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Synthesis and galectin-binding activities of mercaptododecyl glycosides containing a terminal β-galactosyl group

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ABSTRACT

Mercaptododecyl glycosides containing a terminal β-galactosyl group were prepared from D-galactose or from D-lactose via hexa-*O*-acetyl-lactal (**10**) as a key intermediate. Interactions of these glycolipids (5 kinds) and galectins (β-galactoside binding lectins, 6 species) were evaluated by surface plasmon resonance (SPR) method. High binding responses were observed for the lactoside, 2-deoxy-lactoside, and lactosaminide with some galectins (Gal-3, -4, -8), whereas the galactoside and 2,3-dideoxy-lactoside showed low binding activities.

Lectins are a large group of carbohydrate binding proteins widely found in nature including plants, animals, and lower organisms. The galectins,[1] a subfamily of lectins, are defined by shared conserved amino-acid sequence and affinity for β-galactosides. To date, 14 members of the family have been identified in mammals, 10 of which have been detected in human organs and tissues such as lung, liver, and small intestine. Galectins are soluble cytosolic proteins with molecular weight of 14–36 kDa and having only a carbohydrate recognition domain (CRD) and no other functional domains. They appear to be involved in a broad range of biological events such as cell growth and adhesion,[2] inflammation and immune response,[3] apoptosis,[4] and cancer.[5] Galectins display an intriguing combination of intracellular and extracellular activities. However, much work remains to clearly understand the mechanism by which they exert these functions.

Recent biomedical studies have demonstrated the relationships between the galectin species and diseases. Among the family, galectin-1 and -3 have been most extensively studied, and they can be considered as promising tumor markers since their expression has been correlated with tumor progression[6] and metastasis.[7] Galectin-2 was found to be associated with increased risk for myocardial infarction.[8] Strong expression of galectin-4 is induced with a proceeded malignancy of breast and liver cancer,[9] and galectin-8 has been reported on its relevance to prostate and lung cancer.[10] Therefore, highly sensitive and selective detection of each galectin would be desirable in clinical and biomedical area.

In recent years various detection methods of galectins have been developed,[5c,11] for example, enzyme-linked immunosorbent assay (ELISA),[12] western-blotting, fluorescence measurements,[13,14] isothermal titration microcalorimetry,[15] and surface plasmon resonance (SPR) measurement.[16] We focused on the SPR measurement to evaluate galectin–carbohydrate interactions. The advantages of the SPR method are high sensitivity, the elimination of the need for labeling of the analytes, a larger reduction of measurement time, and a real-time monitoring.

Non-specific adsorption of biological species such as protein on solid surfaces is a ubiquitous problem in the measurement. Recently we found that, in the specific interaction of concanavalin A with maltosyl-dodecanethiolate on gold surface, triethylene glycol (TEG)-terminated short-alkane (C6, C8)-thiols effectively suppress the non-specific adsorption of various proteins.[17]

In a preceding paper we have reported a highly sensitive detection (nanomolar level) of galectin-4 and -8 with lactoside-protuberant hybrid monolayer surface.[18] In the present report, to examine the carbohydrate specificity of each galectin, we designed and prepared four kinds of 12-thiododecyl disaccharides composed of a terminal β-galactoside and a spacer sugar (Fig. 1) including non-natural analogues. After construction of their self-assembled monolayers mixed with TEG-hexanethiol on gold chips, their interactions with six members of galectins (-1, -2, -3, -4, -7, and -8) were investigated by the SPR method.

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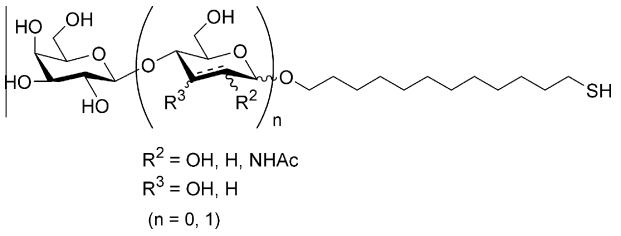


Figure 1. β-Galactoside-terminated dodecanethiols.

