Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 48 (2007) 5055–5060

Hyaluronan-based glycoclusters as probes for chemical glycobiology

Shyam M. Rele,[a,*] Suri S. Iyer[a] and Elliot L. Chaikof[a,b,*]

[a]*Departments of Surgery and Biomedical Engineering, Emory University School of Medicine, 101 Woodruf Circle, Rm 5105, Atlanta, GA 30322, United States*
[b]*School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA 30332, United States*

Received 8 April 2007; revised 14 May 2007; accepted 16 May 2007
Available online 23 May 2007

Abstract—A strategy is described for the synthesis of β-(1,3)-GlcA-GlcNAc dimeric and tetrameric glycoclusters through the conjugation of disaccharide groups onto a diaminodiamide aromatic scaffold by reductive amination.

Hyaluronan (HA, **1**) is a linear non-sulfated high molecular weight glycosoaminoglycan (GAG) composed of repeating disaccharide units of β-D-glucuronic acid (GlcA) and *N*-acetyl glucosamine (GlcNAc) linked by alternating β-(1,3) and β-(1,4) glycosidic linkages (Fig. 1). HA is found in the extracellular matrix, at the cell surface, inside cells, and has been implicated in a variety of biological processes, including inflammation, tumorogenesis, and wound repair.[1] Specifically, the unique biophysical and biological properties of HA appear to have a profound influence on the structural integrity, as well as the biomechanical, and physiological properties of the extracellular and pericellular matrix. In the process, HA serves as a template for the assembly of other macromolecules and interacts directly with cell surface receptors (CD44, RHAMM) that influence a number of physiological events, including cell adhesion, migration, and proliferation.[2–4] Given the potential significance of therapies based on controlling GAG–protein interactions, the chemical preparation of HA and related analogues remains an area of active investigation. In this regard, it is noteworthy that potent modulators of inflammation,[5] chemokine gene expression,[6] angiogenesis,[7] and tumor growth[8] have been derived from low molecular mass HA fragments (3–10 disaccharides).

Carbohydrate–protein recognition events, such as those mediated by HA, are often driven by interactions that generate glycoligand-receptor complexes via a glycoside cluster effect (Fig. 1).[9] As such, the synthesis of multi-antennary saccharide derivatives based upon a variety of multivalent scaffolds, including glycoproteins,[10] calixarenes,[11] cyclodextrins,[12] as well as linear,

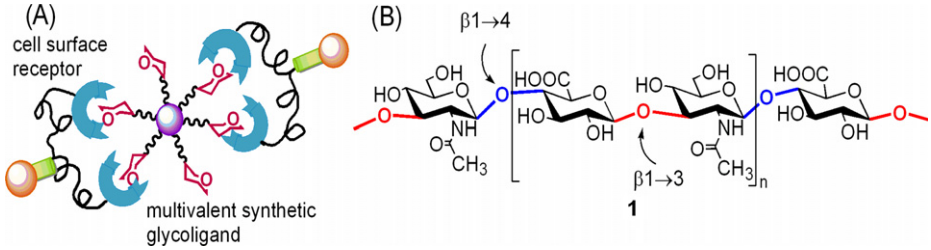

Figure 1. (a) Polyvalent glycoconjugate-receptor molecular recognition. (b) Structure of the hyaluronan repeat unit (**1**).

Keywords: Disaccharides; Hyaluronan; Glycomimetic; Multivalent.
* Corresponding authors. Tel.: +1 404 727 8413; fax: +1 404 727 3660; e-mail addresses: shyamrele@yahoo.com; echaiko@emory.edu

0040-4039/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2007.05.097

branched, and dendrimeric polymers,[13,14] has been reported.

In continuing our effort in the rational design of multivalent glycoforms,[15] we present a convergent strategy for the synthesis of clustered, multivalent HA-mimetics. Our synthetic approach involves three steps: (i) the synthesis of an HA-like β-(1,3)-linked disaccharide with a *n*-pentenyl spacer arm; (ii) the generation of conformationally rigid aromatic diamidodiamine scaffold; and (iii) the chemical conjugation of the disaccharide to the diamide template via reductive amination.

While carbohydrate–protein interactions are primarily dominated by hydrogen bonding and van der Waals interactions, the stacking of aromatic side chains and hydrophobic domains on pyranosyl rings has been reported to play a critical role in enhancing the specificity and stability (enthalpic and entropic contribution) in such interactions.[16] For instance, aromatic glycosides bind to concavalin A more strongly than aliphatic ones, indicating the presence of a hydrophobic region in proximity of the carbohydrate-binding site.[17] All told, designing an appropriate polyvalent ligand for optimized binding activity is influenced by the nature of the scaffold and the length of the tether between the scaffold and the carbohydrate, as well as by the aglycon itself (Fig. 2).

