CHEMISTRY
A EUROPEAN JOURNAL

FULL PAPER

DOI: 10.1002/chem.201201929

Multiple States of Dimeric Aggregates Formed by (Amido–ethynyl)helicene Bidomain Compound and (Amido–ethynyl–amido)helicene Tridomain Compound

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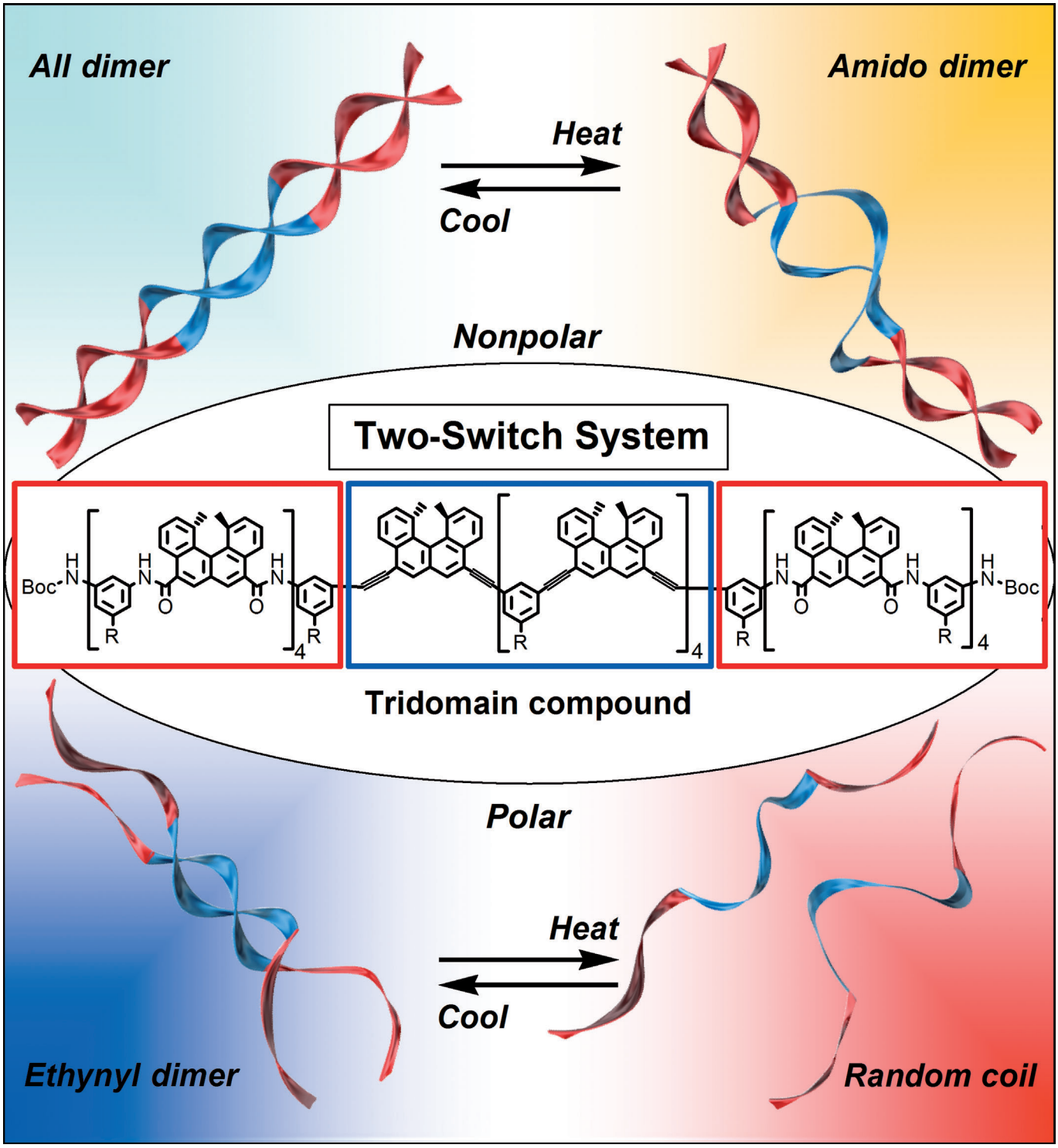

Abstract: An (amido–ethynyl)helicene bidomain compound and an (amido–ethynyl–amido)helicene tridomain compound were synthesized. The multidomain compounds were designed on the basis of previous findings that amido and ethynyl oligomers form dimeric aggregates with properties orthogonal to each other. Four aggregate states of multidomain compounds, namely, all-dimer, amido-dimer, ethynyl-dimer, and random-coil states, were obtained in different solvents, which were analyzed by circular dichroism (CD), UV/Vis, ^{1}H NMR, and IR spectroscopy; vapor pressure osmometry (VPO); dynamic light scattering (DLS); and atomic force microscopy (AFM). The amido and ethynyl domains independently aggregated and disaggregated in a two-state manner. Reversible structural changes occurred for a tridomain compound between the ethynyl-dimer/random-coil state and the all-dimer/amido-dimer state with heating and cooling. Two structural change processes with different properties were obtained using a single compound.

Keywords: aggregation · helical structures · independent structure change · molecular switching · multidomain structures

Introduction

Proteins are organic macromolecules constructed by a linear and sequential connection of amino acids; they fold into programmed three-dimensional structures.[1] Folded proteins contain domains with different structures and properties such as α-helices and β-sheets. Each domain plays important roles in maintaining the three-dimensional structures of proteins and in exhibiting functions such as protein–protein interactions or interactions with small molecules. It is therefore critical to understand the roles of all domains and their combinations in biological functions. Protein folding, however, is a complex process, and its analysis is not straightforward. It is therefore interesting to examine synthetic multidomain compounds with well-defined structures and properties to understand how the combinations of domains can affect the bulk properties of macromolecules. In addition, such multidomain compounds can exhibit functions not observed in single-domain compounds.

Synthetic bidomain compounds with oligomer structures form layers,[2] particles,[3] fibers,[4] and gels,[5] which employ conjugates of peptide/peptide, alkylcarboxylate/carbohydrate, carbohydrate/polymer, alkylcarboxyamide/peptide, and peptide/polymer. In such cases one of the domains has been used as a platform, and the other domain has shown different aggregate properties from the corresponding single-domain compounds. We noted that bidomain compounds, in principle, have four structure states, as each domain can contribute two states. When each domain aggregates and disaggregates independently, multiple molecular-switch systems, such as proteins that respond to external stimuli, can be obtained. Such synthetic compounds, however, are not easily obtained, because the structure at each domain must be well-defined and not perturbed by other domains. Partially cooperatively, various degrees of aggregation, intermediate formation in structural changes, and other undetermined factors complicate structural analysis.

Dimeric aggregate formation, particularly double-helix formation is considered attractive for the development of such well-defined multidomain compounds. This is because double helix formation occurs without forming higher aggregates (selective dimerization). Note that the aggregation and disaggregation of a double helix can occur in a two-state manner, in which no partly aggregated molecular intermediates are formed (two-state structural change). These properties make the design and analysis of double-helix-forming multidomain compounds straightforward. In addition, the structural changes of dimeric aggregates have the advantages of being 1) large, reaching several nanometers; 2) sensitive to the environment; 3) detectable by various methods; and 4) reversible. No such synthetic compounds with a monodispersed multidomain, capable of forming dimeric aggregates at each domain, were examined before.

We previously synthesized oligomers containing optically active helicene and *m*-phenylene that form a double helix in organic solvents. They disaggregate to a random coil with heating,[6] and their double-helix stability is affected by the hardness and softness of aromatic solvents. Amidohelicene oligomers, in which the helicene and *m*-phenylene are connected by an amide linkage, also form dimeric aggregates. They disaggregate in hydrogen-bond-breaking solvents such as 2,2,2-trifluoroethanol or dimethylsulfoxide (DMSO).[7] The stability of helix dimers, however, is not affected by temperature changes. Two helix-dimer-forming compounds that show properties orthogonal to each other were obtained by changing the two-atom linkage from amido to ethynyl groups. The differences were attributed to the different driving forces for the aggregation, π–π interactions in ethynylhelicene oligomers, and hydrogen bonding in amidohelicene oligomers. Also note that these compounds do not form higher aggregates, and aggregate/disaggregate in a two-state manner.

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201201929.

