JOC The Journal of Organic Chemistry

Featured Article

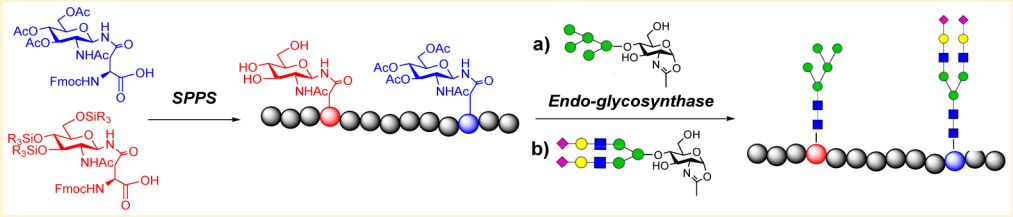
pubs.acs.org/joc

Site-Selective Chemoenzymatic Glycosylation of an HIV-1 Polypeptide Antigen with Two Distinct N-Glycans via an Orthogonal Protecting Group Strategy

Christian Toonstra, Mohammed N. Amin, and Lai-Xi Wang*

Department of Chemistry and Biochemistry, University of Maryland, College Park, Maryland 20742, United States

Supporting Information



ABSTRACT: A convergent chemoenzymatic approach for sequential installation of different N-glycans in a polypeptide is described. The method includes introduction of distinguishably protected GlcNAc-Asn building blocks during automated solid phase peptide synthesis (SPPS), followed by orthogonal deprotection of the GlcNAc primers and site-selective sequential extension of the sugar chains through glycosynthase-catalyzed transglycosylation reactions. It was observed that the protecting groups on one neighboring GlcNAc moiety have an impact on the substrate activity of another GlcNAc acceptor toward some endoglycosynthases in transglycosylation. The usefulness of this synthetic strategy was exemplified by an efficient synthesis of the glycopeptide neutralizing epitope of broadly HIV-neutralizing antibody PG9. The method should be generally applicable for the synthesis of complex glycopeptides carrying multiple different N-glycans.

INTRODUCTION

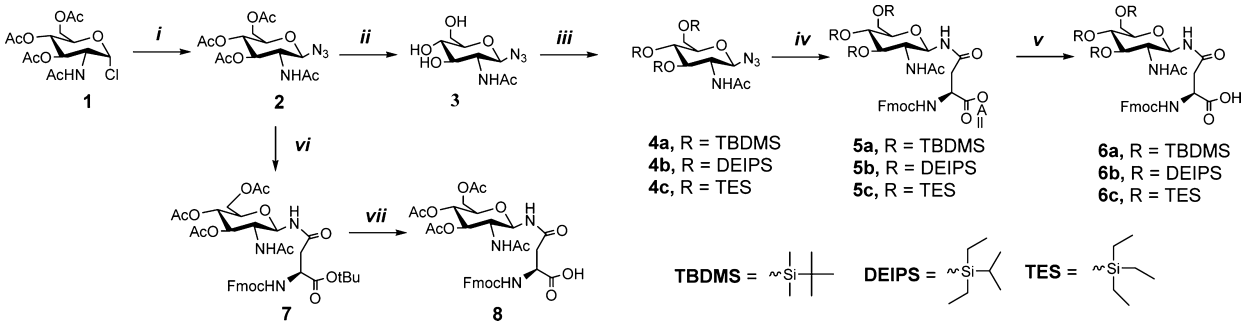
Glycosylation can profoundly affect a protein's structure, stability, intracellular trafficking, and biological functions.[1−4] In the case of the human immunodeficiency virus type 1 (HIV-1), the heavy N-linked glycosylation of the outer envelope glycoprotein gp120 represents a major defense mechanism for the virus to evade host immune attack. The N-glycans assembled by the host synthetic machinery are viewed as "self" and are weakly immunogenic.[5,6] Nevertheless, recent discoveries of a new class of broadly neutralizing antibodies (bNAbs) that recognize both conserved N-glycans and a segment of peptide in the variable (V1 V2 and V3) regions of gp120 as an integrated epitope strongly suggest that the defensive glycan shield of the virus and, in particular, the unique HIV-1 glycopeptide antigens, can serve as important targets for HIV-1 vaccine design.[7−11] PG9 is a broadly neutralizing antibody (bNAb) isolated from HIV-1 infected patients that can neutralize HIV-1 primary strains with significant breadth and potency. Mutational, biochemical and structural studies suggest that PG9 recognizes a strand of peptide and two conserved N-glycans in the V1 V2 domain.[12,13] The PGT series neutralizing antibodies including PGT128 and PGT121 also follow a similar antibody−antigen recognition mode involving targeting unique N-glycans and a protein segment centered at the V3 region.[14−16] These discoveries have stimulated great interests in chemical and chemoenzymatic synthesis of the proposed HIV-1 glycopeptide epitopes aiming at fine characterization and reconstitution of the precise neutralizing epitopes for HIV vaccine design.[17−19]

Major progress has been made in recent years in the total chemical synthesis of large glycopeptides and even homogeneous glycoproteins.[18,20,21] Nevertheless, each complex glycopeptide target could present a special challenge that may require significant optimization of the synthetic schemes in terms of the coupling efficiency for critical ligation steps and the compatibility of protecting group manipulations. On the other hand, the chemoenzymatic approach that exploits the endoglycosynthase-catalyzed transglycosylation for transfer of large oligosaccharide en bloc to a GlcNAc-peptide or protein using a glycan oxazoline as donor substrate is emerging as a promising method for expeditious synthesis of complex glycopeptides and for glycosylation remodeling of glycoproteins as well.[22−30] This method is highly convergent and permits independent manipulations of the glycan and polypeptide portions. We have recently applied this chemoenzymatic method for the synthesis of a series of complex HIV-1 V1 V2 glycopeptides that enabled the characterization of the glycan specificity of antibody PG9.[17] However, construction of complex glycopeptides carrying two or more different N-glycans by this method remains a difficult task, as the endoenzymes usually are unable to distinguish between the GlcNAc acceptors at different sites in a polypeptide. As a result,

Received: May 4, 2016
Published: July 5, 2016

DOI: 10.1021/acs.joc.6b01044
J. Org. Chem. 2016, 81, 6176−6185

Scheme 1. Synthesis of GlcNAc-Asn Building Blocks Carrying Silyl (Acid-Labile) And Acetyl (Base-Labile) Protecting Groups[a]



[a]Reaction conditions: (i) NaN_3, water/CH_2Cl_2, Bu_4NHSO_4, RT; 95%; (ii) (1) cat. MeONa, MeOH; (2) Dowex H^+, RT, quantative in two steps; (iii) Silyl triflate, 2,6-lutidine, 40 °C, quant.; (iv) (1) Pd/C, H_2, MeOH, RT; and (2) Fmoc-Asp-OAll, HATU, DIPEA, DMF, RT, 74% (**5a**), 73% (**5b**), and 38% (**5c**); (v) Pd[$P(Ph)_3$]$_4$, $PhSiH_3$, CH_2Cl_2, RT, 94% (**6a**), 95% (**6b**), and 93% (**6c**); (vi) Fmoc-Asp-OtBu, $P(Et)_3$, CH_2Cl_2, −78 °C to RT, 81%; (vii) 96% formic acid, RT, 100%.

a careful HPLC separation of the partially glycosylated intermediates was required in order to introduce two different N-glycans at the predetermined sites,[17] which was tedious and would be difficult to generalize for other peptides.

To address this fundamental problem, we describe in this paper an orthogonal protecting group strategy for construction of glycopeptides carrying two distinct N-glycans. We reasoned that introduction of two orthogonally protected GlcNAc-Asn building blocks during the automated solid-phase peptide synthesis (SPPS) would allow selective deprotection of the GlcNAc primers at different stages, so that different N-glycans could be sequentially installed in a polypeptide by the glycosynthase-catalyzed transglycosylation. We found that a GlcNAc-Asn building block temporarily protected by O-diethylisopropylsilyl (DEIPS) groups was particularly efficient, which was stable during synthesis but could be readily deprotected simultaneously during acidic global deprotection and retrieval of the peptide from the resin to introduce a free GlcNAc-Asn primer. We demonstrate that the combined use of the DEIPS-protected and O-acetylated GlcNAc-Asn building blocks, coupled with the enzymatic sequential glycosylation, enables a highly efficient and quick synthesis of an array of HIV-1 V1 V2 glycopeptides carrying distinct N-glycans.

RESULTS AND DISCUSSION

Synthesis of Orthogonally Protected GlcNAc-Asn Building Blocks. We envisioned that a GlcNAc-Asn building block carrying an acid-sensitive protecting group such a silyl group could be combined with the common O-acetyl protected GlcNAc-Asn building block to achieve site-selective glycosylation, as an O-silyl group could be simultaneously removed during the global peptide deprotection to provide the free GlcNAc acceptor for the attachment of the first N-glycan via enzymatic transglycosylation, and then the O-acetyl protected GlcNAc could be unmasked to allow the second glycosylation with a different N-glycan. To test the feasibility of this approach, we first synthesized the GlcNAc-Asn building blocks in which the GlcNAc moiety was protected with three different types of silyl groups that are supposed to possess variable acidic sensitivity (Scheme 1). The β-glycosyl azide (**3**) was prepared by treatment of the α-glycosyl chloride (**1**)[31] with sodium azide under a phase-transfer catalysis condition, followed by de-O-acetylation with a catalytic amount of MeONa in MeOH. Protection of the free hydroxy groups in **3** with three types of silyl groups was achieved by treatment of **3** with *tert*-butyldimethylsilyl triflate (TBDMSOTf), diethylisopropylsilyl triflate (DEIPSOTf), or triethylsilyl triflate (TESOTf), respectively, giving the corresponding silyl protected glycosyl azide derivatives (**4a−c**) in quantitative yield. It should be noted that, given the highly hindered nature of the protecting groups, we found that using the highly active silyl triflate was essential for high-yield conversions, as the use of the less active silyl chloride derivatives resulted in incomplete reactions on the secondary hydroxy groups.[32] Conversion of the anomeric azide group of **4a−c** into a primary amine was accomplished via palladium-catalyzed reduction under a hydrogen atmosphere to give the corresponding β-glycosyl amine, which was coupled in situ with Fmoc-Asp-OAll using HATU/DIPEA as the coupling reagent to afford the silyl-protected derivatives (**5a−c**) in moderate to excellent yields. We observed that the TES-protected derivative (**5c**) was much less stable than bulky silyl derivatives (**5a** and **5b**), as partial deprotection of the TES groups occurred during the coupling reactions (a byproduct that missed one TES group was isolated in 33% yield). De-O-allylation at the C-terminal carboxyl groups of **5a−c** was achieved under the catalysis of $Pd(PPh_3)_4/PhSiH_3$ to give the glycosylamino acid building blocks (**6a−c**) in 96%, 95%, and 90% isolated yields, respectively. It should be noted that silica gel purification, while successful for **6a** and **6b**, proved to be too acidic for the TES protected building block (**6c**). Thus, **6c** was purified by LH-20 size-exclusion chromatography using 2:1 DCM/MeOH as the eluent. All glycosylamino acid building blocks and intermediates were characterized by NMR and MS analysis. We observed that the $J_{1,2}$ coupling constants (~5 Hz) of the anomeric protons of the silyl-protected β-glycosyl azides (**4a−c**) were significantly smaller than a typical $J_{1,2}$ coupling constant (usually >9 Hz) found in the acetylated β-glycosyl azide (**2**). This data suggests that the bulky silyl ether protecting groups may induce a conformational change in the sugar ring structures from a typical $^4C_1$ chair conformation to a twisted half-chair or a skew-boat conformation. This observation is consistent with previously reported results for similar silylated glycopyranoside derivatives.[33] On the other hand, the O-acetyl protected building block (**8**) was synthesized by direct coupling of the glycosyl azide (**2**) and an aspartic acid derivative (Fmoc-Asp-OtBu) with triethyl phosphine to give **7**, followed by removal of the *t*-butyl ester group with formic acid (Scheme 1).

