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Research paper

# Design, synthesis of oleanolic acid-saccharide conjugates using click chemistry methodology and study of their anti-influenza activity

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ABSTRACT

The development of entry inhibitors is an emerging approach to the inhibition of influenza virus. In our previous research, oleanolic acid (OA) was discovered as a mild influenza hemagglutinin (HA) inhibitor. Herein, as a further study, we report the preparation of a series of OA-saccharide conjugates via the CuAAC reaction, and the anti-influenza activity of these compounds was evaluated in vitro. Among them, compound **11b**, an OA-glucose conjugate, showed a significantly increased anti-influenza activity with an $IC_{50}$ of 5.47 μM, and no obvious cytotoxic effect on MDCK cells was observed at 100 μM. Hemagglutination inhibition assay and docking experiment indicated that **11b** might interfere with influenza virus infection by acting on HA protein. Broad-spectrum anti-influenza experiments showed **11b** to be robustly potent against 5 different strains, including influenza A and B viruses, with $IC_{50}$ values at the low-micromole level. Overall, this finding further extends the utility of OA-saccharide conjugates in anti-influenza virus drug design.



## 1. Introduction

Influenza A (IAV) and B (IBV) viruses are important causes of upper respiratory tract infections [1]. IAV are classified into different subtypes based on the two large glycoproteins, hemagglutinin (HA) and neuraminidase (NA), which are on the surface of the viral envelope [1]. HA binds to the sialic acid receptor on the target cell membrane and is involved in the initial stage of viral infection. NA hydrolyzes the sialic acid receptor on the cell surface to release the progeny influenza virus from the infected cells. In the past 100 years, influenza virus epidemics, including H1N1 in 1918, H2N2 in 1957, H3N2 in 1968, H5N1 in 2009 and H7N9 in 2013, resulted in serious impact on global morbidity, mortality, and huge economic loss [2–8]. Currently, the main measures to deal with influenza virus infection are vaccination and medical treatment. However, vaccines have certain limitations on the scope of use, and seasonal influenza vaccines are not effective enough to prevent continually mutating influenza viruses. Therefore, drug treatment is the best means of controlling the spread of influenza virus. At present, there are two main classes of anti-influenza drugs approved by FDA. The first class is NA inhibitors, including Tamiflu and Zanamivir, which can inhibit the activity of NA and thereby disable tethered progeny virus from escaping from the host cell [9,10]. The second class is M2 ion channel inhibitors, including Amantadine and Rimantadine, which can inhibit the activity of influenza virus M2 ion channel protein, thereby blocking the exfoliation process of the influenza virus [11]. However, the high mutation rate of the RNA genome of the influenza virus, combined with assortment of its multiple genomic segments, promotes antigenic diversity and new subtypes, allowing viruses to become resistant to existing antiviral drugs [12]. Therefore, it is still urgent to design and develop new anti-influenza drugs.

As the first step to prevent virus infection, entry inhibition presents a favorable strategy for drug discovery which promises an efficient blockage of virus propagation in theory [13,14]. Recently, we found that plant-derived pentacyclic triterpenes and their derivatives have displayed inhibitory activity against influenza A virus entry in vitro, and compound **Y3**, an oleanolic acid (OA)-acetyl galactose conjugate, exhibited robust anti-H1N1 entry activity with an $IC_{50}$ of 4.05 μM [1]. Further study indicated that these triterpene

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derivatives could bind tightly to the pocket of viral envelope HA protein, thus blocking the attachment of viruses to host cells. Therefore, OA became an interesting scaffold for designing novel influenza virus entry inhibitors. Previous reports also showed that the anti-influenza activity can be significantly improved by coupling 17-COOH or 3-OH of OA with oligosaccharides [1,15–17].

Nevertheless, due to the steric hindrance of the 17-COOH, it is hard to obtain OA-di- or tri-saccharide conjugates by the same synthetic strategy employed for **Y3**. So, it is still unclear whether the antiviral activity can be further enhanced by coupling 17-COOH of OA with a disaccharide or trisaccharide. In order to investigate this possibility, we present herein our effort to conjugate different saccharide moieties with 17-COOH of OA through a triazole linker rather than an amide bond via a Cu(I) azide-alkyne cycloaddition reaction (CuAAC). All the synthesized compounds were evaluated for their in vitro anti-influenza A virus (IAV) activities.

## 2. Results and discussion

### 2.1. Chemistry

Click chemistry has recently emerged as one of the most powerful tools in drug discovery. The design and synthesis of pharmacologically relevant heterocyclic molecules have proven to be a promising strategy, and the Cu(I)-catalyzed version of the Huisen 1,3-dipolar cycloaddition reaction between azides and terminal alkynes (CuAAC) for the construction of trizoles is one of the powerful reactions for making carbon–heteroatom–carbon bonds with a wide variety of chemical and biological applications. Therefore, the CuAAC reaction was chosen as the linking method to couple OA with different saccharide molecules. As outlined in Scheme 1, the 17-COOH of OA was firstly activated by TBTU and then reacted with propynylamine to construct the terminal alkyne module. Azido saccharides were synthesized via reported methods and their spectral data were consistent with the data reported previously [18–25]. To perform the CuAAC reaction, OA-propargylamine and an azido saccharide were dissolved in $CH_2Cl_2$–$H_2O$, followed by the addition of sodium L-ascorbate and $CuSO_4 \cdot 5H_2O$. The reaction proceeded smoothly at room temperature to afford the target compounds (**11a**-**20a**) in good yields. Then the *O*-acetyl groups of the saccharide were cleaved with 2 N NaOH/MeOH to give the corresponding deprotected compounds **11b**–**20b**. The $^1H$ and $^{13}C$ NMR spectra of the synthesized compounds **11a**-**20b** are shown in supplementary materials.

### 2.2. Biological assays

#### 2.2.1. In vitro anti-influenza virus activity in MDCK cells

The CellTiter-Glo assay and the cytopathic effect (CPE) reduction assay were employed to evaluate the antiviral activities of compounds (**11a**-**20b**) against the influenza virus strain A/WSN/33, which was propagated in Madin-Darby canine kidney (MDCK) cells [26]. As shown in Fig. 1A, most compounds had no significant cytotoxicity against MDCK cells at a concentration of 100 μM, except for compounds **12a**, **15b** and **16b**. Then, the compounds against the influenza A/WSN/33 virus were evaluated by the CellTiter-Glo assay (Fig. 1B). The inhibition rate was significantly decreased when OA is conjugated with a disaccharide. In addition, when OA is coupled with maltotriose (compounds **20a** and **20b**), the potency was completely abolished. These data suggested that a larger saccharide module at 17-COOH may hinder the effective binding of OA pharmacophore to the target protein, resulting in reduced antiviral activity.

Compounds **11b**, **12a**, **13a**, **14b**, **15b** and **16b** displayed significant anti-A/WSN/33 activity with inhibition rates over 75%, far better than that of OA. However, combined with the data shown in Fig. 1A, the high inhibition rate of compounds **12a**, **15b** and **16b** might reflect the cellular toxicity rather than potency enhancement. These results indicated that the number and configuration of the hydroxyl groups of OA-oligosaccharide conjugates have critical effects on cytotoxicity and anti-viral activity. Compared with DMSO-treated MDCK cells, influenza A/WSN/33 virus causes a severe CPE in MDCK-infected cells. As shown in Fig. 2A, compound **11b** significantly reduced the CPE, indicating that these compounds were able to protect MDCK cells from influenza virus-induced CPE.

Three compounds, **11b 13a**, and **14b**, identified with high inhibition rate (>75%) and low cytotoxicity (cell viability > 75%, Fig. 1), were selected for dose response assays. The results, expressed as $IC_{50}$ (compound concentration required to achieve 50% inhibition of replication of A/WSN/33), $CC_{50}$ (compound concentration required to cause 50% death of uninfected MDCK cells) and SI (selectivity index), are summarized in Table 1, respectively. All the three compounds showed much higher anti-influenza A/WSN/33 activities than OA with $IC_{50}$ at low-micromole level. Compound **11b** showed the strongest inhibitory activity in this study with an $IC_{50}$ value of 5.47 μM. In addition, the $CC_{50}$ of these compounds were all higher than 100 μM.

