Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 339 (2004) 1255–1262
Carbohydrate RESEARCH

Gram-scale syntheses of the (1 → 3)-linked and (1 → 4)-linked hyaluronan disaccharides

Sara L. Adamski-Werner, Bryan K. S. Yeung, Lynne A. Miller-Deist and Peter A. Petillo*

Department of Chemistry, University of Illinois, 600 S. Mathews Ave., Urbana, IL 61801, USA

Received 4 December 2003; accepted 2 March 2004

Available online 10 April 2004

Abstract—The first gram-scale syntheses of two hyaluronan disaccharides are described. Construction of the (1 → 4)-linked disaccharide **12** was achieved in 12% overall yield using 2,3-bis-dimethyl acetal protection in combination with chlorosilane-induced carbamate cleavage methodologies. The uronic acid functionality was installed using TEMPO oxidation with NaOCl as the hypochlorite source. The (1 → 3)-linked disaccharide **18** was achieved in 7% overall yield utilizing acetonide protection in addition to the chlorosilane-induced carbamate cleavage methodology and the TEMPO oxidation.

Keywords: Carbohydrates; Hyaluronan; Oligosaccharides; Large-scale synthesis

1. Introduction

Hyaluronan (HA) is a linear carbohydrate polymer that consists of repeating units of 2-acetamido-2-deoxy-D-glucosamine (GlcNAc) linked β-(1 → 4) to D-glucuronic acid (GlcUA). The disaccharide units are attached β-(1 → 3) to form the linear polymer, which can exist in molecular weights above 1×10^7 Da.[1] HA is a major constituent of the extracellular matrix, connective tissues, and synovial fluid, and the degradation of HA has been implicated in the onset of arthritis.[2] HA has also been implicated in such processes as cell–cell recognition, cell adhesion, cell proliferation as well as cell migration.[3] Studies directed toward understanding the chemical properties of HA, especially NMR spectroscopic investigations and the mechanism of degradation, are best carried out using low-molecular-weight HA oligosaccharides rather than polymeric HA.[4,5] Unfortunately, these investigations are limited by the availability of HA oligosaccharides on a large scale, requiring large-scale syntheses of HA fragments to provide adequate material for the understanding of the biological processes and properties.

The glycochemistry renaissance of the last decade has underscored the importance of efficient, scalable syntheses of biologically important glycoforms and their derivatives. Syntheses of short HA fragments that incorporate the key subunits have been successfully carried out on a small scale, but efficient large-scale syntheses remain elusive. Previous efforts to produce HA oligosaccharides on a small scale include the synthesis by Lerner and co-workers of a (1 → 4)-linked hyaluronan disaccharide containing a methyl β-D-glucopyranosiduronic acid moiety at the reducing end.[6] Blatter and Jacquinet constructed a series of tetra-, hexa-, and octa-saccharide derivatives of HA also containing a methyl β-D-glucopyranosiduronic acid in 20–35 steps for each fragment.[7] Additionally, Slaghek et al. used an *N*-acetylglucosamine residue to construct di-, tri-, and tetra-saccharide derivatives of HA.[4] Yeung et al. constructed two HA trisaccharides incorporating a β-methyl moiety at the reducing end,[8] and most recently, Chaikof and co-workers accomplished the syntheses of two hyaluronan-mimetic gemini disaccharides on a small scale.[9] In addition to chemical syntheses of HA

* Corresponding author at present address: Deciphera Pharmaceuticals, 1505 Wakarusa Drive, Lawrence, KS 66047, USA. Tel.: +1-785-838-3703; e-mail: alchmist@deciphera.com

0008-6215/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2004.03.005

fragments, Jeanloz and Flowers used degradative methanolysis of HA, followed by acetylation, to form the peracetylated methyl ester of the $(1\rightarrow3)$-linked disaccharide of HA,[10] and Asari and co-workers accomplished large-scale depolymerization of HA oligosaccharides.[11] Chemoenzymatic synthesis has also been employed in the formation of disaccharides[12,13] and may show promise for the synthesis of HA oligosaccharides in the future. All of these efforts, while notable in their approaches, only yielded milligram quantities of the final targets in yields ranging from 5% to 30% in 7–35 steps. Here we demonstrate the first scalable chemical syntheses of the $(1\rightarrow3)$- and $(1\rightarrow4)$-linked HA disaccharides.[14]

2. Results and discussion

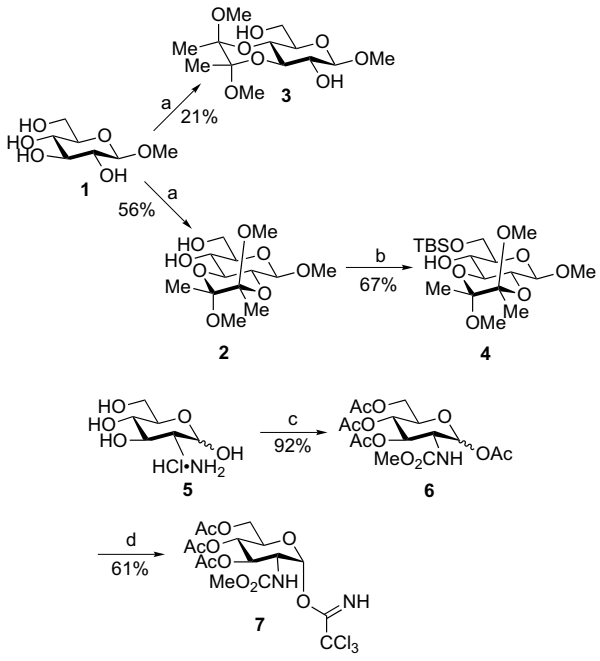
2.1. Construction of the $(1\rightarrow4)$-linked HA disaccharide

The synthesis of the disaccharides was envisioned to incorporate orthogonal protecting groups early in the synthetic scheme to prevent the inclusion of many protection–deprotection steps. In addition, the protecting group strategy was designed to allow simple acid–base deprotection at the conclusion of the synthesis. This goal was accomplished, in part, by the use of a bis-acetal protection of methyl β-D-glucopyranoside as the first step in the synthesis of the acceptor portion of the $(1\rightarrow4)$-linked disaccharide **12**.[15] This protection strategy allowed for the large-scale protection of two adjacent hydroxyl groups, followed by straightforward silica gel separation of the resulting 2,3- and 3,4-protected products in 56% and 21% yields, respectively.

The bulky *tert*-butyldimethylsilyl (TBS) group, which can be removed in the presence of the bis-acetal protecting group, was employed to selectively protect the primary alcohol at C-6. Oxidation and esterification of the C-6 alcohol before glycosylation resulted in poor coupling yields due to the electron-withdrawing nature of the resulting methyl ester, and therefore, oxidation was performed later in the synthesis.[8]

The donor monosaccharide **7** was synthesized in four steps from commercially available and inexpensive glucosamine hydrochloride. Methyl carbamate formation was accomplished with methyl chloroformate in the presence of $NaHCO_3$, followed by acetylation and removal of the C-1 acetate to form the hemiacetal. Reaction of the hemiacetal with trichloroacetonitrile provided 12.60 g of donor **7** in 61% overall yield (Scheme 1).