Glycosylation of alcohols (O-glycosylation) is an essential process for the synthesis of oligosaccharides and glycoconjugates. We have recently reported an efficient synthesis of mercaptoalkyl 1,2-trans-glycosides from sugar peracetates using $ZnCl_2$ as a promoter of the glycosylation.[19] According to this approach, 12-mercaptododecyl β-galactoside **6** and β-lactoside **7** were readily prepared as shown in Scheme 1. Prior to the glycosylation, 12-acetylthio-1-dodecanol **3** was prepared from 12-bromododecanol with potassium thioacetate in DMF in nearly quantitative yield. Treatment of **3** with β-galactose pentaacetate **1** or β-lactose octaacetate **2** in the presence of $ZnCl_2$ in toluene afforded the corresponding β-glycosides **4** or **5**. Deacetylation of **4** and **5** with sodium methoxide in MeOH afforded the thiol-lipids **6** and **7**, respectively.

For the syntheses of other 12-thiododecyl glycosides containing a terminal β-galactosyl group, hexa-*O*-acetyl-lactal was employed as a key intermediate. D-Lactose **8** was conveniently converted to hexa-*O*-acetyl-lactal **10** in three steps according to a literature procedure[20] with slight modification as shown in Scheme 2. Thus, lactose **8** was treated with acetic anhydride in the presence of a catalytic $Cu(OTf)_2$ (solvent-free per-acetylation)[21] to give the octaacetate **2α** (α/β = 9), which was treated in the same flask with HBr (25% in AcOH) to give the anomeric bromide **9**. The reaction was quenched by the addition of sodium acetate, and the mixture was poured into a suspension of zinc in AcOH in the presence of $CuSO_4$ to afford the lactal **10** in high overall yield.

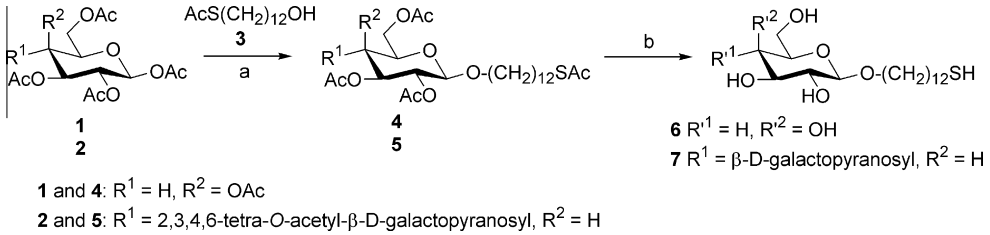
With the lactal in hand, we examined the coupling with acetylthiododecanol **3**. In the acid-catalyzed reactions of O-protected glycals with alcohols, two major reaction pathways are possible: addition of the alcohol to the double bond to form 2-deoxy-O-glycosides[22] and S_N2'-type reaction to form 2,3-dideoxy- and unsaturated glycosides (Ferrier reaction).[23] When *O*-acetyl-protected glycals are employed with a variety of acidic catalysts,[24] 2,3-unsaturated glycosides are generally predominated owing to the presence of reactive allylic acetate group. Indeed, reaction of the lactal **10** and dodecanol **3** in the presence of $BF_3{\cdot}OEt_2$ or I_2[25] gave acetylthiododecyl 2-deoxy-α-lactoside **11** (α/β = 6) in high yield with no 2-deoxy-lactoside (The Table in Scheme 2).

We then explored a direct preparation of 2-deoxy-lactoside from the lactal **10**. Falck reported a selective synthesis of 2-deoxy-glucopyranosides from tri-*O*-acetyl-glucal using triphenylphophine hydrobromide (TPHB) as a catalyst.[26] We tried the TPHB-catalyzed reaction and obtained 2-deoxy-α-lactoside **12α** as a major product in 55% yield. Separable 2-deoxy-β-lactoside **12β** and unsaturated **11** were also obtained in 8% and 20% yield, respectively. Attempts to suppress the formation of **11** were unsuccessful. Deacetylation of **11** and **12α** with sodium methoxide in MeOH afforded the thiol-lipids **13** and **14**, respectively. It is noteworthy that the glycolipids **13** and **14** were more soluble in aqueous EtOH than the lactoside **7**.

