Supporting Information

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Experimental Section

Unless otherwise noted, all compounds and reagents were purchased from Acros or Sigma-Aldrich. All chemicals were purchased as reagent grade and used without further purification. *N*-acetylneuraminic acid aldolase was purchased from Toyobo STC. Reactions were monitored with analytical TLC in EM silica gel 60 F254 plates and visualized under UV (254 nm) and/or staining with acidic ceric ammonium molybdate or ninhydrin. Flash column chromatography was performed on Geduran silica gel 60 (35–75 μm, EM Science). ^{1}H NMR spectra were recorded on a Bruker DRX-400 (400-MHz) spectrometer at 20 °C. Chemical shifts were assigned according to the $CHCl_3$ (δ = 7.24 ppm). ^{13}C NMR spectra were obtained using the attached proton test on a Bruker DRX-400 (100-MHz) spectrometer and were reported using the signal of $CDCl_3$ (δ = 77.0 ppm of central line) for calibration. Mass spectra were obtained by the analytical services of The Scripps Research Institute (ESI-TOF; Agilent) and the Genomics Research Center (Academia Sinica) (LTQ Orbitrap XL ETD). Fluorescence spectra were obtained on a Molecular Devices Spectramax M5 spectrometer. Protease inhibitors were purchased from Roche Applied Sciences; PVDF membranes were from Millipore. NuPAGE Bis-Tris Mini Gels (4–12%), PBS and cell culture media, and reagents were from Invitrogen. Protein concentration was measured by either BCA protein assay (Thermo Scientific) or Bradford assay (Bio-Rad). GM02921 and GM02922 were obtained from the NIGMS Human Genetic Mutant Cell Repository. Chemiluminescence on protein blots was visualized and quantified using the FujiFilm LAS3000 imaging system. Confocal microscopy of sialidase-expressing 293T cells was obtained using Leica TCS-SP5-MP-SMD.

Synthetic Procedures

Methyl 5-(Pent-4-Ynamido)-2,4,7,8,9-Penta-*O*-Acetyl -3,5-Dideoxy-3-Fluoro-D-*Erythro*-α-L-*Manno*-Non-2-Ulopyranosonate (3). A mixture of *N*-4-pentynoylmannosamine (460.0 mg, 1.78 mmol), 3-fluoropyruvic acid (as the sodium salt, 458.2 mg, 3.56 mmol), NaN_3 (1%, 500 μL), and *N*-acetylneuraminic acid aldolase (200 U), in potassium phosphate buffer (pH 7.4, 0.05 mmol/L, 25.0 mL), was incubated at room temperature for 3 d. The mixture was concentrated. The residue was applied to a Dowex column (1 × 2, 200 mesh) and eluted with water and formic acid (0.1–1.0 mol/L) sequentially. Fractions containing the desired product **2** were pooled, and concentrated under reduced pressure. The diastereomeric ratio (axial/equatorial = 7:1 ~3:1) was determined by chemical shift of H-3 (5.03~4.71 in D_2O).

