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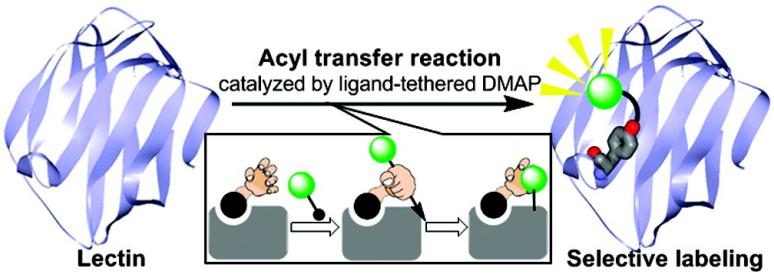

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Target-Specific Chemical Acylation of Lectins by Ligand-Tethered DMAP Catalysts

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Abstract: Because sugar-binding proteins, so-called lectins, play important roles in many biological phenomena, the lectin-selective labeling should be useful for investigating biological processes involving lectins as well as providing molecular tools for analysis of saccharides and these derivatives. We describe herein a new strategy for lectin-selective labeling based on an acyl transfer reaction directed by ligand-tethered DMAP (4-dimethylaminopyridine). DMAP is an effective acyl transfer catalyst, which can activate an acyl ester for its transfer to a nucleophilic residue. To direct the acyl transfer reaction to a lectin of interest, we attached the DMAP to a saccharide ligand specific for the target lectin. It was clearly demonstrated by biochemical analyses that the target-selective labeling of Congerin II, an animal lectin having selective affinity for Lactose/LacNAc (*N*-acetyllactosamine), was achieved in the presence of Lac-tethered DMAPs and acyl donors containing probes such as fluorescent molecules or biotin. Conventional peptide mapping experiments using HPLC and tandem mass−mass analysis revealed that the acyl transfer reaction site-specifically occurred at Tyr 51 of Cong II. This strategy was successfully extended to other lectins by changing the ligand part of the ligand-tethered DMAP. We also demonstrated that this labeling method is applicable not only to purified lectin in test tubes, but also to crude mixtures such as *E. coli* lysates or homogenized animal tissue samples expressing Congerin.

Introduction

Advanced glycobiology and glycochemistry techniques have recently demonstrated that many saccharides and their derivatives play pivotal roles in various biological events by interacting with the corresponding sugar-binding proteins.[1,2] Therefore, characterization of these sugar-binding proteins, so-called lectins or other relevant proteins, is important for understanding the complicated biological phenomena involving sugars.[3,4] Selective labeling of lectins should be useful for investigating the biological roles of these proteins in detail,[5,6] as well as for providing useful molecular tools for analyzing saccharides.[7] For example, employment of a conventional assay based on tight antigen−antibody binding such as pull-down technique is not applicable for the study of the sugar−lectin interactions because the normal sugar−lectin interactions are not sufficiently strong. In such cases, it is expected that selective covalent labeling of lectins can be alternative to these assays, as well as a microarray analysis.[8,9]

For protein labeling, bio-orthogonal organic chemistry toward protein surfaces represents one of the key methodologies. In

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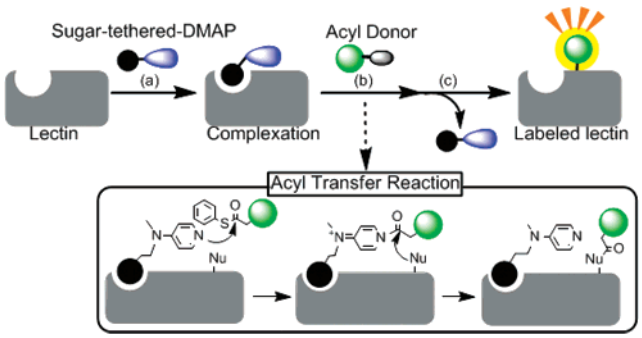
Scheme 1. Scheme of Acyl Transfer Reaction for Lectin[a]



[a] (a) Complex formation between lectin and sugar-tethered DMAP. (b) The acyl transfer reaction catalyzed by DMAP. (c) Removing sugar-tethered DMAP.

addition to classical bioconjugation reactions, several modern reactions have been recently demonstrated to be valid even under biological conditions.[10] Francis and co-workers, for example, developed several unique organometallic reactions and a coenzyme-driven reaction that are elegantly applicable for protein labeling.[11] We previously demonstrated that ligand-directed affinity labeling coupled with subsequent chemical reactions is one of the powerful methods for targeting a specific protein to be functionalized.[12] Here, we propose a new strategy for chemically labeling lectins in a target-selective manner using an acyl transfer reaction catalyzed by ligand-tethered DMAP (4-dimethylaminopyridine). Our idea is shown in Scheme 1. DMAP is a well-established acyl transfer catalyst, which can activate an acyl ester for transfer to a nucleophilic residue.[13] To direct the acyl transfer to a lectin of interest, DMAP was connected to a suitable saccharide ligand having a high affinity to the target lectin. It is reasonably expected that the ligand moiety brings the DMAP unit close to the sugar binding pocket of the lectin, so that the acyl group activated by the anchored DMAP is transferred to a residue of the target lectin on the proximal lectin surface.

