Electronic Supplementary Information

Structural diversification of bola-amphiphilic glycolipid-type supramolecular hydrogelators exhibiting colour changes along with the gel–sol transition

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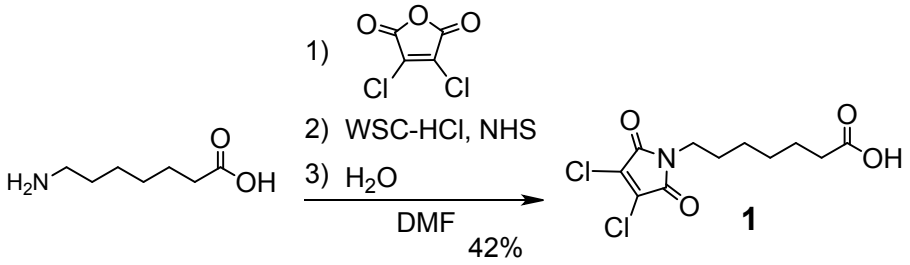
1. Synthesis

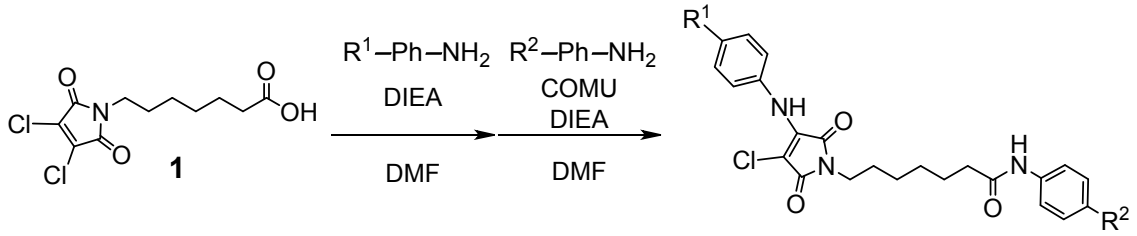
4-Aminophenyl-α/β-galactopyranoside (α/βGal–Ph–NH_2), 4-aminophenyl-α/β-glucopyranoside (α/βGlc–Phe–NH_2), and 4-aminophenyl-α-mannopyranoside (αMan–Ph–NH_2) were synthesized according to previously reported methods.[1] 2-Arylamino-3-chloro-*N*-phenylmaleimide derivatives were synthesized according to previously reported methods.[2]



Scheme S1

Synthesis of compound 1 (Dichloromaleimide–C6–COOH)

2,3-Dichloromaleic anhydride (0.58 g, 3.4 mmol, 1.0 eq.) was added to a solution of 7-aminoheptanoic acid (0.50 g, 3.4 mmol) in dry *N*,*N*-dimethylformamide (DMF, 5 mL), and the mixture was stirred at room temperature overnight under an N_2 atmosphere. 1-[1-(Cyano-2-ethoxy-2-oxoethylideneaminooxy)-dimethylamino-morpholino]-uronium hexafluorophosphate (COMU, 2.95 g, 6.9 mmol, 2.0 eq.) was added to the reaction mixture and stirred at room temperature overnight under an N_2 atmosphere. Distilled water (5 mL) was added to the reaction mixture and stirred at 40 ^oC for 3 hours. The solvent was then evaporated, the residue was dissolved in ethyl acetate (EtOAc, 150 mL), and the solution was washed with 5% aqueous citric acid (100 mL) for three times. The organic layer was collected, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated to dryness, the residue was purified by column chromatography (SiO_2, Hexane:EtOAc = 2:1 to 1:1 (*v*/*v*)), and the residue was further purified by reprecipitation with distilled water for three times. The resulting product was dried under vacuum to give compound **1** (0.42 g, 42%) as a white powder. ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) = 1.29–1.41 (m, 4H), 1.60–1.66 (m, 4H), 2.35 (t, J = 7.5 Hz, 2H), and 3.59 (t, J = 7.5 Hz, 2H). LRMS (ESI-TOF, positive mode): Calcd. for $[M(C_{11}H_{13}Cl_2NO_4) + Na]^+$: *m/z* = 316.0; Found: 316.0.