Glycosylation to provide **8** was performed on a large scale with TMSOTf as the promoter,[8] and removal of the TBS group with tetrabutylammonium fluoride (TBAF) was accomplished cleanly in the presence of the remaining protecting groups (Scheme 2).

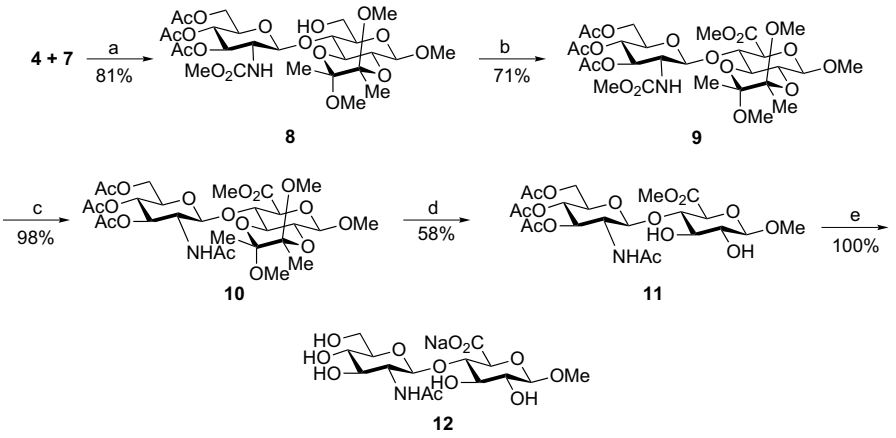


Scheme 1. Reagents and conditions: (a) 2,2,3,3-tetramethoxybutane, trimethylorthoformate, (±)-10-camphorsulfonic acid, MeOH, 60 °C, 19 h. (b) TBSCl, pyridine, 25 °C, 24 h. (c) (1) $ClCO_2Me$, $NaHCO_3$, 1:1 $CHCl_3$–H_2O, 25 °C, 1 h; (2) Ac_2O, pyridine, 25 °C, 12 h. (d) (1) $NH_2NH_2{\cdot}HOAc$, DMF, 25 °C, 4 h; (2) CCl_3CN, DBU, CH_2Cl_2, 25 °C, 12 h.

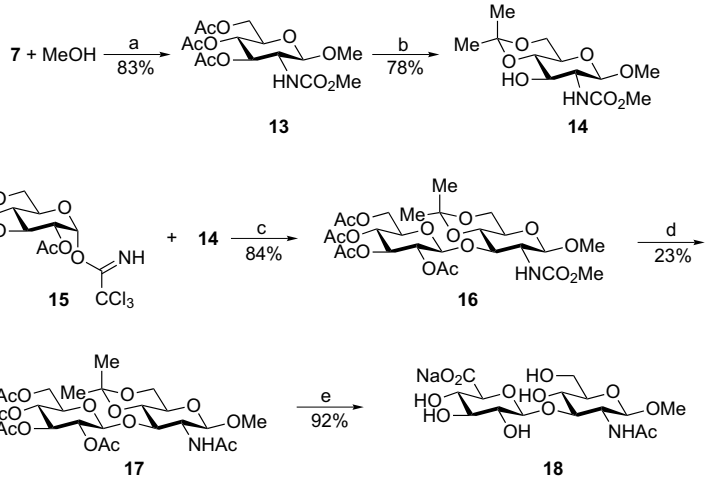
Following glycosylation and desilylation, two primary transformations must occur: (1) the oxidation of the 6-position to form the uronic acid functionality and (2) deprotection of the methyl carbamate and subsequent acetylation to form the acetamido group.[8] The uronic acid functionality was installed utilizing TEMPO[†] oxidation of the primary alcohol,[16] and methylation with diazomethane afforded 4.70 g of methyl ester **9** in 71% yield over two steps.

After installation of the uronic acid moiety, deprotection of the methyl carbamate using $MeSiCl_3$ and Et_3N at 70 °C was accomplished to afford the free amine,[17] and subsequent acetylation to form 3.66 g of the acetamide **10** proceeded in 98% overall yield. Attempts to remove the methyl carbamate in the presence of the TBS group proved unsuccessful, as the product was never formed, perhaps due to loss of the silyl protecting group under the acidic reaction conditions.[18] Removal of the 2,3-bis-dimethyl acetal protecting group with 19:1 TFA–H_2O,[15] followed by saponification with cold 1 N NaOH, afforded 1.18 g of the final deprotected $(1\rightarrow4)$-linked disaccharide **12** in 12% yield over 14 steps.

[†] TEMPO is 2,2,6,6-tetramethyl-1-piperidinyloxy free radical [2564-83-2].



Scheme 2. Reagents and conditions: (a) (1) TMSOTf, 4 Å molecular sieves, CH_2Cl_2, −30 °C, 10 h; (2) TBAF, THF, 0 °C, 12 h. (b) (1) TEMPO, NaBr, TBABr, 5% NaOCl, $NaHCO_3$, 6:1 CH_2Cl_2–H_2O, 0 °C, 30 min; (2) CH_2N_2, Et_2O, 25 °C. (c) (1) $MeSiCl_3$, Et_3N, THF, 70 °C, 22 h; (2) Ac_2O, pyridine, 25 °C, 2 h. (d) (1) 19:1 TFA–H_2O, 25 °C, 17 h. (e) (1) 1 N NaOH, MeOH, pH 12, 0 °C, 14 h; (2) AcOH, pH 6, 25 °C.



Scheme 3. Reagents and conditions: (a) (1) TMSOTf, 4 Å molecular sieves, CH_2Cl_2, −30 °C, 3 h. (b) (1) Na^0, MeOH, 25 °C, 3 h; (2) 2-methylpropene, 25 °C, 15 h. (c) (1) BF_3OEt_2, CH_2Cl_2, −30 °C, 24 h. (d) $MeSiCl_3$, Et_3N, THF, 70 °C, 72 h; (2) Ac_2O, pyridine, 25 °C, 4 h. (e) (1) Na^0, MeOH, 25 °C, 3 h. (2) TEMPO, NaBr, TBABr, 5% NaOCl, $NaHCO_3$, 1:6 CH_2Cl_2–H_2O, 0 °C, 30 min.

2.2. Construction of the (1 → 3)-linked HA disaccharide

As in the case of the (1 → 4)-linked disaccharide, the protecting group strategy for the synthesis of the (1 → 3)-linked disaccharide was envisioned to incorporate appropriate orthogonal protection methods. The first step was accomplished by glycosylation of MeOH with previously discussed trichloroacetimidate **7**.[17] This glycosylation exclusively provided a straightforward and high-yielding route to the methyl β-D-glycoside. The acetonide protecting group was chosen as it allows for simultaneous protection of the C-4 and C-6 hydroxyl groups, and its removal is compatible with the other protecting groups chosen for the (1 → 3)-linked disaccharide. Deacetylation, followed by reaction with 2-methoxypropene, provided 6.02 g of **14**[17](Scheme 3).

