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A Highly Practical Synthesis of Cyclodextrin-Based Glycoclusters Having Enhanced Affinity with Lectins

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Abstract—A simple and highly practical method for the synthesis of cyclodextrin-scaffolded glycoclusters recognized specifically by lectins is described. Nucleophilic displacement of iodide from heptakis 6-deoxy-6-iodo-β-cyclodextrin by unprotected sodium thiolates derived from 3-(3-thioacetyl propionamido)propyl glycosides proceeded smoothly in mild condition and gave novel per-glycosylated cyclodextrins (glycocyclodextrins, glycoCDs) having D-galactose, *N*-acetyl-D-glucosamine, lactose or *N*-acetyllactosamine residues in high yields (78–88%). It was demonstrated that all these per-glycosylated β-cyclodextrins showed amplified inhibitory effects on the erythrocytes agglutination induced by wheat germ (*Triticum vulgaris*) agglutinin (WGA) or *Erythrina corallodendron* lectin (ECorL).

Introduction

Carbohydrates existing on cell surface play important roles in numerous intercellular recognition processes. Interactions between carbohydrates and carbohydrate-binding proteins are crucial steps of the specific biological processes such as cell differentiation, immune response, infection, and cancer metastasis.[1] Although a variety of oligosaccharides as partial structures of glycolipids, glycoproteins, and proteoglycans serve significant biological signals and they can be used as simple inhibitors for testing the glucoconjugates functions, it has been reported that low affinity and broad specificity are often observed in the interactions of these oligosaccharide-based ligands with lectins (carbohydrate binding proteins, CBP).[2] Therefore, it might be indispensable to investigate how such weak and vague interactions can be amplified or controlled in the successful processes for the sugar-recognition and the signal transduction.

The glycoside cluster effect proposed by Lee[3] has attracted considerable attention and promoted extensive efforts by synthetic chemists to design a variety of multivalent glycoligands.[4,5] Cyclodextrin-based glycoclusters[6–13] as well as polymers,[14–17] dendrimers,[18–30] calix[4]allenes,[31–33] and peptides[34–38] are useful tools and reagents to investigate the significance of the multivalency in the carbohydrate–protein interactions. Moreover, macrocyclic carbohydrate-clusters are strongly expected to be specific molecular transporters to the carbohydrate binding proteins existing on the cell surfaces.[32] Since it was suggested that per-glycosylated cyclodextrin (glycoCD) showed much improved ability to form stable complex with an antitumor reagent comparing with the cyclodextrin itself,[39] glycoCD is one of the most potent candidates of specific carriers for the drug delivery systems (DDS) on the basis of cyclodextrin-derived materials.[40] GlycoCDs having oligosaccharides recognized by tissue-specific CBP would be used for the development of a novel class of tailor-made DDS in the medicinal field. Thus, our attention has been focused on the synthesis of cyclodextrin-scaffolded glycoclusters by means of the simple and versatile per-glycosylation strategy.

In the present paper, we describe detailed experimental procedures and results for the synthesis of 6-fully substituted glycoCDs having *N*-acetyl-D-glucosamine, galactose, lactose, and *N*-acetyllactosamine residues. Biological activities of these glycoCDs will also be discussed on the basis of the inhibitory effect on the hemagglutination by wheat germ (*Triticum vulgaris*) agglutinin (WGA) and *Erythrina corallodendron* lectin (ECorL).

Keywords: cluster effect; glycocluster; cyclodextrin; glycocyclodextrin (glycoCD); wheat germ agglutinin (WGA); *Erythrina corallodendron* lectin (ECorL).

