Electronic Supplementary Material (ESI) for Chemical Communications

Supramolecular hydrogels based on bola-amphiphilic glycolipids showing color change in response to glycosidases

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Contents:

1. Experimental.

Generals

Unless stated otherwise, all commercial reagents were used as received. α-Glucosidase (from *S. cerevisiae*, Sigma-Aldrich, cat. no. G0660), β-glucosidase (from almonds, Sigma-Aldrich, cat. no. G4511), β-galactosidase (from *E. coli*, Sigma-Aldrich, cat. no. G5635), and α-mannosidase (from Jack bean, Sigma-Aldrich, cat. no. M7257) were used as received. Chemical reagents were purchased from Tokyo Chemical Industry Co., Ltd. and Wako Pure Chemical Industries, Ltd., and used without further purification. All water used in the experiments refers to ultra pure water obtained from a Millipore system having a specific resistance of 18 MΩ•cm. Thin layer chromatography (TLC) was performed on silica gel $60F_{254}$ (Merck). Column chromatography was performed on silica gel 60N (Kanto Chemical Co., Inc., 40–50 μm). Reverse phase HPLC (RP-HPLC) was conducted with a Hitachi Lachrom instrument equipped with a YMC-Triart C18 column (250 mm × 4.6 mm I.D.) for analysis. ^{1}H NMR spectra were obtained on a Varian Mercury 400 spectrometer with tetramethylsilane (TMS) or residual non-deuterated solvents as the internal references. Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, br = broad. MALDI-TOF mass spectra were recorded using a Bruker autoflexIII. FTMS (ESI) mass spectrometry was performed on a Thermo Scientific Exactive orbitrap mass spectrometer. The absorption spectra were measured using a Shimadzu UV2550. TEM images were acquired using a JEOL JEM-1400 (accelerating voltage: 80 kV) equipped with a CCD camera. FTIR spectra were measured using a Perkin-Elmer Spectra One spectrometer. CD spectra were measured using a JEOL J-720WI.

Preparation of bulk hydrogels: Powders of βGlc-C11, αGlc-C11, βGal-C11, and αMan-C11 (typically, 1.0 mg) were suspended into 200 mM HEPES buffer (100–1000 μL). The suspensions were heated until homogeneous solutions were obtained. The solutions solidified into hydrogels after incubating several minutes at room temperature.

TEM observation of hydrogels: Hydrogels βGlc-C11, αGlc-C11, βGal-C11, and αMan-C11 (0.1 wt%, 5 μL) were dropped on copper TEM grids covered by an elastic carbon-support film (20–25 nm) with a filter paper underneath and the excess solution

were blotted with the filter paper immediately. The TEM grids were washed with H_2O (5 μL) for three times and dried under a reduced pressure for at least 6 h prior to TEM observation.

Measurements of temperature-dependent absorption spectral change of hydrogel βGlc-C11: An aqueous suspension of βGlc-C11 (0.1 wt%, 200 mM HEPES buffer (pH 7.2)) was heated to form a homogeneous solution. This hot solution (100 μL) was transferred into a quartz cell (path length: 1 mm) and stored at room temperature for 10 min to complete gelation. The absorption spectra were measured upon heating from 25 to 83 ºC.

Measurements of temperature-dependent CD observation of hydrogel βGlc-C11: A suspension of βGlc-C11 (0.1 wt%, 200 mM HEPES buffer (pH 7.2)) was heated to form a homogeneous solution. This hot solution (100 μL) was transferred into a quartz cell (path length: 1 mm) and stored at room temperature for 10 min to complete gelation. The CD spectra were measured upon heating from 25 to 90 ºC.

Glycosidase-induced gel-sol transition of bulk gels and product analysis: To gels βGlc-C11, αGlc-C11, βGal-C11, and αMan-C11 (0.1 wt%, 200 mM HEPES (pH 7.4), 100 μL) were added an aqueous solution of glycosidases (120 units/mL, 20 μL) and the resultant gels were incubated at room temperature. After 6 h, acetonitrile (120 μL) was added to dissolve the samples completely. The resultant solutions (20 μL) were subjected to RP-HPLC analysis (YMC-Triart C18 column (250 mm × 4.6 mm I. D.), eluent: 0.1% TFA acetonitrile:0.1% TFA H_2O = 25:75 to 80:20 (over 50 min, linear gradient), flow rate = 1.0 mL/min, detection wavelength = 220 nm).

Measurement of absorption spectral change of hydrogel βGlc-C11 after the addition of β-glucosidase: A gel in a quartz cell was prepared in the same way as described above. After complete gelation, a β-glucosidase solution (120 units/mL, 200 mM HEPES buffer (pH 7.2), 20 μL) was added on the gel. The absorption spectra were measured at room temperature.

TEM observation of hydrogels after the addition of the corresponding glycosidases: Hydrogels 6 h after the addition of the corresponding glycosidases (5 μL) were dropped on copper TEM grids covered by an elastic carbon-support film (20–25 nm) with a filter paper underneath and the excess solution were blotted with the filter paper immediately. The TEM grids were washed with H_2O (5 μL) for three times and dried under a reduced pressure for at least 6 h prior to TEM observation.

