Novel template-assembled oligosaccharide clusters as epitope mimics for HIV-neutralizing antibody 2G12. Design, synthesis, and antibody binding study†

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The synthesis of a new class of template-assembled oligomannose clusters as the mimics of the epitope of the HIV-neutralizing antibody 2G12 is described. The novel oligomannose clusters were successfully assembled on a cyclic decapeptide template using the Cu(I)-catalyzed 1,3-dipolar cycloaddition of azides to alkynes by introducing four units of a synthetic D1 arm tetrasaccharide (Manα1,2Manα1,2Manα1,3Manα-) of high-mannose *N*-glycan on one face of the template and two T-helper epitope peptides on the other face of the template. Their binding to human antibody 2G12 was studied using surface plasmon resonance (SPR) technology. It was found that while the synthetic monomeric D1 arm oligosaccharide and its fluorinated derivative interacted with 2G12 only weakly, the corresponding template-assembled oligosaccharide clusters showed high affinity to antibody 2G12, indicating a clear clustering effect in 2G12 recognition. Interestingly, the fluorinated D1 arm cluster, in which the 6-OH of the terminal mannosyl residue was replaced with a fluorine atom, showed a distinct kinetic model in 2G12 binding as compared with the cluster of the natural D1 arm oligosaccharides. The oligosaccharide clusters with varied length of spacer demonstrated different affinity to 2G12, suggesting that an appropriate spatial orientation of the sugar chains in the cluster was crucial for high affinity binding to the antibody 2G12. It was also found that the introduction of two T-helper epitopes onto the template did not affect the structural integrity of the oligomannose cluster. The novel synthetic glycoconjugates represent a new type of immunogen that may be able to raise carbohydrate-specific neutralizing antibodies against HIV-1.

Introduction

AIDS is caused by the infection of the human immunodeficiency virus (HIV).[1] It is considered that the best hope to control the worldwide epidemic of HIV/AIDS is an effective preventive HIV-1 vaccine. However, development of an effective HIV-1 vaccine has faced many difficulties.[2–4] Under the pressure of host immune surveillance, HIV-1 has evolved a number of defense mechanisms to evade immune attacks, including frequent mutation of neutralizing epitopes; conformational masking of receptor binding sites; heavy glycosylations to form an evolving glycan shield; and formation of envelope glycoprotein complexes to occlude conserved epitopes.[5–8] Therefore, a major challenge in HIV vaccine design is to identify unique neutralizing epitopes on the HIV-1 envelope capable of eliciting broadly neutralizing antibodies to break down the strong viral defense. The existence of a few human monoclonal antibodies such as 2F5, 4E10, b12, and 2G12 that are able to neutralize a broad range of HIV-1 primary isolates suggests the presence of unique neutralizing epitopes on the viral envelope that can be explored for immunogen design.[9] Among them, the human antibody 2G12 was revealed to target a novel oligomannose cluster present on HIV-1 gp120.[10]

Initial biochemical and mutational studies have demonstrated that the epitope of antibody 2G12 involves a novel oligomannose cluster consisting of several high-mannose type *N*-glycans with terminal Manα1,2Man residues.[10–12] Subsequent 2G12-binding studies with defined oligosaccharides and synthetic clusters have indicated that the terminal Manα1,2Man unit was essential for 2G12 recognition but a Manα1,2Man unit alone was not sufficient for an effective binding to 2G12.[13–17] The full-size Man9 was shown to have the highest affinity to 2G12 among several natural high-mannose oligosaccharides.[14] Interestingly, the synthetic mannose tetrasaccharide (Man4) corresponding to the D1 arm of Man9 was revealed to have comparable affinity to the antibody as that of the Man9 moiety.[13] The structural requirement of the oligosaccharide subunit for 2G12 binding was confirmed by recent X-ray structural studies on the 2G12-oligomannose complexes.[18,19] Since 2G12 recognizes an oligomannose cluster with at least two sugar chains, we have initiated a project aiming to create template-assembled oligomannose clusters to mimic the 2G12 epitope found on gp120.[14,15] For example, we have demonstrated that the galactose-based tetravalent Man9-cluster (Tetra-Man9) was 73-fold and 5000-fold more effective in binding to 2G12 than the monomeric $Man_9GlcNAc$ and $Man_6GlcNAc$, respectively.[14]

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† Electronic supplementary information (ESI) available: Experimental details for the synthesis of the fluorinated glycosyl donor **6**, the glycosyl acceptor **11**, and the succinimidyl 8-azido-3,6-dioxaoctanoate **17**, and the ESI-MS and HPLC profiles of the synthetic glycopeptides **21**, **22**, **23**, **24**, **25** and **27**. See DOI: 10.1039/b702961f

These binding data suggest that synthetic oligomannose clusters such as the Tetra-Man9 provides a mimic to the 2G12 epitope. We have recently conjugated the oligomannose cluster Tetra-Man9 to a carrier protein keyhole limpet hemocyanin (KLH), and our immunization studies with the KLH conjugate have demonstrated that the glycoconjugate could induce moderate carbohydrate-specific antibodies in rabbits, which were weakly cross-reactive to HIV-1 gp120.[20] However, it was observed that the majority of antibody responses were directed to the linkers and the template, which drastically dilutes the immune response to the oligomannose cluster. Indeed, the undesired immune responses to the linkers and/or template is of a particular concern when dealing with weakly immunogenic carbohydrate antigens such as those found on HIV and human cancers.[21] As a continuous effort in developing a carbohydrate-based HIV-1 vaccine,[22] we report in this paper the synthesis of a new class of template-assembled oligomannose clusters using a novel cyclic peptide as the template that are expected to have low immunogenicity and the D1 arm tetramannose (Man4) oligosaccharide as the 2G12-recognizing subunits. The oligomannose clusters were efficiently assembled using the Cu(I)-catalyzed 1,3-dipolar cycloaddition between azides and alkynes. The binding of the synthetic clusters and human antibody 2G12 were studied using surface plasmon resonance (SPR) technology.

Results and discussion

Design

Our previous work on the 2G12 binding of synthetic oligomannose clusters has demonstrated that an appropriate template-assembled oligomannose cluster could exhibit much higher affinity to 2G12 than the monomeric oligomannose, showing a clustering effect in the antibody–antigen interactions.[14,15] In addition, our preliminary immunization studies indicated that it was possible to induce carbohydrate-specific antibodies using glycoconjugates containing an oligomannose cluster, but the nature of the linker and template was crucial in determining the immune responses because the maleimide linkers and/or the template in the first generation conjugate vaccines were found to induce predominantly linker/template-specific antibodies, which significantly suppresses the antibody responses to the oligomannose epitope.[20] The immunization data suggest that the choice of appropriate linkers and template is of paramount importance in dealing with weakly immunogenic epitopes such as the self-like high-mannose type oligosaccharides. As a continuous effort in designing a better immunogen, we have chosen the regioselectively addressable functionalized template (RAFT) as the template for oligosaccharide cluster assembling and the D1 arm tetramannose (Man4) oligosaccharide (Manα1,2Manα1,2Manα1,3Manα-) of the high-mannose type *N*-glycan (Fig. 1A) as the 2G12 recognizing subunits. RAFT is a novel cyclic decapeptide containing two proline-glycine moieties as beta-turn inducers that stabilize the template conformations and up to 6 selectively protected lysine residues.[23–26] Thus, the cyclic decapeptide provides two functional faces, one for the attachment of oligosaccharide moieties and the other for the attachment of T-helper peptide epitopes. The conjugation of the oligosaccharide cluster to a T-helper epitope or a carrier protein is important to create an effective immunogen capable of raising long-lasting IgG type antibodies.[27–33] Recent immunization studies have demonstrated that the cyclic peptide template could serve as a non-immunogenic scaffold for assembling multiepitopic anti-cancer vaccines bearing clustered tumor-associated carbohydrate antigens.[33] Based on these considerations, we have designed a novel template-assembled oligomannose cluster immunogen, in which four α-linked Man4 oligosaccharides corresponding to the D1 arm of $Man_9GlcNAc_2$ were introduced at one face of the cyclic peptide template to form a novel cluster, and two universal T-helper epitopes corresponding to the sequence (aa830–844) of tetanus toxoid[34] were introduced at the other face of the cyclic template (Fig. 1B).

The D1 arm tetrasaccharide was chosen because its binding affinity to 2G12 was shown to be comparable to the largest oligomannose $Man_9GlcNAc_2$.[13] X-Ray structural studies have also confirmed that the D1 arm residues account for more than 85% of the 2G12 Fab contacts to $Man_9GlcNAc_2$.[18,19] Moreover, considering the weak immunogenicity of the high-mannose type *N*-glycans that may be regarded as "self" antigens by the immune system, we also decided to selectively modify the terminal mannose residue of the D1 arm Man4 at the 6-position with a fluorine atom, which may still behave as a hydrogen-bonding acceptor but the unnatural modification may enhance the immunogenicity of the oligomannose cluster. To assemble the designed immunogen, we decided to attach the multiple functionality to the

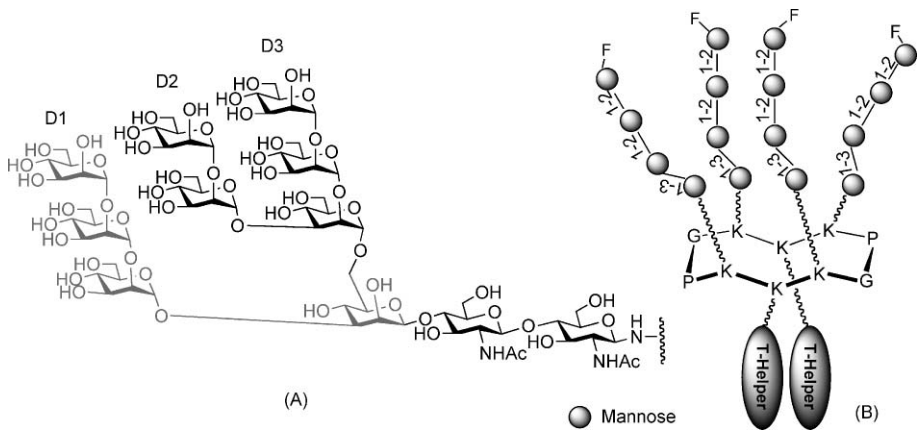
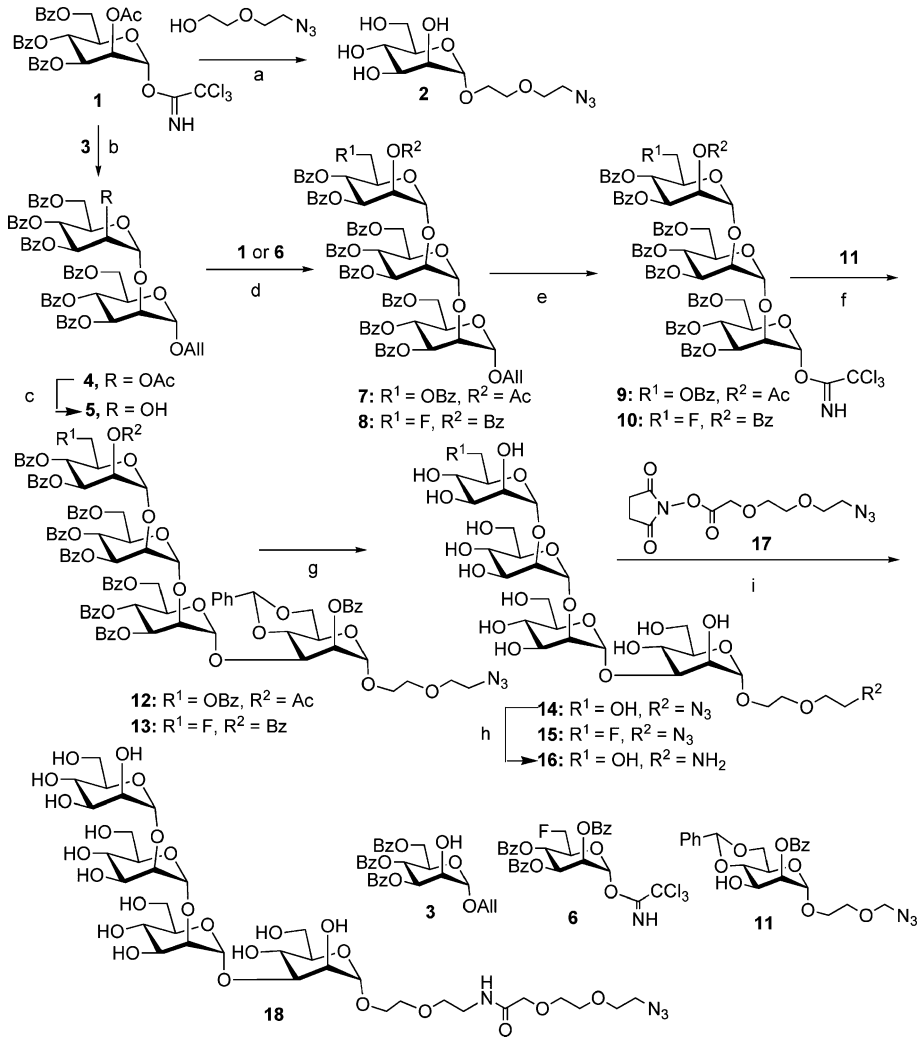


