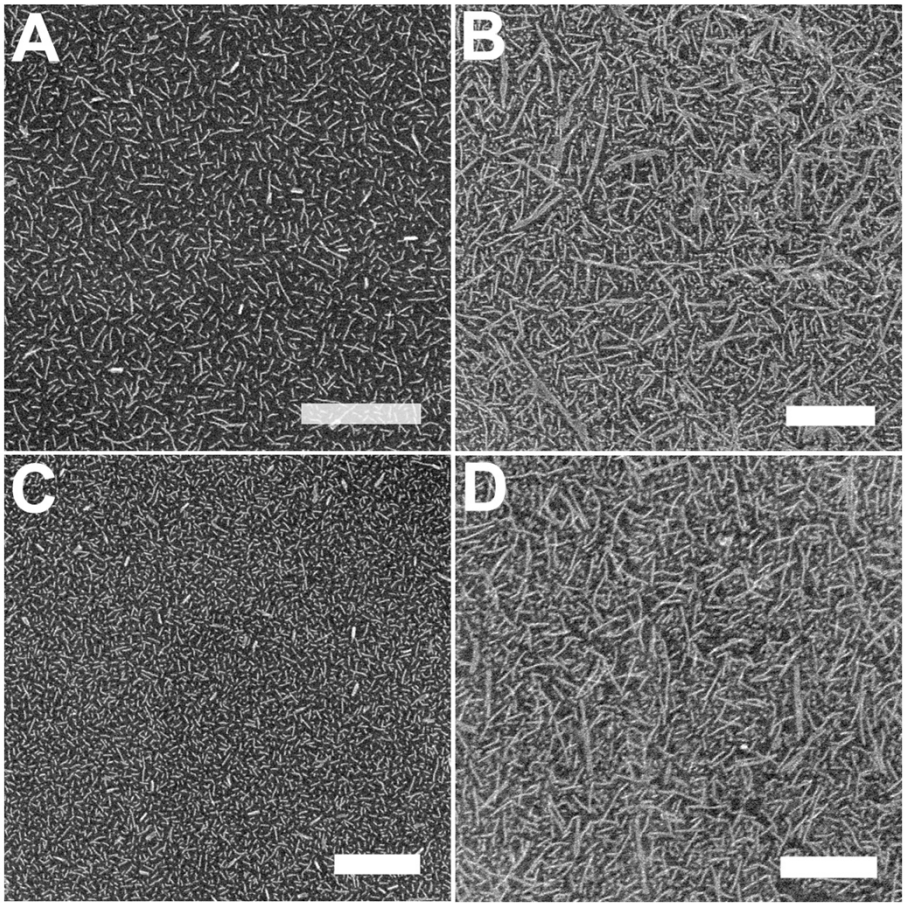


Fig. S4. SEM images of R-TBL (A, B) and S-TBL (C, D) in methanol (A, C) and water (B, D) after 1 day of aging. Concentration = 3.34 x 10^{-5} M. Scale bar 300 nm.

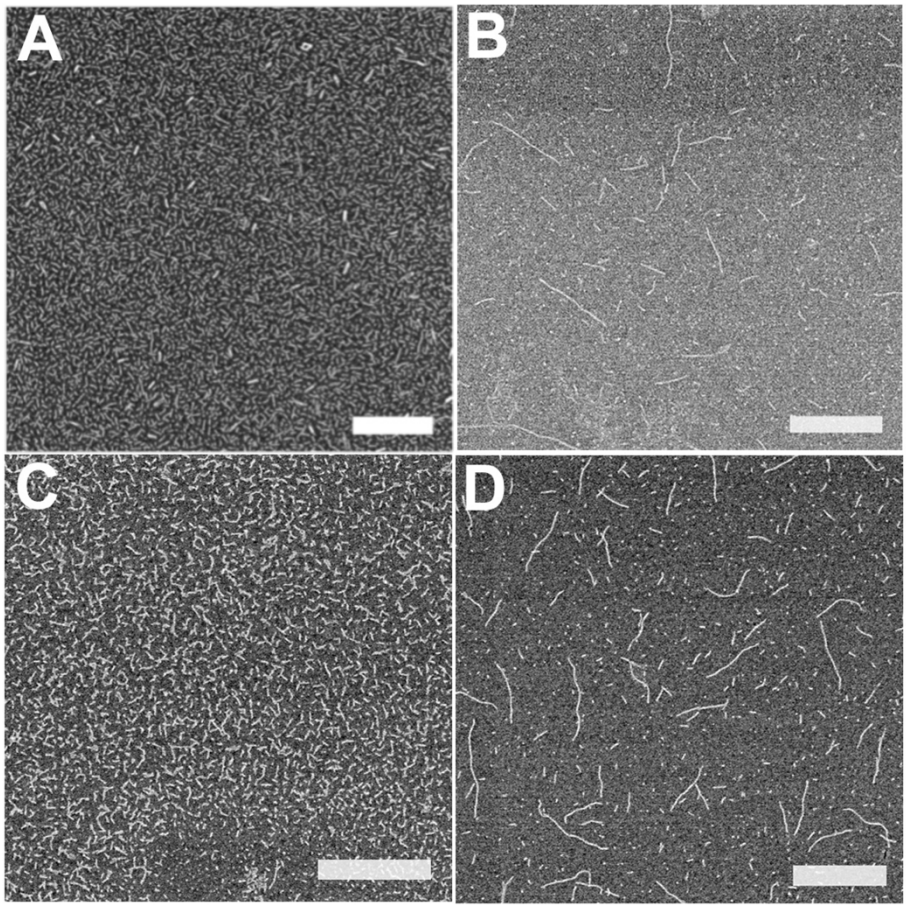


Fig. S5. SEM images of K-C2-TBL (A, B) and K-C4-TBL (C, D) in methanol (A, C) and water (B, D) after 1 day of aging. Concentration = 2.46 x 10^{-5} M. Scale bar 300 nm.