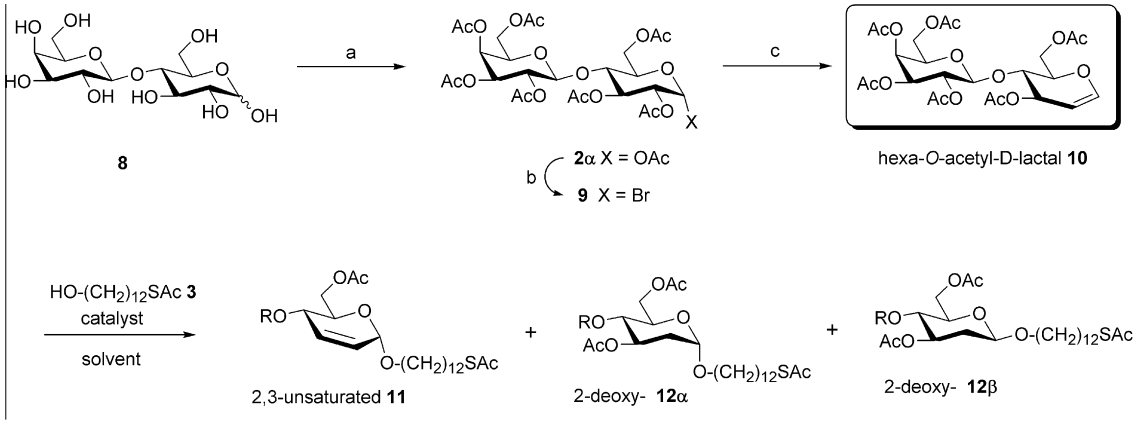
We turned our attention to the synthesis of lactosamine derivatives because galectins strongly bind to *N*-acetyllactosamine.[1] Such compounds would be prepared[27] from the lactal via azido-nitration developed by Lemieux et al.[28] As shown in Scheme 3, lactal **10** was treated with cerium(IV) ammonium nitrate and sodium azide in acetonitrile to give the 2-azide-1-O-nitrates as a stereoisomeric mixture: α-*gluco*-**15α** (14% yield), and less polar β-*gluco* **15β** and α-*manno* **16** isomers (60% combined yield). Since the latter two isomers were inseparable, the mixture of the azido-nitrates was treated with LiBr in CH_3CN[29] to give the α-*gluco*-bromide **17** and α-*manno*-bromide **18**. Although these bromides were separable by chromatography, partial decomposition was observed. Thus the mixture of the bromides after extractive work-up was treated with acetylthiododecanol **3** and silver perchlorate as a promoter to give the coupling products. The products were separated by silica-gel chromatography to give the α-*gluco*-isomer **19** and the α-*manno*-isomer **20** in 42% and 28% yields, respectively. The azide group in **19** was reduced with zinc in acetic acid and acetic anhydride to give the acetamide **21** in high yield. Finally deacetylation with sodium methoxide in MeOH afforded the lactosamine-type thiol-lipid **22**.

To understand the functions of galectins and search for their natural ligands, it is necessary to know their carbohydrate specificity. All members of the galectin family can bind to β-galactoside terminated sugar chains, but their oligosaccharide specificities seem to be different.[1c,30] We designed and synthesized several mercaptododecyl glycosides as described above. The galactoside **6**, lactoside (Lac) **7**, and *N*-acetyllactosaminide (LacNAc) **22** are natural carbohydrates, while 2-deoxy-lactoside (Deoxy) **14** and 2,3-unsaturated glycoside (En) **13** are non-natural disaccharides. Binding inhibition studies have suggested[31] that 4-OH and 6-OH of the galactopyranosyl ring and 3-OH of the glucopyranoside ring in lactose and lactosaminoglycans are primarily responsible for interactions with galectin-1 and -3. The X-ray crystal structures of Gal-1[32a] and -3[32b] in complex with lactose or *N*-acetyllactosamine have confirmed the above interactions via hydrogen bonds with the specific amino acid residues in the carbohydrate binding pocket of galectins. Deoxygenation of the hydroxyl group(s) would affect directly to the binding.

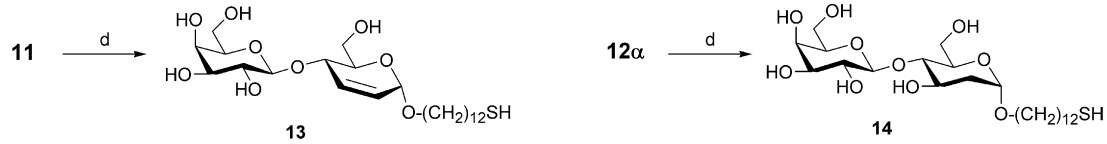
With the glycolipids in hand, we constructed their self-assembled monolayers (SAM) mixed with TEG-hexanethiol on gold chips,[33] and investigated their binding activities with human galectins (Gal-1, -2, -3, -4, -7, and -8)[34] by the SPR method using a BIAcore T100 instrument. The galectin (0.5 or 1.0 μM in HBS-EP[35]) was injected simultaneously to the mixed SAM and the reference monolayer, the latter of which was formed by applying 10 μM TEG-C6SH solution. In our preceding report,[18] the maximum adsorption of Gal-4 was observed for the 4% Lac **7** in TEG-C6SH,



