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Dendritic Cell Lectin-Targeting Sentinel-like Unimolecular Glycoconjugates To Release an Anti-HIV Drug

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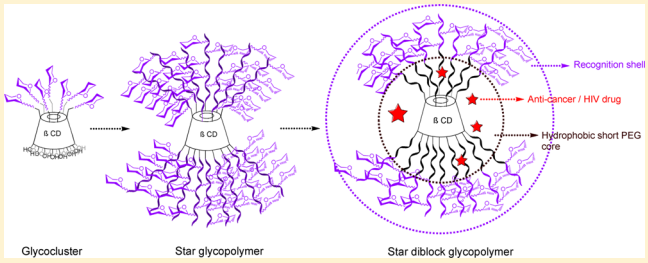
Supporting Information

ABSTRACT: A series of cyclodextrin-based glycoconjugates, including glycoclusters and star glycopolymers, were synthesized via combination of CuAAC Huisgen coupling and copper-mediated living radical polymerization. These glycoconjugates showed high affinity binding to the human transmembrane lectin DC-SIGN and act as inhibitors to prevent the binding of HIV envelope protein gp120 to DC-SIGN at nanomolar concentrations. The star block glycopolymers showed high loading capacity of hydrophobic anticancer and anti-HIV drugs, indicating promising applications in HIV-therapeutic and smart drug delivery.



1. INTRODUCTION

Dendritic cell specific ICAM-3 grabbing nonintegrin (DC-SIGN; CD209) is a C-type lectin present on the surface of dendritic cells that plays a key role in the immune response by modulating the host response to infection and inflammatory stimuli. It can be hijacked by HIV-1 through recognition of oligosaccharides on the viral envelope glycoprotein gp120, and this carbohydrate−protein interaction is considered to be a significant contribution to viral entry via infection of T cells *in trans*.[1] Due to this host receptor-dependent mode of infection, antiadhesion therapy has been developed as an important avenue for anti-HIV therapy, focusing on the application of carbohydrates to interfere with the binding between host lectins and virus.[2−5] Multivalent carbohydrate ligands have proven to yield high-avidity interactions with lectins due to the proximity of glycans in space generating a "Cluster Glycoside Effect".[6] Considerable interest has been generated in cyclodextrin (CD)-based glycoclusters due to both their highly branched carbohydrate-containing structures ideal for protein binding and their potential to act as carriers for the complexation of hydrophobic drug molecules, which can be considered as a means of intelligent drug delivery.[7−9] Different synthetic methodologies have been developed. However, multistep reactions are generally required for the preparation of glycoclusters with complex structures.[10−13]

Glycopolymers, like oligosaccharides, bind tightly to animal lectins and can be synthesized in a relatively facile manner and in significant quantity.[14,15] Well-defined CD-based star polymers can be synthesized via a core-first approach from functional CD initiators for different controlled radical polymerization, including ATRP, SET-LRP, RAFT, and NMP.[16−20] However, reports on synthesis of CD-based glycopolymers are very limited, and RAFT polymerization has not worked well in the past.[21]

The aim of this present study was to synthesize an intelligent drug delivery system as a potential HIV therapeutic with two distinct properties. First, for biorecognition, the macromolecule should have a glycoconjugate structure, either glycocluster or glycopolymer, that specifically binds to DC-SIGN and inhibits its interaction with HIV gp120. Second, it should have an ability to convey an anti-HIV drug, with the potential to deliver the drug to the targeted area and release drug molecules within key cells in a controlled manner.

Herein, we describe a novel approach to the synthesis of β-CD-based glycoclusters and β-CD-based star glycopolymers via the copper(I)-catalyzed Husigen azide−alkyne cycloaddition reaction (CuAAC) and copper(0)-mediated living radical polymerization (Cu(0)-LRP). The binding of different complex glycoconjugates to DC-SIGN was characterized by SPR, to identify high-affinity inhibitors for the HIV gp120/DC-SIGN interaction and to demonstrate interaction with this important