#### 2.2.2. Conjugate *11b* inhibits virus-induced hemagglutination

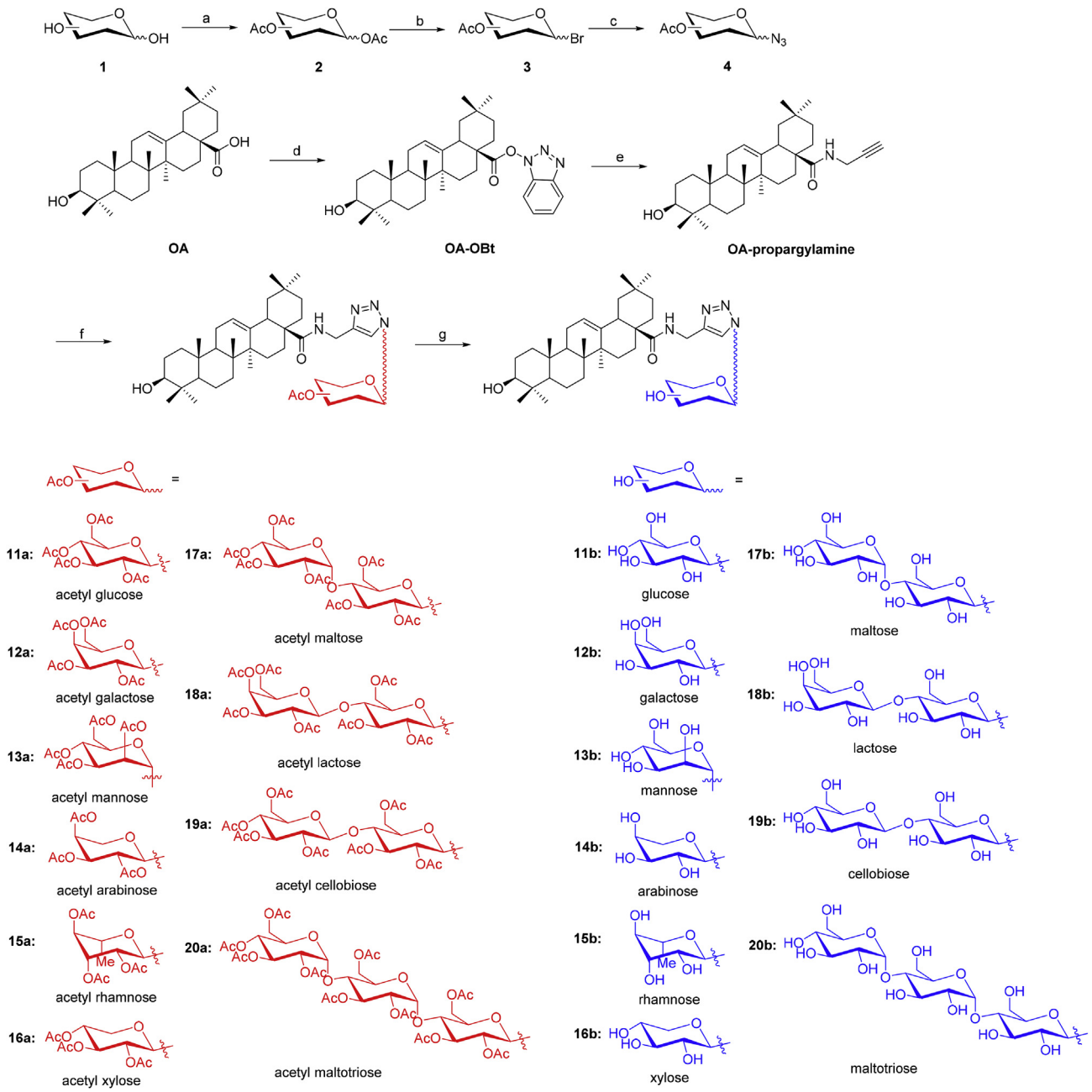
The viral envelope protein HA plays a crucial role in the early stage of influenza virus infection. HA protein can bind to sialic acid on the surface of red blood cells (RBCs), causing agglutination [27]. Antibodies of HA at a sufficient concentration will interfere with the virus attachment to red blood cells and thus inhibit hemagglutination [28,29]. Therefore, hemagglutination inhibition (HI) assay was applied to investigate whether compound **11b** targets HA protein. In this assay, A/WSN/33 virus was pretreated with compound **11b** before the addition of chicken erythrocytes, and a further incubation was conducted at room temperature for 30 min. In parallel, inhibition of hemagglutination by anti-HA antibody was used as the positive control. As shown in Fig. 2B, compound **11b** inhibited hemagglutination with a similar capability as anti-HA antibody. This result suggests that **11b** may have the same target as anti-HA antibody, HA, and thus block the interactions of viruses with target cells.

#### 2.2.3. Molecular docking analysis

We designed molecular docking calculations of compound **11b** with influenza HA protein to further understand the molecular basis of the inhibitory properties of OA-saccharide conjugates. The docked conformations of HA-**11b** were determined based on the minimum free energy analyses using AutoDock 4.2 program (The Scripps research institute, La Jolla, CA, USA).

As shown in Fig. 3, compound **11b** was docked into the binding site of the sialic acid receptor on the HA protein with a binding energy of −10.70 kcal/mol and an inhibition constant (Ki) of 14.44 nM. The corresponding binding energy and inhibition constant of OA were −6.14 kcal/mol and 31.5 nM, respectively, indicating that **11b** binds to HA protein more tightly. In addition, we found that **11b** almost occupied the full site of sialic acid receptor binding pocket on HA, which might explain why the anti-viral activity was significant reduced after coupling OA with di- or trisaccharide.

The modeled structure of HA-**11b** complex indicated that the C3–OH of OA could form a hydrogen bond with Gly225, while the 2′-OH and 3′-OH of the glucose moiety also formed four hydrogen bonds with Arg133, Lys156, and Gly158, respectively. In addition, Ser193 is involved in hydrogen bond interaction with the 28-CO of OA and 6′-OH of the glucose. Moreover, the triazole moiety further enhanced the binding affinity of **11b** to HA by interacting with




**Scheme 1.** Reagents and conditions: (a) $Ac_2O$, DMAP, pyridine, rt, overnight; (b) HBr·AcOH, $CH_2Cl_2$, 0 °C to rt, overnight; (c) $NaN_3$, DMF, rt, overnight; (d) TBTU, DIEA, THF, rt, overnight; (e) 2-propynylamine, $Na_2CO_3$, DMF, rt, 20 min; (f) $CuSO_4·5H_2O$, Na-L-ascorbate, azido-saccharide, $CH_2Cl_2/H_2O$, rt, overnight; (g) 2N NaOH, MeOH, rt, 1 h.

Val155 and Leu194 through π-alkyl stacking interaction (Fig. 3C). Therefore, occupancy of the binding pocket for sialic acid receptor might account for the molecular basis by which **11b** blocks the HA-sialic acid receptor interaction and thus the attachment of viruses to the host cells.

### 2.2.4. Conjugate *11b* exhibits broad-spectrum anti-influenza activity

Given that **11b** occupies the pocket for sialic acid receptor of HA, we inquired whether **11b** exerts broad antiviral spectrum. OSV-p is a highly effective anti-influenza virus inhibitor that inhibits multiple influenza virus subtypes, such as H1N1, H5N1, H9N2. Therefore, OSV-p was used as a positive control. As shown in Table 2, OSV-p and **11b** were evaluated against five strains of influenza A and B viruses in MDCK cells. Three of these strains, which belong to the IAV H3N2 subtype, the Yamagata lineage, and the Victoria lineage of IVB, showed strong OSV-p resistance. Interestingly, compound **11b** exhibited strong inhibitory activity against OSV-p resistant strains A/Victoria/361/2011 (H3N2), and the influenza B virus B/Sichuan/531/2018 (BV) and B/Massachusetts/2/2012 (BV) (with $IC_{50}$ values of 10.42, 26.12 and 11.51 μM, respectively). In addition, **11b** exerted remarkable activity against B/Yamagata/16/88 (BY), which was 4-fold more active than OSV-p ($IC_{50} = 5.54$ μM).

## 3. Conclusion

In summary, we have designed and synthesized a series of OA-saccharide conjugates by using a facile and efficient CuAAC process

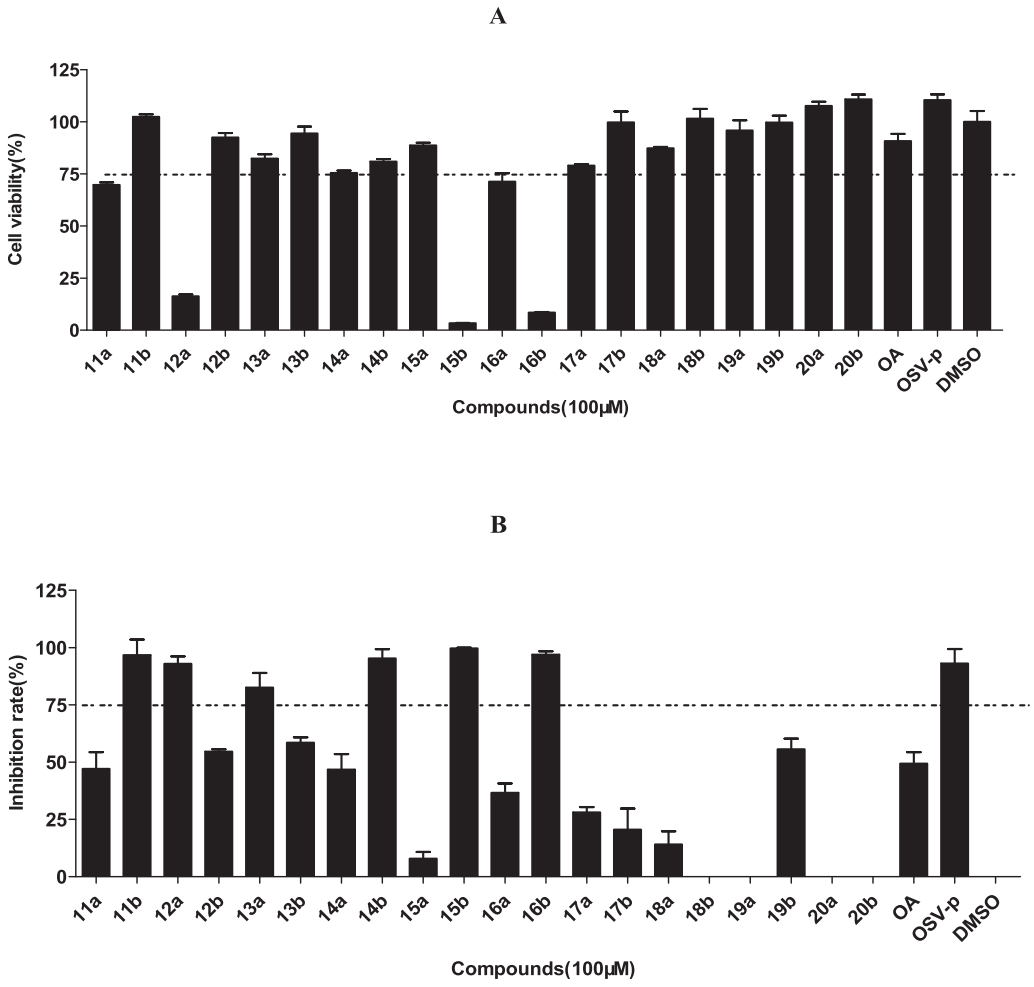



**Fig. 1.** Inhibitory effects of OA-saccharide conjugates against influenza A/WSN/33 (H1N1) virus. (A) The cytotoxic effect-based screen of OA-saccharide conjugates (100 μM) using CellTiter-Glo® assay. (B) The cytopathic effect-based screen of OA-saccharide conjugates (100 μM). MDCK was utilized as the host cell to test A/WSN/33 virus infection; 1% DMSO (final concentration) was used as the negative control, and Oseltamivir phosphate (OSV-p) was used as the positive control. Error bars indicate standard deviations of triplicate experiments.