Results and Discussion

To test this idea, Congerin II (Cong II), an animal lectin having lactose/LacNAc selectivity, was employed.[14] A series of sugar-tethered DMAPs and acyl donors (Figure 1) were synthesized according to Scheme 2. The acceleration of the acyl transfer from the acyl donor **5** to Cong II in the presence of Lac-DMAP **1** was clearly shown by MALDI-TOF mass spectroscopy of the reaction mixture and UV−vis spectroscopy of the purified fluorescein-labeled Cong II (FL-Cong II) (Figure 2 and Table 1). It is considered that hydrolysis of the activated acyl intermediate is a side reaction highly competitive with the acyl transfer, in aqueous solution. Indeed, we found that, in the absence of Cong II, Lac-DMAP **1** induced a significant degree of thioester hydrolysis of **5** (48%), but hydrolysis scarcely occurred without **1** (Table S1). However, the labeling of Cong II by the acyl transfer reaction was remarkably facilitated by Lac-DMAP **1** to give the FL-Cong II in 35% yield. This can be attributed to the active intermediate (acylated DMAP) being produced predominantly in the vicinity of the lectin surface by virtue of the saccharide−lectin interaction and its rapid reaction with a nucleophilic amino acid residue located on the lectin surface, with suppression of the nonproductive hydrolysis. This is supported by the experimental result that the acyl transfer did not occur (less than 1%) in the presence of excess saccharide ligand (50 mM lactose), because of inhibition of the interaction of Lac-DMAP **1** with Cong II.

This acyl transfer reaction directed by Lac-DMAP **1** was optimized by careful selection of the acyl donor structure particularly in the carboxylic ester (acyl group) part and the thiol (leaving group) part. As shown in Table 1, acyl donor **7** containing benzyl thioester was not reactive enough to be activated by DMAP (less than 1%), as compared to thiophenyl esters. Among the thiophenyl esters, on the other hand, acyl donor **6**, which includes an α-amino acid structure, was much too reactive, so that it caused a considerable amount of nonspecific acylation (11%). We found that acyl donor **5**, containing the gamma-amino acid moiety, showed enhanced specificity by reducing nonspecific reactions.[15] The selectivity, that is, the ratio of the yield catalyzed by Lac-DMAP **1** over that of the noncatalyzed reaction, was 22 for the γ-amino thiophenyl ester **5**, a value 20-fold greater than that for the α-amino thiophenyl ester **6**. The labeling site for Cong II using Lac-DMAP **1** and the optimal acyl donor **8**[16] having a coumarin fluorophore was identified by conventional peptide mapping experiments with HPLC and MALDI-TOF analysis. It was confirmed that a single peptide fragment was fluorescent among the trypsin-digested peptides as monitored by HPLC (Figure 3a). The mass/mass experiments for the isolated fluorescent peptide determined that the modified site was Tyr51. Thus, it is clear that the OH group of Tyr51 was the main acyl acceptor in the present labeling reaction. Crystallographic analysis told us that this Tyr is located near the lactose binding pocket (Figure 3b). The basic treatment of the labeled Cong II gave hydrolytic cleavage of the attached fluorophore, which is consistent with the above conclusion (Figure S8).

Restoration of the saccharide binding capability of the modified Cong II was clearly demonstrated by the fluorescent binding assay for various saccharides. The bimolecular fluorescence quenching and recovery technique (BFQR, Figure 4)[9a] was used for fluorescein-labeled Cong II (FL-Cong II) prepared by the Lac-DMAP method. Fluorescence of FL-Cong II was initially quenched by dabcyl-appended lactose (Dab-Lac **10**),

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(15) The hydrolysis tests of acyl donors **5**−**7** were carried out to compare the reactivity of the acyl donors (Table S1). The order of hydrolysis rate without DMAP was in good agreement with the order of the nonspecific modification yield of lectins. In particular, acyl donor **6** was so reactive that it did not need the activation by DMAP in the hydrolysis under the present conditions (95% with Lac-DMAP, 93% without Lac-DMAP). This may cause a considerable amount of the nonspecific acylation for lectin.
(16) MALDI-TOF mass analysis confirmed that selective acylation occurred by acyl donors **8** and **9** like acyl donor **5** (see Supporting Information).