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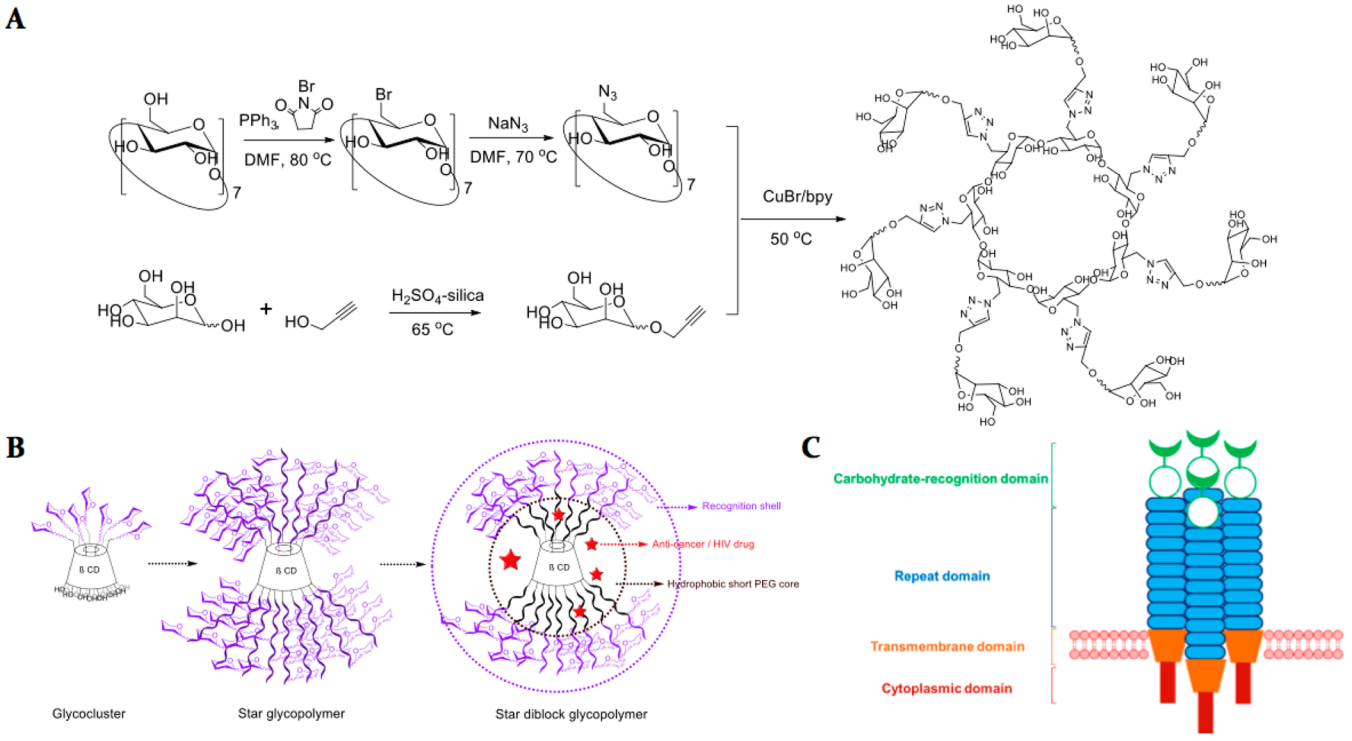
Scheme 1. Scheme Representation for the Synthesis of CD-Based Glycoclusters via CuAAC (A) and the Evolution Route from Glycocluster to Star Diblock Glycopolymer (B) and Schematic Structure of Human DC-SIGN Lectin (C)



dendritic cell lectin. The drug delivery ability of the glycoconjugates was also investigated and revealed high loading capacity for hydrophobic anticancer and anti-HIV drugs.

2. RESULTS AND DISCUSSIONS

2.1. Synthesis and Characterization of Click Glycoclusters. Persubstituted CD-based glycoclusters with multivalent monosaccharides showed binding behavior with different lectins at much lower concentration as compared to monosaccharides.[7,8,13] These promising results inspired us to develop similar structures with glycoclusters on the primary face as recognition sites with an unmodified secondary face such that the CD host retains the ability for drug encapsulation. However, this is a complicated process for the synthesis of CD-based glycoclusters according to the previous synthetic routes, which use multistep organic reactions, protection, deprotection chemistry, and chromatographic purification.[10−13]

The development of facile and efficient "click chemistry" provides routes to simplify this process, allowing for stereospecific, diverse scope, quantitative yields and byproducts that are easily removed by nonchromatographic methods.[22] Different types of click chemistry have been applied to the modification of CD in a relatively facile manner, and we have been inspired to use click chemistry to prepare complex CD-based glycoclusters.[23−26]

In this report, the syntheses of core moiety, per-6-azido-β-CD and its click reaction with alkyne monosaccharides are first presented (Scheme 1A). Starting from β-CD and monosaccharides, target glycoclusters could be obtained in high conversion through four step reactions, which allowed us to avoid the tedious multistep reaction and protection chemistry.

First, the per-6-azido β-CD core was synthesized in 90% yield, according to a modification of the previous method (Scheme 1A).[27] Second, unprotected alkyne monosaccharides were synthesized via a one-pot Fischer type glycosylation reaction using H_2SO_4-silica catalyst (Scheme 1A).[28,29]

The CuAAC of azide CD and alkyne functionalized mannose was first carried out using a CuBr/bpy catalyst in DMSO as solvent to yield the persubstituted β-CD-$(Man)_7$ glycocluster (Scheme 1A). The product was precipitated in methanol and washed with methanol without further chromatographic purification. The final product was characterized to demonstrate total substitution and formation of pure product. The ^{1}H NMR spectra showed the presence of triazole proton at approximately 7.9 ppm and CD residues at 6.0 ppm (OH) and 5.2 ppm (H-1) with the integral ratio consistent with theoretical values, which indicated the success of the CuAAC, Supporting Information S Figure 4. The MALDI-ToF spectrum (Figure 1B) showed peaks corresponding to the fully substituted product and FT-IR (Supporting Information S Figure 5) confirmed the disappearance of azide functionalities at 2100 cm^{-1} following the click reaction. DMF SEC analysis (Figure 1C) revealed the shift of elution traces after reaction due to the change of hydrodynamic volume. All these data support the synthesis of pure β-CD-based mannose glycocluster decorated through 1,2,3-triazole linker with seven mannose units on the primary face and an unmodified secondary face.