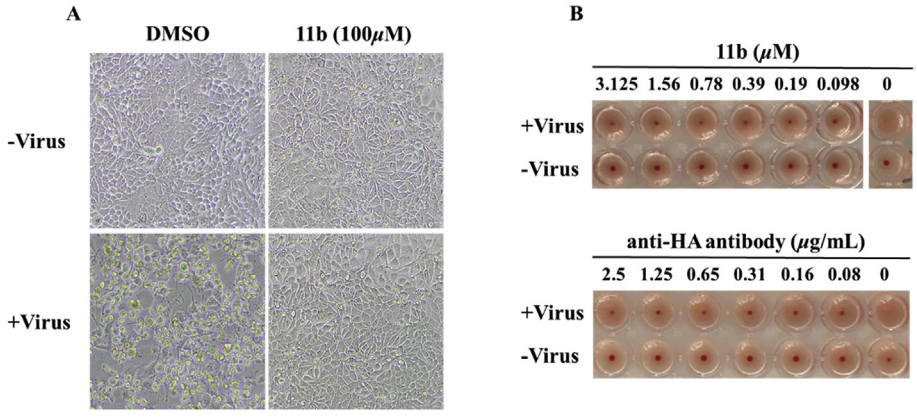



**Fig. 2.** (A) Validation of the protection of MDCK cells from influenza A/WSN/33 virus by compound **11b**. (B) Comparisons of **11b** and anti-HA antibody for inhibiting influenza virus-induced aggregation of chicken erythrocytes. Compound **11b** exerted a similar capability to inhibit hemagglutination to that of anti-HA antibody.

which allows OA to be easily combined with various carbohydrate moieties. The anti-influenza activity of these compounds was evaluated in vitro, where some of them showed desirable potency. Among them, compound **11b** exerted the best inhibitory activity against influenza A/WSN/33 virus, with an $IC_{50}$ value of 5.47 μM. Moreover, **11b** exerted broad spectrum activity even against OSV-p resistant viruses. HI assay and docking simulation indicate that the anti-viral activity of **11b** is potentially due to its high affinity to HA protein, thus blocking the attachment of influenza viruses to host cells, confirming the initial design idea.

**Table 1**
Inhibitory activities of compound **11b**, **13a**, and **14b** against infection of MDCK cells by influenza A/WSN/33 virus.

| Compounds | $CC_{50}$ ($\mu$M)[a] | $IC_{50}$ ($\mu$M)[b] | SI[c] |
|---|---|---|---|
| OSV-p[d] | >100 | 1.82 | >54.94 |
| OA | >100 | 72.2 | >1.38 |
| **11b** | >100 | 5.47 | >18.28 |
| **13a** | >100 | 7.22 | >13.85 |
| **14b** | >100 | 12.93 | >7.73 |

[a] 50% cytotoxicity concentration.
[b] Concentration inhibiting viral replication by 50%. The values are means of at least three independent determinations.
[c] SI: selectivity index as $CC_{50}/IC_{50}$.
[d] Positive control.

## 4. Experimental

### 4.1. Chemistry-general

High Resolution Mass Spectra (HRMS) were obtained with an APEX IV FT_MS (7.0 T) spectrometer (Bruker) in positive ESI mode. NMR spectra were recorded on a Bruker DRX 400 spectrometer at ambient temperature. $^1$H NMR chemical shifts were reported relative to the internal standard TMS ($\delta_H = 0.00$) or the solvent signal ($\delta_H = 7.26$ for the central line of $CDCl_3$, $\delta_H = 3.31$ for the central line of $CD_3OD$). $^{13}$C NMR chemical shifts were referenced to the solvent signal ($\delta_C = 77.16$ for the central line of $CDCl_3$, $\delta_C = 49.00$ for the central line of $CD_3OD$). Reactions were monitored by thin−layer chromatography (TLC) on a precoated silica gel 60 $F_{254}$ plate (layer thickness 0.2 mm; E. Merck, Darmstadt, Germany) and detected by staining with a yellow solution containing $Ce(NH_4)_2(NO_3)_6$ (0.5 g) and $(NH_4)_6Mo_7O_{24}4H_2O$ (24.0 g) in 6%

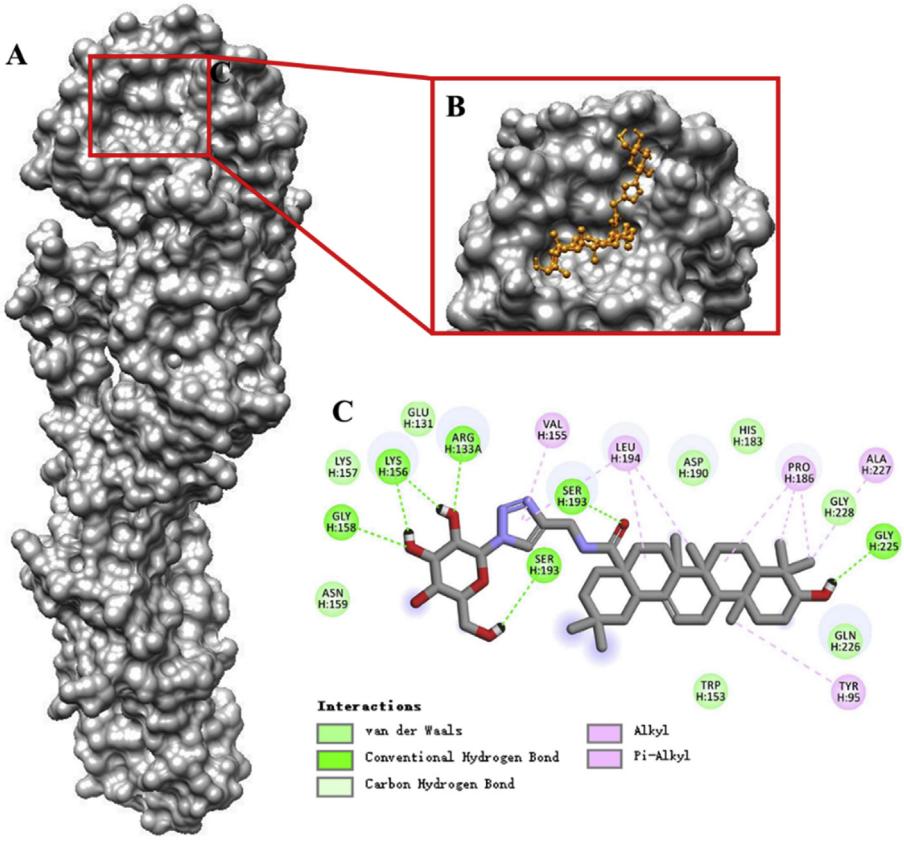



**Fig. 3.** Structural representative of **11b** binding within HA protein (Protein Data Bank: 1RVT) according to blind docking calculation. (A) Overview of HA protein. The inhibitor pocket is highlighted in red square. (B) Closer view of the inhibitor pocket. Protein and compound **11b** are shown as gray surface and orange sticks, respectively. (C) 2D interaction plot with green dashes and pink dashes indicating hydrogen bonds and hydrophobic interactions correspondingly. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

**Table 2**
Comparisons of the anti-influenza virus activity of compound **11b** with that of OSV-p.

| Compounds | $CC_{50}$($\mu$M)[a] | $IC_{50}$($\mu$M)[b] | | | | |
|---|---|---|---|---|---|---|
| | | A/WSN/33 | A/Victoria/361/2011(H3N2) | B/Yamagata/16/88(BY) | B/Sichuan/531/2018(BV) | B/Massachusetts/2/2012(BV) |
| OSV-p | >100 | 1.82 | >100 | 23.45 | >100 | >100 |
| **11b** | >100 | 5.47 | 10.42 | 5.54 | 26.12 | 11.51 |

[a] $CC_{50}$, the concentration required to reduce the viability of normal, non-infected MDCK cells by 50%. Values represent the mean of duplicate samples from three independent experiments.
[b] $IC_{50}$, the concentration required to inhibit viral infection-induced CPE by 50%. Values represent the mean of duplicate samples from three independent experiments.

$H_2SO_4$ (500 mL) followed by heating. Flash column chromatography was performed on silica gel 60 (200 – 300 mesh, Qingdao Haiyang Chemical Co. Ltd).

### 4.2. Synthesis

#### 4.2.1. General procedure a for the CuAAC "click" reaction (**11a-20a**)

To a solution of alkyne (0.16 mmol) and azide (0.19 mmol) in $CH_2Cl_2$ (3 mL) and $H_2O$ (3 mL) was added $CuSO_4 \cdot 5H_2O$ (48 mg, 0.19 mmol) and Na-L-ascorbate (75 mg, 0.38 mmol). The resulting solution was stirred vigorously for 12 h at rt. The reaction mixture was diluted with $H_2O$ (10 mL), then extracted with $CH_2Cl_2$ (10 mL × 3). The combined organic layer was dried over $Na_2SO_4$, filtered, and concentrated. The residue was purified by column chromatography.

