Equilibrium Crossing Exhibited by Ethynylhelicene (M)-Nonamer during the Random-coil to double-helix Thermal Transition in Solution

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Supplementary information

Chemicals
Ethynylhelicene (M)-nonamer (M)-1 was synthesized according to the reference.\[S1\]

1. S-Random-coil state of (M)-1
CD and UV-vis analysis (Figure S1)
(M)-1 was dissolved in bromobenzene (0.1 mM), heated at 40 °C for 1 h, and was subjected to CD and UV-vis analyses. (M)-1 was dissolved in chlorobenzene (0.1 mM), heated at 60 °C for 1 h, and was subjected to CD and UV-vis analyses. S-Random-coil state with Δε +200 cm⁻¹M⁻¹ at 367 nm was determined for both solutions by comparing the shape of the spectra with those of (P)-heptamer.\[S1\]
Figure S1. CD (left) and UV-vis (right) spectra of (M)-I in bromobenzene (40 °C, 0.1 mM, 60 min), chlorobenzene (60 °C, 0.1 mM, 60 min), and those of (P)-heptamer in chloroform (25 °C, 0.005 mM, 15 min).[S1] The CD and UV-vis spectra were obtained at each temperature after allowing the solution to stand for time shown in parentheses.

VPO analysis
A solution of (M)-I (1 mM) in bromobenzene was heated at 100 °C for 1 h, cooled to room temperature, and analyzed immediately by VPO at 60 °C. Apparent molecular weight of (M)-I 4.7 × 10^3; calculated molecular weight 4951, was obtained.

DLS analysis (Figure S2)
A solution of (M)-I in chlorobenzene (0.1 mM) was heated at 60 °C for 1 h, cooled at 20 °C for 1 h, and analyzed by DLS (20 °C), which provided an average dimeter of 1.9 nm. Refractive index 1.52 and UV-vis absorption 0.00 of this solution at 633 nm were obtained, and were used to transform the DLS intensity distribution to the number distribution by the Mie theory.[S2]
Figure S2. Size distribution of (M)-1 obtained by DLS experiments (0.1 mM, 20 °C) in (a) toluene and (b) chlorobenzene.

2. S-Double-helix state of (M)-1 in toluene
HPLC analysis (Figure S3)
A toluene solution of (M)-1 (1 mM) was prepared at 25 °C, and was analyzed by HPLC. The solution was heated at 95 °C for 1 h, cooled to room temperature, and was analyzed immediately by HPLC. Then, the solution was settled at room temperature for 28 h, and was analyzed by HPLC. These solutions were diluted to 0.1 mM just before HPLC analysis. Mobile phase: toluene, flow rate: 0.5 mL/min, UV: 290 nm, column: 7.5 × 300 mm Mesopore. The peak at 13.8 min was assigned to double-helix and 14.6 min random-coil.

Figure S3. HPLC chromatograms of (M)-1 in toluene at 25 °C. (a) (M)-1 in toluene after dissolution. (b) The solution (a) was heated at 95 °C for 1 h, cooled to room temperature, and analyzed immediately. (c) Solution (b) was settled at room temperature for 28 h.

UV-vis analysis (Figure S4)
The above toluene solution of (M)-1 (1 mM) settled for 28 h was diluted to 0.1 mM, and was immediately subjected to CD and UV-vis analyses. Then, the spectra with Δε = –2600 cm⁻¹M⁻¹ at 367 nm are assigned for S-double-helix state, where all the molecules are double-helix.
Crystals of (M)-1 was dissolved in toluene (0.1 mM), and the solution was subjected to CD and UV-vis analyses. The spectra coincided with those of S-double-helix, and it was concluded that all the molecules in crystals are double-helix. It was also determined by DLS that the sample obtained by dissolving crystals in toluene contained dispersed double-helix and not aggregates (Figure S2).
**Figure S4.** CD and UV-vis spectra of (M)-1 (0.1 mM) in toluene at 25 °C. (a) (M)-1 (1 mM) in toluene was heated at 95 °C for 1 h, cooled at room temperature, and settled for 28 h at room temperature. CD spectrum was obtained for the solution diluted to 0.1 mM before analyses. (b) CD spectrum was obtained immediately after dissolution of (M)-1 crystals in toluene.