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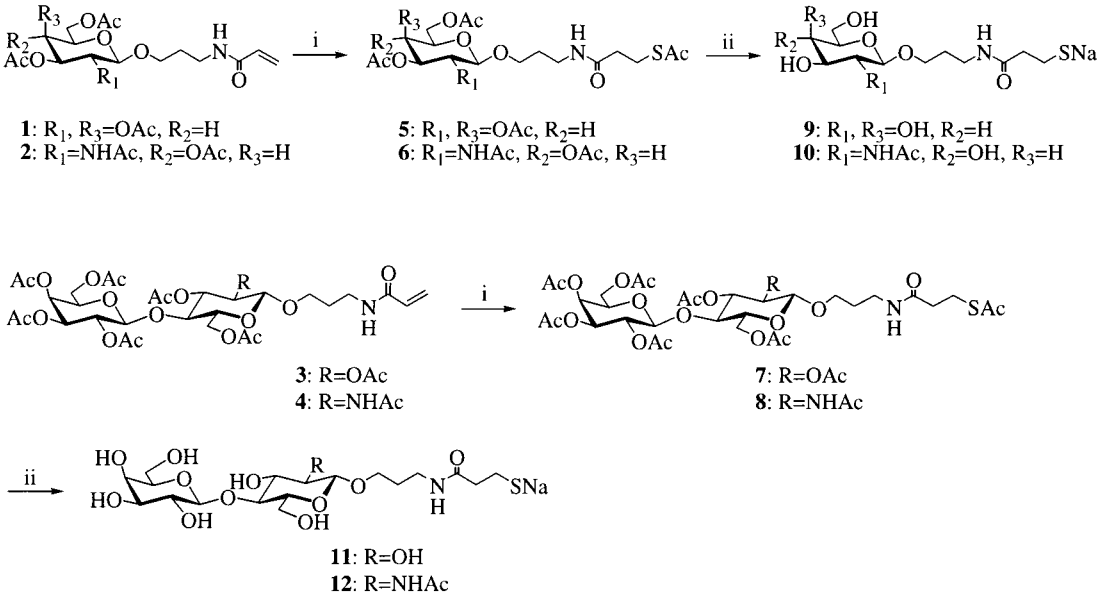
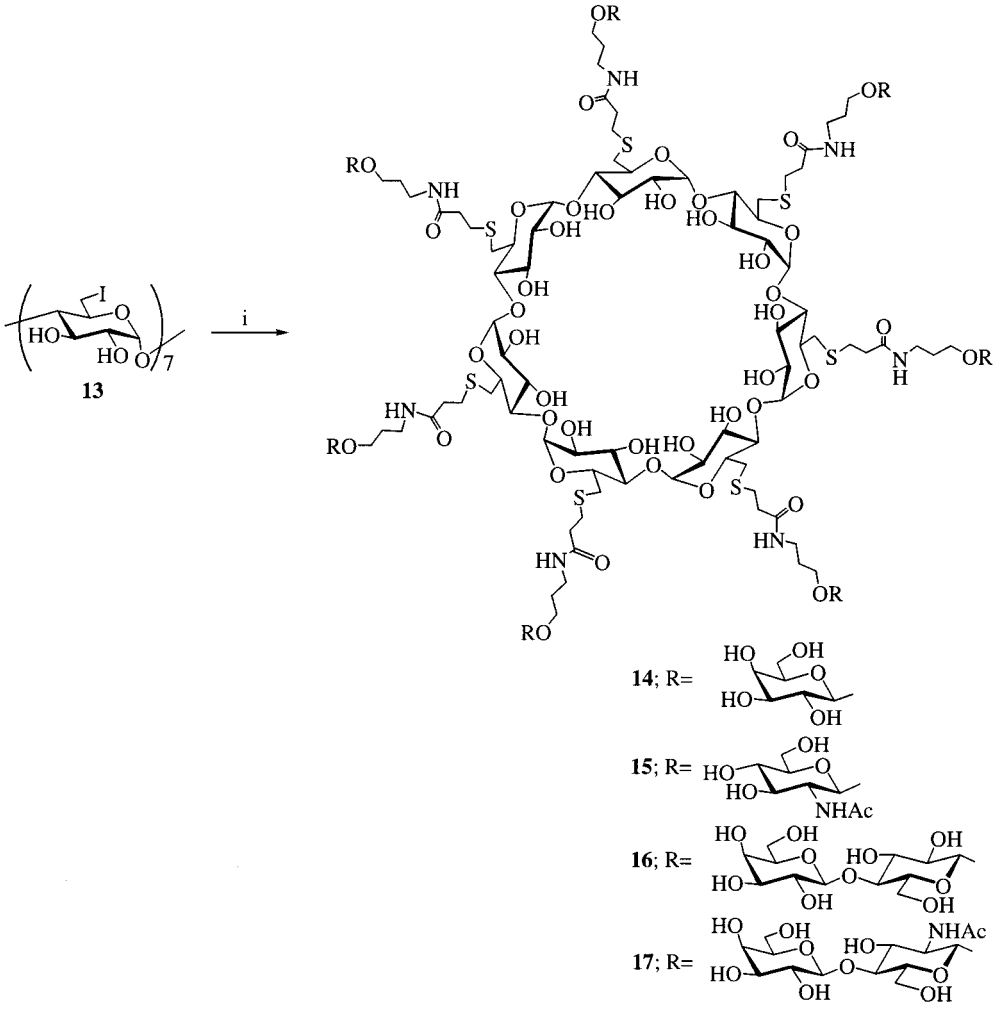


Scheme 1. *Reagents and conditions:* (i) AcSH, Et_3N/EtOAc; (ii) NaOMe/MeOH.

Results and Discussion

Scheme 1 indicates synthetic routes of some glycosides and their derivatives used for the coupling reactions with cyclodextrins. Here, we selected readily available ω-acrylamidoalkyl glycosides[41,42] as convenient precursors, because this type of materials can be easily converted into the reactive glycosides with an appropriate linker moiety by employing



Scheme 2. *Reagents and conditions:* (i) **9**–**12**/ DMF, 70°C, 24 h.