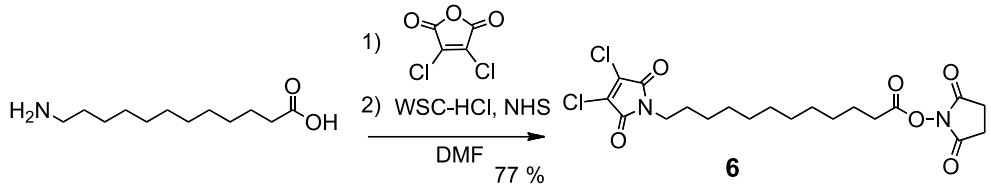
Preparation of gel array: Aqueous suspensions of βGlc-C11, αGlc-C11, βGal-C11, and αMan-C11 (0.5 wt%, 200 mM HEPES (pH 7.2)) were heated to form homogeneous solutions. These hot solutions (10 μL) were spotted on a glass plate (Matsunami, spot diameters were 4 mm (24 spots)) and incubated to complete gelation in a sealed box with high humidity at room temperature for 10 min.

Colorimetric assay of glycosidases using gel array: Glycosidase solutions (120 units/mL, 200 mM HEPES buffer (pH 7.2), 2 μL) were dropped onto each hydrogel spot of the gel array prepared as described above. The photographs of the gel array were collected by using a digital camera (OLYMPUS, PEN E-PL2). The images were analyzed with ImageJ (Ver. 1.46) on a Macintosh PC.

2. Synthesis

α/β-Gal-Ph-NH_2, β-Glc-Phe-NH_2, and α-Man-Ph-NH_2 were synthesized according to the methods reported previously.[1] 2-Arylamino-3-chloro-N-phenylmaleimide derivatives were synthesized according to the methods reported previously.[2]

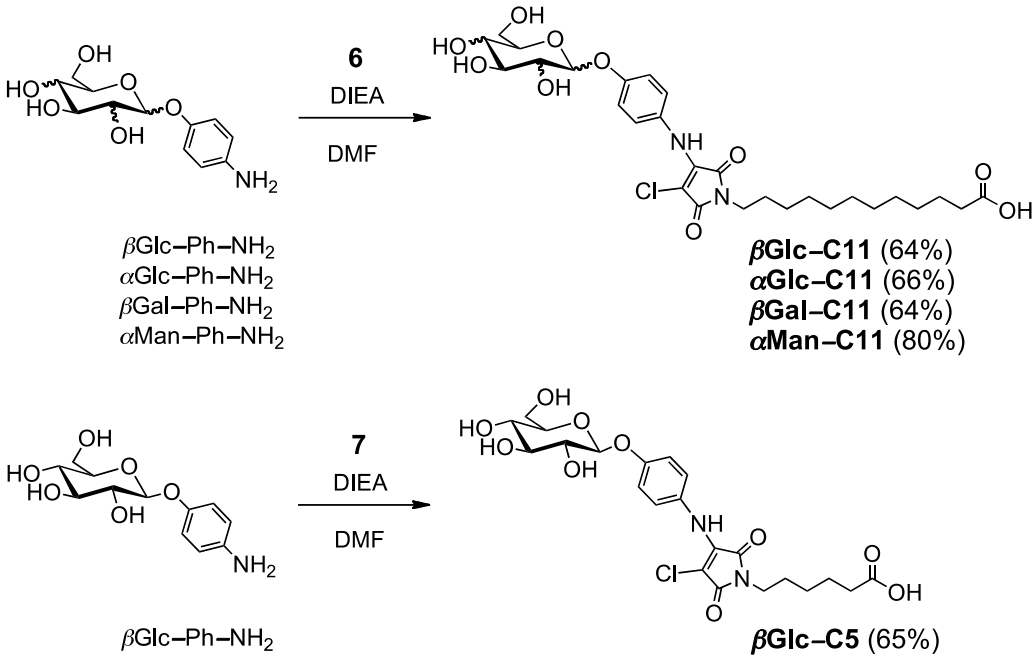
Scheme S1



Synthesis of compound 6 (Dichloromaleimide-C11-COOSu): To a solution of 12-amino-1-dodecanoic acid (1.1 g, 5.0 mmol) in dry *N*,*N*-dimethylformamide (DMF, 10 mL) was added dichloromaleic anhydride (0.88 g, 5.0 mmol, 1.0 eq.), and the mixture was stirred at room temperature for 3 h under Ar atmosphere. After cooling in an ice bath, water soluble carbodiimide hydrochloride (WSC•HCl, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, 1.9 g, 57 mmol, 2.0 eq.) and *N*-hydroxysuccimide (NHS, 0.72 g, 6.3 mmol, 1.3 eq.) were added to the reaction mixture and the mixture was stirred at room temperature overnight under Ar atmosphere. The solvent was then evaporated and the residue was dissolved in ethyl acetate (EtOAc, 200 mL) and the solution was washed with 5% aqueous citric acid (100 mL) for three times. The organic layer was collected and dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated to dryness and the residue was purified by column chromatography (SiO_2, Hexane:EtOAc = 4:1 (v/v)) to give compound **6** (1.8 g, 77%) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.20–1.28 (m, 14H), 1.59–1.64 (m, 4H), 2.31 (t, J = 7.4 Hz, 2H), 2.87 (s, 4H), 3.31 (t, J = 7.4 Hz, 2H), 3.58 ppm (t, J = 7.4 Hz, 2H). MS (MALDI-TOF): Calcd. for $[M(C_{20}H_{26}Cl_2N_2O_6)+H]^+$: m/z = 461.12; Found: 461.08.

