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Biomolecular assembly by iterative oxime ligations

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ABSTRACT

Herein we describe an iterative oxime-based procedure to prepare multivalent bioconjugates. Our approach is illustrated by the assembly of structurally diverse tetravalent and a new generation of hexadecavalent glycoclusters.

The synthesis of new functional biomolecules remains challenging for bioorganic chemists. For this purpose, the assembly of biomolecular devices endowing with specific function (cell recognition, toxicity, detection) constitutes a way for the development of new classes of therapeutics and diagnosis. The major barriers to the chemical synthesis of biomolecular assemblies arise from the incompatibility of the reaction conditions that are respectively involved in the chemistry of peptides, carbohydrates and nucleic acids. Chemoselective ligations are commonly used to overcome this difficulty. Particularly, the oxime ligation is becoming increasingly useful for the preparation of bioconjugates.[1] First developed by Rose for the synthesis of artificial proteins,[2] the oxime bond results from the reaction between an aldehyde and an aminooxy function. It has been shown to be stable in vivo[3] and it was largely exploited, among other applications, to prepare synthetic vaccines,[4] microarrays,[5] imaging agents for monitoring,[6] chemical engineering,[7] combinatorial libraries,[8] therapeutic agents for cancer treatment,[9] and protein mimics.[10] To prepare such complex biomolecular systems, we have recently reported efficient and simple chemical methods.[11]

In the course of our investigations,[12] we report herein a strategy to access to more sophisticated molecules by three successive oxime ligations. To illustrate this strategy, we performed the ligation of biologically relevant carbohydrates and peptides onto a cyclopeptidic scaffold displaying two addressable domains (Fig. 1). Our synthetic procedure first requires the preparation of suitable, unprotected peptide and carbohydrate building blocks that should contain either serine moieties as masked glyoxylyl aldehydes and/or aminooxy functions. By performing successive steps comprising serine oxidation and oxime ligation, a peptide is next coupled to upper side of the template. The carbohydrate cluster is finally introduced on the other side, alternatively on the lysine side chains or through a polylysine dendrimer. We thus obtained tetravalent (Fig. 1A) and hexadecavalent glycocluters respectively (Fig. 1B).

In a first example, a cyclopeptide **7** displaying four serines and two aminooxy functions was prepared on Rink-Amide MBHA® resin. The orthogonally protected decapeptide **2** was assembled from D-glutamic acid following the standard Fmoc protocol (Scheme 1).[13] After removal of allyl and Fmoc protecting groups, the linear peptide **3** was cyclized on the solid support. This crucial step was controlled by both Kaiser and 2,4,6-trinitrobenzene sulfonic acid (TNBS)[14] tests to ensure a complete conversion. RP-HPLC and mass spectrometry analyses of a resin aliquot treated by trifluoroacetic acid (TFA) revealed a clean crude reaction mixture at this stage, showing neither linear peptide nor truncated structures. Further functionalization of the cyclic template **4** was realized sequentially, after regioselective removal of *p*-nitrobenzyloxycarbonyl (*p*NZ) and 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl (Dde) orthogonal protecting groups. To introduce serines, *p*NZ were first removed by treatment with $SnCl_2$[15] and protected serines were then coupled with PyBOP to afford compound **5**. Dde deprotection was carried out with hydrazine[16] and protected aminooxy acetic acid was subsequently introduced on the resulting lysine side chains. The final cleavage by acidolysis and preparative RP-HPLC provided the expected product **7** in 20% yield.

We first attempted to conjugate different classes of biomolecules on both addressable domains of the peptide scaffold **7** (Scheme 1). A cyclo[-RGDfK-] peptide bearing an aldehyde function,[17] that is a ligand of the αvβ3 integrin, was coupled onto **7** through oxime linkages in acidic aqueous buffer. This reaction

