Bioorganic & Medicinal Chemistry Letters 19 (2009) 3775–3778

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Facile synthesis of cyclopeptide-centered multivalent glycoclusters with 'click chemistry' and molecular recognition study by surface plasmon resonance

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ARTICLE INFO

Article history:
Received 2 February 2009
Revised 19 April 2009
Accepted 21 April 2009
Available online 24 April 2009

Keywords:
Glycocluster
Cyclopeptide
Carbohydrate–protein interaction
Click chemistry
SPR

ABSTRACT

A facile synthesis of cyclopeptide-centered multivalent glycoclusters using Cu(I) catalyzed Huisgen 1,3-dipolar cycloaddition of azides and terminal alkynes, so called 'click chemistry', has been developed. The affinities of mannose-specific protein Concanavalin A (Con A) toward two synthetic glycoclusters respectively bearing divalent or tetravalent mannoses were investigated by surface plasmon resonance (SPR). It is founded that the tetravalent glycocluster has 3.0-fold increase in binding affinity relative to the divalent glycoluster (valency-corrected values), which indicates the potential of this system in investigating carbohydrate–protein interactions.

The interactions between carbohydrates and proteins immediate many important biological processes, such as: fertilization, differentiation, development, tissue formation, cell adhesion, antigen/antibody interactions, cancer metastasis, and infection of viruses or bacteria.[1,2] However, monovalent carbohydrate–protein interactions have been characterized by very weak binding affinities, with the K_d value in 10^{-3}–10^{-6} M range.[2] In nature, multivalent presentation of sugar ligands frequently enhances the affinity by simultaneous formation of multiple carbohydrate–protein complexes, which has been referred as 'glycoside cluster effects'.[3,4] In order to facilitate multivalent carbohydrate protein interactions study, multivalent glycoclusters presented on a great variety of well-defined scaffolds such as cyclopeptides,[5–11] polymers,[12] dentrimers,[13] cyclodextrins,[14–18] calix[4]arene, adamantane and benzene scaffolds,[19] monosaccharide,[20,21] and self-assemble supermoleculars[22] have been synthesized. Cyclopeptides might be excellent cores of multivalent glycoclusters, due to the flexibility of the synthetic strategy, easy control of size and spatial orientation, the ease of incorporating amino acids with different functionalities for attachment of linkers or ligands, and their biological compatibility.

It is well known that the efficient attachment of sugar ligands to scaffolds is pivotal for preparation of multivalent glycoclusters. For cyclopeptide scaffolds, different coupling methods have been developed. Dumy's group[6,10] has selected the oxime bond formation for the ligation of carbohydrates and cyclopeptides. Ohta and co-workers[5] have used enzymic method to complete the construction of multivalent glycoconjugates. Wittmann's group[11] has used the formation of urethane bond for the construction of multivalent neoglycopeptides. In recent years, a highly chemoselective and efficient ligation method 'Cu(I) catalyzed Huisgen 1,3-dipolar cycloaddition of azides and terminal alkynes', so called 'click chemistry', has been developed by Sharpless[23] and Meldal[24] group and exploited for the assembling of different glycosyls and various scaffolds.[19,20,25–29] Therefore, we planed to utilize this highly efficient ligation method for assembling cyclopeptide scaffolds and carbohydrate ligands. Since the oligosaccharides containing azido groups are readily accessible,[26,30] the installation of multi alkynes in cyclopeptide side chains has to be considered carefully.

In recent years, Dumy's group[10] has developed lysine-containing cyclodecapeptides called 'regioselectively addressable functionalized templates' (RAFTs) for multivalent presentation of carbohydrates. Based on this cyclic decapeptide scaffold presenting tetra-aldehydes for 'oxime bond formation' ligation strategy, a new cyclopeptide scaffold **1** bearing alkyne groups instead of aldehydes in the side chains of lysine residues was designed (Scheme 1). The amine in the side chain of lysine 9 that has been used to conjugate oligonucleotide in Singh's work[10] would be kept free as further derivation site. In addition, to determine the affinity efficiency of multivalent glycocluster with carbohydrate-binding protein, a cyclopeptide scaffold with di-alkynes **2** would be synthesized. The side chain amines of lysine 5 and 10 in scaffold **2** were capped by acetyl groups.