To verify the versatility of this approach, a β-CD-based fucose glycocluster was also synthesized according to same procedure (see Supporting Information). ^{1}H NMR, MALDI-ToF MS, FT-IR, and SEC analysis revealed similar results as mannose glycocluster and confirmed the right target structure, Figure 1. In summary, persubstituted β-CD-based glycoclusters were prepared by Huisgen 1,3 dipolar cycloaddition of appropriate sugar alkynes with β-CD bearing azide groups on the primary face.

2.2. Synthesis of Cyclodextrin-Based Star Glycopolymers. The CD-based initiator was synthesized via a one-pot

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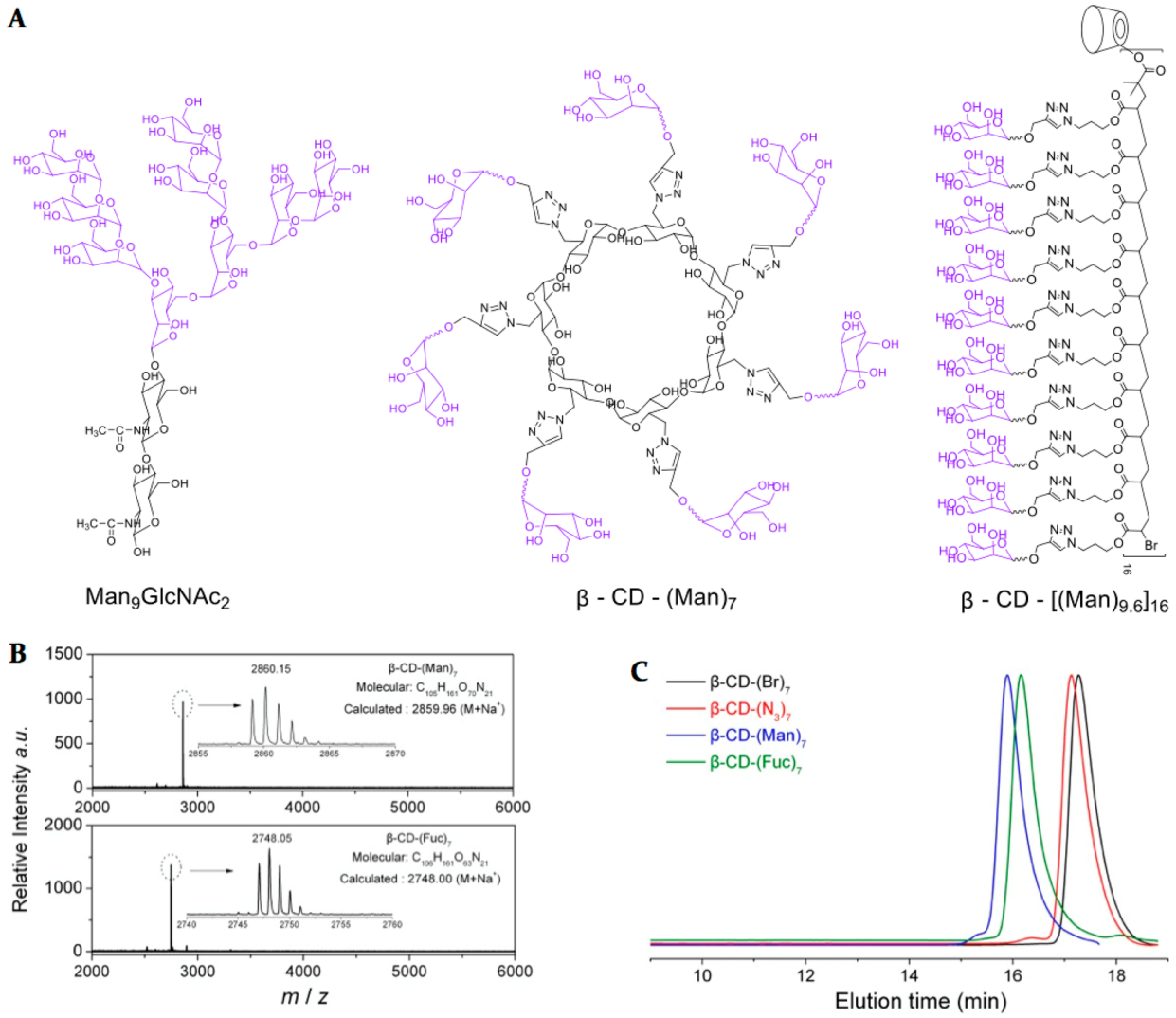

Figure 1. Molecular structure of $Man_9GlcNAc_2$, β-CD-$(Man)_7$, and β-CD-$[(Man)_{9.6}]_{16}$ (A); MALDI-ToF MS (B), and SEC elution traces (C) of β-CD-$(Man)_7$ and β-CD-$(Fuc)_7$ glycoclusters.

