

Synthesis of ABO blood group antigens and functional glycan display on the cell surface

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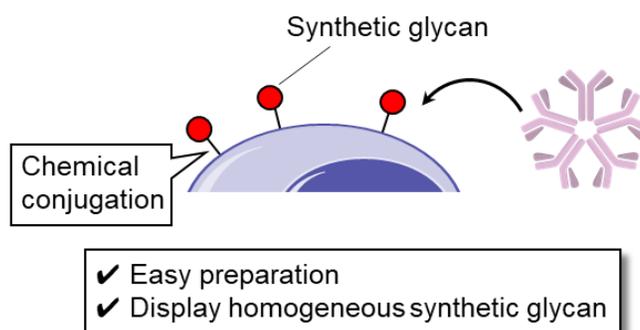
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Abstract

ABO blood group antigens are involved in various biological phenomena, including immune responses and infections. We achieved efficient and scalable synthesis of A, B, and H antigens. Using chemical conjugation, synthesized B antigen was displayed on the surface of living cells and its function as an antigen was confirmed by the IgM antibody recognition. Our results indicate that the multivalent interactions induced by cell surface glycan clustering are crucial in this system. The prepared cells displaying the glycan antigen are expected to be a model cell for investigating ABO antigen function.

< The cell displaying synthetic glycan >

→ Model for investigating of glycan function



Keywords: ABO blood antigens, glycan engineering, IgM antibody, multivalency

Introduction

Glycans are involved in various biological phenomena, including cell migration, cell development, signal transduction, and disease progression.¹⁻³ Glycans cover the cell surface as a glycocalyx, consisting of glycoproteins and glycolipids, and provide the milieu for the first contact with the external environment. Therefore, glycans are closely related to self-recognition and non-self-recognition, such as infections and immune responses. On the other hand, the structural diversity and heterogeneity of glycans make it difficult to elucidate their functions at the molecular level. Thus, chemical synthesis, which provides a certain amount of pure glycans, is a powerful tool for investigating of the biological functions of glycans.

ABO blood types are categorized by the glycan structure on erythrocytes (Figure 1a). Individuals of O, A, and B blood types express H, A, and B antigens, respectively, with H antigen is a precursor of A and B antigens. ABO blood group glycans act as antigens and individuals produce antibodies, mainly IgM antibodies, against the glycans they do not express. The interaction of ABO blood antigens with their antibodies results in blood agglutination.⁴ Such immune responses are extremely important in considering blood transfusions. In addition, ABO blood group glycans are expressed on a wide range of organs, including epithelium, intestine, gastric, and pancreas, and thereby, are closely associated to various diseases.⁵ Some pathogens, such as Norwalk virus and *Helicobacter pylori*, are reported to recognize ABO blood group glycans.⁶⁻⁷ Subsequent to the first synthesis of B antigen by Lemieux et al., many groups have reported the chemical syntheses of ABO blood group glycans.⁸⁻¹⁹

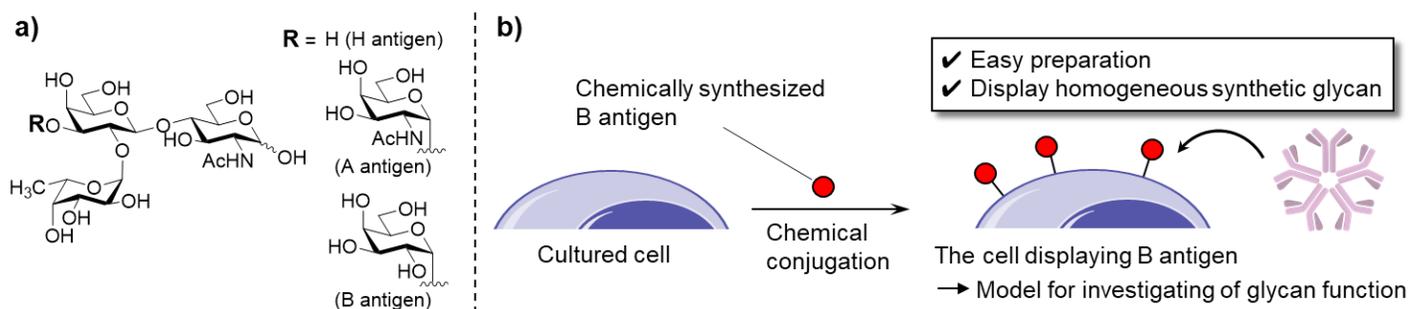


Figure 1. a) Chemical structure of ABO blood group glycans. b) Preparation schematic of cells displaying synthetic antigen glycan using chemical conjugation.

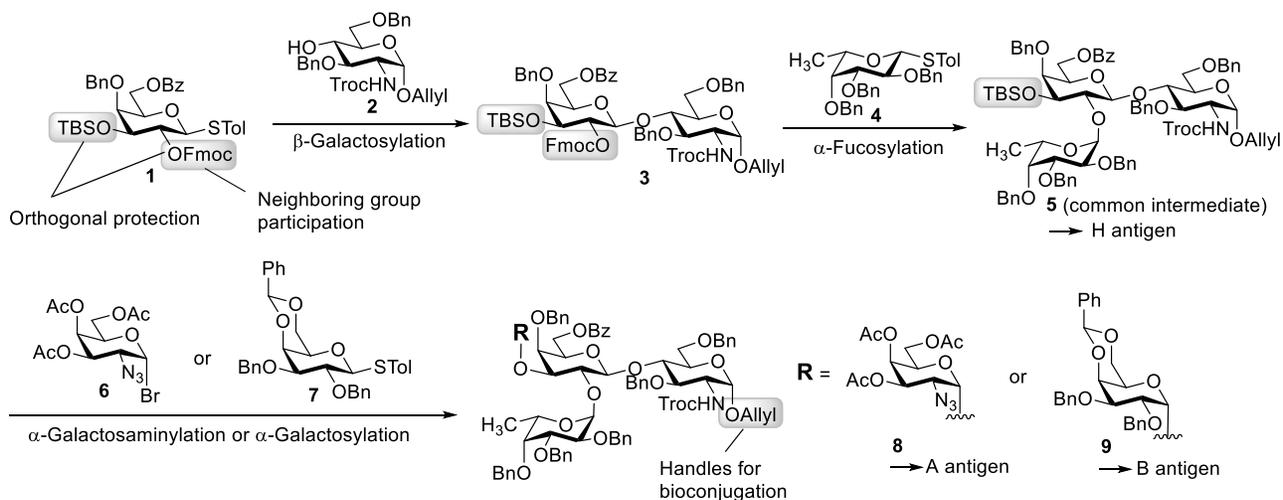
Cell surface glycan engineering is a powerful tool for investigating and revealing glycan functions.²⁰ Genetic approaches, including the knockout or knockdown of glycosyltransferase, are the most classical and versatile methods and have played a pivotal role in elucidating glycan functions. Bertozzi et al. developed metabolic labeling by incorporating unnatural monosaccharide analogs having the reaction handle followed by the bioorthogonal reaction.²¹ This method provides a facile platform for installing new chemical functionality to glycans. Chemical²²⁻²³ and chemoenzymatic²⁴⁻²⁷ glycan engineering have also been reported. In addition, *de novo* glycan display has also been investigated, such as the direct introduction of defined glycan structures into plasma membranes by lipid insertion, liposomal fusion, and tag technology.²⁸⁻³³ Henry et al. reported A and B antigen display on erythrocytes using synthetic glycolipids.²⁹ Palcic et al. reported B antigen introduction onto cell surface by enzymatic labeling.³⁴

In the current study, we synthesized ABO blood group glycans and investigated the display of the synthesized glycans on the cell surface. We achieved efficient and scalable synthesis of ABO blood group

glycans. Synthesized B antigen was introduced onto the surface of living cells by chemical conjugation using *N*-hydroxysuccinimide (NHS) ester (Figure 1b). This approach allowed the display of homogeneous synthetic glycans on the cell surface with easy operation. Furthermore, B antigen displayed by this method was recognized by IgM antibody specific for B antigen glycan. In contrast, the IgM antibody did not interact with B antigen introduced onto the IgG antibody. These results indicate that multivalent interactions through cell surface glycan clustering are crucial for the function of B antigen glycans as an antigen. The prepared cells displaying antigen glycan is expected to be a useful model for investigating ABO antigen function.

Results and Discussion

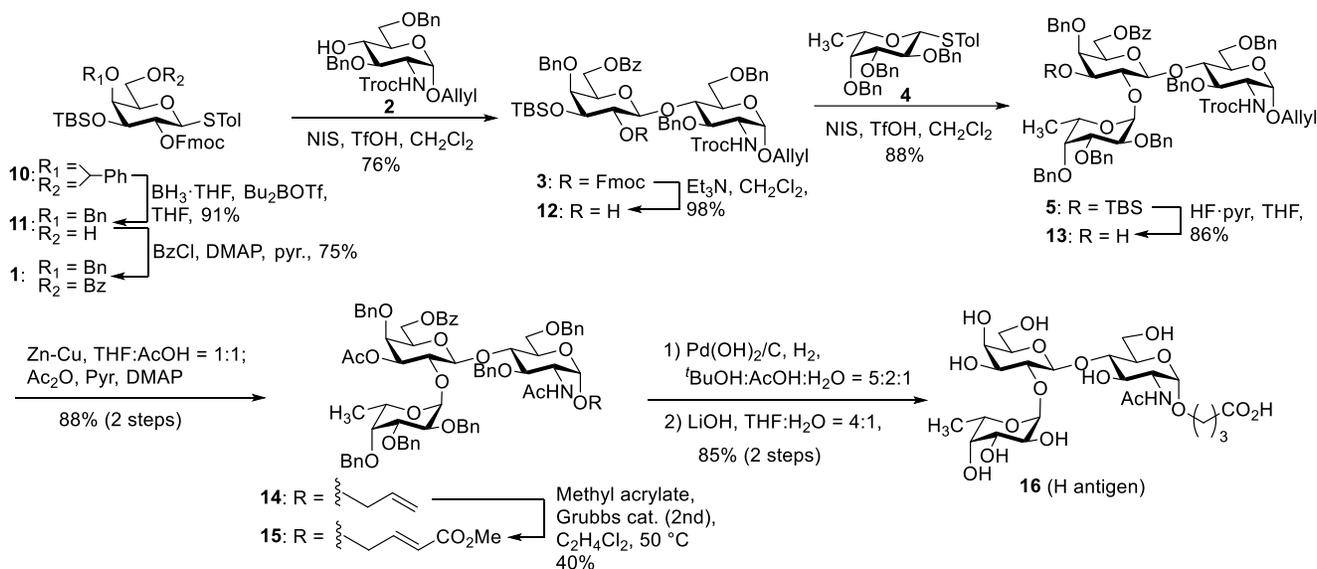
The synthetic plan of H, A, and B antigens is shown in Scheme 1. We synthesized ABO blood group type II antigens, which are widely expressed in the human body. We initiated the synthesis from galactose derivative **1**, which had the orthogonal protecting groups (*tert*-butyl(dimethyl)silyl (TBS) and fluorenylmethoxycarbonyl (Fmoc)) at the glycan elongation sites. Many previous reports on the synthesis of ABO blood group antigens first prepared the disaccharide skeleton and then manipulated the protecting groups.^{9-11, 15, 16, 18, 19} In this study, orthogonally protected galactose fragment **1** was utilized to enable the straightforward synthesis of ABO blood group antigens. β -Selective galactosylation between **1** and **2** using neighboring group participation of Fmoc group yielded disaccharide **3**. After cleavage of Fmoc group, α -fucosylation with **4** produced trisaccharide **5**. Trisaccharide **5** can be used as a common intermediate; H-antigen can be obtained by deprotection, whereas A and B antigens can be obtained by cleavage of TBS group followed by α -galactosaminylation and α -galactosylation, respectively. The allyl group at the reducing termini of **5**, **8**, and **9** can be used as handles for bioconjugation.