Previously reported trichloroacetimidate **15**[19] was synthesized from commercially available and inexpensive D-glucose pentaacetate, and glycosylation using **14** as the acceptor and $BF_3{\cdot}OEt_2$ as the catalyst[‡] provided 7.21 g of the fully protected disaccharide **16** in 84% yield. Deprotection of the methyl carbamate was accomplished using $MeSiCl_3$ and Et_3N, and subsequent acetamide formation was achieved with acetic anhydride to provide 1.85 g of **17**.[17]

The initial carbamate deprotection is extremely clean, with the remainder of the mass balance consisting of the ureido sugar resulting from intermolecular condensation of the quenched amine with unreacted isocyanate.[17] In

[‡] TMSOTf was also an effective catalyst; however, yields of β-linked disaccharide were improved with the use of $BF_3{\cdot}Et_2O$.

all other cases, dilution to concentrations below 0.01 M provided the amine in the absence of urea. However, the isocyanate corresponding to the $(1\rightarrow3)$-disaccharide is remarkably stable and all attempts to dilute the reaction upon workup provided primarily the ureido sugar.

Installation of the uronic acid functionality was initiated by deacetylation under Zemplén conditions, followed by TEMPO oxidation of C-6 to the carboxylic acid.[16] Subsequent removal of the acetonide-protecting group with acidic workup of the TEMPO oxidation provided 1.30 g of the fully deprotected $(1\rightarrow3)$-linked disaccharide **18** in 7% yield over 12 steps.

3. Experimental

3.1. General methods

All starting materials and reagents were obtained from commercial suppliers and were used without further purification unless otherwise noted. Analytical thin-layer chromatography (TLC) was performed using Silica Gel 60 F254 pre-coated plates (E. Merck) with a fluorescent indicator. Visualization of the TLC plates was accomplished by UV illumination, *p*-anisaldehyde solution followed by heat, ninhydrin solution followed by heat, phosphomolybdic acid followed by heat, and ceric ammonium molybdate followed by heat. Flash chromatography was performed using Silica Gel 60 (230–400 mesh) from E. Merck.

NMR spectra were performed on a Varian Unity-400, Varian Unity-500, a Varian Inova-500, or a Varian Inova-750 FT NMR spectrometer. ^{1}H NMR chemical shifts are reported in parts per million (ppm) with reference to $CHCl_3$ in the internal solvent (7.26 ppm) unless otherwise noted. Coupling constants are reported in hertz (Hz). Carbon chemical shifts are reported in parts per million (ppm) with reference to $CHCl_3$ in the internal solvent (77.23 ppm) unless otherwise noted. All mass spectra were obtained on a VG ZAB-SE LRFABMS), a VG 70-SE-4F (HRFABMS), 70-VSE-A (LREIMS, HREIMS).

Dry CH_2Cl_2, Et_3N, CH_3CN, and pyridine were obtained by distillation from calcium hydride prior to use. Dry benzene, THF, and ether were obtained by distillation from sodium benzophenone ketyl prior to use.

3.2. Methyl [(2′*R*,3′*R*)-2,3-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)]-β-D-glucopyranoside (2)[15]

D,L-Camphorsulfonic acid (570 mg, 2.68 mmol) was added to a solution of methyl β-D-glucopyranoside hemihydrate (12.71 g, 62.53 mmol), trimethylorthoformate (19.84 mL, 181.34 mmol), and 2,2,3,3-tetramethoxybutane (13.37 mL, 75.04 mmol) in dry MeOH (80 mL). After 19 h of heating at 60 °C, TLC indicated the absence of starting material, and the reaction was quenched by the addition of solid $NaHCO_3$. The mixture was filtered, concentrated in vacuo, and purified by flash chromatography (20:20:20:1 EtOAC–Et_2O–THF–Et_3N) to afford **2** (10.73 g, 56%) and **3** (4.07 g, 21%) as white foams. ^{1}H NMR of **2** ($CDCl_3$, 500 MHz): δ 4.46 (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 3.89 (1H, *A*BX, J_{AB} 12.0 Hz, J_{AX} 3.4 Hz, J_{BX} 4.7 Hz, ν_a 1944.3 Hz, ν_b 1904.9 Hz, H-6), 3.81 (1H, A*B*X, as above, H-6), 3.74 (1H, t, $J_{3,4} = J_{4,5}$ 9.3 Hz, H-4), 3.69 (1H, t, $J_{2,3} = J_{3,4}$ 9.5 Hz, H-3), 3.53 (3H, s, acetal CH_3), 3.47 (1H, dd, $J_{1,2}$ 9.8 Hz, $J_{2,3}$ 8.0, H-2), 3.40 (1H, AB*X*, ν_x 1699.0 Hz, H-5), 3.28 (3H, s, acetal OCH_3), 3.27 (3H, s, acetal OCH_3), 1.32 (6H, 2×s, acetal CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 101.8 (C-1), 99.7 (acetal Cq), 76.3 (C-5), 72.6 (C-2), 69.4 (C-3), 68.0 (C-4), 62.4 (C-6), 57.2 (anomeric OCH_3), 48.2 (acetal OCH_3), 48.2 (acetal OCH_3), 17.8 (acetal CH_3), 17.8 (acetal CH_3). HRFABMS: Calcd for $C_{18}H_{24}O_8Na$ (M+Na) m/z 331.1370; found m/z 331.1369.

3.3. Methyl [(2′*R*,3′*R*)-2,3-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-(6-*O*-*tert*-butyldimethylsilyl)]-β-D-glucopyranoside (4)

To a solution of **2** (7.20 g, 23.30 mmol) in pyridine (100 mL) was added *tert*-butylchlorodimethylsilane (3.87 g, 25.70 mmol). After stirring at rt for 24 h, TLC indicated the absence of starting material. CH_2Cl_2 (15 mL) was added, and the mixture was washed with a satd aq $CuSO_4$ solution (3×), and H_2O (1×). The organic extract was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography (1:1 hexane–EtOAc) to afford **4** (6.62 g, 67%) as a white foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.33 (1H, d, $J_{1,2}$ 8.1 Hz, H-1), 3.83 (1H, *A*BX, J_{AB} 10.4 Hz, J_{AX} 5.2 Hz, J_{BX} 6.0 Hz, ν_a 1915.8 Hz, ν_b 1872.3 Hz, H-6), 3.74 (1H, A*B*X, as above, H-6), 3.63 (2H, m, H-3 and H-4), 3.42 (3H, s, anomeric OCH_3), 3.39 (1H, dd, $J_{1,2}$ 10.1 Hz, $J_{2,3}$ 8.1 Hz, H-2), 3.31 (1H, AB*X*, ν_x 1655.2 Hz, H-5), 3.22 (3H, s, acetal OCH_3), 3.18 (3H, s, acetal OCH_3), 1.25 (3H, s, acetal CH_3), 1.24 (3H, s, CH_3), 0.80 (9H, s, *t*-Bu CH_3), 0.00 (3H, s, Si–CH_3), −0.01 (3H, s, Si–CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 101.6 (C-1), 99.7 (acetal Cq), 99.6 (acetal Cq), 75.1 (C-5), 72.6 (C-2), 70.4 (C-3), 69.3 (C-4), 64.9 (C-6), 56.9 (anomeric OCH_3), 48.2 (acetal OCH_3), 48.1 (acetal OCH_3), 26.0 (*t*-butyl CH_3), 17.9 (acetal CH_3), 17.8 (acetal OCH_3), −5.3 (Si-CH_3). HRFABMS: Calcd for $C_{19}H_{38}O_8SiNa$ (M+Na) m/z 445.2235; found m/z 445.2234.

