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Design of *N*-acetyl-6-sulfo-β-D-glucosaminide-based inhibitors of influenza virus sialidase

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Abstract—Biological activity of *N*-acetyl-6-sulfo-β-D-glucosaminides (6-sulfo-GlcNAc **1**) having a structural homology to *N*-acetylneuraminic acid (Neu5Ac **2**) and 2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid (Neu5Ac2en **3**) was examined in terms of inhibitory activity against influenza virus sialidase (influenza, A/Memphis/1/71 H3N2). *p*NP 6-Sulfo-GlcNAc **1a** was proved to show substantial activity to inhibit the virus sialidase ($IC_{50}=2.8$ mM), though *p*-nitrophenyl (*p*NP) GlcNAc without 6-sulfo group and *p*NP 6-sulfo-$GlcNH_3^+$ **1b** without 2-NHAc showed little activity ($IC_{50}>50$ mM). The activity was enhanced nearly 100-fold when the *p*NP group of **1a** was converted to *p*-acetamidophenyl one **5** ($IC_{50}=30$ μM) or replaced with 1-naphthyl **6** ($IC_{50}=10$ μM) or *n*-propyl one **8** ($IC_{50}=11$ μM).

1. Introduction

Sialic acids are highly functional molecules bearing a carboxylate anion, an acetamide (or *N*-glycolyl) group, and an acyclic side chain in the nine-carbon skeleton as represented by *N*-acetyl neuraminic acid (Neu5Ac **2**, Fig. 1).[1,2] They are linked to the non-reducing terminal of oligosaccharides in cell surface glycoproteins and glycolipids. The sialyl oligosaccharides are involved in various cell–cell recognition events, which include microbe infection to host cells, fertilization, and metastasis of cancer cells.[1,2] Therefore, the mimic design of sialyl linkages, especially for sialyl $Lewis^X$ and $Lewis^a$ antigens, is thought to lead to the development of saccharide-based medicines and biomaterials.[3]

Much interest is being directed also to the design of inhibitors to viral and bacterial sialidases[4] which play essential roles at the stage of infection and propagation. For example, a *vibrio cholerae* sialidase cleaves sialyl linkages of gangliosides to expose G_{M1} on the host cells. This process allows a *cholerae toxin* to bind and internalize into the host cells.[5a] Influenza viruses utilize sialidases to avoid self-aggregation by their hemagglutinins and to leave from the host cell during budding process.[5b] These microbial events indicate that sialidase inhibitors can become good candidates of anti-influenza medicines. Actually, several sialidase inhibitors with a skeleton of Neu5Ac2en **3** have been developed and applied for anti-influenza therapy like the well-known case of Zanamivir **4**.[6]

Along with our continuous studies on the design of oligosaccharide mimics and their polyvalent models based on the concept of a carbohydrate module,[7] we recently found that an acrylamido copolymer of *N*-acetyl-6-sulfo-β-D-glucosaminide (6-sulfo-GlcNAc) inhibits the binding interaction between sialyl $Lewis^X$ and L-selectin.[8] The result led us to the speculation that the 6-sulfo-GlcNAc module might serve as a mimic of sialyl linkages. We have tried to verify this speculation and

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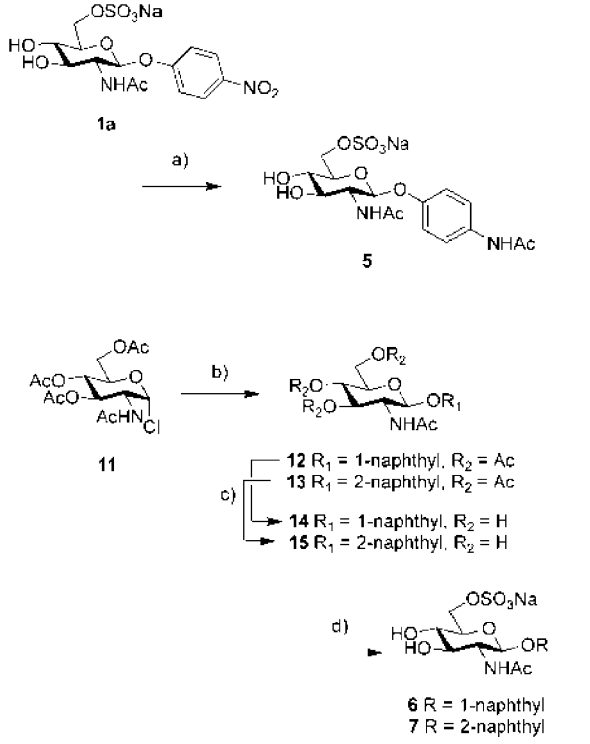
showed the experimental evidence in a preceding study[9] that *p*-nitrophenyl (*p*NP) 6-sulfo-β-D-GlcNAc **1a** has substantial inhibitory activity to *vibrio cholerae* sialidase. In this paper, we describe that the *p*NP 6-sulfo-β-D-GlcNAc **1a** and its derivatives **5–9** (Schemes 1 and 2) display inhibitory activity also to influenza virus sialidase. The results enable us to confirm that the skeleton **1** provides a new class of sialyl mimics giving a common basis to the design of sialidase inhibitors.

2. Results and discussion

2.1. Design and synthesis of 6-sulfo-GlcNAc derivatives 5–9 with various aglycon groups

Though sulfonated glycosaminoglycans[10] and inorganic sulfates[11] are reported to inhibit several sialidases, little structural basis has been provided to rationalize the activity. Our preceding study[9] disclosed that *p*NP 6-sulfo-β-D-GlcNAc **1a** shows structure-dependent activity against the *vibrio cholerae* sialidase ($IC_{50}=2$ mM). Instead of Neu5Ac **2** with a typical $^1C_4$ (chair) conformation, the 2-ene compound **3** is thought to simulate intermediates at the transitional states in sialidase reactions and thus provide a key molecular basis for the inhibitor design.[6] As can be seen in Figure 1, the skeleton of 6-sulfo-GlcNAc **1** and **1a** (R = *p*NP) has a structural homology not only to Neu5Ac **2** but also to Neu5Ac2en **3** in spatial relationship (distance and helicity) between NHAc and anionic groups. The structural homology implies that the simple GlcNAc may provide an alternative structural basis for the design of sialidase inhibitors. Recently, Schwörer and Schmidt also cited a homology between GlcNAc and Neu5Ac **2** for the design of CMP-sialyltransferase inhibitors showing the versatility of this idea.[12]

As depicted in Figure 1, a major difference between **1** and **2** (or **3**) arises at the aglycon moiety of the former (*p*NP group in the case of **1a**) that corresponds to an acyclic side chain in the latter. The difference suggests that introduction of an appropriate aglycon in **1** may induce strong activity. It is also of practical meaning that the difference at the aglycon can be modulated variably in chemical ways. Such expectation prompted us to prepare a series of 6-sulfo-GlcNAc derivatives **5–9** with various groups at the aglycon (phenyl, naphthyl, alkyl, and glycerol) (Schemes 1 and 2) and examine their biological activities against an influenza virus sialidase.