Scheme 1. Synthetic strategy of H, A, and B antigens.

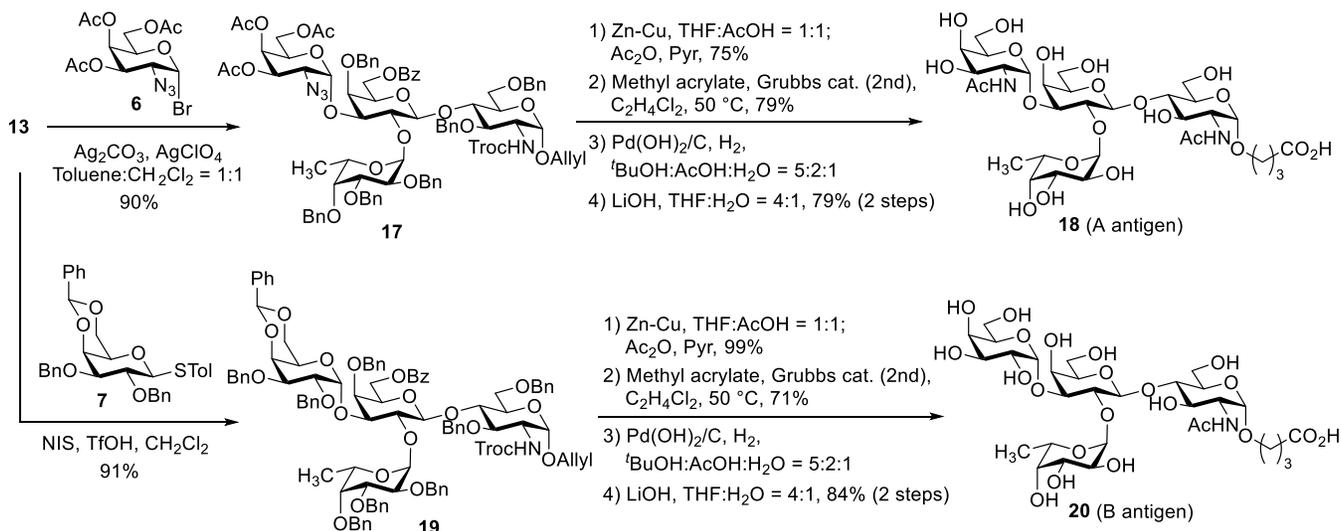
Synthesis of H antigen **16** is shown in Scheme 2. Compound **10**, reported previously by our group,³⁵ was converted to a galactosyl donor **1**. Selective cleavage of benzylidene³⁶ followed by benzoyl protection generated **1**. Glycosylation between **1** and **2**³⁷ using NIS and TfOH³⁸ as activators afforded **3** with perfect β -selectivity due to neighboring group participation. In all case in this study, the stereochemistries produced by glycosylations were determined by the coupling constants at the anomeric positions. Cleavage of Fmoc group resulted **12**, which was then fucosylated with **4**³⁹ to produce **5** in 88% yield with perfect α -selectivity.

With the common intermediate **5** in hand, H antigen **16** was synthesized via deprotection and introduction of the carboxylic acid for bioconjugation. After cleavage of TBS with HF·pyridine, Troc group of **13** was converted to acetamide through the reduction of azide followed by acetylation. In this step, the 3 position of galactose was also acetylated. A carboxy methyl group was then introduced to the reducing terminal of **14** by olefin metathesis reaction with methyl acrylate. A carboxy methyl group was then introduced to the reducing terminal of **14** by olefin metathesis reaction with methyl acrylate to yield **15**. Global deprotection of **15** by hydrogenation and hydrolysis afforded H antigen **16**.



Scheme 2. Synthesis of H antigen **15**.

A antigen **18** and B antigen **20** were synthesized from **13** (Scheme 3). Glycosylation with 2-azido galactosaminyl donor **6**⁴⁰ using AgClO₄⁴¹ gave protected A antigen **17** in 90% with perfect α -selectivity. Synthesis of A antigen **18** was then completed via introduction of carboxylic acid and deprotection, as was done in the synthesis of H antigen **15**. B antigen **20** was similarly synthesized. After α -selective galactosylation with **7**,⁴² the resulting **19** was converted to B antigen **20**.



Scheme 3. Synthesis of A antigen **18** and B antigen **20**.

With ABO blood group antigens in hand, we investigated the introduction of synthetic glycan onto the cell surface (Figure 2). We herein used B-cell lymphoma Raji cells and B antigen. We employed direct chemical conjugation of glycan onto the cell surface proteins using NHS ester, as well as indirect labeling using glycan-antibody conjugates (Figure 2c). In the indirect method, anti-CD20 antibody, which is used for the treatment of B-cell lymphoma, was applied as Raji cell recognition antibody.

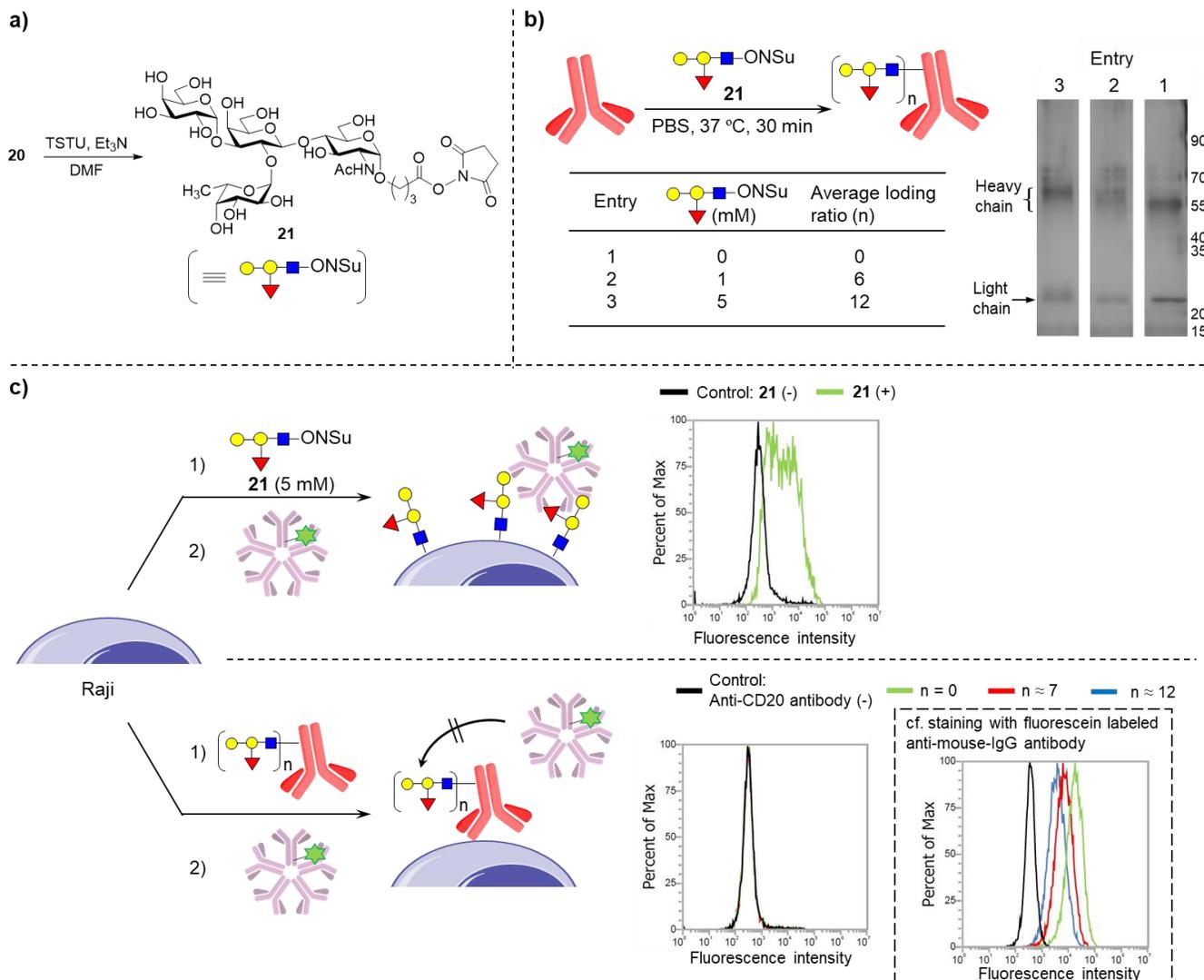


Figure 2. a) Preparation of B antigen NHS ester **20**. b) Preparation of B antigen-antibody conjugate. c) B antigen labeling of cell surfaces and evaluation of the labeled cells using anti-B antigen IgM antibody.

B antigen labeling reagents, B antigen NHS ester **21**, and B antigen-antibody conjugate were readily prepared. Treatment of **20** with *N,N,N',N'*-tetramethyl-*O*-(*N*-succinimidyl)uronium tetrafluoroborate (TSTU) yielded B antigen NHS ester **21** (Figure 2a). Reaction of anti-CD20 antibody with two concentrations of **21** (0, 1.0, and 5.0 mM) in PBS at 4 °C for 30 min afforded the antibodies conjugated with various loading ratios of B antigen (Figure 2b). Based on SDS-PAGE analysis, the loading ratio of B antigen increased as the concentration of **21** was increased. The average loading ratios (*n*) were estimated to be 7 and 12 when 1.0 and 5.0 mM **21** was used, respectively.

After labeling Raji cell with B antigen using B antigen NHS ester **21** or B antigen-antibody conjugate, the interaction with IgM antibody specific for B antigen glycan was determined using flow cytometry to evaluate the function of the B antigen displayed on the cell surface (Figure 2c). The treatment of Raji cell with 5.0 mM **21** in PBS at 37 °C for 30 min did not decrease cell viability, and the treated cells were successfully recognized by the fluorescein-labeled anti-B antigen IgM antibody. In contrast, cells treated with B antigen-antibody conjugates were not recognized by the IgM antibody. Considering the successful staining with the fluorescein-labeled anti-mouse IgG antibody, these results showed that B antigen loaded on anti-CD20 antibody was not recognized by the IgM antibody. In our previous report using α -gal as a glycan antigen, we demonstrated α -gal conjugated to anti-CD20 antibody in the same manner described in the current study was recognized by antibodies against α -gal,⁴³ suggesting that accessibility of the glycan antigen on anti-CD20 antibody does not the issue. The difference in recognition of B antigen and α -gal might be attributed to the binding affinity of the corresponding antibodies as antibodies against α -gal contain a certain amount of IgG antibodies, which exhibit high affinity.