esterification reaction according to a previous report.[30] Under these experimental condition, this esterification reaction cannot reach full conversion and ^{1}H NMR and MALDI-ToF MS analysis confirmed the average degree of substituted hydroxyl groups at the periphery CD was approximately 16, which is given the descriptor β-CD-$(Br)_{16}$. The glycomonomers were synthesized via CuAAC of one azide-functional acrylate with different alkyne-functional monosaccharides.[31]

Cyclodextrin-based star glycopolymers were synthesized via direct Cu(0)-LRP of glyco monomers, Figure 2A. The polymerization of glucose monomer was first conducted at ambient temperature under the catalysis of a Cu(0)/Cu(II)/Me_6TREN system with β-CD-$(Br)_{16}$ as initiator and DMSO as solvent. The polymerization was continued for 24 h, which led to full conversion according to ^{1}H NMR analysis revealed by the disappearance of vinyl groups at 5.8−6.4 ppm. DMF SEC analysis showed a narrow dispersity polymer with M_n = 9300 g·mol^{-1} and M_w/M_n = 1.16, Supporting Information S Figure 6.

It is noted that the M_n as measured by SEC is lower than the theoretical molecular weight, which is ascribed to a lower hydrodynamic volume of a star polymer when compared with a linear polymer of the same molar mass. It is also worth noting that no significant star−star coupling termination was detected even after polymerization to full conversion overnight, which would be observed as a shoulder peak at higher molecular weight. This phenomenon is mainly attributed to the initially added Cu(II) which aids in the preservation of high chain end fidelity.[32,33]

For the test of the ability for chain extension (Figure 2A), a further equivalence of mannose glyco monomer was directly added into the reaction mixture and allowed to polymerize for another 48 h. Following the reaction, ^{1}H NMR analysis showed a conversion of 88% by comparing the integral of triazole proton area at 7.9−8.2 ppm with vinyl groups at 5.9−6.4 ppm.

SEC analysis revealed an increase of M_n to 11 800 g·mol^{-1} and M_w/M_n to 1.17, Supporting Information S Figure 6, which is still a quite narrow dispersity for such a multiarm star polymer. The elution traces almost shift totally, and no coupling peaks were detected at this stage. ^{1}H NMR analysis of the final product showed broad resonance from the triazole protons at 8.1 ppm, overlapped CD and sugar units from 3 to 5 ppm, and typical initiator methyl groups at 1.1 ppm, which proved the structure of CD-based star glycopolymer bearing sugar units with 1, 2, 3-triazole linker, Supporting Information S Figure 6. All of the previous results clearly indicated that CD-based star glycopolymer with controlled structure and high

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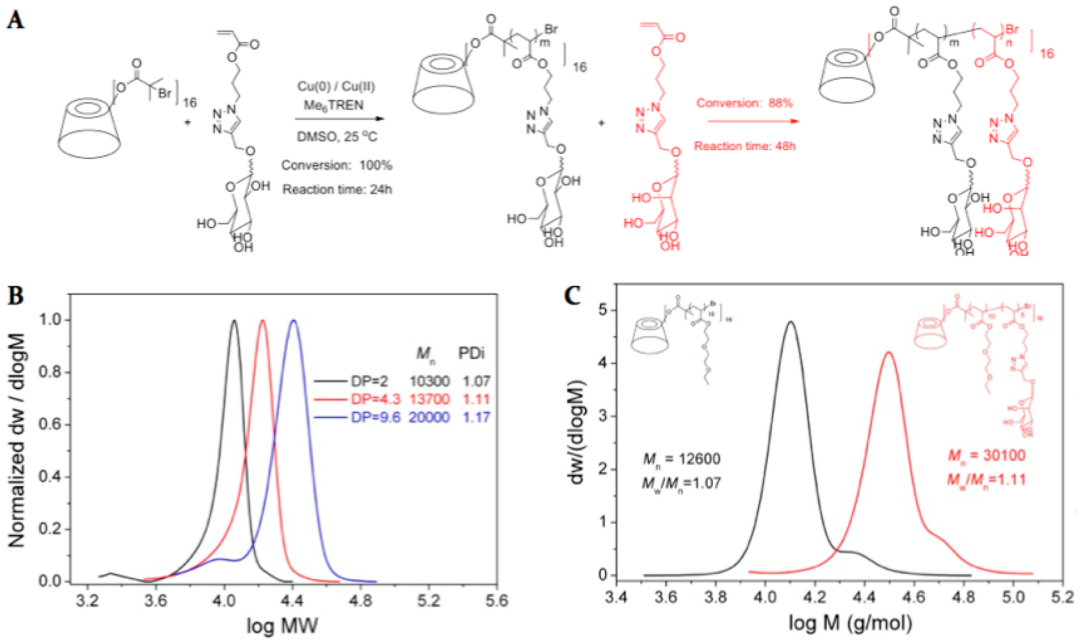

Figure 2. Synthesis of β-CD based glycopolymers via SET-LRP and chain extension reaction (A), SEC traces in DMF (normalized to height, B and C) of CD-based mannose home and diblock glycopolymers obtained by SET-LRP.

chain end fidelity could be synthesized using Cu(0)-LRP (SET LRP).

