Bioconjugate Chemistry

Article

Cite This: Bioconjugate Chem. 2019, 30, 2594−2603

pubs.acs.org/bc

Multivalent Cluster Nanomolecules for Inhibiting Protein−Protein Interactions

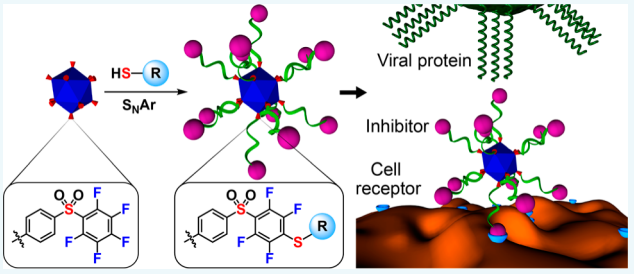
Elaine A. Qian,[†,‡,§] Yanxiao Han,[∥] Marco S. Messina,[†,§] Heather D. Maynard,[†,‡,§] Petr Král,[∥,⊥,#] and Alexander M. Spokoyny*[,†,§]

[†]Department of Chemistry and Biochemistry, [‡]Department of Bioengineering, and [§]California NanoSystems Institute, University of California, Los Angeles, Los Angeles, California 90095, United States

[∥]Department of Chemistry, [⊥]Department of Physics, and [#]Department of Biopharmaceutical Sciences, University of Illinois at Chicago, Chicago, Illinois 60607, United States

Supporting Information

ABSTRACT: Multivalent protein−protein interactions serve central roles in many essential biological processes, ranging from cell signaling and adhesion to pathogen recognition. Uncovering the rules that govern these intricate interactions is important not only to basic biology and chemistry but also to the applied sciences where researchers are interested in developing molecules to promote or inhibit these interactions. Here we report the synthesis and application of atomically precise inorganic cluster nanomolecules consisting of an inorganic core and a covalently linked densely packed layer of saccharides. These hybrid agents are stable under biologically relevant conditions and exhibit multivalent binding capabilities, which enable us to study the complex interactions between glycosylated structures and a dendritic cell lectin receptor. Importantly, we find that subtle changes in the molecular structure lead to significant differences in the nanomolecule's protein-binding properties. Furthermore, we demonstrate an example of using these hybrid nanomolecules to effectively inhibit protein−protein interactions in a human cell line. Ultimately, this work reveals an intricate interplay between the structural design of multivalent agents and their biological activities toward protein surfaces.



Multivalency is a prevalent phenomenon that facilitates many important biological processes in nature.[1] Some of the most fascinating examples are found in our own immune system, where multivalency plays a crucial role in modulating several central functions of the immune cells, including cell signaling, cell−cell interaction, and pathogen recognition.[2−5] A notable example of these intricate interactions takes place between glycoproteins and lectins, whose specificity and affinity toward each other are greatly amplified through multivalency. The important role multivalency plays in nature has fascinated both biologists and chemists alike, who are mutually interested in understanding the fundamental mechanisms behind these supramolecular recognition events as well as developing abiotic tools that are inspired by natural phenomena.[5−9]

An important biological target for studying multivalency is a C-type lectin receptor called dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN).[10] Predominately expressed on the surface of dendritic cells, it organizes into a homotetrameric structure that is critical for the multivalent recognition of pathogens.[11,12] In particular, DC-SIGN is able to bind specific high-mannose glycoproteins and glycolipids on pathogens with high avidity, which activates a sequence of downstream responses including pathogen uptake and degradation as well as subsequent antigen processing and presentation.[13] However, various pathogens such as HIV-1 have been observed to escape the intracellular degradation pathway following DC-SIGN-facilitated uptake.[14] While the mechanism behind this unusual behavior is not well understood, it is clear that DC-SIGN plays an instrumental role in transmitting HIV-1 to the T cells and enhancing the infection in its early stages.[14−16] Therefore, there is significant interest in (1) uncovering the rules that govern the multivalent interactions between DC-SIGN and high-mannose glycoconjugates and (2) inhibiting the DC-SIGN-dependent attachment and uptake of certain pathogens. One of the most promising approaches that can potentially tackle both challenges is centered around building molecules that can mimic the dense multivalent display of carbohydrates on the pathogen surface.[8,9,17−19]

Previously, several promising classes of glycomimetic ligands for DC-SIGN have been designed and synthesized, which include but are not limited to small molecules,[20,21] peptides,[22,23] linear and dendritic polymers,[24−30] fullerenes,[31,32] supramolecular assemblies,[33−35] and hybrid nanoparticles.[36−38] These constructs are capable of engaging DC-

Received: August 2, 2019
Revised: September 4, 2019
Published: September 9, 2019

DOI: 10.1021/acs.bioconjchem.9b00526
Bioconjugate Chem. 2019, 30, 2594−2603

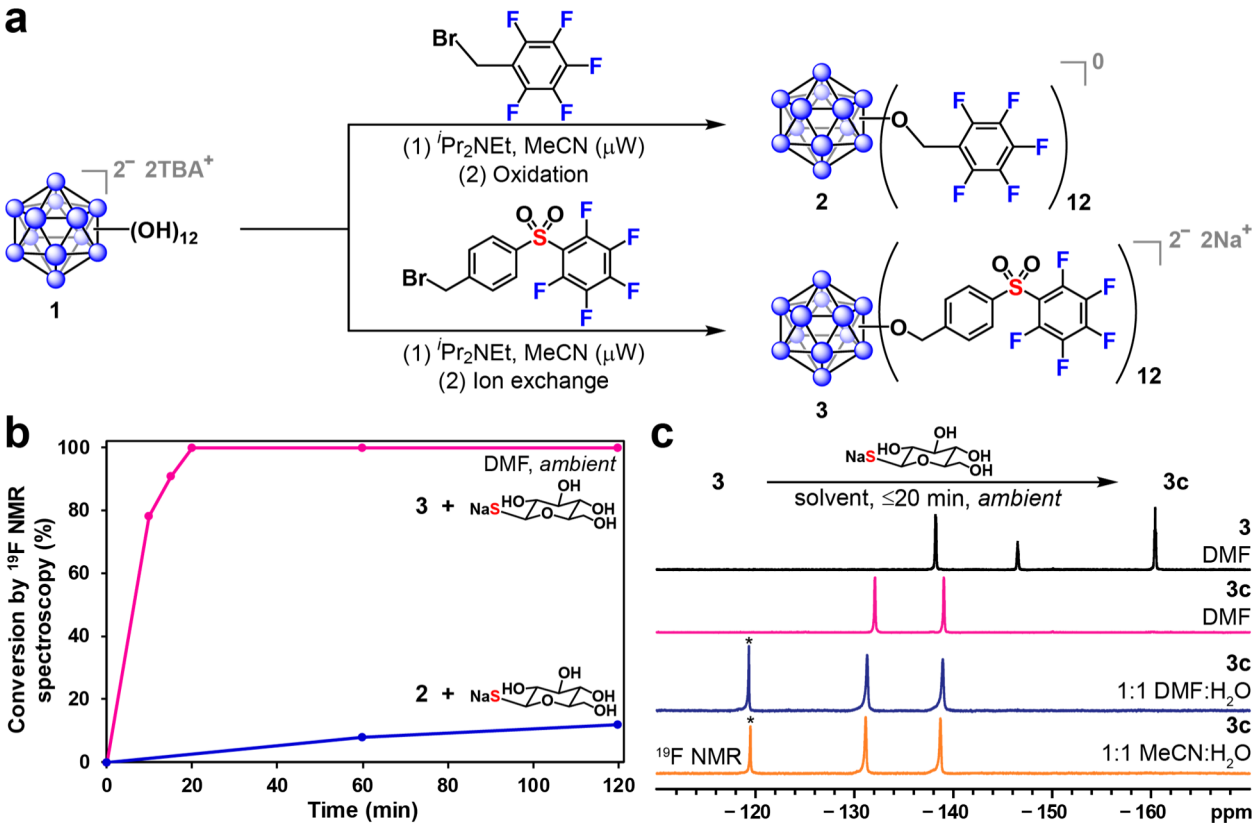


Figure 1. Synthesis of perfluoroaryl-perfunctionalized clusters and their reactivities toward an unprotected thiolated saccharide. (a) Clusters **2** and **3** are readily prepared from **1** with the assistance of a microwave reactor. (b) Conversion rates of S_NAr reactions between **2**/**3** and 1-thio-β-D-glucose sodium salt, as monitored by ^{19}F NMR spectroscopy, reveal the significantly enhanced reactivity of **3** over **2**. (c) ^{19}F NMR spectra of **3** in DMF and **3c** after conjugation with 1-thio-β-D-glucose sodium salt in DMF or mixed aqueous/organic media. *NaF signal.

