Contraction of Supramolecular Double-Threaded Dimer Formed by $\alpha$-Cyclodextrin with the Long Alkyl Chain.

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Experimental Section.

Materials. Dichloromethane was distilled over calcium hydride by trap-to-trap distillation and stored under an argon atmosphere in a dark place. Hexane was distilled over CaH₂ and store under an argon atmosphere. N,N-Dimethylformamide (DMF) was dried over magnesium sulfate and distilled over barium oxide under reduced pressure, then store under an argon atmosphere in the dark. p-Toluenesulfonyl chloride, sodium azide, 28% ammonia solution, N,N'-dicyclohexylcarbodiimide, 1-hydroxybenzotriazole, trifluoroacetic acid and sodium hydrogen carbonate were obtained from Nacalai Tesque, Inc. N-Boc-1,6-diaminohexane, hexamethylene diisocyanate and 4-aminocinnamic acid were obtained from TOKYO KASEI KOGYO Co., Ltd. Triphenylphosphine and sodium 2,4,6-trinitrobenzene-1-sulfonate were obtained from Wako Pure Chemical Industries, Ltd. α-Cyclodextrin (α-CD) were obtained from Junsei Chemical Co., Ltd. These reagents were used without additional purification. 6-p-Toluenesulfonyl-α-CD₁ and 6-amino-α-CD₂ were prepared according to the literature.

References

Measurements. $^1$H NMR, 2D COSY and 2D ROESY spectra were recorded on a JEOL JNM LA-500 spectrometer and $^{13}$C NMR, 2D HMBC and 2D HMQC spectra on VARIAN INOVA 600 spectrometer. Chemical shifts were referenced to the solvent values ($\delta$ 2.50 ppm for DMSO-$d_6$ and $\delta$ 4.70 for HOD). The assignments of $^1$H and $^{13}$C NMR peaks for some complexes were supported by 2D $^1$H-$^1$H NOESY, 2D $^1$H-$^1$H COSY, 2D TOCSY, 2D HMQC and 2D HMBC spectra. The positive-ion matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry was performed on a Shimadzu/KRATOS AXIMA-CFR spectrometer with dihydroxylbenzoic acid (DHBA) as a matrix. Circular dichroism spectra and UV spectra were recorded on a JASCO J820 spectrometer with a 0.1 cm cell at room temperature.
Preparation of Boc-6-(6-aminohexylureido)hexamethylene isocyanate. To a ice-cooled solution of hexamethylene diisocyanate (0.50 mL, 3.1 mmol) in dichloromethane (15 mL) was added a solution of N-Boc-1,6-diaminohexane (0.46 g, 1.5 mmol) in dichloromethane (15 mL) at 0 °C. The mixture was stirred at room temperature under an argon atmosphere for 4 hours. The solution was evaporated and the dried residue was washed with hexane (10 mL) to give 0.64 g of the desired product in 78% yield. $^1$H NMR (DMSO-$d_6$, 500 MHz, 30 °C): δ 6.72 (t, 1H, $J = 4.88$, -NH), 5.69 (m, 2H, -NH), 3.33 (t, 2H, $J = 6.64$, -CH$_2$-), 2.95 (m, 4H, -CH$_2$-), 2.88 (q, 2H, $J = 6.61$, -CH$_2$-), 1.54 (quintet, 2H, $J = 7.02$, -CH$_2$-), 1.37 (s, 9H, -CH$_3$), 1.19-1.39 (m, 16H, -CH$_2$-). MALDI-TOF MS: m/z 422.8 (M+K)$^+$, 405.8 (M+Na)$^+$, 349.7 (M-rBu+Na)$^+$.