In this Letter, we report on a facile synthesis of two cyclopeptide scaffolds presenting di- or tetra-alkynes and the chemoselec-

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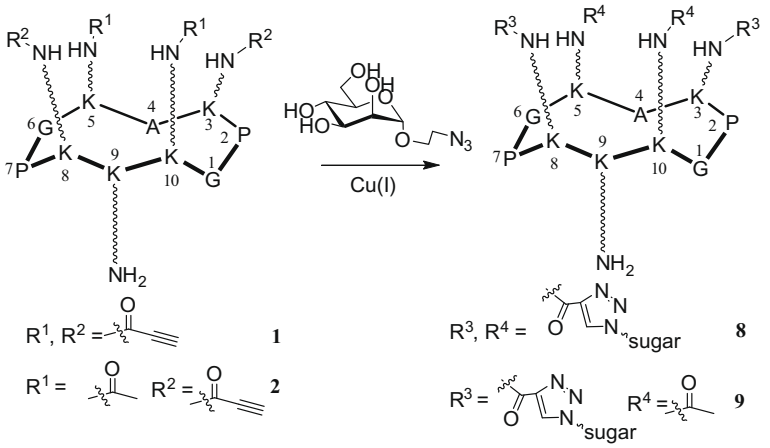
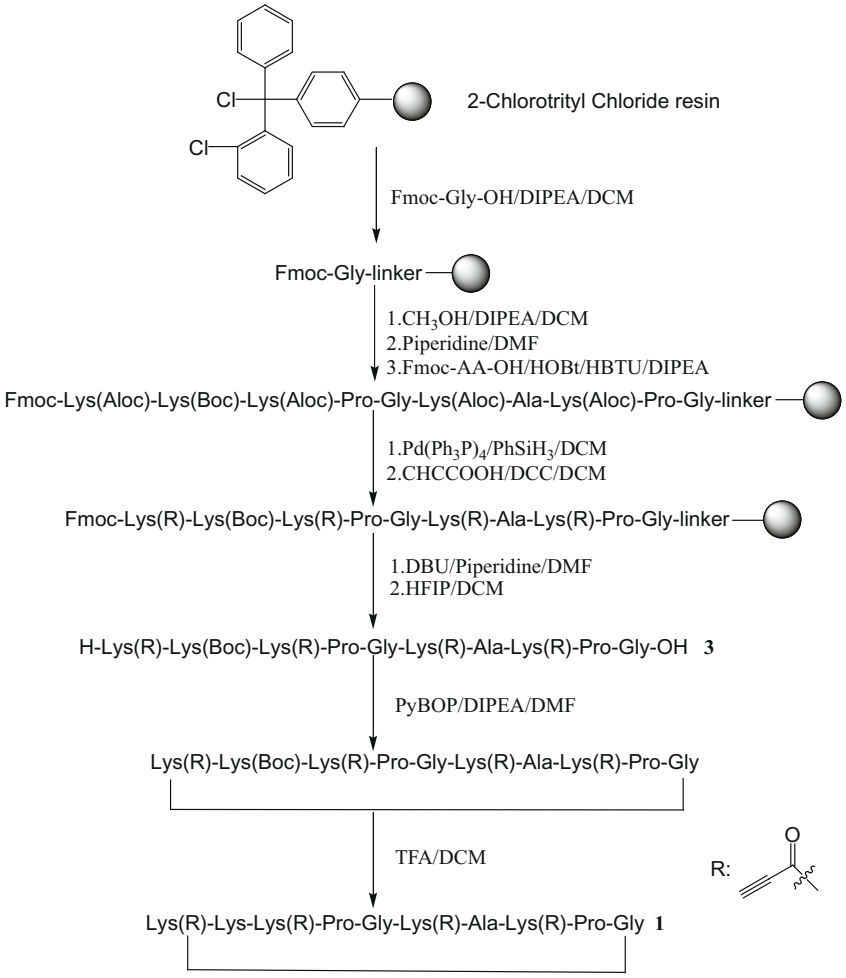
doi:10.1016/j.bmcl.2009.04.090



