Supporting Information for:

Formation of Supramolecular Isomers; Poly[2]rotaxane and Supramolecular Assembly

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Contents

Experimental Section

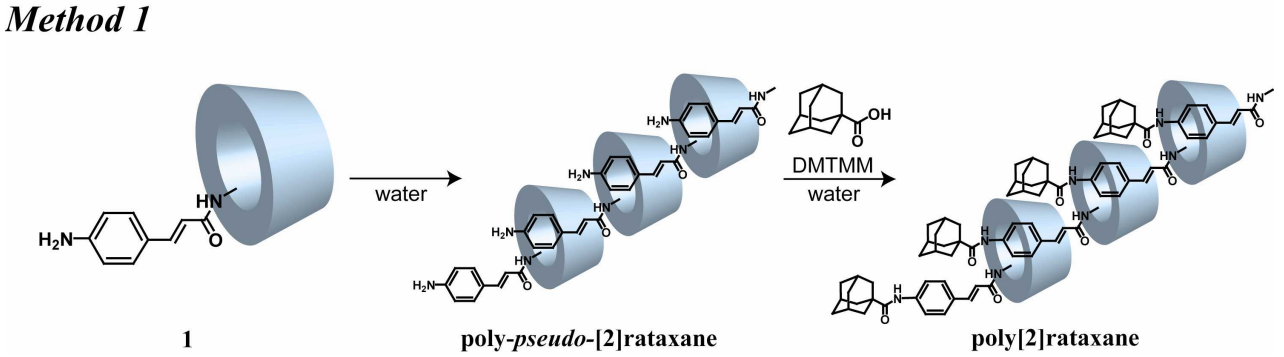
Materials

α-CD (α-Cyclodextrin) was obtained from Junsei Chemical Co., Ltd. *trans-p*-Aminocinnamic acid, *N*-(9-fluorenylmethoxycarbonyl)succinimide, 1-adamantanecarboxylic acid, and 1-adamanatanecarbonyl chloride were obtained from Tokyo Kasei Kogyo, Co., Ltd. *N*, *N'*-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole were obtained from Nacalai Tesque Inc. Piperidine was obtained from Sigma-Aldrich. Dimethyl sulfoxide-d_6 (DMSO-d_6) and D_2O used as solvents for NMR measurements were obtained from Euriso-top. DIAION HP-20® was obtained from Mitsubishi Chemical Corporation. 3^A-Deoxy-3^A-amino-*altro*-α-CD[15] (3-NH_2-α-CD), 4-(4, 6-dimethoxy-1, 3, 5-triazin-2-yl)-4-methylmorpholinium chloride[16] (DMTMM), and **3**[17] were prepared according to the method reported previously.

Synthesis

Preparation of Poly[2]rotaxane

Scheme S1. Preparation of Poly[2]rotaxane.

