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Synthesis of a hyaluronan neoglycopolymer by ring-opening metathesis polymerization†

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A hyaluronan (HA)-derived disaccharide was synthesized bearing an n-pentenyl spacer arm, which facilitated disaccharide derivatization with a norbornene template. Subsequent ring opening metathesis polymerization of the monomer produced an HA-mimetic neoglycopolymer of low polydispersity.

Multivalent interactions of sugar ligands with cell surface receptors modulate a range of biomolecular recognition processes inducing unique cellular responses that are dramatically different from those elicited by monovalent interactions.[1–7] Characteristically, multiple copies of a monovalent glycoligand amplify the binding affinity and specificity due to a chelating effect.[1,2] For example, blocking the binding of influenza virus to red blood cells,[1] L-selectin inhibition[6] during inflammation and chemotactic responses to bacteria[7] can be facilitated and controlled by the interaction of scaffolded multivalent ligands with specific receptor proteins. This observation has led to the development of a number of novel neoglycopolymers that hold significant potential in pharmacotherapy, tissue engineering, and molecular diagnostics.

Hyaluronan (HA, **1**),[8] a linear non-sulfated glycosoaminoglycan (GAG) consisting of alternating β-(1–3) and β-(1–4) linked glucuronic acid (GlcA) and *N*-acetylglucosamine (GlcNAc) residues, influences a variety of cellular processes, such as cell growth, wound healing, and immunological responses, as well as cancer metastasis. Significantly, these effects are mediated by the ability of HA to interact with specific cellular receptors in a polyvalent fashion.[8] Enhancing or limiting these responses in a controlled manner underscores the need to generate multivalent HA-derived ligands that provide insight into relevant protein–carbohydrate binding events. Thus, we postulated that small HA fragments on a polymer backbone may provide a starting point for mimicking selective biological properties normally exhibited by the native polysaccharide (Fig. 1).

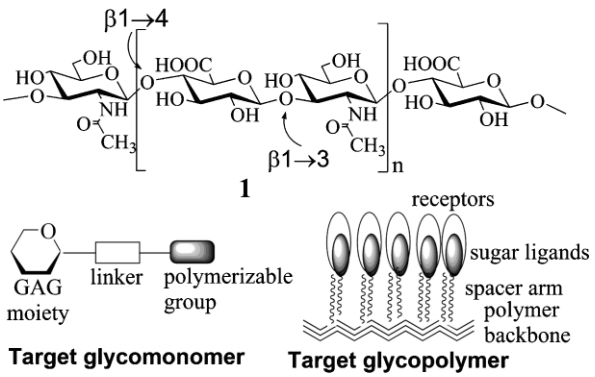

Fig. 1 Design of a hyaluronan-mimetic neoglycopolymer.

† Electronic supplementary information (ESI) available: spectral data for compound **11**, glycomonomer **14** and glycopolymer [**A**]. See http://www.rsc.org/suppdata/cc/b3/b301734f/

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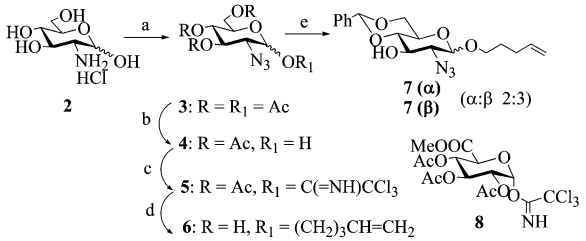
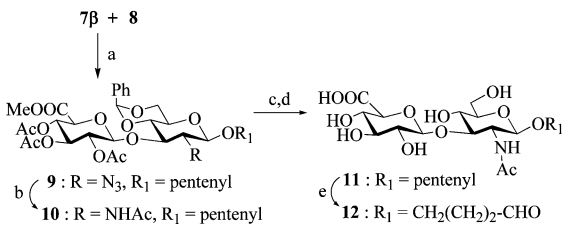
Although a number of chemical and enzymatic routes have been developed for the generation of multivalent ligands,[1,9,10] well-defined linear oligomers have proven more difficult to synthesize. To this end, ring-opening metathesis polymerization (ROMP)[3–5] using Grubbs catalysts[11,12] has been successfully applied to synthesize glycopolymers of defined lengths with unique bioactivities. Moreover, an added advantage of this methodology is its tolerance to a variety of polar functionalities, including hydroxyl, carboxylic acid, and sulfate groups.[3–5,11–13] As such, ROMP provides a facile strategy for the development of a polynorbornene based linear glycopolymer of low polydispersity bearing GlcA-β-(1–3)-GlcNAc pendant carbohydrate residues. This is the first report describing the use of ROMP for generating well-defined, multivalent HA-mimetic neoglycopolymers.

