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Synthesis of sulfated phenyl 2-acetamido-2-deoxy-D-galactopyranosides. 4-*O*-Sulfated phenyl 2-acetamido-2-deoxy-β-D-galactopyranoside is a competitive acceptor that decreases sulfation of chondroitin sulfate by *N*-acetylgalactosamine 4-sulfate 6-*O*-sulfotransferase

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Abstract—We have previously cloned *N*-acetylgalactosamine 4-sulfate 6-*O*-sulfotransferase (GalNAc4S-6ST), which transfers sulfate from 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to the C-6 hydroxyl group of the GalNAc 4-sulfate residue of chondroitin sulfate A and forms chondroitin sulfate E containing GlcA-GalNAc(4,6-SO_4) repeating units. To investigate the function of chondroitin sulfate E, the development of specific inhibitors of GalNAc4S-6ST is important. Because GalNAc4S-6ST requires a sulfate group attached to the C-4 hydroxyl group of the GalNAc residue as the acceptor, the sulfated GalNAc residue is expected to interact with GalNAc4S-6ST and affect its activity. In this study, we synthesized phenyl α- or -β-2-acetamido-2-deoxy-β-D-galactopyranosides containing a sulfate group at the C-3, C-4, or C-6 hydroxyl groups and examined their inhibitory activity against recombinant GalNAc4S-6ST. We found that phenyl β-GalNAc(4SO_4) inhibits GalNAc4S-6ST competitively and also serves as an acceptor. The sulfated product derived from phenyl β-GalNAc(4SO_4) was identical to phenyl β-GalNAc(4,6-SO_4). These observations indicate that derivatives of β-D-GalNAc(4SO_4) are possible specific inhibitors of GalNAc4S-6ST.

Keywords: GalNAc4S-6ST; Chondroitin sulfate; Sulfotransferase; Inhibitor; Sulfated 2-acetamido-2-deoxy-β-D-galactopyranose; Synthesis

1. Introduction

Chondroitin sulfate is a glycosaminoglycan composed of β-D-GlcA-(1→3)-β-D-GalNAc-(1→4) repeating units bearing sulfate moieties on various hydroxyl groups of the sugar residues. CS-E is an isomer in which the C-4 and C-6 hydroxyl groups of the GalNAc residue are sulfated (GlcA-GalNAc(4,6-SO_4)). CS-E is present in

Abbreviations: CS-E, chondroitin sulfate E; CS-A, chondroitin sulfate A; GalNAc4S-6ST, *N*-acetylgalactosamine 4-sulfate 6-*O*-sulfotransferase; C4ST, chondroitin 4-sulfotransferase; C6ST, chondroitin 6-sulfotransferase; GalNAc(4SO_4), 2-acetamido-2-deoxy-4-*O*-sulfonato-D-galactopyranose; GalNAc(6SO_4), 2-acetamido-2-deoxy-6-*O*-sulfonato-D-galactopyranose; GalNAc(4,6-SO_4), 2-acetamido-2-deoxy-4,6-di-*O*-sulfonato-D-galactopyranose; GlcA, D-glucuronic acid; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; ΔDi-0S, 2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-D-galactopyranose; ΔDi-6S, 2-acetamido-2-deoxy-3-*O*-(4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid)-6-*O*-sulfonato-D-galactopyranose; ΔDi-4S, 2-acetamido-2-deoxy-3-*O*-(4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid)-4-*O*-sulfonato-D-galactopyranose; ΔDi-diS_D, 2-acetamido-2-deoxy-3-*O*-(4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid)-6-*O*-sulfonato-D-galactopyranose; ΔDi-diS_E, 2-acetamido-2-deoxy-3-*O*-(4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid)-4,6-di-*O*-sulfonato-D-galactopyranose.

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various cells such as mast cells,[1–4] polymorphonuclear granulocytes,[5,6] monocytes,[7–10] and macrophages[11] and is thought to be involved in the immunological response of mast cells,[12] the binding of platelet factor 4 to neutrophils,[6] the regulation of procoagulant activity,[10] and the binding of lipoprotein lipase to macrophages.[11] CS-E was reported to inhibit the binding of versican to L-selectin or chemokines, and to bind L-selectin or chemokines directly.[13,14] CS-E, as well as a tetrasaccharide containing GlcA-GalNAc(4,6-SO_4) units, was reported to promote neurite outgrowth.[15,16] The presence of nonreducing terminal GalNAc(4,6-SO_4) residues has been reported in chondroitin sulfate attached to aggrecan[17–20] and thrombomodulin.[21] Proportions of the nonreducing terminal GalNAc(4,6-SO_4) residue of aggrecan was found to be decreased in human osteoarthritis.[22]

GalNAc4S-6ST transfers sulfate to the C-6 hydroxyl group of GalNAc(4SO_4) residues of chondroitin sulfate.[23,24] We identified human GalNAc4S-6ST cDNA on the basis of the amino acid sequences of purified squid GalNAc4S-6ST.[25] Recombinant human GalNAc4S-6ST transfers sulfate to the C-6 hydroxyl group of GalNAc(4SO_4) residues located at the nonreducing terminal and internal repeating disaccharide region.[25] We found that a unique nonreducing terminal structure is present in CS-A, and GalNAc4S-6ST should be involved in the production of the nonreducing terminal structure.[26] To obtain information about the function of GalNAc(4,6-SO_4) residues contained in CS-E or located at the nonreducing terminal of CS, it is important to regulate the activity of GalNAc4S-6ST. The enzyme requires a sulfate group attached to the 4-hydroxyl group of the GalNAc residue as the acceptor,[25] suggesting that the sulfated GalNAc residue interacts with GalNAc4S-6ST and affects its enzymatic activity. In this study, we synthesized the α- or β-phenyl glycoside derivatives of 2-acetamido-2-deoxy-D-galactopyranose, which were sulfated at the C-3, C-4, or C-6 hydroxyl groups and examined their inhibitory activity against recombinant GalNAc4S-6ST. We found that phenyl β-GalNAc(4SO_4) inhibited GalNAc4S-6ST activity competitively.

2. Results and discussion

2.1. Synthesis

Strategies with minimal synthetic steps from D-galactosamine were designed for the synthesis of the GalNAc(4SO_4) and GalNAc(3SO_4) derivatives, in which nonsulfated positions of the GalNAc residues were selectively protected. For the synthesis of the β-GalNAc(4SO_4) derivative, D-glucosamine was used as the starting material. This synthetic route is applicable for the synthesis of β-GalNAc(4SO_4) derivatives with various aglycons from a readily available starting material. For the synthesis of GalNAc(6SO_4) derivatives, the highly reactive primary 6-hydroxyl group of GalNAc was sulfated without protection.

Phenyl α-GalNAc(4SO_4) (**7**) was synthesized as depicted in Scheme 1. The reaction of fully acetylated galactosamine **1** with phenol in the presence of $ZnCl_2$ and activated $CaSO_4$ gave phenyl glycoside **2** with α-selectivity. Triol **4** was obtained from **2** after reaction with sodium methoxide in methanol in good yield. Galactopyranoside **5** was synthesized by the selective 3,6-di-*O*-pivaloylation[27] of **4**. Target **7** was then obtained by treatment of **5** with sulfur trioxide–pyridine complex in pyridine, followed by de-*O*-pivaloylation using sodium methoxide in methanol.

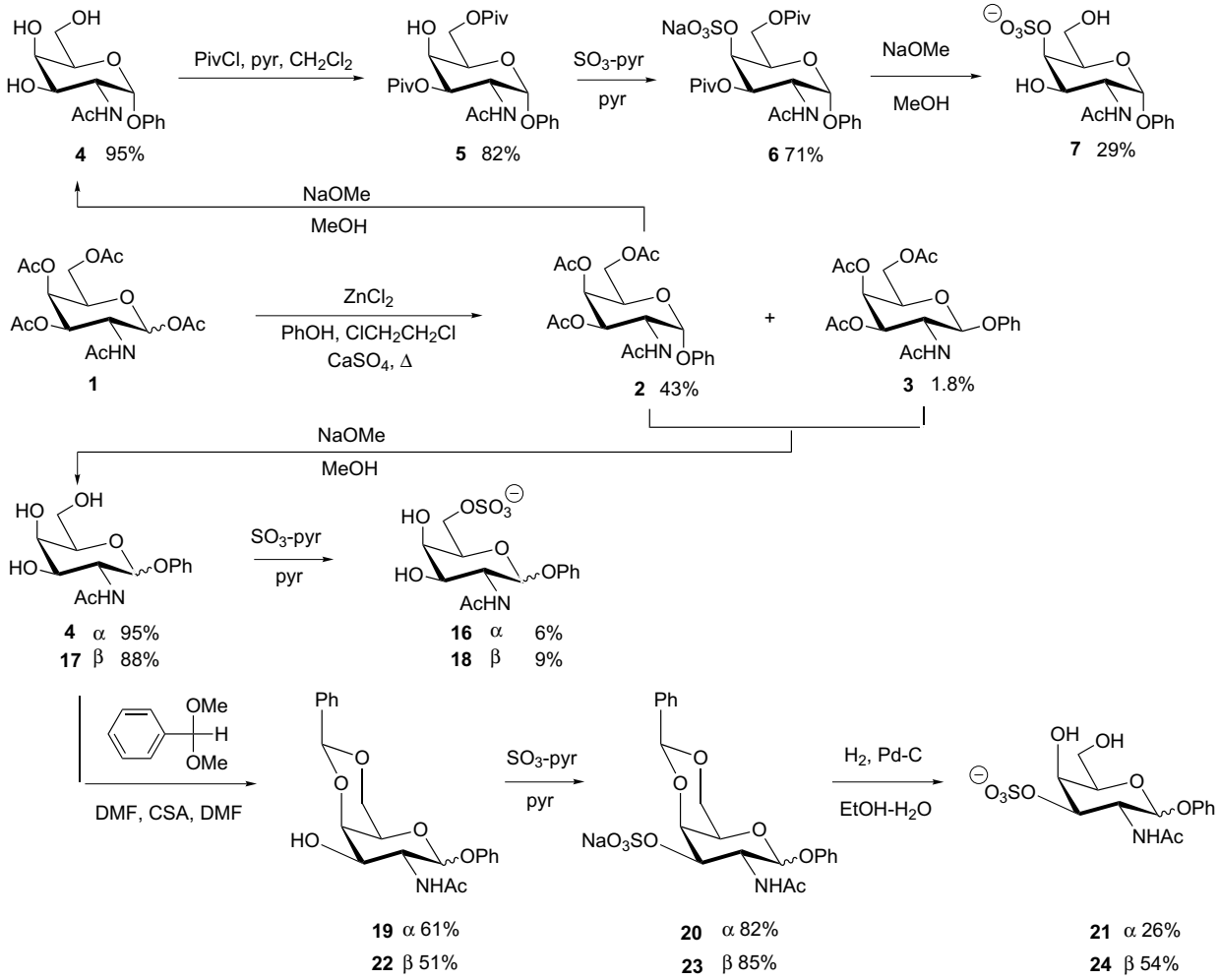
As a starting material for synthesis of phenyl β-GalNAc(4SO_4) **15**, thioglycoside **8** was prepared from D-glucosamine hydrochloride by Ogawa's method (Scheme 2).[28] To invert the 4-position of GlcNPhth, **8** was subjected to mesylation followed by S_N2 reaction by potassium acetate in the presence of 18-crown-6 ether. Thioglycoside **10** thus obtained was converted to galactosyl fluoride **11** in good yield by treating with DAST and NBS in CH_2Cl_2. β-Selective glycosylation of **11** with phenol was carried out by activation of glycosyl fluoride with the Cp_2HfCl_2–$AgClO_4$ promoter system. The phthalimido group of **12** was converted to an acetamido group, and then the acetyl group at the 4-position of the GalNAc residue was removed to give compound **13**. The 4-sulfated target compound **15** was obtained by sulfation with sulfur trioxide followed by removal of the benzyl groups with Pd–C catalyzed hydrogenation.