Fig. 1 Structures of $Man_9GlcNAc_2$ (A) and the designed template-assembled glycopeptide vaccine (B).

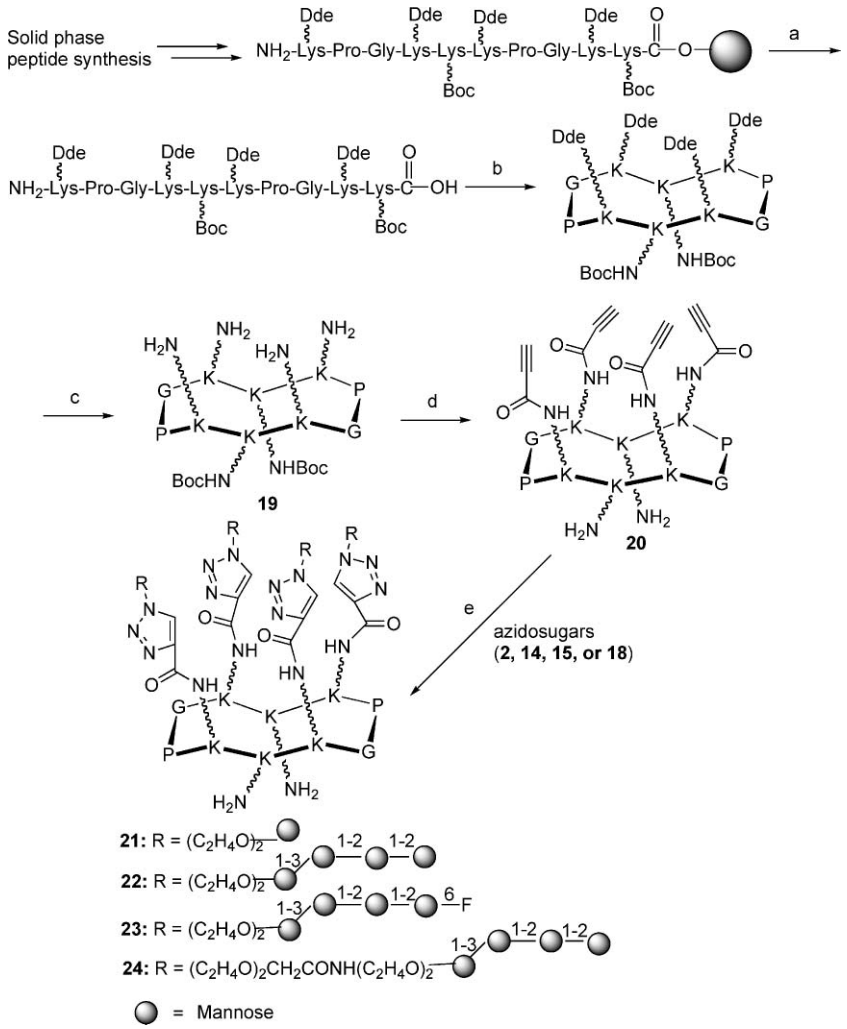
cyclic template by the highly efficient copper(I)-catalyzed 1,3-dipolar azide–alkyne cycloaddition.[35–39] This will introduce the 1,2,3-triazole linkers in the glycoconjugate. Compared to the hydrophobic aminohexyl and maleimide linkers, the hydrophilic 1,2,3-triazole linkers are expected to be less immunogenic. The successful synthesis of such triazole-linked glycoconjugates will allow the evaluation of whether the triazole-linker is truly an immune-compatible linker for conjugate vaccines when applied for immunization studies.

Synthesis

The synthesis of the D1 arm oligosaccharide and its derivatives is summarized in Scheme 1. TMSOTf-catalyzed glycosidation between the mannosyl trichloroacetimidate **1** and acceptor **3** gave the disaccharide **4**. The 2′-*O*-acetyl group in **4** was selectively removed by mild acidic hydrolysis to give the intermediate **5**. Glycosidation of **5** with the mannosyl trichloroacetimidate **1** or the 6-deoxy-6-fluoro-mannosyl trichloroacetimidate **6**, which was prepared in several steps (see ESI†), gave the trisaccharide derivatives **7** and **8**, respectively. After conversion to the corresponding trisaccharide trichloroacetimidates **9** and **10**, these glycosyl donors were coupled with the glycosyl acceptor **11** to afford the tetrasaccharide derivatives **12** and **13**, respectively, with the desired α-glycosidic linkages. Treatment of compounds **12** and **13** with 80% aqueous acetic acid at elevated temperature to remove the benzylidene group, followed by de-*O*-acylation with MeONa–MeOH gave the D1 arm tetrasaccharide **14** and the fluorinated D1 arm tetrasaccharide **15**, respectively. The compounds were purified and further characterized as their per-*O*-acetylated forms. The tetrasaccharide derivatives contain an azido functionality at the aglycon portion that will be used for conjugation to the cyclic peptide template through click chemistry. To prepare a longer spacer at the aglycon for comparative studies, the azido group in compound **14** was reduced to an amino group by hydrogenation to give **16**, which was then coupled with the azido-containing derivative **17** to provide another tetrasaccharide derivative **18** (Scheme 1).



Scheme 1 *Reagents and conditions:* a) 1) TMSOTf, CH_2Cl_2, 82%; 2) MeONa, MeOH, 95%; b) TMSOTf, CH_2Cl_2, 83%; c) CH_3COCl–MeOH, 72%; d) TMSOTf, CH_2Cl_2, 82% for **7**, 78% for **8**; e) 1) $PdCl_2$, MeOH, 90%; 2) CCl_3CN, DBU, 85% for **9**, 88% and 81% for **10**; f) TMSOTf, CH_2Cl_2, 77% for **12**, 73% for **13**; g) 1) 80% AcOH, 60 °C; 2) $NaOCH_3$–CH_3OH; 3) Ac_2O–Py; 4) $NaOCH_3$–CH_3OH, 76% for **14** over 4 steps, 75% for **15** over 4 steps; h) H_2, Pd/C, 98%; i) 0.05M $NaHCO_3$, CH_3CN–H_2O 1 : 1, 74%.



Scheme 2 *Reagents and conditions:* a) 50% AcOH, DCM, r.t., 95%; b) HBTU, DIPEA, 88%; c) 2% hydrazine, DMF, 84%; d) 1) propynoic acid, DCC, 77%; 2) 90% TFA, 93%; e) $CuSO_4$ (0.2 mol. equiv.), sodium ascorbate (0.4 mol. equiv.), *t*-BuOH–H_2O (1 : 1), over 90% for **21**, **22**, **23**, and **24**.

To synthesize the cyclic decapeptide template **19**, a distinctly protected linear decapeptide, in which two Lys residues were protected with Boc groups and the remaining four Lys residues were protected with Dde protecting groups, was prepared by automated peptide synthesizer on an acidic sensitive resin, NovaSyn TGT. After being released from the resin by mild acidic hydrolysis, the fully protected linear peptide was cyclized by treatment with HBTU–DIPEA in DMF to give the corresponding cyclic peptide in excellent yield. Then the Dde protecting groups were selectively removed by treatment with 2% hydrazine[40] in DMF to give the cyclic peptide **19** with four free amino groups on one face of the template. Introduction of alkynyl groups to the template was achieved by coupling four propynoic acid moieties to compound **19** using DCC as the coupling reagent to give the corresponding conjugate in 77% yield, from which the two Boc groups were selectively removed by treatment with TFA to afford the alkynyl derivative **20** in 93% yield (Scheme 2).

After successful preparation of the alkynyl cyclic decapeptide template, the copper(I)-catalyzed dipolar cycloaddition was first examined using the monosaccharide azide derivative **2** in the presence of $CuSO_4$ and sodium ascorbate in a mixed solvent (*t*-BuOH–H_2O, 1 : 1), following the reported procedures.[41,42] The reaction was monitored by HPLC analysis. It was found that the reaction between **2** and **20** at rt proceeded very slowly, resulting in a mixture of mono-, di-, tri- and tetra-substituted products as revealed by ESI-MS analysis of the HPLC peaks (data not shown). Only a fraction of starting materials was consumed even after 2 days. However, it was observed that the cycloaddition proceeded smoothly and quickly when the reaction mixture was heated and stirred at 60 °C. As revealed by HPLC, all of the alkynyl template was consumed in 30 min at 60 °C to give a mixture of di- (peak 2), tri- (peak 3) and tetra-substituted (peak 4) products (Fig. 2). After 6 h, the reaction went to completion to give the single final product (peak 4), which was characterized by MS and NMR as the desired tetra-substituted compound **21**. ^{1}H NMR of **21** revealed only a singlet signal at 8.5 ppm for the proton of the resulting 1,2,3-triazole, suggesting the sole formation of the 1,4-substituted 1,2,3-triazole, rather than a mixture with the 1,5-substituted isomer (which would appear at *ca.* 8.3 ppm as a singlet). The results confirm that the Cu(I)-catalyzed 1,3-dipolar cycloaddition results in a regioselective formation of the 1,4-substituted derivative even at an elevated temperature.[41,43,44] Similarly, the Cu(I)-catalyzed

