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Supplementary Information

Separation of proteins using supramolecular gel electrophoresis

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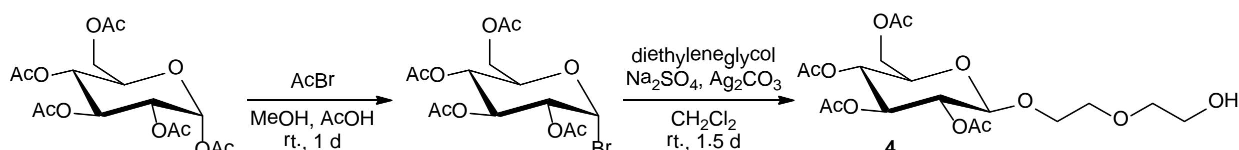
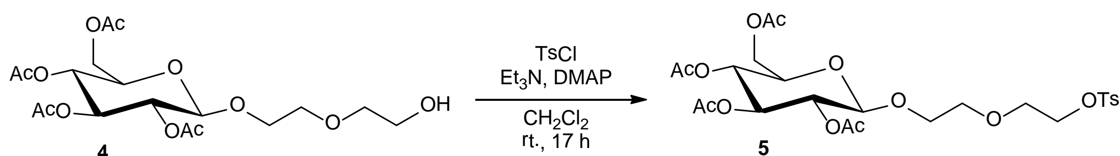
General

Chemicals and solvents were obtained from commercial suppliers. CH_2Cl_2, 1,2-dichloroethane, and Et_3N were distilled from CaH_2 before to use. ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-ECA600 spectrometer. Mass spectra were measured on a JEOL JMS-T100LC AccTOF spectrometer. SEM studies were carried out on a JEOL JSM-6300 spectrometer.

Synthesis and physical properties

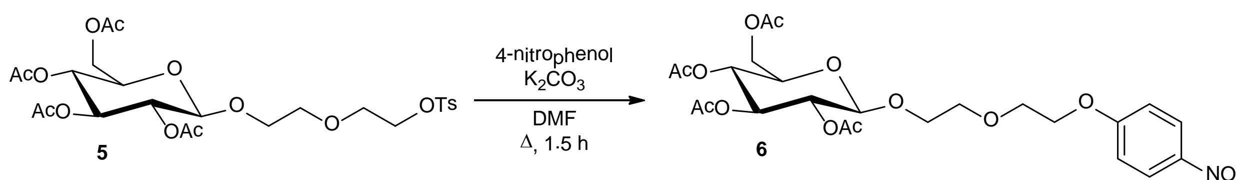


Ac$_4$Glc-DEG (4).[1] To a solution of 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranside (4.00 g, 10.3 mmol) in AcOH (30.7 mL) were added AcBr (2.65 mL, 35.8 mmol) and CH_3OH (0.55 mL, 13.6 mmol), and the reaction mixture was stirred under argon atmosphere at room temperature for 1 day. The solvent was removed under reduced pressure and obtained yellow solid α-glycosyl bromide was afforded to the next reaction without further purification. To the soltion of crude α-glycosyl bromide in CH_2Cl_2 (400 mL) was added Na_2SO_4 (14.6 g, 103 mmol) and diethyleneglycol (10.0 mL, 105 mmol). After stirring at room temperature for 15 min, under argon atmosphere, Ag_2CO_3 (5.89 g, 21.4 mmol) was added to the reaction mixture and stirring was countinued for 1.5 day. The reaction mixture was filtered and washed with water. The organic layer was washed with brine, and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the crude product was purified by column chromatography (SiO_2, hexane/ethyl acetate 1/2 to ethyl acetate). The desired product **4** was obtained as a white solid (2.82 g, 63% 2steps). ^1H NMR (600 MHz, $CDCl_3$) δ 2.01 (s, 3H), 2.03 (s, 3H), 2.06 (s, 3H), 2.09 (s, 3H), 2.21 (t, J = 6.2 Hz, 1H), 3.56-3.61 (m, 2H), 3.66 (t, J = 4.5 Hz, 2H), 3.70-3.76 (m, 4H),3.97 (dt, t, J = 8.9, 5.5 Hz, 1H), 4.15 (dd, J = 12.4, 2.1 Hz, 1H), 4.26 (dd, J = 13.4, 4.8 Hz, 1H), 4.61 (d, J = 7.6 Hz,1H), 5.00 (dd, J = 7.6 and 9.6 Hz, 1H), 5.10 (t, J = 9.6 Hz, 1H), 5.22 (t, J = 6.2 Hz, 1H).

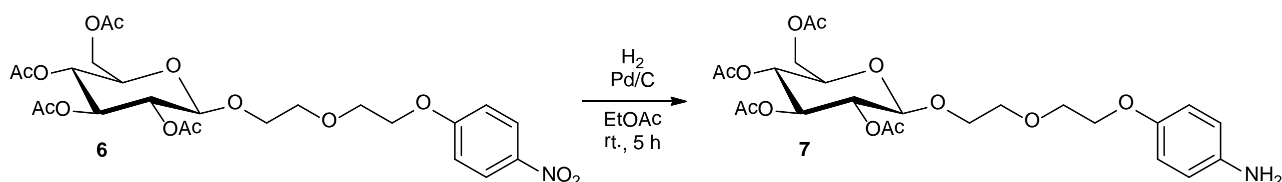


Tosylate (5). To a mixture of *p*-tosyl chloride (1.22 g, 6.39 mmol) and DMAP (24.6 mg, 0.201 mmol) in CH_2Cl_2 (12.5 mL) was added **4** (2.77 g, 6.35 mmol) and anhydrous Et_3N (2.65 mL) under argon atmosphere at 0 °C. The reaction mixture was stirred at room temperature for 17 h and then quenched with saturated NH_4Cl. Separated organic layer was washed with brine, and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the crude product was purified by column