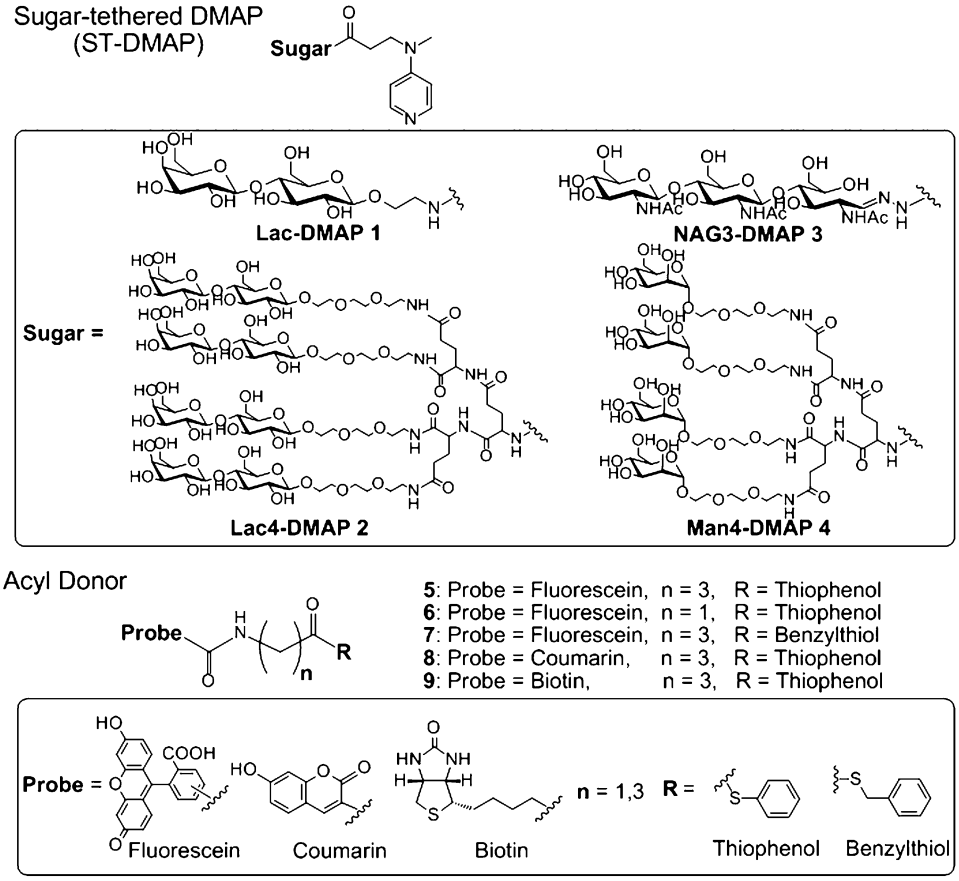
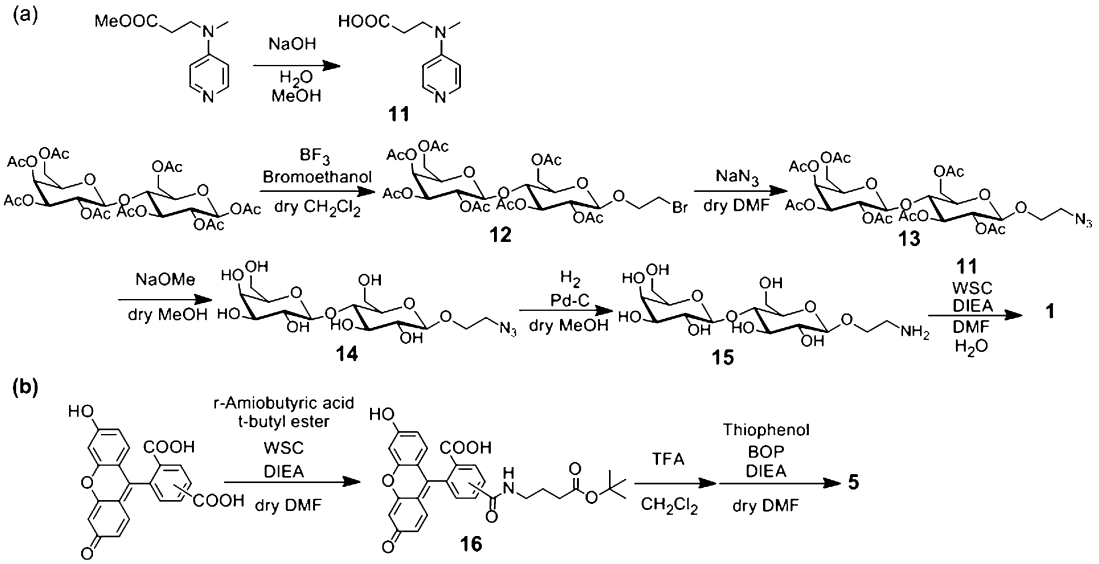


Figure 1. Molecular structures of sugar-tethered DMAPs (**1**−**4**) and acyl donors (**5**−**9**).

Scheme 2. Synthetic Scheme of Lac-DMAP **1** and Acyl Donor **5**



and then various saccharides were added to the quenched solution. Fluorescence intensity was recovered by the addition of lactose or galactose, which can bind to Cong II, indicating that these sugars replace the Dab-Lac. In contrast, maltose and mannose did not cause the fluorescence recovery because of their lack of binding to Cong II. These results indicate that the binding pocket of Cong II was open to retain almost the same saccharide selectivity after labeling.

The present strategy has the advantage that one can flexibly switch the target lectin by changing the saccharide structure of the ligand-tethered DMAP. For example, the acylation of wheat germ agglutinin (WGA, GlcNAc/NeuNAc selective lectin) or concanavalin A (Con A, Man/Glc selective lectin) was successfully carried out using NAG3-DMAP **3** and Man4-DMAP **4**, respectively (see Table 1). The high target-selectivity was clearly confirmed by the experiments with a mixture including three kinds of lectins in the presence of each saccharide-tethered DMAP. As shown in the SDS-PAGE gel images of Figure 5, the fluorescent band of Cong II was clearly observed in lane 3 by the acylation of the lectin mixture catalyzed by Lac4-DMAP

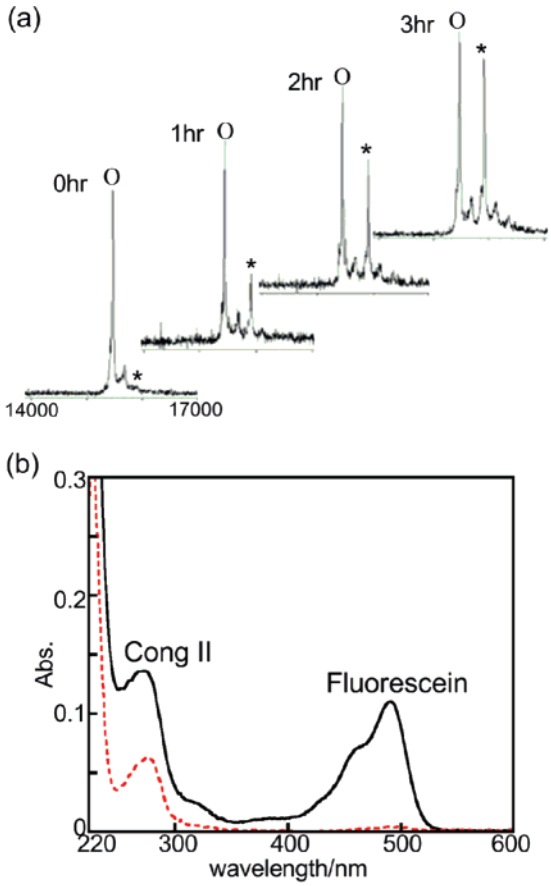


Figure 2. (a) MALDI-TOF mass spectra of reaction mixture of Cong II with sugar-tethered DMAP **1** and acyl donor **5** (○, native Cong II (M_w 15 330); *, FL-Cong II (M_w 15 770)). (b) Absorption spectra of fluorescein-labeled Cong II in the presence (black line) and the absence (red dot line) of sugar-tethered DMAP **1**.