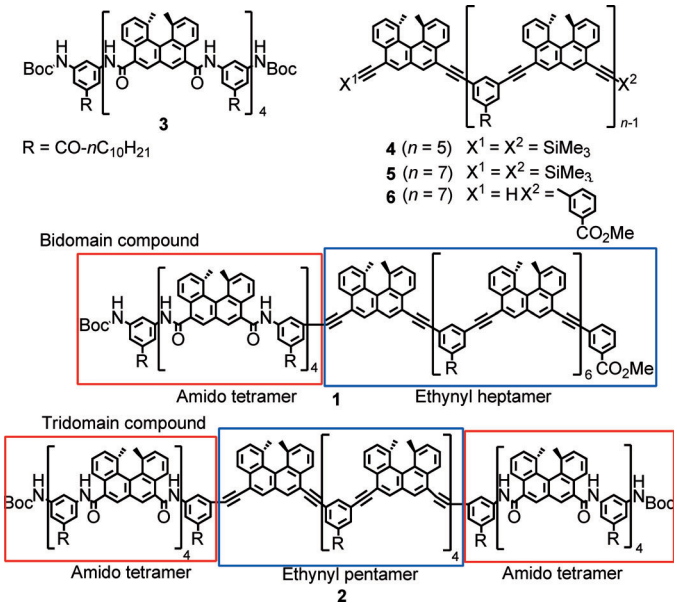


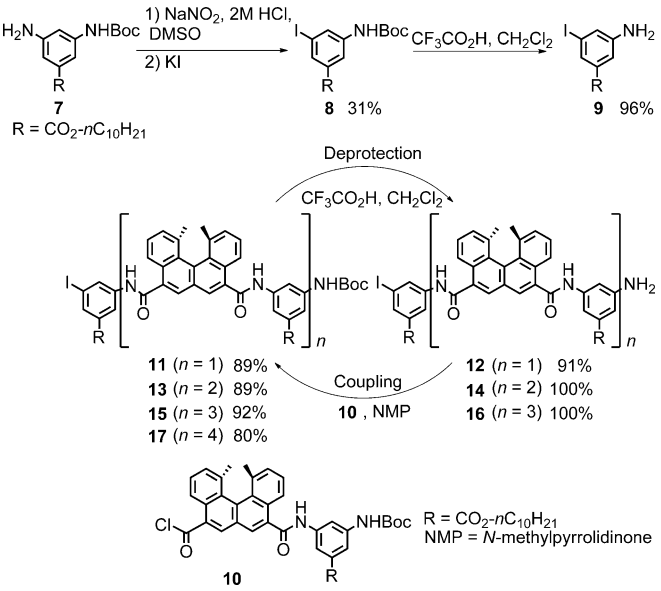
Figure 1. Molecular structure of bidomain **1**, tridomain **2**, amidohelicene oligomer **3**, and ethynylhelicene oligomers **4**–**6**.

In this study, we examined multidomain compounds containing an amido and an ethynyl domain (Figure 1). The bidomain compound **1** was synthesized by the coupling of an amidohelicene tetramer and an ethynylhelicene heptamer, and the tridomain compound **2** was obtained by the coupling of two amidohelicene tetramers and an ethynylhelicene pentamer. Both **1** and **2** formed four well-defined aggregate states, namely, all-dimer, amido-dimer, ethynyl-dimer, and random-coil states. In the all-dimer state, both the amido and ethynyl domains formed dimeric aggregates. In the amido-dimer state, only the amido domain formed dimeric aggregate. In the ethynyl-dimer state, only the ethynyl domain formed dimeric aggregate. The random-coil state was monomeric. Four aggregate states were obtained in different solvents, which were analyzed by circular dichroism (CD), UV/Vis, ^{1}H NMR, and IR spectroscopy; vapor pressure osmometry (VPO); dynamic light scattering (DLS); and atomic force microscopy (AFM). In these multidomain compounds, the amido and ethynyl domains independently aggregated and disaggregated. On the basis of the results of these studies, reversible structure switching between the ethynyl-dimer/random-coil state and the all-dimer/amido-dimer state were conducted for **2**. Two different thermal responses appeared in nonpolar and polar solvents using the single compound **2**, which originated from the well-defined aggregation and disaggregation properties of **2** at the amido domain.

Results and Discussion

Design and synthesis of multidomain compounds: On the basis of previous observations that the ethynylhelicene heptamer **5** and the amidohelicene tetramer **3** formed helix dimers with orthogonal properties,[6,7] the bidomain compound **1** containing the ethynyl heptamer and amido tetramer domains was designed. The tridomain compound **2** with an ethynyl pentamer domain at the central part and an amido tetramer domain at both terminals was also designed. The ethynyl pentamer domain in **2** should aggregate more weakly than the ethynyl heptamer domain in **1**. The design, however, had the advantage of the amido tetramer and ethynyl pentamer domains exhibit comparable Cotton effects with regard intensity, which facilitated the CD analysis of different aggregate states of **2**. It was also considered interesting to induce thermal structural changes of **2** at the central ethynyl pentamer domain without inducing a change at the terminal amido domain.

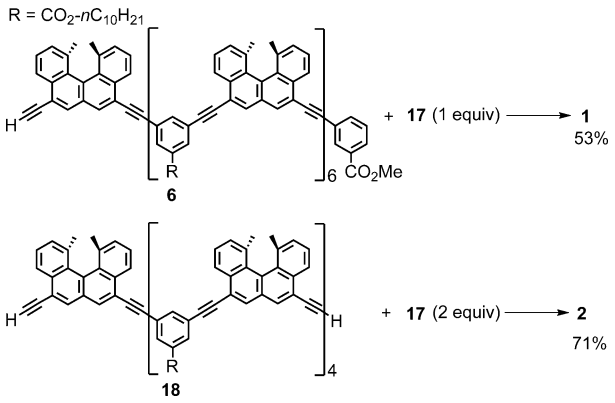
Compounds **1** and **2** were synthesized by the Sonogashira coupling of an amidohelicene oligomer and ethynylhelicene oligomers. The synthesis of the iodo-terminated amidohelicene tetramer **17** was conducted by a one-directional method starting from iodobenzene **9**,[8] which in turn was synthesized from the amine **7**.[7] Compound **9** was subjected to a sequence of couplings with the acid chloride **10** followed by deprotection, giving **17** (Scheme 1).



Scheme 1. Synthesis of iodo-terminated amidohelicene oligomers **11**–**17**.

Then, **1** was obtained by the coupling of an equimolar amount of **17** and the ethynylhelicene heptamer **6**[6] in 53% yield. Polymer **2** was synthesized by the coupling of the dialkyne **18**[6] and two equivalents of **17** in 71% yield (Scheme 2).

The four aggregate states of bidomain compound 1: The bidomain compound **1** formed four aggregate states, that is, all-dimer, amido-dimer, ethynyl-dimer, and random-coil states, in different solvents (Figure 2). In this study, conditions in which the equilibrium was shifted for each structure were explored. For example, the all-dimer state containing



Scheme 2. Synthesis of bidomain compound **1** and tridomain compound **2**.

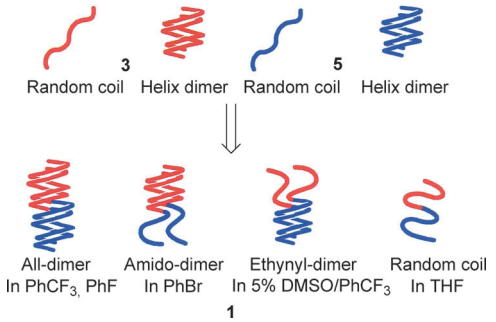


Figure 2. Four aggregate states of **1**.

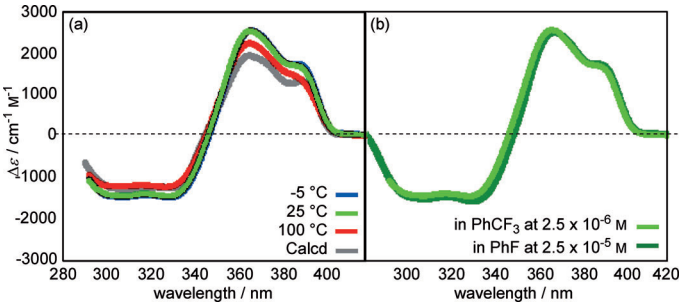


Figure 3. CD spectra of **1** at different a) temperatures (2.5×10^{-6} M, trifluoromethylbenzene) and b) concentrations (25 °C, trifluoromethylbenzene and fluorobenzene). The calculated spectrum (Figure S4 in the Supporting Information) is also shown.

no amido-dimer, ethynyl-dimer, or random-coil state was examined, and such a state was denoted as the S-all-dimer state (S = shifted).