This inspired us to prepare a library of β-CD-based mannose star glycopolymers as inhibitors for the interaction between DC-SIGN and HIV gp120. Three polymerizations with target degree of polymerization of 2, 5, and 10 were conducted, and long reaction times were kept until high conversion values were attained. Under these reaction conditions shown in Supporting Information, polymerization with DP = 2 reached full conversion after 24 h, while conversion of polymerization with DP = 5 and 10 only reached 86% and 96% after 48 h. Sampling of polymerization with DP = 10 revealed that conversion reached around 80% in the first 10 h (see Supporting Information). As expected accompanying the monomer consumption, the reaction rate decreased due to the highly diluted reaction condition. SEC analysis revealed that narrow dispersity star glycopolymers with different chain length have been prepared. M_w/M_n is around 1.1, and the tailing peak in glycopolymer DP = 10 might be caused by sampling during the polymerization, which may have introduced air into the reaction system and caused some termination, Figure 2B. ^{1}H NMR analysis (Supporting Information S Figure 7) revealed the ratio decreased for the integral of initiator methyl groups (H-9) at 1.1 ppm relative to the triazole proton units at 8.1 ppm when the DP increased from 2 to 10, which showed the incorporation of more sugar units. Due to the overlap between initiator residues (H-9), polymer backbone protons (H-1, 2) and monomer units (H-4), calculations from the equivalence of $\int$ H-6$^{7.9-8.3\ ppm}$: $\int$ H-1, 2, 4, 9$^{0.7-2.5\ ppm}$ = DP: (5DP + 6) lead to values of DP = 1.6, 3.0, and 8.5, which are close to the values of DP = 2, 4.3, and 9.6 according to the conversion and thus proved the successful synthesis of star glycopolymers with different chain length. Nevertheless, according to the previous results, three β-CD-based mannose star glycopolymers with defined structures have been successfully prepared for the binding test with lectin.

2.3. Synthesis of Cyclodextrin-Based Diblock Glycopolymer. Water-soluble star shaped polymers have been applied as useful polymeric nanocontainers with high loading capacity of hydrophobic drugs.[34,35] With the synthesis of highly water-soluble CD based star glycopolymers, the CD core became surrounded with dense sugar units at the outside shell, which would make the inclusion of drug molecular into CD host more difficult. To construct an intelligent drug delivery system with carbohydrate recognition sites at the periphery of the sphere, a hydrophobic core area was necessary to be built for drug loading through the host–guest interaction.

A β-CD based diblock glycopolymer was subsequently synthesized by Cu(0)-LRP and one-pot chain extension reaction. First, polymerization of DEGEEA initiated from β-CD-(Br)$_{16}$ with a Cu(0)/Cu(II)/Me$_6$TREN catalyst in DMSO reached almost full conversion (98% according to ^{1}H NMR) in 4 h, after which a part of the solution was removed for characterization and another portion of mannose glyco monomer was directly added into the system for chain extension reaction without purification. After reaction for a further 20 h, conversion was up to 95% and the reaction was then stopped. SEC and NMR analysis confirmed the synthesis of β-CD-[(DEGEEA)$_{10}$]$_{16}$ and β-CD-[(DEGEEA)$_{10}$-*b*-(mannose)$_5$]$_{16}$ featuring narrow molecular weight distributions, Figure 2C and Supporting Information S Figure 9. For the synthesis of first block β-CD-[(DEGEEA)$_{10}$]$_{16}$, it is noted that the dispersity is <1.07 at almost full conversion, even when the small shoulder peak is included which may be caused by the star–star radical coupling or traces of diacrylate impurities from the commercial DEGEEA monomer. Following this chain extension, the SEC elution trace almost shifted totally revealing that high chain end fidelity was maintained throughout the polymerization. The M_n increased from 12 600 to 30 100 g·mol^{-1} and the M_w/M_n had a slight increase from 1.07 to 1.11. Although the shoulder peak at higher molecular weight region became more obvious after reaction overnight, the ratio relative to the main peak was still small. ^{1}H NMR analysis of the final

