Novel mono- and multivalent N-acetylneuraminic acid glycoclusters as potential broad-spectrum entry inhibitors for influenza and coronavirus infection

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Abstract

N-acetylneuraminic acid (Neu5Ac) is a glycan receptor of viruses spread in many eukaryotic cells. The present work aimed to design, synthesis and biological evaluation of a panel of Neu5Ac derivatives based on a cyclodextrin (CD) scaffold for targeting influenza and coronavirus membrane proteins. The multivalent Neu5Ac glycoclusters efficiently inhibited chicken erythrocyte agglutination induced by intact influenza virus in a Neu5Ac density–dependent fashion. Compared with inhibition by Neu5Ac, the multivalent inhibitor with 21 Neu5Ac residues on the primary face of the *β*-CD scaffold afforded 1788-fold higher binding affinity inhibition for influenza virus hemagglutinin with a dissociation constant (K_D) of 3.87×10^{-7} M. It showed moderate binding affinity to influenza virus neuraminidase, but with only about one-thirtieth the potency of that with the HA protein. It also exhibited strong binding affinity to the spike protein of three human coronaviruses (severe acute respiratory syndrome coronavirus, Middle East respiratory syndrome coronavirus, and severe acute respiratory syndrome coronavirus 2), with K_D values in the low micromolar range, which is about 10-time weaker than that of HA. Therefore, these multivalent sialylated CD derivatives have possible therapeutic application as broad-spectrum antiviral entry inhibitors for many viruses by targeting the Neu5Ac of host cells.

Keywords: Neu5Ac, Cyclodextrin, Entry inhibitor, Multivalent effect

1. Introduction

Sialic acids are a class of α-keto acidic nine-carbon acidic sugars with >50 known naturally occurring derivatives, mostly present as glycoprotein and ganglioside terminal residues [1]. The structural and functional diversity of sialic acids mainly arises from the different types of glycosidic bonds present and from several sialic acid–specific modifications, including methylation, acetylation, and sulfation. The most common member of this family is *N*-acetylneuraminic acid (Neu5Ac) (Fig. 1), followed by *N*-glycolylneuraminic acid and 2-keto-3-deoxy-nononic acid. Sialic acids linked to glycoproteins are used by numerous viruses as receptors for specific attachment and entry into cells [2, 3]. Influenza is a major infectious disease caused by influenza A and B viruses. Human or avian influenza viruses are known to use homotrimers of hemagglutinin (HA) to recognize terminal sialic acids attached to galactose residue by either α-2,6 or α-2,3 linkage [4] and thereby initiate infection. Similarly, sialic acids play an important role in human coronavirus (CoV) infection. CoVs commonly use host-sialylated structures for binding and entry. The CoV spike glycoprotein may first bind to the sialoglycan-based receptor via the S_1^A domain and then bind to dipeptidyl peptidase 4 (DPP4) receptor for Middle East respiratory syndrome (MERS)-CoV or to angiotensin-converting enzyme 2 (ACE2) receptor for severe acute respiratory syndrome (SARS)-CoV via the S_1^B domain during entry into cells [5-7]. Additionally, the binding affinity of these viral proteins for sialoglycan-based receptors are reportedly considerably enhanced by multivalent interactions [8]. Therefore, a promising strategy against viral infection is the development of multivalent sialic acid derivatives acting as decoy molecules to bind with viral membrane proteins. Consequently, a series of divalent [9], trivalent [10-12], tetravalent [13] and polyvalent [14, 15] sialosides with various scaffolds [16-18] and linkers [9, 10, 16] have been designed and synthesized. It was found that the density of ligand, the types of scaffold, and the linkers between ligand and scaffold can significantly affect the binding to influenza virus. Whitesides and coworkers have extensively studied α-sialoside copolymers based on polyacrylamide core and found such substances inhibit the hemagglutination of erythrocytes ~10^4-10^5 times more

than methyl α-sialoside.[14, 16] In addition, multivalent sialylated polyglycerols, $LPG_{10}(6'\text{-SLamide})_{0.50}$, showed broad antiviral activity against influenza A viruses.[19]

Cyclodextrins (CDs) have found widespread application as molecular scaffolds and templates for multivalent glycosides [20-22]. However, limited effort has been directed toward preparing sialyl conjugates based on natural α- and β-CD. In 1998, Sukegawa et al published the synthesis of a novel amphiphilic α-CD derivative (**2**) bearing two Neu5Ac residues at the primary face, but the relative positions between the two groups were ambiguous [23]. Two years later, three 2,3-per-*O*-acetylated-6-per-*O*-sialylated-β-CD derivatives (**3–5**) were designed and synthesized by Roy et al.[24] Singh et al fabricated a novel hyper-crosslinked Neu5Ac-β-CD copolymer mixture (**6**) with an average 5.52% content of Neu5Ac to produce a 100 M copolymer solution that contained 18 mol of Neu5Ac [25]. The synthesized Neu5Ac-β-CD/Doxorubicin inclusion complexes demonstrated good drug loading capacity and permeability. More recently, Stellacci group described a new class of 6'-sialyl-*N*-acetyllactosamine (6'SLN) modified β-CDs connected by a long hydrophobic linker with strong broad-spectrum anti-influenza activity (EC_{50}: 42 nM and 56.5 nM for A/Netherlands/602/2009 (H1N1) and A/Singapore/37/2004 (H3N2), respectively) by interacting with HA protein [26, 27].

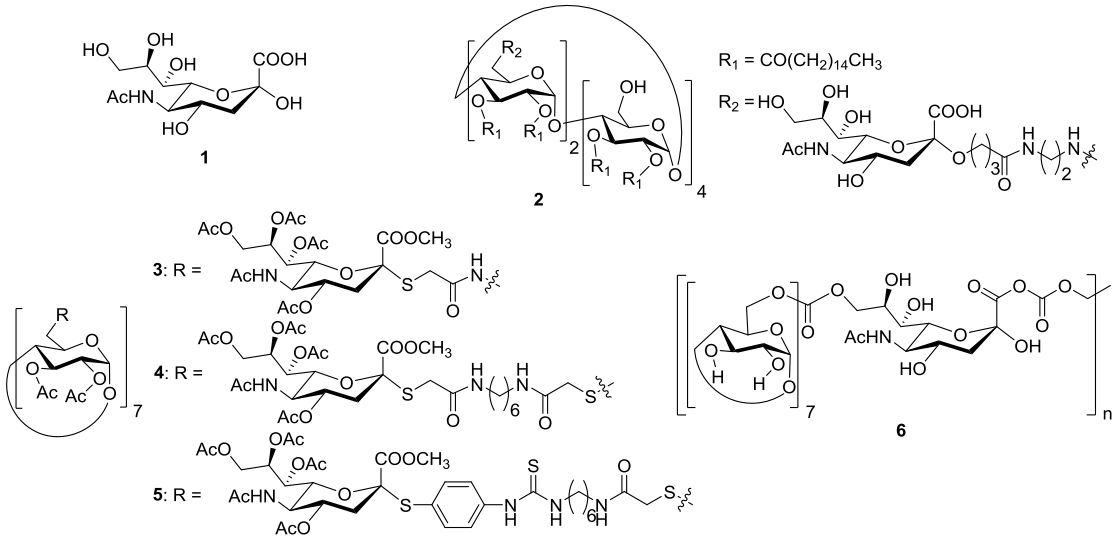


Fig. 1. Chemical structure of Neu5Ac (**1**) and its CD conjugates (**2–6**).

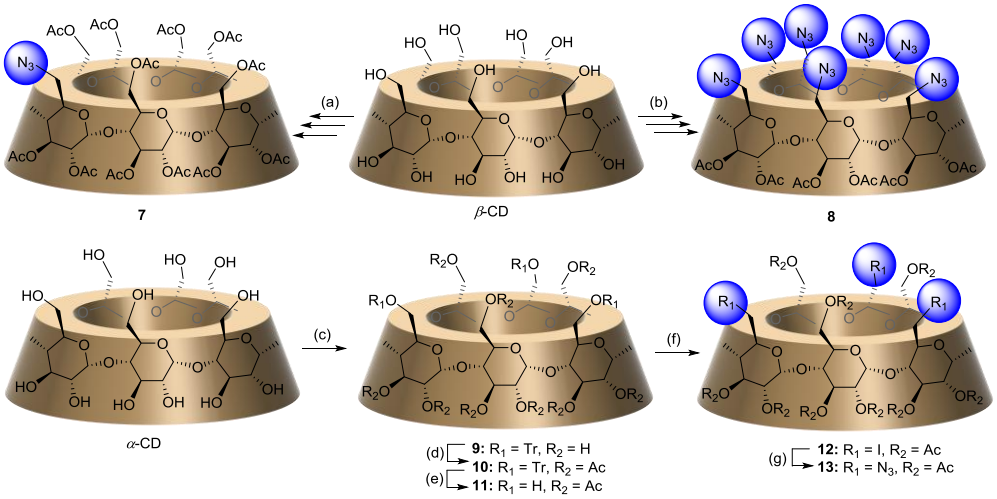
As discussed above, sialic acids linked to terminal galactose and/or *N*-acetyl galactosamine residues in glycoproteins and gangliosides are used by many viruses as a receptor for cell binding and entry in a multivalent fashion. Therefore, many sialylated multivalent constructs have been tested as entry inhibitors for multiple viruses, including influenza virus,[19] adenovirus type 37 (Ad37),[28] coxsackievirus A24 variant (CVA24v),[29] MERS-CoV,[30] and SARS-CoV-2 [31] et al. The dramatic enhancement in avidity and selectivity of multivalent weak interactions is manifested by multivalent sialic acid molecules. It is critical to extensively investigate all factors that can influence the multivalent interactions. To accurately quantify the effects of the sialylated CD derivatives on the influenza and coronavirus infection, precisely and efficiently synthesize structurally well-defined star-shaped sialylated CD derivatives of different valencies, conformations of Neu5Ac, and space lengths are required. We hypothesized that the multiple Neu5Ac ligands based on CD scaffold will simultaneously occupy the sialic acid binding sites of viral attachment proteins, thus block the interactions of influenza and coronavirus to their host cells. In addition, this antagonist effects would be dependent on the valency of Neu5Ac ligands. In this case, we would like to describe the preparation of a panel of mono-, tri-, hepta-, nona-, and 21-valent sialylated α- and β-CD derivatives from two classes of building blocks: terminal alkyne–functionalized sialosides and azide–functionalized CDs using "click chemistry" and the evaluation of their interactions with two important transmembrane glycoproteins (HA and neuraminidase (NA)) of influenza virus and spike glycoproteins of three human-infecting CoVs (SARS-CoV, MERS-CoV, and SARS-CoV-2) by hemagglutination inhibition and surface plasmon resonance assays. Such highly sialylation degree of CD derivatives could be potentially served as novel antiviral entry inhibitors that efficiently targets a broad range of RNA viruses.

2 Results and discussion

2.1 Synthesis of azide-functionalized CD building blocks

Peracetylated monoazido- and heptaazido-β-CD derivatives **7** and **8** were synthesized in a three-step strategy from commercially available β-CD with yields of 11% and 65%, respectively, as described previously [32, 33]. To prepare the

triazido-α-CD derivative **13** (Scheme 1), dried α-CD was reacted with triphenylmethyl chloride (TrCl) to provide the desired C_3-symmetrical $6^{A,C,E}$-*O*-trityl-α-CD **9** (21% yield) together with other mono-, di-, and trisubstituted byproducts [34] following reverse-phase column chromatography with acetonitrile/methanol/water (40:45:15). Full acetylation of the remaining hydroxyl groups of **9** was accomplished using acetic anhydride in pyridine at room temperature with good yield. The *O*-trityl groups were removed from intermediate **10** by treatment with *p*-toluenesulfonic acid, followed by iodination and azidation reactions to produce the peracetylated triazido-α-CD building block **13** (54% yield) in three steps.

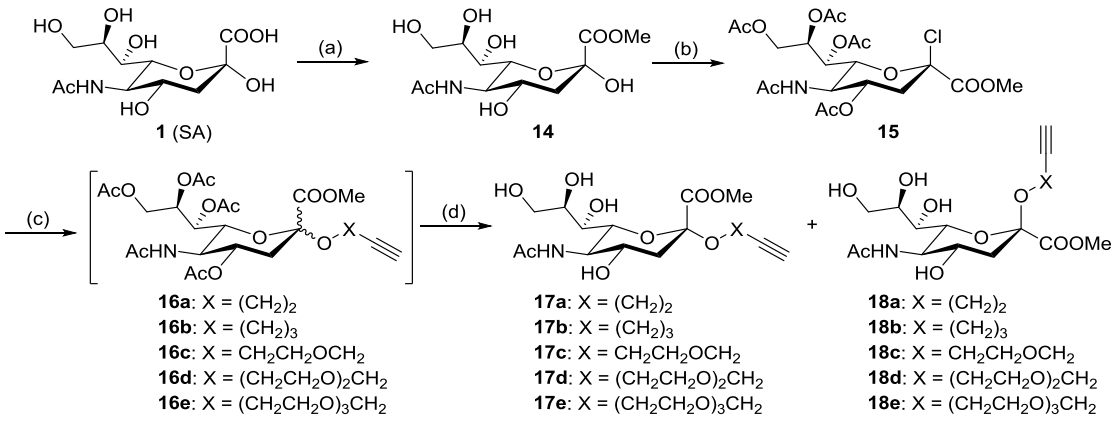


Scheme 1. Synthesis of three azide-functionalized α- and β-CD building blocks **7**, **8**, and **13**. Reagents and conditions: (a) (i) NaOH, TsCl, H_2O, 14%; (ii) NaN_3, DMF, 60°C, 87%; then (iii) pyridine, Ac_2O, DMAP, 89%; (b) (i) Ph_3P, I_2, DMF, 81%; (ii) NaN_3, DMF, 60°C, 82%; then (iii) Ac_2O, pyridine, DMAP, 90%. (c) TrCl, pyridine, 65°C, 36 h, 21%; (d) Ac_2O, pyridine, DMAP, 91%; (e) *p*-TsOH, acetonitrile, 30 min, 81%; (f) Ph_3P, I_2, imidazole, toluene, 70°C, 78%; (g) NaN_3, DMF, 80°C, 86%.

2.2 Synthesis of alkyne-functionalized Neu5Ac building blocks

Neu5Ac bearing an extended linker functionalized with alkyne group at 2-position **17a–17e** (α-anomers) and **18a–18e** (β-anomers) were synthesized according to Scheme 2. In a pilot experiment, under classical Koenigs–Knorr-like conditions, the chloride of acetylated Neu5Ac methyl ester **15**, synthesized from commercially available Neu5Ac in two steps [35], was reacted with 3-butynyl alcohol using silver trifluoromethanesulfonate (AgOTf) as a catalyst to produce a mixture of

the α- and β-anomers **16a** with 40% yield. We observed that the α-anomer was more polar than the β-anomer based on their migratory ability on a thin-layer chromatography plate (silica gel 60 F254, petrol ether/dichloromethane/isopropanol = 10:4:1; α: R_f = 0.20; β: R_f = 0.24). The β-anomer of **16a** was analytically characterized using silica gel column chromatography (Fig. S1). However, the pure α-anomer of **16a** could not be isolated. Therefore, sodium methoxide was added to the crude mixture to remove the *O*-acetyl groups without isolation. In this case, the obtained anomers could be separated via flash column chromatography (CH_2Cl_2/CH_3OH = 8:1; α: R_f = 0.38; β: R_f = 0.26) to produce the desired α-anomer **17a** and β-anomer **18a** at 35% and 46% yield, respectively. Similarly, the other alkyne-functionalized Neu5Ac building blocks **17b–17e** and **18b–18e** were also obtained in moderate yields via intermediates **16b–16e** (Scheme 2).



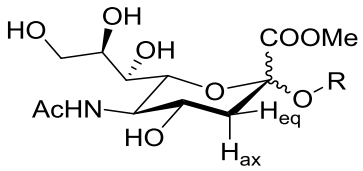
Scheme 2. Synthesis of alkyne-functionalized Neu5Ac building blocks **17a–17e** and **18a–18e**. Reagents and conditions: (a) CH_3OH, ion-exchange resin, 90%; (b) AcCl, 37% HCl, toluene, 95%; (c) Alkynyl alcohol, AgOTf, MeCN, Et_3N, molecular sieves 4 Å, 38%–42%; (d) CH_3ONa/CH_3OH, 35%–45% for **17a–17e**, 40%–51% for **18a–18e**.

The stereochemistry of the anomeric C of the synthesized building blocks was determined by comparison of the chemical shifts and coupling constants of 3-H_{eq} with

the literature [36, 37]. The chemical shift of 3-H_{eq} doublet of doublets (dd) of α-anomer is believed to be between δ 2.5 and 2.8 ppm. For example, in the case of α-anomers **17a** and **17e**, the chemical shifts of 3-H_{eq} were at δ 2.69 and 2.70 ppm, respectively (Fig. S2), while the corresponding peaks for β-anomers **18a** and **18e** were at δ 2.38 and 2.40 ppm, respectively. Compared with the α-anomers, a marked upfield shift of 3-H_{eq} (~0.3 ppm) and small upfield shift of 3-H_{ax} (~0.08 ppm) was commonly observed for β-anomers (Table 1).

Table 1.