Since both **3** and **5** were in the random-coil state in THF,[6,7] the random-coil state of **1** was examined in this solvent. The CD spectrum (THF, 25 °C, 2.5×10^{-6} M) of **1** (Figure S1a in the Supporting Information) coincided with the calculated spectrum (Figure S2 in the Supporting Information) obtained by adding the spectra of **3** (THF, 5×10^{-6} M, 25 °C) and **5** (THF, 5×10^{-6} M, 25 °C).[6,7] The spectrum showed no change with heating at 60 °C or cooling at −5 °C (Figure S1a in the Supporting Information), and the same spectrum was obtained at a higher concentration of 2.5×10^{-5} M (Figure S1b in the Supporting Information). The insensitivity of the CD spectra to temperature and concentration indicated that **1** was in the S-random-coil state in THF at 25 °C between 2.5×10^{-5} and 2.5×10^{-6} M. Then, the CD spectrum obtained here was that of the S-random-coil state of **1**, not containing the all-dimer, amido-dimer, or ethynyl-dimer state.

Trifluoromethylbenzene and fluorobenzene are solvents that promote double-helix formation of the ethynyl heptamer **5**.[6] Moreover, the amidohelicene tetramer **3** exists in the helix dimer state in trifluoromethylbenzene, fluorobenzene, and chloroform (Figure S3 in the Supporting Information).[7] The formation of the all-dimer state of **1**, in which both amido and ethynyl domains formed helix dimers, was examined by CD spectroscopy in these solvents. In trifluoromethylbenzene, the CD spectra (25 °C, 2.5×10^{-6} M) of **1** (Figure 3a) are in good agreement with the calculated spectra (Figure S4 in the Supporting Information) obtained by adding the spectra of **3** (chloroform, 25 °C, 5×10^{-6} M) and the ethynyl heptamer **5** (trifluoromethylbenzene, 25 °C, 5×10^{-6} M), both of which formed helix dimers under the above-mentioned conditions.[6,7] The spectrum did not change by heating at 100 °C or cooling at −5 °C. Since **1** was not soluble in trifluoromethylbenzene concentrations higher than 2.5×10^{-5} M, its CD spectrum at this concentration was obtained in fluorobenzene, which coincided with that in trifluoromethylbenzene at 2.5×10^{-6} M (Figure 3b). These results indicate that **1** is in the S-all-dimer state in trifluoromethylbenzene and fluorobenzene at 25 °C between 2.5×10^{-5} and 2.5×10^{-6} M.

The dimeric aggregation of **1** in the all-dimer state was examined by VPO in chloroform (1×10^{-3} M, 35 °C, benzyl standard). It was confirmed that the same structure was formed in chloroform (1×10^{-4} M, 25 °C) and trifluoromethylbenzene (2.5×10^{-6} M, 25 °C), as indicated in the CD spectrum (Figure S5 in the Supporting Information). The obtained apparent molecular weight of 1.29×10^{4} was in accordance with the calculated molecular weight of the dimeric aggregate, that is, 1.319×10^{4}. The experiment also confirmed the lack of higher-aggregate formation.

It was previously observed that the addition of DMSO promoted the unfolding of the helix dimer of **3**.[7] Therefore, a CD analysis of **1** in the ethynyl-dimer state was conducted in 5% DMSO/trifluoromethylbenzene. The CD spectrum (25 °C, 2.5×10^{-6} M) of **1** in the mixed solvent (Figure 4) was in good agreement with the calculated spectra (Figure S6 in the Supporting Information) obtained by adding the spectrum of **3** in the random-coil state[7] (THF, 25 °C, 5×10^{-6} M) and of **5** in the helix dimer state[6] (trifluoromethylbenzene, 25 °C, 5×10^{-6} M). The CD spectra also did not change between 5 and 60 °C, and the same spectrum was obtained at a higher concentration of 2.5×10^{-5} M (Figure 4b). The spectra did not change by adding DMSO between concentrations of 3 and 10% (Figure S7 in the Supporting Information). The results indicate that **1** is in the S-ethynyl-dimer state in 5%

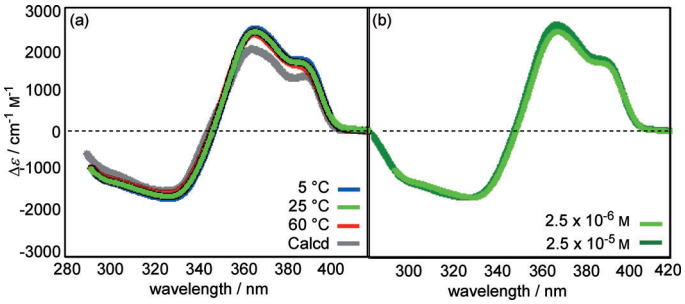


Figure 4. CD spectra of **1** in 5% DMSO/trifluoromethylbenzene at different a) temperatures (2.5×10^{-6} M) and b) concentrations (25 °C). The calculated spectrum (Figure S6 in the Supporting Information) is also shown.

DMSO/trifluoromethylbenzene at 25 °C at concentrations between 2.5×10^{-6} and 2.5×10^{-5} M.

The amido-dimer state of **1** was examined in bromobenzene on the basis of our observations that the ethynylhelicene heptamer **5** was in the random-coil state[6] and the amidohelicene tetramer **3** was in the helix dimer[7] in this solvent (Figure S3 in the Supporting Information). The CD spectrum of **1** (2.5×10^{-6} M, 25 °C) (Figure 5) was similar to

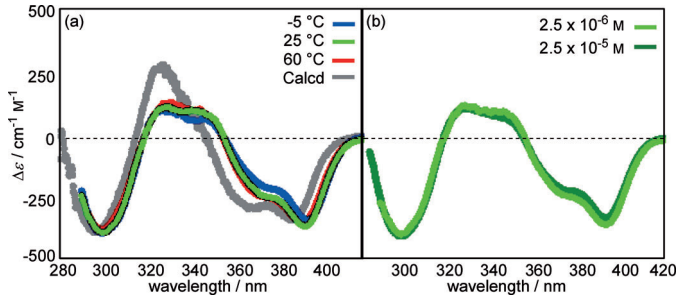


Figure 5. CD spectra of **1** in bromobenzene at different a) temperatures (2.5×10^{-6} M) and b) concentrations (25 °C). The calculated spectrum (Figure S8 in the Supporting Information) is also shown.

the calculated spectrum (Figure S8 in the Supporting Information) obtained from the helix dimer of **3** (chloroform, 25 °C, 5×10^{-6} M) and the random coil of **5** (THF, 25 °C, 5×10^{-6} M), with slight deviations between 320 and 390 nm. Similar deviations in the experimental and calculated spectra for the amido-dimer state were observed in the tridomain compound **2** (vide infra), which could be due to some structural perturbation at the ethynyl domain caused by the dimer formation at the amido domain. The CD spectra did not change between 60 and −5 °C, and the same spectra were obtained at a higher concentration of 2.5×10^{-5} M (Figure 5b). Thus, it is considered that **1** in bromobenzene is in the S-amido-dimer state under these conditions.

Two isomeric structures are conceivable in the amido-dimer state: *syn*, in which the amido- and ethynyl domain were arranged in the same direction and *anti*, in which the two domains were arranged in the opposite direction (Figure 6). The CD spectra of **1** in toluene (2.5×10^{-6} M)

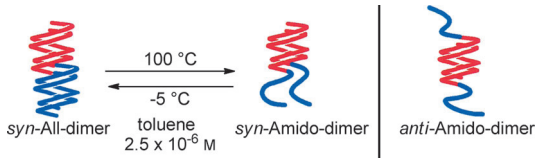


Figure 6. The *syn*-configuration of **1** in the all-dimer and amido-dimer formation.

showed reversible change between all-dimer and amido-dimer on heating and cooling (Figure S9 in the Supporting Information). The all-dimer state should possess the *syn* configuration. Therefore, the amido-dimer state, which interconverted with the all-dimer state, should be the *syn* configuration.