R^1 = *α*Glc, R^2 = *α*Glc	***α*Glc–AAC–C6–*α*Glc** (88%)
R^1 = *β*Glc, R^2 = *β*Glc	***β*Glc–AAC–C6–*β*Glc** (51%)
R^1 = *α*Gal, R^2 = *α*Gal	***α*Gal–AAC–C6–*α*Gal** (67%)
R^1 = *β*Gal, R^2 = *β*Gal	***β*Gal–AAC–C6–*β*Gal** (71%)
R^1 = *α*Man, R^2 = *α*Man	***α*Man–AAC–C6–*α*Man** (40%)
R^1 = *β*Gal, R^2 = *α*Man	***β*Gal–AAC–C6–*α*Man** (34%)
R^1 = *α*Man, R^2 = *β*Gal	***α*Man–AAC–C6–*β*Gal** (15%)

Scheme S2.

Synthesis of *α*Glc–AAC–C6–*α*Glc

*α*Glc–Ph–NH_2 (104 mg, 0.39 mmol, 1.1 eq.) and *N*,*N*-diisopropylethylamine (DIEA, 76.2 μL, 0.44 mmol, 1.25 eq.) were added to a solution of **1** (103 mg, 0.35 mmol, 1.0 eq.) in DMF (4 mL), and the mixture was stirred at room temperature overnight under an N_2 atmosphere. Then, COMU (150 mg, 0.35 mmol, 1.0 eq.), *α*Glc-Ph-NH_2 (104 mg, 0.39 mmol, 1.1 eq.), and DIEA (76.2 μL, 0.44 mmol, 1.25 eq.) were added to the reaction mixture, and the mixture was stirred at room temperature overnight under an N_2 atmosphere. The solvent was then evaporated, and the residue was purified by column chromatography (SiO_2, CH_2Cl_2:MeOH = 6:1 to 3:1 to 2:1 (*v*/*v*)). Then, the residue was further purified by reprecipitation with EtOAc, distilled water, and acetone for two times. The resulting product was dried under vacuum to give compound *α*Glc–AAC–C6–*α*Glc (241 mg, 88%) as a yellow powder. ^{1}H NMR (500 MHz, CD_3OD): δ (ppm) = 1.32–1.46 (m, 4H), 1.60–1.74 (m, 4H), 2.35 (t, J = 7.5 Hz, 2H), 3.52-3.60 (m, 5H), 3.64–3.78 (m, 7H), 3.83–3.88 (m, 2H), 5.40–5.49 (m, 2H), 7.11–7.21 (m, 6H), and 7.44–7.48 (m, 2H). HRMS (ESI-Qq-TOF, positive mode): Calcd. for $[M(C_{35}H_{44}ClN_3O_{15}) + Na]^+$: *m/z* = 804.2353; Found: 804.2379. FT-IR (KBr pellet): ν = 3358.4, 2932.2, 2858.0, 1767.4, 1709.6, 1652.7, 1509.6, 1444.4, 1410.7, 1365.4, 1335.5, 1308.5, 1227.5, 1145.5, 1109.8, 1080.9, 1026.9, 921.8, 869.7, 833.1, 769.5, 742.5 cm^{-1}.

Synthesis of *β*Glc–AAC–C6–*β*Glc

*β*Glc–Ph–NH_2 (298 mg, 1.1 mmol, 2.2 eq.) and DIEA (218 μL, 1.25 mmol, 2.5 eq.) were