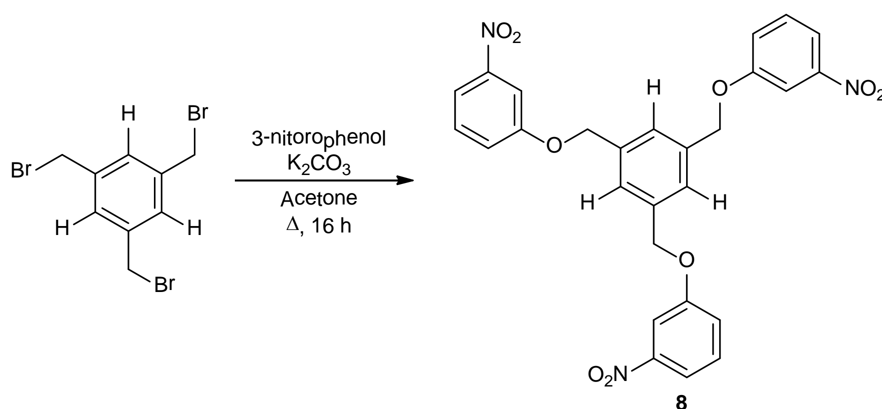
chromatography (SiO_2, hexane/ethyl acetate 1/1 to 1/2). The desired product **5** was obtained as a white solid (3.54 g, 95%). M.p. 92.0−94.1 °C; $[\alpha]_D^{24.0} = -16.7°$ (c = 0.9 in DMSO); ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.00 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.08 (s, 3H), 2.45 (s, 3H), 3.57-3.59 (m, 2H), 3.66 (dt, *J* = 4.8, 1.4 Hz, 2H), 3.68-3.71 (m, 2H), 3.89 (dt, *J* = 9.4, 5.5 Hz, 1H), 4.13-4.15 (m, 3H), 4.26 (dd, *J* = 12.4, 4.8 Hz, 1H), 4.58 (d, *J* = 8.3 Hz, 1H), 4.97 (dd, *J* = 8.2, 9.6 Hz, 1H), 5.08 (t, *J* = 9.4 Hz, 1H), 5.21 (t, *J* = 9.6 Hz, 1H), 7.36 (d, *J* = 8.3 Hz, 2H), 7.80 (d, *J* = 8.2 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 20.72, 20.75, 20.84, 21.74, 62.03, 68.49, 68.90, 69.09, 69.30, 70.51, 71.38, 71.90, 72.91, 100.91, 128.08, 129.97, 133.10, 144.98, 169.49, 169.53, 170.32, 170.74; HRMS (ESI, $M+Na^+$) calcd for $C_{26}H_{34}NaO_{14}S$: 613.1567; found 613.1536.



Nitrophenoxyde (6). To a mixture of 4-nitrophenol (396 mg, 2.85 mmol) and K_2CO_3 (0.91 g, 6.56 mmol) in DMF (25.0 mL) was added **5** (1.29 g, 2.19 mmol) in DMF (11.0 mL) under argon atmosphere at room temperature. Then the mixture was kept at 90 °C for 1.5 h. The reaction mixture was filtered and washed with water. The organic layer was washed with brine, and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the crude product was purified by column chromatography (SiO_2, hexane/ethyl acetate 1/1 to 1/2). The desired product **6** was obtained as a white solid (1.18 g, 97%). M.p. 109.5−110.5 °C; $[\alpha]_D^{19.0} = -9.9°$ (c = 1.0 in DMSO); ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.01 (s, 3H), 2.03 (s, 6H), 2.08 (s, 3H), 3.66-3.77 (m, 4H), 3.87 (t, *J* = 4.8 Hz, 2H), 3.98 (dt, *J* = 10.5, 4.0 Hz, 1H), 4.14 (dd, *J* = 12.4, 2.1 Hz, 1H), 4.19-4.21 (m, 2H), 4.25 (dd, *J* = 12.0, 4.5 Hz, 1H), 4.59 (d, *J* = 7.6 Hz, 1H), 4.99 (t, *J* = 8.9 Hz, 1H), 5.08 (t, *J* = 9.6 Hz, 1H), 5.19 (t, *J* = 9.6 Hz, 1H), 6.99 (d, *J* = 8.9 Hz, 2H), 8.21 (d, *J* = 8.9 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 20.73, 20.75, 20.80, 20.87, 62.10, 68.32, 68.54, 69.22, 69.65, 70.77, 71.43, 71.98, 72.89, 100.98, 114.72, 126.08, 141.84, 163.93, 169.45, 169.56, 170.39, 170.76; HRMS (ESI, $M+Na^+$) calcd for $C_{24}H_{31}NnaO_{14}$; 580.1642; found 580.1681.



Aminophenoxyde (7). A mixture of **6** (1.20 g, 2.15 mmol) and 10% Pd on carbon (120 mg) in ethyl acetate (23.0 mL) was stirred at room temperature for 5 h under hydrogen atmosphere. The reaction mixture was filtered, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (SiO_2, hexane/ethyl acetate 1/2 to1/4). The desired product **7** was obtained as a yellow caramel (1.09 g, 96%). $[\alpha]_D^{21.0} = -21.3°$ (c = 0.9 in DMSO); ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.00 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.08 (s, 3H), 3.43 (s, 2H), 3.67-3.79 (m, 6H), 3.96 (dt, *J* = 11.2, 4.3 Hz, 1H), 4.04 (t, *J* = 4.8 Hz, 2H), 4.13 (dd, *J* = 12.4, 2.7 Hz, 1H), 4.25 (dd, *J* = 12.4, 4.8 Hz, 1H), 4.62 (d, *J* = 8.2 Hz, 1H), 5.00 (dd, *J* = 9.3, 7.9 Hz, 1H), 5.08 (t, *J* = 9.6 Hz, 1H), 5.20 (t, *J* = 9.3 Hz, 1H), 6.64 (d, *J* = 8.9 Hz, 2H), 6.76 (d, *J* = 8.9 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 20.68, 20.70, 20.74, 20.82, 61.99, 68.22, 68.47, 69.17, 70.13, 70.57, 71.33, 71.80, 72.90, 100.89, 115.92, 116.41, 140.38, 151.90, 169.51, 170.35, 170.77; HRMS (ESI, M+Na$^+$) calcd for $C_{24}H_{33}NnaO_{12}$; 550.1900; found 550.1894.