Chemical shifts (δ, ppm) and coupling constants (J, Hz) for 3-H_{eq} of the α-anomers **17a–17e** and β-anomers **18a–18e**.[a]

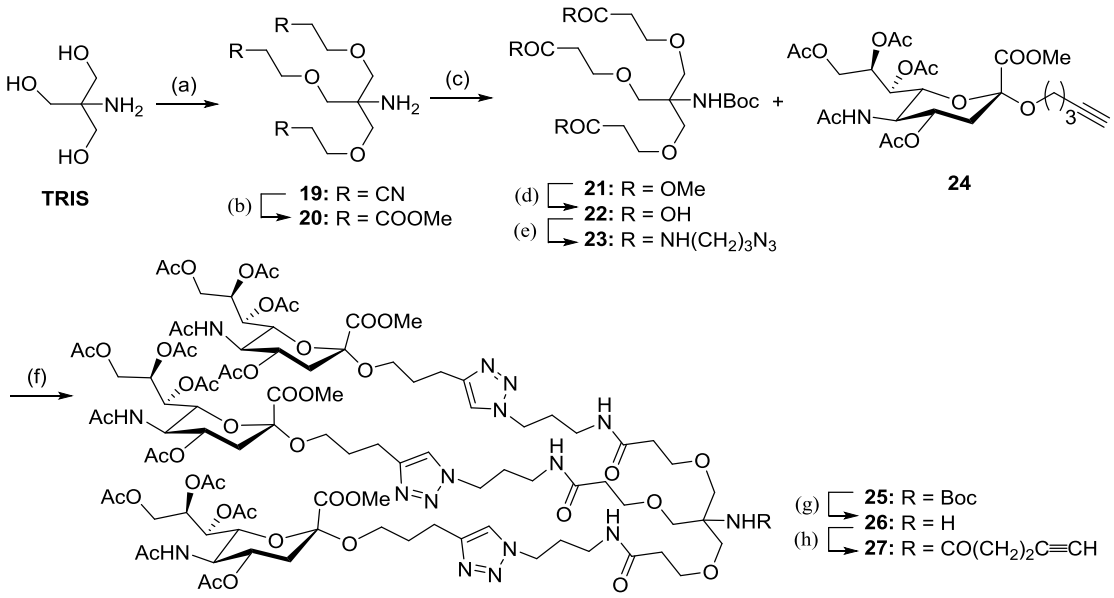


Compound (α-anomer)	3-H_{eq} (δ, J)	3-H_{ax} (δ, J)	Compound (β-anomer)	3-H_{eq} (δ, J)	3-H_{ax} (δ, J)
17a	2.69 (dd, 12.8, 4.6)	1.73 (t, 12.3)	**18a**	2.38 (dd, 13.0, 5.0)	1.66 (dd, 12.9, 11.3)
17b	2.68 (dd, 12.8, 4.6)	1.73 (t, 12.3)	**18b**	2.39 (dd, 12.9, 4.9)	1.63 (dd, 12.9, 11.3)
17c	2.70 (dd, 12.8, 4.6)	1.75 (t, 12.3)	**18c**	2.41 (dd, 13.0, 4.9)	1.66 (dd, 12.9, 11.3)
17d	2.70 (dd, 12.8, 4.6)	1.75 (t, 12.3)	**18d**	2.40 (dd, 13.0, 4.9)	1.66 (dd, 12.8, 11.4)
17e	2.70 (dd, 12.8, 4.6)	1.75 (t, 12.3)	**18e**	2.40 (dd, 12.9, 4.9)	1.66 (dd, 12.6, 11.6)

[a] ^{1}H NMR spectra were measured in CD_3OD at 298 K.

The reaction of di-*tert*-butyl dicarbonate with **20**, which was synthesized from

tris(hydroxymethyl)aminomethane with acrylonitrile via Michael addition followed by hydrolysis and esterification [38], produced the *N*-Boc derivative **21**. Methyl ester **21** was further hydrolyzed with NaOH (0.1 M), and the resulting carboxylic acid was then treated with 3-azidopropylamine to afford triazide **23**, which coupled with **24** yielded the three-arm Neu5Ac intermediate **25**. Removing the Boc group via trifluoroacetic acid (TFA) treatment produced amine **26**. Amide coupling of **26** with 4-pentynoic acid afforded the desired terminal alkyne–functionalized building block **27**.



Scheme 3. Reagents and conditions: (a) acrylonitrile, 40% aq (w/v) KOH, 1,4-dioxane, 81%; (b) sulfuric acid, MeOH, 80°C, 45%; (c) Boc_2O, 1,4-dioxane, TEA, 85%; (d) NaOH (0.1 M), methanol/water (1:1, v/v), 91%; (e) 3-azidopropylamine, EDC, HOBt, DIPEA, 60%; (f) $CuSO_4$, sodium ascorbate, methanol/water (1:1, v/v), 70%; (g) CH_2Cl_2, TFA, 92%; (h) 4-pentynoic acid, EDC, HOBt, DIPEA, 78%.

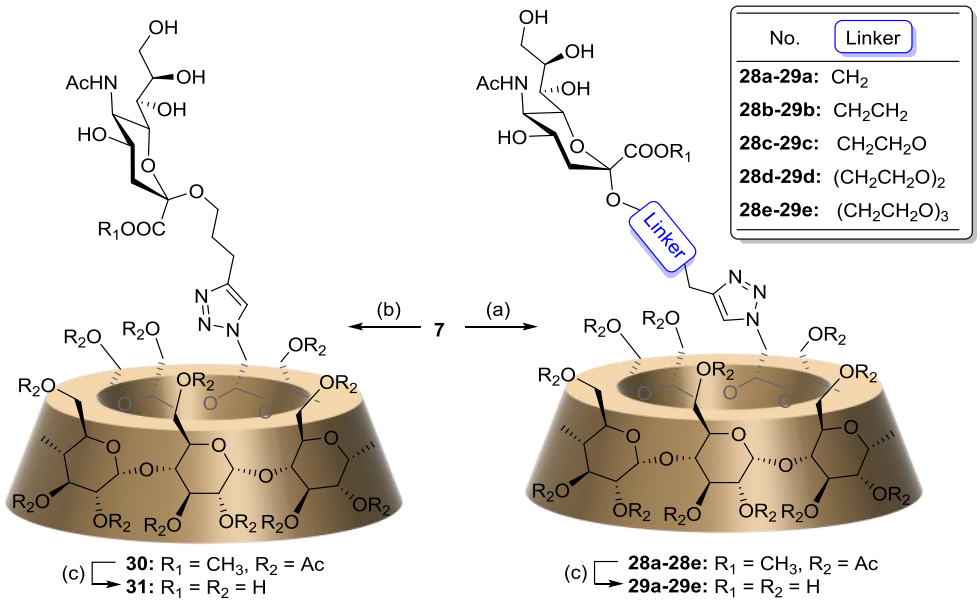
2.3 Synthesis of Neu5Ac glycoclusters based on α- and β-CD scaffold

In a coupled step, chemically diverse Neu5Ac glycoclusters were efficiently generated by combining azide-functionalized CD building blocks (**7**, **8**, and **13**) with alkyne-functionalized Neu5Ac building blocks (**17a–17e**, **18b**, and **27**).

First, the monovalent sialylated *β*-CD conjugates **29a–29e** were synthesized using the click chemistry reaction between monoazide-substituted *β*-CD **7** and terminal

alkyne–substituted sialosides **17a–17e** with 60%–70% yield at room temperature, followed by a full deprotection step to cleave the methyl ester and acetyl moieties with quantitative yield (Scheme 4). In a pilot experiment, a two-step deprotection sequence was used for compound **28a** according to a modified literature procedure [39]. The acetyl groups of **28a** were removed easily under Zemplén saponification condition with 94% yield [40]. ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, and high-resolution mass spectrometry (HRMS) spectra indicated that all 24 *O*-acetyl groups were fully hydrolyzed but that the methyl ester on the Neu5Ac moiety was still present (Fig. S3). Then, the second deprotection step proceeded using 0.2 M NaOH in methanol/water (1:1, v:v) to produce the corresponding product **29a** in almost quantitative yield. Further study showed that both acetolysis and methanolysis could take place in one step using milder alkali (0.1 M NaOH) to produce the desired product **29a**. An unexpected but plausible desialylated *β*-CD byproduct, formed slowly via cleavage of the *α*-*O*-linked sialoside of **29a**, was observed and the ^{1}H and ^{13}C NMR spectra agreed with those in our previous study [41]. According to the integral of 3-H_{eq} of the Neu5Ac moiety, about one-third of **29a** was hydrolyzed at day 90 at 4°C (Fig. 2). Therefore, the solution pH should be carefully adjusted to ~6.5 by the addition of H^{+}-exchange resin to avoid the acid-catalyzed cleavage of the Neu5Ac residue from the monovalent sialylated *β*-CD conjugates **29a–29e**.

To compare the effect of the conformation of the Neu5Ac moiety on binding affinity, a monovalent sialylated *β*-CD conjugate **31** bearing a *β*-anomeric Neu5Ac group was similarly designed and synthesized from building blocks **7** and **18b** with a total yield of 61% over two steps.



Scheme 4. Synthesis of monovalent sialylated *β*-CD conjugates **29a–29e**. Reagents and conditions: (a) **17a–17e**, $CuSO_4$, sodium ascorbate, methanol/water (1:1, v/v), rt, 59%–70%; (b) **18b**, $CuSO_4$, sodium ascorbate, methanol/water (1:1, v/v), rt, 67%; (c) methanol/water (1:1, v/v), NaOH (0.1 M), then H^+-exchange resin, 96%–99%.

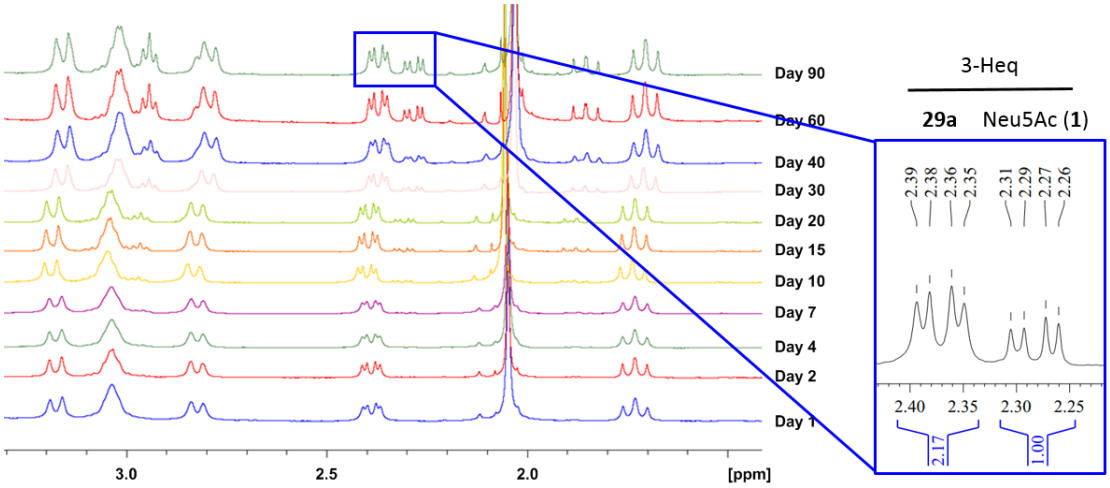
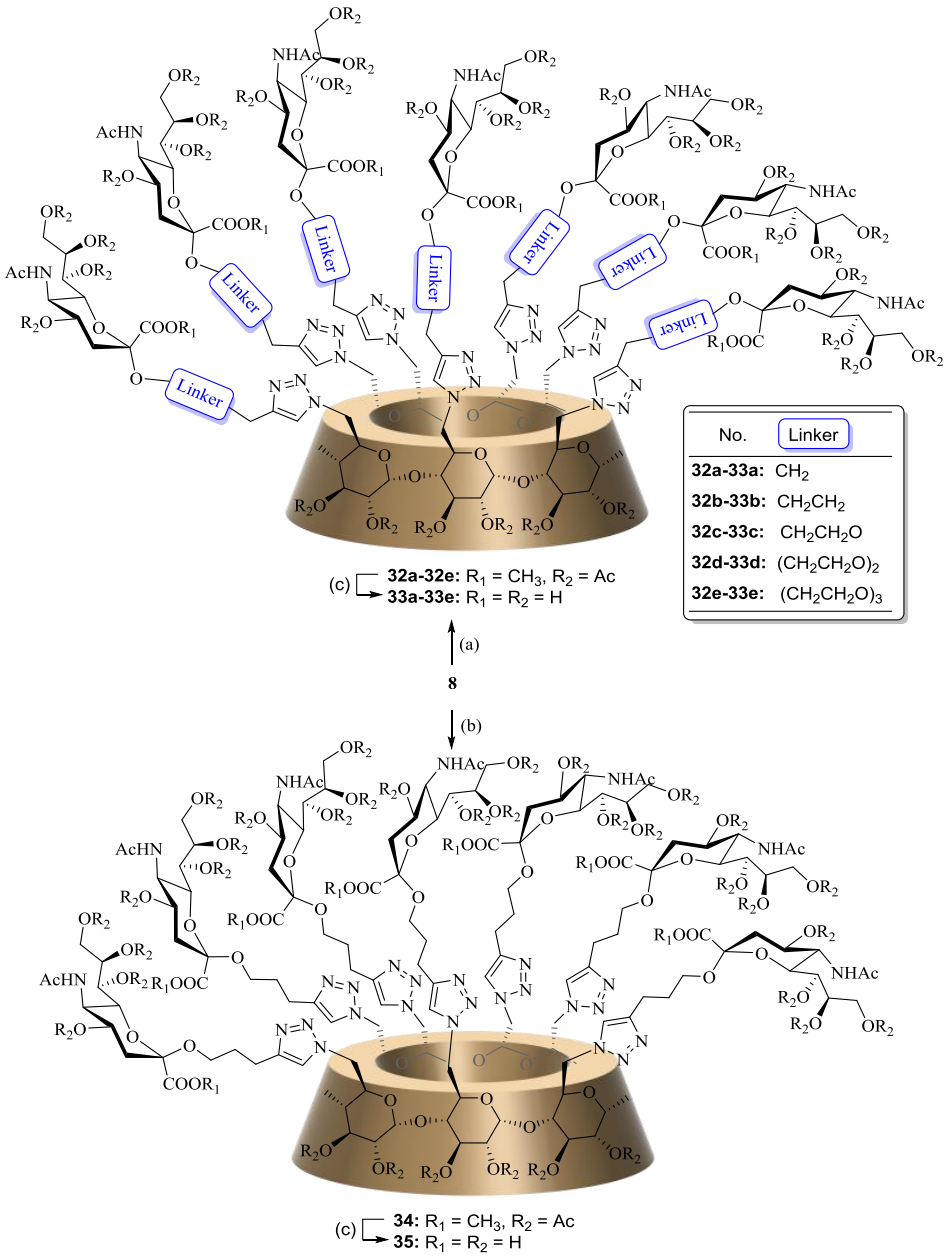


Fig. 2. Hydrolysis of conjugate **29a** at 4°C.

Subsequently, we synthesized heptavalent Neu5Ac glycoclusters. The reaction was initially performed with building blocks **8** and **17a** as a model using the methodology for the monovalent sialylated *β*-CD derivative **28a**, which demonstrated incomplete coupling. Therefore, the click chemistry reaction was performed under microwave-heating conditions; however, separation of the desired product was

difficult because of the multiple polar hydroxyl groups of Neu5Ac. To overcome this, compound **17a** was first reacetylated with acetic anhydride in pyridine, followed by microwave-assisted click reaction with **8** in acetone/water (1:1, v/v) at 100°C for 1 h to produced conjugate **32a** with 46% yield over two steps (Scheme 5). Therefore, conjugates **32b–32e** were prepared under exactly the same conditions with yields ranging from 46% to 56%. In a similar method described for synthesizing conjugates **29a–29e**, both the methyl ester and *O*-acetyl groups of heptavalent sialylated *β*-CD intermediates **32a–32e** were readily hydrolyzed with 0.1 M NaOH to produce the desired heptavalent Neu5Ac glycoclusters **33a–33e** in quantitative yields.

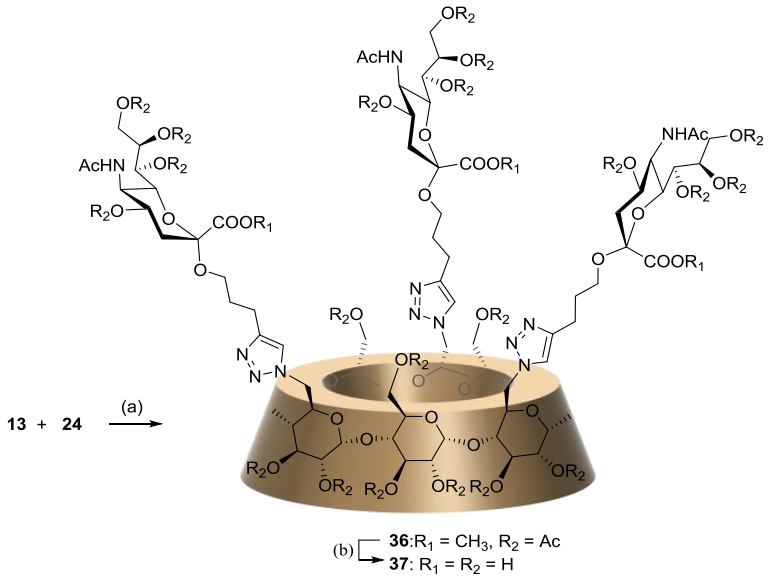
The heptavalent glycocluster **35** with *β*-anomeric Neu5Ac residues was also synthesized from building blocks **8** and **18b** with 55% yield over three steps according to the same procedures described for synthesizing **33a–33e**.



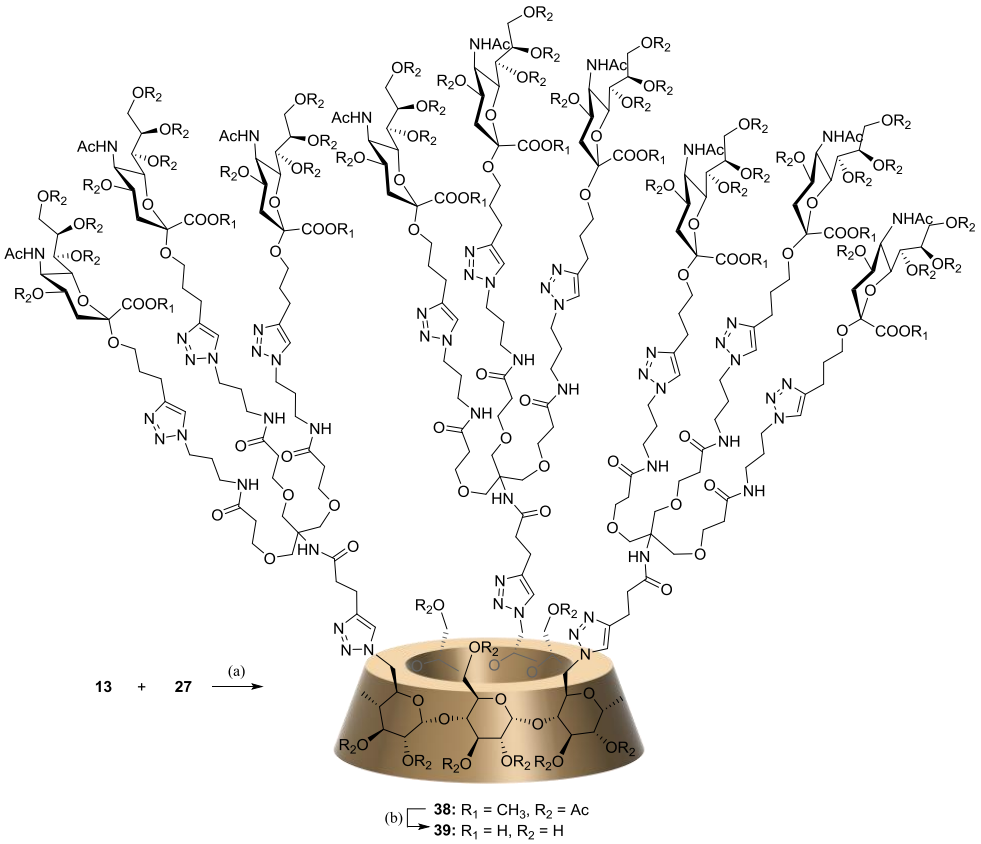
Scheme 5. Microwave-assisted synthesis of heptavalent Neu5Ac glycoclusters **33a–33e** and **35**. Reagents and conditions: (a) **17a–17e**, Ac_2O, pyridine, DMAP; then $CuSO_4$, sodium ascorbate, acetone/water (1:1, v/v), microwave, 100°C, 80 W, 1 h, 46%–56% for two steps; (b) **18b**, Ac_2O, pyridine, DMAP; then $CuSO_4$, sodium ascorbate, acetone/water (1:1, v/v), microwave, 100°C, 80 W, 1 h, 61% for two steps; (c) methanol/water (1:1, v/v), NaOH (0.1 M); then H^+-exchange resin, 78%.