2, whereas the other two bands coming from other lectins were not clearly observed. On the other hand, the fluorescent WGA band (lane 2) or Con A band (lane 1) selectively appeared by the fluorescence imager, where the NAG3- or Man4-tethered DMAP was used as the catalyst, respectively.[17]

This method was also applied to crude mixtures such as cell lysates of *E. coli* cells overexpressing Cong II.[14b] As shown in the SDS-PAGE gel image (Figure 6a), only one band was fluorescent, which is ascribed to the coumarin-labeled Cong II, indicating that the labeling using the ligand-tethered DMAP is selective even in the presence of many other proteins. Interestingly, the in-gel fluorescence intensity was greater when catalyzed by Lac4-DMAP **2**, which has four lactose units (lane 3), than with the mono-valent Lac-DMAP **1** (lane 2), suggesting selective labeling is facilitated by multivalent binding, in such a crude sample.[6a,18] Labeling of Cong II did not take place significantly in the presence of excess amounts of saccharide ligand (50 mM lactose, lane 4) or without DMAP ligands (lane 5).

More interestingly, we can selectively label a lectin in a animal tissue lysate using this method. The lysate of the skin mucus of Conger eel contains two kinds of galectins (Congerin I and II).[14] The Congerin was labeled with biotinylated acyl donor **9** in the presence of the catalyst Lac4-DMAP **2**. SDS-PAGE (Figure 6b) demonstrated that the naturally occurring Congerin was labeled (lane 7), which was analyzed by blotting and the subsequent diaminobenzidine (DAB) staining. In contrast, no labeling occurred in the presence of other DMAP derivatives such as Man4- or NAG3-DMAP (lanes 8, 9), in the coexistence of Lac4-DMAP with excess of saccharide ligand (lane 10), or in the absence of sugar-tethered DMAP (lane 11).

In conclusion, we have developed a novel method for lectin-selective covalent labeling using ligand-directed acyl transfer catalyzed by DMAP. The method was successfully applied not only to a purified lectin, but also to crude mixtures, such as lysates of recombinant bacterial cells or animal tissue expressing the target lectin. Thus, it is expected that this method can be used for profiling lectin-like proteins in cells or in tissues without corresponding antibodies, and fluorescent lectins prepared by this method are useful for the scaffold of biosensor to various saccharides. This chemical strategy may be generally applicable to receptor proteins other than lectin, by changing the ligand part for targeting a protein of interest and/or by replacing the catalyst part for other chemical reactions available. Such investigations are now underway.

Experimental Section

General Comments for Synthesis. All chemical reagents were purchased from commercial suppliers (Aldrich, TCI, or Wako) and used without further purification. All air- or moisture-sensitive reactions were performed with distilled solvents (DMF, dichloromethane, or MeOH) under a nitrogen or argon atmosphere.

Synthesis. Lac-DMAP **1** and acyl donor **5** were synthesized as shown in Scheme 2, and details are described below. Detailed synthetic procedures and characterizations of other sugar-tethered DMAPs **2**−**4**, acyl donors **6**−**9**, and Dab-lac **10** are described in the Supporting Information.

3-(Methyl-4-pyridylamino)propionic Acid (11). 3-(Methyl-4-pyridylamino)propionic acid methyl ester **10** was synthesized as previously reported.[13b] To a solution of **10** (240 mg, 1.23 mmol) in 2 mL of methanol was added 3 mL of 1 N NaOH solution. After being stirred for 8 h at room temperature, the reaction mixture was neutralized with HCl solution. The resulting solution was lyophilized to yield a white solid. Methanol was added to dissolve the crude product, and the insoluble salts were removed by filtration. The filtrate was evaporated under vacuum to afford 3-(methyl-4-pyridylamino)propionic acid **11** as a white solid (280 mg, 1.09 mmol, 89%). ^{1}H NMR (400 MHz, CD_3OD): δ/ppm 2.49 (t, J_H = 7.2 Hz, 2H), 3.20 (s, 3H), 3.84 (t, J_H = 7.2 Hz, 2H), 7.00 (d, J_H = 7.2 Hz, 2H), 8.07 (t, J_H = 7.2 Hz, 2H).

Peracetyl 2-Bromoethyl Lactose (12). 2-Bromoethanol (1.1 mL, 15.0 mmol) and molecular sieves 3A (2.00 g) were added to a solution of peracetyl lactose (5.11 g, 7.50 mmol) in 20 mL of CH_2Cl_2. After being stirred for 30 min at room temperature, boron trifluoride−diethyl ether (4.70 mL, 37.5 mmol) was added to the above solution. The reaction mixture was then stirred for 12 h at room temperature and diluted with 200 mL of ethyl acetate. The organic layer was washed with deionized water and dried over anhydrous Na_2SO_4. The crude residue was purified by flash column chromatography on silica gel (elution: ethyl acetate/hexanes 3:2) to give peracetyl 2-bromoethyl lactose **12** as a white solid (2.17 g, 2.92 mmol, 39%). ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 1.98−2.15 (m, 21H), 3.42−3.46 (m, 2H),

(17) In these two lectins, we did saponification experiments as the case of Cong II to investigate the acylation site. After the incubation at pH 12 for 1 day, the fluorescent band WGA remained clear, whereas the bands for Cong II and Con A almost disappeared (see Supporting Information Figure S8). This suggests that the acylation site of Con A is probably Tyr as the case of Cong II, whereas the acylation site of WGA is not Tyr, but Thr or Ser (for the alkyl ester) or Lys (for the amide).

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Table 1. Modification Yield of Lectins in the Presence or Absence of Sugar-Tethered DMAPs[a]

lectin		Cong II			WGA	Con A
acyl donor		**5**	**6**	**7**	**5**	**5**
modification yield	with ST-DMAP	35 ± 2%	12%	<1%	62%	74%
	without ST-DMAP	1.6 ± 0.2%	11%	<1%	2.2%	11%
ratio of yield with/without DMAP		22	1.1		28	6.7

[a] The yield of Cong II acylated by **5** was averaged by three individual experiments, and other yields were calculated by single experiment.