Four aggregate S-states of **1** were obtained in different solvents exhibiting different CD spectra (Figure 7a). However, the spectrum of the S-ethynyl-dimer state in 5%

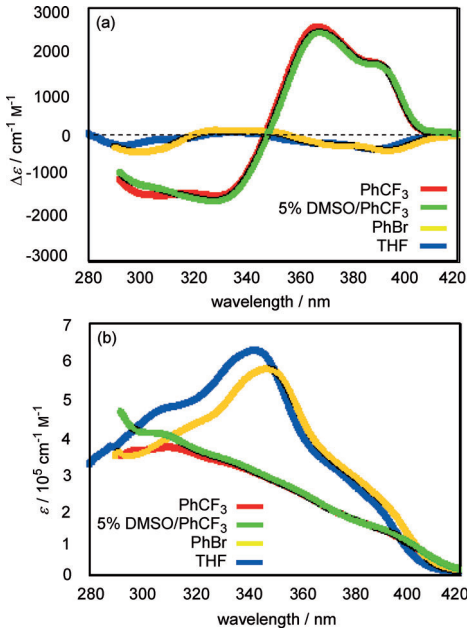


Figure 7. Spectra of four aggregate S-states of **1** (2.5×10^{-6} M, 25 °C): a) CD and b) UV/Vis spectra.

DMSO/trifluoromethylbenzene was similar to that of the S-all-dimer state in trifluoromethylbenzene, and small differences were observed only at the 290–320 nm region. Amidohelicene oligomers showed changes in this region by aggregation and disaggregation,[7] and the small differences between the S-ethynyl-dimer and the S-all-dimer states were due to the extremely strong Cotton effect of the ethynyl heptamer domain on double-helix formation compared with the Cotton effect of the amido tetramer domain. As will be discussed later, apparently different CD spectra of four aggregate S-states were obtained from the tridomain compound **2**, in which the ethynyl pentamer domain instead of the ethynyl heptamer domain was employed to adjust CD

intensity. Four aggregate S-states of **1** were also observed from the UV/Vis spectra (Figure 7b). The absorptions at about 340 nm for the S-amido-dimer (bromobenzene) and S-random-coil (THF) states were enhanced compared with those for the S-all-dimer (trifluoromethylbenzene) and S-ethynyl-dimer (5% DMSO/trifluoromethylbenzene) states. The results were ascribed to disaggregation at the ethynyl domain, in accordance with our previous observations.[6]

Quite different ^{1}H NMR spectra were obtained for **1** in $CDCl_3$, [D_5]bromobenzene, 20% [D_6]DMSO/$CDCl_3$, and [D_8]THF, which reflected different aggregate states (Figure 8). In [D_8]THF (2×10^{-4} M, 25 °C), **1** was in the

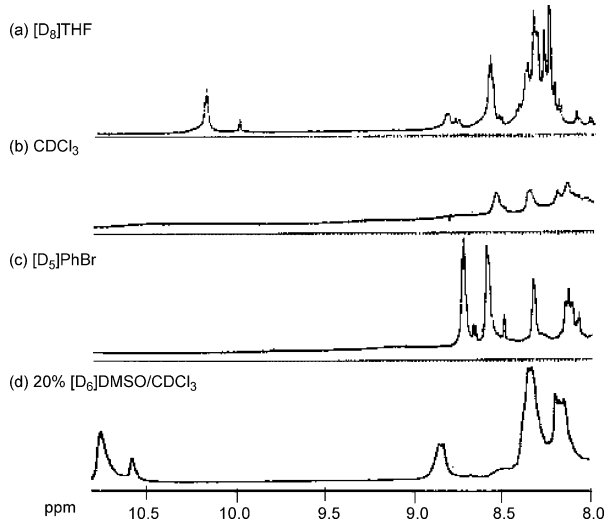


Figure 8. ^{1}H NMR spectra of **1** in different solvents (2×10^{-4} M, 25 °C); a) [D_8]THF, b) $CDCl_3$, c) [D_5]PhBr, and d) 20% [D_6]DMSO/$CDCl_3$.

S-random-coil state, and, accordingly, two sharp amide protons at $\delta = 10.0$ and 10.2 ppm as well as well-resolved aromatic protons were observed owing to rapid molecular motions. In $CDCl_3$ (2×10^{-4} M, 25 °C), **1** was in the S-all-dimer state, as indicated by CD and VPO, and its ^{1}H NMR spectra showed a serious broadening of amide and aromatic protons, which was attributed to restricted molecular motions. Broadening was previously observed for the amido tetramer **3** (Figure S10 in the Supporting Information) and the ethynyl heptamer **5**[6] in $CDCl_3$ (1×10^{-3} M, 25 °C). In [D_5]bromobenzene (2×10^{-4} M, 25 °C), an S-amido-dimer was formed, and the amide protons were again broadened. In 20% [D_6]DMSO/$CDCl_3$ (2×10^{-4} M, 25 °C), two relatively sharp amide peaks were observed at $\delta = 10.3$ and 10.5 ppm, which were in accordance with the S-ethynyl-dimer formation observed by CD spectroscopy (Figure S11 in the Supporting Information). The broadening of amide protons in $CDCl_3$ and [D_5]bromobenzene was ascribed to hydrogen bonding in helix dimer formation, which should be different from hydrogen bonding in solvents such as in [D_8]THF and [D_6]DMSO/$CDCl_3$.

IR analysis of **1** was conducted in solution (3×10^{-3} M) at room temperature. The all-dimer in fluorobenzene and amido-dimer in bromobenzene showed amide carbonyl absorptions at 1653 and 1648 cm^{-1}, respectively (Figure S12a and b in the Supporting Information). In THF, **1** was in random-coil state, and provided a carbonyl absorption at 1682 cm^{-1} (Figure S12c in the Supporting Information). The presence of such a hydrogen-bonded amide carbonyl in the all-dimer and amido-dimer state was previously observed in the helix-dimer formation of amidohelicene tetramer **3**.[7] The aggregation of **1** at the amide domain was confirmed in the all-dimer and amido-dimer states.

AFM analysis was conducted on the aggregates of **1**. The AFM images of the samples prepared from fluorobenzene solution showed particles about 30 nm diameter for all-dimer state. The samples prepared from bromobenzene solution provided 20–30 nm diameter for amido-dimer state (Figure S13 in the Supporting Information). Particles of uniform sizes were formed in wide surfaces for both experiments.

It is shown that four aggregate states, namely, S-all-dimer, S-amido-dimer, S-ethynyl-dimer, and S-random-coil states, were obtained for **1** by the judicious choice of solvents, which indicated that the amido and ethynyl domains of **1** independently aggregated and disaggregated.

The four aggregate states of tridomain compound 2: Four aggregate states of tridomain compound **2**, namely, S-all-dimer, S-amido-dimer, S-ethynyl-dimer, and S-random-coil states, were examined by the same methods. (Figure 9)

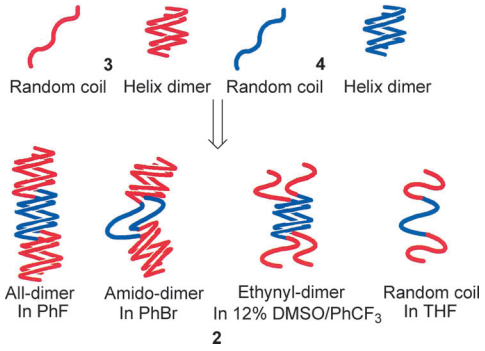


Figure 9. Four aggregate states of **2**.

The CD spectrum of **2** (25 °C, 2.5×10^{-6} M) in THF (Figure S14a in the Supporting Information) coincided with the calculated spectrum (Figure S15 in the Supporting Information) obtained by adding the spectra of the random-coil amido tetramer **3**[7] (THF, 5×10^{-6} M, 25 °C) and the random-coil ethynyl pentamer **4**[6] (chloroform, 5×10^{-6} M, 25 °C) in 2:1 ratio. The spectra did not change by heating at 60 °C or cooling at 5 °C at a higher concentration of 2.5×10^{-5} M (Figure S14b). Hence, compound **2** is in the S-random-coil state in THF under the above-mentioned conditions.

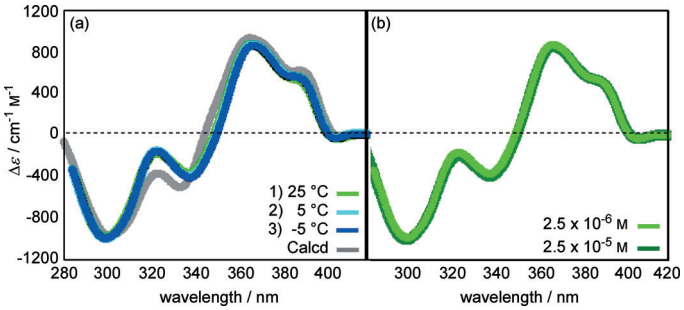


Figure 10. CD spectra of **2** in fluorobenzene at different a) temperatures (2.5×10^{-6} M) and b) concentrations (25 °C). The calculated spectrum (Figure S16 in the Supporting Information) is also shown.