#### 4.2.2. General procedure B for the O-Deacetylation of OA-Saccharide conjugates (**11b**–**20b**)

To the O-pentacetylated compound stirred in methanol was added 2N NaOH (cat.). The mixture was stirred at rt. After completion (TLC), the reaction mixture was neutralized with HCl (1 M). Water was added, and the resulting suspension was filtered. The crude product was purified by column chromatography.

#### 4.2.3. Compound OA-OBt

To OA (912 mg, 2 mmol) and TBTU (963 mg, 3 mmol) stirred in 20 mL THF was added DIEA (0.5 mL, 3 mmol). The mixture was stirred at room temperature overnight. After completion (TLC), the reaction mixture was filtered, and the filtrate was concentrated. The crude filtrate was purified by column chromatography (petroleum ether/AcOEt, 4/1 v/v) to give OA-OBt as a white solid (1.01 g, 86%). Mp 184.4–185.6 °C. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 0.79, 0.85, 0.91, 0.97, 1.00, 1.01, 1.22 (7 × $CH_3$), 0.79–2.30 (m, other aliphatic ring protons), 2.98 (dd, $J$ = 4.08, 13.68 Hz, 1H), 3.23 (d, $J$ = 7.92 Hz, 1H), 5.38 (t, $J$ = 3.48 Hz, 1H), 7.36–7.42 (m, 2H), 7.53 (t, $J$ = 7.86 Hz, 1H), 8.05 (d, $J$ = 8.4 Hz, 1H); $^{13}$C NMR (150 MHz, $CDCl_3$): $\delta$ 15.38, 15.59, 17.27, 18.30, 23.10, 23.45, 23.52, 25.73, 27.14, 28.05, 28.11, 30.65, 32.41, 32.82, 32.93, 33.66, 37.00, 38.48, 38.75, 39.44, 41.57, 41.89, 45.45, 47.54, 47.61, 55.21, 78.98, 108.15, 120.50, 123.90, 124.64, 128.49, 128.74, 142.08, 143.54, 173.66. ESI-HRMS ($m/z$) $[M+H]^+$ calcd for $C_{36}H_{52}N_3O_3$, 574.3964; found, 574.4016.

#### 4.2.4. Compound OA-propargylamine

To OA-OBt (589 mg, 1 mmol) and $Na_2CO_3$ (106 mg, 1 mmol) stirred in 8 mL DMF was added 2-propynylamine (0.1 mL, 1.5 mmol). The mixture was stirred at room temperature for 20 min. After completion (TLC), the solvent was removed under reduced pressure. The mixture was resolved in AcOEt and washed with water and brine twice. The organic layer was dried over $MgSO_4$, then filtered and concentrated. The crude residue was purified by column chromatography (petroleum ether/AcOEt, 2/1 v/v) to give OA-propargylamine as a white solid (473 mg, 93%). Mp 201.3–202.7 °C. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 0.78, 0.79, 0.91, 0.92, 0.99, 1.17 (7 × CH3), 0.73–2.20 (m, other aliphatic ring protons), 2.21 (t, $J$ = 2.52 Hz, 1H), 2.53 (d, $J$ = 9.48 Hz, 1H), 3.22 (dd, $J$ = 4.26, 11.34 Hz, 1H), 3.88–3.92 (m, 1H), 4.04–4.08 (m, 1H), 5.42 (t, $J$ = 3.36 Hz, 1H), 6.11 (t, $J$ = 4.62 Hz, 1H). $^{13}$C NMR (150 MHz, $CDCl_3$): $\delta$ 15.51, 15.69, 17.06, 18.39, 23.65, 23.70, 24.01, 25.87, 27.26, 27.35, 28.20, 29.47, 30.85, 32.32, 32.40, 33.11, 34.17, 37.05, 38.60, 38.88, 39.49, 42.13, 42.24, 46.42, 46.76, 47.63, 55.18, 71.74, 79.05, 79.77, 123.31, 144.87, 178.22.

#### 4.2.5. Compound **11a**

Prepared from OA-propargylamine (78.88 mg, 0.16 mmol), acetyl-glucosyl azide (71 mg, 0.19 mmol) according to general procedure A. The residue was purified by column chromatography (petroleum ether/AcOEt, 1/2 v/v) to give **11a** as a white solid (117 mg, 83%). Mp 129.8–131.7 °C. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 0.67, 0.78, 0.88, 0.89, 0.90, 0.99, 1.16 (7 × $CH_3$), 0.67–1.80 (m, other aliphatic ring protons), 1.88 (s, 3H), 2.04 (s, 3H), 2.07 (s, 3H), 2.10 (s, 3H), 2.55 (d, $J$ = 4.44 Hz, 1H), 3.21 (dd, $J$ = 4.08, 11.22 Hz, 1H), 3.98–4.01 (m, 1H), 4.15 (dd, $J$ = 2.04, 12.66 Hz, 1H), 4.24–4.32 (m, 2H), 4.62(dd, $J$ = 5.64, 15.06 Hz, 1H), 5.25 (t, $J$ = 9.9 Hz, 1H), 5.4–5.46 (m, 3H), 5.83 (d, $J$ = 8.82 Hz, 1H), 6.60 (brs, 1H), 7.78 (s, 1H). $^{13}$C NMR (150 MHz, $CDCl_3$): $\delta$ 15.49, 15.67, 16.73, 18.38, 20.29, 20.65 (2C), 20.81, 20.87, 23.59, 23.71, 24.00, 25.90, 27.23, 27.35, 28.18, 30.81, 32.39, 32.41, 33.09, 34.18, 35.06, 37.03, 38.56, 38.85, 39.45, 42.05, 42.08, 46.31, 46.74, 47.64, 55.17, 61.59, 62.09, 67.68, 70.43, 72.77, 75.22, 79.03, 85.85, 121.23, 123.35, 144.51, 168.82, 169.42, 170.11, 170.63, 178.57. ESI-HRMS ($m/z$) $[M+Na]^+$ calcd for $C_{47}H_{70}N_4O_{11}Na$, 889.4933; found, 889.4939.

#### 4.2.6. Compound **11b**

Prepared from **11a** (78 mg, 0.09 mmol) according to general procedure B. The crude product was washed with water three times without further purification to afford **11b** as a white solid (61 mg, 95%). Mp 190.0–191.8 °C. $^1$H NMR (600 MHz, $CD_3OD$): $\delta$ 0.63, 0.77, 0.90, 0.93, 0.94, 0.96, 1.16 (7 × $CH_3$), 0.63–2.10 (m, other aliphatic ring protons), 2.79 (dd, $J$ = 3.72, 13.38 Hz, 1H), 3.14 (dd, $J$ = 4.62, 11.46 Hz, 1H), 3.47–3.58 (m, 3H), 3.71 (dd, $J$ = 5.52, 12.3 Hz, 1H), 3.83–3.88 (m, 2H), 4.34 (d, $J$ = 15.12 Hz, 1H), 4.44 (d, $J$ = 15.12 Hz, 1H), 5.35 (t, $J$ = 3.3 Hz, 1H), 5.57 (d, $J$ = 9.18 Hz, 1H), 7.99 (s, 1H). $^{13}$C NMR (150 MHz, $CD_3OD$): $\delta$ 16.13, 16.49, 17.79, 19.63, 24.03, 24.18 (2C), 24.21, 24.69, 26.62, 27.99, 28.63, 28.89, 31.76, 33.69, 33.90, 34.26, 35.19, 35.98, 38.26, 39.98, 40.76, 42.68, 43.01, 47.66, 47.77, 56.84, 62.55, 71.04, 74.13, 78.59, 79.83, 81.27, 89.70, 123.91, 124.37, 145.24, 146.27, 180.66. ESI-HRMS ($m/z$) $[M+Na]^+$ calcd for $C_{39}H_{62}N_4O_7Na$, 721.4511; found, 721.4515.

#### 4.2.7. Compound **12a**

Prepared from OA-propargylamine (93 mg, 0.19 mmol), acetyl-galactosyl azide (82 mg, 0.22 mmol) according to general procedure A. The residue was purified by column chromatography (petroleum ether/AcOEt, 1/2 v/v) to give **12a** as a white solid (160 mg, 98%). Mp 145.2–146.8 °C. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 0.68, 0.78, 0.88, 0.89, 0.90, 0.99, 1.16 (7 × $CH_3$), 0.68–1.80 (m, other aliphatic ring protons), 1.88 (s, 3H), 2.01 (s, 3H), 2.05 (s, 3H), 2.24 (s, 3H), 2.56 (dd, $J$ = 3.54, 13.62 Hz, 1H), 3.21 (dd, $J$ = 3.84, 11.16 Hz, 1H), 4.11–4.15 (m, 1H), 4.19–4.25 (m, 3H), 4.64 (dd, $J$ = 6.12, 15.06 Hz, 1H), 5.24 (dd, $J$ = 3.36, 10.26 Hz, 1H), 5.41 (t, $J$ = 3.36 Hz, 1H), 5.54–5.57 (m, 2H), 5.80 (d, $J$ = 9.24 Hz, 1H), 6.60 (t, $J$ = 5.34 Hz, 1H), 7.84 (s, 1H). $^{13}$C NMR (150 MHz, $CDCl_3$): $\delta$ 15.47, 15.67, 16.66, 18.38, 20.36, 20.62, 20.75, 20.80, 23.58, 23.69, 24.04, 25.90, 27.24, 27.34, 28.17, 30.80, 32.37, 32.39, 33.09, 34.18, 34.99, 37.03, 38.55, 38.84, 39.43, 42.03 (2C), 46.26, 46.74, 47.64, 55.16, 61.22, 66.86, 67.95, 70.93, 74.06, 78.99, 86.38, 121.32, 123.29, 144.56, 145.36, 168.91, 169.98, 170.16, 170.39, 178.48. ESI-HRMS ($m/z$) $[M+Na]^+$ calcd for $C_{47}H_{70}N_4O_{11}Na$, 889.4933; found, 889.4935.

