Combinatorial Library of Low Molecular-Weight Organo- and Hydrogelators Based on Glycosylated Amino Acid Derivatives by Solid-Phase Synthesis

Shigeki Kiyonaka,[b] Seiji Shinkai,[b] and Itaru Hamachi*[a]

Abstract: A combinatorial approach for the synthesis of supramolecular gelators as new organic materials is described herein. In the course of the development of a convenient and flexible solid-phase synthesis of the artificial glycolipids, some of these compounds were accidentally found to act as low molecular-weight gelators toward organic solvents. Using this combinatorial solid-phase synthesis of glycosylated amino acetates, screening and optimization of low molecular-weight organo/hydro-gelators were efficiently carried out. We found that an *N*-acetyl-galactosamine-appended amino acid ester (GalNAc-aa) efficiently gelates a broad spectrum of organic solvents. More interestingly, some GalNAc-aa derivatives displayed an excellent hydrogelation capability. Transmission electron microscopy, scanning electron microscopy, confocal laser scanning microscopy, and FT-IR were used for characterization of the gel structure. It is indicated that supramolecular fibers supported by strong hydrogen-bonding networks are entangled so that the resulting spaces can immobilize a number of solvent molecules effectively. In addition, the supramolecular hydrogel consisting of GalNAc-suc-glu(*O*-methyl-*cyc*-pentyl)$_2$ is stable even under high salt concentrations probably due to its nonionic character and as a result, a native protein is successfully entrapped in the gel matrix without denaturation.

Keywords: combinatorial chemistry · gels · glycoconjugates · solid-phase synthesis · supramolecular chemistry

Introduction

Supramolecular chemistry proposed by Lehn has produced an amazing array of non-covalently assembled architectures through elegant control of molecular interactions.[1] In materials science, it is recently anticipated that the supramolecular strategy facilitates to design useful polymeric materials on the basis of small molecules. Engineering of crystal lattices, for instance, successfully leads to zeolite-like organic and inorganic hybrid catalysts[2] and novel electric or magnetic devices.[3] Diverse arrangement of multiple hydrogen-bonding donor and acceptor pairs in organic molecules now provides a new type of liquid crystals,[4] organogels,[5] and supramolecular polymers.[6] These new materials are predominantly developed by the rational design.

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On the other hand, random and/or combinatorial synthesis of functional materials is now attractive as an alternative methodology. Most of the materials obtained by the combinatorial approach have been categorized in inorganic solid materials,[7] organic catalysts,[8] and pharmaceuticals,[9] whereas organic materials[10] have limited availability. To develop functionalized organic materials by such combinatorial strategy, it is required to establish an appropriate solid-phase synthetic method for the corresponding target. As a combinatorial approach to supramolecular materials science, we recently developed convenient methods for solid-phase (glyco)lipid synthesis (SPLS).[11] During the synthesis, some of the artificial glycolipids were accidentally found to act as low molecular-weight gelators toward organic solvents.[11a] Here we describe screening and optimization of low molecular-weight organo-/hydrogelators using the more flexible SPLS method. Interestingly, an *N*-acetyl-galactosamine-appended amino acid (GalNAc-aa) derivative forms organogel toward a broad spectrum of organic solvents. Furthermore, some derivatives displayed excellent hydrogelation capability that have potentials in the application to biomaterials. The structural characteristics of the low molecular-weight organo/hydrogels are examined, indicating that the efficient hydrogen-bonding networks are crucial for the gelation. It is also demonstrated that the selected hydrogel can entrap myoglobin, an oxygen-storage hemoprotein, without any loss of activity.

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Results and Discussion

A general scheme of solid-phase glycolipid synthesis: We previously reported two distinct SPLS schemes based on a module combination strategy[12] as shown in Figure 1a and b. The synthesis gave the target glycolipid in 30–70% of the total isolation yield over 6–7 steps. However, the first one was disadvantageous because a functional group such as carboxylic acid or hydrazide group remained even after the attachment of the lipid to the resin and subsequent cleavage.[11b] The traceless method was developed in SPLS-2, but still the troublesome protection with acetal prior to linking a saccharide moiety to a resin is inevitably required.[11a] As a more flexible synthesis based on our glycolipid scaffold, the improved SPLS-3 was developed (see Figure 1c). In the SPLS-3, the OH groups were not protected with acetyl or acetal units; instead the primary OH group of the saccharide was linked directly to the trityl resin. Thus, the variety of potential saccharides is greatly expanded and the resin can easily be modified with seven distinct glycosyl groups. The corresponding azidoethyl glycoside is mixed and gently shaken with the chlorotrityl resin in pyridine in order to attach the glycosyl moiety, followed by reduction of azide with the Staudinger method (i.e., phosphoimine formation and the subsequent hydrolysis). The amine formed was linked with succinic acid and subsequently the other end of the succinic acid was converted to amide with a variety of amines. The cleavage from the resin was successfully conducted by a mild treatment with TFA to afford an artificial glycolipid family without any traces. The overall yields using the present SPLS-3 are relatively high (20–85%), that is independent of the saccharide structure, as summarized in Table 1.

Screening of organogelators: After cleavage of some glycolipids from the resin, we faced a severe problem in the purification process during development of SPLS-2. The solvent for the silica gel column chromatography was sometimes solidified upon dissolving the crude mixture prior to chromatography. For instance, Glc-suc-glu(*O*-dodecyl)$_2$ shows gelation activity towards hexane, benzene, toluene, ethyl acetate, and acetonitrile. Thus, the gelation capability of the present glycolipid was discovered only accidentally.