The GalNAc(6SO_4) derivatives **16** and **18** were prepared from the corresponding triol **4** and **17**, respectively, by sulfation with SO_3–pyr (Scheme 1).

The GalNAc(3SO_4) derivatives **21** and **24** were prepared from triol **4** and **17**, respectively, by protection of the 4- and 6-hydroxyl groups as benzylidene acetals **19** and **22**, followed by sulfation and removal of benzylidene group (Scheme 1).

Phenyl β-GalNAc(4,6-SO_4) **28** was synthesized from benzylidene acetal **22** (Scheme 3), by first pivaloylation of the 3-hydroxyl group to give **25**, which was then subjected to removal of benzylidene group, sulfation, and deprotection to give phenyl β-GalNAc(4,6-SO_4) **28**.

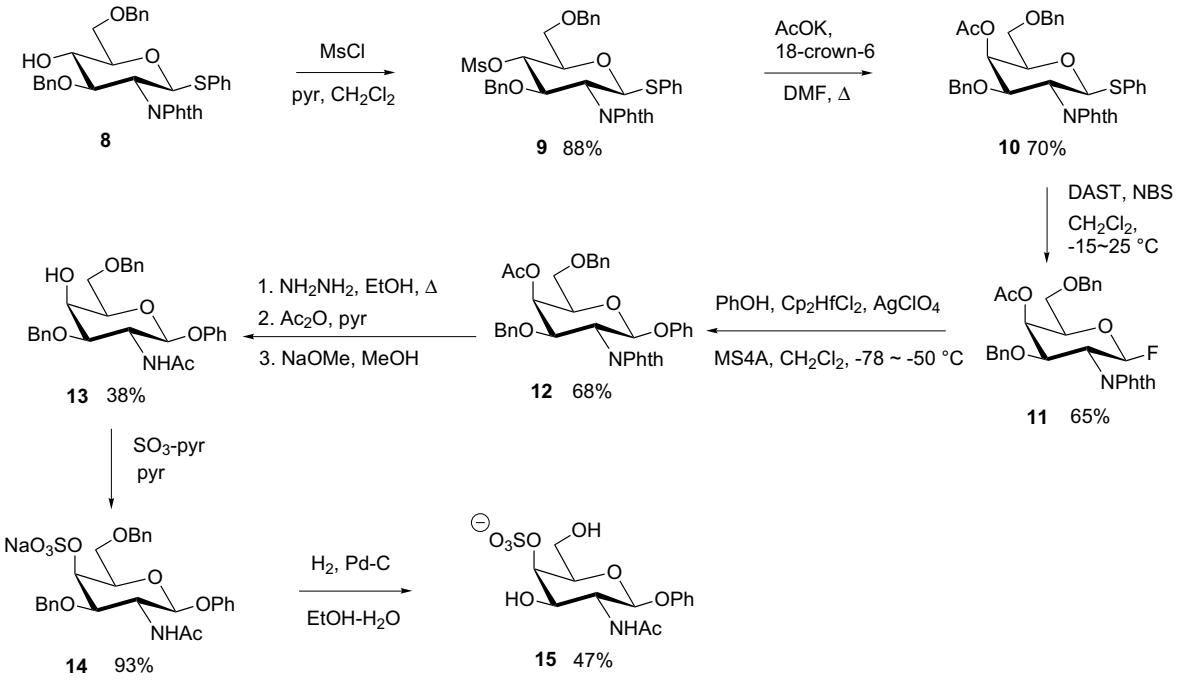
In the ^1H NMR spectrum of the products, the signal for the hydrogens attached to the carbon bearing the *O*-sulfonate group were shifted downfield relative to the corresponding signals in **4** or **17**. For example, the H-4 signal in the spectrum for phenyl β-GalNAc(4SO_4) **15** was shifted by 0.8 ppm relative to the corresponding signal in the spectrum of **17**. Similarly, the downfield shift of the H-3 signal for the α-GalNAc(3SO_4) derivative **21** by 0.65 ppm relative to the corresponding signal for **4** indicates that **21** has *O*-sulfate group at C-3.



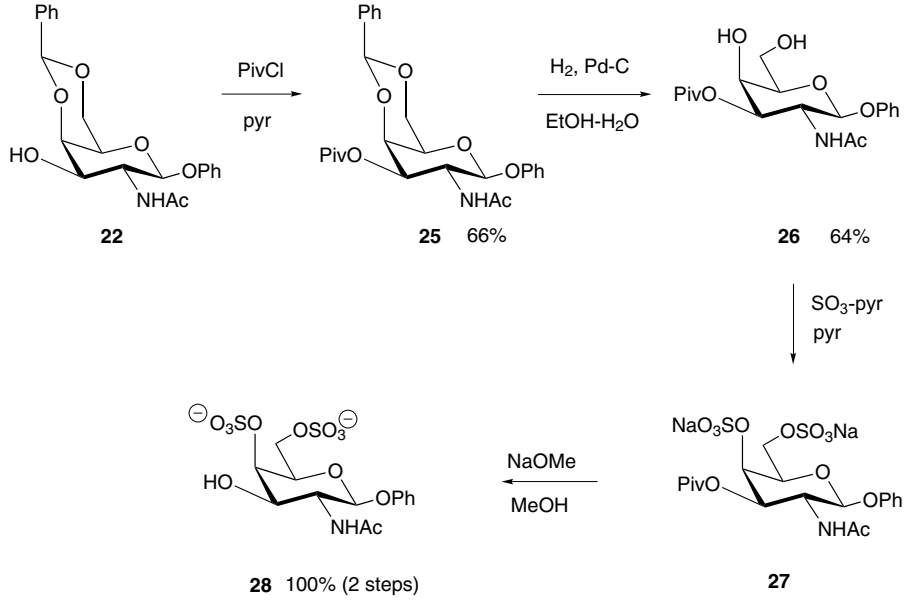
Scheme 1. Synthetic route to **7**, **16**, **18**, **21**, and **24** from **1**.

2.2. Inhibition of GalNAc4S-6ST

Compounds **7**, **15**, **16**, **18**, **21**, and **24** were tested as inhibitors for GalNAc4S-6ST activity. Dose dependent inhibition of GalNAc4S-6ST was observed for compound **15** (phenyl β-GalNAc($4SO_4$)); in the presence of 5 mM of the inhibitor, the GalNAc4S-6ST activity decreased to 24% of the control (Fig. 1). Compound **16** (phenyl α-GalNAc($6SO_4$)) was shown to inhibit rather weakly. GalNAc4S-6ST is thus thought to recognize not only the position of sulfate group on the GalNAc residue but also configuration of the anomeric carbon of the sugar residue. Neither C4ST nor C6ST was inhibited by compound **15** (Fig. 2); indicating that the observed inhibitory activity of **15** is specific to GalNAc4S-6ST. GalNAc4S-6ST transfers sulfate to the C-6 hydroxyl group of GalNAc($4SO_4$) residues located at the nonreducing terminal and in the internal repeating units.[25] We examined whether the degree of inhibition by phenyl β-GalNAc($4SO_4$) (**15**) depends on the location of GalNAc($4SO_4$) residue. Thus, ^{35}S-labeled glycosaminoglycan was digested with chondroitinase ACII and the degradation products were separated by SAX-HPLC. As shown in Figure 3, the proportion of the radioactivity recovered in ΔDi-diS_E, which was derived from the internal repeating disaccharide units, and the radioactivity recovered in GalNAc(4,6-SO_4) and β-GalNAc(4,6-SO_4)-(1→4)-β-GlcA($2SO_4$)-(1→3)-GalNAc-($6SO_4$), which were derived from the nonreducing terminal, was not altered in the presence of the inhibitor. This indicates that sulfation of the 6-hydroxyl group of nonreducing terminal GalNAc($4SO_4$) residue and internal GalNAc($4SO_4$) residues was inhibited equally by phenyl β-GalNAc($4SO_4$) (**15**). GalNAc4S-6ST was found to require the presence of sulfate moiety attached to the C-4 hydroxyl group of GalNAc residue; therefore, phenyl β-GalNAc($4SO_4$) (**15**) may be recognized by the acceptor binding domain of GalNAc4S-6ST. If this is the case, phenyl β-GalNAc($4SO_4$) (**15**) may act as a competitive inhibitor. To clarify the mode of inhibition by phenyl β-GalNAc($4SO_4$) (**15**), the effect of the concentration of the acceptor and the inhibitor was determined. As



Scheme 2. Synthetic route to **15** from **8**.



Scheme 3. Synthetic route to **28** from **22**.

shown in Figure 4, it is evident that phenyl β-GalNAc(4SO$_4$) (**15**) inhibited GalNAc4S-6ST competitively; the K_m for CS-A was 0.038 mM and the K_i for phenyl β-GalNAc(4SO$_4$) (**15**) was 0.98 mM. When phenyl β-GalNAc(4SO$_4$) (**15**) was added to the reaction mixture instead of CS-A, a sulfated product was detected by paper electrophoresis (Fig. 5B). This material behaved identically with phenyl β-GalNAc(4,6-SO$_4$) (**28**) in the SAX-HPLC (Fig. 6), indicating that sulfate was transferred to the C-6 hydroxyl group of the GalNAc(4SO$_4$) residue of phenyl β-GalNAc(4SO$_4$) (**15**). These observations indicate that phenyl β-GalNAc(4SO$_4$) served not only as a competitive inhibitor, but also as an acceptor for GalNAc4S-6ST. Phenyl β-GalNAc(4SO$_4$) (**15**) is thus a competitive acceptor that decreases sulfation of chondroitin sulfate by *N*-acetylgalactosamine 4-sulfate 6-*O*-sulfotransferase. Unlike phenyl β-GalNAc(4SO$_4$) (**15**), phenyl β-GalNAc(4,6-SO$_4$)

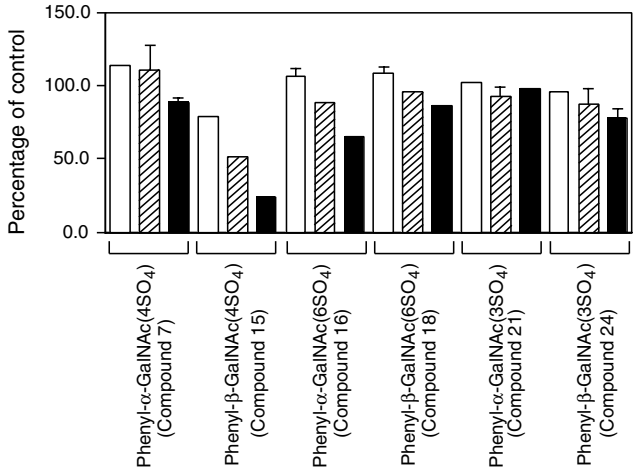


Figure 1. Inhibition of GalNAc4S-6ST activity by various sulfated phenyl GalNAc derivatives. The sulfotransferase reaction was carried out as described in Section 3.3. Sulfated phenyl GalNAc derivatives were added to the reaction mixtures at a final concentration of 1.0 mM (open bar), 2.5 mM (hatched bar), or 5.0 mM (closed bar). Values are averages of three determinations and the standard deviation is indicated by perpendicular lines.