Synthesis of compound 7 (Dichloromaleimide-C5-COOSu): To a solution of 6-amino-hexanoic acid (0.66 g, 5.0 mmol) in dry DMF (10 mL) was added dichloromaleic anhydride (0.88 g, 5.0 mmol, 1.0 eq.), and the mixture was stirred at room temperature for 3 h under Ar atmosphere. After cooling in an ice bath, WSC·HCl (1.9 g, 57 mmol, 2.0 eq.) and NHS (0.72 g, 6.3 mmol, 1.3 eq.) were added to the reaction mixture and the mixture was stirred at room temperature overnight under Ar atmosphere. The solvent was then evaporated and the residue was dissolved in EtOAc (200 mL) and the solution was washed with 5% aqueous citric acid (100 mL) for three times. The organic layer was collected and dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated to dryness and the residue was purified by column chromatography (SiO_2, Hexane:EtOAc = 2:1 (v/v)) to give compound **7** (1.09 g, 58%) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.38–1.44 (m, 2H), 1.58–1.65 (m, 2H), 1.71–1.79 (m, 2H), 2.60 (t, J = 7.2 Hz, 2H), 2.82 (s, 4H), 3.51 ppm (t, J = 7.0 Hz, 2H). MS (MALDI-TOF): Calcd. for $[M(C_{14}H_{14}Cl_2N_2O_6)+Na]^+$: m/z = 399.01; Found: 399.59.

Scheme S2.



Synthesis of *β*Glc-C11: To a solution of **6** (230 mg, 0.50 mmol, 1.0 eq.) in dry DMF (10 mL) was added *β*-Glc-Ph-NH_2 (149 mg, 0.55 mmol, 1.1 eq.) and *N, N*-diisopropylethylamine (DIEPA, 200 μL, 0.75 mmol, 1.5 eq.), and the mixture was stirred at room temperature overnight under Ar atmosphere. The solvent was then evaporated and the residue was purified by column chromatography (SiO_2, $CHCl_3$:MeOH = 1:0 to 8:1 to 4:1 (v/v)) and the residue was further purified by reprecipitation by diethyl ether. The resulting product was dried under vacuum to give compound *β*Glc-C11 (191 mg, 64%) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD): δ = 1.31–1.34 (m, 14H), 1.59–1.61 (m, 4H), 2.29 (t, J = 7.4 Hz, 2H), 3.38–3.49 (m, 4H), 3.51 (t, J = 6.7 Hz, 2H), 3.70 (dd, J = 5.4 and 12.0 Hz, 1H), 3.90 (dd, J = 2.2 and 12.0 Hz, 1H), 4.86–4.88 (m, 1H(overlapped with water), 7.12 ppm (dd, J = 9.4 and 19 Hz, 4H). HR-FTMS (ESI, negative mode): Calcd. for $[M(C_{28}H_{39}ClN_2O_{10})]^-$: *m/z* = 597.2220; Found: 597.2225.

Synthesis of *α*Glc-C11: The title compound was prepared from and compound **6** (206 mg, 0.47 mmol) and *α*-Glc-Ph-NH_2 (140 mg, 0.52 mmol) in the same way as *α*Glc-C11 and was obtained in 66% yield (205 mg) as a yellow powder. ^{1}H NMR (400 MHz, CD_3OD): δ = 1.31–1.36 (m, 14H), 1.59–1.61 (m, 4H), 2.26 (t, J = 7.4 Hz, 2H), 3.41 (t,

J = 9.4 Hz, 1H), 3.51 (t, *J* = 7.0 Hz, 2H), 3.56 (dd, *J* = 3.6 and 9.8 Hz, 1H), 3.66–3.76 (m, 2H), 3.84 (d, *J* = 9.4 Hz, 1H), 5.46 (d, *J* = 3.2 Hz, 1H), 7.16 ppm (dd, *J* = 9.4 and 19 Hz, 4H). HR-FTMS (ESI, negative mode): Calcd. for $[M(C_{28}H_{39}ClN_2O_{10})]^-$: *m/z* = 597.2220; Found: 597.2230.

Synthesis of βGal-C11: The title compound was prepared from and compound **6** (80 mg, 0.17 mmol) and *β*-Gal-Ph-NH_2 (52 mg, 0.19 mmol) in the same way as *β*Gal-C11 and was obtained in 64% yield (73 mg) as a yellow powder. ^{1}H NMR (400 MHz, CD_3OD): *δ* = 1.31–1.36 (m, 14H), 1.59–1.61 (m, 4H), 2.26 (t, *J* = 7.4 Hz, 2H), 3.51 (t, *J* = 7.0 Hz, 2H), 3.58 (dd, *J* = 3.2 and 9.6 Hz, 1H), 3.67–3.69 (m, 1H), 3.75–3.81 (m, 3H), 3.90 (d, *J* = 3.2 Hz, 1H), 4.84–4.86 (m, 1H(overlapped with water), 7.12 ppm (dd, *J* = 9.4 and 19 Hz, 4H). HR-FTMS (ESI, negative mode): Calcd. for $[M(C_{28}H_{39}ClN_2O_{10})]^-$: *m/z* = 597.2220; Found: 597.2228.

Synthesis of αMan-C11: The title compound was prepared from and compound **6** (231 mg, 0.50 mmol) and *α*-Man-Ph-NH_2 (149 mg, 0.55 mmol) in the same way as *α*Man-C11 and was obtained in 80% yield (73 mg) as a yellow powder. ^{1}H NMR (400 MHz, CD_3OD): *δ* = 1.31–1.34 (m, 14H), 1.59–1.61 (m, 4H), 2.26 (t, *J* = 7.4 Hz, 2H), 3.51 (t, *J* = 7.2 Hz, 2H), 3.61–3.63 (m, 1H), 3.69–3.78 (m, 3H), 3.89 (dd, *J* = 3.4 and 9.4 Hz, 1H), 3.99 (m, 1H), 5.46 (d, *J* = 0.8 Hz, 1H), 7.13 ppm (dd, *J* = 9.4 and 19 Hz, 4H). HR-FTMS (ESI, negative mode): Calcd. for $[M(C_{28}H_{39}ClN_2O_{10})–H]^-$: *m/z* = 597.2220; Found: 597.2228.

