“Inverse” thermoresponse: heat-induced double-helix formation of an ethynylhelicene oligomer with tri(ethylene glycol) termini†

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An ethynylhelicene oligomer [$(M)\cdot d\cdot 4\cdot C_{12}-TEG$] with six triethylene glycol (TEG) groups at the termini was synthesized, and double-helix formation was studied using CD, UV-Vis, vapor pressure osmometry, dynamic light scattering, and $^1$H NMR. [$(M)\cdot d\cdot 4\cdot C_{12}-TEG$] reversibly changed its structure between a double helix and a random coil in response to heating and cooling in aromatic solvents, non-aromatic polar organic solvents, and aqueous solvent mixtures of acetone/water/triethylamine. Notably, [$(M)\cdot d\cdot 4\cdot C_{12}-TEG$] in acetone/water/triethylamine (1/2/1) formed a double helix upon heating and disaggregated into random coils upon cooling. The double helix/random coil ratio sharply changed in response to temperature changes. This is an unprecedented “inverse” thermoresponse, which is opposite to the “ordinary” thermoresponse in molecular dimeric aggregate formation. This phenomenon was explained by the dehydration of the terminal TEG groups and the formation of condensed triethylamine domains upon heating.

In contrast, molecules that exhibit the “inverse” thermoresponse in dimeric aggregation are also conceivable, for which heating induces aggregation and cooling induces disaggregation. Hypothetically, this phenomenon can occur in an endothermic process with a positive enthalpy change $\Delta H > 0$, and the process is accompanied by an increase in freedom in molecular mobility with a positive entropy change $\Delta S > 0$. Consequently, the equilibrium moves toward aggregation to form $A_2$ upon heating according to the equations $\Delta G = -RT \ln K$ and $R \ln K = -\Delta H/T + \Delta S$ (Fig. 1a), because of $-\Delta H/T < 0$. However, such a thermoresponse is counter-intuitive. The dimeric aggregation of synthetic molecules exhibiting the “inverse” thermoresponse, being a molecular-level phenomenon, has essentially not been observed.†

The “ordinary” and “inverse” thermoresponses are complementary, and the development of the latter will largely broaden the use of thermoresponsive materials. Here, we report the synthesis of [$(M)\cdot d\cdot 4\cdot C_{12}-TEG$], which is an ethynylhelicene tetramer with triethylene glycol (TEG) groups at its termini (Scheme 1), and it has a notable “inverse” thermoresponse: [$(M)\cdot d\cdot 4\cdot C_{12}-TEG$] aggregated to form a double helix upon heating and disaggregated to give a random coil upon cooling in an aqueous solvent mixture of acetone/water/triethylamine. A sharp transition between the double helix and the random coil occurred due to temperature changes.

Results and discussion

Our previous studies showed that $(M)\cdot d\cdot n$ (ref. 5) exhibited the “ordinary” thermoresponse. [$(M)\cdot d\cdot 4\cdot C_{12}-TEG$], with six TEG...
groups at both termini, was designed in this study to examine aggregation behavior in aqueous solvents. Compound 4 with a TEG moiety was obtained from benzoic acid 1 (ref. 8) in 2 steps, which was then connected to the termini of the ethynylhelicene tetramer \((M)-D-4H\) by Sonogashira coupling (Scheme 1).

\([([M]-D-4)]\)-C\(_{12}\)-TEG showed the “ordinary” thermoresponse in organic solvents; it formed double helices upon cooling and disaggregated into random coils upon heating. Solutions of \([([M]-n-4)]\)-C\(_{12}\)-TEG were heated at 60 °C or 40 °C for disaggregation then cooled, and their circular dichroism (CD) and UV-Vis spectra were obtained. The CD spectra in chloroform (5 × 10\(^{-4}\) M) at 40, 25, and 5 °C showed weak Cotton effects mirror-imaged to the typical random-coil state of \((P)\)-ethynylhelicene tetracene (ESI Fig. S1a†). On the other hand, \([([M]-D-4)]\)-C\(_{12}\)-TEG showed an intense CD as well as a hypochromic shift in the UV-Vis spectrum in trifluoromethylbenzene (1 × 10\(^{-3}\) M) upon cooling to 5 and −10 °C (ESI Fig. S1b†). The apparent molecular weight of the double helix obtained by vapor pressure osmometry (VPO) studies (trifluoromethylbenzene, 40 °C) above 1 × 10\(^{-3}\) M was

