JOC*Article*

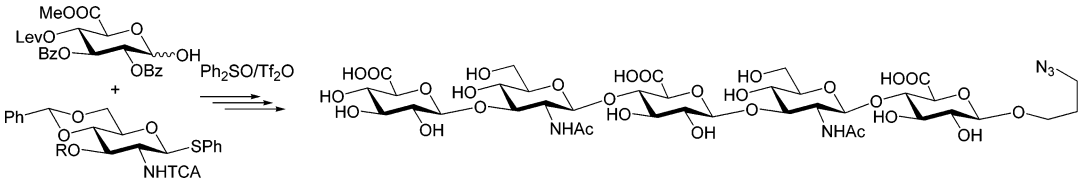
Synthesis of Hyaluronic Acid Oligomers Using Ph_2SO/Tf_2O-Mediated Glycosylations

Jasper Dinkelaar, Jeroen D. C. Codée, Leendert J. van den Bos, Herman S. Overkleeft, and Gijsbert A. van der Marel*

Leiden Institute of Chemistry, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands

marel_g@chem.leidenuniv.nl

Received April 10, 2007



The synthesis of hyaluronic acid (HA) oligomers using a stepwise glycosylation strategy is described. This method employs protected 1-hydroxyuronic acid and 1-phenylthio glucosamine donors, both of which are activated with the Ph_2SO/Tf_2O activator system.

Introduction

Hyaluronan (hyaluronic acid, HA), a negatively charged high molecular weight polymer assembled from β-1,3-linked-2-acetamido-2-deoxy-D-glucose-β-(1−4)-D-glucuronic acid[1] repeating units, is structurally the least complicated member of the glycosaminoglycan (GAG) polysaccharide family. HA is involved in many biological functions, including cell-migration, proliferation, adhesion, recognition,[2] tumor invasion (in particular malignant tumors which show increased levels of HA),[3] and tumor inhibition.[4] Small HA fragments have recently been identified as potent activators of immunocompetent cells that play a critical role in the development of T cell-mediated immune responses.[5] HA oligomers have been found to bind to Toll-like receptor 4 (TLR-4), and it is thought that the immunostimulating properties ascribed to HA oligomers are effected through this receptor. The above-mentioned studies on the immunological properties of HA oligomers made use of compound mixtures containing HA oligomers differing in size ranging from four monosaccharides to at least eight units. The reason for this compound heterogeneity is that HA oligomers are routinely prepared by enzymatic processing of HA polymers, a process that cannot be controlled to such an extent that pure HA oligomers of a distinct molecular weight can be obtained at will. The exact requirements in terms of HA oligomer size for TLR-4 binding and agonizing activity cannot be determined by making use of the available HA oligomer mixtures (this situation is reminiscent of alginate oligomer-mediated TLR-4 stimulation). Well-defined HA oligomers can be obtained through organic synthesis, a strategy that has the advantage that functionalized HA oligomers are within reach as well.

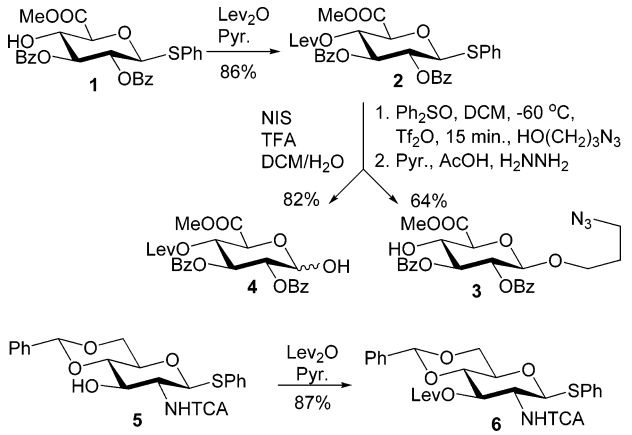
Since the pioneering work of Jeanloz[6] in 1964, several research groups have studied the synthesis of HA oligomers,[7] but HA has received only a little attention from the synthetic carbohydrate community compared to other glycosaminoglycan family members, such as heparan. With the aim to prepare HA oligomers that can be functionalized and used for TLR-4 binding studies, we set out to develop a general synthesis protocol. Here we report our results in the synthesis of a well defined, fully deprotected HA trimer, tetramer, and pentamer, each with a glucuronic acid moiety as the reducing end sugar.

Our synthesis strategy is based on our previous finding that donor 1-hydroxysugars can be chemoselectively condensed with acceptor 1-thioglycosides using Gin's activator system for dehydrative glycosylations (diphenyl sulfoxide/trifluoromethane-

(1) Meyer, K.; Palmer, J. W. *J. Biol. Chem.* **1934**, *107*, 629−634.
(2) Laurent, T. C.; Fraser, J. R. E. *FASEB J.* **1992**, *6*, 2397−2404.
(3) Knudson, C. B.; Knudson, W. *FASEB J.* **1993**, *7*, 1233−1241.
(4) Zeng, C.; Toole, B. P.; Kinney, S. D.; Kuo, J.; Stamenkovic, I. *Int. J. Cancer* **1998**, *77*, 396−401.
(5) Termeer, C.; Benedix, F.; Sleeman, J.; Fieber, C.; Voith, U.; Ahrens, T.; Miyake, K.; Freudenberg, M.; Galanos, C.; Simon, J. C. *J. Exp. Med.* **2002**, *195*, 99−111.
(6) Flowers, H. M.; Jeanloz, R. W. *Biochemistry* **1964**, *3*, 123−125.
(7) (a) Slaghek, T. M.; Hyppönen, T. K.; Kruiskamp, P. H.; Ogawa, T.; Kamerling, J. P.; Vliegenthart, J. F. G. *Tetrahedron Lett.* **1993**, *34*, 7939−7942. (b) Blatter, G.; Jacquinet, J.-C. *Carbohydr. Res.* **1996**, *288*, 109−125. (c) Yeung, B. K. S.; Hill, D. C.; Janicka, M.; Petillo, P. A. *Org. Lett.* **2000**, *2*, 1279−1282. (d) Yeung, B. K. S.; Chong, P. Y. C.; Petillo, P. A. *Carbohydr. Res.* **2002**, *21*, 779−865 (and references cited therein). (e) Iyer, S. S.; Rele, S. M.; Baskaran, S.; Chaikof, E. L. *Tetrahedron* **2003**, *59*, 631−638. (f) Lu, X. A.; Chou, C. H.; Wang, C. C.; Hung, S. C. *Synlett* **2003**, *9*, 1364−1366. (g) Palmacci, E. R.; Seeberger, P. H. *Tetrahedron* **2004**, *60*, 7755−7766. (h) Huang, L.; Huang, X. *Chem. Eur. J.* **2007**, *13*, 529−540.

10.1021/jo070704s CCC: $37.00
Published on Web 06/22/2007

SCHEME 1. Synthesis of Monomer HA Building Blocks



sulfonic anhydride),[8] resulting in the formation of 1-thiodisaccharides amenable for elongation at both the reducing end and nonreducing end.[9] We further elected to mask the reducing glucuronide in our target HA oligomers as the 3-azido-1-propanol glycoside, with the dual advantage of locking the anomeric configuration and enabling functionalization of the azide moiety for future conjugation studies.