#### 4.2.8. Compound **12b**

Prepared from **12a** (70 mg, 0.08 mmol) according to general procedure B. The crude product was washed with water three times without further purification to afford **12b** as a white solid (53 mg, 93%). Mp 189.6–192.0 °C. $^1$H NMR (600 MHz, $CD_3OD$) $\delta$: 0.62, 0.77, 0.90, 0.92, 0.94, 0.96, 1.16 (7 × $CH_3$), 0.62–2.13 (m, other aliphatic ring protons), 2.78 (dd, $J$ = 3.9, 13.38 Hz, 1H), 3.13 (dd, $J$ = 4.56, 11.4 Hz, 1H), 3.68 (dd, $J$ = 3.3, 9.54 Hz, 1H), 3.74 (d, $J$ = 6.9 Hz, 1H), 3.81 (dd, $J$ = 6, 6.72 Hz, 1H), 3.98 (d, $J$ = 2.7 Hz, 1H), 4.13 (t, $J$ = 9.36 Hz, 1H), 4.34 (d, $J$ = 15.12 Hz, 1H), 4.44 (d, $J$ = 15.18 Hz, 1H), 5.35 (t, $J$ = 1.62 Hz, 1H), 5.52 (d, $J$ = 9.18 Hz, 1H), 8.02 (m, 1H). $^{13}$C

NMR (150 MHz, $CD_3OD$) $\delta$: 16.11, 16.49, 17.71, 19.63, 24.20, 24.21, 24.70, 26.63, 28.00, 28.63, 28.88, 31.76 (2C), 33.70, 33.89, 34.24, 35.19, 36.04, 38.26, 39.98 (2C), 40.76, 42.70, 43.00, 47.66, 47.77, 56.85, 62.38, 70.34, 71.47, 75.48, 79.84, 80.01, 90.35, 123.36, 124.39, 145.25, 146.40, 180.62. ESI-HRMS (*m/z*) $[M+Na]^+$ calcd for $C_{39}H_{62}N_4O_7Na$, 721.4511; found, 721.4511.

#### 4.2.9. Compound **13a**

Prepared from OA-propargylamine (80 mg, 0.16 mmol), acetylmannosyl azide (71 mg, 0.19 mmol) according to general procedure A. The residue was purified by column chromatography (petroleum ether/AcOEt, 1/2 v/v) to give **13a** as a white solid (112 mg, 80%). Mp 139.5–141.3 °C. $^1H$ NMR (600 MHz, $CDCl_3$): $\delta$ 0.45, 0.78, 0.87, 0.88, 0.89, 0.98, 1.14 (7 × $CH_3$), 1.99 (s, 3H), 2.09 (s, 3H), 2.11 (s, 3H), 2.17 (s, 3H), 0.45–2.40 (m, other aliphatic ring protons), 2.55 (d, $J$ = 9.48 Hz, 1H), 3.21 (dd, $J$ = 1.86, 7.32 Hz, 1H), 3.94–3.97 (m, 1H), 4.20 (d, $J$ = 12.54 Hz, 1H), 4.29 (dd, $J$ = 5.4, 12.54 Hz, 1H), 4.36–4.46 (m, 2H), 5.28 (dd, $J$ = 2.94, 10.08 Hz, 1H), 5.35–5.40 (m, 2H), 5.56 (s, 1H), 6.17 (s, 1H), 6.68 (t, $J$ = 4.86 Hz, 1H), 7.85 (s, 1H). $^{13}C$ NMR (150 MHz, $CDCl_3$): $\delta$ 15.42, 15.62, 16.45, 18.25, 20.56, 20.70, 20.73, 20.80, 23.60, 23.96, 25.75, 27.06, 27.19, 27.27, 28.14, 30.74, 32.23, 32.44, 33.03, 34.13, 34.95, 36.95, 38.54, 38.80, 39.30, 42.00, 42.02, 46.27, 46.69, 47.54, 55.16, 62.14, 64.90, 68.83, 70.97, 75.63, 78.98, 84.67, 122.13, 123.35, 144.34, 144.83, 169.31, 169.62, 169.91, 170.64, 178.69. ESI-HRMS (*m/z*) $[M+Na]^+$ calcd for $C_{47}H_{70}N_4O_{11}Na$, 889.4933; found, 889.4938.

#### 4.2.10. Compound **13b**

Prepared from **13a** (70 mg, 0.08 mmol) according to general procedure B. The crude product was washed with water three times without further purification to afford **13b** as a white solid (53 mg, 93%). Mp 179.7–181.2 °C. $^1H$ NMR (600 MHz, $CD_3OD$): $\delta$ 0.56, 0.77, 0.90, 0.92, 0.94, 0.96, 1.15 (7 × CH3), 0.56–2.12 (m, other aliphatic ring protons), 2.78 (dd, $J$ = 3.54, 13.2 Hz, 1H), 3.13 (dd, $J$ = 4.5, 11.34 Hz, 1H), 3.51–3.53 (m, 1H), 3.73–3.79 (m, 3H), 3.91 (d, $J$ = 2.16 Hz, 1H), 3.93 (d, $J$ = 2.22 Hz, 1H), 4.10 (s, 1H), 4.34–4.46 (m, 2H), 5.34 (t, $J$ = 3.06 Hz, 1H), 5.9 (s, 1H), 8.16–8.17 (m, 1H). $^{13}C$ NMR (150 MHz, $CD_3OD$): $\delta$ 16.12, 16.48, 17.62, 19.62, 24.18 (2C), 24.68, 26.62, 27.99, 28.61, 28.88, 31.76 (2C), 33.70, 33.90, 34.29, 35.18, 35.77, 38.24, 38.27, 39.95, 39.98, 40.73, 42.70, 42.99, 47.63, 47.73, 56.83, 62.77, 67.88, 72.42, 75.08, 79.82, 81.69, 88.66, 124.38, 124.93, 145.17, 180.61. ESI-HRMS (*m/z*) $[M+Na]^+$ calcd for $C_{39}H_{62}N_4O_7Na$, 721.4511; found, 721.4511.

#### 4.2.11. Compound **14a**

Prepared from OA-propargylamine (79 mg, 0.15 mmol), acetylarabinosyl azide (60 mg, 0.2 mmol) according to general procedure A. The residue was purified by column chromatography (petroleum ether/AcOEt, 1/2 v/v) to give **14a** as a white solid (111 mg, 92%). Mp 152.1–153.9 °C. $^1H$ NMR (600 MHz, $CDCl_3$): $\delta$ 0.63, 0.77, 0.87, 0.88, 0.89, 0.98, 1.15 (7 × $CH_3$), 0.68–1.80 (m, other aliphatic ring protons), 1.89 (s, 3H), 2.04 (s, 3H), 2.23 (s, 3H), 2.58 (dd, $J$ = 3.54, 13.08 Hz, 1H), 3.21 (dd, $J$ = 4.2, 11.22 Hz, 1H), 3.94 (d, $J$ = 13.32 Hz, 1H), 4.16–4.24 (m, 1H), 4.66 (dd, $J$ = 6.18, 15.06 Hz, 1H), 5.24 (dd, $J$ = 3.42, 10.08 Hz, 1H), 5.39 (t, $J$ = 3.3 Hz, 1H), 5.43 (s, 1H), 5.58 (t, $J$ = 9.9 Hz, 1H), 5.71 (d, $J$ = 9.12 Hz, 1H), 6.60 (t, $J$ = 5.34 Hz, 1H), 7.85 (s, 1H) $^{13}C$ NMR (150 MHz, $CDCl_3$): $\delta$ 15.41, 15.68, 16.66, 18.37, 20.37, 20.70, 21.08, 23.54, 23.69, 23.94, 25.90, 27.22, 27.33, 28.17, 30.80, 32.38, 32.52, 33.10, 34.17, 34.92, 37.03, 38.53, 38.84, 39.40, 41.94, 41.98, 46.23, 46.67, 47.63, 55.16, 67.29, 67.76, 68.26, 70.63, 79.01, 86.82, 121.34, 123.21, 144.48, 145.25, 169.00, 170.05, 170.35, 178.40. ESI-HRMS (*m/z*) $[M+Na]^+$ calcd for $C_{44}H_{66}N_4O_9Na$, 817.4722; found, 817.4720.

#### 4.2.12. Compound **14b**

Prepared from **14a** (77 mg, 0.9 mmol) according to general procedure B. The crude product was washed with water three times without further purification to afford **14b** as a white solid (64 mg, 99%). Mp 182.0–183.8 °C. $^1H$ NMR (600 MHz, $CD_3OD$): $\delta$ 0.54, 0.77, 0.90, 0.91, 0.94, 0.95, 1.14 (7 × CH3), 0.54–2.11 (m, other aliphatic ring protons), 2.79 (dd, $J$ = 3.84, 13.26 Hz, 1H), 3.13 (dd, $J$ = 4.38, 11.22 Hz, 1H), 3.70 (dd, $J$ = 3.36, 9.42 Hz, 1H), 3.85 (dd, $J$ = 0.84, 12.6 Hz, 1H), 3.93–3.94 (m, 1H), 4.00 (dd, $J$ = 1.92, 12.66 Hz, 1H), 4.12 (t, $J$ = 9.24 Hz, 1H), 4.36 (d, $J$ = 15.12 Hz, 1H), 4.44 (d, $J$ = 15.06 Hz, 1H), 5.33 (t, $J$ = 3.42 Hz, 1H), 5.45 (d, $J$ = 9.12 Hz, 1H), 8.02 (s, 1H). $^{13}C$ NMR (150 MHz, $CD_3OD$): $\delta$ 16.07, 16.48, 17.71, 19.61, 24.14, 24.20, 24.66, 26.62, 28.00, 28.62, 28.88, 31.77 (2C), 33.71, 33.90, 34.31, 35.19, 35.88, 38.27, 39.96, 39.98, 40.72, 42.69, 42.97, 47.63, 47.73, 56.84, 70.36, 70.93, 71.55, 74.99, 79.84, 90.74, 123.45, 124.35, 145.22, 146.32, 180.55. ESI-HRMS (*m/z*) $[M+Na]^+$ calcd for $C_{38}H_{60}N_4O_6Na$, 691.4405; found, 691.4405.