3.4. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-methoxycarbonylamino-α,β-D-glucopyranoside (6)

Methyl chloroformate (4.30 mL, 55.56 mmol) was added dropwise to a vigorously stirring solution of **5** (10.05 g, 46.58 mmol) and $NaHCO_3$ (11.79 g, 140.33 mmol) in a 1:1 mixture of $CHCl_3$ and H_2O (200 mL). The mixture was allowed to stir for 1 h at rt, after which time it was

neutralized with 1 M HCl and concentrated in vacuo to a white solid. The residue was dissolved in a solution of dry pyridine (150 mL) and Ac_2O (36.0 mL, 381.96 mmol) and allowed to stir under a nitrogen atmosphere at rt. After 12 h, the reaction was quenched with MeOH (50 mL), and the mixture was concentrated in vacuo. The syrupy residue was dissolved in CH_2Cl_2 (100 mL) and washed consecutively with a 2:1 mixture of 10% ammonium sulfate–10% HCl (3×), a satd aq solution of $NaHCO_3$ (1×), and brine (1×) then dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography (1:1 EtOAc–hexane) to afford **6** (17.36 g, 92%) as a white foam and a mixture of anomers. The purified anomeric mixture of **6** was used without further attempts to separate the anomers. HRFABMS: Calcd for $C_{16}H_{23}NO_{11}Na$ (M+Na) m/z 428.1169; found m/z 428.1169.

3.5. 3,4,6-Tri-*O*-acetyl-2-deoxy-2-methoxycarbonylamino-α-D-glucopyranosyl trichloroacetimidate (7)

Hydrazine acetate (4.12 g, 44.78 mmol) was added to a vigorously stirring solution of **6** (16.50 g, 40.71 mmol) in dry DMF (100 mL) under dry nitrogen. After 4 h the reaction was diluted with EtOAc (100 mL) and washed consecutively with H_2O (1×), satd aq $NaHCO_3$ (1×), and brine (1×), then the aqueous layer was reextracted with EtOAc (3×), dried over $MgSO_4$, and concentrated in vacuo.

1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (3.04 mL, 20.36 mmol) was added to a flask containing the crude hemiacetal and trichloroacetonitrile (20.40 mL, 203.55 mmol) in dry CH_2Cl_2 (200 mL) at rt. After 12 h the reaction was concentrated in vacuo, and the residue purified by flash chromatography (1:1 EtOAc–hexane) to afford **7** (12.60 g, 61%) as a light-yellow foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.80 (1H, s, imidate NH), 6.36 (1H, d, $J_{NH,2}$ 3.7 Hz), 5.29 (1H, t, $J_{2,3} = J_{3,4}$ 9.9 Hz, H-3), 5.21 (1H, t, $J_{3,4} = J_{4,5}$ 9.9 Hz, H-4), 4.95 (1H, d, $J_{1,2}$ 9.4 Hz, H-1), 4.24 (2H, m, H-2, and H-6), 4.10 (2H, m, H-5, and H-6), 3.63 (3H, s, carbamate CH_3), 2.05 (3H, s, acetate CH_3), 2.03 (3H, s, acetate CH_3), 2.02 (3H, s, acetate CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.3 (acetate C=O), 170.7 (acetate C=O), 169.4 (acetate C=O), 160.4 (imidate C=O), 156.4 (carbamate C=O), 95.0 (C-1), 90.7 (CCl_3), 70.7 (C-4), 70.3 (C-3), 67.6 (C-5), 61.6 (C-6), 53.6 (C-2), 52.7 (carbamate CH_3), 20.8 (acetate CH_3), 20.7 (acetate CH_3). HRFABMS: Calcd for $C_{16}H_{21}Cl_3N_2O_{10}Na$ (M+Na) m/z 529.0159; found, m/z 529.0160.

3.6. Methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-methoxycarbonylamino-β-D-glucopyranosyl-(1 → 4)-[(2′*R*,3′*R*)-2,3-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)]-β-D-glucopyranoside (8)

To a solution of **7** (11.32 g, 22.29 mmol), 4 Å molecular sieves, and trimethylsilyl trifluoromethanesulfonate (150 μL, 0.83 mmol) in CH_2Cl_2 (20 mL) at 0 °C was added **4** (5.0 g, 11.83 mmol) in CH_2Cl_2 (10 mL) dropwise via cannula. After warming to rt over 10 h, the reaction was quenched with Et_3N (2 mL), filtered through Celite® and concentrated in vacuo to afford the crude disaccharide as a yellow foam.

Tetrabutylammonium fluoride (23.66 mL, 1 M solution in THF, 23.66 mmol) was added to a solution of the crude disaccharide in THF (10 mL) at 0 °C. After warming to rt over 12 h, the mixture was diluted with CH_2Cl_2 and washed with satd aq $NaHCO_3$ (1×), brine (1×), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash chromatography (5:1 EtOAc–hexane) to afford **8** (6.29 g, 81%) as a white foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.63 (1H, d, $J_{NH,2}$ 9.3 Hz, carbamate NH), 5.21 (1H, b t, $J_{3,4} = J_{4,5}$ 10.4 Hz, H-4), 5.05 (1H, t, $J_{2,3} = J_{3,4}$ 9.8 Hz, H-3), 4.77 (1H, b d, $J_{1,2}$ 8.4 Hz, H-1), 4.37 (1H, d, $J_{1',2'}$ 8.0 Hz, H-1′), 4.28 (1H, *A*BX, J_{AB} 12.4 Hz, J_{AX} 3.4 Hz, J_{BX} 2.4 Hz, ν_a 2141.3 Hz, ν_b 2036.3 Hz, H-6), 4.07 (1H, A*B*X, as above, H-6), 3.81 (1H, t, $J_{3',4'} = J_{4',5'}$ 10.4 Hz, H-4′), 3.80 (1H, t, $J_{2',3'} = J_{3',4'}$ 9.5 Hz, H-3′), 3.77 (1H, *A*BX, J_{AB} 12.2 Hz, J_{AX} 1.6 Hz, J_{BX} 3.2 Hz, ν_a 1884.3 Hz, ν_b 1863.8 Hz, H-6′), 3.73 (1H, A*B*X, as above, H-6′), 3.65 (1H, m, H-2), 3.64 (3H, s, carbamate OCH_3), 3.62 (1H, m, H-5), 3.48 (3H, s, anomeric OCH_3), 3.44 (1H, dd, $J_{1',2'}$ 9.6 Hz, $J_{2',3'}$ 8.1 Hz, H-2′), 3.34 (1H, AB*X*, ν_x 1671.3 Hz, H-5′), 3.29 (3H, s, acetal OCH_3), 3.23 (3H, s, acetal OCH_3), 2.03 (3H, s, acetate CH_3), 2.00 (3H, s, acetate CH_3), 1.98 (3H, s, acetate CH_3), 1.28 (3H, s, acetal CH_3), 1.25 (3H, s, acetal CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.8 (acetate C=O), 169.6 (acetate C=O), 156.8 (carbamate C=O), 101.8 (C-1), 101.4 (C-1′), 99.6 (acetal Cq), 99.5 (acetal Cq), 75.7 (C-4′), 75.3 (C-3), 72.8 (C-5′), 71.8 (C-5), 71.5 (C-3′), 69.7 (C-2′), 68.3 (C-4), 62.0 (C-6), 60.8 (C-6′), 57.2 (C-2), 56.5 (anomeric OCH_3), 52.5 (carbamate OCH_3), 48.3 (acetal OCH_3), 48.1 (acetal OCH_3), 20.8 (acetal CH_3), 17.7 (acetate CH_3), 17.7 (acetate CH_3). HRFABMS: Calcd for $C_{27}H_{43}NO_{17}Na$ (M+Na) m/z 676.2432; found m/z 676.2429.