Furthermore, to ascertain the feasibility of synthesizing tri- and nonavalent Neu5Ac glycoclusters based on the CD scaffold, an investigation was performed based on the C_3-symmetrical triazido-α-CD intermediate **13**, as shown in Scheme 6.

Trifunctional *α*-CD **13** was coupled with **24** via click chemistry at room temperature to produce the acetyl-protected sialylated *α*-CD conjugate **36** in 86% yield. As described above, the methyl ester and acetyl groups of **36** were hydrolyzed with NaOH (0.1 M), producing the trivalent sialylated *α*-CD conjugate **37** with excellent yield (96%). Similarly, we performed the click reaction at room temperature to synthesize the nonavalent sialylated *α*-CD derivative **39**, but the reaction was found to be very slow. To mitigate this, we used a microwave-assisted strategy, and the desired nonavalent Neu5Ac glycocluster **39** was successfully synthesized from **13** and **27** with 50% yield over two steps (Scheme 7).

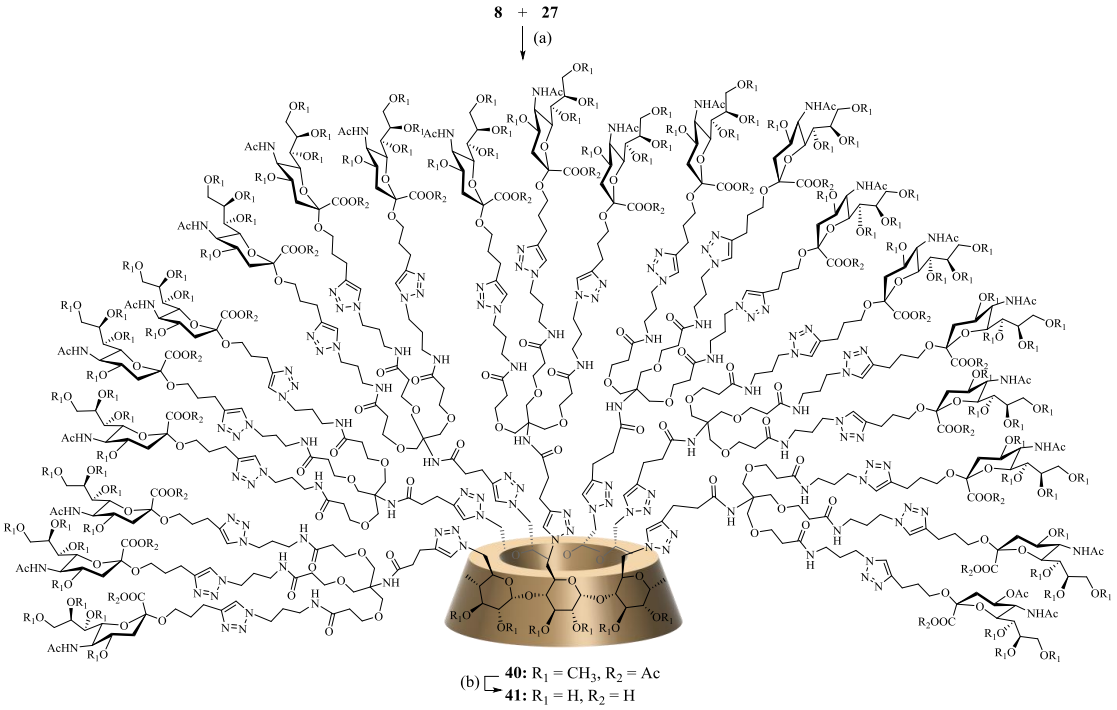


Scheme 6. Synthesis of the trivalent Neu5Ac glycocluster **37**. Reagents and conditions: (a) $CuSO_4$, sodium ascorbate, acetone/water (1:1, v/v), rt, 24 h, 86%; (b) methanol/water (1:1, v/v), NaOH (0.1 M); then H^+-exchange resin, 96%.



Scheme 7. Synthesis of the nonavalent Neu5Ac glycocluster **39**. Reagents and conditions: (a) $CuSO_4$, sodium ascorbate, acetone/water (1:1, v/v), microwave, 100°C, 80 W, 1 h, 51%; (b) methanol/water (1:1, v/v), NaOH (0.1 M); then ion-exchange resin, 98%.

CD scaffold bearing more Neu5Ac units is expected to exhibit improved binding affinity and efficiency with the target protein; therefore, we focused on synthesizing the 21-valent Neu5Ac glycocluster **41** with a total yield of 38% over two steps, following the protocol described above (Scheme 8). Unlike the reaction conditions described in Scheme 7, the reaction was found to be slightly sluggish, and a methanol/water mixture (1:1, v:v) was thus investigated as a reaction solvent. Extending the reaction time from 1 to 2 h afforded a mild increase in product conversion. The optimized conditions for synthesizing the 21-valent Neu5Ac glycocluster **41** were microwaving at 80 W for 2 h in methanol/water (1:1, v:v). Electrospray ionization–quadrupole time-of-flight mass spectrometry (ESI–QTOF MS) exhibited predominant multiply charged ions $[M+4H]^{4+}$ at m/z 3460.7346 (calculated. for $C_{567}H_{907}N_{133}O_{266}$, 3460.7893, Δ = 15.8 ppm).



Scheme 8. Synthesis of the 21-valent Neu5Ac glycocluster **41**. (a) $CuSO_4$, sodium ascorbate, methanol/water (1:1, v/v), microwave, 100°C, 80 W, 2 h, 47%; (b) methanol/water (1:1, v/v), NaOH (0.1 M), then H^+-exchange resin, 97%.

2.4 Binding of influenza virus to Neu5Ac glycoclusters

The hemagglutination inhibition (HI) test is the most widely used test for detecting the effect of sialylated glycopolymers with influenza virus [42, 43]. The influenza virus can agglutinate erythrocytes by binding to their surface HA protein via sialic acid receptors (commonly Neu5Ac). The binding affinity of the synthesized Neu5Ac glycoclusters for HA was determined as the lowest concentration detectable to fully inhibit aggregation (Table 2). This method is widely used to evaluate the binding of molecules with intact influenza virus by targeting HA protein.

Inhibition with unmodified Neu5Ac exhibited relatively weak inhibitory activity with a HI constant (K_i^{HAI}) value of 2.5×10^{-2} M, which was weaker than that of 2,3-sialyllactose ($K_i^{HAI} \approx 2.0 \times 10^{-2}$ M [44]), whereas *β*-CD had no detectable binding to wild-type HA. Furthermore, the K_i^{HAI} values of all monovalent sialylated CDs **29a–29e** were similar to those of natural Neu5Ac (**1**), suggesting that the *β*-CD scaffold had no considerably effect on hemagglutination binding inhibition.

Compared with the binding affinities of Neu5Ac and monovalent **29a–29e** to HA, trivalent **37** and heptavalent **33a–33e** bound HA at sub-mM to mM affinity (K_i^{HAI} = 10^{-3}–10^{-4} M). Maximal inhibition efficiency was achieved with the 21-valent Neu5Ac glycocluster **41**, with K_i^{HAI} = 7.8 × 10^{-5} M (Table 2), representing a 321-fold enhancement compared to monovalent **29a–29e**, and a 15-fold enhancement per Neu5Ac group. Surprisingly, the linkers of the sialylated CDs exhibited no significant effect on binding affinity, and thus only one monovalent **29b** and one heptavalent conjugate **33b** were selected for further SPR assessment.

To assess the effect of Neu5Ac group conformation on HA binding, two sialylated *β*-CD derivatives **31** and **35** bearing one and seven *β*-sialoside groups, respectively, were also synthesized to evaluate their potency as hemagglutination inhibitors. Compounds **31** and **35** exhibited similar binding affinity to monovalent **29a–29e** and heptavalent **33a–33e**, with K_i^{HAI} values of 25 and 0.62 mM, respectively. The previously reported compounds, indicating that the conformation of Neu5Ac residues has no significant effect on binding with influenza A (A/Wisconsin/588/2019 (H1N1)) HA protein.

Table 2.

Hemagglutination inhibition constant (K_i^{HAI}) of Neu5Ac glycoclusters.

Compound	Conformation of Neu5Ac	Number of Neu5Ac residues	K_i^{HAI} (M)	K_i^{HAI} (**1**)/K_i^{HAI}
β-CD	-	0	nd [a]	
1	*α*	1	2.5 × 10^{-2}	1
29a–29e	*α*	1	2.5 × 10^{-2}	1
31	*β*	1	2.5 × 10^{-2}	1
37	*α*	3	1.2 × 10^{-3}	21
33a–33e	*α*	7	6.2 × 10^{-4}	40
35	*β*	7	6.2 × 10^{-4}	40

39	α	9	1.6×10^{-4}	156
41	α	21	7.8×10^{-5}	321

[a] Not determined.

2.5 Binding of HA to Neu5Ac glycoclusters

Direct binding of the synthesized Neu5Ac glycoclusters to HA was further investigated using SPR. Previously, we reported that β-CD did not bind to HA [45], while Neu5Ac only exhibited low binding affinity to HA with an equilibrium dissociation constant (K_D) of 6.92×10^{-4} M [46]. SPR sensorgrams obtained from injecting mono-, tri-, hepta-, nona-, and 21-valent Neu5Ac glycoclusters at eight different concentrations (0.78–100 μM) are shown in Fig. 3. Unsurprisingly, compared with that of the natural Neu5Ac ligand, the binding affinity of monovalent sialoside **29b** to HA reduced to 82% (Table 3), indicating that the relative bulky hydrophilic group of β-CD weakened binding affinity. However, binding affinity considerably increased for the trivalent sialoside **37**, producing a 20-fold decrease in K_D to 341 μM. The affinity of heptavalent **33b** and nonavalent **39** to HA protein increased >100-fold compared with that of Neu5Ac. Notably, the 21-valent Neu5Ac glycocluster **41** had a considerably increased binding affinity for HA protein, with a 1788-fold and 2191-fold decrease in K_D value than that of Neu5Ac and its monovalent derivative **29b**, respectively, indicating that HA binding affinity was markedly enhanced by increasing the Neu5Ac density of glycoclusters. In 2014, a nanomolar trivalent sialylated glycopeptide has been reported to show strong binding to hemagglutinin H5 of avain influenza (K_D = 450 nM).[12] Similarly, it was also reported that the multivalent 6'-sialyllactose-polyamidoamine (6SL-PAMAM) derivative, S3-G4 6SL-PAMAM dendrimer conjugate, with an average of 20.4 6SL ligands on each PAMAM dendrimer G4, showed the strongest binding to a hemagglutinin trimer ($K_D = 1.6 \times 10^{-7}$ M).[47]

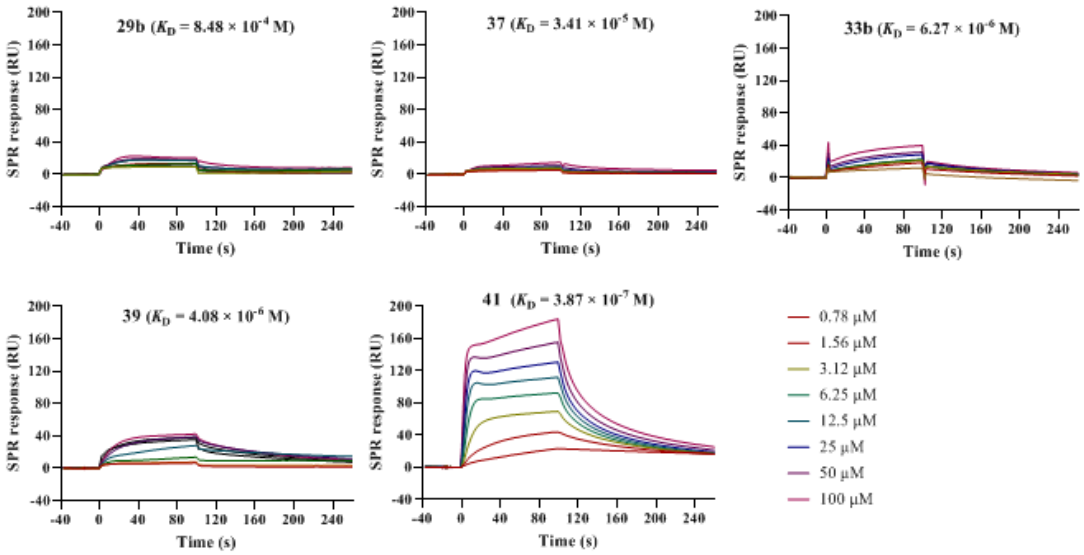


Fig. 3. SPR analyses of the binding kinetics of the mono-, tri-, hepta-, nona-, and 21-valent Neu5Ac glycoclusters with HA protein. Values represent fitted equilibrium dissociation constants (K_D).

Table 3.

Equilibrium dissociation constant (K_D) of Neu5Ac glycoclusters with HA protein.

Compound	Valency	K_D (M)	$K_{D(Neu5Ac)}/K_D$	$K_{D(\mathbf{29b})}/K_D$
β-CD [a]	0	nd [b]	-	-
Neu5Ac [c]	1	6.92×10^{-4}	1.00	-
29b	1	8.48×10^{-4}	0.82	1.00
33	3	3.41×10^{-5}	20.3	24.9
33b	7	6.27×10^{-6}	110	135
39	9	4.08×10^{-6}	169	208
41	21	3.87×10^{-7}	1788	2191

[a] Data reported from Ref [45].

[b] nd: Not determined.

[c] Data reported from Ref [46].

*2.6 Binding of NA to Neu5Ac glycoclusters **41***

NA is another important surface glycoprotein that plays key roles in newly assembled virus particle release by cleaving the sialoside glycosidic linkage. Balance between HA and NA activities has been suggested to be important for virus–receptor binding and virus particles rolling over the receptor surface [48]. Therefore, multiple Neu5Ac groups on **41** may bind to NA, and thereby affect HA–NA balance. To address this, we used SPR to measure the binding affinity of 21-valent Neu5Ac glycoclusters **41** for wild-type NA protein. This afforded a faster association rate constant k_a of 1.63×10^3 M^{-1} s^{-1} and slower dissociation rate constant k_d of 1.80×10^{-2} s^{-1}, yielding an apparent affinity K_D of 1.11×10^{-5} M (Fig. 4A), which is approximately one-thirtieth the potency of that with HA protein. These details of the binding kinetics of **41**–HA and **41**–NA interactions are highly valuable in understanding the role of sialic acid in influenza virus infection and release molecular mechanism. For efficient attachment of influenza virus particle to host cell surface, there is a requirement for a virus to have a rapid and strong avidity to Neu5Ac receptor.

*2.7 Binding of CoV spike protein to Neu5Ac glycocluster **41***

There is increasing evidence that CoV spike proteins bind to sialic acid receptor via the S1 subunit to engage host cells [5, 49, 50]. Recently, Petitjean et al. reported that multivalent sialic acid glycoclusters exhibited potential antiviral activity in SARS-CoV-2-infected A549 cells, with IC_{50} values in the sub-μM range [51]. Therefore, we measured the binding affinity of CoV spike protein interactions with the 21-valent Neu5Ac glycocluster **41** (Fig. 4B-D). Conjugate **41** exhibited high affinity to all three human-infecting CoVs, i.e., SARS-CoV, MERS-CoV, and SARS-CoV-2. The K_D values were in the low nM range, indicating that multivalent Neu5Ac glycoclusters could be potentially inhibit CoV entry into the cell, making them suitable for the early management of COVID-19. Compound **41** exhibited the highest binding affinity for the MERS-CoV spike protein (lower K_D value indicates stronger binding affinity), which was almost twice as potent as that for the SARS-CoV or SARS-CoV-2 spike protein. Additionally, compared with the R_{max} of

SARS-CoV and SARS-CoV-2, a slight drop was observed for the MERS-CoV spike protein R_{max} (50 μM: 58.5 RU *vs.* 100 μM: 88.8 and 81.6 RU, respectively). These results suggest stronger and stable interaction of **41** with MERS-CoV spike protein.

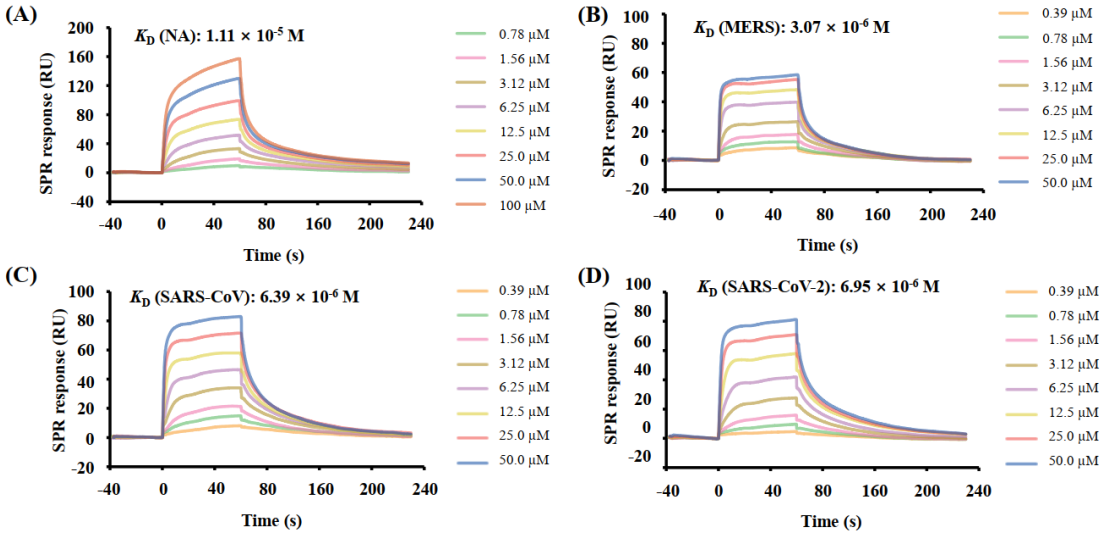


Fig. 4. Binding affinity determination of the 21-valent Neu5Ac glycocluster **41** with influenza virus HA protein (A) and three human-infecting CoV spike protein (B-D). Values shown represent fitted equilibrium dissociation constants.