Results and Discussion

The synthesis of functionalized monosaccharides **3**, **4**, **5**, and **6** that we required for executing our strategy is summarized in Scheme 1. Partially protected 1-phenylthioglucuronide **1**, prepared following our previously reported procedure,[10] was transformed into the corresponding 4-*O*-levulinoyl derivative **2**. Glycosylation with 3-azidopropanol (Ph_2SO, Tf_2O) followed by deblocking of the 4′-hydroxyl function gave reducing end building block **3**, whereas hydrolysis of the thioacetal in **2** provided 1-hydroxy donor **4**.[11] Partially protected glucosamine derivative **5** was prepared as described by Blatter and Jacquinet,[12] and 3-*O*-levulinoylation gave donor thioglycoside **6**.

The synthesis of fully protected HA trimer **8** is depicted in Scheme 2 and commenced with the Ph_2SO/Tf_2O/TTBP-mediated condensation of donor glycoside **4** and acceptor glycoside **5**. The resulting thiodisaccharide **7** was condensed with acceptor glucuronide **3** under the same conditions to provide trisaccharide **8**.

At this stage, we investigated the efficiency in preparing trisaccharide **8** using a one-pot procedure.[13] Accordingly, the reaction mixture containing disaccharide **7**, formed after Tf_2O/Ph_2SO-mediated condensation of 1-hydroxydonor **4** with thioglycoside **5**, was cooled and preactivated with an additional 1 equiv of Tf_2O and TTBP (0.95 equiv with respect to Tf_2O), followed by addition of acceptor glycoside **3** (1 equiv). Following this protocol (Scheme 2), trisaccharide **8** was prepared, but the overall yield (12%) proved to be considerably lower than the combined yield (26%) of the two separate glycosylation steps. The difficulty here in our opinion is that base (TTBP) needs to be introduced in both condensation steps in order to avoid acid-mediated (due to in situ formation of TfOH) cleavage of the benzylidene group in the glucosamine derivative. However, the amount of base should be such that orthoester formation during dehydrative glycosylation and oxazolidine formation upon activation of the thioglucosamine donor is avoided. Indeed, we could not enhance the efficiency of the one-pot procedure by varying the amount of TTBP. We conclude that trisaccharide **8** is best prepared via two individual glycosylation steps, and that the optimal amount of base used in both steps is 0.95 equiv with respect to Tf_2O.

Fully protected HA tetramer **11** and pentamer **13** were prepared starting from trimer **8** as follows (Scheme 3). Deprotection of the levulinoyl group in **8** afforded trisaccharide **9** (hydrazine, pyridine/AcOH). Activation of thioglucosamine **6** using the protocol described above (Ph_2SO, Tf_2O, TTBP) followed by addition of acceptor trisaccharide **9** led to the formation of fully protected HA tetramer **11**. Quenching the reaction at −15 °C improved the yield considerably with respect to quenching at 0 °C, and tetramer **11** was isolated in 62% yield. In a similar fashion, but with phenylthiodisaccharide **7** as the donor, fully protected pentasaccharide **13** (42% yield) was prepared. Finally, the fully deprotected target tri-, tetra-, and pentamers **10**, **12**, and **14** were obtained (Scheme 3) by acid cleavage of the benzylidene group followed by saponification of the ester and amide functionalities with KOH, *N*-acetylation in MeOH with Ac_2O, and purification by gel filtration.

In conclusion, we have demonstrated that HA oligomers that are suitably functionalized for future biological studies can be conveniently prepared by making use of thioglycosides and 1-hydroxyglycosides, in combination with the Ph_2SO/Tf_2O/TTBP activating system. The yields in the glycosidic bond formations are moderate, and the combination of the presence of acid-labile benzylidene protective groups and the propensity of orthoester formation requires that the glycosylations be monitored with care, especially with respect to the amount of base used. However, we believe our general strategy to be convenient, in that useful quantities can be prepared from readily available building blocks. By making use of glucuronic acid building blocks, postglycosylation manipulations can be kept to a minimum. We are therefore confident that our strategy will be of use for the large-scale synthesis of HA oligomers, including compounds with a glucosamine residue at the reducing end and oligomers assembled from more than five monosaccharide residues. These compounds in turn will prove to be useful in the assessment of the biological properties of HA oligomers, such as their TLR-4-mediated immunostimulatory activity.

Experimental Section

Methyl (Phenyl 2,3-di-*O*-benzoyl-4-*O*-levulinoyl-1-thio-*β*-D-glucopyranoside) Uronate (2). To a solution of **1** (3.81 g, 7.49 mmol) in pyridine (75 mL) was added a solution of Lev_2O in dioxane (0.5 M, 37.5 mL, 18.7 mmol). After 18 h the mixture was diluted with EtOAc (200 mL) and washed with 1 M HCl (aq), $NaHCO_3$ (aq), and brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **2** as a colorless oil (3.91 g, 86%). $[\alpha]_D^{22}$: +63 (c = 1,

(8) (a) Codée, J. D. C.; Stubba, B.; Schiattarella, M.; Overkleeft, H. S.; van Boeckel, C. A. A.; van Boom, J. H.; van der Marel, G. A. *J. Am. Chem. Soc.* **2005**, *127*, 3767−3773. (b) Garcia, B. A.; Poole, J. L.; Gin, D. Y. *J. Am. Chem. Soc.* **1997**, *119*, 7597−7598.

(9) (a) Codée, J. D. C.; Litjens, R. E. J. N.; Den Heeten, R.; Overkleeft, H. S.; van Boom, J. H.; van der Marel, G. A. *Org. Lett.* **2003**, *5*, 1519−1522. (b) Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123*, 9015−9020.

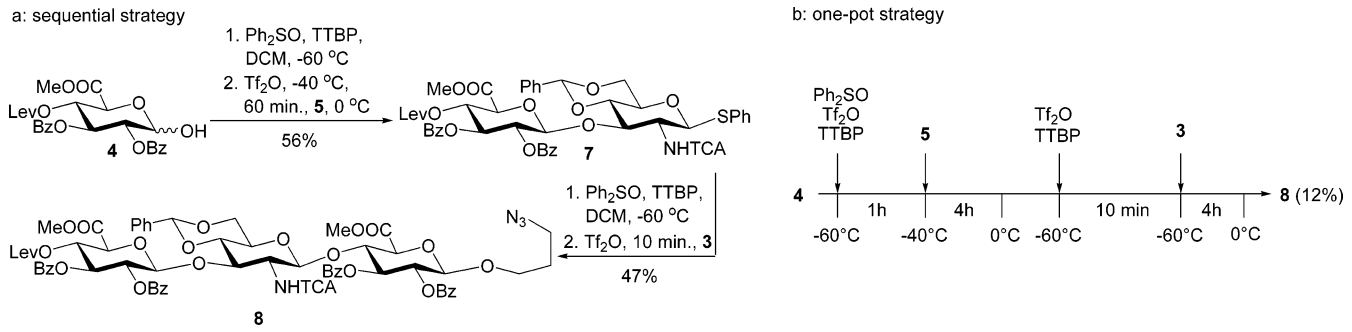
(10) van den Bos, L. J.; Codée, J. D. C.; van Boom, J. H.; Overkleeft, H. S.; van der Marel, G. A. *Org. Lett.* **2004**, *6*, 2165−2168.

(11) Dinkelaar, J.; Witte, M. D.; van den Bos, L. J.; Overkleeft, H. S.; van der Marel, G. A. *Carbohydr. Res.* **2006**, *341*, 1723−1729.

