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Article

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Structure-Based Design, Synthesis of C-1 and C-4 Modified Analogs of Zanamivir as Neuraminidase Inhibitors

Enguang Feng,[†,‖] *Woo-Jin Shin,*[‡,‖] *Xuelian Zhu,*[†,‖] *Jian Li,*[†] *Deju Ye,*[†] *Jiang Wang,*[†] *Mingyue Zheng,*[†] *Jian-Ping Zuo,*[†] *Kyoung Tai No,*[‡] *Xian Liu,*[†] *Weiliang Zhu,*[*,†] *Wei Tang,*[*,†] *Baik-Lin Seong,*[*,‡] *Hualiang Jiang,*[†] *and Hong Liu,*[*,†]

[†] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu chong zhi Road, Shanghai 201203, China

[‡] Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University, Seoul 120-749, South Korea

[‖] These authors contributed equally to this work

E-mail: hliu@mail.shcnc.ac.cn(H. Liu), blseong@yonsei.ac.kr(B.-L. Seong), tangwei@mail.shcnc.ac.cn (W. Tang), wlzhu@mail.shcnc.ac.cn (W. Zhu)

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Abstract

In order to exploit the 430-cavity in the active sites of neuraminidases, 22 zanamivir analogs with C-1 and C-4-modification were synthesized, and their inhibitory activities against both group-1 (H5N1, H1N1) and group-2 neuraminidases (H3N2) were determined. Compound **9f** exerts the most potency, with IC_{50} value of 0.013, 0.001 and 0.09 μM against H3N2, H5N1 and H1N1, which is similar to that of zanamivir (H3N2: IC_{50} 0.0014 μM, H5N1: IC_{50} 0.012 μM, H1N1: IC_{50} 0.001 μM). Pharmacokinetic studies of compound **9f** in rats showed a much longer plasma half-life ($t_{1/2}$) than that of zanamivir following administration (*po* dose). Molecular modeling provided information about the binding model between the new inhibitors and neuraminidase, with the elongated groups at the C-1 position being projected towards the 430-loop region. This study may represent a novel starting point for the future development of improved anti-flu agents.

Introduction

In the face of the persistent threat of human influenza infections, and the potential of new human or avian influenza variants to unleash a pandemic, there is much concern about the shortage in both the number and supply of effective anti-influenza virus agents.[1] Accordingly, the discovery of effective drugs, which are not susceptible to mutation, is an urgent need. Among the antiviral targets, neuraminidase (NA) has been extensively investigated for drug design. The first NA inhibitors of Neu5Ac2en

(DANA) (**1**) and 4-amino-Neu5Ac2en (**2**) were designed based on the principle of the 'transition state analogs'[2, 3]. Further optimization of the structures lead to two potent NA inhibitors, zanamivir (compound **3**) (4-guanidino-Neu5Ac2en, GG167)[4] and oseltamivir (compound **4**) (GS4071 ethyl ester, GS4104, Ro64-0796)[5] (Figure 1), both are currently marketed for the treatment (and prophylaxis) of influenza A and B virus infections.

However, the low oral bioavailability and rapid renal elimination of compound **3**, and the rapid emergence of compound **4**-resistant influenza viruses, have prompted the further development of more potent and longer duration therapeutic drugs to combat potential human influenza pandemics.[6-19] Very recently, a long-acting NA inhibitor, laninamivir (compound **5**), has been approved in Japan. This drug is effective against compound **4**-resistant strains and also effective when using just a single inhaled dose *via* its octanoate prodrug (laninamivir octanoate, CS-8958), demonstrating superior anti-influenza virus activity. [5]

Figure 1

The nine known NAs of influenza A viruses can be divided phylogenetically into two distinct groups: group-1 contains N1, N4, N5 and N8 subtypes, and group-2 contains N2, N3, N6, N7 and N9.[20] Group-1 NAs have a flexible '150-loop' providing a novel, large cavity (150-cavity) adjacent to the active site, which is absent in group-2 NAs. Based on this knowledge, a C-4-modified analog[21] of compound **3** and a C-3-modified sialic acid derivative[22] were recently reported to inhibit group-1 NAs at micromolar level. Further molecular modeling studies and X-ray crystallography

indicated that these two inhibitors occupied the 150-cavity in the open form of group-1 NAs[21, 22]. We have also previously reported a series of C-4-triazole-modified analogs of compound **3** as potential anti-avian influenza virus (AIV) agents, and one compound exerts promising inhibitory activity, shown ~61% protection against AIV (H5N1) infection, while compound **3** was ~86% protective in the same assay.[23] However, our group recently found[24] that the 2009 H1N1 influenza pandemic NA (p09N1) is an atypical group-1 NA with some group-2 like features in its active site (lack of a 150-cavity), implying that neuraminidase inhibitors targeted at the 150-cavity will probably be less effective against the subtype of group-1 variants containing Ile149, as argued in a recent review.[25] Furthermore, it has been reported that certain compound **4**-resistant amino acid substitutions in the NA active site are group-1 specific. Using molecular dynamics (MD) simulations of influenza NA, Amaro et al. observed extended conformational shifts of the 150-loop and 430-loop (Figure 2).[26-29] It was demonstrated that the binding site of NAs can open to a much larger extent than that anticipated from prior X-ray structure analyses, which can be systematically explored for the development of more potent, chemically diverse inhibitors. Recently, a novel small-molecule inhibitor (NSC89853, **6**) has been reported by An and co-workers, and the predicted binding mode of compound **6** to the known H5N1 NA was different to that of compound **3** or compound **4**. They also suggested that the existence of another binding site within the pocket, formed by 430-loop, could be accessed by the new inhibitors.[30] In addition, investigating the seed extract of *Alpinia katsumadain*, Grienke et al. found one of the most promising

constituent, *katsumadain A*, inhibited the NA of H1N1 swine influenza viruses, with IC_{50} values between 0.9 and 1.64 μM.[31] Due to its large molecular volume, the extended conformation of the 430-loop and 245-loop appeared to be essential for the accommodation of the ligand into the active site of NA.

Figure 2

It is revealed that the active site of NAs could be empirically divided into five major sub-sites (S1-S5) which are essential for the interactions with different NA inhibitors (Figure 3).[32] The 1-carboxyl group of compound **3** was shown to form strong hydrogen bonds with three arginine residues (Arg118, Arg292 and Arg371) in the S1 subsite, which is very important for the NA inhibitory activity.[33] So far, little work has been done to synthesize C-1-modified sialic acid derivatives with the exception of some ester prodrugs.[34] Very recently, with the replacement of 1-carboxyl group of compound **3** with phosphonate group, Wong et al. discovered two phosphono anologs of compound **3** which are more potent than compound **3** against the NAs of avian and human influenza viruses, including the compound **4**-resistant strains.[35] Meanwhile, as shown in Figure 2, the flexible 430-loop in both group-1 and group-2 NAs is next to the subsite S1, which can provide a large cavity (430-loop) adjacent to the active site. This suggested new opportunities to design highly effective NA inhibitors that target both 430-cavity and the known active sites (Figure 2 and 3). Based on this knowledge, here we designed and synthesized a series of compound **3** analogs (**7a-g, 8a-e** and **9a-j**, Table 1) with different substitutions at C-4 and C-1 positions to explore the 430-cavity. Their anti-influenza virus activities against both group-1 (H5N1 and

H1N1) and group-2 (H3N2) NAs were tested. Meanwhile, taking compounds **7d**, **8a**, **9c**, **9f, 9g** and **9i** as examples, the interaction models between inhibitors and NAs were also studied.

Figure 3

Results and discussion

Designing analogs of zanamivir. Based on the structural feature of compound **3** and its binding models with group-1 and group-2 NAs (Figure. 3A and Figure. 3B) [32], 22 analogs (**7a-g**, **8a-e** and **9a-j**, Table 1) were designed and synthesized. In order to explore additional interactions with 430-loop as revealed in group-1 and group-2 NAs (Figure 2), a range of substitutions at C-1 and C-4 modified analogs were introduced. In particular, we were interested in using aliphatic acids to modify the 1-carboxyl group to retain the strong hydrogen bonds with three arginine residues (Arg118, Arg292 and Arg371). Initially, by replacing the C-4 position of compound **3** with a hydroxyl group as well as introducing different substituted amides at C-1 carboxyl, we obtained analogs **7a-g**. Further replacement of C-4 hydroxyl group with an amino group led to compounds **8a-e**. In an effort to gain more potency, we subsequently introduced the guanidinium group at the C-4 position as in compound **3** to obtain a series of derivatives **9a-e**. Because the 430-cavity was reported to favor aromatic ring-based compounds,[36] compounds **9f-j** were also obtained by replacing amino acid moieties at the C-1 position with corresponding benzylamine substituents.

Synthesis of target compounds. The synthesis of **7a-g, 8a-e** and **9a-j** is shown in Scheme 1. Compound **9** (*N*-acetyl neuraminic acid, NANA) was esterified with benzyl bromide to protect the 1-carboxyl group. Compound **10** was obtained using acetic anhydride in pyridine with catalytic 4-(dimethyamino)-pyridine (DMAP), then treated with trimethylsiyltrifluoromethanesulfonate (TMSOTf) in acetonitrile at 0 ^{o}C to give compound **12**. The key intermediate **13** was obtained by reducing compound **12**, which reacted with different amines,[37] then hydrolyzed with NaOH in methanol to give compounds **7a-g**. Compound **11** was treated with TMSOTf in ethyl acetate at 52 ^{o}C using a modification of the procedure described by Chandler *et al*.[38] to give **14** in high yield. Compound **14** was hydrolyzed, then reacted with diphenylphosphoryl azide (DPPA) and 1,8-diazabicyclo[5,4,0]undecen-7-ene (DBU) to yield the inverted azide **16**. The key compound **17** was obtained by reduction of **16** using H_2S (gas) in pyridine. Reaction of **17** with Boc-anhydride in methanol produced intermediate **18**, which was hydrogenated over Pd/C (10%) to give compound **19**. Compound **19** was reacted with a range of different amines, deprotected with trifluoroacetic acid (TFA) and then hydrolyzed with NaOH to give the target compounds **8a-f**. Compounds **9a-j** were prepared similarly from compound **22** which was accessed from compound **21** by the method described by Konrad F. *el at*.[39]

Scheme 1

Inhibitory activities against NAs and binding models. All 22 target compounds were evaluated for their NA inhibition using 2'-(4-methylumbelliferyl) -*a*-D-*N*-acetylneuraminic acid (MUNANA) as the substrate. In the hope of achieving uniform activity, we simultaneously selected N1 (H5N1, H1N1) from group-1 and N2 (H3N2) from group-2 as representatives for testing, and the results are summarized in Table 1. Although general structure-activity relationships (SAR) of these compounds is not evident, the bioassay results provide some information for further structural modifications. When the guanidinium group of compound **3** is replaced with a hydroxyl group, only week NA inhibitory activities were observed for compounds **7a-g**, which is similar to that of Neu5Ac2en[4] (DANA, A/Mississippi/1/85(H3N2): IC_{50} 52 μM, H5N1: IC_{50} 0.012 μM, A/Brazilll/78(H1N1): IC_{50} 240 μM). However, as revealed by von Itzstein et al. the acidic residues Glu119, Asp151 and Glu227 in the active sites of NA are favorable of a basic substituent at the C-4 position of the scaffold.[40, 41] Thus, compounds **8a-e** which contain an amino group had significantly improved NA inhibition comparing with compounds **7a-g**. Further replacing the C-4 amino group with a stronger base of guanidine should increase the electrostatic interactions with the acidic residues in the NA active site. Indeed, compounds **9a-j** with a 4-guanidino group showed an enhanced NA inhibition in comparison with **7a-g** and **8a-e**. Generally, the introduction of the amino acid substituent to the carboxyl group at the C-1 position of compound **3** via amide bond did not translate into better potency as shown in compounds **9a-e** compared to compound **3**. Gratefully, compound **9f** bearing a 3-fluorobenzyl amine exerts the greatest potency, with IC_{50}

value of 0.013, 0.001 and 0.09 μM against H3N2, H5N1 and H1N1, which is comparable to that of compound **3** (H3N2: IC_{50} 0.0014 μM, H5N1: IC_{50} 0.012 μM, H1N1: IC_{50} 0.001 μM). The higher NA inhibitory potency may be ascribe to the elongation of the aromatic ring of the molecule to the 430-cavity to form hydrogen bond and strong hydrophobic interactions with NAs. However, further conversion of 3-fluorobenzyl amine into corresponding 2-fluorobenzy amine, 4-fluorobenzy amine, 3-chlorobenzy amine or 4-methylbenzyl amine did not show improved inhibition. The incorporation of fluorine imparts special characteristics that enhance therapeutic efficacy and improved pharmacological properties. As the second smallest substituent, fluorine not only closely mimics hydrogen with respect to steric requirements at enzyme receptor sites, but also leads to increased lipid solubility and hydrophobic interaction with NAs.

