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Synthesis of Two Hyaluronan Trisaccharides

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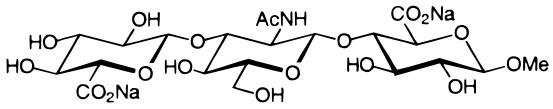
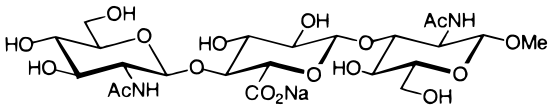
ABSTRACT



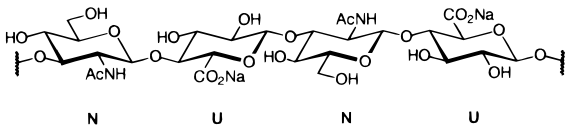
UNU



NUN

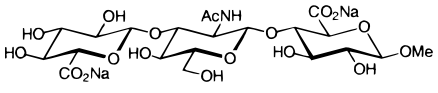
The synthesis of two hyaluronan trisaccharides, methyl *O*-(*β*-D-glucopyranosyluronic acid)-(1,3)-*O*-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl)-(1,4)-*O*-*β*-D-glucopyranosiduronic acid and methyl *O*-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl)-(1,4)-*O*-*β*-D-glycopyranosyluronic acid)-(1,3)-*O*-(2-acetamido-2-deoxy-*β*-D-glucopyranoside, are described. Construction of the target molecules was achieved though a combination of the phenyl sulfoxide and trichloroacetimidate glycosylation methodologies. This is the first report on the synthesis of the *β*-methyl derivatives, which represent the smallest fragments that incorporate all the structural features of polymeric hyaluronan.

Hyaluronan (HA) is a member of the glycosaminoglycan superfamily of bioactive oligosaccharides that are characteristically highly functionalized, linear, and anionically charged. Structurally, hyaluronan (Figure 1) consists of a repeating polymer of 2-acetamido-2-deoxy-D-glucosamine (GlcNAc or N) linked β(1,4) to D-glucuronic acid (GlcUA or U). The disaccharide repeating units are in turn bound through a β(1,3) linkage to form the HA chain. Although HA occurs as a high molecular weight polymer, numerous studies have demonstrated that only short oligosaccharides are necessary for recognition and binding by HA proteins.[1] Because of the nature of these interactions, the chemical synthesis of small HA oligomers permits detailed studies of how structure influences function at a molecular level. In addition, the solution conformations of small, chemically

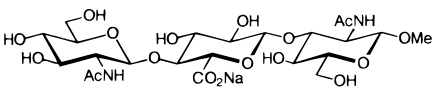


N **U** **N** **U**

(1) polymeric hyaluronan



(2) UNU



(3) NUN

Figure 1. Hyaluronan and the two target trisaccharides that represent the polymer.

(1) (a) Underhill, C. *J. Cell Sci.* **1992**, *103*, 293. (b) Bonnet, F.; Dunham, D. G.; Hardingham, T. E. *Biochem. J.* **1985**, *228*, 77. (c) Tengblad, A. *Biochem. J.* **1981**, *199*, 297. (d) Toole, B. P. *Curr. Opin. Cell Biol.* **1990**, *2*, 839. (e) Christner, J. E.; Brown, M. L.; Dziewiatkowski, D. D. *J. Biol. Chem.* **1979**, *254*, 4624.

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synthesized HA fragments are more easily characterized than that of the native polymeric form.