Scheme 1. Reagents and conditions: (a) (i) $Pd(OH)_2/C$, H_2, H_2O, rt; (ii) Ac_2O, K_2CO_3, H_2O, rt, 80% (2 steps); (b) 1- or 2- naphthol, TBAB, 1N NaOH aq/CH_2Cl_2, rt, 66% (for **12**), 65% (for **13**); (c) NaOMe, MeOH, rt, 99% (for **14**) and 99% (for **15**); (d) Me_3NSO_3, DMF, 50 °C, 40% (for **6**), 45% (for **7**). TBAB = tetrabutylammonium bromide.

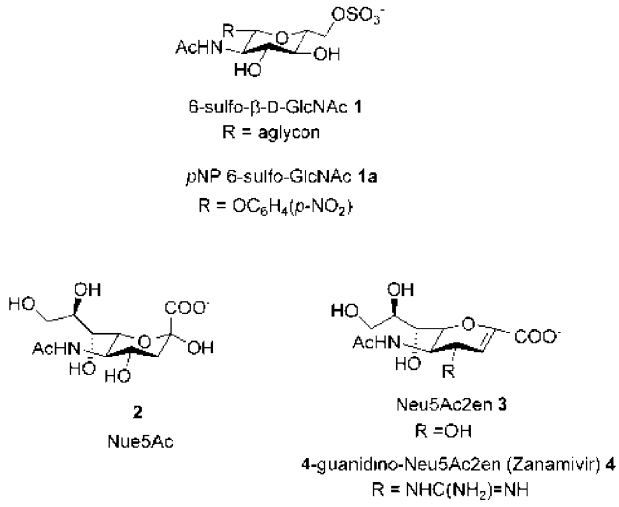
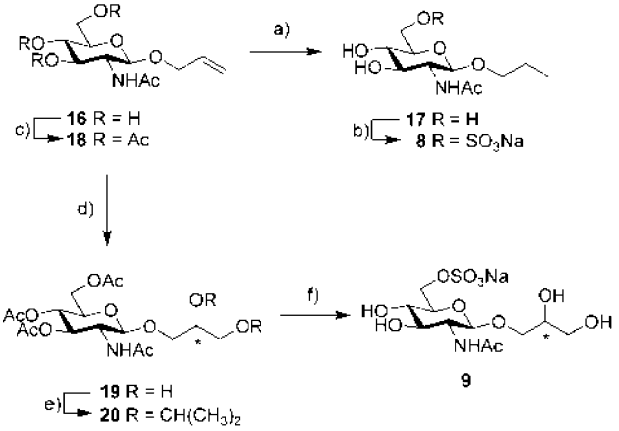


Figure 1. Structure of 6-sulfo-β-D-GlcNAc skeleton **1** having homology to Neu5Ac **2**. Neu5Ac2en **3** is considered as a putative transition state-like analogue in sialidase reaction.



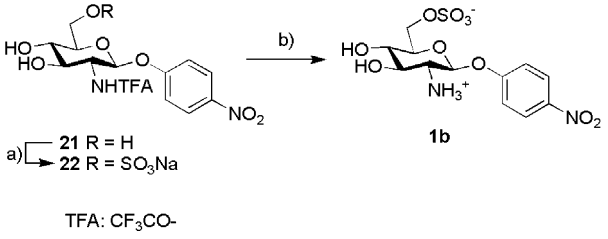
Scheme 2. Reagents and conditions: a) $Pd(OH)_2/C$, H_2, MeOH, rt, 99%; (b) Me_3NSO_3, DMF, 40 °C, 53%; (c) Ac_2O, pyr, rt, 98%; (d) AD-mix, *tert*-BuOH/H_2O, 0 °C 80%; (e) $Me_2C(OMe)_2$, PPTS, DMF, rt, 60%; (f) (i) NaOMe, MeOH, rt; (ii) Me_3NSO_3, DMF, 40 °C; (iii) TFA, H_2O, MeOH, rt, 34% (3 steps). PPTS = pyridinium *p*-toluenesulfonic acid, TFA = trifluoroacetic acid.

Compound **5** with a 4-acetamidophenyl group was derived from **1a** in the conventional way of catalytic hydrogenation in water and *N*-acetylation with acetic anhydride. Here, it should be noted that 4-acetamidophenol in **5** per se is the analgesic and antipyretic medicine called acetaminophen.[13] Considering the possible metabolizing pathway of 6-sulfo-GlcNAc derivatives, we expect that the *p*-acetamidophenyl glycoside may represent a promising skeleton of *O*-glycoside-based medicines. Naphthyl compounds **6** and **7** were prepared from 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride **11**[14] in the glycosylation with either 1- or 2-naphthol under phase-transfer catalytic conditions.[15] Direct *O*-sulfonation using Me_3NSO_3 in DMF[16] successfully gave the corresponding 6-*O*-sulfated GlcNAc derivatives in moderate yields. Thus, 1-*O*-aryl glycosides **1a**, **5**, **6**, and **7** were available in a common way and used as key models to investigate the effect of aryl groups on sialidase inhibitory activity.

n-Propyl glycoside **8** was prepared from allyl *N*-acetyl-β-D-glucosaminide **16**[17] obtained as a major product in the reaction of GlcNAc and allyl alcohol (60–70 °C) in the presence of trimethylsilyl chloride (TMSCl). Here, the TMSCl accelerates the allyl glycosidation by trapping water molecules in the form of TMSOH. The 1,2-diol compound **9** was prepared as a mixture of diastereomers (55: 45, ^{1}H NMR analysis) in the dihydroxylation of tri-*O*-acetyl derivative **18** and AD-mix.[18] A mixture of the diastereomers, which could be hardly separated from each other, was applied to an inhibitory assay to see how the hydrophilic aglycon in **9** works for the activity in comparison with the hydrophobic aglycon in **8** and others.

2.2. Inhibitory activities of 6-sulfo-GlcNAc derivatives against influenza virus sialidase

An influenza virus (A/Memphis/1/71 H3N2) was used to evaluate the biological potential of the synthetic 6-sulfo-GlcNAc derivatives as anti-influenza agents. The assay was conducted with the authentic fluorescent method[19] utilizing 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid as a substrate. Though the substrate is non-fluorescent, the enzymic hydrolysis generates the fluorescent 4-methylumbelliferone detectable at Em 450 nm (Ex 365 nm). As reference compounds, *p*NP GlcNAc (6-OH) without a 6-sulfo group and *p*NP 6-sulfo-GlcNH$_3^+$ **1b** (Scheme 3) without 2-NHAc were also applied to the assay to investigate the role of each of the two functional groups. The assay



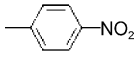
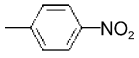
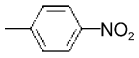
Scheme 3. Reagents and conditions: (a) Me_3NSO_3, DMF, 40 °C, 67%; (b) 0.1 N NaOH aq, rt, 89%.

data summarized in Table 1 indicated that the 6-sulfo-GlcNAc compound **1a** showed substantial activity (IC_{50} = 2.8 mM), while neither *p*NP GlcNAc nor **1b** showed activity. These results, similar to those observed for the *vibrio cholerae* sialidase (IC_{50} = 2 mM) in our preceding studty,[9] allowed us to confirm that 6-sulfo and 2-NHAc groups are recognized as key functional groups also by the influenza virus enzyme. The similar activity of **1a** for both bacterial and viral sialidases may be ascribed to the conserved topology of their catalytic domains in the active site.[4]

The model compounds **5–9** subjected to the same assay had variable activities depending on the aglycon structures (Table 2). Interestingly, compound **5** showed ca. 90 times stronger activity (IC_{50} = 33 μM) than **1a**, in spite of the fact that there was no diversity between the two structures except for the *p*-substituting groups (*p*-nitro and *p*-acetamido). The enhancement should be ascribable to the higher affinity of the *p*-acetamidophenyl group in **5** to this enzyme than that of the *p*-nitrophenyl one in **1a**.