Table 1

To better understand the discrepancy of activity among compounds **7**, **8**, **9** and compound **3**, we compared the 3D binding models of compound **3** with NA to that of our different inhibitors to NA derived from docking simulation. Compounds with different substituents at the C-4 position exhibit varying activity against NAs under experimental conditions. In order to explain the difference in their activities, compounds **7d**, **8a** and **9c** were selected since they are identical with the exception of C-4 substitution. The hydroxyl group of **7d** and the amino group of **8a** are embedded within subsite S2 and form two hydrogen bonds with Glu119 and Asp151 of N2 (Figure 4. A, B, E and F). However, the guanidinium group of **9c** forms five hydrogen

bonds with Glu119, Asp151, Trp178 and Glu227 (Figure 4. C, D and G, H), which is consistent with previous findings.[4, 40] In addition to the hydrogen bond, the charge-charge interaction also plays an important role in increasing the activity of **9c**. The subsite S2 has negative charge properties (Figure 4. E, F and G), which preferentially binds to the positive-charged substituent. The protonated guanidinium group has the greatest positive charge among these three functional groups, followed by protonable amino group, whereas the hydroxyl group has no positive character. Therefore, **9c** is predicted to be the most potent compound, followed by **8a**, and finally **7d**, which is confirmed by the experimental test.

Figure 4

Based on the docking results, the elongated amide groups at the C-1 position were projected towards the 430-loop region (Figure 5. A and C) to form hydrogen bonding and/or hydrophobic interactions with NAs, while the guanidinium group establishes hydrogen bonds and electrostatic interactions within S2 subsite similar to that of compound **3**. This explains the generally higher inhibitory activity of this series against NAs. Among all the synthesized compounds, **9f** has the most potent antiviral activity. The elongated group at the C-1 position in **9f** was projected towards the 430-loop region to form extensive hydrophobic interactions with Val149, Ile427, Pro431 and Thr439, which can partially compensate for weaker activity caused by the absence of electrostatic interactions. Therefore, **9f** still retains inhibitory activity against Influenza neuraminidases, but weaker than compound **3**. When compared **9g**, **9i** with **9f**, it seems the position of the fluorine on the phenyl ring is also very

important, which may be ascribe to the large electronic effect on the molecule's dipole moment and the hydrophobic interaction with the 430-cavity. Figure 5 (B and D) showed that the fluorine substituted phenyl ring of **9g** and **9i** could not form similar hydrophobic interactions as **9f**.

Figure 5

Pharmacokinetic (PK) profiles of the selected anti- influenza agent. It is reported that compound **3** has poor bioavailability and rapidly eliminated (short plasma half-life $t_{1/2}$) following oral administration.[8,9] To gain an initial understanding about the pharmacokinetic parameters of our series, we selected compound **9f** which had the most potent antiviral activity as representative for further investigation following intravenous (*iv*) and oral (*po*) dosing in rats. For comparison, we also listed the parameters of compound **3** and compound **4** as reported in the literature[42] in Table 2. The results showed that **9f** has similar bioavailability following oral dosing to that of compound **3** (1.41% vs 2.7%). However, a much longer plasma $t_{1/2}$ for **9f** was observed after *po* dosing compared to that of compound **3** (2.96 h *vs* 1.8 h). Meanwhile, the maximum concentration (C_{max}) of **9f** in plasma after oral administration was 116 ng/mL (~0.26 μM), which was much higher than compound **3** or compound **4**. These results indicated that **9f** may serve as a possible lead compound for the development of anti-influenza drugs with longer duration of action.

Table 2

Conclusion

In summary, we designed and synthesized 22 analogs of zanamivir with modification at the C-1 position to retain the strong hydrogen bonds with three arginine residues (Arg118, Arg292 and Arg371). To increase the structural diversity of the analogs, we selected C-4-substituted Neu5Ac2en with a hydroxy, amino and guanidinium group. Compound **9f** exerts the most potency, with IC_{50} value of 0.013, 0.001 and 0.09 μM against H3N2, H5N1 and H1N1, which is very similar to that of compound **3** (H3N2: IC_{50} 0.0014 μM, H5N1: IC_{50} 0.012 μM, H1N1: IC_{50} 0.001 μM). Moreover, compound **9f** was shown to have a prolonged plasma half-life following oral dosing in rat. Molecular modeling results revealed information about the binding models between inhibitors and NAs, illustrating that the elongated amide groups at C-1 position were projected towards the 430-loop, which may aid future development of improved inhibitors against both group-1 and group-2 NAs.

Experimental section

General information on synthesis. The reagents (chemicals) were purchased from Lancaster, Sigma, Acros and Shanghai Chemical Reagent Company, and used without further purification. Analytical thin-layer chromatography (TLC) was performed on HSGF 254 (150-200 μm thickness, Yantai Huiyou Company, China). Yields were not optimized. Column chromatography was performed with Combi*Flash*® Companion system (Teledyne Isco, Inc.). Nuclear magnetic resonance (NMR) spectra were performed on a Brucker AMX-400 and AMX-300 NMR (IS as TMS). Chemical shifts were reported in parts per million (ppm, δ) downfield from

tetramethylsilane. Proton coupling patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). LC-MS analysis were conducted on Agilent 1100 Series HPLC with an Agilent Zorbax Eclipse XDB-C18 (4.6 × 50 mm, 5 μm) reversed phase column. Compounds **7a-g, 8a-e** and **9a-j** were confirmed ≥95% purity (Supporting Information, Table S1). The details for purity analyses of compounds **7a-g, 8a-e** and **9a-j** are described in the Supporting Information.

Neuraminidase enzyme inhibitory assay. Influenza virus A/Sydney/5/97 (H3N2), A/Guangdong/376/2001 (H1N1) and recombinant virus containing the HA and NA genes from A/Indonesia/5/2005 (H5N1) virus in the background of X-31 ca virus were propagated in 11-day-old embryonated chicken eggs as described elsewhere.[25,26] Influenza neuraminidase activity was determined by using 2′-(4-methylumbelliferyl)-α-$_{D}$-*N*-acetylneuraminic acid (MUNANA; Sigma Chemical *Co., St. Louis, Mo.*) as a substrate, as described previously with a little modification.[19] The NA activity of each virus was determined before it was used in NA inhibition tests. Briefly, 50 μl of virus was serially diluted in enzyme buffer [sodium acetate buffer 150 mM (pH 7.0) containing 1mM calcium chloride] and 50 μl of substrate in enzyme buffer to give a final MUNANA concentration of 100 μM. The reaction mixtures were incubated at 37 ℃ for 30 min. The reactions were then stopped by addition of 100 μl of 0.014 N NaOH in 83% ethanol to each well. The fluorescence of the released 4-methylumbelliferone was quantified using a fluorescence spectrophotometer (excitation wavelength, 355 nm; emission wavelength, 460 nm).

NA inhibition was assayed by determining the drug concentration required to reduce NA activity to 50% of control NA activity (IC_{50}). Five-fold dilutions ranging from 10 μM to 128 nM were made of the appropriate compound, and 25 μL of each dilution was incubated with 25 μL of virus-containing allantoic fluid at a standard amount of NA activity (150 relative fluorescence units). The mixture was incubated at 37 °C for 1 h, protected from light to allow interaction between the drug and virus. The enzymatic reaction was initiated by adding 50 μL of substrate in enzyme buffer at a final concentration of 100 μM. The reaction was stopped after 30 min of incubation at 37 °C. Standard curves were constructed by plotting the percentage of fluorescence inhibition relative to the activity of controls against the logs of inhibitor concentration. The IC_{50}s were obtained by interpolation of the data.

Pharmacokinetic studies. Six male Sprague-Dawley (SD) rats (~250 g; Shanghai Laboratory Animals Co., Shanghai, China) were housed in two rat cages (three rats for one group) and maintained in an air-conditioned rat room, which were used to determine kinetic profile after oral and *iv* dosing with compound **9f**. In the first group, the tested compound was administered orally at a dose of 10 mg/kg; in the second group, the tested compound was administered intravenously at a dose of 2 mg/kg. Serial specimens (0.5 mL) were collected via the retrobulbar vein at various time points after the compound was given (0, 5, 15, 30 min and 1, 2, 5, 8, 24 h), and quantification was performed by LC/MS/MS (Agilent 1100 PLC and Thermo Finigan $TSQ_{Quantum}$). Pharmacokinetic parameters were calculated from the mean plasma concentration by noncompartmental analysis.

Molecular docking. Molecular docking was performed using Glide (grid-based ligand docking with energetics) program in the Schrödinger suite of software (Maestro, version 7.5.112). Glide uses the position and size of the co-crystallized ligand to determine the binding site. Specifically, the default center of this site was the centroid of the ligand and the default size was defined similar to the ligand. The crystal structures of neuraminidase were retrieved from the Protein Data Bank (PDB entry 3CKZ and 3TIC) for subtype N1 (group-1) and N2 (group-2). It remains controversial whether water molecules should be kept during molecular docking. Two points need to be noticed: First, it is difficult to determine exactly whether a water molecule is conserved; second, the orientation of hydrogen bonds formed between water molecules and different ligands is variable. In consideration of explicit water molecules, current docking programs usually fix the orientation of the water molecules and treat them as part of the receptor environment, which are not beneficial to the assessment of ligand-receptor interaction in many cases. In contrast, Zheng et al. reported a fair correlation between the predicted binding free energies and the experimental inhibitory potencies based on the reasonable binding conformations of 126 NA inhibitors obtained from molecular docking with removing all crystal water molecules.[43] Therefore, all waters and unessential ligands in the structures were deleted for molecular docking in this study. Then the synthesized compounds were docked to the protein in standard-precision (SP) mode, with up to thirty poses saved per compound and other default parameters.

Experimental Procedure

General methods

General procedure for the synthesis of compounds 7a-7g

To a solution of acid **13** (0.2 mmol), EDCI (0.3 mmol), Et_3N (0.3 mmol) in 5 mL dry CH_2Cl_2 was added an equimolar amount of the appropriate amide. The mixture was kept stirring at room temperature for 24 h. After TLC detection to show no starting materials, the reaction mixture was concentrated under reduced pressure and purified by flash column chromatography to obtain intermediates. 200 μL 5% NaOH aqueous solution was added into a solution of intermediates in 5 mL methanol. After the reaction mixture was stirred at r.t. overnight, the pH value of solution was adjusted to 5-6 with positive ion-exchange resin (Dowex 50w x 4-400). The mixture was filtered, and the solvent was removed under vacuum. The residue was lyophilized to obtain the final products **7a-7g**.

(2R,3R,4S)-3-acetamido-4-hydroxy-N-(4-methoxybenzyl)-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carboxamide (7a) The product was obtained as white foam after lyophilization. Yield 50%. ^{1}H NMR (400 MHz, CD_3OD, ppm) δ 7.23 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.6 Hz, 2H), 5.81 (d, *J* = 2.4 Hz, 1H), 4.46-4.37 (m, 3H), 4.18 (d, *J* = 10.8 Hz, 1H), 3.96 (dd, *J* = 10.7, 8.8 Hz, 1H), 3.83-3.77 (m, 2H), 3.77 (d, *J* = 2.6 Hz, 3H), 3.68-3.61 (m, 1H), 3.58 (d, *J* = 9.4 Hz, 1H), 3.34 (d, *J* = 4.8 Hz, 1H), 2.04 (s, 3H); ^{13}C NMR (126 MHz, MeOD) δ 178.62, 162.20, 158.63, 145.82, 129.79, 128.18, 113.07, 107.57, 76.30, 69.06, 67.88, 66.06, 62.82, 53.85, 50.19,

41.63, 22.41. LC-MS m/z 433 $[M+Na]^+$; HRMS (ESI) $[M+Na]^+$ found m/z 433.1569, calcd for $C_{19}H_{26}N_2O_8Na$ 433.1587.

(S)-2-((2R,3R,4S)-3-acetamido-4-hydroxy-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carboxamido)-4-methylpentanoic acid (7b) The product was obtained as white foam after lyophilization. Yield 34%. ^{1}H NMR (400 MHz, CD_3OD, ppm) δ 7.28 (dd, *J* = 13.1, 7.0 Hz, 1H), 5.84 (d, *J* = 2.5 Hz, 1H), 4.48-4.39 (m, 1H), 4.35-4.25 (m, 1H), 4.18 (d, *J* = 10.9 Hz, 1H), 4.02 (dd, *J* = 10.8, 8.7 Hz, 1H), 3.93-3.77 (m, 2H), 3.65 (dd, *J* = 11.8, 5.6 Hz, 1H), 3.61-3.56 (m, 1H), 2.04 (d, *J* = 11.3 Hz, 3H), 1.64-1.46 (m, 1H), 1.27-1.09 (m, 1H), 0.93 (dd, *J* = 14.3, 7.1 Hz, 6H); ^{13}C NMR (126 MHz, MeOD) δ 178.60, 173.42, 161.02, 145.71, 107.64, 76.83, 69.11, 67.99, 66.27, 63.03, 58.94, 50.32, 37.63, 24.20, 22.37, 20.90, 20.73. LC-MS m/z 403 $[M-H]^-$; HRMS (ESI) $[M+2Na-H]^+$ found m/z 449.1492, calcd for $C_{17}H_{27}N_2O_9Na_2$ 449.1512.