added to a solution of **1** (147 mg, 0.50 mmol, 1.0 eq.) in DMF (5 mL), and the mixture was stirred at room temperature overnight under an N_2 atmosphere. Then, COMU (321 mg, 0.75 mmol, 1.5 eq.) was added to the reaction mixture, and the mixture was stirred at room temperature overnight under an N_2 atmosphere. The solvent was then evaporated, and the residue was purified by column chromatography (SiO_2, CH_2Cl_2:MeOH = 6:1 to 3:1 to 2:1 (*v*/*v*)). Then, the residue was further purified by reprecipitation with EtOAc, distilled water, and acetone for two times. The resulting product was dried under vacuum to give compound *β*Glc–AAC–C6–*β*Glc (200 mg, 51%) as a yellow powder. ^{1}H NMR (500 MHz, CD_3OD): δ (ppm) = 1.35-1.45 (m, 4H), 1.60-1.73 (m, 4H), 2.35 (t, J = 7.5 Hz, 2H), 3.38-3.48 (m, 8H), 3.50–3.55 (m, 2H), 3.67–3.73 (m, 2H), 3.86–3.93 (m, 2H), 4.81–4.91 (m, 2H, overlapped with water), 7.03–7.08 (m, 2H), 7.09–7.17 (m, 4H), and 7.42–7.47 (m, 2H). HRMS (ESI-Qq-TOF, positive mode): Calcd. for $[M(C_{35}H_{44}ClN_3O_{15}) + Na]^+$: *m/z* = 804.2353; Found: 804.2380. FT-IR (KBr pellet): ν = 3363.3, 2929.3, 2855.1, 1769.4, 1713.4, 1654.6, 1509.0, 1445.4, 1410.7, 1369.2, 1338.4, 1315.2, 1226.5, 1172.5, 1101.2, 1077.1, 1045.2, 1022.1, 924.7, 904.5, 866.8, 831.2, 769.5, 743.4 cm^{-1}.

Synthesis of *α*Gal–AAC–C6–*α*Gal

*α*Gal–Ph–NH_2 (119 mg, 0.44 mmol, 1.1 eq.) and DIEA (87.1 μL, 0.50 mmol, 1.25 eq.) were added to a solution of **1** (119 mg, 0.40 mmol, 1.0 eq.) in DMF (4 mL), and the mixture was stirred at room temperature overnight under an N_2 atmosphere. Then, COMU (171 mg, 0.40 mmol, 1.0 eq.), *α*Gal–Ph–NH_2 (119 mg, 0.44 mmol, 1.1 eq.), and DIEA (87.1 μL, 0.50 mmol, 1.25 eq.) were added to the reaction mixture, and the mixture was stirred at room temperature overnight under an N_2 atmosphere. The solvent was then evaporated, and the residue was purified by column chromatography (SiO_2, CH_2Cl_2:MeOH = 6:1 to 3:1 to 2:1 (*v*/*v*)). Then, the residue was further purified by reprecipitation with EtOAc, distilled water, diethyl ether (Et_2O), and acetone for two times. The resulting product was dried under vacuum to give compound *α*Gal–AAC–C6–*α*Gal (210 mg, 67%) as a yellow powder. ^{1}H NMR (500 MHz, CD_3OD): δ (ppm) = 1.33–1.46 (m, 4H), 1.60–1.73 (m, 4H), 2.35 (t, J = 7.5 Hz, 2H), 3.50–3.59 (m, 2H), 3.66–3.73 (m, 4H), 3.90–4.00 (m, 8H), 5.44 (d, J = 5 Hz, 1H), 5.49 (d, J = 5 Hz, 1H), 7.09–7.21 (m, 6H), and 7.42–7.48 (m, 2H). HRMS (ESI-Qq-TOF, positive mode): Calcd. for $[M(C_{35}H_{44}ClN_3O_{15}) + Na]^+$: *m/z* = 804.2353; Found: 804.2334. FT-IR (KBr pellet): ν

= 3335.1, 2934.2, 2859.9, 1771.3, 1712.5, 1654.6, 1509.0, 1442.5, 1411.6, 1355.7, 1335.5, 1227.5, 1108.9, 1082.8, 1029.8, 973.9, 957.5, 862.0, 768.5, 740.5 cm^{-1}.