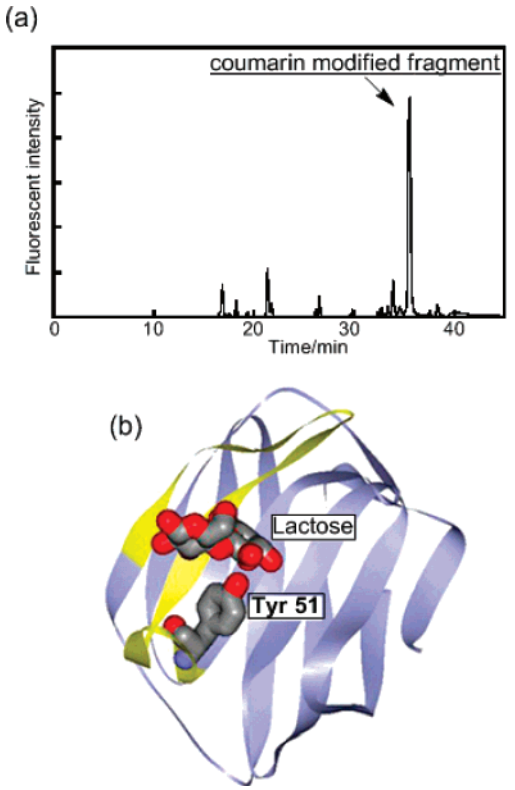

Figure 3. (a) HPLC analysis of coumarin-labeled Cong II fragments monitored by a fluorescence detector (λ_{ex} = 360 nm, λ_{em} = 410 nm). (b) Structural model of the complex of Cong II and lactose (PDB ID: 1is4). The fragment isolated by HPLC is marked in yellow, and the modified amino acid (Tyr 51) is indicated by stick style.

3.62−3.64 (m, 1H), 3.78−3.82 (m, 2H), 3.88 (t, J_H = 7.2 Hz, 1H), 4.08−4.13 (m, 4H), 4.48−4.54 (m, 2H), 4.90−4.98 (m, 3H), 5.12 (dd, J_H = 7.2, 3.6 Hz, 1H), 5.23 (d, J_H = 7.2 Hz, 1H), 5.35 (d, J_H = 3.6 Hz, 1H).

Peracetyl 2-Azidoethyl Lactose (13). To a solution of peracetyl 2-bromoethyl lactose **12** (2.17 g, 2.92 mmol) in 80 mL of DMF was added sodium azide (949 mg, 14.6 mmol). The reaction mixture was stirred for 3 h at 80 °C. After filtration, the filtrate was evaporated under vacuum and diluted with 200 mL of ethyl acetate. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. The solution was filtered and concentrated under vacuum to afford peracetyl 2-azidoethyl lactose **13** as a white solid (2.06 g, 2.17 mmol, 100%). ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 1.97−2.15 (m, 21H), 3.29−3.47 (m, 2H), 3.62−3.70 (m, 2H), 3.80−3.88 (m, 2H), 4.06−4.14 (m, 4H), 4.49−4.57 (m, 2H), 4.90−4.97 (m, 3H), 5.11 (dd, J_H = 7.2, 3.6 Hz, 1H), 5.21 (d, J_H = 7.2 Hz, 1H), 5.36 (d, J_H = 3.6 Hz, 1H).

2-Azidoethyl Lactose (14). To a solution of peracetyl 2-azidoethyl lactose **13** (2.06 g, 2.17 mmol) in 12 mL of methanol was added 181 μL of methanol solution of sodium methoxide (217 μmol). After being stirred for 4 h at room temperature, the reaction was quenched by adding ion-exchange resin (Amberlite IRC-50, 2 g). After filtration, the filtrate was removed under vacuum to afford 2-azidoethyl lactose **14** as a white solid (880 mg, 2.14 mmol, 99%). ^{1}H NMR (400 MHz, CD_3OD): 3.39−3.94 (m, 16H), 4.30 (d, J_H = 7.2 Hz, 1H), 4.34 (d, J_H = 7.2 Hz, 1H).

2-Aminoethyl Lactose (15). To a solution of 2-azidoethyl lactose **14** (880 mg, 2.14 mmol) in 100 mL of methanol was added palladium−carbon (10%, 100 mg). Under H_2 atmosphere, the reaction mixture was stirred for 20 h at room temperature. After the palladium−carbon was removed by filtration, the filtrate was evaporated under vacuum to afford 2-aminoethyl lactose **15** as a white solid (709 mg, 1.84 mmol, 86%). ^{1}H NMR (400 MHz, CD_3OD): 3.38−4.02 (m, 16H), 4.35−4.37 (m, 2H).

Lac-DMAP (1). To a solution of 2-aminoethyl lactose **15** (32 mg, 168 μmol) in 200 μL of DMF were added WSC (16 mg, 84 μmol) and DIEA (20 μL, 168 μmol). A solution of 3-(methyl-4-pyridylamino) propionic acid **11** (10 mg, 56 μmol) in 60 μL of distilled water was added to the above solution. The reaction mixture was then stirred for 10 h at room temperature, and the solution was purified by reversed-phase HPLC. The eluent was lyophilized and redissolved in distilled water to obtain Lac-DMAP **1** as a 300 μL aqueous solution (9.88 mM, 2.96 μmol, 5%). The RP-HPLC was performed using an ODS-A column (YMC, 250 × 10 mm) using a linear gradient of CH_3CN and 10 mM aqueous ammonium acetate from 2/98 to 10/90 over 30 min: flow rate = 3 mL/min, detection at 280 nm. HRMS (FAB) calcd for [M + H^+] ($C_{23}H_{38}N_3O_{12}$), 548.2450; found, 548.2455.