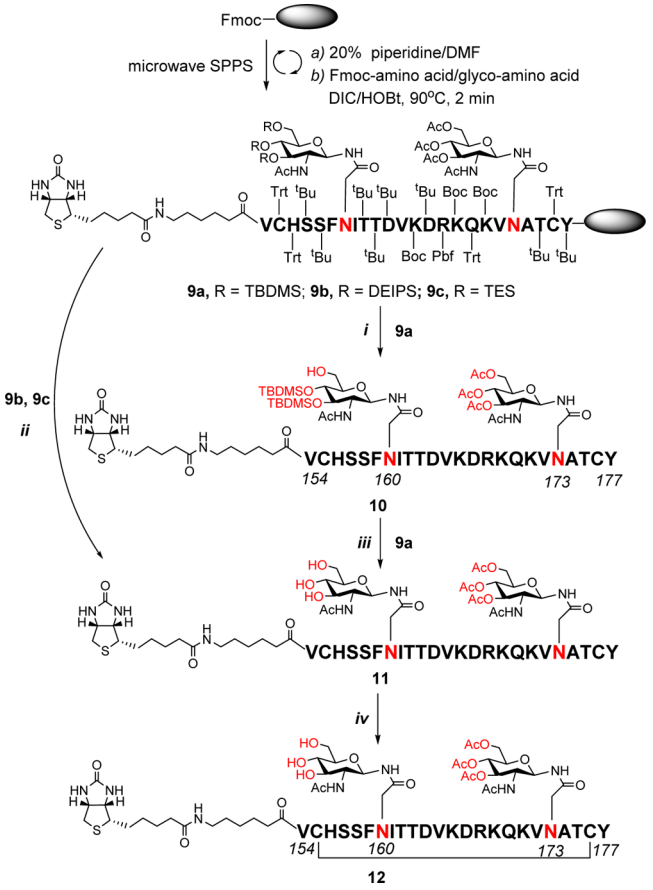
Use of the GlcNAc-Asn Building Blocks for Glycopeptide Synthesis. The usefulness of the orthogonally protected

DOI: 10.1021/acs.joc.6b01044
J. Org. Chem. 2016, 81, 6176−6185

GlcNAc-Asn building blocks (**6** and **8**) for the synthesis of complex glycopeptides carrying different N-glycans was examined by the synthesis of several HIV-1 V1 V2 glycopeptide antigens that were proposed as the neutralizing epitope for broadly neutralizing antibodies PG9.[13,17] We have recently designed and synthesized an array of cyclic 24-mer V1 V2 glycopeptides bearing different glycan structures at the N160 and N173 glycosylation sites.[17] The synthesis coupled with antibody binding studies has enabled the characterization of the fine glycan specificity of antibody PG9 in antigen recognition. That is, a $Man_5GlcNAc_2$ glycan at the N160 site and a sialylated complex type N-glycan at the N173 glycosylation site (for the ZM109 strain) coupled with a segment of V1 V2 peptide constitutes the minimal epitope of PG9. However, the previous chemoenzymatic synthesis of these glycopeptide epitopes had to rely on tedious separation of the monoglycosylated intermediates in order to introduce two distinct N-glycans.[17] Here we sought to add the acid-labile silyl-protected GlcNAc-Asn building block at N160 site first and then the base-labile acetylated GlcNAc-Asn building block at the N173 site to achieve sequential glycosylations with distinct N-glycans at the two sites. Thus, automated solid-phase peptide synthesis (SPPS) incorporating the two distinctly protected GlcNAc-Asn building blocks led to the assembling of the resin-bound fully protected peptide (**9a–c**) (Scheme 2).

A biotin-tag was introduced at the N-terminus to facilitate detection and immobilization for binding studies. The sensitivity of the silyl protecting groups during global peptide deprotection was first examined with cocktail R (TFA/thioanisole/EDT/anisole, 90/5/3/2, v/v), a common cocktail reagent for global deprotection and retrieval of the polypeptide from the resin. Aliquots were taken at intervals and the product was analyzed by HPLC. It was observed that treatment of the TBDMS protected glycopeptide-resin (**9a**) with cocktail R with an extended reaction time (up to 4 h) led to only partial desilylation to give a glycopeptide product with only one of the three TBDMS group being removed (assessed by LC–MS analysis), which was assumed to be glycopeptide **10** with the deprotection at the 6-position. In contrast, deprotection of the DEIPS- and TES-protected glycopeptide-resin, **9b** and **9c**, respectively, was readily achieved using cocktail R or even the milder cocktail K (TFA/phenol/H_2O/thioanisole/EDT, 82.5/5/5/5/2.5, v/v) within 2h to obtain the desired glycopeptide intermediate (**11**), in which a free GlcNAc was installed at the N160 site while the O-acetyl protected GlcNAc at the N173 site was still intact. The resistance of the O-TBDMS groups in **9a** to acid-catalyzed deprotection was unexpected, as previous reports have shown that the TBDMS groups on a corresponding disaccharide moiety could be efficiently removed by a similar acid treatment during peptide deprotection.[32,33] To verify whether the phenomenon was special for the peptide context, we treated the TBDMS-protected GlcNAc-Asn building block (**6a**) with cocktail R and found that the O-TBDMS groups could be completely removed within 1 h (data not shown). This result suggests that the resistance of the O-TBDMS groups in the glycopeptide-resin (**9a**) to acid-catalyzed deprotection is likely specific for the polypeptide context. The reason is not clear. Interestingly, complete desilylation of **10** was readily achieved by its treatment with TBAF in pyridine/AcOH to give **11** in excellent yield. Taken together, the experimental data suggest that the DEIPS protecting group, which is much more stable than the TES group during building block synthesis and can be readily removed during the global

Scheme 2. Synthesis of GlcNAc-Peptide Precursor Using the Orthogonally Protected GlcNAc-Asn Building Blocks[a]



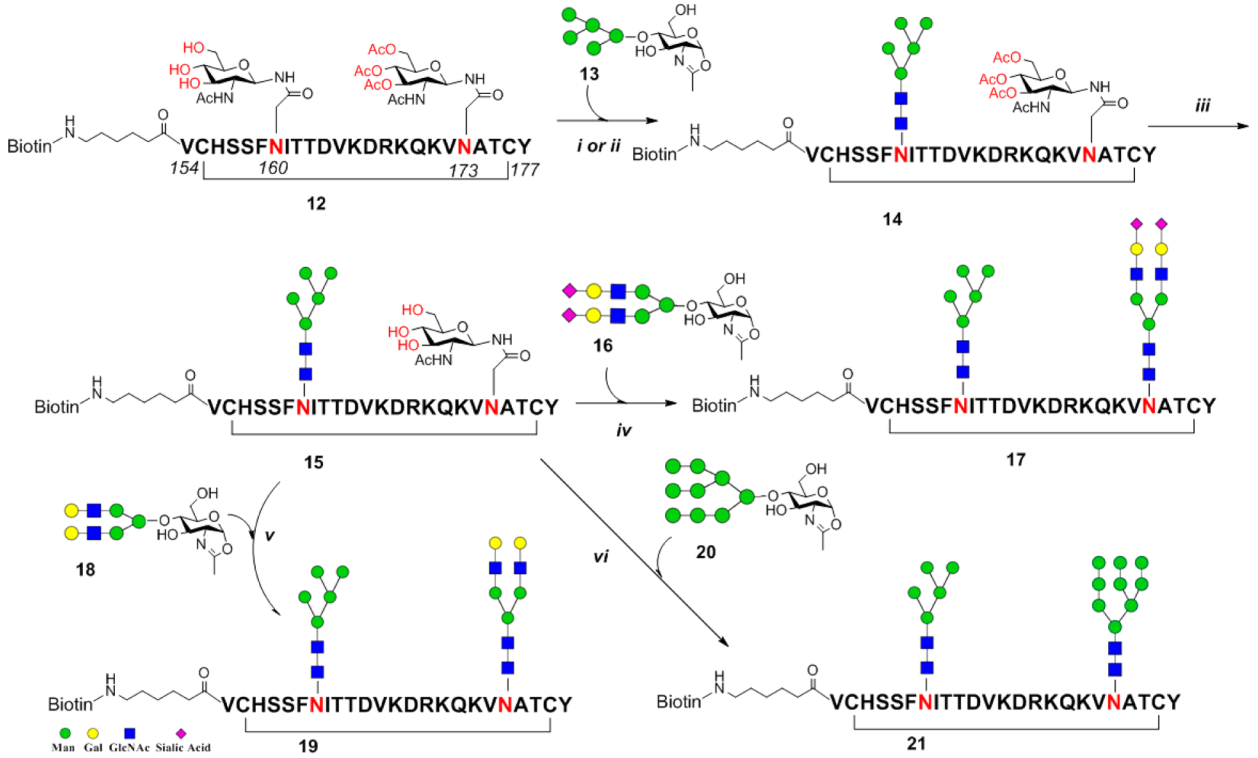
[a]Reaction conditions: (i) Cocktail R, 4 h, 39% (total isolated yield, only one TBDMS removed); (ii) Cocktail R, 2 h, 41% (total isolated yield, complete removal of all three TBDMS groups), or Cocktail K, 38% (total isolated yield, complete removal of all three TBDMS groups); (iii) Bu_4NF, pyridine/AcOH; (iv) phosphate buffer (pH, 7.2), 20% DMSO, 94%

polypeptide deprotection, is the best among the three for introducing a free GlcNAc-Asn building block in SPPS. Finally, cyclization of the linear glycopeptide (**11**) was achieved by treatment with 20% aqueous DMSO to give the cyclic glycopeptide (**12**), where a free GlcNAc was located at N160 and a temporarily acetyl-protected GlcNAc moiety is installed at the N173 site (Scheme 2).