Since **2** was not soluble enough in trifluoromethylbenzene for CD analysis, the experiments on the all-dimer state were conducted in fluorobenzene (25 °C, 2.5×10^{-6} M). The CD spectrum of **2** (Figure 10a) coincided with the calculated spectrum (Figure S16 in the Supporting Information) obtained by adding the spectra of **3** in the helix dimer state[7] (chloroform, 25 °C, 5×10^{-6} M) and of **4** in the double helix state[6] (trifluoromethylbenzene, 25 °C, 1×10^{-3} M) in a ratio of 2:1. The spectra did not change between −5 and 25 °C. However, when the mixture was heated at 80 °C, the Cotton effect was minimized, which is due to partial disaggregation at the central ethynyl pentamer domain (Figure S17 in the Supporting Information). The difference in thermal stability between **1** (Figure 3) and **2** in ethynyl domain aggregation is due to the weaker aggregate formation of the ethynyl pentamer domain of **2** than of the heptamer domain of **1**. The same spectrum of **2** was obtained at concentrations of 2.5×10^{-6} and 2.5×10^{-5} M (Figure 10b). The results indicated that **2** is in the S-all-dimer state in fluorobenzene at 25 °C.

In DMSO/trifluoromethylbenzene, **2** is considered to be in the ethynyl-dimer state. The CD spectrum of **2** (5×10^{-5} M, 25 °C) in 12% DMSO/trifluoromethylbenzene (Figure 11a) coincided in shape with the calculated spectrum (Figure S18 in the Supporting Information), which was obtained by adding the spectra of the random coil of **3**[7] (THF, 5×10^{-6} M, 25 °C) and the helix dimer of **4**[6] (trifluoromethylbenzene, 1×10^{-3} M, 25 °C) in 2:1 ratio. The difference in intensity between the experimental and calculated spectra may be partly due to the relatively unstable nature of the helix dimer of **4**, which contains only five helicenes. The CD spectra did not change between 25 and 5 °C. The intensity, however, considerably decreased with heating at 80 °C, which then recovered with cooling to 5 °C. The similar spectra were obtained at concentrations up to 1×10^{-4} M (25 °C) (Figure 11b). The DMSO content was changed from 6 to 16%, and essentially the same CD spectrum was obtained between 10 and 16% (Figure S19 in the Supporting Information). Since the amount of hydrogen-bonding-breaking solvent had no effect on the CD spectra above 10%, the amido domain should be fully disaggregated in these states. The results indicate the S-ethynyl-dimer state of **2** under the above-mentioned conditions.

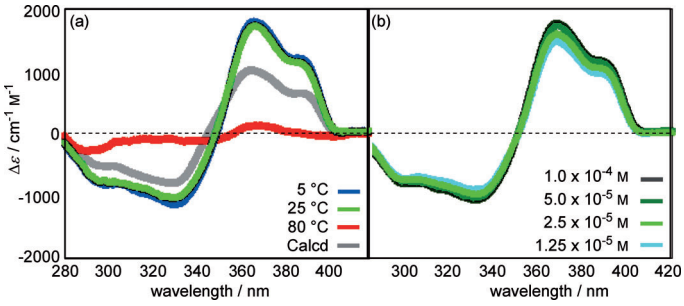


Figure 11. CD spectra of **2** in 12% DMSO/trifluoromethylbenzene at different a) temperatures (5×10^{-5} M) and b) concentrations (25 °C). The calculated spectrum (Figure S18 in the Supporting Information) is also shown.

In bromobenzene, the CD spectrum (2.5×10^{-6} M, 25 °C) of **2** (Figure 12a) was similar to the calculated spectrum (Figure S20) obtained by adding the spectra of the helix dimer of **3**[7] (chloroform, 25 °C, 5×10^{-6} M) and the random coil of **4**[6] (chloroform, 25 °C, 5×10^{-6} M) in 2:1 ratio. Some deviations at 320 and 360 nm, however, were observed as for **1** in bromobenzene (Figure 5a). The CD spectra did not change between 5 and 60 °C, and at a higher concentration at 2.5×10^{-5} M (Figure 12b). Compound **2** is in the S-amido-dimer state in bromobenzene under these conditions.

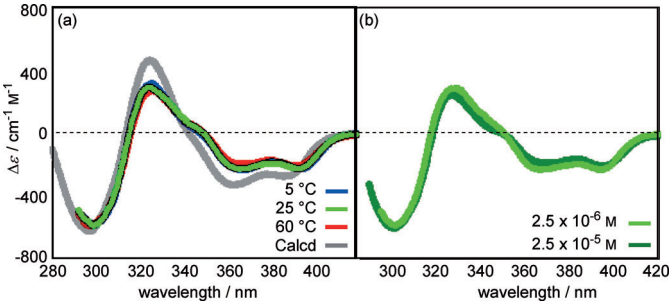


Figure 12. CD spectra of **2** in bromobenzene at different a) temperatures (2.5×10^{-6} M) and b) concentrations (25 °C). The calculated spectrum (Figure S20 in the Supporting Information) is also shown.

The amido and ethynyl domains of **2** aggregated and disaggregated in different solvents, and four aggregate S-states were obtained (Figure 13). Compared with the case of **1**, quite different CD spectra were obtained for **2** in the four S-states (Figure 13a), which were used for the reversible structural changes, as will be described later. Different UV/Vis spectra were also obtained for the four S-states (Figure 13b). A characteristic absorption at about 340 nm appeared for the S-random coil in THF and S-amido-dimer in bromobenzene, in which the ethynyl domain was disaggregated. Stronger absorptions were observed at about 300 nm for the S-ethynyl-dimer in 12% DMSO/trifluoromethylbenzene and the S-random coil in THF, which were disaggregate states at the amido domain.

^{1}H NMR analysis of **2** in [D$_8$]THF at 2×10^{-4} M showed sharp amide peaks at $\delta=10.0$ and 10.2 ppm and well-re-

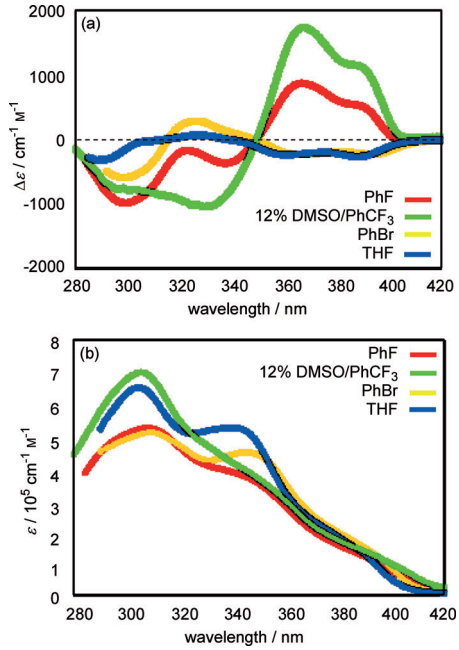


Figure 13. Spectra of four aggregate S-states of **2** (25 °C): a) CD and b) UV/Vis spectra. Concentration of **2** in 12 % DMSO/trifluoromethylbenzene, 5×10^{-6} M, and in other solvents, 2.5×10^{-6} M.