1,3,5-tris(3-nitrophenoxymethyl)benzene (8).[2] To a mixture of 3-nitrophenol (4.68 g, 33.6 mmol) and K_2CO_3 (12.4 g, 89.7 mmol) in acetone (86.0 mL) was added 1,3,5-tris(bromomethyl)benzene (4.00 g, 11.2 mmol) under argon atmosphere at 0 °C. The reaction mixture was stirred at 70 °C for 16 h. Then the reaction mixture was diluted with chloroform, and the precipitate was removed by filtration. The solvent was removed under reduced pressure, and the crude product was purified by reprecipitation from dichloromethane and hexane to give the desired product **8** as a white solid (5.89 g, 99%). M.p. 151-153 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.20 (s, 6H), 7.30 (dd, *J* = 8.3, 2.4 Hz, 3H), 7.45 (dd, *J* = 8.1 Hz, 3H), 7.52 (s, 3H), 7.81 (t, *J* = 2.2 Hz, 3H),7.85 (dd, *J* = 7.8, 1.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 70.00, 109.14, 116.29, 121.91, 126.26, 130.11, 137.17, 149.21, 158.89; HRMS (ESI, M+Na$^+$) calcd for $C_{27}H_{21}N_3NaO_9$: 554.1176; found 554.1192.

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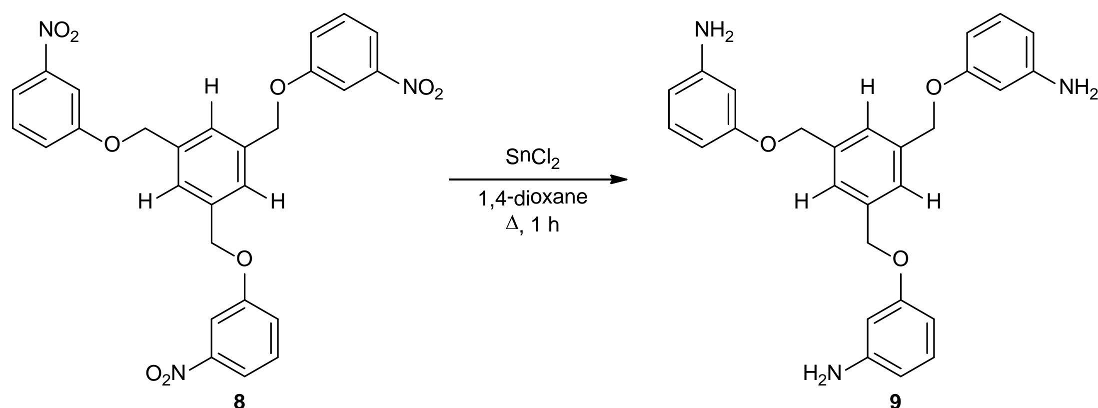
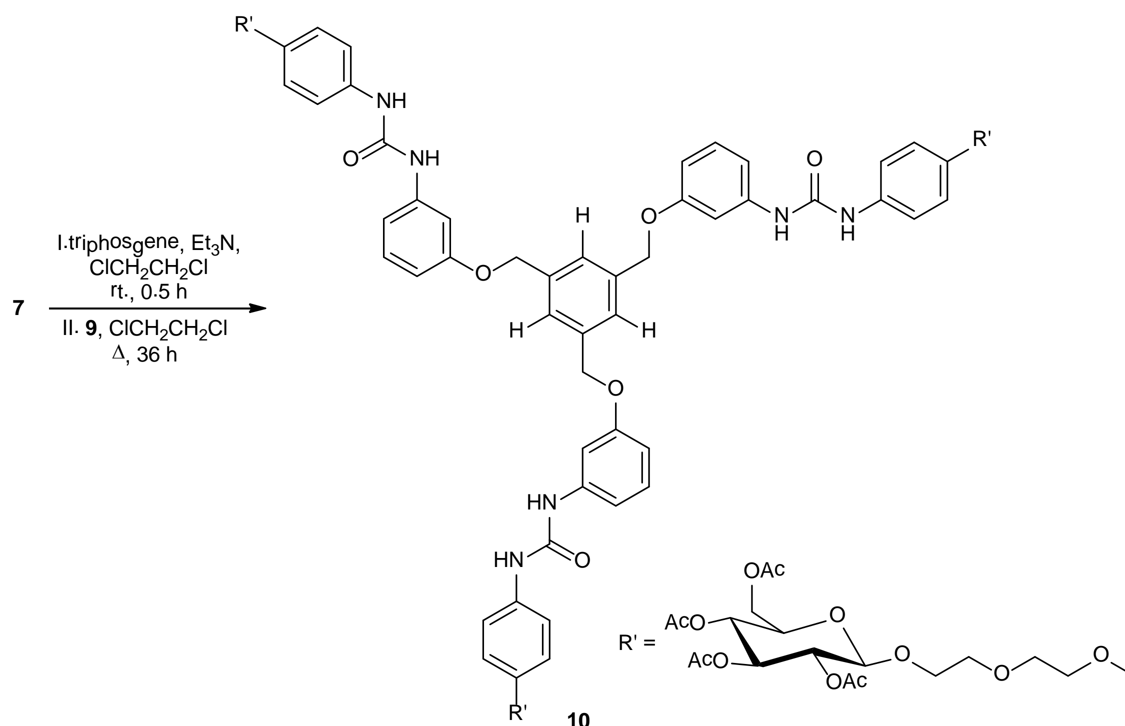