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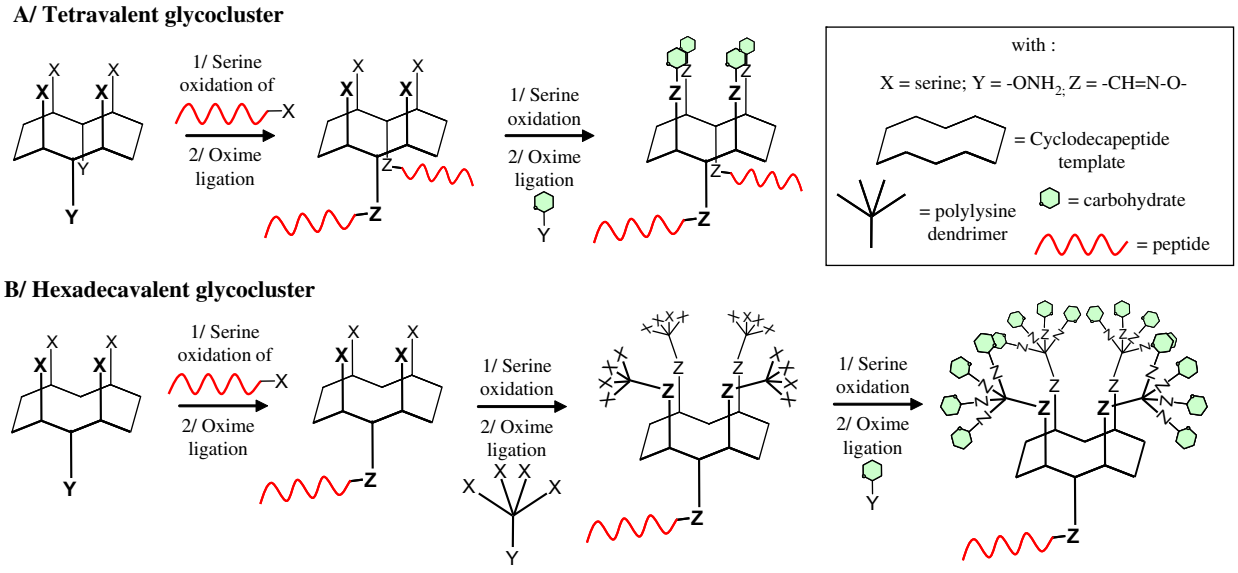
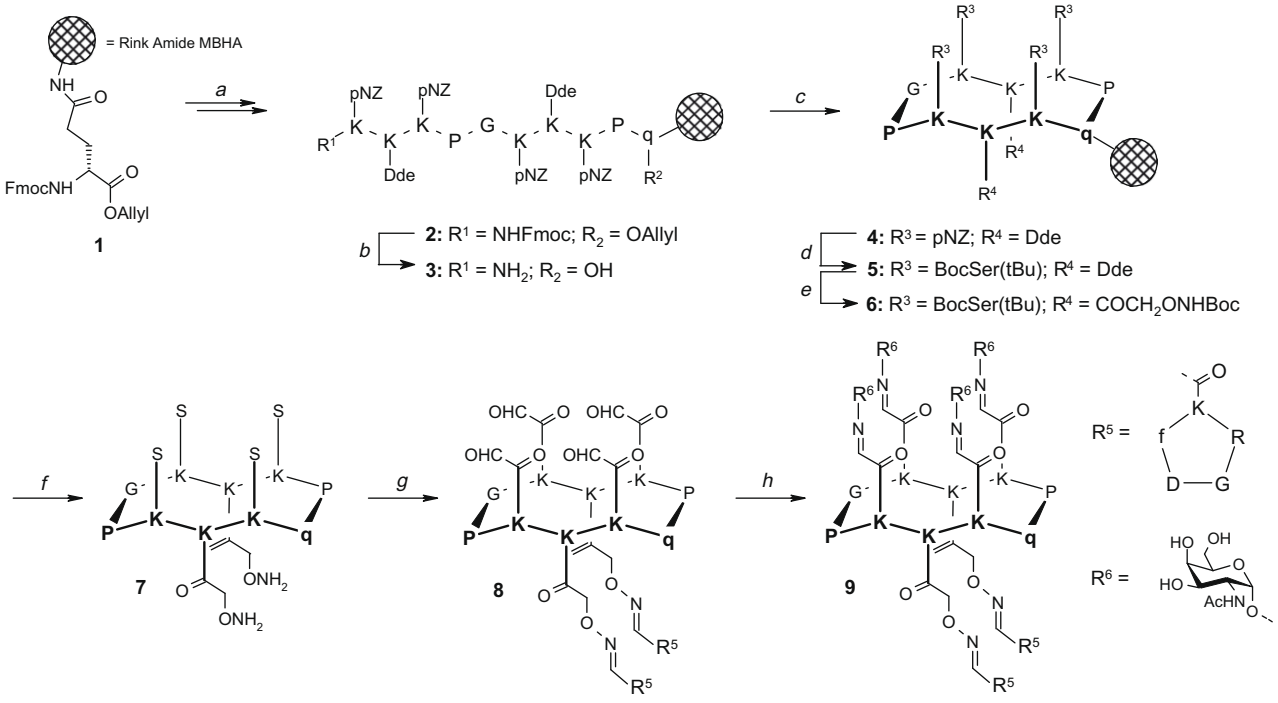