To the crude product **2** were added MeOH (30 mL) and ion exchange resin Amberlite IR 120-H (500 mg). The mixture was stirred at room temperature for 24 h and filtered through a pad of Celite, giving ester product. MeOH was removed, and the residue was treated with pyridine (25 mL), 4-dimethylaminopyridine (DMAP) (10.0 mg) and Ac_2O (10 mL). The mixture was stirred at room temperature for 12 h. After that, pyridine was removed under vacuum first and the residue was taken up in EtOAc (100 mL) and washed with 5% citric acid (×3), 10% $NaHCO_3$ (×3), and brine. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated. The single diastereomer **3** (366.6 mg, 35% overall yield) was obtained as white foam after silica gel column chromatography eluted with EtOAc/hexane (4:1). TLC (EtOAc/hexane = 3:1) R_f = 0.31. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.66 (d, J = 9.0 Hz, 1 H), 5.55 (d, J = 2.4, 10.9, 27.7 Hz, 1 H), 5.34 (dd, J = 1.9, 5.3 Hz, 1 H), 5.11 (m, 1 H), 4.92 (dd, J = 2.4, 49.1 Hz, 1 H), 4.51 (dd, J = 2.4, 12.5 Hz, 1 H), 4.25 (dd, J = 1.3, 10.6 Hz, 1 H), 4.17 (dd, J = 6.4, 12.5 Hz, 1 H), 4.13 (m, 1 H), 2.53–2.39 (m, 2 H), 2.37–2.26 (m, 2 H), 2.16 (s, 3 H), 2.13 (s, 3 H), 2.07 (s, 3 H), 2.01 (s, 3 H), 1.99 (s, 3 H), 1.97 (t, J = 2.5 Hz, 1 H). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 171.2, 170.6, 170.5, 170.3, 170.2, 167.1, 165.1, 95.1 (d, J = 29.0 Hz), 86.9 (d, J = 184.0 Hz), 82.7, 71.6, 71.1, 69.6, 68.2, 68.1, 68.0, 62.1, 53.5, 45.7, 35.4, 20.8 (2 ×), 20.7, 20.5, 14.6. ^{19}F-NMR ($CDCl_3$, 282.4 MHz) δ–209.1 (dd, J = 28.0, 52.0 Hz) HR-ESI MS calcd for $C_{25}H_{33}NO_{14}$ $[M + H]^+$: 548.1774; found: 548.1770.

Methyl 5-(Pent-4-Ynamido)-4,7,8,9-Tetra-*O*-Acetyl-3,5-Dideoxy-3-Fluoro-D-*Erythro*-α-L-*Manno*-2-Non-2-Ulopyranosonate (3). To a solution of compound **3** (165.0 mg, 0.28 mmol) in 10 mL of CH_2Cl_2 was added hydrazine acetate (116.0 mg, 1.26 mmol) in 2.0 mL of MeOH. The mixture was stirred at 0 °C for 8 h, then concentrated under reduced pressure. The product **4** (110.0 mg, 72%) was obtained as an oil after silica gel column chromatography eluted with EtOAc/hexane (4:1). TLC (EtOAc/hexane = 3:1) R_f = 0.31. ^{1}H-NMR (400 MHz, $CDCl_3$: CD_3OD = 3:2) δ 5.46 (dd, J = 2.4, 4.4 Hz, 1 H), 5.39–5.26 (m, 2 H), 4.97 (dd, J = 2.4, 50 Hz, 1 H), 4.76 (m, 1 H), 4.46–4.34 (m, 2 H), 4.20 (dd, J = 7.4, 12.4 Hz, 1 H), 3.85 (s, 3 H), 2.54–2.40 (m, 2 H), 2.36–2.30 (m, 2 H), 2.17 (s, 3 H), 2.11 (s, 3 H), 2.09 (s, 3 H), 2.07–2.05 (m, 4 H). ^{13}C-NMR (100 Hz, $CDCl_3$ + CD_3OD) δ 173.7, 172.4, 171.9, 171.7, 171.6, 168.7, 95.5 (d, J = 20.0 Hz), 88.5 (d, J = 146.0 Hz), 83.5, 72.6, 71.2, 71.1, 71.0, 70.0, 69.3, 63.6, 53.4, 45.4, 36.1, 21.1 (2 ×), 21.0, 15.4. ^{19}F-NMR ($CDCl_3$, 282.4 MHz) δ –205.3 (dd, J = 28.0, 52.0 Hz). HR-ESI MS calcd for $C_{23}H_{31}FNO_{13}$ $[M + H]^+$: 522.1618; found: 522.1211.