We thus decided to explore the enhanced gelators toward various organic solvents based on this glycolipid scaffold. It

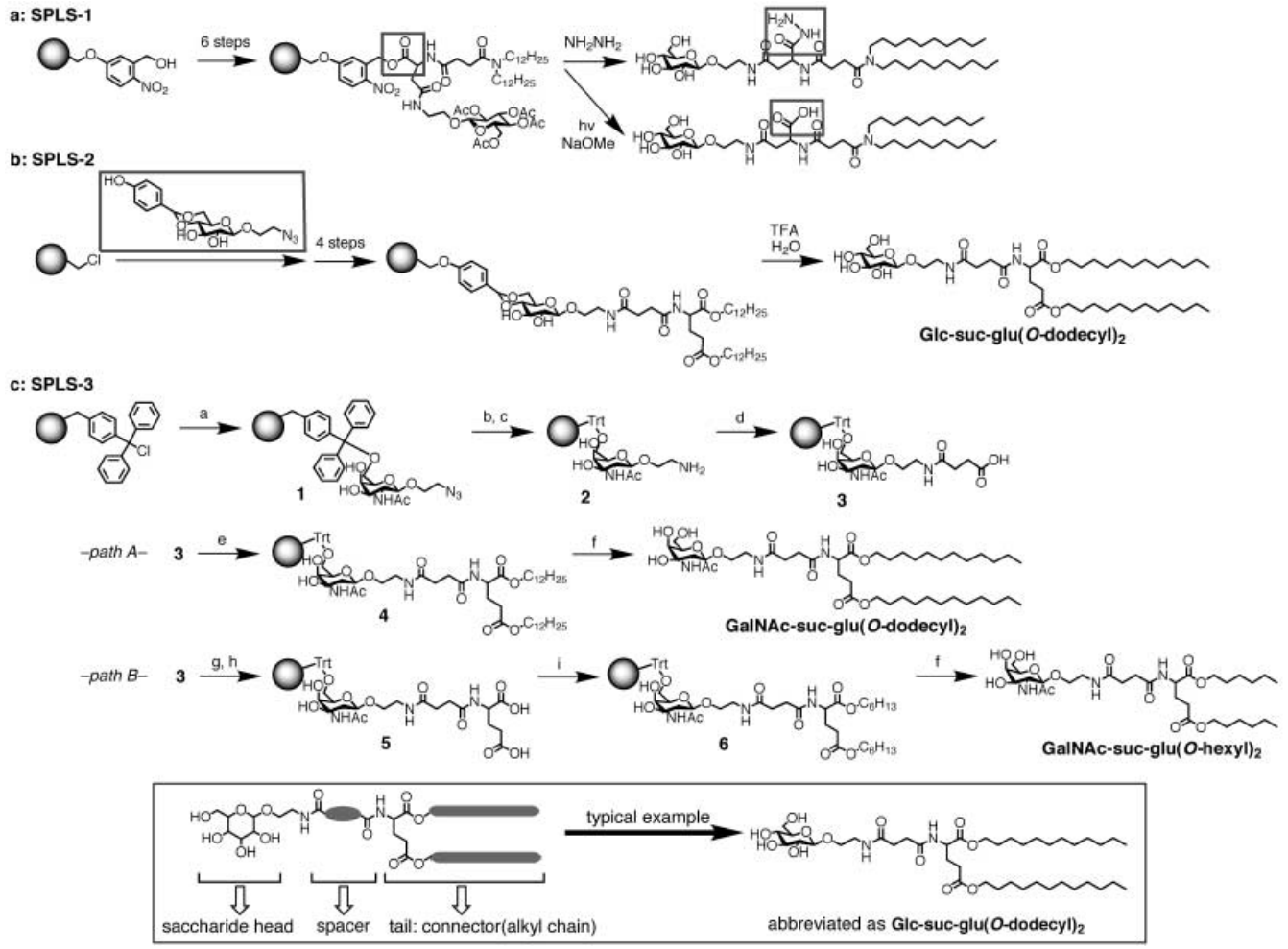

Figure 1. a) SPLS-1 scheme. b) SPLS-2 scheme. c) Synthetic route of SPLS-3. i) 2-azidoethyl β-*N*-acetyl-galactosamine/dry pyridine; ii) PPh_3/dry CH_2Cl_2; iii) 10% H_2O/THF; iv) succinic anhydride, DIEA/DMF; v) H-glu(*O*-dodecyl)$_2$, DPPA, DIEA/DMF; vi) 2% TFA/CH_2Cl_2; vii) $Pd^{II}(OAc)_2$, PPh_3, morpholine/THF; viii) H-glu(*O*-allyl)$_2$, DPPA, DIEA/DMF; ix) *n*-hexanol, HOBt, DMAP, DIC/DMF. d) A typical example of synthetic glycolipid. The glycolipids are abbreviated as "saccharide head-spacer-connector(alkyl chain)".

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Table 1. Synthetic yields of the artificial glycolipids prepared by SPLS-3.

Sugar	Spacer	Tail		Method	Yield
		connector	alkyl chain		[%]
Man	suc	glu	*O*-methyl-*cyc*-hexyl	c: path A	52
Man	suc	glu	*O*-docecyl	c: path A	56
GalNAc	suc	glu	*O*-allyl	c: path A	51
GalNAc	suc	glu	*O*-butyl	c: path A	49
GalNAc	suc	glu	*O*-hexyl	c: path B	20
GalNAc	suc	glu	*O*-*cyc*-hexyl	c: path A	45
GalNAc	suc	glu	*O*-methyl-*cyc*-pentyl	c: path A	61
GalNAc	suc	glu	*O*-methyl-*cyc*-hexyl	c: path A	54
GalNAc	suc	glu	*O*-benzyl	c: path A	64
GalNAc	suc	glu	*O*-hexyl(2Et)	c: path A	49
GalNAc	suc	glu	*O*-octyl(3,7-Me)	c: path A	50
GalNAc	suc	glu	*O*-dodecyl	c: path B	50
GalNAc	suc	glu	*O*-octadecyl	c: path A	43
GalNAc	suc	glu	*NH*-hexyl	c: path B	20
GalNAc	suc	glu	*NH*-docecyl	c: path B	42
GalNAc	suc	asp	*O*-hexyl	c: path A	48
GalNAc	suc	asp	*O*-dodecyl	c: path A	54
GalNAc	suc	aad	*O*-hexyl	c: path A	36
GalNAc	suc	aad	*O*-dodecyl	c: path A	61
GalNAc	suc		*NH*-dodecyl	c: path A	34
GalNAc	suc		*N*-(docecyl)$_2$	c: path A	34

was difficult for us to rationally design such gelators, because of the structural complexity and the poor information about their gelation ability. Therefore, the combinatorial and systematic approach seems suitable using our SPLS schemes. At the first stage, the effect of saccharide structure on gelation ability was investigated. Only the saccharide head moiety was varied with fixing the spacer and tail structures. Figure 2a summarizes the gelation capability of seven glycosyl-suc-glu(*O*-dodecyl)$_2$ glycolipids towards 10 different organic media. Apparently, the gelation ability depends considerably on the saccharide head structure. Among three monosaccharides, Glc and Gal showed almost identical gelation ability, whereas a Man head significantly decreases the gelation. Relative to Glc or Gal-suc-glu(*O*-dodecyl)$_2$, Man-suc-glu(*O*-dodecyl)$_2$ was found to be the more soluble in diverse organic solvents. This may be a result of the α-configuration of the glycosyl bond which decreases the ordered packing of the mannose unit because of the steric hindrance. Compared with Glc and Gal, disaccharides derivatives such as Cel and Mal are poor gelators. Especially, Mal-suc-glu(*O*-dodecyl)$_2$ with α-linkage between two glucose is the less efficient gelator than

