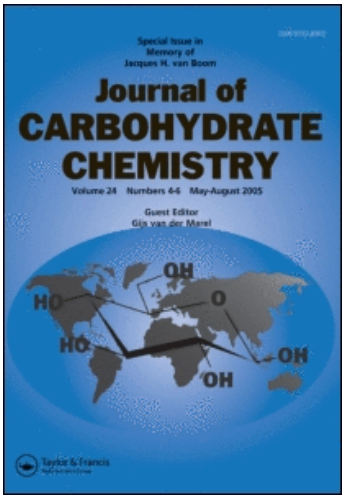

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Synthesis of 3,4,5-Tris(alkyloxy)benzyl Glycosides as Glycolipid Analogues

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CONTENTS

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ABSTRACT

A series of 3,4,5-tris(alkyloxy)benzyl glycosides of D-glucose, D-galactose, D-mannose, *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine were prepared by the trichloroacetimidate procedure. After immobilization on a hydrophobic surface, the affinity of the carbohydrate to a lectin was evaluated using a surface plasmon resonance biosensor. The selective interaction achieved with the lectin showed that the glycosides had potential for use as glycolipid analogues. The 3,4,5-tris(dodecyloxy)benzyl glycosides were soluble in ethanol, and potentially would be useful for cell culture experiments.

Key Words: Synthesis; Glycolipid analogue; Carbohydrate recognition; Surface plasmon resonance; Cell culture.

INTRODUCTION

Glycolipids are known to be involved in many biological events.[1] The recognition of carbohydrates by proteins and cells forms the molecular basis for many physiological events. Glycolipids are often employed in studies of carbohydrate recognition. However, problems exist when natural glycolipids are used, because they are difficult to obtain in large amounts and are heterogeneous in most cases. Therefore, a number of glycolipid analogues, synthetic conjugates of carbohydrates and lipid-like compounds are frequently used. Amphiphilic carbohydrates have been used as tools for molecular recognition in organized systems, such as membranes, micelles, liquid crystals, and vesicles.[2,3] Both natural and artificial glycolipids are known to adhere to a hydrophobic surface and can be used in cell attachment experiments.[4]

We previously proposed a new method for the synthesis of a solution phase library, based on a high molecular weight protecting group.[5] The high molecular weight of the protecting group and even higher molecular weights of the resulting protected compounds permit their easy purification with gel filtration, and the utility of our strategy was demonstrated using peptide synthesis as an example. The protecting group developed is a 3,4,5-tris(octadecyloxy)benzyl group. 3,4,5-Tris(octadecyloxy)benzyl alcohol has a high molecular weight and sufficiently large molecular size for size exclusion chromatography to be used in a purification procedure as an alternative to conventional silica gel column chromatography, which separates compounds mainly based on their molecular polarities. Compounds protected with this 3,4,5-tris(octadecyloxy)benzyl group are eluted almost with the void fraction of Sephadex LH-20 size exclusion chromatography and are easily separated from other compounds of small molecular size in the reaction mixture. The benzyl alcohol can be prepared in 2 steps from commercially available methyl gallate and 1-bromooctadecane.

3,4,5-Tris(octadecyloxy)benzyl alcohol has long alkyl tails, which resemble the fatty acid portions of natural lipids. If the 3,4,5-tris(octadecyloxy)benzyl group is covalently linked to a carbohydrate moiety, the resulting synthetic conjugate can be used as a glycolipid analogue. The synthetic advantage may make it easier to synthesize a series of glycolipid analogues of various carbohydrate structures.

To prove our hypothesis, we synthesized a series of 3,4,5-tris(alkyloxy)benzyl glycosides and examined their applicability as glycolipid analogues. Because the original structure had some drawbacks, we modified the structure slightly to 3,4,5-tris(dodecyloxy)-benzyl. The glycosides behaved like glycolipids and also could be used in biological assays.

RESULTS AND DISCUSSION

In initial experiments, we introduced a monosaccharide moiety to 3,4,5-tris(octadecyloxy)benzyl alcohol by the Schmidt trichloroacetimidate method[6] (Sch. 1). 3,4,5-Tris (octadecyloxy)benzyl was employed as a protecting group in our previous work.[5] Although our strategy worked well in the synthesis of peptides, some practical problems were encountered in the present study.

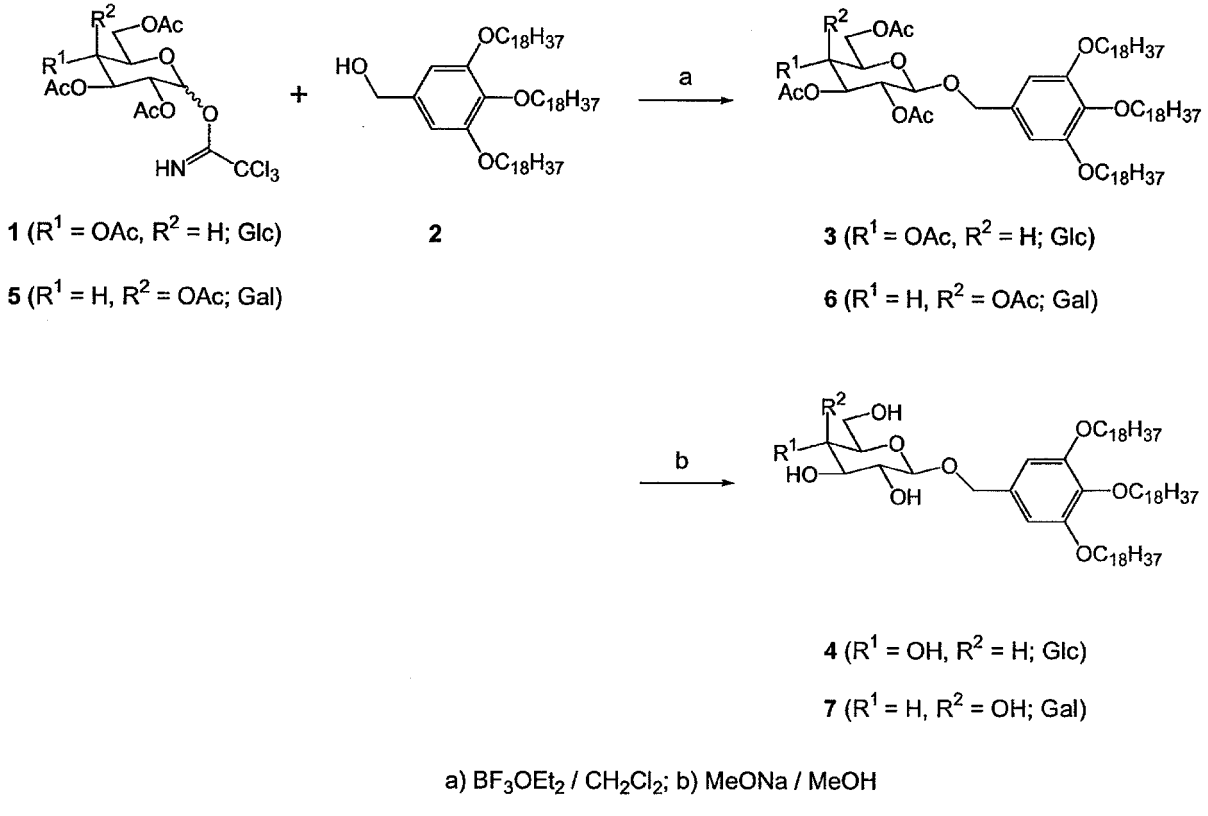
One problem was that it was not possible to carry out the glycosylation reaction at or below 0°C. 3,4,5-Tris(octadecyloxy)benzyl alcohol gradually precipitated from a dichloromethane solution at 0°C. Low-temperature conditions are required in most glycosylation reactions to control the stereochemistry and to prevent side reactions. It was necessary to leave the reaction mixture at room temperature after the addition of a Lewis acid at 0°C, and the efficiency of the reaction was not high.