(2S,3R)-2-((2R,3R,4S)-3-acetamido-4-hydroxy-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carboxamido)-3-methylpentanoic acid (7c) The product was obtained as white foam after lyophilization. Yield 51%. ^{1}H NMR (400 MHz, CD_3OD, ppm) δ 5.83 (d, *J* = 2.5 Hz, 1H), 4.41 (dt, *J* = 6.4, 3.5 Hz, 2H), 4.26-4.12 (m, 1H), 4.01 (dd, *J* = 10.8, 8.7 Hz, 1H), 3.93-3.79 (m, 2H), 3.66 (dd, *J* = 11.3, 5.2 Hz, 1H), 3.61-3.52 (m, 1H), 2.05 (s, 3H), 1.72-1.62 (m, 2H), 1.64-1.48 (m, 1H), 0.95 (dd, *J* = 7.5, 6.2 Hz, 6H); ^{13}C NMR (126 MHz, MeOD) δ 178.53, 173.36, 161.01, 145.74, 107.53, 76.73, 69.00, 67.93, 66.29, 63.00, 52.81, 50.28, 41.82, 24.32, 22.32, 21.91,

20.53. LC-MS m/z 403 $[M-H]^-$; HRMS (ESI) $[M+Na]^+$ found m/z 427.1709, calcd for $C_{17}H_{28}N_2O_9Na$ 427.1693.

(S)-2-((2R,3R,4S)-3-acetamido-4-hydroxy-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carboxamido)-3-methylbutanoic acid (7d) The product was obtained as white foam after lyophilization. Yield 82%. ^{1}H NMR (400 MHz, CD_3OD, ppm) δ 7.38-7.16 (m, 1H), 5.85 (d, *J* = 2.5 Hz, 1H), 4.41 (dd, *J* = 8.6, 2.5 Hz, 1H), 4.29-4.23 (m, 1H), 4.18 (d, *J* = 10.9 Hz, 1H), 4.03 (dd, *J* = 10.8, 8.7 Hz, 1H), 3.86 (ddd, *J* = 16.3, 8.9, 2.7 Hz, 2H), 3.68-3.57 (m, 2H), 2.26-2.15 (m, 1H), 2.05 (s, 3H), 0.95 (dd, *J* = 13.4, 6.9 Hz, 6H); ^{13}C NMR (126 MHz, MeOD) δ 178.53, 173.44, 161.16, 145.73, 107.65, 76.85, 69.09, 68.02, 66.28, 63.08, 59.35, 50.34, 30.98, 22.32, 20.88, 20.72. LC-MS m/z 389 $[M-H]^-$; HRMS (ESI) $[M+Na]^+$ found m/z 413.1546, calcd for $C_{16}H_{26}N_2O_9Na$ 413.1536.

(S)-2-((2R,3R,4S)-3-acetamido-4-hydroxy-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carboxamido)-2-phenylacetic acid (7e) The product was obtained as yellow foam after lyophilization. Yield 75%. ^{1}H NMR (400 MHz, CD_3OD, ppm) δ 7.45 (dd, *J* = 8.2, 7.4 Hz, 2H), 7.35-7.16 (m, 3H), 5.80 (dd, *J* = 14.3, 2.5 Hz, 1H), 5.21 (d, *J* = 16.9 Hz, 1H), 4.48-4.33 (m, 1H), 4.31-4.12 (m, 1H), 4.12-3.98 (m, 1H), 3.97-3.81 (m, 2H), 3.69 (dd, *J* = 11.7, 6.6 Hz, 1H), 3.63-3.54 (m, 1H), 2.04 (s, 3H); LC-MS m/z 423 $[M-H]^-$.

N-((2R,3R,4S)-4-hydroxy-6-(morpholine-4-carbonyl)-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-3-yl)acetamide (7f) The product was obtained as

yellow foam after lyophilization. Yield 38%. ^{1}H NMR (400 MHz, CD_3OD, ppm) δ 5.28 (d, *J* = 2.3 Hz, 1H), 4.38 (dd, *J* = 8.7, 2.3 Hz, 1H), 4.18 (d, *J* = 10.8 Hz, 1H), 4.03-3.96 (m, 1H), 3.82-3.77 (m, 1H), 3.73-3.60 (m, 9H), 3.56 (dd, *J* = 17.4, 8.0 Hz, 2H), 2.04 (s, 3H); ^{13}C NMR (126 MHz, MeOD) δ 173.43, 163.91, 146.96, 106.93, 76.48, 69.20, 68.03, 65.76, 63.05, 50.24, 22.27, 20.81. LC-MS m/z 395 $[M+Cl]^-$; HRMS (ESI) $[M+Na]^+$ found m/z 383.1412, calcd for $C_{15}H_{24}N_2O_8Na$ 383.1430.

(2R,3R,4S)-3-acetamido-N-benzyl-4-hydroxy-2-((1R,2R)-1,2,3-trihydroxypropyl) -3,4-dihydro-2H-pyran-6-carboxamide (7g) The product was obtained as white foam after lyophilization. Yield 68%. ^{1}H NMR (400 MHz, CD_3OD, ppm) δ 7.39-7.15 (m, 5H), 5.83 (d, *J* = 2.3 Hz, 1H), 4.50-4.37 (m, 3H), 4.20 (d, *J* = 10.8 Hz, 1H), 3.98 (dd, *J* = 10.7, 8.7 Hz, 1H), 3.87-3.74 (m, 2H), 3.70-3.53 (m, 2H), 3.34-3.12 (m, 1H), 2.03 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (126 MHz, MeOD) δ 173.37, 162.31, 145.83, 137.79, 127.72, 126.79, 126.46, 107.62, 76.37, 69.03, 67.92, 66.10, 62.89, 50.28, 42.16, 20.85. LC-MS m/z 403 $[M+Na]^+$; HRMS (ESI) $[M+Na]^+$ found m/z 403.1466, calcd for $C_{18}H_{24}N_2O_7Na$ 403.1481.

General procedure for the synthesis of compounds 8a-8e

To a solution of acid **19** (0.2 mmol), EDCI (0.3 mmol), Et_3N (0.3 mmol) in 5 mL dry CH_2Cl_2 was added an equimolar amount of the appropriate amide. The mixture was kept stirring at room temperature for 24 h. After TLC detection to show no starting materials, the reaction mixture was concentrated under reduced pressure and purified by flash column chromatography to obtain intermediates **20a-e**.

5 % NaOH aqueous solution (200 μL) was added into a solution of intermediates in 5 mL methanol. After the reaction mixture was stirred at r.t. overnight, the pH value of solution was adjusted to 5-6 with positive ion-exchange resin (Dowex50WX4-400). The mixture was filtered, and the solvent was removed under vacuum. Then trifluoroacetic acid (50 % in DCM) was added to the porducts above abtained, and stirred at r.t. overnight. After the reaction was completed, the solvent was evaporated to give the product **8a-e** as a salt of trifluoroacetic acid, which was further purified by a column of Dowex50WX8-200 (H^+) resin (2.0 g).

(S)-2-((2R,3R,4S)-3-acetamido-4-amino-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carboxamido)-3-methylbutanoic acid (8a) The product was obtained as wight foam after lyophilization. Yield 80 %. ^{1}H NMR (400 MHz, CD_3OD, ppm) δ 5.81 (s, 1H), 4.69 (t, *J* = 11.5 Hz, 2H), 4.56-4.29 (m, 5H), 4.11 (s, 3H), 3.71 (dd, *J* = 23.8, 9.0 Hz, 2H), 2.24 (s, 1H), 2.05 (s, 4H), 1.05-0.87 (m, 6H); ^{13}C NMR (126 MHz, MeOD) δ 173.02, 172.43, 160.89, 148.46, 99.87, 76.31, 69.57, 67.19, 62.73, 57.22, 49.38, 45.84, 30.13, 21.01, 17.52, 16.51. LC-MS m/z 340 $[M+H]^+$; HRMS (ESI) $[M+Na]^+$ found m/z 412.1683, calcd for $C_{16}H_{27}N_3O_8Na$ 412.1696.

(2S)-2-((3R,4S)-3-acetamido-4-amino-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carboxamido)-4-methylpentanoic acid (8b) The product was obtained as white foam after lyophilization. Yield 78 %. ^{1}H NMR (400 MHz, CD_3OD, ppm) δ 5.80 (d, *J* = 2.0 Hz, 1H), 4.69 (d, *J* = 9.5 Hz, 1H), 4.62-4.48 (m, 2H),

4.39 (dt, *J* = 19.6, 10.7 Hz, 2H), 4.23-4.14 (m, 1H), 4.11 (d, *J* = 6.9 Hz, 1H), 3.72 (d, *J* = 9.8 Hz, 1H), 3.48 (dd, *J* = 14.1, 7.1 Hz, 1H), 2.05 (s, 3H), 1.71 (d, *J* = 6.3 Hz, 3H), 1.27 (d, *J* = 5.5 Hz, 1H), 0.95 (dd, *J* = 8.9, 5.9 Hz, 6H); ^{13}C NMR (126 MHz, MeOD) δ 173.66, 172.99, 160.98, 148.58, 99.68, 76.16, 69.32, 67.20, 62.66, 50.24, 49.32, 47.67, 47.50, 47.33, 47.16, 46.99, 46.82, 46.65, 45.81, 39.85, 24.17, 21.44, 21.01, 20.07. LC-MS m/z 404 $[M+H]^+$; HRMS (ESI) $[M+Na]^+$ found m/z 426.1869, calcd for $C_{17}H_{29}N_3O_8Na$ 426.1852.

1-((2R,3R,4S)-3-acetamido-4-amino-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carbonyl)piperidine-3-carboxylic acid (8c) The product was obtained as white foam after lyophilization. Yield 50%. ^{1}H NMR (400 MHz, CD_3OD, ppm) δ 5.20 (s, 1H), 4.66 (d, *J* = 9.8 Hz, 1H), 4.53-4.22 (m, 3H), 4.18-3.95 (m, 2H), 3.95-3.77 (m, 1H), 3.76-3.62 (m, 1H), 3.51 (dt, *J* = 14.0, 10.5 Hz, 1H), 3.30 (s, 3H), 3.18 (dd, *J* = 31.8, 12.1 Hz, 1H), 2.48 (s, 1H), 2.16-1.95 (m, 4H), 1.77 (s, 2H), 1.54 (s, 1H); ^{13}C NMR (126 MHz, MeOD) δ 174.48, 172.95, 162.99, 150.79, 98.11, 76.06, 71.89, 69.25, 67.31, 62.89, 49.24, 45.68, 43.41, 40.25, 26.44, 24.13, 20.99. LC-MS m/z 424 $[M+Na]^+$; HRMS (ESI) $[M+Na]^+$ found m/z 424.1684, calcd for $C_{17}H_{27}N_3O_8Na$ 424.1696.

2-((3R,4S)-3-acetamido-4-amino-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carboxamido)acetic acid (8d) The product was obtained as white foam after lyophilization. Yield 81%. ^{1}H NMR (400 MHz, CD_3OD, ppm) δ 5.83 (s, 1H), 4.68 (dd, *J* = 11.3, 2.0 Hz, 1H), 4.52 (dd, *J* = 11.3, 5.7 Hz, 1H), 4.46-4.33 (m, 2H),

4.29-4.20 (m, 1H), 4.09 (dd, *J* = 13.5, 6.8 Hz, 1H), 4.01 (s, 2H), 3.95-3.78 (m, 1H), 3.70 (d, *J* = 9.6 Hz, 1H), 3.30 (dd, *J* = 3.1, 1.5 Hz, 3H), 2.05 (s, 3H); ^{13}C NMR (126 MHz, MeOD) δ 172.97, 170.88, 161.36, 148.31, 99.71, 76.16, 69.17, 67.34, 62.77, 49.29, 45.76, 39.94, 21.04. LC-MS m/z 348 $[M+H]^+$; HRMS (ESI) $[M+Na]^+$ found m/z 370.1222, calcd for $C_{13}H_{21}N_3O_8Na$ 370.1226.

1-((2R,3R,4S)-3-acetamido-4-amino-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carbonyl)piperidine-4-carboxylic acid (8e) The product was obtained as white foam after lyophilization. Yield 78%. ^{1}H NMR (400 MHz, CD_3OD, ppm) δ 5.20 (s, 1H), 4.66 (d, *J* = 9.4 Hz, 1H), 4.52-4.45 (m, 1H), 4.38 (dd, *J* = 21.0, 6.3 Hz, 2H), 4.24 (d, *J* = 14.7 Hz, 1H), 4.15-3.97 (m, 3H), 3.90-3.75 (m, 1H), 3.69 (d, *J* = 10.7 Hz, 2H), 3.54-3.43 (m, 1H), 3.27-3.16 (m, 1H), 2.96 (s, 1H), 2.63 (s, 1H), 2.00 (dd, *J* = 21.0, 8.8 Hz, 6H), 1.63 (s, 2H); ^{13}C NMR (126 MHz, MeOD) δ 179.63, 173.14, 162.75, 150.64, 98.10, 76.04, 69.29, 67.23, 62.90, 49.21, 45.71, 41.02, 39.81, 27.05, 21.07. LC-MS m/z 402 $[M+H]^+$; HRMS (ESI) $[M+Na]^+$ found m/z 424.1709, calcd for $C_{17}H_{27}N_3O_8Na$ 424.1696.