The results described above indicate that the method used for glycan displaying is important for synthetic glycans to exhibit their function on the cell surface. Although individual glycan recognition in nature is usually weak, multiple glycans on the cell surface provide multivalent interactions leading to strong avidity.⁴⁴⁻⁴⁵ Such multivalency plays a crucial role in glycan recognition. Glycan-receptor interaction on the cell surface can be enhanced by multivalency of receptor or glycan clustering.⁴⁶⁻⁴⁸ In the presence of a multivalent binding partner, membrane proteins can diffuse in lipid bilayer to form clusters. IgM antibodies have 10 antigen-binding sites and exhibit multivalent interactions.⁴⁹⁻⁵⁰ When B antigen was introduced to the membrane proteins using NHS ester **21**, the clustering mechanism was expected to enhance the interaction between B antigen displayed on the cell surface and IgM antibody. However, B antigen introduced onto the anti-CD20 antibody was not flexible, and thereby, may not provide multivalent interaction with IgM antibody. Multivalent interaction by CD20 clustering might be prevented by steric hindrance of anti-CD20 antibody. These results demonstrated that reconstruction of multivalent interaction is crucial for the functional display of synthetic glycans in biological systems. ABO blood group glycans are known to primarily exist on glycolipids and are also expressed on glycoproteins as *N*-glycans and *O*-glycans. However, their recognition on glycoproteins by the IgM antibodies has not been elucidated. Glycan display using NHS ester **21** is usually directed against amines on proteins to produce pseudo-glycoproteins. Thus, the current study suggests that ABO blood group glycans on glycoproteins can also be recognized by IgM antibodies through their clustering and can induce biological events.

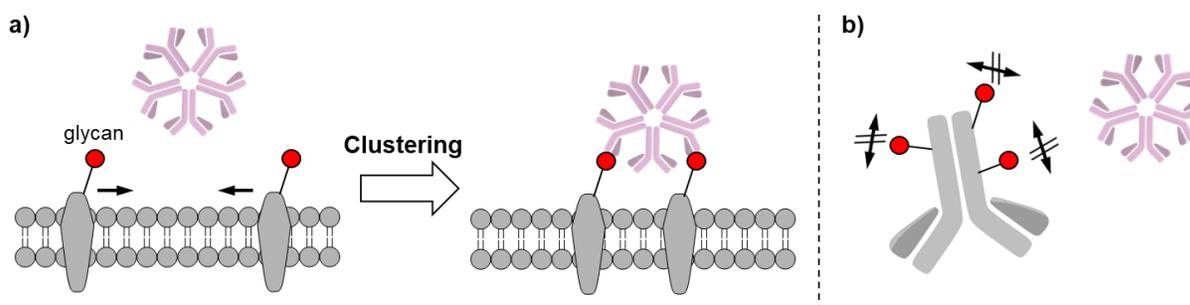


Figure 3. Putative interaction of B antigen with IgM antibody. a) B antigen (red circle) chemically conjugated to the cell surface membrane protein. b) B antigen chemically conjugated to IgG antibody.

Conclusions

We synthesized ABO blood group glycans and achieved functional display of B antigen on the surface of a living cells. Importantly, our synthesis was efficient and scalable. Orthogonal protection strategy enabled the straightforward synthesis of ABO blood group antigens. All glycosylations proceeded in more than 70% yield with perfect stereoselectivity and each glycan (**16**, **18**, and **20**) was procured at quantities greater than 50 mg. Synthesized B antigen was displayed on the cell surface in two ways, direct chemical conjugation using NHS ester **21** and indirect labeling using the glycan-antibody conjugate. B antigen directly conjugated onto cell surface proteins was recognized by IgM antibody, whereas B antigen conjugated onto IgG antibody was not recognized. These results indicate that the multivalent interactions induced by glycan clustering are important for the emergence of proper glycan function on the cell surface. Our future work will include using this newly developed approach for displaying “functional” synthetic glycan antigen on the cell surface to elucidate ABO antigen function in various biological phenomena, including immune responses and pathogen infections.

Experimental Section

General. ^1H and ^{13}C NMR spectra were recorded in an indicated solvent with JEOL ECA 500 MHz spectrometer. Chemical shifts of ^1H and ^{13}C NMR were referenced to the solvent peaks: $\delta=7.26$ and $\delta=77.16$ for CDCl_3 , $\delta=3.30$ and $\delta=49.30$ for CD_3OD . Multiplicities abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. High-resolution mass spectra were recorded on a ESI-LTQ-Orbitrap XL (FTMS) mass spectrometer and ESI-Q-TOF mass spectrometer. Chemical purification was carried out using silica-gel column chromatography. Silica-gel column chromatography was carried out using Silica Gel 60N (Kanto Chemical Co., 40-50 μm or 63-210 μm) at medium pressure (1-4 kg cm^{-2}). Gel permeation chromatography was carried out using Sephadex LH-20 at atmospheric pressure. Silica-gel 60 F254 (Merck Co.) was used for TLC analysis and preparative TLC purification, and compounds were visualized by UV (254 nm), *p*-methoxybenzaldehyde (*p*-anisaldehyde, 0.03% in EtOH- H_2SO_4 -acetic acid buffer). Anhydrous CH_2Cl_2 were distilled in the presence of calcium hydride. Anhydrous THF, DMF, distilled water, and toluene were purchased from FUJIFILM Wako Pure Chemical Corporation, Ltd.. Nonaqueous reactions were carried out under argon atmosphere. Molecular sieve 4A (MS4A) was activated with a microwave and dried in vacuo for 3 times before use. All other commercially available reagents and solvents were used as purchased.

4-Methylphenyl 4-O-benzyl-3-O-tert-butyl dimethylsilyl-2-O-9-fluorenylmethyloxycarbonyl-1-thio- β -D-galactopyranoside (11). To a solution of **10** (20.5 mg, 28.83 μmol) in THF (290 μL) were added 1.0 M $\text{BH}_3\cdot\text{THF}$ (288.3 μL , 288.3 μmol) and 1.0 M $\text{Bu}_2\text{BOTf}\cdot\text{CH}_2\text{Cl}_2$ (28.83 μL , 28.83 μmol) at 0 $^\circ\text{C}$ under Ar atmosphere. After being stirred for 10.5 h at 0 $^\circ\text{C}$ and the reaction was quenched with MeOH and sat. NaHCO_3 aq. and extracted with CHCl_3 . The organic layer was dried over Na_2SO_4 , filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (toluene/EtOAc 25/1) to give **11** (18.7 mg, 91%) as a white solid. ^1H NMR (500 MHz, CDCl_3): δ 7.77 (d, *J* 7.6 Hz, 2H), 7.68 (dd, *J* 12.7, 7.6 Hz, 2H), 7.44-7.37 (m, 4H), 7.37-7.28 (m, 7H), 7.05 (d, *J* 7.9 Hz, 2H), 5.18 (t, *J* 9.7 Hz, 1H), 5.05 (d, *J* 11.6 Hz, 1H), 4.64 (d, *J* 10.0 Hz, 1H), 4.64 (dd, *J* 10.2, 7.0 Hz, 1H), 4.57 (d, *J* 11.6 Hz, 1H), 4.31 (t, *J* 7.0 Hz, 1H), 4.24 (dd, *J* 10.2, 7.0 Hz, 1H), 3.88 (dd, *J* 10.0, 2.7 Hz, 1H), 3.86-3.80 (m, 1H), 3.73 (d, *J* 2.7 Hz, 1H), 3.58-3.51 (m, 2H), 2.35 (s, 1H), 2.30 (s, 3H), 0.88 (s, 9H), 0.16 (s, 3H), 0.11 (s, 3H). ^{13}C NMR (500 MHz, CDCl_3): δ 154.39, 145.65, 143.51, 143.22, 141.29, 141.22, 138.26, 137.76, 132.47, 129.58, 129.51, 129.00, 128.36, 127.96, 127.83, 127.78, 127.75, 127.15, 127.06, 125.47, 125.15,

125.09, 119.99, 119.96, 119.90, 87.00, 78.81, 76.63, 75.56, 74.83, 74.75, 70.08, 62.09, 46.69, 25.56, 21.10, 17.88, -4.17, -5.14. HRMS (ESI-LTQ-Orbitrap) m/z $[M+Na]^+$ calcd for $C_{41}H_{48}O_7SSiNa$, 735.2782, found 735.2781.

4-Methylphenyl 4-O-benzyl-6-O-benzoyl-3-O-tert-butylidimethylsilyl-2-O-9-fluorenylmethyloxycarbonyl-1-thio- β -D-galactopyranoside (1). To a solution of **11** (200.8 mg, 281.6 μ mol) and *N,N*-dimethyl-4-aminopyridine (3.5 mg, 28.16 μ mol) in pyridine (2.81 mL) was added benzoyl chloride (65.4 μ L, 563.3 μ mol) at 0 °C under Ar atmosphere. After being stirred for 3.5 h at rt, to the reaction solution were added *N,N*-dimethyl-4-aminopyridine (3.4 mg, 27.83 μ mol) and benzoyl chloride (65.0 μ L, 559.5 μ mol) at 0 °C. After being stirred for 3.5 h at rt and the reaction was quenched with sat. $NaHCO_3$ aq. and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 , filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (toluene/EtOAc 30/1) to give **1** (173.2 mg, 75%) as a white solid. 1H NMR (500 MHz, $CDCl_3$): δ 8.00 (dd, J 8.3, 1.1 Hz, 2H), 7.77 (d, J 7.6 Hz, 2H), 7.68 (dd, J 15.8, 7.4 Hz, 2H), 7.59 (tt, J 7.4, 1.1 Hz, 1H), 7.48-7.25 (m, 13H), 6.88 (d, J 7.9 Hz, 2H), 5.21 (t, J 9.5 Hz, 1H), 5.11 (d, J 11.3 Hz, 1H), 4.68-4.59 (m, 3H), 4.51 (dd, J 11.5, 7.6 Hz, 1H), 4.40 (dd, J 11.5, 4.6 Hz, 1H), 4.32 (t, J 7.3 Hz, 1H), 4.24 (dd, J 10.3, 7.9 Hz, 1H), 3.91 (dd, J 9.5, 3.5 Hz, 1H), 3.85 (dd, J 7.6, 3.5 Hz, 1H), 3.82 (d-br, J 2.0 Hz, 1H), 2.24 (s, 3H), 0.89 (s, 9H), 0.17 (s, 3H), 0.12 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 166.23, 154.46, 143.56, 143.22, 141.32, 141.24, 138.12, 137.58, 133.13, 132.33, 129.99, 129.81, 129.69, 129.45, 128.37, 128.32, 127.92, 127.85, 127.80, 127.69, 127.17, 127.07, 125.52, 125.16, 120.01, 119.97, 87.26, 76.30, 75.50, 75.03, 74.74, 70.16, 64.09, 46.72, 25.58, 21.08, 17.91, -0.03, -4.15, -5.13.