DLS analysis (Figure S2)
Crystals of (M)-1 were dissolved in toluene (0.1 mM) at 20 °C, and the solution was subjected to DLS analysis, which provided an average diameter 2.4 nm. Refractive index 1.49 and UV-vis absorption 0.00 of this solution at 633 nm were obtained, and were used to transform the DLS intensity distribution to the number distribution by the Mie theory.\(^{[S2]}\)

VPO analysis
A toluene solution of (M)-1 (1 mM) was analyzed immediately after dissolution. Apparent molecular weight (M)-1 by VPO (40 °C) $1.1 \times 10^4$; calculated molecular weight 9902, was obtained.

3. **Equilibrium Crossing**
A toluene solution of (M)-1 (0.1 mM) was heated at 95 °C for 20 min, cooled to 5 °C, and heated to 95 °C at a rate of 1.0 K/min. $\Delta \varepsilon$ value at 367 nm was followed during the cooling and heating process (Figure 3a).

Reproducibility experiment (Figure S5).
In order to examine reproducibility, the same experiments were conducted using different lots of samples.
Figure S5. Δε (367 nm)/temperature profiles of (M)-1 (0.1 mM) in toluene. (a) The solution was heated at 95 °C for 20 min, cooled to 5 °C, and then heated to 95 °C at a rate of 1.0 K/min (Figure 3a). (b) Another sample was used. (c) The same solution with (b) was used. (d) Still other sample was used.

4. Constant-temperature experiment with stop-heating
Experiment 1 (Table S1 and Figure S6)
A toluene solution of (M)-1 (0.1 mM) was heated at 95 °C for 20 min, cooled to 5 °C, and heated to 37 °C at a rate of 1.0 K/min, during which Δε value (367 nm) was followed. Then, the solution was settled at 37 °C for 50 min, during which Δε value changed from –930 to –1020 M⁻¹cm⁻¹. Then, the solution was heated to 42 °C at a rate of 1.0 K/min, and settled for 25 min, during which Δε value changed from –1020 to –1240 M⁻¹cm⁻¹. The solution was heated to following temperatures, and Δε was followed for the time in parentheses: 47 °C (50 min), 52 °C (50 min), 57 °C (20 min), 62 °C (50 min), 67 °C (50 min), 72 °C (50 min), 77 °C (50 min), and 82 °C (50 min).

Table S1. Summary of experiment 1. The initial Δε value, time of settling at the temperature, and increase (upward arrow) or decrease (downward arrow) of Δε at each temperature are shown.
Experiment 2 (Table S2 and Figure S7)
The solution used in Experiment 1 was subjected to the following experiment. The solution was heated at 95 °C for 20 min, cooled to 5 °C, heated to 49 °C at a rate of 1.0 K/min, and settled at 49 °C for 20 min, during which \( \Delta \varepsilon \) was followed. The solution was heated to following temperatures, and \( \Delta \varepsilon \) was followed for the time in parentheses: 54 °C (10 min), 59 °C (10 min), 69 °C (10 min), and 79 °C (10 min).

Table S2. Summary of experiment 2. The initial \( \Delta \varepsilon \) value, time of settling at the temperature, and increase (upward arrow) or decrease (downward arrow) of \( \Delta \varepsilon \) at each temperature are shown.
Figure S7. $\Delta \varepsilon$/temperature profiles of experiment 2.

Experiment 3 (Table S3 and Figure S8)
The solution used in Experiment 2 was subjected to the following experiment. The solution was heated at 95 °C for 20 min, cooled to 5 °C, heated to 49 °C at a rate of 1.0 K/min, and settled at 43 °C for 70 min, during which $\Delta \varepsilon$ was followed. The solution was heated to following temperatures, and $\Delta \varepsilon$ was followed for the time in parenthesis: 55 °C (70 min), 60 °C (70 min), and 70 °C (70 min).

Table 3. Summary of experiment 3. The initial $\Delta \varepsilon$ value, time of settling at the temperature, and increase (upward arrow) and decrease (downward arrow) of $\Delta \varepsilon$ at each temperature are shown.
Figure S8. $\Delta \varepsilon$/temperature profiles of experiment 3.

Figure S9. Summary of constant-temperature experiments and equilibrium curve (black line). Black arrows show the results of the experiments in Figures 4, S12, and S13. The threshold temperature of 50 °C is also shown.