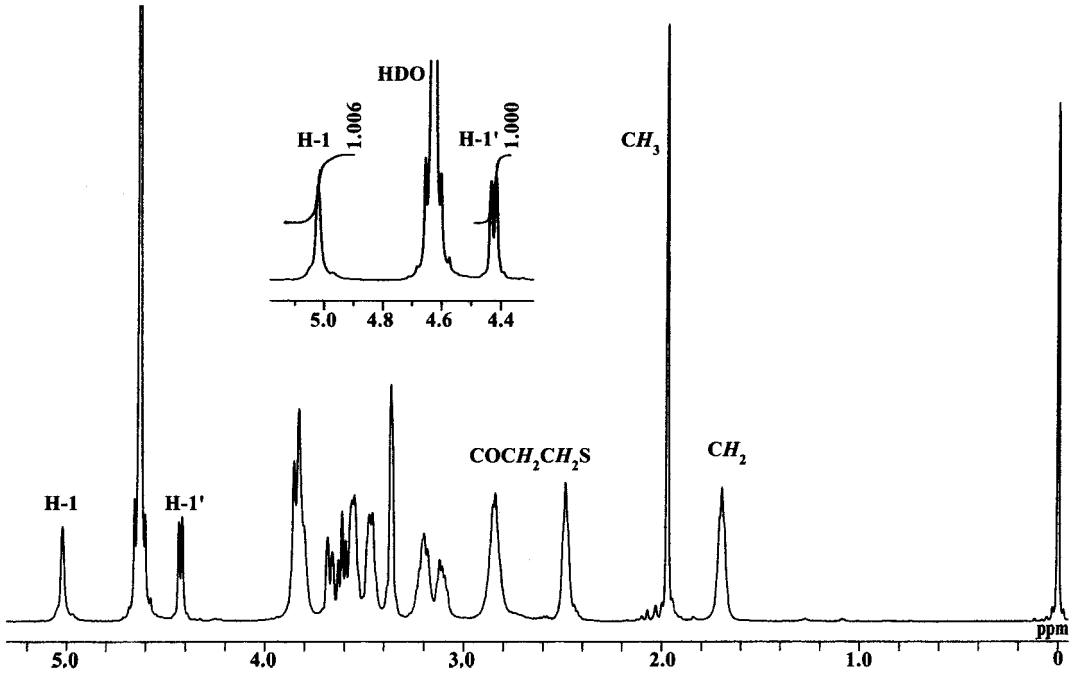


Figure 1. [1]H-NMR spectrum of GlcNAc-CD ligand **15**.

simple Michael addition reactions at the terminal double bond. Glycosides **1**–**4** were employed for the reaction with thioacetic acid to afford 3-(3-thioacetyl propionamido)-propyl glycosides **5**–**8**. After de-*O*,*S*-acetylation, unprotected glycosides having a terminal sodium thiolate **9**–**12** were allowed to react with per-6-iodo-β-CD **13**[43] in DMF at 70°C for 24 h under a nitrogen atmosphere (Scheme 2). The reaction mixture was subjected directly to the size exclusion chromatography on Sephadex G-25 and eluted with H_2O to give the corresponding glycoCDs **14**–**17** in high yields.

All new compounds synthesized here were fully characterized by ^1H and ^{13}C NMR spectroscopy in addition to elemental analyses and mass spectroscopy. For example, Fig. 1 shows fully assigned ^1H NMR spectrum of compound **15**. The signals due to the anomeric protons of D-glucose residues (β-CD) and *N*-acetyl-D-glucosamine residues were observed at δ 5.02 and 4.42 ppm, respectively. It was clearly suggested from the integration data of this spectrum that nucleophilic displacement of iodide from **13** by sodium thiolate **10** proceeded smoothly and provided per-glycosylated CD **14**. Moreover, the ^{13}C NMR spectrum of compound **17** (Fig. 2) exhibits three significant signals at 104.01 (C-1, D-glucose residue), 103.24 (C-1′, *N*-acetyl-D-glucosamine residue), and 105.15 (C-1″, D-galactose residue) due to the anomeric carbons. High-field chemical shift at 35.30 ppm