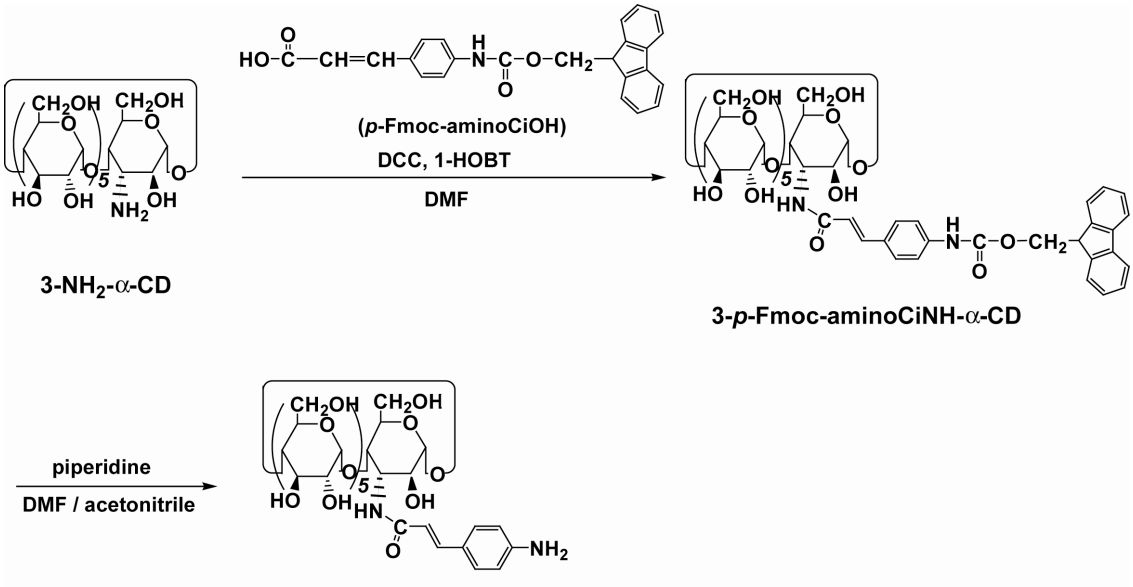


1 was found to form supramolecular polymers as well as 3-cinnamamide-α-CD. To an aqueous solution of **1** (40 mM) was added 1.5 M excess DMTMM and 1.5 M excess 1-adamanatanecarboxylic acid in aqueous solution, and was stirred for 1 day (Method 1). After removal of the insoluble materials by filtration, the filtrate was poured into acetone. The resulting precipitate was collected and washed with acetone.

Preparation of 3^{A}-Deoxy-3^{A}-aminocinnamamide-*altro*-α-CD (1)

1 was synthesized according to Scheme S2.

Scheme S2. Preparation of **1**.



(a) *p*-(9-Fluorenylmethyloxycarbonylamino)cinnamic acid (*p-Fmoc*-AminoCiOH)

To a solution of 9-Fluorenylmethyl *N*-succinimidyl carbonate (2.0 g, 4.4 x 10^{-4} mol) in 20 mL THF was added *trans-p*-aminocinnamic acid (330 mg, 2.2 x 10^{-3} mol). After stirring the THF solution at room temperature for 1 day, the solution became turbid gradually. The precipitate was collected by filtration and washed with dichloromethane to give *p-Fmoc*-AminoCiOH in 47% yield.

^{1}H NMR (DMSO-d_6, 270 MHz): δ 9.89 (s, 1H, N*H*), 7.91-7.31 (m, 13H, Ph part of Fmoc and cinnamic acid, and Ph-C*H*=), 6.38 (d, *J* = 16.0 Hz, 1H, =C*H*CO), 4.51 (d, *J* = 6.5 Hz, 2H, -CH_2- of Fmoc), 4.31 (t, *J* = 6.5 Hz, 1H, -C*H*- of Fmoc). Anal. calcd for $C_{24}H_{19}NO_4$: C, 74.79; H, 4.97; N, 3.63. Found: C, 74.54; H, 4.92; N, 3.82. M.p. 285 ^{o}C.

(b) 3^{A}-Deoxy-3^{A}-aminocinnamamide-*altro*-α-CD (1)

To a solution of 3-NH_2-α-CD (1.0 g, 1.0 x 10^{-4} mol) in 50 mL DMF was added *p*-Fmoc-AminoCiOH (590 mg, 1.6 x 10^{-3} mol). After the solution was cooled down below 0 ^{o}C, *N*,*N'*-dicyclohexylcarbodiimide (DCC) (280 mg, 1.3 x 10^{-3} mol) and 1-hydroxybenzotriazole (1-HOBT) (180 mg, 1.3 x 10^{-3} mol) were added. The resulting mixture was stirred at room temperature for 5 days. After insoluble materials were removed by filtration, the filtrate was poured into acetone (1 L). The precipitate was collected by

filtration and washed with acetone. The crude product was purified by column chromatography on DIAION HP-20® column (elution: water/methanol = from 100/0 to 50/50). After concentration of the eluent (water/methanol = 60/40), the residue was obtained as 3^A-deoxy-3^A-[*p*-(9-fluorenylmethyloxycarbonylamino)cinnamamide-*altro*-α-CD (3-*p*-FmocAminoCiNH-α-CD). To a solution of 3-*p*-FmocAminoCiNH-α-CD in acetonitrile (10 mL) was added piperidine (20 mL). The solution was stirred at room temperature for 2 hours. After concentration of the solution, the residue was resolved in DMF (10 mL). The DMF solution was poured into acetone (500 mL). The precipitate was collected by centrifugation and washed with acetone to give **2** in 60% yield.