SIGN with high avidities (K_D spanning nM to μM), which allowed several of these systems to inhibit viral entry and infection. In particular, rigid three-dimensional (3D) architectures such as thiol-capped gold nanoparticles (AuNPs) are attractive glycomimetic platforms due to the ease of generating tunable and well-defined multivalent agents. Nevertheless, due to the weak bonding interactions between gold and thiol-based ligands, the surface morphology of these systems is poorly defined and highly dynamic, especially under biologically relevant conditions.[39−42] This ultimately hinders researchers' ability to understand the precise structure−activity relationships of these systems with respect to biomolecular recognition and binding events.

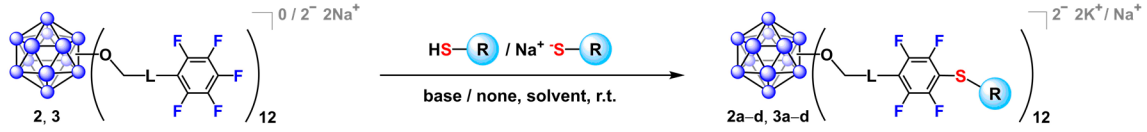
Here we report the synthesis of a family of atomically precise glycosylated cluster nanomolecules featuring robust inorganic cluster scaffolds as nanoparticle core templates. Specifically, we developed conditions that allow the rapid functionalization of perfluoroaryl-based moieties covalently grafted onto a rigid dodecaborate core via "click"-like nucleophilic aromatic substitution (S_NAr) chemistry, thus leading to fully covalent nanomolecules with a densely packed layer of saccharides.[43,44] This chemistry mimics the operational simplicity with which thiol-capped AuNPs are synthesized, yet produces well-defined assemblies that are stable under biologically relevant conditions.[44] Importantly, direct binding studies between these hybrid assemblies and DC-SIGN reveal the multivalency-enhanced avidity in addition to the carbohydrate specificity of the lectin and the structural requirements for the multivalent ligands. Furthermore, competitive binding data suggest the mannose-coated nanomolecules can inhibit the protein−protein interactions between DC-SIGN and an HIV-1 envelope glycoprotein, gp120. Moreover, we found that the nanomolecules exhibit no apparent toxicity to a human lymphoblast-like cell line at 0.5−50 μM concentrations. This allowed us to perform cellular experiments, which revealed that the mannose-functionalized clusters are capable of preventing the cell uptake of gp120 by blocking cell-surface DC-SIGN. Therefore, we demonstrate that easily accessible, precisely engineered hybrid cluster-based nanomolecules can be utilized to not only study the rules governing multivalent recognition but also inhibit protein−protein interactions in cells.

RESULTS AND DISCUSSION

Given our success in installing a wide scope of thiols onto the perfluoroaryl-perfunctionalized clusters using S_NAr chemistry,[44] we hypothesized that this strategy could be applied to generate a library of atomically precise nanomolecules featuring a variety of saccharides densely packed on the rigid 3D surface. Using the perfluoroaryl-perfunctionalized cluster **2** (Figure 1a) and 1-thio-β-D-mannose tetraacetate,[44−47] we performed S_NAr reactions in the presence of base in dimethylformamide (DMF), stirring under a N_2 atmosphere. These test conjugation reactions revealed significant conversions, as determined by ^{19}F NMR spectroscopy. Following efficient optimization facilitated by in situ ^{19}F NMR spectroscopy, we found that employing an excess of the thiol and potassium phosphate (K_3PO_4) allowed the nearly quantitative (≥99%) substitution of **2** with the substrate within 48 h. The

DOI: 10.1021/acs.bioconjchem.9b00526
Bioconjugate Chem. 2019, 30, 2594−2603

Table 1. Glycosylation and PEGylation of Clusters 2 and 3



Entry	Compound	L	R	Time (h)	*In situ* yield[a] (%)	Isolated yield[b] (%)
1	**2a**	none	mannose	48	≥99	80
2	**2b**	none	galactose	48	≥99	84
3	**2c***	none	glucose	24	≥99	65
4	**2d***	none	$-O(CH_2CH_2O)_6-$	24	≥99	76
5	**3a**		mannose	0.3	≥99	83
6	**3b**		galactose	0.3	≥99	67
7	**3c**		glucose	0.3	≥99	77
8	**3d**		$-O(CH_2CH_2O)_6-$	1.5	≥99	84

[a]Yield determined by ^{19}F NMR spectroscopy. [b]Isolated yield after purification. *Previously reported compounds. r.t., room temperature.

product was briefly treated with sodium methoxide (NaOMe) to remove all the acetyl groups, then purified by a desalting centrifugal filter to yield the mannose-coated nanomolecule **2a** (Table 1, entry 1) in 80% isolated yield (see the Supporting Information for experimental details). The purified **2a** was subsequently subjected to characterization via ^{1}H, ^{11}B, and ^{19}F NMR spectroscopy and electrospray ionization-high resolution mass spectrometry (ESI-HRMS), which support the proposed structure and composition (see the Supporting Information for characterization data). Furthermore, we found that a similar strategy could be used to perfunctionalize **2** with 1-thio-β-D-galactose tetraacetate within 48 h,[47,48] giving rise to the purified nanomolecule **2b** (Table 1, entry 2), after isolation in 84% yield (see the Supporting Information for experimental details and characterization data). Additionally, we prepared previously reported glucose- and poly(ethylene glycol) (PEG)-coated structures **2c** and **2d** (Table 1, entries 3 and 4),[44] and notably the isolated yield for **2c** was significantly improved (17−65%) through the new purification strategy (see the Supporting Information for experimental details). Overall, these results demonstrate that perfluoroaryl-thiol S_NAr chemistry can be utilized to assemble a panel of well-defined, multivalent hybrid nanomolecules functionalized with various saccharides including mannose, galactose, and glucose. Moreover, both the glycosylated and PEGylated nanomolecules can be easily purified using desalting centrifugal filters, which streamlines access to the pure materials. Ultimately, these nanomolecules provide us with the ability to evaluate the biological activities of multivalent assemblies as a function of the molecular structure precisely displayed in 3D space.