General procedure for the synthesis of compounds 9a-j

NaOH aqueous solution (5 %, 200 μL) was added into a solution of intermediates **23** in 5 mL methanol. After the reaction mixture was stirred at r.t. overnight, the pH value of solution was adjusted to 5-6 with positive ion-exchange resin (Dowex 50WX4-400). The mixture was filtered, and the solvent was removed under vacuum. Then trifluoroacetic acid (50 % in DCM) was added to the porducts above abtained,

and stirred at r.t. overnight. After the reaction was completed, the solvent was evaporated to give the product **9a-j** as a salt of trifluoroacetic acid, which was further purified by a column of Dowex50WX8-200 (H^+) resin (2.0 g).

(S)-2-((2R,3R,4S)-3-acetamido-4-guanidino-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carboxamido)pentanedioic acid (9a) The product was obtained as light yellow foam after lyophilization. Yield 92 %. ^{1}H NMR (300 MHz, D_2O, ppm) δ 7.95 (m, 1H), 5.85 (s, 1H), 4.57 (m, 1H), 4.40-4.13 (m, 3H), 4.08-3.85 (m, 2H), 3.81 – 3.64 (m, 1H), 2.38-2.21 (m, 2H), 2.11 (m, 2H), 1.96 (s, 3H); ^{13}C NMR (126 MHz, D_2O) δ 181.04, 173.97, 161.96, 156.54, 146.02, 103.90, 75.80, 69.33, 67.41, 62.57, 54.89, 50.23, 47.25, 33.52, 27.58, 22.80, 21.48. LC-MS m/z 462 $[M+H]^+$; HRMS (ESI) $[M+Na]^+$ found m/z 484.1673, calcd for $C_{17}H_{27}N_5O_{10}Na$ 484.1656.

(S)-2-((2R,3R,4S)-3-acetamido-4-guanidino-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carboxamido)-4-methylpentanoic acid (9b) The product was obtained as light yellow foam after lyophilization. Yield 80 %. ^{1}H NMR (400 MHz, D_2O, ppm) δ 5.85 (s, 1H), 4.55 (d, J = 9.9 Hz, 2H), 4.34-4.31 (m, 2H), 4.03-3.90 (m, 2H), 3.80 (d, J = 9.3 Hz, 1H), 3.72-3.67 (m, 1H), 2.08 (s, 3H), 1.69-1.58 (m, 3H), 1.05 – 0.84 (s, 6H); ^{13}C NMR (126 MHz, D_2O) δ 178.84, 173.97, 161.85, 156.53, 146.18, 104.39, 75.99, 69.38, 67.11, 62.45, 53.37, 50.33, 47.25, 40.39, 39.89, 24.12, 21.90, 21.43, 20.50. LC-MS m/z 446 $[M+H]^+$; HRMS (ESI) $[M+Na]^+$ found m/z 468.2052, calcd for $C_{18}H_{31}N_5O_8Na$ 468.2070.

(S)-2-((2R,3R,4S)-3-acetamido-4-guanidino-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carboxamido)-3-methylbutanoic acid (9c) The product was obtained as light yellow foam after lyophilization. Yield 95 %. ^{1}H NMR (400 MHz, D_2O, ppm) δ 5.87 (s, 1H), 4.56 (d, *J* = 10.2 Hz, 2H), 4.35 (t, *J* = 9.9 Hz, 1H), 4.20 (d, *J* = 5.2 Hz, 1H), 3.96 (dd, *J* = 15.4, 8.3 Hz, 2H), 3.81 (d, *J* = 9.3 Hz, 1H), 3.71 (dd, *J* = 10.7, 4.6 Hz, 1H), 2.26-2.17 (m, 1H), 2.10 (d, *J* = 10.6 Hz, 3H), 0.97 (dd, *J* = 14.2, 6.9 Hz, 6H); ^{13}C NMR (126 MHz, D_2O) δ 177.39, 173.97, 161.86, 156.53, 146.08, 104.47, 76.10, 69.52, 67.05, 62.45, 59.93, 50.38, 47.27, 30.26, 21.43, 18.29, 16.62. LC-MS m/z 432 $[M+Na]^+$; HRMS (ESI) $[M+Na]^+$ found m/z 454.1900, calcd for $C_{17}H_2N_5O_8Na$ 454.1914.

(S)-2-((2R,3R,4S)-3-acetamido-4-guanidino-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carboxamido)propanoic acid (9d) The product was obtained as light yellow foam after lyophilization. Yield 89 %. ^{1}H-NMR (400 MHz, D_2O, ppm): *δ* 5.87 (d, *J* = 2.0 Hz, 1H), 4.57 (d, 2H), 4.37-4.29 (m, 2H), 4.02-3.96 (m, 2H), 3.81 (d, *J* = 9.6 Hz, 1H), 3.75 (q, *J* = 6.8 Hz, 1H), 2.78 (s, 1H), 2.11 (s, 3H), 1.45 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, D_2O) δ 181.04, 179.22, 174.08, 161.62, 156.58, 104.14, 75.87, 69.44, 67.25, 62.54, 50.37, 50.26, 47.27, 22.86, 21.49, 16.97. LC-MS m/z 404$[M+H]^+$; HRMS (ESI) $[M+Na]^+$ found m/z 426.1613, calcd for $C_{15}H_{25}N_5O_8Na$ 426.1601.

3-((2R,3R,4S)-3-acetamido-4-guanidino-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carboxamido)propanoic acid (9e) The product was obtained as

light yellow foam after lyophilization. Yield 85 %. ^{1}H NMR (300 MHz, CD_3OD, ppm) δ 7.99 (d, *J* = 10.7 Hz, 1H), 5.72 (s, 1H), 4.57-4.35 (m, 2H), 4.20 (t, *J* = 9.1 Hz, 1H), 4.06-3.91 (m, 1H), 3.75 (dd, *J* = 41.2, 9.2 Hz, 3H), 3.53 (d, *J* = 4.7 Hz, 2H), 2.55 (d, *J* = 6.3 Hz, 2H), 2.01 (s, 3H); ^{13}C NMR (126 MHz, MeOD) δ 173.56, 172.66, 161.55, 157.05, 146.72, 102.96, 76.45, 69.33, 67.81, 62.78, 49.60, 35.15, 34.41, 32.46, 20.80. LC-MS m/z 402 $[M-H]^-$; HRMS (ESI) $[M+H]^+$ found m/z 404.1797, calcd for $C_{15}H_{26}N_5O_8$ 404.1781.

(2R,3R,4S)-3-acetamido-N-(3-fluorobenzyl)-4-guanidino-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carboxamide (9f) The product was obtained as light yellow foam after lyophilization. Yield 85 %. ^{1}H-NMR (400 MHz, D_2O, ppm) δ 7.33-7.30 (m, 1H), 7.13-6.97 (m, 3H), 5.77-5.74 (m, 1H), 4.51-4.42 (m, 4H), 4.24-4.08 (m, 2H), 3.82-3.68 (m, 2H), 2.01 (s, 3H); ^{13}C NMR (126 MHz, MeOD) δ 162.89 (*J* = 248 Hz), 162.23, 161.92, 157.37, 147.27, 141.09, 129.83 (*J* = 7 Hz), 122.90 (*J* = 8 Hz), 113.77 (*J* = 22 Hz), 113.55 (*J* = 21 Hz), 103.56, 76.82, 70.65, 69.70, 68.18, 63.07, 49.96, 42.05, 21.34. LC-MS m/z 440$[M+H]^+$; HRMS (ESI) $[M+H]^+$ found m/z 440.1934, calcd for $C_{19}H_{27}N_5O_6F$ 440.1945.

(2R,3R,4S)-3-acetamido-N-(2-fluorobenzyl)-4-guanidino-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carboxamide (9g) The product was obtained as light yellow foam after lyophilization. Yield 92 %. ^{1}H-NMR (400 MHz, D_2O, ppm) δ 7.35-7.28 (m, 2H), 7.15-7.08 (m, 2H), 5.73 (d, *J* = 2.1 Hz, 1H), 4.53 (s, 2H), 4.46-4.41 (m, 2H), 4.24-4.18 (m, 1H), 3.83-3.78 (m, 2H), 3.71-3.67 (m, 2H), 2.01 (s,

3H); ^{13}C NMR (126 MHz, D_2O) δ 174.36, 162.24 (*J* = 217.98 Hz), 159.42, 156.87, 146.64, 129.47 (*J* = 8 Hz), 129.30 (*J* = 8 Hz), 123.96, 115.22 (*J* = 22 Hz), 104.59, 76.19, 69.60, 67.45, 62.72, 50.52, 47.58, 37.16, 21.76. LC-MS m/z 440$[M+H]^+$; HRMS (ESI) $[M+H]^+$ found m/z 440.1956, calcd for $C_{19}H_{27}N_5O_6F$ 440.1945.

(2R,3R,4S)-3-acetamido-4-guanidino-N-(4-methylbenzyl)-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carboxamide (9h) The product was obtained as light yellow foam after lyophilization. Yield 85 %. ^{1}H-NMR (400 MHz, D_2O, ppm) δ 8.34 (s, 1H), 7.18 (d, *J* = 8.1 Hz, 2H), 7.13 (d, *J* = 8.1 Hz, 2H), 5.74 (d, *J* = 2.4 Hz, 1H), 4.40-4.64 (m, 5H), 4.08-4.24 (m, 3H), 3.65-3.69 (m, 1H), 2.30 (s, 3H), 2.01 (s, 3H); ^{13}C NMR (126 MHz, D_2O) δ 174.36, 163.00, 156.85, 146.72, 137.56, 134.34, 129.20, 127.19, 104.50, 76.19, 69.59, 67.47, 62.73, 50.51, 47.59, 42.54, 21.76, 19.96. LC-MS m/z 436 $[M+H]^+$; HRMS (ESI) $[M+H]^+$ found m/z 436.2213, calcd for $C_{20}H_{30}N_5O_6$ 436.2196.

(2R,3R,4S)-3-acetamido-4-guanidino-N-(4-fluorobenzyl)-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carboxamide (9i) The product was obtained as light yellow foam after lyophilization. Yield 81 %. ^{1}H-NMR (400 MHz, D_2O, ppm) δ 7.30-7.35 (m, 2H), 7.01-7.07 (m, 2H), 5.73 (d, 2.1Hz, 1H), 4.40-4.44 (m, 4H), 4.21-4.24 (m, 1H), 3.79-3.82 (m, 2H), 3.66-3.70 (m, 2H), 2.01 (s, 3H); ^{13}C NMR (125 MHz, MeOD) δ 173.09, 162.54 (*J* = 114.6 Hz), 161.05, 157.39, 147.28, 134.17, 129.16, 129.09, 114.74, 114.57, 103.45, 76.78, 69.65, 68.14, 63.06, 49.91, 47.93,

41.81, 21.12. LC-MS m/z 440 $[M+H]^+$; HRMS (ESI) $[M+H]^+$ found m/z 440.1946, calcd for $C_{19}H_{27}N_5O_6F$ 440.1945.

(2R,3R,4S)-3-acetamido-4-guanidino-N-(3-chlorobenzyl)-2-((1R,2R)-1,2,3-trihydroxypropyl)-3,4-dihydro-2H-pyran-6-carboxamide (9j) The product was obtained as light yellow foam after lyophilization. Yield 83 %. ^{1}H-NMR (400 MHz, D_2O, ppm) δ 7.22-7.32 (m, 4H), 5.74 (s, 1H), 4.42-4.46 (m, 4H), 4.19-4.25 (m, 1H), 3.78-3.85 (m, 2H), 3.66-3.71 (m, 2H), 2.02 (s, 3H); ^{13}C NMR (100 MHz, MeOD) δ 173.08, 162.30, 157.47, 147.26, 140.62, 133.93, 129.66, 127.21, 126.96, 125.63, 103.76, 76.89, 69.69, 68.18, 63.10, 49.95, 48.21, 42.07, 21.23. LC-MS m/z 456 $[M+H]^+$; HRMS (ESI) $[M+H]^+$ found m/z 456.1646, calcd for $C_{19}H_{27}N_5O_6Cl$ 456.1650.

(2S,4S,5R,6R)-benzyl 5-acetamido-2,4-dihydroxy-6-((1R,2R)-1,2,3-trihydroxypropyl)tetrahydro-2H-pyran-1-carboxylate (10) A stirred suspension of *N*-acetylneuraminic acid (10 g, 32.4 mmol) in 10 mL of water was treated with Cs_2CO_3 (5 g), and the pH value of solution was adjusted to 7-8. Then the reaction mixture was concentrated to be a glassy solid under reduced pressure. The glassy solid salt was dissolved in DMF (30 mL) and BnBr (6 mL) was added dropwise. After being stirred for 24 h, the resulting mixture was filtered. Compound 10 crystallized when the filtrate was poured into DCM (1000 mL) with agitate vigorously (10.8 g). ^{1}H-NMR (400 MHz, D_2O, ppm): δ 7.31-7.41 (m,5H), 5.22 (dd, J = 22.4, 12.8 Hz, 2H), 4.04 (m, 1H), 3.99 (dd, J = 10.4, 1.6 Hz, 1H), 3.80 (d, J = 2.8 Hz, 1H), 3.77 (m, 1H), 3.70 (m, 1H), 3.62 (dd, J = 10.8, 5.2 Hz,1H), 3.48 (dd, J = 9.2, 1.2

Hz,1H), 2.22 (dd, *J* = 12.8, 5.2 Hz, 1H), 1.91 (dd, *J* =12.8, 11.2Hz, 1H); LC-MS m/z 398 $[M+H]^+$.