^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.93 (d, *J* = 9.4 Hz, 1H, N*H*) 7.29 (d, *J* = 8.6 Hz, 2H of Ph), 7.23 (d, *J* = 15.4 Hz, 1H, PhC*H*=), 6.54 (d, *J* = 8.4 Hz, 2H of Ph), 6.21 (d, *J* = 15.4 Hz, 1H, =C*H*CO), 5.91-5.11 (m, 13H, NH_2, O(2)*H*, and O(3)*H* of α-CD), 4.91-4.83 (m, 6H, C(1)*H* of α-CD), 4.51-4.40 (m, 8H, O(6)*H* and C(6')*H* of α-CD), 4.15-3.30 (m, overlaps with HOD). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 165.4 (-*C*ONH-), 150.4, 129.2, 116.0, 113.6 (C of Ph), 139.6, 122.2 (-*C*H=*C*H-), 105.0 (C'(1) of α-CD), 102.4, 102.1, 101.9, 101.6, 101.1 (C(1) of α-CD), 84.6, 82.7, 81.8, 81.7, 80.8, 79.9 (C(4) of α-CD), 76.5, 73.6, 73.2, 72.8, 72.5, 72.3, 72.3, 72.1, 72.1, 72.1, 72.0, 71.9. 71.8, 71.8, 71.7, 71.3 (C(3), C(2), and C(5) of α-CD), 60.2, 60.1, 60.1, 59.9, 59.7 (C(6) of α-CD). Positive ion MALDI-TOF Mass *m/z* = 1362 $[M + Na]^+$. IR (KBr, cm^{-1}): 1597 (vs, $^{\nu}$C=O). Anal. calcd for $C_{45}H_{68}N_2O_{30}\cdot 5H_2O$: C, 44.78; H, 6.51; N, 2.32. Found: C, 44.72; H, 6.59; N, 2.56.

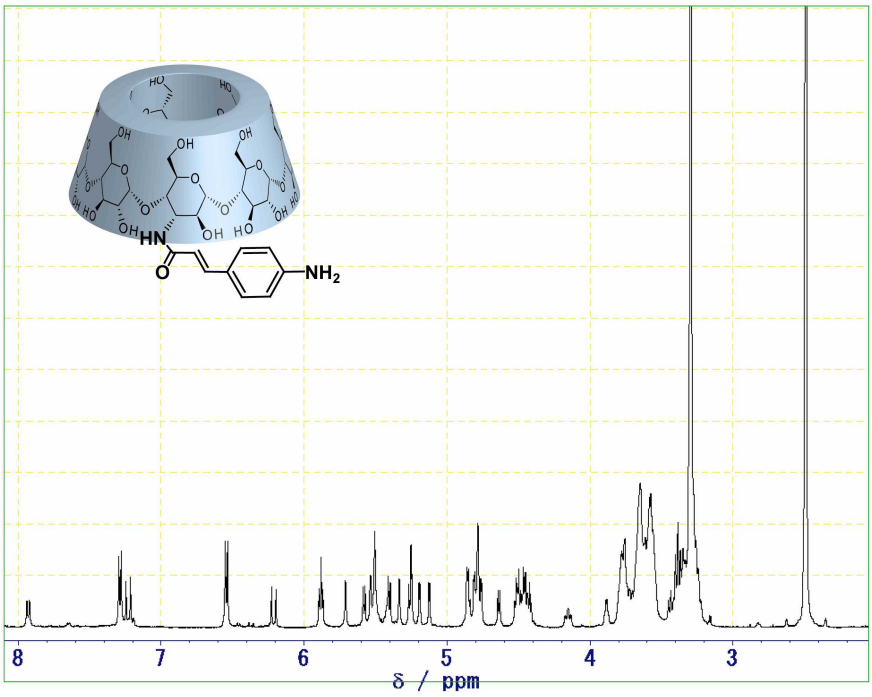


Figure S1. 500 MHz ^{1}H NMR spectrum of **1** in DMSO-d_6.