With the successful synthesis of glycosylated nanomolecules **2a−c**, we sought to build a new generation of multivalent architectures that share the precision and rigidity of the first-generation assemblies but feature a rationally designed linker that will modularly extend the cluster scaffold. We envisioned that the new class of larger-sized glycosylated nanomolecules featuring a distinct multivalent display of saccharides, when studied alongside **2a−c**, will allow us to further investigate the complex relationship between molecular structure and activity in the multivalent constructs. Keeping the downstream biological applications in mind, we set out to find a rigid linker that could ideally lead to water-soluble glycosylated nanomolecules. After testing multiple linker designs, we found a sulfone-bridged biphenyl derivative (Figure 1a) to be the most suitable candidate. The rationale behind choosing this linker was 2-fold: not only could the polar sulfone group promote the overall water solubility of the nanomolecule (our attempt to use a biphenyl motif resulted in a poorly water-soluble glycosylated cluster), but also a similar molecule, decafluorobiphenylsulfone, was recently found to exhibit remarkably fast S_NAr reactivity toward cysteine residues on peptides under aqueous conditions.[49] Therefore, we hypothesized that perfunctionalization of **1** (Figure 1a) with the sulfone-bridged linker could enhance the S_NAr reaction

DOI: 10.1021/acs.bioconjchem.9b00526
Bioconjugate Chem. 2019, 30, 2594−2603

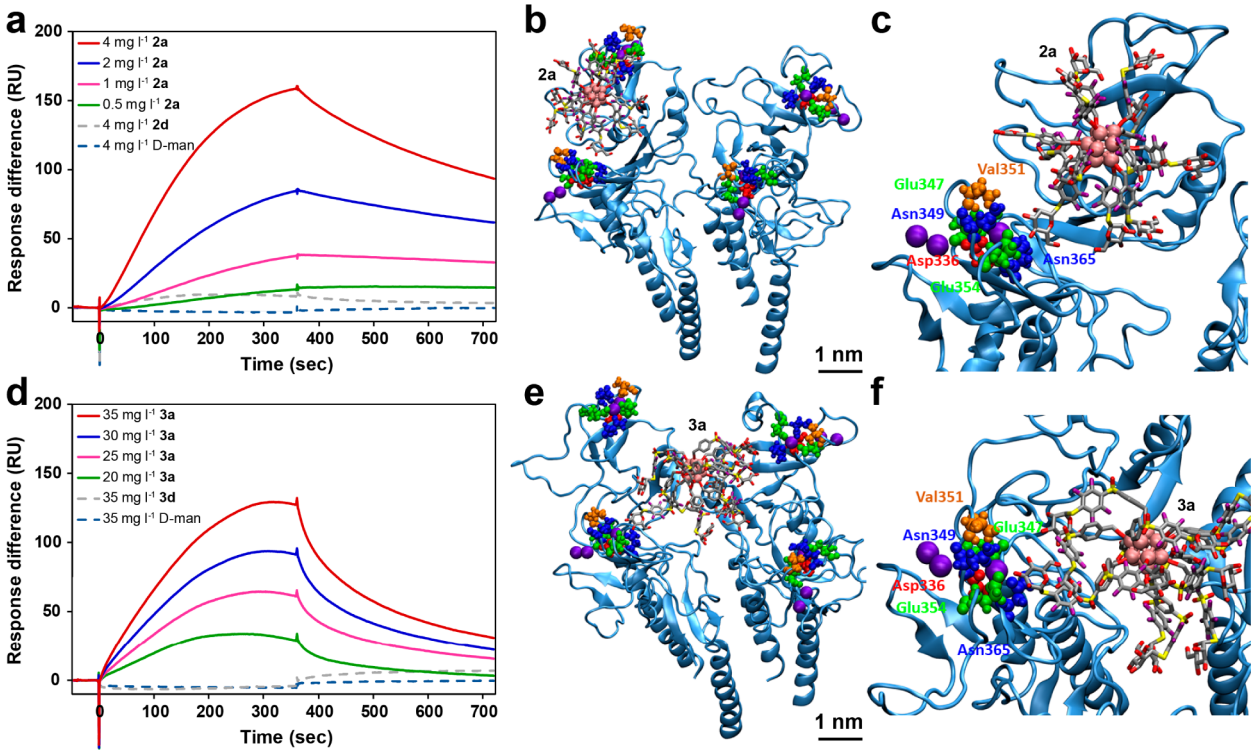


Figure 2. Multivalent binding interactions between mannose-functionalized nanomolecules and DC-SIGN. (a, d) SPR sensorgrams reveal dose-dependent multivalent binding of **2a** and **3a** to DC-SIGN, respectively, while the controls PEGylated clusters (**2d** and **3d**) and D-mannose exhibit minimal to no binding to DC-SIGN. In all SPR experiments, the flow rate is 5 μL/min, and the analytes are injected for 6 min, followed by buffer flow. (b, e) Snapshots after 40 ns of MD simulations of the binding interactions between **2a**/**3a** and DC-SIGN. (c, f) Zoomed-in snapshots reveal each nanomolecule binding to the carbohydrate recognition sites of DC-SIGN. See the Supporting Information for the MD simulation movies.

kinetics and impart aqueous compatibility to the cluster conjugation, resulting in a water-soluble glycosylated species. The target benzyl bromide linker containing a terminal $SO_2C_6F_5$ functional group was synthesized in three steps (see the Supporting Information for experimental details and characterization data). Using a microwave-assisted synthesis method,[50] we observed nearly quantitative conversion of **1** to the perfunctionalized cluster within 30 min, based on ^{11}B NMR spectroscopy and ESI-HRMS. The cluster species was isolated from the residual organic-based starting materials via silica gel chromatography in 94% yield. After the compound was subjected to a sodium ion exchange column, **3** (Figure 1a) was isolated as a light salmon-colored solid (see the Supporting Information for experimental details). ^{1}H, ^{11}B, and ^{19}F NMR spectroscopy (Figure 1c) and ESI-HRMS results of **3** are consistent with the proposed structure and composition of the dodecafunctionalized B_{12}-based cluster (see the Supporting Information for characterization data).

To test whether cluster **3** exhibits enhanced S_NAr reactivity toward thiols, we exposed **3** dissolved in DMF to a stoichiometric amount of an unprotected thiolated saccharide, 1-thio-β-D-glucose sodium salt, and observed by ^{19}F NMR spectroscopy a nearly quantitative (≥99%) conversion to **3c** (Table 1, entry 7) within 20 min (Figure 1b,c). The purified water-soluble **3c** was obtained via a desalting centrifugal filter and was subjected to analysis via ^{1}H, ^{11}B, and ^{19}F NMR spectroscopy and ESI-HRMS, which support the proposed structure and composition (see the Supporting Information for experimental details and characterization data). Notably, due to the rapid kinetics, this reaction did not require a N_2 atmosphere in order to proceed to completion; therefore all subsequent conjugation reactions of **3** were performed under ambient conditions. Parallel experiments monitoring the S_NAr reaction conversion over time of **2** and **3** by ^{19}F NMR spectroscopy revealed the significantly improved conversion rates of **3** over **2** (Figure 1b), which is consistent with our hypothesis. We then proceeded to test whether **3** tolerates water in the conjugation reaction by subjecting **3** to a stoichiometric amount of 1-thio-β-D-glucose sodium salt in 1:1 DMF:water and 1:1 acetonitrile (MeCN)/water mixtures and in both cases observed nearly quantitative (≥99%) conversion to **3c** within 15 min (Figure 1c) (see the Supporting Information for experimental details). These remarkably fast reaction kinetics in mixed aqueous/organic media are consistent with the observations by Kalhor-Monfared et al. and furthermore may be facilitated by the enhanced solubility of 1-thio-β-D-glucose sodium salt in water.[49] Overall, these studies demonstrate that by employing rational linker design, the S_NAr reaction characteristics including kinetics and aqueous compatibility can be dramatically enhanced, allowing for the rapid assembly of atomically precise, densely glycosylated nanomolecules.

On the basis of the successful glycosylation of **2** to yield functionalized nanomolecules **2a**–**c**, we hypothesized that **3** could likewise be glycosylated by mannose and galactose in addition to glucose (vide supra). Treatment of **3** with the sodium salts of 1-thio-α-D-mannose and 1-thio-β-D-galactose in 1:1 DMF/water mixtures resulted in nearly quantitative (≥99%) conversions within 15 min to **3a** and **3b** (Table 1, entries 5 and 6), respectively. Following purification, **3a** and **3b** were subjected to characterization via ^{1}H, ^{11}B, and ^{19}F NMR spectroscopy and ESI-HRMS, which support the proposed

DOI: 10.1021/acs.bioconjchem.9b00526
Bioconjugate Chem. 2019, 30, 2594–2603

structures and compositions (see the Supporting Information for experimental details and characterization data). Furthermore, we were able to fully PEGylate **3** within 90 min, giving rise to purified **3d** (Table 1, entry 8) after isolation in 84% yield (see the Supporting Information for experimental details and characterization data). These experiments demonstrate that cluster **3** can rapidly lead to a library of multivalent hybrid entities featuring diverse functional groups, which allows us to study how the specific surface chemistry affects the protein-binding properties. Ultimately, the family of precisely engineered multivalent nanomolecules (**2a**−**3** and **3a**−**d**, vide supra) creates a framework that can potentially enable us to study the fundamental rules that govern multivalent biological recognition events.