Convergent Synthesis of HIV-1 V1 V2 Glycopeptide Epitopes of Antibody PG9. With the key intermediate (**12**) at hand, we first tested the transfer of a $Man_5GlcNAc$ glycan to the GlcNAc moiety at N160 using mutant endoglycosidase D (N332Q) from *Streptococcus pnuemoniae*.[34] Unexpectedly, incubation of **12** with $Man_5GlcNAc$ oxazoline (**13**) proceeded very inefficiently to give only trace amounts of the transglycosylation product (**14**). This observation was surprising as the N322Q mutant of EndoD was previously found to be highly efficient to transfer $Man_5GlcNAc$ oxazoline (**13**) to a similar cyclic glycopeptide carrying two free GlcNAc moieties to give a doubly glycosylated peptide.[17] In contrast, we found that glycosynthase mutants of EndoM from *Mucor hiemalis*,[35,36] such as the N175Q mutant, were efficient to catalyze the transfer of a $Man_5GlcNAc$ glycan to the free GlcNAc moiety in

DOI: 10.1021/acs.joc.6b01044
J. Org. Chem. 2016, 81, 6176–6185

Scheme 3. Convergert Chemoenzymatic Installation of Two Distinct N-Glycans in the V1 V2 Cyclic Peptide[a]



[a]Reaction conditions: (i) EndoD-N322Q, donor/acceptor = 8:1, Tris buffer (80 mM, pH 7.2), RT, < 5%; (ii) EndoM-N175Q, donor/acceptor = 8:1, Tris buffer (80 mM, pH 7.2), RT, 85%; (iii) 2.5% aq. hydrazine, RT; (iv) EndoM-N175Q, donor/acceptor = 4:1, Tris buffer (80 mM, pH 7.2), RT, 92%; (v) EndoM-N175Q, donor/acceptor = 4:1, Tris buffer (80 mM, pH 7.2), RT, 95%; (vi) EndoM-N175Q, donor/acceptor = 4:1, Tris buffer (80 mM, pH 7.2), RT, 91%

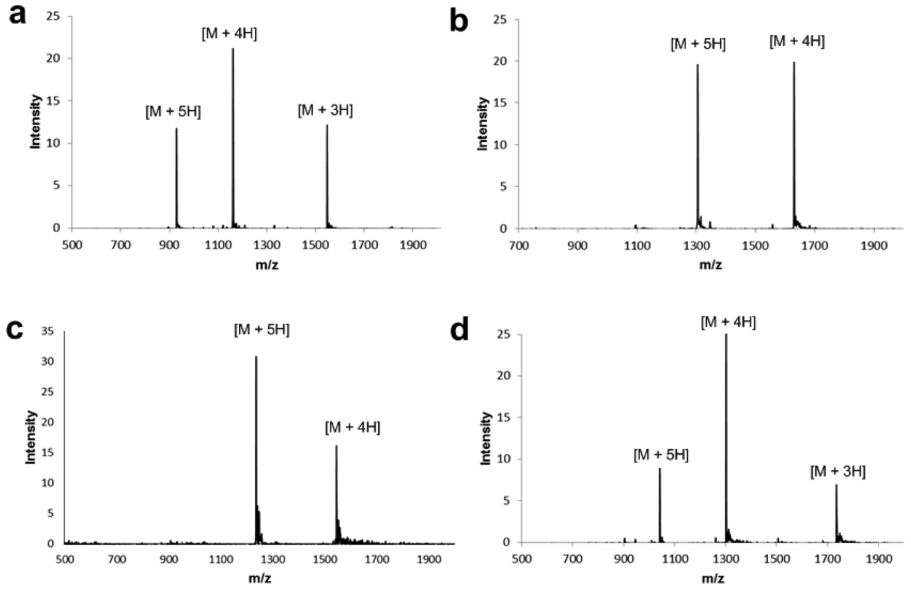


Figure 1. ESI-MS characterization of doubly glycosylated glycopeptides. (a) glycopeptide **15**, (b) glycopeptide **17**, (c) glycopeptide **19**, (d) glycopeptide **21**.

acceptor **12**, giving glycopeptide **14** in excellent yield (Scheme 3).

In order to test if a proximal acetylated GlcNAc moiety caused the low yield in enzymatic transglycosylation with the EndoD mutant (N322Q), we synthesized the corresponding linear peptide (**S-1**) by reduction of **12** with DTT followed by alkylation with iodoacetamide (Scheme S1, Figure S1, Supporting Information). Interestingly, we found that both EndoD-N322Q and EndoM-N175Q mutants now could efficiently glycosylate the unmasked GlcNAc moiety in the linear peptide (**S-1**) to give the corresponding glycopeptide (**S-2**) (Scheme S1 and Figure S2, Supporting Information). This result suggests that the O-acetylated GlcNAc moiety present in the cyclic peptide (**12**) may provide steric hindrance and/or

DOI: 10.1021/acs.joc.6b01044
J. Org. Chem. 2016, 81, 6176−6185

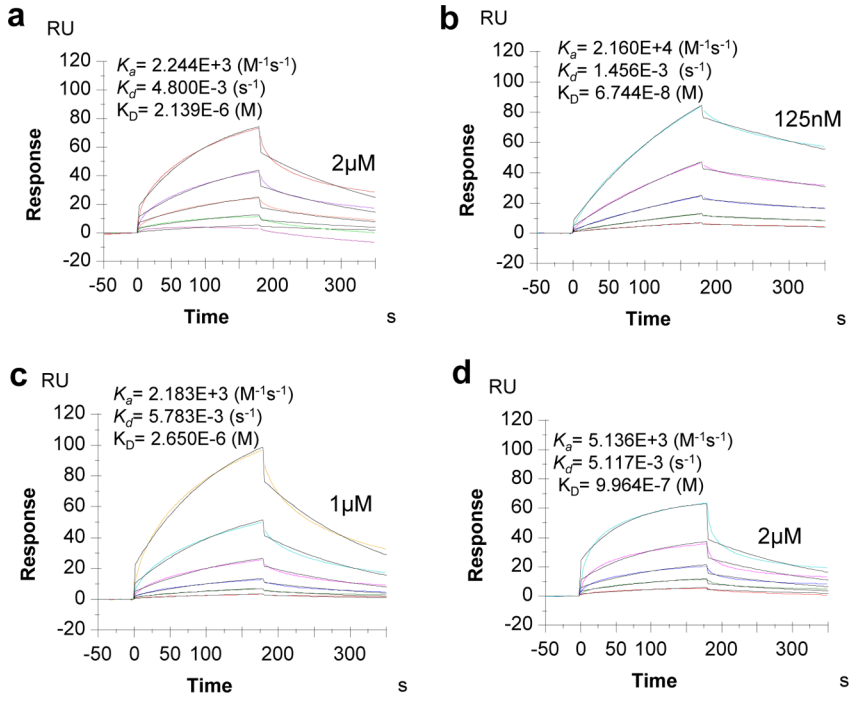


Figure 2. SPR analysis of PG9 IgG recognition of synthetic V1 V2 glycopeptides. Biotinylated glycopeptides were immobilized on streptavidin-coated chips and whole-IgG PG9 was flowed through as the analyte. (a) Compound **15** (N160 = M5/N173 = GN); (b) Compound **17** (N160 = M5/N173 = SCT); (c) Compound **19** (N160 = M5/N173 = CT); (d) Compound **21** (N160 = M5/N173 = M9).

unfavorable hydrophobic interactions with the EndoD mutant, leading to much reduced catalytic activity, while the EndoM mutant seems more flexible for the presence of the bulky/hydrophobic Ac_3GlcNAc-Asn moiety proximal to the free GlcNAc acceptor in enzymatic transglycosylation.

After the installation of the first N-glycan ($Man_5GlcNAc_2$) at the N160 site, the O-acetyl protecting groups on the GlcNAc moiety at the N173 site were then removed by treatment with 2.5% aqueous hydrazine to give glycopeptide **15**, in which the free GlcNAc moiety at the N173 site is now ready for introduction of a different N-glycan. Installation of a sialylated complex type N-glycan at the N173 site was achieved by EndoM-N175Q catalyzed transglycosylation of **15** with sialylated glycan oxazoline **16** to obtain glycopeptide **17**. Transglycosylation of **15** with glycan oxazoline **18**, which lacks the sialic acids, gave the corresponding asialylated glycopeptide (**19**). Moreover, enzymatic transglycosylation with a high mannose type N-glycan oxazoline, the $Man_9GlcNAc$-oxazoline (**20**), gave the glycopeptide (**21**). Thus, this convergent approach permits a quick construction of various glycopeptides from a common precursor. In all the cases, the enzymatic reaction went smoothly and a quantitative conversion was achieved by using an excess of glycan oxazolines, which could be recovered as free N-glycans during HPLC purification and could be converted back into the glycan oxazoline substrate in one step.[37,38] The transglycosylation products were purified by HPLC, and the purity and identity were confirmed by ESI-MS analysis (Figure 1).

SPR Analysis of the Interactions between Antibody PG9 and the Synthetic Glycopeptides. The binding of the synthetic glycopeptides with antibody PG9 was probed by surface plasmon resonance (SPR) analysis through immobilization of the biotinalyted glycopeptides on a streptavidin chip. The results are summarized in Figure 2. The data indicate that the glycopeptide **17**, which carries a $Man_5GlcNAc_2$ glycan at the N160 and a sialylated N-glycan at N173 site, has the highest affinity among all the synthetic V1 V2 glycopeptides, with an apparent K_D of 67 nM. Removal of the sialic acid residues in **17** resulted in more than 39-fold decrease of the affinity for antibody PG9, as demonstrated by a significant increase of the K_D (2.65 μM) for the asialylated glycopeptide (**19**). Interestingly, installation of a full-size, nonsialylated high-mannose glycan at the secondary glycosylation site, as demonstrated by glycopeptide **21**, resulted in drastic (over 1000 fold) decrease of the affinity for PG9 (K_D = 99 μM), suggesting that a bulky N-glycan at the secondary site may conflict the interaction of the antibody with the neutralizing epitope. These results are consistent with the previously reported affinity data where the PG9 antibody Fab fragment was used for the binding analysis,[17] and the more recent crystal structural analysis of PG9 and PG16 in complex with sialylated V1 V2 domain.[39] The experimental data indicate that sialylation of the secondary N-glycan is essential for the high-affinity PG9-glycopeptide epitope interactions.

■ CONCLUSION

A facile chemoenzymatic strategy that permits sequential enzymatic glycosylations to introduce distinct N-glycans into a polypeptide is described. This method exploits two orthogonally protected GlcNAc-Asn building blocks in SPPS. Among several acid-sensitive silyl protecting groups tested, the diethylisopropylsilyl (DEIPS) protecting group was found to be the most efficient, which is stable during building block synthesis but can be easily removed during the global peptide deprotection step, while the O-acetyl protecting groups can be selectively removed at a later stage. The usefulness of this method was exemplified by the efficient synthesis of the HIV-1 V1 V2 glycopeptide neutralizing epitopes carrying two distinct N-glycans. The uniqueness of this synthetic strategy is its high convergence and efficiency. This method should be equably

DOI: 10.1021/acs.joc.6b01044
J. Org. Chem. 2016, 81, 6176−6185

applicable for convergent synthesis of other complex glycopeptides carrying multiple distinct N-glycans.