Synthesis of *β*Gal–AAC–C6–*β*Gal

*β*Gal–Ph–NH_2 (102 mg, 0.37 mmol, 1.1 eq.) and DIEA (74 μL, 0.43 mmol, 1.25 eq.) was added to a solution of **1** (100 mg, 0.34 mmol, 1.0 eq.) in DMF (4 mL), and the mixture was stirred at room temperature overnight under an N_2 atmosphere. Then, COMU (146 mg, 0.34 mmol, 1.0 eq.), *β*Gal–Ph–NH_2 (102 mg, 0.37 mmol, 1.1 eq.), and DIEA (74 μL, 0.43 mmol, 1.25 eq.) were added to the reaction mixture, and the mixture was stirred at room temperature overnight under an N_2 atmosphere. The solvent was then evaporated and the residue was purified by reprecipitation with EtOAc, distilled water, and acetone for two times. The resulting product was dried under vacuum to give compound *β*Gal–AAC–C6–*β*Gal (189 mg, 71%) as a yellow powder. ^{1}H NMR (500 MHz, CD_3OD): δ (ppm) = 1.35–1.45 (m, 4H), 1.60–1.73 (m, 4H), 2.35 (t, J = 7.5 Hz, 2H), 3.52–3.60 (m, 4H), 3.64–3.82 (m, 8H), 3.88–3.91 (m, 2H), 4.79–4.70 (m, 2H, overlapped with water), 7.04–7.08 (m, 2H), 7.10–7.15 (m, 4H), and 7.45 (dd, J_1 = 5 Hz, J_2 = 5 Hz, 2H). HRMS (ESI-Qq-TOF, positive mode): Calcd. for $[M(C_{35}H_{44}ClN_3O_{15}) + Na]^+$: m/z = 804.2353; Found: 804.2387. FT-IR (KBr pellet): ν = 3345.9, 2934.1, 2861.8, 1768.4, 1713.4, 1658.5, 1510.0, 1444.4, 1410.7, 1371.1, 1330.6, 1234.2, 1143.6, 1074.1, 946.9, 918.0, 887.1, 827.3, 743.4 cm^{-1}.

Synthesis of *α*Man–AAC–C6–*α*Man

*α*Man–Ph–NH_2 (221 mg, 0.81 mmol, 2.2 eq.) and DIEA (161 μL, 0.93 mmol, 2.5 eq.) was added to a solution of **1** (111 mg, 0.37 mmol, 1.0 eq.) in DMF (5 mL), and the mixture was stirred at room temperature overnight under an N_2 atmosphere. Then, COMU (238 mg, 0.56 mmol, 1.5 eq.) was added to the reaction mixture, and the mixture was stirred at room temperature overnight under an N_2 atmosphere. The solvent was then evaporated, and the residue was purified by column chromatography (SiO_2, CH_2Cl_2:MeOH = 6:1 to 3:1 to 2:1 (*v*/*v*)). Then, the residue was further purified by reprecipitation with EtOAc, distilled water, and acetone for two time. The resulting product was dried under vacuum to give compound *α*Man–AAC–C6–*α*Man (117 mg, 40%) as a yellow powder. ^{1}H NMR (500 MHz, CD_3OD): δ (ppm) = 1.31–1.45 (m, 4H), 1.60–1.73 (m, 4H), 2.33–2.37 (m, 2H), 3.49–3.55 (m, 2H), 3.57–3.63 (m, 2H), 3.67–3.80 (m, 6H), 3.85–3.92 (m, 2H), 3.96–

4.03 (m, 2H), 5.42 (d, J = 5 Hz, 1H), 5.47 (d, J = 5 Hz, 1H), and 7.04–7.09 (m, 2H), 7.11–7.15 (m, 4H), 7.42–7.47 (m, 2H). HRMS (ESI-Qq-TOF, positive mode): Calcd. for $[M(C_{35}H_{44}ClN_3O_{15}) + Na]^+$: m/z = 804.2353; Found: 804.2320. FT-IR (KBr pellet): ν = 3363.3, 2932.5, 2859.9, 1771.3, 1712.5, 1652.7, 1509.0, 1443.5, 1410.7, 1373.1, 1338.4, 1228.4, 1121.4, 1067.4, 1045.2, 1010.5, 975.8, 884.2, 830.2, 742.5 cm^{-1}.