Methyl 5-(Pent-4-Ynamido)-4,7,8,9-Tetra-*O*-Acetyl-2,3,5-Trideoxy-3-Fluoro-D-*Erythro*-β-L-*Manno*-Non-2-Ulopyranosylonate Fluoride (PDFSA). To a solution of compound **4** (75.0 mg, 0.14 mmol) in 5 mL of CH_2Cl_2 was added 19 μL (0.19 mmol) of diethylaminosulfur trifluoride at –30 °C, and stirred for 5 h. The reaction was quenched by adding a small amount of silica gel and 1.5 mL of MeOH. The mixture was concentrated under reduced pressure. PDFSA (α-anomer, 46.0 mg, 60%) and the β-anomer (23.0 mg, 30%) were isolated by silica gel column chromatography eluted with EtOAc/hexane (5:1). TLC (EtOAc/hexane = 3: 1) R_f = 0.37 (anomer) and 0.33 (PDFSA).

PDFSA (α-anomer). TLC (EtOAc/hexane = 3:1) R_f = 0.33. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.79 (d, J = 8.9 Hz, 1 H), 5.46 (dd, J = 10.7, 25.6 Hz, 1 H), 5.36–5.28 (m, 1 H), 5.10 (ddd, J = 2.6, 2.7, 50.7, 1 H), 4.34 (d, J = 10.9 Hz, 1 H), 4.29 (dd, J = 1.7, 12.4 Hz, 1 H), 4.16 (dd, J = 4.2, 12.4 Hz, 1 H), 4.08 (m, 1 H), 3.87 (s, 3 H), 2.52–2.37 (m, 2 H), 2.36–2.23 (m, 2 H), 2.12 (s, 3 H), 2.08 (s, 3H), 2.06 (s, 3 H), 1.99 (s, 3 H), 1.97 (t, J = 2.5 Hz, 1 H). ^{13}C-NMR (100 Hz, $CDCl_3$) δ 171.3, 170.5 (2 ×), 170.4, 170.2, 164.3 (d, J = 20.0 Hz), 104.5 (dd, J = 13.0, 179.0 Hz), 85.4 (dd, J = 16.0, 154.0 Hz), 82.7, 72.5, 69.6, 69.0, 68.3 (d, J = 5.0 Hz), 67.0, 61.8, 53.7, 45.5 (d, J = 3.0 Hz), 35.4, 20.7, 20.6 (2 ×), 20.5, 14.6. ^{19}F-NMR ($CDCl_3$, 282.4 MHz) δ –123.3 (d, J = 12.0 Hz), –217.1 (ddd, J = 12.0, 24.0, 52.0 Hz). ESI-HR MS calcd for $C_{23}H_{30}F_2NO_{12}$ $[M + H]^+$: 550.1730; found: 550.1736.

PDFSA (β-anomer). TLC (EtOAc/hexane = 3: 1) R_f = 0.37. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.68 (d, J = 9.7 Hz, 1 H), 5.42 (dd, J = 2.1, 6.3 Hz, 1 H), 5.38 (m, 1 H), 5.25 (m, 1 H), 5.10 (dd, J = 2.3, 48.6 Hz, 1 H), 4.48 (dd, J = 2.6, 12.6 Hz, 1 H), 4.43 (dd, J = 10.5, 20.8 Hz, 1 H), 4.32 (d, J = 10.7 Hz, 1 H), 4.11 (dd, J = 10.5, 20.8 Hz, 1 H), 3.86 (s, 3 H), 2.38–2.53 (m, 2 H), 2.23–2.37 (m, 2 H), 2.13 (s, 3 H), 2.09 (s, 3 H), 2.04 (s, 3 H), 2.03 (s, 3 H), 1.99 (t, J = 2.6 Hz,

1H). ^{13}C-NMR (100 Hz, $CDCl_3$) δ 171.1, 170.6, 170.5, 170.2, 169.9, 162.6 (d, J = 21.0 Hz), 105.0 (dd, J = 23.0, 183.0 Hz), 84.6 (dd, J = 35.0, 147.0 Hz), 82.84, 72.7 (d, J = 2.0 HZ), 72.3 (d, J = 2.0 Hz), 69.6, 68.4 (d, J = 14.0 Hz), 67.1, 62.1, 53.7, 44.5, 35.4, 20.7 (3 ×), 20.6, 14.6. ^{19}F-NMR ($CDCl_3$, 282.4 MHz) δ –122.4 (d, J = 20.0 Hz), –207.2 (d, J = 16.0 Hz).