3.7. Methyl [methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-methoxycarbonylamino-β-D-glucopyranosyl-(1 → 4)-(2′*R*,3′*R*)-2,3-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-β-D-glucopyranosid]uronate (9)

A solution of 5% NaOCl (73 mL) and satd aq $NaHCO_3$ (54 mL) was added dropwise to a solution of **8** (6.35 g, 9.71 mmol), tetrabutylammonium bromide (178 mg, 0.55 mmol), sodium bromide (200 mg, 1.94 mmol), and TEMPO (82 mg, 0.52 mmol) in CH_2Cl_2 (120 mL) and H_2O (20 mL) at 0 °C.[16] After stirring at 0 °C for 30 min, the reaction was quenched with MeOH (5 mL) and extracted with $CHCl_3$ (1×). The aqueous layer was acidified with 1 M HCl and extracted once again with $CHCl_3$ (5×). The organic extract was dried over $MgSO_4$ and concentrated in vacuo to afford the crude acid as a yellow syrup.

A solution of Diazald® (7.28 g, 33.60 mmol) in Et_2O (70 mL) was added dropwise to a solution of KOH (7.0 g, 124.76 mmol) in H_2O (11 mL) and EtOH (14 mL). The resulting CH_2N_2 was distilled into a solution of the acid in CH_2Cl_2 (100 mL) and Et_2O (50 mL). Excess CH_2N_2 was allowed to evaporate, and the methyl ester was purified by flash chromatography (3:1 EtOAc–hexane) to afford **9** (4.70 g, 71%) as a white foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.07 (2H, m, H-3 and H-4), 4.73 (1H, b d, $J_{NH,2}$ 7.8 Hz, carbamate NH), 4.64 (1H, d, $J_{1,2}$ 8.2 Hz, H-1), 4.42 (1H, d, $J_{1',2'}$ 8.0 Hz, H-1′), 4.26 (1H, *A*BX, J_{AB} 12.1 Hz, J_{AX} 3.5 Hz, J_{BX} 2.4 Hz, ν_a 2131.8 Hz, ν_b 2055.3 Hz, H-6), 4.11 (1H, A*B*X, as above, H-6), 3.99 (1H, t, $J_{3',4'} = J_{4',5'}$ 9.2 Hz, H-4′), 3.86 (1H, d, $J_{4',5'}$ 9.2 Hz, H-5′), 3.80 (1H, t, $J_{2',3'} = J_{3',4'}$ 9.9 Hz, H-3′), 3.79 (3H, s, ester OCH_3), 3.63 (3H, s, carbamate OCH_3), 3.61 (1H, AB*X*, ν_x 1805.3 Hz, H-5), 3.52 (1H, dd, $J_{1',2'}$ 10.2 Hz, $J_{2',3'}$ 8.1 Hz, H-2′), 3.51 (3H, s, anomeric OCH_3), 3.27 (3H, s, acetal OCH_3), 3.24 (3H, s, acetal OCH_3), 2.04 (3H, s, acetate CH_3), 1.99 (3H, s, acetate CH_3), 1.99 (3H, s, acetate CH_3), 1.29 (3H, s, acetal CH_3), 1.25 (3H, s, acetal CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.9 (acetate C=O), 169.6 (ester C=O), 102.3 (C-1), 102.2 (C-1′), 99.8 (acetal Cq), 99.7 (acetal Cq), 77.9 (C-4′), 74.8 (C-3), 73.0 (C-5′), 71.8 (C-5), 70.6 (C-3′), 69.2 (C-2′), 68.3 (C-4), 62.1 (C-6), 57.7 (anomeric OCH_3), 57.4 (C-2), 53.0 (ester OCH_3), 52.4 (carbamate OCH_3), 48.2 (acetal OCH_3), 20.8 (acetal CH_3), 20.5 (acetal CH_3), 17.7 (acetate CH_3), 17.6 (acetate CH_3). HRFABMS: Calcd for $C_{28}H_{43}NO_{18}Na$ (M+Na) m/z 704.2381; found m/z 704.2378.

3.8. Methyl [methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1 → 4)-(2′*R*,3′*R*)-2,3-*O*-(2′,3′dimethoxybutane-2′,3′-diyl)-β-D-glucopyranosid]-uronate (10)

$MeSiCl_3$ (6.30 mL, 53.70 mmol) was added to a solution of **9** (3.66 g, 5.37 mmol) and Et_3N (7.50 mL, 5.37 mmol) in dry THF (35 mL) at 70 °C. After stirring for 22 h, the reaction was diluted with THF (200 mL), quenched with H_2O (125 mL) at 0 °C, and extracted with CH_2Cl_2 (3×). The aqueous layer was neutralized with satd aq $NaHCO_3$ and extracted with CH_2Cl_2 (3×). The organic extract was dried over Na_2SO_4 and concentrated in vacuo to afford the crude amine as a light-yellow solid.

Ac_2O (5.10 mL, 53.70 mmol) was added to a solution of the crude amine in dry pyridine (50 mL). After stirring at rt for 2 h, the mixture was concentrated in vacuo and purified by flash chromatography (EtOAc) to afford **10** (3.50 g, 98%) as a white foam: mp 176–177 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.60 (1H, d, $J_{NH,2}$ 9.4 Hz, NH), 5.11 (1H, t, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 5.05 (1H, t, $J_{2,3} = J_{3,4}$ 10.3 Hz, H-3), 4.69 (1H, d, $J_{1,2}$ 8.4 Hz, H-1), 4.42 (1H, d, $J_{1',2'}$ 7.9 Hz, H-1′), 4.28 (1H, *A*BX, J_{AB} 12.2 Hz, J_{AX} 3.5 Hz, J_{BX} 2.4 Hz, ν_a 2142.3 Hz, ν_b 2041.3 Hz, H-6), 4.08 (1H, A*B*X, as above, H-6), 3.98 (1H, m, H-2), 3.95 (1H, m, H-5′), 3.91 (1H, t, $J_{3',4'} = J_{4',5'}$ 8.4 Hz, H-4′), 3.81 (1H, dd, $J_{2',3'}$ 10.1 Hz, $J_{3',4'}$ 8.9 Hz, H-3′), 3.79 (3H, s, ester OCH_3), 3.62 (1H, AB*X*, ν_x 1810.3 Hz), 3.52 (1H, dd, $J_{1',2'}$ 9.8 Hz, $J_{2',3'}$ 7.9 Hz, H-2′), 3.51 (3H, s, anomeric OCH_3), 3.28 (3H, s, acetal OCH_3), 3.24 (3H, s, acetal OCH_3), 2.04 (3H, s, acetate CH_3), 2.04 (3H, s, acetate CH_3), 1.99 (3H, s, acetate CH_3), 1.99 (3H, s, acetate CH_3), 1.90 (3H, s, acetate CH_3), 1.29 (3H, s, acetal CH_3), 1.25 (3H, s, acetal CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 171.2 (acetate C=O), 170.9 (acetate C=O), 170.4 (acetate C=O), 169.5 (acetate C=O), 169.4 (ester C=O), 102.0 (C-1), 101.7 (C-1′), 99.8 (acetal Cq), 99.7 (acetal Cq), 77.7 (C-4′), 74.6 (C-3), 73.5 (C-5′), 72.0 (C-5), 71.0 (C-3′), 69.1 (C-2′), 68.1 (C-4), 62.1 (C-6), 57.4 (anomeric OCH_3), 54.5 (C-2), 53.0 (ester OCH_3), 48.2 (acetal OCH_3), 23.4 (acetal CH_3), 20.8 (acetal CH_3), 17.7 (acetate CH_3), 17.6 (acetate CH_3). HRFABMS: Calcd for $C_{28}H_{43}NO_{17}Na$ (M+Na) m/z 688.2426; found m/z 646.2429.