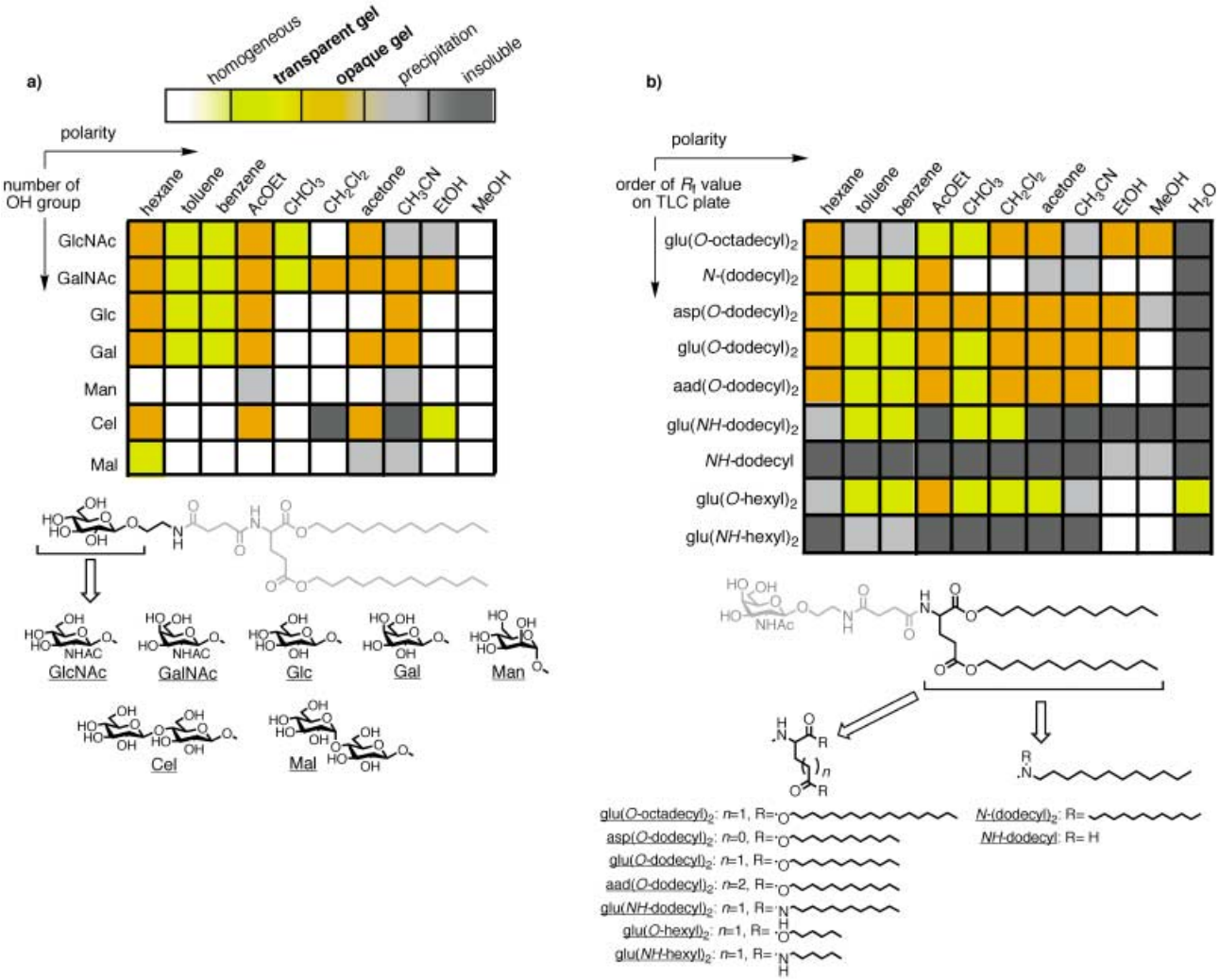

Figure 2. a)Gelation test of glycosyl-suc-glu(*O*-dodecyl)$_2$ in various organic solvents for screening diverse the saccharide head module. [glycolipid] = 25 mM. b) Gelation test of GalNAc-suc-tail in various organic solvents for screening the tail module. The order of R_f value on silica gel TLC plate roughly indicates to the order of the molecular polarity of glycolipids. [glycolipid] = 25 mM.

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Cel-type (β-linkage between two glucose). Similar to the case of Man, the Mal-derivative is much too soluble in many organic solvents to be a gelator. On the other hand, GlcNAc and GalNAc derivatives act as better gelators than the other five derivatives. Especially, GalNAc-suc-glu(O-dodecyl)$_2$ can gelate nine organic solvents with methanol being the only exception among the 10 solvents tested (Figure 3). This implies that the replacement of one OH group with N-acetamido group in the saccharide moiety enhances the gelation ability of the glycolipids. We therefore selected GalNAc as the best modular head for the organogelator.

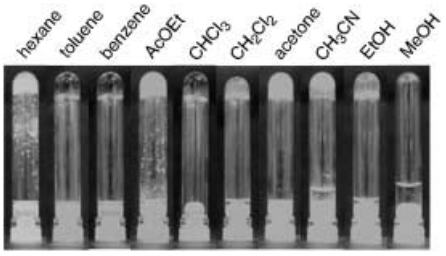

Figure 3. Photograph of organogels of GalNAc-suc-glu(O-dodecyl)$_2$ in 10 different solvents. [GalNAc-suc-glu(O-dodecyl)$_2$] = 25 mM.