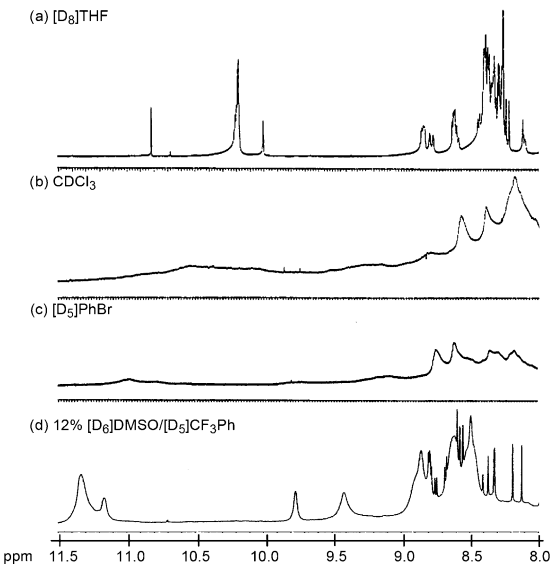


Figure 14. ^{1}H NMR spectra of **2** in different solvents (2×10^{-4} M, 25 °C): a) [D_8]THF, b) $CDCl_3$, c) [D_5]PhBr, and d) 12 % [D_6]DMSO/[D_5]$PhCF_3$.

solved aromatic peaks (Figure 14), which are due to the rapid molecular motions. It was confirmed by CD that the all-dimer state was formed in chloroform and fluorobenzene (Figure 10 and Figure S21 in the Supporting Information). In $CDCl_3$ and [D_5]bromobenzene, broad amide protons were observed by ^{1}H NMR spectroscopy. In 12 % [D_6]DMSO/[D_5]CF_3Ph, in which CD (25 °C, 1×10^{-4} M) analysis indicated ethynyl-dimer formation (Figure 11), the

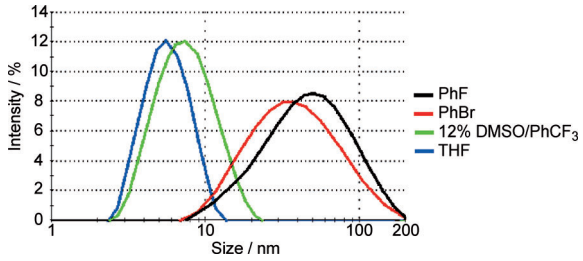


Figure 15. Size distributions of **2** by DLS experiments in different solvents (5×10^{-5} M, 20 °C).

amide peaks were sharp. The ^{1}H NMR analysis of **2** exhibited the same trends as **1**.

Four aggregate states of **2** were examined by DLS (20 °C, 5.0×10^{-5} M) (Figure 15). In THF, an average diameter of 6 nm was obtained for the random-coil state. The ethynyl-dimer state in 12 % DMSO/trifluoromethylbenzene showed a 7 nm diameter, similar to the random-coil state. The all-dimer state in fluorobenzene gave a peak at 50 nm, and the amido-dimer state in bromobenzene gave a peak at 35 nm. The sizes of the random-coil monomer and ethynyl-dimer states for **2** were 6–7 nm, and those of the amido-dimer and all-dimer states were 35–50 nm. This may be due to the rod-like rigid structure of the amido-dimer state, which increased the apparent diameters of aggregates of the all-dimer and amido-dimer states. A larger diameter was obtained for the all-dimer state in fluorobenzene than for the amido-dimer state in bromobenzene. This may be ascribed to the folding of the latter at the disaggregated ethynyl domain.

AFM analysis of the aggregates of **2** was conducted (Figure 16). AFM images of the samples prepared from chloroform showed dispersed particles of about 30 nm diameter, which should indicate an all-dimer state. The samples prepared from bromobenzene provided particles of an amido-dimer of approximately 20 nm diameter. Particles of uniform sizes were formed in wide surfaces for both experiments. The results were in good accordance with those obtained by DLS. The sizes were similar with the all-dimer of **1**, which was confirmed to be dimeric by VPO (vide supra). The observations are consistent with dimeric aggregate formation of **2**. The random-coil and ethynyl-dimer states of **2** prepared in THF and 12 % DMSO/trifluoromethylbenzene solution produced no evident particles, which may be due to the small size of the particles and/or rapid molecular motions.

Compounds **1** and **2** formed four dimeric aggregate states, and the ethynyl and amido domains independently aggregated and disaggregated. It was also noted that **1** and **2** aggregated and disaggregated in a two-state manner. This is a notable example of synthetic multidomain compounds with well-defined aggregate structures.

Structural change of tridomain compound: It was considered interesting to examine the structural switch of **2** between different states, particularly, the thermoresponding structural

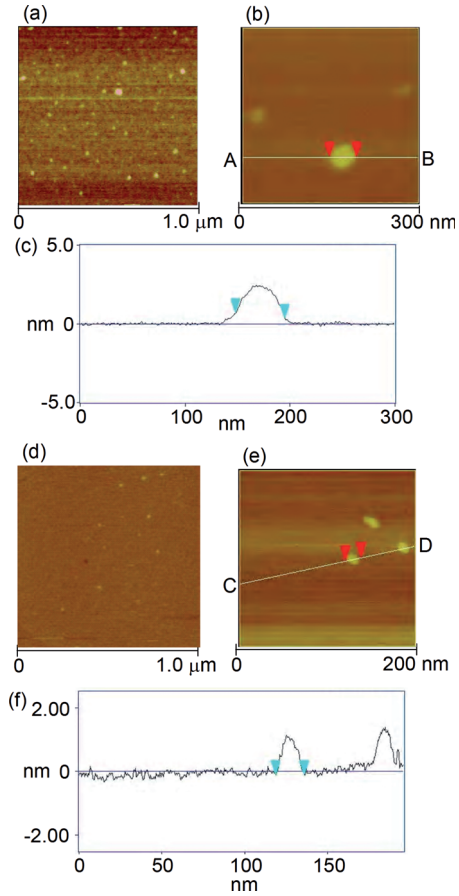


Figure 16. AFM images (height mode) of **2** on mica: a)–c) obtained in chloroform (2.5×10^{-8} M), d)–f) obtained in bromobenzene (2.5×10^{-8} M). c) Section analysis along line A–B. f) Section analysis along line C–D.

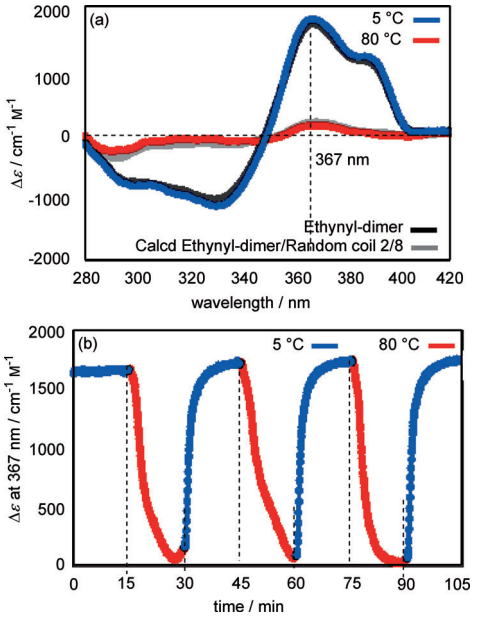


Figure 17. a) CD spectra (12% DMSO/trifluoromethylbenzene, 5.0×10^{-5} M) of **2** at 5 and 80 °C. A calculated spectrum obtained by adding the spectra of 2:10 of the ethynyl-dimer and 8:10 of the random coil is also shown. b) The profiles of $\Delta\varepsilon$ at 367 nm and the time of **2** (12% DMSO/trifluoromethylbenzene, 5.0×10^{-5} M) for repeating cycles of 80/5 °C every 15 min.

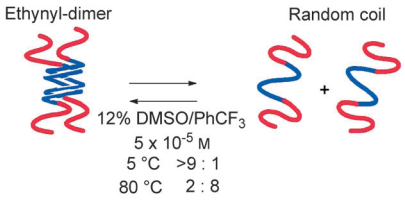


Figure 18. Structure change of **2** between ethynyl-dimer and random coil.

change at the ethynyl domain. As noted in the experiment using 12% DMSO/trifluoromethylbenzene, **2** changed its structure between the ethynyl-dimer and random-coil monomer states on heating (Figure 11), and this structural change was examined in detail. At 5 °C, **2** formed the S-ethynyl-dimer, as indicated by the CD (5×10^{-5} M) with a $\Delta\varepsilon$ value at 367 nm of $+1700\ cm^{-1}M^{-1}$ (Figure 11a). When the solution was heated at 80 °C for 30 min, the $\Delta\varepsilon$ value decreased to $+80\ cm^{-1}M^{-1}$. A ratio of 2:8 of the ethynyl-dimer to the random coil was estimated at 80 °C on the basis of the $\Delta\varepsilon$ value of $+1730\ cm^{-1}M^{-1}$ of the S-ethynyl-dimer (Figure 11) and that of $-221\ cm^{-1}M^{-1}$ of the S-random coil (Figure S14 in the Supporting Information). The calculated CD spectra obtained by adding those of the ethynyl-dimer and the random coil in a 2:8 ratio was in good agreement with the experimental spectra (Figure 17a). When the solution was cooled to 5 °C, the CD spectra returned to the S-ethynyl-dimer state with a $\Delta\varepsilon$ value of $+1700\ cm^{-1}M^{-1}$. The process of heating at 80 °C and cooling at 5 °C was reversible and reproducible, as indicated by the $\Delta\varepsilon$ time profiles (Figure 17b). The results indicate that the structural change involved the ethynyl-dimer and random-coil states, and not the all-dimer or amido-dimer state (Figure 18). In the polar solvent, the amido domain of **2** remained disaggregated, and only two of the four structural states of **2** were mutually switched.