(1S,2R)-1-((2R,3R,4S,6R)-3-acetamido-4,6-diacetoxy-6-(benzyloxycarbonyl)tetrahydro-2H-pyran-2-yl)propane-1,2,3-triyl triacetate (11) A suspension of compound **10** (10.8 g,) in 50 mL of pyridine was treated with catalytic amount of DMAP (350 mg) in ice bath, and then 26 mL of acetic anhydride was added dropwise. The resulting mixture was stirred for 24 h at room temperature. After most of solvent was evaporated under reduced pressure, 300 mL of ethyl acetate was added, and the resulting mixture was washed sequentially by 2 M HCl (100 mL × 3), saturated aqueous sodium bicarbonate (100 mL × 2), and saturated brine (100 mL). The organic layer was dried by $MgSO_4$, and filtered, the solvent was evaporated to give buff-colored syrup, which was further purified by flash column chromatography (ethyl acetate) to yield the expected product **11** (7.4 g). Yield of the above two steps: 37.6 %. ^{1}H-NMR (300 MHz, $CDCl_3$, ppm): δ 7.34 (s, 5H), 5.42-5.36 (m, 2H), 5.25-5.13 (m, 3H), 5.09-5.07 (m, 1H), 4.44 (dd, *J* = 12.3, 2.7 Hz, 1H), 4.17-4.06 (m, 3H), 2.54 (dd, *J* = 13.5, 4.8 Hz, 1H), 2.12 (s, 3H), 2.10 (s, 3H), 2.04-2.03 (m, 1H), 2.01 (s, 9H), 1.88 (s, 3H), 1.82 (br, 1H); LC-MS m/z 610 $[M+H]^+$。

(1S,2R)-1-((2R,3R,4S)-3-acetamido-4-acetoxy-6-(benzyloxycarbonyl)-3,4-dihydro-2H-pyran-2-yl)propane-1,2,3-triyl triacetate (12) Compound **11** (7 g) was dissolved in CH_3CN (30 mL) and 5 mL of TMSOTf was added dropwise under the protection of N_2 at 0 ^oC, and continued to stir for 2.5 h. The reaction mixture was

allowed to pour into a vigorously stirred mixture of ice-cold saturated aqueous sodium bicarbonate (500 mL, including a lot of solid sodium bicarbonate). After 10 min, the solution was filtered and the aqueous phase was extracted with ethyl acetate (100 mL × 2). The combined organic layer was concentrated, and the resulting residue was further purified by flash column chromatography (ethyl acetate) to yield the expected product **12** (3.8 g, yield 60 %). ^{1}H-NMR(400 MHz, CD_3OD, ppm): δ 7.35-7.12 (m, 5H), 6.0 (d, J = 6.9 Hz, 1H), 5.49-5.43 (m, 1H), 5.35-5.32 (m, 1H), 5.20 (s, 2H), 4.55 (dd, J = 3.3, 2.0 Hz, 1H), 4.38-4.32 (m, 2H), 4.15-4.02 (m, 2H), 2.06(s, 3H), 2.04 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H),1.90 (s, 3H); LC-MS m/z 550 $[M+H]^+$.

(2R,3R,4S)-3-acetamido-4-acetoxy-2-((1S,2R)-1,2,3-triacetoxypropyl)-3,4-dihydro-2H-pyran-6-carboxylic acid (13) Compound **12** (3 g) was dissolved in ethanol (20 mL), and 10%Pd/C (300 mg) was added to the reaction mixture. A slow stream of H_2 gas was added to the system. After about 10 min, the H_2 gas was stopped when all compound **12** was consumed as evidenced by TLC. Then the reaction mixture was filtered, and concentrated to obtain the compound **13** under reduced pressure (2.4 g, yield 95 %).

(1S,2R)-1-((3aR,4R,7aR)-6-(benzyloxycarbonyl)-2-methyl-4,7a-dihydro-3aH-pyrano[3,4-d]oxazol-4-yl)propane-1,2,3-triyl triacetate (14) After compound 11 (7.4 g) was dissolved in ethyl acetate (40 mL) and 6.4 mL of TMSOTf was added dropwise under the protection of N_2 at room temperature. Then the temperature was raised to 52 ^{o}C and continued to stir for 2.5 h. The reaction mixture was allowed to

cool and poured into a vigorously stirred mixture of ice-cold saturated aqueous sodium bicarbonate (500 mL, including a lot of solid sodium bicarbonate). After 10 min, the solution was filtered and the aqueous phase was extracted with ethyl acetate (100 mL × 3). The combined organic layer was concentrated, and the resulting residue was directly used in the next step. LC-MS *m/ z*: 512 $[M + Na]^+$.

(1S,2R)-1-((2R,3R,4R)-3-acetamido-6-(benzyloxycarbonyl)-4-hydroxy-3,4-dihydro-2H-pyran-2-yl)propane-1,2,3-triyl triacetate (15) The above resulting product **14** was dissolved in ethyl acetate (30 mL), and acetic acid (50 %, 0.5 mL) was added dropwise at room temperature. After the reaction mixture was stirred for 24 h, ethyl acetate (200 mL) were added to the reaction solution, and the mixture was washed with 5% aqueous sodium hydrogen carbonate (100 mL × 2), and saturated brine (100 mL). The organic layer was dried by Na_2SO_4, and filtered, the solvent was evaporated to give the crude product, which was further purified by flash column chromatography (ethyl acetate) to yield the expected product **15** (2.09 g). Yield 34 %. ^{1}H-NMR (300 MHz, $CDCl_3$, ppm): δ 7.34-7.39 (m, 5H), 6.22 (d, J = 9.6 Hz, 1H), 6.17 (d, J = 5.7 Hz, 1H), 5.44 (dd, J = 3.9, 1.8 Hz, 1H), 5.16-5.31 (m, 4H), 4.73 (dd, J = 12.6, 6 Hz, 1H), 4.30 (m, 1H), 4.17-4.28 (m, 3H), 3.23 (br, 1H), 2.09 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H),1.93 (s, 3H); LC-MS m/z 508 $[M+H]^+$.

(1S,2R)-1-((2R,3R,4S)-3-acetamido-4-azido-6-(benzyloxycarbonyl)-3,4-dihydro-2H-pyran-2-yl)propane-1,2,3-triyl triacetate (16) Compound 15 (2 g, 3.94 mmol) and DPPA (1.3 g) was dissolved in benzene (25 mL), and then DBU (0.7 g) was

added under Ar protection at 0 oC. The reaction mixture was stirred about 5 hours at room temperature, an addition DPPA (0.25 g) and DBU (0.14 g) was added, and the reaction mixture stirred for another 18 hours. Ethyl acetate (200 mL) was added to the reaction solution, and the mixture was washed with 1N HCl (80 mL × 2), and saturated brine (100 mL). The organic layer was dried by Na_2SO_4, and filtered, the solvent was evaporated to give the crude product, which was further purified by flash column chromatography (MeOH/DCM = 1/20) to yield the expected product **16** (1.67 g). Yield 86 %. ^{1}H-NMR (300 MHz, $CDCl_3$, ppm): δ 7.34-7.41 (m, 5H), 6.00 (d, J = 3.0 Hz, 1H), 5.89 (d, J = 8.7 Hz, 1H), 5.46 (dd, J = 5.4, 2.4 Hz, 1H), 5.36-5.31 (m, 1H), 5.24 (d, J = 2.1 Hz, 2H), 4.58 (dd, J = 12.3, 2.7 Hz, 1H), 4.52 (dd, J = 9.9, 2.7 Hz, 1H), 4.48-4.43 (m, 1H), 4.21 (dd, J = 12.3, 6.6 Hz, 1H), 3.87 (dd, J = 18, 8.7 Hz, 1H), 2.12 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H); LC-MS m/z 533 $[M+H]^+$。

(1S,2R)-1-((2R,3R,4S)-3-acetamido-4-amino-6-(benzyloxycarbonyl)-3,4-dihydro-2H-pyran-2-yl)propane-1,2,3-triyl triacetate (17) A 100 mL 3-necked, round-bottomed flask, fitted with gas inlet and outlet tubes, was charged with a solution of compound **16** (1.5 g) in dry pyridine. A slow stream of H_2S gas was added to this system. After 16 h, the system was purged with nitrogen and the reaction mixture was concentrated under reduced pressure to give an orange oil. Then the residue was dissolved in ethyl acetate (100 mL), and washed sequentially with 1 mol/L hydrochloric acid (2 × 100 mL), saturated aq. sodiumhydrogen carbonate (100 mL), and finally brine (100 mL). The organic layer was then dried ($MgSO_4$), and evaporated under reduced pressure at 35-40 °C. The resulting residue was purified by

flash column chromatography (MeOH/DCM = 1/10) to afford the expected product **17** (1.0 g) Yield 70 %. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 7.29-7.37 (m, 5H), 6.19 (br, 1H), 6.00 (d, J = 1.6 Hz, 1H), 5.48 (dd, J = 4, 2.8 Hz, 1H), 5.25-5.29 (m, 1H), 5.19 (dd, J = 22, 12 Hz, 2H), 4.66 (dd, J = 12, 2.4 Hz, 1H), 4.24 (dd, J = 10, 2.4 Hz, 1H), 4.20 (dd, J = 12.4, 8 Hz, 1H), 3.92 (dd, J = 18.8, 9.6 Hz, 1H), 3.53 (d, J = 7.6 Hz, 1H), 2.07 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.95 (s, 3H); LC-MS m/z 507 $[M+H]^+$。

(1S,2R)-1-((2R,3R,4S)-3-acetamido-6-(benzyloxycarbonyl)-4-(tert-butoxycarbonylamino)-3,4-dihydro-2H-pyran-2-yl)propane-1,2,3-triyl triacetate (18) Compound **17** (1 g) was dissolved in methanol (25 mL), and then $(Boc)_2O$ (480 mg) was added. The reaction mixture was stirred about 30 min at room temperature, ethyl acetate (100 mL) was added to the reaction solution, and the mixture was washed with 1N HCl (80 mL × 2), and saturated brine (100 mL). The organic layer was dried by Na_2SO_4, and filtered, the solvent was evaporated to give the crude product, which was further purified by flash column chromatography (MeOH/DCM = 1/20) to yield the expected product **18** (920 mg). Yield 76 %. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.40-7.31 (m, 5H), 6.12 (d, J = 9.2 Hz, 1H), 5.91 (d, J = 2.3 Hz, 1H), 5.46 (dd, J = 4.7, 1.9 Hz, 1H), 5.32-5.26 (m, 1H), 4.84 (d, J = 9.7 Hz, 1H), 4.63 (dd, J = 12.5, 2.6 Hz, 1H), 4.44 (d, J = 2.2 Hz, 1H), 4.28-4.13 (m, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.89 (s, 3H), 1.40 (d, J = 18.2 Hz, 9H); LC-MS m/z 607 $[M+H]^+$。

(2R,3R,4S)-3-acetamido-4-(tert-butoxycarbonylamino)-2-((1S,2R)-1,2,3-triacetoxypropyl)-3,4-dihydro-2H-pyran-6-carboxylic acid (19) Compound **18** (500 mg)

was dissolved in ethanol (20 mL), and 10 % Pd/C (50 mg) was added to the reaction mixture. A slow stream of H_2 gas was added to the system. After about 10 min, the H_2 gas was stopped when all compound **12** was consumed as evidenced by TLC. Then the reaction mixture was filtered, and concentrated to obtain the compound **19** under reduced pressure (404 mg). Yield 95 %. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 5.97 (s, 1H), 5.51-5.22 (m, 2H), 5.11-4.88 (m, 1H), 4.74-4.42 (m, 2H), 4.36-4.01 (m, 3H), 3.95-3.56 (m, 2H), 2.17-2.01 (m, 9H), 1.92 (s, 3H), 1.41 (s, 9H); LC-MS m/z 518 $[M-H]^-$.

(1S,2R)-1-((2R,3R,4S)-3-acetamido-6-(benzyloxycarbonyl)-4-(2,3-bis(tert-butoxy carbonyl)guanidino)-3,4-dihydro-2H-pyran-2-yl)propane-1,2,3-triyl triacetate (21) The compound **17** (2.8 g) was added neat to a solution of triethylamine (1.5 mL) and N,N'-di-boc-N''-trifluoromethanesulfonyl-guanidine (3 g) in DCM (20 mL), and the mixture was stirred at rt until all **17** was consumed as evidenced by TLC. After then, the mixture was diluted with DCM (50 mL) and washed with saturates sodium bicarbonate, and brine. After drying with sodium sulfate and filtering the solvent was removed under reduced presuure. The residue was purified by flash column chromatography (MeOH/DCM = 1/20) to afford the expected product **21** (3.4 g). Yield 82 %. ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 7.39-7.30 (m, 5H), 6.07-6.06 (m, 1H), 5.90 (d, J = 2.0 Hz, 1H), 5.41 (dd, J = 4.8, 1.2 Hz, 1H), 5.31-5.26 (m, 1H), 5.22 (dd, J = 21.6, 12 Hz, 2H), 5.15-5.10 (m, 1H), 4.50 (dd, J = 12.4, 2.8 Hz, 1H), 4.27-4.23 (m, 1H), 4.17 (dd, J = 12, 7.2 Hz, 1H), 2.11 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H), 1.85 (s, 3H), 1.47 (s, 9H),1.46 (s, 9H); LC-MS m/z 749 $[M+H]^+$.

