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Synthesis of biotinylated keratan sulfate repeating disaccharides

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We synthesized four types of keratan and keratan sulfate repeating disaccharides containing non-sulfate, Galβ1-4GlcNAcβ, and three types of sulfates, Gal6Sβ1-4GlcNAcβ, Galβ1-4GlcNAc6Sβ, and Gal6Sβ1-4GlcNAc6Sβ in an efficient and stereo-controlled manner. These disaccharides were conjugated with biotin via a hydrophilic linker at the reducing terminal.

Key words: keratan sulfate; glycosaminoglycan; glycosylation; biotin

Keratan sulfate (KS) is a member of the proteoglycan family that has a linear glycan chain composed of a repeating disaccharide unit, Galβ1-4GlcNAcβ. KS repeating oligosaccharides are often sulfated at *O*-6 of Gal and GlcNAc. A KS repeating disaccharide, the minimum unit of the KS oligosaccharide, has four different types of sulfation patterns, including the non-sulfate. KS oligosaccharides have recently been shown to have an inhibitory effect on axonal regrowth.[1)] However, the relationship between the factors regulating neuronal actions and facile structures, including the sulfation patterns of KS oligosaccharides, in this interaction has not yet been elucidated.

A few studies have investigated the synthesis of KS oligosaccharides. In 1989, Ogawa's group reported the synthesis of the KS tetrasaccharide, GlcNAc6Sβ1-3Gal6Sβ1-4GlcNAc6Sβ1-3Galβ,[2,3)] while Misra's group described the synthesis of the octyl glycosides of *N*-acetyl lactosamine, Gal6Sβ1-4GlcNAcβ, and Galβ1-4GlcNAc6Sβ, in 2004.[4)] However, these synthetic approaches are not suitable for preparing KS conjugates with four different types of sulfation patterns, including the non-sulfate.

We demonstrate in this study the efficient synthesis of KS disaccharides with three possible types of sulfation patterns, as well as one without the sulfate. The target KS disaccharides (**1**–**4**) were attached to biotin for biological use via a hydrophilic linker at the reducing terminal.

Results and discussion

Based on the retrosynthetic analysis depicted in Scheme 1, we attempted to synthesize the target compounds (**1**–**4**) by adopting a common disaccharide unit (**18**). Common disaccharide **18** was, respectively, protected with chemoselectively removable NAP and TBDPS at the position 6 of the Gal and GlcNAc residues.

Scheme 2 shows that all the acetyl groups in known thioglycoside **5** were removed by the Zemplén reaction. The triol was regiospecifically benzylidenated at the 4 and 6 positions with benzaldehyde dimethylacetal under acidic conditions to give **6** in a 78% yield in two steps. The resulting *OH*-3 of **6** was benzylated with NaH and BnBr to give **7** in an 86% yield. The benzylidene acetal of **7** was removed and the primary alcohol was selectively protected with TBDPS to afford **8** in a 69% yield in two steps. The glycosyl acceptor (**8**) was coupled with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (**9**) in the presence of TMSOTf at −20 °C. However, desired disaccharide **10** could only be obtained in a 2% yield under these reaction conditions. The dodecylthio group of **8** was transferred to the anomeric position of **9** to give **12α** and **12β** in respective 34 and 64% yields.

Similar intermolecular transfer of the thio group to the anomeric position of the donor has been reported for glycosylation with a thioglycoside.[5–7)] In 2000, Yu's group reported similar glycosylation with a trichloroacetimidate donor and an acceptor having thioglycoside in the presence of TMSOTf (0.05 eq. vs. the donor) at −10 °C to show intermolecular transfer of the dodecylthio group.[8)] However, the coupling reaction at −78 °C afforded the desired disaccharide in a 92% yield without the formation of a side product.

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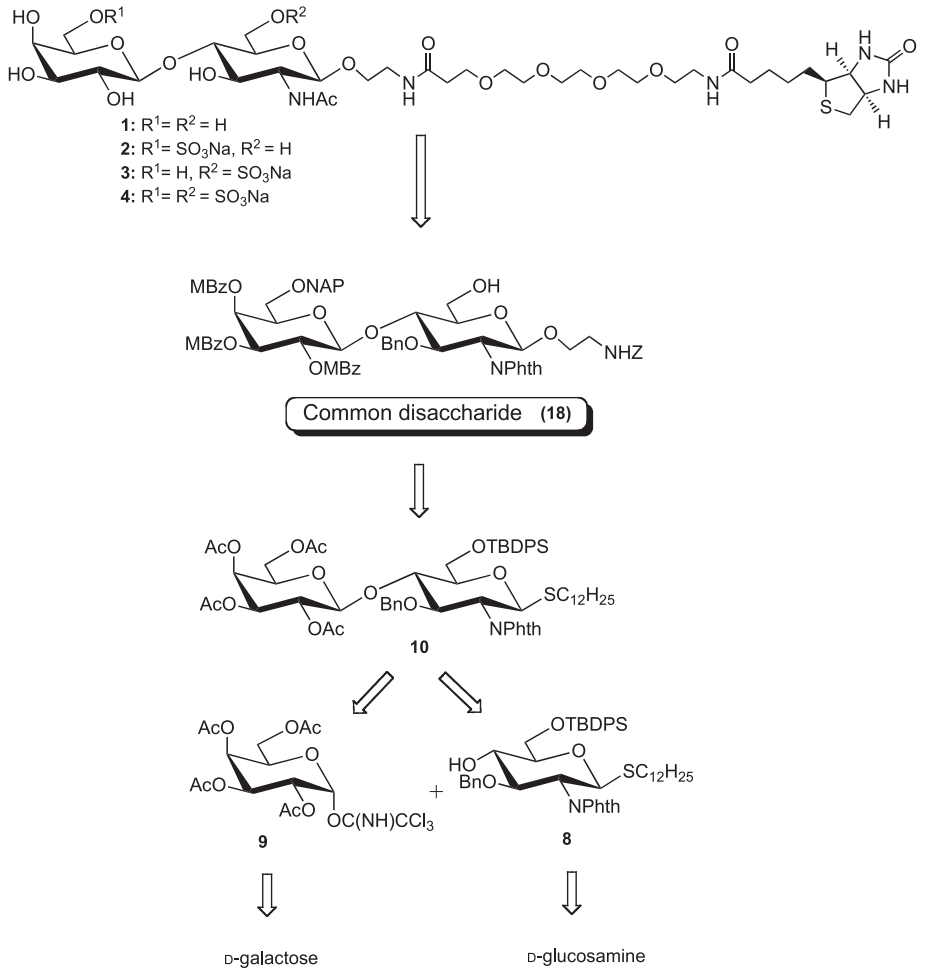
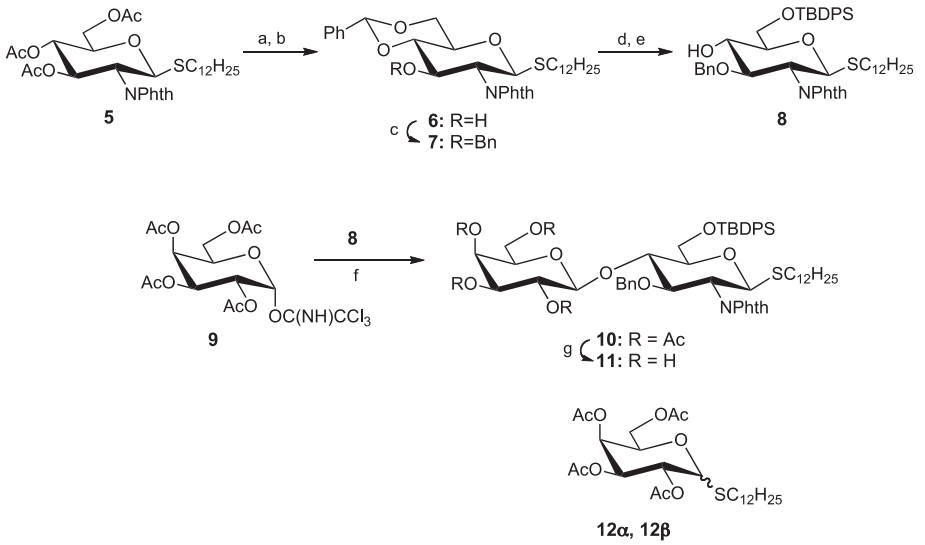
Abbreviations: KS, keratan sulfate; NAP, 2-naphthylmethyl; TBDPS, *tert*-butyldiphenylsilyl; TMSOTf, trimethylsilyl trifluoromethanesulfonate; MBz, 4-methylbenzoyl; Z, benzyloxycarbonyl; Bn, benzyl; NIS, *N*-iodosuccinimide; TfOH, trifluoromethanesulfonic acid; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; Phth, phthaloyl; DMAP; dimethylaminopyridine; THF, tetrahydrofurane; MS4A, molecular sieves 4A.



