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,

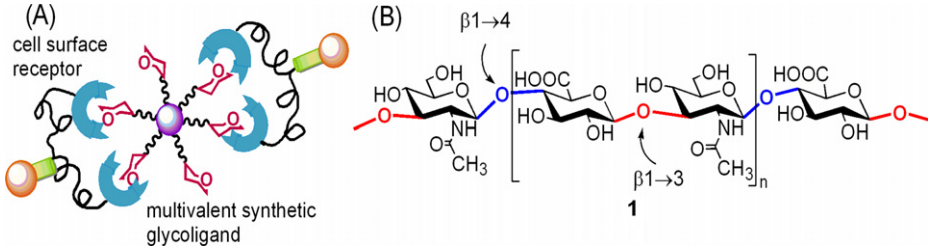


Figure 1. (a) Polyvalent glycoconjugate-receptor molecular recognition. (b) Structure of the hyaluronan repeat unit (**1**).

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

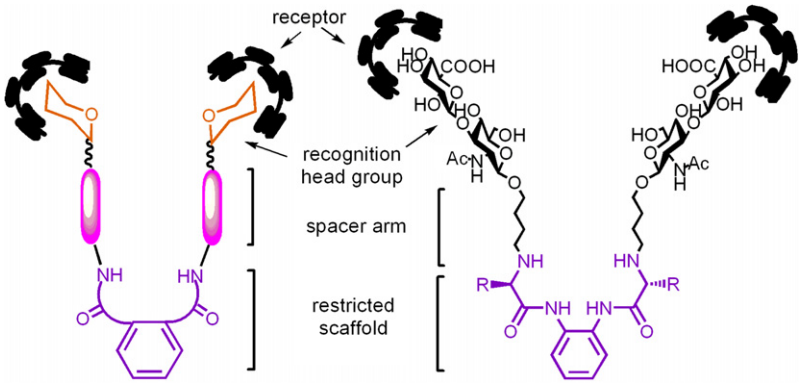
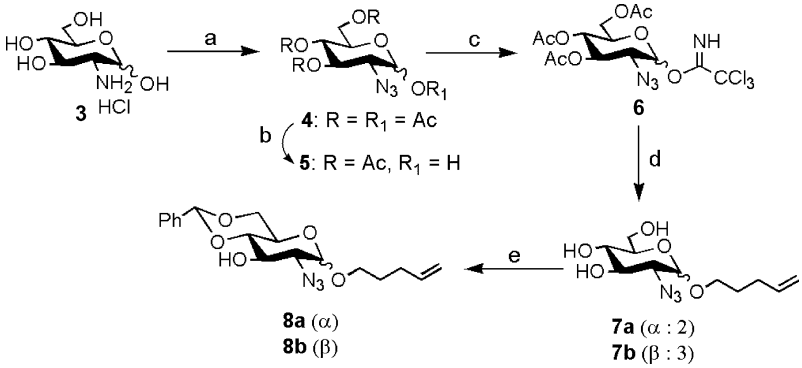
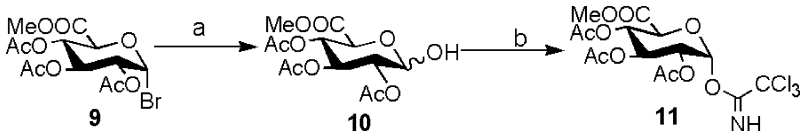


Figure 2. A biantennary HA-glycoconjugate attached to a conformationally rigid scaffold.



Scheme 1. Synthesis of *n*-pentenyl glycoside acceptor **8b**. Reagents and conditions: (a) (i) TfN_3, MeOH, DMAP, 25 °C, 18 h; (ii) Ac_2O, pyridine, 0 °C, 10 h, yield = 75%; (b) $H_2NNH_2{\cdot}AcOH$, DMF, 0 °C to 25 °C, 45 min, yield = 70%; (c) anhydrous K_2CO_3, CCl_3CN, dry CH_2Cl_2, 25 °C, 48 h, yield = 74%; (d) (i) 4 Å Molecular sieves, TMSOTf, 4-pentene-1-ol, dry CH_2Cl_2, 0 °C, 1 h; (ii) MeONa, MeOH, 0 °C to 25 °C, 6 h, yield = 80% over two steps; (e) camphorsulfonic acid, dry THF, $C_6H_5CH(OMe)_2$, reflux, 6 h, yield = 75%.