In spite of this, we were able to obtain the target compounds, but an additional problem was also encountered. The resultant glycolipid analogues were soluble in chloroform-methanol but not in ethanol. To use chloroform-methanol solutions of the compounds for cell culture experiments, it is necessary to use organic-solvent-resistant plastic dishes. Such a dish is expensive and non-transparent, and chloroform vapor is toxic to cells. Therefore, the 3,4,5-tris(octadecyloxy)benzyl glycosides are not an ideal choice for cell culture experiments. If samples were soluble in ethanol, inexpensive transparent polystyrene dishes could be used.

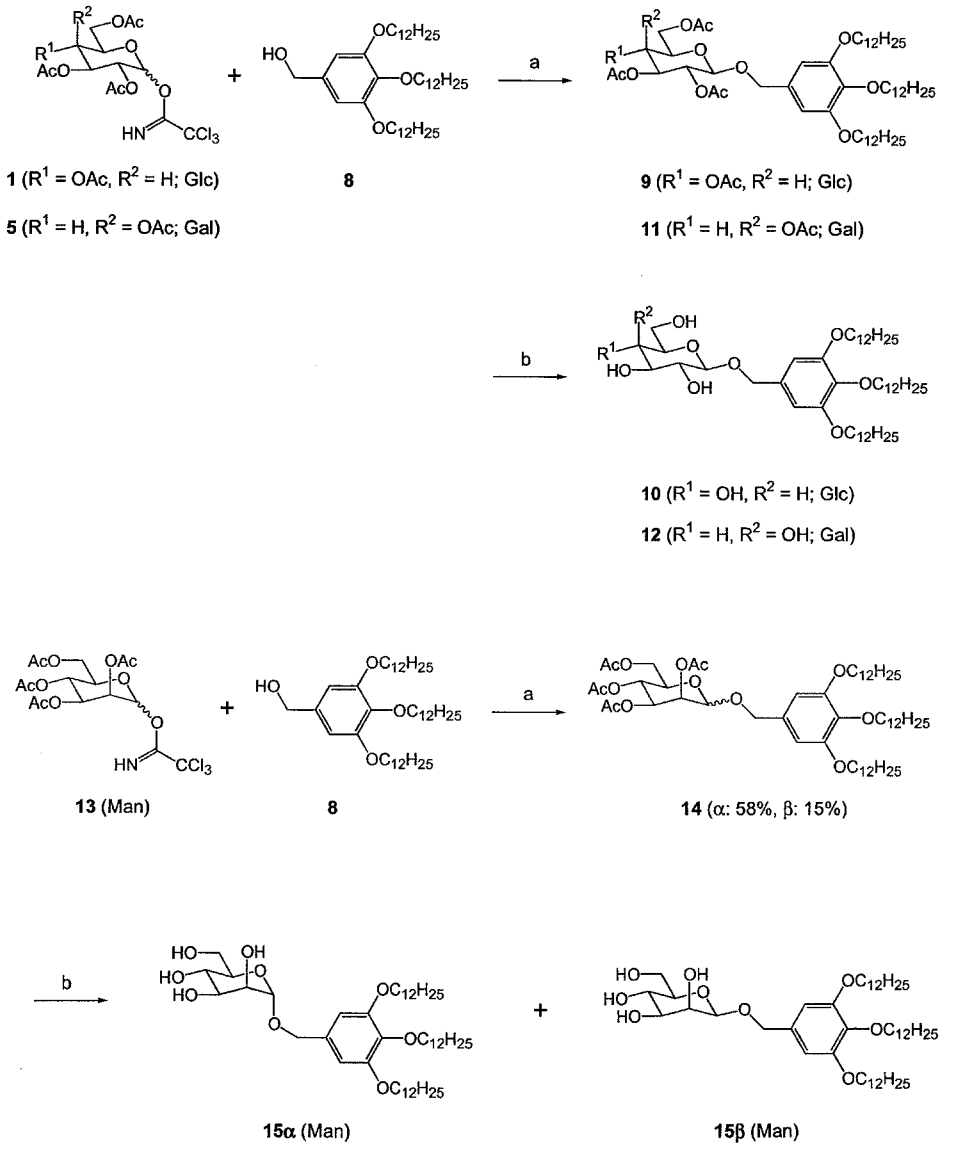
To overcome these problems, we truncated the alkyl chain length of the benzyl alcohol from 18 to 12. In most cases, the alkyl chain length determines the solubility of a synthetic lipid analogue. The shorter chain confers better solubility. If the chain were to be truncated too much, however, the compound may not behave like a lipid. Thus, solubility and the lipid-likeness must be balanced. In a sense, this type of structural flexibility to adjust some properties of compounds is an advantage of using synthetic glycolipid analogues.

The synthetic procedure of 3,4,5-tris(dodecyloxy)benzyl alcohol is the same as the octadecyloxy derivative and was reported previously by Percec and coworkers.[7] Though the molecular size is decreased, the compounds possessing the 3,4,5-tris(dodecyloxy)benzyl group were still eluted almost with the void fraction of size exclusion chromatography, thus allowing separation from smaller molecular size components. The dodecyloxy derivative did not precipitate from dichloromethane solution even at 0°C, and we were able to carry out the glycosylation reaction at 0°C. The resulting glycolipid analogues were soluble in ethanol.

β-Glucose (**10**), β-galactose (**12**), and α- and β-mannose derivatives (**15**) were synthesized (Sch. 2). The chemical yields of glycosides **9** and **11** were improved compared with those of **3** and **6**, from 27% to 35% and from 34% to 56%, respectively, partly because the solubility of the compounds was increased and side reactions analogous to those reported for the oxybenzyl moiety[8] could be minimized.