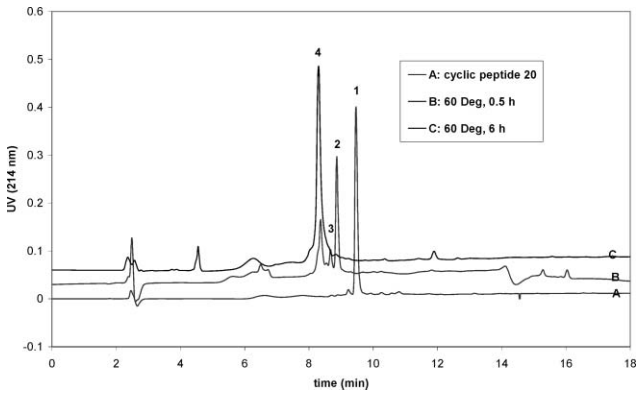


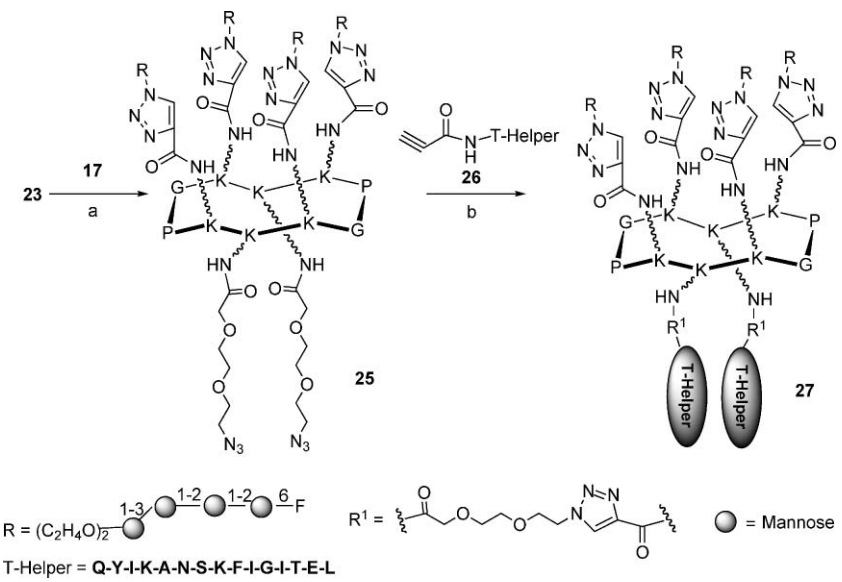
Fig. 2 Monitoring the reaction of 1,3-dipolar cycloaddition between azido sugar **2** and the alkynyl template **20**. Peak 1, cyclic peptide **20**; peaks 2 and 3, reaction intermediates; and peak 4, the final adduct **21**.

cycloaddition of the tetrasaccharide derivatives **14**, **15**, and **18** with the tetra-alkynyl template **20** proceeded very efficiently at an elevated temperature to give the corresponding oligosaccharide clusters **22–24** in more than 90% isolated yields. The results indicate that the Cu(I)-catalyzed dipolar cycloaddition of azides to alkynes provides a neat and highly efficient means for constructing even large oligosaccharide clusters.

Finally, to construct an effective immunogen capable of eliciting T-cell dependent immune responses such as IgG type antibodies, two T-helper peptides derived from tetanus toxoid (aa830–844)[34] were successfully introduced into the fluorinated oligosaccharide cluster **23**. Thus, the oligosaccharide cluster **23** that contains two free amino groups at the down face was reacted with the activated ester **17** to give the azido-containing oligosaccharide cluster **25**. The azido groups in **25** were then reacted with the alkynylated T-helper peptide **26**, again through Cu(I)-catalyzed cycloaddition, affording the fully synthetic vaccine candidate **27** in 70% isolated yield (Scheme 3).

Antibody binding studies

With the synthetic oligosaccharide clusters in hand, their binding to the human antibody 2G12 was analyzed utilizing surface plasmon resonance (SPR) technology. Binding experiments were carried out with a Biacore 3000 system in a HBS-P buffer. The immobilization of antibody 2G12 to the CM5 sensor chip was performed based on a previously reported procedure.[17] Briefly, the chip surface was activated by injection of EDC–NHS for 7 min at 5 μl min^{-1}, followed by injection of 20 μg ml^{-1} 2G12 solution in an acetate buffer (10 mM pH 5.5) until the target level of 10 000 response units (RU) was reached, and then the surface was saturated by 7 min pulse of ethanol amine HCl (1 M, pH 8.5) at 5 μl min^{-1}. A reference surface was prepared by a similar procedure, but without the injection of antibody solution. Each compound was injected to reference and the antibody-activated surface channels, and the binding profile was obtained by an automatic subtraction of the reference surface signal from the 2G12-activated surface signal. The sensor surface between runs was regenerated with a short pulse of 3.5 M $MgCl_2$. It was found that, at 10 μM concentrations, all the synthetic monomeric D1 arm oligosaccharides **14**, **15**, and **18**, as well as the natural high-mannose type *N*-glycan $Man_9GlcNAc_2Asn$, did not show apparent binding to antibody 2G12, with the signal responses being around the detection threshold (below 5 RU). The results are in agreement with the previous observation by Danishefsky and co-workers that synthetic monomeric glycopeptides carrying a single high-mannose type *N*-glycan did not bind to 2G12 at 10 μM as measured by SPR technology.[17] The template-assembled mannose cluster **21** did not show detectable 2G12 binding at 10 μM, either (data not shown). The results indicate that neither single oligomannose nor the cluster of terminal α-mannosides is sufficient for 2G12 binding. However, it was found that the synthetic oligosaccharide clusters carrying four units of the D1 arm tetrasaccharide and its fluorinated derivative, namely clusters **22**, **23**, and **24**, have demonstrated apparent affinity to antibody



Scheme 3 *Reagents and conditions:* a) 0.05 M $NaHCO_3$, CH_3CN–MeOH 1 : 1, 90%; b) $CuSO_4$ (0.2 mol. equiv.), sodium ascorbate (0.4 mol. equiv.), *t*-BuOH–H_2O (1 : 1), 70%.

2G12. Under the measuring conditions (10 μM, at 30 sec), for example, the binding response units (RU) for the oligosaccharide clusters **22**, **23**, and **24** are 105, 43, 142 RU, respectively. The fluorinated derivative **23** also exhibits significant affinity to 2G12, despite at a reduced efficiency in comparison with the natural D1 arm cluster **22**. It was observed that the cluster **24** with an extended spacer showed higher affinity to 2G12 than the cluster **22** that has a relatively short spacer. The data suggest that an appropriate spatial orientation of the sugar chains in the cluster is crucial for high affinity binding to the antibody 2G12.

In order to probe the binding nature of synthetic oligosaccharide clusters, we performed kinetic studies with compounds **22**, **23**, and **24**. The experiments were carried out at six different concentrations at 10.0, 5.00, 2.50, 1.25, 0.625, and 0.312 μM (Fig. 3). The observed binding profiles for compounds **22** and **24** are similar, but both of them showed rather complex kinetics that do not fit into a simple 1 : 1 Langmuir model.[45] The observed profile implies multiple association and dissociation (fast and slow) steps that may involve a required conformational adjustment for the binding of subsequent oligosaccharide chains in the cluster.[46] These experimental data are similar to the kinetic studies for the 2G12-binding of a bivalent $Man_9GlcNAc_2$-containing glycopeptide reported by Danishefsky and co-workers.[17] Interestingly, it was found that the binding profile of the fluorinated oligomannose cluster **23** was different from the non-fluorinated clusters, and could fit perfectly to the 1 : 1 Langmuir model. Kinetic studies gave $R_{max} = 47.2$ RU, Ka = 3.79 e^5, Kd = 2.64 e^{-6}, whereas Chi2 = 0.49. The data suggested the existence of a typical first order kinetic process in the antigen–antibody binding. Considering the fact that the only structural difference between the clusters **22** and **23** is the replacement of the 6-OH group in the terminal mannosyl residue in **22** by a fluorine atom in **23**, the different binding kinetic outcome between **22** and **23** may implicate a different 2G12-binding model for the two compounds. Since fluorine and hydroxyl groups have different capabilities in hydrogen bonding, it is likely that the interaction of the fluorinated compound **23** with antibody 2G12 does not induce significant conformational changes in the antibody, thus resulting in an apparent first order binding, whereas the interaction between the cluster **22** and antibody 2G12 may cause conformational changes in the antibody and shows a complex kinetic process. An alternative explanation for this difference may be the existence of many preexisting antibody conformations (conformational diversity).[46] Thus, different antigens may interact preferably with different conformational isomers with or without induced fit isomerization that would lead to high-affinity interactions.

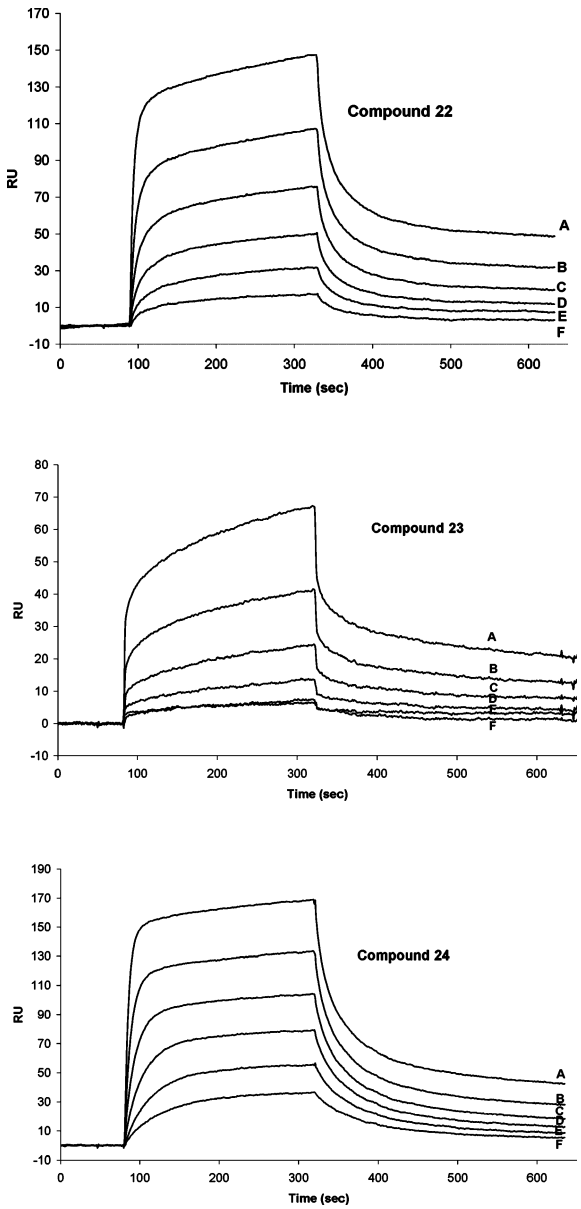


Fig. 3 SPR kinetic studies on the 2G12-binding to compounds **22–24** at 10.0 (A), 5.00 (B), 2.50 (C), 1.25 (D), 0.625 (E), 0.312 (F) μM concentrations, respectively.

