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Homodimerization of hyaluronan and heparan sulfate derivatives by olefin metathesis reaction

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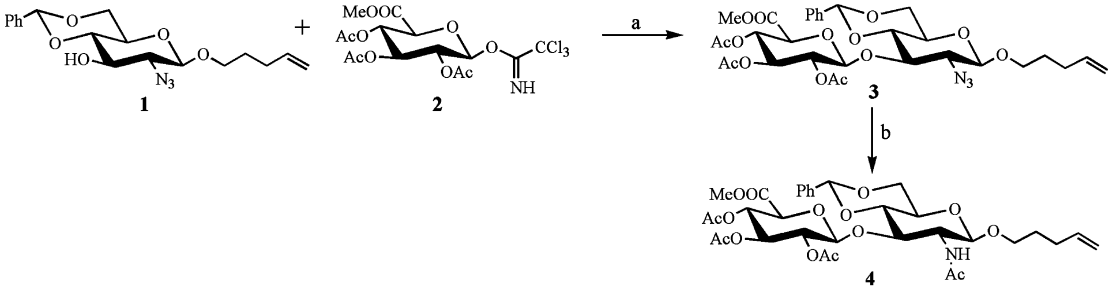
Abstract—Hyaluronan and heparan sulfate disaccharides of the type β-D-glucuronic acid-(1→3)-*N*-acetyl-β-D-glucosamine and α-L-iduronic acid-(1→4)-*N*-acetyl-β-D-glucosamine, respectively, with an *n*-pentenyl group at the reducing end have been synthesized. Homodimerization of these derivatives using Grubbs catalyst furnished dimerized disaccharides separated by a C_8 spacer arm.

Glycosaminoglycans (GAG), such as heparan sulfate (HS) and hyaluronan (HA), sequester and modulate the activity of a variety of soluble, membrane-bound, and matrix proteins and thereby regulate a number of biological processes, such as cell growth, migration and proliferation, bacterial and viral recognition events, as well as anticoagulation and cancer metastasis.[1,2] While the GAG biosynthetic pathway is quite complex, the end result is a family of polysaccharides consisting of repeating disaccharide units. Significantly, the recent identification that smaller oligosaccharide sequences[3,4] may be responsible for many of the unique biological activities of these parent polysaccharides holds the promise of generating relatively small molecule GAG equivalents through a total synthesis strategy.[5] For example,[1,3,4] discrete HS oligosaccharides induce basic fibroblast growth factor (bFGF) dimerization and activation and, likewise, potentiate the activity of antithrombin III (AT-III), a protease inhibitor of blood coagulation. Similarly, HA derived saccharide sequences have been implicated in embryonic development, cancer, as well as angiogenesis and wound healing.[2] With this in mind, we have postulated that the tethering of structurally defined HA and HS based fragments by a neutral molecular spacer might provide a strategy for the synthesis of related molecular agonists and/or antagonists of GAG dependent events.

Olefin metathesis[6,7] has become an attractive method for the construction of complex molecules due to the high reactivity, operational simplicity and remarkable functional group tolerance of Grubbs ruthenium catalyst.[7] For example, as applied to carbohydrate chemistry, olefin cross metathesis has been recently used by Roy et al. to synthesize sialoside monosaccharide derivatives.[6a] In this report, we utilize olefin metathesis



Scheme 1. *Reagents and conditions*: (a) TMSOTf, CH_2Cl_2, 0–25°C, 3.5 h, 80%; (b) CH_3COSH, 25°C, 24 h, 70%.

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as a convenient approach for the homodimerization of more complex HA and HS derived disaccharides synthesized with a pendant pentenyl group. In this manner, a series of fully protected higher ordered dimerized (*Gemini*) disaccharides separated by a C_8 spacer arm were generated.