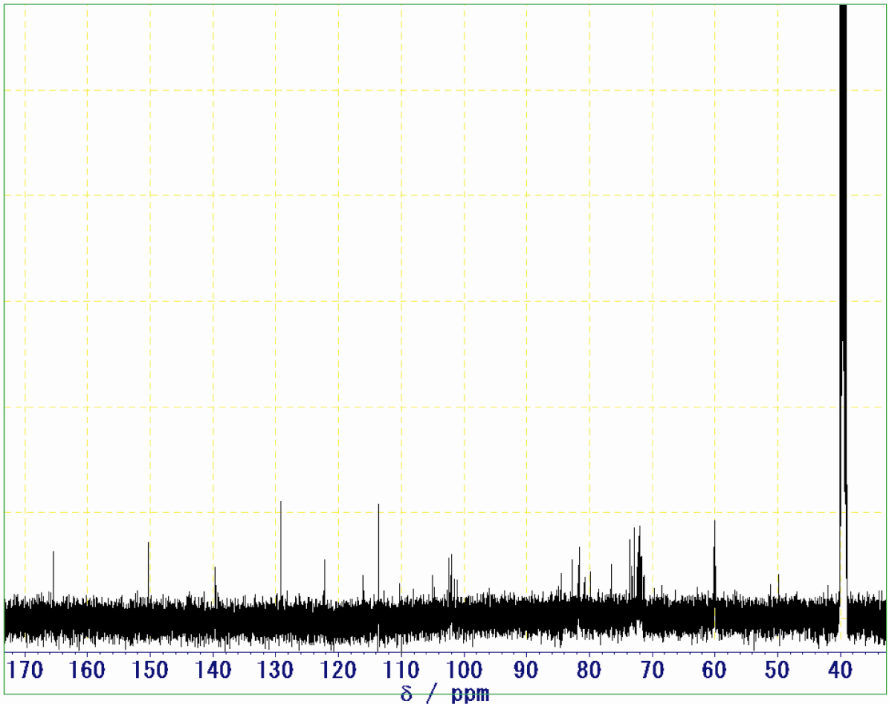
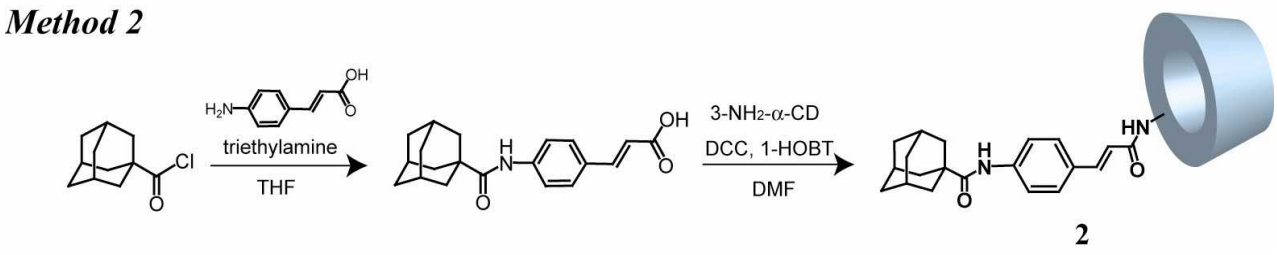


Figure S2. 125 MHz ^{13}C NMR spectrum of **1** in DMSO-d_6.

Preparation of 3^A-Deoxy-3^A-(adamantylcarbonylamino)cinnamamide-*altro*-α-CD (2)

2 was synthesized according to Scheme S3 (Method 2).

Scheme S3. Preparation of **2**.



(a) *p*-Adamantylcarbonylaminocinnamic acid (*p*-AdAminoCiOH)

To a solution of *trans*-*p*-aminocinnamic acid (1.7 g, 10.0 x 10^{-3} mol) and excessive amount of triethylamine in THF was added 1-adamantanecarbonyl chloride (1.0 g, 5.0 x 10^{-3} mol). The solution was stirred at 0 ^{o}C for 2 hours. After removal of the insoluble materials by filtration, the filtrate was concentrated by evaporator. The precipitate was washed by ethyl acetate to give *p*-AdAminoCiOH in 90% yield.

^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.16 (s, 1H, -COO*H*), 9.24 (s, 1H -CON*H*-), 7.72 (d, *J* = 8.7 Hz, 3-H of Ph), 7.59 (d, *J* = 8.7 Hz, 2-H of Ph), 7.50 (d, *J* = 16.0 Hz, 1H, Ph-C*H*=), 6.39 (d, *J* = 15.9 Hz, 1H, =C*H*-CO), 2.01(s, 4H, -C*H*-), 1.91(s, 6H, -CH_2-CH-NH), 1.70 (s, 6H, -CH-CH_2-CH-).