Scheme 1. Retrosynthetic analysis for disaccharides **1–4.**



Scheme 2. Synthesis of disaccharide **11**.

Note: Reagents and conditions: (a) NaOMe, MeOH; (b) $PhCH(OMe)_2$, *p*-TsOH, 78% (2 steps); (c) BnBr, NaH, DMF, 0 °C-rt, 86%; (d) camphorsulfonic acid, CH_2Cl_2-MeOH; (e) TBDPSCl, imidazole, DMF, 69% (2 steps); (f) TMSOTf, CH_2Cl_2, −20 or −78 °C; (g) NaOMe, MeOH, 60% (2 steps).

We rationalized that intermolecular transfer of the dodecylthio group was due to activation of the sulfur atom on the dodecylthio group by the larger amount of TMSOTf (0.5 eq.) and higher temperature (−20 °C). We therefore adopted Yu's conditions, lowered the reaction temperature to −78 °C, and decreased the amount of TMSOTf to 0.05 eq. We subsequently obtained desired disaccharide **10** without intermolecular transfer of the dodecylthio group; however, a portion of the corresponding isomer having an ortho-ester

linkage was generated due to the lower acidity used (data not shown). We finally succeeded in suppressing the ortho-ester formation by increasing the amount of TMSOTf to 0.1 eq. Saponification then removed the four acetyl groups of **10** to give desired tetraol **11** in a 60% yield in two steps.

The tetraol (**11**) was 2-naphthylildenated at the 4 and 6 positions of the Gal residue with 2-naphthaldehyde under acidic conditions to give **13** in an 89% yield (Scheme 3).[9] The resulting diol was protected with MBz to quantitatively give **14**. Reductive ring opening of the naphthylidene acetal using $BH_3{\cdot}NMe_3$, $AlCl_3$, and a catalytic amount of H_2O in THF[10] afforded 6-*O*-(2-naphthyl)methyl ether **15** in a 92% yield, without any formation of the regioisomer. The resulting *OH*-4 of **15** was methylbenzoylated to give **16** in a 98% yield. The disaccharide donor (**16**) was stereoselectively coupled to $HO(CH_2)_2NHZ$ in the presence of NIS and TfOH to quantitatively give **17**. We chemoselectively removed TBDPS to give common disaccharide **18** in a 79% yield.

Scheme 4 shows that the liberated primary position of **18** was protected with an acetyl group, and NAP on the Gal residue was chemoselectively removed with DDQ to give **19** in an 84% yield in two steps. NAP on **18** was similarly removed to give **20** in a 79% yield. The free hydroxyl groups of **19**, **18**, and **20** were sulfated with $SO_3{\cdot}Me_3N$ in DMF to respectively afford corresponding sulfates **21**, **22**, and **23** in 88%, quantitative and 90% yields. Phth, MBz, and Ac of **18**, **21**, **22**, and **23** were removed with 1,3-diaminopropane. The generated free amine was acetylated with Ac_2O and Et_3N in MeOH. Bn, Z, and NAP were removed by hydrogenolysis in the presence of Pd-black. Finally, free amines on the aglycon were biotinylated to quantitatively give the target compounds (**1**–**4**). Selected ^{1}H-NMR data for **1**–**4** are given in Table 1. The chemical shifts of H-6′ (**2**), H-6 (**3**), and of H-6 and 6′ (**4**), which were *O*-sulfated positions, were shifted down-field.

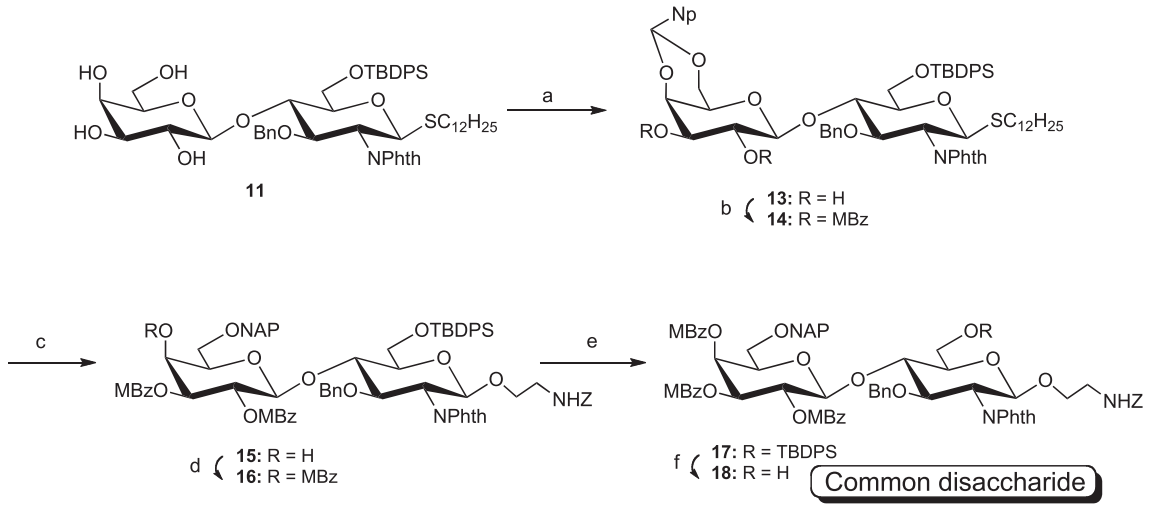
Conclusion

In summary, we synthesized for the first time in an efficient and stereo-controlled manner four types of keratan and KS repeating disaccharides containing a non-sulfate, Galβ1-4GlcNAcβ, as well as three sulfates, Gal6Sβ1-4GlcNAcβ, Galβ1-4GlcNAc6Sβ, and Gal6Sβ1-4GlcNAc6Sβ, using a common disaccharide. These disaccharides were linked to biotin via a hydrophilic linker to successfully give the target compounds (**1-4**).

Experimental

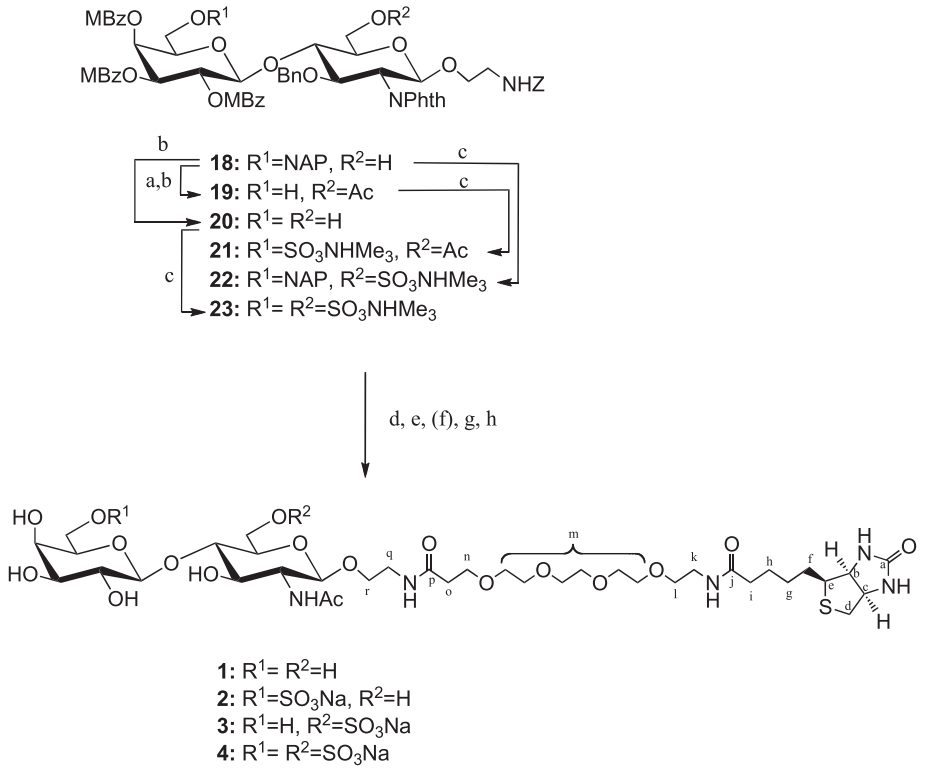
General methods. ^{1}H-NMR assignments were confirmed by two-dimensional HH COSY experiments using Bruker ADVANCE II 600 MHz spectrometers. Silica gel chromatography and analytical TLC were performed in a column of Silica Gel 60 (Merck) and Silica Gel 60 N (spherical neutral; Kanto Kagaku). The gel for size-exclusion chromatography (Sephadex LH-20) was from GE Healthcare. Bond Elut was from Agilent Technologies. Molecular sieves were from GL Science and activated at 200 °C under reduced pressure prior to their use. All reactions in organic solvents were performed in a dry Ar-containing atmosphere. The organic phase of the reaction mixture was successively washed with aq. $NaHCO_3$ and brine, and then dried over anhyd. $MgSO_4$ by the usual work-up.

*Dodecyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**6**).* To a solution of **5** (5.57 g, 8.99 mmol) in MeOH (212 mL) was added 0.5 M NaOMe (9 mL) while stirring at rt. After 2 h, the reaction was quenched with Dowex 50W × 8 (H^+ form), and the mixture was filtered. The volatiles were removed under reduced pressure to give a triol (4.27 g) which was dissolved in CH_3CN (400 mL). To this solution, benzaldehyde dimethylacetal (2.6 mL, 17 mmol)



Scheme 3. Synthesis of common disaccharide **18**.

Note: Reagents and conditions: (a) 2-naphthaldehyde, *p*-TsOH·H_2O, CH_3CN, 89%; (b) MBzCl, DMAP, pyridine-CH_2Cl_2, quant.; (c) $BH_3{\cdot}Me_3$ N, $AlCl_3$, cat. H_2O, THF, 92%; (d) MBzCl, DMAP, pyridine-CH_2Cl_2, 98%; (e) NIS, TfOH, MSAW300, Et_2O-$(CH_2Cl)_2$, quant.; (f) 1 M tetra-*n*-butyl ammonium fluoride, AcOH, THF, 79%.



Scheme 4. Synthesis of target compounds **1–4.**

Note: Reagents and conditions: (a) Ac_2O, pyridine, quant.; (b) DDQ, cat. H_2O, CH_2Cl_2-MeOH, 84% for **19** (2 steps), 78% for **20**; (c) $SO_3 \cdot NMe_3$, DMF, 60 °C, 88% for **21**, quant. for **22**, 90% for **23**; (d) 1,3-diaminopropane, EtOH, reflux; (e) Ac_2O, Et_3N, MeOH; (f) NaOH, MeOH (for **21**, **22**), 50% MeOH (for **23**), reflux; (g) H_2, Pd-black, aq. EtOH + dil. AcOH (for **18**), aq. 2-PrOH + dil. AcOH (for **21**, **22**), dil. AcOH (for **23**); (h) NHS-PEG_4-biotin, 1 M Na_3PO_4, 0.15 M NaCl.

Table 1. Selected chemical shifts (ppm) for **1–4** by 600 MHz ^{1}H-NMR.

	1	2	3	4
H-1	4.48	4.45	4.51	4.49
H-2	3.67	3.75	3.7	3.69
H-6ab	3.75–3.60	3.74, 3.59	4.35, 4.27	4.35, 4.24
H-1′	4.38	4.37	4.51	4.47
H-2′	3.45	3.57	3.46	3.48
H-6′ab	3.90, 3.75	4.13, 3.96	3.85–3.58	4.10, 3.93
NAc	1.96	1.90	1.96	1.94
H-b	4.33	4.27	4.38	4.35
H-c	4.51	4.45	4.54	4.54
H-d	2.90, 2.69	2.85, 2.63	2.91, 2.71	2.93, 2.72
H-e	3.24	3.36	3.28	3.27

and a catalytic amount of *p*-TsOH (pH < 2) were added, while stirring for 3 h. The reaction mixture was neutralized with Et_3N. The volatiles were removed under reduced pressure, and the residue was treated in a column of silica gel (20:1–10:1 toluene-EtOAc) to give **6** (4.06 g) in a 78% yield in two steps. ^{1}H-NMR δ_H ($CDCl_3$): 8.01–7.26 (m, 10H, Ar H), 5.57 (s, 1H, PhC<u>H</u>), 5.39 (d, 1H, $J_{1,2}$ = 10.62 Hz, H-1), 4.56 (br t, 1H, J = 9.57 Hz, H-3), 4.36 (dd, 1H, $J_{5,6a}$ = 5.04 Hz, J_{gem} = 10.44 Hz, H-6a), 4.31 (br t, 1H, J = 10.29 Hz, H-2), 3.80 (br t, 1H, J = 10.26 Hz, H-6b), 3.64 (m, 1H, H-5), 3.61 (br t, 1H, J = 9.15 Hz, H-4), 2.68 (m, 1H, 1/2SCH_2), 2.59 (m, 1H, 1/2SCH_2), 2.54 (s, 1H, 3-OH), 1.30–1.13 [m, 20H, $SCH_2(C\underline{H}_2)_{10}CH_3$], 0.87 (t, 3H, J = 14.10 Hz, CH_3). ESI-HRMS *m/z* [(M + Na)$^+$]: calcd. for $C_{33}H_{43}NO_6SNa$, 604.2703; found, 604.2690.

Dodecyl 3-O-benzyl-4,6-O-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (7). To a suspension of NaH (205.1 mg, 4.70 mmol, 55%) in DMF (3.0 mL) was added **6** (1.37 g, 2.35 mmol) in dry DMF (24 mL) at 0 °C, and the mixture was stirred for 1 h. BnBr was then added. After 1 h, unreacted NaH was decomposed with MeOH, and the reaction mixture was quenched with aq. NH_4Cl and diluted with EtOAc. The organic phase was washed with brine and dried over anhyd. $MgSO_4$. The volatiles were removed under reduced pressure, and the residue was treated in a column of silica gel (7:1–4:1 *n*-hexane-EtOAc) to give **7** (1.36 g) in an 86% yield. ^{1}H-NMR δ_H ($CDCl_3$): 7.86–6.86 (m, 14H, Ar H), 5.63 (s, 1H, PhC<u>H</u>), 5.31 (d, 1H, $J_{1,2}$ = 10.68 Hz, H-1), 4.79, 4.50 (ABq, 2H, J = 12.36 Hz, PhC<u>H</u>$_2$), 4.44 (br t, 1H, J = 9.45 Hz, H-3), 4.41 (dd, 1H, $J_{5,6a}$ = 10.62 Hz, J_{gem} = 4.92 Hz, H-6a), 4.27 (br t, 1H, J = 10.29 Hz, H-2), 3.82 (br t, 1H, J = 10.02 Hz, H-5), 3.80 (br t, 1H, J = 9.09 Hz, H-4), 3.70 (dd, 1H, $J_{5,6b}$ = 9.66 Hz, H-6b), 2.64 (m, 1H, 1/2SCH_2), 2.55 (m, 1H, 1/2SCH_2), 1.51–1.13 [m, 20H, $SCH_2(C\underline{H}_2)_{10}CH_3$], 0.87 (t, 3H, J = 14.16 Hz, CH_3). ESI-HRMS *m/z* [(M + Na)$^+$]: calcd. for $C_{40}H_{49}NO_6SNa$, 694.3173; found, 694.3168.

*Dodecyl 3-O-benzyl-6-O-tert-butyldiphenylsilyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**8**).* To a solution of **7** (5.12 g, 7.62 mmol) in CH_2Cl_2 (48 mL) and MeOH (48 mL), a catalytic amount of camphorsulfonic acid was added while stirring for 4 d. The reaction was quenched with Et_3N. The volatiles were

removed under reduced pressure, and the residue was treated in a column of silica gel (4:1–1:1 toluene-EtOAc) to give a corresponding 4,6-diol (4.10 g) which was dissolved in DMF (100 mL). Imidazole (1.06 g, 15.5 mmol) and TBDPSCl (2.0 mL, 7.7 mmol) were added to the solution while stirring for 5 d. The reaction mixture was diluted with EtOAc. The organic phase was treated as described for general methods, and the residue was then treated in a column of silica gel (10:1–3:1 *n*-hexane-EtOAc) to give **8** (4.35 g) in a 69% yield (two steps) as a syrup. ^{1}H-NMR δ_H ($CDCl_3$): 7.90–6.90 (m, 19H, Ar H), 5.23 (d, 1H, $J_{1,2}$ = 10.38 Hz, H-1), 4.78, 4.55 (ABq, 2H, J = 12.24 Hz, PhC$\underline{H}_2$), 4.29 (dd, 1H, $J_{2,3}$ = 10.26 Hz, $J_{3,4}$ = 8.46 Hz, H-3), 4.21 (br t, 1H, J = 10.35 Hz, H-2), 3.98 (dd, 1H, $J_{5,6a}$ = 4.56 Hz, J_{gem} = 10.62 Hz, H-6a), 3.92 (m, 2H, H-4, 6b), 3.60 (m, 1H, H-5), 3.07 (d, 1H, $J_{4,OH}$ = 2.28 Hz, OH-4), 2.62 (m, 1H, 1/2SCH_2), 2.52 (m, 1H, 1/2SCH_2), 1.43 (m, 2H, $SCH_2C\underline{H}_2$), 1.30–1.13 [m, 18H, $SC_2H_4(C\underline{H}_2)_9CH_3$], 1.09 [s, 9H, $C(CH_3)_3$], 0.86 (t, 3H, J = 14.22 Hz, CH_3). ESI-HRMS *m/z* [(M + Na)$^+$]: calcd. for $C_{49}H_{63}NO_6SSiNa$, 844.4038; found, 844.4026.