#### 4.2.13. Compound **15a**

Prepared from OA-propargylamine (77 mg, 0.15 mmol), acetylrhamnosyl azide (60 mg, 0.19 mmol) according to general procedure A. The residue was purified by column chromatography (petroleum ether/AcOEt, 1/2 v/v) to give **15a** as a white solid (109 mg, 89%). Mp 158.4–160.1 °C. $^1H$ NMR (600 MHz, $CDCl_3$): $\delta$ 0.52, 0.77, 0.86, 0.89, 0.98, 1.14 (7 × $CH_3$), 1.99, 2.10, 2.13 (3 × $CH_3CO$), 0.52–1.90 (m, other aliphatic ring protons), 2.49 (d, $J$ = 9.42 Hz, 1H), 3.19–3.23 (m, 1H), 3.78–3.82 (m, 1H), 4.34 (dd, $J$ = 5.64, 14.94 Hz, 1H), 4.48 (dd, $J$ = 5.28, 15.00 Hz, 1H), 5.16–5.22 (m, 2H), 5.38 (t, $J$ = 3.36 Hz, 1H), 5.65 (dd, $J$ = 1.26, 2.76 Hz, 1H), 6.08 (d, $J$ = 1.20 Hz, 1H), 6.58 (t, $J$ = 5.40 Hz, 1H), 7.78 (s, 1H). $^{13}C$ NMR (150 MHz, $CDCl_3$): $\delta$ 15.45, 15.69, 16.60, 17.64, 18.37, 20.67, 20.77, 20.89, 23.55, 23.71, 23.94, 25.87 (2C), 27.27, 27.34, 28.19, 30.82, 32.42, 32.43, 33.09, 34.16, 34.99, 37.04, 38.55, 38.86, 39.41, 42.05, 42.22, 46.34, 46.76, 47.62, 55.17, 69.23, 69.68, 70.97, 74.13, 79.04, 84.83, 121.87, 123.42, 144.42, 169.45, 169.89, 170.06, 178.59. ESI-HRMS (*m/z*) $[M+Na]^+$ calcd for $C_{45}H_{68}N_4O_9Na$, 831.4879; found, 831.4873.

#### 4.2.14. Compound **15b**

Prepared from **15a** (70 mg, 0.08 mmol) according to general procedure B. The crude product was washed with water three times without further purification to afford **15b** as a white solid (56 mg, 95%). Mp 185.2–186.9 °C. $^1H$ NMR (600 MHz, $CD_3OD$): $\delta$ 0.49, 0.76, 0.90, 0.94, 0.96, 0.95, 1.14 (7 × $CH_3$), 0.49–2.11 (m, other aliphatic ring protons), 1.37 (d, $J$ = 5.94 Hz, 3H), 2.77 (dd, $J$ = 3.54, 13.14 Hz, 1H), 3.13 (dd, $J$ = 4.26, 11.1 Hz, 1H), 3.47–3.56 (m, 2H), 3.67 (dd, $J$ = 3.18, 9.24 Hz, 1H), 4.06 (d, $J$ = 3.06 Hz, 1H), 4.37 (d, $J$ = 11.34 Hz, 1H), 4.44 (d, $J$ = 15.06 Hz, 1H), 5.33 (s, 1H), 5.95 (s, 1H), 8.09 (s, 1H). $^{13}C$ NMR (150 MHz, $CD_3OD$): $\delta$ 16.11, 16.48, 17.68, 18.32, 19.65, 24.16 (2C), 24.20, 24.64, 26.56, 27.98, 28.61, 28.87, 31.76 (2C), 33.68, 33.91, 34.29, 35.19, 35.79, 38.25, 39.94, 39.96, 40.71, 42.73, 43.00, 47.70, 47.75, 56.80, 72.45, 73.23, 74.85, 77.11, 79.79, 88.57, 124.37, 124.74, 145.13, 180.61. ESI-HRMS (*m/z*) $[M+Na]^+$ calcd for $C_{39}H_{62}N_4O_6Na$, 705.4562; found, 705.4566.

#### 4.2.15. Compound **16a**

Prepared from OA-propargylamine (89 mg, 0.17 mmol), acetylxylosyl azide (57 mg, 0.19 mmol) according to general procedure A. The residue was purified by column chromatography (petroleum ether/AcOEt, 1/2 v/v) to give **16a** as a white solid (106 mg, 78%). Mp 142.2–143.3 °C. $^1H$ NMR (600 MHz, $CDCl_3$): $\delta$ 0.55, 0.71, 0.81, 0.82, 0.91, 1.08 (7 × $CH_3$), 0.55–1.80 (m, other aliphatic ring protons), 1.81 (s, 3H), 1.99 (s, 3H), 2.01 (s, 3H), 2.49 (d, $J$ = 8.88 Hz, 1H), 3.14 (dd, $J$ = 4.14, 11.22 Hz, 1H), 3.51 (t, $J$ = 10.56 Hz, 1H), 4.17–4.23 (m, 2H), 4.55 (dd, $J$ = 5.94, 15.18 Hz, 1H), 5.05–5.09 (m, 1H), 5.32–5.35 (m, 3H), 5.67 (d, $J$ = 8.76 Hz, 1H), 6.49 (t, $J$ = 5.28 Hz, 1H), 7.21 (s, 1H),

7.68 (s, 1H). $^{13}$C NMR (150 MHz, $CD_3OD$): δ 14.31, 14.55, 15.58, 17.24, 19.20, 19.61, 19.64, 22.43, 22.57, 22.80, 24.76, 26.11, 26.21, 27.05, 29.69, 31.26, 31.38, 31.98, 33.03, 33.90, 35.91, 37.41, 37.72, 38.29, 40.88, 40.90, 45.15, 45.57, 46.50, 54.04, 64.44, 67.34, 69.38, 71.01, 77.91, 85.26, 120.15, 122.15, 143.36, 144.26, 167.80, 168.71, 168.97, 177.35. ESI-HRMS (*m/z*) $[M+Na]^+$ calcd for $C_{44}H_{66}N_4O_9Na$, 817.4722; found, 817.4724.

*4.2.16. Compound* ***16b***

Prepared from **16a** (55 mg, 0.07 mmol) according to general procedure B. The crude product was washed with water three times without further purification to afford **16b** as a white solid (45 mg, 97%). Mp 185.3–187.0 °C. $^1$H NMR (600 MHz, $CD_3OD$): δ 0.40, 0.67, 0.81, 0.84, 0.86, 1.04 (7 × CH3), 0.40–2.00 (m, other aliphatic ring protons), 2.70 (dd, $J$ = 4.68, 13.74 Hz, 1H), 3.03 (dd, $J$ = 4.38, 11.28 Hz, 1H), 3.35–3.41 (m, 2H), 3.54–3.58 (m, 1H), 3.75 (t, $J$ = 9.18 Hz, 1H), 3.89 (dd, $J$ = 5.46, 11.28 Hz, 1H), 4.25–4.35 (m, 2H), 5.23 (t, $J$ = 3.42 Hz, 1H), 5.39 (d, $J$ = 9.18 Hz, 1H), 7.85 (s, 1H). $^{13}$C NMR (150 MHz, $CD_3OD$): δ 15.90, 15.92, 16.34, 17.66, 19.45, 23.98, 24.02, 24.50, 26.44, 27.84, 28.45, 28.73, 31.61, 33.56, 33.75, 34.20, 35.04, 35.70, 38.11, 39.79, 39.82, 40.56, 42.52, 42.81, 47.47, 47.57, 56.67, 69.81, 70.69, 73.87, 78.59, 79.67, 90.15, 123.69, 124.12, 145.11, 146.15, 180.36. ESI-HRMS (*m/z*) $[M+Na]^+$ calcd for $C_{38}H_{60}N_4O_6Na$, 691.4405; found, 691.4406.