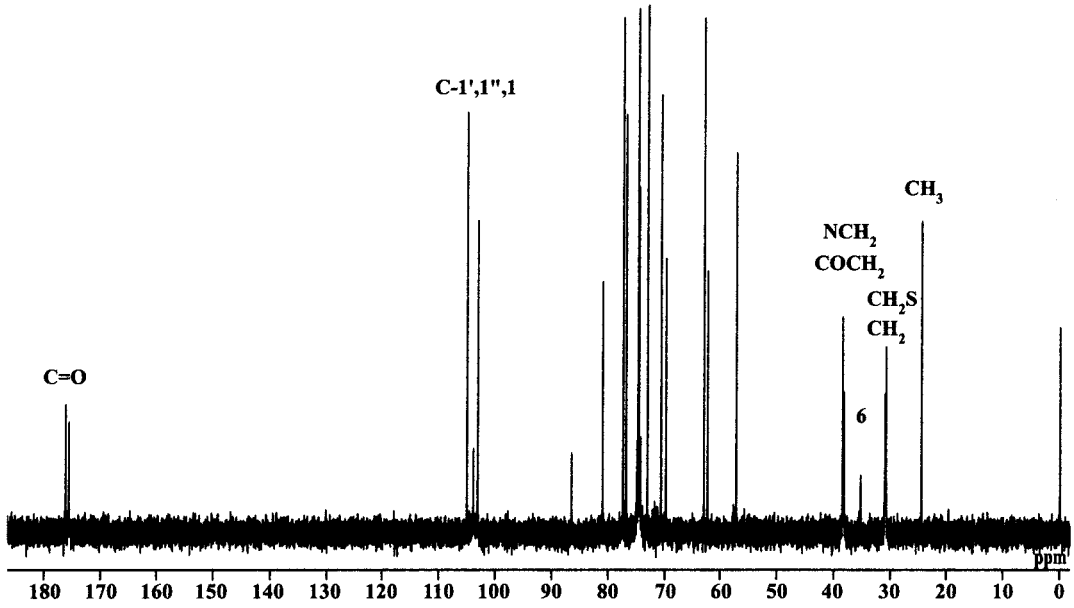


Figure 2. ^{13}C-NMR spectrum of LacNAc-CD ligand **17**.

Table 1. Inhibitory effects on the hemagglutination of WGA ([Lectin]; WGA (50 μg/mL)) by glycoCD **15** and the corresponding sugars (Buffer; 50 mM PBS (pH 7.2))

Compound	MIC (mM)[a]	Relative potency
N-Acetyl-glucosamine	50	1
GlcNAc-CD (**15**)	0.178	280 (40)[b]
β-CD	>10	

[a] MIC: Minimum Inhibitory Concentration
[b] Value in parentheses are on a per-hapten basis.

Table 2. Inhibitory effects on the hemagglutination of ECorL ([Lectin]; ECorL (100 μg/mL)) by glycoCDs **14**, **16**, **17** and their corresponding sugars (Buffer; 50 mM PBS (pH 7.2))

Compound	MIC (mM)[a]	Relative potency
Galactose	10	1
Gal-CD (**14**)	0.714	14 (2)[b]
Lactose	5	1
Lac-CD (**16**)	0.357	14 (2)
N-Acetyllactosamine	2.5	1
LacNAc-CD (**17**)	0.0892	28 (4)
β-CD	>10	

[a] MIC: Minimum Inhibitory Concentration
[b] Value in parentheses are on a per-hapten basis.

due to C-6 positions of CD suggests the presence of the thio-ether bonds. All these signals of the ^{13}C NMR spectra were observed as sharp and simple singlets, representing the seven-fold symmetric branched structures. The satisfactory results of the Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) mass spectra and elemental analyses also supported homogeneity of the products. The molecular masses of glycoCDs **14**–**17** were detected as the peaks of the desired molecular ions in agreement with their $[M+H]^+$ or $[M+Na]^+$ adducts, respectively.

In order to elucidate the effect of the clustering on the biological activity of glycoCDs, the inhibitory activities by compounds **14**–**17** on the hemagglutination were examined by means of wheat germ (*Triticum vulgaris*) agglutinin (WGA)[34–47] and *Erythrina corallodendron* lectin (ECorL)[48–50] which are known as *N*-acetyl-D-glucosamine- and *N*-acetyllactosamine-specific lectins. The results are summarized in Tables 1 and 2 in comparison with those by the corresponding simple mono- and disaccharides. As anticipated, compound **15** showed drastically enhanced affinity against WGA. The minimum inhibitory concentration of compound **15** was 40-fold higher than that of *N*-acetyl-D-glucosamine used as a control. This result suggests that cyclodextrin-based glycoclusters exhibit almost the same inhibitory activity with those found in the cases of macromolecular ligands such as glycol-chitin and glycopolymers having *N*-acetyl-D-glucosamine branches.[41] Similarly, the inhibitory effect of glycoCDs **14**, **16**, **17** on the hemagglutination induced by ECorL was also examined. Compound **17** bearing *N*-acetyllactosamine residues showed the highest potency (MIC=0.0892 mM) among the glycoCDs used in this experiment.

In conclusion, we established a simple and versatile method for the synthesis of per-glycosylated cyclodextrins as novel class of multivalent glycoligands. It should be noted that high reactivity of unprotected sodium thiolates derived from simple ω-acrylamido glycosides permitted high efficiency in the nucleophilic substitution reactions of the functional glycosides with heptakis 6-deoxy-6-iodo-β-cyclodextrin to afford a variety of per-glycosylated cyclodextrins in high yields. It was also demonstrated that per-glycosylated cyclodextrins showed amplified affinity with lectins dependent on the sugar-specificity introduced at C-6 positions. Further enzymic modification studies toward the synthesis of highly specific and convenient glycoclusters such as dendritic glycoCDs and star shape glycoCDs with complicated carbohydrate structures are now under investigation and the results will be reported elsewhere.