1,3,5-tris(3-aminophenoxymethyl)benzene (9).[2] A mixture of **8** (1.46 g, 2.75 mmol) and tin(II)chloride dihydrate (9.36 g, 41.5 mmol) in 1,4-dioxane (16.8 mL) was stirred at room temperature for 1 h. Then the mixture was kept at 50 °C for 1 h. Then the reaction mixture was poured into ice-cooled water, and neutralized with saturated sodium bicarbonate solution. The precipitate was removed by filtration through Celite, and the Celite was washed with ethyl acetate. The organic layer was separated and the aqueous solution was extracted with ethyl acetate. The combined organic layer was washed with brine, and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the crude product was purified by reprecipitation from ethyl acetate and hexane to give the desired product **9** as a white solid (1.12 g, 92%). M.p. 180-182 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.99 (s, 6H), 5.05 (s, 6H), 6.15 (d, J = 7.8 Hz, 6H), 6.21 (s, 3H), 6.89 (t, J = 7.8 Hz, 3H), 7.41 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 69.57, 102.04, 104.92, 108.25, 125.75, 130.14, 138.03, 147.78, 159.90; HRMS (ESI, M+Na$^+$) calcd for $C_{27}H_{27}N_3NaO_3$: 464.1950; found 464.1924.



Tris-urea (10). To a soltion of **7** (562 mg, 1.07 mmol) in 1,2-dichloroethane (2.00 mL) was added triphosgene (317 mg, 1.07 mmol) in 1,2-dichloroethane (2.20 mL) and Et_3N (300 μL, 2.15 mmol), successively under argon atmosphere at 0 °C. The reaction mixture was stirred at room temperature for 0.5 h. The low boiling point compounds were removed under reduced pressure and corresponding isocyanate was obtained as white solid. A solution of **9** (157 mg, 0.355 mmol) in 1,2-dichloroethane (4.20 mL) was added to the isocyanate under argon atmosphere at 0 °C. The reaction mixture was kept at 90 °C for 36 h and then cooled to room temperature and neutralized with saturated aqueous NH_4Cl solution. The organic layer was washed with brine, and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the crude product was purified by re-precipitation (hexane/ethyl acetate). The desired product **10** was obtained as a yellow solid (624 mg, 84%). M.p. 136.7−137.8 °C; $[\alpha]_D^{20.0} = -14.8°$ (c = 1.0 in DMSO); ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.92 (s, 9H), 1.97 (s, 18H), 2.00 (s, 9H), 3.55-3.61 (m, 6H), 3.63-3.66 (m, 3H), 3.69 (t, J = 4.5 Hz, 6H), 3.80-3.83 (m, 3H), 3.96 (ddd, J = 10.0, 5.2, 2.4 Hz, 3H), 4.00-4.02 (m, 9H), 4.16 (dd, J = 12.4, 4.8 Hz, 3H), 4.74 (dd, J = 9.6, 8.2 Hz, 3H), 4.83 (d, J = 8.3 Hz, 3H), 4.89 (t, J = 10.0 Hz, 3H), 5.11 (s, 6H), 5.25 (t, J = 9.6 Hz, 3H), 6.62 (d, J = 8.2 Hz, 3H), 6.84 (d, J = 8.9 Hz, 6H), 6.95 (d, J = 8.3 Hz, 3H), 7.15 (t, J = 8.2 Hz, 3H), 7.24 (s, 3H), 7.32 (t, J = 8.9 Hz, 6H), 7.52 (s, 3H), 8.45, (s, 3H), 8.57 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 20.28, 20.36, 20.38, 20.50, 61.71, 67.28, 68.19, 68.62, 68.94, 69.06, 69.47, 70.57, 70.89, 72.07, 99.51, 104.77, 107.85, 110.75, 114.62, 120.00, 126.32, 129.57, 132.77, 137.66, 141.15, 152.63, 153.63, 158.77, 169.09, 169.29, 169.56, 170.05; HRMS (ESI, M+Na$^+$) calcd for $C_{102}H_{120}N_6NaO_{42}$: 2123.7336; found 2123.7363.