(b) 3^A-Deoxy-3^A-(adamantylcarbonylamino)cinnamamide-*altro*-α-CD (2)

To a solution of 3-NH_2-α-CD (1.0 g, 1.0 x 10^{-4} mol) in 50 mL DMF was added *p*-AdAminoCiOH (340 mg, 1.0 x 10^{-4} mol). After the solution was cooled down below 0 ^{o}C, DCC (276 mg, 1.3 x 10^{-3} mol) and 1-HOBT (178 mg, 1.3 x 10^{-3} mol) were added. The resulting mixture was stirred at room temperature for 5 days. After insoluble materials were removed by filtration, the filtrate was poured into acetone (1 L). The precipitate was purified by preparative reversed phase chromatography (elution: water-acetonitrile) to give **2** in 35% yield.

^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.21 (s, 1H, -CON*H*-Ph), 8.10 (d, *J* = 8.8 Hz, 1H CON*H*-CO-), 7.71 (d, *J* = 8.6 Hz, 3-H of Ph), 7.55 (d, *J* = 8.6 Hz, 2-H of Ph), 7.35 (d, *J* = 15.6 Hz, 1H, Ph-C*H*=), 6.45 (d, *J* = 15.6 Hz, 1H, =C*H*-CO), 5.96-5.15 (m, 11H, O(2)*H* and O(3)*H* of α-CD), 4.91-4.83 (m, 6H, C(1)*H* of α-CD), 4.51-4.40 (m, 8H, O(6)*H* and C(6')*H* of α-CD), 4.15-3.30 (m, overlaps with HOD). ^{13}C NMR (D_2O 150 MHz): δ 180.3 (-*C*ONH-), 168.5 (-*C*ONH-), 140.5 (Ph-*C*H=CH-), 138.6, 131.2, 129.2, 122.9 (C of Ph), 118.2 (-CH=*C*H-CONH-), 105.1 (C'(1) of α-CD), 102.3, 102.2, 102.1, 101.5, 99.9

(C(1) of α-CD), 81.8, 81.6, 80.4 (C(4) of α-CD), 79.4 (C'(4) of α-CD), 76.0, 74.1, 73.9, 73.7, 73.4, 72.5, 72.3, 72.1, 72.0, 71.9, 71.8, 71.7, 71.6 (C(3), C(2), and C(5) of α-CD), 73.1 (C'(2) of α-CD), 71.4 (C'(3) of α-CD), 61.1, 60.5, 60.2 (C(6) of α-CD), 60.2 (C'(6) of α-CD), 50.5 (C'(5) of α-CD), 41.5, 38.7, 36.1, 28.1 (C of adamantyl). Positive ion MALDI-TOF Mass *m/z* = 1302 $[M + Na]^+$. Anal. calcd for $C_{56}H_{82}N_2O_{31} \cdot 5.1H_2O$: C, 47.25; H, 6.94; N, 1.97. Found: C, 47.11; H, 6.77; N, 2.05.