*4.2.17. Compound* ***17a***

Prepared from OA-propargylamine (75 mg, 0.15 mmol), acetylmaltosyl azide (126 mg, 0.19 mmol) according to general procedure A. The residue was purified by column chromatography (petroleum ether/AcOEt, 1/2 v/v) to give **17a** as a white solid (163 mg, 93%). Mp 153.9–154.5 °C. $^1$H NMR (600 MHz, $CDCl_3$): δ 0.66, 0.78, 0.87, 0.89, 0.98, 1.16 (7 × $CH_3$), 0.66–1.80 (m, other aliphatic ring protons), 1.85 (s, 3H), 2.02 (s, 3H), 2.04 (s, 6H), 2.07 (s, 3H), 2.11 (s, 3H), 2.14 (s, 3H), 2.53 (d, $J$ = 9.72 Hz, 1H), 3.21 (dd, $J$ = 3.96, 11.16 Hz, 1H), 3.49 (s, 1H), 3.98 (dd, $J$ = 2.28, 9.48 Hz, 1H), 4.06 (dd, $J$ = 1.56, 12.36 Hz, 1H), 4.14 (t, $J$ = 6.54 Hz, 1H), 4.23–4.27 (m, 3H), 4.48 (dd, $J$ = 1.92, 12.42 Hz, 1H), 4.60 (dd, $J$ = 6, 15.18 Hz, 1H), 4.89 (dd, $J$ = 3.9, 10.5 Hz, 1H), 5.08 (t, $J$ = 9.9 Hz, 1H), 5.31–5.40 (m, 4H), 5.44–5.47 (m, 2H), 5.84 (d, $J$ = 10.98 Hz, 1H), 6.60 (t, $J$ = 5.28 Hz, 1H), 7.72 (s, 1H); $^{13}$C NMR (150 MHz, $CDCl_3$): δ 15.51, 15.68, 16.73, 18.38, 20.32, 20.71 (5C), 20.83, 20.90, 20.96, 23.60, 23.70 (2C), 24.04, 25.89, 27.25, 27.35, 28.18, 30.81, 32.38, 33.09, 34.19, 35.05, 37.04, 38.57, 38.86, 39.45, 42.06, 42.11, 46.31, 46.75, 47.64, 55.17, 61.55, 62.62, 68.00, 68.85, 69.32, 70.11, 71.07, 72.48, 75.25, 75.42, 79.03, 85.38, 96.00, 121.34, 123.38, 144.54, 145.38, 169.14, 169.56, 170.07, 170.13, 170.44, 170.66, 170.69, 178.65. ESI-HRMS (*m/z*) $[M+Na]^+$ calcd for $C_{59}H_{86}N_4O_{19}Na$, 1177.5778; found, 1177.5778.

*4.2.18. Compound* ***17b***

Prepared from **17a** (100 mg, 0.09 mmol) according to general procedure B. The crude product was washed with water three times without further purification to afford **17b** as a white solid (64 mg, 86%). Mp > 250 °C. $^1$H NMR (600 MHz, $CD_3OD$): δ 0.60, 0.77, 0.91, 0.93, 0.94, 0.96, 1.15 (7 × $CH_3$), 0.60–2.11 (m, other aliphatic ring protons), 2.79 (dd, $J$ = 3.72, 13.38 Hz, 1H), 3.14 (dd, $J$ = 4.5, 11.34 Hz, 1H), 3.50 (dd, $J$ = 3.3, 9.72 Hz, 1H), 3.56–3.63 (m, 2H), 3.70–3.75 (m, 3H), 3.77–3.83 (m, 3H), 3.90–3.94 (m, 3H), 4.36–4.46 (m, 3H), 5.35 (t, $J$ = 3.36 Hz, 1H), 5.64 (d, $J$ = 9.18 Hz, 1H), 8.07 (s, 1H) $^{13}$C NMR (150 MHz, $CD_3OD$): δ 16.01, 16.39, 17.63, 19.48, 24.04 (3C), 24.54, 26.46, 27.84, 28.47, 28.74, 31.60, 33.54, 33.74, 34.13, 35.03, 35.66, 38.11, 39.80, 39.83, 40.60, 42.51, 42.85, 47.51, 47.60, 56.68, 61.50, 62.51, 70.28, 72.52, 73.64, 74.79, 76.75, 77.15, 79.55, 79.67 (2C), 89.46, 105.11, 124.09, 124.21, 145.06, 145.92, 180.54. ESI-HRMS (*m/z*) $[M+Na]^+$ calcd for $C_{45}H_{72}N_4O_{12}Na$, 883.5039; found, 883.5038.

*4.2.19. Compound* ***18a***

Prepared from OA-propargylamine (60 mg, 0.12 mmol), acetyllactosyl azide (126 mg, 0.19 mmol) according to general procedure A. The residue was purified by column chromatography (petroleum ether/AcOEt, 1/2 v/v) to give **18a** as a white solid (120 mg, 86%). Mp 148.0–159.5 °C. $^1$H NMR (600 MHz, $CDCl_3$): δ 0.66, 0.78, 0.87, 0.89, 0.99, 1.16 (7 × $CH_3$), 1.87, 1.98, 2.06, 2.07, 2.09, 2.12, 2.17 (7 × $CH_3CO$), 0.66–2.30 (m, other aliphatic ring protons), 2.53 (dd, $J$ = 3.42, 12.90 Hz, 1H), 3.21 (dd, $J$ = 3.78, 11.10 Hz, 1H), 3.89–3.97 (m, 3H), 4.09–4.17 (m, 3H), 4.24 (dd, $J$ = 4.80, 15.12 Hz, 1H), 4.49 (d, $J$ = 11.52 Hz, 1H), 4.53 (d, $J$ = 7.92 Hz, 1H), 4.60 (dd, $J$ = 6.00, 15.12 Hz, 1H), 4.98 (dd, $J$ = 3.42, 10.44 Hz, 1H), 5.12–5.15 (m, 1H), 5.37 (d, $J$ = 3.18 Hz, 1H), 5.40 (dd, $J$ = 2.88, 6.36 Hz, 2H), 5.78 (dd, $J$ = 2.64, 6.42 Hz, 1H), 6.62 (t, $J$ = 5.46 Hz, 1H), 7.7 (s, 1H). $^{13}$C NMR (150 MHz, $CDCl_3$): δ 15.48, 15.68, 16.68, 18.36, 20.34, 20.63, 20.75, 20.77, 20.80, 20.83, 20.92, 22.77, 22.79, 23.58, 23.69, 24.04, 25.88, 28.16, 30.80, 32.33, 33.08, 34.17, 35.01, 37.02, 38.54, 38.85, 39.41, 42.03, 42.07, 46.27, 46.73, 47.62, 55.14, 60.52, 60.92, 61.81, 66.66, 69.09, 70.61, 70.91, 71.00, 72.70, 75.65, 75.95, 78.99, 85.62, 101.23, 121.35, 123.37, 144.53, 145.32, 169.05, 169.22, 169.68, 170.19, 170.24, 170.31, 170.52, 178.64. ESI-HRMS (*m/z*) $[M+Na]^+$ calcd for $C_{59}H_{86}N_4O_{19}Na$, 1177.5778; found, 1177.5778.

*4.2.20. Compound* ***18b***

Prepared from **18a** (80 mg, 0.07 mmol) according to general procedure B. The crude product was washed with water three times without further purification to afford **18b** as a white solid (52 mg, 91%). Mp 190.5–192.0 °C. $^1$H NMR (600 MHz, $CD_3OD$): δ 0.60, 0.77, 0.91, 0.93, 0.94, 0.96, 1.15 (7 × $CH_3$), 0.60–2.11 (m, other aliphatic ring protons), 2.79 (dd, $J$ = 3.72, 13.38 Hz, 1H), 3.14 (dd, $J$ = 4.5, 11.34 Hz, 1H), 3.50 (dd, $J$ = 3.3, 9.72 Hz, 1H), 3.56–3.63 (m, 2H), 3.70–3.75 (m, 3H), 3.77–3.83 (m, 3H), 3.90–3.94 (m, 3H), 4.36–4.46 (m, 3H), 5.35 (t, $J$ = 3.36 Hz, 1H), 5.64 (d, $J$ = 9.18 Hz, 1H), 8.07 (s, 1H). $^{13}$C NMR (150 MHz, $CD_3OD$): δ 16.01, 16.39, 17.63, 19.48, 24.04 (3C), 24.54, 26.46, 27.84, 28.47, 28.74, 31.60, 33.54, 33.74, 34.13, 35.03, 35.66, 38.11, 39.80, 39.83, 40.60, 42.51, 42.85, 47.51, 47.60, 56.68, 61.50, 62.51, 70.28, 72.52, 73.64, 74.79, 76.75, 77.15, 79.55, 79.67 (2C), 89.46, 105.11, 124.09, 124.21, 145.06, 145.92, 180.54. ESI-HRMS (*m/z*) $[M+Na]^+$ calcd for $C_{45}H_{72}N_4O_{12}Na$, 883.5039; found, 883.5036.

*4.2.21. Compound* ***19a***

Prepared from OA-propargylamine (82 mg, 0.16 mmol), acetylcellobiosyl azide (126 mg, 0.19 mmol) according to general procedure A. The residue was purified by column chromatography (petroleum ether/AcOEt, 1/2 v/v) to give **19a** as a white solid (163 mg, 87%). Mp 143.7–145.2 °C. $^1$H NMR (600 MHz, $CDCl_3$): δ 0.66, 0.78, 0.87, 0.89, 0.98, 1.16 (7 × CH3), 0.66–2.20 (m, other aliphatic ring protons), 1.86 (s, 3H), 1.99 (s, 3H), 2.02 (s, 3H), 2.05 (d, 6H), 2.11 (s, 3H), 2.12 (s, 3H), 2.53 (dd, $J$ = 3.33, 12.84 Hz 1H), 3.21 (dd, $J$ = 4.14, 11.28 Hz, 1H), 3.69–3.71 (m, 1H), 3.88–3.96 (m, 2H), 4.07 (dd, $J$ = 1.92, 12.5 Hz, 1H), 4.12 (dd, $J$ = 5.04, 12.36 Hz, 1H), 4.23 (dd, $J$ = 4.8, 15.12 Hz, 1H), 4.40 (dd, $J$ = 4.38, 12.6 Hz, 1H), 4.51 (d, $J$ = 10.92 Hz, 1H), 4.56 (d, $J$ = 7.92 Hz, 1H), 4.60 (dd, $J$ = 6.06, 15.12 Hz, 1H), 4.95 (t, $J$ = 8.22 Hz, 1H), 5.09 (t, $J$ = 9.78 Hz, 1H), 5.17 (t, $J$ = 9.36 Hz, 1H), 5.37–5.43 (m, 3H), 5.77 (d, $J$ = 8.7 Hz, 1H), 7.73 (s, 1H). $^{13}$C NMR (150 MHz, $CDCl_3$): δ 15.49, 15.68, 16.68, 18.37, 19.39, 20.33, 20.57, 20.66, 20.81, 20.92, 23.59, 23.69, 24.04, 25.89, 27.23, 27.32, 28.17, 30.80, 32.33, 32.35, 33.08, 34.18, 35.02, 37.03, 38.55, 38.85, 39.43, 42.04, 42.07, 46.28, 46.74, 47.63, 55.15, 61.62, 61.69, 67.76, 70.54, 71.20, 71.63, 72.21, 72.43, 72.94, 75.89, 75.98, 79.02, 85.68, 100.96, 121.40, 123.38, 144.53, 145.33, 169.03, 169.23, 169.46, 169.74, 170.28, 170.36, 170.64, 178.70. ESI-HRMS (*m/z*) $[M+Na]^+$ calcd for $C_{59}H_{86}N_4O_{19}Na$, 1177.5778; found, 1177.5776.