CD analysis (Figure S10)
CD spectrum in Experiments 1-3 was obtained at each temperature, and an isosbestic point at 349 nm was observed.
Experimental and calculated CD spectra (Figure S10)

Experimental CD spectra in experiment 1 at 37 °C, 42 °C, 67 °C, and 77 °C were compared with calculated spectra. Calculated CD spectra were obtained by adding those of S-double-helix (Figure S4a) and S-random-coil (Figure S1 in bromobenzene) in appropriate ratios. The spectra coincided with the experimental spectra.
Figure S11. Comparison of experimental and calculated CD spectra in experiment 1 at 37 °C, 42 °C, 67 °C, and 77 °C. The calculated spectra were obtained by adding spectrum of S-double-helix (Figure S4a) and that of S-random-coil (bromobenzene, Figure S1) in (a) 7:3, (b) 11:9, (c) 7:3, and (d) 9:1 ratio.

5. Constant-temperature experiment
Experiments at 20, 25, and 30 °C (Figure 4)
A toluene solution of (M)-1 (0.1 mM) was heated at 95 °C for 20 min, and cooled to 20 °C at a rate of 1.0 K/min. Then, Δε value was followed for 2 h.
A toluene solution of (M)-1 (0.1 mM) was heated at 95 °C for 20 min, and cooled to 25 °C at a rate of 1.0 K/min. Then, Δε value was followed for 6 h.
A toluene solution of (M)-1 (0.1 mM) was heated at 95 °C for 20 min, and cooled to 30 °C at a rate of 1.0 K/min. Then, Δε value was followed for 9 h.

Experiments at 70 °C (Figure S11)
A toluene solution of \((M)\)-I (0.1 mM) was heated at 95 °C for 20 min, and immediately cooled to 70 °C. Then, \(\Delta\varepsilon\) value was followed for 60 min. The same experiment was also conducted at 80 °C for 60 min.

**Figure S12.** \(\Delta\varepsilon\)/time profile of \((M)\)-I (0.1 mM) in toluene. The solution was heated at 95 °C for 20 min, and immediately cooled to 80 °C (60 min) or 70 °C (60 min). Then, the \(\Delta\varepsilon\) was followed for the time in parentheses.

Experiment at 30 °C from the other side of equilibrium (Figure S12)
A toluene solution of \((M)\)-I (0.1 mM) was prepared by dissolving crystals, and the temperature was immediately adjusted 30 °C. Then, \(\Delta\varepsilon\) value was followed for 11 h.
Figure S13. Δε/time profile of (M)-1 (0.1 mM) after dissolution in toluene at 30 °C.

6. Kinetic analysis of self-catalysis
Self-catalytic reaction in the structure change between the random-coil A and double-helix B is expressed as follows. $v_{+1}$ and $v_{-1}$ are the reaction rates for the forward and backward reactions.

$$2A + B \xrightleftharpoons{v_{+1}}{v_{-1}} 2B$$  (1)

The reaction rate $v$ of self-catalytic reaction between random-coil A and double-helix B is shown by

$$v = v_{+1} - v_{-1} = k_{+1}[A]^2[B] - k_{-1}[B]^2$$

where $k_{+1}$, $k_{-1}$, [A], and [B] are forward reaction rate constant, backward rate constant, concentration of A, and concentration of B, respectively. Initial concentration of A is defined $[A]_0$

$$[A]_0 = [A] + 2[B].$$

It is used to substitute [B] giving

$$v = k_{+1}[A]^2[B] - k_{-1}[B]^2 = 1/2([A]_0 - [A])(k_{+1}[A]^2 + (k_{-1}/2)[A] - (k_{-1}/2)[A]_0)$$

$$= -(k_{-1}/2)[A]^3 + (k_{+1}[A]_0/2 - k_{-1}/4)[A]^2 + (k_{-1}/2)[A]_0[A] - (k_{-1}/4)[A]_0^2.$$  