Synthesis of *β*Gal–AAC–C6–*α*Man

*β*Gal–Ph–NH_2 (89.5 mg, 0.33 mmol, 1.0 eq.) and DIEA (71.8 µL, 0.41 mmol, 1.25 eq.) was added to a solution of **1** (97.2 mg, 0.33 mmol, 1.0 eq.) in DMF (3 mL), and the mixture was stirred at room temperature overnight under an N_2 atmosphere. Then, COMU (141 mg, 0.33 mmol, 1.0 eq.), *α*Man–Ph–NH_2 (98.5 mg, 0.36 mmol, 1.1 eq.), and DIEA (71.8 µL, 0.41 mmol, 1.25 eq.) were added to the reaction mixture, and the mixture was stirred at room temperature overnight under an N_2 atmosphere. The solvent was then evaporated, and the residue was purified by reprecipitation with EtOAc, distilled water, and diethyl ether for two times. The resulting product was dried under vacuum to give compound *β*Gal–AAC–C6–*α*Man (88.2 mg, 34%) as a yellow powder. ^{1}H NMR (500 MHz, CD_3OD): δ (ppm) = 1.31–1.46 (m, 4H), 1.60–1.74 (m, 4H), 2.32–2.38 (m, 2H), 3.51–3.63 (m, 4H), 3.68-3.82 (m, 7H), 3.87-3.92 (m, 2H), 3.98-4.01 (s, 1H), 4.80-4.91 (m,1H, overlapped with water), 5.42 (d, J = 5 Hz, 1H), 7.06-7.09 (m, 2H), 7.10-7.16 (m, 4H), and 7.43-7.47 (m, 2H). HRMS (ESI-Qq-TOF, positive mode): Calcd. for $[M(C_{35}H_{44}ClN_3O_{15}) + Na]^+$: m/z = 804.2353; Found: 805.2379. FT-IR (KBr pellet): ν = 3356.5, 2937.1, 2859.9, 1771.3, 1712.5, 1655.6, 1509.0, 1444.4, 1409.7, 1373.1, 1338.4, 1316.2, 1229.4, 1172.5, 1123.3, 1070.3, 1016.3, 978.7, 919.9, 885.2, 835.0, 743.4 cm^{-1}.

Synthesis of *α*Man–AAC–C6–*β*Gal

*α*Man–Ph–NH_2 (109 mg, 0.40 mmol, 1.0 eq.) and DIEA (87.1 µL, 0.50 mmol, 1.25 eq.) were added to a solution of **1** (118 mg, 0.40 mmol, 1.0 eq.) in DMF (4 mL), and the mixture was stirred at room temperature overnight under an N_2 atmosphere. Then, COMU (171 mg, 0.40 mmol, 1.0 eq.), *β*Gal–Ph–NH_2 (109 mg, 0.40 mmol, 1.0 eq.), and DIEA (87.1 µL, 0.50 mmol, 1.25 eq.) were added to the reaction mixture, and the mixture was stirred at room temperature overnight under an N_2 atmosphere. The solvent was then evaporated, and the residue was purified by reprecipitation with EtOAc, distilled water,

and acetone. The resulting product was dried under vacuum to give compound *α*Man–AAC–C6–*β*Gal (46 mg, 15%) as a yellow powder. ^{1}H NMR (500 MHz, CD_3OD): δ (ppm) = 1.31–1.46 (m, 4H), 1.58–1.73 (m, 4H), 2.30–2.37 (m, 2H), 3.39–3.63 (m, 4H), 3.68–3.81 (m, 7H), 3.85–3.92 (m, 2H), 3.97–4.02(m, 1H), 4.80–4.91 (m,1H, overlapped with water), 5.47 (d, J = 5 Hz, 1H), 7.04–7.09 (m, 2H), 7.11–7.16 (m, 4H), and 7.42–7.46 (m, 2H). HRMS (ESI-Qq-TOF, positive mode): Calcd. for $[M(C_{35}H_{44}ClN_3O_{15}) + Na]^+$: *m/z* = 804.2353; Found: 804.2362. FT-IR (KBr pellet): ν = 3366.1, 2934.2, 2861.8, 1768.4, 1713.4, 1653.7, 1509.0, 1445.4, 1411.6, 1375.0, 1338.4, 1307.5, 1228.4, 1173.5, 1071.3, 1047.2, 1013.4, 977.7, 917.0, 885.2, 833.1, 771.4, 741.5 cm^{-1}.