Preparation of Boc-4-[6-(6-aminohexylureido)hexylureido]cinnamic acid. To a ice-cooled solution of Boc-6-(6-aminohexylureido)hexamethylene isocyanate (1.4 g, 3.7 mmol) in DMF (25 mL) was added a solution of 4-aminocinnamic acid (0.68 g, 4.2 mmol) in DMF (15 mL) and stirred for 24 hours at room temperature. The mixture was evaporated under reduced pressure after the reaction. The residue was washed 5 times with tetrahydrofuran and recrystallized from methanol to obtain 0.68 g of the product in 34% yield. $^1$H NMR (DMSO-$d_6$, 500 MHz, 30 °C): δ 12.10 (s, 1H, COOH), 8.64 (s, 1H, -
NH\textsubscript{2}), 7.53 (d, 2H, \(J = 8.77\), aromatic), 7.48 (d, 1H, \(J = 15.87\), CH), 7.43 (d, 2H, \(J = 8.77\), aromatic), 6.71 (t, 1H, \(J = 5.11\), -NH\textsubscript{2}), 6.33 (d, 1H, \(J = 15.87\), -CH=CH-Ph), 6.20 (t, 1H, \(J = 5.57\), -NH\textsubscript{2}), 5.70 (m, 2H, -NH\textsubscript{2}), 3.07 (q, 2H, \(J = 6.48\), -CH\textsubscript{2}-), 2.95 (m, 4H), 2.88 (q, 2H, \(J = 6.56\), -CH\textsubscript{2}-), 1.36 (s, 9H, -CH\textsubscript{3}), 1.18-1.46 (m, 16H, -CH\textsubscript{2}-).  $^{13}$C NMR (DMSO-$d_6$, 125 MHz, 30 \(^\circ\)C): \(\delta\) 167.8 (COOH), 158.0 (CO), 155.5 (CO), 154.8 (CO), 143.8 (CH), 142.7 (C), 126.7 (C), 117.3 (aromatic), 116.0 (CH), 77.2 (C), 40.1 (-CH\textsubscript{2}-), 39.8 (-CH\textsubscript{2}-), 39.1 (-CH\textsubscript{2}-), 39.0 (-CH\textsubscript{2}-), 28.2 (-CH\textsubscript{3}), 30.0, 29.6, 29.4, 26.1, 26.0(CH\textsubscript{2}).  MALDI-TOF MS: m/z: 585.5 (M+K)+, 569.1 (M+Na)+, 513.6 (M-tBu+K)+, 486.2 (M-tBu+Na)+, 470.5 (M-Boc+K)+, 448.1 (M-Boc+Na)+.  Elem. anal.: calcd for C\textsubscript{28}H\textsubscript{45}N\textsubscript{5}O\textsubscript{6}: C, 61.40; H, 8.28; N, 12.79.  found: C, 60.94; H, 8.30; N, 12.88.