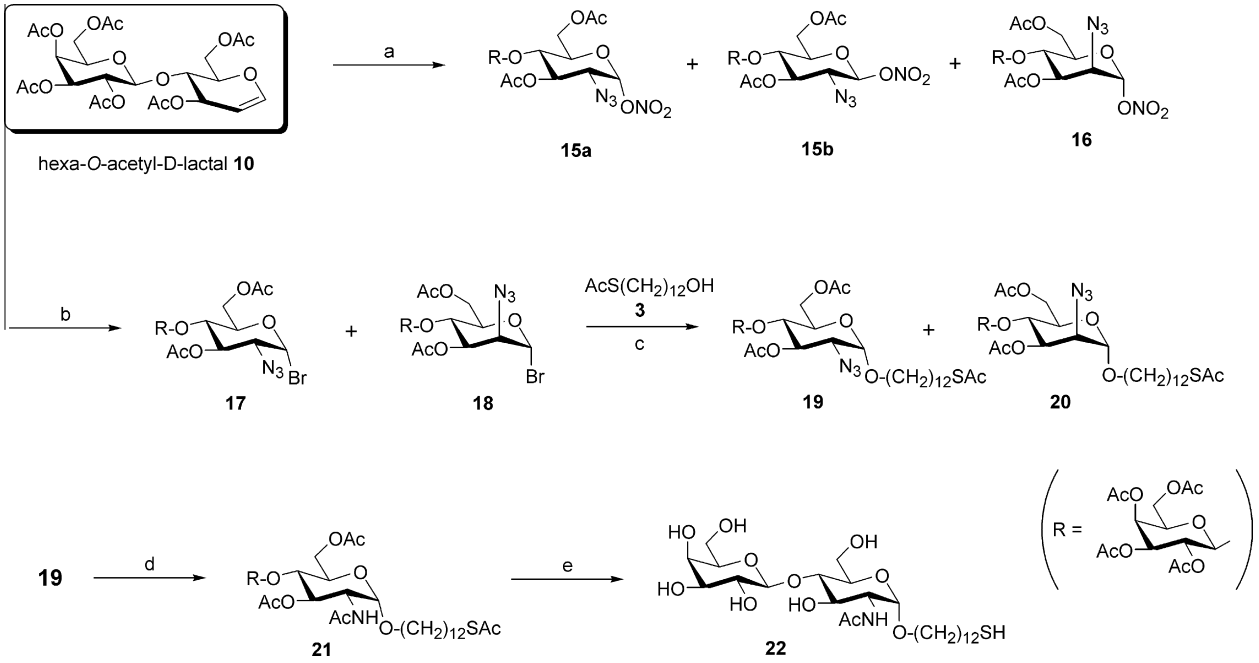
Scheme 1. Reagents and conditions: (a) $ZnCl_2$, toluene, 55–65 °C, 1 h, 52–65%; (b) NaOMe, MeOH, CH_2Cl_2, 5 °C, 75–82%.



catalyst (equiv.)	solvent	conditions	yield (%) 11	12α	12β
$BF_3 \cdot OEt_2$ (0.2)	CH_2Cl_2	5 to 10 °C, 3 h	91	0	0
I_2 (0.15)	CH_2Cl_2	5 °C, 2 h	93	0	0
$HBr \cdot PPh_3$ (0.2)	CH_2Cl_2	r.t., 20 h	20	55	8
$HBr \cdot PPh_3$ (0.1)	toluene	r.t., 20 h	14	52	23



Scheme 2. Reagents and conditions: (a) Ac_2O, $Cu(OTf)_2$, rt, 12 h; (b) HBr, AcOH, 5 °C; (c) Zn, $CuSO_4$, AcOH, NaOAc, H_2O, 5 °C, 90% from **8**; (d) NaOMe, MeOH, CH_2Cl_2, 5 °C, 68–77%.