3.9. Methyl [methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1 → 4)-glucopyranosid]-uronate (11)

Trifluroacetic acid (1 mL) and H_2O (0.05 mL) were added to a solution of **10** in CH_2Cl_2 (20 mL). After stirring at rt for 17 h, the mixture was neutralized with satd aq $NaHCO_3$ and extracted with CH_2Cl_2 (5×). The organic extract was dried over $MgSO_4$, concentrated in vacuo and purified by flash chromatography (10:1 EtOAc–MeOH) to afford **11** (1.58 g, 58%) as a white solid: mp 215 °C (dec). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.74 (d, 1H, $J_{NH,2}$ 9.0 Hz, NH), 5.13 (dd, 1H, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 9.5 Hz, H-3), 5.05 (t, 1H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 4.69 (d, 1H, $J_{1,2}$ 8.6 Hz, H-1), 4.29 (d, 1H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.26 (*A*BX, 1H, J_{AB} 12.5 Hz, J_{AX} 2.4 Hz, J_{BX} 6.1 Hz, ν_a 2129.8 Hz, ν_b 2059.8 Hz, H-6), 4.12 (A*B*X, 1H, as above, H-6), 4.03 (dt, 1H, $J_{NH,2}$ 10.4 Hz, $J_{1,2} = J_{2,3}$ 8.8 Hz, H-2), 3.92 (d, 1H, $J_{4',5'}$ 9.6 Hz, H-5′), 3.81 (AB*X*, 1H, ν_x 1905.3 Hz, H-5), 3.80 (s, 3H, ester OCH_3), 3.75 (dd, 1H, $J_{3',4'}$ 9.5 Hz, $J_{4',5'}$ 8.6 Hz, H-4′), 3.66 (t, 1H, $J_{2',3'} = J_{3',4'}$ 8.9 Hz, H-3′), 3.57 (s, 3H, anomeric OCH_3), 3.44 (dd, 1H, $J_{1',2'}$ 9.3 Hz, $J_{2',3'}$ 8.0 Hz, H-2′), 2.10 (s, 3H, acetate CH_3), 2.03 (s, 3H, acetate CH_3), 2.02 (s, 3H, acetate CH_3), 1.90 (s, 3H, acetate CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 175.8 (acetate C=O), 171.1 (acetate C=O), 170.9 (acetate C=O), 170.4 (ester C=O), 104.0 (C-1′), 102.1 (C-1), 82.4 (C-4′), 74.4 (C-3′), 73.3 (C-5′), 73.1 (C-2′), 72.7 (C-3), 72.4 (C-5), 68.3 (C-4), 62.0 (C-6), 57.8 (anomeric OCH_3), 54.0 (C-2), 53.0 (ester OCH_3), 23.9 (acetate CH_3), 20.8 (acetate CH_3), 20.8 (acetate CH_3). HRFABMS: Calcd for $C_{22}H_{33}NO_{15}Na$ (M+Na) m/z 574.1749; found m/z 574.1748.

3.10. Methyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosiduronate, sodium salt (12)

Cold 1 N NaOH was added dropwise to a solution of **11** in MeOH (50 mL) to pH 10–12. After 14 h of stirring at rt, the reaction was neutralized with glacial HOAc and concentrated in vacuo. The residue was purified by flash chromatography (4:2:2:1 BuOH–EtOH–H_2O–HOAc), followed by size-exclusion chromatography on a Sephadex G-10 column using H_2O as the eluent. The resulting residue was then subjected to reversed-phase chromatography on a C-18 column using H_2O as the eluent to afford **12** (1.18 g, 100%) as a white solid: mp 191 °C (dec). ^{1}H NMR (500 MHz, D_2O): δ 4.55 (d, 1H, $J_{1,2}$ 8.5 Hz), 4.37 (d, 1H, $J_{1',2'}$ 7.7 Hz, H-1′), 3.93 (d, 1H, $J_{5,6}$ 12.3 Hz, H-6), 3.76 (d, 1H, $J_{5,6}$ 12.6 Hz, H-6), 3.74 (m, 1H, H-5), 3.72 (t, 1H, $J_{3',4'} = J_{4',5'}$ 7.1 Hz, H-4′), 3.70 (m, 1H, H-2), 3.58 (t, 1H, $J_{2',3'} = J_{3',4'}$ 9.3 Hz, H-3′), 3.55 (s, 3H, anomeric OCH_3), 3.53 (m, 1H, H-5′), 3.47 (m, 2H, H-3 and H-4), 3.32 (dd, 1H, $J_{1',2'}$ 9.4 Hz, $J_{2',3'}$ 8.1 Hz, H-2′), 2.05 (s, 3H, acetamide CH_3). ^{13}C NMR (D_2O, 125 MHz): δ 175.1 (acid C═O), 174.5 (acetamide C═O), 103.6 (C-1′), 100.9 (C-1), 80.2 (C-5), 76.8 (C-4′), 76.0 (C-3), 74.0 (C-4), 73.9 (C-3′), 72.9 (C-2′), 69.9 (C-5′), 60.7 (C-6), 57.5 (anomeric OCH_3), 55.5 (C-2), 22.7 (acetamide CH_3). HRFABMS: Calcd for $C_{15}H_{24}NO_{12}Na_2$ (M+Na) m/z 456.1095; found m/z 456.1094.