Our synthetic approach involved three major steps *viz.*, i) synthesis of a hyaluronan-derived disaccharide containing an n-pentenyl spacer arm; ii) attachment of the norbornene amine functionality to the disaccharide; and iii) subsequent ROMP of the designed monomer. The details for the synthesis of the HA-derived disaccharide are presented in Schemes 1 and 2, respectively. Significantly, the potential of the n-pentenyl group to serve as a versatile handle by transformation into various functionalities such as aldehyde, carboxylic acid, ester, thioether, thioester or hydroxyl group, enhances the scope and applicability of the strategy in the design and development of novel glycomimetics. Moreover, in contrast to the post-synthetic modification method,[4] initial incorporation of the



Scheme 1 *Reagents and conditions*: a) i) TfN_3, MeOH, DMAP, 25 °C, 18 h, ii) Ac_2O, pyridine, 0 °C, 10 h, 75%; b) H_2NNH_2·AcOH, DMF, 0–25 °C, 45 min, 70%; c) anhyd. K_2CO_3, CCl_3CN, CH_2Cl_2, 25 °C, 48 h, 74%; d) i) 4 Å mol. sieves, TMSOTf, 4-penten-1-ol, CH_2Cl_2, 0 °C, 1 h, ii) MeONa, MeOH, 0–25 °C, 6.0 h, 80% over two steps; e) CSA, THF, $C_6H_5CH(OMe)_2$, reflux, 6 h, 75%.

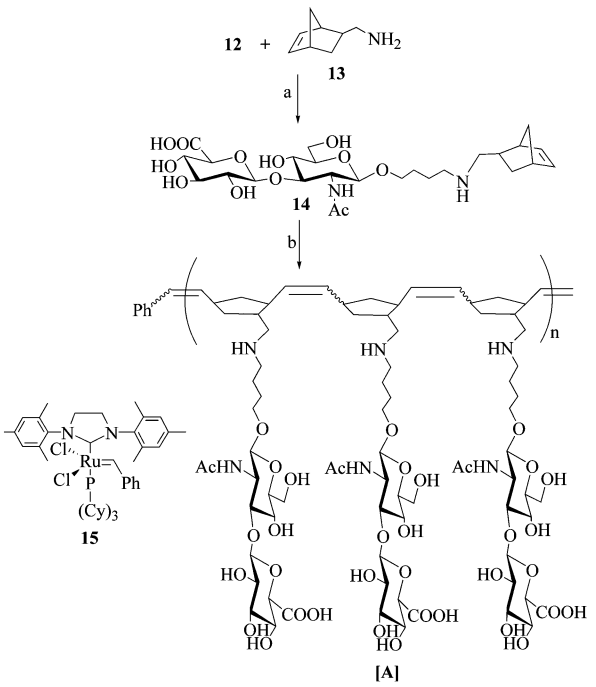


Scheme 2 *Reagents and conditions*: a) TMSOTf, CH_2Cl_2, 0–25 °C, 3.5 h, 78%; b) CH_3COSH, 25 °C, 24 h, 70%; c) TFA–H_2O (2 : 1), CH_2Cl_2, 0 °C, 1 h, 86%; d) 3 M NaOH, 9 : 1 MeOH–H_2O, 25 °C, 2 h, 86%; e) O_3 (−78 °C), then add Me_2S, −78 °C–25 °C, 24 h, 85%.

sugar residues on the polymerizable template prior to the process of ROMP, offers a facile route to generate controlled assembly of evenly distributed HA-based oligosaccharide residues on the polymer backbone. Briefly, the amino group in α-D-glucosamine hydrochloride **2** was converted to the azide[14] and the free hydroxyl groups were subsequently acetylated to give **3**. Hydrolysis of the anomeric acetate using hydrazine acetate afforded **4**, which was then converted to the imidate **5**. Introduction of the n-pentenyl group at the anomeric position using TMSOTf as promoter at 0 °C afforded an anomeric mixture (α : β) in the ratio 2 : 3 (^{1}H NMR). Treatment of this mixture under Zemplen conditions gave the triol **6**, which was then easily converted to the 4,6-benzylidene derivative **7** in 75% yield. The two isomers **7α** and **7β** (ratio 2 : 3) were isolated in pure form at this stage by column chromatography and the β-isomer (**7β**) was used for further synthetic manipulations.