Synthesis of βGlc-C5: The title compound was prepared from and compound **7** (95 mg, 0.25 mmol) and *β*-Glc-Ph-NH_2 (68 mg, 0.25 mmol) in the same way as *β*Glc-C5 and was obtained in 65% yield (84 mg) as a orange powder. ^{1}H NMR (400 MHz, CD_3OD): *δ* = 1.32–1.39 (m, 2H), 1.59–1.68 (m, 4H), 2.29 (t, *J* = 7.4 Hz, 2H), , 3.40–3.48 (m, 4H), 3.52 (t, *J* = 7.2 Hz, 2H), 3.70 (dd, *J* = 5.4 and 12.2 Hz, 1H), 3.90 (dd, *J* = 2.0 and 12.0 Hz, 1H), 4.86–4.88 (m, 1H(overlapped with water), 7.12 ppm (dd, *J* = 9.4 and 19 Hz, 4H). HR-FTMS (ESI, negative mode): Calcd. for $[M(C_{22}H_{27}ClN_2O_{10})]^-$: *m/z* = 514.1354; Found: 514.1350.

3. Hydrogel formation ability of glyco-lipids.

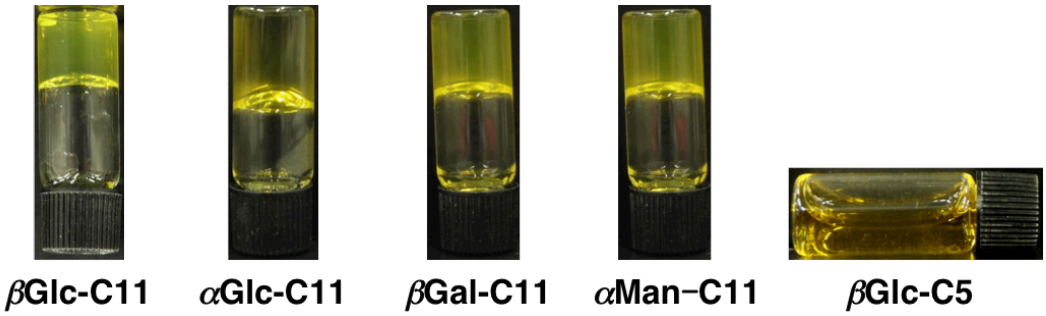


Figure S1. Photographs of gels of βGlc-C11, αGlc-C11, βGal-C11, and αMan-C11 and a sol of βGlc-C5 (0.1 wt%) in 200 mM HEPES (pH 7.2). ([Compounds] = 0.1 wt%, 200 mM HEPES (pH 7.2))

Table S1. Gelation capability (G and S denote gel and sol, respectively) of βGlc-C11, αGlc-C11, βGal-C11, αMan-C11, and βGlc-C5, critical gelation concentration (*CGC*) and sol-gel phase transition temperature (T_{gel}) of the gels, and absorption maxima (λ_{max}) of the gels and the sol βGlc-C5**.** Condition: 200 mM HEPES (pH 7.2)

Compound		*CGC* [wt%]	T_{gel} [°C]	λ_{max}[nm]
βGlc-C11	G	0.10	78	402
αGlc-C11	G	0.10	78	408
βGal-C11	G	0.08	75	404
αMan-C11	G	0.09	63	407
βGlc-C5	S	—	—	410

4. Temperature-dependent absorption spectral change of hydrogel of *β*Glc-C11.

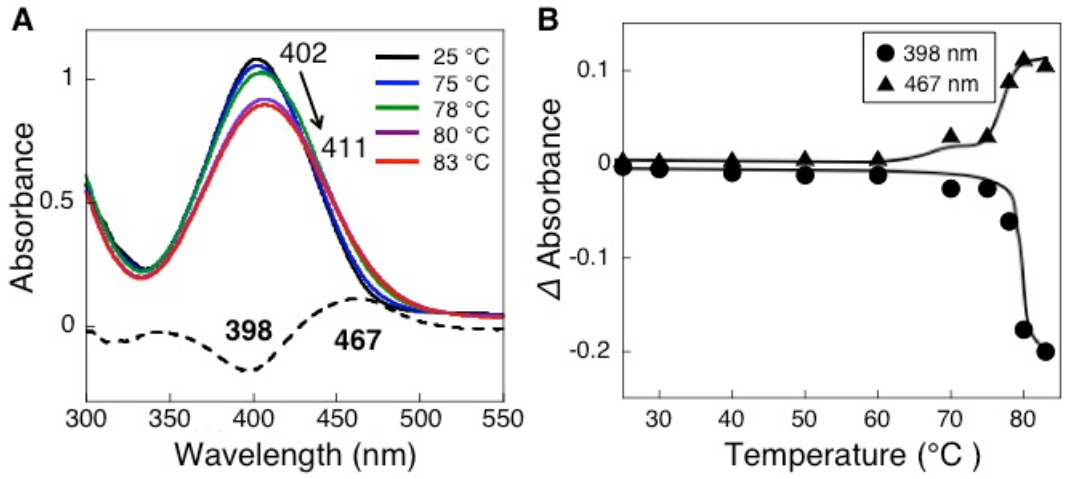


Figure S2. (**A**) Absorption spectral change of hydrogel upon heating (broken line: differential spectrum between 83 ºC and 25 ºC) and (**B**) change of absorbance at 398 and 467 nm of hydrogel upon heating. ([*β*Glc-C11] = 0.1 wt% in 200 mM HEPES buffer (pH 7.2).