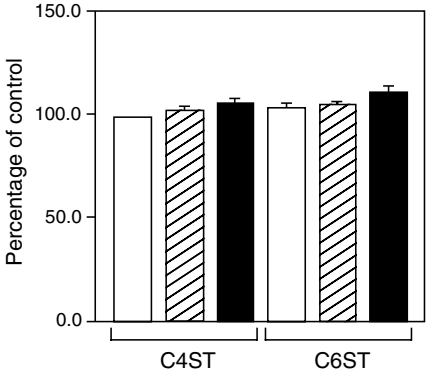


Figure 2. Effect of phenyl β-GalNAc($4SO_4$) (**15**) on the activities of C4ST and C6ST. The sulfotransferase reaction was carried out as described in Section 3.3. Phenyl β-GalNAc($4SO_4$) (**15**) was added to the reaction mixtures at a final concentration of 1.0 mM (open bar), 2.5 mM (hatched bar), or 5.0 mM (closed bar). Values are averages of three determinations and the standard deviation is indicated by perpendicular lines.

(**28**) did not inhibit GalNAc4S-6ST activity at all under the same conditions (data not shown), suggesting that the product of the enzymatic reaction with GalNAc4S-6ST may not have detectable affinity for the active site of the enzyme.

In this letter, we found that phenyl β-GalNAc($4SO_4$) (**15**) inhibited sulfation of CS-A by GalNAc4S-6ST in vitro. Membrane permeable glycosides were found to act as inhibitors of glycosyl transferases in the living cells.[29,30] Therefore, it is possible that β-GalNAc($4SO_4$) derivatives containing various hydrophobic might be able to inhibit CS-E synthesis in living cells.

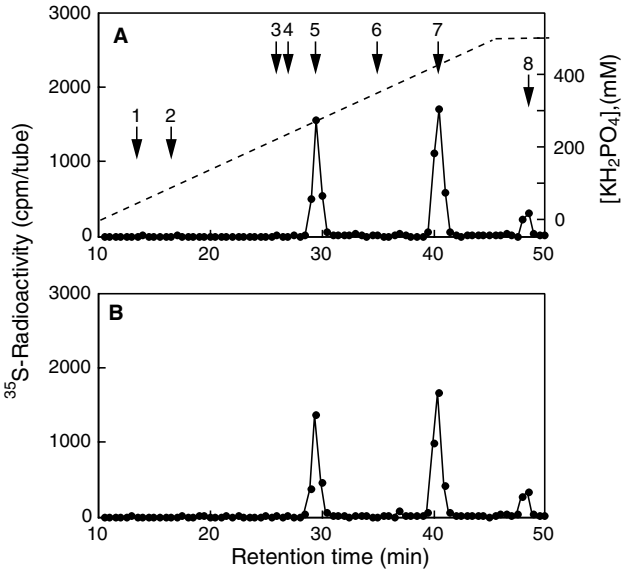


Figure 3. Effect of phenyl β-GalNAc($4SO_4$) (**15**) on the sulfation of internal and nonreducing terminal GalNAc($4SO_4$) residues. The sulfotransferase reaction with GalNAc4S-6ST was carried out as described in Section 3.3 in the absence (A) or presence (B) of 5 mM phenyl β-GalNAc($4SO_4$) (**15**). The ^{35}S-labeled glycosaminoglycans were digested with chondroitinase ACII, and subjected to SAX-HPLC. The arrows indicate the elution position of GalNAc($6SO_4$) (arrow 1); GalNAc($4SO_4$) (arrow 2); ΔDi-6S (arrow 3); ΔDi-4S (arrow 4); GalNAc(4,6-SO_4) (arrow 5); ΔDi-diS_D (arrow 6); ΔDi-diS_E (arrow 7); and β-GalNAc(4,6-SO_4)-(1→4)-β-GlcA($2SO_4$)-(1→3)-GalNAc-($6SO_4$) (arrow 8).

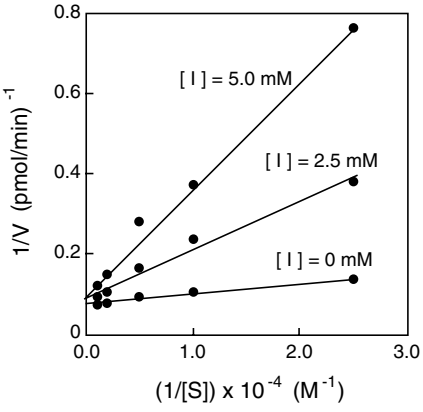


Figure 4. Competitive inhibition of GalNAc4S-6ST by phenyl β-GalNAc($4SO_4$) (**15**). The GalNAc4S-6ST activity was determined at the varying concentration of CS-A and phenyl β-GalNAc($4SO_4$) (**15**). Data are expressed as a Lineweaver–Burk plot.

3. Experimental

3.1. General methods

Structures of synthetic compounds were confirmed by ^{1}H NMR, ^{13}C NMR, and two-dimensional NMR (COSY, HMQC, HMBC). ^{1}H and ^{13}C NMR spectra

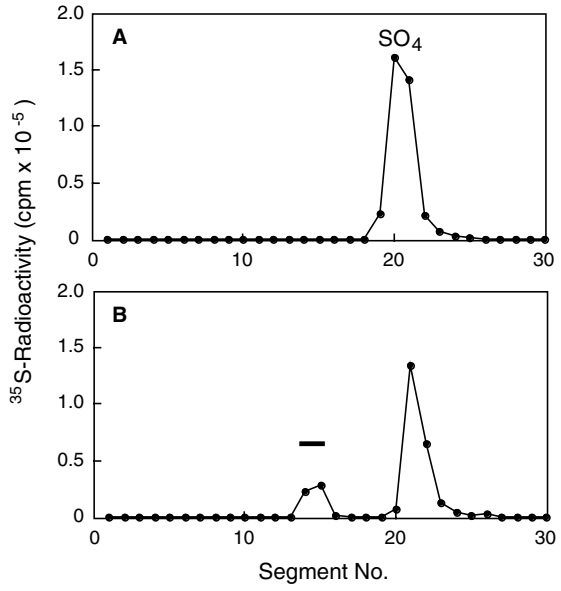


Figure 5. Sulfation of phenyl β-GalNAc($4SO_4$) (**15**) by GalNAc4S-6ST. The sulfotransferase reaction was carried out as described in Section 3.3 using phenyl β-GalNAc($4SO_4$) as the acceptor instead of CS-A. The reaction mixtures without acceptor (A) or with acceptor (B) were subjected to the paper electrophoresis in buffer B after degradation of [^{35}S]PAPS by mild acid hydrolysis. The fractions indicated by a bar in (B) were used for further analysis.

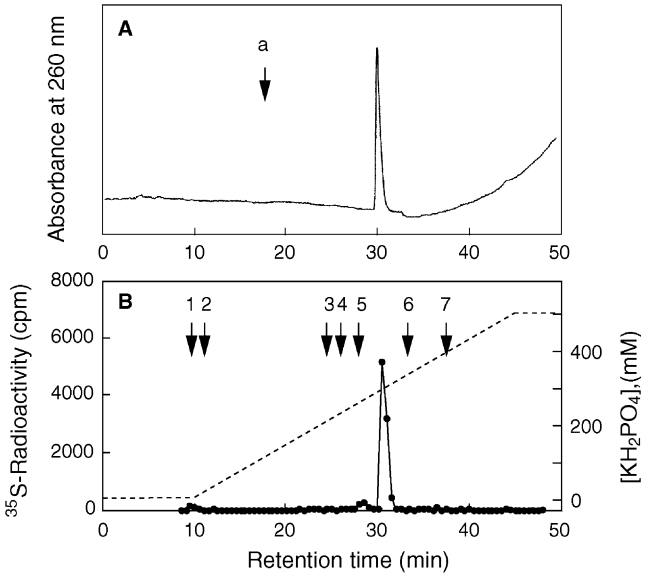


Figure 6. HPLC separation of the reaction product derived from phenyl β-GalNAc($4SO_4$) (**15**) after the reaction with GalNAc4S-6ST. (A) Elution pattern of phenyl β-GalNAc(4,6-SO_4) (**28**). (B) Elution pattern of the reaction product derived from phenyl β-GalNAc($4SO_4$) (**15**) after the reaction with GalNAc4S-6ST. The standards were the same as those described under the legend for Figure 3. Arrow a in (A) indicates the elution position of phenyl β-GalNAc($4SO_4$) (**15**).

were recorded with a JEOL LA-400 spectrometer operating at 399.65 and 100.40 MHz, respectively. Chemical shifts were referenced to TMS. Electrospray ionization mass spectra (ESI) were obtained on a LCQ (Thermoquest) or a LCT (Micromass) spectrometer.

3.2. Preparation of the recombinant human GalNAc4S-6ST, C4ST, and C6ST

Recombinant GalNAc4S-6ST (EC 2.8.2.-), C4ST (EC 2.8.2.5), and C6ST (EC 2.8.2.17) were expressed as fusion proteins with FLAG peptide in COS-7 cells, and were affinity purified as described previously.[25,31]

3.3. Assay of sulfotransferase activity

Sulfotransferase activities of GalNAc4S-6ST, C4ST, and C6ST were assayed by the methods described previously.[25,32,33] The standard assay mixture for GalNAc4S-6ST contained, in a final volume of 50 μL, 2.5 μmol of imidazole–HCl, pH 6.8, 0.5 μmol of $CaCl_2$, 1 μmol of reduced glutathione, 25 nmol (as D-galactosamine) of CS-A, 50 pmol of [^{35}S]PAPS (about 5.0×10^5 cpm), the inhibitors and the enzyme. To determine the activity toward phenyl β-GalNAc($4SO_4$) (**15**), CS-A was replaced with 25 nmol of compound **15**. The standard reaction mixture for C4ST or C6ST contained 2.5 μmol of imidazole–HCl, pH 6.8, 1.25 μg of protamine chloride, 0.1 μmol dithiothreitol, 25 nmol (as D-galactosamine) chondroitin, 50 pmol [^{35}S]PAPS (about 5.0×10^5 cpm), and enzyme in a final volume of 50 μL. The reaction mixtures were incubated at 37 °C for 20 min and the reaction was stopped by immersing the reaction tubes in a boiling waterbath for 1 min. After the reaction was stopped, ^{35}S-labeled glycosaminoglycans were isolated by the precipitation with ethanol followed by gel chromatography with a Fast Desalting Column as described previously,[33] and the radioactivity was determined. When phenyl β-GalNAc($4SO_4$) (**15**) was used as the acceptor, the reaction was stopped by the addition of 30 μL 0.25 M HCl and the mixture was then incubated for an additional 60 min at 37 °C to hydrolyze the remaining [^{35}S]PAPS. Under these hydrolysis conditions, sulfated products formed in the assay were completely stable. Aliquots of the reaction mixtures were applied to paper electrophoresis in buffer B as described below, and the ^{35}S-labeled products were separated from $^{35}SO_4$.