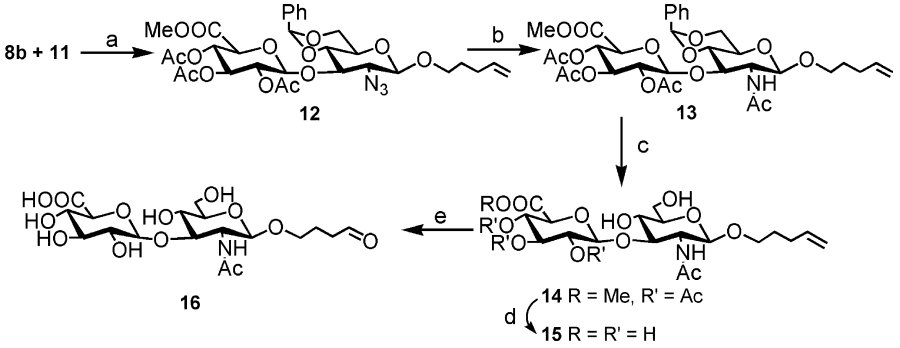


Scheme 2. Synthesis of imidate donor **11**. Reagents and conditions: (a) $CdCO_3$, H_2O (2 equiv), CH_3CN, 75 °C, 4 h, yield = 75%; (b) CCl_3CN, 1,2-dichloroethane, 1,8-DBU, −10 to 0 °C, 1 h, yield = 60%.

afforded the stereospecific β-(1,3) linked disaccharide **12** in 78% yield (Scheme 3).

Characterization of compound **12** by NMR and FAB-MS spectrum was in agreement with the expected structure. The resonances for the anomeric proton were doublets and had coupling constants consistent with assignment of the β-configuration. For example, ^{1}H NMR of **12** displayed the anomeric protons (H-1 and H-1′) as a doublet at δ 4.78 ($J = 8.0$ Hz) characteristic of the 1,2-trans system. Reduction of the azido functionality to the acetamido (NHAc) group was performed using thioacetic acid,[19] which furnished **13** in appreciable yield (70%).

The ^{1}H NMR spectrum of **13** showed a variable doublet between δ 5.8 and 6.3 ($J = 6.8$–9.2 Hz) ppm attributing to the –NH proton of the acetamido group in addition to the acetyl peak at 1.94 ppm. Selective removal of the benzylidene protecting group using aqueous TFA gave **14**, which was subsequently deesterified and deacetylated using 3 M NaOH in MeOH/water (9:1) mixture to yield the completely deprotected disaccharide **15** in 86% yield. The crude product was purified using Sephadex LH-20 with MeOH as the eluant. The synthesized disaccharide fragment obtained is similar to the native β-(1,3) linked region of hyaluronan (**1**) with a pendant *n*-pentenyl functionality as a spacer arm at the anomeric position of the GlcNAc moiety (Fig. 3). The presence of characteristic signals in the ^{13}C NMR spectrum at δ 173.0 (–COOH), 171.0 (–NHCO) along with resonance signals at δ 138.2 (=CH), 114.1 ($=CH_2$), 103.8 and 101.1 (C-1 and C-1′), and 22.0 ($-COCH_3$) ppm further corroborated the formation of compound **15** (FAB-MS $[M^+ + 2Li - H] = 478.2068$).



Scheme 3. Synthesis of HA-mimetic β-(1,3)-linked disaccharide. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, 0 to 25 °C, 3.5 h, yield = 78%; (b) CH_3COSH, 25 °C, 24 h, yield = 70%; (c) 3 mL of TFA/H_2O (2:1), CH_2Cl_2, 0 °C, 1 h, yield = 86%; (d) 3 M NaOH, 9:1 MeOH/H_2O, 25 °C, 2 h, yield = 86%; (e) O_3 (−78 °C), then add Me_2S, −78 °C to 25 °C, 24 h, yield = 85%.