5. Temperature-dependent CD spectral change of hydrogel and CD spectrum of methanol solution of *β*Glc-C11.

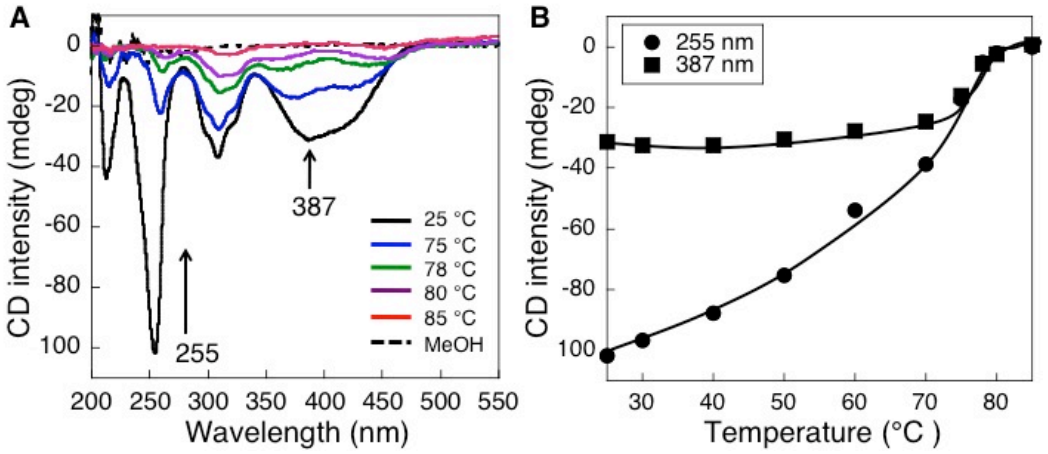


Figure S3. (**A**) CD spectral change of hydrogel (solid lines) upon heating (CD spectrum of methanol solution of *β*Glc-C11 is shown as a black broken line) and (**B**) change of CD intensity at 255 and 387 nm of hydrogel upon heating. (Hydrogel: [*β*-Glc-C11] = 0.1 wt% in 200 mM HEPES buffer (pH 7.2), Sol: [*β*-Glc-C11] = 0.1 wt% in methanol)

6. Typical TEM images of hydrogels.

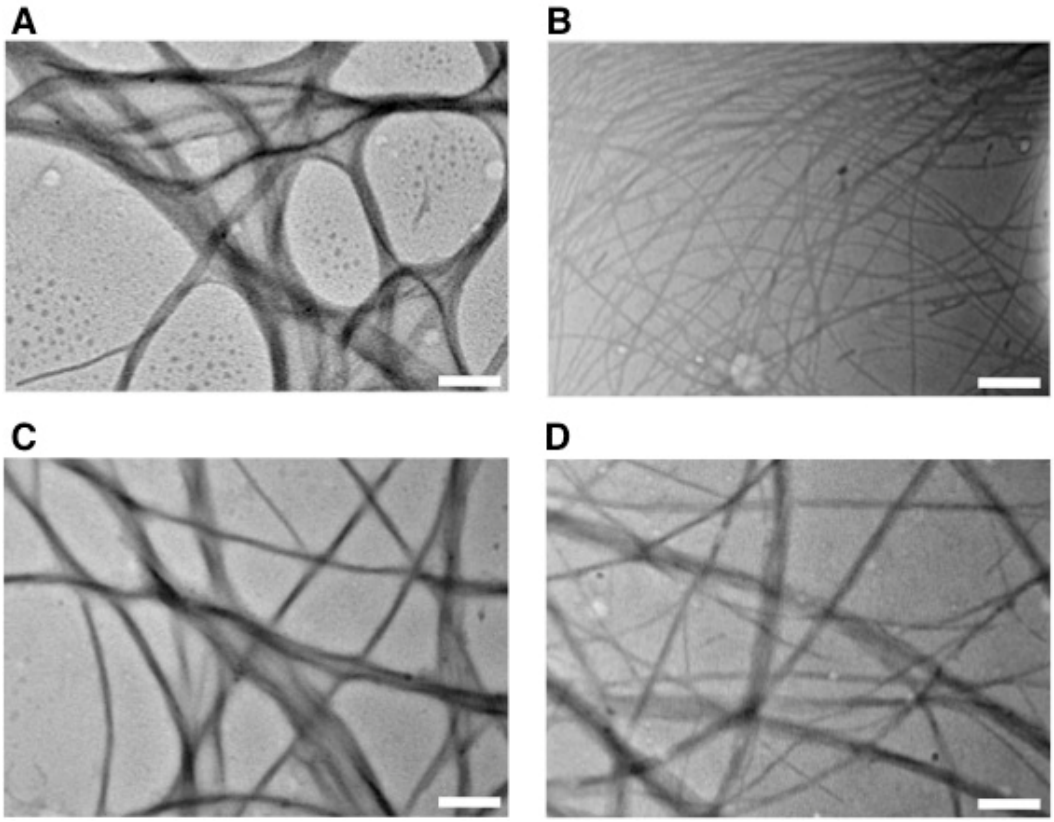


Figure S4. Typical TEM images of hydrogels *β*Glc-C11 (**A**), *α*Glc-C11 (**B**), *β*Gal-C11 (**C**), and *α*Man-C11 (**D**) (0.1 wt%, 200 mM HEPES (pH 7.2)) transferred on an elastic carbon-coated grid. Scale bar is 200 nm.