EXPERIMENTAL SECTION

General Methods and Materials. EndoD^{N322Q} and EndoM^{N175Q} mutants were produced by the reported procedures.[34,35] ^{1}H and ^{13}C NMR spectra were collected on either a 500 or 400 MHz NMR spectrometer. The chemical shifts (δ) were assigned in parts per million (ppm) rounded to the nearest 0.01 for ^{1}H NMR and 0.1 for ^{13}C NMR. Complete proton assignment was determined by two-dimensional correlational experiments (COSY (^{1}H−^{1}H)). The ^{13}C NMR was measured at 100 MHz. Analytical and preparative reverse-phase HPLC (RP-HPLC) purifications were carried out using an HPLC system equipped with a UV detector, using a C18 column at either a flow rate of 0.5 mL/min (analytical) or 4 mL/min (preparative) using MeCN containing 0.1% TFA at 40 °C. Glycopeptides were detected at two wavelengths (214 and 280 nm). Mass spectrometry data for peptides was collected using either a single quadrupole mass spectrometer or a LC−MS tandem quadrupole mass spectrometer. High-resolution (HR) mass spectra were collected with an electrospray ionization time-of-flight (ESI-TOF) instrument. Glycopeptides were analyzed using an analytical C18 column at a flow rate of 0.4 mL/min at 50 °C using MeCN containing 0.1% formic acid at a gradient of 5−95%B in 10 min. Peptide synthesis was performed using a microwave-assisted peptide synthesizer. Surface plasmon resonance (SPR) analysis was performed at 25 °C. Biotinylated glycopeptides were immobilized using a streptavidin-bound sensor chips in a solution of 0.1 M HEPES, 0.15 M NaCl, 0.5% v/v surfactant P20, pH 7.4. The substrates were immobilized manually by injecting the samples until 20−30 RU was achieved (low-loading). PG9 was injected over four cells at 2-fold increasing concentrations at a flow rate of 50 μL/min for 3 min. Dissociation was allowed to occur over a period of 5 min. The chip was regenerated by injection of 3 M $MgCl_2$ at a flow rate of 50 μL/min for 3 min followed by injection of the running buffer for 5 min. Data was collected at a rate of 10 Hz. A 1:1 Langmuir binding model was used for fitting the data.

*2-Acetamido-3,4,6-tri-O-acetyl-1,2-deoxy-β-D-glucopyranosyl azide (**2**).* A solution of **1**[31] (50 g, 137 mmol) in DCM (50 mL) was mixed with a solution of sodium azide (25 g, 391 mmol) and tetrabutylammonium hydrogen sulfate (133 g, 391 mmol) in saturated sodium bicarbonate (50 mL). The biphasic mixture was stirred vigorously for 2 h. Upon completion of the reaction as indicated by TLC, the organic layer was separated, dried with Na_2SO_4, and filtered. The filtrate was concentrated and the residues was crystallized from ethanol/hexane to give **2**[31] (50 g, quant. yield) as a white crystal. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.99, 2.03, 2.04, 2.10 (s each, 3H each, 3 × OAc and NHAc), 3.84 (m, 1H, H-5), 3.95 (m, 1H, H-2), 4.16−4.29 (m, 2H, H-6), 4.85 (d, 1H, J = 9.2 Hz, H-1), 5.10 (t, 1H, J = 7.9 Hz, H-3), 5.12 (t, 1H, J = 8.2 Hz, H-4), 6.22 (d, 1H, J = 9.0 Hz, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.0, 170.9, 170.8, 169.5, 88.6, 74.1, 72.4, 68.4, 62.1, 54.3, 23.4, 20.9, 20.8, 20.8. MS (ESI) m/z [M + H]$^+$ Calcd for $C_{14}H_2N_4O_8$: 372.13, found 373.23. The ^{1}H and ^{13}C NMR data were consistent with the reported data.[31]

*2-Acetamido-1,2-deoxy-β-D-glucopyranosyl azide (**3**).* A solution of **2** (10 g, 27 mmol) in MeOH (20 mL) containing a catalytic amount of MeONa (79 mg, 2.7 mmol) was stirred at 25 °C for 2 h. Then Dowex (H$^+$form) was added, and the mixture was filtered. The filtrate was concentrated to dryness to afford **3** (6.65 g, quant. yield) as a white solid. The product was used for the next step without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.99 (s, 3H, -NHAc), 3.30−3.36 (m, 3H, H-5, H-6), 3.45 (t, J = 8.5 Hz, 1H, H-3), 3.65−3.72 (m, 2H, H-2, H-4), 3.89 (d, J = 12.3 Hz, 1H, H-1), 4.50 (d, J = 9.3 Hz, 1H, -NHAc), 4.84 (s, 3H, 3 × OH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 23.0, 56.9, 62.8, 71.9, 75.9, 80.5, 90.3, 173.9. MS (ESI) m/z [M + H]$^+$ Calcd for $C_8H_{14}N_4O_5$: 246.10, found 246.52. The ^{1}H and ^{13}C NMR data are consistent with the previously reported data.[40]

*2-Acetamido-3,4,6-tri-O-tert-butyldimethylsilyl-1,2-deoxy-β-D-glucopyranosyl azide (**4a**).* To a solution of **3** (1 g, 41 mmol) in 2,6-lutidine (4 mL) at 0 °C was added *tert*-butyldimethylsilyl trifluoromethanesulfonate (1.8 g, 7.4 mmol). The solution was stirred for 1 h on ice, then heated to 40 °C and stirred overnight. The solvent was removed and the product was purified on silica (1:1 hexane: ethyl acetate), yielding **4a** (2 g, quant.) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.03−0.12 (m, 18H, 6 Si-CH_3), 0.87−0.91 (m, 27H, 3 *t*-Bu), 1.93 (s, 3H, NHAc), 3.68 (m, 1H, H-3), 3.75−3.84 (m, 2H, H-6a,b), 3.93 (m, 1H, H-4), 3.98 (m, 1H, H-2), 4.20 (t, J = 9.4 Hz, 1H, H-5), 5.04 (d, J = 5.0 Hz, 1H, H-1), 6.67 (d, J = 9.2 Hz, 1H, -NHAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.1, 18.2, 18.5, 23.6, 25.9, 26.0, 26.1, 50.4, 63.3, 68.6, 70.8, 80.1, 87.5, 168.9. HRMS (ESI-TOF) m/z [M + Na]$^+$ Calcd for $C_{26}H_{56}N_4O_5Si_3Na$: 611.3456, found 611.3447.

*2-Acetamido-3,4,6-tri-O-diethylisopropylsilyl-1,2-deoxy-β-D-glucopyranosyl azide (**4b**).* To a solution of **3** (1 g, 41 mmol) in 2,6-lutidine (4 mL) at 0 °C was added diethyl(isopropyl)-silyltrifluoromethanesulfonate (2.1 g, 7.4 mmol). The solution was stirred for 1 h on ice, then heated to 40 °C and stirred overnight. The solvent was removed and the product purified on silica (1:1 hexane:ethyl acetate), yielding **4b** (2 g, quant.) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.621−0.720 (m, 15H, Si-CH_2, Si-CH), 0.976−1.056 (m, 36H, 4 Si-CH_3), 1.964 (s, 3H, NHAc), 3.801 (m, 1H, H-3), 3.835−3.923 (m, 2H, H-6), 4.036 (m, 1H, H-4), 4.052 (m, 1H, H-2), 4.289 (t, J = 9.35 Hz, 1H, H-5), 5.09 (d, J = 5.0 Hz, 1H, H-1), 6.77 (d, J = 9.35 Hz, 1H, -NHAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ 3.1, 3.2, 3.4, 3.4, 3.5, 4.5, 6.7, 6.9, 6.9, 6.9, 7.0, 12.6, 12.63, 12.7, 13.3, 17.0, 17.2, 17.2, 17.3, 17.3, 23.3, 50.5, 63.1, 68.5, 70.6, 80.1, 87.4, 168.7. HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{29}H_{62}N_4O_5Si_3Na$: 653.3926, found 653.3927.

*2-Acetamido-3,4,6-tri-O-triethylsilyl-1,2-deoxy-β-D-glucopyranosyl azide (**4c**).* To a solution of **3** (1 g, 41 mmol) in 2,6-lutidine (4 mL) at 0 °C was added triethysilyltrifluoromethanesulfonate (1.9 g, 7.4 mmol). The solution was stirred for 1 h on ice, then heated to 40 °C and stirred overnight. The solvent was removed and the product purified on silica (1:1 hexane: ethyl acetate), yielding **4c** as a white solid (2 g, quant.). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.58−0.68 (m, 18H, 6 Si-CH_2CH_3), 0.94−1.00 (m, 27H, 9 Si-CH_2CH_3), 1.96 (s, 3H, NHAc), 3.74 (t, J = 6.4 Hz, 1H, H-3), 3.79−3.81 (m, 2H, H-6), 3.92 (m, 1H, H-4), 3.94 (m, 1H, H-2), 4.16 (t, J = 10.8 Hz, 1H, H-5), 5.02 (d, J = 5.1 Hz, 1H, H-1), 6.59 (d, J = 9.2 Hz, 1H, -NHAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ 4.6, 4.8, 4.8, 6.9, 6.9, 6.9, 23.6, 51.5, 62.8, 68.9, 71.4, 80.2, 87.6, 169.1. HRMS (ESI-TOF) m/z [M + Na]$^+$ Calcd for $C_{26}H_{56}N_4O_5Si_3Na$: 611.3456, found 611.3448.