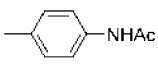
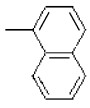
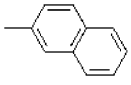
Compound **7** with a 2-naphthyl group was less active than **5**, while compound **6** with a 1-naphthyl group was much more active (IC_{50} = 10 μM) (Fig. 2). A large hydrophobic group at the aglycon may change the morphology of the glycoside to take a supra-molecular structure in aqueous solution. The assembling structure

Table 1. Inhibition activity of *p*NP-GlcNAc derivatives against influenza virus sialidase

Compd	Aglycon	IC_{50} (mM)[a]
1a		2.8
1b (2-NH_3^+)		nd[b]
*p*NP GlcNAc (6-OH)		nd

[a] The mean of duplicate experiments.
[b] Not detected.

Table 2. Inhibition activity of 6-sulfo-GlcNAc derivatives having various aglycons against influenza virus sialidase

Compd	Aglycon	IC_{50} (mM)[a]
5		0.033
6		0.010
7		0.110
8	–$CH_2CH_2CH_3$	0.011
9[b]	–$CH_2CH(OH)CH_2OH$	0.019

[a] The mean of duplicate experiments.
[b] Tested as a racemic mixture.

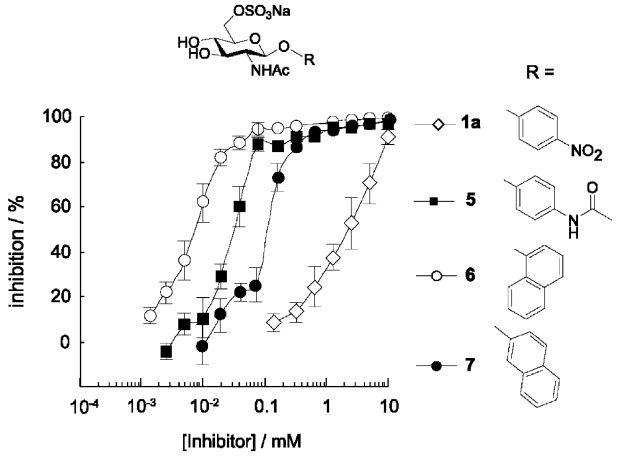


Figure 2. Inhibitory activities of 6-sulfo-GlcNAc derivatives against influenza sialidase. The degree of inhibition (%, deviation ±5%) was obtained as an averaged value for duplicate experiments using an equation as follows. Inhibition (%) = $100 \times F_i/F_o$, where F_i and F_o indicate the strength of fluorescence at Em. 450 nm (Ex. 365 nm) in the presence and the absence of inhibitors, respectively.

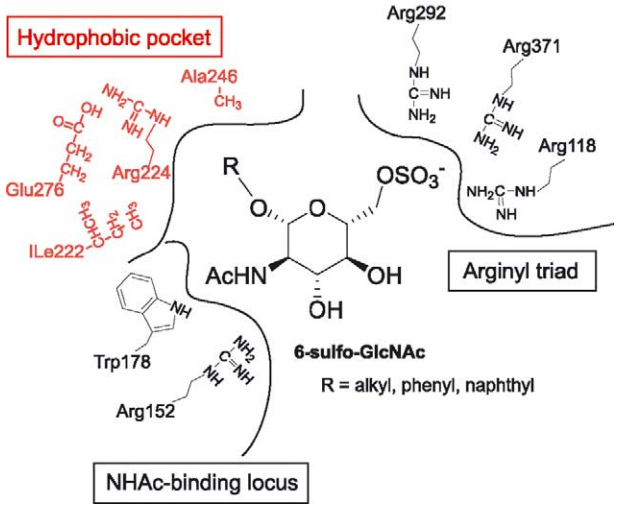


Figure 3. A proposed binding mode of 6-sulfo-GlcNAc derivatives with influenza virus sialidase referred to X-ray data of Varghese et al.[20] and Kim et al.[6c]

often induces strong biological activity due to the effect of a carbohydrate cluster. In the present case, however, the large difference in the activity between **5** and **7**, having analogous hydrophobic groups to each other, is apparently ascribed to the discrimination between the 1- and 2-naphthyl aglycons by this enzyme. It is probable that the enzyme may give a hydrophobic pocket at the position accepting the 1-naphthyl preferentially. Compound **8** with a *n*-propyl group also showed potent activity close to that of **6**, suggesting that the hydrophobic pocket admits both 1-naphthyl and the flexible alkyl groups. The activity was decreased when the hydrophobic aglycon was replaced with the glycerol in compound **9** ($IC_{50} = 19$ μM).

The above results have indicated that introduction of hydrophobic groups into the aglycon of **1** is more effective than that of hydrophilic ones, though natural sialic acids (**2** and **3**) bear a 1,2,3-triol function at the corresponding position. However, as judged from the stronger activity of **9** than that of **5** and **7**, it is obvious that this enzyme provides a hydrophilic domain for the recognition of the acyclic 1,2,3-triol moiety in **2**.

By X-ray crystalline analysis of a complex of influenza sialidase and natural Neu5Ac, Varghese et al.[20] disclosed that the enzyme possesses mainly three binding sites for sialyl acceptor substrates, that is, an arginine triad domain for the carboxylate anion, a hydrophobic pocket for the NHAc group, and a hydrophilic one for the acyclic side chain. More recently, Kim et al. [6c] reported the X-ray crystalline data of a complex of influenza sialidase and shikimic acid-based inhibitor. There, they have shown that the enzyme locates a hydrophobic pocket in the vicinity of the hydrophilic domain and turns to use it for artificial substrates bearing alkyl chains at the corresponding position. These X-ray data well accord with the present experimental data. A binding mode for the 6-sulfo-GlcNAc may be thus illustrated with a cartoon model as shown in Figure 3, where the 6-sulfo group and the hydrophobic aglycon groups are recognized, respectively, by the arginine triad in a way similar to the carboxylate anion and the hydrophobic pocket proposed for the alkyl groups.

The data in Table 2 have also disclosed that every model compound **5**–**9** is more active than the *p*NP derivative **1a**. This fact should mean that the *p*-nitro group is disfavored by the hydrophobic and hydrophilic domains to show seemingly a negative effect on the inhibitory activity. Consequently, among the synthetic models examined here, 1-naphthyl and *n*-propyl glycosides (**6** and **8**) showed the highest activity, indicating that introduction of these hydrophobic groups at the aglycon improves the inhibitory activity significantly.