At the second stage of screening, we varied the tail part of the glycolipid with the fixed GalNAc head group. The gelation ability of nine GalNAc derivatives was screened as shown in Figure 2b. It is clear that the tail structure greatly effects the gelation property, as well as the saccharide head. GalNAc-suc-*NH*-dodecyl bearing the single chain tail with an monoalkylamide does not act as a gelator at all since it is poorly soluble in all of the solvents. GalNAc-suc-*N*-(dodecyl)$_2$, the double alkyl chain derivative, shows the better gelation ability toward hydrocarbon solvents such as hexane, benzene, and toluene than the single alkyl GalNAc-suc-*NH*-dodecyl derivative. The single alkyl chain has intrinsically good crystallization propensity and thus it is not suitable as a gelator. The glutamic acid ester derivatives show a better gelation ability than the simple amide derivatives, whereas the glutamic acid amide derivatives do not show such a good ability. The simple increase in the number of amide bond decreases the solubility of the GalNAc-derivatives and as a result gelation ability is suppressed. This is in sharp contrast to the *N*-acetamido effect on the sugar moiety, indicating the importance of the suitable site-juxtaposition of the functional units. The random incorporation of amide bonds is not necessarily an appropriate strategy. Among glutamic acid ester derivatives (GalNAc-suc-glu(O-alkyl)$_2$), they showed a distinct gelation ability which was dependent on the alkyl chain length. While the dodecyl glutamate derivative can gelate nine organic solvents, seven solvents were gelated by the octadecyl glutamate derivative and six solvents were gelated by the hexyl glutamate derivative. Though the gelation ability was decreased in the case of longer or shorter alkyl chain, they showed unique characteristics. That is, the only derivative which can gelate methanol is GalNAc-suc-glu(O-octadecyl)$_2$; GalNAc-suc-glu(O-hexyl)$_2$ can gelate even H_2O.

On the other hand, replacement of the glutamate with aspartate or adipate unit in the GalNAc-suc-X(O-dodecyl)$_2$ series displays the gelation pattern and abilitiy to various organic solvents similar to those of the glu connector. The subtle difference in the linkage between the saccharide head and the tail does not considerably affect the organogelation ability.

By combinatorial approach, we successfully obtained excellent low molecular-weight gelators for various organic solvents. GalNAc-suc-glu(O-dodecyl)$_2$ was the best one which can gelate organic solvents at low concentrations (for $CHCl_3$: minimal gelator concentration = 0.4 wt%, CH_2Cl_2: 0.8 wt%, toluene: 0.2 wt%, benzene: 0.2 wt%) without any heat treatment (i.e., simple mixing). The gel–sol transition temperature (T_{gel}) of this gelator for each organic solvent was also determined as follows: $CHCl_3$: 55 °C, CH_2Cl_2: 66 °C, toluene: 80 °C, benzene: 84 °C.

Structural analysis of glycosyl amino acetate based organogels: Several entangled fibers are observed by transmission electron microscopy (TEM) of the GalNAc-suc-glu(O-dodecyl)$_2$ in $CHCl_3$ without staining (Figure 4a). A clearer image was obtained by SEM photograph of the freeze-dried sample of benzene gel of GalNAc-suc-glu(O-dodecyl)$_2$ (Figure 4b). It is apparent that the fibrous networks are well developed in the

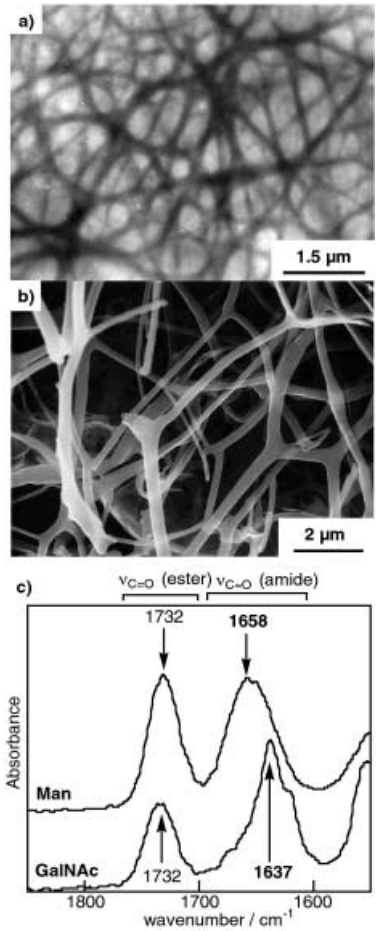

Figure 4. a) A TEM image of the benzene-gel (25 mM) of GalNAc-suc-glu(O-dodecyl)$_2$. b) A SEM image of the freeze-dried sample of GalNAc-suc-glu(O-dodecyl)$_2$ from benzene. c) FT-IR spectra of the $CHCl_3$ gel for GalNAc-suc-glu(O-dodecyl)$_2$ and the $CHCl_3$ solution for Man-suc-glu(O-dodecyl)$_2$. [glycolipid] = 25 mM.

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3D manner so that many microcavities are formed, in which solvent molecules are immobilized as a gel. In contrast, SEM picture of Man-suc-glu(*O*-dodecyl)$_2$ which was freeze-dried from the homogeneous solution in benzene shows an amorphous solid (data not shown[11a]).

Interestingly, XRD data of the xerogel of GalNAc-suc-glu(*O*-dodecyl)$_2$ shows a strong diffraction peak at 5.7 nm, clearly suggesting the regular structure of the gel fibers, which may be attributed to the tilted bimolecule unit of the gelator. When the gel is formed in benzene, it is reasonable that the bimolecular aggregate assembles at the solvophobic saccharide side, instead of the long alkyl chain side. Two broad peaks at 0.40 and 0.45 nm are also observed, which are in good agreement with the saccharide ring thickness. These may be ascribed to the packing of the saccharide ring.

The FT-IR spectra of the gel of GalNAc-suc-glu(*O*-dodecyl)$_2$ formed in $CHCl_3$ displayed a strong peak due to the amide carbonyl stretching at 1637 cm^{-1} (Figure 4c). This value is almost identical to the amide carbonyl stretching of the solid-state sample (1640 cm^{-1}). Similar low-energy-side shift of C=O stretching in IR was observed in the gel of Gal-suc-glu(*O*-dodecyl)$_2$. In the case of Man-suc-glu(*O*-dodecyl)$_2$ dissolved in $CHCl_3$ solution, on the other hand, the stretching peak is assigned at 1658 cm^{-1}. These results suggest that strong hydrogen-bonding networks formed only in the gel fibers.