*N$^{\omega}$-(2-Acetamido-3,4,6-tri-O-tert-butyldimethylsilyl-2-deoxy-β-D-glucopyranosyl-N$^{\alpha}$-(9-fluorenylmethyloxycarbonyl)-L-asparagine-allyl ester (**5a**).* To a solution of **4a** (1 g, 1.7 mmol) in MeOH (40 mL) was added palladium on carbon (8.5 mg). The flask was evacuated and flushed with hydrogen three times, and the mixture was stirred under hydrogen for 1 h at RT, until the reaction was complete as indicated by TLC. The sample was filtered through Celite. The filtrate was concentrated and the resulting clear oil was used immediately in the next step without further purification. The clear oil was dissolved in DMF (25 mL) and Fmoc-Asp-OAll (0.752 g, 1.9 mmol) was added. Diisopropylethylamine (0.66 g, 5.1 mmol) in DMF (1 M) was added with vigorous stirring under argon. HATU (1.94 g, 5.1 mmol) in DMF (0.5 M) was added dropwise. The reaction was stirred for 1 h at RT, until the reaction was complete as indicated by TLC. The reaction was diluted with EtOAc, and washed with *aq.* $NaHCO_3$. The aqueous layer was extracted with EtOAc (3x). The organic extracts were combined, dried over Na_2SO_4, filtered. The filtrate was concentrated. The product was purified by flash silica gel chromatography (20−50% EtOAc in hexane) to give **5a** (1.18 g, 74%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.04−0.16 (m, 18H, 6 Si-CH_3), 0.88−0.95 (t, J = 8.2 Hz, 27H, 9 *t*-Bu), 2.04−2.06 (s, 3H, -NHAc), 2.75−3.02 (dd, 2H, Asn-β-CH_2), 3.84−3.87 (m, 2H, H-6), 3.88−3.90 (m, 1H, H-5), 3.95−3.98 (m, 1H, H-2), 4.24−4.47 (m, 5H, H-3, H-4, Fmoc-CH, Fmoc-CH_2), 4.63−4.64 (m, 1H, α-CH-Asn), 4.65−4.69 (d, J = 4.8 Hz, 2H, O—CH_2—CH═CH_2), 5.19−5.22 (d, J = 10.5 Hz, 1H, OCH_2—CH═C$H_{cis}H_{trans}$), 5.33 (d, J = 17.2 Hz, 1H, OCH_2—CH═$CH_{cis}H_{trans}$), 5.55 (d, J = 7.75 Hz, 1H, H-1), 5.87−5.94 (m, 1H, OCH_2—CH═CH2), 6.04 (d, J = 8.5 Hz, 1H, Asn-α-NH),

DOI: 10.1021/acs.joc.6b01044
J. Org. Chem. 2016, 81, 6176−6185

6.82 (d, J = 7.6 Hz, 1H, NH-Asn), 7.19 (d, J = 8.7 Hz, 1H, -NHAc), 7.30−7.76 (m, 8H, Fmoc-Ar). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5, 170.9, 170.0, 156.4, 144.1, 141.5, 131.9, 127.9, 127.4, 125.5, 120.1, 118.6, 81.7, 72.7, 71.0, 68.2, 67.4, 66.5, 61.5, 51.5, 50.7, 47.3, 38.1, 25.9, 23.8, 4.7. HRMS (ESI-TOF) m/z [M + H]$^+$ Calcd for $C_{48}H_{78}N_3O_{10}Si_3$: 940.4995, found 940.4994.

*N^{ω}-(2-Acetamido-3,4,6-tri-O-diethylisopropyl-2-deoxy-β-D-glucopyranosyl-N^{α}-(9-fluorenylmethyloxycarbonyl)-L-asparagine-allyl ester (**5b**).* To a solution of **4b** (1 g, 1.6 mmol) in MeOH (40 mL), and palladium on carbon (8 mg) was added. The flask was evacuated and flushed with hydrogen three times, and stirred under hydrogen for 1 h at RT, until the reaction was complete by TLC. The sample was filtered through Celite. The filtrate was concentrated and the resulting clear oil was used immediately in the next step without further purification. The clear oil was dissolved in DMF (25 mL) and Fmoc-Asp-OAll (0.709 g, 1.8 mmol) was added. Diisopropylethylamine (0.62 g, 4.8 mmol) in DMF (1 M) was added with vigorous stirring under argon. HATU (1.94 g, 4.8 mmol) in DMF (0.5 M) was added slowly, dropwise. The reaction was stirred for 1 h at RT until the reaction was complete by TLC. After completion, the reaction was diluted with EtOAc, and washed with 50% $NaHCO_3$ (aq.). The aqueous layer was extracted with EtOAc 3x. The organic extracts were combined, dried over Na_2SO_4, filtered, and evaporated in vacuo. The compound was purified by flash silica gel chromatography (20−50% EtOAc in hexane), yielding **5b** (1.15 g, 73%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.63−0.73 (m, 15H, Si-CH_2, Si-CH), 0.98−1.06 (m, 36H, Si-CH_3), 2.06 (s, 3H, -NHAc), 2.77−3.03 (dd, 2H, Asn-β-CH_2), 3.86−3.89 (m, 1H, H-5), 3.93−3.98 (m, 2H, H-6), 4.0−4.03 (m, 1H, H-2), 4.25−4.41 (m, 5H, H-3, H-4, Fmoc-CH, Fmoc-CH_2), 4.63−4.64 (m, 1H, α-CH-Asn), 4.67−4.70 (d, J = 5.4 Hz, 2H, O−CH_2−CH=CH_2), 5.23 (d, J = 10.5 Hz, 1H, OCH_2−CH=$CH_{cis}H_{trans}$), 5.34 (d, J = 17.2 Hz, 1H, OCH_2−CH=$CH_{cis}H_{trans}$), 5.56 (d, J = 8.0 Hz, 1H, H-1), 5.92 (m, 1H, OCH_2−CH=CH_2), 6.09 (d, J = 8.6 Hz, 1H, Asn-α-NH), 6.84 (d, J = 8.0 Hz, 1H, 1-NH), 7.27−7.78 (m, 9H, Fmoc-Ar, -NHAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5, 170.9, 169.9, 156.4, 144.1, 144.1, 141.5, 131.9, 131.9, 127.8, 127.3, 125.5, 125.4, 120.1, 118.6, 118.5, 81.9, 72.0, 70.9, 68.2, 67.5, 66.5, 61.5, 51.7, 50.8, 47.3, 38.1, 23.7, 21.2, 17.6, 17.5, 17.4, 17.4, 12.8, 12.8, 12.8, 7.2, 7.2, 7.2, 7.1, 7.1, 3.6, 3.6, 3.6, 3.5. HRMS (ESI-TOF) m/z [M + H]$^+$ Calcd for $C_{51}H_{84}N_3O_{10}Si_3$: 982.5465, found 982.5466.

*N^{ω}-(2-Acetamido-3,4,6-tri-O-triethylsilyl-2-deoxy-β-D-glucopyranosyl-N^{α}-(9-fluorenylmethyloxycarbonyl)-L-asparagine-allyl ester (**5c**).* Compound **4c** (1 g, 1.7 mmol) was dissolved in methanol (42 mL), and palladium on carbon (8.5 mg) was added. The flask was evacuated and flushed with hydrogen three times, and stirred under hydrogen for 1 h at RT, until the reaction was complete by TLC. The sample was filtered through Celite. The filtrate was concentrated and the resulting clear oil was used immediately in the next step without further purification. The clear oil was dissolved in DMF (25 mL) and Fmoc-Asp-OAll (0.752 g, 1.9 mmol) was added. Diisopropylethylamine (0.66 g, 5.1 mmol) in DMF (1 M) was added with vigorous stirring under argon. HATU (1.94 g, 5.1 mmol) in DMF (0.5 M) was added slowly, dropwise. The reaction was stirred for 1 h at RT, until the reaction was complete by TLC. After completion, the reaction was diluted with EtOAc, and washed with 50% $NaHCO_3$ (aq.). The aqueous layer was extracted with EtOAc. The organic extracts were combined, dried over Na_2SO_4, filtered, and evaporated in vacuo. The compound was purified by flash silica gel chromatography (20−50% EtOAc in hexane), yielding **5c** (0.61 g, 38%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88−1.03 (m, 18H, -Si−CH_2−CH_3), 0.53−0.71 (m, 27H, -Si−CH_2−CH_3), 2.02−2.05 (s, 3H, -NHAc), 2.76−3.03 (dd, 2H, Asn-β-CH_2), 3.81−3.98 (m, 3H, H-2, 5, 6), 4.22−4.43 (m, 5H, H-3, 4, Fmoc-CH, Fmoc-CH_2), 4.62−4.66 (m, 1H, α-CH-Asn), 4.68 (d, J = 5.4 Hz, 1H, OCH_2−CH=CH_2), 5.21 (d, J = 10.5 Hz, 1H, OCH_2−CH=$CH_{cis}H_{trans}$), 5.32 (d, J = 17.2 Hz, 1H, OCH_2−CH=$CH_{cis}H_{trans}$), 5.58 (d, J = 7.95 Hz, 1H, H-1), 5.87−5.94 (m, 1H, OCH_2−CH=CH_2), 6.07 (d, J = 8.65 Hz, 1H, NH-Asn), 6.86 (d, J = 7.95 Hz, 1H, 1-NH), 7.29 (d, J = 8.05 Hz, 1H, -NHAc), 7.27−7.77 (m, 8H, Fmoc-Ar). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5, 170.9, 169.9, 156.3, 144.1, 141.4, 131.9, 129.4, 127.8, 127.2, 125.4, 120.8, 120.0, 81.8, 71.7, 70.9, 68.3, 67.9, 67.4, 66.4, 61.0, 51.6, 47.2, 37.9, 23.7, 6.9, 6.9, 4.6, 4.6, 4.5. HRMS (ESI-TOF) m/z [M + H]$^+$ Calcd for $C_{48}H_{78}N_3O_{10}Si_3$: 940.4995, found 940.4999.

*N^{ω}-(2-Acetamido-3,4,6-tri-O-tert-butyldimethylsilyl-2-deoxy-β-D-glucopyranosyl-N^{α}-(9-fluorenylmethyloxycarbonyl)-L-asparagine (**6a**).* To a solution of **5a** (1 g, 1.1 mmol) in DCM (50 mL) were added tetrakis(triphenylphosphine)palladium(0) (25.4 mg, 0.02 mmol) and phenylsilane (238 mg, 2.2 mmol). The resulting mixture was stirred at RT, under argon, for 45 min. After completion of reaction as indicated by TLC, water (2 mL) was added to quench the reaction, and the biphasic mixture was vigorously stirred for 30 min. The mixture was concentrated to dryness and the residue was purified by flash silica gel column chromatography (0−15% MeOH in DCM containing 0.5% acetic acid) and the crude product was further purified by size-exclusion chromatography (Sephadex LH-20, 2:1 CH_2Cl_2/MeOH) to afford **6a** (0.93 g, 94% yield) as a white powder. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.11−0.16 (m, 18H, Si-Me), 0.88−0.96 (m, 27H, Si-tBu), 2.06 (s, 3H, -NHAc), 2.85−2.98 (m, 2H, Asn-β-CH_2), 3.39−3.48 (m, 3H, H-4, H-6), 3.81 (m, 1H, H-5), 4.06 (m, 1H, H-2), 4.19−4.35 (m, 3H, Fmoc-CH, Fmoc-CH_2), 4.49 (t, J = 11.9 Hz, 1H, H-3), 4.65 (m, 1H, α-CH-Asn), 5.58 (d, J = 6.35 Hz, 1H, H-1), 6.22 (d, J = 6.60 Hz, 1H, Asn-α-NH), 7.27−7.74 (m, 10H, Fmoc-Ar, -NHAc, 1-NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.7, 173.1, 172.8, 156.4, 144.1, 144.0, 141.4, 127.8, 127.3, 125.5, 120.1, 81.3, 71.9, 69.9, 69.3, 67.5, 58.5, 51.0, 47.3, 25.9, 23.5, 18.1, 4.8. HRMS (ESI-TOF) m/z [M + H]$^+$ Calcd for $C_{45}H_{74}N_3O_{10}Si_3$: 900.4682, found 900.4694.