3. Conclusion

We have demonstrated the notable activity of 6-sulfo-GlcNAc derivatives inhibiting an influenza virus sialidase. Though the observed activities are still poorer than that of Zanamivir **4**[6a,b] ($IC_{50} = 0.3$ μM, in our inhibition assay), it is of significance that the 6-sulfo-D-GlcNAc skeleton can be used for the development of sialidase inhibitors. This is because *N*-acetyl-D-glucosamine is one of the most abundant carbohydrate resources in nature. Moreover, the biological activity can be modulated in a variable way by changing the aglycon. It may be also of interest to replace the 6-sulfo group with a 6-sulfamide group possessing higher biological and chemical stability.

We envision the possibility that the inhibitory activity of 6-sulfo-D-GlcNAc has a certain biological meaning. This is because the skeleton is involved widely in mammalian cell surface oligosaccharides (glycosaminoglycans and glycoproteins). The oligosaccharides including 6-sulfo-GlcNAc are associated with GlcNAc-6-*O*-sulfotransferases distributed in human endothelial cells to give 6-sulfo-D-GlcNAc residues in the 6-sulfo-sialyl

Lewis^x motif.[21] Moreover, mammalian tissues are known to express sialidases,[22] which can potentially modulate the biological function of these sialyl glycoconjugates.[23] The behavior of the synthetic 6-sulfo-D-GlcNAc derivatives against the mammalian sialidases may provide key insights into the role of the natural 6-sulfo-D-GlcNAc linkages and the mammalian enzymes. The study along this line presently in progress will be reported elsewhere.

4. Experimental

Reagents of the highest commercial quality were purchased and used without further purification. Unless stated otherwise, reactions were performed in well-dried glassware under an inert atmosphere of N_2 and followed by thin-layer chromatography (TLC) on Merck aluminum roll silica gel 60-F_{254} and/or glass plate RP-18 F_{254} to visualize with UV light and *p*-anisaldehyde-sulfuric acid in EtOH. Merck silica gel 60 (particle size 40–63 μm) and LiChroprep® RP-18 (particle size 40–63 μm) were employed for column chromatography. Dowex® (AG-50W×4, Na^+ form) ion-exchange resin was used for purification on sulfonation steps.

^{1}H NMR (500 MHz and 300 MHz) spectra were recorded at ambient temperature with a Varian Inova 500 (or 300) spectrometer equipped with a Sun workstation. Optical rotations were determined on a JASCO DIP-1000 digital polarimeter at ambient temperature. IR spectra were recorded on a JASCO FT/IR-230 Fourier transform infrared spectrometer in the form of KBr disk. Mass spectra were taken on a JEOL JMS700 mass spectrometer under FAB conditions with an appropriate matrix. Elemental analyses were performed with PE2400 II CHN/O Analyzer. Fluorescence spectra were recorded on a JASCO FP-777 spectrofluorometer.

4.1. 4-Acetamidophenyl 2-acetamido-2-deoxy-6-*O*-sulfonate-β-D-glucopyranoside (5)

To a H_2O (2 mL) solution of *p*NP 6-sulfo-β-D-GlcNAc **1a** (50 mg, 0.11 mmol) was added a catalytic amount of $Pd(OH)_2/C$ and the mixture was stirred under H_2 atmosphere. After stirring at room temperature for 2 h, the mixture was filtered through Celite pad and concentrated in vacuo. The residue was dissolved in H_2O (2 mL) again. K_2CO_3 (46 mg, 0.33 mmol) was added to the solution and the mixture was stirred at 0 °C. Ac_2O (32 μL, 0.33 mmol) was added dropwise to the cool solution and stirred at 0 °C for 3 h. The reaction mixture was concentrated and purified by reverse phase column chromatography (H_2O). After treating the fraction with ion-exchange resin, the residue was lyophilized to afford **5** (41 mg 80%) as a white solid. $[\alpha]_D = -78.9°$ ($c = 0.11$ in H_2O); IR (KBr) 3299 (OH), 1660 and 1548 (amide), 1226 (OSO_3); ^{1}H NMR (500 MHz, D_2O, 30 °C) δ 7.33 (d, 2H, $J = 9.0$ Hz, H_{metha} of phenyl group), 7.06 (d, 2H, $J = 9.0$ Hz, H_{ortho} of phenyl group), 5.12 (d, 1H, $J = 8.5$ Hz, H-1), 4.37 (dd, 1H, $J = 2.0$, 11.5 Hz, H-6$_{proS}$), 4.23 (dd, 1H, $J = 5.5$, 11.5 Hz, H-6$_{proR}$), 3.97 (dd, 1H, $J = 8.0$, 10.5 Hz, H-2), 3.84 (m, 1H, H-5), 3.65 (dd, 1H, $J = 10.0$, 10.0 Hz, H-3), 3.61 (dd, 1H, $J = 9.0$, 9.5 Hz, H-4), 2.13, 2.02 (s×2, 6H, acetamido groups); HRFAB$^+$MS *m*/*z*: 479.0715 (calcd for $C_{16}H_{21}N_2Na_2O_{10}S$ 479.0712 $[M+Na]^+$). Anal. calcd for $C_{16}H_{21}N_2NaO_{10}S{\cdot}2H_2O$: C, 39.03; H, 5.12; N, 5.69. Found: C, 38.34; H, 5.19; N, 5.34.