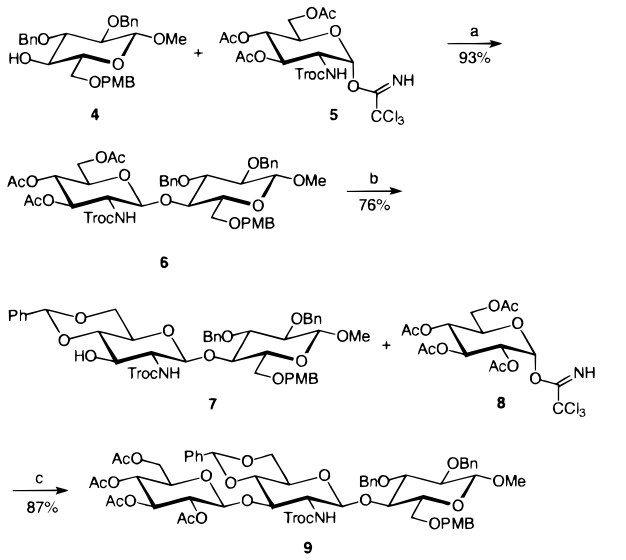
Several syntheses of HA oligosaccharides have been previously reported.[2] Among these, Slaghek and Ogawa constructed a tetrasaccharide derivative with D-glucuronic acid at the reducing end[3] as well as a series of di-, tri-, and tetrasaccharides with *N*-acetyl-D-glucosamine at the reducing end.[4] However, all of the derivatives reported incorporated a *p*-methoxyphenyl ether moiety at C-1 of the reducing sugar that facilitates chain elongation but does not accurately mimic native HA fragments. In this regard, *O*-methyl is a better structural mimic of native HA because (1) *O*-methyl is the smallest functional group available to minimize any potential secondary interactions and (2) anomerization, which significantly complicates any NMR solution study, can be eliminated by capping C-1 of the reducing end sugar.

Among the β-methyl HA derivatives previously reported are the $\beta(1,4)$ disaccharide[5] and a series of tetra-, hexa-, and octasaccharides with D-glucuronic acid at the reducing end.[6] This Letter describes the first report of the β-methyl HA trisaccharides, which represent the smallest fragments incorporating all of the structural features of polymeric hyaluronan.

The UNU Trisaccharide. There are three central considerations in the synthesis of glycosaminoglycans: (1) the mode of glycosylation, or formation of the glycosidic linkages; (2) the installation of the acetamido group; and (3) the oxidation of C-6 on the glucuronic acid precursors. Construction of the UNU trisaccharide utilized TMSOTf-mediated glycosylation of methyl 2,3-di-*O*-benzyl-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (**4**)[5] with the trichloroacetimidate donor **5**[7] to afford the corresponding $\beta(1,4)$ disaccharide, **6** in 87% yield (Scheme 1).

To form the $\beta(1,3)$ linkage, we envisioned removal of the three acetates on **6** followed by formation of the 4,6-benzylidene. Saponification under Zemplén conditions (Na°/MeOH) afforded the corresponding triol; however, in a side reaction, the *N*-trichloroethoxycarbonyl group (TROC) was inadvertently converted into the corresponding methyl carbamate. Consequently, an alternative, milder method was employed utilizing a basic solution of guanidinium nitrate[8] and quantitatively produced the desired triol after 45 min. This procedure is specific for deacetylation in the presence of a TROC carbamate.

Scheme 1[a]

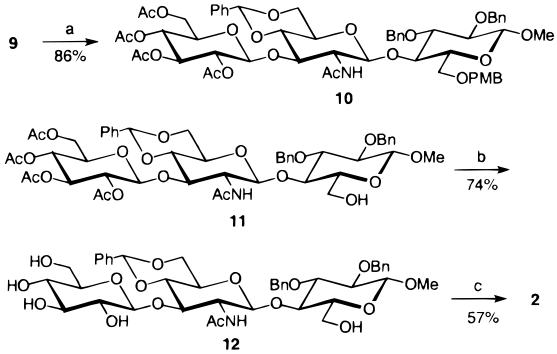


[a] (a) TMSOTf, 4 Å MS, CH_2Cl_2, 0 to 25 °C, 3 h; (b) (1) guanidinium nitrate, 25 °C, 45 min; (2) benzaldehyde dimethyl acetal, *p*-TsOH·H_2O, CH_3CN, 45 min; (c) TMSOTf, 4 Å MS, CH_2Cl_2, −30 to 25 °C, 6 h.

Benzylidenation afforded the disaccharide acceptor, **7**, in 76% yield over two steps. Condensation of **7** with imidate **8** in the presence of a catalytic amount of TMSOTf produced the fully protected trisaccharide **9** in 84% yield.