Finally, the 2G12-binding of the fully synthetic vaccine candidate **27** containing two T-helper peptides was examined. It was found that compound **27** demonstrated almost identical binding profiles and kinetic outcome to those of the corresponding fluorinated oligosaccharide cluster **23** (data not shown). This result suggested that the introduction of the T-helper epitopes onto the cyclic decapeptide template did not affect the structural integrity of the fluorinated oligosaccharide cluster formed at the other face of the template. Therefore, the novel synthetic glycoconjugate represents a valuable immunogen that may be able to raise carbohydrate-specific neutralizing antibodies against HIV-1.

Conclusion

The synthesis of a new class of template-assembled oligomannose clusters as the mimics of the epitope of human antibody 2G12 was described. The experimental data indicate that the Cu(I)-catalyzed 1,3-dipolar cycloaddition of azides and alkynes is highly efficient for constructing conjugate vaccines containing large oligosaccharide clusters and free T-helper epitopes. The binding studies with the synthetic oligosaccharide clusters have revealed an interesting, distinct kinetic outcome for the different synthetic antigens. The data implicate a distinct binding mechanism for the fluorinated and natural D1 arm clusters that may involve antibody conformational changes during the antigen–antibody interactions. Oligosaccharide cluster **24**, with an extended spacer, showed higher affinity to 2G12 than the cluster **22**, that has a relatively short spacer; these results suggest that an appropriate spatial

orientation of the sugar chains in the cluster is crucial for high affinity binding to antibody 2G12. The successful construction of the fully synthetic immunogens that contain both the novel oligosaccharide clusters and T-helper epitopes has now laid the foundation for further immunization studies in animals to evaluate whether the vaccine candidates are able to elicit carbohydrate-specific neutralizing antibodies against HIV-1.

Experimental

General procedures

TLC was performed on aluminium plates coated with silica gel 60 with detection by charring with 10% (v/v) sulfuric acid in methanol or by UV detection. Flash column chromatography was performed on silica gel 60 (230–400 mesh). ^{1}H and ^{13}C NMR, and 2D NMR spectra were recorded on a 500 NMR spectrometer in $CDCl_3$, D_2O, or CD_3OD, as specified. Chemical shifts are expressed in ppm. The ESI-MS spectra were measured on a single quadruple mass spectrometer. Analytical RP-HPLC was carried out on a C18 column (3.9 × 150 mm) at 40 °C. The column was eluted with a linear gradient of 0–90% MeCN containing 0.1% TFA at a flow rate of 1 mL min^{-1} over 25 minutes, unless otherwise specified. Peptides and glycopeptides were detected at double wavelengths (214 and 280 nm). Preparative RP-HPLC was performed on a preparative C18 column (19 × 300 mm). The column was eluted with a suitable gradient of MeCN containing 0.1% TFA at 12 mL min^{-1}.

2-(2-Azidoethoxy)ethyl-α-D-mannopyranoside (2). 2-*O*-Acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate **1**[47] (200 mg, 0.295 mmol) and 2-(2-azidoethoxy)-ethanol (100 mg, 0.76 mmol) were dried together under high vacuum for 2 h. The mixture was dissolved in anhydrous CH_2Cl_2 (20 mL). TMSOTf (10 µl, 0.05 mmol) was added and the mixture was stirred under an argon atmosphere at rt overnight. The mixture was neutralized with triethylamine and concentrated under vacuum. The residue was purified by flash silica gel column chromatography (hexanes–EtOAc 1 : 1) to give a white solid (167 mg). The solid was dissolved in MeOH (10 mL) and a solution of MeONa in MeOH (0.5 M, 0.3 mL) was added. The mixture was stirred at rt for 1 h, then neutralized by adding Dowex 50W (H^+). After filtration, the filtrate was concentrated to give compound **2** (68 mg, 78% over two steps) as a white solid. δ_H (500 MHz, CD_3OD): 4.84 (d, 1 H, J = 1.5 Hz, H-1), 3.91–3.59 (m, 12 H), 3.42 (t, 2 H, J = 5.0 Hz, CH_2N_3); δ_C (CD_3OD, 125 MHz): δ = 100.4, 73.2, 71.2, 70.7, 69.9, 69.8, 67.2, 66.5, 61.5, 50.4.

Allyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranoside (4). To a solution of allyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranoside **3**[48] (532 mg, 1.0 mmol) and 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate **1** (810 mg, 1.20 mmol) in anhydrous CH_2Cl_2 (10 mL) was added TMSOTf (10 µl, 0.05 mmol) under an argon atmosphere. The mixture was stirred at rt for 10 h and neutralized with triethylamine. The mixture was then concentrated under vacuum and the residue was purified by flash silica gel column chromatography (hexanes–EtOAc 5 : 1) to give **4** (870 mg, 83%) as a white foam. δ_H (500 MHz, $CDCl_3$): 8.11–7.33 (m, 30 H, Ph), 6.03 (t, 1 H, J = 9.9 Hz, H-4′), 5.97–5.88 (m, 4 H, -C*H*=CH_2, H-3, H-3′, H-4), 5.76 (t, 1 H, J = 2.5 Hz, H-2′), 5.33 (dd, 1 H, J = 1.0, 17.0 Hz, 1/2 CH_2=CHCH_2), 5.26 (dd, 1 H, J = 1.0, 10.0 Hz, 1/2 CH_2=CHCH_2), 5.21 (d, 1 H, J = 1.7 Hz, H-1′), 5.15 (d, 1 H, J = 1.4 Hz, H-1), 4.70–4.50 (m, 5 H, H-5′, 4 H-6), 4.46–4.40 (m, 2 H, H-2, H-5), 4.20–3.89 (m, 2 H, CH_2CH=CH_2), 2.08 (s, 3H, $COCH_3$); δ_C ($CDCl_3$, 125 MHz): 169.4, 166.4, 166.1, 165.7, 165.6, 165.3, 165.0, 133.6, 133.5, 133.4, 133.3, 133.2, 133.2, 133.2, 133.1, 130.2, 130.0, 130.0, 129.9, 129.9, 129.9, 129.8, 129.8, 129.7, 129.7, 129.3, 129.1, 128.9, 128.8, 128.6, 128.5, 128.5, 128.5, 128.4, 118.1, 99.6, 98.0, 70.9, 69.8, 69.6, 69.0, 68.8, 67.6, 67.2, 63.7, 63.4, 20.6; ESI-MS: m/z: calcd for $C_{59}H_{52}O_{18}$: 1048.3; found: 1049.2 [M + H]$^+$, 1071.2 [M + Na]$^+$.

Allyl 3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranoside (5). To a solution of **4** (500 mg, 0.476 mmol) in anhydrous CH_3OH (30 mL) was added acetyl chloride (1.0 mL). The mixture was stirred at 40 °C for 4 h, when TLC (hexanes–EtOAc 2 : 1) indicated that the selective de-*O*-acetylation was complete to give a less mobile spot. The mixture was concentrated under reduced pressure, and the residue was purified by flash silica gel column chromatography (hexanes–EtOAc 3 : 1) to give **5** (348 mg, 72%) as a white foam. δ_H (500 MHz, $CDCl_3$): 8.14–7.36 (m, 30 H, Ph), 6.05 (t, 1 H, J = 9.9 Hz, H-4′), 5.99 (t, 1 H, J = 9.8 Hz, H-4), 5.94 (m, 1 H, C*H*=CH_2), 5.89 (dd, 1 H, J = 3.1, 9.9 Hz, H-3′), 5.85 (dd, 1 H, J = 3.1, 9.8 Hz, H-3), 5.33 (dd, 1 H, J = 1.0, 17.0 Hz, 1/2 CH_2=CHCH_2), 5.25 (dd, 1 H, J = 1.0, 10.0 Hz, 1/2 CH_2=CHCH_2), 5.24 (d, 1 H, J = 1.1 Hz, H-1′), 5.22 (d, 1 H, J = 1.2 Hz, H-1), 4.68–4.50 (m, 6 H), 4.48 (t, 1 H, J = 3.0 Hz, H-2), 4.45 (m, 1 H, H-5), 4.21–3.91 (m, 2 H, CH_2CH=CH_2); δ_C ($CDCl_3$, 125 MHz): 166.4, 166.2, 165.7, 165.6, 165.4, 165.3, 133.5, 133.4, 133.3, 133.1, 133.1, 129.9, 129.8, 129.8, 129.7, 129.7, 129.3 129.1, 129.0, 129.0, 128.6, 128.5, 128.4, 128.4, 128.3, 128.3, 118.1, 101.6, 98.1, 72.3, 71.4, 69.7, 69.4, 68.9, 68.8, 67.5, 67.0, 63.7, 63.6; ESI-MS: m/z: calcd for $C_{57}H_{50}O_{17}$: 1007.0; found: 1007.5.

Allyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranoside (7). To a solution of **1** (485 mg, 0.72 mmol) and **5** (600 mg, 0.6 mmol) in anhydrous CH_2Cl_2 (20 mL) was added TMSOTf (5 µL, 0.026 mmol). The reaction mixture was stirred at rt overnight and was then neutralized with triethylamine. The mixture was concentrated under reduced pressure, and the residue was subject to flash column chromatography (hexanes–EtOAc 2 : 1) to give compound **7** (750 mg, 82%) as a white foam. δ_H (500 MHz, $CDCl_3$): 8.15–7.30 (m, 45 H, Ph), 6.09–5.90 (m, 3 H, H-3″,H-4′, H-4″), 5.95 (m,1 H, -C*H*=CH_2), 5.90–5.80 (m, 3 H, H-3, H-3′, H-4), 5.63 (t, 1 H, J = 2.0 Hz, H-2″), 5.47 (s,1 H, H-1″), 5.37–5.27 (m, 2 H, CH_2=CHCH_2), 5.20 (s, 1 H, H-1′), 4.92 (s, 1 H, H-1), 4.70–4.30 (m, 10 H), 4.28–3.96 (m, 2 H, CH_2CH=CH_2), 4.18 (m, 1 H, H-6), 2.09 (s, 3 H, $COCH_3$); δ_C ($CDCl_3$, 125 MHz): 169.1, 166.3, 166.2, 165.8, 165.7, 165.6, 165.4, 165.3, 165.2, 165.0, 133.4, 133.3, 133.3, 133.3, 133.2, 133.1, 133.1, 130.1, 130.0, 130.0, 129.7, 129.6, 129.5, 129.1, 129.1, 129.0, 128.9, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.2, 118.0, 100.1, 99.8, 98.0, 71.1, 70.4, 69.7, 69.6, 69.5, 68.9, 68.8, 67.4, 67.0, 63.9, 63.7, 63.4, 20.7; ESI-MS: m/z: calcd for $C_{86}H_{74}O_{26}$: 1522.5; found: 1523.6.