7. Selective gel-sol phase transition of hydrogels toward the corresponding glycosidases.

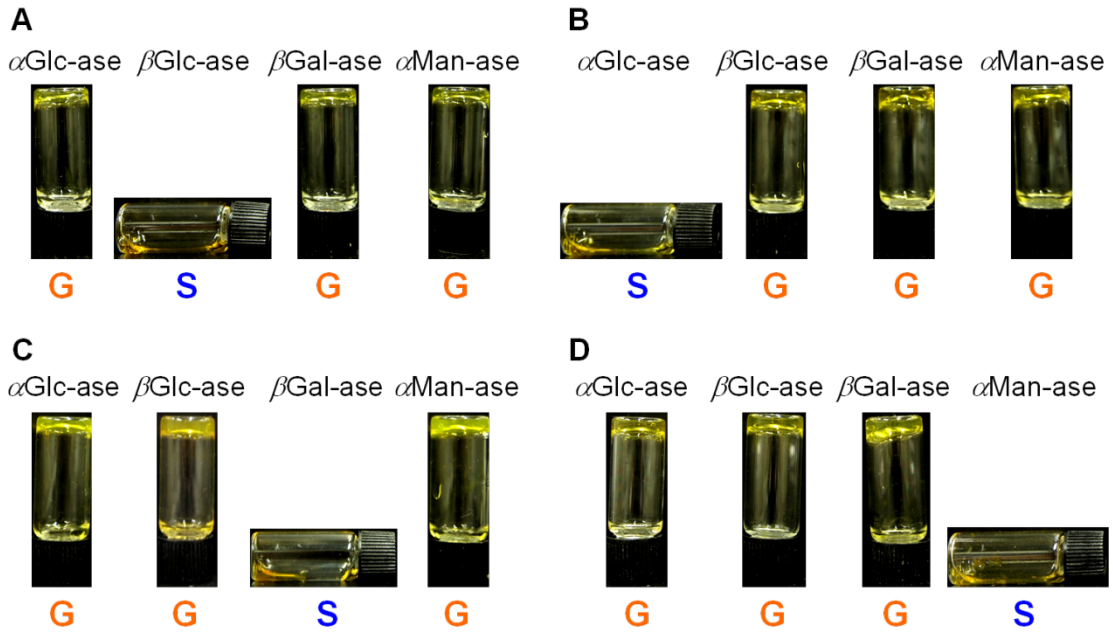


Figure S5. Photographs of hydrogels βGlc-C11 (**A**), αGlc-C11 (**B**), βGal-C11 (**C**), and αMan-C11 (**D**) in 200 mM HEPES (pH 7.2) after the addition of glycosidases. Conditions: (**A**): [βGlc-C11] = 0.1 wt% (100 μL), [α-glucosidase (αGlc-ase), β-glucosidase (βGlc-ase), β-galactosidase (βGal-ase), α-mannosidase (αMan-ase)] = 120 units/mL (20 μL), 200 mM HEPES (pH 7.2), (**B**): [αGlc-C11] = 0.1 wt% (100 μL), [αGlc-ase, βGlc-ase, βGal-ase, αMan-ase] = 120 units/mL (20 μL), (**C**): [βGal-C11] = 0.1 wt% (100 μL), [αGlc-ase, βGlc-ase, βGal-ase, αMan-ase] = 120 units/mL (20 μL), (**D**): [αMan-C11] = 0.1 wt% (100 μL), [αGlc-ase, βGlc-ase, βGal-ase, αMan-ase] = 120 units/mL (20 μL) in 200 mM HEPES (pH 7.2), room temperature (RT), 6 h.