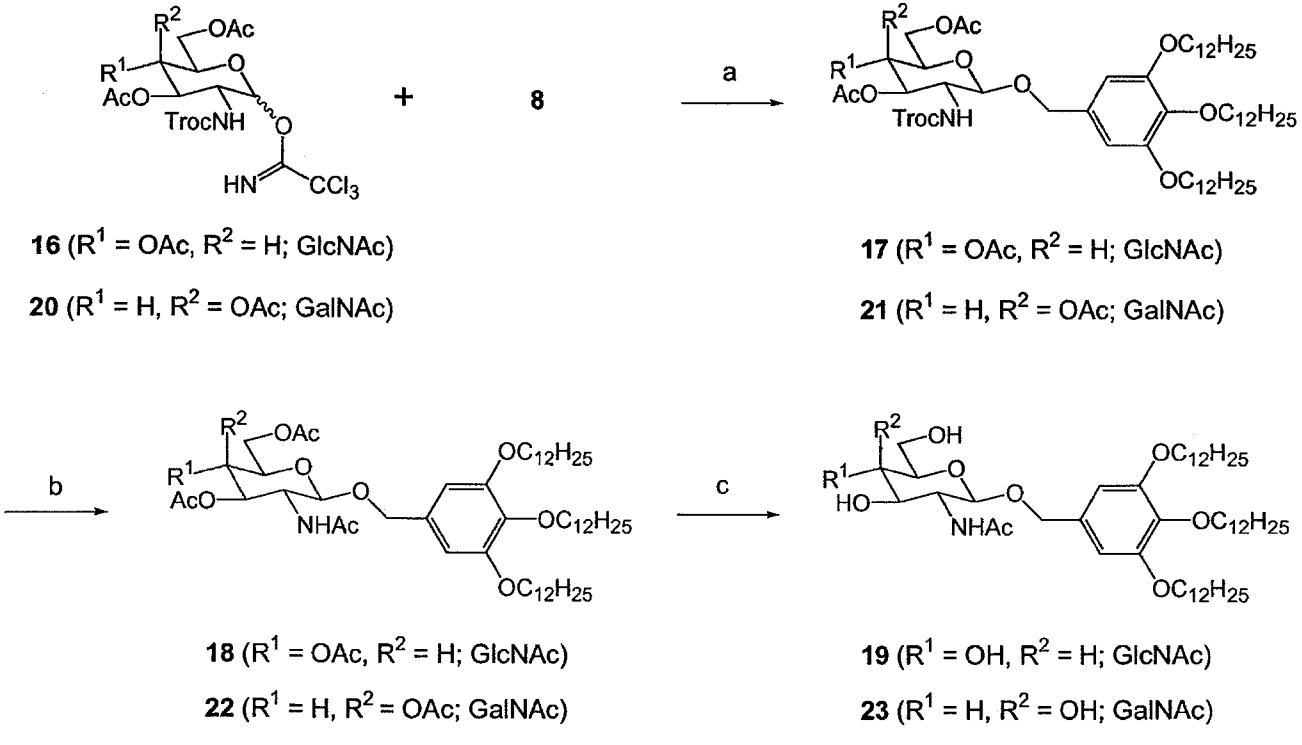


Scheme 1. Synthesis of Glc and Gal derivatives with C18 tails.



Scheme 2. Synthesis of Glc, Gal, and Man derivatives with C12 tails.

β-*N*-Acetyl-D-glucosamine (**19**) and β-*N*-acetyl-D-galactosamine (**23**) derivatives were prepared (Sch. 3). 2,2,2-Trichloroethoxycarbonyl (Troc) was selected as the amino-protecting group, because this blocking group can be removed under relatively mild conditions such as Zn reductive cleavage.[9]



Scheme 3. Synthesis of GlcNAc and GalNAc derivatives with C12 tails.

All the above glycosylations except for the mannose derivative **14** gave the β-anomers as major products after isolation, because of the neighboring group effect of the acetyl or Troc moieties. The β-anomer of **14** was obtained as a minor product in the reaction of **13** with **8**. Some of the deacetylation reactions resulted in low yields, mainly because of the low recovery from silica gel column chromatography, which is probably due to the solvent- and structure-dependent aggregation behavior of the glycolipid analogues.

To examine whether the synthetic compounds behaved like natural glycolipids, with the carbohydrate portion exposed on the hydrophobic surface, their interactions with a lectin were examined using a surface plasmon resonance biosensor. Surface plasmon resonance is one of the best analytical methods for analyzing weak interactions between biological molecules, provided that one of the interacting molecules can be immobilized on the analytical surface of a biosensor.[10]

The glycolipid analogues were immobilized on a hydrophobic cuvette of an IAsys biosensor. The hydrophobic cuvette provides a well-defined hydrophobic surface produced by a self-assembled monolayer of long alkyl chains that are covalently attached to the analytical surface of the cuvette.[11] We assumed that the alkyl portion of the glycolipid analogues interacted with the hydrophobic surface and that the carbohydrate portion was exposed to the aqueous phase.[12]

It was found that the open cuvette-type biosensor, such as an IAsys system, was superior to the flow-type, such as a BiaCore system, in handling our compounds. The sample was applied as a chloroform-methanol solution directly to the open cuvette of the IAsys biosensor. A similar experiment was reported by Mann et al.[13] They employed a mannose glycolipid analogue, which has a lipid structure quite different from ours, and a BiaCore flow surface plasmon resonance biosensor system. For immobilization in the flow system, however, it is necessary to apply the glycolipid analogues as a component of liposomes, because the sample in the flow system must be soluble in water. This immobilization procedure was examined for our compounds using a BiaCore system, but a specific interaction between the carbohydrate and lectin was not detected, probably because the length of the glycolipid analogue was shorter than that of phosphatidylcoline, the main and indispensable component of the liposomes.

Chloroform-methanol solutions of 3,4,5-tris(dodecyloxy)benzyl glycosides, **10**, **12**, **15α**, **15β**, **19**, and **23**, were applied to an IAsys biosensor. The amounts of **10**, **12**, **15α**, **15β**, **19**, and **23** immobilized were 577, 551, 498, 498, 596, and 550 arcsec, respectively. Although the amount of each derivative immobilized deviated by about 20% among the compounds, the difference in the interaction with concanavalin A (Con A) was shown to be far beyond the deviation in the amount immobilized. Specificity with respect to carbohydrate structure was evident (Fig. 1). Con A, a mannose-binding lectin, showed a strong interaction only with the α-anomer of the mannoside (**15α**). The α-mannoside (**15α**) was clearly favored over the β-anomer (**15β**) and the glucoside (**10**), and the response of other glycosides was almost the same, indicating that their apparent $\sim$250 arcsec interaction was non-specific. This confirms that the carbohydrate moieties were exposed to the aqueous phase, as we expected, when immobilized on the hydrophobic surface.