*Dodecyl β-D-galactopyranosyl-(1→4)-3-O-benzyl-6-O-tert-butyldiphenylsilyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**11**).* Method A: A solution of **8** (495.2 mg, 544.7 μmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (**9**, 668.9 mg, 1.36 mmol) in CH_2Cl_2 (55 mL) was stirred over AW300 molecular sieves (1.44 g) for 30 min at rt. To this solution, TMSOTf (173 μL, 679 μmol) was added at −20 °C for 2.5 h. The reaction was quenched with aq. $NaHCO_3$ and diluted with $CHCl_3$. The insoluble materials were filtered through Celite. The organic phase was treated as describe for general methods, and the crude mixture was then treated in an LH-20 gel permeation column (1:1 $CHCl_3$-MeOH) and silica gel column (6:1–5:1 *n*-hexane-EtOAc) to give **10** (9.7 mg, 2%), dodecyl thioglycosides (**12α**, 101.0 mg) and (**12**β, 184.3 mg) in respective 34 and 64% yields.

Method B: 4A molecular sieves (2.93 g) were added to a solution of **8** (2.81 g, 3.42 mmol) and **9** (2.53 g, 5.13 mmol) in CH_2Cl_2 (233 mL), and the mixture was stirred for 30 min at rt. To this mixture TMSOTf (46 μL, 0.26 mmol) was added while stirring at −78 °C for 2 h. The reaction was treated in the same way as that described for Method A. The residue was purified through a column of silica gel (15:1–5:1 toluene-EtOAc) to give **10** (4.26 g). The disaccharide (**10**) was diluted in MeOH (160 mL), and 0.5 M NaOMe (3.4 mL) was added to the solution while stirring at rt. After 4 h, the reaction was quenched with Dowex 50Wx8 (H^+ form), and the mixture was filtered. The volatiles of the filtrate were removed under reduced pressure, and the residue was directly treated in a column of silica gel to give **11** (2.02 g, 60% in two steps). Compound **11**. ^{1}H-NMR δ_H ($CDCl_3$): 7.83–6.88 (m, 19H, Ar H), 5.20 (d, 1H, $J_{1,2}$ = 10.50 Hz, H-1), 4.90, 4.50 (ABq, 2H, J = 12.18 Hz, PhC$\underline{H}_2$), 4.76 (d, 1H, $J_{1',2'}$ = 7.80 Hz, H-1′), 4.42 (br t, 1H, J = 9.48 Hz, H-3), 4.28 (br t, 1H, J = 8.94 Hz, H-4), 4.27 (br t, 1H, J = 10.43 Hz, H-2), 4.22 (dd, 1H, $J_{5,6a}$ = 2.04 Hz, J_{gem} = 11.04 Hz, H-6a), 4.03 (d, 1H, H-6b), 3.94 (d, 1H, $J_{3',4'}$ = 2.76 Hz, H-4′), 3.75 (m, 2H, H-6′ab), 3.69 (br t, 1H, J = 8.61 Hz, H-2′), 3.57 (br d, 1H, J = 9.66 Hz, H-5), 3.41 (dd, 1H, $J_{2',3'}$ = 9.42 Hz, H-3′), 3.34 (br t, 1H, J = 4.11 Hz, H-5′), 2.65 (m, 1H, 1/2SCH_2), 2.58 (m, 1H, 1/2SCH_2), 1.47 (m, 2H, $SCH_2C\underline{H}_2$), 1.28–1.11 [m, 18H, $SC_2H_4(C\underline{H}_2)_9CH_3$], 1.08 [s, 9H, $C(CH_3)_3$], 0.87 (t, 3H, J = 14.16 Hz, CH_3). ESI-HRMS *m/z* [(M + Na)$^+$]: calcd. for $C_{55}H_{73}NO_{11}SSiNa$, 1006.4566; found, 1006.4549.

*Dodecyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-galactopyranoside (**12α**).* ^{1}H-NMR δ_H ($CDCl_3$): 5.71 (d, 1H, $J_{3,4}$ = 5.52 Hz, H-4), 4.45 (d, 1H, $J_{1,2}$ = 3.18 Hz, H-1), 5.26 (dd, 1H, $J_{2,3}$ = 10.86 Hz, H-3), 4.22 (dd, 1H, H-2), 4.59 (br t, 1H, J = 6.44 Hz, H-5), 4.11 (m, 2H, H-6ab), 2.56 (m, 1H, 1/2SCH_2), 2.49 (m, 1H, 1/2SCH_2), 2.15, 2.08, 2.05, 2.00 (4s, each 3H, 4Ac), 1.58 (m, 2H, $SCH_2C\underline{H}_2$), 1.38–1.23 [m, 18H, $SC_2H_4(C\underline{H}_2)_9CH_3$], 0.88 (t, 3H, J = 14.04 Hz, CH_3). ESI-HRMS *m/z* [(M + Na)$^+$]: calcd. for $C_{26}H_{44}O_9SNa$, 555.2598; found, 555.2585.

*Dodecyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranoside (**12β**).* ^{1}H-NMR δ_H ($CDCl_3$): 5.43 (d, 1H, $J_{3,4}$ = 3.30 Hz, H-4), 5.24 (br t, 1H, J = 9.96 Hz H-2), 5.05 (dd, 1H, $J_{2,3}$ = 9.66 Hz, H-3), 4.48 (d, 1H, $J_{1,2}$ = 10.02 Hz, H-1), 4.16 (dd, 1H, $J_{5,6b}$ = 6.60 Hz, J_{gem} = 11.34 Hz, H-6b), 4.11 (dd, 1H, $J_{5,6a}$ = 6.60 Hz, H-6a), 3.93 (t, 1H, H-5), 2.64–2.73 (m, 2H, SCH_2), 2.16, 2.07, 2.05, 2.00 (4s, each 3H, 4Ac), 1.38–1.25 [m, 20H, $SCH_2(C\underline{H}_2)_{10}CH_3$], 0.88 (t, 3H, J = 14.04 Hz, CH_3). ESI-HRMS *m/z* [(M + Na)$^+$]: calcd. for $C_{26}H_{44}O_9SNa$, 555.2598; found, 555.2598.

*Dodecyl 4,6-O-(2-naphthylidene)-β-D-galactopyranosyl-(1→4)-3-O-benzyl-6-O-tert-butyldiphenylsilyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**13**).* To a solution of **11** (1.18 g, 1.20 mmol) in CH_3CN, 2-naphthaldehyde (374.8 mg, 2.40 mmol) and *p*-TsOH (22.8 mg, 0.12 mmol) were added while stirring for 2 h. The reaction mixture was neutralized with Et_3N. The volatiles were removed under reduced pressure, and the residue was treated in a column of silica gel (4:1–2:1 toluene-EtOAc) to give **13** (1.20 g) in an 89% yield which was used without further purification. ^{1}H-NMR δ_H ($CDCl_3$): 7.84–6.80 (m, 26H, Ar H), 5.67 (s, 1H, NpC$\underline{H}$), 5.21 (d, 1H, $J_{1,2}$ = 10.56 Hz, H-1), 5.10, 4.64 (ABq, 2H, J = 12.24 Hz, PhC$\underline{H}_2$), 4.85 (d, 1H, $J_{1',2'}$ = 7.74 Hz, H-1′), 4.45 (dd, 1H, $J_{2,3}$ = 10.02 Hz, $J_{3,4}$ = 8.82 Hz, H-3), 4.40 (d, 1H, J_{gem} = 12.36 Hz, H-6′a), 4.34 (t, 1H, H-4), 4.29 (br d, 2H, J = 10.20 Hz, H-2,6a), 4.20 (d, 1H, $J_{3',4'}$ = 3.66 Hz, H-4′), 4.04 (d, 1H, J_{gem} = 12.36 Hz, H-6b), 4.01 (d, 1H, H-6′b), 3.79 (t, 1H, H-2′), 3.54 (m, 2H, H-5, 3′), 3.36 (s, 1H, H-5′), 2.66 (m, 1H, 1/2SCH_2), 2.58 (m, 2H, 1/2SCH_2, OH-2′), 2.51 (d, 1H, $J_{3',OH}$ = 9.36 Hz, OH-3′), 1.52–1.30 [m, 20H, $SCH_2(C\underline{H}_2)_{10}CH_3$], 1.10 [s, 9H, $C(CH_3)_3$], 0.87 (t, 3H, J = 14.16 Hz, CH_3). ESI-HRMS *m/z* [(M + Na)$^+$]: calcd. for $C_{66}H_{79}NO_{11}SSiNa$, 1144.5035; found, 1144.5013.