*N^{ω}-(2-Acetamido-3,4,6-tri-O-diethylisopropylsilyl-2-deoxy-β-D-glucopyranosyl-N^{α}-(9-fluorenylmethyloxycarbonyl)-L-asparagine (**6b**).* To a solution of **5b** (1 g, 1.0 mmol) in DCM (50 mL), were added tetrakis(triphenylphosphine)palladium(0) (25.4 mg, 0.02 mmol) and phenylsilane (238 mg, 2.2 mmol).The resulting mixture was stirred at RT, under argon, for 45 min. After completion of reaction as indicated by TLC, water (2 mL) was added to quench the reaction, and the biphasic mixture was vigorously stirred for 30 min. The mixture was concentrated to dryness and the residue was purified by flash silica gel chromatography (0−15% MeOH in DCM containing 0.5% acetic acid), and the crude product was further purified followed by size exclusion chromatography (Sephadex LH-20, 2:1 CH_2Cl_2/MeOH) to afford **6b** (0.89 g, 95% yield) as a white powder. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.59−0.69 (m, 15H, Si-CH_2, Si-CH), 0.94−1.01 (m, 36H, Si-CH_3), 2.03 (s, 3H, -NHAc), 2.77−2.88 (m, 2H, Asn-β-CH_2), 3.87−3.96 (m, 5H, H-3, H-4, Fmoc-CH, Fmoc-CH_2), 3.98−4.01 (m, 1H, H-2), 4.17−4.33 (m, 3H, H-5, H-6), 4.59−4.61 (m, 1H, α-CH-Asn), 5.58 (d, J = 7.5 Hz, 1H, H-1), 6.09 (d, J = 6.5 Hz, 1H, Asn-α-NH), 7.19 (d, J = 7.0 Hz, 1H, 1-NH), 7.28 (d, J = 7.0 Hz, 1H, -NHAc), 7.34−7.73 (m, 8H, Fmoc-Ar). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.2, 172.7, 171.2, 155.9, 143.9, 141.2, 127.6, 127.1, 125.3, 119.8, 81.7, 71.7, 71.2, 67.9, 67.2, 61.1, 51.5, 50.5, 47.1, 23.4, 17.3, 12.6, 12.6, 12.5, 6.9. HRMS (ESI-TOF) m/z [M + H]$^+$ Calcd for $C_{48}H_{80}N_3O_{10}Si_3$: 942.5152, found 942.5167.

*N^{ω}-(2-Acetamido-3,4,6-tri-O-triethylsilyl-2-deoxy-β-D-glucopyranosyl-N^{α}-(9-fluorenylmethyloxycarbonyl)-L-asparagine (**6c**).* To a solution of **5c** (1 g, 1.1 mmol) in DCM (50 mL) were added tetrakis(triphenylphosphine)palladium(0) (25.4 mg, 0.02 mmol) and phenylsilane (238 mg, 2.2 mmol). The resulting mixture was stirred at RT, under argon, for 45 min. After completion of reaction by TLC, water (2 mL) was added to quench the reaction, and the biphasic mixture was vigorously stirred for 30 min. The mixture was concentrated to dryness and purified by flash silica gel column chromatography size exclusion chromatography (0−15% MeOH in DCM containing 0.5% acetic acid), the crude product was further purified by size-exclusion chromatography (Sephadex LH-20, 2:1 CH_2Cl_2/MeOH) to afford **6c** (0.92 g, 97% yield) as a white powder (0.92 g, 97% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.54−0.69 (m, 18H, -Si-CH_2-CH_3), 0.89−0.99 (m, 27H, -Si-CH_2−CH_3), 1.95 (s, 3H, -NHAc), 2.63−2.90 (m, 2H, Asn-β-CH_2), 3.57 (m, 1H, H-5), 3.68−3.71 (m, 2H, H-6), 3.82−3.85 (m, 2H, Fmoc-CH_2), 3.95 (m, 1H, H-2), 4.17−4.19 (m, 1H, Fmoc-CH), 4.25−4.29 (m, 1H, H-4), 4.35−4.48 (m, 1H, H-3), 4.58−4.59 (m, 1H, α-CH-Asn), 5.14 (d, J = 8.0 Hz,

DOI: 10.1021/acs.joc.6b01044
J. Org. Chem. 2016, 81, 6176−6185

1H, H-1), 6.24 (d, J = 8.2 Hz, 1H, NH-Asn), 6.58 (d, J = 9.2 Hz, 1H, 1-NH), 7.19 (d, J = 8.4 Hz, 1H, -NHAc), 7.28−7.29 (d, J = 7.35 Hz, 2H, Fmoc-Ar), 7.35−7.38 (t, J = 8.7 Hz, 2H, Fmoc-Ar), 7.57−7.61 (t, J = 8.6 Hz, 2H, Fmoc-Ar), 7.73−7.74 (d, J = 7.6 Hz, 2H, Fmoc-Ar). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.6, 172.0, 171.2, 162.9, 156.4, 141.2, 132.4, 132.2, 128.6, 128.5, 127.1, 119.9, 79.3, 67.4, 62.6, 50.5, 47.1, 36.5, 31.5, 23.2, 6.9, 4.4, 4.0. HRMS (ESI-TOF) m/z $[M + H]^+$ Calcd for $C_{45}H_{74}N_3O_{10}Si_3$: 900.4682, found 900.4689.

N^{ω}-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-N^{α}-(9-fluorenylmethyloxycarbonyl)-L-asparagine tert-butyl ester (7). A solution of **2** (5 g, 13.4 mmol) and Fmoc-Asp-OtBu (4.96 g, 12.1 mmol) in DCM (10 mL) was cooled to −10 °C under an argon atmosphere. A catalytic amount of triethylphosphine (PEt_3) (31.9 mg, 0.27 mmol) was added, and the reaction mixture was stirred to RT overnight. The product (**7**) precipitated as a white solid.[2] The solid was collected and recrystallized from warm CH_2Cl_2 to afford **7** (8.03 g, 81% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.45 (s, 9H, t-Bu), 1.96 (s, 3H, -NHAc), 2.04, 2.05, 2.07 (three s, 9H, 3 × OAc), 2.69−2.87 (m, 2H, Asn-β-CH_2), 3.74−3.76 (m, 1H, H-5), 4.05−4.24 (m, 3H, H-2, 6), 4.28−4.44 (m, 3H, Fmoc-CH_2, Fmoc-CH), 4.52 (m, 1H, Asn-α-CH), 5.06−5.14 (m, 3H, H-1, 3, 4), 5.98 (d, J = 8.5 Hz, 1H, Asn-α-NH), 6.19 (d, J = 8.0 Hz, 1H, -NHAc), 7.22 (d, J = 10 Hz, 1H, 1-NH), 7.30 (t, J = 8.4 Hz, 2H, Fmoc-Ar), 7.39 (t, J = 8.2 Hz, 2H, Fmoc-Ar), 7.60 (d, J = 7.4 Hz, 2H, Fmoc-Ar), 7.75 (t, J = 7.4 Hz, 2H, Fmoc-Ar). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.8, 172.2, 171.5, 171.1, 170.4, 169.7, 156.6, 144.2, 141.7, 141.6, 128.1, 127.5, 125.6, 120.4, 80.7, 80.5, 73.9, 73.2, 68.1, 67.6, 62.1, 53.8, 51.4, 47.5, 38.6, 28.3, 23.5, 21.1, 21.00, 13.9, 0.4. MS (ESI) m/z $[M + H]^+$ Calcd for $C_{37}H_{45}N_3O_{13}$: 739.30, found 740.74. The ^{1}H and ^{13}C NMR data were consistent with the reported data.[41]

N^{ω}-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-N^{α}-(9-fluorenylmethyloxycarbonyl)-L-asparagine (8). Compound **7** (1 g, 1.35 mmol) was dissolved in neat formic acid. The mixture was stirred at RT until complete removal of the tBu group, as indicated by TLC. Formic acid was removed in vacuo to provide **8** (0.922 g, quant.). ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 1.72 (s, 3H, NHAc), 1.89 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.99 (s, 3H, OAc), 2.62−2.67 (m, 2H, Asn-β-CH_2), 3.79−3.95 (m, 3H, H-5, 6), 4.16−4.29 (m, 4H, H-2, Fmoc-CH_2, Fmoc-CH), 4.35−4.39 (m, 1H, α-CH-Asn), 4.79−4.84 (t, J = 8.8 Hz, 1H, H-4), 5.07−5.19 (m, 2H, H-1, 3), 7.33 (t, J = 8.5 Hz, 2H, Fmoc-Ar), 7.41 (t, J = 8.1 Hz, 2H, Fmoc-Ar), 7.51 (d, J = 8.40 Hz, 1H, Asn-α-NH), 7.71 (d, J = 7.60 Hz, 2H, Fmoc-Ar), 7.89 (m, 3H, -NHAc, Fmoc-Ar), 8.61 (d, J = 9.2 Hz, 1H, 1-NH). ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ 173.9, 170.9, 170.7, 170.4, 170.4, 170.2, 163.2, 156.7, 144.7, 144.7, 141.6, 129.8, 128.5, 127.9, 126.2, 126.1, 120.9, 78.9, 74.2, 73.1, 69.2, 66.6, 62.7, 52.9, 50.9, 47.5, 37.8, 36.7, 31.6, 23.5, 21.4, 21.3, 21.2. MS (ESI) m/z $[M + H]^+$ Calcd for $C_{37}H_{45}N_3O_{13}$: 683.23, found 683.24. The ^{1}H and ^{13}C NMR data were consistent with the reported data.[41]

Synthesis of the Precursor Glycopeptides Incorporating the GlcNAc-Asn Building Blocks. All the three precursor glycopeptides were synthesized on a 0.1 mmol scale using 5-fold excess of reagents [0.2 M amino acid solution (in DMF)] with 1 M DIC (in DMF) and 0.5 M HOBt (in DMF). Low-loading Fmoc-PAL-PEG-PS resin (0.17 mmol/g, Life Technologies) was used for the synthesis of the peptides. The glycosylamino acid building blocks (**6a−c** and **8**) were coupled to the growing peptide at 90 °C with a 50 Hz MW power for 10 min, Fmoc-Cys(Trt)-OH and Fmoc-His(Trt)-OH were coupled at 50 °C with a 50 Hz MW power for 2 min. Fmoc-Arg(Pbf)-OH was double coupled (RT without MW for 25 min, followed by 90 °C with 50 Hz MW power for 2 min). All other amino acids were coupled at 90 °C with 50 Hz MW power for 2 min. The deblocking reagent used was 20% piperidine with 0.1 M HOBt (deblocking occurred at 50 °C and 50 Hz MW power). The N-terminus was capped with a biotin tag by treatment with biotin-LC-N-hydroxysuccinimide (3 mol. equivalent) in DIPEA/DMF. Resin cleavage and global peptide deprotection was achieved after washing the resin with DCM (3x) and adding freshly prepared cocktail R (TFA/thioanisole/1,2-EDT/anisole, 90/5/3/2) and shaking for 2 h at RT. The resin was filtered onto ice-cold ethyl ether for precipitation in a 50 mL centrifuge tube. The crude glycopeptides were purified by RP-HPLC and the purity and identity were confirmed by analytical HPLC and LC−MS analysis. Glycopeptide (**10**): Analytical RP-HPLC, t_R = 26 min. (gradient, 0−90% aq. MeCN containing 0.1% TFA for 30 min; flow rate = 0.5 mL/min). ESI-MS: Calcd M = 3856.50, found: 771.53 $[M + 5H]^{5+}$, 964.24 $[M + 4H]^{4+}$, 1285.43 $[M + 3H]^{3+}$. Deconvolution mass: 3856.03 ± 0.15. Glycopeptide (**11**): Analytical RP-HPLC, t_R = 21 min. (gradient, 5−25% aq. MeCN containing 0.1% FA for 30 min; flow rate, 1 mL/min). ESI-MS: Calcd M = 3628.37, found: 726.55 $[M + 5H]^{5+}$, 908.18 $[M + 4H]^{4+}$, 1210.57 $[M + 3H]^{3+}$, 1815.35 $[M + 2H]^{2+}$. Deconvolution mass: 3628.65 ± 0.28.