5-(Pent-4-Ynamido)-2,3,5-Trideoxy-3-Fluoro-D-*Erythro*-β-L-*Manno*-Non-2-Ulopyranosylonic Fluoride (DFSA). To a solution of PDFSA (42.0 mg, 0.076 mmol) in 5 mL of CH_3OH was added Na_2CO_3 (32.4 mg, 0.31 mmol) at room temperature for 1 h. H_2O (1 mL) was added, and the mixture was left at room temperature for 2 h. The mixture was neutralized by ion exchange resin Amberlite IR 120-H and filtered through a pad of Celite. The filtrate was concentrated under reduced pressure, and the crude product was chromatographed on a silica gel 100 reversed-phase C18 column (H_2O to 10% aqueous MeOH) to yield product DFSA (23.7 mg, 85%) as a white foam. ^{1}H-NMR (400 MHz, D_2O) δ 5.24 (ddd, J = 2.5, 2.5, 51.3 Hz, 1 H), 4.12–4.37 (m, 2 H), 3.82–3.93 (m, 3 H), 3.61–3.72 (m, 2 H), 2.51–2.60 (m, 4 H), 2.42 (s, 1H). ^{13}C-NMR (150 Hz, D_2O) δ 175.6, 168.2 (d, J = 40.6 Hz), 106.1 (dd, J = 15.5, 218.1 Hz), 88.4 (dd, J = 18.0, 183.5 Hz), 83.3, 72.7 (d, J = 3.3 Hz), 70.3 (d, J = 5.6 Hz), 68.6 (dd, J = 5.6, 17.8 Hz), 67.8, 63.0, 48.8, 46.8 (d, J = 3.3 Hz), 34.6 (d, J = 7.1 Hz), 14.5. ^{19}F-NMR ($CDCl_3$, 282.4 MHz) δ –121.3 (d, J = 12.0 Hz), –218.0 (ddd, J = 12, 28, 52 Hz). HR-ESI MS calcd for $C_{14}H_{20}F_2NO_8$ $[M + H]^+$: 368.1151; found: 368.1152.

Cloning of Bacterial Sialidases. The sialidases nanA (SP1693), nanB (SP1687), and nanC (SP1326) were amplified by PCR from *Streptococcus pneumonia* TIGR4 genomic DNA (ATCC BAA-334), whereas sialidases nanH (CPF 0985), nanI (CPF 0721), and nanJ (CPF 0532) were cloned into a modified form of pET47b+ (Novagen) from *Clostridium perfringens* NCTC 8237 genomic DNA (ATCC 13124D-5) by specific primers. Several sialidases contain a hydrophobic region at *N*-terminal predicted to be a signal peptide by SignalIP and were not included in the primers during cloning. All these bacterial sialidases were expressed with *N*-terminal His tag for protein purification and antibody identification.