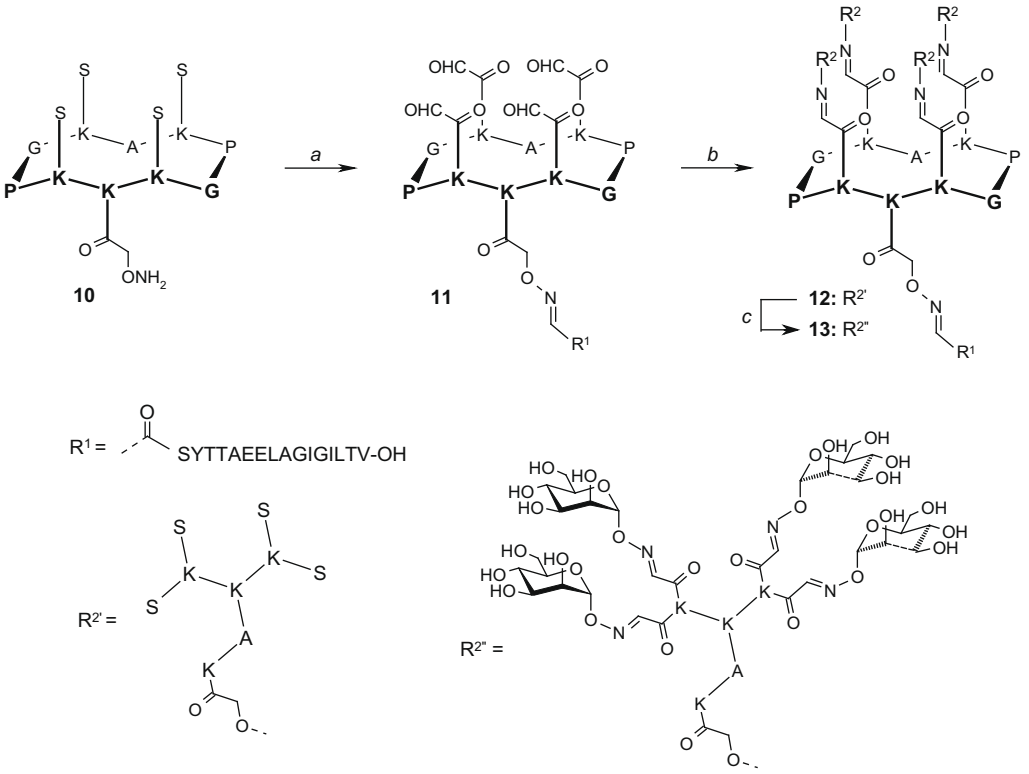
Figure 1. Iterative process (oxidation/oxime ligation) for the assembly of tetravalent glycocluster (A) and hexadecavalent glycocluster (B).



Scheme 1. Reagents and conditions: (a) solid phase peptide synthesis, Fmoc strategy, Fmoc-aa-OH (2 equiv); PyBOP (2 equiv), DIPEA (5 equiv), DMF; (b) (i) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, 2 × 20 min; (ii) 20% (v/v) piperidine:DMF, 1 × 10 min, 2 × 5 min; (c) PyAOP, DIPEA, DMF, 2 × 30 min; (d) (i) $SnCl_2$ 2 M, Phenol 0.01 M, AcOH 1.6 mM, DMF, 3 × 2 h; (ii) BocSer(tBu)OH, PyBOP, DIPEA, DMF; (e) (i) 2% hydrazine, DMF, 4 × 10 min; (ii) BocAoaOSu, DIPEA, DMF, 1 h; (f) TFA/TIS/H_2O (95:2.5:2.5), 2 × 1 h; (g) (i) c[-RGDfK-]-CHO, 0.1 M AcONa pH 4.6/CH_3CN (1:1); (ii) $NaIO_4$, H_2O; (h) GalNAcαONH2, 0.1 M AcONa pH 4.6.