Synthesis of Glycopeptide (12). Compound **11** (50 mg) was dissolved in DMSO (5 mL) and then the solution was diluted with water (20 mL) (final concentration of DMSO, 20%). The solution was shaken overnight at RT. After cyclization was complete (by HPLC analysis), the mixture was lyophilized and the resulting residue was purified by preparative HPLC, yielding **12** (43 mg, 85%) as a white powder. Analytical RP-HPLC, t_R = 33 min. (gradient, 15−30% aq. MeCN containing 0.1% TFA for 30 min; flow rate, 0.5 mL/min). ESI-MS: Calcd M = 3626.21, found: 726.61 $[M + 5H]^{5+}$, 908.04 $[M + 4H]^{4+}$, 1210.08 $[M + 3H]^{3+}$, 1789.79 $[M + 2H]^{2+}$. Deconvolution mass: 3626.63 ± 0.96.

Preparation of $Man_5GlcNAc$ and $Man_9GlcNAc$ Oxazolines (13 and 20). $Man_9GlcNAc_2Asn$ was isolated from soy bean flour, using the method previously reported.[42] For the synthesis of $Man_5GlcNAc$ oxazoline (**13**), a solution of $Man_9GlcNAc_2Asn$ (10 μmol) was dissolved in 50 mM citrate buffer (pH = 5.6) containing 5 mM calcium chloride. α1,2-mannosidase from *Bacteroides thetaiotaomicron* was added to a final concentration of 0.02 mg/mL, the enzymatic reaction was incubated at 37 °C for 8 h, while monitoring by HPAEC-PAD analysis. Once complete conversion was observed, the pH of the solution was adjusted to 7.0, and enzyme EndoA was added to convert $Man_5GlcNAc_2Asn$ to $Man_5GlcNAc$ via enzymatic cleavage of the chitobiose core. $Man_5GlcNAc$ was purified by gel filtration on a Sephadex G-15 (GE Healthcare) column. The carbohydrate positive fractions (assessed by phenol-sulfuric acid analysis) were pooled and lyophilized to obtain the free glycan $Man_5GlcNAc$ (9 mg, 87%). The identity and purity of the $Man_5GlcNAc$ was confirmed by MALDI-TOF and HPAEC-PAD (data not shown). The sugar oxazoline of $Man_5GlcNAc$ was prepared by treatment of a solution of $Man_5GlcNAc$ (9 mg, 87.3 μmol) in water (0.45 mL) with triethylamine (3.9 mmol) and 2-chloro-1,3-dimethylimidazolinium chloride (DMC) (1.3 mmol). The reaction was incubated on ice for 30 min, while monitored by HPAEC-PAD analysis. The glycan oxazoline product was purified by SEC (Sephadex G-10) eluting with 0.1% triethylamine. The carbohydrate containing fractions were pooled and lyophilized to give **13** (8 mg, 91%). The sugar oxazoline of $Man_9GlcNAc$ (**20**) was prepared from soy flour in a similar manner as **13**.

Enzymatic Transglycosylation: Synthesis of Glycopeptide 14 Bearing a $Man_5GlcNAc_2$ Moiety. A solution of the GlcNAc-containing peptide **12** (1 mg, 0.28 μmol) and $Man_5GlcNAc$-oxazoline (**13**) (2.3 mg, 2.24 μmol) in a Tris buffer (40 μL, 80 mM, pH 7.2) was incubated with EndoM-N175Q (final concentration, 0.2 μg/μL) at RT. The reaction was monitored by RP-HPLC. After 30 min, the reaction was quenched by addition of 0.1% aq. TFA. The transglycosylation product was purified by RP-HPLC to afford **14** (1.1 mg, 85%) as a white powder after lyophilization. Analytical RP-HPLC, t_R = 31 min. (gradient, 15−30% aq. MeCN containing 0.1% TFA for 30 min; flow rate = 0.5 mL/min). ESI-MS: Calcd M = 4639.56, found: 929.31 $[M + 5H]^{5+}$, 1161.43 $[M + 4H]^{4+}$, 1548.71 $[M + 3H]^{3+}$. Deconvolution mass: 4640.32 ± 0.84.

Deprotection of Glycopeptide 15. Compound **14** (0.95 mg, 0.21 μmol) was dissolved in 2.5% aqueous hydrazine (final concentration 2 mg/mL). The mixture was shaken at RT for 30 min. When the reaction was complete as indicated by RP-HPLC, the solution was neutralized by addition of glacial acetic acid. The product was purified by RP-HPLC yielding **15** (0.54 mg, 58%) as a white powder. Analytical RP-HPLC, t_R = 28 min. (gradient, 15−30% aq. MeCN containing 0.1% TFA for 30 min; flow rate = 0.5 mL/min).

DOI: 10.1021/acs.joc.6b01044
J. Org. Chem. 2016, 81, 6176−6185

ESI-MS: Calcd M = 4514.63, found: 904.11 $[M + 5H]^{5+}$, 1129.53 $[M+ 4H]^{4+}$, 1506.03 $[M + 3H]^{3+}$. Deconvolution mass: 4514.94.

Enzymatic Transglycosylation: Synthesis of Glycopeptide 17 Bearing a Man5GlcNAc2 at N160 and a Sialylated Glycan at N173 Sites. A solution of the GlcNAc-containing peptide (**15**) (1 mg, 0.22 μmol) and $(NeuGalGlcNAc)_2Man_3GlcNAc$-oxazoline (**16**) (2 mg, 0.9 μmol) in Tris buffer (40 μL, 80 mM, pH 7.2) was incubated with EndoM-N175Q (final concentration, 0.2 μg/μL) at RT. The reaction was monitored by RP-HPLC. After 30 min, the reaction was quenched by addition of 0.1% aq. TFA. The transglycosylation product was purified by RP-HPLC yielding **17** (1.32 mg, 92%) as a white powder. Analytical RP-HPLC, t_R = 22 min. (gradient, 15−30% aq. MeCN containing 0.1% TFA for 30 min; flow rate = 0.5 mL/min). ESI-MS: calcd M = 6517.30, found: 1304.66 $[M + 5H]^{5+}$, 1630.41 $[M + 4H]^{4+}$. Deconvolution mass: 6516.21 ± 1.45.

*Synthesis of Glycopeptide **19**.* The enzymatic transglycosylation of **15** with glycan oxazoline (**18**) under the catalysis of EndoM-N175Q was carried out in the same manner as for the preparation of **17**, giving **19** (95% yield based on HPLC estimation). Analytical RP-HPLC, t_R = 24 min. (gradient, 15−30% aq. MeCN containing 0.1% TFA for 30 min; flow rate = 0.5 mL/min). ESI-MS: Calcd M = 5933.01, found 996.55 $[M + 6H]^{6+}$, 1188.28 $[M + 5H]^{5+}$, 1484.88 $[M + 4H]^{4+}$, 1978.93 $[M + 3H]^{3+}$. Deconvolution mass: 5934.49 ± 1.67.

*Synthesis of Glycopeptide **21**.* The enzymatic transglycosylation of **15** with glycan oxazoline (**20**) under the catalysis of EndoM-N175Q was carried out in the same way as for the preparation of **17**, giving **21** in 95% yield (based on HPLC estimation). Analytical RP-HPLC, t_R = 25 min. (gradient, 15−30% aq. MeCN containing 0.1% TFA for 30 min; flow rate = 0.5 mL/min). ESI-MS: Calcd M = 6175.37, found: 1236.54 $[M + 4H]^{4+}$, 1735.28 $[M + 3H]^{3+}$. Deconvolution mass: 6175.76 ± 0.81.

*Synthesis of the Linear Glycopeptide (**S-1**).* Compound **12** (0.5 mg, 0.14 μmol) was incubated with 0.5 mM dithiothreitol (DTT) for 1 h at 37 °C. Iodoacetamide was added to a final concentration of 180 mM, and the reaction was incubated for 30 min at 37 °C. The reaction was monitored by HPLC. The reaction was purified by HPLC yielding **S-1** (0.47 mg, 90%) as a white powder. Analytical RP-HPLC, t_R = 30 min. (gradient, 15−30% aq. MeCN containing 0.1% TFA for 30 min; flow rate = 0.5 mL/min). ESI-MS: calcd M = 3740.71, found: 748.38 $[M + 5H]^{5+}$, 935.78 $[M + 4H]^{4+}$, 1246.93 $[M + 3H]^{3+}$, 1870. 61 $[M + 2H]^{2+}$. Deconvolution mass: 3740.82 ± 0.92.

Transglycosylation of Linear Glycopeptide S-1 by the EndoD Mutant. Compound **S-1** (0.5 mg, 0.13 μmol) and $Man_5GlcNAc$-oxazoline (**13**) (1.1 mg, 1.05 μmol) in Tris buffer (20 μL, 80 mM, pH 7.2) was incubated with EndoD-N322Q (final concentration, 40 ng/μL) at RT. The reaction was monitored by RP-HPLC. After 30 min, the reaction was quenched by addition of 0.1% aq. TFA. The transglycosylation product was purified by RP-HPLC yielding **S-2** (0.57 mg, 92%) as a white powder. Analytical RP-HPLC, t_R = 26 min. (gradient, 15−30% aq. MeCN containing 0.1% TFA for 30 min; flow rate = 0.5 mL/min). ESI-MS: Calcd M = 4736.06, found: 947.94 $[M + 5H]^{5+}$, 1184.88 $[M + 4H]^{4+}$, 1578.62 $[M + 3H]^{3+}$. Deconvolution mass: 4736.71 ± 0.65.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b01044.

^1H and ^{13}C NMR spectra of the synthetic building blocks, HPLC profiles of glycopeptides. (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: wang518@umd.edu. Phone: (301) 405-7527.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank members of the Wang lab for technical assistance and helpful discussions. The anti-HIV-1 gp120 monoclonal antibody (PG9) was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH. This work was supported by the National Institutes of Health (NIH Grants R01AI113896, R21AI101035, and R01GM080374 to LXW).