Conversion of the TROC carbamate into the acetamido moiety was carried out with Cd dust[9] in DMF:AcOH (2:1) followed by treatment with acetic anhydride in pyridine (Scheme 2) to afford **10** in 86% yield. Removal of the

Scheme 2[a]



[a] (a) (1) Cd dust, DMF:AcOH (2:1), rt, 8 h; (2) Ac_2O, pyridine, 25 °C, 1 h; (b) DDQ, CH_2Cl_2, 2 h; (b) (1) Na°, MeOH, 25 °C, 30 min; (2) TEMPO, NaBr, TBABr, 5% NaOCl, $NaHCO_3$, CH_2Cl_2:H_2O (6:1), 20 min; (c) (1) $Pd(OH)_2$, H_2, MeOH:H_2O (10:1), 18 h.

p-methoxybenzyl ether was achieved with DDQ in CH_3CN at 0 °C and produced the corresponding alcohol **11**, in 84% yield.

Treatment of **11** with NaOMe in methanol provided **12** in quantitative yield. The selective oxidation of the primary

(2) (a) Takanashi, S.; Hirasaka, Y.; Kawada, M. *J. Am. Chem. Soc.* **1962**, *84*, 3029. (b) Jeanloz, R. W.; Flowers, H. M. *J. Am. Chem. Soc.* **1962**, *84*, 3030. (c) Flowers, H. M.; Jeanloz, R. W. *Biochemistry* **1964**, *3*, 123. (d) Walker-Nasir, E.; Jeanloz, R. W. *Carbohydr. Res.* **1979**, *68*, 343. (e) Klaffke, W.; Warren, C. D.; Jeanloz, R. W. *Carbohydr. Res.* **1993**, *244*, 171.

(3) Slaghek, T. M.; Hypponen, T. K.; Ogawa, T.; Kamerling, J. P.; Vliegenthart, J. F. G. *Tetrahedron Lett.* **1993**, *34*, 7939.

(4) (a) Slaghek, T.; Nakahara, Y.; Ogawa, T. *Tetrahedron Lett.* **1992**, *33*, 4971. (b) Slaghek, T. M.; Nakahara, Y.; Ogawa, T.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1994**, *255*, 61.

(5) Carter, M. B.; Petillo, P. A.; Anderson, L.; Lerner, L. E. *Carbohydr. Res.* **1994**, *258*, 299.

(6) Blatter, G.; Jacquinet, J.-C. *Carbohydr. Res.* **1996**, *288*, 109.

(7) Dullenkopf, W.; Castro-Palomino, J. C.; Manzoni, L.; Schmidt, R. R. *Carbohydr. Res.* **1996**, *296*, 135.

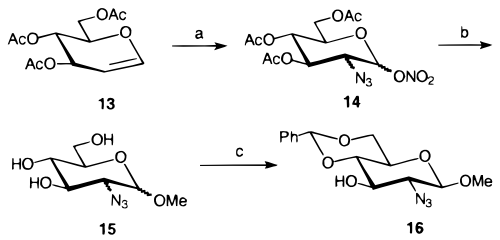
(8) Ellervik, U.; Magnusson, G. *Tetrahedron Lett.* **1997**, *38*, 1627.

(9) Hancock, G.; Galpin, I. J.; Morgan, B. A. *Tetrahedron Lett.* **1982**, *23*, 249.

hydroxyls to the corresponding diacid was achieved using a catalytic amount of 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and 5% aqueous NaOCl as an oxidant.[10] Finally, hydrogenolysis with Pearlman's catalyst (60 psi) and purification on a Sephadex G-10 size-exclusion column, followed by preparative TLC (4:2:2:1 *n*-BuOH, H_2O, EtOH, AcOH) on SiO_2, afforded **2** as a clear foam in 10 steps and 22% overall yield. Complete spectral characterization of **2**, including mass spectral analysis and all ^1H and ^{13}C assignments, is provided in the Supporting Information.

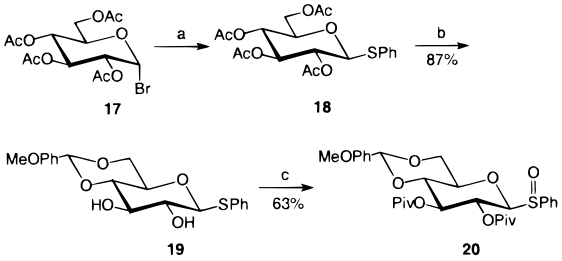
The NUN Trisaccharide. Although the NUN trisaccharide contains the same monosaccharide units as the UNU trisaccharide, alternate monomer precursor units were employed for the glycosylation steps. Of the monomer units used to construct the fully protected trisaccharide, only compound **5** was used in the syntheses of both trisaccharides. Schemes 3 and 4 outline the routes to the other monomer units used in the NUN synthesis to form the β(1,3) linked disaccharide.