In the surface plasmon resonance analysis of intermolecular interactions, not only specific interactions but also non-specific interactions must be evaluated, because small and reproducible non-specific interactions can be used as indispensable controls.

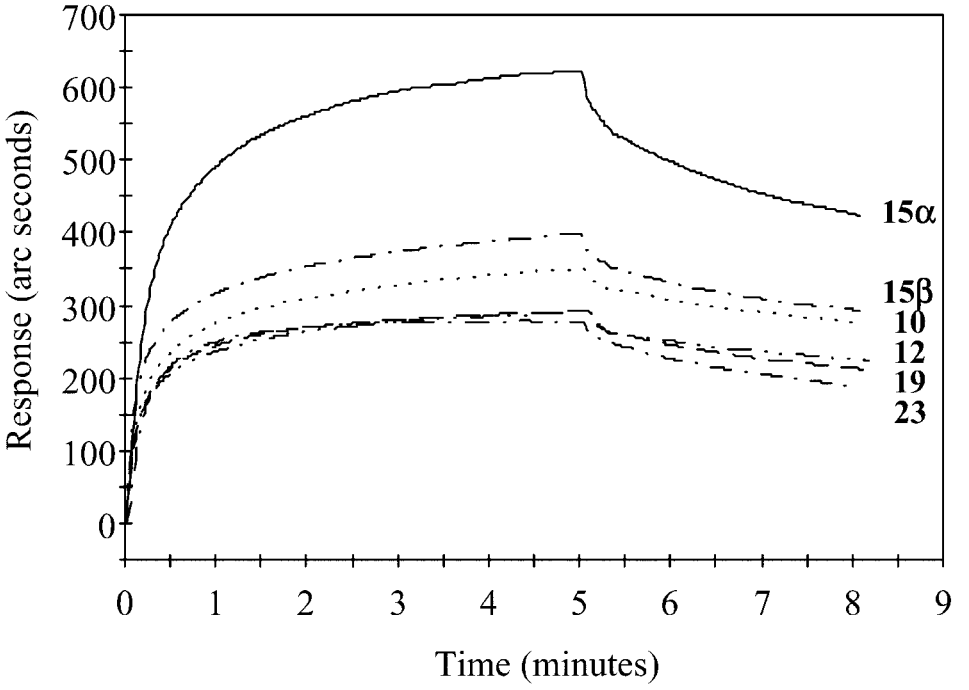


Figure 1. Sensorgrams of the interaction between Con A and immobilized glycolipid analogues: **10** (β-Glc), **12** (β-Gal), **15*α*** (α-Man), **15*β*** (β-Man), **19** (β-GlcNAc), and **23** (β-GalNAc).

Con A usually shows strong non-specific interactions with hydrophobic surfaces. The non-specific interaction of Con A is considerably suppressed by the immobilization of glycolipid analogues, and Fig. 1 indicates that the remaining non-specific interaction is not dependent on the carbohydrate structures that Con A does not recognize. Therefore, each glycolipid analogue can be used as a control compound in the intermolecular interaction study of other glycolipid analogues.

To demonstrate utility of the glycolipid analogues in cell culture experiments, the effect of the carbohydrate structures on the activation of rat hepatocytes was examined. Each glycolipid analogue with C12 tails, **10**, **12**, **15α**, **15β**, **19**, or **23**, was dissolved in ethanol. The ethanol solution was poured onto a plastic dish, and the ethanol was allowed to evaporate to give a dish coated with a glycolipid analogue. Freshly harvested primary rat hepatocytes were cultured on the glycolipid-coated dishes. Among the glycolipid analogues examined, only the galactose derivative specifically activated ammonium consumption activity in the hepatocytes. This indicates that the glyocolipid analogues can be useful in cell culture experiments. Details have been reported elsewhere.[14,15]

In summary, a series of 3,4,5-tris(alkyloxy)benzyl glycosides were synthesized as glycolipid analogues. The exposure of the carbohydrate structure to the surface was confirmed by selective interaction with Con A using a surface plasmon biosensor. The glycolipid analogues were soluble in ethanol and can be used in cell culture experiments.

EXPERIMENTAL

General. All reagents and anhydrous solvents were purchased from commercial suppliers and were used as provided. ^{1}H-NMR spectra were determined on a JNM-EX400 spectrometer (JEOL, Tokyo, Japan). Matrix-assisted laser desorption ionization time-of-flight mass spectra (TOFMS) were obtained on a Voyager-DESTR spectrometer (PerSeptive Biosystems, Framingham, USA), using dithranol as a matrix. Surface plasmon resonance analysis was performed on an IAsys plus optical biosensor (Affinity Sensors, Cambridge, UK). IAsys hydrophobic cuvettes were purchased from Affinity Sensors. The HBS-N buffer (aqueous buffer containing 0.01 M HEPES, pH 7.4, 0.15 M NaCl) was purchased from Biacore AB (Uppsala, Sweden).

3,4,5-Tris(octadecyloxy)benzyl β-D-glucopyranoside (4). An anhydrous CH_2Cl_2 (24 mL) solution of 3,4,5-tris(octadecyloxy)benzyl alcohol (**2**)[5] (0.41 g, 0.44 mmol) and glucopyranosyl trichloroacetimidate (**1**)[6] (0.44 g, 0.88 mmol) was stirred in the presence of molecular sieves AW-300 (0.40 g, powder) for 1 hr under Ar. The solution was cooled to 0°C, and $BF_3 \cdot OEt_2$ (16.8 μL, 0.13 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 0.5 hr, quenched with NEt_3, filtered through Celite, and evaporated. The residue was purified by silica gel column chromatography using 10 : 1 hexane-EtOAc as the eluent to give **3** (0.15 g, 27%). ^{1}H-NMR ($CDCl_3$): δ 6.47 (s, 2H, Ar-H), 5.20 (dd, 1H, $J_{2,3} = 9.3$ Hz, $J_{3,4} = 9.5$ Hz, H-3), 5.11 (dd, 1H, $J_{4,5} = 9.8$ Hz, H-4), 5.06 (dd, 1H, $J_{1,2} = 7.8$ Hz, H-2), 4.80, 4.50 (each d, 1H × 2, $J = 12.0$ Hz, Ar-CH_2), 4.58 (d, 1H, H-1), 4.28 (dd, 1H, $J_{5,6a} = 4.6$ Hz, $J_{6a,6b} = 12.3$ Hz, H-6a), 4.17 (dd, 1H, $J_{5,6b} = 2.4$ Hz, H-6b), 4.06–3.92 (m, 6H, ArO-CH_2), 3.70 (ddd, 1H, H-5), 2.10, 2.03, 2.00, 1.99 (each s, 3H × 4, –OAc), 1.76 (m, 6H, ArOC-CH_2), 1.46 (m, 6H, $ArOC_2$-CH_2), 1.25 (m, 84H, $ArOC_3$-$C_{14}H_{28}$), 0.88 (t, 9H, $J = 7.1$ Hz, terminal CH_3). TOFMS found: m/z 1244. Calcd. for $C_{75}H_{135}O_{13}$: $[M + H]^+$ 1244.