Scheme 1. The assembly of cyclopeptide scaffolds **1** and **2** with azido sugar.

tive and efficient assembly of unprotected α-mannose unit containing azido group to cyclopeptide scaffolds through Cu (I) catalyzed Huisgen 1, 3-dipolar cycloaddition (Scheme 1). Furthermore, these synthesized glycoclusters' binding abilities to the mannose-specific lectin Concanavalin A (Con A) have been evaluated by surface plasmon resonance (SPR), which was proven particularly suitable for the study of carbohydrate–protein interactions[31–33] due to low sample demands, being in needless of fluorescent, radioactive, or enzymatic reporter groups labeling and the ability to evaluate interactions under dynamic conditions.

The cyclopeptide scaffold **1** has been prepared following the facile synthetic route in Scheme 2. Firstly, the linear peptide has been assembled on a highly acid labile 2-chlorotritylchloride resin by standard Fmoc solid phase peptide chemistry with commercially available amino acids referred to Dumy's strategy.[10] One of the advantages of cyclopeptide scaffold for glycoclusters is the ease



Scheme 2. Synthesis route of cyclopeptide scaffold **1** presenting four alkynes.

of incorporating amino acids with orthogonally protected side chains. Therefore, four lysine residues at the positions of 3, 5, 8 and 10 with Aloc protected side chains served as carbohydrates attachment points and the lysine 9 bearing Boc protection group could provide a free amine for further derivation. After the completion of peptide chain elongation, the Aloc groups were removed with a catalytic amount of $Pd(Ph_3P)_4$ and 48 equiv $PhSiH_3$ in dichloromethane (DCM) to give four free amines. Under this neutral condition, both of the Boc group and the linker between peptide and resin were intact. Afterwards, propiolic acids were efficiently coupled with four amines of the peptide on solid support using *N,N′*-dicyclohexylcarbodiimide (DCC) coupling method in DCM, monitored by Kaiser's test. After the deprotection of Fmoc at the N-terminal of peptide by 2% 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)/2% piperidine in dimethylformamide (DMF), the linear peptide **3** was released from resin using 20% hexafluoroisopropanol (HFIP) in DCM instead of the normal cleavage agent (1/1/8 AcOH/TFE/DCM) for this resin, due to trouble in removing acetic acid. Under this cleavage condition, Boc group in the side chain of lysine was intact. After precipitation from diethyl ether, followed by purification with semi-preparative HPLC, the pure linear peptide **3** was obtained. Subsequently, the 'head to tail' cyclization of peptide **3** was performed in DMF at low concentration (less than 0.5 mM) using 1.0 equiv PyBOP and 5.0 equiv diisopropylethylamine (DIPEA). Further deprotection of Boc at lysine 9 by treatment with 50% trifluoroacetic acid (TFA) in DCM followed by purification with semi-preparative HPLC afforded the final cyclopeptide scaffold **1** bearing four alkynes in the 'arms', confirmed by ESI-MS (in the Supplementary data). In addition, the cyclopeptide scaffold **2** bearing di-alkynes was also synthesized following the similar procedures as scaffold **1**. Only the lysine residues with acetyl capped side chains were substituted of lysine residues with Aloc protected at positions of 5 and 10 during the assembling of the corresponding linear peptide **4**. The acetyl groups kept stable in the following synthetic steps.

The unprotected α-mannose unit containing azido group **5** was easily generated (Scheme 3) starting with 2,3,4,6-tetra-*O*-benzoyl-mannopyranosyl trichloroacetimidate **6**[34], which was coupled to the spacer 2-bromoethan-1-ol in DCM using trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a catalyst. Then the substitution of bromo group by azido group from sodium azide in DMF followed by deacetylation afforded 2-azidoethyl α-D-mannopyranoside **5**.