The cubic curve is shown in the $v/[A]$ profiles by blue line (Figure 5), where $v = 0$ at $[A] = [A]_0$ and at

$$[A] = (1/2k_{+1})\{-k_{-1}/2 + (k_{-1}^2/4 + 2k_{+1}k_{-1}[A]_0)^{1/2}\}.$$  

A maximal rate $v_{\text{max}}$ is obtained by the following differential equation

$$\frac{dv_{+1}}{d[A]} = -3k_{+1}/2[A]^2 + (k_{+1}[A]_0 - k_{-1}/2)[A] - (k_{-1}/2)[A]_0 = 0$$

at the concentration $[A]_{\text{max}}$

$$v_{\text{max}} = v_{+1\text{max}} - v_{-1\text{max}} = k_{+1}[A]_{\text{max}}^2[B]_{\text{max}} - k_{-1}[B]_{\text{max}}^2$$

$$[A]_{\text{max}} = (1/3k_{+1})\{k_{+1}[A]_0 - k_{-1}/2 + ((k_{+1}[A]_0 - k_{-1}/2)^2 - 3k_{+1}k_{-1}[A]_0)^{1/2}\}.$$  

Substitution with $2[B]_{\text{max}} + [A]_{\text{max}} = [A]_0$ gives
\( v_{\text{max}} = \frac{1}{2}([A]_0 - [A]_{\text{max}})(k_{+1}[A]_{\text{max}}^2 + (k_{-1}/2)[A]_0[A]_{\text{max}} - (k_{-1}/2)[A]_0). \)

The rate \( v_{\text{max}} \) is separated to \( v_{+1\text{max}} \) and \( v_{-1\text{max}} \), which are rates of the forward and backward reaction at \([A]_{\text{max}}\), respectively, and are exhibited by

\[
\begin{align*}
v_{+1\text{max}} &= k_{+1}[A]_{\text{max}}^2[B]_{\text{max}} \\
v_{-1\text{max}} &= k_{-1}[B]_{\text{max}}^2.
\end{align*}
\]

A numerical analysis is conducted assuming \( k_{+1} = 10^8 \text{ M}^{-2}\text{sec}^{-1} \), \( k_{-1} = 10^2 \text{ M}^{-1}\text{sec}^{-1} \), and \([A]_0 = 10^{-4} \text{ M} \).

\([A]_{\text{max}} = 0.66 \times 10^{-4} \text{ M} \) and \([B]_{\text{max}} = 0.17 \times 10^{-4} \text{ M} \)

\( v_{\text{max}} = 0.0698 \times 10^{-4} \text{ Msec}^{-1} \)

\( v_{+1\text{max}} = 0.070 \times 10^{-4} \text{ Msec}^{-1} \)

\( v_{-1\text{max}} = 0.0002 \times 10^{-4} \text{ Msec}^{-1} \).

Then, the rates \( v_{+1\text{max}} \) and \( v_{-1\text{max}} \) at \([A]_{\text{max}}\) are obtained at the 33\% conversion, where the forward reaction predominates.

\( v_{+1\text{max}}/v_{-1\text{max}} = 350. \)

The initial rates \( v_{+1\text{init}} \) and \( v_{-1\text{init}} \) are calculated at the 1\% conversion, \([A] = 0.99 \times 10^{-4} \text{ M} \) and \([B] = 0.005 \times 10^{-4} \text{ M} \) giving

\( v_{+1\text{init}} = 0.01 \times 10^{-4} \text{ Msec}^{-1} \)

\( v_{-1\text{init}} = 0.000025 \times 10^{-4} \text{ Msec}^{-1} \).

The self-catalysis (reaction 1) provides the rate \( v_{+1\text{max}} \) at the 33\% conversion of \([A] \) to be 7 times larger than the rate \( v_{+1\text{init}} \) at the 1\% conversion.

\( v_{+1\text{max}}/v_{+1\text{init}} = 7.0 \)
Figure S14. Kinetic model for self-catalysis, which is expressed using $v/[A]$ profiles. As the reaction proceeds with the decrease in $[A]$ from $[A]_0$, the reaction rate $v$ increases, providing a maximum rate $v_{\text{max}}$ at the concentration $[A]_{\text{max}}$, which is near the point of equilibrium crossing. $[A]_0$ is the initial concentration of $A$ expressed by $[A]_0 = [A] + 2[B]$.

7. An energetic model

Approximate positions of the states I to V (Figure S13) noted in the mechanistic model (Figure 6) are shown in the hysteresis curve (Figure 3a).

Figure S15. $\Delta\varepsilon$/temperature profiles of equilibrium crossing containing approximate states I to IV.

References