![Scheme 1](image_url)

**Scheme 1** Synthesis of \([([M]-D-4)]\)-C\(_{12}\)-TEG.
twice as large as the calculated molecular weight of [(M)-d-4]-C₁₂-TEG (3726.7) [ESI Fig. S2 and Table S2†]. The results indicated the formation of double helices of [(M)-d-4]-C₁₂-TEG in trifluoromethane. The reversible structural changes between random coils and double helices was examined for \( \Delta \alpha \) at 360 nm (trifluoromethane benzene) by repeating the cycle of heating to 60 °C and cooling to 5 °C [ESI Fig. S4]. \( \Delta \alpha_{360} \) increased upon heating and decreased upon cooling; the “ordinary” thermoresponse was observed in the aromatic solvent.

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[(M)-d-4]-C₁₂-TEG
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was soluble in non-aromatic polar solvents such as acetone and ethyl acetate even at a concentration of \( 1 \times 10^{-3} \text{ M} \), at which conventional ethynylhelicene oligomers without TEG moieties were not soluble. The “ordinary” thermoresponse was observed in the polar solvents as well as in the aromatic solvent. A solution of [(M)-d-4]-C₁₂-TEG in acetone \( (1 \times 10^{-5} \text{ M}) \) showed an increase in the CD intensity and a hypochromic shift in the UV-Vis spectrum upon cooling from 40 to -10 °C (Fig. 2), which indicated the formation of double helices upon cooling. Dimeric aggregate formation in acetone was confirmed by VPO studies (acetone, 45 °C, above \( 4 \times 10^{-3} \text{ M} \) [ESI Fig. S3 and Table S3†]). The CD analysis at a low concentration of \( 1 \times 10^{-5} \text{ M} \) (−10 °C) and at high concentrations of \( 5 \times 10^{-4} \text{ M} \) and \( 1 \times 10^{-3} \text{ M} \) (5 °C) converged on the same spectrum with the \( \Delta \alpha \) of \(-1.1 \times 10^{3} \text{ cm}^{-1} \text{ M}^{-1} \) at a wavelength of 360 nm [ESI Fig. S4†]. It indicated that the spectrum was that of the equilibrium-shifted state to double-helices containing practically no random coils in the solution, namely S-double-helix state. The average diameter determined by dynamic light scattering (DLS) in acetone \( (1 \times 10^{-3} \text{ M}) \) at 5 °C was 5.2 nm [ESI Fig. S5a†], which was consistent with the VPO result showing dimeric aggregate formation, not polymolecular aggregate formation. At a lower concentration \( (1 \times 10^{-4} \text{ M}) \), particles of 3.8 nm and 0.76 nm in diameter were observed at 5 °C, which corresponded to double helices and random coils, respectively (ESI Fig. S5b†). The increase of larger particles and the decrease of smaller particles upon cooling from 25 and 40 °C are consistent with the formation of bimolecular double helices from monomeric random coils. In ethyl acetate, the “ordinary” thermoresponse was also observed in the CD and UV-Vis spectra (ethyl acetate, \( 1.0 \times 10^{-3} \text{ M} \) [ESI Fig. S6†]).

Notably, the “inverse” thermoresponse of [(M)-d-4]-C₁₂-TEG was observed in aqueous solvents. In a mixed solvent of acetone/water/triethylamine \((1/2/1, \text{v/v/v})\), intense Cotton effects \( (1 \times 10^{-5} \text{ M}) \) were observed at 40 °C (Fig. 3a), which coincided with that for the S-double-helix state in acetone [ESI Fig. S7†]. Upon cooling, UV-Vis absorption increased and CD intensity decreased, which indicated disaggregation (Fig. 3a). An isosbestic point at 350 nm indicated an equilibrium between two states, the double helix and the random coil. The average diameters obtained by DLS in acetone/water/triethylamine \((1/2/1, 1 \times 10^{-5} \text{ M})\) were 3.7 nm, 1.7 nm, and 1.1 nm at 40 °C, 25 °C, and 5 °C, respectively (Fig. 3b). The diameter at 40 °C coincided

![Fig. 2](image1.png)

**Fig. 2** CD (top) and UV-Vis (bottom) spectra of [(M)-d-4]-C₁₂-TEG in acetone \( (1 \times 10^{-5} \text{ M}) \) at different temperatures. Arrows show the changes upon cooling.