Screening of hydrogelators: In the optimization process of GalNAc-based organogelators, we noticed that GalNAc-suc-glu(*O*-hexyl)$_2$ acts as a hydrogelator, as well as an organogelator. Hydrogels have a potential application in many fields and most of the reported gelators are based on cross-linked polymers. Although the gelation properties of low molecular-weight hydrogelators should be easily tuned through the molecular design, only a few hydrogelators are available thus far.[13] Therefore, we next attempted to screen good hydrogelators based on the GalNAc-appended amino acid scaffold.

The hydrogelation ability of 11 GalNAc derivatives was examined as shown in Figure 5. The gelation property is again sensitive to the tail structure (Figure 5a). The GalNAc-amino acid derivatives with a longer tail are not soluble in water because of the strong hydrophobicity, thus resulting in precipitation. On the other hand, the derivatives with the shorter tails are too soluble in water to act as gelators. The medium length of the tail is necessary for a low molecular-weight hydrogelator. GalNAc-suc-glu(*O*-methyl-*cyc*-hexyl)$_2$ and GalNAc-suc-glu(*O*-methyl-*cyc*-pentyl)$_2$ can gelate water at very low concentrations, that is, less than 0.25 wt%, displaying their gelation ability superior to GalNAc-suc-glu(*O*-hexyl)$_2$ with linear alkyl chains. Surprisingly the cyclohexyl or benzyl ester derivatives, which have only a slightly different structure from the methyl-cyclohexyl ester, cannot cause the efficient gelation.

In addition to the tail unit, the linker structure is another critical factor (Figure 5b). Replacement of glutamate connector with aspartate induced a simple precipitation in water. The fibers in the hydrogel formed in the case of the adipate connector turned out to be more loosely packed than those from the glu-derivative. Interestingly, this is in sharp contrast

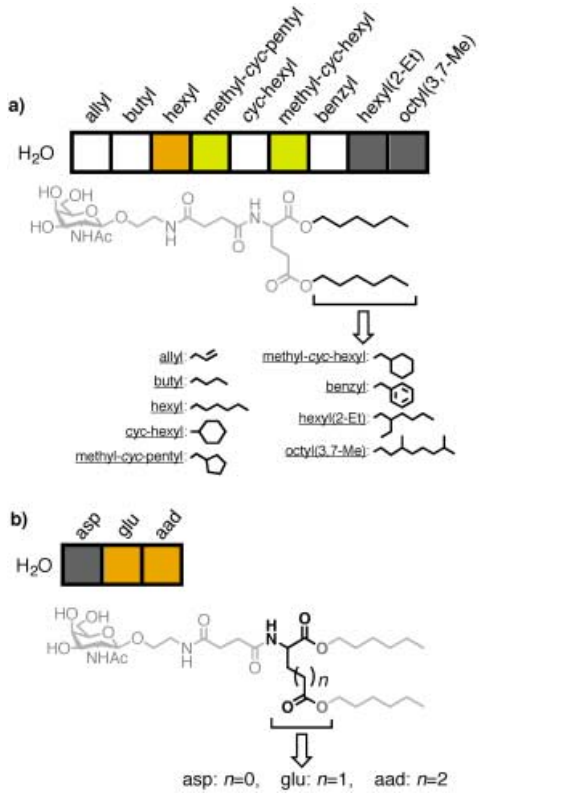

Figure 5. Gelation test of GalNAc derivatives (GalNAc-suc-tail) in H_2O. [GalNAc-suc-tail] = 8 mM. a) For screening an alkyl chain group. b) For screening a connector group.

to the insensitivity of the connector part observed in the case of organogelator. The tail part is solvophobic in the hydrogel, whereas it is solvophilic in the apolar organogel. Therefore, this discrepancy suggests that the packing of the tail part controlled by the connector is indispensable for the hydrogel, but not the case for the organogel.

Structural analysis of glycosyl amino acetate-based hydrogels: Many fibrous networks consisting of GalNAc-suc-glu(*O*-methyl-*cyc*-pentyl)$_2$ are observed by TEM with the higher resolution of the gel dried on a grid (Figure 6a). Without staining, the contrast against the bulk space was restored; the diameters of the fibers can be estimated to range between 40 to 150 nm. The morphology of the hydrogel in the wet state can be obtained by the diffraction mode of confocal laser microscopy (Figure 6b). In this case, we clearly observed entangled fiber networks bearing many spaces without drying the sample. The diameter of these fibers is roughly 10-fold greater than that obtained by TEM, suggesting that thin fibers are assembled so as to form thick fibrils in the hydrogel. It is reasonably considered that water molecules are efficiently entrapped within many cavities.

The FT-IR spectra of the wet hydrogel of GalNAc-suc-glu(*O*-methyl-*cyc*-pentyl)$_2$ formed in D_2O displayed a unique peak due to the amide carbonyl stretching at 1622 cm^{-1} (Figure 6c and d). Compared with the conventional amide carbonyl connected through hydrogen bonding, this value is found to be largely shifted to the lower energy side and comparable to that due to the well-developed hydrogen-

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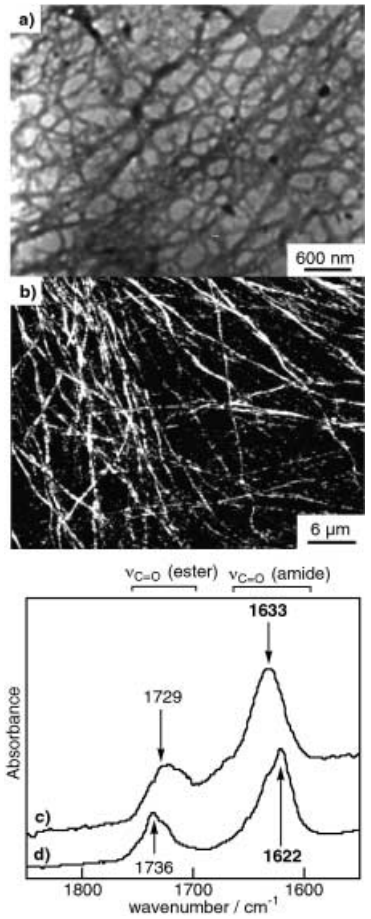

Figure 6. a) A TEM image of the hydrogel of GalNAc-suc-glu(*O*-methyl-*cyc*-pentyl)$_2$. b) A confocal laser scanning micrograph of the hydrogel of GalNAc-suc-glu(*O*-methyl-*cyc*-pentyl)$_2$. [GalNAc-suc-glu(*O*-methyl-*cyc*-pentyl)$_2$] = 4 mM. FT-IR spectra of c) the homogeneous aqueous solution of GalNAc-suc-glu(*O*-butyl)$_2$ and d) the hydrogel of GalNAc-suc-glu(*O*-methyl-*cyc*-pentyl)$_2$. [GalNAc-suc-glu(*O*-R)$_2$] = 32 mM at room temperature.