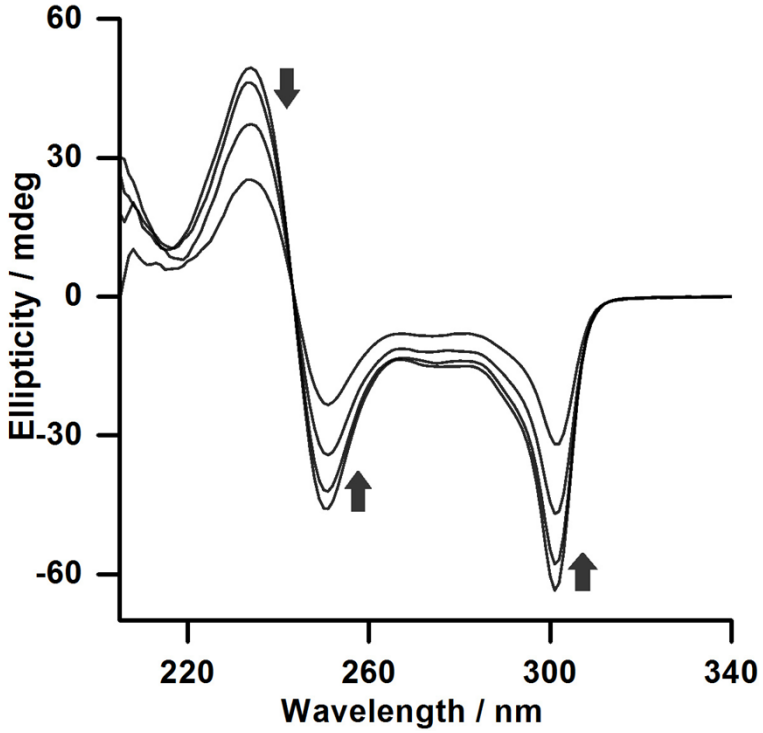


Fig. S6. Variable temperature CD of K-C4-TBL (2.46 x 10^{-5} M) in methanol from 20 °C to 50 °C.

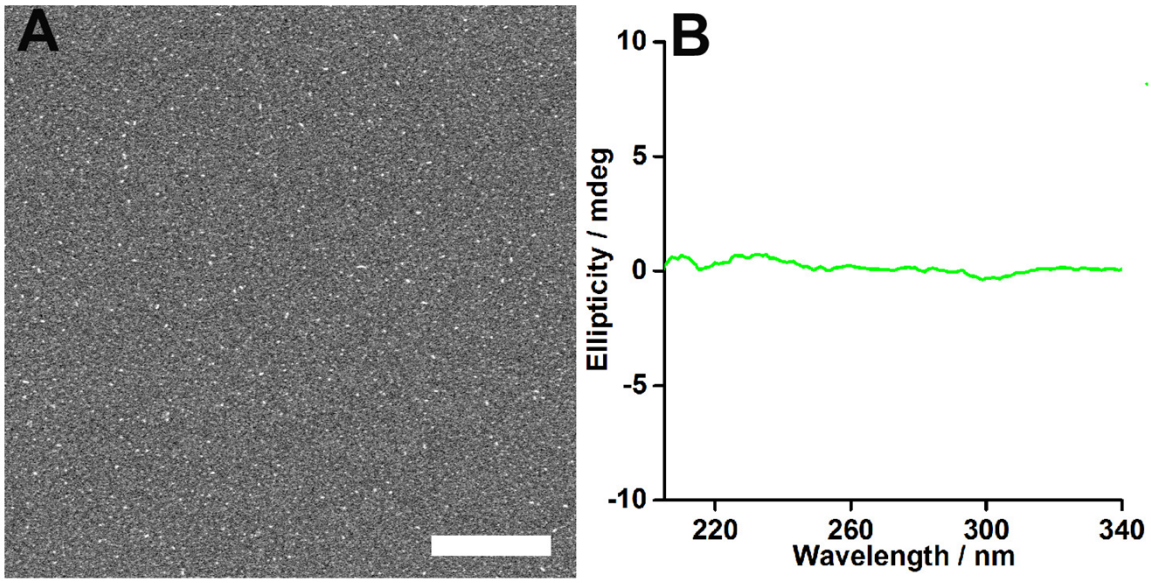


Fig. S7. Self-assembly of K3T in methanol and its corresponding CD profile after 25 days. Scale bar = 300 nm.

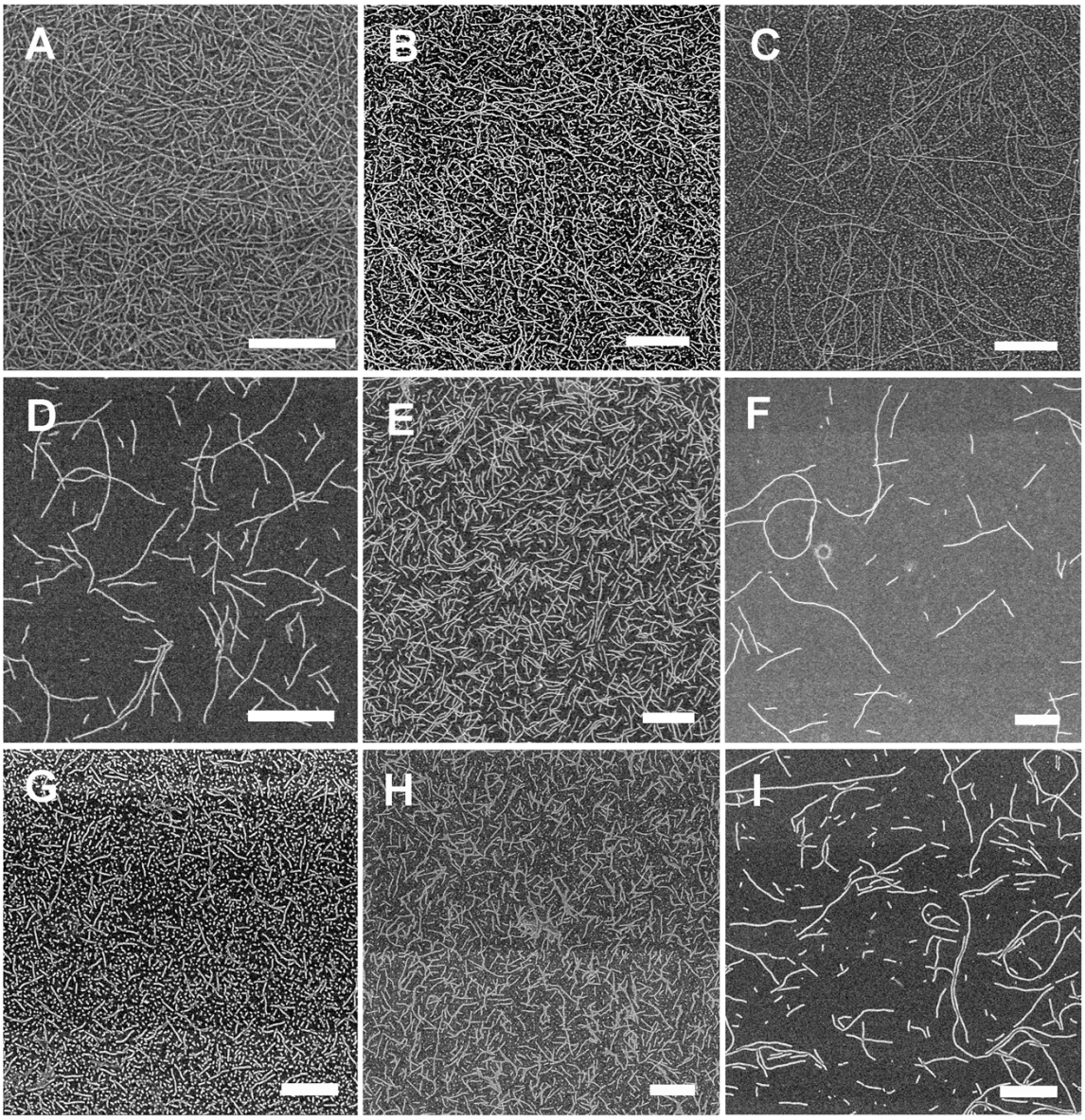
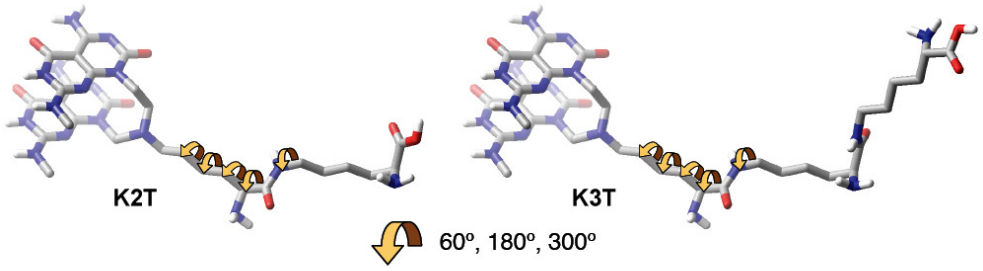