*4.2.22. Compound **19b***

Prepared from **19a** (100 mg, 0.09 mmol) according to general procedure B. The crude product was washed with water three times without further purification to afford **19b** as a white solid (69 mg, 92%). Mp 199.6–201.8 °C. $^1$H NMR (600 MHz, $CD_3OD$): $\delta$ 0.61, 0.78, 0.91, 0.93, 0.94, 0.97, 1.16 (7 × $CH_3$), 0.61–2.18 (m, other aliphatic ring protons), 2.79 (d, $J$ = 9.48 Hz, 1H), 3.14 (dd, $J$ = 4.56, 11.34 Hz, 1H), 3.25–3.28 (m, 1H), 3.32–3.40 (m, 6H), 3.67–3.74 (m, 3H), 3.79 (t, $J$ = 9.30 Hz, 1H), 3.88–3.94 (m, 4H), 4.36 (d, $J$ = 15.18 Hz, 1H), 4.43–4.48 (m, 2H), 5.35 (t, $J$ = 3.42 Hz, 1H), 5.62 (d, $J$ = 9.24 Hz, 1H), 8.00 (s, 1H). $^{13}$C NMR (150 MHz, $CD_3OD$): $\delta$ 16.01, 16.40, 17.63, 19.48, 24.03 (2C), 24.05, 24.54, 26.46, 27.84, 28.46, 28.74, 31.61, 33.55, 33.74, 34.13, 35.04, 35.82, 38.11, 39.80, 39.83, 40.60, 42.51, 42.85, 47.50, 47.61, 49.85, 56.68, 61.45, 62.43, 71.33, 73.70, 74.88, 76.77, 77.82, 78.16, 79.52, 79.67, 89.26, 104.60, 123.77, 124.20, 145.07, 146.14, 180.50. ESI-HRMS ($m/z$) $[M+Na]^+$ calcd for $C_{45}H_{72}N_4O_{12}Na$, 883.5039; found, 883.5031.

*4.2.23. Compound **20a***

Prepared from OA-propargylamine (68 mg, 0.13 mmol), acetylmaltotriosyl azide (180 mg, 0.19 mmol) according to general procedure A. The residue was purified by column chromatography (petroleum ether/AcOEt, 1/2 v/v) to give **20a** as a white solid (163 mg, 87%). Mp 146.0–147.7 °C. $^1$H NMR (600 MHz $CDCl_3$): δ 0.59, 0.74, 0.83, 0.85, 0.94, 1.11 (7 × $CH_3$), 1.97, 1.98, 1.99, 2.00, 2.03, 2.04, 2.07, 2.13 (10 × $CH_3CO$), 0.59–2.30 (m, other aliphatic ring protons), 2.50 (d, $J$ = 9.9 Hz, 1H), 3.16–3.20 (m, 2H), 3.90–4.30 (m, 13H), 4.44 (d, $J$ = 11.04 Hz, 2H), 4.55 (dd, $J$ = 5.94, 15.18 Hz, 1H), 4.73 (dd, $J$ = 3.96, 10.32 Hz, 1H), 4.82 (dd, $J$ = 3.96, 10.5 Hz, 1H), 5.04 (t, $J$ = 9.84 Hz, 1H), 5.27–5.44 (m, 8H), 5.82 (d, $J$ = 9.12 Hz, 1H), 6.69 (t, $J$ = 5.4 Hz, 1H), 7.74 (s, 1H). $^{13}$C NMR 150 MHz, $CDCl_3$): δ 15.44, 15.65, 16.62, 18.31, 20.26, 20.66, 20.75, 20.83, 20.88, 20.97, 23.52, 23.63, 23.86, 25.03, 25.84, 27.07, 27.11, 27.26, 28.12, 30.74, 32.31, 32.32, 33.04, 34.10, 34.90, 36.96, 38.50, 38.79, 39.37, 41.96, 42.02, 46.25, 46.65, 47.56, 50.22, 55.11, 61.41, 62.31, 62.73, 67.89, 68.56, 69.22, 69.38, 70.14, 70.47, 70.54, 71.03, 71.66, 72.47, 73.47, 75.01, 75.25, 79.01, 85.31, 95.71, 96.15, 121.57, 123.34, 144.34, 145.17, 169.18, 169.59, 169.84, 170.01, 170.04, 170.51, 170.53, 170.71, 170.73, 175.06, 178.77. ESI-HRMS ($m/z$) $[M+Na]^+$ calcd for $C_{71}H_{102}N_4O_{27}Na$, 1465.6624; found, 1465.6621.

*4.2.24. Compound **20b***

Prepared from **20a** (110 mg, 0.07 mmol) according to general procedure B. The crude product was washed with water three times without further purification to afford **20b** as a white solid (54 mg, 73%). Mp 203.5–205.8 °C. $^1$H NMR (600 MHz, $CD_3OD$): $\delta$ 0.63, 0.78, 0.91, 0.93, 0.94, 0.97, 1.16 (7 × $CH_3$), 0.63–2.14 (m, other aliphatic ring protons), 2.80 (d, $J$ = 9.54 Hz, 1H), 3.14 (dd, $J$ = 4.5, 11.34 Hz, 1H), 3.28 (t, $J$ = 9.78 Hz, 1H), 3.45 (dd, $J$ = 3.72, 9.72 Hz, 1H), 3.51–3.93 (m, 17H), 4.36 (d, $J$ = 15.18 Hz, 1H), 4.44 (d, $J$ = 15.24 Hz, 1H), 5.16 (d, $J$ = 3.84 Hz, 1H), 5.25 (d, $J$ = 3.78 Hz, 1H), 5.35 (brs, 1H), 5.63 (d, $J$ = 9.06 Hz, 1H), 8.04 (s, 1H). $^{13}$C NMR (150 MHz, $CD_3OD$): $\delta$ 16.01, 16.36, 17.68, 19.49, 24.03 (2C), 24.05, 24.54, 26.45, 27.84, 28.48, 28.73, 31.61, 33.53, 33.75, 34.14, 35.04, 35.77, 38.11, 39.80, 39.83, 40.61, 42.50, 42.85, 47.51, 47.61 (2C), 56.69, 61.83, 62.13, 62.72, 71.46, 73.43, 73.61, 73.78, 74.24, 74.78, 74.93, 75.07, 78.12, 79.61, 79.68, 80.27, 81.32, 89.45, 102.75, 102.92, 123.89, 124.21, 145.07, 146.06, 180.57. ESI-HRMS ($m/z$) $[M+Na]^+$ calcd for $C_{51}H_{82}N_4O_{17}Na$, 1045.5567; found, 1045.5569.

*4.3. Bioassays*

*4.3.1. CPE reduction assay*

MDCK cells were seeded into 96-well plates, incubated overnight and infected with influenza virus (MOI ¼ 0.1). Cells were suspended in DMEM supplemented with 1% FBS, containing test compound and 2 m g/mL TPCK-treated trypsin, and a final DMSO concentration of 1% was added in each well. After 40 h of incubation, CellTiter-Glo reagent (Promega Corp., Madison, WI, USA) was added, and the plates were read using a plate reader (Tecan Infinite M2000 PRO; Tecan Group Ltd., Mannedorf, Switzerland).

*4.3.2. Cytotoxicity test*

Cells were grown in 96-well plates containing 1% FBS overnight and then cultured with increasing amounts of the test compounds for 40 h. Cytotoxicity was assessed with the CellTiter-Glo assay as above described.

*4.3.3. HI assay*

Compound from a 2-fold serial dilution in salinewas mixed with an equal volume of influenza virus (The HA titers of A/WSN/33 (H1N1) virus is 1:2 $^{x\ (x=1)}$) in the V-bottomed 96-well microplates. Subsequently, 50 μL of freshly prepared chicken red blood cells (cRBC) (1% v/v in saline) was added to each well. The mixture was incubated for 30 min at room temperature before observing cRBC aggregation on the plate.

*4.3.4. Docking simulation*

Compound **11b** was docked into the HA protein using AutoDock 4.2 [30]. The structural template of influenza HA (Protein Data Bank: 1RVT [31]) was obtained from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). To carry out blind docking experiments, grids of points covering the whole HA head and partial stem were generated with ADT [32]. Box size: 126 × 126 × 126 points with a standard space of 0.375 Å. Docking simulations of the compound were carried out using the Lamarckian genetic algorithm and through a protocol with a number of 50 GA runs, an initial population of 300 randomly placed individuals, a maximum number of 25 million energy evaluations, a mutation rate of 0.02, and a crossover rate of 0.80. The resulting conformations that differed by less than 2.0 Å in positional root-mean-square deviation (rmsd) were clustered together. Other parameters were set as default. All the relevant torsion angles were treated as rotatable during the docking process, thus allowing a search of the conformational space. After running autogrid and autodock, the possible poses of compounds in HA were obtained.

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## Appendix A. Supplementary data

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