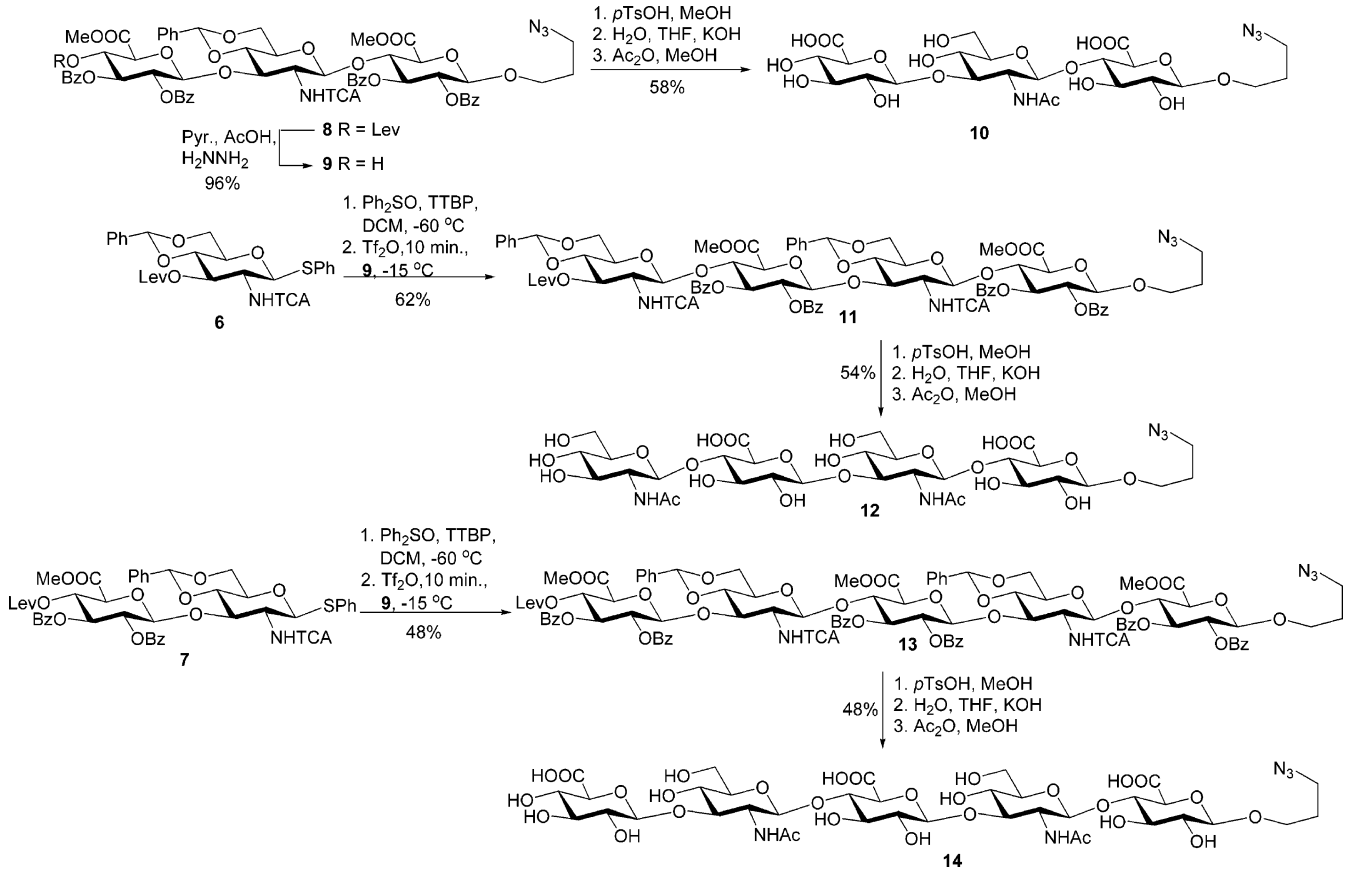
(12) Blatter, G.; Jacquinet, J.-C. *Carbohydr. Res.* **1996**, *288*, 109−125.

(13) Codée, J. D. C.; van den Bos, L. J.; Litjens, R. E. J. N.; Overkleeft, H. S.; van Boom, J. H.; van der Marel, G. A. *Org. Lett.* **2003**, *5*, 1947−1950.

SCHEME 2. Sequential (a) and One-Pot (b) Glycosylation Strategy



SCHEME 3. HA Tetramer and Pentamer Synthesis



$CHCl_3$); IR (neat): 716, 1068, 1263, 1710, 2930 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 2.04 (s, 3H, CH_3 Lev), 2.37−2.58 (m, 4H, 2 × CH_2 Lev), 3.81 (s, 3H, CH_3 COOMe), 4.23 (d, 1H, J = 10.0 Hz, H-5), 4.97 (d, 1H, J = 10.0 Hz, H-1), 5.41 (m, 2H, H-2, H-4), 5.71 (t, 1H, J = 10.0 Hz, H-3),7.29−7.55 (m, 11H, CH_{arom}), 7.87 (d, 2H, J = 7.2 Hz, CH Bz), 7.93 (d, 2H, J = 7.2 Hz, CH Bz); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 27.7 (CH_2 Lev), 29.5 (CH_3 Lev), 37.6 (CH_2 Lev), 53.0 (CH_3 COOMe), 69.5, 70.0 (C-2, C-4), 73.5 (C-3), 76.4 (C-5), 86.6 (C-1), 128.4−128.7 (CH_{arom}), 129.0 ($C_{q\ arom}$), 129.8−130.0 (CH_{arom}), 131.3 ($C_{q\ arom}$), 133.4−133.5 (CH_{arom}), 164.9, 165.6, 166.9, 171.1 (C═O Bz, COOMe, C═O Lev), 205.5 (C═O Lev); HRMS: $C_{32}H_{30}O_{10}S + H^+$ requires 607.16324, found 607.16324.

3-Azidopropyl (Methyl (2,3-di-*O*-benzoyl-*β*-D-glucopyranoside) uronate) (3). A mixture of **2** (1.21 g, 2.00 mmol) and Ph_2SO (0.485 g, 2.40 mmol) were coevaporated with toluene two times to remove traces of water, dissolved in DCM (40 mL), and further dried by stirring over molecular sieves for 15 min. At −60 °C Tf_2O (0.40 mL, 2.4 mmol) was added. After 15 min a solution of 3-azidopropanol (0.608 g, 6.00 mmol) in DCM (15 mL) was slowly added, and the reaction mixture was allowed to warm to 0 °C. Dry Et_3N (1.39 mL, 10 mmol) was added, and the reaction was washed with $NaHCO_3$ (aq). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. This crude concentrate was then dissolved in a mixture of pyridine (16 mL) and AcOH (4 mL), after which hydrazine monohydrate (0.48 mL, 10 mmol) was added. The mixture was stirred for 15 min, diluted with EtOAc (50 mL), and washed with 1 M HCl (aq), $NaHCO_3$ (aq), and brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **3** as a colorless oil (0.639 g, 64%). $[\alpha]_D^{22}$: +56 (c = 1, $CHCl_3$); IR (neat): 709, 1067, 1252, 1717, 2103, 2930 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 1.78 (m, 2H, CH_2 $C_3H_6N_3$), 3.25 (m, 2H, CH_2 $C_3H_6N_3$), 3.37 (d, 1H, J = 2.4 Hz, OH), 3.63 (m, 1H, CH_2 $C_3H_6N_3$), 3.87 (s, 3H, CH_3 COOMe), 4.02 (m, 1H, CH_2 $C_3H_6N_3$), 4.10 (m, 1H, H-3), 4.10 (dt, 1H, J = 2.4 Hz, 9.2 Hz, H-4), 4.75 (d, 1H, J = 7.6 Hz, H-1), 5.43 (dd, 1H, J = 8.0 Hz, 9.6 Hz, H-2), 5.54 (dd, 1H, J = 9.2 Hz, 9.6 Hz, H-5), 7.39 (m, 4H, CH Bz), 7.51 (m, 2H, CH Bz), 7.97 (m, 4H, CH Bz); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 28.9 (CH_2 $C_3H_6N_3$), 47.8 (CH_2 $C_3H_6N_3$), 53.0 (CH_3 COOMe), 66.9 (CH_2 $C_3H_6N_3$), 70.6