![Fig. 3](image2.png)

**Fig. 3** (a) CD (top) and UV-Vis (bottom) spectra and (b) number average size distributions of [(M)-d-4]-C₁₂-TEG in acetone/water/triethylamine \((1/2/1, 1 \times 10^{-5} \text{ M})\) determined by DLS at different temperatures. Arrows show the changes upon cooling.
with that of the double helix in acetonitrile (ESI Fig. S5a†), for which dimeric aggregate formation was confirmed by VPO (ESI Fig. S3f). The decrease in the diameter upon cooling is consistent with the disaggregation from a bimolecular double helix to a monomeric state. Thus, the “inverse” thermoresponsive behavior was observed for [(M)-4]-C12-TEG in acetone/water/triethylamine (1/2/1): the double helix was formed at 40 °C and the random coil at 5 °C. In addition, the “inverse” thermoresponsive behavior in this system was confirmed to be a molecular-level phenomenon in the dispersed state by DLS analysis; it was not caused by polymer aggregation. The spectra reversibly changed in response to changing temperature in a manner opposite to the “ordinary” thermoresponsive behavior (trifluoromethylbenzene, 1 × 10⁻³ M) (Fig. 4).

The “inverse” thermoresponsive behavior was also observed at different concentrations. Temperature-dependent changes in CD and UV-Vis spectra similar to those at 1 × 10⁻⁵ M were observed at 5 × 10⁻⁶ M and 1.5 × 10⁻⁵ M (Fig. S8a and b†). It is notable that dimeric aggregation occurred at these low concentrations. DLS analyses at these concentrations showed that the size of the aggregates was similar to those at 1 × 10⁻⁵ M (Fig. S9a and b†), and the “inverse” thermoresponsive behavior at the molecular level was confirmed. The “inverse” thermoresponsive behavior was observed at a higher concentration such as 3 × 10⁻⁵ M as well, although polymolecular aggregates partially formed below 20 °C, as indicated by CD and DLS (ESI Fig S8c and S9c†).

The CD spectra in acetone/water/triethylamine (1/2/1, 1 × 10⁻⁵ M) at 10°C and 5°C coincide, which show a convergence to a spectrum with a Δr of −3.1 × 10⁻² cm⁻¹ M⁻¹ at 360 nm. The CD spectra at different concentrations, 5 × 10⁻⁶ M and 1.5 × 10⁻⁵ M, also converge to the same spectra at 5°C (ESI Fig. S8a and b†). In the following discussions, the spectrum in acetone/water/triethylamine (1/2/1, 1 × 10⁻⁵ M, 5°C) with a Δr of −3.1 × 10⁻² cm⁻¹ M⁻¹ at 360 nm is defined as the Saq-random-coil state, which is the equilibrium-shifted state to random coils in aqueous media practically containing no double helices. It was noted that the CD spectra of the random-coil state in organic solvents (ESI Fig. S1a†) and the Saq-random-coil state in aqueous solvents (Fig. 3a) are slightly different, which may be due to a specific conformation of TEG moieties in aqueous media.⁹

The “inverse” thermoresponsive behavior also occurred when the amount of acetone was changed to 0.6/2/1 and 0.8/2/1, keeping the concentration of [(M)-4]-C12-TEG at 1 × 10⁻⁵ M (ESI Fig. S10f†).