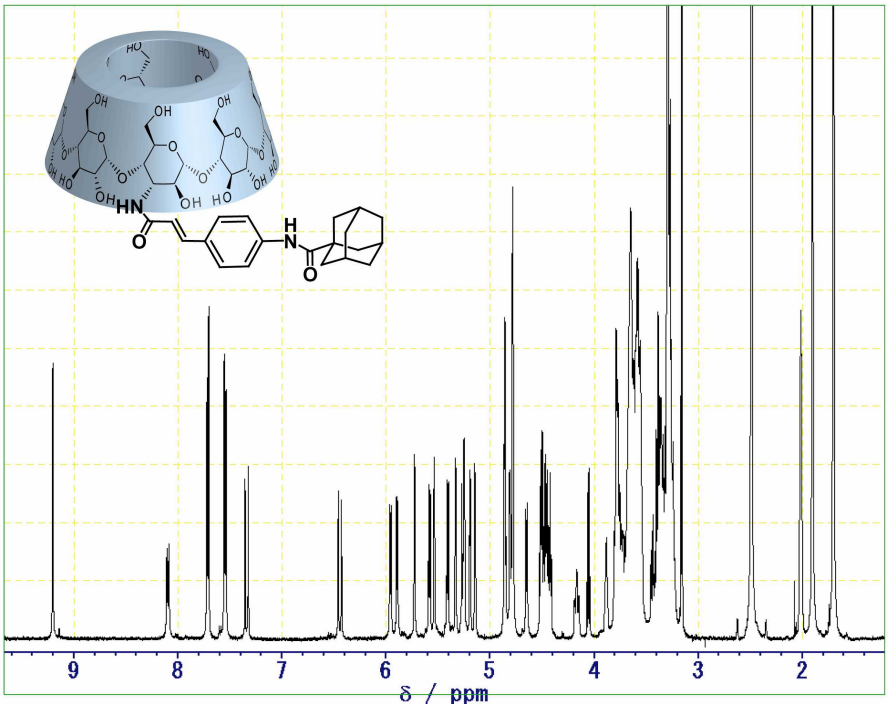


Figure S3. 500 MHz ^{1}H NMR spectrum of **2** in DMSO-d_6.

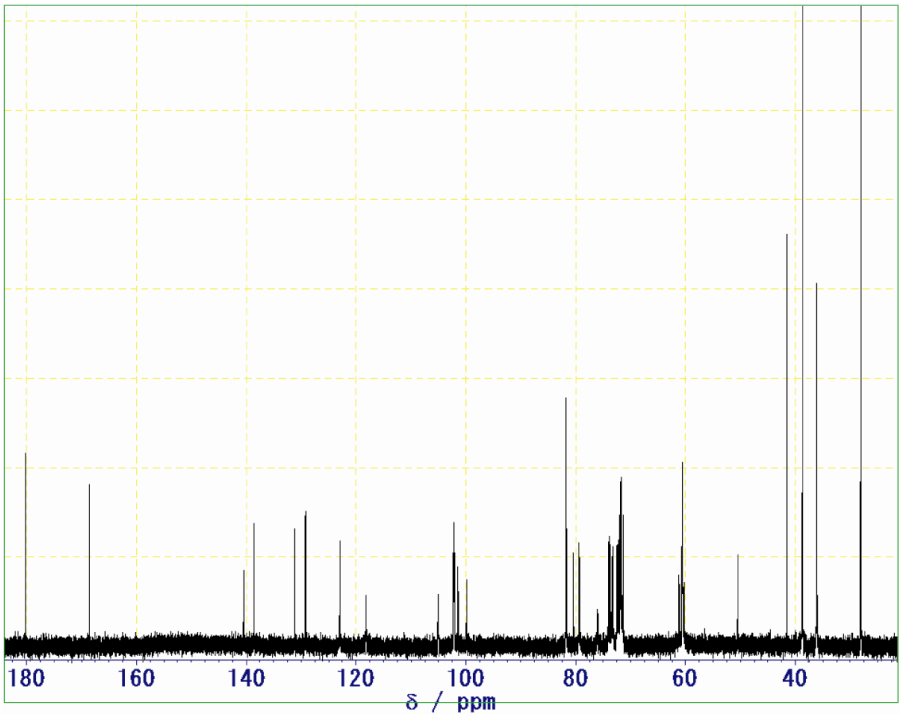


Figure S4. 150 MHz ^{13}C NMR spectrum of **2** in D_2O.

Measurement

The ^{1}H NMR spectra were recorded on a JEOL JNM EX-270 and JEOL JNM LA-500 NMR spectrometer at 30 ^{o}C. Chemical shifts were determined with reference to solvent values (δ 2.49 ppm for DMSO-d_6 and δ 6.45 ppm for D_2O). 2D NMR and pulsed field gradient spin-echo (PFGSE) NMR experiments were obtained with D_2O as the solvent at 30 ^{o}C on a VARIAN-INOVA-600 NMR spectrometer. The PFGSE NMR diffusion measurements were carried out by using the bipolar pulse pair stimulated echo (BPPSTE).[14] The pulsed gradients' strength was increased from 6.36×10^{-1} to 61.7 gauss/cm. The time separation of pulsed field gradients and their duration were 0.10 and 1.0×10^{-3} s. The sample was not spun and the airflow was disconnected. The shape of the gradient pulse was rectangular, and its strength varied automatically during the course of the experiments. The D values where D represents diffusion coefficient were determined from the slope of the regression line $\ln (I / I_o)$ versus G^2, according to Equation (1).