HRMS (ESI-LTQ-Orbitrap) m/z $[M+Na]^+$ calcd for $C_{48}H_{52}O_8SSiNa$, 839.3044, found 839.3046.

Allyl 3,6-di-O-benzyl-4-O-(4-O-benzyl-6-O-benzoyl-3-O-tert-butylidimethylsilyl-2-O-9-fluorenylmethyloxycarbonyl- β -D-galactopyranosyl)-2-(2,2,2-trichloroethoxycarbonylamino)-2-deoxy- α -D-glucopyranoside (3).

To a suspension of donor **1** (170.8 mg, 208.9 μ mol), acceptor **2** (100.1 mg, 174.1 μ mol), *N*-iodosuccinimide (47.9 mg, 208.9 μ mol), and MS4A powder (ca. 300 mg) in CH_2Cl_2 (3.50 mL) was added trifluoromethanesulfonic acid (6.1 μ L, 69.65 μ mol) at -40 °C under Ar atmosphere. After being stirred for 1 h at -40 °C, the reaction was quenched with sat. $NaHCO_3$ aq. and 10% $Na_2S_2O_3$ aq. and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 , filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (toluene/EtOAc 50/1 to 20/1) to give **3** (167.0 mg, 76%) as a white solid. 1H NMR (500 MHz, $CDCl_3$): δ 7.96 (d, J 7.0 Hz, 2H), 7.78 (t, J 7.5 Hz, 2H), 7.60 (dd, J 7.5, 1.1 Hz, 2H), 7.58-7.54 (m, 1H), 7.48-7.39 (m, 4H), 7.33-7.27 (m, 6H), 7.25-7.18 (m, 8H), 7.18-7.11 (m, 3H), 5.84-5.75 (m, 1H), 5.19 (dq, J 17.3, 1.5 Hz, 1H), 5.12 (dt, J 11.7, 1.3 Hz, 1H), 5.07 (d, J 11.3 Hz, 1H), 5.05-4.98 (m, 2H), 4.95 (d, J 9.5 Hz, 1H), 4.89 (d, J 3.6 Hz, 1H), 4.73 (d, J 12.0 Hz, 1H), 4.66 (d, J 12.0 Hz, 1H), 4.61-4.53 (m, 3H), 4.50 (d, J 11.3 Hz, 1H), 4.46 (dd, J 10.5, 6.3 Hz, 1H), 4.36 (d, J 8.0 Hz, 1H), 4.31 (d, J 12.2 Hz, 1H), 4.26-4.20 (m, 2H), 4.10-4.05 (m, 1H), 4.05-3.96 (m, 2H), 3.96-3.89 (m, 2H), 3.69 (dd, J 11.0, 2.9 Hz, 1H), 3.62-3.56 (m, 3H), 3.50 (dd, J 9.7, 2.9 Hz, 1H), 3.45 (dd, J 11.0, 1.6 Hz, 1H), 3.40 (t, J 6.3 Hz, 1H), 0.88 (s, 9H), 0.09 (s, 3H), 0.01 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 166.11, 154.33, 154.18, 143.41, 141.50, 141.38, 138.82, 138.38, 138.18, 133.39, 133.08, 129.85, 129.67, 128.41, 128.36, 128.29, 127.94, 127.92, 127.89, 127.73, 127.67, 127.58, 127.51, 127.45, 127.13, 127.10, 127.05, 124.84, 124.75, 120.10, 120.07, 117.83, 100.72, 96.58, 95.49, 77.71, 77.48, 77.21, 76.88, 75.17, 74.56, 74.18, 72.99, 72.21, 70.71, 69.47, 68.33, 67.65, 62.96, 54.70, 46.83, 25.59, 17.88, -4.23, -5.21. HRMS (ESI-LTQ-Orbitrap) m/z $[M+Na]^+$ calcd for $C_{67}H_{74}Cl_3NO_{15}SiNa$ 1290.3756, found 1290.3781.

Allyl 3,6-di-O-benzyl-4-O-(4-O-benzyl-6-O-benzoyl-3-O-tert-butylidimethylsilyl- β -D-galactopyranosyl)-2-(2,2,2-trichloroethoxycarbonylamino)-2-deoxy- α -D-glucopyranoside (12). To a solution of **3** (2.083 g, 1.643 mmol) in CH_2Cl_2 (20.0 mL) was added triethylamine (5.0 mL) at rt under Ar atmosphere. After being stirred for 16 h at rt, the reaction mixture was diluted with toluene and concentrated *in vacuo*. The crude compound was azeotropic-dried with toluene three times. The residue was purified by silica-gel column chromatography

(Toluene/AcOEt 9/1 to 4/1) to give **12** (1.685 g, 98%) as a light yellow solid. ^1H NMR (500 MHz, CDCl_3): δ 7.89 (d, J 7.2 Hz, 2H), 7.51 (t, J 7.4 Hz, 1H), 7.38-7.29 (m, 8H), 7.29-7.12 (m, 9H), 5.90-5.80 (m, 1H), 5.24 (dq, J 17.2, 1.5 Hz, 1H), 5.19 (dq, J 10.4, 1.2 Hz, 1H), 5.05 (d, J 11.5 Hz, 1H), 4.97 (d, J 10.0 Hz, 1H), 4.94 (d, J 11.5 Hz, 1H), 4.88 (d, J 3.6 Hz, 1H), 4.72-4.63 (m, 4H), 4.57-4.52 (m, 3H), 4.33 (dd, J 11.1, 6.1 Hz, 1H), 4.19 (t, J 5.5 Hz, 1H), 4.17-4.13 (m, 1H), 4.13-4.10 (m, 1H), 4.05 (dd, J 11.5, 3.0 Hz, 1H), 3.99-3.92 (m, 2H), 3.85-3.80 (m, 1H), 3.79-3.71 (m, 2H), 3.69 (dd, J 11.5, 1.7 Hz, 1H), 3.62 (d, J 2.3 Hz, 1H), 3.55 (dd, J 9.5, 2.8 Hz, 1H), 3.51 (t, J 6.2 Hz, 1H), 3.18 (d, J 2.1 Hz, 1H), 0.94 (s, 9H), 0.13 (s, 3H), 0.11 (s, 3H). ^{13}C NMR (500 MHz, CDCl_3): δ 175.66, 169.56, 166.05, 138.93, 137.62, 133.02, 129.82, 129.57, 128.45, 128.34, 128.24, 127.96, 127.79, 127.75, 127.69, 127.51, 127.48, 127.07, 126.53, 126.32, 126.06, 125.84, 125.77, 125.60, 125.56, 102.81, 98.22, 77.38, 77.35, 77.20, 76.91, 75.98, 75.34, 75.08, 74.83, 73.58, 73.21, 72.68, 72.49, 71.14, 70.57, 68.22, 63.66, 63.41, 59.29, 51.87, 26.68, 25.90, 23.99, 18.27, -4.42, -4.47. HRMS (ESI-LTQ-Orbitrap) m/z $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{52}\text{H}_{64}\text{Cl}_3\text{NO}_{13}\text{SiNa}$ 1068.3075, found 1068.3083.

Allyl 3,6-di-O-benzyl-4-O-(4-O-benzyl-2-O-(2,3,4-tri-O-benzyl- α -L-fucopyranosyl)-6-O-benzoyl-3-O-tert-butylidimethylsilyl- β -D-galactopyranosyl)-2-(2,2,2-trichloroethoxycarbonylamino)-2-deoxy- α -D-glucopyranoside (5).

To a suspension of donor **4** (1.438 g, 2.66 mmol), acceptor **12** (926.7 mg, 0.886 mmol), *N*-iodosuccinimide (717.7 mg, 3.191 mmol), and MS4A powder (ca. 2.7 g) in CH_2Cl_2 (17.7 mL) was added trifluoromethanesulfonic acid (31.2 μL , 0.354 mmol) at -40°C under Ar atmosphere. After being stirred at -40°C for 20 mins, the reaction was quenched with sat. NaHCO_3 aq. and 10% $\text{Na}_2\text{S}_2\text{O}_3$ aq. and extracted with CHCl_3 . The organic layer was dried over Na_2SO_4 , filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (Toluene/AcOEt 20/1 to 10/1) to give **5** (1.139 g, 88%) as a white solid. ^1H NMR (500 MHz, CDCl_3): δ 8.01 (d, J 7.3 Hz, 2H), 7.57 (t, J 7.4 Hz, 1H), 7.46 (t, J 7.7 Hz, 2H), 7.40-7.27 (m, 20H), 7.25-7.17 (m, 10H), 5.87-5.78 (m, 1H), 5.50 (d, J 3.3 Hz, 1H), 5.23-5.17 (m, 1H), 5.10 (d, J 10.6 Hz, 1H), 5.03-4.93 (m, 5H), 4.89 (d, J 3.4 Hz, 1H), 4.84-4.59 (m, 9H), 4.54 (d, J 11.0 Hz, 1H), 4.48 (d, J 11.0 Hz, 1H), 4.41 (d, J 12.2 Hz, 1H), 4.37-4.18 (m, 3H), 4.14-4.04 (m, 4H), 4.00-3.83 (m, 4H), 3.69-3.64 (m, 2H), 3.61-3.46 (m, 3H), 1.14 (d, J 6.4 Hz, 3H), 1.02 (d, J 6.6 Hz, 1H), 0.88 (s, 9H), 0.10 (s, 3H), 0.05 (s, 3H). ^{13}C NMR (500 MHz, CDCl_3): δ 176.37, 138.90, 138.65, 138.61, 138.57, 138.46, 138.10, 133.61, 133.08, 130.00, 129.62, 129.48, 128.45, 128.42, 128.34, 128.30, 128.27, 128.24, 128.21, 128.14, 128.12, 128.03, 128.00, 127.92, 127.67, 127.57, 127.53, 127.51, 127.47, 127.41, 127.31, 127.25, 127.13, 117.27, 100.95, 97.40, 93.96, 80.26, 79.15, 77.59, 77.31, 77.20, 77.05, 76.24, 76.09, 74.85, 74.61, 74.59, 74.20, 74.18, 73.28, 72.98, 72.76, 72.04, 71.93, 67.90, 66.72, 66.26, 26.09, 17.90, 16.73, 16.59, -0.02, -3.30, -4.80. HRMS (ESI-LTQ-Orbitrap) m/z $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{79}\text{H}_{92}\text{Cl}_3\text{NO}_{17}\text{SiNa}$, 1484.5063, found 1484.5074.

Allyl 3,6-di-O-benzyl-4-O-(4-O-benzyl-2-O-(2,3,4-tri-O-benzyl- α -L-fucopyranosyl)-6-O-benzoyl- β -D-galactopyranosyl)-2-(2,2,2-trichloroethoxycarbonylamino)-2-deoxy- α -D-glucopyranoside (13).