4.2. Naphthyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosides (12) and (13)

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-glucopyranosyl chloride **11**[14] (200 mg, 0.55 mmol), tetrabutylammonium bromide (176 mg, 0.55 mmol), and 1- or 2-naphthol (157 mg, 1.09 mmol) in CH_2Cl_2 (2.5 mL) and 1 M NaOH aq (2.5 mL) were vigorously stirred at room temperature. After complete consumption of the chloride (50 min), the reaction mixture was worked up by adding ethyl acetate. The organic layer was successively washed with 1M NaOH aq, water and, finally, with saturated brine. The organic phase was dried over $MgSO_4$, concentrated, purified by silica gel column chromatography (hexane: ethyl acetate = 1:2→1:3), and crystallized from EtOH to give respectively 1-naphthyl derivative **12** (171 mg 66%) and 2-naphthyl derivative **13** (169 mg 65%) as white powder. **12**:[15a] mp 210–212 °C; $[\alpha]_D = -63.5°$ ($c = 0.20$ in MeOH); IR (KBr) 1747 and 1232 (*O*-acetyl), 1619 and 1556 (amide); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.17 (m, 1H, H-9 of naphthyl), 7.79 (m, 1H, H-6 of naphthyl), 7.53 (d, 1H, $J = 8.0$ Hz, H-4 of naphthyl), 7.49 (m, 2H, H-7, H-8 of naphthyl) 7.38 (t, 1H, $J = 8.0$, 8.0 Hz, H-3 of naphthyl) 7.03 (d, 1H, $J = 8.0$ Hz, H-2 of naphthyl), 5.57 (d, 1H, $J = 9.5$ Hz, amide), 5.31 (dd, 1H, $J = 10.5$, 10.5 Hz, H-3), 5.24 (d, 1H, $J = 8.5$ Hz, H-1), 5.23 (dd, 1H, $J = 10.5$, 10.5 Hz, H-4), 4.54 (m, 1H, H-2), 4.33 (dd, 1H, $J = 5.5$, 12.5 Hz, H-6$_{proR}$), 4.21 (d, 1H, $J = 2.5$, 12.0 Hz, H-6$_{proS}$), 3.93 (m, 1H, H-5), 2.10, 2.09, 2.07 (s×3, 9H, acetyl), 1.95 (s, 3H, acetamido); FAB$^+$MS: 474 $[M+H]^+$. Anal. calcd for $C_{24}H_{27}NO_9$: C, 60.88; H, 5.75; N, 2.96. Found: C, 60.80; H, 5.71; N, 2.81. **13**:[15a] mp 217–219 °C; $[\alpha]_D = -12.7°$ ($c = 0.20$ in MeOH); IR (KBr) 1749 and 1224 (*O*-acetyl), 1658 and 1535 (amide); ^{1}H NMR (500 MHz, CD_3OD) δ 7.79 (d, 2H, $J = 9.0$ Hz, H-4 and H-9 of naphthyl), 7.76 (d, 1H, $J = 8.0$ Hz, H-6 of naphthyl), 7.44 (m, 1H, H-7 of naphthyl), 7.41 (d, 1H, $J = 2.5$ Hz, H-1 of naphthyl), 7.36 (m, H-8 of naphthyl), 7.18 (dd, 1H, $J = 2.5$, 9.0 Hz, H-3 of naphthyl), 5.45 (d, 1H, $J = 8.0$ Hz, H-1), 5.37 (dd, 1H, $J = 10.5$, 10.5 Hz, H-3), 5.09 (dd, 1H, $J = 10.5$, 10.5 Hz, H-4), 4.33 (dd, 1H, $J = 5.5$, 12.5 Hz, H-6$_{proR}$), 4.16 (m, 2H, H-2 and H-6$_{proS}$), 4.08 (m, 1H, H-5), 2.04, 2.03, 2.02 (s×3, 9H, acetyl), 1.93 (s, 3H, acetamido); FAB$^+$MS: 474 $[M+H]^+$. Anal. calcd for $C_{24}H_{27}NO_9$: C, 60.88; H, 5.75; N, 2.96. Found: C, 60.78; H, 5.70; N, 2.81.

4.3. 1-Naphthyl 2-acetamido-2-deoxy-β-D-glucopyranoside (14)

To a MeOH solution (10 mL) of **12** (140 mg, 0.30 mmol) was added a catalytic amount of NaOMe. After stirring at room temperature for 1 h, the mixture was neutralized with ion exchange resin and filtered and then the filtrate was concentrated in vacuo to give a white powder **14**[15a] (100 mg, 99%). Mp 269–271 °C;

$[\alpha]_D = -68.6°$ ($c = 0.1$ in MeOH); IR (KBr) 3299 (OH), 1619 and 1556 (amide); ^{1}H NMR (500 MHz, CD_3OD) δ 8.17 (m, 1H, H-9 of naphthyl), 7.83 (m, 1H, H-6 of naphthyl), 7.50 (d, 1H, $J = 8.0$ Hz, H-4 of naphthyl), 7.44 (m, 2H, H-7, H-8 of naphthyl) 7.38 (t, 1H, $J = 8.0$, 8.0 Hz, H-3 of naphthyl) 7.18 (d, 1H, $J = 8.0$ Hz, H-2 of naphthyl), 5.11 (d, 1H, $J = 8.5$ Hz, H-1), 4.18 (dd, 1H, $J = 8.5$, 10.0 Hz, H-2), 3.97 (dd, 1H, $J = 3.0$, 12.3 Hz, H-6$_{proS}$), 3.75 (dd, 1H, $J = 5.5$, 12.3 Hz, H-6$_{proR}$), 3.57 (dd, 1H, $J = 10.5$, 10.5 Hz, H-4), 3.53 (m, 1H, H-5), 3.48 (dd, 1H, $J = 10.0$, 10.5 Hz, H-3), 1.95 (s, 3H, acetamido); FAB$^+$MS: 348 $[M+H]^+$. Anal. calcd for $C_{18}H_{21}NO_6 \cdot H_2O$: C, 59.17; H, 6.34; N, 3.83. Found: C, 59.33; H, 6.18; N, 3.70.

4.4. 1-Naphthyl 2-acetamido-2-deoxy-6-*O*-sulfonate-β-D-glucopyranoside sodium salt (6)

To the DMF (4 mL) solution of **14** (100 mg, 0.34 mmol) was added Me_3NSO_3 complex (236 mg, 1.75 mmol) in DMF (8 mL). After stirring at 50 °C for 3 h, the mixture was quenched with MeOH (5 mL) and stirred for 12 h. The residue was concentrated and purified by reverse phase silica gel column chromatography (H_2O to H_2O:MeOH = 3:1). The fraction was treated with ion-exchange resin and lyophilized to afford 6 (60 mg 40%) as a white solid. $[\alpha]_D = -89.6°$ ($c = 0.10$ in H_2O); IR (KBr) 3292 (OH), 1652 and 1552 (amide), 1236 (OSO_3); ^{1}H NMR (500 MHz, D_2O, 30 °C) δ 8.08 (m, 1H, H-9 of naphthyl), 7.93 (m, 1H, H-6 of naphthyl), 7.65 (d, 1H, $J = 8.5$ Hz, H-4 of naphthyl), 7.58 (m, 2H, H-7, H-8 of naphthyl) 7.49 (t, 1H, $J = 8.0$, 8.0 Hz, H-3 of naphthyl) 7.26 (dd, 1H, $J = 0.7$, 7.7 Hz, H-2 of naphthyl), 5.27 (d, 1H, $J = 8.5$ Hz, H-1), 4.44 (d, 1H, $J = 2.0$, 11.5 Hz, H-6$_{proS}$), 4.27 (dd, 1H, $J = 5.5$, 11.5 Hz, H-6$_{proR}$), 4.20 (dd, 1H, $J = 8.0$, 10.5 Hz, H-2), 3.96 (m, 1H, H-5), 3.70 (dd, 1H, $J = 10.5$, 9.5 Hz, H-3), 3.65 (dd, 1H, $J = 9.5$, 9.5 Hz, H-4), 1.93 (s, 3H, acetamido); HRFAB$^+$MS *m/z*: 472.0661 (calcd for $C_{18}H_{20}NNa_2O_9S$ 472.0654 $[M+Na]^+$). Anal. calcd for $C_{18}H_{20}NNaO_4S \cdot 4.5H_2O$: C, 40.75; H, 5.51; N, 2.64. Found: C, 40.47; H, 4.95; N, 2.36.