Glycosylation of acceptor **7β** with the imidate donor **8**[15] afforded the β-(1–3) linked HA disaccharide **9** in 78% yield. Selective reduction of the azido group to acetamido functionality (**10**) was achieved using thiolacetic acid. Finally, debenzylidenation in aqueous TFA and saponification in 3 M NaOH of the protected *N*-acetylated glycoside yielded the deprotected β-(1–3) linked HA disaccharide **11** containing the pendant n-pentenyl spacer.‡ The product obtained was purified using Sephadex LH-20 with methanol as the eluant to give a white foam. In order to convert the terminal olefin to a four carbon aldehyde, the n-pentenyl glycoside **11** was subjected to ozonolysis, which gave the requisite product **12** (Scheme 2). Reductive amination of the glycosidic aldehyde with 5-methylaminobicyclo[2.2.1]hept-2-ene (**13**)[16] using $NaCNBH_3$ resulted in the attachment of the polymerizable (norbornene) template affording the monoalkylated glycomonomer **14** (Scheme 3).‡ The product was purified using Sephadex G-10 gel filtration and lyophilized to give an off-white solid in an overall yield of 10% from **2**.

Polymerization of the glycomonomer **14** with a second generation olefin metathesis Ru-initiator **15**[11–13,17] in the presence of a detergent, dodecyltrimethylammonium bromide (DTAB), under emulsion conditions (dichloroethane–water mixture) at 60 °C for 5 h followed by chain termination with ethyl vinyl ether afforded the HA-glycopolymer [**A**] as a pale brown spongy solid (Scheme 3).‡ The ratio of molecular equivalents of initiator : glycomonomer used was 1 : 20. The polymerization reaction proceeded efficiently, consuming all the monomer (monitored by TLC). The degree of polymerization (DP) of the resultant polymer was assessed by size-exclusion chromatography (SEC) coupled with refractive index (RI) and laser-light scattering (LLS) detectors. Analyses of the glycopolymer revealed that the polydispersity index (M_w/M_n) was 1.17. Furthermore, comparison of the integrated ^{1}H NMR signal of the alkene protons (δ 5.24 and 5.35 ppm) to that of the terminal phenyl ring protons (δ *ca.* 7.25 ppm) indicated a glycopolymer molecular weight (M_n) of 9.0 kDa (n = 15.5 glycomonomer units).



Scheme 3 *Reagents and conditions*: a) MeOH, 25 °C, 3 h, then add $NaCNBH_3$, 25 °C, 15 h, 90%; b) DTAB (1 eq.), **15** (5 mol%), $(CH_2)_2Cl_2$, H_2O, 60 °C, 5 h, 85%.

In summary, an HA-substituted norbornene glycopolymer has been synthesized using ROMP. The present approach will facilitate the production of HA-based compounds for use in engineering of HA-containing tissue equivalents and pharmacotherapy directed at modulating events mediated by HA binding receptors.

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Notes and references

‡ All the compounds have been characterized using spectral data (^{1}H, ^{13}C NMR, HRMSFAB, Maldi-Tof), see ESI.

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15 The compound **8** was prepared from commercially available aceto-bromo-α-D-glucoronic acid methyl ester in 2 steps (50%) including conversion of the C-1 acetyl to hydroxy ($CdCO_3$–CH_3CN, 70 °C, 3 h) followed by introduction of the imidate group (dichloroethane–CCl_3CN–DBU, 0 °C, 1.5 h).
16 Reduction of 5-norbornene-2-carbonitrile (commercially available from Aldrich as a mixture of isomers) using $LiAlH_4$ afforded the amine **13** (yield 62%) as an *endo* : *exo* mixture.
17 Catalyst **15** not only has activity comparable to that of the catalyst $Cl_2(PCy_3)_2Ru{=}CHPh$, used by Kiessling and coworkers,[3–5,7] but also retains its functional group tolerance.[12,13]