occurred quantitatively, showing a clean crude reaction mixture by HPLC. The subsequent periodate oxidation under mild conditions allowed the generation of glycoxylyl aldehyde functions from serines,[18] providing **8** after RP-HPLC purification. Carbohydrate binding motifs were next introduced. This final chemoselective oxime coupling was performed with an excess of αGalNAc modified at the anomer position with an aminooxy function following a similar procedure.[19] We thus obtained the tetravalent glycopeptide **9** with 70% yield. At this stage, it has to be mentioned that undesirable transoximation side reaction was not observed during this successive oxime ligation process.

A similar iterative strategy was next envisioned to prepare a new generation of cyclopeptide-based glycocluster exhibiting sixteen copies of carbohydrates. For that purpose, we designed a hexadecavalent mannosyl cluster containing a peptide fragment of the Melan-A/Mart-1 protein[20] using three successive oxime ligations. This latter peptide bearing a serine at N-terminal end was first oxidized to be introduced onto **10** (Scheme 2). Further serine oxidation of the template provided anchoring sites for chemoselective coupling of a polylysine dendrimer displaying four serines and one oxyamine function.[11d] Resulting template **12** thus bearing sixteen serine residues was subsequently oxidized. The final oxime



Scheme 2. Reagents and conditions: (a) (i) MELAN-CHO, AcOH/H_2O/CH_3CN (0.5:3:1); (ii) $NaIO_4$, H_2O; (b) Polylysine-ONH_2, 10% AcOH:H_2O (v/v); (c) (i) $NaIO_4$, H_2O; (ii) ManαONH2, 10% AcOH:H_2O (v/v).

assembly with aminooxy mannose derivatives[19] yielded to the desired hexadecavalent glycoconjugate **14** containing a total of 21 oxime linkages.

As indicated in Table 1, mass spectrometry analyses have shown molecular weight for each bioconjugates in a good agreement with the expected calculated values. In addition, quantitative conversion was observed by analytical HPLC for each oxidation and oxime coupling steps, confirming the efficiency of our stepwise procedure. However, it has to be mentioned that expected materials **11** and **12** were only partially recovered (43–56% yield) from HPLC purification, presumably due to their low aqueous solubility.

To conclude, we have reported herein a simple and iterative strategy that allowed the syntheses of sophisticated biomolecules from deprotected peptides and carbohydrate building blocks by successive serine oxidation and oxime coupling under mild conditions. In a first part of this study, two different recognition motifs (cycloRGD peptide and αGalNAc residues) have been combined in a multivalent display within the same peptide backbone **9**. Such a bi-functional conjugate can bind simultaneously to both integrins and carbohydrate binding proteins.[21] Secondly, a new generation of hexacavalent glycocluster such as **13**, which display a melanoma tumor antigen and a mannosyl cluster has been assembled. As shown recently, similar molecular vectors were found to induce efficient uptake and presentation of tumor antigens by dendritic cells.[22] We anticipate that the glycocluster-tumor antigen conjugate **13** described in our report might represent a promising model for the design of synthetic vaccines. Biological assays in this perspective are currently investigated and will be reported in due course

Table 1
Yields and MS data for compounds **8–9** and **11–13**

Compds	Isolated yield[a] (%)	ES-MS analysis[b]	
		Calculated	Found
8	61	2801.3	2801.3
9	70	3673.7	3674.0
11	56	2992.4	2992.4
12	43	7006.8	7007.4
13	70	9347.5	9347.0

[a] Yields were not optimized. They are given after preparative RP-HPLC either after oxime ligation for **9**, **12** and **13** or oxime ligation/serine oxidation steps for **8** and **11**.

[b] Mass spectrometry analysis was performed by electrospray ionisation method in positive mode (ESI-MS).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.03.119.

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