Experimental

General

Unless otherwise stated, all commercially available solvents and reagents were used without further purification. Optical rotations were determined with a JASCO P-1010 polarimeter at 25°C and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. ^1H and proton decoupled ^{13}C NMR spectra were recorded at 500 and 125.65 MHz, respectively, on a JEOL ALPHA-500 spectrometer in chloroform-*d* or deuterium oxide and *J*-value are in Hz. Ring-proton assignments in NMR were made by first-order analysis of the spectra and supported by HH-COSY experiments. Reactions were monitored by thin-layer chromatography (t.l.c.) on a precoated plate of silica gel $60F_{254}$ (layer thickness, 0.25 mm; E. Merck, Darmstadt, Germany). Column chromatography was performed on silica gel (Wakogel C-200; 100–200 mesh, Wako Pure Chemical Industries Co. Ltd., Japan). WGA and ECorL were purchased from Sigma Chemical Co.. Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry was carried out on a Finnigan LASEMAT 2000 instrumental using 2,5-dihydroxy benzoic acid. The instrument was operated in the positive ion reflectron mode with an accelerating potential of 20 kV. Solvent extracts were dried with anhydrous magnesium sulfate and concentrated below 40°C under diminished pressure.

3-(3-Thioacetyl propionamido)propyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (5). To a solution of **1** (1.7 g, 3.70 mmol) in EtOAc (20 mL) were added AcSH (0.79 mL, 11.1 mmol) and Et_3N (0.2 mL). The reaction mixture was stirred for 2 h at room temperature under nitrogen atmosphere and concentrated. The residue was dissolved in $CHCl_3$, washed with sat. $NaHCO_3$ aq. and brine, dried and evaporated. The resulting syrup obtained was chromatographed on silica gel with chloroform: methanol (60:1, v/v) as the eluant to afford **5** (1.9 g, 96%) as syrup foam: $[\alpha]_D=-24.4°$ (*c* 0.21, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.02 (brt, 1H, NH), 5.42 (dd, 1H, $J_{4,5}$=1.2 Hz, H-4), 5.19 (dd, 1H, $J_{2,3}$=10.3 Hz, H-2), 5.04 (dd, 1H, $J_{3,4}$=3.7 Hz, H-3), 4.44 (d, 1H, $J_{1,2}$=7.9 Hz, H-1), 4.19 (dd, 1H, $J_{6a,6b}$=11.5 Hz, H-6a), 4.13 (dd, 1H, $J_{5,6b}$=6.7 Hz, H-6b), 3.91 (ddd, 1H, $J_{5,6a}$=6.7 Hz, H-5), 3.99, 3.58, 3.43, 3.30 (4m, 4 H, CH_2), 3.15 (t, 2H, CH_2S), 2.52 (m, 2H, COCH_2), 2.33 (s,

3H, SCOCH_3), 2.17, 2.07, 2.05, 1.99 (all s, 12H, 4OCOCH_3) and 1.79 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$): δ 195.95 (–S*C*O–), 170.73, 170.37, 170.21, 170.06, 169.91(–*C*O–×5), 101.41(C-1), 70.86, 70.83, 70.65, 69.07, 68.95, 67.03, 61.26, 37.57, 35.97, 29.24, 25.00(–SCO*C*H_3), 20.81, 20.67, 20.67 and 20.57(–*C*H_3×4); Anal. Calcd for $C_{22}H_{33}NO_{12}S\cdot0.5CH_3OH$: C, 48.99; H, 6.40; N, 2.54; S, 5.81. Found: C, 49.25; H, 6.05; N, 2.25; S, 5.76.

3-(3-Thioacetyl propionamido)propyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (6). To a solution of **2** (1.0 g, 2.18 mmol) in EtOAc (10 mL) were added AcSH (0.47 mL, 6.54 mmol) and Et_3N (0.1 mL). The mixture was worked up as the procedure for the synthesis of **5** and the residual oil was purified by chromatography on silica gel with chloroform: methanol (30:1, v/v) as the eluant, and crystalline **6** (950 mg, 83%) was obtained from a solution of MeOH–Et_2O: $[\alpha]_D=-20.4°$ (*c* 0.21, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.49 [d, 1H, *J*=8.5 Hz, NH (GlcNAc)], 6.23 (brt, 1H, NH), 5.16 (t, 1H, $J_{3,4}$=9.1 Hz, H-3), 5.10 (t, 1H, $J_{4,5}$=9.7 Hz, H-4), 4.51 (d, 1H, $J_{1,2}$=8.5 Hz, H-1), 4.27 (dd, 1H, $J_{6a,6b}$=12.8 Hz, H-6a), 4.14 (dd, 1H, $J_{5,6b}$=3.0 Hz, H-6b), 4.04 (t, 1H, $J_{2,3}$=10.5 Hz, H-2), 3.99, 3.69, 3.64, (3m, 3H, CH_2), 3.45 (ddd, 1H, $J_{5,6a}$=4.2 Hz, H-5), 3.15 (t, 2H, CH_2S), 3.09 (m, 1H, CH_2), 2.53 (m, 2H, COCH_2), 2.34 (s, 3H, SCOCH_3), 2.08, 2.04, 2.02, 1.95 (all s, 12H, 4OCOCH_3), 1.80 and 1.66 (2m, 2H, CH_2); ^{13}C NMR ($CDCl_3$): δ 196.06 (–S*C*O–), 171.12, 170.96, 170.84, 170.73, 169.36 (–*C*O–×5), 101.55 (C-1), 73.05, 71.97, 68.58, 67.71, 62.12, 54.26, 36.46, 36.13, 29.41, 25.14 (–SCO*C*H_3), 23.25, 20.77, 20.72 and 20.63 (–*C*H_3×4); Anal. Calcd for $C_{22}H_{34}N_2O_{11}S$: C, 49.43; H, 6.41; N, 5.24; S, 6.00. Found: C, 49.29; H, 6.38; N, 5.13; S, 5.94.