To an anhydrous methanol (10 mL) solution of **3** (62 mg, 0.050 mmol), NaOMe (1.4 mg, 0.025 mmol) was added. The reaction mixture was refluxed for 1 hr, neutralized with Amberlite IR-120 (H^+) resin, filtered, and evaporated. The residue was purified by silica gel column chromatography using 10 : 1 $CHCl_3$-methanol as the eluent to give **4** (48 mg, 89%). $[\alpha]_D^{25}$ −1.8° (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ 6.53 (s, 2H, Ar-H), 4.81, 4.47 (each d, 1H × 2, $J = 12.0$ Hz, Ar-CH_2), 4.42 (d, 1H, $J_{1,2} = 7.6$ Hz, H-1), 3.96–3.90 (m, 6H, ArO-CH_2), 3.87–3.82 (m, 2H, H-6a, H-6b), 3.67–3.56 (m, 2H, H-3, H-4), 3.44–3.40 (m, 2H, H-2, H-5), 1.74 (m, 6H, ArOC-CH_2), 1.44 (m, 6H, $ArOC_2$-CH_2), 1.24 (m, 84H, $ArOC_3$-$C_{14}H_{28}$), 0.86 (t, 9H, $J = 6.8$ Hz, terminal CH_3). TOFMS found: m/z 1098. Calcd. for $C_{67}H_{126}O_9Na$: $[M + Na]^+$ 1098.

3,4,5-Tris(octadecyloxy)benzyl β-D-galactopyranoside (7). Similar to the synthesis of **3**, **6** (0.19 g, 34%) was obtained from D-galactose imidate (**5**)[6] (0.44 g, 0.88 mmol) and **2** (0.40 g, 0.44 mmol). $[\alpha]_D^{25}$ + 0.7° (*c* 0.9, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ 6.47 (s, 2H, Ar-H), 5.39 (dd, 1H, $J_{4,5} = 2.7$ Hz, H-4), 5.27 (dd, 1H, $J_{1,2} = 7.8$ Hz, $J_{2,3} = 10.5$ Hz, H-2), 5.00 (dd, 1H, $J_{3,4} = 3.4$ Hz, H-3), 4.81, 4.51 (each d, 1H × 2, $J = 11.6$ Hz, Ar-CH_2), 4.53 (d, 1H, H-1), 4.22 (dd, 1H, $J_{5,6a} = 6.6$ Hz, $J_{6a,6b} = 11.1$ Hz, H-6a), 4.14 (dd, 1H, $J_{5,6b} = 7.0$ Hz, H-6b), 3.96–3.88 (m, 7H, H-5, ArO-CH_2), 2.15, 2.06, 1.99, 1.97 (each s, 3H × 4, –OAc), 1.76 (m, 6H, ArOC-CH_2), 1.46 (m, 6H, $ArOC_2$-CH_2), 1.25 (m, 84H, $ArOC_3$-$C_{14}H_{28}$), 0.88 (t, 9H, $J = 7.1$ Hz, terminal CH_3). TOFMS found: m/z 1244. Calcd. for $C_{75}H_{135}O_{13}$: $[M + H]^+$ 1244.

Similar to the synthesis of **4**, **7** (81 mg, 88%) was obtained from **6** (0.11 g, 0.087 mmol). $[\alpha]_D^{25}$ −4.4° (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ 6.53 (s, 2H, Ar-H), 4.82, 4.47 (each d, 1H × 2, $J = 11.5$ Hz, Ar-CH_2), 4.43 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1), 3.97 (dd, 1H, $J_{3,4} = 3.4$ Hz, H-4), 3.94–3.82 (m, 7H, ArO-CH_2, H-6a), 3.84 (dd, 1H, $J_{5,6b} = 4.6$ Hz, $J_{6a,6b} = 11.9$ Hz, H-6b), 3.66 (dd, 1H, $J_{2,3} = 9.5$ Hz, H-2), 3.54 (dd, 1H, H-3), 3.52 (m, 1H, H-5), 1.72 (m, 6H, ArOC-CH_2), 1.42 (m, 6H, $ArOC_2$-CH_2), 1.24 (m, 84H, $ArOC_3$-$C_{14}H_{28}$), 0.84 (t, 9H, $J = 6.8$ Hz, terminal CH_3). TOFMS found: m/z 1098. Calcd. for $C_{67}H_{126}O_9Na$: $[M + Na]^+$ 1098.

3,4,5-Tris(dodecyloxy)benzyl β-D-glucopyranoside (10). An anhydrous CH_2Cl_2 (24 mL) solution of **1** (0.60 g, 1.2 mmol) and **8**[7] (0.40 g, 0.61 mmol) was stirred in the presence of molecular sieves AW-300 (0.40 g, powder) for 1 hr under Ar. The solution was cooled to 0°C, and $BF_3 \cdot OEt_2$ (11.7 μL, 0.09 mmol) was added dropwise. The reaction mixture was stirred at 0°C for 0.5 hr, quenched with NEt_3, filtered through Celite, and evaporated. The residue was purified by silica gel column chromatography using 4 : 1 hexane-EtOAc as the eluent to give **9** (0.21 g, 35%). $[\alpha]_D^{25}$ −22.8° (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ 6.47 (s, 2H, Ar-H), 5.19 (dd, 1H, $J_{2,3} = 9.3$ Hz, $J_{3,4} = 9.5$ Hz, H-3), 5.12 (dd, 1H, $J_{4,5} = 9.8$ Hz, H-4), 5.06 (dd, 1H, $J_{1,2} = 7.8$ Hz, H-2), 4.80, 4.50 (each d, 1H × 2, $J = 12.0$ Hz, Ar-CH_2), 4.57 (d, 1H, H-1), 4.28 (dd, 1H, $J_{5,6a} = 4.6$ Hz, $J_{6a,6b} = 12.3$ Hz, H-6a), 4.17 (dd, 1H, $J_{5,6b} = 2.4$ Hz, H-6b), 3.96–3.92 (m, 6H, ArO-CH_2), 3.69 (ddd, 1H, H-5), 2.10, 2.02, 2.00, 1.99 (each s, 3H × 4, −OAc), 1.77 (m, 6H, ArOC-CH_2), 1.44 (m, 6H, $ArOC_2$-CH_2), 1.28 (m, 48H, $ArOC_3$-C_8H_{16}), 0.88 (t, 9H, $J = 6.8$ Hz, terminal CH_3). TOFMS found: m/z 1014. Calcd. for $C_{57}H_{98}O_{13}Na$: $[M + Na]^+$ 1014.