2.8 Cytotoxicity assay

The cytotoxicity of all final Neu5Ac glycoclusters (**29a–29e**, **31**, **37**, **33a–33e**, and **35**) was assessed using the CellTiter-Glo® luminescent cell viability assay (Promega) and MDCK cell line, which is widely used for evaluating host–pathogen and host–chemical interactions [52]. None of the conjugates exhibited cytotoxicity for MDCK cells at 100 μM (Fig. S4).

3. Conclusions

In this study, we have presented a facile and highly efficient synthetic strategy to access structurally well-defined Neu5Ac glycoclusters. A series of novel Neu5Ac glycoclusters bearing mono-, tri-, hepta-, nona-, and 21-valent Neu5Ac attached to the primary face of natural α- and β-CDs via different linkers was synthesized, unambiguous characterized and evaluated for their binding affinity with influenza HA and NA proteins and three human-infecting CoV spike proteins. We have proven our hypothesis, having demonstrated a prominent strategy to construct Neu5Ac-based multivalent binders with high affinity to several enveloped virus membrane proteins

for the blocking of viral entry into host cells. Our experimental data strongly suggests that the binding affinities of multivalent sialylated CD derivatives to HA and spike proteins depended on the valency of the Neu5Ac ligand. Among the Neu5Ac glycoclusters, the 21-valent Neu5Ac glycocluster **41** had the highest HA binding affinity, with K_D = 3.87 × 10^{-7} M, which was ~1770-fold more potent than that of Neu5Ac and ~30-fold more potent in binding with the NA protein. Even more important, conjugate **41** had high binding affinity for the spike glycoprotein from SARS-CoV, MERS-CoV, and SARS-CoV-2, with K_D values in the low nM range. Thus, these multivalent Neu5Ac derivatives based on a CD scaffold may have strong potential for blocking the interactions with host cells for many viruses. The study is fundamental for further development not only for influenza viruses and CoVs, which represent two of the most important zoonotic threats, described here, but also, in general, for other viruses which use sialic acids as cellular entry receptors. To sum up, given the importance of multivalent effect in many biological processes, notably for virus-cell interactions, this study may provide a general strategy for rationally designing of novel multivalent sialylated derivatives as broad-spectrum entry inhibitors. Further studies on the antiviral activity and mechanism are underway in our laboratories.

4. Experimental

4.1 Materials

α-CD and *β*-CD were supplied by Adamas Reagent Co., Ltd. (Shanghai, China). Neu5Ac was purchased from Tianjin Heowns Biochemical Technology Co., Ltd. (Tianjin, China). Sodium methoxide, AgOTf, and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide were provided by J&K Chemical Ltd. (Beijing, China). Di-tert-butyl dicarbonate, 1-hydroxybenzotriazole, and 3-azidopropylamine were obtained from Alfa Aesar Chemical Co, Ltd. (Beijing, China) All other chemical reagents and solvents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China) and used as received. Red erythrocytes from chickens for the HI assay were provided by Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. (Beijing, China). Recombinant HA protein (Asp18-Ile530) and NA protein from virus

A/Wisconsin/588/2019 (H1N1) were kindly provided by ACRO Biosystems (Beijing, China) and Sino Biological Inc. (Beijing, China), respectively. Recombinant spike protein from SARS-CoV-2 (Val16-Pro1213), SARS (Ser14-Pro1195) and MERS (Tyr18-Trp1295) were all supplied by ACRO Biosystems (Beijing, China).

Three azido-substituted CD intermediates **7** [32], **8** [33], and **9** [34] were prepared using previously described methods. The terminal alkyne–functionalized Neu5Ac derivatives **17a–17b**, **18a–18b**, and **24** [53] and intermediates **19–22** [38] were prepared according to published methods.

4.2 Measurements

The ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance III 400 or 600 MHz spectrometers. ESI–HRMS and ESI–QTOF MS were recorded on a Bruker Apex-Ultra 7.0T FTMS and Waters Synapt G2-Si mass spectrometer, respectively. Matrix-assisted laser desorption ionization–time-of-flight (TOF) mass spectrometry was recorded on a Sciex 72115 TOF/TOF mass spectrometer. Microwave-assisted synthesis was conducted using an open system in a CEM Discover SP microwave reactor. surface plasmon resonance (SPR) measurements were performed on a Biacore 8K instrument (GE Healthcare).

4.3 Synthesis of mono- and multivalent Neu5Ac glycoclusters

*4.3.1 Synthesis of $6^{A,C,E}$-O-trityl-per-O-acetylated-α-CD (**10**)*

To a well-stirred solution of **9** (600 mg, 0.35 mmol) in 5 mL of pyridine was added 2.5 mL of Ac_2O and 8.6 mg of DMAP (0.07 mmol, 0.2 equiv). After stirring for 18 h at rt, the reaction mixture was concentrated *in vacuo* and the resulting crude was dissolved in 50 mL of CH_2Cl_2, washed successively with water (10 mL) and brine (10 mL). The resulting organic phase was dried over Na_2SO_4, and filtered. The solvent was removed *in vacuo* and the crude product was purified by flash chromatography eluted with petroleum ether/acetone (6:1 to 1:1) to afford **10** (751 mg, 91%) as a white foam. R_f = 0.50 (petroleum ether/acetone = 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40 (d, J = 7.4 Hz, 18H), 7.29 – 7.23 (m, 18H), 7.19 (t, J = 7.2 Hz, 9H), 5.45 (t, J = 9.5 Hz, 3H), 5.29 (dd, J = 9.7, 8.3 Hz, 3H), 5.11 (dt, J = 15.6, 5.0 Hz, 6H), 4.52 (d, J = 3.0 Hz, 3H), 4.46 (dd, J = 10.0, 3.1 Hz, 3H), 4.33 (t, J = 9.2 Hz, 3H), 3.99 – 3.92 (m, 3H),

3.77 – 3.65 (m, 9H), 3.52 – 3.44 (m, 3H), 3.38 – 3.28 (m, 6H), 2.17 – 2.16 (m, 9H), 2.16 (s, 9H), 2.06 (s, 9H), 2.04 (s, 9H), 1.96 (s, 9H), 1.71 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.32, 170.29, 170.18, 169.51, 169.45, 143.55, 128.66, 127.75, 126.92, 98.46, 94.96, 86.33, 76.50, 76.12, 73.46, 71.67, 71.40, 70.99, 70.06, 67.86, 63.23, 61.57, 21.03, 20.80, 20.72, 20.69, 20.36. ESI-HRMS (m/z) $[M + Na]^+$ calcd for $C_{123}H_{132}O_{45}$, 2351.7933, found, 2351.7937.

*4.3.2 Synthesis of 6^{A,C,E}-trihydroxyl-per-O-acetylated-α-CD (**11**)*

To a solution of **10** (50.0 mg, 0.021 mmol) in 3 mL of acetonitrile was added 28 mg of *p*-toluene sulfonic acid (0.16 mmol). After stirring for 30 min at rt and the reaction was monitored by TLC, the reaction mixture was adjusted to pH to 7.0 with ammonia solution. The solution was diluted with 20 mL of CH_2Cl_2 and washed successively with water (10 mL) and brine (10 mL). The resulting organic layer was dried with Na_2SO_4 and filtered. The solvent was removed *in vacuo* and the crude product was purified by flash chromatography eluted with petroleum ether/acetone (2:1 to 1:1) to afford **11** (28 mg, 81%) as a white foam. R_f = 0.15 (petroleum ether/acetone = 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.52 (dd, *J* = 9.1, 8.7 Hz, 3H), 5.45 (t, 3H), 5.13 (d, *J* = 3.6 Hz, 3H), 5.09 (d, *J* = 3.5 Hz, 3H), 4.86 (dd, *J* = 9.9, 3.6 Hz, 3H), 4.76 (dd, *J* = 10.0, 3.5 Hz, 3H), 4.53 (d, *J* = 10.9 Hz, 3H), 4.40 (dd, *J* = 12.3, 4.9 Hz, 3H), 4.29 – 4.21 (m, 3H), 4.13 – 4.03 (m, 3H), 3.97 – 3.74 (m, 12H), 2.17 (s, 9H), 2.08 (d, *J* = 2.6 Hz, 27H), 2.04 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.09, 170.63, 170.60, 169.21, 169.13, 96.37, 96.31, 77.22, 76.12, 72.30, 71.49, 71.01, 70.90, 70.14, 69.52, 63.45, 61.98, 20.84, 20.76, 20.71. ESI-HRMS (m/z) $[M + H]^+$ calcd for $C_{66}H_{90}O_{45}$, 1603.4827, found, 1603.4825.

*4.3.3 Synthesis of 6^{A,C,E}-trideoxy-6^{A,C,E}-triiodo-per-O-acetylated-α-CD (**12**)*

Compound **11** (200 mg, 0.12 mmol) was dissolved in 20 mL of toluene, 103 mg of triphenylphosphine (0.39 mmol), 118 mg of iodine (0.47 mmol) and 8.9 mg of imidazole (0.13 mmol) was added to the reaction mixture. After stirring for 2 h under nitrogen at 70°C, the reaction mixture was quenched by the addition of sodium sulfite solution. Then the solution was diluted with 40 mL of CH_2Cl_2 and washed successively with water (10 mL) and brine (10 mL). The resulting organic layer was dried with

Na_2SO_4 and filtered. The solvent was removed *in vacuo* and the crude product was purified by flash chromatography eluted with petroleum ether/acetone (1:1 to 1:1) to afford **12** (140 mg, 78%) as a white foam. R_f = 0.50 (petroleum ether/acetone = 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.62 (t, *J* = 9.5 Hz, 3H), 5.48 (t, *J* = 8.7 Hz, 3H), 5.15 (d, *J* = 3.6 Hz, 3H), 5.07 (d, *J* = 2.9 Hz, 3H), 4.88 (dd, *J* = 10.3, 3.7 Hz, 3H), 4.77 (dd, *J* = 10.2, 3.2 Hz, 3H), 4.57 (s, 6H), 4.17 (s, 3H), 3.93 (s, 3H), 3.84 (t, *J* = 9.1 Hz, 3H), 3.69 (dd, *J* = 16.2, 8.3 Hz, 6H), 3.58 – 3.47 (m, 3H), 2.22 (s, 9H), 2.09 (s, 9H), 2.08 (s, 9H), 2.06 (s, 9H), 2.02 (d, *J* = 5.3 Hz, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.64, 170.59, 170.48, 169.23, 169.05, 97.00, 96.62, 81.42, 77.57, 71.13, 70.76, 70.65, 70.47, 70.22, 69.42, 63.46, 53.83, 20.99, 20.80, 20.70. ESI-HRMS (m/z) $[M + H]^+$ calcd for $C_{66}H_{88}I_3O_{42}$, 1933.1879, found, 1933.1891, $[M + NH_4]^+$ calcd for $C_{66}H_{91}NI_3O_{42}$, 1950.2150, found, 1950.2153.

*4.3.4 Synthesis of 6^{A,C,E}-triazido-6^{A,C,E}-trideoxy-per-O-acetylated-α-CD (**13**)[54, 55]*

Compound **12** (24 mg, 0.012 mmol) was dissolved in 2 mL of DMF, 8.3 mg of NaN_3 (0.075 mmol) was added and stirred for 8 h at 80 °C. It was diluted with 20 mL of water and then extracted with CH_2Cl_2 (10 mL × 3). The resulting organic layer was dried with Na_2SO_4 and filtered. The solvent was removed *in vacuo* and the crude product was purified by flash chromatography eluted with petroleum ether/acetone (4:1 to 1:1) to afford **13** (18 mg, 86%) as a white foam. R_f = 0.33 (petroleum ether/acetone = 1:1).

*4.3.5 Synthesis of methyl (2-(2-(prop-2-yn-1-yloxy)ethoxy)-5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)onate (**17c**) and methyl (2-(2-(prop-2-yn-1-yloxy)ethoxy)-5-acetamido-3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosyl)onate (**18c**)*

To a well-stirred solution of **16c** (276 mg, 0.48 mmol) was dissolved in 8.5 mL of methanol, a 30 wt% solution of sodium methoxide in methanol (25 μL, 0.19 mmol) was added. After stirring for 6 h at rt, the reaction mixture was neutralized with ion-exchange resin (Amberlite IR-120) (H^+). Filtered the resin in a sintered glass filter and washed with methanol, and the solvent was removed *in vacuo* and the crude

product was purified by flash chromatography eluted with CH_2Cl_2/CH_3OH (10:1 to 8:1). The initial fractions collected to afford *α*-isomer **17c** (78 mg, 40%) and the later fractions to afford *β*-isomer **18c** (88 mg, 45%).

α-Isomer (**17c**): R_f = 0.25 (CH_2Cl_2/CH_3OH = 8:1). ^{1}H NMR (400 MHz, CD_3OD): *δ* 4.17 (d, *J* = 2.4 Hz, 2H), 3.94 (s, 1H), 3.87 – 3.73 (m, 6H), 3.69 – 3.54 (m, 6H), 3.50 (dd, *J* = 8.8, 1.3 Hz, 1H), 2.85 (t, *J* = 2.4 Hz, 1H), 2.70 (dd, *J* = 12.8, 4.6 Hz, 1H), 2.00 (s, 3H), 1.75 (t, *J* = 12.3 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD): *δ* 175.15, 170.83, 100.16, 80.47, 76.00, 74.90, 72.42, 70.20, 69.74, 68.54, 64.73, 64.47, 59.02, 53.77, 53.44, 41.59, 22.69. ESI-HRMS (m/z) $[M + H]^+$ calcd for $C_{17}H_{27}NO_{10}$, 406.1708, found, 406.1711.

β-Isomer (**18c**): R_f = 0.18 (CH_2Cl_2/CH_3OH = 8:1). ^{1}H NMR (400 MHz, CD_3OD): *δ* 4.19 (d, *J* = 2.4 Hz, 2H), 4.08 – 3.99 (m, 1H), 3.93 – 3.76 (m, 8H), 3.71 – 3.60 (m, 3H), 3.54 – 3.42 (m, 2H), 2.86 (t, *J* = 2.4 Hz, 1H), 2.41 (dd, *J* = 13.0, 4.9 Hz, 1H), 2.01 (s, 3H), 1.66 (dd, *J* = 12.9, 11.3 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD): *δ* 174.89, 170.62, 100.02, 80.53, 76.00, 72.47, 71.41, 70.15, 69.78, 67.65, 65.23, 63.76, 59.00, 53.79, 53.17, 41.58, 22.71. ESI-HRMS (m/z) $[M + H]^+$ calcd for $C_{17}H_{27}NO_{10}$, 406.1708, found, 406.1712.

*4.3.6 Synthesis of methyl (2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)-5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)onate **(17d)** and methyl (2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)-5-acetamido-3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosyl)onate **(18d)***

The deacetylation of **16d** (265 mg, 0.43 mmol) was carried out as described for the preparation of **17c** and **17d** to afford, after purified by flash chromatography eluted with CH_2Cl_2/CH_3OH (10:1 to 8:1), *α*-isomer **17d** (82 mg, 37%) and *β*-isomer **18d** (98 mg, 51%).

α-Isomer (**17d**): R_f = 0.25 (CH_2Cl_2/CH_3OH = 8:1). ^{1}H NMR (400 MHz, CD_3OD): *δ* 4.19 (d, *J* = 2.4 Hz, 2H), 3.96 – 3.88 (m, 1H), 3.87 – 3.79 (m, 5H), 3.76 (d, *J* = 10.2 Hz, 1H), 3.69 – 3.54 (m, 10H), 3.50 (dd, *J* = 8.7, 1.5 Hz, 1H), 2.84 (t, *J* = 2.4 Hz, 1H), 2.70 (dd, *J* = 12.8, 4.6 Hz, 1H), 2.00 (s, 3H), 1.81 – 1.69 (t, *J* = 12.3 Hz, 1H). ^{13}C

NMR (101 MHz, CD_3OD): δ 175.19, 170.92, 100.22, 80.63, 75.94, 74.95, 72.49, 71.43, 71.21, 70.25, 70.09, 68.61, 64.78, 64.71, 59.07, 53.82, 53.45, 41.66, 22.70. ESI-HRMS (m/z) $[M + H]^+$ calcd for $C_{19}H_{31}NO_{11}$, 450.1970, found, 450.1977.

β-Isomer (**18d**): R_f = 0.20 (CH_2Cl_2/ CH_3OH = 8:1). ^1H NMR (400 MHz, CD_3OD): δ 4.20 (d, *J* = 2.4 Hz, 2H), 4.10 – 3.97 (m, 1H), 3.92 – 3.75 (m, 8H), 3.72 – 3.58 (m, 7H), 3.53 – 3.41 (m, 2H), 2.87 (t, *J* = 2.4 Hz, 1H), 2.40 (dd, *J* = 13.0, 4.9 Hz, 1H), 2.03 (s, 3H), 1.66 (dd, *J* = 12.8, 11.4 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD): δ 175.00, 170.66, 99.97, 80.61, 76.09, 72.34, 71.43, 71.29, 71.23, 70.18, 70.11, 67.66, 65.23, 63.92, 59.03, 53.89, 53.23, 41.55, 22.69. ESI-HRMS (m/z) $[M + H]^+$ calcd for $C_{19}H_{31}NO_{11}$, 450.1970, found, 450.1977.

*4.3.7 Synthesis of methyl (2-(2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethoxy)-5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)onate (**17e**) and methyl (2-(2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethoxy)-5-acetamido-3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosyl)onate (**18e**)*

The deacetylation of **16e** (325 mg, 0.49 mmol) was carried out as described for the preparation of **17c** and **17d** to afford, after purified by flash chromatography eluted with CH_2Cl_2/CH_3OH (10:1 to 8:1), α-isomer **17e** (103 mg, 42%) and β-isomer **18e** (112 mg, 46%).

α-Isomer (**17e**): R_f = 0.40 (CH_2Cl_2/CH_3OH = 7:1); ^1H NMR (400 MHz, CD_3OD): δ 4.19 (d, *J* = 2.4 Hz, 2H), 3.96 – 3.88 (m, 1H), 3.87 – 3.78 (m, 5H), 3.76 (d, *J* = 10.2 Hz, 1H), 3.71 – 3.54 (m, 14H), 3.50 (dd, *J* = 8.7, 1.4 Hz, 1H), 2.85 (t, *J* = 2.4 Hz, 1H), 2.70 (dd, *J* = 12.8, 4.6 Hz, 1H), 2.00 (s, 3H), 1.75 (t, *J* = 12.3 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD): δ 175.18, 170.91, 100.21, 80.64, 75.95, 74.93, 72.48, 71.59, 71.53, 71.38, 71.22, 70.24, 70.13, 68.59, 64.76, 64.72, 59.05, 53.81, 53.46, 41.65, 22.71. ESI-HRMS (m/z) $[M + H]^+$ calcd for $C_{21}H_{35}NO_{12}$, 494.2232, found, 494.2245.