Fig. S8. SEM images in unbuffered water of freshly prepared samples of (A) K1T (1×10^{-4} M), (B) K2T (8.4×10^{-5} M), (C) K3T (3.3×10^{-5} M), (D) K4T (1.9×10^{-5} M), (E) K5T (5.1×10^{-5} M). SEM images in unbuffered water after one week of aging (F) K6T (4.4×10^{-5} M), (G) K7T (7.9×10^{-5} M), (H) K8T (3.5×10^{-5} M), (I) K9T (3.3×10^{-5} M). Scale bar = 300 nm.

Molecular modeling

Initial conformations (3×3×3×3×3=243) of K2T and K3T were generated by varying five dihedral angles, as indicated below. The carboxylic and amino groups were taken to be neutral such that the motifs have zero net charge.



243 RNTs consisting of 7 twin-rosette stacks with a staggered angle of 30º and stacking distance of 4.0 Å were generated.[2,3] The RNT conformations were optimized by fixing the top two and bottom two rosette stacks as well as all of the G∧C bases to reduce the end effect. The central rosette stack was taken from the optimized RNTs to finally construct RNTs composed of N=1~7 twin-rosette stacks. Due to the steric repulsion, 224 out of 243 K2T motifs and 202 out of 243 K3T motifs were able to form RNTs. The free energy as the sum of internal energy by molecular mechanics and the solvation free energy by 3D-RISM[4] theory with OPLS force field[5] were then calculated. As illustrated in Fig. S9 (A, B), K2T RNT (087) and K3T RNT (024) were determined to be the most stable RNTs in water. The negative association free energies for these RNTs suggest that they would be formed in water (Fig. S9).

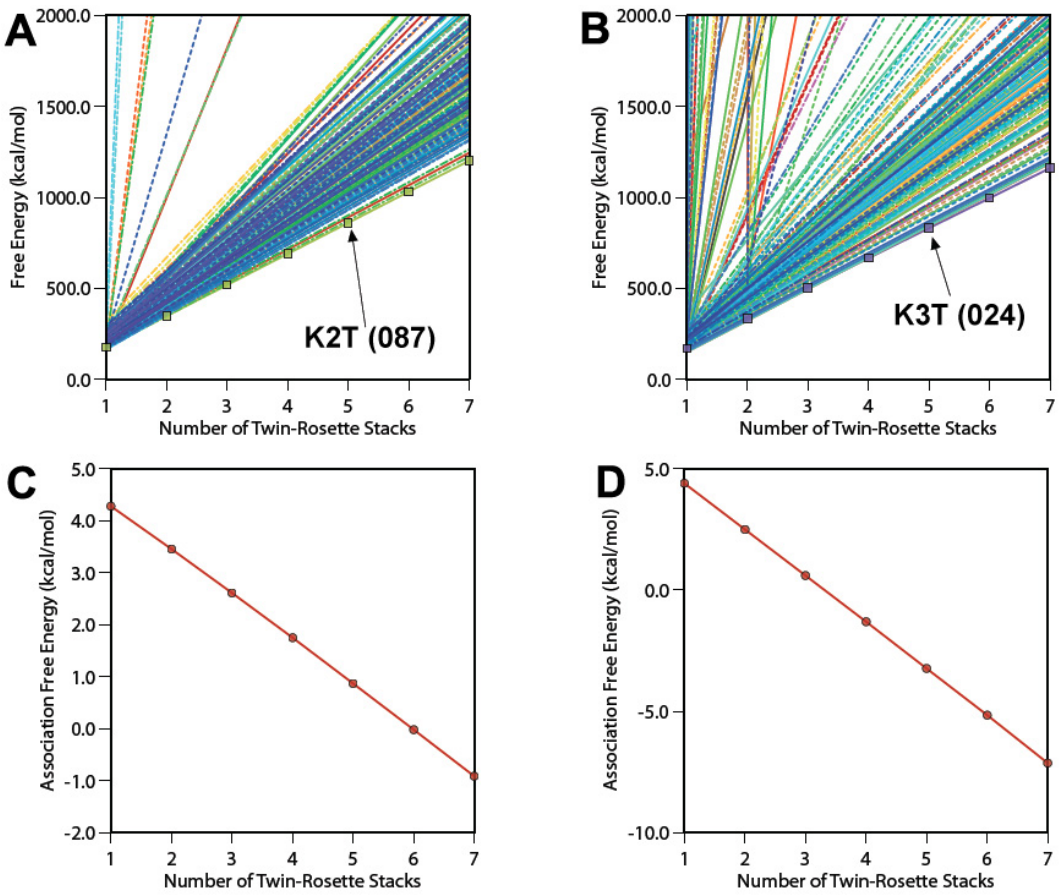


Figure S9. Free energy plots of (A) K2T and (B) K3T illustrating the most stable RNTs 087 and 024 respectively. Association free energy plots of (C) K2T (087) and (D) K3T (024).