3-(3-Thioacetyl propionamido)propyl 4-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (7). To a solution of **3** (1.60 g, 2.14 mmol) in EtOAc (20 mL) were added AcSH (0.46 mL, 6.42 mmol) and Et_3N (0.2 mL). The same manner as described for the synthesis of **5** was carried out, and the residual oil was chromatographed on silica gel with chloroform: methanol (70:1, v/v) as the eluant, and the fractions containing the product were recrystallized from MeOH–Et_2O to afford **7** (1.48 g, 84%): $[\alpha]_D=-27.0°$ (*c* 0.20, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.05 (brt, 1H, NH), 5.35 (d, 1H, $J_{4',5'}$<1.0 Hz, H-4′), 5.21 (t, 1H, $J_{3,4}$=9.1 Hz, H-3), 5.11 (dd, 1H, $J_{2',3'}$=9.7 Hz, H-2′), 4.97 (dd, 1H, H-2), 4.88 (dd, 1H, $J_{3',4'}$=3.0 Hz, H-3′), 4.57 (d, 1H, $J_{6a,6b}$=11.6 Hz, H-6b), 4.51 (d, 1H, $J_{1',2'}$=7.9 Hz, H-1′), 4.45 (d, 1H, $J_{1,2}$=7.9 Hz, H-1), 4.18–4.05 (m, 3H, H-6a, 6′b, and 6′a), 3.92–3.86 (m, 2H, H-5′ and C*H*H), 3.80 (t, 1H, H-4), 3.62–3.55 (m, 2H, H-5 and CH*H*), 3.14 (t, 2H, CH_2S), 2.50 (m, 2H, COCH_2), 2.33 (s, 3H, SCOCH_3), 2.16, 2.13, 2.06, 2.05, 2.05, 2.05, 1.97 (all s, 21H, 7OCOCH_3) and 1.77 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$): δ 196.01 (–S*C*O–), 170.74, 170.42, 170.32, 170.12, 170.05, 169.92, 169.65, 169.04 (–*C*O–×8), 101.08, 100.61 (C-1,1′), 76.16, 72.90, 72.53, 71.69, 70.99, 70.75, 69.19, 68.46, 66.64, 61.71, 60.81, 37.21, 35.99, 30.58, 29.13, 25.03 (–SCO*C*H_3), 20.88, 20.79, 20.74, 20.63, 20.61, 20.61 and 20.50 (–*C*H_3×7); Anal. Calcd for $C_{34}H_{49}NO_{20}S\cdot H_2O$: C, 48.51; H, 6.10; N, 1.66; S, 3.81. Found: C, 48.44; H, 5.93; N, 1.63; S, 3.92.

3-(3-Thioacetyl propionamido)propyl 2-acetamido-4-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-3,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (8). To a solution of **4** (910 mg, 1.22 mmol) in EtOAc (10 mL) were added AcSH (0.26 mL, 3.66 mmol) and Et_3N (0.1 mL). The same manner as described for the synthesis of **5** was carried out, and the residual oil was chromatographed on silica gel with chloroform: methanol (30:1, v/v) as the eluent, and the fractions containing product were recrystallized from MeOH–Et_2O to afford **8** (970 mg, 97%): $[\alpha]_D=-17.5°$ (*c* 0.554, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.38 (brt, 1H, NH), 6.21 (brd, 1H, NH), 5.35 (d, 1H, $J_{4',5'}$<1.0 Hz, H-4′), 5.12 (dd, 1H, $J_{2',3'}$=10.2 Hz, H-2′), 5.07 (t, 1H, $J_{2,3}$=10.1 Hz, $J_{3,4}$=9.1 Hz, H-3), 4.88 (dd, 1H, $J_{3',4'}$=2.9 Hz, H-3′), 4.51 (m, 1H, H-6b), 4.51 (d, 1H, $J_{1',2'}$=8.5 Hz, H-1′), 4.35 (d, 1H, $J_{1,2}$=8.3 Hz, H-1), 4.18–4.02 (m, 4 H, H-2, 6a, 6′b, and 6′a), 3.94 (m, 1H, OCH_2), 3.88 (t, 1H, H-5′), 3.81 (t, 1H, $J_{4,5}$=8.9 Hz, H-4), 3.68–3.56 (m, 2H, OC*H*, C*H*), 3.40 (ddd, 1H, H-5), 3.15 (t, 2H, CH_2S), 3.08 (m, 1H, CH_2), 2.54 (m, 2H, COCH_2), 2.33 (s, 3H, SCOCH_3), 2.15, 2.12, 2.09, 2.06, 2.05, 1.97, 1.96 (all s, 21H, 7OCOCH_3) and 1.76 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$): δ 196.20 (–S*C*O–), 171.11, 170.81, 170.80, 170.51, 170.39, 170.16, 170.09, 169.20 (–*C*O–×8), 101.51, 101.10 (C-1,1′), 76.00, 73.05, 72.75, 70.88, 70.66, 69.09, 67.68, 66.59, 62.06, 60.72, 53.73, 36.48, 36.03, 30.64, 29.33, 25.10 (–SCO*C*H_3), 23.25, 20.93, 20.92, 20.68, 20.67, 20.66 and 20.54(–*C*H_3×7); Anal. Calcd for $C_{34}H_{50}N_2O_{19}S$: C, 49.63; H, 6.13; N, 3.40; S, 3.90. Found: C, 49.60; H, 6.18; N, 3.25; S, 3.79.