Preparation of 6-[Boc-4-[6-(6-aminohexylureido)hexylureido]cinnamide]-\(\alpha\)-CD.  6-Amino-\(\alpha\)-CD (429 mg, 0.441 mmol), Boc-4-[6-(6-aminohexylureido)hexylureido]cinnamic acid (191 mg, 0.349 mmol) and 1-hydroxybenzotriazole (47 mg, 0.34 mmol) were dried under vacuum and dissolved in DMF (40 mL).  To the ice-cooled DMF solution was added \(N,N'\)-dicyclohexylcarbodiimide (114 mg, 0.553 mmol), and then the mixture was stirred for 3 days at 0 \(^\circ\)C.  After the prescribed time, the solution was evaporated under reduced pressure.  The residue was added to methanol to get rid of precipitated 6-amino-\(\alpha\)-CD by centrifugation.  The filtrate of the crude product was purified by HPLC on ODS (65% methanol-water as eluent) to give the product (308 mg) in 59% yield.  $^1$H NMR (DMSO-$d_6$, 500 MHz, 30 \(^\circ\)C): \(\delta\) 8.60 (s, 1H, -NH\textsubscript{2}), 7.82 (t, 1H, \(J = 5.19\), -NH\textsubscript{2}), 7.42 (m, 4H, aromatic), 7.30 (d, 1H, \(J = 15.79\), -CH=CH-Ph), 6.72 (m, 1H, -NH\textsubscript{2}), 6.57 (d, 1H, \(J = 15.79\), -CH=CH-Ph), 6.21 (t, 1H, \(J = 5.53\), -NH\textsubscript{2}), 5.71 (m, 2H, -NH\textsubscript{2}), 5.57-5.41 (m, 12H, OH), 4.91 (d, 1H, \(J = 3.28\), CH of CD), 4.84-4.78 (m, 5H, CH of CD), 4.55-4.46 (m, 5H, OH), 4.42 (t, 1H, \(J = 5.57\), OH), 3.84-3.23 (m, 30H, CH of CD), 3.08 (q, 2H, \(J = 6.48\), -CH\textsubscript{2}-), 2.96 (m, 4H, -CH\textsubscript{2}-), 2.89 (q, 2H, \(J = 6.59\), -CH\textsubscript{2}-), 1.38 (s, 9H, -CH\textsubscript{3}), 1.46-1.19 (m, 16H, -CH\textsubscript{2}-).  MALDI-TOF MS: m/z: 1522.9 [M+Na]+.
Preparation of 6-[4-[6-(6-aminohexylureido)hexylureido]cinnamide]-α-CD (1). 6-[Boc-4-[6-(6-aminohexylureido)hexylureido]cinnamide]-α-CD (308 mg, 0.205 mmol) was dissolved in 90% trifluoroacetic acid-water (1.5 mL) and stirred for 30 minutes at 0 °C. After the prescribed time, the mixture was evaporated to dryness under reduced pressure. The obtained residue was dissolved in 5.0 mL of water, and was neutralized by 0.5 N ammonia solution. The crude product was purified by column chromatography with SP Sephadex C-25 to give the desired product (117 mg) in 41% yield.  

\[ \delta \] H NMR (DMSO-\( d_6 \), 500 MHz, 30 °C): \( \delta \) 8.59 (s, 1H, -NH\( \text{-} \)), 7.81 (m, 1H, -NH\( \text{-} \)), 7.41 (m, 4H, aromatic), 7.30 (d, 1H, J = 15.64, -CH=CH-Ph), 6.72 (m, 1H), 6.56 (d, 1H, J = 15.64, -CH=CH-Ph), 6.19 (m, 1H, -NH\( \text{-} \)), 5.70 (m, 2H, -NH\( \text{-} \)), 5.55-5.40 (m, 12H, OH), 4.90 (d, 1H, J = 3.28, CH), 4.83-4.77 (m, 5H, CH of CD), 4.55-4.45 (m, 4H, OH of CD), 4.40 (t, 1H, J = 5.57, OH of CD), 3.82-3.23 (m, 36H, CH of CD), 3.08 (q, 2H, J = 6.15, -CH\( \text{Z} \)-), 2.96 (m, 4H, -CH\( \text{Z} \)-), 1.46-1.19 (m, 16H, -CH\( \text{Z} \)-). MALDI-TOF MS: m/z: 1424.4 ([M+Na\( \text{+} \)]\(^+\)). Anal. Calcd. for C\(_{59}\)H\(_{96}\)N\(_{6}\)O\(_{32}\)5H\(_2\)O: calcd H 7.16, C 47.51, N 5.63; found H 7.01, C 47.40, N 5.47.
Preparation of 6-[4-[6-(6-(2,4,6-trinitroanilino)hexylureido)hexylureido]cinnamide]-α-CD (2): $\text{1 (12 mg, 8.63 µmol)}$ was dissolved in a 1:1 mixture of methanol and pyridine (2.5 mL). To the solution of $\text{1}$ was added sodium 2,4,6-trinitrobenzene-1-sulfonate (29 mg, 91.7 µmol). After stirring for 24 hours, $\text{2}$ was precipitated by an addition of a large amount of acetone. The precipitate was washed with acetone to give the product (12 mg). $^1$H NMR (DMSO-$d_6$, 500 MHz, 30 °C): δ 8.72 (s, 2H, aromatic), 8.77 (m, 1H, -NH-), 8.56 (s, 1H, -NH-), 7.80 (t, 1H, $J = 5.34$, -NH-), 7.40 (m, 4H, aromatic), 7.29 (d, 1H, $J = 15.72$, -CH=CH-Ph), 6.57 (d, 1H, $J = 15.72$, -CH=CH-Ph), 6.17 (t, 1H, $J = 5.57$, -NH-), 5.67 (m, 2H, -NH-), 5.55-5.40 (m, 12H, OH of CD), 4.90 (d, 1H, $J = 3.43$, CH of CD), 4.83-4.77 (m, 5H, CH of CD), 4.53-4.45 (m, 4H, OH of CD), 4.40 (t, 1H, $J = 5.65$, OH of CD), 3.82-3.31 (m, 36H, CH of CD), 3.07 (q, 2H, $J = 6.54$, -CH$_2$-), 3.01 (m, 2H, -CH$_2$-), 2.94 (m, 4H, -CH$_2$-), 1.62 (m, 2H, -CH$_2$-), 1.45-1.17 (m, 14H, -CH$_2$-). MALDI-TOF MS: 1624.4 ([M+Na]$^+$), 1426.7 (M-TNB+Na)$^+$. 