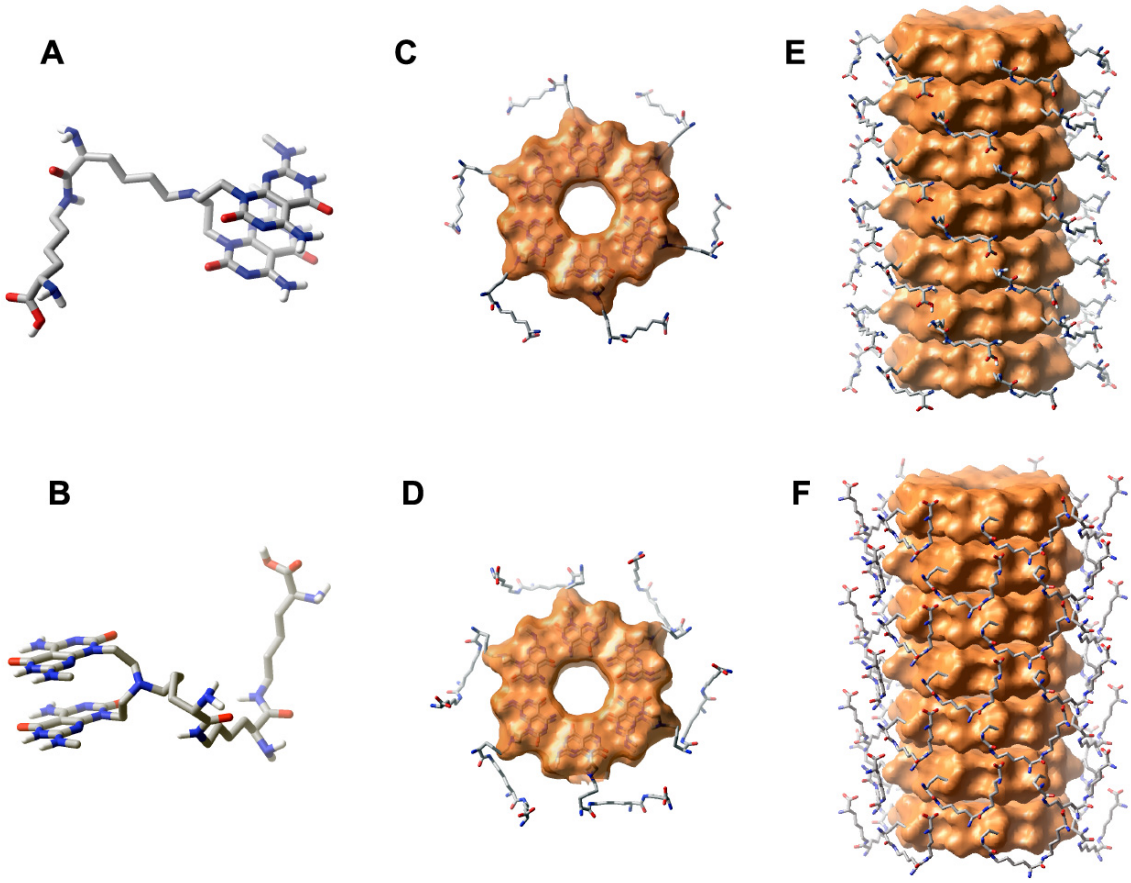


Fig. S10. Modeling showing motif (A, B), rosette (C, D) and RNT (E, F) of K2T (087) and K3T (024) respectively.

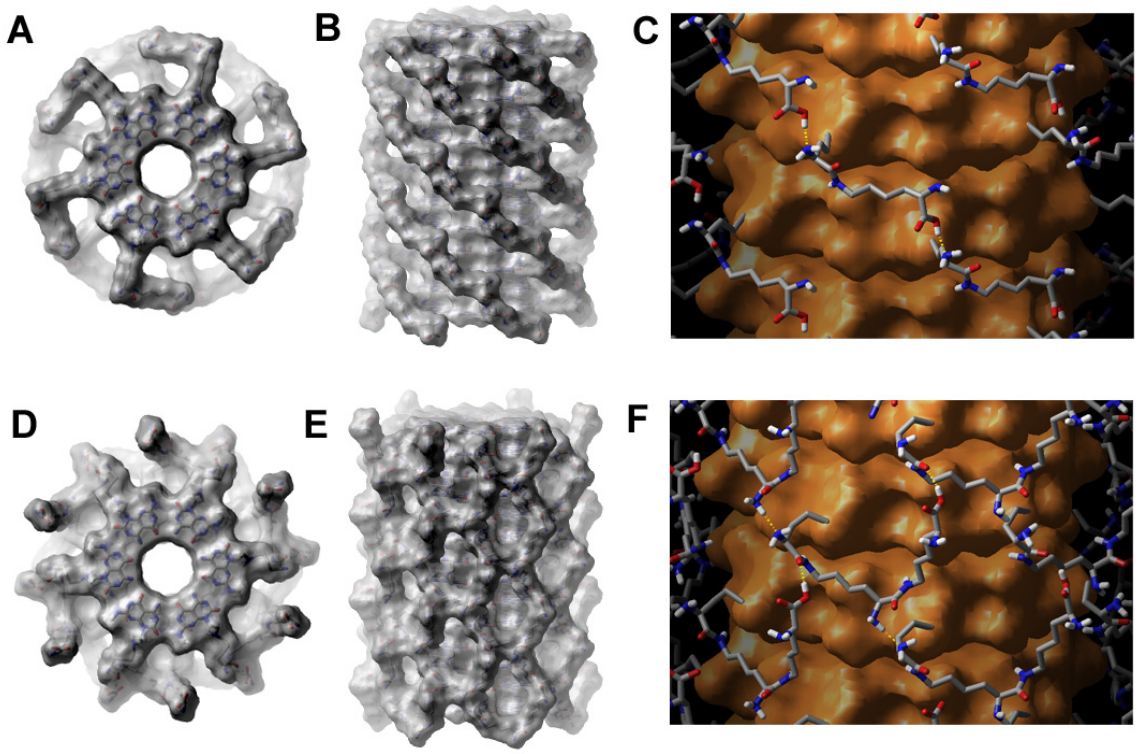


Fig. S11. Molecular models showing rosettes of K2T (A) and K3T (D), helicity of K2T (B) and K3T (E) RNTs due to orientation of L-oligo-lysine chains, and inter-rosette hydrogen-bonding interactions in K2T (C) and K3T (F) RNTs. K2T and K3T motifs have opposite chiralities, which agrees with the CD data.