Note that [(M)-4]-C12-TEG provided an unprecedented example of dimeric aggregation with the “inverse” thermoresponsive behavior, which is in contrast to the other known synthetic double helices that exhibit the “ordinary” thermoresponsive behavior.⁴,⁵

An examination of the solvents revealed the critical roles of water and triethylamine. In a mixed solvent of acetone/triethylamine (3/1, 1 × 10⁻⁵ M), the “ordinary” thermoresponsive behavior was observed: CD and UV-Vis spectra of the random-coil state were obtained at 40°C and 25°C. The spectra changed at 5°C, and those of partial double helices were obtained upon cooling to −10°C (ESI Fig. S11a†). The presence of molecular-level aggregates but not polymolecular aggregates was confirmed by DLS, which showed average diameters from 0.84 to 0.88 nm at 25°C (ESI Fig. S12†). In acetone/water (3/1, 1 × 10⁻⁵ M), the system was opaque, spectra of the random-coil state were obtained by CD at 40, 25, 5, and −10°C (Fig. S11b,§ which may be due to the formation of polymolecular aggregates of random coils. The results indicated that both water and triethylamine are necessary for the “inverse” thermoresponsive behavior of [(M)-4]-C12-TEG.

Variable-temperature ¹H NMR studies (acetone-d₆/D₂O/triethylamine-d₁₅, 1/2/1, 2 × 10⁻⁴ M) were conducted to obtain insight into the thermoresponsive behavior of [(M)-4]-C₁₂-TEG in aqueous solvents. Broad proton signals of the terminal TEG groups were observed between δ 2.4–3.5 at 25°C (ESI Fig. S13†). When the temperature was increased to 40°C, the signals of the TEG groups became sharper and increased in intensity. An upfield shift of the chemical shifts of HD₅ signals was also observed. These results are consistent with the reported hydration/dehydration of poly(ethylene glycol) (PEG) groups.⁸⁻¹¹

Thermodynamic parameters of the double-helix formation with the “inverse” thermoresponsive behavior were experimentally determined using equilibrium constants K (Table S4†) obtained from the CD Δr values at 360 nm in acetone/water/triethylamine (1/2/1, 1 × 10⁻⁵ M), ΔH = +2.4 × 10⁻² kJ mol⁻¹ and ΔS = +9.2 × 10⁻² J mol⁻¹ K⁻¹ (ESI Fig. S14†). Note that both ΔH and ΔS are positive and large. This contrasted with the “ordinary” thermoresponsive behavior of the dimeric aggregation of molecules giving negative ΔH and ΔS values, including conventional ethynylhelicene oligomers in organic solvents.²⁶ The positive ΔH and ΔS consequently induced an increase in the dimerization constant K upon heating according to the equation R ln K = - ΔH/T + ΔS, which appeared as the “inverse” thermoresponsive behavior (Fig. 1b). The result validated the hypothesized discussion in the introduction.

A significant change in the double helix/random coil ratio was observed in response to small temperature changes (Table S4†). For example, the double helix/random coil ratios in acetone/water/triethylamine (1/2/1, 1 × 10⁻⁵ M) at 40°C and 10°C were estimated to be 90%/10% and 3%/97%, respectively. The large ΔH resulted in a substantial “inverse” thermoresponsive behavior.
The positive $\Delta H$ and $\Delta S$ of the “inverse” thermoresponse are counter intuitive in the dimeric molecular aggregation but can be explained by the hydration/dehydration of the PEG moiety. Dehydration upon heating makes the PEG moiety hydrophobic, which reduces the thermodynamic stability of random coils in aqueous solvents ($\Delta H > 0$) and promotes dimeric aggregation. When the PEG moieties are dehydrated, $\Delta S$ increases and overcomes the decrease in $\Delta S$ resulting from dimeric aggregation. It is known that PEG and oligo(ethylene glycol) (OEG) are hydrated in water below the temperature defined as the lower critical solution temperature (LCST), and that dehydration upon heating enhances hydrophobic interactions. Then, polymolecular aggregation and precipitation occur to reduce the molecular surface area exposed to water.11-14 Similar heat-induced aggregation15 and self-assembly16 related to the hydration/dehydration of biological peptide and protein molecules have also been reported.