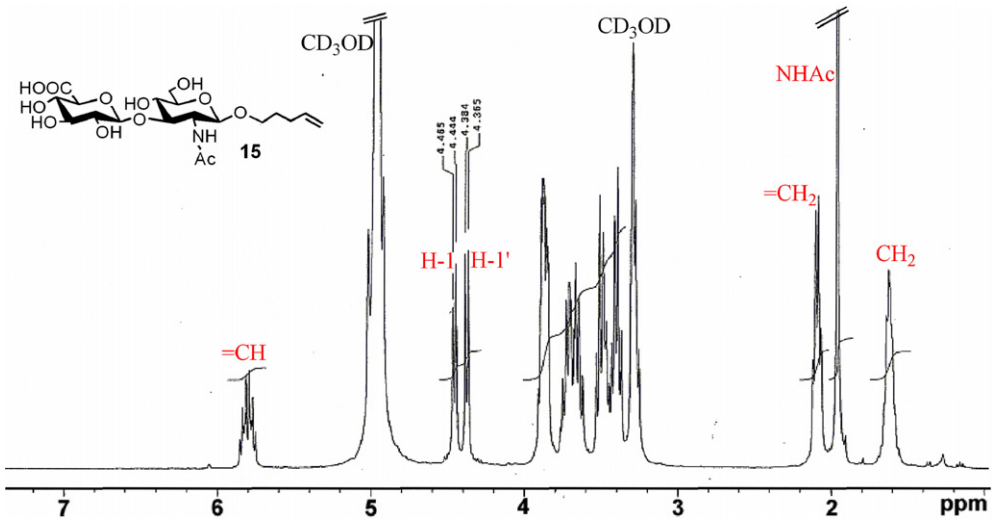
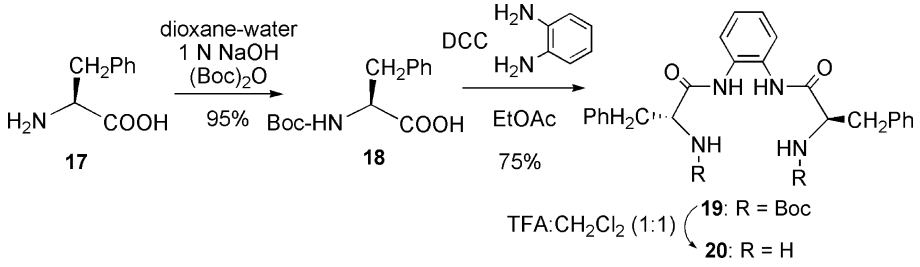


Figure 3. ^{1}H NMR of a HA-mimetic β-(1,3)-linked disaccharide.

The presence of an *n*-pentenyl group in compound **15** potentially serves as a versatile handle by transformation of the terminal olefin to either an aldehyde, carboxylic acid, ester, thioether, thioester or hydroxyl group.[20] To this end, *n*-pentenyl disaccharide **15** was subjected to reductive ozonolysis, which afforded a terminal aldehyde glycoside intermediate **16** in 85% yield (Scheme 3). The appearance of the aldehyde signal at δ 9.63 and 201.2 ppm in the ^{1}H and ^{13}C NMR spectrum, respectively, along with the disappearance of the olefinic double bond peaks at δ 5.81 (δ_C 138.2) and 5.1–4.9 (δ_C 114.1) from the parent compound confirmed the formation of **16** (FAB-MS $[M^{+}+Li] = 474.2550$).

A diamino diamide scaffold was synthesized for the covalent attachment of the carbohydrate epitopes (Scheme 4). *N*-Boc-protected phenylalanine amino acid, which was prepared by standard protection methods,[21] was coupled with *o*-phenylenediamine in a 2:1 ratio using dicyclohexyl carbodiimide (DCC) as a condensating agent at room temperature to yield the corresponding diamide adduct **19**. Subsequently, the carbamate function in **19** was cleaved with a 50% solution of CF_3COOH (TFA) in CH_2Cl_2 to afford diamino diamide scaffold **20**.