Following the assembly and isolation of the glycosylated and PEGylated clusters, we proceeded to uncover the binding characteristics of the various nanomolecules toward an important dendritic cell receptor, DC-SIGN. Among the existing techniques that can experimentally elucidate the binding affinities between complex molecules and biomolecular targets, the surface plasmon resonance (SPR) technology represents a "gold standard" used by researchers in both academic and biotechnology communities.[51,52] Given the ability of the SPR technology to perform real-time, label-free detection of biomolecular interactions with high sensitivity,[52] we decided to use it for studying the binding interactions between the multivalent cluster nanomolecules and DC-SIGN. In the first set of SPR-based direct binding experiments, the tetrameric DC-SIGN extracellular domain (ECD) was immobilized on a commercial sensor chip via standard amide coupling, and the mannose-functionalized nanomolecules **2a** and **3a** were injected over the protein surface for real-time visualization of their respective binding interactions with DC-SIGN (see the Supporting Information for experimental details). The resulting sensorgrams (Figure 2a,d) reflect changes in the refractive index as molecules interact with the lectin surface, and reveal the dose-dependent binding response of **2a** and **3a**, respectively, toward DC-SIGN. By fitting the Langmuir 1:1 binding model to the binding curves of the mannose-coated clusters, we estimated K_D values of 0.11 μM for **2a** and 5.0 μM for **3a**. Compared to D-mannose (low mM affinity),[11] these multivalent systems exhibit avidities 3−4 orders of magnitude higher for DC-SIGN through the cluster glycoside effect.[3] To further understand the dynamics of the multivalent interactions, we performed computational studies using a tetrameric model derived from an X-ray structure of DC-SIGN (see the Supporting Information for experimental details).[12,53] Molecular dynamics (MD) simulations of the interactions between the DC-SIGN model and **2a**/**3a** over 40 ns were conducted, and snapshots were taken at the end of both simulations (Figure 2b,c/e,f, respectively; see the Supporting Information for experimental details and movies). The MD movies and snapshots suggest that consistent with previous reports using monosaccharides and oligosaccharides,[11,12] the equatorial 3-OH and 4-OH groups on the cluster-linked mannose residues engage in Ca^{2+}-mediated binding in the carbohydrate recognition sites. Furthermore, **2a** was observed to stay longer than **3a** near the binding site of the protein model (Figure S16), which agrees with the lower K_D value of **2a** determined from the SPR experiments. A possible explanation for the observed difference in avidity is the flexibility of the linker; while the extended linker in **3a** is still rigid, it allows more flexibility compared to the benzylic linker in **2a**. Although a more flexible linker can relax the requirements for the precise positioning of ligands on a multivalent scaffold, it can also lower the overall affinity for a target protein.[5]

After analyzing the binding interactions of mannose-coated cluster nanomolecules toward DC-SIGN, we hypothesized that the clusters grafted with other saccharides would exhibit different protein-binding behaviors. Therefore, we conducted another set of SPR-based direct binding studies with the glucose-coated nanomolecules (**2c** and **3c**) (Figures S1 and S2), which yielded K_D values of 0.18 and 30 μM, respectively. These similar but slightly higher K_D values compared to the mannose-coated analogs agree with results from previous reports using monosaccharides,[11,54] which suggest the equatorial 3- and 4-OH groups on glucose allow a similar binding interaction with DC-SIGN. In contrast, the galactose-coated species (**2b** and **3b**) were unable to engage DC-SIGN with similar avidities (the estimated K_D values were 0.87 and 96 μM, respectively; Figures S3 and S4). This finding is also consistent with prior reports with monosaccharides and glycopolymers,[11,24,54] since the axial 4-OH group on galactose prevents proper recognition by the carbohydrate-binding sites on DC-SIGN. In contrast, the controls (PEGylated clusters (**2d** and **3d**) and D-mannose) exhibit minimal to no binding to the protein surface when injected at the highest mass concentrations with respect to **2a** and **3a** (Figure 2a,d). Overall, these experiments reveal the dramatically enhanced binding avidities of the glycosylated cluster nanomolecules as a result of multivalency and highlight a potentially intricate relationship between the scaffold flexibility and the binding affinity. Nevertheless, in nature DC-SIGN is known to be a very flexible transmembrane receptor that can reposition its carbohydrate recognition domains to adapt to the ligands,[55] and this dynamic behavior is not fully captured by the immobilized protein setup in the in vitro SPR and in silico MD experiments.

Therefore, we turned to SPR-based competitive binding assays in order to test (1) whether free (vs immobilized) DC-SIGN exhibits different binding characteristics to the cluster nanomolecules and 2) whether the mannose-coated species can inhibit the protein−protein interactions between DC-SIGN and a sub-nM binder, HIV-1 gp120.[24,56] In these competition experiments, 100 nM DC-SIGN and various concentrations of the nanomolecules were co-injected over the surface-immobilized gp120, and the binding response of each injection was compared to that of each preceding injection of DC-SIGN alone for an estimation of the % inhibition of the DC-SIGN−gp120 interaction. As shown in Figure 3, **2a** and **3a** can both inhibit free DC-SIGN from attaching to gp120, with IC_{50} values of 2.0 and 5.2 μM, respectively. These values are over 3 orders of magnitude lower than the reported IC_{50} of monovalent D-mannose (6−9 mM),[54,57] indicating dramatically enhanced inhibition. Notably, compared with the IC_{50} values from a similar SPR-based competition assay using a multivalent third-generation dendrimer (50 μM, 32 mannose residues),[27] these values are an order of magnitude lower. These results suggest that rigid inorganic cluster-based nanomolecules featuring significantly fewer (12) saccharides can serve as more potent inhibitors of this protein−protein interaction. Furthermore, in agreement with the direct binding data, the galactose-coated (**2b**, **3b**) and PEGylated (**2d**, **3d**) nanomolecules as well as D-mannose were less successful at inhibiting this interaction (Figures S5 and S6). Overall, these

DOI: 10.1021/acs.bioconjchem.9b00526
Bioconjugate Chem. 2019, 30, 2594−2603

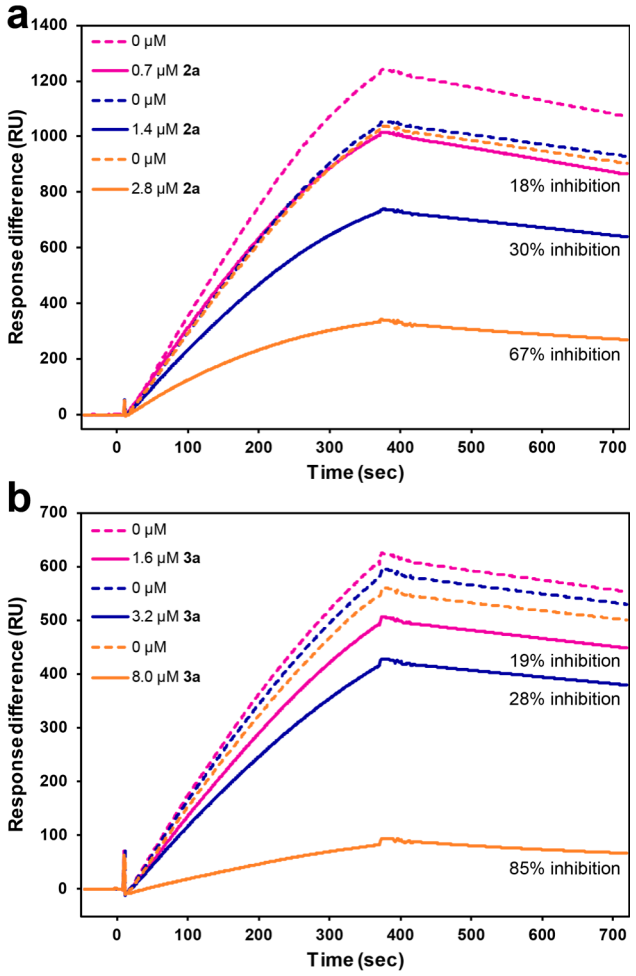


Figure 3. Mannose-functionalized clusters are capable of inhibiting protein−protein interactions. (a, b) SPR-based competitive binding studies suggest that **2a** and **3a** effectively compete against immobilized gp120 to bind free DC-SIGN, which leads to reduced binding responses.