It should be noted here that [(M)-d-4]-C_{12}-TEG formed dimeric aggregates upon heating, not polymolecular aggregates, which is another unusual aspect of the “inverse” thermoresponse phenomenon in this system. Such dimeric aggregate formation of synthetic molecules has not been reported. The result reminds us of peptides and enzymes,15,17 which control their activities by forming dimeric aggregates upon heating and disaggregating to monomers upon cooling. In our system, triethylamine is considered to play a crucial role in the formation of dimeric aggregates of [(M)-d-4]-C_{12}-TEG. Triethylamine and water are known to form hydrogen-bonds at low temperatures, and microscopic phase separation occurs upon heating.18 The resulting triethylamine domains incorporate dehydrated [(M)-d-4]-C_{12}-TEG molecules (Fig. 5). Fewer polar environments made by organic solvents can promote the dimeric aggregate formation of ethynylhelicene oligomer moieties, which is enthalpically driven by $\pi-\pi$ interactions, and does not induce polymolecular aggregation as a result of the hydrophobic interactions. It should be emphasized again that the “inverse” thermoresponse in dimeric aggregate formation shown in this study is a molecular-level phenomenon in the dispersed solution state and is different from the phenomenon in which polymolecular aggregates are formed by hydrophobic interactions above the LCST.

**Materials and methods**

**Synthesis of [(M)-d-4]-C_{12}-TEG**

Under an argon atmosphere, a mixture of 4 (45.0 mg, 0.0451 mmol), tris(dibenzyldieneacetone)dipalladium(0) chloroform adduct (0.82 mg, 0.753 $\mu$mol), cuprous iodide (1.72 mg, 9.03 $\mu$mol), tris[2,4,6-trimethylphenyl]phosphine (1.75 mg, 4.52 $\mu$mol), triphenylphosphine (1.18 mg, 4.52 $\mu$mol), tetrabutyrammonium iodide (22.2 mg, 0.0602 mmol), triethylamine (0.1 mL) and N,N-dimethylformamide (1.0 mL) was freeze-evacuated four times in flask A. In flask B, a mixture of ethynylhelicene tetramer [(M)-d-4H^+] (30.0 mg, 0.0151 mmol) in THF (1.0 mL) was freeze-evacuated four times, and the mixture was slowly added to flask A. The mixture was stirred at 45 °C for 20 min. The reaction was quenched by adding saturated aqueous ammonium chloride, and the organic materials were extracted with ethyl acetate. The organic layer was washed with brine, and dried over sodium sulfate. The solvents were evaporated under reduced pressure, and separation by silica gel chromatography and recycling GPC gave [(M)-d-4]-C_{12}-TEG as a yellow amber solid (42.7 mg, 0.0115 mmol, 72%). M_p: 59-61 °C (chloroform); [z]_D^27 = -1631 (c 0.37, trifluoromethylenbenzene); $^1$H NMR (400 MHz, CDCl3): $\delta$ 0.86 (9H, t, J = 6.8 Hz), 1.25–1.52 (74H, m), 1.72–1.89 (14H, m), 1.97 (12H, s), 2.00 (12H, s), 3.37 (18H, s), 3.52–3.55 (12H, m), 3.62–3.68 (24H, m), 3.70–3.75 (12H, m), 3.80 (4H, t, J = 5.2 Hz), 3.87 (8H, t, J = 5.0 Hz), 4.01 (4H, t, J = 6.6 Hz), 4.18–4.23 (12H, m), 4.28 (4H, t, J = 6.8 Hz), 4.41–4.45 (6H, m), 6.94 (4H, dt, J = 8.8, 1.8 Hz), 7.29 (4H, s), 7.46–7.52 (8H, m), 7.64 (4H, dt, J = 8.8, 1.8 Hz), 7.66–7.77 (8H, m), 8.06 (2H, s), 8.12 (2H, s), 8.16 (4H, s), 8.21 (2H, t, J = 1.6 Hz) 8.22 (1H, t, J = 1.6 Hz), 8.36–8.38 (6H, m), 8.52–8.58 (8H, m); $^{13}$C NMR (100 MHz, CDCl3): $\delta$ 14.1, 22.6, 23.2, 25.96, 26.02, 28.7, 29.2, 29.3, 29.4, 29.5, 29.6, 31.8, 58,97, 59.00, 65.2, 65.8, 68.1, 68.8, 69.0, 70.49, 70.52, 70.6, 70.8, 71.9, 72.4, 86.2, 89.2, 89.3, 89.4, 92.8, 92.97, 92.99, 95.1, 109.0, 114.6, 115.1, 119.6, 119.8, 119.9, 119.8, 120.9, 123.5, 123.6, 123.7, 124.2, 124.2, 124.3, 123.5, 126.2, 126.7, 126.8, 127.0, 128.82, 128.84, 129.1, 129.2, 129.3, 129.8, 129.88, 129.92, 130.9, 131.01, 131.03, 131.2, 131.4, 132.0, 132.2, 132.4, 133.2, 136.76, 136.80, 136.9, 138.3, 142.5, 152.2, 159.4, 165.4, 166.1; IR (KBr): 2924, 1717, 1244, 1111 cm$^{-1}$; UV-Vis (S-random-coil state: CHCl3, $\lambda_{max}$ (3.1 x 10$^5$ cm$^{-1}$ M$^{-1}$); UV-Vis (S-double-helix state: acetone, 1 x 10$^{-3}$ M, 5 °C); $\lambda_{max}$ (340 nm (2.0 x 10$^5$ cm$^{-1}$ M$^{-1}$); CD (S-random-coil state: CHCl3, 5 x 10$^{-4}$ M, 40 °C): $\lambda (\Delta \epsilon)$ 296 nm (+51 cm$^{-1}$ M$^{-1}$), 341 nm (76 cm$^{-1}$ M$^{-1}$), 389 nm (+189 cm$^{-1}$ M$^{-1}$); CD (S-double-helix state: acetone, 1 x 10$^{-3}$ M, 5 °C): $\lambda (\Delta \epsilon)$ 325 nm (+679 cm$^{-1}$ M$^{-1}$), 362 nm (−113 cm$^{-1}$ M$^{-1}$); MALDI-TOF MS (m/z): [M + Na]$^+$ for C$_{239}$H$_{278}$O$_{36}$Na, 3747.0; found, 3746.3; [M + K]$^+$ for C$_{239}$H$_{278}$O$_{36}$K, 3763.0; found, 3763.0; analysis (calcd, found for C$_{239}$H$_{278}$O$_{36}$Na): C (77.03, 76.90), H (7.52, 7.54).