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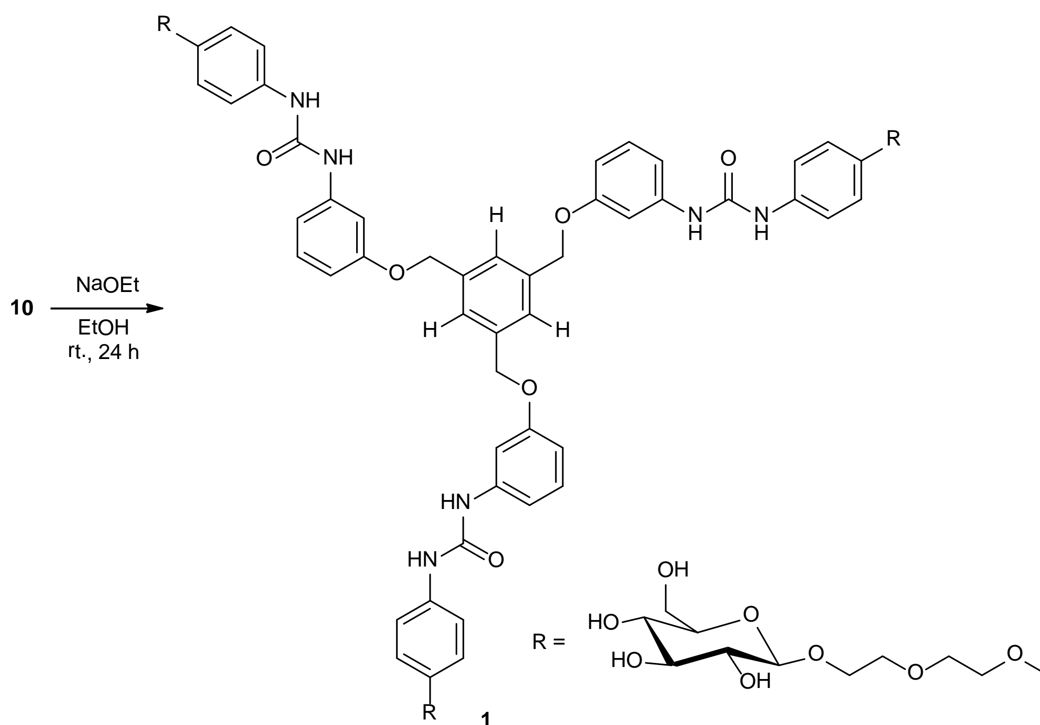
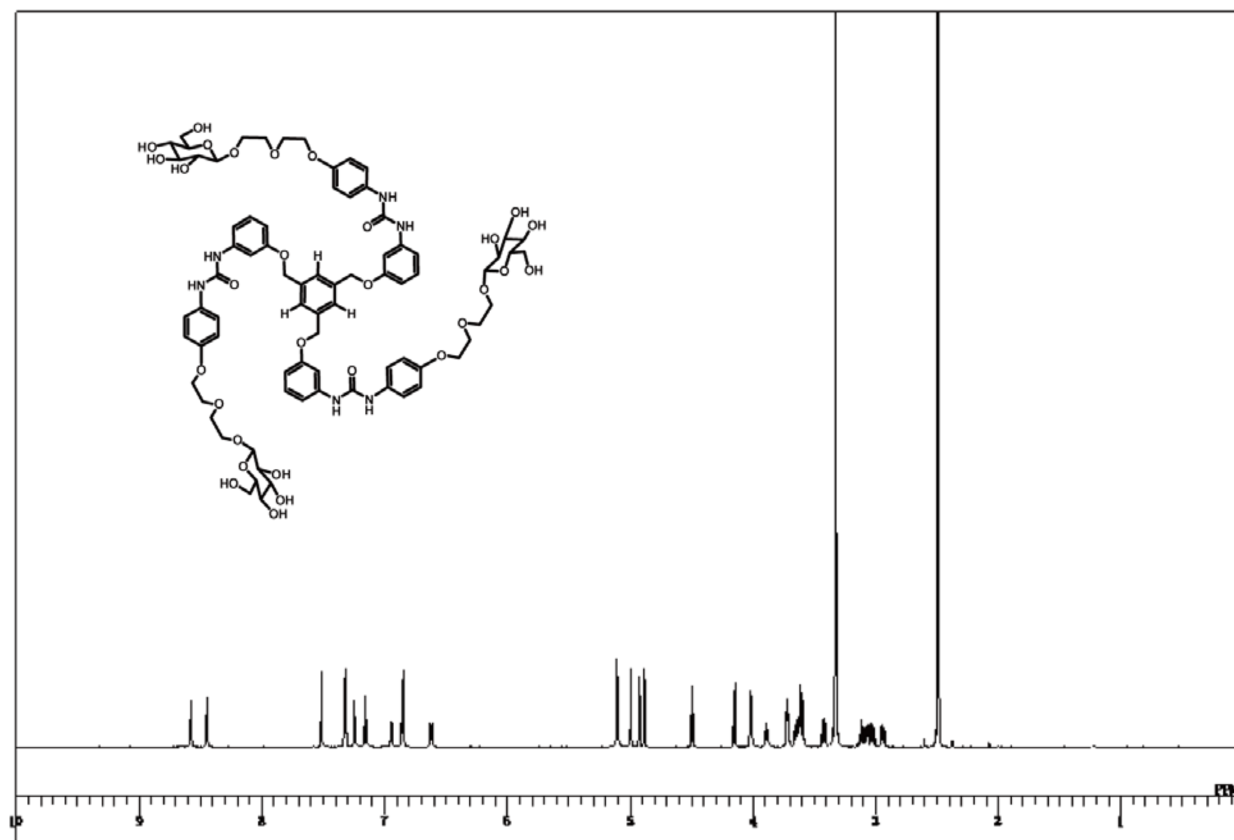
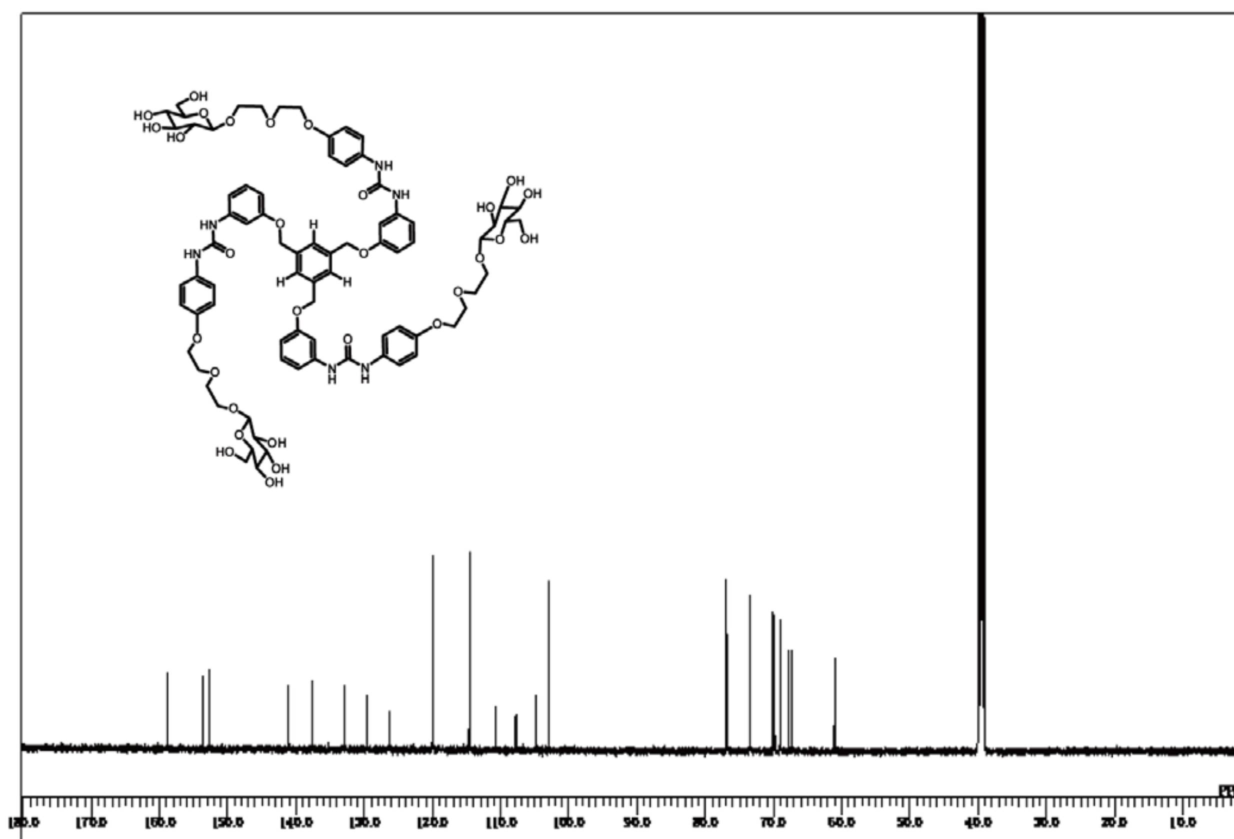