Allyl 2,3,4-tri-*O*-benzoyl-6-deoxy-6-fluoro-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranoside (8). To a solution of **5** (650 mg, 0.646 mmol) and **6** (500 mg, 0.707 mmol) in anhydrous CH_2Cl_2 (20 mL) was added TMSOTf (5 μL, 0.026 mmol). The mixture was stirred at rt under an argon atmosphere overnight and was then neutralized with triethylamine. The mixture was concentrated under reduced pressure, and the residue was purified by flash column chromatography (hexanes–EtOAc 2 : 1) to give compound **8** (750 mg, 78%) as a white foam. δ_H (500 MHz, $CDCl_3$): 8.15–7.20 (m, 45 H, Ph), 6.09–5.90 (m, 5 H, H-2″, H-3″, H-4′, H-4″, C*H*=CH_2), 5.90–5.80 (m, 3 H, H-3, H-3′, H-4), 5.48 (s,1 H, H-1″), 5.37–5.27 (m, 2 H, CH_2=CHCH_2), 5.24 (s, 1 H, H-1′), 4.92 (s, 1 H, H-1), 4.70–4.30 (m, 9 H), 4.57 (m, 2 H, H-6″), 4.28–3.96 (m, 2 H, CH_2CH=CH_2); δ_C ($CDCl_3$, 125 MHz): 166.4, 166.3, 165.8, 165.6, 165.5, 165.4, 165.3, 165.1, 164.9, 133.6, 133.5, 133.3, 133.3, 133.2, 133.1, 133.1, 130.1, 130.0, 130.0, 129.9, 129.8, 129.8, 129.2, 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.2, 118.1, 100.4, 99.8, 98.1, 81.3 (d, J_{C-F} = 175.0 Hz, C-6″), 71.4, 70.5, 70.4, 70.3, 70.0, 69.7, 69.6, 69.0, 68.8, 67.5, 67.4, 65.9, 65.9, 63.7; ESI-MS: *m*/*z*: calcd for $C_{84}H_{71}FO_{24}$: 1482.4; found: 1483.5.

2-*O*-Acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (9). To a solution of **7** (500 mg, 0.33 mmol) in anhydrous CH_3OH (30 mL) was added $PdCl_2$ (0.30 g), the mixture was stirred at 40 °C for 4 h, when TLC (2 : 1 hexanes–EtOAc) indicated the completion of the de-*O*-allylation. The mixture was filtered through a pad of celite. The filtrate was concentrated, and the residue was purified by flash silica gel column chromatography (hexanes–EtOAc 2 : 1) to give the corresponding hemiacetal (440 mg, 90%) as a white foam. δ_H (500 MHz, $CDCl_3$): 8.10–7.29 (m, 45 H, Ph), 6.00–5.88 (m, 3 H, H-3″, H-4′, H-4″), 5.84–5.78 (m, 3 H, H-3, H-3′, H-4), 5.58 (t, 1 H, J = 2.0 Hz, H-2″), 5.54 (s, 1 H, H-1″), 5.42 (s, 1 H, H-1′), 4.90 (s, 1H, H-1), 4.64–4.50 (m, 7 H), 4.50-4.20 (m, 4 H), 2.08 (s, 3 H, $COCH_3$); δ_C ($CDCl_3$, 125 MHz): 169.1, 166.5, 166.2, 166.2, 165.9, 165.7, 165.6, 165.4, 165.3, 165.0, 133.3, 133.2, 133.0, 130.1, 130.0, 130.0, 129.9, 129.8, 129.7, 129.7, 129.6, 129.4, 129.2, 129.1, 129.0, 128.9, 128.6, 128.4, 128.4, 128.3, 100.2, 99.8, 93.3, 69.6, 69.5, 68.9, 67.7, 67.0, 63.9, 63.7, 20.7; ESI-MS: *m*/*z*: calcd for $C_{83}H_{79}O_{26}$: 1482.3; found: 1483.6 $[M + H]^+$, 1505.8 $[M + Na]^+$.

The above prepared hemiacetal (450 mg, 0.303 mmol) was dissolved in CH_2Cl_2 (40 mL), then trichloroacetonitrile (3 mL) and DBU (0.3 mL) were added. The reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated under vacuum and the residue was purified by flash silica gel column chromatography (hexanes–EtOAc 4 : 1) to give imidate **9** (420 mg, 85%) as a white foam. δ_H (500 MHz, $CDCl_3$): 8.79 (s, 1 H, HN=), 8.16–7.29 (m, 45 H, Ph), 6.68 (d, 1 H, J = 2.4 Hz, H-1), 6.12–6.06 (t, 2 H, J = 9.5 Hz, H-4′, H-4″), 6.00 (dd, 1 H, J = 3.5, 10 Hz, H-3″), 5.93 (dd, 1 H, J = 2.5, 8.5 Hz, H-3), 5.88–5.85 (m, 2 H, H-3′, H-4), 5.68 (s, 1 H, H-2″), 5.62 (s, 1 H, H-1″), 5.03 (s, 1 H, H-1′), 4.79 (t, 1 H, J = 3.0 Hz, H-2′), 4.74 (dd, 1 H, J = 2.3, 11.7 Hz, H-6″), 4.70–4.58 (m, 5 H), 4.56 (t, 1 H, J = 2.0 Hz, H-2), 4.45 (m, 1H), 4.32 (m, 1 H, H-5), 4.17 (m, 1H, H-6), 2.08 (s, 3 H, $COCH_3$); δ_C ($CDCl_3$, 125 MHz): 169.1, 166.3, 166.2, 165.9, 165.6, 165.5, 165.4, 165.3, 165.2, 165.0, 160.0, 133.5, 133.4, 133.4, 133.3, 133.2, 133.1, 133.0, 130.0, 130.0, 129.9, 129.9, 129.8, 129.8, 129.7, 129.7, 129.6, 129.3, 129.0, 128.9, 128.9, 128.8, 128.8, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 99.7, 96.3, 90.7, 71.7, 70.5, 70.0, 69.6, 69.5, 69.5, 67.1, 67.0, 63.5, 63.3, 63.2, 20.6.

2,3,4-tri-*O*-Benzoyl-6-deoxy-6-fluoro-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (10). To a solution of **8** (600 mg, 0.40 mmol) in anhydrous MeOH (80 mL) was added $PdCl_2$ (0.30 g) and the mixture was stirred at 40 °C for 4 h, when TLC (hexanes–EtOAc 2 : 1) indicated the completion of de-*O*-allylation. The mixture was filtered through a pad of celite and the filtrate was concentrated. The residue was purified by flash silica gel column chromatography (hexanes–EtOAc 2 : 1) to give the corresponding hemiacetal (510 mg, 88%) as a white foam. δ_H (500 MHz, $CDCl_3$): 8.15–7.20 (m, 45 H, Ph), 6.10–5.80 (m, 7 H, H-2″, H-3, H-3′, H-3″, H-4, H-4′, H-4″), 5.64 (s, 1 H, H-1″), 5.46 (d, 1 H, J = 1.3 Hz, H-1′), 5.04 (d, 1 H, J = 1.5 Hz, H-1), 4.74–4.30 (m, 11 H); δ_C ($CDCl_3$, 125 MHz): 166.5, 166.3, 165.9, 165.7, 165.6, 165.5, 165.3, 165.0, 164.9, 133.6, 133.5, 133.4, 133.3, 133.3, 133.2, 133.1, 133.0, 130.1, 130.0, 130.0, 129.9, 129.8, 129.7, 129.7, 129.6, 129.4, 129.2, 129.1, 129.0, 128.9, 128.6, 128.4, 128.4, 128.3, 100.3, 99.7, 93.5, 81.1 (d, J_{C-F} = 175 Hz, C-6″), 71.1, 70.5, 70.4, 70.0, 69.7, 68.8, 67.4, 67.0, 65.9, 63.7, 60.5.

The above hemiacetal (450 mg, 0.312 mmol) was dissolved in CH_2Cl_2 (20 mL), then trichloroacetonitrile (0.32 mL, 3.12 mmol) and DBU (0.06 mL) were added. The reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated under vacuum and the residue was purified by flash silica gel column chromatography (hexanes–EtOAc 4 : 1) to give imidate **10** (400 mg, 81%) as a white foam. δ_H (500 MHz, $CDCl_3$): 8.78 (s, 1 H, HN=CCCl_3), 8.18–7.20 (m, 45 H, Ph), 6.68 (d, 1 H, J = 2.4 Hz, H-1), 6.17–6.13 (t, 2 H, J = 10.0 Hz, H-4′, H-4″), 6.03 (dd, 1 H, J = 3.5, 10 Hz, H-3″), 6.00 (dd, 1 H, J = 3.5, 9.5 Hz, H-3′), 5.91 (dd, 1 H, J = 3.0, 9.0 Hz, H-3), 5.90 (t, 1H, J = 9.5 Hz, H-4), 5.85 (dd, 1 H, J = 1.3, 3.5 Hz, H-2″), 5.57 (s, 1 H, H-1″), 5.10 (s, 1 H, H-1′), 4.81 (t, 1 H, J = 3.0 Hz, H-2′), 4.77-4.62 (ddd, 2 H, J = 2.3, 11.7, 44.0 Hz, H-6″), 4.76–4.60 (m, 3 H), 4.57 (dd, 1 H, J = 1.0, 3.0 Hz, H-2), 4.48-4.28 (m, 4 H); δ_C ($CDCl_3$, 125 MHz): 166.3, 166.2, 165.8, 165.6, 165.5, 165.3, 165.2, 165.0, 164.9, 160.1, 133.6, 133.5, 133.5, 133.4, 133.2, 133.1, 130.1, 130.0, 130.0, 129.9, 129.9, 129.8, 129.8, 129.7, 129.7, 129.6, 129.3, 129.0, 128.9, 128.9, 128.8, 128.8, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 99.7, 96.3, 90.7, 81.2 (d, J_{C-F} = 175 Hz, C-6″), 71.6, 70.5, 70.0, 70.0, 69.6, 67.1, 65.8, 63.5, 63.5, 63.2.

2-(2-Azidoethoxy)ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)-4,6-*O*-benzylidene-2-*O*-benzoyl-α-D-mannopyranoside (12). To a solution of **9** (197 mg, 0.12 mmol) and **11** (42 mg, 0.0866 mmol) in anhydrous CH_2Cl_2 (10 mL) was added TMSOTf (3 μL, 0.02 mmol). The mixture was stirred under an argon atmosphere at rt overnight and was neutralized by triethylamine. The mixture was concentrated under reduced pressure, and the residue was purified by column chromatography (hexanes–EtOAc 2 : 1) to give compound **12** (130 mg, 77%) as a white foam. δ_H (500 MHz, $CDCl_3$): 8.38–6.90 (m, 55 H, Ph), 6.10–5.95 (m, 2 H, H-4″, H-4‴), 5.93–5.70 (m, 3 H, H-3″, H-3‴, H-4′), 5.83 (s, 1 H, PhC*H*), 5.76 (dd, 1 H, J = 1.0, 3.5 Hz, H-2), 5.63 (s, 1 H, H-1‴), 5.60–5.40

(m, 2 H), 5.39 (s, 1 H, H-1″), 5.15 (s, 1 H, H-1), 4.91 (s, 1 H, H-1′), 4.83–4.60 (m, 5 H), 4.50–3.67 (m, 17 H), 3.44 (t, 2 H, J = 5.0 Hz, CH_2N_3), 2.01 (s, 3 H, CH_3CO); δ_C ($CDCl_3$, 125 MHz): 169.1, 166.4, 166.0, 165.7, 165.5, 165.2, 164.9, 137.0, 133.6, 133.2, 133.2, 133.1, 132.8, 130.1, 130.0, 130.0, 129.9, 129.9, 129.8, 129.8, 129.7, 129.6, 129.4, 129.2, 129.2, 129.1, 129.0, 128.8, 128.7, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.2, 125.8, 110.0, 103.6, 101.7, 99.7, 99.0, 72.1, 71.7, 70.6, 70.3, 70.2, 69.6, 69.5, 69.4, 68.8, 67.0, 66.7, 63.7, 63.6, 63.6, 62.9, 62.9, 50.7, 20.6.