To a solution of **5** (1.13 g, 0.773 mmol) in THF (6.25 mL) was added 70% HF-pyridine (2.50 mL) at rt under Ar atmosphere. After being stirred for 16 h at rt, to the reaction mixture was added 70% HF-pyridine (2.19 mL) at rt. After being stirred for 13.5 h, the reaction mixture was diluted with CHCl_3 , quenched with sat. NaHCO_3 aq., and extracted with CHCl_3 . The organic layer was dried over Na_2SO_4 , filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (Toluene/AcOEt 20/1 to 9/1) to give **13** (894.4 mg, 86%) as a white solid. ^1H NMR (500 MHz, CDCl_3): δ 7.98-7.94 (m, 2H), 7.56 (t, J 7.4 Hz, 1H), 7.46-7.38 (m, 6H), 7.36-7.29 (m, 13H), 7.24-7.13 (m, 13H), 5.86-5.77 (m, 1H), 5.20 (dd, J 17.2, 1.4 Hz, 1H), 5.14 (dd, J 10.4, 1.1 Hz, 1H), 5.00-4.54 (m, 17H), 4.38-4.23 (m, 4H), 4.11-4.04 (m, 3H), 3.96-3.87 (m, 3H), 3.83 (dd, J 10.6, 2.7 Hz, 1H), 3.80-3.75 (m, 2H), 3.73-3.68 (m, 2H), 3.61-3.59 (br m, 1H), 3.57-3.47 (m, 4H), 1.02 (d, J 6.4 Hz, 3H). ^{13}C NMR (500 MHz, CDCl_3): δ 166.03, 154.17, 138.73, 138.63, 138.49, 138.42, 137.81, 137.19, 133.47, 133.07, 129.91, 129.67, 128.77, 128.52, 128.44, 128.41, 128.39, 128.33, 128.28, 128.25, 128.22, 128.19, 128.19, 128.10,

127.84, 127.75, 127.69, 127.54, 127.47, 127.35, 118.05, 101.03, 100.78, 96.47, 95.53, 81.42, 79.35, 77.40, 77.36, 77.22, 77.17, 75.43, 75.12, 74.79, 74.65, 74.61, 74.57, 73.89, 73.50, 72.96, 71.98, 70.59, 68.42, 67.93, 67.65, 62.84, 54.32, 16.79. HRMS (ESI-LTQ-Orbitrap) m/z $[M+Na]^+$ calcd for $C_{73}H_{78}Cl_3NO_{17}Na$ 1370.4198, found 1370.4225.

Allyl 2-acetamide-4-O-(3-O-acetyl-4-O-benzyl-2-O-(2,3,4-tri-O-benzyl- α -L-fucopyranosyl)-6-O-benzoyl- β -D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranoside (14). To a solution of **13** (731.8 mg, 0.543 mmol) in THF (5.4 mL) and AcOH (5.4 mL) was added Zn-Cu complex (ca. 1.40 g) at rt under Ar atmosphere. After being stirred for 1.5 h at rt, the reaction mixture was filtered, and concentrated *in vacuo* to give amine as a crude mixture. This crude mixture was used for next reaction. To a solution of crude product in pyridine (5.0 mL) were added Ac_2O (5.0 mL) and *N,N*-dimethyl-4-aminopyridine (6.7 mg, 54.3 μ mol) at rt under Ar atmosphere. After being stirred for 2.5 d at rt, the reaction mixture was diluted with $CHCl_3$, quenched with 1.0 M HCl aq., and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 , filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (Toluene/AcOEt 7/3) to give **14** (602.0 mg, 88% in 2 steps) as a light yellow oil. 1H NMR (500 MHz, $CDCl_3$): δ 8.01-7.95 (m, 2H), 7.60-7.55 (m, 1H), 7.46 (t, J 7.7 Hz, 2H), 7.38-7.18 (m, 30H), 5.86-5.76 (m, 1H), 5.28 (d, J 8.7 Hz, 1H), 5.28 (d, J 3.6 Hz, 1H), 5.18 (dq, J 17.2, 1.6 Hz, 1H), 5.11 (dq, J 10.5, 1.2 Hz, 1H), 4.99 (d, J 11.7 Hz, 1H), 4.91-4.87 (m, 2H), 4.82 (dd, J 10.1, 3.1 Hz, 2H), 4.80 (d, J 11.6 Hz, 2H), 4.72 (d, J 12.1 Hz, 1H), 4.69-4.63 (m, 4H), 4.57 (d, J 11.6 Hz, 1H), 4.51 (d, J 11.6 Hz, 1H), 4.36 (d, J 12.1 Hz, 1H), 4.30-4.03 (m, 8H), 3.94-3.88 (m, 2H), 3.82-3.74 (m, 2H), 3.62-3.57 (m, 2H), 3.54-3.45 (m, 3H), 1.87 (s, 3H), 1.83 (s, 3H), 1.13 (d, J 6.4 Hz, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 170.22, 169.71, 165.89, 138.89, 138.68, 138.55, 138.53, 137.79, 137.77, 133.74, 133.17, 129.71, 129.66, 129.60, 129.01, 128.57, 128.46, 128.38, 128.34, 128.30, 128.21, 128.17, 128.14, 127.85, 127.83, 127.78, 127.75, 127.62, 127.55, 127.35, 127.24, 125.27, 117.40, 100.71, 98.01, 96.50, 79.19, 77.40, 76.54, 76.36, 75.10, 74.99, 74.68, 74.30, 73.64, 73.42, 73.34, 73.25, 72.61, 71.75, 70.79, 68.13, 67.74, 66.77, 62.33, 51.96, 43.92, 23.32, 20.97, 16.68. HRMS (ESI-LTQ-Orbitrap) m/z $[M+Na]^+$ calcd for $C_{74}H_{81}NO_{17}Na$ 1278.5397, found 1278.5433.

Methyl 4-(2-acetamide-4-O-(3-O-acetyl-4-O-benzyl-2-O-(2,3,4-tri-O-benzyl- α -L-fucopyranosyl)-6-O-benzoyl- β -D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl)-2-ene-butyrates (15). To a solution of **14** (602.0 mg, 0.479 mmol) in $C_2H_4Cl_2$ (9.6 mL) were added methyl acrylate (431 μ L, 4.79 mmol) and Grubbs catalyst 2nd generation (4.4 mg, 5.18 μ mol) at rt under Ar atmosphere. After being stirred for 8.5 h at 50 $^\circ C$, to the reaction mixture was added Grubbs catalyst 2nd generation (8.1 mg, 9.54 μ mol) at rt. After being stirred for 15.5 h at 50 $^\circ C$, to the reaction mixture was added Grubbs catalyst 2nd generation (12.0 mg, 14.1 μ mol) at rt. After being stirred for 23 h at 50 $^\circ C$, the reaction mixture were added Grubbs catalyst 2nd generation (12.6 mg, 14.8 μ mol) and methyl acrylate (210 μ L, 2.22 mmol) at rt. After being stirred for 17.5 h, the reaction mixture was diluted with toluene and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (Toluene/AcOEt 5/1 to 3/2 to 1/1) to give **15** (246.4 mg, 40%) as a light brown solid. 1H NMR (500 MHz, $CDCl_3$): δ 8.00-7.96 (m, 2H), 7.60-7.55 (m, 1H), 7.48-7.43 (m, 2H), 7.38-7.16 (m, 30H), 6.91 (dt, J 15.8, 4.5 Hz, 1H), 5.97 (dt, J 15.8, 1.9 Hz, 1H), 5.26 (d, J 3.6 Hz, 1H), 5.23 (d, J 8.2 Hz, 1H), 4.99 (d, J 11.5 Hz, 1H), 4.94 (d, J 3.6 Hz, 1H), 4.89 (d, J 11.5 Hz, 1H), 4.84 (dd, J 10.0, 3.2 Hz, 1H), 4.81 (d, J 11.6 Hz, 1H), 4.73-4.63 (m, 5H), 4.62 (d, J 11.5 Hz, 1H), 4.58 (d, J 11.6 Hz, 1H), 4.51 (d, J 11.5 Hz, 1H), 4.36 (d, J 12.2 Hz, 1H), 4.31-4.22 (m, 3H), 4.20 (dd, J 4.2, 1.9 Hz, 1H), 4.17-4.04 (m, 6H), 3.88 (d, J 2.9 Hz, 1H), 3.80 (dd, J 11.0, 3.2 Hz, 1H), 3.76 (dd, J 10.2, 2.7 Hz, 1H), 3.72 (s, 3H), 3.63 (d, J 2.1 Hz, 1H), 3.61-3.47 (m, 4H), 1.87 (s, 3H), 1.83 (s, 3H), 1.16 (d, J 6.6 Hz, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 170.22, 169.79, 166.22, 165.88, 143.33, 138.68, 138.65, 138.52, 137.75, 137.69, 133.17, 129.69, 129.64, 128.57, 128.44, 128.37, 128.35, 128.30, 128.27, 128.24, 128.17, 128.13, 127.86, 127.79, 127.73, 127.59, 127.53, 127.51, 127.22, 121.12, 100.83, 98.11, 96.96, 79.17, 77.84, 76.69, 76.46, 76.15, 75.08, 74.94, 74.89, 74.28, 73.90, 73.42, 73.40, 73.33, 72.44, 71.78, 71.14, 67.65, 66.84, 65.85,

62.33, 52.06, 51.67, 23.23, 20.97, 16.66. HRMS (ESI-LTQ-Orbitrap) m/z $[M+Na]^+$ calcd for $C_{76}H_{83}NO_{19}Na$ 1336.5452, found 1336.5451.

4-(2-Acetamide-4-O-(2-O-(α -L-fucopyranosyl)- β -D-galactopyranosyl)-2-deoxy- α -D-glucopyranosyl)butyric acid (16). To a solution of **15** (297.6 mg, 226.4 μ mol) in t BuOH (2.83 mL), AcOH (1.13 mL), and H₂O (566 μ L) was added palladium hydroxide on activated carbon (Pd 20%, wetted with ca.50% water, 158.9 mg, 1.13 mmol) at rt. After being stirred for 8 h under H₂ atmosphere with the pressure of 1.0 MPa, the reaction mixture was filtered through Hyflo Super-Cel[®] and concentrated *in vacuo* to give a crude mixture. This crude mixture was used for next reaction. To a solution of crude product in THF (11.3 mL) and H₂O (11.3 mL) was added LiOH·H₂O (95.0 mg, 2.26 mmol) at 0 °C under Ar atmosphere. After being stirred for 16 h at rt, the reaction mixture was quenched with AcOH (130 μ L), concentrated *in vacuo*, and lyophilized with H₂O. The residue was purified by gel filtration chromatography (MeOH) to give **16** (119.1 mg, 85% in 2 steps) as a white solid. ¹H NMR (500 MHz, CD₃OD): δ 5.24 (d, J 3.3 Hz, 1H, anomeric proton of GlcNAc), 4.76 (d, J 3.6 Hz, 1H, anomeric proton of Fuc), 4.49 (d, J 7.4 Hz, 1H, anomeric proton of Gal), 4.21 (q, J 6.6 Hz, 1H), 3.96 (dd, J 10.7, 3.6 Hz, 1H), 3.87-3.62 (m, 14H), 3.56 (dd, J 7.7, 4.2 Hz, 1H), 3.41 (dt, J 11.8, 4.8 Hz, 1H), 2.31-2.18 (m, 2H), 2.00 (s, 3H), 1.91-1.84 (m, 2H), 1.20 (d, J 6.6 Hz, 3H). ¹³C NMR (500 MHz, CD₃OD): δ 173.67, 166.29, 102.37, 101.62, 98.52, 78.60, 78.32, 77.06, 75.42, 73.55, 72.54, 71.69, 71.00, 70.75, 70.57, 68.92, 68.14, 62.60, 61.53, 55.03, 27.61, 22.56, 16.78. HRMS (ESI-Q-TOF) m/z $[M-H]^-$ calcd for $C_{24}H_{40}NO_{17}$ 614.2291, found 614.2304.