4.5. 2-Naphthyl 2-acetamido-2-deoxy-β-D-glucopyranoside (15)

To a MeOH solution (10 mL) of 13 (150 mg, 0.31 mmol) was added a catalytic amount of NaOMe. After stirring at room temperature for 1 h, the mixture was neutralized with ion exchange resin and filtered and then the filtrate was concentrated in vacuo to give a white powder of **15**[15a] (110 mg, 99%). Mp 245–248 °C; $[\alpha]_D = +16.4°$ ($c = 0.20$ in MeOH); IR (KBr) 3386 (OH), 1658 and 1535 (amide); ^{1}H NMR (500 MHz, CD_3OD) δ 7.76 (m, 3H, H-4, H-9 and H-6 of naphthyl), 7.42 (m, 2H, H-7 and H-1 of naphthyl), 7.34 (m, 1H, H-8 of naphthyl), 7.18 (dd, 1H, $J = 2.5$, 9.0 Hz, H-3 of naphthyl), 5.19 (d, 1H, $J = 8.5$ Hz, H-1), 3.97 (dd, 1H, $J = 8.5$, 10.5 Hz, H-2), 3.95 (dd, 1H, $J = 2.5$, 12.3 Hz, H-6$_{proS}$), 3.74 (dd, 1H, $J = 6.0$, 12.3 Hz, H-6$_{proR}$), 3.60 (dd, 1H, $J = 10.5$, 10.5 Hz, H-3), 3.53 (m, 1H, H-5), 3.44 (dd, 1H, $J = 10.3$, 10.0 Hz, H-4), 1.99 (s, 3H, acetamido); FAB$^+$MS: 348 $[M+H]^+$. Anal. calcd for $C_{18}H_{21}NO_6 \cdot 0.5H_2O$: C, 60.67; H, 6.22; N, 3.93. Found: C, 60.99; H, 5.99; N, 3.78.

4.6. 2-Naphthyl 2-acetamido-2-deoxy-6-*O*-sulfonate-β-D-glucopyranoside sodium salt (7)

To a DMF (4 mL) solution of **15** (123 mg, 0.35 mmol) was added Me_3NSO_3 complex (246 mg, 1.77 mmol) in DMF (8 mL). After stirring at 50 °C for 3 h, the mixture was quenched with MeOH (5 mL) and stirred for 12 h. The residue was concentrated and purified by reverse phase silica gel column chromatography (H_2O to H_2O:MeOH = 3:1). The fraction was treated with ion-exchange resin and lyophilized to afford 7 (72 mg 45%) as a white amorphous. $[\alpha]_D = -80.6°$ ($c = 0.12$ in H_2O); IR (KBr) 3396 (OH), 1656 and 1548 (amide), 1253 (OSO_3); ^{1}H NMR (300 MHz, D_2O, 30 °C) δ 7.64 (m, 3H, H-4, H-9 and H-6 of naphthyl), 7.34 (m, 1H, H-7 of naphthyl), 7.27 (m, 2H, H-8 and H-1 of naphthyl), 7.03 (dd, 1H, $J = 2.4$, 9.0 Hz, H-3 of naphthyl), 5.12 (d, 1H, $J = 8.7$ Hz, H-1), 4.23 (dd, 1H, $J = 2.1$, 11.5 Hz, H-6$_{proS}$), 4.07 (dd, 1H, $J = 5.7$, 11.5 Hz, H-6$_{proR}$), 3.89 (dd, 1H, $J = 8.4$, 9.9 Hz, H-2), 3.67 (m, 1H, H-5), 3.56 (dd, 1H, $J = 9.9$, 9.6 Hz, H-3), 3.44 (dd, 1H, $J = 9.6$, 9.6 Hz, H-4), 1.87 (s, 3H, acetamido); HRFAB$^+$MS *m/z*: 472.0658 (calcd for $C_{18}H_{20}NNa_2O_9S$ 472.0654 $[M+Na]^+$). Anal. calcd for $C_{18}H_{20}NNaO_9S \cdot 0.5H_2O$: C, 47.16; H, 4.62; N, 3.06. Found: C, 47.48; H, 4.74; N, 3.04.

4.7. Allyl 2-acetamido-2-deoxy-β-D-glucopyranoside (16)

To an allylalcohol (30 mL) solution of *N*-acetyl-D-glucosamine (2.0 g, 7.04 mmol) was added TMSCl (0.01 eq for GlcNAc) and the reaction mixture was stirred at 60–80 °C for 30 min. After cooling to room temperature, the reaction mixture was neutralized with Et_3N and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 10:1) and crystallized from EtOH to afford **16**[17] (704 mg, 35%) as a colorless crystal. Mp 160–163 °C; $[\alpha]_D = -28.4°$ ($c = 0.42$ in MeOH); IR (KBr) 3284 (OH), 1652 and 1546 (amide); ^{1}H NMR (500 MHz, CD_3OD) δ 5.88 (m, 1H, =CH in allyl group), 5.25 (ddd, 1H, $J = 1.5$, 3.0, 17.0 Hz, =CH$_{cis}$H$_{trans}$ in allyl group), 5.15 (ddd, 1H, $J = 1.5$, 3.0, 10.5 Hz, =CH$_{cis}$H$_{trans}$ in allyl group), 4.42 (d, 1H, $J = 8.5$ Hz, H-1), 4.32 (m, 1H, OCH_2 in allyl group), 4.06 (m, 1H, OCH_2 in allyl group), 3.86 (dd, 1H, $J = 2.5$, 12.3 Hz, H-6$_{proS}$), 3.65 (dd, 1H, $J = 4.0$, 12.3 Hz, H-6$_{proR}$), 3.32 (dd, 1H, $J = 8.5$, 11.0 Hz, H-2), 3.26 (m, 2H, H-3 and H-4), 3.23 (ddd, $J = 2.5$, 5.0, 10.5 Hz, H-5), 1.96 (s, 3H, acetamido); HRFAB$^+$MS *m/z*: 262.1299 (calcd for $C_{11}H_{20}NO_6$ 262.1291 $[M+Na]^+$). Anal. calcd for $C_{11}H_{21}NO_6$: C, 50.18; H, 8.04; N, 5.32. Found: C, 49.01; H, 8.13; N, 5.05.

4.8. *n*-Propyl 2-acetamido-2-deoxy-β-D-glucopyranoside (17)

To a MeOH (20 mL) solution of **16** (500 mg, 1.90 mmol) was added a catalytic amount of $Pd(OH)_2$/C. After vigorously stirring under H_2 atmosphere for 1.5 h, the mixture was filtered through Celite pad and concentrated in vacuo. The residue was crystallized from

EtOH to afford **17**[24] (495 mg, >99%) as a white crystal. Mp 183–184 °C; $[\alpha]_D = -28.9°$ ($c = 0.64$ in MeOH). IR (KBr) 3270 (OH), 1654 and 1556 (amide); ^{1}H NMR (500 MHz, D_2O, 30 °C) δ 4.44 (d, 1H, $J = 8.5$ Hz, H-1), 3.83 (dd, 1H, $J = 1.5$, 12.5 Hz, H-6$_{proS}$), 3.77 (m, 1H, OCH_2 in propyl group), 3.66 (m, 1H, H-6$_{proR}$), 3.60 (dd, 1H, $J = 8.5$, 10.5 Hz H-2), 3.46–3.36 (m, 4H, H-5, H-3, H-4 and OCH_2 in propyl group), 1.96 (s, 3H, acetamido), 1.47 (m, 2H, CCH_2 in propyl group), 0.79 (t, 1H, $J = 7.5$, 7.5 Hz, CCH_3 in propyl group); FAB$^+$MS 286 $[M+Na]^+$. Anal. calcd for $C_{11}H_{21}NO_6 \cdot 0.1H_2O$: C, 49.84; H, 8.06; N, 5.28. Found: C, 49.69; H, 7.47; N, 5.09.