**Conclusions**

In summary, [(M)-d-4]-C$_{12}$-TEG, an ethynylhelicene oligomer with six tri(ethylene glycol) moieties at its termini, was synthesized. [(M)-d-4]-C$_{12}$-TEG formed double helices in aromatic...
solvents, polar non-aromatic solvents, and the aqueous solution of acetone/water/triethylamine. \([M\{\pi\alpha\}C_{12}\text{-TEG}}\) exhibited the “inverse” thermoresponsive in acetone/water/triethylamine (1/2/1); \([M\{\pi\alpha\}C_{12}\text{-TEG}}\) aggregated and formed double helices upon heating and disaggregated to random coils upon cooling. The double helix/random coil ratio sharply and reversibly changed in response to thermal stimuli. This is an unprecedented molecular-level “inverse” thermoresponsive, in which dimeric aggregates but not polymolecular aggregates are formed. Positive and large \(\Delta H\) and \(\Delta S\) values in the aggregation process were determined, which were explained by the dehydration of terminal TEG groups upon heating and the formation of triethylamine domains that promoted double-helix formation by \(\pi-\pi\) interactions.

**Note added after first publication**

This article replaces the version published on 22nd February 2016, which contained errors in the grant numbers reported in the acknowledgements section.

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**Notes and references**


7 The “inverse” thermoresponse of a synthetic molecule was suggested by the shift of \(^1\)H NMR signals, but the ratio of monomer and dimer was not clarified: A. Brück and K. V. Kilway, *Tetrahedron*, 2001, 57, 7263–7268. In ref. 3a, only one molecule gave a positive \(\Delta H\) in a very limited temperature range.