Allyl 4-O-(3-O-(3,4,5-tri-O-acetyl-2-azide-2-deoxy- α -D-galactopyranosyl)-4-O-benzyl-2-O-(2,3,4-tri-O-benzyl- α -L-fucopyranosyl)-6-O-benzoyl- β -D-galactopyranosyl)-3.6-di-O-benzyl-2-(2,2,2-trichloroethoxycarbonylamino)-2-deoxy- α -D-glucopyranoside (17). To a suspension of donor **6** (60.1 mg, 152.5 μ mol), acceptor **13** (100.9 mg, 74.86 μ mol), and MS4A powder (ca. 300 mg) in CH₂Cl₂ (748.6 μ L, 0.05 M) and toluene (748.6 μ L, 0.05 M) were added Ag₂CO₃ (42.1 mg, 152.7 μ mol) and AgClO₄ (about 9 mg, 43.4 μ mol) at 0 °C under Ar atmosphere. After being stirred for 13.5 h at rt, the reaction mixture was filtered, washed with sat. NaHCO₃ aq., and extracted with CHCl₃. The organic layer was dried over Na₂SO₄, filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (toluene/EtOAc 15/1) to give **17** (111.7 mg, 90%) as a white solid. ¹H NMR (500 MHz, CDCl₃): δ 7.99 (t, J 4.2 Hz, 2H), 7.56 (tt, J 7.4, 1.5 Hz, 1H), 7.50-7.43 (m, 4H), 7.40-7.28 (m, 19H), 7.24-7.12 (m, 9H), 5.90-5.80 (m, 1H), 5.55 (d, J 4.0 Hz, 1H), 5.27-5.17 (m, 4H), 5.15-5.10 (m, 2H), 5.06 (d, J 11.3 Hz, 1H), 5.02-4.98 (m, 2H), 4.91 (d, J 3.6 Hz, 1H), 4.85 (d, J 12.0 Hz, 1H), 4.78-4.72 (m, 3H), 4.69-4.55 (m, 6H), 4.37 (d, J 12.0 Hz, 1H), 4.30 (dd, J 11.2, 6.0 Hz, 1H), 4.26-4.15 (m, 3H), 4.12-3.96 (m, 7H), 3.94-3.86 (m, 2H), 3.82-3.75 (m, 2H), 3.72-3.67 (m, 1H), 3.61-3.46 (m, 5H), 3.37 (t, J 6.2 Hz, 1H), 3.28 (dd, J 10.9, 3.2 Hz, 1H), 2.16 (s, 3H), 2.05 (s, 3H), 1.92 (s, 3H), 1.15 (d, J 6.6 Hz, 3H). ¹³C NMR (500 MHz, CDCl₃): δ 170.87, 169.74, 169.59, 166.08, 154.19, 139.56, 138.74, 138.71, 138.63, 138.56, 137.91, 133.68, 133.11, 129.92, 129.70, 129.03, 128.59, 128.50, 128.46, 128.30, 128.22, 128.11, 128.08, 128.06, 127.90, 127.78, 127.68, 127.64, 127.44, 127.35, 127.27, 127.05, 125.30, 117.60, 100.45, 97.53, 97.47, 96.69, 95.49, 79.95, 77.84, 77.47, 77.23, 76.38, 76.26, 75.63, 75.25, 74.86, 74.68, 74.64, 74.49, 74.38, 73.52, 72.13, 72.02, 70.97, 69.28, 68.28, 67.78, 67.69, 66.30, 62.92, 62.31, 59.32, 54.54, 53.42, 21.45, 20.65, 20.63, 20.46, 16.74. HRMS (ESI-LTQ-Orbitrap) m/z $[M+Na]^+$ calcd for $C_{85}H_{93}Cl_3N_4O_{24}Na$, 1683.5108, found 1683.5104.

Allyl 2-acetamide-4-O-(3-O-(2-acetamide-3,4,5-tri-O-acetyl-2-deoxy- α -D-galactopyranosyl)-4-O-benzyl-2-O-(2,3,4-tri-O-benzyl- α -L-fucopyranosyl)-6-O-benzoyl- β -D-galactopyranosyl)-3.6-di-O-benzyl-2-deoxy- α -D-glucopyranoside (17a). To a solution of **17** (622.2 mg, 0.375 mmol) in THF (3.8 mL) and AcOH (3.8 mL) was added Zn-Cu complex (ca. 1.90 g) at rt under Ar atmosphere. After being stirred for 3 h at rt, the reaction mixture was filtered, and concentrated *in vacuo* to give amine as a crude mixture. This crude mixture was used for next reaction. To a solution of crude product in pyridine (6.0 mL) was added Ac₂O (6.0 mL) at rt under Ar atmosphere. After being stirred for 1.5 d at rt, the reaction mixture was diluted with CHCl₃, quenched with 1.0

M HCl aq., and extracted with CHCl_3 . The organic layer was dried over Na_2SO_4 , filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (Toluene/AcOEt 1/1) to give **17a** (432.7 mg, 75% in 2 steps) as a white solid. ^1H NMR (500 MHz, CDCl_3): δ 7.99 (d, *J* 7.3 Hz, 2H), 7.58 (t, *J* 7.4 Hz, 1H), 7.47 (t, *J* 7.7 Hz, 2H), 7.42-7.27 (m, 12H), 7.25-7.15 (m, 18H), 5.89-5.79 (m, 1H), 5.58-5.45 (m, 2H), 5.32-5.17 (m, 4H), 5.13-5.03 (m, 2H), 5.01-4.87 (m, 5H), 4.70-4.50 (m, 11H), 4.43-4.24 (m, 5H), 4.23-4.13 (m, 3H), 4.08 (dd, *J* 13.2, 5.1 Hz, 1H), 4.00 (br s, 1H), 3.94 (dd, *J* 13.2, 5.7 Hz, 1H), 3.92-3.85 (m, 3H), 3.76-3.54 (m, 6H), 3.04 (brs, 1H), 2.07 (s, 3H), 1.96 (s, 3H), 1.87 (s, 3H), 1.74 (s, 3H), 1.51 (s, 3H). ^{13}C NMR (500 MHz, CDCl_3): δ 170.53, 170.40, 170.13, 169.94, 169.73, 165.99, 139.14, 138.86, 138.45, 138.15, 138.03, 137.64, 133.81, 133.30, 129.66, 129.60, 128.53, 128.48, 128.34, 128.30, 128.26, 128.24, 128.18, 127.92, 127.72, 127.68, 127.58, 127.50, 127.41, 127.18, 127.15, 126.57, 117.06, 100.82, 98.36, 96.46, 80.42, 77.64, 76.82, 75.92, 75.50, 74.65, 73.72, 73.64, 73.47, 72.10, 71.38, 71.07, 68.59, 67.94, 67.65, 67.12, 66.79, 62.85, 52.10, 47.06, 23.29, 22.62, 20.62, 20.60, 20.43, 16.72. HRMS (ESI-LTQ-Orbitrap) *m/z* $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{86}\text{H}_{98}\text{N}_2\text{O}_{24}\text{Na}$, 1565.6402, found 1565.6394.

Methyl 4-((2-acetamide-4-O-(3-O-(2-acetamide-3,4,5-tri-O-acetyl-2-deoxy- α -D-galactopyranosyl)-4-O-benzyl-2-O-(2,3,4-tri-O-benzyl- α -L-fucopyranosyl)-6-O-benzoyl- β -D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl)-2-ene-butyrates (17b). To a solution of **17a** (21.0 mg, 13.6 μmol) in $\text{C}_2\text{H}_4\text{Cl}_2$ (270 μL) were added methyl acrylate (12.26 μL , 136 μmol) and Grubbs catalyst 2nd generation (2.34 mg, 2.76 μmol) at rt under Ar atmosphere. After being stirred for 1 h at 50 $^\circ\text{C}$, the reaction mixture was added methyl acrylate (12.25 μL , 136 μmol) and Grubbs catalyst 2nd generation (2.33 mg, 2.74 μmol) at rt. After being stirred for 11 h at 50 $^\circ\text{C}$, the reaction mixture was heated to 80 $^\circ\text{C}$. After being stirred for 31.5 h at 80 $^\circ\text{C}$, the reaction mixture was diluted with toluene and concentrated *in vacuo*. The residue was purified by preparative layer chromatography ($\text{CHCl}_3/\text{MeOH}$ 3/1) to give **17b** (17.3 mg, 79%) as a brown solid. ^1H NMR (500 MHz, CDCl_3): δ 8.02-7.97 (m, 2H), 7.61-7.56 (m, 1H), 7.47 (t, *J* 7.8 Hz, 2H), 7.41-7.16 (m, 30H), 6.94 (dt, *J* 15.8, 4.4 Hz, 1H), 6.00 (dt, *J* 15.8, 2.0 Hz, 1H), 5.62 (brs, 1H), 5.47 (brs, 1H), 5.25-5.18 (m, 3H), 5.06 (dd, *J* 11.6, 3.2 Hz, 1H), 5.03-4.85 (m, 4H), 4.70-4.49 (m, 10H), 4.46-4.21 (m, 7H), 4.19-4.13 (m, 2H), 4.10 (ddd, *J* 16.1, 4.7, 2.0 Hz, 1H), 4.01 (brs, 1H), 3.93-3.85 (m, 3H), 3.81 (s, 1H), 3.78-3.52 (m, 7H), 3.71 (s, 3H), 2.08 (s, 3H), 1.95 (s, 3H), 1.86 (s, 3H), 1.75 (s, 3H), 1.51 (s, 3H), 1.26 (s, 3H). ^{13}C NMR (500 MHz, CDCl_3): δ 170.60, 170.53, 170.19, 170.01, 169.93, 166.23, 166.04, 143.48, 139.15, 138.68, 138.61, 138.22, 137.97, 137.63, 133.37, 129.69, 129.60, 128.59, 128.54, 128.36, 128.34, 128.29, 128.12, 128.05, 127.78, 127.77, 127.65, 127.58, 127.57, 127.23, 127.18, 126.72, 126.63, 120.99, 100.92, 98.33, 96.84, 80.23, 77.62, 75.86, 75.48, 75.10, 73.80, 73.72, 73.49, 72.10, 71.90, 71.72, 71.04, 68.53, 67.85, 67.65, 67.12, 66.88, 65.68, 62.86, 52.19, 51.77, 47.08, 23.27, 22.65, 20.68, 20.66, 20.49, 16.72. HRMS (ESI-LTQ-Orbitrap) *m/z* $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{88}\text{H}_{100}\text{N}_2\text{O}_{26}\text{Na}$ 1623.6457, found 1623.6506.