Expression of Sialidase in *Escherichia coli* and Purification of the Recombinant Sialidases. All sialidase genes were obtained via PCR from the genomic DNA or cDNA library by respective primers. (nanA: forward-TTCAGGGAGCGATCGCTATGGAAGGGGCAAGTGAGC and reverse-GTCATTACGTTTAAACCTAGTTCGCTTCGGTAGGA; nanB: forward-TTCAGGGAGCGATCGCTATGTCTCCTATTTTTCAAGGAG and reverse-GTCATTACGTTTAAACTTATTTTGTTAAATCATTAATTTCC; nanC: forward-TTCAGGGAGCGATCGCTATGAAAAAAAATATTAAACAATATGTA and reverse-GTCATTACGTTTAAACTTAATTCTTTTTCAGATCTTCAAT; nanI: forward-TTCAGGGAGCGATCGCTAAAGGAAGTACTTTAGACTCAG and reverse-GTCATTACGTTTAAACTTATTTATTAGCTCCACTCTCTA; nanJ: forward-TTCAGGGAGCGATCGCTAATCCTAATGTAAATCATAAGGCA and reverse-GTCATTACGTTTAAACTTACCTAGCAGTTCTTATAGTC; nanH: forward-TTCAGGGAGCGATCGCTATGTATAACAAAAACAACACCTTT and reverse-GTCATTACGTTTAAACTTATTGTTTATTAATTAGTGAGTAAT). The PCR products were ligated into the modified form of pET47b vector and confirmed by DNA sequencing. The plasmids with correct sequence were transformed into ArcticExpress/RIL competent cells by the chemical transformation method. Single colonies were picked and cultured in TB medium with kanamycin overnight. The cell cultures in fresh TB medium were induced by 0.1 mM isopropyl β-D-thiogalactopyranoside (IPTG) and allowed to grow at 16 °C for 24 h. *E. coli* cells were harvested and disrupted in a buffer containing 50 mM sodium phosphate buffer (pH 8.0), 300 mM sodium chloride, and 10 mM imidazole by microfluidizer and clarified by centrifugation. The expressed sialidases were purified by Ni-NTA agarose. The protein concentration was determined by Qubit protein quantitation (Invitrogen), and purity was confirmed by SDS/PAGE.

Cloning of Human Sialidase. The cDNA of human sialidases, Neu1, Neu2, and Neu4 were amplified from MGC clone (clone ID: 40004620 and 40125765, respectively) by PCR and subcloned into the modified form of expression vector, pCMV-Tag 2 (Sigma) with *N*-terminal FLAG tag, whereas Neu1 and Neu4 were also cloned into the plasmide using primers to bring FLAG tags to both the *N*- and *C*-termini (clone ID: 3506824) by primer addition. Neu3 cDNA was synthesized according to its sequence (GenBank accession no. BC144059.1) and cloned as the other three sialidases. All clones were confirmed by DNA sequencing, and sialidase expressions were confirmed by FLAG-specific antibody.

Determination of IC_{50} of DFSA and PDFSA. Sialidase inhibition was determined by mixing inhibitor and neuraminidase (NA) for 10 min at room temperature, followed by the addition of 200 μM of substrate MUNANA. Inhibitor IC_{50} value was determined from the dose–response curves by plotting the percent inhibition of NA activity versus inhibitor concentrations using Graph Pad Prism 4.

Labeling of Bacteria Sialidase, Influenza NA, and Recombinant Human Sialidases. Purified bacteria sialidases (1 μg) were incubated with DFSA (0.1 mM) at room temperature for 1 h and separated on 4–12% NuPAGE (Invitrogen). In addition, 5×10^4 influenza viruses were incubated with DFSA (30 μM) at room temperature for 1 h and separated on 4–12% NuPAGE (Invitrogen). Sialidase transfectant 293T cells were lysed by different lysis buffers: pH 4.5 [1% Nonidet P-40, 100 mM NaOAc, 150 mM NaCl, 3 mM KCl (pH 4.5), 1 × EDTA-free protease inhibitor mixture from Roche]; pH 7.4 [1% Nonidet P-40, 25 mM Tris, 150 mM NaCl, 3 mM KCl (pH 7.4), 1 × EDTA-free protease inhibitor mixture from Roche]; and pH 9.0 [1% Nonidet P-40, 25 mM Tris, 150 mM NaCl, 3 mM KCl (pH 9.0), 1 × EDTA-free protease inhibitor mixture from Roche]. The lysates were collected and incubated with DFSA (0.1 mM) at 37 °C for 1 h. Following incubation, the samples were clarified and protein concentrations were determined by a bicinchoninic acid protein assay kit (Pierce). For each sample, 10 μg total lysate (5 μg of Neu2 total lysate) was separated on 4–12% NuPAGE (Invitrogen).