(2R,3R,4S)-3-acetamido-4-(2,3-bis(tert-butoxycarbonyl)guanidino)-2-((1S,2R)-1, 2,3-triacetoxypropyl)-3,4-dihydro-2H-pyran-6-carboxylic acid (22) Compound **21** (4 g) was dissolved in methanol (20 mL), and 10% Pd/C (500 mg) was added to the reaction mixture. A slow stream of H_2 gas was added to the system. After about 10 min, the H_2 gas was stopped when all compound **21** was consumed as evidenced by TLC. Then the reaction mixture was filtered, and concentrated to obtain the compound **22** under reduced pressure (3.2 g, yield 90 %). LC-MS m/z 659 $[M+H]^+$

General procedure for the synthesis of compounds 23a-j

To a solution of acid **22** (0.2 mmol), EDCI (0.3 mmol), Et_3N (0.3 mmol) in 5 mL dry CH_2Cl_2 was added an equimolar amount of the appropriate amide. The mixture was kept stirring at room temperature for 24 h. After TLC detection to show no starting materials, the reaction mixture was concentrated under reduced pressure and purified by flash column chromatography to obtain intermediates **23a-j**.

(S)-dimethyl2-((2R,3R,4S)-3-acetamido-4-(2,3-bis(tert-butoxycarbonyl)guanid-in o)-2-((1S,2R)-1,2,3-triacetoxypropyl)-3,4-dihydro-2H-pyran-6-carboxamido)pent anedioate (23a) The compound was purified by flash column chromatography with an eluent of MeOH /DCM = 1/25. Yield 75 %. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 11.32 (s, 1H), 8.51 (d, *J* = 8.3 Hz, 1H), 7.13 (d, *J* = 8.1 Hz, 1H), 6.11 (d, *J* = 8.5 Hz, 1H), 5.88 (dd, *J* = 7.5, 2.4 Hz, 1H), 5.49-5.43 (m, 1H), 5.37 (dd, *J* = 8.2, 1.8 Hz, 1H), 5.11 (t, *J* = 7.9 Hz, 1H), 4.58 (td, *J* = 9.0, 5.1 Hz, 1H), 4.35 (dd, *J* = 12.4, 2.6 Hz, 1H), 4.32 – 4.22 (m, 2H), 4.02 (dd, *J* = 12.5, 6.5 Hz, 1H), 3.74 (d, *J* = 3.4 Hz, 3H), 3.70 –

3.69 (m, 3H), 2.47 (td, *J* = 7.4, 3.3 Hz, 2H), 2.40-2.22 (m, 2H), 2.15 (s, 3H), 2.12 (s, 3H), 2.05 (s, 3H), 1.87 (s, 3H), 1.47 (d, *J* = 2.5 Hz, 18H); LC-MS m/z 816 $[M+H]^+$

(1S,2R)-1-((2R,3R,4S)-3-acetamido-4-(2,3-bis(tert-butoxycarbonyl)guanidino)-6-((S)-1-methoxy-4-methyl-1-oxopentan-2-ylcarbamoyl)-3,4-dihydro-2H-pyran-2-yl)propane-1,2,3-triyl triacetate (23b) The compound was purified by flash column chromatography with an eluent of MeOH /DCM = 1/25. Yield 80 %. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 11.32 (s, 1H), 8.52 (d, *J* = 8.1 Hz, 1H), 6.94 (d, *J* = 8.0 Hz, 1H), 6.08 (d, *J* = 8.4 Hz, 1H), 5.87 (d, *J* = 2.3 Hz, 1H), 5.38-5.35 (m, 2H), 5.10-5.04 (m, 1H), 4.55-4.32 (m, 1H), 4.30-4.25 (m, 3H), 4.00 (dd, *J* = 12.4, 6.5 Hz, 1H), 3.72 (s, 3H), 2.16 (s, 3H), 2.13 (s, 3H), 2.04 (s, 3H), 1.87 (s, 3H), 1.73-1.62 (m, 3H), 1.47 (s, 18H), 0.96 (dd, *J* = 12.2, 5.9 Hz, 6H); LC-MS m/z 786 $[M+H]^+$.

(1S,2R)-1-((2R,3R,4S)-3-acetamido-4-(2,3-bis(tert-butoxycarbonyl)guanidino)-6-((S)-1-methoxy-3-methyl-1-oxobutan-2-ylcarbamoyl)-3,4-dihydro-2H-pyran-2-yl) propane-1,2,3-triyl triacetate (23c) The compound was purified by flash column chromatography with an eluent of MeOH /DCM = 1/25. Yield 82 %. ^{1}H-NMR (300 MHz, $CDCl_3$, ppm): *δ* 8.53 (d, *J* = 8.7 Hz, 1H), 7.05(d, *J* = 7.8 Hz, 1H), 5.87 (d, *J* = 2.1 Hz, 1H), 5.37-5.48 (m, 2H), 5.11 (t, *J* = 7.8 Hz, 1H), 4.56 (t, *J* = 7.8 Hz, 1H), 4.27-4.36 (m, 3H), 4.11 (q, *J* = 7.2 Hz, 1H), 4.02 (dd, *J* = 12.6, 6.6 Hz, 1H), 3.73 (s, 3H), 2.14 (s, 3H), 2.12 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.50 (d, *J* = 6.9 Hz, 3H), 1.40 (s, 9H), 1.39(s, 9H); LC-MS m/z 766 $[M+Na]^+$.

(1S,2R)-1-((2R,3R,4S)-3-acetamido-4-(2,3-bis(tert-butoxycarbonyl)guanidino)-6-((S)-1-methoxy-1-oxopropan-2-ylcarbamoyl)-3,4-dihydro-2H-pyran-2-yl)propane-1,2,3-triyl triacetate (23d) The compound was purified by flash column chromatography with an eluent of MeOH /DCM = 1/25. Yield 65 %. ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ 5.87 (s, 1H), 5.40 (dd, *J* = 17.4, 7.4 Hz, 2H), 5.22 (d, *J* = 46.2 Hz, 2H), 4.62 – 4.49 (m, 1H), 4.30 (dd, *J* = 16.6, 8.9 Hz, 3H), 4.02 (dd, *J* = 12.4, 6.4 Hz, 1H), 3.74 (t, *J* = 3.7 Hz, 3H), 2.14 (s, 6H), 2.05 (s, 3H), 1.87 (s, 3H), 1.47 (s, 18H), 1.26 (d, *J* = 8.3 Hz, 3H); LC-MS m/z 744 $[M-H]^-$.

(1S,2R)-1-((2R,3R,4S)-3-acetamido-4-(2,3-bis(tert-butoxycarbonyl)guanidino)-6-(3-methoxy-3-oxopropylcarbamoyl)-3,4-dihydro-2H-pyran-2-yl)propane-1,2,3-triyl triacetate (23e) The compound was purified by flash column chromatography with an eluent of MeOH /DCM = 1/25. Yield 78 %. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 11.33 (d, *J* = 11.1 Hz, 1H), 8.50 (t, *J* = 10.2 Hz, 1H), 7.21-7.05 (m, 1H), 6.19 (dd, *J* = 23.0, 17.5 Hz, 1H), 5.85 (t, *J* = 5.6 Hz, 1H), 5.40 (td, *J* = 7.4, 2.6 Hz, 1H), 5.34-5.28 (m, 1H), 5.14-5.00 (m, 1H), 4.34 (dd, *J* = 12.5, 2.5 Hz, 1H), 4.25 (dd, *J* = 19.6, 9.9 Hz, 1H), 4.20-4.10 (m, 3H), 4.03-3.93 (m, 1H), 3.66-3.54 (m, 2H), 2.59 (dd, *J* = 14.0, 7.1 Hz, 2H), 2.15-2.01 (m, 9H), 1.89-1.80 (m, 3H), 1.53-1.38 (m, 18H), 1.27-1.23 (m, 3H); LC-MS m/z 759 $[M+H]^+$.

(1S,2R)-1-((2R,3R,4S)-3-acetamido-4-(2,3-bis(tert-butoxycarbonyl)guanidino)-6-(3-fluorobenzylcarbamoyl)-3,4-dihydro-2H-pyran-2-yl)propane-1,2,3-triyl triacetate (23f) The compound was purified by flash column chromatography with an

eluent of MeOH /DCM = 1/25. Yield 65 %. ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ11.31 (br, 1H), 8.49 (d, *J* = 7.8 Hz, 1H), 7.44-7.40 (m, 1H), 7.28-7.22 (m, 1H), 7.11-7.03 (m, 2H), 6.95-6.89 (m, 1H), 6.28 (d, *J* = 8.1 Hz, 1H), 5.89 (d, *J* = 2.4 Hz, 1H), 5.38-5.30 (m, 2H), 5.07-5.04 (m, 1H), 4.49-4.45 (m, 2H), 4.37-4.18 (m, 3H), 4.12-4.05 (m, 1H), 3.95-3.88 (m, 1H), 2.11 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.83 (s, 3H), 1.45 (s, 9H), 1.44 (s, 9H); LC-MS m/z 766$[M+H]^+$.

(1S,2R)-1-((2R,3R,4S)-3-acetamido-4-(2,3-bis(tert-butoxycarbonyl)guanidino)-6-(2-fluorobenzylcarbamoyl)-3,4-dihydro-2H-pyran-2-yl)propane-1,2,3-triyl triacetate (23g) The compound was purified by flash column chromatography with an eluent of MeOH /DCM = 1/25. Yield 80 %. ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 11.32 (s, 1H), 8.52 (d, *J* = 8.7 Hz, 1H), 7.40-7.35 (m, 1H), 7.25-7.21 (m, 1H), 7.13-7.00 (m, 2H), 6.27 (d, *J* = 8.7 Hz, 1H), 5.89 (d, *J* = 2.4 Hz, 1H), 5.41-5.31 (m, 2H), 5.13-5.08 (m, 1H), 4.60-4.54 (m, 2H), 4.40-4.35 (m, 1H), 4.29-4.19 (m, 2H), 3.97-3.90 (m, 1H), 2.13 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.85 (s, 3H), 1.47 (s, 9H), 1.46 (s, 9H); LC-MS m/z 766 $[M+H]^+$.

(1S,2R)-1-((2R,3R,4S)-3-acetamido-4-(2,3-bis(tert-butoxycarbonyl)guanidino)-6-(4-methylbenzylcarbamoyl)-3,4-dihydro-2H-pyran-2-yl)propane-1,2,3-triyl triacetate (23h) The compound was purified by flash column chromatography with an eluent of MeOH /DCM = 1/25. Yield 55 %. ^{1}H-NMR (300 MHz $CDCl_3$, ppm) δ 11.33 (s, 1H), 8.54 (d, *J* = 8.7 Hz, 1H), 7.25 (d, *J* = 7.8 Hz, 2H), 7.14 (d, *J* = 7.8 Hz, 2H), 6.16 (d, *J* = 8.7 Hz, 1H), 5.90 (d, *J* = 2.1 Hz, 1H), 5.30-5.38 (m, 2H), 5.07-5.14

(m, 1H), 4.53-4.58 (m, 1H), 4.28-4.41 (m, 3H), 4.21-4.25 (m, 1H), 3.91-3.97 (m, 1H), 2.33 (s, 3H), 2.13 (s, 3H), 2.01 (s, 6H), 1.86 (s, 3H), 1.48 (s, 9H), 1.47(s, 9H); LC-MS m/z 762$[M+H]^+$.

(1S,2R)-1-((2R,3R,4S)-3-acetamido-4-(2,3-bis(tert-butoxycarbonyl)guanidino)-6-(4-fluorobenzylcarbamoyl)-3,4-dihydro-2H-pyran-2-yl)propane-1,2,3-triyl triacetate (23i) The compound was purified by flash column chromatography with an eluent of MeOH /DCM = 1/25. Yield 65 %. ^{1}H-NMR (300 MHz $CDCl_3$, ppm) δ 11.32 (s, 1H), 8.52 (d, *J* = 8.5 Hz, 1H), 7.33 (dd, *J* = 13.5, 5.4 Hz, 2H), 7.00 (t, *J* = 8.7 Hz, 2H), 6.30 (d, *J* = 9.1 Hz, 1H), 5.88 (s, 1H), 5.34 (t, *J* = 9.5 Hz, 2H), 5.10 (t, *J* = 9.0 Hz, 1H), 4.53 – 4.15 (m, 6H), 3.92 (dd, *J* = 12.3, 6.7 Hz, 1H), 2.12 (s, 3H), 2.01 (d, *J* = 2.5 Hz, 6H), 1.85 (s, 3H), 1.46 (d, *J* = 3.2 Hz, 18H); LC-MS m/z 766$[M+H]^+$.