competition studies demonstrate for the first time the ability of multivalent glycosylated cluster nanomolecules to effectively compete against a sub-nM-binding viral glycoprotein for DC-SIGN. This suggests that a rigid cluster scaffold-based multivalent display of carbohydrates that mimics the natural highly glycosylated proteins on the surface of pathogens can be engineered to inhibit the interactions between a cell-based lectin receptor and a viral glycoprotein. Moreover, the similarity in IC_{50} values for **2a** and **3a** in contrast to their different K_D values could be due to a combination of the free (vs immobilized) DC-SIGN better adapting to the more flexible nanomolecule **3a** and the greater receptor surface coverage by the larger nanomolecule **3a**.

To further investigate the ability of the mannose-functionalized cluster nanomolecules to inhibit the protein−protein interactions between DC-SIGN and gp120 in an experimental setup more reminiscent of natural systems, we moved to cell-based studies using a DC-SIGN-expressing human lymphoblast-like cell line (Raji DC-SIGN+ cells) and HIV-1 gp120 (Figure 4a).[58,59] First, in order to gain a better understanding of the biocompatibility of the cluster nanomolecules, we conducted an MTS-based cell proliferation assay (see the Supporting Information for experimental details) and observed no apparent cytotoxic effects of the mannose-coated (**2a**, **3a**) and PEGylated (**2d**, **3d**) clusters toward Raji DC-SIGN+ cells at 0.5−50 μM concentrations (Figure 4b). This finding allowed the evaluation of the nanomolecules' potential biological function in inhibiting the attachment of gp120 to cell-surface DC-SIGN. Fluorescein isothiocyanate-labeled gp120 (gp120-FITC) undergoes significant uptake by Raji DC-SIGN+ cells (Figure 4c), as observed by a confocal laser scanning microscopy-based assay (see the Supporting Information for experimental details and Figures S7−S15). This internalization is DC-SIGN-dependent since no gp120-FITC uptake was observed in a Raji cell line not expressing DC-SIGN (Figure 4c).[60,61] In order to test competitive inhibition, we introduced mixtures of gp120-FITC and mannose-coated clusters **2a**/**3a** to Raji DC-SIGN+ cells and observed reduced gp120-FITC uptake as a function of the cluster concentration (10−25 μM) (Figure 4c). Notably, at the same concentrations, **3a** was more effective than **2a** at preventing the binding and uptake of gp120-FITC. This result suggests that **3a** can bind DC-SIGN in its natural transmembrane conformation better, which could be due to its higher flexibility and larger size. Furthermore, these cell-based studies capture important information about the dynamic receptor-mediated antigen internalization process,[62] thus enabling us to assess both the nanomolecules' binding to DC-SIGN and the inhibition of antigen uptake. Consistent with the presented SPR-based direct and competitive binding data, the control molecules (PEGylated clusters (**2d** and **3d**) and D-mannose) were not able to bind to DC-SIGN and inhibit gp120 uptake at 25 μM (Figure 4c). Overall, the biological studies in cells reveal that biocompatible mannose-functionalized cluster nanomolecules are capable of competing against HIV-1 gp120 for cell-surface DC-SIGN, thereby preventing the receptor-mediated internalization of a viral envelope component.

CONCLUSIONS

We have demonstrated the rapid assembly of multivalent glycosylated inorganic cluster nanomolecules capable of inhibiting protein−protein interactions. Specifically, a dense layer of thiolated saccharides can be grafted on a rigid perfluoroaryl-perfunctionalized B_{12} cluster within 15 min in mixed aqueous/organic media using S_NAr chemistry. The resulting fully covalent glycosylated assemblies can serve as multivalent binders with dramatically enhanced affinity compared to monovalent saccharides toward target lectins. We showed an example of using these hybrid agents for engendering ligand-specific, multivalent recognition with a biologically important dendritic cell receptor, DC-SIGN. Importantly, we demonstrated the ability of the cluster nanomolecules to inhibit protein−protein interactions between DC-SIGN and a sub-nM-binding HIV-1 envelope glycoprotein in a competitive binding study. We further found these clusters to be biocompatible in a human cell line and capable of preventing the internalization of gp120 by DC-SIGN-expressing cells. Notably, we uncovered an intricate interplay between the structural designs of multivalent binders and their biological activities. We strive to further elucidate the structure−activity relationship of well-defined multivalent agents through vertex-differentiated clusters[63] and other types of molecular scaffolds. Ultimately, this work showcases a rare example of the application of tunable, stable inorganic cluster-based nanomolecules as valuable tools for studying the