*Dodecyl 2,3-di-O-(4-methyl)benzoyl-4,6-O-(2-naphthylidene)-β-D-galactopyranosyl-(1→4)-3-O-benzyl-6-O-tert-butyldiphenylsilyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**14**).* Compound **13** (1.10 g, 0.98 mmol) was dissolved in CH_2Cl_2 (5.0 mL) and pyridine (30 mL). *p*-Toluoylchloride (312 μL, 2.35 mmol) and a catalytic amount of DMAP were added to the solution while stirring for 5 d. The reaction mixture was diluted with $CHCl_3$. The organic phase was successively washed with 1 M HCl, aq. $NaHCO_3$ and brine, and dried over anhyd. $MgSO_4$. The volatiles were removed under reduced pressure, and the residue was treated in a column of silica gel (10:1 toluene-EtOAc) to quantitatively give **14** (1.35 g). ^{1}H-NMR δ_H ($CDCl_3$): 7.85–6.75 (m, 34H, Ar H), 5.91 (dd, 1H, $J_{1',2'}$ = 8.04 Hz, $J_{2',3'}$ = 10.44 Hz, H-2′), 5.65 (s, 1H, NpCH), 5.29 (dd, 1H, $J_{3',4'}$ = 3.66 Hz, H-3′), 5.25 (d, 1H, H-1′), 5.14, 4.72 (ABq, 2H, J = 12.30 Hz, PhCH_2), 5.12 (d, 1H, $J_{1,2}$ = 10.26 Hz, H-1), 4.60 (d, 1H, H-4′), 4.53 (d, 1H, J_{gem} = 12.36 Hz, H-6′a), 4.47 (dd, 1H, $J_{3,4}$ = 8.40 Hz, $J_{4,5}$ = 9.72 Hz, H-4), 4.32 (dd, 1H, $J_{2,3}$ = 10.26 Hz, H-3), 4.26 (t, 1H, H-2), 4.10 (d, 1H, H-6b′), 3.92 (d, 1H, J_{gem} = 10.56 Hz, H-6a), 3.83 (d, 1H, H-6b), 3.57 (s, 1H, H-5′), 3.23 (d, 1H, H-5), 2.62 (m, 1H, 1/2SCH_2), 2.53 (m, 1H, 1/2SCH_2), 2.32, 2.29 (2 s, each 3H, 2MePh), 1.48–1.18 [m, 20H, SCH_2(CH_2)$_{10}$CH_3], 1.14 [s, 9H, C(CH_3)$_3$], 0.87 (t, 3H, J = 14.22 Hz, CH_3). ESI-HRMS *m/z* [(M + Na)$^+$]: calcd. for $C_{82}H_{91}NO_{13}SSiNa$, 1380.5873; found, 1380.5852.

*Dodecyl 2,3-di-O-(4-methyl)benzoyl-6-O-(2-naphthylmethyl)-β-D-galactopyranosyl-(1→4)-3-O-benzyl-6-O-tert-butyldiphenylsilyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**15**).* To a solution of **14** (1.35 g, 0.98 mmol) in THF (37 mL) was added BH_3·NMe_3 (427.3 mg, 5.88 mmol) while stirring for 30 min. To this mixture, $AlCl_3$ (786.6 mg, 5.88 mmol) and H_2O (1 drop) were added while stirring overnight. The reaction was quenched with aq. NH_4Cl and diluted with EtOAc. The organic phase was successively washed with aq. $NaHCO_3$ and brine, and dried over anhyd. $MgSO_4$. The volatiles were removed under reduced pressure, and the residue was treated in an LH-20 gel permeation column (1:1 $CHCl_3$-MeOH) to give **15** (1.23 g) in a 92% yield. ^{1}H-NMR δ_H ($CDCl_3$): 7.83–6.79 (m, 34H, Ar H), 5.80 (dd, 1H, $J_{1',2'}$ = 8.04 Hz, $J_{2',3'}$ = 10.32 Hz, H-2′), 5.21 (dd, 1H, $J_{3',4'}$ = 3.18 Hz, H-3′), 5.25 (d, 1H, H-1′), 5.11 (d, 1H, $J_{1,2}$ = 10.02 Hz, H-1), 4.94, 4.60 (ABq, 2H, J = 12.30 Hz, ArCH_2), 4.71, 4.67 (ABq, 2H, J = 12.18 Hz, ArCH_2), 4.44 (dd, 1H, $J_{3,4}$ = 8.40 Hz, $J_{4,5}$ = 9.72 Hz, H-4), 4.42 (dd, 1H, $J_{4',OH}$ = 3.84 Hz, H-4′), 4.32 (br t, 1H, J = 10.20 Hz, H-3), 4.26 (br t, 1H, J = 10.20 Hz, H-2), 3.76 (m, 5H, H-6ab,5′,6′ab), 3.22 (d, 1H, H-5), 2.73 (d, 1H, OH-4′), 2.60 (m, 1H, 1/2SCH_2), 2.52 (m, 1H, 1/2SCH_2), 2.35, 2.29 (2 s, each 3H, 2MePh), 1.47–1.06 [m, 20H, SCH_2(CH_2)$_{10}$CH_3], 1.06 [s, 9H, C(CH_3)$_3$], 0.86 (t, 3H, J = 14.28 Hz, CH_3). ESI-HRMS *m/z* [(M + Na)$^+$]: calcd. for $C_{82}H_{93}NO_{13}SSiNa$, 1382.6029; found, 1382.6003.

*Dodecyl 2,3,4-tri-O-(4-methyl)benzoyl-6-O-(2-naphthylmethyl)-β-D-galactopyranosyl-(1→4)-3-O-benzyl-6-O-tert-butyldiphenylsilyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**16**).* To a solution of **15** (152.2 mg, 111.8 μmol) in CH_2Cl_2 (1.5 mL) and pyridine (1.0 mL) *p*-toluoylchloride (18 μL, 0.13 mmol) and a catalytic amount of DMAP were added while stirring for 6 d. The reaction mixture was treated in the same way for the synthesis of **14**. The crude mixture was treated in an LH-20 gel permeation column (1:1 $CHCl_3$-MeOH) to give **16** (161.2 mg) in a 98% yield. ^{1}H-NMR δ_H ($CDCl_3$): 7.90–6.90 (m, 38H, Ar H), 5.95 (d, 1H, $J_{3',4'}$ = 3.54 Hz, H-4′), 5.76 (dd, 1H, $J_{1',2'}$ = 8.04 Hz, $J_{2',3'}$ = 10.38 Hz, H-2′), 5.47 (dd, 1H, H-3′), 5.25 (d, 1H, H-1′), 5.12 (d, 1H, $J_{1,2}$ = 10.14 Hz, H-1), 4.98, 4.69 (ABq, 2H, J = 11.88 Hz, ArCH_2), 4.67, 4.48 (ABq, 2H, J = 11.58 Hz, ArCH_2), 4.46 (br t, 1H, J = 9.06 Hz, H-4), 4.34 (dd, 1H, $J_{2,3}$ = 10.14 Hz, $J_{3,4}$ = 8.28 Hz, H-3), 4.30 (t, 1H, H-2), 4.08 (br t, 1H, J = 6.39 Hz, H-6′a), 3.85 (br d, 2H, H-6ab), 3.66 (dd, 1H, $J_{5',6'b}$ = 9.66 Hz, J_{gem} = 5.70 Hz, H-6′b), 3.57 (dd, 1H, $J_{5',6'a}$ = 7.68 Hz, H-5′), 3.24 (br d, 1H, J = 9.78 Hz, H-5), 2.63 (m, 1H, 1/2SCH_2), 2.55 (m, 1H, 1/2SCH_2), 2.30, 2.30, 2.29 (3 s, each 3H, 3MePh), 1.52–1.12 [m, 20H, SCH_2(CH_2)$_{10}$CH_3], 1.08 [s, 9H, C(CH_3)$_3$], 0.87 (t, 3H, J = 14.28 Hz, CH_3). ESI-HRMS *m/z* [(M + Na)$^+$]: calcd. for $C_{90}H_{99}NO_{14}SSiNa$, 1500.6448; found, 1500.6411.