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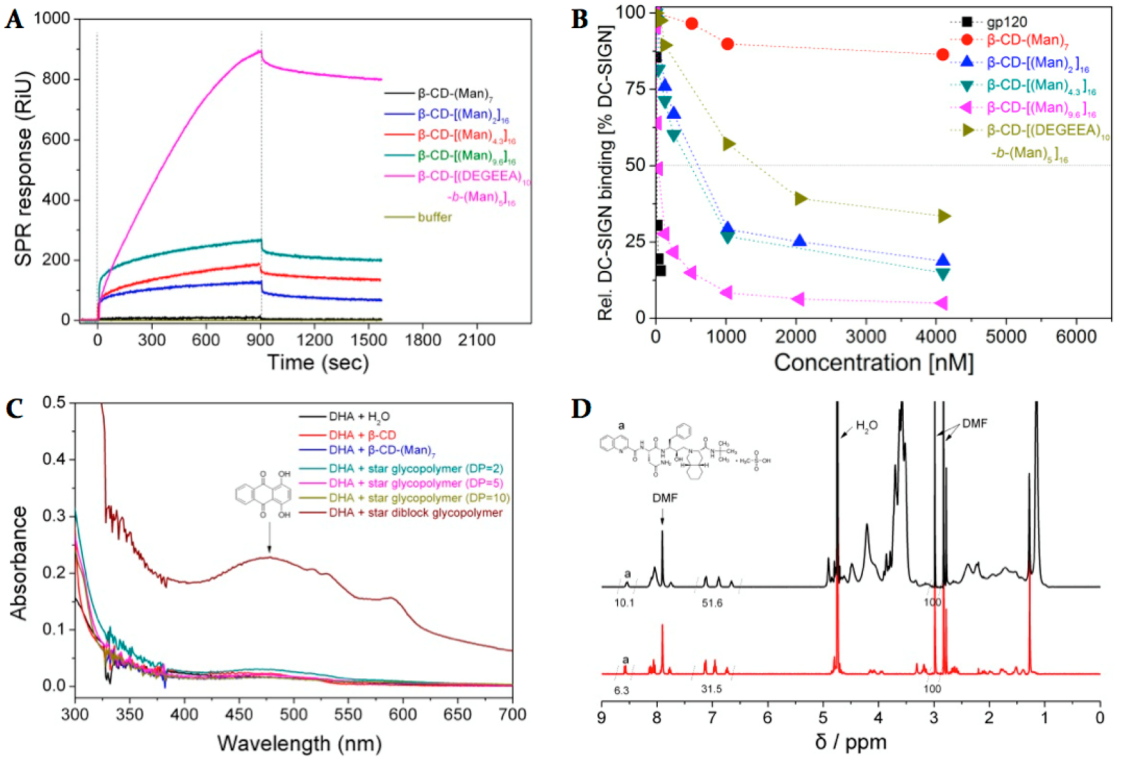

Figure 3. (A) SPR sensorgrams showing the binding of β-CD based glycoconjugates onto DC-SIGN functionalized surfaces. The concentration ranges for β-CD based glycol conjugates were 4096 nM. (B) Competition experiments on gp120 functionalized surface between DC-SIGN and β-CD based glycol conjugates at a concentration range of 0−4096 nM for glycol conjugates and 4 nM DC-SIGN. (C) UV−vis spectra of DHA solution in the presence of different cyclodextrin products. All measurements with β-CD, β-CD-$(Man)_7$ and glycopolymers were performed at a concentration of 1 mg/mL. (D) ^{1}H NMR spectra of saquinavir mesylate (bottom) and of saquinavir mesylate encapsulated into polymer β-CD-$[(DEGEEA)_{10}$-*b*-$(Mannose)_5]_{16}$ (top). Both measurements were performed in D_2O with 10 mM DMF as internal standard. The concentration of β-CD-$[(DEGEEA)_{10}$-*b*-$(Mannose)_5]_{16}$ in D_2O was 0.3 mM (D).

Table 1. Properties of Saquinavir Mesylate Encapsulation Test from CD-Based Glycoconjugates and Binding Kinetics and Inhibition Concentration of CD-Based Glycoconjugates

	encapsulation test			DC-SIGN binding[c]		
sample name	$[CD\ glycoconjugate]_0$ [mM][a]	$[saquinavir\ mesylate]_0$ [mM][b]	K_{on} [M^{-1} s^{-1}]	K_{off} [s^{-1}]	K_D [nM]	IC_{50} [nM]
gp120	/	/	7.3×10^5	7.8×10^{-5}	0.11	11
D_2O	/	1.9	/	/	/	/
β-CD	16	4.0	/	/	/	/
β-CD-$(Man)_7$	1.0	2.3	/	/	/	/
β-CD-$[(Man)_2]_{16}$	0.3	2.0	810	1.7×10^{-4}	210	436
β-CD-$[(Man)_{4.3}]_{16}$	0.3	2.1	500	3.9×10^{-5}	77	389
β-CD-$[(Man)_{9.6}]_{16}$	0.3	2.0	130	2.8×10^{-7}	0.22	30
β-CD-$[(DEGEEA)_{10}$-*b*-$(Man)_5]_{16}$	0.3	3.0	363	1.1×10^{-4}	290	1420

[a]Concentrations of CD or CD-based conjugates were calculated from [mass]/[molecular weight]. The molecular weights of glycopolymer were theoretical value based on conversion from ^{1}H NMR. [b]Solubility of saquinavir mesylate was determined by ^{1}H NMR using DMF (10 mM) as internal standard. [c]Binding kinetics and inhibition concentration of CD-based glycoconjugates were determined by SPR.

product showed resonances from mannose monomer units after chain extension, including triazole protons at 8.1 ppm and typical mannose protons at 4.7 ppm. The FT-IR (Supporting Information S Figure 10) also revealed strong OH absorbance due to the addition of mannose units. All these data clearly proved the successful synthesis of diblock β-CD-$[(DEGEEA)_{10}$-*b*-$(mannose)_5]_{16}$ glycopolymer.

2.4. SPR Interaction Analysis of Cyclodextrin-Based Glycoconjugates to DC-SIGN. C-type (calcium-dependent) lectins are a family of animal lectins that participate in many cell-surface carbohydrate-recognition events.[36] DC-SIGN, a typical family member is composed of a carbohydrate-recognition domain (CRD, common to all C-type lectins), connected to a neck domain and with a cytoplasmic domain at the N-terminus.[37] Polypeptides form homotetramers through interactions between the neck regions (Scheme 1C).