With cyclopeptides bearing alkynes and the azido sugar in hand, we explored the conjugation conditions. $Cu(OAc)_2$ and the reducing agent sodium ascorbate were chosen for the generation of Cu(I) in situ. Cyclopeptide **2** containing two alkynes was firstly investigated. To a solution of **2** and **5** in water and methanol (2:1, v/v) were added 0.4 equiv $Cu(OAc)_2$ and 0.8 equiv sodium ascorbate. To accelerate the conjugation rate, the cycloaddition reaction was carried at 37 °C by ultrasonic irradiation, which is an important technique in organic synthesis in terms of reductions in reaction times, improved yields, relative to traditional thermal heating.[35,36] Recently, Raghunathan[37] group has also reported on the application of ultrasonic irradiation to 1,3-dipolar cycloaddition reactions of azomethine ylides with high efficiency under mild reaction. Our reaction was monitored by analytical HPLC and ESI-MS, which indicated that the tris–triazole adduct **9** was formed quantitatively within 1 h. The yield after semi-preparative HPLC purification was 53%. The analytical HPLC and ESI-MS spectra of **9** are shown in Figures S5 and S6 (Supplementary data). This condition was also suitable for the tetra-alkynyl cyclopeptides **1** to give the corresponding tetravalent glycoclusters **8**. The yield after semi-preparative HPLC purification was 44%. The analytical HPLC and MALDI-TOF-MS spectra of **8** are shown in Figures S7 and S8 (Supplementary data).

The affinities of the prepared cyclopeptide-centered glycoclusters **8** and **9** towards Con A were measured by surface plasmon resonance (SPR) on a Biacore 3000 instrument with CM5 sensor chip. Since the SPR response is proportional to the accumulation of mass on the sensor surface, in an initial attempt we would like to immobilize the synthetic glycoclusters on the sensor chip through the free amine at lysine 9 of cyclopeptide scaffolds. However, the observed non-specific binding between Con A and CM5 sensor chip in the test measurement resulted in the switch to immobilize Con A on the chip instead of glycoconjugates which were found no non-specific binding to chip surface. After Con A was immobilized on one channel of a CM5 sensor chip (3600 RU), each cyclopeptide-centered glycocluster at a series of concentrations was respectively injected over this channel to quantitatively character-

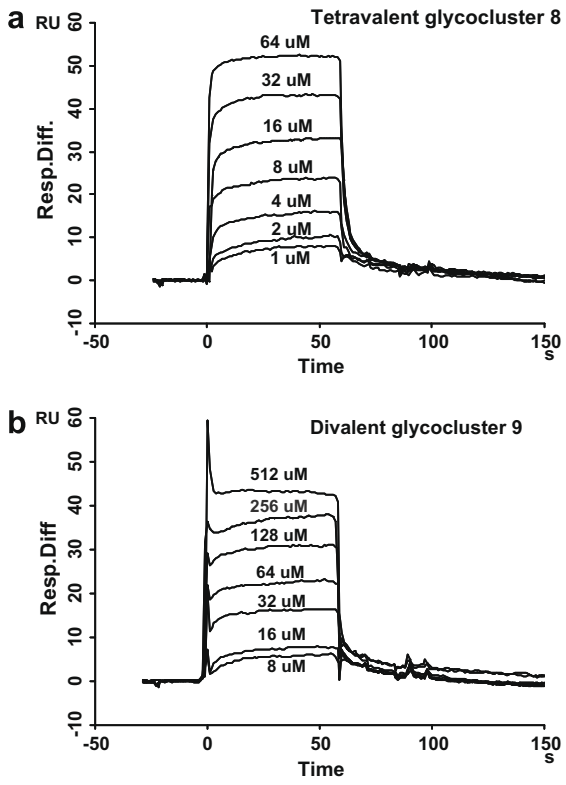
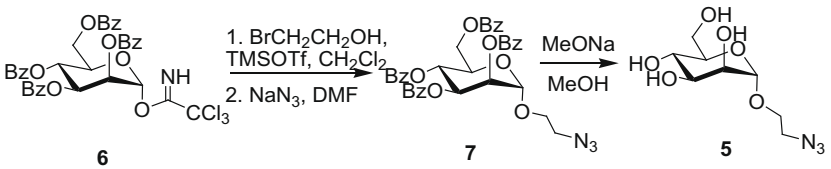


Figure 1. Sensorgrams of binding of glycoclusters at different concentrations over immobilized Con A. (a) for tetravalent glycocluster **8**; (b) for divalent glycocluster **9**.



Scheme 3. Synthesis route of 2-azidoethyl α-D-mannopyranoside **5**.