DOI: 10.1021/acs.bioconjchem.9b00526
Bioconjugate Chem. 2019, 30, 2594−2603

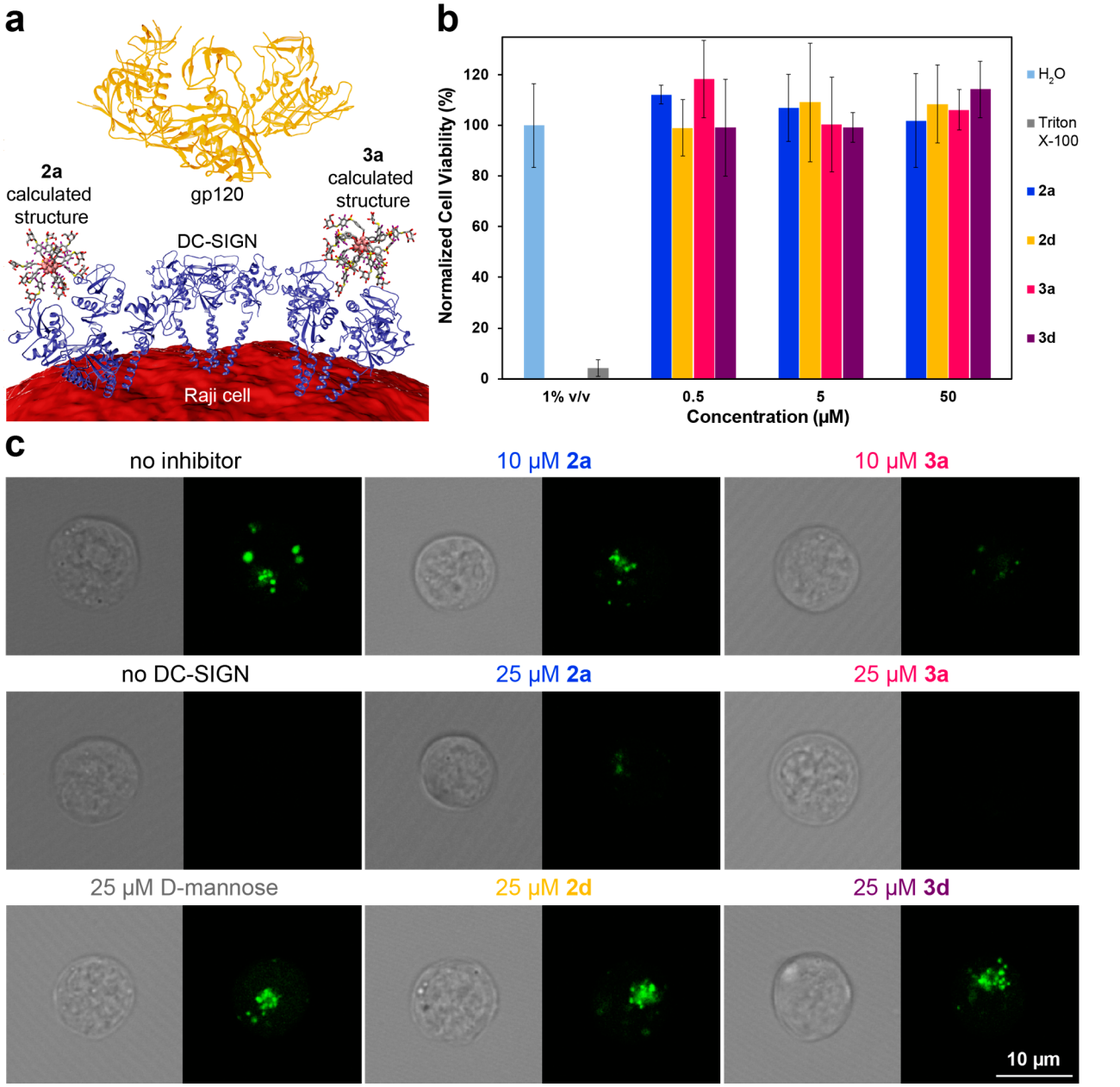


Figure 4. Biocompatible mannose-coated cluster nanomolecules can serve as multivalent inhibitors to prevent the DC-SIGN-mediated cell uptake of gp120. (a) Glycosylated clusters can potentially inhibit the uptake of viral glycoproteins such as gp120 by blocking cell-surface DC-SIGN. Figure is not drawn to scale. (b) Mannose-coated and PEGylated clusters exhibit no apparent toxicity toward Raji DC-SIGN+ cells at least up to 50 μM, as assessed by an MTS assay. (c) DC-SIGN-dependent cell uptake of gp120-FITC is inhibited by mannose-coated clusters (**2a** and **3a**), as indicated by confocal microscopy analysis. However, the controls PEGylated clusters (**2d** and **3d**) and D-mannose do not affect the uptake of gp120-FITC.

rules that govern multivalent interactions and disrupting protein−protein interactions.[64−66]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.bioconjchem.9b00526.

All methods, synthetic procedures, characterization data, and supplementary data (PDF)

MD simulation movies (MP4)

MD simulation movies (MP4)

MD simulation movies (ZIP)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: spokoyny@chem.ucla.edu.

ORCID

Elaine A. Qian: 0000-0002-7111-7383

Marco S. Messina: 0000-0003-2827-118X

Heather D. Maynard: 0000-0003-3692-6289

Petr Král: 0000-0003-2992-9027

Alexander M. Spokoyny: 0000-0002-5683-6240

Notes

The authors declare the following competing financial interest(s): UCLA has patents on several compounds reported in this work from which A.M.S. and current/former co-workers

DOI: 10.1021/acs.bioconjchem.9b00526
Bioconjugate Chem. 2019, 30, 2594−2603

receive royalty payments. Compounds 1 (catalog no. 902209) and 2 (catalog no. 901272) are commercially available through MilliporeSigma catalog.

No unexpected or unusually high safety hazards were encountered.

■ ACKNOWLEDGMENTS

E.A.Q. thanks the NIH for the Predoctoral Training Fellowship through the UCLA Chemistry-Biology Interface Training Program under the National Research Service Award T32GM008496 and the UCLA Graduate Division for the Dissertation Year Fellowship. M.S.M. is grateful to the National Science Foundation (NSF) for the Bridge-to-Doctorate (Grant HRD-1400789) and the Predoctoral (Grant DGE-0707424) Fellowships and UCLA for the Christopher S. Foote Fellowship. H.D.M. thanks the Dr. Myung Ki Hong Endowed Chair in Polymer Science. A.M.S. thanks the UCLA Department of Chemistry and Biochemistry for start-up funds, 3M for a Non-Tenured Faculty Award, the Alfred P. Sloan Foundation for a Fellowship in Chemistry, Research Corporation for Science Advancement (RCSA) for a Cottrell Scholar Award, and the National Institutes of Health (NIH) for a Maximizing Investigators Research Award (MIRA, Grant R35GM124746). We are grateful for the assistance from Dr. Alex I. Wixtrom in optimizing the synthesis of **2**. We thank UCLA Molecular Instrumentation Center for mass spectrometry (NIH Grant 1S10OD016387-01) and NMR spectroscopy. We also thank the UCLA Biochemistry Instrumentation Facility for use of the SPR instrument. The following reagents were obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: pcDNA3-DC-SIGN from Drs. S. Pöhlmann, F. Baribaud, F. Kirchhoff, and R. W. Dom,[67] HIV-1 CM235 gp120 recombinant protein from NIAID, DAIDS, and both Raji and Raji DC-SIGN+ cells from Drs. Li Wu and Vineet N. KewalRamani.[58]

■ REFERENCES

(1) Mammen, M., Choi, S.-K., and Whitesides, G. M. (1998) Polyvalent interactions in biological systems: implications for design and use of multivalent ligands and inhibitors. *Angew. Chem., Int. Ed. 37*, 2754−2794.

(2) Weis, W. I., Taylor, M. E., and Drickamer, K. (1998) The C-type lectin superfamily in the immune system. *Immunol. Rev. 163*, 19−34.

(3) Lundquist, J. J., and Toone, E. J. (2002) The cluster glycoside effect. *Chem. Rev. 102*, 555−578.

(4) Wolfert, M. A., and Boons, G. J. (2013) Adaptive immune activation: glycosylation does matter. *Nat. Chem. Biol. 9*, 776−784.

(5) (2018) *Multivalency*, 1st ed. (Huskens, J., Prins, L. J., Haag, R., and Ravoo, B. J., Eds.) John Wiley & Sons, Hoboken, NJ.

(6) Conn, M. M., and Rebek, J. (1997) Self-assembling capsules. *Chem. Rev. 97*, 1647−1668.

(7) Müller, C., Despras, G., and Lindhorst, T. K. (2016) Organizing multivalency in carbohydrate recognition. *Chem. Soc. Rev. 45*, 3275−3302.

(8) Bernardi, A., Jiménez-Barbero, J., Casnati, A., De Castro, C., Darbre, T., Fieschi, F., Finne, J., Funken, H., Jaeger, K.-E., Lahmann, M., et al. (2013) Multivalent glycoconjugates as anti-pathogenic agents. *Chem. Soc. Rev. 42*, 4709−4727.

(9) Bhatia, S., Camacho, L. C., and Haag, R. (2016) Pathogen inhibition by multivalent ligand architectures. *J. Am. Chem. Soc. 138*, 8654−8666.

(10) Geijtenbeek, T. B. H., Torensma, R., van Vliet, S. J., van Duijnhoven, G. C. F., Adema, G. J., van Kooyk, Y., and Figdor, C. G. (2000) Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell 100*, 575−585.

(11) Mitchell, D. A., Fadden, A. J., and Drickamer, K. (2001) A novel mechanism of carbohydrate recognition by the C-type lectins DC-SIGN and DC-SIGNR. Subunit organization and binding to multivalent ligands. *J. Biol. Chem. 276*, 28939−28945.

(12) Feinberg, H., Mitchell, D. A., Drickamer, K., and Weis, W. I. (2001) Structural basis for selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR. *Science 294*, 2163−2166.

(13) Švajger, U., Anderluh, M., Jeras, M., and Obermajer, N. (2010) C-type lectin DC-SIGN: an adhesion, signalling and antigen-uptake molecule that guides dendritic cells in immunity. *Cell. Signalling 22*, 1397−1405.

(14) van Kooyk, Y., and Geijtenbeek, T. B. H. (2003) DC-SIGN: escape mechanism for pathogens. *Nat. Rev. Immunol. 3*, 697−709.

(15) Geijtenbeek, T. B. H., Kwon, D. S., Torensma, R., Van Vliet, S. J., Van Duijnhoven, G. C. F., Middel, J., Cornelissen, I. L. M. H. A., Nottet, H. S. L. M., KewalRamani, V. N., Littman, D. R., et al. (2000) DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. *Cell 100*, 587−597.