3.4. Superdex 30 chromatography, SAX-HPLC, and paper electrophoresis

A Superdex 30 16/60 column was equilibrated with 0.2 M NH_4HCO_3, and run at a flow rate of 2 mL/min. One-milliliter fractions were collected. SAX-HPLC was carried out using a Whatman Partisil-10 SAX column (4.6 mm × 25 cm) equilibrated with 5 mM KH_2PO_4. The column was developed with 5 mM KH_2PO_4 for

10 min followed by a linear gradient from 5 to 500 mM KH_2PO_4. Fractions (0.5 mL) were collected at a flow rate of 1 mL/min and a column temperature of 40 °C. Disaccharide and monosaccharide standards for SAX-HPLC were obtained from commercial sources. The β-GalNAc(4,6-SO_4)-(1→4)-β-GlcA(2SO_4)-(1→3)-GalNAc(6SO_4) standard was prepared as described.[26] Paper electrophoresis was carried out on Toyo No. 1 paper (20 cm × 60 cm) in buffer A (30 mM NH_4HCO_3) or on Whatman No. 3 paper (2.5 cm × 57 cm) in buffer B (50 mM ammonium acetate, pH 5.0) at 30 V/cm for 40 min.

3.5. Phenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside (2)

A suspension of **1** (1.556 g, 4.00 mmol), phenol (1.88 g, 20.0 mmol), $ZnCl_2$ (2.75 g, 20.2 mmol), and anhydrous $CaSO_4$ (3.05 g) in 1,2-dichloroethane (25 mL) was heated at reflux for 18 h with stirring. After cooling, the reaction was quenched with a satd aq $NaHCO_3$ (40 mL). The mixture was filtered through Celite, washed with satd aq $NaHCO_3$ and a satd aq NaCl successively, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was dissolved in toluene and separated by silica gel chromatography eluting with 35% EtOAc–hexane to give **2** (0.731 g, 43%) as a colorless solid, further elution with 40% EtOAc–hexane to gave **3** (31 mg, 1.8%) as a colorless solid. ^{1}H NMR ($CDCl_3$): δ 1.90 (s, 3H, $COCH_3$), 1.95 (s, 3H, $COCH_3$), 2.02 (s, 3H, $COCH_3$), 2.16 (s, 3H, $COCH_3$), 4.01 (dd, 1H, $J_{6a,6b}$ 12.2, $J_{5,6a}$ 6.5 Hz, H-6a), 4.10 (dd, 1H, $J_{5,6b}$ 6.5 Hz, H-6b), 4.27 (t, 1H, H-5), 4.74 (ddd, 1H, $J_{2,3}$ 11.3, $J_{2,NH}$ 10.2, $J_{1,2}$ 3.6 Hz, H-2), 5.38 (dd, 1H, $J_{3,4}$ 3.4 Hz, H-3), 5.42 (d, 1H, H-4), 5.59 (d, 1H, H-1), 5.73 (br, 1H, NH), 7.02–7.07 (m, 3H, arom. CH), and 7.27–7.32 (m, 2H, arom. CH); ^{13}C NMR ($CDCl_3$): δ 20.53 (q), 20.72 (q), 20.78 (q), 23.30 (q, 2-Ac), 47.91 (d, C-2), 61.64 (t, C-6), 67.22 (d, C-4), 67.56 (d, C-5), 68.22 (d, C-3), 96.31 (d, C-1), 116.57 (d, arom. CH), 123.10 (d, arom. CH), 129.71 (d, arom. CH), 155.96 (s, arom. C), 170.11 (s, 2-*C*OCH_3), 170.30 (s, *C*OCH_3 × 2), and 171.06 (s, 3-*C*OCH_3).

3.6. Phenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranoside (3)

^{1}H NMR ($CDCl_3$): δ 1.93 (s, 3H, $COCH_3$), 2.01 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 2.15 (s, 3H, $COCH_3$), 4.06–4.26 (m, 4H), 5.27 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 5.37–5.41 (m, 2H), 5.59 (br, 1H, NH), 6.98–7.05 (m, 3H, arom. H), and 7.26 (t, 2H, arom. H); ^{13}C NMR ($CDCl_3$): δ 20.65 (q), 20.67 (q), 20.69 (q), 23.47 (q, 2-Ac), 51.73 (d, C-2), 61.52 (t, C-6), 66.63 (d, C-4), 69.60 (d, C-3), 70.93 (d, C-5), 99.19 (d, C-1), 116.88 (d, arom. CH), 123.11 (d, arom. CH), 129.52 (d, arom. CH), 157.07 (s, arom. C), 170.25 (s, 2-*C*OCH_3), 170.39 (s, *C*OCH_3), 170.43 (s, *C*OCH_3), and 170.45 (s, *C*OCH_3).

3.7. Phenyl 2-acetamido-2-deoxy-α-D-galactopyranoside (4)

A solution of **2** (827 mg, 1.95 mmol) in dry CH_3OH (30 mL) was treated with 1 M methanolic $NaOCH_3$ (1.95 mL) for 40 min at room temperature. The mixture was neutralized with Amberlite IRC-50 H^+ resin, and concentrated under reduced pressure to give **4** (552 mg, 95%) as colorless crystals. ^{1}H NMR (CD_3OD): δ 1.99 (s, 3H, NHCO*C*H_3), 3.67 (dd, 1H, $J_{6a,6b}$ 11.2, $J_{5,6a}$ 6.1 Hz, H-6a), 3.71 (dd, 1H, $J_{5,6b}$ 6.1 Hz, H-6b), 3.94 (t, 1H, H-5), 3.98 (d, 1H, $J_{3,4}$ 3.2 Hz, H-4), 4.00 (dd, 1H, $J_{2,3}$ 10.4 Hz, H-3), 4.41 (dd, 1H, $J_{1,2}$ 3.6 Hz, H-2), 5.49 (d, 1H, H-1), 6.97–7.01 (m, 1H, arom. CH), 7.09–7.12 (m, 2H, arom. CH), 7.24–7.29 (m, 2H, arom. CH); ^{13}C NMR (CD_3OD): δ 22.57 (q, CH_3), 51.59 (d, C-2), 62.47 (t, C-6), 69.43 (d, C-3), 70.12 (d, C-4), 73.20 (d, C-5), 98.37 (d, C-1), 118.26 (d, arom. CH), 123.55 (d, arom. CH), 130.48 (d, arom. CH), 158.73 (s, arom. C), 174.10 (s, NH*C*OCH_3).

3.8. Phenyl 2-acetamido-2-deoxy-3,6-di-*O*-pivaloyl-α-D-galactopyranoside (5)

Pivaloyl chloride (0.125 mL, 1.01 mmol) was added to a solution of **4** (103 mg, 0.346 mmol) in dry pyridine (1.05 mL) and CH_2Cl_2 (1.55 mL) at 0 °C, and the mixture was stirred for 15 h at 0 °C. Because the reaction was not complete, more pivaloyl chloride (0.100 mL, 0.812 mmol) was added to the mixture, and the mixture was stirred for 7 h at room temperature. The mixture was then diluted with $CHCl_3$ (50 mL), washed with a satd aq $NaHCO_3$ and a satd aq NaCl, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was separated by silica gel chromatography eluting with 30% EtOAc–hexane to give **5** (132 mg, 82%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.04 (s, 9H, C-6-OCOC(CH_3)$_3$), 1.22 (s, 9H, C-3-OCOC(CH_3)$_3$), 1.92 (s, 3H, NHCOCH_3), 4.04 (br, 1H, H-4), 4.07–4.13 (m, 1H, H-5), 4.17 (dd, 1H, $J_{6a,6b}$ 11.3, $J_{5,6a}$ 7.4 Hz, H-6a), 4.33 (dd, 1H, $J_{5,6b}$ 4.3 Hz, H-6b), 4.86 (ddd, 1H, $J_{2,3}$ 11.2, $J_{2,NH}$ 9.8, $J_{1,2}$ 3.6 Hz, H-2), 5.32 (dd, 1H, $J_{3,4}$ 2.9 Hz, H-3), 5.56 (d, 1H, H-1), 5.76 (d, 1H, NH), 7.00–7.06 (m, 3H, arom. H), and 7.25–7.29 (m, 2H, arom. H); ^{13}C NMR ($CDCl_3$): δ 23.24 (q, NHCO*C*H_3), 26.98 (q, C-6-OCOC(*C*H_3)$_3$), 27.07 (q, C-3-OCOC(*C*H_3)$_3$), 38.62 (s, C-6-OCO*C*(CH$_3$)$_3$), 39.09 (s, C-3-OCO*C*(CH$_3$)$_3$), 47.38 (d, C-2), 63.25 (t, C-6), 67.68 (d, C-4), 69.28 (d, C-5), 70.29 (d, C-3), 96.33 (d, C-1), 116.44 (d, arom. CH), 122.83 (d, arom. CH), 129.67 (d, arom. CH), 156.09 (s, arom. C), 169.92 (s, NH*C*OCH_3), 178.33 (s, C-6-O*C*OC(CH$_3$)$_3$), and 178.60 (s, C-3-O*C*OC(CH$_3$)$_3$).

3.9. Phenyl 2-acetamido-2-deoxy-3,6-di-*O*-pivaloyl-4-*O*-sulfonato-α-D-galactopyranoside sodium salt (6)

A mixture of **5** (110.3 mg, 0.237 mmol) and sulfur trioxide–pyridine complex (76.7 mg, 0.482 mmol) in dry pyridine (5 mL) was stirred for 6 h at 40 °C. CH_3OH (1.5 mL) was then added, and the mixture was passed through the column of Dowex 50W X8 Na^+, and concentrated under reduced pressure. The residue was dissolved in 20% CH_3OH–$CHCl_3$ and separated by silica gel chromatography eluting with 30% CH_3OH–$CHCl_3$ to give **6** (95.8 mg, 71%) as colorless crystals. ^{1}H NMR (CD_3OD): δ 0.98 (s, 9H, C-6-OCOC(CH_3)$_3$), 1.22 (s, 9H, C-3-OCOC(CH_3)$_3$), 1.93 (s, 3H, NHCOCH_3), 4.23–4.29 (m, 2H, H-5, H-6a), 4.40–4.47 (m, 1H, H-6b), 4.79 (dd, 1H, $J_{2,3}$ 11.5, $J_{1,2}$ 3.7 Hz, H-2), 4.93 (d, 1H, $J_{3,4}$ 3.2 Hz, H-4), 5.20 (dd, 1H, H-3), 5.58 (d, 1H, H-1), 6.99–7.02 (m, 1H, arom. H), 7.11–7.15 (m, 2H, arom. H), 7.25–7.30 (m, 2H, arom. H); ^{13}C NMR (CD_3OD): δ 22.47 (q, NHCOCH_3), 27.42 (q, C-6-OCOC(CH_3)$_3$), 27.67 (q, C-3-OCOC(CH_3)$_3$), 39.60 (s, C-6-OCOC(CH_3)$_3$), 39.99 (s, C-3-OCOC(CH_3)$_3$), 48.01 (d, C-2), 65.97 (t, C-6), 70.23 (d, C-3), 70.56 (d, C-5), 73.90 (d, C-4), 97.60 (d, C-1), 117.91 (d, arom. CH), 123.68 (d, arom. CH), 130.61 (d, arom. CH), 158.13 (s, arom. C), 173.37 (s, NHCOCH$_3$), 179.81 (s, C-6-OCOC(CH_3)$_3$), 180.00 (s, C-3-OCOC(CH_3)$_3$).