4.9. *n*-Propyl 2-acetamido-2-deoxy-6-*O*-sulfonate-β-D-glucopyranoside sodium salt (8)

To a DMF (15 mL) solution of **17** (100 mg, 0.38 mmol) was added Me_3NSO_3 complex (318 mg, 2.28 mmol). After stirring at 40 °C for 6 h, the reaction was quenched with MeOH (10 mL). The mixture was stirred at room temperature for 12 h and concentrated in vacuo. The residue was purified by reverse phase silica gel column chromatography (H_2O). Treating the mixture with ion-exchange resin, the residue was lyophilized to afford **8** (74 mg, 53%) as a white solid. $[\alpha]_D = -62.5°$ ($c = 0.17$ in H_2O); IR (KBr) 3295 (OH), 1656 and 1554 (amide), 1251 (OSO_3); ^{1}H NMR (500 MHz, D_2O, 30 °C) δ 4.39 (d, 1H, $J = 8.5$ Hz, H-1), 4.20 (dd, 1H, $J = 1.5$, 12.3 Hz, H-6$_{proS}$), 4.07 (dd, 1H, $J = 5.5$, 12.3 Hz, H-6$_{proR}$), 3.70 (ddd, 1H, $J = 10.0$, 10.0, 10.0, OCH_2 in propyl group), 3.55 (dd, 1H, $J = 8.5$, 10.5 Hz H-2), 3.50 (m, 1H, H-5), 3.42 (m, 2H, H-3, H-4 and OCH_2 in propyl group), 1.89 (s, 3H, acetamido), 1.41 (m, 2H, CCH_2 in propyl group), 0.72 (t, 1H, $J = 7.5$, 7.5 Hz, CCH_3 in propyl group); HRFAB$^+$MS *m/z*: 388.0653 (calcd for $C_{11}H_{20}NNa_2O_9S$ 388.0654 $[M+Na]^+$). Anal. calcd for $C_{11}H_{20}NNaO_9S \cdot 2H_2O$: C, 32.92; H, 6.03; N, 3.49. Found: C, 32.91; H, 5.94; N, 3.44.

4.10. Allyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (18)

To a pyridine (20 mL) solution of **16** (700 mg, 2.44 mmol) was added acetic anhydride (2 mL) at 0 °C and the mixture was stirred overnight. After concentration of the reaction mixture, the residue was diluted with ethyl acetate and the solution was washed with 1 N HCl aq, satd $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (toluene: ethyl acetate = 1:1) to give **18** (940 mg, 98%) as a white powder. $[\alpha]_D = -24.1°$ ($c = 1.00$ in MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 5.88 (m, 1H, =CH in allyl group), 5.25 (ddd, 1H, $J = 1.5$, 3.0, 17.0 Hz, =CH$_{cis}H_{trans}$ in allyl group), 5.20 (dd, 1H, $J = 10.5$, 10.5 Hz, H-3), 5.15 (ddd, 1H, $J = 1.5$, 3.0, 10.5 Hz, =CH$_{cis}$H$_{trans}$ in allyl group), 4.97 (dd, 1H, $J = 10.5$, 10.5 Hz, H-4), 4.65 (d, 1H, $J = 8.5$ Hz, H-1), 4.30 (m, 1H, OCH_2 in allyl group), 4.27 (dd, 1H, $J = 5.0$, 12.3 Hz, H-6$_{proR}$), 4.10 (dd, 1H, $J = 2.5$, 12.3 Hz, H-6$_{proS}$), 4.05 (m, 1H, OCH_2 in allyl group), 3.86 (dd, 1H, $J = 8.5$, 11.0 Hz, H-2), 3.76 (ddd, $J = 2.5$, 5.0, 10.5 Hz, H-5), 2.05, 1.99, 1.97 (s×3, 9H, acetyl), 1.89 (s, 3H, acetamido). Anal. calcd for $C_{17}H_{25}NO_9$: C, 52.71; H, 6.50; N, 3.62. Found: C, 52.77; H, 6.50; N, 3.49

4.11. 1 (and 3)-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glycopyranosyl)-*sn*-glycerol (19)

AD-mix-α (5.41 g) was dissolved in *tert*-BuOH (5 mL) and H_2O (10 mL). Stirring at room temperature produced two clear phases. The mixture was cooled to 0 °C, and **18** (1.5 g, 3.87 mmol) dissolved in *tert*-BuOH (5 mL) was added dropwise to the solution and vigorously stirred at 0 °C for 12 h. While the mixture was stirred at 0 °C, sodium sulfite (5.7 g) was added and the mixture was allowed to warm to room temperature and stirred for 30 min. *n*-BuOH was added to the reaction mixture, and after separation of the layers, the aqueous phase was further extracted with *n*-BuOH. The combined organic layers were dried over $MgSO_4$. The residue was purified by silica gel column chromatography ($CHCl_3$: MeOH = 20:1) to afford the diol **19** (1.3 g, 3.09 mmol) in 80% yield as diastereo mixture (55:45). IR (KBr) 3318 (OH), 1745 and 1232 (acetyl), 1652 and 1546 (amide); ^{1}H NMR (500 MHz, CD_3OD) δ 5.28 (dd×2, 2H, $J = 10.5$, 10.5 Hz, H-3), 5.06 (dd×2, 2H, $J = 10.5$, 10.5 Hz, H-4), 4.63 (d×2, $J = 8.5$ Hz, H-1), 4.26 (dd×2, 1H, $J = 5.0$, 12.5 Hz, H-6$_{proR}$), 4.06 (dd×2, 1H, $J = 2.5$, 12.5 Hz, H-6$_{proS}$), 3.85 (dd×2, 1H, $J = 8.4$, 10.2 Hz H-2), 3.79 (m, 1H, H-5), 3.73–3.46 (m, 5H, glycerol), 2.04, 1.99, 1.96 (s×3, 9H, acetyl), 1.90 (s, 3H, acetamido); HRFAB$^+$MS *m/z*: 422.1656 (calcd for $C_{17}H_{28}NO_{11}$ 422.1662 $[M+H]^+$).

4.12. 1,2 (and 2,3)-*O*-Isopropylidene-3 (and 1)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-*sn*-glycerol (20)

To a DMF (10 mL) solution of **19** (670 mg, 1.59 mmol) was added $Me_2C(OMe)_2$ (390 μL, 3.18 mmol) and pyridinium *p*-toluenesulfonic acid (PPTS) (40 mg 0.16 mmol). After stirring under N_2 atmosphere at room temperature for 2.5 h, the mixture was neutralized with Et_3N. The residue was eluted with EtOAc and the organic layer was washed with brine and H_2O. The solution was dried over $MgSO_4$. The residue was concentrated in vacuo and purified by silica gel column chromatography ($CHCl_3$:MeOH = 10:1) to afford colorless syrup **20** (421 mg, 60%) as diastereo mixture (55:45). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.12 (d×2, 1H, $J = 8.5$ Hz, acetamide), 5.28 (dd×2, 1H, $J = 10.5$, 10.5 Hz, H-3), 5.06 (dd×2, 1H, $J = 10.5$, 10.5 Hz, H-4), 4.77 (d×2, 1H, $J = 8.0$ Hz, H-1), 4.26 (m, 2H, H-6$_{proR}$ and glycerol), 4.06 (dd, 1H, $J = 2.5$, 11.4 Hz, H-6$_{proS}$), 4.02 (m, 1H, glycerol), 3.89 (m, 1H, glycerol), 3.79 (dd×2, 1H, $J = 8.4$, 10.2 Hz H-2), 3.73 (m, 1H, H-5), 3.63 (dd×2, 1H, $J = 6.0$, 10.5 Hz, glycerol), 2.09, 2.03, 2.02 (s×3, 9H, acetyl), 1.95 (s, 3H, acetamido), 1.42, 1.34 (s×2, 6H, isopropylidene); HRFAB$^+$MS *m/z*: 462.1985 (calcd for $C_{20}H_{32}NO_{11}$ 462.1975 $[M+H]^+$).