Further extension of the four carbon aliphatic glycosyl aldehydes by reductive amination of **16** with **20** not only resulted in the formation of symmetrically branched β-(1,3)-linked HA dimer **21** (32%) as the major product, but also furnished the tetrameric derivative **22** (15%), albeit in low yield (Scheme 5).[22] Repeated purification of the crude to separate the individual components using Sephadex G-25 was unsuccessful. The complex crude mixture was subsequently purified using C18 reverse phase chromatography using a CH_3CN/H_2O gradient system in 0.1% TFA (see Supplementary data). The collected fractions were screened for homogeneity by analytical HPLC (C-18 silica column, 120 A μBore) and MALDI-TOF (Fig. 4), which confirmed the formation of dimer **21** (M^{+} 1304.407) and tetramer **22** (M^{+} 2206.521). Furthermore, the number of sugars grafted onto the scaffold could also be calculated by comparing the resonance signals of the aromatic peaks from the diamino diamide unit with that of the acetamido (NHAc), methylene (CH_2), and anomeric peaks of the sugar residues (see Fig. 4). For example, the ratio of the relative intensities of ArH/anomeric H/NHAc/CH_2 yield a signal ratio of 3.52:1.0:1.48:1.96, indicating the formation of the dimeric ligand **21**. As the number of carbohydrates grafted on the scaffold increased, as in the case of tetramer **22**, the resonance signals in the proton NMR appeared to broaden with an observed signal ratio of 1.78:1.0:1.52:2.07.



Scheme 4. Synthesis of a diamido diamine scaffold.

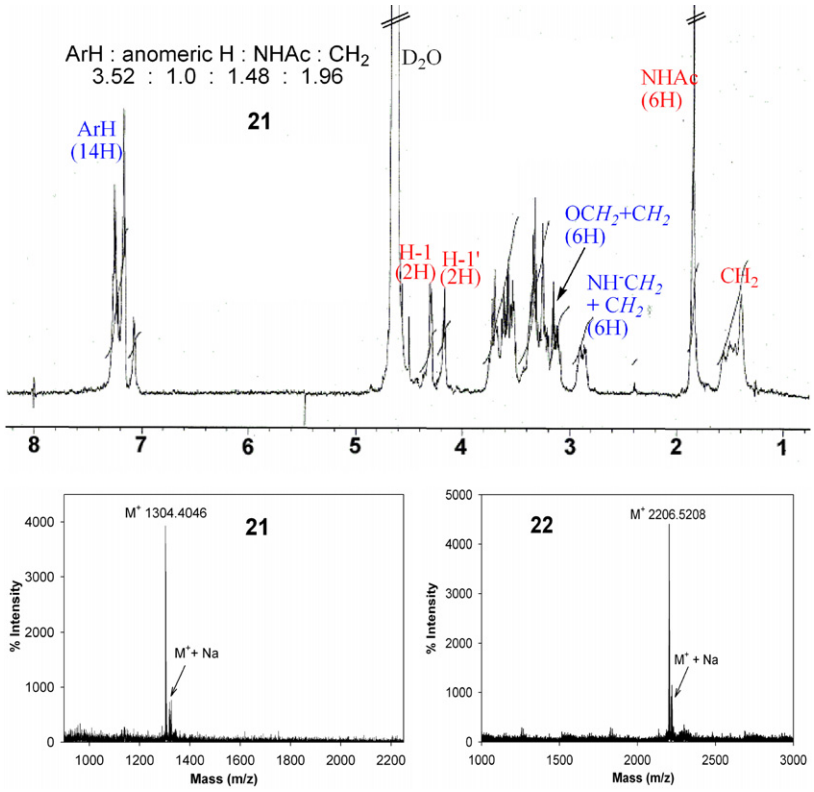
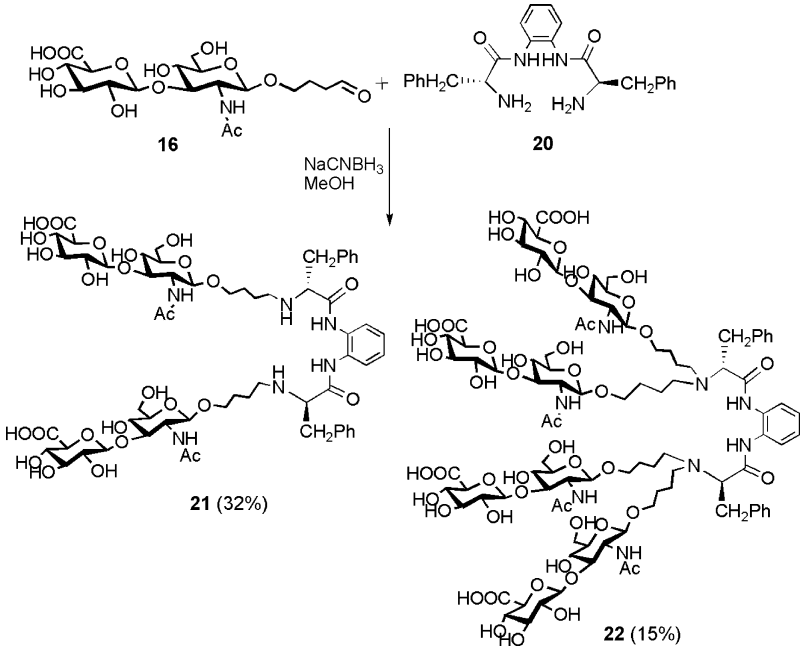