*2-(N-Benzyloxycarbonyl)aminoethyl 2,3,4-tri-O-(4-methyl)benzoyl-6-O-(2-naphthylmethyl)-β-D-galactopyranosyl-(1→4)-3-O-benzyl-6-O-tert-butyldiphenylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**17**).* AW 300 molecular sieves (993.2 mg) were added to a solution of **16** (819.1 mg, 553.9 μmol) and benzyl *N*-(2-hydroxyethyl)carbamate (162.2 mg, 830.9 μmol) in $(CH_2Cl)_2$ (8.5 mL) and Et_2O (29 mL), and the mixture was stirred for 30 min at rt. To this mixture, NIS (249.8 mg, 1.11 mmol) and TfOH (15 μL, 0.16 mmol) were added while stirring for 6 h at rt. The reaction mixture was treated in the same way as that for the synthesis of **11**. The crude mixture was treated in a column of silica gel (20:1–15:1 toluene-EtOAc) to quantitatively give **17** (867.6 mg). ^{1}H-NMR δ_H ($CDCl_3$): 7.77–6.74 (m, 43H, Ar H), 5.95 (d, 1H, $J_{3',4'}$ = 3.36 Hz, H-4′), 5.76 (dd, 1H, $J_{1',2'}$ = 8.04 Hz, $J_{2',3'}$ = 10.38 Hz, H-2′), 5.47 (dd, 1H, H-3′), 5.27 (d, 1H, H-1′), 5.05 (d, 1H, $J_{1,2}$ = 8.58 Hz, H-1), 4.98, 4.67 (ABq, 2H, J = 11.97 Hz, ArCH_2), 4.89 (m, 3H, NH, ArCH_2), 4.68, 4.48 (ABq, 2H, J = 12.24 Hz, ArCH_2), 4.46 (br t, 1H, J = 9.18 Hz, H-4), 4.28 (dd, 1H, $J_{2,3}$ = 10.80 Hz, $J_{3,4}$ = 8.82 Hz, H-3), 4.17 (dd, 1H, H-2), 4.07 (br t, 1H, J = 6.84 Hz, H-5′), 3.89 (br d, 1H, J = 10.38 Hz, H-6a), 3.81 (br d, 1H, J = 11.40 Hz, H-6b), 3.70 (m, 1H, 1/2OCH_2), 3.67 (dd, 1H, $J_{5',6'a}$ = 5.52 Hz, J_{gem} = 9.54 Hz, H-6′a), 3.58 (dd, 1H, $J_{5',6'b}$ = 7.68 Hz, H-6′b), 3.45 (m, 1H, 1/2OCH_2), 3.24 (m, 3H, H-5, NHCH_2), 2.28 (s, 9H, 3MePh), 1.09 [s, 9H, C(CH_3)$_3$]. ESI-HRMS *m/z* [(M + Na)$^+$]: calcd. for $C_{88}H_{86}N_2O_{17}SiNa$, 1493.5588; found, 1493.5549.

*2-(N-Benzyloxycarbonyl)aminoethyl 2,3,4-tri-O-(4-methyl)benzoyl-6-O-(2-naphthylmethyl)-β-D-galacto-pyranosyl-(1→4)-3-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**18**).* Compound **17** (867.6 mg, 553.9 μmol) was dissolved in THF (4.0 mL) and AcOH (320 μL, 5.55 mmol). To this mixture, 1 M TBAF (2.8 mL, 2.8 mmol) was added while stirring for 13 d. The reaction mixture was diluted with $CHCl_3$. The organic phase was washed with brine and dried over $MgSO_4$. The volatiles were removed under reduced pressure, and the residue was treated in an LH-20 gel permeation column (1:1 $CHCl_3$-MeOH) to give **18** (538.4 mg) in a 79% yield. ^{1}H-NMR δ_H ($CDCl_3$): 7.83–6.75 (m, 33H, Ar H), 5.91 (d, 1H, $J_{3',4'}$ = 3.24 Hz, H-4′), 5.91 (dd, 1H, $J_{1',2'}$ = 7.98 Hz, $J_{2',3'}$ = 10.38 Hz, H-2′), 5.56 (dd, 1H, H-3′), 5.06 (d, 1H, $J_{1,2}$ = 8.34 Hz, H-1), 5.06 (s, 1H, NH), 5.04 (d, 1H, H-1′), 4.97, 4.67 (ABq, 2H, J = 12.45 Hz, ArC$\underline{H}_2$), 4.89 (m, 2H, ArC$\underline{H}_2$), 4.59, 4.43 (ABq, 2H, J = 12.36, Hz, ArC$\underline{H}_2$), 4.30 (br t, 1H, J = 9.04 Hz, H-3), 4.15 (br t, 1H, H-5′), 4.12 (m, 1H, H-2), 4.04 (br t, 1H, J = 9.27 Hz, H-4), 3.70 (br s, 2H, H-6ab), 3.34 (m, 1H, 1/2OCH_2), 3.55 (m, 3H, H-6′ab, 1/2OCH_2), 3.28 (m, 2H, H-5, 1/2NCH_2), 3.16 (m, 1H, 1/2NCH_2), 2.37, 2.32, 2.29 (3 s, each 3H, 3$\underline{\text{Me}}$Ph), 1.99 (br s, 1H, OH-6). ESI-HRMS *m/z* [(M + Na)$^+$]: calcd. for $C_{77}H_{68}N_2O_{17}Na$, 1255.4410; found, 1255.4380.

*2-(N-Benzyloxycarbonyl)aminoethyl 2,3,4-tri-O-(4-methyl)benzoyl-β-D-galactopyranosyl-(1→4)-6-O-acetyl-3-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**19**).* To a solution of **18** (130.6 mg, 105.9 μmol) in CH_2Cl_2 (2.0 mL), pyridine (90 μL) and Ac_2O (90 μL) were added while stirring overnight. The volatiles were removed under reduced pressure. To the residue in CH_2Cl_2 (2.1 mL) and MeOH (0.6 mL), DDQ (24.0 mg, 106 μmol) and H_2O (1 drop) were added while stirring for 3 d. The reaction mixture was diluted in $CHCl_3$. The organic phase was treated as described for the general methods. The residue was treated in an LH-20 gel permeation column (1:1 $CHCl_3$-MeOH) to give **19** (84.1 mg) with an 84% yield in two steps. ^{1}H-NMR δ_H ($CDCl_3$): 7.93–6.80 (m, 26H, Ar H), 5.87 (dd, 1H, $J_{1',2'}$ = 7.92 Hz, $J_{2',3'}$ = 10.38 Hz, H-2′), 5.72 (d, 1H, $J_{3',4'}$ = 3.30 Hz, H-4′), 5.50 (dd, 1H, H-3′), 5.07 (d, 1H, $J_{1,2}$ = 8.58 Hz, H-1), 5.07 (br s, 1H, NH), 4.95, 4.90 (ABq, 2H, J = 12.24 Hz, PhC$\underline{H}_2$), 4.89, 4.52 (ABq, 2H, J = 12.06 Hz, PhC$\underline{H}_2$), 4.86 (d, 1H, H-1′), 4.35 (m, 1H, H-6a), 4.31 (m, 1H, H-3), 4.16 (m, 1H, H-6b), 4.11 (m, 1H, H-2), 3.92 (m, 2H, H-4,5′), 3.64 (m, 1H, 1/2OCH_2), 3.53 (m, 2H, H-5, 1/2OCH_2), 3.46 (m, 1H, H-6′a), 3.36 (m, 1H, H-6′b), 3.20 (m, 2H, NCH_2), 2.54 (br s, 1H, 6′-OH), 2.38, 2.37, 2.28 (3 s, each 3H, 3$\underline{\text{Me}}$Ph), 2.01 (s, 3H, Ac). ESI-HRMS *m/z* [(M + Na)$^+$]: calcd. for $C_{63}H_{62}N_2O_{18}Na$, 1157.3890; found, 1157.3862.

*2-(N-Benzyloxycarbonyl)aminoethyl 2,3,4-tri-O-(4-methyl)benzoyl-β-D-galactopyranosyl-(1→4)-3-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**20**).* To a solution of **18** (77.0 mg, 62.4 μmol) in CH_2Cl_2 (1.3 mL) and MeOH (0.4 mL), DDQ (16.8 mg, 74.9 μmol) and H_2O (1 drop) were added while stirring overnight. The reaction mixture was treated in the same way as that for the synthesis of **19**, and the residue was treated in an LH-20 gel permeation column (1:1 $CHCl_3$-MeOH) to give **20** (52.9 mg) in a 78% yield. ^{1}H-NMR δ_H ($CDCl_3$): 7.95–6.78 (m, 26H, Ar H), 5.85 (dd, 1H, $J_{1',2'}$ = 7.95 Hz, $J_{2',3'}$ = 10.32 Hz, H-2′), 5.77 (d, 1H, $J_{3',4'}$ = 3.06 Hz, H-4′), 5.59 (dd, 1H, H-3′), 5.24 (br t, J = 5.46 Hz, NH), 5.16 (d, 1H, $J_{1,2}$ = 7.62 Hz, H-1), 5.15 (d, 1H, H-1′), 4.92, 4.65 (ABq, 2H, J = 12.12 Hz, PhC$\underline{H}_2$), 4.82, 4.66 (ABq, 2H, J = 12.36 Hz, PhC$\underline{H}_2$), 4.27 (m, 1H, H-3), 4.21 (m, 1H, H-2), 4.11 (m, 1H, H-6′a), 3.85 (br d, 1H, J = 12.18 Hz, H-6a), 3.75 (br s, 1H, H-6b), 3.51 (m, 3H, H-5′,6′b, 1/2OCH_2), 3.33 (br d, 1H, J = 6.12 Hz, H-4), 3.20 (m, 1H, 1/2NCH_2), 3.10 (m, 1H, 1/2NCH_2), 2.89 (br s, 1H, H-5), 2.38, 2.33, 2.28 (3 s, each 3H, 3$\underline{\text{Me}}$Ph). ESI-HRMS *m/z* [(M + Na)$^+$]: calcd. for $C_{61}H_{60}N_2O_{17}Na$, 1115.3784; found, 1115.3760.