3-Carboxypropylaminocarbonyl Fluorescein *tert*-Butyl Ester (16). 5,6-Carboxyfluorescein was synthesized as previously reported.[19] To a solution of 5,6-carboxyfluorescein (100 mg, 266 μmol) in 5 mL of DMF were added WSC (78.6 mg, 399 μmol), DIEA (153 μL, 931 μmol), and γ-aminobutyric acid *tert*-butyl ester (104 mg, 532 μmol). The reaction mixture was stirred for 3 h at room temperature, evaporated under vacuum, and diluted with 200 mL of ethyl acetate. The organic layer was washed with 5% aqueous citric acid and brine and dried over anhydrous $MgSO_4$. The crude residue was purified by flash column chromatography on silica gel (elution: ethyl acetate/hexanes 3:1) to give 3-carboxypropylaminocarbonyl fluorescein *tert*-butyl ester **16** as a yellow solid (72 mg, 139 μmol, 59%). 3-Carboxypropylaminocarbonyl fluorescein *tert*-butyl ester **16** was obtained as a mixture of 5′ and 6′ isomers. ^{1}H NMR (400 MHz, CD_3OD): δ/ppm 1.37 (s, 9H(6′)), 1.44 (s, 9H(5′)), 1.79 (quintet, J_H = 7.2 Hz, 2H(6′)), 1.91 (quintet, J_H = 7.2 Hz, 2H(5′)), 2.25 (t, J_H = 7.2 Hz, 2H(6′)), 2.35 (t, J_H = 7.2 Hz, 2H-(5′)), 3.33 (t, J_H = 7.2 Hz, 2H(6′)), 3.46 (t, J_H = 7.2 Hz, 2H(5′)), 6.51−6.61 (m, 4H(5′, 6′)), 6.68−6.69 (m, 2H(5′, 6′)), 7.29 (d, J_H = 8.0 Hz, 1H(5′)), 7.60 (d, J_H = 2.0 Hz, 1H(6′)), 8.07 (d, J_H = 8.0 Hz, 1H(6′)), 8.12 (d, J_H = 8.0 Hz, 1H(6′)), 8.18 (d, J_H = 8.0, 1H(5′)), 8.42 (s, 1H-(5′)).

Acyl Donor (5). 3-Carboxypropylaminocarbonyl fluorescein *tert*-butyl ester **16** was dissolved in 2 mL of CH_2Cl_2 containing 40% trifluoroacetic acid. The reaction mixture was stirred for 1 h at room temperature and evaporated under vacuum to afford 3-carboxypropylaminocarbonyl fluorescein as a yellow solid. To a solution of fluorescein aminobutyric acid in 2 mL of DMF were added BOP (20.5 mg, 46.4 μmol), DIEA (6.3 μL, 46.4 μmol), and thiophenol (7.9 μL, 77.4 μmol). The reaction mixture was stirred for 1 h at room temperature, evaporated under vacuum, and diluted with 200 mL of ethyl acetate. The organic layer was washed with 5% aqueous citric

(19) Ueno, U.; Jiao, G.-S.; Burgess, K. *Synthesis* **2004**, *15*, 2591−2593.

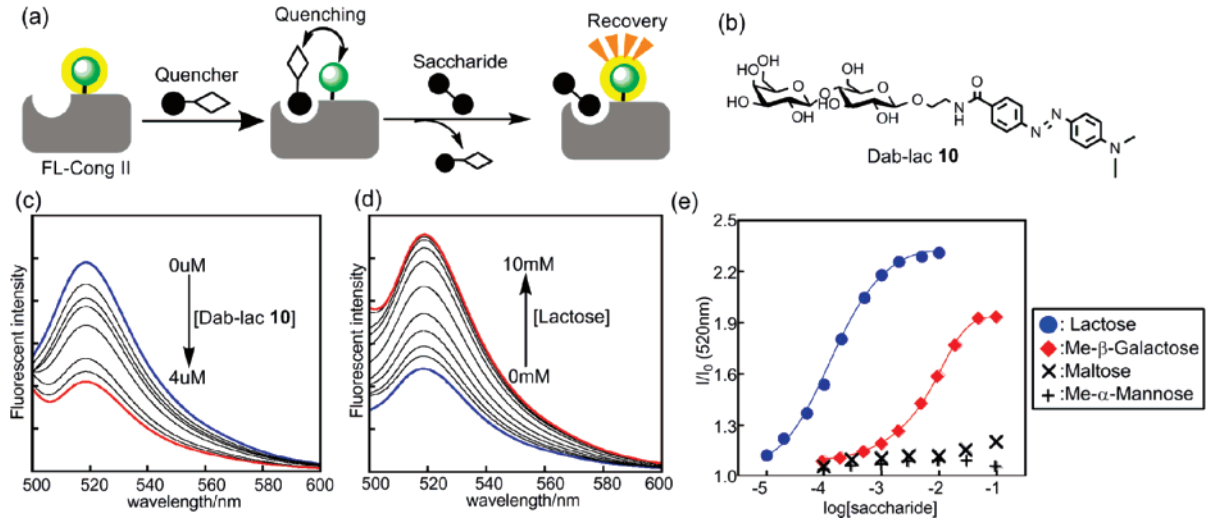

Figure 4. (a) Schematic illustration of bimolecular fluorescence quenching and recovery (BFQR). (b) Molecular structure of Dab-lac **10**. (c) Fluorescence quenching process of FL-Cong II upon addition of **10** (0−4 μM). (d) Fluorescence recovery of FL-Cong II by the addition of lactose (0−10 mM) in the presence of **10** (4 μM). (e) Fluorescence titration plot of the relative intensity (I/I_0) of FL-Cong II versus the saccharide concentration (log[saccharide]). Titration conditions: 0.2 μM FL-Cong II, pH 7.5, 20 °C, λ_{ex} = 488 nm.