Hydrogelator (1). A mixture of **10** (301 mg, 0.143 mmol) and NaOEt (29.1 mg, 0.428 mmol) in ethanol (3.40 mL) was stirred at room temperature for 1 d. The reaction mixture was purified by membrane dialysis, and the solvent was removed under reduced pressure. The desired product **1** was obtained as pale brown solid (221 mg, 97%). M.p. 162.0-165.8 °C; $[\alpha]_D^{19.0} = -14.5°$ (c = 1.0 in DMSO); ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.93-2.96 (m, 3H), 3.01-3.04 (m, 3H), 3.06-3.13 (m, 6H), 3.40-3.44 (m, 3H), 3.59-3.67 (m, 12H), 3.71-3.73 (m, 6H), 3.86-3.91 (m, 3H), 4.02 (t, J = 4.8 Hz, 6H), 4.15 (d, J = 7.6 Hz, 3H), 4.49 (t, J = 5.8 Hz, 3H), 4.88 (d, J = 5.5 Hz, 3H), 4.92 (d, J = 4.8 Hz, 3H), 4.99 (d, J = 4.8 Hz, 3H), 5.11 (s, 6H), 6.62 (d, J = 8.2 Hz, 3H), 6.85 (d, J = 8.9 Hz, 6H), 6.94 (d, J = 8.9 Hz, 3H), 7.15 (t, J = 8.2 Hz, 3H), 7.25 (s, 3H), 7.32 (d, J = 8.9 Hz, 6H), 7.52 (s, 3H), 8.45 (s, 3H), 8.58 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 61.09, 67.29, 67.87, 68.94, 69.04, 69.84, 70.05, 73.41, 76.75, 76.91, 103.01, 104.79, 107.80, 110.78, 114.62, 120.01, 126.33, 129.56, 132.83, 137.67, 141.22, 152.69, 153.62, 158.76; HRMS (ESI, M+Na$^+$) calcd for $C_{78}H_{96}N_6NaO_{30}$; 1619.6069; found 1619.6057.

^{1}H NMR spectrum (600 MHz, DMSO-d_6, 298 K) of LMWHG **1**



^{13}C NMR spectrum (150 MHz, DMSO-d_6, 298 K) of LMWHG **1**



Gelation Experiments: TGS solutionr gel of 1. A mixture of **1** and Tris-Glycine-SDS (TGS) solution (Tris : 25 mM; Glycine : 192 mM; SDS : 0.1%) in test tube (ϕ = 6.2 mm) was heated at 100 °C. Obtained solution was gradually cooled to ambient temperature. Gel formation was evaluated by the inverted tube test. A mixture remaining at the top of an inverted test tube was defined as a gel.

Estimation of gel-sol phase transition temperature (T_{gel}). Temperatures of gel-sol phase transition were determined by the inverse flow method. A sealed test tube containing the gel was immersed upside-down in a thermostated oil bath. The temperature of the bath was raised at a rate of 1 °C/min. T_{gel} was defined as the temperature at which the gel fell down the tube.

Table S1. T_{gel} of TGS solution gel of 1.

1 (wt%)	1.5	2.0	2.5	3.0	4.0
state of gel	transparent	transparent	transparent	transparent	transparent
T_{gel} (°C)	46	92	97	>100	>100

Electrophoresis of color dye conjugated protein marker using TGS solution gel of 1, polyacrylamide gel, and agarose gel.

A capillary (ϕ = 2.0 mm, length = 120 mm) was filled with gel (ca. 80 mm), and color dye conjugated protein marker (DynaMarker® Protein MultiColor III) was applied on a side. The marker contains eight proteins: myosin (229.3 kDa, orange), β-galactosidase (140.4 kDa, blue), phosphorylase-b (95.5 kDa, purple), BSA (64.9 kDa, green), ovalbumin (44.5 kDa, blue), carbonic anhydrase (32.3 kDa, red), trypsin inhibitor (24.9 kDa, orange), and lysozyme (16.0 kDa, blue). The capillary was contributed for electrophoresis using submarine electrophoresis system.