2-(2-Azidoethoxy)ethyl 2,3,4,-tri-*O*-benzoyl-6-deoxy-6-fluoro-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)-4,6-*O*-benzylidene-2-benzoyl-α-D-mannopyranoside (13). To a solution of **10** (240 mg, 0.15 mmol) and **11** (61 mg, 0.125 mmol) in anhydrous CH_2Cl_2 (10 mL) was added TMSOTf (3 μL, 0.02 mmol). The mixture was stirred under an argon atmosphere at rt overnight and was then neutralized by triethylamine. The mixture was concentrated under reduced pressure, and the residue was purified by column chromatography (hexanes–EtOAc 2 : 1) to give compound **13** (175 mg, 73%) as a white foam. δ_H (500 MHz, $CDCl_3$): 8.32–6.82 (m, 55 H, Ph), 6.11–5.98 (m, 2 H, H-4″, H-4‴), 5.97–5.78 (m, 3 H, H-3″, H-3‴, H-4′), 5.79 (s, 1 H, PhC*H*), 5.72 (dd, 1 H, J = 1.0, 3.5 Hz, H-2), 5.64 (s, 1 H, H-1‴), 5.64–5.58 (m, 1 H), 5.42 (s, 1 H, H-1″), 5.14 (s, 1 H, H-1), 4.93 (s, 1 H, H-1′), 4.80–4.56 (m, 5 H), 4.48–3.67 (m, 17 H), 3.44 (t, 2 H, J = 5.0 Hz, CH_2N_3); δ_C ($CDCl_3$, 125 MHz): 166.5, 166.1, 166.0, 165.5, 165.4, 165.3, 165.2, 165.1, 165.0, 164.9, 137.1, 133.6, 133.5, 133.3, 133.2, 133.0, 132.8, 130.1, 130.1, 130.0, 130.0, 129.9, 129.8, 129.7, 129.2, 129.2, 129.2, 129.1, 129.1, 128.9, 128.7, 128.7, 128.6, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.2, 125.9, 101.7, 100.1, 99.7, 99.0, 81.2 (d, $J_{C\text{-}F}$ = 175 Hz, C-6‴), 72.2, 70.5, 70.3, 70.2, 70.0, 69.7, 69.5, 69.4, 68.9, 67.1, 63.7, 63.0, 50.7.

2-(2-Azidoethoxy)ethyl α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 3)-α-D-mannopyranoside (14). A solution of **12** (170 mg, 0.0872 mmol) in aqueous acetic acid (80%, 10 mL) was stirred at 60 °C. After 10 h, TLC indicated the completion of de-benzylidenation. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in MeOH (10 mL), to which a solution of MeONa in MeOH (0.5 M, 0.5 mL) was added. The mixture was stirred at rt overnight. After neutralized with Dowex 50W (H^+), the mixture was filtered and the filtrate was concentrated. The residue was then dissolved into pyridine–Ac_2O (10 mL, 1 : 1) and resulting mixture was stirred overnight. The reaction mixture was concentrated under reduced pressure and the residue was purified by flash chromatography to give the corresponding *O*-acetylated derivative (90 mg, 78%) as a white solid. δ_H (500 MHz, $CDCl_3$): 5.44 (dd, 1 H, J = 3.5, 10.0 Hz, H-3‴), 5.39–5.28 (m, 7 H), 5.19 (dd, 1 H, J = 3.0, 9.5 Hz, H-3″), 5.17 (d, 1 H, J = 1.8 Hz, H-1‴), 5.04 (d, 1 H, J = 2.0 Hz, H-1″), 4.99 (dd, 1 H, J = 1.8 Hz, H-1), 4.93 (dd, 1 H, J = 1.8 Hz, H-1′), 4.34–4.10 (m, 12 H), 4.08–4.02 (m, 2 H), 3.96 (t, J = 2.5 Hz, 1 H), 3.90–3.84 (m, 1 H), 3.76–3.68 (m, 5 H), 3.42 (t, 2 H, J = 5.5 Hz, CH_2N_3), 2.42–2.06 (13 s, 39 H, 13 CH_3CO); δ_C ($CDCl_3$, 125 MHz): 170.9, 170.8, 170.7, 170.5, 170.4, 170.0, 169.9, 169.8, 169.7, 169.7, 169.5, 169.5, 169.4, 100.2, 99.5, 99.3, 97.5, 74.2, 70.7, 70.1, 70.0, 69.9, 69.7, 69.6, 69.5, 69.4, 68.4, 68.3, 68.0, 67.0, 66.5, 66.1, 65.7, 62.6, 62.4, 62.1, 62.0, 50.7, 20.9, 20.8, 20.8, 20.7, 20.7, 20.6; ESI-MS: m/z: calcd for $C_{54}H_{75}N_3O_{35}$: 1326.2, found: 1327.2 $[M + H]^+$.

To a solution of the above compound (80 mg, 0.0603 mmol) in anhydrous MeOH (5 mL) was added a solution of MeONa in MeOH (0.5 M, 0.2 mL). The mixture was stirred at rt for 2 h, and then neutralized by Dowex 50W (H^+). The mixture was filtered and the filtrate was concentrated to give **14** (45 mg, 98%) as a white solid. δ_H (500 MHz, CD_3OD): 5.42 (d, 1 H, J = 1.5 Hz, H-1‴), 5.34 (d, 1 H, J = 1.5 Hz, H-1″), 5.03 (d, 1 H, J = 2.0 Hz, H-1), 4.82 (d, 1 H, J = 1.5 Hz, H-1′), 4.08–4.01 (m, 4 H), 3.98–3.59 (m, 26 H), 3.42 (t, 2 H, J = 5.0 Hz, CH_2N_3); δ_C (CD_3OD, 125 MHz): 102.7, 100.9, 100.6, 100.5, 79.1, 78.9, 78.8, 73.7, 73.6, 73.4, 71.1, 70.7, 70.5, 70.1, 69.9, 67.7, 67.4, 66.6, 66.3, 61.8, 61.6, 61.4, 50.4; ESI-MS: m/z: calcd for $C_{28}H_{49}N_3O_{22}$: 779.7; found: 780.5 $[M + H]^+$, 803.1 $[M + Na]^+$.

2-(2-Azidoethoxy)ethyl 6-deoxy-6-fluoro-α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 3)-α-D-mannopyranoside (15). A solution of **13** (180 mg, 0.0941 mmol) in aqueous acetic acid (80%, 10 mL) was stirred at 60 °C. After 10 h, TLC indicated the completion of de-benzylidenation. The mixture was concentrated under reduced pressure, and the residue was dissolved in MeOH (10 mL), to which a solution of MeONa in MeOH (0.5 M, 0.5 mL) was added. The mixture was stirred overnight and then neutralized with Dowex 50W (H^+). The mixture was filtered and the filtrate was concentrated. The residue was dissolved in pyridine–Ac_2O (10 mL, 1 : 1) and the resulting solution was stirred overnight. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography to give the corresponding *O*-acetylated compound (90 mg, 78%) as a white solid. δ_H (500 MHz, $CDCl_3$): 5.45 (dd, 1 H, J = 3.5, 10.0 Hz, H-3‴), 5.39–5.21 (m, 7 H), 5.21 (dd, 1 H, J = 3.0, 9.5 Hz, H-3″), 5.14 (d, 1 H, J = 1.8 Hz, H-1‴), 5.04 (d, 1 H, J = 2.0 Hz, H-1″), 4.99 (dd, 1 H, J = 1.8 Hz, H-1), 4.92 (dd, 1 H, J = 1.8 Hz. H-1′), 4.56–4.45 (m, 2 H, H-6‴), 4.30–4.08 (m, 10 H), 4.05–3.95 (m, 2 H), 3.90 (t, J = 2.5 Hz, 1 H), 3.90–3.84 (m, 1 H), 3.72–3.68 (m, 5 H), 3.42 (t, 2 H, J = 5.5 Hz, CH_2N_3), 2.42–2.06 (12 s, 36 H, 12 CH_3CO); δ_C ($CDCl_3$, 125 MHz): 170.9, 170.8, 170.7, 170.4, 170.0, 169.9, 169.8, 169.7, 169.7, 169.5, 169.5, 169.4, 100.3, 99.9, 99.3, 97.5, 81.8 (d, $J_{C\text{-}F}$ = 175 Hz, C-6‴), 74.9, 70.8, 70.1, 70.0, 69.8, 69.7, 69.6, 69.5, 69.4, 68.5, 68.3, 67.8, 67.1, 66.2, 65.8, 62.5, 62.2, 62.1, 61.9, 50.7, 20.9, 20.8, 20.8, 20.7, 20.7, 20.6; ESI-MS: m/z: calcd for $C_{52}H_{72}FN3O_{33}$: 1286.1; found: 1286.4 $[M + H]^+$.

To a solution of the *O*-acetylated compound (70 mg, 0.0544 mmol) in anhydrous MeOH (5 mL) was added a solution of MeONa in MeOH (0.5 M, 0.2 mL). The mixture was stirred at rt for 2 h, and then neutralized with Dowex 50W (H^+). The reaction mixture was filtered and the filtrate was concentrated to give **15** (41 mg, 95%) as a white solid. δ_H (500 MHz, CD_3OD): 5.40 (d, 1 H, J = 1.5 Hz, H-1‴), 5.25 (d, 1 H, J = 1.5 Hz, H-1″), 5.05 (d, 1 H, J = 2.0 Hz, H-1), 4.82 (d, 1 H, J = 1.5 Hz, H-1′), 4.72–4.60 (m, 2 H, H-6‴), 4.09–4.01 (m, 4 H), 3.98–3.59 (m, 24 H), 3.42 (t, 2 H, J = 5.0 Hz, CH_2N_3); δ_C (CD_3OD, 125 MHz): 102.8, 101.0, 100.8, 100.5, 82.1 (d, $J_{C\text{-}F}$ = 175 Hz, C-6‴), 79.1, 78.8, 78.8, 73.7, 73.6, 73.5, 72.3, 72.1, 71.1, 70.7, 70.6, 70.4, 70.0, 69.9, 67.8, 67.7, 66.6, 66.3, 66.0, 61.8, 61.6, 61.4, 50.4; ESI-MS: m/z: calcd for $C_{28}H_{48}FN_3O_{21}$: 779.7; found: 781.5 $[M + H]^+$, 804.2 $[M + Na]^+$.

2-(2-Aminoethoxy)ethyl α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 3)-α-D-mannopyranoside (16). A mixture of **14** (20 mg, 0.0257 mmol) and 10% Pd/C (20 mg) in MeOH (100 mL) was stirred under H_2 at 40 PSI for 2 h. The mixture was filtered through a pad of celite, and the filtrate was concentrated under vacuum to give **16** (18 mg, 98%) as a white solid. δ_H (500 MHz, CD_3OD): 5.29 (s, 1 H, H-1‴), 5.24 (d, 1 H, $J = 1.5$ Hz, H-1″), 4.98 (d, 1 H $J = 1.5$ Hz, H-1), 4.81 (d, 1 H $J = 1.2$ Hz, H-1′), 4.04–4.00 (m, 4 H), 3.94–3.56 (m, 28 H); ESI-MS: *m/z*: calcd for $C_{28}H_{51}NO_{22}$: 753.7; found: 754.4 $[M + H]^+$.