4-(2-Acetamide-4-O-(3-O-(2-acetamide-2-deoxy- α -D-galactopyranosyl)-2-O-(α -L-fucopyranosyl)- β -D-galactopyranosyl)-2-deoxy- α -D-glucopyranosyl)butyric acid (18). To a solution of **17b** (171.1 mg, 106.9 μmol) in $t\text{BuOH}$ (1.34 mL), AcOH (535 μL), and H_2O (267 μL) was added palladium hydroxide on activated carbon (Pd 20%, wetted with ca.50% water, 75.02 mg, 534.3 μmol) at rt. After being stirred for 20.5 h under H_2 atmosphere with the pressure of 1.0 MPa, the reaction mixture was filtered through Hyflo Super-Cel[®] and concentrated *in vacuo* to give a crude mixture. This crude mixture was used for next reaction. To a solution of crude product in THF (5.3 mL) and H_2O (5.3 mL) was added LiOH \cdot H_2O (45.2 mg, 1.08 mmol) at 0 $^\circ\text{C}$ under Ar atmosphere. After being stirred for 24.5 h at rt, the reaction mixture was quenched with AcOH (61.1 μL), concentrated *in vacuo*, and lyophilized with H_2O . The residue was purified by gel filtration chromatography (MeOH only) to give **18** (69.4 mg, 79% in 2 steps) as a white solid. ^1H NMR (500 MHz, CD_3OD): δ 5.33 (d, *J* 3.9 Hz, 1H, anomeric proton of GalNAc), 5.16 (d, *J* 3.7 Hz, 1H, anomeric proton of GlcNAc), 4.77 (d, *J* 3.6 Hz, 1H, anomeric proton of Fuc), 4.53 (d, *J* 7.6 Hz, 1H, anomeric proton of Gal), 4.37 (dd, *J* 13.0, 6.3 Hz, 1H), 4.32 (dd, *J*

11.0, 3.6 Hz, 1H), 4.18 (dd, *J* 6.6, 5.0 Hz, 1H), 4.11 (d, *J* 2.6 Hz, 1H), 4.03-3.89 (m, 4H), 3.86-3.61 (m, 14H), 3.54 (dd, *J* 7.2, 4.8 Hz, 1H), 3.42 (dt, *J* 11.6, 4.8 Hz, 1H), 2.37-2.23 (m, 2H), 2.01 (d, *J* 4.2 Hz, 6H), 1.91-1.86 (m, 2H), 1.21 (d, *J* 6.6 Hz, 3H). ¹³C NMR (500 MHz, CD₃OD): δ 180.16, 174.47, 173.62, 102.11, 100.28, 98.53, 93.56, 78.45, 77.93, 76.85, 73.56, 73.47, 72.69, 72.65, 71.83, 71.17, 70.54, 70.01, 69.83, 68.70, 67.71, 64.95, 63.36, 62.54, 61.53, 55.09, 51.27, 49.85, 34.31, 27.23, 22.76, 22.55, 16.68. HRMS (ESI-Q-TOF) *m/z* [M-H]⁻ calcd for C₃₂H₅₃N₂O₂₂ 817.3084, found 817.3113.

Allyl 3,6-di-O-benzyl-4-O-(4-O-benzyl-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-3-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl)-6-O-benzoyl-β-D-galactopyranosyl)-2-(2,2,2-

trichloroethoxycarbonylamino)-2-deoxy-α-D-glucopyranoside (19). To a suspension of donor **7** (26.3 mg, 47.4 μmol), acceptor **13** (20.3 mg, 15.1 μmol), *N*-iodosuccinimide (12.6 mg, 56.0 μmol), and MS4A powder (ca. 63 mg) in CH₂Cl₂ (301.2 μL) was added trifluoromethanesulfonic acid (0.5 μL, 5.66 μmol) at -60 °C under Ar atmosphere. After being stirred for 30 mins at -60 °C, the reaction mixture was diluted with CHCl₃, quenched with sat. NaHCO₃ aq., and extracted with CHCl₃. The organic layer was dried over Na₂SO₄, filtered, and concentrated *in vacuo*. The residue was purified by preparative layer chromatography (toluene/AcOEt 7/1) to give **19** (24.5 mg, 91%) as a white solid.

¹H NMR (500 MHz, CDCl₃): δ 7.97-7.92 (m, 2H), 7.57-7.52 (m, 1H), 7.52-7.49 (m, 2H), 7.46-7.39 (m, 7H), 7.38-7.26 (m, 15H), 7.23-7.06 (m, 23H), 5.87-5.78 (m, 1H), 5.63 (d, *J* 3.9 Hz, 1H), 5.39 (d, *J* 2.6 Hz, 1H), 5.26 (s, 1H), 5.22-5.16 (m, 1H), 5.11-5.07 (m, 1H), 5.01-4.95 (m, 4H), 4.90 (d, *J* 3.4 Hz, 1H), 4.77-4.61 (m, 10H), 4.57 (d, *J* 11.5 Hz, 1H), 4.49 (t, *J* 10.7 Hz, 2H), 4.35-4.25 (m, 4H), 4.21-4.00 (m, 7H), 3.99-3.93 (m, 2H), 3.93-3.87 (m, 1H), 3.85-3.75 (m, 5H), 3.67 (dd, *J* 9.7, 2.6 Hz, 1H), 3.61-3.47 (m, 5H), 3.40 (d, *J* 12.0 Hz, 1H), 3.25 (t, *J* 6.4 Hz, 1H), 1.17 (d, *J* 6.6 Hz, 3H). ¹³C NMR (500 MHz, CDCl₃): δ 165.84, 154.17, 139.18, 138.67, 138.63, 138.60, 138.53, 138.39, 138.10, 137.96, 137.92, 133.64, 130.06, 129.89, 129.66, 128.54, 128.38, 128.29, 128.25, 128.19, 128.10, 128.03, 127.96, 127.94, 127.83, 127.70, 127.64, 127.57, 127.44, 127.34, 127.25, 127.20, 126.33, 117.43, 100.77, 100.51, 97.46, 97.23, 96.53, 95.45, 80.59, 80.23, 76.39, 76.09, 75.69, 74.97, 74.91, 74.78, 74.62, 74.26, 74.19, 74.12, 73.62, 73.51, 72.25, 72.04, 71.33, 71.22, 69.25, 68.11, 67.96, 67.19, 66.20, 63.41, 62.70, 54.49, 33.88, 28.55, 16.70. HRMS (ESI-LTQ-Orbitrap) *m/z* [M+Na]⁺ calcd for C₁₀₀H₁₀₄Cl₃NO₂₂Na, 1801.6012, found 1801.6016.

Allyl 2-acetamide-3,6-di-O-benzyl-4-O-(4-O-benzyl-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-3-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl)-6-O-benzoyl-β-D-galactopyranosyl)-2-deoxy-α-D-

glucopyranoside (19a). To a solution of **19** (77.9 mg, 43.8 μmol) in THF (440 μL) and AcOH (440 μL) was added Zn-Cu complex (ca. 80 mg) at rt under Ar atmosphere. After being stirred for 4.5 h at rt, to the reaction mixture was added Zn-Cu complex (ca. 84 mg). After being stirred for 2.5 h at rt, the reaction mixture was filtered and concentrated *in vacuo* to give amine as a crude mixture. This crude mixture was used for next reaction. To a solution of crude product in pyridine (2.0 mL) was added Ac₂O (2.0 mL) at rt under Ar atmosphere. After being stirred for 6.5 h at rt, the reaction mixture diluted with toluene and concentrated *in vacuo*. The reaction mixture was dissolved in CHCl₃, washed with sat. NaHCO₃ aq., and extracted with CHCl₃. The organic layer was dried over Na₂SO₄, filtered, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (toluene/AcOEt 35/1) to give **19a** (71.0 mg, 99% in 2 steps) as a light yellow oil. ¹H NMR (500 MHz, CDCl₃): δ 7.97-7.93 (m, 2H), 7.57-7.53 (m, 1H), 7.52-7.49 (m, 2H), 7.46-7.39 (m, 7H), 7.38-7.25 (m, 17H), 7.24-7.06 (m, 21H), 5.87-5.77 (m, 1H), 5.64 (d, *J* 3.9 Hz, 1H), 5.40 (d, *J* 2.6 Hz, 1H), 5.29-5.25 (m, 2H), 5.18 (dd, *J* 17.2, 1.6 Hz, 1H), 5.08 (dd, *J* 10.4, 1.6 Hz, 1H), 4.98 (t, *J* 10.1 Hz, 2H), 4.90 (t, *J* 5.2 Hz, 2H), 4.73-4.60 (m, 8H), 4.56 (d, *J* 11.9 Hz, 1H), 4.51 (d, *J* 3.3 Hz, 1H), 4.49 (d, *J* 4.9 Hz, 1H), 4.38-4.25 (m, 5H), 4.21 (dd, *J* 11.0, 6.6 Hz, 1H), 4.17-4.10 (m, 3H), 4.09-4.00 (m, 4H), 3.97-3.91 (m, 2H), 3.87-3.75 (m, 5H), 3.68 (dd, *J* 9.7, 2.6 Hz, 1H), 3.62-3.55 (m, 3H), 3.54-3.47 (m, 1H), 3.44-3.38 (m, 1H), 3.27 (t, *J* 6.7 Hz, 1H), 1.26 (s,

3H), 1.20 (d, *J* 6.6 Hz, 3H). ¹³C NMR (500 MHz, CDCl₃): δ 169.76, 165.89, 139.16, 138.96, 138.70, 138.67, 138.53, 138.40, 138.10, 138.03, 137.97, 137.94, 133.83, 133.08, 129.66, 129.01, 128.84, 128.54, 128.52, 128.42, 128.37, 128.31, 128.23, 128.20, 128.12, 128.08, 128.06, 127.99, 127.82, 127.71, 127.66, 127.63, 127.56, 127.33, 127.28, 127.26, 127.16, 126.33, 117.16, 100.76, 100.59, 97.49, 97.23, 96.47, 80.59, 80.27, 77.36, 77.32, 76.40, 76.10, 75.67, 75.03, 74.90, 74.80, 74.61, 74.56, 74.18, 74.12, 73.69, 73.67, 73.51, 72.29, 72.04, 71.33, 69.28, 69.25, 69.22, 68.05, 68.01, 66.18, 63.41, 62.76, 52.19, 23.32, 21.43, 16.74. HRMS (ESI-LTQ-Orbitrap) *m/z* [M+Na]⁺ calcd for C₉₉H₁₀₅NO₂₁Na, 1667.7105, found 1667.7130.