3.10. Phenyl 2-acetamido-2-deoxy-4-*O*-sulfonato-α-D-galactopyranoside (7)

A solution of **6** (26.4 mg, 0.0465 mmol) in dry CH_3OH (1 mL) was treated with 1 M methanolic $NaOCH_3$ (0.10 mL) for 47 h at room temperature. The mixture was neutralized with Amberlite IRC-50 H^+ resin and concentrated under reduced pressure. The residue was purified by paper electrophoresis in buffer A, and gel filtration with Superdex 30 afforded **7** (5.3 mg, 29% as ammonium salt) as colorless crystals. ^{1}H NMR (D_2O): δ 1.90 (s, 3H, NHCOCH_3), 3.57–3.67 (m, 2H, H-6), 4.07–4.10 (m, 1H, H-5), 4.15 (dd, 1H, $J_{2,3}$ 11.2, $J_{3,4}$ 2.9 Hz, H-3), 4.23 (dd, 1H, $J_{1,2}$ 3.5 Hz, H-2), 4.65–4.70 (m, 1H, H-4), 5.53 (d, 1H, H-1), 6.98–7.03 (m, 3H, arom. H), 7.24–7.38 (m, 2H, arom. H); ^{13}C NMR (D_2O): δ 24.72 (q, NHCOCH_3), 52.98 (d, C-2), 63.87 (t, C-6), 69.55 (d, C-3), 74.02 (d, C-5), 79.43 (d, C-4), 98.84 (d, C-1), 119.98 (d, arom. CH), 126.05 (d, arom. CH), 132.73 (d, arom. CH), 158.92 (s, arom. C), 177.58 (s, NHCOCH$_3$); ESI-MS ($C_{14}H_{18}NO_9S^-$) m/z 376 (M^-).

3.11. Phenyl 3,6-di-*O*-benzyl-2-deoxy-4-*O*-methanesulfonyl-2-phthalimido-1-thio-β-D-glucopyranoside (9)

Methanesulfonyl chloride (0.30 mL, 3.88 mmol) was added to a solution of thioglycoside **8**[28] (0.62 g, 1.07 mmol) in dry CH_2Cl_2 (1.80 mL) and dry pyridine (1.5 mL). The mixture was stirred for 11 h at 40 °C under dry nitrogen, and diluted with $CHCl_3$ (100 mL). The solution was washed with satd aq $NaHCO_3$ (20 mL × 3), water (20 mL × 2), and brine (20 mL × 2). The organic layer was dried over Na_2SO_4 and concentrated. Purification was carried out by silica gel column chromatography eluting with 20% EtOAc–hexane to give **9** (0.62 g, 88%) as colorless needles. ^{1}H NMR ($CDCl_3$): δ 2.93 (s, 3H, CH_3), 3.77 (dd, 1H, $J_{6a,6b}$ 11.0, $J_{5,6a}$ 5.4 Hz, H-6a), 3.86 (ddd, 1H, $J_{4,5}$ 9.9, $J_{5,6b}$ 2.2 Hz, H-5), 3.92 (dd, 1H, H-6b), 4.33 (t, 1H, $J_{1,2}$ 9.9 Hz, H-2), 4.36 (d, 1H, J_{gem} 11.7 Hz, C-3-OCH(H)Ph), 4.52 (t, 1H, $J_{2,3}$ 9.9 Hz, H-3), 4.60 (s, 2H, C-6-OCH_2Ph), 4.71 (t, 1H, $J_{3,4}$ 9.9 Hz, H-4), 4.74 (d, 1H, C-3-OCH(H)Ph), 5.50 (d, 1H, H-1), 6.83–6.87 (m, 1H, arom. H), 6.91–6.95 (m, 2H, arom. H), 7.00–7.02 (m, 2H, arom. H), 7.12–7.23 (m, 3H, arom. H), 7.27–7.37 (m, 7H, arom. H), 7.65–7.78 (m, 4H, arom. H); ^{13}C NMR ($CDCl_3$): δ 38.57 (q, CH_3), 54.81 (d, C-2), 68.85 (t, C-6), 73.58 (t, C-6-OCH$_2$Ph), 75.04 (t, C-3-OCH$_2$Ph), 77.83 (d, C-5), 78.84 (d, C-3), 78.90 (d, C-4), 83.43 (d, C-1), 123.42 (d, arom. CH), 123.63 (d, arom. CH), 127.61 (d, arom. CH), 127.75 (d, arom. CH), 128.00 (d, arom. CH), 128.18 (d, arom. CH), 128.32 (d, arom. CH), 128.85 (d, arom. CH), 131.33 (s, arom. C), 131.41 (s, arom. C), 131.59 (s, arom. C), 132.56 (d, arom. CH), 133.98 (d, arom. CH), 134.08 (d, arom. CH), 136.95 (s, arom. C), 137.96 (s, arom. C), 166.84 (s, C═O), 168.17 (s, C═O).

3.12. Phenyl 4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside (10)

Thioglycoside **9** (102.1 mg, 0.155 mmol) was added to a solution of potassium acetate (150 mg, 1.53 mmol) and a catalytic amount of 18-crown-6 in dry DMF (10 mL). The mixture was stirred at 140 °C for 14 h under dry nitrogen, and diluted with $CHCl_3$ (80 mL). The solution was washed with water (15 mL × 3) and the aqueous layer was extracted with $CHCl_3$ (20 mL × 2). The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was separated by silica gel column chromatography eluting with 30% EtOAc–hexane to give **10** (67.1 mg, 70%) as a pale brown syrup. ^{1}H NMR ($CDCl_3$): δ 2.11 (s, 3H, OCOCH_3), 3.56 (dd, 1H, $J_{6a,6b}$ 9.6, $J_{5,6a}$ 6.5 Hz, H-6a), 3.64 (dd, 1H, $J_{5,6b}$ 6.5 Hz, H-6b), 3.95 (t, 1H, H-5), 4.23 (d, 1H, J_{gem} 12.3 Hz, C-3-OCH(H)Ph), 4.29 (dd, 1H, $J_{2,3}$ 10.6, $J_{3,4}$ 3.2 Hz, H-3), 4.45 (d, 1H, J_{gem} 12.0 Hz, C-6-OCH(H)Ph), 4.49 (t, 1H, $J_{1,2}$ 10.6 Hz, H-2), 4.56 (m, 2H, C-3-OCH(H)Ph + C-6-OCH(H)Ph), 5.55 (d, 1H, H-1), 6.87–6.94 (m, 4H, arom. H), 6.97–7.03 (m, 1H, arom. H), 7.15–7.19 (m, 3H, arom. H), 7.28–7.38 (m, 7H, arom. H), 7.61–7.74 (m, 2H, arom. H); ^{13}C NMR ($CDCl_3$): δ 20.88 (q, C-4-OCOCH$_3$),

51.56 (d, C-2), 66.00 (d, C-4), 68.16 (t, C-6), 70.97 (t, C-3-O*C*H_2Ph), 73.56 (d, C-3), 73.62 (t, C-6-O*C*H_2Ph), 76.24 (d, C-5), 84.22 (d, C-1), 123.25 (d, arom. CH), 123.52 (d, arom. CH), 127.61 (d, arom. CH), 127.65 (d, arom. CH), 127.83 (d, arom. CH), 127.98 (d, arom. CH), 128.05 (d, arom. CH), 128.12 (d, arom. CH), 128.44 (d, arom. CH), 128.78 (d, arom. CH), 131.59 (s, arom. C), 131.68 (s, arom. C), 132.06 (d, arom. CH), 132.67 (s, arom. C), 133.84 (d, arom. CH), 134.01 (d, arom. CH), 137.26 (s, arom. C), 137.67 (s, arom. C), 167.38 (s, C=O), 167.92 (s, C=O), 170.42 (s, C-4-O*C*OCH_3).

3.13. 4-*O*-Acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl fluoride (11)

N-Bromosuccinimide (77.9 mg, 0.438 mmol) and diethylaminosulfur trifluoride (0.120 mL, 0.910 mmol) were added to a solution of **10** (179.8 mg, 0.2883 mmol) in dry CH_2Cl_2 (4.3 mL) at −15 °C, and the mixture was stirred for 1 h under dry nitrogen. The reaction mixture was then gradually warmed to 0 °C and stirred for 4.5 h. The mixture was diluted with $CHCl_3$ (100 mL), and washed with satd aq $NaHCO_3$ (30 mL × 3), water (30 mL × 2), and brine (30 mL × 2). The organic layer was dried over Na_2SO_4 and concentrated. Purification was carried out by silica gel column chromatography eluting with 20% EtOAc–hexane to give fluoride **11** (100.7 mg, 65%) as colorless powder. ^{1}H NMR ($CDCl_3$): δ 2.14 (s, 3H, $COCH_3$), 3.59 (dd, 1H, $J_{6a,6b}$ 9.5, $J_{5,6a}$ 6.8 Hz, H-6a), 3.66 (dd, 1H, $J_{5,6b}$ 5.9 Hz, H-6b), 3.98–4.02 (m, 1H, H-5), 4.23 (d, 1H, J_{gem} 12.4 Hz, C-3-OC*H*(H)Ph), 4.32 (dd, 1H, $J_{2,3}$ 11.2, $J_{3,4}$ 3.0 Hz, H-3), 4.46 (ddd, 1H, $J_{F,2}$ 12.7, $J_{1,2}$ 8.0 Hz, H-2), 4.48 (d, 1H, J_{gem} 11.8 Hz, C-6-OC*H*(H)Ph), 4.58 (d, 1H, C-3-OCH(*H*)Ph), 4.60 (d, 1H, C-6-OCH(*H*)Ph), 5.65 (t, $J_{4,5}$ 3.0 Hz, H-4), 5.83 (dd, 1H, $J_{F,1}$ 53.4 Hz, H-1), 6.89–6.95 (m, 4H, arom. H), 7.00–7.04 (m, 1H, arom. H), 7.27–7.38 (m, 5H, arom. H), 7.72–7.75 (m, 4H, arom. H); ^{13}C NMR ($CDCl_3$): δ 20.83 (q, C-4-OCO*C*H_3), 52.91 (d, $J_{F,2}$ 21.5 Hz, C-2), 65.24 (d, C-4), 67.51 (t, C-6), 71.24 (t, C-3-O*C*H_2Ph), 72.16 (d, $J_{F,3}$ 9.9 Hz, C-3), 72.48 (d, $J_{F,5}$ 5.7 Hz, C-5), 73.73 (t, C-6-O*C*H_2Ph), 104.93 (d, $J_{F,1}$ 214.2 Hz, C-1), 123.45 (d, arom. CH), 127.73 (d, arom. CH), 127.98 (d, arom. CH), 128.05 (d, arom. CH), 128.08 (d, arom. CH), 128.19 (d, arom. CH), 128.52 (d, arom. CH), 131.55 (s, arom. C), 134.08 (d, arom. CH), 137.07 (s, arom. C), 137.35 (s, arom. C), 167.50 (s, C=O), 170.25 (s, C-4-O*C*OCH_3).