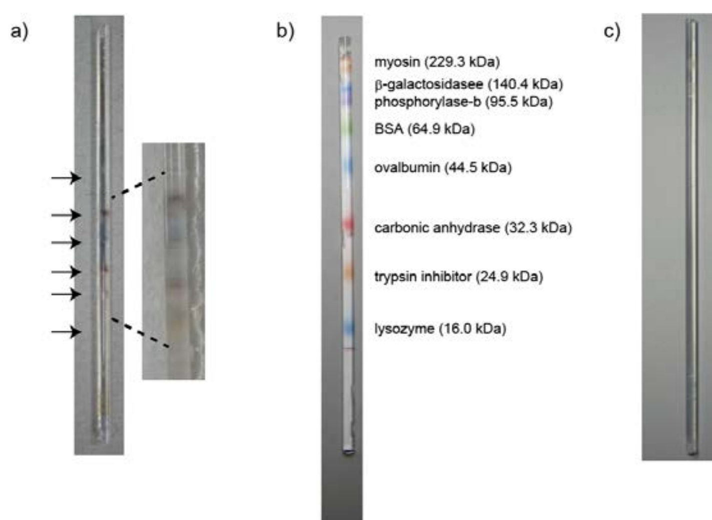


Fig. S1 Photographs of the capillary after electrophoresis using a) 2.0 wt% TGS solution gel of **1** (100 V, 40 min.), b) 12 wt% polyacrylamide gel (135 V, 40 min.), c) 2.0 wt% agarose gel (100 V, 40 min.).

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Preparation of 1-AG gel

A mixture of **1** (60 mg), agarose (60 mg), and TGS solution (3.0 mL) in test tube was dispersed by test tube mixer, and heated using microwave oven (500 W). Obtained solution was gelled at ambient temperature.

Collection of protein from 1-AG gel by centrifugation

I. A capillary (ϕ = 2.0 mm) was filled with **1**-AG gel (10 mm).

II. Protein sample (6.08 μg of ovalbumin or 3.04 μg of lysozyme in sample-loading solution) was placed on an end of **1**-AG gel.

III. Then both ends of the capillary were filled with agarose gel (2 wt%).

IV. The capillary was sunk in TGS solution of submarine electrophoresis system, and electrophoresed at 100 V for 30 min.

V. The electrophoresed **1**-AG gel was taken out from the capillary, and put in a microtube.

VI. The **1**-AG gel in the microtube was centrifuged (14,100 G, 30 min.). Extraction from the precipitate with sample-loading solution was repeated two times.

VII. The supernatant was applied to SDS-PAGE together with weighted references.

VIII. Electrophoresed polyacrylamide gel was stained with CBB.

IX. The CBB stained polyacrylamide gel was analyzed by ImageJ.

Prodedure of SDS-SUGE and the analysis using SDS-PAGE

I. A capillary (ϕ = 2 mm, length = 120 mm) was filled with **1**-AG gel (80 mm).

II. Proteins in sample-loading solution were placed on an end of **1**-AG gel.

III. Then both ends of the capillary were filled with agarose gel (2 wt%).

IV. The capillary was sunk in TGS solution of submarine electrophoresis system, and electrophoresed by optional voltage and time.

V. The electrophoresed **1**-AG gel was taken out from the capillary, and divided into eight equal parts (numbered 1 to 8 from much moved anode side).

VI. Each fragment was put in a microtube, and extracted.

VII. Extracted solutions were analyzed by typical SDS-PAGE and CBB staining.

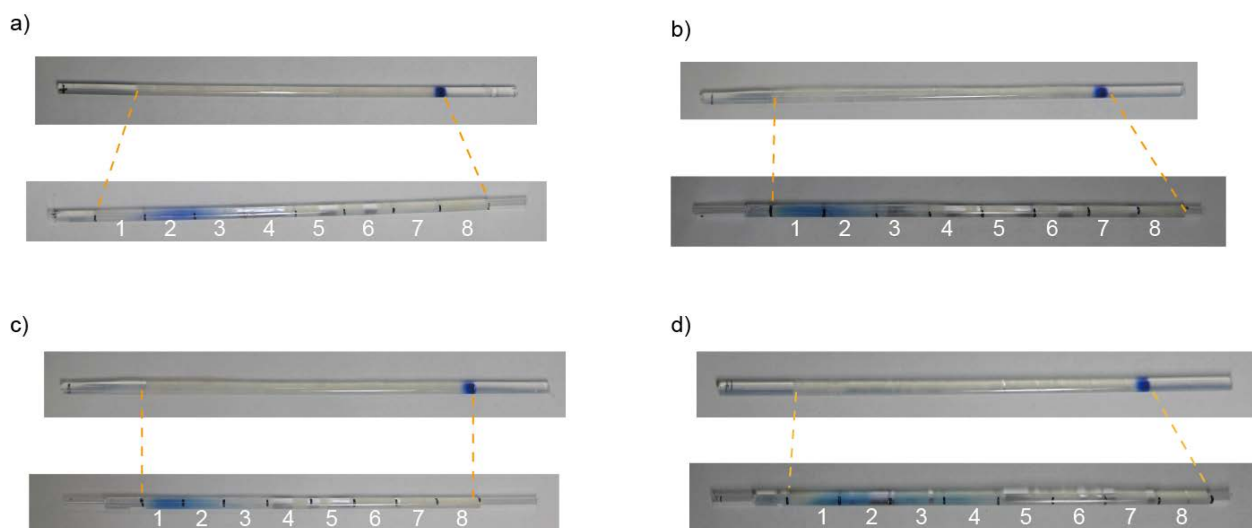

Fig. S2 Photographs of the capillary before (top) and after (bottom) electrophoresis. a) β-galactosidase (116 kDa) and ovalbumin (45 kDa) (100 V, 120 min.); b) ovalbumin and lysozyme (14.4 kDa) (100 V, 170 min.); c) ovalbumin and aprotinin (6.5 kDa) (100 V, 150 min.); d) lysozyme and aprotinin (100 V, 180 min.).