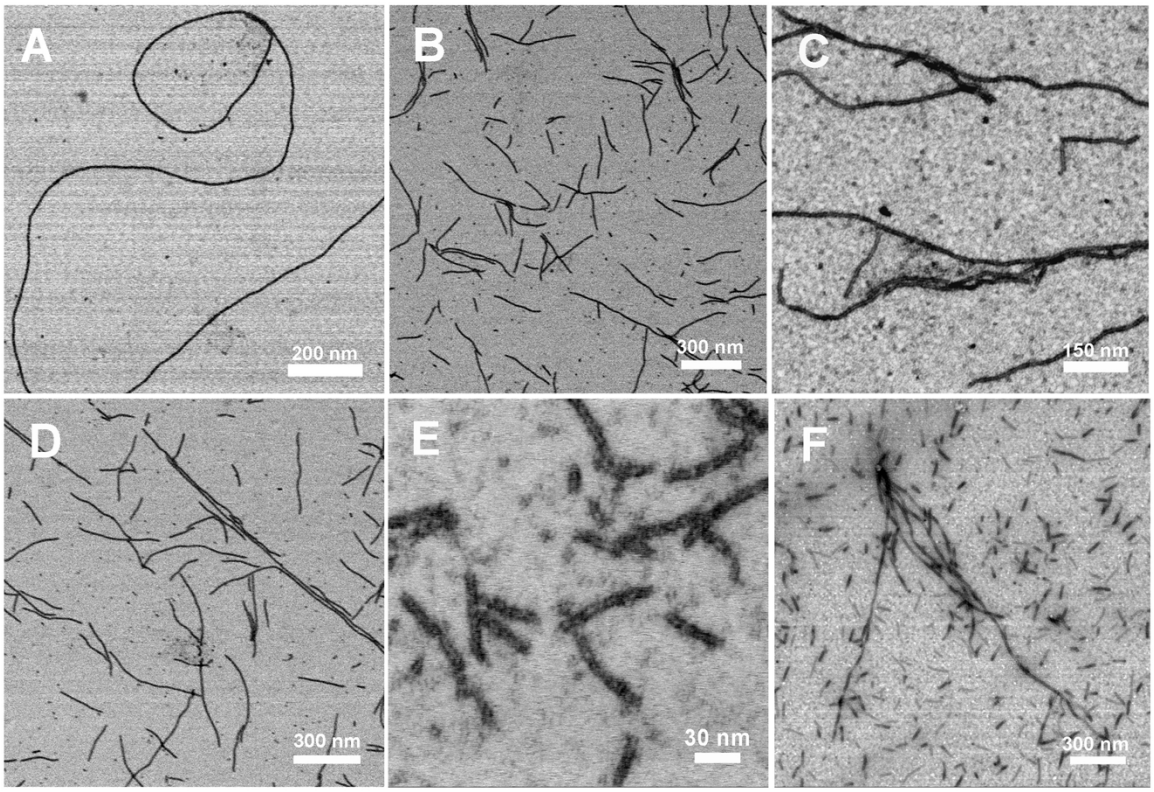


Fig. S12. STEM images of negatively stained samples using uranyl acetate. Samples were aged for 2 weeks before casting onto the grids. (A) K10T (8.8×10^{-5} M), pH 10, (B) K11T (5.4×10^{-5} M), pH 10 (C) K12T (6.9×10^{-5} M), pH 10 (D) K13T (4.9×10^{-5} M), pH 11 (E) K14T (3.1×10^{-5} M), pH 11 (F) K15T (5.7×10^{-5} M), pH 11.