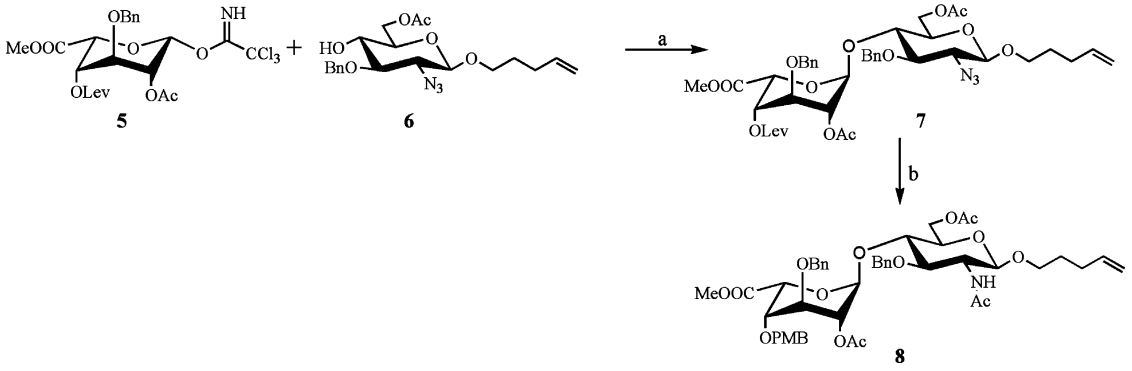
The synthesis of the desired hyaluronan disaccharide containing a pentenyl group at the anomeric position was accomplished in 65% yield over two steps starting from the β-D-glucuronic acceptor **1**[8] (Scheme 1). Briefly, condensation of the β-D-glucosamine acceptor **1** with the β-D-glucuronic acid donor **2** in the presence of a catalytic amount of TMSOTf afforded the fully protected disaccharide **3** in 80% yield. The azido group was reduced to the *N*-acetyl group using thiolacetic acid and the product **4**[9] was obtained in 70% yield.

The synthesis of the fully protected heparan sulfate disaccharide is shown in Scheme 2. The iduronic acid donor **5** was synthesized in 14 steps starting from 1,3 D-glucuronolactone.[3b,10] Condensation of **5** with the β-D-glucosamine acceptor **6** in the presence of 5 mol% of TMSOTf resulted in the formation of the fully protected disaccharide **7** in 55% yield. Further transformation included replacement of the levulinyl group by a *p*-methoxybenzyl moiety.[3a] This substitution was critical since delevulinylation prevents further elongation of the disaccharide through the levulinyl end and, as a consequence, provides selective control over the process of olefenic homodimerization via the *n*-pentenyl spacer arm. Subsequent reduction of the azido functionality to acetamido using CH_3COSH afforded the disaccharide **8**[9] in 39% yield over three steps.

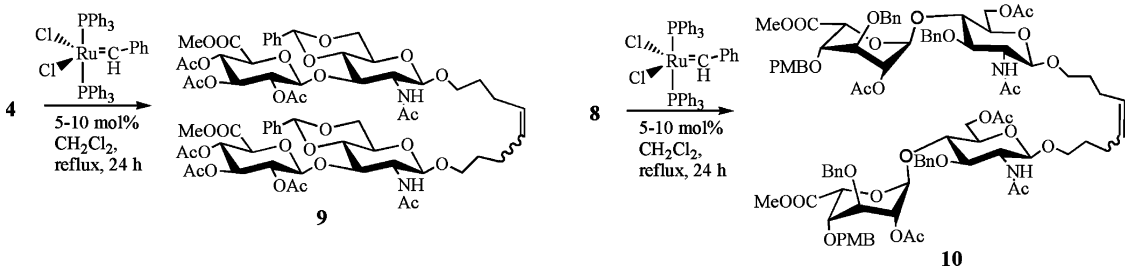
With the requisite disaccharides in hand, we proceeded with the cross metathesis reaction. The protected disaccharides **4** and **8** were refluxed in dichloromethane with 5–10 mol% of Grubbs catalyst for 24 h under an argon atmosphere. After quenching with ethyl vinyl ether, the products were flash chromatographed which provided the resulting divalent disaccharides **9** and **10** in 90% yield, respectively, as shown in Scheme 3.

The *E*:*Z* ratio could not be determined in the ^1H NMR spectrum due to overlapping resonance signals around 5.0 ppm. However, HMQC experiments of **9** revealed two peaks at 128.0 and 126.5 ppm that corresponded to the *E*- and *Z*-isomers, respectively.[11] The area under the peaks was calculated and corresponded to an *E*:*Z* ratio of 4:1. A similar result was obtained for the divalent disaccharide **10**.

In conclusion, the synthesis of disaccharides of hyaluronan and heparan sulfate possessing a *n*-pentenyl group at the reducing glucosamine end has been accomplished. Olefin metathesis of these protected GAG (HA and HS) derivatives has been performed for the first time using Grubbs catalyst under mild conditions to yield novel and complex divalent disaccharides linked by a C_8 spacer.



Scheme 2. *Reagents and conditions*: (a) TMSOTf, CH_2Cl_2, –20 to 25°C, 3.5 h, 55%; (b) i. $NH_2NH_2{\cdot}HOAc$, pyridine, 15 min, 90%, ii. $CH_3OC_6H_4CH_2OC(=NH)CCl_3$, TfOH, CH_2Cl_2, 2 h, 60%, iii. CH_3COSH, 25°C, 24 h, 70%.