Scheme 3[a]



[a] (a) Ceric(IV) ammonium nitrate, NaN_3, CH_3CN, −20 °C, 8 h; (b) Na°, MeOH, 25 °C, 2 h; (c) benzaldehyde dimethyl acetal, *p*-TsOH·H_2O, CH_3CN, 25 °C, 6 h.

Scheme 4[a]



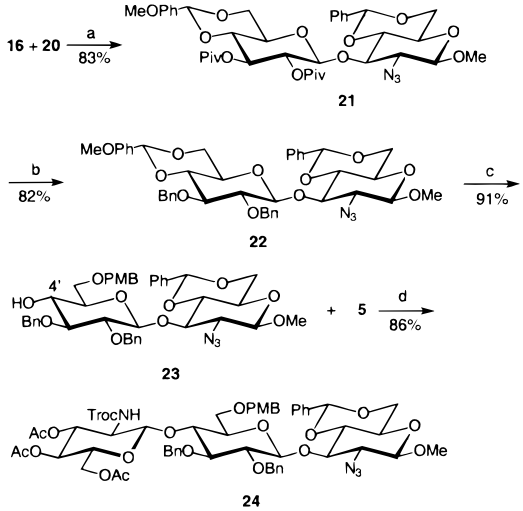
[a] (a) PhSH, tetrabutylammonium hydrogen sulfate, 1 M Na_2CO_3, CH_2Cl_2, 25 °C, 45 min; (b) (1) Na°, MeOH, 25 °C, 2 h; (2) *p*-methoxybenzaldehyde dimethyl acetal, p-TsOH·H_2O, DMF, 50 °C, 3 h; (c) PivCl, DMAP, pyridine, reflux, overnight; (d) *m*-CPBA, CH_2Cl_2, −78 °C, 40 min.

Starting from tri-*O*-acetyl-D-glucal (**13**), treatment with ceric(IV) ammonium nitrate (CAN) and sodium azide afforded a mixture of 2-deoxy-2-azido-1-nitrate derivatives (**14**).[11] Conversion of the nitrate group to the corresponding methyl glycoside was achieved with methanolic sodium methoxide and provided **15** as a mixture of diastereomers. Treatment of **15** with benzaldehyde dimethyl acetal and *p*-toluenesulfonic acid afforded the corresponding benzylidene derivatives. The desired diastereomer was purified on silica gel to afford pure **16**[12] in 12% yield over five steps. The advantage of this method lies in the single chromatographic step at the conclusion of the sequence, which essentially makes this a one-pot procedure.[13]

The use of the sulfoxide glycosylation methodology developed by Kahne[14] required the preparation of sulfoxide **20** (Scheme 4). Phase transfer catalysis of glycosyl bromide **17** in the presence of thiophenol afforded the corresponding thiophenylglycoside **18** in 87% yield.[15] Subsequent deacetylation and formation of the *p*-methoxybenzylidine afforded diol **19** (88%), which was esterified with PivCl in pyridine (92%) and then subsequently oxidized with *m*-CPBA to afford sulfoxide **20** in 69% yield.

With the desired monomer building blocks in hand, condensation of **16** and **20** in the presence catalytic trifluoromethanesulfonic anhydride (Tf_2O) afforded β(1,3) disaccharide **21** in 86% yield (Scheme 5). Prior to unmasking the hydroxyl on C-4′ by a selective reduction of the 4,6-*O*-*p*-methoxybenzylidene, it was necessary to exchange the pivaloyl esters with benzyl ethers (**22**). The lower reactivity of the C-4′ hydroxyl coupled with the steric bulk of the

Scheme 5[a]



[a] (a) Tf_2O, DTBMP, CH_2Cl_2, 4 Å MS, −60 to 25 °C, 1.5 h; (b) (1) LiOH·H_2O, MeOH:THF:H_2O (3:2:1), 50 °C, 24 h; (2) BnBr, NaH, DMF, 25 °C, 6 h; (c) $NaBH_3CN$, TFA, 3 Å MS, DMF, 25 °C, overnight; (d) TMSOTf, CH_2Cl_2, 4 Å MS, 0 °C, 3 h.