2. Preceding experiment

Table S1. Gelation ability (pG: partial gel, oG: opaque gel, Ppt: Precipitation, Sol: solution), critical gelation concentration (CGC), and gelation time at room temperature (around 23 °C), and sol–gel phase transition temperature (T_{gel}) of bola-amphiphilic glycolipids. Conditions: 200 mM HEPES–NaOH buffer (pH 7.2). The compounds were synthesized by the same procedure as C6-type compounds.

Compound		CGC [wt%]	T_{gel} [°C]	Gelation time
*β*Gal–AAC–C11–*β*Gal	pG	(1.7)	—	(~10 min)
*β*Gal–AAC–C6–*β*Gal	oG	0.5	92	~5 min
*β*Gal–AAC–C5–*α*Gal	pG	(4.0)	—	(~1 day)
*β*Gal–AAC–C3–*β*Gal	Ppt	—	—	—

3. Hydrogel formation ability of compounds (C6-type).

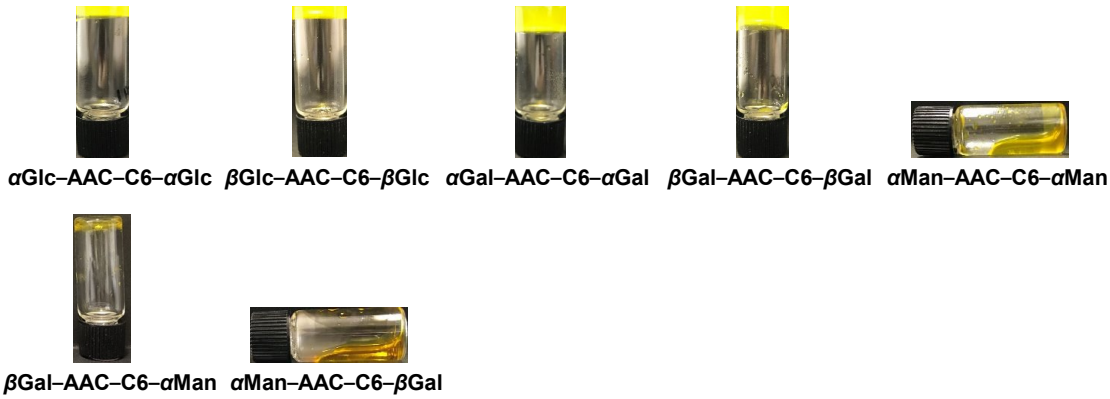


Figure S1. Photographs of hydrogels and solutions of compounds at room temperature (around 23 °C). For the hydrogels, the concentrations were set to CGC ([*α*Glc–AAC–C6–*α*Glc] = [*β*Glc–AAC–C6–*β*Glc] = [*β*Gal–AAC–C6–*α*Man] = 1.6 wt%, [*α*Gal–AAC–C6–*α*Gal] = [*β*Gal–AAC–C6–*β*Gal] = 0.5 wt% in 200 mM HEPES–NaOH buffer (pH 7.2). For the sols, the concentrations were [*α*Man–AAC–C6–*α*Man] = [*α*Man–AAC–C6–*β*Gal] = 2.0 wt% in 200 mM HEPES–NaOH buffer (pH 7.2).