(C-4), 71.2 (C-2), 74.6 (C-5), 74.9 (C-3), 101.4 (C-1), 128.4 (CH Bz), 128.4 (C_q Bz), 128.9 (C_q Bz), 129.7 (CH Bz), 129.9 (CH Bz), 133.4 (CH Bz), 165.1, 166.6, 169.1 (C═O Bz, COOMe); HRMS: $C_{24}H_{25}N_3O_9 + Na^+$ requires 522.14830, found 522.14827.

Methyl (2,3-Di-*O*-benzoyl-4-*O*-levulinoyl-D-glucopyranose) Uronate (4). To a vigorously stirred solution of **2** (0.30 g, 0.50 mmol) in CH_2Cl_2 (5 mL) and H_2O (0.5 mL) were added at 0 °C NIS (112 mg, 0.50 mmol) and TFA (39 μL, 0.50 mmol). After TLC analysis showed complete consumption of starting material, the reaction was quenched with $Na_2S_2O_3$ (aq) and washed with $NaHCO_3$ (aq). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **4** as a colorless oil (0.21 g, 82%). Spectral data of the major anomer α. IR (neat): 711, 1264, 1722, 2343, 2361, 2927, 3440 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 2.03 (s, 3H, CH_3 Lev), 2.40 (m, 1H, CH_2 Lev), 2.60 (m, 3H, CH_2 Lev), 3.74 (s, 3H, CH_3 COOMe), 4.75 (d, 1H, J = 10.0 Hz, H-5), 5.08 (d, 1H, J = 4.4 Hz, OH), 5.24 (dd, 1H, J = 3.2 Hz, 10 Hz, H-2), 5.43 (dd, 1H, J = 10.0 Hz, 9.6 Hz, H-4), 5.78 (d, 1H, J = 3.6 Hz, H-1), 6.06 (dd, 1H, J = 10.0 Hz, 9.6 Hz, H-3), 7.33 (m, 4H, CH Bz), 7.48 (m, 2H, CH Bz), 7.94 (m, 4H, CH Bz); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 27.6 (CH_2 Lev), 29.3 (CH_3 Lev), 37.5 (CH_2 Lev), 52.8 (CH_3 COOMe), 67.9 (C-5), 69.5 (C-3, C-4), 71.6 (C-2), 90.2 (C-1), 128.2 (CHBz), 128.7 (C_qBz), 129.6 (CH Bz), 133.3 (CH Bz), 165.6, 165.8, 168.6, 171.3 (C═O Bz, C═O COOMe, C═O Lev), 206.2 (C═O Lev); HRMS: $C_{26}H_{26}O_{11} + H^+$ requires 515.15479, found 515.15500.

Phenyl 4,6-*O*-Benzylidene-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-1-thio-β-D-glucopyranoside (6). To a solution of **5** (0.505 g, 1.00 mmol) in pyridine (10 mL) was added a solution of Lev_2O in dioxane (0.5 M, 5.0 mL, 2.5 mmol). After 18 h the mixture was diluted with EtOAc (mL) and washed with 1 M HCl (aq), $NaHCO_3$ (aq), and brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **6** as a off-white solid (0.523 g, 87%). $[\alpha]_D^{22}$: −47 (c = 1, $CHCl_3$); IR (neat): 750, 824, 1081, 1534, 1688, 2882, 3312 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 2.08 (s, 3H, CH_3 Lev), 2.54 (m, 2H, CH_2 Lev), 2.67 (m, 2H, CH_2 Lev), 3.52 (dd, 1H, J = 9.6 Hz, 4.8 Hz, H-5), 3.69 (m, 2H, m, H-4, H-6), 4.04 (dd, 1H, J = 10.0 Hz, 9.6 Hz, H-2), 4.11 (dd, 1H, J = 10.0 Hz, 4.8 Hz, H-6), 4.92 (d, 1H, J = 10.4 Hz, H-1), 5.50 (m, 2H, H-3, C*H*Ph), 7.23 (d, 1H, J = 9.6 Hz, NH), 7.24 (m, 6H, CH_{arom}), 7.46 (m, 4H, CH_{arom}); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 28.0 (CH_2 Lev), 29.6 (CH_3 Lev), 37.9 (CH_2 Lev), 55.0 (C-2), 68.2 (C-6), 70.6 (C-5), 72.4 (C-3), 78.3 (C-4), 87.1 (C-1), 92.3 (CCl_3), 101.1 (*C*HPh), 126.0−129.1 (CH_{arom}), 132.0 (C_q SPh), 132.8 (CH_{arom}), 136.8 (C_q CHPh), 161.8, 173.1 (C═O TCA, C═O Lev), 205.6 (C═O Lev); HRMS: $C_{26}H_{26}Cl_3NO_7S + Na^+$ requires 624.03878, found 624.03870.

Phenyl (4,6-*O*-Benzylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-levulinoyl-β-D-glucopyranosyl) uronate)-1-thio-β-D-glucopyranoside (7). A mixture of 1-hydroxy donor **4** (0.514 g, 1.00 mmol), Ph_2SO (0.485 g, 2.40 mmol), and TTBP (0.248 g, 1.00 mmol) was coevaporated with toluene two times to remove traces of water, dissolved in DCM (20 mL), and further dried by stirring over molecular sieves for 15 min. At −60 °C Tf_2O (0.177 mL, 1.05 mmol) was added and the temperature was raised to −40 °C. After 1 h. a solution of acceptor **5** (0.505 g, 1.00 mmol) in DCM (20 mL) was slowly added, and the reaction mixture was allowed to warm to 0 °C. Dry Et_3N (1.35 mL, 10 mmol) was added, the reaction was washed with $NaHCO_3$ (aq), and the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **7** as a white solid (0.314 g, 56%). IR (neat): 709, 1090, 1271, 1718, 2360, 2930, 3334 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 2.01 (s, 3H, CH_3 Lev), 2.34 (m, 1H, CH_2 Lev), 2.50 (m, 3H, CH_2 Lev), 3.45 (m, 1H, H-2), 3.62 (dt, 1H, J = 4.8 Hz, 9.6 Hz, H-5), 3.66 (s, 3H, CH_3 COOMe), 3.81 (m, 3H, H-4, H-5′, H-6), 4.35 (dd, 1H, J = 5.2 Hz, 10.8 Hz, H-6), 4.69 (t, 1H, J = 9.2 Hz, H-3), 5.02 (d, 1H, J = 7.6 Hz, H-1′), 5.37 (m, 2H, H-2′, H-4′), 5.44 (d, 1H, J = 10.4 Hz, H-1), 5.51 (s, 1H, C*H*Ph), 5.54 (t, 1H, J = 9.6 Hz, H-3′), 6.98 (d, 1H, J = 6.8 Hz, NH), 7.29−7.43 (m, 16H, CH_{arom}), 7.83 (m, 4H, CH_{arom}); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 27.6 (CH_2 Lev), 29.6 (CH_3 Lev), 37.5 (CH_2 Lev), 52.9 (CH_3 COOMe), 57.4 (C-2), 68.6 (C-6), 69.3 (C-2′), 70.5 (C-5), 71.9 (C-4′), 72.0 (C-5′), 72.5 (C-3′), 77.0 (C-3), 79.7 (C-4), 84.1 (C-1), 99.3 (C-1′), 101.5 (*C*HPh), 126.0 (CH_{arom}), 128.4−128.7 (CH_{arom}), 128.8 ($C_{q\ arom}$),129.2−129.9 (CH_{arom}), 131.1 ($C_{q\ arom}$), 133.4−133.5 (CH_{arom}), 136.9 ($C_{q\ arom}$), 161.7, 164.9, 165.5, 167.0, 171.1 (C═O TCA, C═O Bz, C═O COOMe, C═O lev), 205.5 (C═O lev); HRMS: $C_{47}H_{44}Cl_3NO_{15}S + NH_4^+$ requires 1017.18355, found 1017.18301.