After electrophoresis, the gels were blotted onto PVDF membranes (Millipore). Click reactions were performed on the PVDF membranes, and labeling signals were processed and analyzed by chemiluminescence detector.

Sialidase Activity Assays. Fibroblasts (from D551, GM02921, and GM02922) were fed with PDFSA (0.2 mM) at 37 °C for 15 h. Fibroblasts were lysed by lysis buffer [1% Nonidet P-40, 100 mM NaOAc, 150 mM NaCl, 3 mM KCl (pH 4.5), 1 × EDTA-free protease inhibitor mixture from Roche] and then incubated on ice for 15 min. Following incubation, the samples were spun at 18,000 × *g* for 15 min. The supernatants were collected; then, 100-μg total lysates in a total volume of 0.1 mL were incubated with MUNANA (0.1 mM) at 37 °C for 1 h. The reaction was terminated with 0.1 mL of 0.85 M glycine–carbonate buffer (pH 9.3) and kept at 4 °C before reading fluorescence. Fluorescence was determined on a fluorometer with excitation at 365 nm and emission at 450 nm.

Visualization of Flu-Infected Cells Using DFSA. Cells from the human kidney cell line MDCK were seeded onto six-well plates (3×10^5/2 mL per well) containing glass coverslips and were cultivated in 2% FCS/DMEM and 1% P/S antibiotic–antimycotic. Cells were infected with 0.03 multiplicity of infection of flu virus for 20 h at

35 °C and treated with 30 μM of DFSA for 1 h at 35 °C. Cells on coverslips were fixed with methanol for 3 min, then permeabilized with 0.05% Triton X-100 in PBS for 1 min. Cells were subjected to the probe labeling reaction (0.1 mM azide-biotin probe, 0.1 mM Tris-triazole ligand, 1 mM $CuSO_4$, and 2 mM sodium ascorbate in PBS) at room temperature for 30 min. Subsequently, the fixed and labeled cells were rinsed with PBS and stained with anti-nucleoprotein monoclonal antibody (500-fold dilution in PBS), streptavidin-DyLight 488 (2 μg/mL in 5% BSA/PBS), and 0.6 μg/mL of Alexa Fluor 594 labeled goat anti-mouse IgG (Invitrogen; no. A11020) at room temperature for 30 min. 4′6-Diamidino-2-phenylindole (10 μg/mL in PBS) was used to stain nuclei. Fluorescent images were captured by Leica TCS-SP5-MP-SMD.

Quick Detection of Oseltamivir Acid Susceptibility of Influenza Viruses on Membrane. PVDF membrane mounted on Bio-Dot SF (Bio-Rad) was wetted with methanol. Influenza viral samples that were previously treated for 1 h with either 30 μM DFSA or 30 μM DFSA plus oseltamivir acid (OS) were introduced to neighboring slots by suction. The membranes were blotted using PBS with 3% BSA and then PBS with streptavidin, 5 μg/mL, to lower the endogenous biotin noise. Following the click reaction, the membranes were incubated with horseradish peroxidase-conjugated streptavidin (KPL) according to the manufacturer's instructions. After additional washing using PBS with 0.05% Tween-20, horseradish peroxidase substrate ECL (Calbiochem) was added for chemiluminescent development.

Mass Spectrometric Analyses of Tryptic Peptides of DFSA-Labeled Sialidases. DFSA-labeled sialidases (5 μg) were dissolved in 100 mM ammonium bicarbonate and 8 mM DTT and incubated at 65 °C for 1 h. The DTT treated protein solution was subsequently treated with 4μL of 40 mM iodoacetamide for 1 h at dark and then added with 1μL of 40 mM DTT for 1 h at room temperature. The sialidase samples were treated with trypsin at neutral pH for 17 h, heated to inactivate trypsin, and dried for MS analysis.