The CRD is a tightly folded modular unit with a diameter of ~4 nm.[38,39] Individual CRDs in the tetramers possess a high affinity for mannose-containing oligosaccharides by binding to mannose residues spaced at appropriate distances.[40] The neck domain comprises seven and a half tandem repeats of 23 amino acids with a total length of ~20−30 nm.[39] Although the neck does not bind carbohydrates directly, it plays an important role in stabilizing the tetramer, allowing the presentation of multiple

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binding sites for carbohydrate ligands and increasing the avidity of interactions.[37]

In previous work, $Man_9GlcNac_2$ (Figure 1A) has been used as a model oligosaccharide for binding to the CRD of DC-SIGN, and a 130-fold increase relative to the binding of a single mannose has been shown.[39−41] Compared with the branched $Man_9GlcNac_2$, β-CD-$(Man)_7$ (Figure 1A) also has a cluster structure but notably possesses different linker length and spacing for the terminal mannose. β-CD-$(Man)_7$ showed relatively weak binding to DC-SIGN (Figure 3A) and was a poor inhibitor of gp120 binding to DC-SIGN with only ∼12% reduction in binding at the highest concentration tested (4096 nM β-CD-$(Man)_7$; Figure 3B). β-CD-$(Fuc)_7$ also showed very weak binding with DC-SIGN (S Figure 21). Thus, the combination of a limited number of monosaccharide units, the relatively crowded structure, and the short linker between monosaccharide units means that the β-CD moiety is suboptimal for binding, probably due to relatively few saccharide residues in the glycocluster are correctly positioned to access the binding sites on the lectin. Thus, to get good binding, we can learn from the $Man_9GlcNac_2$ to construct a branched glycocluster onto a cyclodextrin core, which may have to use complex and multistep organic synthesis. Therefore, star glycopolymers could be a wise option as its star-shape simulates the branched structure and the multivalent carbohydrate units may have sufficient chance to interact with the binding sites of DC-SIGN lectin (Scheme 1B).

For the CD-based star glycopolymer, even with only DP = 2, the binding affinity for DC-SIGN is much higher than that of the CD-based glycocluster, Figure 3A and Table 1, with the total mannose units increased to ∼32, 4- to 5-fold greater than the number of mannose units in β-CD-$(Man)_7$. It should be noted that, for each chain of the star glycopolymer, an average DP = 2 means that each chain is likely to contain between 1 and 4 mannose units, increasing its similarity to the structure of $Man_9GlcNac_2$ leading to more efficient binding.

With an increase of DP to ∼4, the binding signal increased (a higher RU value and decreased K_D value from 210 to 77 nM) indicating that more of the polymer binds to DC-SIGN; however, the IC_{50} value decreased only slightly from 436 to 389 nM (Figure 3B), revealing that the change in DP from 2 to 4 did not greatly improve inhibition of the DC-SIGN/gp120 interaction.

With an increase of DP to ∼10, a further increase in polymer binding was observed compared to both DP = 2 and DP = 4. β-CD-$[(Man)_{9.6}]_{16}$ showed a K_D value of 0.22 nM, which is only 2 times the K_D value of gp120, suggesting the high affinity of star glycopolymer for DC-SIGN. β-CD-$[(Man)_{9.6}]_{16}$ showed IC_{50} values as low as ∼30 nM (Figure 3B), which is only slightly higher than that of gp120 competing itself (∼11 nM, Figure 3B) under the same test conditions, indicating that this polymer is a potent inhibitor of DC-SIGN binding to gp120. The dramatic difference in activity indicates that the sugar number, linker length, and type of spacer may contribute for the binding with DC-SIGN. The complex $Man_9GlcNAc_2$ clustering on gp120 could be seen as a precise key for interaction with DC-SIGN that opens the gateway into cells. In contrast, glycopolymers ostensibly work through brute force via presentation of multimeric carbohydrate units, at high density, in order to break the lock. This finding is also consistent with the known properties of DC-SIGN in which there are multiple binding sites for mannose on the DC-SIGN CRDs and tandem mannose units in a cluster structure with enough density are necessary to increase the likelihood of blocking all the binding sites.

Interestingly, the block star polymer β-CD-$[(DEGEEA)_{10}$-*b*-$(mannose)_5]_{16}$, which bears a similar number of mannose units as β-CD-$[(Man)_{4.3}]_{16}$, showed weaker inhibition with a much higher IC50 value (∼1420 nM), Figure 3B. Addition of the poly(DEGEEA) core increases the spacing of the mannose units between each chain, which clearly does not favor binding. In summary, these SPR experiments demonstrate that CD-based conjugates bind with high affinity to DC-SIGN to inhibit the interaction with HIV gp120. The low IC_{50} value of β-CD-$[(Man)_{9.6}]_{16}$ of ∼30 nM is particularly impressive, with the desirable binding and inhibition properties of a HIV therapeutic.