To an anhydrous methanol (15 mL) solution of **9** (0.21 g, 0.21 mmol), NaOMe (6.0 mg, 0.11 mmol) was added. The reaction mixture was stirred at room temperature for 1 hr, neutralized with Amberlite IR-120 (H^+) resin, filtered, and evaporated. The residue was purified by silica gel column chromatography using 10 : 1 $CHCl_3$-methanol as the eluent to give **10** (0.15 g, 84%). $[\alpha]_D^{25}$ −0.9° (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ 6.57 (s, 2H, Ar-H), 4.84, 4.54 (each d, 1H × 2, $J = 11.5$ Hz, Ar-CH_2), 4.46 (d, 1H, $J_{1,2} = 7.6$ Hz, H-1), 3.99–3.93 (m, 6H, ArO-CH_2), 3.92–3.85 (m, 2H, H-6a, H-6b), 3.68–3.58 (m, 2H, H-3, H-4), 3.48–3.40 (m, 2H, H-2, H-5), 1.80 (m, 6H, ArOC-CH_2), 1.45 (m, 6H, $ArOC_2$-CH_2), 1.23 (m, 48H, $ArOC_3$-C_8H_{16}), 0.89 (t, 9H, $J = 6.8$ Hz, terminal CH_3). TOFMS found: m/z 846. Calcd. for $C_{49}H_{90}O_9Na$: $[M + Na]^+$ 846.

3,4,5-Tris(dodecyloxy)benzyl β-D-galactopyranoside (12). Similar to the synthesis of **9**, **11** (0.17 g, 56%) was obtained from **5** (0.30 g, 0.61 mmol) and **8** (0.20 g, 0.30 mmol). $[\alpha]_D^{25}$ −4.8° (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ 6.48 (s, 2H, Ar-H), 5.40 (dd, 1H, $J_{3,4} = 3.4$ Hz, H-4), 5.28 (dd, 1H, $J_{1,2} = 8.1$ Hz, $J_{2,3} = 10.5$ Hz, H-2), 5.01 (dd, 1H, H-3), 4.82, 4.52 (each d, 1H × 2, $J = 12.0$ Hz, Ar-CH_2), 4.54 (d, 1H, H-1), 4.22 (dd, 1H, $J_{5,6a} = 6.4$ Hz, $J_{6a,6b} = 11.2$ Hz, H-6a), 4.15 (dd, 1H, $J_{5,6b} = 7.0$ Hz, H-6b), 3.97–3.89 (m, 7H, ArO-CH_2, H-5), 2.16, 2.07, 2.00, 1.99 (each s, 3H × 4, −OAc), 1.77 (m, 6H, ArOC-CH_2), 1.47 (m, 6H, $ArOC_2$-CH_2), 1.27 (m, 48H, $ArOC_3$-C_8H_{16}), 0.88 (t, 9H, $J = 6.8$ Hz, terminal CH_3). TOFMS found: m/z 1014. Calcd. for $C_{57}H_{98}O_{13}Na$: $[M + Na]^+$ 1014.

Similar to the synthesis of **10**, **12** (0.12 g, 45%) was obtained from **11** (0.33 g, 0.33 mmol). $[\alpha]_D^{25}$ −18.2° (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ 6.58 (s, 2H, Ar-H), 4.84, 4.50 (each d, 1H × 2, $J = 12.0$ Hz, Ar-CH_2), 4.33 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1),

3.97–3.91 (m, 7H, ArO-CH_2, H-4), 3.89 (dd, 1H, $J_{5,6a} = 6.0$ Hz, $J_{6a,6b} = 11.7$ Hz, H-6a), 3.83 (dd, 1H, $J_{5,6b} = 9.4$ Hz, H-6b), 3.66 (dd, 1H, $J_{2,3} = 9.4$ Hz, H-2), 3.53 (dd, 1H, $J_{3,4} = 3.4$ Hz, H-3), 3.50 (m, 1H, H-5), 1.76 (m, 6H, ArOC-CH_2), 1.44 (m, 6H, $ArOC_2$-CH_2), 1.28 (m, 48H, $ArOC_3$-C_8H_{16}), 0.88 (t, 9H, $J = 7.0$ Hz, terminal CH_3). TOFMS found: m/z 846. Calcd. for $C_{49}H_{90}O_9Na$: $[M + Na]^+$ 846.

3,4,5-Tris(dodecyloxy)benzyl α- and β-D-mannopyranoside (15). Similar to the synthesis of **9**, the α-anomer of **14** (**14α**; 0.17 g, 58%) and the β-anomer of **14** (**14β**; 46 mg, 15%) were obtained from **13**[6] (0.30 g, 0.61 mmol) and **8** (0.20 g, 0.30 mmol). **14α**: $[\alpha]_D^{25}$ +1.0° (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ 6.51 (s, 2H, Ar-H), 5.38 (dd, 1H, $J_{2,3} = 3.4$ Hz, $J_{3,4} = 10.1$ Hz, H-3), 5.30 (t, 1H, $J_{4,5} = 10.1$ Hz, H-4), 5.28 (dd, 1H, $J_{1,2} = 1.7$ Hz, H-2), 4.86 (d, 1H, H-1), 4.60, 4.44 (each d, 1H × 2, $J = 12.0$ Hz, Ar-CH_2), 4.31 (dd, 1H, $J_{5,6a} = 5.1$ Hz, $J_{6a,6b} = 12.2$ Hz, H-6a), 4.09 (dd, 1H, $J_{5,6b} = 2.4$ Hz, H-6b), 4.02 (ddd, 1H, H-5), 3.99–3.93 (m, 6H, ArO-CH_2), 2.15, 2.12, 2.04, 1.99 (each s, 3H × 4, –OAc), 1.77 (m, 6H, ArOC-CH_2), 1.45 (m, 6H, $ArOC_2$-CH_2), 1.28 (m, 48H, $ArOC_3$-C_8H_{16}), 0.88 (t, 9H, $J = 6.8$ Hz, terminal CH_3). TOFMS found: m/z 991. Calcd. for $C_{57}H_{99}O_{13}$: $[M + H]^+$ 992. **14β**: $[\alpha]_D^{25}$ +1.1° (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ 6.48 (s, 2H, Ar-H), 5.46 (d, 1H, $J_{2,3} = 3.0$ Hz, H-2), 5.27 (t, 1H, $J_{3,4} = J_{4,5} = 10.1$ Hz, H-4), 5.01 (dd, 1H, H-3), 4.77, 4.53 (each d, 1H × 2, $J = 12.0$ Hz, Ar-CH_2), 4.62 (s, 1H, H-1), 4.33 (dd, 1H, $J_{5,6a} = 5.4$ Hz, $J_{6a,6b} = 12.2$ Hz, H-6a), 4.17 (dd, 1H, $J_{5,6b} = 2.4$ Hz, H-6b), 3.95 (t, 6H, ArO-CH_2), 3.63 (ddd, 1H, H-5), 2.20, 2.12, 2.04, 1.99 (each s, 3H × 4, –OAc), 1.77 (m, 6H, ArOC-CH_2), 1.45 (m, 6H, $ArOC_2$-CH_2), 1.26 (m, 48H, $ArOC_3$-C_8H_{16}), 0.88 (t, 9H, $J = 6.7$ Hz, terminal CH_3). TOFMS found: m/z 991. Calcd. for $C_{57}H_{99}O_{13}$: $[M + H]^+$ 992.