3-Azidopropyl (Methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-levulinoyl-β-D-glucopyranosyl) uronate)-β-d-glucopyranosyl)-β-D-glucopyranoside) Uronate (8). A mixture of 1-thio donor **7** (0.314 g, 0.314 mmol), Ph_2SO (0.070 g, 0.345 mmol), and TTBP (0.078 g, 0.314 mmol) was coevaporated with toluene two times to remove traces of water, dissolved in DCM (6 mL), and further dried by stirring over molecular sieves for 15 min. At −60 °C Tf_2O (55 μL, 0.329 mmol) was added, and after 15 min at −60 °C a solution of acceptor **3** (0.191 g, 0.377 mmol) in DCM (3 mL) was slowly added and the reaction mixture was allowed to warm to 0 °C. Dry Et_3N (0.44 mL, 3.14 mmol) was added, the reaction was washed with $NaHCO_3$ (aq), and the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **8** as a colorless oil (0.204 g, 47%). $[\alpha]_D^{22}$ +31 (c = 1, $CHCl_3$); IR (neat): 709, 1027, 1045, 1267, 1726, 2099, 2361, 2927, 3338 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 1.75 (m, 2H, CH_2 $C_3H_6N_3$), 2.01 (s, 3H, CH_3 Lev), 2.32 (m, 1H, CH_2 Lev), 2.51 (m, 3H, CH_2 Lev, H-6′), 3.26 (m, 2H, CH_2 $C_3H_6N_3$), 3.32 (dt, 1H, J = 4.8 Hz, 9.6 Hz, H-5′), 3.37 (q, 1H, J = 9.6 Hz, H-2′), 3.46 (t, 1H, J = 9.0 Hz, H-4′), 3.59 (m, 1H, CH_2 $C_3H_6N_3$), 3.63 (s, 3H, CH_3 COOMe), 3.69 (dd, 1H, J = 4.8 Hz, 10.8 Hz, H-6′), 3.79 (s, 3H, CH_3 COOMe), 3.79 (d, 1H, J = 9.6 Hz, H-5″), 3.94 (m, 1H, CH_2 $C_3H_6N_3$), 4.04 (d, 1H, J = 9.6 Hz, H-5), 4.34 (t, 1H, J = 9.0 Hz, H-4), 4.40 (t, 1H, J = 9.6 Hz, H-3′), 4.70 (d, 1H, J = 7.2 Hz, H-1″), 4.93 (d, 1H, J = 7.8 Hz, H-1), 5.11 (d, 1H, J = 8.4 Hz, H-1′), 5.14 (s, 1H, C*H*Ph), 5.34 (m, 3H, H-2, H-2″, H-3″), 5.49 (t, 1H, J = 9.6 Hz, H-4″), 5.57 (t, 1H, J = 9.6 Hz, H-3), 6.74 (d, 1H, J = 7.8 Hz, NH), 7.31−7.58 (m, 17H, CH_{arom}), 7.81 (d, 2H, J = 7.2 Hz, CH Bz), 7.85 (d, 2H, J = 7.2 Hz, CH Bz), 7.93 (d, 2H, J = 7.2 Hz, CH Bz), 7.99 (d, 2H, J = 7.2 Hz, CH Bz); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 27.6 (CH_2 Lev), 28.9 (CH_2 $C_3H_6N_3$), 29.6 (CH_3 Lev), 37.6 (CH_2 Lev), 47.8 (CH_2 $C_3H_6N_3$), 52.8 (CH_3 COOMe), 53.2 (CH_3 COOMe), 58.4 (C-2′), 65.9 (C-5′), 66.9 (CH_2 $C_3H_6N_3$), 67.7 (C-6′), 69.4 (C-3″), 71.5, 71.9 (C-2, C-2″), 72.1 (C-5″), 72.4 (C-3), 72.6 (C-4″), 74.0 (C-5), 75.8 (C-4), 76.4 (C-3′), 79.6 (C-4′), 98.5 (C-1′), 99.6 (C-1), 101.2 (*C*HPh), 101.4 (C-1″), 126.1 (CH_{arom}), 128.3−128.9 (CH_{arom}), 128.9−129.2 ($C_{q\ arom}$), 129.8−130.0 (CH_{arom}), 133.3−133.4 (CH_{arom}), 136.9 ($C_{q\ arom}$), 161.4, 164.9, 165.2, 165.3, 165.6, 167.0, 168.3, 171.1 (C═O TCA, C═O Bz, C═O COOMe, C═O lev), 205.7 (C═O Lev); HRMS: $C_{65}H_{63}Cl_3N_4O_{24} + H^+$ requires 1389.29706, found 1389.29504.

3-Azidopropyl (Methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-β-D-glucopyranosyl) uronate)-β-D-glucopyranosyl)-β-D-glucopyranoside) uronate) (9). HA-trimer (**8**) (204 mg, 0.147 mmol) was dissolved in a mixture of pyridine (2.35 mL) and AcOH (0.58 mL), after which hydrazine monohydrate (0.036 mL, 0.735 mmol) was added. The mixture was stirred for 15 min and diluted with EtOAc (20 mL) and washed with 1 M HCl (aq), $NaHCO_3$ (aq), and brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **9** as a white solid (182 mg, 96%). IR (neat): 705, 1027, 1091, 1264, 1711, 1734, 2098, 2343, 2360, 2890, 3374, 3503 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 1.76 (m, 2H, CH_2 $C_3H_6N_3$), 2.56 (t, 1H, J = 10.4 Hz, H-6′), 3.16 (d, 1H, J = 3.2 Hz, OH), 3.28 (m, 2H, CH_2 $C_3H_6N_3$), 3.34 (dd, 1H, J = 4.8 Hz, 9.6 Hz, H-5′), 3.42 (m, 2H, H-4′ H-2′), 3.59 (m, 1H, CH_2 $C_3H_6N_3$), 3.71