3.11. Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-2-deoxy-4,6-*O*-isopropylidene-2-methoxycarbonylamino-β-D-glucopyranoside (16)

A solution of **14** (4.0 g, 13.73 mmol) in CH_2Cl_2 (10 mL) was added dropwise via cannula to a mixture of **15** (18.0 g, 36.53 mmol) and $BF_3{\cdot}Et_2O$ (1.40 mL, 10.96 mmol) in CH_2Cl_2 (20 mL) at −30 °C. The reaction was allowed to warm to rt after 24 h, then quenched with Et_3N (5 mL) and concentrated in vacuo. The residue was purified by flash chromatography (2.5:1 EtOAc–hexane) to afford **16** (7.21 g, 84%) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.18 (1H, b d, $J_{1',2'}$ 7.1 Hz, H-1′), 5.09 (1H, t, $J_{2,3} = J_{3,4}$ 9.2 Hz, H-3), 5.05 (1H, t, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4), 4.88 (1H, t, $J_{1,2} = J_{2,3}$ 8.0 Hz, H-2), 4.70 (1H, d, $J_{1,2}$ 7.8 Hz, H-1), 4.64 (1H, b s, H-3′), 4.20 (1H, dd, $J_{6,6}$ 12.3 Hz, $J_{5,6}$ 4.3 Hz, H-6), 4.08 (1H, dd, $J_{6,6}$ 11.8 Hz, $J_{5,6}$ 2.6 Hz, H-6), 3.87 (1H, dd, $J_{6',6'}$ 11.1 Hz, $J_{5',6'}$ 5.7 Hz, H-6′), 3.72 (1H, t, $J_{6',6'} = J_{5',6'}$ 10.4 Hz, H-6), 3.64 (1H, t, $J_{3',4'} = J_{4',5'}$ 9.2 Hz, H-4′), 3.61 (3H, s, carbamate OCH_3), 3.58 (1H, m, H-5), 3.41 (3H, s, anomeric OCH_3), 3.23 (1H, td, $J_{4',5'}$ 9.9 Hz, $J_{5',6'} = J_{6',6'}$ 5.4 Hz, H-5′), 3.14 (1H, b s, H-2′), 2.03 (3H, s, acetate CH_3), 1.97 (3H, s, acetate CH_3), 1.96 (3H, s, acetate CH_3), 1.93 (3H, s, acetate CH_3), 1.45 (3H, s, acetonide CH_3), 1.33 (3H, s, acetonide CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): 170.8 (acetate C═O), 170.4 (acetate C═O, 169.5 (acetate C═O), 156.4 (carbamate C═O), 100.1 (C-1), 99.4 (acetonide Cq), 73.2 (C-3), 73.2 (C-2), 71.9 (C-4), 71.8 (C-5′), 68.3 (C-4′), 66.6 (C-5), 62.2 (C-6), 62.0 (C-6′), 57.6 (carbamate OCH_3), 57.2 (anomeric OCH_3), 29.2 (acetonide CH_3), 20.8 (acetate CH_3), 20.7 (acetate CH_3), 20.6 (acetate CH_3), 19.1 (acetonide CH_3). HRFABMS: Calcd for $C_{26}H_{39}$ $NO_{16}Na$ (M+Na) m/z 644.2169; found m/z 644.2167.

3.12. Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (17)

$MeSiCl_3$ (3.08 mL, 26.22 mmol) was added to a solution of **16** (8.15 g, 13.11 mmol) and Et_3N (7.31 mL, 52.44 mmol) in dry THF (64 mL) at 70 °C. After 72 h, the reaction was diluted with THF (300 mL) and Et_3N (20 mL), poured into a mixture of THF (1 L) and H_2O (300 mL) and extracted with CH_2Cl_2 (3×). The organic layer was dried over Na_2SO_4, and concentrated in vacuo to afford the crude amine as a dark-yellow foam.

Ac_2O (3.71 mL, 39.32 mmol) was added to a solution of the free amine in dry pyridine (50 mL). After stirring at rt for 4 h, the reaction was concentrated in vacuo and purified by flash chromatography (EtOAc) to afford **17** (1.85 g, 23%) as a white solid: mp 201–202 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.92 (1H, d, $J_{NH,2}$ 7.0 Hz, NH), 5.15 (1H, t, $J_{2,3} = J_{3,4}$ 9.5 Hz, H-3), 5.08 (1H, t, $J_{3,4} = J_{4,5}$ 9.7 Hz, H-4), 5.01 (1H, d, $J_{1',2'}$ 8.2 Hz, H-1′), 4.93 (1H, t, $J_{1,2} = J_{2,3}$ 8.0 Hz, H-2), 4.89 (1H, d, $J_{1,2}$ 7.8 Hz, H-1), 4.49 (1H, t, $J_{2',3'} = J_{3',4'}$ 9.3 Hz, H-3′), 4.23 (1H, *A*BX, J_{AB} 12.3 Hz, J_{AX} 4.7 Hz, J_{BX} 2.5 Hz, ν_a 2116.8 Hz, ν_b 2072.8 Hz, H-6), 4.15 (1H, A*B*X, as above, H-6), 3.92 (1H, dd, $J_{6',6'}$ 10.9 Hz, $J_{5',6'}$ 5.3 Hz, H-6′), 3.76 (1H, t, $J_{6',6'}J_{5',6'}$ 10.7 Hz, H-6′), 3.70 (1H, t, $J_{3',4'} = J_{4',5'}$ 9.3 Hz, H-4′), 3.63 (1H, AB*X*, ν_x 1815.5 Hz), 3.46 (3H, s, anomeric OCH_3), 3.35 (1H, td, $J_{5',6'} = J_{5',6'}$ 10.3 Hz, $J_{4',5'}$ 5.6 Hz, H-5′), 3.03 (1H, m, H-2′), 2.07 (3H, s, acetate CH_3), 2.05 (3H, s, acetate CH_3), 2.01 (3H, s, acetate CH_3), 1.99 (3H, s, acetate CH_3), 1.97 (3H, s, acetate CH_3), 1.50 (3H, s, acetonide CH_3), 1.39 (3H, s, acetonide CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 171.0 (acetate C═O), 170.9 (acetate C═O), 170.5 (acetate C═O), 169.8 (acetate C═O), 169.6 (acetate C═O), 100.5 (C-1′), 99.5 (acetonide Cq), 99.2 (C-1), 76.6 (C-3′), 73.7 (C-4′), 73.2 (C-3), 72.2 (C-2), 72.1 (C-5), 68.5 (C-4), 66.6 (C-5′), 62.6 (C-6), 62.3 (C-6′), 58.4 (anomeric OCH_3), 57.4 (C-2′), 29.4 (acetonide CH_3), 21.0 (acetate CH_3), 21.0 (acetate CH_3), 20.8 (acetate CH_3), 19.2 (acetate CH_3). HRFABMS: Calcd for $C_{26}H_{39}NO_{15}Na$ (M+Na) m/z 628.2217; found m/z 628.2217.

3.13. Methyl (sodium β-D-glucopyranosyluronate)-(1 → 3)-(2-acetamido-2-deoxy-β-D-glucopyranoside (18)

A catalytic amount of Na^0 was added to a solution of **17**(1.85 g, 3.05 mmol) in dry MeOH (20 mL). The

mixture was allowed to stir at rt under nitrogen for 3 h, after which time Dowex 50W-X8 cation-exchange resin was added to neutralize the reaction mixture. The resin was filtered, and the filtrate was concentrated in vacuo to quantitatively afford the corresponding triol.