Similar to the synthesis of **10**, **15α** (77 mg, 53%) was obtained from **14α** (0.17 g, 0.18 mmol). $[\alpha]_D^{25}$ –29.0° (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ 6.49 (s, 1H, Ar-H), 4.90 (s, 1H, H-1), 4.57, 4.38 (each d, 1H × 2, $J = 12.0$ Hz, Ar-CH_2), 3.96–3.91 (m, 9H), 3.86 (m, 1H), 3.78 (m, 1H), 3.58 (m, 1H), 1.76 (m, 6H, ArOC-CH_2), 1.45 (m, 6H, $ArOC_2$-CH_2), 1.26 (m, 48H, $ArOC_3$-C_8H_{16}), 0.88 (t, 9H, $J = 6.8$ Hz, terminal CH_3). TOFMS found: m/z 846. Calcd. for $C_{49}H_{90}O_9Na$: $[M + Na]^+$ 846.

Similar to the synthesis of **10**, **15β** (40 mg, 39%) was obtained from **14β** (0.12 g, 0.12 mmol). $[\alpha]_D^{25}$ –35.9° (*c* 0.5, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ 6.51 (s, 2H, Ar-H), 4.77, 4.51 (each d, 1H × 2, $J = 12.0$ Hz, Ar-CH_2), 4.48 (s, 1H, H-1), 3.94–3.83 (m, 10H), 3.46 (m, 1H), 3.14 (m, 1H), 1.74 (m, 6H, ArOC-CH_2), 1.46 (m, 6H, $ArOC_2$-CH_2), 1.27 (m, 48H, $ArOC_3$-C_8H_{16}), 0.87 (t, 9H, $J = 6.8$ Hz, terminal CH_3). TOFMS found: m/z 846. Calcd. for $C_{49}H_{90}O_9Na$: $[M + Na]^+$ 846.

3,4,5-Tris(dodecyloxy)benzyl 2-acetamido-2-deoxy-β-D-glucopyranoside (19). Similar to the synthesis of **9**, **17** (0.49 g, 88%) was obtained from **16**[7] (0.62 g, 0.99 mmol) and **8** (0.33 g, 0.50 mmol). $[\alpha]_D^{25}$ –11.7° (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ 6.49 (s, 2H, Ar-H), 5.26 (t, 1H, $J_{2,3} = J_{3,4} = 10.0$ Hz, H-3), 5.10 (t, 1H, $J_{4,5} = 9.8$ Hz, H-4), 5.08 (d, 1H, $J = 9.5$ Hz, –N*H*Troc), 4.81, 4.50 (each d, 1H × 2, $J = 11.7$ Hz, Ar-CH_2), 4.79, 4.61 (each d, 1H × 2, $J = 12.2$ Hz, CCl_3-CH_2), 4.65 (d, 1H, $J_{1,2} = 8.3$ Hz, H-1), 4.30 (dd, 1H, $J_{5,6a} = 4.4$ Hz, $J_{6a,6b} = 12.2$ Hz, H-6a), 4.17 (dd, 1H, $J_{5,6b} = 2.2$ Hz, H-6b), 3.96–3.92 (m, 6H, ArO-CH_2), 3.75–3.66 (m, 2H, H-2, H-5), 2.11, 2.03, 2.02 (each s, 3H × 3, –OAc), 1.77 (m, 6H, ArOC-CH_2), 1.46 (m, 6H, $ArOC_2$-CH_2), 1.28 (m, 48H, $ArOC_3$-C_8H_{16}), 0.88 (t, 9H, $J = 6.8$ Hz, terminal CH_3). TOFMS found: m/z 1145. Calcd. for $C_{58}H_{98}Cl_3NO_{13}Na$: $[M + Na]^+$ 1145.

The Troc-protected amine **17** (0.67 g, 0.59 mmol) was dissolved in 3 : 2 : 1 THF –AcOH-Ac_2O (36 mL), and activated zinc dust (0.80 g) was added. The reaction mixture was stirred at room temperature for 3 hr under Ar, filtered through Celite, and evaporated. The residue was diluted with CH_2Cl_2, and washed with H_2O, sat. aqueous $NaHCO_3$ and brine. The organic layer was dried with Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel column chromatography using 1 : 1 hexane-EtOAc as the eluent to give **18** (0.38 g, 65%). $[\alpha]_D^{25}$ –23.6° (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ 6.49 (s, 2H, Ar-H), 5.33 (d, 1H, $J_{2,2N} = 9.0$ Hz, –N*H*Ac), 5.20 (t, 1H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 5.10 (t, 1H, $J_{4,5} = 10.0$ Hz, H-4), 4.79, 4.48 (each d, 1H × 2, $J = 12.0$ Hz, Ar-CH_2), 4.66 (d, 1H, $J_{1,2} = 8.6$ Hz, H-1), 4.29 (dd, 1H, $J_{5,6a} = 4.5$ Hz, $J_{6a,6b} = 12.3$ Hz, H-6a), 4.17 (dd, 1H, $J_{5,6b} = 2.3$ Hz, H-6b), 3.96–3.92 (m, 7H, H-2, ArO-CH_2), 3.68 (ddd, 1H, H-5), 2.10, 2.02 × 2, 1.90 (each s, 3H × 4, –OAc, –NAc), 1.77 (m, 6H, ArOC-CH_2), 1.46 (m, 6H, $ArOC_2$-CH_2), 1.28 (m, 48H, $ArOC_3$-C_8H_{16}), 0.88 (t, 9H, $J = 6.8$ Hz, terminal CH_3). TOFMS found: m/z 1013. Calcd. for $C_{57}H_{99}NO_{12}Na$: $[M + Na]^+$ 1013.

Similar to the synthesis of **10**, **19** (0.33 g, quant) was obtained from **18** (0.38 g, 0.38 mmol). $[\alpha]_D^{25}$ –22.0° (*c* 0.9, 3 : 1 $CHCl_3$-MeOH). ^{1}H-NMR ($CDCl_3$): δ 6.52 (2H, s, Ar-H), 4.77, 4.48 (each d, 1H × 2, $J = 12.0$ Hz, Ar-CH_2), 4.50 (d, 1H, $J_{1,2} = 7.6$ Hz, H-1), 3.97–3.89 (m, 7H, H-6a, ArO-CH_2), 3.81 (dd, 1H, $J_{5,6b} = 4.6$ Hz, $J_{6a,6b} = 12.0$ Hz, H-6b), 3.60–3.48 (m, 3H, H-2, H-3, H-4), 3.32 (m, 1H, H-5), 1.96 (s, 3H, –NAc), 1.76 (m, 6H, ArOC-CH_2), 1.44 (m, 6H, $ArOC_2$-CH_2), 1.28 (m, 48H, $ArOC_3$-C_8H_{16}), 0.88 (t, 9H, $J = 6.8$ Hz, terminal CH_3). TOFMS found: m/z 887. Calcd. for $C_{51}H_{93}NO_9Na$: $[M + Na]^+$ 887.