2.5. Encapsulation Ability of the Cyclodextrin-Based Glycoconjugates. Molecular encapsulation behaviors of obtained cyclodextrin-based glycoconjugates were first characterized by UV−vis spectroscopy using DHA as a guest molecule, which is considered as a potential anticancer chemotherapeutic agent.[42] DHA was also chosen as a model compound for CD encapsulation test since this hydrophobic drug possesses a sensitive UV−vis absorbance.[43] Due to the low solubility in water, only very weak absorbance could be detected for DHA in water without addition of CD conjugates. After addition of β-CD, β-CD-$(Man)_7$, β-CD-$[(Man)_2]_{16}$, β-CD-$[(Man)_5]_{16}$, and β-CD-$[(Man)_{10}]_{16}$, absorbance values are still at the same level and no significant increase could be detected, which revealed that no stable inclusion could be formed between DHA and β-CD, β-CD-$(Man)_7$ and β-CD-based homo glycopolymers. Upon addition of DHA to the aqueous solution of β-CD-$[(DEGEEA)_{10}$-*b*-$(mannose)_5]_{16}$, the absorption at 479 nm (λ_{max}) significantly increased (Figure 3C), indicating the encapsulation and solubilization of DHA by the diblock copolymer. It is assumed that the core of β-CD-$[(DEGEEA)_{10}$-*b*-$(mannose)_5]_{16}$ acted as a hydrophobic area at ambient temperature in aqueous solution and could encapsulate hydrophobic molecular based on the host−guest interaction. Due to the larger hydrophobic core area compared with β-CD, β-CD-based glycocluster, and homo glycopolymers, the star block copolymer could encapsulate more guest molecules and thus showed more significant absorbance on UV−vis spectroscopy.

On the basis of these encouraging results, we have investigated the delivery of an anti-HIV drug. Saquinavir is an important class of archetypal HIV protease inhibitor; however, its poor aqueous solubility results in low and variable bioavailability.[44] β-CD derivatives have been applied to enhance the aqueous solubility of Saquinavir base and mesylate salt.[44] Thus Saquinavir mesylate was also used as guest molecules to test the encapsulation ability of CD-based glycoconjugates.

For the quantitative evaluation of the encapsulated drug by ^1H NMR spectroscopy, several solutions of CD-based glycoconjugates were prepared in D_2O, with excess of the guest Saquinavir mesylate and DMF as internal standard (10 mM). First, control measurement was performed in order to detect the intrinsic water solubility of drug (C_i) in the absence of glycoconjugates, which revealed a water solubility of Saquinavir mesylate of C_i = 1.9 mM (Figure 3D). With the addition of β-CD and β-CD-$(Man)_7$, the solubility of Saquinavir mesylate was also increased, suggesting that stable inclusion of CD host with Saquinavir mesylate could be formed. After addition of β-CD-based homo glycopolymer, the solubility of Saquinavir mesylate was almost at the same level,

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indicating that larger hydrophobic core area was needed in order to increase the solubility even with low concentration of polymer. Following the addition of the block copolymer, the peak integrals of Saquinavir mesylate increased compared with DMF standard (Figure 3D), indicating that the solubility of Saquinavir mesylate could be increased due to the encapsulation behavior of star block copolymer. The solubility of Saquinavir mesylate increased to 3.0 mM as calculated from the ratio of the integrated DMF signal with the characteristic peaks of guest molecule. Followed by a subtraction of the intrinsic concentration in water, the encapsulated drug concentration was calculated as 1.1 mM. The maximum loading capacity of the block copolymer was then determined as 4 by dividing the mole ratio of drug with the mole ratio of polymer, which means that each star copolymer can encapsulate up to four Saquinavir mesylate molecules on average.

3. CONCLUSION

Cyclodextrin-based mannose and fucose clusters were synthesized via the CuAAC reaction of azide-functionalized CD with relative alkyne sugar following precipitation in methanol without the need of protection chemistry and column chromatography purification. For the design of high affinity lectin-glycoprotein binding inhibitor, star-shaped glycopolymers containing cyclodextrin core and oligosaccharide chains were synthesized by direct Cu(0) mediated LRP of glycomonomers from CD-based initiator, which also allowed facile synthesis of diblock glycopolymer via in situ monomer addition.

SPR analysis revealed that these glycoconjugates bind with high affinity to DC-SIGN and could be used as inhibitors to prevent the binding of HIV envelope protein gp120 to DC-SIGN at nanomolar concentrations. From different binding phenomena of glycoclusters and star glycopolymers with varying DP, it can be concluded that star glycopolymers with enough carbohydrate units could work as an efficient inhibitor for the binding of gp120 with CRDs of DC-SIGN.

An encapsulation test of these glycoconjugates via UV/vis and NMR revealed that star diblock glycopolymer bearing a hydrophobic core area showed high loading capacity of hydrophobic anticancer and anti-HIV drugs, indicating promising application in HIV-therapeutic and smart drug delivery, potentially utilizing versatile endocytic lectins such as DC-SIGN.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and supplementary data including NMR, SEC, UV/vis, FTIR, and MALDI-ToF MS. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare the following competing financial interest(s): D. M. Haddleton is a Director of Warwick Effect Polymers Ltd.

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