Figure 4. ^{1}H NMR of the dimeric HA-mimetic **21** and MALDI-TOF of the dimeric (**21**) and tetrameric (**22**) glycoclusters.



Scheme 5. Synthesis of HA multidentate glycoclusters.

In summary, we have designed and synthesized dimeric and tetrameric HA-mimetic glycoclusters by grafting β-(1,3) GlcA-GlcNAc disaccharides onto an aromatic scaffold. The biological evaluation of these compounds and their potential as novel glycotherapeutics is ongoing.

Acknowledgments

The authors thank Dr. J. Pohl, M. Slamova, and P. Svobada (Microchemical Facility, Emory University) for assistance in conducting HPLC.

Supplementary data

Experimental procedures and spectral data are provided for compounds **8b**, **12**–**16**, and **18**–**22**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.05.097.

References and notes

1. (a) Lapcik, L., Jr.; Lapcik, L.; Smedt, S.; Demeester, J.; Chabrecek, P. *Chem. Rev.* **1998**, *98*, 663–2684; (b) Underhill, C. B. In *The Biology of Hyaluronan*; Foundation, C., Ed.; John Wiley: Chichester, UK, 1989; pp 87–106; (c) Laurent, T. C. *The Chemistry Biology and Medical Applications of Hyaluronan and its Derivatives*; Portland: London, 1998.
2. Toole, B. P. *J. Clin. Invest.* **2000**, *106*, 335–336.
3. (a) Entwistle, J.; Hall, C. I.; Turley, E. A. *J. Cell Biochem.* **1996**, *61*, 569–577; (b) Evanko, S. P.; Wight, T. N. *J. Histochem. Cytochem.* **1999**, *47*, 1331–1342.
4. Travis, J. A.; Hughes, M. G.; Wong, J. M.; Wagner, W. D.; Geary, R. L. *Circ. Res.* **2001**, *88*, 77–83.
5. (a) McKee, C.; Penno, M.; Cowman, M.; Burdick, M.; Strieter, R.; Bao, C.; Noble, P. *J. Clin. Invest.* **1996**, *98*, 2403–2413; (b) McKee, C.; Lowenstein, C.; Horton, M.; Wu, J.; Bao, C.; Chin, B.; Choi, A.; Noble, P. *J. Biol. Chem.* **1997**, *272*, 8013–8018; (c) Hodge-Dufour, J.; Noble, P.; Horton, M. R.; Bao, C.; Wysoka, M.; Burdick, M. D.; Strieter, R. M.; Trinchieri, G.; Pure', E. *J. Immunol.* **1997**, *15*, 2492–2500.
6. McKee, C. M.; Penno, M. B.; Cowman, M.; Burdick, M. D.; Strieter, R. M.; Bao, C.; Noble, P. W. *J. Clin. Invest.* **1996**, *98*, 2403–2413.
7. (a) Lees, V. C.; Fan, T. P.; West, D. C. *Lab. Invest.* **1995**, *73*, 259–266; (b) West, D.; Hampson, I.; Arnold, F.; Kumar, S. *Science* **1985**, *228*, 1324–1326.
8. Zeng, C.; Toole, B. P.; Kinney, S. D.; Kuo, J. W.; Stamenkovic, I. *Int. J. Cancer* **1996**, *77*, 396–401.
9. (a) Lundquist, J. J.; Toone, E. *J. Chem. Rev.* **2002**, *102*, 555–578; (b) Mammen, M.; Choi, S.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2754–2794.