3.14. Phenyl 4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (12)

A mixture of **11** (99.1 mg, 0.186 mmol), phenol (106.0 mg, 1.126 mmol), and activated molecular sieves 4 Å (85.5 mg) in dry CH_2Cl_2 (3 mL) was stirred for 15 min at room temperature under dry nitrogen. Hafnocene dichloride (74.1 mg, 0.195 mmol) and silver perchlorate (78.1 mg, 0.377 mmol) were added to the mixture at −78 °C. The mixture was stirred for 1 h at this temperature, then stirred for 23 h at −50 °C, and diluted with $CHCl_3$ (50 mL). The mixture was washed with satd aq $NaHCO_3$ (10 mL × 3), water (10 mL × 3), and brine (10 mL × 2). The organic layer was dried over Na_2SO_4 and concentrated. Purification was carried out by silica gel column chromatography eluting with 10% EtOAc–hexane to give **12** (77.3 mg, 68%) as a pale brown oil. ^{1}H NMR ($CDCl_3$): δ 2.15 (s, 3H, C-4-OCOCH_3), 3.62 (dd, 1H, $J_{6a,6b}$ 9.5, $J_{5,6a}$ 6.5 Hz, H-6a), 3.65 (dd, 1H, $J_{5,6b}$ 6.5 Hz, H-6b), 4.06 (t, 1H, H-5), 4.39 (dd, 1H, $J_{2,3}$ 11.0, $J_{3,4}$ 3.6 Hz, H-3), 4.66 (dd, 1H, $J_{1,2}$ 8.5 Hz, H-2), 5.70 (d, 1H, H-4), 5.77 (d, 1H, H-1), 6.79–7.05 (m, 7H, arom. H), 7.12–7.35 (m, 8H, arom. H), and 7.58–7.86 (m, 4H, arom. H); ^{13}C NMR ($CDCl_3$): δ 20.93 (q, COC*H*$_3$), 52.73 (d, C-2), 65.76 (d), 68.03 (t, C-6), 71.18 (t, OCH_2), 72.76 (d), 72.82 (d), 73.68 (t, OCH_2), 96.52 (d, C-1), 116.83 (d, arom. CH), 122.77 (d, arom. CH), 123.24 (d, arom. CH), 123.48 (d, arom. CH), 127.64 (d, arom. CH), 127.84 (d, arom. CH), 127.96 (d, arom. CH), 128.04 (d, arom. CH), 128.16 (d, arom. CH), 128.45 (d, arom. CH), 129.34 (d, arom. CH), 131.57 (s, arom. C), 133.83 (d, arom. CH), 134.01 (d, arom. CH), 137.29 (s, arom. C), 137.62 (s, arom. C), 156.66 (s, arom. C), 167.59 (s, C=O), 168.20 (s, C=O), and 170.46 (s, C-4-O*C*OCH_3).

3.15. Phenyl 2-acetamido-4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranoside (13)

A solution of **12** (105.4 mg, 0.1735 mmol) and hydrazine monohydrate (0.085 mL) in ethanol (5 mL) was heated at reflux for 2 h. After cooling, the mixture was concentrated under reduced pressure. The residue was treated with dry pyridine (4 mL) and Ac_2O (2 mL). The mixture was stirred for 15 h at room temperature, diluted with $CHCl_3$ (100 mL), and washed with water (20 mL × 2), 3% HCl aq (20 mL × 3), satd aq $NaHCO_3$ (20 mL × 3), water (20 mL × 2), and brine (20 mL × 2). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. A solution of the residue in dry CH_3OH (5 mL) was treated with 1 M methanolic $NaOCH_3$ (0.90 mL) for 4 h at room temperature. The mixture was neutralized with Amberlite IRC-50 H^+ resin and concentrated under reduced pressure. Purification was carried out by silica gel column chromatography eluting with 50% EtOAc–hexane to give **13** (31.4 mg, 38%) as colorless crystals. ^{1}H NMR ($CDCl_3$): δ 1.89 (s, 3H, OCOCH_3), 3.61–3.68 (m, 1H, H-2), 3.73–3.84 (m, 3H, H-6+H-3), 4.12 (t,

1H, $J_{3,4}$ 3.0 Hz, H-4), 4.33 (dd, 1H, $J_{2,3}$ 10.6 Hz, H-3), 4.52–4.55 (m, 1H, C-3-OC*H*(H)Ph), 4.55 (s, 2H, C-6-OCH_2Ph), 4.70 (d, 1H, J_{gem} 11.7 Hz, C-3-OCH(*H*)Ph), 5.54 (d, 1H, $J_{1,2}$ 8.3 Hz, H-1), 5.61 (d, 1H, $J_{2,NH}$ 7.1 Hz, NH), 6.97–7.01 (m, 3H, arom. H), and 7.20–7.37 (m, 12H, arom. H); ^{13}C NMR ($CDCl_3$): δ 23.69 (q, OCO*C*H_3), 54.41 (d, C-2), 65.88 (d, C-4), 69.28 (t, C-6), 71.88 (t, C-3-O*C*H_2Ph), 73.45 (d, C-5), 73.68 (C-6-O*C*H_2Ph), 76.10 (d, C-3), 97.81 (d, C-1), 117.10 (d, arom. CH), 122.66 (d, arom. CH), 127.71 (d, arom. CH), 128.10 (d, arom. CH), 128.18 (d, arom. CH), 128.40 (d, arom. CH), 128.61 (d, arom. CH), 129.40 (d, arom. CH), 137.61 (s, arom. C), 137.95 (s, arom. C), 157.25 (s, arom. C), and 170.81 (s, C=O).

3.16. Phenyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-sulfonato-β-D-galactopyranoside sodium salt (14)

Sulfur trioxide–pyridine complex (66.3 mg, 0.417 mmol) was added to a solution of **13** (21.6 mg, 0.0452 mmol) in dry pyridine (5 mL), and the mixture was stirred for 18 h at room temperature. CH_3OH (5 mL) was added to the reaction mixture, and the resulting solution was subjected to Dowex 50W X8 Na^+ resin. The effluent was concentrated under reduced pressure. The residue was dissolved in 20% CH_3OH–$CHCl_3$, and separated by silica gel column chromatography eluting with 30% CH_3OH–$CHCl_3$ to give **14** (24.4 mg, 93%) as colorless crystals. ^{1}H NMR (CD_3OD): δ 1.85 (s, 3H, NHCOC*H*$_3$), 3.86–3.91 (m, 3H, H-3, H-5, H-6a), 3.97–4.00 (m, 1H, H-6b), 4.04–4.09 (m, 1H, H-2), 4.42 (d, 1H, J_{gem} 11.5 Hz, C-3-OC*H*(H)Ph), 4.54 (d, 1H, J_{gem} 11.3 Hz, C-6-OC*H*(H)Ph), 4.58 (d, 1H, C-6-OCH(*H*)Ph), 4.95 (d, 1H, C-3-OCH(*H*)Ph), 4.96 (d, 1H, $J_{3,4}$ 3.4 Hz, H-4), 5.20 (d, 1H, $J_{1,2}$ 8.3 Hz, H-1), 6.94–7.03 (m, 3H, arom. H), 7.16–7.40 (m, 12H, arom. H); ^{13}C NMR (CD_3OD): δ 23.00 (q, CH_3), 53.91 (d, C-2), 71.87 (t, C-6), 72.26 (t, C-3-O*C*H_2Ph), 73.01 (d, C-4), 74.41 (t, C-6-O*C*H_2Ph), 75.56 (d, C-5), 77.96 (d, C-3), 100.51 (d, C-1), 117.94 (d, arom. CH), 123.42 (d, arom. CH), 128.46 (d, arom. CH), 128.50 (d, arom. CH), 128.89 (d, arom. CH), 129.17 (d, arom. CH), 129.26 (d, arom. CH), 129.31 (d, arom. CH), 130.39 (d, arom. CH), 139.94 (s, arom. C), 140.04 (s, arom. C), 159.17 (s, arom. C), and 173.57 (s, C=O).

3.17. Phenyl 2-acetamido-2-deoxy-4-*O*-sulfonato-β-D-galactopyranoside (15)

To a solution of **14** (16.2 mg, 0.0279 mmol) in 95% ethanol–water (2.0 mL) was added 10% Pd–C 30.4 mg and the mixture was stirred for 21 h at 40 °C under hydrogen. The reaction mixture was filtered through Celite and concentrated under reduced pressure. The residue was dissolved in water, and purified by SAX-HPLC and Superdex 30 chromatography to give **15** (5.2 mg, 47% as ammonium salt) as colorless crystals. ^{1}H NMR (D_2O): δ 1.89 (s, 3H, NHCOC*H*$_3$), 3.66–3.75 (m, 2H, H-6), 3.83–3.88 (m, 2H, H-3, H-5), 4.04 (dd, 1H, $J_{2,3}$ 10.9, $J_{1,2}$ 8.4 Hz, H-2), 4.65–4.70 (m, 1H, H-4), 5.02 (d, 1H, H-1), 6.95–7.03 (m, 3H, arom. H), 7.23–7.27 (m, 2H, arom. H); ^{13}C NMR (D_2O): δ 24.99 (q, NHCOC*H*$_3$), 55.59 (d, C-2), 63.65 (t, C-6), 72.61 (d, C-3), 77.57 (d, C-5), 78.33 (d, C-4), 102.79 (d, C-1), 119.59 (d, arom. CH), 126.34 (d, arom. CH), 132.80 (d, arom. CH), 159.57 (s, arom. C), 177.92 (s, NH*C*OCH$_3$); ESI-MS ($C_{14}H_{18}NO_9S^-$) *m*/*z* 376 (M^-).

3.18. Phenyl 2-acetamido-2-deoxy-6-*O*-sulfonato-α-D-galactopyranoside (16)

A mixture of **4** (50.8 mg, 0.171 mmol) and sulfur trioxide–pyridine complex (56.8 mg, 0.357 mmol) in dry pyridine (2 mL) was stirred for 6 h at 40 °C. CH_3OH (2 mL) was then added, and the mixture was passed through the column of Dowex 50W X8 Na^+, and concentrated under reduced pressure. The residue was dissolved in water and purified successively by gel filtration with Superdex 30, paper electrophoresis in buffer A, SAX-HPLC and Superdex 30 chromatography to give the target compound **16** (4.0 mg, 6% as ammonium salt) as colorless crystals. ^{1}H NMR (D_2O): δ 1.92 (s, 3H, NHCOC*H*$_3$), 3.97–4.10 (m, 4H), 4.23–4.27 (m, 2H), 5.44 (d, $J_{1,2}$ 3.7 Hz, H-1), 7.00–7.04 (m, 3H, arom. H), 7.24–7.28 (m, 2H, arom. H); ^{13}C NMR (CD_3OD): δ 22.57 (q, NHCOC*H*$_3$), 51.55 (d, C-2), 67.34 (t, C-6), 69.08 (d, C-3), 69.58 (d, C-4), 71.04 (d, C-5), 98.77 (d, C-1), 118.59 (d, arom. CH), 123.73 (d, arom. CH), 130.53 (d, arom. CH), 158.84 (s, arom. C), and 174.04 (s, NH*C*OCH$_3$); ESI-MS ($C_{14}H_{18}NO_9S^-$) *m*/*z* 376 (M^-).