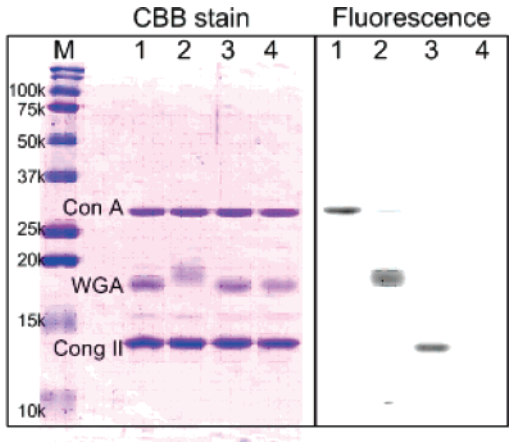

Figure 5. Selective acylation in lectin mixture. Protein modification was detected by using SDS-PAGE: left, CBB staining; right, coumarin fluorescence image. Lane M, protein marker; 1, Man4-DMAP **4**; 2, NAG3-DMAP **3**; 3, Lac4-DMAP **2**; 4, no DMAP. Reaction conditions: 0.1 mg/mL lectins (Con A, WGA, and Cong II), 50 μM sugar-tethered DMAPs **2**−**4**, 50 μM acyl donor **8**, pH 8.0, 3 h, 25 °C.

acid and brine and dried over anhydrous $MgSO_4$. The crude residue was purified by flash column chromatography on silica gel (elution: ethyl acetate/hexanes 3:1) to give acyl donor **5** as a yellow solid (17.3 mg, 31.3 μmol, 81%). Acyl donor **5** was obtained as a mixture of 5′ and 6′ isomers. ^{1}H NMR (400 MHz, CD_3OD): δ/ppm 1.91 (quintet, J_H = 7.2 Hz, 2H(6′)), 2.06 (quintet, J_H = 7.2 Hz, 2H(5′)), 2.69 (t, J_H = 7.2 Hz, 2H(6′)), 2.81 (t, J_H = 7.2 Hz, 2H(5′)), 3.36 (t, J_H = 7.2 Hz, 2H(6′)), 3.49 (t, J_H = 7.2 Hz, 2H(5′)), 6.50−6.70 (m, 4H(5′, 6′)), 6.68−6.69 (m, 2H(5′, 6′)), 7.26−7.40 (m, 5H(5′, 6′)+1H(5′)), 7.61 (d, J_H = 2.0 Hz, 1H(6′)), 8.06 (d, J_H = 8.0 Hz, 1H(6′)), 8.12 (d, J_H = 8.0 Hz, 1H(6′)), 8.18 (J_H = 8.0 Hz, 1H(5′)), 8.41 (s, 1H(5′)). HRMS (FAB) calcd for [M + H$^+$] ($C_{31}H_{24}NO_7S$), 554.1268; found, 554.1285.

General Comments for Biochemical Assays. Recombinant Congerin II (Cong II) was expressed in *E. coli* strain JM109/pTV-Cong II and purified as previously described.[12b] Concanavalin A (Con A) and Wheat Germ Agglutinin (WGA) were purchased from Funakoshi and used without further purification. The concentration of lectins (Cong II, ϵ_{280nm} = 11 500; Con A, ϵ_{280nm} = 35 000; and WGA, ϵ_{280nm} = 25 500/M^{-1} cm^{-1}), sugar-tethered DMAPs **1**−**4** (ϵ_{280nm} = 18 000), and acyl donors **5**−**8** (**5**−**7**, ϵ_{494nm} = 75 000 (pH 9); **8**, ϵ_{419nm} = 36 000 (in MeCN))[20] was determined spectrophotometrically.

Chemical Acylation of Lectins in Test Tubes. Acyl transfer reactions were carried out under the following conditions: 10 μM lectin, 50 μM acyl donor, and 50 μM sugar-tethered DMAP in 50 mM HEPES buffer (pH 8.0). The reaction was initiated by the addition of acyl donor and incubated at 25 °C. For MALDI-TOF mass measurements shown in Figure 2a, aliquots were taken at every hour, immediately purified by ZipTip (Millipore), and mixed with a matrix solution (sinapinic acid). After 3 h incubation, the reaction solution was subjected to gel filtration (TOYOPEARL HW-40F) using 50 mM acetate buffer (pH 5.0), and the eluent was dialyzed against 50 mM HEPES buffer (pH 7.5). Modification yields shown in Table 1 were calculated by the absorbance at 464 nm to that at 280 nm using the molar extinction coefficients of acyl donors **5**−**7** (ϵ_{464nm} = 26 000, ϵ_{280nm} = 16 000 (pH 7.5)) and lectins.

Acyl transfer reactions in a lectin mixture were carried out in 50 mM HEPES buffer (pH 8.0) containing 0.1 mg/mL of each lectin (Cong II, Con A, and WGA), 50 μM acyl donor **8**, and 50 μM sugar-tethered DMAPs **2**−**4** as described above. After 3 h incubation at 25 °C, the reaction mixture was fractionated by 15% SDS-PAGE in a reducing condition. The gel was analyzed by a fluorescent gel imager ChemiDoc XRS (BioRad) and stained by Coomasie brilliant blue.

Determination of Modification Sites. The coumarin-modified Cong II prepared as described above was diluted in 50 mM HEPES buffer (pH 8.0) containing 2 M urea. Trypsin was added to the solution to a trypsin/substrate ratio of 1/10 (w/w), and the digestion was allowed to proceed for 3 h at 37 °C. The digested peptides were separated by reversed-phase HPLC using an ODS-A column (YMC, 250 × 4.6 mm), and each fraction was analyzed by MALDI-TOF mass spectrometry. The HPLC condition used was as follows: CH_3CN/H_2O (both containing 0.1% TFA) 5/95−50/50 (linear gradient over 45 min), flow rate = 1 mL/min, detection by UV absorption (220 nm) and fluorescence (λ_{ex} = 360 nm, λ_{em} = 410 nm). The most fluorescent fraction was collected, lyophilized, and subjected to mass−mass analysis (Shimadzu, AXIMA QIT (MALDI-QIT-TOF)) to determine the modified amino acid.