$$\ln (I / I_o) = - \gamma^2 G^2 \delta^2 (\Delta - \delta / 3 - \tau / 2) D \qquad (1)$$

Where (I / I_o) is (observed spin echo intensity / intensity without gradients), G is gradient strength, Δ is delay between the midpoints of the gradients, δ is gradient length, and τ is 90 ^{o}-180 ^{o} pulse distance. The calibration of the gradients was carried out by a diffusion measurement of H_2O ($D_{H2O} = 2.30 \times 10^{-9}$ m^2 s^{-1})[18] at 25 ^{o}C. The preparative reversed phase chromatography was carried out with a Waters Delta 600 system (column: SunFireTM Prep C18 19 × 150 mm). Positive-ion matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry experiments were performed using a Shimadzu / KRATOS Axima CFR Ver.2.2.3 mass spectrometer calibrated by α-cyano-4-hydroxycinnamic acid and insulin. Electrospray ionization mass spectrometry measurements were performed by using a JASCO Q-Tof Premier system mass spectrometer.

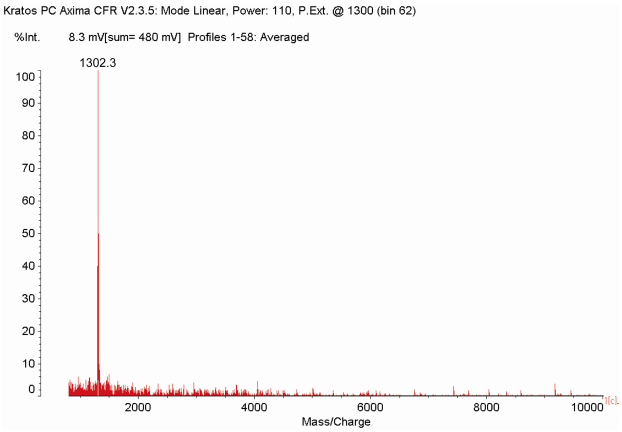


Figure S5. MALDI TOF mass spectrum of **2**.

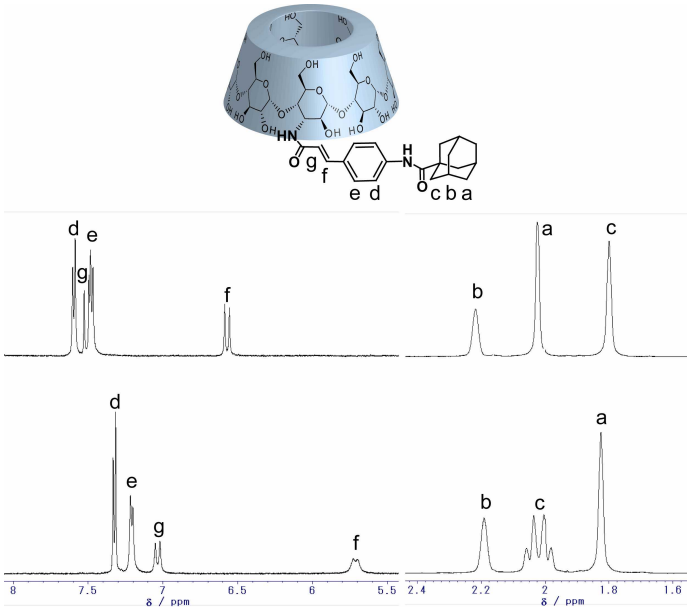


Figure S6. Partial 500 MHz ^{1}H NMR spectra of **2** at 5mM (lower) and addition of β-CD (upper) in D_2O.

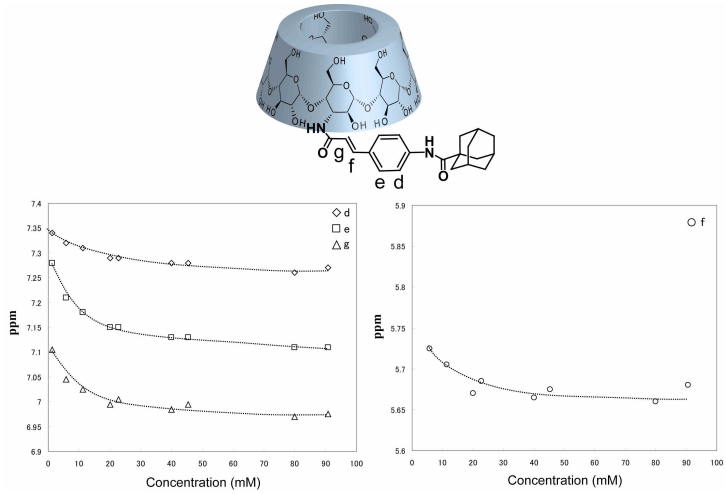


Figure S7. Chemical shifts of aromatic protons for **2** as a function of concentration.