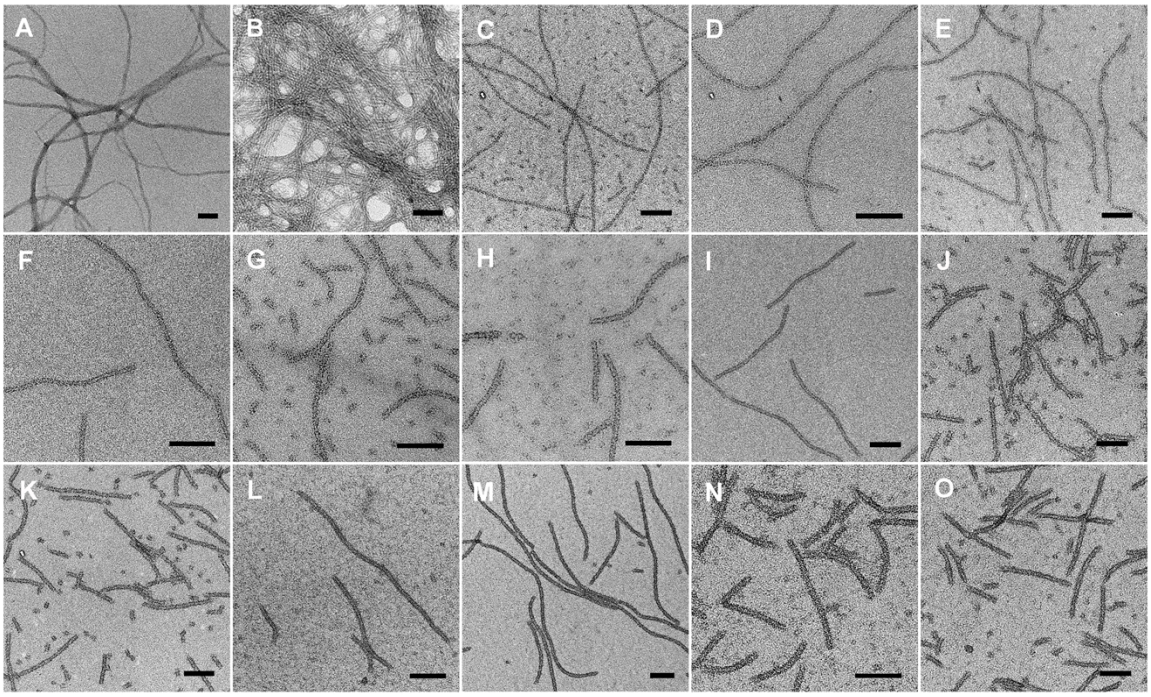


Fig. S13. TEM images negatively stained with uranyl acetate of (A) K1T (8.4×10^{-5} M), (B) K2T (8.4×10^{-5} M), (C) K3T (1.7×10^{-5} M), (D) K4T (2.9×10^{-5} M), (E) K5T (1.3×10^{-5} M), (F) K6T (4.4×10^{-5} M), (G) K7T (1.2×10^{-5} M), (H) K8T (3.5×10^{-5} M), (I) K9T (3.3×10^{-5} M). (J) K10T (1.9×10^{-5} M), pH 10 (K) K11T (1.8×10^{-5} M), pH 10 (L) K12T (6.9×10^{-5} M), pH 10 (M) K13T (1.6×10^{-5} M), pH 11 (N) K14T (3.1×10^{-5} M), pH 11 (O) K15T (5.7×10^{-5} M), pH 11. Scale bar = 50 nm.

Table 1. Diameter of RNTs, measured by TEM.

Modules	Diameter (nm)	Modules	Diameter (nm)
K1T	3.3 ± 0.2	K9T	6.3 ± 0.2
K2T	4.1 ± 0.5	K10T	7.1 ± 0.2
K3T	4.4 ± 0.5	K11T	7.2 ± 0.2
K4T	5.0 ± 0.5	K12T	7.4 ± 0.2
K5T	5.1 ± 0.2	K13T	8.1 ± 0.2
K6T	5.2 ± 0.2	K14T	8.2 ± 0.2
K7T	5.4 ± 0.2	K15T	8.7 ± 0.3
K8T	6.1 ± 0.1		

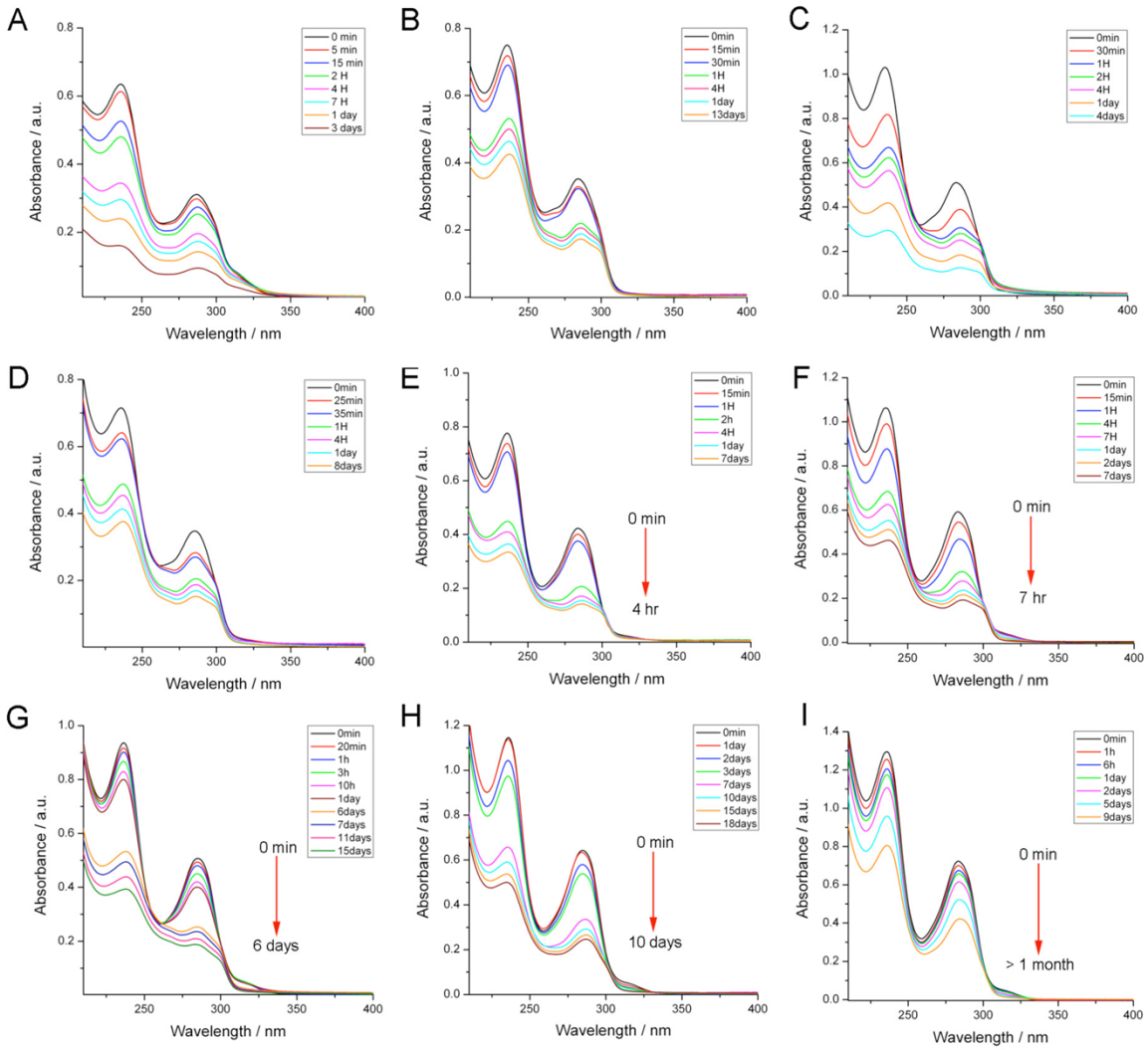


Fig. S14. UV-vis spectroscopy of RNTs in unbuffered water (2.2 x 10^{-5} M), monitored at room temperature over time: (A) K1T (B) K2T (C) K3T (D) K4T (E) K5T (F) K6T (G) K7T (H) K8T (I) K9T. All of these motifs show a hypochromic shift, indicating self-assembly. Red arrows for K5T – K9T indicate the time required for a 50% change in absorbance, which is shown to increase from 4 hr to > 1 month as the length of the peptide increases.