(1S,2R)-1-((2R,3R,4S)-3-acetamido-4-(2,3-bis(tert-butoxycarbonyl)guanidino)-6-(3-chlorobenzylcarbamoyl)-3,4-dihydro-2H-pyran-2-yl)propane-1,2,3-triyl triacetate (23j) The compound was purified by flash column chromatography with an eluent of MeOH /DCM = 1/25. Yield 57 %. ^{1}H-NMR (300 MHz $CDCl_3$, ppm) δ 11.35 (s, 1H), 8.51-8.54 (m, 1H), 7.47-7.51 (m, 1H), 7.37 (s, 1H), 6.55-6.58 (m, 1H), 5.92 (m, 2.1Hz, 1H), 5.36-5.45 (m, 2H), 5.09-5.15 (m, 1H), 4.49 (d, 6.3Hz, 2H), 4.11-4.41 (m, 3H), 3.93-4.00 (m, 1H), 2.15 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 1.87 (s, 3H), 1.49 (s, 9H), 1.48 (s, 9H); LC-MS m/z 782$[M+H]^+$.

Supporting information

Table S1 and experimental details associated with this article can be available via the internet at http://pubs.acs.org.

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Author information

***Corresponding Author**. For H. Liu: Phone: +86-21-50807042. Fax: +86-21-50807042. E-mail: hliu@mail.shcnc.ac.cn. For B.L. Seong: Phone: (82-2)2123-2885. Fax: (82-2)362-7265. E-mail: blseong@yonsei.ac.kr. For W. Tang: Phone: +86-21-50805896. Fax: +86-21-50805896. E-mail: tangwei@mail.shcnc.ac.cn. For W.L. Zhu: Phone: +86-21-50805020. Fax: +86-21-50807088. E-mail: wlzhu@mail.shcnc.ac.cn.

Abbreviation Used

NA, neuraminidase; DANA, Neu5Ac2en; AIV, anti-avian influenza virus; MD, molecular dynamics; NANA, *N*-acetyl neuraminic acid; DMAP, 4-(dimethy

-amino)-pyridine; TMSOTf, trimethylsiyltrifluoromethanesulfonate; DPPA, diphenyl -phosphoryl azide; DBU, 1,8-diazabicyclo[5,4,0]undecen-7-ene; TFA, trifluoroacetic acid; MUNANA, 2'-(4-methylumbelliferyl)-*a*-$_{D}$-*N*-acetylneuraminic acid; SAR, structure-activity relationships; PK，pharmacokinetic；TLC, thin-layer chromate -graphy; SD, Sprague-Dawley.

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Figures and Tables

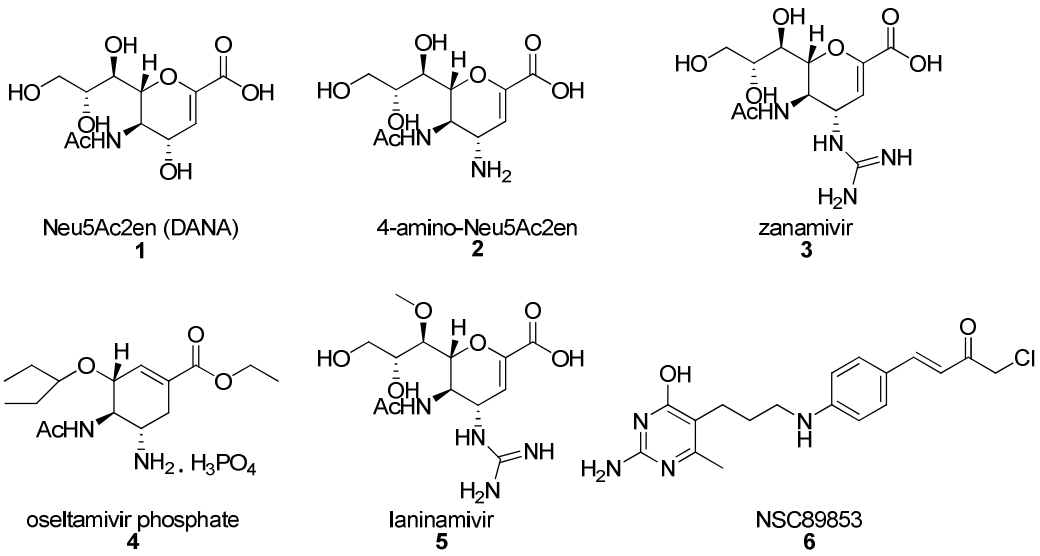


Figure 1. Structures of Neu5Ac2en, 4-amino-Neu5Ac2en, compound **3**, compound **4** phosphate, compound **5** and compound **6**.

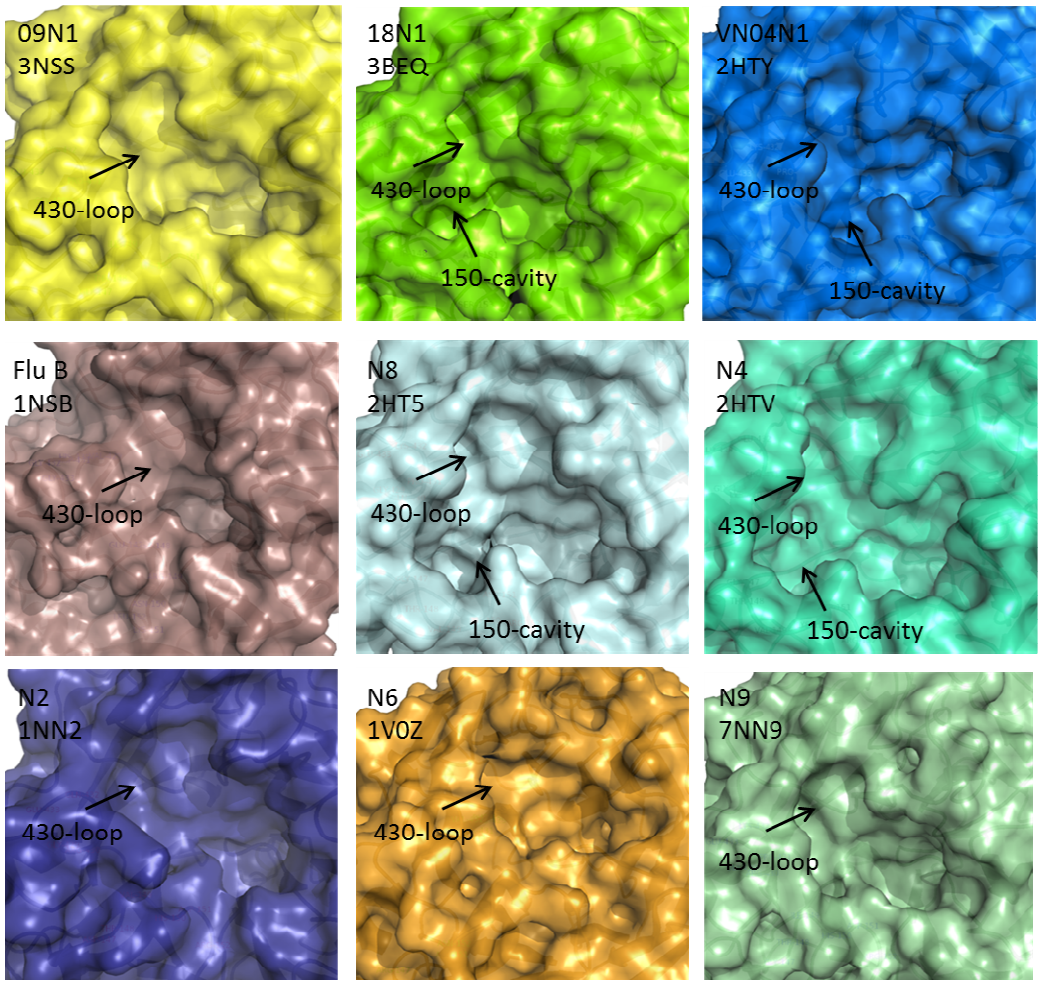


Figure 2. Comparison of the active cavity in all neuraminidase subtypes for which structures have been solved. Structures of 09N1 (PDB 3NSS), 18N1 (PDB 3BEQ), VN04N1 (PDB 2HTY), influenza B (flu B) neuraminidase (B/Beijing/1/87; PDB 1NSB), N8 (PDB 2HT5), N4 (PDB 2HTV), N2 (PDB 1NN2), N6 (PDB 1V0Z) and N9 (PDB 7NN9) are in surface representation. This shows that 09N1, along with members of group 2 neuraminidases (N2, N6 and N9) have no 150-cavity but 18N1, VN04N1 and other members of group 1 (N4 and N8) have the cavity. The flexible '430-loop' in group-1 and group-2 NAs can provide a large cavity adjacent to the active site.

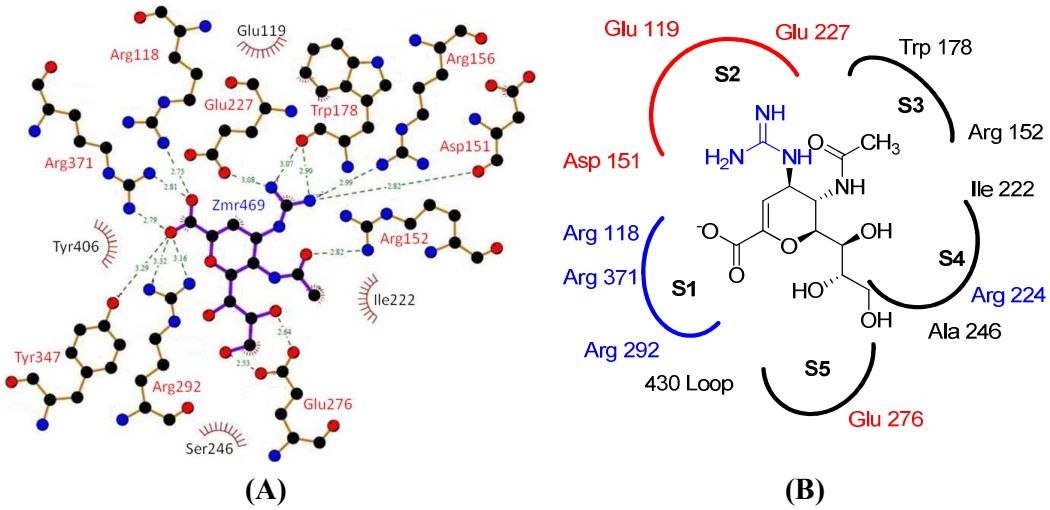
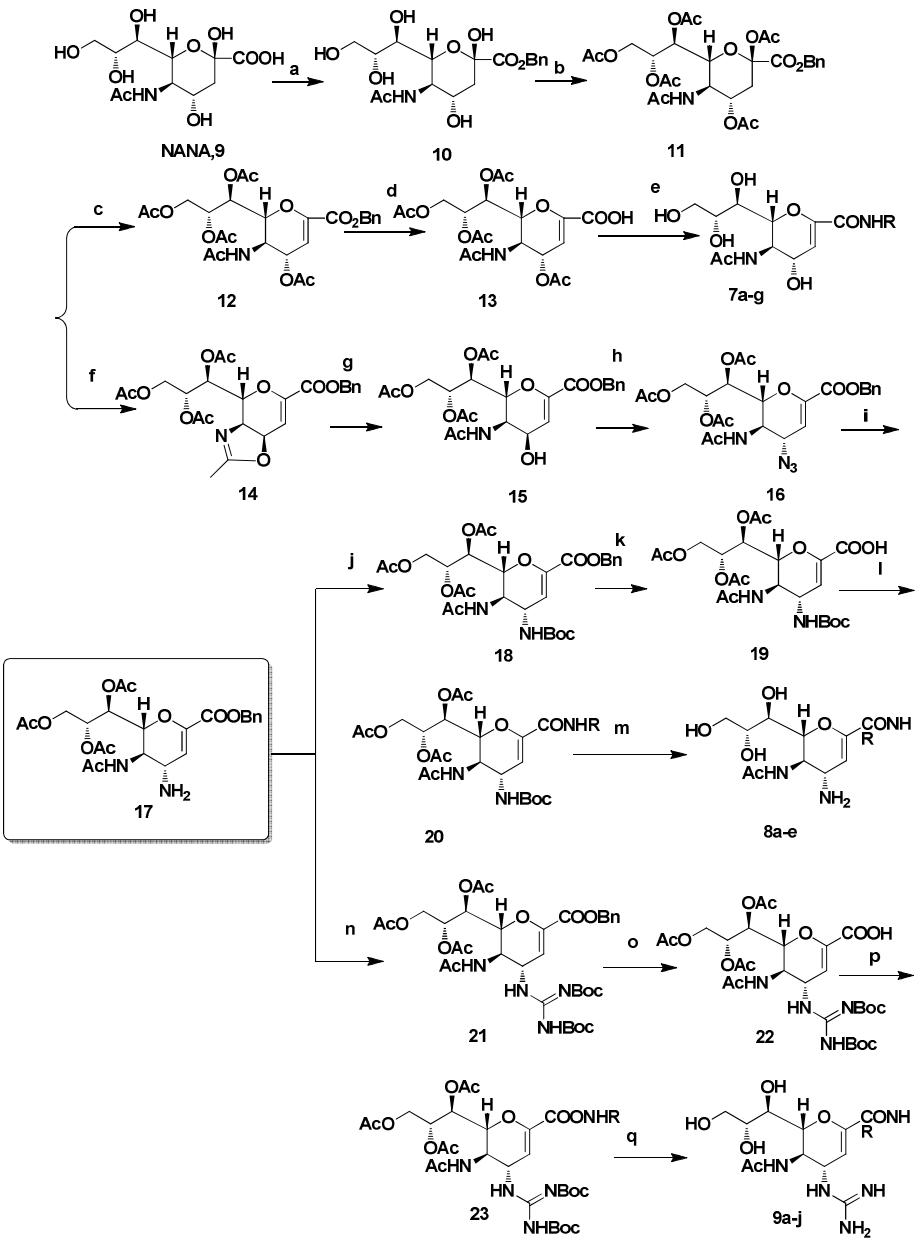
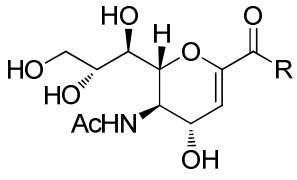


Figure 3. Perspective view into the active site of the sialidase (Neuraminidase PDB entry 3CKZ) complexed with compound **3**. Left A: 2D interactions map between zanamivier and the active pocket of N1. Right B: S1-S5 of NA active sites.