β-Isomer (**18e**): R_f = 0.28 (CH_2Cl_2/ CH_3OH = 7:1); ^1H NMR (400 MHz, CD_3OD): δ 4.20 (d, *J* = 2.3 Hz, 2H), 4.10 – 3.95 (m, 1H), 3.93 – 3.75 (m, 8H), 3.73 – 3.55 (m, 11H), 3.47 (dd, *J* = 18.1, 9.9 Hz, 2H), 2.88 (t, *J* = 2.3 Hz, 1H), 2.40 (dd, *J* = 12.9, 4.9 Hz, 1H), 2.02 (s, 3H), 1.66 (dd, *J* = 12.6, 11.6 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD):

δ 174.81, 170.61, 99.87, 80.56, 76.09, 72.30, 71.40, 71.37, 71.34, 71.17, 71.13, 70.12, 70.00, 67.54, 65.16, 63.83, 58.96, 53.71, 53.22, 41.49, 22.78. ESI-HRMS (m/z) $[M + H]^+$ calcd for $C_{21}H_{35}NO_{12}$, 494.2232, found, 494.2240.

*4.3.8 Synthesis of tert-butyl N-tri((3-((3-azidopropyl)amino)-3-oxopropoxy)methyl)carbamate (**23**)*

To an ice-cooled (0°C) solution of **22** (167 mg, 0.38 mmol) in 2 mL DMF was added 236 mg of EDC (1.52 mmol) and 206 mg of HOBt (1.52 mmol). After stirring for 30 min at 0°C, 174 mg of 3-azidopropanamine (1.52 mmol) and 197 mg of DIPEA (1.52 mmol) were added. The resulting mixture was allowed to warm to room temperature and continuously stirred for 6 h. The reaction mixture was concentrated *in vacuo* and the resulting crude was dissolved in 30 mL of CH_2Cl_2, washed successively with water (10 mL) and brine (10 mL). The resulting organic phase was dried over Na_2SO_4, and filtered. The solvent was removed *in vacuo* and the crude product was purified by flash chromatography eluted with petroleum ether/acetone (2:1 to 1:1) to provide **23** (157 mg, 60%) as an oil. R_f = 0.45 (CH_2Cl_2/MeOH = 9:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.93 (t, *J* = 5.8 Hz, 3H), 5.02 (s, 1H), 3.65 (t, *J* = 5.7 Hz, 6H), 3.57 (s, 6H), 3.36 – 3.23 (m, 12H), 2.37 (t, *J* = 5.7 Hz, 6H), 1.76 (p, *J* = 6.7 Hz, 6H), 1.37 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.58, 154.93, 79.38, 69.61, 67.29, 58.43, 49.13, 36.84, 36.65, 28.77, 28.31. ESI-HRMS (m/z) $[M + H]^+$ calcd for $C_{27}H_{50}N_{13}O_8$, 684.3900, found, 684.3887.

*4.3.9 Synthesis of tert-butyl N-(tri(9-(4-(3-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)prop-1-yl)-1H-1,2,3-triazol-1-yl)-2-oxa-5-oxo-6-aza-nona-1-yl)methyl)carbamate (**25**)*

Copper (II) sulfate (6.4 mg, 0.04 mmol) and sodium ascorbate (20 mg, 0.10 mmol) was added to a solution of **23** (95 mg, 0.17 mmol) and **24** (39 mg, 0.057 mmol) in 1:1 methanol/water mixture (5 mL). The reaction mixture was stirred vigorously for 10 h at rt and was then diluted with 20 mL of CH_2Cl_2, and washed successively with water (5 mL) and brine (5 mL). The resulting organic phase was dried over Na_2SO_4, and filtered. The solvent was removed *in vacuo* and the crude product was purified by flash

chromatography eluted with CH_2Cl_2/CH_3OH (30:1 to 10:1) to afford **25** (95 mg, 70%) as an oil. R_f = 0.30 (CH_2Cl_2/CH_3OH = 9:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47 (s, 3H), 5.70 (d, *J* = 9.5 Hz, 3H), 5.43 – 5.26 (m, 6H), 4.83 (ddd, *J* = 12.4, 9.9, 4.6 Hz, 3H), 4.38 (t, *J* = 6.7 Hz, 6H), 4.31 (dd, *J* = 12.4, 2.1 Hz, 3H), 4.14 – 3.99 (m, 9H), 3.80 (dd, *J* = 10.7, 4.8 Hz, 3H), 3.76 (s, 9H), 3.70 – 3.64 (m, 6H), 3.64 (s, 6H), 3.35 – 3.18 (m, 9H), 2.83 – 2.67 (m, 6H), 2.58 (dd, *J* = 12.8, 4.6 Hz, 3H), 2.41 (t, *J* = 5.5 Hz, 6H), 2.12 (s, 9H), 2.12 (s, 9H), 2.07 (dd, *J* = 11.6, 4.6 Hz, 6H), 2.02 (s, 9H), 2.01 (s, 9H), 1.91 (dd, *J* = 15.7, 9.5 Hz, 9H), 1.86 (s, 9H), 1.33 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.01, 170.93, 170.74, 170.44, 170.16, 168.46, 155.01, 147.32, 121.50, 98.79, 79.31, 72.42, 69.62, 69.14, 68.69, 67.43, 64.11, 62.38, 58.67, 52.72, 49.31, 47.61, 37.98, 36.77, 36.28, 30.27, 29.27, 28.32, 23.10, 22.12, 21.09, 20.84, 20.83, 20.76. ESI-HRMS (m/z) $[M + H]^+$ calcd for $C_{102}H_{155}N_{16}O_{47}$, 2356.0225, found, 2356.0227.

*4.3.10 Synthesis of N-tri(9-(4-(3-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)prop-1-yl)-1H-1,2,3-triazol-1-yl)-2-oxa-5-oxo-6-aza-nona-1-yl)methyl pent-4-ynamide (**27**)*

To a solution of **25** (49 mg, 0.02 mmol) in 2 mL of CH_2Cl_2 was added 0.5 mL of TFA dropwise. After stirring for 30 min at rt, the reaction mixture was concentrated *in vacuo* and the crude product **26** was used directly for the next reaction withour further purification.

To a ice-cooled solution (0°C) of 4-pentynoic acid (3.4 mg, 0.03 mmol) in 2 mL of DMF was added 4.7 mg of EDC (0.03 mmol) and 4.0 mg of HOBt (0.03 mmol). After stirring at 0°C for 30 min, 47 mg of amine intermediate (0.02 mmol) and 3.8 mg of DIPEA (0.03 mmol) were added. The resulting mixture was allowed to warm to room temperature and continuously stirred for 6 h. The reaction mixture was concentrated *in vacuo* and the resulting crude was dissolved in 30 mL of CH_2Cl_2, washed successively with water (10 mL) and brine (10 mL). The resulting organic phase was dried over Na_2SO_4, and filtered. The solvent was removed *in vacuo* and the crude product was purified by flash chromatography eluted with CH_2Cl_2/CH_3OH (30:1 to 10:1) to afford **27** (38 mg, 78%) as an oil. R_f = 0.28 (CH_2Cl_2/CH_3OH = 9:1). ^{1}H NMR (400 MHz,

$CDCl_3$): δ 7.46 (s, 3H), 7.17 (d, J = 6.0 Hz, 3H), 6.74 (s, 1H), 5.79 – 5.54 (m, 3H), 5.40 – 5.30 (m, 6H), 4.92 – 4.75 (m, 3H), 4.39 (t, J = 6.7 Hz, 6H), 4.32 (dd, J = 12.3, 2.0 Hz, 3H), 4.17 – 3.99 (m, 9H), 3.87 – 3.78 (m, 3H), 3.77 (s, 9H), 3.70 (s, 6H), 3.66 (t, J = 5.3 Hz, 6H), 3.34 – 3.21 (m, 9H), 2.82 – 2.68 (m, 6H), 2.59 (dd, J = 12.8, 4.4 Hz, 3H), 2.46 – 2.37 (m, 8H), 2.37 – 2.26 (m, 2H), 2.13 (s, 18H), 2.11 – 2.06 (m, 6H), 2.03 (s, 9H), 2.02 (s, 9H), 2.01 – 1.99 (m, 1H), 1.97 – 1.88 (m, 9H), 1.87 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.98, 171.61, 170.92, 170.72, 170.37, 170.12, 168.45, 147.63, 121.41, 98.79, 83.14, 72.43, 70.53, 69.58, 69.14, 68.62, 67.42, 64.12, 62.37, 59.90, 52.71, 49.35, 47.64, 38.00, 36.65, 36.36, 35.54, 30.30, 29.28, 23.13, 22.13, 21.10, 20.84, 20.77, 14.78. ESI-QTOF-MS (m/z) $[M + H]^{1+}$ calcd for $C_{102}H_{151}N_{16}O_{46}$, 2335.9968, found 2335.9553.

*4.3.11 Synthesis of 6^A-deoxy-6^A-(4-((methyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)eth-2-yl)-1 H-1,2,3-triazol-1-yl)-per-O-acetylated-β-CD (**28a**)*

Copper (II) sulfate (3.2 mg, 0.02 mmol) and sodium ascorbate (20 mg, 0.10 mmol) was added to a solution of **7** (140 mg, 0.072 mmol) and **17a** (30 mg, 0.079 mmol) in 1:1 methanol/water mixture (5 mL). The reaction mixture was stirred vigorously for 6 h at rt and was then diluted with 20 mL of CH_2Cl_2, and washed successively with water (5 mL) and brine (5 mL). The resulting organic phase was dried over Na_2SO_4, and filtered. The solvent was removed *in vacuo* and the crude product was purified by flash chromatography eluted with CH_2Cl_2/CH_3OH (15:1 to 10:1) to afford **28a** (120 mg, 70%) as a white foam. R_f = 0.27 (CH_2Cl_2/CH_3OH = 8:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.54 (s, 1H), 5.60 (d, J = 3.5 Hz, 1H), 5.43 – 5.20 (m, 8H), 5.18 (d, J = 3.9 Hz, 1H), 5.14 (d, J = 4.0 Hz, 1H), 5.10 (d, J = 4.0 Hz, 1H), 5.07 (d, J = 3.5 Hz, 2H), 5.04 (d, J = 3.7 Hz, 1H), 4.91 (dd, J = 8.9, 3.8 Hz, 1H), 4.83 (td, J = 9.8, 3.9 Hz, 3H), 4.76 (ddd, J = 9.8, 7.9, 3.7 Hz, 2H), 4.71 (d, J = 11.5 Hz, 1H), 4.65 – 4.46 (m, 8H), 4.37 – 4.17 (m, 10H), 4.15 – 4.03 (m, 4H), 3.87 (d, J = 9.9 Hz, 2H), 3.82 (s, 3H), 3.80 – 3.49 (m, 16H), 3.43 (d, J = 10.4 Hz, 1H), 3.03 – 2.91 (m, 2H), 2.77 – 2.73 (m, 1H), 2.17 – 2.00 (m, 63H), 1.83 (dd, J = 15.3, 9.2 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 173.42, 171.31, 171.28, 171.05, 170.95, 170.92, 170.90, 170.80,170.78, 170.75, 170.53, 169.87, 169.80, 169.69, 169.61,

169.59, 169.57, 169.54,169.49, 144.61,125.30, 98.84, 97.29, 96.95, 96.87, 96.84, 96.70, 96.64, 96.61, 77.36, 77.32, 77.24, 77.07, 77.01, 76.76, 76.47, 75.94, 74.34, 71.71, 71.50, 71.46, 71.19, 71.01, 70.53, 70.43, 70.37, 70.23, 70.08, 70.00, 69.95, 69.82, 69.76, 69.69, 69.61, 69.41, 68.29, 64.51, 63.11, 63.07, 62.90, 62.76, 62.68, 62.40, 53.37, 53.27, 49.36, 40.67, 26.50, 23.28, 21.05, 21.03, 21.02, 21.00, 20.97, 20.96, 20.95, 20.91, 20.86, 20.83. ESI-HRMS calcd for $C_{98}H_{134}N_4NaO_{63}$ $[M + Na]^+$: 2397.7302, found 2397.7371.

*4.3.12 Synthesis of 6^A-deoxy-6^A-(4-((methyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)prop-3-yl)-1H-1,2,3-triazol-1-yl)-per-O-acetylated-β-CD (**28b**)*

The click reaction between **7** (235 mg, 0.12 mmol) and **17b** (52 mg, 0.13 mmol) was carried out as described for the preparation of **28a** to provide, after purified by flash chromatography eluted with CH_2Cl_2/CH_3OH (15:1 to 8:1), **28b** (148 mg, 62%) as a white foam. R_f = 0.32 (CH_2Cl_2/CH_3OH = 8:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.47 (s, 1H), 6.40 (d, *J* = 7.1 Hz, 1H), 5.61 (d, *J* = 3.7 Hz, 1H), 5.30 (tt, *J* = 17.6, 9.2 Hz, 6H), 5.21 (t, *J* = 7.9 Hz, 1H), 5.18 – 5.13 (m, 2H), 5.12 (d, *J* = 4.0 Hz, 1H), 5.09 (d, *J* = 4.0 Hz, 1H), 5.07 – 5.04 (m, 2H), 5.02 (d, *J* = 3.6 Hz, 1H), 4.90 (dd, *J* = 8.8, 3.9 Hz, 1H), 4.83 (dt, *J* = 8.8, 3.4 Hz, 4H), 4.78 – 4.72 (m, 2H), 4.70 (d, *J* = 11.3 Hz, 1H), 4.62 (dd, *J* = 14.7, 2.7 Hz, 1H), 4.59 – 4.50 (m, 5H), 4.45 (d, *J* = 11.4 Hz, 1H), 4.35 – 4.13 (m, 10H), 4.13 – 4.03 (m, 3H), 3.92 – 3.84 (m, 2H), 3.84 – 3.68 (m, 13H), 3.68 – 3.61 (m, 2H), 3.54 (t, *J* = 9.1 Hz, 2H), 3.46 (d, *J* = 10.4 Hz, 1H), 3.37 (dt, *J* = 9.4, 5.7 Hz, 1H), 2.81 (dt, *J* = 14.7, 7.3 Hz, 1H), 2.76 – 2.69 (m, 3H), 2.14 – 2.00 (m, 60H), 1.92 (s, 4H), 1.88 – 1.81 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 173.53, 171.01, 170.91, 170.88, 170.83, 170.77, 170.74, 170.71, 170.53, 170.48, 170.09, 169.74, 169.62, 169.56, 169.53, 169.47, 169.46, 169.42, 147.11, 124.47, 98.77,97.20, 97.08, 96.92, 96.84, 96.74, 96.67, 96.57, 77.53, 77.36, 77.12, 77.00, 76.74, 76.58, 75.97, 74.03, 71.58, 71.48, 71.40, 71.11, 71.03, 70.96, 70.65, 70.55, 70.51, 70.39, 70.20, 70.12, 70.06, 69.94, 69.80, 69.77, 69.74, 69.68, 69.65, 69.43, 68.39, 64.47, 63.04, 62.86, 62.79, 62.75, 62.61, 62.41, 58.54, 53.31, 53.27, 49.34, 40.41, 29.20, 23.21, 21.98, 21.01,

21.00, 20.99, 20.96, 20.94, 20.90, 20.88, 20.87, 20.85, 20.83, 20.80. ESI-HRMS (m/z) $[M + H]^+$ calcd for $C_{99}H_{136}N_4O_{63}$, 2411.7453, found, 2411.7441.

*4.3.13 Synthesis of 6^A-deoxy-6^A-(4-((methyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)ethoxymethyl)-1H-1,2,3-triazol-1-yl)-per-O-acetylated-β-CD (**28c**)*

The click reaction between **7** (80 mg, 0.04 mmol) and **17c** (29 mg, 0.044 mmol) was carried out as described for the preparation of **28a** to afford, after purified by flash chromatography eluted with CH_2Cl_2/CH_3OH (9:1), **28c** (50 mg, 69%) as a white foam. R_f = 0.25 (CH_2Cl_2/CH_3OH = 9:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.72 (s, 1H), 6.11 (s, 1H), 5.59 (d, *J* = 3.6 Hz, 1H), 5.39 – 5.24 (m, 6H), 5.24 – 5.14 (m, 3H), 5.11 (dd, *J* = 9.7, 4.0 Hz, 2H), 5.09 – 5.02 (m, 3H), 4.93 (dd, *J* = 8.6, 3.8 Hz, 1H), 4.88 – 4.80 (m, 3H), 4.76 (ddd, *J* = 9.6, 5.8, 3.8 Hz, 3H), 4.73 – 4.63 (m, 4H), 4.56 (t, *J* = 14.0 Hz, 5H), 4.45 (d, *J* = 11.5 Hz, 1H), 4.37 – 4.17 (m, 9H), 4.16 – 4.04 (m, 4H), 4.00 – 3.85 (m, 3H), 3.85 – 3.60 (m, 15H), 3.60 – 3.50 (m, 3H), 3.44 (d, *J* = 10.4 Hz, 1H), 2.78 (dd, *J* = 12.9, 4.3 Hz, 1H), 2.26 – 1.94 (m, 63H), 1.93 – 1.85 (m, 1H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 173.42, 171.00, 170.96, 170.83, 170.78, 170.68, 170.65, 170.63, 170.44, 170.41, 169.79, 169.65, 169.58, 169.48, 169.44, 169.39, 169.35, 169.33, 165.84, 144.79, 125.84, 98.70, 97.12, 96.91, 96.83, 96.65, 96.60, 96.53, 77.27, 77.18, 77.02, 76.97, 76.65, 76.59, 75.95, 74.24, 71.49, 71.39, 71.02, 70.88, 70.84, 70.65, 70.44, 70.41, 70.28, 70.09, 70.05, 69.92, 69.83, 69.73, 69.70, 69.65, 69.56, 69.32, 69.15, 68.18, 64.52, 64.37, 63.80, 62.83, 62.75, 62.69, 62.67, 62.57, 62.31, 53.29, 53.24, 49.43, 40.41, 23.17, 20.95, 20.93, 20.92, 20.90, 20.89, 20.87, 20.82, 20.80, 20.79, 20.77, 20.75, 20.72. ESI-HRMS (m/z) $[M + Na]^+$ calcd for $C_{99}H_{136}N_4O_{64}$, 2427.7403, found, 2427.7393.