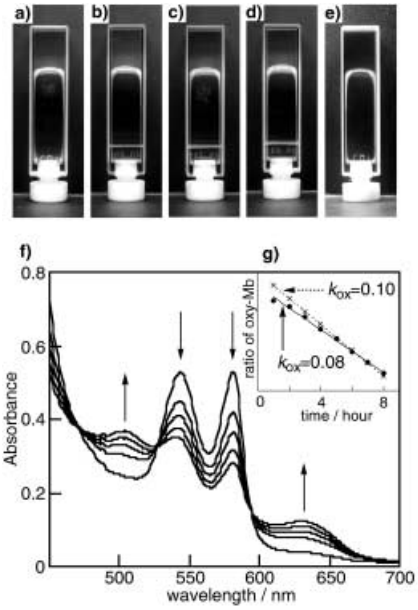

Figure 7. Photographs of hydrogels of GalNAc-suc-glu(*O*-methyl-*cyc*-pentyl)$_2$ under various conditions. a) H_2O; b) 250 mM NaCl solution; c) 25 mM acetate buffer (pH 5.0); d) 25 mM phosphate buffer (pH 8.0). [GalNAc-suc-glu(*O*-methyl-*cyc*-pentyl)$_2$] = 8 mM. e) The hydrogel containing Mb. f) An UV-visible spectral change of oxy-Mb in the hydrogel. g) Time courses of the auto-oxidation of oxy-Mb. The solid line corresponds to decrease of oxy-Mb in hydrogel, and the dotted line corresponds to that of oxy-Mb in buffer solution. [GalNAc-suc-glu(*O*-methyl-*cyc*-pentyl)$_2$] = 8 mM, [oxy-Mb] = 30 μM, 25 mM phosphate buffer (pH 6.0).

bonding network of β-sheet in natural proteins.[14] Such a shift is almost comparable to the amorphous solid state of GalNAc-suc-glu(*O*-methyl-*cyc*-pentyl)$_2$ (1624 cm^{-1}). On the other hand, the non-gelated aqueous solution of GalNAc-suc-glu(*O*-butyl)$_2$ gave the C=O stretching at 1633 cm^{-1}. Thus, it is concluded that the well-developed hydrogen-bonding network is formed only in the hydrogel state.

Immobilization of a hemoprotein in the hydrogel: Figure 7a–d show the stability of the present hydrogel against different salt concentrations and pH changes. The hydrogel consisting of GalNAc-suc-glu(*O*-methyl-*cyc*-pentyl)$_2$ is stable between 0 mM (i.e., pure water) and 250 mM of NaCl addition. In addition to the insensitivity to the salt concentration, the gel is stable between pH 5 and 8. Such a behavior is in contrast to the conventional ionic hydrogels; this is mainly ascribed to their nonionic feature of these glycosylated hydrogel. The T_{gel} value was also determined as 50 °C in pure water. It is reasonable to expect that the present hydrogels are potentially useful as biomaterials under physiological conditions.

As a proof-of-principle study, we subsequently immobilized myoglobin (Mb), an oxygen-storage hemoprotein, in the hydrogel matrix. In order to investigate the activity, the oxygen-binding form of Mb (oxy-Mb) was entrapped in the hydrogel matrix of GalNAc-suc-glu(*O*-methyl-*cyc*-pentyl)$_2$. Since it is well known that the active state of Mb (i.e., oxy-Mb) gradually changes to the resting form (i.e., ferric-Mb) by a auto-oxidation reaction, the oxidation rate monitored by UV/Vis spectroscopy indicates the lifetime of the active state of Mb. After oxy-Mb was dissolved in an aqueous solution, it is treated with a solution of GalNAc-suc-glu(*O*-methyl-*cyc*-pentyl)$_2$; the mixture rapidly turned into the reddish hydrogel (Figure 7e) upon cooling. Figure 7f displays the visible spectrum of oxy-Mb in the hydrogel. Two absorption bands at 540 and 580 nm are identical to those of native oxy-Mb and these slowly change to the other two bands (505 and 635 nm, characteristic of ferric-Mb) with two isosbestic points at 525 and 595 nm. This change obeys a typical single-exponential decay against the time course and the semilog plots are linear (Figure 7g). Using the linear relationship, the half lifetime of oxy-Mb is estimated to be 8.7 h in the hydrogel and 6.9 h in aqueous solution, respectively. The lifetime of the active state of Mb in the gel is comparable to or rather more stable than that of Mb in homogeneous aqueous solution. The leakage rate of Mb in the hydrogel was also determined spectrophotometrically. The Mb-immobilized hydrogel was placed at the bottom of the sample tube, and a buffer solution was added on the hydrogel. We continuously monitored the leaking Mb concentration to the supernatant from the gel. The leakage takes place nonlinearly, showing that 24 % of Mb leaked after 24 h, 30 % after 48 h, and 35 % after 96 h. Clearly, the present hydrogel is capable of entrapping Mb with an perfectly active

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form, whereas the partial loss of the proteins activity is often claimed upon immobilization by the conventional polymer gel matrix.[15] A controlled slow releasing system of proteins may be accomplished by fine-tuning many factors such as the gel density and others.

Conclusion

This paper clearly demonstrates the use of a combinatorial approach for the development of supramolecular organic materials based on solid-phase synthesis. It is especially useful in the case where controlling factors to a desired function are not simply elucidated because of the structural and functional complexity of the target organic materials. In the present study, we have discovered unique organogelator and hydrogelators based on nonionic glycosylated amino acid ester derivatives. When the basic motif can be divided into several modules, this approach becomes powerful tool for screening even the organic compounds. In addition, the present hydrogelators consist of saccharide, amino acetate, and alcohol modules, all of which are easily biodegradable. Therefore it can reasonably anticipate that the hydrogelators thus obtained may become highly biocompatible materials. We are currently conducting studies to establish structure–function relationships of these supramolecular gelators and various applications of these gelators.