(m, 2H, H-6′, H-5″), 3.73 (s, 3H, CH_3 COOMe), 3.79 (s, 3H, CH_3 COOMe), 3.94 (m, 1H, CH_2 $C_3H_6N_3$), 4.05 (d, 1H, J = 9.2 Hz, H-5), 4.09 (dd, 1H, J = 3.2 Hz, 9.2 Hz, H-4″), 4.34 (t, 1H, J = 9.2 Hz, H-4), 4.38 (t, 1H, J = 9.2 Hz, H-3′), 4.70 (d, 1H, J = 7.2 Hz, H-1″), 4.93 (d, 1H, J = 7.2 Hz, H-1), 5.10 (d, 1H, J = 8.4 Hz, H-1′), 5.18 (s, 1H, C*H*Ph), 5.34 (m, 3H, H-2, H-2″, H-3″), 5.58 (t, 1H, J = 9.2 Hz, H-3), 6.72 (d, 1H, J = 8.0 Hz, NH), 7.31−7.58 (m, 17H, CH_{arom}), 7.88 (m, 4H, CH Bz), 7.93 (m, 2H, CH Bz), 7.99 (m, 2H, CH Bz); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 28.9 (CH_2 $C_3H_6N_3$), 47.8 (CH_2 $C_3H_6N_3$), 52.7 (CH_3 COOMe), 53.2 (CH_3 COOMe), 58.3 (C-2′), 65.9 (C-5′), 66.9 (CH_2 $C_3H_6N_3$), 67.7 (C-6′), 70.2 (C-4″), 71.5, 71.7 (C-2, C-2″), 72.5 (C-3), 74.0 (C-5), 74.1 (C-5″), 75.1 (C-3″), 75.9 (C-4), 76.2 (C-3′), 79.5 (C-4′), 98.8 (C-1′), 99.5 (C-1″), 101.2 (C-1), 101.4 (*C*HPh), 125.9 (CH_{arom}), 128.3−128.4 (CH_{arom}), 128.9−129.2 ($C_{q\ arom}$), 129.7−130.0 (CH_{arom}), 133.3−133.4 (CH_{arom}), 137.0 ($C_{q\ arom}$), 161.4, 165.0, 165.1, 165.2, 166.4, 168.4, 169.0 (C═O TCA, C═O Bz, C═O COOMe); HRMS: $C_{60}H_{57}Cl_3N_4O_{22}$ + NH_4^+ requires 1308.28683, found 1308.28478.

3-Azidopropyl (Methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-<bold>β-D-glucopyranosyl)-β-D-glucopyranosyl) uronate)-β-d-glucopyranosyl)-β-D-glucopyranoside) uronate) (11). A mixture of 1-thio donor **6** (0.081 g, 0.135 mmol), Ph_2SO (0.030 g, 0.148 mmol), and TTBP (0.034 g, 0.135 mmol) was coevaporated with toluene two times to remove traces of water, dissolved in DCM (2.7 mL), and further dried by stirring over molecular sieves for 15 min. At −60 °C Tf_2O (24 μL, 0.141 mmol) was added, and after 15 min at −60 °C a solution of trisaccharide acceptor **9** (0.145 g, 0.112 mmol) in DCM (1.1 mL) was slowly added and the reaction mixture was allowed to warm to −15 °C. Dry Et_3N (0.2 mL, 1.3 mmol) was added, and the reaction was washed with $NaHCO_3$ (aq). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **11** as a colorless oil (0.124 g, 62%). IR (neat): 708, 1027, 1070, 1265, 1718, 2100, 2342, 2360, 2926 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.75 (m, 2H, CH_2 $C_3H_6N_3$), 2.10 (s, 3H, CH_3 Lev), 2.45 (t, 1H, J = 10.4 Hz, H-6‴), 2.54 (m, 3H, CH_2 Lev, H-6′), 2.66 (m, 2H, CH_2 Lev), 3.23 (m, 2H, CH_2 $C_3H_6N_3$), 3.29 (m, 2H, H-5′, H-5‴), 3.39 (m, 2H, H-4′, H-4‴), 3.46 (m, 2H, H-2′, H-6‴), 3.61 (m, 1H, CH_2 $C_3H_6N_3$), 3.65 (s, 3H, CH_3 COOMe), 3.69 (dd, 1H, J = 4.4 Hz, 10.4 Hz, H-6′), 3.81 (s, 3H, CH_3 COOMe), 3.87 (m, 2H, H-2‴, H-5), 3.94 (m, 1H, CH_2 $C_3H_6N_3$), 4.05 (d, 1H, J = 9.2 Hz, H-5″), 4.16 (t, 1H, J = 9.2 Hz, H-4), 4.32 (t, 1H, J = 9.6 Hz, H-3′), 4.33 (t, 1H, J = 9.6 Hz, H-4″), 4.71 (d, 1H, J = 7.2 Hz, H-1″), 4.87 (d, 1H, J = 8.4 Hz, H-1‴), 4.96 (d, 1H, J = 6.8 Hz, H-1), 5.06 (d, 1H, J = 8.0 Hz, H-1′), 5.14 (s, 1H, C*H*Ph), 5.18 (s, 1H, C*H*Ph), 5.22 (t, 1H, J = 10.0 Hz, H-3‴), 5.26 (t, 1H, J = 6.8 Hz, H-2), 5.57 (dd, 1H, J = 7.6 Hz, 9.6 Hz, H-2″), 5.48 (t, 1H, J = 9.6 Hz, H-3), 5.58 (t, 1H, J = 9.6 Hz, H-3″), 6.71 (d, 1H, J = 8.0 Hz, NH), 6.88 (d, 1H, J = 9.2 Hz, NH), 7.31−7.58 (m, 22H, CH_{arom}), 7.85 (d, 2H, J = 7.6 Hz, CH Bz), 7.91 (m, 4H, CH Bz), 7.99 (d, 2H, J = 7.2 Hz, CH Bz); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 28.0 (CH_2 Lev), 28.8 (CH_2 $C_3H_6N_3$), 29.7 (CH_3 Lev), 37.9 (CH_2 Lev), 47.8 (CH_2 $C_3H_6N_3$), 52.8 (CH_3 COOMe), 53.2 (CH_3 COOMe), 56.1 (C-2‴), 58.1 (C-2′), 65.9 (C-5′), 66.1 (C-5‴), 66.9 (CH_2 $C_3H_6N_3$), 67.4 (C-6‴), 68.0 (C-6′), 71.4 (C-2″), 71.7 (C-3″), 72.2 (C-3), 72.3 (C-2), 72.4 (C-3″), 73.4 (C-5), 73.9 (C-5″), 76.0 (C-3′), 76.1 (C-4″), 77.0 (C-4), 78.0 (C-4‴), 79.1 (C-4′), 92.3 (C_q TCA), 99.0 (C-1′), 99.6 (C-1), 100.8 (*C*HPh), 101.0 (*C*HPh), 101.0 (C-1‴), 101.4 (C-1″), 125.7−126.1 (CH_{arom}), 128.2−128.4 (CH_{arom}), 128.9−129.2 ($C_{q\ arom}$), 129.6−129.9 (CH_{arom}), 133.2−133.4 (CH_{arom}), 136.7 ($C_{q\ arom}$), 137.0 ($C_{q\ arom}$), 161.4, 161.6, 164.9−165.1, 168.5, 168.9, 172.3 (C═O TCA, C═O Bz, C═O COOMe, C═O lev), 205.8 (C═O Lev); HRMS: $C_{80}H_{77}Cl_6N_5O_{29}$ + H^+ requires 1782.29081, found 1782.29053.