*4.3.14 Synthesis of 6^A-deoxy-6^A-(4-(((methyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)ethoxyl)ethoxymethyl)-1H-1,2,3-triazol-1-yl)-per-O-acetylated-β-CD (**28d**)*

The click reaction between **7** (100 mg, 0.05 mmol) and **17c** (25 mg, 0.055 mmol) was carried out as described for the preparation of **28a** to afford, after purified by flash chromatography eluted with CH_2Cl_2/CH_3OH (9:1), **28d** (75 mg, 61%) as a white foam.

R_f = 0.20 (CH_2Cl_2/CH_3OH = 10:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 (s, 1H), 6.92 (d, *J* = 6.2 Hz, 1H), 5.60 (d, *J* = 3.7 Hz, 1H), 5.39 – 5.18 (m, 7H), 5.14 (d, *J* = 4.0 Hz, 1H), 5.09 (d, *J* = 4.0 Hz, 1H), 5.07 (d, *J* = 4.0 Hz, 1H), 5.04 (d, *J* = 3.6 Hz, 1H), 5.02 (d, *J* = 3.7 Hz, 1H), 4.99 (d, *J* = 3.6 Hz, 1H), 4.86 (dd, *J* = 9.0, 3.8 Hz, 1H), 4.83 – 4.77 (m, 3H), 4.73 (dd, *J* = 9.8, 3.4 Hz, 2H), 4.70 – 4.59 (m, 4H), 4.60 – 4.46 (m, 5H), 4.42 (d, *J* = 11.3 Hz, 1H), 4.36 – 4.22 (m, 5H), 4.22 – 4.14 (m, 4H), 4.14 – 4.04 (m, 4H), 3.95 – 3.45 (m, 29H), 3.45 – 3.31 (m, 1H), 2.79 – 2.53 (m, 1H), 2.23 – 1.92 (m, 63H), 1.88 – 1.70 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 173.78, 170.83, 170.77, 170.73, 170.70, 170.59, 170.57, 170.40, 170.38, 169.80, 169.63, 169.55, 169.53, 169.43, 169.41, 169.39, 144.60, 125.88, 98.77, 97.02, 96.93, 96.77, 96.70, 96.56, 96.42, 77.12, 76.93, 76.77, 76.55, 76.45, 75.89, 73.99, 71.42, 71.35, 71.29, 70.98, 70.88, 70.52, 70.47, 70.42, 70.33, 70.16, 70.02, 69.94, 69.82, 69.79, 69.68, 69.60, 69.54, 69.43, 69.26, 67.64, 64.30, 64.06, 63.50, 62.78, 62.59, 62.56, 62.26, 53.22, 52.99, 49.33, 40.07, 22.91, 20.85, 20.83, 20.79, 20.78, 20.73, 20.67, 20.65. ESI-HRMS (m/z) $[M + H]^+$ calcd for $C_{101}H_{140}N_4O_{65}$, 2449.7845, found, 2449.7834.

*4.3.15 Synthesis of 6^A-deoxy-6^A-(4-(((methyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)ethoxyl)ethoxyl)ethoxymethyl)-1H-1,2,3-triazol-1-yl)-per-O-acetylated-β-CD (**28e**)*

The click reaction between **7** (200 mg, 0.10 mmol) and **17c** (59 mg, 0.11 mmol) was carried out as described for the preparation of **28a** to afford, after purified by flash column chromatography on silica gel (CH_2Cl_2/CH_3OH, 9:1), 147 mg **28e** (59%) as a white foam. R_f = 0.40 (CH_2Cl_2/CH_3OH = 8:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (s, 1H), 7.05 (d, *J* = 7.4 Hz, 1H), 5.59 (d, *J* = 3.7 Hz, 1H), 5.40 – 5.20 (m, 7H), 5.20 – 5.11 (m, 2H), 5.09 (d, *J* = 4.0 Hz, 1H), 5.07 – 5.00 (m, 3H), 4.98 (d, *J* = 3.6 Hz, 1H), 4.86 (dd, *J* = 9.1, 3.8 Hz, 1H), 4.79 (ddd, *J* = 7.4, 3.7, 1.7 Hz, 3H), 4.75 – 4.69 (m, 2H), 4.65 (d, *J* = 14.3 Hz, 4H), 4.53 (d, *J* = 11.1 Hz, 3H), 4.48 (dd, *J* = 10.3, 3.6 Hz, 2H), 4.43 (d, *J* = 11.4 Hz, 1H), 4.34 – 4.11 (m, 10H), 4.11 – 4.00 (m, 3H), 3.94 – 3.80 (m, 4H), 3.80 – 3.49 (m, 26H), 3.49 – 3.35 (m, 3H), 2.71 (d, *J* = 8.9 Hz, 1H), 2.20 – 1.90 (m, 63H), 1.85 (t, *J* = 11.9 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 173.80, 170.82, 170.78, 170.73, 170.71, 170.67, 170.59, 170.58, 170.52, 170.41, 170.37, 169.79, 169.64, 169.52,

169.43, 169.41, 169.38, 144.50, 125.91, 98.70, 97.04, 96.95, 96.75, 96.68, 96.61, 96.46, 96.42, 76.99, 76.93, 76.78, 76.65, 76.36, 75.84, 73.97, 71.30, 71.12, 70.94, 70.86, 70.80, 70.51, 70.45, 70.41, 70.33, 70.26, 70.18, 70.03, 69.86, 69.82, 69.72, 69.62, 69.57, 69.53, 69.47, 69.33, 69.23, 67.57, 64.23, 64.03, 63.42, 62.76, 62.62, 62.58, 62.45, 62.28, 53.17, 52.93, 49.30, 40.13, 22.86, 20.83, 20.81, 20.77, 20.76, 20.71, 20.65, 20.63. ESI-HRMS (m/z) $[M + H]^+$ calcd for $C_{103}H_{144}N_4O_{66}$, 2493.8107, found, 2493.8096.

*4.3.16 Synthesis of 6^A-deoxy-6^A-(4-((5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl onate)eth-2-yl)-1H-1,2,3-triazol-1-yl)-β-CD (**29a**)*

To a solution of **28a** (72 mg, 0.03 mmol) was dissolved in 1:1 methanol/water mixture (5 mL), and 8 mg sodium hydroxide (0.2 mmol) was added. After stirring at room temperature overnight, the reaction mixture was neutralized with ion-exchange resin (Amberlite IR-120) (H^+). Filtered the resin in a sintered glass filter and washed with methanol, and the solvent was removed *in vacuo* to afford **28a** (45 mg, 99%) as a white foam. R_f = 0.70 (isopropanol/NH_3·H_2O/H_2O = 5:5:2). ^{1}H NMR (400 MHz, D_2O): δ 7.83 (s, 1H), 5.08 (d, J = 3.3 Hz, 1H), 4.98 (d, J = 3.3 Hz, 4H), 4.91 (dd, J = 10.1, 3.3 Hz, 3H), 4.49 (dd, J = 14.4, 9.4 Hz, 1H), 4.12 (t, J = 9.1 Hz, 1H), 4.01 – 3.66 (m, 26H), 3.65 – 3.37 (m, 20H), 3.19 (d, J = 12.3 Hz, 1H), 3.01 (s, 2H), 2.73 (dd, J = 20.4, 8.0 Hz, 2H), 2.04 (s, 3H), 1.71 (t, J = 12.1 Hz, 1H). ^{13}C NMR (101 MHz, D_2O): δ 175.16, 172.87, 144.75, 125.99, 102.06, 101.92, 101.84, 101.79, 101.46, 100.18, 83.19, 81.21, 81.10, 81.07, 80.75, 73.07, 72.80, 72.06, 71.97, 71.84, 71.76, 71.71, 71.52, 71.42, 70.46, 68.25, 67.90, 63.14, 62.73, 60.24, 59.05, 51.85, 51.20, 39.97, 25.52, 22.02. ESI-HRMS (m/z) $[M + H]^+$ calcd for $C_{57}H_{92}N_4O_{43}$, 1521.5208, found, 1521.5208.

*4.3.17 Synthesis of 6^A-deoxy-6^A-(4-((5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl onate)prop-4-yl)-1H-1,2,3-triazol-1-yl)-β-CD (**29b**)*

The deprotection of **28b** (61 mg, 0.026 mmol) was carried out as described for the preparation of **29a** to afford **29b** (38 mg, 96%) as a white foam. R_f = 0.41 (isopropanol/NH_3·H_2O/H_2O = 5:5:2). ^{1}H NMR (600 MHz, D_2O): δ 7.86 (s, 1H), 5.19 (d,

J = 3.7 Hz, 1H), 5.09 (dd, *J* = 7.1, 3.5 Hz, 4H), 5.01 (dd, *J* = 17.1, 3.7 Hz, 3H), 4.58 (dd, *J* = 14.5, 9.6 Hz, 1H), 4.21 (t, *J* = 9.6 Hz, 1H), 4.09 – 3.74 (m, 26H), 3.74 – 3.42 (m, 20H), 3.17 (d, *J* = 11.8 Hz, 1H), 2.89 – 2.70 (m, 4H), 2.04 (s, 3H), 1.97 – 1.91 (m, 2H), 1.67 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (151 MHz, D_2O): *δ* 176.32, 174.85, 148.80, 126.15, 103.28, 103.17, 103.13, 103.04, 102.58, 101.79, 84.45, 82.46, 82.27, 82.24, 81.81, 74.27, 74.24, 74.22, 74.00, 73.80, 73.26, 73.23, 73.18, 73.14, 73.05, 72.98, 72.95, 72.89, 72.67, 71.83, 69.48, 69.40, 64.97, 63.77, 61.51, 61.39, 61.33, 60.11, 53.12, 52.30, 41.63, 29.83, 23.21, 22.47. ESI-HRMS (m/z) $[M + H]^+$ calcd for $C_{58}H_{94}N_4O_{43}$, 1535.5365, found, 1535.5366.

*4.3.18 Synthesis of 6^A-deoxy-6^A-(4-((5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl onate)ethoxymethyl)-1H-1,2,3-triazol-1-yl)-β-CD (**29c**)*

The deprotection of **28c** (33 mg, 0.013 mmol) was carried out as described for the preparation of **29a** to afford **29c** (21 mg, 98%) as a white foam. R_f = 0.70 (isopropanol/$NH_3 \cdot H_2O$/H_2O = 5:5:2). ^{1}H NMR (400 MHz, D_2O): *δ* 7.97 (s, 1H), 5.04 (s, 1H), 5.00 – 4.81 (m, 7H), 4.61 – 4.43 (m, 3H), 4.06 (t, *J* = 9.3 Hz, 1H), 3.92 – 3.31 (m, 48H), 3.06 (d, *J* = 12.2 Hz, 1H), 2.64 (d, *J* = 12.1 Hz, 1H), 2.60 – 2.50 (m, 1H), 1.89 (s, 3H), 1.66 (t, *J* = 12.1 Hz, 1H). ^{13}C NMR (101 MHz, D_2O): *δ* 175.07, 171.49, 143.71, 126.94, 102.05, 101.92, 101.84, 101.42, 99.11, 83.14, 81.22, 81.13, 81.06, 80.66, 73.08, 73.02, 72.86, 72.80, 72.75, 72.04, 72.00, 71.95, 71.91, 71.81, 71.75, 71.42, 70.95, 70.47, 68.89, 68.28, 67.43, 63.28, 62.93, 62.90, 60.29, 60.20, 59.18, 51.68, 51.24, 39.38, 22.03. ESI-HRMS (m/z) $[M + H]^+$ calcd for $C_{58}H_{94}N_4O_{44}$, 1551.5314, found, 1551.5314.

*4.3.19 Synthesis of 6^A-deoxy-6^A-(4-(((5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosy lonate)ethoxyl)ethoxymethyl)-1H-1,2,3-triazol-1-yl)-β-CD (**29d**)*

The deprotection of **28d** (36 mg, 0.015 mmol) was carried out as described for the preparation of **29a** to afford **29d** (23 mg, 99%) as a white foam. R_f = 0.68 (isopropanol/$NH_3 \cdot H_2O$/H_2O = 5:5:2). ^{1}H NMR (400 MHz, D_2O): *δ* 7.98 (s, 1H), 5.08 (d, *J* = 3.1 Hz, 1H), 4.96 (s, 4H), 4.93 – 4.83 (m, 2H), 4.64 – 4.47 (m, 3H), 4.10 (dd, *J*

= 17.2, 6.9 Hz, 1H), 3.97 – 3.35 (m, 53H), 3.07 (d, *J* = 12.2 Hz, 1H), 2.73 (d, *J* = 12.3 Hz, 1H), 2.61 (dd, *J* = 12.5, 4.1 Hz, 1H), 1.92 (s, 3H), 1.65 (t, *J* = 12.1 Hz, 1H). ^{13}C NMR (101 MHz, D_2O): *δ* 175.03, 171.98, 143.86, 126.72, 102.05, 101.92, 101.87, 101.48, 99.49, 83.09, 81.75, 81.24, 81.08, 81.05, 80.74, 73.09, 73.07, 72.85, 72.78, 72.06, 71.96, 71.92, 71.79, 71.41, 71.15, 70.53, 69.52, 69.04, 68.29, 67.67, 63.30, 63.04, 62.86, 60.20, 59.14, 51.80, 51.21, 39.57, 22.05. ESI-HRMS (m/z) $[M + H]^+$ calcd for $C_{60}H_{98}N_4O_{45}$, 1595.5576, found, 1595.5569.

*4.3.20 Synthesis of 6^A-deoxy-6^A-(4-((((5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)ethoxyl)ethoxyl)ethoxymethyl)-1H-1,2,3-triazol-1-yl)-β-CD (**29e**)*

The deprotection of **28e** (40 mg, 0.016 mmol) was carried out as described for the preparation of **29a** to afford **29e** (26 mg, 99%) as a white foam. R_f = 0.63 (isopropanol/$NH_3 \cdot H_2O$/H_2O = 5:5:2). ^{1}H NMR (400 MHz, D_2O): *δ* 7.99 (s, 1H), 5.07 (d, *J* = 3.3 Hz, 1H), 5.02 – 4.84 (m, 6H), 4.63 – 4.46 (m, 3H), 4.10 (t, *J* = 10.0 Hz, 1H), 3.97 – 3.32 (m, 57H), 3.05 (d, *J* = 12.0 Hz, 1H), 2.71 (d, *J* = 11.9 Hz, 1H), 2.60 (dd, *J* = 12.5, 4.3 Hz, 1H), 1.91 (s, 3H), 1.61 (t, *J* = 12.1 Hz, 1H). ^{13}C NMR (101 MHz, D_2O): *δ* 175.03, 172.45, 143.96, 126.83, 102.03, 101.90, 101.85, 101.45, 83.09, 81.73, 81.23, 81.07, 81.03, 80.73, 73.07, 73.04, 72.82, 72.73, 72.68, 72.06, 72.02, 71.96, 71.91, 71.76, 71.72, 71.38, 70.50, 69.52, 69.07, 68.26, 67.91, 63.25, 63.03, 62.75, 60.20, 59.12, 51.82, 51.21, 39.86, 22.03. ESI-HRMS (m/z) $[M + H]^+$ calcd for $C_{62}H_{102}N_4O_{46}$, 1639.5838, found, 1639.5835.

*4.3.21 Synthesis of 6^A-deoxy-6^A-(4-((methyl 5-acetamido-3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosylonate)prop-3-yl)-1H-1,2,3-triazol-1-yl)-per-O-acetylated-β-CD (**30**)*

The click reaction of **7** (199 mg, 0.10 mmol) and **18b** (27 mg, 0.070 mmol) was carried out as described for the preparation of **28a** to afford, after purified by flash chromatography eluted with CH_2Cl_2/CH_3OH (15:1 to 8:1), **30** (107 mg, 65%) as a white foam. R_f = 0.36 (CH_2Cl_2/ CH_3OH = 8:1). ^{1}H NMR (600 MHz, $CDCl_3$): *δ* 7.45 (s, 1H), 5.55 (d, *J* = 3.7 Hz, 1H), 5.37 – 5.30 (m, 4H), 5.30 – 5.21 (m, 4H), 5.17 (d, *J* = 3.9 Hz, 1H), 5.13 (d, *J* = 4.0 Hz, 1H), 5.09 (d, *J* = 3.9 Hz, 1H), 5.07 – 5.01 (m, 3H), 4.99 (d, *J* =

3.5 Hz, 1H), 4.86 (dd, *J* = 9.0, 3.9 Hz, 1H), 4.82 (ddd, *J* = 13.4, 6.7, 2.6 Hz, 3H), 4.78 – 4.73 (m, 3H), 4.70 (dd, *J* = 14.5, 2.5 Hz, 1H), 4.62 – 4.49 (m, 5H), 4.39 (d, *J* = 11.6 Hz, 1H), 4.36 – 4.14 (m, 10H), 4.14 – 4.09 (m, 2H), 4.07 (d, *J* = 9.2 Hz, 2H), 3.93 – 3.80 (m, 6H), 3.80 – 3.68 (m, 12H), 3.65 – 3.55 (m, 2H), 3.52 (t, *J* = 9.1 Hz, 1H), 3.32 (dt, *J* = 9.0, 5.5 Hz, 1H), 2.91 – 2.80 (m, 1H), 2.80 – 2.73 (m, 1H), 2.40 (dd, *J* = 12.6, 3.5 Hz, 1H), 2.15 – 2.00 (m, 63H), 1.99 – 1.95 (m, 1H), 1.95 – 1.87 (m, 1H), 1.73 – 1.67 (m, 1H). ^{13}C NMR (151 MHz, $CDCl_3$): *δ* 173.62, 171.13 171.00, 170.98, 170.85, 170.83, 170.78, 170.75, 170.73, 170.70, 170.57, 170.52, 169.66, 169.62, 169.57, 169.53, 169.51, 169.48, 169.39, 169.05, 147.70, 124.45, 98.76, 97.21, 96.98, 96.90, 96.86, 96.80, 96.71, 96.57, 77.60, 77.36, 77.33, 77.06, 76.73, 76.42, 75.94, 71.67, 71.29, 71.22, 71.09, 70.79, 70.50, 70.45, 70.32, 70.26, 70.10, 70.05, 70.02, 69.96, 69.90, 69.83, 69.76, 69.59, 69.44, 67.21, 64.86, 62.94, 62.84, 62.72, 62.68, 62.64, 62.41, 58.52, 53.56, 53.34, 52.74, 49.61, 40.41, 29.81, 29.77, 28.96, 23.14, 22.46, 21.06, 21.01, 20.96, 20.93, 20.91, 20.88, 20.84, 20.83, 20.80. ESI-HRMS (m/z) $[M + H]^+$ calcd for $C_{99}H_{136}N_4O_{63}$, 2411.7453, found, 2411.7439.