4. Absorption spectra of the compounds.

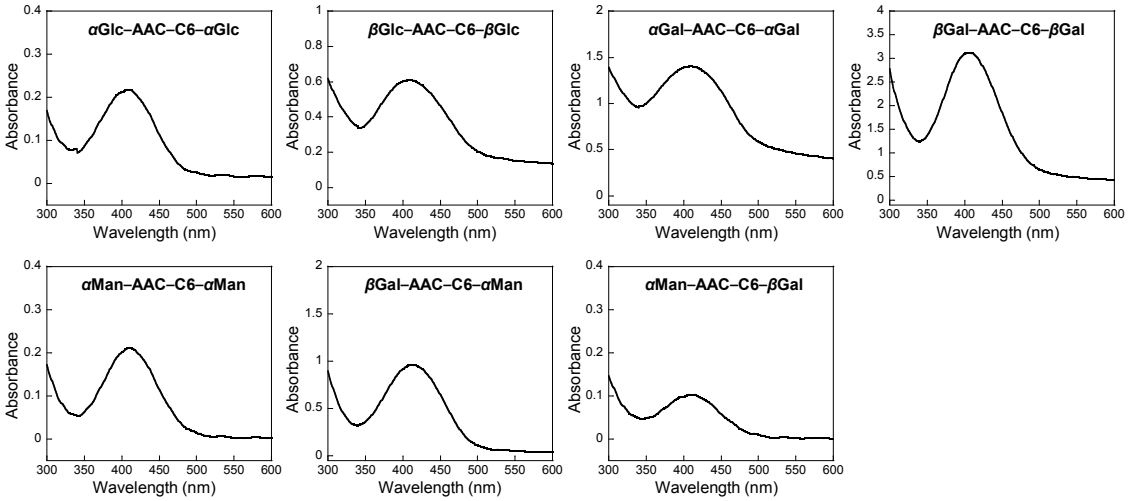


Figure S2. Absorption spectra of the compounds in 200 mM HEPES–NaOH buffer (pH 7.2) at room temperature. For the gels, the concentrations were set to *CGC* ([*α*Glc–AAC–C6–*α*Glc] = [*β*Glc–AAC–C6–*β*Glc] = [*β*Gal–AAC–C6–*α*Man] = 1.6 wt%, [*α*Gal–AAC–C6–*α*Gal] = [*β*Gal–AAC–C6–*β*Gal] = 0.5 wt%). For the sols, the concentrations were [*α*Man–AAC–C6–*α*Man] = [*α*Man–AAC–C6–*β*Gal] = 2.0 wt%.

5. Gel sate and solution phase of the bulk hydrogels.

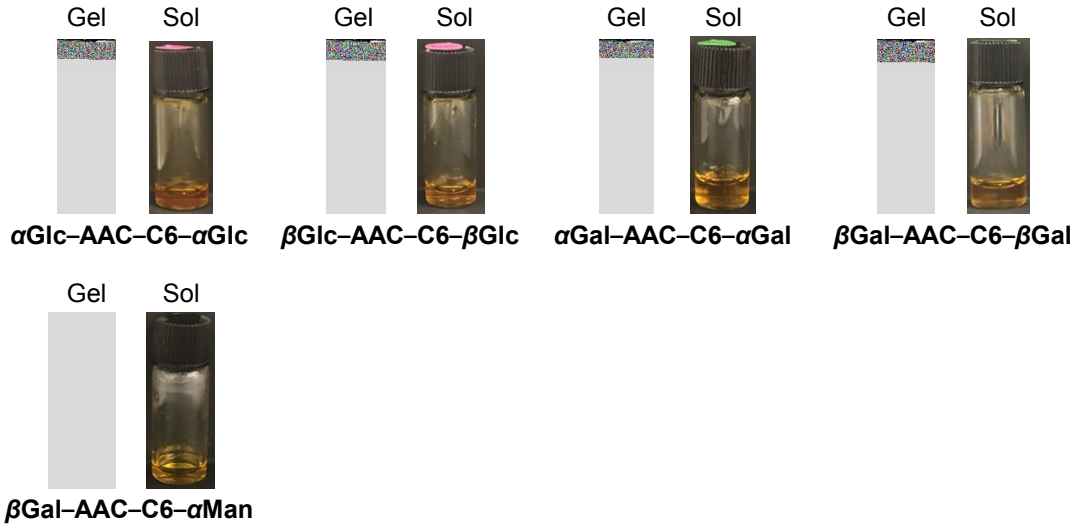


Figure S3. Photographs of gel state (Gel) and solution state (Sol) of the bulk hydrogels. The Sol samples were obtained by heating of the Gel samples. The concentrations were set to CGC ([*α*Glc–AAC–C6–*α*Glc] = [*β*Glc–AAC–C6–*β*Glc] = [*β*Gal–AAC–C6–*α*Man] = 1.6 wt%, [*α*Gal–AAC–C6–*α*Gal] = [*β*Gal–AAC–C6–*β*Gal] = 0.5 wt% in 200 mM HEPES–NaOH buffer (pH 7.2).