To this end, the rationale for selecting an aromatic diamide scaffold for the assembly of head-to-head HA-derived oligomers was two fold. First, we postulated that glycoligand–protein binding affinity would be further augmented by the potential for secondary hydrophobic and hydrogen-bonding interactions provided, respectively, by the aromatic and amide/amine groups in the short peptide skeleton. In addition, by incorporating different R substituents in the scaffold backbone, the spatial presentation and conformational mobility of the pendant saccharides could be altered as additional structural variables for optimized binding. Second, we envisioned that the incorporation of HA disaccharide epitopes onto a restricted scaffold via a flexible aliphatic linker would minimize direct steric interactions between carbohydrate residues and the diamide scaffold. Since ligand affinity and specificity is often dependant upon the proper spacing and orientation of the carbohydrate residues, we anticipate that such compounds would be able to preorganize and fold into a conformation suitable for facilitating clustering, while concomitantly promoting protein–protein interactions to a greater degree than monovalent counterparts. Structure-based design of such clustered analogues with distinct pharmacophoric sugar recognition domains can easily be screened and could provide a mechanism to facilitate receptor recognition resulting in the identification of binding agonists or inhibitors (Fig. 2).

Based on our initial assessment,[15a] the general methodology for the synthesis of *n*-pentenyl terminated glycoside acceptor **8** is summarized in Scheme 1. Initially, the azido group was incorporated at the 2-position of D-glucosamine hydrochloride using triflic azide (TfN_3) followed by acetylation affording 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-D-glucopyranosyl acetate **4**.[18] Selective hydrolysis of the anomeric acetate to hydroxyl group **5** was performed using hydrazine acetate, which was then converted to the activated imidate derivative **6** in 74% yield. The installation of the *n*-pentenyl group as a spacer at the C-1 anomeric position was accomplished using catalytic amount of TMSOTf as a promoter at 0 °C. Deacetylation of the mixture using NaOMe subsequently realized triol **7** in 80% yield. Benzylidenation of **7** with benzaldehyde dimethylacetal in the presence of a catalytic amount of camphorsulfonic acid yielded the corresponding *n*-pentenyl glycoside acceptor **8** (α:β = 70:30 mixture), which served as the key building block for generating β-(1,3) linked HA disaccharide-like glycoclusters. The α- and β-isomers were separated using column chromatography and the β-isomer (**8b**) was further elaborated. The glucuronic acid imidate donor served as the other glycosylating coupling partner and was synthesized in 50% yield in two steps from commercially available acetobromo-α-D-glucuronic acid methyl ester **9** (Scheme 2).[15a] Specifically, **9** was converted to its corresponding free hemiacetal **10** using $CdCO_3/H_2O$, which was subsequently converted to the active α-imidate derivative **11** using trichloroacetonitrile and the initiator 1,8-diazabicyclo [5,4,0]undec-7-ene, as a base. Glycosylation of *n*-pentenyl glycoside acceptor **8b** with trichloroacetimidate donor **11** in the presence of a Lewis catalyst TMSOTf

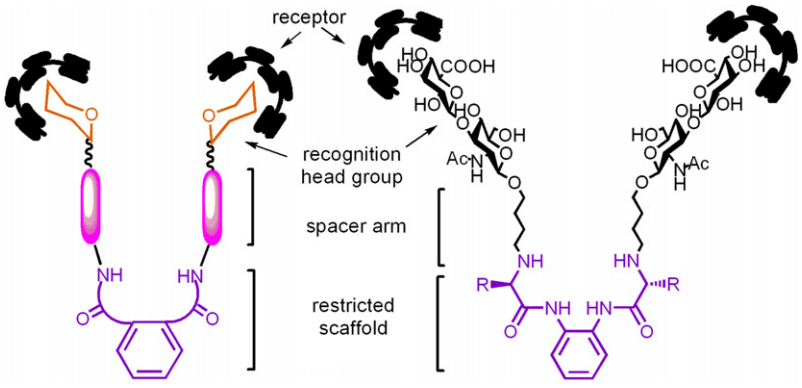

Figure 2. A biantennary HA-glycoconjugate attached to a conformationally rigid scaffold.