*4.3.22 Synthesis of 6^A-deoxy-6^A-(4-((5-acetamido-3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosyl onate)prop-3-yl)-1H-1,2,3-triazol-1-yl)-β-CD (**31**)*

The deprotection of **30** (61 mg, 0.026 mmol) was carried out as described for the preparation of **29a** to afford **31** (38 mg, 96%) as a white foam. R_f = 0.49 (isopropanol/$NH_3 \cdot H_2O$/H_2O = 5:5:2). ^{1}H NMR (600 MHz, D_2O): *δ* 7.86 (s, 1H), 5.18 (d, *J* = 3.6 Hz, 1H), 5.08 (dd, *J* = 7.8, 4.1 Hz, 4H), 5.01 (dd, *J* = 12.2, 3.3 Hz, 3H), 4.63 – 4.53 (m, 1H), 4.19 (dd, *J* = 16.1, 6.5 Hz, 1H), 4.11 – 4.05 (m, 1H), 4.05 – 3.80 (m, 25H), 3.79 – 3.74 (m, 1H), 3.73 – 3.46 (m, 18H), 3.41 – 3.33 (m, 1H), 3.18 (d, *J* = 11.9 Hz, 1H), 2.91 – 2.76 (m, 3H), 2.39 (dd, *J* = 13.1, 4.9 Hz, 1H), 2.06 (s, 3H), 2.02 – 1.92 (m, 2H), 1.71 (dd, *J* = 12.9, 11.7 Hz, 1H); ^{13}C NMR (151 MHz, D_2O): *δ* 175.88, 175.11, 148.74, 126.06, 103.19, 103.06, 102.98, 102.58, 100.41, 84.27, 82.35, 82.18, 82.15, 81.74, 74.16, 74.11, 73.90, 73.85, 73.14, 73.10, 73.03, 73.02, 72.93, 72.86, 72.77, 72.55, 71.73, 71.41, 70.97, 69.23, 67.86, 64.57, 63.45, 61.40, 61.28, 61.22, 60.03,

53.09, 52.26, 40.86, 29.56, 23.21, 22.56. ESI-HRMS (m/z) $[M + H]^+$ calcd for $C_{58}H_{94}N_4O_{43}$, 1535.5365, found, 1535.5355.

*4.3.23 Synthesis of 6^{A-F}-deoxy-6^{A-F}-hepta(4-((methyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)eth-2-yl)-1 H-1,2,3-triazol-1-yl)-per-O-acetylated-β-CD (**32a**)*

To a well-stirred solution of **17a** (45 mg, 0.12 mmol) in 2 mL of pyridine was added 0.5 mL of Ac_2O and catalytic amount of DMAP at rt. After stirring for 18 h at rt, the reaction mixture was concentrated *in vacuo* and then resolved in 1:1 acetone/water mixture (4 mL). 26 mg of **8** (0.014 mmol) was added, followed by copper sulfate (20 mg, 0.12 mmol) and sodium ascorbate (50 mg, 0.25 mmol). The reaction vessel was placed in a Discover SP microwave reactor (CEM) and heated to 100 °C and stirred for 1 h. The crude mixture was diluted with 30 mL of CH_2Cl_2, and washed successively with water (10 mL) and brine (10 mL). The resulting organic phase was dried over Na_2SO_4, and filtered. The solvent was removed *in vacuo* and the crude product was purified by flash chromatography eluted with petroleum ether/dichloromethane/methanol (5:5:1 to 5:5:2) to afford **32a** (45 mg, 46%) as a white foam. R_f = 0.22 (CH_2Cl_2/CH_3OH = 9:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.66 (s, 7H), 5.70 (s, 7H), 5.57 (s, 7H), 5.34 (s, 21H), 4.91 (d, *J* = 27.7 Hz, 14H), 4.84 – 4.75 (m, 7H), 4.72 (d, *J* = 8.1 Hz, 7H), 4.46 (s, 7H), 4.20 (d, *J* = 11.8 Hz, 7H), 4.15 – 3.99 (m, 21H), 3.94 (d, *J* = 7.9 Hz, 7H), 3.72 (s, 21H), 3.58 – 3.44 (m, 14H), 2.87 (s, 14H), 2.57 (dd, *J* = 12.3, 3.9 Hz, 7H), 2.13 (s, 21H), 2.07 – 1.95 (m, 105H), 1.95 – 1.88 (m, 7H), 1.85 (s, 21H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.97, 170.83, 170.41, 170.03, 169.92, 169.43, 168.49, 144.54, 124.46, 98.91, 96.26, 76.23, 72.47, 70.89, 70.02, 69.83, 69.20, 68.24, 67.11, 63.68, 62.12, 52.84, 49.88, 49.39, 37.92, 26.35, 23.18, 21.08, 20.93, 20.89, 20.82, 20.79, 20.74. MALDI-TOF MS (m/z) $[M + Na]^+$ calcd for $C_{238}H_{322}N_{28}NaO_{133}$, 5722.91, found 5722.99.

*4.3.24 Synthesis of 6^{A-F}-deoxy-6^{A-F}-hepta(4-((methyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)prop-3-yl)-1H-1,2,3-triazol-1-yl)-per-O-acetylated-β-CD (**32b**)*

The click reaction between **8** (23 mg, 0.012 mmol) and **17b** (39 mg, 0.10 mmol)

was carried out as described for the preparation of **32a** to afford, after purified by flash chromatography eluted with petroleum ether/dichloromethane/methanol (5:5:1 to 5:5:2), **32b** (38 mg, 46%) as a white foam. R_f = 0.23 (CH_2Cl_2/CH_3OH = 9:1). ^1H NMR (600 MHz, $CDCl_3$): δ 7.48 (s, 7H), 5.56 (s, 7H), 5.49 (d, *J* = 9.1 Hz, 7H), 5.41 – 5.22 (m, 21H), 5.05 – 4.88 (m, 7H), 4.88 – 4.77 (m, 14H), 4.72 (d, *J* = 7.1 Hz, 7H), 4.46 (s, 7H), 4.35 – 4.22 (m, 7H), 4.16 – 3.99 (m, 21H), 3.90 – 3.73 (m, 28H), 3.56 – 3.41 (m, 7H), 3.32 – 3.13 (m, 7H), 2.85 – 2.46 (m, 21H), 2.15 – 2.08 (m, 42H), 2.07 – 1.98 (m, 84H), 1.97 – 1.90 (m, 14H), 1.90 – 1.83 (m, 28H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 171.04, 170.79, 170.27, 170.11, 169.94, 169.50, 168.47, 147.58, 123.74, 98.83, 96.22, 76.19, 72.38, 70.79, 69.82, 69.34, 69.25, 68.45, 67.25, 64.21, 62.26, 52.85, 49.84, 49.34, 38.14, 29.25, 23.20, 22.12, 21.17, 20.90, 20.89, 20.82, 20.80, 20.75. MALDI-TOF MS (m/z) $[M + Na]^+$ calcd for $C_{245}H_{336}N_{28}NaO_{133}$, 5821.0, found 5821.8.

*4.3.25 Synthesis of 6^{A-F}-deoxy-6^{A-F}-hepta(4-((methyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)ethoxymethyl)-1H-1,2,3-triazol-1-yl)-per-O-acetylated-β-CD (**32b**)*

The click reaction between **8** (36 mg, 0.018 mmol) and **17c** (74 mg, 0.18 mmol) was carried out as described for the preparation of **32a** to afford, after purified by flash chromatography eluted with petroleum ether/dichloromethane/methanol (5:5:1 to 5:5:2), **32c** (53 mg, 49%) as a white foam. R_f = 0.25 (CH_2Cl_2/CH_3OH = 9:1). ^1H NMR (400 MHz, $CDCl_3$): δ 7.85 (s, 7H), 5.88 – 5.67 (m, 7H), 5.67 – 5.47 (m, 7H), 5.44 – 5.31 (m, 21H), 5.02 – 4.82 (m, 14H), 4.81 – 4.69 (m, 7H), 4.69 – 4.39 (m, 21H), 4.29 (d, *J* = 12.0 Hz, 7H), 4.17 – 3.98 (m, 21H), 3.87 (s, 7H), 3.82 – 3.39 (m, 56H), 2.73 – 2.55 (m, 7H), 2.14 (d, 42H), 2.10 – 1.91 (m, 91H), 1.88 (s, 21H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.89, 170.68, 170.33, 170.06, 170.02, 169.49, 168.30, 145.04, 125.56, 98.94, 96.30, 76.27, 72.43, 70.82, 69.69, 69.14, 68.55, 67.30, 64.44, 64.32, 62.27, 52.82, 49.91, 49.36, 37.94, 23.15, 21.13, 20.85, 20.76. ESI-QTOF-MS (m/z) $[M + 4H]^{4+}$ calcd for $C_{245}H_{336}N_{28}O_{140}$, 1478.5086, found 1478.5067.

*4.3.26 Synthesis of 6^{A-F}-deoxy-6^{A-F}-hepta(4-(((methyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)ethoxyl)ethoxymethyl)-1H-1,2,3-triazol-1-yl)-per-O-acetylated-β-CD (**32d**)*

The click reaction between **8** (60 mg, 0.03 mmol) and **17d** (102 mg, 0.23mmol) was carried out as described for the preparation of **32a** to afford, after purified by flash chromatography eluted with petroleum ether/dichloromethane/methanol (5:5:1 to 5:5:2), **32d** (100 mg, 53%) as a white foam. R_f = 0.20 (CH_2Cl_2/CH_3OH = 10:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.74 (s, 7H), 5.85 – 5.56 (m, 7H), 5.56 – 5.40 (m, 7H), 5.39 – 5.21 (m, 21H), 4.94 – 4.76 (m, 14H), 4.76 – 4.62 (m, 14H), 4.58 – 4.36 (m, 21H), 4.30 – 4.16 (m, 7H), 4.08 – 3.94 (m, 21H), 3.87 – 3.76 (m, 7H), 3.76 – 3.67 (m, 21H), 3.66 – 3.45 (m, 49H), 3.45 – 3.35 (m, 7H), 2.57 (dd, *J* = 12.4, 4.0 Hz, 7H), 2.07 (s, 21H), 2.05 (s, 21H), 2.03 – 1.93 (m, 84H), 1.90 (t, *J* = 12.6 Hz, 7H), 1.82 (s, 21H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.84, 170.62, 170.35, 170.30, 170.03, 169.52, 168.17, 144.75, 125.62, 98.77, 96.18, 76.31, 72.34, 70.63, 70.24, 69.95, 69.83, 69.57, 69.11, 68.59, 67.25, 64.31, 64.25, 62.23, 52.73, 49.89, 49.15, 37.82, 23.03, 21.03, 20.76, 20.75, 20.69, 20.63. ESI-QTOF-MS (m/z) $[M + 2H]^{2+}$ calcd for $C_{259}H_{364}N_{28}O_{147}$, 3110.1012, found 3110.0706.

*4.3.27 Synthesis of 6^{A-F}-deoxy-6^{A-F}-hepta(4-(((methyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)ethoxyl)ethoxyl)ethoxymethyl)-1H-1,2,3-triazol-1-yl)-per-O-acetylated-β-CD (**32e**)*

The click reaction between **8** (50 mg, 0.025 mmol) and **17b** (99 mg, 0.20 mmol) was carried out as described for the preparation of **32a** to afford, after purified by flash chromatography eluted with petroleum ether/dichloromethane/methanol (5:5:1 to 5:5:2), **32e** (90 mg, 55%) as a white foam. R_f = 0.30 (CH_2Cl_2/CH_3OH = 10:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.74 (s, 7H), 5.65 – 5.42 (m, 14H), 5.42 – 5.32 (m, 14H), 5.32 – 5.26 (m, 7H), 4.97 – 4.78 (m, 14H), 4.78 – 4.64 (m, 14H), 4.64 – 4.38 (m, 21H), 4.34 – 4.21 (m, 7H), 4.11 – 3.99 (m, 21H), 3.90 – 3.81 (m, 7H), 3.81 – 3.72 (m, 21H), 3.72 – 3.47 (m, 77H), 3.47 – 3.38 (m, 7H), 2.60 (dd, *J* = 12.7, 4.4 Hz, 7H), 2.15 – 2.08 (m, 42H), 2.07 – 1.97 (m, 84H), 1.94 (t, *J* = 12.6 Hz, 7H), 1.86 (s, 21H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.95, 170.67, 170.31, 170.11, 170.01, 169.58, 168.29,

144.88, 125.71, 98.84, 96.23, 76.40, 72.42, 70.48, 70.45, 70.38, 70.06, 69.97, 69.18, 68.49, 67.30, 64.49, 64.40, 62.35, 52.80, 49.97, 49.32, 37.98, 23.17, 21.13, 20.88, 20.87, 20.79, 20.72. ESI-QTOF-MS (m/z) $[M + 2H]^{2+}$ calcd for $C_{273}H_{392}N_{28}O_{154}$, 3264.1929, found 3264.1636.

*4.3.28 Synthesis of 6^{A-F}-deoxy-6^{A-F}-hepta(4-((methyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)eth-2-yl)-1 H-1,2,3-triazol-1-yl)-β-CD (**33a**)*

The deprotection of **32a** (90 mg, 0.016 mmol) was carried out as described for the preparation of **29a** to afford **33a** (59 mg, 97%) as a white foam. ^{1}H NMR (400 MHz, D_2O): δ 7.77 (s, 7H), 5.15 (s, 7H), 4.48 (d, J = 12.3 Hz, 7H), 4.33 – 4.11 (m, 14H), 4.00 (t, J = 9.0 Hz, 7H), 3.91 – 3.77 (m, 21H), 3.74 (d, J = 10.1 Hz, 7H), 3.70 – 3.45 (m, 42H), 3.45 – 3.32 (m, 7H), 2.86 – 2.71 (m, 7H), 2.65 (dd, J = 12.0, 3.8 Hz, 14H), 2.00 (s, 21H), 1.55 (t, J = 11.9 Hz, 7H). ^{13}C NMR (101 MHz, D_2O): δ 174.28, 172.69, 144.04, 125.08, 101.00, 99.86, 81.75, 71.86, 71.66, 70.97, 69.21, 67.56, 61.92, 51.21, 49.36, 39.50, 24.74, 21.36. ESI-QTOF-MS (m/z) $[M + H]^+$ calcd for $C_{147}H_{224}N_{28}O_{91}$, 3838.3838, found 3838.4514. $[M + 2H]^{2+}$ 1919.6958, found 1919.6742. $[M + 3H]^{3+}$ 1280.1331, found 1280.1245.

*4.3.29 Synthesis of 6^{A-F}-deoxy-6^{A-F}-hepta(4-((5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulop yranosylonate)prop-3-yl)-1H-1,2,3-triazol-1-yl)-β-CD (**33b**)*

The deprotection of **32b** (75 mg, 0.013 mmol) was carried out as described for the preparation of **29a** to afford **33b** (50 mg, 98%) as a white foam. ^{1}H NMR (400 MHz, D_2O): δ 7.83 (s, 7H), 5.12 (s, 7H), 4.46 – 4.27 (m, 7H), 4.27 – 4.09 (m, 14H), 3.98 (s, 7H), 3.80 (t, J = 9.0 Hz, 21H), 3.75 – 3.49 (m, 42H), 3.34 (dd, J = 40.1, 15.9 Hz, 14H), 2.62 (d, J = 9.1 Hz, 7H), 2.48 (s, 14H), 2.00 (s, 21H), 1.66 (s, 21H). ^{13}C NMR (101 MHz, D_2O): δ 175.03, 171.79, 146.60, 125.74, 101.83, 99.30, 72.81, 72.27, 71.67, 71.17, 69.91, 68.24, 67.58, 63.43, 62.85, 51.84, 50.69, 39.61, 28.13, 22.05, 21.08, 20.77. ESI-QTOF-MS (m/z) $[M + 2H]^{2+}$ calcd for $C_{154}H_{238}N_{28}O_{91}$, 1968.7506, found 1968.7501.

4.3.30 Synthesis of

*6^{A-F}-deoxy-6^{A-F}-hepta(4-((5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)ethoxymethyl)-1H-1,2,3-triazol-1-yl)-β-CD (**33c**)*

The deprotection of **32c** (61 mg, 0.01 mmol) was carried out as described for the preparation of **29a** to afford **33c** (40 mg, 96%) as a white foam. ^{1}H NMR (400 MHz, D_2O): δ 7.94 (s, 7H), 5.05 (s, 7H), 4.50 – 4.01 (m, 35H), 3.99 – 3.85 (m, 7H), 3.84 – 3.67 (m, 28H), 3.67 – 3.56 (m, 14H), 3.56 – 3.37 (m, 42H), 3.37 – 3.21 (m, 7H), 2.57 (d, J = 9.3 Hz, 7H), 1.92 (s, 21H), 1.60 (t, J = 11.5 Hz, 7H). ^{13}C NMR (101 MHz, D_2O): δ 174.99, 171.89, 144.31, 126.77, 101.62, 99.52, 82.66, 72.73, 72.35, 71.65, 71.14, 69.93, 69.06, 68.28, 67.69, 63.31, 62.98, 62.83, 51.81, 50.32, 39.70, 22.06. ESI-QTOF-MS (m/z) $[M + 2H]^{2+}$ calcd for $C_{154}H_{238}N_{28}O_{98}$, 2024.7328, found 2024.7323.

*4.3.31 Synthesis of 6^{A-F}-deoxy-6^{A-F}-hepta(4-(((5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)ethoxyl)ethoxymethyl)-1H-1,2,3-triazol-1-yl)-β-CD (**33d**)*

The deprotection of **32d** (75 mg, 0.012 mmol) was carried out as described for the preparation of **29a** to afford **33d** (51 mg, 99%) as a white foam. ^{1}H NMR (400 MHz, D_2O): δ 8.02 (s, 7H), 5.13 (s, 7H), 4.52 – 4.31 (m, 21H), 4.31 – 4.13 (m, 14H), 4.00 (t, J = 9.3 Hz, 7H), 3.93 – 3.78 (m, 28H), 3.78 – 3.68 (m, 14H), 3.67 – 3.46 (m, 70H), 3.38 (t, J = 8.6 Hz, 7H), 2.67 (dd, J = 12.5, 4.2 Hz, 7H), 2.01 (s, 21H), 1.73 (t, J = 12.1 Hz, 7H). ^{13}C NMR (101 MHz, D_2O): δ 174.98, 171.56, 143.82, 126.79, 101.66, 99.22, 82.52, 72.80, 72.35, 71.65, 71.02, 69.89, 69.49, 69.11, 68.28, 67.55, 63.27, 62.89, 51.82, 50.35, 39.47, 22.08. ESI-QTOF-MS (m/z) $[M + H]^+$ calcd for $C_{168}H_{266}N_{28}O_{105}$, 4356.6414, found 4356.6387.