3-Azidopropyl (Methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-levulinoyl-β-D-glucopyranosyl) uronate)-β-D-glucopyranosyl)-β-D-glucopyranosyl) uronate)-β-D-glucopyranosyl)-β-D-glucopyranoside) uronate) (13). A mixture of 1-thio donor **7** (0.164 g, 0.164 mmol), Ph_2SO (0.036 g, 0.177 mmol), and TTBP (0.039 g, 0.157 mmol) was coevaporated with toluene two times to remove traces of water, dissolved in DCM (3.3 mL), and further dried by stirring over molecular sieves for 15 min. At −60 °C Tf_2O (29 μL, 0.171 mmol) was added, and after 15 min at −60 °C a solution of trisaccharide acceptor **9** (0.143 g, 0.110 mmol) in DCM (1.1 mL) was slowly added and the reaction mixture was allowed to warm to −15 °C. Dry Et_3N (0.25 mL, 1.6 mmol) was added, the reaction was washed with $NaHCO_3$ (aq), and the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **13** as a colorless oil (0.116 g, 48%). IR (neat): 709, 1027, 1069, 1267, 1728, 2100, 2342, 2360, 2926 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.75 (m, 2H, CH_2 $C_3H_6N_3$), 2.01 (s, 3H, CH_3 Lev), 2.45 (t, 1H, J = 6.8 Hz, H-6′ or H-6‴), 2.45 (t, 1H, J = 6.4 Hz, H-6′ or H-6‴), 2.35 (m, 1H, CH_2 Lev), 2.51 (m, 3H, CH_2 Lev), 3.25 (m, 5H, CH_2 $C_3H_6N_3$, H-5′, H-5‴, H-2‴), 3.39 (m, 3H, H-2′, H-4′, H-4‴), 3.59 (m, 1H, CH_2 $C_3H_6N_3$), 3.62 (s, 3H, CH_3 COOMe), 3.63 (s, 3H, CH_3 COOMe), 3.67 (m, 2H, H-6′, H-6‴), 3.78 (m, 2H, H-5, H-5‴), 3.80 (s, 3H, CH_3 COOMe), 3.85 (m, 1H, CH_2 $C_3H_6N_3$), 4.05 (d, 1H, J = 9.6 Hz, H-5″), 4.30 (m, 3H, H-3‴, H-4, H-4″), 4.43 (t, 1H, J = 9.2 Hz, H-3′), 4.71 (d, 1H, J = 7.2 Hz, H-1), 4.90 (d, 1H, J = 8.0 Hz, H-1″ or H-1⁗), 4.92 (d, 1H, J = 8.0 Hz, H-1″ or H-1⁗), 5.04 (d, 1H, J = 8.0 Hz, H-1′), 5.08 (d, 1H, J = 8.4 Hz, H-1‴), 5.10 (s, 1H, C*H*Ph), 5.17 (s, 1H, C*H*Ph), 5.22 (dd, 1H, J = 6.8 Hz, 9.6 Hz, H-2⁗), 5.34 (m, 3H, H-2, H-2″, H-4⁗), 5.39 (t, 1H, J = 9.2 Hz, H-3⁗), 5.48 (t, 1H, J = 9.2 Hz, H-3), 5.57 (t, 1H, J = 9.2 Hz, H-3″), 6.66 (d, 1H, J = 7.2 Hz, NH), 6.72 (d, 1H, J = 8.0 Hz, NH), 7.27−7.57 (m, 28H, CH_{arom}), 7.78−7.98 (m, 12H, CH Bz); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 27.6 (CH_2 Lev), 28.9 (CH_2 $C_3H_6N_3$), 29.6 (CH_3 Lev), 37.5 (CH_2 Lev), 47.8 (CH_2 $C_3H_6N_3$), 52.8 (CH_3 COOMe), 52.9 (CH_3 COOMe), 53.2 (CH_3 COOMe). 58.1 (C-2′), 58.5 (C-2‴), 65.8, 65.9 (C-5′, C-5‴), 66.9 (CH_2 $C_3H_6N_3$), 67.6 (C-6′ and C-6‴), 69.3, 71.4, 71.9, 72.1, 72.2, 72.4, 72.6 (C-2, C-2″, C-2⁗, C-3, C-3″, C-3⁗), 73.8, 74.0 (C-5, C-5″, C-5⁗), 75.4, 76.0, 76.2 (C-3′, C-3‴, C-4, C-4″, C-4⁗), 79.3, 79.5 (C-4′, C-4‴), 92.3 (C_q TCA), 98.4 (C-1‴), 99.0 (C-1′), 99.5 (C-1″), 99.8 (C-1⁗), 100.8 (*C*HPh), 101.1 (*C*HPh), 101.4 (C-1), 125.8−126.2 (CH_{arom}), 128.3−128.4 (CH_{arom}), 128.7−129.4 ($C_{q\ arom}$), 129.7−123.0 (CH_{arom}), 133.3−133.3 (CH_{arom}), 136.9 ($C_{q\ arom}$), 137.0 ($C_{q\ arom}$), 161.4, 161.4, 164.8−165.5, 166.9, 168.1, 168.5, 171.1 (C═O TCA, C═O Bz, C═O COOMe, C═O lev), 205.6 (C═O Lev); HRMS: $C_{101}H_{95}Cl_6N_5O_{37}$ + NH_4^+ + Na^+ requires 1110.20338, found 1110.20361.

3-Azidopropyl (4-*O*-(2-Deoxy-2-acetamido-3-*O*-(β-D-glucopyranuronic acid)-β-D-glucopyranosyl)-β-D-glucopyranuronic Acid (10). Trisaccharide **8** (44 mg, 0.032 mmol) was dissolved in MeOH (5 mL), and a catalytic amount of *p*-toluenesulfonic acid was added. The reaction mixture was stirred for 15 h, quenched with Et_3N (0.1 mL), and concentrated *in vacuo*. The remaining syrup was taken up in a mixture of THF and H_2O (6 mL, 1/1 v/v), and a 0.5 M solution of KOH in H_2O (0.64 mL, 0.32 mmol) was added stepwise (1 equiv) over a period of 48 h. The reaction mixture was stirred for 4 days after which it was quenched with Amberlite H^+, concentrated *in vacuo*, and desalted by gel filtration. The resulting sugar was then taken up in MeOH (5 mL) and Ac_2O (0.25 mL). After 2 h this mixture was coevaporated three times with MeOH and toluene (1/1 v/v) and concentrated *in vacuo*. Purification by gel filtration and lyophilization yielded **10** as a white solid (12 mg, 58%). ^{1}H NMR (600 MHz, D_2O): δ = 1.83 (m, 2H, CH_2 $C_3H_6N_3$), 1.97 (s, 3H, CH_3 NHAc), 3.26 (m, 2H, H-2, H-2″), 3.85 (t, 2H, J = 7.2 Hz, CH_2 $C_3H_6N_3$), 3.43−3.44 (m, 2H), 3.47−3.54 (m, 2H), 3.63−3.74 (m, 7H), 3.79 (t, 1H, J = 8.4 Hz, H-2′), 3.86 (d, 1H, J = 10.8 Hz, H-6′), 3.91 (m, 1H, CH_2 $C_3H_6N_3$), 4.40 (m, 2H, H-1,

H-1″), 4.51 (d, 1H, J = 8.4 Hz, H-1′); ^{13}C NMR (150 MHz, D_2O) δ = 23.4 (CH_3 NHAc), 29.2 (CH_2 $C_3H_6N_3$), 48.8 (CH_2 $C_3H_6N_3$), 55.2 (C-2′), 61.5 (C-6′), 68.5 (CH_2 $C_3H_6N_3$), 69.4, 72.7, 73.6, 73.7, 74.8, 76.2, 76.3, 76.9, 77.6, 81.1, 83.9 (C-2, C-2″, C-3, C-3′, C-3″, C-4, C-4′, C-4″, C-5, C-5′, C-5″), 101.6 (C-1′), 103.4 (C-1″), 104.0 (C-1), 175.2, 175.9, 176.5 (C═O COOH, C═O NHAc); HRMS: $C_{23}H_{36}N_4O_{18}$ + H^+ requires 657.20974, found 657.20997.