Preparation of 3: $\text{1 (32 mg, 22.6 µmol)}$ was dissolved in the 0.1 M sodium bicarbonate aqueous solution (6.5 mL). A solution of $\text{1}$ was stirred for 24 h to form the double threaded dimmer and sodium 2,4,6-trinitrobenzene-1-sulfonate (61 mg, 194 µmol) added. After stirring for 24 h, the resulting
precipitate was collected by centrifuge and the crude product was purified by HPLC on ODS (65% methanol-water as eluent) to give the product (10 mg). $^1$H NMR (DMSO-$d_6$, 500 MHz, 30 °C): δ 8.92 (s, 2H, aromatic CH), δ 8.79 (m, 1H, -NH-), δ 8.54 (s, 1H, -NH-), δ 7.59 (m, 1H, -NH-), δ 7.18 (d, 2H, $J$ = 8.32, aromatic), δ 7.15 (d, 1H, $J$ = 15.79, -CH=CH-Ph), δ 7.05 (d, 2H, $J$ = 8.32, aromatic), δ 6.30 (d, 1H, $J$ = 15.79, -CH=CH-Ph), δ 5.87 (m, 2H, -NH-), δ 5.85-5.46 (m, 12H, OH of CD), δ 4.95-4.77 (m, 5H, CH of CD), δ 4.63-4.38 (m, 5H, OH of CD), δ 3.84-3.33 (m, 36H, CH of CD), δ 3.16-2.90 (m, 8H, -CH$_2$-), δ 1.68-1.19 (m, 16H, -CH$_2$-). MALDI-TOF MS: m/z: 3247.7 ([M+Na]$^+$).

Comment: It is possible to enlarge the preparation scale, whereas the purification of 3 in large scale is difficult because of the use of HPLC.
Characterization of 1: The $^1$H NMR spectra of 1 were measured at varying concentrations from 0.31 mM to 4.9 mM. Figure S1 shows the partial $^1$H NMR spectra focusing on the cinnamamide group in the long alkyl chain group. The peaks of olefin protons (c and d) shifted to higher magnetic fields with increasing concentration. The peaks of phenyl protons (a and b) changed from doublet peaks to broad peaks. These spectral changes in the long alkyl chain group were caused by the formation of supramolecular complexes. We supposed that 1 formed a supramolecular polymer or a double-threaded dimer in aqueous solutions.