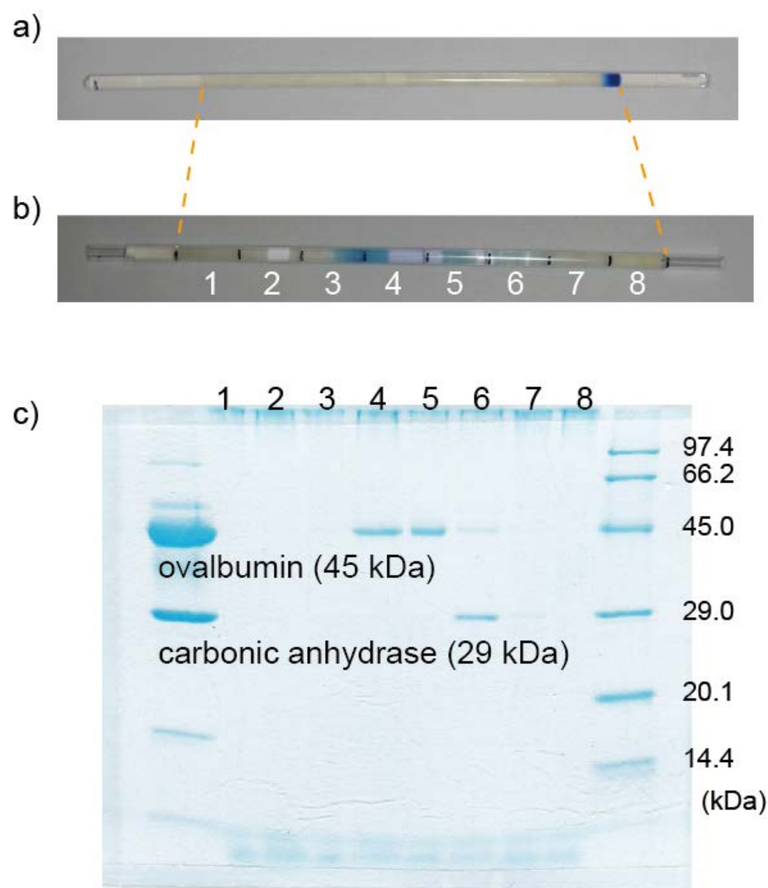

Fig. S3 Photographs of the capillary a) before electrophoresis, b) after electrophoresis of ovalbumin (45 kDa) and carbonic anhydrase (29 kDa) (100 V, 170 min.). c) SDS-PAGE (12% polyacrylamide gel) analysis of SDS-SUGE (**1**-AG gel) separation of ovalbumin and carbonic anhydrase.

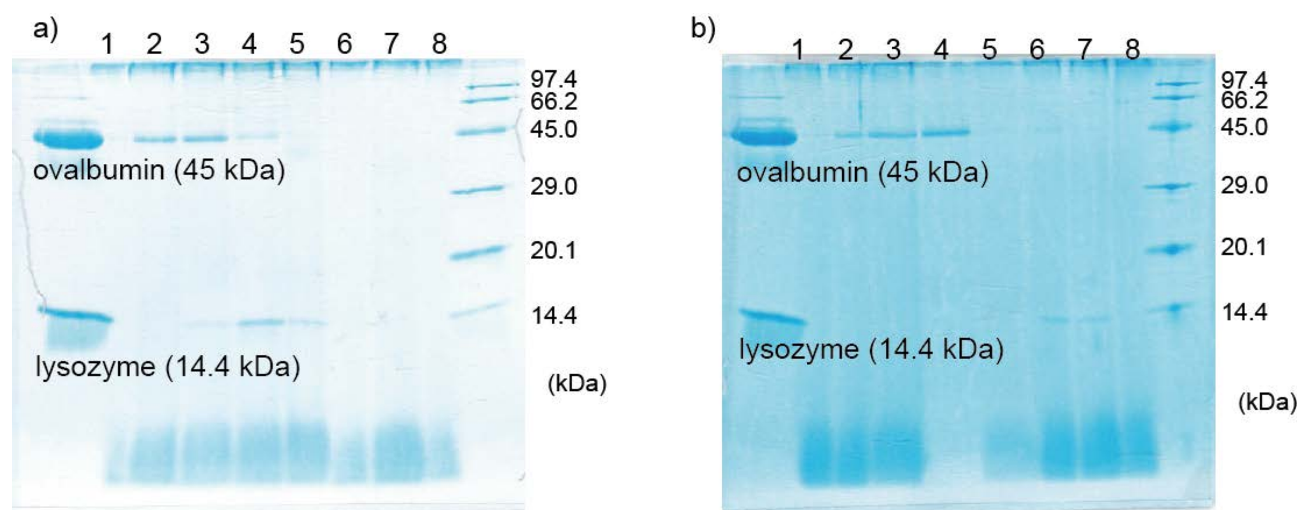

Fig. S4 SDS-PAGE (15% polyacrylamide gel) analyses of SDS-SUGE of ovalbumin (45 kDa) and lysozyme (14.4 kDa) using agarose (2 wt%) mixed TGS solution gel of **1**. a) 1.5 wt% of **1**, b) 3.0 wt% of **1**.

References

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(2) M. Yamanaka, T. Nakagawa, R. Aoyama, T. Nakamura, *Tetrahedron* **2008**, *64*, 11558-11567.