BFQR Measurement. Fluorescence spectra were measured by a Perkin-Elmer LS55 fluorescence spectrometer. The slit widths for excitation and emission were set at 15 and 10 nm, respectively. The excitation wavelength was 488 nm. In fluorescence quenching processes, FL-Cong II solution prepared as described above was diluted

(20) Haugland, R. P. *Handbook of Fluorescent Probe and Research Chemicals*, 9th ed.; Molecular Probes, Inc.: Eugene, OR, 2002.

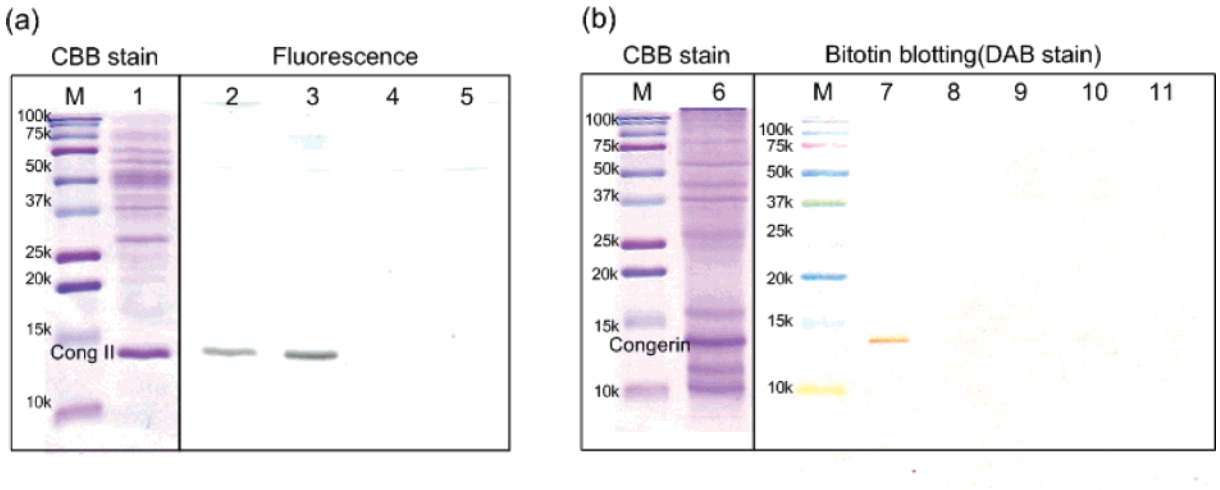

Figure 6. Selective acylation of Congerin in crude mixtures. Protein modification was detected by SDS-PAGE: CBB staining (lanes M, 1, 6); coumarin fluorescence image (lanes 2−5); biotin blotting (lanes 7−11). (a) Coumarin labeling in cell lysates of *E. coli* overexpressing Cong II. (b) Biotin labeling in tissue lysates of conger skin mucus. Lane M, protein marker; 1, whole proteins of *E. coli*; 2, Lac-DMAP **1**; 3, Lac4-DMAP **2**; 4, Lac4-DMAP **2** with 50 mM lactose; 5, in the absence of DMAP; 6, whole proteins of conger skin mucus; 7, Lac4-DMAP **2**; 8, Man4-DMAP **4**; 9, NAG3-DMAP **3**; 10, Lac4-DMAP **2** with 50 mM lactose; 11, in the absence of DMAP. Reaction conditions: 50 μM sugar-tethered DMAPs, 50 μM acyl donor, pH 8.0, 3 h, 25 °C.

to 0.2 μM with 50 mM HEPES buffer (pH 7.5), and Dab-lac **10** was added to this solution. In fluorescence recovery processes, to a solution of 0.2 μM FL-Cong II with 4 μM Dab-lac **10** were added various saccharide solutions. The structure of saccharides used for this BFQR study was shown in the Supporting Information.

Selective Acylation of Cong II in *E. coli* lysates. *E. coli* cells expressing Cong II (JM109/pTV-Cong II) were cultured in 2 × YT medium at 37 °C for 20 h, harvested, and lysed by sonication using a Sonifier 450 (Branson). After the insoluble materials were removed by centrifugation, the soluble fraction was dialyzed agains 50 mM HEPES buffer (pH 8.0) containing 0.1 M NaCl and incubated with 1 mM *N*-maleoyl-β-alanine to mask free cysteine residues at 25 °C overnight. To the lysate solution were added acyl donor **6** (50 μM) and sugar-tethered DMAP **1** or **2** (50 μM). After 3 h incubation at 25 °C, the reaction mixtures were fractionated by 15% SDS-PAGE in a reducing condition and analyzed as described above.

Congerin Acylation in Skin Mucous Extracts. Skin mucous extracts of conger myriaster were prepared according to the procedure reported.[14a] The extracts were dialyzed against 50 mM HEPES buffer (pH 8.0) containing 0.1 M NaCl and incubated with 1 mM *N*-maleoyl-β-alanine and 1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride (protease inhibitor) at 25 °C overnight. To the extract were added acyl donor **7** (50 μM) and each sugar-tethered DMAP **2**−**4** (50 μM). After 3 h incubation at 25 °C, the reaction mixtures were fractionated by 15% SDS-PAGE as described above and blotted onto a PVDF membrane. The biotinylated products were detected with avidin−peroxidase conjugate (Sigma) using diaminobenzidine (DAB) staining.

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Supporting Information Available: Synthesis of sugar-tethered DMAPs (**2**−**4**) and acyl donors (**6**−**9**) and Dab-lac **10**, mass and absorption spectra of labeled lectins, mass−mass spectrum of modified fragment, complete ref 8h, and molecular structures of saccharides used in BFQR measurement. This material is available free of charge via the Internet at http://pubs.acs.org.

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