![Figure S1](image)

*Figure S1. The $^1$H NMR spectra of 1 as a fraction of concentration in D$_2$O.*

Characterization of 2 and 3: Figure S2 shows the $^1$H NMR spectra of 1, 2 and 3 in DMSO-$d_6$ at 30 °C. The spectrum of 1 is similar to that of 2 (Figures S2(a) and 2(b)). However, the spectra of 2 and 3 are different. Comparing the $^1$H NMR spectra of 2 with 3, the peaks of cinnamamide protons and alkyl protons of 3 are shifted to higher magnetic fields. (Figure S2(c)) Moreover, a single species of the magnetic resonance for the end groups of the double-threaded dimer was observed, indicating that the double-threaded dimer formed a symmetric structure. On the basis of these data, addition of TNBS to 1 in a sodium carbonate buffer gives a double-threaded dimer (3).
Figure S2. The $^1$H NMR spectra of 1 (a), 2 (b) and 3 (c) in DMSO-$d_6$ at 30 °C.
The MALDI-TOF MS confirmed the formation of the double-threaded dimer species (Figure S3). The MALDI-TOF mass spectrum of 3, which was prepared in a sodium carbonate, showed the double-threaded topology species (Figure S3, red line), indicating the formation of a supramolecular dimer. The peaks of the supramolecular polymers of a degree of polymerization of more than two were not observed. On the basis of the $^1$H NMR data and those of the MALDI-TOF mass spectra, 3 formed double-threaded dimer. The formation of supramolecular complex from 1 in an aqueous solution could be expected by the $^1$H NMR spectroscopy. However, supramolecular complexes could not be observed by the MALDI-TOF mass spectroscopy (Figure S3, green line). The compound 1, which does not have the stopper molecules at the end group, would dissociate into the monomer unit by the irradiation of laser. The MALDI-TOF mass spectrum of 2 (a model compound), which was prepared in pyridine-methanol, also showed only the monomer species (Figure S3, blue line).

![Figure S3](image-url)

**Figure S3.** The MALDI-TOF mass spectra of 1 (green line), 2 (blue line) and 3 with dihydroxy bezoic acid as a matrix.
**Figure S4.** Circular dichroism spectra of 2 in DMSO at 25 °C.

A monomer model compound (2) exhibited no induced Cotton band at the maximum absorption wavelength of the cinnamamide group (324 nm) in DMSO.
Determination of Diffusion Coefficient:

The Pulse Field Gradient Spin-Echo (PFGSE) NMR spectra were recorded at 600 MHz in D₂O on a VARIAN INOVA NMR spectrometer at 30.0 °C. The bipolar pulse pair stimulated echo (BPPSTE) sequence was applied for PFGSE NMR measurement. The pulsed gradients’ strength was increased from $6.36 \times 10^{-3}$ to 43.1 (gauss/cm). The time separation of pulsed field gradients and their duration were 0.10 and $1.0 \times 10^{-3}$ (s). The sample was not spun and the airflow was discontinued. The shape of the gradient pulse was rectangular, and its strength varied automatically during the course of the experiments. The $D$ values were determined from the slope of the regression line $\ln \left( I / I_0 \right)$ versus $G^2$, according to the Stejskal and Tanner’s Equation (1).

$$\ln \left( I / I_0 \right) = - \gamma^2 G^2 \delta^2 \left( \Delta - \delta/3 - \tau/2 \right) D \tag{1}$$

$I / I_0$: observed spin echo intensity / intensity without gradients,
$G$: gradient strength,
$\Delta$: delay between the midpoints of the gradients,
$D$: diffusion coefficient,
$\delta$: gradient length
$\tau$: 90°-180° pulse distance.

The calibration of the gradients was carried out by a diffusion measurement of H₂O ($D_{\text{H}_2\text{O}} = 2.30 \times 10^{-9}$ m² s⁻¹) at 25 °C. According to the Stejskal and Tanner’s reports,²,³ when $\ln I / I_0$ versus $g^2$ were plotted, where $I$ and $g$ are the echo intensity and (pulsed) gradient strength, respectively, the slope of the line given by $D/(\Delta - \delta/3)\gamma^2\delta^2$. 
Figure S5. Stejskal-Tanner plots $\ln(I/I_0)$ vs. $g^2$ of 3 at 30 °C in DMSO-$d_6$ (filled rhombic) and DMSO-$d_6$-D$_2$O (filled square).
Figure S6. The ROESY spectra of 3 in DMSO-$d_6$ (a) and in DMSO-$d_6$/H$_2$O (b). To clarify the illustration, an another long alkyl chain was omitted.