3.19. Phenyl 2-acetamido-2-deoxy-β-D-galactopyranoside (17)

A solution of **3** (226.6 mg, 0.535 mmol) in dry CH_3OH (8 mL) was treated with 1 M methanolic $NaOCH_3$ (0.54 mL) for 1 h at room temperature. The mixture was neutralized with Amberlite IRC-50 H^+ resin, and concentrated under reduced pressure to give **17** (139.8 mg, 88%) as colorless crystals. ^{1}H NMR (CD_3OD): δ 1.97 (s, 3H, NHCOC*H*$_3$), 3.65 (t, 1H, $J_{5,6a}$ 6.1 Hz, H-5), 3.71 (dd, 1H, $J_{2,3}$ 10.5, $J_{3,4}$ 2.7 Hz, H-3), 3.76 (dd, 1H, $J_{6a,6b}$ 11.5 Hz, H-6a), 3.80 (dd, 1H, $J_{5,6b}$ 6.1 Hz, H-6b), 3.90 (d, 1H, H-4), 4.17 (dd, 1H, $J_{1,2}$ 8.5 Hz, H-2), 5.00 (d, 1H, H-1), 6.96–7.03 (m, 3H, arom. H), and 7.23–7.89 (m, 2H, arom. H); ^{13}C NMR (CD_3OD): δ 22.98 (q, CH_3), 54.32 (d, C-2), 62.44 (t,

C-6), 69.59 (d, C-4), 72.98 (d, C-3), 77.06 (d, C-5), 101.28 (d, C-1), 117.75 (d, arom. CH), 123.45 (d, arom. CH), 130.43 (d, arom. CH), and 159.25 (s, arom. C), 174.23 (s, C=O).

3.20. Phenyl 2-acetamido-2-deoxy-6-*O*-sulfonato-β-D-galactopyranoside (18)

A mixture of **17** (40.4 mg, 0.136 mmol) and sulfur trioxide–pyridine complex (44.5 mg, 0.278 mmol) in dry pyridine (2 mL) was stirred for 6 h at 40 °C. CH_3OH (1.5 mL) was then added, and the mixture was passed through the column of Dowex 50W X8 Na^+, and concentrated under reduced pressure. The residue was dissolved in water, and purified by paper electrophoresis in buffer A, SAX-HPLC and Superdex 30 chromatography to give the target compound **18** (5.0 mg, 9% ammonium salt) as colorless crystals. ^{1}H NMR (D_2O): δ 1.88 (s, 3H, NHCOCH_3), 3.70–3.73 (m, 1H, H-3), 3.93 (s, 1H, H-4), 3.97–4.15 (m, 4H, H-2, H-5, H-6), 4.95 (d, 1H, $J_{1,2}$ 8.3 Hz, H-1), 6.94–7.03 (m, 3H, arom. H), and 7.22–7.83 (m, 2H, arom. H); ^{13}C NMR (CD_3OD): δ 22.97 (q, NHCOCH_3), 54.10 (d, C-2), 67.56 (t, C-6), 69.17 (d, C-4), 72.90 (d, C-3), 74.56 (d, C-5), 101.37 (d, C-1), 117.80 (d, arom. CH), 123.46 (d, arom. CH), 130.47 (d, arom. CH), 159.25 (s, arom. C), and 174.16 (s, NH*C*OCH$_3$); ESI-MS ($C_{14}H_{18}NO_9S^-$) *m/z* 376 (M^-).

3.21. Phenyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (19)

A mixture of benzaldehyde dimethyl acetal (0.13 mL, 0.866 mmol), compound **4** (100.4 mg, 0.338 mmol), and D-camphor-10-sulfonic acid (34.9 mg, 0.150 mmol) was stirred at 40 °C for 24 h under reduced pressure. Benzaldehyde dimethyl acetal (0.20 mL, 1.33 mmol) and D-camphor-10-sulfonic acid (68.5 mg, 0.295 mmol) was added to the mixture, and then it was stirred at 40 °C for 17 h under reduced pressure. The mixture was diluted with $CHCl_3$ (50 mL), and washed with satd aq $NaHCO_3$ (20 mL × 3), water (20 mL × 2), and brine (20 mL × 2). The organic layer was dried over Na_2SO_4, and concentrated under reduced pressure. Purification was carried out by silica gel column chromatography eluting with EtOAc to give **19** (79.6 mg, 61%) as colorless needles. ^{1}H NMR (CD_3OD): δ 1.99 (s, 3H, NHCOCH_3), 3.85 (s, 1H, H-5), 4.11 (s, 2H, H-6), 4.19 (dd, 1H, $J_{2,3}$ 11.2, $J_{3,4}$ 3.4 Hz, H-3), 4.34 (d, 1H, H-4), 4.52 (dd, 1H, $J_{1,2}$ 3.4 Hz, H-2), 5.65 (d, 1H, H-1), 6.99–7.03 (m, 1H, arom. H), 7.09–7.12 (m, 2H, arom. H), 7.26–7.39 (m, 5H, arom. H), and 7.55–7.58 (m, 2H, arom. H); ^{13}C NMR (CD_3OD): δ 22.56 (q, CH_3), 51.62 (d, C-2), 65.29 (d, C-5), 67.90 (d, C-3), 70.27 (t, C-6), 77.17 (d, C-4), 98.38 (d, C-2), 102.33 (d, CH), 117.83 (d, arom. CH), 123.58 (d, arom. CH), 127.63 (d, arom. CH), 129.05 (d, arom. CH), 129.94 (d, arom. CH), 130.60 (d, arom. CH), 139.69 (s, arom. C), 158.45 (s, arom. C), and 174.14 (s, C=O).

3.22. Phenyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-sulfonato-α-D-galactopyranoside sodium salt (20)

Sulfur trioxide–pyridine complex (134.3 mg, 0.844 mmol) was added to a solution of **19** (30.4 mg, 0.0789 mmol) in dry pyridine (2 mL), and the mixture was stirred for 20.5 h at 50 °C. CH_3OH (2 mL) was added to the reaction mixture, and the resulting solution was subjected to Dowex 50W X8 Na^+ resin. The effluent was concentrated under reduced pressure. The residue was dissolved in 20% CH_3OH–$CHCl_3$, and separated by silica gel column chromatography eluting with 30% CH_3OH–$CHCl_3$ to give **20** (31.5 mg, 82%) as colorless crystals. ^{1}H NMR (CD_3OD): δ 1.98 (s, 3H, NHCOCH_3), 3.89 (s, 1H, H-5), 4.09 (dd, 1H, $J_{6a,6b}$ 12.6, $J_{5,6a}$ 1.6 Hz, H-6a), 4.13 (dd, 1H, $J_{5,6b}$ 1.6 Hz, H-6b), 4.64 (dd, 1H, $J_{2,3}$ 11.5, $J_{1,2}$ 3.2 Hz, H-2), 4.71 (d, 1H, $J_{3,4}$ 3.1 Hz, H-4), 4.94 (dd, 1H, H-3), 5.66 (s, 1H, CH), 5.78 (d, 1H, H-1), 7.00–7.04 (m, 1H, arom. H), 7.10–7.13 (m, 2H, arom. H), 7.28–7.37 (m, 5H, arom. H), 7.52–7.55 (m, 2H, arom. H); ^{13}C NMR (CD_3OD): δ 22.72 (q, CH_3), 50.09 (d, C-2), 65.33 (d, C-5), 70.19 (t, C-6), 73.57 (d, C-3), 75.53 (d, C-4), 98.23 (d, C-1), 102.04 (d, CH), 117.85 (d, arom. CH), 123.69 (d, arom. CH), 127.54 (d, arom. CH), 128.98 (d, arom. CH), 129.86 (d, arom. CH), 130.64 (d, arom. CH), 139.63 (s, arom. C), 158.31 (s, arom. C), 173.97 (s, NH*C*OCH$_3$).

3.23. Phenyl 2-acetamido-2-deoxy-3-*O*-sulfonato-α-D-galactopyranoside (21)

To a solution of **20** (18.5 mg, 0.0380 mmol) in 95% ethanol–water (2.0 mL) was added 10% Pd–C (21.2 mg) and the mixture was stirred for 9 h at 40 °C under hydrogen. The reaction mixture was filtered through Celite and concentrated under reduced pressure. The residue was dissolved in water, and purified by SAX-HPLC and Superdex 30 chromatography to give the target compound **21** (3.9 mg, 26%, ammonium salt) as colorless crystals. ^{1}H NMR (D_2O): δ 1.91 (s, 3H, NHCOCH_3), 3.60 (d, 2H, $J_{5,6}$ 6.2 Hz, H-6), 4.01 (t, 1H, H-5), 4.26 (d, $J_{3,4}$ 3.2 Hz, H-4), 4.43 (dd, 1H, $J_{2,3}$ 10.7, $J_{1,2}$ 4.0 Hz, H-2), 4.65 (dd, 1H, H-3), 5.55 (d, 1H, H-1), 6.99–7.06 (m, 3H, arom. H), 7.25–7.28 (m, 2H, arom. H); ^{13}C NMR (D_2O): δ 24.82 (q, NHCO*C*H$_3$), 50.65 (d, C-2), 63.76 (t, C-6), 69.75 (d, C-4), 74.41 (d, C-5), 78.50 (d, C-5), 99.09 (d, C-1), 120.03 (d, arom. CH), 126.06 (d, arom. CH), 132.72 (d, arom. CH), 158.75 (s, arom. C),

177.61 (s, NH*C*OCH$_3$); ESI-MS ($C_{14}H_{18}NO_9S^-$) *m*/*z* 376 (M^-).