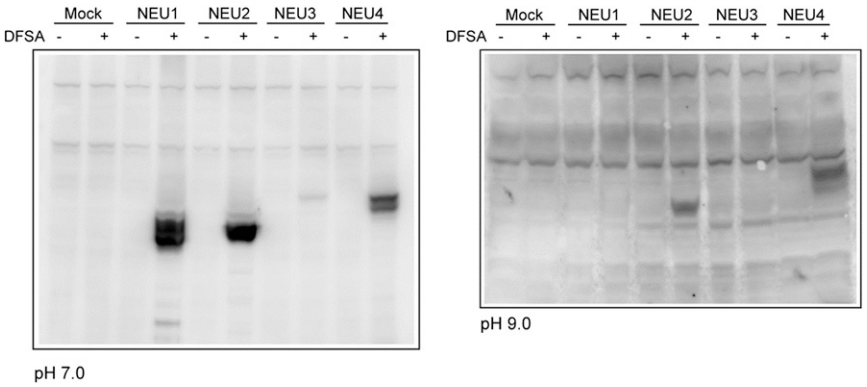


Fig. S1. Labeling of human sialidases in vitro is pH-dependent. The sialidase-overexpressing cells were collected in buffers with different pH (7.0 or 9.0) and labeled with DFSA (100 μM). The samples were processed for sialidase detections as described in Fig. 2.

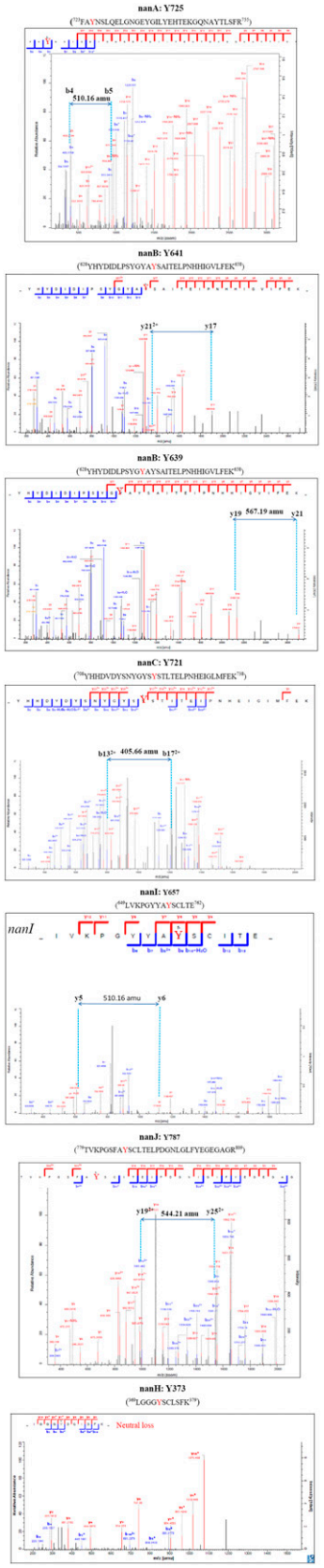


Fig. S2. Liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) analyses of tryptic peptides of DFSA-labeled sialidases.

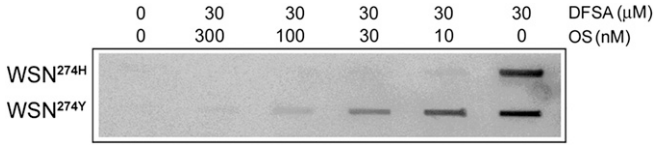


Fig. S3. Detection of influenza virus with DFSA on PVDF membrane. Differentiation of OS-sensitive (WSN^{274H}) and OS-resistant (WSN^{274Y}) influenza viruses by DFSA staining in the presence of competing OS. Influenza viruses with OS-sensitive (wild type NA with histidine at residue no. 274) or OS-resistant NA at the same titles ($1*10^5$ PFU) were spotted on PVDF membrane along with DFSA and OS at different concentrations as shown. For OS-sensitive influenza virus, the DFSA-mediated detection is apparent only in the absence of the competitive OS, while for the OS-resistant influenza virus, the virus can be detected in the presence of OS also.