6. Temperature-dependent absorption spectral change of the *β*Gal–AAC–C6–*β*Gal hydrogel.

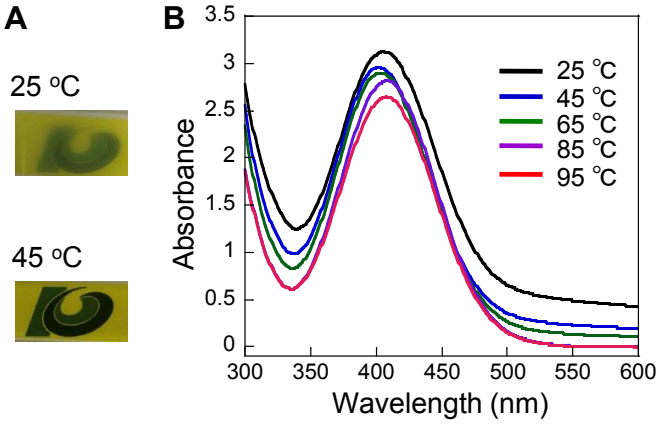


Figure S4. (**A**) Photograph of the *β*Gal–AAC–C6–*β*Gal hydrogel in a quartz cell (path length: 1 mm) at 25 and 45 °C. (**B**) Absorption spectral changes of the *β*Gal–AAC–C6–*β*Gal hydrogel upon heating ([*β*Gal–AAC–C6–*β*Gal] = 0.5 wt% in 200 mM HEPES–NaOH buffer (pH 7.2)). Baseline rise at lower temperature, especially 25 °C, was observed, which would be due to the increased sample turbidity.

7. Temperature-dependent absorption spectral change of the *α*Man–AAC–C6–*α*Man solution.

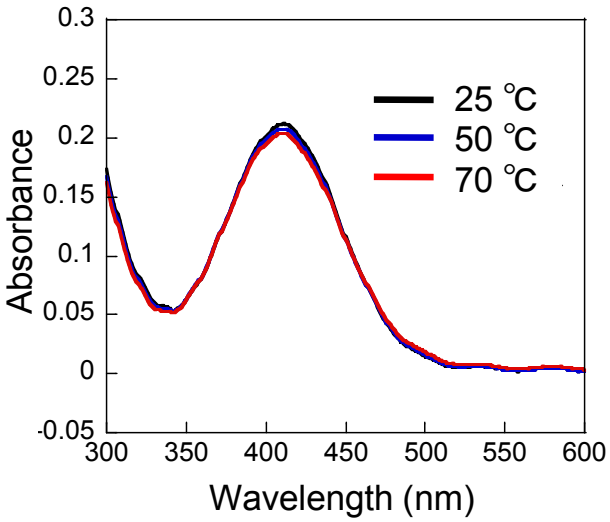


Figure S5. Absorption spectral changes of *α*Man–AAC–C6–*α*Man solution upon heating ([*α*Man–AAC–C6–*α*Man] = 2.0 wt% in 200 mM HEPES–NaOH buffer (pH 7.2) in a quartz cell (path length: 0.1 mm)).

References

1 J. H. Jung, S. Shinkai and T. Shimizu, *Chem. –Eur. J.* 2002, **8**, 2684.

2 (a) L. Hanaineh-Abdelnour, S. Bayyuk and R. Theodorie, *Tetrahedron*, 1999, **55**, 11859; (b) R. Ochi, K. Kurotani, M. Ikeda, S. Kiyonaka and I. Hamachi, *Chem. Commun.*, 2013, **49**, 2115.