(10) (a) Davis, N. J.; Flitsch, S. L. *Tetrahedron Lett.* **1993**, *34*, 1181. (b) Garegg, P. J.; Oscarson, S.; Tedebark, U. *J. Carbohydr. Chem.* **1988**, *17*, 587.

(11) Lemieux, R. U.; Ratcliffe, R. M. *Can. J. Chem.* **1979**, *57*, 1244.

(12) (a) Pozsgay, V.; Coxon, B. *Carbohydr. Res.* **1995**, *277*, 171. (b) Pozsgay, V.; Coxon, B. *Carbohydr. Res.* **1994**, *257*, 189.

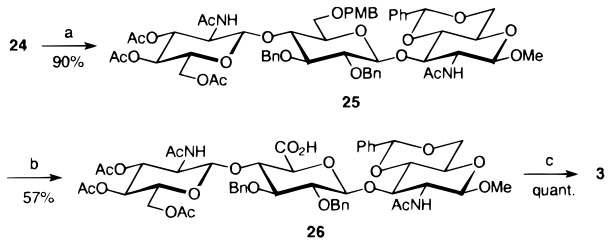
(13) Compound **15** was reported as an undesired side product in ref 12b.

(14) (a) Kahne, D.; Walker, S.; Cheng, Y.; van Engen, D. *J. Am. Chem. Soc.* **1989**, *111*, 6881. (b) Yan, L.; Kahne, D. *J. Am. Chem. Soc.* **1996**, *118*, 9239.

pivaloyl ester at C-3′ precluded the formation of the β(1,4) linkage with **5**. Treatment of **22** with TFA and sodium cyanoborohydride produced glycosyl acceptor **23** in 91% yield, and subsequent glycosylation with imidate **5** in the presence of catalytic TMSOTf afforded 86% of the fully protected trisaccharide **24**.

Conversion of the TROC carbamate and the azide to the corresponding acetamido group proceeded smoothly as a one-pot three-step process (Scheme 6). Reduction of the TROC group with Cd (dust) in DMF:AcOH was followed by reduction of the azide with thiolacetic acid in pyridine. The crude reaction mixture was subsequently treated with acetic anhydride to afford the diacetamido trisaccharide, **25**, in 90% yield. Removal of the *p*-methoxybenzyl group with CAN afforded the free alcohol. Subsequent oxidation of the alcohol to the corresponding carboxylic acid with catalytic TEMPO and 5% aqueous NaOCl afforded **26** in 57% yield over two steps.[10] Following aqueous workup, the crude reaction mixture was subjected to hydrogenolysis with $Pd(OH)_2$ and saponification with lithium hydroxide monohydrate. The final purification step was carried out on Sephadex G-10 using water as an eluent and, upon lyophilization, afforded the target trisaccharide, **3**, as a white solid in 11 steps and 18% overall yield from the monosaccharides. Complete spectral characterization of **3**, including mass spectral analysis and all ^{1}H and ^{13}C assignments, is provided in the Supporting Information.

Scheme 6 [a]



[a] (a) (1) Cd (dust), DMF:AcOH (1:1), 25 °C, 8 h; (2) AcSH, pyridine, 25 °C, 12 h; (3) Ac_2O, pyridine, 2 h; (b) (1) CAN, CH_2Cl_2:H_2O (9:1), 2 h; (2) TEMPO, NaBr, TBABr, 5% NaOCl, $NaHCO_3$, CH_2Cl_2:H_2O (6:1), 30 min; (c) (1) $Pd(OH)_2$, H_2 (60 atm), MeOH:H_2O (10:1), 18 h; (2) LiOH·H_2O, H_2O:THF (2:1), 25 °C, 8 h.

Acknowledgment. This research was funded by the Petroleum Research Fund, the National Institutes of Health, and the American Heart Association.

Supporting Information Available: Experimental procedures and complete spectroscopic data for **2**, **3**, and all key intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0057075

(15) Tropper, F. D.; Andersson, F. O.; Grand-Maitre, C.; Roy, R. *Synthesis* **1991**, 734.