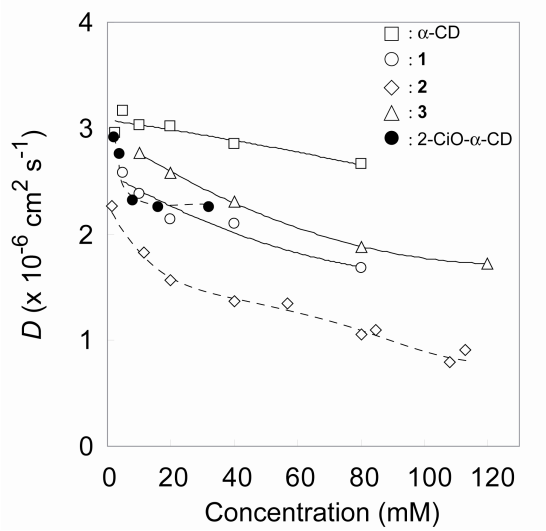


Figure S8. Diffusion coefficients (*Ds*) of **1** (circle), **2** (rhombic), and **3** (triangle) at various concentrations in D_2O.

Table S1. Diffusion coefficients of **2** and HDO.

Conc. [mM]		1.3	20	40	57	80	85	108	113
Ds (2)	[$\times 10^{-6}$cm^2·s^{-1}]	2.27	1.57	1.36	1.35	1.05	1.10	0.80	0.91
Ds' (2)	[$\times 10^{-6}$cm^2·s^{-1}]		1.65	1.51	1.56	1.25	1.31	1.00	1.12
Ds (HDO)	[$\times 10^{-6}$cm^2·s^{-1}]	20.4	19.4	18.3	17.2	16.6	16.4	15.9	15.8

Ds ; experimentally estimated value Ds' ; corrected value

$Ds' = Ds \times (2 - Ds_{(HDO\_x\ mM)} / Ds_{(HDO\_1.3\ mM)})$ [x = 20, 40, 57, 80, 85, 108, 113]

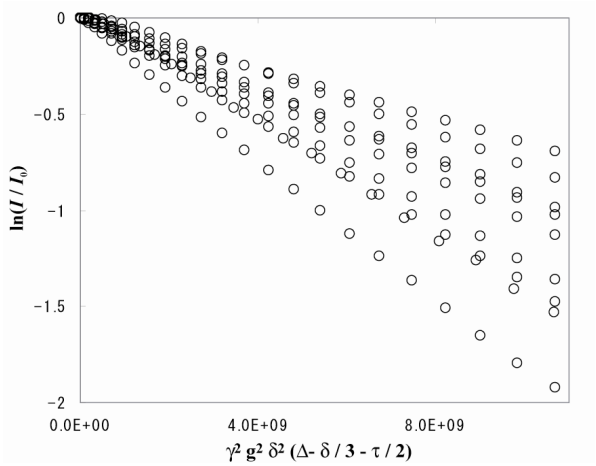


Figure S9. Signal intensity decay carves of **2** as a function of the pulsed field gradient strength.

References

15 (a) K. Takahashi, K. Hattori, F. Toda, *Tetrahedron Lett*., 1984, **25**, 3331. (b) H. Ikeda, Y. Nagano, Y. Du, T. Ikeda, F. Toda, *Tetrahedron Lett*., 1990, **31**, 5045.

16 M. Kunishima, C. Kawachi, J. Morita, K. Terao, F. Iwasaki, S, Tani, *Tetrahedron*., 1999, **55**, 13159.

17 M. Miyauchi, A. Harada, *J. Am. Chem. Soc*., 2004, **126**, 11418.

18 H. *Z.* Weingartner, *Phys. Chem. NF (Leipzig)*., 1982, **132**, 129.