General procedure for the preparation of sodium thiolate 9–12 from the intermediates

NaOMe (28.4 mg, 0.526 mmol) was added to the solution of 3-(3-thioacetyl propionamido)propyl sugars **5**–**8** (0.478 mmol) in MeOH (10 mL). The reaction mixture was stirred for 1 h under nitrogen atmosphere. After concentration, the obtained sodium thiolates **9**–**12** were used for the next reaction without further purification.

General procedure for the preparation of GlycoCDs 14–17

To a solution of hexakis 6-deoxy-6-iodo-β-cyclodextrin **13**[42] (100 mg, 52.5 μmol) in DMF (10 mL) was added the crude sodium thiolate described above (0.478 mmol, 9.1 equiv.; 1.3 equiv. per iodo-group), and the suspension was stirred at 70°C for 24 h under nitrogen atmosphere. After concentration, the residue was subjected to a column of Sephadex G-25 and eluted with H_2O as an eluent. The fractions containing product were concentrated, the crude solid was washed with acetone or MeOH, and dissolved in H_2O. The water solution was finally lyophilized to afford powdery glycoCD. The individual physical properties are described below.

Heptakis{6-deoxy-6-*S*-[7-(β-D-galactopyranosyloxyl)-4-aza-3-oxo-heptano-1-yl]-6-thio}-cyclomaltoheptaose (14). (145 mg, 84%): $[\alpha]_D=+55.5°$ (*c* 0.543, H_2O); ^{1}H NMR (D_2O): δ 5.03(s, 1H, H-1), 4.31 (d, 1H, $J_{1',2'}$=7.5 Hz, H-1′), 2.87 (brt, 2H, CH_2S), 2.50 (brs, 2H, COCH_2) and 1.78 (brt, 2H, CH_2); ^{13}C NMR (D_2O): δ 175.52 (C=O), 104.71 (C-1′), 103.66 (C-1), 86.23 (C-4),

76.86, 74.70 (C-5′, 3′), 74.68, 74.02, 73.96 (C-3, 5, 2), 72.66, 70.47, 69.44 (C-2′, 4′, OCH_2), 62.74 (C-6′), 38.20, 37.92 (NCH_2, $COCH_2$), 34.98 (C-6), 30.62 and 30.55 (CH_2-S, CH_2); Anal. Calcd for $C_{126}H_{217}N_7O_{77}S_7 \cdot 3H_2O$: C, 45.30; H, 6.73; N, 2.94; S, 6.72. Found: C, 45.40; H, 6.77; N, 2.65; S, 6.85; MALDI-TOF MS. Calcd for $C_{126}H_{217}N_7O_{77}S_7$ 3286.59. Found *m/z* 3287.6 for $[M+H]^+$.