10. (a) Baigude, H.; Katsuraya, K.; Okuyama, K.; Tokunaga, S.; Uryu, T. *Macromolecules* **2003**, *36*, 7100–7106; (b) Veprek, P.; Jezek, J. *J. Pept. Sci.* **1999**, *5*, 5–23.
11. Hayashida, O.; Mizuki, K.; Akagi, K.; Matsuo, A.; Kanamori, T.; Nakai, T.; Sando, S.; Aoyama, Y. *J. Am. Chem. Soc.* **2003**, *125*, 594–601.
12. Benito, J. M.; Gomez-Garcia, M.; Mellet, C.; Baussanne, I.; Defaye, J.; Fernandez, J. M. *J. Am. Chem. Soc.* **2004**, *66*, 7786–7795.
13. (a) Kobayashi, K.; Tsuchida, A.; Usui, T.; Akaike, T. *Macromolecules* **1997**, *30*, 2016–2020; (b) Choi, S. K.; Mammen, N.; Whitesides, G. M. *J. Am. Chem. Soc.* **1997**, *119*, 4103–4111; (c) Zanini, D.; Roy, R. *J. Org. Chem.* **1998**, *63*, 3486–3491.
14. (a) Ashton, P. R.; Boyd, S. E.; Brown, C. L.; Jayaraman, N.; Nepogodiev, S. A.; Stoddart, J. F. *Chem. Eur. J.* **1996**, *2*, 1115–1128; (b) Kensinger, R.; Yowler, B.; Benesi, A.; Schengrund, C.-L. *Bioconjugate Chem.* **2004**, *15*, 349–358.
15. (a) Iyer, S. S.; Rele, S. M.; Baskaran, S.; Chaikof, E. L. *Tetrahedron* **2003**, *59*, 631–638; (b) Rele, S. M.; Iyer, S. S.; Baskaran, S.; Chaikof, E. L. *J. Org. Chem.* **2004**, *69*, 9159–9170; (c) Rele, S. M.; Cui, W.; Hou, S.; Barr-Zarse, G.; Tatton, D.; Gnanou, Y.; Esko, J.; Chaikof, E. L. *J. Am. Chem. Soc.* **2005**, *127*, 10132–10133; (d) Iyer, S. S.; Rele, S. M.; Nolan, S.; Grasa, G.; Chaikof, E. L. *Chem. Commun.* **2003**, 1518–1519; (e) Rele, S. M.; Iyer, S. S.; Chaikof, E. L. *Tetrahedron Lett.* **2003**, *44*, 89–91.
16. (a) Quiocho, F. A. *Annu. Rev. Biochem.* **1986**, *55*, 287–315; (b) Weis, W. I.; Drickamer, K. *Annu. Rev. Biochem.* **1996**, *65*, 441–473; (c) Miyauchi, H.; Yuri, M.; Tanaka, M.; Kawamura, N.; Hayashi, M. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 989; (d) Davis, B. G. *J. Chem. Soc., Perkin Trans. 1* **1999**, 3215–3237.
17. Poretz, R. D.; Goldstein, I. *J. Biochem. Pharmacol.* **1971**, *20*, 2727.
18. Vasella, A.; Witzig, C.; Chiara, J.-L.; Martin-Lomas, M. *Helv. Chim. Acta* **1991**, *74*, 2073–2077.
19. Jacquinet, J. C. *Carbohydr. Res.* **1990**, *199*, 153–181.
20. Buskas, T.; Soderbegh, E.; Konradsson, P.; Fraser-Reid, B. *J. Org. Chem.* **2000**, *65*, 958–963.
21. Greene, T. W.; Wuts, P. G. *Protecting Groups in Organic Synthesis*, 3rd ed.; Wiley: New York, 1999, Chapter 7, p 494.
22. A small percentage (<5%) of mono and tri functionalized glycoligands could also be observed in the MALDI-TOF spectrum of the crude mixture, however no attempts were made to purify the compounds.