*4.3.32 Synthesis of 6^{A-F}-deoxy-6^{A-F}-hepta(4-((((5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)ethoxyl)ethoxyl)ethoxymethyl)-1H-1,2,3-triazol-1-yl)-β-CD (**33e**)*

The deprotection of **32e** (67 mg, 0.010 mmol) was carried out as described for the preparation of **29a** to afford **33e** (47 mg, 98%) as a white foam. ^{1}H NMR (400 MHz, D_2O): δ 7.91 (s, 7H), 5.02 (s, 7H), 4.49 – 4.20 (m, 21H), 4.20 – 4.00 (m, 14H), 3.97 – 3.83 (m, 7H), 3.83 – 3.66 (m, 35H), 3.66 – 3.35 (m, 105H), 3.34 – 3.14 (m, 7H), 2.58

(d, *J* = 10.5 Hz, 7H), 1.89 (s, 21H), 1.59 (t, *J* = 12.0 Hz, 7H). ^{13}C NMR (101 MHz, D_2O): *δ* 174.99, 172.43, 143.85, 126.82, 101.66, 99.83, 82.47, 72.68, 72.34, 71.65, 71.33, 69.90, 69.50, 69.12, 68.27, 67.89, 63.24, 62.94, 62.76, 51.85, 50.32, 39.84, 22.06. ESI-QTOF-MS (m/z) $[M + 2H]^{2+}$ calcd for $C_{182}H_{294}N_{28}O_{112}$, 2332.9163, found 2332.8635.

*4.3.33 Synthesis of 6^{A-F}-deoxy-6^{A-F}-hepta(4-((methyl 5-acetamido-3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosylonate)prop-3-yl)-1H-1,2,3-triazol-1-yl)-per-O-acetylated-β-CD (**34**)*

The click reaction between **8** (29 mg, 0.015 mmol) and **18b** (50 mg, 0. 13 mmol) was carried out as described for the preparation of **32a** to afford, after purified by flash chromatography eluted with petroleum ether/dichloromethane/methanol (5:5:1 to 5:5:2), **34** (60 mg, 56%) as a white foam. R_f = 0.20 (CH_2Cl_2/CH_3OH = 11:1). ^{1}H NMR (600 MHz, $CDCl_3$): *δ* 7.60 (s, 7H), 6.61 (s, 7H), 5.55 (s, 7H), 5.47 – 5.30 (m, 14H), 5.27 – 5.06 (m, 14H), 5.02 – 4.60 (m, 28H), 4.47 (s, 7H), 4.24 – 4.03 (m, 14H), 4.02 – 3.87 (m, 7H), 3.77 (s, 21H), 3.68 – 3.45 (m, 14H), 3.40 (s, 7H), 2.70 (s, 14H), 2.52 – 2.33 (m, 7H), 2.15 – 2.11 (m, 21H), 2.08 – 1.95 (m, 105H), 1.94 – 1.84 (m, 35H), 1.83 – 1.72 (m, 7H); ^{13}C NMR (151 MHz, $CDCl_3$): *δ* 170.74, 170.68, 170.59, 170.49, 170.26, 169.42, 167.67, 147.37, 124.18, 98.63, 96.26, 76.54, 71.54, 71.45, 70.66, 70.01, 69.85, 69.29, 68.46, 63.53, 62.62, 52.73, 49.98, 48.80, 37.40, 29.18, 23.10, 22.47, 21.02, 20.97, 20.89, 20.84, 20.81, 20.75. MALDI-TOF MS (m/z) $[M + H]^+$ calcd for $C_{245}H_{337}N_{28}O_{133}$, 5799.0, found 5798.8; $[M + Na + H]^+$ calcd for $C_{245}H_{337}N_{28}NaO_{133}$, 5822.0, found 5822.5.

*4.3.34 Synthesis of 6^{A-F}-deoxy-6^{A-F}-(4-((5-acetamido-3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyrano sylonate)prop-3-yl)-1H-1,2,3-triazol-1-yl)-β-CD (**35**)*

The deprotection of **34** (75 mg, 0.013 mmol) was carried out as described for the preparation of **29a** to afford **35** (50 mg, 98%) as a white foam. ^{1}H NMR (400 MHz, D_2O): *δ* 7.75 (s, 7H), 5.10 (s, 7H), 4.51 – 4.32 (m, 7H), 4.32 – 4.08 (m, 14H), 4.08 – 3.90 (m, 14H), 3.90 – 3.66 (m, 28H), 3.66 – 3.43 (m, 28H), 3.43 – 3.26 (m, 7H), 3.26 – 3.06 (m, 7H), 2.49 (s, 14H), 2.31 (d, *J* = 8.9 Hz, 7H), 1.97 (s, 21H), 1.77 – 1.52 (m,

21H). ^{13}C NMR (101 MHz, D_2O): δ 174.76, 172.53, 147.21, 125.14, 101.81, 98.80, 82.61, 72.27, 71.64, 70.46, 69.81, 67.98, 66.47, 63.39, 62.43, 51.89, 50.41, 39.53, 28.14, 22.12, 21.18. ESI-QTOF-MS (m/z) $[M + 2H]^{2+}$ calcd for $C_{154}H_{238}N_{28}O_{91}$, 1968.7506, found 1968.7494.

*4.3.35 Synthesis of 6^{A,C,E}-trideoxy-6^{A,C,E}-tri(4-((methyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)prop-3-yl)-1H-1,2,3-triazol-1-yl)-per-O-acetylated-α-CD (**36**)*

The click reaction between **13** (22 mg, 0.013 mmol) and **24** (34 mg, 0.059 mmol) was carried out as described for the preparation of **28a** to afford, after purified by flash chromatography eluted with CH_2Cl_2/CH_3OH (30:1 to 10:1), **36** (38 mg, 86%) as a white foam. R_f = 0.35 (EA/CH_3OH = 10:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44 (s, 3H), 5.60 (d, *J* = 3.7 Hz, 3H), 5.59 – 5.45 (m, 6H), 5.45 – 5.26 (m, 9H), 5.13 (d, *J* = 17.5 Hz, 3H), 5.06 (d, *J* = 3.2 Hz, 3H), 4.94 (dd, *J* = 10.2, 3.5 Hz, 3H), 4.90 – 4.78 (m, 3H), 4.64 (d, *J* = 12.9 Hz, 3H), 4.53 (dd, *J* = 10.5, 3.3 Hz, 3H), 4.43 – 4.25 (m, 12H), 4.18 – 4.00 (m, 9H), 3.90 – 3.71 (m, 15H), 3.68 – 3.57 (m, 3H), 3.38 – 3.23 (m, 3H), 2.86 – 2.67 (m, 6H), 2.61 (dd, *J* = 12.7, 4.5 Hz, 3H), 2.16 (s, 9H), 2.15 (s, 9H), 2.14 (s, 9H), 2.14 (s, 9H), 2.06 (s, 9H), 2.05 (s, 9H), 2.05 (s, 9H), 2.04 (s, 9H), 2.03 (s, 9H), 2.01 – 1.90 (m, 9H), 1.88 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.04, 170.82, 170.70, 170.38, 170.30, 170.20, 170.08, 169.55, 169.24, 168.51, 147.47, 123.75, 98.81, 97.05, 96.32, 77.33, 76.97, 72.45, 71.31, 70.62, 70.45, 70.23, 69.18, 68.75, 67.38, 64.26, 63.15, 62.31, 52.73, 50.15, 49.43, 38.02, 29.17, 23.16, 22.14, 21.10, 20.87, 20.84, 20.77, 20.73. ESI-QTOF-MS (m/z) $[M + H]^+$ calcd for $C_{141}H_{193}N_{12}O_{81}$, 3350.1352, found 3350.0889.

*4.3.36 Synthesis of 6^{A,C,E}-trideoxy-6^{A,C,E}-tri(4-((5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)prop-3-yl)-1H-1,2,3-triazol-1-yl)-α-CD (**37**)*

The deprotection of **36** (50 mg, 0.015 mmol) was carried out as described for the preparation of **29a** to afford **37** (31 mg, 96%) as a white foam. ^{1}H NMR (600 MHz, D_2O): δ 7.83 (s, 3H), 5.06 (d, *J* = 3.2 Hz, 3H), 4.96 (d, *J* = 3.0 Hz, 3H), 4.85 (d, *J* = 13.2 Hz, 3H), 4.62 (dd, *J* = 14.5, 7.8 Hz, 3H), 4.18 – 4.07 (m, 3H), 4.01 – 3.86 (m, 6H), 3.79

– 3.75 (m, 3H), 3.74 – 3.68 (m, 9H), 3.67 – 3.63 (m, 3H), 3.63 – 3.46 (m, 21H), 3.39 – 3.32 (m, 9H), 2.97 (d, *J* = 10.9 Hz, 3H), 2.64 – 2.58 (m, 9H), 1.95 (s, 9H), 1.79 (t, *J* = 6.9 Hz, 6H), 1.50 (t, *J* = 12.1 Hz, 3H); ^{13}C NMR (151 MHz, D_2O): δ 175.08, 173.62, 147.72, 124.93, 101.54, 101.30, 100.52, 82.69, 80.88, 73.11, 72.87, 72.56, 71.87, 71.66, 71.51, 71.45, 70.43, 68.24, 68.19, 63.69, 62.53, 59.30, 51.87, 50.86, 40.37, 28.72, 21.99, 21.25. ESI-QTOF-MS (m/z) $[M + H]^+$ calcd for $C_{84}H_{133}N_{12}O_{54}$, 2173.7930, found 2173.8030.

*4.3.37 Synthesis of 6^{A,C,E}-trideoxy-6^{A,C,E}-tri(4-((N-tri(9-(4-(3-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)prop-1-yl)-1H-1,2,3-triazol-1-yl)-2-oxa-5-oxo-6-aza-nona-1-yl)methylamino)-3-oxopropyl)-1H-1,2,3-triazol-1-yl)-per-O-acetylated-α-CD (**38**)*

The click reaction between **13** (43 mg, 0.025 mmol) and **27** (239 mg, 0.10 mmol) was carried out as described for the preparation of **32a** to afford, after purified by flash chromatography eluted with CH_2Cl_2/CH_3OH (30:1 to 5:1), **38** (113 mg, 51%) as a white foam. R_f = 0.45 (ethyl acetate/methanol = 5:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48 (s, 9H), 5.37 – 5.23 (m, 18H), 4.86 – 4.73 (m, 9H), 4.44 – 4.30 (m, 18H), 4.27 (d, *J* = 11.8 Hz, 9H), 4.15 – 3.94 (m, 27H), 3.84 – 3.67 (m, 36H), 3.67 – 3.45 (m, 36H), 3.33 – 3.12 (m, 27H), 2.71 (s, 18H), 2.55 (d, *J* = 8.6 Hz, 9H), 2.36 (s, 18H), 2.09 (s, 27H), 2.08 (s, 27H), 2.06 – 2.00 (m, 18H), 1.98 (s, 27H), 1.97 (s, 27H), 1.92 – 1.84 (m, 27H), 1.83 (s, 27H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.06, 170.80, 170.68, 170.43, 170.08, 168.43, 147.40, 121.75, 98.78, 72.39, 69.18, 68.67, 67.43, 64.05, 62.34, 52.70, 49.24, 47.77, 37.95, 36.64, 36.41, 30.27, 29.12, 23.04, 22.13, 21.05, 20.79, 20.71. ESI-QTOF-MS (m/z) $[M + 6H]^{6+}$ calcd for $C_{372}H_{543}N_{57}O_{180}$, 1448.0848, found 1448.2487.

*4.3.38 Synthesis of 6^{A,C,E}-trideoxy-6^{A,C,E}-tri(4-((N-tri(9-(4-(3-(5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)prop-1-yl)-1H-1,2,3-triazol-1-yl)-2-oxa-5-oxo-6-aza-nona-1-yl)methylamino)-3-oxopropyl)-1H-1,2,3-triazol-1-yl)-α-CD (**39**)*

The deprotection of **39** (40 mg, 0.0046 mmol) was carried out as described for the preparation of **29a** to afford **39** (29 mg, 98%) as a white foam. ^{1}H NMR (400 MHz,

D_2O): δ 7.75 (s, 9H), 4.34 – 4.14 (m, 18H), 3.71 – 3.50 (m, 36H), 3.50 – 3.34 (m, 72H), 3.34 – 3.22 (m, 9H), 3.07 – 2.92 (m, 18H), 2.69 – 2.55 (m, 27H), 2.30 – 2.12 (m, 18H), 2.01 – 1.88 (m, 18H), 1.84 (s, 27H), 1.80 – 1.64 (m, 18H), 1.49 (t, J = 11.5 Hz, 9H); ^{13}C NMR (101 MHz, D_2O): δ 175.00, 173.92, 124.15, 72.72, 71.37, 68.45, 68.22, 67.79, 67.44, 63.49, 62.75, 60.14, 51.87, 49.69, 48.41, 39.83, 36.33, 36.14, 28.94, 28.33, 22.06, 20.95. ESI-QTOF-MS (m/z) $[M + 2H]^{2+}$ calcd for $C_{261}H_{419}N_{57}O_{129}$, 3207.3950, found 3207.9033.

*4.3.39 Synthesis of 6^{A-F}-trideoxy-6^{A-F}-hepta(4-((N-tri(9-(4-(3-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)prop-1-yl)-1H-1,2,3-triazol-1-yl)-2-oxa-5-oxo-6-aza-nona-1-yl)methylamino)-3-oxopropyl)-1H-1,2,3-triazol-1-yl)-per-O-acetylated-α-CD (**40**)*

The click reaction between **8** (3.6 mg, 0.018 mmol) and **27** (44 mg, 0.019 mmol) was carried out as described for the preparation of **32a** to afford, after purified by flash chromatography eluted with CH_2Cl_2/CH_3OH (30:1 to 5:1), **40** (16 mg, 47%) as a white foam. R_f = 0.55 (CH_2Cl_2/CH_3OH = 5:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (s, 21H), 5.46 – 5.22 (m, 42H), 4.97 – 4.76 (m, 21H), 4.49 – 4.34 (m, 42H), 4.30 (d, J = 11.7 Hz, 21H), 4.20 – 3.97 (m, 63H), 3.91 – 3.70 (m, 84H), 3.69 – 3.46 (m, 84H), 3.36 – 3.11 (m, 63H), 2.84 – 2.64 (m, 42H), 2.59 (d, J = 8.9 Hz, 21H), 2.49 – 2.28 (m, 42H), 2.12 (s, 63H), 2.11 (s, 63H), 2.09 – 2.04 (m, 42H), 2.02 (s, 63H), 2.01 (s, 63H), 1.95 – 1.88 (m, 63H), 1.87 (s, 63H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.34, 170.86, 170.81, 170.54, 170.17, 170.10, 168.50, 147.43, 121.68, 98.82, 72.39, 69.23, 68.64, 67.44, 64.05, 62.34, 60.11, 52.71, 49.27, 47.77, 37.98, 36.49, 30.17, 29.65, 29.18, 23.06, 22.06, 21.07, 20.81, 20.73. ESI-QTOF-MS (m/z) $[M + 8H]^{8+}$ calcd for $C_{784}H_{1149}N_{133}O_{364}$, 2281.3185, found 2281.3225.

*4.3.40 Synthesis of 6^{A-F}-trideoxy-6^{A-F}-hepta(4-((N-tri(9-(4-(3-(5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)prop-1-yl)-1H-1,2,3-triazol-1-yl)-2-oxa-5-oxo-6-aza-nona-1-yl)methylamino)-3-oxopropyl)-1H-1,2,3-triazol-1-yl)-α-CD (**41**)*

The deprotection of **40** (60 mg, 0.0033 mmol) was carried out as described for the preparation of **29a** to afford **41** (44 mg, 97%) as a white foam. ^{1}H NMR (400 MHz, D_2O): δ 7.61 (s, 21H), 4.20 (s, 42H), 3.64 (dd, J = 19.3, 10.8 Hz, 84H), 3.53 – 3.33 (m, 168H), 3.33 – 3.19 (m, 21H), 3.07 – 2.89 (m, 42H), 2.63 – 2.56 (m, 63H), 2.23 (s, 42H), 1.95 – 1.86 (m, 42H), 1.85 (s, 63H), 1.79 – 1.61 (m, 42H), 1.46 (t, J = 11.7 Hz, 21H). ^{13}C NMR (101 MHz, D_2O): δ 174.99, 173.77, 173.53, 147.63, 123.21, 100.59, 72.54, 71.74, 68.52, 68.30, 68.19, 67.47, 63.66, 62.54, 60.11, 51.95, 47.79, 40.44, 36.43, 36.19, 29.17, 28.75, 22.06, 21.28. ESI-QTOF-MS (m/z) $[M + 4H]^{4+}$ calcd for $C_{567}H_{907}N_{133}O_{266}$, 3460.7346, found 3460.7893.

4.4 Hemagglutination inhibition assay

Hemagglutination inhibition (HI) assay was used to analyze the binding of inhibitor to influenza virus. Inhibitors were two-fold serially diluted with phosphate-buffered saline (PBS) buffer (pH 7.4). A/Wisconsin/588/2019 (H1N1) was added to all wells in a 96-well plate and then incubated at 4°C for 30 min. Chicken erythrocyte solution (50 μL of 1% solution) was added and incubated at 37°C for 60 min. The inhibition constant K_i^{HAI} represents the lowest inhibitor concentration that fully inhibits virus-induced hemagglutination.

4.5 SPR assay

Binding affinity of the synthesized Neu5Ac glycoclusters toward influenza virus HA and NA proteins and three CoV spike proteins was assessed using a Biacore 8K instrument (GE Healthcare), as previously described.[56] Proteins were immobilized using an amine-coupling kit on a carboxymethyl dextran–coated sensor chip (CM5) to ~15000 RU with running buffer PBS-P (20 mM PBS, 2.7 mM KCl, 137 mM NaCl, 0.05% surfactant P20, pH 7.4). Test conjugates (0.39–100 μM) containing 5% DMSO were individually injected and spread over the chip surface. Binding kinetics were analyzed with the Biacore 8K Evaluation Software using the Langmuir 1:1 binding model.

4.6 MDCK cell viability assay

Cytotoxicity of the synthesized Neu5Ac glycoclusters was evaluated using the CellTiter-Glo® kit. Briefly, Madin–Darby canine kidney (MDCK) cells were seeded

into 96-well cell culture plates (1×10^4 cells/well) in Dulbecco's modified Eagle medium supplemented with 1% fetal bovine serum and incubated overnight at 37°C with 5% CO_2. Then, the culture media were removed and replaced with fresh medium containing 100 μM conjugates in the sample wells, 5 μM paclitaxel in the positive-control wells, and 1% DMSO in the negative-control wells. All samples were in triplicate. Cells were incubated for 36 h at 37°C with 5% CO_2. Cell viability was assessed using CellTiter-Glo® reagent according to the manufacturer's instructions. A Tecan Infinite M2000 PRO microplate reader (Tecan Group Ltd., Mannedorf, Switzerland) was used to quantify luminescence.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/

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