■ REFERENCES

(1) Varki, A. *Glycobiology* **1993**, *3*, 97.
(2) Helenius, A.; Aebi, M. *Science* **2001**, *291*, 2364.
(3) Jefferis, R. *Nat. Rev. Drug Discovery* **2009**, *8*, 226.
(4) Dalziel, M.; Crispin, M.; Scanlan, C. N.; Zitzmann, N.; Dwek, R. A. *Science* **2014**, *343*, 1235681.
(5) Reitter, J. N.; Means, R. E.; Desrosiers, R. C. *Nat. Med.* **1998**, *4*, 679.
(6) Wei, X.; Decker, J. M.; Wang, S.; Hui, H.; Kappes, J. C.; Wu, X.; Salazar-Gonzalez, J. F.; Salazar, M. G.; Kilby, J. M.; Saag, M. S.; Komarova, N. L.; Nowak, M. A.; Hahn, B. H.; Kwong, P. D.; Shaw, G. M. *Nature* **2003**, *422*, 307.
(7) Walker, L. M.; Phogat, S. K.; Chan-Hui, P. Y.; Wagner, D.; Phung, P.; Goss, J. L.; Wrin, T.; Simek, M. D.; Fling, S.; Mitcham, J. L.; Lehrman, J. K.; Priddy, F. H.; Olsen, O. A.; Frey, S. M.; Hammond, P. W.; Kaminsky, S.; Zamb, T.; Moyle, M.; Koff, W. C.; Poignard, P.; Burton, D. R. *Science* **2009**, *326*, 285.
(8) Walker, L. M.; Huber, M.; Doores, K. J.; Falkowska, E.; Pejchal, R.; Julien, J. P.; Wang, S. K.; Ramos, A.; Chan-Hui, P. Y.; Moyle, M.; Mitcham, J. L.; Hammond, P. W.; Olsen, O. A.; Phung, P.; Fling, S.; Wong, C. H.; Phogat, S.; Wrin, T.; Simek, M. D.; Principal Investigators, P. G.; Koff, W. C.; Wilson, I. A.; Burton, D. R.; Poignard, P. *Nature* **2011**, *477*, 466.
(9) Kwong, P. D.; Mascola, J. R.; Nabel, G. J. *Nat. Rev. Immunol.* **2013**, *13*, 693.
(10) Wang, L. X. *Curr. Opin. Chem. Biol.* **2013**, *17*, 997.
(11) Doores, K. J. *FEBS J.* **2015**, *282*, 4679.
(12) Doores, K. J.; Burton, D. R. *J. Virol.* **2010**, *84*, 10510.
(13) McLellan, J. S.; Pancera, M.; Carrico, C.; Gorman, J.; Julien, J. P.; Khayat, R.; Louder, R.; Pejchal, R.; Sastry, M.; Dai, K.; O'Dell, S.; Patel, N.; Shahzad-ul-Hussan, S.; Yang, Y.; Zhang, B.; Zhou, T.; Zhu, J.; Boyington, J. C.; Chuang, G. Y.; Diwanji, D.; Georgiev, I.; Kwon, Y. D.; Lee, D.; Louder, M. K.; Moquin, S.; Schmidt, S. D.; Yang, Z. Y.; Bonsignori, M.; Crump, J. A.; Kapiga, S. H.; Sam, N. E.; Haynes, B. F.; Burton, D. R.; Koff, W. C.; Walker, L. M.; Phogat, S.; Wyatt, R.; Orwenyo, J.; Wang, L. X.; Arthos, J.; Bewley, C. A.; Mascola, J. R.; Nabel, G. J.; Schief, W. R.; Ward, A. B.; Wilson, I. A.; Kwong, P. D. *Nature* **2011**, *480*, 336.
(14) Pejchal, R.; Doores, K. J.; Walker, L. M.; Khayat, R.; Huang, P. S.; Wang, S. K.; Stanfield, R. L.; Julien, J. P.; Ramos, A.; Crispin, M.; Depetris, R.; Katpally, U.; Marozsan, A.; Cupo, A.; Maloveste, S.; Liu, Y.; McBride, R.; Ito, Y.; Sanders, R. W.; Ogohara, C.; Paulson, J. C.; Feizi, T.; Scanlan, C. N.; Wong, C. H.; Moore, J. P.; Olson, W. C.; Ward, A. B.; Poignard, P.; Schief, W. R.; Burton, D. R.; Wilson, I. A. *Science* **2011**, *334*, 1097.
(15) Mouquet, H.; Scharf, L.; Euler, Z.; Liu, Y.; Eden, C.; Scheid, J. F.; Halper-Stromberg, A.; Gnanapragasam, P. N.; Spencer, D. I.; Seaman, M. S.; Schuitemaker, H.; Feizi, T.; Nussenzweig, M. C.; Bjorkman, P. J. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, E3268.
(16) Garces, F.; Sok, D.; Kong, L.; McBride, R.; Kim, H. J.; Saye-Francisco, K. F.; Julien, J. P.; Hua, Y.; Cupo, A.; Moore, J. P.; Paulson, J. C.; Ward, A. B.; Burton, D. R.; Wilson, I. A. *Cell* **2014**, *159*, 69.
(17) Amin, M. N.; McLellan, J. S.; Huang, W.; Orwenyo, J.; Burton, D. R.; Koff, W. C.; Kwong, P. D.; Wang, L. X. *Nat. Chem. Biol.* **2013**, *9*, 521.
(18) Aussedat, B.; Vohra, Y.; Park, P. K.; Fernandez-Tejada, A.; Alam, S. M.; Dennison, S. M.; Jaeger, F. H.; Anasti, K.; Stewart, S.; Blinn, J. H.; Liao, H. X.; Sodroski, J. G.; Haynes, B. F.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2013**, *135*, 13113.

DOI: 10.1021/acs.joc.6b01044
J. Org. Chem. 2016, 81, 6176−6185

(19) Alam, S. M.; Dennison, S. M.; Aussedat, B.; Vohra, Y.; Park, P. K.; Fernandez-Tejada, A.; Stewart, S.; Jaeger, F. H.; Anasti, K.; Blinn, J. H.; Kepler, T. B.; Bonsignori, M.; Liao, H. X.; Sodroski, J. G.; Danishefsky, S. J.; Haynes, B. F. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 18214.
(20) Unverzagt, C.; Kajihara, Y. *Chem. Soc. Rev.* **2013**, *42*, 4408.
(21) Wang, P.; Dong, S.; Shieh, J. H.; Peguero, E.; Hendrickson, R.; Moore, M. A.; Danishefsky, S. J. *Science* **2013**, *342*, 1357.
(22) Wang, L. X.; Amin, M. N. *Chem. Biol.* **2014**, *21*, 51.
(23) Li, B.; Zeng, Y.; Hauser, S.; Song, H.; Wang, L. X. *J. Am. Chem. Soc.* **2005**, *127*, 9692.
(24) Li, H.; Li, B.; Song, H.; Breydo, L.; Baskakov, I. V.; Wang, L. X. *J. Org. Chem.* **2005**, *70*, 9990.
(25) Ochiai, H.; Huang, W.; Wang, L. X. *J. Am. Chem. Soc.* **2008**, *130*, 13790.
(26) Huang, W.; Li, C.; Li, B.; Umekawa, M.; Yamamoto, K.; Zhang, X.; Wang, L. X. *J. Am. Chem. Soc.* **2009**, *131*, 2214.
(27) Amin, M. N.; Huang, W.; Mizanur, R. M.; Wang, L. X. *J. Am. Chem. Soc.* **2011**, *133*, 14404.
(28) Huang, W.; Zhang, X.; Ju, T.; Cummings, R. D.; Wang, L. X. *Org. Biomol. Chem.* **2010**, *8*, 5224.
(29) Huang, W.; Giddens, J.; Fan, S. Q.; Toonstra, C.; Wang, L. X. *J. Am. Chem. Soc.* **2012**, *134*, 12308.
(30) Giddens, J. P.; Lomino, J. V.; Amin, M. N.; Wang, L. X. *J. Biol. Chem.* **2016**, *291*, 9356.
(31) Tropper, F. D.; Andersson, F. O.; Braun, S.; Roy, R. *Synthesis* **1992**, *7*, 618.
(32) Hackenberger, C. P.; Friel, C. T.; Radford, S. E.; Imperiali, B. *J. Am. Chem. Soc.* **2005**, *127*, 12882.
(33) Holm, B.; Linse, S.; Kihlberg, J. *Tetrahedron* **1998**, *54*, 11995.
(34) Fan, S. Q.; Huang, W.; Wang, L. X. *J. Biol. Chem.* **2012**, *287*, 11272.
(35) Umekawa, M.; Huang, W.; Li, B.; Fujita, K.; Ashida, H.; Wang, L. X.; Yamamoto, K. *J. Biol. Chem.* **2008**, *283*, 4469.
(36) Umekawa, M.; Li, C.; Higashiyama, T.; Huang, W.; Ashida, H.; Yamamoto, K.; Wang, L. X. *J. Biol. Chem.* **2010**, *285*, 511.
(37) Noguchi, M.; Tanaka, T.; Gyakushi, H.; Kobayashi, A.; Shoda, S. I. *J. Org. Chem.* **2009**, *74*, 2210.
(38) Huang, W.; Yang, Q.; Umekawa, M.; Yamamoto, K.; Wang, L. X. *ChemBioChem* **2010**, *11*, 1350.
(39) Pancera, M.; Shahzad-Ul-Hussan, S.; Doria-Rose, N. A.; McLellan, J. S.; Bailer, R. T.; Dai, K.; Loesgen, S.; Louder, M. K.; Staupe, R. P.; Yang, Y.; Zhang, B.; Parks, R.; Eudailey, J.; Lloyd, K. E.; Blinn, J.; Alam, S. M.; Haynes, B. F.; Amin, M. N.; Wang, L. X.; Burton, D. R.; Koff, W. C.; Nabel, G. J.; Mascola, J. R.; Bewley, C. A.; Kwong, P. D. *Nat. Struct. Mol. Biol.* **2013**, *20*, 804.
(40) Hong, S. Y.; Tobias, G.; Ballesteros, B.; El Oualid, F.; Errey, J. C.; Doores, K. J.; Kirkland, A. I.; Nellist, P. D.; Green, M. L.; Davis, B. G. *J. Am. Chem. Soc.* **2007**, *129*, 10966.
(41) Mizuno, M.; Muramoto, I.; Kobayashi, K.; Yaginuma, H.; Inazu, T. *Synthesis* **1999**, *1999*, 162.
(42) Wang, L. X.; Ni, J.; Singh, S.; Li, H. *Chem. Biol.* **2004**, *11*, 127.

DOI: 10.1021/acs.joc.6b01044
J. Org. Chem. 2016, 81, 6176−6185