Table S1. IC_{50} values (μM) of DFSA, 3-fluorosialyl fluoride, DANA, and PDFSA for sialidase inhibition

Sialidase*	DFSA	3-Fluorosialyl fluoride	DANA†	PDFSA
NA	51 ± 24	0.35 ± 0.08	5.4 ± 1.6	N.D.‡
Neu1	10.4 ± 2.3	>100	>100	N.D.
Neu2	26.1 ± 12.8	12.1 ± 3.8	24 ± 2.8	N.D.
Neu3	5.3 ± 3.2	0.18 ± 0.05	2.4 ± 0.6	N.D.
Neu4	59.8 ± 1.3	1.0 ± 0.8	4.3 ± 1.4	N.D.
nanA	0.3 ± 0.1	0.15 ± 0.05	9.3	N.D.
nanB	82 ± 22	>100	25.4	N.D.
nanC	42.8 ± 17.5	>100	>100	N.D.
nanJ	34.9 ± 4.2	0.1 ± 0.01	4.5	N.D.
nanI	25.5 ± 0.6	0.1	1.9	N.D.
nanH	354 ± 146	0.7	15.7	N.D.

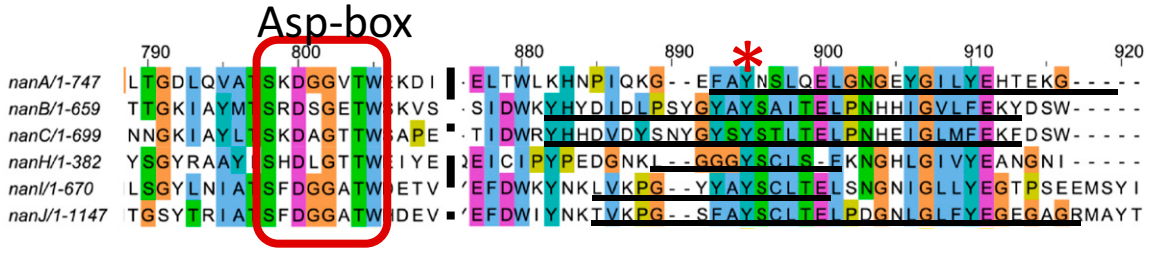
DANA, 2,3-didehydro-2-deoxy-*N*-acetylneuraminic acid; N.D., not detectable.
*The sialidases used in this study are NA (influenza neuraminidase from A/WSN/1933/H1N1), Neu1–Neu4 (recombinant sialidases from human), nanA–nanC (recombinant sialidases of *Streptococcus pneumoniae*), and nanJ–nanH (recombinant sialidases from *C. perfringens*).
†A fluorescent substrate, 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid (MUNANA), was used to determine the IC_{50} values that are concentrations causing 50% inhibition of different sialidases.
‡Not detectable.

Table S2. DFSA-labeled tryptic peptides from different sialidases

Sialidase	Tryptic peptide	Modified sites
nanA	^{723}FAYNSLQELGNGEYGILYEHTEKGQNAYTLSFR^{755}	Y725
nanB	^{628}YHYDIDLPSYGYAYSAITELPNHHIGVLFEK^{658}	Y639 and Y641*
nanC	^{708}YHHDVDYSNYGYSYSTLTELPNHEIGLMFEK^{738}	Y721
nanI	^{649}LVKPGYYAYSCLTE^{762}	Y657
nanJ	^{779}TVKPGSFAYSCLTELPDGNLGLFYEGEGAGR^{809}	Y787
nanH	^{369}LGGGYSCLSFK^{379}	Y373

*The LC-MS/MS analysis showed that DFSA was covalently linked to Tyr639 and Tyr641 in a ratio of 85:15.

Table S3. Sequence alignment of bacterial sialidases



*3-Fluorosialyl-labeled tyrosine residue. The Asp-box labeled with the red box is located near the catalytic site.