(16) Chung, N. P. Y., Breun, S. K. J., Bashirova, A., Baumann, J. G., Martin, T. D., Karamchandani, J. M., Rausch, J. W., Le Grice, S. F. J., Wu, L., Carrington, M., et al. (2010) HIV-1 transmission by dendritic cell-specific ICAM-3-grabbing nonintegrin (DC-SIGN) is regulated by determinants in the carbohydrate recognition domain that are absent in liver/lymph node-sign (L-SIGN). *J. Biol. Chem. 285*, 2100−2112.

(17) Lepenies, B., Lee, J., and Sonkaria, S. (2013) Targeting C-type lectin receptors with multivalent carbohydrate ligands. *Adv. Drug Delivery Rev. 65*, 1271−1281.

(18) Kamiya, N., Tominaga, M., Sato, S., and Fujita, M. (2007) Saccharide-coated $M_{12}L_{24}$ molecular spheres that form aggregates by multi-interaction with proteins. *J. Am. Chem. Soc. 129*, 3816−3817.

(19) Zhang, Q., Savagatrup, S., Kaplonek, P., Seeberger, P. H., and Swager, T. M. (2017) Janus emulsions for the detection of bacteria. *ACS Cent. Sci. 3*, 309−313.

(20) Sattin, S., Daghetti, A., Thépaut, M., Berzi, A., Sánchez-Navarro, M., Tabarani, G., Rojo, J., Fieschi, F., Clerici, M., and Bernardi, A. (2010) Inhibition of DC-SIGN-mediated HIV infection by a linear trimannoside mimic in a tetravalent presentation. *ACS Chem. Biol. 5*, 301−312.

(21) Borrok, M. J., and Kiessling, L. L. (2007) Non-carbohydrate inhibitors of the lectin DC-SIGN. *J. Am. Chem. Soc. 129*, 12780−12785.

(22) Frison, N., Taylor, M. E., Soilleux, E., Bousser, M.-T., Mayer, R., Monsigny, M., Drickamer, K., and Roche, A.-C. (2003) Oligolysine-based oligosaccharide clusters. *J. Biol. Chem. 278*, 23922−23929.

(23) Ng, S., Bennett, N. J., Schulze, J., Gao, N., Rademacher, C., and Derda, R. (2018) Genetically-encoded fragment-based discovery of glycopeptide ligands for DC-SIGN. *Bioorg. Med. Chem. 26*, 5368−5377.

(24) Becer, C. R., Gibson, M. I., Geng, J., Ilyas, R., Wallis, R., Mitchell, D. A., and Haddleton, D. M. (2010) High-affinity glycopolymer binding to human DC-SIGN and disruption of DC-SIGN interactions with HIV envelope glycoprotein. *J. Am. Chem. Soc. 132*, 15130−15132.

(25) Turnbull, W. B., and Stoddart, J. F. (2002) Design and synthesis of glycodendrimers. *Rev. Mol. Biotechnol. 90*, 231−255.

(26) Lasala, F., Arce, E., Otero, J. R., Rojo, J., and Delgado, R. (2003) Mannosyl glycodendritic structure inhibits DC-SIGN-mediated Ebola virus infection in cis and in trans. *Antimicrob. Agents Chemother. 47*, 3970−3972.

(27) Tabarani, G., Reina, J. J., Ebel, C., Vivès, C., Lortat-Jacob, H., Rojo, J., and Fieschi, F. (2006) Mannose hyperbranched dendritic polymers interact with clustered organization of DC-SIGN and inhibit gp120 binding. *FEBS Lett. 580*, 2402−2408.

(28) Luczkowiak, J., Sattin, S., Sutkevičiute, I., Reina, J. J., Sánchez-Navarro, M., Thépaut, M., Martínez-Prats, L., Daghetti, A., Fieschi, F.,