Experimental Section

Materials and methods: All chemical reagents were obtained from Aldrich, Sigma, TCI, or Watanabe chemical industries. Commercially available reagents were used without further purification. Solvents were dried according to standard procedures. ^{1}H NMR spectra were obtained on Bruker DRX-600 (600 MHz). The peak assignments of ^{1}H NMR spectra were made by the double-resonance technique COSY. Mass spectra were recorded on MALDI-TOF-Mass spectrometer (PE Biosystems Voyager DE-RP).

General procedure for SPLS-3 (path A): A typical synthetic example is described for GalNAc-suc-glu(*O*-dodecyl)$_2$. The synthetic yield was calculated as a total yield for six steps.

Synthesis of azido ethyl glycosyl trityl resin 1:[16] Chlorotrityl resin (4.77 g, 1.05 mmol g^{-1}, 5.0 mmol), and 2-azidoethyl β-*N*-acetyl-galactosamine[17, 18] (1.60 g, 1.1 equiv) were suspended in dry pyridine (45 mL) under N_2 atmosphere. The reaction mixture was stirred for 24 h at 65 °C. After the solution was drained through a cannula capped with a filter, the obtained resin was washed with DMF (20 mL × 5), and CH_2Cl_2 (20 mL × 5). Compound **1** was obtained by drying in vacuo.

Synthesis of amino ethyl glycosyl trityl resin 2:[19] Compound **1** (5.68 g, 0.83 mmol g^{-1}, 4.71 mmol) and triphenylphosphine (3.71 g, 3.0 equiv) were suspended in dry CH_2Cl_2 (80 mL) under N_2 atmosphere. The reaction mixture was heated under reflux for 8 h. After the solution was drained through a cannula capped with a filter, the resin was washed with DMF (20 mL × 5), and then CH_2Cl_2 (20 mL × 5). Then, the resulting resin was suspended in H_2O (10 mL) and THF (90 mL) under N_2 atmosphere. The reaction mixture was heated under reflux overnight. After the solution was drained through a cannula capped with a filter, the resultant resin was washed with DMF (20 mL × 5), and CH_2Cl_2 (20 mL × 5) and then dried in vacuo.

Synthesis of succinyl glycosyl trityl resin 3: Compound **2** (5.70 g, 0.85 mmol g^{-1}, 4.83 mmol) was suspended in DMF (80 mL) under N_2 atmosphere. Succinic anhydride (0.05 g, 0.1 equiv) was added several times, and the reaction mixture was stirred for 1 h at room temperature. The Kaiser color test[20] was used for the reaction monitoring. After the test was negative, the reaction was stopped by addition of succinic anhydride. Subsequently the solution was drained through cannula capped with a filter, the collected resin was washed with DMF (20 mL × 5), and CH_2Cl_2 (20 mL × 5). After drying in vacuo, **3** was obtained.

Synthesis of glycolipid appended trityl resin 4: Compound **3** (0.75 g, 0.71 mmol g^{-1}, 0.54 mmol), didodecyl glutamate (1.04 g, 4.0 equiv), diphenylphosphoryl azide (DPPA) (0.34 mL, 3.0 equiv), and *N*,*N*′-diisopropylethylamine (DIEA) (0.56 mL, 6.0 equiv) were suspended in DMF (8.0 mL) under N_2 atmosphere. The reaction mixture was shaken overnight at room temperature. After the solution was drained by cannula capped with a filter, the resin was washed with DMF (20 mL × 5), CH_2Cl_2 (20 mL × 5) and dried in vacuo to afford **4**.

Synthesis of GalNAc-suc-glu(*O*-dodecyl)$_2$:[16] Compound **4** (0.85 g, 0.57 mmol g^{-1}, 0.48 mmol) was suspended in CH_2Cl_2 (7.0 mL) under N_2 atmosphere, and TFA (0.14 mL) was subsequently added. The reaction mixture was stirred for 1 h at room temperature. After neutralization of the reaction solution by addition of anion-exchange resin (Amberlite IRA96SB) (1.0 g), the solids were removed by filtration. The filtrate was concentrated and the residue was purified by column chromatography (silica gel, $CHCl_3$/CH_3OH 7:1 → 4:1) to obtain white solid of GalNAc-suc-glu(*O*-dodecyl)$_2$ (210 mg, overall yield: 50 %). ^{1}H NMR (600 MHz, $CDCl_3$/CD_3OD 5:1): δ = 4.49 (m, 1H, CH), 4.44 (d, 1H, J = 8.2, H-1), 4.16–4.04 (m, 4H, $CH_3(CH_2)_{10}CH_2OCO$), 3.93 (m, 1H, H-2), 3.87–3.82 (m, 4H, H-4, H-6 $NHCH_2CH_2O$), 3.82–3.71 (m, 2H, $NHCH_2CH_2O$, H-3), 3.59 (m, 1H, H-5), 3.43–3.35 (m, 2H, $NHCH_2CH_2O$), 2.61–2.51 (m, 4H, $COCH_2CH_2CO$), 2.43 (m, 2H, $CHCH_2CH_2COO$), 2.15 (m, 1H, $CHCH_2CH_2COO$), 2.05 (s, 3H, CH_3CONH), 2.01 (m, 1H, $CHCH_2CH_2COO$), 1.62 (m, 4H, $CH_3(CH_2)_9CH_2CH_2OCO$), 1.27 (m, 36H, $CH_3(CH_2)_9CH_2CH_2OCO$), 0.88 (t, 6H, J = 6.8, $CH_3(CH_2)_9CH_2$); MALDI-TOF-MS: *m*/*z*: calcd for $C_{43}H_{79}N_2O_{12}$: 830.11; found: 853.00 $[M+Na]^+$, 868.96 $[M+K]^+$; elemental analysis calcd (%) for $C_{43}H_{79}N_2O_{12}\cdot 0.5\,H_2O$: C 61.55, H 9.61, N 5.01; found: C 61.54, H 9.66, N 4.85.

General procedure for SPLS-3 (path B): A typical synthetic example is described for (GalNAc-suc-glu(*O*-hexyl)$_2$). The synthetic yield was calculated as a total yield for eight steps.