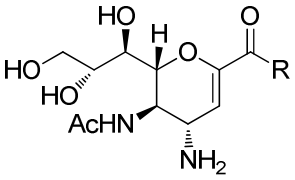


Scheme 1. Reagents and conditions: a) (i) Cs_2CO_3; (ii) BnBr, DMF (90%); (b) Pyridine, Ac_2O, DMAP (87%); c) TMSOTf, CH_3CN, 0 ^oC, Ar (85%); d) H_2, 10% Pd/C, C_2H_5OH (95%); e) (i) EDCI, DMAP, NH_2R (75%); (ii) NaOH; (iii) H^+ resin; f) TMSOTf, EtOAc, Ar, 52 ^oC (70%); g) 50% AcOH, EtOAc, H_2O (50%); h) DPPA, DBU, C_6H_6 (80%); i) H_2S, Py. (95%); j) $(Boc)_2O$, CH_3OH (85%); k) H_2, 10% Pa/C (70%); l) EDCI, DMAP, NH_2R, TEA (70%); m) (i) TFA, CH_2Cl_2 (80%); (ii) 10% NaOH, CH_3OH; (iii) H^+ resin (70%); n) *N*,*N*'-di-Boc-*N*''-trifluoromethane-sulfonyl-guanidine, TEA, CH_2Cl_2 (70%); o) H_2, 10%Pa/C (70%); p) EDCI, DMAP, NH_2R, TEA (70%); q) (i) TFA, CH_2Cl_2 (80%); (ii) 10% NaOH, CH_3OH; (iii) H^+ resin (70%).

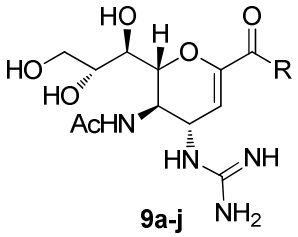
Table 1 Chemical structures of compounds **7**, **8**, **9** and their anti-influenza virus activities



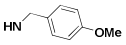
7a-g

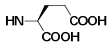


8a-e



9a-j

Compound	R	Sialidase inhibition assay IC_{50} (μM) [a]		
		H3N2 [b]	H5N1 [c]	H1N1 [d]
7a		857.74 ± 34.18	> 1000	> 1000
7b		917.85 ± 28.31	> 1000	> 1000
7c		790.00 ± 29.27	938.87 ± 3.24	> 1000
7d		688.38 ± 36.09	792.62 ± 12.74	> 1000
7e		60.03 ± 5.24	74.38 ± 2.89	60.49 ± 4.88
7f		52.95 ± 7.61	66.67 ± 5.37	98.95 ± 13.21
7g		707.14 ± 87.72	822.36 ± 54.76	142.64 ± 13.23
8a		5.15 ± 0.95	4.30 ± 0.59	2.25 ± 0.26
8b		646.69 ± 44.25	668.85 ± 39.51	159.42 ± 35.26

8c		146.32 ± 95.81	162.22 ± 120.46	103.41 ± 12.04
8d		16.74 ± 9.63	8.67 ± 0.14	13.89 ± 5.8
8e		5.86 ± 1.16	5.76 ± 0.04	6.33 ± 0.74
9a		0.81 ± 0.14	0.16 ± 0.06	0.45 ± 0.07
9b		22.50 ± 2.76	3.51 ± 0.36	5.11 ± 0.33
9c		0.107 ± 0.103	4.19 ± 0.18	2.14 ± 1.68
9d		0.41 ± 0.03	0.063 ± 0.003	0.1 ± 0.02
9e		0.50 ± 0.05	0.070 ± 0.005	0.36 ± 0.14
9f		0.013 ± 0.0002	0.001 ± 0.0005	0.09 ± 0.01
9g		0.89 ± 0.08	0.26 ± 0.02	0.24 ± 0.02
9h		0.85 ± 0.05	0.28 ± 0.04	0.26 ± 0.08
9i		NA	NA	54.79% [e]
9j		NA	NA	50.98% [e]
compound **3**		0.0014 ± 0.0001	0.012	0.001 ± 0

[a] NA inhibition was assayed by determining the drug concentration required to reduce NA activity to 50% of control NA activity (IC_{50}); [b] H3N2 (A/Sydney/5/97); [c] X-31 ca virus based on H5N1; [d] H1N1 (A/Guangdong/376/2001); [e] The inhibition rate was tested at 1 μM H1N1 (A/Guangdong/376/2001).

Table 2. Comparision of pharmacokinectic parameters for compound **9f** and compound **3** in rat

Parameter	**9f** [a]		compound **3** [j]		compound **4** [j]	
	iv dose	po dose	iv dose	po dose	iv dose	po dose
dose (mg/Kg)	2	10	10	10	10	10
C_{max} (ng/mL) [b]	8796 ± 572	116 ± 22.4	-	60 ± 0.01		30 ± 0
T_{max} (h) [c]	-	1.0 ± 0	-	1.2 ± 0.7		4.0 ± 1.6
$t_{1/2}$ (h) [d]	0.69 ± 0.05	2.96 ± 0.74	1.1 ± 0.3	1.8 ± 0.6	1.6 ± 0.4	10.6 ± 5.5
$AUC_{0\text{-}8h}$ (ng·h/mL) [e]	5380 ± 873	378 ± 45.2	-	-		
MRT (h) [f]	0.65 ± 0.04	6.29 ± 2.25	-	-		
Cl (L/h/kg) [g]	0.35 ± 0.05	-	1.9 ± 0.6		1.5 ± 0.3	
V_{ss} (L/kg) [h]	0.23 ± 0.02	-	0.8 ± 0.1	-	1.3 ± 0.6	
F_{po} (%) [i]	-	1.41 ± 0.17	-	3.7 ± 2.3		4.3 ± 1.6

[a] Data are means of four independent experiments; [b] The maximum plasma concentration (C_{max}); [c] The time to reach C_{max} (T_{max}); [d] Terminal half-life ($t_{1/2}$); [e] The area under the concentration-time curve ($AUC_{0\text{-}t}$); [f] Mean resident time (MRT); [g] Clearance (Cl); [h] Volume of distribution at steady state (V_{ss}); [i] Percentage bioavailability (F (%)); [j] *Antimicrob. Agents Chemother.* **1998**, *42(3)*: 647-653.

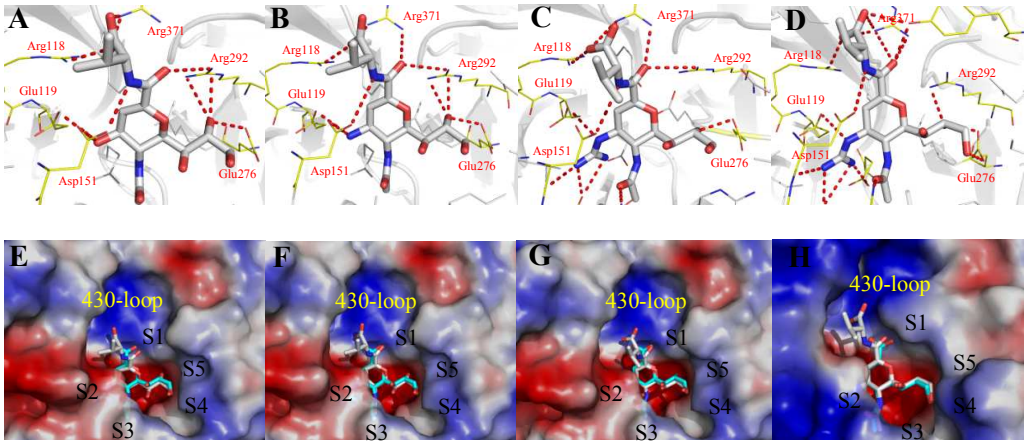


Figure 4. Detailed interactions of **7d** (A, E), **8a** (B, F) and **9c** (C, D, G and H) binding to the active sites of group-1 neuraminidase (N1: D, H; PDB 3CKZ) and group-2 neuraminidase (N2: A, B, C, E, F, G; PDB 3TIC). The neuraminidases are shown in grey (A-D), whereas the key residues in the active site are labeled and highlighted in atom-color (yellow) model. The compounds **7d**, **8a** and **9c** are shown in sticks mode with C atoms colored in white. Hydrogen-bonds are represented by red dotted lines. The pocket surface (E-H) is colored by their electrostatic properties, with red color representing negative charge, blue color for positive charge. Compound **3** (sticks mode) is colored cyan. S1-S5 represents the active site of NAs. 430-loop is also highlighted. This figure is done with PYMOL.

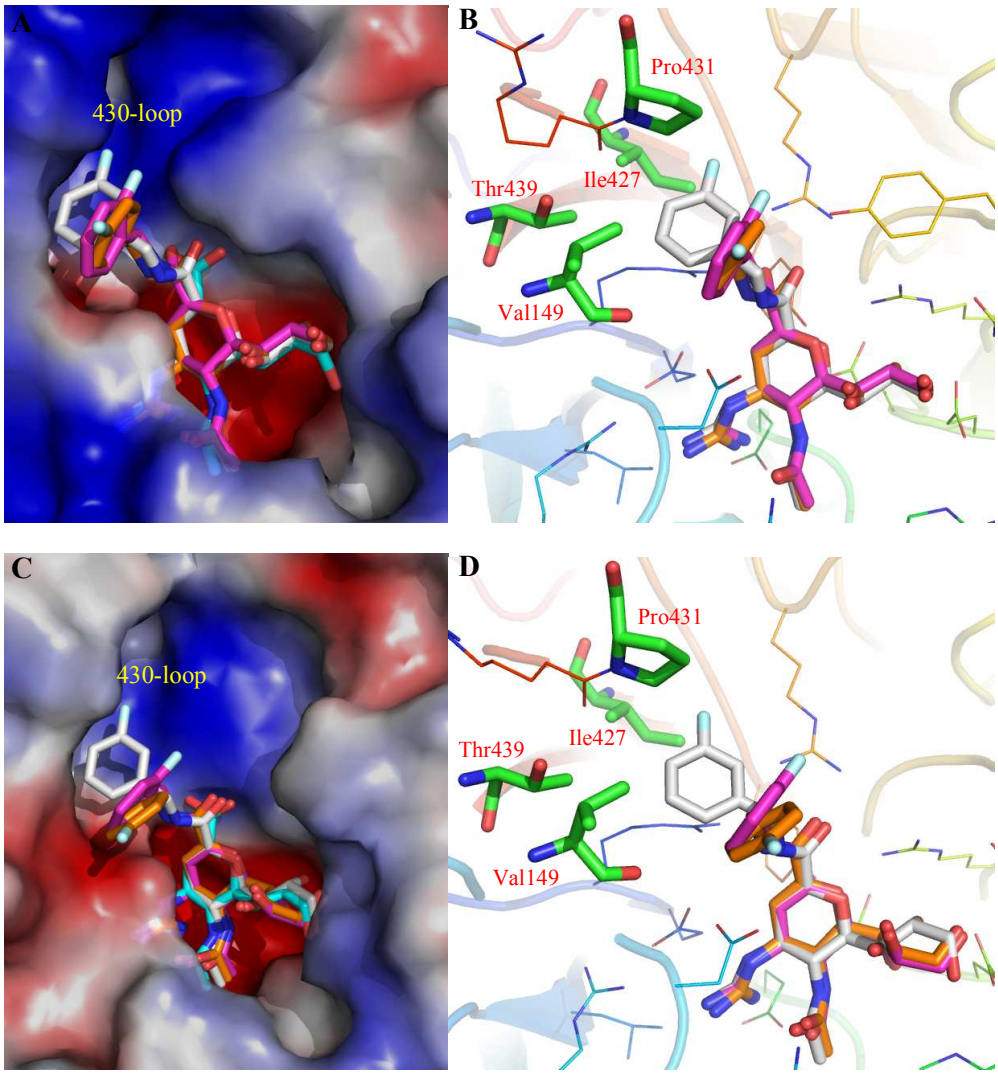


Figure 5. The binding models of compounds **9f** (white)**, 9g** (magenta) and **9i** (orange) with NAs. A and C represents the binding pocket of N1 (PDB 3CKZ) and N2 (PDB 3TIC), respectively. Compounds **9f, 9g**, **9i** and **3** (cyan) are superposed together within the active pocket in sticks mode; B (N1) and D (N2) separately illustrate the detailed interactions between these three compounds and NAs. The key residues (Val149, Ile427, Pro431 and Thr439) involved in hydrophobic interactions are also highlighted with sticks mode (green). This figure is done with PYMOL.

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