DOI: 10.1021/acs.bioconjchem.9b00526
Bioconjugate Chem. 2019, 30, 2594−2603

Delgado, R., et al. (2011) Pseudosaccharide functionalized dendrimers as potent inhibitors of DC-SIGN dependent Ebola pseudotyped viral infection. *Bioconjugate Chem. 22*, 1354−1365.
(29) Garcia-Vallejo, J. J., Koning, N., Ambrosini, M., Kalay, H., Vuist, I., Sarrami-Forooshani, R., Geijtenbeek, T. B. H., and van Kooyk, Y. (2013) Glycodendrimers prevent HIV transmission via DC-SIGN on dendritic cells. *Int. Immunol. 25*, 221−233.
(30) Ordanini, S., Varga, N., Porkolab, V., Thépaut, M., Belvisi, L., Bertaglia, A., Palmioli, A., Berzi, A., Trabattoni, D., Clerici, M., et al. (2015) Designing nanomolar antagonists of DC-SIGN-mediated HIV infection: ligand presentation using molecular rods. *Chem. Commun. 51*, 3816−3819.
(31) Luczkowiak, J., Muñoz, A., Sánchez-Navarro, M. A., Ribeiro-viana, R., Ginieis, A., Illescas, B. M., Martín, N., Delgado, R., and Rojo, J. (2013) Glycofullerenes inhibit viral infection. *Biomacromolecules 14*, 431−437.
(32) Muñoz, A., Sigwalt, D., Illescas, B. M., Luczkowiak, J., Rodríguez-Pérez, L., Nierengarten, I., Holler, M., Remy, J. S., Buffet, K., Vincent, S. P., et al. (2016) Synthesis of giant globular multivalent glycofullerenes as potent inhibitors in a model of Ebola virus infection. *Nat. Chem. 8*, 50−57.
(33) Delbianco, M., Bharate, P., Varela-Aramburu, S., and Seeberger, P. H. (2016) Carbohydrates in supramolecular chemistry. *Chem. Rev. 116*, 1693−1752.
(34) Zhang, Q., Su, L., Collins, J., Chen, G., Wallis, R., Mitchell, D. A., Haddleton, D. M., and Becer, C. R. (2014) Dendritic cell lectin-targeting sentinel-like unimolecular glycoconjugates to release an anti-HIV drug. *J. Am. Chem. Soc. 136*, 4325−4332.
(35) Morbioli, I., Porkolab, V., Magini, A., Casnati, A., Fieschi, F., and Sansone, F. (2017) Mannosylcalix[n]arenes as multivalent ligands for DC-SIGN. *Carbohydr. Res. 453−454*, 36−43.
(36) Adak, A. K., Lin, H. J., and Lin, C. C. (2014) Multivalent glycosylated nanoparticles for studying carbohydrate-protein interactions. *Org. Biomol. Chem. 12*, 5563−5573.
(37) Martínez-Ávila, O., Hijazi, K., Marradi, M., Clavel, C., Campion, C., Kelly, C., and Penadés, S. (2009) Gold manno-glyconanoparticles: multivalent systems to block HIV-1 gp120 binding to the lectin DC-SIGN. *Chem. - Eur. J. 15*, 9874−9888.
(38) Ribeiro-Viana, R., Sánchez-Navarro, M., Luczkowiak, J., Koeppe, J. R., Delgado, R., Rojo, J., and Davis, B. G. (2012) Virus-like glycodendrinanoparticles displaying quasi-equivalent nested polyvalency upon glycoprotein platforms potently block viral infection. *Nat. Commun. 3*, 1303.
(39) Hostetler, M. J., Templeton, A. C., and Murray, R. W. (1999) Dynamics of place-exchange reactions on monolayer-protected gold cluster molecules. *Langmuir 15*, 3782−3789.
(40) Daniel, M., and Astruc, D. (2004) Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chem. Rev. 104*, 293−346.
(41) Love, J. C., Estroff, L. A., Kriebel, J. K., Nuzzo, R. G., and Whitesides, G. M. (2005) Self-assembled monolayers of thiolates on metals as a form of nanotechnology. *Chem. Rev. 105*, 1103−1170.
(42) MacLeod, M. J., and Johnson, J. A. (2015) PEGylated N-heterocyclic carbene anchors designed to stabilize gold nanoparticles in biologically relevant media. *J. Am. Chem. Soc. 137*, 7974−7977.
(43) Becer, C. R., Hoogenboom, R., and Schubert, U. S. (2009) Click chemistry beyond metal-catalyzed cycloaddition. *Angew. Chem., Int. Ed. 48*, 4900−4908.
(44) Qian, E. A., Wixtrom, A. I., Axtell, J. C., Saebi, A., Jung, D., Rehak, P., Han, Y., Moully, E. H., Mosallaei, D., Chow, S., et al. (2017) Atomically precise organomimetic cluster nanomolecules assembled via perfluoroaryl-thiol S_NAr chemistry. *Nat. Chem. 9*, 333−340.
(45) Messina, M. S., Axtell, J. C., Wang, Y., Chong, P., Wixtrom, A. I., Kirlikovali, K. O., Upton, B. M., Hunter, B. M., Shafaat, O. S., Khan, S. I., et al. (2016) Visible-light-induced olefin activation using 3D aromatic boron-rich cluster photooxidants. *J. Am. Chem. Soc. 138*, 6952−6955.
(46) Floyd, N., Vijayakrishnan, B., Koeppe, J. R., and Davis, B. G. (2009) Thiyl glycosylation of olefinic proteins: S-linked glycoconjugate synthesis. *Angew. Chem., Int. Ed. 48*, 7798−7802.
(47) Pelegri-O'Day, E. M., Paluck, S. J., and Maynard, H. D. (2017) Substituted polyesters by thiol-ene modification: rapid diversification for therapeutic protein stabilization. *J. Am. Chem. Soc. 139*, 1145−1154.
(48) Zhu, S. J., Ying, H. Z., Wu, Y., Qiu, N., Liu, T., Yang, B., Dong, X. W., and Hu, Y. Z. (2015) Design, synthesis and biological evaluation of novel podophyllotoxin derivatives bearing 4β-disulfide/trisulfide bond as cytotoxic agents. *RSC Adv. 5*, 103172−103183.
(49) Kalhor-Monfared, S., Jafari, M. R., Patterson, J. T., Kitov, P. I., Dwyer, J. J., Nuss, J. M., and Derda, R. (2016) Rapid biocompatible macrocyclization of peptides with decafluoro-diphenylsulfone. *Chem. Sci. 7*, 3785−3790.
(50) Wixtrom, A. I., Shao, Y., Jung, D., Machan, C. W., Kevork, S. N., Qian, E. A., Axtell, J. C., Khan, S. I., Kubiak, C. P., and Spokoyny, A. M. (2016) Rapid synthesis of redox-active dodecaborane $B_{12}(OR)_{12}$ clusters under ambient conditions. *Inorg. Chem. Front. 3*, 711−717.
(51) Hill, R. T. (2015) Plasmonic biosensors. *Wiley Interdiscip. Rev. Nanomedicine Nanobiotechnology 7*, 152−168.
(52) Olaru, A., Bala, C., Jaffrezic-Renault, N., and Aboul-Enein, H. Y. (2015) Surface plasmon resonance (SPR) biosensors in pharmaceutical analysis. *Crit. Rev. Anal. Chem. 45*, 97−105.
(53) Tabarani, G., Thépaut, M., Stroebel, D., Ebel, C., Vivès, C., Vachette, P., Durand, D., and Fieschi, F. (2009) DC-SIGN neck domain is a pH-sensor controlling oligomerization. SAXS and hydrodynamic studies of extracellular domain. *J. Biol. Chem. 284*, 21229−21240.
(54) Su, S. V., Hong, P., Baik, S., Negrete, O. A., Gurney, K. B., and Lee, B. (2004) DC-SIGN binds to HIV-1 glycoprotein 120 in a distinct but overlapping fashion compared with ICAM-2 and ICAM-3. *J. Biol. Chem. 279*, 19122−19132.
(55) Menon, S., Rosenberg, K., Graham, S. A., Ward, E. M., Taylor, M. E., Drickamer, K., and Leckband, D. E. (2009) Binding-site geometry and flexibility in DC-SIGN demonstrated with surface force measurements. *Proc. Natl. Acad. Sci. U. S. A. 106*, 11524−11529.
(56) Zhang, Q., Collins, J., Anastasaki, A., Wallis, R., Mitchell, D. A., Becer, C. R., and Haddleton, D. M. (2013) Sequence-controlled multi-block glycopolymers to inhibit DC-SIGN-gp120 binding. *Angew. Chem., Int. Ed. 52*, 4435−4439.
(57) Wang, S.-K., Liang, P.-H., Astronomo, R. D., Hsu, T.-L., Hsieh, S.-L., Burton, D. R., and Wong, C.-H. (2008) Targeting the carbohydrates on HIV-1: interaction of oligomannose dendrons with human monoclonal antibody 2G12 and DC-SIGN. *Proc. Natl. Acad. Sci. U. S. A. 105*, 3690−3695.
(58) Wu, L., Martin, T. D., Carrington, M., and KewalRamani, V. N. (2004) Raji B cells, misidentified as THP-1 cells, stimulate DC-SIGN-mediated HIV transmission. *Virology 318*, 17−23.
(59) Liu, J., Bartesaghi, A., Borgnia, M. J., Sapiro, G., and Subramaniam, S. (2008) Molecular architecture of native HIV-1 gp120 trimers. *Nature 455*, 109−113.
(60) Mangold, S. L., Prost, L. R., and Kiessling, L. L. (2012) Quinoxalinone inhibitors of the lectin DC-SIGN. *Chem. Sci. 3*, 772−777.
(61) Arnáiz, B., Martínez-Ávila, O., Falcon-Perez, J. M., and Penadés, S. (2012) Cellular uptake of gold nanoparticles bearing HIV gp120 oligomannosides. *Bioconjugate Chem. 23*, 814−825.
(62) Boulant, S., Stanifer, M., and Lozach, P. Y. (2015) Dynamics of virus-receptor interactions in virus binding, signaling, and endocytosis. *Viruses 7*, 2794−2815.
(63) Wixtrom, A. I., Parvez, Z. A., Savage, M. D., Qian, E. A., Jung, D., Khan, S. I., Rheingold, A. L., and Spokoyny, A. M. (2018) Tuning the electrochemical potential of perfunctionalized dodecaborate clusters through vertex differentiation. *Chem. Commun. 54*, 5867−5870.
(64) Řezáčová, P., Pokorná, J., Brynda, J., Kožíšek, M., Cígler, P., Lepšík, M., Fanfrlík, J., Řezáč, J., Grantz Šašková, K., Sieglová, I., et al.

DOI: 10.1021/acs.bioconjchem.9b00526
Bioconjugate Chem. 2019, 30, 2594−2603

(2009) Design of HIV protease inhibitors based on inorganic polyhedral metallacarboranes. *J. Med. Chem. 52*, 7132−7141.
(65) Lo Conte, M., Staderini, S., Chambery, A., Berthet, N., Dumy, P., Renaudet, O., Marra, A., and Dondoni, A. (2012) Glycoside and peptide clustering around the octasilsesquioxane scaffold via photo-induced free-radical thiol-ene coupling. The observation of a striking glycoside cluster effect. *Org. Biomol. Chem. 10*, 3269−3277.
(66) Levine, D. J., Stöhr, J., Falese, L. E., Ollesch, J., Wille, H., Prusiner, S. B., and Long, J. R. (2015) Mechanism of scrapie prion precipitation with phosphotungstate anions. *ACS Chem. Biol. 10*, 1269−1277.
(67) Pöhlmann, S., Baribaud, F., Lee, B., Leslie, G. J., Sanchez, M. D., Hiebenthal-Millow, K., Münch, J., Kirchhoff, F., and Doms, R. W. (2001) DC-SIGN interactions with human immunodeficiency virus type 1 and 2 and simian immunodeficiency virus. *J. Virol. 75*, 4664−4672.

DOI: 10.1021/acs.bioconjchem.9b00526
Bioconjugate Chem. 2019, 30, 2594−2603