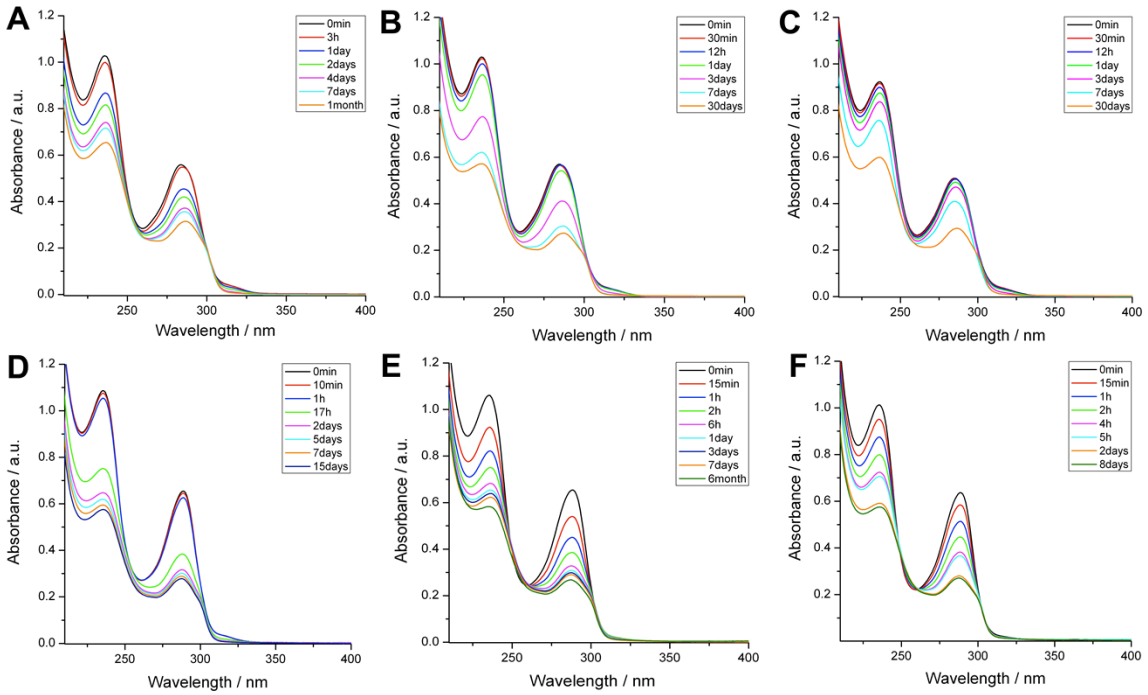


Fig. S15. UV-vis spectroscopy of RNTs in buffered conditions (2.2 x 10^{-5} M), monitored at room temperature over time: (A) K10T, pH 10 (B) K11T, pH 10 (C) K12T, pH 10 (D) K13T, pH 11 (E) K14T, pH 11 (F) K15T, pH 11. All of these motifs show a hypochromic shift, indicating self-assembly.

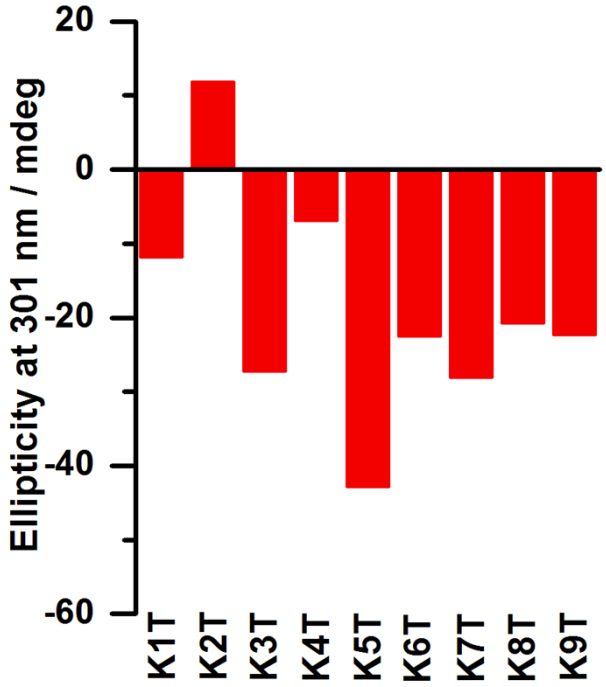


Fig. S16. Ellipticity at 301 nm of a 4.0 x10^{-5} M solution of K1T – K9T at pH = 7

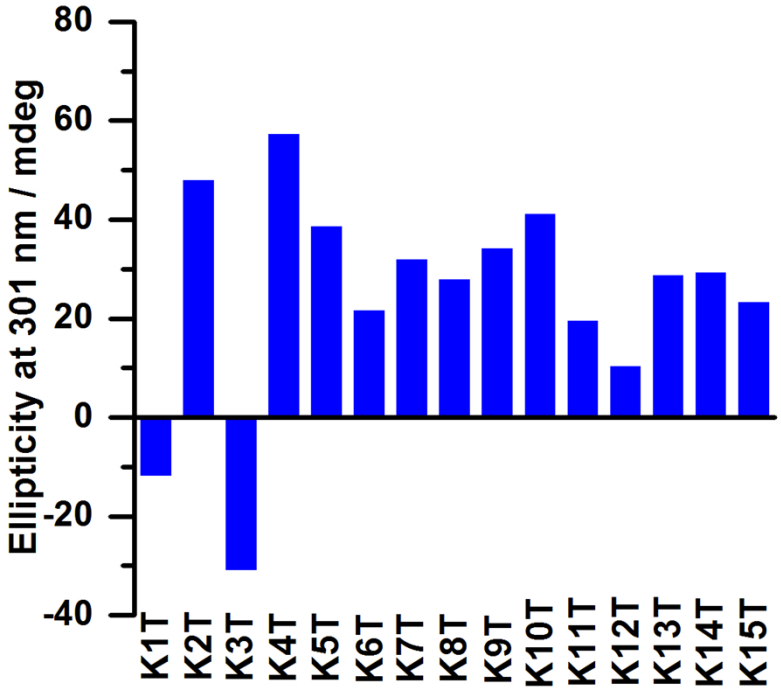


Fig. S17. Ellipticity at 301 nm of a 4.0 x 10^{-5} M solution of K1T – K15T at pH = 11