2-(2-(8-Azido-3,6-dioxaoctanamido)ethoxy)ethyl-α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 3)-α-D-mannopyranoside (18). Compound **16** (5.6 mg, 0.0076 mmol) was dissolved in aqueous $NaHCO_3$ (50 mM, 2 mL) containing MeCN (1 mL) and MeOH (0.1 mL). To the solution was added a solution of **17** (5 mg, 0.0175 mmol) in MeCN (1 mL). The mixture was stirred at rt for 3 h and then lyophilized. The residue was loaded onto a column of Sephadex G-10 and eluted with 0.1 M HOAc. The fractions containing the product were pooled and lyophilized to give **18** (5.2 mg, 74%) as a white foam. δ_H (500 MHz, CD_3OD): 5.29 (d, 1 H, $J =$ 1.5 Hz, H-1‴), 5.24 (d, 1 H, $J = 1.5$ Hz, H-1″), 4.98 (d, 1 H, $J =$ 1.5 Hz, H-1), 4.81 (d, 1 H, $J = 1.5$ Hz, H-1′), 4.05–3.97 (m, 6 H), 3.94–3.56 (m, 32 H), 3.46 (t, 2 H, $J = 5.0$ Hz,), 3.41 (t, 2 H, $J = 5.0$ Hz, CH_2N_3); ESI-MS: *m/z*: calcd for $C_{34}H_{60}N_4O_{25}$: 924.8; found: 925.6 $[M + H]^+$.

The orthogonally protected linear decapeptide. Peptide synthesis was performed on an automatic peptide synthesizer using distinctly protected fluorenylmethyloxycarbonyl (Fmoc)-amino acids as building blocks, HATU as the coupling reagent, and Fmoc-Lys(Boc)-NovaSyn TGT acidic sensitive resin as the solid support. The linear peptide was synthesized on a 0.4 mmole scale and cleaved from resin by treatment with 50% acetic acid in DCM for 2 h at rt. The crude peptide was precipitated by cold ethyl ether and purified by preparative RP-HPLC. The column was eluted with 30% MeCN containing 0.1% TFA in 40 min to give the linear decapeptide (810 mg, 95%). ESI-MS calcd. for $C_{112}H_{182}N_{16}O_{23}$: M = 2120.74; found: 2121.83 $(M + H)^{1+}$, 1061.36 $(M + 2H)^{2+}$, 707.88 $(M + 3H)^{3+}$, 874.49 $(M - Boc + 3H)^{3+}$, 641.17 $(M - 2Boc + 3H)^{3+}$.

The fully protected cyclic decapeptide. The linear peptide obtained above (30 mg, 14 μmol) was dissolved in 40 mL acetonitrile. To the solution was added 0.5 M 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HBTU) (28 μmol) in DMF and 1.0 M diisopropylethylamine (DIPEA). The mixture was gently shaken at rt for 2 h. The reaction mixture was diluted with 30 mL water and lyophilized. The crude cyclic peptide was subjected to purification by preparative RP-HPLC. The peptide was eluted with 30% MeCN containing 0.1% TFA in 40 min to give the fully protected cyclic decapeptide (26 mg, 88%). ESI-MS calcd. for $C_{112}H_{180}N_{16}O_{22}$: M = 2102.72; found: 2103.59 $(M + H)^{1+}$, 1052.32 $(M + 2H)^{2+}$, 668.47 $(M - Boc + 3H)^{3+}$, 635.15 $(M - 2Boc + 3H)^{3+}$.

The selectively deprotected cyclic decapeptide 19. The above-obtained cyclic peptide (29 mg, 0.014 mmol) was dissolved in 10 mL 2% hydrazine in DMF, and gently shaken at rt for 20 min to selectively remove the Dde protecting groups. The reaction mixture was neutralized with acetic acid to pH 7.0, diluted with 40 mL water, and lyophilized. The crude de-protected cyclic peptide was purified by RP-HPLC to give the compound **19** (15 mg, 84%). ESI-MS calcd. for $C_{60}H_{108}N_{16}O_{14}$: M = 1277.60; found: 1278.03 $(M + H)^+$, 639.40 $(M + 2H)^{2+}$, 589.55 $(M - Boc + 2H)^{2+}$, 539.39 $(M - 2Boc + 2H)^{2+}$, 359.93 $(M - 2Boc + 3H)^{3+}$.

***c*[Lys(-propynoic amide)-Lys-Lys (-propynoic amide)-Pro-Gly-Lys(-propynoic amide)-Lys-Lys (propynoic amide)-Pro-Gly] (20).** To a solution of cyclic peptide **19** (26 mg, 0.02 mmol) in DMF (5 mL), were added propynoic acid (11.2 mg, 0.16 mmol) and dicyclohexylcarbodiimide (DCC) (32 mg, 0.16 mmol). The resulting mixture was shaken at rt for 30 min. After dilution with water (50 mL), the suspension was filtered and the filtrate was lyophilized. The crude acylated cyclic peptide was subject to preparative RP-HPLC to give the corresponding acylated cyclic decapeptide (23 mg, 77%). ESI-MS calcd for $C_{72}H_{108}N_{16}O_{18}$: 1484.80; found: 1485.67 $[M + H]^+$, 743.52 $[M + 2H]^{2+}$, 693.35 $[M - Boc + 2H]^{2+}$, 642.73 $[M - 2Boc + 2H]^{2+}$, 428.62 $[M - 2Boc + 3H]^{3+}$.

To selectively remove the Boc protective groups, the above acylated cyclic peptide (23 mg, 0.015 mmol) was treated with 90% TFA in DCM at rt for 10 min, then the solution was diluted with water and lyophilized. The product was purified by preparative RP-HPLC to give the alkynylized cyclic decapeptide **20** (18 mg, 93%). ESI-MS calcd. for $C_{62}H_{92}N_{16}O_{14}$: 1284.70; found: 1285.64 $[M + H]^+$, 643.32 $[M + 2H]^{2+}$.

Synthesis of glycopeptides 21–24. General procedures. To a solution of the individual azido-tagged oligosaccharide (**2**, **14**, **15**, or **18**) (5.0 mol. equiv), the alkynyl-substituted cyclic peptide **20** (1.0 mg, 0.78 μmol, 1 mol. equiv.) in an aqueous *t*-BuOH (50%, 0.95 mL) were added $CuSO_4$ (10 mM in 1 : 1 *t*-BuOH–H_2O, 15 μl, 0.15 μmol, 0.2 mol. equiv.) and sodium ascorbate (20 mM in 1 : 1 *t*-BuOH–H_2O, 15 μl, 0.30 μmol, 0.4 mol. equiv.). The reaction mixture was stirred at 60 °C. The reaction was monitored by RP-HPLC. After 6–12 h, HPLC indicated the completion of the 1,3-dipolar cycloaddition to give a single product. The product was easily purified by preparative RP-HPLC to give the template-assembled oligosaccharide cluster (**21–24**).

Glycopeptide 21. δ_H (500 MHz, D_2O): 8.48 (s, 4 H, H-triazole), 4.94 (s, 4 H, 4 H-1); ESI-MS: *m/z*: calcd for $C_{102}H_{168}N_{28}O_{42}$: 2457.62; found: 1230.3 $[M + 2H]^{2+}$, 841.5 $[M + 3Na]^{3+}$, 820.7 $[M + 3H]^{3+}$, 766.6 $[M + 3H\text{-Man}]^{3+}$, 712.6 $[M + 3H\text{-2Man}]^{3+}$, 658.6 $[M + 3H\text{-3Man}]^{3+}$.

Glycopeptide 22. δ_H (500 MHz, D_2O): 8.48 (s, 4 H, H-triazole), 5.45 (s, 4 H, 4 H-1‴), 5.36 (s, 4 H, 4 H-1″), 5.05 (s, 4 H, 4 H-1), 4.83 (s, 4 H, 4 H-1′); ESI-MS: *m/z*: calcd for $C_{174}H_{288}N_{28}O_{102}$: 4403.5; found: 1469.2 $[M + 3H]^{3+}$, 1115.6 $[M + 2H + 2Na]^{4+}$.

Glycopeptide 23. δ_H (500 MHz, D_2O): 8.48 (s, 4 H, H-triazole), 5.41 (s, 4 H, 4 H-1‴), 5.27 (s, 4 H, 4 H-1″), 5.07 (s, 4 H, 4 H-1), 4.83 (s, 4 H, 4 H-1′); ESI-MS: *m/z*: calcd for $C_{174}H_{284}N_{28}O_{98}F_4$: 4411.5; found: 1471.9 $[M + 3H]^{3+}$, 1118.0 $[M + H + 3Na]^{4+}$, 1113.8 $[M + 2H + 2Na]^{4+}$, 1109.8 $[M + 3H + Na]^{4+}$.

Glycopeptide 24. δ_H (500 MHz, D_2O): 8.48 (s, 4 H, H-triazole), 5.41 (s, 4 H, 4 H-1‴), 5.27 (s, 4 H, 4 H-1″), 5.07 (s, 4 H, 4 H-1), 4.83 (s, 4 H, 4 H-1′); ESI-MS: *m/z*: calcd for $C_{198}H_{332}N_{32}O_{114}$: 4984.1;

found: 1662.9 $[M + 3H]^{3+}$, 1260.8 $[M + H + 3Na]^{4+}$, 1257.0 $[M + 2H + 2Na]^{4+}$, 1253.6 $[M + 3H + Na]^{4+}$.

Glycopeptide 25. Compound **23** (2.0 mg, 0.45 μmol) was dissolved in 2 mL 0.05 M $NaHCO_3$, 1 mL CH_3CN and 0.1 mL methanol. To this solution, compound **17** (5 mg, 0.0175 mmol) in 1 mL CH_3CN was added, and the reaction mixture was stirred at room temperature for 1 h. The mixture was purified by RP-HPLC and lyophilized to give compound **25** as a white foam (1.9 mg, 90%). ESI-MS: *m/z*: calcd for $C_{186}H_{302}N_{34}O_{104}F_4$: 4753.5; found: 1586 $[M + 3H]^{3+}$, 1423.5 $[M + 3H\text{-}FMan3]^{3+}$, 1369.2 $[M + 3H\text{-}FMan4]^{3+}$, 1260.3 $[M + 3H\text{-}2FMan3]^{3+}$, 1206.0 $[M + 3H\text{-}FMan3\text{-}FMan4]^{3+}$, 1098.0 $[M + 3H\text{-}3FMan3]^{3+}$, 1039.6 $[M + 3H\text{-}2FMan3\text{-}FMan4]^{3+}$.