*O-Sulfation of **19**, **18** and **20**.* The starting material was dissolved in DMF (2.0 mL per 50 mg of the starting material). To this solution, $SO_3{\cdot}Me_3N$ (20 equiv. per hydroxyl group) was added while stirring at 60 °C for 1–3 h. The reaction mixture was treated in an LH-20 gel permeation column (1:1 $CHCl_3$-MeOH) to give the corresponding sulfate (**21**, **22**, and **23**) in respective 88%, quantitative and 90% yields.

*2-(N-Benzyloxycarbonyl)aminoethyl 2,3,4-tri-O-(4-methyl)benzoyl-6-O-sulfo-β-D-galactopyranosyl-(1→4)-6-O-acetyl-3-O-benzyl-2-deoxy-2-phthalimido-β-D-gluco-pyranoside, trimethylamine salt (**21**).* ^{1}H-NMR δ_H ($CDCl_3$): 7.91–6.85 (m, 26H, Ar H), 5.93 (br s, 1H, H-4′), 5.78 (dd, 1H, $J_{1',2'}$ = 7.92 Hz, $J_{2',3'}$ = 10.32 Hz, H-2′), 5.46 (dd, 1H, $J_{3',4'}$ = 2.94 Hz, H-3′), 5.06 (d, 2H, $J_{1,2}$ = 12.06 Hz, H-1, NH), 5.00, 4.59 (ABq, 2H, J = 12.06 Hz, PhC$\underline{H}_2$), 4.94, 4.90 (ABq, 1H, J = 12.30 Hz, PhC$\underline{H}_2$), 4.92 (d, 1H, H-1′), 4.39 (m, 1H, H-5′), 4.33 (d, 1H, J_{gem} = 11.28 Hz, H-6b), 4.27 (m, 2H, H-3,6′a), 4.17 (dd, 1H, $J_{5,6a}$ = 4.32 Hz, H-6a), 4.15 (m, 1H, H-6′b), 3.96 (m, 2H, H-2,4), 3.62 (m, 1H, 1/2CH_2), 3.53 (m, 1H, 1/2CH_2), 3.48 (m, 2H, H-5,5′), 3.21 (br s, 2H, CH_2), 2.77 (t, 9 H, NMe_3), 2.37, 2.32, 2.29 (3 s, each 3H, 3$\underline{\text{Me}}$Ph), 2.05 (s, 3H, Ac). ESI-HRMS *m/z* [(M + Na)$^+$]: calcd. for $C_{63}H_{61}N_2O_{21}SNa_{1,2}$259.3277; found, 1259.3248.

*2-(N-Benzyloxycarbonyl)aminoethyl 2,3,4-tri-O-(4-methyl)benzoyl-6-O-(2-naphthylmethyl)-β-D-galactopyra-nosyl-(1→4)-3-O-benzyl-2-deoxy-2-phthalimido-6-O-sulfo-β-D-glucopyranoside, trimethylamine salt (**22**).* ^{1}H-NMR δ_H (CD_3OD): 7.96–6.56 (m, 33H, Ar H), 5.84 (d, 1H, $J_{3',4'}$ = 3.36 Hz, H-4′), 5.64 (dd, 1H, $J_{1',2'}$ = 7.80 Hz, $J_{2',3'}$ = 10.26 Hz, H-2′), 5.56 (dd, 1H, H-3′), 5.37 (d, 1H, H-1′), 5.01 (d, 1H, $J_{1,2}$ = 8.52 Hz, H-1), 4.89, 4.47 (ABq, 1H, J = 11.64 Hz, ArC$\underline{H}_2$), 4.69 (s, 2H, ArC$\underline{H}_2$), 4.61, 4.36 (ABq, 2H, J = 12.18 Hz, ArC$\underline{H}_2$), 4.35 (d, 1H, J_{gem} = 10.50 Hz, H-6a), 4.33 (br t, J = 6.60 Hz, H-5′), 4.17 (dd, 1H, $J_{2,3}$ = 10.50 Hz, $J_{3,4}$ = 9.18 Hz, H-3), 4.09 (br t, 1H, J = 9.42 Hz, H-4), 4.03 (d, 1H, H-6b), 3.92 (dd, 1H, H-2), 3.60 (m, 1H, 1/2CH_2), 3.55 (dd, 1H, $J_{5',6'a}$ = 5.82 Hz, J_{gem} = 9.54 Hz, H-6′a), 3.49 (dd, 1H, $J_{5',6'b}$ = 7.50 Hz, H-6′b), 3.37 (m,

2H, 1/2CH_2, H-5), 3.30 (m, 2H, CH_2), 2.77 (s, 9 H, NMe_3), 2.27, 2.18, 2.15 (3 s, each 3H, 3MePh). ESI-HRMS *m/z* [$(M + Na)^+$]: calcd. for $C_{72}H_{68}N_2O_{20}SNa$, 1357.3798; found, 1357.3782.

*2-(N-Benzyloxycarbonyl)aminoethyl 2,3,4-tri-O-(4-methyl)benzoyl-6-O-sulfo-β-D-galactopyranosyl-(1→4)-3-O-benzyl-2-deoxy-2-phthalimido-6-O-sulfo-β-D-glucopyranoside, bistrimethylamine salt (**23**).* ^{1}H-NMR δ_H (CD_3OD): 7.95–6.75 (m, 26H, Ar H), 5.83 (d, 1H, $J_{3',4'}$ = 3.18 Hz, H-4′), 5.67 (dd, 1H, $J_{1',2'}$ = 7.92 Hz, $J_{2',3'}$ = 10.26 Hz, H-2′), 5.56 (dd, 1H, H-3′), 5.40 (d, 1H, H-1′), 5.01 (d, 1H, $J_{1,2}$ = 8.58 Hz, H-1), 4.92, 4.57 (ABq, 1H, J = 12.00 Hz, $PhCH_2$), 4.69 (s, 2H, $PhCH_2$), 4.39 (br t, 1H, J = 6.63 Hz, H-5′), 4.35 (br d, 1H, J = 9.30 Hz, H-6a), 4.14 (m, 2H, H-3,6′a), 4.01 (m, 3H, H-4,6b,6′b), 3.86 (dd, 1H, $J_{2,3}$ = 10.44 Hz, H-2), 3.57 (m, 1H, 1/2OCH_2), 3.36 (m, 1H, 1/2OCH_2), 3.35 (d, 1H, $J_{5,6a}$ = 9.96 Hz, H-5), 2.99 (t, 2H, NCH_2), 2.82 (s, 18 H, 2NMe_3), 2.25, 2.18, 2.17 (3 s, each 3H, 3MePh). ESI-HRMS *m/z* [$(M + Na)^+$]: calcd. for $C_{61}H_{58}N_2O_{23}S_2Na_{1,\ 3}$319.2559; found, 1319.2536.

*Biotinylated KS (0S) disaccharide (**1**).* To a solution of **18** (57.2 mg, 46.4 μmol) in EtOH (2.4 mL) was added 1,3-diaminopropane (232 μL, 2.8 mmol). The reaction mixture was heated overnight under reflux, and then the volatiles were removed under reduced pressure. To the residue in MeOH (5.0 mL), Ac_2O (0.2 mL) and Et_3N (0.2 mL) were added while stirring overnight. The volatiles were removed again, and the residue was subjected to reverse-phase chromatography in a Bond Elut C8 column (0–90% MeOH). The product was dissolved in 50% aq. EtOH (2.0 mL) and then hydrogenated in the presence of a catalytic amount of Pd-black in an H_2 atomosphere, while stirring for 2 d. H_2O (1.0 mL) and AcOH (1 drop) were added to the reaction mixture, and the reaction was continued for 4 d. The insoluble materials were removed on Celite, and the volatiles were removed under reduced pressure. The product having free amine at the end of the linker was dissolved in 1 M Na_3PO_4 and 0.15 M NaCl (1.5 mL). To this solution, NHS-PEG_4-biotin (60.4 mg, 99.7 μmol) was added while stirring overnight. The reaction mixture was treated in an LH-20 gel permeation column (H_2O) to quantitatively give **1** from **18**. ^{1}H-NMR δ_H (D_2O, selected): 4.51 (dd, 1H, $J_{b,c}$ = 7.92 Hz, $J_{c,d}$ = 4.86 Hz, H-c), 4.48 (d, 1H, $J_{1,2}$ = 8.37 Hz, H-1), 4.38 (d, 1H, $J_{1',2'}$ = 7.86 Hz, H-1′), 4.33 (dd, 1H, $J_{b,e}$ = 4.50 Hz, H-b), 4.00 (m, 1H, H-r), 3.90 (dd, 1H, $J_{5',6'a}$ = 2.16 Hz, J_{gem} = 12.18 Hz, H-6′a), 3.84 (d, 1H, $J_{3',4'}$ = 3.36 Hz, H-4′), 3.79 (m, 1H, H-r), 3.75 (dd, 1H, $J_{5',6'b}$ = 5.10 Hz, H-6′b), 3.75–3.60 (m, 2H, H-6ab), 3.67 (m, 2H, H-2,3), 3.58 (dd, 1H, $J_{2',3'}$ = 9.90 Hz, H-3′), 3.52 (m, 3H, H-4,5,5′), 3.45 (dd, 1H, H-2′), 3.24 (m, 1H, H-e), 3.17 (m, 2H, H-q), 2.90 (dd, 1H, J_{gem} = 13.02 Hz, H-d), 2.69 (d, 1H, H-d′), 1.96 (s, 3H, NAc). ESI-HRMS *m/z* [$(M + Na)^+$]: calcd. for $C_{37}H_{65}N_5O_{18}SNa$, 922.3938; found, 922.3925.