As noted before, **2** with the all-dimer state changed its structure on heating (Figure S17 in the Supporting Information). When **2** in fluorobenzene (2.5×10^{-6} M) was heated at 80 °C and then cooled to 5 °C, a $\Delta\varepsilon$ value of $+720\ cm^{-1}M^{-1}$ at 367 nm was obtained. Using the $\Delta\varepsilon$ values of the all-dimer ($+871\ cm^{-1}M^{-1}$) and amido-dimer ($-228\ cm^{-1}M^{-1}$) states, which were obtained from the CD spectra in fluorobenzene and bromobenzene, respectively (Figure 13), the mixture was calculated to be an 8:2 mixture of the all-dimer and amido-dimer states. The experimental CD spectra (2.5×10^{-6} M, 25 °C) were in good agreement with the calculated spectra obtained by adding those of the all-dimer and amido-dimer states in an 8:2 ratio (Figure 19a). When the mixture was heated to 80 °C, the $\Delta\varepsilon$ value rapidly decreased, reaching a steady state within 5 min with a $\Delta\varepsilon$ value of $+270\ cm^{-1}M^{-1}$. The equilibrium ratio of the all-dimer state to the amido-dimer state was calculated to be 4:6, and the calculated spectra were in good agreement with the experi-

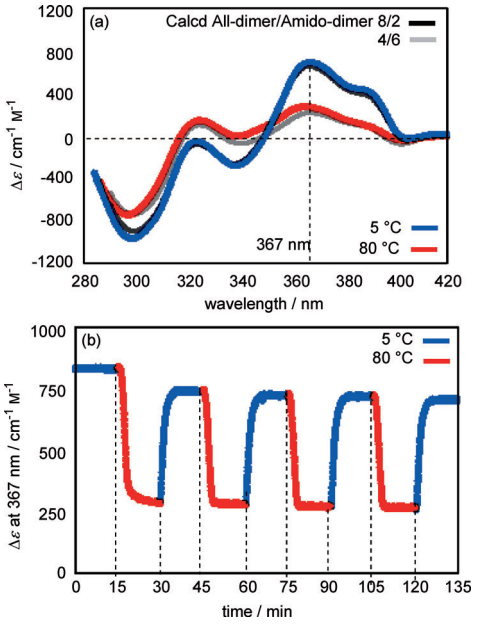


Figure 19. a) CD spectra (fluorobenzene, 2.5×10^{-6} M) of **2** at 5 and 80 °C. The calculated spectra, obtained by adding the spectra of 8:10 of the all-dimer state and 2:10 of the amido-dimer state, and 4:10 of the all-dimer state and 6:10 of the amido-dimer state, are also shown. b) The profiles of $\Delta\varepsilon$ at 367 nm and the time of **2** (fluorobenzene, 2.5×10^{-6} M) for repeating cycles of 80/5 °C every 15 min.

mental results (Figure 19a). The heating–cooling cycle between 80 and 5 °C was repeated, and $\Delta\varepsilon$/time profiles were obtained in a reproducible manner (Figure 19b). The results showed that the thermal process involved the all-dimer and amido-dimer states, not the ethynyl-dimer or random-coil state (Figure 20). The amido domain remained aggregated

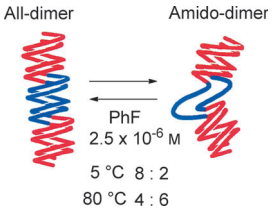


Figure 20. Structure change of **2** between all-dimer and amido-dimer.

during the process in the nonpolar solvent. The all-dimer/amido-dimer change is a switching phenomenon between the double helix and random-coil states at the central domain with aggregation at both terminal domains. As suggested by the results of the DLS (Figure 15) and AFM (Figure 16) measurements, this heating and cooling process should induce the structure change of **2** on a 10 nm scale. The ethynyl-dimer/random-coil switch in the polar solvent (Figure 17) and all-dimer/amido-dimer switch in the nonpolar solvent between 5 and 80 °C (Figure 19) exhibited different dynamic properties. The CD spectral changes in the processes were quite different, and the thermal response rates, concentration effects, and aggregate sizes were also different. In other words, two types of thermal response appeared using a single compound, **2**. The differences originated from the well-defined aggregate and disaggregate states at the amido domain. It may be worth noting that these two structural changes are orthogonal to each other, and that each process proceeds only between two structures and does not involve other structures.

Conclusion

In summary, the bidomain compound **1** and the tridomain compound **2** were designed and synthesized. Both **1** and **2** form four well-defined aggregate states, namely, all-dimer, amido-dimer, ethynyl-dimer, and random-coil states. The domains in **1** and **2** independently aggregate and disaggregate in a two-state manner. The reversible and reproducible structural changes of **2** between the ethynyl-dimer/random-coil state and the all-dimer/amido-dimer state occur with heating and cooling. The well-defined aggregate and disaggregate states at the amido domain provide two different thermal processes using a single compound, **2** (Figure 21). Since ethynyl oligomers exhibit various phenomena such as gelation[9] and vesicle formation,[10] multidomain compounds should exhibit various switch behavior in higher-assembly formation.

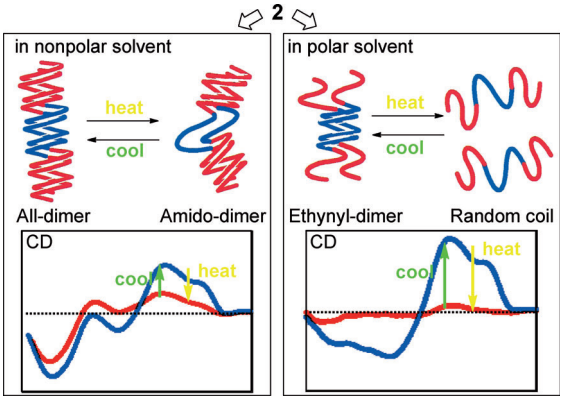


Figure 21. Two structure change systems of **2** between all-dimer/amido-dimer and ethynyl-dimer/random coil.

Experimental Section

Bidomain oligomer 1: Under an argon atmosphere, a mixture of **17** (9 mg, 0.0031 mmol), a tris(dibenzylideneacetone)dipalladium(0) chloroform adduct (0.81 mg, 0.00078 mmol), cuprous iodide (1.7 mg, 0.0093 mol), trimesitylphosphine (1.8 mg, 0.0047 mol), tetrabutylammonium iodide (11 mg, 0.031 mmol), triethylamine (0.3 mL), and *N,N*-dimethylformamide (3 mL) was also freeze-evacuated three times. A solution of **6** (12 mg, 0.0031 mmol) in THF (3 mL) was freeze–evacuated three times and then added dropwise to the above solution. The mixture was stirred for 9 h at 45 °C. The reaction was quenched by adding saturated aqueous ammonium chloride (20 mL), and the organic materials were extracted with toluene (30 mL). The organic layer was washed with water (15 mL) and brine (15 mL), and dried over magnesium sulfate. The solvents were