8. Product analysis by HPLC.

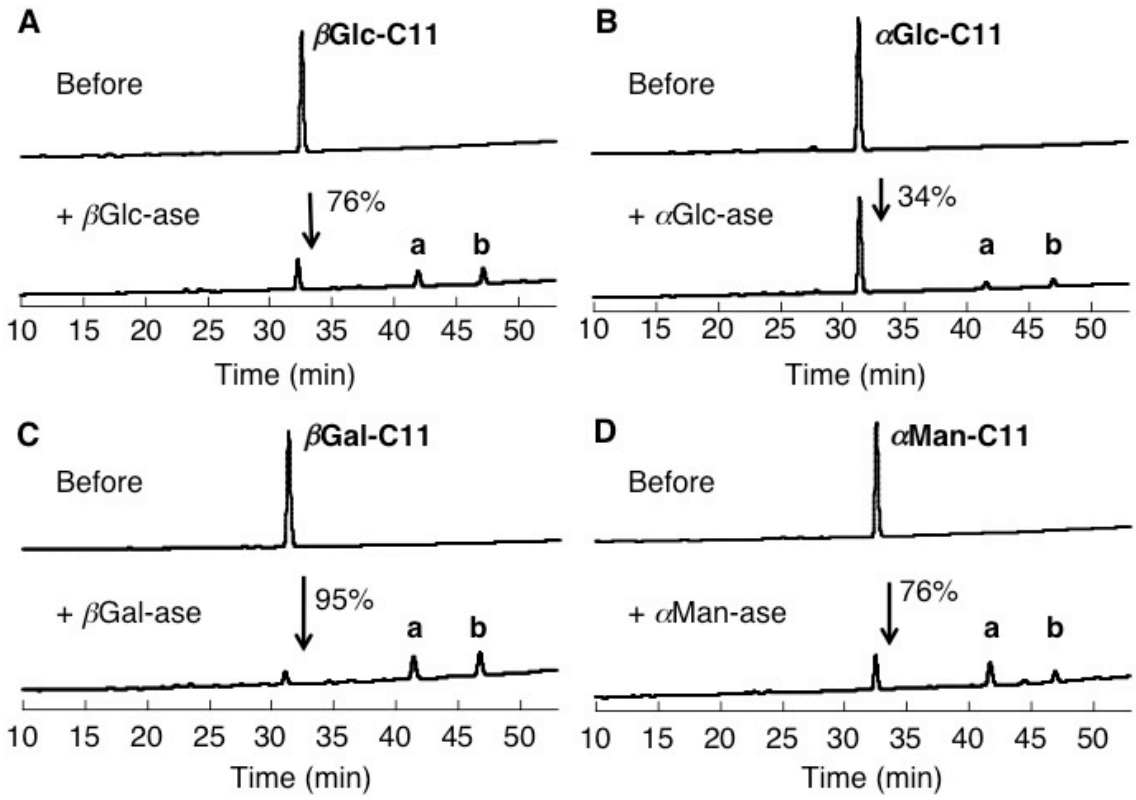


Figure S6. HPLC analysis of hydrogels βGlc-C11 (**A**), αGlc-C11 (**B**), βGal-C11 (**C**), and αMan-C11 (**D**) before and 6 h after the addition of glycosidases. Conditions: (**A**): [βGlc-C11] = 0.1 wt% (100 μL), [βGlc-ase] = 120 units/mL (20 μL), 200 mM HEPES (pH 7.2), (**B**): [αGlc-C11] = 0.1 wt% (100 μL), [αGlc-ase] = 120 units/mL (20 μL), (**C**): [βGal-C11] = 0.1 wt% (100 μL), [βGal-ase] = 120 units/mL (20 μL), (**D**): [αMan-C11] = 0.1 wt% (100 μL), [αMan-ase] = 120 units/mL (20 μL) in 200 mM HEPES (pH 7.2), RT, RP-HPLC (column: YMC-Triart C18 column (250 mm × 4.6 mm I. D.), eluent: 0.1% TFA acetonitrile:0.1% TFA H_2O = 25:75 to 80:20 (over 50 min, linear gradient), flow rate = 1.0 mL/min, detection wavelength = 220 nm)).

9. ESI MS of the products after the addition of βGlc-ase to hydrogel βGlc-C11 and proposed scheme of the reaction.

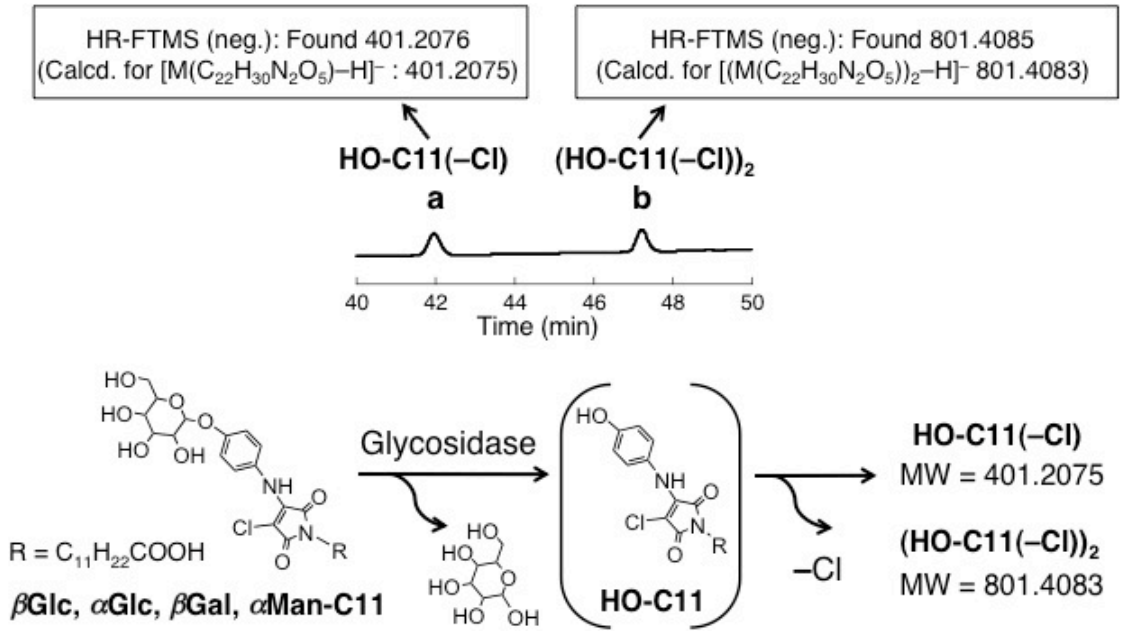


Figure S7. HPLC trace of gel **1** after addition of βGlc-ase and ESI-MS data of the two main peaks. The ESI-MS data suggest that two main peaks can be assigned as a dechlorinated compound of the *N*-alkyl-2-anilino-3-chloromaleimide (AAC) moiety.