*Biotinylated KS (6'S) disaccharide (**2**).* To a solution of **21** (80.9 mg, 65.4 μmol) in EtOH (3.5 mL) was added 1,3-diaminopropane (290 μL, 3.9 mmol). The reaction mixture was heated overnight under reflux, and then the volatiles were removed under reduced pressure. To the residue in MeOH (2.3 mL), Ac_2O (0.3 mL) and Et_3N (0.3 mL) were added while stirring overnight. The volatiles were removed again, and the residue was subjected to reverse-phase chromatography in a Bond Elut C8 column (0–90% MeOH). To a solution of the product in MeOH (0.6 mL) was added 0.5 M NaOH (250 μL). The reaction mixture was heated under reflux for 9 h and then quenched with 1% AcOH. The volatiles were removed under reduced pressure, and the residue was purified through a Bond Elut C8 column (0–90% MeOH) as already described. The product was dissolved in 50% aq. 2-PrOH (1.0 mL) and then hydrogenated in the presence of a catalytic amount of Pd-black in an H_2 atomosphere while stirring for 1 d. H_2O (1.0 mL) and AcOH (1 drop) were added to the reaction mixture, and the reaction was continued for 2 d. The insoluble materials were removed on Celite, and the volatiles were removed under reduced pressure. The product was dissolved in 1 M Na_3PO_4 and 0.15 M NaCl (0.8 mL). To this solution, NHS-PEG_4-biotin (27.7 mg, 47.1 μmol) was added while stirring overnight. The reaction mixture was treated in an LH-20 gel permeation column (H_2O) to quantitatively give **2** from **21**. ^{1}H-NMR δ_H (D_2O, selected): 4.45 (m, 2H, H-c, 1), 4.37 (d, 1H, $J_{1',2'}$ = 8.37 Hz, H-1′), 4.27 (m, 1H, H-b), 4.13 (d, 1H, J_{gem} = 4.74 Hz, H-6′a), 3.96 (d, 1H, H-6′b), 3.75 (m, 1H, H-2), 3.74–3.59 (m, 2H, H-6ab), 3.57 (m, 1H, H-2′), 3.36 (m, 1H, H-e), 2.85 (dd, 1H, $J_{c,d}$ = 5.10 Hz, J_{gem} = 13.02 Hz, H-d), 2.63 (d, 1H, H-d′), 1.90 (s, 3H, NAc). ESI-HRMS *m/z* [$(M + Na)^+$]: calcd. for $C_{37}H_{64}N_5O_{21}S_2Na_2$, 1024.3325; found, 1024.3331.

*Biotinylated KS (6S) disaccharide (**3**).* To a solution of **22** (64.1 mg, 46.7 μmol) in EtOH (2.7 mL) was added 1,3-diaminopropane (234 μL, 2.8 mmol). The reaction mixture was heated overnight under reflux, and then the volatiles were removed under reduced pressure. To the residue in MeOH (1.5 mL) were added Ac_2O (0.2 mL) and Et_3N (0.2 mL) while stirring overnight. The volatiles were removed again, and the residue was subjected to reverse-phase chromatography in a Bond Elut C8 column (0–90% MeOH). To a solution of the product in MeOH (0.6 mL) was added 0.5 M NaOH (250 μL). The reaction mixture was heated under reflux for 6 h and then quenched with 1% AcOH. The volatiles were removed under reduced pressure, and the residue was purified through Bond Elut C8 (0–90% MeOH) as already described. The product was dissolved in 50% 2-PrOH (1.0 mL) and then hydrogenated in the presence of a catalytic amount of Pd-black in an H_2 atmosphere while stirring for 2 d. H_2O (1.0 mL) and AcOH (1 drop) were added to the reaction mixture, and the reaction was continued for 4 d. The insoluble materials were removed on Celite, and the volatiles were removed under reduced pressure. The product having free amine at the end of the linker was dissolved in 1 M Na_3PO_4 and 0.15 M NaCl (0.8 mL). To this solution, NHS-PEG_4-biotin (9.9 mg, 18.5 μmol) was added while stirring overnight. The reaction

mixture was treated in an LH-20 gel permeation column (H_2O) to quantitatively give **3** from **22**. ^{1}H-NMR δ_H (D_2O, selected): 4.54 (m, 1H, H-c), 4.51 (m, 2H, H-1,1′), 4.38 (m, 1H, H-b), 4.35 (m, 1H, H-6a), 4.27 (br s, 1H, H-6b), 3.86 (br d, 1H, $J_{3',4'}$ = 3.48 Hz, H-4′), 3.85–3.58 (m, 2H, H-6′ab),3.70 (m, 1H, H-2), 3.68 (m, 1H, H-3), 3.62 (m, 1H, H-3′), 3.63 [dd, 1H, $J_{1',2'}$, $J_{2',3'}$ = 9.87, 7.98 Hz (reversible), H-2′], 3.28 (m, 1H, H-e), 2.91 (dd, 1H, $J_{c,d}$ = 4.95 Hz, J_{gem} = 13.02 Hz, H-d), 2.71 (d, 1H, H-d′), 1.96 (s, 3H, NAc). ESI-HRMS *m/z* [$(M + Na)^+$]: calcd. for $C_{37}H_{64}N_5O_{21}S_2Na_{2,\ 10}$24.3325; found, 1024.3325.

*Biotinylated KS (6,6′-diS) disaccharide (**4**).* To a solution of **23** (67.1 mg, 49.0 μmol) in EtOH (2.8 mL) was added 1,3-diaminopropane (245 μL, 2.9 mmol). The reaction mixture was heated overnight under reflux, and then the volatiles were removed under reduced pressure. To the residue in MeOH (1.5 mL), Ac_2O (0.2 mL) and Et_3N (0.2 mL) were added while stirring overnight. The volatiles were removed again, and the residue was subjected to Bond Elut C8 reverse-phase chromatography (0–90% MeOH). To a solution of the product in 50% aq. MeOH (1.5 mL) was added 0.5 M NaOH (200 μL). The reaction mixture was heated under reflux for 8 h and then quenched with 1% AcOH. The volatiles were removed under reduced pressure, and the residue was purified through Bond Elut C8 (0–90% MeOH) as already described. The product was dissolved in H_2O (1.5 mL) and AcOH (1 drop) and then hydrogenated in the presence of a catalytic amount of Pd-black in an H_2 atmosphere while stirring for 4 d. The insoluble materials were removed on Celite, and the volatiles were removed under reduced pressure. The product having free amine at the end of the linker was dissolved in 1 M Na_3PO_4 and 0.15 M NaCl (0.3 mL). To this was added NHS-PEG_4-biotin (2.6 mg, 4.6 μmol) while stirring overnight. The reaction mixture was treated in an LH-20 gel permeation column (H_2O) to quantitatively give **4** from **23**. ^{1}H-NMR δ_H (D_2O, selected): 4.54 (dd, 1H, $J_{b,c}$ = 7.92 Hz, $J_{c,d}$ = 4.86 Hz, H-c), 4.49 (d, 1H, $J_{1,2}$ = 7.92 Hz, H-1), 4.47 (d, 1H, $J_{1',2'}$ = 7.86 Hz, H-1′), 4.35 (m, 2H, H-b,6a), 4.24 (m, 1H, H-6b), 4.10 (m, 1H, H-6′a), 3.94 (d, 1H, $J_{3',4'}$ = 3.06 Hz, H-4′), 3.93 (dd, 1H, H-6′b), 3.84 (m, 1H, H-r), 3.70 (m, 1H, H-3), 3.69 (br t, 1H, J = 5.94 Hz, H-2), 3.63 (m, 1H, H-3′), 3.48 (dd, 1H, $J_{2',3'}$ = 10.02 Hz, H-2′), 3.33 (m, 2H, H-q), 3.27 (m, 1H, H-e), 2.93 (dd, 1H, J_{gem} = 13.02 Hz, H-d), 2.72 (d, 1H, H-d′), 1.94 (s, 3H, NAc). ESI-HRMS *m/z* [$(M + Na)^+$]: calcd. for $C_{37}H_{63}N_5O_{24}S_3Na_{1,\ 3}$126.2713; found, 1126.2700.

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