evaporated under reduced pressure. Separation by recycling GPC and silica gel chromatography gave **1** (11 mg, 0.0016 mmol, 53%). M.p.: 222–226°C (hexane/chloroform); $[\alpha]_D^{26} = +2790$ ($c = 0.18$ in chloroform within 1 h after dissolution); ^{1}H NMR (600 MHz, [D$_8$]THF, 1 mM at 60°C): $\delta =$ 0.85 (t, $J = 6$ Hz, 33H), 1.27–1.53 (m, 154H), 1.75–1.87 (m, 22H), 1.98 (s, 66H), 3.91 (s, 3H), 4.27 (quint, $J = 6$ Hz, 4H), 4.33 (m, 6H), 4.41 (m, 12H), 7.47 (d, $J = 5$ Hz, 8H), 7.51 (d, $J = 5$ Hz, 14H), 7.60–7.74 (m, 22H), 7.89 (d, $J = 7$ Hz, 1H), 7.93 (s, 1H), 8.03 (d, $J = 7$ Hz, 1H), 8.07 (s, 1H), 8.11 (s, 1H), 8.17–8.40 (m, 40H), 8.53 (d, $J = 8$ Hz, 1H), 8.58 (d, $J = 8$ Hz, 12H), 8.70 (s, 1H), 8.80 (s, 2H), 8.81 (d, $J = 9$ Hz, 1H), 9.81 (s, 1H), 10.0 ppm (s, 8H); ^{13}C NMR (151 MHz, [D$_8$]THF): $\delta = 14.4$, 23.4, 23.5 (2 peaks), 27.0, 27.8, 28.7, 29.7, 29.8, 30.2 (2 peaks), 30.3, 30.5, 32.8 (2 peaks), 52.3, 65.5, 65.7, 66.3, 80.0, 90.2, 93.7, 93.8, 115.4, 115.8, 115.8, 116.9, 120.9, 121.0, 124.1, 124.6, 125.4, (2 peaks) 127.7 (2 peaks), 127.9, 128.0, 128.4, 129.6, 129.7, 129.8, 130.9, 131.1, 131.2, 132.0, 132.2, 132.3, 132.6, 132.8, 133.0, 133.3, 136.2, 136.3 (3 peaks), 136.4 (2 peaks), 136.5 137.6 (2 peaks), 137.7, 137.8 (2 peaks), 138.9, 139.0, 141.2, 141.4, 141.6, 153.7, 165.4, 166.5 (2 peaks), 168.1 ppm; IR (KBr): $\tilde{\nu} = 3284$, 2922, 2852, 2206, 1724, 1681, 1651 cm^{-1}; UV/Vis (trifluoromethylbenzene, 2.5×10^{-6} M): λ_{max} (ε) = 310 nm (3.8×10^5 cm^{-1}M^{-1}); CD (trifluoromethylbenzene, 2.5×10^{-6} M): λ ($\Delta\varepsilon$) = 307 (−1519), 328 (−1523), 366 nm (+2663 cm^{-1}M^{-1}); MS (MALDI-TOF, α-cyano-4-hydroxycinnamic acid): m/z calcd for $^{12}C_{452}{}^{13}C_4H_{443}N_9O_{34}$: 6592.33; found: 6590.57 $[M-H]^-$; elemental analysis calcd (%) for $C_{456}H_{443}N_9O_{34}$: C 83.07, H 6.77, N 1.91; found: C 82.95, H 6.96, N 2.07.

Tridomain oligomer 2: Under an argon atmosphere, a mixture of **17** (25 mg, 0.0086 mmol), a tris(dibenzylideneacetone)dipalladium(0) chloroform adduct (2.3 mg, 0.0043 mmol), cuprous iodide (4.8 mg, 0.052 mmol), trimesitylphosphine (5.0 mg, 0.026 mmol), triphenylphosphine (3.4 mg, 0.026 mmol), tetrabutylammonium iodide (16 mg, 0.043 mmol), triethylamine (0.1 mL), and *N,N*-dimethylformamide (1 mL) was freeze-evacuated three times. A solution of **6** (11 mg, 0.0043 mmol) in THF (1 mL) was also freeze-evacuated three times and then added dropwise to the above solution. The mixture was stirred for 5 h at 45°C. The reaction was quenched by adding saturated aqueous ammonium chloride (20 mL), and the organic materials were extracted with ethyl acetate (20 mL) and toluene (20 mL). The organic layer was washed with water (20 mL) and brine (20 mL), and dried over magnesium sulfate. The solvents were evaporated under reduced pressure. Separation by recycling GPC and silica gel chromatography gave **2** (25 mg, 0.0031 mmol, 71%). M.p.: 251–255°C (hexane/dichloromethane); $[\alpha]_D^{27} = -349$ ($c = 0.25$ in THF); ^{1}H NMR (400 MHz, [D$_8$]THF): $\delta = 0.84$ (m, 42H), 1.27–1.51 (m, 196H), 1.76–1.89 (m, 28H), 1.96 (s, 39H), 1.97 (s, 39H), 4.26–4.43 (m, 28H), 7.48–7.55 (m, 26H), 7.62–7.70 (m, 16H), 7.71–7.78 (m, 10H), 7.97 (s, 2H), 8.10 (s, 2H), 8.11 (s, 2H), 8.22–8.45 (m, 66H), 8.58–8.63 (m, 9H), 8.78–8.85 (m, 9H) 10.02 (s, 2H), 10.20 ppm (s, 16H); ^{13}C NMR (100 MHz, [D$_8$]THF): $\delta = 14.4$, 23.5, 23.5, 23.6, 27.0, 28.6, 29.7 (2 peaks), 29.8, 30.3 (2 peaks), 30.5, 32.8, 32.9, 65.5, 65.8, 66.0, 66.3, 80.0, 89.1, 90.1, 90.2, 93.7, 93.8, 94.8, 114.0, 115.1, 115.6, 116.7, 120.8, 120.9, 121.1, 121.4, 124.1, 124.6, 125.1, 125.4, 127.1, 127.5, 127.7 (2 peaks), 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 129.8, 130.3, 130.7, 130.9, 131.0, 131.1 (2 peaks), 131.9, 132.1, 132.2, 132.3, 132.4, 132.6, 132.7, 132.8, 132.9, 133.0, 133.2 (2 peaks), 133.3, 135.8, 136.2 (2 peaks), 136.3, 137.5 (2 peaks), 137.8 (2 peaks), 139.0, 141.2, 141.4, 141.6, 153.6, 165.4, 165.8, 166.5 (2 peaks), 167.9, 168.1, 168.2 ppm; IR (KBr): $\tilde{\nu} = 3319$, 2924, 2852, 2207, 1724, 1681, 1652 cm^{-1}; UV/Vis (fluorobenzene, 2.5×10^{-6} M): λ_{max} (ε) = 308 nm (5.3×10^5 cm^{-1}M^{-1}); CD (fluorobenzene, 2.5×10^{-6} M): λ ($\Delta\varepsilon$) = 300 (−980), 323 (−169), 337 (−365), 367 nm (+871 cm^{-1}M^{-1}); MS (MALDI-TOF, α-cyano-4-hydroxycinnamic acid): m/z calcd for $^{12}C_{539}{}^{13}C_5H_{554}N_{18}O_{48}$: 8111.16; found: 8110.47 $[M-H]^-$; elemental analysis calcd (%) for $C_{544}H_{554}N_{18}O_{48}$: C 80.54, H 6.88, N 3.11; found: C 80.40, H 7.07, N 2.96.

Acknowledgements

We thank Professor Hiroyuki Isobe (Department of Chemistry, Graduate School of Sciences, Tohoku University) for DLS use. This work was financially supported by a Grant-in-Aid for Scientific Research (No. 21229001). Financial support and a grant for research assistantship from the WPI-AIMR Fusion Research and the G-COE program from JSPS are acknowledged. W. Ichinose thanks the JSPS for a Fellowship for Young Japanese Scientists.

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Received: May 31, 2012
Published online: ■■ ■■, 0000

Molecular Switching

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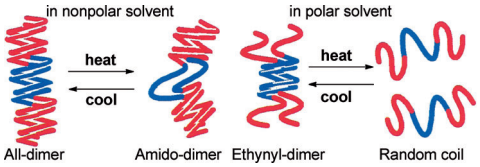
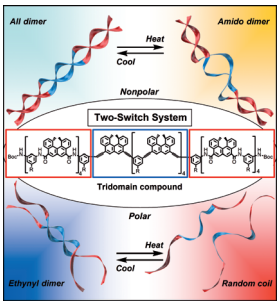
Multiple States of Dimeric Aggregates Formed by (Amido–ethynyl)helicene Bidomain Compound and (Amido–ethynyl–amido)helicene Tridomain Compound



Domain switching! An (amido–ethynyl)helicene bidomain compound and an (amido–ethynyl–amido)helicene tridomain compound were synthesized. Four aggregate states of multidomain compounds, namely, all-dimer, amido-dimer, ethynyl-dimer, and random-coil states, were obtained in different solvents. Each domain independently aggregated and disaggregated in a two-state manner. Two different reversible structural changes occurred between the ethynyl-dimer/random-coil and the all-dimer/amido-dimer with heating and cooling.



Molecular Switching

Well-defined aggregate formation of multidomain linear oligomers is described by M. Yamaguchi et al. in their Full Paper on page ■■ ff. Multidomain compounds designed on the basis of amido and ethynyl oligomers to form dimeric aggregates with properties orthogonal to each other. Four aggregate states and two reversible structural change processes were obtained using a single compound.