Scheme 3.

Acknowledgements

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8. The acceptor **1** was synthesized from D-glucosamine hydrochloride over eight steps: ^{1}H NMR (400 MHz, $CDCl_3$) δ ^{1}H NMR ($CDCl_3$) δ 7.48–7.45 (m, 2H, Ph), 7.38–7.26 (m, 3H, Ph), 5.83–5.79 (m, 1H, C*H*=CH_2), 5.56 (s, 1H, C*H*Ph), 5.06–4.97 (m, 2H, CH=CH_2), 4.33 (d, 1H, H-1β, J=7.8 Hz), 4.29 (m, 1H), 3.89 (m, 1H), 3.73 (t, 1H, J=10.0 Hz), 3.56–3.48 (m, 3H), 3.45–3.38 (m, 2H), 2.65 (br s, 1H, -OH) 2.17–2.15 (m, 2H), 1.76–1.73 (m, 2H).
9. All compounds gave satisfactory NMR and mass spectral data. Selected spectral data for some of the compounds is reported below. For **4**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45–7.30 (m, 5H, C_6H_5), 6.20 (d, 1H, J=9.0 Hz, N*H*Ac), 5.70 (m, 1H, C*H*=CH_2), 5.51 (1H, s, C*H*Ph), 5.17 (m, 2H, CH=CH_2), 5.01–4.92 (m, 3H), 4.88 (d, 1H, H-1′, J=7.8 Hz), 4.78 (d, 1H, H-1, J=3.6 Hz), 4.32–4.19 (m, 2H), 4.02–3.96 (m, 1H), 3.79–3.60 (m, 6H), 3.55 (s, 3H, CO_2CH_3), 3.45–3.34 (m, 1H), 2.18–2.10 (m, 2H, CH_2CH_2CH=CH_2), 2.01 (s, 3H, $COCH_3$), 1.99 (s, 3H, $COCH_3$), 1.96 (s, 3H, $COCH_3$), 1.94 (s, 3H, NH-$COCH_3$), 1.84–1.78 (m, 2H, CH_2CH_2CH=CH_2); HRMS (FAB) calcd for $C_{33}H_{43}O_{15}N$ (M^++Li) 700.2793, found 700.2795. For **8**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50–7.25 (10H, m, C_6H_5), 7.09 (2H, d, J=8.4 Hz, $C_6H_4OCH_3$), 6.8 (2H, d, J=8.4 Hz, $C_6H_4OCH_3$), 5.85 (1H, d, J=8.4 Hz, N*H*) 5.81 (1H, m, C*H*=CH_2), 5.23 (1H, d, J=4.8 Hz, H-1′), 5.05–4.95 (2H, m, CH=CH_2), 4.84 (m, 1H), 4.71–4.64 (m, 4H), 4.42–4.38 (3H, m), 4.26 (1H, d, H-1, J=7.5 Hz), 4.19 (2H, m), 3.93–3.81 (3H, m), 3.79 (3H, s, $C_6H_4OCH_3$), 3.73 (1H, m), 3.53 (3H, s, CO_2CH_3), 3.47–3.36 (3H, m), 2.15 (m, 2H, CH_2CH_2CH=CH_2), 2.05 (3H, s, CO_2CH_3), 2.01 (3H, s, CO_2CH_3), 1.73 (m, 2H, CH_2CH_2CH=CH_2); HRMS (FAB) calcd for $C_{46}H_{57}O_{15}N$ (M^++H) 864.00, found 864.5179. For **9**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39–7.30 (m, 10H), 6.11(d, 2H, J=7.2 Hz), 5.40–5.36 (m, 4H), 5.19–5.06 (m, 4H), 4.97–4.91 (vt, 2H, J=8.1 Hz), 4.80 (d, 2H, J=7.8 Hz), 4.66 (vt, 2H, J=9.3 Hz), 4.30 (dd, 2H, J=10.3 Hz, J=4.5 Hz), 3.85–3.63 (m, 10H), 3.58 (s, 6H), 3.56–3.47(m, 4H), 3.07 (q, 2H, J=8.7 Hz), 2.04 (m, 2H), 1.98 (s, 18 H), 1.95–19.3 (s, br, 6H), 1.80 (m, 2H) 1.75 (m, 2H), 1.60 (m, 2H), 1.23 (m, 2H); HRMS (FAB) calcd for $C_{64}H_{82}O_{30}N_2$ (M^++Na) 1381.00, found 1381.50.
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11. The peaks for the *E*-isomer are generally accepted to resonate downfield to that of the *Z*-isomer. See Ref. 6a.