3-Azidopropyl (4-*O*-(2-Deoxy-2-acetamido-3-*O*-(4-*O*-(2-deoxy-2-acetamido-*β*-D-glucopyranosy l)-*β*-D-glucopyranuronic acid)-*β*-D-glucopyranosyl)-*β*-D-glucopyranuronic Acid (12). Tetrasaccharide **11** (80 mg, 0.045 mmol) was dissolved in MeOH (5 mL), and a catalytic amount of *p*-toluenesulfonic acid was added. The reaction mixture was stirred for 15 h, quenched with Et_3N (0.1 mL), and concentrated *in vacuo*. The remaining syrup was taken up in a mixture of THF and H_2O (9 mL, 1/1 v/v), and a 0.5 M solution of KOH in H_2O (1 mL, 0.5 mmol) was added stepwise (1 equiv) over a period of 48 h. The reaction mixture was stirred for 7 days after which it was quenched with Amberlite H^+, concentrated *in vacuo*, and desalted by gel filtration. The resulting sugar was then taken up in MeOH (9 mL) and Ac_2O (0.25 mL). After 2 h this mixture was coevaporated three times with toluene and concentrated *in vacuo*. Purification by gel filtration and lyophilization yielded **12** as a white solid (21 mg, 54%). ^{1}H NMR (600 MHz, D_2O): δ = 1.83 (m, 2H, CH_2 $C_3H_6N_3$), 1.96 (s, 3H, CH_3 NHAc), 1.99 (s, 3H, CH_3 NHAc), 3.25−3.30 (m, 2H, H-2, H-2″), 3.37−3.40 (m, 4H), 3.43−3.48 (m, 2H), 3.50−3.54 (m, 2H), 3.62−3.72 (m, 10H), 3.77−3.80 (m, 1H), 3.85−3.87 (m, 2H, H-6′, H-6‴), 3.91 (m, 1H, CH_2 $C_3H_6N_3$), 4.40 (m, 2H, H-1, H-1″), 4.47 (d, 1H, J = 8.4 Hz, H-1′ or H-1‴), 4.50 (d, 1H, J = 8.4 Hz, H-1′ or H-1‴); ^{13}C NMR (150 MHz, D_2O) δ = 23.3 (CH_3 NHAc), 23.4 (CH_3 NHAc), 29.2 (CH_2 $C_3H_6N_3$), 48.8 (CH_2 $C_3H_6N_3$), 55.2, 56.3, 61.4 (C-2′, C-2‴, C-6′, C-‴), 68.5 (CH_2 $C_3H_6N_3$), 69.4, 70.6, 73.4, 73.7, 74.5, 74.7, 76.3, 76.8, 77.2, 77.6, 80.7, 81.1, 83.4 (C-2, C-2″, C-3, C-3′, C-3″, C-3‴, C-4, C-4′, C-4″, C-4‴, C-5, C-5′, C-5″, C-5‴), 101.6 (C-1′ and C-1‴), 103.4 (C-1 or C-1″), 104.1 (C-1 or C-1″), 175.1, 175.2, 175.8, 175.9 (C═O COOH, C═O NHAc); HRMS: $C_{31}H_{49}N_5O_{23}$ + H^+ requires 860.28911, found 860.28932.

3-Azidopropyl (4-*O*-(2-Deoxy-2-acetamido-3-*O*-(4-*O*-(2-deoxy-2-acetamido-3-*O*-(*β*-D-glucopyranonic acid)-*β*-D-glucopyranosyl)-*β*-D-glucopyranuronic acid)-*β*-D-glucopyranosyl)-*β*-D-glucopyranuronic Acid (14). Pentasaccharide **13** (53 mg, 0.024 mmol) was dissolved in MeOH (5 mL), and a catalytic amount of *p*-toluenesulfonic acid was added. The reaction mixture was stirred for 15 h, quenched with Et_3N (0.1 mL), and concentrated *in vacuo*. The remaining syrup was taken up in a mixture of THF and H_2O (8 mL, 1/1 v/v), and a 0.5 M solution of KOH in H_2O (0.72 mL, 0.36 mmol) was added stepwise (1 equiv) over a period of 64 h. The reaction mixture was stirred for 12 days after which it was quenched with Amberlite H^+, concentrated *in vacuo*, and desalted by gel filtration. The resulting sugar was then taken up in MeOH (5 mL) and Ac_2O (0.25 mL). After 2 h this mixture was coevaporated three times with toluene and concentrated *in vacuo*. Purification by gel filtration and lyophilization yielded **14** as a white solid (12 mg, 48%). ^{1}H NMR (600 MHz, D_2O): δ = 1.84 (m, 2H, CH_2 $C_3H_6N_3$), 1.96 (s, 3H, CH_3 NHAc), 1.97 (s, 3H, CH_3 NHAc), 3.25−3.30 (m, 2H, H-2, H-2″, H-2⁗), 3.85 (m, 2H, CH_2 $C_3H_6N_3$), 3.43−3.45 (m, 4H), 3.46−3.50 (m, 2H), 3.51−3.54 (m, 2H), 3.61−3.73 (m, 10H), 3.78 (m, 2H, H-2′, H-2‴), 3.85 (m, 2H, H-6′, H-6‴), 3.91 (m, 1H, CH_2 $C_3H_6N_3$), 4.40 (m, 3H, H-1, H-1″, H-1⁗), 4.50 (m, 2H, H-1′, H-1‴); ^{13}C NMR (150 MHz, D_2O) δ = 23.4 (CH_3 NHAc), 29.2 (CH_2 $C_3H_6N_3$), 48.8 (CH_2 $C_3H_6N_3$), 55.2 (C-2′, C-2‴), 61.4 (C-6′, C-6‴), 68.4 (CH_2 $C_3H_6N_3$), 69.4, 69.5, 72.7, 73.4, 73.7, 74.5, 74.8, 76.2, 76.3, 76.3, 76.7, 77.3, 77.7, 80.8, 81.1, 83.5, 84.0 (C-2, C-2″, C-2⁗, C-3, C-3′, C-3″, C-3‴, C-3⁗, C-4, C-4′, C-4″, C-4‴, C-4⁗, C-5, C-5′, C-5″, C-5‴, C-5⁗), 101.5, 101.6 (C-1′, C-1‴), 103.4, 104.0, 104.2 (C-1, C-1″, C-1⁗), 175.1, 175.9, 176.5 (C═O COOH, C═O NHAc); HRMS: $C_{37}H_{57}N_5O_{29}$ + H^+ requires 1036.32120, found 1036.32094.

Supporting Information Available: General experimental and NMR spectra of all new compounds. This information is available free of charge via the Internet at http://pubs.acs.org.

JO070704S