Compound **20** was dissolved in MeOH (10 mL) and a catalytic amount of NaOMe was added in the solution. After stirring at room temperature for 1 h, the mixture was neutralized with ion exchange resin and filtered and

then the filtrate was concentrated in vacuo to give deacetylated one as a white powder [320 mg, 90%, diastereo mixture (55:45)]. This compound was used to the next reaction without further purification.

4.13. 1 (and 3)-*O*-(2-acetamide-2-deoxy-6-*O*-sulfonate-β-D-glucopyranosyl)-*sn*-glycerol sodium salt (9)

To a DMF (10 mL) solution of the deacetylated compound (244 mg, 0.73 mmol) was added Me_3NSO_3 complex (303 mg, 2.19 mmol). After stirring at 40 °C for 2 h, the mixture was quenched with MeOH (8 mL) and stirred for 12 h. The residue was concentrated and purified by silica gel column chromatography ($CHCl_3$:MeOH = 3:1) to afford sulfated compound (100 mg) as a syrup. The syrup was dissolved in TFA:H_2O:MeOH = 1:12:12 (5 mL) and the mixture stirred at room temperature for 1 h and concentrated. The residue was dissolved in H_2O, treated with ion-exchange resin and lyophilized to afford **9** (100 mg, 34%) as diastereo mixture (55:45). IR (KBr) 3399 (OH), 1656 and 1558 (amide), 1218 (OSO_3); ^{1}H NMR (500 MHz, D_2O, 30 °C) δ 4.39 (d×2, 1H, J = 8.5 Hz H-1), 4.19 (dd, 1H, J = 2.0, 11.4 Hz, H-6$_{proS}$), 4.08 (dd, 1H, J = 5.5, 11.4 Hz, H-6$_{proR}$), 3.70 (m, 2H), 3.30–3.58 (m, 7H), 1.89 (s, 3H, acetamido); HRFAB$^+$MS m/z: 420.0535 (calcd for $C_{11}H_{20}NNa_2O_{11}S$ 420.0552 $[M+Na]^+$).

4.14. *p*-Nitrophenyl 2-deoxy-6-*O*-sulfonate-sulfo-2-trifluoroacetamido-β-D-glucopyranoside sodium salt (22)

To a DMF (6 mL) solution of *p*-nitrophenyl 2-deoxy-2-trifluoroacetamido-β-D-glucopyranoside **21**[25] (200 mg, 0.50 mmol) was added Me_3NSO_3 complex (210 mg, 1.50 mmol) in DMF (4 mL). After stirring at 40 °C for 3 h, the mixture was quenched with MeOH (4 mL) and stirred at room temperature for 12 h. The residue was concentrated and purified by reverse phase silica gel column chromatography (H_2O). After treating the fraction with ion-exchange resin, the residue was lyophilized to afford **22** (166 mg 67%) as a white solid. $[\alpha]_D = -21.2°$ (c = 0.07 in H_2O); IR (KBr) 3448 (OH), 1716 and 1600 (amide), 1245 (OSO_3); ^{1}H NMR (500 MHz, D_2O, 30 °C) δ 8.18 (d, 2H, J = 9.0 Hz, H_{metha} of phenyl group), 7.13 (d, 2H, J = 9.0 Hz, H_{ortho} of phenyl group), 5.34 (d, 1H, J = 8.5 Hz, H-1), 4.34 (d, 1H, J = 2.0, 11.5 Hz, H-6$_{proS}$), 4.19 (dd, 1H, J = 6.0, 11.5 Hz, H-6$_{proR}$), 4.08 (dd, 1H, J = 8.0, 10.5 Hz, H-2), 3.90 (m, 1H, H-5), 3.73 (dd, 1H, J = 10.5, 9.0 Hz, H-3), 3.58 (dd, 1H, J = 9.0, 10.0 Hz, H-4); HRFAB$^+$MS m/z: 521.0073 (calcd for $C_{14}H_{14}F_3N_2Na_2O_{11}S$ 521.0066 $[M+Na]^+$).

4.15. *p*-Nitrophenyl 2-amino-2-deoxy-6-*O*-sulfonate-β-D-glucopyranoside (1b)

Compound **22** (150 mg, 0.30 mmol) was dissolved in 0.1N NaOH aq (10 mL) and the mixture was stirred at room temperature for 2 h. After neutralizing the mixture with 0.1 N HCl aq, the solution was concentrated and purified by reverse phase silica gel column chromatography (H_2O). The residue was lyophilized to afford **1b** (100 mg 89%) as a white solid. $[\alpha]_D = -77.8°$ (c = 0.06 in H_2O); IR (disk) 3442 (OH), 1247 (OSO_3); ^{1}H NMR (500 MHz, D_2O, 30 °C) δ 8.21 (d, 2H, J = 9.0 Hz, H_{metha} of phenyl group), 7.21 (d, 2H, J = 9.0 Hz, H_{ortho} of phenyl group), 5.20 (d, 1H, J = 8.5 Hz, H-1), 4.32 (d, 1H, J = 2.0, 11.5 Hz, H-6$_{proS}$), 4.17 (dd, 1H, J = 6.0, 11.5 Hz, H-6$_{proR}$), 3.88 (m, 1H, H-5), 3.52 (m, 2H, H-3 and H-4), 3.03 (m, 1H, H-2); HRFAB$^+$MS m/z: 381.0602 (calcd for $C_{12}H_{17}N_2O_{10}S$ 381.0604 $[M+H]^+$). Anal. calcd for $C_{12}H_{16}N_2O_{10}S{\cdot}2H_2O$: C, 34.62; H, 4.84; N, 6.73. Found: C, 34.36; H, 4.90; N, 6.46.

4.16. Sialidase inhibition assay using influenza virus

The inhibition assay was carried out with 100 mM sodium acetate buffer (pH 5.0) in a micro tube (the number of replicates = 2). A buffer solution (5 μL, 10 μg/mL based on protein volume) of influenza virus (A/Memphis/1/71 H3N2)[26] was incubated with 4 mM 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid (SIGMA, M8639) (5 μL) and inhibitor (5 μL) prepared at appropriate concentration at 37 °C for 30 min. The reaction was stopped with 1 mL of cold 0.1 M sodium carbonate (pH 11.5). The fluorescence of released 4-methylumbelliferone was detected on a spectrofluorometer (Jasco FP-777) with excitation at 365 nm and emission at 450 nm.

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