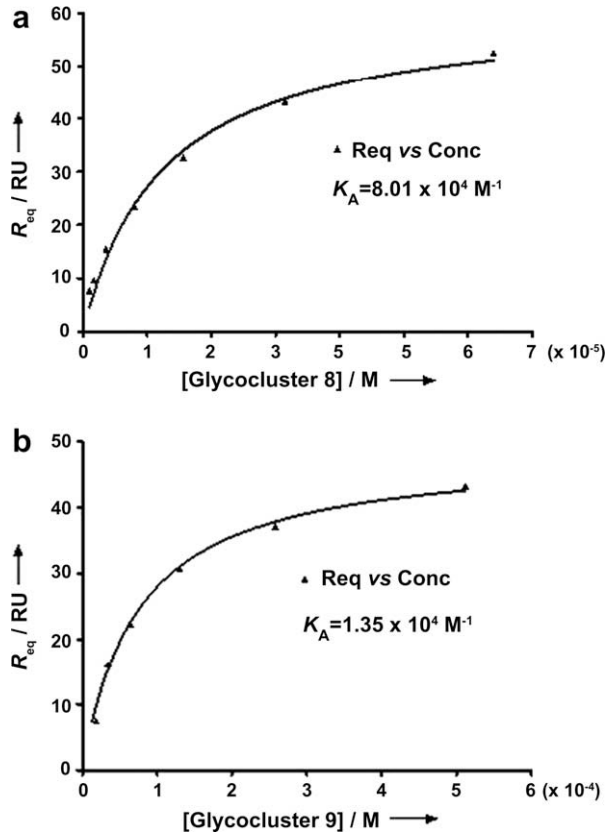


Figure 2. Equilibrium response as a function of glycoclusters concentration. (a) for tetravalent glycocluster **8**; (b) for divalent glycocluster **9**. Experimental data (black squares) were fitted by using the steady state model to obtain values for the thermodynamic association constants.

ize its interaction with Con A. The concentration-dependent overlay sensorgrams of **8** and **9** were recorded in Figure 1. It is shown that the mannose—Con A interactions are rapid association and dissociation processes and a plateau was reached before the dissociation phase for each injection. Thus, affinity constants for both of two glycocluster were calculated by using a general steady state model that assumes the system achieved equilibrium during sample injections.[31] A plot of the response signal at equilibrium as a function of concentration for each of glycoclusters is adjusted to a hyperbolic equation from which the thermodynamic affinity constants can be obtained as described in Figure 2. The K_A values of divalent and tetravalent glycoclusters were respectively $1.35 \times 10^4\ M^{-1}$ and $8.01 \times 10^4\ M^{-1}$, indicative of 3.0-fold increases in binding affinity for tetravalent **8** relative to divalent **9** (valency-corrected values). So, it can be inferred that this tetravalent glycocluster is effective in improving the weak carbohydrate–protein interaction through 'glycoside cluster effects'.

In summary, we have developed a facile strategy for the synthesis of cyclopeptide-centered glycoclusters. The cyclopeptide scaffolds bearing di- or tetra-alkynes have been synthesized for highly chemoselective and efficient attachment of azido monomannose through Cu(I) catalyzed Huisgen 1,3-dipolar cycloaddition. Furthermore, the affinity of these two synthetic multivalent glycoclusters **8** and **9** to Con A have been determined by SPR technique. It is observed that tetravalent glycocluster **8** has 3.0-fold increase in binding affinity relative to divalent glycocluster **9** (valency-corrected values), which indicates the potential of this system in investigating carbohydrate–protein interactions. The multivalent cyclopeptide-centered glycoclusters bearing more complex oligosaccharides have been also prepared through this strategy in our lab using the readily accessible azido oligosaccharides, which will be reported in due time. In addition, the flexible peptide synthesis provides the chance to develop analogous cyclopeptide scaffolds with various size and spatial orientation for presenting multivalent carbohydrates to be suitable for the corresponding carbohydrate-binding proteins.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Projects 20532020). We would thank Ms. Chen Yuanyuan (Institution of Biophysics, Chinese Academic of Science) for SPR support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.04.090.

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