3.24. Phenyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (22)

A mixture of benzaldehyde dimethyl acetal (0.15 mL, 0.999 mmol), compound **17** (50.5 mg, 0.170 mmol), and D-camphor-10-sulfonic acid (42.3 mg, 0.182 mmol) was stirred at 40 °C for 22.5 h under reduced pressure. Additional benzaldehyde dimethyl acetal (0.10 mL, 0.666 mmol) and D-camphor-10-sulfonic acid (40.3 mg, 0.173 mmol) was added to the mixture, and then it was stirred at 40 °C for 9 h under reduced pressure. The mixture was diluted with $CHCl_3$ (50 mL), and washed with satd aq $NaHCO_3$ (20 mL × 3), water (20 mL × 2), and brine (20 mL × 2). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification was carried out by silica gel column chromatography eluting with EtOAc to give **22** (33.7 mg, 51%) as colorless needles. ^{1}H NMR (CD_3OD): δ 1.97 (s, 3H, NHCOC*H*$_3$), 3.73 (q, 1H, *J* 1.4 Hz, H-5), 3.90 (dd, 1H, $J_{2,3}$ 11.1, $J_{3,4}$ 3.5 Hz, H-3), 4.18 (dd, 1H, $J_{6a,6b}$ 12.3, $J_{5,6a}$ 1.4 Hz, H-6a), 4.24 (dd, 1H, $J_{5,6b}$ 1.4 Hz, H-6b), 4.24–4.28 (m, 2H, H-3, H-4), 5.15 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 5.67 (s, 1H, CH), 6.98–7.06 (m, 3H, arom. CH), 7.25–7.30 (m, 2H, arom. CH), 7.33–7.38 (m, 3H, arom. CH), and 7.56–7.59 (m, 2H, arom. CH); ^{13}C NMR (CD_3OD): δ 22.98 (CH_3), 54.16 (C-2), 68.19, 70.22, 71.49, 78.31, 100.96, 102.52, 117.88, 123.61, 127.73, 129.03, 129.95, 130.49, 139.64, 159.10, and 174.23 (s, NH*C*OCH$_3$).

3.25. Phenyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-sulfonato-β-D-galactopyranoside sodium salt (23)

Sulfur trioxide–pyridine complex (98.7 mg, 0.620 mmol) was added to a solution of **22** (23.7 mg, 0.0615 mmol) in dry pyridine (2 mL), and the mixture was stirred for 13 h at 40 °C. CH_3OH (2 mL) was added to the reaction mixture, and the resulting solution was subjected to Dowex 50W X8 Na^+ resin. The effluent was concentrated under reduced pressure. The residue was separated by silica gel column chromatography eluting with 30% CH_3OH–$CHCl_3$ to give **23** (25.4 mg, 85%) as colorless crystals. ^{1}H NMR (CD_3OD): δ 1.96 (s, 3H, NHCOC*H*$_3$), 3.77 (t, 1H, *J* 1.7 Hz, H-5), 4.18 (dd, 1H, $J_{6a,6b}$ 12.6, $J_{5,6a}$ 1.7 Hz, H-6a), 4.23 (dd, 1H, $J_{5,6b}$ 1.7 Hz, H-6b), 4.41 (dd, 1H, $J_{2,3}$ 10.9, $J_{1,2}$ 8.4 Hz, H-2), 4.62 (dd, 1H, $J_{3,4}$ 3.4 Hz, H-3), 4.65 (d, 1H, H-4), 5.29 (d, 1H, H-1), 5.66 (s, 1H, CH), 6.98–7.06 (m, 3H, arom. CH), 7.25–7.37 (m, 5H, arom. CH), and 7.54–7.57 (m, 2H, arom. CH); ^{13}C NMR (CD_3OD): δ 23.11 (q, CH_3), 51.99 (d, C-2), 68.04 (d, C-5), 70.19 (t, C-6), 75.16 (d, C-4), 76.32 (d, C-3), 101.08 (d, C-1), 102.30 (d, CH), 117.91 (d, arom. CH), 123.60 (d, arom. CH), 127.68 (d, arom. CH), 128.94 (d, arom. CH), 129.82 (d, arom. CH), 130.47 (d, arom. CH), 139.64 (s, arom. C), 159.10 (s, arom. C), and 174.07 (s, C=O).

3.26. Phenyl 2-acetamido-2-deoxy-3-*O*-sulfonato-β-D-galactopyranoside (24)

To a solution of **23** (25.4 mg, 0.0521 mmol) in 95% ethanol–water (2.0 mL) was added 10% Pd–C (36.2 mg) and the mixture was stirred for 35 h at 40 °C under hydrogen. The reaction mixture was filtered through Celite and concentrated under reduced pressure. The residue was dissolved in water, and purified by SAX-HPLC and Superdex 30 chromatography to give the target compound **21** (11.2 mg, 54% as ammonium salt) as colorless crystals. ^{1}H NMR (D_2O): δ 1.87 (s, 3H, NHCOC*H*$_3$), 3.68–3.70 (m, 2H, H-6), 3.76–3.79 (m, 1H, H-5), 4.18–4.20 (m, 2H, H-2, H-4), 4.37–4.40 (m, 1H, H-3), 5.11 (d, 1H, $J_{1,2}$ 9.5 Hz, β-H-1), 6.96–7.03 (m, 3H, arom. H), and 7.24–7.28 (m, 2H, arom. H); ^{13}C NMR (D_2O): δ 25.07 (q, NHCOC*H*$_3$), 53.48 (d, C-2), 63.56 (t, C-6), 69.03 (d, C-4), 77.95 (d, C-5), 80.37 (d, C-3), 102.55 (d, C-1), 119.65 (d, arom. CH), 126.37 (d, arom. CH), 132.84 (d, arom. CH), 159.61 (s, arom. C), and 177.85 (s, NH*C*OCH$_3$); ESI-MS ($C_{14}H_{18}NO_9S^-$) *m*/*z* 376 (M^-).

3.27. Phenyl 2-acetamido-4,6-*O*-benzilidene-2-deoxy-3-*O*-pivaloyl-β-D-galactopyranoside (25)

Pivaloyl chloride (0.050 mL, 0.406 mmol) was added to a solution of **22** (15.9 mg, 0.0413 mmol) in dry pyridine (0.5 mL) and CH_2Cl_2 (0.5 mL), and the mixture was stirred for 15 h at room temperature. The mixture was diluted with $CHCl_3$ (60 mL), washed with satd aq $NaHCO_3$ (20 mL × 3), water (20 mL × 2), and brine (20 mL × 2), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was dissolved in toluene, and separated by silica gel chromatography eluting with 30% EtOAc–hexane to give **25** (12.8 mg, 66%) as colorless crystals. ^{1}H NMR ($CDCl_3$): δ 1.19 (s, 9H, C(C*H*$_3$)$_3$), 1.91 (s, 3H, NHCOC*H*$_3$), 3.68 (s, 1H, H-5), 4.07 (dd, 1H, $J_{6a,6b}$ 12.4, $J_{5,6a}$ 1.7 Hz, H-6a), 4.22 (dt, 1H, $J_{2,3}$ 11.3, $J_{1,2}$ 8.2 Hz, H-2), 4.35 (dd, 1H, $J_{5,6b}$ 1.2 Hz, H-6b), 4.42 (d, 1H, $J_{3,4}$ 3.4 Hz, H-4), 5.47 (dd, 1H, $J_{2,3}$ 11.3 Hz, H-3), 5.49 (d, 1H, H-1), 5.50 (d, 1H, $J_{NH,2}$ 8.2 Hz, N-H), 5.54 (s, 1H, CH), 6.99–7.03 (m, 3H, arom. H), 7.23–7.36 (m, 5H, arom. H), and 7.48–7.50 (m, 2H, arom. H); ^{13}C NMR ($CDCl_3$): δ 23.44 (q, CH_3), 27.01 (q, C(*C*H$_3$)$_3$), 39.02 (s, *C*(CH$_3$)$_3$), 51.93 (d, C-2), 66.54 (d), 69.07 (t, C-6), 69.88 (d), 72.94 (d), 98.67 (d), 100.50 (d), 117.30 (d, arom. CH), 122.85 (d, arom. CH), 126.06 (d, arom. CH), 128.07 (d, arom. CH), 128.81 (d, arom. CH), 129.41 (d, arom. CH), 137.65 (s, arom. C), 157.29 (s, arom. C), 170.22 (s, C=O), and 178.23 (s, C=O).

3.28. Phenyl 2-acetamido-2-deoxy-3-*O*-pivaloyl-β-D-galactopyranoside (26)

To a solution of **25** (10.6 mg, 0.0226 mmol) in 95% ethanol–water (2.0 mL) was added 10% Pd–C (19.5 mg), and the mixture was stirred for 2 days at 40 °C under hydrogen. The reaction mixture was filtered through Celite, and concentrated under reduced pressure. The residue was purified by silica gel chromatography eluting with EtOAc to give **26** (5.5 mg, 64%) as colorless crystals. ^{1}H NMR (CD_3OD): δ 1.21 (s, 9H, C-3-OCOC(CH_3)$_3$), 1.90 (s, 3H, NHCOCH_3), 3.71–3.82 (m, 3H, H-5, H-6), 4.05 (d, 1H, $J_{3,4}$ 3.2 Hz, H-4), 4.51 (dd, 1H, $J_{2,3}$ 11.2, $J_{1,2}$ 8.5 Hz, H-2), 4.94 (dd, 1H, H-3), 5.10 (d, 1H, H-1), 6.97–7.04 (m, 3H, arom. H), 7.24–7.29 (m, 2H, arom. H); ^{13}C NMR (CD_3OD): δ 22.85 (q, NHCOCH_3), 27.49 (q, C-3-OCOC(CH_3)$_3$), 39.95 (s, C-3-OCOC(CH_3)$_3$), 51.47 (d, C-2), 62.16 (t, C-6), 67.12 (d, C-4), 74.62 (d, C-3), 76.98 (d, C-5), 101.19 (d, C-1), 117.79 (d, arom. CH), 123.61 (d, arom. CH), 130.49 (d, arom. CH), 159.14 (s, arom. C), 173.43 (s, NHCOCH$_3$), 179.52 (s, C-3-OCOC(CH_3)$_3$).

3.29. Phenyl 2-acetamido-2-deoxy-4,6-di-*O*-sulfonato-β-D-galactopyranoside (28)

Sulfur trioxide–pyridine complex (22.2 mg, 0.139 mmol) was added to a solution of **26** (3.8 mg, 0.00996 mmol) in dry pyridine (2 mL), and the mixture was stirred for 17 h at 40 °C. CH_3OH (2 mL) was added to the reaction mixture and the resulting solution was subjected to Dowex 50W X8 Na^+ resin. The effluent was concentrated under reduced pressure. A solution of crude **27** (12.8 mg) in dry CH_3OH (1.5 mL) was treated with 1 M methanolic $NaOCH_3$ (0.50 mL) for 41 h at room temperature. The mixture was neutralized with Amberlite IRC-50 H^+ resin and concentrated under reduced pressure. The residue was purified by SAX-HPLC and Superdex 30 chromatography to give the target compound **28** (5.0 mg, 100% as ammonium salt) as colorless crystals. ^{1}H NMR (D_2O): δ 1.85 (s, 3H, NHCOCH_3), 3.84 (dd, 1H, $J_{2,3}$ 11.0, $J_{3,4}$ 2.9 Hz, H-3), 3.99–4.11 (m, 3H, H-2, H-5, H-6a), 4.19 (d, 1H, J 9.3 Hz, H-6b), 4.96 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 6.92–6.99 (m, 3H, arom. H), 7.20–7.23 (m, 2H, arom. H); ^{13}C NMR (D_2O): δ 25.03 (q, NHCOCH_3), 55.43 (d, C-2), 70.48 (t, C-6), 72.50 (d, C-3), 75.34 (d, C-5), 78.19 (d, C-4), 102.82 (d, C-1), 119.57 (d, arom. CH), 126.37 (d, arom. CH), 132.85 (d, arom. CH), 159.64 (s, arom. C), 177.99 (s, NHCOCH$_3$); ESI-MS ($C_{14}H_{18}NO_{12}S_2^-$) m/z 456 (M^-).

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