Alkynyl-tagged T-helper peptide 26. Peptide synthesis was performed on a Pioneer automatic peptide synthesizer (Applied Biosystems) using fluorenylmethyloxycarbonyl (Fmoc)-protected amino acids as building blocks, HATU as the coupling reagent, and PAL-PEG-PS resin as the solid support. The peptide was synthesized on a 0.4 mmole scale. After removal of the Fmoc in the last amino acid at the N-terminus, propynoic acid was reacted with the peptide resin in DMF using HATU as coupling reagent. The resin was treated with 95% TFA to remove the protecting groups and retrieve the peptide from the resin. The crude peptide was precipitated in ethyl ether and purified through RP-HPLC to give the desired compound **26**. ESI-MS: *m/z*: calcd for: $C_{83}H_{129}N_{19}O_{23}$: 1776.0; found: 1776.9 $[M + H]^+$, 889.0 $[M + 2H]^{2+}$.

Glycopeptide 27. To a solution of compound **26** (1.0 mg, 0.56 μmol) and oligosaccharide cluster **25** (0.50 mg, 0.105 μmol) in aqueous *t*-BuOH (50%, 0.25 mL) were added $CuSO_4$ (10 mM in 1 : 1 *t*-BuOH–H_2O, 2 μl, 0.02 μmol, 0.2 mol. equiv.) and sodium ascorbate (20 mM in 1 : 1 *t*-BuOH–H_2O, 2 μl, 0.40 μmol, 0.4 mol. equiv.). The reaction mixture was stirred at 60 °C for 24 h and the product was purified by preparative RP-HPLC to give **27** (0.6 mg, 70%) as a white foam. ESI-MS: *m/z*: calcd for $C_{352}H_{560}N_{72}O_{150}F_4$: 8305.5; found: 1662.7 $[M + 5H]^{5+}$, 1385.6 $[M + 6H]^{6+}$.

Binding studies

Surface plasmon resonance (SPR) technology was used for analyzing the binding of the synthetic oligomannoses and their clusters to monoclonal antibody (mAb) 2G12. The experiments were carried out with a biacore 3000 system in a HBS-P buffer (10 mM Hepes, 150 mM NaCl, 0.005% surfactant P20, pH 7.4) at 25 °C. For coupling of mAb 2G12 to CM5 sensor chip, the chip surface was activated by injection of EDC–NHS for 7 min at 5 μL min^{-1}, followed by injection of 20 μg mL^{-1} mAb 2G12 solution in an acetate buffer (10 mM pH 5.5) until the target level of 10 000 response units (RU) was reached. The reaction was then quenched by injection of ethanolamine HCl (1 M, pH 8.5) for 7 min at 5 μL min^{-1}. A reference surface was prepared by a similar procedure, but without the injection of antibody solution. Each synthetic compound was injected to reference and mAb 2G12 channels, and a binding profile was obtained by subtraction of the reference surface signal from the mAb 2G12 surface signal. For a kinetic study, three analytes, compounds **22–24**, at six different concentrations were injected at 30 μL min^{-1} for 4 min, followed by 5 min dissociation. The censor surface was regenerated with a short pulse of 3.5 M $MgCl_2$ with a flow rate of 100 μL min^{-1} for 30 seconds.

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References

1 R. C. Gallo and L. Montagnier, *N. Engl. J. Med.*, 2003, **349**, 2283–2285.
2 A. J. McMichael and T. Hanke, *Nat. Med.*, 2003, **9**, 874–880.
3 G. Pantaleo and R. A. Koup, *Nat. Med.*, 2004, **10**, 806–810.
4 R. C. Gallo, *Lancet*, 2005, **366**, 1894–1898.
5 R. Wyatt and J. Sodroski, *Science*, 1998, **280**, 1884–1888.
6 P. D. Kwong, M. L. Doyle, D. J. Casper, C. Cicala, S. A. Leavitt, S. Majeed, T. D. Steenbeke, M. Venturi, I. Chaiken, M. Fung, H. Katinger, P. W. Parren, J. Robinson, D. Van Ryk, L. Wang, D. R. Burton, E. Freire, R. Wyatt, J. Sodroski, W. A. Hendrickson and J. Arthos, *Nature*, 2002, **420**, 678–682.
7 J. N. Reitter, R. E. Means and R. C. Desrosiers, *Nat. Med.*, 1998, **4**, 679–684.
8 X. Wei, J. M. Decker, S. Wang, H. Hui, J. C. Kappes, X. Wu, J. F. Salazar-Gonzalez, M. G. Salazar, J. M. Kilby, M. S. Saag, N. L. Komarova, M. A. Nowak, B. H. Hahn, P. D. Kwong and G. M. Shaw, *Nature*, 2003, **422**, 307–312.
9 D. R. Burton, R. C. Desrosiers, R. W. Doms, W. C. Koff, P. D. Kwong, J. P. Moore, G. J. Nabel, J. Sodroski, I. A. Wilson and R. T. Wyatt, *Nat. Immunol.*, 2004, **5**, 233–236.
10 A. Trkola, M. Purtscher, T. Muster, C. Ballaun, A. Buchacher, N. Sullivan, K. Srinivasan, J. Sodroski, J. P. Moore and H. Katinger, *J. Virol.*, 1996, **70**, 1100–1108.
11 C. N. Scanlan, R. Pantophlet, M. R. Wormald, E. Ollmann Saphire, R. Stanfield, I. A. Wilson, H. Katinger, R. A. Dwek, P. M. Rudd and D. R. Burton, *J. Virol.*, 2002, **76**, 7306–7321.
12 R. W. Sanders, M. Venturi, L. Schiffner, R. Kalyanaraman, H. Katinger, K. O. Lloyd, P. D. Kwong and J. P. Moore, *J. Virol.*, 2002, **76**, 7293–7305.
13 H. K. Lee, C. N. Scanlan, C. Y. Huang, A. Y. Chang, D. A. Calarese, R. A. Dwek, P. M. Rudd, D. R. Burton, I. A. Wilson and C. H. Wong, *Angew. Chem., Int. Ed.*, 2004, **43**, 1000–1003.
14 L. X. Wang, J. Ni, S. Singh and H. Li, *Chem. Biol.*, 2004, **11**, 127–134.
15 H. Li and L. X. Wang, *Org. Biomol. Chem.*, 2004, **2**, 483–488.
16 E. W. Adams, D. M. Ratner, H. R. Bokesch, J. B. McMahon, B. R. O'Keefe and P. H. Seeberger, *Chem. Biol.*, 2004, **11**, 875–881.
17 V. Y. Dudkin, M. Orlova, X. Geng, M. Mandal, W. C. Olson and S. J. Danishefsky, *J. Am. Chem. Soc.*, 2004, **126**, 9560–9562.
18 D. A. Calarese, C. N. Scanlan, M. B. Zwick, S. Deechongkit, Y. Mimura, R. Kunert, P. Zhu, M. R. Wormald, R. L. Stanfield, K. H. Roux, J. W. Kelly, P. M. Rudd, R. A. Dwek, H. Katinger, D. R. Burton and I. A. Wilson, *Science*, 2003, **300**, 2065–2071.
19 D. A. Calarese, H. K. Lee, C. Y. Huang, M. D. Best, R. D. Astronomo, R. L. Stanfield, H. Katinger, D. R. Burton, C. H. Wong and I. A. Wilson, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 13372–13377.
20 J. Ni, H. Song, Y. Wang, N. M. Stamatos and L. X. Wang, *Bioconjugate Chem.*, 2006, **17**, 493–500.
21 T. Buskas, Y. Li and G. J. Boons, *Chem.–Eur. J.*, 2004, **10**, 3517–3524.
22 L. X. Wang, *Curr. Opin. Drug Discovery Dev.*, 2006, **9**, 194–206.
23 G. Tuchscherer, C. Servis, G. Corradin, U. Blum, J. Rivier and M. Mutter, *Protein Sci.*, 1992, **1**, 1377–1386.
24 G. Tuchscherer, D. Grell, M. Mathieu and M. Mutter, *J. Pept. Res.*, 1999, **54**, 185–194.
25 S. Peluso, T. Ruckle, C. Lehmann, M. Mutter, C. Peggion and M. Crisma, *ChemBioChem*, 2001, **2**, 432–437.
26 O. Renaudet and P. Dumy, *Org. Lett.*, 2003, **5**, 243–246.
27 S. J. Danishefsky and J. R. Allen, *Angew. Chem., Int. Ed.*, 2000, **39**, 836–863.
28 S. Vichier-Guerre, R. Lo-Man, L. BenMohamed, E. Deriaud, S. Kovats, C. Leclerc and S. Bay, *J. Pept. Res.*, 2003, **62**, 117–124.
29 R. Lo-Man, S. Vichier-Guerre, R. Perraut, E. Deriaud, V. Huteau, L. BenMohamed, O. M. Diop, P. O. Livingston, S. Bay and C. Leclerc, *Cancer Res.*, 2004, **64**, 4987–4994.

30 T. Buskas, Y. Li and G. J. Boons, *Chem.–Eur. J.*, 2005, **11**, 5457–5467.
31 J. Alexander, M. F. del Guercio, A. Maewal, L. Qiao, J. Fikes, R. W. Chesnut, J. Paulson, D. R. Bundle, S. DeFrees and A. Sette, *J. Immunol.*, 2000, **164**, 1625–1633.
32 O. Renaudet and P. Dumy, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 3619–3622.
33 S. Grigalevicius, S. Chierici, O. Renaudet, R. Lo-Man, E. Deriaud, C. Leclerc and P. Dumy, *Bioconjugate Chem.*, 2005, **16**, 1149–1159.
34 P. Panina-Bordignon, A. Tan, A. Termijtelen, S. Demotz, G. Corradin and A. Lanzavecchia, *Eur. J. Immunol.*, 1989, **19**, 2237–2242.
35 H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004–2021.
36 H. C. Kolb and K. B. Sharpless, *Drug Discovery Today*, 2003, **8**, 1128–1137.
37 F. Perez-Balderas, M. Ortega-Munoz, J. Morales-Sanfrutos, F. Hernandez-Mateo, F. G. Calvo-Flores, J. A. Calvo-Asin, J. Isac-Garcia and F. Santoyo-Gonzalez, *Org. Lett.*, 2003, **5**, 1951–1954.
38 D. Giguere, R. Patnam, M. A. Bellefleur, C. St-Pierre, S. Sato and R. Roy, *Chem. Commun.*, 2006, 2379–2380.
39 A. Dondoni and A. Marra, *J. Org. Chem.*, 2006, **71**, 7546–7557.
40 B. Rohwedder, Y. Mutti, P. Dumy and M. Mutter, *Tetrahedron Lett.*, 1998, **39**, 1175–1178.
41 V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596–2599.
42 P. Wu, A. K. Feldman, A. K. Nugent, C. J. Hawker, A. Scheel, B. Voit, J. Pyun, J. M. Frechet, K. B. Sharpless and V. V. Fokin, *Angew. Chem., Int. Ed.*, 2004, **43**, 3928–3932.
43 C. W. Tornoe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057–3064.
44 A. K. Feldman, B. Colasson and V. V. Fokin, *Org. Lett.*, 2004, **6**, 3897–3899.
45 R. L. Rich and D. G. Myszka, *Trends Microbiol.*, 2003, **11**, 124–133.
46 L. C. James, P. Roversi and D. S. Tawfik, *Science*, 2003, **299**, 1362–1367.
47 L. Heng, J. Ning and F. Kong, *J. Carbohydr. Chem.*, 2001, **20**, 283–296.
48 Y. Xing and J. Ning, *Tetrahedron: Asymmetry*, 2003, **14**, 1275–1283.