Scheme 3. Reagents and conditions: (a) $Ce(NH_4)_2(NO_3)_6$, NaN_3, CH_3CN, −20 °C, 10 h, 74%; (b) LiBr, CH_3CN, rt, 10 h; (c) $AgClO_4$, MS4A, CH_2Cl_2, rt, 2 h, 70%; (d) Zn, AcOH, Ac_2O, THF, 5 °C, 2 h, 82%; (e) NaOMe, MeOH, CH_2Cl_2, 5 °C, 72%.

whereas the response was very low with 100% Lac **7**. Thus the binding activities were measured using the mixed SAM containing 4–6% glycolipid.[36]

Figure 2 shows the SPR sensorgrams of galectin binding to the LacNAc **22** presenting surface. The 'Diff. Response (RU)' curve was obtained after subtraction of the reference curve (100%

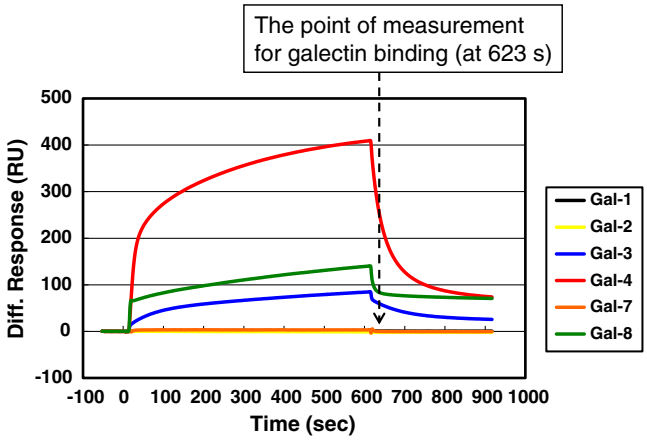


Figure 2. Differential SPR sensorgrams of galectin (Gal-1, -2, -3, -4, -7 and -8; 0.2 μM) binding to the LacNAc **22** presenting surface. (The sensorgrams of Gal-1, -2, -7 have overlapped.)

TEG-C6SH). Specific binding to LacNAc **22** was observed for Gal-3, Gal-4, and Gal-8. We defined the point for determining the amount of bound galectin to be 23 s after the change to dissociation phase (arrow in Fig. 2). The amounts of bound Gal-4, -8, and -3 were 274 RU, 85.5 RU, and 61.2 RU, respectively. Other galectins (Gal-1, -2, -7) showed little or no differential response.

The binding studies were carried out with each glycolipid, and the bound amounts of the galectins are summarized in Table 1. The galactoside **6** showed much lower affinities to most galectins than the Lac **7**. The presence of the glucose residue in lactose would be advantageous for binding to the CRD pocket by formation of multiple hydrogen bondings. Gal-3, -4, and -8 bound to the Lac **7** and LacNAc **22** surfaces in much larger amounts than other galectins (Gal-1, -2, -7) did. Especially, the bound amount of Gal-4 to **7** was five times larger than that of Gal-3, and that of Gal-4 to **22** was three times larger than that of Gal-3 or Gal-8. The results also indicate the feasibility of the Lac **7** and LacNAc **22** surfaces for the specific and sensitive detection of Gal-4. Gal-3, -4, and -8 showed good affinities to the Deoxy **14**, but they showed negligible specific binding to the En **13**. The result suggests that the 3-OH in the adjacent sugar would be significant for the galectin recognition, being consistent with the previous reports.[30]

In the precedent paper,[18] we fitted the SPR response curves to the binding models by using the BIAcore T100 evaluation software to estimate the most appropriate binding model for Gal-3, -4 and -8 with the lactoside **7**. It was indicated that the Gal-3 curve fitted to the simple 1:1 binding model, whereas the Gal-4 and -8 fitted to the heterogeneous ligand model, which has two or more binding sites with different affinities. Herein we fitted the SPR response curves of Gal-3, -4, -8 with the Deoxy **14** and LacNAc **22** to the binding models by using the evaluation software. The results were similar; the Gal-3 curves fitted to the 1:1 binding model, and the Gal-4 and -8 curves fitted to the heterogeneous ligand model. These results as well as the binding activities shown in Table 1 may be correlated to the structures of the galectins: Gal-1, -2, and -7 are simple proto-type dimers; Gal-3 is chimera-type having a single CRD; Gal-4 and -8 are tandem-repeat type having two heterogeneous CRDs.