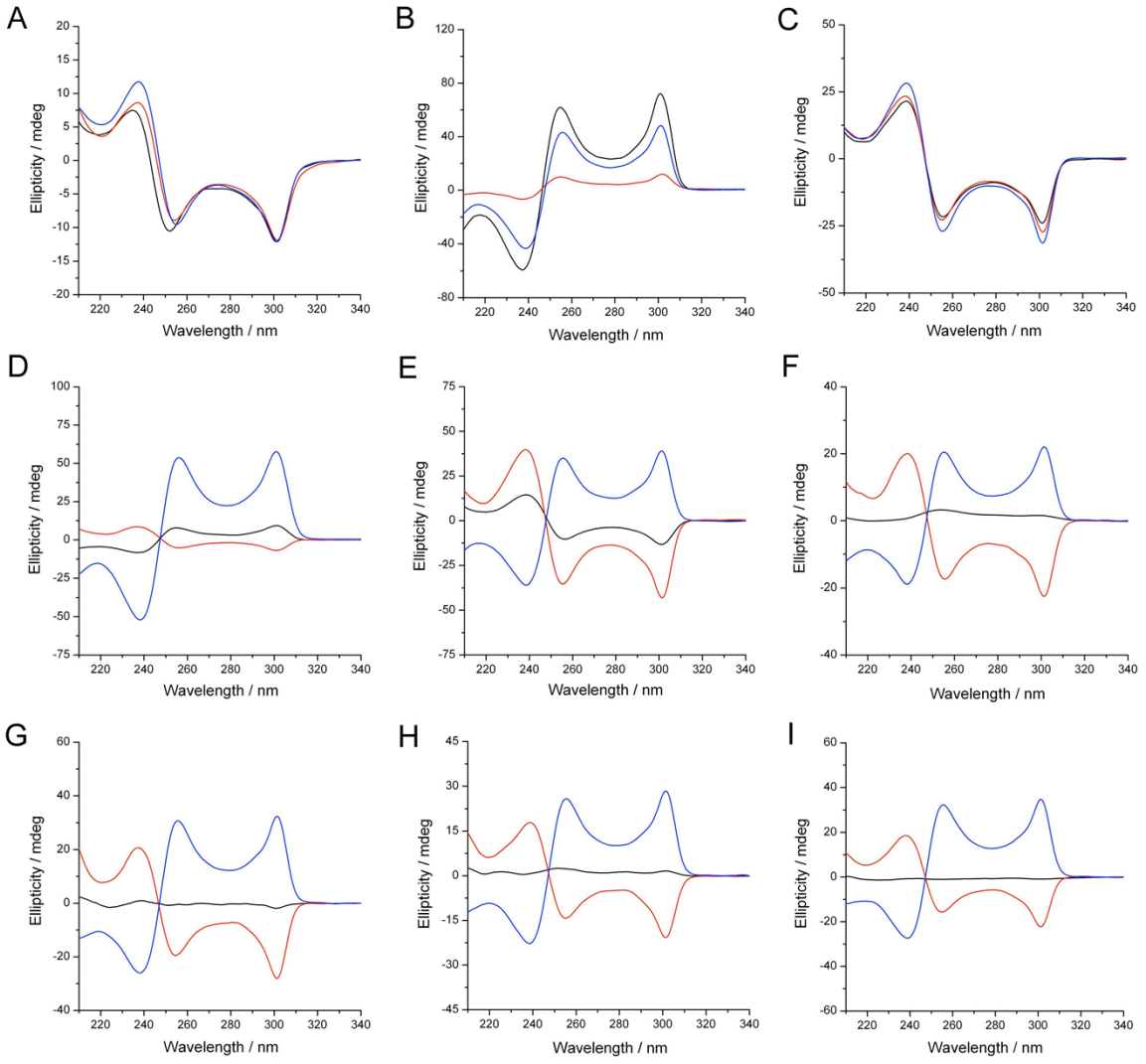


Fig. S18. CD spectra at 4.0 x 10^{-5} M of (A) K1T (B) K2T (C) K3T (D) K4T (E) K5T (F) K6T (G) K7T (H) K8T (I) K9T in unbuffered water (black), pH 7 (0.05 HEPES buffer, red) and pH 11 (0.05 CAPS buffer, blue).

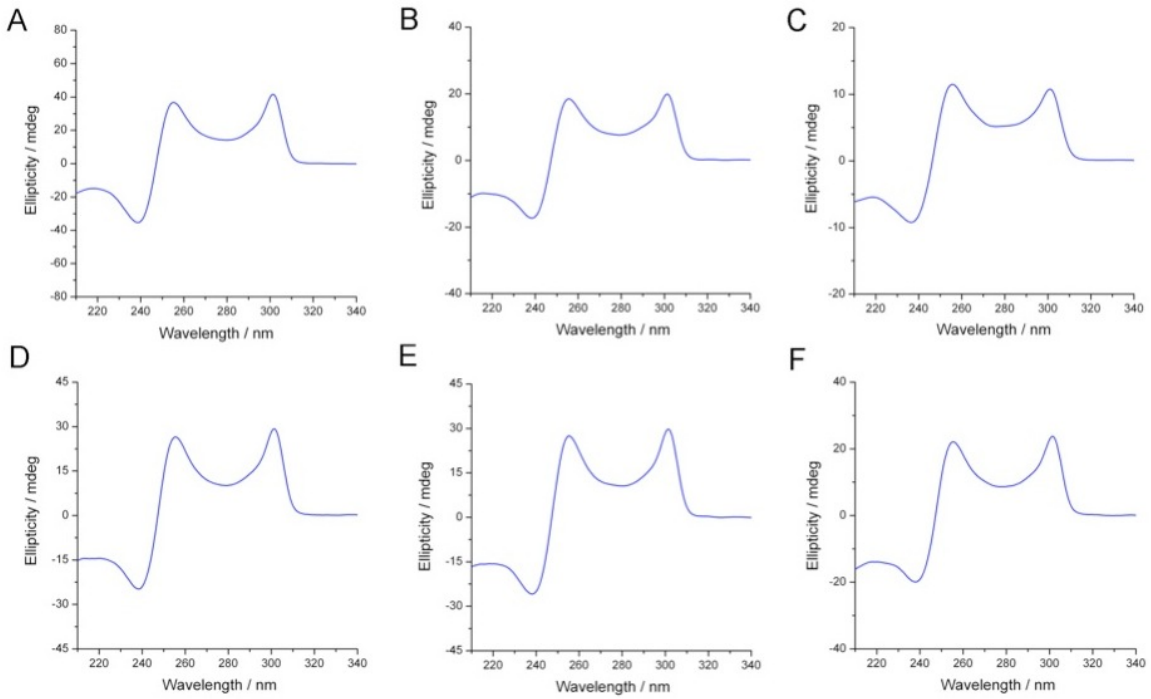


Fig. S19. CD spectra at 4.0 x 10^{-5} M of (A) K10T (B) K11T (C) K12T (D) K13T (E) K14T (F) K1T at pH 11 (0.05 CAPS buffer).

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