Methyl 4-((2-acetamide-3,6-di-*O*-benzyl-4-*O*-(4-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl- α -L-fucopyranosyl)-3-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene- α -D-galactopyranosyl)-6-*O*-benzoyl- β -D-galactopyranosyl)-2-deoxy- α -D-glucopyranosyl)-2-ene-butyrate (19b). To a solution of **19a** (20.2 mg, 12.3 μ mol) in C₂H₄Cl₂ (246 μ L, 0.05 M) were added methyl acrylate (11.0 μ L, 123 μ mol) and Grubbs catalyst 2nd generation (1.03 mg, 1.21 μ mol) at rt under Ar atmosphere. After being stirred for 1.5 h at 50 °C, to the reaction mixture was added methyl acrylate (11.0 μ L, 123 μ mol) and Grubbs catalyst 2nd generation (1.12 mg, 1.32 μ mol) at rt. After being stirred for 30 mins at 50 °C, the reaction mixture was diluted with toluene and concentrated *in vacuo*. The residue was purified by preparative layer chromatography (toluene/AcOEt 1/4) to give **19b** (14.9 mg, 71%) as a light yellow solid. ¹H NMR (500 MHz, CDCl₃): δ 7.97-7.93 (m, 2H), 7.58-7.53 (m, 1H), 7.52-7.48 (m, 2H), 7.46-7.39 (m, 6H), 7.37-7.32 (m, 7H), 7.30-7.06 (m, 32H), 6.93 (dt, *J* 15.8, 4.4 Hz, 1H), 5.98 (dt, *J* 15.8, 1.9 Hz, 1H), 5.62 (d, *J* 3.9 Hz, 1H), 5.39 (d, *J* 2.9 Hz, 1H), 5.25 (s, 1H), 5.17 (d, *J* 8.2 Hz, 1H), 5.01-4.94 (m, 3H), 4.89 (d, *J* 11.9 Hz, 1H), 4.72-4.61 (m, 7H), 4.57 (d, *J* 11.9 Hz, 1H), 4.48 (t, *J* 11.4 Hz, 2H), 4.34 (q, *J* 6.5 Hz, 1H), 4.29 (d, *J* 8.4 Hz, 2H), 4.27 (d, *J* 8.6 Hz, 1H), 4.25-4.19 (m, 2H), 4.17-4.02 (m, 5H), 4.00 (dd, *J* 10.2, 3.9 Hz, 1H), 3.93 (d, *J* 2.4 Hz, 1H), 3.87-3.76 (m, 5H), 3.71 (s, 2H), 3.71-3.67 (m, 1H), 3.66-3.64 (m, 1H), 3.59 (dd, *J* 11.1, 1.4 Hz, 1H), 3.57-3.47 (m, 3H), 3.38 (d, *J* 11.6 Hz, 1H), 3.28 (t, *J* 6.5 Hz, 1H), 1.86 (s, 3H), 1.56 (s, 3H), 1.26 (d, *J* 6.5 Hz, 3H). ¹³C NMR (500 MHz, CDCl₃): δ 169.83, 166.20, 165.88, 143.41, 139.17, 138.87, 138.76, 138.62, 138.54, 138.38, 138.09, 137.95, 137.93, 133.09, 129.86, 129.64, 128.82, 128.53, 128.41, 128.33, 128.29, 128.22, 128.19, 128.07, 127.97, 127.83, 127.71, 127.67, 127.63, 127.51, 127.50, 127.47, 127.42, 127.31, 127.23, 127.17, 126.31, 120.91, 100.73, 100.69, 97.49, 97.15, 96.81, 80.51, 80.14, 78.03, 76.35, 75.97, 75.65, 75.00, 74.87, 74.84, 74.75, 74.53, 74.18, 74.12, 73.75, 73.60, 73.51, 72.07, 72.01, 71.70, 71.31, 69.21, 67.94, 66.23, 65.71, 63.39, 62.77, 52.29, 51.70, 23.24, 16.72. HRMS (ESI-Q-TOF) *m/z* [M+Na]⁺ calcd for C₁₀₁H₁₀₇NO₂₃Na, 1724.7126, found 1724.7167.

4-(2-Acetamide-4-*O*-(2-*O*-(α -L-fucopyranosyl)-3-*O*-(α -D-galactopyranosyl)- β -D-galactopyranosyl)-2-deoxy- α -D-glucopyranosyl)butyric acid (20). To a solution of **19b** (300.7 mg, 177 μ mol) in ^tBuOH (2.21 mL), AcOH (880 μ L), and H₂O (440 μ L) was added palladium hydroxide on activated carbon (Pd 20%, wetted with ca.50% water, 124.1 mg, 884 μ mol) at rt. After being stirred for 12 h under H₂ atmosphere with the pressure of 1.0 MPa, the reaction mixture was filtered through Hyflo Super-Cel[®] and concentrated *in vacuo* to give a crude mixture. This crude mixture was used for next reaction. To a solution of crude product in THF (9.0 mL) and H₂O (9.0 mL) was added LiOH·H₂O (74.9 mg, 1.79 mmol) at 0 °C under Ar atmosphere. After being stirred for 16.5 h at rt, the reaction mixture was quenched with AcOH (100 μ L), concentrated *in vacuo*, and lyophilized with H₂O. The residue was purified by gel filtration chromatography (MeOH only) to give **20** (115.4 mg, 84%) as a white solid. ¹H NMR (500 MHz, CD₃OD): δ 5.32 (d, *J* 3.9 Hz, 1H, anomeric proton of Gal (non-reducing terminal)), 5.16 (s, 1H, anomeric proton of GlcNAc), 4.76 (d, *J* 3.4 Hz, 1H, anomeric proton of Fuc), 4.54 (d, *J* 7.4 Hz, 1H, anomeric proton of Gal), 4.35 (q, *J* 6.5 Hz, 1H), 4.17-4.11 (m, 2H), 4.02-3.89 (m, 4H), 3.88-3.55 (m, 16H), 3.45-3.38 (m, 1H), 2.33-2.21 (m, 2H), 2.00 (s, 3H), 1.91-1.84 (m, 2H), 1.20 (d, *J* 6.5 Hz, 3H). ¹³C NMR (500 MHz, CD₃OD): δ 180.79, 173.65, 102.19, 100.36, 98.59, 96.10, 79.76, 78.58, 76.68, 73.69, 73.62, 73.12, 72.65, 71.84, 71.44, 71.33, 71.18, 70.06, 69.93, 68.88, 67.70, 65.82, 63.35, 62.67, 61.58, 55.06, 49.90, 34.87, 27.51, 22.59, 16.70. HRMS (ESI-Q-TOF) *m/z* [M-H]⁻ calcd for C₃₀H₅₀NO₂₂, 776.2819, found 776.2836.

Materials and methods for bio assay

Materials and cells. Raji cells were purchased from JCRB Cell Bank (JCRB9012). Roswell Park Memorial Institute (RPMI-1640; 189-02025), penicillin-Streptomycin Solution ($\times 100$; 168-23191), SDS-PAGE sample buffer solution (2ME+, $\times 4$; 191-13272) were purchased from FUJIFILM Wako Pure Chemical Corporation, Ltd. Fetal Bovine Serum (FBS; 10270-106), Trypan Blue Stain (0.4%; 15250-061) were purchased from Thermo Fisher Scientific. 10% gel (acrylamide gel, for SDS-PAGE) was purchased from Bio Craft (SDG521). 2D-Silver Stain-II and reagents for electrophoresis were purchased from Cosmo Bio Co., LTD (423413). Purified anti-human CD20 antibody (Isotype: Mouse IgG2b) was purchased from BioLegend, Inc (302302), Anti-B antigen antibody (sc-69952, Isotype: IgM) and anti-B antigen antibody fluorescently labeled with FITC (sc-69952 FITC, Isotype: IgM) was purchased from Santa Cruz Biotechnology. Thermo Fisher Scientific Attune NxT Acoustic Focusing Cytometer was used for flow cytometry analysis.

B antigen Labeling of cells using B antigen NHS ester 21. Raji cells (ca. 3.6×10^6 cells/tube) were incubated with two concentrations (0, 5.0 mM) of B antigen NHS ester **21** in PBS buffer (pH 7.4) at 37 °C for 30 mins under a CO₂ atmosphere. The suspension was centrifuged at $1600 \times g$ for 3 mins at rt, and the cells were washed three times with PBS buffer.

Protocol for B antigen-antibody conjugates using B antigen NHS ester 21. Anti-CD20 antibody (80 μ L, 40 μ g) was transferred into 50 kDa Amicon ultra (0.5 mL) and PBS (300 μ L) was added. The solution was centrifuged under $14,000 \times g$ for 10 mins at 4 °C. To the supernatant was then added PBS (300 μ L). The step of centrifugation and PBS addition was repeated for three times. After the antibody was divided equally into 3 tubes (10 μ g in 50 μ L PBS buffer), the B antigen NHS ester **21** at two concentrations (final concentrations: 0, 1.0 and 5.0 mM) were added to antibody solution and mixed by pipetting. After being incubated for 30 mins at 4 °C, the reaction mixture (in 50 kDa Amicon ultra) was centrifuged under $14,000 \times g$ for 10 mins at 4 °C. To the supernatant was then added PBS (350 μ L). The step of centrifugation and PBS addition was repeated for five times to give B antigen-antibody conjugates. These antibodies were stored at 4 °C for short term (1-2 weeks).

SDS PAGE analysis. 10% gel (acrylamide gel) was used. To 0.4 μ g of sample (antibody alone or B antigen-antibody conjugates) in 13 μ L PBS buffer were added 5 μ L SDS-PAGE sample buffer (4 \times) and 2 μ L 1.0 M dithiothreitol solution in PBS buffer, and heated at 100 °C for 5 mins. 3 μ L standard protein marker was used. After loading the samples to the gels, SDS-PAGE were run using Tris-Glycine buffer (25 mM Tris, 192 mM glycine and 0.1% SDS) at 200 V for 1 h. The gels were immediately stained with silver stain kit reagents (2D-Silver Stain-II) and scanned using ImageQuant™ LAS 500. After measuring the distance from the top of the gel to each MW marker band (210, 140, 90, 70, 55, 40, 35, 20 kDa), MW was plotted against the distance and a logarithmic approximation curve was drawn. The MW of target band was estimated using this curve.

Flow cytometry. <Raji cells labeled with B antigen using NHS ester **21**> Raji cells (ca. 3.0×10^5 cells/tube) were incubated with anti-B antigen antibody fluorescently labeled with FITC (clone: Z5H-2, 2 μ L in 98 μ L PBS buffer, 1:50) on ice for 1 h. The cells were washed twice and suspended in 0.3 mL of PBS buffer and analyzed with flow cytometer.

<Raji cells labeled with B antigen-antibody conjugates> Raji cells (ca. 3.0×10^5 cells/tube) were incubated with B antigen-antibody conjugates (0.1 μ g in 100 μ L PBS buffer) or PBS buffer on ice for 1 h. The cells were washed three times with PBS buffer and incubated with Alexa Fluor®488-goat anti-mouse IgG antibody (0.05 μ L in 100 μ L PBS buffer, 1:2000) or anti-B antigen antibody fluorescently labeled with FITC (clone: Z5H-2, 2 μ L in 98 μ L PBS buffer, 1:50) on ice for 1 h. The cells were washed twice and suspended in 0.3 mL of PBS buffer and analyzed with flow cytometer.

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Supplementary Material

Supplementary data (¹H and ¹³C NMR spectra) can be found in the online version.

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