Heptakis{6-deoxy-6-*S*-[7-(2-acetamido-2-deoxy-β-D-glucopyranosyloxyl)-4-aza-3-oxo-heptano-1-yl]-6-thio}-cyclomaltoheptaose (15). (165 mg, 88%): $[\alpha]_D=+42.6°$ (*c* 0.519, H_2O); ^1H NMR (D_2O): δ 5.02 (d, 1H, $J_{1,2}=2.4$ Hz, H-1), 4.42 (d, 1H, $J_{1',2'}=7.9$ Hz, H-1′), 2.85 (brt, 2H, CH_2S), 2.48 (brs, 2H, $COCH_2$), 1.97 (s, 3H, $NHCOCH_3$) and 1.69 (brt, 2H, CH_2); ^{13}C NMR (D_2O): δ 175.80 [C=O (GlcNAc)], 175.15 [C=O(aglycon)], 103.39 (C-1), 102.67 (C-1′), 85.99 (C-4), 77.47, 75.48 (C-5′, 3′), 74.39, 73.87, 73.76 (C-3, 5, 2), 71.66, 69.21, 62.46, 57.21 (C-4′, $-OCH_2$, 6′, 2′), 37.88, 37.64 (NCH_2, $COCH_2$), 34.63 (C-6), 30.33, 30.25 (CH_2-S, CH_2) and 23.86 (CH_3); Anal. Calcd for $C_{140}H_{238}N_{14}O_{77}S_7 \cdot 5H_2O$: C, 45.89; H, 6.82; N, 5.36; S, 6.13. Found: C, 45.89; H, 6.81; N, 5.05; S, 6.10; MALDI-TOF MS. Calcd for $C_{140}H_{238}N_{14}O_{77}S_7$ 3573.96. Found: *m/z* 3575.0 $[M+H]^+$.

Heptakis{6-deoxy-6-*S*-[7-(4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyloxyl)-4-aza-3-oxo-heptano-1-yl]-6-thio}-cyclomaltoheptaose (16). (180 mg, 78%): $[\alpha]_D=+36.8°$ (*c* 0.562, H_2O); ^1H NMR (D_2O): δ 5.03 (s, 1H, H-1), 4.39 (d, 1H, $J_{1',2'}=7.9$ Hz, H-1′), 4.38 (d, 1H, $J_{1'',2''}=7.5$ Hz, H-1″), 2.87 (brs, 2H, CH_2S), 2.50 (brs, 2H, $COCH_2$) and 1.77 (brt, 2H, CH_2); ^{13}C NMR (D_2O): δ 175.44 (C=O), 104.77 (C-1″), 103.94 (C-1′), 103.57 (C-1), 86.25 (C-4), 80.57, 77.09, 76.54, 76.27, 74.66, 74.43, 74.40, 74.06, 73.95, 72.76, 70.35, 69.54, 62.74, 62.09, 38.15, 37.86, 34.89 (C-6), 30.56 and 30.48; Anal. Calcd for $C_{168}H_{287}N_7O_{112}S_7 \cdot 9H_2O$: C, 44.02; H, 6.71; N, 2.14; S, 4.90. Found: C, 43.64; H, 6.77; N, 1.90; S, 4.79; MALDI-TOF MS. Calcd for $C_{168}H_{287}N_7O_{112}S_7$ 4421.66. Found: *m/z* 4445.3 $[M+Na]^+$.

Heptakis{6-deoxy-6-*S*-[7-(2-acetamido-4-*O*-(β-D-galacto-pyranosyl)-2-deoxy-β-D-glucopyranosyl-oxyl)-4-aza-3-oxo-heptano-1-yl]-6-thio}-cyclomaltoheptaose (17). (205 mg, 83%): $[\alpha]_D=+23.5°$ (*c* 0.553, H_2O); ^1H NMR (D_2O): δ 5.07(s, 1H, H-1), 4.48 (d, 1H, $J_{1'',2''}=7.5$ Hz, H-1″), 4.44 (d, 1H, $J_{1',2'}=7.9$ Hz, H-1′), 2.89 (brs, 2H, CH_2S), 2.53 (brs, 2H, $COCH_2$), 2.01 (s, 3H, $NHCOCH_3$) and 1.74 (brs, 2H, CH_2); ^{13}C NMR (D_2O): δ 176.34, 175.75 (C=O), 105.15 (C-1″), 104.01 (C-1), 103.24 (C-1′), 86.63 (C-4), 81.12, 77.50, 76.96, 75.01, 74.82, 74.66, 74.49, 74.37, 73.17, 70.75, 69.87, 63.12, 62.48, 57.32, 38.45, 38.25, 35.30 (C-6), 30.99, 30.84 and 24.51; Anal. Calcd for $C_{182}H_{308}N_{14}O_{112}S_7 \cdot 10H_2O$: C, 44.22; H, 6.69; N, 3.97; S, 4.54. Found: C, 44.20; H, 6.62; N, 3.71; S, 4.69; MALDI-TOF MS. Calcd for $C_{182}H_{308}N_{14}O_{112}S_7$ 4709.03. Found: *m/z* 4732.4 $[M+Na]^+$.

Inhibition assay of hemagglutination by lectins

The inhibitory activity of the synthetic glycoCDs was tested using a fresh suspension [ca. 2% (v/v)] of human type O red blood cells in 0.05 M phosphate buffered saline (PBS, pH 7.2). WGA and ECorL were used at a concentration of 50 μg/mL and 100 μg/mL, respectively, in 0.05 M PBS solution. The procedure was as follows: the human blood cells (10 μL) were added to a solution of the test inhibitors (10 μL) and lectins (10 μL). The mixture was incubated for 30 min at room temperature before visual examination of hemagglutination activity. The minimum concentrations of the sample required to inhibit the aggregation were determined by 2-fold serial dilutions of each glycoCD ligand.

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