A solution of 5% NaOCl (16 mL) and satd aq $NaHCO_3$ (12 mL) was added dropwise to a solution of the crude triol, tetrabutylammonium bromide (56 mg, 0.17 mmol), sodium bromide (63 mg, 0.61 mmol), and TEMPO (25 mg, 0.16 mmol) in CH_2Cl_2 (4 mL) and H_2O (24 mL) at 0 °C.[16] After stirring at 0 °C for 30 min, the reaction was quenched with MeOH (5 mL) and concentrated in vacuo. The residue was purified by flash chromatography (4:2:2:1 BuOH–EtOH–H_2O–HOAc), followed by size-exclusion chromatography on a Sephadex LH-20 column using H_2O as the eluent. The resulting residue was then subjected to reversed-phase chromatography on a C-18 column using H_2O as the eluent to afford **18** (1.30 g, 98%) as a white solid: mp 135 °C (dec). ^{1}H NMR (500 MHz, D_2O): δ 4.66 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1), 4.59 (d, 1H, $J_{1',2'}$ 8.3 Hz, H-1′), 4.04 (d, 1H, $J_{4,5}$ 9.3 Hz, H-5) 4.02 (d, 1H, $J_{6',5'}$ 12.5 Hz, H-6′), 3.93 (dd, 1H, $J_{1',2'}$ 10.1 Hz, $J_{2',3'}$ 8.3 Hz, H-2′), 3.87 (m, 2H, H-3′, and H-6′), 3.65 (m, 2H, H-3, and H-4), 3.63 (m, 1H, H-5′), 3.60 (s, 3H, anomeric OCH_3), 3.59 (t, 1H, $J_{3',4'} = J_{4',5'}$ 8.4 Hz, H-4′), 3.43 (t, 1H, $J_{1,2} = J_{2,3}$ 8.1 Hz, H-2), 2.12 (s, 3H, acetamide CH_3). ^{13}C NMR (125 MHz, D_2O): δ 175.2 (acid C=O), 175.1 (acetamide C=O), 103.0 (C-1′, 102.0 (C-1), 83.1 (C-2′), 75.6 (C-5), 75.4 (C-4), 72.8 (C-4′), 71.6 (C-3), 68.9 (C-5′), 60.9 (C-6′), 57.6 (anomeric OCH_3), 54.5 (C-2), 22.7 (acetamide CH_3). HRFABMS: Calcd for $C_{16}H_{24}NO_{12}Na_2$ (M+Na) m/z 456.1095; found m/z 456.1094.

4. Conclusions

Starting from commercially available and inexpensive starting materials, we provided a scalable route to the syntheses of the $(1\rightarrow4)$-linked and $(1\rightarrow3)$-linked HA disaccharides. Large scale availability of such compounds will allow more facile methods by which to acquire and study the biological properties of hyaluronan and hyaluronan oligomers.

Acknowledgements

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

References

1. Lapčík, L., Jr.; Lapčík, L.; De Smedt, S.; Demeester, J.; Chabreček, P. *Chem. Rev.* **1998**, *98*, 2663–2684.
2. Flugge, L. A.; Miller-Deist, L. A.; Petillo, P. A. *Chem. Biol.* **1999**, *6*, R157–R166.
3. (a) Yeung, B. K. S.; Chong, P. Y.; Petillo, P. A. The Synthesis of Glycosaminoglycans. In *Glycochemistry: Principles, Synthesis and Applications*; Bertozzi, C., Wang, G., Eds.; Marcel Dekker: New York, 2001; (b) Yeung, B. K. S.; Chong, P. Y. C.; Petillo, P. A. *J. Carbohydr. Chem.* **2002**, *21*, 799–865.
4. Slaghek, T. M.; Nakahara, Y.; Ogawa, T.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1994**, *255*, 61–85.
5. Sičinska, W.; Adams, B.; Lerner, L. *Carbohydr. Res.* **1993**, *242*, 29–51.
6. Carter, M. B.; Petillo, P. A.; Anderson, L.; Lerner, L. E. *Carbohydr. Res.* **1994**, *258*, 299–306.
7. Blatter, G.; Jacquinet, J.-C. *Carbohydr. Res.* **1996**, *288*, 109–125.
8. Yeung, B. K. S.; Hill, D. C.; Janicka, M.; Petillo, P. A. *Org. Lett.* **2000**, *2*, 1279–1282.
9. Iyer, S. S.; Rele, S. M.; Baskaran, S.; Chaikof, E. L. *Tetrahedron* **2003**, *59*, 631–638.
10. Jeanloz, R. W.; Flowers, H. M. *J. Am. Chem. Soc.* **1962**, *84*, 3030–3030.
11. Tawada, A.; Masa, T.; Oonuki, Y.; Watanabe, A.; Matsuzaki, Y.; Asari, A. *Glycobiology* **2002**, *12*, 421–426.
12. Haynie, S. L.; Whitesides, G. M. *Appl. Biochem. Biotechnol.* **1990**, *23*, 155–170.
13. For extensive reviews on the chemoenzymatic synthesis of carbohydrates and the application to carbohydrate recognition, see: Wong, C.-H. *Acta Chem. Scand.* **1996**, *50*, 211–218; Toone, E. J.; Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *Tetrahedron* **1989**, *45*, 5365–5422.
14. Nonenzymatic large-scale syntheses of key glycoforms have been reported, for example: (a) Urban, F. J.; Breitenbach, R.; Buzon, R. A.; Daniels, P. J.; Dunn, P. J.; Gut, S.; Lehner, R. S.; Orrill, S. L.; Watson, H. A., Jr. *Org. Process Res. Dev.* **1998**, *2*, 66–70; (b) Smith, A. B., III; Beauchamp, T. J.; LaMarche, M. J.; Kaufman, M. D.; Qiu, Y.; Arimoto, H.; Jones, D. R.; Kobayashi, K. *J. Am. Chem. Soc.* **2000**, *122*, 8654–8664; (c) Choi, S.-K.; Lee, S.; Whitesides, G. M. *J. Org. Chem.* **1996**, *61*, 8739–8745; (d) Martichonok, V.; Whitesides, G. M. *J. Am. Chem. Soc.* **1996**, *118*, 8187–8191; (e) Martichonok, V.; Whitesides, G. M. *J. Org. Chem.* **1996**, *61*, 1702–1706; (f) Martichonok, V.; Whitesides, G. M. *Carbohydr. Res.* **1997**, *302*, 123–129; (g) Adelhorst, K.; Whitesides, G. M. *Carbohydr. Res.* **1993**, *242*, 69–76; (h) Heidlas, J. E.; Williams, K. W.; Whitesides, G. M. *Acc. Chem. Res.* **1992**, *25*, 307–314.
15. Montchamp, J.-L.; Tian, F.; Hart, M. E.; Frost, J. W. *J. Org. Chem.* **1996**, *61*, 3897–3899.
16. Garegg, P. J.; Oscarson, S.; Tedebark, U. *J. Carbohydr. Chem.* **1998**, *17*, 587–594.
17. Yeung, B. K. S.; Adamski-Werner, S. L.; Bernard, J. B.; Poulenat, G.; Petillo, P. A. *Org. Lett.* **2000**, *2*, 3135–3138.
18. (a) Green, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*. 2nd ed.; John Wiley: New York, 1991, pp 127–141; (b) Kocienski, P. J. *Protecting Groups*; Georg Thiem: Stuttgart, 1994; pp 33–40.
19. (a) Schmidt, R. R.; Stumpp, M. *Liebigs Ann. Chem.* **1983**, 1249–1256; (b) Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21–123.