3,4,5-Tris(dodecyloxy)benzyl 2-acetamido-2-deoxy-β-D-galactopyranoside (23). Similar to the synthesis of **9**, **21** (0.45 g, 90%) was obtained from **20**[7] (0.51 g, 0.82 mmol) and **8** (0.28 g, 0.41 mmol). $[\alpha]_D^{25}$ –15.3° (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ 6.50 (s, 2H, Ar-H), 5.38 (d, 1H, $J_{3,4} = 2.7$ Hz, H-4), 5.19 (bd, 1H, $J_{2,3} = 10.0$ Hz, H-3), 5.00 (bd, $J_{2,2N} = 6.8$ Hz, –N*H*Troc), 4.82, 4.51 (each d, 1H × 2, $J = 11.9$ Hz, Ar-CH_2), 4.79, 4.61 (each d, 1H × 2, $J = 12.6$ Hz, CCl_3-CH_2), 4.65 (d, 1H, $J_{1,2} = 8.1$ Hz, H-1), 4.22 (dd, 1H, $J_{5,6a} = 6.6$ Hz, $J_{6a,6b} = 11.2$ Hz, H-6a), 4.15 (dd, 1H, $J_{5,6b} = 6.8$ Hz, H-6b), 3.96–3.86 (m, 8H, H-2, H-5, ArO-CH_2), 2.16, 2.07, 1.99 (each s, 3H × 3, –OAc), 1.77 (m, 6H, ArOC-CH_2), 1.46 (m, 6H, $ArOC_2$-CH_2), 1.28 (m, 48H, $ArOC_3$-C_8H_{16}), 0.88 (t, 9H, $J = 6.8$ Hz, terminal CH_3). TOFMS found: m/z 1145. Calcd. for $C_{58}H_{98}Cl_3NO_{13}Na$: $[M + Na]^+$ 1145.

Similar to the synthesis of **18**, **22** (0.16 g, 72%) was obtained from **21** (0.25 g, 0.22 mmol). $[\alpha]_D^{25}$ –25.1° (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ 6.50 (s, 2H, Ar-H), 5.36 (d, 1H, $J = 2.4$ Hz, H-4), 5.28 (d, 1H, $J = 8.8$ Hz, –N*H*Ac), 5.21 (dd, 1H, $J_{3,4} = 3.4$ Hz, H-3), 4.81, 4.49 (each d, 1H × 2, $J = 12.0$ Hz, Ar-CH_2), 4.69 (d, 1H, $J_{1,2} = 8.3$ Hz), 4.22 (dd, 1H, $J_{5,6a} = 6.6$ Hz, $J_{6a,6b} = 11.2$ Hz, H-6a), 4.15 (dd, 1H, $J_{5,6b} = 6.8$ Hz, H-6b), 4.08 (dt, 1H, $J_{2,3} = 11.2$ Hz, H-2), 3.97–3.89 (m, 7H, H-5, ArO-CH_2), 2.16, 2.07, 2.00, 1.90 (each s, 3H × 4, –OAc, –NAc), 1.77 (m, 6H, ArOC-CH_2), 1.45 (m, 6H, $ArOC_2$-CH_2), 1.28 (m, 48H, $ArOC_3$-C_8H_{16}), 0.88 (t, 9H, $J = 6.8$ Hz, terminal CH_3). TOFMS found: m/z 1013. Calcd. for $C_{57}H_{99}NO_{12}Na$: $[M + Na]^+$ 1013.

Similar to the synthesis of **10**, **23** (0.19 g, 83%) was obtained from **22** (0.26 g, 0.26 mmol). $[\alpha]_D^{25}$ –15.1° (*c* 1.0, 3 : 1 $CHCl_3$-MeOH). ^{1}H-NMR ($CDCl_3$): δ 6.53 (s, 2H, Ar-H), 4.80, 4.49 (each d, 1H × 2, $J = 12.0$ Hz, Ar-CH_2), 4.41

(d, 1H, $J_{1,2} = 8.3$ Hz, H-1), 3.97–3.91 (m, 8H, H-4, H-6a, ArO-CH_2), 3.86 (dd, 1H, $J_{5,6b} = 4.9$ Hz, $J_{6a,6b} = 11.7$ Hz, H-6b), 3.79 (dd, 1H, $J_{2,3} = 10.0$ Hz, H-2), 3.65 (dd, 1H, $J_{3,4} = 3.2$ Hz, H-3), 3.52 (t, 1H, $J_{5,6a} = J_{5,6b} = 5.2$ Hz, H-5), 1.97 (s, 3H, –NAc), 1.77 (m, 6H, ArOC-CH_2), 1.45 (m, 6H, ArOC$_2$-CH_2), 1.28 (m, 48H, ArOC$_3$-C$_8H_{16}$), 0.88 (t, 9H, $J = 6.7$ Hz, terminal CH_3). TOFMS found: m/z 887. Calcd. for $C_{51}H_{93}NO_9Na$: $[M + Na]^+$ 887.

Immobilization of the Glycolipid Analogues on Hydrophobic Cuvettes. All IAsys experiments were carried out at 25°C. Prior to the immobilization of a glycolipid analogue, a hydrophobic cuvette was washed with a 20 mM aqueous solution of 3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate, HBS-N buffer, and 2-propanol. Each glycolipid analogue was immobilized by adding 20 μL of a 5 mM 1 : 3 $CHCl_3$-methanol solution to 80 μL of 2-propanol in a cuvette. The immobilized glycolipid analogue layer was washed with HBS-N buffer, 0.1 M HCl, and 10 mM NaOH for stabilization. The difference in the response before and after the immobilization procedure was taken as the amount of material immobilized for each glycolipid analogue.

Interaction between the Glycolipid Analogues and Con A. To prevent non-specific interactions 1.5 mg/mL bovine serum albumin (Wako Pure Chemical Industries, Osaka, Japan) solution was incubated in a cuvette to which a glycolipid analogue had been immobilized. After washing with HBS-N buffer, a 10 μM solution of Con A (Vector Laboratories, Burlingame, USA) was added. The Con A solution was incubated for 5 min to allow for the binding interaction to proceed. The binding buffer was then replaced with HBS-N buffer to obtain the dissociation curve of Con A.

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