Table 1
The bound amounts of galectins to the glycolipid surfaces[a]

Glycolipid	Gal-1	Gal-2	Gal-3	Gal-4	Gal-7	Gal-8
6	3.4	3.8	Nb[b]	Nsb[c]	Nb[b]	34.1
7 (Lac)	9.5	2.0	82.3	457.0	4.2	368.2
13 (En)	25.1	13.4	5.1	Nsb[c]	Nb[b]	Nsb[c]
14 (Deoxy)	17.8	11.0	135.1	345.0	Nb[b]	74.5
22 (LacNAc)	4.1	Nb[b]	166.9	462.3	0.8	163.3

[a] The values are the RUs (resonance units) of galectin binding to the carbohydrates. Concentration of each galectin used for the binding experiment was 1 μM, except for LacNAc **22** experiment (0.5 μM).
[b] Nb: No binding detected in both the carbohydrate and the reference surface.
[c] Nsb: Non-specific binding to the reference surface was too large to detect specific galectin binding to the carbohydrate.

Table 2
Dissociation constants (K_D) of galectins for interaction with the glycolipid surfaces[a]

Binding model	Gal-3 1:1 Binding	Gal-4 Heterogeneous ligand		Gal-8 Heterogeneous ligand	
		(1)	(2)	(1)	(2)
7 (Lac)	1.06	0.14	0.085	0.0016	0.025
14 (Deoxy)	1.43	0.30	0.058	0.037	0.031
22 (LacNAc)	2.20	1.26	0.78	0.00023	0.049

[a] $K_D = k_d/k_a$ (μM).

The kinetic parameters [association rate constant (k_a (1/M s)), dissociation rate constant (k_d (1/s)), and dissociation constant (K_D (M))] were calculated from the best-fitted model curves, which were obtained from the measurements at lower concentrations (1–1000 nM) of the galectins (see Supplementary Tables S1–S3). The dissociation constants (K_D) are shown in Table 2, in which interactions of Gal-4 (-8) with the glycosides consist of two components: (1) and (2). The component (1) is in slow association and slow dissociation, and the other (2) is in faster association and dissociation. In the complexes of Gal-8, the slow component (1) appears to contribute largely to the stability. These kinetic data indicate the order of the affinities to the glycoside surfaces: Gal-8 > -4 > -3.

In summary, 12-mercaptododecyl β-D-galactoside and four kinds of mercaptododecyl disaccharides composed of a terminal β-galactoside and a spacer sugar were prepared. Through construction of their self-assembled monolayers mixed with TEG-C6SH on gold chips, their binding activities with the galectins, promising tumor markers, were evaluated by the SPR method. High binding responses were observed for the lactoside, 2-deoxy-lactoside, and *N*-acetyllactosaminide with Gal-3, -4, and -8. These findings would lead to the rational design of novel potent ligands with selectivity for the galectin species toward cancer diagnosis.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.049.

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33. The galactoside presenting monolayers were formed by applying the glycolipid solution (1 μM, in phosphate buffered saline (PBS; pH 7.2) added with 20% ethanol) to the bare gold surface of the sensor chip (BIAcore SIA Kit Au, GE Healthcare) for a brief time (20 s at 10 μL/min × 1–3 times) and following with the TEG-C6SH solution (10 μM, in PBS added with 20% ethanol) for 3600 s at 1 μL/min. The amounts of the glycolipid adsorbed on Au were controlled by changing the application time. The chip surface was washed sequentially with PBS and HBS-EP[35] before the galectin binding measurement.
34. Human galectins (Gal-1, -2, -3, -4, -7 and -8) were obtained from R&D Systems, Inc. (Minneapolis, USA).
35. HBS-EP: 10 mM HEPES buffer, 0.15 mM NaCl, 3 mM EDTA, 0.05% Tween 20, pH 7.4. HBS-EP was used as the running buffer for all SPR measurements.
36. The glycolipid solution was applied repeatedly (1–3 times) until the total amount of the adsorbed thiol reached to around 100 Resonance Unit (1 RU = 1 pg/mm^2) by monitoring the SPR angle shift change. By this procedure, a mixed monolayer containing 4–6% carbohydrate ligand filled with TEG-C6SH was obtained.