10. Typical TEM images of hydrogels after the addition of the corresponding glycosidases.

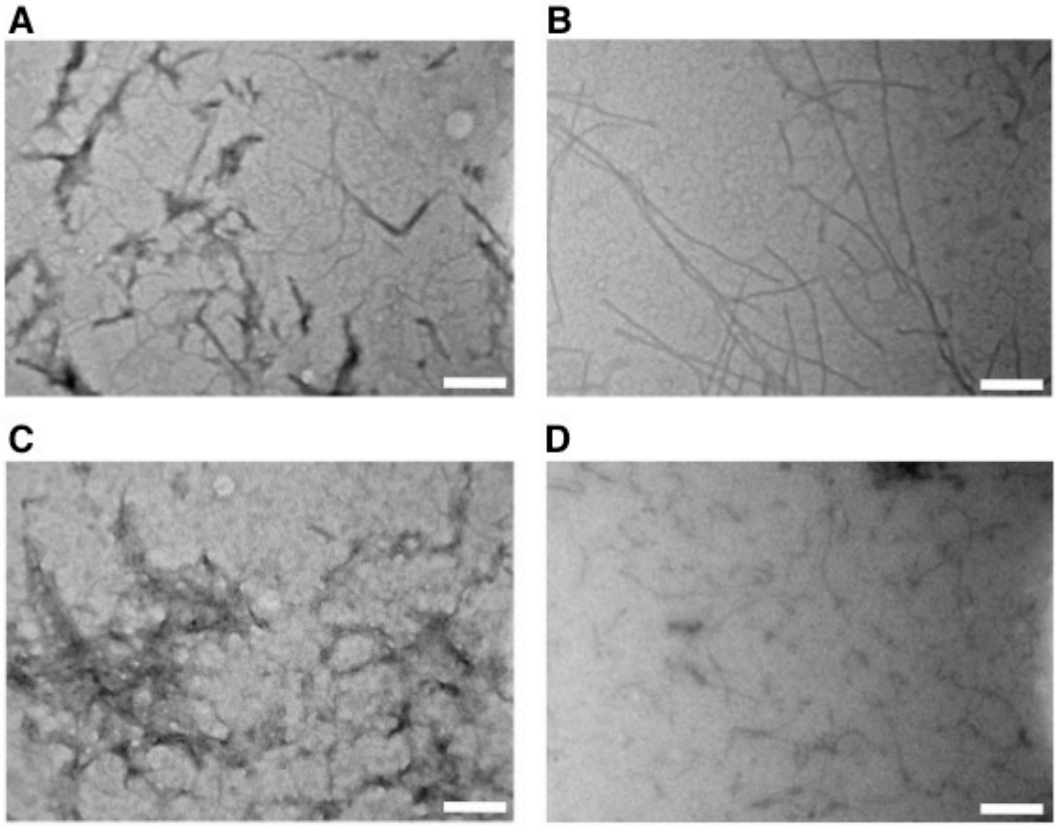


Figure S8. Typical TEM images of hydrogels βGlc-C11 (**A**), αGlc-C11 (**B**), βGal-C11 (**C**), and αMan-C11 (**D**) 6 h after the addition of the corresponding glycosidases transferred on an elastic carbon-coated grid. Scale bar is 200 nm. ([Gelators] = 0.1 wt% (100 µL), [Glycosidases] = 120 units/mL (20 µL), in 200 mM HEPES (pH 7.2))

11. Colorimetric assay of *β*Glc-ase using gel array of *β*Glc-C11.

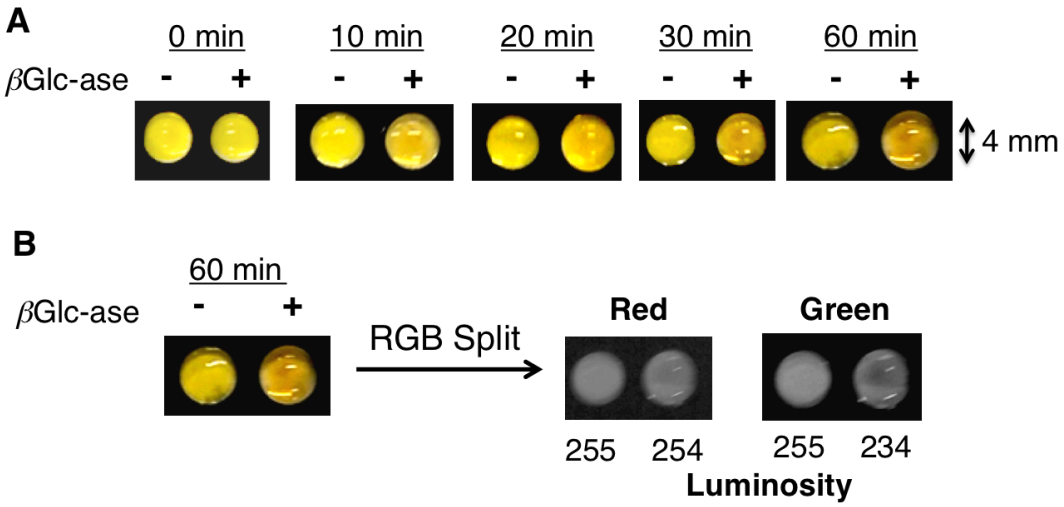


Figure S9. (**A**) Photographs of the supramolecular hydrogel array of ***β*Glc-C11** after the addition of *β*Glc-ase**.** ([*β*Glc-C11] = 0.1 wt% (10 μL), (**B**) RGB split image of the photograph of hydrogel array 60 min after the addition of *β*Glc-ase. The split images (red and green channels) were shown in gray scale ([*β*Glc-ase] = 120 units/mL (2 μL), 200 mM HEPES (pH 7.2)).

References

(1) J. H. Jung, S. Shinkai, and T. Shimizu, *Chem. –Eur. J.* **2002**, *8*, 2684–2690.

(2) L. Hanaineh-Abdelnour, S. Bayyuk, and R. Theodorie, *Tetrahedron*, **1999**, *55*, 11859–11870.