Synthesis of glutamic acid attached to the trityl resin 5:[21] Compound **3** (2.80 g, 0.53 mmol g^{-1}, 1.48 mmol), di-allyl glutamate (1.01 g, 3.0 equiv), DPPA (0.64 mL, 2.0 equiv), and DIEA (1.03 mL, 4.0 equiv) were suspended in DMF (30 mL) under N_2 atmosphere. The reaction mixture was stirred overnight at room temperature. After the solution was drained through a cannula capped a filter, the obtained resin was washed with DMF (20 mL × 5), CH_2Cl_2 (20 mL × 5). The crude resin, PPh_3 (0.78 g, 2.0 equiv), morphorine (0.39 mL, 3.0 equiv), and $Pd^{II}(OAc)_2$ (27 mg, 0.08 equiv) were suspended in THF (30 mL) under N_2 atmosphere. The reaction mixture was stirred for 12 h at 40 °C. After the solution was drained through a cannula capped a filter, the collected resin was washed with DMF (20 mL × 5), CH_2Cl_2 (20 mL × 5). After drying in vacuo, **5** was obtained.

Synthesis of glycolipid appended trityl resin 6: Compound **5** (0.70 g, 0.53 mmol g^{-1}, 0.37 mmol), *n*-hexyl alcohol (0.93 mL, 20 equiv), HOBt·H_2O (0.23 g, 4.0 equiv), *N*,*N*′-dimethylaminopyridine (DMAP) (0.18 g, 4.0 equiv), and *N*,*N*′-diisopropylcarbodiimide (DIC) (0.23 mL, 4.0 equiv) were suspended in DMF (6.0 mL). The reaction mixture was shaken overnight at room temperature. After the solution was drained through a cannula capped with filter, the obtained resin was washed with DMF (20 mL × 5) and CH_2Cl_2 (20 mL × 5) and then dried in vacuo to afford **6**.

Synthesis of GalNAc-suc-glu(*O*-hexyl)$_2$: GalNAc-suc-glu(*O*-hexyl)$_2$ was obtained from **6** according to the same method as that of GalNAc-suc-glu(*O*-dodecyl)$_2$. Overall yield: 20 % as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$:CD_3OD 5:1): δ = 7.75 (d, 1H, J = 7.6, NH), 7.56 (t, 1H, NH), 4.48 (m, 1H, $COCH(NH)CH_2CH_2CO$), 4.38 (d, 1H, J = 8.4, H-1), 4.12, 4.08 (m, 4H, $COOCH_2CH_2(CH_2)_3CH_3$), 3.88–3.59 (m, 6H, H-2, H-3, H-4, H-5, H-6), 3.79, 3.71 (m, 2H, OCH_2CH_2NH), 3.37 (m, 2H, OCH_2CH_2NH), 2.58–2.48 (m, 4H, $COCH_2CH_2CO$), 2.40 (m, 2H, $COCH(NH)CH_2CH_2CO$), 2.16,1.98 (m, 2H, $COCH(NH)CH_2CH_2CO$), 2.03 (s, 3H, CH_3CONH), 1.64 (m, 4H, $CH_2CH_2(CH_2)_3CH_3$), 1.31 (m, 12H, $CH_2CH_2(CH_2)_3CH_3$), 0.90 (t, 1H, J = 7.0, $CH_2CH_2(CH_2)_3CH_3$); MALDI-TOF-MS: calcd for $C_{31}H_{55}N_3O_{12}$: 661.78; found: 684.80 $[M+Na]^+$, 700.75 $[M+K]^+$; elemental analysis calcd (%) for $C_{31}H_{55}N_3O_{12}\cdot H_2O$: C 54.77, H 8.45, N 6.18; found: C 54.77, H 8.15, N 6.14.

Gelation test: The gelator and the corresponding solvent were mixed in a test tube and heated until the solid was dissolved. The solution was

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subsequently cooled to room temperature, and stored at 25 °C overnight. The sample that had no fluid solvent when the vial was inverted was defined as a gel state.

Observation of gel structure by microscopy: Transmission electron microscopy (TEM) measurements were carried out with Hitachi H-600 electron microscopy. A piece of the gel was added onto a carbon-coated copper grid, and dried for 3 h under vacuum without staining. The grid was examined with an accelerating voltage 90 kV. Scanning electron microscopy (SEM) measurement was conducted with Hitachi S-900. The benzene gel (2 mL) was frozen in liquid nitrogen and then dried under vacuum for 24 h at −5 °C. The dry sample (xerogel) thus obtained was coated with platinum. The accelerating voltage was 25 kV. Confocal laser scanning microscopy observation was carried out with BIO-RAD Radiance 2000 AGR3.

X-ray powder diffraction: X-ray powder diffraction was obtained with MAC Science M18XHF. The hydrogel (5 mL) was frozen in liquid nitrogen. The frozen specimen was dried under vacuum for 24 h at −5 °C. The obtained xerogel was put into a glass capillary tube (∅ = 0.7 mm). X-ray diffractogram was recorded on a imaging plate using Cu radiation (1.54178 Å) at a distance of 15 cm.

FT-IR measurement of organogels and hydrogels: FT-IR was measured with Perkin–Elmer spectrometer with a universal ATR unit so that all FT-IR measurement of gels were performed on attenuated total reflection (ATR) mode. Organogels from $CHCl_3$ and hydrogels from D_2O were measured on the plate of universal ATR.

Immobilization of oxy-Mb in the hydrogel: GalNAc-suc-glu(*O*-methyl-*cyc*-pentyl)$_2$ (7.9 mg) was suspended in a 25 mM phosphate buffer (1.5 mL, pH 6.0), and the suspension was heated until the solution turned homogeneous. The heated solution was cooled to room temperature, then the aqueous solution of oxy-Mb[22] (30 μM in phosphate buffer) was added. After the solution was quickly stored at 4 °C for 5 min, the oxy-Mb gel was obtained. The auto-oxidation reaction was monitored by UV/Vis spectroscopy Beckman Coulter DU 7400 at 25 °C.

Leakage experiment of immobilized Mb into the hydrogel: The Mb-immobilized hydrogel (2 mL) was placed at the bottom of the sample tube (∅ = 1.8 cm), and same volume of the buffer solution was gently added on the hydrogel. Leakage of the immobilized Mb from the gel was spectrophotometrically determined by the Mb concentration of the supernatant at 25 °C. $[Mb]_0$ (the initial concentration) = 170 μM, [GalNAc-suc-glu(*O*-methyl-*cyc*-pentyl)$_2$] = 8 mM, 25 mM phosphate buffer (pH 6.0).

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